GIDRM SATELLITE MEETING

Under the auspices of GIRM (SCI)

Saturday 10th July, Aula Magna, University of Florence

P.zza San Marco 4, Florence

09.00 Welcome Address

Chairperson: To be selected

09.15 Neri Niccolai Università di Siena

Protein Surface Accessibility & Dynamic Drug Design

10.00 Carla Marchioro GlaxoSmithKline, Verona

Investigation of the Rotational Barrier of Amide C–N bonds in a New Class of Orexin Antagonists and Impact on their Biological Activity

10.45 Coffee Break

Chairperson: To be selected

11.15 Andrea Mele Politecnico di Milano

Heteronuclear NOE and Diffusion Measurements for the Assessment of the Local Structure of Li-Doped Ionic Liquids of Electrochemical Interest

11.40 Luca Venturi Università di Bologna

Methods for Fast Acquisition of Spatially Resolved 2D T_1-T_2 Relaxation Spectra

12.05 Mariano Casu Università di Cagliari

Myoglobin, a Structural and Dynamical Paradigm of Complexity

12.30 Klaus Müller Università di Trento

Selected Applications of Solid-State NMR in Materials Science

13.00 Lunch

Chairperson: To be selected

14.30 Alceo Macchioni Università di Perugia

Diffusion and NOE NMR Studies on the Self-Aggregation Tendency and Intermolecular Structure of Zirconocenium and Ammonium Salts in Low Polar Solvents

14.55 Maristella Gussoni Politecnico di Milano

Can a Protein be Used 'Alone' to Solve Biological Problems by ^{1}H-NMR? Myoglobin Dressing 'Old' and 'New' Clothes

15.20 Luca Varani IRB, Bellinzona

NMR Epitope Mapping: Experimentally Validated Computational Biology

15.45 Coffee Break

16.15 GIDRM Assembly

17.45 GIRM Assembly

PROTEIN SURFACE ACCESSIBILITY & DYNAMIC DRUG DESIGN

Neri Niccolai

Dipartimento di Biologia Molecolare, Università di Siena, Via Fiorentina 1, 53100 Siena, Italy

E-mail: niccolai@unisi.it

Targeting protein-protein interactions for a therapeutic purpose is an attractive idea that has proved to be extremely challenging in practice. This is due to the large and flat landscape of most contact surfaces that make them less amenable to intervention by a small molecule. However, in recent years a growing body of evidence has demonstrated that small molecules can disrupt such large and complex protein interactions by binding to interface "hotspots" with drug-like potencies [1]. The fact that small molecules bind targeted proteins in surface pockets which are not present in protein-protein interfaces indicates that conventional drug design procedures, based on the available structural information, are not suitable to discover molecules which can interfere with the protein-protein interaction process. Thus, we are developing criteria and algorithms to search for transient pockets on the protein surface which can accommodate molecules disrupting protein-protein adducts. Molecular Dynamics simulations, rather than static molecular structures, are used as a rational basis for Dynamic Drug Design.

Protein-protein interactions involving chemokines are of primary relevance, as the immune system relies on chemokine signaling to direct lymphocyte homing, orchestrate inflammatory responses, and stimulate wound healing. Outside of these normal functions, chemokines and their receptors also participate in numerous disease states, including HIV/AIDS, asthma, autoimmune diseases, and cancer. We have investigated the transient pocket formation on the surface of SDF1-α, the chemokine stromal cell-derived factor 1, a structurally resolved protein [2] which directs stem cell homing and breast cancer metastasis.

The already established acceptor capability of hydrogen bonds exhibited by TEMPOL towards free backbone amides [3] is proposed as an experimental filter to identify surface hotspots among all the predicted transient pockets.

References

[1] Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. Nature. 2007 Dec 13;450(7172):1001-1009.

[2] Veldkamp CT, Ziarek JJ, Su J, Basnet H, Lennertz R, Weiner JJ, Peterson FC, Baker JE, Volkman BF. Monomeric structure of the cardioprotective chemokine SDF-1/CXCL12. Protein Sci. 2009 Jul;18(7):1359-1369.

[3] Bernini A, Spiga O, Venditti V, Prischi F, Bracci L, Tong AP, Wong WT, Niccolai N. NMR studies of lysozyme surface accessibility by using different paramagnetic relaxation probes. J Am Chem Soc. 2006 Jul 26;128(29):9290-9291.

INVESTIGATION OF THE ROTATIONAL BARRIER OF AMIDE C–N BONDS IN A NEW CLASS OF OREXIN ANTAGONISTS AND IMPACT ON THEIR BIOLOGICAL ACTIVITY

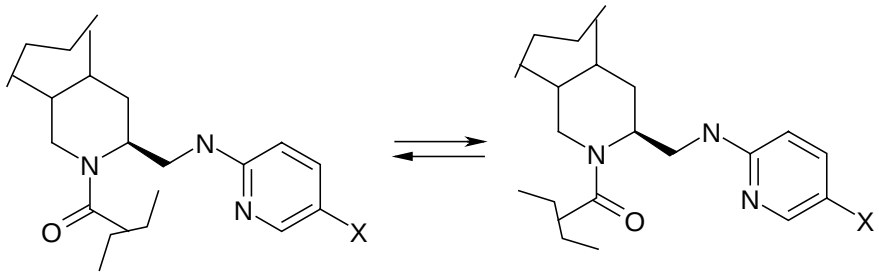
Carla Marchioro

Analytical Chemistry, GlaxoSmithKline Medicines Research Centre, Via Fleming, 4, 37135 Verona

E-mail: carla. marchioro@gsk.com, marchioro.carla@alice.it

Tertiary amides are well known being involved in conformational equilibrium characterized by slow inter-conversion among the *E/Z* forms. In some cases, restricted rotation confers to the two species high stability to be separated by chromatography, to show different physico-chemical behaviour and also different biological activity.

This work will illustrate how NMR techniques have been used in order to study the *E/Z* inter-conversion on compounds active as Orexin antagonists with general structure as reported below.



E ***Z***

Kinetics and thermodynamics of the *E*/*Z* inter-conversion in solution have been investigated. Values of half-life, free activation energy and relative thermodynamic stability have been evaluated in function of different physico-chemical conditions such as sample preparation, dissolution solvent, temperature and pH.

In conclusion, the analytical approach for the separation of the two species will be discussed and some highlights will be given in relation to biological activity.

HETERONUCLEAR NOE AND DIFFUSION MEASUREMENTS FOR THE ASSESSMENT OF THE LOCAL STRUCTURE OF LI DOPED IONIC LIQUIDS OF ELECTROCHEMICAL INTEREST

Franca Castiglione[1], Enzio Ragg[2], Andrea Mele[1*], Giovanni Battista Appetecchi[3], Maria Montanino[3], Stefano Passerini[3,4]

1) Dipartimento di Chimica, Materiali e Ingegneria Chimica "G. Natta", Politecnico di Milano, I-20131 Milano, Italy

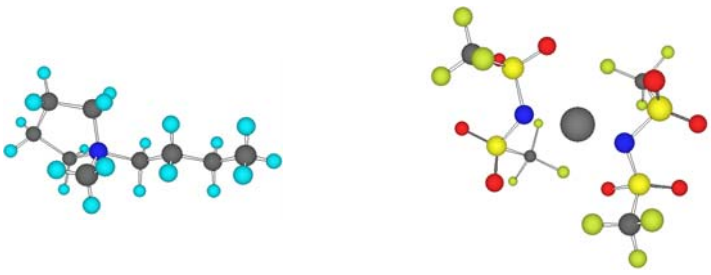
2) Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Via Celoria, 2 I-20133 Milano (Italy)

3) ENEA, Italian Agency for the New Technology, Energy, and the Environment, TER Dept., Via Anguillarese 301, I-00123 Roma, Italy

4) Institute of Physical Chemistry, University of Münster, Corrensstr. 28/30, D48149 Münster, Germany

E-mail: andrea.mele@polimi.it

A new class of liquid electrolytes for lithium batteries has been recently developed by doping suitable ionic liquids with Li salts. In this communication we present a study of the local structure and transport properties of the ionic liquid N-methyl-N-butylpyrrolidinium bis(trifluoromethanesulfonyl)imide [(PYR_{14})TSFI] in the presence of LiTFSI. The mixture composition was 0.9 PYR_{14}TFSI-0.1 LiTFSI. Heteronuclear NOE (HOESY) and pulsed field gradient spin-echo (PFGSE) techniques were used together with viscosity and conductivity measurements. (^{1}H-^{19}F)- and (^{1}H-^{7}Li)-HOESY gave information on cation-anion and dopant (Li^+)-cation (PYR_{14}^+) interactions, respectively. Similar NOEs were observed for both $TFSI^-$ and Li^+ towards PYR_{14}^+, thus giving evidence of a strict interaction, possibly coordination, of Li^+ and $TFSI^-$. These data are consistent with the existence of the complex $[Li(TFSI)_2]^-$ in solution, as already postulated [1]. The geometries, the energies of the possible ion cluster were examined using theoretical DFT calculations and a minimum energy structure for the complex was found to be more stable then the isolated ions (Figure 1).



$[PYR_{14}]^+$ $[Li(TFSI)_2]^-$

Figure 1

The self-diffusion coefficients D for the individual ions in the mixture were measured and the results compared with those of pure $PYR_{14}TSFI$ [2]. The temperature dependency of diffusivity and viscosity allowed us to obtain the related activation energies E_a. PYR_{14}^+ and $TFSI^-$ in the Li-doped mixture gave similar $E_a[D]$ values, comparable with those measured in the pure $PYR_{14}TFSI$[2]. The $E_a[D]$ value for Li^+ turned out to be remarkably higher than those measured for PYR_{14}^+ and $TFSI^-$, indicating a different transport mechanism for Li^+ with respect to the other ionic species.

References

[1] J.C. Lassegues, et al. J. Phys. Chem. A 113 (2009), 305.

[2] F. Castiglione, et al. J. Phys. Chem. B 113 (2009), 10750.

METHODS TO FAST ACQUISITION OF SPATIALLY RESOLVED 2D T_1-T_2 RELAXATION SPECTRA

Luca Venturi

IRCCS San Giovanni di Dio Fatebenefratelli, Lab. LENITEM, Brescia, Italy

E-mail: luca.unibo@gmail.com

2D T_1-T_2 relaxation spectra based on the fast 2D inverse Laplace transformation algorithm developed by Song and Hurlimann [1,2] have proved invaluable as microstructural probes of a wide variety of complex, heterogeneous systems including porous rocks, cellular tissue, protein systems and even nano-structured synthetic hydrogels.

Besides its application in materials science, 2D relaxometry also has potential as a non-invasive image "biomarker" for clinical diagnosis in magnetic resonance imaging (MRI). However, for routine clinical diagnosis, it will be necessary to not only volume-select but also minimise the acquisition time to just a few minutes.

A number of approaches to fast acquisition of spatially localised T_1-T_2 relaxation spectra [3] will be presented, including reducing the recovery delay, multislicing [4] and steady-state methods based on the periodic inversion of longitudinal magnetisation [5].

References

[1] Y.Q.Song et al., **T_1-T_2 correlation spectra obtained using a fast 2D Laplace Inversion,** J. Magn. Reson. 154 (2002) 261-268.

[2] M.D. Hurlimann and L. Venkataramanan, **Quantitative measurements of 2D distribution functions of diffusion and relaxation in grossly inhomogeneous fields,** *J.Magn. Reson. 157 (2002) 31-42.*

[3] L. Venturi and B. Hills (2010). ***Spatially Resolved 2-D Cross-Correlation Relaxometry.*** *J. Magn Reson. Imag. 28 (2), 171-177.*

[4] L. Venturi, K. Wright and B. Hills (2010). ***Ultrafast T_1-T_2 relaxometry using FLOP sequences.*** *J. Magn. Reson. (In Press).*

[5] L. Venturi, J. Warner and B. Hills (2010). ***Multisliced Ultrafast 2-D Relaxometry.*** *J. Magn Reson. Imag. (In Press).*

MYOGLOBIN
A STRUCTURAL AND DYNAMICAL PARADIGM OF COMPLEXITY

Mariano Casu[a], Roberto Anedda[b], Mariano Andrea Scorciapino[a], Arturo Robertazzi[c], Paolo Ruggerone[d] and Matteo Ceccarelli[d]

[a]*Department of Chemical Sciences, University of Cagliari, Monserrato-Sestu Km 0.700 I-09042, Monserrato (CA), Italy*
[b]*Porto Conte Ricerche S.r.l., NMR/MRI facilities, SP 55 Porto Conte/Capo Caccia Km 8.400, Loc Tramariglio 07041 Alghero (SS), Italy*
[c]*Institute of Chemistry und Biochemistry, Freie Universitaet, Fabeckstr. 36a, D-14195, Berlin-Dahlem, Germany*
[d]*CNR-INFM SLACS, Department of Physics, University of Cagliari, S.P. Monserrato-Sestu Km 0.700, I-09042 Monserrato (CA), Italy*

E-mail: mcasu@unica.it

Myoglobin, since its three-dimensional structure was solved 50 years ago by Kendrew et al. (*Nature* 1960, *185*, 422), still represents a model system to unveil how structure and dynamics contribute to the protein function. X-ray structures of myoglobins from different species and in many mutated forms are by now well characterized to a very precise extent, and the molecular and electronic structures of these proteins have been extensively studied by using numerous complementary techniques. This protein reversibly binds gas ligands at the active site buried in the protein matrix, and the internal cavities are sites in which ligand molecules can reside. The breathing motions of these cavities, of relatively small volume, play a specific role in controlling protein functions, e.g., ligand migration and binding. Nowadays, still under discussion is how a ligand finds its migration pathways between the internal cavities and the solvent.
Aiming to address some of these issues (i.e., structure of internal hydrophobic cavities, movements of ligands inside the protein matrix and the role of surface waters) two tools were employed, Nuclear Magnetic Resonance and all-atom MD simulations, which complement well each other providing an overview of some structural and dynamical aspects of the protein.
Here, we will focus on the use of ^{129}Xe NMR chemical shifts and spin-lattice relaxation time in solutions of myoglobin to extract structural information on the internal cavities. The relevance of guest-host interactions in xenon complexes with myoglobins is thoroughly analyzed, and the use of xenon to detect and characterize voids within flexible biomolecules is critically discussed.
The existence of hydrophobic sites can also be inferred from the determination of the influence of xenon concentration on the protein ^{1}H chemical shifts. Solution ^{1}H NMR studies of paramagnetic myoglobin provide a wealth of insights into electronic, magnetic and molecular properties of the hydrophobic cavities close to the active site and consequently into the interaction with the xenon atom.
To pinpoint intrinsic internal pathways between cavities a statistical approach was applied, on the MD trajectories with special emphasis on the pathway that joins the ligand binding site and the other cavities. Our study points out the remarkable dynamical behavior of some cavities, whose “breathing motions” may facilitate migration of ligands. Moreover, the MD-based analysis of the solvent waters residing long around/inside the protein reveals peculiar roles of the solvent and enables *portal* for ligands to enter/escape the protein to be identified.

SELECTED APPLICATIONS OF SOLID-STATE NMR IN MATERIALS SCIENCE

Klaus Mueller

Dipartimento di Ingegneria dei Materiali e Tecnologie Industriali, Università degli Studi di Trento, Via Mesiano 77, I-38123 Trento

E-mail: klaus.mueller@ing.unitn.it

Solid-state NMR spectroscopy is used to evaluate the structural and dynamic features in different types of solid materials. Such information is a prerequisite for the correlation of the molecular behaviour and distinct bulk properties of the materials under investigation, and puts the basis for a directed development of new materials with specific functional properties.

Representative examples are shown which include solid-state NMR investigations on (i) polymer membranes for low temperature fuel cells, (ii) hydrogen storage materials, (iii) protective sol-gel coatings for wood and paper, and (iv) guest-host systems.

In addition, a new, generally applicable computer simulation programme for dynamic solid-state NMR experiments will be briefly discussed.

DIFFUSION AND NOE NMR STUDIES ON THE SELF-AGGREGATION TENDENCY AND INTERMOLECULAR STRUCTURE OF ZIRCONOCENIUM AND AMMONIUM SALTS IN LOW POLAR SOLVENTS

Luca Rocchigiani, Gianfranco Bellachioma, Gianluca Ciancaleoni, Stefano Crocchianti, Antonio Laganà, Cristiano Zuccaccia, Daniele Zuccaccia and Alceo Macchioni

Dipartimento di Chimica, Università di Perugia, Via Elce di Sotto, 8, 06123 Perugia, Italy

E-mail: alceo@unipg.it

The results of ^{1}H- and ^{19}F-diffusion and ^{19}F,^{1}H-HOESY NMR studies on the self-aggregation tendency of zirconocenium salts ([Zr][X]) with alkylic chains (miming the growth of a polymeryl chain) of variable length [1] and di-long chain quaternary ammonium salts ([$(C_{18}H_{37})_2Me_2N$][X]) with different X^- counterions are reported. It is shown that the tendency to self-aggregate of [Zr][X] results enhanced enormously in cyclohexane (with respect to that in benzene) to the point that the percentage of ion quadruples is noticeable even at the concentration level used in the olefin polymerizations. The effect of X^- on the tendency of [$(C_{18}H_{37})_2Me_2N$][X] to form both ion quadruples and higher aggregates is quantified by applying several models of indefinite aggregation. Mathematical fitting of their appropriate formulations allows realistic thermodynamic parameters of the self-aggregation processes to be evaluated.

References

[1] Rocchigiani, L.; Zuccaccia, C.; Zuccaccia, D.; Macchioni, A. *Chem. Eur. J.*, **2008**, 14, 6589.

CAN A PROTEIN BE USED *'ALONE'* TO SOLVE BIOLOGICAL PROBLEMS BY ^{1}H-NMR? *MYOGLOBIN DRESSING 'OLD' AND 'NEW' CLOTHES*

M. Gussoni[1], F. Greco[3], A. Vezzoli[4], L. Zetta[3] and M. A. Cremonini[2]

[1] *Università di Milano, Dipartimento di Scienze e Tecnologie Biomediche. Segrate (MI), I-20090 Italy*
[2] *Università di Bologna, Dipartimento di Scienze degli Alimenti - Cesena, I-47023 Italy*
[3] *CNR, Lab. NMR, Istituto per lo Studio delle Macromolecole. Milan, I-20133 Italy*
[4] *CNR, Istituto di Bioimmagini e di Fisiologia Molecolare. Segrate (MI), I-20090 Italy*

E-mail: maristella.gussoni@unimi.it

Main challenge of biomedicine resides into the possibility of non-invasively performing measurements returning absolute quantitative metabolic levels. It is just under this respect that NMR was early detected as particularly suited to quantitatively assess metabolite signal levels in tissues. Myoglobin (Mb), an O_2 binding protein, plays an essential role in maintaining aerobic metabolism throughout its widely accepted function in cellular O_2 transport and buffering. However, Mb has been recently reported to dress 'new clothes'[1] like a scavenger of nitric oxide and O_2 free radicals produced under oxidative stress conditions. In this talk we will be discussing the old and new clothes of Mb, reporting about two relevant biomedical problems that have been faced by NMR, namely the quantitative measure of in vivo PO_2 and the relationship between the amount of human isoforms of Mb and muscle efficiency in high altitude natives. ***a)*** the well known oxygen binding equation: $k = 1 / [PO_2]_{50} = [\text{oxy-Mb}] / [\text{deoxy-Mb}]$ PO_2, is a simple direct method to assess muscle intra-cellular oxygenation once that oxy-Mb and deoxy-Mb concentrations are obtained from the correspondent ^{1}H-NMR peak areas[2]. This was attained by first choosing *'in vitro'* a couple of NMR sequences that could each maximize the signal to noise ratio of the oxy and deoxy-Mb NMR signals, while avoiding baseline rolling and still sufficiently suppressing the water signal; then an external calibration curve was built from a number of deoxy- and met-Mb solution samples. Finally, these sequences and calibration curves were employed to estimate for the first time the actual concentrations of both forms of bioactive Mb under increasing hypoxia and calculate the intracellular PO_2 in Arenicola Marina, a hypoxia tolerant lungworm widely used as a living model to study metabolic adaptation in oxy-conformers tissues; ***b)*** human Mb is characterized by five isoforms: the more expressed Mb I (~75%) and Mb II (~15-20%) differ only by a single substitution (Lys (K) vs Glu (E)) at position 54 respectively, playing relevant influence on the superficial charge: pI = 7.29 (54E) vs pI = 8.57 (54K). The observation that high altitude natives are characterized by a greater muscle Mb level (+ 20%), totally attributable to the increase of the 54E isoform (+ 100%)[3] jointed to a better metabolic efficiency[4], supported the hypothesis of a cause-effect relationship between the two findings, ascribable probably to the free radicals scavenging role of Mb, accounted by the greater oxidative stress beard by high altitude populations. A comparative analysis of the two isoforms,

never recombined nor studied before by NMR, was developed to gain insight into the structure to function relationship in the attempt to find an answer to the physiological question. However the preliminary study did not highlighted significant structural differences.

References

[1] Cossins A, Berenbrink M. Nature, 2008, 454, 416-417.

[2] Gussoni M, Cremonini MA, Vezzoli A, Greco F, Zetta L. Analytical Biochem., 2010, 400, 33-45.

[3] Gelfi C, De Palma S, Ripamonti M, Wait R, Eberini I, Bajracharya A, Marconi C, Schneider A, Hoppeler H, Cerretelli P. Faseb J., 2004, 18, 612-614.

[4] Marconi C, Marzorati M, Sciuto D, Ferri A, Cerretelli P. J. Physiol., 2005, 569, 667-675.

NMR EPITOPE MAPPING: EXPERIMENTALLY VALIDATED COMPUTATIONAL BIOLOGY

Luca Varani

Institute for Research in Biomedicine, Via Vela 6, 6500 Bellinzona, Switzerland

E-mail: luca.varani@irb.unisi.ch

If we understand the structural rules governing antibody/antigen interactions in a given virus, then we have the molecular basis to attempt to design and synthesize new epitopes to be used as vaccines or optimize the antibodies themselves for passive immunization. Comparing the binding of several different antibodies to related antigens should also further our understanding of general principles of recognition. To obtain and compare the three-dimensional structure of a large number of different complexes, however, we need a faster method than traditional experimental techniques. While biocomputational docking is fast, its results might not be accurate. Combining experimental validation with computational prediction may be a solution.

As a proof of concept, here we isolated a monoclonal antibody from the blood of a human donor recovered from Dengue virus infection, characterized its immunological properties, and identified its epitope on domain III of Dengue virus E protein through simple and rapid NMR chemical shift mapping experiments. We then obtained the three-dimensional structure of the complexes between the antibody and different antigens by computational docking, using the NMR data to drive and validate the results. In an attempt to represent the multiple conformations available to flexible antibody loops, we docked several different starting models and present the result as an ensemble of models equally agreeing with the experimental data. The antibody was shown to bind a region accessible only in part on the viral surface, explaining why it cannot effectively neutralize the virus.