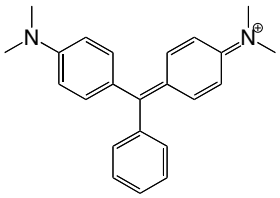
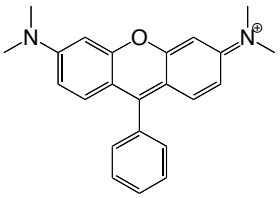
Chemistry 391 - Problem Set #6 **Name**_____

Due Thursday 10/25/18 in class

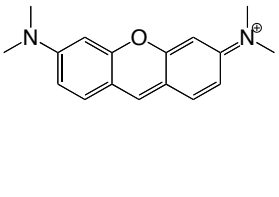
1. A 38-nucleotide RNA aptamer has been developed to bind malachite green, one of a group of compounds referred to as the triphenylmethane dyes. The x-ray crystal structure has been solved of the RNA aptamer bound to tetramethylrosamine (ROS). You can download a PyMOL session file, **ROS.pse**, from the Chem 391 web site. The structures of three dyes and their K_d's are shown below. It will help to examine ligand binding more carefully by zooming in on the **bind** object in ROS.pse.



Malachite Green
K_d = 800 nM



Tetramethylrosamine
K_d = 40 nM



Pyronin Y
K_d = 225 nM

+8.3 kcal/mol +10.2 kcal/mol +9.2 kcal/mol

a. What is the ΔG_{diss}° for each of the ligands above (in kcal/mol)? Please write the value under the K_d's in the space above, showing one sample calculation in this space.

$\Delta G^{\circ} = -(0.001987 \text{ kcal/mol•K})(298 \text{ K})\ln(800 * 10^{-9} \text{ M}) = 8.3 \text{ kcal/mol}$

b. What is the structural basis for the difference in ΔG°_{diss} for pyronin Y vs. tetramethylrosamine? Provide an entropic and/or enthalpic rationale for the difference in free energy of dissociation.

The extra phenyl ring of rosamine stacks against adenine at position 30 of the aptamer. That would lose the enthalpic benefit of vdW interactions and the entropic benefit of the hydrophobic effect by burying the phenyl ring surface.

c. Note that the ring oxygen of tetramethylrosamine does not interact with any atom groups in the RNA. Suggest an enthalpic and/or entropic reason for the difference in ΔG°_{diss} between it and malachite green. Think rotamers…

Enthalpically MG may bind more weakly do to a lack of favorable electrostatic interactions with the ring oxygen. Entropically, MG may gain more entropy upon dissociation because the phenyl rings are not constrained by the bridging oxygen.

d. How to the exocylic dimethylamino groups contribute to binding affinity to the aptamer? Note that they are equivalent by resonance (hint: think about charge-charge interactions.)

Phosphate oxygens in vicinity provide ion/ion interactions.

3. Consider a receptor that binds two ligands in a sequential fashion. For example, ligand "L" must be present before ligand "M" can bind. In dissociation reactions, it would look like this.

$R{\bullet}L{\bullet}M \Leftrightarrow R{\bullet}L + M$ with equilibrium constant K_{dM}

$R{\bullet}L \Leftrightarrow R + L$ with equilibrium constant K_{dL}

a. Derive an equation that relates the fraction $[R{\bullet}L{\bullet}M]/[R]_{tot}$ to the concentrations of free L and free M.

b. In terms of the dissociation constant, K_{dM}, what concentration of ligand "M" will lead to 50% of the receptor existing in the R•L•M state when $[L] = 10 \times K_{dL}$?

$$\frac{[R{\bullet}L{\bullet}M]}{[R]_{tot}} = \frac{[R{\bullet}L{\bullet}M]}{[R]+[R{\bullet}L]+[R{\bullet}L{\bullet}M]}$$

$$\frac{[R{\bullet}L{\bullet}M]}{[R]_{tot}} = \frac{[R][L][M]/K_{dL}{\bullet}K_{dM}}{[R]+[R][L]/K_{dL}+[R][L][M]/K_{dL}{\bullet}K_{dM}}$$

$$\frac{[R{\bullet}L{\bullet}M]}{[R]_{tot}} = \frac{[L][M]}{K_{dL}{\bullet}K_{dM}+[L]{\bullet}K_{dM}+[L][M]}$$

$$\frac{[L][M]}{K_{dL}{\bullet}K_{dM}+[L]{\bullet}K_{dM}+[L][M]} = 0.5$$

$$\frac{10K_{dL}[M]}{K_{dL}{\bullet}K_{dM}+10K_{dL}{\bullet}K_{dM}+10K_{dL}[M]} = 0.5$$

$$[10K_{dL}][M] = 0.5\left(K_{dL}{\bullet}K_{dM}+10K_{dL}{\bullet}K_{dM}+10K_{dL}[M]\right)$$

$$[M] = 0.05\left(K_{dM}+10{\bullet}K_{dM}+10[M]\right)$$

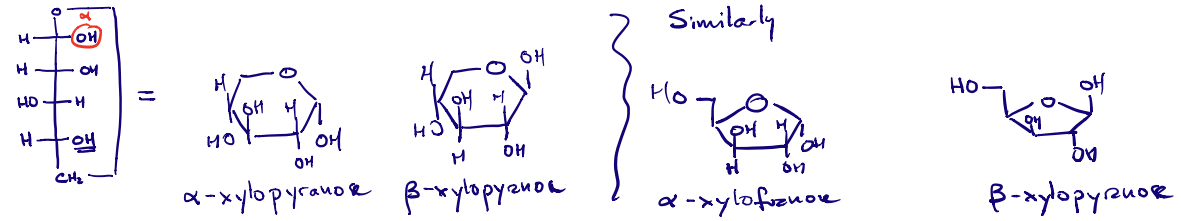
$$[M] = 0.55K_{dM}+0.5[M]$$

$$0.5[M] = 0.55K_{dM}$$

$$[M] = 1.1K_{dM}$$

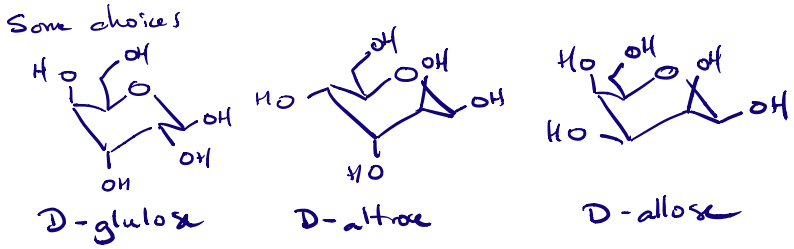
4. Something sweet for break.

a. Draw all furanose and pyranose forms of D-xylose, labeling α- and β- anomers. Special praise to those who can justify anomeric labels and don't just google them.

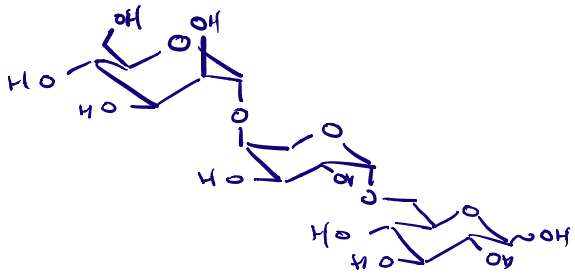


b. Glucose forms a pyranose with only equatorial substituents. Identify two D-hexaldoses would have the most possible axial substituents in their stable pyranose conformation, identifying the anomers as well. (Note glucose could have 6 axial substituents, but that wouldn't be a stable conformation.)

Important – hexoses in pyranose conf. have 5 substituents. Only 2 can be axial. If 3, the alternate chair would dominate.



c. Draw Man(α 1→4)Gal(α 1→6)Glc. Circle the reducing end of the trisaccharide.



Paper of the week: Wesener et al. (2015) Recognition of microbial glycans by human intelectin-1, *Nat Struct Mol. Biol.* **22**, 603-610. A PyMOL session file is available on the website.

First some question for you to work on independently

1. What is the guiding question or hypothesis in this work and why might it matter?

Human intelection-1 is a member of a family of lectins implicated in a variety of important functions. The goal of this work is to determine the ligand specificity of HintL-1 and, hopefully, in so doing, identify its function.

2. The crystal structure of hIntL-1 bound to βGal*f* is described in this paper:

a. Cite three statistics that indicate the good quality of data used in this study.

Resolution is good (1.6 Å). The redundancy is high (much greater than three observations per measurement). The data are complete (100%). Also it has high signal to noise - $I/\sigma(I)$ and to really push it the disagreement between measurements (R_{xym}) is less than 10%.

b. Indicate a statistic that shows how well the model agrees with the data.

R_{work} is 15.5% and R_{free} is 18%

c. Indicate information that can be used to evaluate the quality of this model in comparison to ideal molecular geometry.

Rms deviations in bond length and angle are low (0.010 Å and 1.12°).

d. Note that two different subunits appear in the crystal structure with slightly different conformations. This is common in crystallography and provides redundancy of observations. Are the two subunits similar to each other in structure? How is that evaluated?

The rms deviation in C_α atoms is 0.65 Å. That indicates "similar, though non-identical" structures. The dissimilarity is not large and generally shows that the structures are correct. Note that there are good reasons why two copies of the same protein may not look exactly alike.

3. Summarize the findings of this paper in 1-2 sentences.

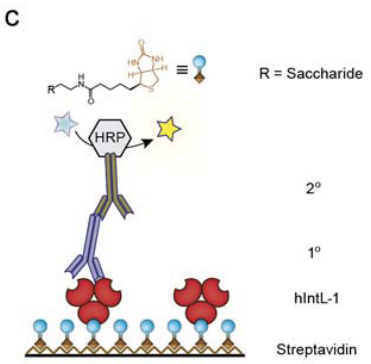
"Data from glycan microarrays reveal that hIntL-1 recognizes multiple microbial glycan epitopes yet paradoxically can discriminate between microbial and mammalian glycans… The five saccharide epitopes identified as recognition motifs (Gal*f*, phosphoglycerol, *glycero*-d-*manno*-heptose, KDO and KO) share a common feature: a terminal acyclic 1,2-diol. The hIntL-1 X-ray structure indicates that these terminal vicinal hydroxyl groups can coordinate to a protein-bound calcium ion."

Of equal note, HIntL does not bind human glycans with a 1,2 diol.

Group Questions

4. Lots of stuff got bound. This is high-tech ligand detection.

a. What is ELISA. In this specific study, explain how the sugar "panel" is presented to intelectin in terms of the components of the ELISA and how binding of hIntL-1 is detected. Drawings will help (and a good guide is supplemental Figure 1)



hIntL-1 binding assay schematic.

Surface is coated with streptavidin. Sugars are linked to biotin, which binds to avidin. Then hIntL is added. If it binds, then it can be detected with a primary antibody, which can be in turn detected with a secondary antibody that is linked to horseradish peroxidase (HRP). HRP can be used to catalyze a color-generating reaction which goes back to saying that hIntL bound there. What a bunch of steps.

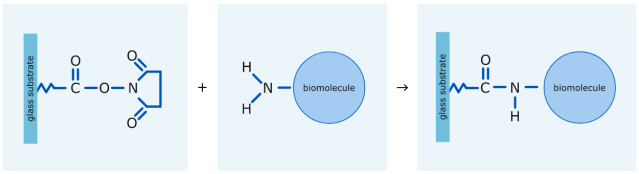
b. Explain how the sugars were presented in SPR (see experimental). Explain the plots shown in figure 1 for βGal*f* and compare to those with ribofuranose.

The chip is purchased with an avidin coating. Sugars were covalently attached to biotin which then binds to the avidin coating.

When HIntL is added to a chip coated with βGal*f* there is a change in the resonant angle being monitored in SPR. No such change is observed with ribofuranose. That indicates that HIntL binds to the βGal*f*, but not ribofuranose.

c. What is a microarray and how it prepared? See the experimental on the furanoside glycan array. It may be useful to show how N-hydroxysuccinimide reacts with amines.
See here: https://www.us.schott.com/nexterion/english/products/functional-coatings/3d-polymer-coating.html

A microarray is a "printed" grid of some target class of compounds (often DNA, but here glycans) where each printed spot contains a unique compound. Here, glycans were connected via glycosidic linkages to a primary amine that could react with N-hydroxysuccinimide esters.



d. How is microarray binding detected? What form of hIntL-1 is used and why? What was learned?

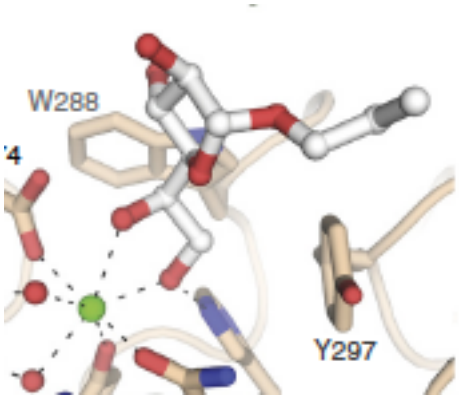
HIntL was produced as a "fusion" with avidin. That is, one protein chain contains both the sequence of HIntL and avidin. HIntL will bind to the cognate glycans. Then a fluorescent antibody that binds avidin was used to find bound protein on the array.

5. How was hIntL-1 binding to *Streptococcus* detected. What determinants of specificity were uncovered and how are these consistent with the in vitro work?

Essentially the same procedure as in the microarray. Consistent with in vitro work, the cell surfaces with glycans that possess either a diol from glycerol or from βGal*f* bind.

6. From the structural work (the PyMOL file may assist here).

a. Describe how βGal*f* interacts with intelectin. What portions of the molecule interact with the protein (and its bound Ca^{2+} ion)?

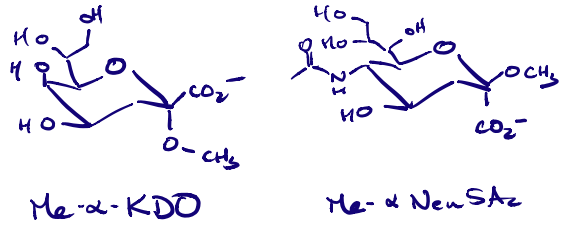


The chief interactions are an Hbond from His263 to the primary alcohol as well as the coordination of the Ca^{2+} by the vicinal hydroxyl groups of the diol.

b. What feature of the binding site restricts affinity to diols that include a primary alcohol?

Trp288 and Y297 form a "wall" that blocks addition of other substituents (beyond the -OH group) to primary alcohol carbon.

c. Draw methyl-α-NeuAc and methyl-α-KDO in their preferred chair forms. Why can one bind and not the other, despite each having a C1 carboxylate? What evolutionary pressure would there be on hIntL-1 to develop that selectivity?



Note the opposite orientations of carboxylate. In Neu5Ac that creates electrostatic repulsion and the N-acetyl group clashes elsewhere.

Since alpha-Neu5Ac is found in human glycans, you don't want HIntL-1 binding it and targeting self-proteins.