Protein-Level Proteorhodopsin Expression Patterns in Aquatic Microbes

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Anoxygenic forms of phototrophy – which do not split water and are not necessarily coupled to carbon fixation – are increasingly recognized as important contributors to the energy budgets of microbial communities. One form of anoxygenic phototrophy, which has garnered attention for its apparent prevalence in a wide variety of both environments and taxa [1], is based on proteorhodopsin (PR), a light-activated, retinal-containing proton pump. PR generates a proton gradient that microbes can potentially use for ATP synthesis, flagellar movement, or nutrient transport, but its physiological role in marine microbes and its contribution to the overall energy budget of marine microbial communities is not yet clear. Previously, PR and its vertical distributions have been estimated using metagenomics [2] and retinal concentrations [3], but not yet using direct quantification of the protein itself, principally due to challenges in detecting this integral membrane protein using proteomic mass spectrometry. Understanding protein-level gene expression patterns of PR and which microbes are its main users in the field is a key missing piece of information to determine the role of PR-based phototrophy in aquatic microbial communities.

Here we quantify protein-level PR expression using mass spectrometry-based proteomics. We developed new techniques for extracting and quantifying PR using *in vitro* isotopic peptide labeling [4]. Expression of PR in several model aquatic microbes was monitored under a range of carbon and light conditions, including varying availability of single and complex carbon substrates, to explore the wild-type physiological role of PR. We also applied these techniques to detection and quantification of PR expression in aquatic microbial communities, to assess which groups of organisms contribute to anoxygenic phototrophy under natural conditions.

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