17th Bijvoet Tutorial Symposium

18-19 April 2011

Kontakt der Kontinenten
Soesterberg




Universiteit Utrecht

Monday 18 April 2011

08:30		**Arrival / Check-in**	
08:45	09:15	**Registration *(Coffee & Tea)***	
09:15	09:20	**Welcome**	Albert Heck
09.20		Morning session	**Chair: Afonso Duarte**
09:20	09:40	*Novel insight into ion channel structure and function in lipid bilayers*	Deepak Nand (NMR)
09:40	10:00	*"There are some enterprises in which a careful disorderliness is the true method" - does this apply to protein-structure refinement?*	Tom Burnley (CSC)
10:00	10:20	*A novel ultra-sensitive HILIC strategy allows in-depth proteome analysis of minute amounts of tissue stem cells*	Serena Di Palma (BMS)
10:20	10:50	***Break (Coffee & Tea, Mounting posters)***	
10:50	11:10	*Production of pharmaceutical proteins in mushrooms*	Elsa Berends (ME)
11:10	11:30	*How Transmembrane Model Peptides Affect Lipid Head Group Orientation: An Application Of 14N NMR*	Jacques Doux (BM)
11:30	12:00	**Poster-Flash session 1 (posters 1-19)**	
12:00	12:30	Check-in hotel guests	
12:30	14:30	***Lunch and Posters (posters 1-26)***	
14.30		Afternoon session	**Chair: Tom Burnley**
14:30	14:50	*Molecular plasticity of Hsp90 chaperone machine.*	Tanja Didenko (CPC)
14:50	15:10	*Mimicking protein-protein self-assembly with small peptides*	Steffen van der Wal (MC)
15:10	15:30	*Role of a PWWP domain in the coordination of chromatin modification and transcription regulation*	Rick van Nuland (MCR)
15:30	16:00	***Break (Coffee & Tea)***	
16:00	16:25	*Quantitative interaction proteomics and genome wide profiling of epigenetic chromatin marks and their readers*	Michiel Vermeulen (UMCU, Associate Member)
16:25	16:50	*Regulation of RapGEFs*	Holger Rehmann (UMCU, Associate Member)
16:50	17:15	*Cancer missense mutations induce formation of a structurally destabilized, aggregation-prone Axin scaffold protein with oncogenic activity in cells*	Madelon Maurice (UMCU, Associate Member)
17.15	18:15	**SAB meets with Bijvoet Groupleaders**	
17:15	18:30	***Drinks in the poster room***	
18:30	20:30	***Dinner***	
20:30	21:00	***After Dinner Talk:*** *"40 years of structural biology: a personal perspective"*	Jack Johnson, The Scripps Research Institute, La Jolla, California

Tuesday 19 April 2011

08:00	09:00	***Breakfast***	
09:00	09:20	Coffee &Tea	
09:20	10:00	*In vitro and in vivo regulation of TNF-induced necroptosis* Peter Vandenabeele, VIB, Ghent, Belgium	Chair: Reinout Raijmakers
10:00	10:40	*Structural insights into RNA polymerase III transcription* Christoph Müller, European Molecular Biology Laboratory, Heidelberg, Germany	Chair: Albert Heck
10:40	11:10	***Break (Coffee & Tea)***	
11:10	11:50	*Folding and aggregation of macromolecules; lessons from the artificial world* Bert Meijer, Eindhoven University of Technology, Eindhoven	Chair: Ineke Braakman
11:50	12:20	**Poster-Flash session 2 (posters 27-45)**	
12:20	14:10	**Lunch and Posters (27-53)**	
13:40	14:10	**SAB meets Bijvoet PhD students**	
14:10	14:50	**Elif Karagöz** PhD student of the year 2010	Chair: Stefan Rüdiger
14:50	15:30	*Under the hood: Single-molecule studies of DNA replication* Antoine van Oijen, Groningen University, Groningen	Chair: Piet Gros
15:30	16:00	***Break (Coffee & Tea)***	
16:00	16:40	*Using Protein Domain Microarrays to Read the Histone Code* Mark T. Bedford, MD Anderson Cancer Center, Department of Molecular Carcinogenesis, Smithville, Texas	Chair: Marc Timmers
16:40	17:00	Poster Award and Closure	
17:00	...	***Drinks***	

Posters (A-G)

Number	Name and Title
15	Ballering, Joost *A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor*
6	Benevento, Marco *Proteome dynamics during cellular reprogramming*
43	Bereszczak, Jessica *Composition, Stability and Conformation of different P-protein Complexes studied by Native Mass Spectrometry*
16	Beringer, Dennis *towards structural insights into the interaction mechanism of fibronectin typeI domains with cross-beta structure*
24	Burnley, Tom *Time-Averaged Crystallographic Refinement: Revisited*
29	Cabukusta, Birol *Mapping Ceramide Trafficking Machinery at the ER-mitochondrial Interface: A Thin Line between Cell Death and Survival*
30	Cruijsen, Elwin van der *Dynamics of a membrane-embedded potassium channel in an open-inactivated state as seen by solid-state NMR*
51	Cukkemane, Abhishek *Structural polymorphism correlates with functional landscape of a cyclic nucleotide binding-domain*
33	Di Palma, Serena *ZIC-HILIC and ZIC-cHILIC provide high resolution separation and increase sensitivity in proteome analysis*
45	Didenko, Tatiana *Interaction of molecular protein Hsp90 with protein kinase A.*
9	Diebolder, Christoph *Dual axis cryo-electron tomography on membrane bound immune complexes*
21	Dijk, Marc van *WeNMR: A Worldwide e-Infrastructure for NMR and structural biology.*
50	Duarte, Afonso *Analyses of the ATP cycle of molecular chaperone Hsp90 by NMR spectroscopy*
39	Faridounnia, Maryam *Unknown*
34	Feitsma, Louris *Conserved binding sites in collagen-receptors involved in immunity & platelet activation*
52	Forneris, Federico *Formation of the Complement C3 Convertase*
2	Galih, Augustinus *Mapping Ceramide Trafficking Machinery at the ER-mitochondrial Interface: A Thin Line between Cell Death and Survival*

(G-N) Posters

Number	Name and Title
5	Gradmann, Sabine *Understanding the functioning of the Nuclear Pore Complex: A structural study on FG-nucleoporin hydrogels via solid-state NMR*
31	Gremme, Sabine *Analysis and characterization of the ER folding machinery: Impact on LDL receptor folding*
22	Haberkant, Per *Bifunctional Lipids - A novel tool to study lipids*
18	Halff, Els *Structural and functional characterisation of Nod-Like Receptors and their interaction partners*
12	Hennrich, Hanka *The flip-side of ion transport: Molecular dissection of a lipid pump*
26	Houben, Klaartje *NMR structure of the abiotic stress related membrane protein pmp3*
20	Ingen, Hugo van *Architecture of the chromatin factor HMGN2 – nucleosome complex by methyl-TROSY NMR*
11	Karaca, Ezgi *A multi-domain flexible docking approach to deal with large conformational changes in the modeling of biomolecular complexes*
10	Kastritis, Panagiotis *STRUCTURAL DETERMINANTS FOR THE AFFINITY OF PROTEIN-PROTEIN COMPLEXES*
25	Kol, Matthijs *Membrane-embedded enzymes and their lipid substrates*
27	Kooijman, Laurens *What are the crucial interactions of the first membrane spanning domain to the cytosolic domains in the (early) folding of CFTR?*
36	Krumpochova, Petra *Control of mitochondrial apoptosis by a ceramide sensor in the ER*
38	Li, Xin *Unknown*
47	Madoori, Pramod *Attempts to improve X-ray diffraction of human a2-macroglobulin*
49	Melquiond, Adrien *A butterfly effect in E2-E3 interactions: dynamic control of selectivity by an ASP to GLU mutation*
37	Michels, Tine *Regulatory mechanism for balancing bilayer and non-bilayer lipids in yeast*
7	Minde, David *Structural mechanism of APC*
14	Nand, Deepak *Unknown*

Posters (P-Z)

Number	Name and Title
19	Panatala Narendranath, Radhakrishnan *Towards non-genetic manipulation of P4 ATPase-catalyzed lipid transport: delving into the significance and inner workings of flippases*
35	Pandey, Abhinav *The effect of GRP94 on protein folding and transport*
17	Peng, Weng Chuan *Structural study of clusterin*
32	Peng, Mao *Combining different enzymes for proteome identification and quantification*
46	Peters, Floor *Uncoupling of the primary and downstream folding defects within F508del CFTR*
28	Pronker, Matti *Towards structural characterization of recognition elements in VWC domains*
40	Quandte, Matthias *HIV 1 Envelope glycoprotein maturation - Exploiting the host cell folding machinery*
3	Remers, Niels *Unknown*
44	Rodrigues, João *Clustering of multi-body docking solutions based on contact analysis*
4	Rosati, Sara *Antibody-Antigen Binding Monitored by Native Mass Spectrometry*
23	Rose, Rebecca *Ticking of a Bacterial Clock: Insights into the Kai Circadian System from Mass Spectrometry*
48	Schwend, Thomas *Identification of novel methionine aminopeptidase 2 substrates using LC-MS/MS*
42	Sinnige, Tessa *Structure and function of the outer membrane protein BamA studied by solid-state NMR*
1	Swart, Leoni *Cell-Free Translation of Integral Membrane Proeteins into Unilamelar Liposomes*
53	Thompson, Natalie *Analysis of DegP Oligomers by Native Mass Spectrometry*
13	Veer, Inge van 't *Unknown*
8	Wu, Jin *Structure studies on regulation of complement activation*
41	Zoumaro-Djayoon, Adja *Investigating the role of FGF-2 in stem cell maintenance by global phosphoproteomics profiling*

Poster Flash Presentations

Session 1, April 18, 11.30-12.00

Name	#
Swart, Leoni	1
Galih, Augustinus	2
Remers, Niels	3
Rosati, Sara	4
Gradmann, Sabine	5
Benevento, Marco	6
Minde, David	7
Wu, Jin	8
Diebolder, Christoph	9
Kastritis, Panagiotis	10
Karaca, Ezgi	11
Hennrich, Hanka	12
Veer, Inge van 't	13
Nand, Deepak	14
Ballering, Joost	15
Beringer, Dennis	16
Peng, Weng Chuan	17
Halff, Els	18
Panatala Narendranath	19

Session 2, April 19, 11.50-12.20

Name	#
Kooijman, Laurens	27
Pronker, Matti28	
Cabukusta, Birol	29
Cruijsen, Elwin van der	30
Gremme, Sabine	31
Peng, Mao	32
Di Palma, Serena	33
Feitsma, Louris	34
Pandey, Abhinav	35
Krumpochova, Petra	36
Michels, Tine	37
Li, Xin	38
Faridounnia, Maryam	39
Quandte, Matthias	40
Zoumaro-Djayoon, Adja	41
Sinnige, Tessa	42
Bereszczak, Jessica	43
Rodrigues, João	44
Didenko, Tatiana	45

Peter Vandenabeele

Flanders Insitute for Biotechnology,
Ghent, Belgium

Prof. Dr. Peter Vandenabeele obtained his PhD from the University of Ghent in Ghent, Belgium in 1990. He became a groupleader at the Flanders Institute for Biotechnology (VIB) in 1996 and is head of the Molecular Signaling and Cell Death Unit.

Selected publications

Vanden Berghe T, Vanlangenakker N, Parthoens E, Deckers W, Devos M, Festjens N, Guerin C, Brunk U, Declercq W, Vandenabeele P (2010) Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features *Cell Death And Differentiation*, 17, 922-30, 2010

Declercq W, Vanden Berghe T, Vandenabeele P (2009) RIP kinases at the crossroads of cell death and survival. *Cell*, 138, 229-32, 2009

In vitro and in vivo regulation of TNF-induced necroptosis

Tumor necrosis factor (TNF) is a pleiotropic molecule that plays a crucial role in cell death and inflammation during infection, tissue damage, and cancer. TNF signaling in L929 cells leads to three major cellular responses initiated by different signalling complexes: survival and inflammatory gene induction, apoptosis, or necrosis. The signalling pathways leading to necrosis or apoptosis are mutually interacting. Overexpression of CrmA, knocking down caspase-8 or FLIPL sensitizes necroptosis, suggesting an anti-necroptotic function of FLIPL/caspase-8 in complex II. Knockdown of RIP1 prevents TNF-induced necrosis, but sensitizes a switch to caspase-8-mediated apoptosis. RIP3 knockdown completely protects against both types of TNF-induced cell death, suggesting that the presence of RIP3 determines the sensitivity to TNF-induced cell death irrespective the type of death.

The balance between cell death and survival is also reflected in vivo by administration of TNF to mice. We examined the contributions of these two cell death modalities in TNF-induced systemic inflammatory response syndrome (SIRS), a sterile sepsis model. We demonstrate that deletion of apoptotic executioner or inflammatory caspases had no impact on lethal SIRS. In contrast, deletion of the RIP3 gene conferred complete protection against mortality, reflected in the levels of circulating damage associated molecular patterns (DAMPs). Pretreatment with the RIP1 kinase inhibitor, necrostatin-1, had a similar effect. These results demonstrate that RIP1/RIP3-mediated necroptosis plays an indispensable role in TNF-induced SIRS as the determinant of life and death, and that components of the necroptotic cell death pathway are potential therapeutic targets for treatment of SIRS.

Christoph Müller

European Molecular Biology Laboratory,
Heidelberg, Germany

Structural insights into RNA polymerase III transcription

Among the three eukaryotic RNA polymerases, RNA polymerase (Pol) I and III are responsible for producing non-coding RNAs. While Pol I is synthesizing pre-rRNA that is subsequently processed into 18S, 5.8S and 28S rRNA to form the RNA backbone of the ribosome, Pol III synthesizes small non-translated RNAs such as tRNAs, 5S rRNA, splicing U6 RNA and signal recognition particle 7SL RNA. Together, Pol I and Pol III contribute up to 80% to the total transcriptional activity in growing cells and need to be tightly regulated. In the last years there has been also increasing awareness that deregulation of Pol I and Pol III transcription is associated with different types of cancer.
Pol III is the largest and most complex among the eukaryotic RNA polymerases and consists of 17 different subunits with an overall mass of 0.7 MDa. The recent electron microscopy structure of the free Pol III enzyme at 10 Å resolution provides an accurate framework to better understand the overall architecture and the structural organization and functional role of two Pol III-specific subcomplexes. The cryo-EM structure of elongating Pol III bound to a DNA/RNA scaffold shows the rearrangement of the Pol III-specific subcomplexes that enclose incoming DNA. Downstream DNA and newly transcribed RNA can be followed over considerably longer distances as in a previous crystal structure of elongating Pol II. The recruitment of Pol III to the transcription start site requires binding of the general transcription factors TFIIIB and TFIIIC. TFIIIC is a large DNA-binding complex (Mr = 0.6 MDa) composed of six polypeptides. TFIIIC can be subdivided into two subcomplexes that initiate Pol III transcription by binding to two internal promoter sites in tRNA genes named A¬¬¬- and B-box. During the last years our group has determined crystal structures of subunits and subcomplexes corresponding to ~2/3 of the entire TFIIIC complex and obtained a low-resolution EM reconstruction of TFIIIC that serves as a starting point to assemble the entire complex. Recent insights resulting from the structural and functional characterization of Pol III and TFIIIC will be presented.

Dr. Christoph Müller obtained his PhD in 1991 from th University of Freiburg, Germany. He worked as a postdoctoral researchers at Harvard University, Cambridge, USA and has moved to the EMBL in Grenoble in 1995. Since 2007, he is joint Head of Unit at EMBL Heidelberg since 2007 and has a joint appointment with the Genome Biology Unit.

Selected publications

Lane, L.A., Fernandez-Tornero, C., Zhou, M., Morgner, N., Ptchelkine, D., Steuerwald, U., Politis, A., Lindner, D., Gvozdenovic, J., Gavin, A.C., Muller, C.W. & Robinson, (2011) Mass Spectrometry Reveals Stable Modules in holo and apo RNA Polymerases I and III. C.V. *Structure.* Jan 12;19(1):90-100

Fernandez-Tornero, C., Bottcher, B., Rashid, U.J., Steuerwald, U., Florchinger, B., Devos, D.P., Lindner, D. & Muller, C.W. (2010) Conformational flexibility of RNA polymerase III during transcriptional elongation. *EMBO J.* Nov 17;29(22):3762-72

Bert Meijer

Eindhoven University of Technology, Eindhoven, The Netherlands

Professor E.W. (Bert) Meijer his PhD degree cum laude from Groningen University in 1982. From 1989 till 1992 he was appointed as head of the department "New Materials" at DSM Research in Geleen, the Netherlands. Since 1992, Bert Meijer has as professor at the Eindhoven University of Technology (TU/e) and since 2008 Bert Meijer is Scientific Director of the Institute for Complex Molecular Systems (TU/e).

Folding and aggregation of macromolecules; lessons from the artificial world

The folding of proteins as well as the self-assembly of proteins into fibrillar and beta-amyloidal structures is the results of specific secondary interactions within a polymer chain or between polymer chains. The diversity in protein structures and the complexity of the processes involved make studies to folding and assembly of proteins challenging research objectives. In the lecture, a number of simple artificial structures will be introduced that are studied in great detail for their self-assembly processes in both organic solvents and water. In a particular example, meta-stable folded single-chain macromolecules will be used as a catalyst.

An attempt will be made to elucidate the differences and similarities between these simple artificial structures and complex proteins to arrive at a few general statements on folding and assembly of (macro)molecules. Both kinetic and thermodynamic studies will be used to show some remarkable similarities in behavior of artificial structures in organic solvents and proteins in water.

Selected publications

S. Cantekin, D.W.R. Balkenende, M.M.J. Smulders, A.R.A. Palmans and E.W. Meijer (2011) The effect of isotopic substitution on the chirality of a self-assembled helix, *Nature Chemistry* 3, 42-46

E.B. Berda, E.J. Foster and E.W. Meijer (2010) Toward controlling folding in synthetic polymers: Fabricating and characterizing supramolecular single-chain nanoparticles, *Macromolecules* 43, 1430-1437

Groningen University, Groningen, The Netherlands

Antoine van Oijen

Under the hood: Single-molecule studies of DNA replication

Novel nanomanipulation and imaging methods have made it possible to study biochemical reactions at the level of individual proteins. In a biological context, most of these proteins function in concert with others in multi-protein complexes, so an important future direction is the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies. I will discuss our single-molecule studies of the replisome, the multi-protein machinery that is responsible for replication of DNA. I will present experiments that rely on the readout of mechanical DNA properties as well as single-molecule fluorescence imaging to obtain information on the catalytic activity and dynamic composition of the bacterial replisome. Further, I will discuss new results on the study of individual eukaryotic replisomes in Xenopus cellular extracts.

Prof. Dr. Antoine van Oijen completed his graduate studies in physics at Leiden University, after which he to the Boston area in 2001 for a postdoctoral fellowship in Sunney Xie's lab at Harvard Chemistry. In 2004 he started his own lab in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and he recently returned to the Netherlands to continue his research as a full professor at the Rijksuniversiteit Groningen.

Selected publications

A. Tafvizi, F. Huang, A.R. Fersht, L.A. Mirny, A.M. van Oijen (2011) A single-molecule characterization of p53 search on DNA. *Proc. Natl. Acad. Sci. USA* 108: 563-8

H. Yardimci, A.B. Loveland, S. Habuchi, A.M. van Oijen, J.C. Walter (2010) Uncoupling of sister replisomes during eukaryotic DNA replication *Mol. Cell* 40: 834-40

Mark T. Bedford

The University of Texas, MD Anderson Cancer Center, Austin, USA

Dr. Mark T. Bedford obtained his PhD degree at the Weizmann Institute of Science in Israel in the field of Developmental Biology. From 1996 till 2000 he was a postdoctoral Fellowship at Harvard Medical School in Boston in the group of Dr. Philip Leder. He currently is Associate Professor at the Department of Carcinogenesis of the University of Texas MD Anderson Cancer Center and the Center for Molecular and Cellular Toxicology of the University of Texas.

Selected publications

Spannhoff A, Kim YK, Raynal NJ, Gharibyan V, Su MB, Zhou YY, Li J, Castellano S, Sbardella G, Issa JP, Bedford MT.(2011) Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO Rep.* 12(3):238-43.

Yang Y, Lu Y, Espejo A, Wu J, Xu W, Liang S, Bedford MT. (2010) TDRD3 is an effector molecule for arginine-methylated histone marks. *Mol Cell.* 40(6):1016-23.

Using Protein Domain Microarrays to Read the Histone Code

Introduction. For cells to survive, differentiate, and grow, information has to be transferred from the cell surface to the nucleus. This process is referred to as signal transduction. A hallmark of cancer is the deregulation of signal transduction pathways. Signaling events in eukaryotic cells involve the assembly and disassembly of large protein-protein complexes. These diverse associations are mediated through interactions of a limited number of modular signaling units or protein-domains. Protein interactions involving domains are often regulated by post-translational modification (PTM – like phosphorylation, methylation and acetylation) of the smaller protein motif within the ligand. We have developed a chip-size protein microarray that harbors a display of over 300 modular protein-interacting domains including SH2, SH3, PDZ, FHA, WW, Chromo, Tudor, PHD and MBT domains.
Objectives. In the emerging proteomic era mass spectrometric approaches are detecting numerous posttranslational modification on proteins. Many of these posttranslational modifications likely generate docking sites for protein modules. Microarray technology will help identify proteins that can interact with motifs that are either methylated or phosphorylated. This high-throughput approach facilitates the rapid identification of protein-protein interactions in vitro. Further in vivo studies are needed to confirm that these interactions do indeed occur in biological systems.
Methods. Protein domains are cloned into a GST expression vector, and recombinant protein is produced in bacteria. These fusion proteins are then arrayed onto nitrocellulose coated glass slides using a robot. These slides are probed with biotinylated peptides that are pre-conjugated to streptavidin-Cy3. The peptides used in this experiment are synthesized as 15 mers, and both the modified and unmodified forms of the peptides are tested on the array.
Impact. More and more posttranslational modifications are being discovered on proteins. The roles of many of these methylation and phosphorylation events often remain obscure. This approach provides an easy way for a researcher to identify potential binding partners for their favorite proteins. These arrays thus offer researchers tools to get at "mechanism". Once investigators know that they are working with a clearly functional PTM, they can proceed with confidence to generate modification specific antibodies and interrogate the signaling pathway that is engaged by the identified PTM-driven protein-protein interaction.

G. Elif Karagöz

The Hsp90 chaperone in action: following the ATP cycle of a molecular machine

Protein folding in the cell is assisted by molecular chaperones. The major chaperones are ATP-controlled machines, one of which is Hsp90. Hsp90 differs from the other main chaperone systems in being rather selective in choosing its substrates. The two key features that determine this selectivity are unknown: where is Hsp90's substrate binding site, and how is the interaction with substrate controlled by the ATPase cycle. Here we show that ATP induces an intimate complex of Hsp90 with substrate, involving its N-terminal and middle domain. We used NMR spectroscopy to follow Hsp90 interaction with its substrate Tau throughout its ATP cycle. The mode of Hsp90's interaction with substrate strongly depended on its nucleotide state. In the absence of nucleotide, only the N-terminal domain was involved in substrate recognition. We anticipate that Hsp90 inhibiting drugs block the cycle by not allowing Hsp90 to form the intimate complex with the substrate.

G. Elif Karagöz[1,], Afonso M.S. Duarte[1], Hans Ippel[2] , Charlotte Uetrecht[3,4,] Tessa Sinnige[1], Martijn van Rosmalen[1], Jens Hausmann[1,¶], Eckhard Mandelkow[5], Albert J.R. Heck[3,4], Rolf Boelens[2] and Stefan G.D. Rüdiger[1]

1 Cellular Protein Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands
2 Biomolecular NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands
3 Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands
4 Netherlands Proteomics Centre, Utrecht, The Netherlands
5 Max Planck Research Unit for Structural Molecular Biology at DESY, Hamburg, Germany
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Notes

Table of Contents


How viruses enter their host cel