SUPPLEMENTAL MATERIAL

Experimental procedures

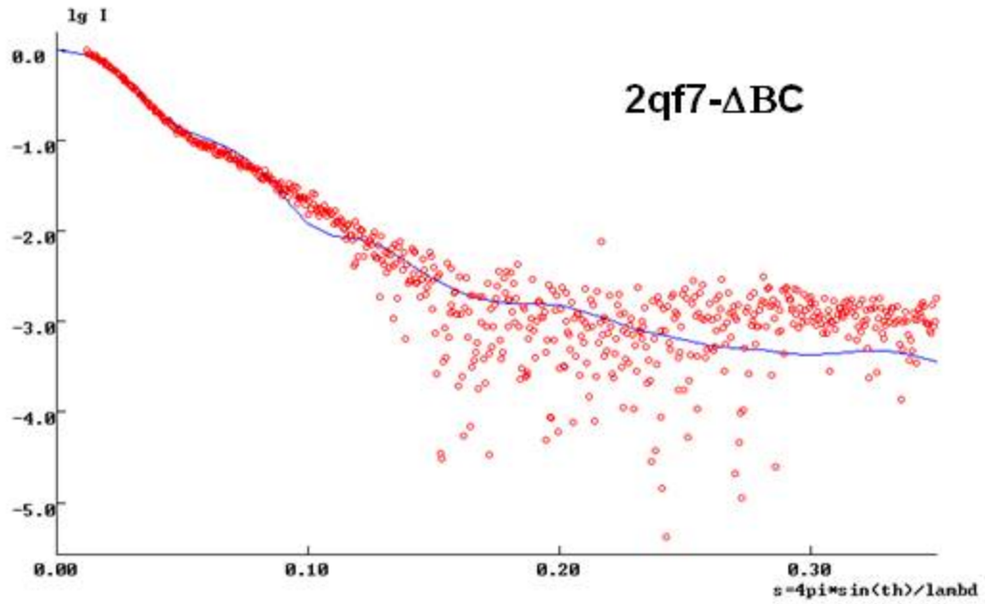
Protein purification- Briefly, protein expression was induced with 0.1 mM IPTG in cells grown at 37 °C .The cell pellets of constructs with a His-tag were resuspended in 20 mM Tris-HCl pH 7.9, 300 mM NaCl, 10 % glycerol, 2 mM β-mercaptoethanol and Complete-protease inhibitor (Roche). After cell disruption and centrifugation of cell debris and membranes, the soluble fraction was supplemented with 10 mM imidazol and loaded onto a metal chelating column. Washing was done with 60 mM imidazol and elution with 500 mM imidazol. The protein was further purified by size exclusion chromatography on a Superdex200 column. Fractions were pooled, concentrated and stored at -80 °C. The cell pellets of constructs without a His-tag, were resuspended in 50 mM Bis-Tris pH 6.9 buffer, 2 mM mercaptoethanol and Complete-protease inhibitor (Roche). The soluble fractions after centrifugation of disrupted cells were loaded on a Resource Q column and eluted with a salt gradient from 10-500 mM NaCl. The fractions containing the protein were pooled and further purified on a Superdex200 column in 20 mM Tris 7.6, 150 mM NaCl, 2 mM β-mercaptoethanol buffer. Point mutations on the Oad-γ' subunit of the Oad-α/γ' (Figure 1, f) were prepared according to (1). The polymerase chain reaction (PCR) was done with Phusion Hot Start High-Fidelity DNA Polymerase (Finnzymes) and pET16b-VcOadG'A-2 as template (2), using the oligonucleotide primers 5'-AGGTGAATCGTCCGCGGTGCTCACGGCCGCTGTCC-3' (G'ALLtoSSfw) and 5'GCGGACGATTCACCTTGGTTTTGATTTGGGTTGGCTTTG-3' (G'ALLtoSSrv). The recombinant Oad-γ' protein was supplied with a His-tag at the N-terminus. Purification was performed as described above. A construct was made in which the *oadGAB-2* genes were amplified by PCR using pET24-VcOadGAB-2 as template (3) and subcloned into a pET28a vector that contributes a His-tag to *oadG-2* gene product (periplamic N-terminus of Oad-γ). C43(Rossetta) cells expressing the OAD complex were resuspended in 50 mM Tris-HCl 8.0, 250 mM NaCl, 10 % glycerol and 1 Complete-protease buffer. After cells and discard cell debris, membranes were collected by centrifugation and solubilised with 2 % Triton X-100. After centrifugation of insolubilized material, the soluble fraction was applied onto a monomeric avidin-Sepharose column and elution was perfomed with 5 mM biotin and 0.05 % Brij-58 (3). Membranes from *E. coli* cells overexpressing the Oad-2 complex (cloned in pET28a that provides a His-tag to the N-terminus of Oad-γ) were collected from the cleared supernatant by centrifugation at 100,000 x *g* for 1 h at 4 °C. The membrane fractions were solubilised in 20 mM Tris-HCl 7.6, 300 mM NaCl and 1% Triton X-100 at 4 °C for 10 min and the complex was purified as previously described (3) using a SoftLink™ Soft Release Avidin Resin (Promega).

Suppl. Fig. 1. Comparison of the theoretical scattering curves of PC (depleted of the BC domains) with the experimental SAXS data from Oad-α/γ'. Note that the PC structures have been completed by homology modelling due to the fact that some loops and BCC proteins are missing in the deposited PDB structures. 3bg3, human PC; 3bg5, *Staphylococcus Aureus* PC; 2qf7, *Rhizobium etli* PC

1. Nohr, J., and Kristiansen, K. (2003) Site-Directed Mutagenesis. in *Protein misfolding and disease: Principles and protocols. Methods in Molecular Biology* (Bross, P., and Gregersen, N. eds.), Humana Press Inc., Totowa, NJ. pp
2. Dahinden, P., Pos, K. M., and Dimroth, P. (2005) *Febs Journal* **272**, 846-855
3. Dahinden, P., Auchli, Y., Granjon, T., Taralczak, M., Wild, M., and Dimroth, P. (2005) *Archives of Microbiology* **183**, 121-129

Supplemental

A



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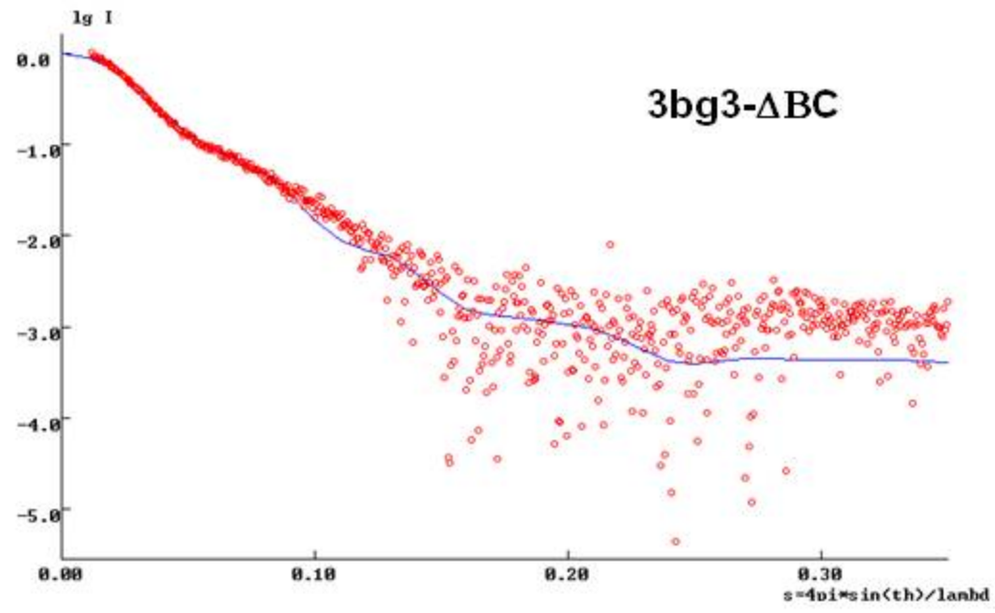

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