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Workshop

PRINCIPLES OF LIGHT-INDUCED CHARGE TRANSFER FOR OPTOGENETICS

July 3-5, 2023 | Modena (IT)

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2nd Edition of the workshop
Principles of Light-induced charge transfer for optogenetics

July 3-5, 2023
Modena | Italy

Index

Presentation

This book of abstracts includes the program of the Workshop of the Nanoscience Institute of the National Research Council (Cnr Nano), the University of Aquila (Univaq), and the University of Southern California that takes place in Modena on July 3-5, 2023. It also lists the participants to the workshop, the abstract of the oral presentations and offers a list of the organizing committee.

The program brings together leading experts in the experimental and theoretical investigation of photoactive proteins that find application in the field of optogenetics.

The workshop emphasizes new results and point out new directions, challenges, and opportunities in the following fields:

- Charge transfer processes in light-sensitive proteins,
- Excited state properties of biological matter,
- Photoreceptor thermodynamics and photocycle kinetics,
- Interplay between photoexcitation and protein conformations.

Program

Monday, July 3		
Session 1		
14:30	15:00	Registration
15:00	15:15	Opening
15:15	16:00	Invited | Massimo Olivucci | University of Siena & Bowling Green State University
		From Photon To Neuron: The Molecular Mechanism Of The Primary Event In Vision
16:00	16:20	Katharina Spies | Karlsruhe Institute of Technology
		Active Site Structure And Absorption Spectrum Of The Channelrhodopsin Chrimson – Wild Type And Mutants
16:20	16:45	Coffee break
16:45	17:30	Invited | John Kennis | Vrije Universiteit Amsterdam
		Isomeric Switching Near The Conical Intersection In Bestrhodopsin, An Unusual Red-Absorbing Microbial Rhodopsin
17:30	17:50	Raffaella Polito | Sapienza University of Rome
		Mid-IR Spectroscopy To Probe Conformational Changes Of Bacteriorhodopsin At The Nanoscale
17:50	18:10	Maria Eleonora Temperini | Sapienza University of Rome
		A New IR Spectroscopy Platform To Study The Effect Of Static Electric Fields On Biomolecules
18:10	18:30	Thanh Nhut Do | Vrije Universiteit Amsterdam
		Excitation-Fluence Dependent Two-Photon Induced Photoionization Of Bacterial Phytochrome

Tuesday, July 4		
Session 2		
09:00	09:45	Invited | Nadia Rega | University of Napoli Federico II & Scuola Superiore Meridionale
		Photoinduced Charge Transfer Non-Equilibrium Processes: Theory And Modeling Strategies
09:45	10:05	Daniele Narzi | University of L'Aquila
		Mechanism Of The Light-Induced Water Oxidation Reaction Occurring In The Natural Oxygenic Photosynthesis
10:05	10:25	Abhishek Sirohiwal | Stockholm University
		Primary Events In Reaction Centre Of Photosystem II
10:25	10:45	Coffee break
10:45	11:30	Invited | Ciro A. Guido | Università del Piemonte Orientale
		Dispersion Interactions At The Excited State: Influence On Light-Responsive Properties Of Biosystems
11:30	11:50	Sinjini Bhattacharjee | Max-Planck-Institut
		Multiscale Modeling Of Genetic Variants Of Photosystem II
11:50	12:35	Invited | Lyudmila Slipchenko | Purdue University
		Triplet Energy Transfer In The Fenna-Matthews-Olson (FMO) Pigment-Protein Complex
12:35	12:55	Pavel Rukin | CNR - Istituto Nanoscienze
		Theoretical Study Of Vibrational-Mediated Interlayer Charge Transfer In A Cobalt Phthalocyanine-Graphene Heterojunction
12:55	14:30	Lunch break

Session 3		
14:30	15:15	Invited | Jochen Blumberger | University College London
		Currents Of Bacterial Life Probed By Molecular Simulation And Pump-Probe Spectroscopy
15:15	15:35	Matteo Capone | University of L'Aquila
		Multiscale Modeling Of Photo-Induced Stereoselective Radical Cyclization In A Flavoenzyme
15:35	15:55	Lorenzo Cupellini | University of Pisa
		How Simulations Uncover The Photoactivation Mechanism Of Appa Bluf
15:55	16:15	Coffee break
16:15	17:00	Invited | Sharon Hammes-Schiffer | Yale University
		Nonequilibrium Excited State Dynamics Of Proton-Coupled Electron Transfer In Bluf Photoreceptor Proteins
17:00	17:20	Laura Pedraza-Gonzales | University of Pisa
		How The pH Controls Photoprotection In The Light-Harvesting Complex Of Mosses
17:20	18:05	Invited | James Boedicker | USC Dornsife
		Optogenetic Tools To Control Charge Transfer Within Bacteria
18:05	18:30	Discussion
19:30		Social Dinner

Wednesday, July 5		
Session 4		
09:00	09:45	Invited | Gloria Mazzone | Università della Calabria
		Light Induced Charge Transfer For Enhanced Photodynamic Therapy Action
09:45	10:05	Colin Coane | University of Southern California
		Unraveling The Mechanism Of Tip-Enhanced Molecular Energy Transfer
10:05	10:25	Giovanni Parolin | University of Padova
		Modelling Plexcitonic States With Single-Molecule Resolution
10:25	10:45	Coffee break
10:45	11:30	Invited | Ksenia Bravaya | Boston University
		Predictive Methods For Simulating Charge Transfer And Redox Processes In Proteins
11:30	11:50	Davide Accomasso | University of Pisa
		Uncovering A Carotenoid Quencher State In The CP29 Light-Harvesting Complex Of Plants
11:50	12:10	Matteo Bruschi | University of Padova
		Simulating Action-2D Electronic Spectroscopy From Molecular Dimers To Photosynthetic Antennas
12:10	12:30	Stefano Scoditti | Università della Calabria
		Unveiling The Photocatalytic Reduction Of Platinum(IV) Complexes By Riboflavin: Insights From Computational Analysis
12:30	14:00	Lunch break

Session 5		
14:00	14:45	Invited | Igor Schapiro | The Hebrew University of Jersualem
		Insight Into The Photochemistry Of Cyanobacteriochromes By Qm/Mm Simulations
14:45	15:05	Leonardo Barneschi | University of Siena
		Mechanism Of Fluorescence Enhancement In Rhodopsin Optogenetic Reporters
15:05	15:25	Giacomo Salvadori | University of Pisa
		Transient Intermediates In A Bacteriophytochrome Photocycle Revealed By Multiscale Simulations
15:25	15:45	Coffee break
15:45	16:05	Federico Gallina | University of Padova
		Simulating 2D Spectroscopic Responses Of Optical Systems With Digital Quantum Computers
16:05	16:50	Invited | Samer Gozem | Georgia State University
		Electrostatic Tuning Maps And Average Protein Configurations: Tools To Aid In Studying Flavoproteins
16:50	17:00	Closing

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Abstract | Oral presentations

FROM PHOTON TO NEURON: THE MOLECULAR MECHANISM OF THE PRIMARY EVENT IN VISION

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The activation of rhodopsin, the light-sensitive G-protein coupled receptor responsible for dim-light vision in vertebrates, is driven by an ultrafast excited state double-bond isomerization with a quantum efficiency ($\Phi_{cis\text{-}trans}$) of almost 70%. The origin of such a high light sensitivity, ultimately allowing the human eye to detect even single photons, is not understood. A key unanswered question is whether and how the level of synchronization between different receptor vibrational modes controls the $\Phi_{cis\text{-}trans}$ value. Here, we employ hundreds of quantum-classical trajectories to show that, 15 femtoseconds after photon absorption the excited state population of rhodopsin split into subpopulations reacting with different velocities and leading to distinct contributions to Φcis-trans. We find that each subpopulation and $\Phi_{cis\text{-}trans}$ contribution, is associated with a different phase relationship between specific critical vibrational modes. We also show that the population splitting is modulated by the protein electrostatics, thus linking amino acid sequence variations to $\Phi_{cis\text{-}trans}$ modulation.

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ACTIVE SITE STRUCTURE AND ABSORPTION SPECTRUM OF THE CHANNELRHODOPSIN CHRIMSON – WILD TYPE AND MUTANTS

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Due to its red-light activation and the associated low phototoxicity for cells, the channelrhodopsin Chrim son is widely used in optogenetic studies, e.g. for vision and hearing restoration. With our computational study based on the Chrimson crystal structure (PDB-ID: 5ZIH) [1], we aim to identify residues and structural conformations responsible for Chrimson's color tuning. We are specially interested in the structure of the active site, which includes the chromophore retinal with a positively charged Schiff base in the ground state structure and its counterions, the glutamate E165 and the aspartate D295.

We performed quantum mechanics/molecular mechanics (QM/MM) simulations of the Chrimson wild type and various mutants using the computationally favorable density functional tight binding method (DFTB3) as the QM method [2, 3]. In addition, we calculated the excitation energies of a large ensemble of QM/MM trajectory snapshots to compare the absorption spectrum to other rhodopsins, such as channelrhodopsin 2 and bacteriorhodopsin. To obtain a valid computational model, we simulated multiple models with different protonation states of the counterions in the active site and the glutamates in the putative ion pathway, and compared the structural and spectroscopic properties in detail.

We confirm the experimental studies [1, 4, 5] that the counterion E165 is protonated in Chrimson, which is an unique configuration among channelrhodopsins, and we also identify the protonation states of the glutamates in the central and outer gates of Chrimson. Our computed excitation energies are qualitatively in the correct range and the absorption spectra of structures with mutated residues in and near the active site also agree well with the experimental results. At least two stable configurations have been observed in the active site: the distance between the counterions is very small and the proton of the counterion E165 is shared by both, or the distance increases and a water molecule is present between the counterions. Furthermore, the orientation of the residues in the active site is highly interdependent, changing the hydrogen bonding network towards the retinal Schiff base and the counterion configuration. We were able to correlate the excitation energies with the structural motifs, and the absorption of Chrimson is shifted to red light when the shared proton motif is present in the active site.

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ISOMERIC SWITCHING NEAR THE CONICAL INTERSECTION IN BESTRHODOPSIN, AN UNUSUAL RED-ABSORBING MICROBIAL RHODOPSIN

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Bestrhodopsins are a newly discovered class of light-regulated ion channels that consist of two rhodopsins in tandem fused with a bestrophin ion channel domain. Bestrhodopsin of the marina alga *Phaeocystis antarctica* binds all-trans retinal Schiff-base (RSB) that absorbs at 660 nm, conveying excellent potential for optogenetic applications. Red light illumination of the tandem domain results in a metastable green-absorbing state P540, which corresponds to an unusual 11-cis RSB isomer rather than the canonical 13-cis isomer. Transient absorption (TA) spectroscopy showed that a primary photoproduct P690 is formed 1 ps at about 10% quantum yield, which evolves to a secondary product P670 in 550 ps. Next, P670 establishes an equilibrium with P590 in 1 μs after which it evolves to the metastable P540 species in 42 μs. Femtosecond stimulated Raman spectroscopy (FSRS) showed that P690 corresponds to a mixture of 11-cis and 13-cis RSB isomers, directly formed from the excited state. Strikingly, upon evolution from P690 to P670 in 550 ps, the fraction of 11-cis species converts to 13-cis, indicating that P670 fully corresponds to the 13-cis species. In contrast, P590, which exists in equilibrium with P670 in 1 μs again corresponds to 11-cis RSB, which is finally stabilized in the P540 species in 42 μs. Hence, extensive isomeric switching on the ground state potential energy surface occurs on the sub-ns to microsecond time scale before bestrhodopsin finally settles on a stable 11-cis photoproduct. FSRS further indicates that P690 and P670 correspond to unusually highly distorted polyene backbones of RSB, while P590 and P540 are more relaxed. We propose that the observed phenomenology relates to trapping of the early photoproducts P690 and P670 high up the ground-state potential energy surface (PES) after passing through the conical intersections that result in 11-cis and 13-cis RSB, caused by steric clashes with amino acid side chains. Co-rotation of C11=C12 and C13=C14 double bonds and adjacent single bonds upon passing through the CIs and subsequent trapping results in a constricted conformational landscape on the ground state PES that allows thermal switching between 11-cis and 13-cis species of highly strained RSB chromophores. On the microsecond timescale, protein relaxation may take place that releases the strain on the RSB chromophore allowing it to finally evolve to a stable 11-cis isomeric configuration. Hence, we have observed structural intermediates close to the conical intersection in a microbial rhodopsin.

MID-IR SPECTROSCOPY TO PROBE CONFORMATIONAL CHANGES OF BACTERIORHODOPSIN AT THE NANOSCALE

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The analysis of protein conformation and structural modifications provides a detailed insight into their functional mechanisms. Mid-infrared (IR) spectroscopy is a well-established tool for biomolecules structural analysis, relying on vibrational transitions measure, which are extremely sensitive to changes in the chemical bond in length and angle and to extramolecular interaction. In the last decade, a breakthrough in the analysis of protein conformation well beyond the diffraction limit has been achieved with the novel IR nanospectroscopy technique (also called AFM-IR) based on an atomic force microscope (AFM) illuminated by an IR laser, detecting photothermal expansion under the AFM tip (fig. 1a) [1].
Here we apply the AFM-IR technique to monitor the functional activity of rhodopsins, relying on cyclic and subtle conformational changes referred to as the photocycle and triggered by visible light [2]. For the first demonstration we probed the light-induced structural changes of the prototype proton pump Bacteriorhodopsin (BR) embedded in cell membrane patches. We leveraged on a difference nanospectroscopy approach, i.e., by acquiring AFM-IR spectra under green (565 nm) and blue (420 nm) visible light, which respectively starts the BR photocycle and brings the proteins back to a resting state. In fig. 1b we report the relative difference absorption spectra ΔA/A obtained on a 1μm-thick films of cell membranes and on a 10 nm-thick stack of two membrane patches deposited on gold surfaces. The differences between the two curves, marked with 1), 2) and 3), have been attributed to a branching of the photocycle that partially inhibits the proton transport as effect of the direct contact of proteins with metals. These results are relevant for protein-based bioelectronic devices and optogenetic applications where proteins adhere to metallic electrods [3].

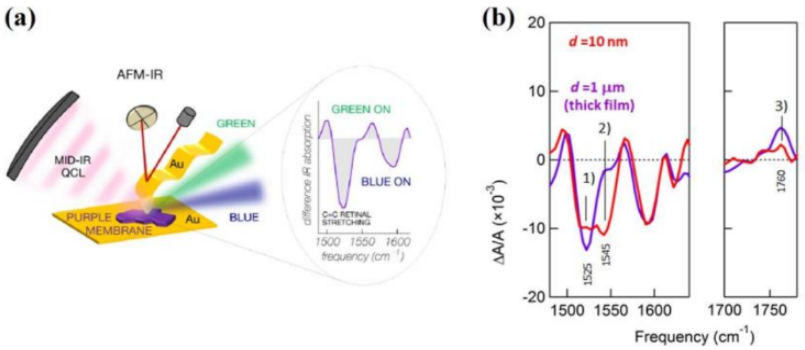

Fig. 1. (a) Sketch of the AFM-IR setup. (b) Relative difference spectra ΔA/A obtained on a 1 μm-thick film of cell membrane patches and on a 10 nm-thick stack of two membrane patches containing BR proteins.

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A NEW IR SPECTROSCOPY PLATFORM TO STUDY THE EFFECT OF STATIC ELECTRIC FIELDS ON BIOMOLECULES

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Infrared (IR) spectroscopy in difference-absorption mode permits to investigate the functional conformational changes of light-sensitive proteins, relying on the vibrational spectroscopy measure of modifications that occur after the protein photoactivation [1]. However, conventional IR spectroscopy is limited in terms of sensitivity preventing its application at the level of the single cell membrane. Here we present an innovative IR difference-spectroscopy approach at the nanoscale, also called AFM-IR [2], and we apply it to the study of light-sensitive transmembrane proteins, i.e. the prototype proton-pump bacteriorhodopsin (BR) and the light-gated ion channel Channelrhodopsin2 (ChR2). The experimental setup consists in an atomic force microscope (AFM) coupled to a mid-IR pulsed quantum cascade laser (QCL): the local IR response is obtained by monitoring the sample thermal expansion induced by the absorption of the IR radiation with the AFM probe tip (fig.1a). Using this approach with a gold-coated AFM tip allows one to collect IR spectra from a sample volume much smaller than the diffraction limit, confining the temperature increase in the volume below the tip apex. Furthermore, sensitivity down to the molecular monolayer can be reached by exploiting the plasmonic field enhancement in the nanogap between the gold-coated AFM tip and a metallic surface used as support for the monolayer sample (fig.1b). By combining this plasmonic field-enhancement approach with a difference-nanospectroscopy mode, we have pioneered the use of the AFM-IR technique for the study of functional conformational changes of light-sensitive membrane proteins at the single membrane patch level (fig.1c) [3,4]. These results open the way to a novel experimental paradigm where one can envision to combine the electrical and spectroscopic capabilities of AFM to tackle the still unresolved issue of monitoring the dependence of membrane protein conformational changes on an applied membrane potential. Recently, we have customized our AFM-IR platform in order to perform IR nano-spectroscopy while applying a DC bias between the metallic AFM tip and the metallic sample support. Preliminary results of vibrational Stark effect obtained at the nanoscale on polymeric samples will be also presented.

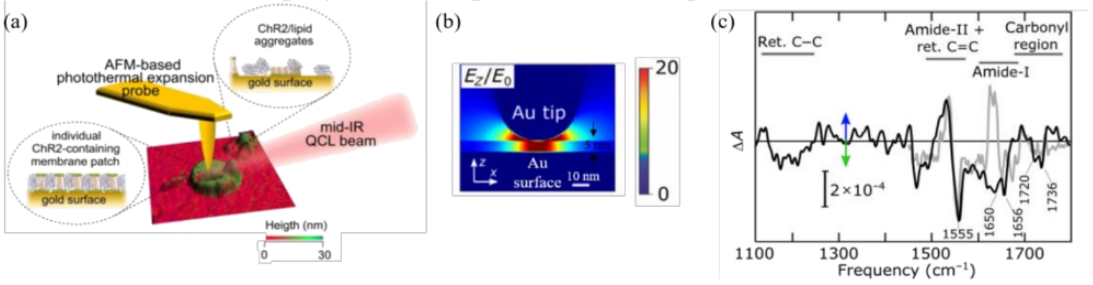

Fig.1(a) Sketch of the AFM-IR setup. (b) EM simulation of the IR enhancement. (c) ΔA IR results on ChR2.

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EXCITATION-FLUENCE DEPENDENT TWO-PHOTON INDUCED PHOTOIONIZATION OF BACTERIAL PHYTOCHROME

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Phytochromes constitute a family of photosensory proteins utilized to regulate several biophysical processes in bacteria and plants [1]. Phytochromes bind a bilin pigment that switches its isomeric state when absorbing red or far-red photons, leading to conformational changes in the protein. Recently, the ultrafast dynamics of the pigment in bacterial phytochrome was resolved to atomistic detail by time-resolved femtosecond X-ray diffraction, showing extensive changes in its molecular conformation [2]. However, the large excitation fluence (in the scale of mJ/mm^2) used in such experiments gives rise to concerns about the validity of the observed dynamics. In this work, we present the excitation-dependent study to verify the sensitivity of bacterial phytochrome to the excitation fluence.

An additional sub-picosecond (sub-ps) dynamic is observed even under moderate excitation densities (tens of µJ/mm^2). This feature can be observed by comparing the 0.4-ps evolution-associated different spectra (EADS) between the three panels in Figure 1. Compared with the main excited-state population decay in 30-40 ps, observed under the low fluence and assigned to the *bona fide* biliverdin isomerization process, the amplitude of the sub-ps component grows non-linearly with respect to the excitation fluence. Together with the fact that the sub-ps dynamic is observed mainly in the 650-700 nm spectral window coinciding with a pronounced excited-state absorption feature, we attribute the sub-ps component to the resonantly enhanced two-photon excited population, which subsequently relaxes to the lowest excited state on the sub-ps timescale. Strikingly, in addition to the native isomerized biliverdin photoproduct, spectral signatures of a biliverdin cation and solvated electron are observed after excited-state decay, indicative of photoionization during the transient population of higher-lying excited states. Thus, excessive excitation densities result in non-native ionic biliverdin species in phytochrome, which may contribute to the observation of structural changes in femtosecond X-ray crystallography [2].

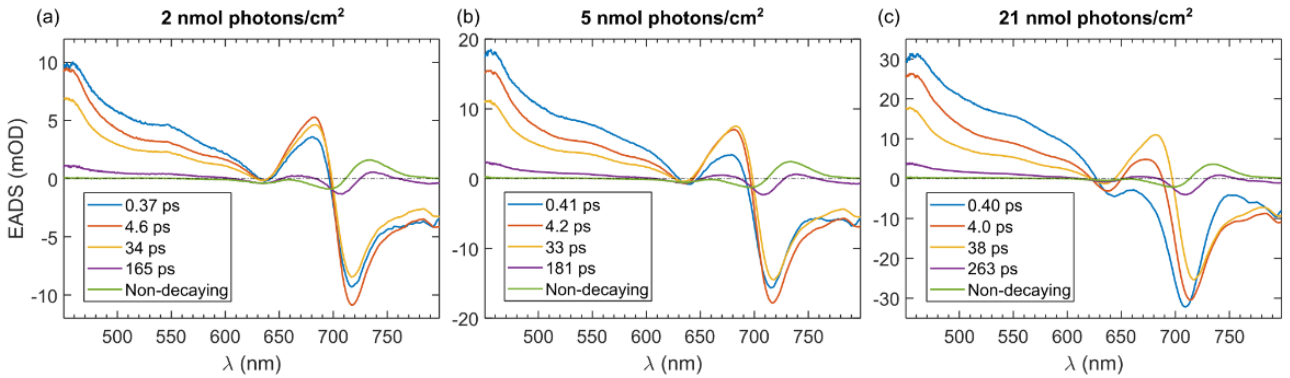

Fig. 1 Power-dependent global analysis results of transient absorption spectra of bacterial phytochrome.

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PHOTOINDUCED CHARGE TRANSFER NON-EQUILIBRIUM PROCESSES: THEORY AND MODELING STRATEGIES

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A wide range of photoinduced reactions and far from equilibrium processes are based on intra- and inter-molecular charge transfer. To date, modern vibrational and optical spectroscopies (such as Femtosecond Stimulated Raman, Transient spectroscopy, 2D techniques) [1] provide a plethora of data on ultrafast time scales (fs/ps) relating molecular motion and electronic density relaxation, although a detailed interpretation in terms of dynamics can be still difficult. In this context, the atomistic-level description, combining semiclassical theory, ab-initio dynamics, hybrid quantum/molecular mechanics potentials and accurate solvation methods, [2,3] can be mandatory for a reliable theoretical analysis of experimental insights. In this contribution, we investigate the electronic density and the nuclear reorganization following the photoexcitation, thanks to an accurate molecular modeling of the molecular dynamics. This protocol is critical to understand how nuclear motion can mediate the photo-dynamics of several photo-active systems, such as light-driven rotary molecular motors [4] and p-stacked charge transfer complexes [5] in solution. The relaxation channels of the photoexcited systems are unveiled thanks to a detailed time-resolved analysis of key activated vibrational modes [6,7]. The retention of temporal resolution of the analyzed modes is obtained via multiresolution Wavelet Transform. We are able in this way to disentangle the molecular deactivation pathways, characterizing the excited state vibrational relaxation also including the quantification of anharmonic couplings. The acquired knowledge about the photo chemical/physical features in excited electronic states can be employed to guide and modulate the rational design of photosensitive materials. Examples of applications will be discussed regarding charge transfer complexes, molecular rotors, photoacids in solution and protein-DNA photoinduced crosslinking models. Perspectives will be given as conclusions.

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MECHANISM OF THE LIGHT-INDUCED WATER OXIDATION REACTION OCCURRING IN THE NATURAL OXYGENIC PHOTOSYNTHESIS

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A key step in natural photosynthesis is the water-splitting reaction into molecular oxygen, electrons, and hydrogen equivalents. Understanding the molecular mechanisms behind this photoreaction will unravel the secrets of solar energy conversion in biochemistry and may inspire the design of artificial bio-mimetic materials for green energy production. Photosynthetic water oxidation occurs in the Mn_4Ca core of the Photosystem II complex where, through five subsequent steps of the catalytic cycle, four electrons are sequentially removed until the oxidation of two water molecules. Here, using multiscale atomistic calculations, we investigated the molecular mechanism of the O2 formation and release in Photosystem II. Our results, over the last ten years, provided a clear structural and energetic description of the different steps of the catalytic cycle,[1-5] finally identifying the slowest step in photosynthetic O_2 evolution consisting of a reactive oxygen radical formation occurring in a single-electron multi-proton transfer event.

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PRIMARY EVENTS IN REACTION CENTRE OF PHOTOSYSTEM II

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Photosystem II is a multi-subunit pigment-protein complex that utilizes sunlight to trigger charge-separation and catalyse water oxidation. The charge separation cascade is initiated in the reaction centre (RC), which is composed of six pigments (four Chlorophyll a and two Pheophytin a) arranged symmetrically along the D1 and D2 core poly-peptides (Figure 1). Biological evolution favoured productive electron transfer only along the D1 side with the precise nature of the initial excitation event(s) remaining under debate. In our work,[1-3] we employ multiscale quantum mechanics/molecular mechanics (QM/MM) coupled with high-level computations (full time-dependent density functional theory with range-separated functionals benchmarked against coupled cluster theory) to investigate the excited state profile of the RC (Figure 1). Our results describe at a fundamental electronic structure level precisely how differential protein electrostatics create the observed excitation asymmetry within the RC. By simultaneous quantum chemical treatment of multimeric pigment assemblies, we identify the critical pairs of RC pigments associated with low-lying charge-transfer states and we eventually propose a novel model to describe excitation of Photosystem II RC based on two parallel charge-separation pathways. Among others, our new model explains the triggering of charge separation by direct absorption of far-red photons (700-800 nm), i.e. beyond the known "red-limit" (680 nm) of oxygenic photosynthesis.

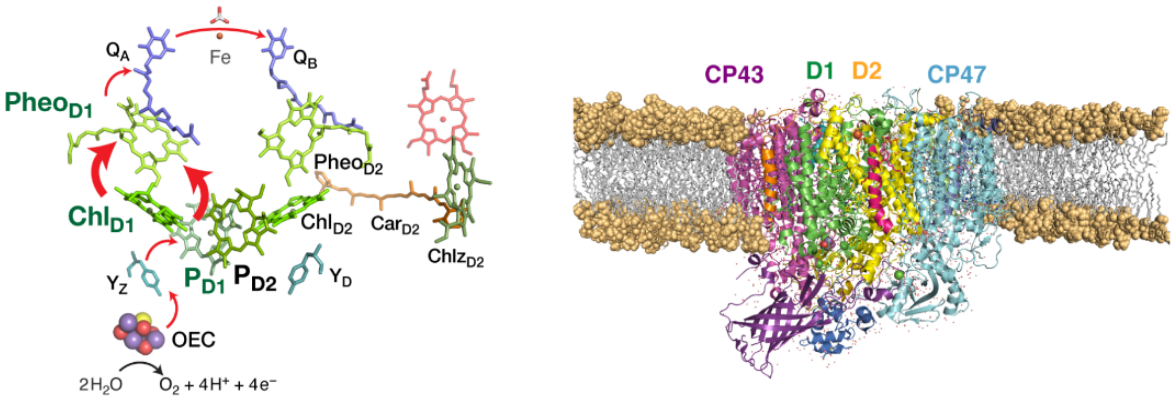

Figure 1. (*left*) Overview of symmetrically arranged pigments in the reaction centre along with key redox-active components of Photosystem II and (*right*) molecular-mechanics based model of the lipid-bilayer bound Photosystem II.

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DISPERSION INTERACTIONS AT THE EXCITED STATE: INFLUENCE ON LIGHT-RESPONSIVE PROPERTIES OF BIOSYSTEMS

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To understand the biological reactions of natural light-sensitive receptors, which are crucial for optogenetics, research focus on the targeted expression of specific microbial rhodopsins that undergo photochemical reactions upon light absorption, allowing precise control of ion currents across cell membranes and regulation of neuronal action potentials. [1,2] The relaxation dynamics of the initial excited state involved in the photoisomerization process of chromophores are highly influenced by their surrounding environment. [3] Since computational schemes exclusively accounting for the effect of the protein atomic charges can lead to a qualitatively wrong picture, [4] modeling a responsive protein environment is fundamental to successfully capture its tuning effects on the chromophore. However, our current knowledge of how dispersion affects excited chromophores interacting with the surrounding environment remains limited. [5]
In this talk, we introduce [6] a customized state-specific model designed specifically to investigate excited systems using a quantum/classical hybrid multiscale protocol [7] (Fig.1): the atomistic polarizable environment that includes dispersive interactions responds to the excited state electron density of the chromophore. We demonstrate the effectiveness of this innovative methodology by examining a chromophore in a solvent with low polarity and providing insights into the significant role of dispersion forces in modulating the light-induced properties of large biological systems, such as photosynthetic antenna complexes.

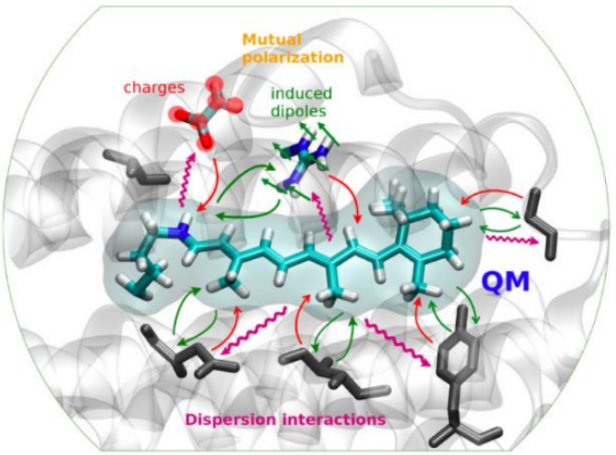

Figure 1: Schematization of responsive environment including mutual polarization and state-specific dispersion interactions.

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MULTISCALE MODELING OF GENETIC VARIANTS OF PHOTOSYSTEM II

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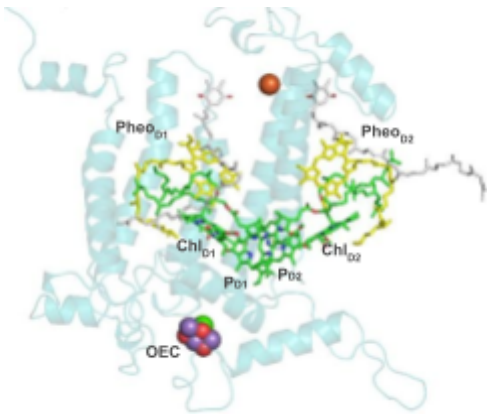
Photosystem II (PSII) is a protein-pigment complex that utilizes sunlight to catalyze water oxidation and plastoquinone reduction, thereby initiating the electron transfer chain in photosynthesis. Among the 17 transmembrane subunits in PSII, D1 and D2 are most important because they bind all the redox active components involved in primary charge separation and electron transfer, especially the Reaction Center (RC) and the Oxygen Evolving Complex (OEC).[1] Under high-light conditions, the abundance of molecular O_2 makes the D1 protein most prone to oxidative photodamage, and the repair cycle likely involves regulation at the genetic level. In the thermophilic cyanobacterium *T. elongatus*, the D1 protein is encoded by the *psbA* gene family (*psbA*$_{1–3}$). Interestingly, the *psbA* genes encode three different D1 isoforms which are expressed depending upon environmental conditions. [2-3] From a molecular perspective, a large number of these differences in D1 isoforms are present in close proximity to the active-branch RC pigments P_{D1}, P_{D2}, Chl_{D1} and $Pheo_{D1}$ (**Figure 1**). It is well established that excitonic asymmetry and light-induced charge separation in the RC arises exclusively due to local protein electrostatics and pigment-pigment interactions.[4] However, an atomistic description of how specific protein mutations might influence primary charge separation and electron transfer processes in the RC, remains incomplete. Towards this goal, we combine large- scale MD simulations with QM/MM calculations on the membrane-bound PSII monomer of each *psbA* variant (*psbA*$_{1–3}$).[5] We compare the optical properties (local and charge-transfer excitations) of RC pigments using TDDFT methods and identify which variable amino acid residues are responsible for specific effects on distinct pigments and pigment groups. This work provides the first atomistic description of how regulation at the genetic level can modulate protein electrostatics and influence primary electron transfer processes in photosynthetic reaction centers.

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psbA1|Chain_A    MTTTLQRRESANLWERFCNWVTSTDNRLYVGWFGVIMIPTLLAATICFVIAFIAAPPVDI   60
psbA2|Chain_A    MTTVLQRRQTANLWERFCDWITSTENRLYIGWFGVIMIPTLLAATICFVIAFIAAPPVDI   60
psbA3|Chain_A    MTTVLQRREQLNLWEQFCSWVTSTNNRLYVGWFGVLMIPTLLAATICFVIAFIAAPPVDI   60
                 ***.****:  ****:**.*:***:****:*****:**********************

psbA1|Chain_A    DGIREPVSGSLLYGNNIITGAVVPSSNAIGLHFYPIWEAASLDEWLYNGGPYQLIIFHFL   120
psbA2|Chain_A    DGIREPVSGSLLYGNNIITAAVVPSSNAIGLHLYPIWDAASLDEWLYNGGPYQLIIFHFL   120
psbA3|Chain_A    DGIREPVSGSLLYGNNIITGAVVPSSNAIGLHFYPIWEAASLDEWLYNGGPYQLIIFHFL   120
                 ********************.************:****:**********************

psbA1|Chain_A    LGASCYMGRQWELSYRLGMRPWICVAYSAPLASAFAVFLIYPIGQGSFSDGMPLGISGTF   180
psbA2|Chain_A    IGIFCYMGREWELSYRLGMRPWIPVAFSAPVAAATAVLLIYPIGQGSFSDGLMLGISGTF   180
psbA3|Chain_A    IGVFCYMGREWELSYRLGMRPWICVAYSAPVAAATAVFLIYPIGQGSFSDGMPLGISGTF   180
                 :*  *****:************* **:***:*:* **:************: *******

psbA1|Chain_A    NFMIVFQAEHNILMHPFHQLGVAGVFGGALFCAMHGSLVTSSLIRETTETESANYGYKFG   240
psbA2|Chain_A    NFMIVFQAEHNILMHPFHMLGVAGVFGGALFAAMHGSLVTSSLIRETTETESTNYGYKFG   240
psbA3|Chain_A    NFMLVFQAEHNILMHPFHQLGVAGVFGGALFSAMHGSLVTSSLIRETTETESANYGYKFG   240
                 ***:************** ************.*********************:*******

psbA1|Chain_A    QEEETYNIVAAHGYFGRLIFQYASFNNSRSLHFFLAAWPVVGVWFAALGISTMAFNLNGF   300
psbA2|Chain_A    QEEETYNIVAAHGYFGRLIFQYASFNNSRSLHFFLAAWPVVGIWFAALGISTMAFNLNGF   300
psbA3|Chain_A    QEEETYNIVAAHGYFGRLIFQYASFNNSRALHFFLAAWPVIGIWFTALGISTMAFNLNGF   300
                 ******************************:**********:*:**:**************

psbA1|Chain_A    NFNHSVIDAKGNVINTWADIINRANLGMEVMHERNAHNFPLDLA----------------   344
psbA2|Chain_A    NFNHSVVDAQGNVINTWADIINRANIGIEVMHERNAHNFPLDLASGELAPVAMIAPSIEA   360
psbA3|Chain_A    NFNHSVVDAQGNVINTWADIINRANLGMEVMHERNAHNFPLDLASAESAPVAMIAPSING   360
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Figure 1. (a) The D1 protein in PSII with associated RC pigments. (b) Comparison of *Psb*A1, *Psb*A2 and *Psb*A3 amino acid sequences from *T. elongatus*.

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TRIPLET ENERGY TRANSFER IN THE FENNA-MATTHEWS-OLSON (FMO) PIGMENT-PROTEIN COMPLEX

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This talk will discuss new experimental data and multiscale molecular modeling revealing the dynamics of the triplet energy transfer and dissipation in the Fenna-Matthews-Olson (FMO) pigment-protein complex. Triplet states generated in photosynthetic complexes present a hazard as they can readily generate highly-reactive singlet oxygen. Nature has developed mechanisms to safely dissipate triplet-state energy, such as incorporating carotenoids into photosynthetic complexes. However, the photoprotection mechanism in FMO that lacks carotenoids is still unknown. Our modeling shows that a possible photoprotection mechanism could be a lowering of the triplet state energy of BChl *a* pigments in FMO below the energy of singlet oxygen. An additional benefit of studying the triplet state dynamics lies in the fact that this allows us to mimic knock-out mutations without actually removing BChl *a* from the system. A pigment in the triplet state is effectively excluded from excitonically interacting pools of pigments. We have measured triplet state dynamics by time-resolved absorption/CD spectroscopy and used these data in conjunction with computational modeling to reveal the detailed excitonic structure of this protein and its mutants. The developed methodology provides structure-function relations for energy transfer in FMO and is generalizable to other photosynthetic complexes.

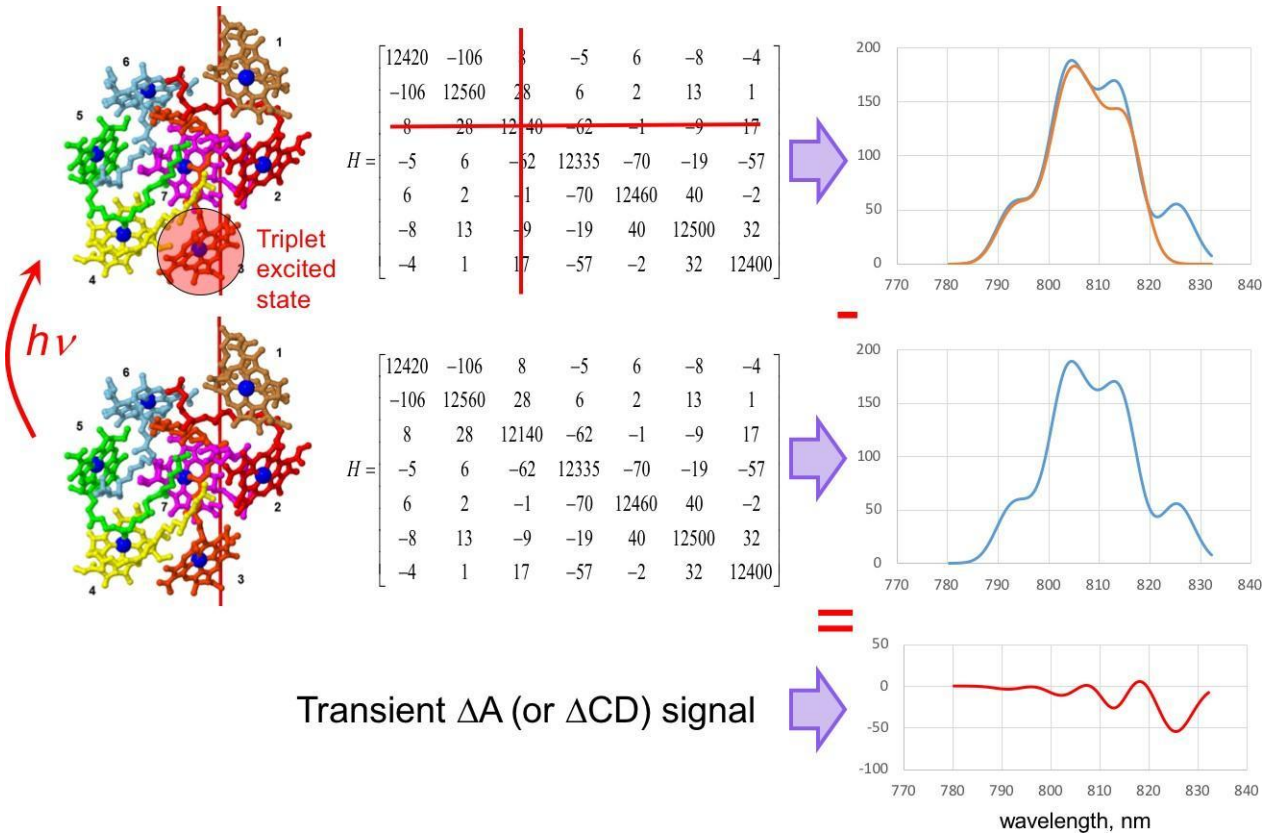

Figure 1. Schematic modeling of the excitonic absorption spectrum of the FMO complex when one of the BChl *a* pigments is in the triplet state.

THEORETICAL STUDY OF VIBRATIONAL-MEDIATED INTERLAYER CHARGE TRANSFER IN A COBALT PHTHALOCYANINE-GRAPHENE HETEROJUNCTION

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We employed time-dependent density functional theory to characterize the nature of the charge transfer (CT) process at the donor-acceptor interface between a localized thin layer of cobalt phthalocyanine (CoPc) and a graphene flake (Gr), placed onto an insulating Si 3 N 4 substrate (see Figure 1). By employing optimally tuned range-separated hybrid functional (OT-RSH) [1] we described excited energy levels of the CoPc - Gr which has complex open-shell nature. Two types of the states were found to be relevant: a low oscillator strength bright state (LB) that shows a marked π-π coupling with the substrate, and a CT state that has mostly intra-molecular character (involving electron density transfer to the Co atom). By using per-mode reorganization energy analysis [2,3] of the excited states, we shed light on the role of specific vibrational modes in dynamically coupling the molecule to the substrate. In particular we identified a set of molecular vibrations mainly responsible for driving the charge transfer at the interface.

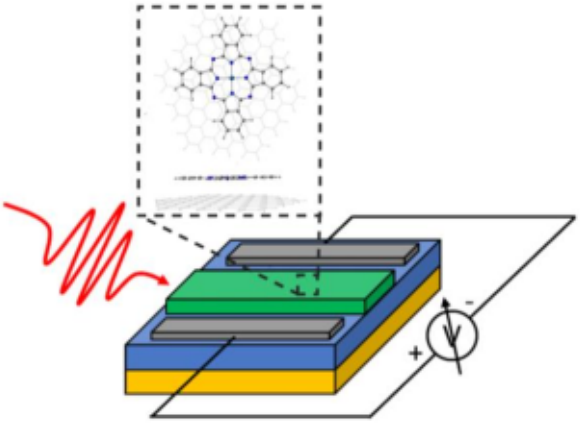

Figure 1. Pictorial representation of the CoPc-Gr sample. The molecules are localized in the central region, while the electrodes are in contact with the graphene layer, from which the signal is collected.

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Currents of Bacterial Life Probed by Molecular Simulation and Pump-probe Spectroscopy

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Nature has evolved remarkable biological structures that shuttle electrons over length scales of more than 10 micrometers. An intriguing example are multiheme cytochromes (MHCs) which arrange a large number of densely spaced redox-active c-type Fe-heme groups in wire-like chains within their protein frame. Experimental determination of heme-heme ET rates in these proteins has been a great challenge for many years because of the identical chemical nature of the cofactors. In my talk I will first review extensive molecular dynamics and DFT calculations that our group has carried out to predict electron transfer rates and electron fluxes through these intriguing structures.[1] I will then explain in detail recent pump-probe transient absorption measurements that our collaborators have carried out to determine ET kinetics experimentally [2,3]. In these experiments the protein was photosensitised by site-selective labeling with a Ru (II)(bipyridine)3 dye and the dynamics of light-driven electron transfer reported by a spectrally unique His/Met reporter heme. Both simulation and experiments show that heme-to-heme electron transfer rates in these proteins can reach magnitudes of up to 10^9 s^{-1} (4 Angstrom edge-edge distance). These rates are among the highest reported for ground-state electron transfer in biology. Yet, some fall 2 to 3 orders of magnitude below the Moser–Dutton ruler because electron transfer at these short heme-heme distances is through space and therefore associated with a higher tunneling barrier than the through-protein tunneling scenario that is usual at longer distances. We also find that
His/Met-ligated hemes create an electron sink that stabilizes the charge separated state (oxidized dye/reduced protein) on the 100-μs time scale. This feature could be exploited in future designs of multiheme cytochromes as components of versatile photosynthetic biohybrid assemblies.

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MULTISCALE MODELING OF PHOTO-INDUCED STEREOSELECTIVE RADICAL CYCLIZATION IN A FLAVOENZYME

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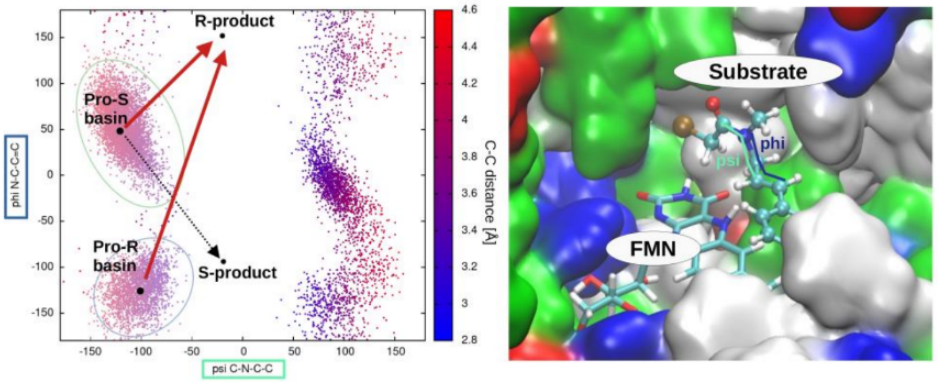
Light absorption is a powerful and efficient switch for chemical reactivity, allowing reactions that are precluded in the ground state. An emergent field in the in-vitro photo-activated reactions is the catalysis based on Electron-Donor-Acceptor-Complexes (EDACs). EDACs are transient species composed by two molecules, namely an electron donor and an electron acceptor, that undergo charge-transfer under light irradiation [1]. EDA-based catalysis can be easily coupled with enzymes, using the protein cofactor as a reaction partner. In this way, it is possible to exploit their intrinsic regio- and stereo-selectivity to perform chiral synthesis. Remarkable examples are Flavin mononucleotide (FMN) ene-reductases (ERs) because their cavity allows for a large substrate variability and the active charge-transfer excited states are accessible through visible-light. The system here considered is the best performing Gluconobacter Oxidans ER (GluER) mutant (T36A-K317M-Y343F) named "G6" that showed an enantiomeric excess (e.e.) up to 96:4 (R:S) and a yield of 92% for the radicalic cyclization of 2-chloro-N-cinnamyl-N-methylacetamide [2]. To rationalize this high enantioselectivity we studied the first steps of the reaction, i.e., the chlorine anion release after the charge-transfer (mesolytic cleavage) and the cyclization, by means of a multiscale approach based on the Perturbed Matrix Method, full quantum-mechanics (QM) gas-phase calculations and all-atom Molecular Dynamics (MD) simulations [3]. Our results indicate that the protein environment strongly favours both the mesolytic-cleavage and cyclization steps, in terms of free energy barriers. More importantly, we observed that the cyclization enantioselectivity strongly depends on the configuration of an internal degree of freedom of the amidic substrate, namely the C-N-C-C dihedral angle (psi): if it is negative, the R-product is favoured, while if it is positive, the S-product is favoured. In the studied G6 enzyme configurations with a positive psi angle are shown to be favoured, thus explaining the experimentally observed enantiomeric excess.



Representative structure of the active site (right) and possible distributions of the two diehdrals defining the prochirality of the cyclization (left).

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HOW SIMULATIONS UNCOVER THE PHOTOACTIVATION MECHANISM OF APPA BLUF

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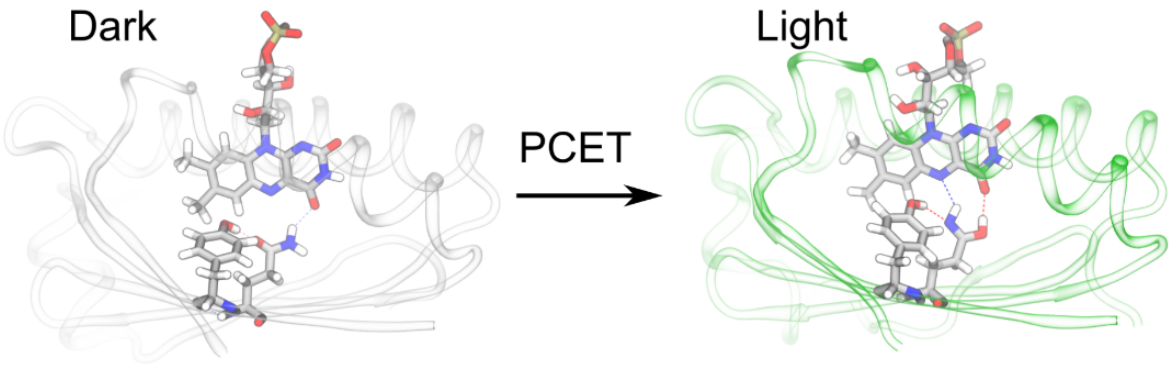
Several photoreceptor classes use flavin as a chromophore [1]. Among these, blue light-using flavin (BLUF) domains possibly have the most intriguing photoactivation mechanism, which results in no apparent chemical change to the flavin [2]. What is more, conflicting structures, differing in the active site composition, were resolved for the BLUF domain of the AppA protein, complicating the quest for an activation mechanism. Spectroscopic evidence and computational models supported a proton-coupled electron transfer (PCET) mechanism for BLUF domains, involving the conserved active site Tyr and Gln residues. Again, contrasting evidence was found for $AppA_{BLUF}$, leading researchers to consider it an exception [3], and to propose alternative mechanisms.

Here we show how integrated computational methods can solve these conundrums, and help uncover the photoactivation mechanism of $AppA_{BLUF}$. First, microsecond classical molecular dynamics (MD) simulations, combined with multiscale calculations of NMR, IR, and UV-Vis spectroscopic features allowed us to unequivocally establish the resting state structure of $AppA_{BLUF}$ among the conflicting hypotheses [4]. Secondly, we investigated the photochemical step with multiscale polarizable QM/MM MD simulations, revealing that AppA undergoes the PCET mechanism, resulting in tautomerization of the active-site Gln63 [5]. Finally, we explored the light-induced structure of AppA with MD simulations on the microsecond time scale, connecting the observed spectroscopic changes to the tautomerization of Gln63 [6]. This not only validated the proposed light-induced structure, but also revealed the subtle structural changes occurring in the BLUF domains after photoactivation.



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NONEQUILIBRIUM EXCITED STATE DYNAMICS OF PROTON-COUPLED ELECTRON TRANSFER IN BLUF PHOTORECEPTOR PROTEINS

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Blue light using flavin (BLUF) photoreceptor proteins are critical for the light regulation of many physiologically important processes. Photoexcitation of the flavin chromophore induces proton-coupled electron transfer (PCET), as well as conformational changes that propagate and drive distal chemical or physical changes. In the Slr1694 BLUF photoreceptor, photoexcitation to a locally excited state within the flavin instigates electron transfer from a tyrosine to the flavin, followed by proton transfer from this tyrosine to the flavin and then a reverse PCET that produces the light-adapted signaling state. Excited state quantum mechanical/molecular mechanical (QM/MM) molecular dynamics simulations using time-dependent density functional theory elucidate the complete photocycle and the roles of protein dynamics, conformational changes, and electrostatics. After initial photoexcitation, protein reorganization drives electron transfer from the tyrosine to the flavin [1], followed by sequential double proton transfer from tyrosine to the flavin via the intervening glutamine [2]. The imidic acid tautomer of the glutamine generated by this forward PCET rotates to allow a reverse PCET that retains this tautomeric form. In the resulting purported light-adapted state, the glutamine tautomer forms a hydrogen bond with the flavin carbonyl group [3]. Ensemble-averaged QM/MM calculations of the dark-adapted and purported light-adapted states demonstrate that the light-adapted state with the imidic acid glutamine tautomer reproduces the experimentally observed red shifts in the flavin electronic absorption and carbonyl stretch infrared spectra for the light-adapted state [4]. These simulations provide insights into the nonequilibrium dynamics of photoinduced PCET in the BLUF photocycle as well as the nature of the elusive light-adapted state.

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HOW THE PH CONTROLS PHOTOPROTECTION IN THE LIGHT-HARVESTING COMPLEX OF MOSSES

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In response to varying light conditions, light-harvesting complexes (LHCs) switch from a light-harvesting state to a quenched state to protect the photosynthetic organism from excessive light irradiation in a strategy known as nonphotochemical quenching (NPQ). NPQ is activated by an acidification of the thylakoid lumen, which is sensed directly or indirectly by the LHC, resulting in a conformational change of the complex that leads to the quenched state. The conformational changes responsible for NPQ activation and their connection to specific quenching mechanisms are still unknown. Here we present a multistep computational protocol to investigate the pH-triggered conformational changes in the light-harvesting complex stress-related (LHCSR) of mosses[1]. By combining constant-pH molecular dynamics and enhanced sampling techniques, we find that the pH sensitivity of the complex is driven by the coupled protonation of three key residues (i.e., E114, E227 and E233) modulating the conformation of the short amphipathic helix placed at the lumen side of the embedding membrane. Combining these results with quantum mechanics/molecular mechanics calculations, we show that the quenching mechanism sensitive to the pH goes through a charge-transfer (CT) between a carotenoid (L1-Lut) and an excited chlorophyll (*a*Chl 612), which is controlled by the protein conformation (Figure 1).

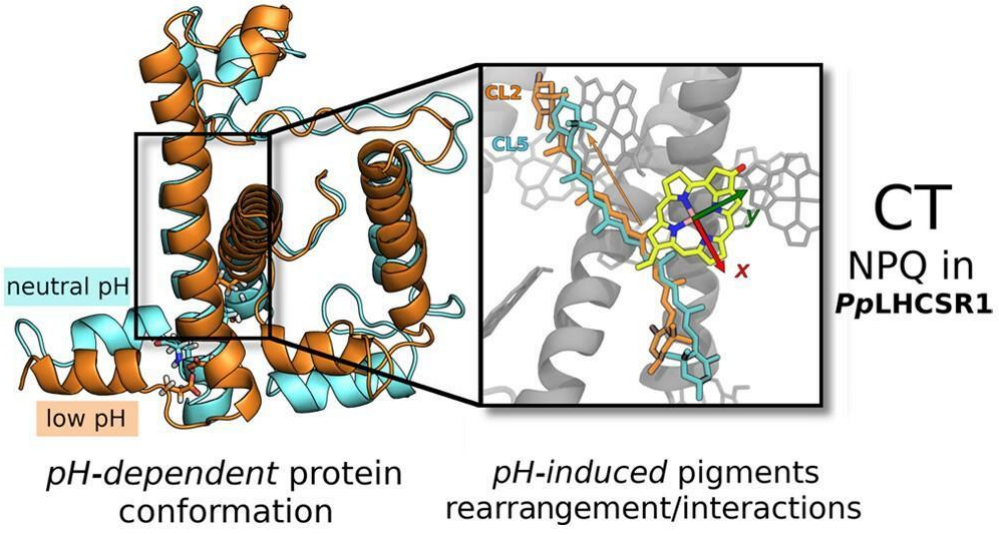

Figure 1. pH-dependent "soft switch" for increasing/decreasing the amount of quenching in LHCSR1. (left) Conformations are explored at low pH (orange cartoons) that are not accessible at neutral pH (cyan cartoons), suggesting that a pH drop can exert a profound influence on the conformation. (right) A CT-based quenching mechanism may be tuned through conformational changes (low pH) located near helix D inducing a displacement of lutein in the site L1 toward the stroma.

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OPTOGENETIC TOOLS TO CONTROL CHARGE TRANSFER WITHIN BACTERIA

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Many bacterial species are naturally capable of transferring electrons to external surfaces, leading to long-distance electron transport and the formation of electrically conductive films of cells on solid substrates. *Shewanella oneidensis* is one such exoelectrogenic organism, which uses a network of multiheme c-type cytochromes to transfer electrons from the cellular interior to electrodes, metals, or even adjacent cells. Our work has implemented the tools of optogenetics to control such electron transfer within bacteria. Strategies for light-induced gene expression were adapted for use in *Shewanella oneidensis*, resulting in optical control of current generation within cell biofilms. Light was used to pattern cells grown on top of electronic devices, improving the interface between biological and inorganic components and leading to fundamental insights into bacterial electron transfer pathways. Our group has also utilized such electron transfer pathways for the biogenic synthesis of inorganic nanomaterials, including photoactive quantum dots. Engineering live cells to produce quantum dots enables new approaches for light-induced charge transfer in living systems, as excited electrons could be directed towards electron transfer pathways and enzymatic reactions.

LIGHT INDUCED CHARGE TRANSFER FOR ENHANCED PHOTODYNAMIC THERAPY ACTION

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The medical techniques based on the use of light for activating the drug are occupying a prominent place in the cancer treatment due to their selectivity that contributes to reduce undesirable side effects of conventional chemotherapy. Among these therapeutic treatments, photodynamic therapy (PDT) and photoactivated chemotherapy (PACT) are emerging as complementary approaches for selective destruction of neoplastic tissue through direct cellular damage. Both techniques rely on the employment of a molecule, photosensitizer PS, able to absorb within the so-called therapeutic window. Thus, the exposure to light of otherwise inert molecules promotes the population of excited states of the drug, that in PDT are able to produce the cytotoxic species, such as $^1O_2$ and other ROS, in PACT are deputed to the active species release or formation. Many photosensitizers were proposed and explored as anticancer agents for applications in different medical approaches in order to improve their chemical, biological and photophysical properties, including organic molecules as well as metal complexes particularly important for PACT application [1]. In this field, also cofactors like riboflavin, essential for numerous reactions catalysed by flavoproteins, including activation reactions of other vitamins, could play a crucial role, as it could able to promote the photoactivation of well-designed molecules to be inert until they reach the target [2].
Depending on the chemical structure, beside the photogeneration of cytotoxic species, the action as anticancer drugs of the photosensitizers can include reversible DNA interactions by intercalation. The establishment of non-covalent π-π stacking interactions between the drug and two base pairs in the DNA major and minor groove can alter the cellular functions ultimately lead to cell death. DNA intercalative interactions of aromatic moieties are usually associated with both bathochromism and hypochromism of the absorption bands thanks to the charge transfer from DNA to the drug [3-5], leading to a possible shift of the drug absorption band within the therapeutic window, thus enhancing PDT efficacy. In this field, several crucial characteristics of candidate PSs can be accurately predicted from first principle [3-7], contributing to the understanding of the entire photochemical pathways involved that can help in improving the efficiency of PS.

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UNRAVELING THE MECHANISM OF TIP-ENHANCED MOLECULAR ENERGY TRANSFER

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Electronic Energy Transfer (EET) between chromophores is fundamental in many natural light-harvesting complexes, serving as a critical step for solar energy funneling in photosynthetic plants and bacteria. The complicated role of the environment in mediating this process in natural architectures has been addressed by recent scanning tunneling microscope (STM) experiments involving EET between two molecules supported on a solid substrate [1]. These measurements demonstrated that EET in such conditions has peculiar features, such as a steep dependence on the donor-acceptor distance, reminiscent of a short-range mechanism more than of a Förster-like process. By using state of the art hybrid *ab initio*/electromagnetic modeling, here we provide a comprehensive theoretical analysis of tip-enhanced EET. In particular, we show that this process can be understood as a complex interplay of electromagnetic-based molecular plasmonic processes, whose result may effectively mimic short range effects. Therefore, the established identification of an exponential decay with Dexter-like effects does not hold for tip-enhanced EET, and accurate electromagnetic modeling is needed to identify the EET mechanism.

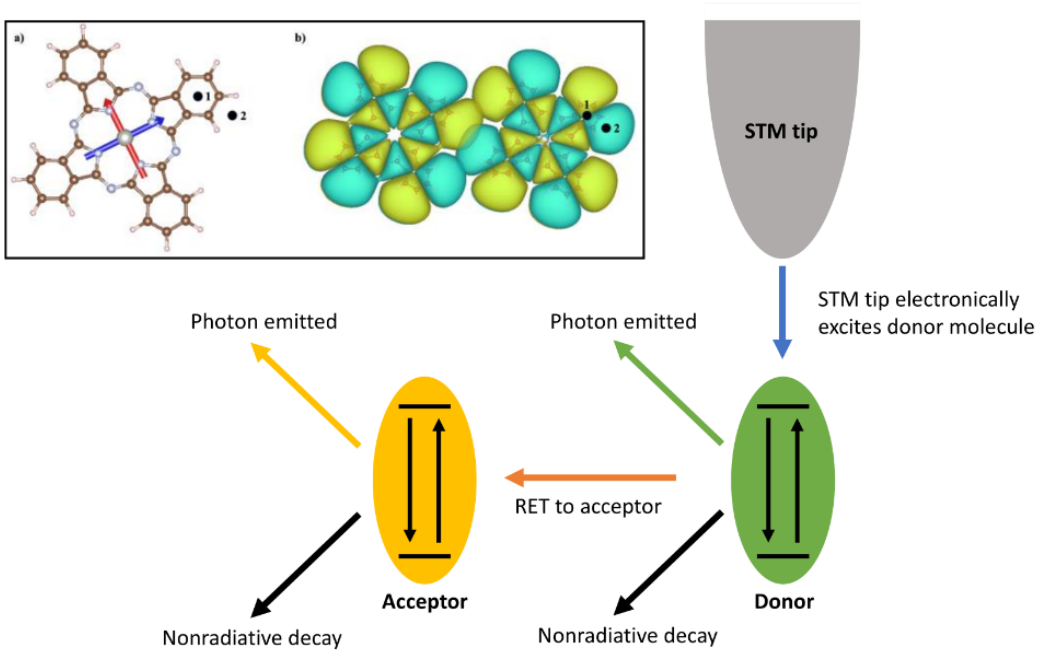

Figure 1: Schematic diagram of energy transfer processes in the tip-donor-acceptor system studied. (a) Transition dipoles of the donor molecule when excited by a tip above point 1. (b) Computed HOMO density of both donor and acceptor molecules, displaying overlap between the two.

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MODELLING PLEXCITONIC STATES WITH SINGLE-MOLECULE RESOLUTION

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Plexcitons are light-matter states that can originate from the hybridization of plasmon and molecular excitations, in presence of an efficient interaction that couples the individual subsystems [1,2]. Besides the strength of interaction, a key factor is represented by the energy difference between the states that are to be coupled. The formation of plexcitonic states give rise to characteristic optical responses, such as a Rabi splitting in the linear absorption spectrum of the overall system.
In a previous work, a supramolecular plexcitonic system has been prepared and characterized, showing two Rabi-splitted absorption bands, which can be related to the hybridization of the plasmon resonance with the J-type excitonic state of a molecular aggregate and with a single-molecule transition [3]. The latter evidence appears particularly interesting, as aggregates usually appear to be best suited in achieving an efficient coupling with the plasmon, due to their larger dipole moment.
Therefore, we decided to investigate further this peculiar signature of plexcitonic coupling. Besides improving our understanding of the experimental results, we also wanted to address the fundamental question of the local contribution of each individual excitation to the resulting delocalized hybrid states. We tried to keep the highest possible detail within the molecular subsystem, by explicitly taking into account all plasmon-molecule and molecule-molecule interactions.
Our results clearly showed that differences in the position of molecules around the nanostructures, and therefore in the alignment with the energetically closer plasmon mode, are reflected in the different local contributions to the delocalized plexcitonic hybrids. In other words, the molecular character of the letters is not uniformly distributed over a blurred, structureless entity. Moreover, since some extent of plasmon hybridization through nanoparticle association is required to approach resonance with the molecular transitions, nano-sized gaps are confirmed as the most relevant sites [4]. Finally, we demonstrated that even highly detuned molecular excitations can be combined in the resulting plexcitonic states: this means that, through coupling with a common plasmon mode, different molecular levels can be effectively coupled in a common photophysical dynamics.

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PREDICTIVE METHODS FOR SIMULATING CHARGE TRANSFER AND REDOX PROCESSES IN PROTEINS

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Simulating charge transfer and redox processes in proteins is a challenging task as multiple factors, such protein structural fluctuations, specific short-range and long-range electrostatic interactions, should be reliably taken into account. Moreover, advanced computational studies require initial mechanistic input to identify the most electronically important part of the system that directly participates in the process and should be treated quantum-mechanically. In this talk I will discuss the models and software that allow for (i) identification of the efficient electron transfer pathways in proteins based on their crystal structure, and, thus, can serve as input for more advanced simulations; and (ii) qualitative description of observables such as redox potentials or charge transfer rate constants. I will introduce eMap software (https:emap.bu.edu) that predicts efficient electron transfer pathways in proteins using their crystal structure as an input [1]. Once the key players of redox or electron transfer processes are identified the next step is qualitative evaluation of observables that can be directly compared to the experimental observables such as redox potentials or electron transfer rates. I will show that the environment polarization can be crucial for the accurate evaluation of the energies of charge-transfer states and vertical ionization/attachment energies needed to compute redox potentials. By considering a plant cryptochrome protein I will show that the QM/polarizable MM based protocol is capable of accurate characterization of redox [2] and charge transfer processes in proteins.

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UNCOVERING A CAROTENOID QUENCHER STATE IN THE CP29 LIGHT-HARVESTING COMPLEX OF PLANTS

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Light-harvesting complexes (LHCs) of oxygenic photosynthetic organisms, such as plants and algae, are pigment-protein assemblies serving a dual function in photosynthesis. Specifically, under low-light conditions LHCs absorb sunlight and transfer the excitation energy to the reaction center, whereas in high-light situations they switch to a quenched state, dissipating the excess of the absorbed energy as heat to avoid photodamage. While the processes underlying their light-harvesting function are now rather well understood, the quenching mechanisms of LHCs are still under debate. In CP29, a minor LHC of plants (Figure 1), spectroscopic investigations have recently proposed that quenching mainly occurs via excitation energy transfer from specific chlorophylls to a carotenoid S^* state [1,2]. Although S^* was attributed to a specific carotenoid [1], namely lutein, the detailed nature of this state is still unclear.
To uncover the carotenoid quencher state S^*, we performed simulations of the nonadiabatic excited-state dynamics for lutein in CP29. In these calculations, we used a semiempirical quantum mechanics/molecular mechanics (QM/MM) scheme and the surface hopping method [3]. Our simulations indicated that one carotenoid conformation (s-trans) has the characteristics of the spectroscopic S^* state: (i) a shorter excited-state lifetime, and (ii) a blue-shifted excited-state absorption peak, compared to the dominant carotenoid conformer (s-cis). These findings allow us to better understand the nature of the quencher state S^* in CP29 and to associate it with a specific carotenoid conformation.

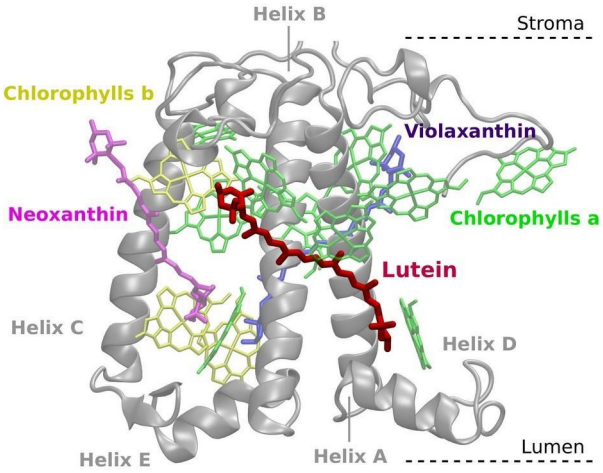

Figure 1: Side-view of the CP29 light-harvesting complex.

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SIMULATING ACTION-2D ELECTRONIC SPECTROSCOPY FROM MOLECULAR DIMERS TO PHOTOSYNTHETIC ANTENNAS

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Action-2D Electronic Spectroscopy is emerging as a powerful technique to probe exciton and charge transfer in molecular aggregates and nanostructures. While highlighting the coherent dynamics induced by the interaction with four laser pulses, action detection relies on measuring an incoherent signal proportional to excited-state populations (Fig. 1a), i.e., fluorescence [1] or photocurrent [2], paving the way for studying systems in *operando* conditions. Despite its advantages, the origin of certain spectral features still remains ambiguous, i.e., cross peaks at early waiting-time [3] and incoherent mixing [4], calling for the aid of numerical simulations.

We set up and test a numerical protocol to simulate the action response of weakly-interacting chromophores using a non-perturbative treatment of the light-matter interaction [5]. Starting from a molecular dimer, we establish the correspondence between the optical response and a kinetic scheme for populations in the one- and two-particle representations [6]. On this basis, we propose a unified framework that reconciles incoherent mixing with the theory of non-linear optical response in the presence of exciton-exciton annihilation or other multi-exciton interactions. Then, we address the scaling of incoherent mixing with the system size, pointing out how these contributions may completely hide coherent features in the case of large photosynthetic systems.

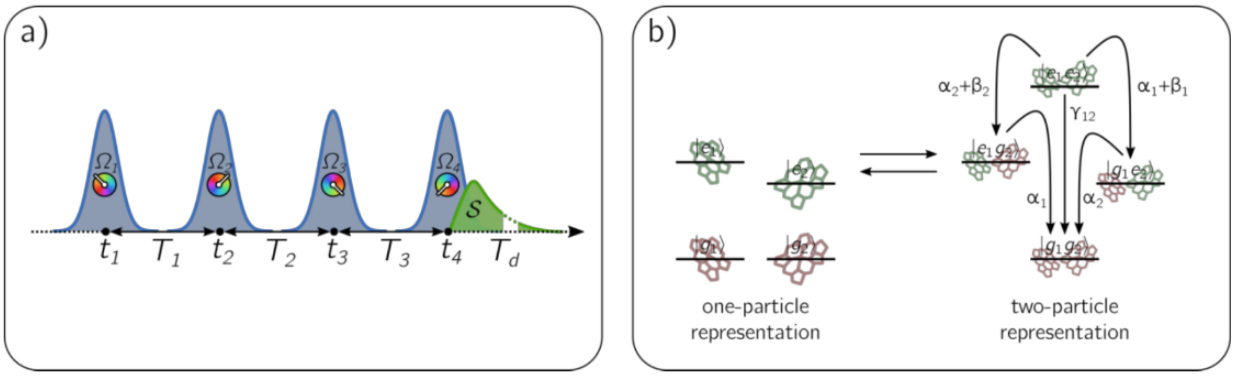

Figure 1: a) Schematic representation of a train of four laser pulses (blue) and the emitted incoherent signal (green). b) One- and two-particle representations of a molecular dimer.

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UNVEILING THE PHOTOCATALYTIC REDUCTION OF PLATINUM(IV) COMPLEXES BY RIBOFLAVIN: INSIGHTS FROM COMPUTATIONAL ANALYSIS

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The ability of riboflavin (Rf) and its derivatives to photocatalyze $1e^-$ and $2e^-$ organic redox reactions is well acknowledged in the literature.[1] The oxidative triplet excited state of Rf can promote efficient electron transfer from substrates and electron donors to ultimately afford oxidation and reduction products. Recently, Salassa and co-workers have demonstrated that flavins (FL), like riboflavin, and flavoproteins are able to catalyse the reduction of Pt^{IV} complexes under light irradiation, releasing clinically-approved cisplatin and carboplatin.[2] In comparison with metal catalysts, biogenic organic photocatalysts are more attractive for *in vivo* applications with excellent biocompatibility.
With the use of density functional theory (DFT), the flavin-catalyzed reduction mechanism has been investigated. Firstly, the hydride-transfer mechanism between NADH and riboflavin in both dark and under light irradiation has been investigated. Next, the reduction mechanism of Pt(IV) complexes with the reduced Rf has been studied and the reaction has been compared with other reducing agents such NADH and ascorbate in order to establish the conversion efficiency respect to the use of classic bioreductants.

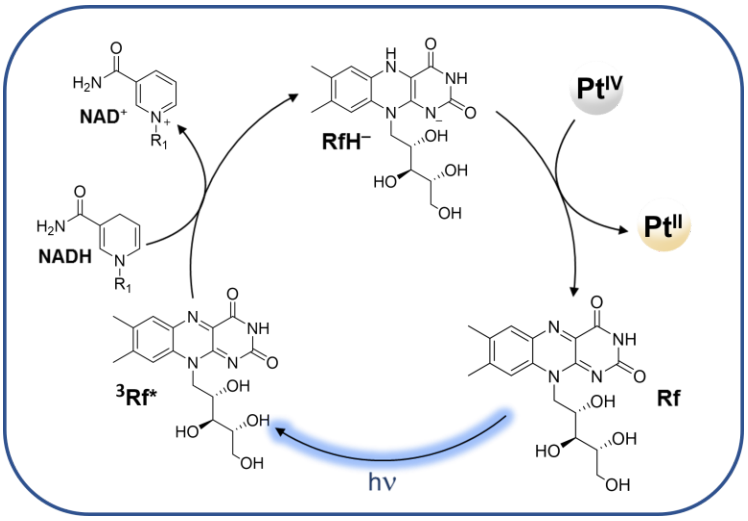

Figure 1: Mechanism of photocatalytic activation of Pt(IV) prodrugs by flavins

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INSIGHT INTO THE PHOTOCHEMISTRY OF CYANOBACTERIOCHROMES BY QM/MM SIMULATIONS

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Cyanobacteriochromes (CBCRs) are recently discovered light-sensitive proteins which are members of the phytochrome superfamily.[1] Like phytochromes they bind a linear tetrapyrrole as a chromophore but in contrast to their complex protein architecture, CBCRs require only the GAF domain for their function. There are at least four subfamilies known in CBCRs that are distinct by their photochemical reactivity and the color of the absorbed light. In this contribution, I will address the red/green CBCRs, which have a red-absorbing reactant and a green-absorbing photoproduct state. Two aspects of their photochemistry will be covered.

The first part, I will address the origin of the spectral tuning from red to green. Using QM/MM simulation of the spectra and subsequent analysis of the results a ring twist was identified that is shortening the conjugation length of the chromophore in the green absorbing state.[2]

In the second part, I will address the photoisomerization mechanism, which occurs upon light illumination. The chromophore in the red/green CBCR undergoes a reversible photoisomerization around the C15=C16 double bond with a concomitant rotation of the D-ring. However, we found that the structure of the protein is heterogeneous.[3] Therefore, various products are formed with different structural characteristics. Further, we have studied the photoisomerization mechanism in chromophore models which are limited to two pyrrole rings. This truncation allowed us to apply XMS-CASPT2 level of theory to optimize each point of the relaxed scan to derive the mechanism of the double bond isomerization.

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MECHANISM OF FLUORESCENCE ENHANCEMENT IN RHODOPSIN OPTOGENETIC REPORTERS

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The imaging of neural activity requires bright fluorescent probes localized in the neuron membrane. Rhodopsins are membrane proteins that can be expressed in neurons and used for triggering, silencing and reporting on neuronal action potentials [1]. The prototypical fluorescent reporter is Archaerhodopsin-3 (Arch3), an archaeal rhodopsin from Halorubrum Sodomense [2]. The fluorescence of Arch3 is extremely dim; its quantum yield (FQY) of ca. $1.1 \cdot 10^{-4}$ is almost four order of magnitude lower than that of the green fluorescent protein [3]. In order to achieve better reporters, Arch3 has been engineered via directed evolutionary approaches and random mutagenesis. These activities culminated in the discovery of a set of variants with improved FQY such as the QuasArs [1], Arch5 and Arch7 [4]. These variants feature a brighter fluorescence enabling applications in living mammals and invertebrates [5]. It has also been shown that the enhanced fluorescence originates from a one-photon excitation and must therefore come from the dark-adapted (DA) state and not from excitation of a photocycle intermediate [1]. However, the FQYs are still in the range of 10^{-3}-10^{-2} and therefore not yet as bright as desirable. Here we report on a computational model that shows how one-photon FQY is controlled by the protein electrostatic field, and therefore by the sequence of specific Arch3 variants (**Figure 1A**). We also show that, to be exploited in practical predictive calculations, the model entails the characterization of the photoisomerization channel (i.e. a sloped conical intersection with a nearby intermediate) of each variant (**Figure 1B**) and, therefore, of transient molecular structures that can be promptly computed, but whose systematic experimental study is presently impossible [5].

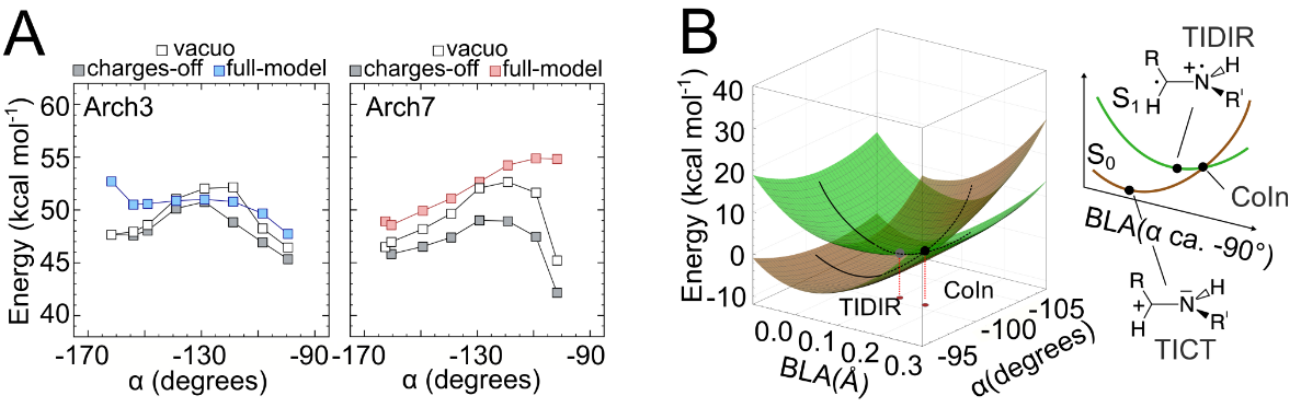

Figure 1 (A) Effect of the opsin charges on the isomerization energy profile for Arch3 (left) and Arch 7 (right). (B) Schematic representation of the Conical Intersection (CoIn) region of Arch3 including the twisted diradical intermediate (TIDIR) along the relevant components of the reaction coordinate.

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TRANSIENT INTERMEDIATES IN A BACTERIOPHYTOCHROME PHOTOCYCLE REVEALED BY MULTISCALE SIMULATIONS

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Phytochromes are photoreceptors responsible for sensing red and far-red light in plants, fungi, and bacteria[1,2]. They can switch between the resting state (Pr) and the biologically active state (Pfr), which differ in both chromophore stereochemistry and protein structure (Figure 1). Their photoactivation is initiated by the photoisomerization of an embedded chromophore, which triggers large conformational changes in the entire protein[3]. Despite decades of studies[4-7], a comprehensive understanding of the photoactivation mechanism of phytochromes is still far from being achieved.

In previous computational work[7], we obtained atomistic details on the photoactivation mechanism of *Deinococcus radiodurans* bacteriophytochrome through an integrated multiscale approach of nonadiabatic and adiabatic molecular dynamics and IR spectroscopy simulation. In particular, we showed the ps-timescale mechanism of chromophore photoisomerization and how a histidine residue can control the kinetics of the process. The initial photoproduct evolves rapidly into an early intermediate (early Lumi-R) that relaxes on the ns-to-μs scale to a late intermediate (late Lumi-R), characterized by a more disordered binding pocket (Figure 1).

The transition to the Meta-R intermediate occurs on a timescale of tens of microseconds[5,6]. Therefore, we relied on enhanced-sampling methods to investigate such a mechanism and the role of the environment. Our simulations show how the stability of our putative Meta-R is strongly influenced by the interactions between the chromophore and the nearby residues.

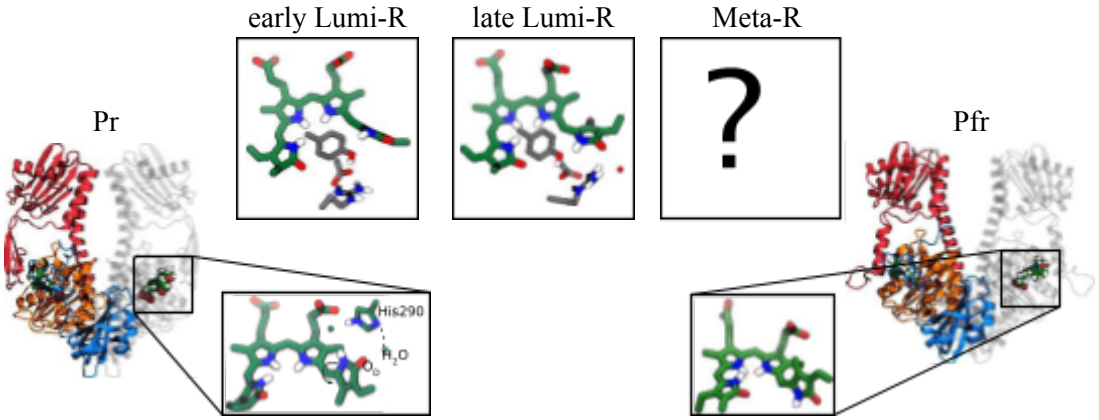

Figure 1 Representation of the Pr and Pfr photoproducts, with a zoom on the chromophore. The structures of the chromophore and the nearby residues for the early and late Lumi-R intermediates are reported (top panels).

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SIMULATING 2D SPECTROSCOPIC RESPONSES OF OPTICAL SYSTEMS WITH DIGITAL QUANTUM COMPUTERS

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2D electronic spectroscopy techniques have proven to be an effective method for tracking ultrafast coherent and incoherent processes in energy and charge transport in biological complexes or artificial materials [1]. Because of the high information content of the spectra, numerical simulations of the material response are of primary importance to assist the interpretation of the spectral signatures [2]. The complexity of the investigated systems, often comprising an overwhelming number of degrees of freedom, and of their quantum dynamics calls for novel simulation strategies. For this reason, in this talk we will discuss the opportunity of using digital quantum computers for the simulation of the optical response of a non-linear spectroscopy experiment [3] (Figure a). Quantum computers are emerging technologies harnessing quantum coherence for computational purposes. Simulating physical (inherently quantum) systems is one of the founding ideas and among the most promising fields of application where quantum computers are considered to be potentially disruptive [4]. Our approach merges quantum dynamical simulation [5] with the double-sided Feynman diagram representation of non-linear response theory [6], which represent the 4-time correlation function of the dipole moment. We exploit the similarity between the diagrammatic approach and quantum circuit representation [7] to devise a dedicated algorithm for the simulation of the optical response (Figure b). The output of the computation is a time signal whose Fourier transform is a map giving us spectroscopic information of the system (Figure c). The potential quantum advantage stems from the possibility of explicitly including the environment in the computation of large chromophore systems, unleashing the power of quantum computing.

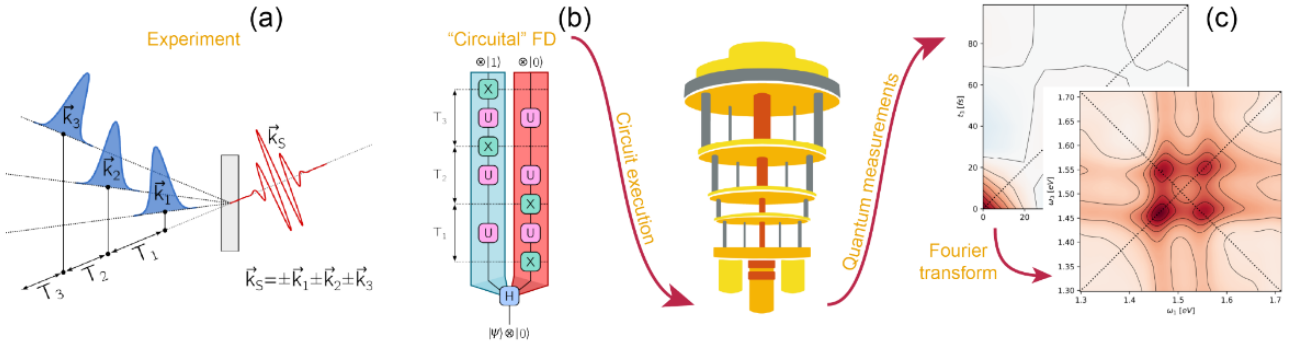

Figure: Pictorial representation of the workflow: from target experiment (a), to theory (b) and simulation (c).

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ELECTROSTATIC TUNING MAPS AND AVERAGE PROTEIN CONFIGURATIONS: TOOLS TO AID IN STUDYING FLAVOPROTEINS

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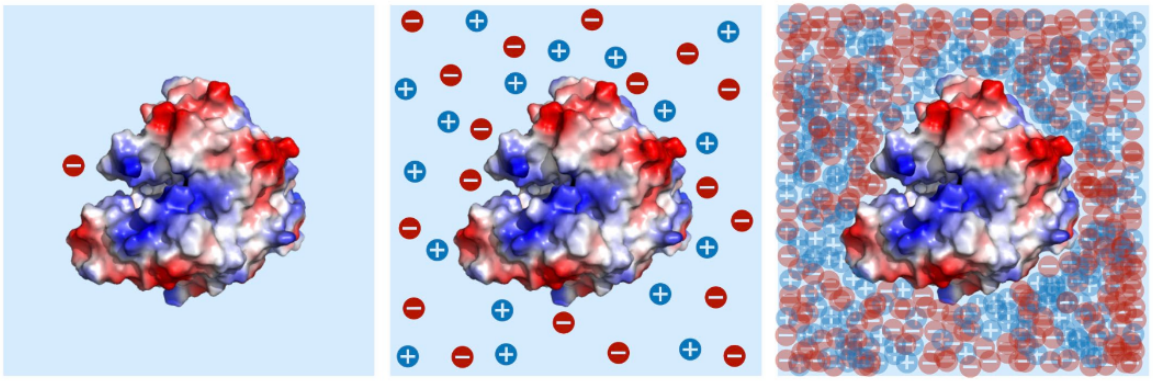
Abstract

Flavins can undergo photoredox, proton-coupled electron transfer, fluorescence, intersystem crossing, and/or photochemical reactions, depending on the protein hosting the flavin. We are missing a fundamental understanding of how a protein tunes the excited-state properties and chemistry of flavin so that it selectively undergoes some of those processes in different systems. We are developing and applying computational tools to study the spectroscopy, photophysics, and photochemistry of flavins. I will introduce two such tools we have been using: Electrostatic tuning maps [1-2] and average protein (or solvent) electrostatic configurations (ASEC). I will also describe their recent application to biological systems. In the first application [3], we studied how introducing a charge embedded in the active site of an enzyme affects the UV-visible absorption spectrum of the bound flavin cofactor. In the second application, we investigated spectral tuning mechanisms of the flavin-binding fluorescent protein iLOV [4]. In the third application, we study the effect of solvation on the ionization energies of bio-mimetic molecular switches.[5] In all three applications, we find that ASEC is well suited to capture the effect of long-range electrostatic interactions in an averaged way (see figure).



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