EMBO Practical Course

Multidimensional NMR in Structural Biology

August 10 - 15, 2014

Joachimsthal, Germany

Organizers:

C. Griesinger, Göttingen
R. Boelens, Utrecht
H. Oschkinat, Berlin

Program EMBO Course 2014
Multidimensional NMR in Structural Biology

Sunday, August 10th			
09:00 - 19:00	Arrival of the participants		
19:30 - 21:00	Welcome Dinner		
21:00 - open end	Poster Session		
Monday, August 11th			
09:00 - 10:30	**Lecture**	**Product Operators**	**C. Griesinger**
10:30 - 11:00	Break		
11:00 - 12:30	**Lecture**	**Phase cycles and gradients**	**D. Nietlispach**
12:30 - 14:00	Lunch		
14:00 - 16:00	Exercises		
16:00 - 16:30	Coffee break		
16:30 - 17:30	Exercises		
17:30 - 19:00	**Lecture**	**Introduction to 2 and 3D NMR**	**R. Boelens**
19:00 - 20:00	**Lecture**	**Average Hamiltonian Theory, optimal control** / Principles of Solid State NMR I	**B. Reif**
20:00 - 21:00	Dinner		
21:00 - open end	Poster Session		
Tuesday, August 12th			
09:00 - 10:30	**Lecture**	**Principles of Solid State NMR**	**B. Reif**
10:30 - 11:00	Break		
11:00 - 12:30	**Lecture**	**Relaxation I**	**A. Palmer**
12:30 - 14:00	Lunch		
14:00 - 16:00	Exercises		
16:00 - 16:30	Coffee break		
16:30 - 17:30	Exercises		
17:30 - 19:00	**Lecture**	**Paramagnetic NMR**	**I. Felli**
19:00 - 20:15	**Lecture**	**Labelling, assignments and distance measurements in solid-state NMR**	**H. Oschkinat**
20:15 - 21:15	Dinner		

Wednesday, August 13th			
09:00 - 10:30	**Lecture**	**Relaxation II**	**A. Palmer**
10:30 - 11:00	Break		
11:00 - 12:30	Exercises		
12:30 - 13:00	Lunch packages		
13:00 - 19:00	Self-organized social event		
20:00 - 21:00	Conference Dinner		
Thursday, August 14th			
09:00 - 10:30	**Lecture**	**Alignment: dipolar couplings**	**C. Griesinger**
10:30 - 11:00	Break		
11:00 - 12:30	**Lecture**	**TROSY & Cross correlated relaxation**	**C. Griesinger**
12:30 - 14:00	Lunch		
14:00 - 16:30	Exercises		
16:30 - 17:00	Coffee break		
17:00 - 18:00	**Lecture**	**Computational aspects of structure determination by NMR**	**A. Bonvin**
18:00 - 19:30	**Lecture**	**Enhancement schemes in NMR**	**R. Kaptein**
20:00 - 22:00	Scientific Dinner	**Application of hyperpolarization**	**M. Lelli**
Friday, August 15th			
09:00 - 10:30	**Lecture**	**NMR on RNA**	**T. Carlomagno**
10:30 - 11:00	Break		
11:00 - 12:30	Scientific round table		
12:30 - 14:00	Departure, lunch package		

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Speakers	Topics
Boelens	**Introduction to 2 and 3D NMR**
Bonvin	**Computational aspects of structure determination by NMR**
Carlomagno	**NMR on RNA**
Felli	**Paramagnetic NMR**
Griesinger I	**Product Operators**
Griesinger II	**Alignment: dipolar couplings**
Griesinger III	**TROSY & Cross correlated relaxation**
Kaptein	**Enhancement schemes in NMR**
Lelli	**Application of hyperpolarization**
Nietlispach	**Phase cycles and gradients**
Oschkinat	**Labelling, assignments and distance measurements in solid-state**
Palmer I	**Relaxation I**
Palmer II	**Relaxation II**
Reif I	**Average Hamiltonian Theory, optimal control**
Reif II	**Principles of Solid State NMR**

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Posters

RNA-recognition motif RRM2 in TDP-43: oxidative stress leads to conformational change, proteolysis, formation of inclusion bodies, and neurodegenerative disease

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TDP-43 is 414-amino-acid protein which plays an important (but only partially understood) role in RNA control and transcription.[1] Seven years ago it has been discovered that TDP-43 constitutes a major component of the inclusion bodies formed in the brains of the patients with frontotemporal lobar degeneration (FTLD).[2] FTLD is currently believed to be third or fourth most common dementia; in the age group 40-65 it is second only to Alzheimer disease.[3] The analysis of inclusion bodies demonstrated that they are largely comprised of the C-terminal fragments of TDP-43 (CTF TDP-43).[4,5]

As it turns out, formation of C-terminal fragments is a consequence of proteolytic cleavage at multiple sites within the RRM2 domain of TDP-43. RRM2 is a 65-residue globular domain for which the solution NMR structure is available (PDB ID 1WF0). Proteolytic cleavage within this domain leads to the loss of Nuclear Localization Signal (NLS) located at the N-terminus of TDP-43 and exposure of Nuclear Export Sequence (NES) encoded in the RRM2.[6,7] Consequently, CTF TDP-43 are exported from nucleus into the cytoplasm, where they form the inclusion bodies.

We hypothesize that proteolytic cleavage in RRM2 occurs as a result of (transient) unfolding caused by oxidative stress. To investigate the details of this mechanism, we prepared the isotopically labeled sample of RRM2 and used the standard suite of triple-resonance experiments to obtain spectral assignment (data collected at the Center for Magnetic Resonance in St. Petersburg State University; results deposited in BMRB, entry 19922). In order to model the effect of oxidative stress on RRM2 we resort to H_2O_2 treatment. As a result of this treatment, many peaks in ^1H-^{15}N HSQC spectrum become significantly attenuated or disappear altogether. The affected residues are spatially localized around the exposed residue C244, suggesting that RRM2 undergoes dimerization through formation of the disulfide bridge at this site. The disappearance of the spectral peaks suggests that the protein structure becomes significantly perturbed at the dimeric interface. Currently H/D exchange experiments are underway to test this hypothesis. The partial destabilization of protein structure due to structural strain associated with intermolecular disulfide bridge is an interesting mechanism that can potentially explain the vulnerability of many proteins to proteolytic cleavage under the conditions of oxidative stress.

Literature:

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