Electronic Supplementary Information

for

Competition Between Two Cysteines in Covalent Binding of Biliverdin

to Phytochrome Domains

Maria G. Khrenova,[a,b] Anna M. Kulakova,[a] Alexander V. Nemukhin [a,c]

[a] Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow, 119991, Russian Federation

[b] Federal Research Center of Biotechnology, Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskiy Prospect 33, 119071 Moscow, Russian Federation

[c] Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Kosygina 4, Moscow, 119334, Russian Federation
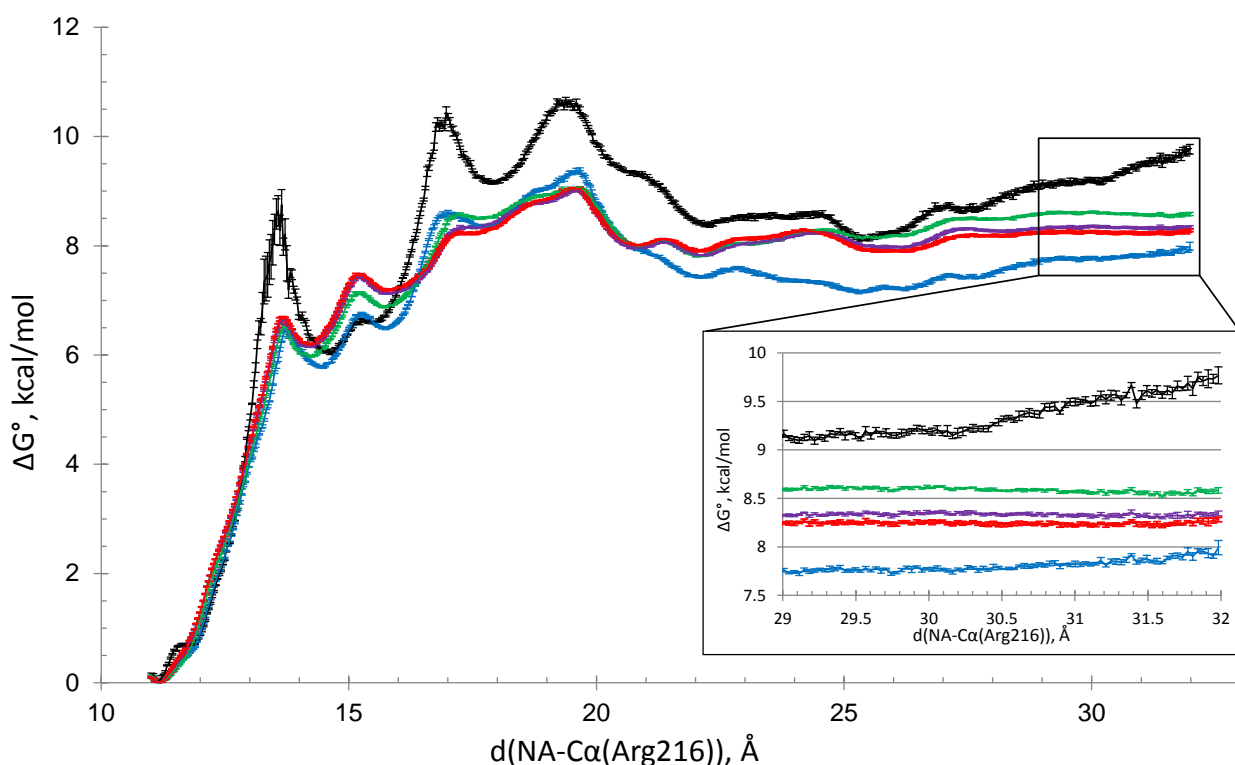

* Corresponding author:

Prof. Alexander Nemukhin, Chemistry Department, M.V. Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow, 119991, Russian Federation

Phone: +7-495-939-10-96

E-mail: anemukhin@yahoo.com or anem@lcc.chem.msu.ru

Section S1. Convergence of the replica exchange umbrella sampling molecular dynamics calculations of the Gibbs free energy profile (see subsection "Classical molecular dynamics simulations of noncovalent BV binding to PAS-GAF" of Methods).

Convergence of the procedure was checked by comparing the graphs computed by a gradual increase of the simulation time from 4 ns to 15 ns. When analyzing the data we exclude the values obtained during the first 2 ns in every run. The data shown in Fig. S1 demonstrate that the convergence is reached for trajectories of the 15 ns length in total used in estimates of the dissociation constant.

Figure S1. Computed free energy profiles corresponding to the following simulation times: 2.0 ns (black), 5.0 ns (blue), 10.0 ns (green), 11.5 ns (violet), and 13.0 ns (red) per replica (the data from the first 2 ns in every run are not shown). Statistical errors are estimated by the Monte Carlo Bootstrap Error Analysis.

Section S2. Resonance forms of BV in the computed model systems.

Numerous resonance forms can be considered for the BV molecule as it is a large conjugated π-system with the delocalized positive charge. The single-determinant DFT wave function describes the average system being a superposition of different resonance structures. If one of the resonance structures dominates, it should be well recognized in the electron density. Therefore we calculated the Mulliken charges of the NH fragments of all four rings in all minima on the potential energy surfaces shown in Fig. 3 of the main text. In Table S1 we present the computed charges. We found that variations in these charges differ do not exceed 0.11 e. Even if these minor differences are considered to be significant, we conclude that the positive charge is more likely localized on the NH fragment of D ring in the reagents and A or D rings in the products of both A and B pathways.

Table S1. Mulliken charges of the NH fragments of the A, B, C and D rings of BV.

BV ring	Reag	I1-A	I2-A	I3-A	I4-A	I1-B
A	-0.11	-0.09	-0.08	-0.07	-0.04	-0.03
B	-0.10	-0.12	-0.17	-0.17	-0.11	-0.14
C	-0.10	-0.11	-0.11	-0.10	-0.08	-0.06
D	-0.05	-0.04	-0.06	-0.06	-0.05	-0.05