Supplementary Information (ESI) for Chemical Communication

Engineered Carbohydrate-Binding Module (CBM) Protein-suspended Single-walled Carbon Nanotubes (SWNTs) in Water

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Electronic Supplementary Information

1. Protein sequence analysis and overexpression of the recombinant protein, *Ct*CBM4.

The parameters for molecular weight, theoretical pI, amino acid composition, and extinction coefficient were calculated by the ProtParam tool: http://www.expasy.org/tools/protparam.html. The sequence of the *CelK* gene isolated from *Clostridium thermocellum* was obtained from the Carbohydrate-Active Enzymes server. Its GenBank accession number is ABN51650 (CAZy website is [http://afmb.cnrs-mrs.fr/_pedro/CAZY/db.html]). The domain of CBM4 derived from *CelK* (termed as *Ct*CBM4) was amplified by PCR (polymerase chain reaction) templated by *C. thermocellum* genomic DNA. The PCR resulting fragment was subcloned into the *E. coli* plasmid, pET28b. The fusion protein product containing the engineered 6xhis-tag at the *C* terminus was overexpressed in *E. coli* BL21(DE3) under 0.1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside; Sigma-Aldrich, St. Louis, MO) induction at 37°C. Recombinant proteins were purified using the QIAexpress Ni-NTA protein purification system (Qiagen; Valencia, CA) in 50 mM Tris, pH 8.0 buffer supplemented with 300 mM NaCl and 0.03 % sodium azide. The purity of the fractionated proteins was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In order to remove the possibly remaining DNA and RNA, the purified protein was treated with deoxyribonuclease (DNAase from bovine pancreas; Sigma-Aldrich) and ribonuclease (DNAase and RNAase from bovine pancreas; Sigma-Aldrich). Final

working solutions of the purified protein were prepared by several buffer exchanges using the 5 kD macromolecular membrane from Millipore (Bedford, MA). The recombinant protein was stored in the purification buffer at 4°C.

2. Measuring the SWNT content in the *Ct*CBM4/SWNTs suspension.

The 500 μL *Ct*CBM4/SWNTs suspension was added to four carefully weighed Eppendorf tubes. The filled tubes were then incubated at 105°C overnight. These tubes were capped using the provided covers, which were perforated to prevent the dried nanotubes from escaping. After the solution completely dried, the weight of the tubes was measured. The net weight of *Ct*CBM4/SWNTs conjugates was then calculated and averaged. To provide a control, the weight of the same *Ct*CBM4 solution without nanotubes was measured in the same way described above. After subtracting *Ct*CBM4 protein and salts in buffer, the SWNTs concentration in the solution was determined. The data are summarized in Table S1.

Table S1. Determination of the SWNTs Concentration in the *Ct*CBM4/SWNTs Suspension

Sample	Weight of Empty Tubes (g)	Volume (μL)	Weight of Dried Tubes (g)	Net weight (mg)	Average (mg)	Concentration of SWNTs(mg/mL)[a]
*Ct*CBM4/	1.0343	500	1.0387	4.4	4.52	1.48±0.38[b]
SWNTs	1.0363	500	1.0408	4.5		
Suspension	1.0344	500	1.0388	4.4		
	1.0338	500	1.0386	4.8		
*Ct*CBM4	1.0345	500	1.0382	3.7	3.78	
Solution	1.0541	500	1.0576	3.5		
Without	1.0337	500	1.0376	3.9		
SWNTs	1.0413	500	1.0453	4.0		

[a] Concentration of nanotubes = (Weight of *Ct*CBM4/SWNTs – Weight of *Ct*CBM4)/Volume.

[b] calculated by $STDV = \sqrt{\frac{\sum (x - \bar{x})^2}{(n-1)}}$

At the same experimental condition, the solubility of SWNTs in SDBS was also measured. However, the weight of SDBS and SWNTs was measured to be ~8.5 mg/mL, while the weight of SDBS in 1% wt solution was measured to be ~9.5 mg/mL (closed to the calculated concentration 10 mg/mL). It is likely that part of SDBS molecules precipitate with big nanotube bundles and the remaining SDBS amount in the final solution cannot be determined precisely. Similarly, portion of *Ct*CBM4 protein molecules may also bind to nanotube bundles and precipitate out with centrifugation. Therefore, the above calculated 1.48 mg/mL represents the minimum value, and the real solubility of SWNTs in *Ct*CBM4 is equal to or bigger than this value.

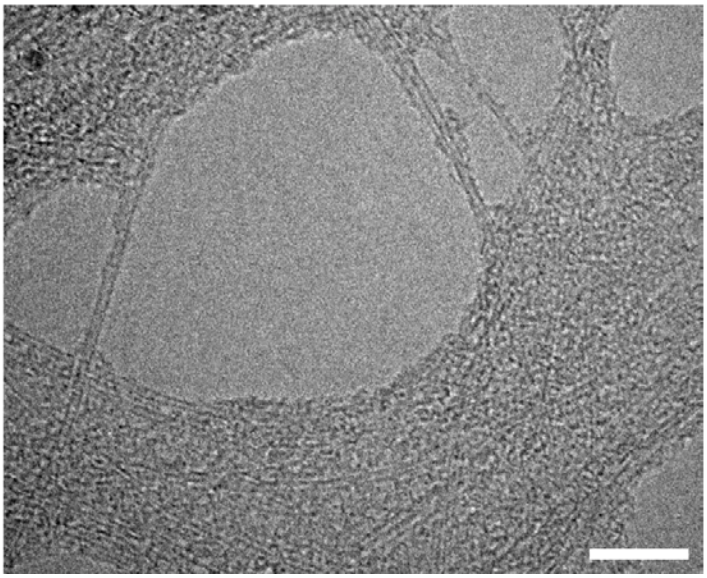

Figure S1. TEM image of *Ct*CBM4/SWNTs, showing individual and small bundles of isolated SWNTs. (scale bar = 10 nm)

3. Preparation of TEM sample.

High-resolution transmission electron microscopy was performed on a FEI Tecnai G^2 microscope operated at 200 kV. To increase the contrast, a calcium staining method for

preparing the TEM sample was employed which followed a previously published procedure.[1] Ten μL the *Ct*CBM4/SWNTs suspension was dropped onto a 300 mesh copper grid containing a lacey formvar/carbon support film (Ted Pella; Redding, CA). The drop was allowed to remain for 10 min. The remaining solution was removed with a Kimwipe and the sample was dried overnight in a fume hood. Five μL of the 10mM $CaCl_2$ solution was dropped on one side of the grid and 5 μL of a 5 mM Na_2HPO_4 solution was dropped on the other side. Ten min later, the remaining solution was wicked away. The sample was then rinsed by water and dried completely in a fume hood. Figure S1 is a typical HRTEM image of *Ct*CBM4/SWNTs showing a larger area. Both individual and small bundles of isolated SWNTs are clearly seen.

4. 2D-PL spectra of SDBS/SWNTs Suspension.

1% wt/wt SDBS and water solution was used to suspend the SWNTs under the same sonication and centrifugation conditions as described in text for preparation of the *Ct*CBM4/SWNTs suspension. The 2D-PL contour map of the SDBS/SWNTs is shown in Figure S2. The specific types of nanotubes observed was assigned using the work of Weisman et al.,[2] and labeled, (n,m). The E11, E22 values and the maximum PL intensity of each individual nanotube identified in the 2D-PL spectra of the two samples are summarized in Table S2. The ratio of the maximum intensity of each type of nanotube from the *Ct*CBM4/SWNTs suspension relative to the maximum intensity of the SDBS/SWNTs suspension is also calculated and listed. The ratio was found to be roughly the same for all measurable features on the spectra.

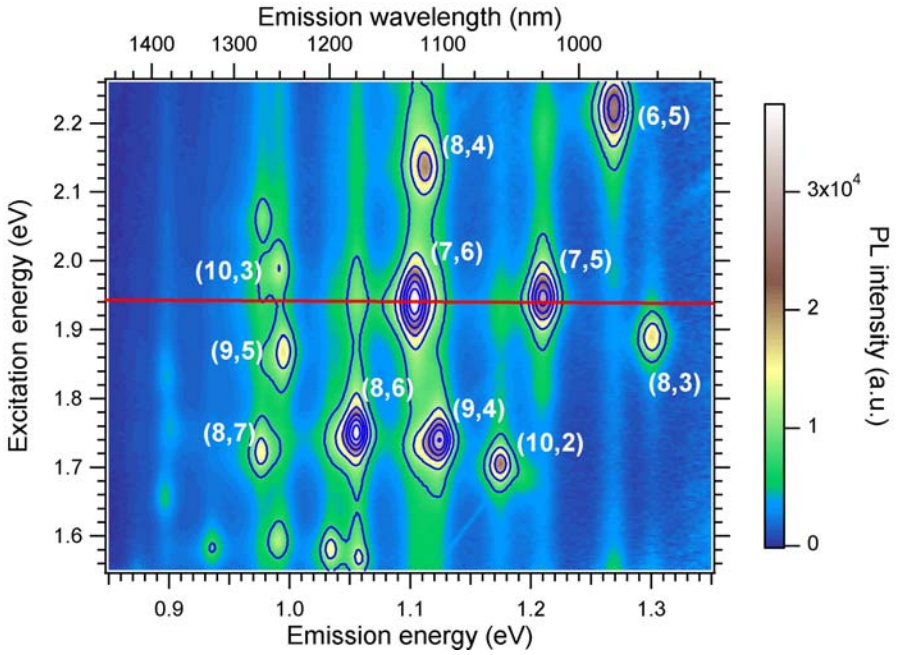

Figure S2. Two-dimensional excitation versus emission photoluminescence contour map of SDBS/SWNTs suspension. The separated semiconducting SWNT species are identified and labeled.

Table S2. Spectral Data from the 2D-PL Maps of the *Ct*CBM4/SWNTs and SDBS/SWNTs Suspensions.[a]

(n,m)	SDBS/SWNTs			*Ct*CBM4/SWNTs			Ratio[c]	Diameter[b] (nm)
	E11 (eV)	E22 (eV)	Intensity (a.u.)	E11 (eV)	E22 (eV)	Intensity (a.u.)		
(6,5)	1.269	2.222	2.514E+04	1.245	2.192	4.710E+03	0.187	0.757
(8,3)	1.301	1.889	1.605E+04	1.276	1.868	3.692E+03	0.230	0.782
(7,5)	1.210	1.946	2.899E+04	1.187	1.923	5.535E+03	0.191	0.829
(8,4)	1.112	2.137	1.943E+04	1.090	2.112	3.954E+03	0.203	0.84
(10,2)	1.175	1.705	2.019E+04	1.152	1.680	4.590E+03	0.227	0.884
(7,6)	1.104	1.941	3.526E+04	1.081	1.914	6.745E+03	0.191	0.895
(9,4)	1.123	1.740	3.120E+04	1.099	1.716	6.365E+03	0.204	0.916
(10,3)	0.991	1.990	1.257E+04	0.971	1.959	2.881E+03	0.229	0.936
(8,6)	1.055	1.751	3.445E+04	1.032	1.728	6.705E+03	0.195	0.966
(9,5)	0.995	1.869	1.610E+04	0.973	1.843	3.420E+03	0.212	0.976
(8,7)	0.976	1.721	1.521E+04	0.958	1.702	2.937E+3	0.193	1.032

[a]The spectral parameters were obtained by the curve fitting with a Lorentzian function. [b]From Weisman et al.[2]. [c]The ratio corresponds to the intensity of each type of nanotube in the *Ct*CBM4/SWNTs sample divided by the intensity of the same type of nanotube in the SDBS/SWNTs sample.

References

[1] L. S. Witus, J. D. R. Rocha, V. M. Yuwono, S. E. Paramonov, R. B. Weisman and J. D. Hartgerink, J. Mater. Chem., 2007, **17**, 1909.

[2] R. B. Weisman and S. M. Bachilo, *Nano Lett.,* 2003, ***3,*** 1235.