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AcrB et al.: Obstinate contaminants in a picogram scale. One more bottleneck in the membrane protein structure pipeline

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Supplementary figures

S1. MALDI-TOF identification of AcrB (**A**) and SdhA (**B**), from the tryptic digestion of the corresponding protein bands in Fig. 1. Colored in blue the peptide sequences which were identified.

S2. Detergent screening of GluP-RGS(His)₆ comprising BLR(DE3) *E. coli* membrane fractions and (NH₄)₂SO₄ solubilization of AcrB. 250-400 µg of inner membranes containing GluP-RGS(His)₆, were solubilized in 50 mM Tris-HCl pH 8.5, 10% (v/v) glycerol, 2 mM β-mercaptoethanol in the presence of the selected detergent. Solubilization mixtures were incubated at 4 °C for 3-16 hrs with mixing (1000 rpm). The samples were centrifuged at 30 psi for 15 min using an air-centrifuge (Beckman, -200,000 g). Solubilized (S) and pelleted (P) fractions were analyzed by SDS-PAGE gel electrophoresis (30 µg - **A**), Western Blotting (10 µg - **B**) and Dot-Blots (10 µg - **C**). (**A**) Detergent based solubilization of AcrB (open arrow) and of GluP (closed arrow). The following detergents were used: n-dodecyl-β-D-maltoside (DDM) 1.5% (w/v), lauryl-dimethyl-amine oxide (LDAO) 2.0% (v/v), n-octyl-β-D-glucopyranoside (OG) 2.0% (w/v), polyoxyethylene-9-laurylether (C₁₂E₉) 3.0% (w/v), Triton X-100 (TX-100) 3.35% (v/v), octanoyl-n-methylglucamide (MEGA₈) 100 mM, decanoyl-n-methylglucamide (MEGA₁₀) 2.4 mM, n-undecyl-β-D-maltoside (NUM) 3.0 mM, n-decyl-D-glucoside (DG) 10 mM, n-decyl-β-D-maltoside (DM) 2.0% (w/v). Only the optimal detergent concentrations are shown. All detergents were purchased from Applichem. CONTR: Control solubilization (buffer without detergent). (**B**) Western blot of the fractions in **A** using the anti-RGS(His)₅ antibody (Qiagen). (**C**) Dot blot on selected fractions from **A**, including n-decyl-β-D-maltoside (DM), and using the anti-

(His)₅ antibody (Qiagen). **(D)** Selective solubilization of AcrB (lane 2) from BLR(DE3) *E. coli* membrane fractions harbouring GluP (lane 1), in the presence of 0.2 M (NH₄)₂SO₄. Coomassie brilliant blue staining (lane 3) and silver staining (lane 4) of purified GluP-RGS(His)₆ following the 0.2 M (NH₄)₂SO₄ washing.

S3. Purification of GluP(His)₆ from mixed membrane fractions of *E. coli* K-12 cells, on 12% Coomassie Blue stained SDS-PAGE gels. Lane M, molecular weight markers (Sigma-Aldrich, 2 µg); Lane 1, *E. coli* JM110 membranes containing GluP(His)₆ (30 µg); Lane 2, detergent extract of *E. coli* JM110/ GluP(His)₆ (30 µg); Lanes 3-4, purified GluP(His)₆ (30 µg) from *E. coli* JM110 cells; Lane 5, purified GluP(His)₆ (15 µg) from RE707 *E. coli* cells. *E. coli* JM110 cells were grown in minimal media (M9) and RE707 cells in rich media (LB). Open arrows show the position of SdhA (Lanes 3-4) and of AcrB (Lane 5). Closed black arrows show the position of GluP(His)₆. Closed red arrows indicate the presence of a small aggregated population of GluP(His)₆ migrating at the position of a dimer.