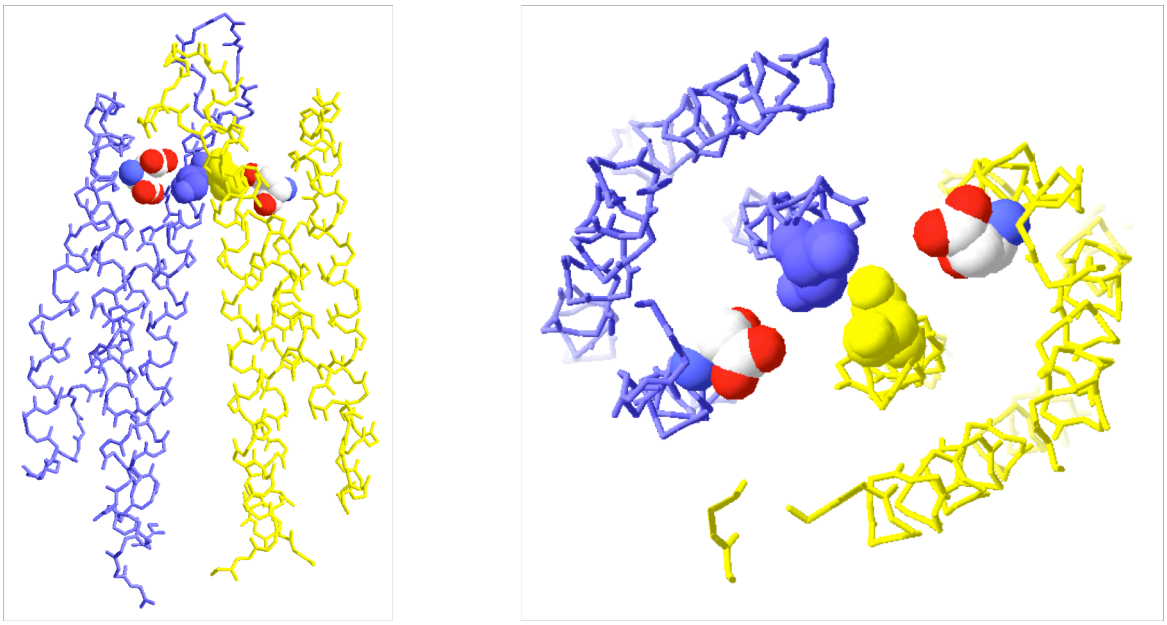
Cooperative Ligand Binding in a Dimeric Protein

Origin_Assign_6_Binding_Data.xlsx contains the results of ligand binding experiments conducted with the *Salmonella typhimurium* aspartate receptor, a transmembrane receptor protein, which is organized as a homodimer. The dimer of the ligand-binding domain has two nonoverlapping aspartate binding sites. This problem set explores the properties of cooperative ligand binding – whether the binding of ligand at site one influences the binding at site two.

In addition to the wildtype (*wt*) protein, three mutants were studied in which a residue in the ligand-binding domain at the domain interface, serine-68, was substituted with alanine (S68A), cysteine (S68C), or isoleucine (S68I).



The aspartate receptor ligand binding domain crystal structure (1*vat*). The two views of the domain dimer show one aspartate molecule bound per subunit, rendered in a space-filling representation (red, white & blue). The helical backbones of each subunit are rendered in blue and yellow. Serine-68 is rendered in space-filling representation with the side chain displayed. *Left*: side view emphasizes the four-helix bundle organization of the domain. *Right*: a slab view from the top shows the relative positions of the bound aspartates and serine 68 with respect to the domain interface. Structure reference: Yeh, JI, HP Biemann, GG Prive, J Pandit, DE Koshland, Jr, & S-H Kim. 1996. High-resolution structures of the ligand-binding domain of the wild-type bacterial aspartate receptor. *J. Mol. Biol.* **262:**186-201.

Procedure

1. Generate Scatchard plots of the binding data for the *wt* dimer and the three mutants. What do you conclude about the cooperativity of aspartate binding through visual inspection of the plots, and from what you know of the structural organization? (Qualitative analysis.)

2. Plot the binding isotherms ($\bar{v}$ as a function of $[asp]_{free}$), and fit the data to the single-set-of-sites model, the Hill equation and the Sequential model to generate estimates of the following variables. Include representative examples of the plots and tabulate all the data including estimates and their uncertainties.

a. Single set of sites model
   i. total number of sites (N_{tot})
   ii. intrinsic association constant (K_A)
b. Hill equation
   i. total number of sites (N_{tot})
   ii. intrinsic association constant (K_A)
   iii. the Hill coefficient (n_H)
c. Sequential model for two binding sites on a protein dimer
   i. intrinsic association constants (K_1, K_2)
   ii. adjustment factor (α), to allow for uncertainty in the total protein concentration (and/or aspartate concentration).

3. Discuss these data critically, and address how the fits by the different models provide support for, or are consistent with, the presence and nature of the cooperative interactions.

4. What provides more compelling evidence of cooperative interactions: qualitative trends in the Scatchard plots, or goodness of fit statistics (residuals, reduced Chi-Sqr, Adj. R-Square)?

5. Speculate on the structural basis for the changes in cooperative interactions produced by the mutations.