Synthetic de novo designed polypeptides for control of nanoparticle assembly and biosensing

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Abstract

This contribution describes how de novo designed synthetic helix–loop–helix polypeptides are utilized to control the assembly of gold nanoparticles and as scaffolds for biosensing. The synthetic polypeptides are designed to fold into a four-helix bundle upon dimerization. When immobilized on gold nanoparticles, dimerization and folding occur between peptides on neighbouring particles as an effect of particle aggregation and the folded polypeptides are rigid enough to keep the particles separated at a distance corresponding to the size of the four-helix bundle. Moreover, peptide dimerization offers a convenient route to assemble nanoparticles into hybrid multilayers on planar substrates. The drastic change in the resonance conditions of the localized nanoparticle surface plasmon upon particle aggregation is shown to be useful for optical detection of biomolecular interactions.

Introduction

Synthetic, de novo designed polypeptides are robust molecular building blocks with a high degree of structural and chemically versatility, ideal for bottom-up nanofabrication. In combination with metal nanoparticles, complex materials with novel properties can be obtained, e.g. for bioanalytical applications [1,2]. Peptides immobilized on the particle surface can be exploited to control particle assembly and as molecular scaffolds for the design of artificial bioreceptors.

The present study is based on synthetic 42-amino-acid helix–loop–helix polypeptides, designed to fold into four-helix bundles upon dimerization [3]. Folding is mainly driven by the formation of the hydrophobic core made up by the apolar faces of the amphiphilic helices, but electrostatic interactions are also important. A cysteine residue in the loop is used to facilitate site-specific and -directed immobilization on to various substrates.

Gold nanoparticles have a pronounced molar absorption coefficient maximum close to 520 nm. The position of this LSPR (localized surface plasmon resonance) peak is very sensitive to particle aggregation and changes in refractive index close to the particle surface. In case of particle aggregation, near-field coupling causes a huge red-shift and a broadening of the LSPR peak. The smaller the interparticle distance and the larger the aggregates, the more red-shifted is the LSPR peak [4,5].

Key words: biosensing, folding, four-helix bundle, gold nanoparticle, nanoparticle assembly, polypeptide scaffold.

Abbreviations used: HCAII, human carbonic anhydrase II; LSPR, localized surface plasmon resonance; TEM, transmission electron microscopy.

Aggregation-induced folding: homodimerization

Two glutamic acid residue-rich polypeptides, JR2EC and JR2ECref, were immobilized as monomers on gold nanoparticles with an average diameter of 13 nm. JR2EC is made up of L-amino acids and has a net charge at neutral pH of –5. At pH <6, the decrease in net charge, caused by protonation of glutamic acid residues, allows for JR2EC to homodimerize and fold in solution. The primary sequence of JR2ECref is identical with that of JR2EC, but in JR2ECref all L-alanine residues are replaced by D-alanine, which fully prevents folding in solution at any pH.

The peptide-functionalized nanoparticles are very stable at neutral pH. When lowering the pH close to the calculated pI (∼4.6) of the peptides, the low charge repulsion between the particles causes them to aggregate. At pH close to pH 3.5, the peptides adopt a positive net charge large enough to disperse the particles. Reversible particle aggregation can therefore be induced in a relatively narrow pH interval. Aggregation is also observed at neutral pH when introducing zinc ions. Zinc ions bind specifically to the peptides, the low charge repulsion between the particles causes them to aggregate. At pH close to pH 3.5, the peptides adopt a positive net charge large enough to disperse the particles. Reversible particle aggregation can therefore be induced in a relatively narrow pH interval.

For the JR2ECref-functionalized particles the red-shift of the LSPR peak upon particle aggregation at pH 4 is approx. 50 nm, while for particles functionalized with JR2EC the shift is considerably smaller, approx. 30 nm. TEM (transmission electron microscopy) micrographs of aggregated particles on carbon-coated TEM grids show no apparent differences in aggregate size but the interparticle distance varies: 1.4 ± 0.1 and 2.3 ± 0.1 nm for JR2ECref- and JR2EC-coated particles respectively. The thickness of a monolayer of folded and dimerized JR2EC immobilized on planar gold substrates is ∼2.2 nm, as reported previously [6].

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Figure 1 | Aggregation-induced polypeptide folding

(A) TEM micrograph of aggregated JR2EC-functionalized particles. (B) UV/visible spectra of JR2EC-functionalized particles. Aggregation occurs at pH 4–4.5 seen as a red-shift of the LSPR peak. (C, D) Aggregation of JR2EC-modified particles induces folding of the immobilized peptides.

Our interpretation of these observations is that JR2EC immobilized on neighbouring particles dimerizes and folds when brought in close proximity as an effect of particle aggregation (Figure 1). The folded peptides are rigid enough to keep the particles separated at a distance corresponding to the size of the four-helix bundle, therefore giving rise to a smaller red-shift of the LSPR peak compared with the JR2ECref-functionalized particles. JR2ECref is unable to dimerize and fold at any pH resulting in a smaller average interparticle distance [7].

Particle assembly on surfaces: heterodimerization

JR2KC is a 42-amino-acid polypeptide with a positive net charge at neutral pH that forms heterodimeric four-helix bundles with JR2EC at neutral pH in solution and on planar gold substrates [6]. By covalently linking JR2KC to a mixed SAM (self-assembled monolayer) of maleimide- and hydroxy-terminated tri(ethylene glycol) disulfides, the assembly of JR2EC-functionalized gold nanoparticles on the surface can be studied. Subsequent incubations with JR2KC- and JR2EC-functionalized particles are used to assemble multilayers of particles. Up to six layers of particles were bound to the surface resulting in a linear red-shift of the LSPR peak.

Aggregation-based biosensing

The large red-shift upon particle aggregation and the sensitivity towards small differences in interparticle distance were utilized in the design of an aggregation-based biosensor for HCAII (human carbonic anhydrase II). The helix–loop–helix polypeptide KE2C, which folds into a homodimeric four-helix bundle in solution, was modified with an aromatic sulfonamide in position 34 to form KE2C-C6. In a previous study, HCAII has been shown to bind with nanomolar affinity to an almost identical peptide construct through interactions with the sulfonamide moiety [8].

KE2C-C6 was immobilized on gold nanoparticles and when inducing aggregation by evaporating the buffer, the red-shift is dramatic in the absence of HCAII or in the presence...
of HSA (human serum albumin). In the presence of a small amount of HCAII the shift is clearly reduced, indicating a larger interparticle distance apparently due to binding of HCAII to KE2C-C6. The negative control measurement with KE2C resulted in a substantially larger red-shift (Figure 2).

In this proof-of-principle demonstration, specific binding of a target protein to a small ligand, covalently bound to the immobilized polypeptide scaffold, prevents aggregation of the particles, whereas aggregation occurred in the absence of target protein.

**Conclusions**

We have shown how synthetic, designed polypeptides can be used as structural and functional elements in nano-engineering. Folding of immobilized polypeptides offers a novel approach of controlling the aggregation state of nanoparticles. Polypeptide-functionalized nanoparticles are a new class of hybrid materials for biosensors, with many exciting properties yet to be examined.

**References**


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