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).

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 ($\bar{\upsilon}$ 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.

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.)*

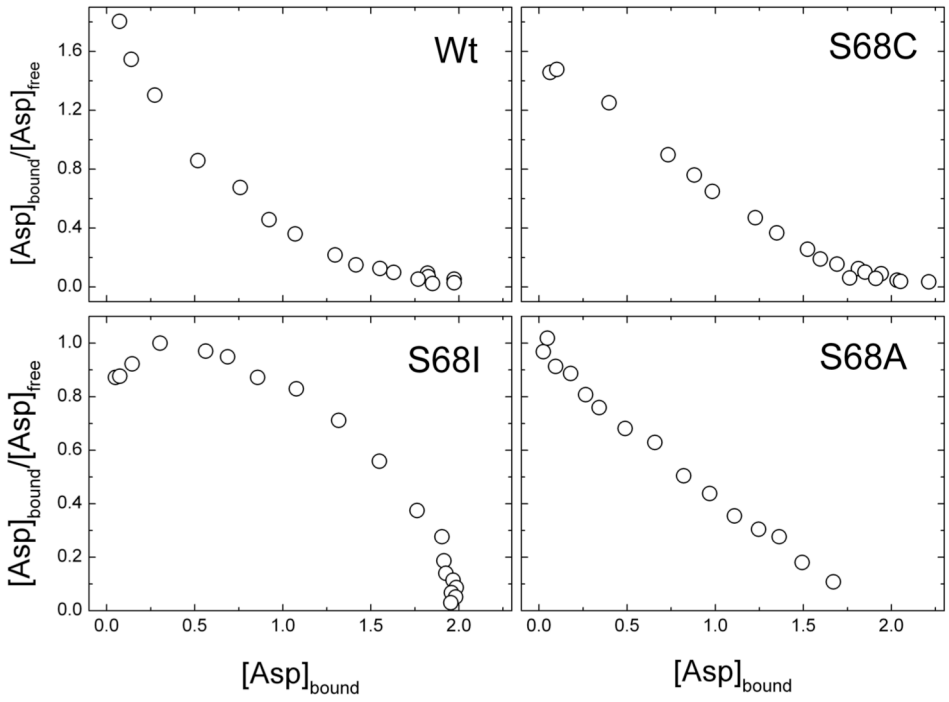

Figure 1. Scatchard plots of the Wt and the three S68 ligand binding domain mutants.

The Scatchard plots of Figure 1 provide good evidence that the side chain of residue 68 can affect the aspartate binding significantly. The protein is organized as a homodimer with two non-overlapping sites of aspartate binding. Further, if it is assumed that the dimers are symmetric, then the two binding sites are probably equivalent in the absence of ligand, we can say that the Wt protein, and to a lesser degree the S68C mutant, both display negative cooperativity. (If the sites were not equivalent, which might be the case in an asymmetric homodimer, the concave upward plot could reflect binding to two independent and nonequivalent sites or, conceivably, positive cooperativity between two sites with different intrinsic affinities.

The Scatchard plot of the S68I mutant exhibits aspartate binding that is positively cooperative. The S68A mutation results in a protein that is neither strongly positive nor negative cooperative in aspartate binding.

2. *Plot the binding isotherms ($\bar{\upsilon}$ as a function of $[asp]_{free}$), and fit the data to the Single-Set-of-Sites Model, the Hill Equation and the Sequential Model. Include representative examples of the plots and tabulate all the data including estimates and their uncertainties.*

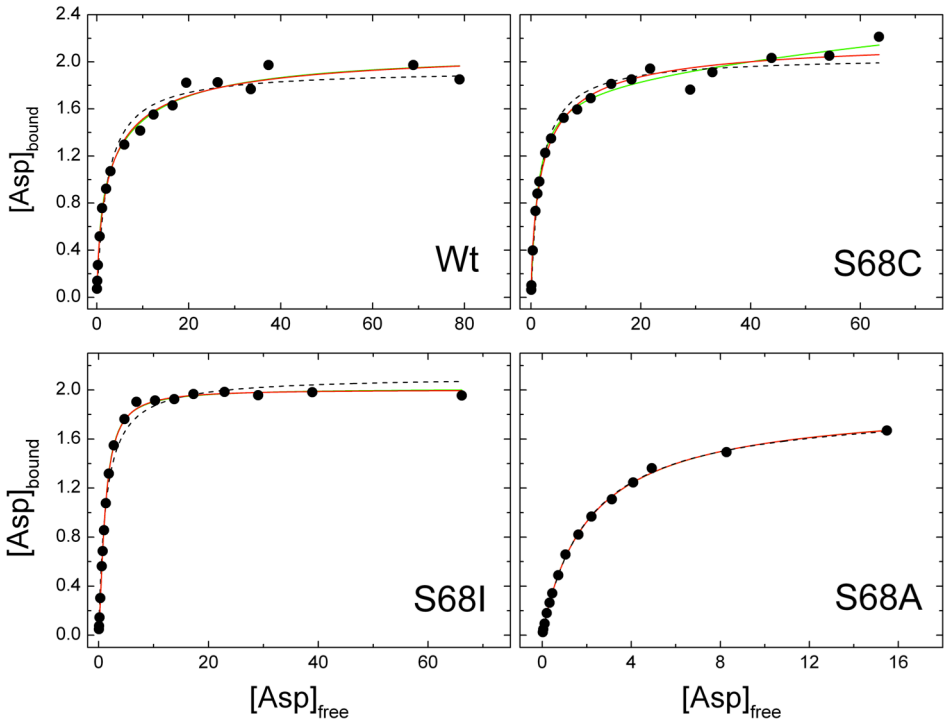

Figure 2. The binding isotherms of the Wt aspartate receptor, and the three mutants receptor proteins with substitutions at position 68. The differences in binding of ligand are subtle in this plot format, which is partly due to the fact that the x-and y-axis scales are different. Each set of binding data were fit to a single-set-of-sites model (black dashed lines), the Hill equation (red line), or the KNF sequential model for a dimer (green line). Note that the three fit lines are nearly superimposable in the plot of the S68A data.

The fit parameters, generated in Origin, are tabulated below. The fits provide additional evidence of cooperativity, whether it's from the Hill equation (n_H) or from the relative magnitudes of the intrinsic binding constants in the sequential model (K_1, K_2).

Protein	**Cooperativity**	n_H	K_1, K_2
Wt	Most Negative	< 1	$K_1 > K_2$
S68C	Negative	< 1	$K_1 > K_2$
S68A	Independent	~ 1	$K_1 \approx K_2$
S68I	Positive	> 1	$K_1 < K_2$

Table 1. Single-Set-of-Sites Model, $(N_{tot}K_A[L])/(1 + N_{tot}K_A[L])$

Protein	N_{tot}	K_A (uM^{-1})	Red. χ^2	Adj. R^2
Wt	1.93 ± 0.04	0.451 ± 0.054	0.00888	0.97993
S68C	2.04 ± 0.04	0.584 ± 0.055	0.00755	0.98316
S68I	2.11 ± 0.04	0.775 ± 0.059	0.00623	0.98899
S68A	1.88 ± 0.02	0.488 ± 0.012	0.000245	0.99923

Table 2. Hill Equation, $N_{tot}K_A{}^{nH}[L]^{nH}/(1 + K_A{}^{nH}[L]^{nH})$

Protein	N_{tot}	K_A (uM^{-1})	n_H	Red. χ^2	Adj. R^2
Wt	2.15 ± 0.09	0.333 ± 0.054	0.722 ± 0.061	0.00436	0.9901
S68C	2.22 ± 0.08	0.466 ± 0.060	0.755 ± 0.063	0.00466	0.9896
S68I	2.00 ± 0.01	0.870 ± 0.017	1.384 ± 0.037	0.000609	0.9989
S68A	1.91 ± 0.03	0.467 ± 0.021	0.970 ± 0.023	0.000234	0.9993

Table 3. Two-Site Sequential Model, $\alpha(2K_1[L] + 2K_1K_2[L]^2)/(1 + 2K_1[L] + K_1K_2[L]^2)$

Protein	α	K_1 (uM^{-1})	K_2 (uM^{-1})	Red. χ^2	Adj. R^2
Wt	1.045 ± 0.033	0.721 ± 0.095	0.191 ± 0.047	0.00466	0.9896
S68C	1.783 ± 0.058	0.413 ± 0.041	0.009 ± 0.002	0.00299	0.9933
S68I	1.007 ± 0.005	0.356 ± 0.030	2.071 ± 0.050	0.000560	0.9990
S68A	0.956 ± 0.016	0.501 ± 0.016	0.438 ± 0.042	0.000236	0.9993

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.*

The single set of sites model describes the properties of the S68A ligand-binding domain, since no cooperative interactions are present (in agreement with Scatchard plots) at display cooperative interactions (Wt, S68C and S68I).

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)?*

A combination of approaches provides the best evaluation. The Scatchard plot shows the qualitative distinctions very well. The fit statistics provide factor that show how the fit improves as the best fit is approached, Chi-Sqr, but some caution must be exercised in drawing comparisons between different data sets and to a degree different models. The Hill equation and the sequential model are in agreement with each other and the expected relationships amongst variables that indicate negative, positive or no cooperative interactions. The structure and organization of the protein subunits are known, this information too is used to rule out some binding scenarios: multiple sites without cooperative interactions and positive cooperative interactions among intrinsically different sites.

In addition, plots of the residuals allow one to see whether the Hill Equation and Sequential Model explain that data better.

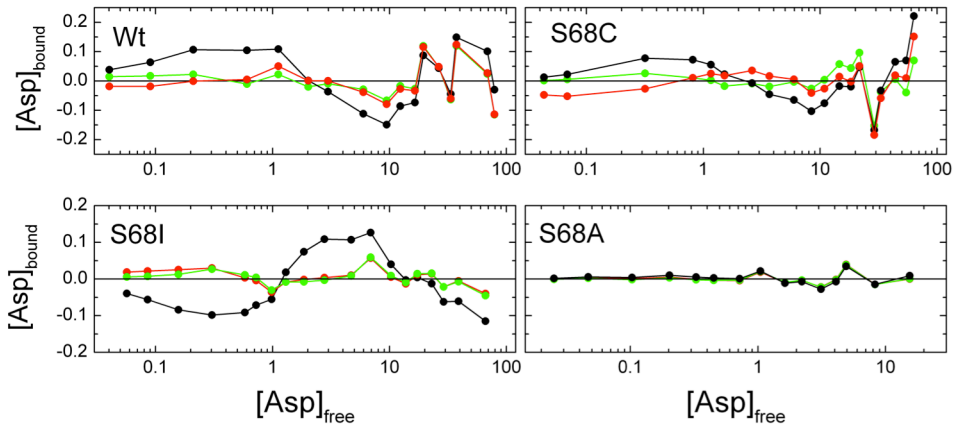

Figure 3. Residual plots of the four ligand binding domains fit to the Single-Set-of-Sites Model (black), the Hill Equation (red) and the Sequential Model (green).

From these plots it can be seen that the single-set-of-sites model does a generally poorer job at explaining the data of the proteins that are cooperative (Wt, S68C, S68I). The residuals lessen significantly for these when the data were fit to either the Hill equation or the two-site sequential model. Structural data, as much as anything else, ruled out a model based on two sets of independent sites. The fit of the data to a two sets model, would also likely lead to a reduction in the residuals. Notably for S68A, neither the Hill equation nor the two-site sequential model improved the residuals, implying that the simplest model (single set of sites) is adequate.

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

Structural analysis of the ligand-binding domain places the S68 residue at the domain interface, and the data demonstrate that S68 has a major role in these cooperative interactions. It is tempting to say that the side chain volume is relevant. The S68C mutation replaces the serine side chain with that of cysteine, which is most similar in volume and chemistry of the three, and both residues produce domains with negative cooperativity. The larger volume sidechain (isoleucine) is characterized by positive cooperativity, and the small volume sidechain of alanine results in no cooperative interactions. More information is warranted to know whether the correlation holds up; this is a subset of all the data and the structural correlation.

See the paper at the course website: Andrew F. Kolodziej, Thomas Tan, and Daniel E. Koshland, Jr. 1996. Producing positive, negative, and no cooperativity by mutations at a single residue located at the subunit interface in the aspartate receptor of *Salmonella typhimurium*. *Biochemistry* **35,** 14782-14792.