Luminescent iridium(III)-containing block copolymers: self-assembly into biotin-labeled micelles for biodetection assays.

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1. Experimental section: Reagents, methods and equipment

Reagents and supplies. All reagents and solvents were purchased from Sigma-Aldrich Chemicals and used without further purification. TEM grids were purchased from Electron Microscopy Sciences. Streptavidin-coated magnetic beads were Promega Magnesphere Magnetic Particles. Streptavidin-Dylight 405 was purchased from VWR. Poly(dimethylsiloxane) (PDMS) polymer and poly(dimethylhydrosilane) cross linker (Sylgard 184) were from Dow Corning. Blocking agent (poly-L-lysine grafted with poly(ethylene glycol)) (PLL-PEG) was purchased from Surface Solution. Anti-fade mounting medium was purchased from Electron Microscopy Sciences.

Equipment and methods. Unless otherwise stated, all synthetic reactions were carried out under an atmosphere of nitrogen at room temperature. All monomers were prepared according to literature procedures.² Column chromatography was carried out using Silica 60A (particle size 35-70 µm, Fisher, UK) as the stationary phase, and TLC was performed on precoated silica gel plates (0.25 mm thick, 60 F₂₅₄, Merck, Germany) and observed under UV light. Petrol refers to the fraction of petroleum ether boiling between 40 °C - 60 °C. IPA refers to isopropyl alcohol. ¹H and ¹³C spectra were recorded on a Variant Oxford 400 instrument. Chemical shifts are
reported in parts per million (ppm) from low to high frequency and referenced to the residual solvent resonance. Coupling constants (J) are reported in hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: s = singlet, d = doublet, t = triplet, dd = double doublet, q = quartet, m = multiplet, b = broad.

Self-assembly experiments were performed by dissolving the polymer in acetonitrile at a concentration of 10 mg/mL and slowly adding water dropwise, with stirring, to a final water content of 90% and a final polymer concentration of 1 mg/mL. Transmission electron microscopy (TEM) samples were prepared by placing a small drop onto a carbon-coated 400-mesh copper grid. The drop was wicked off after a few seconds and the grid was left to air dry in a fume hood. Images were recorded on a Tecnai 12 instrument operating at 120 kV. The images were analyzed using Sigma Scan software, and between 1500 and 2000 particles over several pictures and sample preparations were measured to get the average diameters of the micelles by TEM. The diameters by TEM were 20 ± 7 nm for polymer 6 and 7, and 28 ± 16 nm for polymer 8. For Atomic Force Microscopy (AFM) imaging, a drop of micelle solution was placed on a mica surface and allowed to air dry. The samples were imaged at room temperature in air using ScanAsyst®, on a MultiMode 8 instrument equipped with a Nanoscope V controller (Bruker), and processed using Nanoscope Analysis 1.4 software. Silicon nitride probes having a nominal spring constant of ~0.4 N/m, a resident frequency of ~70 kHz, and a tip radius of <20 nm were purchased from Bruker. Images were acquired with a resolution of 512 x 512 and a scan rate of ~1 Hz. Dynamic light scattering (DLS) measurements were performed on a Brookhaven Instruments Corp. system equipped with a BI-200SM goniometer, a BI-9000AT digital correlator, and a Compass 315-150 CW laser light source from Coherent Inc. operating at 532 nm (150 mW). DLS vials were purchased from Canadawide Scientific. DLS measurements were consistent with aggregates of 40-50 nm in addition to larger particles than we expected based on TEM data. Size determination based on DLS data can over-represent larger particles in the mixture, and the presence of a minor proportion of large compound micelles in the solution can skew the DLS results.

Absorbance measurements were recorded on a Cary 5000 UV-Vis-NIR Spectrometer. Most fluorescence spectra were obtained using a Jobin Yvon FluoroMax-2 fluorimeter. Quantum yields were calculated using Ir(ppy)$_3$ in dichloromethane as a reference (Φ=0.4). For quantum yield measurements, the absorbance and fluorescence of aliquots of increasing sample concentration were recorded, where the absorbance in the MLCT region (~375 nm) was always less than 0.1. The area of the emission peak was plotted against the absorbance at 375 nm. The quantum yields were calculated using the equation Φ(sample)= Φ(ref) x (slope(sample)/slope(ref)) x (η$^2$(sample)/η$^2$(ref)), where “ref” is the values for the Ir(ppy) reference and η is the refractive index of the solvent.

For typical experiments with magnetic beads, 2 µL of polymer micelle solution (1 mg/mL) was mixed with 40 µL of bead suspension (1 mg/mL, pre-washed), in 108 µL of PBS (+0.05%
Tween 20) (total reaction volume of 150 µL PBS (+0.05% Tween 20)). (Control solutions were composed of 2 µL micelle sample in 148 µL PBS (+0.05% Tween 20)). Samples were gently mixed on a tube inverter for 30 min. The reaction vials were placed in a magnetic rack and the reaction solution was pipetted out and into a new vial. Beads were washed with 0.1% Tween 20 (aq.), and the wash solution was concentrated using 30K MWCO Microcon filters. These reaction and wash solutions were transferred to a black 96-well plate and their fluorescence spectra were recorded on a Biotek Synergy H4 Microplate reader.

PDMS stamps with striped patterns were generated by pouring a mixture of ethylene-terminated PDMS polymer and poly(dimethylhydrosilane) cross linker (10:1) over a Si master wafer containing the line patterns. The mixture was degassed for 20 min and cured overnight at 60°C. The individual stamps were cut from the bulk cured PDMS. Cover slips for surface-binding measurements were prepared by washing in piranha solution (75:25 H₂SO₄:H₂O₂) for 10 min, followed by washing and reaction with 1% (3-aminopropyl)trimethoxy silane (APTES) in acetone (5 min). After rinsing, the slides were treated overnight with glutaraldehyde (2.5% in PBS). Just prior to use, cover slips were rinsed with water and dried under a stream of nitrogen. Stamps were incubated with STV-Dylight 405 by placing a drop (4 µL, 125 µg/mL) and spreading it by placing an untreated coverslip on top for 5 min. After incubation, the stamp was lightly rinsed with PBS and water, dried under a stream of nitrogen, and the STV-Dylight 405 printed by carefully placing it onto the treated cover slip. The stamp was left approximately 5 minutes before being carefully removed. The stamped cover slip was then treated with a blocking agent (PLL-PEG, 10 µg/mL in PBS) for 30 min, then rinsed with PBS (+0.1% Tween 20) and water. A droplet (1-2 µL) of a solution of micelles of polymer 8 (diluted 1:4 in water) was added to the stamped region and incubated ~20 min. After the cover slip was rinsed, it was mounted on a glass microscope slide, using mounting medium with anti-fade agent (1,4-phenylenediamine). The fluorescence was imaged on a Nikon TE-2000 microscope, using a 20x objective and filter blocks from Chroma. The micelles of polymer 8 were imaged using a 350/50 nm excitation filter, a 400 nm long pass dichroic mirror, and a 535/50 nm emission filter. The streptavidin-Dylight 405 was imaged using a 350/50 nm excitation filter, a 400 nm long pass dichroic mirror, and a 460/50 nm emission filter.

2. Synthesis of PEG-Biotin ROMP monomer

A solution of tosyl chloride (381 mg, 2 mmol, 1 equiv.) in dry CH$_2$Cl$_2$ (20 mL) was added dropwise via syringe pump (4 hours) to a solution of PEG 2000 (4 g, 2 mmol, 1 equiv.), NEt$_3$ (1.4 mL, 10 mmol, 5 equiv.) and DMAP (cat. amount) in dry CH$_2$Cl$_2$ (200 mL). The reaction mixture was stirred for 8 hours at room temperature. The solution was washed with 1M HCl (100 mL), with water (2 x 100 mL) and concentrated under vacuo. The residue was dissolved in MeOH (20 mL) and precipitated with Et$_2$O to afford 2 as a pure waxy white solid (3.88 g, 94%).

$^1$H-NMR (400 MHz, CD$_2$Cl$_2$, 298 K): $\delta = 7.76$ (d, $J = 6.5$ Hz, 2H, $H_c$), 7.31 (d, $J = 7.4$ Hz, 2H, $H_b$), 4.12 (bt, 2H, $H_d$), 3.38-3.82 (m, 178H, $H_e$, $H_f$, and $H_g$), (2.42 s, 3H, $H_a$).

A suspension of 2 (3.3 g, 1.51 mmol, 1 equiv.), 3 (250.0 mg, 1.51 mmol, 1 equiv.) and K$_2$CO$_3$ (2.1 g, 15.1 mmol, 10 equiv.) in butanone (20 mL) was stirred at 80 °C for 8 hours. The reaction
mixture was allowed to cool down before the solvent was removed under reduced pressure. The residue was extracted with a mixture of CHCl₃/IPA 3:1 (50 mL) and washed with 1M HCl (50 mL) and then with water (50 mL). The organic phase was dried (MgSO₄), filtered and concentrated under vacuo to afford 4 as a white waxy solid (3.1 g, 87%). ¹H-NMR (400 MHz, CD₂Cl₂, 298 K): δ = 6.49 (s, 2H, Ha), 5.23 (s, 2H, Hb), 3.79 (bt, 2H, He), 3.40-3.85 (m, 178H, Hd, Hf and Hg), 2.84 (s, 2H, Hc), 2.62 (bs, 1H, Hh).

DCC (57.8 mg, 0.28 mmol, 1.2 equiv.) was added to a solution of 4 (500 mg, 0.23 mmol, 1 equiv.), biotin (68.4 mg, 0.28 mmol, 1.2 equiv.) and DMAP (cat. amount) in CH₂Cl₂ (5 mL). The reaction mixture was stir at room temperature for 6 hours. The solvent was removed under reduced pressure and the residue dissolved in a mixture of CHCl₃/IPA 3:1 (10 mL). The organic phase was washed with NaHCO₃ sat. (3 × 50 mL), water (50 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The obtained oil was dissolved in MeOH (3 mL) and 5 was precipitated with Et₂O as a white solid. ¹H-NMR (400 MHz, CDCl₃/CD₃OD 99:1, 298 K): δ = 6.46 (s, 2H, Ha), 5.18 (s, 2H, Hb), 4.52 (dd, J = 4.8, 7.9 Hz, 1H, Ho or Hn), 4.30 (dd, J = 4.3, 8.1 Hz, 1H, Ho or Hn), 3.75 (t, J = 4.6 Hz, 2H, Hb), 3.48-3.65 (m, 178H, Hd, He and Hf), 3.39 (bt, J = 4.7 Hz, 1H, Hj), 3.31 (bs, 2H, Hm), 2.81 (s, 2H, Hc), 2.31 (s, 2H, Hb), 1.54-1.70 (m, 4H, Hi and Hk), 1.40 (q, J = 8.6 Hz, 2H, Hj).

3. General procedure for polymerisation
A dry Schlenk tube was charged with Grubb’s catalyst 3rd generation (10.0 mg, 0.011 mmol, 1 equiv.) under an argon atmosphere. Dry CH₂Cl₂ (1 mL) was added and the mixture was stirred at room temperature for 5 minutes. Addition of each monomer in dry CH₂Cl₂ was followed by 30 minutes stirring at room temperature. The polymerisation was quenched using ethyl vinyl ester (1 mL, excess) and the polymer was precipitated using petroleum ether.

4. Polymer 6 : (PEG)₃-(Ir)₁₀-(nBu)₁₀

Following the general procedure with Grubbs III (10.0 mg, 0.011 mmol, 1 equiv.), PEG-monomer (71.3 mg, 0.033 mmol, 3 equiv.), Ir-monomer (119.0 mg, 0.11 mmol, 10 equiv.) and nBu-monomer (24.3 mg, 0.11 mmol, 10 equiv.). The polymer was obtained as a yellow solid (161 mg, 75%). ¹H-NMR (400 MHz, CD₂Cl₂, 298 K): δ = 8.61 (bs, 10H, Hk’), 8.53 (bs, 10H, Hj’), 7.86-8.02 (m, 30H, Hi’), 7.65-7.86 (m, 40H, Hf’ and Hg’), 7.18-7.60 (m, 85H, Hq’, Ht’, Hn’, Hs’ and Ho’), 6.96-7.12 (m, 20H, Hh’ and Hm’), 6.86-6.96 (m, 20H, Hp’), 6.76 (d, J = 7.6 Hz, 1H, Hd), 6.52 (m, 1H, He), 6.32 (d, J = 6.5 Hz, 21H, Hp), 6.08 (bs, 21H, Hc), 5.80 (bs, 3H, Hi and Hı), 4.86-5.08 (m, 40H, Hn’ and Hı’), 4.73 (s, 20H, Hg’), 4.32-4.54 (m, 46H, Hc and Hı), 3.40-3.72 (m, 540H, Hs, Hb, Hc and Hı), 3.35 (bs, 9H, Hı), 2.58 (bs, 30H, Hı’), 1.48-1.90 (m, 126H, Hı’), Hc’, Hı’, Hı’, Hı’ and Hı’), 1.20-1.48 (m, 40H, Hı’ and Hı’), 0.93 (bt, J = 6.8 Hz, 30H, Hı’).
5. Polymer 7 : (Bio)1-(PEG)3-(Ir)10-(nBu)10

Following the general procedure with Grubbs III (10.0 mg, 0.011 mmol, 1 equiv.), Biotin- monomer (4.8 mg, 0.011 mmol, 1 equiv.), PEG-monomer (71.3 mg, 0.033 mmol, 3 equiv.), Ir- monomer (119.0 mg, 0.11 mmol, 10 equiv.) and nBu-monomer (24.3 mg, 0.11 mmol, 10 equiv.). The polymer was obtained as a yellow solid (161 mg, 75%). $^1$H-NMR (400 MHz, CD$_2$Cl$_2$, 298 K): $\delta = 8.62$ (bs, 10H, $H_k$'), 8.54 (bs, 10H, $H_j$'), 7.86-8.02 (m, 30H, $H_i$', $H_n$' and $H_o$'), 7.65-7.86 (m, 40H, $H_r$' and $H_s$'), 7.18-7.60 (m, 85H, $H_q$', $H_t$', $H_u$', $H_v$', $H_A$, $H_B$ and $H_C$), 6.96-7.12 (m, 20H, $H_h$' and $H_m$'), 6.86-6.94 (m, 20H, $H_e$'), 6.76 (d, $J = 7.6$ Hz, 1H, $H_D$), 6.52 (m, 1H, $H_E$), 6.32 (d, $J = 6.3$ Hz, 21H, $H_F$), 6.08 (bs, 21H, $H_G$), 5.80 (bs, 3H, $H_H$ and $H_I$), 4.86-5.08 (m, 42H, $H_k$' and $H_o$'), 4.73 (s, 20H, $H_g$'), 4.32-4.54 (m, 48H, $H_a$, $H_b$, $H_c$ and $H_d$'), 4.31 (bt, 2H, $H_c$'), 3.40-3.72 (m, 543H, $H_a$, $H_b$, $H_c$, $H_d$, $H_a$' and $H_g$'), 3.35 (bs, 9H, $H_e$), 2.91(m, 2H, $H_{r'}$), 2.65 (m, 2H, $H_{b'}$), 2.58 (bs, 30H, $H_{f'}$), 1.48-1.90 (m, 132H, $H_{b'}$, $H_{c'}$, $H_v$, $H_{b'}$, $H_{c'}$, $H_{a'}$, $H_{e'}$ and $H_{r'}$), 1.20-1.48 (m, 40H, $H_{c'}$ and $H_{d'}$), 0.93 (bt, $J = 6.8$ Hz, 30H, $H_{f'}$).
6. Polymer 8 : (BioPEG)₃-(Ir)₁₀-(nBu)₁₀

Following the general procedure with Grubbs III (10.0 mg, 0.011 mmol, 1 equiv.), Biotin-decorated-PEG-monomer (78.8 mg, 0.033 mmol, 3 equiv.), Ir-monomer (119.0 mg, 0.11 mmol, 10 equiv.) and nBu-monomer (24.3 mg, 0.11 mmol, 10 equiv.). The polymer was obtained as a yellow solid. ^1^H-NMR (400 MHz, CD₂Cl₂, 298 K): δ = 8.62 (bs, 10H, H_k), 8.53 (bs, 10H, H_j), 7.86-8.02 (m, 30H, H_i, H_n and H_o), 7.65-7.86 (m, 40H, H_i and H_s), 7.18-7.60 (m, 85H, H_q, H_t, H_v, H_u, H_A, H_B and H_C), 6.96-7.12 (m, 20H, H_p and H_m), 6.86-6.94 (m, 20H, H_p), 6.77 (d, J = 7.6 Hz, 1H, H_D), 6.52 (m, 1H, H_E), 6.32 (d, J = 6.1 Hz, 21H, H_F), 6.07 (bs, 21H, H_G), 5.79 (bs, 3H, H_H and H_I), 4.86-5.08 (m, 42H, H_a and H_b), 4.73 (s, 20H, H_g), 4.32-4.54 (m, 48H, H_a, H_b, H_I and H_m), 4.31 (bt, 2H, H_f), 3.40-3.72 (m, 542H, H_a, H_b, H_c, H_d, H), 3.35 (bs, 9H, H_c), 2.91(m, 2H, H_k), 2.58 (bs, 30H, H_i), 1.48-1.90 (m, 132H, H_b, H_c, H_d), 0.93 (bt, J = 6.8 Hz, 30H, H_d).
7. TEM images of self-assembled polymers 6-8.

Figure S1. TEM images of micelles of 6 (A), 7 (B) and 8 (C) in 90% water/10% acetonitrile. Part of 1C is expanded in 1D. Scale bars for A-D are 100 nm, 100 nm, 500 nm, and 500 nm, respectively.

8. AFM images of polymers
Figure S2. AFM images of 6. Next to each image is the height profile of the trace indicated by the white line in the image.
9. Quantification of amount of polymer bound to Streptavidin-coated magnetic beads:

First, by measuring the fluorescence decrease of the micelle solution upon adding increasing amounts of magnetic beads, we determined a working range over which the reduction in fluorescence signal (and hence, micelle binding) scaled linearly with the amount of beads added. Our typical conjugation conditions (40 µL of bead suspension) fell within this linear range. Second, by adding polymer micelles to PBS (+ 0.05% Tween 20) in known concentrations, we were able to establish a calibration curve for the fluorescence signal obtained for a particular polymer concentration. Using the slope of this second calibration curve, the fluorescence signals obtained in the first set of calibration experiments could be converted to polymer concentrations, and subsequently the weight of polymer bound to a particular volume of beads added to solution was calculated.

a. Calibration curve for the fluorescence of the reaction solution after adding increasing amounts of beads.

3 different experiments shown - shows decrease in fluorescence signal per unit of beads added.

![Calibration curve for the fluorescence of the reaction solution after adding increasing amounts of beads.](image)
b. Example of calibration curve for fluorescence signal vs polymer concentration.

![Calibration curve for fluorescence signal vs polymer concentration](image)

Apply the results of curve b to the data in curve a to calculate weight of polymer removed per unit of beads added. The slope of the linear range is $2.2 \times 10^{-5}$ mg polymer removed per $\mu$L of 1 mg/mL bead suspension (for this particular data set, graph shown below), which corresponds to 22 $\mu$g of polymer removed (bound) per 1 mL of beads. Repeated experiments gave an average of 24 $\mu$g of polymer removed (bound) per 1 mL of beads. The approximate MW of the polymer is 20 382 g/mol, so 24 $\mu$g = 1.2 nmol.
10. Scheme S1. Microcontact printing of streptavidin onto glass surfaces.

1. Cast PDMS on a mold with stripe features
2. Incubate PDMS with fluorescent dye
3. Microcontact print stripes of dye
4. Block with PLL-PEG, and incubate with nickel- biotin