Compact Peptoid Molecular Brushes for Nanoparticle Stabilization

Shih-Ting Wang, Honghu Zhang, Sunting Xuan, Dmytro Nykypanchuk, Yugang Zhang, Guillaume Freychet, Benjamin M. Ocko, Ronald N. Zuckermann, Nevena Todorova, and Oleg Gang*

**ABSTRACT:** Controlling the interfaces and interactions of colloidal nanoparticles (NPs) via tethered molecular moieties is crucial for NP applications in engineered nanomaterials, optics, catalysis, and nanomedicine. Despite a broad range of molecular types explored, there is a need for a flexible approach to rationally vary the chemistry and structure of these interfacial molecules for controlling NP stability in diverse environments, while maintaining a small size of the NP molecular shell. Here, we demonstrate that low-molecular-weight, bifunctional comb-shaped, and sequence-defined peptoids can effectively stabilize gold NPs (AuNPs). The generality of this robust functionalization strategy was also demonstrated by coating of silver, platinum, and iron oxide NPs with designed peptoids. Each peptoid (PE) is designed with varied arrangements of a multivalent AuNP-binding domain and a solvation domain consisting of oligo-ethylene glycol (EG) branches. Among designs, a peptoid (PES) with a diblock structure is demonstrated to provide a superior nanocolloidal stability in diverse aqueous solutions while forming a compact shell (~1.5 nm) on the AuNP surface. We demonstrate by experiments and molecular dynamics simulations that PES-coated AuNPs (PES/AuNPs) are stable in select organic solvents owing to the strong PES (amine)–Au binding and solubility of the oligo-EG motifs. At the vapor–aqueous interface, we show that PES/AuNPs remain stable and can self-assemble into ordered 2D lattices. The NP films exhibit strong near-field plasmonic coupling when transferred to solid substrates.

**INTRODUCTION**

Colloidal nanoparticles (NPs) have demonstrated wide functionality in electronics,1 optoelectronics,2 spintronic devices,3 catalysis,4,5 and nanomedicine.6 To control NP stability, degree of dispersion, chemical functionalization, interaction, and self-assembly behavior, a wide range of molecular coatings, including polymers,6,7 surfactants,8 ionic liquids,9,10 and biomacromolecules (e.g., peptides11,12 proteins,13 DNA,14,15 aptamers,16−20 and polysaccharides21−24), have been developed.25−27 Despite these advancements, flexible variation of NP coating in a systematic and pre-determined way while maintaining minimal thickness remains limited. The development of new classes of stabilizing agents with such properties may enable multifunctional NPs, stability in different media, and compatibility with biomedical and green industrial processes.

Materials for NP stabilization primarily consist of two functional domains for creating an ionic or physical barrier between NPs: (1) an NP-binding domain and (2) a solvating domain suitable for target solvents. Extensive studies on the synthesis and functionalization of NPs have provided rationales for tailoring the compositions and surface structures of coating materials for the preparation of stable NP systems,27,28 and robust NP stabilization has been achieved by the control of grafting density and size (e.g., degree of polymerization) of covalently and linearly tethered chains.29−32 However, it remains a challenge for the current approaches to establish a structure–property (i.e., stability and functionality) relationship, in particular, due to the complex interactions of coating materials with both NPs and solvents, intrinsic polarity and conformational preferences of the molecular backbone, and varied surface characteristics derived from the shape of the attached molecular moieties. Thus, there is a need for materials that can be designed with defined molecular architectures that enable precise controls over the NP and solvent interfaces.

Stable NPs in physiological media or organic solvents typically feature a high surface grafting density and high molar mass of the coating molecules, and for linear polymers, formation of a densely packed “brush” shell is accompanied by an overlay of adjacent chains and stretching orthogonally to the NP surface.31,33 However, this polymer topology may

**Received:** January 20, 2022  
**Published:** April 22, 2022
Here, our aim is to synthesize and characterize a novel class of sequence-defined branched oligomers for stabilizing NPs in varied solution environments and at the interfaces while minimizing a molecular shell thickness (Figure 1). We designed several comb-branched architectures that mimic the chemical properties of poly(ethylene glycol) (PEG), a widely used neutral, solvophilic synthetic polymer for a variety of materials and biomedical applications. To implement control over the sequence, length, and molecular structure of our molecular stabilizers for colloidal NPs, we employed peptoids, oligomers of N-substituted glycine, which offer high designability via controlled and efficient synthesis processes. Compared to natural peptides, peptoids’ side chains are appended to the backbone nitrogen atoms rather than the α-carbons. The absence of hydrogen-bond donors and chiral centers along the backbone of peptoids implies that the peptoids’ main chains are more flexible than peptides’, which makes them less likely to adopt preferentially folded conformations through intramolecular hydrogen bonds. Previously, peptoids have shown promise to passivate NPs and surfaces and confer effective biofouling resistance. Recently, we demonstrated a series of bifunctional peptoids, with polycationic character and branched oligo-EG motifs, for stabilizing negatively charged DNA nanostructures in ionic solutions. We showed that these peptoids bind strongly to DNA and that the EG motifs stabilized both duplex DNA and 3D nanostructures without altering their shape. Inspired by these findings, here, we explore this class of branched peptoid architectures for controlling the size, stability, surface properties, and interfacial assembly of gold NPs (AuNPs).

Our peptoid sequences comprised two types of functional monomers with primary amine and triethylene glycol (TEG) side chains in different numeric ratios and arrangements (Figures 1 and 2). We selected citrate-capped AuNPs (5–20 nm in diameter) for our study since they have been extensively investigated in biological and chemical sensing and optical applications. Amines and heterocyclic nitrogens are known to bind to Au surfaces via weak covalent bonds and to the citrate adlayer via electrostatic interactions, providing a rapid and simple attachment method. The affinity to AuNPs is also known to increase with the number of amines per chain. Thus, to stabilize AuNPs in compact surface coating, we designed peptoids with short-chain lengths. Here, the comb-branched architectures were composed of 6 or 12 primary amines, inspired by consecutive polycationic (e.g.,...
histidine) residues in nature, and based on considerations related to minimizing distances between NP surfaces. The selected designs are also suitable for exploring multidentate interactions with AuNPs and for achieving a high density of ether motifs on AuNP surfaces in aqueous and organic solvents and at the vapor–aqueous interface, as required for the stabilization. Such thin shells can perform comparably to AuNPs where the shells are formed by covalently grafted PEG of an unbranched and linear structure. To compare NP stability and functionality using linear PEG and branched EG, we selected widely used and commercially available thiolated PEG (avg. $M_n = 2000$ g/mol, $\sim 47$ EG per chain) with a linear architecture and with similar numbers of EG per chain to our designed peptoids (Figure 1).

As we will discuss below, certain designs of peptoids (PEs) form a thin coating layer on AuNP surfaces and are able to stabilize the NPs in various aqueous environments. This high stability of PE-coated AuNPs (PE/AuNPs) is also realized in organic solvents through phase transfer, and the cognate conformational preferences and mechanisms of interactions of PEs in different solvents are studied using molecular dynamics (MD) simulations. Beyond bulk solutions, we further probe PE/AuNP stability and self-assembly properties at the vapor–aqueous interface using in situ specular X-ray reflectivity (XR), grazing-incidence small-angle X-ray scattering (GISAXS), and modeling. Plasmonic properties of the assembled films transferred to solid substrates are analyzed. Our study illustrates the potential of this class of designable and

Figure 2. Concentration-dependent interaction of designed peptoids with citrate-capped gold nanoparticles (AuNPs). (a) Peptoid architectures in this study. Nae: N-(2-aminoethyl)glycine, Nte: N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)glycine, and Nme: N-(2-methoxyethyl)glycine. (b–e) UV–vis analysis of 10 and 20 nm AuNPs mixed with peptoids (PEs) in phosphate buffer (PB, 10 mM, pH 7.0). (b) Spectra of 10 nm AuNPs (7.5 nM) with PE at 12.5 $\mu$M. (c) Plot of absorbance ratios at 520 and 620 nm ($A_{520}$/$A_{620}$) of 10 nm AuNPs for varied PE concentrations. (d) Spectra of 20 nm AuNPs (0.75 nM) with PE at 12.5 $\mu$M. (e) Plot of $A_{520}$/$A_{620}$ of 20 nm AuNPs for varied PE concentrations. In (c) and (e), results were averaged from three independent experiments. Expanded views of $[\text{PE}] = 0.03-12.5$ $\mu$M are plotted in Figure S1.
structurally well-defined peptoid molecules for providing stabilized NPs with broad application prospects.

**RESULTS AND DISCUSSION**

**Peptoid Design and Interactions with Gold Nanoparticles (AuNPs).** Our designed peptoid (PE) had a comb-branched architecture with regularly spaced side chains consisting of positively charged N-(2-aminoethyl)glycine (Nae) monomers for binding to the AuNP surface and the citrate adlayer and neutral N-2-(2-methoxyethoxy)ethoxyethylglycine (Nte) monomers that contained TEG moieties for shielding the AuNP surfaces (Figure 2a).

Two types of PE architectures were used to coat AuNPs, including brush types (PE1: (Nae−Nte)$_6$, PE2: (Nae−Nte)$_{12}$, PE3: (Nae−Nte−Nme−Nte)$_6$, Nme: N-(2-methoxyethyl)glycine), where charged Nae and neutral Nte moieties were arranged alternately, and block types (PE4: (Nae$_6$−Nte$_6$), and PE5: (Nae$_6$−Nte$_{12}$)), where the Nae and Nte moieties were arranged in separate blocks. To compare the strength of PE–AuNP interaction, we also designed PE6 (Nme$_6$−Nte$_{12}$) that only had a single amino group at the N-terminus.

We first analyzed PE–AuNP interactions by assessing the NP aggregation in aqueous solutions. The peptoids (PE1–PE5) of varying concentrations were mixed with 10 nm (7.5 mM) and 20 nm (0.75 nM) AuNPs in phosphate buffer (PB, 10 mM, pH 7.0), and the optical red shift, as an indication of NP aggregation in the AuNP optical absorption spectra, was monitored. We note that the pristine AuNPs prior to PE coating are stabilized by citrate, which contains carboxyl groups weakly interacting with the Au surfaces, and these pristine AuNPs are denoted as “uncoated”. The PEs can exchange with citrate ligands over time, and this results in PE-coated AuNPs.

Aggregation behavior was parametrized by the absorbance ratios between 520 and 620 nm ($A_{520}/A_{620}$), where the ratio is ~2.5 for stable NPs and ~1 for irreversibly aggregated NPs. Figure 2b,c shows that 10 nm AuNPs remained stable with peptoids present, except for PE2, which caused aggregation at 0.1–3 μM, as indicated by a red-shift of the surface plasmon resonance, which was likely due to a larger number of amino groups per molecule. Further increase in the PE2 concentration appeared to saturate the adsorption, exhibiting polyelectrolyte behavior, likely due to screening of the AuNP surface charge.

Larger AuNPs (20 nm), which had a stronger core−core van der Waals attraction (Figure S2), were more prone to aggregation after PE coating than the 10 nm AuNPs (Figures 2d,e and S1). Brush-type PE1 and PE2 showed similar aggregation behavior with PE2 bearing 12 Nae monomers aggregating NPs at lower concentration (~50 nM, Figure S1). Compared to PE1 and PE2, increasing the number of Nte relative to Nae in PE3 reduced the extent of aggregation, as indicated by an increased $A_{520}/A_{620}$ (Figure 2e). Similarly, in the block-type peptoids (PE4 and PE5), increasing from 6 to 12 Nte in PE5 effectively inhibited NP aggregation across a wide range of concentrations, suggesting that PE5 was best suited for stable binding and steric repulsion for stabilizing AuNPs.

**Stability of Peptoid (PE5)-Coated AuNPs in Aqueous Solutions.** We further investigated the properties of PE-coated AuNPs in varied aqueous solutions. Although for each designed PE, the PE to NP ratios and salt concentrations can be explored, we selected PE5-coated AuNPs (PE5/AuNPs) for further detailed investigation due to its highest colloidal stability it shows for AuNPs among our designs (Figure 2). We first determined the PE5 grafting density on citrate-capped AuNPs (10 nm) by monitoring free PEs in the coating reaction (methods are detailed in Figure S3). In brief, different PE5 concentrations (2.63–37.5 μM) were mixed with AuNPs (10 nm, 7.5 nM), and the unbound PE5 was removed by centrifugation and quantitated using 4-phenylsulfonfuran-2(3H,1'-phthalan)-3,3'-dione (or fluorescamine$^{79}$). The emission intensity of fluorescent derivatives formed by fluorescamine reaction with the primary amines of Nae and the N-terminus showed a linear relationship with increasing PE5 concentration. The grafting density of PE5 was estimated to be ~0.12 chains/nm$^2$ (Figure S3). To enable the long-term stability of PE5-coated AuNPs, we used a higher starting PE5 concentration for AuNP coating and removed excess by centrifugations (Figure S4).

We next coated PE5 onto different sized (i.e., 5, 15, and 20 nm) citrate-capped AuNPs in addition to the 10 nm AuNPs discussed above (Figure 3a,b). In all cases, the absorption spectra showed a 1–5 nm red shift compared to uncoated NPs, indicating successful PE5 adsorption on AuNP surfaces (Figure S5). Remarkably, a consistent PE5 shell thickness ($h$) of 1–1.5 nm was observed for these AuNPs, as determined by dynamic light scattering (DLS) and confirmed by negative-stained transmission electron microscopy (TEM) imaging (Figure 3).

This reveals the formation of a compact PE5 shell for all sizes of AuNPs studied. PE5 shielding of NP surface charge was also...
confirmed by ζ-potential measurements, showing neutralization of the negative AuNP charge (Table S1).

The high stability of PES/AuNPs is thought to result from the high EG density (36 EG units presented in 12 Nte monomers per PES chain) forming a compact shell. To compare the stability to a linear PEG chain, we grafted AuNPs monomers per PEG chain) forming a compact shell. To compare the stability to a linear PEG chain, we grafted AuNPs (10 nm) with thiolated PEG (avg. M = 2000 g/mol, equivalent to ~47 EG per chain), using a similar preparation procedure as that of PES/AuNPs (see the Supporting Information). Compared to PES/AuNPs, PEG2k/AuNPs were also stable but formed a thicker shell (h ~5 nm), as determined by DLS (Figure 3c) and consistent with previous studies.80 Negative-stained TEM imaging also confirmed a compact PES layer (1.4 ± 0.2 nm, 65 NPs) and a larger cloud-like shell for PEG2k/AuNPs around the 10 nm NP cores (Figures 3d,e, S6, and S7).

Since EG moieties are known to impart protein resistance by creating a stable solvation layer on surfaces and preventing protein attachment,81 we examined this property by comparing PEG2k- (only unbranched EG chains) and PES-coated AuNPs. We selected bovine serum albumin (BSA) and lysozyme, which are abundant proteins in serum and secretions, respectively, and known to bind strongly to AuNPs.82 Meanwhile, PEG2k/AuNPs completely prevented proteins from adsorption, and the repelling effects of PE5 coating showed neutralization of the negative AuNP charge (Table S1).

Figure 4. Stability of PES/AuNPs in varied ionic environments. Effect of PES coating on the stability of AuNPs (10 nm) in the presence of (a) different heavy metal ions (2 mM) and (b) monovalent and bivalent salts (50 mM). (c, d) Stability of PES/AuNPs in the presence of different concentrations of NaCl. In (d), the plot of the absorbance ratio A320/A620 versus NaCl concentrations showed no aggregation. The “+” and “−” signs in the inset photographs denote the presence and absence of PE5, respectively. (e, f) Stability of PES/AuNPs in PB (10 mM) at different pH values. In (f), the plot of A320/A620 versus pH values showed no aggregation between pH 3 and 13, while aggregation was observed at pH 1 and 2 due to spontaneous protonation of the positively charged groups.

The stability of the PE5/AuNPs to pH was demonstrated by monitoring their UV−vis spectra in PB solutions ranging from pH = 1 to 13 for 1 h. Figure 4e,f shows that PE5/AuNPs remained stable above pH = 3, while aggregation was observed at pH = 1 and 2, likely due to protonation of the amino groups that resulted in desorption of PE5 from the AuNP surface. Finally, we showed that PE5/AuNPs remained stable after freezing at −20 °C and thawing at room temperature owing to the EG motifs, which are known to inhibit formation of ice...
crystals, while uncoated AuNPs exhibited an irreversible aggregation (Figure S11).

**Aqueous-to-Organic Phase Transfer of PE5/AuNPs.**

Beyond stabilization in aqueous solutions of various ionic environments, we explored the stability and solvation properties of PE5/AuNPs in organic solvents, as repeating EG motifs are known to have dual solubility in water and certain organic solvents.91,92 The ability to synthesize AuNPs in their optimal solvent (aqueous) and use them in organic solvents is desirable for simplifying NP synthetic steps and for use, for example, in NP recycling and catalysis.93 We selected EG compatible solvents of varied polarity indices (P), such as chloroform (P = 4.1), dichloromethane (P = 3.1), and toluene (P = 2.4).94

To allow an aqueous-to-organic phase transfer, the addition of a common solvent such as methanol that is miscible in both phases is required to reduce tension at the organic/water interface.95,96 Methanol can also compete with water to form hydrogen bonds with EG moieties and subsequently decrease an energy barrier for NP migration to the organic phase, as reported previously,97 which was observed by our molecular dynamics (MD) simulations described below. Here, our experiments showed that PE5/AuNPs in PB (10 mM, pH 7.0) could be transferred to chloroform, dichloromethane, and toluene in the presence of methanol, as evidenced by the solution color change (Figure 5a). We focused on the chloroform–methanol–PB system due to the high solubility of EG in chloroform.92,97

We first estimated the partition coefficient of free PE5 (without NP) using the fluorescamine assay (Figure S12). In the absence of methanol, ~10% of PE5 partitioned in chloroform, while ~90% of PE5 equilibrated in the organic phase with methanol present (chloroform/methanol/PB = 1:1:1 v–v:v) despite the charged Nae motifs, suggesting that PE5 was more soluble in chloroform via the Nte domain. We note that methanol was mostly partitioned in the aqueous phase, as indicated by the small change of chloroform volume (measured by relative heights, Figure S13) and agreed with the phase diagrams in previous reports.

To explore the role of methanol in the PE5/AuNP phase-transfer system, we added varied volumes of methanol in a mixed solvent of PB and chloroform (1:1 v–v) (Figures 5b and S14). We measured up to 50 vol % of methanol, where PB and chloroform were no longer miscible (i.e., the biphasic regime), and PE5/AuNPs partitioned in the water-rich and chloroform-rich phases. Figures 5b and S14 show that PE5/AuNPs migrated to chloroform with 20–45 vol % methanol while partial or no transfer at or below that; in this regime, UV–vis-detected NPs dispersed in chloroform without

---

**Figure 5.** Aqueous–organic phase transfer of PE5/AuNPs. (a) Photographs showing migration of PE5/AuNPs from PB (10 mM, pH 7.0) to organic solvents in the presence of methanol (MeOH) at equal volumes. PB: phosphate buffer, CHCl3: chloroform, and DCM: dichloromethane. (b, c) Photographs of phase transfer of PE5/AuNPs from PB to CHCl3 by (b) adding a varied amount of MeOH, followed by the (c) addition of sodium chloride (NaCl, 100 mM). Brown arrows indicate complete phase transfers, and light-yellow arrows indicate partial PE5/AuNP transfers. (d) Truncated CHCl3–MeOH–H2O phase diagram98 is plotted for our obtained data, where a black dashed line separates the monophasic and biphasic regimes, showing AuNP phase transfer occurred in the biphasic regime and required at least 10 wt % of MeOH (red dashed line) for PE5/AuNPs to migrate across the liquid–liquid interface.
aggregation (Figure S15). We also observed that phase transfer could be achieved using as low as 5 vol % methanol by adding NaCl (100 mM) (Figures 5c and S14). This was attributed to the interruption of hydrogen-bond networks (i.e., salt−solvent competing with PE5−solvent) in the aqueous phase and suggested that the PE5 solubility could be mediated by salt.

We further varied compositions of the ternary solvent mixture and plotted in Bligh’s and Dyer’s chloroform−methanol−water phase diagram (shown in wt %) (Figure 5d). We note that the studied PE5/AuNP systems contained a low concentration of phosphate (10 mM, denoted as PB) in the aqueous phase to maintain pH = 7. At compositions with low methanol, particularly below x:0.5:y:v:w:v, where x and y are volumes of chloroform and PB, respectively, no phase transfer was observed and PE5/AuNPs appeared unstable. Our results suggested that at least 10 wt % of methanol was required for maintaining the ternary solvent mixture in a biphasic regime while lowering the chloroform−PB interfacial tensions for the PE5/AuNP phase transfer. This amount of methanol was reported to significantly lower (by ∼40−50%) interfacial tensions of several binary organic liquid−water systems (e.g., benzene and pentane, which have interfacial tensions with respect to water without methanol of ∼34 and ∼49 mN/m, respectively, close to chloroform of ∼32 mN/m), supporting our observations.

**Molecular Dynamics (MD) Simulations of PE5 on the Au(111) Surface in Aqueous and Organic Phases.** To further understand the interactions of PE5/AuNP with solvents, we performed all-atom MD simulations of PE5 on the Au(111) surface in water, water/methanol mixture, and chloroform, commensurate to the experimental conditions. The simulations involved a step-wise procedure, where PE5 was first modeled in water and allowed to spontaneously adsorb to the Au(111) surface from solution. Subsequently, PE5/Au(111) was solvated in a water/methanol mixture (1:0.9 v−v)
mixture and finally in chloroform (see the Supporting Information for detail).

In water, PE5 exhibited free adsorption to the Au(111) surface and remained attached due to favorable Nae−Au interactions, where at least three or more Nae moieties (out of six) of single PE5 were bound to the Au surface for 78% of the total 600 ns contact time analyzed. PE5 remained adsorbed to the Au surface in subsequent water/methanol and chloroform with persistent binding of three or more Nae chains for 95 and 74% of the time, respectively. Clustering analysis showed that PE5 is most conformationally dynamic in water among the solvents (Figure S16). Despite the difference in conformational entropy, the most frequently sampled PE5 structures in each solvent shared a similar elongated conformation with the majority of Nte exposed to the solvent (Figures 6a−c and S16). Indeed, the average radius of gyration ($R_g$) of PE5 in each solvent calculated as a measure of the peptoid’s compactness at the Au surface showed similar conformational features and globular shapes (Table S2).

Nonetheless, PE5 exhibited differences in the solvent accessible surface area (SASA) and the interfacial (contact) area with Au(111) between the three phases (Table S2). The average SASA for surface-bound PE5 was calculated at 26.1 ± 1.6 nm$^2$ (water/methanol) < 26.9 ± 2.0 nm$^2$ (water) < 28.2 ± 1.9 nm$^2$ (chloroform). In contrast, PE5 had a larger contact area with Au(111) in water/methanol (9.48 ± 1.04 nm$^2$) compared to water (7.83 ± 1.56 nm$^2$), suggesting that with methanol present, PE5 became less dynamic on the Au(111) surface (Figure S16). Interestingly, PE5 was most solvent-exposed in chloroform while exhibiting a similar contact area (8.09 ± 0.77 nm$^2$) with Au(111) to that in water, confirming favorable solvent interactions for an aqueous-to-chloroform phase transfer observed in Figure 5.

The peptoid−solvent interactions were evaluated using hydrogen-bonding analysis and radial distribution functions.
(RDFs) over the equilibrated regions of each trajectory (Figures 6d, 6e, S17, and Table S3). The simulations of PES/Au(111) in water and water/methanol showed similar water structuring, where three distinct hydration peaks appeared in both RDFs of PES’s Nte acceptors (O) to solvent hydrogens (H) (Figure 6d) and its Nae donors (NH) to solvent oxygens (O) (Figure 6e). The first two peaks represent the first hydration shell (r ~0.18 and ~0.32 nm, distance from PES(O) or (NH)), and the third peak as the second hydration shell (r ~0.47 nm), indicating hydrogen bonding between PES and both solvents. We note that the overall lower RDF peaks in Figure 6d than in Figure 6e were likely due to the fluctuating solvent exposure and dynamic nature of Nte moieties, compared to the Au surface-bound Nae, causing diminished interactions between PES’s Nte and the solvents. This was supported by a shorter hydrogen-bond lifetime for water and methanol bound to the Nte moieties compared to PES as a whole (Table S3). On average, we observed a higher number of hydrogen bonds formed between PES and water (37.2 ± 3.2) versus water/methanol (31.5 ± 3.1). The higher RDF peaks in water/methanol indicated an increase in solvent density near PES with a longer hydrogen-bond lifetime binding to Nte (Table S3), suggesting methanol’s competitiveness for binding to PES. This competition was also supported by the overlapping initial RDF peaks for the deconvoluted water and methanol interactions with PES (Figure S17).

By replacing water/methanol with chloroform, we observed a significant enhancement in the RDF peak at r ~0.25 nm (Figure 6d), which was attributed to the bifurcated hydrogen bonds (C–H···O bond, 0.19–0.25 nm) formed between the ether oxygens and chloroform, as reported previously. This indicated a strong interaction between chloroform and PES, which was supported by the highest solvent accessible area observed for PES in chloroform. We note that dispersion attractions or steric repulsions might also contribute to the distant interactions between chloroform and PES, due to the large size of a chloroform molecule (Figure 6d). Our results suggested that solvent structuring and accessibility to the PES layer on the Au surface are the key determinants of the favorable condition for aqueous-to-chloroform phase transfer as observed experimentally.

Finally, we used MD simulations to confirm the spontaneous formation of the PES monolayer on Au(111) surfaces in a water environment, with a PES thickness of ~1.3 nm (Figures 6f and S18), in agreement with our experiments (Figure 3). To estimate the monolayer density on the Au(111) surface, peptoids were added until a saturation point was reached. Independently from the starting orientations of PES, the Nae amines ensured binding to Au(111), where a majority of EG motifs remained solvent-exposed. The maximum peptoid density obtained using this approach was 0.08 chains/nm², comparable to a value of ~0.12 chains/nm² (Figure S3) obtained experimentally. Our MD simulations provided the atomistic insights into the conformational features and manifold range of interactions responsible for the experimentally observed NP stability and phase transfer of the peptoid system. We expect future studies will use these findings to rationally tailor the grafting density through peptoid design (e.g., Nae–Nte ratios) and salt modulation.

**Self-assembly of PES/AuNPs into Monolayers of High NP Number Density at the Vapor–Aqueous Interface Probed by In Situ X-ray Scattering.** Beyond investigating the stability of PES/AuNPs in bulk solutions, we studied the interfacial properties of PES/AuNPs by exploring their self-assembly at the vapor–aqueous interface. Two-dimensional NP monolayers spontaneously formed at vapor–aqueous interfaces are free-standing, defect-correcting across macroscopic length scales, and of great interest for both the fundamental understanding of assembly processes and bottom-up fabrications of nanomaterials. Interfacial energies and ligand’s salt-dependent behaviors have been reported to drive the formation of NPs into close-packed monolayers.

Here, we asked whether the PES’s branched oligo-EG motifs would exhibit similar salt-dependent properties as PEG-coated NPs and to what degree the PES shell of AuNPs could influence the formation of interfacial NP organizations. We added salts, including NaCl and PB to the aqueous PES/AuNP solutions, to mediate a subphase and the salt-dependent EG hydrophobicity of the PES coating layer. The self-assembly of PES/AuNPs was probed *in situ* by specular X-ray reflectivity (XR) for determining the surface-normal electron density profile of adsorbed species at the interface, and grazing-incidence small-angle X-ray scattering (GISAXS) for investigating the organization of AuNPs within the plane of the interface. Figure 7a illustrates the setup of this experiment, where aqueous samples of PES/AuNPs were loaded into a rectangular trough.

Figure 7b shows the normalized XR (R(Qz)/R(Q0)) of the vapor–aqueous interface, as measured for PES/AuNPs (20 nM) in water, where Qz is the z-component of the scattering vector (see the Supporting Information), R is the intensity of the reflected beam relative to the intensity of the incident beam as a function of Qz, and R0 is the Fresnel reflectivity of an ideally flat and sharp water surface. The R(Qz)/R(Q0) curve exhibited weak oscillations (or Kiessig fringes), suggesting spontaneous adsorption of PES/AuNPs at the vapor–aqueous interface (i.e., Gibbs monolayer). Adding NaCl (10 mM), phosphate (10 mM, pH 7.0), or a mixture of both salts into the subphase strongly enhanced the reflectivity oscillations, a typical signature of an increased density of adsorbed PES/AuNPs (Figure 7b). The use of phosphate buffer (PB) strongly enhanced the interfacial surface adsorption and self-assembly, as indicated by the first maxima in the R/R0 curves, which increased by a factor of 10.

The interfacial electron density (ED) profiles for PES/AuNPs systems with and without salt were further extracted by fitting XR curves using Parratt’s recursive method using simple density models (see the Supporting Information). The ED profiles were dominated by a stratum at the interface with an extended depth of ~100 Å (Figure 7c), consistent with the size of the 10 nm NP cores, suggesting that PES/AuNPs self-assembled into an NP monolayer. With salts added to the subphase, the peak in the ED at the interface was enhanced, while the layer thickness was preserved, indicating salt-induced additional adsorption of NPs within the monolayer. Indeed, in PB, the average area per NP at the interface (A_{XR}, calculated by a simple space-filling model (see the Supporting Information and Figure S19), decreased from ~870 to ~210 nm² corresponding to about a 4-fold increase in the areal density of the AuNP monolayer.

The in-plane arrangement of the self-assembled PES/AuNP Gibbs monolayers was investigated by GISAXS. For ordered monolayers, the scattering pattern exhibits diffuse rods of scattering along Qz at discrete in-plane vectors Qy. The positions of the peaks along Qy and the peak widths are related to the symmetry and correlation length, respectively.
(see the definition of scattering vectors in the Supporting Information). As shown in Figures 7d and S20, for the systems with PB or NaCl/PB subphases, $Q_{001}$ exhibited sharp peaks at 0.046, 0.080, and 0.092 Å$^{-1}$ with associated diffuse rods of scattering along $Q_{x}$, indicating a scattering pattern characteristic of an ordered interface; thus, NP lattice emerged from these subphases. In contrast, only broad peaks were observed along $Q_{xy}$ using water and NaCl subphases, indicating short-range order. In the case of a water subphase, the broad scattering at $Q_{xy} = 0.022$ Å$^{-1}$ was resulted from an isolated (uncorrelated) surface PE5/AuNP and was used to derive the form factor of surface-bound NPs (sector-shaped and arched features). As noted above, the ordering was significantly improved by salt (NaCl, PB, NaCl/PB) additions, particularly in PB and NaCl/PB, where long-range ordered crystalline structures were manifested by the appearance of high-order diffraction peaks. Such structural evolutions were evidenced by horizontal line-cut profiles from the GISAXS maps (Figure 7e). From the subphase of water to NaCl, to PB and NaCl/PB, the primary peak progressively shifted to higher $Q_{xy}$, suggesting a corresponding decrease in interparticle distance. We note that the interparticle distance in the PE5/AuNP monolayers could also be tailored by varying the NP concentrations (Figure S21). In a typical PB subphase, a scattering profile of the crystalline assembly exhibited peak positions at relative ratios of $Q_{n}/Q_{1} = 1, \sqrt{3}, \sqrt{4}$, and $\sqrt{7}$ with $Q_{1} = 0.0459$ Å$^{-1}$, consistent with a 2D hexagonal lattice with a nearest-neighbor lattice constant of $a_{L} = 15.8$ nm. We estimated the surface coverage of this 2D superlattice by comparing the area per NP in the lattice ($A_{\text{hex}} = \frac{\sqrt{3}a_{L}^{2}}{2}$, see Figure S22) to $A_{\text{XR}}$, a measure of the average coverage. The excellent agreement between $A_{\text{hex}}$ and $A_{\text{XR}}$ suggests that the PE5/AuNP crystalline phase fully covers the liquid surface. We also performed a GISAAXS simulation using a 2D hexagonal NP superlattice model with crystalline domains randomly rotated on the water surface using BornAgain software, and the simulated GISAXS patterns agreed well with our experimental observations (Figure 7).

To elucidate the role of a compact PE5 coating in the interfacial NP self-assembly, we compared the results discussed above to those obtained using PEG2k/AuNPs under the same subphase conditions. Similar to PE5/AuNPs, we observed the formation of Gibbs monolayers and 2D hexagonal superlattices of PEG2k/AuNPs in the presence of salts (Figure S23), consistent with previous works.110,111 Interestingly, the smaller size of PE5/AuNPs (Figure 3c) enabled a larger number of NPs to migrate from the subphase to the interface, leading to a higher surface number density of NPs, higher ED, smaller layer thickness, and smaller lattice constant compared to PEG2k/AuNPs (Figures S24 and S25). For instance, in PB, the surfacetosurface distance between NP cores at the interfaces was $d_{ss} = a_{L} − D = 6.8$ nm for the PE5/AuNP lattice, while $d_{ss} = 15.3$ nm for the PEG2k/AuNP lattice (NP core size $D = 9.0 ± 1.0$ nm, see the Supporting Information and Figure S26). At such a $d_{ss}$ value, the PE5/AuNP lattice approached a saturated interfacial accumulation as no change in the lattice constant was observed with additional NaCl. On the contrary, the PEG2k/AuNP lattice continued to evolve upon adding NaCl, although PEG2k has a similar molecule weight to PE5.

The compact PE5 coating is advantageous, for example, for optical applications of AuNP assemblies since the plasmonic coupling increases significantly with the decrease in $d_{ss}$.116 To illustrate this effect, the 2D assemblies of PE5/AuNPs and PEG2k/AuNPs (AuNP size of 20 nm) were transferred to a solid substrate for optical characterization using a dark-field microscope (Figure S27). Although the transfer to a solid support and drying processes affected the integrity of AuNP monolayers, the small interparticle separations resulting from the thin PE5 shells led to strong near-field interactions between the AuNPs. Indeed, the PE5/AuNP film exhibited a strong near-field coupling, as evident from the dark-field scattering spectra showing a significant red-shift of the plasmon band ($\lambda_{\text{max}} = 690$ nm) from the original peak of well-dispersed
An AuNP ($\lambda_{\text{max}} = 520$ nm), while the plasmonic coupling in the PEG2k/AuNP film was weaker ($\lambda_{\text{max}} = 620$ nm).

**Functionalization of Multiple Types of NPs with Peptoids.** As presented above, we have demonstrated the designability of peptoids for controlling AuNP interactions in bulk and at the interfaces. Here, we explored the general applicability of NP functionalization by peptoids for different types of NPs, including silver, platinum, and iron oxide NPs. First, we investigated silver (Ag, 10 nm) and platinum (Pt, 5 nm) noble metal NPs by applying PE5 coating. In these cases, PE5 grafting to the NP surface was supported by amines, which bound similarly to AuNPs. The UV–vis spectra (Figure 8a,d) indicated that PE5-coated AgNPs and PtNPs were stable, and no signs of aggregation were present, as evident by the absence of absorption peaks at high wavelengths. Similar to PE5/AuNPs (Figure S5), a 3 nm red shift was observed in the surface plasmon resonance of PE5/AgNPs versus uncoated AgNPs, confirming PE5 coating; no SPR was observed for PtNPs in this UV–vis range due to their small sizes. DLS confirmed the thin PE5 coating layers on AgNPs and PtNPs, exhibiting increases in hydrodynamic sizes of 1.5 and 2 nm, respectively (Figure 8b,e), which are consistent with the PE5/AuNP results (Figure 3a). Similar to AuNP phase transfer (Figure 5), the PE5-coated AgNPs and PtNPs could also be transferred from aqueous buffer to chloroform (Figure 8c,f). No color change was observed after the transfer, suggesting that the NPs were stable in chloroform.

We further designed PE7 ($N_{\text{c}} = 6$ (2-carboxyethyl)glycine) for coating of magnetic iron oxide NPs (IONPs). Here, the six primary amines ($N_{\text{a}}$) of PE5 were replaced by six carboxyl groups ($N_{\text{c}}$), which could bind to IONPs through interactions with iron atoms or the surface hydroxyl groups.117,118 The oleic acid-stabilized IONPs (0.2% w/v, 0.001–0.002 M, pH 10) were added to the NPs (see the Supporting Information for detail). We showed in Figure S28 that IONPs could be transferred from chloroform to water in the presence of a small amount of methanol (chloroform/methanol/water = 1:0.067:1.5 v:v:v) to slightly lower the interfacial tension at the chloroform–water interface.

**CONCLUSIONS**

In summary, we designed and synthesized a series of bifunctional comb-branched peptoid architectures and demonstrated their ability to stabilize AuNPs in ionic aqueous solutions in a compact monolayer shell ($\sim$1.5 nm), further to enable efficient transfer to the organic phase, and to control an interfacial assembly for creating high-density NP monolayers.

Among the peptoids, coating PE5 ($N_{\text{a}} = 6$) of a diblock structure to AuNPs rendered their stability under high salt concentration ($\sim$1 M), freeze/thaw treatment, over a wide range of pH, and resisted protein adsorption comparable to AuNPs. The large chemical library of solid-phase synthesis and combinatorial methods for stabilizing and functionalizing various types of NPs. To this end, we demonstrated peptoid functionalization of noble metal (Au, Pt, Ag) NPs and magnetic IONPs. We anticipate that such development will provide molecular-level controls over NP interfaces, which will facilitate their applications in materials science, nanotechnology, and nanomedicine.
The authors thank the support from the Center for Functional Molecule-Semiconductor Nanoparticle Interface. Acknowledgments

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.2c00743

Author Contributions

Notes

The authors declare no competing financial interest.

REFERENCES

(22) Xiao, Z.; Farokhzad, O. C. APTamer-Functionalized Nanoparticles for Medical Applications: Challenges and Opportunities. ACS Nano 2012, 6, 3670–3676.
Polyelectrolyte adsorption, interparticle forces, and colloidal aggregation.

Analysis

Science

Novel one-step synthesis of amine-stabilized aqueous colloidal gold nanoparticles.


Recommended by ACS

Molecular Recognition by Gold Nanoparticle-Based Receptors as Defined through Surface Morphology and Pockets Fingerprint
Laura Riccardi, Marco De Vivo, et al.
JUNE 10, 2021
THE JOURNAL OF PHYSICAL CHEMISTRY LETTERS

Toward Smaller Aqueous-Phase Plasmonic Gold Nanoparticles: High-Stability Thiolate-Protected 4.5 nm Cores
M. Mozammel Hoque, Robert L. Whetten, et al.
JULY 12, 2019
LANGMUIR

Ultrastable PEGylated Calixarene-Coated Gold Nanoparticles with a Tunable Bioconjugation Density for Biosensing Applications
Maurice Retout, Gilles Bruylants, et al.
JANUARY 13, 2021
BIOCONJUGATE CHEMISTRY

Size-Dependent Phase Transfer Functionalization of Gold Nanoparticles To Promote Well-Ordered Self-Assembly
Florian Schulz, Holger Lange, et al.
DECEMBER 01, 2017
LANGMUIR

Get More Suggestions >