

BMT-72106 CELLULAR BIOPHYSICS

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ABSTRACT

This week's exercise is about the general concept of the mechanotransduction, including the protein quaternary, ECM and the tool AFM. In calculation part, the Molecular dissociation constant is of our interest. The binding between avidin biotin pair and antibodies antigen pair are compared by calculation.

1. EXERCISE 1

a) Protein quaternary structure: Protein quaternary structure is the number and arrangement of multiple folded protein subunits in a multi-subunit complex. It includes organisations from simple dimers to large homooligomers and complexes with defined or variable numbers of subunits. It can also refer to biomolecular complexes of proteins with nucleic acids and other cofactors.

b) Young's modulus: Young's modulus or Young modulus is a mechanical property that measures the stiffness of a solid material. It defines the relationship between stress (force per unit area) and strain (proportional deformation) in a material in the linear elasticity regime of a uniaxial deformation:

$$E = \sigma/\epsilon, \text{ where}$$

σ is the uniaxial stress, or uniaxial force per unit surface, in pascal. ϵ is the strain, or proportional deformation (change in length divided by original length) (nondimensional). In practice, Young's moduli are given in megapascals (MPa or N/mm^2) or gigapascals (GPa or kN/mm^2).

c) Mechanotransduction: Mechanotransduction means the process which allows cells to sense and alter the physical properties of the environment (rigidity, dimensionality, elasticity, etc.). It is the conversion of extracellular matrix (ECM).

2. EXERCISE 2

Extracellular matrix (ECM) forms the extracellular part of tissues. ECM consists of many kinds of molecular components. Which are the main molecular components in ECM? Describe their structure and main functions. 1) Collagens: Thick and thin fibres. It works as the tensile strength.

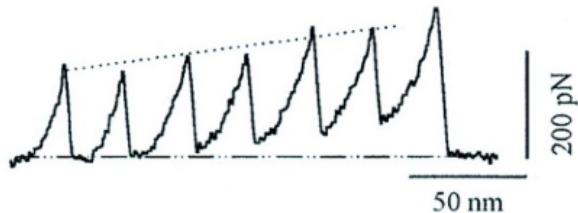


Figure 1: Result plot from AFM measurements.

Fig. 1. Results plot from AFM measurements.

2) Elastin/Microfibrillar proteins: Elastic fibers of skin, lung, blood vessels. It works as the elasticity. 3) Proteoglycan: Space between the proteins. It works as the stiffness. 4) Fibronectin/Laminins: Cell-ECM contracts. They are cell attachments, working as the bridge between cell and ECM.

3. EXERCISE 3

Explain the working principle of atomic force microscope (AFM). How it can be used to study the protein folding and interactions between proteins? Further, explain what is happening in the result plot from AFM below, including what the axes describe. AFM is a very-high-resolution type of scanning probe microscopy (SPM), with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. Force spectroscopy has emerged as a new tool to study protein folding, in which force replaces the chemical denaturant used in traditional folding experiments. A protein molecule is tethered between a probe (e.g. an AFM cantilever tip) and another surface. The probe is manipulated to apply force to the protein under study. It reports on both the force applied to the protein molecule and the overall end-to-end extension of the system, allowing real-time monitoring of the conformation of the molecule. Thus, in the figure 1, the perpendicular axis stands for the force applied on the protein (pN as units) and the horizontal axis refers to the extension of the system (with nm as units). The peaks along the perpendicular axis represent the individual interactions between the probe and the protein, while the overall shape of the curve represents the collective behavior of the protein molecule.

lar axis are the results of the tips unfolding the material, the value along horizontal axis shows the extension of the protein by everytime to total extension. The force is proportion to the extension generally.

4. EXERCISE 4

Molecular dissociation constant can be calculated as where

$$K_d = \frac{k_r}{k_f} = \exp\left(-\frac{\Delta E + F\Delta x}{k_B T}\right),$$

Fig. 2. Molecular dissociation constant.

kr is the unbinding reaction rate and kf is the binding reaction rate. Avidin protein binds biotin extremely efficiently, $K_d[\text{biotin-avidin}] = 10^{14}$. Antibodies bind to their epitopes also really well with $K_d[\text{Ab-epitope}] = 10^{10}$. However, when the force needed plain why this sounds strange and what the possible cause is for this by using the above equation and schematic figures below

According to the K_d , compare to the antibodies, the avidin binds to biotin extremely efficiently with the molecular dissociation constant 10^{-4} times to the one of antibodies. However, the force needed for unbinding for biotin-avidin is 1/4 of the one for antibody-antigen pair. Ignoring the energy related to drag coefficient, the length change of the biotin-avidin is $2*\ln 10/25$ minus the length change of the antibody-antigen pair. In contrast, according to the figure, the distance change of the biotin-avidin seems to be smaller than the antibody-antigen pair.

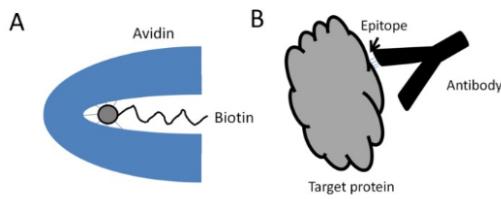


Figure 2: Schematic presentation of A) biotin-avidin binding and B) antibody-epitope binding.

Fig. 3. folding.