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Native State Dynamics of Human Neuroserpin Investigated with Hydrogen/Deuterium Exchange and Molecular Dynamics Simulations

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Wild-type human neuroserpin, a member of the serine protease inhibitor superfamily, is expressed in neurons of the central and peripheral nervous system, as well as in the adult brain. Polymerization of certain mutants of neuroserpin is associated with dementia caused by familial encephalopathy. We have performed hydrogen/deuterium exchange-mass spectrometry in order to monitor the structural stability and flexibility of different regions of the neuroserpin structure. We find that beta-sheet A, a critical region thought to be involved in polymerization, is less stable and more labile in neuroserpin than in other serpins such as alpha-1 antitrypsin and antithrombin. This may explain why wild-type neuroserpin is more susceptible to polymerization than other serpins. Molecular dynamics simulations also indicate that Wild Type neuroserpin shows increases flexibility on the nanosecond timescale as compared with alpha-1 antitrypsin. In the simulations, a novel 2 stranded beta-sheet was formed between the N terminal portion of the reactive center loop and the loop connecting strand 3A to beta-sheet C. This phenomenon occurred repeatedly in multiple independent simulations. If such an interaction in fact occurs in solution, it could contribute to the relatively poor inhibitory efficiency of neuroserpin compared to other serpins by retarding the insertion of the reactive center loop into sheet A after proteolytic cleavage. Simulations of a pathological mutant of neuroserpin showed distortions near the top of the central beta-sheet A, a critical site for polymer formation. This distortion may help explain why the mutant is more prone to polymerize than wild type.

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Optimized Quantitation from Proteomic Datasets - Application to Lamin Knockdown and Isoform Modulation During Stem Cell Differentiation

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Label-free mass spectrometry is rapidly emerging as an alternative to antibody-based methods, although there is continued need in either case to maximize measurement accuracy. Here we describe Spectral Ion Fingerprint Recognition (SpIFR) for optimized identification of 'protein fingerprints' within proteomic datasets derived from liquid chromatography tandem mass spectrometry (LC-MS/MS). We focus initially on siRNA knockdown of nuclear lamina proteins that are low in abundance and tightly regulated in stem cells, cancer, and aging. Sets of optimal peptides show that intermediate MS signal - rather than strong signal - is often best for quantitation and produce a high level of accuracy ($\pm 6\%$) while also providing global assessments of protein changes. Surprisingly, lamin-A and -C spliceoforms are knocked down to different extents despite siRNA targeting of a shared sequence, and the results also allow the first calculations of ratios for lamin-A/C to lamin-B. From the MS-derived proteomes, SpIFR computes an invariant reference set of 'housekeeping' peptides and enables mapping of species-specific peptides encountered in embryonic stem cell cultures. Based on the measured knockdown in reference cells, large differences in lamin isoform abundance between stem cell types are calculated, thus highlighting major differences in nuclear architecture as well as broad application of the optimized quantitation.

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Characterization of the Metal-Binding Sites of Metallothioneins and Structural Changes Between Metal-Free and Metal-Bound Forms by Ion Mobility-Mass Spectrometry (IM-MS)

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Metal ions play important roles in many chemical and biochemical processes, such as oxidation, oxygen transport, and electron transfer. The cellular trafficking of metal ions is controlled by a group of low molecular weight (6-10 kDa), cysteine-rich proteins (~30%) referred to as metallothioneins (MTs). Despite intensive research of MTs for more than 50 years, the precise physiological function of MTs has not yet been identified. X-ray crystallography and NMR studies of fully cadmium-saturated human MTs have revealed two binding domains in Cd_7-MT: the N-terminal β-domain $Cd_3(Cys)_9$ and the C-terminal α-domain $Cd_4(Cys)_{11}$. Although the structure of fully-metallated MT was published 30 years ago; the structures of metal-free and partially-metallated MTs are extremely difficult to determine and the metal-binding mechanism remains unclear. To understand the structural change of MT upon metal binding, we performed titration experiments and ion mobility-mass spectrometry (IM-MS). Titration experiments revealed preferential formation of Cd_4-MT with addition of <4 eq Cd^{2+}, suggesting the cooperative binding of four Cd^{2+} to MT. IM-MS ATD for apo-MT are composed of a single, broad peak, consistent with multiple conformers, possibly disordered structure, whereas the ATD for Cd7-MT is composed of 2-3 distinct peaks. Moreover, shifts in the ATD as MT accumulates metals, suggest the formation of more compact structures upon binding of metals. The results from these studies underscore the potential of IM-MS for monitoring structural changes that occur as the number of metals ions bind to MT, both in terms of increasing and decreasing metal content. In addition, proposed conformers of partially and fully metallated species and preferences for metal ion binding can be examined by using combining ion mobility with tandem mass spectrometry and hydrogen/deuterium exchange (HDX) chemistry.

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First Crystal Structure of Na-ASP-1, a Two-Domain Cap (Cysteine-Rich Secretory Protein / Antigen 5 / Pathogenesis Related-1) protein

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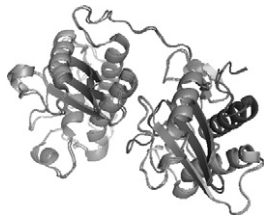

The major proteins secreted by the infective larval stage hookworms upon host entry, are Ancylostoma secreted proteins (ASPs) which are characterized by one or two CAP (cysteine-rich secretory protein / antigen 5 / pathogenesis related-1) domains. The CAP domain has been reported in a diversity of proteins unrelated by phylogeny and isolated from bacteria, plants, as well as animals but it has no confirmed function. The first structure of a two-CAP domain protein, Na-ASP-1, from the major human hookworm parasite Necator americanus was refined to a resolution limit of 2.2 Å. The structure reveals two CAP domains linked by a short loop. Overall the carboxyl terminus CAP domain is more similar structurally to one-domain ASPs than to the amino terminus CAP domain. This first structure of a two-domain CAP is a useful template for the homology modeling of other two-domain CAP proteins.

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Structure of a Prokaryotic Lipoxygenase Bound to a Phospholipid

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Lipoxygenases are non-heme iron enzymes essential in eukaryotes, where they catalyze formation of the fatty acid hydroperoxides that are required by diverse biological (and also pathological) processes. In prokaryotes, most of them totally lacking in polyunsaturated fatty acids, the possible biological roles of lipoxygenases have remained obscure.

To date, the available structures of lipoxygenases were only of eukaryotic origin. Here we report the first crystal structure of a prokaryotic lipoxygenase, from *Pseudomonas aeruginosa* (*Pa*_LOX). The structure shows major differences with respect to the eukaryotic enzymes, while retaining much of the overall lipoxygenase fold. The most striking difference is an insertion in the catalytic domain of a pair of long antiparallel alpha-helices, contributing to an enlarged binding pocket in *Pa*_LOX containing a bound phospholipid: a phosphatidylethanolamine with well defined chains of 18 and 14 carbons in length. The insertion acts as a lid that apparently needs to be opened to allow entrance of the phospholipid into the substrate-binding pocket. This idea is supported by the variability observed for this two-helix motif in a second crystal form and by the flexibility of the residues linking the motif to the protein. The mobility of the two-helix motif, together with the specific interactions observed between the phospholipid polar head and the protein residues, suggest a mechanism by which the enzymatic activity of secreted Pa_LOX can be exerted directly on target cell membranes.

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Crystal Structure of the C-Terminal Domain of the Hypoxia Regulator Ofd1

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Sre1, the fission yeast homologue of the mammalian sterol regulatory element binding protein, SREBP, is a hypoxic transcription factor required for sterol homeostasis and low oxygen growth. The level of the N-terminal transcription factor domain of Sre1, Sre1N, is regulated by Ofd1-Nro1 in an oxygen-dependent manner. Ofd1 is a putative prolyl hydroxylase of the