JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Self-Assembly of a Catalytic Multivalent Peptide–Nanoparticle Complex

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Supporting Information

ABSTRACT: Catalytically active peptide–nanoparticle complexes were obtained by assembling small peptide sequences on the surface of cationic self-assembled monolayers on gold nanoparticles. When bound to the surface, the peptides accelerate the transesterification of the *p*-nitrophenyl ester of *N*-carboxybenzylphenylalanine by more than 2 orders of magnitude. The gold nanoparticle serves as a multivalent scaffold for bringing the catalyst and substrate into close proximity but also creates a local microenvironment that further enhances the catalysis. The supramolecular nature of the ensemble permits the catalytic activity of the system to be modulated in situ.

The catalytic efficiency, mechanistic pathways, and structural complexity displayed by enzymes make them a tremendous source of inspiration for chemists involved in catalyst development.[1,2] Nature has evolved enzymes as large multi-kilodalton complex structures in which even units that are remote from the actual active site may profoundly affect the activity of the enzyme.[3] The much lower complexity of artificial enzyme mimics may be an important reason for their typical modest performances with respect to enzymes. This awareness has led to an interest in catalysts based on multivalent scaffolds, such as dendrimers,[4] micelles,[5] and nanoparticles,[6] with the idea of increasing the structural complexity of the synthetic system. A key challenge is straightforward access to synthetic catalysts that can match up to the size and complexity of enzymes. The necessity for multistep synthesis can be overcome by relying on self-assembly for the formation of the multivalent structure. In particular, the self-assembly of catalytic monolayers on the surface of gold nanoparticles (Au NPs) to give gold monolayer-protected clusters (Au MPCs) is emerging as an attractive strategy.[7,8] Nonetheless, although they rely on self-assembly, the composition of self-assembled monolayers (SAMs) on Au NPs is typically still of rather low complexity.[9] This mainly originates from the use of synthetic protocols for mixed SAM formation (e.g., place exchange), which do not give full control over the final composition, require purification of each single NP system, and suffer from issues related to the characterization of mixed SAMs both in terms of composition and morphology. For that reason, we recently started to study the formation of heterofunctionalized multivalent structures relying on the self-assembly of small peptides on the surface of Au MPCs.[10] Those studies extended the seminal contributions by Rotello and co-workers, which amply demonstrated the attractiveness of cationic Au MPCs as a construction element for the development of innovative biosensors.[11−13] Here we now show that the self-assembly of histidine-containing peptides $\mathbf{H_1}$–$\mathbf{H_3}$ on the surface of Au MPC **1** triggers their esterolytic activity (Figure 1), resulting in

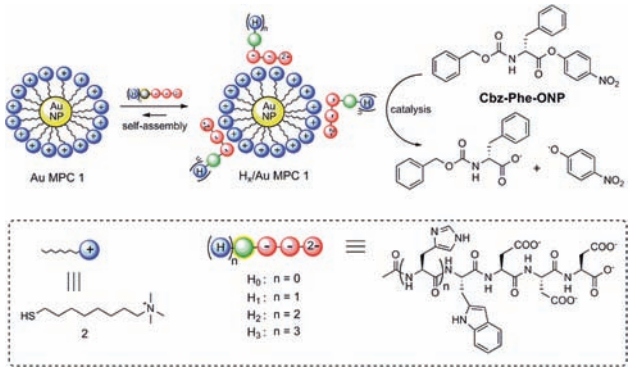

Figure 1. Self-assembly of peptides $\mathbf{H_0}$–$\mathbf{H_3}$ on the surface of Au MPC **1**, resulting in the formation of $\mathbf{H}_x$/Au MPC **1** complexes that (for x = 1–3) can catalyze the transesterification of the substrate **Cbz-Phe-ONP**.

nanostructures that can induce a more than 100-fold rate acceleration. Self-assembly is a prerequisite for catalysis, as the peptides are not catalytically active at all in the absence of the Au MPCs. Importantly, the multivalent surface plays a crucial role in tuning the catalytic activity. The surface not only brings the substrate and catalyst in close proximity but also generates a microenvironment with an enhanced local pH that further activates the catalytic peptide.

We previously showed that the (covalent) insertion of imidazole residues in a SAM generates nanosystems that act as transesterification catalysts.[14] It occurred to us that cationic Au MPCs would be an excellent multivalent platform for bringing such catalytic units together through self-assembly. Peptides $\mathbf{H_1}$–$\mathbf{H_3}$ were designed taking into consideration three issues: (a) the presence of three aspartic acid residues for binding to Au MPCs **1**, (b) the presence of a fluorescent tryptophan residue to verify the binding, and (c) the presence of one or more histidine residues for catalysis. Peptide $\mathbf{H_0}$ lacking histidine units was added as an inert reference compound. Au MPCs **1** (1.8 ± 0.3 nm diameter) containing trimethylammonium headgroups were prepared and characterized as described

Received: March 21, 2012
Published: May 4, 2012

dx.doi.org/10.1021/ja302754h | *J. Am. Chem. Soc.* 2012, 134, 8396–8399

previously.[10,15] Binding of peptides to Au MPCs **1** was studied using fluorescence titrations, relying on the ability of the Au NPs to quench the fluorescence of bound fluorophores.[16] Thus, the fluorescence intensity of tryptophan emission was measured as a function of the amount of peptide $\mathbf{H_0}$–$\mathbf{H}_3$ added to a solution of Au MPC **1** ([headgroup] = 60 μM) in water buffered at pH 7.0. The obtained profiles (Figure 2) are

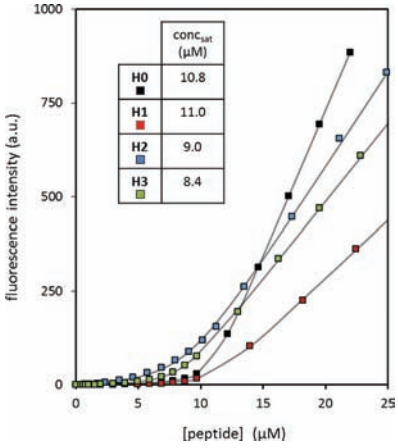

Figure 2. Tryptophan fluorescence intensity at 360 nm as a function of the concentration of peptides $\mathbf{H_0}$– $\mathbf{H_3}$ in the presence of Au MPC **1** ([headgroup] = 60 μM). Conditions: pH 7.0; [HEPES] = 10 mM; H_2O/CH_3CN = 90:10; T = 37 °C). The solid lines represent best fits to a binding model used to determine the surface saturation concentration ($conc_{sat}$) values shown in the inset.[11] The slightly different slopes in the linear parts of the curves result from the different intrinsic fluorescence properties of the tryptophan residues in $\mathbf{H_0}$–$\mathbf{H_3}$ (see the SI).

indicative of strong complex formation between the peptides and Au MPC **1**. From the binding isotherms, a surface saturation concentration ($conc_{sat}$) was determined using a procedure reported previously.[10] The surface saturation concentration defines the maximum loading of the peptide on the Au MPC surface (Figure 2 inset).[17]

Subsequently, we tested the catalytic activity of the obtained systems in the transesterification of the *p*-nitrophenyl ester of *N*-carboxybenzylphenylalanine (**Cbz-Phe-ONP**).[18] Kinetic experiments were performed by adding the substrate (10 μM) to a solution of Au MPC **1** and the peptides $\mathbf{H_1}$–$\mathbf{H_3}$ at their respective surface saturation concentrations in 9:1 H_2O/CH_3CN buffered at pH 7.0 at 37 °C. The presence of 10% CH_3CN was required for solubilization of the substrate. The kinetics of hydrolysis were followed by measuring the increase in absorbance at 400 nm originating from the release of the *p*-nitrophenolate anion (PNP). We were excited to observe that all of the complexes $\mathbf{H_{1-3}}$/Au MPC **1** gave rise to rate accelerations of at least 2 orders of magnitude for the cleavage of **Cbz-Phe-ONP** relative to the background reaction (Figure 3a,b). The observed change in absorbance of 0.1 units at 400 nm corresponded to that expected for the complete hydrolysis of 10 μM substrate. Importantly, the addition of any one of the peptides $\mathbf{H_1}$–$\mathbf{H_3}$ at the same concentrations but in the absence of Au MPC **1** did not result in any rate acceleration over the background (see the SI). The obtained rate constants were corrected for the surface saturation concentration in order to compare the contributions to catalysis by the various peptides. A plot of the obtained second-order rate constants as a function of the number of histidines present in the catalyst gave a

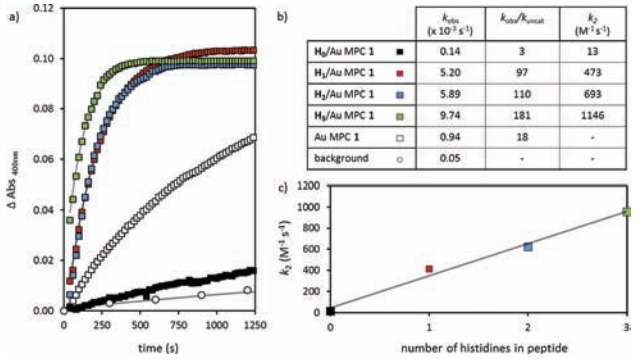

Figure 3. (a) Changes in the absorbance at 400 nm upon the addition of **Cbz-Phe-ONP** (10 μM) to solutions of Au MPC **1** (□), $\mathbf{H_0}$/Au MPC **1** (black ■), $\mathbf{H_1}$/Au MPC **1** (red ■), $\mathbf{H_2}$/Au MPC **1** (blue ■), and $\mathbf{H_3}$/Au MPC **1** (green ■). Included also are data for the background hydrolysis of **Cbz-Phe-ONP** at the same concentration (○). Solid lines represent the best fits to a kinetic model (see the SI). Conditions: [Au MPC **1** headgroup] = 60 μM; [$\mathbf{H_0}$] = 11 μM, [$\mathbf{H_1}$] = 11 μM, [$\mathbf{H_2}$] = 8.5 μM, [$\mathbf{H_3}$] = 8.5 μM; [HEPES] = 10 mM; H_2O/CH_3CN = 90:10; pH 7.0; T = 37 °C. (b) Observed rate constants (k_{obs}), rate accelerations relative to background (k_{obs}/k_{uncat}), and rate constants corrected for the surface saturation concentration of peptide $\mathbf{H_0}$–$\mathbf{H_3}$ (k_2). (c) Plot of the second-order rate constant k_2 as a function of the number of histidines present in peptides $\mathbf{H_0}$–$\mathbf{H_3}$.

straight line (Figure 3c). This shows that the histidine residues are indeed at the origin of the catalytic effect. Furthermore, the linear correlation indicates that the catalysis does not originate from the cooperative action between two histidine residues in the same probe.

Regrettably, the low solubility of the substrate did not permit us to perform catalytic experiments using large excesses of substrate. To show that the catalytic system $\mathbf{H_1}$/Au MPC **1** indeed works under turnover conditions, this problem was solved by sequentially adding batches of **Cbz-Phe-ONP** substrate at the saturation concentration of 10 μM. The amount of PNP released was measured as a function of time after each addition, yielding the profile depicted in Figure 4. Since $\mathbf{H_1}$ was present at a concentration of 5 μM (see below), the total release of 40 μM PNP shows that $\mathbf{H_1}$/Au MPC **1** indeed operates under turnover conditions. The slight decrease in reactivity observed after each addition presumably originates from competitive binding of the reaction products. The

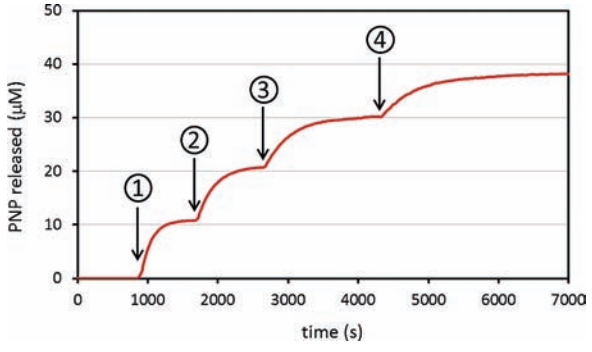

Figure 4. Amounts of PNP released upon four consecutive additions of **Cbz-Phe-ONP** substrate (10 μM) to a solution of $\mathbf{H_1}$/Au MPC **1** as a function of time. Conditions: [Au MPC **1** headgroup] = 60 μM; [$\mathbf{H_1}$] = 5 μM; [HEPES] = 10 mM; H_2O/CH_3CN = 90:10; pH 7.0; T = 37 °C.

dx.doi.org/10.1021/ja302754h | J. Am. Chem. Soc. 2012, 134, 8396–8399

experiments also show that there is no accumulation of intermediates during the reaction.

To understand the origin of the catalytic effect, a series of experiments were performed. Interestingly, it was observed that the simple addition of Au MPC **1** to **Cbz-Phe-ONP** also resulted in a 20-fold enhancement in the hydrolysis rate (□ in Figure 3a), which points at a catalytic role of the cationic headgroups. The addition of tetramethylammonium chloride at the same concentration had no effect at all (see the SI). Similar effects have also been observed in cationic micelles and ascribed to an increase in the local pH where the substrate binds.[19−21] Experimental evidence that the pH at the monolayer surface is indeed higher than the bulk pH was obtained by adding the pH indicator bromothymol blue (30 μM) to a solution of Au MPC **1** under the exact conditions of the catalytic experiments (i.e., pH 7.0). Bromothymol blue (pK_a = 7.1) has two negative charges under basic conditions. This, combined with the presence of a large hydrophobic aromatic portion, was expected to generate a significant binding affinity for Au MPC **1**. Comparison of the absorption spectra in the presence and absence of Au MPC **1** indeed showed a significant increase of 0.29 units in the absorption maximum at 620 nm, corresponding to an apparent local pH of 7.7 (see the SI). Importantly, the catalytic effect of Au MPC **1** is to a large extent suppressed upon addition of the reference peptide $\mathbf{H_0}$ at the surface saturation concentration of 11.0 μM (■ in Figure 3a). Thus, the reference compound $\mathbf{H_0}$ neutralizes the effect of the trimethylammonium groups on the pH. These observations were confirmed by an experiment in which peptides $\mathbf{H_0}$ and $\mathbf{H_1}$ were added 300 s after mixing Au MPC **1** and the substrate (Figure 5a). Relative to the control (no addition), the resulting profiles clearly indicate the accelerating effect of $\mathbf{H_1}$ and the inhibitory effect of $\mathbf{H_0}$ on the catalysis. In addition, this experiment also illustrates an important aspect of supramolecular catalysts, which is the ability to modulate the activity in situ as a function of the type of components added.

The high local pH indicates that cooperativity between imidazoles localized on different probes, as is frequently observed in multivalent imidazole-containing catalysts,[14,22,23] is unlikely to be the source of the observed catalytic activity, as this would require both protonated and unprotonated imidazoles. This was indeed confirmed by measuring the rate of hydrolysis of **Cbz-Phe-ONP** as a function of the amount of $\mathbf{H_1}$ added to Au MPC **1** over the range from 0 to 11 μM. In the case of positive cooperativity, an exponental profile would be expected, corresponding to the exponential formation of catalytic sites composed of two imidazoles.[24,25] Rather, the obtained profile was exactly the opposite of that, showing a strong increase in k_{obs} at low $\mathbf{H_1}$ concentrations (up to 4 μM) and constant values afterward (red ■ in Figure 5b). Evidently, the highest catalytic activity *per histidine residue* is obtained not at maximum surface saturation of Au MPC **1** by $\mathbf{H_1}$ but rather in a situation in which the peptide is isolated on the surface. Thus, the obtained profile indicates an active contribution of the cationic SAM to the catalysis. Indeed, the results are fully consistent with the hypothesis that the presence of a higher local pH on the SAM affects the concentration of deprotonated nucleophilic imidazole. As the peptide covers the surface, its carboxylates replace the OH^- counterions of the ammonium headgroups, thus decreasing the local pH.[21] This in turn increases the concentration of protonated imidazole. Evidence for this hypothesis was obtained by an analogous experiment in which the contribution of the monolayer was suppressed by

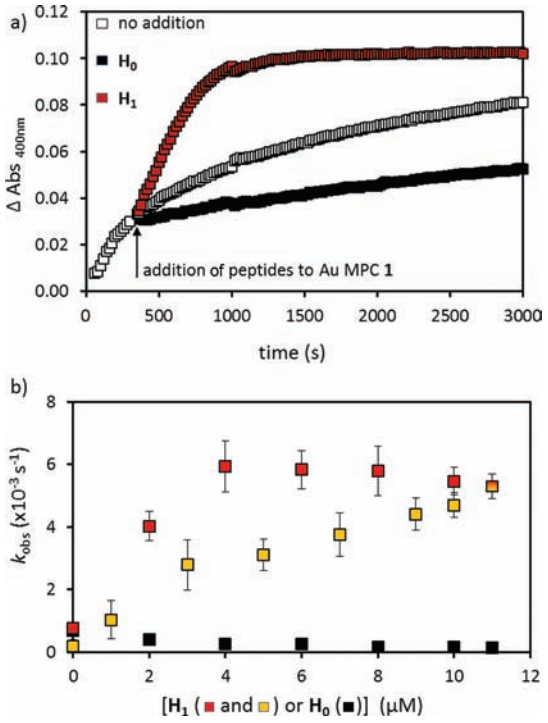

Figure 5. (a) Changes in the absorbance at 400 nm as functions of time for the experiments in which either $\mathbf{H_0}$ (11 μM, black ■) or $\mathbf{H_1}$ (11 μM, red ■) was added 300 s after mixing of Au MPC **1** (60 μM, □) and **Cbz-Phe-ONP** (10 μM). Conditions: [HEPES] = 10 mM; H_2O/CH_3CN = 90:10; pH 7.0; T = 37 °C. (b) Observed rate constants (k_{obs}) as functions of the concentrations of peptide $\mathbf{H_1}$ (red and yellow ■) or $\mathbf{H_0}$ (black ■) at a constant concentration of Au MPC **1** (60 μM) and **Cbz-Phe-ONP** (10 μM). The yellow symbols were obtained in the experiment performed with [$\mathbf{H_0}$] + [$\mathbf{H_1}$] held constant at 11 μM. Conditions: [HEPES] = 10 mM; H_2O/CH_3CN = 90:10; pH 7.0; T = 37 °C.

performing the measurements in the presence of a compensating amount of $\mathbf{H_0}$ (i.e., [$\mathbf{H_0}$] + [$\mathbf{H_1}$] held constant at 11 μM; yellow ■ in Figure 5b). In this case, the increase in k_{obs} as a function of [$\mathbf{H_1}$] was linear over the full interval explored, but the slope was smaller than that observed in the first part of the previous plot, accounting for a lower deprotonated imidazole concentration in this case. Also, the observation that the reaction rate decreased when the pH was lowered (see the SI) is consistent with this explanation, since a cooperative mechanism would have led to an increase in the rate resulting from the presence of both protonated and nonprotonated imidazoles. Similar changes in the reactivity of imidazoles because of a different local chemical environment have also been observed in other multivalent systems.[26,27]

The above experiment indicates that the increase in local pH due to the cationic monolayer can explain the higher activity of $\mathbf{H_1}$ at lower surface loadings but not at the surface saturation concentration, where this effect is largely suppressed. A final piece of information came from measurements of the initial reaction rate as a function of **Cbz-Phe-ONP** concentration (Figure 6). Although the solubility of the substrate permitted only a relatively small range (1−10 μM) to be studied, the nonlinearity of the plot of the initial rate versus substrate concentration gave clear evidence of substrate binding to Au MPC **1**. We hypothesize that this binding is driven by hydrophobic interactions between the highly apolar substrate and the hydrophobic part of the monolayer. Interactions of this

dx.doi.org/10.1021/ja302754h | *J. Am. Chem. Soc.* 2012, 134, 8396−8399

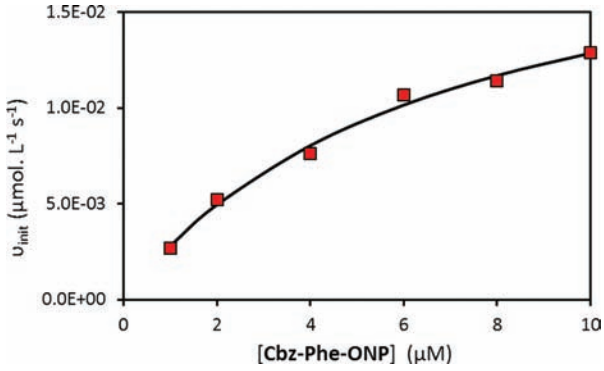

Figure 6. Initial rate as a function of the concentration of **Cbz-Phe-ONP**. Conditions: [Au MPC **1** headgroup] = 60 μM; [$\mathbf{H_1}$] = 10 μM; [HEPES] = 10 mM; H_2O/CH_3CN = 90:10; pH 7.0; T = 37 °C.

type between small molecules and SAMs have also been reported by Mancin and co-workers.[28] The contemporaneous binding of both $\mathbf{H_1}$ and the substrate on the multivalent surface of Au MPC **1** at low micromolar concentrations results in an enormous increase in the effective molarity, which, along with the above-mentioned pH effect, appears to be the main reason for the observed high catalytic activity.

In conclusion, we have developed a supramolecular catalytic system formed through the self-assembly of small peptides on the surface of Au MPCs. Self-assembly is a prerequisite for the catalysis, since the catalytic peptides do not display any activity in the absence of Au MPCs. Assembly on the surface of Au MPC **1** results in a rate acceleration of at least 2 orders of magnitude in a transesterification reaction. The multivalent surface is essential for bringing the substrate and catalyst into close proximity but, importantly, also generates a local chemical environment that enhances the reactivity of the catalytic unit. In this way, the system mimics some of the key features of enzymes. The system presented here allows for a series of exciting possibilities, such as the possibility of fine-tuning the catalytic properties simply by altering the catalytic peptide sequence or the possibility of turning the catalytic activity on or off in situ as a result of the addition of inhibitors/activators. In our opinion, this approach has the potential to be exploited for the development of synthetic systems able to match up to the complexity displayed by enzymes simply by combining various small fragments.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of $\mathbf{H_0}$−$\mathbf{H_3}$, procedures for the kinetic experiments, background rates and control experiments, titration with bromothymol blue, and reaction rate as a function of pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Alessandro Cazzolaro is gratefully acknowledged for the synthesis of Au MPC **1**. Financial support from the ERC (StG-239898), COST (CM0703 and CM0905), and MIUR is acknowledged.

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