Supporting information

for

Solution structure of a cucurbit[8]uril induced compact supramolecular protein dimer

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Construction of plasmids and design of proteins

The monomeric fluorescent yellow protein mYFP with N-terminal FGG motif was generated by using the N-terminal intein from plasmid pTWIN-1 (New England). This monomeric fluorescent protein was additionally provided with a flexible linker consisting of a Strep-tag (mYFP), also used for purification purposes. The linker ensures flexible reorientation of the protein upon cucurbit[8]uril binding. The DNA encoding for FGG-mYFP is described in literature.[1] The plasmids for MGG-mYFP was generated from the FGG-mYFP template by using a QuickChange site-directed mutagenesis kit (Qiagen) to replace F with M.

Protein expression and purification

The plasmids were transformed into *E. coli* strain BL21(DE3) (New England BioLabs). The bacteria were cultured in lysogeny broth (LB) medium containing 100 µg/ml of ampicillin and the cells were grown at 37 ^{o}C and shaken at 250 rpm to an OD_{600} of 0.5 - 0.7. Subsequently, IPTG was added to a final concentration of 0.4 mM. The cells were continuously incubated overnight at 15 ^{o}C under shaking at 180 rpm until harvested. The pellet was resuspended into the bugBuster protein extraction reagent plus benzonase nuclease, and the insoluble material was removed by centrifugation at 20,000 rpm for 40 minutes at 4 ^{o}C. The soluble fraction was applied to a column filled with chitin beads (New England Biolabs) through gravity flow, and the column was washed with 40 volumes of sodium phosphate buffer (10 mM H_3PO_4, 100 mM of sodium chloride, pH 7) and subsequently incubated overnight at room temperature to cleave off the intein. The intein cleaved proteins were then collected in the flow-through using the phosphate buffer. The target proteins were analyzed by SDS-PAGE and LC-ESI-MS which confirmed their identity and purity.

Dynamic light scattering

Dynamic light scattering measurements were carried out on a Zetasizer µV (Malvern Instruments Limited, UK). All samples were dissolved in 10 mM sodium phosphate, pH 7 to a protein concentration of 40 µM. Samples were filtered via a 0.1 µm syringe filter (Anotop 10, Whatman, UK) into a clean cuvette (UV-transparent disposable cuvettes, Sarstedt, Germany). The cuvette was then inserted into the unit and left to equilibrate for 2 minutes at 20 $^{\circ}$C before the measurement. Cucurbit[8]uril was added at 20 µM. Memantine was added in excess at 40 µM.

Small angle X-ray scattering (SAXS)

SAXS measurements were done with a SAXSess[2] camera (Anton-Paar, Graz, Austria), which was connected to an X-ray generator (Panalytical PW3830) operating at 40 kV and 50 mA with a sealed-tube Cu anode. A Göbel mirror was used to convert the divergent polychromatic X-ray beam into an intense focused line-shaped beam of Cu Kα radiation (wavelength λ = 0.154 nm). The scattering pattern was recorded on a one dimensional silicon strip detector operating in single-photon counting mode (Mythen 1K by Dectris, Switzerland). This detector features 1280 pixels with a height of 50 µm each (active area: 64 x 8 mm²). Cosmic ray removal was done on the raw data before absorption correction and background subtraction. The data were converted into one-dimensional scattering patterns I(q) as a function of q, where q is the magnitude of the scattering vector defined by $q = (4\pi/\lambda)\sin(\theta/2)$. Here θ is the scattering angle.

The samples were filled into a quartz capillary (diameter 1 mm, wall thickness 0.01 mm) kept in a temperature controlled sample holder (20 ± 0.1 ^{o}C) and placed in the evacuated SAXS instrument. The samples were exposed for 1 hour (6 frames of 10 min). The individual frames were averaged and the standard deviation was calculated. Experiments on FGG-mYFP (910 µM), FGG-mYFP:cucurbit[8]uril at two different concentrations (857:428 µM / 214:107 µM) on cucurbit[8]uril alone and only solution were performed in 10mM sodium phosphate buffer at pH 7. Background scattering files were subtracted from the sample scattering after transmission correction.

The program BUNCH,[3] which is part of the ATSAS software package,[4] was used for data analysis. BUNCH performs modelling of multidomain proteins against SAXS data using a combined rigid body and *ab initio* modelling approach. The program allows for the determination of the three-dimensional domain structure of proteins when the high resolution structure of individual domains are available, for instance from protein crystallography. A simulated annealing algorithm was employed to find the optimal positions and orientations of the available high resolution coordinates of the domains with known structure, while the portion or amino acid residues with unknown secondary structure is represented as a chain of dummy residues. Here, the YFP beta-barrel monomers were represented by the structure published as 1YFP in the protein data base[5] provided with an additional N-terminal flexible chain, with a terminal FGG motif. An additional boundary condition was defined to take into account only those dimeric complexes wherein the 3 dummy residues at the N-termini (FGG) are in close proximity. The calculations were performed several times on the same data set to get information about both the structure and flexibility of the complex.

Reference list

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