

Integrated Use of AFM in Cell Biology and Biophysics: Probing Matrix Elasticity for Stem Cells and Protein Structure for Mechanism

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Cells generate force and are also exposed to external forces, but whether molecular rearrangements occur or not has not been clear. Do proteins within cells unfold under force and change in tertiary structure, and/or do the proteins dissociate from each other with changes in quaternary structure due to stress? We have developed a suite of nano-mechanical and chemical approaches to address these questions. Coupled AFM nano-mechano-chemical schemes with purified proteins [1] establish a general methodology for cell studies, and the strong temperature dependence of biomolecular transitions must be appreciated – not only for the special challenges it presents at the single molecule scale [2]. The nanomechanical probing has been further used to characterize the compliance of substrates that cells adhere to and apply stresses to (in proportion to substrate compliance) [3], and this fact together with the entire set of experimental/computational methods and ideas can be extended to help identify – within living cells – proteins and their sites that indeed unfold and dissociate under stress [4].

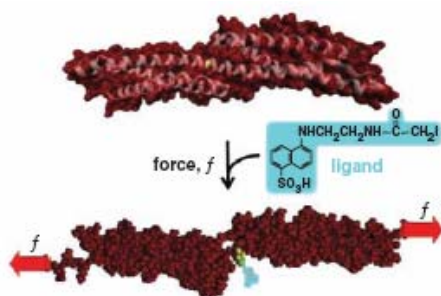


Figure 1: Force-induced changes in protein structure are hypothesized to expose novel binding sites for ligands. This example of a molecular dynamics simulation shows that Cysteine1167 in β -spectrin exposes 0 \AA^2 surface area (of 224 \AA^2) until forced extension exposes Cysteine's thiol group for reaction.

References

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