NAME: _____ Exam 1

You may use a computer and any electronic resources to complete this exam. You may not use the computer or any electronic device to communicate with other individuals either enrolled in the course or outside the course. Violation of this policy will result in your immediate and permanent removal from this course.

All references to original data, structures, and authors found in this exam are from:
McCorvy, J.D.; Wacker, D.; Wang, S.; Agegnehu, B.; Liu, J.; Lansu, K.; Tribo, A.R.; Olsen, R.H.J.; Che, T.; Jin, J.; and Roth, Bryan. (2018). Structural Determinants of 5-HT_{2B} Receptor Activation and Biased Agonism. *Nature Structural and Molecular Biology* **25:** 787-796.

The authors report the cellular calcium flux in response to various concentrations of 5-HT (serotonin). The data are summarized below and are also available on the course Moodle page. Sigmoid activation data as this are normally fit to the following equation:

Log [5-HT] (M)	% Calcium Flux
-12	0
-11.5	2
-11	3
-10.5	4
-10	3
-9.5	18.75
-9	45
-8.5	62.5
-8	81.25
-7.5	95
-7	105
-6.5	102
-6	100
-5.5	98
-5	96

$y = 100 \text{ x } \left(\frac{10^x}{(10^x)+(10^b)}\right)$; where "y" is percent calcium flux; "x" is the log of the 5-HT concentration; and "b" is the inflection point and only fit parameter.

1. **Using Excel and Solver, determine the value of the fit parameter "b". Report:**

   a. **The unrounded best-fit value of b:**

   b. **The unrounded Standard Deviation of b:**

   c. **The unrounded Standard Error of b:**

   d. **The correctly rounded value of b with the correctly rounded standard error:**

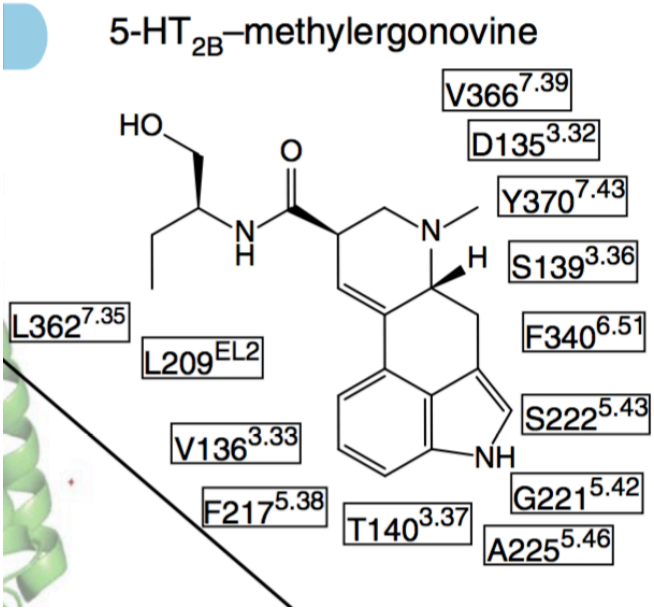
The fit-parameter "b" is related to the equilibrium constant (K_{eq}) for binding 5-HT: $K_{eq} = 10^b$. In this case, K_{eq} tells you what concentration of 5-HT is required to have half the maximum cellular stimulation.

e. **Determine the value (without error) of the K_{eq} for binding 5-HT.** The units are Molar.

f. **What is so amazing about this number?**

2. The authors determined the crystal structure of the 5-HT_{2B} receptor in complex with the agonist methylergonovine (PDB ID: 6DRY). In Figure 1c, the authors present the following image.

Figure 1c



a. Draw the sidechain of Asp as it would appear at pH 7.

b. Based on the authors' statement: "Methylergonovine forms a salt bridge with Asp135...", correct the ionization state in the adjacent representation of methylergonovine.

c. The authors determine that the interaction between Thr140 and the indole nitrogen of methylergonovine is critical for the agonistic action. What is the distance between the hydroxyl oxygen of Thr140 and the indole nitrogen of methylergonovine? What type of intermolecular interaction(s) do expect? [You need more detail than van der Waals]

d. For the structure of the receptor bound to methylergonovine, generate the vacuum electrostatics protein contact potential. In conjunction with the cartoon representation, estimate the width (across the membrane) of the transmembrane region.

e. How many transmembrane α-helices are in the 5-HT_{2B} receptor?

Leu362 is particularly important in regulating the position of transmembrane helix 7 and biasing the activity between Calcium flux or β-arrestin recruitment.

f. Using the structure containing lisuride, determine the distance between Leu362 and the closest position on lisuride.

3. For the myoglobin stability lab, we started with 1.5 L of 50 mM phosphate buffer at pH 7.0. The relevant pKa of phosphate is 7.21. Biological phosphate buffers are prepared from solid powder NaH_2PO_4 and Na_2HPO_4.

    a. Which phosphate species will predominate in the buffer?

    $$H_2PO_4^- \qquad\qquad HPO_4^{2-}$$

    b. How many grams of NaH_2PO_4 are required to produce 1.5 L of 50 mM phosphate buffer at pH 7.0? [Show your work]

    c. How many grams of Na_2HPO_4 are required to produce 1.5 L of 50 mM phosphate buffer at pH 7.0? [Show your work]