Systems *Bioengineering* Laboratory

Fluorescent Labeling of Proteins

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Materials

- Sodium bicarbonate (Fisher Scientific, Cat: S78284)
- Anhydrous sodium carbonate (Fisher Scientific, Cat: S263)
- Amine-reactive dye, Texas Red (Thermo-Fischer, Cat: T6134)
- N, N, -Dimethylformamide, DMF (Fisher Scientific, Cat: Fl-05-1105)
- Proteins of interest:
  - Fibronectin from human plasma (BD, Cat: 356008) dissolved in diH_2O at 1 mg/ml
  - Poly-L-Lysine (70-150kD) hydrobromide (Sigma, Cat: P6282) dissolved in borate buffer
  - 0.1 M borate buffer: 1.24 g boric acid (Sigma, Cat: B1934), 1.9 g sodium tetraborate (Sigma, Cat: 229946), 400 mL diH_2O, adjusted to 8.5 pH, sterile filtered
  - Albumin from human serum (Sigma Aldrich, CAT: 70024-90-7)
- Dialysis tubes and float, Slide-A-Lyzer Mini Unit 3.5KMWCO (Thermo Scientific, Cat: 66333)
- Aluminum foil
- Large beaker
- Stirrer/stir bar

Methods

1. Prepare 0.1M Bicarbonate Solution (makes 400mL).
   a. Dissolve 2.2g anhydrous sodium carbonate into 100mL of deionized water.
   b. Dissolve 1.68g sodium bicarbonate into 100mL of deionized water.
   c. Combine 4mL of carbonate solution from 1a with 46mL of bicarbonate solution from 1b.
   d. In a large enough container, bring the solution from 1c to 400mL with 250mL of deionized water.
2. Dissolve 2.5mg of protein into 500uL bicarbonate solution from Step 1. Leave on shaker for about an hour or until protein is completely dissolved.
3. Wrap a small tube in aluminum foil. Dissolve 0.2mg Texas-Red Dye into 20uL DMF via vortex immediately before conjugation. Do not dissolve before conjugation. The dye is not stable in solution and must be kept at <-20ºC if not used immediately.
4. Calculate the appropriate volume for the mixture of a 7:1 molecular ratio between the dye and the protein. For this, use the molecular weight of the dye and the protein to compute the molarities and match accordingly.

   Example: Human Serum Albumin (HSA): 68,000MW; Texas Red (dye): 817MW. If a dye:protein ratio of 7:1 is desired, 11.87ug of albumin is required for each ug of dye. Using the solution concentrations from steps 2 and 3, add 80uL dissolved dye per 2mL dissolved HSA.
5. Wrap the tube containing the protein solution from step 2 in foil. While stirring the solution, slowly add the reactive dye solution from step 3. Incubate the reaction for approximately one hour with continuous stirring.

   Example: for 2.5mL HSA solution (5mg/mL), add 100uL dissolved dye.
6. Wrap the tube in foil and incubate the reaction at room temperature for approximately one hour with continuous stirring using the shaker.
7. Prepare 800mL of bicarbonate solution from step 1.

8. Label dialysis cassette(s) and transfer the protein-dye solution into the dialysis cassette (s). Make sure not to touch the filters at the sides of the cassettes with your fingers or the gauge needle.
9. Place the cassettes into a beaker with at least 800mL of bicarbonate solution. Cover the beaker with foil and store the beaker at 4ºC overnight (>12 hours). Longer diffusion times may be needed for larger MW proteins.
10. The next day, aliquot labeled proteins into small volumes and store the conjugated protein at the same temperature as you would the parent protein. For long term storage, add sodium azide (2mM final concentration) as a preservative.

    Example: HSA is stored at 4ºC.