

Electronic Supplementary Information

A novel bifunctional protein supramolecule for construction of carbon nanotube-titanium hybrid material.

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Supplementary materials, methods and results

Construction and purification of CDT1 and DT

To obtain the synthetic gene encoding CDT1, the DNA fragment was amplified by whole vector PCR using primers (5'-tttggatcgc aattcgagct ccgtcg-3' and 5'-tttggatcct tacgcatcgc gaagtttgcg catttctaatt ggagcttttc-3') and pET20b-NHBP-LiDps plasmid¹ as a template. The pET20b-NHBP-LiDps plasmid contained the gene encoding *Listeria innocua* Dps fused with only NHBP-1 at the N-terminal region (NHBP-LiDps). The PCR product was digested with *Bam*HI and the resulting fragment was self-ligated. pET-CDT1 plasmid encoding CDT1 was obtained. In addition, pET-DT encoding Dps fused with minTBP-1 in a C-terminal region (Dps-Titanium binding peptide, DT) was also constructed following the procedures described above. To obtain the pET-DT plasmid, the PCR product was amplified by using the primers (5'-tttcatatgt atatctcctt cttaaagtta aac-3' and 5'-tttcatatga tgaacaacaat caactcagta g-3') and pET-CDT1 as template. The PCR product was degraded with *Nde*I and was self-ligated. Those plasmids encoding Dps-variants were introduced into *Escherichia coli* BL21(DE3), respectively. Transformants were cultivated in LB medium with ampicillin at 37 °C for 24 hours. CDT1 and DT were purified from bacteria using a previously reported modified *L. innocua* Dps purification method (Fig. S1).² NHBP-LiDps was purified by a protocol previously reported.¹ To purify CDT1, BL21(DE3)/pET-CDT1 was disrupted by ultrasonication and was centrifuged at 6,000 rpm for 5 min to remove precipitates. The supernatant was treated at 60 °C for 20 min, and was centrifuged at 6,000 rpm for 5 min. The supernatant of heat treated sample was applied to gel filtrated column (HiPrep 26/60 Sephacryl S-300 High resolution, GE healthcare, USA) with 50 mM TrisHCl buffer (pH8.0) containing 150 mM NaCl. The gel filtrated sample was applied to an anion-exchanger column (HiLoad 26/10 Q-Sepharose High Performance, GE healthcare, USA). CDT1 was eluted with a NaCl gradient in 50 mM TrisHCl buffer (pH8.0). The size of obtained CDT1 was analyzed by size-exclusion chromatography (Fig. S3). The purified CDT1 was finally stored in water and was used for experiments.

Biominingeralization of iron nanoparticle in the interior cavity of CDT1.

The solution containing 0.5 mg/ml CDT1 and 1 mM ammonium iron sulfate in 80 mM HEPES buffer (pH7.5) was incubated at 4 °C for 3 hours. After incubation, the solution including CDT1 with an iron oxide nanoparticle core (Fe-CDT1) was centrifuged at 15,000 rpm for 5 min to remove bulk precipitates. The supernatant containing Fe-CDT1s was concentrated by centrifugal ultrafiltration (Amicon Ultra, Millipore, MA), and was replaced with 50 mM Tris-HCl buffer, 150 mM NaCl (pH8.0). Several micro liters of the solution containing Fe-CDT1s was put on the carbon film coated TEM mesh, and excessive solution was removed. The Fe-CDT1s were stained with 3 % phosphotungstic acid (PTA) and were analyzed by TEM (Fig. S2b, JEM-2200FS, JEOL, Japan).

Determination of kinetic parameters of Dps variants

The adsorption of Dps-variants on SWNTs or TiO₂ was analyzed by a QCM technique (Affinix QNμ, Initium Inc., Japan). The resonance frequency of a sensor was measured with a resolution of 1 Hz at a sampling interval of 0.1 s. The AQUA software (ver. 3, Initium Inc., Japan) was then used to determine the kinetic parameters. The change of resonance frequency (*f*) was described by following equation³;

$$\Delta f_t = \Delta f_{\text{Max}} \cdot \{1 - \exp(-k_{\text{obs}}t)\}$$

$$k_{\text{obs}} = [P] \cdot k_{\text{on}} + k_{\text{off}}$$

Δf_t : the resonance frequency decrease at time *t*, Δf_{Max} : Maximum of the resonance frequency decrease, [P]: the

12-metric protein concentration in reaction buffer (CDT1; 246 kDa, NHBP-LiDps; 236 kDa, and DT; 226 kDa), k_{on} : binding rate constant, k_{off} : dissociate rate constant.

Construction of SWNT-CDT1 conjugate at various pH conditions

It was anticipated that the pH condition would be critical for CDT1-SWNT interaction. Solution pH greatly affects the electrostatic interaction among CDT1. Too strong electrostatic repulsive force will result in the SWNTs being covered sparsely by CDT1 and too weak leads to aggregation of CDT1s. Therefore, we tried to mix SWNT and CDT1 under various solution pHs and observed the interaction behaviors of CDT1s and SWNTs by TEM. The SWNTs in 50 mM citrate buffer pH 4.0, 50 mM potassium phosphate buffer pH 6.0, 50 mM HEPES buffer pH 7.5, 50 mM Tris-HCl buffer pH 9.0 and 50 mM glycine buffer pH 10.5 were prepared. The SWNT solutions were mixed with CDT1 and made to a final concentration of 0.5 mg/ml CDT1 and 0.3 mg/ml SWNT. The mixtures were treated by ultrasonic sonication (1 s on/3 s off, amplitude 20%, Digital Sonifier 450, Branson, USA) in an ice bath for 5 min. After ultrasonic treatment, the mixtures were centrifuged at 15,000 rpm for 5 min to remove unreacted SWNTs. The supernatant was retrieved and aliquots were observed with TEM. The adsorption of CDT1 with SWNTs was confirmed in buffers of pH 6.0, pH 7.5, and pH 9.0. As pH value increased from 6 to 9, CDT1s showed a tendency to bind with not only SWNT, but also each other. Therefore, CDT1s could bind around SWNT efficiently in the pH 6.0 buffer. In contrast, the CDT1 was not able to bind SWNTs in buffers at pH 4.0 and pH 10.5. We concluded that the best condition for making conjugates of CDT1 and SWNT was 50 mM potassium phosphate buffer at pH 6.0. In contrast, complexes of SWNT and DT protein lacking the NHBP-1 aptamer could not be obtained.

Mineralization activity of SWNT.

A 0.3 mg of SWNT was dispersed in 0.95 ml of 53 mM potassium phosphate buffer (pH6.0). The 50 μ l aliquots of a 50 %wt solution of titanium (IV) bis-(ammonium lactato)-dihydroxide (Ti[BALDH], Sigma Aldrich, MO, USA) was added to 0.95 ml of the SWNT suspension with mixing and incubated at 24°C for 16 hour. After the incubation step, the solution was centrifuged for 5 minutes at 15,000 rpm. The pellet was recovered and washed 3 times with distilled water. The product was stained with 3 % PTA and was analyzed by TEM (Fig. S4). The SWNTs were not coated by titanium-layer.

Supplementary figures

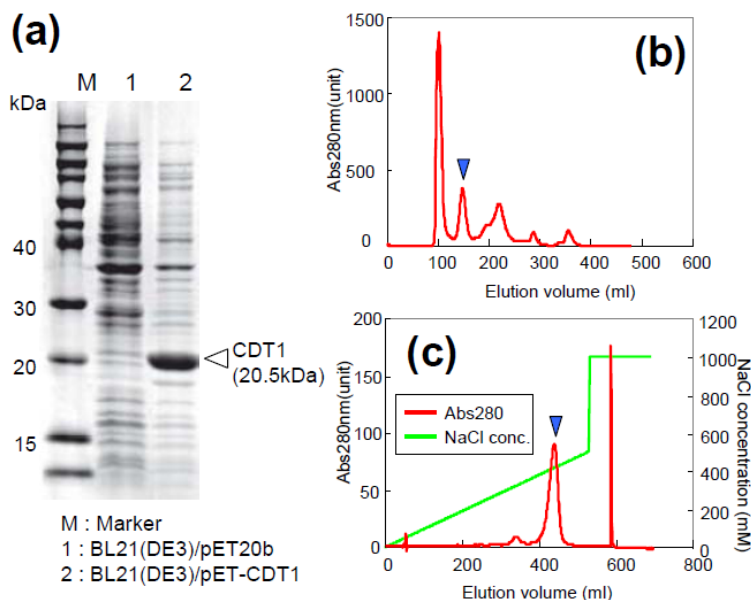


Fig. S1 Expression and purification of CDT1. (a) Expression of protein was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using TGX gel (Any kD, Bio-Rad, USA) followed by coomassie brilliant blue staining. Lane M; size marker, lane 1; broth of BL21(DE3)/pET20b, lane 2; broth of BL21(DE3)/pET-CDT1. (b) Purification of CDT1 by gel filtrated column. BL21(DE3)/pET-CDT1 was disrupted by ultrasonication and heat treatment. The Supernatant of sample was applied to gel filtrated column

(HiPrep 26/60 Sephacryl S-300 High resolution) with 50 mM TrisHCl buffer (pH8.0) containing 150 mM NaCl. (c) Purification of CDT1 by anion-exchanger column. The gel filtrated protein was applied to an anion-exchanger column (HiLoad 26/10 Q-Sepharose High Performance). The CDT1 eluted with a NaCl gradient in 50 mM TrisHCl buffer (pH8.0). The fraction indicated by blue arrow contained CDT1.

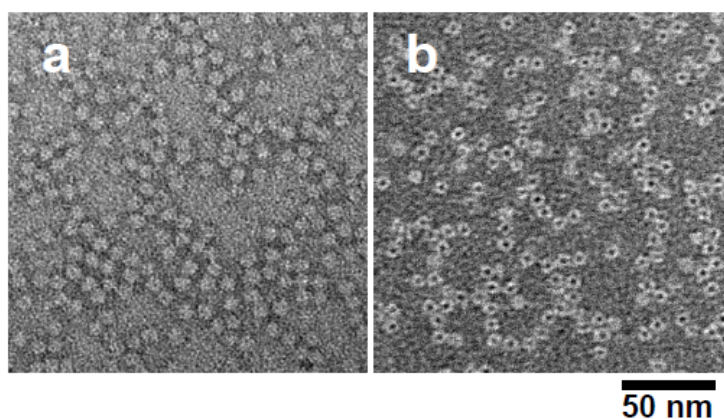


Fig. S2 TEM image of (a) apo-CDT1, and (b) Fe-cored CDT1. The ratio of CDT1s which had iron core was approximately 90 % of all CDT1s. The samples were stained with 3 % phosphotungstic acid (PTA). Since native Dps is capable of forming various kinds of metal NPs within its cavity where source ions go through the channels in the protein shell,² cores should be formed in the variant cavity, if the variant cage was complete. We tried iron oxide NP core synthesis in the interior of CDT1 using ammonium iron sulfate solution. We clearly observed via TEM that iron oxide NPs 4–5 nm in diameter were formed inside the CDT1. It was apparent that the mineralization activity of Dps was not hampered by addition of NHBP-1 and minTBP-1 at both terminal ends.

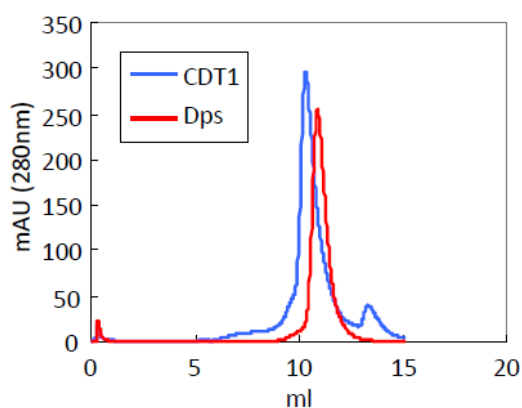


Fig. S3 The CDT1 and Dps were applied to gel filtrated column (TSK-GEL BioAssist G4SWXL 7.8 mm x 300 mm, TOSOH, Japan) with 50 mM TrisHCl buffer (pH8.0) containing 150 mM NaCl, respectively. The elution volume of CDT1 was slightly smaller than that of Dps. The result indicated that the size of CDT1 is slightly larger than that of Dps.

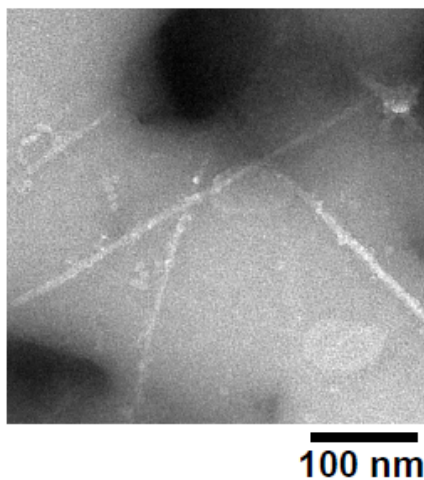


Fig. S4 TEM image of SWNT mixed with titanium precursor. The SWNT-titanium conjugate could not be obtained.

Supplementary reference

1. M. Kobayashi, S. Kumagai, B. Zheng, Y. Uraoka, T. Douglas and I. Yamashita, *Chem. Commun.*, 2011, **47**, 3475-3477.
2. K. Iwahori, T. Enomoto, H. Furusho, A. Miura, K. Nishio, Y. Mishima and I. Yamashita, *Chem. Mater.*, 2007, **19**, 3105-3111.
3. Y. Ebara, K. Itakura and Y. Okahata, *Langmuir*, 1996, **12**, 5165-5170.