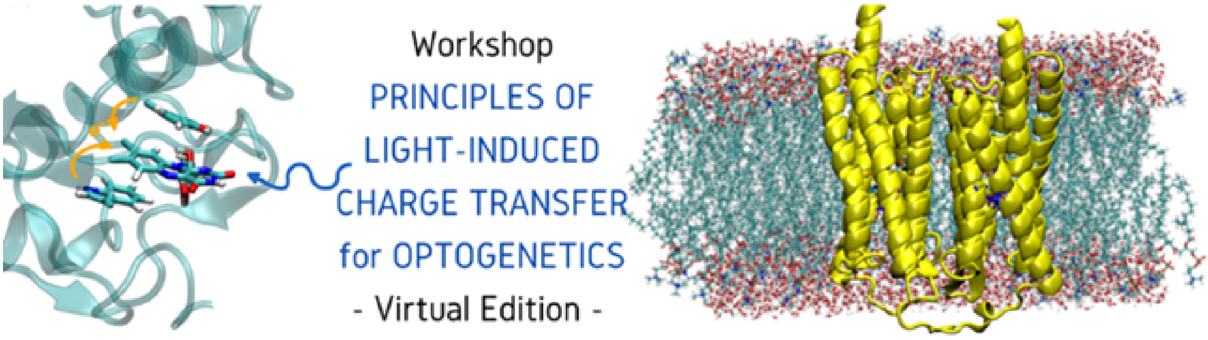

Principles of light-induced charge transfer for optogenetics

CT4OPTO REPORT

June 14-16, 2021

Document information

Workshop acronym:	CT4OPTO
Workshop full title:	Principles of light-induced charge transfer for optogenetics
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Disclaimer:

SUMMARY

INTRODUCTION

The "**Principles of light-induced charge transfer for optogenetics**" workshop was held online from June 14 to June 16, 2021.
The workshop focused on the discussion of state-of-the-art theoretical approaches to treat photoinduced charge transfer processes in light-sensitive proteins that are currently exploited in optogenetic tools. Attention was also devoted to the experimental probing of such events.
Leading scientists from the fields of excited state properties of biological matter and charge transfer processes in proteins, and in particular in rhodopsins and flavin-containing domains, shared and discussed their theoretical and experimental approaches.
Current optogenetic challenges were faced from a chemicophysical perspective, highlighting the main achievements and open questions in this timely and stimulating research field.

1. TOPICS

A number of natural photoreceptors, in which a chromophore is bound to a protein, utilize light absorption for the regulation of many biological processes.
Recent advances in the understanding of photo-activated proteins have enabled researchers to control biological signaling with unprecedented spatial and temporal resolution. The field of optogenetics exploits these photoreceptors using light and genetic engineering to systematically target and manipulate cellular systems in living organisms controlling physiological processes.
Optogenetic approaches are currently receiving a huge interest as promising tools for neuroscience, and the understanding of the molecular mechanisms of photo-activated proteins is a prerequisite for the functional design of optogenetics tools in the future.
Photoinduced charge transfer reactions are key steps along the receptor photocycle, and their investigation was the focus of this workshop.
Main **CT4OPTO topics** were:

- Optical properties: A crucial point for the development of optogenetic tools is the tuning of their light sensitivity range. The investigation of the optical properties of photoactive proteins is therefore of paramount importance, as well as the comprehension of the variability, and possible tuning, of light absorption depending on the environmental conditions.
- Excited state dynamics: Photoinduced charge transfer processes occur out of equilibrium, and the significant changes induced by the photoexcitation in both the electronic and nuclear structure of the reactants have to be considered to model the system dynamics. Strategies and challenges in the theoretical investigation of the excited state dynamics of complex systems were therefore discussed.
- Proton and electron transfer reactions: Theoretical approaches to treat ET, PT and proton coupled electron transfer (PCET), based both on purely quantum and on QM/MM methods, were discussed. Experimental techniques to investigate the thermodynamics and kinetics of charge transfer events in photoreceptors were presented. The investigation of the protein role and response to charge transfer was also addressed.
- Photoreceptors of current optogenetic interest: The first and most widely used optogenetic tools are light-controlled rhodopsins. More recently, blue-light photoreceptors with flavin chromophores, like LOV (Light Oxygen-Voltage) and BLUF (Blue Light Using Flavin) domains, have been also recruited for optogenetic applications.

2. ORGANIZATION

The CT4OPTO workshop was based on the work carried out by a scientific committee, an organizing committee and partners agencies and institutions. The workshop was financed by Psi-k.
The online platform used to manage the workshop was Zoom. Guidelines for participating were issued in order to facilitate interaction among the participants.
The Organizing Committee also set up a mailing list managed through mailchimp in order to collect registrations to the workshop, and keep participants up-to-date with the development of the event.

2.1. CT4OPTO Committees

The CT4OPTO **Scientific Committee** was composed by:

- Isabella Daidone | University of L'Aquila | isabella.daidone@univaq.it
- Rosa Di Felice | University of Southern California and CNR Nano Modena | rosa.difelice@nano.cnr.it
- Laura Zanetti Polzi | CNR Nano Modena | laura.zanettipolzi@nano.cnr.it

The CT4OPTO Organizing Committee | ct4opto@nano.cnr.it, was composed by:

- Laura Zanetti Polzi | CNR Nano Modena
- Mara Di Berardo | CNR Nano Modena
- Maria Bartolacelli | CNR Nano Modena
- Emanuela Bertini| Alchimie Digitali

Organizing institutions were the following:

- CNR Institute Nanoscience
- Università degli Studi dell'Aquila
- University of Southern California

Sponsor of the event was Psi-k:

The event received the **Patronage** of Cecam-It-Simul.

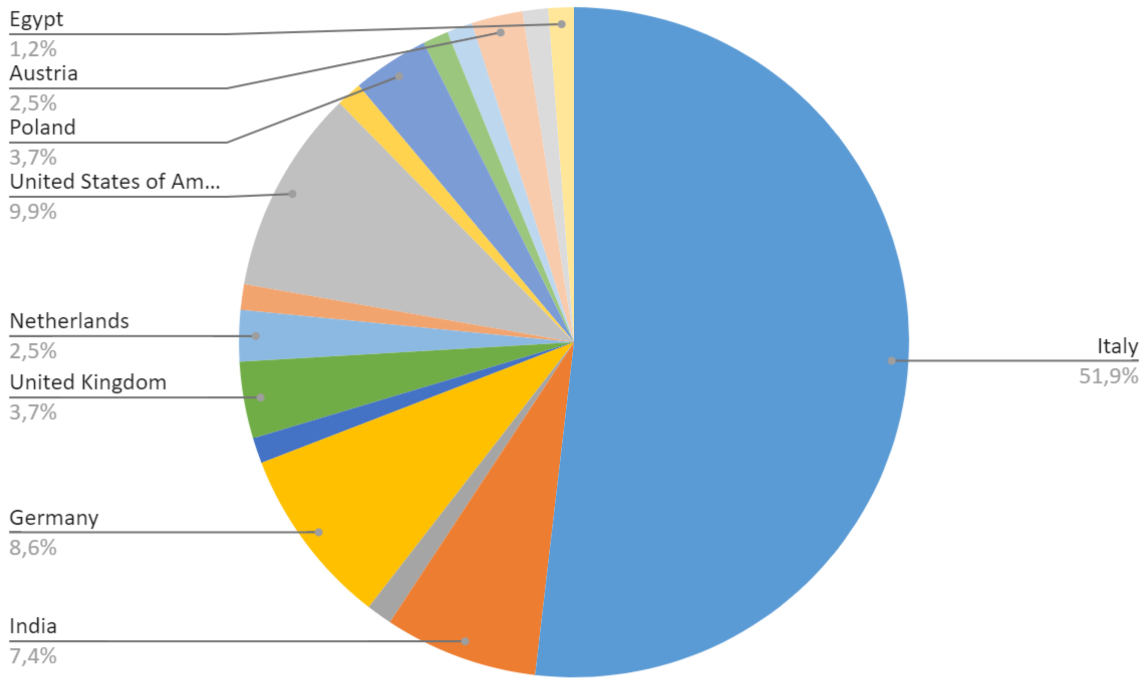
Partner of the event was **Gruppo Alchimie**, supporting the live platform.

2.2. Registrations and participation

The mailing list managed through mailchimp collected 100 registered participants. Registration deadline was the end of May, 2021.

Participants

The total number of participants is 81 coming from 16 countries, as specified: Italy: 42; United States of America: 8; Germany: 7; India: 6; United Kingdom: 3; Poland: 3; Netherlands: 2; Austria: 2; Hungary: 1; Czech Republic: 1; Cameroon: 1; China: 1; Belgium: 1; France: 1; Israel: 1; Egypt: 1.



14 participants were invited speakers, 17 were contributed speakers, 3 were members of the CT4OPTO scientific committee, and 3 of the organizing committee.
The list of participants is available in Annex 1. During the workshop, a sign-it sheet was defined and updated by the organizers. The total number of access is 230, divided as follows:

- June 14, 2021: 63
- June 15, 2021: 56
- June 16, 2021 (morning): 52
- June 16, 2021 (afternoon): 59

Registration Management

A welcome message was set for registered participants from April.

April, 2021 (1)

Single welcome email — Archived — 59.0% Opens — 15.4% Clicks
Single Email Welcome · CT4OPTO
Archived on **mar, giugno 22nd 4:14 PM**

The following campaigns were sent before, during and after the workshop:

		Opens	Clicks
Thank you for joining our CT4OPTO workshop Regular · CT4OPTO Sent **gio, giugno 17th 10:26 AM** to 100 recipients by you	Sent	60.0%	9.0%
CT4OPTO June 16 Agenda and link to connect Regular · CT4OPTO Sent **mer, giugno 16th 9:30 AM** to 100 recipients by you	Sent	71.0%	50.0%
CT4OPTO June 15 Agenda and link to connect Regular · CT4OPTO Sent **mar, giugno 15th 10:11 AM** to 100 recipients by you	Sent	78.0%	46.0%
CT4OPTO June 14 Agenda and link to connect Regular · CT4OPTO Sent **lun, giugno 14th 10:15 AM** to 99 recipients by you	Sent	80.8%	62.6%

Campaign	Status	Opens	Clicks
Book of Abstracts and guidelines Regular · CT4OPTO Sent **ven, giugno 11th 1:31 PM** to 98 recipients by you	Sent	73.5% Opens	57.1% Clicks
CT4OPTO Agenda Regular · CT4OPTO Sent **gio, giugno 3rd 4:21 PM** to 94 recipients by you	Sent	74.5% Opens	53.2% Clicks

May, 2021 (2)

Campaign	Status	Opens	Clicks
Abstract submission deadline extended! Regular · CT4OPTO Sent **lun, maggio 17th 11:33 AM** to 65 recipients by you	Sent	56.9% Opens	12.3% Clicks
CT4OPTO abstract submission deadline Regular · CT4OPTO Sent **lun, maggio 10th 2:14 PM** to 54 recipients by you	Sent	55.6% Opens	18.5% Clicks

A final e-mail containing the links to the videos and the present report will be sent soon after publication, and will close the workshop.

The performances of the campaigns sent so far are the following:

Audience performance

Average open rate 68.0%

Average click rate

Average subscribe rate 3,200.0%

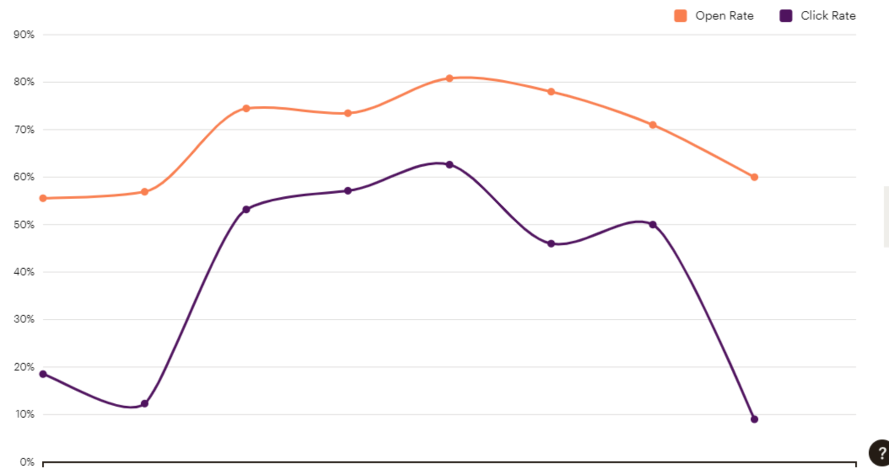
Average unsubscribe rate

Email marketing engagement

Your subscribers, broken down by how often they open and click your emails.

57%	**Often** Your percentage of subscribers who are highly engaged and often open and click your emails.
9%	**Sometimes** Your percentage of subscribers who are moderately engaged and sometimes open and click your emails.
6%	**Rarely** Your percentage of subscribers who are not very engaged and rarely open and click your emails.

Campaign performance



Top locations

Italy	49.0%
Other	17.0%
USA	9.0%
India	7.0%
Germany	6.0%

2.3. Guidelines for participating

The Guidelines sent to the participants and published on the workshop website are the following.

The “Principles of Light Induced Charge Transfer for Optogenetics” virtual workshop will use the Zoom platform. The easiest way to join the workshop is to have the latest version of Zoom installed on your computer and an updated profile. However, it is not mandatory to have a Zoom account to join the workshop or to have the Zoom app installed, you can join the meeting via browser.

Joining the workshop

Before the workshop, you will receive an email from the organizers with the workshop URL link. Once you join the workshop, you will enter the Zoom Waiting Room and the workshop organizers will let you enter the workshop if your name is on the expected list of participants. This prevents unauthorised parties from trying to enter the workshop. If your name does not appear on the participant list, then unfortunately you won’t be granted admission. It is important to ensure your Zoom screen name is the name you used to register for the workshop and not a nickname or, for example, “John’s iPad” or “Mary’s Phone”. Given the high number of registered participants, the organizers might need some minutes to let you in. If you disconnect during the workshop, try rejoining and the workshop organizers will let you in from the waiting room. If you are still having difficulty, email ct4opto@nano.cnr.it.
During the workshop, keep your microphone muted unless you’re speaking. That will prevent unwanted interruptions due to background noise. We encourage you to use your video, so that we can create the feel of a workshop.

Questions & Answers

After each invited talk and after each contributed talks session (i.e., four 5 minutes contributed talks) there will be 5 minutes for asking questions to speakers.
To ask or answer a question or be part of a discussion you can either:

- use the “Raise hand” tool: click on the icon at the bottom of the Participant list and this places a raised hand next to the participant’s name. When asked from the workshop organizers, you then unmute and speak. Remember to mute again when you have finished.
- type your request to ask a question in the private chat with the workshop organizers. When asked to speak, you then unmute. Remember to mute again when you have finished.

Breakout rooms

There will be breakout rooms during coffee and lunch breaks (lunch break is scheduled only on June 16th).
During the breakout room time, participants will have the opportunity to discuss, share ideas and deepen the topics discussed during the sessions and to meet other participants. We warmly encourage you to join the breakout room during the workshop breaks. Upon request, additional breakout rooms can be created by the organizers to allow discussion within a smaller group of participants.

- Joining breakout rooms: you will see an invitation to join – click to accept.
- Who is in the room: you will only see and speak with/listen to your room members.
- Asking for help: you can ask the workshop organizers to join the room for help.
- Leaving the room: you can leave the room at any time. The workshop organizers will close the rooms at the end of the break. This will be indicated by a message box with a countdown from 60 seconds.

Privacy

Participants are required to respect the privacy of others and refrain from any unauthorized or unwelcomed recording or photography, including recording of any direct or indirect interactions or electronic messaging. Participants are not allowed to take photos, videos or screenshots of presentation materials shown. Participants are not allowed to post images or videos online without permission.

3. AGENDA

The CT4OPTO agenda[1] for the three days is the following.

CEST	Monday, June 14
2:45 PM	Main zoom room opens
Session 1/1	Investigating optical properties (1)
3:00 – 3:10 PM	Opening remarks
3:10 – 3:50 PM	**Massimo Olivucci** *University of Siena & Bowling Green State University* "On the fluorescence enhancement of arch neuronal optogenetic reporters"
3:50 - 4:30 PM	**Dongping Zhong** *The Ohio State University* "Light-induced charge transfer triggers dimer dissociation of UVR8 photoreceptor for possible optogenetics"
4:30 – 4:55 PM	**Contributed talks:** **Nadja K. Singer** *University of Vienna* "From taco to banana: turn-on mechanism of a fluorescent probe for imaging $GABA_A$ receptors" **Laura Pedraza-González** *University of Siena* "Automated QM/MM model screening of rhodopsin variants displaying enhanced fluorescence" **Volha Chukhutsina** *Imperial College London* "The keto group in β2 of the carotenoid tunes the orange carotenoid protein photocycle kinetics" **Ciro A. Guido** *University of Padova* "Exploring the spatial features of electronic transitions in biomolecular systems by swift electrons"
4:55 – 5:25 PM	Coffee break & breakout rooms
Session 1/2	Investigating optical properties (2)
5:25 – 6:05 PM	**Igor Schapiro** *The Hebrew University of Jerusalem* "Insight into the spectral tuning mechanism of retinal proteins"
6:05 – 6:45 PM	**Roberta Croce** *Vrije Universiteit Amsterdam* "Breaking the red-limit: driving oxygenic photosynthesis with far-red light"

[1] The .pdf version of the agenda can be downloaded from the workshop website at https://optogenetics.nano.cnr.it/.

	Tuesday, June 15
2:45 PM	Main zoom room opens
Session 2/1	**Interplay between CT events and environmental factors**
3:00 – 3:40 PM	**Petra Imhof** *Freie Universität Berlin* "Interplay of hydration, water mobility, and proton transfer in cytochrome c oxidase"
3:40 – 4:20 PM	**Andrea Amadei** *University of Rome "Tor Vergata"* "On the modeling of charge transfer processes in complex chemical systems"
4:20 – 4:50 PM	**Contributed talks**
	Puja Goyal *State University of New York* "Modulation of adenosylcobalamin photochemistry by the CarH photoreceptor protein"
	Bryan Kudisch *Princeton University* "Active-site environmental factors customize the photophysics of photoenzymatic old yellow enzymes"
	Matteo Capone *University of L'Aquila* "Multiscale modelling of the photoactivation of electron donor acceptor complexes in ene reductases"
	Ruibin Liang *Texas Tech University* "Light-activation mechanism of Channelrhodopsin 2"
	Fulvio Perrella *University of Naples Federico II* "Proton transfer in fluorescent proteins: a dynamical viewpoint on hydrogen bonds networks"
4:50 – 5:25 PM	Coffee break & breakout rooms
Session 2/2	**Excited states dynamics (1)**
5:25 – 6:05 PM	**Gregory Scholes** *Princeton University* "Electron transfer reactions: vibration and dielectric tuning"
6:05 – 6:45 PM	**Benedetta Mennucci** *University of Pisa* "From the light absorption by the embedded chromophore to the conformational change of the protein: can we simulate such a long travel in space and time?"

	Wednesday, June 16
10:45 AM	Main zoom room opens
Session 3/1	**Excited states dynamics (2)**
11:00 – 11:40 AM	**Nadia Rega** *University Federico II of Napoli & Center for Advanced Biomaterials for Healthcare* "Probing relaxation mechanisms of photoinduced charge transfer phenomena: combining time-resolved vibrational analysis and ab-initio molecular dynamics"
11:40 – 12:20 AM	**Basile Curchod** *Durham University* "In silico photochemical experiments with non-Born-Oppenheimer molecular dynamics"
12:20 – 12:45 AM	**Contributed talks** **Uriel N. Morzan** *International Centre for Theoretical Physics* "Optical signature of strong hydrogen bonds" **James Green** *CNR-IBB* "A fragment based approach to the quantum dynamics of multichromophoric systems: application to the GC DNA base pair" **F. Di Maiolo** *Goethe Universität* "Quantum molecular dynamics in out of equilibrium environments: redfield-smoluchowski and hydrodynamic approaches" **Pavel S. Rukin** *CNR-S3 Institute of Nanoscience* "Theoretical study of internal conversion between B and Q bands in a functionalized porphyrin"
12:45 AM – 2:30 PM	Lunch break (breakout rooms 12:45 AM - 1:15 PM)

Session 3/2	Retinal and flavin based systems (1)
2:30 – 3:10 PM	**Marco Garavelli** University of Bologna "Modelling accurate photoinduced events and transient spectroscopies in biomolecules: the paradigmatic case of retinal systems"
3:10 - 3:50 PM	**Sharon Hammes-Schiffer** *Yale University* "Nonequilibrium excited state dynamics of proton-coupled electron transfer in BLUF photoreceptor proteins"
3:50 – 4:15 PM	**Contributed talks** **Valeria Giliberti** *Istituto Italiano di Tecnologia* "Conformational changes of light-sensitive membrane proteins determined by infrared difference nanospectroscopy" **Luca Bellucci** *CNR-NEST Institute of Nanoscience* "Relating retinal isomerization and deprotonation mechanism in Channelrhodopsin-2" **Himanshu Bansal** *Dayalbagh Educational Institute* "Improved optogenetic retinal prostheses with Chrmine" **Xiankun Li** *Princeton University* "Ultrafast dynamics of light-induced charge transfer in Lactate Monooxygenase"
4:15 – 4:45 PM	Coffee break & breakout rooms
Session 3/3	**Retinal and flavin based systems (2)**
4:45 – 5:25 PM	**Ana-Nicoleta Bondar** *Freie Universität Berlin* "Proton transfers with dynamic hydrogen-bond networks"
5:25 – 6:05 PM	**Andreas Möglich** *University of Bayreuth* "Interplay of signals in Light-Oxygen-Voltage receptors"
18:05 – 18:30 PM	Prizes and closing remarks

Video playlists for each day are available on the CT4OPTO youtube channel:

- June 14: https://www.youtube.com/playlist?list=PLNk5urUzDFo81YbCq3HIOoNyzIkOTIJin;
- June 15, 2021: https://www.youtube.com/playlist?list=PLNk5urUzDFo8Jn2gTs9_z15Yof0TPs514;
- June 16, 2021: https://www.youtube.com/playlist?list=PLNk5urUzDFo8V6PjGFqEFm2KM04CGWif4.

4. INVITED SPEAKERS

CT4OPTO invited speakers were the following:

- Andrea Amadei, University of Rome “Tor Vergata”, “On the modeling of charge transfer processes in complex chemical systems”;
  video: https://www.youtube.com/watch?v=9aXotG1sQ6g;
- Ana-Nicoleta Bondar, Freie Universität Berlin, “Proton transfers with dynamic hydrogen-bond networks”;
  video: https://www.youtube.com/watch?v=IPDXACz-8iQ;
- Roberta Croce, Vrije Universiteit Amsterdam, “Breaking the red-limit: driving oxygenic photosynthesis with far-red light”;
- Basile Curchod, Durham University, “In silico photochemical experiments with non-Born-Oppenheimer molecular dynamics”;
  video: https://www.youtube.com/watch?v=bUWZn8fAg9o;
- Marco Garavelli, University of Bologna, “Modelling accurate photoinduced events and transient spectroscopies in biomolecules: the paradigmatic case of retinal systems”;
- Sharon Hammes-Schiffer, Yale University, “Nonequilibrium excited state dynamics of proton-coupled electron transfer in BLUF photoreceptor proteins”;
  video: https://www.youtube.com/watch?v=6DumPi16Q64;
- Petra Imhof, Freie Universität Berlin, “Interplay of hydration, water mobility, and proton transfer in cytochrome c oxidase”;
  video: https://www.youtube.com/watch?v=0LExUvSrGKw;
- Benedetta Mennucci, University of Pisa, “From the light absorption by the embedded chromophore to the conformational change of the protein: can we simulate such a long travel in space and time?”;
  video: https://www.youtube.com/watch?v=Vy_xxt0z90Y;
- Andreas Möglich, University of Bayreuth, “Interplay of signals in Light-Oxygen-Voltage receptors”;
- Massimo Olivucci, University of Siena & Bowling Green State University, ““On the fluorescence enhancement of arch neuronal optogenetic reporters”;
- Nadia Rega, University of Naples “Federico II”, “Probing relaxation mechanisms of photoinduced charge transfer phenomena: combining time-resolved vibrational analysis and ab-initio molecular dynamics”;
  video: https://www.youtube.com/watch?v=IwFi0LM8COk;
- Igor Schapiro, The Hebrew University of Jerusalem, “Insight into the spectral tuning mechanism of retinal proteins”;
- Gregory Scholes, Princeton University, “Electron transfer reactions: vibration and dielectric tuning”;
  video: https://www.youtube.com/watch?v=8QMSs6mRF08;
- Dongping Zhong, The Ohio State University, “Light-induced charge transfer triggers dimer dissociation of UVR8 photoreceptor for possible optogenetics”;
  video: ttps://www.youtube.com/watch?v=v5wv28ISfQMh.

The abstracts, collected in the CT4OPTO Book of Abstracts[2], are reported below.

[2] The Ct4OPTO Book of abstracts is available on the workshop website at https://optogenetics.nano.cnr.it/.

Permission for recording and publishing the speech was requested. The videos were uploaded to a specific youtube channel, CT4OPTO, opened after the workshop. A playlist with the videos of the invited speakers can be found here: https://www.youtube.com/watch?v=9aXotG1sQ6g&list=PLNk5urUzDFo99SFFg0gakLZY_R0TPKDfF&index=1.

4.1. On the modeling of charge transfer processes in complex chemical systems

Andrea Amadei

University of Rome “Tor Vergata”
andrea.amadei@uniroma2.it

In this talk we present a theoretical-computational methodology specifically aimed at describing charge transfer processes from atomistic (semiclassical) simulations and, hence, very suitable for treating complex atomic-molecular systems. The core of the presented approach is the evaluation of the diabatic perturbed energy surfaces of a portion of the whole system, treated at the quantum level and therefore preventively selected, in semi-classical interaction with the atomic-molecular environment. Subsequently, the estimation of the diabatic energy surfaces perturbed by the atomic-molecular environment and their coupling allows to obtain a properly designed kinetic model. Such an approach allows the reconstruction of the whole phenomenology directly comparable to the experimental (typically kinetic) data.
Application to different systems has demonstrated that the proposed approach can represent a valuable tool, somewhat complementary to other methods based on explicit quantum-dynamical approaches, for the theoretical-computational investigations of large and complex atomic molecular systems.

4.2. Proton transfers with dynamic hydrogen-bond networks

Ana-Nicoleta Bondar

University of Bucharest, Faculty of Physics, Str. Atomiştilor 405, Bucureşti-Măgurele 077125 & Forschungszentrum Jülich, Institute for Advanced Simulations, Computational Biomedicine (IAS-5/INM/9), Wilhelm-Johnen-Straße, 5428 Jülic
nbondar@zedat.fu-berlin.de

Retinal proteins absorb light to initiate a reaction cycle that couples protonation change to changes in protein conformational dynamics. Description of the mechanisms by which retinal photo-isomerization couples to events in the protein matrix can guide the selection of mutant proteins for optogenetics applications. Particularly important here is to evaluate how internal protein-water hydrogen-bond networks respond to retinal photo-isomerization and protonation binding.
We have recently developed graph-based algorithms for efficient analyses of dynamic hydrogen-bond networks of membrane proteins, and applied these algorithms to dissect conformational dynamics of retinal proteins. Internal hydrogen-bond networks can respond rapidly to mutation, rearranging to allow sampling of long-distance hydrogen-bond connections that would have been difficult to predict based on static coordinate snapshots of a wild-type protein. To evaluate the relative importance of protein groups in an internal hydrogen-bond network we use measures of centrality, and probe the response of the protein to mutation.

References

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Bondar A-N, Lemieux J. Reactions at membrane interfaces. Chemical Reviews 119, 6162-6183 (2019).
Harris A, Lazaratos M, Siemers M, Watt E, Hoang A, Tomida S, Schubert L, Saitta M, Heberle J, Furutani Y, Kandori H, Bondar A-N, Brown LS. Mechanism of inward proton pumping in an Anarctic microbial rhodopsin. Journal of Physical Chemistry B 124, 4851-4872 (2020).
Lazaratos M, Karathanou K, Bondar A-N. Graphs of dynamic H-bond networks: from model proteins to protein complexes in cell signaling. Current Opinion in Structural Biology 64, 79-87 (2020).
Siemers M, Lazaratos M, Karathanou K, Brown LS, Bondar A-N. Bridge: A graph-based algorithm to analyze dynamic H-bond networks in membrane proteins. Journal of Chemical Theory and Computation 15, 6781-6798, doi 10.1021/acs.jctc.9b00697 (2019).

4.3. Breaking the red-limit: driving oxygenic photosynthesis with far-red light

Roberta Croce

Vrije Univesiteit Amsterdam, Amsterdam, the Netherlands
r.croce@vu.nl

Photosynthetic organisms use sunlight energy to fix CO2 into carbohydrates, in this way sustaining almost all life on our planet. The capacity of these organisms to harvest light is a crucial factor in the photosynthetic process, especially in light-limited conditions, which occur in greenhouses and canopies. However, plants and algae only use the visible part of the solar spectrum, discarding more than 50% of the photons reaching the surface of the Earth. This is because their photosynthetic proteins bind as main pigments chlorophyll (Chl) a and b, which have intense absorption in the red and the blue regions of the electromagnetic spectrum but do not absorb above 700 nm. For a long time, it was believed that cyanobacteria, the prokaryotic ancestors of plant chloroplasts, also could only use visible light to drive photosynthesis. The discovery of species containing Chl d and Chl f, which absorb in the far-red region of the spectrum, has shown that this is not the case. However, due to their different energetics, Chl d and f are expected to alter the excited state dynamics of the photosynthetic units and, ultimately, their performances. How can thus cyanobacteria use far-red light for efficient photochemistry?

To answer this question we use a combination of biochemistry and spectroscopic measurements on intact cells and isolated complexes. We show that chlorophyll f insertion marginally affects the charge separation efficiency of Photosystem I [1] but decreases significantly that of Photosystem II [2]. The difference between the two photosystems and the possibility to introduce Chl d and f in plant photosynthetic complexes to extend the photosynthetic active radiation in crops will be discussed.

References

[1] Tros M, Mascoli V, Shen G, Ho MY, Bersanini L, Gisriel CJ, Bryant DA, Croce R, Chem, 7, 155-173 (2021).

[2] Mascoli V, Bersanini L, Croce R, Nature Plants, 6, 1044-1053 (2020).

4.4. In silico photochemical experiments with non-born-oppenheimer molecular dynamics

Basile F.E. Curchod

Department of Chemistry, Durham University, South Road, Durham DH1 3LE, United Kingdom
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What happens to a molecule once it has absorbed UV or visible light? How does the molecule release or convert the extra energy it just received? Answering these questions clearly goes beyond a pure theoretical curiosity, as photochemical and photophysical processes are central to numerous domains like energy conversion and storage, radiation damages in DNA, or atmospheric chemistry.

Different theoretical tools have been developed to address these questions by simulating the excited-state dynamics of molecules [1]. Two examples of such methods include ab initio multiple spawning (AIMS) and trajectory surface hopping (TSH). AIMS describes the dynamics of nuclear wavepackets using adaptive linear combinations of traveling frozen Gaussians [2]. TSH portrays the nuclear dynamics with a swarm of independent classical trajectories that can hop between potential energy surfaces for this task [3].

In this talk, I intend to survey some of our recent work aiming at understanding the approximations underlying AIMS [4] and developing new approximate techniques based on the multiple spawning framework [5]. These new methods dramatically reduce the cost of a multiple spawning simulation while preserving a rigorous description of nonadiabatic transitions.

References

[1] F. Agostini and B. F. E. Curchod, WIREs Comput. Mol. Sci. 2019, 9, e1417.
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4.5. Modelling accurate photoinduced events and transient spectroscopies in biomolecules: the paradigmatic case of retinal

Marco Garavelli

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The use of the computer to simulate light induced phenomena in photoactive molecular materials has given access to a detailed description of the molecular motions and mechanisms underlying the reactivity of organic and bio-organic chromophores in realistic conditions. Thus, different computational strategies and tools can now be operated like a “virtual spectrometer” to characterize and understand the photoinduced dynamics and reactivity of a given dye, allowing for an accurate description of photochemical/photobiological processes and a rational of the corresponding properties including time-resolved spectroscopy over a wide spectral regime, spanning the NIR-VIS-UV-Xray spectral window.
This contribution reviews our recent advances in the field, by presenting methodological developments and applications in modelling the photochemistry and photophysics of complex photoactive molecular architectures (e.g., retinal systems and visual proteins, including artificial rhodopsin mimics), including their multi-pulse transient spectroscopy [1-3]. Non-adiabatic semiclassical trajectories by hybrid QM/MM calculations at the multireference perturbative (RASPT2) QM level will be shown to be an elective tool for modelling photoinduced dynamics on large molecular materials, including tuning/controlling effects of the environment. The simulations are facilitated by our program package COBRAMM [4], that is able to integrate some specialized softwares and acts as a flexible computational environment. We report a remarkable agreement with state-of-the-art transient absorption spectroscopy measurements, which allows us to resolve the fate of the investigated systems and disclose environment effects. Results on other photoactive molecular materials will be show.

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4.6. 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 and serve as a prototype for photoinduced proton-coupled electron transfer (PCET) in proteins. Photoexcitation of the flavin chromophore induces PCET, as well as local conformational changes that propagate to distal parts of the protein and drive other chemical and physical changes. In the Slr1694 BLUF photoreceptor, experiments indicate that 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 photoexcitation to the locally excited state of the flavin, protein reorganization drives electron transfer from the tyrosine to the flavin, followed by sequential double proton transfer from tyrosine to the flavin via the intervening glutamine. 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. 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 in the light-adapted state. 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.

4.7. Interplay of hydration, water mobility, and proton transfer in cytochrome c oxidase

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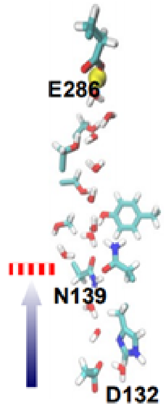

Cytochrome c Odixase (CcO) also known as complex IV in the respiratory chain is a protein that uses the energy from the reduction of oxygen to water to pump (further) protons through the membrane. For CcO to act as an oxidase and a proton pump, these processes have to be highly regulated. Proton uptake from the inner side of the membrane to the chemical redox centre takes place through two so-called channels, named D or K, after an important Asp or Lys residue, respectively.

Our simulations show that the protonation state of the two channels has an impact on the hydration level within the two channels [1] and of the communication within and between the two channels [2]. For the D-channel, the hydration level is lower when the proton has already reached E286 at the end of the channel. This can be explained by a hydrogen-bonded network pointing from E286 to the so-called asparagine gate (formed by N139 and N121), favouring a “closed” conformation [1]. The thus prevented water passage also blocks the most favourable pathway [3] for proton transfer from the channel entrance to its terminus.

The D-channel can thus be regarded as auto-regulated, allowing proton passage only when required, that is the proton has not arrived at the upper end of the channel, yet. In the K-channel, the hydration level depends even more critically on the position of the excess proton, suggesting that the proton drags its own hydration sphere with it. Likewise, the conformation of residue E101 at the entrance and K362 in the middle of the channel, are predominantly in an “up” conformation, when protonated [2]. The directionality of the hydrogen-bonded networks and the probabilities for proton transfer are coupled to the conformation of K362 [4]. Proton transfer through the entire channel in both directions is feasible only in the “down” conformation and unlikely in the “up” conformation [4]. Similar to the D-channel, this interplay can be regarded as an auto-regulation, preventing back leakage and the transfer of an extra charge, once the proton has reached the upper part of the channel and is therefore close to the redox centre.

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4.8. From the light absorption by the embedded chromophore to the conformational change of the protein: can we simulate such a long travel in space and time?

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Organisms of all domains of life are capable of sensing, using and responding to light. The molecular mechanisms used are diverse, but most commonly the starting event is an electronic excitation localized on a chromophoric unit bound to the protein matrix. The initial excitation rapidly “travels” across space and time finally leading to the protein conformational change required to complete the biological function. Here we discuss the main theoretical and methodological challenges of the modeling of such a multiscale problem, and we present possible strategies based on the integration of quantum chemistry and molecular dynamics.

4.9. Interplay of signals in light-oxygen-voltage receptors

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As one class of sensory photoreceptors, light-oxygen-voltage (LOV) receptors harness flavin nucleotide chromophores to sense blue light and elicit diverse physiological responses. Upon blue-light-driven electronic excitation and progression through short-lived intermediates, a covalent bond forms between the flavin C4a atom and a conserved cysteine in the LOV protein. The resultant protonation at the flavin N5 atom prompts hydrogen-bonding changes and conformational transitions permeating the LOV module. In case of the paradigmatic LOV2 domain from Avena sativa phototropin 1, the most extensively studied system, commonly denoted AsLOV2, blue-light exposure culminates in the reversible unfolding of two α helices. Crystal structures of AsLOV2 in its dark-adapted and light-adapted states at 1 Ångström resolution reveal in unprecedented detail how photochemical events are coupled to the protein scaffold. The light-dependent helical unfolding of AsLOV2 has been leveraged for the regulation of protein activity in multiple and ingenious ways. As a case in point, we subjected the activity of the RNA-guided endonuclease Cas9 to blue light via insertion of the AsLOV2 module in a surface-exposed loop.

4.10. On the fluorescence enhancement of arch neuronal optogenetic reporters

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The lack of a theory capable of connecting the amino acid sequence of a light-absorbing protein with its fluorescence brightness is hampering the developments of tools for understanding neuronal communications. Here we demonstrate that a theory can be established by constructing the quantum chemical models of a set of established Archaerhodopsin reporters in their excited state. We found that the experimentally observed increase in fluorescence quantum yield is proportional to the computed decrease in energy difference between the fluorescent state and a nearby photoisomerization channel. This finding is important because, ultimately, it will make possible to develop technologies for searching novel fluorescent rhodopsin variants and unveil electrostatic changes that make light emission brighter and brighter.

4.11. Probing relaxation mechanisms of photoinduced charge transfer phenomena: combining time-resolved vibrational analysis and ab-initio molecular dynamics

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Vibrational spectroscopy is a key instrument to understand structural changes and chemical reactivity at the molecular scale. Nowadays, time-resolved Infrared and Raman spectroscopies [1] allow to catch the temporal evolution of vibrational bands, disentangling couplings among modes beyond harmonic approximation and changes in bands positions and intensity. The interpretation of the time resolved vibrational spectra can be very challenging to unravel, in this scenario the atomistic-level description provided by tailored theoretical protocols can be a valuable aid in interpretation and further clarification of experimental evidence. In this contribution we show the capabilities of a developed and successfully implemented theoretical-computational approach based on ab-initio molecular dynamics simulations [2] for both ground and excited state integrated with a Wavelet transform based vibrational analysis.[3] Spectra computed through this approach allow to retain the temporal evolution of vibrational bands, allowing to simultaneously catch time-dependent bands couplings, frequency shifts and changes in intensity. Precious molecular insights can be achieved through this approach, where molecular motions can be observed on-the-fly through the direct access to their vibrational band evolution. Moreover, this analysis allows to tune the resolution with whom the spectrum is simulated, allowing to adapt it to the frequency content of the signal. The wavelet transform is successfully applied to both generalized normal modes or structural quantities such as bond distances or dihedral angles. The relaxation channels of the photoexcited systems are unveiled thanks to a detailed time-resolved analysis of key activated vibrational modes [4,5]. 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 involved light-induced reactivity and charge transfer phenomena. Perspectives will be given as conclusions.

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4.12. Insight into the spectral tuning mechanism of retinal proteins

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Retinal proteins are used for various biotechnological applications due to their favorable properties. These proteins have the retinal chromophore in common but the specific interaction with the altering protein environment can alter the absorption maximum. The so called spectral tuning mechanism is responsible for covering a wide range of the visible spectrum. In this contribution we will present two case studies of the spectral tuning found in proteorhodopsin and neorhodopsin.
Proteorhodopsin (PR) is a photoactive proton pump found within marine bacteria which was first discovered in 2000 [1]. PR has been suggested to play a large role in marine photoactivated processes due to their wide presence in marine life and their unique ability to absorb sunlight [2, 3]. PR has two major variants which exhibit an environmental adaptation in their absorption maximum to the ocean's depth. The green-absorbing PR (GPR, λmax = 520 nm) is mainly found in microbes at the surface of water whereas the blue-absorbing PR (BPR, λmax = 490 nm) is distributed at the deeper region in the ocean [4, 5]. The amino acid at position 105 controls the color tuning of the two variants, where an L to Q substitution causes a ~25 nm green to blue color-shift in addition to affecting the geometric properties of the retinal chromophore [6–8]. In this work the green-blue shift was investigated with QM/MM simulations using a polarizable embedding scheme. The L to Q mutation produces a positive electrostatic interaction near C14-C15 of retinal, which in turn destabilizes the S1 state leading to the observed green to blue shift.
Neorhodopsin is the most red-shifted retinal protein with the absorption in the near infrared (NeoR λmax = 690 nm) [9]. It is bistable and has the second stable form absorbing in the UV. In a combination of site-specific mutagenesis and hybrid QM/MM simulations we have demonstrated that the strong red-shift is due to a unique counterion triad composed of two glutamic and one aspartic acids. These findings substantially expand our understanding of the natural potential and limitations of spectral tuning in rhodopsin photoreceptors.

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4.13. Electron transfer reactions: vibration and dielectric tuning

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We have recently studied ultrafast electron transfer (ET) dynamics of three Old Yellow Enzymes. Despite their overall large degree of structural homology, these enzymes display distinct ultrafast ET dynamics. Using a Marcus ET framework, we explained that the differences observed between their photoinduced ET pathways depend on an interplay between the driving forces for photoinduced and back ET with their reorganization energies. In the case of OPR1, the reorganization energy is large enough and the driving forces are suitable to induce inverted ET kinetics. I will debate how the heterogeneous dielectric environment in OY enzymes provides a biological handle for optimizing the protein function.
Vibrations enable a dramatic speed up for some ET reactions, or control of ET by suppressing and enhancing reaction paths. Despite these, and other, compelling examples of the function of vibrations in ET reactions, experimental resolution of the mechanism of interplay of ET with vibrations has eluded researchers. Here I report ultrafast coherence experiments that resolve how quantum vibrations participate during an ET reaction. We observe generation—by the ET reaction, not the laser pulse—of a new coherence along a reaction coordinate in a mode associated with the reaction product. This surprising spontaneous launch of a vibrational wavepacket shows that coherence can be generated by a separation of timescales in chemical dynamics, and not solely by pulsed laser photoexcitation.

4.14. Light-induced charge transfer triggers dimer dissociation of uvr8 photoreceptor for possible optogenetics

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UVR8 (UV RESISTANCE LOCUS 8) proteins are a class of UV-B photoreceptors in high plants. UVR8 is a homodimer that dissociates into monomers upon UV-B irradiation (280 to 315 nm), which triggers various protective mechanisms against UV damages. Uniquely, UVR8 does not contain any external chromophores and utilizes the natural amino acid tryptophan (Trp) to perceive UV-B light. Each UVR8 monomer has 14 tryptophan residues. However, only the epicenter two Trp (W285 W233) residues are critical to the light-induced dimer-to-monomer transformation. Here, combining time-resolved spectroscopy and extensive site-directed mutations, we have revealed the entire dynamics of UV perception to lead to monomerization, including a series of critical dynamical processes of a striking energy-flow network, exciton charge separation and recombination, charge neutralization, salt-bridge zipping and protein solvation, providing a complete molecular picture of the initial biological function and thus a potential candidate for optogenetics.

5. CONTRIBUTED SPEAKERS

CT4OPTO contributed speakers were the following:

- Luca Bellucci CNR-NEST Institute of Nanoscience, “Relating retinal isomerization and deprotonation mechanism in Channelrhodopsin-2”;
  video: https://www.youtube.com/watch?v=2Rag-bevIEA;
- Himanshu Bansal, Dayalbagh Educational Institute, “Improved optogenetic retinal prostheses with Chrmine”;
  video: https://www.youtube.com/watch?v=etrT7iZM4Ng;
- Matteo Capone, University of L’Aquila, “Multiscale modelling of the photoactivation of electron donor acceptor complexes in ene-reductases”;
  video: https://www.youtube.com/watch?v=__sT5SNasOI;
- Volha Chukhutsina, Imperial College London, “The keto group in β2 of the carotenoid tunes the orange carotenoid protein photocycle kinetics”;
- Francesco Di Maiolo, Goethe Universität, “Quantum molecular dynamics in out of equilibrium environments: redfield-smoluchowski and hydrodynamic approaches”;
  video: https://www.youtube.com/watch?v=vsqdTVwNt88;
- Valeria Giliberti Istituto Italiano di Tecnologia. “Conformational changes of light-sensitive membrane proteins determined by infrared difference nanospectroscopy”;
  video: https://www.youtube.com/watch?v=2JpF-SX5n2U;
- Puja Goyal, State University of New York, “Modulation of adenosylcobalamin photochemistry by the CarH photoreceptor protein”;
  video: https://www.youtube.com/watch?v=XxyC_0Cqewg;
- James Green, CNR-IBB, “A fragment based approach to the quantum dynamics of multichromophoric systems: application to the GC DNA base pair”;
  video: https://www.youtube.com/watch?v=oKQ_-ZtpjIA;
- Ciro A. Guido, University of Padova, “Exploring the spatial features of electronic transitions in biomolecular systems by swift electrons”;
  video: https://www.youtube.com/watch?v=vLJShC3AXQc;
- Bryan Kudisch, Princeton University, “Active-site environmental factors customize the photophysics of photoenzymatic old yellow enzymes”;
  video: https://www.youtube.com/watch?v=s0LuHnTfOX8;
- Xiankun Li, Princeton University, “Ultrafast dynamics of light-induced charge transfer in lactate monooxygenase”;
  video: https://www.youtube.com/watch?v=HafEd5mUU28;
- Ruibin Liang, Texas Tech University, “Light-activation mechanism of Channelrhodopsin 2”;
  video: https://www.youtube.com/watch?v=yUetkDCTDyk;
- Uriel N. Morzan, International Centre for Theoretical Physics, “Optical signature of strong hydrogen bonds”;
  video: https://www.youtube.com/watch?v=8HgHk6W9aKM;
- Laura Pedraza-González, University of Siena, “Automated QM/MM model screening of rhodopsin variants displaying enhanced fluorescence”;
  video: https://www.youtube.com/watch?v=cTiQ9vjiJfs;

- Fulvio Perrella, University of Naples Federico II, "Proton transfer in fluorescent proteins:a dynamical viewpoint on hydrogen bonds networks";
  video: https://www.youtube.com/watch?v=MBhvj1f5xWg;
- Pavel S. Rukin, CNR-S3 Institute of Nanoscience, "Theoretical study of internal conversion between B and Q bands in a functionalized porphyrin";
  video: https://www.youtube.com/watch?v=Bz5OJpfsg5g;
- Nadja K.Singer, University of Vienna, "From taco to banana: turn-on mechanism of a fluorescent probe for imaging GABAA receptors";
  video: https://www.youtube.com/watch?v=wcB2YLUiSvs.

The abstracts, collected in the CT4OPTO Book of Abstracts[3], are reported below.
Permission for recording and publishing the speech was requested. A playlist with all the contributed videos can be found here:
https://www.youtube.com/playlist?list=PLNk5urUzDFo9svo6OVYK8r1FPjIXYY8e1.

[3] The Ct4OPTO Book of abstracts is available on the workshop website at https://optogenetics.nano.cnr.it/.

5.1. Relating retinal isomerization and deprotonation mechanism in channelrhodopsin-2

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The light-gated ion channel channelrhodopsin 2 (ChR2) is the most widely used optogenetic tool. ChR2 is structurally similar to other microbial rhodopsins and undergoes a well characterized photocycle starting with the retinal isomerization from the all-trans to 13-cis state. Nonetheless, the relationship between the light-induced structural changes and the channel opening are still under debate. In particular, fully dark-adapted and non-dark-adapted experiments provided controversial data on the protonation state of the central gate residue E90 along the photocycle. Recently, a possible solution to this controversy was proposed by hypothesizing a branched photocycle with C=N-anti and C=N-syn retinal conformations. Within the anti-cycle E90 stays protonated and two conducting states with different ions selectivity are observed. Within the syn cycle E90 is deprotonated and proton conductance is promoted [1].
Here, we propose a computational protocol to investigate the linkage between the C=N-anti to C=N-syn retinal isomerization and the thermodynamics of E90 deprotonation to clarify the molecular mechanisms at the basis of the hypothesized branched photocycle. Starting from a recently resolved X-ray structure of ChR2 [2], we perform extended atomistic molecular dynamics simulation of four systems, comprising both retinal isomerization states with the protonated and unprotonated E90. The thermodynamics of E90 deprotonation is investigated by using a multiscale hybrid quantum-classical approach based on the Perturbed Matrix Method (PMM), which has been recently applied to investigate the thermodynamics of proton transfer reactions [3]. This approach allows us to investigate how the intricate hydrogen-bonding network in the neighborhood of the retinal affects the protonation/deprotonation of E90.

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5.2. Improved optogenetic retinal prostheses with chrmine

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Optogenetics with unprecedented spatiotemporal resolution and minimal invasiveness has emerged as a promising technique for retinal prostheses [1]. New opsins with high light-sensitivity and improved kinetics have provided low-power, high-frequency, and minimally invasive deep tissue excitation of neurons [2-5]. However, these new opsins have not been studied for retinal prostheses. The recently discovered marine opsin gene, named ChRmine from Tiarina fusus, results in a very large photocurrent at red-shifted excitable wavelength and at very low-power [6]. A detailed theoretical analysis of optogenetic excitation of retinal ganglion neurons with ChRmine has been carried out and compared with other experimentally studied opsins, namely, ChR2, ReaChR and ChrimsonR. The theoretical model formulated for the first time that is in excellent agreement with reported experimental results also provides new insights for optogenetic control.
The study reveals that ChRmine can evoke high-fidelity spiking upto 40 Hz, while ChR2 and eaChR both fail above 10 Hz. Although ChrimsonR leads to high-fidelity spiking upto 100 Hz, the required light power of each light pulse with ChRmine is at least two orders of magnitude lower than other opsins. Spike latency in ChRmine-expressing neurons is also shorter by an order of magnitude in comparison to other opsins. The present study highlights the potential of ChRmine for optogenetic retinal prostheses.

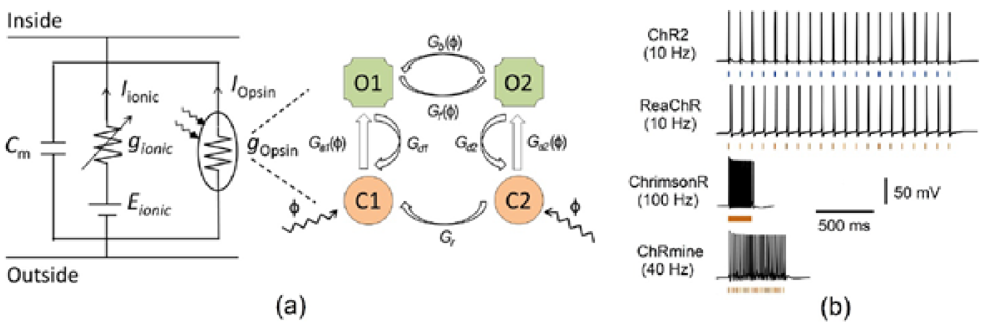

Fig. 1 (a) Schematic of integrated neuron circuit model of opsin-expressing retinal ganglion neuron, where IIonic = INa + I, + IK- + IKA + ICa * + IKCa. (b) High-frequency limit of high-fidelity optogenetic spiking in opsin-expressing retinal ganglion neurons, under photostimulation protocol of 20 pulses each of 2.5 ms and 5 mW/mm2 for ChR2, 1 ms and 5 mW/mm2 for ReaChR, 1.2 ms and 2.8 mW/mm2 for ChrimsonR, and 0.93 ms and 0.013 mW/mm2 for ChRmine at wavelength of 460 nm for ChR2 and 590 nm for others.

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5.3. Multi-scale modeling of the photoactivation of the electron donor acceptor complexes in ene-reductases

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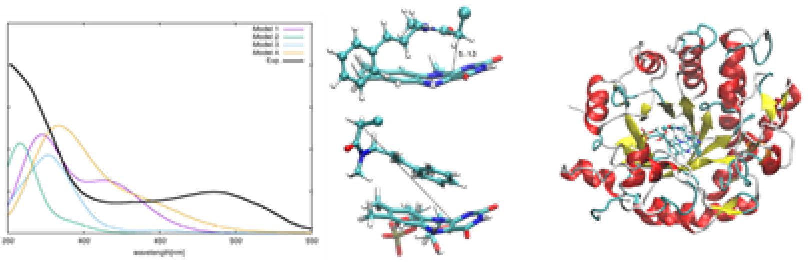
An Electron Donor Acceptor Complex (EDAC) is a transient species formed by two molecules, one electron donor and the other electron acceptor. Excitation under visible light to charge-transfer excited states of an EDAC can be used to catalyze chemical functionalization on non-activated substrates [1]. A protein-based EDAC is an ideal kind of catalyst due to the intrinsic regio- and stereo-selectivity. Additionally, in the excited state the protein-based EDA complexes can fulfill reactions that are precluded to the ground state of the protein, as for example in flavin-dependent “ene"-reductases [2]. In addition to the transient nature and large structural variability of an EDAC, the protein anisotropic contribution of the environment increases the complexity of its characterization at an electronic level. Herein we characterize the photoexcitation of an EDAC between the substrate α-chloroacetamide-(1) and the FMN enereductase GluER [2] which promotes an asymmetric radical cyclization of the susbstrate. To this aim we employ a methodology based on quantum-chemical calculations, molecular dynamics simulation and the Perturbed Matrix Method (PMM) [3,4] which allows the inclusion of the effects of the complex protein environment in the characterization of the excited states of a large number of substrate-FMN configurations. With this approach we find that the substrate adopts a large number of local arrangements in the active site of the enzyme, thus forming transient complexes. Moreover, the protein dynamics affect the character and distribution of the excited states of the different substrate-FMN complexes observed, therefore perturbing the accessibility to charge-transfer excited states. These observations would have been precluded if a canonical QM/MM methodology was used for the description of such catalytic complexes. The next goal is to characterize at the electronic level the subsequent EDAC catalytic mechanism of the ciclization reaction that is still poorly understood.



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5.4. The keto group in β2 of the carotenoid tunes the orange carotenoid protein photocycle kinetics

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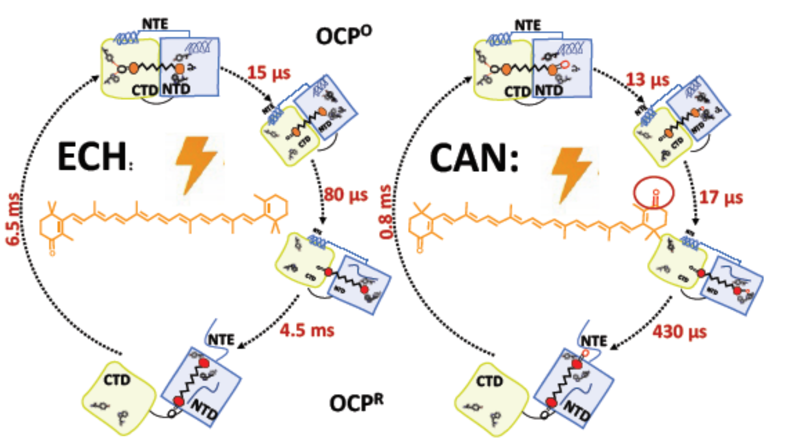
All vital biological processes, including photosynthesis, rely on the function of various photoreceptors. In cyanobacteria light-harvesting of the photosynthetic machinery is controlled by a unique photosensitive protein, Orange Carotenoid Protein (OCP). Being found only among cyanobacteria, OCP is the only known photoreceptor which uses a carotenoid for its light activation.

Understanding and, therefore, gaining control over OCP light-activation has a broad range of applications from improving photosynthesis productivity to potentially revolutionizing the field of optogenetics.

OCP is a water-soluble protein composed of the two domains: an all-helical N-terminal domain (NTD) and a C- terminal domain (CTD). CTD resembles blue-light responsive BLUF and LOV domains, which also contain a core β-sheet surrounded by helices. Absorption of blue–green light by the carotenoid induces conformational changes, converting stable inactive OCPO (the so-called orange form) into unstable but active OCPR (red form), which are also followed by drastic changes in the OCP absorption. The OCP has been shown to bind and be activated by various keto-carotenoids (3-hydroxyl-echinenone, hECN; echinenone, ECN; and canthaxanthin, CAN). The conserved keto group of the β1-ring of the keto-carotenoid is hydrogen bonded to Tyr201 and Trp288 of CTD. The other ring (β2) is nestled within a group of conserved aromatic residues (Trp41, Tyr44, Trp110) in NTD. The structure of β2-ring is actually specific for different keto-carotenoids. For example, CAN contains a second keto-group at the 4’ position, which is absent in ECH. Up to now, the mechanism of OCP light activation is considered to be independent on the keto- carotenoid bound to the OCP. Particularly this statement holds on the general idea that OCP light-activation is initiated by hydrogen bonded rupture between the keto group of the β1 ring and Tyr201 and Trp288[1]. Therefore, the photocycle intermediates resolved on OCPECH is considered to be characteristic for OCP in general [2]. In this study we for the first time to our knowledge study the effect of the keto group in the β2 ring on the OCP photocycle intermediates and activation energies. For that OCP constructs with ECH and CAN in their structure were studied. Us-ms time-resolved spectroscopy and Arrhenius



temperature dependence measurements were performed. The results indicate different activation energies and photocycle intermediate rates for the OCP with different carotenoids imbedded. The OCPCAN demonstrates lower activation energies both for OCPO-OCPR and OCPO-OCPR reactions. As a result, the photocycle rates are also faster for OCPCAN. These results indicate that β2 ring plays an important role in OCP photocycle and can be tuned by the presence of a keto group.

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5.5. Quantum molecular dynamics in out of equilibrium environments: redfield-smoluchowski and hydrodynamic approaches

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The description of quantum molecular dynamics as influenced by a polarizable and dynamically evolving environment is critical to understand the nature of various physical processes, from solvation phenomena to photobiological processes in protein environments, and transport of charge carriers and excitons in nanostructures. However, the typically used dielectric continuum picture for the environment [1-2] is likely to fail when dealing with nonequilibrium solvation effects. On the other hand, fully atomistic first principles quantum calculations are hardly feasible due to the large number of environmental degrees of freedom.
Against this background, we present the effect of a dynamic polar environment on a time-evolving molecular system, using two different approaches, namely the Multistate Redfield-Smoluchowski Equation (MRSE) [3] and the Quantum-Classical Reduced Hydrodynamic (QCRH) approach [4-5]. Both approaches can describe molecular relaxation in condensed dynamic phases, complementing typically used dielectric continuum models for the environment.

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5.6. Conformational changes of light-sensitive membrane proteins determined by infrared difference nanospectroscopy

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Infrared (IR) vibrational spectroscopy (wavelength in the range 20 – 5 μm) is sensitive to the chemical bonds and functional groups constituting the skeleton of the protein structure, thus providing fundamental insights into the functional conformational changes of light-sensitive transmembrane proteins typically used for optogenetic applications. Here we push the sensitivity capabilities of IR spectroscopy, in terms of number of probed molecules, well beyond the state-of-the-art of IR functional study of light-sensitive proteins. We apply the photothermal expansion IR nanospectroscopy technique (see Fig. 1a), based on the coupling of an atomic force microscope (AFM) and a tunable mid-IR laser (also named AFM-IR), to investigate the functional conformational changes of the prototype protein bacteriorhodopsin (BR) [1] and of the optogenetic gate channelrhodopsin (ChR) [2], both embedded in individual membrane patches (see Fig. 1b). In order to probe the protein functional conformational changes, we implement the AFM-IR nanospectroscopy platform with a visible illumination setup so as to perform difference IR nanospectroscopy by acquiring the difference absorption spectra ΔA=AON-Adark (visible light ON - visible light OFF). In Fig. 1c we report representative results obtained on BR samples. We obtain an excellent agreement with the reference curve acquired by conventional Fourier transform IR spectroscopy (FTIR) on a huge ensemble of membrane patches containing BR (~109). The unprecedented sensitivity of 102 protein molecules reached by AFM-IR opens the way towards the combination of IR spectroscopic capabilities with electrical probing at the nanoscale, which is also possible with AFM, and it is of crucial importance for transmembrane proteins.

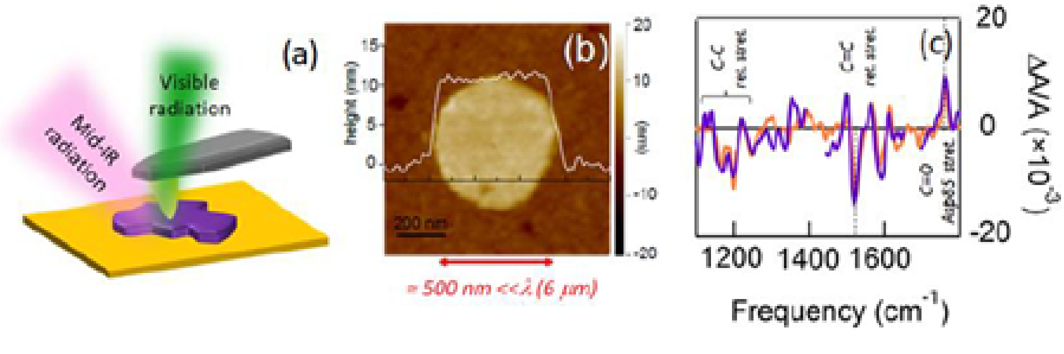

Fig. 1 (a) Sketch of the AFM-IR platform. (b) AFM topography map of an individual 5 nm-thick membrane patch embedding light-sensitive BR. (c) Purple curve: relative AFM-IR difference spectrum ΔA/A obtained on membrane patch containing BR. The positive and negative peaks are related to light-induced structural modifications of the protein backbone and of the retinal. Orange curve: reference FTIR ΔA/A curve.

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5.7. Modulation of adenosylcobalamin photochemistry by the carh photoreceptor protein

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Recently, coenzyme B12 has been studied experimentally as a light sensor in the photoreceptor protein CarH, a bacterial transcriptional repressor that controls biosynthesis of carotenoids upon light excitation [1,2]. Previously, the only known function of B12 derivatives, or cobalamins, was as a cofactor to thermally driven enzymes. Experimental transient absorption spectroscopic studies have shown a distinct difference between the photochemistry of coenzyme B12 (5'-deoxy-5'-adenosylcobalamin, AdoCbl) in the CarH environment compared to that in aqueous solution and enzymes. In contrast to aqueous solution and enzymes, photolysis of the Co-C bond in AdoCbl in CarH shows large excited state charge separation between cobalt and adenosyl with possible heterolytic cleavage of the Co-C bond. While experimental studies have yielded insights into the photochemical mechanisms of B12 derivatives, numerous details remain unclear. Our studies employ molecular dynamics (MD), quantum mechanical (QM), and hybrid quantum mechanical/molecular mechanical (QM/MM) methods to investigate the modulation of AdoCbl excited states by the CarH protein environment. We examine this relationship through adequate protein conformational sampling during MD simulations and through thorough testing of the effect of environment (gas phase, aqueous solution, protein) on AdoCbl excited states using QM and QM/MM calculations. Our results reveal the stabilization of certain charge transfer excited states of AdoCbl facilitated by specific amino acid residues and environmental factors in CarH, a combination of which is absent in aqueous solution and AdoCbl-containing enzymes. Our study lays the foundation for further investigations aimed at understanding the mechanism of AdoCbl photochemistry in CarH using experimental and computational methods, which has implications for the design of effective B12-based optogenetic tools for biological applications.

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5.8. A fragment based approach to the quantum dynamics of multichromophoric systems: application to the gc dna base pair

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A fragment diabatisation scheme is described, based on recent works [1-4], that can parameterise a linear vibronic coupling (LVC) Hamiltonian in a relatively automated fashion, for use with quantum dynamics calculations. It can treat internal conversion on individual chromophores, such as between bright ππ* and dark nπ* states, on the same footing as excitonic and charge transfer (CT) dynamics between chromophores. As an initial test, the method is applied to the guanine-cytosine (GC) Watson-Crick DNA base pair, an archetypal example of a multi-chromophoric species with individual local excitation structure. We compute the dynamics with ML-MCTDH and illustrate how strong electronic coupling of the ππ* states on G and C to the G→C CT state, combined with the large vibrational reorganisation energy of the G→C CT state leads to its efficient ultrafast population. We also show how formation of the GC pair leads to suppression of the population of the nπ* states. We believe the method can be useful for other multi-chromophoric species, such as light harvesting systems, and we will soon expand the approach to include the effect of solvent/environment.

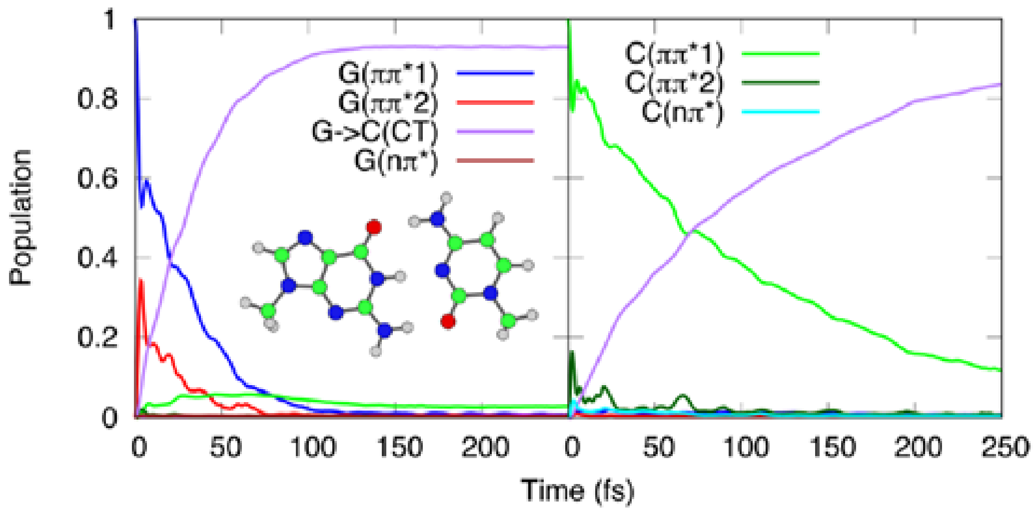

Fig. 1 Population dynamics following initial excitation to left: G(ππ*1) and right: C(ππ*1)

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5.9. Exploring the spatial features of electronic transitions in biomolecular systems by swift electrons

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In this contribution a conceived new kind of experiment (Fig. 1) will be introduced, that extends the technology of electron energy loss spectroscopy (EELS) to probe (supra-)molecular systems [1]. Indeed, understanding the electronic structure of matter is a formidable task that largely made use of optical spectroscopies and their corresponding selection rules, but not all the electronic transitions can be probed: for instance, a long debate in the literature is still ongoing on the possible role of charge transfer (CT) states in photosynthetic mechanisms: being dark, it can only be indirectly probed [2]. On the other hand, electron-beam spectroscopies are now emerging as probing techniques to study optical excitations with combined space, energy, and time resolution [3]. Performed in a scanning transmission electron microscope, EELS is based on inelastic scattering of fast electrons in a thin specimen and, very recently, new electron optics configuration has been introduced [4], opening the way to the analysis of the single components of orbital angular momentum (OAM) [5] of the outcoming electrons [6]. Physical insight into the proposed experiment is provided by means of a rigorous model to obtain the transition rate and the selection rule. Numerical simulations of DNA G-quadruplexes and other biomolecular systems, based on time dependent density functional theory calculations, point out that the conceived new technique can probe the multipolar components and even the chirality of molecular transitions, superseding the usual optical spectroscopies for those cases that are problematic, such as dipole-forbidden transitions, at a very high spatial resolution.

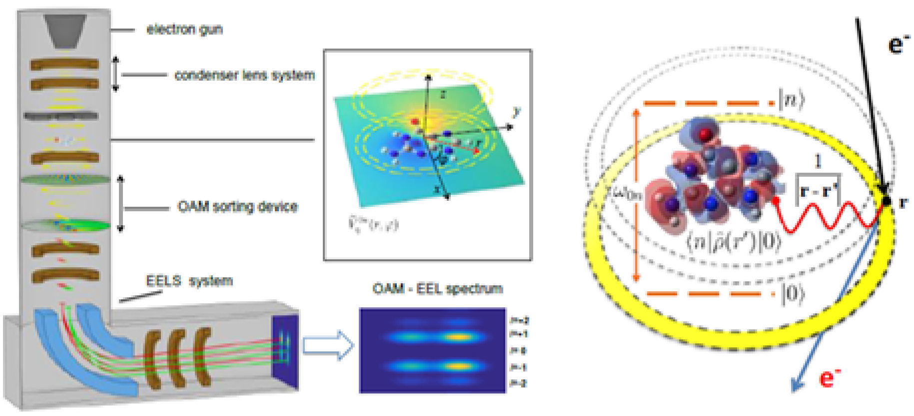

Fig. 1 Scheme of an OAM-resolved EELS experiment to investigate a molecular system.

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5.10. Active-site environmental factors customize the photophysics of photoenzymatic old yellow enzymes

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The development of non-natural photoenzymatic systems has reinvigorated the study of photoinduced electron transfer (ET) within protein active sites, providing new and unique platforms for understanding how biological environments affect photochemical processes. In this work, we use ultrafast spectroscopy to compare the photoinduced electron transfer in known photoenzymes. 12-Oxophytodienoate reductase 1 (OPR1) is compared to Old Yellow Enzyme 1 (OYE1) and morphinone reductase (MR), which all have flavin-based cofactors naturally used for ene-reductase chemistry and have been recently shown to engage in photoenzymatic catalysis with non-natural substrates [1-3]. The latter enzymes are structurally homologous to OPR1. We find that slight differences in the amino acid composition of the active sites of these proteins determine their distinct electron-transfer dynamics. Despite the high degree of structural homology between these proteins, their generation of amino acid radicals at the active site and the rates of charge recombination all distinctly differ from one another. Our work suggests that the inside of a protein active site is a complex/heterogeneous dielectric network where genetically programmed heterogeneity near the site of biological ET can significantly affect the presence and lifetime of various intermediate states. Our work motivates additional tunability of Old Yellow Enzyme active-site reorganization energy and electron transfer energetics that could be leveraged for photoenzymatic redox approaches.

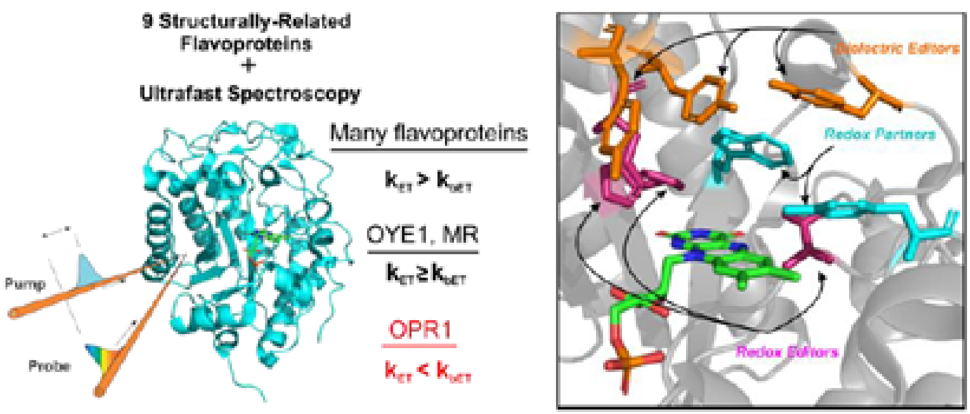

Fig. 1 Overview of this work. Ultrafast spectroscopy of nine structurally-related Old Yellow Enzymes reveals an unexpected complexity and diversity in electron transfer dynamics which are hypothesized to originate from a modulation of the reorganization energy by nearby tyrosine residues in the active site.

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5.11. Ultrafast dynamics of light-induced charge transfer in lactate monooxygenase

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Light-induced charge transfer is essential in various light-mediated biological processes including blue-light signalling [1], DNA repair by photolyases [2] and the recently reported decarboxylation of fatty acids by fatty acid photodecarboxylase (FAP) [3]. An important purpose of learning from the nature is to design light-controlled systems with broader functions. Besides applying photoreceptors to optogenetics, the directed evolution of existing flavoenzymes to non-natural photoenzymatic systems capable of catalyzing challenging stereoselective reactions is another promising direction [4].
Lactate monooxygenase (LMO) is a flavoenzyme that uses oxygen to convert L-lactate to acetate, CO2, and water [5].

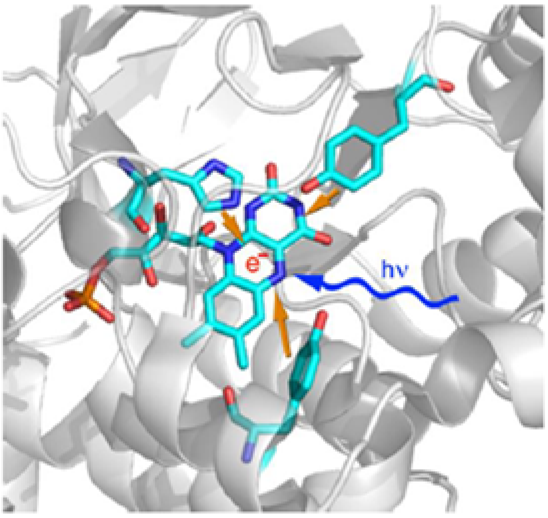

Fig. 1 FMN biding site of M. smegmatis LMO with proposed electron transfer pathways labeled with orange arrows.

Its photodecarboxylation activity towards various carboxylic acids [6] makes LMO an ideal platform for oxidative photoredox catalysis.
Here, we performed ultrafast spectroscopy on LMO from M. smegmatis and revealed the ultrafast dynamics of photo-initiated electron transfer (ET) at the flavin mononucleotide (FMN) binding site (Fig. 1). Forward electron transfer from nearby Tyrosine or Histidine residues to the excited FMN occurs in picoseconds, followed by backward ET to return ground state. ET from pyruvate substrate to FMN is slower than the intrinsic ET between FMN and amino acid sidechains. Our results provide mechanistic insights into photochemistry of LMO, facilitating future protein engineering for novel photoenzymatic catalysis.

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5.12. Light-activation mechanism of channelrhodopsin 2

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The channelrhodopsin 2 (ChR2) is a light-gated ion channel and a widely used tool in optogenetics. The photoisomerization of the retinal protonated Schiff base (RPSB) in ChR2 triggers the channel opening and firing of neuronal signals. Despite the importance of the ChR2, its light activation mechanism is still not fully understood in atomistic detail. In this work [1,2], we combine quantum dynamics, classical dynamics, electronic structure, and free energy calculations to comprehensively characterize the light activation mechanism of ChR2. Nonadiabatic dynamics simulations of both the wild type (WT) ChR2 and its E123T mutant are carried out using the ab initio multiple spawning (AIMS) method in a QM/MM setting, where spin-restricted ensemble-referenced Kohn-Sham (REKS) method is used to describe the QM region. Our simulations [1,2] agree well with the experiments and highlight the interplay between the photochemical reaction and the surrounding protein environment: (1) the E123T mutation changes the protein's electrostatic environment around the RPSB, and significantly slows down its photoisomerization; (2) the photoisomerization facilitates its subsequent deprotonation and the hydration of the ion channel. This work presents the first simulation of the photodynamics of ChR2 with a correlated first-principles electronic structure method and provides design principles for new optogenetic tools. [1,2]

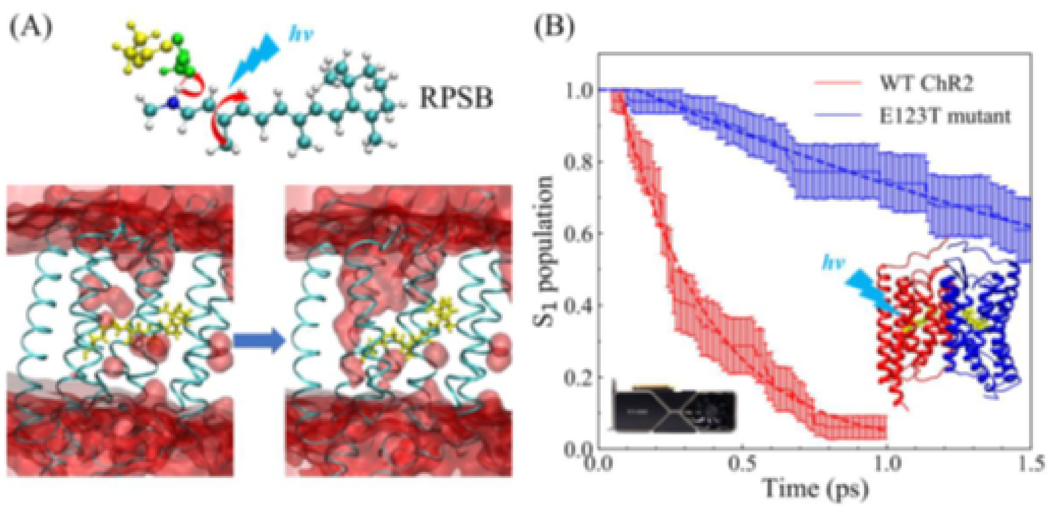

Fig. 1 (A) The RPSB photoisomerization in ChR2 facilitates its deprotonation and increases the channel's hydration level.
(B) The E123T mutation of ChR2 slows down the decay of S1 state population.

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5.13. Optical signature of strong hydrogen bonds

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Hydrogen bonds are usually considered as electrostatic interactions, considerably weaker than covalent bonds. Nevertheless, in some cases they can have a stronger and shorter three-centre covalent character. Here we employ our TDDFT-based Trajectory Surface Hopping implementation to show that strong hydrogen bonds can delay the passage through a conical intersection between the excited and the ground states, significantly increasing the fluorescent yield of non-aromatic molecules [1]. Furthermore, our simulations demonstrate that the delayed conical intersection is a unique signature that can be captured with ultrafast X-Ray absorption spectroscopy, offering new possibilities to study the dynamics of strong hydrogen bonds, both in the gas and condensed phases.

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5.14. Automated qm/mm model screening of rhodopsin variants displaying enhanced fluorescence

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We present a computational protocol for the fast and fully automatic construction of excited-states QM/MM models of rhodopsins and their subsequent screening as fluorescent proves, by extending our previously reported Automatic Rhodopsin Modeling protocol (a-ARM) [1]. The so-called “a-ARM rhodopsin fluorescence screening protocol”, is implemented through a driver written into the new Python-based ARM package software. Such a driver incorporates three sequential automated phases, each acting as an independent filter to categorize rhodopsin variants as dim-fluorescent or enhanced fluorescent systems, with respect to their wild-type form (Fig. 1). It has also a “one-click” command-line architecture capable of executing all phases without any user decision/intervention beyond the provided input (a list of target rhodopsin variants along with their ground-state a-ARM QM/MM models) and generate the output (a list containing the selected potentially enhanced-fluorescent candidates). The performance of the protocol and its implementation is assessed using a set of 10 microbial (Archaea) rhodopsin variants (wild type Archeorhodospin-3 and 9 of its mutants) with available experimental data on photophysical properties related to their fluorescent behavior, such as emission wavelength (λfmax), excited state lifetime (ESL) and (indirectly) fluorescence quantum yield (φf). It is also shown that the protocol successfully reproduced trends in λfmax and, therefore, was able to select the most likely enhanced fluorescent candidates in agreement with experimental evidence. Furthermore, in order to apply the protocol as a predictive tool we evaluate a set of 16 Anabaena Sensory Rhodopsin (ASR)-based variants that have not been experimentally investigated in terms of fluorescence behavior. We found that 9/16 candidates can be proposed as possible fluorescent candidates to be proven experimentally. Finally, we demonstrate that our proposed screening protocol is, therefore, suitable not only for identifying fluorescent candidates already tested experimentally, but also for proposing in silico new fluorescent candidates to be experimentally investigated.

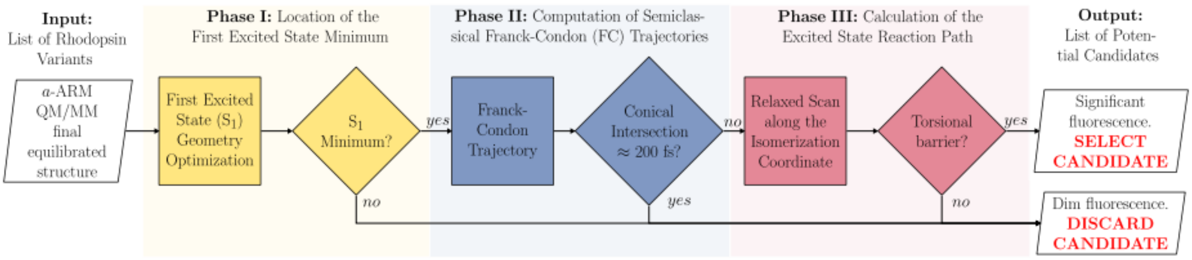

Fig. 1 This diagram displays the methodology for automatic searching of fluorescent rhodopsins. The protocol is composed of three phases: I) Location of the first excited state minimum, II) Franck-Condon trajectory calculation, and III) Relaxed scan along the isomerization path; each of these phases serves as a criterion to select/discard possible fluorescent candidates.

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5.15. Proton transfer in fluorescent proteins: a dynamical viewpoint on hydrogen bonds networks

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The Green Fluorescent Protein (GFP) is the prototype of fluorescent proteins, exhibiting a characteristic fluorescence emission following Excited State Proton Transfer (ESPT). [1] GFP chromophore (4-(p-hydroxybenzylidene)-imidazolid-5-one, HBDI) can exist in a neutral or anionic protonation state. Starting from GFP A form, predominant at the ground state, ESPT event leads towards the anionic B form. An intermediate (I*) state, anionic but retaining the A structure, is the immediate product of the proton transfer, then deactivating and evolving to the B one through conformational relaxation. [2,3] The three GFP forms show different chromophore cavity structural arrangements. In particular, distinct volumes and hydrogen bond networks are found. Fluctuations of H-bonds in the ground-state structures along the PT process (A, I and B) have been characterized from a dynamical point of view through ab initio molecular dynamics simulations. In particular, a cross-correlation analysis allows to show the concerted nature of H-bonds fluctuations. Such correlated residues interacting with the HBDI are often not close in space. Collective motions seem therefore to control the extensive H-bond network in the protein matrix around the chromophore. [4]
Moreover, the absorption properties of HBDI in two different solvents (water and methanol) have been modeled at the TD-DFT level by an ab initio MD sampling employing an innovative hybrid implicit/explicit solvent model, able to correctly reproduce the solute microsolvation. [5]

References

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[4] F. Coppola, F. Perrella et al. Front. Mol. Biosci. 7, 569990 (2020).
[5] U Raucci et al. J. Comput. Chem. 41, 2228-2239 (2020).

5.16. Theoretical study of internal conversion between b and q bands in a functionalized porphyrin

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The nature of internal conversion of 5-Ethoxycarbonyl-10-mesityl-15-carboxymethylbenzene porphyrin (1P) [1] is investigated using DFT and TDDFT with the range-separated hybrid CAM-B3LYP functional and the 6-311G(d,p) basis set. The solvent effects (THF) are taken into account within the polarized continuum model.
In addition to the excited states referred to classical Gouterman four-orbital model this study takes into account two additional dark states. One of them has similar orbital nature as the lowest dark state in case of the bare porphyrin [2]. However, another one is particular for 1P and involves the orbital on the substituent. It is shown that both of these states have intersection with the states of B band. Active normal modes that contribute to Internal Conversion due to vibrational relaxation are defined [3]. It is shown that the oscillator strengths of the Q band states along one of the active modes are increasing faster than for others. This is accompanied by the intersection of the Q band states. No crossing is obtained for other active modes. This leads to an assumption that this intersection may play an important role in the internal conversion process.

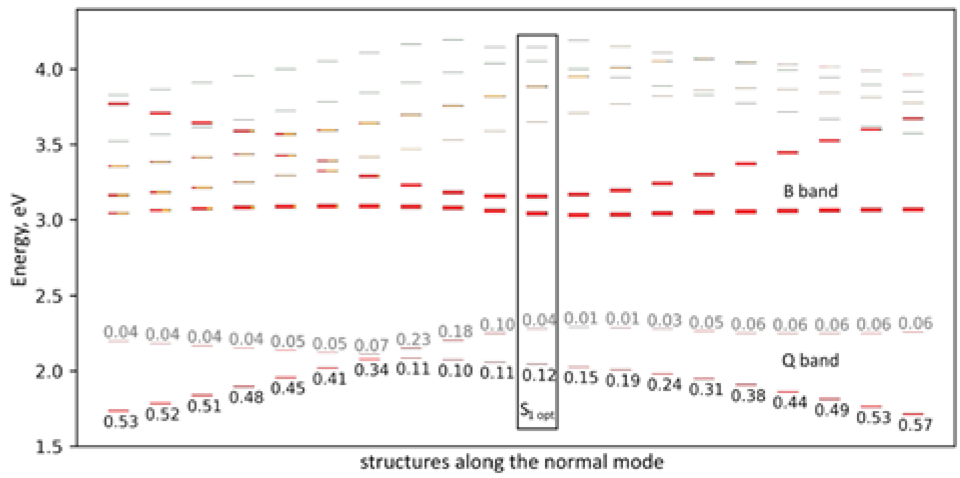

Fig. 1 Trajectory along the active mode of the system with respect to the ground state of each structure. Numbers on the plot show the oscillator strength of the 0-1 and 0-2 transitions. Thickness of the lines correlates with an oscillator strength.

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[2] K. Falahati, C. Hamerla, M. Huix-Rotllant, I. Burghardt, Ultrafast photochemistry of free-base porphyrin: A theoretical investigation of B→ Q internal conversion mediated by dark states, Phys. Chem. Chem. Phys., 2018, 20, 12483–12492.
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5.17. From taco to banana: turn-on mechanism of a fluorescent probe for imaging gabaa receptors

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Human GABAA receptors are a large and complex family of drug targets [1]. However, investigation of the multiple binding sites at the receptor is challenging and often needs the help of new research tools, especially optical ones. Here we report the turn-on mechanism of a novel imaging probe for monitoring allosterism in GABAA receptors [2]. We used classical molecular dynamics to find the binding mode of the probe and identify structural differences between the water and the biological receptor environment. Further, a multiscale quantum mechanics/molecular mechanics (QM/MM) approach was employed to calculate excited state properties related to fluorescence. Our calculations show that the probe undergoes drastic conformational changes upon binding to the receptor. While in water solution the probe adopts a 'taco-like' structure (Fig. 1), when binding to the GABAA receptor the 'taco' is required to unclench resulting in a 'banana-like' structure (Fig. 1B). Such conformational changes are the key to unlock the different electronic responses of the probe. In the "taco-like" conformation the probe retains intramolecular ππ-stacking interactions that quench fluorescence in solution. In contrast, in the biological environment, unclenching removes these intramolecular ππ-stacking interactions and generates fluorescence (Fig. 1C).

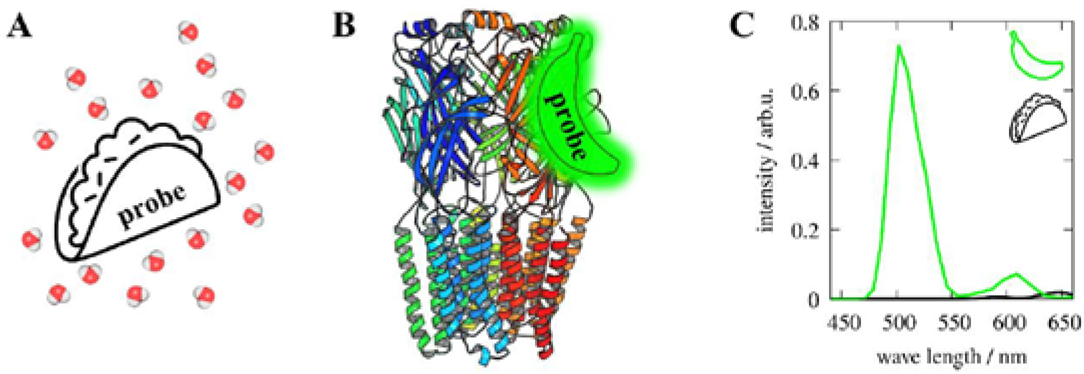

Fig. 1 Schematic representation of the turn-on effect of the probe. In solution the probe has a 'taco-like' structure (A) but upon binding to the GABAA receptor it adopts a 'banana shape' (B). The emission spectrum of the probe in solution (black) and in the receptor environment (green) is shown in panel (C).

References

[1] M. Ernst, F. Steudle, K. Bampali, eLS, 1–12 (2018).
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6. BEST ORAL CONTRIBUTION AWARDS

The two best flash presentations of the workshop were recognized with the "**Best Oral Contribution Award**".

The award recipients were announced during the closing session. A cash award and a certificate stating the award name, the name of the presenting author, and the title of the presentation were sent to the award recipient after the workshop. The "Best Oral Contribution Awards" are financed by Psi-k Network.

6.1. Selection Criteria

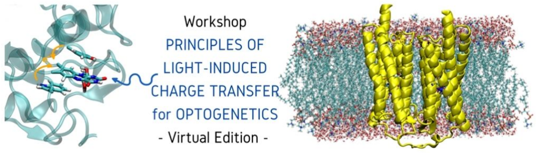
The criteria to define the winners were the following:

- Excellence of the candidate and presentation
- Relevance to the theme of the workshop
- Juniority
- Experimental/theoretical balance
- Gender balance
- Geographical balance

6.2. Winners

The winners of the "Best Oral Contribution Award" are Laura Pedraza-González from the Department of Biotechnology, Chemistry and Pharmacy, University of Siena, and Bryan Kudisch, from the Department of Chemistry, Princeton University, with the following contributions:

- Pedraza-González, L., Barneschi, L., Marszałek, M., Valentini, A., Padula, D., De Vico, L., Olivucci, M. (2021), "Automated QM/MM model screening of rhodopsin variants displaying enhanced fluorescence".
- Kudisch, B., Oblinsky, D. G., Black, M. J., Zieleniewska, A., Emmanuel, M. A., Rumbles, G., Hyster, T. K., Scholes, G. D. (2021), "Active-site environmental factors customize the photophysics of photoenzymatic old yellow enzymes".



This is to certify that

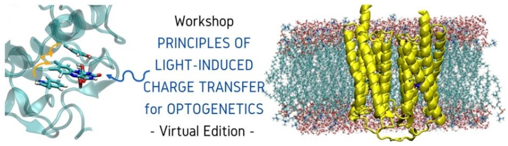
LAURA PEDRAZA-GONZÁLEZ

DEPARTMENT OF BIOTECHNOLOGY, CHEMISTRY AND PHARMACY, UNIVERSITY OF SIENA

has been recognized with the **"Best Oral Contribution Award"** in the "**Principles of Light-Induced charge transfer for Optogenetics**" workshop, held online on June 14-16, 2021, with the following contribution: Pedraza-González, L., Barneschi, L., Marszałek, M., Valentini, A., Padula, D., De Vico, L., Olivucci, M. (2021), "**Automated QM/MM model screening of rhodopsin variants displaying enhanced fluorescence**".

June 16, 2021

LAURA ZANETTI-POLZI
Workshop Coordinator



This is to certify that

BRYAN KUDISCH

DEPARTMENT OF CHEMISTRY, PRINCETON UNIVERSITY

has been recognized with the **"Best Oral Contribution Award"** in the "**Principles of Light-Induced charge transfer for Optogenetics**" workshop, held online on June 14-16, 2021, with the following contribution: Kudisch, B., Oblinsky, D. G., Black, M. J., Zieleniewska, A., Emmanuel, M. A., Rumbles, G., Hyster, T. K., Scholes, G. D. (2021), "**Active-site environmental factors customize the photophysics of photoenzymatic old yellow enzymes**".

June 16, 2021

LAURA ZANETTI-POLZI
Workshop Coordinator

CONCLUSIONS

During the whole workshop all participants showed a very high level of interest and keen participation. Despite the online format, which does not facilitate the speakers-audience interaction, the discussion was always lively and fruitful, showing the high competence of the participants in the workshop topics. The invited speakers delivered very interesting and inspiring talks, but we were also impressed by the high quality of the contributed talks. Overall, the whole workshop was characterized by a fruitful exchange of ideas that will surely motivate new research on the topic.

The workshop was undoubtedly successful: the number of applicants exceeded our expectations, the invited speakers presented very well their excellent research work, the contributed speakers delivered well-prepared and interesting talks. We are sure that all the speakers gave very interesting inputs to the participants, but we also think that very interesting inputs from the audience came out as well. We noticed that research on these topics has reached impressive levels, both from an experimental and from a theoretical point of view. We also believe that some possible routes for further developments in the field emerged during the workshop.

Annex 1. PARTICIPANTS LIST

First Name	Last Name
Davide	Accomasso
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Emanuela	Bertini
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Sofia	Canola
willMatteo	Capone
Volha	Chukhutsina
Sergio	Ciuchi
Roberta	Croce
Basile	Curchod
Isabella	Daidone
Giulia	Dall'Osto
Chintu	Das
Luca	De Vico
Sara	Del Galdo
Mara	Di Berardo
Rosa	Di Felice
Francesco	Di Maiolo
Jean Baptiste	Fankam Fankam
Elisa	Fresch
Marco	Garavelli
Valeria	Giliberti
Puja	Goyal
James	Green
Ciro Achille	Guido
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Petra	Imhof
Antonia	Intze
Francesco	Iori
XiuWen	Kang
john	Kennis
Tatiana	Korona

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Xiankun	Li
Ruibin	Liang
Giacomo	Londi
Emanuele	Marsili
Margherita	Marsili
Benedetta	Mennucci
Alessio	Milanesi
Andreas	Moeglich
Carla	Molteni
Uriel	Morzan
Som	Narayan
Daniele	Narzi
Isabelle	Navizet
Massimo	Olivucci
Michele	Ortolani
Daniele	Padula
Riccardo	Palombo
Dimitrios	Pantazis
Laura Milena	Pedraza Gonzalez
Fulvio	Perrella
Alessio	Petrone
Sandra	Pinto
Gur	Pyari
Xiaofeng	Qian
Nadia	Rega
Marco	Romanelli
Sukhdev	Roy
Carlo Andrea	Rozzi
Pavel	Rukin
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Supriyo	Santra
Igor	Schapiro
Gregory	Scholes
Yasmine	Shalaby
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Abhishek	Sirohiwal
Maria Eleonora	Temperini
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Laura	Zanetti-Polzi

First Name	Last Name
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