intermediates to various enzyme partners. Structures of apo-, holo-, and saturated acyl-ACPs indicate that the acyl groups are housed inside a hydrophobic binding cavity between three largely parallel helices. Molecular dynamics (MD) simulations have previously been used to illustrate the behaviour of various saturated acyl chains attached to ACP, in excellent agreement with experimental data. However, relatively little is known about the fatty acyl derivatives formed during the elongation of the acyl chains, namely the β-ketoacyl-, β-hydroxyacyl-, and enoyl-intermediates. ACP is one of the most abundant proteins in *Escherichia coli* and possesses a large number of interacting enzymes. As such, we seek to understand how the enzymes of fatty acid biosynthesis recognize which derivative is bound by ACP. To address this, we conducted numerous MD simulations of *E. coli* ACP with β-ketoacyl-, β-hydroxyacyl-, and enoyl- intermediates. The unconstrained MD simulations were set up with the attachment either in a solvent exposed or a solvent shielded conformation inside the hydrophobic binding pocket of ACP. We investigate a range of acyl group derivatives attached to ACP spanning from four to eighteen carbon groups in length to develop our understanding of the differences imparted on ACP by various acyl chain lengths. The results of the simulations of fatty acyl derivatives will provide a first look at the manner in which ACP accommodates these binding groups. What these data contribute to the understanding of enzyme:ACP interactions and substrate recognition by fatty acid synthase enzymes will be discussed.

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The Dependence Of Coiled-coil Chirality On Elastic Energy

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Coiled coils are proteins that consist of two or more alpha-helices that wrap around each other to form a super-helical structure. Using a continuum elastic model, a recent paper [1] has shown that the chirality of the super-helical twist is dictated by the chirality of the pattern of hydrophobic residues on each helix only when the bending and twisting energy of each helix is considered. In the absence of any energy cost due to the flexible motions of each helix, they showed that there is a family of structures which are consistent with the hydrophobic pattern. Using a coarse-grained atomistic model for coiled coils that includes the flexible degrees of freedom for each helix, we have carried out monte-carlo simulations to examine how the energy and chirality of coiled coils depends on the strength of the elastic energy. We find that there is an optimal weighting of the elastic energy that leads to the coiled coils adopting the same chirality as the hydrophobic pattern on each helix. We then explored how the chirality of the coiled coil changed under the application of an applied force or an applied torque. Our findings are compared to recent measurements on the mechanics of coiled-coils from single-molecule studies.

[1] S. Neukirch, A. Goriely and A.C. Hausrath, PRL, 100, 038105 (2008).

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Computational Modeling of the Structural Mechanism Linking Ligand and Corepressor Binding to Thyroid Hormone Receptors

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Thyroid hormone receptors (TRs) are nuclear receptors with two functional states. Upon binding to the thyroid hormone, TRs recruit coactivator proteins and activate gene transcription. Without the hormone ligands, TRs interact with corepressor proteins and repress transcription. Due to the importance of the thyroid hormone action in embryo development, metabolism, heart rhythm and cholesterol level, the molecular mechanism behind the functional switch has been studied extensively. However, the current available crystal structures of thyroid hormone receptor ligand binding domains (LBDs) are all in the ligand-bound (holo) form, with no revelation on ligand-free (apo) form or transcription corepressor bound form. In order to elucidate the complete apo to holo switch process, we constructed homology models of apo TR from the available apo structures of interrelated nuclear receptors: retinoid acid receptor (RXR) and peroxisome proliferators activated receptor (PPAR). Both models were subjected to energy minimization followed by molecular dynamic (MD) simulation. Analysis of the MD simulations proved that the model based on PPAR was more stable than the model based on RXR. As a result, unlike the prevailing idea that TRs would exert a major structural change in the C-terminal activation helix (AF2) domain upon ligand binding, TRs exhibit only subtle changes at the AF2 domain. Our model predicts that the recruitment of corepressor proteins, which require the relocation of the AF2 domain, is more appropriately portrayed as an induced fit process. Additionally, we constructed homology models of TR LBD in complex with the nuclear receptor interacting domains of corepressor proteins. Molecular dynamic simulation of this receptor-correpressor complex system in the absence or presence of the ligand thyroid hormone has identified correlated conformational changes that may be important for the functional switch.

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Effects Of Reactive Oxygen Species On Cyan Fluorescent Protein

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Recent advances in microscopy techniques and the development of many different colour variants of the GFP family of proteins allow for a more direct analysis of protein function in live cells. The advantage of genetically coded fluorescent protein probes is often offset by their photophysical properties which usually make them very sensitive to cellular environmental changes. Among these, reactive oxygen species (ROS) are an essential part of key cellular processes (mitochondria respiration, apoptosis) and are also involved in the pathogenesis of various diseases (cancer, atherosclerosis, Alzheimer's disease, etc.). We studied the effects of ROS on the cyan fluorescent protein (CFP) *in vitro,* as this fluorescent protein is currently one of the most widely used in protein interaction studies. We studied the fluorescence and absorption changes of recombinant CFP protein using γ-radiolysis for ROS production. γ-radiolysis ROS production allowed us to have an exact control over the radical concentrations delivered unto the protein samples. The radicals used in this study were OH°, O_2^{-} or a mixture of OH° and O_2^{-}. We also determined the chemical modifications that take place upon ROS induced protein oxidation by mass spectrometry. We show that the targets of oxidation are one tyrosine and four methionine residues located on the protein surface and that the chromophore is not likely modified through these oxidation processes.

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Thermodynamic Intermediates of the Alkaline III→IV Transition in Ferricytochrome c Probed by 695 nm Charge Transfer Band

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The 695 nm band in the spectrum of the native state (III) of ferricytochrome c has recently been shown to be composed of different sub-bands which reflect different conformational substates (CS) of the functional pivotal Fe-M80 linkage. In order to explore the influence of the alkaline III→IV transition on this CSs we measured and analyzed the absorption and CD profile of this band as a function of pH between pH 7 and 10 at high (50mM) and low anion concentration for horse heart (hh) cytochrome c. Additional measurements on yeast cytochrome c were performed at low anion concentrations. The titration curves of the two dominating sub-bands are clearly biphasic at low anion concentrations and reflect effective pK-values of 8.5 and 9.65. On the contrary, the titrations curves obtained at high anion concentrations are monophasic; the effective pK being 9.23. The thermodynamic parameters (i.e. pK and the Hill coefficient as a measure of cooperativity) are slightly different for the two CSs. We fitted the data obtained at low anion concentration with a model, which assigns the two effective pK-values to two different alkaline states IV1 and IV2, in which M80 is replaced by K73 and K79, respectively. This model did not reproduce our data well in contrast to an alternative model which assumes only one species with two protonation sites. This suggests that the state populated upon the pK= 8.5 protonation can be interpreted as a thermodynamic intermediate of the III→IV transition. This notion is consistent with our observation, that the 695 nm titration curves of the y-cytochrome c mutants K79R and K73V are still mostly biphasic at low ionic strength.

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X-ray Footprinting at Beamline X28C: A National Resource for Studying Macromolecular Structure and Dynamics

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X-ray footprinting employs intense X-rays produced by synchrotron radiation to generate hydroxyl radicals in solution on microseconds-milliseconds timescales. These hydroxyls radicals undergo stable reaction with solvent accessible sites of macromolecule and produce covalent modifications, which are appropriate to probing macromolecule dynamics under physiological condition. For nucleic acids, one analyzes the pattern of fragments after X-ray exposure by gel electrophoresis; the protected sections that are not cleaved yield a ''footprint''. For proteins, the exposed samples are digested with proteases and analyzed by liquid chromatography- and tandem-mass spectrometry to determine the extent and sites of modification. The data provide detailed structural information to map tertiary contacts of macromolecular interactions, which can subsequently be used as constraints for molecular modeling to generate high-resolution