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Comments on 'Analysis and parameter resolution in highly cooperative systems' by S.J. Gill et al. (Biophys. Chem. 30 (1988) 133)

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In a recent paper S.J. Gill et al. [1] analyzed binding data for a system in which the binding unit had four sites for binding a ligand. They considered three cases: noncooperative binding (case A), cooperative (case B) and highly cooperative binding (case C). It is with the latter that I shall be concerned here.

For all cases they compared the parameters obtained by fitting with 'overall' binding constants, β_i, according to the binding polynomial (grand partition function)

$$\Xi(x) = 1 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \beta_4 x^4 \qquad (1)$$

and the corresponding equation using stepwise constants K_i:

$$\Xi(x) = 1 + K_1 x + K_1 K_2 x^2 + K_1 K_2 K_3 x^3 + K_1 K_2 K_3 K_4 x^4 \qquad (2)$$

Their 'data' $A(x)$ were produced using the equation

$$A(x) = A(0) + [A(\infty) - A(0)]\,\theta(x) \qquad (3)$$

with

$$\theta(x) = \tfrac{1}{4}\frac{\mathrm{d}\ln \Xi(x)}{\mathrm{d}\ln x} \qquad (4)$$

For each case they generated 100 data sets by adding a random normal error to the exact values of $\theta(x)$. For case C the reported (mean) values of the binding constants obtained were grossly in error, the latter sometimes amounting to 2000%. The authors conclude that for such a case "the bias in the binding parameters... invalidates even qualitative mechanistic interpretation of the underlying process" (ref. 1, p. 140). This conclusion, if true, is certainly very discouraging for anyone concerned with binding problems. For this reason I have attempted to reproduce their results and – luckily! – failed.

The problem facing the experimenter is the following: given a set of binding curves, with a maximum binding capacity of four sites per binding unit, what can be inferred about the binding constants and the underlying processes? An answer to these questions can be obtained if a model, with the appropriate (minimum) number of parameters, can be successfully fitted to the data. This is the point of view adopted in the present note. The position of Gill et al. [1] is that in highly cooperative systems, this may not always lead to meaningful answers when using conventional fitting procedures.

First of all I fully concur with Gill et al. that the use of eq. 2 with stepwise binding constants is inferior to using eq. 1, since in the former equation considerable statistical interdependence of the constants is introduced by having the parameters appear as products. However, quite reasonable results were obtained. I generated the data (with

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Table 1

Exact and fitted binding parameters

The experimental parameters $A(0)$ and $A(\infty)$ were fixed at 0.1 and 1.1, respectively. The values given in parentheses were obtained using data with tighter spacing on the steep part of the isotherm (see text).

	True	Fitted			
		Mean	σ	% bias	% error
Case C, ref. 1					
K_1	0.535	0.534	0.0291	0.2	5.4
K_2	0.0660	0.0737	0.0761	−11.7	103.2
K_3	0.476	2.22	48.7	−361.4	2193.7
K_4	59.5	41.0	235.0	31.1	573.2
Case C, this work (weighted)					
K_1	0.535	0.508	0.0078	5.0	1.5
		(0.522)	(0.0073)	(2.4)	(1.4)
K_2	0.0660	0.098	0.023	−48.5	23.5
		(0.079)	(0.017)	(−19.7)	(21.5)
K_3	0.476	0.485	0.241	−1.9	49.5
		(0.456)	(0.208)	(4.2)	(45.6)
K_4	59.5	38.3	11.0	35.6	28.7
		(49.7)	(12.9)	(16.5)	(26.0)
Case C, this work (unweighted)					
K_1	0.535	0.509	0.0039	4.86	0.8
K_2	0.0660	0.098	0.0035	−48.4	5.1
K_3	0.476	0.491	0.206	−3.2	4.2
K_4	59.5	41.3	2.27	30.6	5.5
Positive cooperativity, this work					
K_1	0.535	0.481	0.0129	10.1	2.7
K_2	2.006	2.293	0.186	−14.3	8.1
K_3	8.961	9.955	0.685	−11.1	6.9
K_4	33.438	30.544	0.478	8.7	1.6

fixed values of $A(0)$ ($=0.1$) and $A(\infty)$ ($=1.1$)) for 10 runs by the same procedure as in ref. 1. The parameters obtained from each run were weighted by their reciprocal variances, and the weighted means and the associated accumulated S.E. are displayed in table 1. For comparison, the results from ref. 1 are also shown. It is noted that the largest % error and % bias obtained here were 49.5 and 48.5, respectively, as compared to 2194 and 361 obtained by Gill et al. Also shown are the unweighted means and S.E. calculated as if the individual determinations are data, showing that the individual determinations of binding constants do not differ much between runs. The exact distribution of species with different degrees of ligation

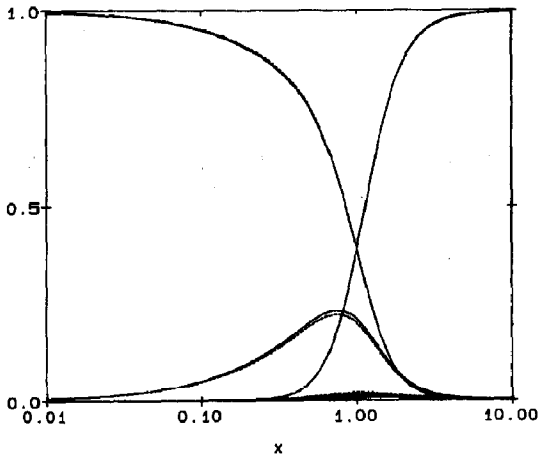

Fig. 1. A comparison of the exact (full lines) and fitted (broken lines) distribution of species, using the values from table 1 (this work).

is compared with that obtained from the fitted parameters in fig. 1. It appears that the fitting procedure yields quite acceptable results. In addition, a Wilk-Shapiro test for each parameter distribution indicates that there are no grounds for rejecting the hypothesis that the distributions are normal (at a significance level of 0.05). But of course here it should be stressed that only 10 sets of constants are considered.

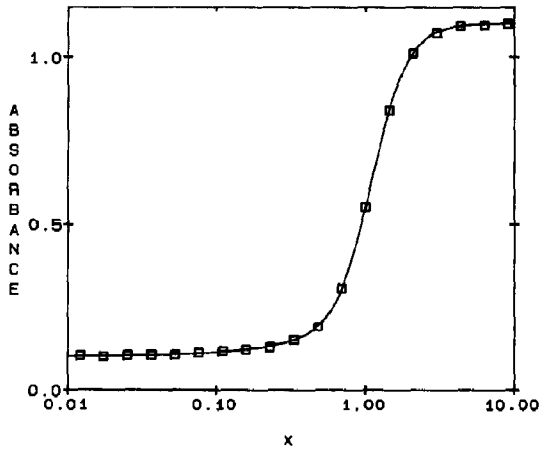

Fig. 2. A fit to a set of simulated experimental points using the two-parameter binding polynomial: $\Xi(x) = 1 + C_1 x + C_1 C_4 x^4$. The mean values ± S.E. obtained for C_1 and C_4 in this case were $C_1 = 0.583 \pm 0.0078$ and $C_4 = 1.748 \pm 0.020$.

Nevertheless, the values of the bias are quite large. This situation may be somewhat improved if the spacing of the experimental points is such that about 2/3 of the points are on the steeply rising part of the isotherm, as they are in the noncooperative case (see fig. 1 of ref. 1). The parameters found for these cases are shown in parenthesis in table 1.

However, from the statistical indicators calculated during the fitting procedure, it is clear that two of the constants, K_2 and K_3 in this case, are redundant and should be left out of the model. In other words, the doubly and triply ligated species do not contribute to the experimental data. A fit to the data, using only two parameters, i.e., omitting the squared and the cubed terms in eq. 2, is shown in fig. 2. The fit seems excellent.

But this is valuable mechanistic information: The model, having four binding sites in all, is such that doubly and triply ligated species are (almost) not observed, as seen from the exact curves in fig. 1. If the binding data are practically devoid of information regarding these species, one should not expect to obtain reliable estimates of their binding constants. The mechanism is hence best described by the two stepwise equilibria:

$$P + X = PX$$
$$PX + 3X = PX_4$$

It is, probably, a rather unusual case: the second and third ligand experiences strong negative cooperativity, while the fourth binds in a strongly positive cooperative manner. On general grounds [2] it is doubtful whether such a case would be met in practice.

Gill et al. found that with the values obtained for the binding constants K_i, the resulting binding polynomial greatly overestimated the doubly and triply ligated species. This, together with the unreasonably high bias in the individual binding constants, led to their conclusion, cited above, that for highly cooperative cases the results of the fitting were useless for mechanistic interpretations. This seems rather overstated in view of the results presented here.

To provide further support for the notion that cooperativity per se does not create difficulties in the fitting procedures, I present results from a calculation on a model showing strong positive cooperativity in binding. The binding sites are presumed to be arranged with a tetragonal geometry, since this case ensures the most 'dense' degree of interaction [2], in the sense that any bound ligand molecule interacts with all the others. The values assumed in the model correspond to a nearest-neighbor interaction energy of -5.7 kJ/mol at 25°C. The data were produced in the same manner as described above. Ten runs were calculated. The parameters obtained are shown in table 1, and the comparison between the exact and the fitted distribution of species is displayed in fig. 3. It is seen that the fit is quite good.

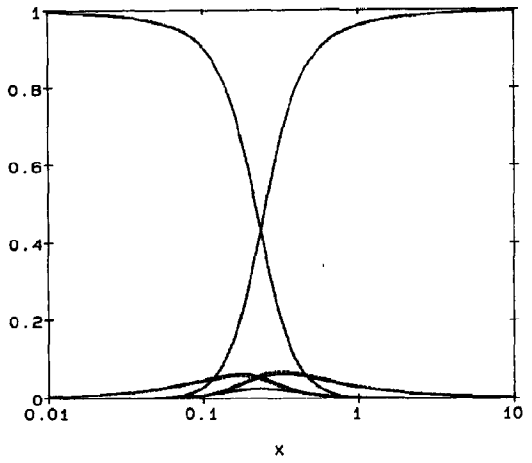

Fig. 3. Distribution of species for the positive cooperativity case discussed in the text. Full lines are the exact values, while the broken lines are those calculated using the fitted binding constants from table 1 (final entries).

The calculations described in this note were performed using the software package RS/1 from BBN Software Products Corp., Cambridge, MA, installed on a PC-AT.

References

1 S.J. Gill, P.R. Connelly, E. Di Cera and C.H. Robert, Biophys. Chem. 30 (1988) 133.
2 T.L. Hill, Cooperativity theory in biochemistry (Springer, Berlin, 1985).