SUPPORTING INFORMATION

Sandwich Antibody Arrays

Using Recombinant Antibody-Binding Protein L

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Materials and Methods

Chemicals and solvents were purchased from Aldrich. All solvents were dried by passage through a column of activated alumina. Bis-sulfosuccinimidylsuberate (BS^3) and mSeries LifterSlip coverslips were purchased from Thermo Scientific. The High Throughput Wash Station, SuperAmine 2 glass slides, and SuperEpoxy 2 were obtained from Arrayit. Alexa-680 carboxylic acid, succinimidyl ester was purchased from Invitrogen. The hybridization chamber was from CORNING. The NAP-5 column was purchased from GE Healthcare. Antibodies were purchased from Invitrogen or Rockland Immunochemicals. TLC was performed on Merck silica gel 60 GF254 aluminum plates. Silica gel column chromatography was performed using silica gel 230-400 mesh. Cellulose chromatography was performed using Whatman CF-11 fibrous cellulose. Cellulose TLC was performed on cellulose F 0.1 mm plastic sheets and visualized using sulfosalicylic acid and ferric chloride stains. Reactions involving air/moisture sensitive materials were conducted under nitrogen. Fluorescence intensities were measured with a Typhoon 8600 phosphoimager (GE Healthcare). Mass spectra were measured on a Quattro-II triple-quadrupole mass spectrometer in the University of Utah Mass Spectrometry and Proteomics Core Facility. NMR spectra were recorded on a Varian Unity Inova FT NMR spectrometer 300 MHz (^{1}H NMR), 75 MHz (^{13}C NMR) or 121 MHz (^{31}P NMR).

Posttranslational Modification of ProL-CVIA. ProL-CVIA was prepared as described previously. ProL-CVIA (20 μL, 350 μM) and propargyl farnesyl diphosphate (PFPP) (6 μL, 1.8 mM) were incubated in 156 μL of 25 mM phosphate buffer, pH 7.0, containing 10 mM $MgCl_2$, and 10 μM $ZnSO_4$ at 30 °C. After 10 min, 10 μL of yeast PFTase (250 nM) were added. After 1.5 h at 30 °C an additional 10 μL of PFTase were added and the incubation was continued for 1.5 h. The samples were concentrated using Centricon YM-10 (Millipore), and if

need, excess PFPP was removed on a NAP-5 column. Protein concentrations were determined by Bradford analysis. The modified protein was stored at -80 ℃.

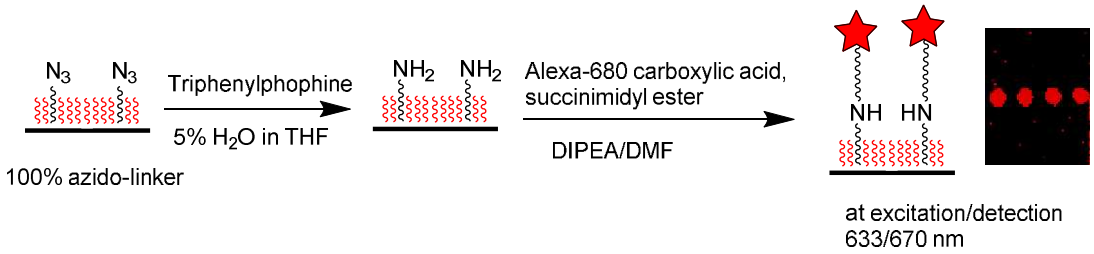
Preparation of Azido-modified Slides. *N,N'*-Disuccinimidyl carbonate (DSC, 25.8 mg, 0.101 mmol) was dissolved in 2.7 mL of *N*,*N*-dimethylformamide (DMF) and 0.303 mL of Hunig's base (*N*,*N*-diisopropylethylamine, 1.74 mmol) was added. The solution was mixed to completely dissolved DSC. The DSC-DIPEA solution (3 mL/slide) was added the surface of the glass slide placed face up in a reaction chamber that is kept under N_2. After the chamber was shaken at rt overnight at 60~90 rpm, the glass slide was washed 3 times with 100 mL of DMF at rt for 20 min. The slide treated with azido linker (60 µL of 11 mM Linker 1) or mixture of a 1:9 solution azido linker (Linker 1):unreactive linker (Linker 2) (31 µL of 11 mM Linker 1 and 146 µL of 21 mM Linker 2) and 27.9 µL of Hunig's base in 3 mL of DMF. The slide was shaken overnight at 60~90 rpm; washed with three times with 100 mL of DMF at rt for 20 min as described above; The slide was capped with 40 µL of ethanolamine in 3 mL of DMF at rt overnight at 60-90 rpm and and washed with three times 100 mL portions of DMF at rt for 20 min, dried under N_2.

Preparation of Huisgen Cycloaddition Buffer. Buffer for the Huisgen cycloaddition was prepared immediately before use by mixing 20 µL of $CuSO_4$ (100 mM, 16 mg/mL in deionized water), 20 µL of tris(carboxyethyl)phosphine (TCEP, 100 mM, 14.3 mg/0.5 mL in deionized water), 100 µL of tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 10 mM, 5.3 mg/mL of 4:1 t-BuOH:DMSO) and 1.8 mL of PBS (Phosphate Buffered Saline, pH 7.4).

Immobilization of ProL-CVIApf on Epoxy-modified Slides. An epoxy-modified glass slide was rinsed with PBS and deionized water and dried under N_2. A silicone mat was attached and 0.33 µM to 333 µM proL-CVIApf (1.5 µL)

was added to each well, followed by addition of 3.5 µL of "Huisgen buffer". The final concentration of proL-CVIApf was serially diluted into 0.1 µM to 100 µM with PBS (pH 7.4). The slide was placed in the hybridization chamber and shaken (60~90 rpm) at rt for 2 h. After the silicone mat was carefully removed, the slide was washed twice with PBST (PBS buffer containing 0.1% Tween 20, pH 7.2) at rt in a High Throughput Wash Station. 3 mL of a blocking solution (1% BSA in PBST) was added and shaken at rt for 1 h.

Preparation of Standards for Alexa-680.



An azido-linker modified glass slide was rinsed with deionized water and acetone and dried under N_2. A silicone mat was attached and 1 M triphenylphosphine (10 µL) in 5% H_2O/THF was added to individual wells. The slide was swirled at 80 rpm at rt for 4 h. The solution was removed, and the individual wells were washed twice with deionized water, THF, and DMF. A 5 µL solution of Alexa-680 carboxylic acid, succinimidyl ester (0.1 M) and Hunig's base in DMF was added to individual wells. The slide was swirled at 80 rpm at rt for 1 h. After the silicone mat was carefully removed, the slide was washed twice with THF and DMF at rt in a chamber.

AFM analysis of proL-CVIApf Immobilized on the Glass Slide.

AFM measurments were performed on a Bruker Dimension Icon PT instrument in ScanAsyst in air mode using SCANASYST-AIR tips with nominal spring constant of 0.4 N/m and resonance frequency of 70 kHz.

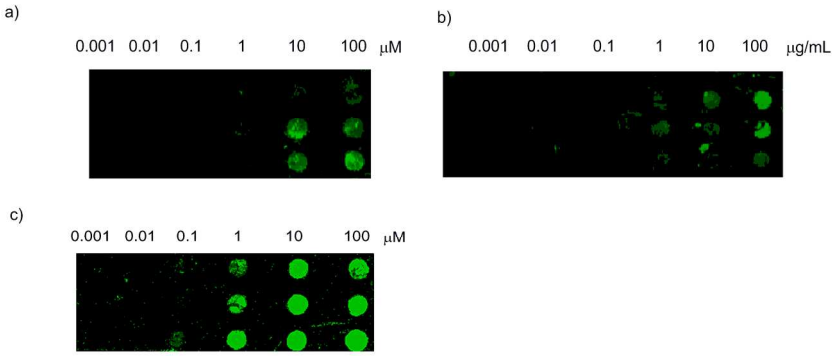
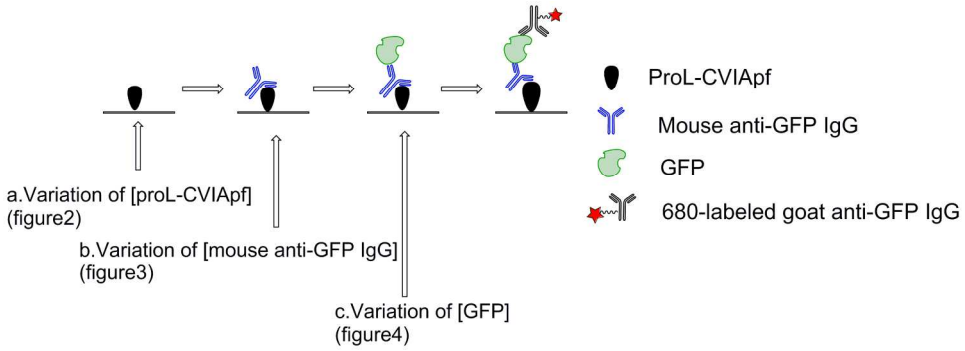


Figure S1. Visualization of GFP in the "Sandwich" antibody arrays described in Scheme S1. Slide a – varied concentrations of proL GFP, mouse anti-GFP IgG (55 μL, 1 mg/mL), GFP (20 μM), and DyLight 680-labeled goat anti-GFP IgG (1.0 μg/mL); slide b - varied concentrations of mouse anti-GFP IgG, 100 μM proL-CVIApf, GFP (20 μM), and DyLight 680-labeled goat anti-GFP IgG (1.0 μg/mL); slide c – varied concentrations of GFP, 100 μM proL-CVIApf, mouse anti-GFP IgG (55 μL, 1 mg/mL), and DyLight 680-labeled goat anti-GFP IgG (1.0 μg/mL). Fluorescence intensities were measured by excitation/detection at 532/526 nm (GFP).

Scheme S1. Fluorescent "sandwich" antibody array.



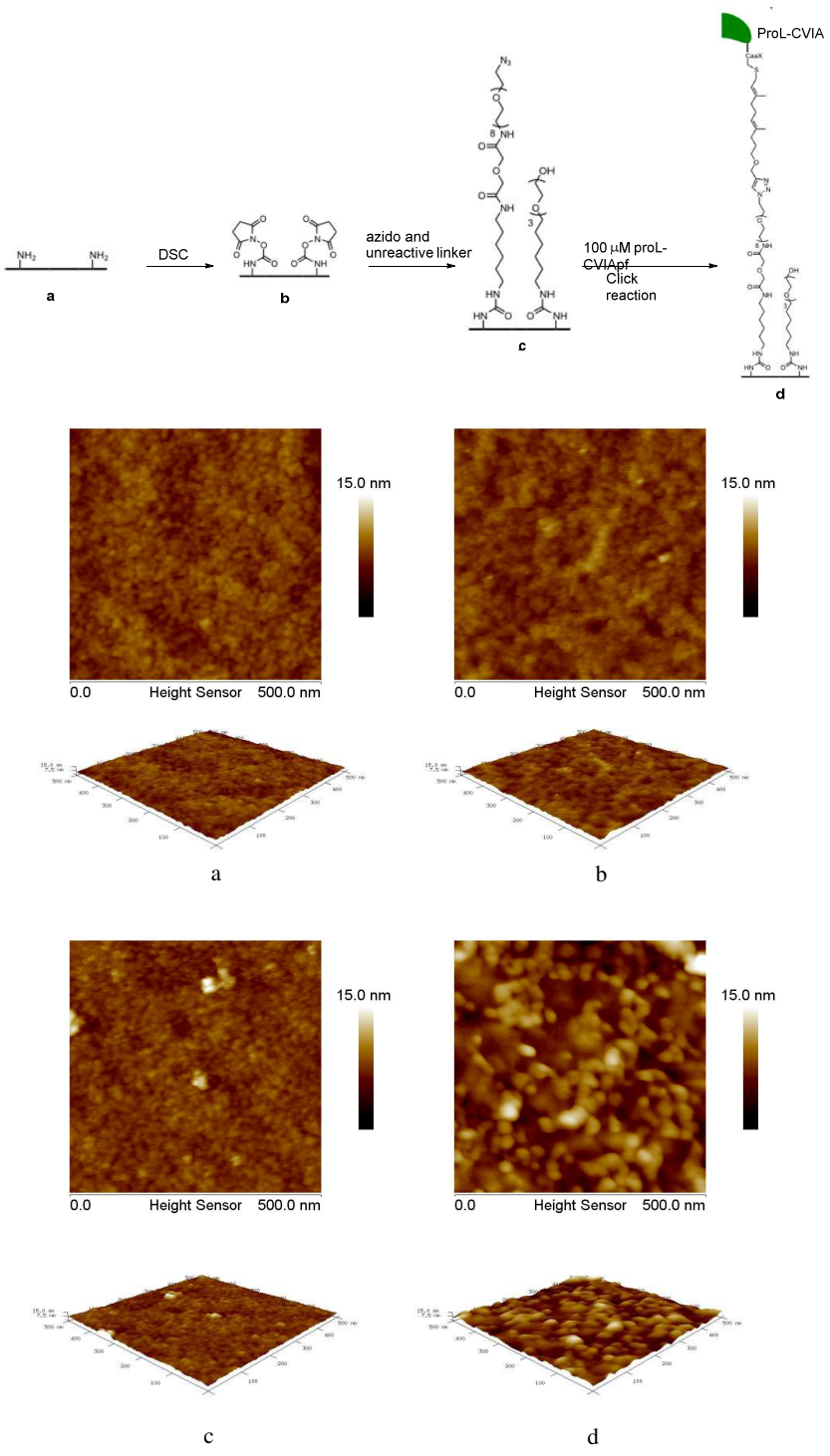


Figure S2. AFM images with 0-15 nm vertical scales of modified silica slides from each step in the immobilization of protein L and slide with proL-CVIApf. The images were acquired at 1 Hz rate infor 500 x 500 nm areas. (a) An amine slide. R, root mean square roughness (RMS) = 0.82 nm; (b) a succinimidyl ester slide, RMS = 0.87; (c) an azido-PEG modified slide, RMS = 0.89; (d)

a slide coated with proL-CVIApf, RMS = 1.82. The images (a) and (b) before modification with azido-PEG linker show uniform surfaces. The image (c) and is less uniform, perhaps reflecting the azido-PEG linker, and the roughness of the surface in image (d) is consistent with surface immobilized proL.

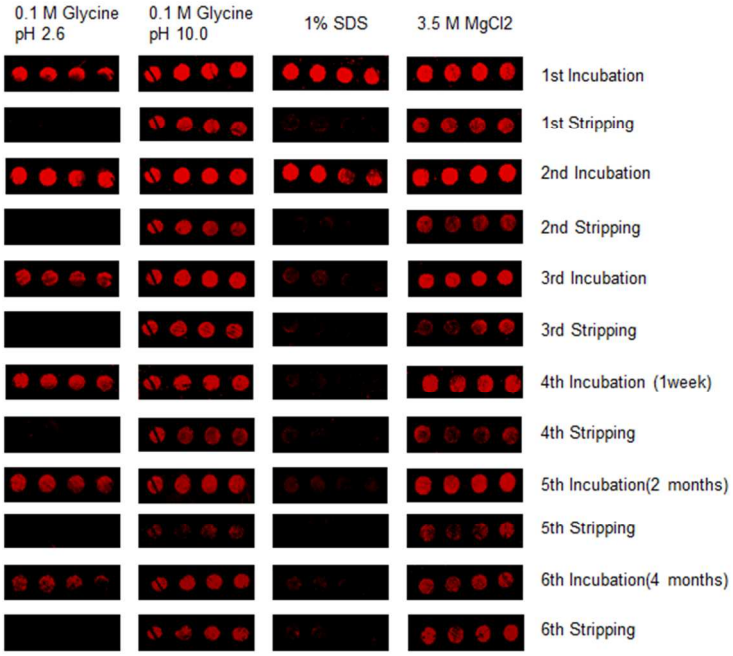


Figure S3. Regeneration of proL-CVIApf slides (Figure 6). After incubation of Texas Red-labeled monoclonal mouse anti-GFP IgG (1 µg/mL) in proL-CVIApf-coated slide, these slides were treated several times with 0.1 M glycine at pH 2.6, 0.1 M glycine at pH 10.0, 1% SDS, or 3.5 M $MgCl_2$ for 4 months. Fluorescence intensity was measured at 532 nm at the end of each cycle.

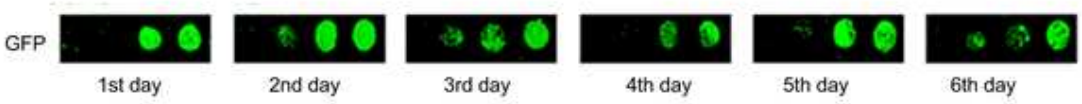


Figure S4. GFP fluorescence during regeneration (Figure 7). The proL-CVIApf-mouse anti-GFP IgG coated slide was spotted using GFP with various concentrations (0.01 to 10 μM) in wells. Fluorescence intensity was measured at 532 nm at the each cycle.

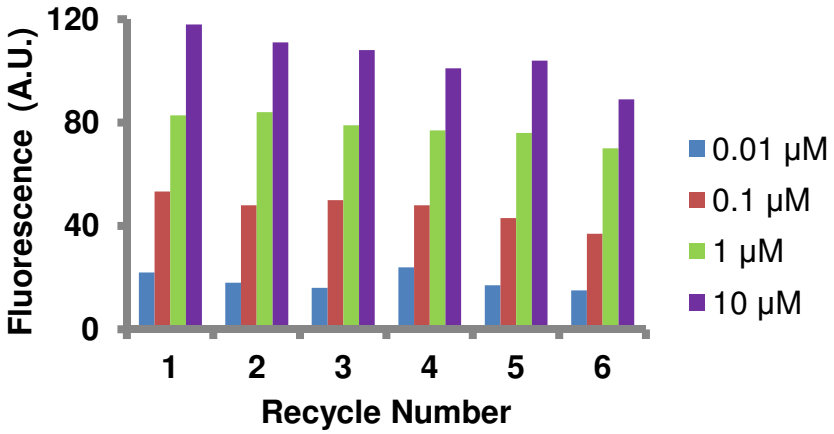


Figure S5. Reuse multicycles of proL-CVIApf in "Sandwich" antibody array (Figure 7). A plot of relative fluorescence intensity versus recycle number. Fluorescence intensities were measured by 633/670 (DyLight 680-labeled goat anti-GFP IgG) nm.