Matrix-assisted polymer pen lithography induced Staudinger Ligation

Shudan Bian,¹ Kevin B. Schesing,¹ and Adam B. Braunschweig^{1,*}

¹Department of Chemistry and The Molecular Design Institute, New York University, 100 Washington

Square East, Room 1001, New York, NY 10003.

E-mail: adamb@nyu.edu

^{*}To whom correspondence should be addressed

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1. Organic Synthesis.

General methods. All solvents were dried prior to use with Pure Solv MD-6 Solvent purification system (Innovative Technology, Inc.). All reagents and starting materials were purchased from Aldrich or VWR and used without further purification unless otherwise noted. Solutions were prepared from nanopure water purified from Milli-Q plus system (Millipore Co.), with a resistivity over 18 M Ω cm⁻¹. Compound **3**,¹**4**,²**5**² and 11-azidoundecane-1-thiol³ were prepared according to published literature procedures. Thinlayer chromatography was carried out using aluminum sheets precoated with silica gel 60 (EMD 40 - 60 mm, 230 - 400 mesh with 254 nm dye). All reactions were carried out under an inert atmosphere of N₂ using standard Schlenk techniques or an inert-atmosphere glovebox unless otherwise noted. Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. and used as received. Azide-terminated Au⁴ and glass surfaces⁵ were prepared according to published literature protocols. NMR spectra were obtained on Bruker AVANCE 400 and 500 MHz spectrometers. All chemical shifts were reported in ppm units and referenced with the internal solvent peaks for ¹H and ¹³C chemical shifts. ³¹P spectra were referenced using 85% H₃PO₄. High-resolution mass spectral analyses were carried out on an Agilent 6200 LC/MSD TOF system.



Scheme S1. Preparation of ink molecules 1, 2 and 11-azido-undecane-1-thiol.

Rhodamine phosphine (1). *N*,*N*-Diisopropylethylamine (0.096 mL, 1.1 mmol) was added to a stirring solution of **5** (100 mg, 0.27 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'* -tetramethyluronium hexafluorophosphate (100 mg, 0.28 mmol) in 2.6 mL dry DMF in a glove box at room temperature. **3** (160 mg, 0.31 mmol) was added to the reaction mixture which was subsequently stirred for 14 h at room temperature in the dark. The crude product mixture was concentrated in vacuo and washed with water (50 mL) and EtOAc (100 mL). The layers were separated and the organic layer extract was washed with 100 mL 0.1 M HCl, 40 ml saturated NaHCO₃, 70 ml saturated NaCl, and dried over anhydrous MgSO₄. After filtration, the solvent was removed under reduced pressure to provide a purple solid mixture. The mixture

was subsequently purified by preparative TLC on S4 silica gel plates (MeOH:CH₂Cl₂ (1:20)) as the eluting solvent to provide a purple solid (0.080 g, 34%). ¹H NMR (500 MHz, CD₂Cl₂): δ 1.21 (t, *J*=10 Hz, 12H), 3.04-3.49 (8H), 3.64 (br, 8H), 3.82 (s, 3H), 6.83 (s, 2H), 6.90-6.92 (d, *J*=10, 3H), 7.23(s,1H), 7.24-7.38 (m, 10H), 7.42-7.50 (m, 3H), 7.58(s,1H), 7.77 (m, 2H), 8.10 (s, 1H). ¹³C NMR (400 MHz, CD₂Cl₂): δ 12.33, 46.09, 52.13, 77.60, 96.21, 113.73, 113.89, 127.57, 128.59, 128.84, 130.10, 130.43, 130.97, 131.69, 131.97, 132.54, 133.72, 133.93, 135.00, 135.69, 135.89, 137.51, 137.62, 137.97, 140.83, 141.12, 155.71, 155.89, 157.81, 166.46, 168.82. ³¹P NMR (CDCl₃, 400 MHz): δ –3.79. HRMS m/z calcd for C₅₃H₅₄N₄O₅P⁺ 857.3832, found 857.3852.

Ferrocene phosphine (2). 4-Dimethylaminopyridine (1.7 mg, 0.014 mmol) was added to a stirring solution of ferrocenemethanol (100 mg, 0.46 mmol) and **5** (170 mg, 0.46 mmol) in CH₂Cl₂ (1.8 mL) under N₂. The solution was stirred for 1 hour before Dicyclohexyl carbodiimide (DCC) was added dropwise to the reaction mixture, which was subsequently stirred for 8 hours, and a white precipitate was observed. The solution was filtered to remove the white precipitate, the precipitate was rinsed with CH₂Cl₂ (1.5 mL), the liquid phase were combined, the solvent was removed in vacuo, and the crude product was purified twice by flash chromatography (SiO₂: 3:1 EtOAc : CH₂Cl₂) to afford **2** as a yellow solid (0.089 g, 34%). ¹H NMR (400 MHz, CDCl₃): δ 3.66 (br, 3H), 4.02 (s, 5H), 4.09 (br, 4H), 4.93 (br, 2H), 7.17-7.26 (m, 10H), 7.55 (dd, *J*=1.4, 3.8 Hz, 1H), 7.94-8.01 (m, 2H). ¹³C NMR (500 MHz, CDCl₃): δ 52.30, 63.60, 63.66, 68.17, 68.68, 69.47, 81.17, 128.54, 128.64, 128.99, 130.65, 133.03, 133.85, 135.20, 137.21, 138.00, 165.27, 166.68. ³¹P NMR (CDCl₃, 400 MHz): δ -4.1. HRMS, m/z calcd for [C₃₂H₂₇FeO₄P+H]⁺ 563.1030, found 563.1075.



2. NMR and high resolution mass spectra.

Figure S1. ¹H NMR spectrum of 1.



Figure S2. ¹³C NMR spectrum of 1.



Figure S3. ³¹P NMR spectrum of 1.



Figure S4. High resolution mass spectrum of 1.



Figure S5. ¹H NMR spectrum of 2.



Figure S7. ³¹P NMR spectrum of 2.



Figure S8. High resolution mass spectrum of 2.

3. Monolayer Preparation.

General method. Microscope glass slides were purchased from VWR. SuperAmineTM slides were purchased from Arrayit, Corp. Disuccinimidyl glutaric dicarboxylate was purchased from ProteoChem, Inc. 3-Azidopropylamine was purchased from Primetech, LLC. All other chemicals and materials were purchased from Aldrich. All chemicals were used as received. Metals were evaporated using Bal-Tec MED 020 Coating System.



Scheme S9. Preparation of azide-terminated (a) Au and (b) glass surfaces.

Azide-terminated Au substrates.⁴ Au substrates were prepared by thermal evaporation of 10 nm Cr followed by 150 nm Au onto microscope glass slides, which were pre-cleaned by sonication in EtOH for 20 min prior to metal deposition. The freshly obtained Au substrates were cleaned by immersion in piranha solution (1:3 v:v H_2O_2 : H_2SO_4) for 1 min, and then rinsed with copious amount of deionized H_2O followed by a quick rinse in HCl (conc'd). The Au substrates were again washed with copious amount of deionized H_2O and EtOH. The cleaned Au substrates were immediately immersed in an ethanolic solution of 11-azidoundecane-1-thiol (1 mM) for 24 h. The functionalized Au substrates were then rinsed in EtOH and deionized H_2O to remove excessive absorbents, and dried in an N_2 stream. The azido-functionalized Au substrates were characterized by contact angle measurements (Table S1).

Azide-terminated glass substrates.⁵ The SuperAmine slides were immersed for 24 h in each of the following solutions: 0.1 M phosphate buffer (pH=8), then anhydrous DMF containing disuccinimidyl glutaric dicarboxylate (10 mM) and *N*,*N*-diisopropylethylamine (100 mM), and finally anhydrous DMF containing 3-azidopropylamine (10 mM) and *N*,*N*-diisopropylethylamine (100 mM). The slides were rinsed liberally with deionized H₂O and dried in a N₂ stream after each step. The azido-functionalized glass substrates were characterized by contact angle measurements (Table S1).

Contact angle measurements. Contact angles were measured on a custom goniometer equipped with a 48 M/M EFL microscope (Gaertner Scientific Corp.) by a sessile drop method at ambient temperature and humidity. These results were consistent with those reported previously in the literature. ⁶

Surfaces	Cleaned Au	Azido Au	Amino glass	Azido glass
Contact Angle (°)	62±4	80±3	58±1	75±2

Table S1. Contact angle measurements of different functionalized surfaces.

4. Polymer Pen Lithography.

PPL Tip Array Fabrication. Polymer pen tip moulds with 80 μ m and 120 μ m spacing between tips were prepared following previously reported literature procedures.⁷ The PDMS precursor was prepared by mixing 5.0 g vinylmethylsiloxane-dimethylsiloxane copolymer (Gelest, Inc.), 2.0 μ L Pt divinyl tetramethyl disiloxane (Gelest, Inc.) and 0.20 μ L 2,4,6,8-tetramethyl-2,4,6,8-tetravinylcyclotetrasiloxane (Gelest, Inc.) followed by stirring for 3 days. The final elastomer mixture to form the pen arrays was prepared by mixing 3.4 g of the PDMS precursor with 1.0 g 25-35% (methylhydrosiloxane) 65-70% dimethylsiloxane) copolymer (Gelest, Inc.). The mixture was stirred, degassed, and poured over the center of the master. A glass slide was placed over PDMS and the whole assembly was placed in an oven at 78°C for 36 h to cure the PDMS mixture.

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To prepare the pen arrays for inking, they were exposed to O_2 plasma (Harrick PDC-001, 40 s, medium power) to render the surfaces of the pen-arrays hydrophilic. Subsequently, 4 drops of the ink solution, comprised of **1** (1.3 mg, 1.5 mmol) and PEG (2000 g mol⁻¹, 2.5 mg mL⁻¹) in 2 mL 80:20 THF:H₂O that was sonicated to ensure solution homogeneity, were spin coated (2000 rpm, 2 min) onto the pen PPL array. A Park XE-150 Scanning probe microscope equipped with a PPL head (Park Systems Corp.), custom lithography software and an environmental chamber capable of controlling humidity were used for PPL writing at a humidity of 85%-90% at room temperature. The tip array was leveled by optical methods⁷ or force methods⁸ with respect to the substrate surface using an xy tilting stage. The ink mixture was left on the surface to react 48 hours, washed with THF (50 ml) and H₂O (50 ml), and dried with an N₂ stream.

Control Experiments.

1 (1.3 mg, 1.5 mmol) dissolved in a PEG matrix (2000 g mol⁻¹, 2.5 mg mL⁻¹) was deposited onto amine terminated glass slide by PPL following the same procedure described above. The surface showed clear fluorescent patterns before washing (Figure S10a). However, no fluorescent pattern was visible rinsing with 50 mL THF and 50 mL H₂O (Figure S10b), indicating that the Staudinger ligation had not occurred on amine terminated surfaces.



Figure S10. (a) Fluorescence microscope image of 1 / PEG on amine coated glass slide ($\lambda_{ex} = 532-587$ nm, $\lambda_{obs} = 608-683$ nm) before washing, and (b) after washing with THF and H₂O.

Fluorescence Intensity Profile. Fluorescence intensity profiles (Figure S11) of the array were obtained from a Nikon Eclipse Ti fluorescence microscope ($\lambda_{ex} = 532-587$ nm, $\lambda_{obs} = 608-683$ nm), and extracted by NIS-elements software (Nikon Instruments, Inc.). These patterns show that, regardless of feature size, the signal-to-noise ratios all are in the range of 1.4-1.7, indicating monolayer coverage in all spots.



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Figure S11. Fluorescence intensity profiles of **1** immobilized onto an azide-terminated glass slide produced by PPL using a 8,000-tip PPL arrays with 80 μ m tip-to-tip spacing under 85% humidity at room temperature using -10μ m extension and varying dwell times: (a) 20 s; (b) 10 s; (c) 2 s; (d) 1 s; (e) 0.5 s; (f) 0.1s; (g) 0.05s; (h) 0.02s.

Surface electrochemistry.

Surface redox chemistry of monolayers of 2. To measure the redox properties of 2 on a surface, a control experiment was carried out by immersing an azide-terminated Au surface in an aqueous solution of 2 (1.5 mM) for 16 h to completely cover the surface with 2. The solution was prepared by dissolving 2 (1.7 mg, 3.0 mmol) in 0.1 mL THF followed by mixing the THF solution with 4.9 mL H₂O. The azide coated Au surface was immersed in this solution for 24 hours, washed with THF (50 mL) and H₂O (50 mL) to produce a fc coated substrate. The surface was then dried in a N₂ stream and subsequently characterized by cyclic voltammetry (Figure S12). The cover density of fc, Γ_{fc} , was calculated to be (2.23 ± 0.02) x 10¹⁴ cm⁻² (see calculation of fc Cover Density for details).



Figure S12. (a) Cyclic Voltammetry (CV) characterization of **2**-bearing Au using a Pt counter electrode and Ag/AgCl/1M KCl reference electrode in 1M HClO₄ (aq). Different colored curves indicate different scan rates (0.05, 0.10, 0.15, 0.20, 0.25, 0.30 V/s from black to dark yellow). (b) The linear relationship between peak current and scan rate indicates a surface immobilized species.

Calculation of Fc Cover Density. The cover density of fc, Γ_{fc} , was calculated using Eq (1).⁹ $\Gamma_{fc} = Q_{fc} / neA$ (Eq. 1)

 $Q_{\rm fc}$, the total charge passed in the redox reaction, was calculated by dividing the integral of the redox peak (Figure S13) by the corresponding scan rate. The $Q_{\rm fc}$ for the PPL deposited fc was (3.99 ± 0.07) x 10⁻⁶ C. A, the surface area of the working electrode, was calculated by taking the average feature area and multiplying by the total number of features that could fit within the area of the bore in the Teflon surface cell. For the PPL deposited **2**, A=0.13 cm². The calculated cover density for the PPL deposited **2** was found to be (1.99 ± 0.03) x 10¹⁴ cm⁻². Using the same calculation method, the cover density of **2** on the surface exposed to a solution of **2** rather than by PPL patterning was (2.23 ± 0.02) x10¹⁴ cm⁻², which was close to the theoretical maximum cover density of self-assembled monolayer of fc species — 2.7 x 10¹⁴ cm⁻².

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Figure S13. Cyclic voltammograms (1M HClO₄, Ag/AgCl reference electrode, Pt counter electrode, 0.2 V/s scan rate) of **2** bearing Au surface (red line). The red line demarcates the baseline of the CV of the fc bearing Au surface used to calculate Γ_{fc} .

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