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Nanoparticle Assembly

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Peptide-Directed Synthesis and Assembly of Hollow Spherical CoPt Nanoparticle Superstructures**

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Controlling the directed assembly of nanoparticles into well-defined nanoparticle superstructures is a significant challenge,[1] and our goal is to develop a general methodology to address this issue. The success and broad applicability of a particular nanoparticle-assembly methodology should be assessed according to the following important criteria: 1) diverse structural scope, 2) ability to tune and tailor superstructure metrics (e.g., nanoparticle size, nanoparticle shape, interparticle distances, and superstructure diameter), and 3) diverse compositional scope. Only through rational control of structure, metrics, and composition can diverse collections of nanoparticle superstructures with highly specific and tailorable properties ultimately be designed and prepared.

We recently introduced and developed a peptide-based methodology for directing the synthesis and assembly of gold nanoparticles.[2] This methodology relies on carefully designed peptide-conjugate molecules that control the synthesis of individual nanoparticles and direct their assembly into complex nanoparticle superstructures. We successfully demonstrated that this methodology addresses criteria 1 and 2 listed above. Specifically, we used this methodology to prepare a diverse set of complex gold nanoparticle superstructures, including double helices,[2] linear belts,[3] and hollow spherical structures,[4] and to tune the metrics of the superstructures.[5] In this contribution, we begin to address criterion 3 listed above.

Peptide conjugates are the centerpiece molecules in this methodology. They consist of two components: an inorganic-binding peptide and an organic moiety tethered to the peptide terminus. The peptide portion binds to the nanoparticle surface while the organic moiety influences the assembly of the peptide and therefore the assembly of the nanoparticles. Numerous peptides exist which have been evolved and selected, naturally or unnaturally, to adhere to specific inorganic surfaces.[6]

Thus far, we have only utilized a gold-binding peptide in this methodology.[7] In principle, however, we could choose any inorganic-binding peptide, which would allow us to target and prepare nanoparticle superstructures of variable composition.

Here, we introduce, describe, and demonstrate how our peptide-based methodology can be adapted to target and prepare CoPt nanoparticle superstructures. It is established that CoPt nanoparticles are potentially useful in nanomedicine as magnetic resonance imaging (MRI) contrast agents[8] and as electrocatalysts.[9] For these applications, individual CoPt nanoparticles are typically employed. We sought to assemble CoPt nanoparticles into hollow spherical sub-100 nm superstructures, reasoning that they may ultimately be useful as multifunctional bionanomaterials capable of serving both as MRI imaging[8] and delivery[10] agents (e.g., drug, biomolecule, labeling, etc.) or as high surface area electrocatalysts.[9] As a first step in the latter direction, we show herein that the assembled CoPt superstructures can serve as electrocatalysts for the oxidation of methanol.

To prepare hollow spherical CoPt nanoparticle superstructures, we first selected the Co-binding peptide HYPTLPLGSSTY (Co1-P10), which was isolated by Naik et al.[11] and is hereafter referred to as PEP_{Co}. PEP_{Co} was used previously by Naik et al. to prepare both Co nanoparticles and CoPt alloy nanoparticles.[11] The reported syntheses are straightforward and are performed in aqueous media at a neutral pH value. In order to prepare hollow spherical superstructures, we decided to utilize the conjugate BP-PEP_{Co} ($C_{12}H_9$CO-HYPTLPLGSSTY, BP = biphenyl; see the Supporting Information for synthetic details), because our previous results with biphenyl-based PEP_{Au} conjugates resulted in hollow spherical gold nanoparticle superstructures.[12] BP-PEP_{Co}, upon dissolution and subsequent incubation in HEPES buffer (0.1 M, HEPES = 4-(2-hydroxyethyl)-piperazineethanesulfonic acid), assembles into well-defined spherical structures ((30.9 ± 4.5) nm), as evidenced by transmission electron microscopy (TEM) studies (Figure S3 in the Supporting Information).

Encouraged by these results, we next adapted the reported synthesis for CoPt nanoparticles by replacing PEP_{Co} with BP-PEP_{Co} to determine whether BP-PEP_{Co} could serve the dual purpose of directing both the synthesis of CoPt nanoparticles and their assembly into spherical superstructures (Scheme 1). Specifically, a 6.5:1 mixture of cobalt acetate and BP-PEP_{Co} was incubated for four hours at room temperature in HEPES buffer to give a colorless solution. Thereafter, aliquots of sodium borohydride

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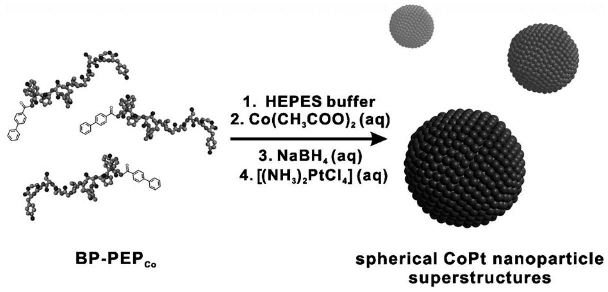

Scheme 1. Preparation of hollow spherical CoPt nanoparticle superstructures (small gray spheres = CoPt nanoparticles).

($NaBH_4$) and ammonium tetrachloroplatinate [$(NH_4)_2(PtCl_4)$] were added in sequence. Black precipitate appeared in the solution immediately after the addition of $NaBH_4$, indicating the reduction of the cobalt and platinum ions. After the reaction, a sample of the precipitate was collected and analyzed using transmission electron microscopy (TEM).

Examination of the TEM images indicated the formation of spherical nanoparticle superstructures ((53.9 ± 7.9) nm; Figure 1 a–c and Figure S4) that consist of individual nanoparticles ((3.3 ± 0.5) nm; Figure S5). These structures have dark edges and light cores, which suggests the formation of "hollow" spherical nanoparticle assemblies. The average diameter of the lighter inner core was (29.5 ± 5.5) nm (Figure S5), consistent with the size of the self-assembled peptide structures. Larger hollow spheres were constructed by halving the concentration of BP-PEP$_{Co}$ in the synthesis (Figure S6), thus indicating that the dimension of the CoPt nanoparticle superstructures can possibly be tuned using this methodology. Energy-dispersive X-ray spectroscopy (EDS) showed a Co:Pt ratio of 45:55 for the assembled superstructures (Figure 1 d). To further confirm the composition of the individual nanoparticles, we used high-resolution TEM to measure their atomic lattice spacing. The measured distance (0.218 nm; Figure 1 e) is consistent with reported results[11,13] as well as X-ray diffraction data[14] (Figure S7) collected for the assembled superstructures. When we performed the same reaction using PEP$_{Co}$ instead of BP-PEP$_{Co}$, random aggregates composed of individual nanoparticles ((2.61 ± 0.43) nm) were observed (Figure S8).

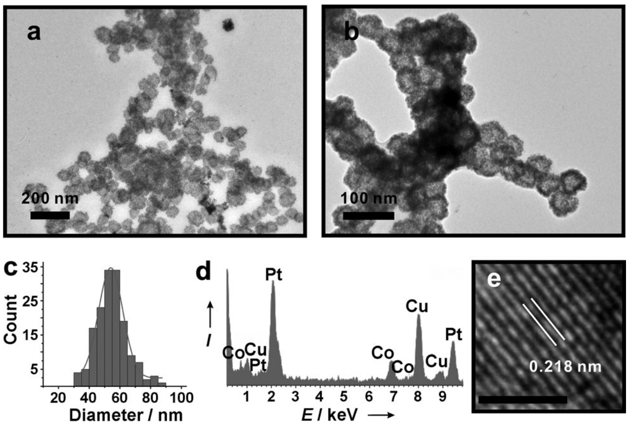

Figure 1. a, b) TEM images of hollow spherical CoPt nanoparticle superstructures. c) Diameter distribution of CoPt nanoparticle superstructures ((53.9 ± 7.9) nm; based on 158 counts). d) EDS data for CoPt nanoparticle superstructures (Cu is from the TEM grid). e) HRTEM of a single CoPt nanoparticle showing the lattice fringes (scale bar: 2 nm).

Other groups reported the synthesis of continuous CoPt nanoshells through a galvanic displacement reaction of Co nanoparticles with Pt salts.[14–15] Our superstructures are distinguished from these reported materials, because they are composed of non-fused discrete nanoparticles. However, given their similarity to the reported nanoshells, we decided to confirm that our structures were indeed the product of a peptide-conjugate-directed synthesis rather than a galvanic displacement reaction. Specifically, we performed the synthesis described above in the absence of BP-PEP$_{Co}$. After the addition of $NaBH_4$, we observed, by TEM, the formation of large Co nanoparticles ((99.5 ± 19.0) nm; Figure S9). The addition of [$(NH_4)_2(PtCl_4)$] to the reaction mixture resulted in the formation of large hollow structures consisting of a continuous shell ((96.7 ± 7.4) nm; Figure S9), which were similar to those reported by Schaak et al.[14] These results are markedly different from what we observed for our synthesis. Based on these comparison studies, we conclude that our structures are formed through BP-PEP$_{Co}$-directed synthesis and assembly rather than galvanic displacement. Detailed studies of the mechanistic aspects of superstructure formation are in progress and will be reported in due course.

Peptide-capped Pd nanoparticles have been successfully utilized for catalysis.[16] We envision that the 'hollow' spherical CoPt nanoparticle superstructures reported herein could potentially be used as magnetically separable reaction vessels for heterogeneous catalysis.[9a,15,17] When placed in a magnetic field, the superstructures can be drawn to the wall of a reaction flask (Figure S10). The superstructures can also serve as methanol oxidation catalysts. We monitored the oxidation of methanol in a cyclic voltammetry (CV) experiment (Figure 2). When the surface of a glassy carbon disk electrode was loaded with the hollow CoPt nanoparticle superstruc-

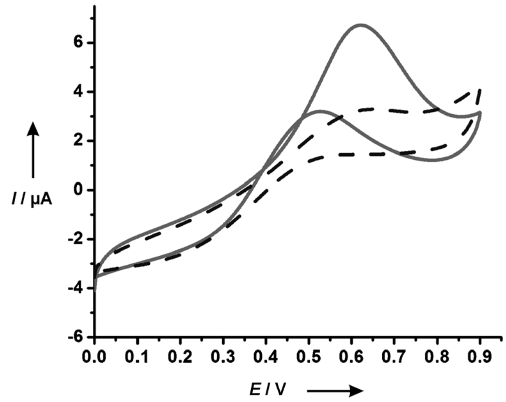

Figure 2. Cyclic voltammograms of hollow spherical CoPt nanoparticle superstructures (solid line) and CoPt nanoparticles (dashed line) in H_2SO_4 (0.5 M) containing methanol (0.6 M). Note: sweep rate = 0.05 V s^{-1}; loading ≈ 7.5 μg.

tures, significant anodic current (6.72 μA) was observed with an oxidation peak at 0.62 V (vs. Ag/AgCl), which is consistent with reported values for the oxidation of methanol.[18] In comparison, only weak anodic current (1.94 μA) was observed when the electrode was instead coated with an identical loading of PEP_{Co}-capped CoPt nanoparticles (Figure 2). To confirm that the CoPt superstructures and CoPt nanoparticles remain intact in the H_2SO_4/MeOH solutions used for the electrocatalysis experiments, we soaked samples of the CoPt superstructures and CoPt nanoparticles in a H_2SO_4/MeOH solution and then examined the structures using TEM. We found that the superstructures remained intact and retained their spherical shape. The CoPt nanoparticles also remained intact. However, the Co:Pt ratio of the superstructures changed from 45:55 to 20:80 after soaking in the acidic methanol, as evidenced by EDS (Figure S11); clearly, Co leached from the structures in the acidic environment. EDS showed that the Co:Pt ratio of the PEP_{Co}-capped nanoparticles (22:78) is similar to that of superstructures after soaking in the acidic methanol (Figure S12). We note that Co leaching in acidic media will affect the magnetic properties of the superstructures and may therefore affect our ability to magnetically separate them from solution. However, not all potential catalytic reactions require acidic conditions, and as long as a significant percentage of the Co remains, the catalysts should remain magnetically separable. Our data suggest that CoPt nanoparticles assembled into hollow spherical superstructures exhibit greater electrocatalytic activity toward the oxidation of methanol compared to non-assembled CoPt nanoparticles. Our current work is focused on examining this enhanced activity in more detail through both tuning and controlling the diameters of the assembled structures and by developing an understanding of the mechanism of the electrocatalytic activity.

We have demonstrated that designed peptide-conjugate molecules can be used to direct the synthesis and assembly of CoPt nanoparticles into hollow spherical magnetic superstructures, which can serve as catalysts for the oxidation of methanol. This work is important from a methodology standpoint because it demonstrates that functional nanoparticle superstructures of arbitrary composition can be targeted and prepared, provided that the proper peptide is identified and the proper peptide conjugate is designed.

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