

## Protein Gradients

*Substrate Preparation.* Microscope slides were cut to 1 cm x 1.5 cm and cleaned in piranha (4:1 H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub>) cleaning solution. (CAUTION: *Piranha is a vigorous oxidant and should be used with extreme caution.*) An Au film, 50 nm thick, was vapor deposited on the slides as either a 10 mm x 3 mm or 6 mm x 0.5 mm strip. For the 6 mm x 0.5 mm strip, thick pads (200-250 nm) separated by 3 mm were deposited over the strip. These pads define an active 3 mm x 1.5 mm region for gradient formation. An adhesion layer of Cr (1 nm) was deposited under each layer of Au. Samples were stored under N<sub>2</sub> before use. Prior to thiol assembly, the films were exposed to O<sub>3</sub> for 30 min, rinsed thoroughly with EtOH, and dried with N<sub>2</sub>. Samples were then immersed in a 1mM ethanolic solution of thiol for at least 1 hour, rinsed with EtOH, and dried with N<sub>2</sub>. Substrates for surface plasmon resonance measurements were prepared by evaporating 47 nm of Au with a 1 nm Cr layer directly on an SF-10 prism. Substrates for FTIR measurements have a 5 cm x 1 cm area of 50 nm Au and 1 nm Cr.

*Fibronectin Immobilization.* Gold films containing a thiol SAM were immersed in a freshly-prepared aqueous solution of 75 mM EDC and 15 mM NHS for 15 min. After rinsing with water, samples were exposed to FN at a concentration of 20 µg/mL in 10 mM phosphate buffer (pH 6.0). The samples were then placed in a petri dish in 0.1 M NaOH on an orbital shaker at a speed of 40-50 rpm for 1 hour to remove physisorbed FN. In order to characterize the FN layer, a two-step tagging procedure was performed. Samples were exposed to anti-FN (10 µg/mL with 10% goat serum in pH 6.0 10 mM PB and 100 mM NaCl (PBS) for 1 hr and then FITC-conjugated IgG (10 µg/mL with 10% goat serum in pH 6.0 PBS for 1 hr, with water rinses between solutions. Samples were stored in pH 7.4 PBS unless used immediately.

*Gradient Formation.* Gradients were produced by assembling a thiol SAM in 1 mM ethanolic solution for at least 1 hr onto Au films with thick connection pads. Au wire press connections were made to the pads and the sample was placed in a Teflon electrochemical cell containing an Ag/AgCl reference electrode and a Pt wire counter electrode, with 0.5 M methanolic KOH as the electrolyte. The solution was purged for 15 minutes prior to use and during the application of potential. A potential window was applied to the Au film through the wire press connections by a Pine Instruments model AFCBP1 bipotentiostat. The potential window width,  $\Delta V$ , and the center value of the potential window,  $V_0$ , characterized the applied potential window. Potential was applied for 1 min., after which the film was immediately removed from the electrochemical cell and rinsed thoroughly with MeOH and EtOH. The film was immediately placed in a 1 mM ethanolic solution of a second thiol to backfill the bare Au regions produced by forming the gradient in the first thiol. After a 1 min assembly time, the sample was rinsed with EtOH and dried with N<sub>2</sub>. Samples were stored in water until ready to use.

## Peptide Gradients

*Substrate Preparation.* Substrates for Au deposition were either glass slides, for FTIR study, or single-polished silicon (100) wafers, for electrochemical stripping

analysis of gradients. Prior to metal deposition, slides and wafers were cut into 12 × 50mm pieces and cleaned in a freshly prepared Piranha solution (4:1 H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub>; *Caution: Piranha is a vigorous oxidant and should be used with extreme caution*), rinsed thoroughly with doubly deionized H<sub>2</sub>O and 2-propanol and blown dry with N<sub>2</sub>. The samples were then immediately transferred to the evaporation chamber. Chromium (1nm) was evaporated to promote adhesion of Au on both glass and silicon substrates, followed by evaporation of 50nm of Au. Samples were stored under N<sub>2</sub> before use.

*Gradient Formation.* SAMs of **1** or HDT were prepared by immersing Au films in either 20μM **1** in 1:1 EtOH:H<sub>2</sub>O for 24h or 1mM EtOH solution of HDT for 1h, respectively. A bipotentiostat (Pine Instruments Model AFCBPI) employing a Ag/AgCl reference electrode and a Pt mesh counter electrode was used to apply the desired electrochemical potentials to the two ends of the working electrode in deaerated 0.25M KOH/MeOH. The in-plane electrochemical potential presented by the Au film has been shown to be a linear function of position. Typically, SAMs of HDT and **1** were electrolyzed for 1 min and 30 min, respectively, to achieve one-component gradients. After electrolysis samples were quickly removed from the electrolyte solution, rinsed with MeOH and reimmersed in a solution of a second thiol (MUD or MPA) for 1min to form two-component gradients. Gradients composed of **1**/MUD and HDT/MPA were prepared in this manner.