supported by Hamiltonian Replica Exchange (H-REUS) along the root mean square deviation of a set of selected distances (dRMSD) between protein backbone atoms. For a segment length of ten amino acids the approach allowed reasonable convergence of the calculated free energy change along the reaction coordinate and rapid scanning of protein segments. The method was applied to intrinsically disordered protein segments of the protein Axin which adopts an alpha-helical structure in the interaction regions upon binding to protein partners. The predicted regions of enhanced tendency to adopt alpha-helical conformations were in good agreement with available experimental data. The approach could be useful to characterize transiently stable motifs in disordered proteins and to estimate the free energy associated with the transition to a target conformation.

1592-Pos Board B543
Computational Study of Ubiquitin Recruitment and Transport of the 26S Proteasome
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Protein degradation is vital for a variety of essential cellular processes like apoptosis and transcription. Its malfunction is associated with severe diseases including cancer and neurodegenerative diseases. In eukaryotes, protein degradation is regulated by the ubiquitin-proteasome pathway, in which the 26S proteasome acts as its executive key player. The 26S proteasome is a 2.5 MDa multi-subunit molecular machine, which recruits, unfolds, and degrades tetra-ubiquitin tagged proteins. Recently, a near atomic model of the complete 26S proteasome has been obtained (Unverdorben et al. 2014, PNAS, 111:5544 5549), providing a structural context for understanding its function.

Here, we investigate the first functional processes of the degradation cycle by the use of molecular dynamics (MD) simulations coupled with enhanced sampling methods. We focus on the recruitment of a poly ubiquitin tag by the flexible arm of Rpn10 and its transport to the active site of de ubiquitylating enzyme Rpn11. We refined the near-atomic model of Rpn10 by closing mechanistically relevant structural gaps, which have been inaccessible to structural experiments due to high flexibility or multi conformational states. In simulations, Rpn10’s flexible arm covers a wide conformational space, making a number of transient contacts to proteasome subunits. The observation that Rpn10 can reach the de ubiquitylating enzyme Rpn11 confirms its ability to deliver the substrate to Rpn11 and the ATPase module where substrate is unfolded. The dynamic insights on ubiquitin recruitment and transport gained by MD simulations are a basic prerequisite to understand the complex functional cycle of protein recognition and degradation of the 26S proteasome at the atomic level and will motivate further experiments.

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Atomistic Mechanism of Peptide Unfolding and Translocation by AAA+ Unfolding Chaperones Clp
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Atomistic simulations of peptide unfolding and translocation by AAA+ biological nanomachines Clp ATPases are members of the AAA+ superfamily that associate with their peptidase partner to degrade proteins. Structurally, Clp ATPases are homohexameric rings that enclose a narrow central channel. During repetitive cycles driven by ATP hydrolysis, conserved central channel loops undergo axial motions to effect substrate protein unfolding and translocation to the peptidase compartment. Structural and biochemical studies of AAA+ ATPases proteins have suggested several models (stochastic or ordered) of the progression of allosteric transition around the ring. We use atomistic molecular dynamics simulations to investigate the mechanism of peptide unfolding and translocation by the ClpY ATPase. Substrates with diverse secondary structure (alpha helix/beta turn/random coil) are covalently attached to a degradation tag (ssrA). We find that substrate gripping is mediated by strong interaction at Tyr91 site of the conserved central pore loop. Axial motions of the Tyr91 side chain towards the distal side result in strong forces that drive substrate translocation. Mutations of Tyr91 site results in change of unfolding and translocation efficacy. Detailed analysis of the Tyr91-substrate interaction indicate formation of transient hydrogen bonds of Tyr91 to the substrate backbone and strong interaction of the Tyr91 aromatic ring with the substrate.

Translocation takes place in stepwise manner and involves 5-6 amino acid per step, in accord with single molecule experiments. Structured peptides (prion helix 1 and hairpin fragment of GB1 protein) undergo highly cooperative unfolding and translocation by ClpY with partial preservation of secondary structure. The most efficient translocation mechanism is found to involve clockwise intra-ring allostery that maximally benefits from the torque applied by the central channel loop onto the substrate.

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Mechanism of Inhibition of Glycoside Hydrolases Investigated by Molecular Dynamics Simulations
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Biofuels are a well-known alternative to the largely used fossil-derived fuels. One of the main challenges for the production of the second-generation biofuels, produced by enzymatic degradation of agricultural waste, is the inhibition of the glycoside hydrolases enzymes by non-suitable substrates or by their own product. Enzymes show molecular dynamics (MD) simulations, we investigated how two enzymes of the glycoside hydrolases family are affected by a non-suitable substrate (Man5B) or by their own product (CEL7A). Combined with previous experimental results, our simulations reveal that the reduction of Man5B enzymatic efficiency in the presence of a poor substrate (gluco-oligosaccharides) is associated with a loss of the enzyme’s flexibility, the latter being required to bind new substrate, while the presence of a more suitable substrate (manno-oligosaccharides) does not pose this problem. The C-terminal domain of the glycoside hydrolase enzyme CEL7A interacting with cellulose substrate of different lengths in position –1 to –7 offers a detailed view of its enzymatic mechanism and also on how the enzyme dynamics is affected by substrate. Employing steered molecular dynamics (SMD) we were able to analyze the difference in free-energy when a substrate is moving through CEL7A’s catalytic tunnel under different conditions. All together our results are indicating that the inhibition of the CEL7A by its product is directly related to the cellubiose (enzymatic product) that was just cleaved and does not leave the exit pocket because of the already high concentration of cellubiose in solution. References:

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Structural Determinants of Hybrid State Intermediates of the Bacterial Ribosome
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Hybrid state formation is a crucial step of the RNA translocation process. After peptide bond formation, a deacetylated tRNA in the P site (here tRNA-fMet) moves to a hybrid P/E configuration while the A-site tRNA (here tRNA-Val) stays in its classical A/A state. In this process the CCA tail of the tRNA-fMet must move by about 4.3 nm and the elbow region moves by ~2.5 nm forming contact with the L1 stalk. The anticodon stem-loop remains almost fixed at its 30S position, the 30S body and head undergo a slight (~5°) rotation relative to their classical state. The atomic detailed pathway of this transition is still unresolved. Here, starting from structures obtained from previous equilibrium simulations, we performed a series of 200ns all-atom MD simulations in which we drive the system using a biasing potential that maximizes correlation to the target state ensemble. Analysis of the trajectories in terms of the applied biasing potential and the dwelling time in configurational space appears to indicate the existence of several intermediate states separated by barriers along the hybrid state formation pathway. Interestingly, most of the bottlenecks along the trajectories are found to involve interactions between the CCA tail of the tRNA-fMet and specific 50S residues. The CCA tail interacts initially with the P-loop of helix 80 of the 23S RNA, it then contacts helix 93 and 12076 of helix 75, finally reaching the E site through a different set of interactions which include His19 of ribosomal protein L28. In contrast, the other large scale motions of the L1 stalk and 30S subunit appear to be less restricted along the pathway.

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Molecular Dynamics Studio of Poly(VINYL Alcohol) Mechanical Properties for its Incorporation in Bones Structures as a PVA-PLA Substrate for Tissue Regeneration
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Materials may be strong or ductile, but rarely both at once. Recent studies have shown that nanostructured material may lead to a unique combination of exceptionally high strength and ductility. The analysis and characterization of these properties will open new paths for many applications. Using the technique down to top, we can generate new structures that can be used on many applications for example in bone regeneration. One way for achieving this is combining PLA,