Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting information for

Spectral deciphering of the interaction between an intramolecular hydrogen bonded ESIPT drug, 3,5-dichlorosalicylic acid and a model transport protein

Bijan Kumar Paul*, Debarati Ray, Nikhil Guchhait*

Department of Chemistry, University of Calcutta, 92 A. P. C. Road, Calcutta-700009, India.

*Author for correspondence. Tel.: 91-33-2350-8386. Fax: +91-33-2351-9755. E-mail: nguchhait@yahoo.com (N. Guchhait).

Contents **Page Nos.**

S1. Blind docking simulation protocol

The molecular geometry of 3,5-dichlorosalicylic acid (3,5DCSA) was first optimized on Gaussian 03W suite of programs[1,2] using Density Functional Theoretical (DFT) method with the B3LYP functional, i.e., a combination of the Becke's three-parameter (B3) hybrid exchange functional[3] and Lee-Yang-Parr (LYP) nonlocal correlation functional.[4] In our calculation particular emphasis is delivered on the 6-311++G(d,p) basis set because this basis set is of triple -ζ quality[1,2,5] for valence electrons with diffuse functions which are useful in calculations for anions and structures with lone pair electrons.[1,2,5-8] The geometrical constraints were not imposed in equilibrium geometry optimizations. Vibrational frequency calculations were carried out for the optimized structures in order to assess the nature of stationary points. The characteristic of local minimum was verified by establishing that matrices of energy second derivatives (Hessian) have no imaginary frequency.[1-8]

The native structure of HSA was taken from the Protein Data Bank having PDB ID:1AO6[9] (http://www.pdb.org/pdb/explore.do?structureId=1ao6). BSA was generated from it by performing necessary additions at the N-terminal as well as some mutations in the required regions as no PDB is available for BSA.[10] Docking studies were performed with AutoDock 4.2 suite of programs which utilizes the Lamarckian Genetic Algorithm (LGA) implemented therein (http://autodock.scripps.edu/). For docking of HM with BSA, the required file (corresponding to the three-dimensional structure of HM) for the ligand (HM) was created through combined use of Gaussian 03W[1,2] and AutoDock 4.2[17,18] software packages. The optimized geometry of 3,5DCSA was (DFT//B3LYP/6-311++G(d,p)) read in AutoDock 4.2 software in compatible file format, from which the

required file was generated in AutoDock 4.2. At the beginning of docking study all water molecules were removed and hydrogens were added followed by computing Gasteiger charges, as required in Lamarckian Genetic Algorithm.[11,12] The grid size was set to 126, 126, and 126 along *X*-, *Y*-, and *Z*-axis with 0.375 Å grid spacing, i.e. in order to recognize the binding site of 3,5DCSA in BSA blind docking was performed. The AutoDocking parameters used were as follows: GA population size = 150; maximum number of energy evaluations = 250000; GA crossover mode = two points. The lowest binding energy conformer was searched out of 30 different conformers for each docking simulation and the resultant one was used for further analysis. The PyMOL software package was used for visualization of the docked conformations.[13]

S2. Time-resolved fluorescence anisotropy decay: Two-Step and Wobbling-in-Cone Model

Under the framework of the *two-step and wobbling-in-cone* model the fluorescence depolarization can be the result of three independent motions: (a) wobbling of the probe ($r_W(t)$) with a time constant τ_W, (b) translational motion of the probe ($r_D(t)$) along the surface of the protein with time constant τ_D, and (c) overall rotation of the protein ($r_P(t)$) with a time constant τ_P. Thus r(t) can be decomposed into a product of three independent motions as follows[14-23]:

$$r(t) = r_W(t) r_D(t) r_P(t) \quad \text{(S1)}$$

Again, r(t) may be expressed in terms of the generalized order parameter S as[14-23]:

$$r(t) = r_0\left[S^2 + \left(1 - S^2\right)\exp\left(-t/\tau_W\right)\right]\exp\left[-t\left(1/\tau_D + 1/\tau_P\right)\right] \quad \text{(S2)}$$

In the wobbling-in-cone model, S is related to the semicone angle θ_w as follows[14-23]:

$$S = \frac{1}{2}\cos\theta_w\left(1 + \cos\theta_w\right) \quad \text{(S3)}$$

The order parameter S is a measure of the spatial restriction having values ranging from 0 (corresponding to unrestricted motion) to 1 (for complete restriction on the motion).

Again the functional form of the biexponential anisotropy decay, r(t), can be represented as[14-19]:

$$r(t) = r_0 \times \left[\alpha_{1r}\exp(-t/\tau_{1r}) + \alpha_{2r}\exp(-t/\tau_{2r})\right] \quad \text{(S4)}$$

in which r_0 is the limiting anisotropy that describes the inherent depolarization of the fluorophore and α_{ir} is the pre-exponent that provides the fraction of the i^{th} rotational relaxation time i.e. τ_{ir}.

Comparing equations S3 and S4 the following relations are obtained[14-23]:

$$S^2 = \alpha_{2r} \tag{S5}$$

$$\frac{1}{\tau_{2r}} = \frac{1}{\tau_D} + \frac{1}{\tau_P} \tag{S6}$$

$$\frac{1}{\tau_{1r}} = \frac{1}{\tau_W} + \frac{1}{\tau_{2r}} \tag{S7}$$

Here, τ_{1r} and τ_{2r} are, respectively, the observed fast and slow components of the anisotropy decay. The time constant for the overall rotation of the protein has been determined by applying the Stokes-Einstein-Debye equation[14-23]:

$$\tau_P = \frac{\eta V}{k_B T} \tag{S8}$$

in which η is the viscosity of the solution, V is the volume of BSA, k_B is the Boltzmann constant and T is the Kelvin temperature.

The volume of BSA has been calculated from one reported model – prolate ellipsoid of dimension 140 × 40 × 40 Å.[24] The calculated time constant for overall tumbling motion of BSA is found to be (τ_P = 54.58 ns) significantly larger compared to the slower component of the time constant (τ_{2r}) in the anisotropy decay. Hence the condition $\tau_P \rangle\rangle \tau_{2r}$, implies that the slower component τ_{2r} essentially represents τ_D, suggesting that lateral diffusion comprises an important component in the anisotropy decay of the probe. The data compiled in Table 3 and 4 of the text clearly reflects that τ_D and τ_{2r} values are reasonably close to each other. At the same time, an increasing magnitude of the order parameter from 0.44 in 40 × 10^{-5} M BSA to 0.47 in 60 × 10^{-5} M BSA is consistent with the idea of increasing degree of motional restriction imposed on the probe. This is further manifested in decreasing value of the semicone angle θ_w with increasing protein

concentration (i.e. the treatment of the so-obtained rotational dynamical parameters under the provision of two-step and wobbling-in-cone model is found to faithfully indicate an increasing degree of motional restriction imposed upon the probe molecule in the protein environment with respect to the bulk aqueous phase). The calculated dynamical parameters are tabulated in Table 4 of the text. The wobbling diffusion coefficient (D_W) for the probe in BSA is obtained from the following equations[14-23]:

$$D_W = \frac{7\theta^2}{24\tau_W} \quad \text{for } \theta_w \leq 30^o \tag{S9}$$

while for $\theta_w \geq 30^o$ D_W is given as:

$$D_W = \left\{\left(1-s^2\right)\tau_W\right\}^{-1}\left[\frac{x^2(1+x)^2}{2(x-1)}\left\{\ln\left(\frac{1+x}{2}\right)+\left(\frac{1-x}{2}\right)\right\}+\left(\frac{1-x}{24}\right)\left(6+8x-x^2-12x^3-7x^4\right)\right] \tag{S10}$$

where $x = \cos\theta_w$.

Since in the present case θ_w is found to be $\geq 30^o$ (Table 4 of the text), we have applied equation S10 to calculate the values of the wobbling diffusion coefficient (D_W) and the calculated values are summarized in Table 4 of the text.

S3. References:

1. Gaussian 03, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 2003.

2. J. B. Foresman, Æ. Frisch, *Exploring Chemistry with Electronic Structure Methods*, 2nd ed. (Gaussian, Inc., Pittsburgh, PA, 1996).

3. A. D. Becke, *J. Chem. Phys.* 1993, **98**, 5648–5652.

4. C. Lee, W. Yang, and R. G. Parr, *Phys. Rev. B* 1988, **37**, 785–789.

5. W. J. Hehre, L. Radom, P. v. Schleyer, and J. A. Pople, *Ab Initio Molecular Orbital Theory*; Wiley: New York, (1986).

6. B. K. Paul, and N. Guchhait, *J. Lumin.* 2011, **131**, 1918-1926.

7. B. K. Paul, and N. Guchhait, *Comput. Theor. Chem.* 2011, **966**, 250-258.

8. B. K. Paul, S. Mahanta, R. B. Singh, and N. Guchhait, *J. Phys. Chem. A*, 2010, **114**, 2618–2627.

9. S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, *Protein Eng.* 1999, **12**, 439-446.

10. B. K. Paul, A. Samanta, and N. Guchhait, *J. Phys. Chem. B*, 2010, **114**, 6183–6196.

11. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, and A. J. Olson, *J. Comput. Chem.* 1998, **19**, 1639-1662.

12. C. Hetenyi, and D. van der Spoel, *FEBS Letters* 2006, **580**, 1447-1450.

13. W. L. De Lano, The PyMoL Molecular Graphics System (2002), De Lano Scientific, San Carlos, CA, USA.

14. B. K. Paul, and N. Guchhait, *J. Phys. Chem. B* 2011, **115**, 10322-10334.

15. B. K. Paul, and N. Guchhait, *J. Phys. Chem. B* 2011, **115**, 11938-11949.

16. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, **1999**, Plenum, New York.

17. *Topics in Fluorescence Spectroscopy*; J. R. Lakowicz, Ed.; Plennum Press: New York, 1994; Vol 2.

18. A. Chakraborty, D. Seth, P. Setua, and N. Sarkar, *J. Chem. Phys.*, 2008, **128**, 204510-204519.

19. G. B. Dutt, *J. Chem. Phys.*, 2003, **119**, 11971-11976.

20. F. S. Ariola, D. J. Mudaliar, R. P. Walvick, and A. A. Heikal, *Phys. Chem. Chem. Phys.*, 2006, **8**, 4517–4529.

21. K. Kinosita, S. Kawato, and A. Ikegami, *Biophys. J.*, 1977, **20**, 289-305.

22. H. Pottel, B. W. van der Meer, and W. Herreman, *Biochim. Biophys. Acta*, 1983, **730**, 181-186.

23. E. L. Quitevis, A. H. Marcus, and M. D. Fayer, *J. Chem. Phys.*, 1993, **97**, 5792-5769.

24. F. N. Castellano, J. D. Dattelbaum, and J. R. Lakowicz, *Anal. Biochem.*, 1998, **255**, 165-170.