CCPN Conference 2017

University of Stirling, Stirling, Scotland

13th-15th July 2017

Thursday 13th July

CCPN Training workshops will take place from 9:00 on Thursday morning.

12:00 Registration (Pathfoot Building) / Check-in (Houses/Halls 1-8 – see map for location)

12:30 Lunch

13:30 **Brian Smith**: Introduction

Session 1: NMR in drug design: small molecules

Session Chair : Matt Crump (University of Bristol)

13:40 **Ben Davis** (Vernalis)
Applications of NMR in (Fragment Based) Drug Discovery

14:20 **Richard Taylor** (UCB)
Developments in NMR Fragment Screening at UCB

14:50 Break (Tea/Coffee)

15:20 **Judith Schlagnitweit** (Karolinska Institute)
Monitoring Changes of Dynamics of Biomolecules in Response to Drugs

15:50 **Christopher Waudby** (UCL)
TITAN: New Developments in Two-Dimensional NMR Lineshape Analysis

16:20 Discussion

16:50 **Geerten Vuister** (University of Leicester)
"CCPN : New Developments" and "UK NMR infrastructure"

17:45 **Rasmus Fogh**
CCPN Assembly Meeting

18:15 Poster Session (Main Hall)

19:00 Dinner

Friday 14th July

8:00 Breakfast

8:55 Meeting Starts

Session 2 : NMR in drug design: biologics

Session Chair : Helen Mott (University of Cambridge)

9:00 **Mark Carr** (University of Leicester)

NMR in Therapeutic Antibody Development and Antibody-Assisted Drug Discovery

9:40 **Chun-Wa Chung** (GSK)

Addressing biopharmaceutical questions using NMR and Biophysics

10:10 **Andrew Jamieson** (University of Glasgow)

Application of NMR in the Design of Peptide Tools for Chemical Biology and Drug Discovery

10:40 Break (Tea /Coffee)

11:10 **Antje Wolter** (Goethe University Frankfurt)

Dissecting ligand recognition and folding of a structurally complex aptamer-GTP-complex by solution NMR

11:40 Discussion

13:00 Walk (Meet: TBD)

18:00 Poster Session

19:15 Conference Dinner and Poster Prize Presentation (Stirling Court Hotel)

Saturday 15th July

8:00 Breakfast

8:55 Meeting Starts

Session 3 : Nucleic Acid NMR

Session Chair : Katja Petzold (Karolinska Institute)

9:00 **Janosch Hennig** (EMBL, Heidelberg)

NMR in integrated structural biology of ribonucleoprotein complexes

9:40 **Lukas Trantirek** (Central European Institute of Technology, Brno)

In-cell NMR spectroscopy of nucleic acids

10:10 **Malgorzata Duszczyk** (ETH, Zurich)

De novo structure determination of a 27.5 kDa protein-RNA complex: a Dead End for classical NMR approaches?

10:40 Break (Tea /Coffee)

11:10 **Thomas Wiegand** (ETH, Zurich)

Protein-nucleotide interactions detected by solid-state NMR

11:40 Discussion

12:00 Discussion Overall Meeting

12:30 Closing Remarks

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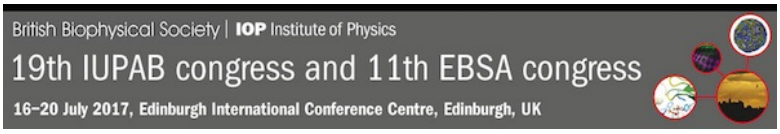

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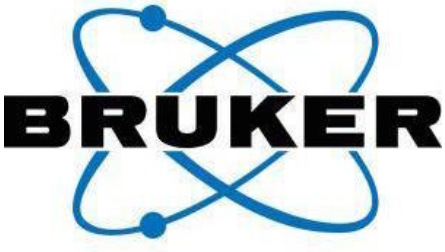

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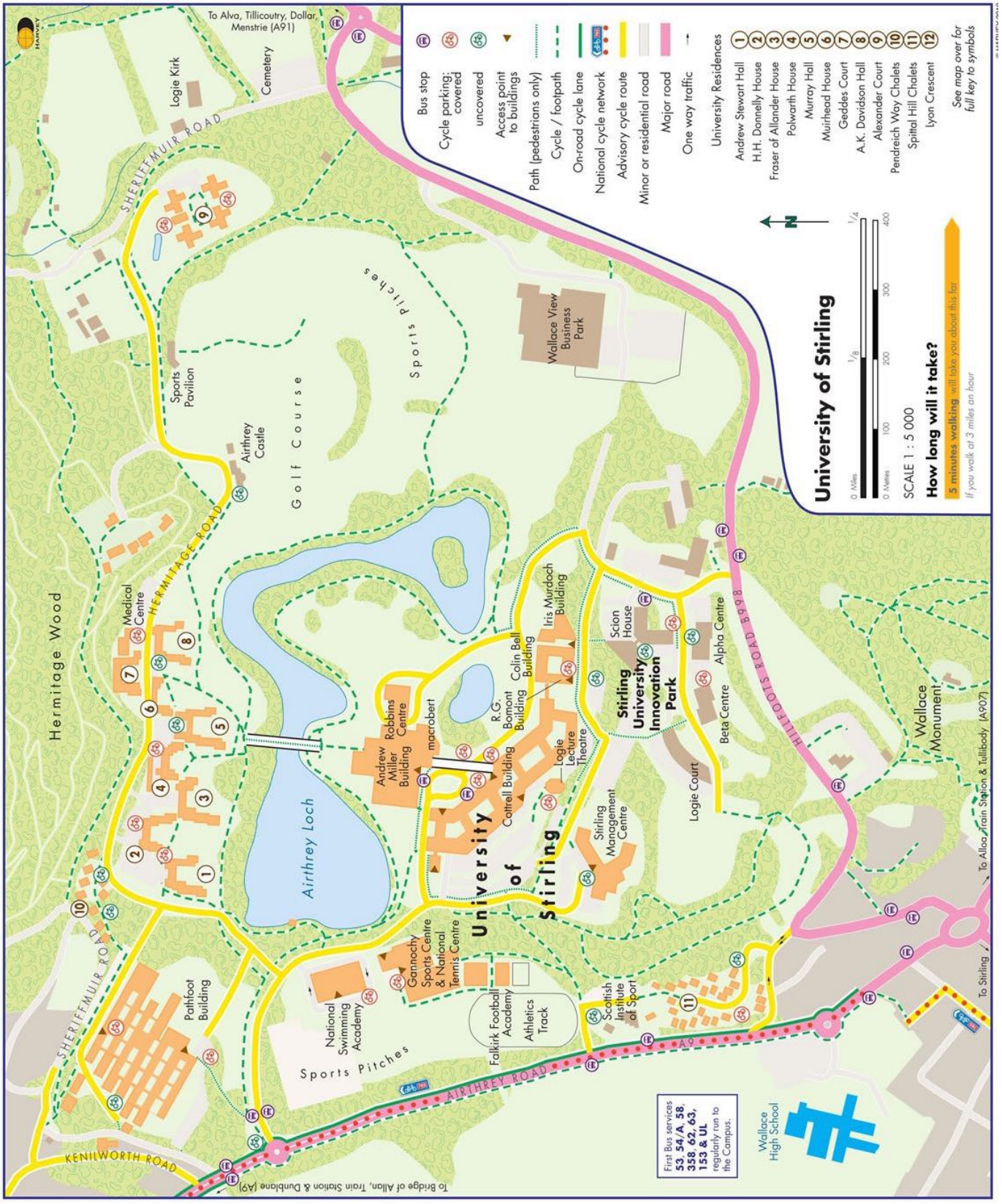
Map of Conference Venue



Session 1: NMR in drug design: small molecules

Applications of NMR in (Fragment Based) Drug Discovery

Ben Davis

In Fragment-Based Drug Discovery, small (and typically low affinity) ligands are identified as start-points for evolution into potent hits and leads. Detecting these weak interactions can push screening technologies to the limit of their sensitivity. It is therefore vitally important to have a high level of confidence in the initial fragment hits in order to minimize the risk of pursuing potentially misleading artefacts.

I will discuss the role of NMR in this process and in particular the practicalities and applications of NMR both as a primary screening method, and as a tool for validating and characterizing putative fragment hits. I will discuss examples of various issues which can arise when working with a range of experimental systems, and of how NMR can provide possible solutions to these problems. I will also discuss how simple NMR data can be used to "guide" the generation of structural models, allowing medicinal chemists to progress a chemical series rapidly in the absence of high resolution solution or crystal structures.

Developments in NMR Fragment Screening at UCB

Richard Taylor

In this talk we will cover the NMR workflow for fragment screening at UCB including the application of ligand observe and protein observe experiments. In this context we will share our recent work on addressing the two critical issues in applying this approach to new target proteins, namely the amount of protein required for screening and the experimental time required to acquire the data. We will show how the choice of NMR hardware, advanced data acquisition strategies and buffer optimisation can result in a tangible reduction in protein consumption and data collection timeframes.

Monitoring Changes of Dynamics of Biomolecules in Response to Drugs

Judith Schlagnitweit[a], *Emilie Steiner*[a], *Hampus Karlsson*[a], *Patrik Lundström*[b], *Katja Petzold*[a]

[a]Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

[b]Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

Changes in molecular structure are essential for the function of biomolecules. This has lead to an increased interest in studying not only static structures but also the interchange of structures into different conformers, i.e. the dynamics of the system. The equilibrium between these various conformations can be perturbed by a variety of molecules e.g. proteins and drugs, or altered in response to cellular cues, such as changes in ion concentration or pH.

$R_{1\rho}$ relaxation dispersion (RD) experiments are very well suited to monitor conformational exchange processes that involve otherwise difficult to detect short-lived (μs to ms) and low-populated, so-called excited states. ^{13}C, ^{15}N and ^{1}H nuclei can be used as probes to monitor conformational dynamics of the biomolecule of interest in its native state or upon titration with a drug molecule.

TITAN: New Developments in Two-Dimensional NMR Lineshape Analysis

Christopher Waudby

NMR titration experiments are a rich source of structural, mechanistic, thermodynamic and kinetic information on biomolecular interactions and conformational exchange processes, which can be extracted by the quantitative analysis of resonance lineshapes. However, applications of such analyses are frequently limited by peak overlap inherent to complex biomolecular systems. Moreover, systematic errors may arise due to the analysis of two-dimensional data using theoretical frameworks developed for one-dimensional experiments.

We have previously introduced a more accurate and convenient method for the analysis of such data, based on the direct quantum mechanical simulation of pulse sequences and fitting of entire two-dimensional experiments, which we implement in a new software tool, TITAN (www.nmr-titan.com) [1]. This method can be used to fully resolve even highly overlapped clusters of residues, greatly extending the general applicability of lineshape analysis. Moreover, as calculations account for differential relaxation during the execution of the pulse sequence, normalisation of individual spectra is not required and resonance intensities can therefore be powerful additional constraints on exchange behaviour, resulting in more accurate and precise results than can be achieved by one-dimensional methods.

We will present a discussion of 'fast' and 'slow' chemical exchange regimes, derived from one-dimensional experiments, in the context of two dimensional experiments. Particular care is needed in the case of HMQC experiments, in which magnetisation is encoded as a mixture of zero and double quantum coherences, and we will present a new analysis of coalescence points and exchange regimes in these experiments. We will also present a new experiment, SIM-H(Z/D)QC, for the simultaneous and sensitive acquisition of zero and double quantum coherence correlation spectra, and discuss the utility of this experiment for the analysis of protein–ligand interactions.

[1] Waudby, C. A., Ramos, A., Cabrita, L. D. & Christodoulou, J. Sci Rep 6, 24826 (2016).

Session 2: NMR in drug design: biologics

Dissecting ligand recognition and folding of a structurally complex aptamer-GTP-complex by solution NMR

Antje C. Wolter[1], A. Katharina Weickhmann[1], Amir H. Nasiri[1], Katharina Hantke[1], Oliver Ohlenschläger[2], Christoph H. Wunderlich[3], Christoph Kreutz[3], Elke Duchardt-Ferner[1], Jens Wöhnert[1]

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[3]Institute of Organic Chemistry, Center for Molecular Biosciences CMBI, University of Innsbruck, Innsbruck, Austria

Aptamers are short, artificially created nucleic acids that bind their cognate ligands with high affinity and specificity. They derive from in vitro selection procedures called SELEX, in which aptamers for very diverse targets can be isolated. Aptamers are versatile molecules that can be used for e.g. therapeutic applications. However, their intricate folds make them highly interesting for structural investigations.

The GTP class II aptamer is one of 11 structurally and sequentially diverse GTP-binding RNA aptamers. Despite its small size (34 nt) it binds GTP with a KD in the nanomolar range[1]. We initiated

structural studies of the aptamer GTP-complex by solution NMR in order to gain insight into the structural diversity of GTP recognition by different aptamers[2].

We solved the structure of the RNA-GTP complex by solution NMR with a low overall rmsd (0.5 Å). GTP binds to the apical loop of the aptamer and is recognized in a base triplet containing an intermolecular Watson-Crick base-pair and a sugar edge interaction with an adenine. The base of the ligand binding site consists of an intramolecular base triplet with a similar architecture. A base quartet is located adjacent to this intramolecular base triplet, where a guanosine imino group forms a hydrogen bond to a phosphate group oxygen. Moreover, an adenosine is stably protonated at its N1 position with a NMR-observable imino proton resonance. The pKa for the protonation is ~ 9.0 and thus shifted by more than five pH units compared to free adenosine (3.7). Interestingly, the free RNA exists as a partially pre-folded conformational ensemble already containing the protonated adenine and the base quartet at pH 5.3 in the presence of Mg2+. This suggests that the ligand selects the prefolded, protonated state of the aptamer for binding and stabilizes its structure even further. Structural and thermodynamic studies of several mutants and ligand analogs show the role of individual residues for the intricate structure and the interplay of hydrogen bonding interactions of the RNA-ligand complex, respectively.

[1] Carothers, J. M.; Oestreich, S. C.; Davis, J. H.; Szostak, J. W. J. Am. Chem. Soc., 126, 5130-5137 (2004)

[2] Wolter, A. C.; Weickhmann, K. A.; Nasiri, A. H.; Hantke, K; Nasiri, A. H.; Hantke, K.; Ohlenschäger, O; Wunderlich, C. H.; Kreutz, C.; Duchardt-Ferner, E.; Wöhnert, Angew. Chem. Int. Ed. Engl. doi: 10.1002/anie.201609184R1

Session 3: Nucleic Acid NMR

NMR in integrated structural biology of ribonucleoprotein complexes

Janosch Hennig

RNA binding proteins (RBPs) are key factors for the regulation of gene expression. However, it is still poorly understood exactly how these trans-acting RBPs recognize their cognate RNAs (base- or structure- specific) to regulate gene expression. Thus, structural studies are crucial to obtain detailed mechanistic insights. This is especially important for ribonucleoprotein (RNP) complexes featuring more than one protein, which form possibly transient and larger complexes consisting also of flexible regions complicate the utilization of available standard methods.

In this lecture, I will attempt to provide a short introduction into NMR of nucleic acids and protein-RNA complexes and how data of both complement each other to obtain structural insights into RNA recognition. This will include the identification of cognate RNA motifs for a specific RBP, RNA assignment (as an introduction to following speakers), intermolecular NOEs (protein-RNA), and paramagnetic relaxation enhancements.

This is followed by an overview about how this data can be integrated with data from other sources (X-ray, SAXS, and SANS) to enable structure determination of larger complexes.

In-cell NMR spectroscopy of nucleic acids

Lukas Trantirek

Central European Institute of Technology, Brno, Czech Republic

I will present our latest results on development of in-cell NMR spectroscopic methods allowing characterization of structure and stability of nucleic acids (NA) and NA-drug complexes, respectively, under native physiological conditions *in vivo*. Among the specific results the following points will be discussed: 1) Difficulties in formulation of buffering systems for in vitro NMR studies of NA, 2) Limitations of *Xenopus laevis* oocyte, an established eukaryotic model, for in-cell NMR studies of NA, and 3) advantages and limitations of newly developed in-cell NMR-based approach for characterization of NA structure in nucleus of living mammalian cells.

References:

[1] Krafciková M, Dzatko S, Fiala R,Granzhan A, Tuleaude-Fishou M.-P, Carone C, Fessl T, Hänsel-Hertch R, Foldynova-Trantírkova S, Trantirek L. Monitoring of DNA-ligand interactions under native conditions in nucleus of mammalian cell using in-cell NMR spectroscopy. *in preparation*, **2017**

[2] Hänsel R, Foldynová-Trantírková S, Dötsch V, Trantírek L. Investigation of quadruplex structure under physiological conditions using in-cell NMR. *Top Curr Chem*. **2013**, 330:47-65.

[3] Hänsel R, Löhr F, Foldynová-Trantírková S, Bamberg E, Trantírek L, Dötsch V. The parallel G-quadruplex structure of vertebrate telomeric repeat sequences is not the preferred folding topology under physiological conditions. *Nucleic Acids Res.* **2011**, 39(13):5768-75.

[4] Fiala R, Spacková N, Foldynová-Trantírková S, Sponer J, Sklenár V, Trantírek L. NMR cross-correlated relaxation rates reveal ion coordination sites in DNA. *J Am Chem Soc*. **2011,** 133(35):13790-3.

[5] Hänsel R1, Foldynová-Trantírková S, Löhr F, Buck J, Bongartz E, Bamberg E, Schwalbe H, Dötsch V, Trantírek L. Evaluation of parameters critical for observing nucleic acids inside living Xenopus laevis oocytes by in-cell NMR spectroscopy. *J Am Chem Soc*. **2009** 131(43):15761-8.

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Protein-nucleotide interactions detected by solid-state NMR

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In this contribution solid-state NMR based strategies to characterize protein-nucleotide interactions in large biological assemblies will be presented using the example of a (double-) hexameric DnaB helicase from *Helicobacter pylori*. The binding of nucleotides (ATP-analogues and single-stranded DNA, ssDNA) to the helicase is monitored by 31P cross-polarization experiments. The observed chemical-shift values allow for a clear discrimination between the different nucleotides used in this work and reveal that two nucleotides of bound ssDNA can be structurally distinguished. 15N,13C correlation experiments highlight the role of arginine sidechains in binding to single- stranded DNA.

We further present dipolar-coupling based polarization-transfer experiments from 31P spins of bound nucleotides to 13C or 15N spins of the protein (or vice versa) to describe nucleotide binding at the atomic level. Residues involved in protein-nucleotide interactions are identified in CHHP and NHHP correlation experiments.

While conventional NMR experiments on such large protein assemblies typically suffer from low signal-to-noise ratios and thus long experimental measurements times, we used Dynamic Nuclear Polarization (DNP) enhanced MAS experiments to increase the sensitivity. The samples for DNP were prepared in the absence of glycerol which results in the highest sensitivity for DnaB. The obtained spectra allow to identify spin systems of the helicase in the neighbourhood of 31P spins of the nucleotides. Despite the extensive line-broadening at cryogenic temperatures, the sparsity of peaks in the obtained spectra allows to identify residue types involved in protein-nucleotide binding.