

The Synthesis of Fluorescently-labeled Polymers and their use in Single-Molecule Imaging

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Supporting Information

General information. The 3,4,9,10-perylenetetracarboxylic anhydride, imidazole, L-isoleucine (99% ee), triphenyl phosphine, diethyl azodicarboxylate, and solvents were purchased from Aldrich and used as received. The 2,2,5-trimethyl-3-(1-(4'-hydroxymethyl)phenylethoxy)-4-phenyl-3-azahexane was a gift from Craig J. Hawker of the IBM Almaden Research Center. The GPCs were run on a Waters 510 using THF as the eluent and calibrated with polystyrene standards.

Synthesis of the perylene diimide (2). In a round bottom flask 6.34 g (0.016 mole) of 3,4,9,10-perylenetetracarboxylic anhydride was added to 50 g of imidazole and 4.68 g (0.036 mole) of L-isoleucine. The contents were pumped down and backfilled with nitrogen 5 times. The round bottom was maintained under a slight positive pressure of nitrogen as it was heated at 160°C for 4 h. The contents were cooled and added to 300 mL of water with 200 mL of concentrated HCl. The contents were stirred for 20 min,

filtered, and washed with copious amounts of water. The contents were dried under vacuum.

The product was cleaned by running a column in silica gel with 2% Et₃N/10% CH₃OH/88% CH₂Cl₂ as the eluent. A red solid was isolated and added to HCl/H₂O to separate the product from the Et₃N. The product was pumped dry to yield 7.31 g (73% yield).

The product was analyzed by low resolution mass spectrometry (619.0 g/mole). The product had limited solubility; thus, we were not able to obtain a NMR spectrum. The product was carried on to the next step without further analysis.

Synthesis of the monoalkoxyamine and dialkoxyamine perylene diimides (3 and 4).

To a round bottom flask under nitrogen 1.31 g (2.1 mmole) of the perylene diimide (2) were added. Dry, O₂ free THF (250 mL) was added. The 2,2,5-trimethyl-3-(1-(4'-hydroxymethyl)phenylethoxy)-4-phenyl-3-azahexane (1.50 g, 4.2 mmole) was added and the contents were stirred for an hour. The flask was cooled with an ice bath, and the triphenyl phosphine (1.66 g, 6.3 mmole) and diethyl azodicarboxylate (1.10 g, 6.3 mmole) were added. The reaction was allowed to warm to room temperature and stirred for 6.5 h. The reaction was rotovapped to dryness.

The red, solid products were isolated by chromatography in silica gel with a graded eluent of 100% CH₂Cl₂ to 5% MeOH/95% CH₂Cl₂. The reaction yielded 1.35 g of dialkoxyamine perylene diimide (4) and 0.2 g of monoalkoxyamine perylene diimide (3).

Characterization of dialkoxyamine perylene diimide (4). The product was isolated as a mixture of diastereomers due to the six stereogenic centers. Two of the centers were derived from the L-isoleucine and the other four stereocenters were derived from the hydroxyalkoxyamine; this combination lead to ten diastereomers in the mixture. ^1H -NMR (400 MHz, CDCl_3 , 298K) δ (ppm): 8.58 (m, 4H), 8.41 (m, 4H), 7.05-7.39 (m 18H), 5.50-5.56 (m 2H), 5.20-5.30 (m, 4H), 4.82-4.85 (m, 2H), 3.75 (m, 0.5H), 3.35 (d of d, 1H, $^3J_{\text{HH}} = 2.2$ Hz, 10.5 Hz), 3.23 (m, 1H), 2.74 (m, 2H), 2.25 (m, 1H), 2.02 (m, 1H), 1.86 (m, 0.5H), 1.23-1.60 (m of m, 16.4H), 1.04 (m, 5.8), 0.97 (s, 5.8H), 0.78-0.91 (m, 9.6H), 0.67 (m, 8.6H), 0.50 (d, 2.8H, $^3J_{\text{HH}} = 6.5$ Hz), 0.09 (d, 1H, $^3J_{\text{HH}} = 6.7$ Hz), 0.06 (d, 1H, $^3J_{\text{HH}} = 6.4$ Hz). ^{13}C -NMR (400 MHz, CDCl_3 , 298K) δ (ppm): 170.0, 169.8, 163.3, 145.9, (2x)145.8, 145.1, 142.6, 142.5, 142.3, 134.8, 134.2, 132.0, 131.1, 131.0, 129.6, 128.7, (2x)128.4, (2x) 128.1, 127.5, 127.3, 127.1, 126.5, 126.4, 126.3, 126.2, 123.3, 123.0, 83.3, 83.2, 82.5, 82.3, 72.3, 72.2, 67.1, 60.6, 60.5, 58.8, 57.5, 34.1, 34.0, 32.1, 31.8, 31.7, 28.5, 28.3, 27.3, 25.5, 24.8, 23.3, 23.2, 22.2, 22.1, 21.3, 21.1, 21.0, 18.2, 15.2, 11.5, 11.3
HRMS calculated for MH^+ : 1293.6892, found: 1293.6908

Characterization of monoalkoxyamine perylene diimide (3). The product was isolated as a mixture of diastereomers due to the four stereogenic centers. Two of the centers were derived from the L-isoleucine and the other two centers were derived from the hydroxyalkoxyamine; this combination lead to four diastereomers in the mixture. ^1H -NMR (400 MHz, CDCl_3 , 298K) δ (ppm): 8.50 (broad, 4H), 8.27 (broad, 4H), 7.05-7.40 (m, 9H), 5.50-5.56 (m, 2H), 5.21-5.31 (m, 2H), 4.80-4.84 (m, 1H), 3.30 (d, 0.5H, $^3J_{\text{HH}} = 10.4$ Hz), 3.21 (d, 0.5H, $^3J_{\text{HH}} = 11.0$ Hz), 2.68 (m, 2H), 2.23 (m, 0.5H), 2.01 (m, 1H),

1.20-1.51 (m, 10.5H), 0.77-1.03 (m, 17H), 0.65 (d, 4.5H, $^3J_{\text{HH}} = 3.0\text{Hz}$), 0.47 (d, 1H, $^3J_{\text{HH}} = 6.4\text{ Hz}$), 0.06 (m, 0.5H). ^{13}C -NMR (300 MHz, CDCl_3 , 298K) 174.8, 170.2, 170.0, 163.3, 146.0, 145.3, 142.6, 142.4, 134.9, 134.4, 132.1, 131.1, 129.3, 128.3, 127.6, 127.4, 126.5, 126.0, 123.1, 122.9, 122.6, 83.5, 83.2, 82.5, 72.4, 72.2, 67.4, 60.7, 60.6, 58.9, 57.7, 34.1, 33.8, 32.2, 31.8, 28.6, 28.5, 28.4, 27.7, 27.4, 25.5, 24.9, 24.8, 23.3, 22.2, 22.1, 21.3, 18.3, 15.3, 15.2, 11.7, 11.5, 11.3 HRMS calculated for MH^+ : 956.4486, found: 956.4475

Polymerization Conditions for the Synthesis of Polyisoprene, Polystyrene, and Polybutylacrylate. The ratio of the monomer to the alkoxyamine determined the molecular weight of the polymer. Based on work by others, we assumed that the polymerizations would consume 80% of the monomer. Thus, the molecular weights of the polymers could be predicted with reasonable accuracy.

The monomer was added to a glass vial. The alkoxyamine (typically 100 mg of the alkoxyamine was used in each polymerization) was added to the glass vial. For the isoprene polymerizations glass vials with thicknesses of 2 mm were used; for the styrene and butyl acrylate polymerizations glass vials with thicknesses of approximately 0.5 mm were used. The glass vial was frozen in LN_2 , evacuated, and thawed. This process was repeated three times. The vial was flame sealed. The vial was heated to 125°C for eight hours (for the styrene), sixteen hours (for the butyl acrylate), and thirty-two hours (for the isoprene). The vial was allowed to cool after the polymerization and the contents were added to methanol. The polymer was isolated, redissolved in minimal CH_2Cl_2 , and precipitated from methanol.

Polymerization Conditions for the Synthesis of Polybutadiene. Butadiene has a low boiling point (-4.5°C) and could not be polymerized in the glass vials. A 300 mL stirred metal reactor (Parr Instrument Company) were used for these polymerizations. The alkoxyamine was added to the Parr reactor and placed under N₂. Approximately 40 mL of dry, O₂ free xylenes were transferred into the parr reactor. The reactor was cooled with dry ice and butadiene was condensed into it. Typically 60 g of butadiene were used in each polymerization; this amount represents a large excess of butadiene. The molecular weight of the polybutadiene was determined not by the ratio of alkoxyamine to monomer but by the duration of the polymerization. After condensing the butadiene into the reactor it was heated to 125°C. After the polymerization was complete the polymer was precipitated into methanol and isolated.

Single-Molecule Imaging. Samples were imaged using an epifluorescence geometry in an inverted microscope (Nikon Diaphot 200, NA 1.4 oil immersion objective). Laser radiation at 488 nm was focused onto the sample and controlled in polarization by passage through an electro-optic modulator (Conoptics M350-50 modulator) at a rate of 5 Hz. A dichroic beamsplitter (Omega 488DRLP) was used to reflect the excitation light towards the sample, and the resulting red-shifted fluorescence was transmitted through the dichroic beamsplitter followed by a bandpass filter (Omega 535DF55) before being imaged onto a back illuminated frame transfer CCD camera (Princeton Instruments MicroMax) which collected data at 10 Hz. The vertical axis in Figures 1c, 2a, and 2c corresponds to ADC counts. The in-plane angle with respect to the x-axis was computed using

$$\phi = \tan^{-1}[(I_y/I_x)^{1/2}]$$

where I_y and I_x are the integrated detected emission signals for y and x polarized pumping, respectively. For a reference to the derivation of this and related equations see: Forkey, J. N.; Quinlan, M. E.; Goldman, Y. E. "Protein structural dynamics by single-molecule fluorescence polarization" *Progress in Biophysics & Molecular Biology*, **2000**, *74*(1-2), 1-35.