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# Ultra fast MAS solid-state NMR studies of protein-protein interactions in the bacterial replisome

M Felletti
*Ecole Normale Superieure de Lyon*

S Jehle
*Ecole Normale Superieure de Lyon*

Y Wang
*University of Wollongong*, yw974@uowmail.edu.au

Kiyoshi Ozawa
*University of Wollongong*, ozawa@uow.edu.au

S Jergic
*University of Wollongong*, jergic@uow.edu.au

***See next page for additional authors***



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version of human muscle acylphosphatase into oligomers with varying heparan sulfate and protein concentrations in a remarkably reproducible manner. We also analyzed mutants of the 15 basic amino acids of acylphosphatase, identifying the residues primarily involved in heparan sulfate–induced oligomerization of this protein and resolving the process with unprecedented molecular detail. Finally, we showed the general applicability of our method to other protein systems.

## P20-212
## Ultra fast MAS solid-state NMR studies of protein–protein interactions in the bacterial replisome

**M. Felletti**[1], S. Jehle[1], Y. Wang[2], K. Ozawa[3], S. Jergic[2], G. Otting[3], L. Emsley[1], A. Lesage[1], N. Dixon[2] and G. Pintacuda[1]
*[1]Ecole Normale Supérieure de Lyon, CRMN, Villeurbanne, France, [2]School of Chemistry, University of Wollongong, Wollongong, NSW, Australia, [3]Research School of Chemistry, Australian National University, Canberra, ACT, Australia*

Replication of genomic DNA in bacteria involves multiple stable and transient interactions among protein subunits constituting the replisome. Here we show how ultra-fast magic angle spinning (MAS) solid-state NMR (ssNMR) and $^{1}$H-detection on either deutereted or fully protonated samples, can give new information about protein-protein interactions in the *E. coli* replisome. We focused on two fundamental interactions: the first one between the C-terminal domain of the single-stranded (ss) DNA binding protein and its own DNA-binding site, and the second one between the DNA polymerase III subunits $\alpha$ and $\tau$. SSB (a tetramer of 79 kDa) has a structurally well-defined ssDNA binding domain (OB-domain) and an intrinsically disordered C-terminal (Ct) domain. Its extreme Ct acidic motif is known to mediate the binding of SSB to different DNA processing enzymes and scaffold proteins. ssNMR provides, for the first time, residue-specific evidence for interaction, in multiple heterogeneous conformations, of SSB-Ct with its own ssDNA-binding site. In particular we compared spectra from the native protein and from a deletion mutant lacking the extreme Ct. This interaction acts as a switch that directs recruitment of SSB-binding proteins specifically to SSB only when it is bound to ssDNA. Moreover we investigated the interaction between the C-terminal domains of $\alpha$ ($\alpha_{CTS}$ 22.5 kDa) and $\tau$ ($\tau_{C16}$ 16 kDa). The structure of the complex is so far unknown. We used cell free protein synthesis to produce $\alpha_{CTS}$ in the presence of $\tau_{C16}$. We analyzed two different samples in which each the interacting partners is separately $^{2}$H,$^{13}$C,$^{15}$N labelled. The complete resonance assignment of the proteins in the complex opens the way to mapping the protein-protein contacts and to the determination of the overall structure.

## P20-213
## Investigation of a critical radical SAM enzyme involved in co-factor biosynthesis in Actinobacteria

L. Decamps[1], A. Benjdia[2], R. H. White[3] and **O. Berteau**[1]
*[1]INRA, Institut Micalis, UMR 1319 Inra-Agroparistech, Domaine de Vilvert, jouy en josas, France, [2]Max-Planck Institute for Medical Research, Department of Biomolecular Mechanisms, Heidelberg, Germany, [3]Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA*

F420 is a major coenzyme in Archea and Actinobacteria, including *Mycobacterium tuberculosis* [1, 2]. This cofactor is involved in many metabolic pathways; notably, its reduced form protects Mycobacteria from nitrosative stress in macrophages during infection [3]. Hence, the F420 biosynthesis pathway represents an attractive target for drug development against Mycobacteria.

We aimed to characterize the penultimate reaction of F420 biosynthesis catalyzed by the enzyme FO-synthase, which is part of the superfamily of radical S-adenosylmethionine (SAM) enzymes. These enzymes catalyze the reductive cleavage of SAM bound to their [4Fe-4S] cluster held by a characteristic SAM radical motif: CxxxCxxC. In Mycobacteria, FO-synthase contains two radical SAM motifs whereas in methanogenic Archea, FO synthase is composed of two subunits. This implies that two radical species are likely generated during catalysis to perform the complex reaction catalyzed by FO synthase. Using physico-chemical techniques approaches combined with mutagenesis study we investigated in details the reaction mechanism of this new radical SAM enzyme. The knowledge gained on the mechanism of this enzyme not only brings new insight into the radical SAM enzyme superfamily but also on the physiology of Archae and Actinobacteria, including the human pathogen *M. tuberculosis*.

**Références**

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## P20-214
## The effects of ligand binding on protein structure and rigidity: studies with HIV-1 protease and cyclophilin A

**J. W. Heal**[1], E. Jimenez-Roldan[2], S. Wells[2], R. Freedman[3] and R. Roemer[2]
*[1]The University of Warwick, Coventry, UK, [2]Department of Physics, University of Warwick, Coventry, UK, [3]Department of Life Sciences, University of Warwick, Coventry, UK*

For certain proteins there are a large number of crystal structures available, making them appropriate as data sources for studying the effects of ligand binding on their structure and function. The rigidity analysis software FIRST divides a protein into rigid and flexible regions based on a bond network inferred from a crystal structure. FIRST is computationally inexpensive, allowing the investigation of multiple structures in tandem. We have evaluated 206 high-resolution (≤2 Å) X-ray crystal structures of HIV-1 protease and used FIRST to compare the effects of different inhibitors on the rigidity of the enzyme. We find that inhibitor binding has little effect on the overall rigidity of the protein homodimer, including the rigidity of the active site and that the principal effect of inhibitor binding on rigidity is to constrain the flexibility of the beta-hairpin flaps, which move to allow access to the active site of the enzyme. We show that commercially available antiviral drugs which target HIV-1 protease can be divided into two classes, those which significantly affect flap rigidity and those which do not. The non-peptidic inhibitor tipranavir is distinctive in its consistently strong effect on flap rigidity. We have also evaluated the rigidity of 54 structures of the peptidyl prolyl cis-trans isomerase cyclophilin A and are currently comparing the results of these rigidity analyses with experimental findings. The techniques used include HD exchange, fluorimetry, and circular dichroism. Initial results are presented in addition to details of currently ongoing experiments.