

Molecular Design for Precise Sequence Control and Functions of Alternating Copolymers

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GENERAL INTRODUCTION

Background

Peptides are the copolymers made of 20 kinds of comonomers, i.e., amino acids. The significant difference from synthetic copolymers is that the order of composed amino acid units (that is “sequence”) can be perfectly defined. In other words, there is no distribution of sequence as well as composition ratio among chains for one type of peptide. Thus, one-to-one relationship exists between sequence and functions. It is noteworthy that the template system on the basis of central dogma allows the perfect sequence control, although reactivity for the aminolysis reaction between amine and carboxylic acid is basically identical regardless of the amino acid residue.¹

Radical polymerization² is one of chain-growth polymerizations and very useful for the progress of copolymerization combining various comonomers, but the synthetic process is absolutely no match for the peptide production in nature in terms of structural control. For example, it would be reckless to attempt sequence control for many types of acrylate units having different pendant groups via the chain-growth mechanism. From a more realistic viewpoint, we should begin by sequence control for two types of units. Figure 1 shows representative sequence patterns made of two comonomers and herein the averaged composition ratio is 1:1 in every case. For example, radical copolymerization of two simple alkyl acrylate monomers would give “random copolymer”, unless there is special interaction and/or steric hinderance between the comonomers. On the other hand, when methacrylate and acrylate are copolymerized via reversible deactivation radical polymerization (RDRP),³ “gradient copolymer” could be obtained due to the higher reactivity of methacrylate, which is not the case via free radical polymerization. The specific example is “block copolymer” that

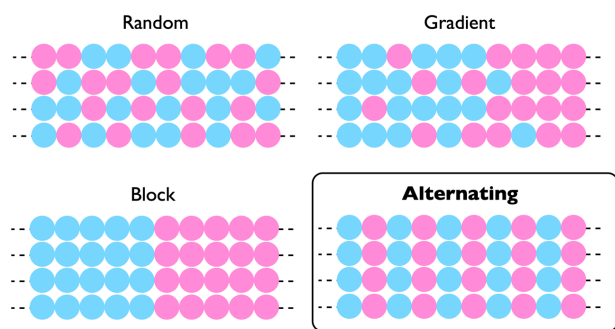


Figure 1. Four types of copolymers with 1:1 composition ratio.

can be synthesized via sequential monomer addition with RDRP or coupling between terminal groups of two polymer chains. When the compatibility between the two segments is poor, periodic aggregation structures are spontaneously induced to reduce the surface energy between the immiscible domains.

Unlike the three types of copolymers, “alternating copolymer” is distinctly unique, because it contains periodicity in the sequence and no consecutive connection of same unit. The first paper on synthesis of alternating copolymer dates back to 1930⁴ when alternating radical copolymerization of stilbene with maleic anhydride was reported. Despite the discovery long time ago, the mechanism of the cross-over propagation has been not fully understood and mainly complex participation model and penultimate unit model have been proposed.⁵ The comonomer combination leading to alternating sequence is limited, which is the cause of a few material applications. However, as mentioned below, some methodologies to synthesize alternating copolymers have been recently developed. The significant feature of the alternating copolymer is no sequence distribution as well as the periodicity. No consecutive sequence of identical unit (AA, AAA, AAAA etc.) is also characteristic of the alternating copolymers, which is different from the corresponding random or gradient copolymers. The periodic structure in the scale of repeating unit might afford to give smaller domain via the unique self-assembly than block copolymers.

In this section, the four topics are reviewed for alternating copolymers synthesized via chain-growth polymerization: (1) how to control alternating sequence; (2) sequence characterization; (3) self-assembly; (4) functions.

1. How to control Alternating Sequence

In the theory of copolymerization, a comonomer pair whose monomer reactivity ratios are almost zero would afford an alternating copolymer. In this case, the active species derived from the both monomers favor another comonomer and thus cross-over propagation takes place. The most well-known example is radical copolymerization of the pair of styrene or vinyl ether and maleic anhydride or maleimide (Figure 2a).⁶ The combination of electron-donating and -withdrawing monomers is important for the selective cross-over propagation in alternating radical copolymerization. Such alternating propagation has been also reported with other chain-growth polymerizations. For example, Aoshima's group have recently found ethanesulfonic acid (EtSO₃H)/GaCl₃ initiating system in conjunction with 1,4-dioxane as an

added base is effective for alternating cationic copolymerization of vinyl ether (VE) with benzaldehyde (BzA) (Figure 2b).⁷ The appropriate combination of substituents on VE and BzA is important to realize the alternating copolymerization as well as to suppress cyclic trimer formation of BzA. The resultant alternating copolymers are interesting as degradable polymers (see below).

Sampson et al. reported specific combinations of cyclic monomers to progress of alternating copolymerization by ruthenium-mediated ring-opening metathesis polymerization (ROMP).⁸ The typical pair is 1-cyclobutene carboxylic acid ester and cyclohexene (Figure 2c). The alternating propagation is attributed to the formation of chelated enoic ruthenium carbene species derived from the cyclobutene monomer and the subsequent metathesis reaction for cyclohexene. Several cyclobutene-based bicyclic esters and amides are also available as the comonomer and functional groups can be embedded at the appropriate position in both comonomers.

Except for the examples on the cross-over propagation on the basis of electronic mechanism, some approaches to control alternating sequence relying on external factors or special molecular design have been reported. They are roughly classified into four categories: (a) increased difference in electron density; (b) steric hindrance; (c) pseudo connection; (d) pre-sequenced monomer (Figure 3). For the first approach, electron-donating or electron-withdrawing substituent is introduced onto the pendant groups of each comonomer to enhance the difference in electron density of the double bond between two comonomers (Figure 3a). The electron density can be also tuned through the interaction with an additive. A steric

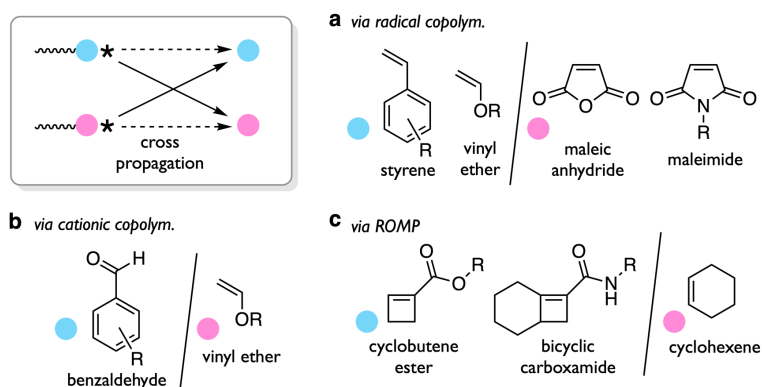


Figure 2. The monomer design for making the difference in monomer reactivities induced selective cross-over propagation at (a) radical copolymerization, (b) cationic copolymerization, and (c) ROMP.

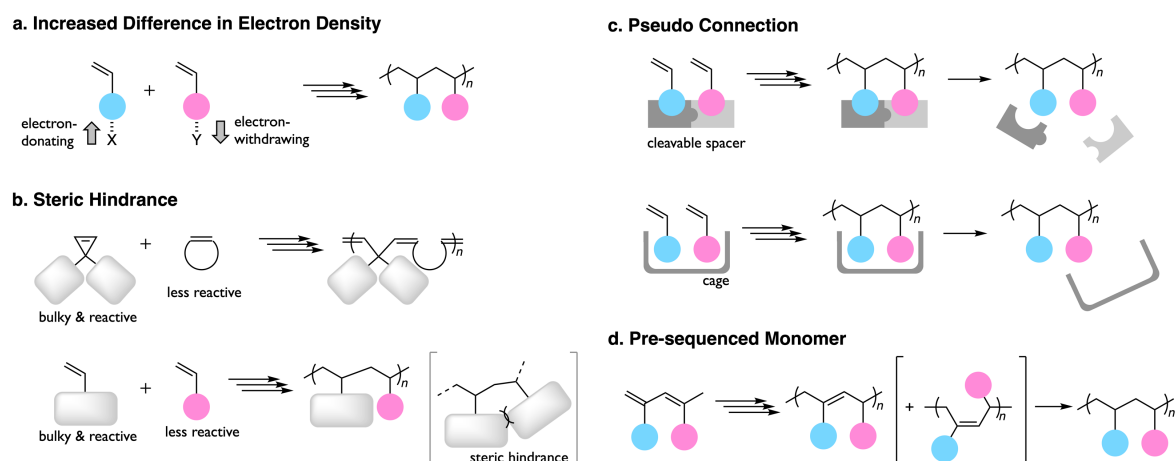


Figure 3. Synthetic approaches for controlling alternating sequence by utilizing (a) increased difference in electron density between comonomers, (b) steric hindrance of monomers, (c) pseudo connection of comonomers, and (d) pre-sequenced monomer.

hindrance is useful to suppress the propagation of same monomer and thus a combination with an appropriate comonomer could allow alternating sequence (Figure 3b). In the pseudo connection approach, comonomers are connected between the pendant groups via cleavable bonds (Figure 3c). The selective cyclopolymerization is controlled and subsequently the cyclopendant is cleaved to obtain alternating copolymers. Utilizing a cage to make comonomers close to each other also falls into the category. Information of sequence can be pre-embedded into the conjugated diene monomer (Figure 3d). The regiospecific polymerization of the pre-sequenced monomer followed by hydrogenation affords polymers equivalent of alternating copolymer made of two vinyl comonomer units.

1-1. Increased Difference in Electron Density. Radical copolymerization of methyl methacrylate (MMA) with styrene tends to give alternating-rich random sequence inherently, but the addition of $\text{AlEt}_{1.5}\text{Cl}_{1.5}$ in the copolymerization allowed the further increase in degree of alternating sequence (DAS) of the resultant copolymer (Figure 4a).⁹ Herein, the Lewis acid interacts with MMA via the coordination on the carbonyl group to decrease the electron density of the double bond to the same degree as that of maleic anhydride. Then alternating propagation takes place with styrene. Subsequently, some studies have shown that Lewis acids such as AlEtCl_2 , ZnCl_2 or SnCl_4 increased the DAS even for other comonomer pairs (styrene/acetonitrile, isoprene/MMA).¹⁰⁻¹² Several RDRP techniques, such as atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), iodide

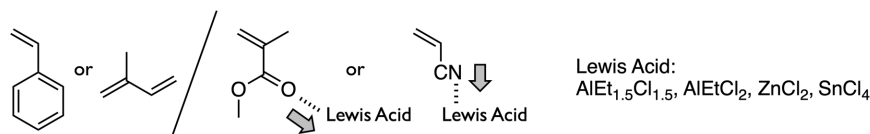
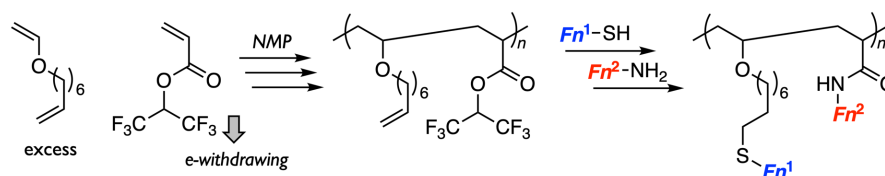
a. External Addition**b. Introduction of Substituent and Functionalization**

Figure 4. (a) External Addition for controlling alternating sequence by utilizing difference in electron density between comonomers and (b) synthetic scheme of functionalization via post-reaction of alternating copolymers bearing activated esters.

degenerative transfer polymerization (IDTP) and reversible addition-fragmentation chain transfer (RAFT) polymerization, in the presence of Lewis acid were also studied for copolymerization of MMA and styrene for simultaneous control both molecular weight and sequence.¹³

Higher DAS was also obtained via the copolymerization of an electron deficient acrylate in conjunction with an excess of vinyl ether (Figure 4b).¹⁴ 1,1,1,3,3,3-Hexafluoro-isopropyl pendant acrylate (HEIPA) was used and the pendant is transformable into amide via aminolysis due to the activated ester property. One of the reactivity ratios is not so low [$r_1 = 0.12$ and $r_2 = 0.028$ ($M_1 = \text{HEIPA}$ and $M_2 = n\text{-butyl vinyl ether}$)], which is the reason why an excess of vinyl ether is required to obtain the alternating copolymer.

1-2. Steric Hindrance. If bulky substitutes are introduced into monomers whose polymerizable groups (e.g., vinyl groups) are inherently reactive enough for polymerization, the homopolymerization ability could be lowered due to the steric hindrance. In this case, a possibility arises that alternating copolymerization proceeds with the bulky monomer in conjunction with a suitable comonomer.

1,1-Diphenylethylene (DPE) is known to show no homopolymerization ability in anionic polymerization due to steric hindrance of two phenyl groups, but the double bond is active for nucleophilic species and the highly resonance-stabilized carbanion species can propagate with other conjugated monomers. Using excess of DPE coupled with a slightly lower reactive

monomer such as butadiene derivatives,¹⁵⁻¹⁷ styrene derivatives,^{18,19} and isoprene²⁰ allows the progress of the alternating copolymerization (Figure 5a).

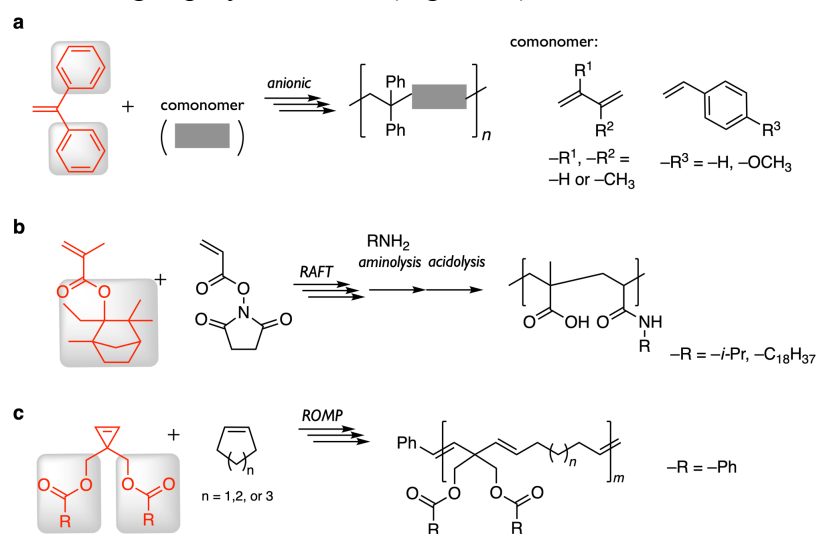


Figure 5. Synthetic schemes of alternating copolymers by utilizing steric hindrance between bulky monomers: (a) 1,1-diphenylethylene, (b) bulky *tert*-alkyl methacrylate carrying ethyl and fenchol substituents, and (c) cyclopropane with bulky substituents.

An extremely bulky *tert*-alkyl methacrylate carrying ethyl and fenchol substituents is available as the comonomer for control of alternating sequence via radical polymerization (Figure 5b).²¹ An electron-deficient acrylate carrying a *N*-succinimide substituent is appropriate to the comonomer for the bulky methacrylate monomer. Importantly, after the copolymerization, the both monomer units are transformable into methacrylic acid and acrylamide via acidolysis and aminolysis, respectively. Thus, various types of alternating copolymers of methacrylic acid with alkyl acrylamide can be synthesized.

The bulky monomer concept is available even with ring-opening metathesis polymerization (ROMP) (Figure 5c). For example, cyclopropane (CP) is highly strained and thus very reactive for ROMP. As the monomer becomes bulkier by introducing substituents, the homopolymerization ability become lower due to the steric hindrance between the monomers. However, the bulky monomer can be copolymerized with lower reactive and less bulky monomer such as cyclohexene to give the alternating copolymer.²²

1-3. Pseudo Connection. If two comonomers can be temporary connected before the copolymerization, there is a potential for occurrence of alternating propagation of the two comonomers beyond the inherent copolymerization ability predicted by the monomer reactivity

ratios. Some examples on the “pseudo connection” approach have been reported (Figure 6). For instance, two vinyl monomers are connected via a cleavable spacer and cyclopolymerization of the divinyl monomer is performed without cross-linking reaction. The embedded cleavable spacer of the resultant cyclopolymer is cleaved to lead to an alternating copolymer. The selectivity of cyclic active species to two vinyl groups in the divinyl monomer is crucial to enhance the DAS. The selectivity is higher as the r_2 value is lower (herein left monomer: M_1 ; right monomer: M_2 in Figure 6a). The design of divinyl monomer consisting of two different monomers is distinguished from those designed in conventional cyclopolymerization, where two same monomers is symmetrically introduced on a rigid scaffold.²³

As a cleavable bond between two vinyl monomers, ester or hemiacetal ester bond was used to cleave into carboxylic acid and hydroxy pendant groups after the cyclopolymerization. Thus, alternating copolymers made of methacrylic acid/acrylic acid,²⁴ methacrylic acid/2-hydroxyethyl acrylate,²⁵ and acrylic acid/2-hydroxyethyl vinyl ether²⁶ have been synthesized. In these cases, the resultant pendant groups after cleavage are limited to the carboxylic acid or hydroxy group. On the other hand, utilizing an activated ester allows incorporation of various groups into alternating copolymers at the cleavage of the bond because the activated ester bond can be converted into amide groups via the substitution-reaction (i.e., aminolysis by amine). A methacrylate-acrylate divinyl monomer carrying the activated ester and a tertiary ester was available in this concept to give alternating copolymer of methacrylic acid (MAA) and *N*-isopropyl acrylamide (NIPAM).²⁷

2,3,4,5,6-Pentafluorostyrene (PFS) is known to interact with styrene via quadrupole

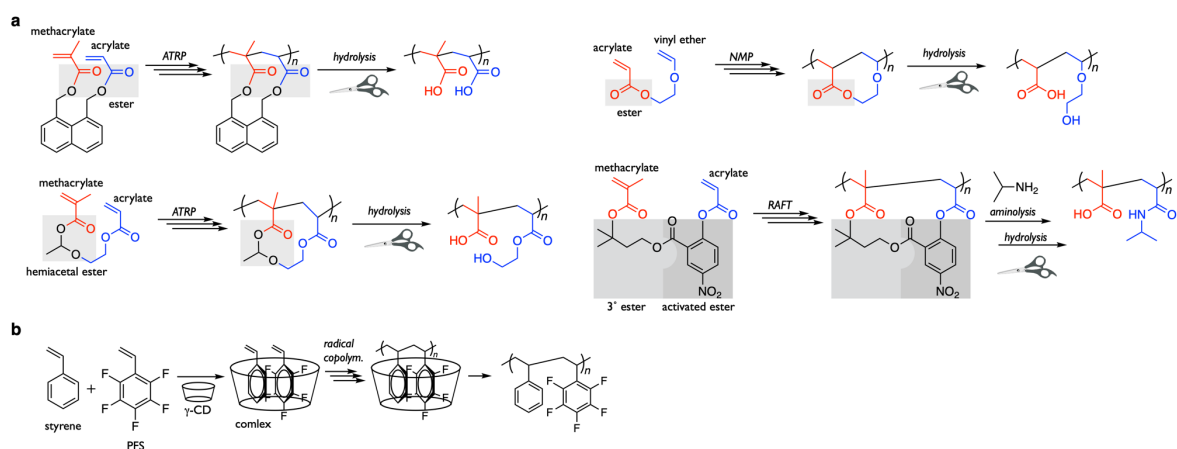


Figure 6. “Pseudo connection” approaches for control alternating sequence.

interaction.²⁸ The dynamic interaction was wrapped by γ -cyclodextrin for the tight immobilization and the resultant complex was polymerized as a pseudo divinyl monomer (Figure 6b).²⁹ In this case, the monomer reactivity ratios were slightly decreased in the presence of γ -CD: $r_1 \sim 0.2$ and $r_2 \sim 0.8$ (M_1 = styrene and M_2 = PFS) in the presence of γ -CD. Considering the reactivity ratios, the DAS of the copolymer was likely not so high.

1-4. Pre-Sequenced Monomer. A mono- or di-substituted 1,3-butadiene is available as a pre-sequenced monomer to obtain alternating copolymer (Figure 7). Herein, control of 1,4-addition-based propagation is required to obtain alternating copolymers. For example, poly(1,3-pentadiene) and poly(isoprene) obtained via 1,4-addition type propagation and subsequent hydrogenation are regarded as an alternating copolymer of ethylene and propylene, and the stereostructures of the copolymers were investigated in the 1970s.^{30,31} The selective 1,4-additions were achieved by design of the electron density and/or the bulkiness of substituents depending on the polymerization system (anionic, cationic, or radical). Particularly, the substituent on the fourth carbon is essential to determine the system. One interesting example is synthesis of alternating copolymer of styrene and vinyl alcohol.³² The copolymer is not accessible with general methodologies: the vinyl alcohol derivatives such as vinyl acetate and vinyl ether cannot be copolymerized with styrene via radical polymerization nor cationic polymerization respectively owing to the copolymerization abilities of these monomers with styrene are extremely low. Some substituted 1,3-butadiene derivatives have been designed to obtain alternating copolymers of comonomer combinations, such as styrene/ethylene,³³ ethylene/vinyl alcohol,³⁴ and vinyl ether/acrylate.³⁵

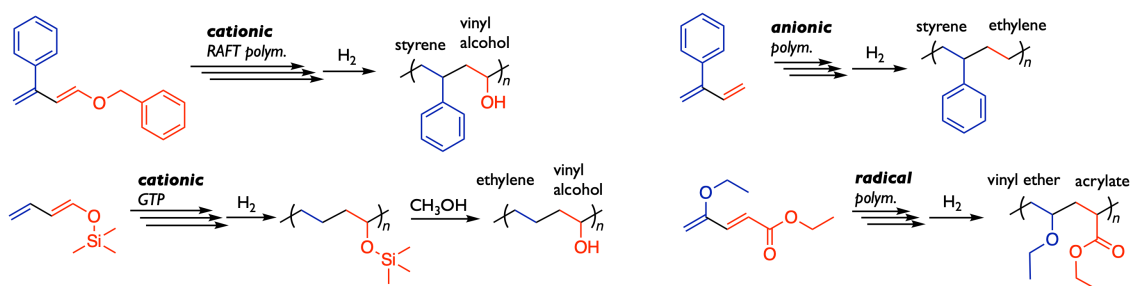


Figure 7. Synthetic scheme of alternating copolymers utilizing pre-sequenced monomers.

2. Sequence Characterization

Sequence of natural polymers such as DNA and proteins can be determined via so called sequencing,^{36,37} which is attributed to that the backbone can be selectively cleaved by enzyme. Lutz et al., have synthesized sequence-defined polymers whose backbone is designed to be cleavable into monomer units to allow the sequencing.³⁸⁻⁴¹ The sequencing process is a kind of reading sequence information and such sequence-defined polymers are expected for application of molecular tag. On the other hand, carbon-carbon based synthetic polymers are not cleavable and thus the sequence analysis is extremely difficult. Reactivity ratios and monomer consumption behaviors in living polymerization are certainly beneficial for prediction of sequence but the direct sequence characterization for obtained polymers is more desirable. Here, some attempts to analyze sequence for synthetic polymers are introduced.

2-1. Nuclear Magnetic Resonance (NMR). Since an alternating copolymer has the periodic structure where each unit is arranged adjacently, the NMR spectrum is different from that of the corresponding 1:1 random copolymer statistically containing consecutive sequence of identical unit. However, the peak identification is not so straightforward because the tacticity also affects the peak shape and pattern. For copolymers of methyl methacrylate (MMA) and methyl acrylate (MA), several peaks derived from α -methyl protons of the MMA unit in ^1H NMR are assigned depending on both tacticity and sequence.⁴² The peak analysis is available for DAS evaluation of the MMA-MA copolymer obtained by the pseudo connection approach with naphthalene spacer (see above).

An alternating sequence of copolymer can be also proved by 2D-NMR, such as ^1H - ^1H COSY,²² ^1H - ^{13}C HSQC,³² ^1H - ^{13}C HMBC,⁴³ and ^1H - ^{13}C HSQC-TOCSY.⁴³ For example, in the case with the alternating copolymer of 1,1-disubstituted cyclopropane and cyclohexane that was synthesized via ROMP (see above), ^1H - ^1H COSY clearly showed strong cross peaks corresponding to the alternating dyad.²² The sequence of alternating copolymer with MMA and isobutyl vinyl ether have been also evaluated by ^1H - ^{13}C HMBC and ^1H - ^{13}C HSQC-TOCSY.⁴³

Sequence for the copolymer of styrene and maleic anhydride was analyzed by DEPT NMR.⁴⁴ The DEPT technique allows a complicated ^{13}C NMR spectrum of the copolymer to be assigned into three types of subspectra (CH_n : $n = 1, 2$ and 3) by combination of three spectra obtained at each flip angle ($\theta = \pi/4, \pi/2$ and $3\pi/4$), which made sequence analysis easier. Herein, only the peaks of methylene carbons ($-\text{CH}_2-$) are extracted from the complicated ^{13}C

NMR spectra where the peaks derived from methylene and methine carbons were overlapped, and the extracted peaks were assigned depending on sequence as well as tacticity. The sequence of alternating copolymer with styrene and acrylonitrile was similarly analyzed.⁴⁴

An isotopic labelling by a deuterated monomer is also useful for sequence analysis. Indeed, evaluation of DAS by this methodology was reported for the copolymer of substituted cyclobutene (monomer A) with cyclohexene (monomer B) via ROMP (Figure 8).⁴⁵ For example, when deuterated monomer B is incorporated into the ideal alternating copolymer, the peaks derived from the protons of AB joints at $\delta = 5.4$ and 6.8 ppm are reduced by 100% and 50%, respectively. Namely, AA dyads in the copolymer can be detected by this methodology.

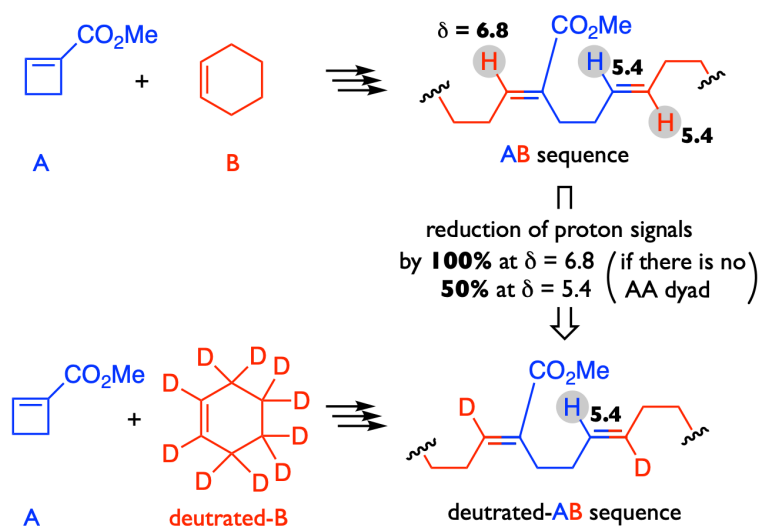


Figure 8. Isotopic labelling approach for sequence analysis of alternating copolymer.

2-2. Mass Spectrometry. MALDI-TOF-MS⁴⁶ is a powerful tool to evaluate the sequence for alternating copolymer synthesized by living polymerization. In the case of an ideal alternating copolymer, the copolymer would provide a series peaks whose mass of peak interval is alternating with each molecular weight of the comonomers or their sum. The mass values of the peaks would be consistent with the copolymers made of both units of less than one unit difference. In most cases the spectrum is more complicated and we need to discuss the errors for alternating sequence from the non-ideal peak series. For example, sequence of the copolymer synthesized via living anionic copolymerization of styrene and 1,1-diphenylethylene (DPE) was evaluated by MALDI-TOF-MS.⁴⁷ The analysis revealed that the copolymerization of styrene and DPE was found to give nearly perfect alternating copolymer in polar solvent

(THF) when the feed ratio is 1:1. On the other hand, the copolymerization in nonpolar solvent such as benzene or toluene resulted in less alternating sequence even if excess of non-polymerizable DPE was injected.

Although the application is limited to low molecular weight oligomers, ESI-MS/MS is also effective to evaluate DAS. DAS of alternating co-oligomer with styrene and maleic anhydride synthesized via NMP ($m/z < 1500$) was evaluated by ESI-MS/MS with HCD.⁴⁸ In daughter ion spectra of the alternating oligomers, HCD fragmentation peaks of the alternating oligomers were observed and the intervals of the peaks are corresponded to the molecular weights of each monomer.

3. Self-Assembly of Alternating Copolymers

The periodicity of alternating polymers could afford an order in the chain conformation and the aggregate structure. Thus, unique self-assembly behaviors different from block copolymers are expected. Here, some studies in self-assembled structures derived from the alternating sequence are introduced.

3-1. Vesicle. Alternating copolymers of *N*-alkyl maleimide with glucose-pendant vinyl ether spontaneously formed unilamellar vesicles with thin bilayer (1.7 ~ 2.6 nm) in water and the thickness depended on the alkyl length (Figure 9a).⁴⁹ Unlike amphiphilic block copolymers where immiscible long chains are connected, immiscible monomer units can be alternately aligned along the backbone for alternating copolymers. Herein, the two units can be segregated laterally along the backbone. The lateral amphiphilicity of the alternating copolymer allows forming relatively small unilamellar vesicle with thin bilayer as if lipid bilayer membranes in nature.

3-2. Flower Micelle. An alternating copolymer of sodium maleate with dodecyl vinyl ether (a kind of so-called polysoap⁵⁰) synthesized via alternating copolymerization of maleic anhydride and dodecyl vinyl ether followed by hydrolysis, formed flower micelles in NaCl aqueous solution (0.05 M) at pH 10 (Figure 9b).^{51,52} The resultant micelles have hydrophobic core mainly formed by dodecyl groups to make minimum-sized hydrophilic loops. They

showed uncore-multicore transition depending on the molecular weight of the copolymer, which was observed by light scattering, fluorescence measurements, and SAXS. The two transitional micelle structures were well fitted with the flower-micelle and -necklace model respectively. Interestingly, the model indicated 75% of hydrophobic dodecyl groups are located in the hydrophilic loops (i.e., the petal part) familiar with the water phase.

3-3. Wormlike Objects. An alternating copolymer of a dendron-pendant maleimide derivative with another type of dendron-pendant styrene gave hexagonally packed core-shell cylinder structure in bulk (Figure 9c).⁵³ Herein the maleimide side chains form the core; whereas the long alkyl chains of the styrene pendant do the shell to cover the core giving wormlike objects. As the generation of the dendron increased, the diameters of the cylinders were larger.

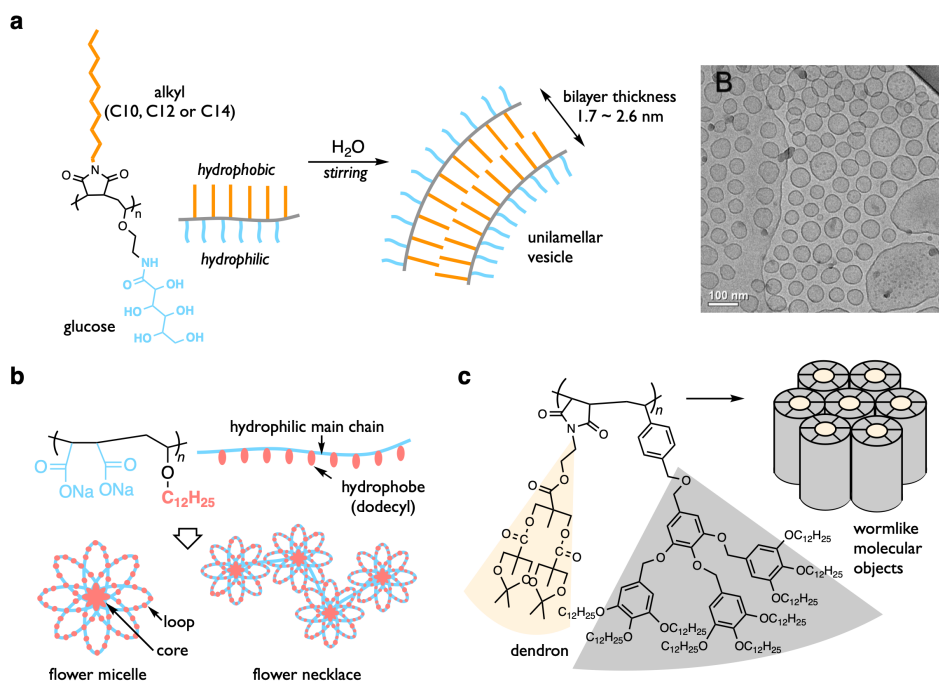


Figure 9. Unique self-assembly behaviors of alternating copolymers: (a) unilamellar vesicles, (b) flower micelle and necklace, and (c) wormlike objects: Adapted with permission from ref. 49 (Copyright American Chemical Chemistry).

4. Alternating Sequence-Driven Functions

The striking structural feature of alternating copolymers is two units are adjacently placed without consecutive sequence. Thus, unique properties/functions different from random and block copolymers are expected. The functions derived from alternating sequence are introduced in this part.

4-1. Fluorescence/Solvatochromism. Alternating copolymer of maleic anhydride and vinyl acetate exhibited fluorescence and solvatochromism in electron-rich solvents (e.g. THF, NMP, DMSO, and DMF) (Figure 10a).⁵⁴ The anhydride units in the alternating sequence can form the cluster through each other's interaction in a "through space" manner due to the rigid structure of the copolymer. The labile interaction between carbonyl groups of the two units induced solvatochromism in electron-rich solvents.

The fluorescence properties of the alternating copolymers have been also reported with the pair of styrene derivative and maleimide derivatives (Figure 10b).⁵⁵ It is assumed that the fluorescent property is attributed to the "through space" π - π interactions between the phenyl rings from styrene derivatives and carbonyl groups of the maleimide derivatives.⁵⁶ Similar fluorescence properties were also observed from the stilbene-maleic anhydride-based alternating copolymers and the fluorescence intensities of the copolymers were much higher than those of the styrene-based alternating copolymers (Figure 10c).⁵⁷ The extra phenyl

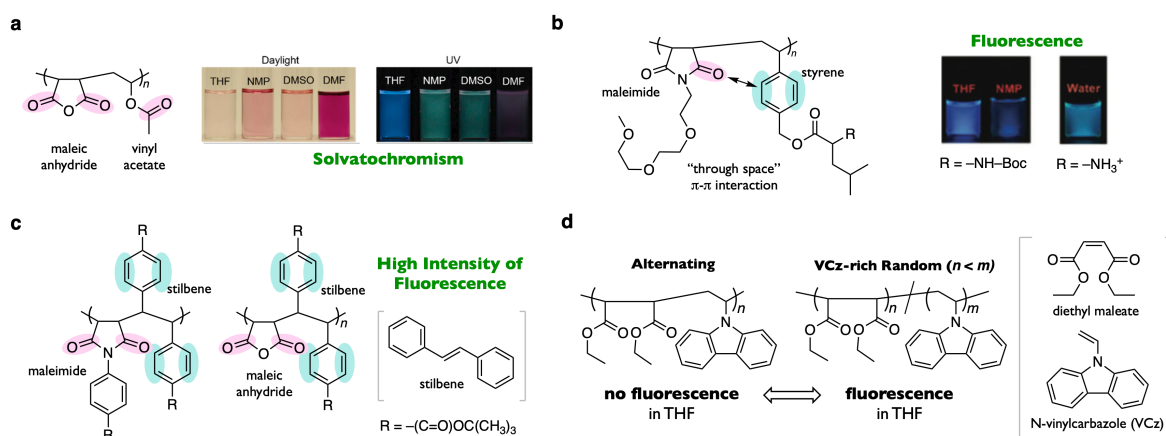


Figure 10. Fluorescence and solvatochromism properties of alternating copolymers: Adapted with permission from ref. 54 (Copyright 2015 American Chemical Society) and ref. 55 (Copyright 2016 Royal Society of Chemistry).

groups on stilbene units likely contribute to formation of the “through space” conjugate and as well as making the polymer backbone more rigid due to the steric hindrance.

On the contrary, an alternating copolymer of diethyl malate with *N*-vinylcarbazole showed no fluorescence property, in contrast to the corresponding random copolymer showing fluorescence property (Figure 10d).⁵⁸ This is due to that the consecutive sequence of *N*-vinylcarbazole is the chromophore.

4-2. Catalysis. An alternating copolymer showing sequence-oriented catalysis has been reported. The copolymer was obtained via orthogonal post reactions, i.e., copper-catalyzed azide-alkyne cycloaddition and fluoroarene-thiol coupling, for the alternating copolymer of 4-azidomethylstyrene with 2,3,4,5,6-pentafluorostyrene (Figure 11).⁵⁹ Thus, the two repeating units in alternating sequence were decorated with sodium sulfonate ($-\text{SO}_3\text{Na}$) and amine ($-\text{NH}_2$) respectively. The resultant acid/base-embedded alternating copolymer showed catalytic activity for Henry reaction of benzaldehyde with nitromethane. The catalytic activity was higher than those of the two alternating copolymers in which either unit is functionalized as well as the mixture of them.

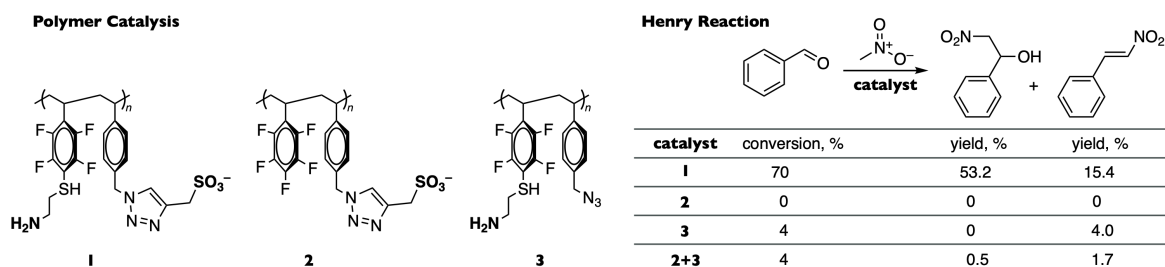


Figure 11. The acid/base-embedded alternating copolymer showed catalytic activity for Henry reaction of benzaldehyde and nitromethane.

4-3. Thermal Response in Solution. The alternating copolymer of methacrylic acid (MAA) with 2-hydroxyethylacrylate (HEA), which was synthesized with the pseudo connection methodology (see above), showed LCST-type solubility in 1,2-dimethoxyethane (DME) (Figure 12).²⁵ The thermal response behavior was not observed with the corresponding statistical copolymer of 1:1 composition ratio. Such LCST behavior in organic solvent is

relatively unique. A recent study revealed that the absence of consecutive HEA sequence is related to the unique thermal response behavior.⁶⁰

The alternating copolymer of MAA with *N*-isopropylacrylamide (NIPAM) (see above) also showed sequence-specific thermal response in water (Figure 12).²⁷ The copolymer was soluble in water at lower temperature, but the solution turned into turbid as the solution was heated. Unlike PNIPAM showing quick thermal response at narrow temperature range ($\Delta T < 2^\circ\text{C}$ for $1^\circ\text{C}/\text{min}$ heating), the turbidity of the alternating copolymer was gradually increased at wider range ($\Delta T \sim 25^\circ\text{C}$). The thermal responses were different from the corresponding 1:1 statistical copolymer which did not give turbid solution but particle precipitation keeping the transparency of the solution. The thermal responsibility of the alternating copolymer was tunable by pH because the MAA unit shows pH response.

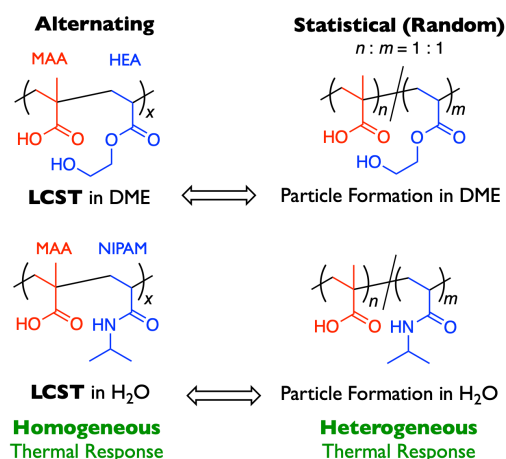


Figure 12. The difference in thermal response in solution between alternating and random copolymers.

4-4. Miscibility in Bulk. Block or statistical (random) copolymer generally works as a compatibilizer for the blend of homopolymers. It was reported that an alternating copolymer of styrene and MMA enhanced the miscibility of PMMA and PSt as the compatibilizer and the effect was greater than the random copolymer.⁶¹ The additive effect was observed by DSC measurement: only one T_g was detected in the 1:1 mixture of PMMA with the alternating copolymer, whereas two T_g s were observed at the 1:1 mixture with the random copolymer. The MMA-St random copolymer is known to have relatively alternating-rich sequence giving only 10% of consecutive triad sequence (MMM and SSS, M: MMA, S: St).⁶² It is interesting

that the slight difference in sequence greatly affected the miscibility behavior.

4-5. Water Repellent. As explained earlier, the pair of bicyclo[4.2.0]oct-1(8)-ene-8-carboxamide derivatives and cyclohexene allows alternating propagation in ROMP. Syntheses of sequence-defined oligomers made of two derivatives in alternating sequence were achieved via sequential stoichiometric addition of the two comonomers for the ruthenium carbene species. For example, two bicyclo monomer derivatives carrying propyl and ester group respectively (**1a** and **1b**) were used in conjunction with cyclohexene (**2**) for the spacer unit between the two monomer units to prepare several types of alternating-based sequence-defined oligomers (Figure 13).⁶³ The sequence pattern greatly affected the contact angles of water. The alternating oligomer, poly[(**1a-alt-2**)₁-block-(**1b-alt-2**)₁]₆, showed the smallest angle value (57°) among several analogues, whereas another type of copolymer, poly[(**1a-alt-2**)₂-block-(**1b-alt-2**)₂]₃, did the largest (66°). It is interesting that the slight difference in sequence appears in the macroscopic physical property even though the composition ratio is totally same as each other.

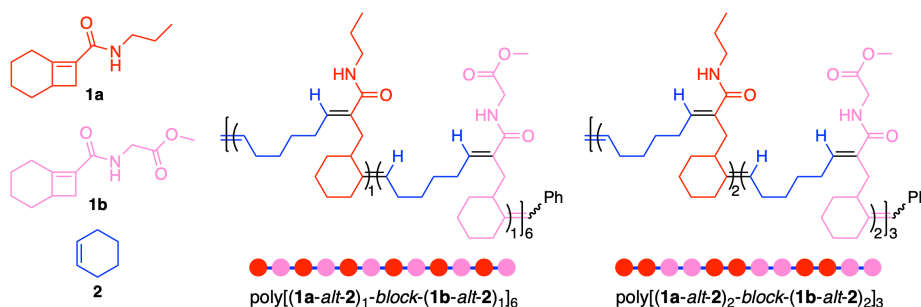


Figure 13. The structure of alternating copolymers showed the difference in water repellent properties.

4-6. Degradation. The alternating copolymer of vinyl ether and benzaldehyde (see above) has the acid-labile acetal linkage in the main chain periodically.⁷ The alternating copolymer can be decomposed under acidic condition and the alternating sequence allows decomposition into low molecular weight compounds. The vinyl ether pendant group can be designed for additional properties of the degradable alternating copolymers, such as thermoresponsivity, pH sensitivity, and hydrophilicity (Figure 14a).⁶⁴⁻⁶⁶

Another type of degradable alternating copolymer was also reported via RAFT copolymerization of cyclic ketene acetal with *N*-ethylmaleimide (Figure 14b).⁶⁷ The acetal

monomer is an electron-rich monomer like vinyl ether and thus suitable as the comonomer for the maleimide monomer in radical alternating copolymerization. The radical species is isomerized to give ester bond and styrene-based radical species favoring the maleimide comonomer. Therefore, the copolymerization proceeds in an alternating fashion. Since the resultant copolymer carries ester bond in the backbone, it can be decomposed under basic condition.

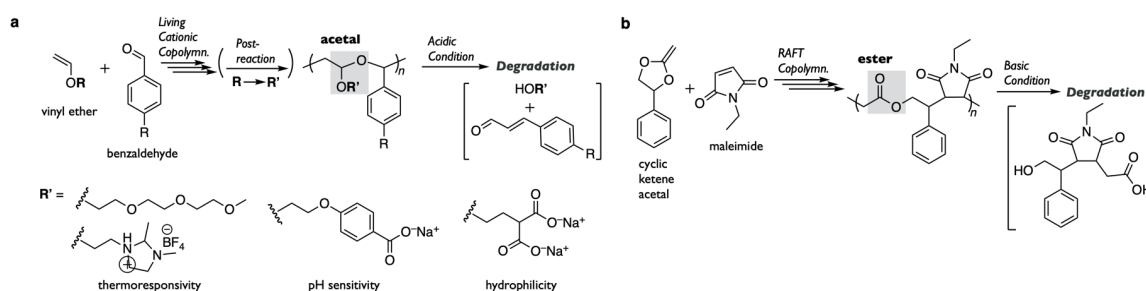


Figure 14. Synthetic and degradation schemes of alternating copolymers synthesized via (a) cationic and (b) RAFT polymerization.

4-7. Chain Mobility. The chain mobility specific to alternating sequence has been studied.^{68,69} For example, T_g of the alternating copolymer of styrene with MMA (103 °C) is slightly lower than that of the corresponding random copolymer (106 °C).⁶⁸ The lower T_g of the alternating copolymer indicates the higher chain mobility. The reason was explained as follows: the rotation of the ester groups in MMA is promoted by the periodic insertion of styrene units between MMA and thereby the dipole interaction between MMA units is shielded.

As shown above, strategic monomer design as well as advanced control for the initiation/propagation process have allows syntheses of various types of AB alternating copolymers. They can exhibit specific self-assemble behaviors and properties to the sequence, and in some cases the features are totally different from conventional copolymers such as random and block counterparts. Thus, novel applications can be expected. Syntheses of alternating copolymers of more complicated sequence pattern such as AAB has been also achieved,⁷⁰⁻⁷³ although they are not mentioned in this section. Alternating copolymers would open the door to development of advanced functional materials in the future.

Objectives

As described above, sequence control methods for synthesis of various alternating copolymers (design of electron density or bulkiness of monomers, using templates, and incorporating alternating sequences into monomers in advance) have been developed, which have allowed to synthesis alternating copolymers that could not be synthesized by conventional methods. However, some of these synthetic methods are not suitable for large scale synthesis due to the need special monomer designs or dilute conditions. Moreover, there are still challenges to be solved in development of alternating copolymers as materials, such as few techniques for analyzing alternating sequence, and few studies of self-assembly or function based on alternating sequence.

In this thesis, the author addressed molecular design for precise alternating sequence control and functions of alternating copolymers. The thesis consists of the following four topics based on alternating copolymerization of styrene and maleimide derivatives with various substituents: **(1)** Precise Alternating Sequence Control, **(2)** Sequence Analysis, **(3)** Sequence-Driven Self-Assembly, and **(4)** Sequence-Driven Functions (Figure 15).

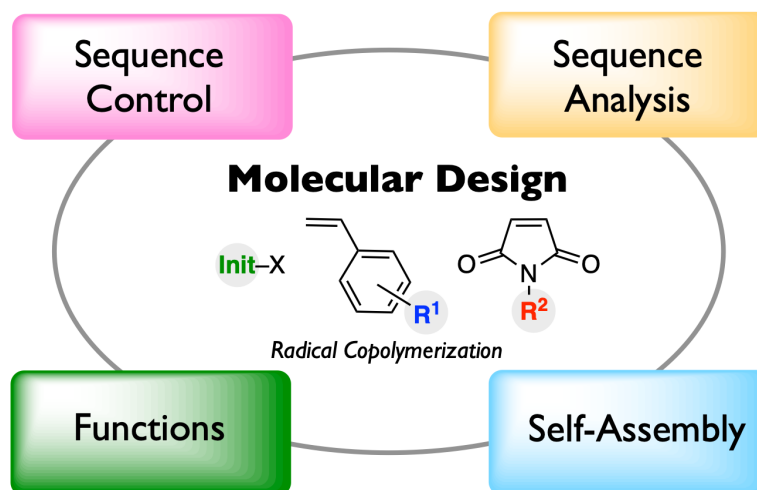


Figure 15. Objectives of this thesis.

Outline of This Study

This thesis consists of three parts including eight chapters: **Part I** (Chapters 1 – 3) deals with electron-poorer initiator and maleimide derivatives for sequence analysis and precise sequence control of alternating radical copolymerization with styrene and maleimide derivatives. **Part II** (Chapters 4 - 7) focused on alternating copolymers bearing long alkyl chains and phenol (or catechol) pendants for structure analysis and functions. **Part III** (Chapters 8) deals with thermoresponsive telechelic polymer for IgG purification.

In Part I, **Chapter 1** focuses on malonate-based bromide initiator where two electron-withdrawing ester groups are attached on the carbon neighboring to bromide. Thanks to the lower electron density of the radical species, the initiator showed styrene selective initiation at alternating radical copolymerization of styrene and maleimide derivatives, which determined the α -end group of the copolymers. The well-defined α -end allows the alternating copolymers to be more simply analyzed by MALDI-TOF-MS.

Chapter 2 deals with design of electron-poorer maleimides for precise sequence control of alternating sequence for radical copolymerization with styrene. The copolymer of styrene and *N*-ethyl maleimide synthesized by using the initiator reported in *chapter 1* via ruthenium-catalyzed living radical polymerization gave complicated peak patterns at MALDI-TOF-MS

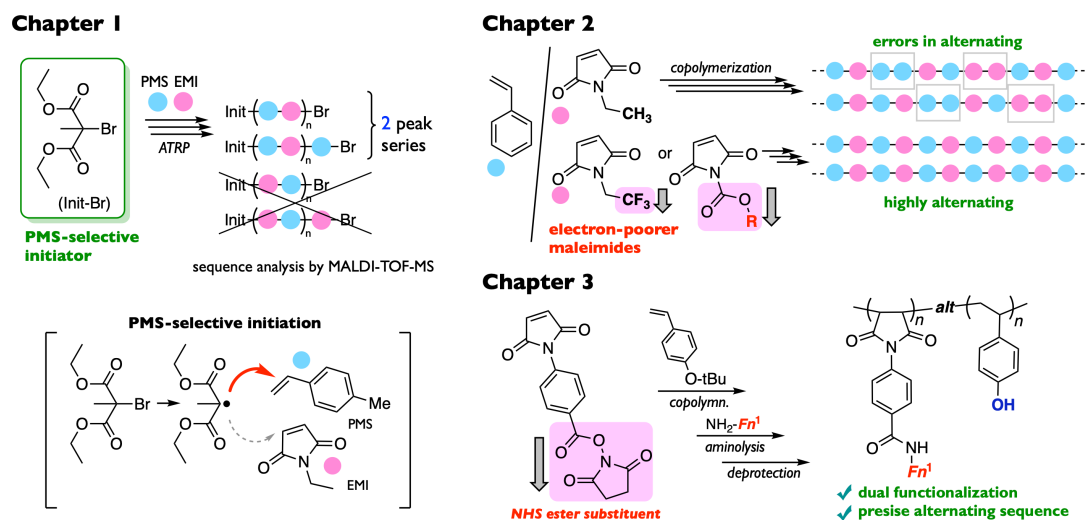


Figure 16. Control of styrene selective initiation for sequence analysis by MALDI-TOF-MS (**Chapter 1**), and design of electron-poorer maleimides for precise alternating sequence control (**Chapter 2**) and functionalization (**Chapter 3**) of copolymer with styrene derivatives.

spectra indicating low degree of alternating sequence, in contrary to expectation from the reactivity ratios (almost zero). The electron-poorer maleimides containing electron-withdrawing groups such as 2,2,2-trifluoroethyl ($-\text{CH}_2\text{CF}_3$) and *N*-alkoxycarbonyl groups [$\text{ROC}(=\text{O})\text{N}-$; R = substituent] in their side chains gave higher alternating sequences for the copolymerizations with styrene. Further, the latter maleimide also allowed to incorporate functional side chains into the highly alternating sequence copolymers.

In **Chapter 3**, I designed an *N*-phenyl maleimide derivative bearing *N*-hydroxy succinimide (NHS) ester in the side chain for precise alternating sequence control and dual functionalization of the copolymer with styrene derivatives. The NHS ester which is an electron-withdrawing substituent and can be converted into various amide groups is expected to allow precise sequence control and functionalization of the copolymers at the same time. The maleimide monomer was copolymerized with *tert*-butoxystyrene and the resultant copolymer had high degree of alternating sequence, which was confirmed by MALDI-TOF-MS. The dual functionalization of the alternating copolymer was accomplished by aminolysis and followed by deprotection reaction to give alternating sequences of phenol and various amide pendants.

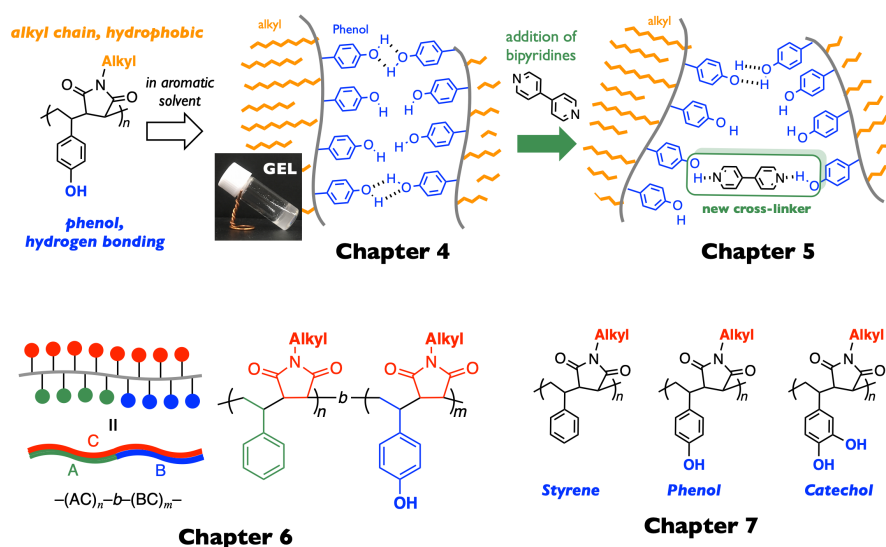


Figure 17. Alternating copolymers bearing long alkyl and phenol pendants formed organogel in aromatic solvents (**Chapter 4**) and bipyridines changed the gel property (**Chapter 5**). Alternating copolymer-based diblock copolymer for novel phase separation behavior (**Chapter 6**). The alternating copolymer bearing alkyl and catechol pendants showed adhesion and water-repellent properties (**Chapter 7**).

In Part II, **Chapter 4** studied the thermoresponsive physical gels of the alternating copolymer bearing long alkyl and phenol pendants in aromatic solvents. By structure analysis by ^1H NMR, SANS and cryo-TEM, the gel was found to consist of the entangled core-shell fibrillar nanostructure, where hydrogen bond-based phenol core is segregated from the alkyl pendant shell in the alternating sequence. The gel properties such as viscoelasticity, gelation temperature and critical gelation concentration can be tunable by alkyl length and molecular weight of the copolymers.

In **Chapter 5**, addition of bipyridines into the gel in chapter 4 was found to give new gelation behaviors or properties reflecting bipyridine structures. By addition of bipyridines, the elastic modulus of the gel was decreased but the gel showed self-healing property due to the dynamic interaction between phenol and pyridine units. Further, fibrillar nanostructures and their higher order aggregates reflecting bipyridine structures were observed by cryo-TEM.

Chapter 6 studied phase separation of alternating copolymer-based diblock copolymers consisting of two alternating sequence segments with *N*-alkyl maleimide and styrene derivatives (styrene or 4-vinylphenol).

In **Chapter 7**, alternating copolymers bearing long alkyl and catechol (or phenol) pendants were found to have high adhesion property on glass plates and water-repellent properties, which were evaluated by cross cut method and contact angle measurements. These properties were attributed to cohesion power of alkyl chain by hydrophobic interaction and high adhesive property of catechol units.

In Part III, **Chapter 8** developed antibody purification materials that can switch

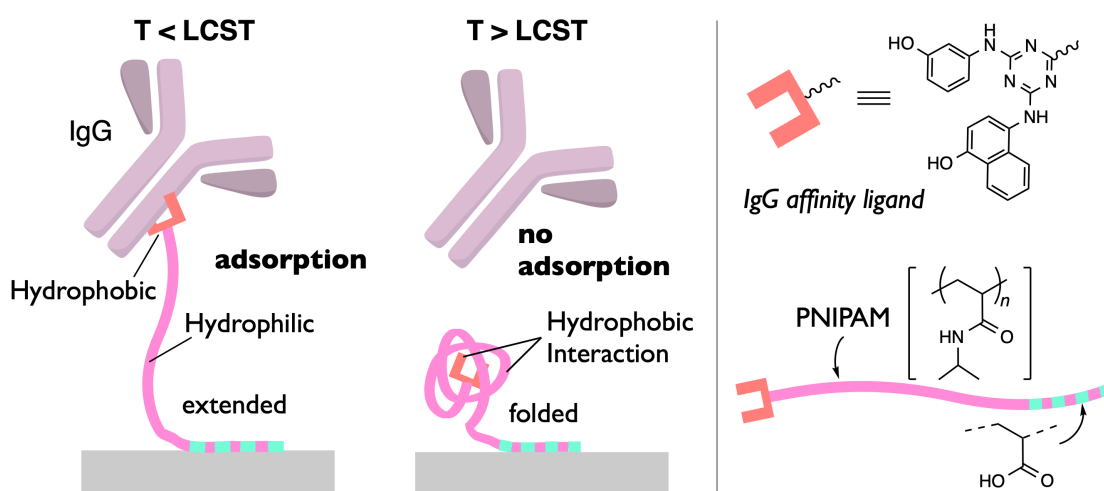


Figure 18. Polymer design for thermoresponsive antibody purification materials (**Chapter 8**).

adsorption/desorption of IgG by temperature by synthesis of telechelic thermoresponsive copolymer via living radical polymerization and followed by polymer reactions (Figure 18). The adsorption/desorption behavior by changing temperature was evaluated by SPR.

In summary, this thesis comprehensively presents the precise sequence control, sequence analysis, self-assembly and functions of alternating copolymers. I hope that this thesis contributes to further developments of alternating copolymer toward material application as well as polymer chemistry.

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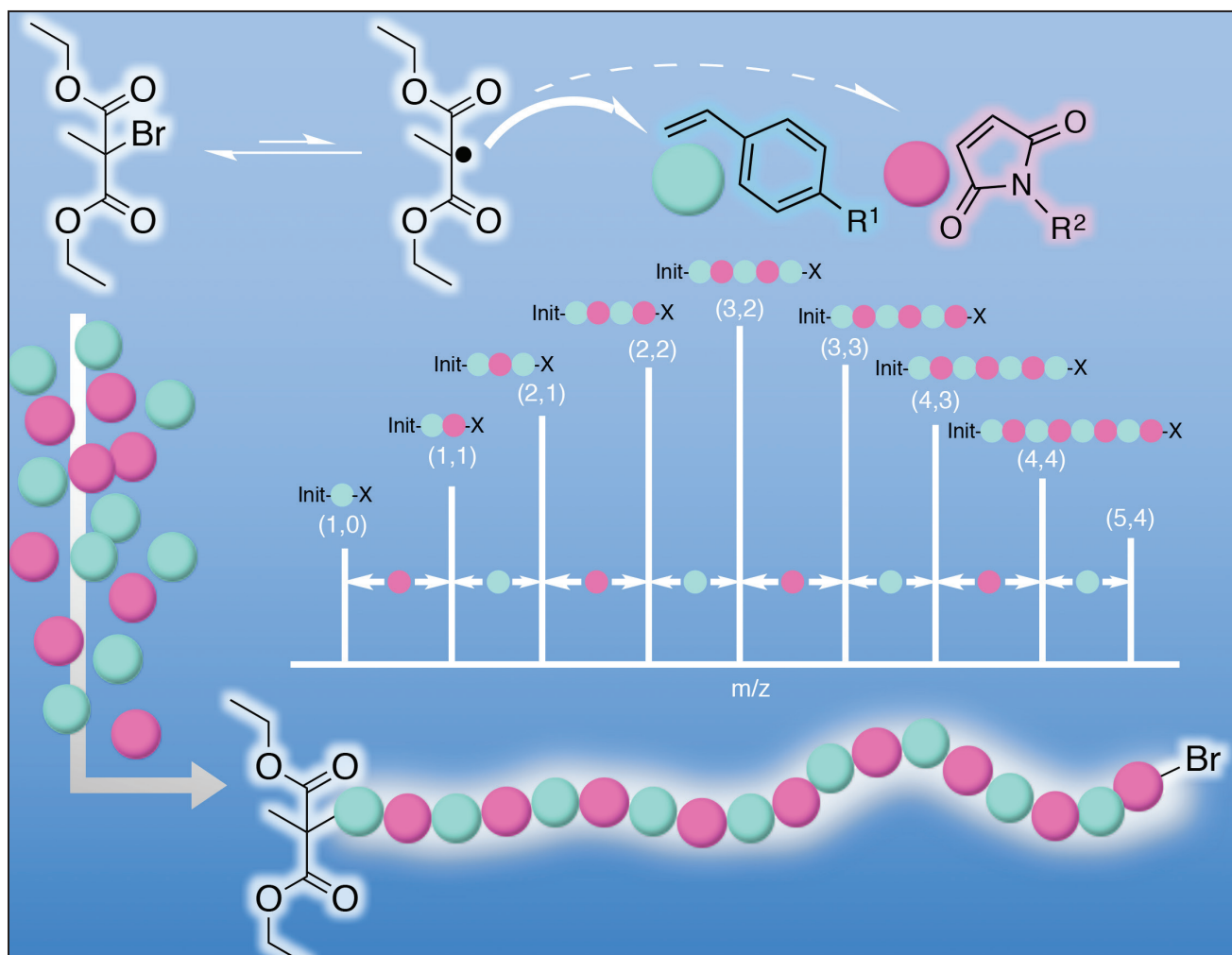
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PART I

Molecular Design for Sequence Analysis and Precise Sequence Control of Alternating Copolymers



Macromolecular Rapid Communications

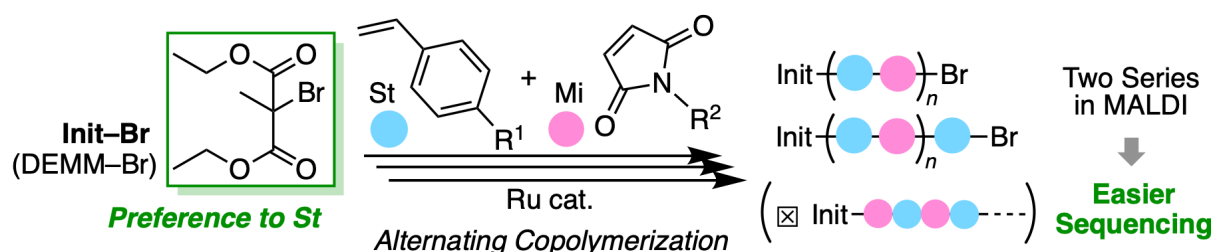
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Chapter 1

Sequence Analysis for Alternating Copolymers by MALDI-TOF-MS: Importance of Initiator Selectivity for Comonomer Pair

Abstract

A special initiator for metal-catalyzed living radical polymerization facilitated sequence analyses by MALDI-TOF-MS of alternating copolymers from styrene and maleimide derivatives. The initiator is a malonate-based alkyl halide (DEMM-Br), in which two ester groups are attached on the carbon neighboring to bromide, and poor electron density of the radical species allowed determination of next unit to the initiator in resultant alternating copolymers owing to the selective initiation to styrene derivative. Thanks to the well-defined α -end group, sequence of the oligomeric products via radical copolymerization of *p*-methylstyrene (PMS) and *N*-ethyl maleimide (EMI) with DEMM-Br can be more simply analyzed by MALDI-TOF-MS, and indeed the followings were clarified: the cross-over propagation is almost perfectly controlled regardless of the injection ratio; a minor error event of the disordered alternating sequence containing St-St sequential unit could take place; the minor error can be suppressed with an excess amount of maleimide.



Introduction

Sequence is a most essential structural factor for natural polymers such as DNA and peptides, and importantly we can identify their sequence with sequencer (i.e., sequencing), where the main chains are fragmented into lower molecular pieces for mass analyses.^{1,2} Quite recently, sequence control for synthetic macromolecules has attracted attentions,^{3,4} but the issue to be solved in this research area is how to analyze or prove the sequence of resultant molecules.⁵ Especially, it is still challenging to prove the accuracy in sequence of vinyl polymers even though it is controlled, because the C–C bond in main chain is too tough to be selectively cleaved unlike the natural polymers. Lutz et al. designed a new type of sequence-controlled macromolecules with a view to sequencing, in which repeating units are connected via carbon-TEMPO bond so as to realize homolytically fragmentation for sequencing.⁶ Molecular design considering sequencing as well as functions will become even more important in the near future.

Alternating copolymerization with a pair of electron-rich and electron-poor monomers, such as vinyl ether and maleic anhydride, is a very unique radical copolymerization, because cross propagation selectively occurs without special treatments to give alternating sequence.^{7,8} The conventional characterization of the alternating sequence is based on that composition ratio of resultant copolymers is 1:1 regardless of the feed ratio. The periodic AB-alternating sequence is most simple sequence pattern, but the direct evidence of the sequence has been little developed.

Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS)⁹ could be one option to characterize sequence of alternating copolymers. In general, some peak series are observed in MALDI-TOF-MS spectrum even for homopolymers, in sharp contrast to peptides giving single peak, because statistical propagation and side reactions take place in chain-growth polymerization. However, when living polymerization is utilized, one series of peaks is observed in which the peak interval is same as mass of repeating unit or monomer, because terminal groups of each chain are defined though the number of repeating unit is distributed.¹⁰ As for alternating copolymers prepared via living polymerization, it is assumed that three series peaks (basically 4 types of structure as for mass) are observed depending on either comonomer is attached neighboring to both terminal groups (Figure 1A). The variation in peak series would cause difficulty in the sequence analyses for obtained copolymers. One exceptional example to realize MALDI-TOF-MS characterization of periodic sequence was reported by Kamigaito and Satoh, in which the unique peak series from

the periodic AAB sequence can be observed due to living polymerization.¹¹ As for sequence analyses with MALDI-TOF-MS, Hutchings et al., also demonstrated with styrene-based copolymer with diphenylethylene.¹²

In this paper, to approach sequence analysis of copolymers by alternating copolymerization, we focused on the selectivity of radical species from the initiator for comonomer pair, i.e., styrene and maleimide derivatives. Some halogen-based initiators for ruthenium-catalyzed living radical polymerization were studied to find a selective initiator to either comonomer and it was actually used as the initiator for alternating copolymerization to analyze the sequence. If the preference of initiator for the pair could be directed to either, the series number could be reduced to two in principle (Figure 1B), which would help analysis of accuracy of alternating sequence. Herein, lower molecular weight or oligomer is targeted because of easier analyses of structures as well as our next purpose such as syntheses of unique multiblock copolymers consisting of various short-alternating segments.

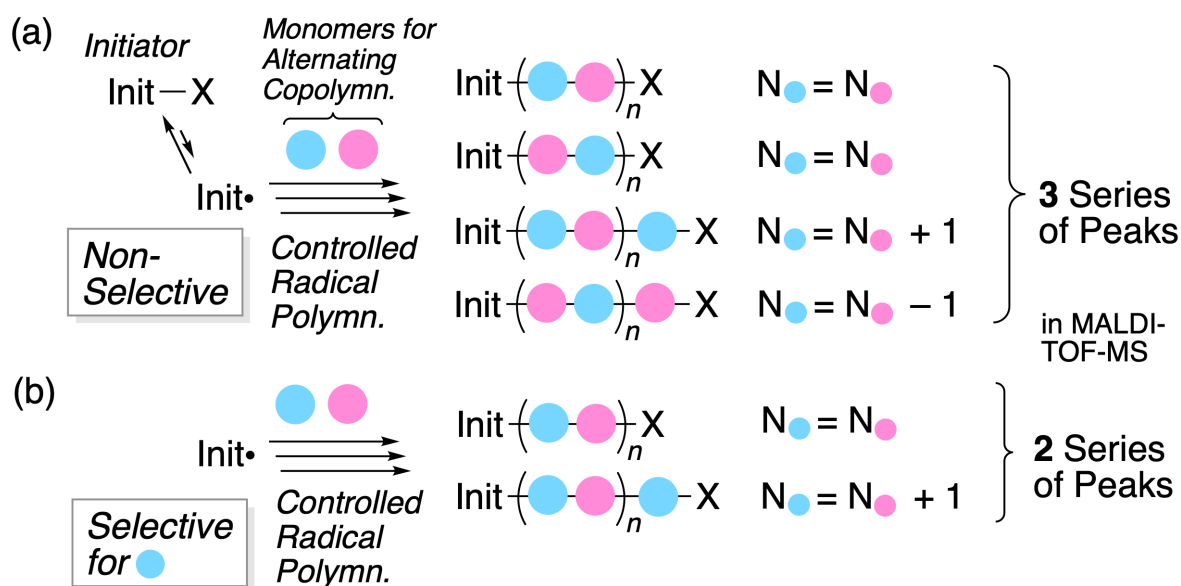


Figure 1. Concept: monomer selectivity of radical initiator could reduce sequence series and make MALDI-TOF-MS analysis easier.

Experimental

Materials

p-Methystyrene (PMS, TCI; purity > 96%) was washed by 10wt% NaOH aq. and H₂O and dried overnight over Na₂SO₄, followed by distillation under reduced pressure over calcium hydride. Styrene (St, TCI; purity > 99%) was dried overnight over calcium chloride and purified by distillation under reduced pressure over calcium hydride. Diethyl 2-bromo-2-methyl malonate (DEMM-Br, TCI; purity > 98%), and 4-vinylphenyl acetate (StOAc, TCI; purity > 98%) was diluted with dry toluene and bubbled with dry nitrogen for 15 minutes. *N*-ethyl maleimide (EMI, TCI; purity > 98%), [Cp**Ru*(CH₃CN)₃]⁺PF₆⁻ (Sigma Aldrich), triphenylphosphine (Sigma Aldrich; purity > 99%), dithranol (Sigma Aldrich; purity > 98%) were used without any purification. *N*-dodecyl maleimide (C12MI) was synthesized as reported.¹³ 1,2,3,4-Tetrahydro-naphthalene (tetralin, Kisida Chemical; purity > 98%), an internal standard in ¹H NMR, was dried over calcium chloride overnight and distilled twice over calcium hydride. Toluene (Kishida Kagaku, Osaka, Japan; purity 99.5%) was dried and purified by passing through purification columns (Solvent Dispensing System, SG Water USA, Nashua, NH; Glass Counter).

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

SEC (THF). MWD curves, *M_n* and *M_w/M_n* ratio of the polymers were also measured by SEC in THF as an eluent at 40 °C on three polystyrene gel columns (Shodex LF-404; exclusion limit = 2×10⁶ g/mol; particle size = 6 μm; pore size, < 3000 Å; 0.46 cm i.d. 25 cm; flow rate 0.3 mL/min) connected to a DU-H2000 pump, a 74S-RI refractive index detector, and a 41-UV ultraviolet detector (all from Shodex; Shodex GPC-104). The columns were calibrated against 12 standard polystyrene samples (TOSOH, PS-Oligomer Kit, *M_w* = 500–1,110,000; *M_w/M_n* = 1.01–1.16).

MALDI-TOF-MS. MALDI-TOF-MS analyses were performed on an ultraflex III (MALDI-TOF mass spectrometer, Bruker Daltonics) equipped with 337 nm nitrogen laser with dithranol as matrix and sodium trifluoroacetate as the cationizing agent.

Polymer Synthesis

Competitive radical reactions or oligomerizations (polymerizations) were carried out by

the syringe technique under dry argon in baked glass tubes equipped with three-way stopcocks. A typical procedure for copolymerization of PMS and EMI with DEMM-Br was as follows. To a Schlenk tube, $[\text{Cp}^*\text{Ru}(\text{CH}_3\text{CN})_3]^+\text{PF}_6^-$ (10.1 mg, 2.0×10^{-5} mol) and triphenylphosphine (10.4 mg, 4.0×10^{-5} mol) were added in 0.6 mL of CH_2Cl_2 under dry argon at 0 °C and stirred for 10 h, followed by solvent replacement with toluene (1.39 mL) via evaporation of CH_2Cl_2 . A solution of PMS (0.48 mL of 415 mM in toluene, 2.0×10^{-4} mol), EMI (25.0 mg, 2.0×10^{-4} mol), tetralin (0.04 mL), and DEMM-Br (0.087 mL of 458.755 mM in toluene, 4.0×10^{-5} mol) were combined to the tube containing the ruthenium complex at 0 °C (total volume: 2.0 mL). Immediately after mixing, the tube was placed in an oil bath at 60 °C. In predetermined intervals, 0.2-0.5 mL of solution was sampled by syringe under dry argon and the polymerization was terminated by bubbling with air and cooling the reaction mixture to -78 °C in dry ice/methanol. Monomer conversion was determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomer with tetralin as an internal standard.

Result and Discussion

Evaluation of Initiator Selectivity for Comonomer Pair

We first studied competitive radical addition reaction of *p*-methylstyrene (PMS)¹⁴ and *N*-ethyl maleimide (EMI) with different types of bromine-initiator (DEMM-Br, EMA-Br, MMA₂-Br, and St-Br) for metal-catalyzed living radical polymerization to estimate selectivity of the initiator to the two comonomers (Figure 2). The combination of styrene and maleimide comonomers is interesting for alternating copolymerization because many derivatives could be used.¹⁵ An equimolar amount of initiator and each comonomer (PMS and EMI) were injected in toluene in conjunction with ruthenium catalyst $[\text{RuCp}^*(\text{Cl})(\text{PPh}_3)_2]$ and amine cocatalyst (*n*-Bu₂NH) to be heated to 60 °C: $[\text{PMS}]_0 = [\text{EMI}]_0 = [\text{Initiator}]_0 = 40$ mM, $[\text{RuCp}^*(\text{Cl})(\text{PPh}_3)_2]_0 = 4.0$ mM, $[\text{n-Bu}_2\text{NH}]_0 = 40$ mM. The consumption rates of the two monomers were monitored with ^1H NMR for earlier reaction stage (before 10 min) and the semi logarithmic plot of $\ln([M]_0/[M])$ against time was prepared to estimate apparent rate constant of each monomer (k_{St} , k_{Mi}) from the slope. The rate constant ratio ($k_{\text{St}}/k_{\text{Mi}}$) was calculated to discuss the selectivity to styrene over maleimide.

When DEMM-Br was used as the initiator, k_{St} was much higher than k_{Mi} ($k_{\text{St}} = 13$, $k_{\text{Mi}} = 0.6$) and high styrene-selectivity ($k_{\text{St}}/k_{\text{Mi}} = 22$) was observed (Figure 2a). On the other hand, almost same rate constant was obtained with EMA-Br (b) or MMA₂-Br (c). The higher styrene-selectivity of DEMM-Br is likely due to that the carbon attaching bromine affords

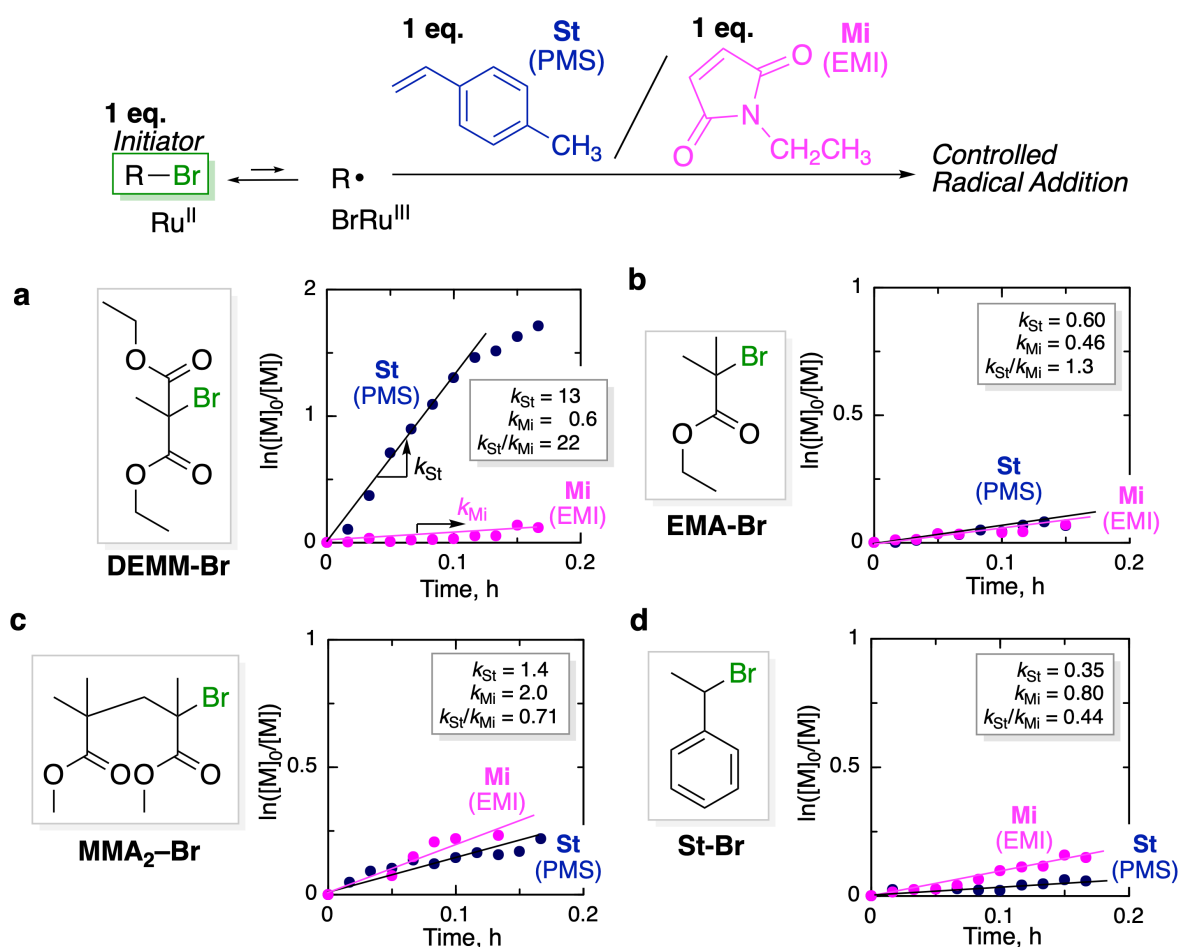


Figure 2. $\ln([M]_0/[M])$ -time plots for double monomer radical addition of PMS (black circle) and EMI (gray circle) with several initiators[(a) DEMM-Br, (b) EMA-Br, (c) MMA₂-Br and (d) St-Br]: $[PMS]_0/[EMI]_0/[Initiator]_0/[RuCp^*Cl(PPh_3)_2]_0/[n-Bu_2NH]_0 = 40/40/40/4/40$ mM in toluene at 60 °C.

giving styrene-favorite radical like maleimide-derived species, because two electron-withdrawing substituents (i.e., ester) are attached on the carbon. The electron-withdrawing effect for EMA-Br and MMA₂-Br with one electron-withdrawing substituent seems to be too weak to realize selective addition of monomer. In contrast, the styrene-derived initiator (St-Br) showed maleimide selectivity ($k_{Mi} > k_{St}$) but the selectivity degree and the rate constants were not so high as DEMM-Br (d). Thus, from the competitive radical addition reactions, it was assumed that DEMM-Br is the suitable initiator to analyze alternating propagation with MALDI-TOF-MS due to higher selectivity and smoother initiation.

Structural Analyses of the Alternating Copolymer with DEMM-Br

Then, DEMM-Br was used as the initiator for ruthenium-catalyzed living radical polymerization of PMS and EMI. To ensure peak detection, lower molecular weight (DP_n) was targeted: $[PMS]_0 = [EMI]_0 = 100$ mM; $[DEMM-Br]_0 = 20$ mM; $[Ru-catalyst]_0 = 10$ mM in toluene at 60°C. The halogen-free ruthenium complex, $[(Cp^*Ru(CH_3CN)(PPh_3)_2]^+PF_6^-$),¹⁶ was used as the catalyst to avoid halogen exchange which would complicate the mass spectrum.

Due to selective initiation of the initiator to PMS, conversion of PMS was higher than that of EMI at earlier stage: $Conv_{PMS}/Conv_{EMI} = 28/11\%$ in 1 hour (Figure 3a). The difference in conversion ($Conv_{PMS} - Conv_{EMI} = 17\%$) was almost comparable to the conversion assuming preferential addition of PMS with the initiator ($20/100 \times 100 = 20\%$). The molecular weight distributions of obtained polymers were narrow (Figure 3b), indicating the polymerization was controlled. Then, sequence of obtained copolymer was analyzed with MALDI-TOF-MS. Herein, as the information of terminal structures was required for the peak analyses, 1H NMR was also measured together. Thus, the measurement copolymer was separately prepared in larger amount under same condition, followed by purification with preparative SEC ($M_n = 1100$, $M_w/M_n = 1.14$ by SEC after purification).

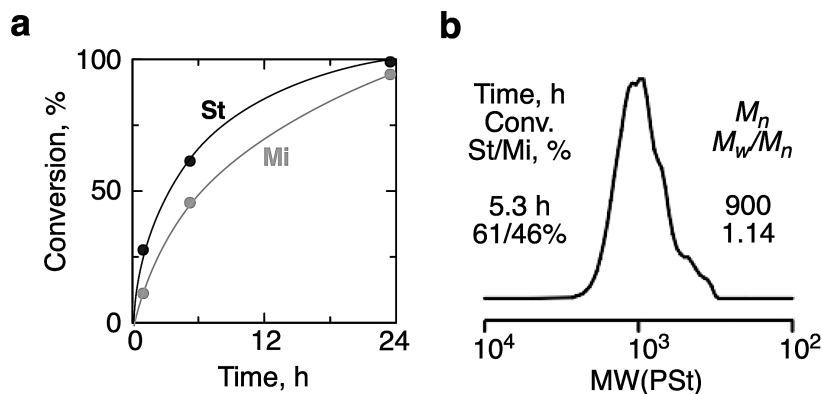


Figure 3. (a) Conversion-time plot and (b) SEC curve for copolymerization of PMS and EMI: $[PMS]_0/[EMI]_0/[DEMM-Br]_0/[(Cp^*Ru(CH_3CN)_3]^+PF_6^-]_0/[PPh_3]_0 = 100/100/20/10/20$ mM in toluene at 60°C.

In the 1H NMR spectrum (Figure 4a), in addition to main signals derived from PMS and EMI units, minor ones from terminal groups were detected: methine proton signal from styrene terminal neighboring to Br (*a*) and from maleimide terminal (*b*); methylene protons of ester group from the initiator unit (*k*). The integration ratio of ω -end terminal protons (*a* + *b*) to α -

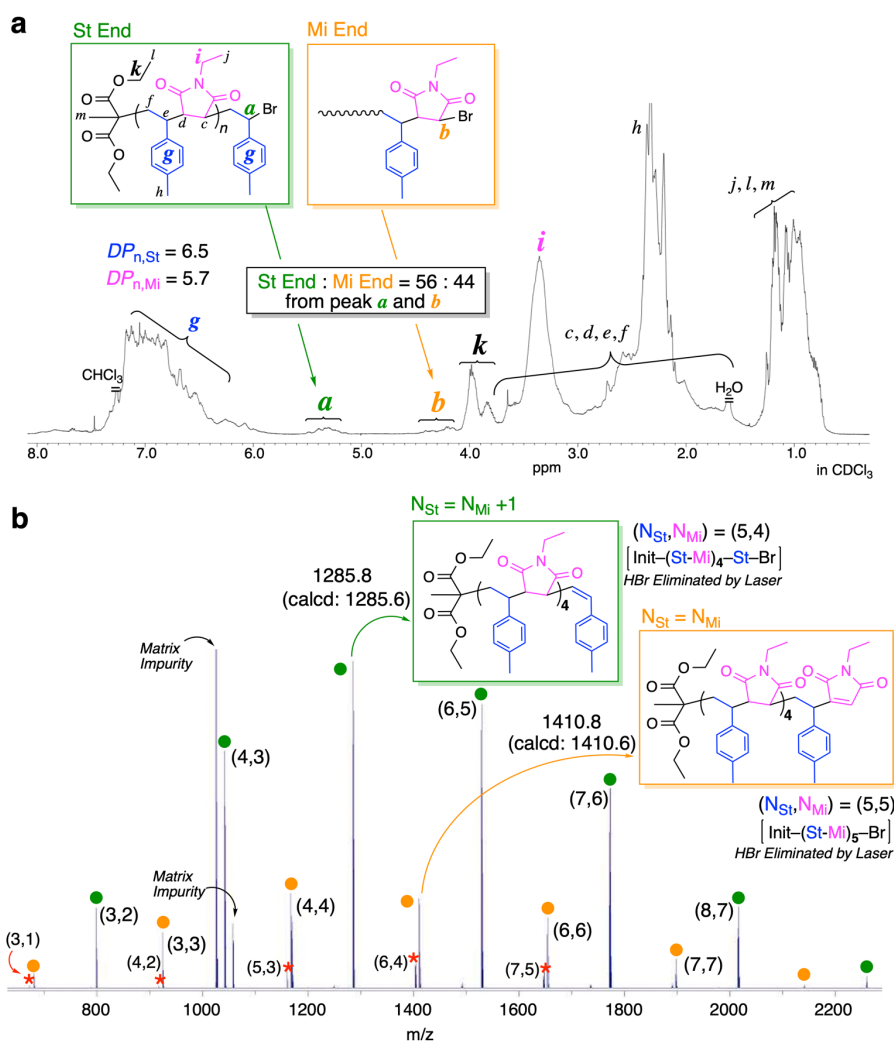


Figure 4. (a) ^1H NMR spectrum (CDCl₃, room temperature) and (b) MALDI-TOF-MS spectrum of the copolymer of PMS and EMI: $[\text{PMS}]_0/[\text{EMI}]_0/[\text{DEMM-Br}]_0/[\text{Cp}^*\text{Ru}(\text{CH}_3\text{CN})_3]^+\text{PF}_6^-/[\text{PPh}_3]_0 = 100/100/20/10/20$ mM in toluene at 60 °C. Two peaks around $m/z = 1000$ were derived from impurities from dithranol used as a matrix.

end (*k*) was consistent with the ideal structure $[(a + b): k = 1: 4]$. It is important to clarify the end group for the MALDI analysis, and both end groups (i.e., St End and Mi End) existed for obtained polymer chains. The ratio was calculated as 56% (St End) and 44% (Mi End), respectively from the integration ratio. Each of DP_n ($DP_{n,\text{St}}$, $DP_{n,\text{Mi}}$) was calculated from the signal intensity ratio of aromatic protons (*g*, from PMS) or ethyl protons (*i*, from EMI) to ethyl ester protons (*k* from DEMM-Br): $DP_{n,\text{St}} = 6.5$, $DP_{n,\text{Mi}} = 5.7$. The higher $DP_{n,\text{St}}$ was due to the styrene-selective initiation.

In MALDI-TOF-MS spectrum, there were two major series whose molecular weight intervals agreed with the sum of molecular weights of PMS and EMI (Figure 4b). Given by the ^1H NMR analysis and the selective initiation to PMS, the larger peak series (shown by black circle) are from the alternating copolymer terminated after styrene addition, though the terminal halogen was eliminated by laser during measurement.¹⁰ Namely, the series is from the alternating copolymer having PMS unit one more than EMI unit (**St End**: $N_{\text{St}} = N_{\text{Mi}} + 1$). On the other hand, the alternating copolymer of maleimide terminal was observed for the smaller series (**Mi End**: $N_{\text{St}} = N_{\text{Mi}}$, gray circle) (The MALDI-TOF-MS analysis is not quantitative but qualitative. Therefore, the peak intensity was not related to abundance ratio). Another minor series (star) of copolymer with two more PMS unit than EMI unit ($N_{\text{St}} = N_{\text{Mi}} + 2$) was also observed and the series is from the copolymer having one set of PMS sequential units, indicating an error in alternating propagation could be contained.

Effects of Comonomer Ratio on Alternating Sequence

From long time ago, alternating copolymerization was speculated by that number-averaged composition ratio of obtained copolymer is 1:1 regardless of comonomer ratio. This logic would be correct in terms of the averaged analyses of all polymer chains, but details of sequence in each chain are unknown. Herein, we also performed copolymerization of PMS and EMI with DEMM-Br under conditions with an excess amount of either of comonomers to study effects of comonomer ratio on the alternating sequence by MALDI-TOF-MS.

Under the styrene-rich condition (Figure 5b: $[\text{PMS}]_0 > [\text{EMI}]_0$), the error series from PMS sequential units was relatively increased, but rather curiously, no other error series including from more than one set of PMS sequential unit were observed. Given by that the copolymerization proceeded with living polymerization, the analyses likely indicate followings: the error took place only at earlier polymerization stage; the error frequency was slightly increased when an excess styrene was used; no further errors occurred during propagation even under the styrene-rich condition. As the error timing is specific to earlier stage, it is plausible that it takes place soon after initiation to give an error sequence of Init–PMS–PMS–EMI.... instead of Init–PMS–EMI–PMS.... Another feature of the PMS-rich condition is that the **Mi End** series was relatively decreased, which is due to that chance of halogen capping to PMS radical was increased.

On the other hand, when the injection ratio of EMI was increased (Figure 5c: $[\text{PMS}]_0 < [\text{EMI}]_0$), the error series or others were not observed at all. The irregular PMS-PMS sequential

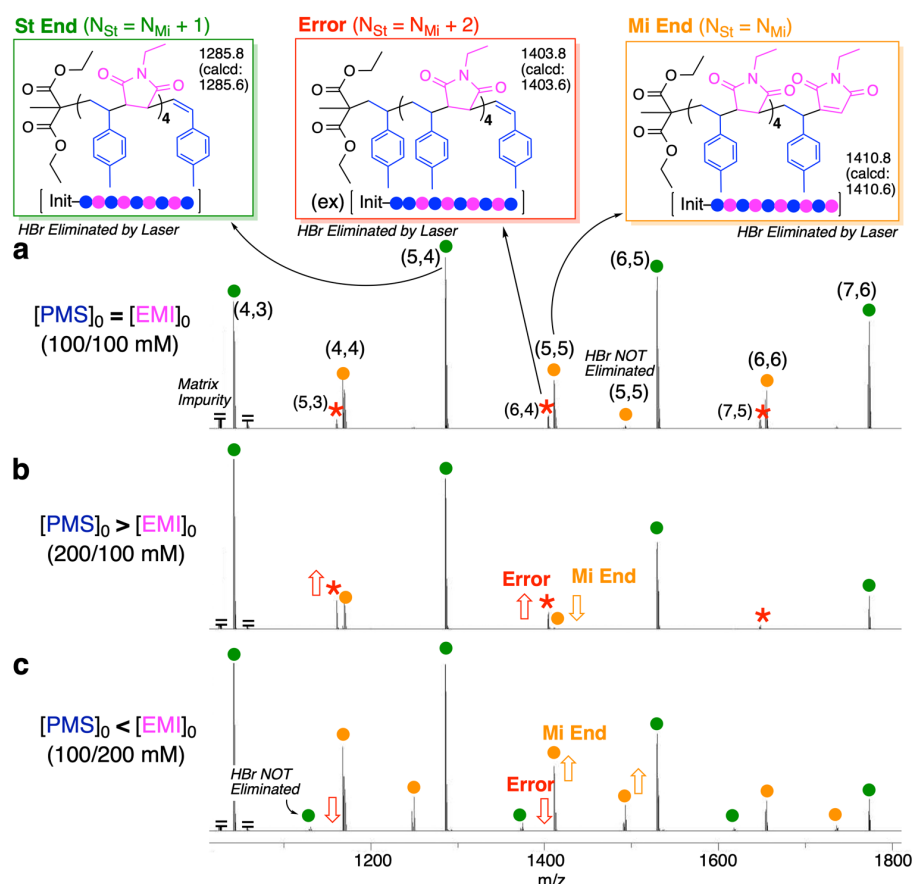


Figure 5. MALDI-TOF-MS spectra of the copolymer of PMS and EMI: $[DEMM-Br]_0/[Cp^*Ru(CH_3CN)_3]^+PF_6^-]_0/[PPh_3]_0 = 20/10/20$ mM in toluene at 60 °C. (a) $[PMS]_0 = [EMI]_0 = 100$ mM, (b) $[PMS]_0 = 200$ mM, $[EMI]_0 = 100$ mM, (c) $[PMS]_0 = 100$ mM, $[EMI]_0 = 200$ mM.

propagation at earlier polymerization seemed to be suppressed due to the presence of excess EMI. The error of EMI-EMI sequential propagation would be less accessible, since the homopolymerization ability of EMI is low. Contrary to the PMS-rich condition, the **Mi End** series was clearly increased due to the same reason.

From the MALDI-TOF-MS analyses, the followings are clarified: the cross-over propagation is almost perfectly controlled regardless of the injection ratio under the relatively diluted condition; the disordered alternating sequence containing St-St sequential unit is observed, although it is a very minor event.

We also did copolymerizations for some combinations of styrene and maleimide (St/EMI, AcOSt/EMI, and PMS/C12MI) with DEMM-Br under the diluted condition and

analyzed the resultant alternating sequence by MALDI-TOF-MS (Figure 6). Similar tendency was obtained from the three copolymerizations.

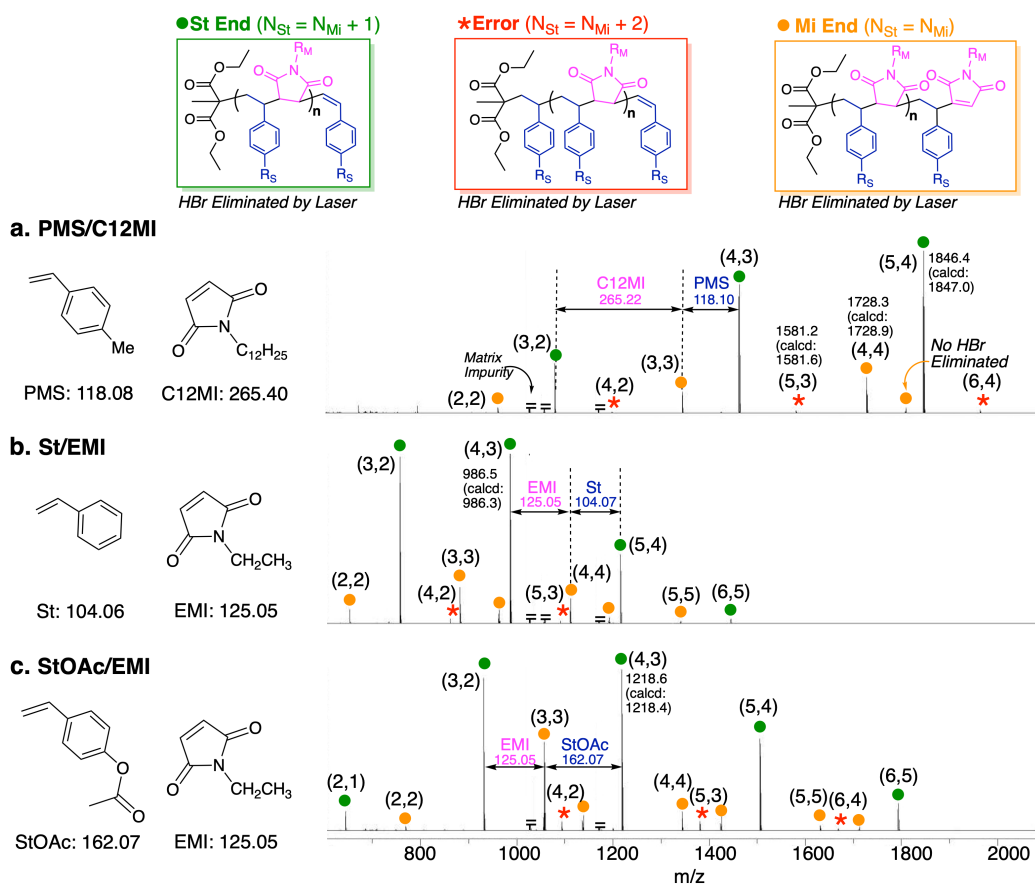


Figure 6. MALDI-TOF-MS spectra of the copolymer of styrene and maleimide derivatives: [St derivative]₀/[Mi derivative]₀/[DEMM-Br]₀/[[Cp^{*}Ru(CH₃CN)₃]⁺PF₆⁻]₀/[PPh₃]₀ = 100/100/20/10/20 mM in toluene at 60 °C: (a) PMS/C12MI, (b) St/EMI and (c) StOAc/EMI.

Conclusion

Sequence of copolymers obtained via alternating radical copolymerization of styrene and maleimide derivatives were characterized by MALDI-TOF-MS. A special initiator carrying two ester groups on the carbon neighboring to halogen (i.e., DEMM-Br) for metal-catalyzed living radical polymerization selectively reacted with the electron-rich monomer (styrene) over electron-poor one (maleimide). The selective initiation facilitated sequence characterization of resultant copolymers by MALDI-TOF-MS, because the unit next to initiator could be defined in resultant alternating copolymers. Indeed, sequence of the oligomeric product by ruthenium-catalyzed radical copolymerization of PMS and EMI using DEMM-Br

as an initiator was characterized to clarify that an error could take place at the earlier stage with equimolar comonomers resulting in contiguous sequence of styrene. However, the error could be suppressed under EMI-rich condition. The alternating propagation was almost controlled under the diluted condition as well as the selective initiation, and thus the optimization could lead to syntheses of perfectly well-defined alternating copolymers.

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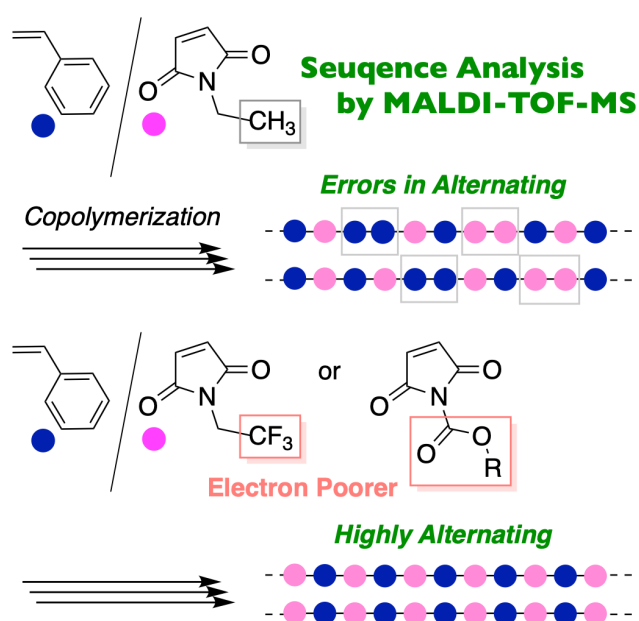
Chapter 2

Design of Maleimide Monomer for Higher Level of Alternating Sequence in Radical Copolymerization with Styrene

Abstract

This work deals with design of maleimide monomer toward more precise control of alternating sequence for radical copolymerization with styrene. Crucial in this study is sequence analysis by MALDI-TOF-MS for resultant copolymers that was obtained via ruthenium-catalyzed living radical copolymerization with a malonate-based alkyl halide initiator showing selective initiation ability. The copolymers of a simple *N*-alkyl maleimide [e.g., *N*-ethyl maleimide (EMI)] with styrene gave complicated peak patterns for the MALDI-

TOF-MS spectra indicating low degree of alternating sequence, in contrary to expectation from the reactivity ratios (almost zero). A simple substitution of methyl group (CH_3) of EMI with trifluoromethyl (CF_3 ; $\text{CF}_3\text{-MI}$) made the peak patterns much simpler giving the copolymer with higher alternating sequence. More interestingly, the peak interval of the copolymer at earlier polymerization stage was equal to sum of the molecular weights of $\text{CF}_3\text{-MI}$ and styrene, suggesting possibility of the pair propagation of the monomers. Indeed, ^1H NMR analyses of the mixture of maleimide with styrene suggested stronger interaction of $\text{CF}_3\text{-MI}$ than EMI. Based on the results, maleimide derivatives carrying a substituent-designable electron-withdrawing group [$\text{ROC}(\text{=O})\text{N-}$; R = substituent] were newly designed toward incorporation of functional side chains. They also gave higher alternating sequence for the copolymerization with styrene.



Introduction

Radical copolymerization is useful polymerization to tune properties or functions of the resultant polymers. The composition ratio can be predicted to some extent from the monomer reactivity ratio (r_1 , r_2), which is estimated by the composition ratio of obtained copolymers at the early stage and comonomer feed ratio (e.g., Fineman-Ross method).¹ For example, when both monomer reactivity ratios are almost one, there is no selectivity of the two types of propagating species to the comonomers, resulting in totally random copolymers. On the other hand, in the case with the both ratios are close to zero, a cross propagation preferentially occurs to give alternating copolymers.

The event of the alternating growth is specific to the comonomer pair, particularly the combination of electron-rich and -poor monomers, typically such as vinyl ether and maleic anhydride.² The selective alternating growth despite the violent reactivity of the growing species (i.e., radical) is interesting for creation of sequence-oriented functions as well as more complicated pattern on the basis of alternating sequence.³⁻⁸ Some approaches have been reported to enhance the alternating sequence for a comonomer pair of inherently lower alternating growth ability (i.e., reactivity ratios are not absolutely zero), such as addition of Lewis acid⁹ and design of pendant group,¹⁰ etc. As the special case, control of AAB alternating sequence was presented by Satoh and Kamigaito et al.: herein fluoroalcohol solvent was used for the copolymerization of *N*-phenyl maleimide (A) and limonene (B) to lead to unique bimolecular complex of the maleimide.¹¹ Some examples exist to control sequence based on reactivity ratio, however, two issues remain. First, examples on alternating-oriented functions are still limited.¹²⁻¹⁴ Second, sequence analysis^{11,15-18} have been unexplored in most cases, and characterization of the alternating sequence needs to rely on reactivity ratios. To approach alternating-oriented functions, precise control of alternating growth and molecular design are required based on detailed sequence analyses.

In chapter 1, I have demonstrated sequence analyses by using MALDI-TOF-MS for copolymers of *para*-methyl styrene and *N*-ethyl maleimide (EMI).¹⁹ The copolymers were synthesized with a malonate-based bromine initiator via ruthenium-catalyzed living radical polymerization. Crucial to the sequence analysis is the selective initiation from the initiator to the styrene over the maleimide, which makes the peak patterns simpler due to the well-defined unit (i.e., the styrene unit) neighboring to the initiator moiety.

Herein some maleimide derivatives were designed for precise control of alternating sequence in the copolymerization with styrene and for this purpose, sequence of the copolymers

was analyzed by MALDI-TOF-MS. First, I analyzed sequence for the copolymers of styrene and EMI that was obtained via ruthenium-catalyzed living radical copolymerization with the styrene-selective bromine initiator. Targeting polymerization degree was higher ($DP_n = 100$ for both comonomers) than that in chapter 1 ($DP_n = 20$)¹⁹ to ensure the sequence analyses with MALDI-TOF-MS. As a result, contrary to expectation from the monomer reactivity ratio, the degree of alternating sequence was not so high for the resultant copolymers. Then, the pendant of maleimide was modified with trifluoromethyl group ($-CF_3$), degree of alternating sequence was greatly enhanced and much simpler peak pattern was obtained in MALDI-TOF-MS spectrum. This is likely due to that cross propagation enhanced by interaction with styrene and CF_3 -MI. Finally, a new motif of maleimide monomer was designed for incorporation of various functional groups into highly alternating sequence copolymer.

Experimental

Materials

Styrene (TCI; purity >99%) was dried over calcium chloride overnight and distilled over calcium hydride. Triethylamine (TCI; purity >99%) was distilled before use. *N*-Ethyl maleimide (EMI, TCI; purity >98%), $[Cp^*Ru(CH_3CN)_3]^+PF_6^-$ (Sigma Aldrich), triphenylphosphine (Sigma Aldrich; purity 99%), Maleic anhydride (TCI; purity > 99%), propyl chloroformate (TCI; purity >95%), benzyl chloroformate (TCI; purity >96%), 2-ethylhexyl chloroformate (TCI; purity >98%), hexadecyl chloroformate (TCI; purity >98%), 2, 2, 2-trifluoroethylamine (TCI; purity >97%), tetrahydrofuran (Wako; purity > 99%, super dehydrated) and dithranol (Sigma Aldrich; purity >98%) were used without any purification. 1,2,3,4-Tetrahydronaphthalene (tetralin, Kisida Chemical; purity >98%), an internal standard in 1H NMR, was dried over calcium chloride overnight and distilled twice over calcium hydride. Toluene (Kishida Kagaku, Osaka, Japan; purity 99.5%) was dried and purified by passing through purification columns (Solvent Dispensing System, SG Water USA, Nashua, NH; Glass Counter).

Characterization

1H NMR. 1H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

SEC (THF). MWD curves, M_n and M_w/M_n ratio of the polymers were also measured by

SEC in THF as an eluent at 40 °C on three polystyrene gel columns (Shodex LF-404; exclusion limit = 2×10^6 g/mol; particle size = 6 μm ; pore size, < 3000 Å; 0.46 cm i.d. 25 cm; flow rate 0.3 mL/min) connected to a DU-H2000 pump, a 74S-RI refractive index detector, and a 41-UV ultraviolet detector (all from Shodex; Shodex GPC-104). The columns were calibrated against 12 standard polystyrene samples (TOSOH, PS-Oligomer Kit, M_w = 500–1,110,000; M_w/M_n = 1.01–1.16).

MALDI-TOF-MS. MALDI-TOF-MS analyses were performed on an ultraflex III (MALDI-TOF mass spectrometer, Bruker Daltonics) equipped with 337 nm nitrogen laser with dithranol or DCTB as matrix and sodium trifluoroacetate as the cationizing agent.

Polymerization

Polymerization was carried out by the syringe technique under dry argon in baked glass tubes equipped with three-way stopcock. A typical procedure for copolymerization of styrene and EMI with bromine initiator was as follows. To a Schlenk tube, $[\text{Cp}^*\text{Ru}(\text{CH}_3\text{CN})_3]^+\text{PF}_6^-$ (5.04 mg, 1.0×10^{-5} mol) and triphenylphosphine (5.24 mg, 2.0×10^{-5} mol) were added in 1.8 mL of toluene and the solution was treated by ultrasonic agitation for 5 min. To the other Schlenk tube, St (0.114 mL, 1.0×10^{-3} mol), EMI (125 mg, 1.0×10^{-3} mol), tetralin (0.02 mL), and the ruthenium complex solution were added and finally a solution of bromine initiator (0.051 mL of 391.255×10^{-3} M in toluene, 2.0×10^{-5} mol) was combined at room temperature (total volume: 2.0 mL). Immediately after mixing, the tube was placed in oil bath at 60 °C. In predetermined intervals, 0.2 – 0.5 mL of solution was sampled by syringe under dry argon and the polymerization was terminated by bubbling with air and cooling the reaction mixture to –78 °C in dry ice/methanol. Monomer conversion was determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomer with tetralin as an internal standard.

Synthesis of *N*-(2,2,2-trifluoroethyl) maleimide ($\text{CF}_3\text{-MI}$)

Maleic Anhydride (2.12 g, 21.6 mmol) was dissolved in 9 mL of THF. A solution of 2,2,2-trifluoroethylamine (1.78 g, 18.0 mmol) in 15 mL of THF was added dropwise to the solution of maleic anhydride at 0 °C and the mixture was stirred at room temperature for 16 h. After stirring, the solution was evaporated in vacuo and the yellow crude was washed by chloroform (20 mL $\times$ 3) and dried overnight in vacuo to give *N*-(2,2,2-trifluoroethyl) maleamic acid as white solid (2.94 g, yield 83%). *N*-(2,2,2-trifluoroethyl) maleamic acid (2.94 g, 14.9 mmol) and sodium acetate (1.96 g, 23.9 mmol) were dissolved in 54 mL of acetic anhydride

and the solution was stirred at 100 °C for 17 h. The solution was evaporated in vacuo and the crude was dissolved in chloroform. The residue was removed by filtration and the filtrate was concentrated and purified by column chromatography on silica gel (eluent: hexane/ethyl acetate = 3/1, R_f = 0.32) to give CF₃-MI (707 mg, 3.94 mmol, yield 27%) as white solid. ¹H NMR (500 MHz, CDCl₃, δ): 6.83 (s, 2H, CH), 4.13 (q, 2H, CH₂); ¹³C NMR (125 MHz, CDCl₃, δ): 168.95, 134.71, 123.08, 38.83 (Figure 1).

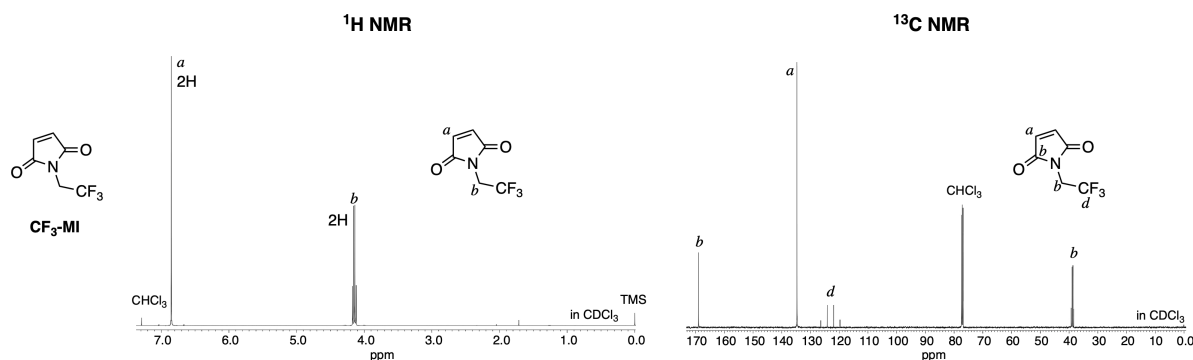


Figure 1. ¹H and ¹³C NMR spectra of CF₃-MI in CDCl₃.

Synthesis of *N*-Propoxycarbonyl maleimide (Pr-CMI)

Maleimide (412 mg, 4.24 mmol, 1 eq.) was dissolved in 8.4 mL of dichloromethane. Propyl chloroformate (780 mg, 6.36 mmol, 1.5 eq.) and triethylamine (860 mg, 8.48 mmol, 2 eq.) were added dropwise to the solution at –30 ~ –40 °C. After stirring for 7 min, the reaction was quenched by adding 20 mL of water. The organic layer was collected and water layer was extracted by ethyl acetate (40 mL × 2). Combined organic extracts were washed by 3% HCl aq. (30 mL × 3) and brine (30 mL × 3). After washing, the organic solution was dried over MgSO₄ for 15 min, filtered, and evaporated in vacuo. The crude product was purified by column chromatography on neutral silica gel (eluent: hexane/ethyl acetate = 3/1 to 1/1, R_f = 0.28 to 0.58) to give Pr-CMI (403 mg, 2.20 mmol, yield 52%) as white solid. ¹H NMR (500 MHz, CDCl₃, δ): 6.85 (s, 2H, CH), 4.32 (t, 2H, CH₂), 1.79 (m, 2H, CH₂), 1.02 (t, 3H, CH₃); ¹³C NMR (125 MHz, CDCl₃, δ): 165.83, 147.72, 135.29, 69.42, 21.84, 10.25 (Figure 2a).

Synthesis of *N*-((2-ethylhexyl)oxy)carbonyl maleimide (2EH-CMI)

Maleimide (412 mg, 4.24 mmol, 1 eq.) was dissolved in 8.4 mL of dichloromethane. 2-Ethylhexyl chloroformate (1.23 g, 6.36 mmol, 1.5 eq.) and triethylamine (860 mg, 8.48 mmol,

2 eq.) were added dropwise to the solution at $-30 \sim -40\text{ }^{\circ}\text{C}$. After stirring for 7 min, the reaction was quenched by adding 20 mL of water. The organic layer was collected and water layer was extracted by ethyl acetate ($40\text{ mL} \times 2$). Combined organic extracts were washed by 3% HCl aq. ($30\text{ mL} \times 3$) and brine ($30\text{ mL} \times 3$). After washing, the organic solution was dried over MgSO_4 for 15 min, filtered, and evaporated in vacuo. The crude product was purified by column chromatography on neutral silica gel (eluent: hexane/ethyl acetate = 85/15 to 70/30, R_f = 0.15 to 0.53) to give 2EH-CMI (787 mg, 3.11 mmol, yield 73%) as pale-yellow liquid. ^1H NMR (500 MHz, CDCl_3 , δ): 6.83 (s, 2H, CH), 4.28 (m, 2H, CH_2), 1.69 (m, 1H, CH), 1.50-1.26 (m, 8H, CH_2), 0.93-0.88 (m, 6H, CH_3); ^{13}C NMR (125 MHz, CDCl_3 , δ): 165.82, 147.86, 135.29, 70.12, 38.76, 30.14, 28.89, 23.59, 22.97, 14.09, 11.01 (Figure 2b).

Synthesis of *N*-benzyloxycarbonyl maleimide (Bz-CMI)

Maleimide (412 mg, 4.24 mmol, 1 eq.) was dissolved in 8.4 mL of dichloromethane. Benzyl chloroformate (1.08 g, 6.36 mmol, 1.5 eq.) and triethylamine (860 mg, 8.48 mmol, 2 eq.) were added dropwise to the solution at $-30 \sim -40\text{ }^{\circ}\text{C}$. After stirring for 7 min, the reaction was quenched by adding 20 mL of water. The organic layer was collected and water layer was extracted by ethyl acetate ($40\text{ mL} \times 2$). Combined organic extracts were washed by 3% HCl aq. ($30\text{ mL} \times 3$) and brine ($30\text{ mL} \times 3$). After washing, the organic solution was dried over MgSO_4 for 15 min, filtered, and evaporated in vacuo. The crude product was purified by column chromatography on neutral silica gel (eluent: hexane/ethyl acetate = 3/1 to 2/1, R_f = 0.16 to 0.41) to give Bz-CMI (464 mg, 2.01 mmol, yield 47%) as white solid. ^1H NMR (500 MHz, CDCl_3 , δ): 7.48-7.33 (m, 5H, Ph), 6.83 (s, 2H, CH), 5.40 (t, 2H, CH_2); ^{13}C NMR (125 MHz, CDCl_3 , δ): 165.67, 147.57, 135.35, 134.45, 128.76, 128.43, 69.17 (Figure 2c).

Synthesis of *N*-hexadecyloxycarbonyl maleimide (C16-CMI)

Maleimide (515 mg, 5.30 mmol, 1 eq.) was dissolved in 10.5 mL of dichloromethane. Hexadecyl chloroformate (2.42 g, 7.95 mmol, 1.5 eq.) and triethylamine (1.08 g, 10.6 mmol, 2 eq.) were added dropwise to the solution at $-30 \sim -40\text{ }^{\circ}\text{C}$. After stirring for 8 min, the reaction was quenched by adding 20 mL of water. The organic layer was collected and water layer was extracted by ethyl acetate ($40\text{ mL} \times 2$). Combined organic extracts were washed by 3% HCl aq. ($30\text{ mL} \times 3$) and brine ($30\text{ mL} \times 3$). After washing, the organic solution was dried over MgSO_4 for 15 min, filtered, and evaporated in vacuo. The crude product was purified by column chromatography on neutral silica gel (eluent: hexane/ethyl acetate = 4/1, R_f = 0.29) to

give C16-CMI (1.14 g, 3.12 mmol, yield 59%) as white solid. ^1H NMR (500 MHz, CDCl_3 , δ): 6.82 (s, 2H, CH), 4.35 (t, 2H, CH_2), 1.75 (m, 2H, CH_2), 1.41 (m, 2H, CH_2), 1.29 (m, 2H, CH_2), 1.25 (m, 22H, CH_2), 0.88 (t, 3H, CH_3); ^{13}C NMR (125 MHz, CDCl_3 , δ): 165.85, 147.78, 135.31, 68.17, 32.04, 29.81, 29.78, 29.75, 29.67, 29.58, 29.48, 29.25, 28.48, 25.74, 22.81, 14.23 (Figure 2d).

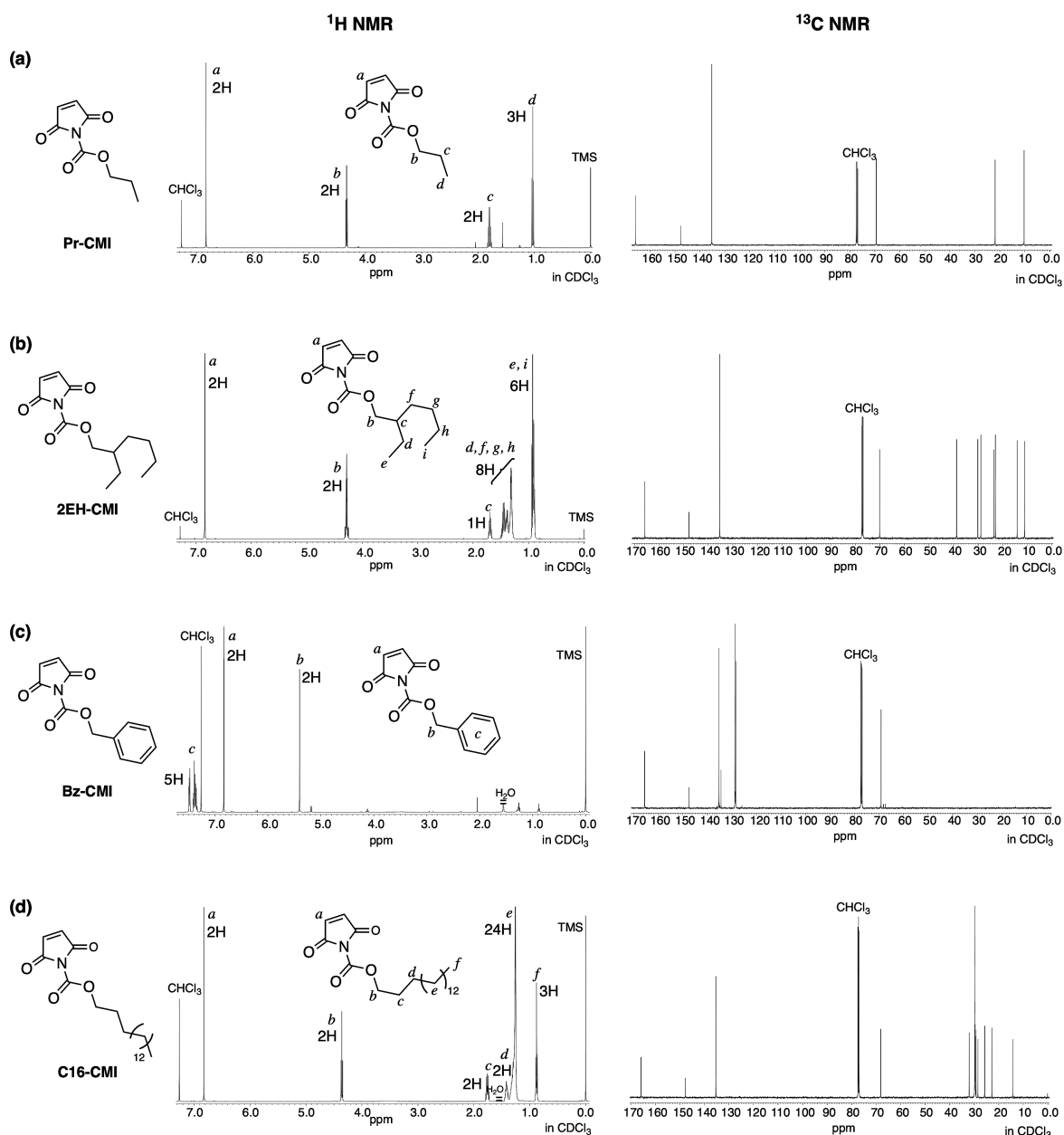


Figure 2. ^1H and ^{13}C NMR spectra of (a) Pr-CMI, (b) 2EH-CMI, (c) Bz-CMI, and (d) C16-CMI in CDCl_3 .

Result and Discussion

Copolymerization of Styrene and EMI

The copolymerization of styrene (S) and EMI (M) was conducted via ruthenium-catalyzed living radical polymerization with the malonate-based bromine initiator (Figure 3). The specific initiator was used to fascinate sequence analyses of resultant copolymers by MALDI-TOF-MS, since the radical species would react with styrene selectively giving only two series of alternating copolymers in MALDI-TOF-MS spectra in the case of no error in alternating propagation, which is described in chapter 1 (Figure 3a).¹⁹ A cationic ruthenium complex free from halogen²⁰ was used as the catalyst, instead of typical chlorine-based ruthenium catalysts²¹ to avoid halogen exchange with the bromine from the initiator incurring complicated mass spectrum. Thus, 50 eq. comonomers were injected for the styrene-selective initiator in conjunction with the ruthenium catalyst in toluene, and the solution was heated at 60 °C to initiate the polymerization: $[\text{styrene}]_0 = [\text{EMI}]_0 = 500 \text{ mM}$, $[\text{initiator}]_0 = 10 \text{ mM}$, $[\text{Cp}^*\text{Ru}(\text{CH}_3\text{CN})_3]^+\text{PF}_6^-]_0 = 5 \text{ mM}$, $[\text{PPh}_3]_0 = 10 \text{ mM}$. The conversions of the comonomers, which were monitored by ^1H NMR, were smoothly increased, and they were almost the same during the copolymerization (Figure 3b). The resultant copolymers showed unimodal SEC curves to give narrow molecular weight distributions (finally $M_w/M_n = 1.13$: Figure 3c), indicating that the initiation and propagation was well controlled. The copolymer at the highest conversion (96%, 97%, respectively) was purified through preparative SEC to remove low molecules (i.e., monomer and catalyst). Then the averaged polymerization degrees of the two monomer units and terminal structure of the purified copolymer were characterized by ^1H NMR (Figure 3d). In the spectrum, the signal derived from the ester methylene protons of the initiator moiety (*b*) was clearly detected. $DP_{n,S}$ and $DP_{n,M}$ (DP_n for styrene and EMI units, respectively) were calculated from the integration ratio of *b* and those from the repeating units (*h* from styrene and *i* from EMI): $DP_{n,S} = 44$, $DP_{n,M} = 45$. They were almost same as the ideal $DP_{n,S}$, which are estimated from the conversions and injection ratios. Thus, the consumption behaviors of the two monomers and the ^1H NMR analysis of the obtained copolymer certainly supported occurrence of alternating copolymerization.

Then, MALDI-TOF-MS was measured for the obtained copolymers to analyze the sequence in more detail. Figure 4 shows spectra of the copolymers obtained at early (conv. = 9/7%), middle (38/38%) and late stage (96/97%) of the copolymerization. Mass to charge ratios (*m/z*) from most peak series provided structures of the copolymers whose terminal groups are the malonate moiety and unsaturated unit [the observed mass is the adduct of sodium ion

(Na⁺)). The unsaturated structure is likely due to the halogen elimination by the laser during mass analysis. In the case that no error for alternating propagation take place as well as the styrene selective initiation, the peak series where number of styrene (N_S) is one more than that of EMI (N_M) or same as it [(N_S, N_M) = (n+1, n) or (n, n)], showed the ideal alternating

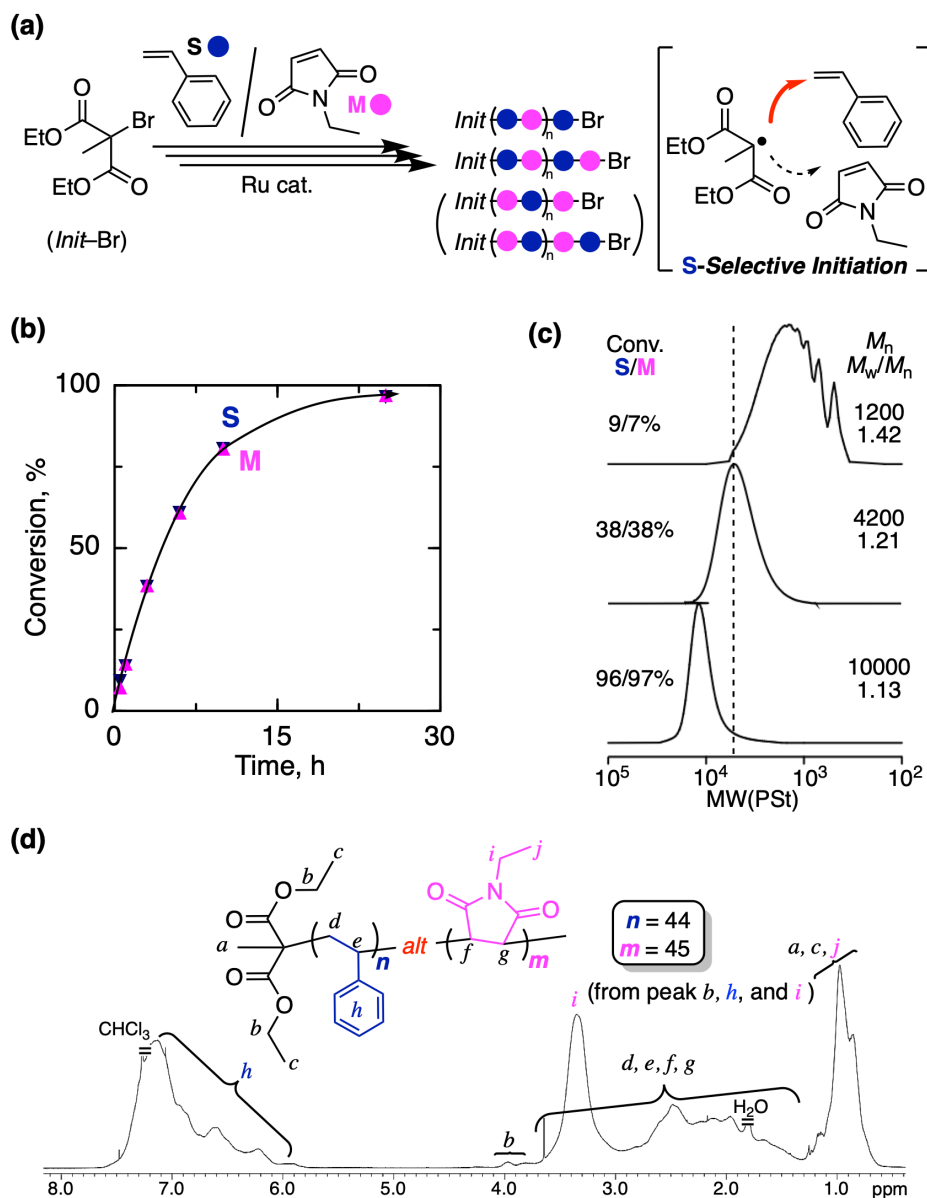


Figure 3. Copolymerization of styrene (S) and EMI (M) via ruthenium-catalyzed living radical polymerization with a malonate-based bromine initiator: [styrene]₀/[EMI]₀/[Initiator]₀/[[Cp^{*}Ru(CH₃CN)₃]⁺PF₆⁻]₀/[PPh₃]₀ = 500/500/10/5/10 mM in toluene at 60 °C. (a) Scheme of the copolymerization, (b) time-conversion plot, (c) SEC curves, and (d) ¹H NMR spectrum of the resultant copolymer [conv. = 96/97% (S/M)].

copolymers. In addition to the main series, minor series were also observed, in which N_M was one more than N_S [$(N_S, N_M) = (n, n+1)$] or N_S was two more than N_M [$(N_S, N_M) = (n+2, n)$] even at early stage of the copolymerization (The former minor series are perhaps derived from an initiation to maleimide (i.e. unexpected initiation) followed by perfect alternating propagation.). Detection of the minor series indicated some errors for alternating growth resulting in the successive same units in the chain: that is, the alternation in the propagation was imperfect. As the copolymerization further proceeded, the error peaks were remarkably observed and eventually the series from the ideal alternating sequence were no longer predominant over the error series. Styrene-rich errors were dominant over maleimide-rich counterparts at the middle stage, whereas the tendency seemed to be reversed at the late stage. One plausible explanation for the complicated trend is that remained comonomer ratio is fluctuated as errors for alternating propagation are accumulated during copolymerization.

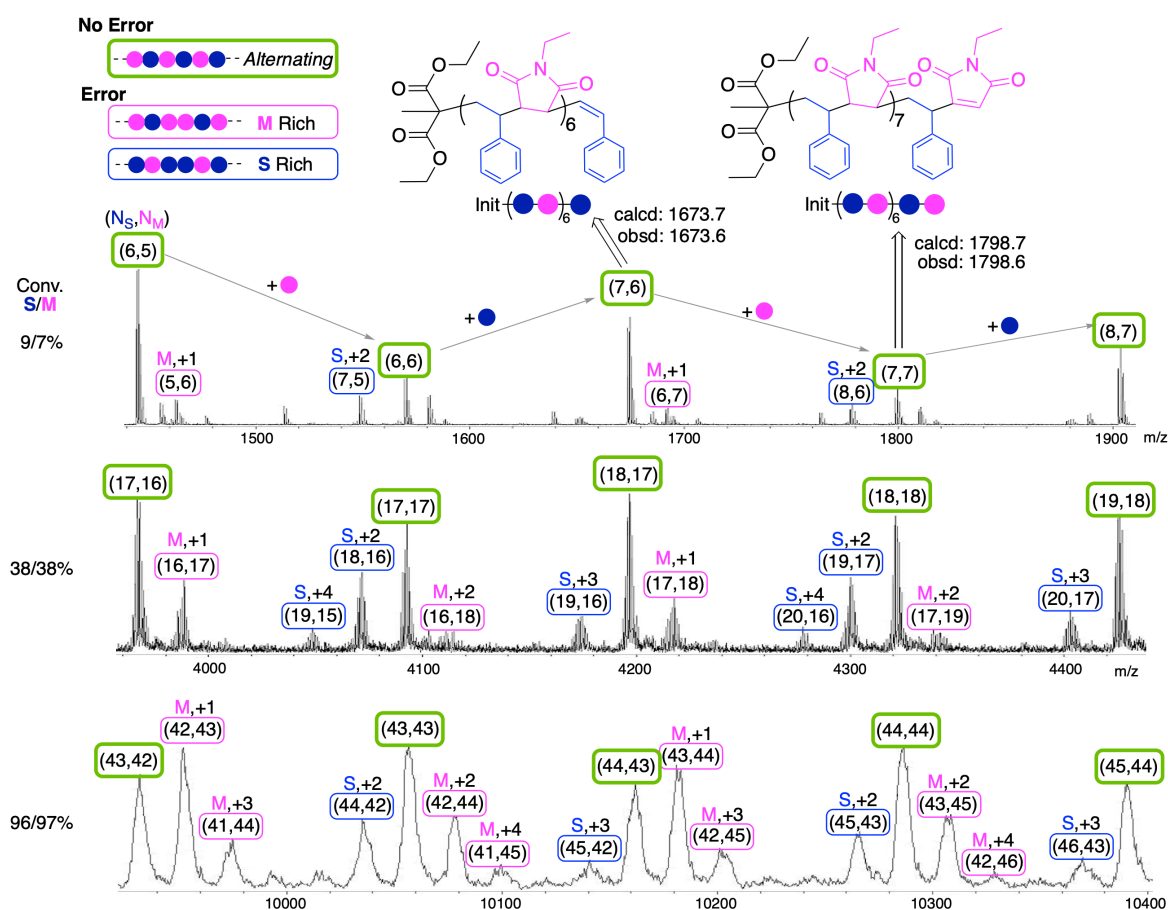


Figure 4. MALDI-TOF-MS spectra of the copolymer with styrene (S) and EMI (M) at early, middle, and late stage of copolymerization.

Anyhow the occurrence of errors at early stage would cause complicated peak patterns of finally obtained copolymers. The error or the successive propagation of same monomer likely took place in a certain proportion from the beginning and this was accumulated to result in various peak series. Thus, the peak series of the green square could be partially derived from the chain carrying same number of SS and MM successive units.

I indeed calculated the reactivity ratios of styrene and EMI with from the free radical copolymerization with various injection ratios by using both Fineman-Ross method and non-linear least square fitting method: $r_1 = 0.04$, $r_2 = 0.09$ (M_1 = styrene, M_2 = EMI, Figure 5). Both the values as well as the multiplication were almost zero, and the theoretical prediction from the reactivity ratios supports progress of alternating copolymerization. However, it was found that sequence of the resultant copolymer chains was imperfectly alternating from the MALDI-TOF-MS analyses.

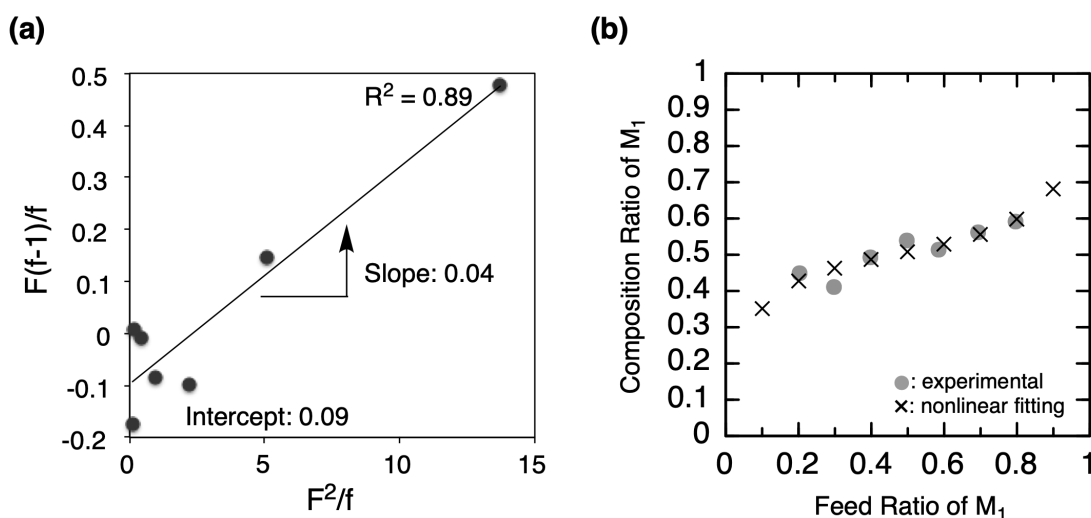


Figure 5. (a) Fineman-Ross plot and (b) copolymer composition curve fitted by non-linear least square method of copolymerization with styrene and EMI; $[\text{styrene}]_0 + [\text{EMI}]_0 / [\text{AIBN}]_0 = 1000/10$ mM in toluene at 60 °C, the copolymerization was quenched at early polymerization stage where monomer conversions were low (~15%).

Design of Maleimide toward Higher Precise Alternating Sequence

Given by the result of poorer alternating sequence for the pair of styrene and EMI, another type of maleimide was designed toward higher precise alternating sequence. More precisely alternating sequence can be expected, as the difference in electron density between

the two comonomers is greater. Thus, a trifluoromethyl group ($-\text{CF}_3$) was introduced instead of the methyl group ($-\text{CH}_3$) in EMI to design $\text{CF}_3\text{-MI}$ (Figure 6). Effect of the electron-withdrawing group ($-\text{CF}_3$) was confirmed by ^{13}C NMR and ^1H NMR spectra: the peaks derived from carbons and protons in the double bond were observed at lower magnetic field than those of EMI. Thus, the CF_3 group likely withdraws the electron of the double bond to decrease the electron density of double bond.

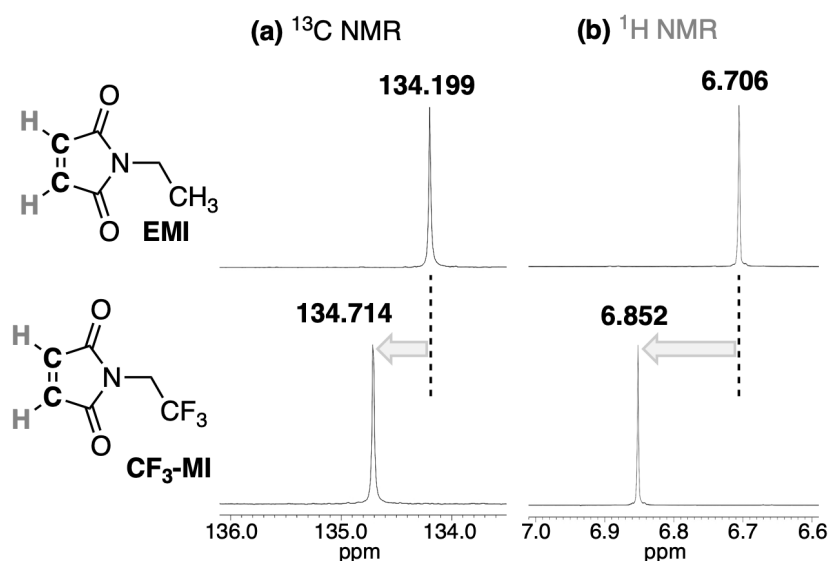


Figure 6. (a) ^{13}C NMR and (b) ^1H NMR spectra of EMI and $\text{CF}_3\text{-MI}$ at 500 mM in CDCl_3 .

Sequence Analysis of the Copolymer with Styrene and $\text{CF}_3\text{-MI}$

The newly designed maleimide monomer, $\text{CF}_3\text{-MI}$, was copolymerized with styrene via ruthenium-catalyzed living radical polymerization with malonate-based bromine initiator under the same condition as for the pair of styrene and EMI: $[\text{styrene}]_0 = [\text{CF}_3\text{-MI}]_0 = 500 \text{ mM}$, $[\text{initiator}]_0 = 10 \text{ mM}$, $[[\text{Cp}^*\text{Ru}(\text{CH}_3\text{CN})_3]^+\text{PF}_6^-]_0 = 5 \text{ mM}$, $[\text{PPh}_3]_0 = 10 \text{ mM}$ in toluene at 60°C . The conversions of the comonomers were also almost the same during the copolymerization and the SEC curves of the resultant copolymers were unimodal giving narrow molecular weight distribution (finally $M_n = 9700$, $M_w/M_n = 1.16$). The copolymer at high conversion (81%, 79%, respectively) was purified by preparative SEC and the composition ratio of the copolymer was measured by ^1H NMR. Although the averaged polymerization degrees of the two monomer units were not estimated due to overlap between peaks from the terminal moieties and the repeating units, the composition ratio was calculated from the integration ratio of the peaks of aromatic protons from styrene and methylene protons from $\text{CF}_3\text{-MI}$: $DP_{n,S} : DP_{n,M} = 49 : 51$.

Monomer reactivity ratios were almost zero [$r_1 = 0.12$, $r_2 = 0.08$ ($M_1 = \text{styrene}$, $M_2 = \text{CF}_3\text{-MI}$), Figure 7] similar to the ratios at the pair of styrene and EMI.

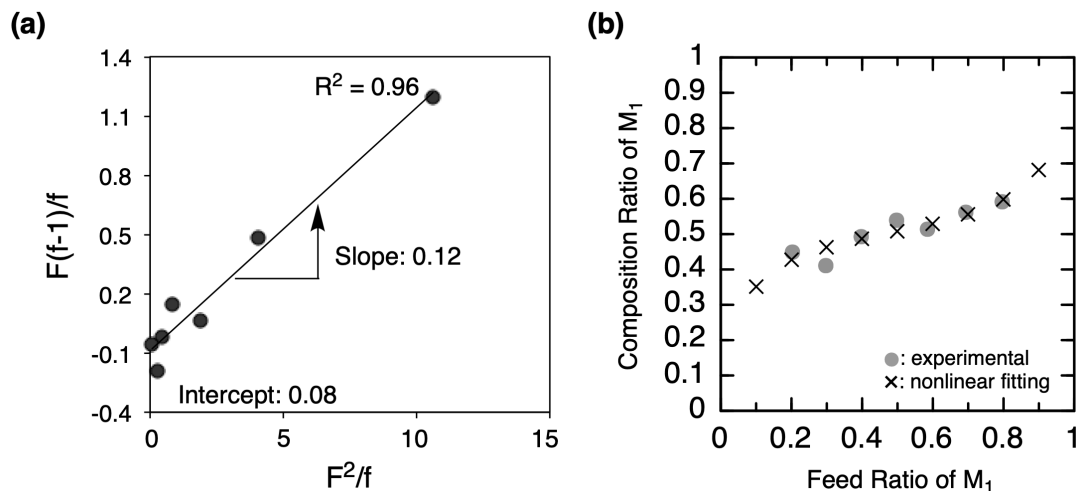


Figure 7. (a) Fineman-Ross plot and (b) copolymer composition curve fitted by non-linear least square method of copolymerization with styrene and $\text{CF}_3\text{-MI}$; $[\text{styrene}]_0 + [\text{CF}_3\text{-MI}]_0 / [\text{AIBN}]_0 = 1000/10$ mM in toluene at 60°C , the copolymerization was quenched at early polymerization stage where monomer conversions were low ($\sim 15\%$).

Sequence of the obtained copolymers was examined by MALDI-TOF-MS. Figure 8 shows the spectra of the copolymers at early (conv. = 26/25%), middle (35/33%), and late stages (81/79%) of the copolymerization. The number of peak series was apparently decreased in comparison with the copolymers of styrene and EMI (Figure 4). Especially, for the early stage of the copolymerization, there was only one peak series. The mass is almost equal to for a sodium adduct of the alternating copolymer and the polymerization degree of styrene was one more than that of maleimide $[(N_S, N_M) = (n+1, n)]$. Interestingly, the interval of the peak series corresponded to the sum of molecular weight of the styrene and $\text{CF}_3\text{-MI}$, and the peak series from same polymerization degree $[(N_S, N_M) = (n, n)]$ was not detected. The result suggests both of the first unit (i.e., initiation) and the last at ω -end are identical among all chains. Assuming that the selective initiation to styrene quantitatively occurs, the one peak series $[(N_S, N_M) = (n+1, n)]$ would be explained as follows: the dormant or the active species after one unit

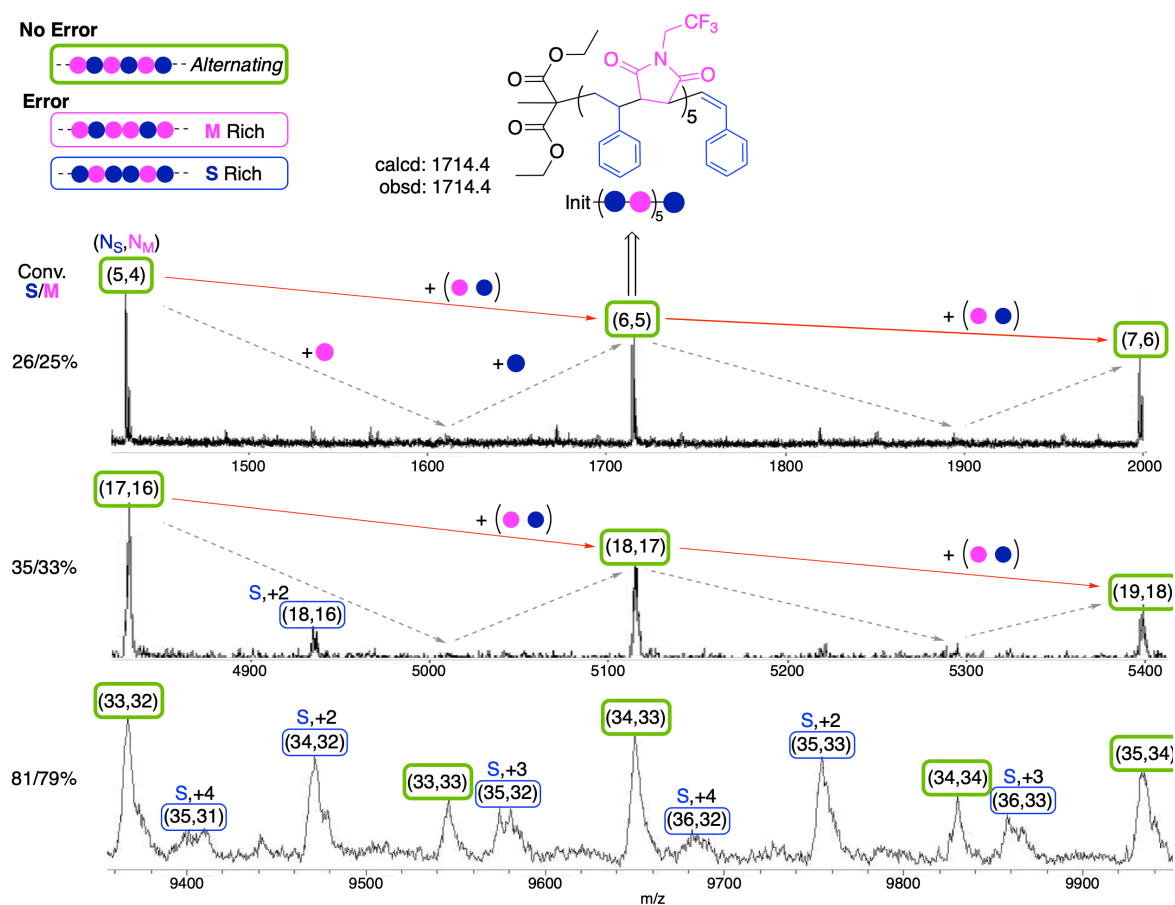


Figure 8. MALDI-TOF-MS spectra of the copolymer with styrene (S) and CF₃-MI (M) at early, middle, and late stage of copolymerization.

of styrene addition to the initiator would propagate with the pair of the comonomer at once to give alternating copolymer carrying the styrene unit one more than the maleimide [Initiator–S–(M–S)_n–Br]. Indeed, some literatures suggest that the comonomer complex can propagate to give alternating copolymer.^{2,22-24}

Evaluation of Interaction between Styrene and Maleimide Derivatives by ¹H NMR

The interaction of CF₃-MI with styrene was evaluated with ¹H NMR in comparison with EMI, where the equimolar mixtures were measured at some concentrations (50, 100, 200, and 500 mM in CDCl₃). Figure 9 shows the spectra from 5.2 – 7.4 ppm for the mixture (upper), along with styrene (middle) and maleimide (lower) at 500 mM (a: CF₃-MI; b: EMI). The peak derived from olefinic protons of CF₃-MI shifted to higher magnetic by 8.6×10^{-2} ppm. Same tendency was also observed for EMI, however, the peak shift value [Δ = chemical shift

(monomer in mixture) – chemical shift (monomer)] was not so large as with CF₃-MI (EMI: $\Delta = 6.1 \times 10^{-2}$ ppm). The difference indicates stronger interaction of CF₃-MI with St than EMI. As the solution was diluted, the peak shift value became smaller (for 200, 100, 50 mM). From the plots of the peak shift values against the concentration (Figure 9c), it turned out that the advantage difference of CF₃-MI over EMI became more significant as the solution was concentrated. On the other hand, the peaks derived from olefinic or aromatic protons of styrene were hardly shifted even though the solution was concentrated, which is likely because of the broader conjugated structure of styrene. The stronger interaction of CF₃-MI with styrene would be related to the simpler pattern as well as the peak interval corresponded to the sum of the both monomer molecular weights in MALDI-TOF-MS spectra (Figure 8). The difference was less significant as the concentration was lower, which would be related to the results the peak pattern in MALDI-TOF-MS became complicated for the copolymers at the late stage. The comonomer interaction was also studied with same solvent (toluene-d₈) as for the copolymerization. However, the peak shift was hardly observed in toluene-d₈. Interestingly, the peak from double bond of CF₃-MI appeared at unusually higher magnetic field in toluene-d₈ (5.8 ppm vs. 6.8 ppm in CDCl₃ or acetone-d₆), indicating some interaction with the toluene molecule. This may be related to the little peak shift but the detailed mechanism is unclear. Some mechanisms for alternating copolymerization have been proposed with monomer complex^{2,22-25} and penultimate model.^{26,27} It is still controversial to conclude but the analyses with MALDI-TOF-MS of resulting copolymer for observation of peak interval and ¹H NMR of the mixture of comonomers for observation of monomer interaction is important for understanding the mechanism.

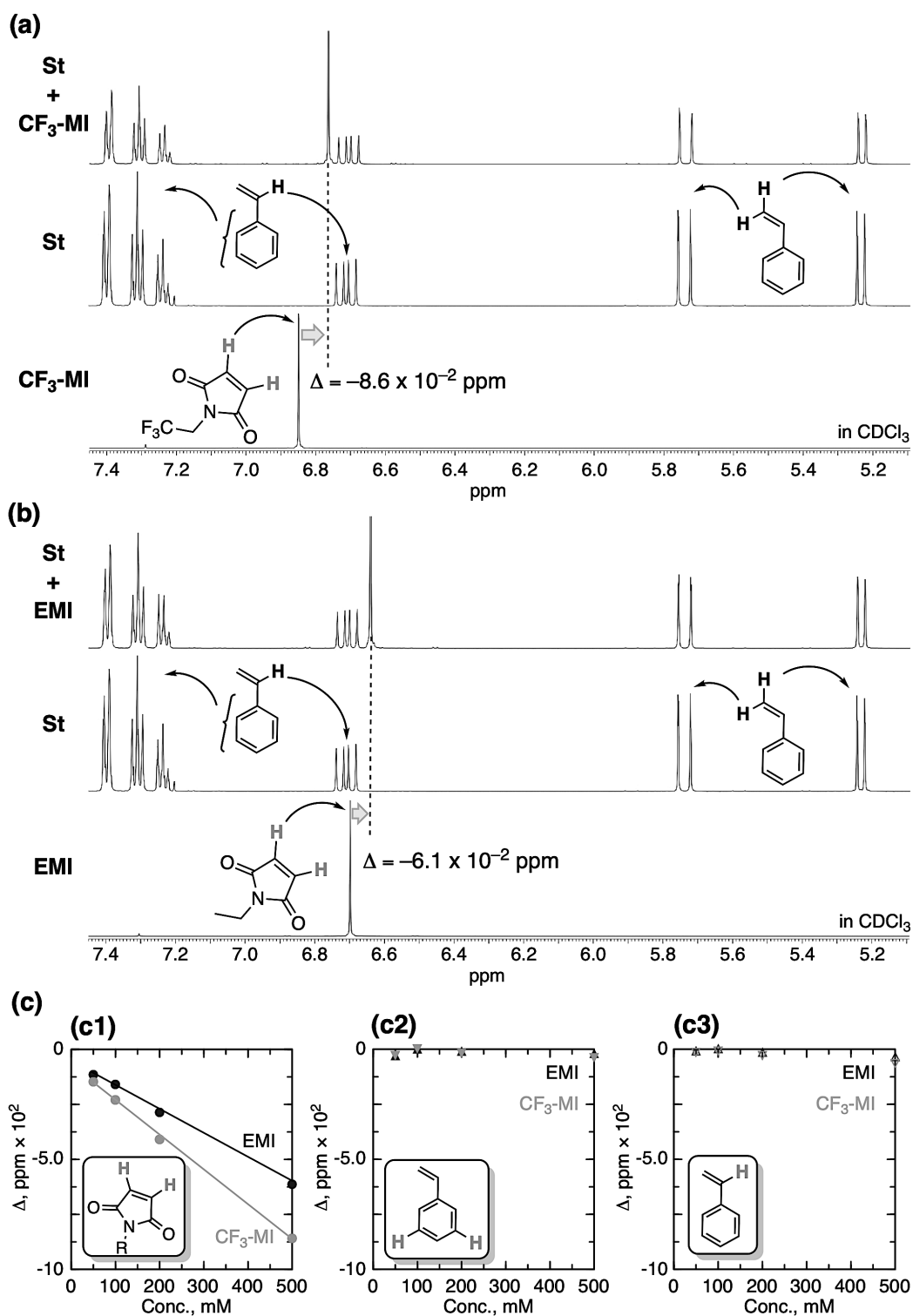


Figure 9. (a, b) ¹H NMR spectra for mixture of styrene (S) and maleimide (M: CF₃-MI or EMI); [S]/[M] = 500/500 mM (upper), [S]/[M] = 500/0 mM (middle), and [S]/[M] = 0/500 mM (lower) in CDCl₃, (c) peak shift Δ – concentration plots of olefinic protons of maleimide (c1), aromatic protons of styrene (c2), and olefinic proton of styrene (c3).

Design of Maleimide for Incorporation of Functional Groups into Highly Precise Alternating Copolymers

The structure of CF₃-MI is not suitable for further incorporation of pendant group, and thus a new type of maleimide derivatives was studied to allow introduction of various functional groups as well as highly precise alternating sequence. Hence, *N*-alkoxycarbonyl maleimide (R-CMI) was newly synthesized via the reaction of maleic anhydride with alkyl chloroformates [alkyl (R): propyl (Pr), benzyl (Bz), 2-ethylhexyl (2EH), hexadecyl (C16)]²⁸ (Figure 10). The NMR analyses for the hexadecyl derivative (C16-CMI) certainly supported lower electron density of the double bond and stronger interaction with styrene than EMI, similar to CF₃-MI: the peaks derived from carbons and protons of the double bond in C16-CMI were observed at 135.263 ppm (¹³C NMR) and 6.854 ppm (¹H NMR) respectively.

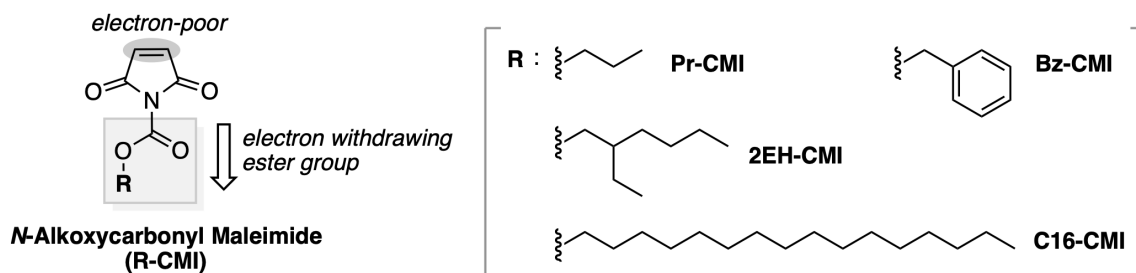


Figure 10. Structures of *N*-alkoxycarbonyl maleimides (R-CMI).

The Sequence Analysis of the Copolymers with Styrene and *N*-Alkoxycarbonyl Maleimides

The *N*-alkoxycarbonyl maleimides were also copolymerized with styrene via ruthenium-catalyzed living radical polymerization with the malonate-based bromine initiator. The copolymerization results (i.e., conversions, SEC curves, and structural analyses by ¹H NMR) supported controlled alternating copolymerizations similar to with EMI and CF₃-MI (finally $M_w/M_n < 1.25$, and composition ratios are almost 1:1). Figure 11 shows MALDI-TOF-MS spectra for the copolymers at the early stage of the copolymerizations. Although there were minor peaks whose structures cannot be identified, the main series were observed at regular mass intervals that were equal to sum of molecular weights of R-CMI and styrene. Thus, even the *N*-alkoxycarbonyl maleimides, the copolymerization with styrene would mainly proceed via alternating propagation. The pendant of the maleimides could be modified with various groups to functionalize the units in the alternating sequence.

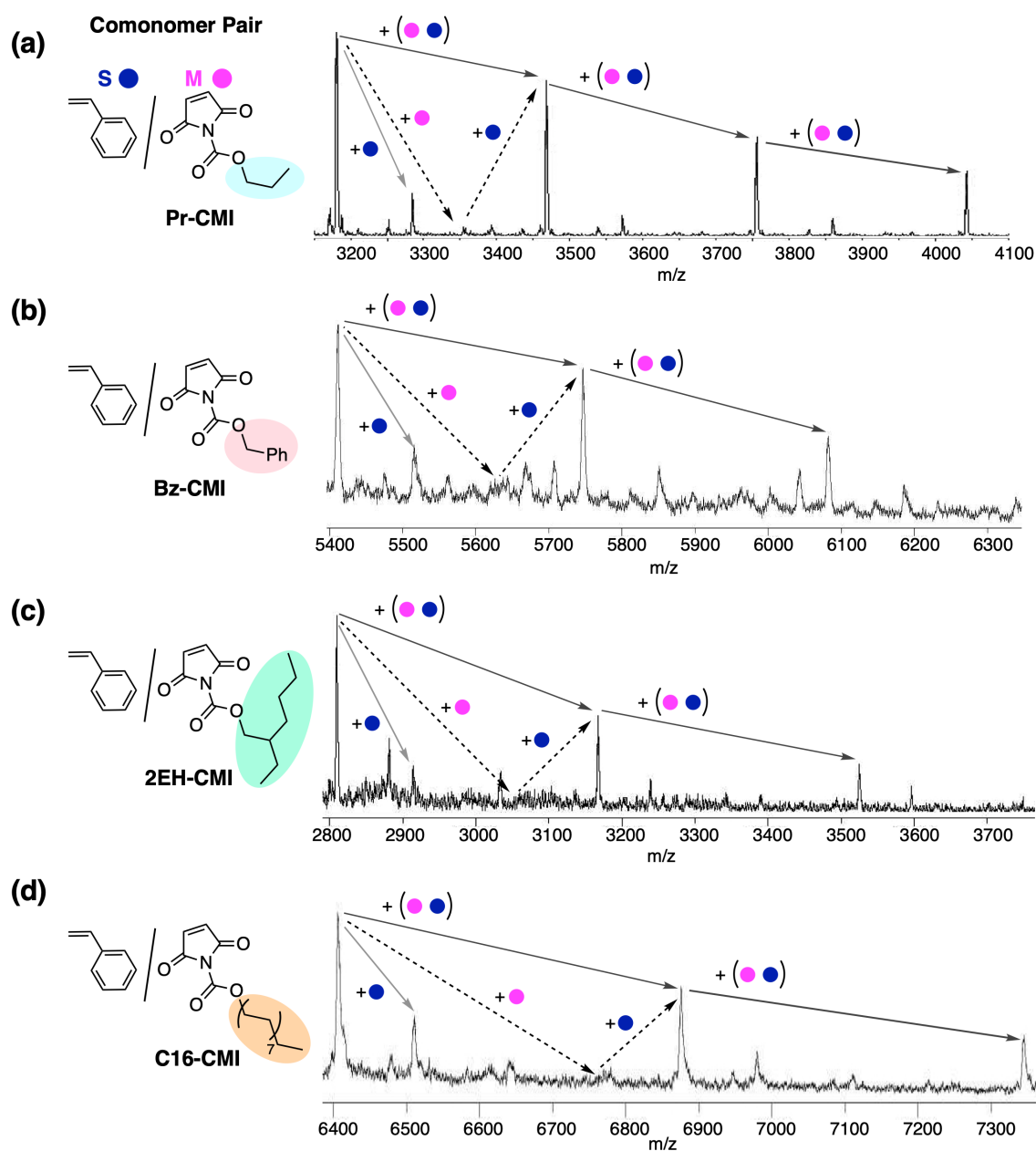


Figure 11. MALDI-TOF-MS spectra of copolymers with styrene (S) and R-CMIs (M) synthesized via ruthenium-catalyzed living radical polymerization with a malonate-based bromine initiator: [styrene]₀/[R-CMI]₀/[Initiator]₀/[[Cp**Ru*(CH₃CN)₃]⁺PF₆⁻]₀/[PPh₃]₀ = 500/500/10/5/10 mM in toluene at 60 °C. (a) Pr-CMI [conv. = 23/17% (S/M), *M*_n = 3300, *M*_w/*M*_n = 1.88], (b) Bz-CMI [conv. = 59/63% (S/M), *M*_n = 7500, *M*_w/*M*_n = 1.57], (c) 2EH-CMI [conv. = 22/25% (S/M), *M*_n = 4700, *M*_w/*M*_n = 2.12], and (d) C16-CMI [conv. = 39/41% (S/M), *M*_n = 10,000, *M*_w/*M*_n = 1.78].

Conclusion

MALDI-TOF-MS sequence analysis revealed that the radical copolymerization of styrene and EMI does not give perfect alternating sequence. On the other hand, when a more electron-deficient maleimide CF₃-MI was used instead of EMI, accuracy of the alternating sequence was dramatically improved: the MALDI-TOF-MS spectra turned into simpler than the case with EMI; the interval of main peaks corresponded to sum of molecular weight of styrene and CF₃-MI. This is due to stronger interaction between styrene with CF₃-MI than with EMI, as supported by ¹H NMR analysis. *N*-Alkoxycarbonyl maleimide derivatives (R-CMI) were also useful as the electron-deficient comonomers for giving high alternating sequence as well as introduction of various pendant groups (R-). The result is significant toward alternating sequence-oriented functions in conjunction with styrene derivatives.

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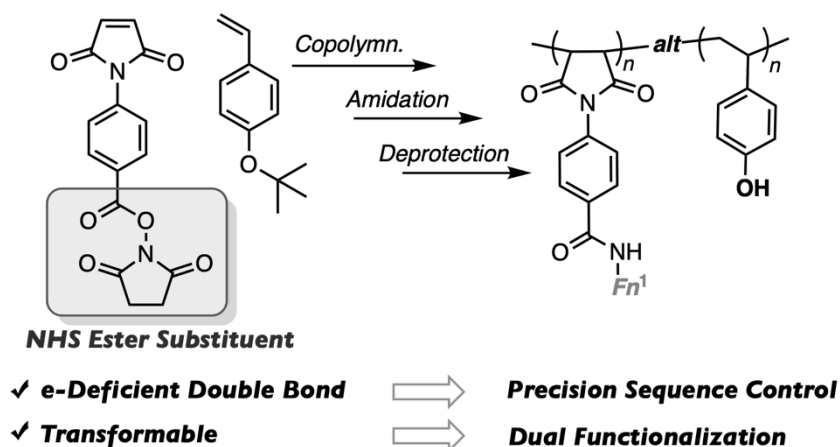
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Chapter 3

Design of Maleimide Monomer to Achieve Precise Sequence Control and Functionalization for Alternating Copolymer with Vinyl Phenol

Abstract

We designed an *N*-phenyl maleimide derivative bearing an activated NHS ester (NHS-PhMI) for radical copolymerization with *tert*-butoxystyrene (tBOS) to achieve both precise alternating sequence control and functionalization. Essential of the monomer design is the electron-deficient carbon-carbon double bond for precise alternating propagation as well as the facile substitution reaction for the activated ester pendant. The precision of alternating sequence was evaluated by MALDI-TOF-MS analysis in comparison with a series of copolymers. Some amine compounds were quantitatively incorporated on the maleimide side chain through the aminolysis reaction, whereas the tBOS units were transformed into vinyl phenol counterparts via the deprotection under acidic condition. The resultant vinyl phenol-based alternating copolymers showed unique solubilities and thermal properties depending on the substituent of the maleimide units.



Introduction

Sequence control for vinyl copolymers made of some repeating units derived from the olefinic comonomer has received considerable attention for development of novel functional polymer materials, and some methodologies have been proposed in the last decade.¹⁻³ However, they often require particular molecules synthesized via many steps and/or diluted condition in polymerization to ensure some selectivity, which often poses difficulties in the large scale synthesis as well as in the property evaluation toward material applications.

Alternating radical copolymerization with the specific comonomer combination has long been known⁴ and the process allows relatively facile synthesis of sequence-controlled copolymer. The alternating propagation is known to occur when an electron-withdrawing monomer (i.e., maleic anhydride) is combined with an electron-donating monomer (i.e., vinyl ether), although the mechanism of the selective cross-over propagation is still controversial.⁵ An importance to enhance the difference in electron densities of the double bonds in monomers for realization of alternating propagation has been implied in some literatures. For example, Hirooka et al. demonstrated that an addition of Lewis acid induced alternating copolymerization of styrene (St) and MMA by decreasing the electron density of the latter monomer through coordination on the carbonyl group.⁶ Studer et al. have recently reported an acrylate carrying hexafluoro-isopropyl group proceeded alternating copolymerization with vinyl ethers. Herein, the electron-withdrawing substituent allows decreasing in electron density of the double bond in the acrylate as well as undergoing post functionalization of the side chain after the copolymerization.⁷ It has been also reported that other factors such as bulkiness and resonance of monomer pendant affect sequence of resultant copolymers in radical copolymerization.^{8,9}

Since alternating copolymer consists of two monomer units in periodic sequence, sequence-driven properties/functions on the basis of the neighboring effect are expected. Indeed, some properties derived from alternating sequence have been reported, such as solvatochromism,¹⁰ fluorescence,^{11,12} catalyst¹³, degradation^{14,15} and thermal response in solvent.¹⁶⁻¹⁸ Our group has recently reported thermal response and physical gelation of alternating copolymers bearing phenol and long alkyl pendants, which was synthesized via radical copolymerization of *tert*-butoxystyrene (tBOS) with *N*-long alkyl maleimide and followed deprotection reaction of *tert*-butoxy groups, in aromatic solvents such as toluene (Figure 1a).¹⁹ The diluted solution (1wt%) of the copolymer showed UCST-type thermal response, and as the concentration was increased (> 5wt%), the gel of core-shell fibrillar nano-structure was formed upon cooling. An incorporation of vinyl phenol unit in alternating

sequence is of interest for material applications due to the periodic array of the characteristic feature, such as hydrophilicity under basic condition and interaction site via hydrogen bonding. Thus, the vinyl phenol-based alternating copolymers that are prepared in conjunction with maleimides carrying other types of pendant groups are fascinating.

We have recently revealed that the degree of alternating sequence of the copolymer with styrene (St) and *N*-alkyl maleimide (i.e. *N*-ethyl maleimide) was found less precise than expected from the reactivity ratios through the structural analyses by MALDI-TOF-MS.^{20,21} We have also reported that an introduction of electron-withdrawing groups into the side chain of MI was effective to give higher precise alternating sequence.²¹ Therefore, it is required to strike the balance between electron-withdrawing feature and functionalization for the MI monomer design toward construction of precisely alternating copolymers having functional groups on the MI units.

From the background described above, in this work, we have newly designed the MI monomer for alternating radical copolymerization with tBOS to achieve precise alternating sequence control as well as dual functionalization of resultant alternating copolymers, that is phenol and another functional group (Figure 1b). We then focused on an *N*-phenyl maleimide (PhMI) derivative as the fundamental framework because PhMI has been often used as the comonomer for copolymerization with St counterparts and the phenyl group is helpful for an introduction of substituent (–R). If orthogonal functionalization (–Fn and phenol) via precise

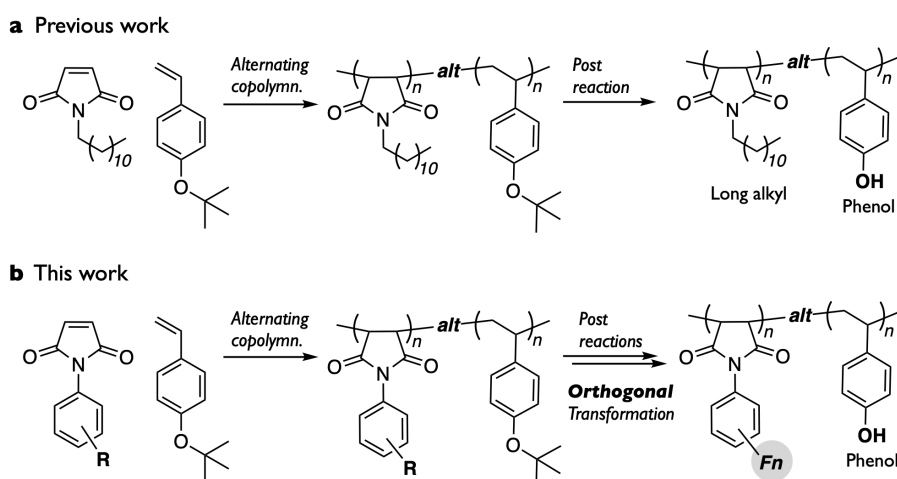


Figure 1. (a) Radical copolymerization of *N*-alkyl maleimide with tBOS followed by deprotection of the *tert*-butoxy group for synthesis of alternating copolymer bearing phenol and long alkyl pendants (our previous work¹⁹). (b) Radical copolymerization of transformable *N*-phenyl maleimide with tBOS followed by orthogonal post reactions for synthesis of alternating copolymer bearing phenol and functional pendants (this work).

alternating copolymerization followed by post-reactions could be performed, it would be useful to explore functions of the phenol-based alternating copolymers. Then, we first designed PhMI derivative to satisfy both high precision of alternating sequence and introduction of diverse functional groups. Second, a series of alternating copolymer consisting of tBOS and the maleimide was synthesized and the precision of alternating sequence was evaluated by MALDI-TOF-MS. Finally, alternating copolymers bearing phenol and various functional groups were obtained via post-reactions and physical properties of the copolymers were investigated.

Experimental

Materials

Maleic anhydride (Wako; purity >99%), *N*-hydroxysuccinimide (TCI; purity >98%), 4-aminobenzoic acid (TCI; purity >99%), 2,4,6-trimethylpyridine (*sym*-collidine, TCI; purity >98%), trifluoroacetic anhydride (TFAA, TCI; purity >98%), *N*-phenyl maleimide (PhMI, TCI; purity >98%), dithranol (Sigma Aldrich; purity >98%), trans-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB, TCI; >98%), sodium trifluoroacetate (TFANa, TCI; purity >98%) 2,2'-azobis(isobutyronitrile) (AIBN, TCI; purity >98%), 2-cyano-2-propyl dodecyl trithiocarbonate (CTA, Sigma Aldrich; purity 97%), *i*-propylamine (TCI; purity >99%), 4-picolylamine (TCI; purity >98%), *N,N*-dimethyl-1,3-propanediamine (TCI; purity >99%), *n*-dodecylamine (TCI; purity >97%), *N,N*-dimethylformamide (DMF, Wako; purity 99.8%), dichloromethane (CH₂Cl₂, Wako, purity >99.5%) were used without purification. Styrene (TCI; purity >99%), *tert*-butoxystyrene (tBOS, Sigma Aldrich; purity 99%) were passed through inhibitor remover (Sigma Aldrich) column to remove inhibitor before use. 1,2,3,4-Tetrahydronaphthalene (tetralin, Kishida Chemical; purity 99.5%), an internal standard in ¹H NMR, was dried over calcium chloride and distilled twice over calcium hydride. Acetonitrile (CH₃CN, Wako; purity >99.8%), tetrahydrofuran (THF, Wako; purity > 99.5%), toluene (Wako; purity > 99%) were dried by Molecular Sieves 3A (Wako) before use.

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operating at 500 MHz.

SEC (DMF). Molecular weight distribution (MWD) curves, number-average molecular weight (M_n) and M_w/M_n ratio of the polymers were measured by size exclusion chromatography

(SEC) in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L; exclusion limit = 4×10^6 g/mol; particle size = 10 μm ; pore size < 5000 Å; 0.8 cm i.d. $\times$ 30 cm) that were connected to a Jasco PU-2080 precision pump, a Jasco RI-2031 refractive index detector and a Jasco UV-2075 UV/vis detector set at 270 nm. The columns were calibrated against 11 standard PMMA samples (PSS, Polymer Standard Service GmbH, Ready Cal Kit: $M_p = 2,380 - 2,200,000$).

SEC (THF). MWD curves, M_n and M_w/M_n ratio of the polymers were also measured by SEC in THF as an eluent at 40 °C on three polystyrene gel columns (Shodex LF-404; exclusion limit = 2×10^6 g/mol; particle size = 6 μm ; pore size, < 3000 Å; 0.46 cm i.d. 25 cm; flow rate 0.3 mL/min) connected to a DU-H2000 pump, a 74S-RI refractive index detector, and a 41-UV ultraviolet detector (all from Shodex; Shodex GPC-104). The columns were calibrated against 12 standard polystyrene samples (TOSOH, PS-Oligomer Kit, $M_w = 500 - 1,110,000$; $M_w/M_n = 1.01 - 1.16$).

MALDI-TOF-MS. MALDI-TOF-MS analyses were performed on an ultraflex III (MALDI-TOF mass spectrometer, Bruker Daltonics) equipped with 337 nm nitrogen laser with dithranol or DCTB as matrix and sodium trifluoroacetate as cationizing agent.

FT-IR. FT-IR spectra of freeze-dried alternating copolymers were measured by Agilent Cary 630 FTIR spectrometer (Agilent Technologies).

DSC. DSC measurements (ca. 2.0 mg weighed into an aluminum pan) were performed on a DSC Q200 calorimeter (TA instruments) equipped with a RCS 90 electric freezing machine under dry nitrogen flow at a heating or cooling rate of 10 °C/min.

Synthesis of NHS-PhMI.^{22,23} Maleic anhydride (2.06 g, 10.5 mmol) was dissolved in 20 mL of ethyl acetate. A solution of 4-aminobenzoic acid (2.74 g, 10 mmol) in ethyl acetate (50 mL) was added dropwise to the solution of maleic anhydride at 0 °C and the mixture was stirred at room temperature for 24 h. After stirring, the solution was evaporated in vacuo to obtain 4-(3-carboxyacrylamido)benzoic acid (4.73 g, yellow solid).

In a flask A, a solution of 4-(3-carboxyacrylamido)benzoic acid (3.76 g, 16 mmol) in 80 mL of DMF was stirred at 0 °C and *sym*-collidine (4.48 mL, 33.6 mmol) was added dropwise. In a flask B, a solution of *N*-hydroxysuccinimide (7.36 g, 64 mmol) in 80 mL of DMF was stirred at 0 °C and TFAA (8.96 mL, 64 mmol) was added dropwise. The mixture was stirred for 10 min and *sym*-collidine (8.48 mL, 64 mmol) was added dropwise. After the mixture was stirred for 10 min, the solution in flask B was added dropwise to the solution in flask A. After

the addition, the reaction mixture was stirred for 20 h at room temperature. The mixture was diluted with 100 mL of CH_2Cl_2 and the solution was washed by 1.0 M HCl aq. ($150\text{ mL} \times 3$) and the organic layer was dried by MgSO_4 and evaporated to yield pale brown solid. In a crude solution in CH_2Cl_2 , silica gel was added and the mixture was evaporated to get dried pale brown silica gel. The dried silica gel was put on the pure silica gel column. The product (pale yellow solid, 2.1 g, yield 42%) was eluted by hexane/ethyl acetate = 5/5. ^1H NMR (500 MHz, CDCl_3 , δ): 8.24 (m, 2H, aromatic), 7.65 (m, 2H, aromatic), 6.91 (s, 2H, $\text{CH}=\text{CH}$), 2.91 (s, 4H, CH_2); ^{13}C NMR (125 MHz, CDCl_3 , δ): 169.24, 169.74, 161.25, 137.42, 134.62, 131.52, 125.31, 123.86, 25.77 (Figure 2).

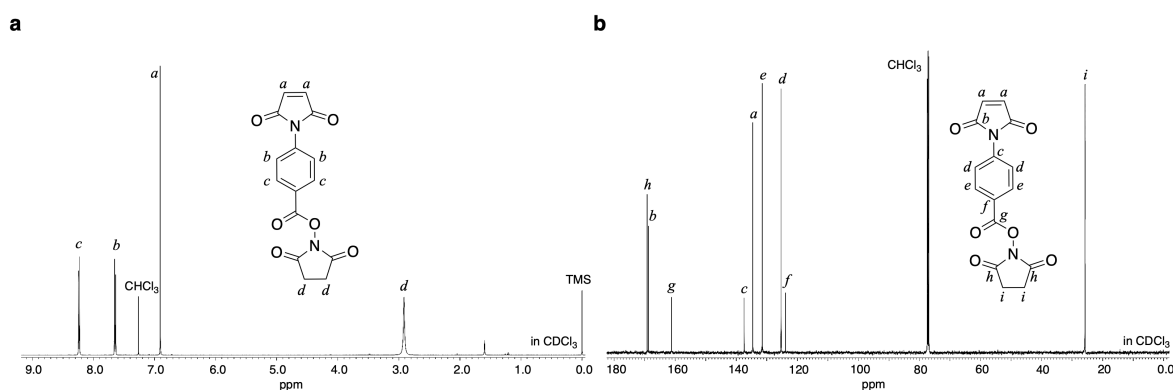


Figure 2. (a) ^1H and (b) ^{13}C NMR spectra of NHS-PhMI in CDCl_3 .

Alternating Copolymerization of tBOS and NHS-PhMI via RAFT Polymerization

Polymerization was carried out by the syringe technique under dry argon in glass tubes equipped with three-way stopcocks. Typical procedure for alternating copolymerization of tBOS and NHS-PhMI was as follows. To a Schlenk tube, NHS-PhMI (756 mg, 2.4 mmol) and tBOS (423 mg, 2.4 mmol) were added and the tube was degassed using a vacuum pump at $-78\text{ }^\circ\text{C}$. Then, CH_3CN (10.8 mL), tetralin (0.14 mL), CTA (1.19 mL of 101.66 mM in toluene, 0.12 mmol) and AIBN (0.1 mL of 120 mM in toluene, 0.012 mmol) were added (tBOS/NHS-PhMI/CTA/AIBN = 200/200/10/1 mM) under a dry argon flow. Immediately after mixing, the tube was placed in an oil bath at $60\text{ }^\circ\text{C}$ for 4.5 h. The copolymerization was terminated by bubbling with air. Monomer conversions were determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomers with tetralin as an internal standard (conversion: tBOS/NHS-PhMI = 84/88%). The solution was added dropwise to 300 mL of methanol with stirring. The precipitate was filtered and washed by methanol. The solid was dried in vacuo overnight to yield the alternating copolymer as white solid (726 mg): $M_n =$

10,100, $M_w/M_n = 1.43$ (from SEC in DMF calibrated by PMMA), $M_n = 7300$, $M_w/M_n = 1.20$ (from SEC in THF calibrated by PSt), composition ratio tBOS/NHS-PhMI = 50/50 (from ^1H NMR).

Aminolysis of NHS-Activated Esther in NHS-PhMI Units. The representative procedure of aminolysis for tBOS/NHS-PhMI alternating copolymer is as follows (with *i*-propylamine): In a flask, the tBOS/NHS-PhMI alternating copolymer (100 mg, 0.2 mmol of NHS ester units) was dissolved in 2 mL of super dehydrated DMF. To the solution, *i*-propylamine (0.17 mL, 2.0 mmol) was added dropwise at room temperature with stirring. After the addition, the flask was put in an oil bath at 50 °C for 16 h. After cooling to room temperature, the solution was dialyzed in methanol for 1 day and evaporated in vacuo to yield an alternating copolymer of tBOS and *i*-propylamide-PhMI (78 mg). The same procedure was performed in the case with other amines (*N,N*-dimethyl-1,3-propanediamine, 4-picolylamine, and *n*-dodecylamine) except the solvent for dialysis. Solvent of dialysis was changed according to the solubility of the resultant copolymer [methanol, methanol/THF (2/8, v/v), or THF].

Deprotection of *tert*-Butoxy Group in tBOS Units. The representative procedure of *tert*-butoxy group deprotection in the alternating copolymer after the aminolysis is as follows (after aminolysis with *i*-propylamine): In a flask, the alternating copolymer with tBOS and *i*-propylamide-PhMI (40 mg) was dissolved in 1.5 mL of THF. To the solution, 0.3 mL of 37% HCl aq. was added in dropwise at room temperature with stirring. After mixing, the solution was refluxed for 20 h. The solution was dialyzed in THF for 1 day and freeze dried to obtain an alternating copolymer with 4-vinylphenol and *i*-propylamide-PhMI (25 mg). The same procedure was performed except for the solvent for dialysis. Solvent of dialysis was changed according to the solubility of the resultant copolymer [water, methanol/water (8/2, v/v), and THF].

Synthesis of poly(*N,N*-dimethyl-propylamide-PhMI)

Polymerization was carried out by the syringe technique under dry argon in glass tubes equipped with three-way stopcocks. To a Schlenk tube, NHS-PhMI (472 mg, 1.50 mmol) and AIBN (4.9 mg, 0.03 mmol) were added and the tube was degassed using a vacuum pump at –78 °C. Then, 0.06 mL of tetralin and 2.94 mL of CH₃CN were added (NHS-PhMI/AIBN = 500/10 mM) under a dry argon flow. Immediately after mixing, the tube was placed in an oil bath at 60 °C. The polymerization was terminated by bubbling with air. Monomer

conversions were 86% in 96 h. The polymer solution was diluted with DMF and added dropwise into 300 mL of methanol to precipitate the polymer. The precipitate was filtered and washed by THF to obtain poly(NHS-PhMI) as pink solid (247 mg): $M_n = 7600$, $M_w/M_n = 1.56$ (from SEC in DMF calibrated by PMMA). In a flask, 247 mg of poly (NHS-PhMI) (0.78 mmol of NHS ester units) was dissolved in 5 mL of DMF. *N,N*-dimethyl-propyldiamine (0.98 mL, 7.8 mmol) was added dropwise to the solution at r.t. During the addition, the color of the polymer solution was changed to dark red from orange-pink. After the addition, the flask was heated at 50 °C for 22 h. After cooling, the solution was dialyzed in methanol for 1 day. After dialysis, the solution was evaporated in vacuo and dissolved in 1,4-dioxane. The solution was freeze dried to obtain poly(*N,N*-dimethyl-propylamide-PhMI) as dark red solid (126 mg): $M_n = 4800$, $M_w/M_n = 1.60$ (from SEC in DMF calibrated by PMMA) (Figure 3a).

Synthesis of poly(4-vinylphenol)

Polymerization was carried out by the syringe technique under dry argon in glass tubes equipped with three-way stopcocks. In a Schlenk tube, AIBN (26.3 mg, 0.16 mmol) was added and the tube was degassed using a vacuum pump at -78 °C. Then, tBOS (3.01 mL, 16.0 mmol), 0.1 mL of tetralin and 4.9 mL of toluene were added (tBOS/AIBN = 2000/20 mM) under a dry argon flow. Immediately after mixing, the tube was placed in an oil bath at 60 °C. The polymerization was terminated after 24 h by bubbling with air. Monomer conversion was 80% in 24 h. The polymer solution was diluted with THF and added dropwise in 800 mL of methanol to precipitate the polymer. The precipitate was filtered and washed by methanol to yield poly(tBOS) as white solid (2.22 g): $M_n = 20700$, $M_w/M_n = 1.59$ (from SEC in THF calibrated by PSt). In a flask, 2.22 g of poly(tBOS) was dissolved in 80 mL of THF. The solution was degassed by bubbling with dry nitrogen. After bubbling, 16.2 mL of 37% HCl aq. was added to the solution. Then, the solution was refluxed for 21 h. After cooling, the

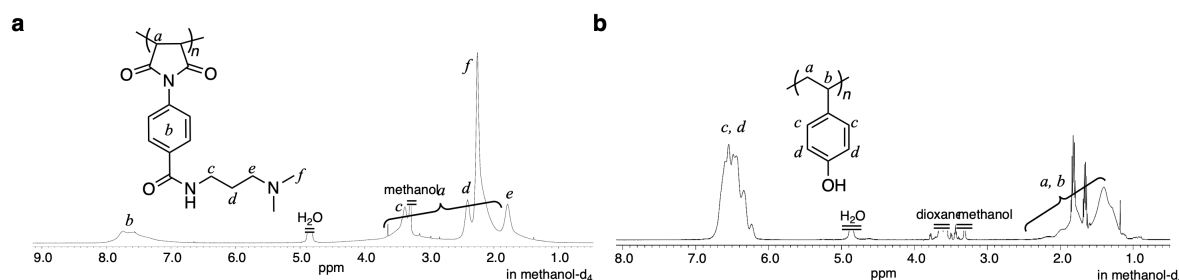


Figure 3. ^1H NMR spectra of (a) poly(*N,N*-dimethylpropylamide-PhMI), and (b) poly(4-vinylphenol) in methanol- d_4 .

solution was added dropwise in 900 mL of water at 0 °C. The precipitate was filtered and washed by water. The solid was dissolved in 1,4-dioxane and freeze dried to obtain poly(4-vinylphenol) as white solid (1.25 g): $M_n = 28100$, $M_w/M_n = 2.27$ (from SEC in DMF calibrated by PMMA) (Figure 3b).

Result and Discussion

Design of *N*-Phenyl Maleimide Derivative for Precise Alternating Sequence Control and Functionalization

As described above, an introduction of electron-withdrawing group on a side chain of maleimide monomer is known effective for precision alternating sequence control through the copolymerization with St.²¹ With the aim of compatibility with functionalization, *N*-hydroxysuccinimide (NHS) ester available as an activated ester was installed at the *para*-position of a phenyl ring in PhMI (NHS-PhMI, Figure 4).^{22,23} The design is rational, since Matsuomoto et al., has recently reported the substituent at the *para*-position of PhMI has an impact on sequence resultant copolymers.⁸ The compound has been used as a linker to connect phospholipid and peptide for synthesis of phospholipopeptides via thiol-ene reaction and aminolysis²³ but there has been no report on the utility as a monomer for polymerization. The NHS activated ester is easily converted into an amide group by just mixing with an amine compound under mild condition owing to the highly electron-withdrawing feature and has been

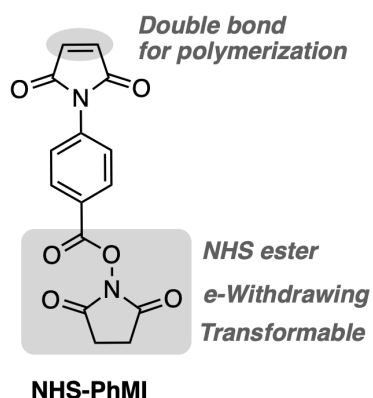


Figure 4. Design of NHS-PhMI as a comonomer of radical copolymerization with tBOS for precise alternating sequence control and dual functionalization of the resultant alternating copolymer.

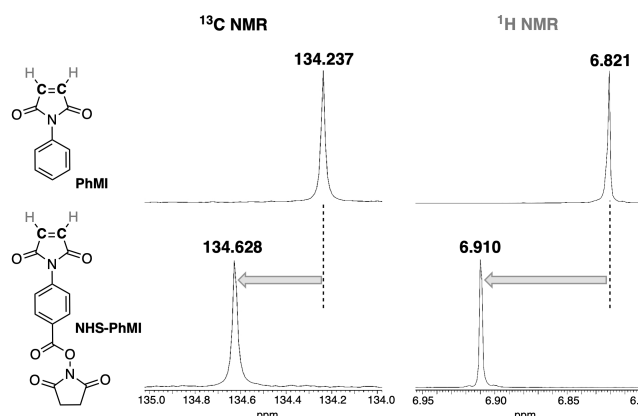


Figure 5. ¹³C and ¹H NMR (CDCl₃) peaks derived from olefinic carbon and proton of NHS-PhMI in comparison with those of PhMI.

used for functionalization of copolymers through the post reaction.²⁴ A peak of the olefinic carbons in NHS-PhMI was observed at lower magnetic field than that of PhMI by ^{13}C NMR in CDCl_3 (Figure 5), indicating the electron density was poorer than that of PhMI. DFT calculations of SOMO energies of the maleimide radicals at the UB3LYP/6-31G level of theory also supported the trend [SOMO energy (eV): -0.23331 for PhMI, and -0.24762 for NHS-PhMI].

Alternating Copolymerization of NHS-PhMI with tBOS via RAFT Polymerization

The copolymerization of NHS-PhMI with tBOS was conducted via RAFT polymerization with a chain transfer agent (CTA): $[\text{tBOS}]_0 = [\text{NHS-PhMI}]_0 = 200 \text{ mM}$, $[\text{CTA}]_0 = 10 \text{ mM}$ and $[\text{AIBN}]_0 = 1 \text{ mM}$ in acetonitrile at 60°C (Figure 6a). Herein, copolymerizations of St/PhMI and St/NHS-PhMI were also performed under the same condition for comparison. The conversions of the monomers were almost the same as each other during the copolymerization (Figure 6b). The copolymerization was faster (conversions: >90% in 4 h) than those of St/PhMI and St/NHS-PhMI due to the bigger difference in electron densities of two comonomers thus facilitating the cross-over propagation. The resultant copolymer showed unimodal SEC curve but the molecular distribution was broad ($M_w/M_n = 1.45$, Figure 6c) compared with the copolymers of St/PhMI and St/NHS-PhMI ($M_w/M_n = 1.21$, and 1.16, respectively, Figure 7). Judging from the faster polymerization, tuning the polymerization condition would be required to realize more precise control narrower molecular weight distribution.

The obtained copolymers [Conv.(tBOS) = 88%, Conv.(NHS-PhMI) = 90%] were purified by reprecipitation in methanol to remove low molecular weight compounds (e.g., unreacted monomer) and the structure of the purified copolymer was analyzed by ^1H NMR (Figure 6d). In the ^1H NMR spectrum, the peculiar peak to the NHS activated ester unit (*i*) was observed at around 2.8 ppm. The number-averaged polymerization degrees of tBOS and NHS-PhMI units [n and m , respectively] were calculated by integration ratio of the observed peaks derived from aromatic protons of tBOS (*c*) and NHS-PhMI (*g* and *h*): $n = 17$; $m = 17$. The values well agreed with the theoretical values calculated from the conversions and the 1:1 ratio satisfied the minimum requirement of the alternating sequence.

Sequence Analysis of tBOS/NHS-PhMI Alternating Copolymer by MALDI-TOF-MS

Figure 8 shows the MALDI-TOF-MS spectra of the copolymer of tBOS/NHS-PhMI in comparison with those of St/PhMI, and St/NHS-PhMI synthesized under the same conditions.

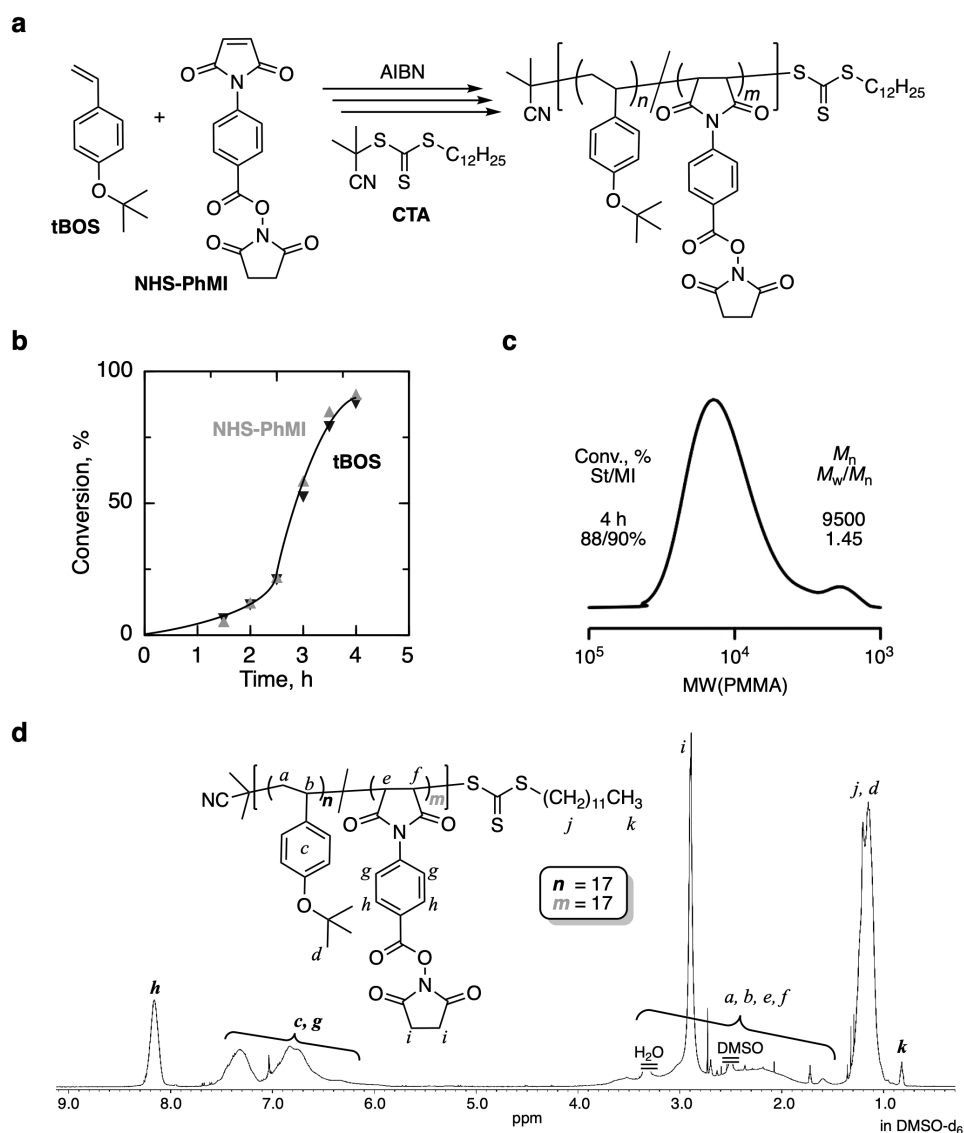


Figure 6. (a) Synthetic scheme of alternating copolymerization of NHS-PhMI with tBOS via RAFT copolymerization, (b) time-conversion plot, (c) a SEC curve of the resultant copolymer eluted in DMF with 10 mM LiBr, and (d) ¹H NMR spectrum of the resultant copolymer after purification in DMSO-d₆.

Here, because the initiations in the copolymerizations were less selective, the peak series possible to indicate alternating sequence are derived from the copolymers where the difference in N_{St} and N_{MI} ($|N_{St} - N_{MI}|$) was no more than one (square).²⁵⁻²⁷ The MALDI-TOF-MS spectrum of the copolymer of tBOS/NHS-PhMI mainly gave the “ideal” peak series. On the other hand, in the spectra of the copolymer of St/PhMI or St/NHS-PhMI, peaks whose $|N_{St} - N_{MI}|$ was more than one, were also observed (star). The relatively high degree of

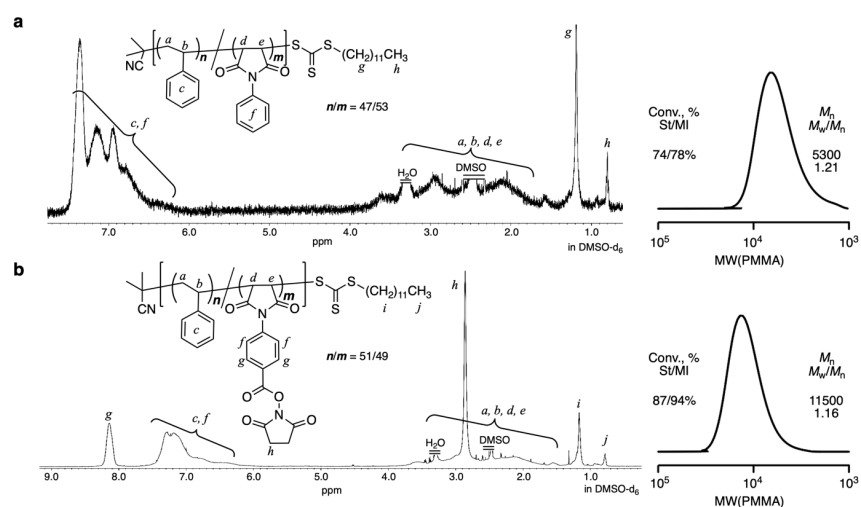


Figure 7. ^1H NMR spectra and DMF SEC curves of alternating copolymers of (a) St/PhMI, and (b) St/NHS-PhMI synthesized via RAFT polymerization after purification: $[\text{styrene}]_0/[\text{PhMI derivative}]_0/[\text{CTA}]_0/[\text{AIBN}]_0 = 200/200/10/1$ mM in acetonitrile at 60°C .

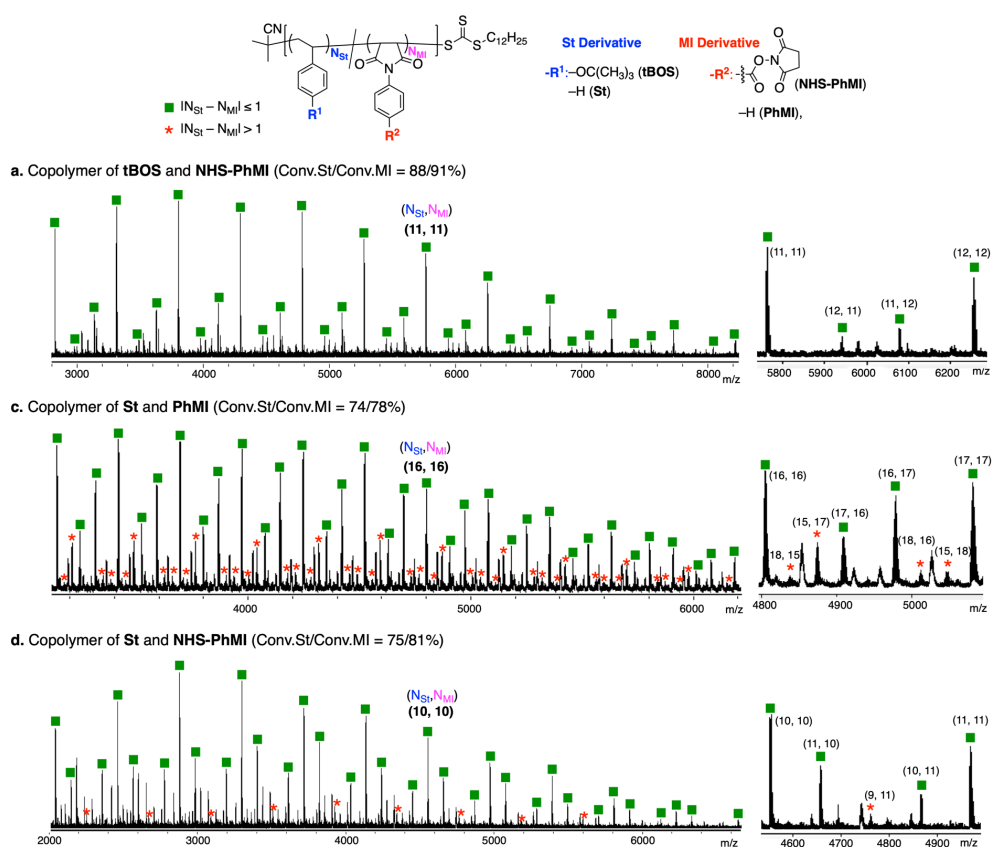


Figure 8. The MALDI-TOF-MS spectra of the copolymers synthesized via RAFT polymerization with a pair of (a) tBOS/NHS-PhMI, (b) St/PhMI, and (c) St/NHS-PhMI.

alternating sequence of the tBOS/NHS-PhMI copolymer is likely attributed to both the electron-withdrawing NHS ester group of NHS-PhMI and the electron-donating *tert*-butoxy group of tBOS.²⁸

Dual Functionalization of the tBOS/NHS-PhMI Alternating Copolymer

Dual functionalization for the two units in alternating copolymer has attracted attentions for development of functional materials. For instance, Weck et al., has demonstrated orthogonal functionalization for an alternating copolymer of 2,3,4,5,6-pentafluorostyrene with propargyl-containing styrene derivative through the copper-catalyzed 3 + 2 cycloaddition between azide and the alkyne of the propargyl groups.²⁹ The catalytic reaction using a dual functionalized alternating copolymer as a catalyst has been also reported by Rodionov et al.¹³ Regarding the copolymer in this work, one unit is fixed to one functional group, i.e., phenol, but the dual functionalization in conjunction with another unit (i.e., maleimide) via post-functionalization could lead to syntheses of alternating copolymers exhibiting interesting

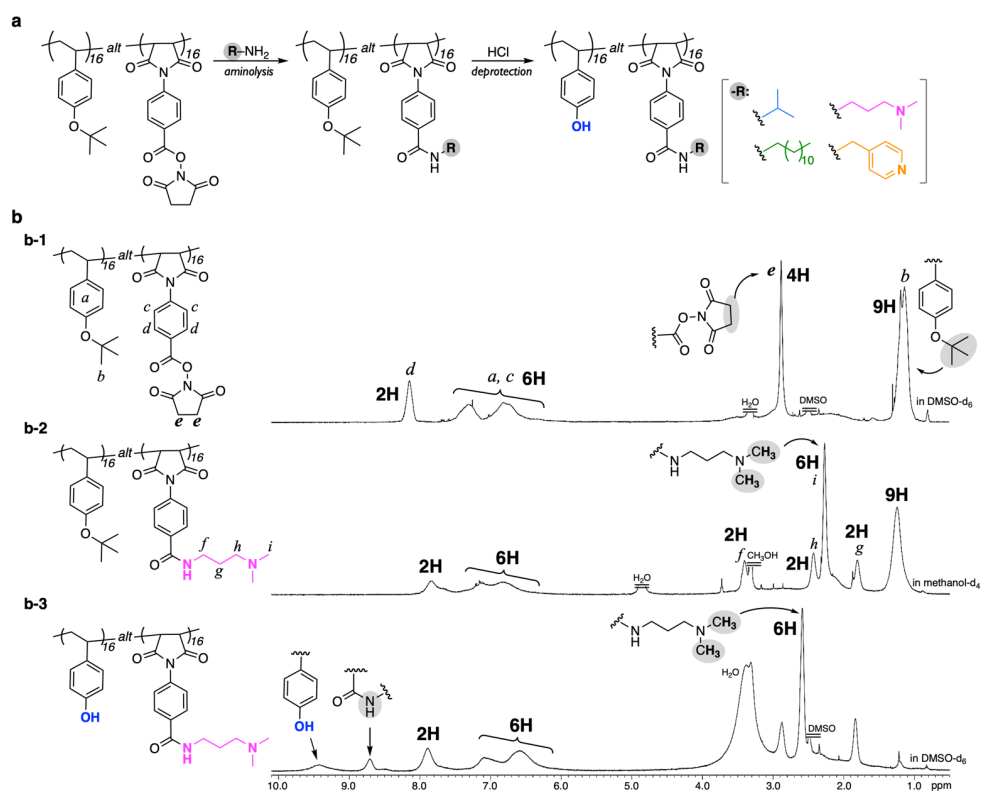


Figure 9. (a) Synthetic scheme for synthesis of vinylphenol-based alternating copolymer via aminolysis and deprotection reactions of tBOS–NHS-PhMI alternating copolymer. (b) ¹H NMR spectra of the copolymers before aminolysis (b-1), after aminolysis with *N,N*-dimethyl-propyldiamine (b-2), and after deprotection reaction (b-3).

physical properties.

For the tBOS/NHS-PhMI alternating copolymer [after purification, $M_n = 10,100$, $M_w/M_n = 1.43$ (calibrated by PMMA), $DP_n(\text{tBOS, NMR}) = 16$, $DP_n(\text{NHS-PhMI, NMR}) = 16$], aminolysis were conducted with various primary amines (*i*-propylamine, *n*-dodecylamine, 4-picolylamine, and *N,N*-dimethyl-propyldiamine) (Figure 9a). 10 eq. of amine was treated in DMF and the reaction was performed at 50 °C for overnight, followed by dialysis to remove low molecular weight compounds such as unreacted amine and NHS. Figure 9b shows the ^1H NMR spectra of the resultant copolymer carrying *N,N*-dimethyl-propyldiamine as the representative example (b-2) along with the starting alternating copolymer (b-1). After aminolysis, the characteristic peak (*e*) derived from NHS activated ester around 2.8-2.9 ppm completely disappeared, and instead new peaks derived from the amine (*f*, *g*, *h*, and *i*) was observed giving the integration ratio reasonable for the quantitative transformations. The transformation was also confirmed by FT-IR (Figure 10). In addition, the SEC curve shape of the obtained copolymer was almost the same as before the aminolysis reaction (Figure 11), which indicated no extra reactions such as cross-linking took place. The quantitative aminolysis reactions with other amines were also confirmed by ^1H NMR and FT-IR (Figure 12 and Figure 10).

Subsequently, the *tert*-butoxy groups in the series of copolymers after the aminolysis were deprotected under acidic condition. Figure 9b-3 shows the ^1H NMR spectrum of the copolymer carrying *N,N*-dimethyl-propyldiamine after deprotection reaction. The peak from

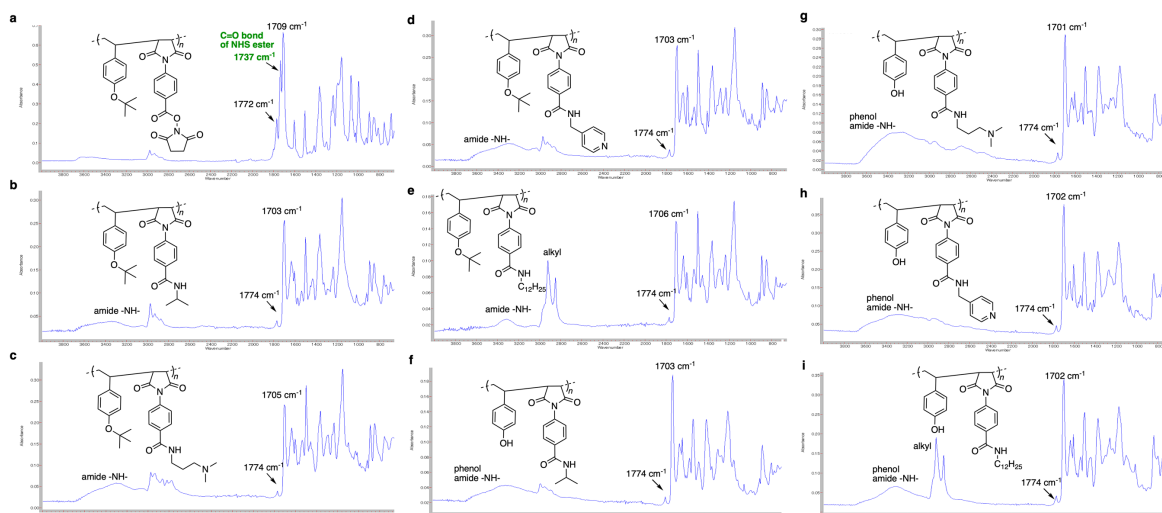


Figure 10. FT-IR spectra of the alternating copolymers of tBOS and NHS-PhMI (a), after aminolysis reaction (b-e), and after deprotection reaction (f-i).

tert-butoxy protons (*b*) at 1.2 ppm vanished and instead the peak from phenol proton appeared at around 9.5 ppm. Similar peak transitions in ^1H NMR were observed in the other copolymers (Figure 13), and the deprotection was also confirmed by FT-IR (Figure 10). The shapes of SEC curves of the copolymers were not changed much through the deprotection reactions (Figure 11).

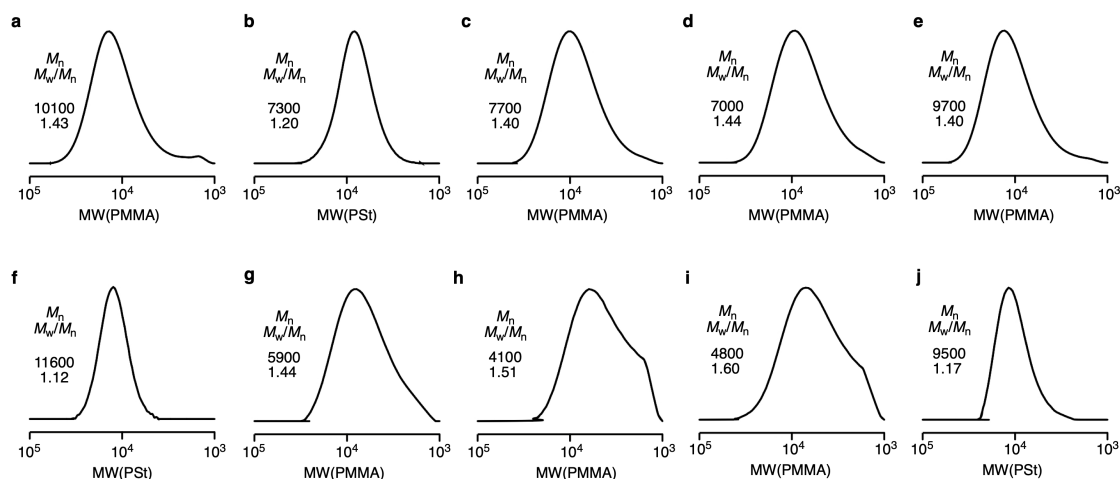


Figure 11. SEC curves of purified alternating copolymers of tBOS and NHS-PhMI synthesized via RAFT before aminolysis (a, b), after aminolysis with (c) *i*-propylamine, (d) *N,N*-dimethyl-1,3-propanediamine, (e) 4-picolylamine, and (f) *n*-dodecylamine, and after deprotection reaction of *tert*-butoxy groups in the copolymers treated with (g) *i*-propylamine, (h) *N,N*-dimethyl-propyldiamine, (i) 4-picolylamine, and (j) *n*-dodecylamine : (a,c,d,e,g,h,i) and (b, f, j) were eluted in DMF and THF, respectively.

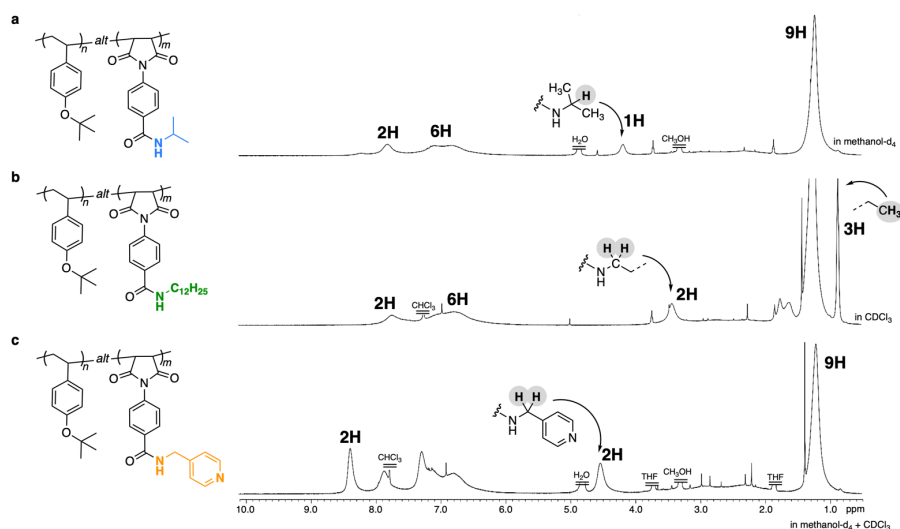


Figure 12. ^1H NMR spectra of alternating copolymers after aminolysis by (a) *i*-propylamine, (b) *n*-dodecylamine, and (c) 4-picolylamine.

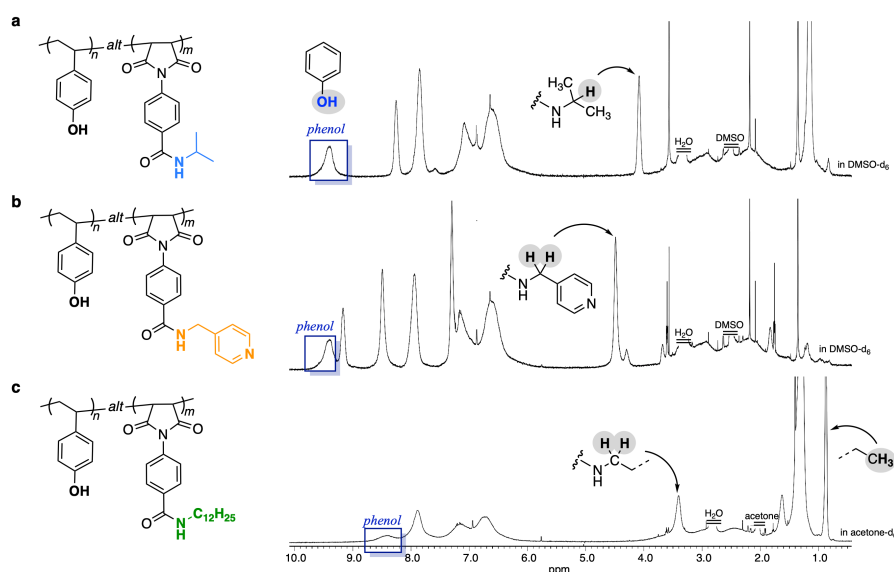


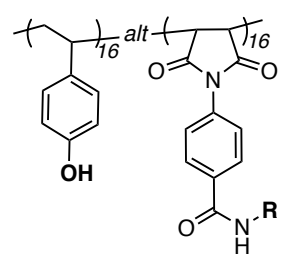
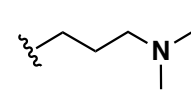
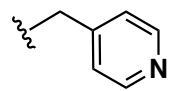
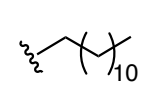
Figure 13. ^1H NMR spectra of the copolymers after deprotection reaction of *tert*-butoxy groups in the copolymers treated with (a) *i*-propylamine, (b) 4-picolylamine, and (c) *n*-dodecylamine.

Solubilities and Thermal Properties of the Dual Functionalized Alternating Copolymers

The four series of alternating copolymers before and after deprotection showed different solubilities and thermal properties depending on the substituent of the PhMI (Table 1, Figure 14 and 15). The dodecyl pendant copolymer was expected to show UCST-type thermal response in aromatic solvents because of its similarity to the alternating copolymer of 4-vinylphenol and *N*-long alkyl maleimide in our previous report. However, the copolymer was insoluble in toluene at r.t. and even after heating. The copolymer was soluble in THF but insoluble even in 1.0 M NaOH solution, and the trend was totally different from the other three copolymers. The *i*-propyl derivative was specifically soluble in methanol. Thus, the installed substituent greatly affected the solubility of the alternating copolymer.

Especially, the solubility of the *N,N*-dimethylamine derivative was very unique: it was insoluble in methanol but soluble in neutral water. The trend was in sharp contrast to the three other copolymers. The insolubility in methanol is due to the interaction between phenol and *N,N*-dimethylamine which was supported by the fact that each of the homopolymers (Figure 3) were soluble but the mixture were insoluble and precipitated in methanol. In water, the acidic phenol and the basic *N,N*-dimethylamine were supposed to form the ionic pair, which likely facilitated to be soluble in the aqueous media. Further, each of the homopolymers and the

Table 1. Solubility of Copolymers After Deprotection^a

Solvent				
	-R:	-R:	-R:	-R:
				
water	<i>Insol</i>	<i>Sol</i>	<i>Insol</i>	<i>Insol</i>
NaOH aq.	<i>Sol</i>	<i>Sol</i>	<i>Sol</i>	<i>Insol</i>
MeOH	<i>Sol</i>	<i>Insol</i>	<i>Insol</i>	<i>Insol</i>
DMF	<i>Sol</i>	<i>Sol</i>	<i>Sol</i>	<i>Sol</i>
CHCl ₃	<i>Insol</i>	<i>Insol</i>	<i>Insol</i>	<i>Insol</i>
THF	<i>Insol</i>	<i>Insol</i>	<i>Insol</i>	<i>Sol</i>
toluene	<i>Insol</i>	<i>Insol</i>	<i>Insol</i>	<i>Insol</i>

Sol: Soluble, *Insol*: Insoluble, ^a Polymer concentration: 1wt%.

mixture were not soluble completely in neutral water (Figure 14). Alternating sequence of phenol and *N,N*-dimethyl-propylamine units presumably enhanced the chance of interaction between the two units, leading to solubilizing the copolymer in neutral water. The pyridine derivative was also expected the similar interaction with the phenol unit in water, but the copolymer was not soluble in water. This is likely attributed to higher hydrophobicity of the side chain spacer.

Finally, thermal properties of the copolymers were investigated by DSC. The copolymers bearing dodecyl or *N,N*-dimethylamine units gave no peaks derived from the glass transition before and after deprotection, but those with *i*-propyl or pyridine clearly showed the transition (Figure 15). After conversion of *tert*-butoxy groups into hydroxyl group by deprotection, *T_g*s of the two copolymers obviously increased. As poly(4-vinylphenol) is known to show higher *T_g* than polystyrene due to the hydrogen bonding between phenol units,²⁵ the increase is presumably due to the hydrogen bonding. Interestingly, the pyridine copolymer gave an increase by about 80 °C via the deprotection and the rising value was much higher than the *i*-propyl counterpart (~40 °C). The remarkable increase is likely ascribed to the interaction between phenol and pyridine in addition to the that between phenols. In the related paper, it

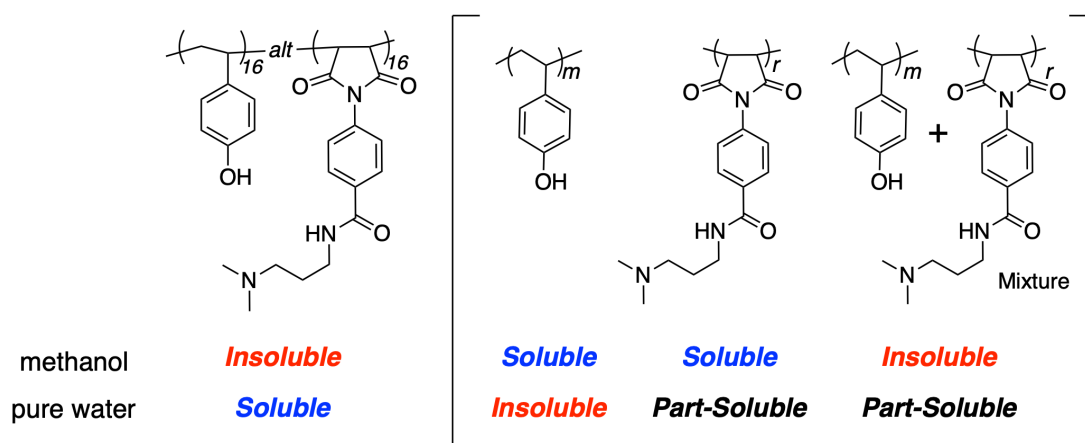


Figure 14. Solubilities of the alternating copolymer consisting of vinyl phenol and *N,N*-dimethyl-propylamine-pendant phenyl maleimide for methanol and water (total concentration 1wt%) in comparison with the homopolymers and the blend.

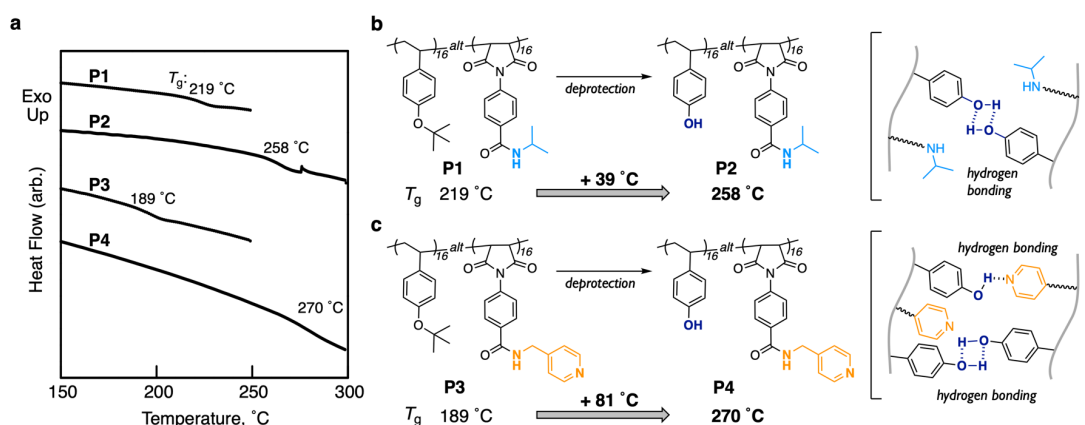


Figure 15. DSC data (a) and remarkable increases in T_g of the alternating copolymers via deprotection of *tert*-butoxy groups into hydroxyl group: (b) *i*-propyl- and (cb) pyridine-based alternating copolymer.

is reported that the blend of poly(4-vinylphenol) and poly(4-vinylpyridine) shows higher T_g than their respective homopolymers and the increase in T_g is maximum when the composition ratio is 1:1.²⁶ In addition, the block copolymers consisting of the two homopolymer had higher T_g than the blends of the homopolymers. Thus, the alternating sequence of phenol and pyridine might enhance the intra- or inter-molecular interaction between the chains to lead to the increased in T_g . Unfortunately, it is difficult to discuss effects of the alternating sequence on the thermal behavior, since the corresponding block or random copolymers cannot be synthesized.

Conclusion

In this work, an *N*-phenyl maleimide derivative bearing activated NHS ester (NHS-PhMI) was synthesized as the comonomer in radical copolymerization with tBOS for the precise alternating sequence control as well as functionalization of the resultant phenol-based alternating copolymer. The combination of NHS-PhMI with tBOS yielded the copolymer with relatively high degree of alternating sequence, which was characterized by MALDI-TOF-MS in comparison with other comonomer combinations. The quantitative and dual functionalization for the two units in alternating sequence was achieved via aminolysis for the activated ester, followed by deprotection of the *tert*-butoxy group under acidic condition. Thus, syntheses of four series of vinyl phenol-based alternating copolymers were demonstrated, and they showed different solubilities and thermal properties according to the amide substituents.

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PART II

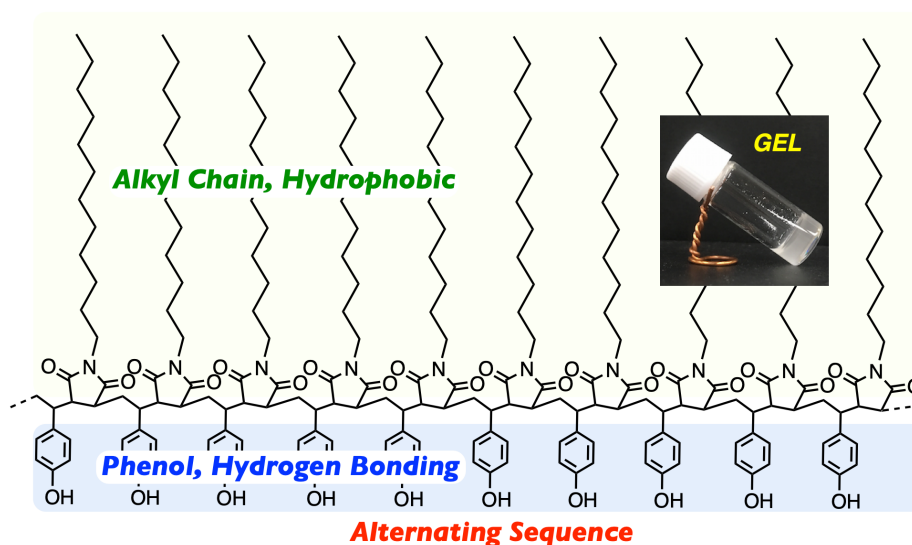
Self-Assembly and Functions of Alternating Copolymers

Chapter 4

Physical Gelation of AB-Alternating Copolymers Made of Vinyl Phenol and Maleimide Units: Cooperation Between Precisely Incorporated Phenol and Long Alkyl Pendant Groups

Abstract

The alternating copolymers consisting vinyl phenol and *n*-long alkyl ($C_{12}H_{25}$ - or $C_{18}H_{37}$ -) maleimide formed UCST-type thermo-responsive organogels in aromatic solvents. The copolymers were synthesized via radical copolymerization of a protected styrene derivative with a functional maleimide monomer followed by the deprotection. Hydrogen bonding of the phenol units is crucial for the gelation and the gelation temperature and stiffness were tuneable by varying concentration, solvent and polymerization degree. Analyses by 1H -NMR, linear rheology, WAXD, SANS and cryo-TEM gave the picture of vermicular self-assembled nano-objects formed through segregated and hydrogen-bonded packing by the precisely incorporated two units in alternating sequence.



Introduction

Radical copolymerization¹ is useful for the tuning properties of polymers, and indeed copolymers have been widely used as polymer materials. The arrangement of comonomer units in each chain and the composition distribution among chains are significant for understanding the aggregation behaviors and properties, and the occurrence of particular sequences in copolymer chains can be predicted to a certain degree from the reactivity ratios and the polymerization methodology (non-living vs. living). For example, when two comonomers of reactivity ratios $r_1 = r_2 = 1$ are employed in copolymerization, the product is an “ideal random copolymer” (Figure 1A). In this case, the two units (A and B) are randomly distributed in chains and composition distribution among chains exists although not so broad, regardless of polymerization methodology. On the other hand, when the reactivity ratios are deviated from 1, the resultant sequence depends on polymerization methodology: copolymerization with a living polymerization system could give a “gradient copolymer” (B), in contrast to a non-living system resulting in broad composition distribution, i.e., mixed copolymer chains of various composition ratios (C). Combinations with reactivity ratios of extremely different values (e.g., styrene and vinyl acetate) would hardly give copolymers. An exceptional but interesting combination is the comonomer combination of zero reactivity ratios

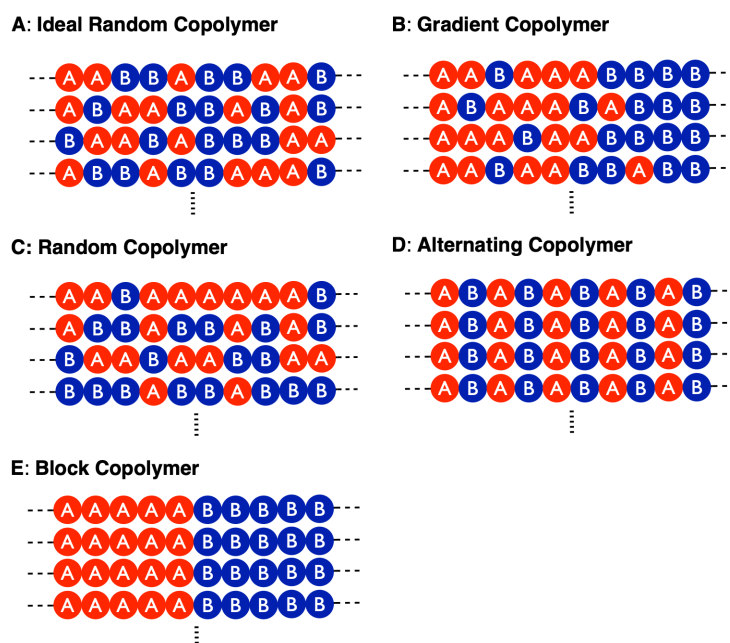


Figure 1. Various copolymers: (A) ideal random copolymer; (B) gradient copolymer; (C) random copolymer of broad composition distribution; (D) alternating copolymer; (E) block copolymer.

($r_1 = r_2 = 0$). Such comonomers give an “alternating copolymer” (D), and most importantly, the resultant chains are uniform in terms of sequence (i.e., no distribution of composition ratio).^{2,3}

On the other hand, block copolymers form another category of copolymers (E), and they have been used for material applications, such as elastomers, in drug delivery, etc. Self-assembly is induced under appropriate conditions giving periodic structures in bulk (e.g., lamella, cylinder, and sphere) or solution (e.g., micelle, liposome, and bilayer sheet) so as to decrease the surface energy between different segments.⁴ Crucial to such a self-assembly is compatibility or interaction between the same chain segments (in other words, incompatibility between different segments). In general, such a self-assembly is hardly expected using random copolymers, because cohesion by randomly distributed comonomer units is much lower and weaker than with block segments. An exceptional example has been reported on the self-assembly with a random copolymer: methacrylate-based amphiphilic random copolymers carrying hydrophilic oligo(ethylene glycol) and hydrophobic crystalline alkyl pendants can undergo phase separation in the bulk state due to the extremely different characters of the two units.⁵

In this work, we studied the self-assembly of an alternating copolymer on the basis of the hypothesis that alternating copolymer chains of totally different properties (affinity, polarity, interaction, etc.) can effectively undergo cohesion or self-assembly. We thus selected a combination of *tert*-butoxy styrene (tBOS) with alkyl-pendant maleimide as the comonomers for radical copolymerization (Figure 2). Styrene (Sty) and its derivatives are known to induce alternating copolymerization with highly electron poor monomers such as maleic anhydride⁶ (MANh) or maleimide derivatives.⁷ This combination was used recently to synthesize well

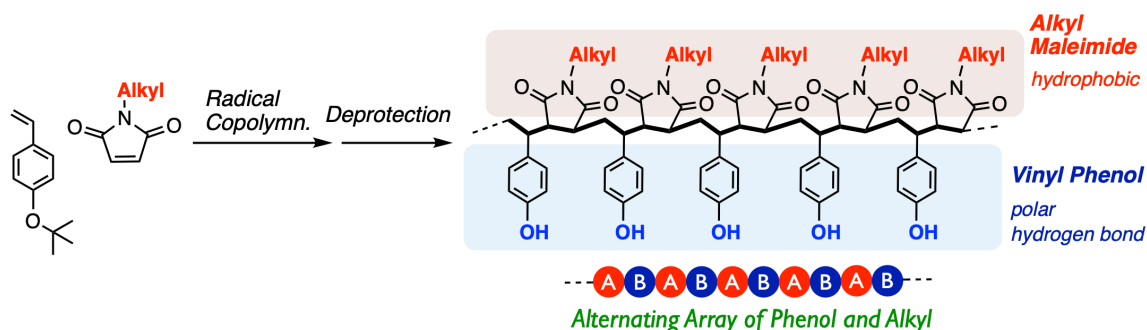


Figure 2. Synthesis of the alternating copolymer consisting of vinyl phenol and alkyl chain-substituted maleimide via radical copolymerization and deprotection for the styrene substituent.

defined graft copolymers by post-functionalization of poly(Sty-*alt*-MAnh).⁸ The alkoxy substituent on styrene is an electron-donating group, which is preferable for crossover propagation.⁹ The *tert*-butoxy group can be deprotected under acidic conditions, giving rise to a phenol (i.e., vinyl phenol) that is more polar and capable of hydrogen-bonding interaction, in contrast to the hydrophobic and non-polar alkyl pendant on the maleimide units. In addition, the rigid maleimide unit may be useful for segregation of the two different pendant groups. Thus, in this work, a series of alkyl maleimide-tBOS copolymers was synthesized in expectation of a unique self-assembly.

Consequently, alternating copolymers consisting of vinyl phenol and alkyl chain (C₁₂H₂₅ or C₁₈H₃₇)-substituted maleimide were found to give a physical gel in aromatic solvents. The fundamental feature for gelation is hydrogen bonding between regularly aligned phenol pendant groups, which was clarified by ¹H NMR and stoichiometric addition of pyridine. Effects of molecular weight and viscoelastic behaviors were also studied.

Experimental

Materials

Maleic anhydride (Wako; purity 99%), *N*-octylamine (TCI; purity >98%), *N*-dodecyl amine (TCI; purity 97%), *N*-octadecyl amine (Aldrich; purity 97%), acetic anhydride (Wako; purity >97%), sodium acetate (Wako; purity >98.5%), 2,2'-azobisisobutyronitrile (AIBN, Wako; purity 98%), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanol (CTA, Aldrich; purity >96.5%), 1,4-dioxane (Wako, purity >99.5%), *N*-ethylmaleimide (EMI, TCI; purity >98%), cumene (abcr GmbH; purity 99%), *p*-cymene (abcr GmbH; purity 96%), anisole (Aldrich; purity 99%), xylene (Alfa Aesar, mixture of isomers; purity 98.5%), 1,2,4-trichlorobenzene (Carlo Erba; purity >98.5%), nitrobenzene (Rectapur; purity 99.5%), pyridine (Wako; purity >99.5%) and hydrochloric acid (Wako; 35–37%) were used without purification. *p*-*tert*-Butoxystyrene (tBOS, Wako; purity >98%) was distilled over calcium hydride before use. 1,2,3,4-Tetrahydronaphthalene (tetralin, Kishida Chemical; purity >98%), an internal standard in ¹H NMR, was dried over calcium chloride overnight and distilled twice over calcium hydride. Toluene (Kishida Kagaku, Osaka, Japan; purity 99.5%) was dried and purified by passing through purification columns (Solvent Dispensing System, SG Water USA, Nashua, NH; Glass Counter).

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

SEC (THF). MWD curves, M_n and M_w/M_n ratio of the polymers were measured by SEC in THF as an eluent at 40 °C on three polystyrene gel columns (Shodex LF-404; exclusion limit = 2×10^6 g/mol; particle size = 6 μ m; pore size, < 3000 Å; 0.46 cm i.d. 25 cm; flow rate 0.3 mL/min) connected to a DU-H2000 pump, a 74S-RI refractive index detector, and a 41-UV ultraviolet detector (all from Shodex; Shodex GPC-104). The columns were calibrated against 12 standard polystyrene samples (TOSOH, PS-Oligomer Kit, M_w = 500–1,110,000; M_w/M_n = 1.01–1.16).

FT-IR. FT-IR spectra of freeze-dried alternating copolymers were measured using an Agilent Cary 630 FTIR spectrometer (Agilent Technologies).

MALDI-TOF-MS. MALDI-TOF-MS analyses were performed on an ultraflex III (MALDI-TOF mass spectrometer, Bruker Daltonics) equipped with 337 nm nitrogen laser with dithranol or DCTB as matrix and sodium trifluoroacetate as the cationizing agent.

Turbidity measurement. Turbidity of the polymer solutions in toluene was measured on a UV-1800 (Shimadzu, optical path length = 10 mm, λ = 670 nm, heating/cooling rate: 1 °C min⁻¹, temperature range: 30–90 °C).

DSC. DSC measurements (ca. 2.0 mg weighed into an aluminum pan) were performed on a DSC Q200 calorimeter (TA instruments) equipped with a RCS 90 electric freezing machine under dry nitrogen flow at a heating or cooling rate of 10 °C/min.

Rheology. Samples of 10wt% **Alt-C12** or **Alt-C18** solutions in 1,2,4-trichlorobenzene were placed at 90 °C in the gap of an Anton Paar Physica MCR 501 rheometer equipped with a 50 mm diameter cone-plate geometry placed under a solvent trap and operated in the standard oscillatory mode. Samples were first annealed for 10 minutes at 90 °C (**Alt-C12**) or 50 °C (**Alt-C18**), then cooled down to the desired temperature and equilibrated for 10 minutes. First, 1 rad s⁻¹ oscillations were applied in the 0.01 to 10% strain range. Frequency sweeps were then recorded in the linear viscoelastic regime (typically 1% deformations) in the same isothermal conditions using ω = 100 to 0.01 rad s⁻¹ oscillations. Samples were annealed again at 90 °C (**Alt-C12**) or 50 °C (**Alt-C18**) and equilibrated as described above between measurements at two different temperatures. Additional viscosity measurements have been performed using an Anton Paar Lovis 2000 M microviscometer equipped with a 1.8 mm thick capillary and a steel ball. Temperature was controlled at 25 °C, and viscosity was measured

at 13 different angles covering the 80–270 s⁻¹ shear rate range.

Small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD). Simultaneous SAXS-WAXD measurements were conducted at the DND-CAT beamline at the Advanced Photon Source in Argonne National Laboratory (Argonne, IL) operated at 17 keV (wavelength: $\lambda = 0.7293 \text{ \AA}$; q -range: 0.00236–4.46 $\text{\AA}^{-1}$). Freeze-dried samples were investigated using custom-made aluminum washers (OD: 13 mm, ID: 4 mm, and thickness: 1 mm) covered with kapton windows on both sides. Solution for temperature-controlled experiments was investigated in 1 mm thick capillaries.

Small-angle neutron scattering (SANS). SANS experiments were performed on line D22 at the Institute Laue Langevin, Grenoble. Monochromatic ($\lambda = 6 \text{ \AA}$) radiation was used. Samples were prepared via dissolution in toluene-d₈ at 5wt% concentration. The solutions and pure solvent were held in amorphous silica (“quartz”) Hellma cells (optical path: 1 mm, cell volume = 300 μL). The temperature was imposed by a circulating fluid in the cells’ holder rack. Data collection at two sample-to-detector distances $D = 1.25 \text{ m}$ and $D = 5.60 \text{ m}$ allowed to cover the scattering wave vector range from $q = 0.01$ to $0.67 \text{ \AA}^{-1}$. Reduced SANS data were corrected from incoherent scattering of the solvent and solute by introducing an adjustable background parameter in the fitting procedure. Fitting was performed using the flexible_cylinder model function provided in the SasView 4.1.2 package.

Cryo-transmission electron microscopy (Cryo-TEM). The morphologies of organogels were examined using a LaB6 JEOL JEM 2100 transmission electron microscope operated at 200 kV with a low-dose system (minimum dose system, MDS). The solutions were maintained at 90 °C for 1 h. Then, they were spread on a Quantifoil holey-carbon-coated grid (Quantifoil Micro Tools) and quickly frozen by plunging the grid into liquid ethane. Images were recorded with an Ultrascan 2k $\times$ 2k CCD Gatan camera. Dimensions were measured on cryo-TEM images using the ImageJ software.

Synthesis of *N*-Alkyl Maleimide¹⁰

Synthesis of *N*-Octyl Maleimide (C8MI). The solution of *N*-octylamine (5.58 g, 43.2 mmol) in 80 mL of ethyl acetate was added dropwise to the solution of maleic anhydride (5.08 g, 51.8 mmol) in 50 mL of ethyl acetate at 0 °C. The mixture was stirred for 20 h at 25 °C. After stirring, the mixture was evaporated in vacuo to obtain *N*-octyl maleamic acid (9.57 g). In a flask, *N*-octyl maleamic acid (9.57 g, 42.1 mmol) and sodium acetate (3.54 g, 43.1 mmol) and 100 mL of acetic anhydride were added and the mixture was heated at 100 °C for 5 h.

After cooling, the mixture was poured into 1000 mL of water and stirred for 2 h at 0 °C. The solution was extracted by ethyl acetate and the organic layer was evaporated in vacuo to obtain brown liquid. The crude was purified by column chromatography using hexane/ethyl acetate (85/15, v/v) as the eluent. The product was purified again by recrystallization in hexane/ethyl acetate (85/15, v/v) to obtain white solid (3.33 g, yield 37%, $R_f = 0.33$). ^1H NMR (500 MHz, CDCl_3 , δ): 6.68 (s, 2H, CH=CH), 3.51 (t, 2H, -N-CH₂-), 1.57 (t, 2H, -CH₂-), 1.3-1.2 (m, 10H, -CH₂-), 0.87 (t, 3H, -CH₃) (Figure 3a).

Synthesis of *N*-Dodecyl Maleimide (C12MI). The solution of *N*-dodecylamine (16.2 g, 87.4 mmol) in 200 mL of ethyl acetate was added dropwise to the solution of maleic anhydride (10.33 g, 105 mmol) in 120 mL of ethyl acetate at 0 °C. The mixture was stirred for 20 h at 25 °C. After stirring, the mixture was evaporated in vacuo to obtain *N*-dodecyl maleamic acid (26.0 g, 91.7 mmol). In a flask, *N*-dodecyl maleamic acid (12.0 g, 42.3 mmol) and sodium acetate (5.36 g, 65.3 mmol) and 200 mL of acetic anhydride were added and the mixture was heated at 100 °C for 18 h. After cooling, the mixture was poured into 1500 mL of water and stirred for 1 h at 0 °C. The brown precipitate was collected by filtration under reduced

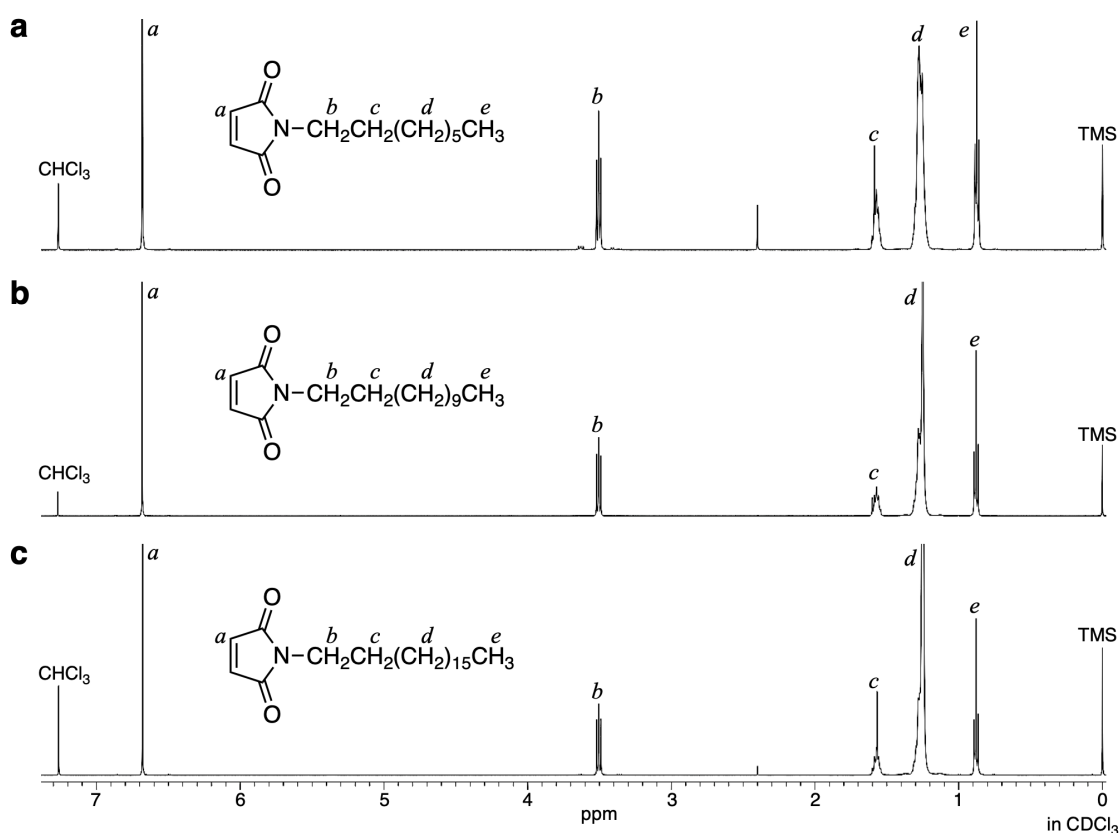


Figure 3. ^1H NMR spectra of (a) C8MI, (b) C12MI, and (c) C18MI in CDCl_3 .

pressure. The crude was purified by column chromatography using hexane/ethyl acetate (90/10, v/v) as the eluent to obtain white solid (5.3 g, yield 47%, $R_f = 0.26$). ^1H NMR (500 MHz, CDCl_3 , δ): 6.68 (s, 2H, $\text{CH}=\text{CH}$), 3.50 (t, 2H, $-\text{N}-\text{CH}_2-$), 1.57 (t, 2H, $-\text{CH}_2-$), 1.3-1.2 (m, 18H, $-\text{CH}_2-$), 0.88 (t, 3H, $-\text{CH}_3$) (Figure 3b).

Synthesis of *N*-Octadecyl Maleimide (C18MI). The solution of maleic anhydride (5.08 g, 51.8 mmol) in 50 mL of ethyl acetate was added dropwise to the solution of *N*-octadecylamine (11.65 g, 43.2 mmol) in 80 mL of ethyl acetate at 0 °C. The mixture was stirred for 20 h at 25 °C. After stirring, the mixture was evaporated in vacuo to obtain *N*-octadecyl maleamic acid (15.9 g, 43.3 mmol). In a flask, *N*-octadecyl maleamic acid (16.5 g, 44.8 mmol) and sodium acetate (5.82 g, 70.9 mmol) and 250 mL of acetic anhydride were added and the mixture was heated at 100 °C for 22 h. After cooling, the mixture was poured into 1500 mL of water and stirred for 1 h at 0 °C. The brown precipitate was collected by filtration under reduced pressure. The crude was purified by column chromatography using hexane/ethyl acetate (90/10, v/v) as the eluent to obtain white solid (5.7 g, yield 36%, $R_f = 0.28$). ^1H NMR (500 MHz, CDCl_3 , δ): 6.68 (s, 2H, $\text{CH}=\text{CH}$), 3.50 (t, 2H, $-\text{N}-\text{CH}_2-$), 1.57 (t, 2H, $-\text{CH}_2-$), 1.3-1.2 (m, 30H, $-\text{CH}_2-$), 0.88 (t, 3H, $-\text{CH}_3$) (Figure 3c).

Polymer Synthesis

Polymerization was carried out by the syringe technique under dry argon in baked glass tubes equipped with three-way stopped cock. Typical procedures for synthesis of the copolymer with tBOS and C12MI via free or living radical polymerization were as follows.

Synthesis of Alt-C12 via free radical polymerization. To a Schlenk tube, C12MI (2.654 g, 1.0×10^{-2} mol) and AIBN (32.84 mg, 2.0×10^{-4} mol) were added and degassed using a vacuum pump at -78 °C. Then, tBOS (1.88 mL, 1.0×10^{-2} mol), tetralin (0.2 mL) and toluene (7.92 mL) were added under a dry argon flow (concentration: tBOS/C12MI/AIBN = 1000/1000/20 mM). Immediately after mixing, the tube was placed in an oil bath at 60 °C for 1 h, and copolymerization was terminated by bubbling with air. Monomer conversion was determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomers with tetralin as an internal standard (conversion: tBOS/C12MI = 98/99%). The solution was diluted by THF (total volume of the diluted solution was about 60 mL), and the solution was added dropwise to 2 L of methanol with stirring. The precipitate was filtered and washed by methanol. The filtered solid was dissolved in 1,4-dioxane, and the solution was freeze-dried to yield the alternating copolymer [poly(tBOS-*alt*-C12MI)] as white solid (4.16 g): $M_n = 91900$,

$M_w/M_n = 6.45$ (from THF SEC, after purification). The composition ratio of the purified copolymer was determined by ^1H NMR from the integrated peak area of the aromatic protons of tBOS and ethylene protons neighboring the nitrogen atom of maleimide (tBOS/C12MI = 49/51). In a flask, 4.0 g of poly(tBOS-*alt*-C12MI) was dissolved in 100 mL of THF. Then, 13.3 mL of 37% HCl aq. was added slowly to the solution with stirring. The solution was heated at reflux with a condenser and stirring for 20 h. After cooling, the solution was added dropwise to 3 L of water with stirring. The precipitate was filtered and washed by pure water several times until the pH of the filtered water became 7. Then, the filtered solid was dissolved in 1,4-dioxane, and the solution was freeze-dried to yield poly(4-vinylphenol-*alt*-C12MI) (**Alt-C12**) as white solid (3.41 g): $M_n = 88000$, $M_w/M_n = 6.61$ (from THF SEC). The progress of deprotection was confirmed by ^1H NMR.

Synthesis of Alt-C12 via RAFT polymerization. To a Schlenk tube, C12MI (1.327 g, 5.0×10^{-3} mol) and AIBN (0.1 mL of 0.82wt% in toluene, 5.0×10^{-6} mol) were added and degassed using a vacuum pump at -78°C . Then, tBOS (0.941 mL, 5.0×10^{-3} mol), tetralin (0.1 mL), toluene (3.68 mL) and CTA (0.277 mL of 90.001 mM in toluene, 2.5×10^{-5} mol) were added (concentration: tBOS/C12MI/CTA/AIBN = 1000/1000/5/1 mM) under a dry argon flow. Immediately after mixing, the tube was placed in an oil bath at 60°C for 4 h, and copolymerization was terminated by bubbling with air. Monomer conversion was determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomers with tetralin as an internal standard (conversion: tBOS/C12MI = 95/97%). Then, the solution was diluted by THF and was added dropwise to 1 L of methanol with stirring. The precipitate was filtered and washed by methanol. The filtered solid was dissolved in 1,4-dioxane, and the solution was freeze-dried to yield the alternating copolymer [poly(tBOS-*alt*-C12MI) ($n = 190$)] as white solid (2.25 g): $M_n = 57100$, $M_w/M_n = 1.28$ (from THF SEC, after purification), composition ratio tBOS/C12MI = 49/51 (from ^1H NMR, after purification). In a flask, 1.1 g of poly(tBOS-*alt*-C12MI) was dissolved in 41 mL of THF. Then, 8 mL of 37% HCl aq. was added slowly to the solution with stirring. The solution was heated at reflux with a condenser and stirring for 20 h. After cooling, the solution was added dropwise to 1 L of water with stirring. The precipitate was filtered and washed by pure water several times until the pH of the filtered water became 7. Then, the filtered solid was dissolved by 1,4-dioxane, and the solution was freeze-dried to yield poly(4-vinylphenol-*alt*-C12MI) as white solid (730 mg): $M_n = 54600$, $M_w/M_n = 1.29$ (from THF SEC). The progress of deprotection was confirmed by ^1H NMR and FT-IR.

Preparation of Organogel

In a vial, the freeze-dried **Alt-C12** (100 mg) was dissolved in 2mL of an aromatic solvent at 80–90 °C using a heat gun (5wt% solution). Then, the solution was cooled to room temperature to form a turbid gel.

Result and Discussion

Syntheses and Characterizations of Alternating Copolymers

Free radical copolymerizations of tBOS with *N*-alkylmaleimide (alkyl: –C₂H₅, –C₈H₁₇, –C₁₂H₂₅, –C₁₈H₃₇) were conducted with 2,2'-azobisisobutyronitrile (AIBN) as the initiator. The resultant copolymers were treated with HCl for deprotection of the repeating *tert*-butoxy groups giving rise to alternating copolymers of vinyl phenol and *N*-alkylmaleimides (Figure 4a). Both of the comonomers were consumed at same rate and the conversions reached > 90% in several hours. Figure 4b summarizes data of M_n , M_w/M_n , and composition ratio (x/y) of the resultant copolymers after deprotection (**Alt-C2**, **-C8**, **-C12**, **-C18**). Molecular weights of all the copolymers are enough high and composition ratios were almost 1:1. Figure 4c shows ¹H NMR spectra before and after deprotection of the copolymer with *N*-dodecylmaleimide (C12MI). From the spectrum before deprotection, the composition ratio (x/y) was determined by using three peaks (*a*, *c* and *e*) to determine x/y = 49/51. The deprotection of *tert*-butoxy groups in the copolymer was done under reflux of the THF solution in the presence of HCl. A peak *b* from *tert*-butoxy groups clearly disappeared and instead a new peak derived from the hydroxy proton (*f*) appeared, indicating quantitative deprotection. The deprotection reaction was also confirmed by FT-IR (Figure 4d). The radical copolymerizations were performed with various injection ratios of the comonomer pair tBOS and C12MI to determine reactivity ratios by non-linear fitting method. The values were almost zero [$r_1 = 0.017$, $r_2 = 0.000$ ($M_1 = \text{tBOS}$, $M_2 = \text{C12MI}$); Figure 5a]. Thus selective cross propagation likely took place to give highly alternating copolymer under condition of 1:1 injection ratio. The alternating sequence was also supported by MALDI-TOF-MS for the copolymer sample synthesized by reversible addition fragmentation transfer (RAFT) polymerization¹¹ (Figure 5b).

Solubility of the Copolymers

Solubilities of the vinyl phenol-alkyl maleimide alternating copolymers (**Alt-C2**, **-C8**, **-C12**, **-C18**) in various solvents were investigated (1 wt% at 25 °C, Table 1). The copolymers showed almost same trend in solubility: soluble in THF, ethyl acetate, acetone and DMF,

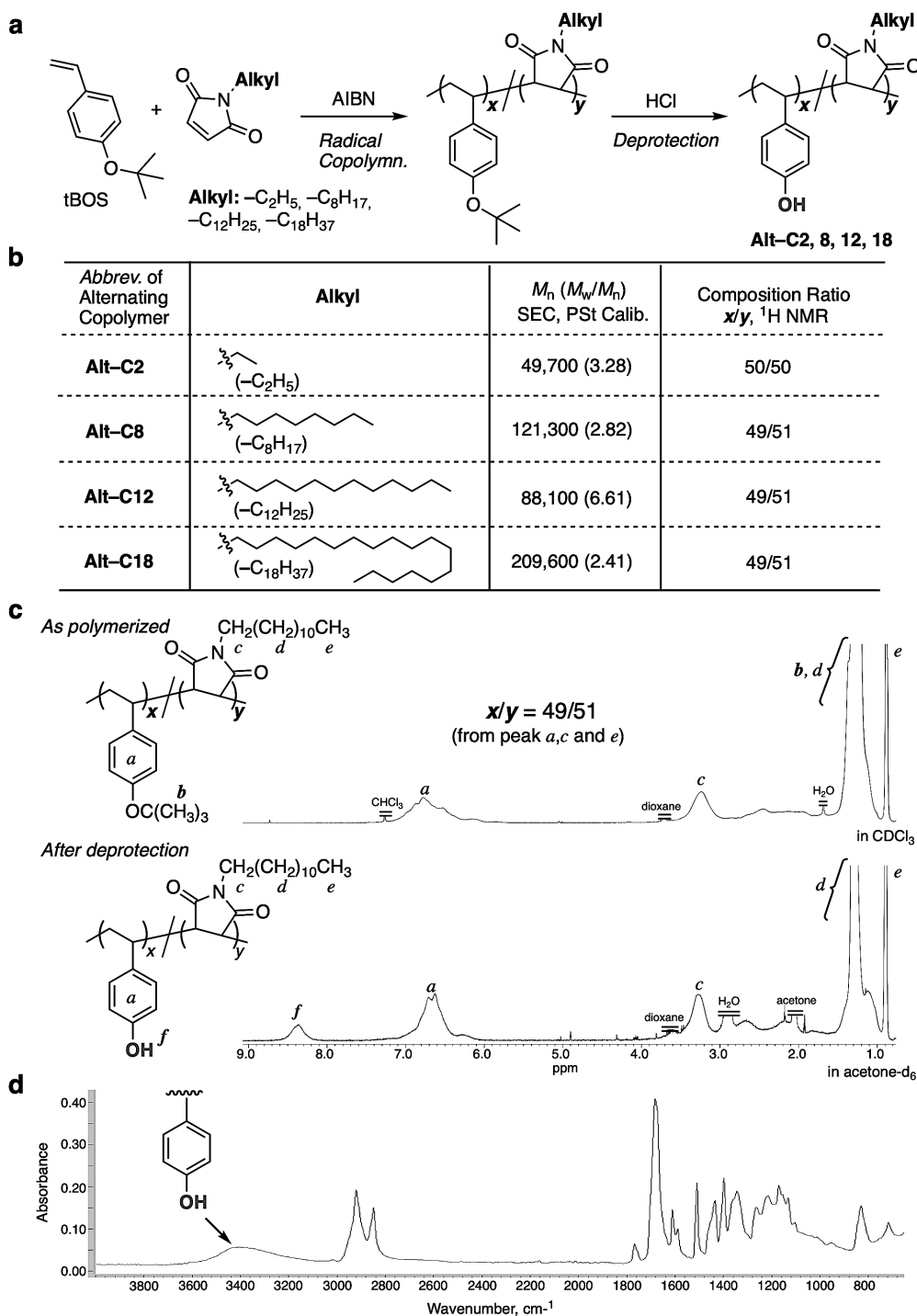


Figure 4. (a) Synthetic scheme of alternating copolymers with 4-vinylphenol and *N*-alkyl maleimide: $[\text{tBOS}]_0/[\text{N-alkyl maleimide}]_0/[\text{AIBN}]_0 = 1000/1000/20$ mM in toluene at 60 °C, (b) table of molecular weight and composition of copolymers after deprotection reaction, (c) ^1H NMR spectra of the copolymer with tBOS and C12MI (upper) and the copolymer after deprotection reaction (lower) and (d) FT-IR spectrum of the copolymer with 4-vinylphenol and *N*-dodecyl maleimide.

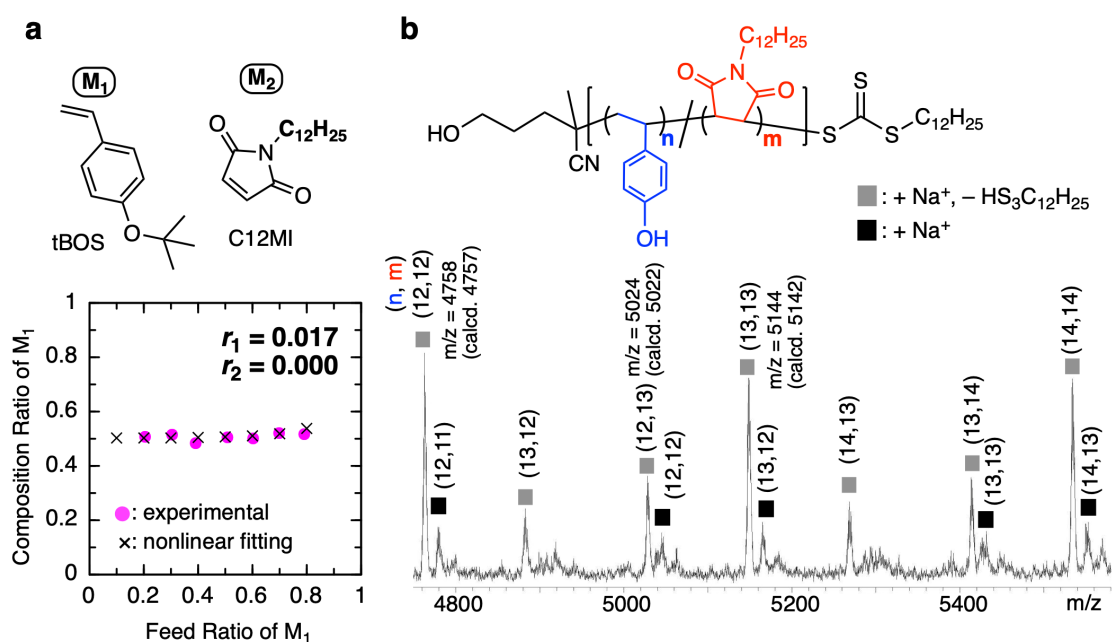


Figure 5. (a) Monomer reactivity ratios of tBOS (M_1) and C12MI (M_2) calculated from non-linear method, and (b) MALDI-TOF-MS spectrum of Alt-C12 ($M_n = 18,200$, $M_w/M_n = 1.22$) synthesized via RAFT copolymerization.

Table 1. Solubility of the Copolymers at 25 °C^a

Solvent	Alt-C2	Alt-C8	Alt-C12	Alt-C18
Hexane	IS	IS	IS	IS
Toluene	IS	IS	Swollen ^b	Swollen ^b
THF	S	S	S	S
Chloroform	IS	S	S	S
Ethyl Acetate	S	S	S	S
Acetone	S	S	S	S _{part}
Methanol	IS	IS ^c	IS	IS
DMF	S	S	S	S _{part}
DMSO	S	IS ^c	S _{part}	IS
Water	IS	IS	S _{part}	IS
NaOH aq.	S	IS	IS	IS

S: soluble, IS.: insoluble, S_{part}: partially soluble, Swollen: insoluble and swollen. ^a Polymer concentration: 1wt%. ^b Soluble at 80 °C. ^c Soluble at 60 °C.

insoluble in hexane and methanol. As for chloroform, toluene, DMSO and NaOH aq., the solubilities depend on the alkyl chain. Thus **Alt-C8**, **Alt-C12** and **Alt-C18** are all totally insoluble in water, whereas **Alt-C2** is soluble at high pH and reversely **Alt-C8**, **Alt-C12** and **Alt-C18** are all soluble in chloroform whereas **Alt-C2** is not. Thus by constitution, the alternated copolymers present an amphiphilic structure: on one end, the lipophilic side chains have more affinity for weakly polar solvents whereas lipophobic phenol units show better affinity for water and polar solvents thus featuring an alternating lipophilic/lipophobic polyamphiphile structure whose lipophilic/lipophobic balance may be tuned by the length of the alkyl side chains.

Interestingly, when toluene was added to the two alternating copolymers carrying long alkyl chains (**Alt-C12** and **Alt-C18**) at 25 °C, these white powder samples swelled to jelly. However, when they were heated to 80 °C, the polymers turned into soluble giving fluid solutions. The homogeneous and transparent solution of **Alt-C18** in toluene (1wt%) that was prepared at 80 °C turned into viscous via the cooling process to ambient temperature while remaining optically transparent. On the other hand, in the case with **Alt-C12**, a more pronounced turbidity was generated through the cooling process. The thermal response behavior of **Alt-C12** was further studied with turbidity measurements. Figure 6 shows the turbidity measurement (wavelength $\lambda = 670$ nm) on the cooling from 80 °C to 25 °C and the reheating to 80 °C. The solution started to become turbid around 60 °C on the cooling process and the transmittance was almost lost below around 50 °C. The thermal response was reversible with a few degrees hysteresis and similar transmittance curve was obtained on the heating.

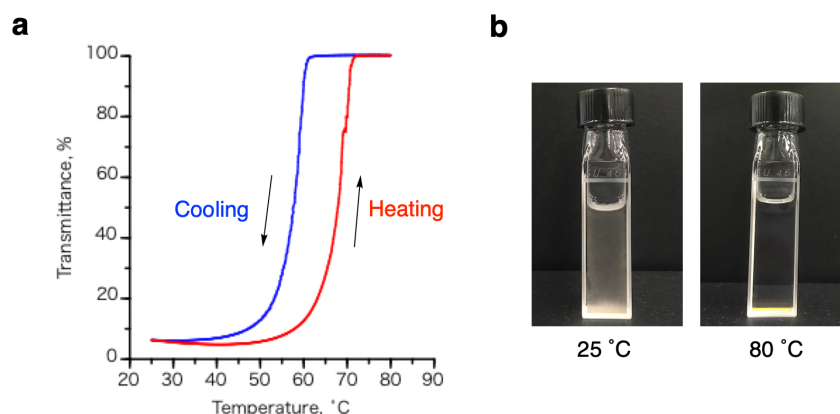


Figure 6. (a) Turbidity measurement of 1wt% **Alt-C12** solution in toluene: heating speed = 0.5 °C/min, wavelength $\lambda = 670$ nm, and (b) pictures of the 1wt% **Alt-C12** solution in toluene at 25 °C and 80 °C.

Physical Gel

As described above, the long alkyl chain-pendant alternating copolymers (**Alt-C12** and **Alt-C18**) were found to show unique thermal response behaviors in diluted toluene solution (1wt%). We then prepared more concentrated solutions in toluene (5wt%) to study the thermal response behaviors. Even the concentrated solutions of both copolymers became soluble at 80 °C giving homogeneous and fluid solutions but they turned into viscous via cooling process. Finally, the flowability was completely lost to yield gels at ambient temperature. The gels were again soluble at 80 °C and gelled again via the cooling process indicating they are thermally responsive physical gels. Interestingly, these copolymers gelled in other aromatic solvents, such as anisole, *p*-cymene, cumene, 1,2,4-trichlorobenzene, nitrobenzene (Figure 7). The turbidity and hardness of the resultant organogels depended on the solvent as well as the alkyl substituent, which is likely due to differences in their structures and morphologies. For instance, both of the copolymers gave obviously harder gels with 1,2,4-trichlorobenzene than other aromatic solvents, and **Alt-C18** did not give gel in nitrobenzene specifically. Detailed study in effects of aromatic solvent on the gelation behaviors is now under investigation.

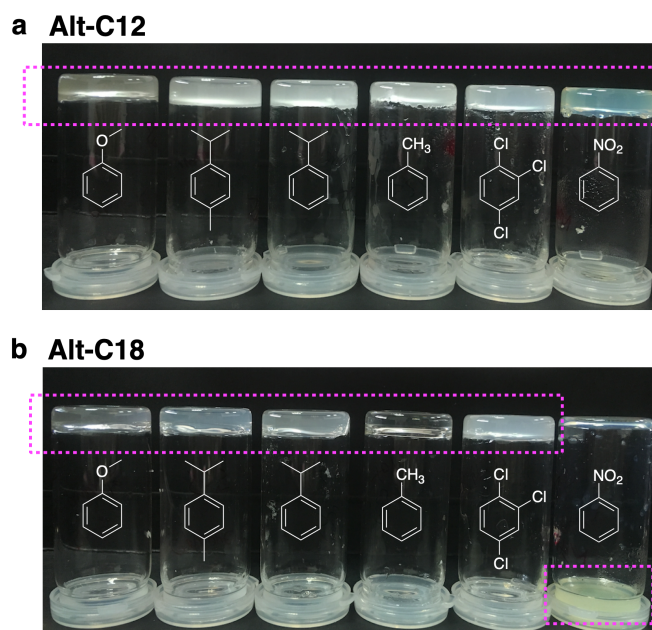


Figure 7. Appearances of 5wt% solutions of (a) **Alt-C12** and (b) **Alt-C18** in various aromatic solvents at ambient temperature (~25 °C) after cooling from 80 °C. The sample tubes were turned upside down for demonstration of gel formation (from left to right: anisole, *p*-cymene, cumene, toluene, 1,2,4-trichlorobenzene, nitrobenzene solvents).

Detailed Study on Physical Gels in Toluene

To investigate the gelation mechanism in toluene, the solution of **Alt-C12** in toluene- d_8 (5wt%) was prepared at 80 °C for variable temperature ^1H NMR measurements (Figure 8a). In the spectrum measured at 25 °C, there is no peak from aromatic protons of vinyl phenol units around 7 ppm but a broad and very small peak at 1-3 ppm that is likely from alkyl pendant and back bone protons was observed. When it was measured at 60 °C, the peaks from the C₁₂ alkyl pendant of the maleimide unit (*d*, *e*) became larger, and further heating up to 80 °C made the intensities stronger. The vinyl phenol peak from aromatic protons of vinyl phenol units was observed only after the temperature was heated to 80 °C. The similar trend was obtained with **Alt-C18** (Figure 8b) but with overall better resolved peaks, meaning overall higher mobility with this longer chain. Thus, for instance, the characteristic signals of the C₁₈ pendant (*d*, *e*) are well defined at 25 °C and that of the vinyl phenol (*a*) is already detected at 60 °C.

From the variable temperature ^1H NMR measurements, it is evident that the phenol groups and the hydrocarbon side chains become mobile in two different temperature ranges hence indicating that these fragments are segregated in the gel structure. Hydrogen bonding between phenol units is supposed to play a part in the gelation behavior, in competition with solubility and mobility brought by alkyl chains. The hydrogen bonding interaction works well

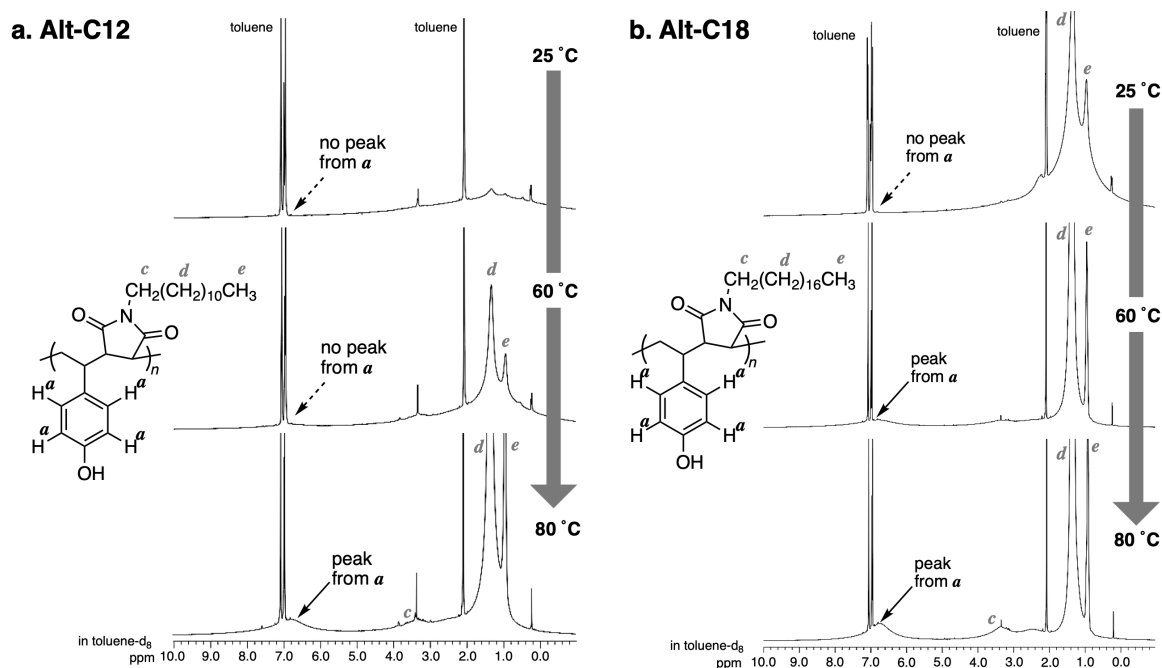


Figure 8. ^1H NMR spectra of 5wt% (a) **Alt-C12** or (b) **Alt-C18** gel in toluene- d_8 at 25, 60, and 80 °C.

at low temperature but it becomes weaker on heating, which corresponds to the thermal response behavior observed. Indeed, the alternating copolymer of C12MI with styrene instead of vinyl phenol was soluble in toluene and no gelation occurred (Figure 9a). To further confirm the hydrogen bond-driven gelation, a tiny drop of pyridine was added into the gel of **Alt-C12** in toluene. Consequently, the gel turned into soluble to give a fluid solution. Interestingly, the transition is sensitive to the pyridine amount: for example, addition of half molar quantity of pyridine (with respect to the amount of phenol groups in the alternating copolymer) made the gel softer, the perfect solubilization took place once a stoichiometric molar amount of pyridine was added but the viscosity was still beyond the range of the falling ball viscometer. After addition of two equivalents of pyridine, the measured viscosity was as low as 8.4 mPa·s, *i.e.* only ten times higher than the one of pure toluene (0.6 mPa·s) and comparable to the viscosity of the THF solution (10.4 mPa·s). The solubilization by the pyridine addition was also supported by ^1H NMR (Figure 9b): some copolymer peaks were clearly observed even at 25 °C after addition of a stoichiometric molar amount of pyridine, similar to that at 80 °C. These experiments nicely confirm the role of hydrogen bonding in gelation.

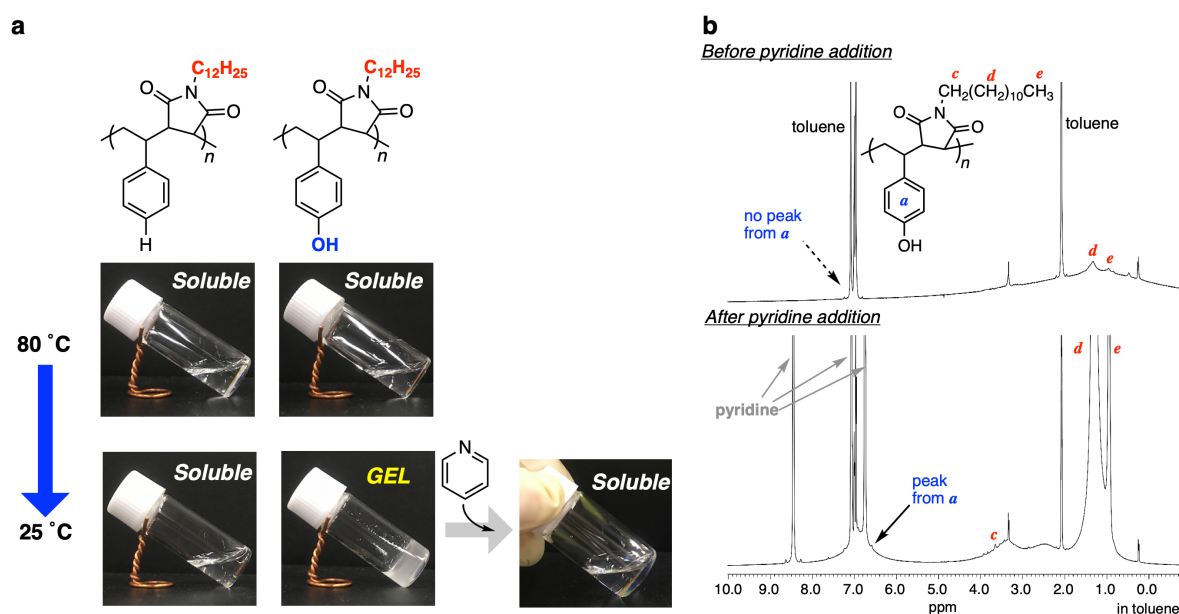


Figure 9. (a) Relation between polymer structures and solubilities and (b) ^1H NMR spectra of 5wt% **Alt-C12** gel in toluene- d_8 (upper) and after addition of pyridine (lower): [phenol unit in gel] = [added pyridine].

Control of Molecular Weight for Study on Molecular Weight Dependence

To examine effects of molecular weight on gelation, the alternating copolymers of vinyl phenol with C12MI having various molecular weights were synthesized via RAFT polymerization. The 1:1 copolymerization of tBOS and C12MI was conducted with 4-cyano-4-[(dodecylsulfanylthiocarbonyl) sulfanyl]pentanol as a chain transfer agent (CTA) in conjunction with AIBN as an initiator. The molecular weight was controlled by changing the ratio of the comonomers to CTA: $[\text{each comonomer}]_0/[\text{CTA}]_0 = 1000/5, 1000/10, 500/10, 300/10, \text{ and } 100/10 \text{ mM}$. The two comonomers were consumed at same rate under every condition, and the conversions reached to over 90% (Table 2). The resultant 5 copolymer samples underwent HCl treatment for the *tert*-butoxy group deprotection, similar to the copolymers synthesized via free radical polymerization. The quantitative deprotection was confirmed by ^1H NMR and the molecular weights were characterized by SEC. The molecular weights of the copolymers showed a clear dependence on the ratio, and the molecular weight distributions were narrow enough ($M_w/M_n < 1.30$, Figure 10a). For lower molecular weight copolymers, the number-averaged polymerization degree for the two units (n) that was characterized by ^1H NMR well agreed with the value calculated from the injection ratio and conversion. The ^1H NMR characterization was not accessible to the higher molecular weight polymers, so calculated values for n were adopted for all the 5 samples in this work ($n = 10, 29, 45, 95, \text{ and } 190$).

Solubilities and gelation behaviors in toluene were studied with the 5 copolymers of different molecular weights. Figure 10b shows dependence of gelation on polymerization degree and concentration. For example, the shortest copolymer ($n = 10$) never gelled at any concentration, whereas the longest one ($n = 190$) did at lower concentration (i.e., 3wt%). As shown in Figure 10b, the critical gelation concentration (CGC) obviously depends on the molecular weight of the copolymer.

The thermal response behaviors were also studied for the 1wt% solutions in toluene. The lower molecular weight copolymers ($n = 10, 29$) were soluble in toluene even at lower temperature. On the other hand, the solutions of the higher molecular weight samples ($n = 45, 95 \text{ and } 190$) became cloudy at lower temperature. Figure 10c shows turbidity measurement of the 1wt% solution in toluene. The transition temperature depended on the molecular weight: it increased as the molecular weight was higher.

The alkyl group of maleimide is basically soluble in a hydrophobic solvent (i.e., toluene) due to the high hydrophobicity. Thus, the periodically embedded hydrophobic maleimide

Table 2. Characterization of Alternating Copolymers with tBOS and *N*-Dodecylmaleimide (C12MI) Synthesized via RAFT Polymerization

Entry ^a	Conc., mM		Conv., % ^b		DP ^c (calcd.)	Composition Ratio ^d tBOS/C12MI	M_n^e	M_w/M_n^e
	tBOS	C12MI	tBOS	C12MI				
1	1000	1000	95	97	190	49/51	54300	1.32
2	1000	1000	94	96	95	48/52	31000	1.23
3	500	500	90	91	45	49/51	18700	1.20
4	300	300	96	99	29	48/52	12100	1.17
5	100	100	100	100	10	50/50	5100	1.11

^a Conditions: [CTA]₀/[AIBN]₀ = 5/1 mM (Entry 1) and 10/1 mM (Entries 2-5) in toluene at 60 °C. ^b Conversions of tBOS and C12MI were determined by ¹H NMR. ^c The average DPs of tBOS and C12MI in copolymers were determined by monomer conversions.

^d Composition Ratio of tBOS and C12MI was determined by ¹H NMR after purification.

^e Determined by SEC in THF with PSt standard calibration (before deprotection reaction).

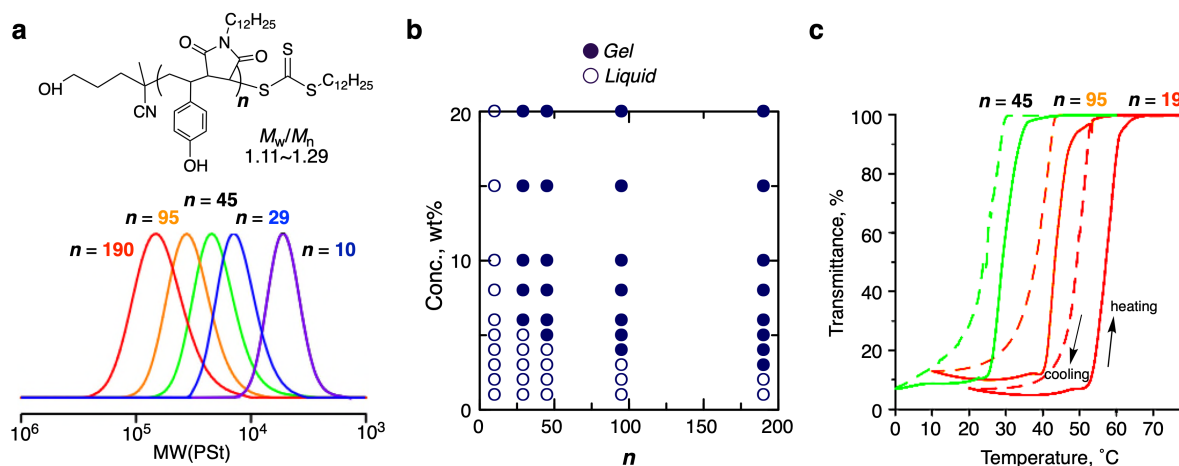


Figure 10. (a) SEC curves of copolymers synthesized via RAFT polymerization and deprotection reaction: [tBOS]₀/[C12MI]₀/[CTA]₀/[AIBN]₀ = 1000/1000/5/1 mM ($n = 190$), 1000/1000/10/1 mM ($n = 95$), 500/500/10/1 mM ($n = 45$), 300/300/10/1 mM ($n = 29$), 100/100/10/1 mM ($n = 10$) in toluene at 60 °C, (b) polymerization degree (n)–concentration plot: (filled circle: gel, open circle: liquid) and (c) turbidity measurements for 1wt% copolymer solutions in toluene ($n = 45, 95, 190$) (heating speed = 0.5 °C/min, wavelength $\lambda = 670$ nm).

units would be helpful for expanding chain due to the high affinity for solvent as well as the rigidity, and the phenol units dynamically interact with each other via hydrogen bonding to give a non-covalent network. The alternating sequence therefore contributes to the balance of the two effects. For inducing self-assembly or gelation, a certain number of phenol groups in one alternating copolymer chain should be necessary, as demonstrated by the dependence of gelation on the polymerization degree.

Rheology Measurement of the Gel

Temperature-variable rheology was investigated with the physical gel samples of the alternating copolymers. For the measurement, 1,2,4-trichlorobenzene was used as the solvent due to the higher boiling point (213 °C) to avoid evaporation during measurement. The 10wt% solutions of **Alt-C12** or **Alt-C18** in 1,2,4-trichlorobenzene were first prepared at 90 °C and they were converted into the physical gels by cooling to ambient temperature. Here, 5wt% gels were not suitable for the measurement of rheology because of big syneresis at low temperature. Figure 11 shows isothermal ω -sweeps for the samples of **Alt-C12** and **Alt-C18** at different temperatures. At 60 °C, **Alt-C18** showed the response of a viscoelastic liquid: the relaxation time, estimated by the angular frequency of the $G' = G''$ cross-over point, was of about 0.1 s; the terminal regime ($G' \approx \omega^2$; $G'' \approx \omega$) was evident in the low frequency region. At lower temperatures, the crossover point was shifted to significantly longer relaxation times (about 3 s at 50 °C, 10 s at 40 °C and outside the measurable range at 25 °C), whereas the terminal regime disappeared and the absolute values of the elastic modulus increased (100 kPa

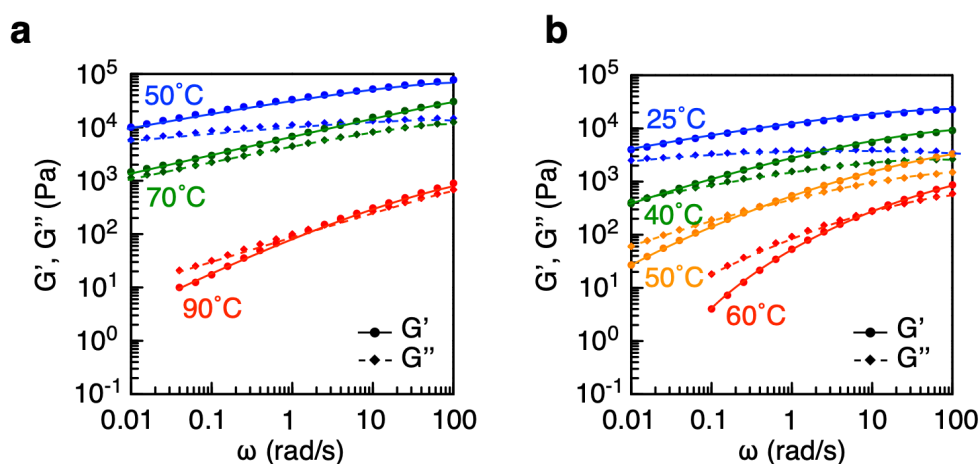


Figure 11. Rheology of 10wt% organogels in 1,2,4-trichlorobenzene ($\gamma = 1$): (a) **Alt-C12** and (b) **Alt-C18**.

at 1 Hz) thus giving the image of a rather stiff transient network or entangled system with very long relaxation time at room temperature. For **Alt-C12**, the general behavior was comparable but the terminal regime was hardly visible at 90 °C. The relaxation time was about 2s at 90 °C and already outside the measurable range at 70 °C. Absolute values of moduli were higher (about 300 kPa at 1 Hz) fully consistent with the lower mobility found by ^1H NMR in the organogel of **Alt-C12** compared with that of **Alt-C18**.

Structure Analysis in the Bulk and Organogel State

Before structure analysis of the physical gels, the structures of **Alt-C12** and **Alt-C18** at solid state were analysed by DSC and wide angle X-ray diffraction (WAXD) because these characterization would be helpful for understanding the gel structure. Figure 12 shows DSC thermograms of the bulk samples with **Alt-C12** and **Alt-C18** recorded at a heating rate of 10 °C/min. For **Alt-C18**, two distinct endothermic signals were detected around 150 °C ($\Delta H_m = 0.8$ J/g) and 200 °C ($\Delta H_m = 4.6$ J/g). On the other hand, a broad endothermic signal ($\Delta H_m \approx 6$ J/g) was observed between 150 and 200 °C with **Alt-C12**. Such values should be compared to the melting enthalpy of reference materials featuring the same type of crystallizable fragments. Thus, for instance, paraffin wax has a melting enthalpy of about 200 J/g,¹² and recently synthesized poly(*Sty-alt*-MA nh) derivatives with C_{22} alkyl side chains⁸ showed a melting enthalpy of about 50 J/g hence indicating by comparison a very low level of

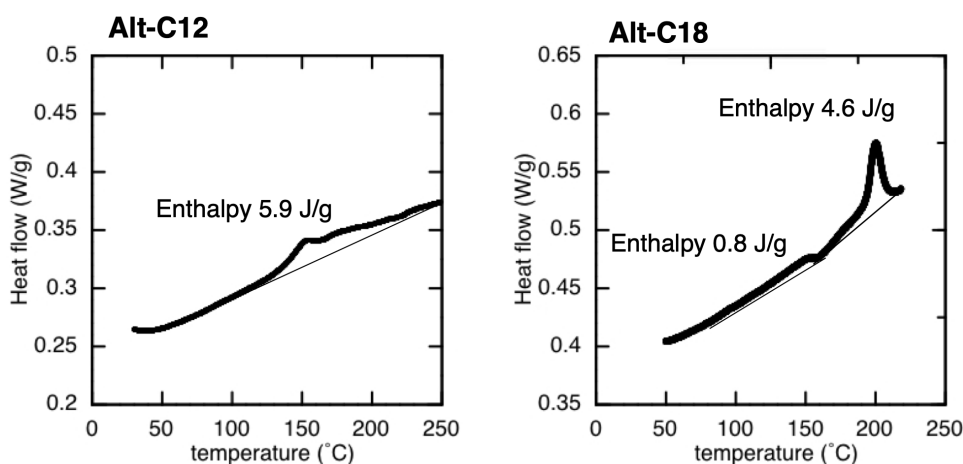


Figure 12. DSC thermograms recorded at 2nd heating process for bulk samples of (a) **Alt-C12** and (b) **Alt-C18**. Temperature program: 1st heating at 10 °C/min from 20 °C to 250 °C; 5 min holding at 250 °C; 1st cooling at 10 °C/min from 250 °C to 20 °C; 2nd heating at 10 °C/min from 20 °C to 250 °C.

crystallinity in our compounds.

WAXD patterns of bulk **Alt-C12** and **Alt-C18** are presented in Figure 13. In Figure 13ab, the same data is plotted as a function of $q = 4\pi (\sin\theta)/\lambda$. In the wide q region, a broad diffraction peak was detected with a maximum at the same position, $q = 1.38 \text{ \AA}^{-1}$ ($2\theta = 9.1^\circ$) for both samples, which corresponds to an average spacing of $2\pi/q = 4.6 \text{ \AA}$. Such a signal is ubiquitous in molten polymers and amphiphiles and corresponds to the amorphous packing of hydrocarbon chains.¹³ At lower q values, another signal was detected for both compounds but its position depends on the length of the alkyl side chains: the maximum arose at $q = 0.248 \text{ \AA}^{-1}$ ($2\theta = 1.63^\circ$) for **Alt-C12** and at $q = 0.192 \text{ \AA}^{-1}$ ($2\theta = 1.31^\circ$) for **Alt-C18**. This corresponds to an average spacing of $2\pi/q = 25 \text{ \AA}$ for **Alt-C12** and $2\pi/q = 33 \text{ \AA}$ for **Alt-C18**. In Figure 14, the author reports calculated dimensions of the repeat unit with alkyl side chains in the fully extended conformation. The maximum possible distance between terminal CH_3 and OH groups is $l = 22 \text{ \AA}$ for **Alt-C12** and $l = 29 \text{ \AA}$ for **Alt-C18**. To account for such distances, intermediate between once and twice the molecular dimensions, and considering the analogy between the repeating unit and an amphiphile, we propose a packing of inverse micelle-like cylinders composed of hydrogen bonded phenol groups in the core surrounded by radically distributed alkyl chains.¹⁴ Starting from above calculated distances, the local order was considered to be close to a hexagonal packing¹⁵ giving rise to a Bragg spacing of $2l/\sqrt{3} = 25.4 \text{ \AA}$ for **Alt-C12** and $2l/\sqrt{3} = 33.5 \text{ \AA}$ for **Alt-C18**, which matches the measured values.

In both compounds, the low- q Bragg peak appears to be rather broad (Figure 13cd), showing a full width at half maximum of $\Delta 2\theta \approx 0.6^\circ$ (**Alt-C12**) and $\Delta 2\theta \approx 0.5^\circ$ (**Alt-C18**). These values are evidently much larger than the experimental resolution ($\Delta 2\theta$ of integrated bins $\approx 0.01^\circ$) and the crystal size L determined through the Scherrer equation¹⁶ $L = \lambda/[(\Delta 2\theta) \cos\theta]$ is of about 70-90 $\text{\AA}$, thus confirming the picture of a very defective semi-crystalline arrangement.

After swelling with a solvent, the low- q Bragg peak disappears for both compounds. The SANS data of **Alt-C12** and **Alt-C18** in toluene- d_8 are shown for several temperatures in Figure 15. For both compounds, the logarithmic plot of data in the 0.01 to 1 $\text{\AA}^{-1}$ region reveals several slopes. Whilst the high- q region is very similar for all spectra, we observe an upturn of scattered intensity at low q when temperature is decreased, consistent with the growth of large percolating objects and indeed it is in the same temperature range (from 90 to 25 $^\circ\text{C}$) that gelation is observed. Regarding the high- q region, similar spectra have been reported in the literature of gel-forming systems: T. Lodge et al. analyzed aqueous solutions of high molecular weight methylcellulose;¹⁷ similar structures were also found by Tung et al. for mixtures of low

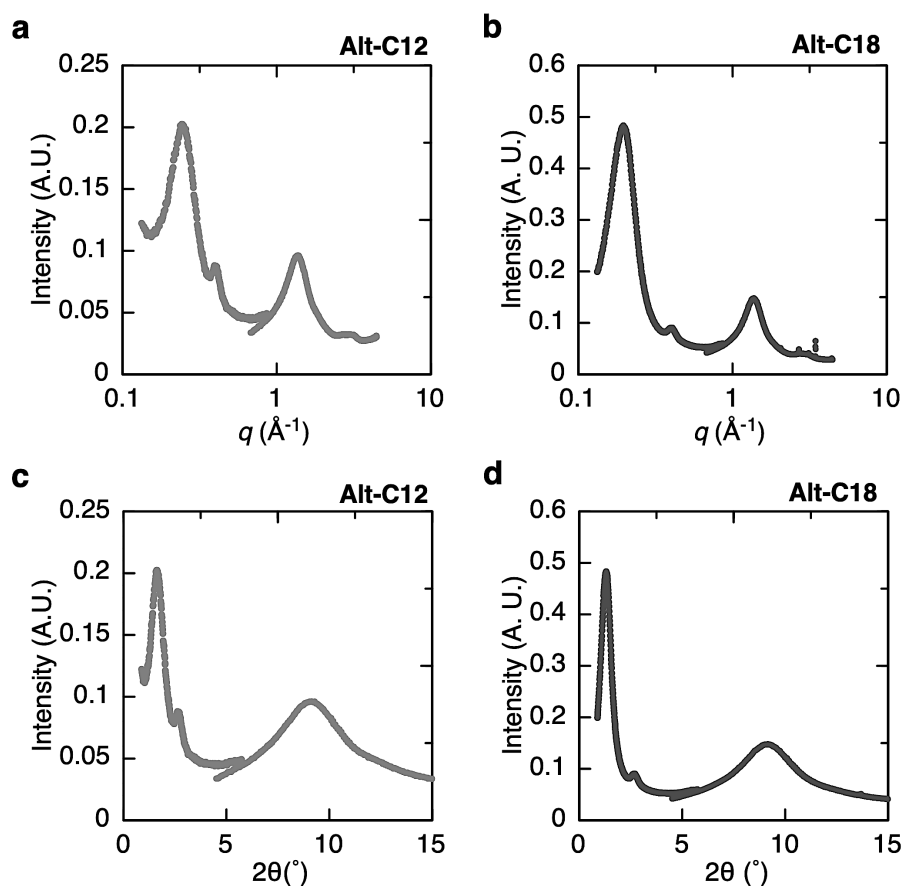


Figure 13. Wide angle X-ray diffraction patterns of freeze-dried bulk **Alt-C12** (a, c) and **Alt-C18** (b, d). The peak arising at $q = 0.4 \text{ \AA}^{-1}$ ($2\theta = 2.7^\circ$) is due to Kapton windows.

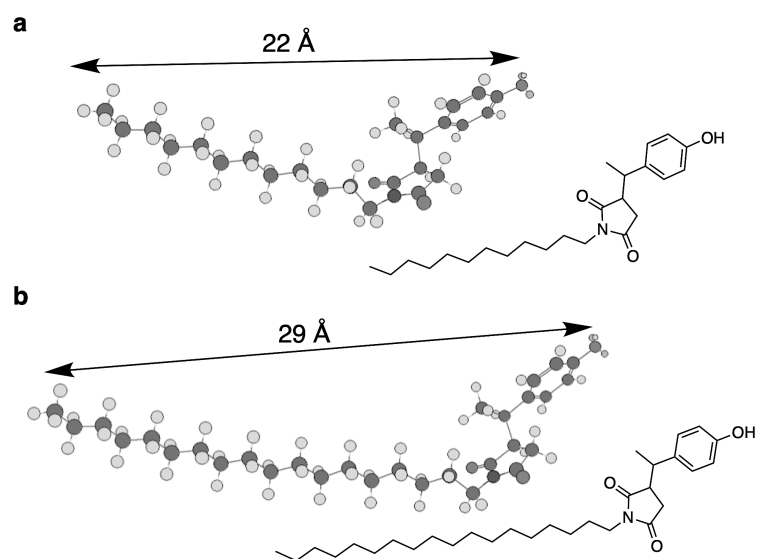


Figure 14. Chem3D Molecular models of dimers of 4-vinylphenol and *N*-alkylmaleimide (a, C12MI; b, C18MI) with alkyl chains in the fully extended conformation.

molecular lipids in aqueous solutions.¹⁸ Following these references, we found that SANS data recorded at 85 °C could be well fitted with a flexible cylinder model.¹⁹ The details of fitting are reported in Figure 16, a summary of results is presented in Table 3. Interestingly, this model based on SANS data in the organogel is reminiscent of the solid state structure found above by WAXD. In particular the diameter of the cylinders is increasing with the lengths of the alkyl side chains. When applying the same treatment to SANS data recorded at lower

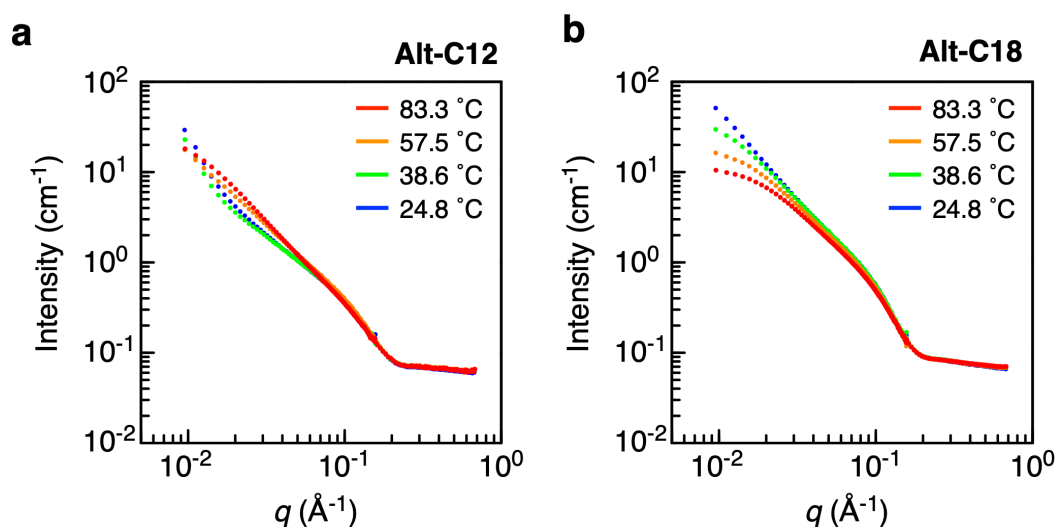


Figure 15. SANS profiles of 5wt% **Alt-C12** (a) and **Alt-C18** (b) in toluene- d_8 at various temperatures.

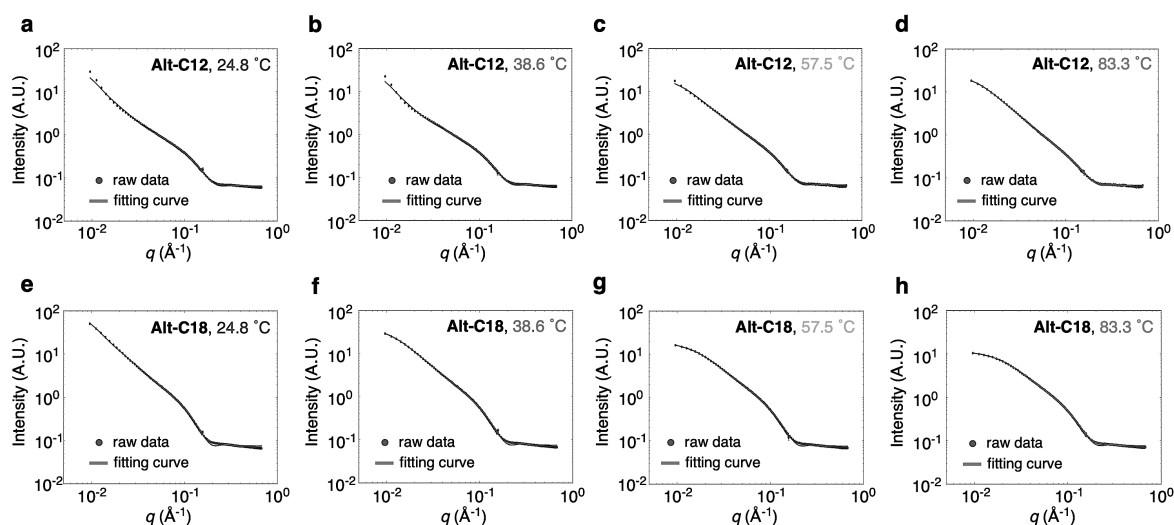
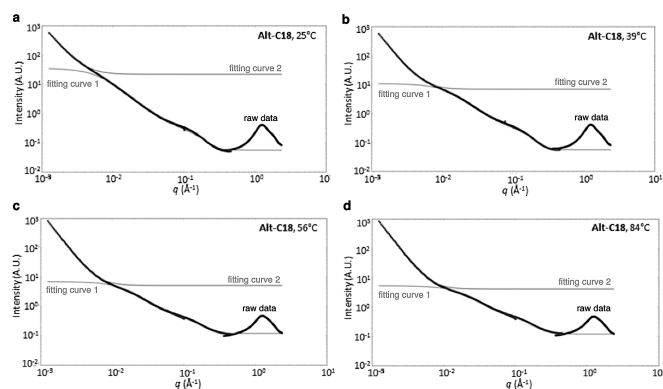


Figure 16. SANS data with fitting curves for **Alt-C12** (a-d) and **Alt-C18** (e-h).

Table 3. Fitting Parameters from Modeling of SANS Data Recorded at Various Temperatures: 5wt% Solutions in Toluene-d₈

Sample	Temp. (°C)	Av. Radius (Å)	Kuhn length (Å)	Contour length (Å)	Background (cm ⁻¹)
Alt-C12	83.3	14.7	44.3	2250	0.0661
	57.5	15.3	79.4	1470	0.0663
	38.6	15.0	135.0	53800	0.0653
	24.8	15.0	99.4	122000	0.0640
Alt-C18	83.3	17.2	77.0	447	0.0755
	57.5	17.4	73.4	688	0.0741
	38.6	17.6	65.3	1510	0.0743
	24.8	17.9	66.9	12300	0.0734

**Figure 17.** SAXS data with fitting curves for **Alt-C18** at different temperatures. The fitting curve 1 correspond to a flexible cylinder model fitted from 0.01 to 0.5 Å⁻¹. The fitting curve 2 correspond to a shape unique power law model fitted from 0.001 to 0.01 Å⁻¹.**Table 4.** Fitting Parameters from Modeling of SAXS Data Recorded at Various Temperatures of 5wt% **Alt-C18** Solutions in Toluene-d₈

Temp. (°C)	Flexible Cylinder				Power Law	
	Av. Radius (Å)	Kuhn length (Å)	Contour length (Å)	Background (cm ⁻¹)	power	Background (cm ⁻¹)
84	8.48	140.4	429.0	0.12	3.19	4.11
56	8.51	103.4	634.6	0.12	3.17	5.23
39	8.50	76.0	1092.5	0.05	3.03	6.87
25	8.50	58.3	4000.0	0.05	2.80	21.96

temperatures, we found that the same model remains valid with some variations in the parameters: the average radius is essentially constant, the Kuhn length is also almost constant for **Alt-C18** but more fluctuating for **Alt-C12**, may be due to more pronounced aggregation, but always remains within 1 to 5 times the average diameter of the cylinders thus giving the picture of curly noddle like objects. Finally, the contour length was found to increase dramatically (as a result of low- q upturn) when temperature was decreased, which is fully consistent with the establishment of a percolating gel of extended objects. In addition, investigations in an extended q range were performed by SAXS for **Alt-C18** (Figure 17 and Table 4). Basically, SAXS data in the 0.01 to 0.5 $\text{\AA}^{-1}$ range were well fitted with the same model as above. The parameters of the fit appear slightly different, probably due to the different origin of contrast for neutrons and X-rays, but their temperature dependence show the same trend: a major increase of the contour length when temperature is decreased. In addition, scattering data in the 0.001 to 0.01 $\text{\AA}^{-1}$ range show a power law dependence: $I(q) \approx q^{-3}$ which indicates the presence of large aggregates similar to what has already been observed in water-soluble fibrillar gelling systems.²⁰

Cryo-TEM images of the **Alt-C18** gel deposited from a 5 wt% cumene solution are presented in Figure 18. The left picture leaves the image of a rather uniform film featuring a densely packed felt morphology with a mesh size in the range of a few nanometers. Due to the small contrast between the solvent and the polymer, visible features may arise from the

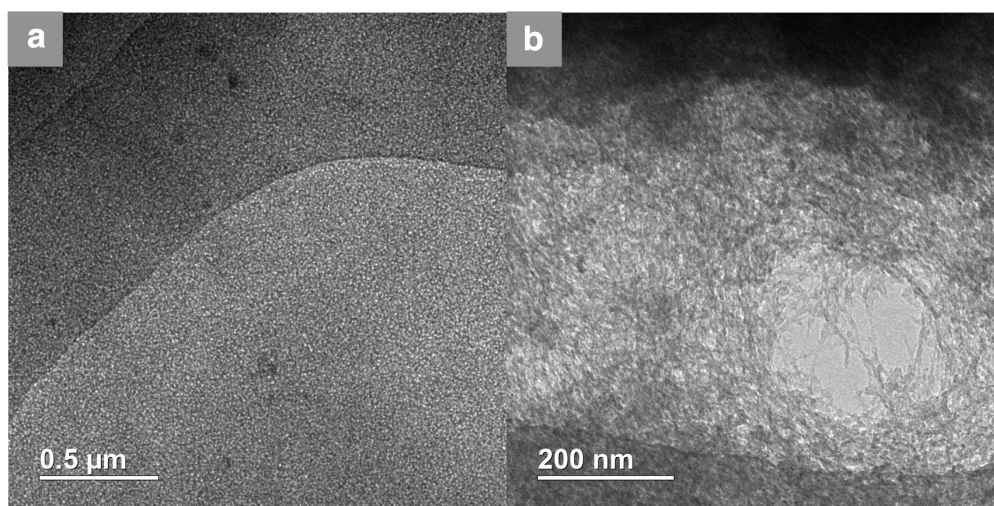


Figure 18. Cryo-TEM images of **Alt-C18** at 5wt% in cumene. The left picture (a) is taken at the edge of the carbon grid. In the right picture (b), the fibrillar morphology is revealed inside a tear defect.

surface rather than the bulk. The inner structure is more clearly revealed at the edge of a defect. The image on the right shows a tear of the film, caused by the radiation, and there is clearly an interlace of fibers impregnated with the solvent. The smallest features' width as measured from the picture is of about 4 nm, in good agreement with the diameter given by the flexible cylinder model in SAXS analysis.

Thus, the morphologies revealed in this study illustrate the self-assembly induced by the alternating structure. In toluene, the solvophobic phenol groups segregate into elongated subdomains where they strongly associate via hydrogen bonds. The aggregates thereby formed, must have a helical or calixarene like structure,²¹ compatible with the connectivity of the polymer chain. Hydrocarbon chains, which have in contrast an excellent affinity with the solvent, occupy the periphery giving rise to an inverse micelle-like cylindrical morphology. At a larger scale, these elongated micelles appear highly agglomerated and interconnected giving rise to a felt like gel morphology in which the excess solvent is trapped.

Conclusion

A series of alternating copolymers consisting of vinyl phenol and alkyl-pendant maleimide were synthesized via radical copolymerization and deprotection. Introduction of long alkyl chain into the maleimide unit gave thermosensitive solubility for diluted solution in aromatic solvent and hard gels when the concentration was increased. The gelation temperature and stiffness of the organogel are tuneable by varying concentration, solvent, molar mass and the length of the alkyl side chains. Some structural analyses with ¹H NMR, X-ray, neutron scattering experiments and cryo-TEM gave the image of a fibrillar nanostructure with segregation phenol fragments from the hydrocarbon in the alternating sequence, in which the hydrogen bond interactions between phenol units are crucial.

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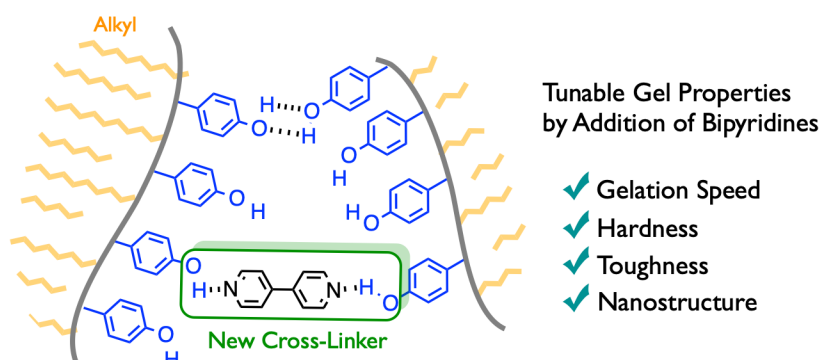
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Chapter 5

Tuning Properties of Organogels Made by Phenolic Alternating Copolymers Through Addition of Bipyridine Derivatives

Abstract

Gel properties of organogels made by 4-vinyl phenol/*N*-alkyl maleimide alternating copolymers in aromatic solvent were tuned by addition of a tiny amount of bipyridine derivatives, i.e., 4,4'-bipyridine, 4,4'-trimethylenedipyridine, and 4,4'-(1,4-phenylene)bipyridine. Interestingly, the slight difference in structure of the bipyridine as well as the amount greatly affected the gel properties and structures, such as gelation speed, hardness, toughness and nanostructures of the gels. The dynamic interaction between bipyridine and phenol units in the gels likely increased gel toughness. Moreover, unique nanostructures such as leaf-like structure and bicontinuous phase formed depending on the spacer structure of the bipyridine additive.



Introduction

If small amounts of additives can change or improve structures or properties of polymer materials, it is significant and interesting from the viewpoint of further development of existing materials. In familiar examples, the addition of bittern or rennet to soymilk or milk produces delicious tofu and cheese, respectively. In the case of tofu, calcium and magnesium ions in bittern neutralize the protein particles in soymilk, which induces the aggregation of the particles to form tofu.^{2,3}

As for synthetic polymer materials, dual cross linked gel⁴⁻⁶ and nanocomposite gel⁷⁻¹⁰ in which addition of low molecules or inorganic particles (silica or clay) improved the mechanical properties of the hydrogels have been reported. Here, these additives allow to improve the strength and toughness of the gels by dynamic interactions with existing gel networks to form additional new networks (so-called sacrificial bonds¹¹). In a broad sense, double network gel^{11,12} also can be said to be an example of improving mechanical strength by addition of a new network.

The author previously discovered the physical gelation behavior of alternating copolymers bearing long alkyl and phenol pendants in aromatic solvents (in Chapter 4).¹³ Hydrogen bonding between phenol groups is crucial for the gelation and the segregated hydrophilic phenol and hydrophobic alkyl units induced fibrillar nanostructure consisting of phenol core and alkyl shell in aromatic solvents. The gel turned into soluble to give a fluid solution by addition of mono-pyridine that interacts with phenol units of the gels. The transition was sensitive to the amount of mono-pyridine and the perfect solubilization took place when a stoichiometric molar amount of pyridine was added.

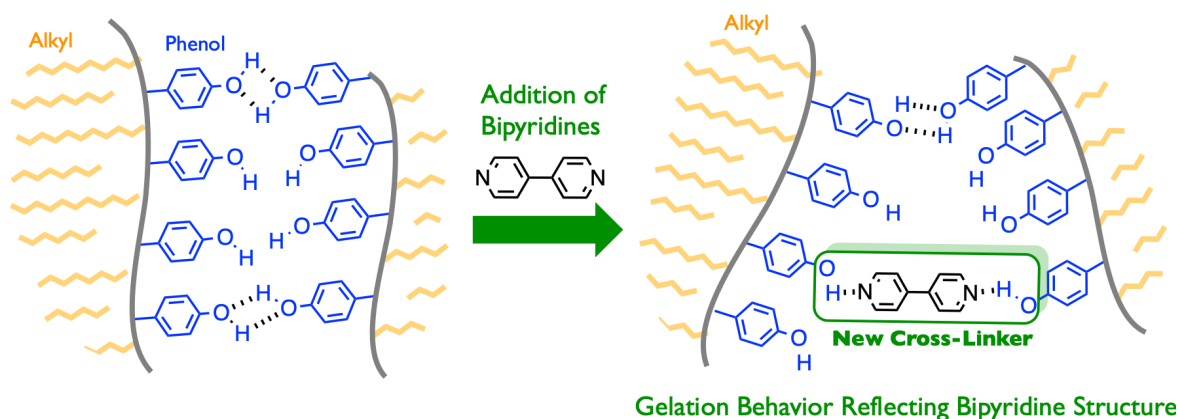


Figure 1. Concept of this work: the author expected new properties of the gels reflecting bipyridine structure.

In this chapter, the author investigated the gelation behaviors and properties of the alternating copolymers after each addition of three types of bipyridine to expect unique gelation behavior reflecting their molecular structure because bipyridines are supposed to interact with phenol at either side and behave as a new cross-linker in the gelation process (Figure 1). The author also expected increasing of gel toughness because the interaction between pyridine and phenol is an equilibrium.¹⁴ If the gel properties can be tunable by small amounts of additives, it would help development of soft materials.

Experimental

Materials

Cumene (abcr GmbH; purity 99%), 1,2,4-trichlorobenzene (TCB, Carlo Erba; purity >98.5%), pyridine (Wako; purity >99.5%), toluene (Wako; purity >99%), 4,4'-bipyridine (**Bipy-1**, TCI; purity), 4,4'-trimethylenedipyridine (**Bipy-2**, Aldrich; purity 98%), 4,4'-(1,4-phenylene)bipyridine (**Bipy-3**, TCI; purity >95%) were used without purification. Poly[(4-vinylphenol)-*alt*-C12MI] (**Alt-C12**) and poly[(4-vinylphenol)-*alt*-C18MI] (**Alt-C18**) were synthesized via free radical polymerization and subsequent deprotection reaction according to Chapter 4.

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

Turbidity measurement. Turbidity of the polymer solutions in toluene was measured on a UV-1800 (Shimadzu, optical path length = 10 mm, $\lambda = 670$ nm, heating/cooling rate: 1 °C min⁻¹, temperature range: 30–90 °C).

Rheology. Samples of 10wt% **Alt-C12** or **Alt-C18** solutions in 1,2,4-trichlorobenzene were placed at 90 °C in the gap of an Anton Paar Physica MCR 302 rheometer equipped with a 50 mm diameter cone-plate geometry placed under a solvent trap and operated in the standard oscillatory mode. Samples were first annealed for 10 minutes at 90 °C (**Alt-C12**) or 60 °C (**Alt-C18**), then cooled down to the desired temperature and equilibrated for 10 minutes. First, 1 rad s⁻¹ oscillations were applied in the 0.01 to 10% strain range. Frequency sweeps were then recorded in the linear viscoelastic regime (typically 1% deformations) in the same isothermal conditions using $\omega = 100$ to 0.01 rad s⁻¹ oscillations. Samples were annealed again at 90 °C (**Alt-C12**) or 50 °C (**Alt-C18**) and equilibrated as described above between

measurements at two different temperatures.

Cryo-transmission electron microscopy (Cryo-TEM). The morphologies of organogels were examined using a LaB6 JEOL JEM 2100 transmission electron microscope operated at 200 kV with a low-dose system (minimum dose system, MDS). The gels were heated at 90 °C to make fluid solutions just before use. Then, they were spread on a Quantifoil holey-carbon-coated grid (Quantifoil Micro Tools) and quickly frozen by plunging the grid into liquid ethane. Images were recorded with an Ultrascan 2k × 2k CCD Gatan camera. Dimensions were measured on cryo-TEM images using the ImageJ software.

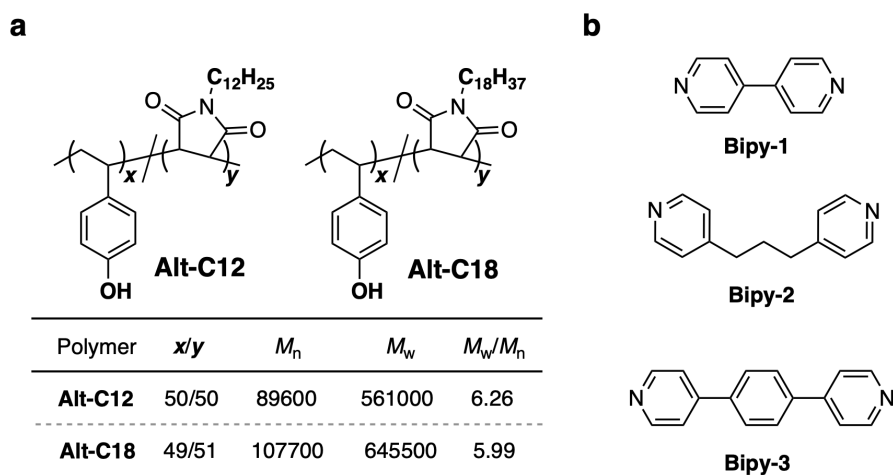


Figure 2. (a) Polymer samples (**Alt-C12** and **Alt-C18**) and (b) bipyridines (**Bipy-1**, **-2** and **-3**) in this work.

Preparation of Organogel with Bipyridines

In a vial, the freeze-dried **Alt-C12** (200 mg, 0.52 mmol of repeating units) and 1/4 molar amount of **Bipy-1** (20.3 mg, 0.13 mmol) with respect to the amount of phenol groups in the vial (the amount of pyridine units is therefore 1/2 equivalent with respect to phenol groups in the gel) were dissolved in 2mL of an aromatic solvent at 80–90 °C using a heat gun (10wt% solution). Then, the solution was cooled at ambient temperature to form a turbid gel after a few minutes.

Result and Discussion

Sample Preparation

The alternating copolymers (**Alt-C12**, **Alt-C18**) bearing long alkyl chain (C12 or C18)

and phenol pendant were synthesized via free radical copolymerization of *N*-alkyl maleimides (C12MI or C18MI) and *tert*-butoxystyrene and subsequent deprotection reactions according to Chapter 4. The three types of bipyridine (**Bipy-1**, **-2** and **-3**) were purchased. The information about molecular weights of the copolymers and chemical structures of the bipyridines is shown in Figure 2.

Gelation Behaviors after addition of Bipyridine

All gel samples are prepared as follows.: In a vial, copolymer, bipyridine, and solvent were mixed well with heating and the homogeneous solution was cooled at 25 °C (Figure 3a). As shown in Chapter 4, the **Alt-C12** and **Alt-C18** gels were sensitive to amount of mono-pyridine and 1 molar amount of mono-pyridine respect to the phenol units in the gels was necessary to break the gels (Figure 3b). However, when 1/2 molar amount of **Bipy-1** (1 molar amount of pyridine units) was added to the **Alt-C12** gel, the mixture after reheating and cooling was still gel state (Figure 3c). From this comparison, **Bipy-1** was found to interact with phenol units to behave as a new linker of the gel network. Further, I investigated the sensitivity of the amount of **Bipy-1** on the gelation behaviors (at 1/8, 1/4, 1/2, or 1 molar amount). At each after addition of **Bipy-1**, the mixtures were reheated and cooled to ambient temperature (Figure 3d). As the amount of **Bipy-1** was increased, the gel was gradually weaker and chewier, while the original gel without bipyridine was strong and brittle. When 1 molar amount of **Bipy-1** (two times amount of pyridine units respect to the phenol units in the gel) was added to the gel, the gel was broken to become a fluid solution. From these results, the gel was found to be sensitive to the amount of bipyridine and excess bipyridine were needed to break the gel.

Next, effects of bipyridine structures on the gelation behaviors such as gelation speed and appearance after addition of each bipyridine (**Bipy-1**, **-2** or **-3**) were investigated (Figure 4). Here, 1/4 molar amount of each bipyridine was added to 10wt% polymer solution in TCB with heating and the mixture was cooled at 25 °C for two days to prepare six samples. The gelation behaviors depended on bipyridine and polymer structures. At **Alt-C12** samples, all three solutions after addition of each bipyridine became turbid gels just after cooling at ambient temperature and the sol-gel transitions were fast (~3 min). Interestingly, the gels with **Bipy-1** or **-3** became chewier than original one which is brittle, while the gels with **Bipy-2** was not chewy. On the contrary, at **Alt-C18** solutions, it took more time to gelate than **Alt-C12** ones. All three **Alt-C18** solutions after addition of each bipyridine were still liquid just after cooling. However, one day later, the **Alt-C18** solutions with **Bipy-1** or **-3** became weak gels while the

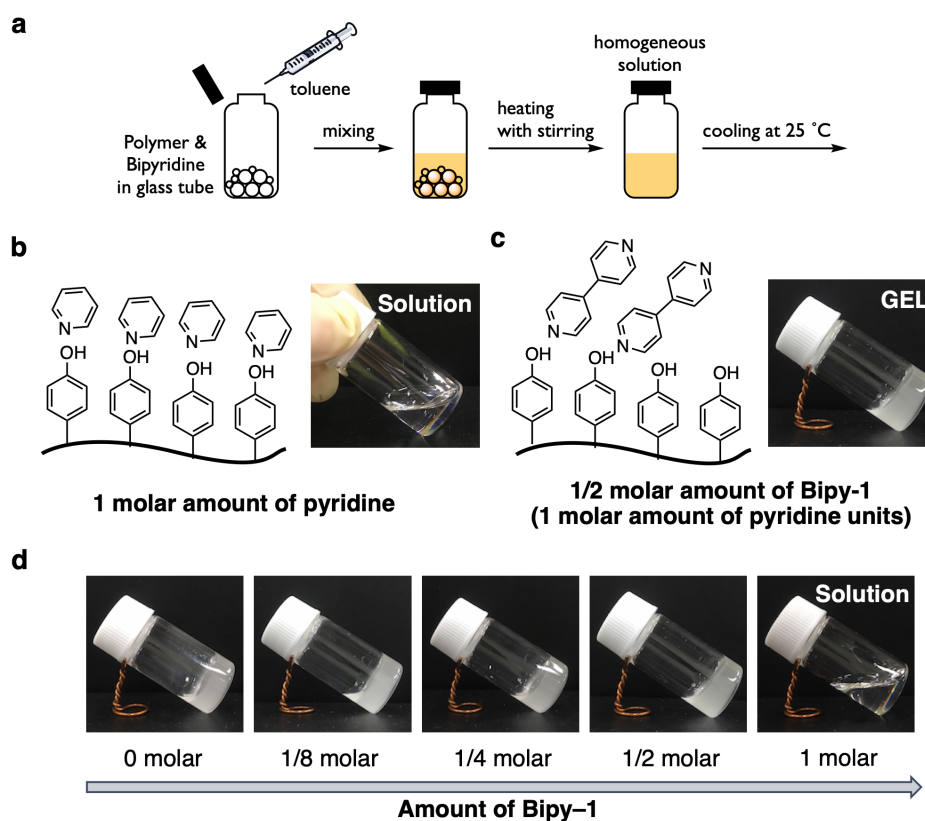


Figure 3. (a) How to make organogel. Pictures of the 5wt% **Alt-C12** gels in TCB after addition of (b) 1 molar amount of pyridine, (c) 1/2 molar amount of **Bipy-1**, and (d) each amount of **Bipy-1** (0, 1/8, 1/4, 1/2, or 1 molar amount) respect to the phenol units in the gel.

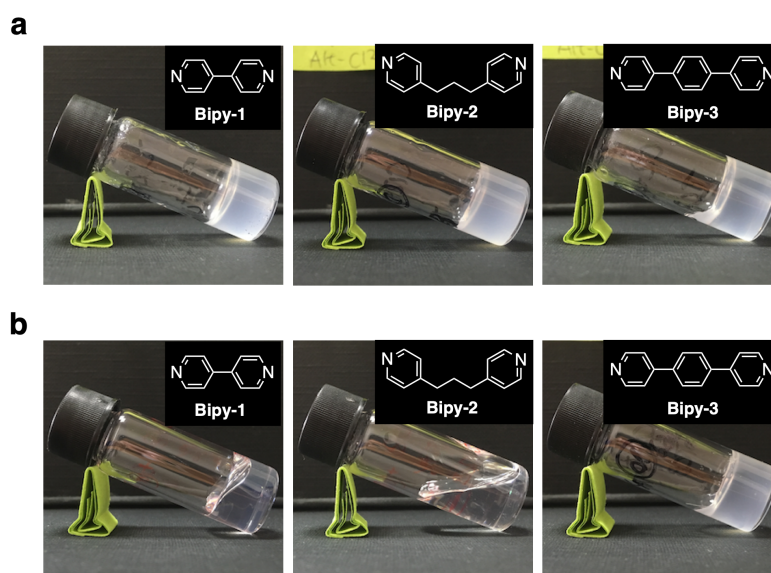


Figure 4. The pictures of 10wt% solution in TCB of (a) **Alt-C12** and (b) **Alt-C18** after addition of 1/4 molar amount of **Bipy-1**, **-2** and **-3** with respect to the molar amount of phenol units in the solutions (after cooling for two days).

solution with **Bipy-2** was still high viscous liquids. Further, only two **Alt-C18** samples with **Bipy-1** or **-2** were transparent. From these results, it is assumed that bipyridines broke hydrogen bonds between phenol units as well as formed bigger spaces in the gel network by interacting with phenol units as new linkers to result the weaker or chewier gels. The gelation behaviors are depended on bipyridine and polymer structure, which are likely due to the compatibility of both structures.

Rheology Measurement of the Gel after Addition of Bipyridines

To further investigate the effect of the amount of bipyridine on the gel properties, variable temperature rheology measurements (ω - and γ -sweeps) were performed with the 10wt% **Alt-C12** gels in TCB after addition of **Bipy-1** (1/8, 1/4 or 1/2 molar amount) or mono-pyridine (1/2 molar amount) at 25, 50 and 70 °C (Detailed rheology data at each amount of **Bipy-1**, at each temperature is shown below of this chapter in Figure S1). Figure 5ab and 5cd showed the rheology data of the **Alt-C12** gel with 1/4 molar amount of **Bipy-1** at each temperature and with each amount of **Bipy-1** at 25 °C, respectively. ω -Sweeps measurements revealed that both G' and G'' were decreased as the amount of **Bipy-1** or the temperature was increased (Figure 5a and 5c). This result was almost corresponded to the gelation behaviors described above. In Figure 5ef, the two rheology data were compared with the one of original gel at 25 °C: the rheology of the gels after addition of 1/4 molar amount of **Bipy-1** and 1/2 molar amount of mono-pyridine (Both of the gels contain the same molar amount of pyridine units). The decreases of G' and G'' by **Bipy-1** were smaller than those by mono-pyridine (Figure 5e) at high frequency region. However, the decreases by **Bipy-1** were not small despite the bipyridine is expected to behave as a new linker of the gel. The big decreases are likely due to both the dynamic interaction between bipyridine and phenol units and increasing of space size of gel network by insertion of bipyridines.

At γ -sweeps measurements, interesting behaviors were observed. Normally, both G' and G'' of the original **Alt-C12** gel without bipyridine were started to decrease at around $\gamma = 3$ at 25 °C (Figure 5d). However, as the amount of **Bipy-1** or temperature was increased, the flat region of both G' and G'' were expanded (Figure 5bd). This phenomenon was not observed at the gel with 1/2 molar amount of mono-pyridine (Figure 5f). It implies that **Bipy-1** as a new linker healed the gel by dynamic interaction with phenol units just after breaking of the gel by the γ -sweeps measurement and the toughness was enhanced as the temperature was increased because the interaction between phenol and pyridine units is

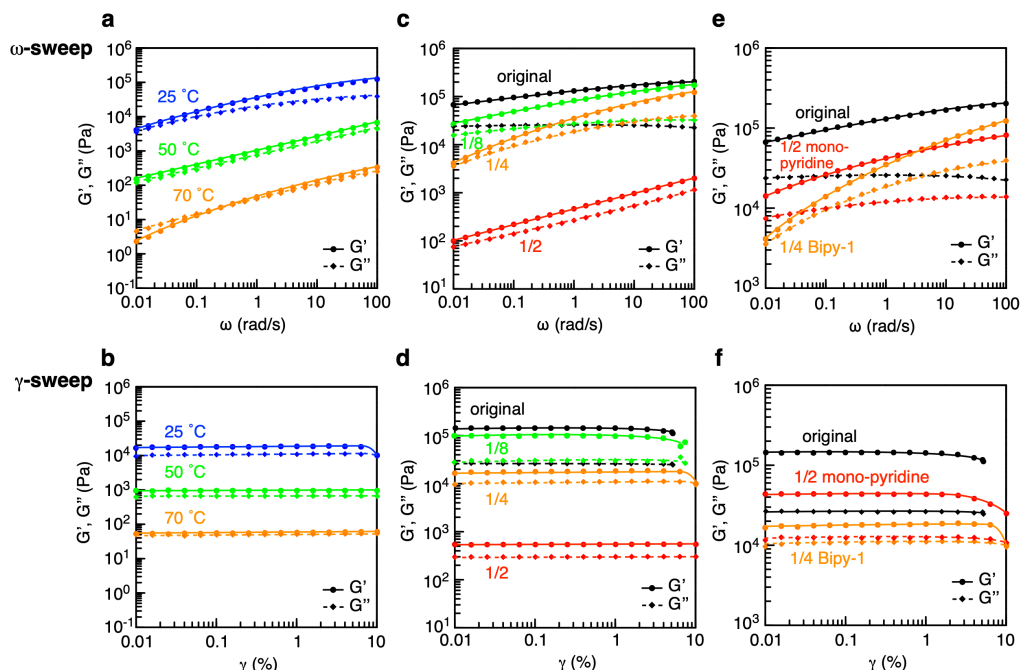


Figure 5. Rheology data of 10wt% **Alt-C12** gel in TCB after addition of (a and b) 1/4 molar amount of **Bipy-1** at each temperature (25, 50 and 70 °C), (c and d) each molar amount of **Bipy-1** (0, 1/8, 1/4 or 1/2) at 25 °C, and (e and f) 1/4 molar amount of **Bipy-1** or 1/2 molar amount of mono-pyridine (mono) at 25 °C.

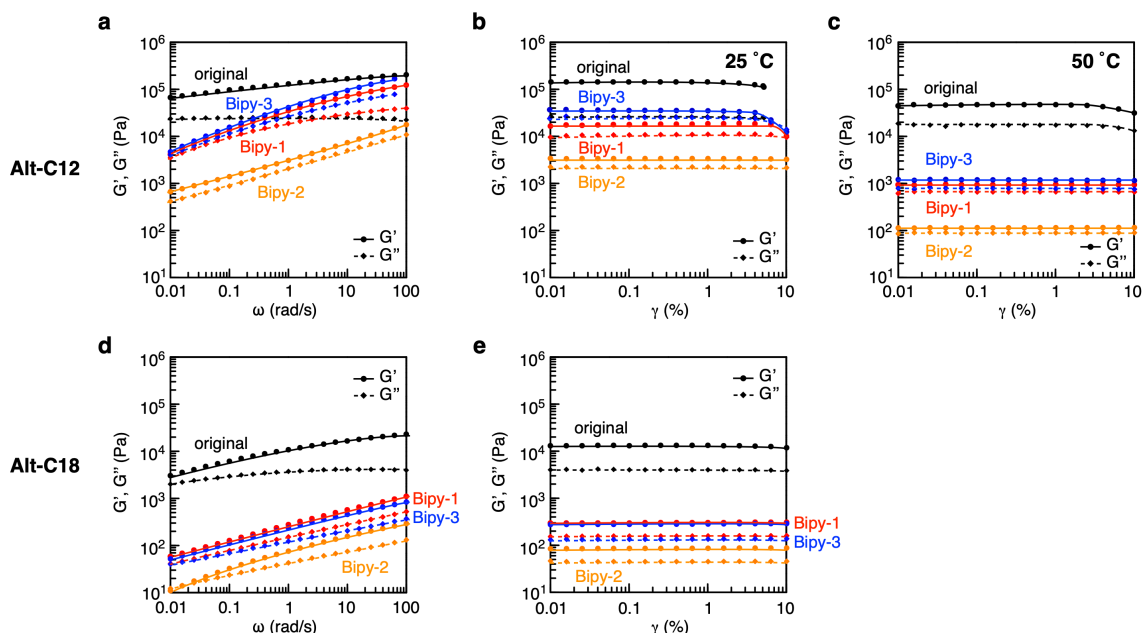


Figure 6. ω - and γ -sweeps measurements of 10wt% gels in TCB after addition of 1/4 molar amount of **Bipy-1**, -2, or -3: (a and b) **Alt-C12** at 25 °C, (c) **Alt-C12** at 50 °C and (d and e) **Alt-C18** at 25 °C.

equilibrium interaction. At the **Alt-C18** samples, decreasing of G' and G'' depends on the amount of **Bipy-1** were also observed, but increasing of gel toughness was not due to that the original **Alt-C18** gel was tough enough to tolerant whole γ region of the γ -sweep measurement (Detailed data is shown below of this chapter as Figure S2).

The author also investigated the effect of spacer structures of bipyridines on the gel rheology. Variable temperature rheology measurements (ω - and γ -sweeps) were conducted with the 10wt% **Alt-C12** or **Alt-C18** gels in TCB after addition of 1/4 molar amount of **Bipy-1**, **-2**, or **-3** (Figure 6). Figure 6a shows the comparison of bipyridine structures at isothermal ω -sweeps measurements for the **Alt-C12** gels at 25 °C. After addition of each bipyridine, all G' and G'' of the gels were decreased and the G' values of the gels after addition of bipyridines depended on the bipyridine structures. For example, when **Bipy-2** bearing relatively flexible three methylene groups between two pyridine units was used, the decrease of G' was biggest among three bipyridines. On the contrary, when **Bipy-3** was used, the decrease of G' was smallest likely owing to the rigid structure containing a benzene ring between two pyridine units. The gel with **Bipy-1** was slightly weaker than that with **Bipy-3**. At γ -sweeps measurements of **Alt-C12** gels at 25 °C (Figure 6b), only **Bipy-2** made the flat region of both G' and G'' expanded, while **Bipy-1** and **-3** did not. However, as the temperature was increased, the flat regions after addition of **Bipy-1** or **-3** were elongated over $\gamma = 10\%$ (Figure 6c). Almost the same tendencies were observed at the **Alt-C18** gels (Figure 6d and 6e). From these results, all three bipyridines were found to make the gels weaker but increased toughness of the gels. Further, the elastic modulus of the gels depended on the sizes and rigidities of bipyridines. Namely, macroscopic gel properties reflecting bipyridine structures were observed.

Calculation of Phenol–Pyridine Interaction

To predict how much bipyridine interacted with phenol as linkers in the gels, the ratios of the phenol units interacted with mono-pyridine in 10wt% **Alt-C12** gel after addition of each amount of mono-pyridine (1/4, 1/2, 1, 2 molar amount) were calculated (Table 1). Herein, the equilibrium constant of *p*-methylphenol and mono-pyridine ($K = 42$ at 20 °C)¹⁴ is used for the calculation. When 1 molar amount of mono-pyridine is used, 74% of phenol units was found to interact with mono-pyridine. Considering that 1 molar amount of mono-pyridine is

Table 1. Amount of Interaction between Phenol (Phe.) and Mono-Pyridine (Pyr.) in 10wt% **Alt-C12** Gels ($[Phe.]_0 = 0.26$ M) after Addition of Each Amount of Mono-Pyridine (1/4, 1/2, 1 and 2 Molar Amount) Calculated by Using the Equilibrium Constant of the Interaction between *p*-Methylphenol and Mono-Pyridine ($K = 42$).

Amount of Pyr., molar amount	$[Pyr.]_0$, M	Ratio of Phe. interacting with Pyr., %	$[Phe. - Pyr.]$ Interaction, M
1/4	0.065	22	0.06
1/2	0.13	43	0.11
1	0.26	74	0.19
2	0.52	92	0.24

necessary to break the gels, it was found that 74% of phenol units have to interact with pyridine units to break the gel. Here, when 1 molar amount of bipyridines (2 molar amounts of mono-pyridine units) is used, the gel is broken (See above) and 92% of phenol units is found to interact with pyridine units at the time. It means that more than 74% of phenol units would contribute to interact with only one pyridine unit of bipyridines and less than 18% of phenol units ($92 - 74 = 18\%$) would do to make new linkers with bipyridines. From the calculation, one of the reasons of the big decreases of G' and G'' is likely due to that most of phenol units ($74\% <$) interacted only with the one side of bipyridines to make the gel weaker and only less than 18% of phenol would contribute to make linkers with bipyridines.

Detailed Study on the Structure of Organogels after Addition of Bipyridine

To investigate the interaction between bipyridine and phenol units of the gel, the solution of 5wt% **Alt-C12** or **Alt-C18** in toluene- d_8 with 1/4 molar amount of **Bipy-1** was prepared at 90 °C for variable temperature 1H NMR measurements (Figure 7). Before the addition of **Bipy-1**, there is no peak from aromatic protons of phenol units at around 7 ppm and only the broad peak from alkyl chain was observed at 1-3 ppm at 25 °C, but as the temperature was increased, the alkyl peak became shaper and bigger and the aromatic peak was observed at 60 and 80 °C (See Chapter 4). The same trend was also observed after addition of **Bipy-1**.

The intensity and position of the peaks from **Bipy-1** also changed by temperature. At **Alt-C18** sample in Figure 7a, a broad and small peak derived from the aromatic protons (*f*) neighboring to nitrogen atom of **Bipy-1** was observed at around 8.4-8.7 ppm at 25 °C. The

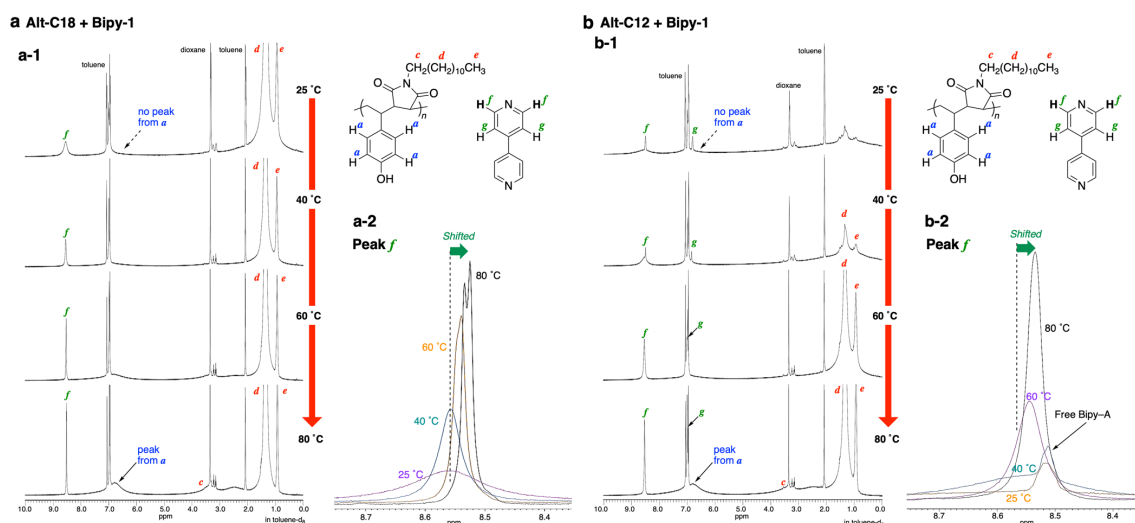


Figure 7. ^1H NMR spectra of 5wt% (a) **Alt-C18** and (b) **Alt-C12** solution in toluene- d_8 after addition of 1/4 molar amount of **Bipy-1**.

broad peak is supposed to be derived from the pyridine units interacting with phenol in the phenol core of the gels because **Bipy-1** in the core should be hidden and freezed by the alkyl shell and the gel network, respectively. The peak position was also supporting this prediction: **Bipy-1** get hydrogen from phenol to interact each other, which make the peak of **Bipy-1** shifted to lower magnetic field compared to the one derived from free **Bipy-1**. As the temperature was increased, the broad peak became bigger and sharper. From the variable temperature ^1H NMR measurements, it is assumed that almost all of **Bipy-1** interacted with phenol units inside the core of the gel at 25 °C and the equilibrium was shifted to increase the amount of free **Bipy-1** as the temperature was increased above 40 °C. At the **Alt-C12** gel, the peak from free **Bipy-1** was appeared at 25 and 40 °C, but the almost same tendency was observed (Figure 7b).

Finally, cryo-TEM images of the 5wt% **Alt-C12** or **Alt-C18** solutions in cumene after addition of 1/4 molar amount of each bipyridine (**Bipy-1**, **-2**, and **-3**) are shown in Figure 8. The structures with entangled fibrils like a felt were observed at both **Alt-C12** and **Alt-C18** original gels without bipyridine. However, the gel structures changed dramatically each after addition of bipyridines: mixture of fibril and large size structures were observed. The size of each fibril (length and diameter) was found to depend on the bipyridine structures through the measurement by imageJ software (Table 2). For example, the **Alt-C18** gel with **Bipy-1** had long fibrils (~1000 nm) but the gels with **Bipy-2** or **-3** had short ones (~200 nm). In the cases of **Alt-C12**, such long fibril was not observed, but the averaged diameters of the observed fibrils increased after any addition of bipyridines, and the order of the fibrillar sizes was **1**<**2**<**3**.

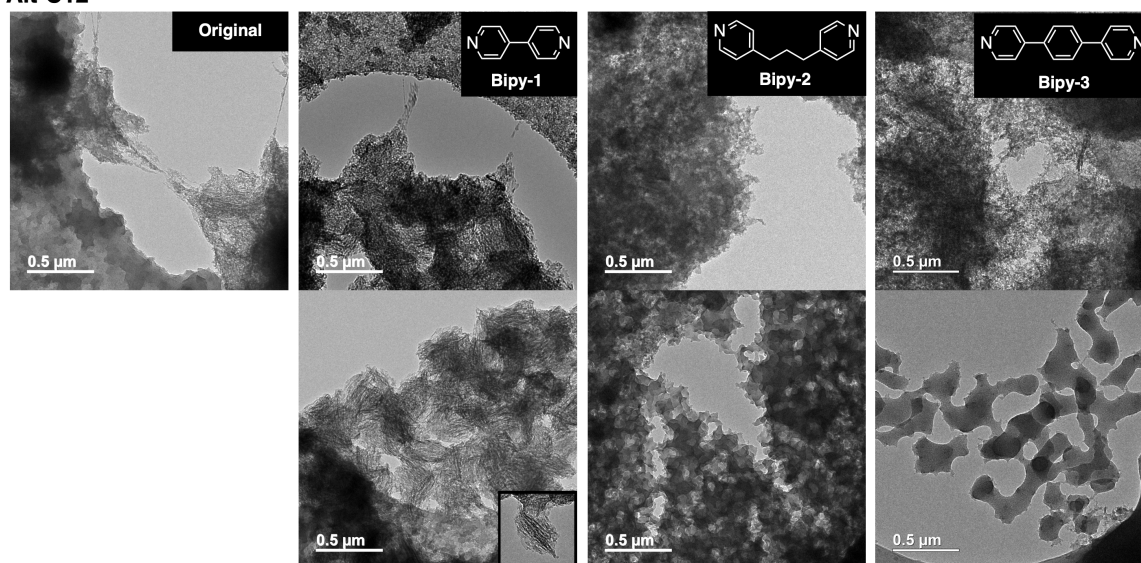
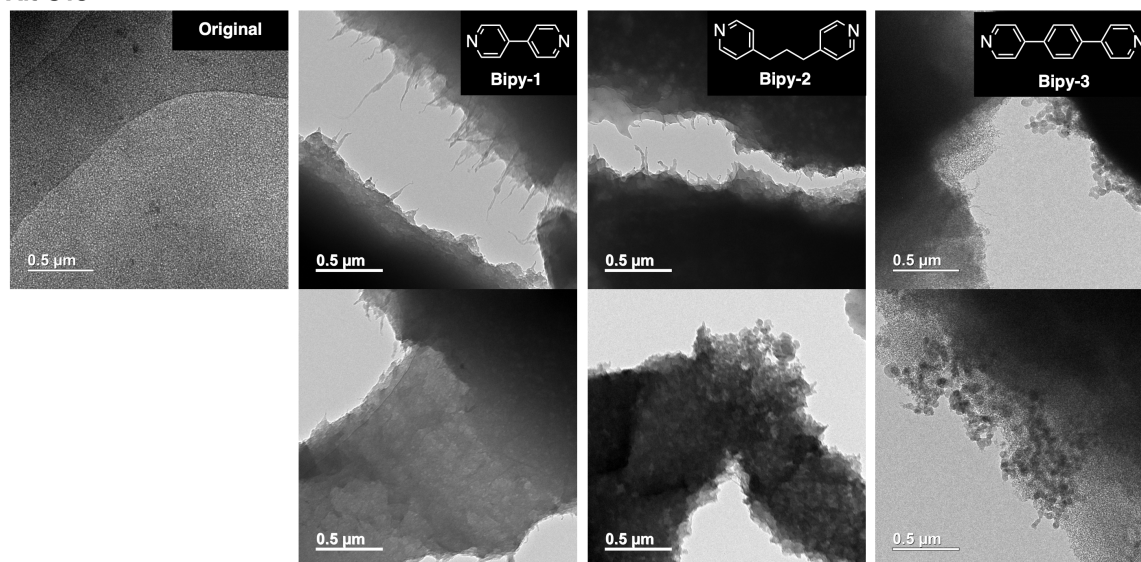
a Alt-C12**b Alt-C18**

Figure 8. Cryo-TEM images of 5wt% (a) **Alt-C12** or (b) **Alt-C18** solution in cumene after addition of 1/4 molar amount of each bipyridine (**Bipy-1**, **-2** and **-3**).

From this result, the fibrillar diameters are supposed to reflecting the spacer of bipyridines such as a trimethylene group in **Bipy-2** and a benzene ring of **Bipy-3**. However, **Alt-C18** did not show such a tendency to increase in diameter, which is likely due to the compatibility of polymer structure and bipyridines. Moreover, interestingly, several unique large size structures were observed in addition to fibrillar structure. For example, the **Alt-C12** gel with **Bipy-1** showed the structure like overlapping leaves with black veins. The leaf-like structure where fibrils were bundled in one direction are expected to have some crystal structure due to

Table 2. The Length of Fibrillar Nanostructure after Addition of 1/4 Molar Amount of Bipyridine (**Bipy-1**, **-2**, and **-3**) Measured by ImageJ Software.

Polymer Sample	Bipyridine	Averaged Fibrillar Diameter, nm
Alt-C12	–	6.0
	Bipy-1	8.5
	Bipy-2	9.0
	Bipy-3	12.7
Alt-C18	–	5.2
	Bipy-1	8.9
	Bipy-2	11.1
	Bipy-3	7.5

the regularity. Further, **Alt-C12** and **Alt-C18** gels with **Bipy-2** or **Bipy-3** formed bicontinuous phase. Further investigations by SANS or SAXS will reveal the detailed gel structures.

Conclusion

The effects of bipyridines as additives on the gelation behavior/property of the alternating copolymers bearing long alkyl and phenol pendants in aromatic solvents were investigated. The bipyridines interacted with phenol units in the core of the gel to break the hydrogen bondings between phenol units as well as to behave as cross-linkers. The equilibrium cross-linking between pyridine and phenol units increased gel toughness, which was enhanced as the temperature increased. However, due to the dynamic interaction between phenol and pyridine units, hardness of the resultant gel was decreased. Cryo-TEM measurements revealed unique gel nanostructures depending on bipyridine structures such as leaf-like structure, bicontinuous phase, and longer and thicker entangled fibrils. Particularly, the averaged fibrillar diameters were reflected by the spacer structures of bipyridines. Future investigations such as SANS analyses will help more detailed analysis of the gel structures.

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Supporting Rheology Data

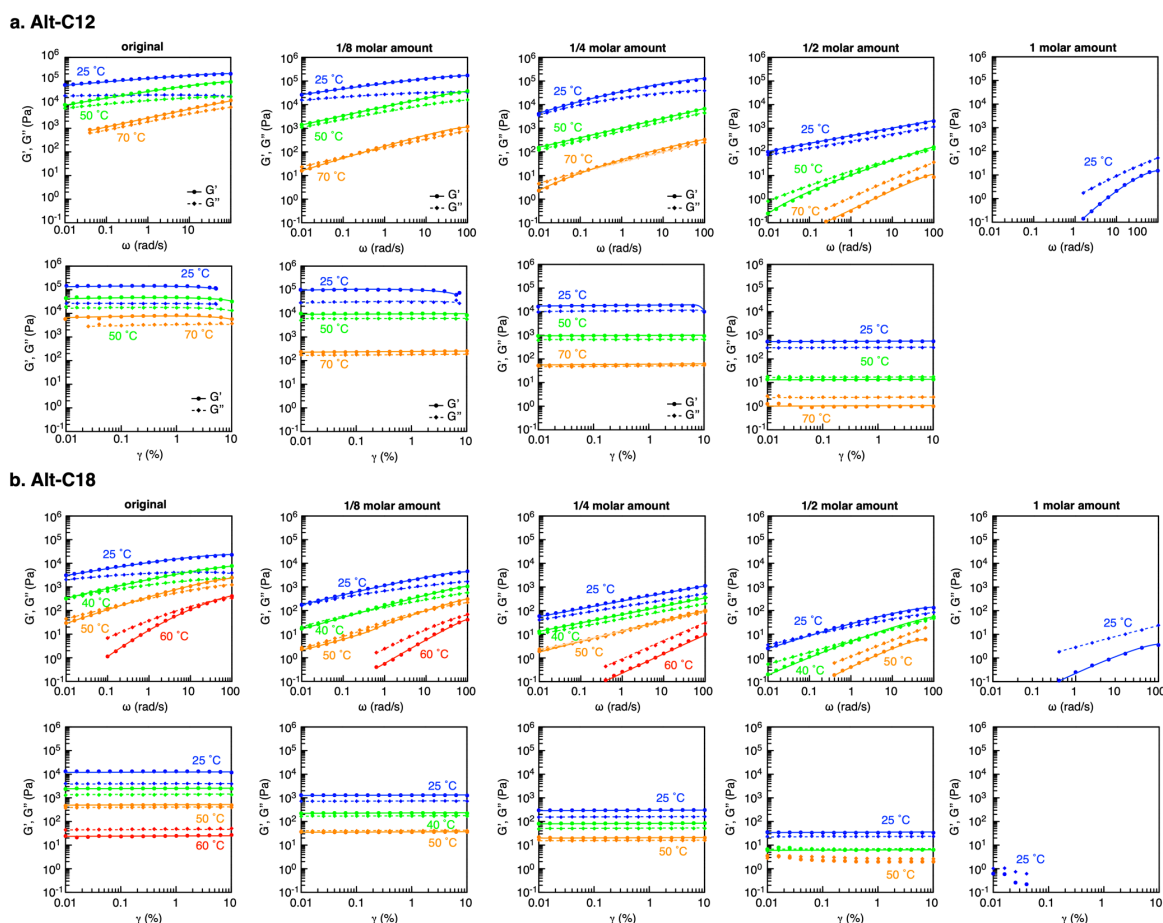
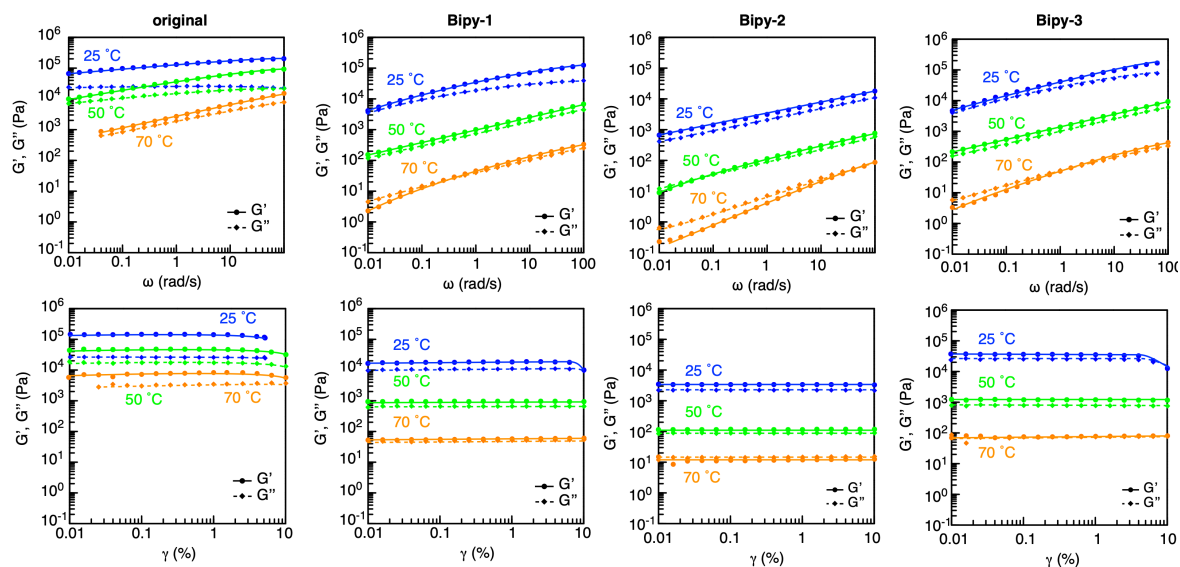


Figure S1. ω - and γ -sweeps measurements of 10wt% gels of (a) Alt-C12 or (b) Alt-C18 in TCB after addition of each amount of **Bipy-1** (0, 1/8, 1/4, 1/2, and 1 molar amount) at each temperature.

a. Alt-C12



b. Alt-C18

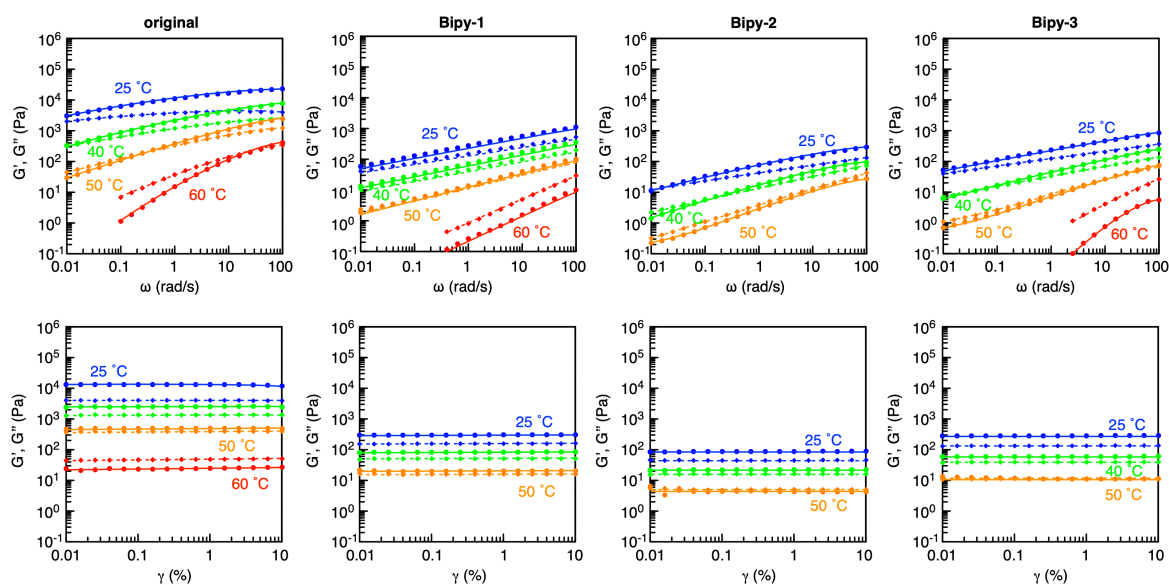


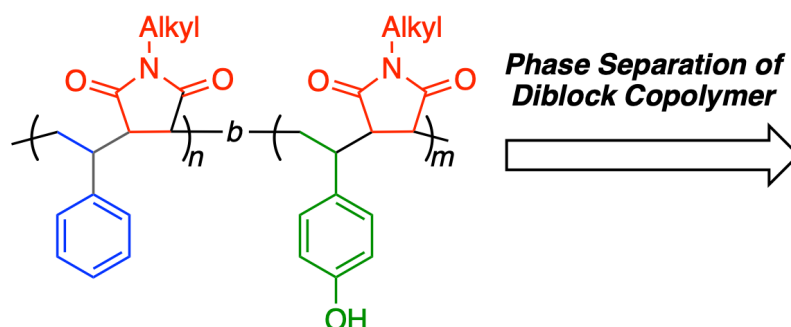
Figure S2. ω - and γ -sweeps measurements of 10wt% gels of (a) **Alt-C12** or (b) **Alt-C18** in TCB after addition of 1/4 molar amount of each bipyridine (**Bipy-1**, **Bipy-2**, and **Bipy-3**) at each temperature.

Chapter 6

Syntheses of Alternating Copolymer-Based Diblock Copolymers Toward Novel Phase Separation Behaviors

Abstract

Alternating copolymer-based diblock copolymers, in which an alternating copolymer of styrene/*N*-alkyl maleimide (MI_{alkyl}) and that of 4-vinylphenol/MI_{alkyl} are connected, were synthesized via sequential RAFT copolymerizations and the phase separation behaviors in the bulk state were investigated. Alkyl length of the copolymer affected the phase separation: the diblock copolymer with a short alkyl chain (C2) maleimide induced phase separation derived from segregation of the two blocks. On the contrary, other diblock copolymers with longer alkyl chains (C12 and C18) maleimide did not enable such phase separations. Probably, in these cases, the two blocks became more miscible due to the strong aggregation force of the long alkyl chains.



Introduction

Several types of copolymers consisting of two comonomers have been synthesized according to the monomer sequence, such as block, random, gradient, and alternating. Among them, an AB-diblock copolymer is interesting for material applications because it affords some types of nano-ordered structures such as sphere, lamella, and cylinder depending on the block combination and the ratio in solution or bulk via phase separation.¹ Herein, the self-assembly occurs for decrease in surface energy between the immiscible segments. When the block increases by one to be a ABC-triblock copolymer, the number of possible ordered structures expands dramatically, because the compatibility balance among three segments becomes more complicated.²

In radical copolymerization, the particular combination of electron-rich comonomer (e.g., styrene and vinyl ether) with electron-poor one (e.g., maleic anhydride and maleimide) is known to give alternating copolymers. Such alternating copolymers may also give ordered architectures via self-assembly depending on the design of the units or the pendant groups. Indeed, some papers have been reported on formations of vesicle,³ flower micelle,^{4,5} and fibrillar structure⁶ from alternating copolymers in the appropriate solution. For example, Co et al. introduced hydrophilic sugar and hydrophobic alkyl chain in the side chain of vinyl ether-maleimide alternating copolymer to prepare the vesicle in water.³ However, to my knowledge, the self-assemblies rely on solution, and few studies on phase separation in bulk state have been known.⁷

Given by these backgrounds, the author synthesized alternating copolymer-based block copolymers toward formations of novel ordered structures via phase separation in bulk. For this purpose, she focused her attention on a pair of styrene and maleimide derivatives in radical copolymerization, because the side chains of the comonomers are designable and the reversible deactivation radical polymerization (RDRP)⁸ would be available for syntheses of the desired block copolymers. In principle, it is possible to prepare several types of diblock copolymers such as $(AB)_n-(CD)_m$ or $(AB)_n-(AC)_m$ (A–D: comonomers) by changing the combination of the comonomer pairs. In this chapter, alkyl maleimide (MI_{alkyl}) derivatives were combined with styrene (ST) or *tert*-butoxy styrene (TBOS) in RAFT polymerization to synthesize $(AB)_n-(AC)_m$ -type block copolymers (Figure 1a). TBOS was used to convert into 4-vinylphenol (VP) unit via the acidolysis after polymerization for introduction of higher polarity units. Such diblock copolymers consisting of three comonomers are expected to give some nano-objectives via phase separation which are different from the linear and miktoarm triblock

copolymers (Figure 1b). The common MI_{alkyl} unit (A) in the two segments may work to increase the compatibility, but the ST (B) and VP (C) units are enough immiscible to each other because phase separation of the block copolymer of ST and VP easily occurs.⁹ In addition, we have recently reported alternating copolymer of MI_{alkyl} with VP causes the phase separation in aromatic solvent, and thus A and C units would be immiscible enough.⁶ Thus, unique segregation among the three units is expected in thus-designed alternating copolymer-based block copolymers.

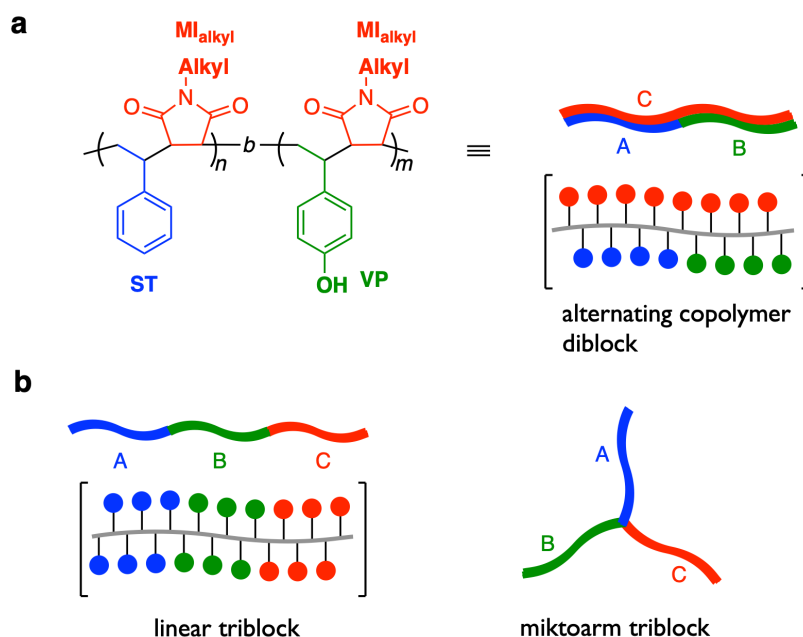


Figure 1. (a) Alternating copolymer-based diblock copolymer; $-(AC)_n-b-(BC)_m-$, (b) ABC-linear triblock copolymer, and (c) ABC-miktoarm triblock copolymer.

Experimental

Materials

Styrene (St, TCI; purity >99%), and 4-*tert*-butoxystyrene (tBOS, Sigma Aldrich; purity 99%) were passed through inhibitor remover (Sigma Aldrich) before use. HCl aq. (Wako; 35-37%), *N*-ethyl maleimide (MI_{C2} , TCI; purity >98%), 4-cyano-4-[(dodecylsulfanyl thiocarbonyl)sulfanyl]pentanol (CTA, Aldrich; purity >96.5%), and 2,2-azobisisobutyronitrile (AIBN, Wako; purity >98%) were used without purification. Toluene (Wako; purity >99.5%) was dried by Molecular sieves 4A before use. 1,2,3,4-Tetrahydronaphthalene (tetralin, Kisida Chemical; purity >98%), an internal standard in 1H NMR, was dried over calcium chloride

overnight and distilled twice over calcium hydride. *N*-dodecyl maleimide (MI_{C12}) and *N*-octadecyl maleimide (MI_{C18}) were synthesized according to Chapter 4.

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

SEC (DMF). Molecular weight distribution (MWD) curves, number-average molecular weight (M_n) and M_w/M_n ratio of the polymers were measured by size exclusion chromatography (SEC) in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L; exclusion limit = 4×10^6 g/mol; particle size = 10 μm; pore size < 5000 Å; 0.8 cm i.d. × 30 cm) that were connected to a Jasco PU-2080 precision pump, a Jasco RI-2031 refractive index detector and a Jasco UV-2075 UV/vis detector set at 270 nm. The columns were calibrated against 11 standard PMMA samples (PSS, Polymer Standard Service GmbH, Ready Cal Kit: M_p = 2,380 – 2,200,000).

SEC (THF). MWD curves, M_n and M_w/M_n ratio of the polymers were also measured by SEC in THF as an eluent at 40 °C on three polystyrene gel columns (Shodex LF-404; exclusion limit = 2×10^6 g/mol; particle size = 6 μm; pore size, < 3000 Å; 0.46 cm i.d. 25 cm; flow rate 0.3 mL/min) connected to a DU-H2000 pump, a 74S-RI refractive index detector, and a 41-UV ultraviolet detector (all from Shodex; Shodex GPC-104). The columns were calibrated against 12 standard polystyrene samples (TOSOH, PS-Oligomer Kit, M_w = 500–1,110,000; M_w/M_n = 1.01–1.16).

DSC. Differential scanning calorimetry of polymers (ca. 2.0 mg weighed into an aluminum pan) was performed on a DSC Q200 calorimeter (TA instruments) equipped with a RCS 90 electric freezing machine under dry nitrogen flow (heating or cooling rate = 10 °C/min).

Synthesis of Diblock Copolymers

Polymerization was carried out by the syringe technique under dry argon in glass tubes equipped with three-way stopcocks. Typical procedure for synthesis of diblock copolymer Copoly(ST–MI_{C2})-*b*-Copoly(VP–MI_{C2}) (**P–C2**) was as follows.

Synthesis of 1st Block. To a Schlenk tube, MI_{C2} (625 mg, 5.0 mmol), St (0.572 mL, 5.0 mmol), 3.95 mL of toluene, 0.1 mL of tetralin, CTA (0.278 mL of 90 mM solution in toluene, 0.025 mmol), and AIBN (0.1 mL of 50 mM toluene solution, 0.005 mmol) were added (total volume: 5.0 mL, $[St]_0/[EMI]_0/[CTA]_0/[AIBN]_0$ = 1000/1000/5/1 mM in toluene).

Immediately after mixing, the tube was placed in an oil bath at 60 °C. After 6 h, the solution was bubbled with air for termination of the copolymerization. Monomer conversions were determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomers with tetralin as an internal standard. The resultant copolymer [Copoly(ST-MI_{C2}), conversion: St/EMI = 88/89% after 6 h] was purified by reprecipitation in methanol to remove unreacted monomers.: $M_n = 29800$, $M_w/M_n = 1.17$ (from SEC in THF calibrated by PSt, after purification), composition ratio St/EMI = 49/51 (from ^1H NMR, after purification).

Synthesis of 2nd Block. To a Schlenk tube, MI_{C2} (250 mg, 2.0 mmol), tBOS (0.376 mL, 2.0 mmol), 1.55 mL of toluene, 0.04 mL of tetralin, macroCTA [Copoly(ST-MI_{C2}), 400 mg], and AIBN (0.04 mL of 50 mM toluene solution, 0.002 mmol) were added (total volume: 2.0 mL, [tBOS]₀/[EMI]₀/[macroCTA]₀/[AIBN]₀ = 1000/1000/5/1 mM in toluene). Immediately after mixing, the tube was placed in an oil bath at 60 °C. After 5 h, the solution was bubbled with air for termination of the copolymerization. Monomer conversions were determined by ^1H NMR from the integrated peak area of the olefinic protons of the monomers with tetralin as an internal standard. The resultant copolymer [Copoly(ST-MI_{C2})-*b*-Copoly(tBOS-MI_{C2}), conversion: St/EMI = 96/98% after 5 h] was purified by reprecipitation in methanol to remove unreacted monomers.: $M_n = 59200$, $M_w/M_n = 1.26$ (from SEC in THF calibrated by PSt, after purification), composition ratio St/EMI = 51/49 (from ^1H NMR, after purification).

Deprotection reaction. In a flask, 586 mg of Copoly(ST-MI_{C2})-*b*-Copoly(tBOS-MI_{C2}) was dissolved in 21 mL of THF. Then, 4.2 mL of 37% HCl aq. was added slowly to the solution with stirring. The solution was heated at reflux with a condenser and stirring for 24 h. After cooling, the solution was added dropwise to 400 mL of water with stirring. The precipitate was filtered and washed by pure water several times until the pH of the filtered water became 7. Then, the filtered solid was dissolved in 1,4-dioxane, and the solution was freeze-dried to yield Copoly(ST-MI_{C2})-*b*-Copoly(VP-MI_{C2}) (**P-C2**) as white solid (470 mg): $M_n = 79500$, $M_w/M_n = 1.49$ (from SEC in DMF). The progress of deprotection was confirmed by ^1H NMR.

Synthesis of Alternating Copolymers

Alternating copolymers for comparison were synthesized via free radical copolymerization according to the Chapter 4 and Chapter 7.

Thermal Annealing of Polymer Samples

All polymer samples were prepared by thermal annealing at 240 °C for 3 days under

vacuum, followed by quenched to room temperature.

Result and Discussion

Synthesis and Characterization of Diblock Copolymers

Three components-diblock copolymers consisting of two alternating sequence segments $[-(AC)_n-b-(BC)_m-]$ were synthesized by sequential addition of monomers via RAFT copolymerizations and followed by deprotection with acid (Figure 2a). Alternating copolymer of *N*-alkyl maleimide [MI_{alkyl} , alkyl = $-C_2H_5$ (C2), $-C_{12}H_{25}$ (C12), and $-C_{18}H_{37}$ (C18)] and styrene (ST) was synthesized with 4-cyano-4-[(dodecylsulfanyl

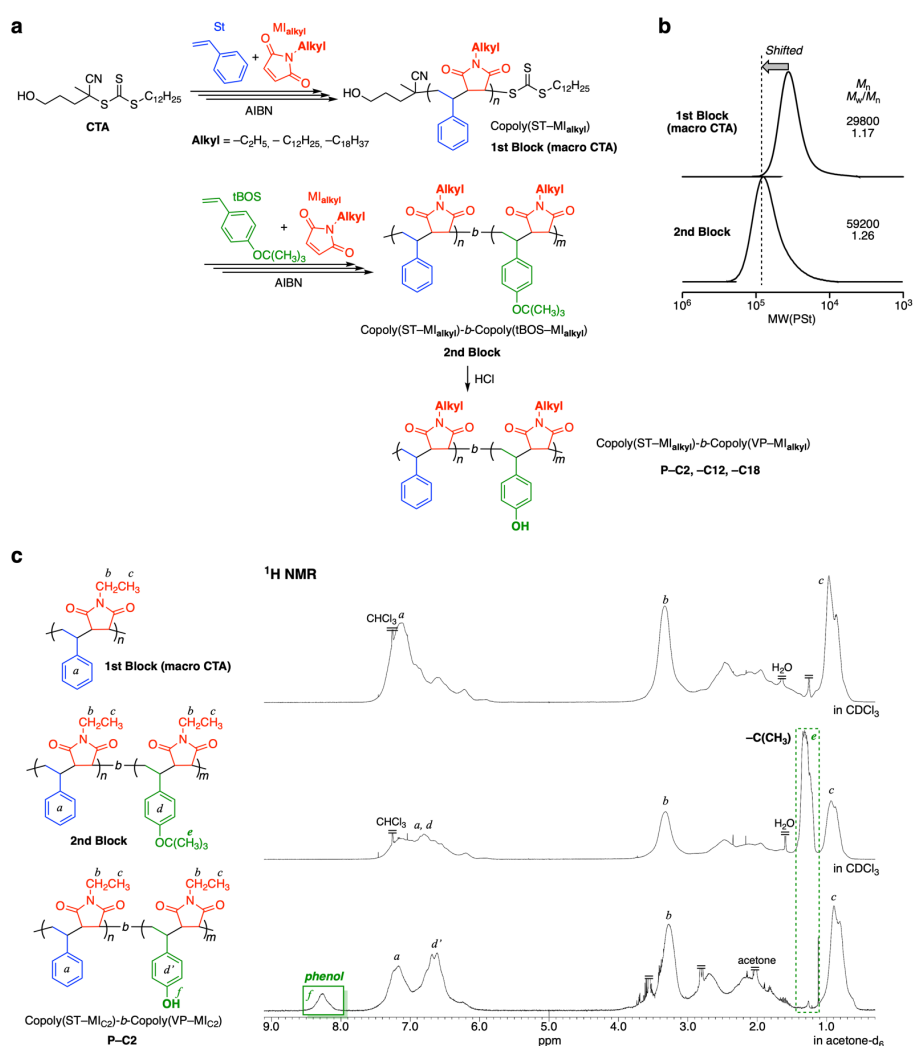


Figure 2. (a) Synthetic scheme of diblock copolymer of this work, (b) SEC curves of macro CTA and resultant copolymer with MI_{C2} , and (c) 1H NMR spectra of copolymers with MI_{C2} : macro CTA, resultant block copolymer, and the copolymer after deprotection reaction.

thiocarbonyl)sulfanyl]pentanol as a CTA via RAFT copolymerization and the resultant copolymer was purified by reprecipitation in methanol to subsequently use as a macro CTA for alternating copolymerization of MI_{alkyl} and *tert*-butoxystyrene (tBOS). At each RAFT copolymerization, both monomers were consumed at the same rate and the conversions of the monomers were reached over 90% in a few hours. The SEC curves shown in Figure 2b revealed a shift of peak top of block copolymer with *N*-ethylmaleimide (MI_{C2}) from that of the macro CTA, indicating an increasing in molecular weight of the resultant block copolymer. Moreover, molecular weight distributions (M_w/M_n) of all the copolymers were narrow and the composition ratios were almost 1:1. Figure 2c shows ^1H NMR spectra before and after deprotection of the copolymer with MI_{C2} . From the spectra after each copolymerization, the composition ratios and the ratios of 1st and 2nd block lengths were determined by using three peaks (*a*, *c*, and *d*). After the copolymerization, the resultant copolymer was treated with HCl for deprotection of *tert*-butoxy groups giving rise to three components-diblock copolymer where a copolymer of ST and MI_{alkyl} and that of VP and MI_{alkyl} were connected. The

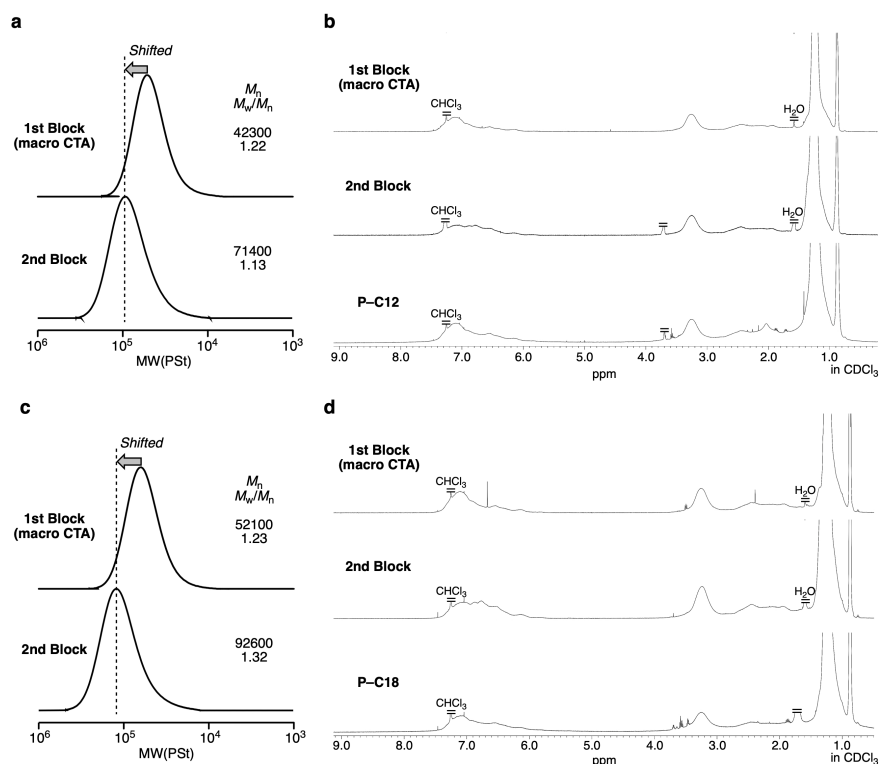


Figure 3. (a and c) SEC curves of macro CTA and resultant copolymer with MI_{C12} or MI_{C18} , and (b and d) ^1H NMR spectra of copolymers with MI_{C12} or MI_{C18} : macro CTA, resultant block copolymer, and the copolymer after deprotection reaction.

Table 1. Characterization of Copolymers after Deprotection Reaction^a

Sample Name	1st Block, <i>n</i> (St/MI _{alkyl}) ^b	2nd Block, <i>m</i> (VP/MI _{alkyl}) ^b	<i>n/m</i>	<i>M_n</i> ^c	<i>M_w/M_n</i> ^c
P–C2	176 (49/51)	224 (51/49)	44/56	79500 ^d	1.49 ^d
P–C12	158 (49/51)	196 (48/52)	45/55	75300	1.42
P–C18	162 (49/51)	193 (50/50)	46/54	90200	1.34

^a Copolymerization conditions: (1st block) [St]₀/[MI_{alkyl}]₀/[CTA]₀/[AIBN]₀ = 1000/1000/5/1 mM in toluene at 60 °C, and (2nd block) [tBOS]₀/[MI_{alkyl}]₀/[CTA]₀/[AIBN]₀ = 1000/1000/5/1 mM in toluene at 60 °C. ^bThe average DPs of monomers and composition ratios in copolymers were calculated from monomer conversions measured by ¹H NMR.

^c*M_n* and *M_w/M_n* were determined by SEC in THF with PSt standard calibration. ^d*M_n* and *M_w/M_n* were determined by SEC in DMF with 10 mM LiBr with PMMA standard calibration.

deprotection reaction was confirmed by ¹H NMR (Figure 2c) and the SEC curve of the copolymer after deprotection reaction was still unimodal. **P–C12** and **P–C18** were also synthesized by the same ways (Figure 3), and Table 1 summarizes data of *M_n*, *M_w/M_n*, and composition ratio [MI_{alkyl}/ST and MI_{alkyl}/4-vinylphenol (VP)] of the resultant copolymers after deprotection (**P–C2**, **P–C12**, and **P–C18**).

Analysis of Phase Separation

For phase separation of the copolymers, all copolymers were thermal annealed at 240 °C for 3 days under vacuum. Then, thermal properties of copolymers were analyzed by DSC (Figure 4). Here, six types of alternating copolymers corresponding to 1st or 2nd block were synthesized and also analyzed by DSC for comparison of *T_g*s. Depending on alkyl length of the diblock copolymer, thermal properties were changed. At a DSC diagram of **P–C2** (Figure 4b), two *T_g*s corresponding to those of alternating copolymers [Copoly(ST–MI_{C2}) and Copoly(VP–MI_{C2})] (176 °C and 223 °C, respectively) were clearly detected, indicating the phase separation of **P–C2** derived from segregation of the two blocks. **P–C18** was also found to have one *T_g* (Figure 4d), and the position of *T_g*s was similar to that of Copoly(VP–MI_{C18}). It is likely due to that octadecyl groups of **P–C18** suppressed the phase separation between ST and VP segments. On the contrary, there was no *T_g* detected in a diagram of **P–C12** (Figure 4c), which is likely due to the fusion of ST and VP segments by dodecyl groups in **P–C12**.

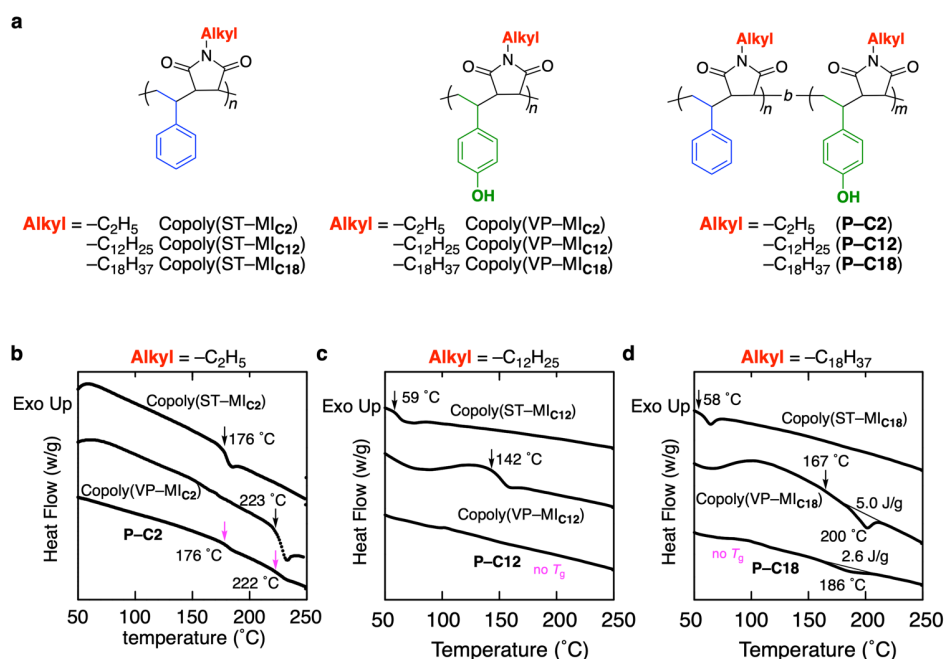


Figure 4. (a) Polymer Structures and (b-d) DSC diagram of 2nd heating of diblock copolymers [(b) P-C2, (c) P-C12, and (d) P-C18] and alternating copolymers corresponding to 1st or 2nd block: heating rate = 10 °C, heating range = -80 – 250 °C.

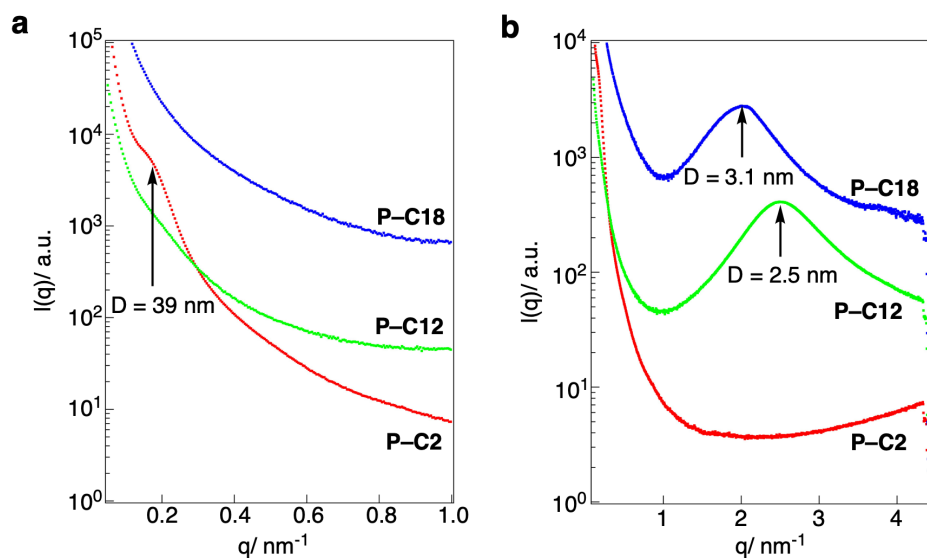


Figure 5. SAXS profiles of diblock copolymers (P-C2, P-C12, and P-C18) after thermal annealing at low q region (a) and high q region (b).

Phase separation of the diblock copolymers were investigated in detail by SAXS. Figure 5 shows SAXS profiles of diblock copolymers after thermal annealing for 3 days at 240

°C. At the profile of **P-C2**, a small peak derived from the phase separation of block segments was detected at low q region ($q = 0.16$, $D = 2\pi/q = 39$ nm), but there was no other peak derived from segregation between side chains. Perhaps, ethyl groups were likely too low hydrophobic for segregation with phenol units. On the contrary, at the profiles of **P-C18** or **P-C12**, only the peaks derived from the fully extended conformation of repeating units of long alkyl maleimide and 4-vinylphenol were detected at high q region (**P-C12**: $q = 2.51$, $D = 2\pi/q = 2.5$ nm; **P-C18**: $q = 2.02$, $D = 2\pi/q = 3.1$ nm), and no other peaks derived from the phase separation of block segments was observed. At **P-C18** or **P-C12**, only the segregation between long alkyl chains and phenol units was induced but the phase separations of block segments were likely suppressed owing to the strong aggregation force of the long alkyl chain. Further studies on effect of side chain in maleimide such as fluorocarbons (e.g. $-\text{CH}_2\text{CF}_3$) will be necessary to investigate phase separation behaviors.

Conclusion

In this Chapter, three components-diblock copolymers where two alternating copolymers of ST/MI_{alkyl} and VP/MI_{alkyl} were connected were synthesized via sequential RAFT copolymerizations. Three diblock copolymers which have different alkyl chains (**P-C2**, **P-C12**, and **P-C18**) were thermal annealed and the phase separations were analyzed by DSC and SAXS. **P-C2** was found to induce phase separation derived from segregation of block segments, which was confirmed by both of DSC and SAXS analysis. On the contrary, at **P-C12** and **P-C18** with long alkyl chains, only the segregation of phenol and long alkyl chains was detected by SAXS and the peak from phase separation of block segments were not observed, which is likely due to that the strong aggregation force of long alkyl chain suppressed the phase separation of the block segments. Further studies on effect of side chain in maleimide will be required to investigate phase separation behaviors.

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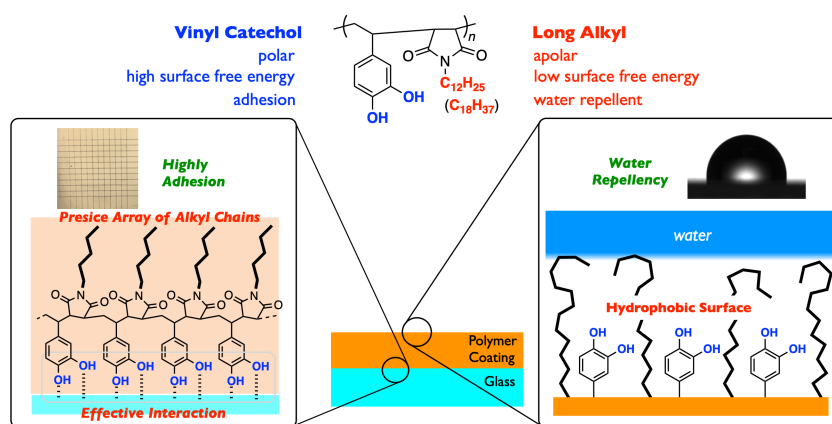
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Chapter 7

Alternating Copolymers of Vinyl Catechol or Vinyl Phenol with Alkyl Maleimide for Adhesive and Water Repellent Coating Materials

Abstract

It is of great challenge to balance both adhesion and water repellent properties for coating materials, because conflicting molecular characters are required. In this work, we synthesized a series of alternating copolymers of styrene derivatives (styrene, vinyl phenol, and vinyl catechol) with *N*-substituted maleimide derivatives via radical copolymerization followed by deprotection toward such unique materials. It was found that VC- and VP-based alternating copolymers with long alkyl chain-substituted maleimide might be available for such unique materials through evaluations of the adhesive property with the cross cut testing and the water repellent property under static and dynamic conditions for the coating on the glass plate. The unique features presumably stem from the chain behaviors on the surface specific to the alternating array of hydrophilic and hydrophobic pendant groups along the polymer backbone.



Introduction

Catechol is an aromatic compound having two hydroxy groups at the ortho-position and is known as a functional group essential to adhesive properties of proteins in nature. Indeed, proteins expressing an adhesive function, such as mussel adhesive proteins, contain the unit of 3,4-dihydroxy-L-phenylalanine (so called L-dopa).¹ It is assumed that some periodic sequence including the dopa unit is essential for the adhesive function,² but the detailed relationship between sequence and adhesive mechanism is unknown.

From the viewpoint of biomimetics, development of adhesive materials has attracted attentions, and polymers carrying catechol units have been studied.³⁻⁵ One straightforward strategy for adhesive materials is synthesis of statistical copolymers via radical copolymerization of a catechol-embedded monomer (or the protected) with some comonomer. Here, various structural factors affect the adhesive function, such as hydrophobic/hydrophilic, soft/rigid, cationic/anionic, molecular weight, terminal groups, and sequence.⁶ Now reversible deactivation radical polymerization (RDRP)⁷ methodologies allows chain growth from the desired position as well as a control of the molecular weight and terminal groups, and such controlled system is actually important for development of adhesive materials: for instance RDRP is useful for surface modification. However, toward drastic improvement in the adhesive properties, sequence control for some substitutes including catechol would be important, as far as inspired by nature. Recently, Matsumoto et al. reported synthesis of alternating copolymer of vinyl catechol with 2-ethylhexyl maleimide and referred the adhesion property in this paper.⁸ However, the detailed study such as the comparison with similar alternating copolymers to clarify the function of catechol unit was not performed.

In the last decade, many attempts have been paid to control sequence for synthetic polymers toward advanced properties and functions.⁹⁻¹¹ Sequence-controlled polymers are categorized into two classes: periodic and aperiodic.¹² Control of the aperiodic sequence is more challenging, and an iterative monomer addition process is required. However, the process is currently less practical for large scale syntheses toward material applications. On the other hand, copolymers of periodic sequence are more realistic to be synthesized, and particularly, AB alternating copolymers synthesized via radical copolymerization have been known from long time ago.¹³ In the copolymers, two kinds of pendant groups derived from the comonomer units can be periodically aligned along the backbone. The most striking feature is no composition distribution among chains, unlike general random copolymers. Some sequence-specific properties of alternating copolymers have been reported, such as fluorescence,^{14,15}

solvatochromism,¹⁶ degradation^{17,18} and thermoresponsibility.^{19,20}

A surface tension property of polymers is related to various properties/behaviors such as adhesion, wettability, and dispersibility. An interesting example that sequence in polymer chain affects such macroscopic properties have been reported by Sampson et al. They synthesized a variety of amphiphilic alternating copolymers via iterative stoichiometric addition of two types of comonomers in ring-opening metathesis polymerization (ROMP) to study effects of sequence on water repellent property.²¹ Consequently, sequence pattern was found to affect the value of water contact angle. The “sequence effect” on the water repellent property would be attributed to the combination of opposite character, i.e. hydrophobic and hydrophilic, in the periodic sequence.

Quite recently, we have synthesized a series of alternating copolymers of vinyl phenol with *N*-long alkyl maleimide derivatives via free radical polymerization or reversible addition fragmentation chain-transfer (RAFT) radical polymerization, followed by deprotection of the protected vinyl phenol (VP) units.²² The vinyl phenol unit is not fully hydrophilic but definitely immiscible to the hydrophobic alkyl chain. The copolymers formed inverse-micelle fibrillar nanostructure in aromatic solvents by segregation of phenol units against long alkyl chains. The synthetic process can be expanded to syntheses of alternating copolymers containing vinyl catechol instead of vinyl phenol.

In this work, a series of alternating copolymers of hydrophilic vinyl catechol (VC) and hydrophobic alkyl maleimide as well as the VP-based were synthesized and some properties such as adhesion on glass plates, water repellent, and coating properties were studied. Specifically, 3,4-dimethoxystyrene (dMOS) and 4-*tert*-butoxystyrene (tBOS) were used as the protected monomers for VC and VP respectively. The electron-donating alkoxy groups are helpful for high degree of control of alternating sequence.²³ These styrene-based monomer were copolymerized with *N*-alkyl maleimide (RMI), and subsequently the alkoxy groups were deprotected to make a series of functional alternating copolymers (Figure 1a). Styrene-based alternating copolymers were also synthesized to clarify effects of catechol or phenol and the physical properties were studied through the comparison among the series of alternating copolymers (Figure 1b). Estimation of the surface free energies as well as measurement of dynamic contact angles of the polymer coatings on glass plates were also performed to predict the surface structures.

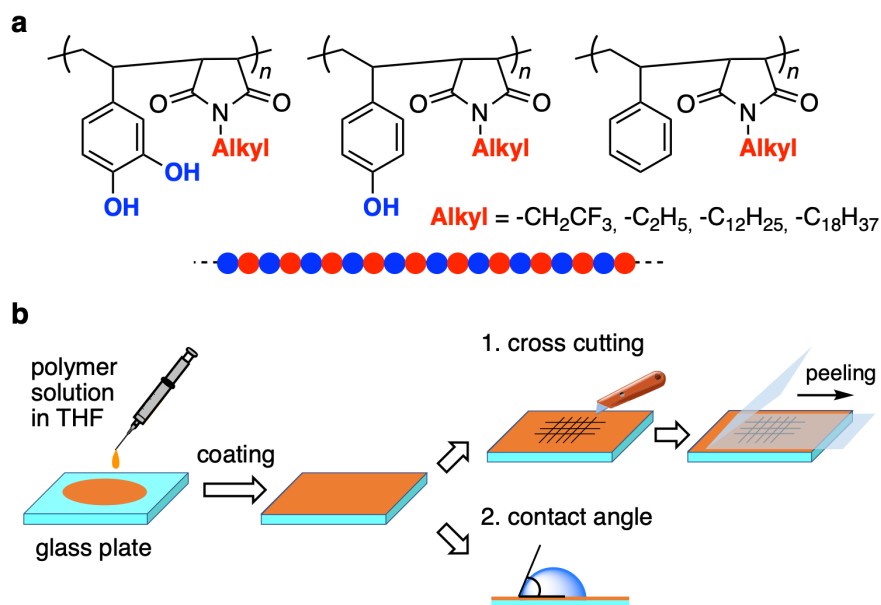


Figure 1. (a) alternating copolymers in this work. (b) sample preparation for evaluation of adhesive and water repellent properties.

Experimental

Materials

Styrene (St, TCI; purity >99%), 4-*tert*-butoxystyrene (tBOS, Sigma Aldrich; purity 99%), 3,4,-dimethoxystyrene (dMOS, Aldrich; technical grade) were passed through inhibitor remover (Sigma Aldrich) before use. Boron Tribromide (BBr₃, TCI; 1M in CH₂Cl₂), HCl aq. (Wako; 35-37%), *N*-ethylmaleimide (EMI, TCI; purity >98%), and 2,2-azobisisobutyronitrile (AIBN, Wako; purity >98%) were used without purification. Toluene (Wako; purity >99.5%) was dried by Molecular sieves 4A before use. 1,2,3,4-Tetrahydronaphthalene (tetralin, Kisida Chemical; purity >98%), an internal standard in ¹H NMR, was dried over calcium chloride overnight and distilled twice over calcium hydride. Glass substrates (76 × 52 mm, thickness: 1.2 mm; Matsunami Glass Ind., Ltd., Kishiwada, Japan) was used after sonication in ethanol for 10 min.

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

SEC (DMF). Molecular weight distribution (MWD) curves, number-average molecular

weight (M_n) and M_w/M_n ratio of the polymers were measured by size exclusion chromatography (SEC) in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L; exclusion limit = 4×10^6 g/mol; particle size = 10 μ m; pore size < 5000 Å; 0.8 cm i.d. $\times$ 30 cm) that were connected to a Jasco PU-2080 precision pump, a Jasco RI-2031 refractive index detector and a Jasco UV-2075 UV/vis detector set at 270 nm. The columns were calibrated against 11 standard PMMA samples (PSS, Polymer Standard Service GmbH, Ready Cal Kit: M_p = 2,380 – 2,200,000).

SEC (THF). MWD curves, M_n and M_w/M_n ratio of the polymers were also measured by SEC in THF as an eluent at 40 °C on three polystyrene gel columns (Shodex LF-404; exclusion limit = 2×10^6 g/mol; particle size = 6 μ m; pore size, < 3000 Å; 0.46 cm i.d. 25 cm; flow rate 0.3 mL/min) connected to a DU-H2000 pump, a 74S-RI refractive index detector, and a 41-UV ultraviolet detector (all from Shodex; Shodex GPC-104). The columns were calibrated against 12 standard polystyrene samples (TOSOH, PS-Oligomer Kit, M_w = 500–1,110,000; M_w/M_n = 1.01–1.16).

MALDI-TOF-MS. MALDI-TOF-MS analyses were performed on an ultraflex III (MALDI-TOF mass spectrometer, Bruker Daltonics) equipped with 337 nm nitrogen laser with dithranol as matrix and sodium trifluoroacetate as the cationizing agent.

FT-IR. FT-IR spectra of freeze-dried alternating copolymers were measured by Agilent Cary 630 FTIR spectrometer (Agilent Technologies).

SPM. The surface morphologies of polymer coatings were measured in air by SPM-9700HT (Shimadzu Corp., Kyoto, Japan) at dynamic mode of atomic force microscopy with an OMCL-AC200TS cantilever (Spring Constant 9 N/m, Olympus, Tokyo, Japan).

DSC. DSC measurements (ca. 2.0 mg weighed into an aluminum pan) were performed on a DSC Q200 calorimeter (TA instruments) equipped with a RCS 90 electric freezing machine under dry nitrogen flow at a heating or cooling rate of 10 °C/min.

Turbidity measurement. Turbidity of the polymer solutions in toluene was measured on a UV-1800 (Shimadzu, optical path length = 10 mm, λ = 670 nm, heating/cooling rate: 1 °C min⁻¹, temperature range: 40–80 °C).

Preparation of polymer coatings on glass plates by drop cast method. Polymer membranes were made by drop cast method. 0.4 mL of the polymer solutions (5wt% in THF) were prepared and spread on glass plates (3 $\times$ 3 cm). The glass plates were dried in vacuo overnight to prepare thin polymer coatings.

Preparation of polymer coatings on glass plates by spin coating method. 0.6 mL of

1wt% polymer solution in THF was spread across the surface of a glass plate (5×5 cm). Subsequently, the plate was rotated by a spin coater (Opticoat, Mikasa) to make flat polymer coatings.

Membrane thickness measurements. Before measurement, the membrane on a glass plate was scratched by spatula to expose glass plate. The membrane thickness was measured by a stylus-type surface profiler as a difference between surface of the membrane and that of glass plate.

Contact angle measurements. A droplet of water or diiodomethane (5 μ L) was dropped onto each polymer coating. The contact angles were measured using a contact angle meter (Kyowa), and contact angle hysteresis was measured by increasing and decreasing the droplet volume to extract the advancing and receding contact angles.

Synthesis of *N*-Alkyl Maleimide

N-(2,2,2-trifluoroethyl) maleimide (CF₃-MI), *N*-dodecyl maleimide (C12MI) and *N*-octadecyl maleimide (C18MI) were synthesized according to the Chapter 2 and Chapter 4.

Synthesis of Copoly(VC–MI_{Alkyl})

Polymerization was carried out by syringe technique under dry argon in baked glass tubes equipped with three-way stopped cocks. Typical procedures for synthesis of Copoly(VC–MI_{Alkyl}) via free radical polymerization followed by deprotection reaction of methoxy groups was as follows.

To a Schlenk tube, C18MI (2.45 g, 7.0 mmol) and AIBN (23 mg, 0.14 mmol) were added and degassed by vacuum pump at -78 °C. Then, dMOS (2.86 mL in 2242.534 mM in toluene, 7.0 mmol), 40 mL of toluene and 0.1 mL of tetralin were added under dry argon flow (concentration: dMOS/C18MI/AIBN = 1000/1000/20 mM). After mixing, the tube was placed in an oil bath at 60 °C for 2 h and the copolymerization was terminated by bubbling with air. Monomer conversion was determined by ¹H NMR from the integrated peak area of the olefinic protons of the monomers with tetralin as an internal standard (conversion: dMOS/C18MI = 94/100%). The solution was diluted by THF (total volume: 50 mL) and the solution was added dropwise in 0.8 L of methanol with stirring to precipitate the copolymer. The precipitate was filtered and washed by methanol. The filtered solid was dried in vacuo to yield the alternating copolymer [poly(dMOS-*alt*-C18MI)] as white solid (3.37 g): $M_n = 150700$, $M_w/M_n = 3.82$ (from SEC in THF, after purification). The composition ratio of the purified

copolymer was determined by ^1H NMR from the integrated peak area of the aromatic protons of dMOS and ethylene protons neighboring to nitrogen atom of maleimide (dMOS/C18MI = 50/50). In a flask, 3.37 g of poly(dMOS-*alt*-C18MI) (Methoxy groups: 13 mmol) was dissolved in 64 mL of CH_2Cl_2 . To the solution, BBr_3 (26.2 mL of 1.0 M in CH_2Cl_2 , 26 mmol) was added dropwise to the solution with stirring under dry argon flow at 0°C . After mixing well for 30 min at 0°C , the flask was placed at room temperature for 20 h. After mixing, the solution was added dropwise in 1 L of 1.0 M HCl aq. with stirring to precipitate the copolymer at 0°C . After mixing for 3 h, the precipitate was filtered and dissolved in THF again and the solution was added dropwise in HCl aq. again. The precipitate was filtered and washed by water for several times until the pH of the filtered water became 7. The filtered solid was

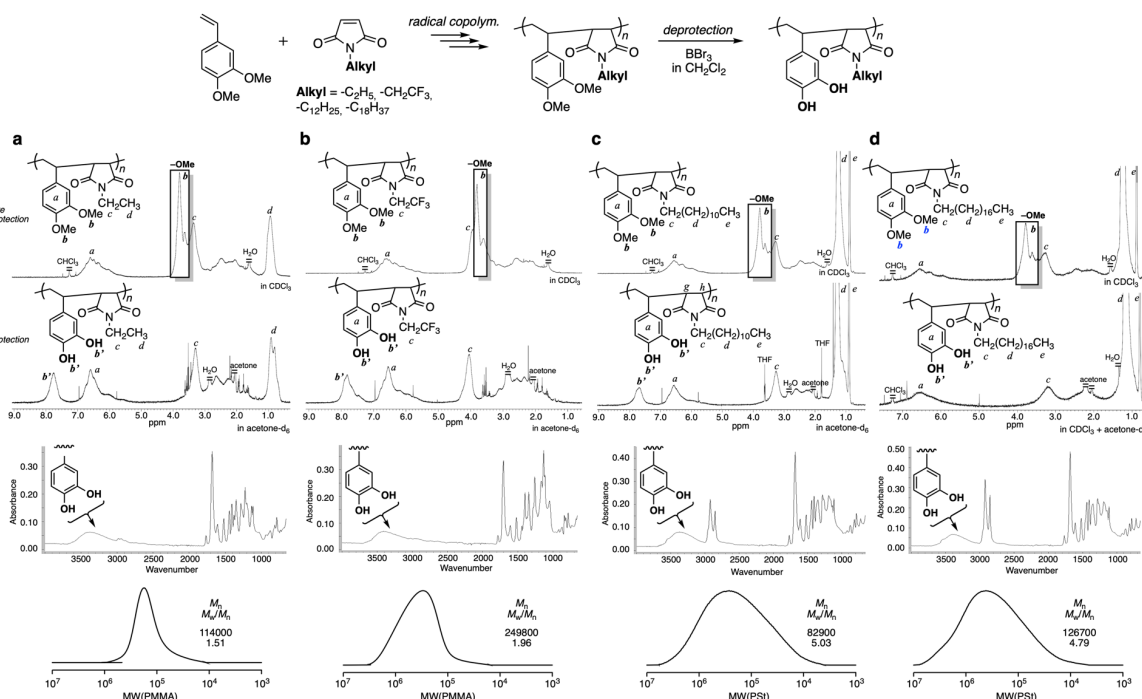


Figure 2. Characterization of copolymers before and after deprotection by ^1H NMR, FT-IR, and SEC (THF or DMF): (a) Copoly(VC-MI_{c2}), (b) Copoly(VC-MI_{cF3}), (c) Copoly(VC-MI_{c12}), and (d) Copoly(VC-MI_{c18}).

dissolved in 1,4-dioxane and freeze-dried to yield the Copoly(VC-MI_{c18}) as orange solid (2.58 g): $M_n = 126700$, $M_w/M_n = 4.79$ (from SEC in THF, after purification). The deprotection reaction was confirmed by ^1H NMR and FT-IR (Figure 2d).

Other alternating copolymers of VC and *N*-alkyl maleimide were synthesized by the

same method. The characterizations of the copolymers by ^1H NMR, FT-IR, and SEC are shown in Figure 2abc.

Synthesis of Copoly(VP-MI_{Alkyl})

Copoly(VP-MI_{Alkyl}) were synthesized via free radical polymerization followed by deprotection reaction of *tert*-butoxy groups according to the Chapter 4.

Synthesis of Copoly(ST-MI_{Alkyl})

Copoly(ST-MI_{Alkyl}) were synthesized via free radical polymerization as described above. The characterizations of the copolymers by ^1H NMR, FT-IR, and SEC are shown in Figure 3.

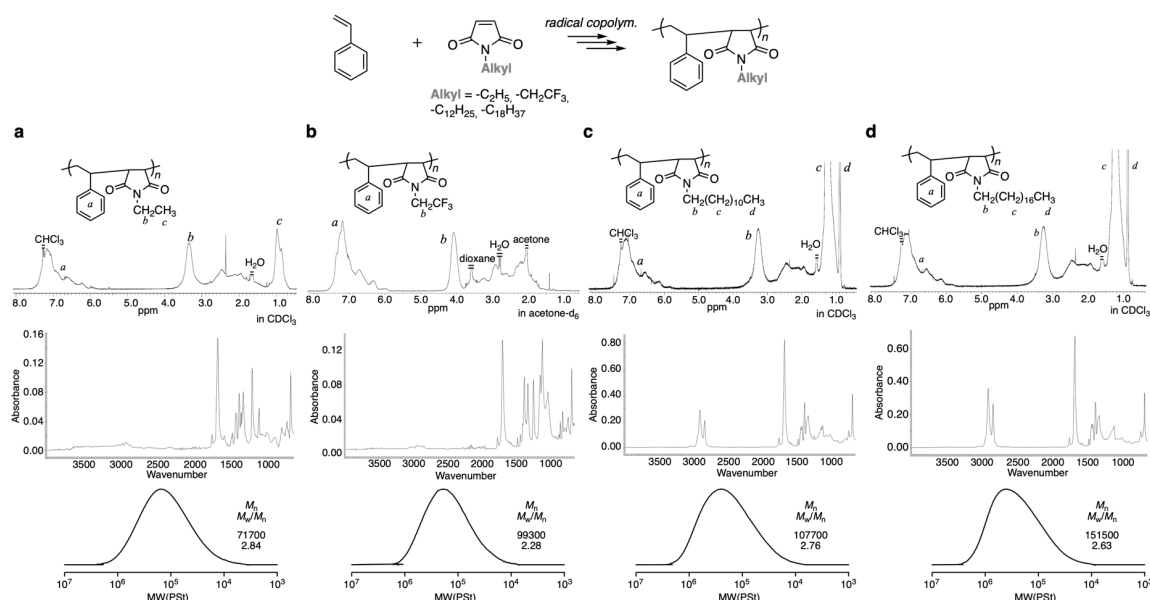


Figure 3. Characterization of copolymers by ^1H NMR, FT-IR, and SEC (THF): (a) Copoly(ST-MI_{c2}), (b) Copoly(ST-MI_{CF3}), (c) Copoly(ST-MI_{c12}), and (d) Copoly(ST-MI_{c18}).

Synthesis of Homopolymers

PolyST, PolyVP, and PolyVC were also synthesized via free radical polymerization and followed deprotection reactions as described above. The characterizations of the polymers by ^1H NMR, FT-IR, and SEC are shown in Figure 4.

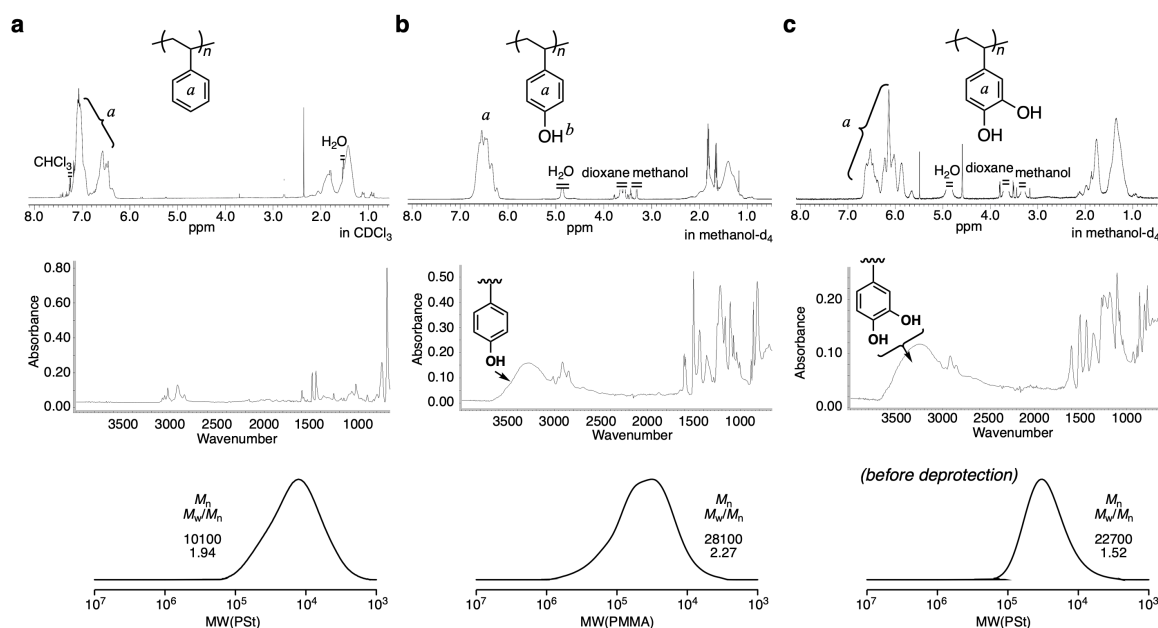


Figure 4. Characterization of copolymers by ^1H NMR, FT-IR, and SEC (THF or DMF): (a) PolyST, (b) PolyVP, and (c) PolyVC. [The SEC curve of Poly(dMOS) was shown instead of that of PolyVC due to that PolyVC was not eluted in DMF nor THF.]

Result and Discussion

Synthesis and Characterization of Alternating Copolymers

All copolymers were synthesized via free radical copolymerization of styrene derivatives (dMOS, tBOS or styrene) with *N*-alkyl maleimides (MI_{Alkyl}) and subsequent deprotection reactions (when the protected styrene was used). Herein, to study effects of hydrophobicity and fluororous on the surface properties, four kinds of alkyl chains were studied for MI_{Alkyl} : ethyl ($-\text{CH}_2\text{CH}_3$, MI_{C_2}), 2,2,2-trifluoroethyl ($-\text{CH}_2\text{CF}_3$, MI_{CF_3}), dodecyl ($-\text{C}_{12}\text{H}_{25}$, $\text{MI}_{\text{C}_{12}}$), and octadecyl ($-\text{C}_{18}\text{H}_{37}$, $\text{MI}_{\text{C}_{18}}$). The quantitative deprotection reactions and 1:1 composition ratios of the copolymers were confirmed by ^1H NMR and FT-IR (Figure 2 and 3). As a comparison, homopolymers of vinylcatechol (VC), 4-vinylphenol (VP), and styrene (ST) [PolyVC, PolyVP, and PolyST, respectively] were also synthesized (Figure 4). The structures of the synthesized polymers are listed in Figure 5, and the molecular weights, polydispersities (M_w/M_n) and composition ratios are summarized in Table 1. Here, the alternating copolymers of VC or VP or ST with MI_{Alkyl} are represented as Copoly(VC- MI_{Alkyl}), Copoly(VP- MI_{Alkyl}) and Copoly(ST- MI_{Alkyl}), respectively.

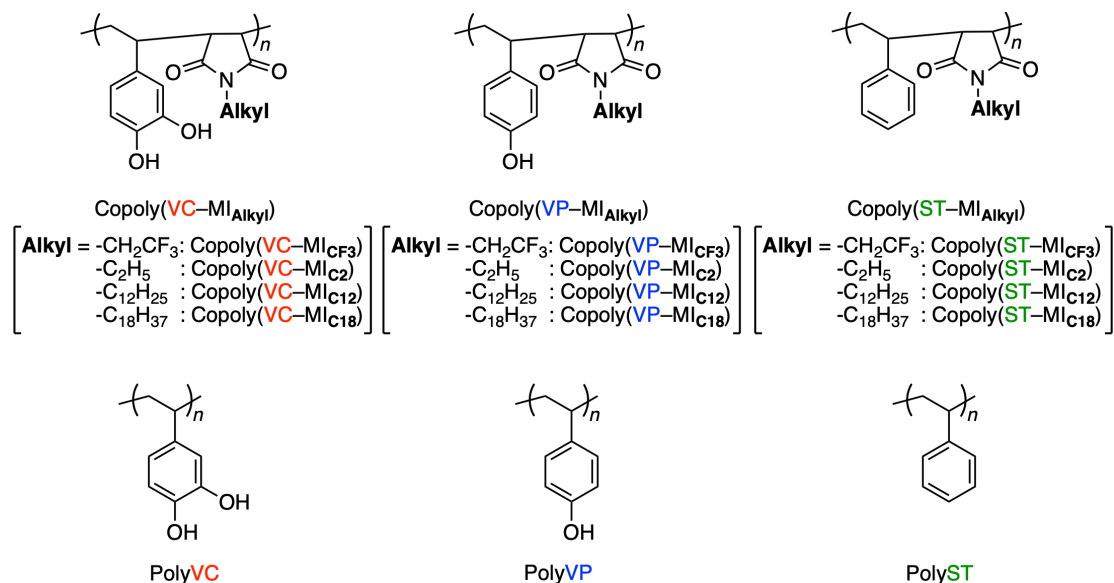


Figure 5. Structures and codes of (co)polymers in this work.

Solubility and Thermal Property of the Alternating Copolymers

The solubility of the VP- or VC-based alternating copolymers is interesting because the phenolic group show opposite polarity to the alkyl group of the maleimide unit. The solubility trend would be helpful for discussion on the surface properties as well as the coating properties toward practical applications. The solubilities of VP-based copolymers were reported in our last study (Table 2), and here those of VC-based counterparts were investigated (Table 3, for concentration of 1wt%). The alkyl chain of maleimide unit affected solubility of the alternating copolymers. For instance, C18-pendant copolymer Copoly(VC-MI_{C18}) was insoluble in acetone, whereas the others were soluble. The basic water did not dissolve the long alkyl chain-substituted copolymers, but did the shorter alkyl derivatives. As already reported, vinyl phenol copolymers with long alkyl chain [Copoly(VP-MI_{C12}) and Copoly(VP-MI_{C18})] show UCST-type thermal responses in the aromatic solvents such as toluene: they are insoluble at ambient temperature but become soluble by heating. However, the series of Copoly(VC-MI_{Alkyl}) were swelled in toluene but totally insoluble regardless of temperature. Such a solubility difference was also observed in chloroform. Probably, the hydrogen bonding between catechol units is stronger than that between phenol units. Interestingly, the four copolymers tended to show UCST-type thermal responses in the alcoholic solvents such as ethanol, *i*-propanol, and butanol. As the typical example, Figure 6a shows the temperature-variable turbidity measurement for Copoly(VC-MI_{C2}) in ethanol, *i*-propanol, and butanol.

Table 1. Characterization of Alternating Copolymers of Styrene Derivatives with *N*-Alkyl Maleimides

Code ^a	Monomer Pair		M_n ^b	M_w/M_n ^b	Composition
	St	MI _{Alkyl}			Ratio ^c St/MI
Copoly(VC–MI _{C2})	dMOS	MI _{C2}	114000 ^e	1.51 ^e	50/50
Copoly(VC–MI _{CF3})	dMOS	MI _{CF3}	249800 ^e	1.96 ^e	49/51
Copoly(VC–MI _{C12})	dMOS	MI _{C12}	82900	5.03	49/51
Copoly(VC–MI _{C18})	dMOS	MI _{C18}	126700	4.79	50/50
Copoly(VP–MI _{C2})	tBOS	MI _{C2}	49700	3.28	50/50
Copoly(VP–MI _{CF3})	tBOS	MI _{CF3}	179200 ^e	2.44 ^e	51/49
Copoly(VP–MI _{C12})	tBOS	MI _{C12}	77400	6.41	50/50
Copoly(VP–MI _{C18})	tBOS	MI _{C18}	95200	4.50	49/51
Copoly(ST–MI _{C2})	ST	MI _{C2}	71700	2.84	49/51
Copoly(ST–MI _{CF3})	ST	MI _{CF3}	99300	2.28	51/49
Copoly(ST–MI _{C12})	ST	MI _{C12}	107700	2.76	49/51
Copoly(ST–MI _{C18})	ST	MI _{C18}	151500	2.63	50/50
PolyVC	VC	–	– ^d	– ^d	–
PolyVP	VP	–	28100 ^e	2.27 ^e	–
PolyST	ST	–	10100	1.94	–

^a Conditions: [St derivative]₀/[*N*-alkyl maleimide]₀/[AIBN]₀ = 1000/1000/20 mM in toluene at 60 °C or [St derivative]₀/[AIBN]₀ = 2000/20 mM in toluene at 80 °C for synthesis of alternating copolymers and homopolymers, respectively. ^b Molecular weight of the copolymers after deprotection and purification determined by SEC in THF with PSt standard calibration. ^c Composition Ratios of styrene derivatives and *N*-alkyl maleimide were determined by ¹H NMR before deprotection reactions. ^d The polymers were not eluted by SEC in THF nor DMF. ^e The polymers were eluted by SEC in DMF containing 10 mM LiBr.

Turbidities of the solutions were monitored on cooling processes ([copolymer] = 10 mg/mL, cooling rate = 1 °C/min from 80 to 40 °C). Upon cooling, all three solutions responded to

Table 3. Solubility of Copoly(VC-MI_{Alkyl}) at r.t. ^a

Solvent	MI _{C2}	MI _{C12}	MI _{C18}	MI _{CF3}
Hexane	IS	IS	IS	IS
Toluene	IS	IS	IS	IS
THF	S	S	S	S
Chloroform	IS	IS	IS	IS
Ethyl Acetate	IS	S _{part}	S _{part}	S
Acetone	S	S	IS	S
Butanol	IS/TR	IS ^c	IS ^c	IS ^c
<i>i</i> -Propanol	IS/TR	IS ^c	IS/TR	IS/TR
Ethanol	IS/TR	IS ^c	IS	S
Methanol	IS/CP	IS/CP	IS	S
DMF	S	S _{part}	S _{part}	S
DMSO	S _{part}	IS/CP	IS	S
H ₂ O	IS.	IS	IS	IS
1M NaOH aq.	S.	IS	IS	S

S: soluble, S_{part}: partially soluble, IS: insoluble, IS/TR: Insoluble at r.t., but showing UCST-type thermal response, IS/CP: insoluble but compatible with solvent. ^a Polymer concentration: 1wt%. ^b Soluble at r.t. after heating at 75 °C.

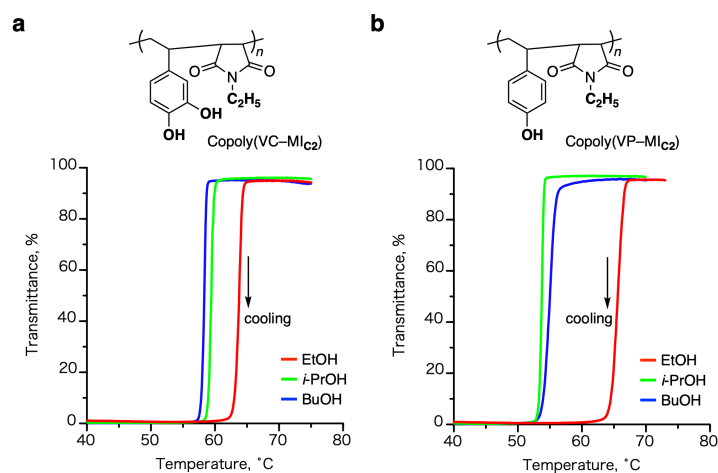


Figure 6. Turbidity measurement of 1wt% (a) Copoly(VC-MI_{C2}) and (b) Copoly(VP-MI_{C2}) in alcohols (butanol, *i*-propanol, and ethanol): heating speed = 1.0 °C/min, wavelength λ = 670 nm.

temperature and became turbid around 60 °C: 58 °C in ethanol, 59 °C in *i*-propanol, and 65 °C in butanol. Probably, the chains are dissociated at higher temperature thanks to the hydrogen bond interaction between catechol and solvent but aggregated at lower temperature due to that the hydrogen bond among chains becomes dominant. Similar UCST-type solubilities were observed with vinyl phenol-based copolymers (Figure 6b).

Poly(4-vinylphenol) is known to exhibit higher glass transition temperature (T_g) than polystyrene due to the hydrogen bond interaction among chains.²⁴ Here, for three types of MI_{C2} -based alternating copolymers, the T_g s were compared (Figure 7a). As expected, T_g increased as the following order: Copoly(VC- MI_{C2}) (229 °C) > Copoly(VP- MI_{C2}) (223 °C) > Copoly(ST- MI_{C2}) (176 °C). The trend also implies existence of hydrogen bond between the phenolic units. Thus, these alternating copolymers showed high heat resistance owing to both hydrogen bond and cyclic backbone derived from maleimide. DSC data for other copolymers are shown in Figure 7b-d. As shown there, the T_g s of VC and VP-based copolymers tend to be higher than those of general vinyl polymers for adhesive applications such as polyacrylates. The thermal stabilities could contribute to the development of heat-resistant adhesive materials.

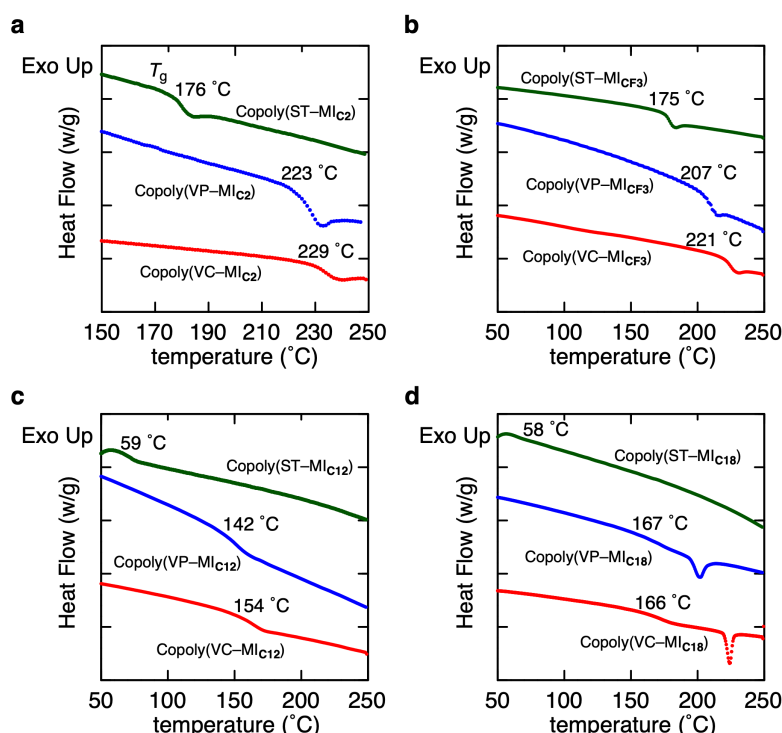


Figure 7. DSC data of copolymers with (a) MI_{C2} , (b) MI_{CF3} , (c) MI_{C12} , and (d) MI_{C18} : heating rate = 10 °C/min, 2nd heating, heating range = -80 to 250 °C.

Evaluation of Adhesive Properties

The clear difference in the adhesive behavior between the two series is due to presence or absence of the hydrophilic catechol unit that can interact with the glass surface. Herein, it is noteworthy that all the series of Copoly(VC-MI_{Alkyl}) showed no less high adhesive properties for the glass plate than the VC homopolymer, although the half units of the copolymer carry the hydrophobic alkyl chain or fluorocarbon causing low surface free energies. These adhesion properties are probably due to the intrinsic ability of catechol group. On the other hand, surprisingly, the phenol-based copolymers with longer alkyl chain-substituted copolymers [*i.e.*, Copoly(VP-MI_{C18}) and Copoly(VP-MI_{C12})] also afforded high adhesive properties, whereas the homopolymer (PolyVP) resulted in poor adhesion property. The adhesive property of the VC-based copolymers was due to the inherent character of catechol, whereas that of the VP-based longer alkyl chain-substituted copolymers to formation of peculiar structure. Most probably, the significant difference is possibly caused by regular alignment of the phenol unit along the glass surfaces made via self-assembly of the alkyl chains thanks to the alternating sequence. The phenol-based copolymers might be applicable for adhesive materials, because phenol is more stable against oxidation²⁵ and more inexpensive than catechol.

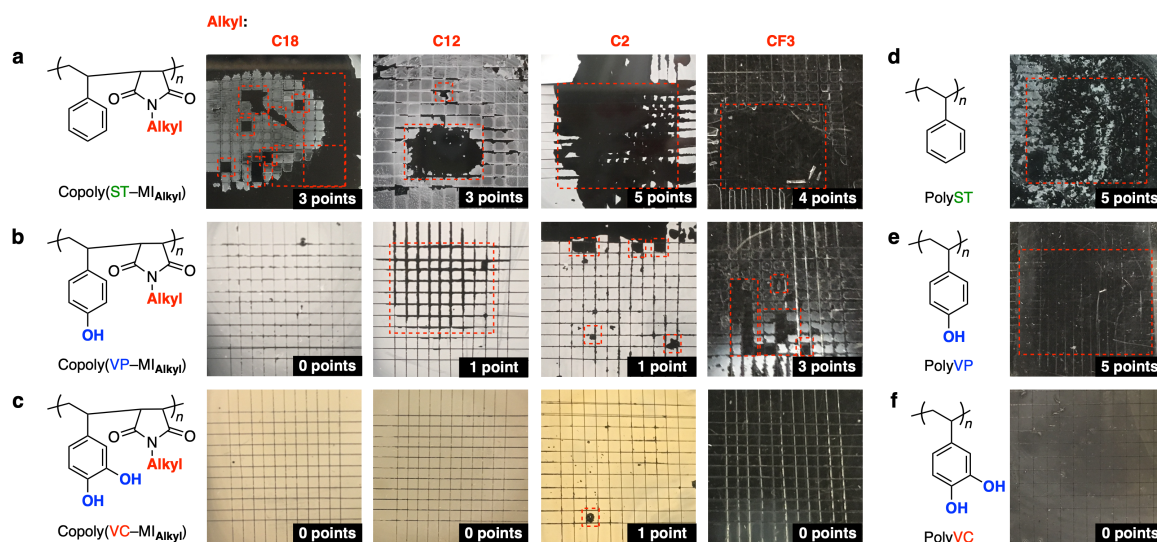


Figure 8. Pictures of the thin polymer coatings on glass plates after cross cut testing: (a) Copoly(VC-MI_{Alkyl}), (b) Copoly(VP-MI_{Alkyl}), (c) Copoly(ST-MI_{Alkyl}), (d) PolyVC, (e) PolyVP, and (f) PolyST.

Static Contact Angles of the Copolymers

The difference in the adhesion property among the series of copolymers is probably attributed to the chain arrangement on the glass surface depending on the substituents and the combinations. For example, the chains of Copoly(VC–MI_{Alkyl}) and Copoly(VP–MI_{Alkyl}) with longer alkyl chain might be stacked to give hydrophobic and hydrophilic layers alternately, and such chain migrations on the surface would affect their wettability.²⁶ Thus, we assumed the measurement of contact angel is helpful to predict such organized structure and thus measured the contact angles of two probe liquids (water and diiodomethane) on the series of polymer coatings (Figure 10). Surface free energies of the polymer coatings were then estimated by Owens-Wendt model.²⁷ The coatings were prepared by spin coating method. The thickness of the coatings and the smooth surfaces were characterized by stylus-type surface profiler and SPM, respectively (Table 4 and Figure 9).

Figure 10 shows the data of contact angles for water and diiodomethane. The trend was observed that the values for both of water and diiodomethane were increased as the alkyl chain of MI_{Alkyl} unit was longer. The CF₃-substituted copolymers exhibited a little higher water contact angles than the ethyl-substituted counterparts likely due to the inherent low surface energy of fluoroalkyl group. However, the trifluoro group effect was smaller than expected. Most probably, the number of the fluorine atom in one pendant is too low to cover the

Table 4. Thickness and Roughness of Polymer Coatings on Glass Plates Measured by Stylus-Type Surface Profiler

Sample	Thickness, nm
Copoly(VC–MI _{C2})	157
Copoly(VC–MI _{C12})	158
Copoly(VC–MI _{C18})	170
Copoly(VP–MI _{C2})	121
Copoly(VP–MI _{C12})	139
Copoly(VP–MI _{C18})	185
Copoly(ST–MI _{C2})	145
Copoly(ST–MI _{C12})	150
Copoly(ST–MI _{C18})	116
PolyVC	106
PolyVP	75
PolyST	151

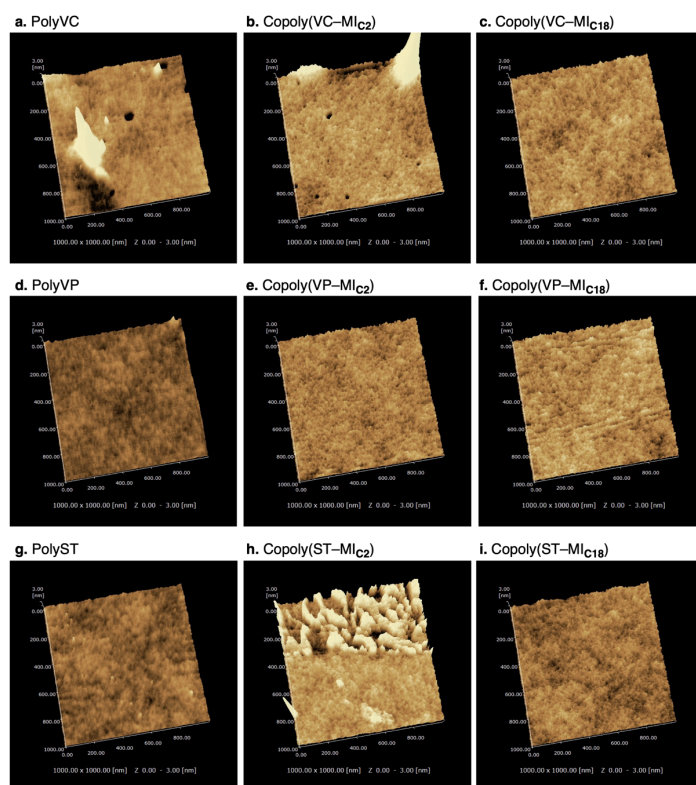


Figure 9. SPM images of the coatings of (a-c) Copoly(VC-MI_{Alkyl}), (d-f) Copoly(VP-MI_{Alkyl}), and (g-i) Copoly(ST-MI_{Alkyl}).

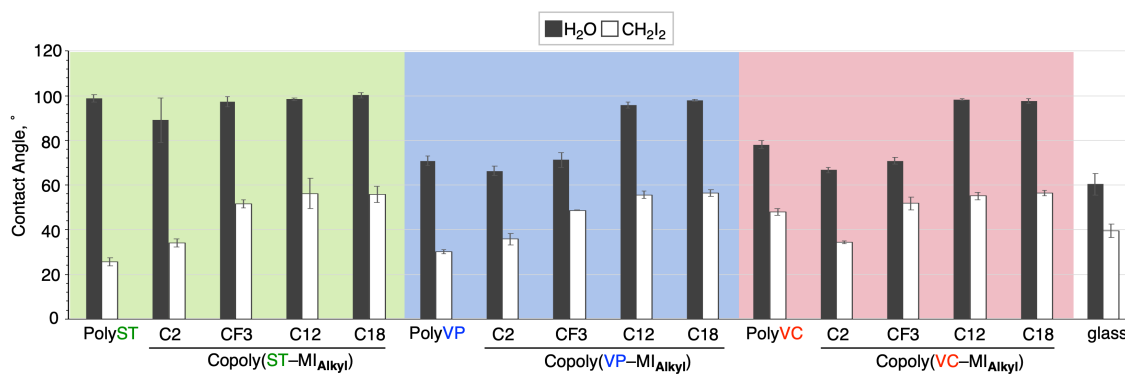


Figure 10. Static contact angles of water (black) and diiodomethane (white) measured for copolymer membranes coated on glass plates: $n = 5$, mean $\pm$ S.D..

hydrophilic units with the hydrophobic ones.

It is anticipated that water contact angle is decreased as hydrophilic moiety is introduced, but the VC and VP copolymers with long alkyl chain maleimide (C12 or C18) gave relatively

high values comparable to the ST-based counterparts. On the contrary, the copolymers with shorter alkyl chain maleimide (C2 or CF3) resulted in lower values than the corresponding ST-derivatives. It is possible that such ethyl-based chains are too short to suppress the migration of the hydrophilic hydroxy groups (*i.e.*, catechol or phenol). On the other hand, the longer alkyl chain could effectively suppress the migration of neighboring hydrophilic unit in alternating sequence, affording the hydrophobic surface with water contact angle $> 90^\circ$.

Surface Free Energies

To discuss the polymer structure at surface, the surface free energies of the coatings were then calculated by using Owens-Wendt method (Equation 1-4).²⁷ Here, the surface free energy data of water and diiodomethane (Table 5)²⁷ were used for the calculation. The total surface free energies tended to decrease as the alkyl length of maleimide unit was longer (Figure 11), as described above. Considering that the surface free energies of the copolymers with long alkyl chains are close to those of saturated hydrocarbons (C6-C16, 18~27) and aromatic compounds (benzene and toluene, 28),²⁸ it is supported that the alkyl chains and/or aromatic parts were exposed and the hydroxy groups faced inside on the surface of the coatings made of the copolymers. Furthermore, the higher values of γ^h of the short alkyl chain (C2 or CF3) copolymers support the prediction.

Equations of Owens-Wendt Method

(Equation 1) $\gamma_S = \gamma_L \cos\theta + \gamma_{SL}$

(Equation 2) $\gamma_L = \gamma_L^d + \gamma_L^h$

(Equation 3) $\gamma_{SL} = \gamma_S + \gamma_L - 2(\gamma_S^d \gamma_L^d)^{1/2} - 2(\gamma_S^h \gamma_L^h)^{1/2}$

(Equation 4) $\gamma_L(1 + \cos\theta)/2 = (\gamma_S^d \gamma_L^d)^{1/2} - (\gamma_S^h \gamma_L^h)^{1/2}$

Where γ^d : surface free energy contributed from dispersion force; γ^h : surface free energy contributed from hydrogen bond.

Table 5. Surface Free Energies of Liquids

Liquid	γ_L^d (mJ/m ²)	γ_L^h (mJ/m ²)	γ_L (mJ/m ²)
H ₂ O	21.8	51.0	72.8
CH ₂ I ₂	49.5	1.3	50.8

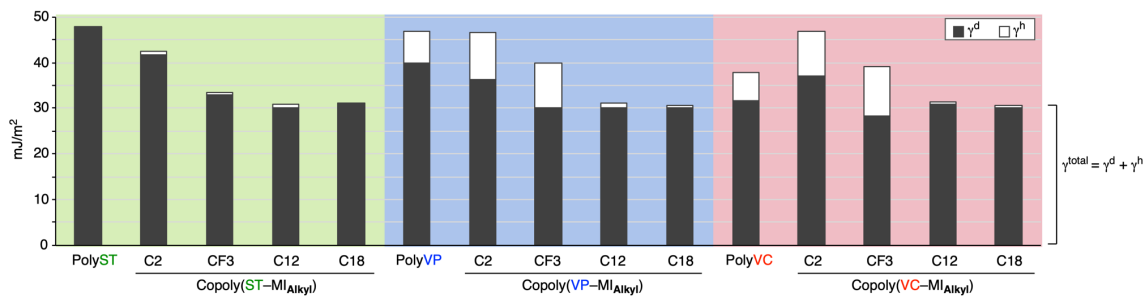


Figure 11. Surface free energies of copolymer membranes coated on glass plates calculated from Owens-Wendt method.

Dynamic Contact Angles

To further investigate surface structures, dynamic water contact angles of the polymer coatings were evaluated with an extension/contraction method. In this method, a water droplet is first placed on the sample for making the stable equilibrium state. A tiny amount of water was then re-injected to expand the droplet and the contact angle for the expanded droplet was measured [advancing contact angle (θ_A), Figure 12a]. Afterward, a partial water is inhaled to shrink the droplet until just before the destruction and the minimum contact angle

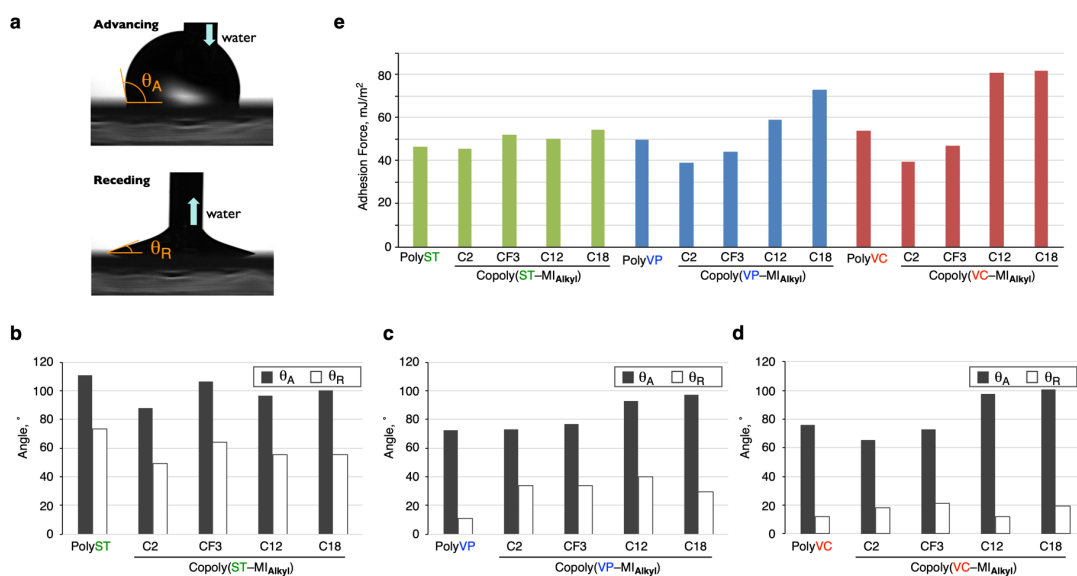


Figure 12. (a) picture of advancing (θ_A) and receding (θ_R) contact angles of Copoly(VC-MI_{C18}) membrane, (b-d) advancing and receding contact angles of water measured for the coatings, and (e) the adhesive forces of coatings against water droplet.

(Equation 5) $f = \gamma_L(\cos\theta_R - \cos\theta_A)$

Where f : adhesion force of surface against liquid per unit length of interface, γ_L : surface free energy of water, θ_A : advancing contact angle, θ_R : receding contact angle.

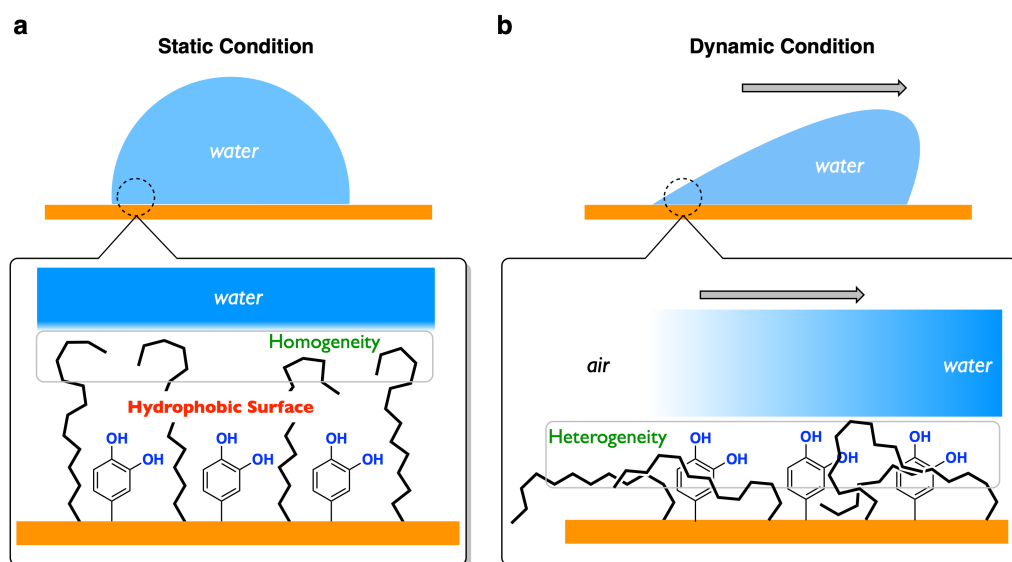


Figure 13. The plausible interface between water droplet and polymer coating with Copoly(VC-MI_{C18}) under static (a) and dynamic (b) condition.

was measured [receding contact angle (θ_R)]. The measurement of the difference between θ_A and θ_R (i.e. hysteresis) is helpful for assumption of the homogeneity of surface free energy as well as the dynamic behavior of polymer chain. The homogeneity of surface free energy of the coating was estimated with the calculation of adhesive force from the hysteresis by Furmidge Equation²⁹ (Equation 5). Figure 12b-d summarizes the dynamic contact angles for water on the series of coatings, whereas Figure 12e shows the adhesive forces of the coatings against water droplets. Copoly(VC-MI_{Alkyl}) with long alkyl chains (C12 and C18) showed relatively high adhesive force and the VP copolymer with C18-substituted MI [Copoly(VP-MI_{C18})] was also comparable to the VC-based copolymers. The trend was rather unexpected from the result of the static contact angle measurement indicating exposure of the hydrophobic long alkyl chains giving hydrophobic surface. We thus presumed the surface of the coating with these copolymers as follows: the long alkyl chain can block the contact of hydrophilic parts (i.e. catechol or phenol) with water droplet under static condition (Figure 13a); the alkyl chain can fluctuate under dynamic condition leading to exposure of the hydrophilic parts

(Figure 13b). The chain motility induced the heterogeneity of the surface free energy, which consequently caused the high adhesive force.

Conclusion

We have synthesized a series of alternating copolymers of vinyl catechol (VC) [or vinyl phenol (VP), or styrene (ST)] and *N*-alkyl maleimide ($-C_2H_5$, $-C_{12}H_{25}$, $-C_{18}H_{37}$, and $-CH_2CF_3$) for functional coating materials. Hydrogen bonding of hydroxyl groups in the copolymers with VC or VP affected the solubilities and induced UCST-type thermal responses in alcoholic solvents such as ethanol, *i*-propanol, and butanol. The copolymers of VC or VP and *N*-long alkyl maleimide (C12 and C18) were found to exhibit high adhesive properties on glass plates as well as high water repellencies, although the half units are made of either non-polar or polar pendant groups. The water repellency is likely due to that the long alkyl chains suppressed the migration of hydrophilic units under the static condition. There have been not many examples on such materials exhibiting both adhesiveness and water repellent. One notable example has been recently presented by Nishino et al.: they achieved the balanced properties by using the (meth)acrylate-based copolymers containing both perfluoroalkyl groups and hydrophilic poly(ethylene oxide) side chains, although the sequence is random.³⁰ Most probably, the design of amphiphilic copolymer made of quite different polarity or affinity to realize unit segregation in nano-scale is crucial to balance the coating properties requiring the conflicting characters. Unfortunately, contribution of alternating sequence cannot be discussed in this work, because it is impossible to synthesize the corresponding random copolymers of VC or VP with *N*-long alkyl maleimide for the comparison. We believe sequence control for copolymer is the key to improve the coating properties.

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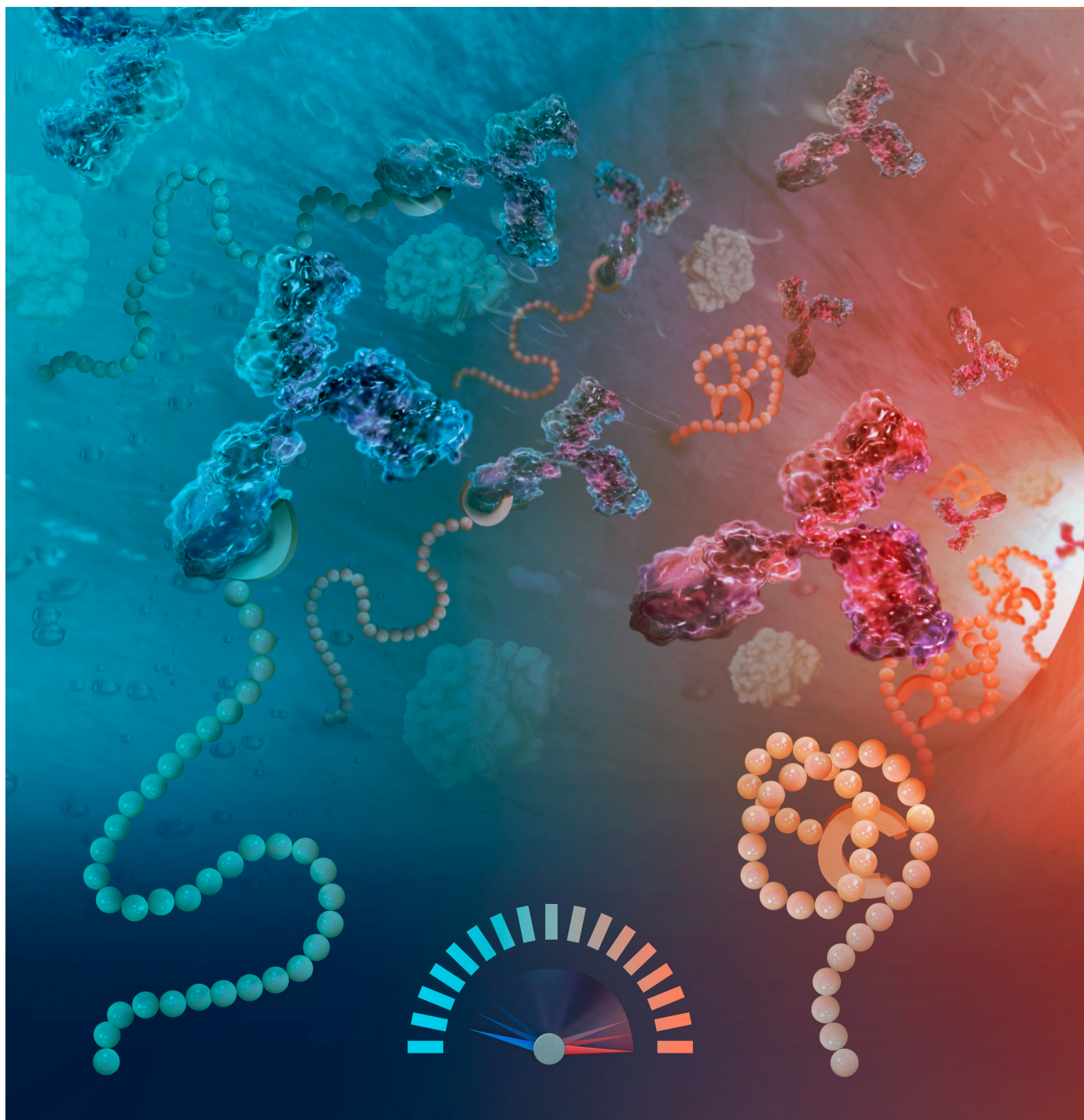
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PART III

Precision Synthesis of Copolymer for Biomaterial Application

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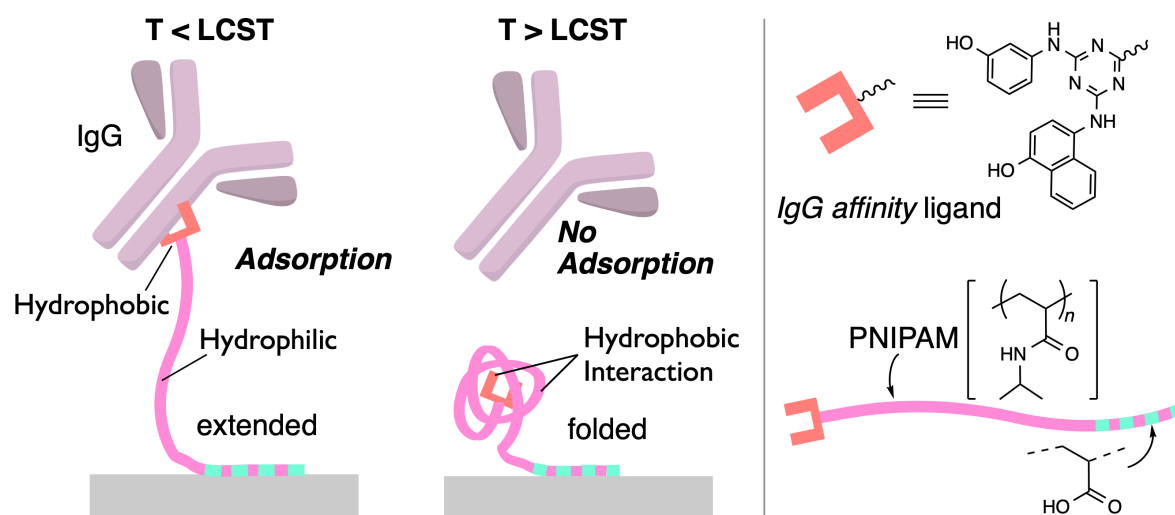
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Chapter 8

Design of Thermoresponsive Polymers Toward Antibody Purification

Abstract

The author proposed a concept of a poly(*N*-isopropylacrylamide) (PNIPAM)-based thermoresponsive antibody adsorbing material toward antibody purification; the end-functionalized PNIPAM chain can afford to adsorb antibodies below LCST and desorb upon heating due to the thermal response feature. To approach the concept, I synthesized telechelic PNIPAM derivatives through atom transfer radical polymerization (ATRP) and post reactions; an immunoglobulin G (IgG) binding ligand was incorporated at the α -terminal and some carboxylic acid groups for immobilization were embedded at another terminal. The thermoresponsive behaviors were characterized by a temperature-variable turbidity measurement and surface plasmon resonance (SPR) measurement.



Introduction

There is a growing demand on affinity materials to purify antibodies from cell extracts containing a wide variety of impurities toward application for antibody drugs and diagnostic drugs.¹ A standard strategy of antibody purification employs affinity materials for the adsorption of antibodies, from which antibodies are released with some stimulus triggers. Protein A, which specifically interacts with immunoglobulin G (IgG), has been widely used for antibody adsorption.²⁻⁴ However, there is a risk that antibodies aggregate or deteriorate during the desorption process requiring a low pH condition.⁵ To solve the problem, antibody purification materials applicable under moderate condition without relying on harsh pH trigger are in great demand.⁶

The author thus focuses on a temperature trigger for the release of antibody instead of pH stimulus. Poly(*N*-isopropylacrylamide) (PNIPAM)⁷ is a physiological thermoresponsive polymer, which is in a hydrophilic elongated form under lower critical solution temperature (LCST) and in a hydrophobic shrunk form over LCST. One advantage of the PNIPAM-based material is that the chain can be precisely modified by using a reversible deactivation radical polymerization (RDRP).⁸ Now, the controlled chain-growth polymerization systems allow to introduce functional groups for purpose in the main chain via the initiator/terminator or comonomer, which is crucial for high designability of functional materials. The author thus introduced an IgG-binding ligand at one terminal of PNIPAM via the initiator and several carboxylic acid groups at the other terminal through copolymerization (Figure 1a). Her prospect for the temperature-trigger antibody adsorption/desorption is as follows. The PNIPAM chains, which are anchored on a basic surface material through electrostatic interactions, are hydrophilic and thus expanded at below LCST to be able to dynamically adsorb IgG from cell extracts. As temperature is increased, the equilibrium should be shifted to desorption of IgG because a hydrophobic interaction of the ligand with PNIPAM chain becomes dominant at above the LCST, leading to release of the adsorbed IgG via the chain folding (Figure 1b). Herein, a protein A mimetic ligand (ligand 22/8,⁹ Figure 1c) was used to develop the IgG adsorption material because of the moderate affinity with IgG ($K_d = 7.1 \times 10^{-6}$) compared with protein A ($K_d = 2.74 \times 10^{-6}$) as well as the easy introduction by reaction with primary amine. Recently, Barner-Kowollik and Franzreb et al. have reported the development of antibody adsorption resin by utilizing thermo-responsibility of PNIPAM.¹⁰ They prepared PNIPAM-grafted agarose microparticles and introduced 4-mercaptoethylpyridine (ligand) at the outer terminal of PNIPAM to interact with IgG. Here the ligand is capable of adsorbing

IgG under a concentrated condition above the critical hydrophobicity.¹¹ The hydrophilic PNIPAM chains at a lower temperature turn into hydrophobic upon heating to interact with IgG. On the other hand, desorption takes place at a lower temperature ($< \text{LCST}$) because the apparent concentration of the ligand is decreased due to the extended chain. Her strategy differs from this report, in that one PNIPAM chain is related to both the adsorption and desorption without relying on the critical hydrophobicity. Her concept could be potentially applied to various combinations of proteins and their ligands.

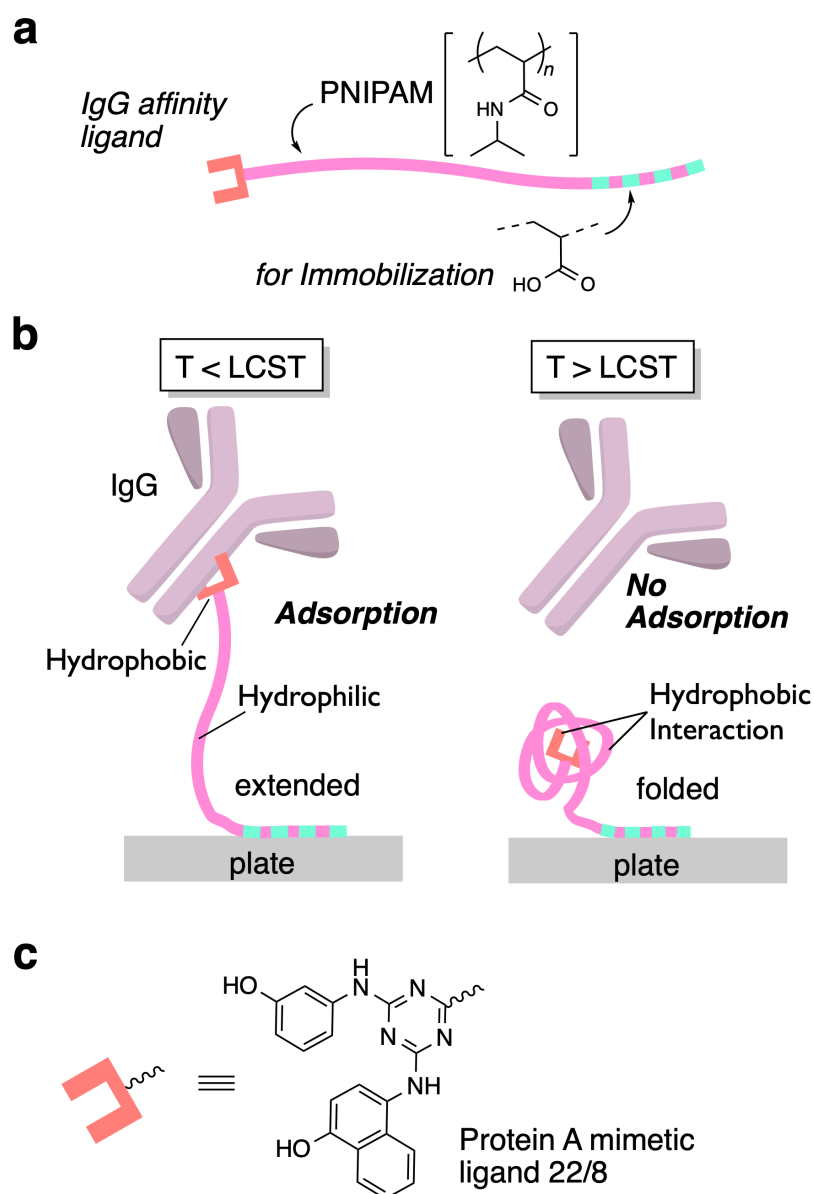


Figure 1. Concept (a) Polymer design for IgG adsorption material; (b) envisioned mechanism of switching IgG adsorption by temperature; (c) structure of ligand 22/8.

Experimental

Materials

N-Isopropylacrylamide (NIPAM, TCI; purity >98%) was recrystallized twice in acetone before use. Tris[2-(dimethylamino)ethyl]amine (Me₆TREN, Sigma Aldrich; purity 97%) was degassed by bubbling dried nitrogen for 15 min. Copper chloride (I) (CuCl, Wako; purity >99.9%), *N*-Boc-ethylenediamine (Sigma Aldrich; purity >98%), 2-chloropropanoyl chloride (TCI; purity >95%), trifluoroacetic acid (TFA, Wako; purity >98%), 1,4-dioxane (Wako, purity >99.5%), protein A mimetic ligand precursor **1** (intellim; purity >95%), human γ -globulin (NICHIIYAKU; 450 mg/3mL), were used without purification. *tert*-Butyl acrylate (TBA, Wako; purity >97%), triethylamine (Et₃N, TCI; purity >99%) were distilled before use.

Characterization

¹H NMR. ¹H NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer, operated at 500 MHz.

SEC (DMF). Molecular weight distribution (MWD) curves, number-average molecular weight (M_n) and M_w/M_n ratio of the polymers were measured by size exclusion chromatography (SEC) in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L; exclusion limit = 4×10^6 g/mol; particle size = 10 μ m; pore size < 5000 Å; 0.8 cm i.d. $\times$ 30 cm) that were connected to a Jasco PU-2080 precision pump, a Jasco RI-2031 refractive index detector and a Jasco UV-2075 UV/vis detector set at 270 nm. The columns were calibrated against 11 standard PMMA samples (PSS, Polymer Standard Service GmbH, Ready Cal Kit: M_p = 2,380 – 2,200,000).

Turbidity measurement. Temperature variable turbidity profiles were recorded in 5mM PBS buffer (pH = 7.4) on UV-1800 (Shimadzu, optical path length = 1.0 cm) at λ = 670 nm upon heating ([Polymer] = 0.5 to 1.0 mg/mL, heating speed = 1 °C/min).

HPLC. Chemical conversion of PNIPAM derivatives was monitored using high performance liquid chromatography (HPLC) equipped with a reverse-phase HPLC column (Nacalai Tesque, Cosmosil Protein-R) under a gradient elution condition (H₂O/CH₃CN with 0.1% trifluoroacetic acid).

SPR. For the surface plasmon resonance (SPR) measurement, self-assembled monolayer (SAM) of thiol-terminated poly (ethylene glycol) (PEG) was prepared on the gold surface. The PEGs (C445 and H354) were purchased from Dojindo Molecular Technologies, Inc. The adsorption and desorption of the polymers (**P2** and **P3**) and IgG were monitored by a

commercial SPR system (MP-SPR NAVITM 210A, BioNavis). 20 mM PBS buffer (pH = 7.4) was used as an elution buffer and the flow rate was 20 $\mu\text{L}/\text{min}$. The flow of the SPR experiment was as follows. First, the solution of the polymer (0.02 wt%) was applied to the SAM-modified surface at 20 $^{\circ}\text{C}$ for 10 min, and then the surface was rinsed for 90 min at 20 $^{\circ}\text{C}$. The adsorption and desorption were measured in the PBS buffer solution. Then, the PBS solution of IgG (1.0 μM) was applied to the surface at 20 $^{\circ}\text{C}$ or 36 $^{\circ}\text{C}$ for 10 min and the amount of adsorption of IgG was evaluated.

Synthesis of Initiator **2**¹²

N-Boc-ethylenediamine (984 μL , 6.25 mmol) and 20 mL of THF were added in a flask under dried argon atmosphere. Et_3N (0.91 mL, 6.6 mmol) was added with stirring and the solution was cooled to -78 $^{\circ}\text{C}$. Then, 2-chloropropanoyl chloride (0.653 mL, 6.6 mmol) was added in a dropwise for 10 min and the solution was left stirring at room temperature for 18 h. The precipitate was removed by filter and the solution was evaporated under reduced pressure. The obtained white solid was dissolved in methanol and precipitated into saturated Na_2CO_3 aq. The solvent was removed by filter and the precipitate was dissolved in methanol. The solution was filtrated, evaporated under reduced pressure and dried overnight to yield white solid (670 mg, yield 43%). ^1H NMR (500 MHz, CDCl_3): δ 4.39 (q, 1H, CH), δ 3.39 (m, 2H, CH_2), δ 3.32 (m, 2H, CH_2), δ 1.73 (d, 3H, CH_3), δ 1.45 (s, 9H, $\text{C}(\text{CH}_3)_3$). (Figure 2)

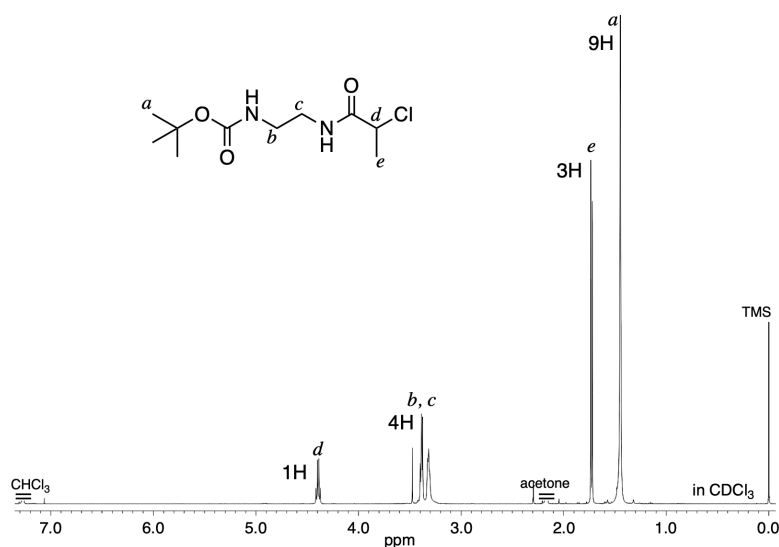


Figure 2. ^1H NMR spectrum of initiator **2** in CDCl_3 .

Polymerization and Characterization

ATRP of NIPAM with the initiator **2** was performed in 2-propanol at 25 °C: $[\text{NIPAM}]_0 = 3500 \text{ mM}$; $[\mathbf{2}]_0 = [\text{CuCl}]_0 = [\text{Me}_6\text{TREN}]_0 = 35 \text{ mM}$.¹³ The conversion of NIPAM reached 47% in 1 hour, and at this point 60 eq. (for polymer chain or initiator) of TBA was added into the solution. Herein, the amount of added TBA was roughly calculated from the remained amount of NIPAM and reactivity ratios ($r_1 = 0.4$, $r_2 = 0.8$ for *N*-propyl acrylamide (M_1) and butyl acrylate (M_2) as the model monomers for NIPAM and TBA, respectively) to make the copolymer segment of approximately 1:1 composition ratio. The polymerization was quenched in 20 min: TBA was slightly consumed ($\text{Conv.}_{\text{TBA}} = 8\%$) and NIPAM was further consumed together with TBA ($\text{Conv.}_{\text{NIPAM}} = 48\%$). Detailed process is shown below.

Polymerization was carried out by the syringe technique under dry argon in baked glass tubes equipped with three-way stopcocks. To a Schlenk tube A, NIPAM (5.00 g, 4.44×10^{-2} mol, 100 eq.), CuCl (43.7 mg, 4.44×10^{-4} mol, 1 eq.) and stirrer bar were added in 8.33 mL of 2-propanol and 0.20 mL of tetralin. Then, Me₆TREN (0.12 mL, 4.44×10^{-4} mol, 1 eq.) was added and the solution was stirred for 20 min at room temperature to form complex with CuCl and Me₆TREN completely. To an another Schlenk tube B, initiator **2** (111.7 mg, 4.44×10^{-4} mol, 1 eq.) was added in 4.20 mL of 2-propanol. Then, all the solution in the tube B was added to the tube A at room temperature. After mixing, the tube was immediately placed in water bath at 25 °C. After 60 min, 8.88 mL of the solution was sampled by a syringe under dry argon and the polymerization was terminated by bubbling with air to obtain **P4**. After sampling, degassed TBA (1.92 mL, 13.3×10^{-2} mol, 60 eq. for polymer chain or initiator) was added immediately to the tube B. After 20 min, the three-way stopcock was removed and the copolymerization was terminated by bubbling with air to obtain **P1**. Monomer conversion was determined by ¹H NMR from the integrated peak area of the olefinic protons of the monomer with tetralin as an internal standard. The two monomer conversions (NIPAM/TBA) were 47%/0% at **P4** and 48%/8% at **P1** respectively. The SEC curve of the resultant polymer was unimodal ($M_w/M_n = 1.10$) and shifted to higher molecular weight than that for before the TBA addition, indicating that copolymerization of the two comonomers likely took place from the PNIPAM chain (Figure 3). Each polymer solution was evaporated in vacuo and the polymers were dissolved in methanol. Then, the solutions were dialyzed using membrane (Spectra/Por®7, MWCO 500) in methanol for 3 days to remove unreacted monomers and Cu catalysis. After dialysis, the solutions were evaporated and freeze-dried in 1,4-dioxane to yield white powder (**P4**: 600 mg, $M_n = 8000$, $M_w/M_n = 1.08$, **P1**: 800 mg, $M_n = 9200$, $M_w/M_n =$

1.10).

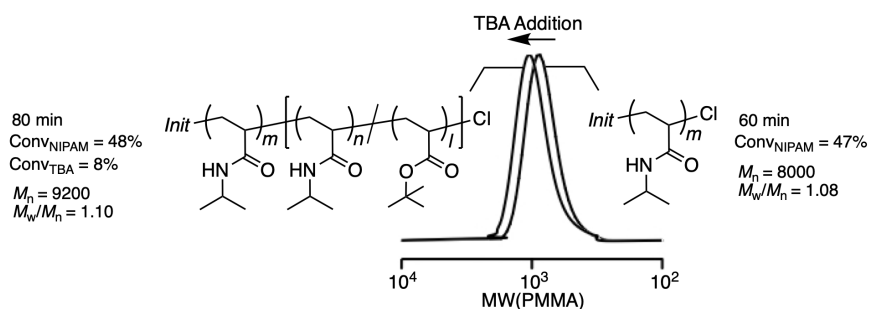


Figure 3. SEC curves of the copolymer **P1** and **P4** in THF.

The polymer structure was evaluated by ^1H NMR after dialysis for removal of low molecular weight compounds. For the polymer before the TBA addition, peaks derived from initiator moieties (a' , b' , and c' in Figure 4b), whose positions were close to those for the initiator **2** (a , b , and c in Figure 4a), were observed along with those from PNIPAM (f , g , h in Figure S2b). The number-averaged polymerization degree (m) for the PNIPAM was estimated to be 71. The value is different from the ideal value ($m = 48$) calculated by the conversion (48%). Part of the initiator molecules likely suffered from irreversible side reactions at the initial stage and thus the number of actual initiator was decreased. For the polymer after TBA addition, the signal derived from the *tert*-butyl group (i in Figure 4c) were apparently observed in addition to the signals derived from the PNIPAM before the TBA addition. Each number-averaged polymerization degree ($DP_{n,\text{NIPAM}}$ and $DP_{n,\text{TBA}}$) was similarly calculated: $DP_{n,\text{NIPAM}} = 78$ and $DP_{n,\text{TBA}} = 6$. Thus, the polymer consists of homopolymer segment of NIPAM ($m = 71$) and random copolymer segment of NIPAM and TBA ($n = 7$, $l = 6$: n and l represent number-averaged polymerization degree of NIPAM and TBA in the copolymer segment, respectively).

Introduction of Ligand via Post Reaction in **P1**

In a flask equipped with a three-way stopped cock, **P1** (723 mg) and stirrer bar were added in 15 mL of dichloromethane. Then, 15 mL of TFA was added in dropwise for 5 min at 0°C and the solution was stirred at room temperature. After 5 h, 1.5 mL of distilled water was added and the solution was stirred for 18 h. The solution was evaporated in vacuo and the residue was dissolved in water and Na_2CO_3 aq. was added to the solution to reach $\text{pH} = 10$. After dialysis in water for 3 days, the solution was evaporated in vacuo and the residue was freeze-dried in 1,4-dioxane to obtain **P2** as white powder. The ^1H NMR signal derived from the NBoc and *tert*-butyl groups (a'' , i in Figure S2c) disappeared, indicating the deprotection

was completed (Figure 4d). Deprotection of NBoc and *tert*-butyl ester was also confirmed by HPLC (Figure 5).

In a flask equipped with a three-way stopped cock, **P2** (500 mg, $M_n = 9200$, 5.4×10^{-5} mol) and ligand precursor **1** (190 mg, 5.0×10^{-4} mol) were dissolved in 17 mL of 1,4-dioxane and 6 mL of distilled water under dry argon. Then, 1.2 mL of Et_3N was added to the solution and the solution was heated in an oil bath at 100 °C for 23 h. After cooling at room temperature, the solution was evaporated in vacuo and the residue was purified by dialysis in

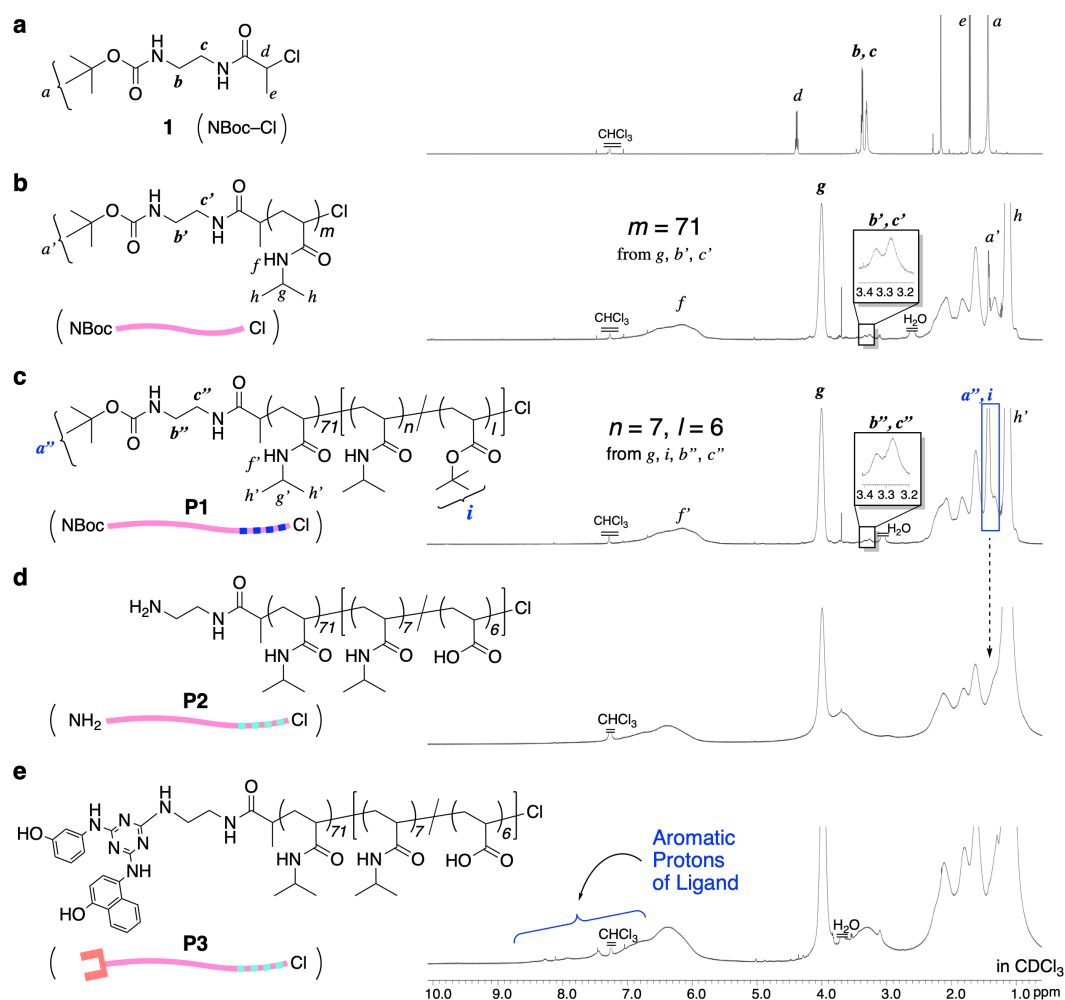


Figure 4. ^1H NMR spectra of initiator and obtained copolymers s: (a) initiator, (b) the polymer before addition of TBA (**P4**), (c) the polymer after addition of TBA (**P1**), (d) after deprotection (**P2**) and (e) after incorporation of ligand (**P3**). Polymerization: $[\text{NIPAM}]_0/[\mathbf{2}]_0/[\text{CuCl}]_0/[\text{Me}_6\text{TREN}]_0 = 3500/35/35/35$ mM in 2-propanol at 25 °C, $[\text{TBA}]_{\text{add}} = 2100$ mM.

ethanol for 1 week to remove unreacted ligand. After dialysis, the solution was evaporated and freeze-dried in 1,4-dioxane to yield **P3** as pale brown powder (355 mg). In Figure 4e, small signals were observed at lower magnetic field (around 6.0-9.0 ppm). The peaks are likely derived from the ligand, and the increase in the integration ratio supported quantitative introduction of the ligand. Induction of the ligand was also confirmed by HPLC (Figure 5).

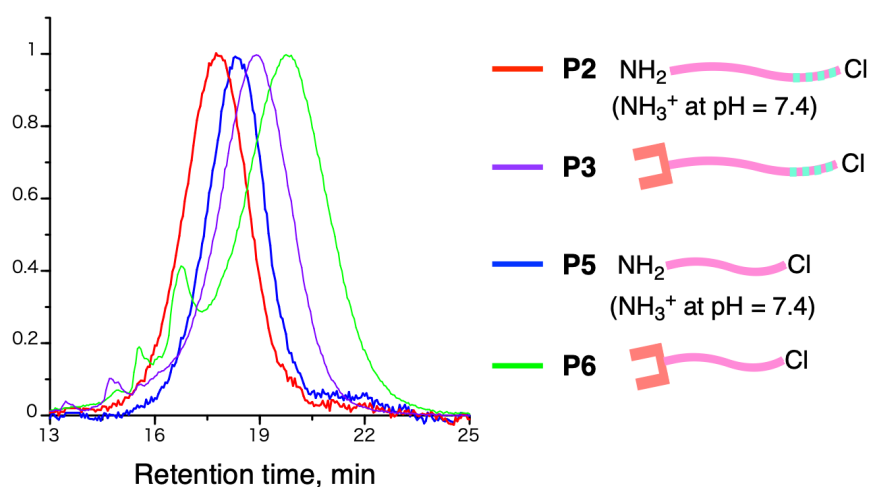


Figure 5. HPLC chromatograms of **P2**, **P3**, **P5** and **P6**. Elution condition: H₂O/CH₃CN = 90/10 to 10/90 (linear gradient, 0~20 min) + 0.1% TFA, wave length = 220 nm.

Introduction of Ligand via Post Reaction in P4

In a flask equipped with a three-way stopped cock, **P4** (548 mg) and stirrer bar were added in 11 mL of dichloromethane. Then, 11 mL of TFA was added in dropwise for 5 min at 0 °C and the solution was stirred at room temperature. After 5 h, 1.2 mL of distilled water was added and the solution was stirred for 18 h. The solution was evaporated in vacuo and the residue was dissolved in water. Then, Na₂CO₃ aq. was added to the solution to reach pH = 10. After dialysis in water for 3 days, the solution was evaporated in vacuo and the residue was freeze-dried in 1,4-dioxane to obtain **P5** as white powder. Deprotection of NBoc was confirmed by ¹H NMR (Figure 6) and HPLC (Figure 5).

In a flask equipped with a three-way stopped cock, **P5** (400 mg, $M_n = 8000$, 5.0×10^{-5} mol) and ligand precursor **1** (85 mg, 2.2×10^{-4} mol) were added in 14 mL of 1,4-dioxane under dry argon. Then, 1 mL of Et₃N was added to the solution and the solution was heated in an

oil bath at 100 °C for 19 h. After cooling at room temperature, the solution was evaporated in vacuo and the residue was purified by dialysis in ethanol for 1 week to remove unreacted ligand. After dialysis, the solution was evaporated and freeze-dried in 1,4-dioxane to yield **P6** as pale brown powder (240 mg). Introduction of the ligand was confirmed by ^1H NMR and HPLC (Figure 6 and Figure 5).

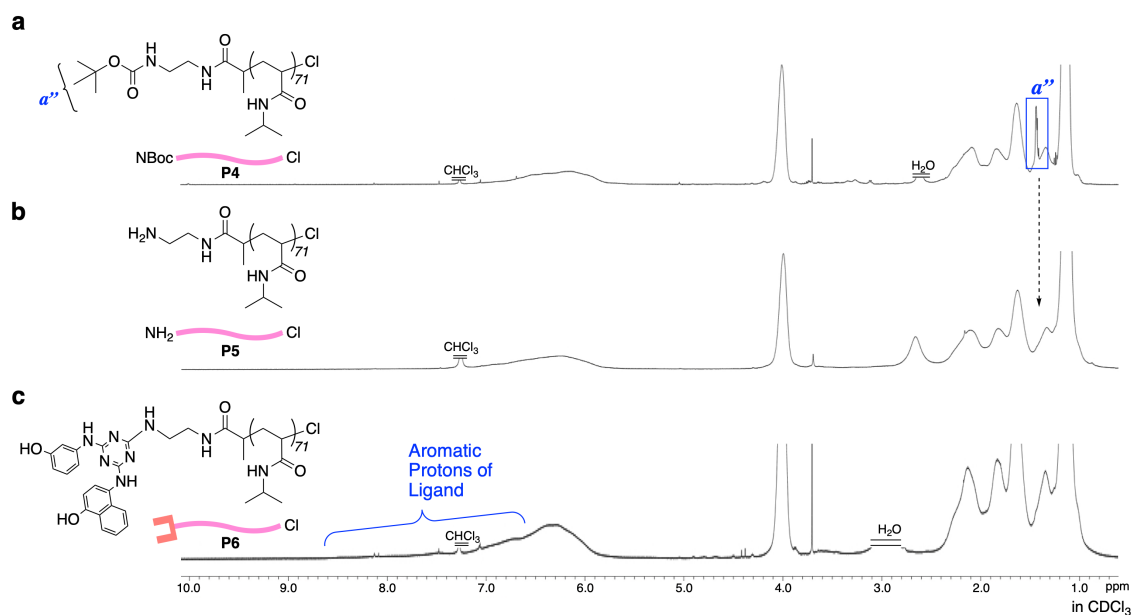


Figure 6. ^1H NMR spectra of the polymers in CDCl_3 : (a) **P4**, (b) **P5** and (c) **P6**.

Result and Discussion

Precision Synthesis of Telechelic Copolymer via ATRP and Post Reactions

The design of the target PNIPAM-based chain is as follows. A precursor of the protein A mimetic ligand (**1**, Figure 7a), where chlorine is substituted on the triazine ring, was used for the installation at the end group of PNIPAM via substitution reaction with the primary amine group. Thus, an ATRP-initiator carrying an amine protecting group (**2**)¹² was used for synthesis of PNIPAM. In the middle of the ATRP of NIPAM with **2**, *tert*-butyl acrylate (TBA) was added for incorporation of some *tert*-butyl ester pendants at the ω -end (Figure 7b). The polymerization was quenched for the NIPAM-TBA copolymer segment to be not as long as the main chain of PNIPAM. The *tert*-butyl ester can be converted into carboxylic acid by TFA together with the deprotection of the *tert*-butoxycarbonyl group on nitrogen (NBoc) at another terminal. Finally, **1** was reacted with the amine-terminated polymer for incorporation of ligand 22/8 at the terminal.

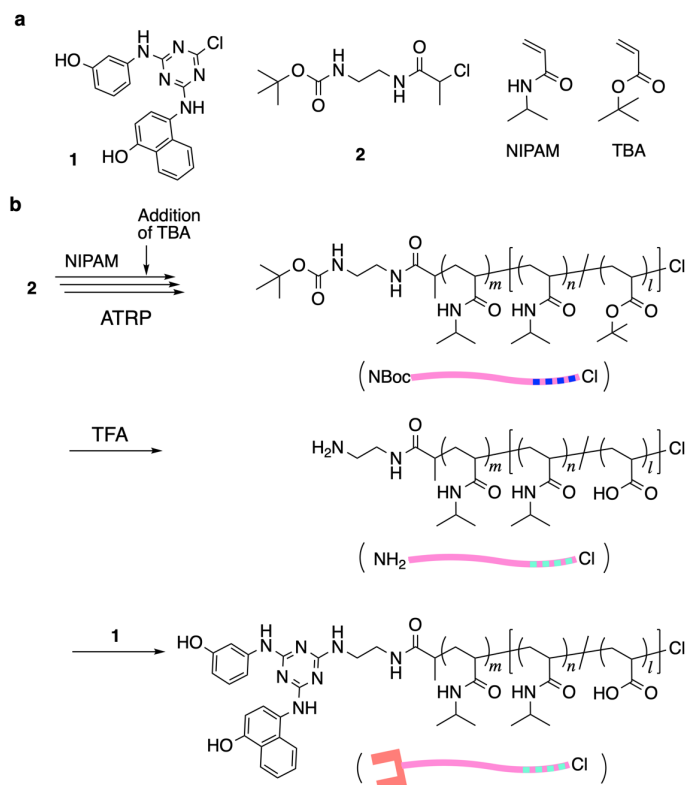


Figure 7. (a) Molecules in this chapter, chlorine-substituted ligand precursor (**1**), initiator (**2**), NIPAM and TBA, and (b) synthetic scheme for PNIPAM-based chain toward antibody purification.

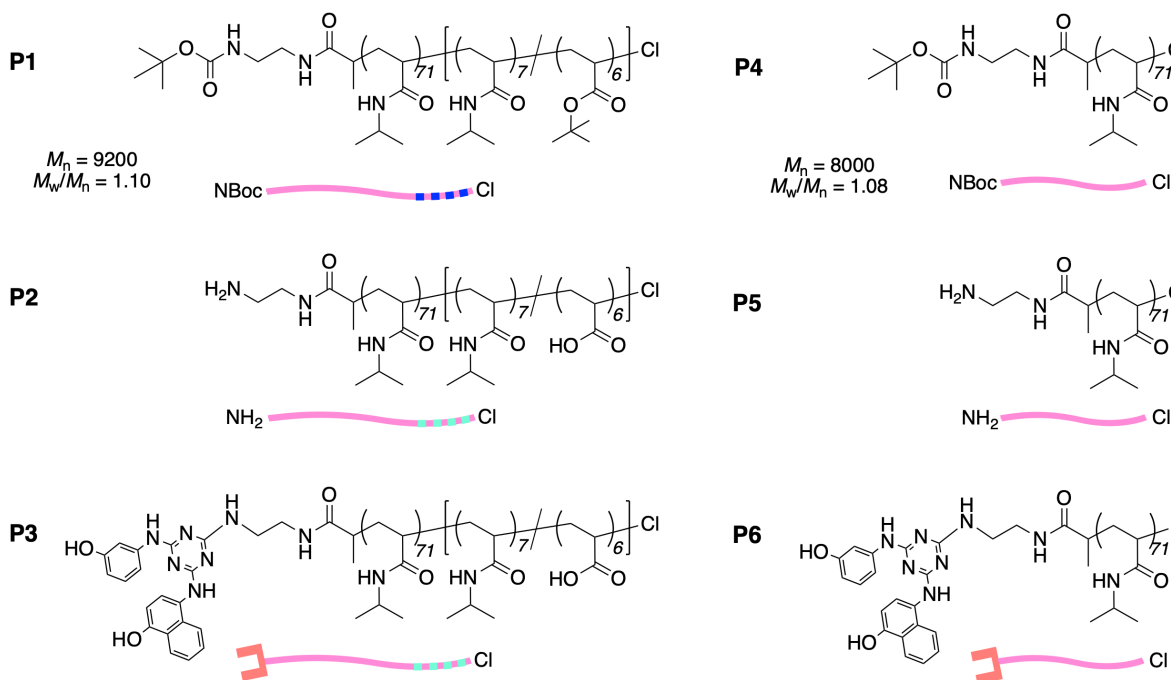


Figure 8. List of copolymers in this chapter.

Figure 8 shows 6 types of PNIPAM-based copolymers (**P1–P6**) synthesized via Atom transfer radical polymerization (ATRP)¹³ and post reactions. Here, the homopolymers (**P4–P6**) were synthesized by the same method, which was evaluated by ¹H NMR and HPLC (Figure 4-6) for evaluation of thermal response because carboxylic acid groups for immobilization in **P1–P3** would affect the turbidity measurement due to the hydrophilicity (Figure 9).

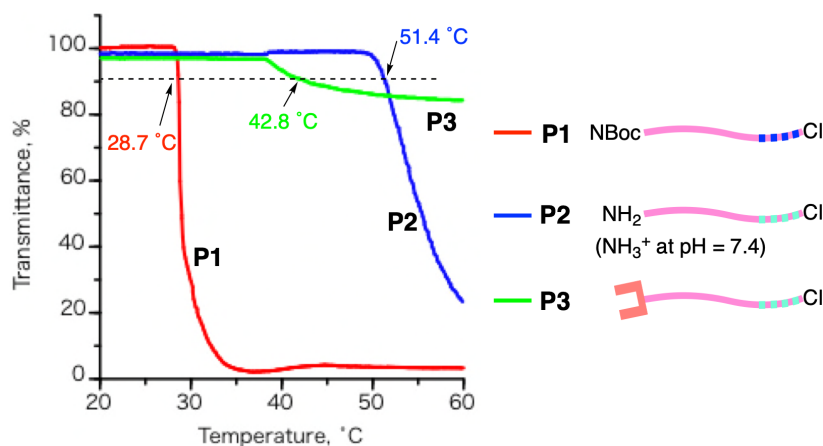


Figure 9. Temperature variable turbidity measurement for PBS buffer solutions of **P1**, **P2** and **P3**: [polymer] = 1.0 mg/mL in 5 mM PBS buffer (pH = 7.4) (heating rate: 1 °C/min).

Evaluation of Thermoresponsive Property of Copolymers by Turbidity Measurements

Thermoresponsive properties of PNIPAM chains bearing ligand 22/8 were then evaluated using model polymers (**P4–P6**) by a temperature variable turbidity measurement. The polymers were perfectly dissolved in 5 mM PBS buffer (pH = 7.4) at 5 °C and kept in a refrigerator overnight before the measurement. Turbidity of the solutions was monitored on a heating process ([polymer] = 0.5 mg/mL, heating rate = 1 °C/min, 30 – 60 °C, and λ = 670 nm). Here, the cloud point (Cp) was defined as the temperature at which the transmittance fell to 90%. Upon heating, all three polymers responded to temperature (Figure 10). The polymers before introduction of the ligand, **P4** and **P5**, showed a sharp response like general PNIPAM samples, and Cp of **P5** was higher than that of **P4** [Cp = 42.7 °C (**P4**) and 47.3 °C (**P5**)]. The higher Cp of **P5** is caused by hydrophilicity of the amine terminal. On the other hand, Cp of the ligand-ended polymer (**P6**) was lower than those of the two polymers due to the hydrophobic terminal, which agreed with the results by HPLC (Figure 5). The polymer showed a blunt and diphasic response, which was quite different from the two polymers. The

unique thermal response is not well understood, but it is rather important that the polymer showed thermal response even though such a hydrophobic and giant ligand is incorporated at the end.

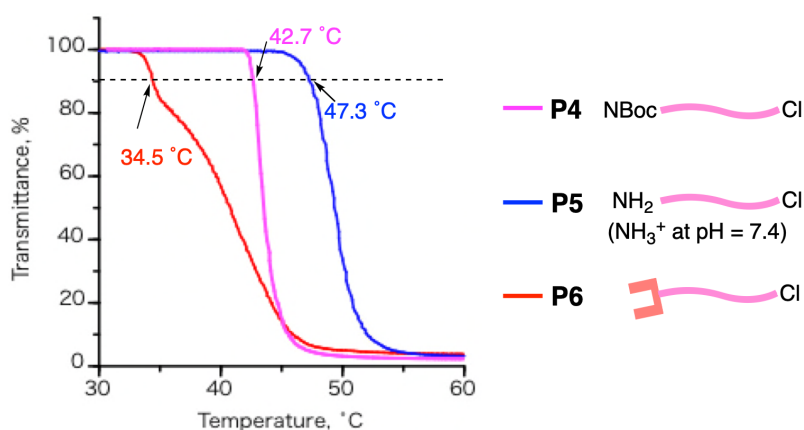


Figure 10. Turbidity measurement for PBS buffer solutions of the copolymers (**P4–P6**), [polymer] = 0.5 mg/mL in 5 mM PBS buffer (pH = 7.4) (heating rate, 1 °C/ min).

To reveal the interaction between the ligand and IgG, the thermal-response of the copolymers was evaluated in the presence of IgG. Here, about 1.3 equiv. of IgG was dissolved for one polymer chain ([polymer] = 0.5 mg/mL and [IgG] = 10 mg/mL in 5 mM PBS buffer (pH = 7.4)), and the thermal-response was evaluated in a similar manner (Figure 11abc). Interestingly, almost the same thermal-response was observed for **P4** and **P5** (a,b); whereas the transmittance curve for **P6** clearly shifted to a lower temperature (Cp was decreased by 2.5 °C) while keeping the blunt and diphasic response transition (c). The results suggest that the ligand at the end of **P6** interacts with IgG to change the transition temperature. Assuming the value of the dissociation constant ($K_d = 7.1 \times 10^{-6}$) between the ligand and IgG reported by literature,⁹ it is estimated that 79% of the polymer chains is bound to IgG in the solution. The interaction is likely strong enough to change the thermal response behavior.

Evaluation of Temperature-Dependent IgG Adsorption onto Immobilized Copolymers by SPR

Finally, the temperature-dependent behavior of IgG adsorption onto **P2** and **P3** immobilized on the amino- modified surface was evaluated by the surface plasmon resonance (SPR) analysis. The SPR analysis enables monitoring of the amount of immobilization and

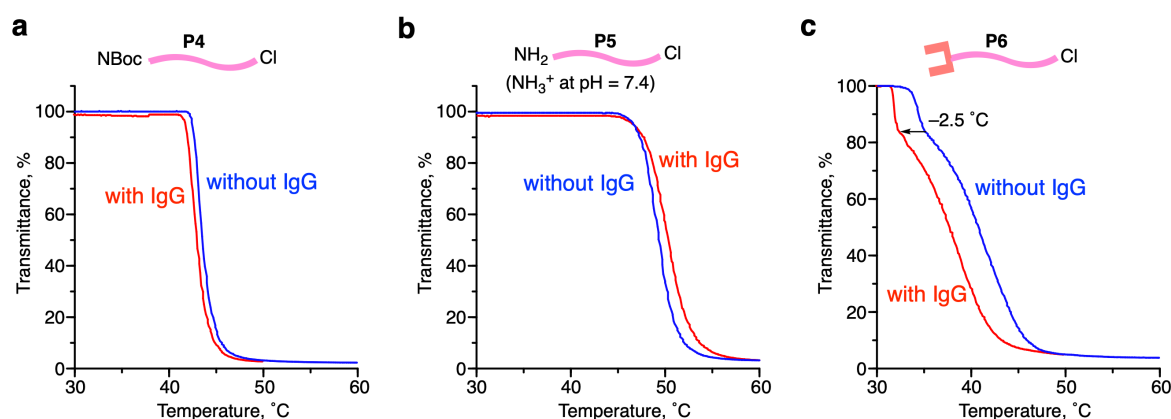


Figure 11. Effects of IgG on thermal response of (a) **P4**, (b) **P5** and (c) **P6**: [polymer] = 0.5 mg/mL and [IgG] = 10 mg/mL in 5 mM PBS buffer (pH = 7.4) (heating rate, 1 °C/min).

adsorption of these molecules in the range of pg/mm^2 .¹⁴ For the preparation of the amino-modified surface, a self-assembled monolayer (SAM) of thiol-terminated poly(ethylene glycol) [PEG, having amino groups (C445) or hydroxy groups (H354) at the other end] was prepared on the gold surface.¹⁵ H354 was used so as to prevent nonspecific adsorption of IgG on the surface.

Figure 12a shows sensorgrams of the adsorption of **P2** and **P3** on the amino-modified surface. In the measurement, the polymer solution (0.02 wt %) was applied to the surface for 10 min, and then the surface was rinsed for 90 min. The adsorption and desorption were measured in the PBS buffer solution. Both polymers showed an increase in $\Delta\theta$, which is a measure proportional to the amount of the polymer adsorbed. In the PBS buffer (pH = 7.4), the adsorption took place mainly through the electrostatic interaction between the protonated amino group on the surface and the negatively charged carboxyl group in the polymer. On the other hand, hydrophobic interaction between the ligand and the SAM would have to be considered for the adsorption because a larger amount of adsorption and desorption (at rinse) was observed for **P3** (with the ligand) compared with that for **P2** (without the ligand). The asymptotic values of polymer adsorption for **P2** and **P3** after rinse were 0.5 and 1.3 ng/mm^2 , respectively, assuming that 1 RU in $\Delta\theta$ corresponds to the adsorption of 1 pg/mm^2 .¹⁶

Figure 12b shows the amount of IgG molecules adsorbed on **P2** and **P3** immobilized on the SAM at 20 and 36 °C. In this experiment, the PBS solution of IgG (1.0 μM) was applied to the surface for 10 min, and the amount of IgG adsorption was evaluated. A larger amount of IgG (160 pg/mm^2) was adsorbed on the **P3**-immobilized surface at 20 °C. Furthermore, when the temperature was increased to 36 °C (above LCST), the amount of adsorption fell in

the same range as those on the **P2**-immobilized surfaces (70–100 pg/mm²). These results suggest that the ligand strongly captures IgG molecules below LCST but cannot capture them above LCST, probably because the hydrophobic ligand interacts with hydrophobic PNIPAM chain above LCST so as to prevent interaction with IgG. From the thermodynamic point of view, the adsorption ability is increased ($S_{\text{ads}} > 0$) as temperature rises, since the interaction between the ligand and IgG is hydrophobic. However, overcoming the inherent feature, the amount of adsorption was decreased on the **P3**-immobilized surface upon heating. This would support the validity of the concept relying on thermal response effect of PNIPAM. These SPR results indicate that **P3** functions properly, as desired; the polymer can be fixed on the surface of the substrate and manipulated to control the IgG adsorption through the thermal stimulus that results in the phase transition of the PNIPAM at LCST. These functions were realized by the molecular design with ATRP, one of the powerful tools for the syntheses of polymers with the controlled molecular structure.

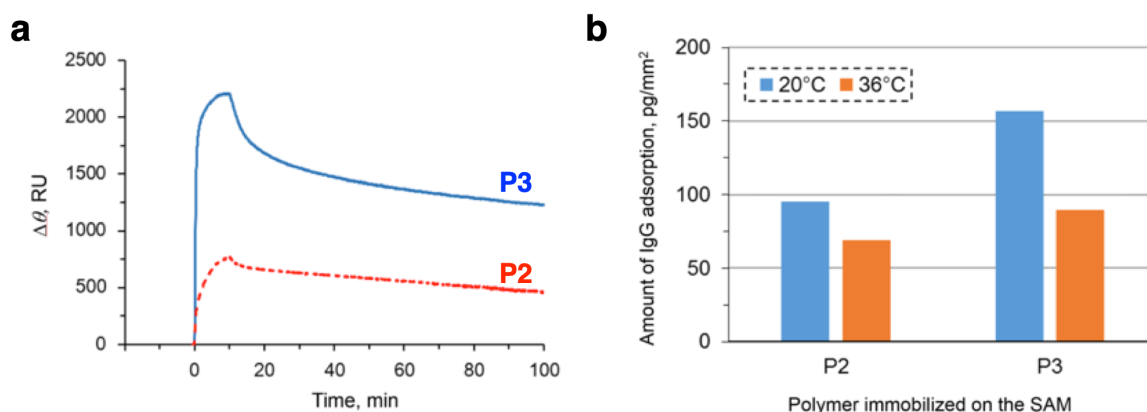


Figure 12. (a) Sensorgrams of adsorption of **P2** or **P3** on the SAM, and (b) the amount of IgG adsorbed on a polymer-immobilized surface.

Conclusion

In conclusion, a novel concept of thermoresponsive IgG adsorbing material was successfully demonstrated. The IgG-binding ligand was precisely introduced into one terminal of a PNIPAM chain, and some carboxylic acid groups were embedded into another terminal of the polymer by ATRP and post reactions. The ligand-ended PNIPAM showed LCST-type thermal responsibility (C_p , 34.5 °C), and the interaction with IgG was confirmed by the significant change in C_p in the presence of IgG. The polymer was immobilized on an amino-modified plate via ionic interaction, and IgG adsorption on the immobilized polymer

was evaluated by SPR. A trend that the ligand-ended PNIPAM on the surface effectively adsorbed IgG below LCST and the adsorption was clearly weakened above LCST was justified through my polymer design.

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List of Publications

General Introduction

“AB-Alternating Copolymers via Chain-Growth Polymerization: Synthesis, Characterization, Self-Assembly, and Functions”

Kana Nishimori, and Makoto Ouchi

Chem. Commun. **2020** *in press*.

(General introduction is the unedited Author’s version of a submitted work that was subsequently accepted for publication in *Chemical Communications*, copyright © The Royal Society of Chemistry after peer review.)

Part I

Chapter 1

“Sequence Analysis for Alternating Copolymers by MALDI-TOF-MS: Importance of Initiator Selectivity for Comonomer Pair”

Kana Nishimori, Makoto Ouchi, and Mitsuo Sawamoto

Macromol. Rapid Commun., **2016**, 37, 1414-1420.

(Chapter 1 is the unedited Author’s version of a submitted work that was subsequently accepted for publication in *Macromolecular Rapid Communications*, copyright © The Wiley Library after peer review. To access the final edited and published work see the following website: <https://doi.org/10.1002/marc.201600251>)

Chapter 2

“Design of Maleimide Monomer for Higher Level of Alternating Sequence in Radical Copolymerization with Styrene”

Kana Nishimori, Mitsuo Sawamoto, and Makoto Ouchi

J. Polym. Sci. Part A: Polym. Chem., **2019**, 57, 367-375.

(Chapter 2 is the unedited Author’s version of a submitted work that was subsequently accepted for publication in *Journal of Polymer Science Part A Polymer Chemistry*, copyright © The Wiley Library after peer review. To access the final edited and published work see the following website: <https://doi.org/10.1002/pola.29191>)

Chapter 3

“Design of Substituted Phenyl Maleimide Monomer for Radical Alternating Copolymerization with a Styrene Derivative to Achieve Compatibility of Precision Sequence Control and Side-Chain Dual Functionalization”

Kana Nishimori, and Makoto Ouchi

Submitted.

(Chapter 3 is the Author’s version of a submitted work for publication.)

Part II

Chapter 4

“Physical Gelation of AB-Alternating Copolymers Made of Vinyl Phenol and Maleimide Units: Cooperation between Precisely Incorporated Phenol and Long Alkyl Pendant Groups”

Kana Nishimori, Esther Cazares-Cortes, Jean-Michel Guigner, Francois Tournilhac, and Makoto Ouchi

Polym. Chem., **2019**, 10, 2327-2336.

(Chapter 4 is the unedited Author’s version of a submitted work that was subsequently accepted for publication in *Polymer Chemistry*, copyright © The Royal Society of Chemistry after peer review. To access the final edited and published work see the following website: <https://doi.org/10.1039/C9PY00329K>)

Chapter 5

“Tuning Gel Properties of Organogels Made by Phenolic Alternating Copolymers Through Addition of Bipyridine Derivatives”

Kana Nishimori, Esther Cazares-Cortes, Jean-Michel Guigner, Francois Tournilhac, and Makoto Ouchi

To be submitted.

(Chapter 5 is the Author’s version of an unsubmitted work that will be submitted for publication.)

Chapter 6

“Syntheses of Alternating Copolymer-Based Diblock Copolymers Toward Novel Phase Separation Behaviors”

Kana Nishimori, Mikihiro Takenaka, and Makoto Ouchi

To be submitted.

(Chapter 6 is the Author’s version of an unsubmitted work that will be submitted for publication.)

Chapter 7

“Alternating Copolymers of Vinyl Catechol or Vinyl Phenol with Alkyl Maleimide for Adhesive and Water Repellent Coating Materials”

Kana Nishimori, Mizuki Tenjimbayashi, Masanobu Naito and Makoto Ouchi

Submitted.

(Chapter 7 is the Author’s version of a submitted work for publication.)

Part III

Chapter 8

“Design of Thermo-Responsive Polymers Toward Antibody Purification”

Kana Nishimori, Masashi Maruyama, Yuzuru Shimazaki, Makoto Ouchi, and Hiroshi Yoshida

ACS Appl. Polym. Mater. **2019**, *1*, 1925-1929.

(Chapter 8 is the unedited Author’s version of a submitted work that was subsequently accepted for publication in *ACS Applied Polymer Materials*, copyright © American Chemical Society after peer review. To access the final edited and published work see the following website: <https://doi.org/10.1021/acsapm.9b00137>)

Other Publications

1 “Polymethacrylic Acid Shows Thermoresponsivity in an Organic Solvent”

Esther Cazares-Cortes, Benjamin C. Baker, Kana Nishimori, Makoto Ouchi, and Francois Tournilhac

Macromolecules, **2019**, 52, 5995–6004.

(This publication was accepted for publication in *Macromolecules*, copyright © American Chemical Society after peer review. To access the final edited and published work see the following website: <https://doi.org/10.1021/acs.macromol.9b00412>)

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