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Reply to the comment by Plesner

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In his comment on our paper 'Analysis and parameter resolution in highly cooperative systems' [1], Plesner claims that "cooperativity per se does not create difficulties in the fitting procedures" and suggests there is no inherent difficulty in parameter resolvability for such systems [2]. His claim is based on the results of 10 simulated runs using stepwise equilibrium constants Ks. In our paper [1] we have shown that when the cooperativity increases, the partition function, and hence the fitting equation, should be cast in terms of overall equilibrium constants βs and not in terms of the Ks, since the resolvability of the Ks becomes severely ill-conditioned at high cooperativity. Our conclusion was drawn on the basis of a Monte Carlo simulation of 100 runs [1]. We have now run a Monte Carlo simulation of 10 000 binding curves for case C of ref. 1 and the results are shown in the accompanying table. Two sets of 10 000 runs each were generated using different seeds for the number generator. The bias on the overall constants βs is found to be negligible and the parameter errors are within acceptable limits. In the case of the stepwise constants Ks the bias is significant but, most importantly, parameter errors are such that K_3 and K_4 are poorly resolved. These results are in complete agreement with those obtained in the case of 100 runs [1]. Although the values of the bias on all parameters are reduced by increasing the number of runs, nevertheless parameter errors consistently indicate that the analysis cast in terms of overall constants should be preferred to the one based on the stepwise constant, which may yield meaningless results. The robustness of our Monte Carlo study can be appreciated by the similarity of the values obtained in the two cases simulated with different seeds. Meaningful Monte Carlo simulations must rely on such a robustness and demand a proper choice of the population size [3–5]. In order to show how critical is this choice, we have also simulated two cases of 10 runs each and the results are given in table 1. In the first case, we have found %bias and %error values of the Ks similar to those reported by Plesner, but in the second case meaningless values of %bias were obtained for both K_3 and K_4.

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References

1 S.J. Gill, P.R. Connelly, E. Di Cera and C.H. Robert, Biophys. Chem. 30 (1988) 133.
2 I. Plesner, Biophys. Chem. 36 (1990) 91.
3 D.A. Ratkowski, Non-linear regression modeling (Dekker, New York, 1983).
4 Y. Bard, Nonlinear parameter estimation (Academic Press, New York, 1974).
5 W.H. Press, B.P. Flannery, S.A. Teukolsky and W.T. Vetterling, Numerical recipes (Cambridge University Press, New York, 1986).

Table 1

Monte Carlo simulation of parameter resolvability in highly cooperative systems

m and σ are the fitted mean and standard deviation of the distributions obtained. %bias and %error were calculated as (100(true$-m$)/true and $100\sigma/m$, respectively. Cases 1 and 2 were obtained by starting the Monte carlo simulations with two different seeds of the random number generator. Optical parameters $A(0)$ and $A(\infty)$ were included in the data fitting procedure but for brevity the results are not given.

	True	m	σ	%bias	%error
10 000 runs: case 1					
β_1	0.5350	0.5350	0.0097	0.0	1.8
β_2	0.0353	0.0353	0.0125	0.0	35.4
β_3	0.0168	0.0168	0.0084	0.0	50.0
β_4	1.0000	1.0000	0.0030	0.0	0.3
K_1	0.5350	0.5350	0.0097	0.0	1.8
K_2	0.0660	0.0664	0.0244	−0.6	36.7
K_3	0.4760	0.5596	16.307	−17.6	2 914.0
K_4	59.500	57.220	196.80	3.8	343.9
10 000 runs: case 2					
β_1	0.5350	0.5353	0.0097	−0.1	1.8
β_2	0.0353	0.0353	0.0124	0.0	35.1
β_3	0.0168	0.0167	0.0083	0.1	49.7
β_4	1.0000	1.0001	0.0031	0.0	0.3
K_1	0.5350	0.5353	0.0097	−0.1	1.8
K_2	0.0660	0.0663	0.0243	−0.4	36.6
K_3	0.4760	0.3676	9.6940	22.3	2 637.1
K_4	59.500	61.863	186.85	−4.0	302.0
10 runs: case 1					
β_1	0.5350	0.5349	0.0073	0.0	1.4
β_2	0.0353	0.0363	0.0091	−2.8	25.1
β_3	0.0168	0.0159	0.0054	5.7	34.0
β_4	1.0000	1.0011	0.0021	0.1	0.2
K_1	0.5350	0.5349	0.0073	0.0	1.4
K_2	0.0660	0.0681	0.0179	−3.2	26.3
K_3	0.4760	0.5059	0.2755	−6.3	54.4
K_4	59.500	73.690	33.190	−23.8	45.0
10 runs: case 2					
β_1	0.5350	0.5377	0.0102	−0.5	1.9
β_2	0.0353	0.0320	0.0167	9.3	52.2
β_3	0.0168	0.0196	0.0125	−16.7	63.8
β_4	1.0000	0.9994	0.0038	0.1	0.4
K_1	0.5350	0.5377	0.0102	−0.5	1.9
K_2	0.0660	0.0601	0.0325	8.9	54.1
K_3	0.4760	6.6860	18.192	−1304.6	272.1
K_4	59.500	569.58	1066.6	−857.3	187.3