Lecture 10 - Exercises

Question 1: Biomolecular Interactions

Select a statement that is **TRUE**:

a) Protein-protein interactions are based on:
- Low desolvation energy contribution to binding
- Hydrophobic interactions created by the water molecules trapped in the interface
- Chemically and geometrically complementary molecular surface features
- Identical surface properties in the binding partners

b) Protein-DNA interactions:
- Always involve Watson-Crick base pairing between amino-acids and nucleotides
- Require an optimal distribution of charged and polar amino-acids on the protein side
- Require exclusively helical protein domains because DNA is also a helix
- Are entropically disfavored due to desolvation

c) Electrostatic (ionic) interactions:
- Are created between charged amino acids and phosphate groups in DNA backbone
- Contribute less energy in biomolecular interactions than a single hydrogen bond
- Do not depend on distance between charged groups
- Are formed between Lewis bases in nucleotides and positively charged amino acids

d) When it comes to biomolecule interactions between two binding partners:
- Enthalpy is the primary contributing factor to k_{on} rate while entropy affects k_{off}
- The displacement of surface-trapped water molecules is entropically favorable for binding
- Hydrophobic interactions are the main contributor to enthalpy but do not affect system entropy
- Dissociation constant does not depend on temperature.

e) Determining affinity between two binding partners:
- Also tells you about the type of interactions at their interface
- Allows to infer potential conformational changes that the binding event induces
- Is always based on measurements of thermodynamic properties
- Allows to estimate the fraction of bound and unbound material under different conditions

Question 2: Experimental methods for affinity measurements

Select a statement that is **FALSE**:

a) Nuclear Magnetic Resonance (NMR):
- Can be used to measure entropy and enthalpy contributions to total binding energy
- Allows to map the potential interacting surface between the binding partners
- Is used to measure affinity between binding partners based on changes in chemical shifts
- Is a spectroscopic method for measuring binding affinity

b) Isothermal Titration Calorimetry (ITC):
- Is based on spectroscopic measurements of heat changes upon addition of binding partner
- Allows to determine the thermodynamic parameters of binding
- Can be applied to measure affinity at different temperatures (T)
- Allows to experimentally measure the stoichiometry of binding (n)

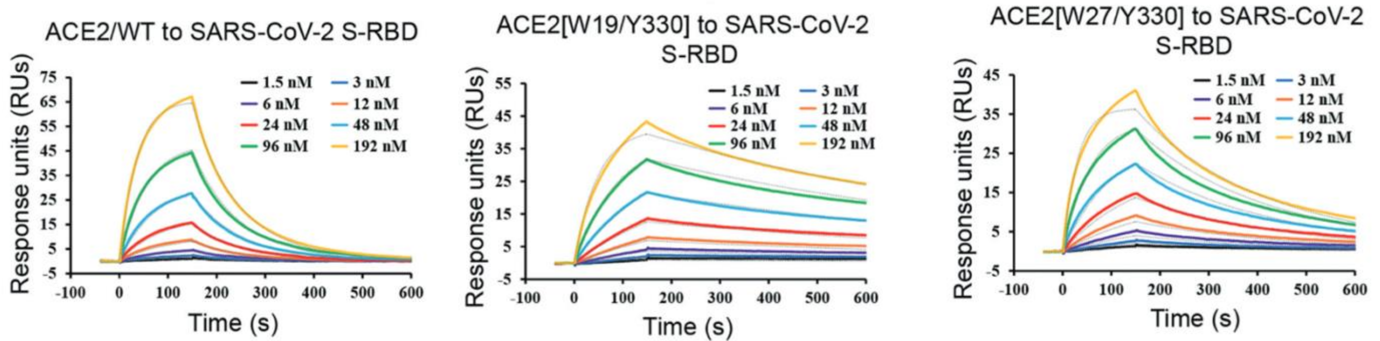
c) Fluorescent polarization (FP):
- Requires fluorescent labeling of one or both binding partners in most cases
- Is based on differing molecular weights of bound versus unbound molecules
- Can be used to measure if two or more ligands compete for the same binding site
- Allows to experimentally measure the stoichiometry of binding (n)

d) Surface plasmon resonance (SPR) is unique compared to other methods because:
- It allows to measure both, the association (K_a) and dissociation constants (K_d)
- It is based on spectroscopic measurement of light reflection at liquid-solid interface
- It can be used to measure kinetic parameters of binding (k_a and k_d rates)
- It requires surface immobilization of molecules for the method to work.

e) When it comes to different experimentally-determined thermodynamic parameters:
- Lower dissociation constants (K_d) indicates stronger binding between two molecules
- Greater reduction in Gibbs free energy indicates stronger affinity between two molecules
- Higher association rate (k_a) automatically indicates lower dissociation rate (k_d).
- For strong binding it is necessary to have high association (k_a) and low dissociation rates (k_d)

Question 3: Application to virus-receptor interactions

During the COVID pandemic scientists spent quite some effort to understand how SARS-CoV-2 virus infects cells. Upon discovery that the ACE2 protein serves as a cellular receptor to which the virus attaches, one research group tested if different mutations in ACE2 would have an effect on binding to the virus. The results for the unmutated ACE2 (WT) as well as the two mutants (W19/Y330 and W27/Y330) are shown below. For each mutant variant multiple concentrations of ACE2 were tested (from 1.5nM to 192nM), while the concentration of SARS-CoV-2 spike was kept constant at 200nM.



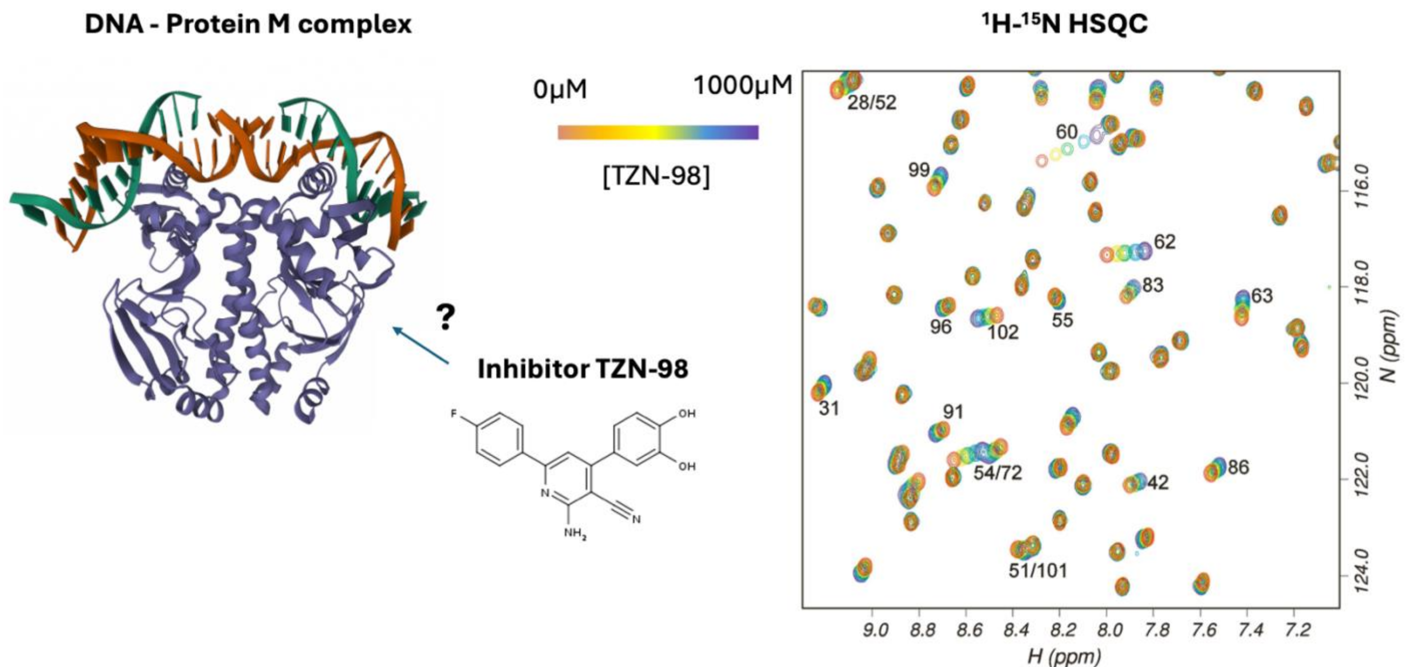
a) Can you identify what method was used to test the binding? Can you assign what different portions of the binding response curves refer to?

b) Why is there an increase in maximum signal with increasing concentration of ACE2 in each of the 3 cases?

c) By comparing the binding response curves corresponding to equivalent ACE2 concentration in 3 mutants (use the highest concentrations since they are the easiest), can you rank the mutants based on the association rate (k_a) from highest to lowest? Can you rank them based on dissociation rates (k_d)?

Question 4: Inhibitor characterization

You screened a large library of compounds and discovered a new molecule (**Inhibitor TZN-98**) with potent antibiotic activity against diverse bacterial strains (even the pan-antibiotic resistant ones). Based on computational predictions, you suspect that the molecular target of this molecule is **Protein M**, which is a DNA binding protein essential for bacterial life cycle. Now you wish to confirm that Protein M and TZN-98 interact, and you perform ^{1}H-^{15}N HSQC characterization of Protein M (isotope labelled with ^{15}N) in the presence of increasing concentrations of TZN-98 (ranging from 0-1000μM concentration). The overlay of ^{1}H-^{15}N HSQC spectra obtained at different protein/inhibitor ratios are shown below in different color. The corresponding residue IDs are indicated next to different peaks.



a) Based on the overlay of HSQC spectra under different conditions do you think that there is interaction between TZN-98 and Protein M? How can you tell?

b) Why did some peaks move in the spectra in response to TZN-98 addition and the others did not?

c) How can you use the data above to calculate the Kd of this interaction? What would you plot?

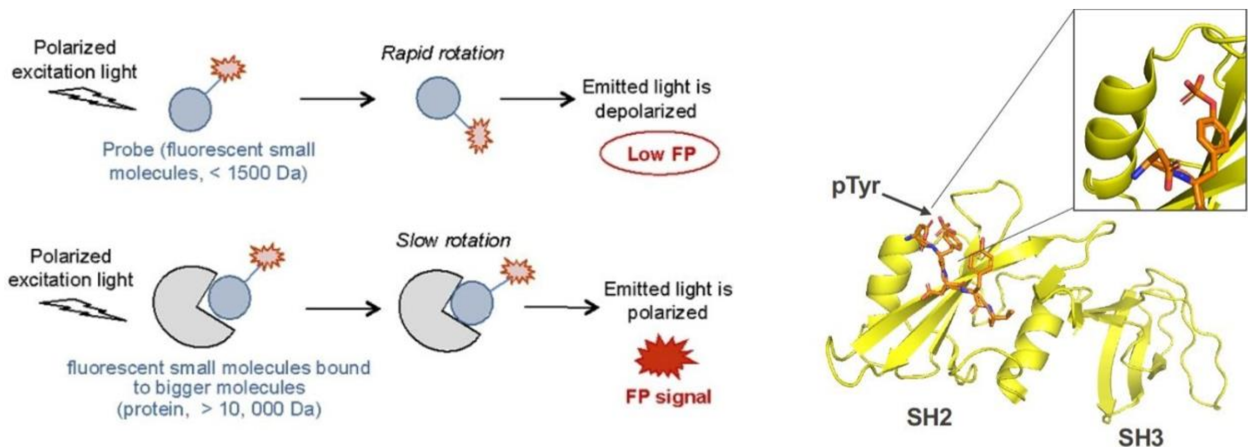
d) Why are some peaks shifting more and others less? Is this an indication of Kd values differing between amino-acids?

e) You suspect that the inhibitor may act by binding at the same site as the DNA molecule and preventing the formation of DNA – Protein M complexes. The structure of this complex is already known. How can you use the NMR data above to see if there is overlap in binding sites?

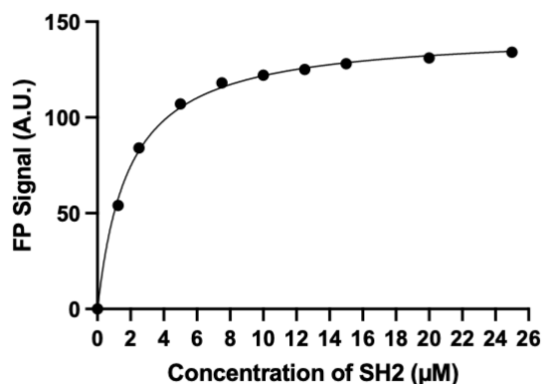
f) Which experiment could you use to directly check if there is competition between DNA and TZN-98 for the same binding site? Describe the details of how the experiment can be performed.

Question 5: Fluorescent polarization to study protein-peptide interactions

You want to determine the binding affinity of the SH2 domain of the Lck kinase to a peptide that contains a phospho-tyrosine residue. You synthesize the phospho-tyrosine peptide and include a fluorescent dye at the N-terminus. You measure fluorescent polarization (FP) in the absence and in increasing concentrations of SH2 domain.



a) The FP plot looks as shown below. Can you estimate the Kd from this graph? Approximate numbers are acceptable but please explain the process.



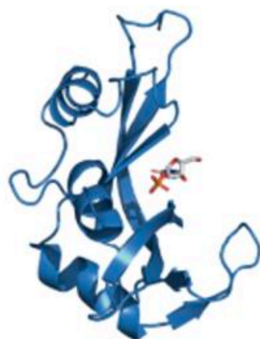
b) You have isolated two monoclonal antibodies that bind to SH2 with very high affinity (~1nM). Now you wish to explore if they compete with the peptide so you perform an FP assay, as above. You pre-mix the SH2 domain (at 25μM) with each antibody separately (at 50μM). After the incubation you add the fluorescent peptide and perform the measurement. For reference you also performed the measurement without antibodies in solution. You obtain the following FP signal values (in arbitrary units):

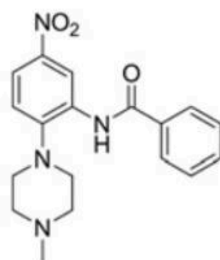
25 μM SH2	**148**
25μM SH2 +50 μM Antibody 1	**52**
25μM SH2 +50 μM Antibody 2	**560**

Why is the FP value for SH2 + Antibody 1 lower than for SH2 alone?
Why is the FP value for SH2 + Antibody 2 higher than for SH2 alone?

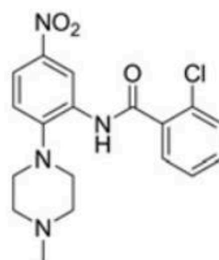
Question 6: Comparing protein inhibitors

WDR5 (WD40 repeat protein 5) plays key roles in development and is abnormally expressed in many cancers. Recent studies discovered small molecules that can disrupt the interaction between WDR5 and peptides from the catalytic domain of MLL (mixed-lineage leukemia protein) and are promising inhibitors for development of drugs for MLL-rearranged leukemias and other cancers. These are three compounds within this class:

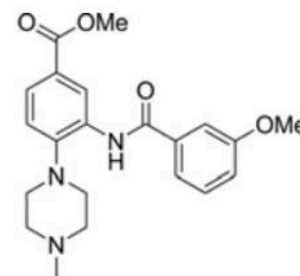




WDR5-0101

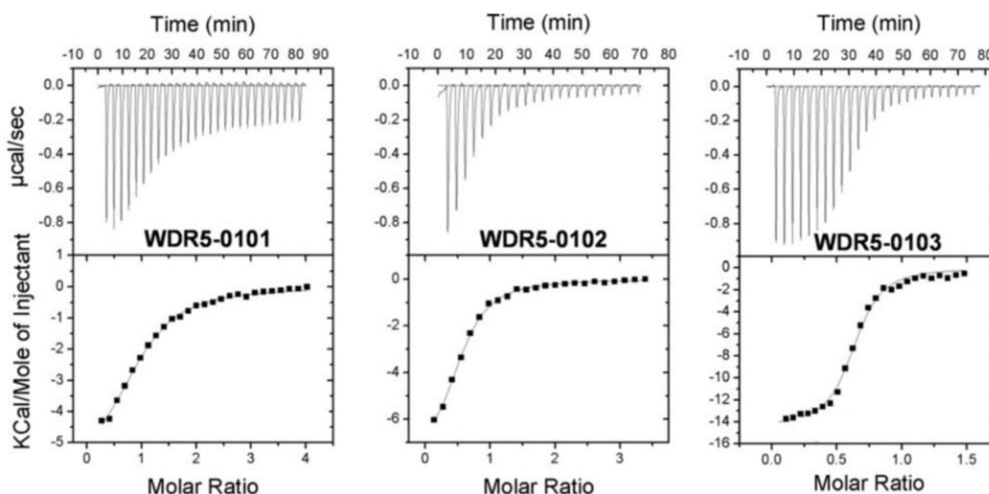


WDR5-0102



WDR5-0103

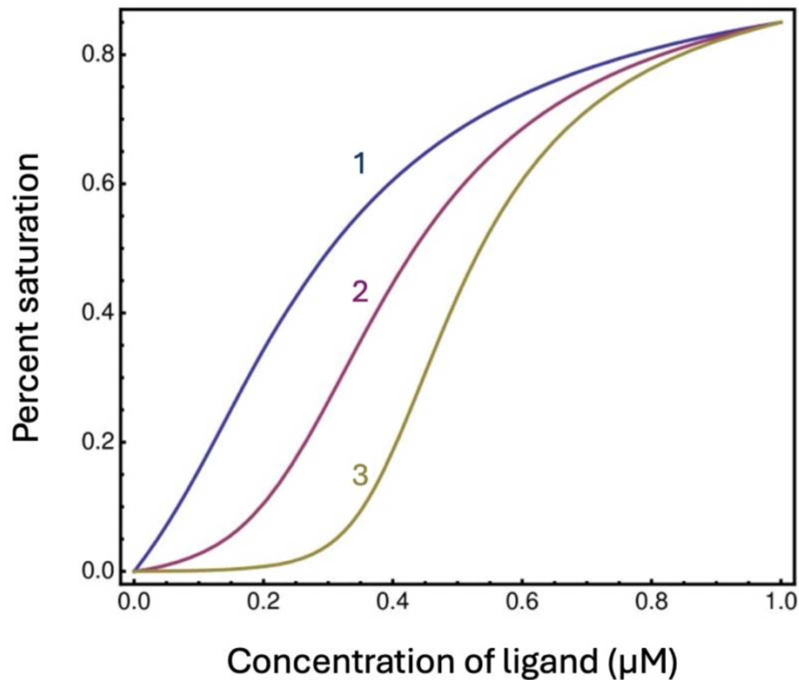
a) Scientists performed an assay to determine their binding affinity. See the results below obtained at 300 K. What is the technique used here? Which compound has the highest affinity (greatest Ka – lowest Kd)? Use the graph below to estimate the enthalpy and stoichiometry of binding for this compound? Note that the X and Y axes have different relative values in 3 cases which makes the comparison a bit trickier.



b) Assume that the most potent compound from the example above has a Kd of 0.45 µM. What is the free energy of binding (dG) and its entropic contribution (dS) for this compound? Hint: You will need to use some data from answer a).

Question 7: Cooperative binding

Below are 3 curves showing the response of different mutant variants (1,2,3) of a mutimeric sensor protein to the same ligand.



a) Rank the protein variants based on the degree of cooperativity displayed (from most to least cooperative) ?

b) If the Hill coefficient for the Sample # 2 (magenta) is 1.5, what is the ratio of K_{d2} and K_{d1} values for the two cooperative binding states in this variant?

c) Would you expect the Hill coefficient to be higher or lower for Sample # 1 (blue) ? What about Samples # 3 ?