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Stabilization of α-helices by the self-assembly of macrocyclic peptides on the surface of gold nanoparticles for molecular recognition†

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A novel strategy to stabilize the α-helical secondary structures of peptides upon binding to gold nanoparticles is described. Using a model protein–protein interaction system, we showed that AuNPs decorated with stabilized p53 α-helix peptides can mediate specific molecular recognition with their target protein.

Peptides have the same chemical structure as proteins but are much shorter in length, which allows facile chemical synthesis and easy manipulation. However, one problem that may arise when using peptides is that they are typically disordered owing to their inherent thermodynamic instability, which significantly limits their molecular recognition capability. The α-helix is one of the most famous and essential secondary structures in proteins. The α helices comprise more than 30% of the secondary structures that are found in globular proteins. Importantly, many α helices play important roles in biomacromolecular interactions, such as protein–protein and protein–nucleic acid interactions.[1–4] Although α-helical structures are well stabilized in the context of intact proteins, they tend to be unstructured when isolated from the protein as monomeric peptides because of their inherent thermodynamic instability.[5] To circumvent this problem, many attempts have been made to stabilize α-helical structures in the framework of monomeric peptides[6–8] or self-assembled peptide nanostructures,[9–11] which is critical for achieving high affinity and specificity.

Gold nanoparticles (AuNPs) have drawn substantial interest because of their advantageous physical and chemical properties. AuNPs are also useful bionanomaterials, in part because of the facile formation of a covalent bond between gold and sulfur, which facilitates the functionalization of AuNPs with a wide variety of biomolecules that contain a sulfhydryl (SH) functional group.[12] Biohybrids composed of AuNPs and biomolecules, such as DNA, RNA, proteins, peptides, and carbohydrates, have been prepared and utilized in many applications, including biosensing, gene delivery, drug delivery, medical therapy, and sophisticated biohybrid nanostructure formation.[13,14]

In this paper, we present the development of self-assembled peptide–AuNP hybrid nanostructures, in which the α-helical conformation of the peptide is well stabilized for molecular recognition. In order to achieve this goal, we first ask the question of whether the α-helical structure can be stabilized by the molecular constraining effect, followed by the binding of the macrocyclic peptide on the gold surface. To test this, we designed and synthesized macrocyclic peptides that contain three different functional units, namely a potential α-helix segment, a cysteine-rich segment, and linker segments (Fig. 1).

The peptide scaffold was designed to be cyclic to constrain the peptide, which may aid in stabilizing the overall peptide structure by reducing both the entropy of the unfolded state and conformational heterogeneity.[15,16] We hypothesized that the

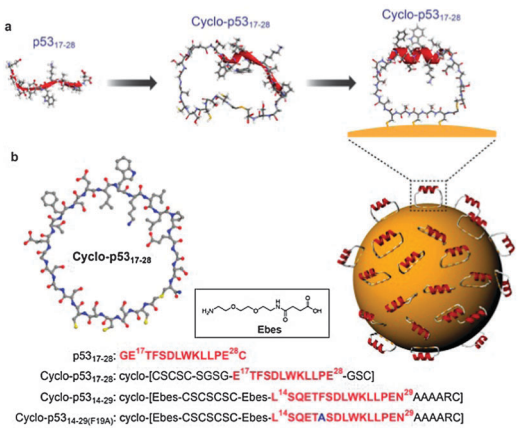

Fig. 1 A macrocyclic strategy to construct bioactive α-helix-decorated AuNP biohybrids. (a) Molecular models depicting AuNP binding-induced stabilization of the p53 α-helix. (b) Sequences of the peptides used and a representative chemical structure of the cyclo-p53$_{17-28}$ peptide.

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partially stabilized α-helical structures within the constrained macrocyclic scaffold might be further constrained and stabilized due to the flexible coil to rigid rod transition of the cysteine-rich segment after its binding onto the rigid gold support (Fig. 1a). The cysteine-rich segment has multiple cysteine residues and is used to facilitate the formation of multiple gold–thiolate bonds. The linker segment was inserted to connect the cysteine-rich and the potentially helical segments. The potentially helical segment was derived from the transactivation domain of the p53 tumor suppressor protein.[3]

p53 induces cell cycle arrest and apoptosis in response to cellular stress and damage; thus, it has an important role in protecting cells from malignant transformation.[17] The p53 peptide binds to its negative regulator, MDM2, in an α-helical conformation.[3] The binding event suppresses the p53-mediated apoptotic pathway and targets p53 to the ubiquitin-proteasome pathway for degradation.[18] Therefore, a stabilized p53 peptide, as an inhibitor or a decoy, may reactivate p53 tumor suppressor activity.

The head-to-tail peptide cyclization reaction was performed with the protected peptide still bound to the resin for a pseudo-dilution effect and to reduce the entropic penalty associated with intramolecular cyclization.[19] We first examined the conformation of a linear $p53_{17–28}$ peptide that includes the most critical residues of p53 (amino acids 17 to 28)[20] for MDM2 recognition using circular dichroism spectroscopy (CD). As expected, the linear $p53_{17–28}$ peptide did not have a clearly defined secondary structure and had a pronounced negative ellipticity minimum at 199 nm (Fig. 2a). CD analysis of the cyclo-$p53_{17–28}$ peptide showed that the negative minimum was slightly red-shifted to 202 nm and that the level of negative ellipticity at 222 nm, which is a signature of the α-helix conformation, was slightly increased compared with that of the $p53_{17–28}$ peptide (Fig. 2b). Therefore, although helicity was increased by constraining the peptide within a cyclic structure, the level of stabilization was minimal.

We next asked whether the multiple gold–thiolate bonds formed between the cyclic peptides and the AuNPs influenced the peptide conformation. The cyclo-$p53_{17–28}$ peptide was mixed with an AuNP colloid solution, which facilitated gold–thiolate bonds formation *via* the multiple cysteine residues in the peptide. Before binding of the peptide to the AuNPs, the peptide was dissolved in a 30% (v/v) 2,2,2-trifluoroethanol (TFE)–water mixture to preform the peptide's α-helical structure. TFE is a well-known cosolvent that stabilizes α-helical structures in proteins and peptides.[21] The peptide and AuNPs were mixed in a 30% TFE solution to maintain the peptide's α-helical structure during AuNP binding. The nanoparticle surface was then passivated with triethylene glycol mono-11-mercaptoundecyl ether (TGMUE). The cyclo-$p53_{17–28}$ peptide–AuNP biohybrids were purified by gel filtration chromatography with water as the eluent. Remarkably, the CD spectrum of the biohybrid showed signatures of a well-stabilized helical structure with broad but distinct double minima at 209 nm and 222 nm (Fig. 2c). In contrast, AuNPs treated only with TGMUE did not show any evidence of α-helical conformation (Fig. 2c). Therefore, the results from this study suggest that the α-helical structure of a peptide with high helix-forming propensity can be stabilized using this macrocyclic scaffold approach.

We further confirmed this idea using a macrocyclic peptide that was similar to the cyclo-$p53_{17–28}$ peptide which had structural variations. The cyclo-$p53_{14–29}$ peptide encompasses more residues from the native p53 sequence than the cyclo-$p53_{17–28}$ peptide and has additional residues (four alanines and one arginine) at the C-terminus to stabilize helix macrodipole.[22] To accommodate this enlarged helical segment within the macrocyclic scaffold, the lengths of the cysteine-rich and linker segments were increased concomitantly. As shown in Fig. 2d, the cyclo-$p53_{14–29}$ peptide had a minimal α-helical content. However, binding of the peptide to a gold surface could significantly stabilize the helical conformation, as evidenced by the distinct double minima at 207 nm and 224 nm (Fig. 2f). The shapes of cyclic peptides–AuNP biohybrids were characterized using atomic force microscopy (AFM). As shown in Fig. 2c and f (insets), the biohybrid nanoparticles were discrete and spherical in shape. Therefore, all these results clearly indicate that this macrocyclic scaffold approach can be utilized in the development of stabilized α-helical peptide–AuNP biohybrids. Taking all these into consideration, the conformational transition from a coil to a rigid structure in the cysteine-rich segment is likely responsible for the observed α-helix stabilization on the gold surface.[8,10]

To determine whether the p53 cyclic peptide–AuNP biohybrids can specifically recognize their protein target, MDM2, we developed a novel colorimetric assay based on the molecular recognition between differently functionalized AuNPs (Fig. 3a). MDM2 was expressed with a hexa histidine-tag (His-tag), and AuNPs derivatized with Ni(II)–NTA were employed for this assay. Because the Ni^{2+} ion is immobilized on the AuNPs *via* chelation by nitrilotriacetic acid (NTA), it forms strong coordination bonds with the His-tag, and mixing the His-tagged MDM2 fusion protein with Ni(II)–NTA–AuNPs resulted in the formation of MDM2-coated AuNPs. Excess MDM2 was removed by centrifugation. When the AuNPs coated with p53 peptides interact with the MDM2-coated AuNPs, multiple binding events between the p53 peptides and MDM2 should drive the aggregation of both

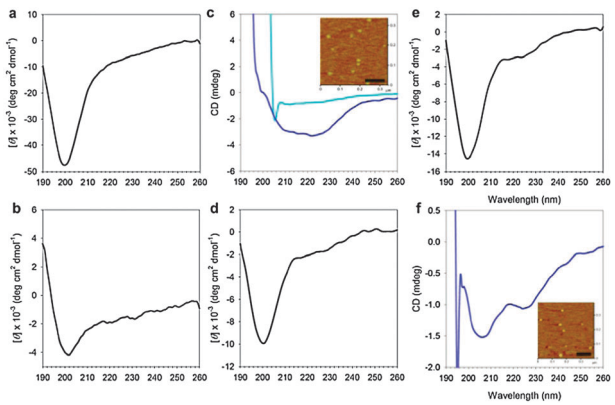

Fig. 2 Induced stabilization of an α-helix through macrocyclic peptide binding on AuNPs. CD spectra of (a) the linear $p53_{17–28}$ peptide, (b) the cyclo-$p53_{17–28}$ peptide, (c) cyclo-$p53_{17–28}$ peptide–AuNP biohybrids (blue) and AuNPs without the peptide (light blue), (d) the cyclo-$p53_{14–29}$ peptide, (e) the cyclo-$p53_{14–29(F19A)}$ peptide (mutant), and (f) cyclo-$p53_{14–29}$ peptide–AuNP biohybrids. The peptide concentration was typically 1–5 μM in water. CD spectra were measured at 25 °C. Insets: AFM images of (c) cyclo-$p53_{17–28}$ peptide–AuNP biohybrids and (f) cyclo-$p53_{14–29}$ peptide–AuNP biohybrids. Bar = 100 nm.

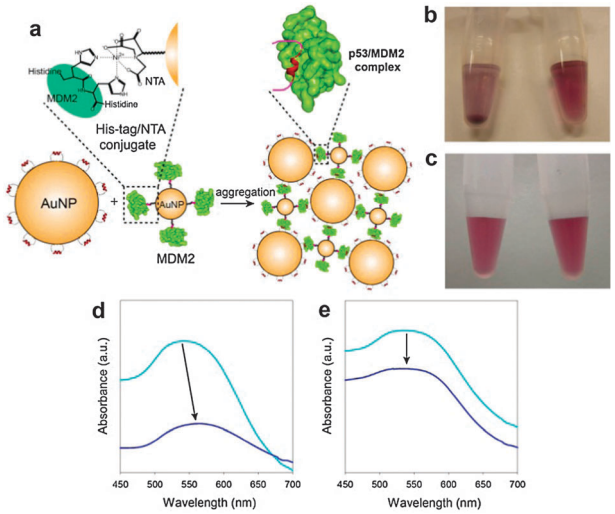

Fig. 3 Recognition of MDM2-coated AuNPs by cyclic peptide–AuNP biohybrids. (a) A scheme depicting the colorimetric assay based on molecular recognition between differently functionalized AuNPs. (b) Left: mixture of cyclo-$p53_{14–29}$ peptide–AuNP biohybrids and MDM2/Ni(II)–NTA–AuNP conjugates (mixture 1). Right: mixture of cyclo-$p53_{14–29}$ peptide–AuNP biohybrids and Ni(II)–NTA–AuNP conjugates (mixture 2). (c) The same experiment as in (b) except that a cyclo-$p53_{14–29(F19A)}$ peptide was used. (d) UV/Vis absorption spectra. Blue: mixture 1, light blue: mixture 2. (e) The same experiment as in (d) except that the cyclo-$p53_{14–29(F19A)}$ peptide was used.

types of AuNPs, resulting in the formation of a precipitate and a shift in the surface plasmon band (Fig. 3a).

When MDM2/Ni(II)–NTA–AuNP conjugates were added to the cyclo-$p53_{14–29}$ peptide–AuNP biohybrid suspension (mixture 1), the color of the suspension darkened, and a precipitate formed (Fig. 3b). This phenomenon indicates that multiple interactions between p53 peptides and MDM2 occurred, driving the formation of AuNP aggregates. In contrast, no such change was observed when Ni(II)–NTA–AuNPs (without MDM2) were added to the biohybrid suspension (mixture 2). In addition, the UV/Vis absorption spectra (Fig. 3d) revealed a concomitant shift in the plasmon bands from 545 nm (mixture 2) to 560 nm (mixture 1). To confirm the specificity of the binding events, we mutated Phe-19, which was previously reported to be crucial for the binding of p53 to MDM2, to Ala (F19A mutant).[6] As shown in Fig. 3c and e, the color of the biohybrid suspension did not change, and almost no shift in the surface plasmon band was evident. Thus, these results clearly indicate that the stabilized p53 α-helix on AuNPs can participate in specific peptide–protein interactions with MDM2. We also tested the ability of cyclo-$p53_{17–28}$ peptide–AuNP biohybrids for molecular recognition of MDM2/Ni(II)–NTA–AuNP conjugates. Although the magnitudes of both phenomena were lower than those obtained with the cyclo-$p53_{14–29}$ peptide–AuNP biohybrids, the cyclo-$p53_{17–28}$ biohybrid could also induce AuNP precipitation and a plasmon band shift (Fig. S3, ESI†).

In conclusion, we first demonstrated that α-helix secondary structures can be stabilized upon binding to AuNPs when using peptides with cyclic topology. Second, a biohybrid comprising a stabilized peptide and AuNPs specifically recognize the peptide's target. This method allows the fabrication of organic–inorganic biohybrids with specific molecular recognition capabilities. These biohybrids combine the molecular recognition ability of the biomolecule and the physicochemical properties of the gold nanoparticles. Considering the prevalence of α-helical structures in biological systems, this type of biohybrid may become a widely applicable platform for developing artificial hybrid nanostructures that can sense and modulate biomacromolecular interactions.

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