Abstract:

Title of Project: Elucidating Cause-Effect Relationships in Light-Gated Enzymes Based on the Vibrational Stark Effect. A Theoretical Approach.

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Co-supervisor 2: Peter Hildebrandt, TU Berlin

Electrostatic interactions are essential for controlling protein structure and function. Changes of these interactions may occur upon light absorption of protein-bound cofactors in biological photosensors such as phytochromes and may thus constitute the starting point for subsequent relaxation processes and structural changes of the photosensor protein which eventually lead to the activation of coupled enzymatic processes. The central objective of this project is to elucidate the structural consequences of electric field changes as an important step towards understanding cause-effect relationships in photoenzymes. Here theoretical methods will be employed to achieve a consistent description of local electric fields in the photoreceptors and their changes during the photoinduced processes. The theoretical methodology will be tested and validated in model systems and subsequently extended to phytochromes, using as experimental reference the vibrational Stark effect (VSE) that results from the field-induced perturbation of vibrational transitions.

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1. Overall goal of the project

Electrostatic interactions are essential for controlling protein structure and function. The underlying forces are attractive or repulsive forces between charged residues and dipoles as well as hydrogen bonds. Changes of these interactions may occur, for instance, upon light absorption of protein-bound cofactors in biological photosensors such as phytochromes [1] leading to isomerization of the chromophore that in turn causes a re-orientation of charged or polar groups within the protein matrix. These alterations of the electrostatics around the chromophore may thus constitute the starting point for subsequent relaxation processes that are translated to more global structural changes of the photosensor protein and eventually lead to the activation of coupled enzymatic processes. However, predictions about the consequences of local changes of the electrostatics on protein structure and reactions are hardly possible or at least associated with substantial uncertainties. The central objective of this project is, therefore, to elucidate the structural consequences of electric field changes as an important step towards understanding cause-effect relationships in photoenzymes. The project utilizes the vibrational Stark effect (VSE) that results from the field-induced perturbation of vibrational transitions. The experimental data from VSE reporter groups in phytochrome and model systems, obtained within collaborations, will be used as a reference for theoretical calculations to achieve a consistent description of local electric fields in the photoreceptors and their changes during the photoinduced processes.

2. State of the art

The VSE has been widely used to assess local electric field in proteins by incorporation of appropriate VSE reporter groups [2]. Among them, the nitrile function is of particular importance since its field-sensitive stretching mode appears in a spectral region, free of any other vibrational modes of the protein. It can thus be readily detected by IR spectroscopy. Site-specific incorporation of nitrile functions into proteins can be accomplished by genetic engineering, either by the substitution with non-canonical amino acids, or by generation of single-Cyt variants in which the Cys thiol is subsequently modified to afford a nitrile function [3]. The extraction of electric field values from the frequency shifts of the VSE reporter groups was so far based on molecular dynamics (MD) simulations combined with electrostatic calculations [2]. However, these approaches suffer from the limitations of the classical force fields, which introduces additional inaccuracies to the inevitable uncertainties of electric field determinations that results from fluctuations of amino acid side chains and water molecules. Furthermore, the frequencies of nitrile groups do not only depend on the electric field projected along the C≡N bond but also on hydrogen-bonding (H-bonding) interactions. To overcome this specific drawback of these VSE reporter groups, previous studies attempted to separate H-bonding and electric field effects on the basis of empirical correlations using models systems with water as H-bonding partner. Recently results by First et al. [4] and from our group, however, suggest that these correlations are not applicable to H-bonding interactions in restricted geometries, as it is typically the case for reporter groups buried inside the protein. Thus, the theoretical analysis of the VSE frequencies is therefore a challenge that has not been mastered so far.

3. Specific aims and how they may be reached:

This project is primarily addressed to candidates with a sound background in theoretical or computational chemistry and basic knowledge on protein structures and functions as well as spectroscopy. The project is divided in 3 work packages. All facilities required for carrying out this project are available in the Mroginski (theory) and Hildebrandt group (experiments). Collaborations include inter alia the groups of R. Schlesinger (FU Berlin) and T. Friedrich (TU Berlin) in protein modification) and P. Scheerer (Charité) in protein structure analysis.

WP1. Development of the methodology on the basis of model systems

The non-canonical amino acids p-cyano-phenylalanine (p-CN-Phe) and 5-cyano-tryptophan (CN-Trp) that will be introduced into the target proteins will be first systematically studied in various solvents and solvent mixtures in which both the polarity and hydrogen-bonding (H-bonding) interactions will be varied. H-bonding interactions will be modified not only by water but also by organic molecules with different steric requirements (e.g., secondary amines) that impose restrictions to the H-bonding interactions with the nitrile function. The experiments will carried out as a function of the temperature since the contribution of H-bonding but not the electric field on the C≡N stretching frequency varies with the temperature. The experimental data, obtained by IR spectroscopy, will be then compared with theoretical calculations using ab initio molecular dynamics (MD) [5] and quantum-mechanics/molecular mechanics (QM/MM) methods in combination with classical MD simulations to achieve a consistent description of the electric field in non-H-bonding and H-bonding systems [6].

WP2. Validation and optimization of the theoretical approach

The theoretical methodology will then be extended to VSE reporter groups buried in model proteins which have been analysed in previous studies both experimentally and theoretically on the basis of empirical force fields.[2,4] If required, the experimental data set from the literature may be expanded by own experiments on selected model proteins, to be carried out in collaboration. The objective is to validate and eventually optimize the theoretical approach.

WP3. Extending the theoretical methodology to phytochrome

This WP focuses on the bathy phytochrome Agp2 for which first experimental results (substitution of Y165 by CN-Phe) are already available. Ongoing experiments will include further Tyr residues in the vicinity of or more remote from the chromophore. In addition, singly-VSE-labeled variants may constitute the basis for generating further site-specific substitutions that alter the electrostatic in the protein. The calculations will carried out for all states for which reliable structural models are available [1], starting with the parent state Pfr.

References:

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