

Infrared nano-spectroscopy study of the heterogeneity of protein conformation in purple membranes

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The macroscopic properties of matter often derive by the short-range arrangement of its constituents, this holding for both organic and inorganic matter. Cell membranes are indeed intrinsically heterogeneous, as the local protein and lipid distribution is critical to physiological processes. In particular, the conformational changes of the membrane proteins are one of the key functions of cell signaling, and indeed they are at the base of novel biotechnology methods for the external control of cell signaling such as optogenetics. Also in template systems, that host a single type of proteins, there can be an heterogeneous response to an external stimulus or to environmental factors.

Here we report on the heterogeneity of protein conformation of bacteriorhodopsin hosted in native purple membranes. Infrared nano-spectroscopy [1] is here used to identify conformational changes connected to the hydration state of the transmembrane proteins contained in a 30 nm diameter cell membrane area, without the need for fluorescent labels. In dried purple membrane monolayers, areas with fully hydrated proteins are found among large numbers of molecules with randomly distributed hydration states [2].

[1] F. Lu, M. Jin and M.A. Belkin, *Nat. Phot.* **8**, 307 (2014).

[2] V. Giliberti et al., *Small* *accepted for publication*