

## Exercise 2

*Read through the entire exercise before you begin*

You received a new project to purify the following protein for the first time:

MSDTEAPVEVQEDFEVVEEFTPVVLATPIPEEVQQAQTEIKLFNKWSFEEVEVKDAS  
LVDYVQVRQPIFVAHTAGRYANKRFRKAQCP I IERLTNSLMMNGRNNGKCLKAVRI I  
KHTLDI INVLTDQNP IQVVDAITNTGPREDTTRVGGGGAARRQAVDVSPLRRVNQA  
IALLTIGAREAAFRNIKTIAETLAEELINAAGSSTSYA I KKKDELERVAKSNR

1. Gather the:
  - Name & function of the protein (any structure available? If so, give the PDB code)
  - MW (attach relevant screen shots of the analysis)
  - Theoretical pI (attach relevant screen shots of the analysis)
  - Biological source
  - Possible disulfide bonds (attach relevant screen shots of the analysis)
2. a) Which organism will you choose for the protein purification?  
b) Your PI tells you to first isolate the complex from the rest of the cell content and only then dissociate the above protein and purify it. How would you dissociate the protein complex?
3. Incorporating your answer to Q2b, give details to the purification process from the point of a cell suspension. Briefly detail and explain the following steps:
  - Lysis method
  - Centrifugations specifics: how many centrifugation steps are needed, the g force (RCF) and time of each centrifugation (see attached table for aid)
  - The buffer used in each step
4. You realize that the only available rotor is the Beckman's 42.2Ti rotor – which RPM will you use for each step?
5. Before continuing your experiments, you want to determine the protein concentration of your 3.5 mL protein sample. You measure the absorbance of the purified protein at a 1:60 dilution, 280 nm wavelength, and get a value of 0.546. How much protein have you purified?

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Table 1. Sedimentation of Some Sub-cellular Components

R.C.F. <i>g</i>	Sedimentation coefficient, S	Components	Comments
<200	>10 <sup>7</sup>	Whole cells connective tissue, clumps of cells, and large debris	Components depend on method of disruption
600–800	4 × 10 <sup>6</sup> –10 <sup>7</sup>	Nuclei	Little agreement on best method of purification
7 000	2–7 × 10 <sup>4</sup>	Mitochondria	Do not use sucrose density gradient (hypertonicity damages mitochondria)
105 000	10 <sup>2</sup> –1.5 × 10 <sup>4</sup> 30–80	'Microsomes' Ribosomes and ribosomal sub-units	5 mM Mg <sup>++</sup> required to prevent dissociation into ribosomal sub-units
105 000– 400 000	up to 120 4–50	DNA RNA	Cannot be adequately separated from the microsomal fraction and each other by differential centrifugation
400 000 +	2–25	Proteins from 'supernatant' fraction	Better results could probably be obtained using gel filtration or other methods