A Biological Porin Engineered into a Molecular, Nanofluidic Diode

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Supporting Information

Mutant OmpF. The plasmid used, pGompF, encodes the OmpF protein behind the PhoE leader sequence (Prilipov et al., 1998). In order to achieve high expression in inclusion bodies, the leader sequence has been removed and a ribosome-binding site and start codon are inserted directly prior to the sequence encoding mature OmpF. This plasmid, pGompF-mature, was used as template in the QuikChange®XL protocol from STRATAGENE (La Jolla, CA). First, the template was duplicated using reverse complement primers, containing the mutation of interest, resulting in mutated plasmid with staggered nicks. Then, the template was degraded and the mutated plasmid (with staggered nicks) was transformed to *E. coli*. Sequencing the DNA verified that the resulting mutated genes were only mutated in the intended codon and did not contain additional mutations.

OmpF isolation and purification. One liter cell cultures of *E. coli* Omp5 (Prilipov et al., 1998), containing the desired plasmid were grown overnight in TY-media supplemented with ampicillin (100 μg/ml). Cells were lysed with 1 M NaCl and 1% Triton X-100, in 50 mM phosphate buffer, pH 7.5. After sonication (3x30 s), inclusion bodies were pelleted and washed with phosphate buffer + 1% Triton X-100, followed by two wash steps with buffer without detergent. After centrifugation, the pellet was dissolved in 8 M urea and diluted to a final protein concentration of 0.2 mg/ml in refolding buffer (containing 20 mM sodium phosphate buffer, pH=6.5; 1 mM dodecylmaltoside and 1mM TCEP). After overnight refolding at room temperature, dimer-to-monomer conversion was accomplished by heating to 70 °C for 1 hour. Subsequent degradation of the monomers was induced by the addition of trypsin (trypsin/protein=1/100 w/w). Final purification of trimer protein was achieved by PD-10 column purification with phosphate buffer, supplemented with 1% OPOE as eluent. Typical total yield was ~50 mg for WT and 10-50 mg for mutant protein.

Prilipov,A. et al., *FEMS Microbiol. Lett.* **1998**, 163, 65-72.