Supporting Information

Lectin-Carbohydrate Interactions on Nanoporous Gold Monoliths

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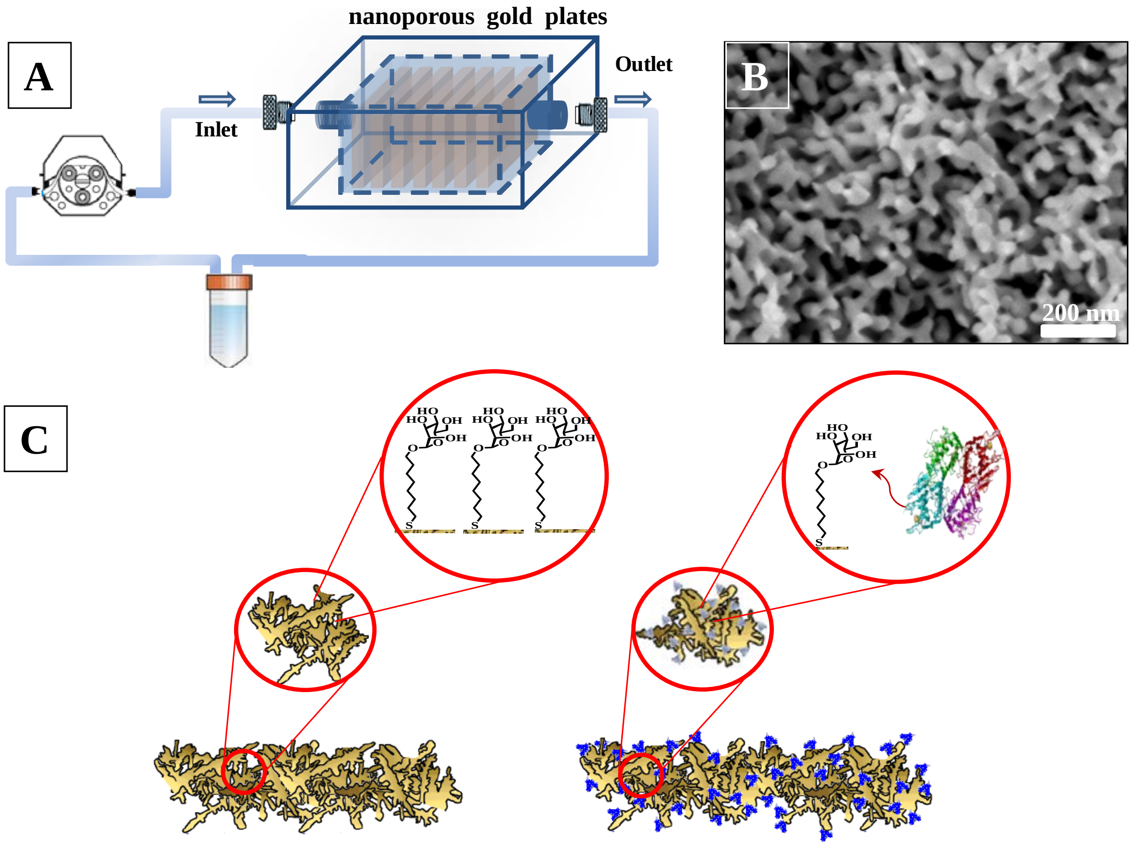

Figure S1. (A) Schematic of the flow system utilized to assist protein passage into the nanoporous gold monoliths for immobilization. (B) Top right, a SEM image of a typical de-alloyed nanoporous gold. (C) Cartoon in lower half represents the 8-mercaptooctyl α-D-mannopyranoside functionalized gold surfaces before and after protein Con A binding.

Thermogravimetric Analysis of the Loading of 1-Octadecanethiol onto Nanoporous Gold

Figure S2 shows TGA data for the mass loading of 1-octadecanethiol (C_{18}-SH) onto np-Au monoliths as a function of time. Two approaches were used to functionalize C_{18}-SH on np-Au, static incubation and loading under flow, over various times. The np-Au used in this analysis was batch de-alloyed. A stock solution of 6.0 mM C_{18}-SH was prepared in HPLC grade ethanol solution for static or flow-through experiments. Given the volume of the flow cell system of 8.0 mL, this corresponds to a total of 13.76 mg of C_{18}-SH initially in circulation (1.72 mg mL^{-1}). The concentration of C_{18}-SH is selected to be high enough to allow for complete modification of the np-Au monolith (estimated capacity of 0.9290 mg) and to not significantly deplete the solution concentration. The np-Au functionalized with C_{18}-SH was rinsed with ethanol at the end of each reaction time point and dried prior to performing TGA. A hexane rinsing step was added to the static incubated np-Au in an attempt to removed physisorbed thiolated residues. Comparison of Figures S2A and S2B shows that the mass of the C_{18}-SH functionalized onto the np-Au, using the static and flow methods are very different, during the first eight hour period. The mass loading of C_{18}-SH molecules onto the np-Au surfaces differs significantly depending upon whether it is conducted under static or flow conditions, with a greater mass loading occurring under static conditions when measured after the 2, 4, 6, and 8 hour time points, most notably so after the 2 and 4 hour time points. The static method is likely to lead to a greater extent of thiolate species non-specifically bound to the gold surfaces, in addition to those bound to Au through strong Au-thiolate interactions, while the flow through method should be more likely to lead to the non-specifically bound species being swept away and out of the np-Au structure. Both the static and flow methods appear to ultimately result in very similar mass loading, suggesting that the alkanethiolates reorganize over time and achieve a similar surface coverage, and that physisorbed molecules that accumulate under static incubation are swept away during the rinsing steps. However, further investigation of the packing density, the crystalline order, the molecular orientation and the presence of defects in SAMs on np-Au is required. Various surface characterization, microscopies, diffraction techniques and vibrational spectroscopies (IR, FTIR, IRRAS, SFG, HREELS, Raman spectroscopy), may be applied to reveal the degree of packing of the monolayers on the surface.[1-8] It has been reported that the thiolate head groups have an affinity towards the gold surface of ~ 40.0 kcal mol^{-1}.[9, 10]

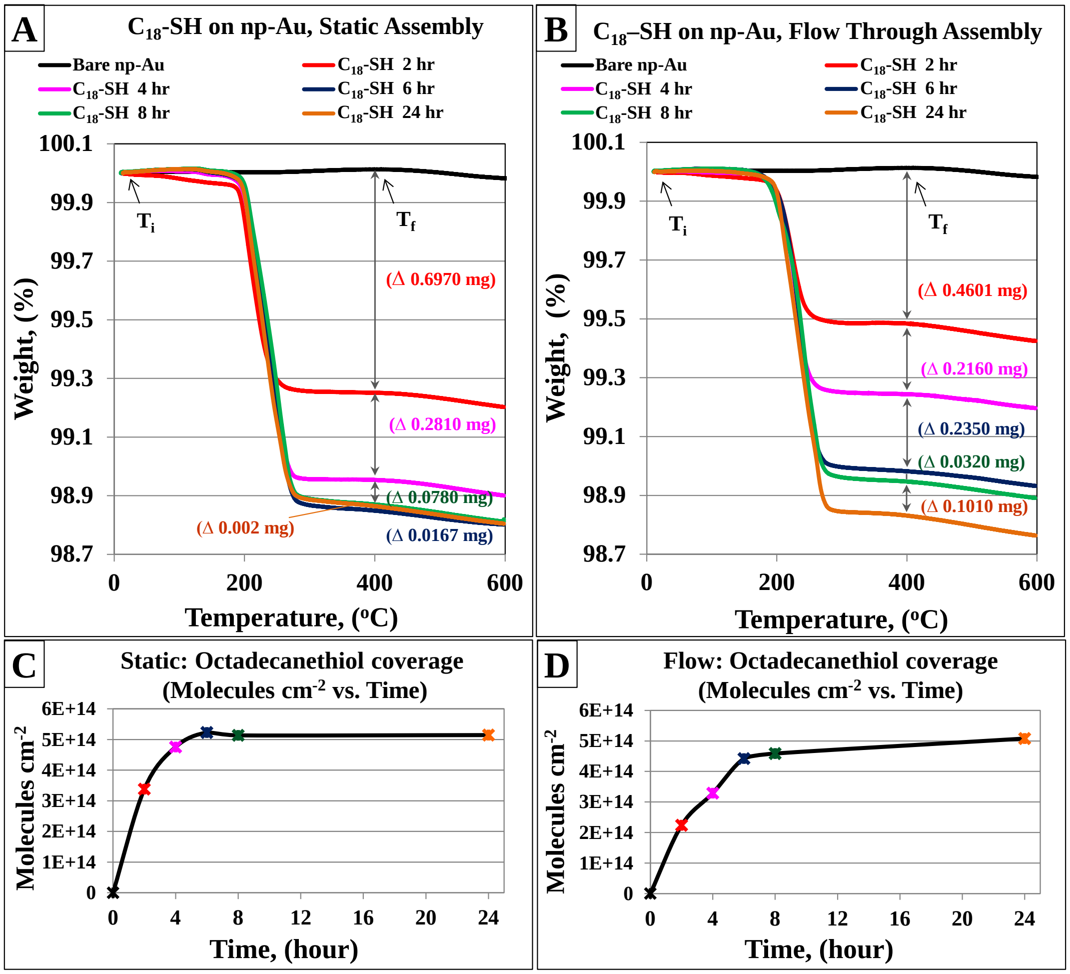

Figure S2. Thermogravimetric analysis of C_{18}-SH adsorbed on np-Au via flow-through and static incubation. (A) Mass loss of C_{18}-SH passivated onto the gold surface of np-Au under static incubation at 2 (red), 4 (pink), 6 (blue), 8 (green) and 24 hour (orange) time points. (B) Mass loss determined by TGA when the flow-through method was used to immobilize C_{18}-SH onto np-Au. TGA analysis was carried out by ramping the temperature at 5 ^{o}C min^{-1} from room temperature to 600 ^{o}C. (C) Static assembly conditions, and (D) Flow-through conditions, represent the adsorption kinetics of C_{18}-SH, in terms of molecules cm^{-2} vs. time, onto np-Au.

Figure S2A (static incubation) shows a mass loss of 0.75% (equivalent to 0.6974 mg) of C_{18}-SH from np-Au at the 2 hour (red line) time point, for the sample modified under static conditions. A mass loss of 0.9800 mg is observed at the 4 hour time point (pink line), but the mass loss of C_{18}-SH was then not observed to increase significantly for the 6, 8 and 24 hour time points (blue, green and orange lines, respectively). The total mass of the C_{18}-SH on the np-Au sample is approximately 1.1 mg at these three time points. The initial physisorption of C_{18}-SH is greater at the earlier time points under static conditions than it is under flow conditions. Figure S2C and S2D show the kinetics of the C_{18}-SH adsorption onto the gold surface of the np-Au monoliths. A faster accumulation of C_{18}-SH within np-Au was observed for the static incubation, Figure S2C, when compared to the flow through experiment shown in Figure S2D, at similar time points.

Effect of Thermogravimetric Analysis Temperature Ramp on Nanoporous Gold Morphology

During the temperature ramp process, the np-Au spends some time at or above temperatures at which it would be expected to anneal and show an increase in pore/ligament size and a decrease in surface area.[11-13] It is not intended that the np-Au be re-used after TGA analysis, and thus the fact that some annealing occurs is not of consequence in that regard. The np-Au could be reclaimed and used to produce new alloys for producing np-Au again if so desired. The total time spent by the sample in the range of 100 ^{o}C – 800 ^{o}C is 140 minutes, and that spent between 100 ^{o}C and 400 ^{o}C where the measured mass loss occurs is 1 hour. Annealing would become progressively more rapid as the temperature increases. The possible interplay of an annealing process ongoing together with an SAM and possible macromolecule decomposition process may pose some interesting questions, such as whether the rate of thermal annealing of np-Au is affected by the presence of surface bound species, and possibly slowed by the thiolates bound to surface gold atoms as they are attempting to diffuse. Once most of the SAM is removed by decomposition, thermal annealing should proceed normally although under a temperature ramp. The scan rate used in TGA is another variable that could affect these concurrent processes. Bare np-Au was examined after a temperature ramp from room temperature to 800 ^{o}C at 5 ^{o}C minute^{-1}. Subsequent SEM imaging of the np-Au removed from the TGA apparatus is shown in Figure S3, and shows a significant increased ligament diameter and pore size. The SEM images in Figure S4, which show np-Au before and after solution flow but with no temperature excursions experienced by the sample can be used as a comparison.

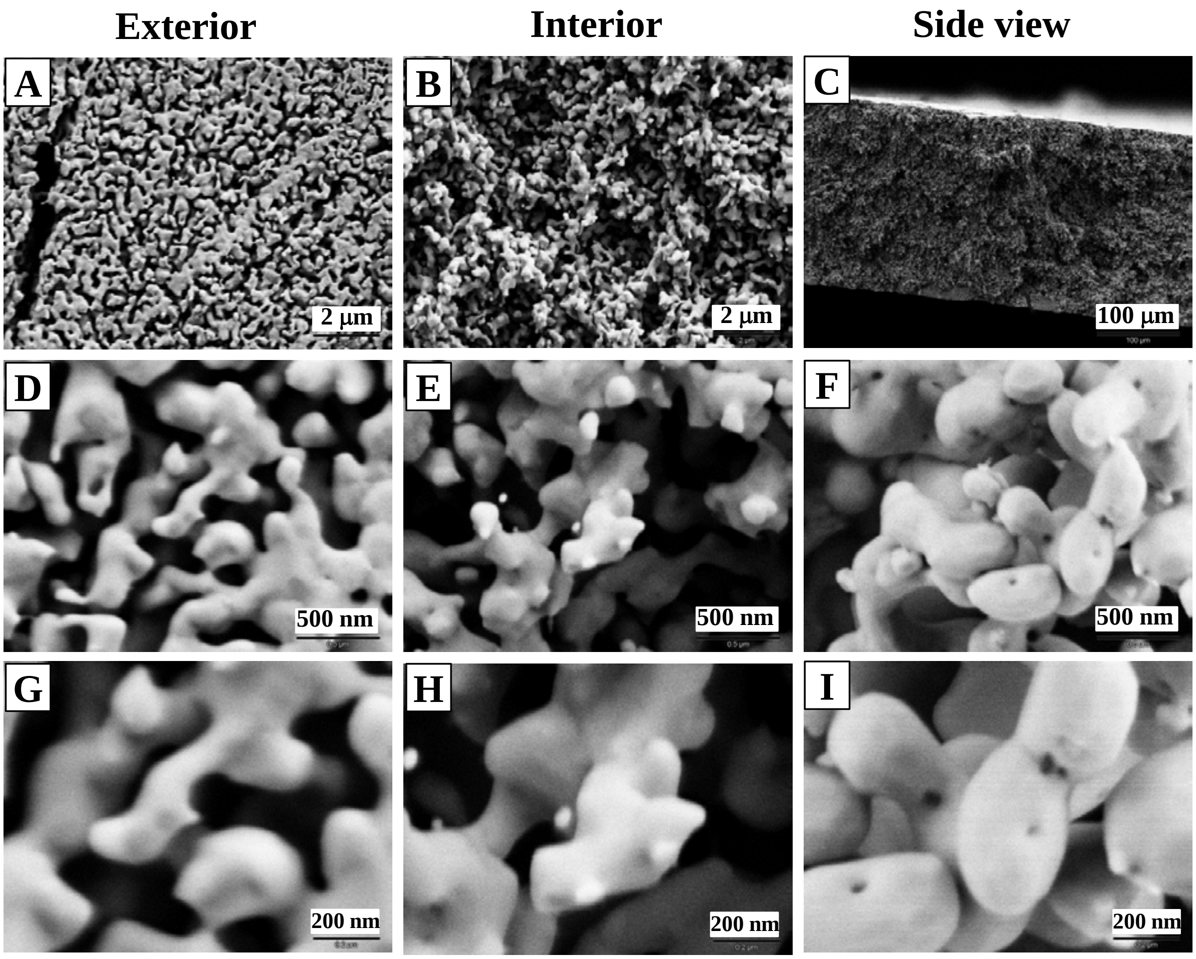

Figure S3. Morphology of nanoporous gold after thermogravimetric analysis. (A) SEM image of the exterior of nanoporous gold after TA analysis. The np-Au was fit into a platinum weighing boat and heated from room temperature to 800 ^{o}C at a ramping rate of 5 ^{o}C min^{-1}. Higher resolution images are shown in (D) 50 kX and (G) 100 kX. Similar pores and ligament networks morphologies were observed for the interior of the nanoporous gold, when compared to the exterior, (B). (E) 50 kX and (H) 100 kX, show the morphology of the interior of np-Au. (C) a side view of the np-Au was captured, the porous and ligament networks were similar to those observed in (A) and (B). The pores and ligaments do not appeared to fuse together or close up, after ramping the temperature to 800 ^{o}C. Images (F) and (I) are the higher magnification images of (C).

Examination of the Effect of Solution Flow on Nanoporous Gold Morphology

An np-Au monolith was examined before and after flow-through assembly of an SAM of octanethiol onto its surface using the conditions used throughout these studies, to check if any change in morphology as a consequence of flow would be observed. Figure S4 confirms that exposure of the np-Au monolith to flow at 1.0 mL min^{-1} has no effect on the np-Au morphology.

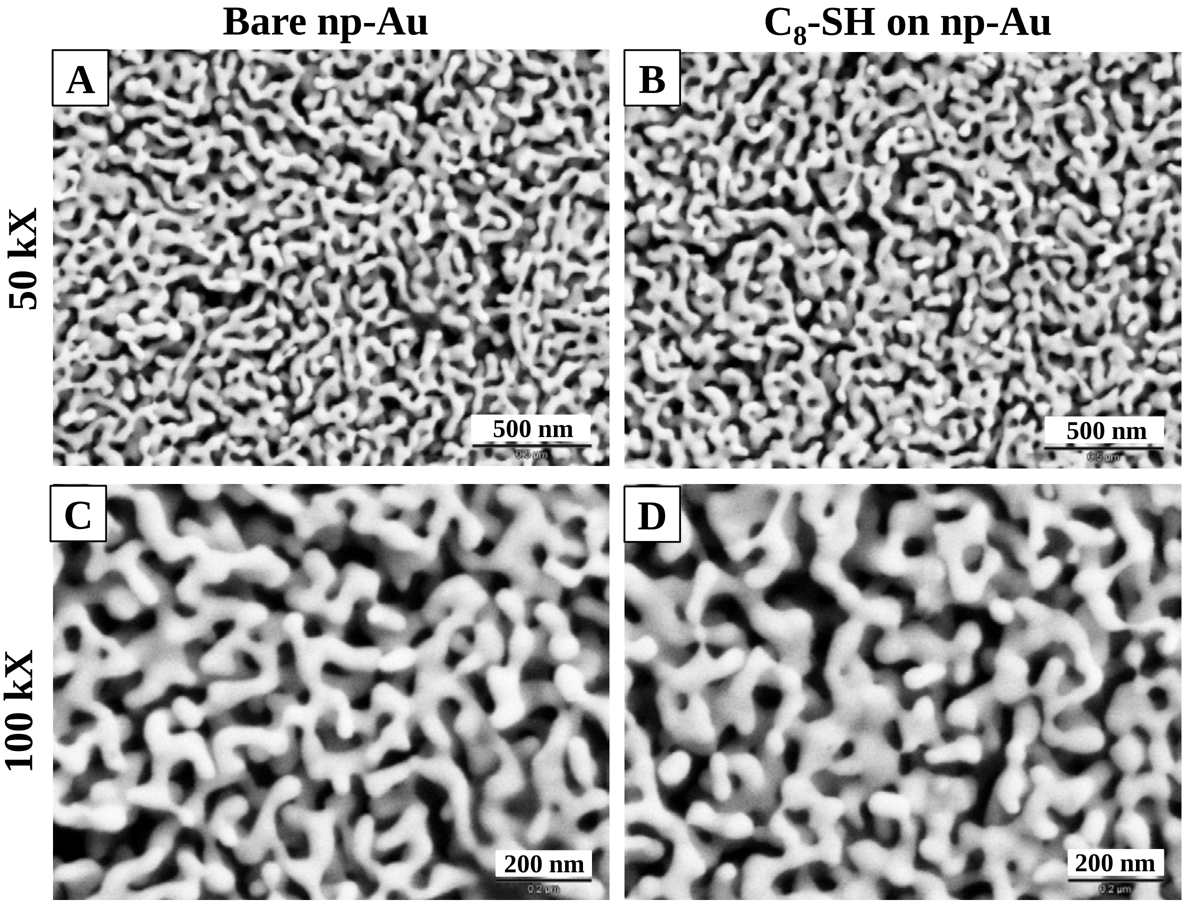

Figure S4. Morphology of nanoporous gold modified with alkanethiolates. (A) SEM images of nanoporous gold, 50 kX, prior to modification with octanethiol. (B) nanoporous gold after modification of octanethiol (5.0 mM, ethanol) using flow through conditions at 1.0 mL min^{-1}. No apparent changes in morphology were observed as a consequence of solution flow through np-Au. (C) and (D) are higher magnification images of (A) and (B), respectively.

Binding of the Lectin Concanavalin A to Modified np-Au under Static Conditions

The mixed SAM of αMan-C$_8$-SH and HO-PEG$_2$-SH was prepared on np-Au under flow of a 1:4 solution molar ratio of these two compounds, and was then removed from the flow cell apparatus. The modified np-Au was immersed in the Con A solution (1.0 mg mL^{-1}) for 3.5 hours, followed by rinsing with Milli-Q water and then subjected to TGA, the results of which are shown in Figure S5. The goal of these experiments was to compare the extent of Con A binding to the same mixed SAM on np-Au and for the same time period and Con A solution concentration under flow versus static conditions. The TGA data in Figure S5 indicate binding of 0.0377 mg of Con A under the stated conditions, which is less than one-third that found under flow conditions (see main text).

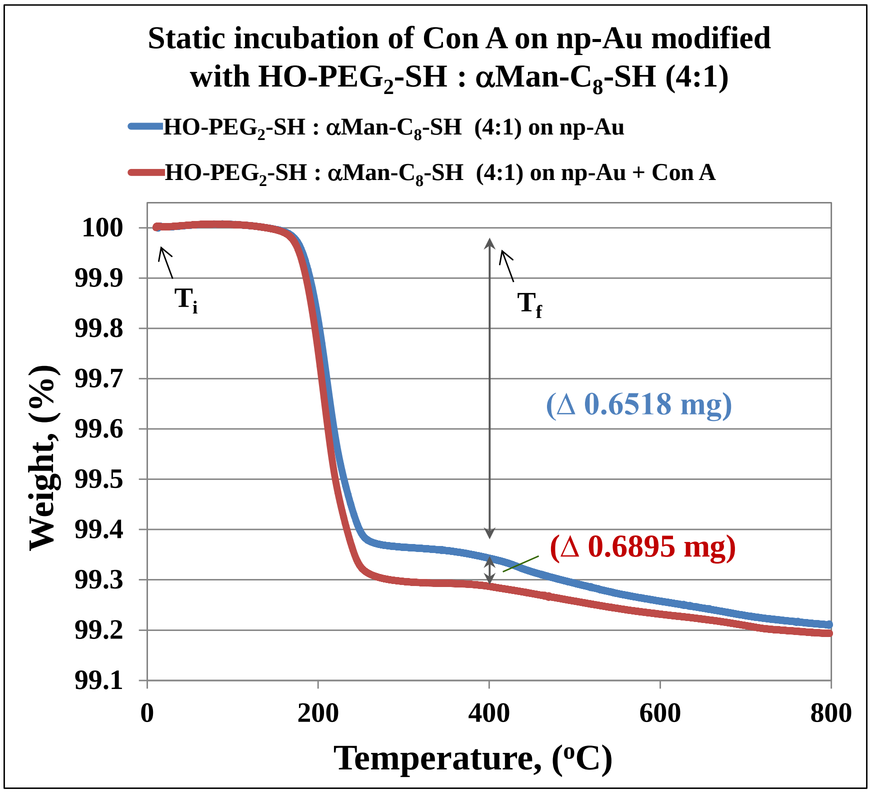

Figure S5. Thermogravimetric analysis of Con A bound onto np-Au monolith modified with mixed SAMs of 8-mercapto-3,6-dioxaoctanol and 8-mercaptooctyl α-D-mannopyranoside. Nanoporous gold modified with αMan-C$_8$-SH and HO-PEG$_2$-SH and (1:4, 5 mM, in ethanol) was exposed to lectin concanavalin A using a static incubation approach. The blue line represents the mass loss for the np-Au modified with the mixed SAMs. The red line represents the mass loss from the specific interaction of Con A with αMan-C$_8$-SH as observed for a np-Au monolith modified with these mixed SAMs. Due to the low penetration depth of the Con A toward the interior of the porous gold environment, a much lower amount (0.04 mg) of Con A was adsorbed onto np-Au modified with these mixed SAMs of αMan-C_8-SH and HO-PEG_2-SH.

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