Supporting Information for:

Drug-Drug Interactions within Protein Cavities Probed by Triplet-Triplet Energy Transfer

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Computational Methods and Results

The calculations have been performed using a combined set of three techniques aimed at finding the binding site of CIN and PPN at HAAG. The structure of HAAG (2868 atoms) has been taken from the recent determination by Nishi et al. [1].

In a first stage, a Monte-Carlo approach, using the GULP code [2], has been used in order to explore a large conformational space of binding sites of -separately- CIN and PPN at HAAG. Starting with experimental location of some drugs [1], a large area of the protein around those binding sites has been explored in order to find stable binding sites for CIN and PPN. As usually, the Monte-Carlo technique takes into account both the protein and the binding molecules as rigid bodies. Although this limits the calculation accuracy, it is convenient to save computer time and hence to explore many millions of random moves and to calculate their corresponding binding energies. The energy expression has been considered according to the Universal Force Field [3]. From all the configurations, those more energetically favorable have been selected for the next steps of the calculation.

In the second stage, a full lattice energy minimization technique [4,5] has been employed by optimizing all the atoms of the system using the Universal Force Field, with 1000 cycles of geometry optimization using a steepest descent algorithm. For this task the Avogadro software [6] has been used. The aim of this approach is to make a low level geometry optimization of the full system so that, in particular, the geometry of the binding site is optimized.

Finally, in the third stage, a quantum semiempirical PM7 [7] partial geometry optimization has been performed within the MOPAC2012 code [8]. This is a considerable step forward to improve the quality of the geometry optimization and to obtain a reliable atomic charge distribution of all the atoms of the system. In addition, this takes into account short and long range interactions with a quantum approach, much more accurate than the atomistic approaches. In this case, taking into account that the whole system includes more than 2000 atoms, a different approach for the geometry optimization has been considered. All atoms in the protein located at a distance shorter than a predefined threshold to any drug atom have been

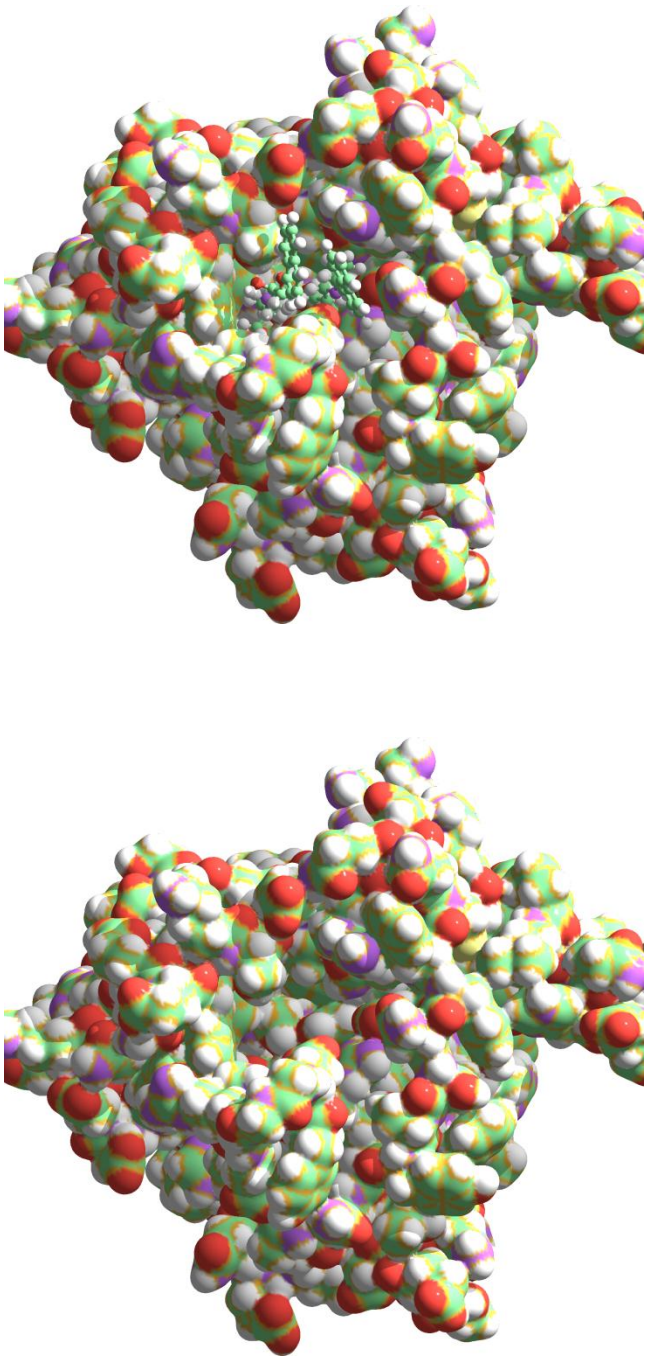
marked for geometry optimization. Also, the atoms of the drugs (CIN and PPN) have been marked for optimization. After a given number of steps, the new list of 'close atoms' has been updated and a new set of geometry optimization cycles have been performed. In this way, a reliable final geometry has been obtained for HAAG, CIN and PPN, using a threshold distance of 8 Å and 300 cycles of geometry optimization.

The full approach of three stages can then be repeated or stopped as many times as needed until convergence of the results. The final geometry is shown in Figure S1. The quantum semiempirical PM7 results indicate a binding energy of -46.3 kJ/mol between the two associated drugs (CIN+PPN) and HAAG. This indicates that there is sufficient space on the protein binding site to accommodate both drugs. An analysis of the energetic interaction between CIN and PPN leads to the value of -14.2 kJ/mol, showing a favorable interaction between the two drugs. Both negative energies are required in order to explain the association of the two drugs in the binding site of HAAG.

After finding the geometry of CIN and PPN at HAAG, single point calculations have been performed by means of a DFT approach, using the hybrid M062X [9] functional and Def2-TZVP [10] basis set and the Gaussian09 code [11]. With this methodology, the energetic interaction between CIN and PPN gives -36.0 kJ/mol, confirming that there is a considerable association between the two drugs. With this first-principles method, and taking the previously optimized geometry of CIN and PPN at HAAG, we have calculated the feasibility of the energy transfer from the excited triplet of PPN to the ground state of CIN. This has been evaluated in terms of the overlap between the involved molecular orbitals and the corresponding energy difference. The M062X functional has been shown particularly accurate to describe the singlet to triplet electronic transitions [12].

With the single point geometry, the electronic structure (M062X/Def2-TZVP) of the PPN triplet and the CIN ground state have been optimized and then the molecular orbital energies and spatial distribution have been extracted. Two simultaneous electron exchanges have been considered for the TTET mechanism: from SOMO(PPN) to LUMO(CIN), and from HOMO(CIN) to SOMO-1(PPN).

A small energy difference, +0.109 eV, has been found between SOMO(PPN) and LUMO(CIN), with the former being higher in energy. Figure S2 (top) shows a massive overlap between SOMO(PPN) and LUMO(CIN). In the case of HOMO(CIN) and SOMO-1(PPN), the energy difference is even smaller, 0.013 eV, which favors the electronic exchange. Here, a massive overlap between the two orbitals has also been found (Figure S2, bottom). The feasibility of both electronic exchange steps demonstrates that a TTET mechanism is indeed possible between PPN(triplet) and CIN(ground state).



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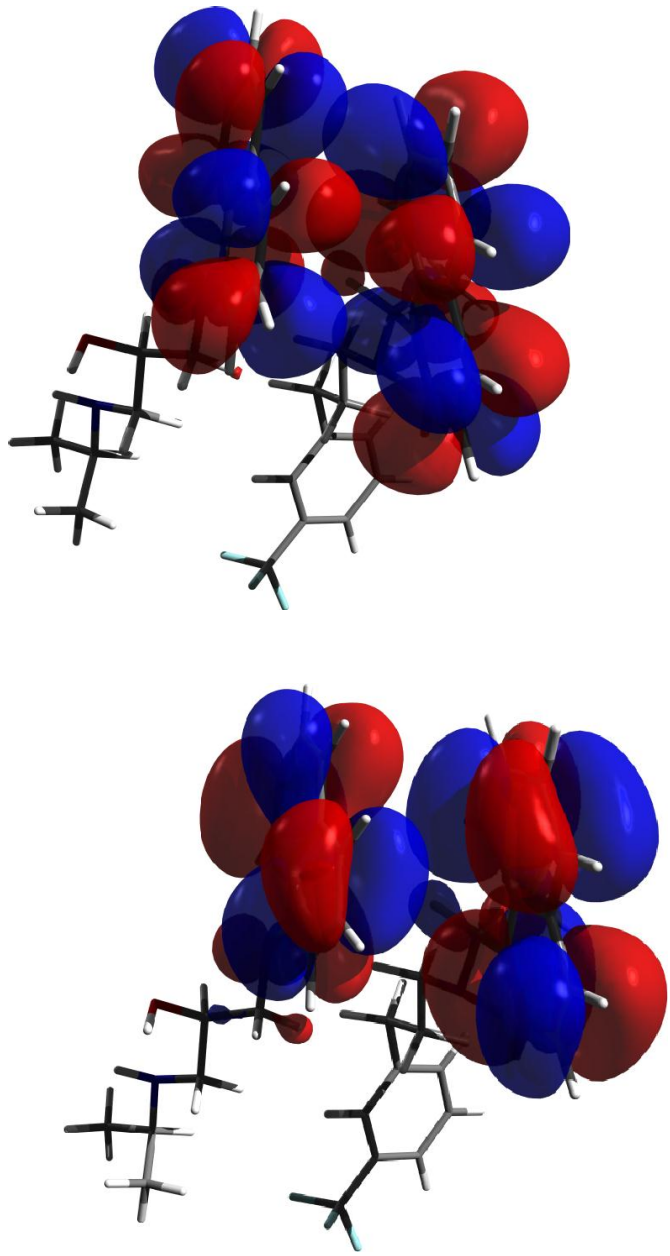

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