Insulin Project Proposal

Langmuir Monolayer Studies

- Pure insulin
    - Applied to subphases consisting of $ZnCl_2$, VCl_2, and $CuCl_2$
        - This would allow us to see if there are any differences in the compression isotherm for these three divalent cations and see if they affect the fluidity of the insulin monolayer
    - Applied to subphases consisting of the aforementioned divalent cations in addition to coordinating anions (Cl^- and Cl^-+ phenol)
        - This would allow us to study how changes in insulin's conformation affect the overall fluidity of the insulin hexamer monolayer
- Modified insulin
    - Additional studies could be done where the cations/anions mentioned previously would be directly added into the insulin solution instead of the subphase to see if similar results are found
- Hysteresis
    - All of the pure insulin trials previously mentioned could be ran using a hysteresis method where the available area of the insulin is reduced and increased until specific surface pressures are attained
        - This would allow us to see how each of the insulin conformations and different cations affect the stability of the insulin monolayer
        - This could also be used to gain an insight into the aggregation/dissociation of the insulin by adding a chelating agent into the subphase
        - We could take a thermodynamical approach with this and use the data to calculate the free energy of hysteresis, free energy of compression, free energy of expansion, configurational entropy of hysteresis, and the enthalpy of hysteresis

Isothermal Titration Calorimetry (If we can do this)

- Measures the heat transfer during binding of biomolecules (insulin and divalent cation)
- Able to determine from this:
    - Binding constants
    - Reaction stoichiometry
    - Enthalpy
    - Entropy
- The use of this set of experiments would be two fold:
    - First, we would perform titrations of EDTA into a solution of insulin and one of three cations: Zn, V, and Cu

        - This would allow us to study the binding affinities and differences of these cations for insulin
    - Second, we would repeat the aforementioned experiment but add additional coordinating anions (Cl^- and Cl^-+ phenol)
        - This would allow us to note any differences between the T_6, T_3R_3, and R_3 conformations on insulin

Brewster Angle Microscopy

- Brewster angle microscopy will be used to visualize the films of the previously mentioned insulin trials to try to discern any differences in aggregation patterns

Molecular Dynamics or DFT Calculations (Possibly)

- Use one or both of these methods to calculate interactions and thermodynamics for our systems

Potential Additional Studies (Future Plans)

- pH dependence of insulin aggregation
    - Perform trials on subphases at (5.4), below (3.4) and above (7.4) insulin's isoelectric point to see how the protonated or deprotonated forms of insulin affect the aggregation and gain an insight into aggregation at physiological pH.
- Temperature dependence of insulin aggregation
    - Trials would be performed at room temperature (25 C) and at body temperature (37 C) to see how thermal changes affect insulin aggregation.
- Insulin-lipid interactions
    - One study could be performed with insulin and various constituents of adipose tissue to gain insight into how insulin may react during injection in diabetic patients
    - Another study could be done by changing lipid's head and tail groups to see how specific groups affect insulin's aggregation. Possible lipids could be:
        - DOPE
        - DOPC
        - DMPC
- Kyle's idea about lipid rafts