

## **227e Using the Peptide Toolbox to Create Protein-like Nanoparticles for Applications in Bionanotechnology**

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Biological sciences is the field in which nanotechnology promises to have its most immediate impact. Quantum dot fluorescence has been shown to be a viable alternative to traditional fluorophores in the labeling of cells, and the light scattering and absorption properties of Au and Ag nanoparticles have proven to be useful in the detection of DNA hybridization and antibody/antigen interactions. A new photothermal detection technique enables single-particle detection of metallic nanoparticles as small as five nanometers in diameter. Compared to the 30 to 40 nm Au and Ag nanoparticles necessary for single-particle detection using dark-field microscopy, this development places metallic nanoparticles on a much more even playing field with fluorescent quantum dots for sensing applications within the cell or on the cell surface. In addition to using nanoparticles to detect biological molecules, biological molecules can be used to self-assemble nanoparticles into patterns that are not otherwise thermodynamically or kinetically favored. In this case, stoichiometric control of the molecules on the nanoparticle surface is the crucial difference between forming dimers, trimers, and other controlled, discrete structures and forming uncontrolled, three-dimensional agglomerates of nanoparticles.

Based on protein folding considerations, a pentapeptide ligand, which converts citrate-stabilized gold and silver nanoparticles into extremely stable, water-soluble nanoparticles with some chemical properties comparable to those of proteins, has been designed. These protein-like nanoparticles can be freeze-dried and stored as powders that can be subsequently redispersed to yield stable aqueous dispersions. Electrophoresis, various types of chromatography (size-exclusion, ion-exchange, hydrophobic interaction, and immobilized metal affinity), centrifugation, and filtration can be applied to these particles with negligible loss of material. The effect of 58 different peptide sequences on the electrolyte-induced aggregation of these nanoparticles was also studied. The stabilities conferred by these peptide ligands depended on their length, hydrophobicity, and charge. The combinatorial approach also yielded detailed design criteria for peptide capping ligands. In particular, cohesive interactions between adjacent peptide chains through hydrophobic interactions or hydrogen bonding appear to be crucial for maintaining stability, even at high electrolyte concentration.

The excellent stability provided by the pentapeptide and the reduced lower limit of detection provided by the photothermal technique has shifted the focus from nanoparticle stabilization to functionalization. Recently, peptide-derivatized nanoparticles have been used to label cells via attachment to membrane-bound receptor proteins and to enter the cell through receptor-mediated endocytosis in order to target specific subcellular compartments, including the nucleus. The difference highlighted in this work is that the nanoparticles are entirely stabilized by peptides through an instantaneous exchange reaction with the citrate molecules on the nanoparticle surface, and the pentapeptide can be used as a matrix peptide to ensure particle stability while a small percentage of functionalized peptide can be used to confer specific recognition properties. Essentially, the interdependence of functionalization and stabilization has been deconvolved while maintaining their preparative simultaneity. The nanoparticle surface can be functionalized with biotin, streptavidin, Strep-Tag II, histidine tag, NTA, DNA, heparan sulfate, and various phage-display peptides that recognize carbon nanotubes and other inorganic surfaces. Nanoparticle functionalization can be extended to more than one peptide, or a single peptide can be synthesized with multiple functional groups. Combined with chromatographic separation techniques this procedure enables precise stoichiometric control of the nanoparticle surface, an integral ingredient for quantitative detection of analytes and the formation of controlled, discrete nanostructures. The simple derivatization procedure and the versatile chemical properties of peptides open the route to a number of applications for bioanalytical sensors and nanotechnology.