Supporting Online Material

Self-assembly of proteins into designed networks

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Experimental Details

Materials

The genes of RhuA and PGAL were available from previous experiments (JS, 2S). Expression vectors pET3b, pKK223-3 and pUC18 were from Pharmacia (Freiburg) and Stratagene (La Jolla). Point mutations were performed with QuikChange (Stratagene). Oligonucleotides were from MWG (Göttingen). DNA was sequenced by SeqLab (Göttingen). Ready-To-Go T4 DNA ligase was from Amersham (Freiburg). PCR was performed with Pwo polymerase from Sawadi (Erlangen). Restriction enzymes were from MBI-Fermentas (St. Leon-Rot). Bug-Buster-Protein-Extraction reagent was from Novagen (Darmstadt). Centricon concentrators were from Millipore (Schwalbach). Streptavidin and dioleoyl phosphatidylcholine (DOPC) were from Sigma (Steinheim), biotin-HPDP (N-[6-(biotinamido)hexyl]-3’-(2’-pyridyl-dithio)propionamide) and tris[2-carboxyethyl] phosphine hydrochloride (TCEP) immobilized on agarose was from Pierce (Rockford), 2-(bis-carboxymethyl-amino)-6-[2-(1, 3-di-O-oleyl-glyceroxy)-acetyl-amino] hexanoic acid (Ni-NTA-DOGA) was a gift from P. Schultz (Illkirch). Ni-NTA spin columns were from Qiagen (Hilden).

Mutagenesis and Vector Design
The RhuA gene cloned in pKK223-3 was modified to yield the triple mutant N133C-K261C-C126S with a His\textsubscript{6}-tag before the stop codon. The modified gene was transferred into plasmid pET3b for expression in \textit{E. coli}.

In the \textit{PGAL} gene, mutation I467L created a unique \textit{XhoI} restriction site before the stop codon (Fig. 1S) which was then used to insert the genes of beta-helix and linker. The beta-helix gene was synthesized as four overlapping oligonucleotides that were annealed to opened pUC18 and ligated with the Ready-To-Go kit. This gene was then inserted at the \textit{XhoI} site of pET3b-I to yield the fusion \textit{PGAL-beta}, which was subsequently excised with \textit{NdeI} and inserted into the original pET3b to yield pET3b-II with the tandem construct \textit{PGAL-beta-PGAL}. The resulting deviations from the native sequences are given at the bottom of Fig. 1S. They are unlikely to cause any disturbance. The applied oligonucleotides are specified in Fig. 2S All mutations and constructs were verified by restriction analyses and DNA sequencing.

\textbf{Protein Expression and Labeling}

RhuA was expressed in a 30 mL culture of \textit{E. coli} BL21(DE3) (Invitrogen, Groningen), extracted with the Bug-Buster reagent and loaded onto a Ni-NTA-spin column. The column was washed with 10 mM imidazole in buffer A (5 mM Tris-HCl, 50 mM NaH\textsubscript{2}PO\textsubscript{4} pH 8.0, 300 mM NaCl) until no further protein could be eluted.

Biotin-labeling was performed on immobilized RhuA by incubating several times in 200 \textmu M biotin-HPDP in buffer A at room temperature over 10 min. Excess reagent was removed by washing. RhuA was eluted with 200-400 mM imidazole in buffer A and the remaining free thiols were determined by applying 200 \textmu M Ellman’s reagent and monitoring 2-nitro-5-thiobenzoate (\textit{e}_{412} = 13600 \text{ M}^{-1}\text{cm}^{-1}) production. The yield was about 10 mg \textit{bR} per liter of culture. Excess imidazole was removed by dialyzing against 1000 volumes of buffer B (20 mM Tris-HCl pH 7.5, 300 mM NaCl). Protein concentrations were established photometrically (\textit{e}_{280} = 46400 \text{ M}^{-1}\text{cm}^{-1} for RhuA, \textit{e}_{280} = 41820 \text{ M}^{-1}\text{cm}^{-1} for streptavidin).
The labeling reaction missed about 30% of the thiols as determined with Ellman's reagent. Moreover, titrating a given amount of $^b$R with streptavidin (S) and monitoring with SDS-PAGE (1% SDS at 50°C that denatures $^b$R but leaves streptavidin intact) showed that the peak of unmodified $^b$R subunits decreased to about 10% of its original value as to be expected for 30% randomly missing biotin labels.

Bis-biotin-labeled streptavidin was prepared by decorating streptavidin with biotin-HPDP and incubating the product with tethered biotin carrying a free thiol (Fig. 3S). The latter was produced by exposing 260 µM biotin-HPDP in buffer B to an equal volume of a 50% (v/v) disulfide-reducing TCEP gel slurry for 20 min and subsequent gel removal by centrifugation. In the reaction 1 mg/mL streptavidin with bound biotin-HPDP was incubated for 30 min at room temperature with an equal volume of 170 µM tethered biotin(thiol). Excess biotin compounds were removed by repeated washing with buffer B using a Centricon (30 kDa cut-off).

$PGAL$-$beta$-$PGAL$ was expressed in $E. coli$ BL21(DE3)Star and purified as described (2S), except that all buffers were devoid of EDTA. The yield was about 40 mg pure protein per liter of culture.

**Electron Microscopy and Self-assembly**

Protein solutions (0.05-0.10 mg/mL) were deposited onto glow discharge hydrophilized carbon films on a copper grid (SCI Science, München). The loaded grids were blotted with filter paper and washed three times with water and then negatively stained using 1-2% uranyl acetate in water, blotted again and dried in air. Electron microscopy was carried out with a LEO CEM 912 transmission electron microscope operating at 120 keV and equipped with a slow-scan CCD camera. Single particle image averaging was performed manually. Fourier-filtering was performed with the program Esivision (SIS, Münster).
The formation of two-dimensional networks can be facilitated by binding to a preformed planar membrane (3S, 4S). Similarly, the four-fold His\textsubscript{6}-tag at the 7×7 nm\textsuperscript{2} top-face of block bR can be used to bind bR to a lipid layer containing a Ni-NTA lipid (5S). This was demonstrated by the association of dissolved bR to a Ni-NTA-presenting lipid monolayer at an air/water interface forming single layer crystals (Fig. 3S). The lipid adsorption was used in network formation. For self-assembly, a 15 µL drop of 0.1 mg/mL bR or 0.05 mg/mL bR\textsubscript{S\textsubscript{4}} in buffer B was transferred into a teflon well (4 mm in diameter, 0.5 mm deep). A 0.5 µL drop of a lipid mixture containing 0.5 mg/mL 1:3 (mol/mol) Ni-NTA-DOGA: DOPC in 1:1 (v/v) chloroform: hexane was deposited on top of the protein solution to form a monolayer. Incubation at room temperature for 2 hours allowed for the adsorption of bR or bR\textsubscript{S\textsubscript{4}} to the Ni-NTA lipids. Further proteins were injected below the monolayer using a Hamilton syringe. The monolayer was picked up by depositing for 1 min a hydrophobic carbon film (on a copper grid) on the surface.

References


FIGURE LEGENDS

Figure 1S.

Construction of *PGAL-beta-PGAL*. The gene for serralysin residues 327-382 (beta-helix) was produced from four synthetic oligonucleotides in pUC18 (top left). Vector pET3b containing the *PGAL* gene (2S) was subjected to the mutation I467L to yield a *Xho*I site before the stop codon in pET3b-I (top right). The beta-helix gene beta* and a synthetic linker with an *Nde*I site were inserted between the *Xho*I and the *BamH*I sites, the *PGAL-beta* fusion gene was cut out and inserted at the *Nde*I site of pET3b to yield the tandem construct in pET3b-II. The amino acid sequence of the PGAL-beta-PGAL fusion is given at the bottom, underlining the deviations from the native sequences.

Figure 2S.

Oligonucleotides for mutations and cloning. Mutations and restriction sites are marked by underlines, the overlapping region within the beta* unit of Fig. 1S is bold. The antisense oligonucleotides for the mutations are not given.

Figure 3S.

(A) The structures of the biotin variants and the production of ^b^R and ^b^b^S from engineered RhuA (X = RhuA) and from streptavidin decorated with biotin-HPDP (X = tethered biotin), respectively. The reactions were photometrically monitored using the released pyridine-2-thione (ε_{343} = 8080 M^{-1}cm^{-1}).

(B) Electron micrograph of negatively stained ^b^R forming a two-dimensional crystal at a Ni-NTA-lipid containing monolayer (left). A Fourier transform yielded diffraction spots up to 4 nm resolution. A filtered image shows the square-shaped tetramers of RhuA (right).
Figure 1S       Ringler & Schulz
RhuA:
N133C: 5'-GC ATT AAA GCC ACC TGC GGC AAA GAT CGG-3'
K261C: 5'-GCG CTC GGC TGC CGT TTC GGC-3'
C126S: 5'-CAC TCC CTG TCC CAC AGC GAG CGC ATT AAA GCC-3'
His6: 5'-pTC GAG CAT CAC CAT CAC CAT CAC TAA A-3'
His6: 5'-pAG CTT TTA GTG ATG GTG ATG GTG C-3'
PCR: 5'-CCG GAA TTC CTC GAG CAT ATG CAA AAC ATT ACT CAG TCC TGG TTT-3'
PCR: 5'-CGG GCC TCC TTA GTG ATG GTG ATG GTG ATG CTC G-3'

PGAL:
XhoI: 5'-CC AGT GCG CTG GCG CTG GAG GGA TCA AAC AGG ATC C-3'
I467L: 5'-GCA GAA ACT CAA GTG CTC GAG TAA TAT CAC CTG AGA ATG-3'
Linker: 5'-pTC GAG GGT GGT CAT ATG TTC GAA GGT TAC CTG GGT TGC TAA G-3'
Linker: 5'-pGA TCC TTA GCA ACC AGC GTA ACC TTC GAA CAT ATG ACC ACC ACC C-3'
β-helix: 5'-pAA TTC CAT ATG CTC GAG ACC ATT GAA AAC GCG ATT GGC GCC AGC GGC
      AAC GAT GTG ATT GTG GGC AAC GCG GGC AAC AAC GTG CTG AAA-3'
      5'-p ACC CGC GCC GCC TTT CAG CAC GTT GTT CGC CGC GTT GCC AAC AAT CAC ATC
      CTT GCC GTT GCC AAT CGC CTT TTC AAT GGT CTC GAG CAT ATG G-3'
      5'-p GGC GCC GCG GGT AAC GAT GTG CTG TTT GCC GGC GCC GTT GCG GAT GAA CTG
      TGG GCC GTT GCC GGC AAA GAT ATT TTT GTG TTT AGC GCC GCC AGC TGC TAAAG-3'
      5'-p GA TCC TTA GCA GCT GCC GCC GCT AAA CAC AAA AAT ATC TTT GCC GCC ACC GCC
      CCA CAG TTC ATC CGC ACC GCC GCC GCC AAA CAG CAC ATC GTT-3'
sequencing: 5'-CG AAC AAC GTG CTG AAA GCC GCC GCG GGT AAC GAT GTG C-3'

Figure 2S  Ringler & Schulz
Figure 3S   Ringler & Schulz