Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Surface-tethered protein switches

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Materials: 11-Amino-1-undecanethiol hydrochloride (99%), 11-Hydroxy-1-undecanethiol (97%), glutaraldehyde, nickel (II) sulphate hexahydrate (99%), maltose and sucrose were purchased from Sigma-Aldrich. Ethylenediaminetetraacetic acid (EDTA) was purchased from Fisher Biotech. N-(5-amino-1-carboxypentyl)iminodiacetic acid (AB-NTA) was purchased from Dojindo. Nitrocefin was purchased from BD Biosciences. All chemicals were used as supplied without further purification. Ultrapure water from Milli-Q was used for all experiments.

Preparation of template-stripped gold (TSG) slides: TSG slides were fabricated by evaporation of 50 nm gold onto (20 mm × 20 mm) p-Si (100) wafer (University Wafers). The Si wafers were cleaned with distilled water, followed by acetone and isopropanol prior to use. After gold evaporation, a microscope glass slide covered with epoxy glue (EPO-TEK 364, Epoxy Technology, Billerica, MA) was placed on the gold surface. The sample was cured in an oven at 120 ˚C for 15 min. The silicon wafer was removed immediately prior to use.

Surface modification: The gold slides were incubated in a mixture of 11-amino-1-undecanethiol hydrochloride and 11-hydroxy-1-undecanethiol in ethanol overnight. The gold slides were then rinsed sequentially in ethanol and water. Stock solutions of thiols (1 mM in absolute ethanol) were combined to give mixture with ratio of 0.1. The amine-terminated thiols in the self-assembled monolayer were reacted with 10% (v/v) glutaraldehyde in 0.05 M HEPES buffer (pH 7.4) for 1 h, followed by rinsing with HEPES buffer. The glutaraldehyde-modified surfaces were incubated for 1 h in 2 mM AB-NTA in 0.02 M HEPES buffer (pH 7.4) and then washed with fresh buffer solution. The AB-NTA-modified slides were immersed in 1% (w/v) $NiSO_4 \cdot 6H_2O$ in water for 1h, and rinsed in water prior to immobilization of the his-tagged RG13 protein switch.

Tethering the protein switch: To immobilize the His-tagged RG13 protein switch, the Ni-NTA-modified gold slides were reacted with 0.15 mg mL^{-1} RG13 in 0.02 M HEPES buffer (pH=7.3) for 1 h at room temperature. The modified slides were washed with buffer to yield the RG13-functionalized gold slides.

To check the reversibility of binding of the His-tagged switch, RG13-modified slides were treated with 0.2 M EDTA for 30 min. The NTA-modified surface was regenerated by incubating with Ni(II) and then with a solution containing the His-tagged RG13 switch.

Switch response: The enzymatic activity of the surface-tethered RG13 switch was measured through reaction with the colorimetric substrate nitrocefin (NCF), which undergoes a color change from yellow (λ_{max} = 390 nm, pH 7.4) to red (λ_{max} = 486 nm, pH 7.4). A teflon cell was placed on the modified gold slides with a viton o-ring to expose an area of 1.13 cm^2. 250 μL of 50 μM NCF was placed in the cell in the dark for up to 60 minutes. In some cases, either 10 mM maltose or 10 mM sucrose were added to the NCF solution. Next, the NCF solution was removed from the cell and absorbance spectra recorded using a Cary50 UV-VIS

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spectrophotometer. The response of the tethered switch is reported as an absorbance change, defined as the absorbance (at 500 nm) of NCF after incubation with the tethered switch in the presence or absence of maltose minus the absorbance of NCF solution at the same concentration.

To measure the influence of maltose concentration on the response of the surface-tethered RG13 switch, 250 μL of 50 μM NCF was placed in the cell with the addition of maltose (5×10^{-5} M - 20×10^{-3} M) in the dark. The absorbance change upon nitrocefin hydrolysis was recorded after 20 minutes incubation.

The absorbance of NCF at 500 nm after incubation with the surface tethered switch, with or without maltose, was reproducible. However, some differences were observed for different batches of the His-tag switch. Variations were also observed for different batches of NCF and 11-amino-1-undecanethiol hydrochloride.

Switch activity: The enzymatic kinetics of the switch can be measured from the initial rate of nitrocefin hydrolysis. β-lactam hydrolysis by β-lactamases can be described by three steps involving the formation of a complex between the enzyme and the substrate (substrate binding), formation of the covalent enzyme-substrate acyl intermediate, and the subsequent release of the hydrolyzed product and enzyme. The last two steps are the catalytic steps. The scheme is written as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \xrightarrow{k_2} EAc \xrightarrow{k_3} E + P$$

where E, S, ES, EAc, and P are the enzyme, substrate, non-covalent enzyme-substrate complex, covalent enzyme-substrate acyl intermediate, and hydrolyzed product, respectively. Although this is not a Michaelis-Menten mechanism, the kinetics can nonetheless be described by Michaels-Menten kinetics. According to Michaelis-Menten kinetics, the initial rate of reaction V_0 can be approximated by:

$$V_0 = \frac{k_{cat}[E][S]}{K_m + [S]}$$

where [E] is the enzyme concentration, [S] is the substrate concentration, K_m $((k_{-1}+k_2)/k_1)\cdot(k_3/(k_2+k_3))$ is the Michaelis-Menten constant, and $k_{cat} = k_2k_3/(k_2+k_3)$ is the catalytic constant. The effect of maltose on the catalytic activity of the switch (i.e. switching activity) can be measured as V_0(maltose)/V_0(no maltose). The initial rates after 2 min incubation of the surface tethered switch with nitrocefin were obtained from the absorbance change versus time curve in Figure 1b.

Control Experiments:

Non-specific adsorption. To confirm that switch activity was from the tethered RG13 switch on the surface and not from of non-specifically absorbed RG13 switch we performed two experiments. (1) When NTA-functionalized surface was not treated with Ni(II) prior to incubation with RG13, no NCF absorbance change was detected (Figure S1). (2) No changes in NCF absorbance were observed after exposure to a functionalized surface without RG13 (Figure S1).

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Specificity of ligand activation. In the presence of sucrose, the absorbance change for NCF in the presence of the tethered RG13 switch was the same as the absorbance change in the absence of maltose (Figure S1). This experiment confirms that a response is seen only for sugars that are known to bind to MBP

Regeneration of the sensor platform. To demonstrate that the sensor platform can be regenerated, we removed the RG13/His-tag complex from the surface by incubation in EDTA (see above for details). We then reattached the switch by incubating the surface in Ni(II) solution and then solution containing the His-tagged RG13 switch. The absorbance change for NCF in the presence of the regenerated surface was the same as for the original surface (Figure S1), indicating that this platform can be reused with different His-tagged switches.

Maltose mass transport. To confirm that nitrocefin hydrolysis does not depend on the mass transport of maltose, the surface-tethered RG13 switch was pre-incubated with 250 μL of 50 μM NCF in the dark for 30 min prior to adding maltose to give a concentration of 10 mM. The absorbance of NCF 20 minutes after adding the maltose was the same as for a surface-tethered RG13 switch without pre-incubation in NCF, confirming that the response is not limited by maltose transport to the switch.

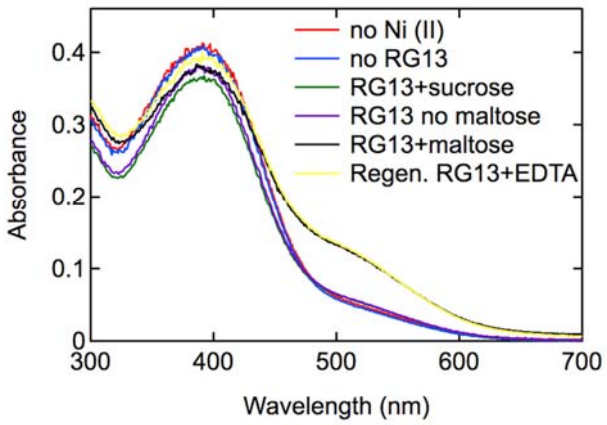

Figure S1. Absorbance spectra for control experiments. **(no Ni (II))** - 50 μM NCF, 10 mM maltose incubated with an NTA-modified gold surface. The NTA-modified surface was incubated with the RG13 switch and rinsed prior to the experiment. **(no RG13)** - 50 μM NCF and 10 mM maltose incubated with an NTA/Ni(II)-modified gold surface. The NTA/Ni(II)-modified gold surface was not incubated with the RG13 switch prior to the experiment. **(RG13+sucrose)** - 50 μM NCF and 10 mM sucrose incubated with the tethered RG13 switch. **(RG13 no maltose)** - 50 μM NCF incubated with the tethered RG13 switch. **(RG1+maltose)** - 50 μM NCF and 10 mM maltose incubated with the tethered RG13 switch. **(Regen. RG13+EDTA)** - 50 μM NCF and 10 mM maltose incubated with a regenerated surface-tethered RG13 switch after removing the original Ni(II)-switch complex by incubation with EDTA. All spectra were recorded in the dark after 20 min incubation.

Protein switch expression and purification: A hexa-histidine tag was added to the C-terminus of RG13 via a flexible GGSG amino acid linker. DNA coding for the linker-6xHis

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tag was PCR-amplified using high fidelity Phusion polymerase (NEB). The desired PCR product was inserted at the C-terminus of RG13 DNA (present on pDIM-C8 plasmid (Cm^R) under the control of *tac* promoter) using unique restriction sites to create DNA encoding the His-tagged version of RG13 (plasmid pDIM-C8-RG13H (Figure S1)). The construct was confirmed by DNA sequencing.

A single colony of K12 TB1 *E. coli* (NEB) harboring pDIM-C8-RG13H was used to inoculate 10 mL of LB containing glucose (1.0 % m/v final) and Cm (50 μg mL^{-1}). The inoculated tube was incubated overnight at 37°C. LB (500 mL) was inoculated with 2 % overnight culture, glucose (1.0 %) and Cm (50 μg/ml) in a 2 L beveled flask and the culture was incubated at 37 °C and 200 rpm. At A_{600nm}= 0.6, RG13H expression was induced by the addition of isopropyl β-D-1-thiogalactopyranosid (IPTG) to 1 mM and the culture shaken at 20 °C for another 16 hours. The cells were pelleted by centrifugation (20 minutes at 4500 x g) and resuspended in 25 mL TBS_{100} (20 mM Tris-HCl, 100 mM NaCl (pH 7.0)) buffer containing 50 μL of protease inhibitor cocktail (Sigma). The cells were lysed using French Press (20,000 psi) and the lysate collected on ice. From this point onwards the samples were kept at 4 °C. The cell lysate was clarified by centrifugation (3 times for 20 minutes each at 14,000 x g) and the supernatant was recovered. The supernatant was filtered using 0.45 μm filters (Corning) and the filtered supernatant passed over a TBS_{100} equilibrated amylose resin gravity column (NEB). The protein-loaded column was washed with TBS_{100} and RG13H protein eluted with 10 mM maltose in TBS_{100}. The elution fraction was confirmed to contain His-tagged RG13 by analysis of the sample on SDS-PAGE gel (Invitrogen) using His-tag staining (Invitrogen). The RG13H (70.9 kD) elution fraction was dialyzed at 4 °C against 300 volumes of 25 mM HEPES buffer (pH 7.0) for two hours and then twice against 1000 volumes of the same buffer for 12 h each using 20 kD dialysis cassette (Pierce). The dialyzed protein was judged by Coomassie staining of SDS-PAGE gel (Invitrogen) to be > 95% pure (Figure S3). The molar extinction coefficient of RG13H at 280 nM was calculated to be 94,310 M^{-1} cm^{-1}. RG13H concentration was determined using its extinction coefficient and the protein stored in aliquots at -20 °C.

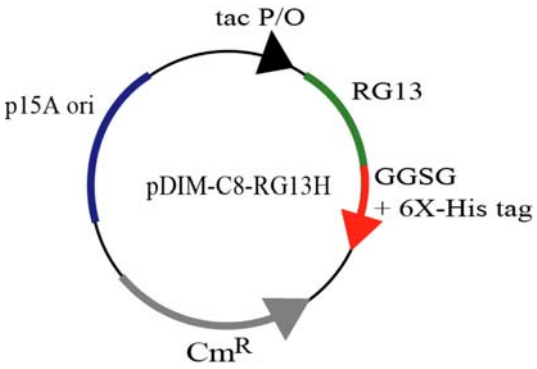

Figure S2. Plasmid map of His-tagged RG13 (pDIM-C8-RG13H). The C-terminus of RG13 is linked to a 6x histidine tag using a flexible GGSG amino acid linker. RG13H is present on a medium copy, chloramphenicol resistant plasmid (pDIM-C8) with a p15A origin of replication. RG13H is under the control of the *tac* promoter that is inducible by IPTG.

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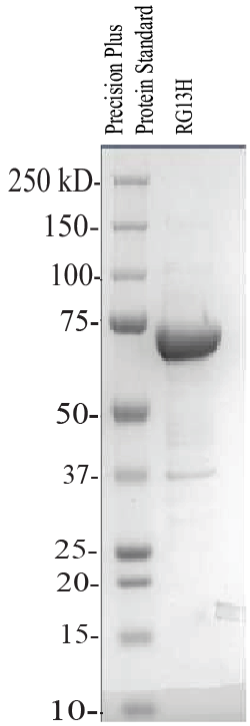

Figure S3. Coomassie stained SDS-PAGE gel of dialyzed RG13H. RG13H (70.9 kD) was judged to be more than 95% pure. Precision Plus Protein Standard was purchased from Bio-Rad.