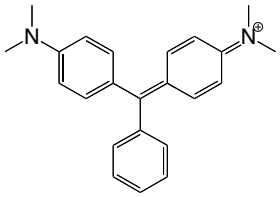
Chemistry 391 - Problem Set #6 **Name**_____

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

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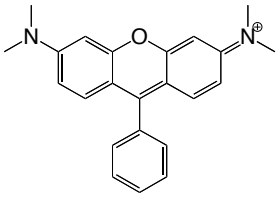

Tetramethylrosamine
K_d = 40 nM

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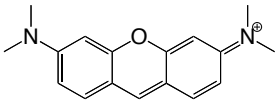

Pyronin Y
K_d = 225 nM

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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.

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.

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 the it and malachite green. Think rotamers…

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.)

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.

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

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

a. Derive an equation that relates the fraction $[R•L•M]/[R]_{tot}$ 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 $[L] = 10 \times K_{dL}$?

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 gluose could have 6 axial substituents, but that wouldn't be a stable conformation.)

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?

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.

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

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

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?

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

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)

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.

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

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

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?

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)?

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

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?