Refolding on Column Standard Operating Procedure

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Reference: Oganesyan, N., Kim, S. –H., Kim, R. (2004) On-column Chemical Refolding of Proteins. PharmaGenomics **4**: 22-26.

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Materials/Reagents/Equipment	Vendor
Resin: Ni-NTA Agarose (store at 2-8^{0}C)	QIAGEN Catalog # 30230
Urea Solution is made fresh each time.	Mol. Biol.Grade, Research Organics Catalog # 9692U MW: 60.6g
β-cyclodextrin	Sigma C4767 MW:1135 g
Triton X-100 **1 CMC: 0.23 mM=0.015%**	Sigma T8787 MW: 647 g

Purpose: Refolding of proteins that are expressed as inclusion bodies.
This is a procedure for His tagged Proteins.

Note: Urea should be made fresh each time.
Preparation of 8 M urea: Weigh urea and place in a beaker, bring to 20 mM Tris, pH 8. Place beaker in a larger container containing water and heat over low heat until dissolved using a magnetic stir bar. DO NOT heat urea to high temperatures. Bring solution to desired final volume, pH of the solution is ~ pH 8.2. Allow solution to cool, do not place in the refrigerator (it will crystallize).

If protein has 1 or more cysteines: add 20 mM beta-mercaptoethanol (βME) in step 1. <u>Before binding the protein to Ni-NTA, dilute the sample 1:1 to bring it to 10 mM βME.</u>

1. **Dissolve inclusion body (IB) protein at a ratio of 1 gm IB/3 ml of 8 M urea/20 mM Tris, pH 8 (Buffer A). Put over a magnetic stirrer for 1 hr at room temperature, stirring slowly. If sample is not going into solution and large particles remain, sonicate 3 x 10 seconds. Spin 15,000 rpm, 20 min in Sorvall centrifuge. Keep supernatant, there should be not much of a pellet.**

2. **Equilibrate Ni-NTA resin with Buffer A (calculate amount of resin needed by using a ratio of 5 mg target protein/ml resin).**

3. **Bind protein to Ni-NTA overnight at room temp in a batch mode, rocking, in a 50 ml plastic conical tube. It is important to incubate overnight for good binding. After binding, pour resin into a BioRad Econo column.**

4. **Wash column with buffer A (5 CV) +/- 10 mM βME, followed by 5 CV buffer A/20 mM imidazole. Keep 10 mM βME in all buffers if cysteines are present.**

5. **Wash column with 10 CV of 0.1% Triton X100/20 mM Tris, pH 7.5/0.5 M NaCl or a detergent of your choice. Let it drip slowly.**

6. **Slowly wash column with 10 CV of 5 mM β-cyclodextrin/20 mM Tris, pH 7.5/0.1 M NaCl. The cyclodextrin removes all the detergent from the protein.**

7. **Wash column with 10 CV of 0.1 M NaCl/20 mM Tris, pH 7.5.**

8. **Elute target with 300/600 mM imidazole /20 mM Tris, pH 7.5/0.1 M NaCl or buffer of your choice.**

9. **Concentrate protein and run size exclusion chromatography column, do dynamic light scattering assay, and run sample on native gel, SDS/PAGE. Perform circular dichroism measurement. If protein is not pure enough, perform ion exchange chromatography on the sample.**