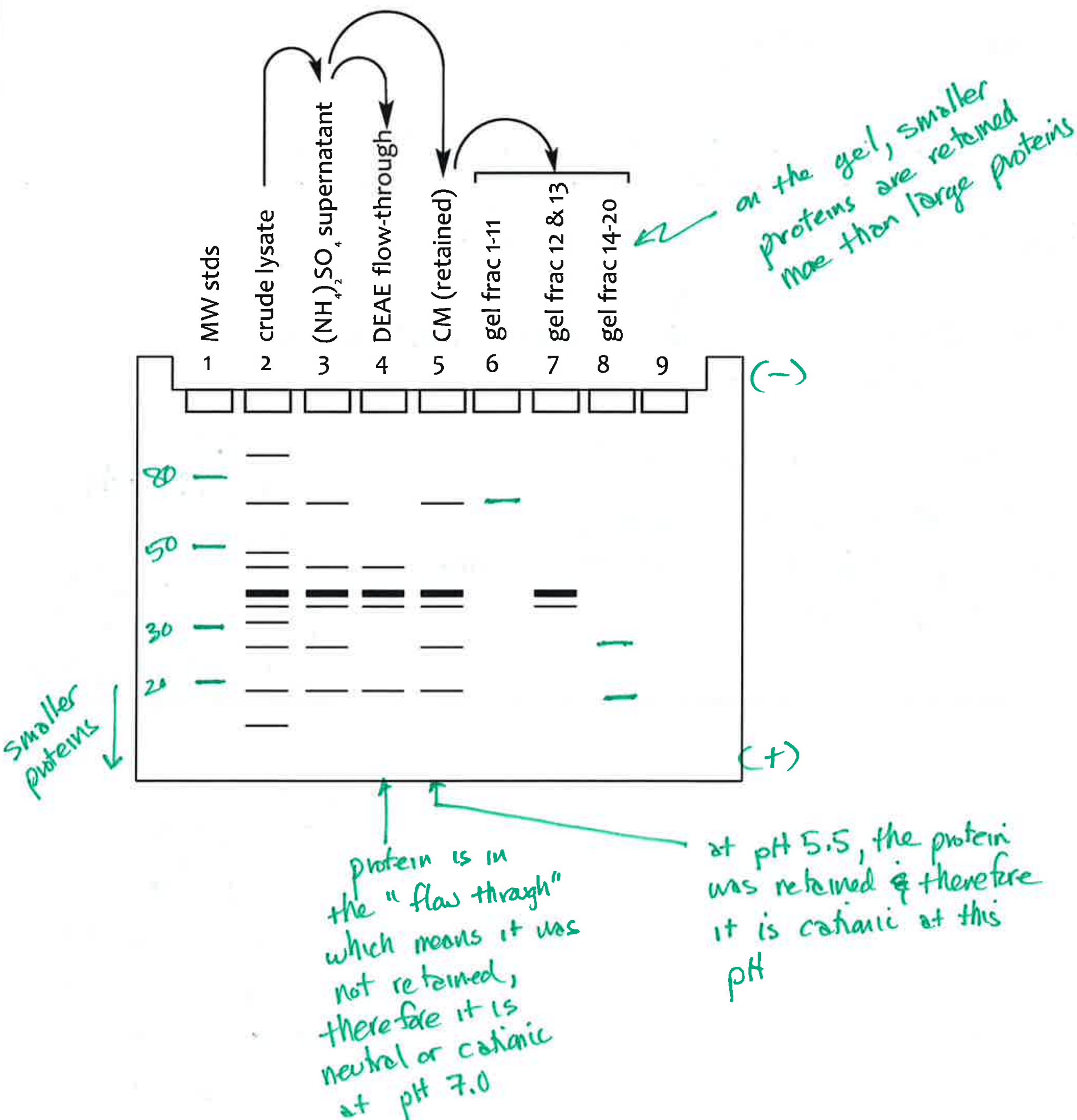
Protein Purification Challenge

Imagine you are developing a purification scheme for a 40 kDa protein associated with photosynthesis in cyanobacteria. You use the following procedures:

1. Ammonium sulfate precipitation of the crude lysate. You find that your protein is in the supernatant.
2. Anion exchange using DEAE resin at pH 7.0. Your protein was not retained at all. At this pH, the protein is neutral or cationic
3. Since anion exchange was not successful, cation exchange on CM resin was performed at pH 5.5 on the ammonium sulfate supernatant, with elution using concentrated NaCl.
4. Gel filtration on the CM-retained proteins. Twenty fractions were collected; your protein appeared in fractions 12 & 13.
5. To evaluate your progress up to this point, you decide to use SDS-PAGE on all your samples. The dark band is your 40 kDa target protein. The gel is shown below.



Complete the picture of the resulting gel by drawing:

1. The charge on the electrodes at the top and bottom of the gel.
2. Molecular weight standards of 20 kDa, 30 kDa, 50 kDa and 80 kDa.
3. Fill in lane 6.
4. Fill in lane 8.

Answer the following questions.

1. Explain what you can conclude with the observation that your protein *was not* retained at step 2.

   It is neutral or cationic at pH 7.0

2. What do you know about the proteins that *were* retained at step 2?

   They are anionic at pH 7.0

3. Explain what you can conclude because your protein was retained at step 3.

   It must be cationic at pH 5.5

4. What is the pI of your protein (report a range).

   Between the two expts, we know the protein is cationic at pH 5.5 and below. Therefore the pI > 5.5

5. Fractions 12 & 13 are reasonably clean but not quite pure. What do you know about the contaminant?

   It weighs slightly less than the protein of interest.

6. If the contaminant is related to your target protein, how might it differ structurally? Give your reasoning.

   It weighs less, so it is missing something, perhaps a light subunit or cofactor of some type.

   Since SDS PAGE is denaturing, another possibility is that the two bands belong together in the functional, intact protein: 2 subunits of unequal but similar mass. But one band is stronger, so there must be unequal numbers of the subunits

7. Suggest another experiment that might separate the target from the contaminant.

   (Gel filtration will <u>not</u> be helpful since the mass is so similar.
   Perhaps isoelectric focussing, or ion-exchange at a different pH.