# Facile Preparation of Near Infrared-Luminescent Protein Complexes with Conjugated Polymers Consisting of Boron Azobenzene Units

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**Kazuo Tanaka**

Kazuo Tanaka received his Ph.D. degree in 2004 from Kyoto University, and worked in Stanford University, USA, Kyoto University, and RIKEN as a postdoctoral fellow. In 2007, he has moved to the Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, and in 2018, he was promoted to Professor. His research projects especially focus on design of new functional materials relating optics and nanotechnology based on the heteroatom-containing conjugated polymers and organic-inorganic polymer hybrids.

**Abstract**

We report herein facile preparation for near infrared (NIR)-luminescent protein complexes with conjugated polymers. We have discovered that solid-state luminescence in the NIR region can be obtained from the series of conjugated polymers consisting of boron azobenzene complexes. We demonstrate in this paper that protein molecules can be modified through adsorption with the boron azobenzene-containing conjugated polymers simply by mixing in the aqueous buffer and subsequently purification with filtration followed by freeze drying. The modified protein complexes can exhibit NIR emission in the buffer and high dispersibility. In particular, comparing to the complex with indocyanine green (ICG), which is a conventional NIR-luminescent dye, polymer-modified protein complexes showed higher resistance to photobleaching. Finally, by using lipase as a scaffold, we confirmed that enzymatic activity can be detected after polymer modification.

**Keywords:** Conjugated Polymer; Near-Infrared Luminescence; Azobenzene; Protein Complex

## 1. Introduction

By employing albumin as a carrier, efficient drug delivery can be realized. This methodology is called as the nanoparticle albumin-bound (nab) technology and suitable especially for transporting hydrophobic molecules, such as paclitaxel and dyes.[1–3] Since protein complexes can be prepared through adsorption, further modifications to both protein and loaded molecules are not necessary. In addition, large amounts of molecules can be loaded and transported through the nab technology. These properties in the nab technology are also advantageous for developing intense emissive materials.[4] Because of advantageous physical properties of NIR light, such as high permeability through vital bodies, NIR-luminescent dyes are the essential building-block for constructing bio-sensors and bio-probes.[5] Therefore, the development of NIR-luminescent materials is of importance for realizing bioimaging and biosensing at deep spots inside bodies.[6,7] To apply organic dyes under biological conditions, high stability and biocompatibility are generally required. However, due to expanded π-conjugated systems, which can generate NIR light originated from narrow energy gaps between frontier molecular orbitals, especially conventional dyes show poor solubility in buffer. Moreover, in solid, luminescent properties are often spoiled due to concentration quenching caused by non-specific intermolecular interactions in the condensed state.

To overcome these problems, we have recently proposed the new idea for NIR-luminescent material designs without expansion of π-conjugated systems.[8] By replacing the skeletal carbon to nitrogen at an isolated lowest unoccupied molecular orbital (LUMO) where only LUMO is distributed, narrow energy gaps, followed by NIR emission, can be obtained. Based on this concept, we have designed deep-red and/or NIR-luminescent molecules and polymers with narrow-energy gaps from the small molecules.[9–12] As a typical example, it was shown that luminescent polymers containing the boron azomethine units can be transformed into NIR-luminescent materials involving the boron azobenzene units by inserting nitrogen at the isolated LUMO positions into boron azomethine in the polymer main-chains.[13,14] Finally, owing to the limited size of π-conjugated systems, we can suppress emission annihilation caused by concentration quenching even in polymer films.[15] As a consequence, we obtained a series of NIR-luminescent polymers with solid-state luminescent properties based on boron azobenzene units.[15–17] Furthermore, some of azobenzene derivatives can show permeability through blood brain barrier.[18] As a consequence, from Kanai and coworkers, it was reported that the photo-responsive agent which can decompose protein aggregation triggered by light irradiation can be successfully obtained.[18] Thus, it can be said that azobenzene-relating materials including polymers have abundant possibilities for the application of biomaterials although azobenzene derivatives have been utilized in material science so far. On the basis of the molecular design with the nitrogen substitution, the energy level of the highest occupied molecular orbital (HOMO) is hardly influenced. Therefore, it is presumed that the nitrogen-substituted molecules can maintain high resistance toward oxidative decomposition and other degradation. To verify this assumption, we designed the experimental scheme based on the conventional manner with our materials.

Herein, we report preparation of protein complexes with NIR-luminescent conjugated polymers involving boron azobenzene units. Initially, based on the nab technology, conjugated polymers were accumulated onto protein molecules by titrating the tetrahydrofuran (THF) solution of each polymer

to the aqueous solution of protein. The synthesized complexes exhibited NIR emission in the buffer. In particular, their emission properties of the protein complexes showed higher durability toward UV irradiation, comparing to that of the indocyanine green (**ICG**)-adsorbed protein. Furthermore, by using lipase as a protein scaffold, we confirmed that the modified protein can show enzymatic activity. We demonstrate here the facile method for preparing NIR-luminescent enzyme without chemical modification to polymers as well as proteins.

## 2. Results and Discussion

Figure 1 shows the NIR-luminescent materials adsorbed onto proteins. To improve the adsorption ability onto protein surfaces and to demonstrate generality of this protocol, the conventional comonomers, such as alkyl-modified fluorene and bithiophene, were used in the polymer synthesis. In order to suppress concentration quenching, the phenyl-substituted boron complex, **BAz-Ph**, was also used.[16] The **BAz-F** monomer having bis-bromine groups was prepared according to the previous report (Schemes S1–S3).[14] The syntheses of conjugated polymers were conducted through Migita–Kosugi–Stille cross-coupling polymerizations in a catalytic system involving $Pd_2(dba)_3$ (dba = dibenzylideneacetone) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos) according to the previous reports.[15] The polymer properties were determined from a gel permeation chromatography (GPC) with polystyrene standards (Table S1). All polymers have almost same chain lengths and similar solubility in organic solvents including THF. The polymers showed very poor solubility in aqueous media, suggesting that adsorption onto protein should proceed efficiently in aqueous media.

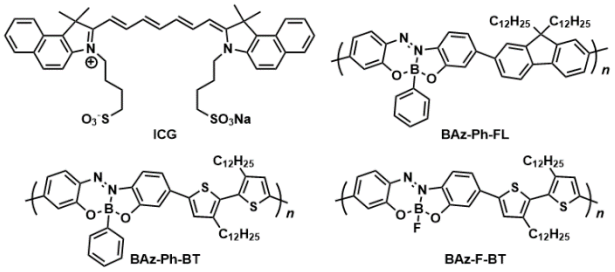
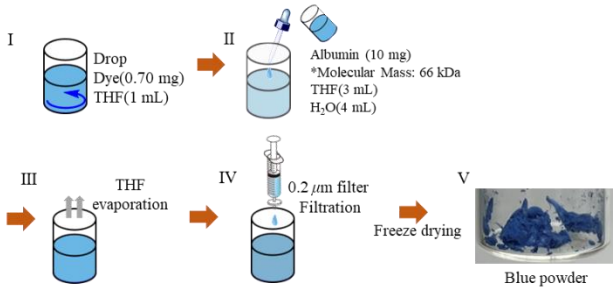



**Figure 1.** Chemical structures of NIR-luminescent materials used in this study.

Next, the protein complexes with conjugated polymers, **BAz-Ph-FL**, **BAz-Ph-BT**, **BAz-F-BT**, and **ICG** were prepared (Figure 1). The general protocol for the preparation of the protein complexes is as follows (Scheme 1); Initially, for preparing stock solutions, 0.7 mg of each polymer and **ICG** was dissolved in 1 mL of THF. Then, 3 mL of the stock solution in THF was injected under sonication into 4 mL of deionized water containing 10 mg protein (albumin or lipase used in this study). Subsequently, THF was removed by evaporation under vacuum with a rotary evaporator, followed by filtration through a membrane filter with 0.2 $\mu$m cutoff. After freeze-drying, the protein complex was obtained as a blue solid (Table S2). The complexes showed good dispersibility in aqueous media including deionized water and the PBS buffer. Comparing to the pristine state, almost same hydrodynamic radii were observed from the complexes (Figure S1), clearly supporting good dispersion in aqueous media. The complexes were able to be stored at room temperature in powder for a week and even in water dispersion at least for several days without any precipitation and loss of optical properties. Stability of the products indicates that fusion of protein complexes as well as separation of each component should be suppressed in the complexes.




**Scheme 1.** Schematic illustration of the preparation for protein complexes.

The amounts of polymers adsorbed onto protein were evaluated (Table 1). From the dispersion of each complex in PBS, the similar shape of the UV–vis absorption spectrum with the solution of the corresponding polymer was obtained, clearly indicating that adsorption can proceed (Figures S2–S4). From the calculation of stoichiometry, it was shown that several chains should be included in the single complex molecule (Table 1). **BAz-Ph-BT** showed the highest amount of adsorption, followed by **BAz-F-BT**, meaning that the BT comonomer is favorable for enhancing protein adsorption. The planar structure of the BT unit could be responsible for improving the adsorption amount.[19,20]

**Table 1.** Spectroscopic data of polymers in solution

| Compounds | Solvents | $\lambda_{abs}$ /nm | $\lambda_{PL}$ /nm | $\Phi_{PL}$ [c] /% | Adsorption mass /$\mu$g | Adsorption mole per 1 mg of albumin /$10^{-2}$ $\mu$mol[e] |
|---|---|---|---|---|---|---|
| **ICG** | PBS[a] | 779 | 810 | 1.3 | 30.4 | 3.92 |
| | PBS[b] | 797 | 822 | 3.3 | | |
| **BAz-Ph-FL** | $CHCl_3$[a] | 616 | 663 | 2.3 | 0.3 | 0.03 |
| | PBS[b] | 618 | 677 | –[d] | | |
| **BAz-Ph-BT** | $CHCl_3$[a] | 646 | 771 | 8.4 | 32.9 | 4.04 |
| | PBS[b] | 646 | 802 | 2.5 | | |
| **BAz-F-BT** | $CHCl_3$[a] | 639 | 795 | 6.9 | 10.8 | 1.32 |
| | PBS[b] | 639 | 850 | 1.8 | | |

[a]$1.0\times10^{-5}$ M. [b]0.5 mg(albumin-polymer)/1 mL(PBS buffer). [c]Absolute PL quantum efficiency excited at $\lambda_{abs}$. [d]Not detected. [e]Albumin (66,000 Da) 1 mg = $1.5\times10^{-2}$ $\mu$mol.

Luminescent properties of the protein complexes were evaluated (Figure 2 and Table 1). The photoluminescence (PL) spectra were compared among each polymer in the diluted chloroform solution ($1.0\times10^{-5}$ M), in film and the protein complex in PBS (0.5 mg/mL). Compared to the PL spectra of Figures 2a and 2b, red-shifted spectra were observed from all complexes similarly to those in film, suggesting that the chromophore units should be aggregated at the protein surface (Figure S5 and Table S3). Although the emission quantum yields ($\Phi_{PLS}$) decreased, it is clearly demonstrated that the protein complexes containing **BAz-Ph-BT** and **BAz-F-BT** can provide NIR emission. It should be mentioned that larger Stokes shifts, which is advantageous to avoid the noise caused by autofluorescence, were observed from these complexes than that of **ICG**. Because of strong electron-donating ability of the **BT** unit, NIR emission with large Stokes shifts should be induced. This property is advantageous for reducing noises generated by light scattering of excitation light in the microscopic observation.

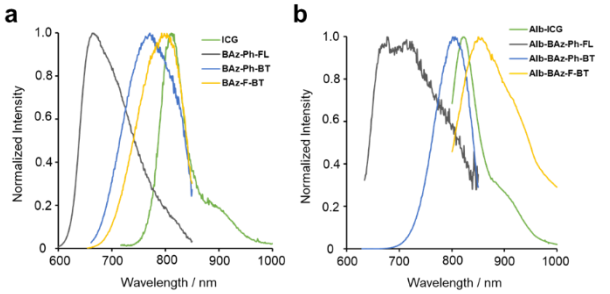



**Figure 2.** PL spectra of (a) **BAz** polymers and **ICG** in the diluted chloroform solutions ($1.0\times10^{-5}$ M) and (b) protein complexes in PBS (0.5 mg/mL).

Organic dyes commonly suffer from photobleaching caused by continuous light irradiation during microscopic observations. Moreover, in the case of luminescent dyes, reduction of emission intensity is critically induced by excitation light. Since these losses of optical properties caused by light irradiation led to decrease in detection sensitivity of optical probes, enhancement of photo-stability of luminescent materials is of great significance.[21,22] We examined the enhancement of photo-stability of polymers by the complexation with protein (Figures 3, S6–S10). We prepared the samples containing each protein complex and **ICG** in PBS and monitored changes in absorption properties under UV irradiation (254 nm). The averaged values were obtained from the data sets of the independent five times. Accordingly, the absorbance value of **ICG** drastically dropped within 10 min, and approximately 90% of the absorption property was lost after 20 min. In contrast, it should be noted that almost same levels of absorption properties of the protein complexes can be maintained even after 60 min irradiation. Moreover, NIR emission can be observed from the protein complexes containing **BAz-Ph-BT** and **BAz-F-BT** after 60 min irradiation. These data clearly indicate that optical properties can be preserved from photo-degradation by the protein complexation. Furthermore, it can be said that robust NIR-luminescent materials can be obtained. Since almost same degree of degradation proceeded in the diluted sample containing **Alb-ICG**, aggregation of the complexes should be negligible (Figure S11). Therefore, it can be said that durability of polymers should play a key role in high robustness.

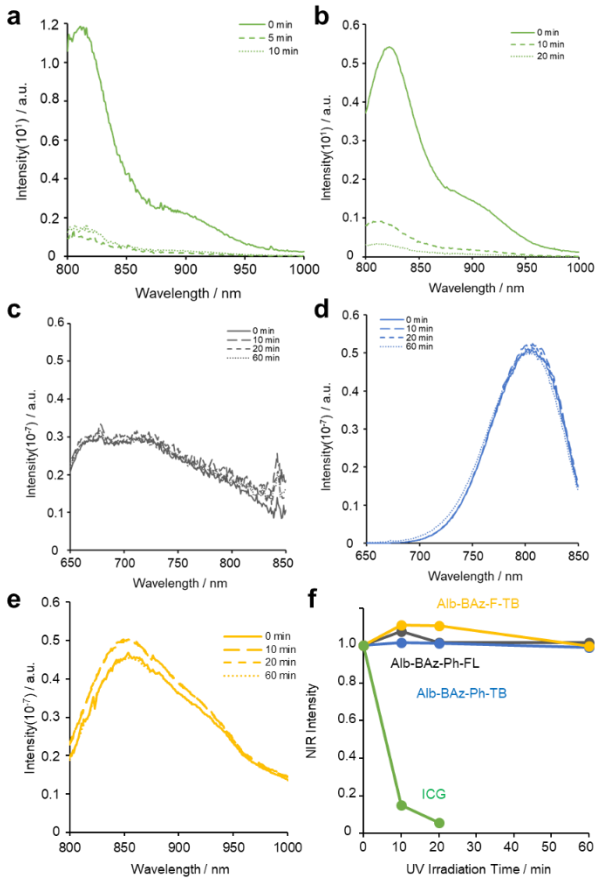



**Figure 3.** Time courses in PL spectra of (a) **ICG**, (b) **Alb-ICG**, (c) **Alb-BAz-Ph-FL**, (d) **Alb-BAz-Ph-BT** and (e) **Alb-BAz-F-BT** in PBS (0.5 mg/mL) under UV irradiation.

Finally, to investigate influence of polymer adsorption on polymer functions, we prepared protein complexes with lipase and evaluate enzymatic activity (Tables S4 and S5 and Figures S12–S17). We fabricated the protein complexes with lipase through the same method described above, and the enzymatic activity was estimated from the decomposition rate of coumarin ester. Although reaction rates were drastically lowered by the protein complexation (Table S6), we confirmed that enzymatic reactions can proceed in the presence of the protein complexes. Since larger amounts of polymers can be adsorbed onto lipase protein, accessibility of the substrate to the reaction pocket followed by enzymatic reactions should be restricted.

## 4. Conclusion

We demonstrate facile preparation of NIR-luminescent protein complexes with conjugated polymers. Through simple mixing with protein and a polymer in a mixture solvent, protein labelling can be achieved without covalent bonds. By replacing the type of polymers or protein, it is easy to tune material properties. The synthesized luminescent complexes can show high durability toward light irradiation. Moreover, it was demonstrated that enzymatic properties can be obtained from the complex. Finally, on the basis of our study, not only robust NIR-luminescent protein complexes but also NIR-luminescent enzyme can be obtained. By the combination with optoelectronic polymers, development of advanced materials could be expected.

**Acknowledgement**

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biomedical engineering (for M.G.) and a Grant-in-Aid for Scientific Research (B) (for M.G.) (JP22H02130) and for Scientific Research (B) (for K.T.) (JP21H02001).

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**Graphical Abstract**

<Title>
Facile Preparation of Near Infrared-Luminescent Protein Complexes with Conjugated Polymers Consisting of Boron Azobenzene Units

<Authors' names>
Dahye Yoo, Masashi Nakamura, Misato Kanjo, Masayuki Gon, Hiroyuki Watanabe, Hiroshi Kita and Kazuo Tanaka*

<Summary> 50-60 words
Near infrared-emissive protein complexes with conjugated polymers were prepared. It was revealed that their properties were complementary depending on the substitution site. The C-substituted compound showed AIE-active ICT emission, and the B-substituted one showed AIE-inactive LE emission. Various experiments including optical measurements, single-crystal X-ray diffractions, and DFT calculations fully supported our results.

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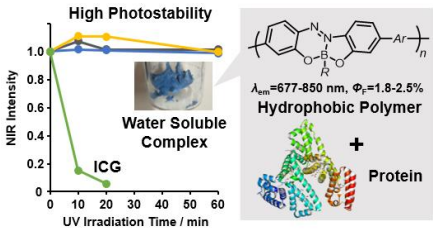

# Supporting Information

**Facile Preparation of Near Infrared-Luminescent Protein Complexes with Conjugated Polymers Consisting of Boron Azobenzene Units**

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## General

$^1$H NMR spectra was recorded on a JEOL AL400 at 400 MHz in $CDCl_3$. The chemical shift values were expressed relative to $Me_4Si$ for $^1$H NMR as an internal standard in $CDCl_3$. Gel permeation chromatography (GPC) was carried out on a SHIMADZU Prominence system equipped with three consecutive polystyrene gel columns (TSK gels: α-4000, α-3000, α-2500). System was operated at 40 °C and a flow rate of 1.0 mL/min with $CHCl_3$ as an eluent. Polystyrene standards were employed for calibration. Recyclable preparative high performance liquid chromatography (HPLC) was carried out on Japan Analytical Industry Model LaboACE LC-5060 (JAIGEL-2.5H and 3HH columns) using $CHCl_3$ as an eluent. The protein complexes of polymers were dried on EYELA FDU-2100 freeze drier for 18 h. UV–vis absorption spectra were recorded on a SHIMADZU UV-3600i plus spectrophotometer, and samples were analyzed at room temperature. Fluorescence emission spectra were recorded on a HORIBA Scientific Fluorolog-3 spectrofluorometer and samples were analyzed at room temperature with PMT P928 (250~810 nm) and DSS-IGA (810~1550 nm) as detectors. Absolute fluorescence (FL) quantum efficiency ($\Phi_{FL}$) was recorded on a Hamamatsu Photonics Quantaurus-QY Plus C13534-01 equipped with infrared measurement unit C13684-01 using an integrating sphere. Irradiation was performed by transilluminator (3UV LMS-20E, 254 nm) for photobleaching test.

## Materials

Commercially available compounds used without purification:

$Pd_2(dba)_3$ (dba = dibenzylideneacetone) (Tokyo Chemical Industry Co, Ltd.)

2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (Strem Chemicals, Inc.)

Commercially available solvents:

MeOH (FUJIFILM Wako Pure Chemical Corporation.), toluene (deoxidized grade, FUJIFILM Wako Pure Chemical Corporation), $CHCl_3$ (FUJIFILM Wako Pure Chemical Corporation.), THF (Stabilizer free grade, FUJIFILM Wako Pure Chemical Corporation.), Phosphate buffered saline solution (PBS) (10x, pH 7.4, Nacalai Tesque, Inc.) used without purification.

Deionized water was obtained from a Merck Elix-Essential-3 instrument with a Progard TS2 Pretreatment Pack.

Compounds prepared as described in the literatures:

5,5′-Bis(trimethylstannyl)-3,3′-didodecyl-2,2′-bithiophene (**BT**) [1,2]

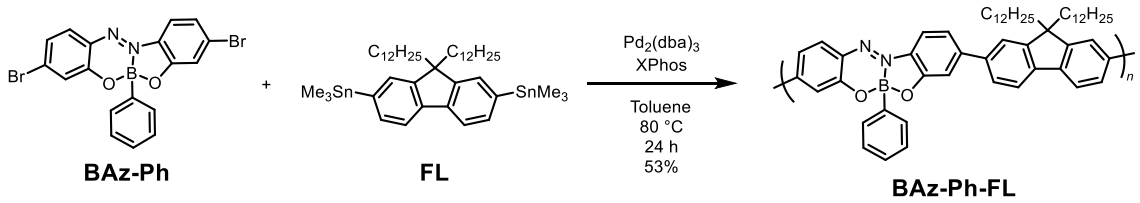
9,9-didodecyl-9H-fluorene-2,7-diyl)bis[trimethylstannane] (**FL**) [3]

**BAz-F-BT** [3]

**BAz-Ph** [4]

## Synthetic Procedures and Characterization

### Synthesis of BAz-Ph-FL




**Scheme S1.** Synthesis of **BAz-Ph-FL**.

A mixture of **BAz-Ph** (45.8 mg, 0.100 mmol), (9,9-didodecyl-*9H*-fluorene-2,7-diyl)bis[trimethylstannane] (**FL**) (82.8 mg, 0.100 mmol), $Pd_2(dba)_3$ (2.7 mg, 0.003 mmol), XPhos (2.9 mg, 0.006 mmol) was placed in a round-bottom flask equipped with a magnetic stirring bar. After degassing and filling $N_2$ three times, toluene (2.0 mL) was added to the mixture. The reaction was carried out at 80 °C for 24 h. After the reaction, the obtained polymer was redissolved in a small amount of $CHCl_3$, and then the product was reprecipitated from MeOH. The polymer collected by filtration was dried in vacuo to afford **BAz-Ph-FL** (63.2 mg, 53%) as a blue solid.

**BAz-Ph-FL**: $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.91 (d, $J$ = 8.2 Hz, 1H), 7.75–7.66 (br, 6H), 7.58 (s, 1H), 7.52 (s, 1H), 7.46–7.41 (br, 6H), 7.21–7.20 (br, 4H), 2.09 (br, 6H), 1.26–1.09 (br, 70H), 0.86–0.83 (br, 11H), 0.72 (br 4H) ppm.

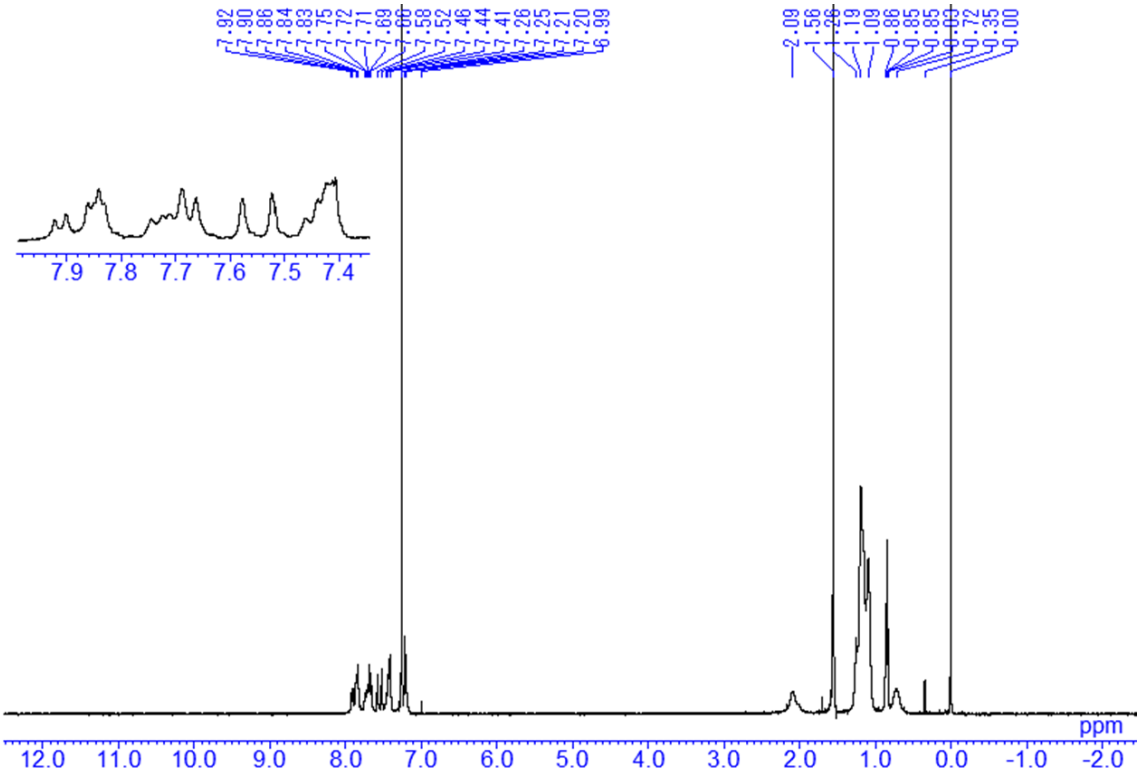
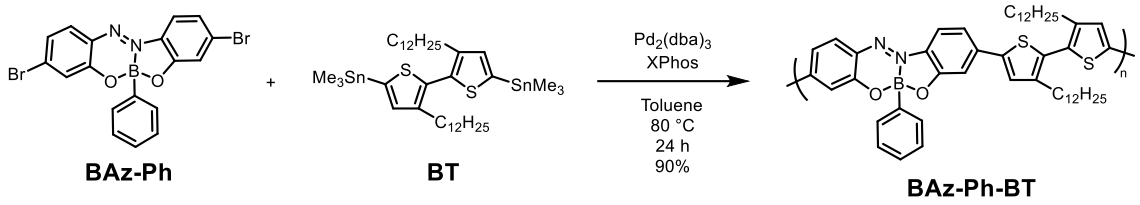



**Chart S1.** $^{1}$H NMR spectrum of **BAz-Ph-FL** in $CDCl_3$.

**Synthesis of BAz-Ph-BT**




**Scheme S2.** Synthesis of **BAz-Ph-BT**.

A mixture of **BAz-Ph** (45.8 mg, 0.100 mmol), 5,5′-bis(trimethylstannyl)-3,3′-didodecyl-2,2′-bithiophene (**BT**) (82.9 mg, 0.100 mmol), $Pd_2(dba)_3$ (2.7 mg, 0.003 mmol), XPhos (2.9 mg, 0.006 mmol) was placed in a round-bottom flask equipped with a magnetic stirring bar. After degassing and filling $N_2$ three times, toluene (2.0 mL) was added to the mixture. The reaction was carried out at 80 °C for 24 h. After the reaction, the obtained polymer was redissolved in a small amount of $CHCl_3$, and then the product was reprecipitated from MeOH. The polymer collected by filtration was dried in vacuo to afford **BAz-Ph-BT** (72.2 mg, 90%) as a blue solid.

**BAz-Ph-BT**: $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, *J* = 8.3 Hz, 1H), 7.70 (d, *J* = 8.5 Hz, 1H), 7.46–7.39 (br, 4H), 7.36–7.32 (br, 4H), 7.20–7.14 (br, 3H), 2.56 (br, 5H), 1.63 (br, 4H), 1.23 (br, 36H), 0.86 (t, J = 9.8 Hz, 8H) ppm.

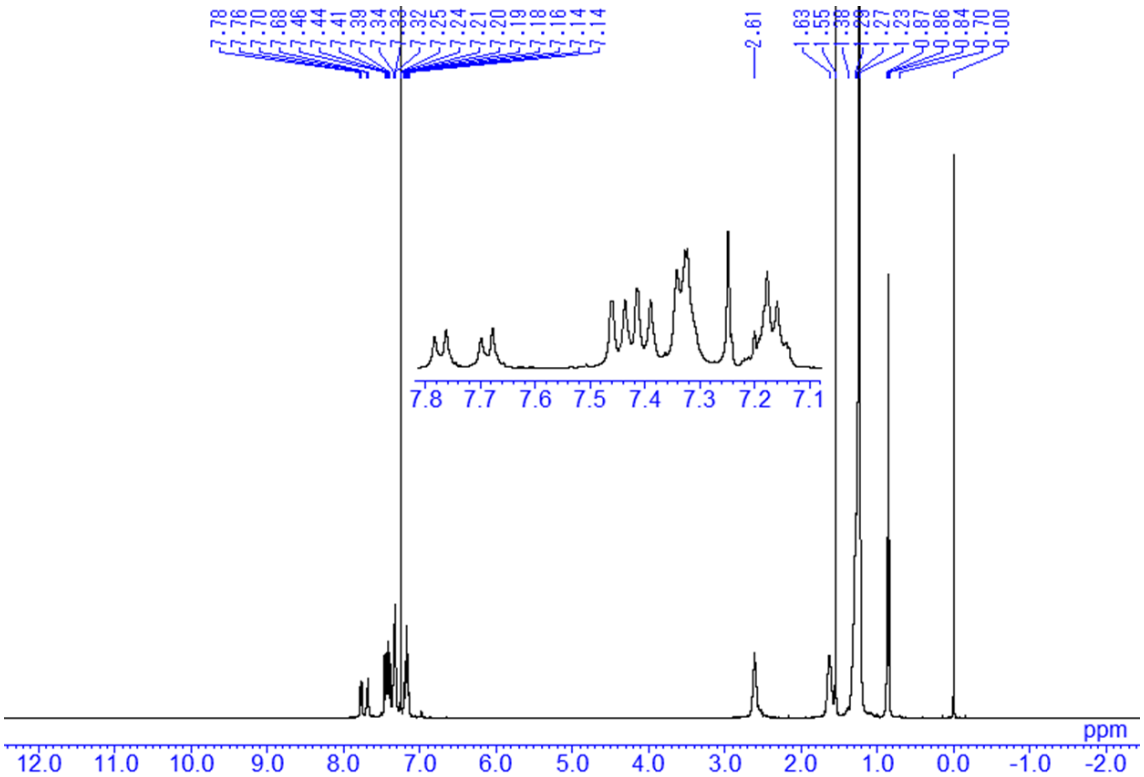



**Chart S2**. $^{1}$H NMR spectrum of **BAz-Ph-BT** in $CDCl_3$.

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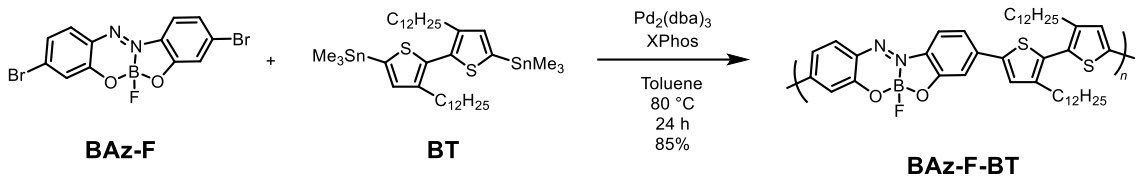
**Synthesis of BAz-F-BT**




**Scheme S3.** Synthesis of **BAz-F-BT**.

According to the previous work (Gon M, Tanaka K, Chujo Y. Angew Chem Int Ed. 2018;57(22):6546−6551)

**BAz-F-BT**: $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.92 (d, $J$ = 8.3 Hz, 1H), 7.81 (d, $J$ = 8.4 Hz, 1H), 7.52−7.37 (m, 6H), 2.63 (br, 4H), 1.64 (br, 3H), 1.56(s, 5H), 1.24 (br, 45H), 0.87 (t, $J$ = 6.8 Hz, 6H) ppm.

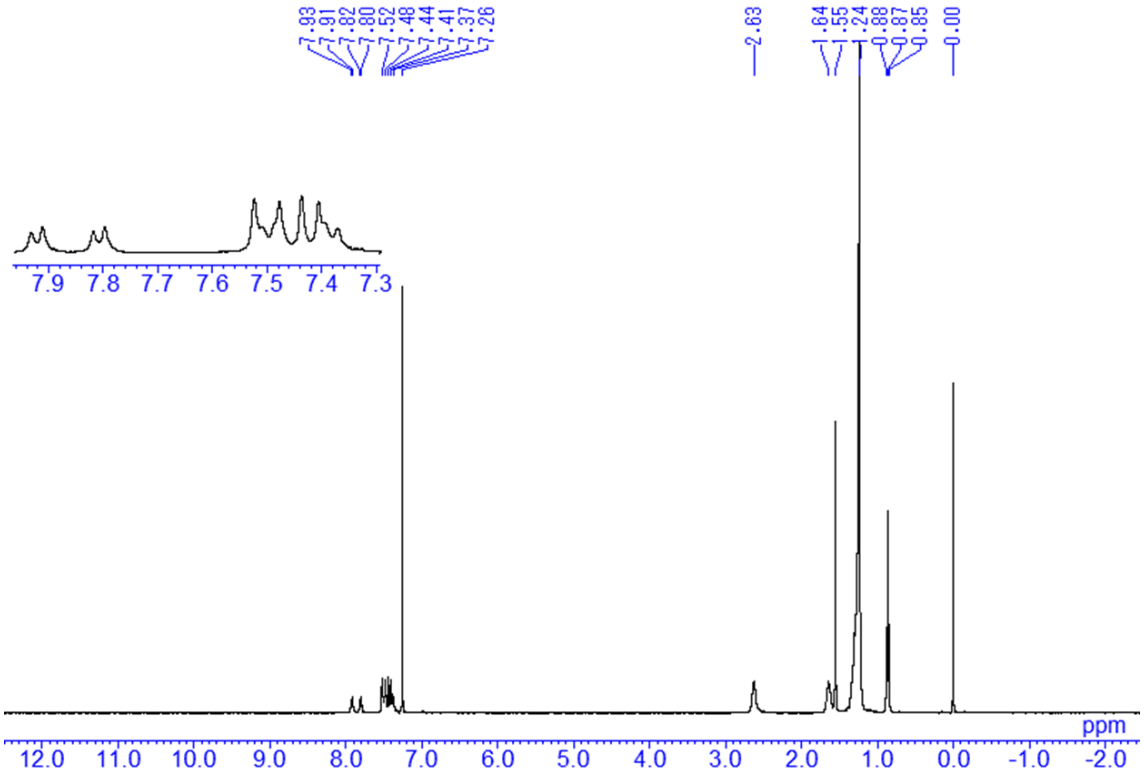



**Chart S3.** $^{1}$H NMR spectrum of **BAz-F-BT** in $CDCl_3$.

**Polymer properties**

**Table S1.** Properties of the fractionated polymers by HPLC

| Compounds | $M_n$ [a] /kDa | $M_w$ [a] /kDa | PDI | $n$ [b] |
|---|---|---|---|---|
| **BAz-Ph-FL** | 15,000 | 19,000 | 1.3 | 18 |
| **BAz-Ph-BT** | 13,000 | 30,000 | 2.4 | 16 |
| **BAz-F-BT** | 19,000 | 36,000 | 1.9 | 25 |

[a] Determined by a gel permeation chromatography (GPC) with polystyrene standards. [b] Number-average degree of polymerization.

**Protein adsorption**

**Table S2.** Properties of the protein complexes with albumin

| Compounds | Pictures of protein complexes | Amount /mg | Hydro dynamic radius (nm)[a,b] |
|---|---|---|---|
| **BAz-Ph-FL** |  | 8.6 | 8.4 ± 5.2 |
| **BAz-Ph-BT** |  | 9.3 | 67.4 ± 23.8 |
| **BAz-F-BT** |  | 8.7 | 62.4 ± 15.7 |

[a]The pristine albumin: 16.2 ± 5.2 nm. [b]Determined in PBS.

**DLS measurements**

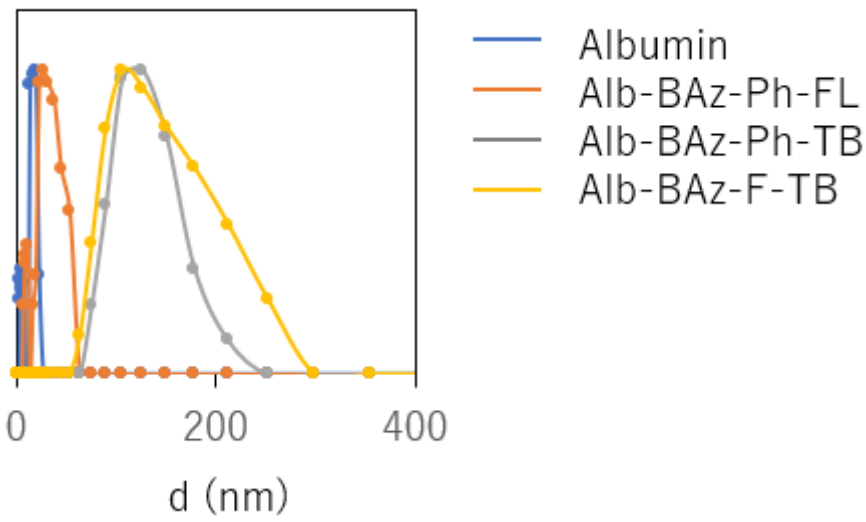



**Figure S1.** DLS data of albumin and complexes.

**UV–vis absorption and PL spectra**

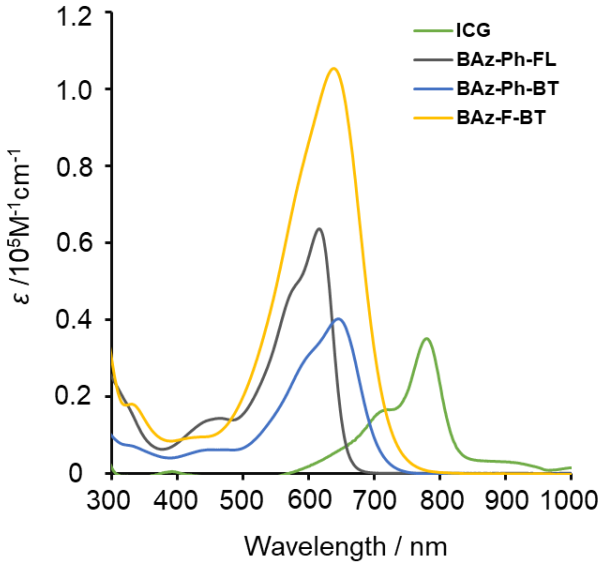



**Figure S2.** UV–vis absorption spectra of **BAz** polymers in the diluted chloroform solution and **ICG** in PBS buffer solution ($1.0\times10^{-5}$ M).

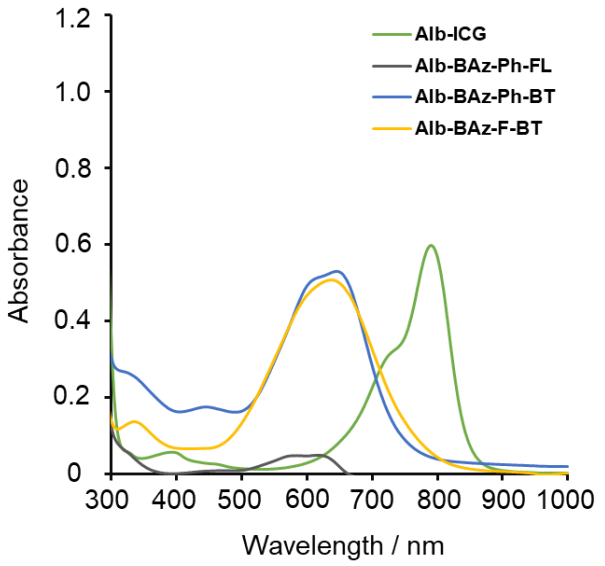



**Figure S3**. UV–vis absorption spectra of protein complexes of **BAz** polymers and **ICG** in PBS buffer solution (0.5 mg/mL).

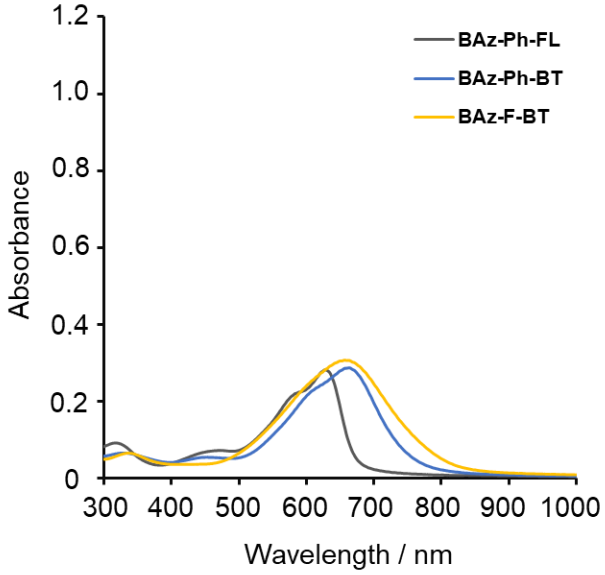



**Figure S4.** UV–vis absorption spectra of **BAz** polymers in film.

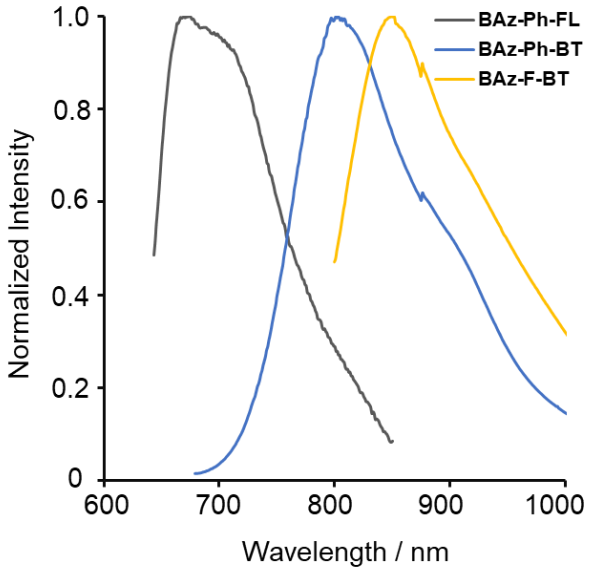



**Figure S5.** PL spectra of **BAz** polymers in film.

**Optical data of BAz polymers in film**

**Table S3**. Optical data of **BAz** polymers in film

| Compound | $\lambda_{abs}$ [a] /nm | $\lambda_{PL}$ [b] /nm | $\Phi_{PL}$ [c] /% |
|---|---|---|---|
| **BAz-Ph-FL** | 628 | 669 | 3.3 |
| **BAz-Ph-BT** | 664 | 812 | 8.9 |
| **BAz-F-BT** | 657 | 849 | 4.6 |

[a] Spin-coated films on the quartz substrate (0.9 × 5 $cm^2$) prepared from chloroform solutions (0.10 mL, 1000 rpm, concentration: 1.0 mg 0.30 $mL^{-1}$). [b] Excited at $\lambda_{abs}$ for PL. [c] Absolute PL quantum efficiency excited at $\lambda_{abs}$.

**Photobleaching of protein complexes of BAz polymers and ICG**

Protein complexes of the polymers and **ICG** were prepared respectively. Dissolving 2 mg of the polymer complexes in 4 mL of PBS solution and filtrated through a membrane filter with 0.2 mm cutoff. For **ICG**, the sample was prepared to the same concentration as the samples used for optical measurements. After placing the samples in five 2 mL eppendorf tubes, they were irradiated with 254 nm UV for 10 minutes using a UV transilluminator. Considering the error due to the position of the UV light exposure, the average value of the five tubes was used. This procedure was repeated and the UV–vis absorption spectrum and photoluminescence spectra were also measured after 20 minutes. Since the **BAz** polymers showed little change after 20 minutes, the UV light was kept on for up to 60 minutes.

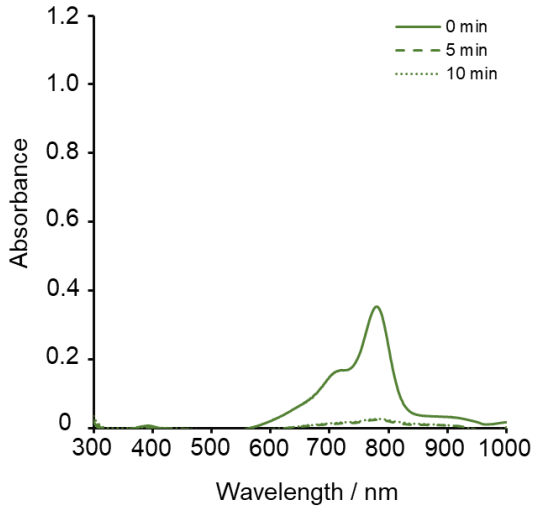



**Figure S6.** Time courses in UV–vis absorption spectra of **ICG** in PBS buffer solution (0.5 mg/mL).

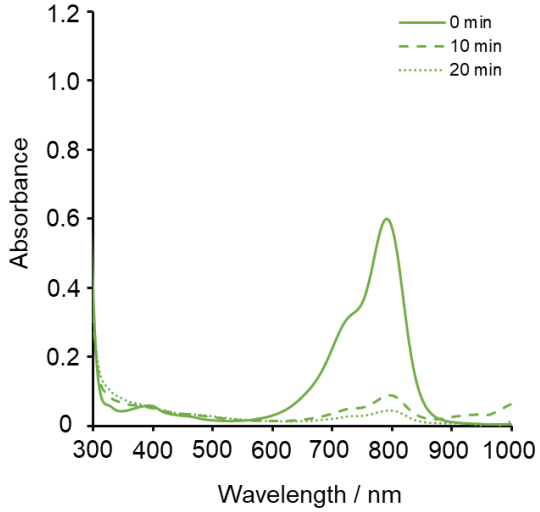



**Figure S7.** Time courses in UV–vis absorption spectra of **Alb-ICG** complex in PBS buffer solution (0.5 mg/mL).

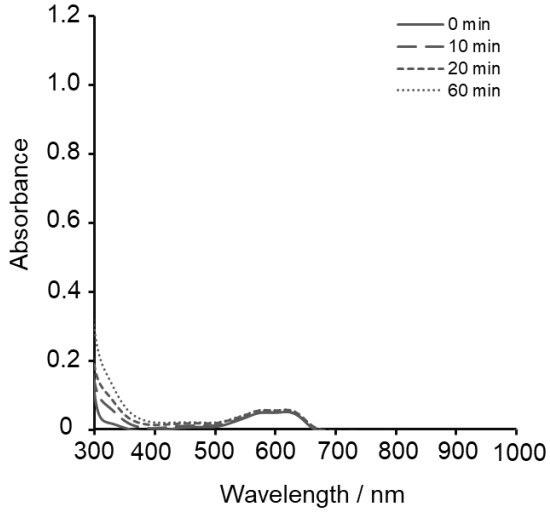



**Figure S8.** Time courses in UV–vis absorption spectra of **Alb-BAz-Ph-FL** complex in PBS buffer solution (0.5 mg/mL).

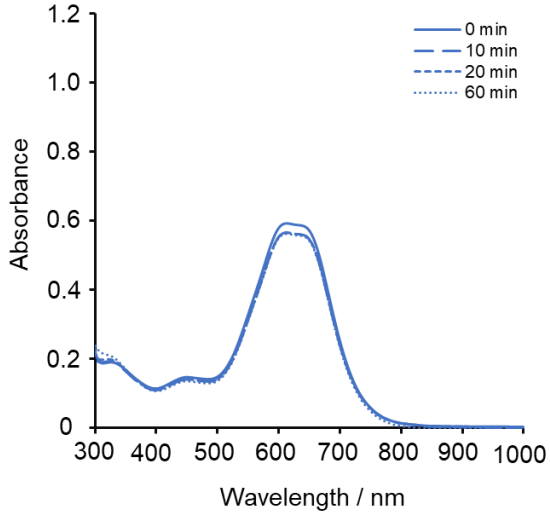



**Figure S9.** Time courses in UV–vis absorption spectra of **Alb-BAz-Ph-BT** complex in PBS buffer solution (0.5 mg/mL).

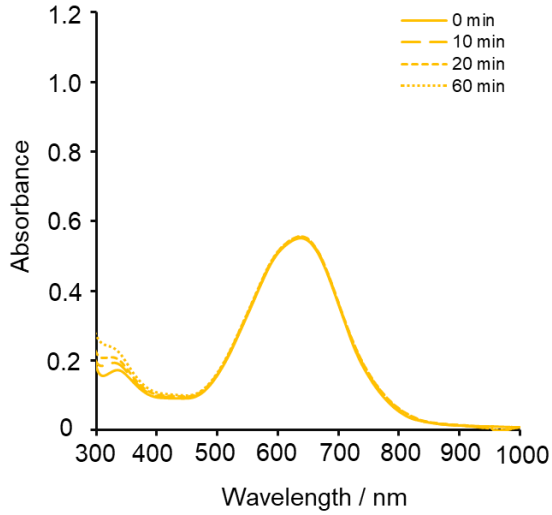



**Figure S10.** Time courses in UV–vis absorption spectra of **Alb-BAz-F-BT** complex in PBS buffer solution (0.5 mg/mL).

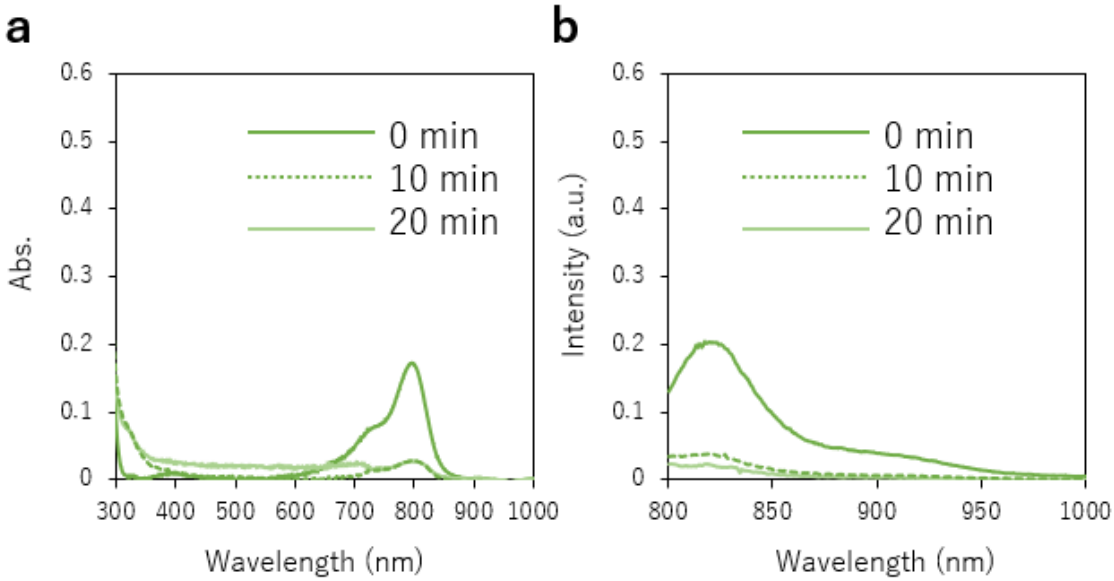



**Figure S11.** Time courses in (a) UV–vis absorption and (b) PL spectra of **Alb-ICG** complex in PBS buffer solution (0.25 mg/mL).

**Evaluation of enzyme-substrate interaction**

The substrate **4MUH** is hydrolyzed by lipase and an absorption wavelength is observed around 362 nm. We evaluated the degree of substrate degradation ability after adsorption of **BAz** polymer and **ICG** on the lipase protein. The composition of the solutions was performed according to Table S5. The solutions were warmed in a heat block to 37 °C. Each sample solution was prepared in triplicate and the average value was used. The measurement time was measured from 1 hour to 96 hours later.

**Table S4.** Amounts of polymers on the lipase complexes

| Compounds | Adsorption mass per 1 mg of lipase /$\mu$g | Adsorption mole per 1 mg of lipaase /$10^{-2}\mu$mol[a] |
|---|---|---|
| **ICG** | 27.9 | 3.59 |
| **BAz-Ph-FL** | 2.45 | 0.30 |
| **BAz-Ph-BT** | 39.2 | 4.82 |
| **BAz-F-BT** | 16.9 | 2.07 |

[a]Lipase (45,000 Da) 1 mg = $2.2\times10^{-2}$ $\mu$mol.

**Table S5.** Composition of the enzymatic reaction solution

| Total /μL | Water /μL | 4MUH[1 mM] /μL | PBS[× $10^{-1}$] /μL | Lipase[5 mg $mL^{-1}$] /$\mu$L |
|---|---|---|---|---|
| 500 | 200 | 50 | 50 | 20 |

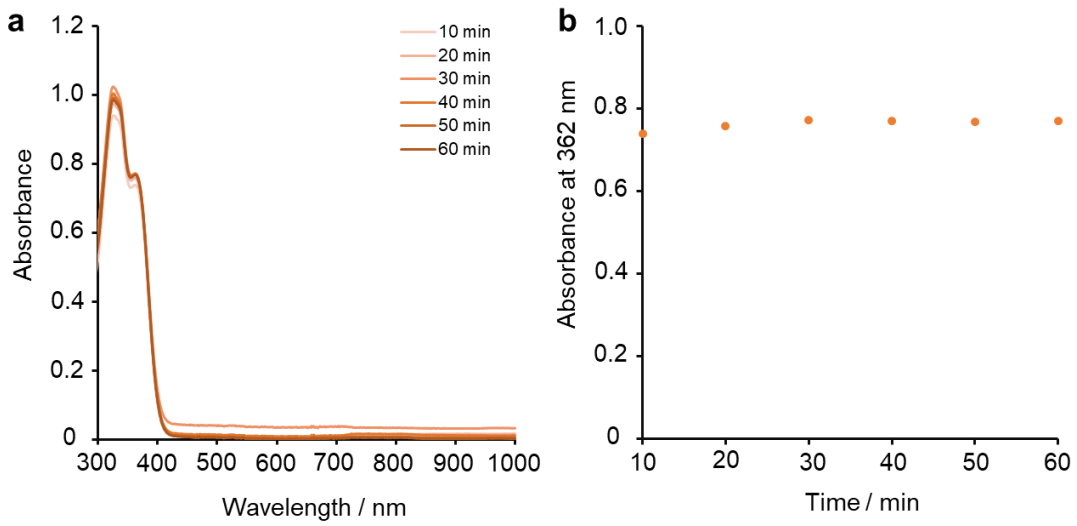



**Figure S12.** (a) Time courses in UV–vis absorption spectra and (b) absorbance at 362 nm of **lipase-4MUH** from 10 min to 60 min.

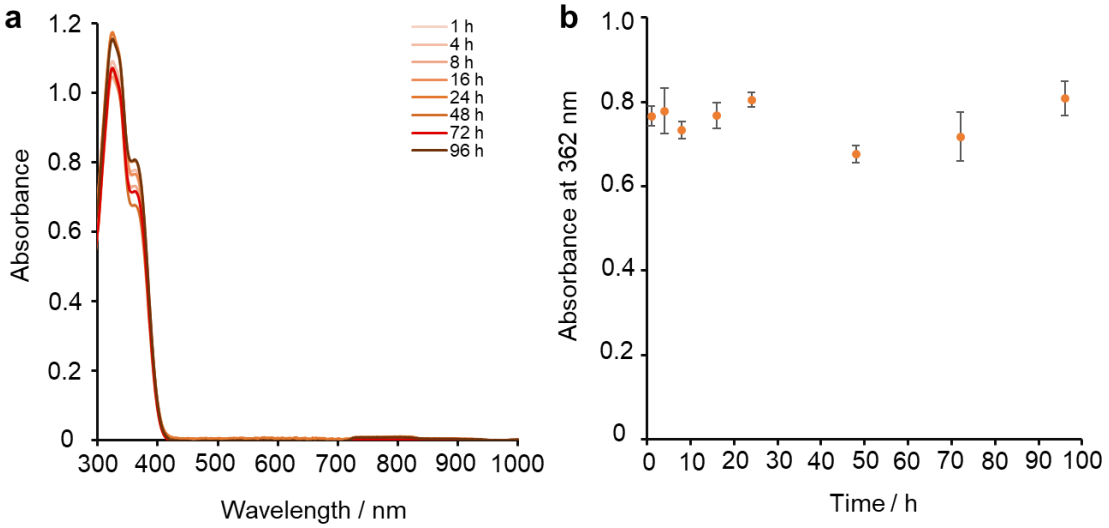



**Figure S13.** (a) Time courses in UV–vis absorption spectra and (b) absorbance at 362 nm of **lipase-4MUH** from 1 h to 96 h.

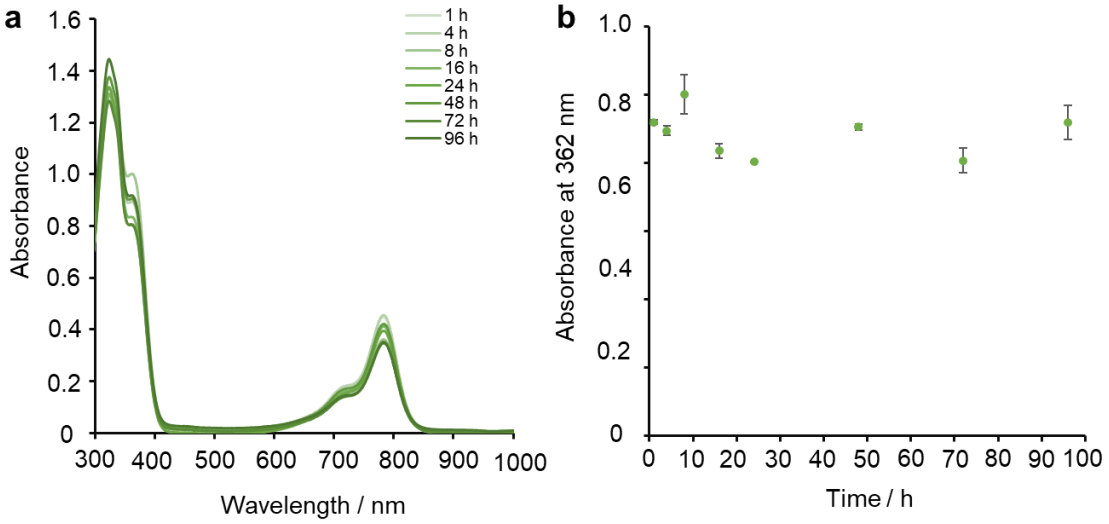



**Figure S14.** (a) Time courses in UV–vis absorption spectra and (b) absorbance at 362 nm of **lipase-ICG-4MUH** from 1 h to 96 h.

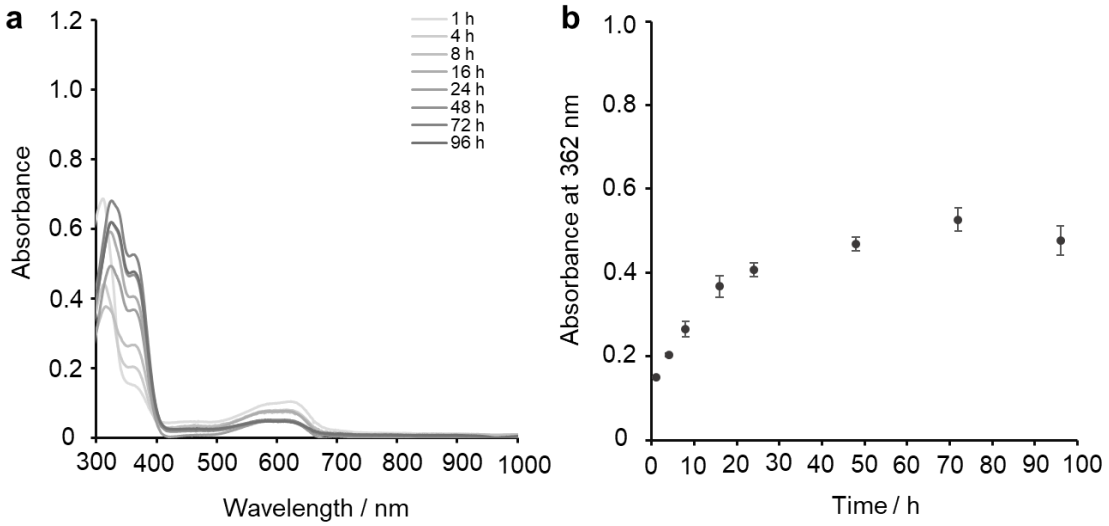



**Figure S15.** (a) Time courses in UV–vis absorption spectra and (b) absorbance at 362 nm of **lipase-Ph-FL-4MUH** from 1 h to 96 h.

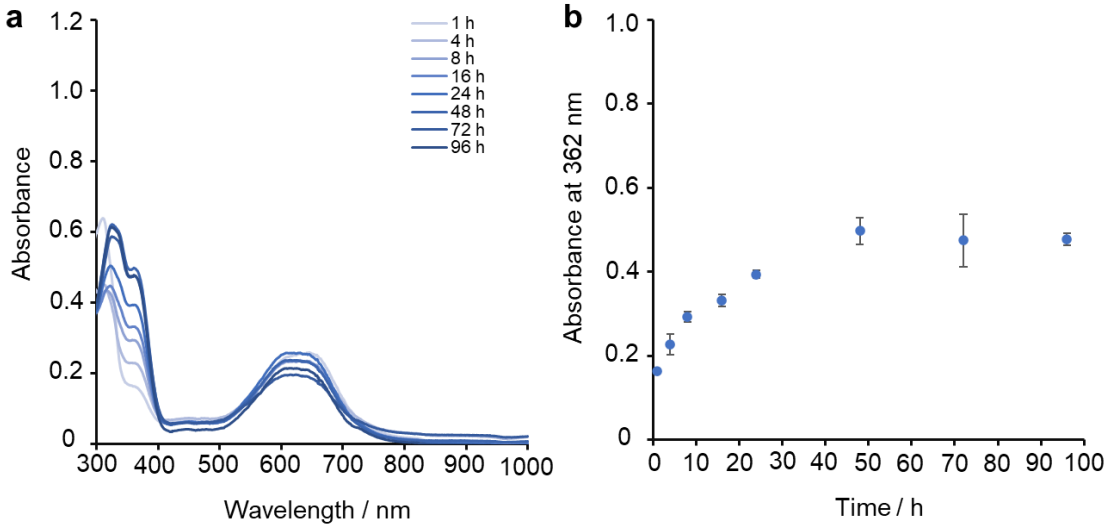



**Figure S16.** (a) Time courses in UV–vis absorption spectra and (b) absorbance at 362 nm of **lipase-Ph-BT-4MUH** from 1 h to 96 h.

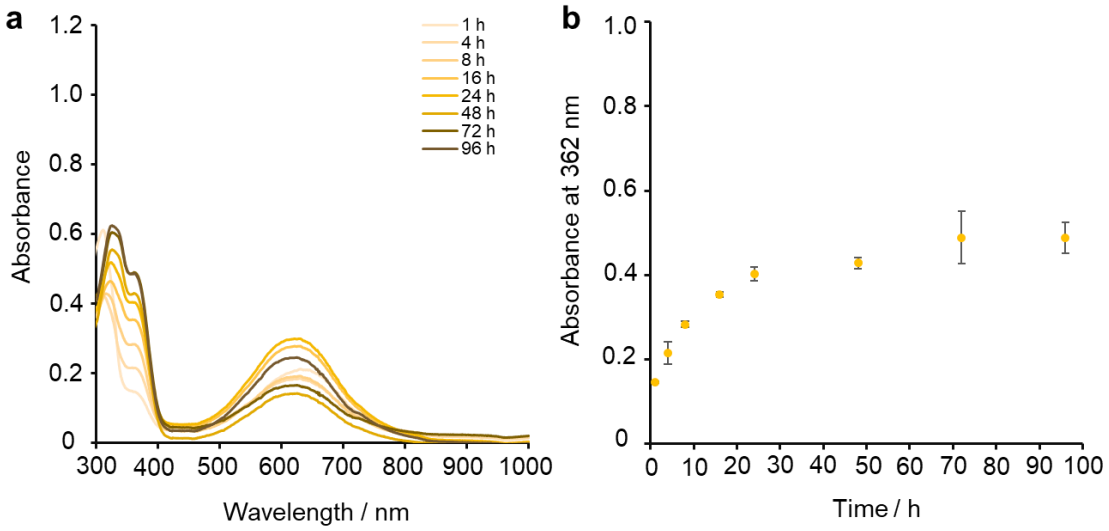



**Figure S17.** (a) Time courses in UV–vis absorption spectra and (b) absorbance at 362 nm of **lipase-F-BT-4MUH** from 1 h to 96 h.

**Table S6.** Kinetics of enzymatic reactions[a]

| Compounds | Decomposition rate of substrate/ mol・$L^{-1}$・$s^{-1}$ |
|---|---|
| **lipase-Ph-FL-4MUH** | $10.9\times10^{-5}$ |
| **lipase-Ph-BT-4MUH** | $11.8\times10^{-5}$ |
| **lipase-F-BT-4MUH** | $8.13\times10^{-5}$ |

[a]Due to rapid reactions with the pristine lipase and **lipase-ICG**, the approximate values were obtained (at least $20 \times 10^{-3}$ mol/L s).

## References

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3. Gon M, Tanaka K, Chujo Y. *Angew Chem Int Ed*. 2018;57(22):6546−6551.

4. Gon M, Junko W, Masashi N, Tanaka K, Chujo Y. *Macromol. Rapid Commun*. 2021;42:2000566.