Emerging Trends in NMR of Biological Solids

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Ann McDermott, Columbia University

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TF6 "SCIENCE DRIVERS": *IN SITU* NMR FOR CHEMISTRY, CATALYSIS, STRUCTURAL, MOLECULAR, AND CELL BIOLOGY

- Structural biology of medically and fundamentally important bio-macromolecular systems and metabolic processes, in cell, in situ, in realistic environments, including complex assemblies, disordered proteins, and metabolomics

- Studies of catalytic systems and novel materials under realistic conditions, where resolution and sensitivity of detection are at a premium

- Key contribution of NMR in both cases is mechanistic dynamic information on chemistry and conformational exchange

- *Technical discussions related to experiments involving improved detection modalities that will be also be significantly enabled by high field (e.g. new nuclei, DNP etc.).. In other words the high field provides multiple dividends.*

Structural Biology

- Impacts fundamental understanding of cell biology
- Impacts drug and diagnostic development
- ***NMR:*** improve molecularly detailed knowledge of specific important categories of understudied macromolecules such as medically important disordered or aggregated systems, intrinsic membrane proteins in membrane environments, native oligomeric assemblies…
- ***NMR:*** relate available structures to biological mechanisms *via* rich and complex dynamical information
- ***Key challenge:*** relate *in vitro* structures to *in vivo* structures dynamics and binding events

- ***Key challenge:*** all of these systems require the best possible spectral resolution and detection sensitivity and hence ***highest possible magnetic fields***.

Some Landmarks in SSNMR Amyloid Structure Determination

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Atomistic structural information for broad range of environments and structures with ***emergent properties and biomedical importance*** : amyloids and prions, membrane proteins in bilayers (liposomes or in whole cells), whole viral particles…..

Complementary to other emerging tools, crystallography, cryo-EM, optical, calorimetric and computational methods are considerable.

*Emerging **Field Dependent** Tools for Studying Dynamics: Site Specific, Structural Signatures, Broad Range of Timescales*

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Krushelnitsky, A and Reichert, D. *Prog Nuc Magn Reson Spectro.* (2005) **47**, 1-25

Mechanistic studies and drug discovery efforts have at their crux a dynamical event.

NMR is unique in providing atom – specific probe of dynamics and thermodynamics, integrated with information on structure, binding, and chemistry

SSNMR exquisitely sensitive to dynamics through powerful tensorial interactions to probe extended timescales.

Emerging computational strategies allow improved ability to interpret these complex experimental observables.

*Allosteric Coupling Depends on **Membranes***

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Coupling in bilayers not observed in detergents

Atom specific probes of structural and dynamical perturbation: we obtain insights as to which residues constitute important players in allostery. Mutations support these conclusions, for example here we removed bulky F103, in hinge of TM2, between two binding sites, and strongly diminished coupling. Computational tests

Imai, Osawa, Takeuchi, Shimada PNAS 2010 V76 Methyl-TROSY, KcsA in DDM Micelles
Bhate, Xu, McDermott et al, submit. MAS SSNMR

Structure Function and Dynamics of IMP

Image Redacted

See recent advances in magic angle spinning solid state NMR of membrane proteins
Shenlin Wanga, b, , Vladimir Ladizhansky PMR 2014

High Field Enables Study of Complex Proteins

11.7, 17.6 and 21.1 Tesla 500, 750, 900 MHz: Resolution in the C-C region increases by 42% for DsbA

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Congestion due to repetitive amino acids, and helical secondary structure means that the highest possible magnetic field strengths are very important.

High Resolution NMR Spectroscopy of Nanocrystalline Proteins at Ultra-High Magnetic Field

Lindsay J. Sperling, Andrew J. Nieuwkoop, Andrew S. Lipton, Deborah A. Berthold, and Chad M. Rienstra

Emerging Proton Spectroscopy of Bio-solids Requires High Field, and expected to Benefit from Utrahigh fields

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Proteorhodopsin 800 MHz
CONH Plane
bR Homology Model
Chemical exchange study

Ward Ladhizhansky et al JACS 2011

Suite of 1H detected 3D experiments
For efficient parrtailly automated
Assignments of proteins up to 30 kDa

Barbet-Massin…. Pintacuda JACS 2014

Review of applications of 1H NMR of solids at high fields S Brown Soli State NMR 2012

^{17}O and other Quadrupolar Nuclei in Biomedical Applications

- *Kong… Gang Wu et al J Phys Chem B 2013*

Image Redacted

Other Important Experimental Modalities in Biomolecular Studies Also Benefit from Higher Field

- Paramagnetic Probes
- Aligned samples, PISEMA

Image Redacted

See Phospholamban
Regulator of cardiac
ATPase activityVeradari G. Veglia
Et al PNAS

Higher Magnetic Fields: Progress in Studies of Median or Larger Sized Proteins

- Resolution improvements from 500 to 900 are transformative for studies of proteins up to about 300 residues

- Improvements in 1.2 T and 1.5 T spectra are expected to be even more powerful, enabling studies of much more complex oligomeric systems, essential for today's challenges in fundamental science and technology

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TIG March 2000, volume 16, No. 3

Future Improvements: Studies in Cells, Tissues, Intact Virus

- Many laboratories have shown important progress towards meaningful studies of signaling and protein function, structure binding and dynamics in the cell– tests of emergent phenomena compared with divide and conquer. Especially important where the *in vitro* experiments are still complex and hint to new mechanisms: IDP, etc.

- Sensitivity requirements depend on available appropriate expression level under meaningful conditions. For some problems involving abundant proteins (prokaryotes, copy number > 10^6), today's benchmark sensitivity could be sufficient. For other situations it may be necessary to improve sensitivity by orders of magnitude.

Discussion Points

- Spectroscopic contexts where biomedical and fundamental biological studies are made possible by the threshold shift from 900 MHz to 1.2 or 1.5 GHz: disordered proteins, cytoskeleton assemblies, virus particles,