

Magnetic Control of Cells by Chemical Fabrication of Melanin

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ABSTRACT: Melanin is an organic material biosynthesized from tyrosine in pigment-producing cells. The present study reports a simple method to generate tailored functional materials in mammalian cells by chemically fabricating intracellular melanin. Our approach exploits synthetic tyrosine derivatives to hijack the melanin biosynthesis pathway in pigment-producing cells. Its application was exemplified by synthesizing and using a paramagnetic tyrosine derivative, m-YR, which endowed melanoma cells with responsiveness to external magnetic fields. The mechanical force generated by the magnet-responsive melanin forced the cells to elongate and align parallel to the magnetic power lines. Critically, even non-pigment cells were similarly remote-controlled by external magnetic fields once engineered to express tyrosinase and treated with m-YR, suggesting the versatility of the approach. The present methodology may potentially provide a new avenue for mechanobiology and magnetogenetic studies and a framework for magnetic control of specific cells.

Melanin is a biologically produced organic material found across all families of living organisms including plants, microorganisms, and animals.¹ Its major functions in animal hair, skin, and neurosensory tissues include UV protection, coloration, and radical scavenging.^{2,3} Animal melanin is synthesized in melanosome, a cellular organelle specialized for melanin synthesis in pigment-producing cells. The major precursor of melanin is tyrosine, which is oxidized by the enzyme tyrosinase triggering a polymerization reaction. Tyrosinase, amyloid fibrils, and substrates are usually confined in melanosomes for regulated cellular melanin synthesis.^{1,4}

The unique properties of this biopolymer have attracted attention in the field of materials science. As a result, a number of melanin-mimicking materials have been developed by implementing tyrosinase as a catalyst for oxidative polymerization.^{5–7} The cellular tyrosinase in melanoma cells has recently been exploited for intracellular synthesis of bioactive peptide polymers for potential therapy.^{8,9} These studies have demonstrated that tyrosinase accepts a wide range of tyrosine-containing substrates *in vitro* and *in cellulo* for oxidative polymerization. In the present study, we envisioned designing synthetic tyrosine derivatives that hijack the melanin biosynthesis pathway and copolymerize with tyrosine to generate tailored functional materials in mammalian cells (Figure 1).

To test the concept, we generated a tyrosine probe linked to an azide handle that selectively reacts with a click-reaction reagent. The resulting probe, Y-N₃ (Figure 2A), was mixed with tyrosinase *in vitro* and allowed for the reaction for 24 h. The polymerization reaction readily proceeded, yielding a water-insoluble dark precipitate (Figure S1). In contrast, a phenylalanine version of the probe, F-N₃, failed to form precipitates, underscoring the importance of the phenolic group for polymerization. When a 1:1 mixture of Y-N₃ and tyrosine was treated with tyrosinase, we were able to detect the formation of an intermediate whose molecular mass is

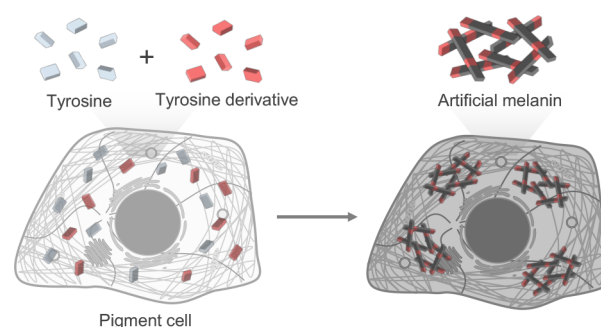
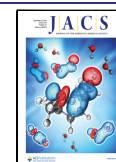


Figure 1. Chemical fabrication of intracellular melanin with synthetic tyrosine derivatives.

consistent with that of an oxidized covalent heterotrimer (Figure S2), suggesting the copolymerization of Y-N₃ and tyrosine. The copolymerization was further supported by the optical properties of the tyrosinase-treated products of the 1:1 mixture (Figure S3). Interestingly, the Y-N₃ homopolymer was found to exhibit autofluorescence in 525 nm confocal images due to unknown reasons. When mixed with the non-fluorescent tyrosine polymer, mosaic-like fluorescent materials were observed under a confocal microscope. No such fluorescence was detected in the tyrosinase-treated products of the 1:1 mixture, indicating that this product is not a simple mixture of two homopolymers but most likely a copolymer.

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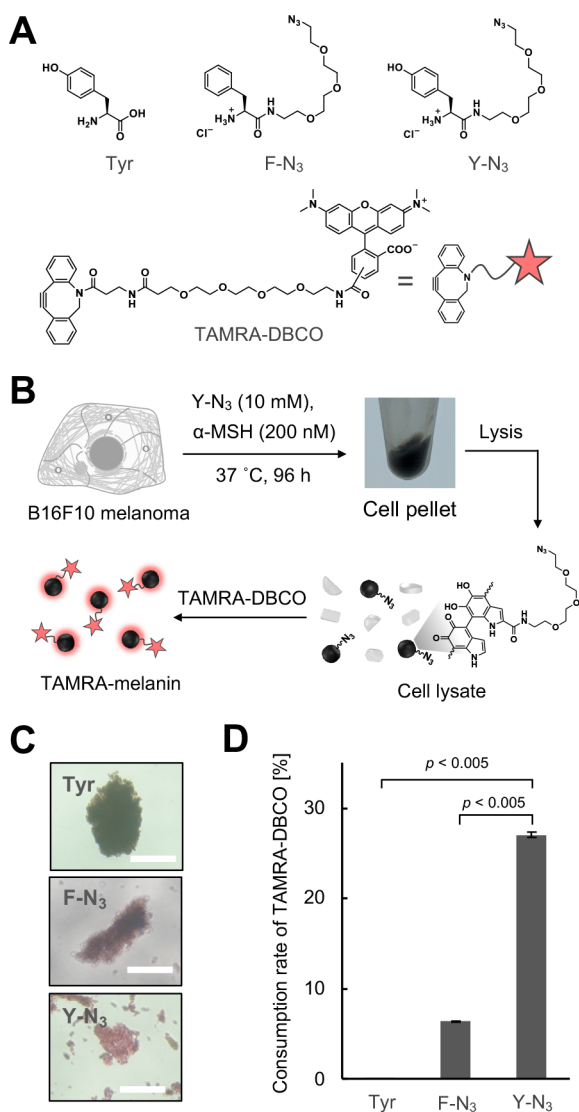


Figure 2. Chemical fabrication of intracellular melanin with a tyrosine click chemistry probe. (A) Chemical structures of monomers and a TAMARA-conjugated click reagent. (B) Experimental workflow of the melanin fabrication in B16F10 melanoma cells. (C) Macroscopic images of isolated melanin after the TAMRA-DBCO treatment. Scale bar: 50 μ m. (D) Consumption rates of TAMRA-DBCO. Experiments were replicated at least in duplicate. $n = 3$. Data represent mean $\pm$ SD. Significance was determined using an unpaired two-tailed Student's t test. * $p < 0.005$.

These results collectively support our notion that tyrosinase copolymerizes Y-N₃ and tyrosine.

To examine whether Y-N₃ can be incorporated into intracellular melanin, we added Y-N₃ to the culture medium of B16F10 melanoma cells and induced melanin biosynthesis by adding α -MSH, a peptide hormone that stimulates melanocytes. After 96 h, the cells were collected, lysed, and treated with TAMRA-DBCO, a red dye-conjugated click-reaction reagent (Figure 2B). Melanin polymers were purified from the lysates by centrifugation in RIPA buffer. As expected, the insoluble melanin particles displayed red color, while the addition of tyrosine or F-N₃ led to the formation of the blacker pigments (Figure 2C). Spectrophotometric quantification of TAMRA-DBCO remaining in the supernatant showed that melanin from the Y-N₃-treated cells consumed more TAMRA-

DBCO than those from the tyrosine or F-N₃-treated cells (Figure 2D). To gain insights into the subcellular localization of the fabricated melanin, the Y-N₃-treated cells were fixed and subsequently reacted with TAMRA-DBCO. Confocal microscopic imaging of the cells revealed that the fabricated melanin co-localized with gp100, a melanosome marker (Figure S4). These results demonstrate that the cellular machinery of melanin biosynthesis recognized Y-N₃ as a building block and integrated it into cellular melanin. Intriguingly, they further indicate that cellular melanin can be chemically fabricated by simply treating pigment cells with tyrosine derivatives.

We then set out to confer mammalian cells with a non-natural capability by chemically fabricating intracellular melanin. We envisioned that a proof of concept of this capability would be the responsiveness of the ensuing cells to magnetic fields. Magnetic actuation on cells is appealing because magnetic fields provide easy, rapid, and non-invasive control.^{10,11} Paramagnetic molecules with unpaired electrons often become highly responsive to magnetic fields when accumulated at high densities. The covalent condensation of paramagnetic tyrosine derivatives in intracellular melanin may impart melanin and its host cells with magnetic responsiveness (Figure 3A). Toward this goal, we synthesized a water-soluble paramagnetic tyrosine derivative, m-YR (Figure 3B), by conjugating a tyrosine-arginine dipeptide with *N,N'*-bis-(salicylidene)ethylenediamine-iron(III) complex, an organo-iron compound often used in organocatalysis¹² and magnetic hyperthermia anti-cancer therapy.^{13–17} When mixed with tyrosinase *in vitro*, m-YR formed a water-insoluble dark precipitate (poly(m-YR)), which was attracted by a 315-mT neodymium magnet (Figure 3C). Without tyrosinase, in contrast, m-YR displayed no detectable levels of magnetically attracted materials. The phenylalanine counterpart of m-YR (m-FR) failed to generate any melanin-like polymer even after tyrosinase treatment (Figure S5). These results indicate that tyrosinase is capable of accepting m-YR as a substrate to produce magnet-responsive melanin-like polymers *in vitro*.

Under a cellular environment, m-YR is supposed to form copolymers with endogenous tyrosine. Tyrosinase treatment of equal amounts of m-YR and tyrosine *in vitro* yielded a black precipitate that was attracted by a 315-mT magnet, whereas co-treatment of tyrosine and m-FR formed a precipitate that exhibited no detectable response to the magnet (Figure S6). The magnetic properties of the m-YR-tyrosine copolymer were quantified by a superconducting quantum interference device (SQUID) (Figure 3D). The m-YR-tyrosine copolymer displayed a positive and linear magnetization-magnetic field relationship with a calculated magnetic susceptibility (X_m) of 1.54×10^{-5} emu g⁻¹ Oe⁻¹, confirming its paramagnetic nature. On the other hand, the melanin polymer consisting of tyrosine alone displayed little magnetic susceptibility as low as -4.42×10^{-7} emu g⁻¹ Oe⁻¹, indicating that natural melanin polymer is magnetically inactive.

Encouraged by the *in vitro* results, we examined the effects of m-YR on α -MSH-stimulated B16F10 melanoma cells. Cell viability assays showed that 96-h incubation with high concentrations of m-YR decreased cell proliferation with the maximum tolerated dose of 100 μ M (Figure S7). RT-PCR analysis of a panel of 84 cell death-linked genes indicated that the treatment with 100 μ M m-YR had little effect on the expression of the cell-death genes, except for only a subset of the genes related to DNA-damaging responses (Figure S8). We therefore used 100 μ M m-YR to examine its ability to confer

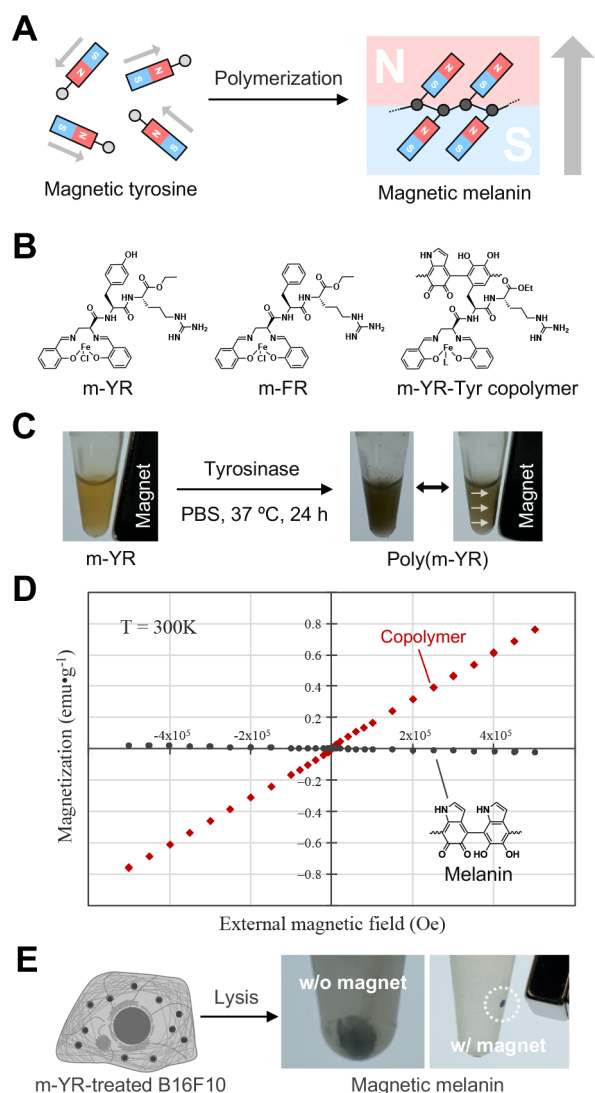


Figure 3. Generation of magnetic melanin *in vitro* and *in cellulo*. (A) Enzymatic polymerization of paramagnetic tyrosines to produce magnet-responsive melanin. (B) Chemical structures of m-YR, m-FR, and a copolymer of m-YR and tyrosine. (C) Magnet responsiveness of m-YR and *in vitro*-prepared poly(m-YR). (D) Magnetization measurement of melanin and a copolymer of m-YR and tyrosine. (E) Magnet responsiveness of m-YR-fabricated cellular melanin. B16F10 melanoma cells were treated with m-YR for 96 h and lysed. Cellular melanin was purified and suspended in water. Macroscopic images of the melanin particles were captured with or without neodymium magnets. The magnetic flux density of the neodymium magnet is 315 mT.

magnetic responsiveness to the cellular melanin. Melanoma cells were treated with 100 μ M m-YR in the presence of α -MSH for 96 h. The resulting cellular melanin polymers were purified and subsequently resuspended in water. When a 315-mT neodymium magnet was placed close to the test tube, the purified melanin particles were attracted toward the magnet (Figure 3E and supplementary movie), whereas m-FR-treated cells failed to generate magnetizable melanin particles. Quantification of iron contents in the polymer indicated the incorporation of 6.47 μ g iron per 1 mg of the melanin polymer purified from m-YR-treated cells, which is translated to the replacement of approximately 8.83% of tyrosine by m-YR. In contrast, lower levels of iron ions were found in the melanin

samples from the cells treated with m-FR (100 μ M), a lower concentration of m-YR (10 μ M), or tyrosine (100 μ M) (Figure S9). These results indicate that m-YR is incorporated into intracellular melanin through tyrosine oxidation, conferring magnetic responsiveness to cellular melanin.

Magnetic materials, such as iron sand, align parallel to the direction of the magnetic power lines. Cultured melanoma cells, in which melanin is fabricated with m-YR, could similarly be aligned by external magnetic fields (Figure 4A). To explore

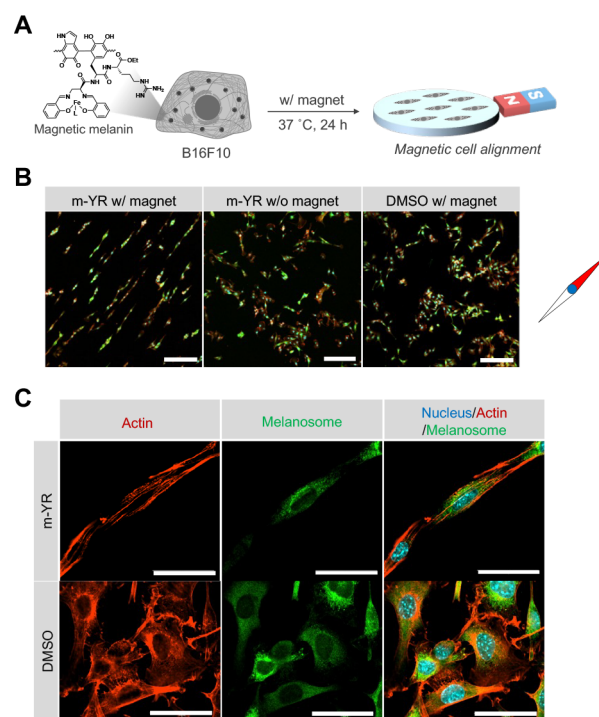


Figure 4. Magnetic control of melanoma cells. (A) Schematic illustration of the experiments. (B) Confocal images of B16F10 melanoma cells in the presence of magnetic fields. The cells were treated with m-YR or DMSO in the presence or absence of magnets. Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst33258, respectively, while melanosome was immunostained with an anti-melanoma gp100 antibody (green). Scale bar: 257 μ m. (C) Enlarged images of the cells. Scale bar: 50 μ m.

this possibility, we treated B16F10 melanoma cells with m-YR (100 μ M) and α -MSH, reseeded them in culture dishes, and cultured them in the presence of an array of ten 315-mT neodymium magnets. After 24-h incubation, the cells were fixed and observed under a confocal microscope. The cells in the vicinity of the magnet were aligned along the magnetic power lines (Figure 4B). When the orientation of the cells was image-analyzed, 64.7% of the m-YR-treated cells were aligned within ± 10 degrees of the magnetic power lines, whereas no preferential orientation (8.59%) was observed in the DMSO-treated cells (Figure S10). No apparent alignment of cells was detectable in regions where the magnetic field was less than 40 mT (Figure S11). The samples with m-FR or in the absence of a magnet or α -MSH also displayed no detectable preference in orientation (Figures S12 and S13). These results demonstrate that the cell alignment requires both copolymerization and magnetic fields. Magnified images of the cells showed an extended shape in which the actin cytoskeleton elongated in the direction of the magnetic power lines (Figure 4C).

Melanosomes, where melanin synthesis takes place, appeared to be distributed throughout the cell as detected by gp100, a melanosome marker. How the magnetized melanin alters the entire cell shape remains unknown. Nevertheless, these results suggest that the mechanical force generated by the magnet-responsive melanin forced the cells to elongate and align themselves parallel to the magnetic power lines.

We next attempted to manipulate the magnetic arrangement of cells by shaping the external magnetic fields. A culture plate was sandwiched by two magnet arrays facing each other with different poles or the same poles to form magnetic power lines of attraction or repulsion between the magnets (Figure 5A).

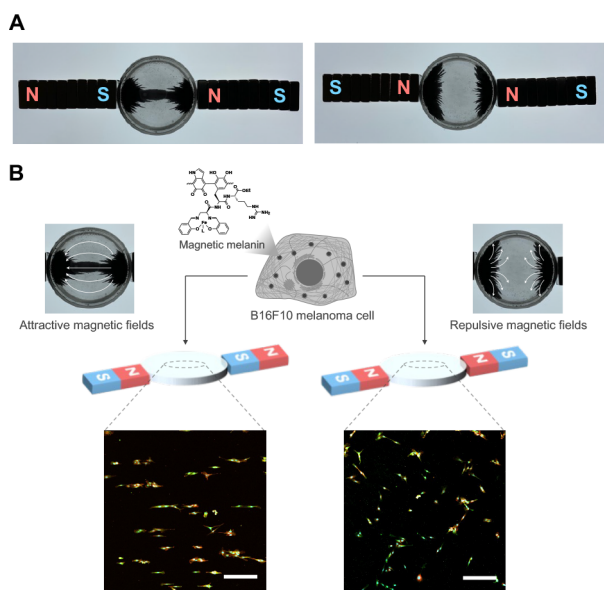


Figure 5. Magnetic field-dependent cell alignments. (A) Magnetic alignments of iron sand under attractive (left) or repulsive magnetic fields (right). (B) Control of magnetic cell alignments by shaping the magnetic fields. B16F10 melanoma cells were treated with m-YR under attractive (left) or repulsive magnetic fields (right). Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst33258, respectively, while melanosome was immunostained with an anti-melanoma gp100 antibody (green). Scale bar: 250 μm .

These two distinct external magnetic fields had distinct effects on the m-YR-treated melanoma cells at the center of the culture dish (Figure 5B). The magnetic field generated by different poles lined up 60.6% of the cells parallel to the magnetic power lines within ± 10 degrees (Figure S14). In contrast, the magnetic fields generated by the same poles had little effect on the cells at the center of the culture dish (7.75% of the cells within ± 10 degrees; Figure S15), where the intensity of the magnetization was found to be essentially 0 mT (Figure S13).

A limitation of our methodology is that it can only be applied to melanin-producing pigment cells. One way to address this issue is overexpression of tyrosinase in non-pigment cells. It has been reported that ectopic overexpression of tyrosinase achieves high levels of melanin biosynthesis even in non-pigment cells.^{18,19} Indeed, we showed that, when a tyrosinase expression plasmid was transfected into human HEK293 cells, the transfected cells acquired the ability to biosynthesize melanin (Figure S16). Following treatment of the tyrosinase-overexpressing HEK293 cells with m-YR, the cells turned black and produced magnetic melanin (Figure

6A). Furthermore, when m-YR-treated HEK293 cells were cultured in the presence of magnets, 35.1% of tyrosinase-

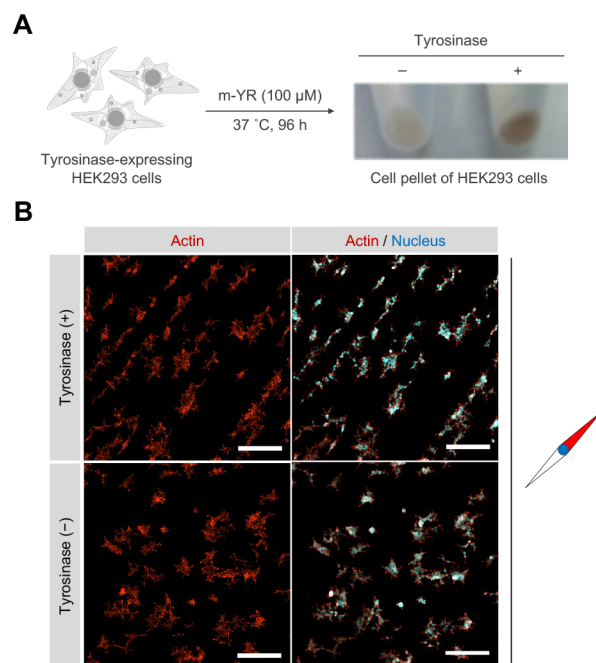


Figure 6. Magnetic control of tyrosinase-expressing HEK293 cells. (A) Images of cell pellets after the m-YR treatment. The tyrosinase-expressing cells displayed darker color than parental cells. (B) Confocal images of tyrosinase-expressing (above) or parental (bottom) HEK293 cells under magnetic fields. The cells were treated with m-YR (100 μM) before reseeding in the presence of magnetic fields. Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst33258, respectively. Scale bar: 260 μm .

expressing HEK293 cells were aligned ± 10 -degree parallel to the magnetic power lines as observed for melanoma cells, while parental HEK293 cells exhibited no significant preferential alignment (8.71%) (Figures 6B and S17). These results indicate that our approach is extendable to non-pigment cells engineered to express tyrosinase, suggestive of the versatility of the approach.

In conclusion, we have developed a simple method to chemically fabricate intracellular melanin by using synthetic tyrosine derivatives. Its application was exemplified by a paramagnetic tyrosine derivative, m-YR, which allowed magnetic control of cultured cells. Mechanical forces are involved in various biological events and pathological conditions.^{20–22} The present method for enzyme-mediated intracellular formation of magnetic materials may potentially contribute to mechanobiology and magnetogenetics investigations. For instance, genetic manipulation of localized tyrosinase expression might permit magnetic control of specific organelles and cell lineages. One drawback of our methodology is the relatively weak magnetization of the m-YR-modified melanin compared with that of the exogenous magnetic nanoparticles used in recent magnetogenetic studies.^{23–31} Work is underway to further optimize the magnetic tyrosine derivatives, with the goal of increasing the magnetization levels of intracellularly formed melanin materials. Such molecules would enable magnetic control of the dynamics and localization of specific cells *in vivo* and find their uses in regenerative therapy and basic developmental biology research.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.2c06555>.

Supplemental Figures S1–S17, details of the supplemental movie, synthetic procedures, spectral characterization, biological experiment details, and additional data (PDF)

Supplemental movie, showing magnet responsiveness of m-YR-fabricated cellular melanin (MOV)

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Notes

The authors declare no competing financial interest.

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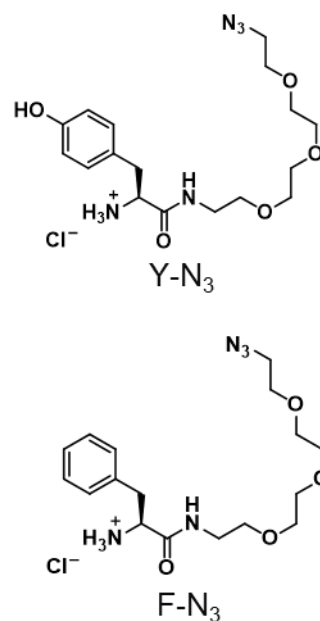
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0.2 mg/mL Tyrosinase

w/o	w/	w/o	w/
Y-N ₃ (1.0 mM)		F-N ₃ (1.0 mM)	



S2

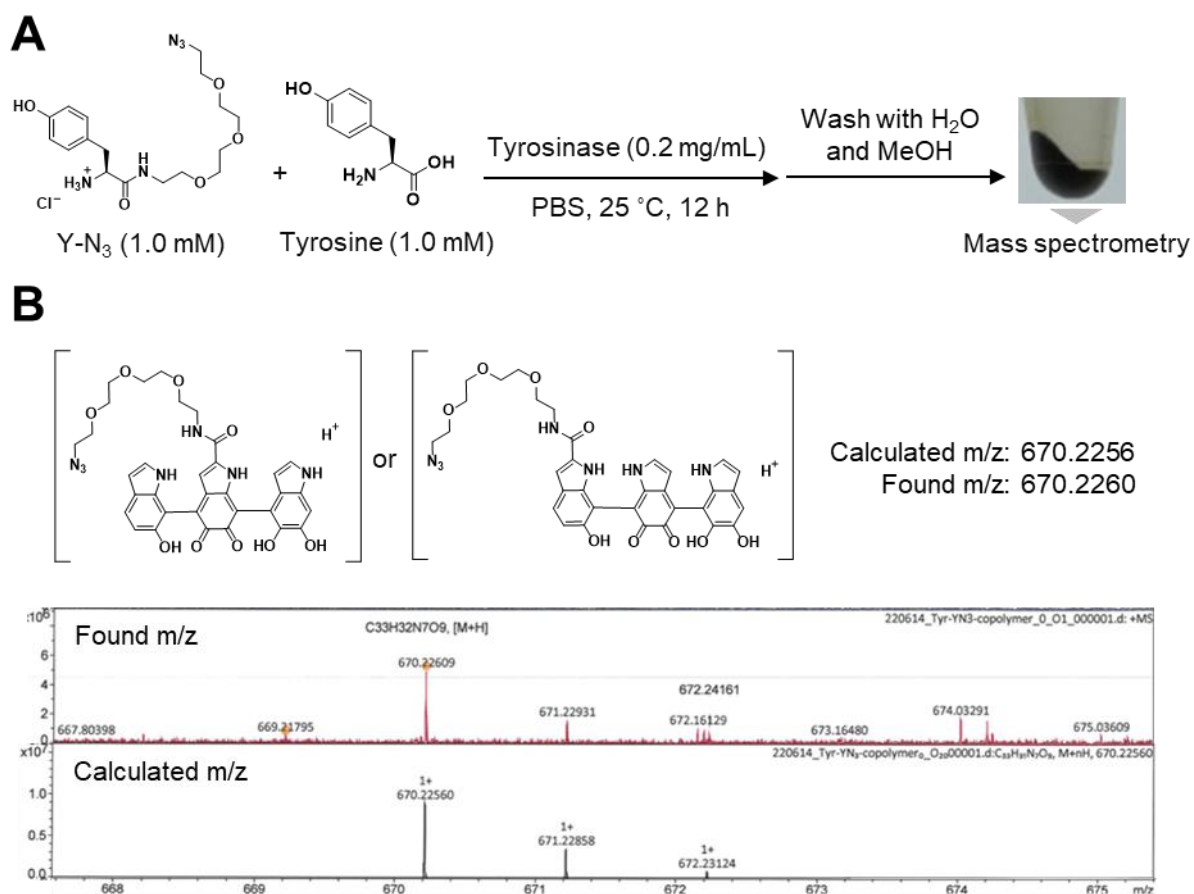


Figure S2. Mass spectrometry analysis of a heterotrimer of tyrosine and Y-N₃. (A) The sample was prepared by mixing tyrosinase (0.2 mg/mL) with tyrosine (1.0 mM) and Y-N₃ (1.0 mM) in PBS. The reaction mixture was stirred at 25 °C for 12 h. The precipitate was collected by centrifugation and analyzed with MALDI-TOF-MS. (B) Possible chemical structure and m/z of an oxidized intermediate in the copolymerization.

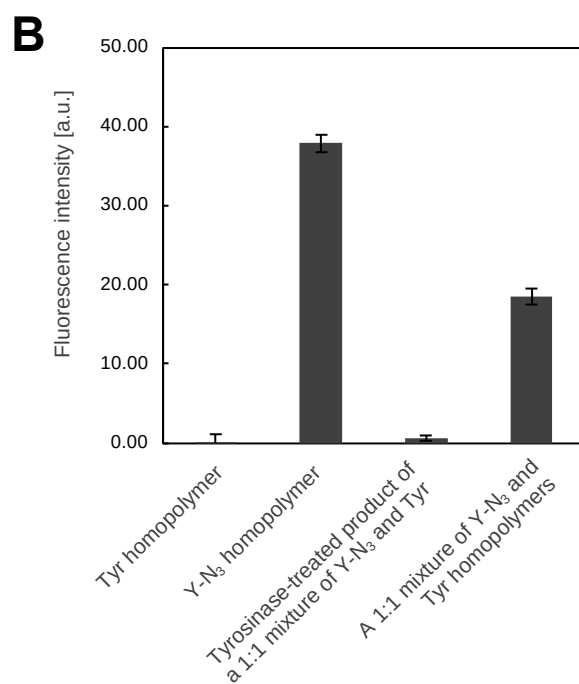
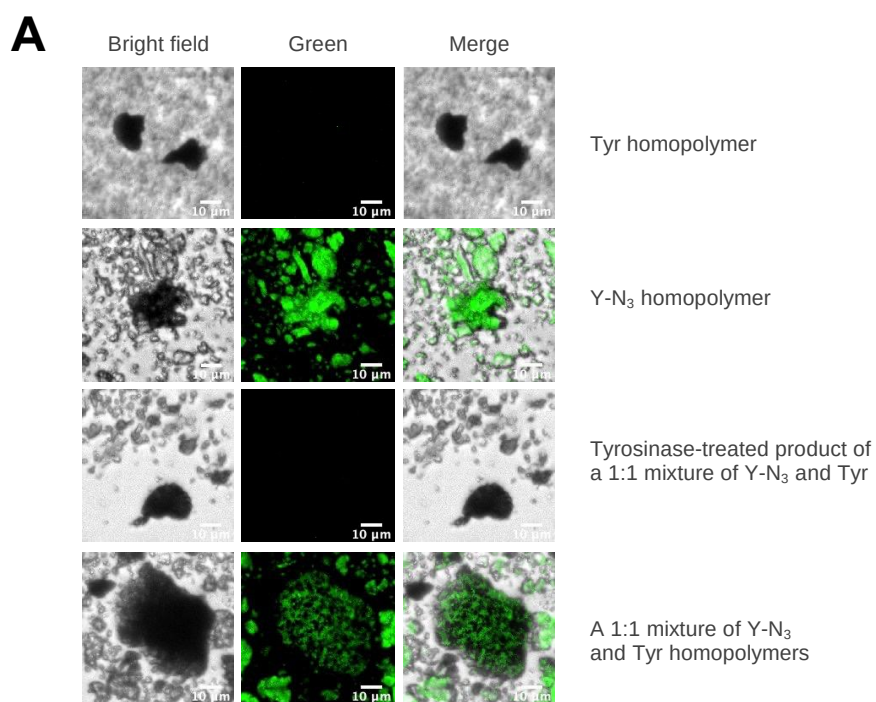


Figure S3. Autofluorescence of the melanin polymers. (A) Confocal imaging of the Tyr and Y-N₃ homopolymers, the product of a 1:1 mixture of Tyr and Y-N₃ following tyrosinase treatment, and a 1:1 mixture of homopolymers. Scale bar: 10 μ m. (B) Fluorescence intensities of melanin polymers.

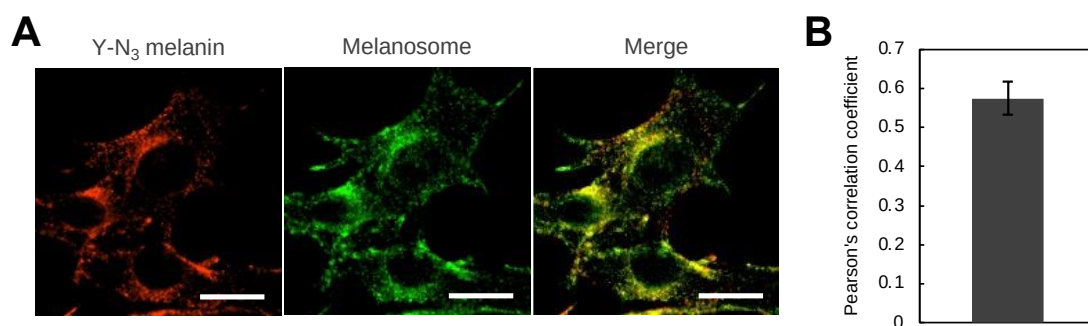


Figure S4. Intracellular localization of azide-conjugated melanin. (A) Confocal imaging of azide-conjugated melanin. B16F10 melanoma cells were treated with Y-N₃ (10 mM) and α -MSH (100 nM) for 96 h. Azide-conjugated melanin was stained with TAMRA-DBCO (red) through click reaction, while melanosomes were immunostained with an anti-melanoma gp100 antibody (green). Scale bar: 25 μ m. (B) Pearson's correlation coefficient for colocalization of Y-N₃ melanin with melanosomes. Pearson's correlation coefficient was calculated on individual cells with ImageJ plug-in colocalization finder. n = 10.

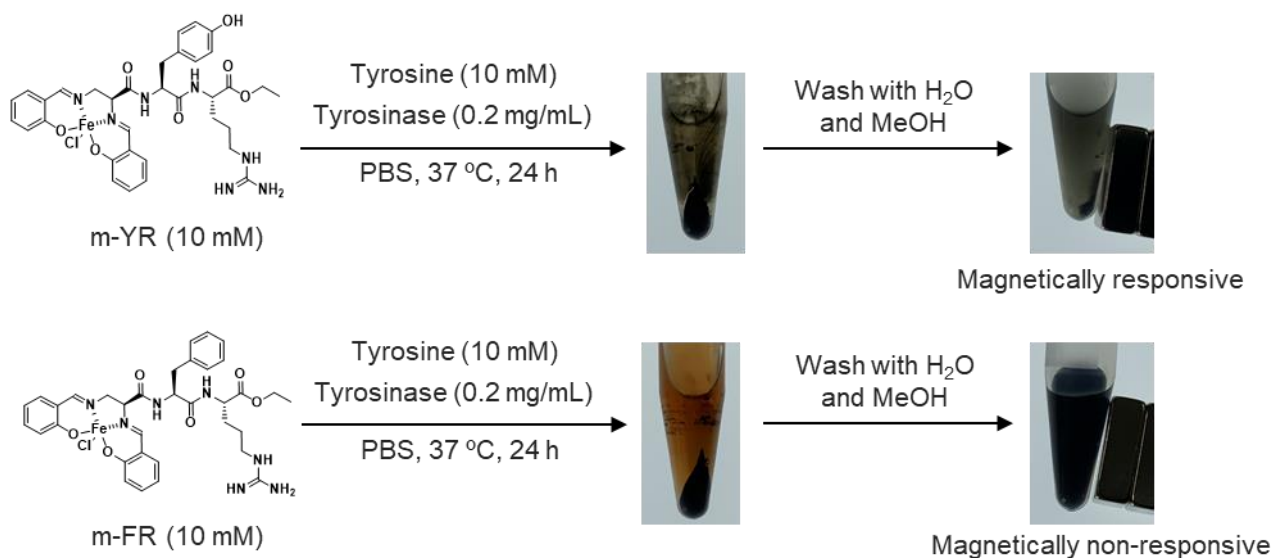


Figure S6. Enzymatic copolymerization of tyrosine and m-YR *in vitro*. Tyrosine (10 mM) and m-YR (10 mM) were mixed in PBS and incubated with tyrosinase for 24 h. The resulting black melanin precipitates were washed with water and methanol and subsequently resuspended in water. The copolymer was attracted by magnets, whereas co-treatment of tyrosine and m-FR formed a precipitate that exhibited no detectable response to the magnets.

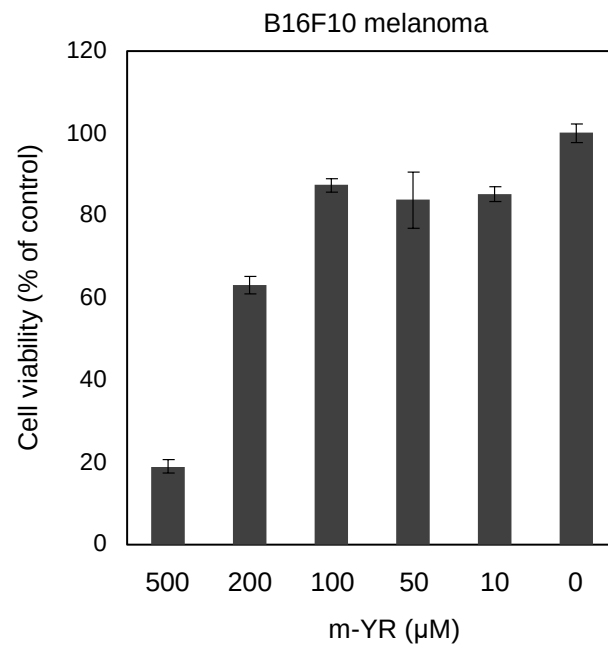


Figure S7. Effects of m-YR on the cell viability of α -MSH-stimulated B16F10 melanoma. The cells were treated with varying concentrations of m-YR and incubated for 96 h. Cell viability was measured with a WST-8 assay.

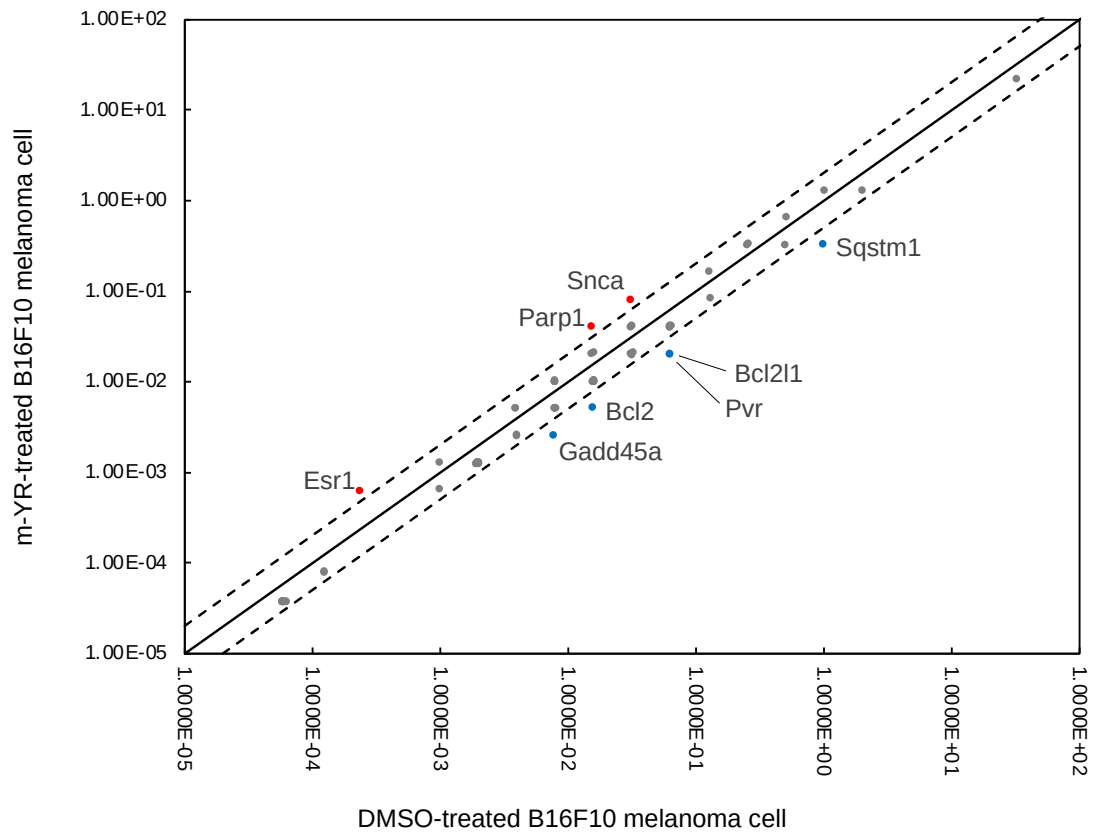


Figure S8. Scatter plot analysis for the correlation of gene expression levels between m-YR- and DMSO-treated B16F10 melanoma cells. The RT2 Profiler PCR Array (Qiagen) was used to examine the expression patterns of 84 genes involved in cellular death. The black line indicates fold changes of 1. The dashed lines represent 2-fold changes.

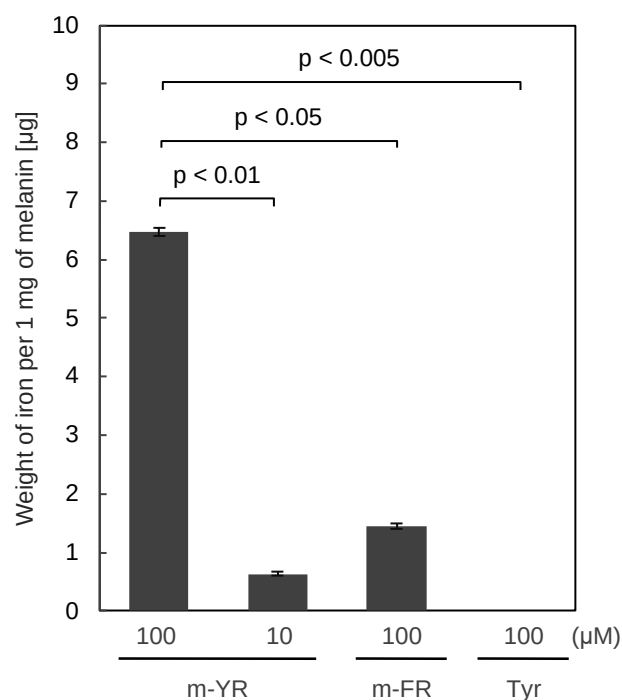


Figure S9. Determination of the iron content of magnetic melanin. Nitroso-PSAP was used as a coloration reagent for quantification. B16F10 melanoma cells were treated with tyrosine (100 μ M), m-YR (100 or 10 μ M), or m-FR (100 μ M), and the cellular melanin polymers were purified from the cell lysates and subjected to analysis. Experiments were replicated at least in duplicate. $n = 3$. Data represent mean $\pm$ SD. Significance was determined using an unpaired two-tailed Student's t-test.

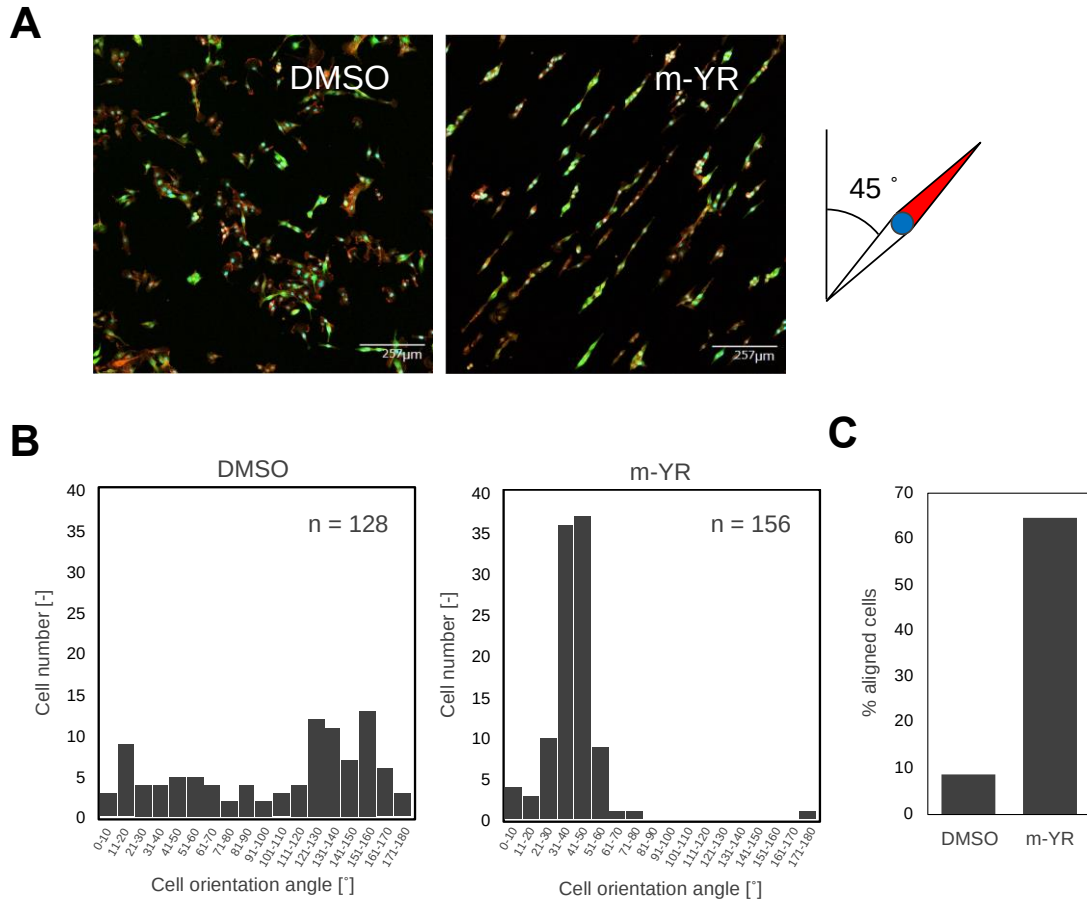


Figure S10. Cell alignment quantification of B16F10 melanoma cells. (A) Confocal imaging of B16F10 melanoma cells in the presence of a magnetic field. The cells were treated with DMSO or m-YR. Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst 33258, respectively, while melanosomes were immunostained with an anti-melanoma gp100 antibody (green). Scale bar: 257 μm . The magnet was placed at a 45° angle to the vertical. (B) The distribution of orientation angles. $n = 128$ cells (DMSO); $n = 156$ cells (m-YR). The angles were automatically calculated by the ImageJ plug-in OrientationJ. (C) Percentages of the magnetically aligned cells. The cells aligned along with the magnetic field are defined as the cells with angles between the orientation axis and the direction of the magnetic field $<10^\circ$.

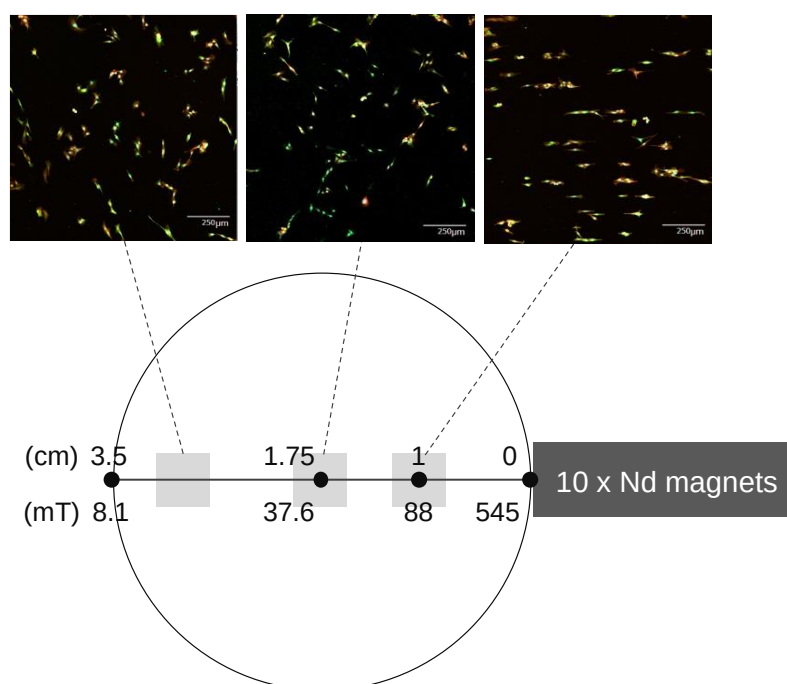


Figure S11. Effects of the magnet distance on the cell alignment. Magnetic field intensities were measured with a Handy Tesla meter MG-801 (MAGNA).

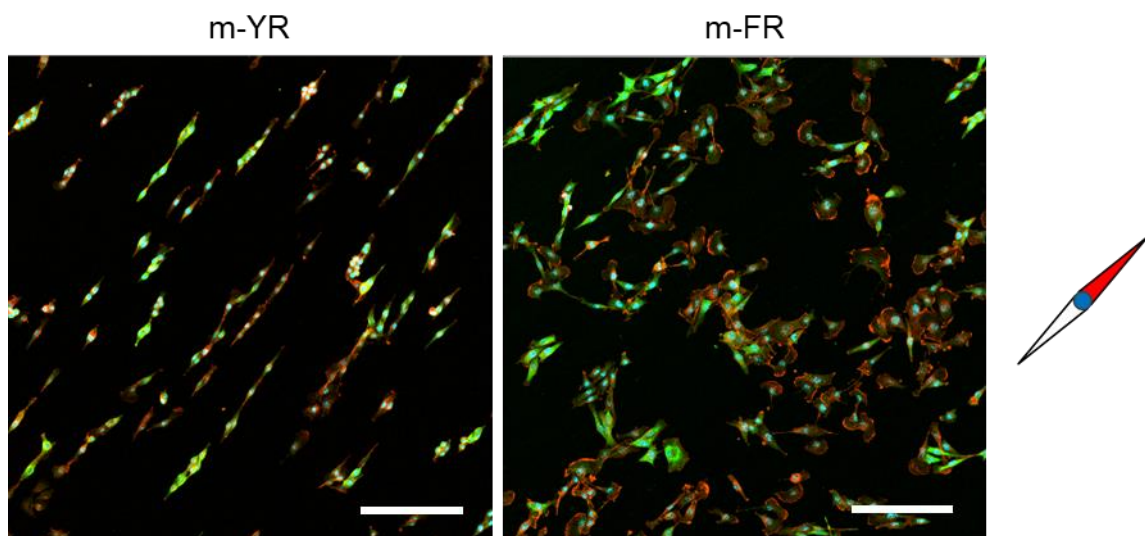


Figure S12. Confocal images of B16F10 melanoma cells treated with m-YR or m-FR in the presence of magnetic fields. Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst 33258, respectively, while melanosome was immunostained with an anti-melanoma gp100 antibody (green). Scale bar: 257 μm .

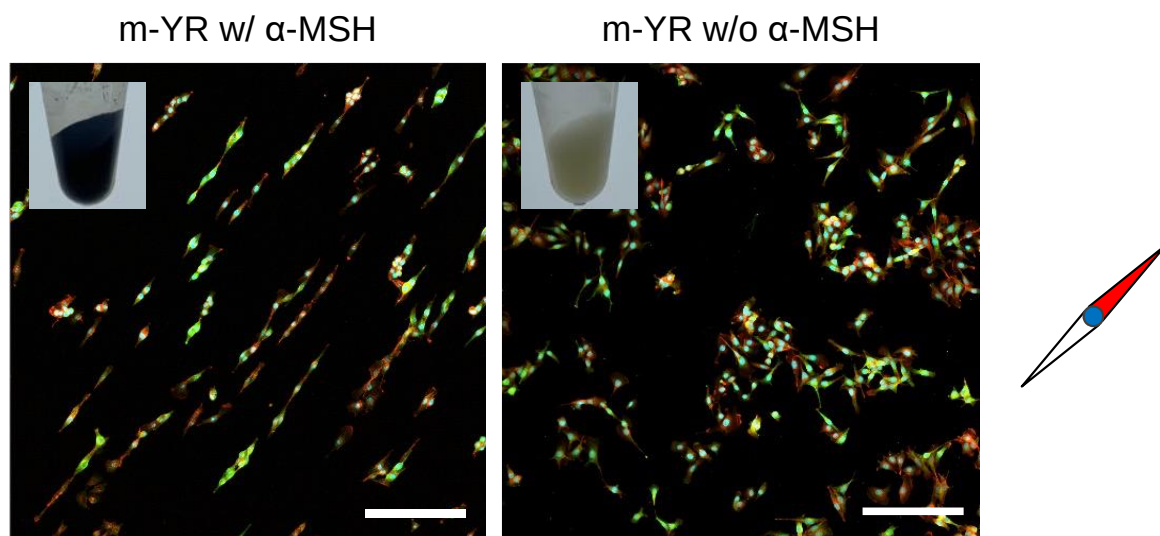


Figure S13. Effects of melanogenesis on magnetic cell alignment. B16F10 melanoma cells were treated with or without α -MSH (100 nM) in the presence of m-YR (100 μ M). After a 96-h incubation, magnetic fields were applied to cells. Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst 33258, respectively, while melanosomes were immunostained with an anti-melanoma gp100 antibody (green) for confocal imaging. Scale bar: 257 μ m.

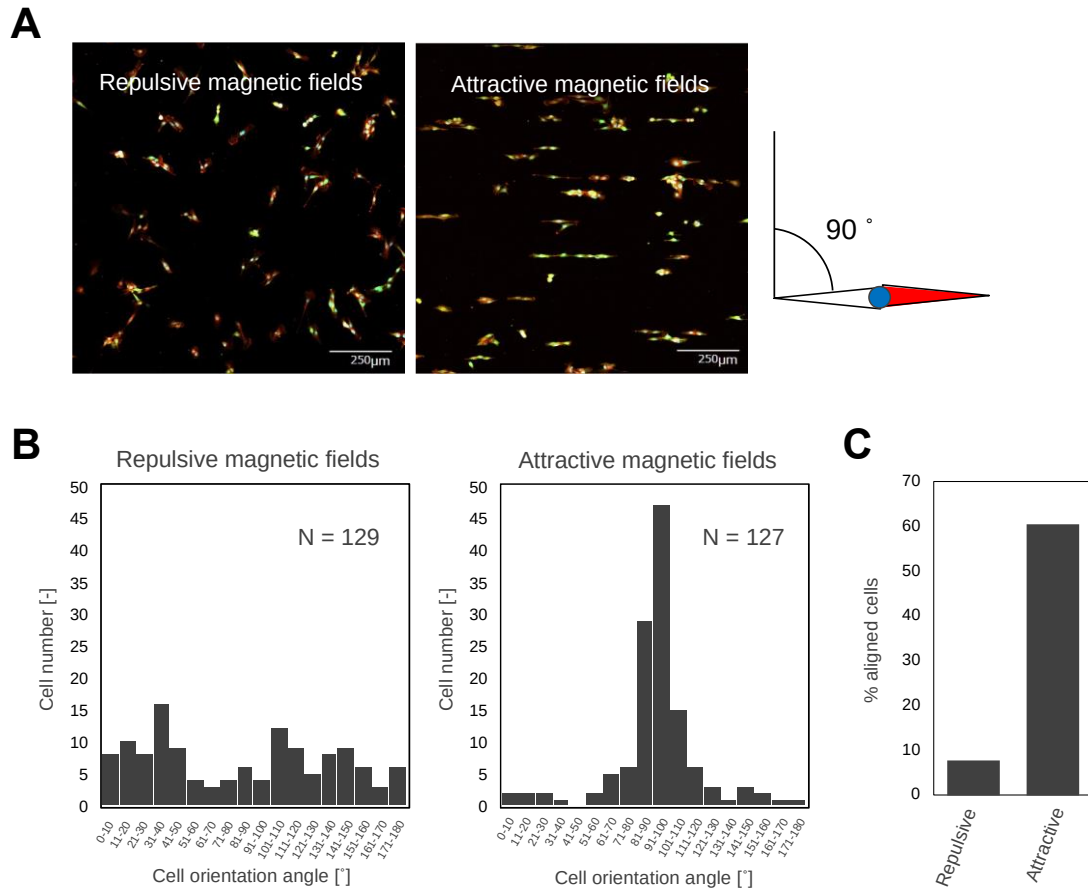


Figure S14. Cell alignment quantification of B16F10 melanoma cells under repulsive or attractive magnetic fields. (A) Confocal images of B16F10 melanoma cells under the different shapes of magnetic fields. The cells were treated with m-YR under repulsive or attractive magnetic fields. Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst 33258, respectively, while melanosome was immunostained with an anti-melanoma gp100 antibody (green). Scale bar: 250 μm . The magnet was placed at a 90° angle to the vertical. (B) The distribution of orientation angles. $n = 129$ cells in repulsive magnetic fields; $n = 127$ cells in attractive magnetic fields. The angles were automatically calculated by the ImageJ plug-in OrientationJ. (C) Percentages of the magnetically aligned cells. The cells aligned along with the magnetic field are defined as the cells with angles between the orientation axis and the direction of the magnetic field $<10^\circ$.

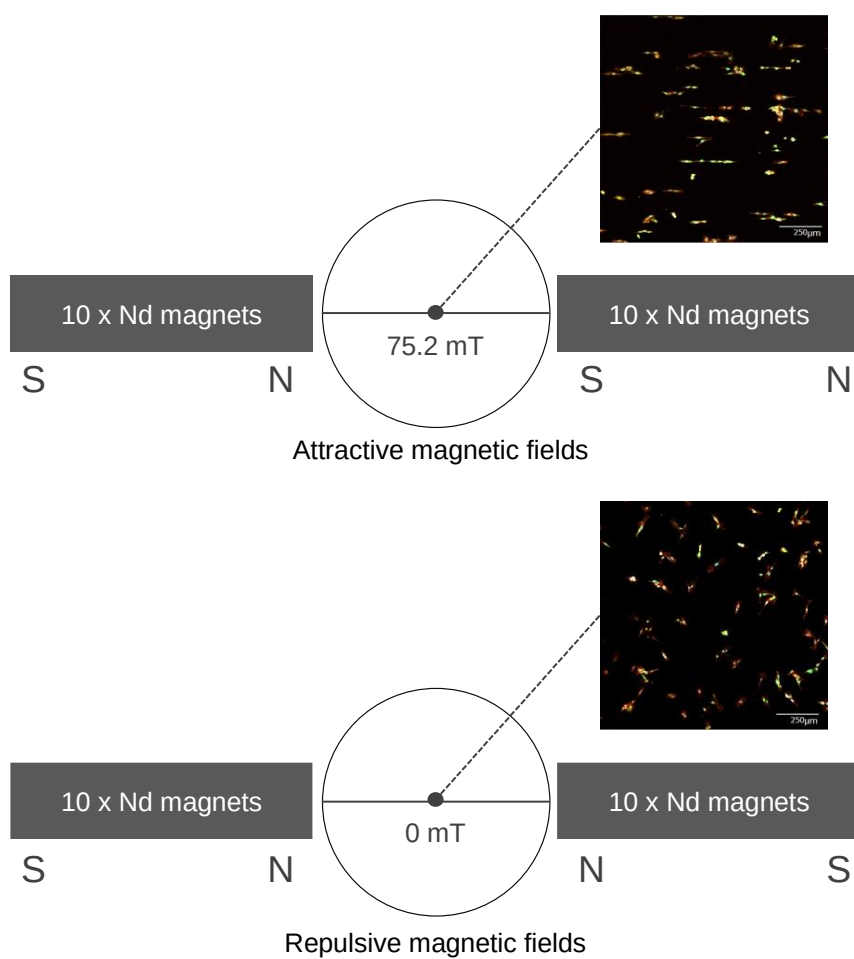


Figure S15. Magnetization intensities under attractive or repulsive magnetic fields. Intensities of the magnetic fields were measured on the center of the dish with a Handy Tesla meter MG-801 (MAGNA).

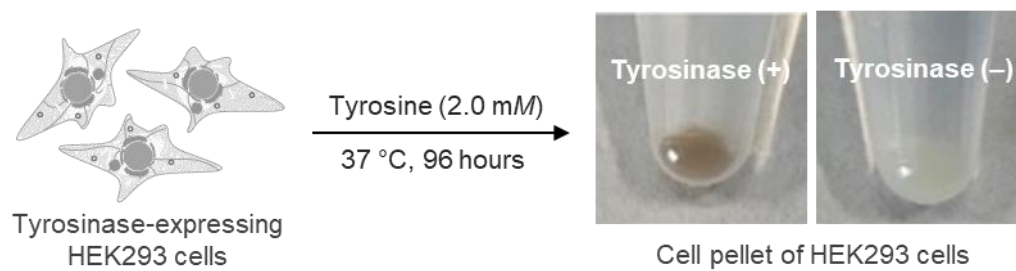


Figure S16. Melanin biosynthesis in HEK293 cells. The cells transfected with a tyrosinase-encoding plasmid displayed darker color than the non-transfected cells.

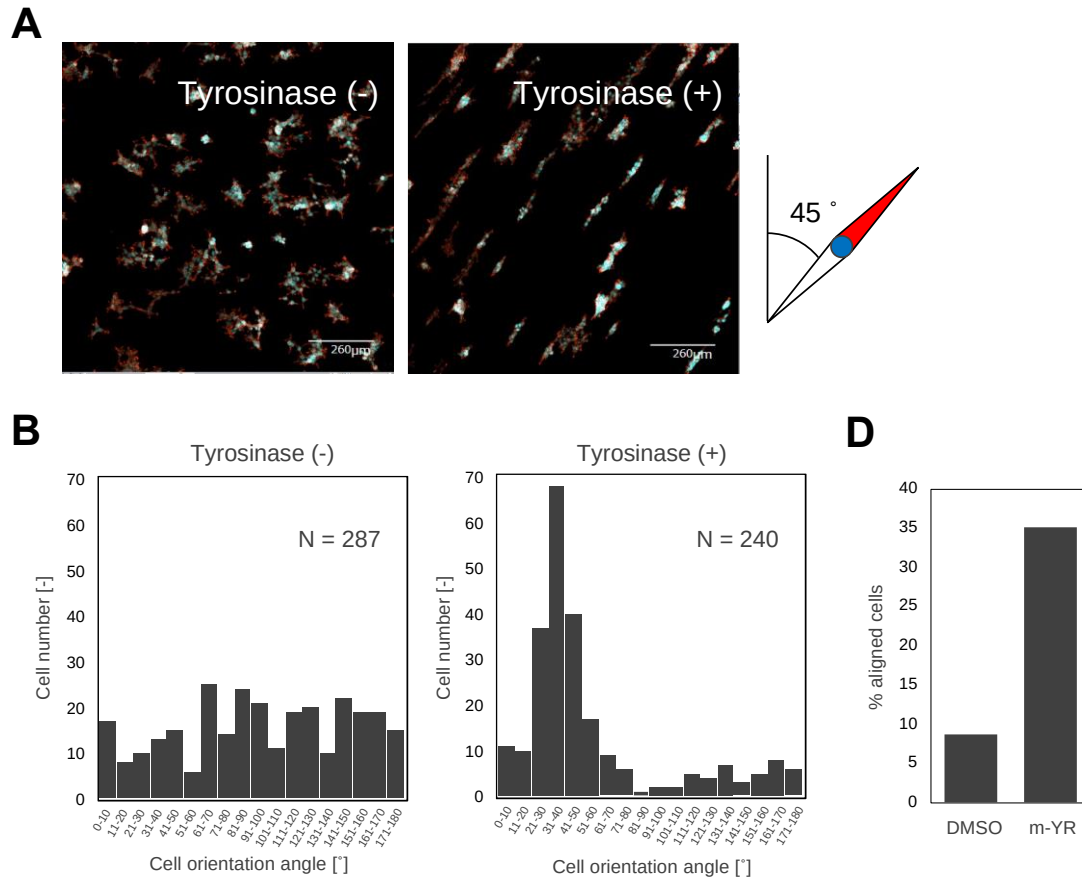


Figure S17. Cell alignment quantification of tyrosinase-expressing HEK293 cells. (A) Confocal images of tyrosinase-expressing HEK293 cells in the presence of magnetic fields. The parental or tyrosinase-expressing HEK293 cells were treated m-YR (100 μ M). Actin (red) and nuclei (cyan) were stained with rhodamine-phalloidin and Hoechst 33258, respectively. Scale bar: 260 μ m. The magnet was placed at a 45° angle to the vertical. (B) The distribution of orientation angles. $n = 287$ cells (DMSO); $n = 240$ cells (m-YR). The angles were automatically calculated by the ImageJ plug-in OrientationJ. (C) Percentages of the magnetically aligned cells. The cells aligned along with the magnetic field are defined as the cells with angles between the orientation axis and the direction of the magnetic field $<10^\circ$.

Supplementary movie

Magnet responsiveness of m-YR-fabricated cellular melanin. B16F10 melanoma cells were treated with m-YR (100 μ M in DMEM) for 96 h and lysed. Cellular melanin was purified and suspended in water. Then, an external magnetic field was applied to purified melanin with a neodymium magnet, whose magnetic flux density is 315 mT.

2) Experimental procedures

Cell culture

HEK293 cells and B16F10 melanoma cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, GIBCO) supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin sulfate (Nacalai Tesque) and 10% fetal bovine serum (FBS, Biosera) at 37°C in a humidified 5% CO₂ incubator.

Materials

The solvents and chemicals for chemical synthesis were used as purchased with no further purification. All solvents and chemicals for chemical synthesis were purchased from Wako Pure Chemicals, TCI, or Sigma-Aldrich. Analytical thin-layer chromatography (TLC) was performed on Merck 60G silica gel glass plates coated with fluorescent indicator F254. Silica gel column chromatography was performed using silica gel 60, spherical, neutra 75µm (Nacalai Tesque). ¹H and ¹³C NMR spectra were collected by a JEOL 600-MHz JNM-ECP NMR spectrometer. High-resolution mass spectra (HRMS) were obtained using a JEOL MStation MS-700V. MALDI-TOF mass spectra were obtained using a microflex LRF (Bruker).

In vitro enzymatic polymerization of tyrosine derivatives

Tyrosine derivatives (1.0 mmol) were dissolved in 900 µL phosphate buffer saline (PBS, pH 7.4, 10010-023, Thermo Fisher Scientific). Tyrosinase (100 µL, 2.0 mg/mL in PBS) was then added to trigger oxidative polymerization. The reaction mixture was incubated at 37 °C for 24 h. To synthesize the tyrosinase-treated product of a 1:1 mixture of tyrosine derivative and Tyr, both monomers were dissolved at 1 mM or 10 mM in PBS buffer. After 24-h co-incubation with tyrosinase (0.2 mg/mL), the precipitate of the copolymer was washed by centrifugation in deionized water (1 mL) and methanol (1 mL) three times, respectively. To prepare a 1:1 mixture of Y-N₃ and Tyr homopolymers, the homopolymers were mixed in PBS buffer and incubated for 24 h. Each homopolymer was prepared by incubating its monomer with tyrosinase (0.2 mg/mL). The resulting homopolymer sample was washed by centrifugation in deionized water (1 mL) and methanol (1 mL) three times, respectively, before use. The fluorescence images were captured with CellVoyager™ CQ1 (Yokogawa Electrical Corporation). Fluorescence intensities of melanin polymers were quantified by ImageJ.

Magnetization measurement

The magnetic susceptibility of samples was measured with a Quantum Design MPMS SQUID magnetometer. The field-dependence magnetization data were collected at 300 K, respectively by applying a magnetic field from -50 kOe to +50 kOe.

Biosynthesis of azide-conjugated melanin and click reaction with TAMRA-DBCO

B16F10 melanoma cells (2.0×10^4) were seeded onto a 10-cm dish. After incubation for a day, the medium was replaced with the medium containing 10 mM Y-N₃ and 100 nM α-MSH (PEPTIDE INSTITUTE, Inc.). After incubation for 96 h, the cells were washed with fresh medium and PBS buffer. Cells were trypsinized, collected, and washed with PBS buffer by centrifugation. The resulting cell pellet (5.0×10^6 cells) was suspended in 1 mL RIPA buffer (Nacalai, product no. 08714-04) and lysed by sonication (4 °C, 10 sec). 10 µL of 1.0 mM TAMRA-DBCO (BroadPharm) in DMSO was added to the cell lysate and incubated at 25 °C for 30 min. The melanin polymer was collected by centrifugation (5,000 g, 4 °C, 10 min), and washed with RIPA buffer, water, and methanol three times, respectively. Purified melanin polymers were observed with a microscope. The supernatants were used for the calculation of consumption rates of TAMRA-DBCO, following the equation below.

Consumption rate of TMRA-DBCO [%]

$$= \frac{(\text{Absorbance}_{555\text{nm}} \text{ of } 10 \mu\text{M TMRA-DBCO in RIPA buffer}) - (\text{Absorbance}_{555\text{nm}} \text{ of supernatant})}{(\text{Absorbance}_{555\text{nm}} \text{ of } 10 \mu\text{M TMRA-DBCO in RIPA buffer})} \times 100$$

Fluorescence imaging of intracellular azide-conjugated melanin

B16F10 melanoma cells (2.0×10^4) were seeded onto a 10-cm dish. After incubation for 24 h, the medium was replaced with a medium containing 10 mM Y-N₃ and 100 nM α -MSH. After incubation for 96 h, the cells were washed with fresh medium and PBS buffer. The cells were trypsinized, collected, and washed with PBS buffer by centrifugation. The Y-N₃-treated B16F10 melanoma cells (5.0×10^3 cells) were reseeded onto a glass-bottom 96-well plate (IWAKI). After 12-h incubation, the cells were fixed with 4% formaldehyde (Muto Pure Chemicals Co., Ltd) for 10 min, permeabilized by 0.1% Triton X-100 solution for 5 min, followed by blocking with 2% BSA solution for 1 h. The resulting samples were treated with a 50 nM TAMRA-DBCO solution in PBS for 30 min. After the click reaction, the cells were incubated with the primary antibody (rabbit monoclonal anti-melanoma gp 100 antibody, ab137078, Abcam) at room temperature for 1 h. After washing with PBS, the samples were exposed to the secondary antibody (AlexaFluor® 488 conjugated anti-rabbit IgG, # A32731, Invitrogen) at 25 °C for 30 min. The samples were washed with PBS after each step. The cell images were captured with CellVoyager™ CQ1 (Yokogawa Electrical Corporation). Individual cells were selected manually in ImageJ, and then Pearson's correlation coefficient was calculated with ImageJ plug-in colocalization finder.

WST-8 assay

B16F10 melanoma (1.0×10^4) cells were seeded onto a 96-well plate. Next day, the cells were treated with chemicals in the presence of α -MSH (100 nM) at 37 °C for 96 h. Then, 10 μ L of Cell Counting Kit-8 solution (DOJINDO) was added. After 1 h, the absorbance (450 nm) was measured by an MTP-880 Lab Microplate Reader (CORONA).

RT-PCR

Total RNA from DMSO-treated or m-YR(100 μ M)-treated B16F10 melanoma cells was prepared using ISOGEN (NIPPON GENE) according to the manufacturer's protocol. Then cDNAs were synthesized using PrimeScript 1st strand cDNA Synthesis Kit (Takara Bio), according to the manufacturer's protocol. The RT2 Profiler PCR Array (Qiagen) was used to examine the expression patterns of 84 genes involved in cellular death. The manufacturer's instructions were strictly followed. Gene expression levels were analyzed by using the web-based software RT2 Profiler PCR Array Data Analysis version 3.5.

Biosynthesis of magnetic melanin in B16F10 melanoma cells

B16F10 melanoma cells (2.0×10^4) were seeded onto a 10-cm dish. The next day, the medium was replaced with a fresh medium containing m-YR (100 μ M) and α -MSH (100 nM). After 96-h incubation, the cells were washed with fresh medium and PBS buffer. The cell pellet was then prepared by trypsinization and centrifugation (5,000 g, 5 min, 25 °C). The cells were further washed by centrifugation in PBS buffer. The resulting cell pellet was used for further experiments.

Purification of magnetic melanin from m-YR-treated B16F10 melanoma cells

m-YR-treated B16F10 melanoma cells (5.0×10^6 cells) were suspended in RIPA buffer and lysed with sonication (4 °C, 10 sec). Melanin polymer was precipitated by centrifugation (5,000 g, 4 °C, 10 min) and washed by centrifugation in RIPA buffer, deionized water, and methanol three times, respectively. The magnetic property was then evaluated by using a 315-mT neodymium magnet (15 $\times$ 10 $\times$ 5 mm, Narika).

Nitroso-PSAP iron assay

The iron content of magnetic melanin was determined by Nitroso-PSAP iron assay.¹ Purified melanin from B16F10 melanoma cells treated with m-YR, m-FR, or tyrosine was dissolved in concentrated hydrochloric acid at 1.0 mg/mL. The solution was then sonicated for 1 h. The resulting suspension was centrifuged (5,000 g, 10 min, 4 °C). The supernatant was collected and diluted with deionized water to adjust the acidity of the sample to pH 2. The resultant solution was subjected to iron measurement by Nitroso-PSAP (DOJINDO), as previously reported.²⁻⁵ The absorbance of iron(II) complex at 750 nm was determined by using Hitachi U-3010 Spectrometer.

Magnetic control of m-YR-treated B16F10 melanoma cells

m-YR-treated B16F10 melanoma cells (2.0×10^4 cells) were reseeded onto a 35-mm dish (μ -Dish, 35-mm high, ibidi) and placed on an artificial magnetic field generated by an array of ten 315-mT neodymium magnets. After 24-h incubation, the cells were fixed with 4% formaldehyde (Muto Pure Chemicals Co., Ltd) for 10 min, permeabilized by 0.1% Triton X-100 solution for 5 min, followed by blocking with 2% BSA solution for 1 h. The resulting samples were incubated with the primary antibody (rabbit monoclonal anti-melanoma gp 100 antibody, ab137078, Abcam) at room temperature for 1 h. After washing with PBS, the samples were exposed to the secondary antibody (AlexaFluor[®] 488 conjugated anti-rabbit IgG, # A32731, Invitrogen) at 25 °C for 30 min, followed by actin and nuclei staining with rhodamine-phalloidin (300 unit, Thermo Fisher Scientific) and Hoechst 33258 (1 μ g/mL, Thermo Fisher Scientific) for 10 min, respectively. The samples were washed with PBS after each step. The cell images were captured with CellVoyager[™] CQ1 (Yokogawa Electrical Corporation).

Magnetic field-dependent cell alignments

m-YR-treated B16F10 melanoma cells (2.0×10^4 cells) were seeded onto a 35-mm dish and placed on attractive or repulsive magnetic fields. Attractive magnetic fields were formed by sandwiching a culture plate with two arrays of ten 315-mT neodymium magnets facing each other in different poles, whereas repulsive magnetic fields were in the same poles. After 24-h incubation, the cells underwent the fluorescent staining procedures for actin and nuclei as described above. The confocal images of cells were captured with a CellVoyager[™] CQ1 (Yokogawa Electrical Corporation).

Quantification of magnetically aligned cells

Individual cells were selected manually in ImageJ. The angles were automatically calculated by the ImageJ plug-in OrientationJ. The cells aligned along with the magnetic field are defined as the cells with angles between the orientation axis and the direction of the magnetic field $<10^\circ$.

Plasmid Construction

Total RNA from cultured B16F10 melanoma cells was prepared using ISOGEN (NIPPON GENE) according to the manufacturer's protocol. The tyrosinase cDNA was then amplified by PCR using forward infusion primer 5' CGGGCTGCAGGAATTCATGTTCTTGGCTGTTTTGT and reverse infusion primer 5' GCTTGATATCGAATTTACAGATGGCTCTGATAC. The amplified tyrosinase cDNA was inserted into pCMV-3Tag-1a (Agilent) at the EcoRI site by In-Fusion[®] HD Cloning Kit (Takara Bio). The PCR amplified sequence was verified by DNA sequencing. The *Escherichia coli* competent cells used for plasmid construction are Competent Quick DH5 α competent cells (TOYOBO). The cells were stored at -80°C upon receipt and thawed on ice just before transformation. The bacterial cells were grown in LB (Lennox, Nacalai Tesque) growth media at 37°C.

Tyrosinase transfection and biosynthesis of magnetic melanin in HEK293 cells

HEK293 cells (1.0×10^5) were seeded onto a 6-well plate. The next day, the cells were transfected

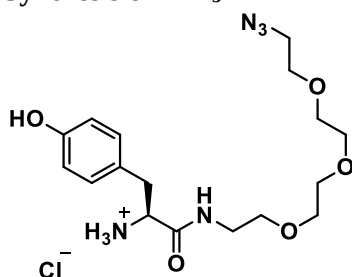
with an expression plasmid encoding Tyrosinase-3xFLAG using FUGENE® HD Reagent (Thermo Fisher Scientific) and incubated for 24 h. Cells were then trypsinized and transferred to 35-mm dish at a cell density of 50,000 cells/dish. The next day, the medium was replaced with fresh medium containing m-YR (100 μ M). After 96-h incubation, the cells were washed with fresh medium and PBS buffer. A cell pellet was then prepared by trypsinization and centrifugation. The cells were further washed by centrifugation in PBS buffer. The resulting cell pellet was used for further experiments.

Magnetic control of m-YR-treated tyrosinase-expressing HEK293 cells

m-YR-treated tyrosinase-expressing HEK293 cells (5.0×10^4 cells) were seeded onto a 35-mm dish and placed on an artificial magnetic field generated by an array of ten 315-mT neodymium magnets. After 48-h incubation, the cells were fixed with 4% formaldehyde for 10 min, permeabilized by 0.1% Triton X-100 solution for 5 min, followed by actin and nuclei staining with rhodamine-phalloidin and Hoechst 33258 for 10 min, respectively. The samples were washed with PBS after each step. The cell images were captured with CellVoyager™ CQ1 (Yokogawa Electrical Corporation).

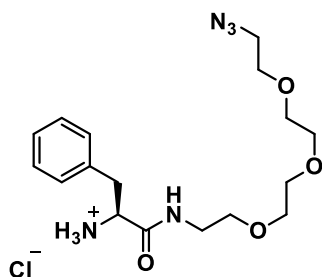
3) Chemical Synthesis

Synthesis of Y-N₃



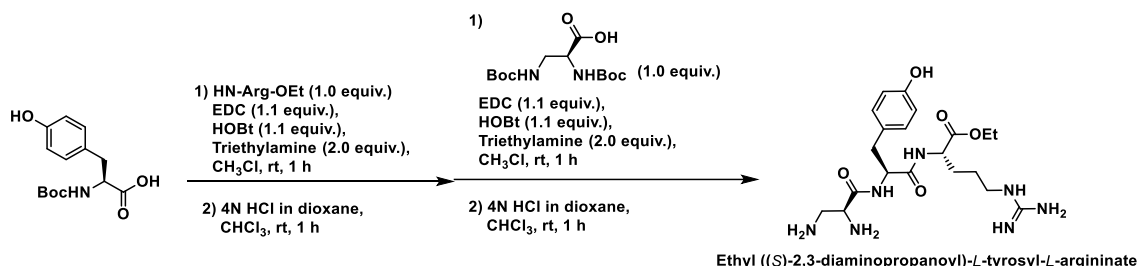
Y-N₃ was synthesized by coupling tyrosine with 11-Azido-3,6,9-trioxaundecan-1-amine via amidation. The coupling reaction was performed with EDC·HCl and HOBt in acetonitrile. To the solution of *N*-Boc-protected tyrosine (281 mg, 1.00 mmol) in 10 mL acetonitrile were added EDC·HCl (210 mg, 1.10 mmol) and HOBt (148 mg, 1.10 mmol). After stirring at 25 °C for 15 min, 11-azido-3,6,9-trioxaundecan-1-amine (240 mg, 1.10 mmol) was added to the mixture, which was stirred at 25 °C for 1 hour under nitrogen atmosphere. For purification, the solvent was removed by evaporation under reduced pressure, and the residual crude product was dissolved in dichloromethane, washed with water twice and brine, dried over Na₂SO₄. The product was purified by silica gel column chromatography using a gradient of dichloromethane and methanol (dichloromethane:methanol = 100:1 to 50:1 v/v), affording the product as a white powder. Following a deprotection using 4N HCl in dioxane (1 mL) for 1 h, the crude white precipitate was evaporated under reduced pressure, then dissolved in ethanol and precipitated in diethylether three times. The titled molecule (Y-N₃) was obtained as a white powder with 34.9% yield for 2 steps. ¹H NMR (600 MHz, DMSO-d₆) δ 2.91 (sep, 2H, *J* = 6.8 Hz), 3.11-3.16 (m, 1H), 3.27-3.32 (m, 2H), 3.35-3.39 (m, 4H), 3.48 (t, 2H, *J* = 5.1 Hz), 3.51-3.55 (m, 5H), 3.58 (t, 2H, *J* = 5.1 Hz), 3.89 (t, 1H, *J* = .6 9 Hz), 6.70 (d, 2H, *J* = 8.4 Hz), 7.02 (d, 2H, *J* = 8.4 Hz), 8.24 (s, 3H), 8.55 (t, 1H, 2.7 Hz), 9.41 (s, 1H); ¹³C NMR (151 MHz, DMSO-d₆) δ 36.07, 38.55, 49.86, 53.51, 68.64, 69.13, 69.50, 69.56, 69.63, 69.67, 115.13, 124.70, 130.36, 156.43, 167.89; ESI-HRMS (*m/z*): calculated for [C₁₇H₂₈N₅O₅]⁺ [M+H]⁺: 382.2085, found 382.2087

Synthesis of F-N₃



F-N₃ was synthesized by coupling phenylalanine with 11-azido-3,6,9-trioxaundecan-1-amine via amidation, following the aforementioned procedure for Y-N₃. The titled molecule (F-N₃) was obtained as a white powder with 54.6% yield for 2 steps. ¹H NMR (600 MHz, DMSO-d₆) δ 3.04 (d, 2H, *J* = 6.6 Hz), 3.10-3.15 (m, 1H), 3.26-3.31 (m, 2H), 3.35-3.39 (m, 4H), 3.47 (t, 2H, *J* = 5.1 Hz), 3.51-3.55 (m, 5H), 3.58 (t, 2H, *J* = 5.1 Hz), 3.99 (br, 1H), 7.24-7.33 (m, 5H), 8.35 (br, 2H), 8.61 (t, 1H, 5.4 Hz); ¹³C NMR (151 MHz, DMSO-d₆) δ 36.79, 38.55, 49.86, 53.23, 68.61, 69.14, 69.48, 69.57, 69.63, 69.68, 126.94, 128.31, 129.43, 134.88, 167.89; ESI-HRMS (*m/z*): calculated for [C₁₇H₂₈N₅O₄]⁺ [M+H]⁺: 336.2136, found 366.2138

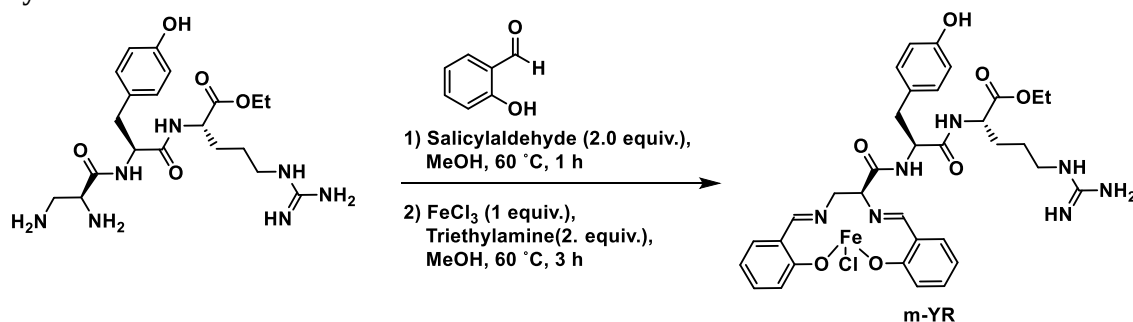
Synthesis of Ethyl ((S)-2,3-diaminopropanoyl)-L-tyrosyl-L-argininate (amino-AYR)



Ethyl ((S)-2,3-diaminopropanoyl)-L-tyrosyl-L-argininate (amino-AYR) was synthesized through 4 steps of amidations and boc-deprotections. To the solution of *N*-Boc-protected tyrosine (281 mg, 1.00 mmol) in 10 mL CHCl₃ were added EDC·HCl (210 mg, 1.10 mmol) and HOBt (148 mg, 1.10 mmol). After stirring at 25 °C for 15 min, L-arginine ethyl ester dihydrochloride (275 mg, 1.00 mmol) and triethylamine (118 mg, 2.00 mmol) were added to the mixture, which was stirred for 1 h under nitrogen atmosphere. The reaction mixture was washed with water twice and brine, dried over Na₂SO₄, and filtered through a pad of silica gel with a mixture of CH₃Cl-methanol (5:1 v/v) as an eluent. The filtrate was concentrated under reduced pressure. The residual material was dissolved in 10 mL CHCl₃, followed by the addition of 4N HCl in dioxane (2 mL). After 1-h stirring at 25 °C, the reaction mixture was concentrated and dried *in vacuo* to yield tyrosine-arginine dipeptide as a white powder. The tyrosine-arginine dipeptide was used for the next reaction without further purification. The dipeptide and triethylamine (2.00 mmol) were added to a reaction mixture of EDC·HCl (1.10 mmol), HOBt (1.10 mmol) and (S)-2,3-bis((tert-butoxycarbonyl)amino)propanoic acid (304 mg, 1.00 mmol) in CHCl₃. After stirring at 25 °C for 1 h under a nitrogen atmosphere, the reaction mixture was washed with water twice and brine, dried over Na₂SO₄, filtered, and evaporated. The resulting residue was applied to a silica gel column chromatography with a gradient of dichloromethane and methanol from 100:1 to 5:1 v/v, affording the product as a white powder. The product was dissolved in CH₃Cl (10 mL), followed by the addition of 4N HCl in dioxane (2 mL). After stirring for 1 h, solvents were removed by evaporation under reduced pressure, affording a white solid of crude product. The crude product

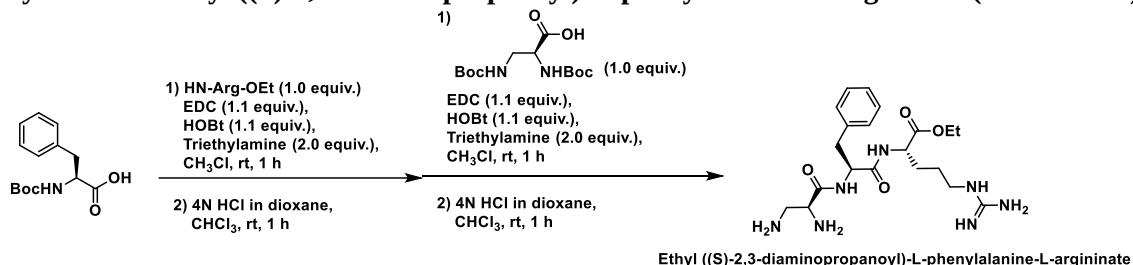
was dissolved in ethanol and precipitated in diethylether three times. Then the precipitate was dissolved in ethanol (1 mL), followed by the addition of triethylamine (505 mg, 5.00 mmol). The reaction mixture was stirred at 25 °C for 12 h. After that, triethylamine hydrochloride was precipitated by the addition of ethyl acetate (10 mL) and removed by filtration. The filtrate was concentrated, and excess triethylamine was removed under reduced pressure. The product was obtained as a white powder with 73.1% yield for 4 steps. ^1H NMR (600 MHz, DMSO- d_6) δ 1.19 (t, 3H, J = 7.2 Hz), 1.56 (quin, 2H, J = 7.5 Hz), 1.64-1.83 (m, 2H), 2.08 (s, 1H), 2.69-2.76 (m, 1H), 2.81-3.25 (m, 5H), 4.07-4.15 (m, 2H), 4.22-4.31 (m, 1H), 4.50-4.53 (m, 1H), 6.67-6.71 (m, 2H), 7.11 (d, 1H, J = 8.4 Hz), 7.96 (t, 1H, J = 5.4 Hz), 8.20 (br, 1H), 8.66 (br, 4H), 9.13 (ddd, 2H, J = 11.7, 7.2 Hz), 9.31-9.41 (m, 1H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 13.94, 13.96, 25.07, 30.60, 37.45, 40.13, 49.70, 52.11, 53.34, 55.64, 60.60, 114.85, 130.20, 130.46, 155.97, 156.90, 168.33, 171.33, 172.02; ESI-HRMS (m/z): calculated for $[\text{C}_{20}\text{H}_{34}\text{N}_7\text{O}_5]^+$ $[\text{M}+\text{H}]^+$: 452.2616, found 452.2617

Synthesis of m-YR



Iron complexes were synthesized following a general procedure previously reported.^{6,7} Under a nitrogen atmosphere, to a suspension of salicylaldehyde (12.2 mg, 1.00 mmol) in dry methanol (10 mL) was added amino-AYR (22.5 mg, 0.50 mmol). The mixture was stirred at 60 °C for 1 h. After 1-h stirring, a solution of triethylamine (59.1 mg, 1.00 mmol) in dry methanol (5 mL) was syringed into the reaction mixture. A solution of FeCl_3 (81.1 mg, 0.50 mmol) in dry methanol (5 mL) was then syringed into the reaction mixture. The resulting mixture was stirred at 60 °C for 3 h. After that, the solvent was removed under reduced pressure, and the resulting residue was dissolved in CHCl_3 . The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and evaporated. The residue was reprecipitated with methanol and diethylether three times to afford m-YR (154 mg, 41.3% yield for 2 steps) as a brown powder. ESI-HRMS (m/z): calculated for $[\text{C}_{34}\text{H}_{40}\text{FeN}_7\text{O}_7]^{2+}$ $[\text{M}-\text{Cl}+\text{H}]^{2+}$: 357.1164, found 357.1163, calculated for $[\text{C}_{34}\text{H}_{40}\text{FeN}_7\text{O}_6]^+$ $[\text{M}+\text{H}]^+$: 749.2022, found 749.2021.

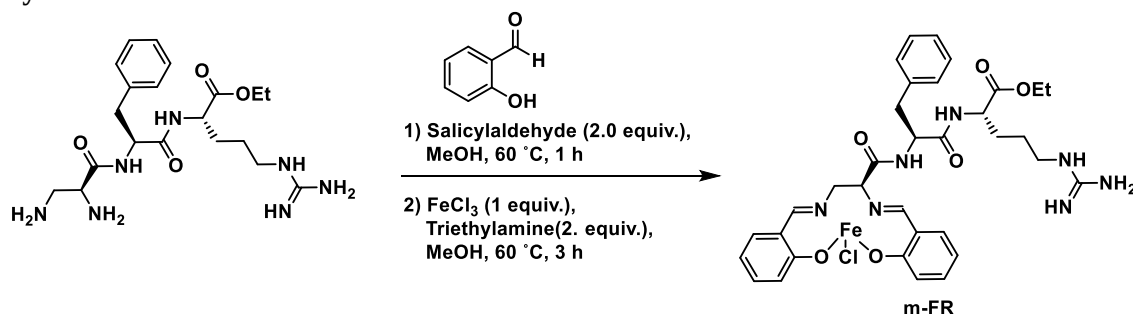
Synthesis of Ethyl ((S)-2,3-diaminopropanoyl)-L-phenylalanine-L-argininate (amino-AFR)



Ethyl ((S)-2,3-diaminopropanoyl)-L-phenylalanine-L-argininate (amino-AFR) was synthesized through the modified procedure for amino-AYR by using *N*-Boc-protected phenyl alanine (265 mg, 1.00 mmol) instead of *N*-Boc-protected tyrosine. The product was obtained as a white powder with 70.2% yield for 4 steps. ^1H NMR (600 MHz, DMSO- d_6) δ 1.19 (t, 3H, J = 7.2 Hz), 1.57 (quin, 2H, J = 7.6 Hz), 1.69-1.75 (m, 1H), 1.78-1.84 (m, 1H), 2.08 (s, 1H), 2.84 (t, 1H, J = 6.2

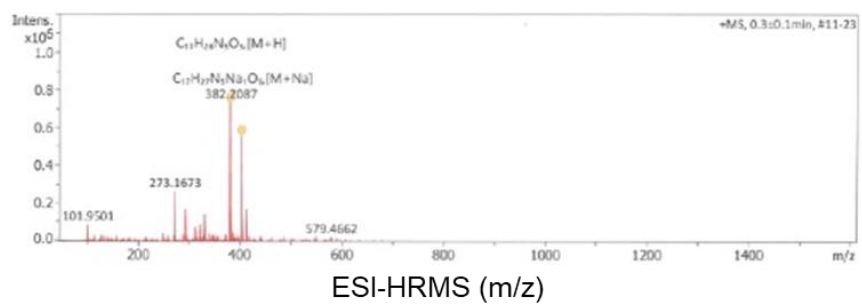
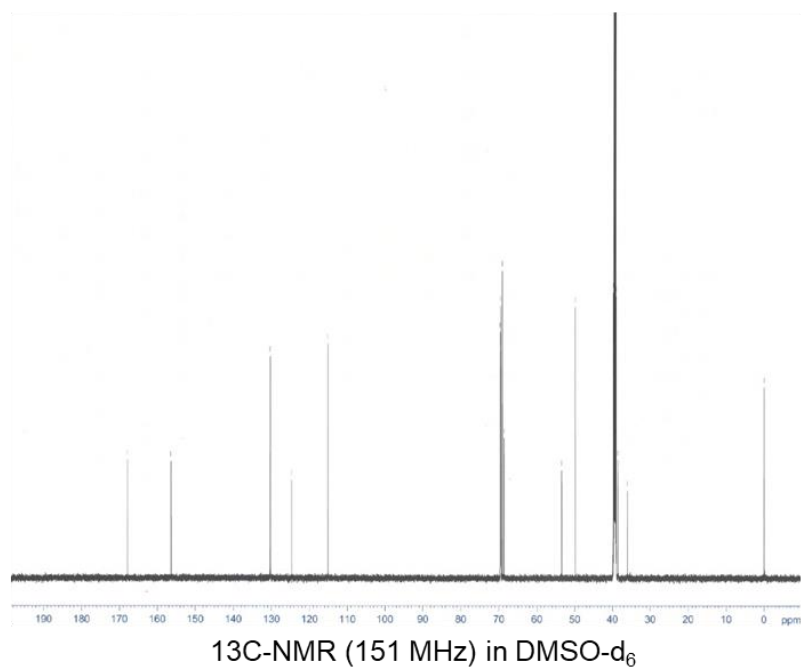
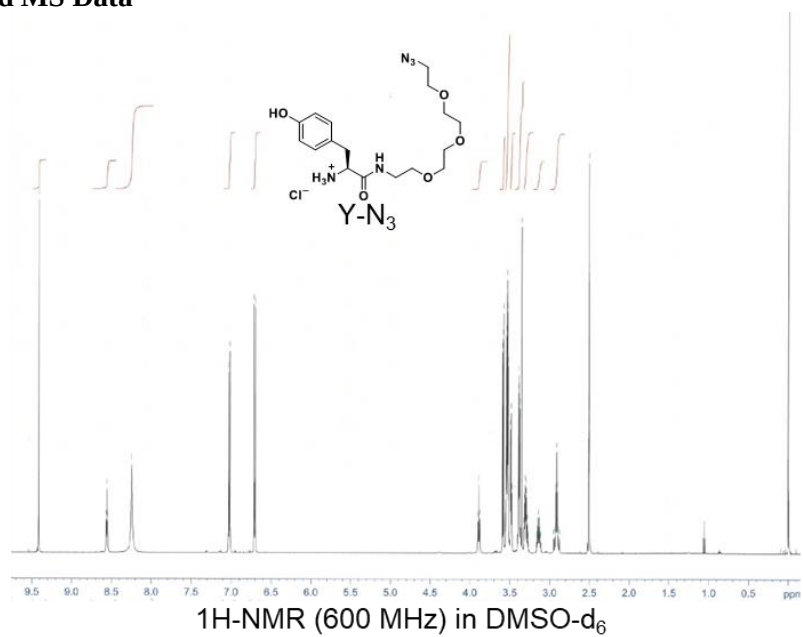
Hz), 3.12-3.42 (m, 6H), 3.56 (s, 1H), 4.07-4.16 (m, 2H), 4.23-4.31 (m, 1H), 4.59-4.62 (m, 1H), 7.02 (br, 2H), 7.22 (t, 1H, $J = 6.9$ Hz), 7.29 (t, 2H, $J = 7.5$ Hz), 7.52 (d, 2H, $J = 7.2$ Hz), 7.98 (t, 1H, $J = 5.7$ Hz), 8.68 (br, 2H), 9.21 (t, 2H, $J = 4.7$ Hz); ^{13}C NMR (151 MHz, DMSO- d_6) δ 13.97, 25.09, 30.60, 36.49, 38.40, 40.13, 52.16, 55.33, 60.61, 66.24, 126.44, 128.04, 129.31, 137.35, 156.93, 167.89, 171.33, 171.87.; ESI-HRMS (m/z): calculated for $[\text{C}_{20}\text{H}_{34}\text{N}_7\text{O}_4]^+$ $[\text{M}+\text{H}]^+$: 436.2667, found 436.2669

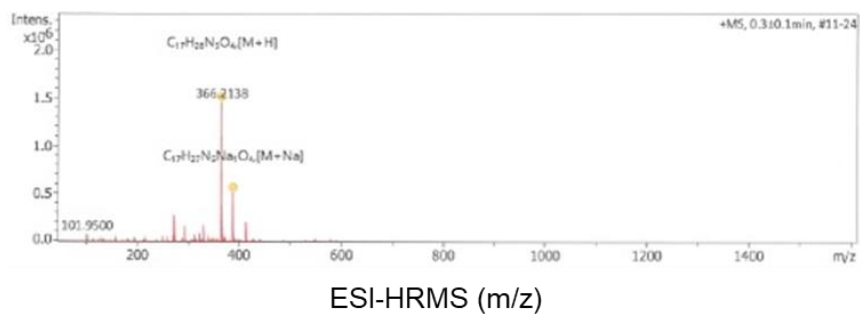
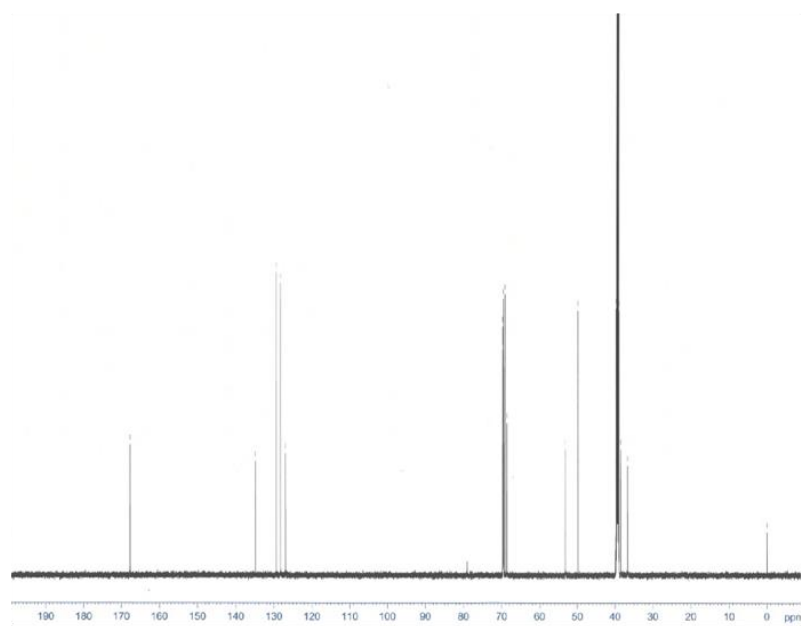
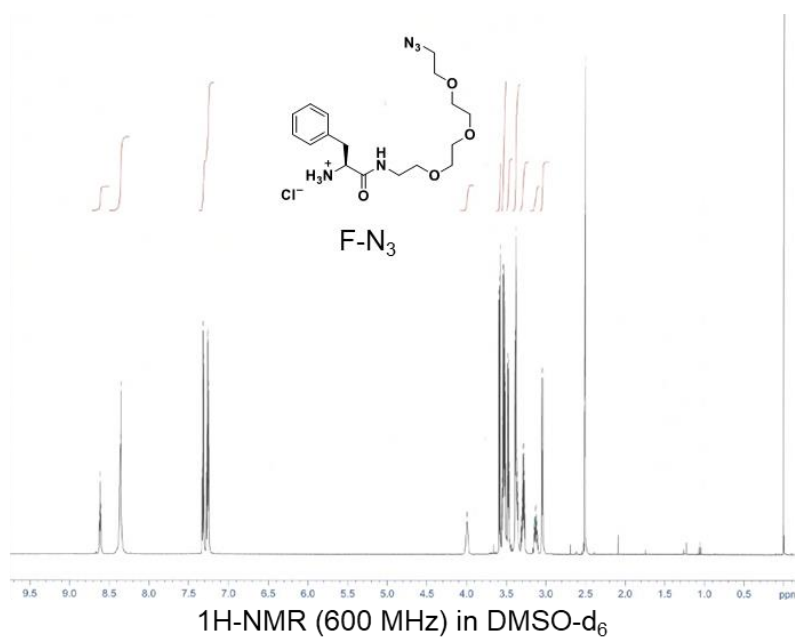
Synthesis of m-FR

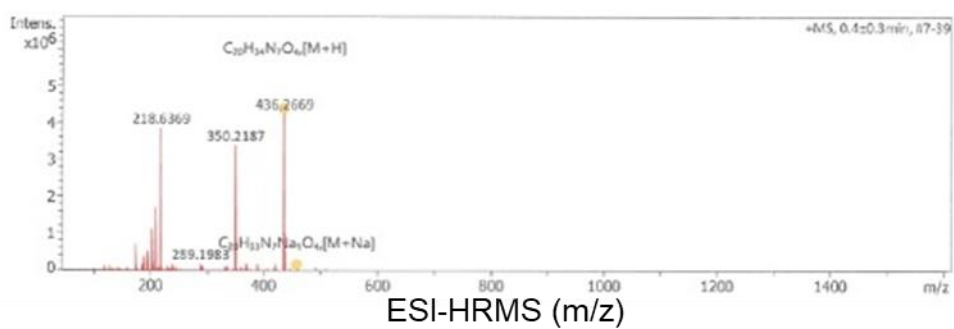
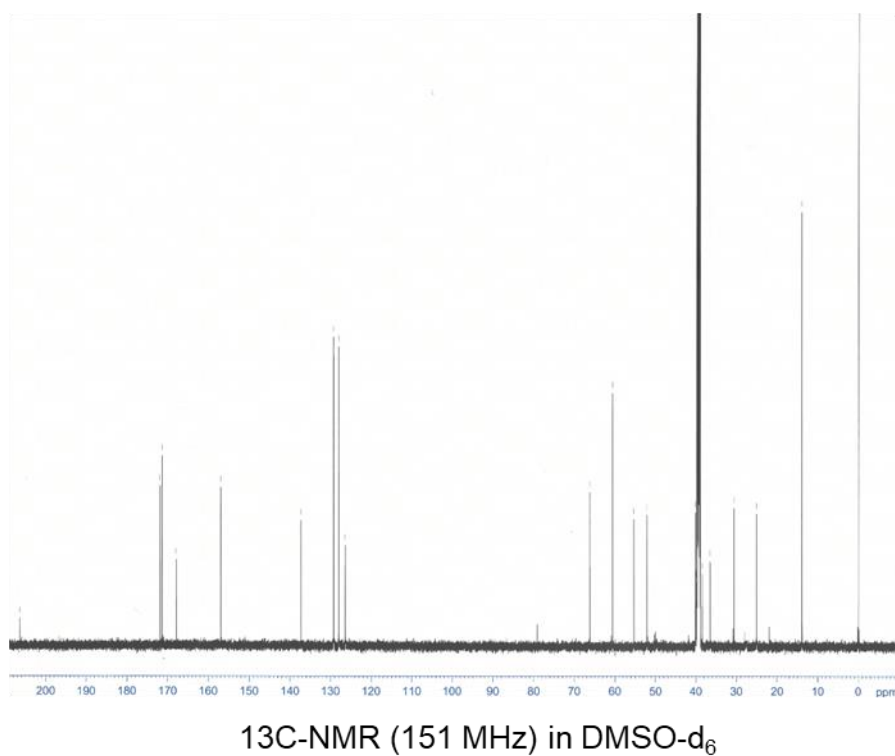
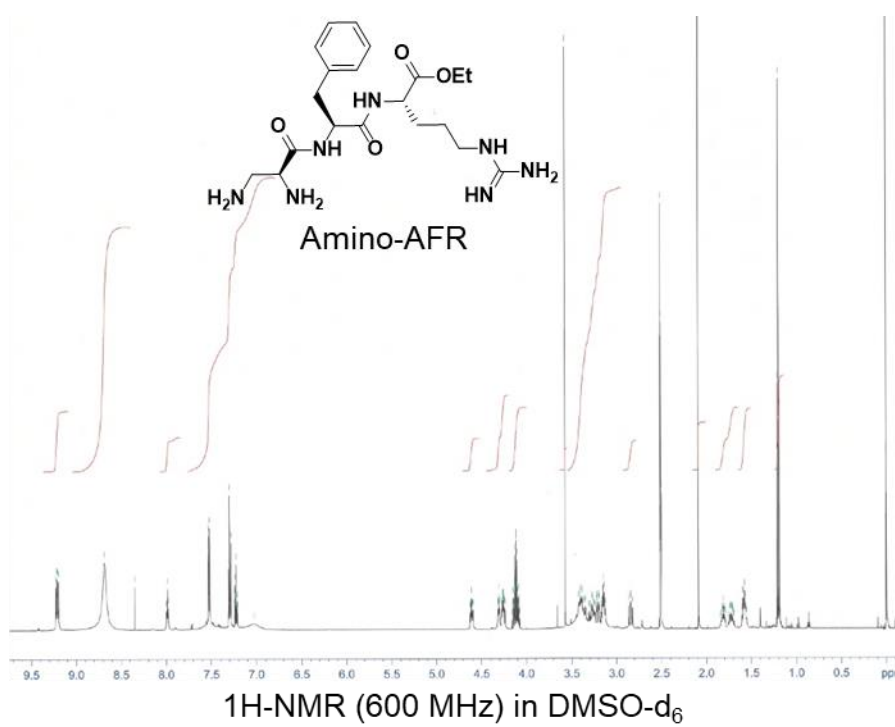


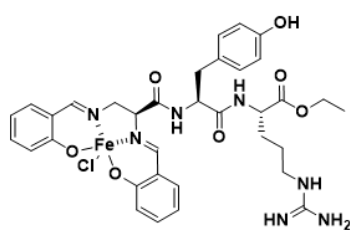
Under a nitrogen atmosphere, to a suspension of salicylaldehyde (12.2 mg, 1.00 mmol) in dry methanol (10 mL) was added amino-AFR (22.5 mg, 0.50 mmol). The mixture was stirred at 60 °C for 1 h. After 1-h stirring, a solution of triethylamine (59.1 mg, 1.00 mmol) in dry methanol (5 mL) was syringed into the reaction mixture. A solution of FeCl_3 (81.1 mg, 0.50 mmol) in dry methanol (5 mL) was then syringed into the reaction mixture. The resulting mixture was stirred at 60 °C for 3 h. After that, the solvent was removed under reduced pressure, and the resulting residue was dissolved in CHCl_3 . The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and evaporated. The residue was reprecipitated with methanol and diethylether three times to afford m-FR (208 mg, 56.9% yield for 2 steps) as a brown powder. ESI-HRMS (m/z): calculated for $[\text{C}_{34}\text{H}_{40}\text{FeN}_7\text{O}_6]^{2+}$ $[\text{M}-\text{Cl}+\text{H}]^{2+}$: 349.1190, found 349.1190, calculated for $[\text{C}_{34}\text{H}_{40}\text{FeN}_7\text{O}_6]^+$ $[\text{M}+\text{H}]^+$: 733.2073, found 733.2068.

4) NMR and MS Data

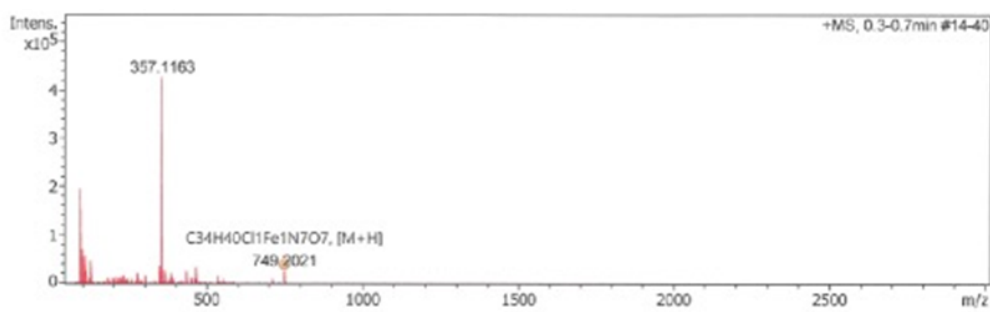




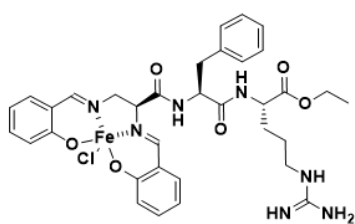




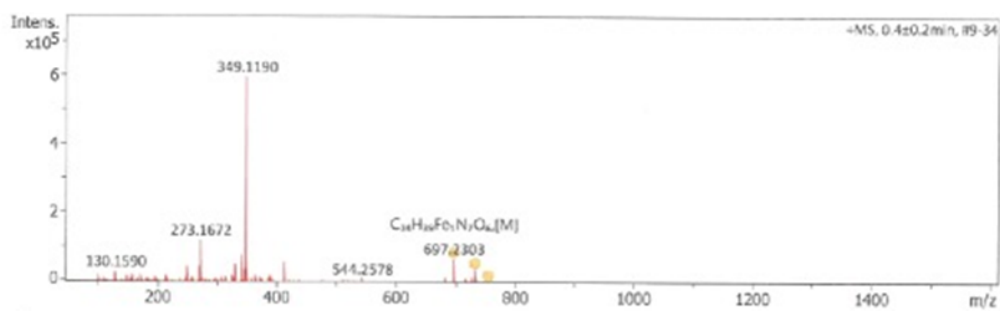
m-YR



ESI-HRMS (m/z)



m-FR



ESI-HRMS (m/z)

5) Reference

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