Spin-Dependent Site-Specific Charge Injection: A Novel Approach to Allosteric Regulation

Maria-Cristina Ghetu, Remi Casier, Dorit Levy, David Scheerer, Ron Naaman, Gilad Haran

Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot 76100, Israel

Funded by the European Union

Charge reorganization allostery

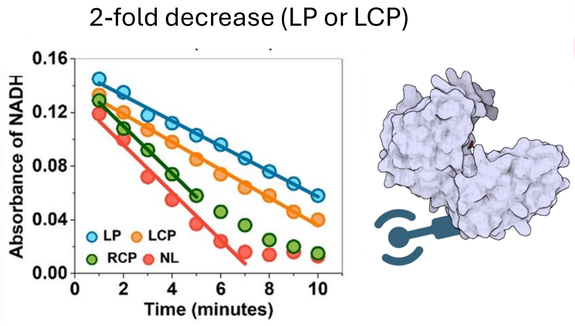
Allosteric regulation is an essential mechanism that controls protein function, typically explained by conformational changes or alterations in protein dynamics. However, recent research involving the chiral induced spin selectivity (CISS) effect has highlighted charge redistribution and spin polarization as significant contributors to protein function, a process referred to as charge-reorganization allostery (CRA).

Background

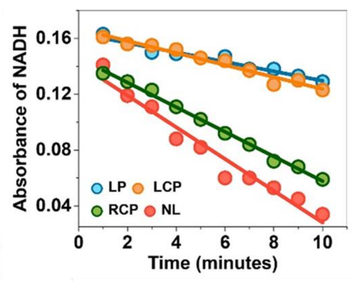
In our previous study, we examined two positions on PGK under various conditions, including no light (NL), linearly polarized light (LP), left circularly polarized light (LCP), and right circularly polarized light (RCP).

- **Position 9**

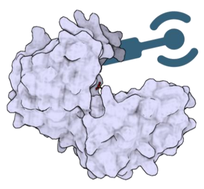
2-fold decrease (LP or LCP)



- **Position 290**



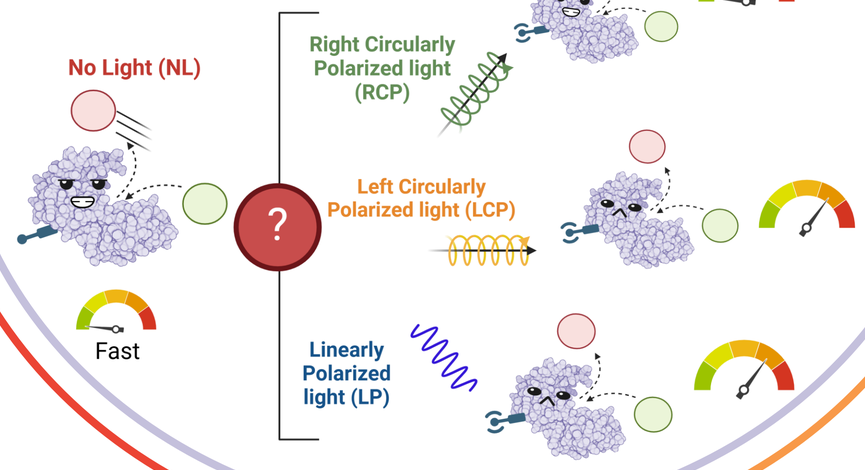
3-fold decrease (LP or LCP)



Proc Natl Acad Sci U S A. 2022 Aug 30;119(35):e2204735119.

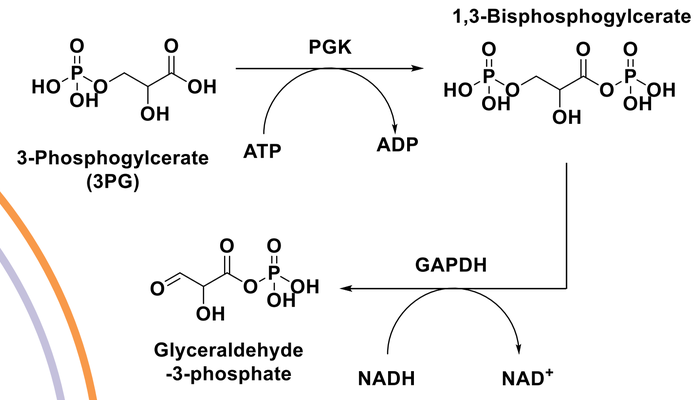
Aim

Our aim is to map information transfer in complex biological systems. We use 3-phosphoglycerate kinase (PGK) as a model to investigate how site-specific charge injection from a photosensitizer affects enzymatic activity.

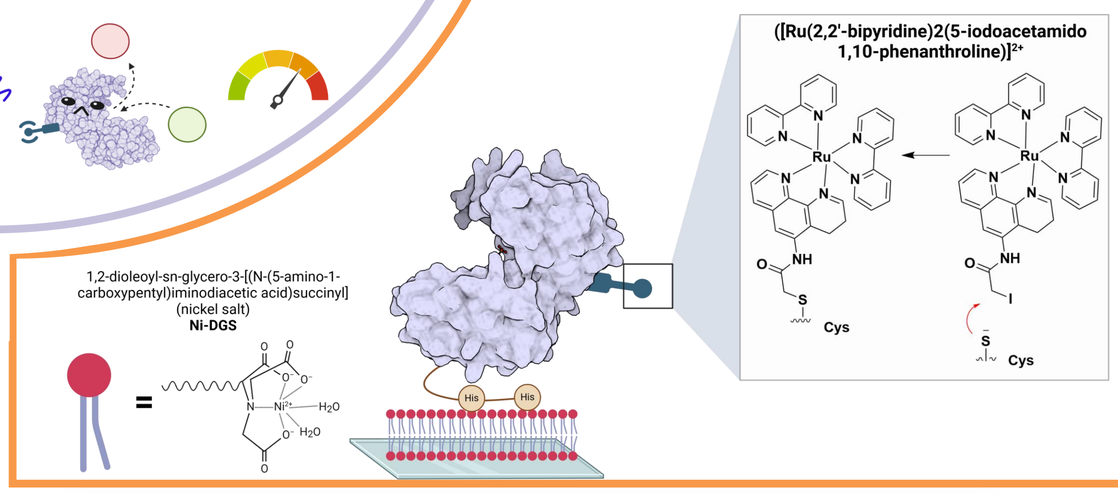


Methodology

PGK activity is assessed using a coupled assay with glyceraldehyde 3-phosphate dehydrogenase (GAPDH), where the reaction progress is monitored by tracking the consumption of nicotinamide adenine dinucleotide (NADH) at 340 nm. The variation in enzymatic activity under different conditions (with and without illumination) is determined by analyzing the slope of the NADH absorbance over time.



PGK, fused with a poly(histidine)-tag, is immobilized on a lipid-modified glass surface. The photosensitizer is covalently attached to specific sites via alkylation at targeted cysteine point mutations.

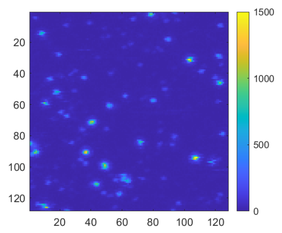


Optimization of the immobilization procedure

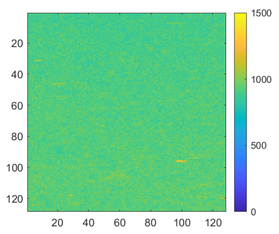
- **Lipid bilayer composition**

Fluorescent images of glass surfaces modified with either pure chelating lipids or a lipid mixture were taken to evaluate the impact of lipid bilayer composition on bilayer formation.

Ni-DGS
15 x 15 µm



Egg-PC - Ni-DGS
15 x 15 µm

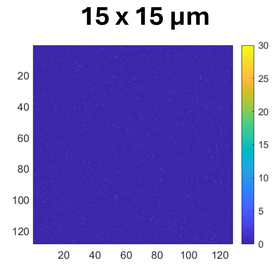


- Egg - PC - L-α-phosphatidylcholine

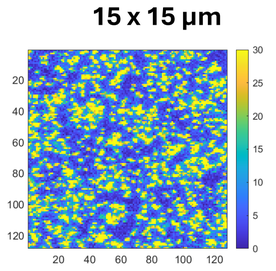
- **Is PGK specifically bound?**

We assessed the immobilization of PGK labeled with a fluorescent probe on the lipid mixture. Following protein binding, the sample was washed with imidazole to test the specificity of the immobilization, resulting in the elution of nearly all the protein, confirming the specificity of the process.

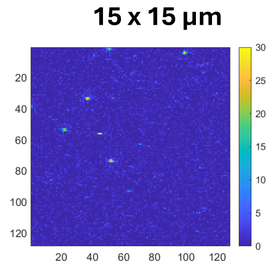
1. Before immobilization
15 x 15 µm



2. After immobilization
15 x 15 µm

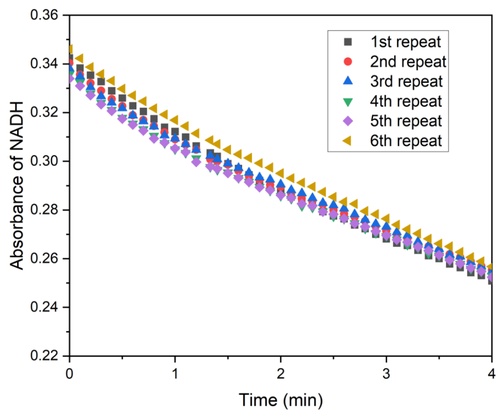


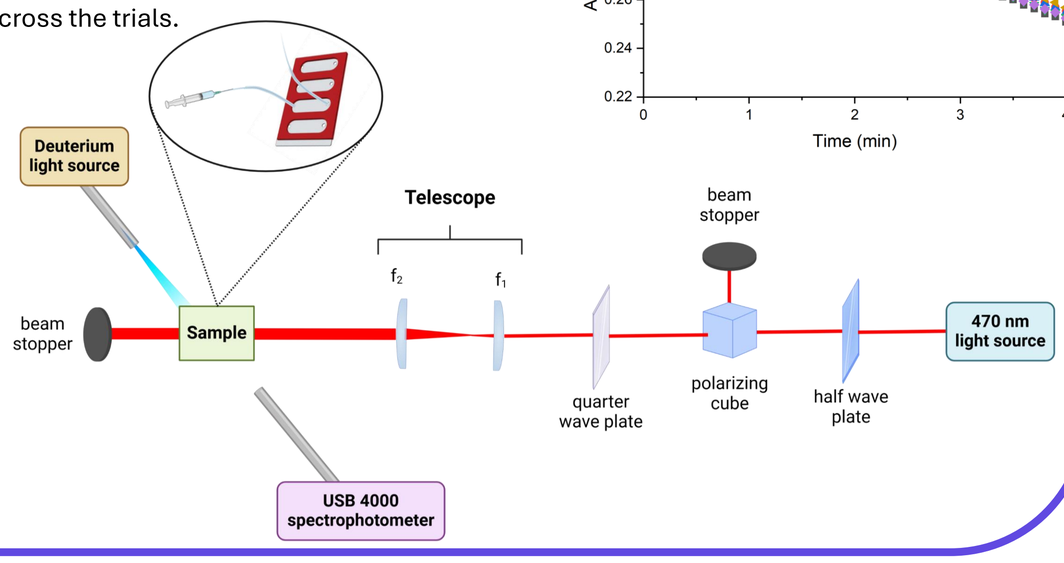
3. After imidazole wash
15 x 15 µm



Setup for in-situ excitation and measurement

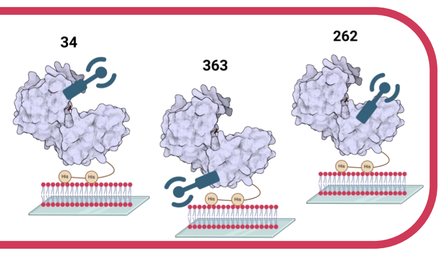
To maximize data collection while minimizing system disturbance, we developed a semi-automated optical setup for in situ measurement and excitation. Using this setup, we conducted preliminary enzyme kinetics studies and achieved good reproducibility across the trials.





What's next?

We plan to attach the photosensitizer at different positions on PGK and map the involvement of allosteric pathways initiated from different charge injection points



Funded by the European Union, in the frame of the Marie Skłodowska-Curie Actions (MSCA), Doctoral Network (DN) CISSE project n° 101071886. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.