Supporting Information

Co-localization of proteins with defined sequential order and controlled stoichiometric ratio on magnetic nanoparticles

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Materials

Unless especially mentioned, all chemical reagents and enzymes that we used in the experiments were purchased from Commercial Companies (Novon, Sigma-Aldrich, Roche, NEB, Takara, Amresco, BioRoYee, Biomed) and used as received without further purification. γ- Methacryloxypropyltrimetho xy-silane (MPS) was bought from TCL Development Co., Ltd.. 1-Vinylimidazole (VIM) was obtained from Adamas Reagent Co.. N, N-methylenebis(acrylamide) (MBA) was supplied by Sigma-Aldrich and recrystallized from acetone. 2, 2-azobis (isobutyronitrile) (AIBN) was provided by Shanghai Titan Scientific Co., Ltd. and recrystallized from ethanol.

Characterization

The instruments and the corresponding test conditions used in this study were shown in Table S1.

Table S1. The instruments used for characterization in this work

test item	instrument type	manufacturer	test condition
TEM	H-800	Hitachi	Accelerating Voltage: 200 kV
XRD	2500VB2+PC	Rigaku	operating at 40 kV and 40 mA
TGA	TG209C	Netzsch, Germany	Nitrogen Flow: 40 mL/min temperature: heating from100 to 800 ℃ at a rate of 20 ℃/min
FTIR	Nicoiet8700	Thermo Electron, US	Scanning Range: 400-4000 cm^{-1}
ZETA	EanoZS	Malvern, Briton	Dispersant: water
Fluorescence Intensity	F-320	Gangdong Sci&Tech., China	Excitation Wavelength: GFP: 475 nm unless especially mentioned DsRed: 558 nm Emission Wavelength: GFP: 499 nm DsRed: 583 nm

Synthesis of Fe_3O_4/PVIM-Ni^{2+}

The Fe_3O_4/PVIM-Ni^{2+} magnetic nanoparticles were prepared according to the reported method. Briefly, 1.350 g of $FeCl_3$ $6H_2O$, 3.854 g of NH_4Ac, and 0.4 g of sodium citrate were added to the flask containing 70 mL of ethylene glycol. The mixture was dissolved under stirring. The homogeneous solution was stirred vigorously at 170 ℃ for 1 h and then transferred into a 100 mL Teflon-lined stainless-steel autoclave. The autoclave was heated to 200 ℃ and maintained for 16 h. The black solution obtained was cooled to room temperature slowly. The nanoparticles were precipitated from the reaction mixture by a small magnet and washed three times with ethanol. The obtained Fe_3O_4 nanoparticles were dried in a vacuum oven at 40 ℃ for 12h. 0.3 g of Fe_3O_4 were added to the flask containing 40 mL of ethanol, 10 mL of deionized water, 1.5 mL of $NH_3 \cdot H_2O$, and the mixture was vigorously stirred for 24 h at 70 ℃ while 0.6 g of MPS was added dropwise. Fe_3O_4/MPS was obtained by a magnet and

washed with ethanol for three times, then was dried in a vacuum oven at 40 ℃ for 12h. 100 mg of Fe_3O_4/MPS was dispersed in a dried 150 mL single-necked flask containing 80 mL acetonitrile. After that, 400 mg of VIM, 100 mg of MBA, and 10 mg of AIBN were added to the flask and heated from ambient temperature to the boiling state within 20 min, and the mixture was maintained at 110 ℃ for 10 min. The obtained Fe_3O_4/PVIM nanoparticles were precipitated by a magnet and washed with ethanol and water for several times. The obtained Fe_3O_4/ PVIM nanoparticles were dried in a vacuum oven at 40 ℃ for 12h. 50 mg of Fe_3O_4/PVIM was added to the flask containing 20 mL $NiCl_2$ $6H_2O$ solution (0.1 M) and stirred for 0.5 h at room temperature. The nanoparticles were separated by a magnet from the solution and washed for several times with water. The Fe_3O_4/PVIM-Ni^{2+} nanoparticles were dried in a vacuum oven at 40 ℃ for 12h and stored for further use.

Plasmid constructions

E. coli BL21 (DE3) (Biomed, Beijing, China) was used for over-expression of GFP-docA, DsRed-docC, scaffoldin ACA and scaffoldin CAC. *Clostridium cellulolyticum* DSM 5812 and the genomic DNA of *C. cellulovorans* DSM 3052 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Plasmid pRS424-HXT7p-GFP-HXT7t was kindly supplied by Dr Huimin Zhao (Department of Chemistry, University of Illinois at Urbana-Champaign). Plasmid pUC19 was bought from BBI Life Science Co.,Ltd.. Plasmids pET22b and pETDuet-1 were purchased from Novagen (Merck, Germany), while pYD1 and pDsRed2-1 were from Biovector (Beijing, China). The genes of GFP and DsRed were cloned by using the plasmids of pRS424-HXT7p-GFP-HXT7t and pDsRed2-1 as templates, respectively. The gene of GFP was inserted into plasmid pYD1

to create GFP-GS which was then transferred into plasmid pET22b. The recombinant plasmids pET22b-GFP-GS-docA and pET22b-GFP-GS-docC were obtained by insertion of docA and docC at the C-terminus of pET22b-GFP-GS. Then, the GFP gene in pET22b-GFP-GS-docC was substituted by DsRed, yielding pET22b-DsRed-GS-docC. The genes of C and A were inserted into vector pUC19 successively and then the generated fusion C-A was inserted into pYD1. Subsequently, the gene of A was ligated to the fragment C-A to create A-C-A and it was then transported into plasmid pETDuet-1 to obtain an N-terminal his-tag. The plasmid of pETDuet1-C-A-C was generated in the same way (Figure S1). The sequences of the primers used in this study were shown in Table S2, and the synthesis service was provided by Beijing Genomics Institute (BGI).

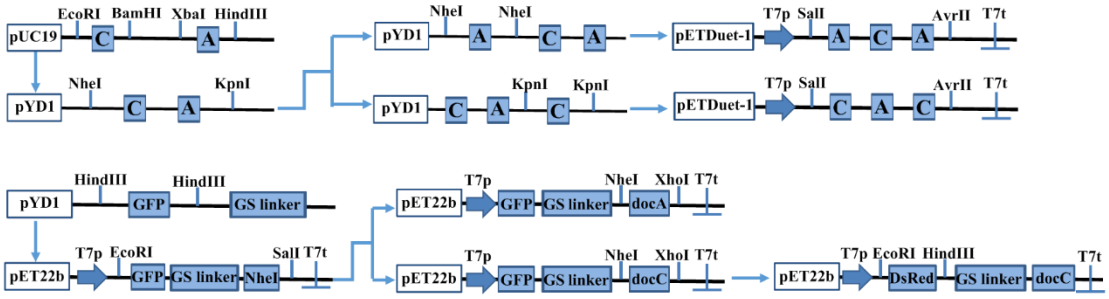

Figure S1. Construction of recombinant plasmids for expression of scaffoldins and the dockerin-containing fluorescent proteins.

Table S2. The primers used in this study

Name	**Sequence**
GFP-Hind III-F	5’-CCAAGCTTAGTAAAGGAGAAGAACTTTT-3’
GFP-Hind III-R	5’-CCAAGCTTGCATGGATGAACTATACAAA-3’
GFP-GS-EcoRI-F	5’-CGGAATTCAGTAAAGGAGAAGAACTTTT-3’
GFP-GS-SalI-R	5’-GCGTCGACGTTCTGGTGGTGGTGGTTCT-3’
docA-NheI-F	5’-CGGCTAGCTTCATTTCAACCTTTGTTAACT-3’
docA-XhoI-R	5’-CCCTCGAGAGGCCAAATTAATAGCTTCTAA-3’
docC-NheI-F	5’-CGGCTAGCTAATTGTATATGGAGATTATAACA-3’
docC-XhoI-R	5’-CCGCTCGAGAGTAAGCTTCCAAGCAACTAA-3’
DsRed-EcoRI-F	5’-CCGGAATTCATGGCCTCCTCCGAGAAC-3’

DsRed-HindIII-R	5’-CCCAAGCTTCACCACCTGTTCCTGTAG-3’
C-EcoRI-F	5’- CGGAATTCGGCGATTCTCTTAAAGTTACA-3’
C-BamHI-R	5’- CGGGATCCTTGAGTACCAGGATCTATAGT-3’
A-XbaI-F	5’- TCCTCTAGAACACCAGTTGAAGCTGTAAC-3’
A-HindIII-R	5’- CCCAAGCTTACCCAGGAACAGTAACTATC-3’
C-A-NheI-F	5’-CTAGCTAGCGGCGATTCTCTTAAAGTT-3’
C-A-KpnI-R	5’-GGGGTACCTTGATAGTTACTGTTCCTG-3’
A- NheI-F	5’-CTAGCTAGCACACCAGTTGAAGCTGTAACAGCTA-3’
A -NheI-R	5’-CTAGCTAGCGATAGTTACTGTTCCTGGGTTAACT-3’
A-C-A-SalI-F	5’-GCGTCGACTATGTTTTTAAGCTTCTGCAGGCTAGT-3’
A-C-A-AvrII-R	5’-CAGCCTAGGTTACACCACACTGGATCCTGGTAC-3’
C-KpnI-F	5’-GGGGTACCAGGCGATTCTCTTAAAGTT-3’
C-KpnI-R	5’-GGGGTACCTTTTGAGTACCAGGATCTA-3’
C-A-C- SalI-F	5’-GCGTCGACTATGTTTTTAAGCTTCTGCAGGCTAGT-3’
C-A-C-AvrII-R	5’-CAGCCTAGGTTACACCACACTGGATCCTGGTAC-3’

Protein over-expression

E. coli strains used for expressing scaffoldins and fluorescent proteins were grown at 37 ^{o}C in Luria-Bertani medium (1% tryptone, 0.5% yeast extract, 1% NaCl) supplemented with ampicillin (100 mg/ml). Subsequently, *E. coli* strains for scaffoldin production were induced with IPTG at 37 ^{o}C for 3h, while those for fluorescent proteins were induced with 0.4 mM IPTG at 20 ^{o}C for 12h. After centrifugation at 8000 rpm for 6 min, cells were washed once with water and resuspended in PBS buffer (pH7.4). Then, the target proteins were obtained after ultrasonication and centrifugation (10000 rpm, 15min). *E. coli* strains carrying plasmids pETDuet-1 or pET22b were used as the control. SDS-PAGE and BandScan analysis were performed to calculate the content of the target proteins (Figure S2). The concentrations of the target proteins were obtained after the total protein levels were determined by the brilliant blue G-250 method.

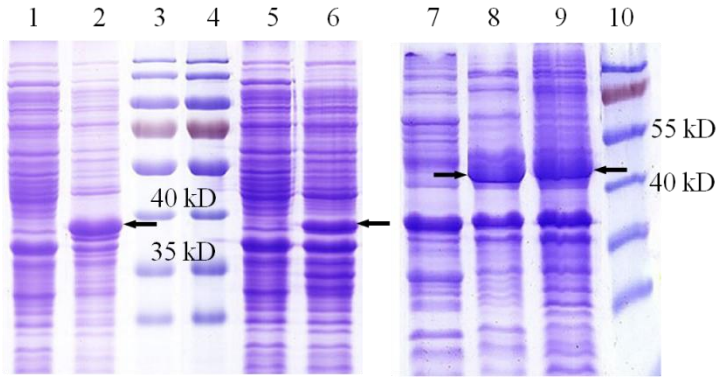

Figure S2. SDS-PAGE analysis. Lanes 3, 4 and 10: marker; Lanes 1 and 5: cells harboring pET22b; Lane 2: cells harboring pET22b-GFP-GS-docA; Lane 6: cells harboring pET22b-DsRed-GS-docC; Lane 7: cells harboring pETDuet-1; Lane 8: cells harboring pETDuet1-A-C-A; Lane 9: cells harboring pETDuet1-C-A-C.

Immobilization of proteins

2 mg of Fe_3O_4/PVIM-Ni^{2+} was added into 4mL scaffoldin or BSA solution and incubated at 13 °C for 5h. The nanoparticles were precipitated by a magnet and rinsed once with PBS 1.2 mL of GFP-docA or DsRed-docC containing 10 mM $CaCl_2$ was then added to the obtained support and incubated for 12h at 4 °C. By comparing the fluorescence intensity of supernatant, the proteins loaded on nanoparticles could be calculated according to Figure S3.

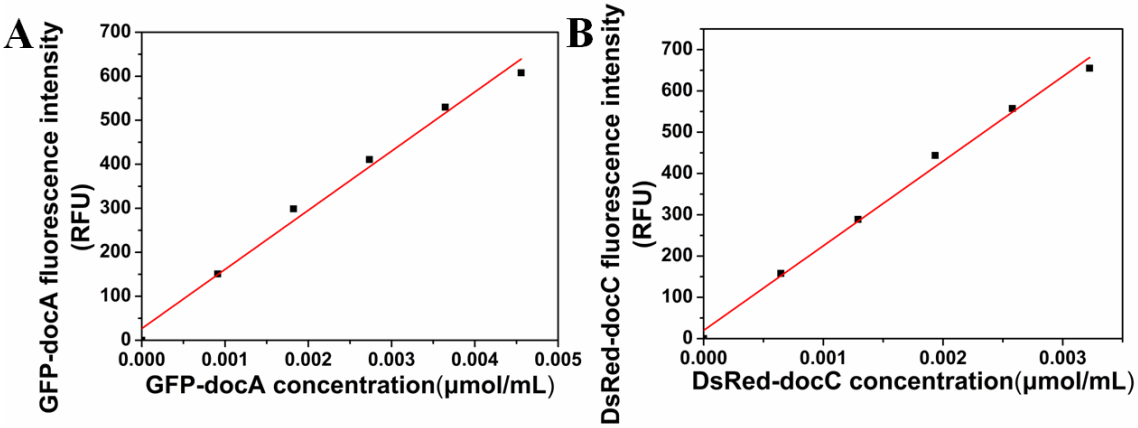

Figure S3. Standard curves of fluorescence intensity versus fluorescent protein concentration. (A) GFP-docA, and (B) DsRed-docC.