Experimental Approaches in Molecular and Cell Biology

Homework questions for "Protein purification and characterization"

Due day: Sep. 27, 2021 to Hanna S. Yuan

(100 points)

Assuming that you are asked to express and purify a full-length human protein XYZ with a His-tag in *E. coli*. XYZ is a mitochondrial DNA-binding repair enzyme with a calculated pI of 9.5 and a molecular weight of ~40 kD. Please answer the following questions with a brief explanation and turn in the typed printed answer of about five pages on Sep. 27, 2021.

(1) The cDNA of XYZ was cloned into a pET28a expression vector by a former lab member, however, you notice that its expression in BL21(DE3)pLysS (Novagen) was low. You therefore plan to express the protein in Rosetta 2(DE3)pLysS strain. How is the protein expression regulated and induced in Rosetta 2(DE3)pLysS and what are the advantages to change the expression host to Rosetta? (30/100 points)

(2) Suggest two types of column chromatography for the purification of XYZ. Briefly explain why these two types of columns can be used. (20/100 points)

(3) How do you know that the purified protein has a high homogeneity and it is indeed XYZ? (15/100 points)

(4) How do you know that the recombinant protein is a well-folded functional protein? (15/100 points)

(5) XYZ forms a disulfide cross-linked dimer in oxidized conditions with a molecular weight of about ~80 kD. Suggest a method to characterize the disulfide linkages and the oxidized Cys residues in the oxidized XYZ. (20/100 points)