DNA-proteins crosslink reactions simulation with quantum-mechanics/molecular mechanics (QM/MM) techniques

RESEARCHE REPORT

Mainly the project was aimed to the modelling of the laser induced crosslink between proteins and DNA in living cells by irradiating them with ultra short UV laser pulses. The way in which we have established to concretize the ideas basically follow two opposite roots. The first one corresponds to the reductionist approach to face the crosslink characterization problem i.e. the study of the monomeric components of the macromolecules involved in the excitation and relaxation processes. In particular, we have focused our attention to the simulation of the 5-benziluracile (5BU) photocyclisation reaction in fig. 1, model of real crosslink reaction.

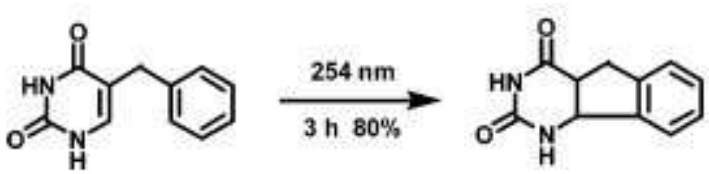

Fig.1: The 5-benzyluracile photocyclisation reaction stimulated by UV light

The 5BU is not casually formed of benzene and uracile as two distinct fragments linked by a CH_2 group that in this model are thought to be the active sides of DNA basis and amminoacid. The CH_2 bridge is the model of the proximity constrain due to the intermolecular electrostatic interaction between the macromolecules involved in alive systems.
If correct enough, a full quantum molecular dynamics simulation of the excited 5BU will lead to the proton transfer and the new covalent bond formation experimentally observed[1]. With this simulation will be possible to:

- Gain experience on how molecular dynamics techniques can be applied to face the problem of the “photo-induction of new covalent bonds formation” study: the failures, problems, successes and possibilities.

[1] G. Sun, C. Fecko, R. B. Nicewonger, W. W. Webb and Tadhg P. Begley, DNA-Protein Cross-Linking: Model Systems for Pyrimidine-Aromatic Amino Acid Cross-Linking, Organic Letters, 2006 Vol. 8, No. 4 681 683

- Obtain the reaction time resolved pathway, an estimation of its quantum yield and the reaction time; Analyze the potential barriers that the system needs to cross for the crosslink formation.
- Get useful information about the relative orientation of the fragments leading to the crosslink when photo-excited.
- Give indications and predictions to be validated with experimental measurements (e.g. time resolved spectroscopy)

Finally, many other future modeling steps could be thought as improvement of this simulation by growing the system complexity and/or adding a solvent.
The final stage following this route would be a simulation involving a real alive system, or a part of it, in which the active pairs (DNA base-amminoacid) would be treated quantum mechanically and the remaining part of the system with classical mechanics. The use of a QCMD (Quantum Classical Molecular Dynamic) method would be hence necessary at that point.

The work made on this topic has been structured in the following way:

- First month:
  Overview on the possible approaches and the available techniques to face the problem: the molecular systems to focus on and the possible simulations to investigate have been selected.
- Second month:

  Characterization of the electronic excited states of the 5BU in the two nuclear conformation already available in the reaction path of the molecular system (the photocyclisation process).
  Predictions relative to different available functional used in the LR-TDDFT method have been obtained. The orbital assignment and the resonance energies predictions have been deeply tested and compared to their equivalent obtained with wave function methods at RI-CC2 and CASSCF theory level.

  Mainly, the attention has been focused to the following test functionals: B3LYP, CAM-B3LYP, M06 and PBE (fig.2). Results show strong similarity of RI-CC2 and M06 predictions while the PBE functional underestimate the excitation energies; the assignment of the orbitals involved in the excitations remains however mostly unchanged for all the cases. The CASSCF wave function analysis moreover has shown that the system multiconfigurational

nature could not be crucial for a correct excited states PES description and single reference methods have good chances to give correct predictions (at least qualitatively)

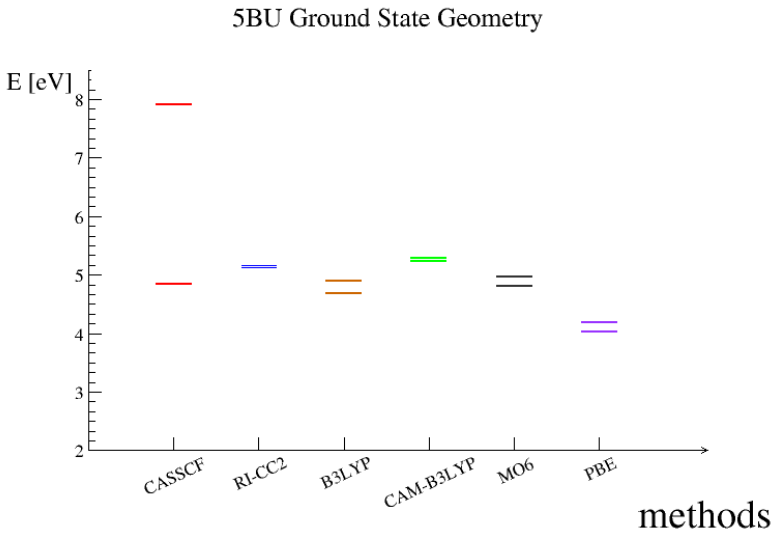

Fig.2: 5-benzyluracil excitation energies predictions according to CASSCF, RI-CC2 and TDDFT with different functionals.

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- Third month:
  Full quantum molecular dynamics simulation setup. The used method is the Born Oppenheimer (BO) excited state molecular dynamics with TDDFT electron energy calculation on the fly and the Tully's Trajectory Surface Hopping (TSH) method to cover non adiabatic effects.

  The system has been equilibrated (thermalized) in gas phase at different temperatures with the use of a preliminary ground state BO molecular dynamics in which has been implemented the Nosé and Hoover thermostat for the temperature control.

  After equilibration a first excited state trajectory has been obtained at 100 K temperature. The preliminary results obtained are encouraging: the photo-excited system in fact, also if very cold, is able to go over potential barriers in the ground state energy profile impossible to be crossed with any reasonable thermal agitation (fig.3).

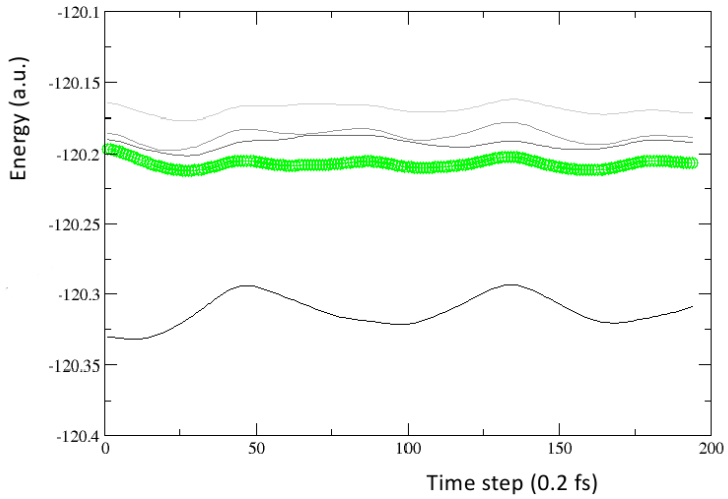

Fig.3: 5-benzyluracil PES profile for the first 50 fs dynamics trajectory.

The second root, opposite of the previous reductionist one, consists in the interrogation of the PDB (Protein Data Bank), the data bank of protein structures obtained experimentally with the NMR or the X ray crystallization, in order to find good candidates of active couples of proteins and DNA monomers leading to crosslink formation in nature.

The collection of real examples of interacting fragments of DNA-proteins structures represent in fact the obvious statistic ensemble to test or speculate possible crosslink formation mechanisms, estimate number and frequency of possible crosslink events and compare the obtained prediction with experimental data.

The work made so far in this direction consists essentially in the writing the general searching program (a perl routine) able to sample the PDB and we got a first collection of structure images in which typical cromophores (DNA bases and aromatic amminoacids) are close. Much work must however still be made on this side.