Nanotechnology

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Genetic Engineering of Biomolecular Scaffolds for the Fabrication of Organic and Metallic Nanowires**

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Biological scaffolds serve as excellent templates for the fabrication of nanowires. In several pioneering studies, metals and other inorganic materials were deposited on natural biological scaffolds to form well-ordered wires at the nanoscale.[1–6] However, the potential application of biological fibrils may be extended further, as the powerful tools of genetic engineering enable fiber functionality, binding selectivity, and material composition to be predefined and genetically encoded. Herein, we report the fabrication of genetically engineered metal-binding 5 nm thick cytoskeletal filaments on the basis of the self-assembly of an FtsZ protein redesigned to accommodate short binding peptides. The spontaneously organized protein filament was designed as a tailor-made scaffold to promote both the self-assembly of the wires and the specific anchoring of inorganic materials and biomolecules. The fusion of gold-binding and silver-reducing peptides to FtsZ monomers enabled the construction of protein-based gold and silver wires. Furthermore, the biotinylated FtsZ filament was used as a self-positioning wire to connect avidin magnetic beads. The enhancement of natural filaments with variable peptide motifs offers a new bioinspired platform for nanotechnologies.

Among various objectives, synthetic biology aims to use biological molecules for the design of novel materials on the scales relevant for sensing, catalysis, photonics, and electronics applications.[7,8] The engineering of hybrid components in general, and nanowires in particular, poses several challenges. First, as the miniaturization of photolithography methods is limited,[9] new fabrication technologies must be developed. Second, future in vivo wires must interface efficiently with biological environments. Finally, the manipulation of hybrid wires must be addressed. To meet these challenges, biological macromolecules, including nucleic acids,[10] virus particles,[11] fungal cells,[12] plant viruses,[13] peptides,[5,14,15] and proteins,[16] have been utilized as platforms for solid-state, inorganic nanostructures.[17]

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Although attempts have been made to harness protein-based fibers in contrived nanosystems,[18,19] researchers have only begun to apply the inexhaustible reservoir of structures, catalysis mechanisms, and biological recognition motifs to nanotechnology. For example, in a pioneering study, Willner and co-workers demonstrated the adenosine-5′-triphosphate-fuelled motility of gold-labeled actin filaments on a myosin interface.[16] However, chemical manipulation of the protein or target material is often required.

Short peptides were also shown to be useful in nanotechnology. Peptide nanotubes, self-assembled from fragments as short as two amino acids, were shown to form ordered nanotubes with remarkable properties,[20] and were further used as a casting mold for the fabrication of metallic wires.[5] Moreover, peptide motifs selected by in vitro evolution for the specific binding and mineralization of inorganic materials[21–23] have been utilized as affinity elements in ferritin-based protein mineralization cages,[24] lithium-ion batteries,[25] and cell-adhesion enhancers,[26] and recently, peptide-based nanoparticle nucleation was used to synthesize ordered gold helices.[14] To our knowledge, however, the integration of peptide motifs with naturally occurring protein fibers for the fabrication of inorganic materials has not yet been explored.

In this study, with the aim of developing new tools for the fabrication of organic and inorganic materials, we used short peptide motifs to design cytoskeletal fibers with specific binding affinities. The prokaryotic tubulin homologue, filamentous temperature-sensitive protein Z (FtsZ), served as a convenient model scaffold for the demonstration of our technique. The crystal structure of FtsZ has been solved, and the controlled assembly of FtsZ in vitro by guanosine-5′-triphosphate (GTP) or cation induction has been studied comprehensively.[27,28] Notably, the system presented herein can be readily applied to any well-ordered protein assembly.

Figure 1 outlines the assembly of versatile binding fibers containing a gold-binding motif, a silver-reducing motif, or a biotinylation motif (see the Supporting Information for full experimental details). First, by using a standard *Escherichia coli* pTrcHis-TOPO overexpression vector, the selected peptide was genetically fused to the FtsZ gene (the FtsZ gene was provided by J. Mingorance, UAM, Spain). Following expression induced by isopropyl β-D-1-thiogalactopyranoside, the FtsZ–peptide construct was purified by precipitation with calcium chloride and ion-exchange chromatography on Q Sepharose. The in vitro assembly of FtsZ monomers by GTP or calcium induction resulted in nanometer-scale fibers, each of which contained multiple-affinity peptides. Finally, the specific adherence of inorganic particles to the protein fibers gave rise to hybrid protein–inorganic wires. Thus,

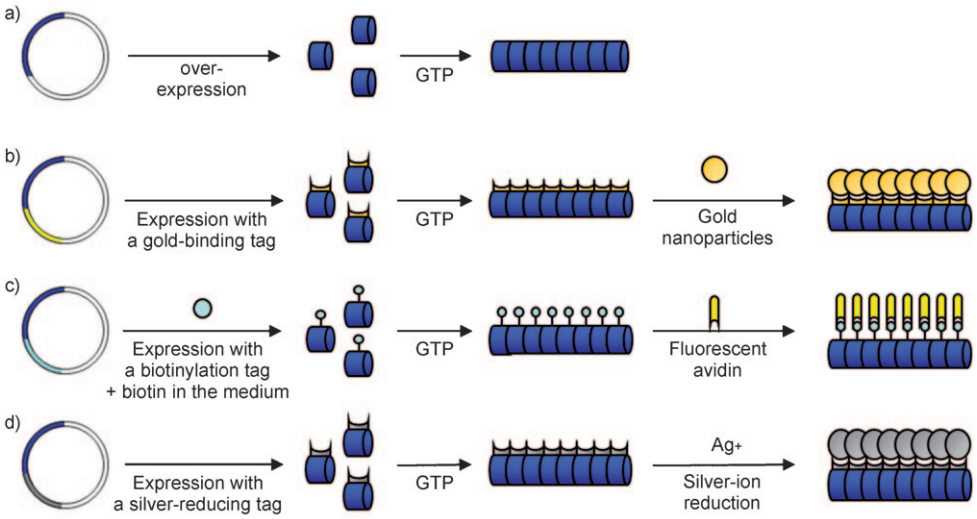

Figure 1. Schematic illustration of the fabrication of hybrid protein-based nanowires. a) The self-assembly of wild-type FtsZ monomers is induced by GTP. b–d) Peptide motifs are genetically fused to FtsZ monomers to form a gold-binding wire (b), a biotinylated wire (c), or a silver-reducing wire (d).

simple genetic modification provides all the information needed for the facile production of many identical protein subunits; no further chemical processing is required prior to self-assembly.

We designed and tested several FtsZ variants, each with a different N-terminal peptide motif, as scaffolds for ordered inorganic assembly (Figure 2). A short serine–glycine flexible fragment separated the peptide, with a length of 13–21 amino acids, from the FtsZ gene (see the Supporting Information). The purified protein monomers were analyzed by spectroscopy and microscopy. Far-UV circular dichroism (CD) and transmission electron microscopy (TEM) were used to confirm that all variants maintained their secondary structure and formed typical GTP-induced filaments (see Figures 4–5 in the Supporting Information). We then investigated the unique binding features of each engineered FtsZ variant in vitro.

The peptide NPSSLFRYLPSD was previously evolved by phage-display methodology to reduce silver ions to solid silver particles.[21] The GTP-induced assembly in the presence of silver nitrate of FtsZ monomers bearing this peptide resulted in highly specific silver coating of the filaments (Figure 2c,d; see also Figure 1 in the Supporting Information). Silver particles were clearly observed by TEM, and their presence was further confirmed by energy-dispersive X-ray analysis (EDX; Figure 2c, inset). Although highly specific and abundant, the nucleation of metallic silver was not entirely continuous: small gaps of uncoated protein remained. We are currently optimizing the coating efficiency (see below).

We also fabricated gold–FtsZ hybrid wires by conjugation of the peptide VSGSSPDS, which was reported to have specific gold-binding properties.[23] We tested the gold-binding efficiency of the conjugated peptide and wires by adding 2.5 nm gold nanoparticles to the polymerization solution. Upon GTP induction, gold-binding FtsZ monomers assembled to form highly specific gold-binding filaments, as confirmed by TEM (Figure 2e,f; see also Figure 2 in the Supporting Information). Both discrete filaments and elaborate gold-binding fibrous networks were observed; however, not all fibers displayed a continuous coating. We are currently investigating improvements in coating efficiency (see below).

Next, we tested a peptide with six N-terminal histidine residues for patterned nickel binding. When GTP calcium ions were added to the polymerization solution, His-FtsZ monomers assembled into highly ordered sheets of aligned filaments at the microscale. The formation of these structures is consistent with previous reports[28] on higher-order FtsZ assembly. In the presence of a nickel sulfate solution, the specific coordination of nickel ions onto the ordered sheets was observed, as evident by a characteristic green coloration (Figure 2b). We envision that FtsZ-based nickel-coordinated sheets could be used for the fabrication of metallic nickel plates.

We are currently investigating several strategies to improve the persistence and uniformity of the metal coating. First, coating enhancement by standard protocols can be applied to bound metallic particles.[29] We are also testing the characterization and optimization of the experimental conditions and wire conductivity. Although our technique requires biologically relevant conditions for assembly, we hypothesize that the final architecture could be maintained even after degradation of the protein scaffold. We are currently exploring the feasibility of this approach. Although FtsZ structures seem less uniform than DNA and peptide assemblies,[10,14] we believe this method may be highly suitable for future applications, as incomparable variability in scaffold architectures and functionality is possible, well-established engineering techniques are employed, and the resulting structures should serve as versatile biocompatible interfaces with multiple cellular structures.

Much like the ordered alignment of inorganic particles, the patterning of active biomolecules along a predefined path is a significant challenge; however, it is critical for sensing, analytical, imaging, and tissue-engineering applications.[7] Therefore, we explored the anchoring of biological moieties onto FtsZ scaffolds on the basis of the biotin–avidin interaction, which is widely used for protein immobilization.[30] FtsZ monomers were biotinylated by fusion of the peptide motif GLNDIFEAQKIEWHE, which is commonly used for in vivo protein biotinylation.[30] Biotinylated monomers not only maintained their assembly properties but also directed the specific binding of fluorescent avidin molecules

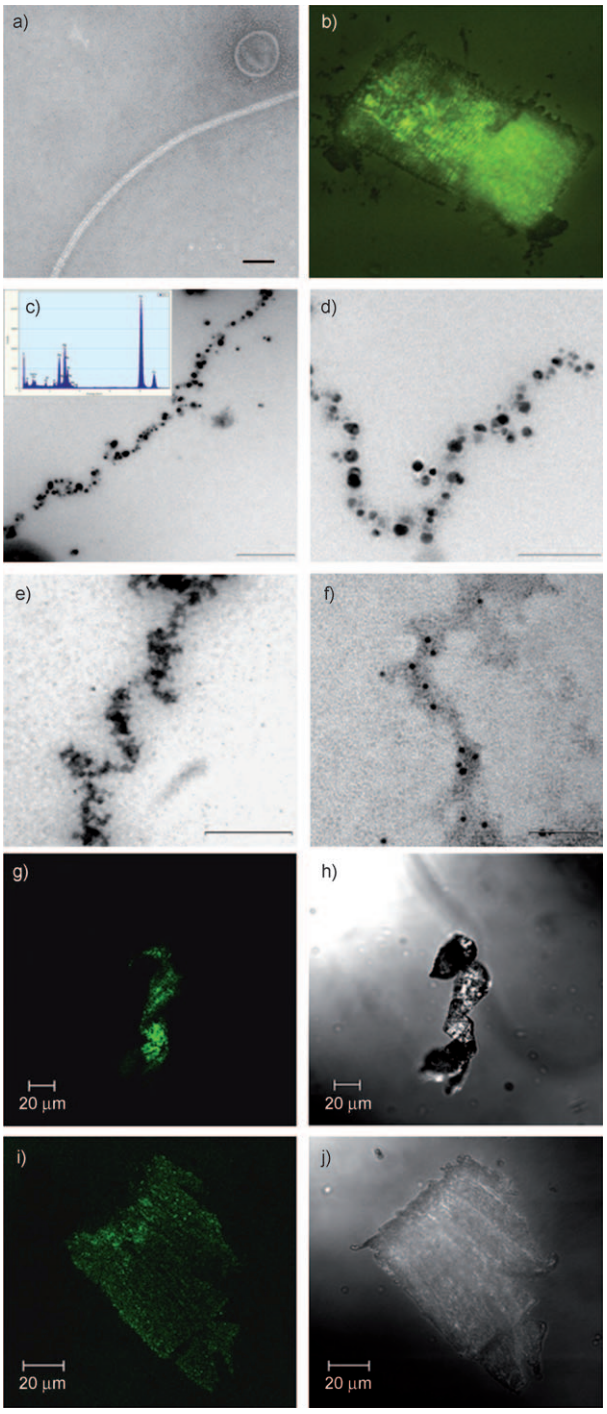

Figure 2. Specific binding of organic and inorganic materials by FtsZ-based self-assembled structures. a) TEM image of filaments and a miniring of wild-type *E. coli* FtsZ. Scale bar: 50 nm. b) Light-microscope image of a His-FtsZ sheet coordinated to nickel ions (×10 magnification). c,d) TEM images of silver-reducing FtsZ filaments coated with silver particles. Inset: EDX material analysis. No negative stain was used for sample preparation. Scale bars: c) 200 nm, d) 100 nm. e,f) TEM images of gold-coated FtsZ filaments. Scale bars: e) 200 nm, f) 50 nm. g,h) Confocal images showing fluorescent avidin specifically bound to biotinylated FtsZ structures.

to FtsZ helices and surfaces, as observed by confocal microscopy (Figure 2 g–j). More elaborate biological molecules, such as receptor and antibody molecules, could readily be patterned in the same way.

In the absence of an external manipulation device, an alternative route for spatial control of the assembly process is necessary. Remarkably, this problem can be addressed by harnessing the existing functionality of FtsZ–peptide building blocks without additional manipulation. Biotinylated FtsZ fibers were shown to anchor spontaneously to avidin-coated magnetic beads upon polymerization (Figure 3 a). By electron microscopy, we observed both singly anchored fibers that extended outward from a single bead (Figure 3 c; see also Figure 3 in the Supporting Information), and doubly anchored fibers, whereby two beads were essentially connected by a fiber anchored to both (Figure 3 d–g; see also Figure 3 in the Supporting Information). The abundance and unique directionality of singly anchored fibers, as observed by TEM analysis, provide evidence for filament anchoring, as non-anchored FtsZ filaments tended to align with one another.[28] The filaments were 2–4 nm thick and 200–800 nm in length. Interestingly, the doubly anchored elements included both single filaments (Figure 3 f,g) and aligned sheets (Figure 3 d,e). We intend to use this technique to study the anchoring of additional biological constructs for biomimetic applications, such as tissue engineering and biofilm formation.

Taken together, our results show FtsZ filaments to be efficient soft scaffolds for the robust, inexpensive, and highly specific assembly of inorganic materials and biomolecules. Readily adaptable to other scaffolds, this effective fabrication technique could also be used to construct multiple-component fibers through the assembly of versatile biointeractive building blocks. We are rapidly approaching the era of programmed materials assembly at the nanometer scale, whereby the convergence of technologies in the nano- and biological sciences can provide novel materials for molecular-scale medicine and computation. Such interdisciplinary research requires a profound understanding of the structure and dynamic behavior of molecular building blocks. Thus, the exploitation of protein scaffolds and recombinant DNA technology is of key importance for bottom-up fabrication techniques.

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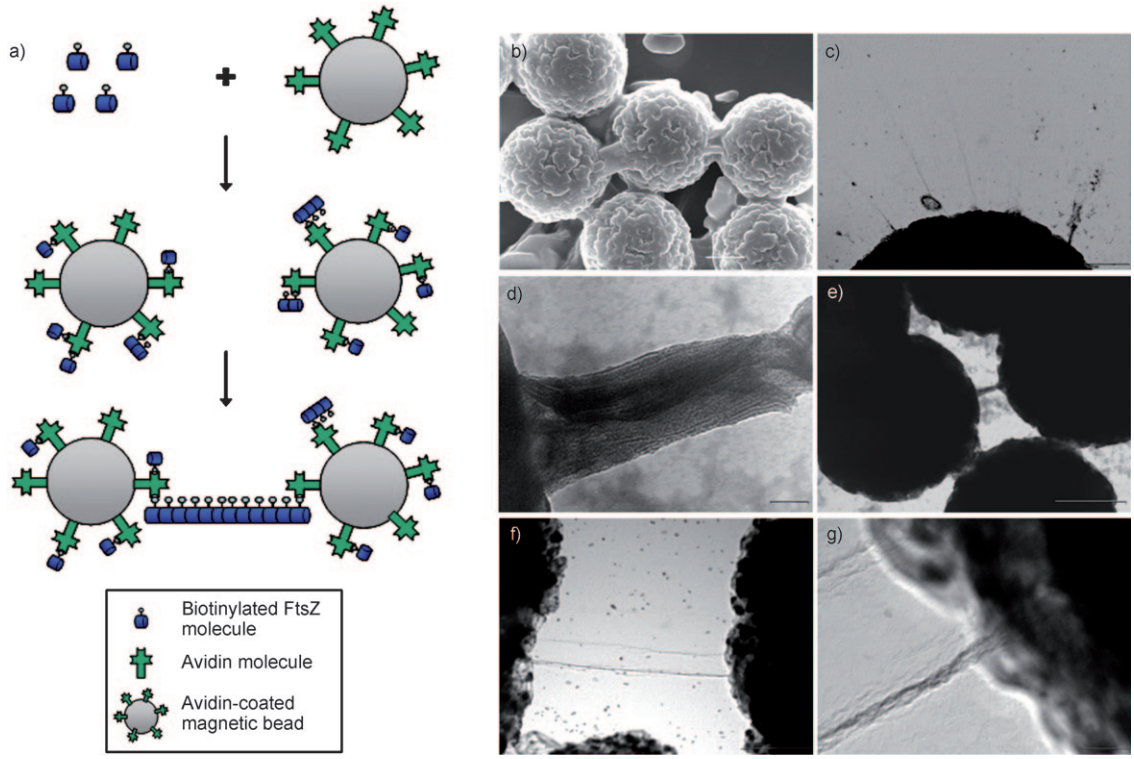

Figure 3. Biotinylated FtsZ filaments as self-positioning wires. a) Schematic illustration of the formation of a biotinylated FtsZ connecting wire. Biotinylated FtsZ molecules are added to avidin-coated magnetic beads in solution. The monomers bind the beads through the biotin–avidin interaction. Upon the addition of GTP, FtsZ monomers spontaneously self-assemble to form a connective wire between two beads. b) SEM and c–e) TEM images of biotinlyated FtsZ filaments anchored to avidin-coated magnetic beads. Scale bars: b) 1 μm, c) 500 nm, d) 20 nm, e) 500 nm, f) 200 nm, g) 20 nm.

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