Communication

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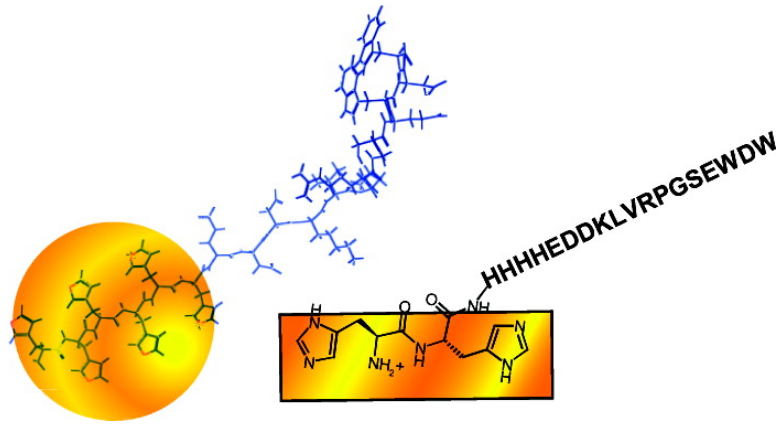

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Single Peptide Assembly onto a 1.5 nm Au Surface via a Histidine Tag

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Biological molecules appended to nanoparticles (NPs) enhance cellular transport,[1] cellular imaging,[2] and bioassay performance;[3] however, conjugation of proteins to a NP can perturb protein function.[4] A more routine method with high regiospecificity would provide a universal interface for protein attachment to NPs, particularly for metalloproteins where nitrilotriacetic acid chelation[5] by Ni(II) would be inapplicable. The ubiquity of the hexa-histidine (His_6) tag in biotechnology prompted us to investigate the direct coupling of His_6 to a AuNP using a set of synthetic peptide sequences and a larger His-tagged protein, namely IdeR, a metal-activated transcriptional regulator in *Mycobacterium tuberculosis*.[6] The use of His_6 simplifies the labeling chemistry and allows for controlled appendage with high regiospecificity. The binding is evidenced by NMR and FT-IR analysis on His_6 residues.

The microscopic binding interactions for identical polypeptide sequences (XEDDKLVPRGSEWDW) containing a His_6, Cys, or Ser N-terminal "headgroup" demonstrates the selectivity of the His_6 strategy for assembly onto AuNPs. Binding of a separate peptide sequence (His_n-AAAAKAAAQGGW) with variable His terminal sequences ($n = 2, 6, 10$) and a His_6-IdeR protein confirm the high binding affinity for the His sequence on a AuNP. AuNPs (1.5 nm) passivated by bis(ρ-sulfonatophenyl)phenylphosphine (bSPP) or triphenylphosphine (TPP) were prepared following literature protocols.[7] The peptide was ligand exchanged either directly onto bSPP-AuNP in phosphate buffered saline (125 μM in 20 mM phosphate buffer, pH 7.5, 50 mM NaCl; PBS) for 30 min at room temperature or through a biphasic process onto TPP-AuNP in CH_2Cl_2/PBS buffer. The biphasic exchange was monitored by aqueous phase absorbance at 420 nm (AuNP). In both methods, the aqueous sample was purified by gel filtration (G-25 Sephadex) to remove unbound peptide.

The peptide/AuNP stoichiometry was determined from the A_{280}/A_{420} intensity ratio assuming that A_{280} arises from the peptide Trp (W) residues plus AuNP, whereas A_{420} is from AuNP alone. This approach yielded stoichiometries of 38:1, 1:1, and 100:1 for the Cys, His_6, and Ser headgroups, respectively (Supplemental Figure 1). The 38:1 Cys-peptide stoichiometry is consistent with 32 to 45 alkylthiols bound on a 1.5 nm AuNP surface[7] and implies that nearly every possible binding site at the AuNP surface is occupied by a Cys-peptide. The unit stoichiometry of the His_6-peptide reflects the larger molecular "footprint" for the multiple His interactions at the AuNP surface. The 100:1 binding ratio for the Ser-peptide is inconsistent with direct binding of the peptide to the AuNP and suggests interactions with the backbone.

Evidence for electrostatic driven assembly was analyzed using a SET molecular beacon to assay fluorescence quenching of the Trp by the AuNP.[8] The preassembled AuNP−peptide conjugates exhibited Trp fluorescence of 53 ± 5% (Cys), 55 ± 3% (His_6), and 46 ± 2% (Ser) relative to the free peptide, suggesting that the Trp distance to the AuNP surface are similar. Treating each peptide−AuNP complex with up to 2.5 M guanidine hydrochloride equally reduced but did not eliminate the quenching (Supplemental Figure 2) indicating electrostatics plays a minor role in assembly and implying that backbone and/or side chains play a more dominant role in assembly.

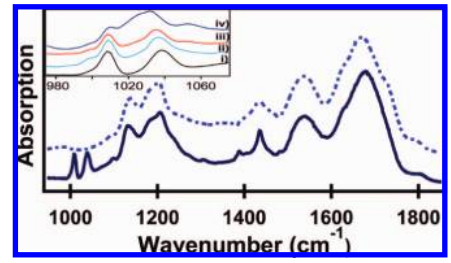

Figure 1. Diffuse reflectance FT-IR of free (dashed) and AuNP-bound (solid) His_6-EDDKLVPRGSEWDW. The inset shows the appearance of His peak feature at 1008 and 1038 cm^{-1} for (i) His_6 shown and (ii−iv) His_n-AAAKAAAQGGW, where $n = 10$ (ii), 6 (iii), and 2 (iv).

The molecular aspects of the peptide−AuNP assembly were analyzed by FT-IR of lyophilized peptide−AuNP samples mixed with KBr (Figure 1). Spectra and tentative assignments for all peptide−AuNP complexes are included in the Supporting Information (Supplemental Figure 3−5, Supplemental Tables 1−3). Assembly of the His_6 onto the AuNP results in the appearance of new features at 1008 and 1038 cm^{-1}, which can be assigned to Nϵ−Au interactions.[9] The appearance of these new features were observed for the binding of His_n-AAAAKAAAQGGW), Figure 1 (Supplemental Figure 6). The intensity of the new IR features for the His-tag AuNP binding scale with the number of His residues.

There are no significant changes for the His_6−AuNP observed in the 1100−1500 cm^{-1} region, which contains vibrations associated with amino acid side chain functional groups. The observation of the imidazole−AuNP interactions with minimal side chain changes for the His_6 peptide provides strong evidence of a direct interaction between the imidazole ring(s) and the AuNP. FT-IR changes for the Ser- and Cys-peptide are consistent with the Cys binding at the thiol (loss of 2550 cm^{-1}) and no significant Ser interaction. In the side chain region, the Ser peptide exhibits no changes, while large changes in the spectrum of the Cys-peptide are observed.

The observed Amide I frequency for the His_6-peptide (1678 cm^{-1}) suggests a more extended conformation on the AuNP than what is observed for the Cys- (1655 cm^{-1}) and Ser-peptides (1661 cm^{-1}). No changes in the Amide II resonance are observed for the His_6-peptide, as expected for a single peptide appended on the AuNP surface. Spectral shifts are observed for Cys−AuNP and Ser−AuNP in the Amide II region reflecting the higher loading levels of the peptides. The FT-IR results support a binding motif where the Cys headgroup binds via a Au−SH[10] interaction while the His_6 coordinates through the imidazole ring(s). Ser exhibits no headgroup involvement implying assembly may occur through the side chains.[11] The large changes in the side chain for Cys but not

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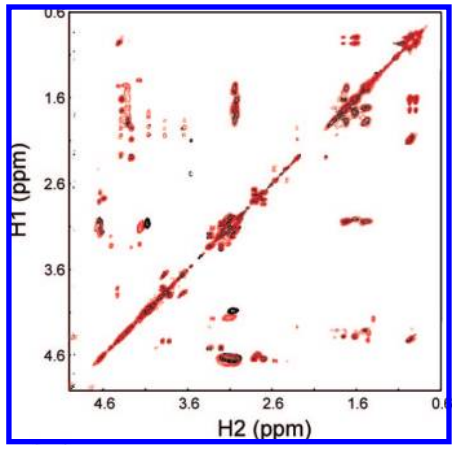

Figure 2. $^1H-^1H$ TOCSY spectrum of the unbound His_6 peptide (black) and His_6 peptide bound to 1.5nm AuNP (red).

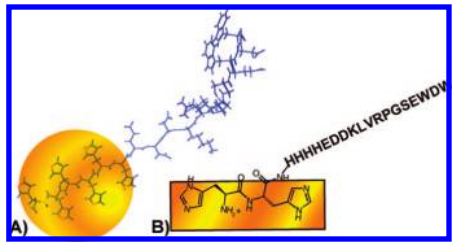

Figure 3. (A) Model of His_6 peptide assembled onto 1.5 nm AuNP. (B) Nϵ imidazole side chain coordination to the AuNP surface for two of the six His residues (for clarity).

His_6 can be interpreted in terms of the higher occupancy of Cys-peptide relative to His_6-peptide on the AuNP surface.

Deeper insight into the molecular interactions is gained via analysis of the aliphatic portion of the $^1H-^1H$ TOCSY spectrum of the His_6-peptide (Figure 2). The only signficant impact on the spectrum is a shift in the His Hα (4.1 ppm)$-$Hβ (3.1 ppm) cross-peak and a pronounced downfield shift ($\Delta\delta = \sim 0.2$ ppm) in the CHϵ (8.1 ppm) and CHδ (7.1 ppm) of all imidazole resonances upon complexation (Supplemental Figure 7), consistent with the FT-IR for the Nϵ vibration. The changes in the aliphatic and aromatic region for His_6-AuNP implies minimal impact on the side chain and backbone, with the His imidazole ring complexing to the AuNP. In comparison, the aliphatic and aromatic portions of the TOCSY spectra of the Cys (Supplementary Figure 8) and Ser (Supplementary Figure 9) peptides change substantially upon complexation with the AuNP, particularly in the aliphatic region, indicating large effects on the peptide side chains arising from higher peptide loading levels for Ser and Cys peptides.

Extrapolation of these results to His_6-IdeR suggests that the His-tag should bind to the AuNP without inhibition of protein$-$DNA binding. Adding AuNP to His_6-IdeR quenched the fluorescence of the two IdeR tryptophans (Supplemental Figure 10), but the His$-$Au signature in the FT-IR is not visible due to the large number of $-CH_2$ vibrations in the protein (Supplemental Figure 11). The nanometal surface energy transfer molecular beacon assay[7] in which AuNP-labeled His_6-IdeR is added to a 33bp duplex DNA sequence containing a *mbtA* iron-box[6a,b] sequence with AF647 appended to the 5′ end was developed. Binding of the IdeR$-$AuNP to the *mbtA* DNA sequence results in emission quenching of the AF647 by 75% (Supplemental Figure 12) indicating that the DNA binding function of His_6-IdeR with an attached AuNP is retained and confirms protein function. A more complete structure and function study for a His_6 protein system is currently underway.

Our results demonstrate direct attachment of a single His_6-peptide to AuNP through the imidazole of a His residue(s) (Figure 3). The data suggest a model where multiple His chelation events occur per AuNP, limiting the number of peptides on the surface. Given the ubiquity of the His_6 moiety for recombinant protein expression, this approach eliminates the need for elaborate surface modifications for appending to AuNPs and, thus, could have a significant impact on the use of 1.5 nm AuNPs to deliver proteins.

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Supporting Information Available: Absorbance spectra for His_6-Au, Cys-Au, Ser-Au, and bSPP-Au, fluorescence quenching of Trp by AuNP with addition of GuHCl, full FT-IR spectra and assignments for all native and AuNP assembled peptides, FT-IR of His_2, His_6, and His_{10} peptides, $^1H-^1H$ 2-D TOCSY NMR spectra for the His_6 aromatic, aromatic and aliphatic regions of Cys and Ser peptides, FT-IR spectra of His_6-IdeR and His_6-IdeR + AuNP, and fluorescence quenching of dye-labeled dsDNA with IdeRAu. This material is available free of charge via the Internet at http://pubs.acs.org.

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