1199-Plat

A Transformation for the Mechanical Fingerprints of Complex Biomolecular Interactions

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Biological processes are carried out through conformational transitions, ranging from the structural changes within biomolecules to the formation of macromolecular complexes and the associations between the complexes themselves. These transitions cover a vast range of timescales and are governed by a tangled network of molecular interactions. The resulting hierarchy of interactions, in turn, becomes encoded in the experimentally measurable "mechanical fingerprints" of the biomolecules, their force-extension curves. How can we decode these fingerprints so that they reveal the kinetic barriers and the associated timescales of a biological process? Here, we show [1] that this can be accomplished with a simple, model-free transformation that is general enough to be applicable to molecular interactions involving an arbitrarily large number of barriers. Specifically, the transformation converts the mechanical fingerprints of the system directly into a map of force-dependent rate constants. This map reveals the kinetics of the multitude of rate processes beyond what is typically accessible to direct measurements. With the contributions from individual barriers to the interaction network now "untangled", the map is straightforward to analyze in terms of the barriers and timescales.

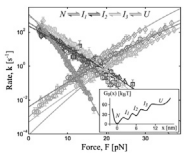

Fig. A rate map obtained by applying the transformation to the mechanical fingerprints of a system with a cascade of barriers along the dissociation pathway.

[1] Y. Zhang and O.K. Dudko, PNAS 2013, doi:10.1073/pnas.1309101110.

1200-Plat

Long-Range Correlated Motion Changes with Protein-Ligand Binding

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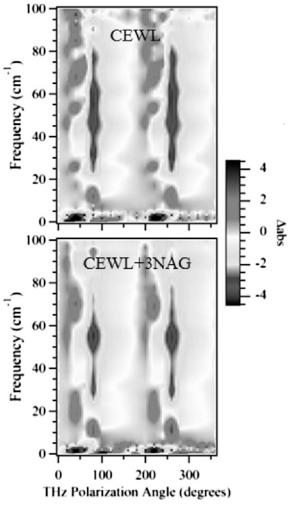
Molecular dynamics calculations have long predicted large scale protein structural vibrations lie in the terahertz (THz) frequency range (5-100 cm^{-1}), and these vibrations are related to protein function. Measuring these vibrational modes has been challenged by the large glass-like contribution from solvent and side chain librational motions. We remove this isotropic background, using anisotropic THz near field microscopy measurements of protein crystals. The technique reveals for the first time narrow band protein excitations in this frequency range. To determine if these features arise from the internal molecular motions, we measured ligand binding dependence using a faster data acquisition technique. The measurements performed on tetragonal chicken-egg white lysozyme (CEWL) single crystals and tetragonal CEWL tri-N-acetylglucosamine inhibitor bound crystals (CEWL+3NAG) show reproducible spectra that change dramatically with inhibitor binding. The large shifts observed indicate the features arise from the protein intramolecular motions and not from crystal phonons, which would have frequency shifts of only $\leq$ 2% with binding. The results validate that the technique can be used to determine ligand binding for inhibitor screening and to understand the role of intramolecular motions in protein function. This work supported by NSF MRI^2 grant DBI295998.



1201-Plat

Proteome-Wide Characterization of Protein Localization Dynamics in Escherichia Coli

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Bacteria exhibit a surprising complexity of subcellular organization despite the absence of membrane-bound organelles and cytoskeletal motor proteins. To capture and analyze localization dynamics throughout the cell cycle at a proteome-wide scale, we combine time-lapse fluorescence microscopy and automated image analysis to capture the cell-cycle localization dynamics of nearly every protein in *E. coli* with non-diffuse localization. For each protein, we capture hundreds of complete cell cycles that facilitates both the quantitative analysis of cell-cycle dynamics and cell-to-cell variation in protein localization. Global analysis of the localization patterns not only recapitulates well-established localization patterns, but also reveals many significant variations in localization both spatially and temporally. We briefly explore one new global insight into protein localization that we believe will be of universal interest: Asymmetric partitioning of proteins at cell division. Although cell division in E. coli was long believed to be essentially symmetric, we have discovered a significant number of transcription factors that partition asymmetrically. This observation in E. coli suggests that processes like asymmetric cell division, which plays a central role in development, have primitive precursors in bacterial cells with even the simplest life cycles.

Platform: Membrane Receptors and Signal Transduction II

1202-Plat

Flim- FRET, a Structural Tool for ErbB Receptor Studies in the Living Cell

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The association state(s) and activities of the ErbB receptor family members in intact living cells differ widely depending upon expression levels and their distribution and interaction partners. There are contradictory views in the literature about the aggregation states and presumed structures of the receptors in the cell membrane. Fixation artifacts may account for apparent quantitative discrepancies. We obtained biophysical FRET/FLIM data on living cells that reveal structural features of ErbB1 (EGFR) and ErbB2 as well as the effects of EGF and various kinase inhibitors on these structures.

We constructed transgenes in which an acyl carrier protein sequence was introduced between the signal peptide and the mature receptor protein sequence. ACP-ErbB1 behaves similarly to wild type ErbB1 with respect to EGF binding, activation and internalization. The ACP-ErbB2 lacks the capacity for binding ligands but can be transactivated as a heterodimer with ErbB1 or ErbB3. Enzymatic labeling of the specific serine in the ACP tag by fluorescent CoA substrates served as donors. The FRET acceptor was the novel membrane probe, NR12S, which is confined exclusively to the outer leaflet of the plasma membrane.

Addition of NR12S to the cells led to a dramatic reduction in the fluorescence lifetime of the donor, indicating a close proximity of the N-terminus of the ErbB1 ectodomain to the plasma membrane, supporting the published autoinhibited structure. EGF addition caused a time-dependent increase in the donor lifetime (reduced FRET), in accordance with the extended dimeric ectodomain structure oberved by Xray-crystallography. The effects of kinase inhibitors on these states and on ensuing endocytosis were also studied. The influence(s) of ErB2 density and antibodies interfering with receptor dimerization were additional topics addressed in this study. TCSPC lifetime images were analyzed with Mathematica software developed for these studies.

1203-Plat

Clustering of H-Ras on the Plasmamembrane of Living Cells

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The composition of the plasma membrane has long been modeled as a fluid mosaic. Studies in the last few years have identified microdomains like lipid rafts and caveolae and membrane-skeleton related fences that constrain membrane proteins within a small region of the cellular plasma membrane. These domains facilitate anchoring of different signaling proteins, like the Ras family of proteins, that has been shown to co-localize with nano domains upon activation by single-molecule tracking studies. It is believed that these nanodomains function as important platforms for a multitude of signalling cascades that are initiated at the plasma membrane. Given that many of the transmembrane signals will need a coordinated domain organization, it is of importance to investigate properties like size, shape, stability and their mutual interaction in a live-cell setting.

Here we transfected 3T3-cells to express the membrane anchors of H-Ras, N-Ras and K-Ras, respectively, when fused to the photoswitchable protein mEos2. Photo-activated localization microscopy (PALM) was used to make super-resolution images of Ras-anchor distributions on the apical membrane of the cells. The spatial distributions were tested against a homogeneous distribution by means of Ripley's analysis. Data showed that for all membrane anchors, the distributions deviated significantly from purely