Improvisations in phototrophy. Protein engineering and functional investigation of rhodopsin proton-pumps

Ganapathy, S.

Summary

Solar energy is the most abundant source of renewable energy available on our planet, which is utilized by plants and certain microorganisms to fuel their growth and survival. Photosynthetic organisms use sunlight to fix atmospheric carbon dioxide and generate chemical energy, which can potentially be channeled towards the sustainable production of biofuels, platform chemicals, secondary metabolites and biomass in bioeconomy applications. However, the industrial applicability of photosynthesis is limited by the yield of the reaction. A significant part of the photon energy utilized by photosynthetic organisms to sustain their growth and maintenance is required to drive the photosynthetic reaction itself. Furthermore, the near-infrared region of the solar spectrum, which accounts for around half of the available photons at the earth surface, is essentially unexploited by photosynthesis.

In this thesis, we propose the use of microbial rhodopsins as an alternative photosystem in a complementary approach towards more efficient use of the photons in the solar spectrum. Microbial rhodopsins are photosensitive pigments implemented in the growth and adaptation of a large population of microorganisms. These relatively simple, tunable photosystems use a molecule of retinal as a chromophore to facilitate the conversion of sunlight to chemical energy. Retinal-based phototrophy is believed to sustain the phototrophic balance of various biospheres and has several important biotechnological applications. In this thesis, we describe the adaptation of two rhodopsin proton-pumps, namely proteorhodopsin (PR) and *Gloeobacter* rhodopsin (GR), to shift their action spectrum into the near-infrared region. In **Chapter 1**, we present a brief introduction to these proton-pumps and their underlying mechanism. We further describe our core research goal, namely, to generate and use near-infrared active proton-pumps as a complement to natural photosynthesis.

In **Chapter 2**, we discuss the first steps towards the spectral modulation of PR and GR using a combination of protein and chromophore engineering. We

describe the use of retinal analogs with different structural and electronic properties. These analogs are further combined with specific protein mutations, which were shown to have a complementary effect on the spectral properties of the analog pigments. Thus, we were able to generate both red and blue shifts in the spectral bands of PR and GR, with a minimal effect on the proton pumping function. From this chapter, we delineate the red-shifting analog retinal A2, which contains an extended conjugation in its ring element, as a template for further chromophore modification.

We focused on increasing the red-shifting potential of retinal A2 in **Chapter 3** with further modifications. The ring conjugation of A2 was extended by introducing electron withdrawing groups at the C3 position on the retinal. Of all analogs tested, one particular analog containing a 3-monomethylamino substituent, namely MMAR, was shown to induce the largest red-shift. MMAR pigments obtained with PR and GR were shown to be active upon illumination with 730 nm light, thereby representing the first near-infrared active proton pumps generated thus far. Quite remarkably, MMAR is very sensitive to the electrostatic environment of the retinal binding pocket, as demonstrated by a ~220 nm red-shift in the absorbance band upon acidification. A similar shift is also effectuated by a specific binding pocket mutation. The corresponding PR-D212N,F234S:MMAR pigment has an absorbance band peaking around 750 nm and thereby is the most red-shifted active microbial rhodopsin reported thus far.

The influence of a variety of site-specific mutations on the absorbance profile and activity of PR and GR were tested over the course of this research. However, a disadvantage inherent in this approach is that it is almost impossible to predict *de novo* whether and how a particular mutation will affect the spectral properties and/or the function of these proteins. To overcome this drawback, we designed a directed evolution based approach to evolve spectral variants of PR and GR, as detailed in **Chapter 4.** We tested various selection criteria, of which bacterial chemotaxis came out as the most efficient. We describe the construction of a novel chemotaxis-based directed

evolution assay, and the initial results obtained are very promising. This assay thus has the potential to evolve rhodopsin proton-pumps with spectral variations and/or augmented function.

The various mutant and analog pigments described in this thesis are ideal templates for further detailed biophysical characterization. Due to the insoluble nature of these membrane proteins, their detergent or lipid microenvironment often has an impact on their structure and dynamics. We assessed the influence of several detergent and lipid complexes on the stability, spectral properties and oligomeric assembly of PR and GR, as described in Chapter 5. We conclude that, ultimately, the choice of a suitable microenvironment depends upon the research question and corresponding methodology. We further provide examples of biophysical characterization of PR and GR made possible using different membrane-mimetic systems.

Chapter 6 presents the future outlook of this thesis. In summary, we have made significant progress towards the generation and characterization of near-infrared active rhodopsin proton-pumps. Follow-up studies involving the theoretical and biophysical characterization of these pigments have already been initiated. The chemotaxis assay described in this thesis has the potential for further evolution of these pigments, and in general, can also be applied towards other energy generating phototrophic systems. In conclusion, the near-infrared active proton-pumps generated in this thesis have important prospects in a number of biotechnological fields such as phototrophy mimetics, optogenetics and membrane sensor technology.