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52 Tubulin bond energies and microtubule biomechanics determined from nanoindentation in silico

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51 A region in the middle domain of *E. coli* Hsp90 is important for collaboration with DnaK

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Molecular chaperones are critical members of the cellular protein quality control system that use the energy from ATP hydrolysis to assist in protein remodeling activities. Heat shock protein 90 (Hsp90) is a widely conserved and highly abundant molecular chaperone that assists in the folding and reactivation of a diverse set of client proteins. Since many of these client proteins have been linked to cancer, inhibition of Hsp90 is of interest for cancer therapy. Hsp90 assembles as a highly flexible homodimer and undergoes large-scale structural rearrangements due to ATP binding and hydrolysis in order to remodel client proteins at various stages of folding. Several cochaperones have also been shown to interact with Hsp90 to modulate ATPase activity.

In *E. coli*, the DnaK chaperone system (homologous to the eukaryotic Hsp70 system) has been shown to collaborate and directly interact with Hsp90 ($Hsp90_{Ec}$) (Genest, Hoskins, Camberg, Doyle, & Wickner, 2011). We identified several residues of $Hsp90_{Ec}$ that are important for interaction with DnaK by making random substitutions in $Hsp90_{Ec}$ and screening for loss of interaction with DnaK by using a bacterial two hybrid assay. Additional mutants in nearby surface exposed residues were also constructed. The $Hsp90_{Ec}$ variants were purified and tested *in vitro* for ATPase activity and client protein remodeling activity in collaboration with the DnaK system. Our results indicate that a surface exposed region on the middle domain of $Hsp90_{Ec}$ is important for collaboration with DnaK. In order to determine whether this region is functionally conserved, we made a homologous substitution in yeast Hsp90 (Hsp82). The wild type and mutant proteins were purified and compared in protein reactivation assays *in vitro*. They were also tested in ATPase assays in the absence and presence of several yeast co-chaperones and client proteins. The results indicate a lower rate of client reactivation by the Hsp82 mutant. The mutant also exhibited defective ATPase activity in the presence of some cochaperones, suggesting this region is involved in an important protein-protein interaction or a conformational change. We are currently using molecular modeling to further explore the potential direct interaction between DnaK and the middle domain region of $Hsp90_{Ec}$.

Reference

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52 Tubulin bond energies and microtubule biomechanics determined from nanoindentation *in silico*

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Microtubules (MTs) play essential roles in health and viability of eukaryotic cells. MTs are stabilized by longitudinal and lateral non-covalent bonds between the αβ-tubulin subunits. However, the thermodynamics of these bonds and the MT physico-chemical properties are poorly understood. In this study, we explore the biomechanics of MT polymers using multiscale computational modeling and nanoindentations *in silico* of a contiguous MT fragment. Our approach is based on a combination of the Self-Organized Polymer model and all-atom Molecular Dynamic simulations of the MT fragment, accelerated on Graphic Processing Units (Zhmurov et al. 2010). Good agreement between the simulated and experimental force-deformation spectra (de Pablo et al. 2003) enabled us to correlate the MT biomechanics with dynamic structural transitions at the nanoscale. Our mechanical testing revealed that the compressed MT behaves as a system of

rigid elements interconnected through a network of lateral and longitudinal elastic bonds. The initial regime of continuous elastic deformation of the MT is followed by discrete structural transitions, which include first the reversible dissociation of lateral bonds and then irreversible dissociation of the longitudinal bonds. From our simulations we have determined the free energies of dissociation of the lateral (6.9 ± 0.4 kcal/mol) and longitudinal (14.9 ± 1.5 kcal/mol) tubulin-tubulin bonds. These values, in conjunction with the large flexural rigidity of tubulin protofilaments we obtained (18,000- 26,000 $pN{\cdot}nm^2$), support the idea that the disassembling MT is capable of generating a large mechanical force to move chromosomes during cell division. Our computational modeling offers a comprehensive quantitative platform to link molecular tubulin characteristics with the physiological behavior of MTs. The developed *in silico* nanoindentation method provides a powerful tool for the exploration of biomechanical properties of other cytoskeletal and multiprotein assemblies (Kononova et al. 2014).

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53 Ion channels: from structure refinement and remodeling to functional mechanisms

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54 Protein folding simulation using temperature based cascade MD

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Protein folding is a multi micro second time scale event and involves many conformational transitions. Large conformational transitions important for biological functions are difficult to capture using super-computers.