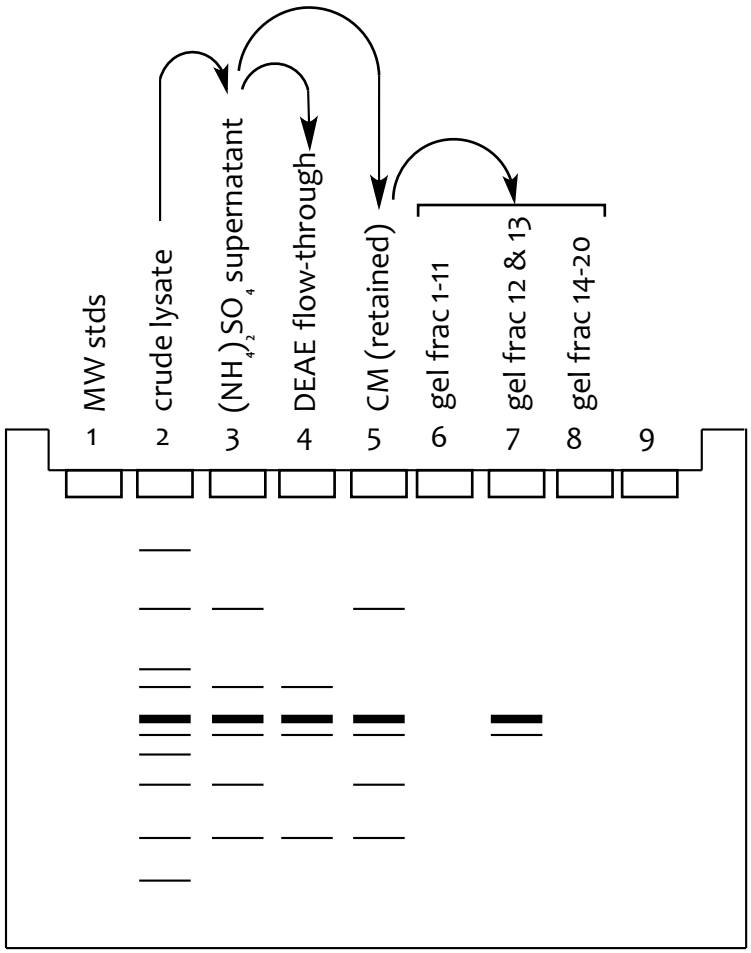
Protein Purification Challenge

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.
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.

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

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

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

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

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

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