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Protein-coated nanofibers for promotion of T cell activity†

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We have demonstrated that stable Concanavalin A (Con A)-coated supramolecular nanofibers can be constructed by complexation of self-assembled mannose nanofibers with Con A. The extended multivalent Con A nanofibers bind strongly to T cell surfaces and promote T cell activation.

In the immune system of the human body, activation of T cells is an important process in the initiation and control of immune responses. T cells are generally activated by recognizing foreign antigens *via* T cell receptors (TCRs). When antigen peptides are loaded onto major histocompatibility complex (MHC) in antigen-presenting cells (APCs), peptide–major histocompatibility complex (pMHC) ligands are recognized by the T cell receptor (TCR) and the pMHC activates the T cells through the ligand-induced deformation and clustering of TCRs.[1] Following this step, TCR clusters can induce intracellular signal transduction and trigger immune response systems, suggesting that the regulation of TCR clusters can play an important role in immune function.[2,3] In current understanding, the permissive clustering model of TCRs shows that pMHC dimers are minimal binding species for the activation of TCRs and pMHC oligomers can also induce stronger T cell activation.[1,4] Various scaffolds for multivalent interactions, such as protein conjugates,[5] beads,[2,6,7] surface,[8,9] and polymers,[10,11] have been used as a manipulating tool for inducing cell receptor clustering. These architectures based on covalent bonds, however, could not be accessible to fit a suitable conformation for dynamic cell and their environments.[12] For example, because the beads and the surface-based multivalent ligands are immobilized differently from cell receptors, it is difficult to control their densities.[13] Moreover, most polymer chains and dendrimers adopt a globular shape rather than an extended linear conformation. This globular shape might not be adaptable for multivalent interactions with extended surfaces such as cell membranes (Fig. S1, ESI†).[14] In this regard, we envisioned that extended supramolecular nanofibers coated by the proteins using self-assembly could overcome this limitation. In fact, many biological processes are driven by self-assembly of unimolecules as follows: formation of cell membrane, DNA double helix, tubulins, and various protein tertiary or quaternary structures.[15] In addition, dynamic and reconstructive self-assembled multivalent architectures from homogeneous building blocks would be better biocompatible and biomimetic materials than traditional covalent multivalent scaffolds.[12,14,15]

We have reported that carbohydrate-functionalized multivalent ligands could be constructed by the self-assembly of rod–coil block molecules.[16–19] These supramolecular ligands agglutinated effectively specific bacterial cells through carbohydrate-mediated multivalent interactions. Here, we describe protein-coated nanofibers that regulate T cell activities *via* multivalent interactions. The molecules that form the bioactive nanofibers consist of a laterally grafted rod–coil molecular architecture containing hydrophilic carbohydrate segments which can bind to a lectin protein, Con A (Fig. 1). The synthesis of laterally grafted rod amphiphiles **1** and **2** has been reported in a previous study.[19] These molecules self-assembled into elongated fiber-like objects in aqueous solution, which is consistent with the reported result (Fig. 2a and b).

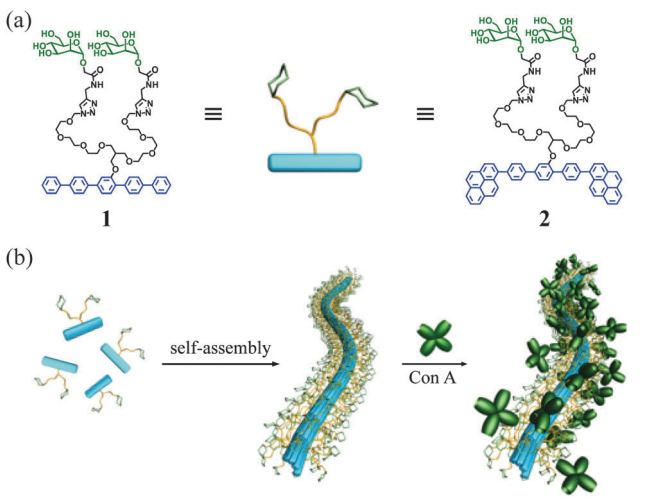

Fig. 1 (a) The chemical structure of amphiphiles **1** and **2**. (b) Representation of protein-coated nanofibers.

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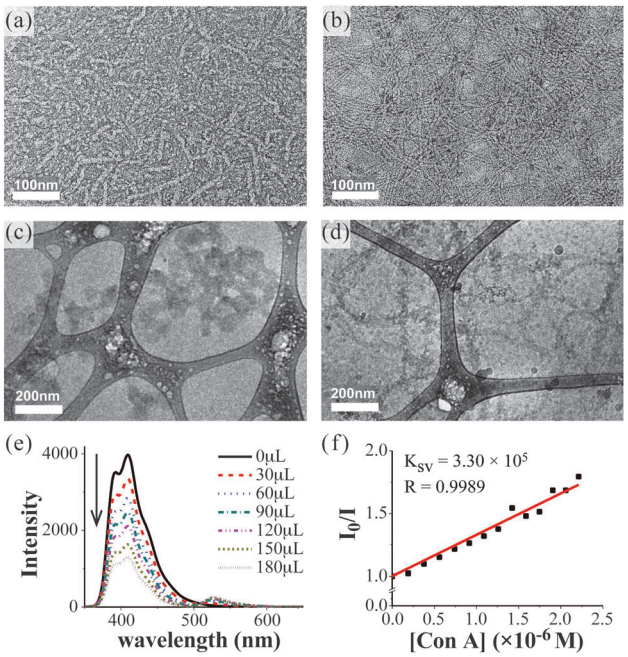

Fig. 2 TEM images of (a) short fibers of **1** and (b) long fibers of **2** in aqueous solution. Cryo-TEM images of (c) an irregular assembly of **1** with Con A, and (d) Con A-coated nanofibers of **2**. The results of FRET experiments upon the addition of fluorescein-labeled Con A (2 mg per mL), respectively, to a solution containing amphiphile **1**; (e) the whole fluorescence spectra and (f) a Stern–Volmer plot of the fluorescence signal, λ_{ex} = 409 nm.

The negatively stained sample of **1** showed the formation of short nanofibers with lengths of 100–200 nm and a width of around 8 nm. While molecule **2** containing pyrene units at both ends of ter(*p*-phenylene) aggregated into much longer nanofibers than molecule **1**, with a uniform width of around 6 nm and a length of over 2 μm (Fig. 2b). Because the pyrene units triggered a stronger π–π stacking interaction between aromatic segments than the simple oligophenylene unit, **2** could self-assemble into extended nanofibers with enhanced conformational stability rather than **1**. These nanofibers consisting of hydrophilic exterior with a high density of mannose units are able to function as multivalent ligands binding lectin protein, Con A. To investigate the multivalent interactions of the nanofibers, we have added Con A (2 mg per mL, 0.2 equiv. of mannose residues) into the solutions of **1** and **2** (1.2 mM mannose residues), respectively, resulting in Con A-tethered nanofibers. Cryogenic transmission electron microscopy (cyro-TEM) revealed that **1** formed irregular aggregates with the addition of Con A, indicating that the nanofibers of **1** are not stable in a Con A solution. The strong carbohydrate–protein interactions of **1** seemed to frustrate the weak self-assembly of loosely packed penta-*p*-phenylene units of the molecule. In great contrast, the nanofibers of **2** showed an unchanged fibrillar shape even after the addition of Con A. Cryo-TEM images of **2** with Con A revealed that the length of the fibers is several micrometers with a thickness of ~40 nm. Considering that the size of Con A is ~10 nm, this dimension of the thickness indicates that the nanofibers are coated with lectin proteins on their exterior while maintaining their fibrillar structures. This result demonstrates that the packing arrangements of the aromatic segments based on the pyrene units within internal cores play a critical role in forming protein-tethered stable nanostructures.

Fluorescence resonance energy transfer (FRET) could provide one of the good methods to monitor the formation of soluble Con A–ligand complexes.[20] Subsequently, FRET experiments were carried out with the mixtures of fluorescein-labeled Con A and amphiphiles **1** and **2**, respectively. As the amount of labeled Con A in a solution containing amphiphile **1** increased, the intensity of fluorescence emission associated with the pentaphenylene segments decreased (Fig. 2e). Similar to **1**, **2** also showed the fluorescence quenching of the aromatic segments upon the addition of the fluorescein-labeled Con A (Fig. S2a, ESI†). On the basis of the fluorescence titration spectra, the Stern–Volmer constants (K_{sv}) of amphiphiles **1** and **2** by Con A were found to be 3.30×10^5 and 3.72×10^5, respectively, predominantly through static quenching (Fig. 2f and Fig. S2b, ESI†).[21] It demonstrated that the binding affinity was not significantly different between each sample with Con A. This result indicates that the carbohydrate ligands effectively bind Con A through specific ligand–protein interactions.

Con A-coated nanofibers are expected to bind T cell surfaces through multivalent interactions between the carbohydrates on the cell surfaces and multivalent lectin proteins to activate T cells. Along this line, the effect of supramolecular multivalent protein nanofibers on live T cells was evaluated using ELISA assay for the induction of interleukin 2 (IL-2) in the Jurkat cells (human T lymphocyte). Upon the treatment of Con A and Con A-coated nanostructures, the Jurkat cells were activated and released IL-2. Con A could induce intracellular signal transduction and cause Jurkat cells to release 100 to 300 times as much IL-2 as lectin-stimulated normal human peripheral blood lymphocytes.[22] In our experiment, Jurkat cells (1.0×10^5 per mL) were incubated with a preincubated solution of mannose-functionalized nanofibers and Con A at 37 °C and 5% CO_2. In the cytotoxicity test, the value of viability is not significantly different between each sample, demonstrating that Con A-tethered nanostructures are cytocompatible without sacrificing cell viability (Fig. 3a). The activation of mitogen-stimulated T cells can be measured by secreted IL-2 after 2 to 4 hours and mainly occurs during the first 48 hours.[23,24] After 24 hours of cell culture, cell media were harvested and were used for the test in the IL-2 ELISA assay. Fig. 3b shows that the in the premixed solution of **2** and Con A there is increased production of IL-2, approximately 80% than Con A alone. In the case of **1** and Con A, however, the amount of IL-2 secretion displays similar values to the mannose fiber of **2** without Con A, indicating no activation of T cells. This result suggests the enhanced binding affinity of multivalent Con A-coated nanofibers which can surround more receptors on the cell surface than other extracellular materials. The extended and long nanofibers formed from the self-assembly of amphiphile **2** could induce stronger Con A–receptor multivalent interactions for receptors clustering than Con A tetramers alone (Fig. 3c and e).

Subsequently, the clustering of the receptors triggers intracellular signal transduction and secretion of IL-2. In contrast, short nanofibers formed, from the self-assembly of amphiphile **1**, would probably disrupt multivalent systems due to the destruction of supramolecular nanofibers and irregular aggregation after forming the lectin–mannose complex. Consequently, the binding pockets of Con A would be blocked by mannose-coated irregular aggregates to inhibit the binding activity to cell surfaces (Fig. 3d). This is also reflected in the formation of discrete aggregates upon the addition of

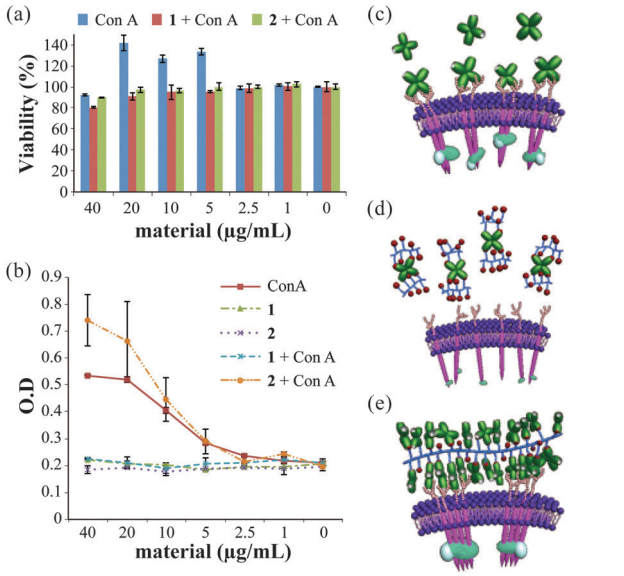

Fig. 3 (a) The viability test using the water-soluble tetrazolium salt (WST) method of Jurkat cells grown with Con A, **1** + Con A and **2** + Con A for one day. (b) Comparison of IL-2 production by Con A and scaffolded Con A. The error bars in (a) and (b) indicate the standard deviations from three experiments performed in duplicate, and some are smaller than the symbols. Schematic representation of IL-2 production depending on the fiber length. (c) Con A alone, (d) **1** + Con A and (e) **2** + Con A.

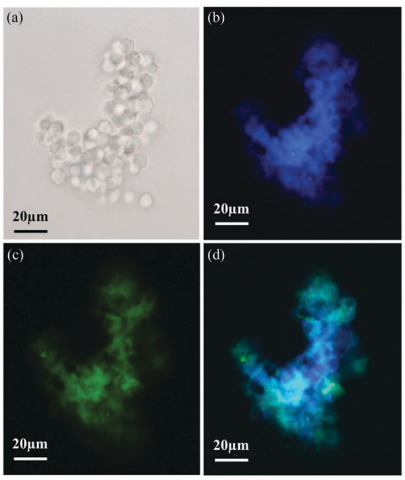

Fig. 4 Co-localization studies of the Con A-coated nanofibers with the Jurkat cells. (a) Optical images of the Jurkat cells, (b) blue fluorescence image derived from amphiphile **2**, (c) green fluorescence image derived from fluorescein-labelled Con A and (d) fluorescence image merged (b) with (c).

Con A into the solution of **1** (Fig. 2c). To further address this issue, we have performed fluorescence optical microscopy (FOM) experiments with the solutions when they are incubated with Jurkat cells (Fig. 4). The amphiphiles **1** and **2** contain aromatic rods which can emit fluorescence light by absorbing light from a suitable excitation source. When labelled Con A-tethered nanofibers were bound to the T cell membrane, the fluorescence emission would be observed in cell surfaces and visualized using FOM. Upon adding the premixed solution of compound **2** and labelled Con A into the Jurkat cell culture, the fluorescence emission of the nanofibers was observed on the cell surfaces with cell aggregation, indicating that the Con A-coated nanofibers were placed on the cell surfaces through multivalent interactions. In great contrast, apparent fluorescence emission was absent on the cell surfaces with the irregular aggregates of **1**, indicating the aggregates of **1** do not bind to the cell surfaces. These results demonstrate that the construction of multivalent protein ligands can be an essential tool to promote T cell activation.

In summary, we have demonstrated that controlling the conformation and stability of the nanofibers tethered by lectin proteins can regulate T cell activation. The lengths as well as stability of the protein-coated supramolecular nanofibers could be manipulated by a small variation in the rod–coil molecular structure. Elongated nanofibers with stronger aromatic interactions maintained their fibrillar shape even after tethering Con A, which was not possible with the short and weakly assembled nanofibers. Notably, extended and long multivalent ligands promote T cell activation compared with monovalent Con A ligands, observed by the secretion of IL-2 and the fluorescence emission. These long and flexible supramolecular multivalent architectures could arrange the desired receptors regularly and be adapted to the mobile cytoskeleton simultaneously. A self-assembled multivalent architecture based on aromatic rod–dendritic coil can offer many opportunities for developing biocompatible and bio-responsive systems.

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