

## Peptide-Directed Co-Assembly of Nanoprobes on Multimaterial Patterned Solid Surfaces

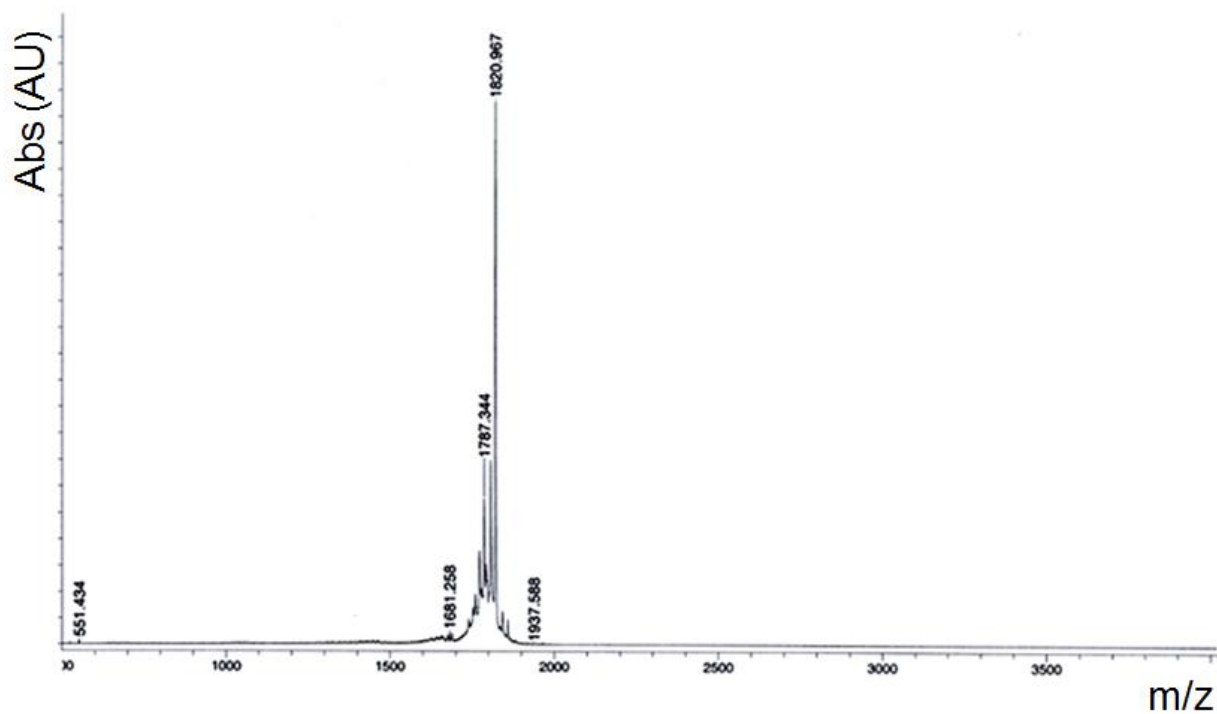
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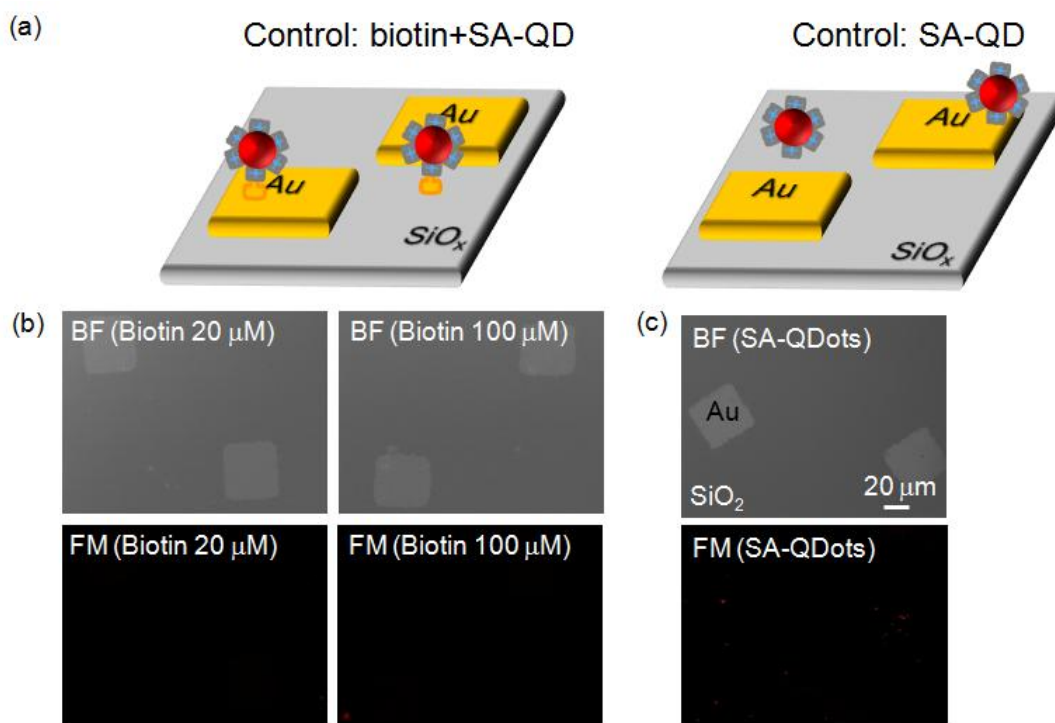
Supplementary Information:

**Fig. S1:** Maldi-TOF spectra of biotinylated gold-binding peptides AuBP2-bio.

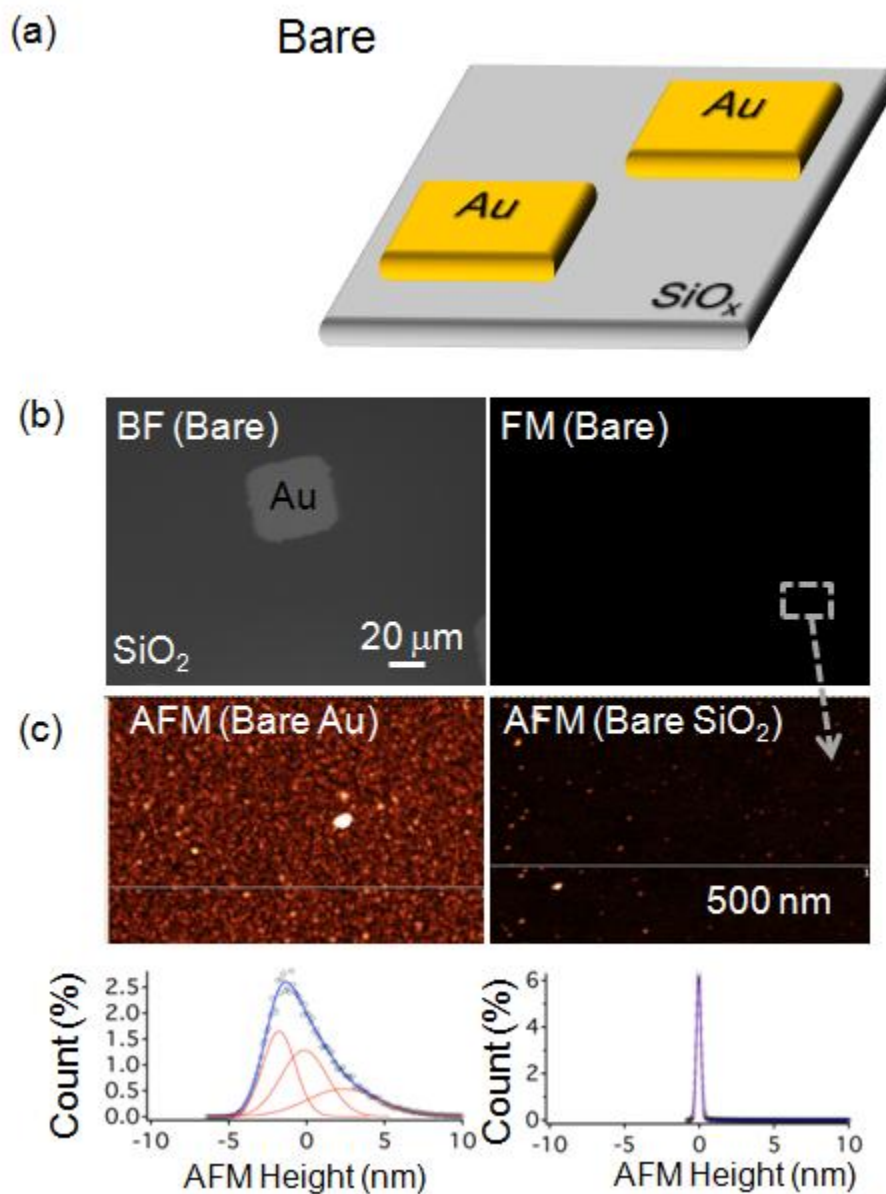
Peptide	Sequence	[M-H] <sup>+</sup> Theoretical Mass	[M-H] <sup>+</sup> Experimental Mass
AuBP2-bio	WALRRSIRRQSY-bio	1819.1	1820.9



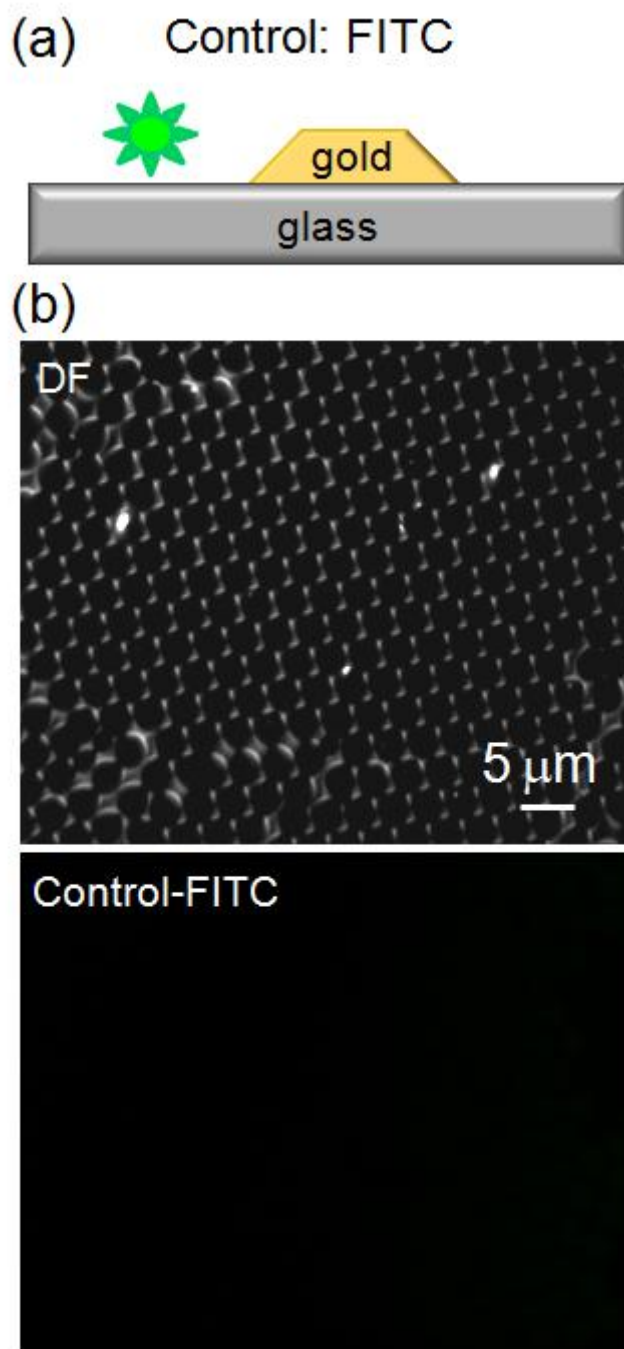
**Fig. S2:** Non-specific immobilization of QDots onto gold and silica micro-patterned surface. (a) Schematic of illustration of non-specific immobilization of streptavidin-QDots (SA-QDots) onto gold and silica regions. (b) Bright field (BF) and fluorescence microscopy images (FM) of QDots non-specifically immobilized onto gold and silica region after incubation with biotin (20 and 100  $\mu\text{M}$ ) and SA-QDots (no peptide present). (c) Bright field (BF) and fluorescence microscopy images (FM) of QDots non-specifically immobilized onto gold and silica region after incubation with SA-QDots only (no peptide present).



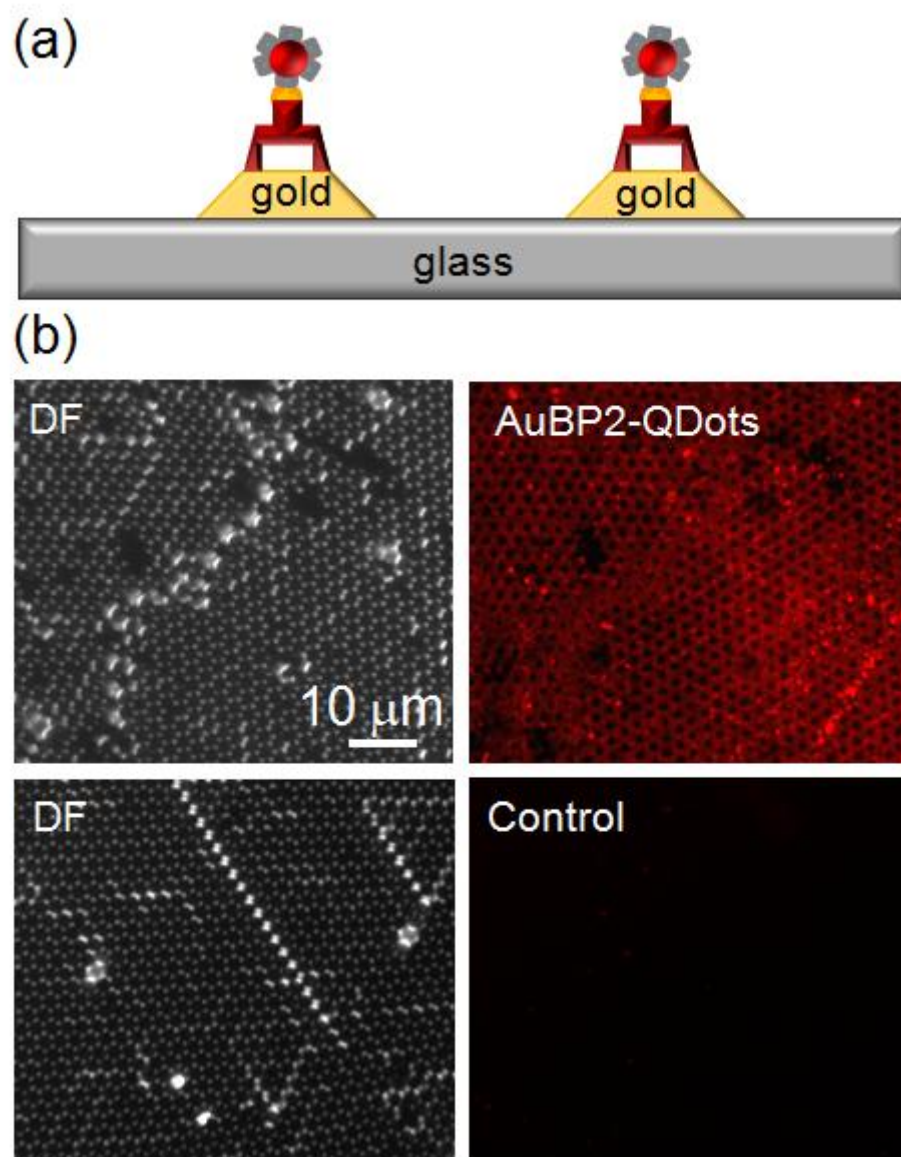
**Fig. S3:** Bare gold and silica micro-patterned surface. (a) Schematic of illustration of bare gold and silica regions. (b) Bright field (BF) and fluorescence microscopy images (FM) of bare gold and silica region. (c) Representative AFM scans and respective histograms generated from micropatterned surfaces from (b) fitted with multiple Gaussian functions characteristic of the bare surfaces.



**Fig. S4:** Non-specific immobilization of FITC probe onto gold and silica NSL surface. (a) Schematic of illustration of non-specific immobilization of FITC probe onto NSL surface. (b) Dark field (DF) and fluorescence microscopy images (FM) of FITC non-specifically immobilized onto NSL surface (no peptide present but only FITC probe alone).

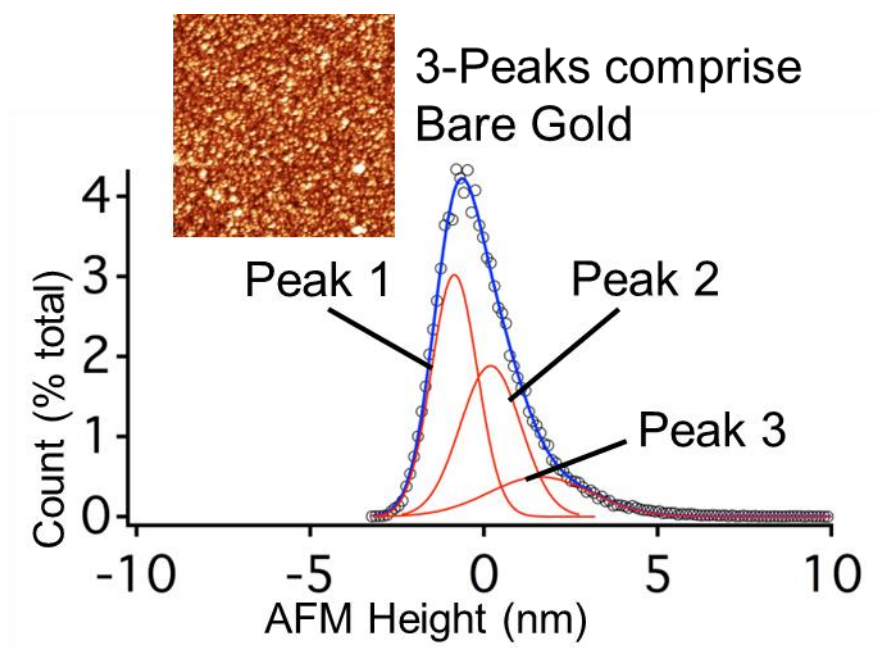


**Fig. S5:** Peptide-directed immobilization of QDots nanostructures on NSL substrate (1.5  $\mu\text{m}$  bead mask). (a) Schematic of immobilization of SA-QDots onto gold regions through gold-binding peptide. (b) Fluorescence image of immobilized SA-QDots through gold-binding peptide (AuBP2) on gold (right top corner) with respective dark field image (left top); control experiment: no peptide but SA-QDots present (right bottom); respective dark field image (left bottom).



**Quantification of QDot Surface Coverage:** The surface coverage of immobilized QDots to gold and silica surfaces were quantified by deconvoluting the original AFM image histogram into separate peaks (Igor Pro 6.12, Lake Oswego, OR) which are then assigned to specific surface features. The steps in this analysis are as follows: 1) bare AFM scans of silica and gold surfaces are analyzed for characteristic peak shapes, where silica is typically fitted with a single normal distribution while evaporated gold surfaces yield an asymmetric peak comprised of three decreasing peaks due to the surface roughness (See Fig. S6). 2) To measure the surface coverage of SA-QDots, histograms of peptide-targeted surfaces are compared to the corresponding bare surface. In all cases, SA-QDots manifest as an additional broad peak located 5-10 nm from the bare surface. 3) The surface coverage is then taken as the area under the SA-QDots peak (Fig S6, red line) over the area under the total fitted histogram (Fig. S6, blue line). For SA-QDots on silica surfaces, height distributions of both materials were sufficiently distinctive to measure separately (see Fig. S3). However, for SA-QDots on gold, there was significant overlap between the asymmetric bare gold peak and the SA-QDots layer (see Main text, Fig 3e). To quantify these images, we first establish that the characteristic three peaks comprising bare gold typically exist at fixed ratios with respect to their intensity: Peak 1/Peak 2, 0.785, Peak 2/Peak 3, 0.34, and Peak 1/Peak 3, 0.27. These are shown in Fig. S6, gathered from 4 representative bare gold images. Using these ratios, we can estimate the contribution of bare surface pixels by using the first histogram peak from a sample with bound SA-QDots, likely to be from bare gold, to calculate subsequent peak heights which are inflated by an SA-QDots layer. Therefore, coverage of SA-QDots on Au is calculated by subtracting the estimated area from the bare surface from the total fitted histogram.

**Fig. S6:** Representative asymmetric height distribution from an AFM scan of bare evaporated gold. Peaks are observed to exist as ratios of each other, statistically sampled in the table below. Using these ratios, we estimate the contribution of the bare surface while being covered by SA-QDots and calculate the surface QDots coverage in AFM histograms of peptide-bound QDots in Fig. 3.



	Peak 1	Peak 2	Peak 3	Peak 1 / Peak 2	Peak 2 / Peak 3	Peak 1 / Peak 3
Bare 1	0.79	0.65	0.27	0.82	0.42	0.34
Bare 2	1.405	1.22	0.43	0.87	0.35	0.306
Bare 3	1.09	0.83	0.2	0.76	0.24	0.183
Bare 4	0.78	0.54	0.183	0.69	0.34	0.235
<b>Ave.</b>				<b>0.785</b>	<b>0.34</b>	<b>0.27</b>



For NSL images, pyramid features are removed from images by first applying an erosion filter to the image, removing SA-QDots or other bound features, (8 pixel structuring element, Gwyddion 2.20) and subtracting it from the original image as seen in Fig. S7. This image is then measured for surface coverage using the same procedure as above.

**Fig. S7:** Image processing to remove NSL pyramid features for SA-QDots coverage quantification. Left, original image of SA-QDots bound to pyramid and Right, image used for coverage analysis.

