Engineering light-gated, transmembrane proteins for use in hybrid systems

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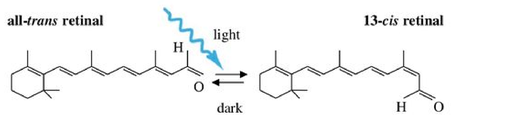
SAND2015-2919C

ABSTRACT

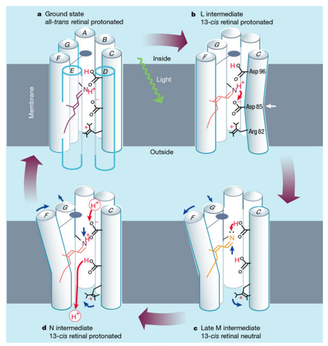
Nature provides a wide array of molecular machines that not only perform important biological functions, but also serve as potential building blocks for hybrid materials, devices, and systems. For example, bacteriorhodopsin (bR) is a transmembrane protein that functions as a light-gated proton pump as part of ATP synthesis and has been used in engineered systems for optical memory storage. Similarly, channelrhodopsin (cR) is a light-gated cation channel that has recently been used in optogenetic applications, such as remote control of neurons. Because both of these proteins use well-controlled photo-current generation, they could potentially serve as functional components of hybrid materials with enhanced optical and electrical properties. Central to this objective is the ability to engineer these proteins with specific characteristics to enable better understanding of the fundamental nature of these proteins as well as provide seamless integration into artificial environments. To this end, site-directed mutagenesis (SDM) was used to create mutations involving single amino acids that are to achieve novel function of these proteins in hybrid nanomaterials. This presentation will discuss the selection, modification, and function of specific mutations in both bR and cR. More specifically, mutational changes to bR will be discussed in terms of creating unique "chemical handles" for directed orientation bR on functionalized polycarbonate surfaces, as well as a mutation (Asp-85 residue with Thr) that enables bR to pump chlorine ions inward and protons outward under green light and reversibly, proton pumping inward under white light. The use of SDM in cR to mutate residues Cys-128 and Asp-156, which have a large effect on the conducting state and photocycle kinetics of cR, will also be presented.

BACKGROUND

- Retinal in both channelrhodopsin (cR) and bacteriorhodipsin (bR) is optically activated
- C128S and D156A mutations create a step-function tool in cR to allow for switching between "on" and "off"
- D85T mutation in bR converts proton pump into a chlorine pump regulated by green and white light



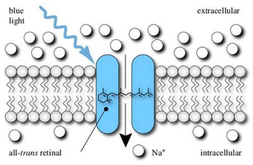
Bacteriorhodopsin (bR)



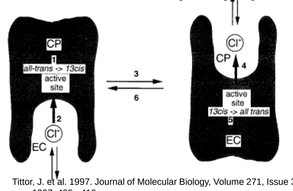
Kühlbrandt, W. 2000. Nature 406, 569-570

Light induced isomerization of al-*trans* retinal triggers conformation change in bR for proton pumping

Channelrhodopsin (cR)



Bacteriorhodopsin (bR)



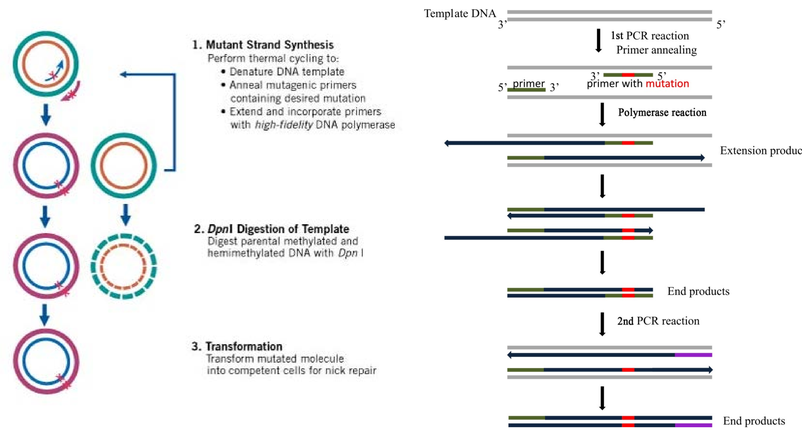
Tittor, J. et al. 1997. Journal of Molecular Biology, Volume 271, Issue 3, 1997, 405 - 416

METHODS

Channelrhodopsin

Site Directed Mutagenesis (SDM)

- SDM was utilized to achieve single amino acid mutations in cR, specifically, Cystein-128 with Serine and Aspartamine-156 with Alanine
- Beginning with our double-stranded DNA vector we designed an insert replacing the single amino acid of interest
- Two synthetic oligonucleotide primers, each containing the desired mutation and complimentary to the vector, were added prior to temperature cycling
- During PCR, the primers are extended into a full mutated plasmid
- Following PCR, the sample is treated with Dpn I endonuclease digestion to destroy parental DNA template and ensure selection of synthesized DNA
- The mutated plasmid is then transformed into XL10-Gold ultra-competent cells for verification



Verification and Purification

- Mutations were verified by sequencing chromosomal DNA of the selected XL10-Gold colony, as was the proper integration into the genome checked by PCR.
- The verified plasmid construct was transformed into *Pichia pastoralis* yeast cells and subsequently scaled up for full growth. After growth and expression of *P. pastoralis* with the incorporated mutants (D156A, C128S), cells were lysed and cR was purified using Ni-NTA Agarose affinity chromatography matrix.
- SDS-PAGE Analysis was performed to confirm presence of purified cR.

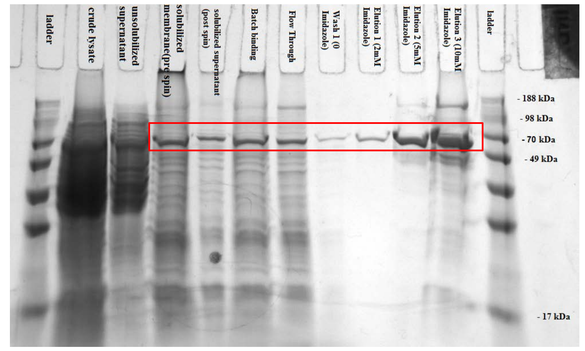
Bacteriorhodopsin

Transformation by Homologous Recombination

- *Halobacterium salinarum* cells were spheroplasted and combined with the D85T plasmid construct. After regeneration and recovery, the cells were plated to select for integrants.
- Successful transformations were scaled up for maximum growth over several weeks before being spun down and harvested.

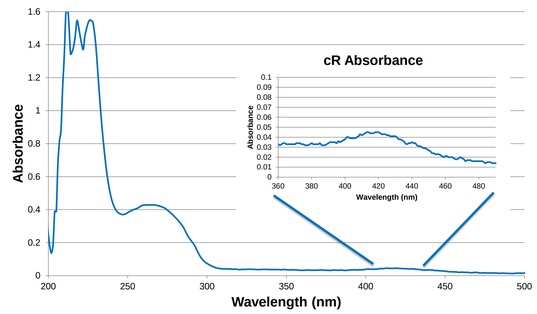
RESULTS

SDS PAGE cR



- cR dimer ~66kDa
- Protein visible from lysis through elution

cR Absorbance



- Expected protein peak at 280nm
- cR absorbs at 480nm

CONCLUSIONS

- Light gated transmembrane proteins like bacteriorhodospin and channelrhodopsin can be engineered for integration into artificial environments
- Discovery of additional useful point mutations in both of these proteins will provide further, novel application to the fields of photocurrent generation and optogenetics

ACKNOWLEDGEMENTS

UNM ACS for travel funding. Research support through Sandia National Laboratories' Laboratory Directed Research and Development office.

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000