Fabrication and Self-Optimization of Multivalent Receptors on Nanoparticle Scaffolds

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Molecular recognition processes are crucial in the function of both natural and man-made systems.[1] In biological systems, combinations of multiple noncovalent bonding interactions such as hydrogen-bonding, π-stacking, and electrostatic interactions provide exquisite control of key interactions such as protein−ligand and protein−protein interactions.[2] Multivalent recognition processes are also important in man-made systems, where these events serve to provide structure and function in synthetic molecular devices and materials.[3]

Synthetic host−guest systems provide a powerful method for the study and application of multivalent recognition processes.[4] A wide range of molecular scaffolds have been developed to serve as hosts, allowing the exploration of the interplay of multiple intermolecular interactions.[5] One potentially versatile scaffold for the preparation of multivalent receptors is provided by self-assembled monolayer (SAM) functionalized gold colloids (SAM-colloids).[6] In these systems the number and kind of recognition elements on the colloid surface can be readily controlled during colloid formation, and subsequently via place-displacement methods,[7] providing a method for the divergent fabrication of multivalent hosts. Additionally, the mobility of thiols on the SAM surface[8] presents the possibility of creating environmentally responsive systems. These systems would use maximization of binding enthalpy to dynamically optimize host−guest interactions. We present here the fabrication of multivalent colloid-based receptors (Figure 1B), as well as dynamic systems where self-assembly of multivalent flavin binding sites on SAM-functionalized gold nanoparticles provides *templated* guest recognition (Figure 1C).[9]

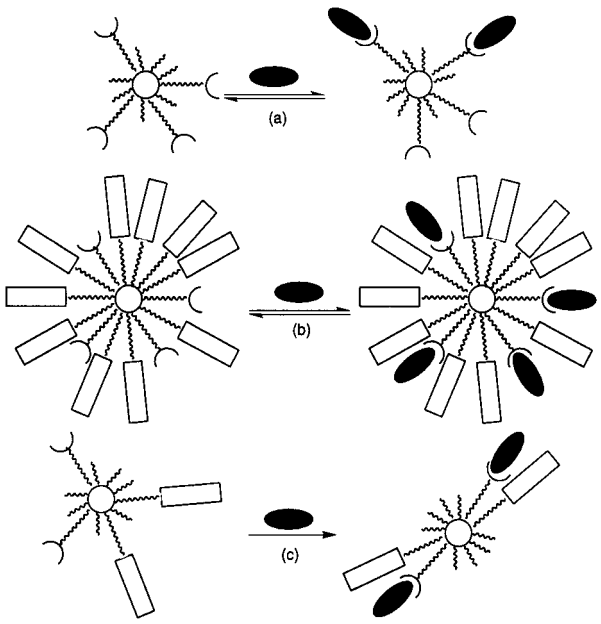

Figure 1. Illustrative representation of (a) monovalent host−guest interactions with a colloid-bound host (b) preformed multivalent binding site from a bifunctional monolayer and (c) templation of a polyvalent binding site with a trifunctional monolayer.

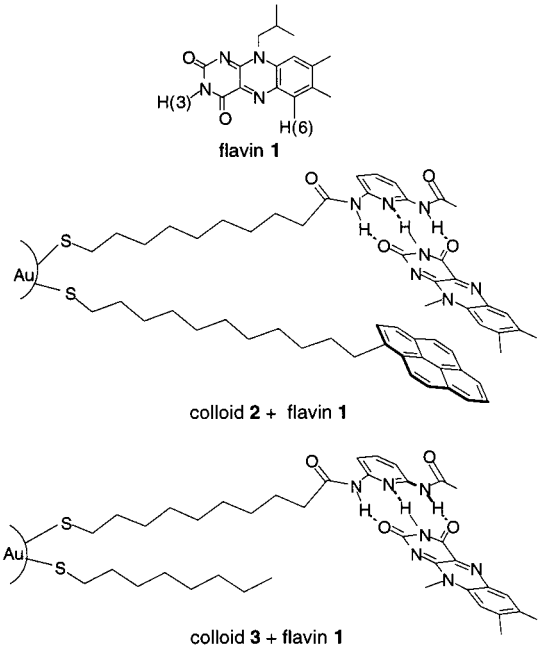

Figure 2. Schematic illustration of the binding of flavin **1** to diaminopyridine/pyrene-functionalized colloid **2** and diaminopyridine-functionalized colloid **3.**[11]

To provide a system capable of multivalent recognition, thiols featuring diacyldiaminopyridine hydrogen-bonding moieties were place-exchanged into a colloid functionalized with pyrene aromatic stacking elements to provide colloid **2**.[10,11] Colloid **2** is

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(10) See Supporting Information for full synthetic details.

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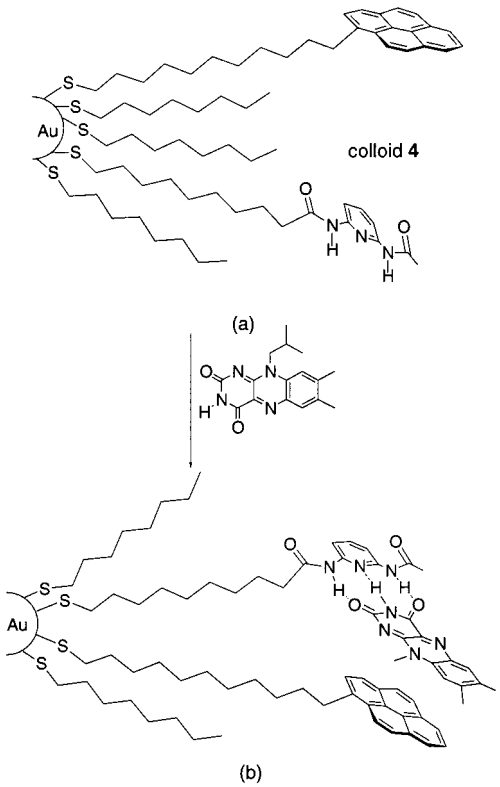

Figure 3. Schematic illustration of the self-assembly of the hydrogen bonding/π stacking binding site on colloid **4**. (a) Prior to incubation with flavin **1**, the initial SAM has both types of recognition elements distributed on the surface. (b) After incubation with flavin **1** the recognition elements assemble into a binding pocket. [11]

capable of both hydrogen bonding and aromatic stacking with flavin **1**.[12] This multivalent interaction was demonstrated by ^{1}H NMR titration in $CDCl_3$, where the observed K_a of colloid **2** with flavin **1** was $K_a = 323 \pm 20$ M^{-1}. This is nearly 2-fold that observed for colloid **3**−flavin **1** binding process ($K_a = 193 \pm 8$ M^{-1}),[6d] where only hydrogen bonding can occur. As expected for the colloid **2**−flavin **1** system, an upfield shift was observed for the aromatic protons of flavin **1**, indicative of aromatic−aromatic stacking of the flavin with the pyrene moieties of colloid **2**.[13] Concurrently, a downfield shift diagnostic of hydrogen bonding was observed for the flavin N(3)H upon binding to colloid **2**[14](Figure 2).

With multivalent recognition demonstrated for colloid **2**, we next explored the use of colloid−flavin binding to self-assemble preorganized binding sites on the nanoparticle surface. To examine the dynamic response of these systems, we prepared colloid **4** (Figure 3), a trifunctional system with dilute hydrogen bonding and aromatic stacking recognition elements in an octanethiol supporting monolayer.[15]

The templation of colloid **4** binding sites by flavin **1** was demonstrated by time-course NMR experiments in $CDCl_3$. In these

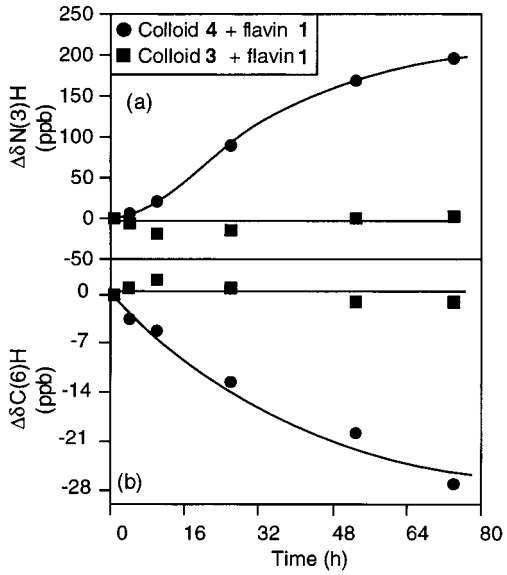

Figure 4. (a) Change in the chemical shift ($\Delta\delta$) of the flavin **1** N(3)H proton, and (b) change in the chemical shift ($\Delta\delta$) of the flavin **1** N(6)H proton as a function of time in the presence of colloid **4** and colloid **3**. $CDCl_3$, $T = 298$ K; [**1**] = 5 mM; [**2**] = [**3**] = 5.9 mM (based on diaminopyridine/pyrene equiv).

studies, chemical shifts of flavin protons were monitored as a function of time in the presence of colloid **4**, with colloid **3** used as a control. As seen in Figure 4, the N(3)H flavin chemical shift in the colloid **4**−flavin **1** sample moved smoothly downfield by ~200 ppb over the 73 h, approaching a limiting value. As aromatic stacking was shown to have little effect on the chemical shift of this proton,[14] this downfield movement is directly correlated to an increase in colloid−flavin recognition. Algebraic determination of the binding constant from the chemical shift data indicates an increase in K_a from 168 to 235 M^{-1}, a 71% enhancement.[16] As expected, there was a concomitant upfield shift of the aromatic protons of flavin **1**, indicative of the enhanced aromatic stacking provided by binding site optimization.

In summary, we have demonstrated the creation of a multivalent host system using colloidal gold nanoparticles as scaffolding. More significantly, we have demonstrated that these systems are environmentally responsive, using the enthalpy of binding to create imprinted binding sites. The application of this methodology to recognition of biomolecular systems, including protein surfaces and cell surface receptors is underway, and will be reported in due course.

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Supporting Information Available: Full synthetic and characterization data for all organic and colloidal compounds, NMR titration data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Pyrene elements have the possibility of binding to both faces of the flavin, providing what would formally be a tritopic binding process. We are currently exploring the use of this structural motif for the creation of "tweezer" functionality.

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(14) The maximum calculated shift of the colloid **2**−flavin **1** system (13.2 ppm) was virtually identical to that observed for the colloid **3**−flavin **1** system (13.0 ppm), indicating the aromatic stacking does not effect the shift of this proton and facilitates direct comparison of the two.

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(16) This enhancement is less than the 4−20-fold enhancement due to aromatic stacking observed in our previous small molecule-based flavin receptors.[12] The lower degree of enhancement observed in the current system is due in part to the relatively long chain length of the recognition elements. Additionally, since imprinting is a dynamically controlled equilibrium process, the templation process is controlled through template molecule concentration, an area we are currently exploring.