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5.36 Biochemistry Laboratory
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SESSION 6 (lab open 1-5 pm)

1.) Preparation of an SDS page gel

SDS-PAGE gel analysis will allow you to check the identity (by molecular weight) and purity of the H396P Abl kinase domain. In an SDS gel, proteins are separated based on their molecular weights. This occurs because the SDS (a detergent) binds to all denatured proteins in an amount approximately proportional to the size of the protein. This results in all proteins having an equal charge to mass ratios, since the negatively-charged SDS masks any charges from the protein side chains. The protein size is thus the sole factor that affects its migration speed through the pores of the polymerized gel. The molecular weight of each protein can be deduced by comparing the migration distance of the protein bands to that of a protein ladder, which is comprised of protein samples with known molecular weights.

Assembly of your SDS-PAGE gel

I. Make up the 12 % running gel. In a 15-mL conical tube, combine 3 mL 40% acrylamide/ bisacrylamide solution, 2.4 mL 1.0 M Tris-HCl (pH 8.8), 1.2 mL 50% sucrose, 3 mL water, and 100 µL 10% SDS. **Wait until a TA is by your side and watching before you add the APS or TEMED**. Once you are ready to pour the gel and your TA is watching you, add 250 µL of 10% APS solution and 2.5 µL of TEMED.
II. Mix the solution by inverting 4-6 times, and then immediately transfer enough solution to fill the gel cassette part way using a Pasteur pipette. Your TA will demonstrate how high the running gel should be to leave sufficient room for the stacking gel and the gel comb. Once the gel is poured, add a thin layer of water to the top of the running gel to keep the top level.
III. Allow the gel to polymerize, which can take up to 30 minutes. While the gel polymerizes, you can prepare the stacking solution (step IV) minus the APS and TEMED, which should not be added until just before the stacking gel is poured. Once the running gel has solidified, decant the water layer and use filter paper to absorb any residual water.
IV. Pour the stacking gel. In a 15 mL conical tube, combine 500 µL 40% acrylamide/ bisacrylamide solution, 1.7 mL 0.38 M Tris-HCl (pH 6.8), 2.5 mL water, and 50 µL 10% SDS. Next add 400 µL 10% APS solution and 2.5 µL TEMED. Mix the solution by inverting 4-6 times, and pour the solution on top of the running gel to the top of each cassette.
V. Quickly insert a comb into the stacking gel. The gel may take up to 30 minutes to polymerize. The purpose of the stacking gel is to concentrate the protein samples as they enter the running gel.
VI. Once the gels have polymerized, place the cassettes in a plastic container with 1X Tank buffer. Wrap the container in saran wrap to prevent the gel from drying out and store the gel at 4 ºC. You will use your gel in Session 7.

2.) Preparation of SDS-PAGE samples

You should prepare the following gel samples: pre-induction, post-induction, and all seven Ni-NTA column elution samples.

To prepare the pre- and post-induction samples, thaw the saved pellets at room temperature, and add 24 μL of the 6X sample loading buffer to each pellet. For the elution samples, combine 20 μL of each sample with 4 μL of the 6X gel loading buffer in 1.5-mL Eppendorf tubes. Boil all of the samples for 3 to 5 minutes to fully denature the proteins. The samples can be stored at room temperature until they are used in Session 7.