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Aggregation-Induced Folding of a De Novo Designed Polypeptide Immobilized on Gold Nanoparticles

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Self-assembly as a tool for nanofabrication is of great interest in the development of novel functional materials and devices. Gold nanoparticles are promising building blocks for the construction of such architectures due to the possibility to chemically alter and control their functionality at the molecular level.[1−3] They also possess fascinating optical properties, making them interesting in biosensor design.[4] This communication reports the first steps in the construction of a novel, nanoparticle-based hybrid material for biomimetic and biosensor applications. Gold nanoparticles were modified with synthetic polypeptides to enable control of the particle aggregation state in a switchable manner, and particle aggregation was, in turn, found to induce folding of the immobilized peptides (Figure 1). Aggregation-induced folding is a novel way of controlling the conformation of designed polypeptides and is envisioned as a convenient tool for turning "on/off" catalytic reactions and ligand binding phenomena that are dependent on a native polypeptide fold.

Synthetic polypeptides are attractive components in hybrid designs since they are chemically and physically robust, functionally and structurally versatile, and can be produced at moderate cost and effort by well-established methods. Importantly, the introduction of cysteine residues provides a straightforward means of thiol-dependent, covalent immobilization onto gold substrates.[5]

The design of the glutamic acid-rich, 42-residue L-polypeptide JR2EC (Chart 1) was based on the sequence of SA-42, a de novo designed helix−loop−helix polypeptide that dimerizes in solution to form four-helix bundles.[6,7] The main driving force for folding is the formation of the protein core made up by the hydrophobic faces of the amphiphilic helices, but electrostatic interactions are also important. At neutral pH, JR2EC has a net charge of −5 and repulsive charge−charge interactions prevent homodimerization.[8] Below pH 6, however, folding is observed due to protonation of glutamic acid residues (Figure 2 inset). In addition, a polypeptide with the same primary sequence as JR2EC but without folding ability was designed by replacing all L-Ala residues in JR2EC with D-Ala. This reference peptide is referred to as JR2ECref. In both peptides, a cysteine residue was incorporated in position 22 (within the loop region of JR2EC) to allow for immobilization in a nonhelix disruptive fashion.

Solid-phase peptide synthesis was made on a Pioneer automated synthesizer (Applied Biosystems) using a standard fluorenylmethoxycarbonyl (Fmoc) chemistry protocol. The peptides were cleaved from the resin with 95% trifluoroacetic acid, purified by reversed phase HPLC, and identified from the MALDI-TOF spectra. Citrate-stabilized gold nanoparticles with an average diameter of 13 nm were a kind gift from professor Chad Mirkin. Peptide immobilization was carried out by incubating the particles (∼10 nM) in a 100 μM peptide solution in 10 mM citrate buffer pH 6.0 for 12 h followed by five subsequent washes and change of buffer to 30 mM Bis-Tris pH 7.0 to ensure particle stability and to reduce the concentration of remaining nonimmobilized peptide. The estimated surface density of the immobilized peptide, based on previously published data, is about 60% of a full monolayer.[9] To study the pH-dependent aggregation, the peptide-functionalized particles were diluted 1:100 in 10 mM acetate or glycine buffers at the requested pH.

The collective electron excitations, or particle plasmon resonance, give rise to a strong absorption peak, which for the dispersed particles has a maximum close to 520 nm. When the pH of peptide-functionalized nanoparticles was lowered below pH 5, a distinct red-shift of the plasmon resonance maximum was observed. This red-shift is due to near-field coupling, which occurs when the interparticle distance decreases, indicating a large degree of

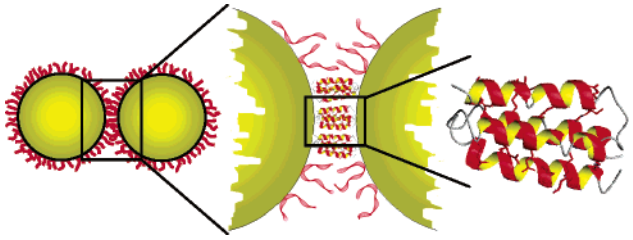

Figure 1. Schematic representation of the interaction of two polypeptide-modified nanoparticles. Under conditions described in the text, particle aggregation induces folding of the immobilized peptides.

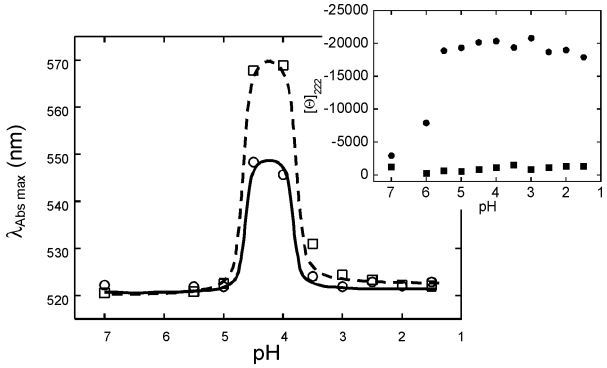

Figure 2. The position of the absorption maximum versus pH of JR2EC (—) and JR2ECref (- - -) modified particles; lines drawn as a guide for the eye. (Inset) Molar ellipticity at 222 nm versus pH for 100 μM JR2EC (●) and JR2ECref (■) in solution.

Chart 1

1 19
H_3N^+-N-A-A-D-L-E-K-A-I-E-A-L-E-K-H-L-E-A-K
20 23
-G-P-C-D
42 24
$^-$OOC-G-A-R-E-F-A-E-F-A-Q-E-L-Q-K-E-L-Q-A-A

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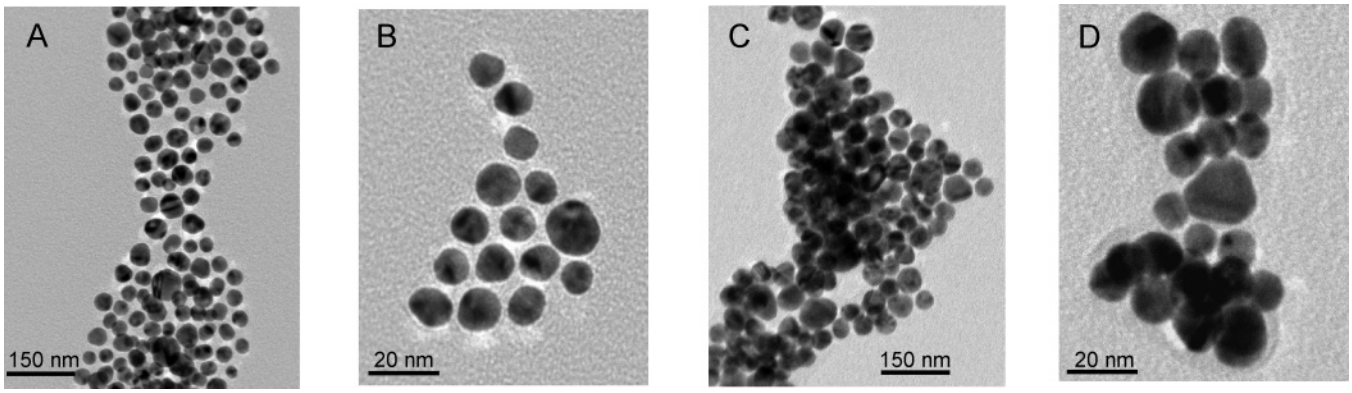

Figure 3. Representative TEM images of peptide-decorated nanoparticles supported on carbon grids. (A and B): Aggregates of JR2EC-modified particles at pH 4.0. (C and D): Aggregates of JR2ECref-modified particles at pH 4.0. No significant differences in aggregate size could be observed for the different peptides by visual inspection. TEM was performed on a Philips CM20 Ultra-Twin lens high-resolution microscope operating at 200 kV.

aggregation.[10,11] The smaller the interparticle distance, the larger the red-shift. At pH 4.5, protonation of glutamic acid residues allows the charge-induced repulsive forces observed in the interval between pH 7 and 5 to be overcome by attractive interactions, which leads to particle aggregation. At pH < 4, positively charged residues outnumber remaining unprotonated acidic residues, and the peptides therefore acquire a positive net charge. At pH 3.5, the electrostatic repulsion is strong enough to interrupt the aggregates, and the position of the resonance absorption maximum shifts back close to the value at pH 7. The narrow pH interval allowing particle aggregation is in good agreement with the JR2EC pI of 4.56 generated from the Expasy ProtParam tool,[12] illustrating the importance of surface charge to particle stability. At pH 4.0, the shifts were about 30 and 50 nm in the case of JR2EC- and JR2ECref-modified particles, respectively (Figure 2). The interparticle distance within the aggregates was measured from electron micrographs (Figure 3) in areas where only one layer of particles was observed and was found to be 2.3 ± 0.1 nm for JR2EC and 1.4 ± 0.1 nm for JR2ECref.[13] Although JR2EC is fully folded at pH < 6 when free in solution, it should not be able to dimerize into four-helix bundles when immobilized as long as the particles are dispersed since dimerization between peptide molecules on the same particle is sterically impossible. However, when aggregation occurs at pH 4.5, some JR2EC monomers on proximate particles will be located close enough in space to dimerize and fold (Figure 1). The significantly lower red-shift upon aggregation of the JR2EC-modified particles compared to those modified with the reference peptide is due to a larger average distance between the particles within the aggregates, impaired by the folded scaffold. The reference peptide does not form any ordered secondary structure at any pH when free in solution (Figure 2 inset), and when immobilized, it is not expected to behave any differently in that sense but to "collapse" as a random coil on the surface. This gives rise to a considerably smaller average interparticle distance and, as a consequence thereof, a larger shift of the absorbance maximum.

In a previous study, immobilization of JR2EC was investigated on planar gold surfaces.[9] It was found that JR2EC was immobilized as an unstructured monomer from a neutral solution and as a homodimer from acidic solutions (pH 4−5). The reported thickness of a monolayer of folded JR2EC immobilized on planar gold surfaces, 2.2 nm,[9] is indeed in very good agreement with the average distance between JR2EC-modified particles at pH 4.0. Provided with the right pH and sterical conditions, JR2EC is thus able to dimerize and fold on surfaces, and the four-helix bundle scaffold is rigid enough to keep the particles separated.

In addition to JR2EC, a charge complementary polypeptide has been designed, JR2KC, able to heterodimerize with JR2EC. Investigations performed on planar surfaces indicated that heterodimerization can be induced on the surface,[9] and the next step in the construction of this nanosized Lego will be to investigate the interaction between JR2EC- and JR2KC-functionalized nanoparticles. Utilizing synthetic polypeptides to lock colloidal building blocks into three-dimensional structures in a switchable manner is a novel way of controlling the assembly of nanoparticles. Not only does it reveal important information about how polypeptides fold and interact at surfaces, but the versatility of possible polypeptide designs in terms of folding and dimerization properties allows for self-assembling multicompetent nanostructured materials to be created.

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(13) The number of measured distances was $n = 200$ for both the JR2EC- and JR2ECref-modified particles. The confidence level is 99%.

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