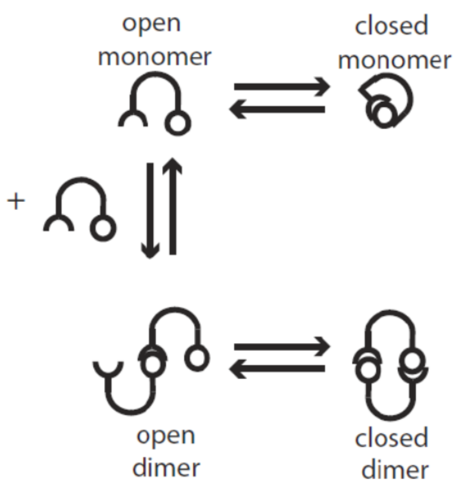
Question 1

"It is more difficult to find commercially available antibodies for immunoprecipitation (that actually work), than antibodies for western blots. This company sells an antibody that works even for a native western blot – not a common technique. But the same antibody does not work well for immunoprecipitation of the protein", bemoaned a researcher. Do you think the researcher is correct? Explain your answer.

Guidelines for answer: Discuss what immunoprecipitation, western blotting and native western blotting are. Schematics of the procedures will help. Follow this up with your explanation. The schematics may provide clues.

Question 2



Consider a monomer that is self-complementary, such that it can either add another monomer or cyclize, as shown in the figure. Let Kd be the dissociation constant of an open dimer going to two open monomers. Let [L]eff,1 be the effective concentration of the two ends for each other in the monomer. Let [L]eff,2 be the effective concentration of the two ends for each other in the open dimer.

1. Assuming that the only four species that can exist are those in the figure above, solve for the ratio of the equilibrium concentration of closed dimer to closed monomer as a function of the effective concentrations, the dissociation constant, and the concentration of open monomer.

2. Using your result from part 1), state conditions (limits) for which [closed dimer] >> [closed monomer]. How could each be achieved in practice?

Question 3)

Consider the binding interaction of a molecule A with molecule B (**Figure 3-1**). A has two identical subunits ('a') that are linked by a flexible linker of length 100 A°. Each subunit can bind B with K_D = 100 nM ($k_{on} = 10^5\ M^{-1}\ s^{-1}$).

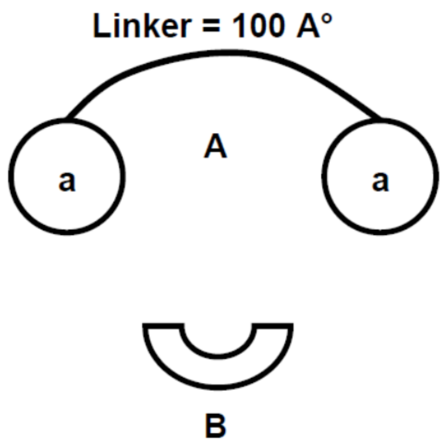


Figure 3-1: Each subunit of A is identical and binds B

(a) Consider a cell, with radius 10 µm, that expresses 10^5 copies of B on the cell surface. 10^4 cells are incubated with a solution containing 100 nM A. Calculate the fraction of cell-surface B that is bound to A (*Hint*: Consider Figure 3.16 in Ch.3 very carefully).

(b) Estimate the half-life of dissociation of molecule A from the surface of the cell.

(c) In pioneering work, a lab has managed to immobilize B on a flat surface in a precise geometric pattern as shown in **Figure 3-2**.

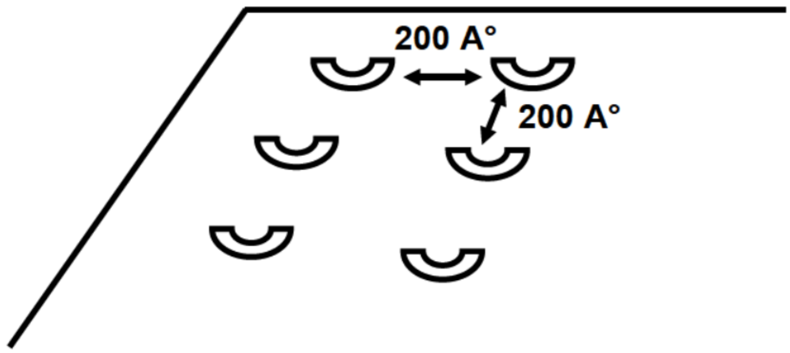


Figure 3-2: Surface with B immobilized in a precise geometric pattern

The graduate student (who actually did the work) is confident that the functionality of B is perfectly maintained upon immobilization. Assessing the binding to A was thought of as a good assay to test the functionality of B.

Accordingly, two experiments were carried out. First, the surface (10 mm^2) was incubated with a large volume of solution containing 100 nM A, and the fraction of surface-bound B was estimated. Second, the half-life of dissociation of A from the surface was determined.

Assuming that immobilized B is functional, what would be the outcome of these experiments? What should the graduate student's conclusions be about the functionality of immobilized B, if one or both the experiments described in (c) give vastly different results from those in (a) and (b).