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Mechanical mapping of single proteins at submolecular resolution

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The capacity of proteins to carry out different functions is related to their ability to undergo changes in their structural conformation, which are directly related to the flexibility of protein domains. There are various techniques to estimate the flexibility of proteins, such as estimation of *B*-factors from crystallographic analysis[1], thermal fluctuations from neutron scattering[2] and force measurements using atomic force microscopy (AFM)[3,4]. However, *B*-factors and thermal fluctuations do not provide direct measure of the mechanical stability. Classical AFM force spectroscopy offers little information about the flexibility of the folded protein. And finally, torsional mode AFM does not control the applied force and provides limited resolution[4]. In this work, we applied an imaging mode based on indentation force spectroscopy to map quantitatively the flexibility of individual membrane proteins in their native, folded state at unprecedented submolecular resolution. Our results enabled us to correlate protein flexibility with crystal structure and showed that α-helices are stiff structures that may contribute importantly to the mechanical stability of membrane proteins, while inter-helical loops appeared more flexible, allowing conformational changes related to function. Flexibility maps allowed us to estimate the elastic energetic cost of protein conformational changes during the bacteriorhodopsin photocycle.

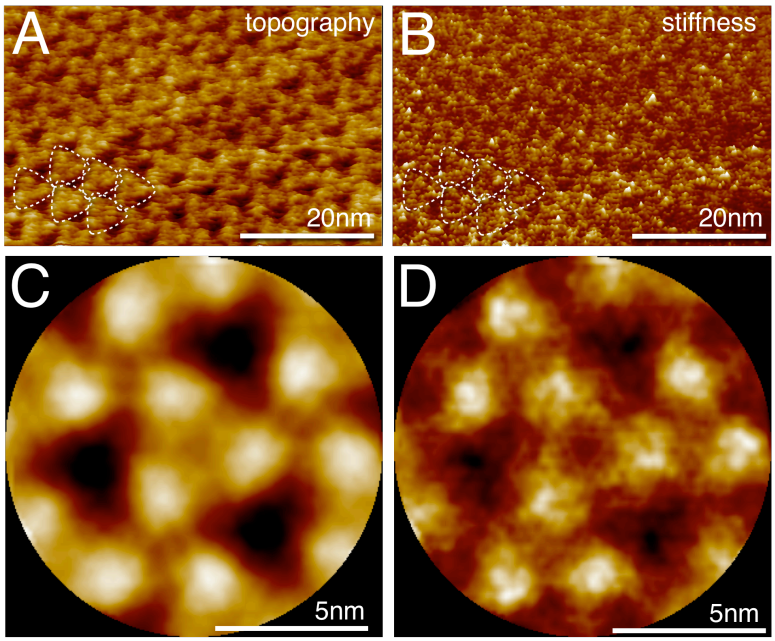

Fig. 1: *High-resolution images of topography (A) and stiffness (B) of bacteriorhodopsin. Individual trimers are encircled. Three-fold symetrized correlation average topograph (C) and stiffness (D) calculated from 13 bacteriorhodopsin trimers from A and B.*

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