Trace Fluorescent Labeling for Protein Crystallization Screening

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Hands-on Practical Outline.

I. Introduction to covalent modification of proteins.
   A. Several groups are commonly available as targets.
      1. Amines – side chain and N-terminal.
      2. Sulfhydryls – less commonly available.
      3. Carboxyls
   B. Easiest is Amines – use pH control to select type.
      1. pH ~7.5, N-terminal amines
      2. pH ~>8.5, side chain amines.
      3. I prefer side chain, less chance of systematic effects.

II. Solutions & Materials needed.
   A. Reaction buffer – 0.05M NaBorate, pH 8.75 used here. Must be free of primary and secondary amines (tertiary OK)!
   B. Reactive probe solution – carboxyrhodamine-SE used (5 mg in 1 mL DMF).
   C. Centrifugal desalting column – 0.5 mL (Pierce). Note – max speed is 1500xG.
   D. Crystallization buffer solution – whatever your protein is equilibrated in. I'm using 0.05 M NaHepes, 0.1 M NaCl, pH 7.5.

III. Procedure
   A. Equilibrate desalting column in reaction buffer (2-3 passes).
   B. Prepare protein solution – demo uses 0.8 mLs at 15 mg/mL as the stock solution.
   C. Pass 80 uL of protein solution thru desalting column.
   D. Add 0.5 uL of reactive probe solution.
   E. Interlude – dismantle microscope and show parts.
   F. Re-equilibrate column in crystallization buffer (2-3 passes).
   G. Pass derivatized protein solution thru desalting column.
   H. Add protein back to stock solution.
   I. Bring volume up to 1.0 mLs.
   J. Use microscope to verify fluorescence.
      1. Solution should be slightly colored at most.
      2. Should see more color in the desalting column – the unbound probe.
      3. From experience – this results in ~0.25% of the protein being labeled.
   K. Use protein as normal in setting up crystallization plates.

When viewing the plates – the crystals may show some slight color to those with more discerning eyes (not mine). Crystals are obvious as they give a much brighter emission than the solution or amorphous precipitate. For leads, look for precipitate that has 'bright spots', that cannot be obviously determined to be crystals.