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

Original Citation


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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 (NH$_4$)$_2$SO$_4$ solibilization 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 (C$_{12}$E$_9$) 3.0% (w/v), Triton X-100 (TX-100) 3.35% (v/v), octanoyl-n-methylglucamide (MEGA$_8$) 100 mM, decanoyl-n-methylglucamide (MEGA$_{10}$) 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 (NH$_4$)$_2$SO$_4$. Coomassie brilliant blue staining (lane 3) and silver staining (lane 4) of purified GluP-RGS(His)$_6$ following the 0.2 M (NH$_4$)$_2$SO$_4$ 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.