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Gold nanoparticle self-assembly promoted by a non-covalent, charge-complemented coiled-coil peptide†

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Non-covalent interactions between cationic gold nanoparticles and an anionic coiled-coil peptide were harnessed for coiled-coil mediated self-assembly of gold nanoparticles (GNPs).

Strategies for the self-assembly of nanoparticles have the potential to provide new systems for biosensors, catalysts, and molecular electronics.[1] The unique electronic[2] and optical[3] properties of gold nanoparticles (GNPs) make them attractive building blocks for the creation of hybrid assemblies and materials. The exquisite structural specificity inherent in biological assembly provides the opportunity to tune such nanoparticle assemblies. For instance, GNP assemblies have been generated with a range of covalently linked biomolecular scaffolds, such as peptides,[4] oligonucleotides[5] and protein/ligand interactions.[6] Recently, cationic GNPs have been shown to template an α-helical conformation within a tetra-aspartate peptide through electrostatic charge complementarity.[7] In this communication we describe harnessing non-covalent peptide–GNP recognition to mediate the specific assembly of GNPs.

Our design criteria focused on a few salient features (Fig. 1). Firstly, charge complementarity between anionic, random coil peptides and cationic GNPs should lead to helical peptide–GNP complexes. If the sequence of the anionic peptide allowed for the formation of an amphiphilic helix upon cationic GNP binding, such binding would reveal a hydrophobic peptide surface that could be satisfied by binding to another GNP-bound, amphiphilic peptide. A continuation of this process would promote the aggregation of gold nanoparticles due to the assembly of amphiphilic peptides.[8] The coiled-coil region of GCN4, **GCN4-p1**, was the starting point for the peptide design.

The leucine zipper region of the transcription factor GCN4 is a well studied peptide that contains a hydrophobic heptad repeat and has been shown to adopt a parallel, dimeric coiled-coil.[9] In the **GCN4-p1** peptide, positions a and d of the heptad repeat are mainly composed of hydrophobic residues. We designed an anionic variant of **GCN4-p1** (**GCN4-E**) in which the hydrophobic core was unchanged, but five residues on the side opposite to the hydrophobic face (solvent exposed f and b positions) were modified to Glu, and two residues in the c position were modified to Ala (Fig. 2). These changes were designed to maximize electrostatic repulsion between residues and thereby diminish the helical content of the peptide at neutral pH, a scenario that we envisioned would be reversed upon binding to cationic GNPs. An anionic control peptide, lacking the hydrophobic interface and the grouping of Glu residues, was also designed by scrambling the residues of **GCN4-E** to produce **GCN4-X**. This peptide would allow us to assess if GNP self-assembly was due to a specific coiled-coil interaction or simply neutralization of GNP charge.

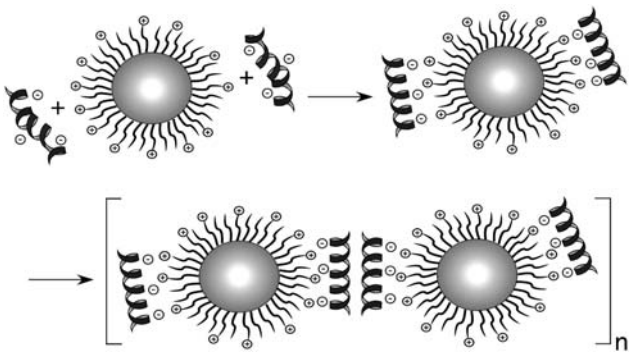

Fig. 1 Schematic of the design of cationic GNP self-assembly promoted by non-covalent interactions with anionic, coiled-coil peptides.

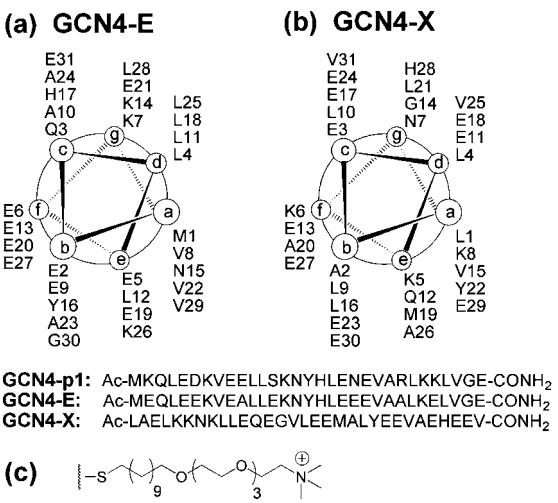

Fig. 2 Helical wheel representation of the designed peptides used in this study: (a) **GCN4-E** and (b) **GCN4-X** with their sequences as compared to **GCN4-p1**, and (c) the ligand used for GNP modification.

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The peptides were synthesized on the H-Rink Amide ChemMatrix solid support using Fmoc-based protection and HBTU coupling chemistry. The peptides were purified to homogeneity by reverse phase HPLC and analyzed by MALDI-TOF mass spectrometry and amino acid analysis. The GNPs used were functionalized by the Murray place exchange method[10] with a cationic ligand (Fig. 2c) to obtain an overall 12 nm particle (6 nm core and ~3 nm ligand[11]) or 8 nm particle for CD studies (2 nm core). The ligand attached to the GNP was terminated by a trimethylammonium cation to provide an overall positive charge.

Circular dichroism (CD) spectroscopy indicated that **GCN4-E** undergoes a conformational shift from random coil at neutral pH to helical upon acidification whereas **GCN4-X** mostly maintained a random coil conformation under both conditions.[12] These data suggest that neutralization of the negative charge on the glutamate residues can generate a helical conformation in this peptide, and potentially dimeric coiled-coil formation when the residues are arranged to promote amphiphilicity. With evidence that the peptide helicity could be manipulated by charge neutralization, we next evaluated the conformation of both peptides in the presence of cationic GNPs (2 nm core particles were used due to strong absorbance of the 6 nm core particles beyond 1 μM). **GCN4-E** (15 μM) was found to adopt a more helical conformation upon increasing the concentration of GNPs (maximum of 60% helicity with 6 μM GNP) at pH 7.4, whereas **GCN4-X** (15 μM) maintained mostly a random coil conformation with a maximum of 20% helicity at 8 μM GNP (Fig. 3a, see ESI†). These data support an interaction between the peptide and GNPs that reduces the electrostatic repulsion in the **GCN4-E** peptide and promotes helicity.[7] A Job titration was also performed *via* CD to identify the binding stoichiometry of GNPs to **GCN4-E**. Maximum helicity was obtained at a 0.7 mole fraction, which corresponds to approximately 2–3 peptides bound to each GNP (Fig. 3b).

GNPs have a characteristic surface plasmon resonance that is found to undergo a red shift upon aggregation.[13] UV-Vis spectroscopy measurements were performed to determine if such aggregation was occurring in the presence of the peptides. Adding increasing amounts of **GCN4-E** to the GNPs (6 nm core) was found to induce a red shift in the spectrum from 527 nm to 550 nm, with a maximum shift occurring at a GNP/peptide molar ratio of 1 : 15 (Fig. 4a). There was only a very slight red shift observed in the spectra for the control peptides, **GCN4-X** (530 nm) or **GCN4-p1** (529 nm), which suggests a role for the coiled-coil interface and cofacial anionic charge in GNP aggregation.

Dynamic light scattering (DLS) was used to measure the size of the aggregates in solution. The cationic GNPs were determined to have a hydrodynamic radius, R_h, of 5.9 ± 0.5 nm, a value that correlates well with the predicted 12 nm particle size (Fig. 4b). Addition of 3 equivalents of **GCN4-E** to the GNPs had no effect on R_h, whereas increasing the equivalents of **GCN4-E** to ten resulted in a significant increase in R_h (~350 nm), a value that remained unchanged with increasing amounts of peptide (up to 25 equivalents). The addition of up to 25 equivalents of **GCN4-X** or **GCN4-p1** to the cationic GNPs

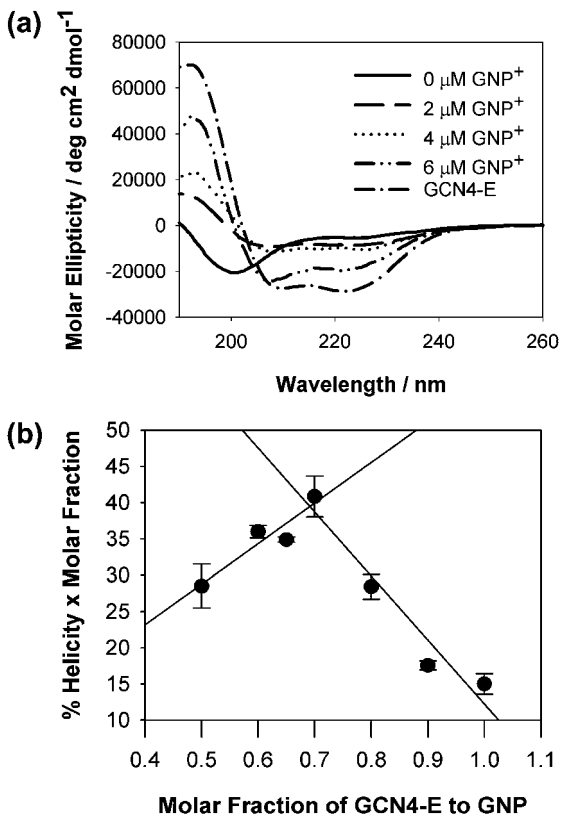

Fig. 3 Circular dichroism analysis with cationic GNPs. (a) Wavelength scan and (b) Job titration of **GCN4-E** with cationic GNPs (2 nm core) indicating ~1 : 2.3 GNPs.

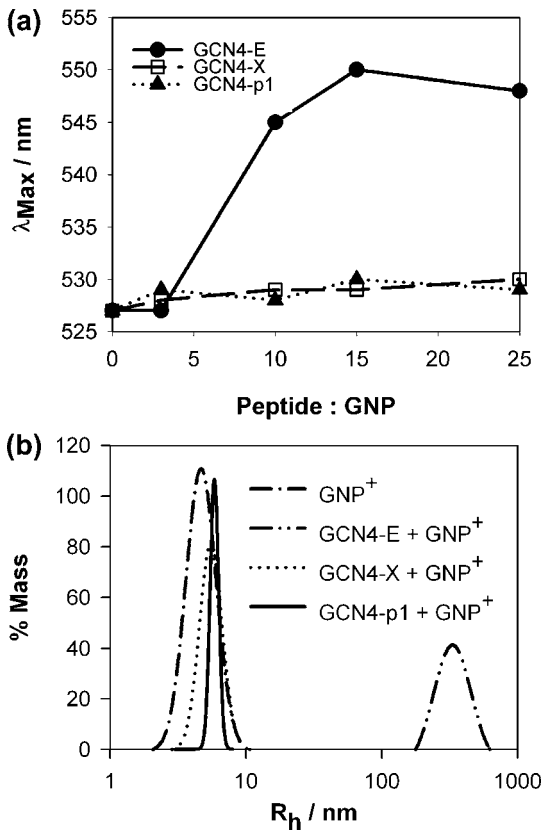

Fig. 4 (a) Change in the maximum surface plasmon resonance band upon increasing the amount of peptide as detected by UV-Vis spectroscopy and (b) dynamic light scattering size distributions of the cationic GNPs, and **GCN4-E**, **GCN4-X**, **GCN4-p1** (25 μM) with cationic GNPs.

resulted in no increase in size of the particles as measured by DLS (6.6 ± 0.8 nm and 5.9 ± 0.9, respectively). These results confirm GNP assembly with the coiled-coil peptide, but not the scrambled sequence, and correlate well with the optimum molar ratio determined for assembly by UV-Vis spectroscopy.

The specific assembly pattern was visualized by transmission electron microscopy (TEM). Before the addition of **GCN4-E**, the GNPs were detected as mostly individual particles that were randomly and evenly spread on the carbon-coated copper grid (Fig. 5a). However upon the addition of **GCN4-E**, clustering of nanoparticle assemblies was observed and significant aggregates were detected after 90 min incubation (Fig. 5b and c). TEM visualization of the GNPs after 90 min incubation with the control peptides, **GCN4-X** and **GCN4-p1**, displayed little difference as compared to the GNPs alone (Fig. 5d and e respectively). These images are a clear indication that specific GNP self-assembly is occurring in the presence of the cofacial anionic coiled-coil peptide, but not the scrambled sequence.

To further examine how the particles are assembling in solution, a TEM time course experiment was performed (Fig. 6). Five minutes after the addition of **GCN4-E**, small chain like clusters were present surrounding a larger collection of GNPs. The GNPs continued to increase in clustering size as time increased. After 90 minutes, there were darker regions present, suggestive of higher ordering. Due to the GNP–peptide design, GNPs would be able to self-assemble three-dimensionally, which was verified by TEM.

Small-angle X-ray scattering (SAXS) was performed to determine if higher order was present in this system. An interparticle distance of 9.1 nm was obtained which agrees with the predicted distance separating the particles with GNP ligand (6 nm) and peptide (2 nm) (see ESI†). Although long range ordering was not observed by SAXS, the GNPs are assembling with one another *via* non-covalent and electrostatic interactions of a specific coiled-coil peptide, **GCN4-E**. We sought to determine if the assembly can be reversed upon masking the charges responsible for the electrostatic interactions. With this in mind we treated the GNP–peptide complexes with 50–70 mM NaCl. DLS measurements confirmed disassembly of the assemblies as a R_h of 5.8 ± 0.2 nm was observed (see ESI†). Therefore this system has the potential to be reversible, a favorable characteristic for devices.

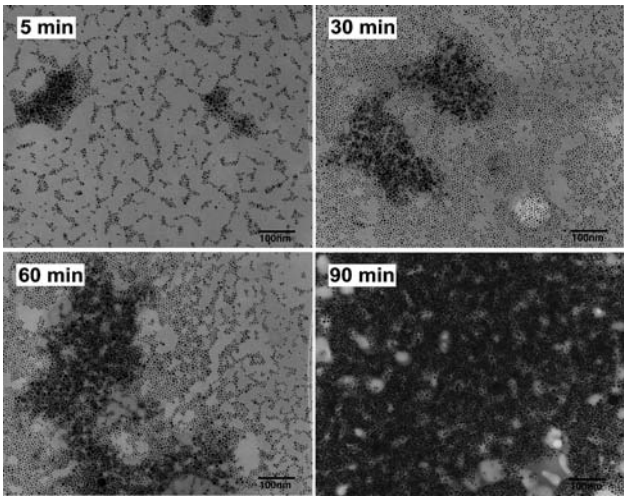

Fig. 6 TEM images depicting various time points during self-assembly of cationic GNPs (6 nm core) and **GCN4-E** (1 : 15 ratio of GNPs to peptide).

In conclusion, we have designed a system that can undergo nanoparticle assembly *via* a non-covalently bound coiled-coil peptide. Hydrophobic forces were found to play a significant role in assembly, as a peptide with an equal overall negative charge as **GCN4-E**, but lacking an ordered hydrophobic face, had no effect on GNP assembly. We recently described a role for cationic GNPs in peptide ligation,[14] and it will be interesting to further explore the interplay between peptide ligation and GNP self-assembly in future designs.

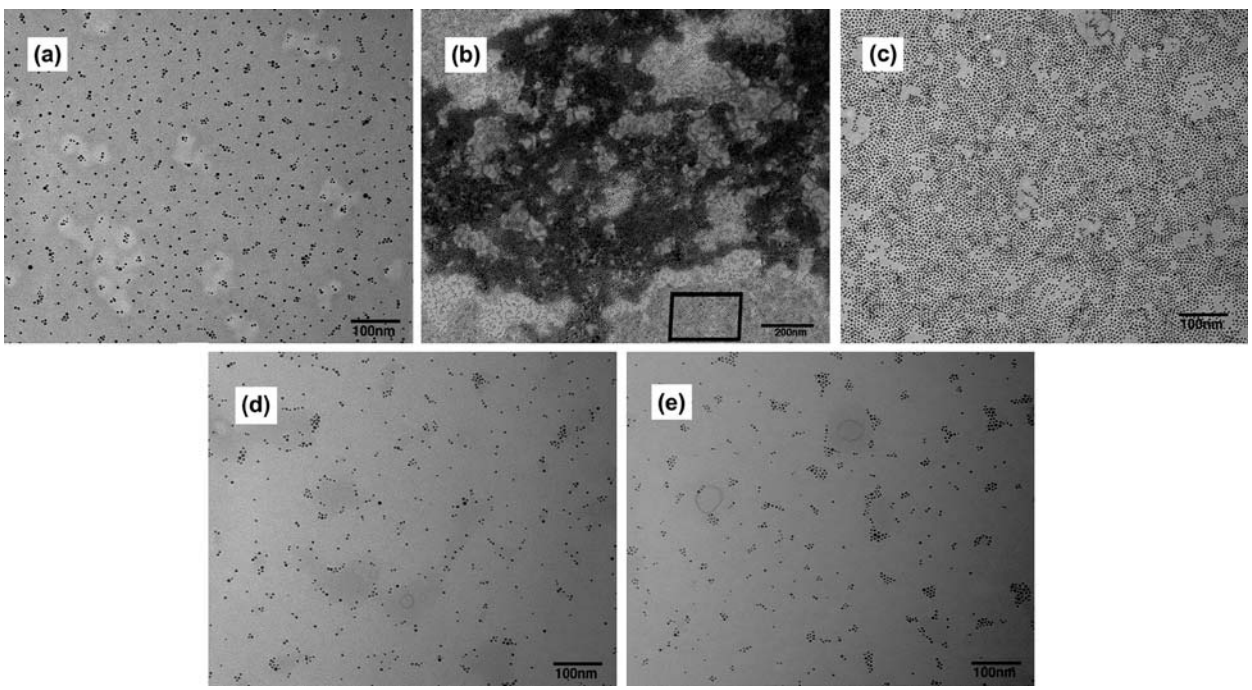

Fig. 5 TEM images of 6 nm core cationic GNPs: (a) alone or with added peptide (1 : 15 ratio of GNPs to peptide with 90 min incubation), (b) **GCN4-E**, (c) zoom in on the monolayer in box (b), (d) **GCN4-X**, and (e) **GCN4-p1**.

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