Shifting Shapes: NMR Studies of Conformational Flexibility in Light Harvesting Complexes

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Project Goals: (see abstract)

Control of the optical properties is a key feature in the function of light harvesting proteins, including maximum wavelength of absorption. The protein environment has an important role in controlling these properties, through the hydrogen bonding, charge interactions and control of the chromophores internal degrees of freedom. NMR is a powerful method for analyzing protein ligand interactions. The structure, the plasticity and heterogeneity are expected to be important for optimal exciton lifetime and transfer efficiencies. Extensive NMR spectral assignments have been determined in my laboratory for LHC1. We previously assigned the backbone and sidechain protein signals for the intrinsic membrane portion of the light harvesting complex I from Rhodobacter Sphaeroides, in absence of the reaction center. Also, early all of the carbons of bacteriochlorophyll a in LH1 from Rhodobacter sphaeroides were assigned in situ using solid state NMR experiments. Two kinds of Bchl acyclic chromophores were detected that differ principally at the exocyclic acetyl group, a functionality that has previous been proposed to modulate the optical properties of the chromophore. This analysis indicates the presence of specialized environments and plasticity at key BChl exocyclic positions.

References

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Under DOE sponsored research, CFD Research Corporation (CFDRC) is currently developing predictive computational tools to address the goals of Kbase towards characterizing the response biological organisms to environmental stimuli that serve as inputs and predicting phenotypes most likely to be observed. Figure 1 shows a schematic of the framework being developed by CFDRC. Drawing upon available databases, our approach relies on the construction of mechanistic Systems Biology based and data-driven models of the differentially regulated cellular pathways. The complex pathway models are then analyzed without requiring information on the kinetics of various biochemical interactions. This enables the discovery and ranking of targets (for example genes, proteins or metabolites) for potential modification and the prediction of their response when these modifications are implemented. This approach thus offers the potential to inform experiments for the development of strains efficient at generating the desired phenotype such as algae strains that can produce biofuels at a higher rate.

As part of the ongoing Phase I study, we are developing a prototype of the software toolkit using transcriptional data to construct and analyze complex pathway networks in an extensible SBML format (Hucka et al., 2003) that will be enhanced to analyze other omic data types in future. Development of these tools will enable researchers to analyze pathways that play important roles in sensing and responding to the external conditions in an integrated manner. These tools are important to understand the organism’s behavior in the modified environment including its survival and in predicting the associated phenotypes. Towards this goal, we are studying the yeast environmental stress response to various external conditions as a test case to test and validate the model. We are also in active discussions with different organizations to demonstrate the technology for microbial systems of DoE interest e.g., identification of targets for genetic engineering of algal strains for higher quantity and quality biofuel production.

References


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Evolution Alters the Social Interactions in a Model Microbial Consortium

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There is great interest in engineering microbial consortia for a wide range of industrial applications from biofuel production to toxin degradation. Such consortia will often require finely balanced microbial interactions, and hence are likely to be highly sensitive to evolutionary change. Through a combination of engineering and experimental evolution we created a model microbial consortia consisting of an auxotrophic Escherichia coli (E. coli) and Salmonella enterica serovar typhimurium (S. typhimurium). When grown in lactose, E. coli provides a carbon source for S. typhimurium, while the S. typhimurium excretes the methionine needed by E. coli. Over 280 generations of evolution on agarose plates the productivity of this consortia decreased slightly as two E. coli strategies evolved. The numerically dominant E. coli evolved to increase efficiency by releasing less carbon byproducts into the media, thereby slowing growth of the community as a whole. A subpopulation of E. coli in each community took the opposite strategy and increased excretion of high-energy galactose; an adaptation that increases consortium growth. The growth phenotype of these two strategies is correctly predicted by dynamic flux balance analysis (dFBA) in a spatially structured environment. This work demonstrates that selection for increased growth rate can pleiotropically affect species interactions, and that in a community context increasing efficiency can be a selfish strategy.

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The GreenCut Resource, a Phylogenomically Derived Inventory of Proteins Specific To The Plant Lineage

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The plastid is a defining structure of photosynthetic eukaryotes and houses many plant specific processes, including the light reactions, carbon fixation, pigment synthesis, and other primary metabolic processes. Identifying proteins associated with catalytic, structural, and regulatory functions that are unique to plastid-containing organisms is necessary to fully define the scope of plant biochemistry. We performed phylogenomics on 20 genomes to compile a new inventory of 597 nucleus-encoded proteins conserved in plants and green algae but not in non-photosynthetic organisms. At the time of analysis, 286 of these proteins were of known function, whereas 311 are not characterized. This inventory was validated as applicable and relevant to diverse photosynthetic eukaryotes using an additional eight genomes from distantly related plants (including Micromonas, Selaginella, and soybean). Manual curation of the known proteins in the inventory established its importance to plastid biochemistry. To predict functions for the 52% of proteins of unknown function, we used sequence motifs, subcellular localization, co-expression analysis, and RNA abundance data. About 18% of the proteins in the inventory have functions outside the plastid and/or beyond green tissues. Although 32% of proteins in the inventory have homologs in all cyanobacteria, unexpectedly, 30% are eukaryote-specific. Finally, 8% of the proteins of unknown function share no similarity to any characterized protein and are plant lineage-specific. We have initiated functional analyses of the eukaryote-specific proteins and we present phenotypes for loss of function mutations in some of the unknown GreenCut genes.

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The Ins and Outs of Algal Metal Transport

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Metal transporters are a central component in the interaction of algae with their environment. They represent the first line of defense to cellular perturbations in metal concentration, and by analyzing algal metal transporter repertoires, we can get insight into a fundamental aspect of algal biology. The ability of individual algae to thrive in environments with unique geochemistry, compared to non-algal species commonly used as reference organisms for metal homeostasis, provides an opportunity to broaden our understanding of biological metal requirements, preferences and trafficking. *Chlamydomonas reinhardtii* is the best developed reference organism for the study of algal biology, especially with respect to metal metabolism; however, the diversity of algal niches necessitates a comparative genomic analysis of all sequenced algal genomes. A comparison between known and putative proteins in animals, plants, fungi and algae using protein similarity networks has revealed the presence of novel metal metabolism components in *Chlamydomonas* including new iron and copper transporters. This analysis also supports the concept that, in terms of metal metabolism, algae from similar niches are more related to one another than to algae from the same phylogenetic clade.