

Interaction of a new antiviral and antitumor photosensitizer hypericin with human serum albumin: molecular modeling study

Jozef Hritz,¹ Jozef Ulicny,¹ and Pavol Miskovsky^{†,1,2}

¹ Department of Biophysics, P. J. Safarik University, Jesenna 5, 041 54 Kosice, Slovak Republic

² International Laser Center, Ilkovicova 3, 812 19 Bratislava, Slovak Republic

ABSTRACT. Molecular modeling has been employed to study the interaction of hypericin (Hyp) with human serum albumin (HSA). The structural model for Hyp/HSA complex is presented. Our results indicate that Hyp is bound in II A subdomain of HSA close to the tryptophan 214 (Trp214) (distance 5.12 Å between the centers of masses). In the presented model the carbonyl group of Hyp is hydrogen bonded to the Asn458. Another two candidates for hydrogen bonds have been identified between the bay-region hydroxyl group of Hyp and the carbonyl group of the Trp214 peptidic link and between the peri-region hydroxyl group of Hyp and Asn458 carbonyl group.

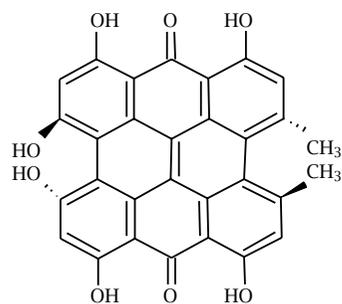
Abbreviations:

Hyp-Hypericin, HSA-Human Serum Albumin, BSA-Bovine Serum Albumin, RSA-Rat Serum Albumin, Trp-Tryptophan, Asn-Asparagine, Arg-Arginine, Lys-Lysine, His-Histidine, Tyr-Tyrosine, Phe-Phenylalanine, Leu-Leucine, Ala-Alanine, Ser-Serine, HIV- Human Immunodeficiency Virus, DMSO- Dimethylsulfoxide, DFT-Density Functional Theory, SERS - Surface Enhanced Raman Spectroscopy

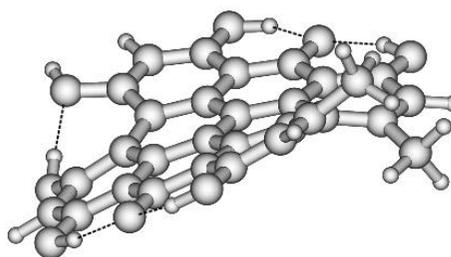
1. INTRODUCTION

Hypericin (Figure 1) is a natural photosensitizing polycyclic aromatic dione, which can be extracted from plants of the *Hypericum* genus [1]. It displays virucidal activity against several types of viruses, including the human immunodeficiency virus (HIV), [2-4] as well as antiproliferative and cytotoxic effects on tumor cells [5-7]. Its virucidal activity is enhanced in the presence of light [4, 8]. Other important biological properties of Hyp have been described, such as potent antidepressive activity [9], light-dependent inhibition of protein kinase C [5, 10], tyrosine kinase [11] and mitochondrial succinoxidase activity [12]. Hyp, like many other anti-cancer drugs also induces apoptosis [7]. Biological activity of Hyp and related compounds has been reviewed by Diwu *et al.* [13].

Serum albumins as the most abundant plasma proteins contribute significantly to many transport and regulatory processes. These proteins bind a wide variety of substrates like metals, fatty acids, amino acids, hormones and impressive spectrum of therapeutic drugs [14]. Serum albumins are known to contain two primary binding sites. The analyses of the crystal



(a)



(b)

Figure 1. (a) Two-dimensional structure of hypericin. (b) Three-dimensional structure of hypericin.

structure have revealed that the specialized drug binding cavities of serum albumins are located in subdomains IIA and IIIA [15]. Because of the clinical and pharmaceutical importance, interaction of serum albumins with a variety of ligands has been studied extensively [16-26]. The binding of Hyp to HSA helps to overcome difficulties in solubilization and disper-

[†]E-mail: misko@kosice.upjs.sk

sion of Hyp in aqueous physiological solution. The HSA-hypericin interaction results in dissociation into the monomeric form of Hyp, which is aggregated in the aqueous phase. The dissociation of Hyp to the monomeric form appears to be crucial for its virucidal action [22].

The studies of Falk *et al.* have suggested that the binding site of Hyp is in the III A subdomain of the HSA, where the Hyp is not completely shielded from the outer environment [24, 25]. However, in our previous study, by means of surface enhanced Raman (SERS) and resonance Raman spectroscopy (RRS) techniques, we have identified the Hyp binding site in the II A subdomain [26]. Observed experimental results indicated the interaction of the carbonyl group of Hyp with a hydrogen bond donor of albumin, leading to a protonated-like carbonyl in the drug and a change of H-bonding at the N1 – H side of tryptophan residue (Trp) in the protein. From these results we have proposed a model in which the hydrophobic interaction of Hyp with HSA leads to such Hyp position in which the formation of hydrogen bond between the carbonyl group of Hyp and the N1 – H position of Trp214, located in the IIA subdomain of the HSA structure, could be formed [26]. Time-resolved spectroscopy has been used in our later study of the HSA-hypericin (Hyp/HSA) complex, in which we have shown that Hyp is rigidly held in the IIA subdomain and is situated very close to Trp214 [16].

In this study a molecular modeling (MM) method has been used with the aim to construct a plausible model of the Hyp/HSA complex. The molecular modeling of the problem of this size represents a great computational challenge. By utilization of the experimental data, obtained in our previous studies [16, 26] a dramatical reduction of the problem has been achieved.

2. MATERIALS AND METHODS

The molecular structure of HSA was taken from 1BM0 structure deposited at PDB database [27]. From the copolymer consisting of two protein units and four water molecules, single unit was extracted with one structural water molecule preserved. The initial structure was completed by adding explicit hydrogens at standard positions. No further manipulations were performed, particularly no water environment nor unresolved residues 1–4 and 583–585 were added. In order to equilibrate the hydrogen positions (it means to relax minor sterical strains induced by adding explicit hydrogens), a short simulated annealing cycle [28] was utilized. Rapid simulated annealing consisting of two phases was used. Initially, 200 fs molecular dynamics was run with the time step of 0.1 fs. In order to avoid numerical instabilities due to long integration timestep, all the atomic masses were scaled by an effective factor 10. An all atom force field CHARMM22 [29] was used

throughout the simulation. All computational work was done using the molecular package tinker [30]. In the following step, 2 ps equilibration of the previous structure was performed, with linear cooling protocol, starting from 1000K and the 1 fs time step. In all simulations modified Beeman algorithm was used as implemented by module anneal.x of the tinker package. No restrictions on particular degrees of freedom were imposed. The results were visualized using Molden, VMD molecular visualizers and povray renderer [31–33]. In spite of the absence of explicit solvent, no signs of structural instability was observed during the simulation. The observed changes of the geometry were insignificant, in particular the differences between the crystal structure and the relaxed structure in the neighborhood of Trp214 residuum. Thus, it is reasonable to expect sufficiently realistic environment of the Trp214 of the free HSA in our simulation.

The constructed charge model of Hyp used in our molecular modeling, was based on effective electrostatic potential model of Merz and Kollman [34] with charge distribution evaluated by DFT computations done at B3LYP/6-31G* level on the neutral form of Hyp. The force field parameters for Hyp molecule were constructed from similar molecular fragments parametrized in standard CHARMM22 force field. The original force field parameters were transferred and adjusted to reproduce properly the equilibrium geometry of the dominant tautomer in propellor conformation (Figure 1).

The Trp residuum forms a part of the interior of the deep pocket within the IIA subdomain of HSA [35] (Figure 2). Near the entrance of this pocket several positively charged residues are placed, namely Arg257, Arg222, Arg218, Lys195, Lys199 and His242, which can have important consequences during the process of molecular docking. While the pocket containing Trp residuum is sufficiently large to accommodate the Hyp molecule, sterical constraints prevent the mutual orientation of Hyp and Trp postulated in the previous hypothetical model, where both Hyp and Trp are placed in the same plane [26]. However, the close inspection of the pocket interior suggests the parallel arrangement of the approximate planes of Hyp and Trp as the sterically most favourable. Accordingly, the Hyp molecule was placed inside the pocket, oriented with the hydrophobic part (consisting of the two sterically constrained methyl groups) pointed toward the interior of the pocket. After that the obtained initial structure was allowed to relax into the energetically more favourable conformation in the series of simulated annealing cycles until no significant conformational changes were observed in the following cycles. In each cycle, the initial structure was brought from 500 K temperature to the 0 K in 2.5 ps, using the linear cooling protocol and the modified Beeman integrator with the timestep of 1 fs. All aminoacid

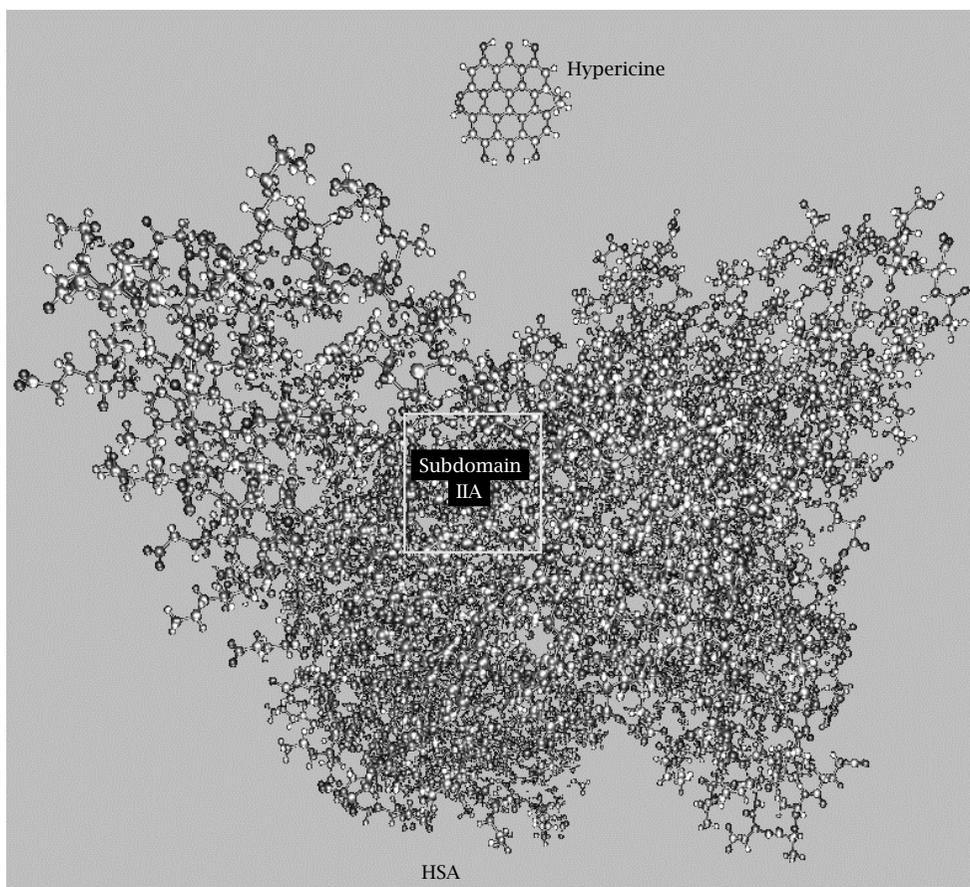


Figure 2. HSA and Hyp molecule. The IIA subdomain is marked by white rectangular (see [38] for details).

residua having at least three atoms within 20 Å from the centre of mass of Hyp were allowed to move, the rest of the HSA was fixed.

3. RESULTS

The obtained experimental data indicate that Hyp is placed in the II A subdomain of HSA [16, 26]. This is why for molecular modeling only this part of HSA has been considered. Hyp molecule is clearly capable to enter the deep Trp214 residuum containing pocket, of the IIA subdomain of HSA (Figures 2 and 3). The most favourable arrangement of Hyp relative to Trp is characterized by a nearly parallel orientation of the Hyp and the Trp residuum aromatic rings. (Figure 4). The characteristic distance between the Hyp and Trp planes, measured as the distance between the centres of mass of the corresponding fragments, is 5.12 Å. In order to achieve this arrangement, changes of the χ^1 (from -176° in the free HSA to -74° in the complex) and the $\chi^{2,1}$ (from 840 in free HSA to 69° in the complex) dihedral angles of Trp were required. The position of Hyp within the pocket is rigid. This rigid position

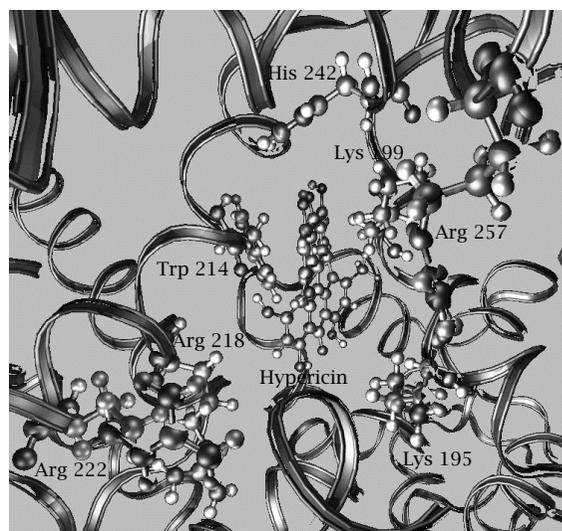


Figure 3. The IIA subdomain of HSA with Hyp molecule docked into the Trp214 containing pocket (see [38] for details).

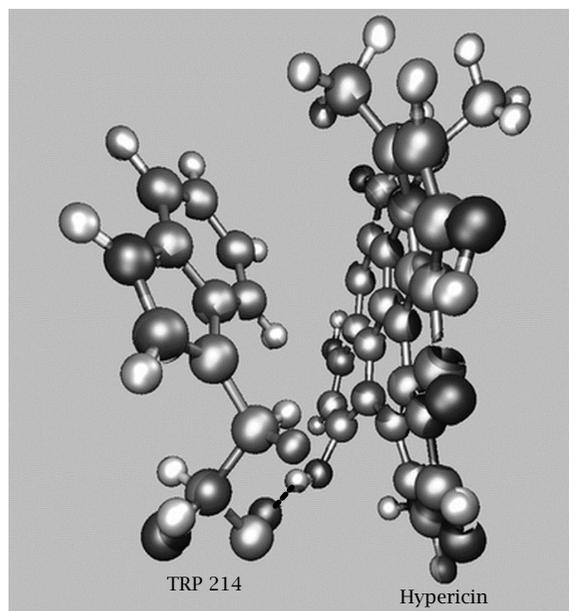


Figure 4. Detailed view of the mutual orientation of Trp214 and Hyp. The mutual distance between the centres of masses is 5.12 Å. (see [38] for details).

of Hyp is determined by a hydrophobic interaction of the Hyp with IIA hydrophobic pocket and by the formation of several intermolecular hydrogen bonds (Figure 5). Structural data from our simulation suggest the formation of three hydrogen bonds: i) between the central carbonyl group of Hyp in the peri-region and the Asn458 residuum (2.77 Å between the hydrogen bond donor to acceptor distance), ii) between the neighboring OH group of Hyp and the Asn458 carbonyl group (2.84 Å), and iii) between the bay hydroxyl group of Hyp and the carbonyl group of the Trp214 peptidic link (2.71 Å), and two eventual hydrogen bonds competing for the favourable geometry with the previous ones: i) between the hydroxyl group of the opposite peri-region of Hyp and the carbonyl group of the Ala210 peptidic link (2.87 Å, but not forming a favourable angle for a strong hydrogen bond formation), and ii) between the second hydroxyl group in the same peri Hyp region and the carbonyl group of the Phe211 peptidic link (3.08 Å).

The results obtained from resonance Raman spectroscopy indicate an increase of the hydrogen bonding at the N1 – H site of Trp [26]. However, no direct candidate for this type of interaction was found in the model. The closest carbonyl oxygen of Ala210 - possible H-bond acceptor is 3.90 Å from the N1 of Trp214.

4. DISCUSSION

The SERS, resonance Raman spectroscopy and time resolved spectroscopy of the Hyp/HSA complex have

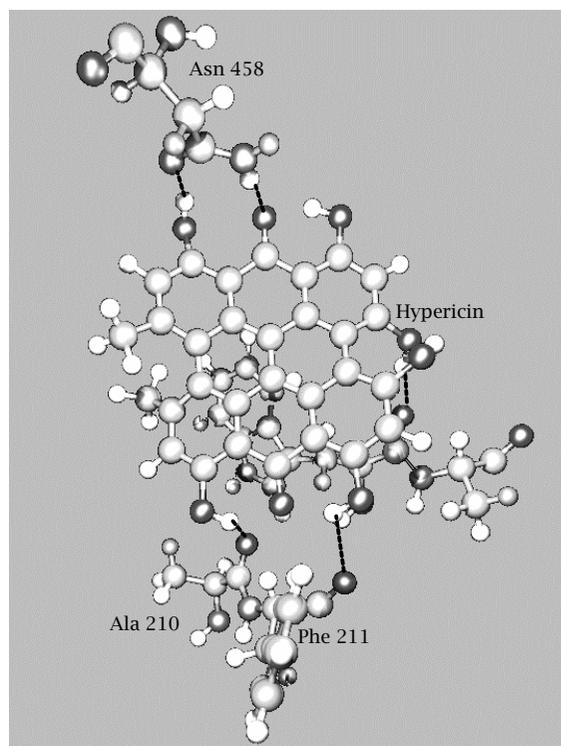


Figure 5. Hydrogen bonds stabilizing Hyp in IIA subdomain of HSA. The interatomic distances connecting hydrogen bond donor-acceptor pairs are marked by dash lines (see [38] for details).

been used in our previous experimental studies [16, 26] with the aim to determine a binding place of Hyp in HSA. SERS quenches the fluorescence of Hyp and consequently a behaviour of Hyp in the complex with HSA can be detected. On the other hand, a resonance enhancement of Trp vibrations in using resonance Raman spectroscopy allows us to detect an interaction of the drug with Trp situated in IIA subdomain of HSA [26]. Apart from this, the data obtained from polarized fluorescence decay of Hyp show that Hyp is tightly bound to HSA and an effective energy transfer from Trp to Hyp indicates that Hyp is placed very close to Trp214 [16]. By this way input data for molecular modeling have been prepared. In addition to the model presentation, there is also another (perhaps more general) interesting result presented in this paper. It is the problem of an agreement between the data obtained from experiment and molecular modeling. It means the question: to what extent the structural model obtained from the molecular modeling corresponds to our previously proposed model based on experimental results obtained from Raman [26] and time resolved spectroscopy [16]?

The results from SERS spectroscopy indicate that the Hyp site of binding in albumin may be placed in the inner part of the protein, since its accessibility to the

metallic surface is markedly limited when forming part of the complex [26]. The deep localization of Hyp in IIA subdomain was also found in our molecular model. Here Hyp is deeply inserted behind the IIA subdomain entrance aminoacids (Figure 3). The results from the resonance Raman spectroscopy [26] indicate the position of Hyp close to the Trp 214. The localization of Hyp in IIA subdomain near the Trp was also confirmed in our previous work by the energy transfer measurement [16]. As we can see from Figures 3 and 4, our results from molecular modeling proved the feasibility of the Hyp localization in the IIA subdomain, in a close proximity of the Trp214 residuum (the distance between the centres of mass of the corresponding fragments is 5.12 Å.).

A single-exponential rotational diffusion time of 31 ns (identical to the rotational time of the whole protein) was measured for Hyp bound to HSA in our previous work [16]. This result indicates that the drug is very rigidly held into the IIA subdomain of HSA. This rigid position of Hyp is determined by a hydrophobic interaction of the Hyp with IIA hydrophobic pocket and by the formation of several intermolecular hydrogen bonds as it was detected by SERS and resonance Raman spectroscopy as well as found by molecular modeling (Figure 5). Our simulation suggests the formation of three fairly strong and two weak hydrogen bonds between Hyp and aminoacids placed near to the drug. Thus, the experimental and computational data are mutually supporting each other.

All hydrogen bonds of Hyp presented on Figure 5, are important not only for the stabilization of the Hyp position in the IIA subdomain but very probably, these hydrogen bonds are responsible for the excited-state Hyp proton transfer blocking. The excited-state proton transfer is considered to be the major primary photochemical process in Hyp in organic solvents [36, 37], but no proton transfer in Hyp/HSA 1:1 molar ratio complex was observed in our previous work [16]. This result was interpreted as a consequence of the hydrogen bond formation between carbonyl group of Hyp and a proton donor in HSA. As it was found by molecular modeling this proton donor is most probably Asn458 (Figure 5) and not Trp214 as we proposed previously for the model based only on experimental results [16, 26].

The resonance Raman spectra indicate a conformational change of the Trp214 side chain [26]. The amide I Trp vibration varies as a function of $\chi^{2,1}$ torsion angle about the $C_{\alpha}-C_{\beta}-C3-C2$ linkage [26]. A change of the $\chi^{2,1}$ torsion angle has been found also in our structural model (Figure 4). On the other hand, the resonance Raman spectra indicate an increase of the hydrogen bonding at the N1 – H site of Trp. It was a reason for which we have previously proposed a hydrogen bonding of the carbonyl group of Hyp with N1 – H position of Trp214. Here we did not find a direct agreement between the

experimental and the computational results. There are several possibilities to be explored. Due to a restricted range of our geometry relaxation, we cannot exclude more extensive rearrangement of the pocket, bringing the Trp to a closer contact with neighbours. This explanation is, however less likely than the possible presence of a single water molecule, mediating the contact between the Ala210 and Trp214 molecule.

ACKNOWLEDGEMENTS

This work has been granted by: the Slovak Ministry of Education, grant No. 1/6027/99

References

- [1] N. Duran and P.-S. Song, *Photochem. Photobiol.* **43** (1986), 677.
- [2] D. Meruelo, G. Lavie, and D. Lavie, *Proc. Natl. Acad. Sci. USA* **85** (1988), 5230.
- [3] I. Lopez-Bazzocchi, J. B. Hudson, and G. H. N. Towers, *Photochem. Photobiol.* **54** (1991), 95.
- [4] J. Lenard, A. Rabson, and R. Vanderoef, *Proc. Natl. Acad. Sci. USA* **90** (1993), 158.
- [5] I. Takahashi, S. Nakanishi, E. Kobayashi, H. Nakano, K. Suzuki, and T. Tamaoki, *Biochem. Biophys. Res. Commun.* **165** (1989), 1207.
- [6] A. Andreoni, A. Colasanti, P. Colasanti, M. Mastrocinque, P. Riccio, and G. Roberti, *Photochem. Photobiol.* **59** (1994), 529.
- [7] W. T. Couldwell, R. Gopalakrishna, D. R. Hinton, S. He, M. H. Weiss, R. E. Law, and M. L. J. Apuzzo, *Neurosurgery* **35** (1994), 705.
- [8] G. A. Kraus, W. Zhang, M. J. Fehr, J. W. Petrich, Y. Wannemuehler, and S. Carpenter, *Chem. Rev.* **96** (1996), 523.
- [9] S. N. Okpanyi and M. L. Weischer, *Arzneimittel-Forschung* **37** (1987), 10.
- [10] T. Utsumi, M. Okuma, T. Utsumi, T. Kanno, T. Yashuda, H. Kobuchi, A. A. Horton, and K. Utsumi, *Arch. Biochem. Biophys.* **316** (1995), 493.
- [11] P. De Witte, P. Agostinis, P. J. Van Lint, W. Merlevede, and J. R. Vandenheede, *Biochem. Pharmacol.* **46** (1993), 1929.
- [12] C. Thomas, R. S. MacGill, G. C. Miller, and R. S. Pardini, *Photochem. Photobiol.* **55** (1992), 47.
- [13] Z. Diwu, *Photochem. Photobiol.* **61** (1995), 529.
- [14] T. Peters, *Advances in Protein Chemistry* **37** (1985), 161.
- [15] X. M. He and D. C. Carter, *Nature* **358** (1992), 209.
- [16] K. Das, A. V. Smirnov, J. Wen, P. Miskovsky, and J. W. Petrich, *Photochem. Photobiol.* **69**(6) (1999), 633.
- [17] K. J. Fehske, W. E. Müller, and U. Wollertt, *Mol. Pharmacology* **16** (1979), 778.

- [18] I. Sjöholm, B. Erman, A. Kober, I. Ljungstedt-Pahlman, B. Seiving, and T. Sjödin, *Mol. Pharmacology* **16** (1979), 767.
- [19] U. Kragh-Hansen, *Mol. Pharmacology* **34** (1988), 160.
- [20] J. Davila and A. Harriman, *J. Am. Chem. Soc.* **112** (1990), 2686.
- [21] M. Rotenberg, S. Cohen, and R. Margalit, *Photochem. and Photobiol.* **46** (1987), 689.
- [22] G. Lavie, Y. Mazur, D. Lavie, A. M. Prince, D. Pascual, L. Liebes, B. Levin, and D. Meruelo, *Transfusion* **35** (1995), 392.
- [23] V. Senthil, J. W. Longworth, C. A. Ghiron, and L. I. Grossweiner, *Biochem. Biophys. Acta* **1115** (1992), 192.
- [24] H. Falk and J. Meyer, *Monatsh. Chem.* **125** (1994), 753.
- [25] M. Köhler, J. Gafret, J. Friedrich, H. Falk, and J. Meyer, *J. Phys. Chem.* **100** (1996), 8567.
- [26] P. Miskovsky, D. Jancura, S. Sánchez-Cortés, E. Kocisova, and L. Chinsky, *J. Am. Chem. Soc.* **120** (1998), 6374.
- [27] S. Sugio, A. Kashima, S. Mochizuki, M. Noda, and K. Kobayashi, *Protein Eng.* **12** (1998), 439.
- [28] C. D. Kirkpatrick, Jr. Gelatt, and M. P. Vecchi, *Science* **220** (1983), 671.
- [29] A. D. Jr. MacKerell, D. Bashford, M. Bellott, R. L. Jr. Dunbrack, J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. J. McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. III Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiorkiewicz-Kuczera, D. Yin, and M. Karplus, *J. Phys. Chem. B* **102** (1998), 3586.
- [30] M. J. Dudek, K. Ramnarayan, and J. W. Ponder, *J. Comput. Chem.* **19** (1998), 548.
- [31] G. Schaftenaar and J. H. Noordik, *J. Comput.-Aided Mol. Design* **14** (2000), 123.
- [32] W. Humphrey, A. Dalke, and K. Schulten, *J. Molec. Graphics* **14** (1996), 33.
- [33] The povray program was obtained from <http://www.povray.org>
- [34] U. C. Singh and P. A. Kollman, *J. Comput. Chem.* **5** (1984), 129.
- [35] D. C. Carter and J. X. Ho, *Adv. Protein Chem.* **45** (1994), 153.
- [36] J. W. Petrich, *Int. Rev. Phys. Chem.* **19** (2000), 479.
- [37] M. J. Fehr, M. C. McCloskey, and J. W. Petrich, *J. Am. Chem. Soc.* **117** (1995), 1833.
- [38] P. Miskovsky, J. Hritz, S. Sanchez-Cortes, G. Fabriciova, J. Ulicny, and L. Chinsky, *Photochem. Photobiol.* **74**(2) (2001), in press



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

