Electronic Supplementary Material (ESI) for Chemical Communications

Supplemental Information

A Generalized Strategy for Immobilizing Uniformly Oriented Membrane Proteins at Solid Interfaces

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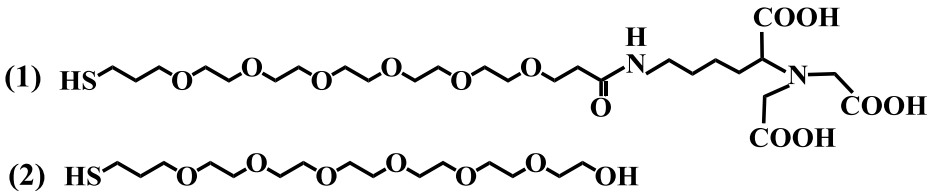
Experimental Section‡

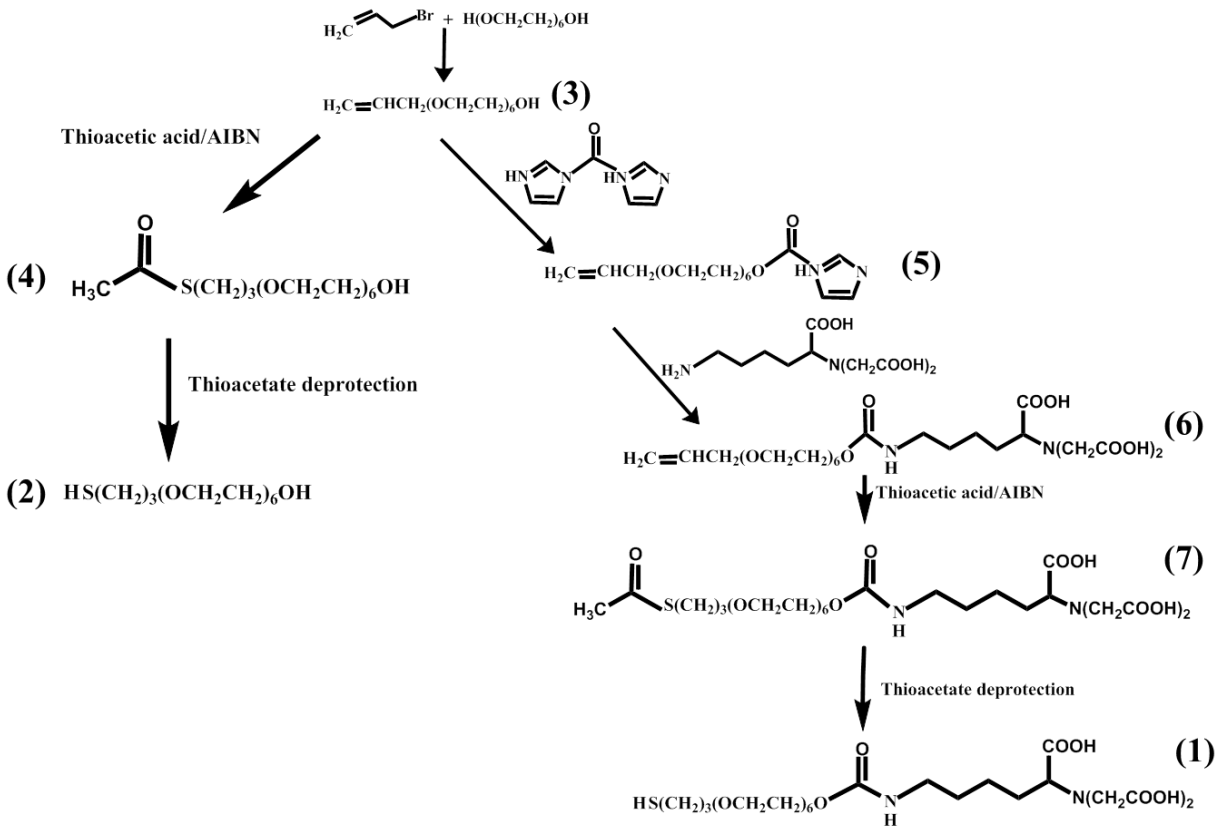
Materials. Hexaethylene glycol, allyl bromide, carbonyldiimidazole, (*S*)-*N*-(5-Amino-1-carboxypentyl)iminodiacetic acid, azobisisobutyronitrile (AIBN), sodium thiomethoxide, and polyhistidine (His)-tagged membrane scaffold protein (MSP1E3D1) were purchased from Sigma-Aldrich (St. Louis, MO). Human peripheral cannabinoid receptor (CB_2) was expressed in *E.coli* with a C-terminal His-tag and purified according to the previously published work.[1] The detergents cholamidopropyl dimethylammonio-1-propanesulfonate (CHAPS), cholesteryl hemisuccinate (CHS), and dodecyl maltoside (DDM) were purchased from Anatrace (Maumee, OH). CP-55,940 was obtained from PerkinElmer (Cambridge, MA).

Synthesis of Thiol Molecules.



‡ Certain trade names and company products are identified in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products are necessarily the best for the purpose.

Scheme S1. Synthesis of oligo(ethylene glycol)-Terminated Thiols (1) and (2)



Synthesis of NTA-Terminated Thiol (1).

Preparation of **3**: 1-allylhexa(ethylene oxide) [IUPAC name: 3,6,9,12,15,18-hexaoxahenicos-20-en-1-ol]

To a stirred mixture of 2.1 g NaH (88 mmol) in 30 mL tetrahydrofuran (THF) was added 24.84 g (88 mmol) hexa(ethylene oxide) in 50 mL THF at 0^{o} C. The heterogeneous mixture was warmed to ≈ 50^{o} C for 15 min. After cooling to ≈ 30^{o} C 10 mL (118 mmol) allyl bromide was added drop wise then refluxed for 3 h. Chromatography (SiO_2) gave 13.07 g (46 %) pure **3**:

^{1}H NMR (270 MHz, $CDCl_3$) δ 5.92 (ddt, 1H, CH_ACH_B=C**H**$_C$$CH_{2(D)}$O-, where H_A and H_B are *cis* and *trans* to H_C, respectively, J_{CA} = 10.4 Hz, J_{CB} = 17.1 Hz, J_{CD} = 5.7 Hz), 5.27 (dd, 1H, CH_AC**H**$_B$=$CH_CCH_{2(D)}$O-, J_{BD} = 1.7 Hz, J_{BC} = 17.1 Hz), 5.18 (dd, 1H, C**H**$_A$$CH_B$=$CH_CCH_{2(D)}$O-, J_{AD} = 1.7 Hz, J_{AC} = 10.4 Hz), 4.03 (dt, 2H, CH_ACH_B=CH_CC**H**$_{2(D)}$O-, J_{DC} = 5.7 Hz, $J_{D(A\ and\ B)}$ = 1.7 Hz), 3.75 to 3.58 [m, (C**H**$_2$C**H**$_2$O)$_6$]. LRMS (FAB): m/z 323 (M + H).

Preparation of **5**:

A solution of 3.78 g (12 mmol) **3** and 3.8 g (24 mmol) carbonyldiimidazole in 35 mL of methylene chloride was stirred at room temperature for 2.5 h. Chromatography (SiO_2) gave 4.3 g (86 %) of pure **5**.

Preparation of **6**:

A solution of 5 g (19 mmol) (*S*)-*N*-(5-Amino-1-carboxypentyl)iminodiacetic acid in 70 mL of water was titrated to pH 10 using 12 mol/L (N) sodium hydroxide, followed by drop wise addition of a solution of 2.61 g (6.26 mmol) **5** in 7 mL of dimethylformamide. The solution was then stirred for 12 h at room temperature. 400 mL of water was added to this solution followed by washing with 200 mL of ethyl acetate. The aqueous phase of this extraction was acidified to

pH 1.5 using 6 N hydrochloric acid and further extracted with 4 x 200 mL of ethyl acetate. After removal of solvents under reduced pressure, 2.28 g of oily liquid **6** was obtained. (**6** was prepared according to previously published work[2])

Preparation of **7**: A solution of 2.28 g (3.73 mmol) of **6**, 3.3 g (31 mmol) thiolacetic acid, and 0.5 g (3 mmol) AIBN in 15 mL of distilled tetrahydrofuran was irradiated with a high pressure Hg lamp (Ace Glass) for 4.5 h at a distance of 4 cm at room temperature. The sample was dried under reduced pressure, followed by hexane trituration which gave 1.2 g of oily liquid **7.**

Preparation of **1**: Deprotection of thioacetate **7** was carried out by dissolving 0.5 g (0.73 mmol) of **7** in 200 mL of methanol in the presence of 0.52 g (7.4 mmol) sodium thiomethoxide stirred for 30 mins under nitrogen environment. 400 mL of 0.1 N aqueous hydrochloric acid solution was added to this solution followed by four times extraction with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate and the solvents removed under reduced pressure to yield 0.35 g of oily liquid. This material was purified by reversed-phase chromatography (Varian ProStar HPLC, XTerra C18 prep column) with water (pH 9) and acetonitrile (70% acetonitrile and 30% water) as the mobile phases. The sample was injected in a 200 µL volume of 5 mg/mL aqueous solution of the material. HPLC purification gave 0.2 g (30% yield of deprotected **7**) of pure **1**:

^{1}H NMR (270 MHz, $CDCl_3$) δ 3.70-3.5 (m, 24 H, m, ($C\mathbf{H}_2C\mathbf{H}_2O)_6OH$), 2.63 (q, J = 6 Hz, $HSC\mathbf{H}_2CH_2$), 1.88 (pentet, 2H, $CH_2C\mathbf{H}_2CH_2$), 1.84 (t, J = 7 Hz $\mathbf{H}SCH_2CH_2$). LRMS (FAB): m/z 645 (M + H).

Synthesis of C_3EO_6H (2).

Preparation of **4**: 1-(3-thioacetylpropanyl)hexa(ethylene oxide) [IUPAC name: S-(1-hydroxy-4,7,10,13,16,19-hexaoxahenicosyl) ethanethioate]

A solution of 1.68 g (5 mmol) **3**, 2.3 g (31 mmol) thiolacetic acid, and 0.03 g (0.18 mmol) AIBN in 20 mL of methanol was irradiated with a high pressure mercury lamp for 7 h at room temperature. Chromatography (SiO_2) gave 1.57g (75 %) of pure **4**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.70-3.5 (m, 24 H, m, ($CH_2CH_2O)_6OH$), 3.52 (t, J = 6 Hz, $CH_2CH_2O(CH_2CH_2O)_6OH$), 2.94 (t, 2H, J = 6 Hz, CH_3COSCH_2), 2.55 (br. t, O**H**), 2.33 (s, 3H, CH_3COS), 1.85 (pentet, 2H, $CH_2CH_2CH_2$).

Preparation of **2**: 1-(3-thiopropanyl)hexa(ethylene oxide) [IUPAC name: 21-thio-3,6,9,12,15,18-hexaoxahenicosanol). A solution of 1.57 g (4.0 mmol) **4** in 30 mL 0.1 N hydrochloric acid/methanol was refluxed for 15 h. Removal of methanol and chromatography (SiO_2) gave 1.30 g (89% yield of deprotected **4**) pure **2**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.70-3.5 (m, 24 H, m, ($CH_2CH_2O)_6OH$), 2.63 (q, J = 6 Hz, $HSCH_2CH_2$), 1.88 (pentet, 2H, $CH_2CH_2CH_2$), 1.84 (t, J = 7 Hz $HSCH_2CH_2$).

Self-Assembled Monolayer (SAM) Preparation

SAMs were prepared on 100 nm thick Au surfaces [on 5 nm adhesion layer of Ti on Si substrates (Platypus Technologies, Madison, WI)] by immersion in 0.2 mmol/L (mM) aqueous solutions of **1** or **1** and **2**, in different molar ratios, for 18 h. Unless otherwise specified, 3 % acetic acid was added to the thiol solutions during SAM formation using **1**. The SAMs were then extensively rinsed with deionized water followed by drying under a stream of nitrogen and used for X-ray photoelectron spectroscopy (XPS), spectroscopic ellipsometry (SE), and surface plasmon resonance (SPR) measurements.

Surface Characterization

All Au substrates were cleaned with piranha solution** (70 % sulfuric acid and 30 % hydrogen peroxide) before SAM formation. SAM formation was carried out in the presence and absence of 3% of acetic acid in the immersion solutions. X-ray photoelectron spectroscopy (XPS) analysis was performed using a Kratos Axis Ultra DLD XPS system (Kratos Analytical, Chestnut Ridge, NY) with a monochromated Al Kα x-ray source (20 mA, 14 kV) and a spot size of 300 μm × 700 μm. High-resolution spectra of the C 1s, N 1s, O 1s, S 2p, and Au 4f regions were acquired at pass energy of 40 eV, step size 0.1 eV at three spots for each condition, and average intensities were used for calculations. In addition, low resolution survey spectra were acquired for all spots (pass energy 160 eV, step size 0.5 eV.) All binding energies were referenced to the Au $4f_{7/2}$ peak at 84.0 eV and Casa XPS software (v. 2.3.16 pre-rel 1.4) was used to analyze XPS spectra.

**Warning: Piranha solution must be handled with caution: it is extremely oxidizing, reacts violently with organics, and should only be stored in loosely tightened containers to avoid pressure buildup.

Assuming that the adsorption of **1** and **2** to the Au surface is irreversible and follows first order Langmuir kinetics, and that activity coefficients in solution are constant at a dilute thiol concentration (0.2 mM), the following expression can be used to correlate the solution mole fractions of thiols **1** and **2**, $\Theta_{1,solu}$ and $\Theta_{2,solu}$ to their mole fractions on the Au surfaces, $\Theta_{1,surf}$ and $\Theta_{2,surf}$, respectively.[3,4]

$$\frac{\Theta_{1,surf}}{\Theta_{2,surf}} = K\left(\frac{\Theta_{1,solu}}{\Theta_{2,solu}}\right) \quad (1)$$

where K is the ratio of the adsorption rate constants of **1** and **2** on the Au surfaces.

By assuming $\Theta_{1,\,solu}+\Theta_{2,\,solu}=1$ in a dilute thiol solution, and $\Theta_{1,\,surf}+\Theta_{2,\,surf}=1$ when all the Au occupancy sites are occupied by thiols, we can rewrite (1) to correlate the mole fraction of compound **1** on the surface to its mole fraction in solution as

$$\Theta_{1,\,surf} = \frac{K\Theta_{1,solu}}{1+(K-1)\Theta_{1,solu}} \quad (2)$$

where $\Theta_{1,}$surf is calculated using the following expression,

$$\Theta_{1,\,surf} = \frac{n_s(\text{SAM of } \mathbf{1}) * I_N(\text{SAM of } \mathbf{1} \text{ and } \mathbf{2}) \,/\, I_N(\text{SAM of } \mathbf{1})}{n_S(\text{SAM of } \mathbf{1} \text{ and } \mathbf{2})} \quad (3)$$

where I_N(SAM of **1**) and I_N(SAM of **1** and **2**) are the intensities of the N 1s nitrogen peaks in the high resolution XPS spectra for a SAM of only **1** and for mixed SAMs of **1** and **2** of different compositions, respectively. The n_s are the surface sulfur coverages, which can be determined from (4) as discussed by Petrovykh *et al.*[5]

$$n_S = \left(\frac{I_S}{I_{Au}}\right)\left(\frac{T_{Au}}{T_S} L_{Au}^{Q} N_{Au}\right) \quad (4)$$

where $\boldsymbol{I_S}$ and $\boldsymbol{I_{Au}}$ are the experimental Au 4f and S 2p intensities scaled by their Kratos relative sensitivity factors including a correction for instrument transmission function. The value of the second term in (4) is a constant, 9.98×10^{15} cm^{-2}. In eq. 3, n_s (SAM of **1**) and n_s (SAM of **1** and **2**) are the surface sulfur coverages of the SAM of only **1** and the mixed SAM (**1** and **2**) of different compositions, respectively.

After substituting (4) in (3), we obtained (5)

$$\Theta_{1,\ surf} = \frac{\frac{I_S}{I_{Au}}(\text{SAM of } \mathbf{1}) * I_N(\text{SAM of } \mathbf{1} \text{ and } \mathbf{2}) \Big/ I_N(\text{SAM of } \mathbf{1})}{\frac{I_S}{I_{Au}}(\text{SAM of } \mathbf{1} \text{ and } \mathbf{2})} \qquad (5)$$

The $\Theta_{1,surf}$ values (eq. 5) as a function of solution molar fractions of **1** are given in Table S1.

Table S1. Surface mole fraction of **1** ($\Theta_{1,\mathrm{surf}}$) for SAMs at different **1** and **2** solution compositions*

$\Theta_{1,\mathrm{solu}}$	I_{Au} (CPSeV)	I_S (CPSeV)	I_N (CPSeV)	$\Theta_{1,\mathrm{surf}}$
1	46785 ± 371	1994 ± 66	4744 ± 40	1
0.75	48661 ± 276	1933 ± 29	2745 ± 94	0.59
0.50	47664 ± 314	1778 ± 65	1572 ± 56	0.36
0.25	52284 ± 498	1777 ± 83	836 ± 52	0.20
0.10	57678 ± 240	1885 ± 101	533 ± 34	0.11
0	59882 ± 249	2004 ± 51	0	0

***** SAMs prepared from solutions containing 3% acetic acid, and I_{Au} (CPSeV), I_S (CPSeV), and I_N (CPSeV) are the Au, S, and N average intensities from the CASA XPS software, respectively, in different samples. Error (±) represents the standard error of the mean based on three measurements per sample.

Data from Table S1 is used in Figure 2 for plotting $\Theta_{1,surf}$ vs. $\Theta_{1,solu}$ and eq. 2 was used to fit the data to determine K.

SAM ellipsometric thickness measurements [spectroscopic ellipsometry (M-2000-DI spectroscopic ellipsometer, J. A. Woollam Co., Inc., Lincoln, NE)] revealed that the mixed SAM ellipsometric thickness increases nonlinearly with increasing NTA mole fraction in the thiol forming solution, as shown in Figure S1. We observed that the SAM ellipsometric thickness of the samples prepared by only **1** in acidic condition is approximately 20 % higher than the SAM prepared by only **1** in water (17 Å) (data not shown).

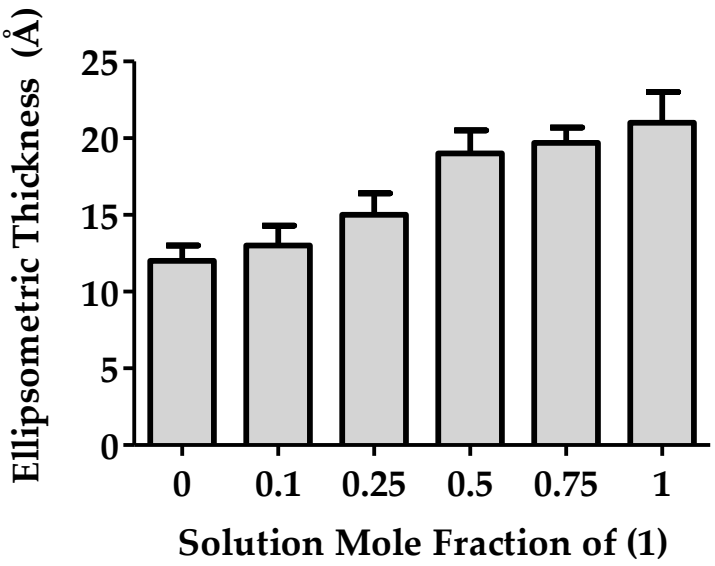


Figure S1. Characterization of self-assembled monolayer formation using a spectroscopic ellipsometer. Error bars represent the standard error of the mean based on three measurements.

Surface Plasmon Resonance Measurements

SPR (Biacore T-100) was employed to monitor His-tagged protein binding to the functionalized surfaces. SAMs were formed on Au surfaces (BIACORE SIA kit Au, GE, New York) as described above. After mounting the substrates into the flow cell, either buffer A (50 mM Tris-HCl at pH 7.5, 200 mM NaCl, 30% glycerol, 0.5% CHAPS, 0.1% CHS, 0.1% DDM, and 10 μM CP-55,940) or Tris-HCl buffer (50 mM Tris, pH 7.4, 100 mM NaCl) were pumped through the flow cell at a rate of 5 μL/min until a stable baseline reading was obtained. The NTA-functionalized surfaces were activated by injecting 40 mM $NiCl_2$ aqueous solution for 3 min. Subsequently, protein either His-tagged CB_2 (0.1 mg/mL in buffer A) or MSP1E3D1 (0.1 mg/mL in Tris buffer) was injected. After 10 min, imidazole (500 mM) was injected for 3 min to displace the His-tagged proteins. All measurements were carried out either at 8^o C or 25^o C as indicated. Figure S2. shows representative SPR binding response sensograms.

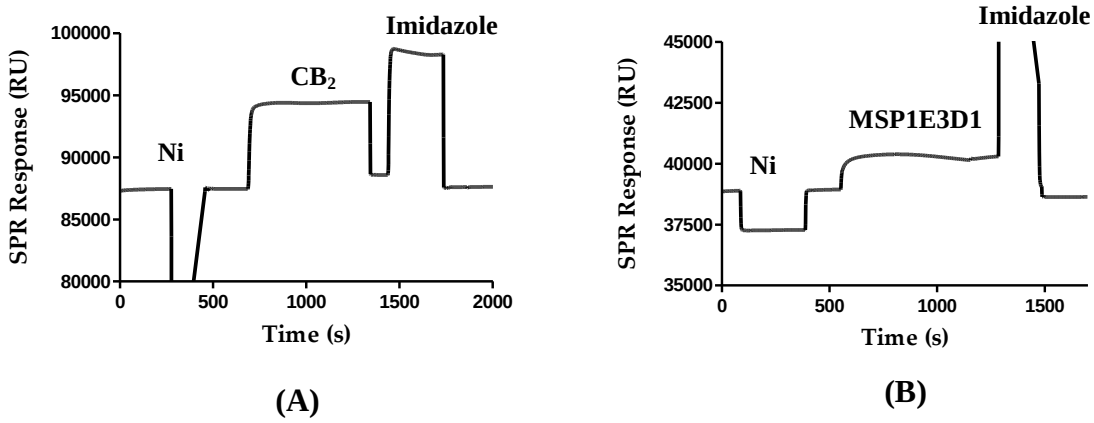


Figure S2. SPR response sensograms illustrating capture/release of protein on NTA-functionalized surfaces (prepared from a $\Theta_{1,solu}$ = 0.1 solution) **(A)** 0.1 mg/mL His-tagged CB_2 in buffer A solution at 8^o C, **(B)** 0.1 mg/mL His-tagged MSP1E3D1 protein in Tris buffer at 25^o C.

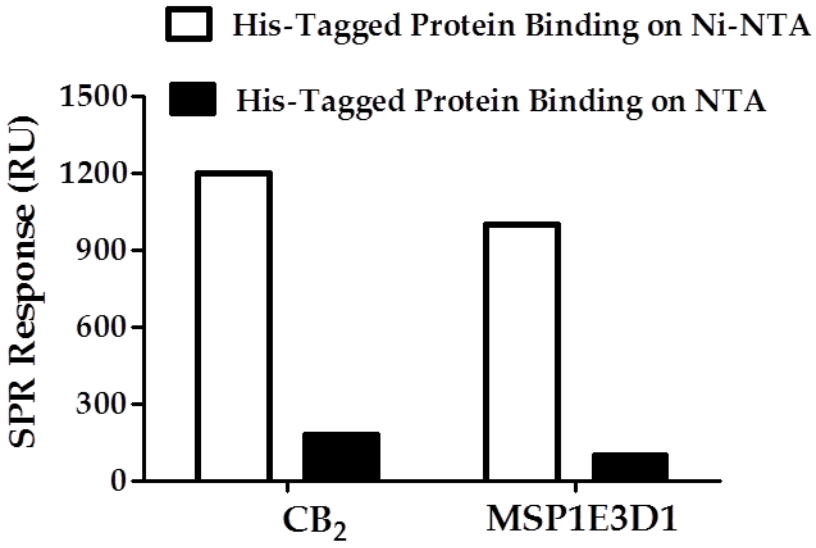


Figure S3. His-tagged proteins binding on NTA-functionalized surfaces [prepared from a $\Theta_{1,solu}$ = 0.2 solution] (i) in the presence of nickel on the surfaces (unfilled bars) and (ii) in the absence of nickel on the surfaces (filled bars). Introduction of His-tagged CB_2 (0.1 mg/mL in buffer A containing 20 mM EDTA) or MSP1E3D1 (0.1 mg/mL in Tris buffer containing 20 mM EDTA) on NTA-functionalized surfaces resulted in minimal SPR response. In contrast, these His-tagged proteins show much higher SPR response on the nickel-activated NTA-functionalized surfaces indicating this is a specific binding. (EDTA was used in the protein solution to scavenge potential metal ions)

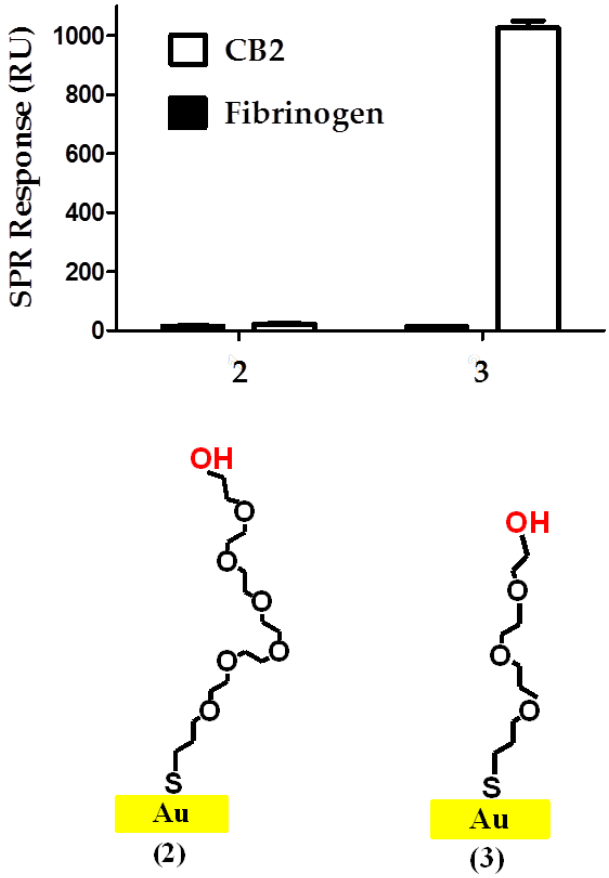


Figure S4. Nonspecific binding histograms of detergent-solubilized His-tagged CB_2 (0.1 mg/mL in buffer A) and fibrinogen (1 mg/ml in Tris buffer), on tri- and hexa-ethylene glycol (EG)-terminated SAMs. SAMs of hexa-EG molecule [**2**, numbering system in the paper] was resistant to both, whereas the SAMs of the tri-EG compound, **3** [synthesis of **3** is similar to **2** except using tri(ethylene oxide) (unpublished data)] failed in resisting the adsorption of CB_2. Error bars represent the standard error of the mean based on two measurements.

Determination of Equilibrium Binding Constant of His-tag/NTA Interaction

The Langmuir-isotherm model was used to determine the binding constant, K_D, of the His-tagged MSP1E3D1 to the Ni-activated NTA surface:[6]

$$R = R_{max}\frac{c}{K_D+c} \qquad (6)$$

Here, R_{max} represents the maximum SPR binding response at saturation, c is the concentration of His-tagged MSP1E3D1 in solution. Here, the Langmuir model assumes that the binding is reversible and K_D is the equilibrium dissociation constant of the interaction. The K_D can be obtained by fitting the response data to eq.6.

Figure S5 shows the adsorption isotherm obtained on a NTA-functionalized surface (prepared from a $\Theta_{1,solu}$ = 0.05 solution). The solid line corresponds to a Langmuir adsorption isotherm to the data using a one-site specific binding nonlinear regression solver (GraphPad Prism, La Jolla, CA). The presence of a saturation binding isotherm with K_D = 31.2nM for the interaction between **1** and His-tagged MSP1E3D1 indicates that not only was this a specific binding, but also His-tagged proteins were stably bound to the surfaces.

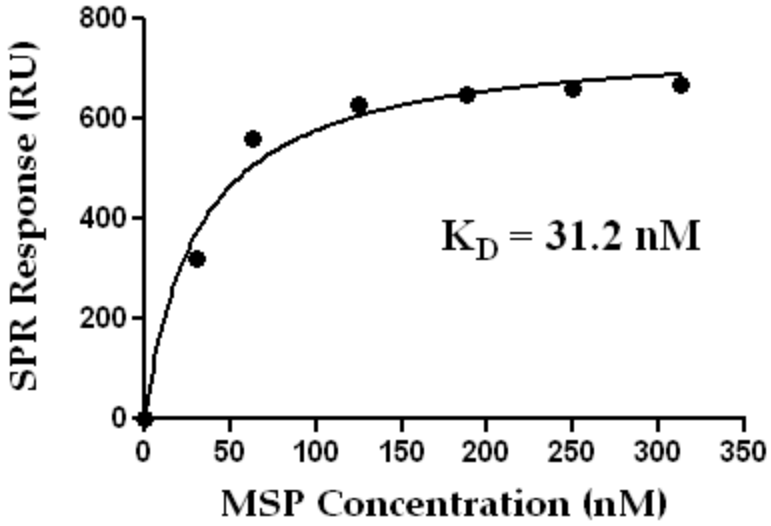


Figure S5. SPR response to His-tagged MSP as a function of MSP concentration. The symbols represent the experimental results and the solid line represents the fit to the data. The best fit value for K_D is 31.2 nM with a standard error of 6.89 nM (R^2 = 0.98).

References

(1) A. Yeliseev, L. Zoubak and K. Gawrisch, *Protein Expression Purif.*, 2007, **53**, 153.
(2) G. B. Sigal, C. Bamdad, A. Barberis, J. Strominger and G. M. Whitesides, *Anal. Chem.*, 1996, **68**, 490.
(3) C. D. Bain, J. Evall and G. M. Whitesides, *J. Am. Chem. Soc.*, 1989, **111**, 7155.
(4) K. E. Nelson, L. Gamble, L. S. Jung, M. S. Boeckl, E. Naeemi, S. L. Golledge, T. Sasaki, D. G. Castner, C. T. Campbell and P. S. Stayton, *Langmuir*, 2001, **17**, 2807.
(5) D. Y. Petrovykh, H. Kimura-Suda, A. Opdahl, L. J. Richter, M. J. Tarlov and L. J. Whitman, *Langmuir*, 2006, **22**, 2578.
(6) G. B. Sigal, M. Mrksich and G. M. Whitesides, *Langmuir*, 1997, **13**, 2749.