Lecture #5
The Cell as a Machine

Biochemistry of Mitosis

Organization of the spindle

Microtubule Polarity and the Motors

Microtubule dynamics and kinetochore capture

Force generation and the signal for chromosome separation (Nicklas, 1997)

Protein-DNA Binding

Summary; In considering the function of the histones, they need to both bind strongly and be able to get out of the way when replication occurs. The physical chemical basis of the histone-DNA interactions and dynamics are important for our understanding of how the system works. We will consider the basic elements of protein-protein interactions and how to create a system that satisfies the engineering (practical) requirements.

Histone-DNA versus Replication Initiation Factor-DNA Binding

Within the nucleus, the majority of the DNA is bound to histones and it is important to understand what that means in quantitative as well as qualitative terms. The DNA needs to be accessible for the activation of replication or transcription; therefore, the proteins that initiate transcription must replace histones to bind to the DNA. Two major mechanisms can be employed to enable the replication initiation factors to bind to the DNA: 1st, the replication initiation factors could be activated and then they wait for the initiation sites to come free during the normal dynamics of the histone-DNA interaction, or 2nd, the activated factors could participate in an active screening process, which would entail transitory disruption of the histone-DNA complexes. Protein-DNA associations can be described by classical physical chemical equations that describe all equilibrium interactions. The dynamics of the DNA in the nucleus requires the input of energy but what it is modifying is the short-term equilibrium that occurs between the components. In thermodynamic terms, the approach to a true equilibrium state can take an arbitrarily long time. Since all biological cells contain non-

Protein-Protein Association

Lecture Notes, Part 1: Protein-protein association, the role of subunit entropy, and the nature of the protein-protein bond as described by Chothia and Janin.

Protein-protein bonds involved in oligomers (e.g., hemoglobin tetramers) and self assembly of larger polymers (actin, microtubules) are non-covalent. Bond formation is freely reversible, and the reaction can be characterized by an equilibrium constant and associated free energy. Consider the association of two subunits to form a dimer.

$$P_1 + P_2 \text{<-------> } P_1\text{-}P_2 \qquad K_D = \frac{[P_1][P_2]}{[P_1\text{-}P_2]} = 1/K_A$$

Note that the units of K_D are M. This is convenient because it expresses the strength of a reaction in units that can be compared directly with the concentration of the reactants. One can set up a quadratic equation to solve for $[P_1\text{-}P_2]$ for any starting concentrations of P_1 and P_2.

The analysis is simplified if we assume that one reactant, say P_1, is present in great excess, so that its concentration is not significantly altered by formation of the complex. Then we have a simple relation for the fraction of P_2 in complex

$$\frac{[P_1\text{-}P_2]}{[P_2]} = \frac{[P_1]}{K_D} = \text{Ratio of } P_2 \text{ bound/free}$$

If $[P_1] << K_D$, there will be relatively little complex; most of the P_2 will remain free monomer.
If $[P_1] = K_D$, the P subunits will be 50% free and 50% in complex.
If $[P_1] >> K_D$, most of the P_2 subunits will be complexed to P_1.

Note that it is the concentration of the species in excess, P_1, relative to K_D, that determines the relative amount of the dilute species that is complexed or free. Of course, the top equation is completely general, and with some additional mathematics the concentrations of reactants can be determined for any conditions.

The dissociation constant, K_D, is especially useful because it allows a quick comparison with the molar concentration of the reactants. The reaction can also be described by an association constant, $K_A = 1/K_D$. For most of the lecture, and in the paper on cooperativity, I use the association constant, K_A. The advantage of discussing association in terms of K_A is that a stronger association corresponds to a larger K_A. The equilibrium constant is related to the free energy by this standard equation:

1. $$RT \ln K_A = -\Delta G_A = -(\Delta H_A - T\Delta S_A)$$

(Note that favorable association requires a negative free energy of association.)
This free energy describes all of the chemical and energetic factors involved in the association reaction. It is extremely useful to break this term down into two opposing energies.

ENZYME KINETICS

Classic Michaelis Menton kinetics is used to describe reactions of a substrate (S) with an enzyme (E).

$$S + E \underset{k_{-1}}{\overset{k_1}{====}} SE \overset{k_{cat}}{------} P + E$$

Where k_1 and k_{-1} are the forward and reverse rate constants for the formation of the bound intermediate (SE) and k_{cat} is the rate constant for the catalysis of the reaction of substrate to product (P). The rate of formation of product is described by

$$d[P]/dt = k_{cat}\,[SE]$$

and

$$k_{-1}[SE] = k_1[S][E]$$

where [E] is enzyme without substrate or more commonly expressed as total enzyme [E_t] minus [ES] or [E] = [E_t] - [ES]

thus

$$d[P]/dt = k_{cat}\,k_1[S][E_t]/(k_{-1} + k_1[S])$$

and this is the Michaelis-Menton equation. In the limit of high concentrations of substrate, all of the enzyme is in complex with substrate; therefore, the rate of formation of product levels off at $k_{cat}[E_t]$. In the limit of low concentration of substrate, the equation can be reduced to a complex constant times the concentration of substrate and enzyme (in the problems below, we will fix the enzyme concentration and describe the kinetics as a constant times the substrate concentration).

Question:

1. Read the review on mitosis (Nicklas, 1997) and tell me which of the two models for the block to chromosome separation is physically possible. A) The first model suggests that the concentration of a small molecule has to be greater than 1 μm. We postulate that the small molecule is produced by all chromosomes under tension (synthesis rate of X was 40,000 molecules/chromosome/minute) and degraded by chromosomes not under tension (degradation rate was given by the formula $d[X]/dt = -k[X]$, where $k = 10^{-4}\ s^{-1}$). The volume of the cell is 4000 μm^3 and there are 10 chromosomes.
   B) In the second model, another metabolite Y is being made by chromosomes under tension (the synthesis rate for Y is the same rate as for X above) and is remodeled by the chromosomes not under tension (synthesis rate is $d[Z]/dt = k_s[Y]$ where $k_s = 10^{-1}\ s^{-1}$) to produce the final produce Z which blocks

chromosome separation. Z has a half-life of 10 minutes in cytoplasm and must be less than 10^{-7} M.

Nicklas, R.B. 1997. How cells get the right chromosomes. *Science*. 275:632-7.