Lectures - Saturday, March 14, Session VII

L27

QUO VADIS? NMR AS AN EMINENT TOOL FOR STRUCTURAL AND SYSTEMS BIOLOGY

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During past twenty years, NMR has established as a prominent technique of chemical and biomedical research and as an eminent tool of structural biology. Compared to X-ray diffraction, NMR, by going beyond the static picture of the 3D structure, provides more than just structural information. Functionally relevant data can be retrieved about molecular dynamics on time scales spanning over 14 orders of magnitude, ranging from 10^{-12} sec to 10^{2} sec, as well as on weak and transient intermolecular interactions within large molecular assemblies. By NMR, also systems that do not tend to crystallize, such as intrinsically unstructured protein, large protein aggregates, and protein-nucleic acid complexes, can be closely investigated; moreover, in physiologically relevant states, where pH, temperature, and salt concentrations can be matched to the conditions in the living cell. The lecture will review the current developments of bio-NMR technology using examples of recently published results. NMR potential of making important contributions to the development of systems biology will be briefly outlined.

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OLIGOMERIZATION OF NON-MYRISTOYLATED M-PMV MATRIX PROTEIN

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The self-assembly of viral particle is based on Gag–Gag interactions mediated by homo-interactions of its three domains, i. e. matrix, capsid and nucleocapsid proteins. As the N-terminal part of Gag polyprotein, matrix protein (MA) targets Gag proteins to the site of virus assembly and it directly interacts with plasma membrane, thus initiating budding of viral particles. MAs of most retroviruses are myristoylated on their N termini, which supports the above-mentioned MA functions and strongly promotes MA–MA interactions.

Oligomerization of isolated MA was most extensively studied in Human immunodeficiency virus 1 (HIV-1). It was found that it forms trimers in solution in its myristoylated (myr(+)) rather than non-myristoylated (myr(–)) form [1–3]. Similar oligomerization behaviour was observed in a closely related, though naturally non-myristoylated Equine infectious anaemia virus (EIAV) MA [4].

We studied oligomerization properties of the myr(–) form of Mason-Pfizer monkey virus (M-PMV) matrix protein by means of NMR spectroscopy. Based on dilution-induced chemical shift perturbation data we observed the oligomerization and mapped out the interaction interface of the molecule. A combined quantification of concentration-dependent chemical shifts and translational diffusion coeffcients allowed us to prove for the first time that M-PMV MA exists in a monomer–dimer–trimer equilibrium in solution, and to calculate the respective equilibrium constants. The chemical shift data were further used to calculate structural models of MA oligomers in HADDOCK, revealing a close similarity of M-PMV and HIV-1 MA trimers. The mechanism and possible consequences of M-PMV myr(–)-MA oligomerization will be discussed with respect to the behaviour of HIV-1 and EIAV MAs.

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OCTAHEDRAL PARTICLES OF BACTERIOPHAGE MS2 COAT PROTEIN MUTANT

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A covalent dimer of the bacteriophage MS2 coat protein was created by genetic fusion of two copies of the gene while removing the stop codon of the first gene. The dimer was crystallized in the cubic F432 space group. The organization of the asymmetric unit together with the F432 symmetry results in an arrangement of subunits that corresponds to T=3 octahedral particles. To form an octahedral particle with closed surface, the dimer subunits interact at sharper angles than in the icosahedral arrangement. The main differences in the subunit packing between the octahedral and icosahedral arrangements are located close to the four-fold and five-fold symmetry axes where different sets of loops mediate the contacts. The octahedral particles are probably artefacts created by the particular crystal packing. We have also determined 4.7 ? resolution X-ray electron density of icosahedral particle assembled from covalent coat protein dimers. At the available resolution the structure corresponds to the wild type MS2 virion except for the intersubunit linker regions. The covalent dimer orientation is random with respect to both icosahedral twofold and quasi-twofold symmetry axes. The inter-subunit contacts around icosahedral three and fivefold symmetry axes are mediated by so called FG loops. The MS2 FG loop was shown to have a function in particle assembly and stability. We have created two covalent dimer mutants, one with substitution and the other with 50 residue insertion, in only one of the two FG loops. The mutations if present in a single subunit would obviate its ability to form capsids. The covalent dimer FG loop mutants were able to form virus-like particles. One FG loop in the covalent dimer was sufficient for particle assembly, but the mutant particles had lowered temperature stability. The formation of virus-like particles by the FG-loop insertion mutant shows possibility for the use of the covalent dimer variants as epitope carriers.

L30

BUQUOY´S „FUNDAMENTAL LAWS OF PHENOMENON OF HEAT" AND THEIR MEANING

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The article „Fundamental laws of phemomenon of heat and their meaning (conceived in the spirit of dynamic-mathematic view, without the acceptance of heat impulse" appeared in the second edition of the book „Sketches of the code book of Nature" in 1818 [1]. It was several times re-publised, namely in Isis 1825 [2] where the subtitle "based on empirical observations" was added. Hovever, any reflection to Carnot´s work from 1824 [3] is not mentioned. It is extension of pages 195-206 of [1], however, for the full understanding also the pages 165-195 need to be read.

As for the heat itself, the article starts by general description of heat processes. Heat state is proportional to its internal tendency to acquire certain volume in the space, eigenene volumizierung which in its part may be exerted to another body as mitteilende volumisierung. The later may be both negative and positive. Eigene volumizierung may be decreased but its values 0 and negative are only fictive, may be observed in nature. I modern terms mittheilende volumisierung is approximately mapped to enthalpy and eigene volumisierung to Gibbs energy. This view in itself contains the first law and joint second and first law of thermodynamic. These concepts are further described in detailed and illustrated by Buquoy´s own experiments. One of the conclusion is the indirect observation of absolute temperature and of the third law and correct statement for efficiency of the heat process. The later is described in most general way.

The main technical difference between Buquoys point of view and our contemporary lies in the fact that Buquoy considered heat moments and conservation of moments as the leading tendency. Contemporary themodynamics is energetic. Despite to it, one must admit that in all aspects Buquoy´s view of thermodynamic processes is more general than the textbook one, since it lacks the dominant reference to ideal gas and includes state transitions. In fact all Buquoy´s experiments were done in condensed phase.

For completeness, one should say that the second law is not explicitly mentioned from energetic point of view, but it is mentioned as the general tendency in the nature in the chapter "Combinatorismus" where the general tendency to mixing is clearly distinguished from chemical change. (Chemical change is by Buquoy understood as change in chemical harmony as analogy to resonance of strings. In chemistry, moment is exchanged in distinct amount. Does it sound familiar to quantum mechanics?)

Besides that, in Buquoy´s work is, at least conceptually correctly and mathematically consistently, expressed the equivalence of heat and light radiation and heat conductance.

Is Buquoys themodynamics contemporary?

The phenomenological themodynamics – as distinguished from themodynamics based on statistical physics – is not a dead field. The latest big unification comes from the 2001 when the Lieb and Yngvasson article [4] appeared. Certainly the mathematic formalism introduced later is substantially more advanced, Buquoy´s thermodynamics is that of practical physical chemist of condensed phase.

Most contemporary, and exclusive, is Buquoy´s concept of infinite reservoir. This was first re-introduced (uniquely) by MacDonald in 1995 [5] and brings a natural definition of entropy as maximal heat that the system absorbs upon terminating the given state change. In Buquoy we read " ...besides that, it (the metal ball) accepts the same type of calorisation manifestation as the surrounding ...without heating it." According to [4] MacDonald´s theory suffers of unstated assumptions about differentiability of entropy which, however, seems to be the only objection against it. Buquoy used this concept for objective definition of absolute temperature. But we know that - in case that the only adiabatic work is the mechanical one. In the moment-based themodynamics this concept may, actually, be quite natural.

As a general concept, Buquoy considers phenomenon of heat as one of major principles governing the world, besides mechanical movement, field (he discusses gravitation field) and chemical changes. He lists distinctions between these phenomena but also considers analogy between partially elastic collision and thermal phenomena.

What would thermodynamic be if Buquoy´s theories were broadly accepted?

Starting from the end of the above argumentation, we may have had a conceptually different thermodynamic theory which naturally brings about the third law. The experiments would show different absolute zero entropies, there are indices to this notion in [1] and [2]. Joint first and second law, in mathematical expression the Gibbs or Helmholtz energies (well, related moments) would have been used from the very beginning. Would we really need entropy after all? Or, in another words, do we understand macroscopic temperature in description of non-equilibrium energy-based thermodynamics?

In practice, there would never be dominancy of ideal gas state equation in our reasoning. For Buquoy, the (moment) state equation was always unknown function to be determined experimentally. Actually, Buquoy´s experimentally devised state equations did not survive the test of time. As he expected in statement "….as far as we may say with our rather imprecise thermometers….".

Finally, a bit outside of this article, Buquoy´s view of heat conductance and diffusion is certainly the one which is used in modern textbooks. Highly visionary is his chemistry and theory of light. For all this, his moment-based view of world was of a big help. At least it is interesting reading for critical minds.

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Lectures - Saturday, March 14, Session VIII

L31

TRANSCRIPTION TERMINATION OF NONPOLYADENYLATED TRANSCRIPTS

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RNA polymerases produce RNA transcripts by initiating and terminating transcription in response to specific sequence elements. Signals that terminate transcription reside either in the DNA template sequence or, alternatively, in the nascent RNA transcript. We will present our functional and structural data related to transcription termination of nonpolyadenylated transcripts that is mediated by the Nrd1 complex.

L32

PROTEOSYNTHETIC ELONGATION FACTOR EF-Tu DOMAINS: THERMAL STABILITY, ADAPTATION AND FUNCTIONS

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Elongation factors EF-Tu are essential components of the bacterial protein synthesis machinery. They are members of the family of GTP-binding proteins, their activity is regulated by guanine nucleotides GDP and GTP. Binding with GTP induces an active-GTP conformation rendering EF-Tu capable of binding aminoacyl-tRNA (aa-tRNA) and transferring it to the ribosomal A-site during mRNA decoding. Hydrolysis of the bound GTP to GDP by the EF-Tu intrinsic GTPase center, activated by the codon-anticodon contact, induces a great conformational change in EF-Tu resulting in an "inactive"-GDP conformation. EF-Tu.GDP leaves the ribosome because its affinity for aa-tRNA is lost, thus allowing a proper accommodation of aa-tRNA at the ribosomal A-site. All elongation factors EF-Tu are composed of a ~400 amino acid residue long polypeptide chain folded into three clearly distinct domains. The N-terminal domain 1 (~200 residues) has an alpha/beta structure. It is called a catalytic domain as it is the site of GDP/GTP binding and GTPase activity. The middle domain 2 and the C-terminal domain 3 (each composed of ~100 residues) are beta barrels. They have no known binding or catalytic activity.

The two guanine nucleotide-induced EF-Tu conformations that determine EF-Tu functional state (as described above) are the results of both interdomain and intradomain rearrangements. To better understand how individual domains are involved in the building of conformational stability and functions of the EF-Tu we compared, in several assays, the properties of isolated domains 1 and full length EF-Tus from mesophilic, Gram- *Escherichia coli* (Ec), growing at 37°C, and thermophilic, Gram+ *Bacillus stearothermophilus* (Bst), growing at 60 °C and six chimeric forms of EF-Tu constructed by combination of domains of Ec and Bst EF-Tus. We found that 1) thermal stability of both EF-Tu proteins in both conformations resulted from domain 1 and domains 2+3 positive cooperation. Domain 1 set up a "basal" level (~20 °C higher with Bst domain 1 than with Ec domain 1) of the thermostability of either EF-Tu and domains 2+3 (mainly domain 3) finally raised the thermostability to the level consistent with the optimal growth temperature of either organism by stabilization of alpha-helical regions of domain 1 (as revealed by CD spectroscopy); 2) non catalytic domains 2+3 of Ec and Bst origin principally differed in their contributions to GDP and GTP binding and GTPase hydrolyzing activities of their respective EF-Tus: (i) Whereas in EcEF-Tu, the physiological GTP/GDP binding activity was attainable only in the presence of all three domains, in BstEF-Tu this activity appeared to be intrinsic to the G-domain alone. Here, the affinity for GTP/GDP was not affected by removal of domains 2+3. (ii) Domains 2+3 (mainly domain 2) of EcEF-Tu suppressed whereas domains 2+3 (mainly do-

main 2) of BstEF-Tu stimulated the GTPase activity of their respective G-domains. These results suggest that despite a high degree of structural homology (~ 80%) EF-Tu proteins developed, in various bacteria, a different inter-domain mechanics to fulfil the same functions. The non-catalytic domains of EF-Tu proteins might represent new potential tools for selective manipulation of G+ and G- bacteria.