**Question 2 (25 points)**

The serotonin receptor (SR) is a ligand-gated channel involved in rapid signal transduction in the peripheral nervous system. Although this receptor is a member of the acetylcholine receptor family, the reason why agonists (i.e., substrates) induce the channel to open, while antagonists (inhibitors) do not, is unknown. To begin to answer these questions a group of investigators synthesize a fluorescent derivative of an antagonist, granisetron (G-F), and studied the kinetics of binding of this molecule to a fluorescent antagonist to the SR. The data for these studies is shown below.

The reaction scheme is:

$$SR + G\text{-}F \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} SR\bullet G\text{-}F$$

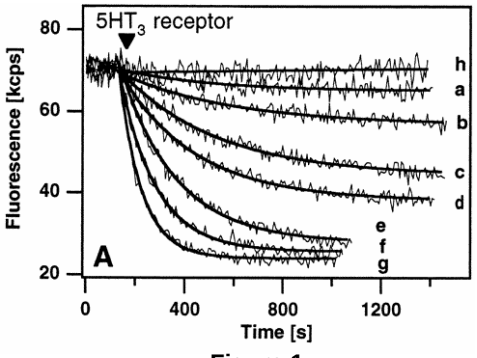


Figure 1

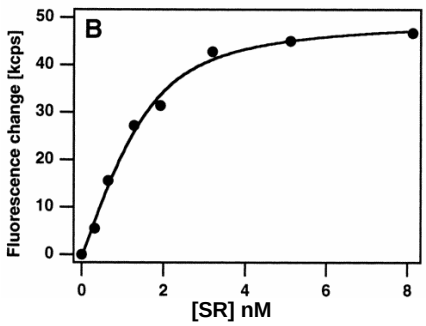


Figure 2

**Table 1**

| [SR] nM | v (M-1s-1) |
|---|---|
| 0.1 | 1.17X10-4 |
| 0.2 | 2.34X10-4 |
| 0.4 | 4.68X10-4 |
| 0.8 | 9.36X10-4 |
| 2 | 2.34X10-3 |
| 4 | 4.68X10-3 |
| 8 | 9.36X10-3 |

**Table 2**

| Temp (°C) | k ($M^{-1}s^{-1}$) |
|---|---|
| 8 | 4.85 X 10+5 |
| 12 | 6.18 X 10+5 |
| 16 | 8.23 X10+5 |
| 20 | 1.05X 10+6 |
| 25 | 1.4 X 10+6 |
| 30 | 1.95X10+6 |

**Table 3**

| Temp (°C) | $K_A$ ($M^{-1}$) |
|---|---|
| 12 | 4.596X10+9 |
| 16 | 4.104X10+9 |
| 20 | 3.69 X 10+9 |
| 25 | 3.23 X 10+9 |
| 30 | 2.85 X 10+9 |

**a.** Figure 1 depicts the effect of adding increasing concentrations (traces a-g) of SR to G-F. What type of data can be extracted from these plots?

**b.** Give the rate law for the binding of G-F to SR. (Hint: in deciding the form of the rate law, be sure you account for the observation that the total fluorescence change increases with increasing [SR] (see Figure 2) and for these expts [SR]>>>[G-F]). Justify your answer.

**c.** From the data in Tables 1, 2 and 3 calculate (Note, for kinetic expt in Table 1 [G-F]=constant=0.1 nM) $k_{on}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the forward reaction (binding of SR to G-F).

**d.** Calculate $k_{off}$ at 20°C and the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the reverse reaction.

**e.** The binding of an agonist (substrate) locks the SR into the open position. Considering the thermodynamic parameters for the forward and reverse reactions, speculate as to why the binding of antagonist (inhibitor) prevent channel opening.