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OmpX Micro-Scale Refolding Protocol

December, 2008

Version 1.0

Summary:

This protocol is currently being used at the JCIMPT laboratory of Professor Kurt Wüthrich to prepare NMR samples of [¹⁵N,²H]-OmpX for the assessment of the conformational state and the solvation of OmpX reconstituted in mixed micelles with different detergent in aqueous solution. In combination with MicroProbe technology this protocol reduces the volume required per NMR sample by two orders of magnitude.

The protocol has two major steps: OmpX reconstitution and buffer exchange. The current refolding protocol takes about 10 hours. The final product consists of 45 µl of a solution containing OmpX/detergent mixed micelles, which can be studied using TROSY-NMR with a MicroProbe.

Materials:

1. Reagents

0.6 mM OmpX in 6 M urea.

Refolding buffer (20 mM Tris-HCl at pH 8.5, 5 mM EDTA, 600 mM L-Arg, 2% (w/v) detergent).

NMR buffer (20 mM sodium phosphate at pH 6.8, 100 mM NaCl, 0.3 % NaN₃, 10% D₂O, adjustable amount of detergent).

2. Equipment

Centrifuge.

Step by step for reconstitution of OmpX into Detergent Micelles for NMR Spectroscopy:

OmpX Refolding

- Mix 100 µl of unfolded OmpX (0.6 mM in 6 M urea) with 600 µl of detergent-containing refolding buffer and stir overnight at 4° C.



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- b) Centrifuge at 8,000 g for 30 min. Recover solubilized OmpX from supernatant. Depending on the detergent used, some OmpX may have precipitated; in this case OmpX may be recovered in 6 M urea by repeating step a).
- c) Concentrate to 50 μ l.

Buffer exchange

- d) Add 150 μ l of NMR buffer and concentrate back to 50 μ l, using a Vivaspin concentrator.
- e) Repeat step d) 6 times.
- f) After 6 repeats, recover solubilized OmpX supernatant. Precipitated OmpX (see above) may be recovered by dissolving in 6 M urea and repeating steps a)-e).

References:

Q. Zhang, R. Horst, M. Geralt, X. Ma, W. X. Hong, M. G. Finn, R. C. Stevens and K. Wuthrich (2008) Microscale NMR screening of new detergents for membrane protein structural biology J Am Chem Soc 130: 7357-63 ;  PubMed

Please send comments, suggestions, and/or questions to Professor Ray Stevens (stevens@scripps.edu)