Supporting Information

The Problem of Diminished cRGD Surface Activity and What Can Be Done About It

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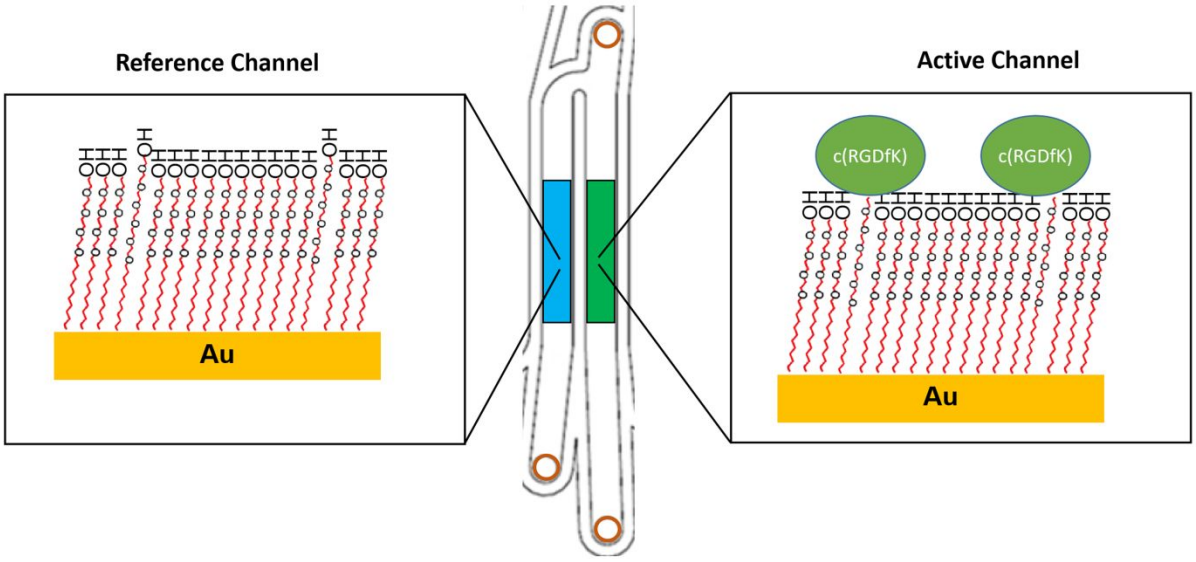

Figure S1: Biacore 8K SPR fluid cell overview. Each flow cell consists of two nearly identically prepared surfaces/channels: an active channel that is functionalized with cRGD and a reference channel without cRGD. The circles indicate microfluidic input and output ports.

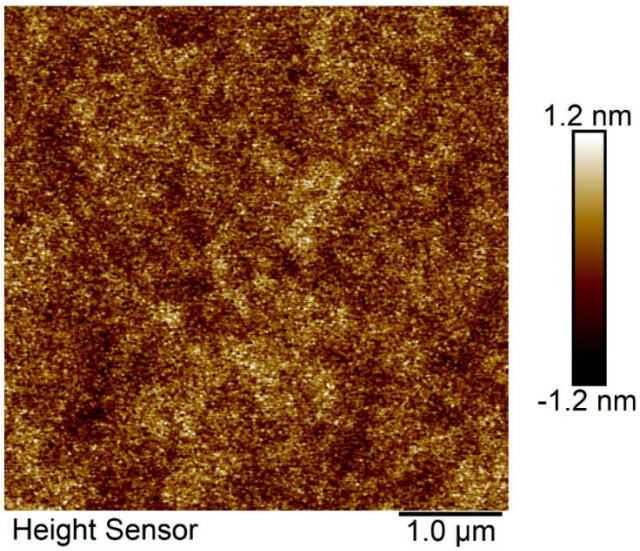

Figure S2. Atomic Force Microscope (AFM) image of Au coated glass coverslip, showing smooth surfaces of roughness $R_a \approx 5$Å. AFM scan taken in tapping mode at 1024x1024 on a Bruker Dimension Fastscan in ambient conditions with a Fastscan-A tip (Bruker).

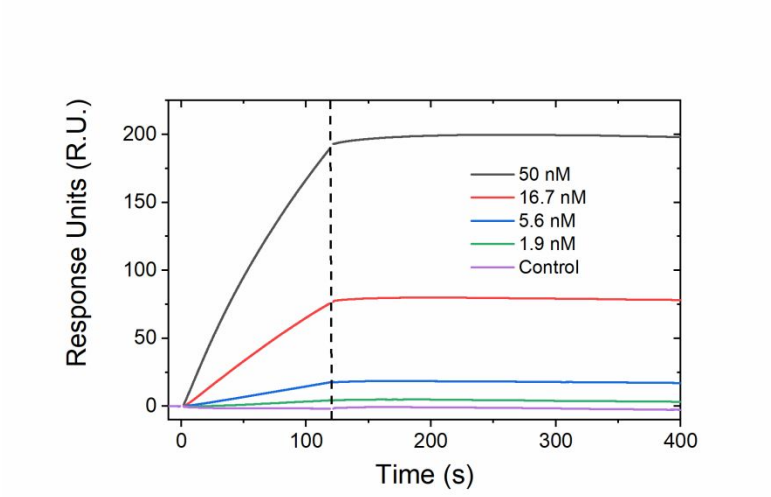

Figure S3: SPR concentration study of recombinant $\alpha_V\beta_3$ integrins on 1:25 cRGD functionalized chips. The control for binding specificity consisting of 50 nM $\alpha_V\beta_3$ recombinant integrins premixed with 1 mM cRGD. The vertical dashed line separates the association phase (left) in which the integrin solution is flowing over the surface from the dissociation phase (right) in which running buffer flows over the surface. The flow rate was 30 µL/min for all steps and the running buffer was TBS + 0.5 mM $MnCl_2$.

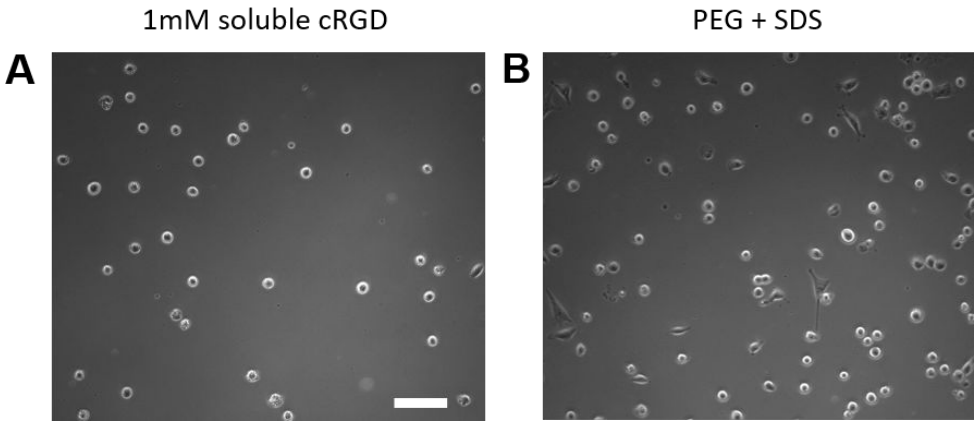

Figure S4. In vitro cRGD control data. A) MDA-MB-231 cells were incubated in serum free media with 1mM soluble cRGD for 1 hr prior to being plated on cRGD functionalized Au surfaces. Cell adhesion was extremely poor and spreading almost non-existent. B) Cells seeded on cRGD functionalized Au surface first blocked by 1mg/mL PLL-g-PEG, then exposed to 0.25% SDS to assess cap-less regeneration. Cell adhesion was poor with little spreading. Measurements taken 6 hrs after plating, scale bar = 100µm.

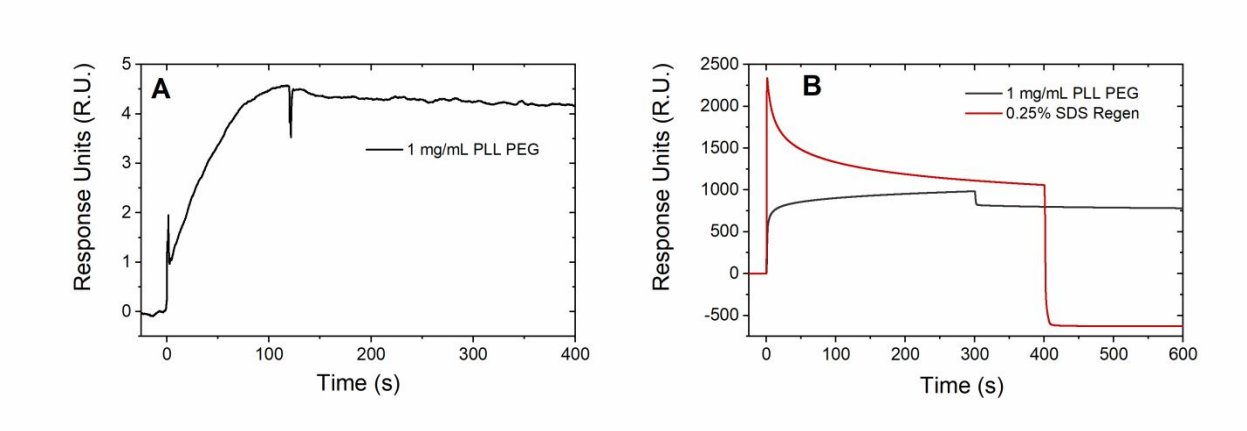

Figure S5: Cell-free cRGD control data (SPR). As with Figure S4, to assess cap-less regeneration the cRGD functionalized Au surface was (A) first blocked with 1mg/mL PLL-g-PEG (black), then exposed to 0.25% SDS (red) and (B) exposed to 15 nM $\alpha_V\beta_3$ recombinant integrins. The low response in (B) is indicative of a heavily blocked surface.

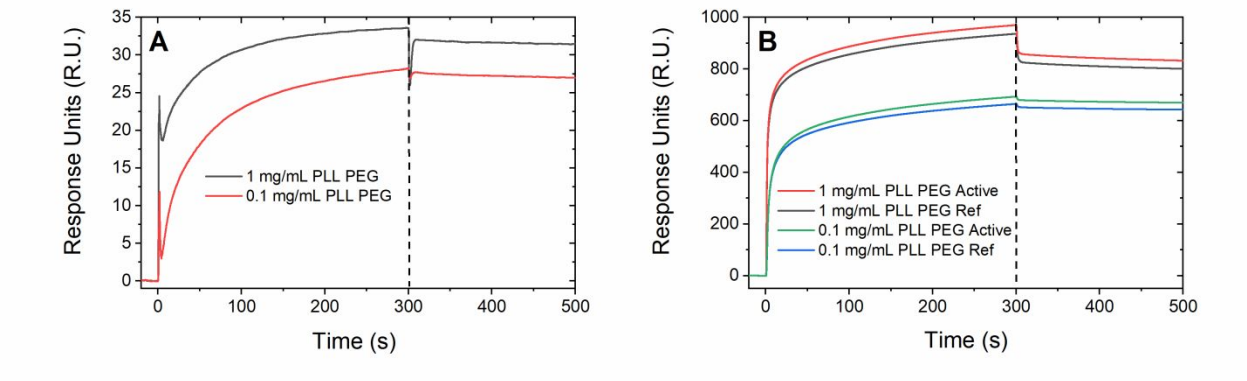

Figure S6: SPR comparison of the active (cRGD functionalized) vs reference (SPO only) channels for 1 mg/mL and 0.1 mg/mL PLL-g-PEG. (A) Reference subtracted data (B) Active and reference channels separately plotted.

cRGD Activity Ratio Calculation

In a SPR measurement, the surface conjugated ligand activity (i.e. cRGD) can be calculated by

$$A_i = \frac{X_{a,i} m_l}{X_l m_a}$$

Where $X_{a,i}$ and X_l are the instrumental response units (R.U.) to analyte (integrins) and ligand (cRGD), respectively. Similarly, m_a and m_l are the analyte and ligand molecular weight. The index i represents one

of two possible activity measurements: $i = c$ for the control case in which no blocking molecules are present and $i = b$ for the case when blocking molecules are present.

For the same cRGD functionalized surface, we take the ratio of these two cases and all terms cancel except for the $X_{a,i}$ terms, giving the activity ratio:

$$\frac{A_b}{A_c} = \frac{X_{a,b}}{X_{a,c}}$$

Ideally the $X_{a,i}$ data would be the result of introducing a saturating concentration of analyte, $[\alpha_V\beta_3] \gg K_D$, where K_D is the equilibrium dissociation rate constant, and waiting for the system to reach equilibrium. This condition, however, requires an inordinate amount of analyte material, so in this study we have taken the ratio at $t = 200\ s$ and with $[\alpha_V\beta_3] = 15$ nM.

Normalized % Spread Cells Calculation

For any given *in vitro* experiment, 2-3 separate samples and thus different surface conditions were conducted in tandem, with one surface always being an adhesion control (i.e. only functionalized with cRGD to measure "full" cellular adhesion to the 1:25 cRGD:OH surface). To quantify cell adhesion of each surface condition (i.e. blocked with 1mg/mL PLL-g-PEG, or capped with integrins and regenerated), the amount of spread cells of each condition are directly compared with that of the control for that particular experiment:

$$\text{Normalized \% spread cells} = \frac{n_S^i / N_T^i}{n_S^C / N_T^C}$$

Where n_s is the total number of spread cells per condition and N_T is the total number of cells measured per condition, the index *i* signifies the condition of blocked *b* or regenerated *r*, and superscript *C* indicates the condition of experimental control.

cRGD Mean-distance Calculation

The self-assembled monolayers (SAM) of thiols on Au surfaces has been extensively studied both theoretically and experimentally. Theoretically, thiol density is typically calculated based on the lattice spacing of the (111) Au surface[1]. However, given that most gold thin films used in experimental biology are polycrystalline and present a variety of orientations it is important to consider a range based on direct experimental measurements as well[2].

Given these considerations, a reasonable range of occupied area per thiolate is

$$0.22\ nm^2 < area\ per\ thiol < 0.42\ nm^2$$

The most appropriate model for determining cRGD density by the 1:25 SPC:SPO ratio and the random nature of adsorption on the au surface during SAM formation is a probabilistic 2D nearest-neighbor model based on a homogeneous Poisson process[3], giving a probability density function (pdf) of

$$g(w) = 2\rho\pi w\, exp\big(-\rho\pi w^2\big)\,,$$

where ρ is the density of SPC and w is the nearest neighbor distance random variable. The mean nearest neighbor distance is then given by:

$$E[w] = 1/(2\sqrt{\rho})$$

Given the above range in area per thiolate, the mean distance between cRGD can range from

$$1.2\ nm < E[w] < 1.7\ nm$$

We emphasize that even under the most extreme assumptions of cRGD conjugation efficiency (< 10%) and thiol density, the calculated mean distance between cRGD peptides is less than 5 nm.

REFERENCES

1. Schreiber, F., Structure and growth of self-assembling monolayers. *Progress in surface science* **2000,** *65* (5-8), 151-257.
2. Nelson, K. E.; Gamble, L.; Jung, L. S.; Boeckl, M. S.; Naeemi, E.; Golledge, S. L.; Sasaki, T.; Castner, D. G.; Campbell, C. T.; Stayton, P. S., Surface characterization of mixed self-assembled monolayers designed for streptavidin immobilization. *Langmuir* **2001,** *17* (9), 2807-2816.
3. Dixon, P. M., Nearest Neighbor Methods. *Encyclopedia of Environmetrics* **2001,** *3*, 1370.