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Noncovalent Modification of Carbon Nanotubes with Proteins via Biotinylated Peptides Having a Binary Pattern within the Sequences

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We describe peptide-based solubilization and functionalization of multiwalled carbon nanotubes (MWNTs), in which biotinylated peptides that are physically adsorbed onto the sidewalls of MWNTs can anchor proteins of interest via specific interactions.

Carbon nanotubes (CNTs) possess unique optical, electrical, and mechanical properties and have potential biological applications such as in biosensors, drug delivery, and tissue support to artificial muscles.[1,2] To date, functionalization of the sidewalls of CNTs with biological molecules has been demonstrated, in which *c*-type heme proteins and ferritins were immobilized onto the sidewalls of carboxylate-functionalized CNTs,[3] plasmid DNA onto amino-functionalized CNTs,[4] and streptavidin onto covalently biotinylated CNTs.[5] However, in those cases, the sidewalls of the CNTs were chemically modified under harsh conditions, probably causing disruption of the electronic conjunction in the CNTs, and requiring nonenvironmentally friendly production conditions for CNT functionalization.

Several noncovalent approaches for rapid manufacturing have been achieved for the dispersion of CNTs in water by low-molecular-weight surfactants (e.g., lithium dodecyl sulfate),[6] pyrene-carrying ammonium ions (e.g., trimethyl(2-oxo-2-pyren-1-ylethyl)ammonium bromide),[7] large molecules such as polystyrene sulfonate[8] and poly(L-lysine),[9] and designed peptides.[10–13] However, protein alignments along CNTs have been limitedly reported so far. Balavoine et al. demonstrated helical crystallization of proteins on the sidewall of a CNT, in which CNT dispersion and direct adsorption of proteins onto CNT surface were attempted in organic solvent-containing aqueous solution.[14] Chen et al. reported nonspecifically functionalized CNT with proteins via covalent amide bonds provided by a reaction of 1-pyrenebutanoic acid succinimidyl ester adsorbed onto the CNT sidewall and an amino group of the protein surface.[15] However, these methods sometimes cause denaturation and inactivation of proteins.

Very recently, Yamashita and co-workers reported noncovalent alignment of *Listeria innocua* Dps protein conjugated with a carbonaceous material-binding sequence (NHBP-LiDps), in which CNTs were sonicated in the presence of NHBP-LiDps in water for the solubilization and functionalization of CNTs at the same time.[16] Although this approach is quite straightforward for functionalizing CNTs, it is possible that protein structures are damaged by sonication. Thus, development of methods solubilizing CNTs in water and functionalizing their sidewalls with water-soluble small molecules, which are easy to design and synthesize and enable to site-specifically immobilize proteins by a tag-system, is still attractive. Here, we describe peptide-based solubilization and functionalization of CNTs, in which biotinylated peptides that are physically adsorbed onto the sidewalls of CNTs can anchor proteins of interest via specific interactions.

Previously, peptides named **RU-001** and **RU-002**, each a nine-residue β-sheet-forming peptide, were designed and synthesized to examine their self-assembled nanostructures in water.[17] The β-sheet structure of peptides allows us to align hydrophobic and hydrophilic amino acids alternately to form a binary pattern within the sequence.[18] This structural property seems promising to solubilize CNTs by hydrophobic interactions and π–π stacking with aliphatic/aromatic side chains at one side of the β-sheet-forming peptide, and to disperse them stably by charged side chains facing the bulk aqueous media. Biotinylation of the peptide is expected to anchor proteins of interest, including anti-biotin antibody (anti-biotin IgG) and streptavidin, via specific interaction(s) (tag-system).

In this paper, we designed eight new amphiphilic peptides based on **RU-002**,[17] and demonstrated functionalization of multiwalled carbon nanotubes (MWNTs) with anti-biotin IgG carrying gold nanoparticles (AuP), in which CNT–protein conjugation was visualized by monitoring AuP localization with transmission electron microscopy (TEM) (Figure 1). MWNT (5–13 nm in o.d. $\times$ 2.5–20 μm in length) was chosen in this study to clearly discriminate CNTs from other fibrous materials, for example, peptide nanofibers constructed by β-sheet-forming peptides during CNT dispersion in TEM observations.

RU-003 was designed to have two isoleucines (Ile) and a 2-naphthylalanine [Nal(2)] at one face, which provide the driving force for adsorption onto the sidewalls of MWNTs through hydrophobic interactions and π–π stacking (Figure 2). Alanines (Ala) and glutamic acids were placed at the other side to make the CNT–peptide conjugates water-soluble. A Lys residue at the hydrophobic face was expected to interact with MWNTs through cation–π interactions as seen in the solubili-

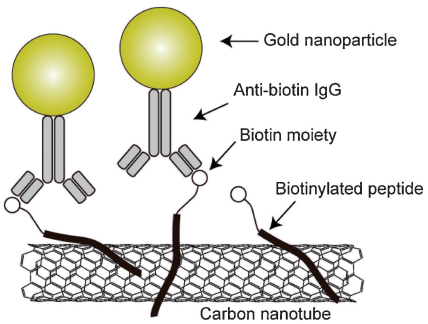
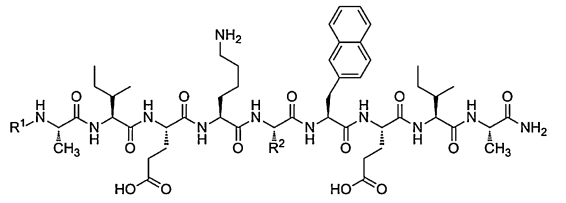
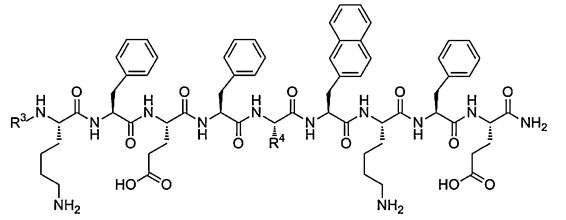


Figure 1. Representative illustration of alignment of anti-biotin antibodies along a CNT via biotinylated peptides. Protein localization is visualized by monitoring gold nanoparticles on TEM.



Compound	R^1	R^2
RU-003	Ac-	CH_3-
RU-010	Biotin-$(Aeea)_2$-	CH_3-
RU-021	Biotin-$(Aeea)_4$-	CH_3-
RU-022	Biotin-$(Aeea)_6$-	CH_3-
RU-020	Ac-	Biotin-$(Aeea)_2$-$NH(CH_2)_4$-



Compound	R^3	R^4
RU-035	Ac-	CH_3-
RU-036	Biotin-$(Aeea)_2$-	CH_3-
RU-037	Ac-	Biotin-$(Aeea)_2$-$NH(CH_2)_4$-

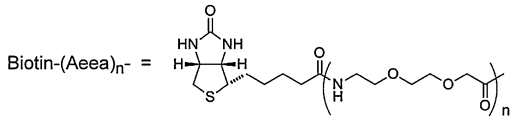


Figure 2. Amino acid sequences of peptides examined in this study.

zation of CNTs with poly(L-lysine).[9] Biotinylation of the *N*-terminus of **RU-003** via two, four, and six repeats of [2-(2-aminoethoxy)ethoxy]acetic acid (Aeea) was designed as **RU-010**, **RU-021**, and **RU-022**, respectively. **RU-020** with a biotin moiety at the hydrophilic center of **RU-003** through a Lys side chain was also designed. Furthermore, a peptide with phenylalanines (Phe) and Lys instead of Ile and Ala residues in **RU-003** was designed as **RU-035** to increase aromaticity and water solubility of the peptide, respectively. Biotinylation of the *N*-terminus and hydrophilic center of **RU-035** via two repeats of Aeea was also used to design **RU-036** and **RU-037**, respectively. Peptide synthesis and sample preparations for measurements are detailed in Supporting Information.[19]

First, we analyzed the secondary structures of eight different peptides by ATR-FTIR measurements (Figure S1). ATR-FTIR spectra (amide I region) of peptide films revealed that all the peptides exhibited a strong amide I band around 1625 cm^{-1} corresponding to a β-sheet conformation.[20,21] However, with increasing the number of repeats of an Aeea unit in **RU-003**-based peptides **RU-010**, **RU-021**, and **RU-022**, the contents of a random coil structure (peak around 1655 cm^{-1} in ATR-FTIR spectra) increased, due to steric hindrance by long spacers for a self-assembly.

With such structural information in hands, we attempted to utilize **RU-003**-based peptides to align anti-biotin IgGs along

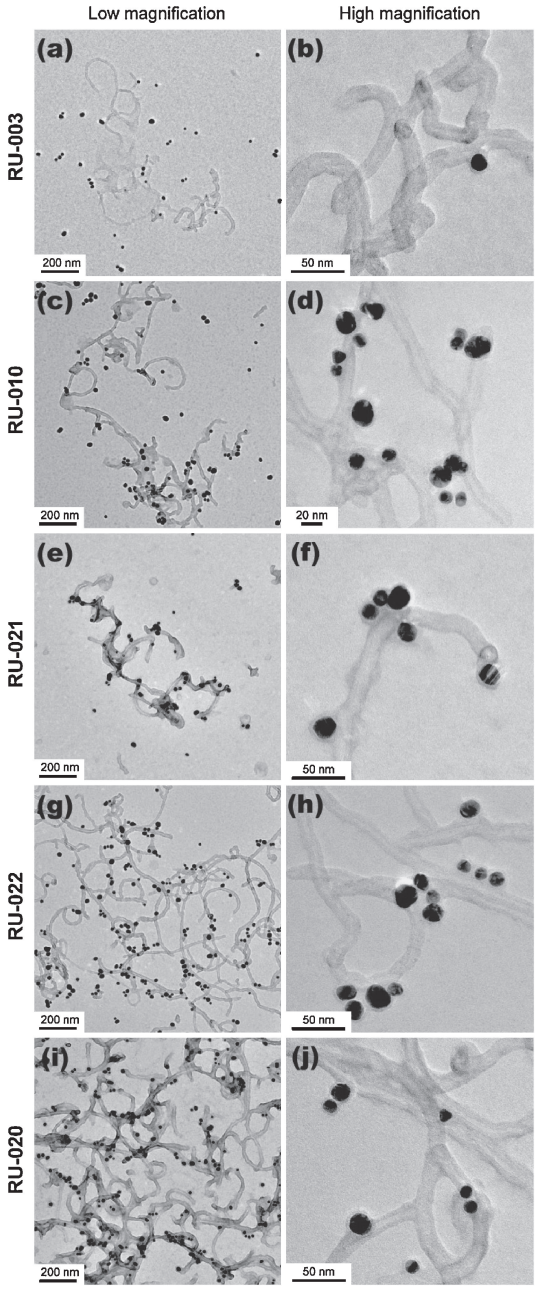


Figure 3. TEM images of MWNT decorated with gold nanoparticle-conjugated anti-biotin IgG via **RU-003** (a, b), **RU-010** (c, d), **RU-021** (e, f), **RU-022** (g, h), and **RU-020** (i, j), respectively (right column: high magnification; left column: low magnification).

MWNTs via specific interactions (Figure 3). TEM observations revealed that AuPs were scarcely bound to MWNT–**RU-003** conjugates (low background signal), probably due to lacking a biotin moiety in the sequence. Conversely, many AuPs were observed on MWNTs solubilized with each biotinylated peptide, **RU-010**, **RU-021**, **RU-022**, and **RU-020**. Average distances between gold nanoparticles on MWNTs were estimated to be ca. 90, 90, 70, and 70 nm for **RU-010**, **RU-021**, **RU-022**, and **RU-020**, respectively. However, (i) the length of the Aeea spacer between a biotin moiety and the nine-residue peptide sequence,

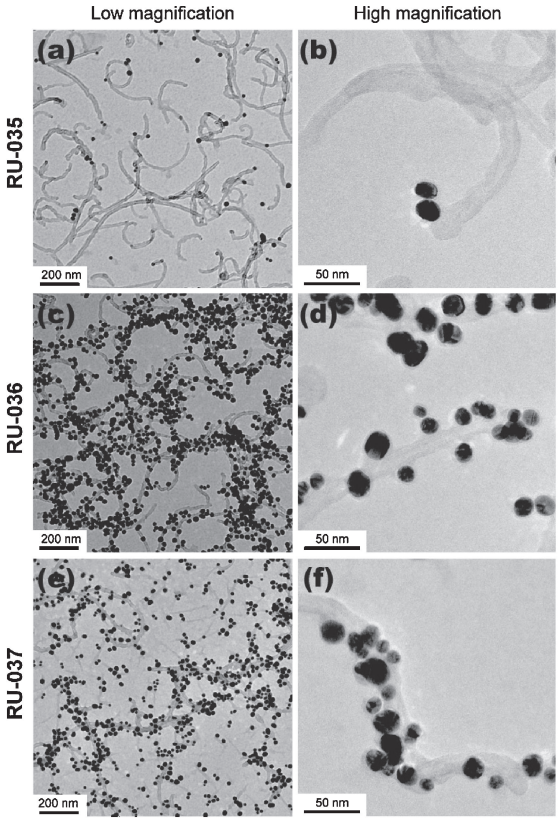


Figure 4. TEM images of MWNT decorated with gold nanoparticle-conjugated anti-biotin IgG via **RU-035** (a, b), **RU-036** (c, d), and **RU-037** (e, f), respectively (right column: high magnification; left column: low magnification).

and (ii) the biotinylating site (*N*-terminus or hydrophilic center) slightly affected the numbers of gold nanoparticles as observed in the TEM images for **RU-010**, **RU-020**, **RU-021**, and **RU-022** (Figure 3).

Meanwhile, the peptides with increased aromaticity, **RU-035**–**RU-037** compared with **RU-003**-based peptides, were examined to align anti-biotin IgGs along MWNTs. Figure 4 shows that **RU-035** lacking a biotin moiety anchored very few AuPs in the TEM image, probably due to lacking a specific interaction mechanism. Both **RU-036** and **RU-037** exhibited markedly increased numbers of AuPs in their TEM images compared with **RU-035**. Average distances between AuPs on MWNTs were estimated to be ca. 30 nm for both **RU-036** and **RU-037**. These resulted in no great differences in the number of AuPs bound to the sidewalls of MWNTs, indicating that the position of the biotin moiety in **RU-036** and **RU-037** sequences had a limited effect on the accessibility of anti-biotin IgG to the MWNT surfaces. In the TEM images of MWNT–**RU-037** conjugate decorated with AuPs (Figure 4e), MWNTs coexisted with some peptide nanofibers, the formation of which might compete against peptide adsorption onto MWNTs, and which was less reactive than peptides on the MWNTs.

Surprisingly, great differences between the numbers of AuPs on MWNTs anchored by **RU-010** and **RU-036** were observed as shown in Figures 3c and 4c, in which the number of repeats of Aeea was the same at $n = 2$ and the β-sheet contents in ATR-FTIR spectra were not significantly different. These results might suggest that two repeating Aeea molecules are far enough from the sidewalls of MWNTs for immunoreaction between biotin and antibodies and that the number of biotinylated peptides adsorbed onto MWNTs is an important factor for alignment of proteins along CNTs. However, peptide conformations on the MWNT sidewalls are still unclear, which must be addressed in the near future.

In conclusion, we developed biotinylated peptide-based CNT dispersion in water and the alignment of IgGs along CNTs. This platform is potentially useful to align desired proteins that are conjugated with anti-biotin IgG or streptavidin, meeting the desire for a sustainable, environmentally friendly fabrication process.

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