collagen in these tissues corresponds to type I collagen, which forms heavily crosslinked protein fibers. Much is known about collagen's mechanical properties from experiments and simulations. However, the preferred modes of covalent bond scission events and subsequent biochemical processes remain elusive.

To investigate how physiological load on collagen relates to the forces in individual molecular bonds of collagen and which bonds are subjected to the highest forces we employed Force Probe Molecular Dynamics simulations. First, we built a full-atom model of collagen fibril using an integrative structural biology approach. Then, we reconstructed covalent crosslinks at conserved lysine residues to represent physiological collagen as realistically as possible. This resulted in the first large-scale (1.8 Mio atoms including water) and yet atomistically resolved structural model of crosslinked collagen.

Force Probe Molecular Dynamics simulations and internal stress distribution in collagen determined by Force Distribution Analysis suggested lysine-based crosslinks as the most susceptible sites to bond rupture. Quantum calculations and electron paramagnetic resonance experiments showed that the bond rupture leads to formation of radicals, which are known to act as signaling molecules. This suggests collagen to play a hitherto unknown role as an intrinsic force gauge by successive and specific rupture of covalent bonds. Our results have interesting implications for the role of the different extent and nature of crosslinks in tissues of different kind and age.

1963-Plat

Investigating Chemokine Receptor CCR2 Dynamics and Druggability by Ensemble Based Approaches

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Chemokine receptor CCR2 is implicated in a wide range of diseases such as inflammation, autoimmunity, and cancer, and thus is an important drug target in the pharmaceutical industry. However, CCR2 antagonists have been largely unsuccessful to date. Recently CCR2 was crystallized for the first time by our collaborator Prof. Handel, opening up new opportunities for rational drug design. While crystal structures provide valuable snapshots of proteins and protein complexes, they lack the ability to reveal chemokine receptor dynamics at a level that is required to develop new drug (lead) molecules. Therefore, key challenges are: (i) understanding how small molecule antagonists modulate the protein dynamics, and (ii) developing compounds that bind to novel (allosteric) binding sites on the receptor. Here, we present our novel approach to understanding molecular recognition and the inactivation mechanism of CCR2, using long-timescale molecular dynamics (MD) simulations and differential dynamics Markov state modeling (ddMSMs). By comparing the difference in conformational ensembles (differential dynamics), we are developing new insights into molecular recognition for CCR2, which will identify unique druggable pockets and ultimately facilitate the design of new potential therapeutic compounds for inflammatory and autoimmune diseases.

1964-Plat

MHC Class II Complexes Sample Intermediate States along the Antigenic Peptide Exchange Pathway

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The presentation of antigenic peptide-MHCII complexes (pMHCIIs) for surveillance by T cells is a well-known immunological concept in vertebrates, yet the conformational dynamics of antigen exchange remain elusive. By combining NMR-detected H/D exchange with Markov modelling analysis of an aggregate of milliseconds of adaptive molecular dynamics simulations, we reveal that a stable pMHCII spontaneously samples intermediate conformations that are both relevant for peptide exchange and consistent with X-ray crystallography. More specifically, these conformations represent two major peptide exchange pathways: the kinetic stability of a pMHCII's ground state defines its propensity for intrinsic peptide exchange, while the population of a rare, intermediate conformation correlates with the propensity of the HLA-DM-catalysed pathway. Helix-destabilizing mutants designed based on our model shift the exchange behavior towards the HLA-DM-catalysed pathway and further allow us to conceptualize how allelic variation can shape an individual's MHC restricted immune response. This research used resources of the Oak Ridge Leadership Computing Facility at the Oak Ridge National Laboratory (project IDs: BIP103 and BIP149), which is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Workshop: Probing Atomic Single Sites in Cells and Bio-Assemblies: Advances in In-Cell NMR

1965-Wkshp

In Cell NMR: Its Contribution for Understanding Functional Processes

Lucia Banci.
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In-cell NMR, i.e. the collection of high resolution NMR spectra of biomolecules in intact, living cells, represents one of the highest impact applications of magnetic resonance.

These experiments allow us to obtain information on the conformational and functional properties of biomolecules at atomic resolution in conditions as closer as possible to the physiological ones.

Methodological aspects and innovations will be discussed and a few examples of the striking power of this approach presented. Particular focus will be on the observed meaningful differences of properties of biological molecules in living cells with respect to those in vitro.

Barbieri L, Luchinat E and Banci L . In-cell NMR spectroscopy in HEK293T cells: a protocol to characterize proteins in their physiological environment. *Nature Protocols* 11: 1101, 2016

Luchinat E, Barbieri L, Rubino JT, Kozyreva T, Cantini F and Banci L. In-cell NMR reveals potential precursor of toxic species from SOD1 fALS mutants. *Nat Commun* 5: 5502, 2014

Banci L, Barbieri L, Bertini I, Luchinat E, Secci E, Zhao Y and Aricescu AR. Atomic-resolution monitoring of protein maturation in live human cells by NMR. *Nat Chem Biol* 9: 297-299, 2013.

1966-Wkshp

Studying Proteins Inside Eukaryotic Cells in NMR

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In-cell NMR spectroscopy is a method used to observe isotopically labeled molecules within living cells. The first in-cell NMR experiment was performed with an E. coli overexpressing a ^{15}N-labeled protein. For the first application of the in-cell NMR method with eukaryotic cells, isotopically labeled target proteins were introduced, by microinjection, into Xenopus laevis oocytes, and a cell-penetrating tag was also utilized. Our group reported an in-cell NMR method for mammalian cells; we used a pore-forming toxin, streptolysin O (SLO), to introduce target proteins by diffusion. By using these methods, protein-drug interactions and intracellular post-translational modifications, such as phosphorylation and acetylation, were successfully detected in vivo. However, the major limitation of the in-cell NMR experiments is the occurrence of cell death during the NMR measurement. As the suspension contains a high density of cells, nutrient depletion occurs rapidly in the anaerobic environment within the NMR tube, thus causing the deterioration of conditions and resulting in cell death during NMR measurements. Therefore, in-cell NMR experiments for eukaryotic cells currently have limited applications, such as for obtaining a single NMR spectrum measured within a very short time. Although sparse sampling methods have been utilized to shorten the time required to acquire multidimensional NMR spectra, many existing in vitro NMR experiments that are used to provide information regarding dynamics and protein interactions take several hours to perform. In this study, we will show a bioreactor system for in-cell NMR, which we developed, to suppress the cell death during NMR measurements, and its recent application in our lab.

1967-Wkshp

Cellular Solid-State NMR Applied to Bacterial and Human cells

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Solid-state NMR (ssNMR) is a method that can be applied to gain atomic-level insight into heterogeneous molecular systems. Over the last 15 years, ssNMR has seen strong methodological and instrumental developments that have allowed for the characterization of complex molecules including membrane

proteins, amyloid fibrils or protein biopolymers with remarkable structural accuracy and comprehensiveness. More recently, ssNMR has profited from revolutionary enhancements in sensitivity, mainly due to the advent of Dynamic Nuclear Polarization (DNP) and it has seen significant progress in the field of ssNMR ^{1}H detection. In our contribution we show how to make combined use of such methods to probe biomolecules in bacterial and human cells. Applications include protein translocation and insertion machines in bacteria and extend to membrane associated as well as soluble protein complexes in human cells. We also describe how to combine such ssNMR-based studies with modalities such as cryo-electron tomography (CET) and Fluorescence microscopy to obtain structural insight into cell organization from the atomic to sub-micrometer scale.

1968-Wkshp
In-cell NMR Spectroscopy for the Investigation of the Conformation of Macromolecules
Volker Dotsch.
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The non-invasive character of NMR spectroscopy allows researchers to investigate the conformation and dynamics of biological macromolecules in their natural environment, for example the cytoplasm of cells. In the past we have used in-cell NMR investigations to study several proteins as well as the behavior of telomeric DNA in different cellular systems. We will show data on two different systems: The telomeric G-overhang is the 3' single stranded protrusion of double stranded telomeres and consists of repeating d(TTAGGG)n elements. These elements form G-quadruplexes, which however under different in vitro conditions can adopt several different conformations. In order to investigate which of these conformations is the biologically relevant conformation we have injected quadruplexes of different length into Xenopus oocytes or investigated them in oocytes extracts. These investigations revealed that G4 units coexist in two conformations, the hybrid-2 and the 2-tetrad antiparallel basket. In addition, we have used in-cell NMR to investigate the behavior of Pin-1, a peptidyl-prolyl isomerase and show that the protein uses its WW domain to nonspecifically investigate other proteins as potential substrates. This non-specific interaction can be blocked by phosphorylation in the WW domain.

1969-Wkshp
Dissecting Bacteria and Mammalian Cells by Whole-Cell NMR: Cell Walls, Ribosomes, Nuclei, Oh My!
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Our research program is inspired by the challenge and importance of elucidating chemical structure and function in complex biological systems and introducing new strategies to prevent and treat human disease. We integrate solid-state NMR spectroscopy with biochemical and microbiological methods to determine atomic- and molecular-level detail in macromolecular assemblies, intact cells, and bacterial biofilms. In this presentation, I will present our efforts to quantify and identify differences in bacterial cell wall composition by whole-cell NMR and to characterize the modes of action of antibiotics in intact bacterial cell samples. Traditional biochemical methods have delivered most of the information to date regarding antibiotic modes of action, but can be accompanied by molecular ambiguities. A new mode of action has even been recently ascribed to beta-lactams such as penicillin. Solid-state NMR methods enable a comprehensive analysis of cell-wall and cellular pools of carbon and nitrogen and can be used to measure distances between labeled small molecules and labeled sites in the bacterial cell. We have identified differential spectral signatures to quantify cell-wall parameters and to quantify the balance of peptidoglycan and teichoic acid in the bacterial cell wall, even in the absence of specific isotopic labeling, and have examined inhibitors with alternate modes of action, such as ribosome-targeting antibiotics. Towards a view of what carbon pools make up a cell, I will also compare and contrast compositional pools among bacteria and mammalian cells.

Workshop: Atoms to Cells: Modeling Biological Complexity

1970-Wkshp
Biomolecular Simulation for All
Ron O. Dror.
Stanford University, Stanford, CA, USA.
Molecular dynamics simulations are increasingly recognized as a powerful complement to experimental structural biology, but their use has historically been limited to a relatively small group of specialists. I will discuss progress toward making atomic-level simulation a broadly accessible tool for molecular biology, together with key remaining challenges.

1971-Wkshp
Crowded and Complex: Molecular Simulations of Biological Membranes
Mark S.P. Sansom[1], Anna L. Duncan[1], Matthieu Chavent[1,2].
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Biological membranes are complex in terms of their lipid composition and crowded with proteins, which occupy between 25 to 40 % protein of the membrane area. Furthermore many membrane proteins form strong and specific interactions with selected lipids. Coarse-grained molecular dynamics (CG-MD) simulations can be used to explore the consequences of this in terms of the emergent dynamic organization of realistic models of cell membranes. These simulations highlight the role of lipid-mediated interactions between membrane proteins. Recent applications include models of bacterial [1], mammalian [2], and viral membranes. The results of very large scale CG-MD simulations may be used to parameterize 'meso' scale models which allow a more direct link to be made between simulations and experimental data from e.g. single particle tracking studies [3].
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[2] Koldsø, H., & Sansom, M.S.P. (2015) Organization and dynamics of receptor proteins in a plasma membrane. *J. Amer. Chem. Soc.* 137: 14694-14704.
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1972-Wkshp
Ras Signaling: Allostery, Conformation, and Function
Ruth Nussinov, Hyunbum Jang.
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Ras is a small GTPase, controlling signal transduction pathways and promoting cell proliferation and survival. KRAS is frequently mutated in cancer. Ras consists of highly homologous catalytic domains and flexible C-terminal hypervariable regions (HVRs) that differ significantly across Ras isoforms. Ras activation is regulated by guanine nucleotide exchange factors that catalyze the exchange of GDP by GTP, and inactivation is terminated by GTPase-activating proteins that accelerate the intrinsic GTP hydrolysis rate by orders of magnitude. Ras has multiple partners, signals through several key pathways and fulfills critical functions in the cell life. Mutations in Ras are common in a variety of cancers; yet it is still undruggable. Elucidation of Ras conformational ensembles including the ligand-bound conformations, the activated (or inactivated) allosteric modulated states, post-translationally modified states, mutational states, transition states, and nonfunctional states are essential for deciphering Ras functions from its conformational landscapes. Our recent works highlight how these may help in elucidating vital mechanistic questions in Ras biology and hopefully contribute to therapeutic strategies. Funded by Frederick National Laboratory for Cancer Research, National Institutes of Health, under contract HHSN261200800001E.

1973-Wkshp
Allostery and Conformational Dynamics in Protein Evolution
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The critical role of protein dynamics has become well recognized in various biological functions, including allosteric signaling and protein ligand recognition electron transfer. Likewise, in protein evolution, the classical view of the one sequence-one structure-one function paradigm is now being extended to a new view: an ensemble of conformations in equilibrium that can evolve new functions. Therefore, understanding inherent structural dynamics are crucial to obtain a more complete picture of protein evolution. A small local structural change due to a single mutation can lead to a