Proton release and uptake in a membrane protein traced with microsecond resolution by a pH-sensitive vibrational probe

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Infrared (IR) spectroscopy has been successfully used in the past to probe the dynamics of internal proton transfer reactions during the functional mechanism of many membrane proteins, but has remained mostly silent to protonation changes in the aqueous medium. In the present work we have overcome such limitation and resolved proton release and uptake events in the light-driven proton-pump bacteriorhodopsin (BR) by selectively monitoring vibrational changes of buffer molecules with 6 µs resolution. As a pH-sensitive vibrational probe we used 2-(*N*-morpholino)ethanesulfonic acid, MES, and its perdeuterated form. Thus, internal and external proton transfer reactions of BR and potentially in other proteins can be now simultaneously probed in a single time-resolved FT-IR experiment, allowing for the comparison of proton release and uptake events with other molecular processes within the protein at unprecedented detail.

We exploited this technical achievement to test our current understanding of the proton pumping mechanism of BR, in particular regarding the proton release and uptake steps. We demonstrate for the first time that the so-called *continuum* band, extending from 2300 to well below 1700 cm^{-1}, consists of two independent spectral contributions. The first contribution corresponds to deprotonation of the proton release complex (PRC), a complex in the EC domain where an excess proton is shared by internal water molecules. From kinetic and spectral considerations we tentatively assign the second component of the *continuum* band to the proton uptake complex (PUC), a complex with an excess proton reminiscent to the PRC but located in the cytoplasmic (CP) domain. Integrating additional results, we propose a revised version of the proton transfer reactions responsible for the light-driven vectorial proton translocation by BR that challenges the current standard model.