several of the histone fold helices in free H3.3/H4 complexes. In sum, we have found that DAXX stabilizes a pre-nucleosomal folded form of H3.3/H4 that differs from its final nucleosome form or its form free in solution. While the steps in the folding pathway we studied are certainly tied to the mechanism of chromatin assembly, so to is the massive destabilization we predict occurs to DAXX [HBD] upon releasing H3.3/H4 dimers during nucleosome assembly.

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Nup153 in Nuclear Transport: Plasticity of Nucleoporin/Transport-Receptor Complexes

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The MDa sized nuclear pore complexes (NPCs) are among the largest molecular machines in eukaryotic cells and constitute a vital transport conduit between nucleoplasm and cytoplasm. Intrinsically disordered and phenylalanine glycine rich nucleoporins (FG-Nups) form a selective permeability barrier in the center of the NPC, through which large molecules can only pass when piggybacked by nuclear transport receptors (NTRs) that specifically interact with FG-motifs. FG-Nups constitute a complex and distinct non-random amino acid composition of these FG-motifs and inter-FG linkers, but how such heterogeneous sequence composition relates to function and how homotypic interactions between disordered stretches, and transient heterotypic interactions with folded transport receptors could give rise to a transport mechanism is still unclear. This holds true in particular since NTRs seem to bind tightly to FG-Nups (K_d in the nanomolar range), but at the same time move through a pore that is densely filled with FG-motifs (> 50 mM FG) within only a few milliseconds. We have now developed an integrated chemical biology-fluorescence approach that allows us to study the molecular plasticity of FG-Nups on the single-molecule level using multi-parameter fluorescence spectroscopy. Despite its inhomogeneous primary sequence, the FG-domain of Nup153 displays a collapsed coil behavior across its entire amino acid sequence. Surprisingly, it retains this collapsed conformation even when bound to NTRs as shown by single-molecule Förster resonance energy transfer and fluorescence anisotropy. However, ultrasensitive photo-induced electron transfer experiments combined with picosecond resolved fluorescence fluctuation analysis revealed the formation of very flexible and dynamic complexes. Screening different labeling sites allowed us to detect differential binding modes of the Nup153·NTR interaction. These results have wide implications on how nuclear transport can pursue specifically and fast inside the nuclear pore complex.

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Molecular Recognition through Concerted Ubiquitin Backbone and Side Chain Motion Determined from NMR and MD Simulations

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Motion is involved in a large number of protein functions. For ubiquitin, residual dipolar coupling (RDC) derived ensembles have suggested it recognizes binding partners via conformational selection through motions occurring on the supra tau-c (>4 ns) timescale [1]. Subsequent relaxation dispersion (RD) studies identified microsecond fluctuations for six backbone atoms [2,3]. However, it has not been clear if these motions are independent or collective, and what role side chains play. To address those questions, we have conducted an in-depth RD analysis of the backbone and side chain methyl groups. This survey showed a large number of atoms (>30) with microsecond fluctuations. These atoms are distributed throughout the structure and nearly all show the same exchange rate, which suggests that ubiquitin undergoes collective motion involving both the backbone and side chains. The presence of microsecond side chain motion agrees with previous RDC measurements of methyl groups within ubiquitin [4]. Furthermore, comparison of different methyl nuclei indicates that the nature of the side chain fluctuations is almost entirely due to changes in rotamer populations. This is borne out in molecular dynamics simulations of ubiquitin trapped in different conformational states by binding partners [5]. Residues showing RD were significantly more likely to show changes in rotamer populations in the simulations. Put together, this leads to a model where collective microsecond backbone motion is coupled to redistribution of side chain rotamer populations. The correlation with binding partner simulations implicates this motion in conformational selection.

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Coupling of Loop Closure and Chemistry in Protein Tyrosine Phosphatases

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Protein tyrosine phosphatases (PTPs) are a class of enzymes that catalyze the dephosphorylation of tyrosine residues. PTPs share a common fold with an active site geometry consisting of two loops a P-loop that is responsible for phosphate binding and a WPD-loop, named for its consensus WPD sequence, which includes a critical Asp residue that functions as a general acid/base during catalysis. Comparison of uncomplexed crystal structures with those bound to oxyanion ligands shows that the WPD-loop is flexible, alternating between ''open'' and ''closed'' conformations. In the closed form, the catalytic Asp residue is within hydrogen bonding distance of the oxyanion; loop closure is essential for catalysis.

Two PTPs studied here are YopH a critical virulence factor from *Yersinia* and human PTP1B. Each enzyme possesses homologous WPD loops and the chemical step is fully rate limiting yet surprisingly their k_{cat} values differ by an order of magnitude, with YopH being 10-fold faster. Using NMR spectroscopy (at pH=6.5, 293K) we have addressed the role of loop motion in the catalytic activity of these two PTPs. The first of these, PTP1B is a less powerful catalyst than YopH with $k_{cat} \sim 17 s^{-1}$. NMR CPMG relaxation dispersion studies remarkably show that the WPD loop in apo PTP1B closes with $k_{close} = 17 \pm 8\ s^{-1}$. YopH catalyzes phosphate hydrolysis with a k_{cat} ~100 - 200 s^{-1}. Estimates of the WPD loop exchange rate constant, were made from from Hahn echo experiments and $^1H^N$-$R_{1\rho}$ dispersion profiles that suggest $k_{close} = 400 \pm 200\ s^{-1}$.

Thus, differences in loop closing rates closely correlates with the respective k_{cat} values in these two enzymes and further suggests a role for the frequency of WPD-loop motion in the catalytic function of protein tyrosine phosphatases.

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Steady State Dynamics of Enzyme Catalytic Action in Solution

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Wide-angle X-ray scattering (WAXS) and neutron spin-echo (NSE) spectroscopy were used to study of the dynamics of adenylate kinase (Adk) as it undergoes steady state catalytic cycling. Analysis of the WAXS data indicates that it cannot be completely explained as a combination of open and closed states and suggests that ADP-bound Adk in solution mostly populates a closed conformation as demonstrated in earlier NMR studies [1,2,3]. The presence of other intermediates must be hypothesized to explain the observed data. Correlated internal dynamics on ps-ns timescales for the protein during active cycling appear to be slower than comparable dynamics in the open and closed states at certain length scales and faster at others. Our NSE results on active enzyme catalysis are consistent with the picture of energetic counterweight balancing on substrate binding. The enzyme appears to shift mobile regions during active catalysis for subsequent substrate release [4]. These methods will help define functional motions in proteins. Supported by DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494)

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