

3. **(1 point)** ELISA tests are used to detect the presence of HIV antibodies in serum. Usually, an antigen (e.g. modified HIV) is immobilized to the substrate. Design a competitive ELISA kit in which an anti-HIV antibody is immobilized to the substrate. In your kit design indicate (A) the reagents that you will include in the kit, (B) the basis for detection of a positive response, and (C) general instructions for the user of the kit.

4. **(2 points)** Interactomes. (A) What is an interactome? What are bait and prey proteins? (B) Describe in detail two approaches that are used to identify partners in an interactome. Draw diagrams that illustrate the key features of each approach.

5. **(1 point)** Design a yeast two-hybrid system experiment that aims to discover proteins interacting with leptin, a protein associated with obesity.

6. (1.5 points) *Seitz. et al., Proteomics, 2006, 6, 5132–5139* describe a functional protein microarray based on the immobilization of His-Tag fusion proteins (prey proteins) onto a plate. To identify bait-prey interactions, they added bait proteins (e.g. GSH-cacyclin) and used fluorescently labeled antibodies against GSH.

Another use of GSH (= glutathione S-transferase) is for immobilization of GSH fusion proteins to glutathione covered plates. **REDESIGN THIS EXPERIMENT** using GSH-fusion proteins as prey. Indicate how the prey proteins would be prepared, immobilized, and used to capture the bait proteins. How will you identify the positions in the array where interacting partners are present?

7. **(1.5 points)** Wikström *et al.*, *Analytical Biochemistry*, 2007, 362, 98-107 show that SPR can be used to monitor interactions occurring at biomembranes. Propose an SPR experiment to investigate the kinetics of lipases (i.e. enzymes that hydrolyze lipids). Include in your proposal comments on the preparation of the SPR surface, measurements, appearance of the SPR data, how to determine the rate constants, and any control experiments that may be needed.