

Chemical Biology Cume

January 18, 2018

100 points

Study reference: L. Xue, I.A. Karpenko, J. Hiblot, K. Johnsson "Imaging and manipulating proteins in live cells through covalent labeling" (2015) *Nat. Chem. Biol.* 11, 917-923.

1) (40) The study reference provided for this exam described methods and applications of covalently labeling proteins with small molecules in or on living cells. Three (3) strategies for protein labeling were discussed: 1) self-labeling tags that target recombinant fusion proteins; 2) reaction of small molecule labels with non-natural amino acids incorporated into protein primary sequences; and 3) self-labeling tags that target endogenous proteins.

Please briefly describe each of these methods. In your answer, list the necessary components for each technique (use schematic drawings and/or chemical structures); provide at least one specific example of each technique; and give at least one potential advantage and one potential disadvantage of each method.

2) (30) A major motivation for *in cellulo* protein labeling with small molecules is the need for fluorophores that perform better than genetically encoded fluorescent proteins, particularly for applications like single molecule or super-resolution imaging. However, most successful examples of small molecule imaging probes tag proteins on the cell surface rather than in the interior of living cells.

- a) Explain why cell surface labeling has generally been more successful than intracellular labeling.
- b) Explain how the challenges of intracellular protein labeling have been addressed using silicon-rhodamine (SiR) fluorophores. In your answer, show the structure of SiR and describe the chemical and photophysical aspects of SiR that provide distinct advantages for intracellular labeling.

3) (30) Another application of small molecule protein labels is to permit the chemical or photochemical control of protein function as a means of understanding biological mechanisms.

Describe and schematically draw one of the semi-synthetic protein switches or sensors discussed in the review article.