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Photocontrolled reversible morphology conversion of protein nanowires mediated by an azobenzene-cored dendrimer†

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A novel strategy to construct photocontrolled protein nanowires with reversible morphology was reported through photoisomerizable azobenzene-cored dendrimer evoked protein self-assembly. Furthermore, the curvature of the protein nanowires could be switched by alternatively irradiating with visible light and ultraviolet light.

Self-assembly strategies of proteins have provided strong motivation for the exploration of pathological mechanisms and the development of high-performance nanobiomaterials.[1] Various protein architectures, such as nanowires, nanotubes, vesicles, sheets and three-dimensional (3D) crystals, have been successfully developed by accurately manipulating the protein–protein interactions.[2] However, manipulating the interaction behaviour of natural proteins can control the tissue differentiation and functions in an organism.[3] How to reversibly control protein assembly morphologies *in vitro* by dynamically regulating the protein–protein interactions for their potential applications in switchable drug/gene delivery, chemical signal transportation, and disease diagnosis/treatment is a challenging topic.[4]

Controllable protein self-assembly is an attractive approach for constructing stimuli-responsive nanobiomaterials due to their response to various external stimuli, such as pH, temperature, light, ligands, and enzymes.[5] However, most studies are focused on the assembly/disassembly process, while the reversible morphology interconversion of protein assemblies between two or more states has rarely been reported, for few proteins can exhibit reversible conformational isomerization under specified conditions.[6] Organic molecule mediated protein self-assembly may provide a new strategy for realizing the reversible and repetitive morphology conversion of protein nanostructures.

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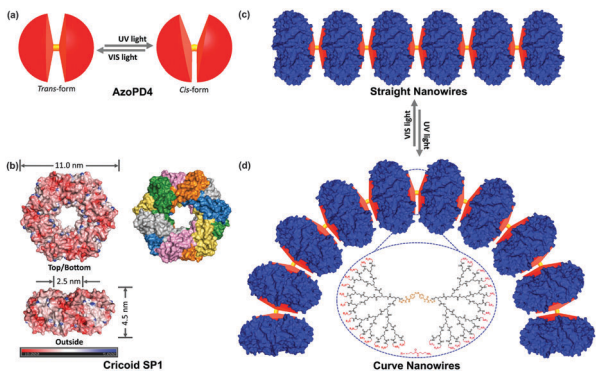
† Electronic supplementary information (ESI) available: Details of the synthesis and characterization (NMR and MS) of AzoPD4 dendrimers; self-assembly strategy; dynamic light scattering, atomic force microscopy, and transmission electron microscopy of free SP1 and assemblies. See DOI: 10.1039/c6cc01730d

Recently, we have demonstrated that a globular poly(amidoamine) (PAMAM) dendrimer can direct the assembly behaviour of SP1 protein,[7] a cricoid protein with twelve monomers bound together to afford a double-layered structure with a height, inner diameter and outer diameter of about 4.5 nm, 2.5 nm and 11 nm, respectively.[8] The dendrimer structure makes it possible for two SP1 rings to interact with a dendrimer in opposite orientations to form an axisymmetric sandwich structure, further assembling into one-dimensional linear protein nanoarrays. This research arouses our curiosity about what will happen if the dendrimer has a "V"-shaped structure. Environmental stimuli triggered molecular isomerization can reversibly interconvert molecules between two conformations. Among all kinds of external stimuli, light is considered to be the optimum for its noninvasive, clean and remote controlling properties.[9] Herein, we developed a novel strategy for the construction of photocontrolled protein nanowires with reversible morphology through photoisomerizable azobenzene-cored PAMAM dendrimer (AzoPD) evoked SP1 protein self-assembly (Scheme 1). The curvature of the protein nanowires



Scheme 1 Structure-based design of photocontrollable protein nanowires. (a) Photoisomerization of AzoPD4 which can be reversibly interconverted between the *trans*-form and *cis*-form. (b) The surface potential distribution and physical dimensions of SP1. The protein assembly can be interconverted between straight nanowires (c) and curved nanowires (d) by irradiating with UV light and VIS light.

could also be switched by alternatively irradiating with visible (VIS) light and ultraviolet (UV) light.

For reversibly manipulating the assembly behaviour of protein nanowires, the AzoPD must satisfy the following three key prerequisites: (1) the diameter of the AzoPD must be larger than the inner cavity of SP1 to ensure interaction with two SP1 rings along the C_6 symmetry axis simultaneously; (2) the steric hindrance effect of bilateral dendrons should be as small as possible to avoid isomerization-induced intramolecular repulsion; and (3) the bridging fragment of the dendrimer should be rigid enough to change the interspace angle of the bilateral dendrons. 1,4-Disubstituted 1,2,3-triazoles formed through Cu^I-catalyzed alkyne–azide "click" cycloaddition reactions with high efficiency are employed as a rigid bridging fragment to covalently connect bilateral dendrons through azobenzene (Fig. S1–S6, ESI†).[10] We have found that small dendrimers could cause SP1 proteins to randomly aggregate, but those with higher generations were compacted into globular structures. The fourth generation AzoPD (AzoPD4), with 64 terminal amino groups around the surface, satisfied the above prerequisites and therefore was employed to manipulate the assembly behaviour of SP1 (Fig. S7, ESI†).

The optimized AzoPD4 structure showed bilateral dendrons could adjust to a "V"-shaped structure when the azobenzene core was switched to the *cis*-isomer (Fig. 1). It is vital to investigate the reversibility and repetitiveness of the photocontrolled isomerization. AzoPD4 showed a maximum absorption at 367 nm in the UV-vis spectra for the absorption of the *trans*-azobenzene segment.[11] When irradiated with UV light for 10 min, the absorption intensity of the AzoPD4 solution at 367 nm (1.166) was gradually reduced to 0.520, indicating that AzoPD4 had isomerized to the *cis*-isomer (Fig. 1b). The *cis*-AzoPD4 could also spontaneously switch to its original state completely when exposed to VIS light for another 10 min (Fig. 1b, inset). We further measured the absorption intensity at 367 nm with alternative irradiation under UV light and VIS light. Significantly, the cycle can be repeated multiple times with no macroscopic retrogression (Fig. 1c). All the results indicated that the AzoPD4 dendrimer afforded good structural reversibility and repetitiveness for the light triggered isomerization process, which laid the foundation for reversibly controlling the protein nanostructures.

AzoPD4 mediated SP1 self-assembly was first investigated using dynamic light scattering (DLS) and agarose gel electrophoresis (Fig. 2). Free SP1 existed as a dodecameric ring with a hydrodynamic diameter (D_h) of about 10.1 nm, close to its crystal size (11 nm). However, the 1 : 1 complexation of AzoPD4 and SP1 through electrostatic interactions could form larger supramolecular aggregates with the D_h rapidly increasing to 190 nm. The free SP1 protein afforded fast electrophoretic mobility (lane 1), but the addition of AzoPD4 to SP1 obviously restricted its mobility, and a new band with slow electrophoretic mobility was gradually observed. Smearing towards the anode was observed when a large excess of AzoPD4 was added to the SP1 solution (lanes 7 and 8).[12] In addition, the AzoPD4–SP1 electrostatic interactions could be tailored by controlling the Debye screening length (adjusting NaCl concentration) and the pH (Fig. S8, ESI†). All these results verified the formation of large binary hybrids.

Tapping-mode atomic force microscopy (AFM) and transmission electron microscopy (TEM) were employed to observe the topological morphology (Fig. 3). SP1 itself afforded a dot-like structure, while it was able to self-assemble into randomly dispersed straight nanowires when an equimolar ratio of AzoPD4 was added (Fig. S9, ESI†). The AFM images showed AzoPD4 could control the SP1 self-assemby into longer straight nanowires with the length close to one micrometer and the height reaching 9.7 ± 0.5 nm, a height close to the outer diameter of the crystal structure of the SP1 ring. The 3D topological image clearly showed the linear structure. The TEM image gave us further favourable evidence for the protein self-assembly behaviour. The free SP1 protein stained with phosphotungstic acid aqueous solution affords a ring structure with an outer diameter of about 10 nm. However, extended protein chains composed of regular repeating SP1 units were formed after AzoPD4 mediated

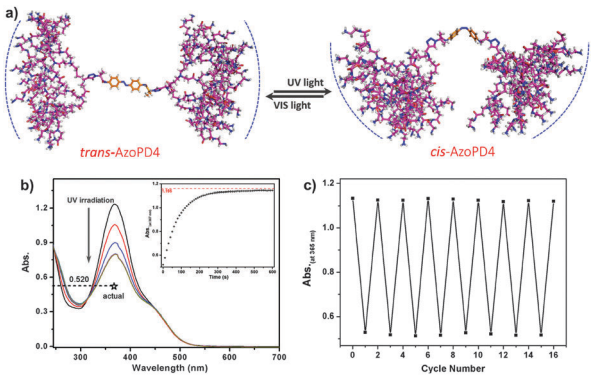


Fig. 1 The photoisomerization of AzoPD4. (a) AzoPD4 structure of the *trans*- and *cis*-isomers, optimized with GaussView. (b) UV-Vis spectra under UV light irradiation, and the inset shows the absorption changes at 367 nm. (c) The absorption at 367 nm with alternative UV light and VIS light irradiation.

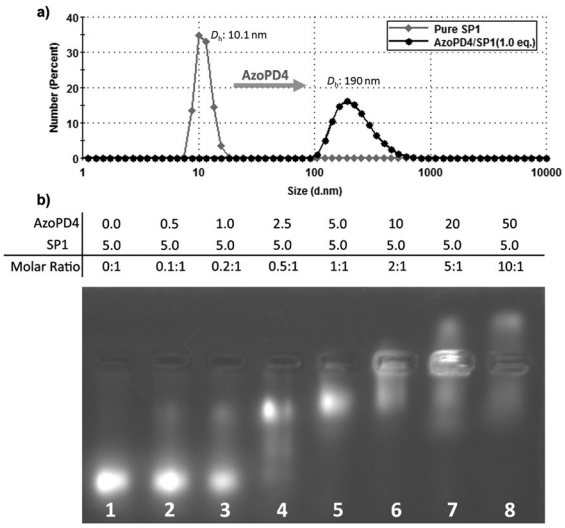


Fig. 2 Formation of AzoPD4/SP1 binary hybrids characterized with dynamic light scattering (DLS) (a) and agarose gel electrophoresis (b).

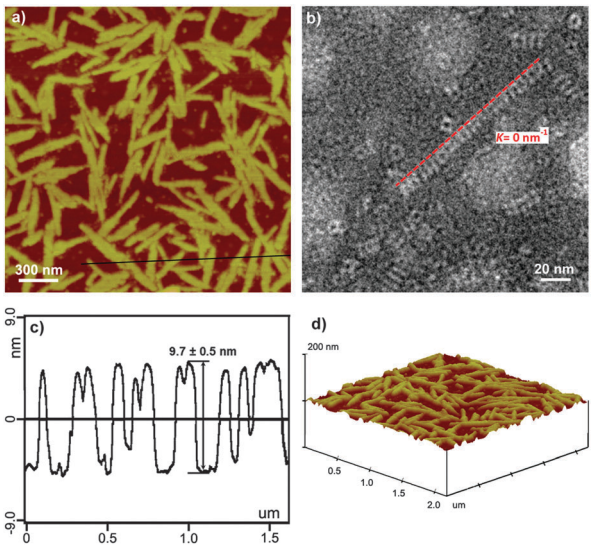


Fig. 3 The topological morphology of the protein nanowires before treating with UV light. AFM (a) and TEM (b) images of the nanowires. (c) Height profile along the black line in (a). (d) The 3D topological image of (a).

SP1 self-assembly. Clearly, *trans*-formed AzoPD4 could manipulate the SP1 proteins into a parallel arrangement along its C_6 symmetry axis face to face to form linear protein chains, further growing into "rigid" straight nanowires.[7,13]

After UV-induced isomerization of AzoPD4 for several minutes, *cis*-AzoPD4 adopted a "V"-shaped structure between two bilateral dendrons. Interestingly, it could control the SP1 self-assembly in a nonlinear orientation to afford curved nanowires, which was quite different from the *trans*-AzoPD4 under VIS light. As shown in Fig. 4a, a great many short protein nanoarcs formed originally when *cis*-AzoPD4 mediated the SP1 assembly. These short nanoarcs could further assemble into curved nanowires if incubated under UV light for a long time (Fig. 4b and c). The topographic phase image showed more clearly that the curved nanowires interlaced each other to form rough-and-tumble reticulate structures, which looked like "soft" curved nanowires. We were also surprised to find out that a few protein nanowires were uniformly distorted in one direction to form half-rings or closed rings with an average diameter of about 50 nm (Fig. 4c, inset).

The TEM images showed that the SP1 rings were not damaged under UV light (Fig. S10, ESI†), but *cis*-AzoPD4 could manipulate them into self-assembling face to face to form standing protein chains. But unlike the *trans*-isomer, the *cis*-isomer could control the SP1 alignment with a certain interspace angle. We further calculated the degree of curvature of the arcs and showed that the average interspace angle (θ) of the adjacent SP1 rings was $10.5^\circ \pm 0.5^\circ$ (Fig. 4d). Because there was no synergic interaction to cooperate with the multiple electrostatic interactions between AzoPD4 and SP1 to accurately control the orientation of the protein, SP1 could randomly orientate in different directions, making the nanoarcs further assemble into "S"-shaped curved structures (Fig. 4e). We further measured the curvature radius (r_i) of each arc and showed that their values were 25 nm, 40 nm and 45 nm, and their corresponding inner curvature (K_i) values were 0.040 nm^{-1}, 0.025 nm^{-1}, and 0.022 nm^{-1}, respectively. We analyzed the composition of the nearly closed supramolecular rings in Fig. 4c and found that the curvature of the rings was 0.040 nm^{-1}, and each ring was composed of nearly 34 SP1 proteins (Fig. S11, ESI†).

Photoisomerizable AzoPD4 could manipulate the assembly behaviour of SP1 in two different manners: *trans*-AzoPD4 was a near-globular molecule and it could manipulate SP1 into a parallel arrangement to form "rigid" straight nanowires, while *cis*-AzoPD4 could control SP1 into "soft"-like curved nanowires due to the "V"-shaped structure of the bilateral dendrons.

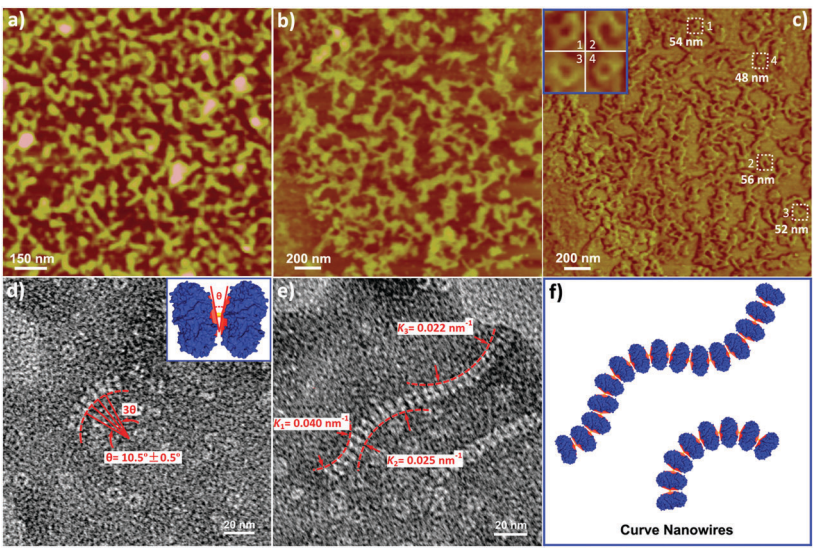


Fig. 4 Topological morphology of the AzoPD4/SP1 nanowires after treating with UV light. AFM images at the initial (a) and final (b) stage, and the phase image of image b (c), inset is the enlarged view as mentioned. TEM images of the nanoarcs (d, inset is assembly model) and curved nanowires (e). (f) Assembly model of the nanowires after UV irradiation.

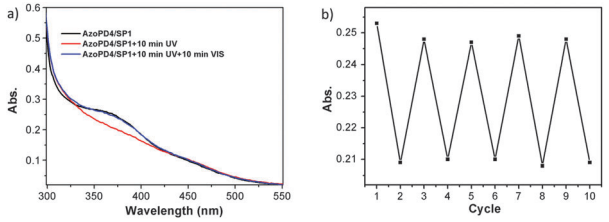


Fig. 5 (a) UV-Vis spectra of AzoPD4/SP1 nanowires. (b) Reversible change between the straight and curved states of the protein nanowires.

UV-vis spectroscopy was employed to monitor the reversibility of the photocontrolled interconversion of the protein nanowires. As shown in Fig. 5, AzoPD4/SP1 could also be isomerized to the *cis*-form upon UV light irradiation for 10 min. After being further exposed to VIS light for another 10 min, the absorbance peak at 367 nm gradually recovered to the original value. Also, the cycle can be repeated several times. Therefore, the photoisomerization process could also happen after the co-assembly with SP1 proteins.

The AFM images further gave clear evidence for the interconversion between the straight and the curved nanowires (Fig. S12, ESI†). The curved nanowires could be switched to straight after exposure to VIS light. Also, the straight nanowires will be transformed to curved if they are irradiated with UV light. Also, the percentage of curved nanowires could recover from the initial 80% to above 65% after alternative irradiation with VIS light and UV light. We presented a straightforward and previously unused method for the construction of protein nanowires that could reversibly interconvert between disparate straight and curved nanowires triggered by noninvasive light. If supplemented with a synergic interaction to cooperate with the multiple electrostatic interactions to accurately control the deflecting direction, we would have reason to believe that a photocontrollable interconvertible protein nanostructure which could interconvert between nanowires and nanorings or nanospirals could be designed, which is meaningful for the construction of function-controllable protein nanostructures.

In summary, photocontrolled protein nanowires with reversible morphology were developed through photoisomerizable azobenzene-cored dendrimer evoked protein self-assembly. AzoPD4 could manipulate SP1 into a parallel arrangement to form straight nanowires. However, when isomerized to the *cis*-form under UV light, *cis*-AzoPD4 could manipulate SP1 obliquely into arranging to afford protein nanoarcs and the average angle of the adjacent SP1 rings was nearly $10.5^\circ \pm 0.5^\circ$. Each nanoarc could further connect to form long "S"-shaped curved nanowires with an inner curvature value up to 0.040 nm^{-1}. The curvature of the protein nanowires could be switched by alternatively irradiating with VIS light and UV light, which provided a new strategy for the design of intelligent protein-based nanobiomaterials.

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