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Fiber Recruiting Peptides: Noncovalent Decoration of an Engineered Protein Scaffold

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An ability to engineer functional materials from the bottom-up would have an impact on nanoscale science and technology.[1a] The exploration of biomolecular systems in this area is especially attractive for the following reasons: natural biomolecular assemblies exhibit exquisite selectivity and specificity, they adopt a wide variety of structures that perform an array of functions, and finally, they are based on various chemistries (nucleic acids, peptides and proteins, lipids, and carbohydrates) that promote self-assembly in water at neutral pH and ambient temperatures. These features have led to major research efforts on natural biomaterials and bioinspired designed materials.[1b,c] Our work has focused on peptide-based assemblies. In particular, we have employed leucine-zipper-type peptides to construct novel structures.[2] Like others,[3] we have developed a self-assembling fiber (SAF) peptide/protein system as a potential scaffold for applications in nanobiotechnology.[2b]

Our system comprises two complementary leucine-zipper[4] peptides, SAF-p1 and SAF-p2.[2b] These co-assemble to give a sticky-ended dimer with complementary overhanging ends, which promote longitudinal assembly to give unbranched fibrous structures 50−70 nm thick and tens of micrometers in length. We have built upon this SAF concept in a number of ways. Specifically, we have designed and synthesized variants (specials) of the standard SAF-p1 and SAF-p2 peptides that co-assemble with the standards to introduce branches and kinks into the fibers.[5] This indicates that SAFs tolerate additional nonstandard peptide units that exhibit demonstrable and desired effects on fiber morphology.

Thus, SAFs constitute engineered fibrous scaffolds that can be fabricated from the bottom-up in water and could be of use in functionalizing surfaces with active biomolecules for the development of diagnostics[6a,b] and as biocompatible scaffolds for cell and tissue engineering.[6b,c] An issue in these areas is that the SAFs are currently "bare" scaffolds, and they will have to be specifically decorated with bioactive macromolecules to introduce a function.[6d] In this respect, it has been demonstrated that multivalent synthetic polymers can be used to recruit folded proteins[7a] and subsequently cells.[7b] Here we describe an approach in which folded and active proteins are recruited to the SAFs using molecular baits introduced into the SAF peptides during peptide synthesis. We call these constructs Fiber Recruiting (FiRe) peptides (Figure 1).

We demonstrate the concept by visualizing FiRe-containing SAFs decorated with gold nanoparticles using transmission electron microscopy (TEM). In related studies, others have shown covalent, sulfur-mediated decoration of amyloid-like protein fibers,[8a] and they use this as a basis for fabricating conducting Au/Ag nanoscale wires.[8b] In our case, the molecular baits had to be small enough to be tolerated during fiber assembly, but available for recognition by the proteins being targeted for recruitment to the matured fibers. Thus, to test the concept, we chose two established noncovalent, protein-recognition systems, namely biotin−streptavidin and peptide antigen−antibody interactions (Figure 1). These have been technologically proven as biomolecular connectors in protein purification,[9a,b] surface

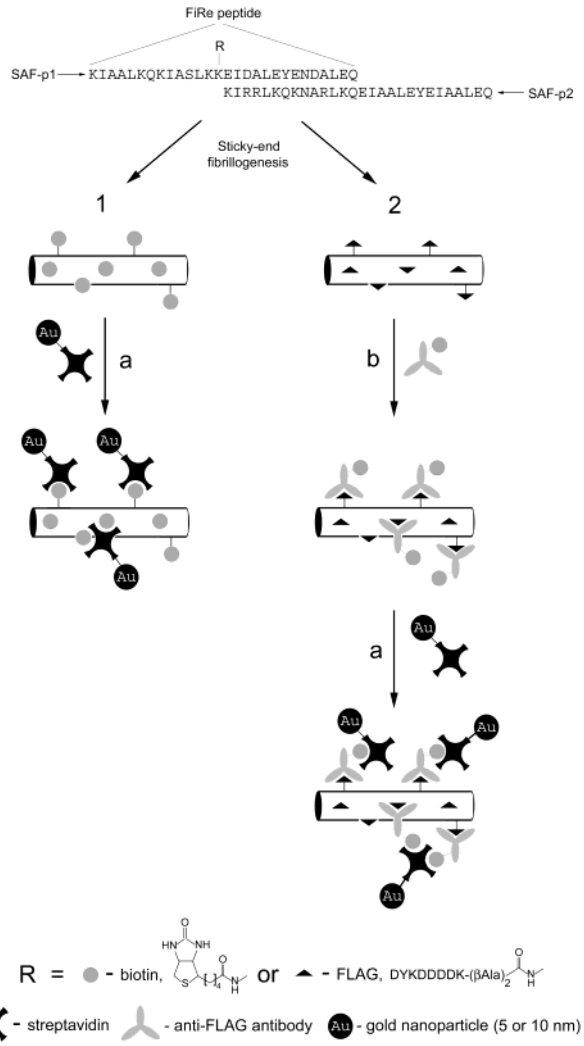

Figure 1. Scheme illustrating the concept of fiber recruiting. After SAF fibrillogenesis, two independent experimental design routes (1 and 2) are shown, which utilize the two different molecular-recognition processes (a) protein−ligand and (b) antigen−antibody interactions.

patterning,[9c] and self-assembling scaffolds.[9d] For our study, we modified the SAF peptides by attaching biotin, or the so-called FLAG octapeptide (DYKDDDDK)[9a] to the ϵ-amino group of a lysine at a solvent-exposed ***f*** position of the leucine-zipper repeat.[2b,5b]

For route 1 (Figure 1), the SAF peptides were biotinylated during peptide synthesis to give the FiRe peptides, SAF-p1-biotin and SAF-p2-biotin. In independent experiments, these peptides readily co-assembled with their standard SAF companion peptides to form normal fibers under our usual assembly conditions (pH 7, 100 μM, 20 °C); i.e., copies of either biotinylated peptide substituted for

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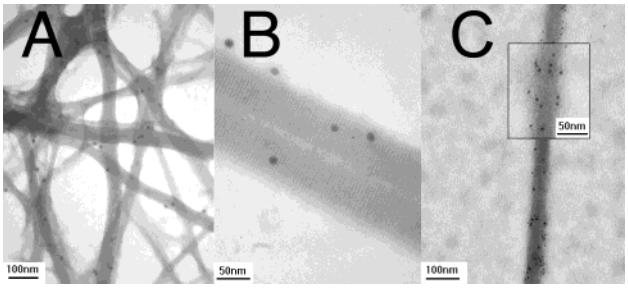

Figure 2. Representative transmission electron micrographs of peptide fibers coated with streptavidin-gold nanoparticles. (A and B) The particles were recruited directly via biotin incorporated into one of the peptide building blocks of the fiber (SAF-p1-biotin). (C) Decoration was achieved in two steps: (1) recruitment of a biotinylated anti-peptide antibody and (2) binding of the streptavidin−gold conjugate. The boxed section in C is enlarged by a factor of two. In A and B, 10-nm particles were used, and 5-nm particles were used in C.

the standards in fiber assembly. To prevent nonspecific protein adsorption on scaffold surfaces, polyoxyethylenesorbitan monolaureate and phosphorocholine chloride were used in the following recruitment experiments (Supporting Information).[10] As revealed by TEM, mixing the fibers with streptavidin conjugated with 10 nm of gold particles (SG10 at 5 μM) resulted in the specific capture of the conjugate on the fibers (Figure 2A,B). Gratifyingly, the nanoparticles were separated from the fiber surfaces by ∼5 nm (Figure 2B and Figure 1S), which corresponds to the approximate size of streptavidin. Fibers assembled from SAF-p1-biotin and SAF-p2-biotin without standard peptides gave comparable coating (Figure 1S). However, samples of streptavidin labeled with smaller 5 nm particles (SG5) gave higher coverage, suggesting that nanoparticles may sterically block binding to biotinylated sites (Figure 1S). Similarly, it is possible that the fibers themselves limit access to biotin and, therefore, reduce streptavidin−biotin binding compared with that in solution. We note that SAFs have ultrastructure (Figure 2B) which may influence recruitment; although at the coverages we observed, there was no patterning of the nanoparticles reflecting this structure.

Streptavidin is known to exhibit at least partial nonspecific binding.[11] To assess this in our system, we compared TEM images obtained for standard (SAF only) fibers treated with SG5 with those for SAF-biotin fibers treated in the same way. At concentrations of SG5 above 5 μM, background binding was not eliminated completely by using surfactants or by washing. At the higher concentrations, nonspecific binding was approximately one-third of that for the biotin-decorated fibers (Figure 2S).

To extend the FiRe concept, we modified SAF-p1 with the FLAG tag to make SAF-p1-FLAG. This peptide was not as well-tolerated in assembly with SAF-p2 as the corresponding biotinylated FiRe peptide: the resulting fibers were shorter and rarer than normally observed. It is likely that fiber assembly was affected by the additional negative charge of the FLAG tag. Nevertheless, treatment of the resulting fibers with a biotinylated anti-FLAG antibody (anti-FLAG Bio-M2), followed by SG5 gave fibers densely coated with gold nanoparticles (Figure 2C and Figure 1S). No coating was observed for SAF-only fibers (Figure 1S). Thus, the decoration of fibers by incorporating FLAG-tagged FiRe peptide followed by treatment with an anti-FLAG-tag antibody and SG5 appeared to reduce nonspecific binding.

In summary, we have introduced the concept of fiber recruiting peptides: peptides conjugated with small molecules are first co-assembled into fibers to present the small molecules on the surface of the fibers; the small molecules are then used as bait to recruit folded and functional proteins to the surfaces. We demonstrated this by recruiting protein-bound gold nanoparticles that were directly visualized by TEM. Two types of bait were tested, namely, biotin and a small peptide antigen. In both cases, reproducible recruitment was observed. Furthermore, a range of coverage, from nanometer to tens-of-nanometer spacings of nanoparticles, was achieved. This offers possibilities for the bottom-up assembly of functional materials with nanometer to micrometer-scale tolerance. For instance, the system presents a starting point for the development of artificial fibrous scaffolds that mimic natural systems, such as collagen and fibrin, which communicate with their cellular environments through active peptides and proteins presented at their surfaces.

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Supporting Information Available: Experimental details, additional experiments, and TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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