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Repliements protéiques dans les maladies infectieuses et neurodégénératives

Protein folds in infectious and neurodegenerative diseases

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Repliements protéiques dans les maladies infectieuses et neurodégénératives

Organisée par Alasdair Steven, Président et Andrey Kajava, vice-Président

La conférence que nous avons organisée était consacrée à des thèmes situés à l'interface de la biologie fondamentale et des applications en santé. Aujourd'hui, l'Europe et la France sont affectées par des menaces infectieuses émergentes et par les maladies neurodégénératives liées au vieillissement d'une population dont l'espérance de vie ne cesse de croître. Les dernières années ont vu apparaître une efflorescence d'informations sur les structures 3D des facteurs de virulence, de toxines et d'adhésines de bactéries pathogènes et de virus, ainsi que sur celles des fibrilles de prions et d'amyloïdes. On peut attribuer l'apparition de ces informations structurales à l'amélioration de l'expression des protéines et des stratégies de cristallisation aussi bien qu'à l'application de nouvelles techniques expérimentales émergentes, telles que la cryomicroscopie électronique, la microscopie électronique en transmission à balayage, la spectroscopie RMN à l'état solide, et la spectroscopie par résonance paramagnétique électronique. Il est aussi devenu évident que de nombreux polypeptides sont non repliés à l'état natif dans les conditions physiologiques, et cette classe de polypeptides inclut les précurseurs de nombreuses des protéines pathogéniques définies plus haut.

En accord avec ces tendances, cette conférence portait sur les structures, la stabilité, les fonctions et la pathogénicité de ces repliements, ainsi que sur les stratégies nécessaires au développement de thérapies et de vaccins. Elle a permis de réunir une masse critique d'experts de renommée internationale dans ce domaine et de mener à des discussions visant à mettre en lumière les particularités structurales caractéristiques des repliements pathogènes, de répertorier leur diversité notable, de disséquer leurs relations séquence-structure et enfin d'explorer de nouvelles voies vers la découverte de médicaments et de vaccins.

La combinaison d'orientations scientifiques différentes mais superposées nous a permis de rassembler des chercheurs qui d'habitude ne participent pas aux mêmes conférences. Il en a résulté que cette conférence a fourni une excellente opportunité d'échange d'idées et d'établissement de nouvelles collaborations et a suscité un grand intérêt au sein de la communauté scientifique. La Conférence a engendré une atmosphère étonnante, marquée par un niveau élevé de présentations scientifiques et par des discussions constructives, amicales et productives. Elle a été hautement appréciée par les participants qui ont massivement exprimé leur soutien à l'organisation d'une autre conférence dans 3 ans. Andrey Kajava (Vice-président de cette conférence et Président de la prochaine) a accepté de présenter une demande pour la prochaine Conférence. Au cours de la dernière soirée de la Conférence, Robert Seckler a été élu de façon unanime en tant que prochain Vice-président et a accepté la charge de cette fonction.

Le succès de l'organisation de la Conférence est également dû au soutien décisif, financier et administratif du CNRS, aux très bonnes compétences pratiques de Madame Lidoreau, secrétaire de la conférence, ainsi qu'à l'hospitalité du personnel du Centre Paul Langevin.

Administrative report

The conference took place at the Conference Center Paul-Langevin of CNRS in Aussois which is a ski resort situated in French Alps. A total of 89 persons from 20 countries attended the conference.

- Australia (1)
- Belgium (6)
- Finland (1)
- France (32)
- Germany (7)
- Greece (3)
- Holland (1)
- Hungary (3)
- Israel (3)
- Italy (2)
- Korea (2)
- Russia (5)
- Singapore (1)
- Slovenia (1)
- Spain (4)
- Sweden (1)
- Switzerland (3)
- Tunisia (1)
- United Kingdom (5)
- USA (7)

The total number of speakers was 41 including 23 invited speakers with 30 minute talks and 18 speakers with 20 minute talks which were selected from the abstracts submitted to the Conference.
The other participants presented their work in the format of posters that were on display during two afternoon sessions of 2h30min and 1h30 min, correspondingly.

Among the participants, there were 18 PhD students representing 20% of the participants.

SCIENTIFIC SUMMARY

Background

The past few years have seen an efflorescence of information on the 3D structures of virulence factors, toxins, and adhesins of pathogenic bacteria and viruses, as well as of prion and amyloid fibrils. The emergence of this structural information can be attributed to the improved protein expression and crystallization strategies as well as applications of emerging experimental techniques, including cryo-electron microscopy, scanning transmission electron microscopy, solid-state NMR spectroscopy, and electron paramagnetic resonance spectroscopy. It has also become apparent that many polypeptides are "natively" unfolded under physiological conditions, and this class includes precursors to many of the pathogenic proteins outlined above. In addition to the recent revolutionary progress on a conceptual level, this area of research has a vitally important medical dimension related to the fact that the structures of pathogenic folds are key to developing new therapeutics for neurodegenerative disease and infectious diseases of bacterial and viral origins.

In line with these trends, our conference focused on the structures, stability, functions and pathogenicity of these protein folds as well as strategies for developing therapeutics and vaccines. We were able to bring together a critical mass of internationally recognized experts who led discussions that allowed to distinguish the characteristic structural features of the pathogenic folds, chart their notable diversity, dissect their sequence-structure relationships, and explore novel pathways to drug and vaccine discovery.

The conference was subdivided into four sessions, focusing respectively on:

(1) The structures of virulence- and infectivity-associated proteins of microbial pathogens and their commonality with amyloid and prion fibrils; structure-based strategies of drug and vaccine development.

(2) The process and end-products of amyloidogenesis; new approaches to obtain atomic-level structural details of amyloid fibrils, toxicity mechanisms of amyloid-forming peptides.

(3) Viral surface proteins.

(4) The properties of natively unfolded protein regions as precursors of disease-related folds.

The combination of the session themes which, on the one hand, corresponded to the different scientific directions, on the other hand, began to overlap, allowed to bring together researchers who normally do not tend to attend the same meetings. As a result, the conference provided an excellent opportunity for exchange of ideas and creation of new collaborations and generated a large interest among scientific community.

Report on the meeting proceedings

<u>Session 1.</u> Virulence factors with non-globular, fibrillar folds.

Many pathogenic bacteria colonize host tissues by binding specifically to the surfaces of susceptible human cells. These interactions are, in many cases, affected by bacterial adhesion molecules that bind to receptors on host cells. In order to be displayed on the bacterial surface, the adhesion molecules have to be secreted through the envelope including the cell wall. In gram-negative bacteria, many virulence factors of are secreted by the Type V secretion system via the autotransporter (AT) and two-partner secretion (TPS) pathways. AT proteins comprise three functional domains: the leader sequence; the secreted mature protein (passenger domain); and the carboxy-terminal translocator domain. It has recently emerged that majority of the secreted AT domains assume rigid linear fibrous beta-structural folds.

Susan BUCHANAN *(Bethesda, USA)* reported new data that allow a better understanding of the AT secretion and cleavage mechanism. She described a recently determined crystal structure of the post-cleavage state of the translocator domain of EspP, an autotransporter produced by E. coli O157:H7. The structure reveals an unprecedented intrabarrel cleavage mechanism and suggests conformational changes that occur in the translocator domain after cleavage.

Peter VAN ULSEN *(Amsterdam, The Netherlands)* showed that the extent of periplasmic folding of ATs is influenced by the signal sequence. They fused different types of signal sequences to NalP, an autotransporter of *N. meningitidis.* In wild type *N. meningitidis* cells, heterologous signal sequences resulted in periplasmic degradation of the passenger. However, secretion of the passenger could be rescued by knocking out a periplasmic chaperone/folding catalyst. Apparently, the timing of periplasmic folding of the AT is important for secretion and the signal sequence directs this timing.

Adrian GOLDMAN *(Helsinki, Finland)* presented latest results on functional and structural studies of trimeric ATs. They have solved the crystal structure of the passenger domains of two trimeric ATs : YadA, a collagen-binding protein from *Y. enterocolitica* and EibD, one of the *E. coli* immunoglobulin-binding proteins (Eibs). The monomeric structures of the trimers have a left-handed β-roll fold, followed by an extended coiled-coil stalk.They have demonstrated that YadA binds promiscuously to collagen segments with a high hydroxyproline to charged residue ratio. Their binding studies were also revealing the IgA and IgG binding motifs within the EibD structure.

Patricia L. CLARK *(Notre Dame, USA)* showed that almost all autotransporter proteins contain parallel beta-helix structure. They tested two otherwise unrelated autotransporter proteins and showed that each include a stable core structure located at the C-terminus of the beta-helix domain. Intriguingly, this C-terminus is the first portion of the autotransporter virulence protein to cross the outer membrane, and the

stable core beta-helix structure is formed on the cell surface prior to the completion of outer membrane secretion, suggesting that the energy released upon folding of the beta-helix can contribute to efficient secretion.

Alexander EBERTH *(Braunschweig, Germany)* reported study on structural characterization of major subunit csgA of Curli, which are extracellular proteinaceous fibrillar structures produced by many enterobacteria like *Escherichia coli* or *Salmonella spp*. They set out to elucidate structural details about csgA in its fibrillar state by using quenched Hydrogen/Deuterium exchange NMR. The fivefold repetitive primary sequence suggests that the csgA amyloid core consists of five repetitive, stacked β-structural units, analogous to what has been found for the fibril structure of prion HET-s.

Anna MITRAKI *(Heraklion, Greece)* presented study on short synthetic peptides corresponding to the repetitive elements of sequences found in natural β-structured fibrous folds and amyloid-forming peptides. They studied amyloidogenic octapeptides from natural fibrous proteins that present sequence homologies with amyloid-forming regions of the Alzheimer's beta amyloid peptide and the human islet amyloid polypeptide with the following techniques: TEM (Transmission Electron Microscopy), X-ray fiber diffraction, and Raman spectroscopy. Their results led to a better understanding of the sequence determinants underlying folding and assembly of β-structural fibrous structures.

Robert SECKLER *(Postdam-Golm, Germany)* described oligomeric β-helix proteins from bacteriophages. Work of his group has focused on the right-handed parallel β-helix fold and, in particular, on trimeric β-helical "tailspike" endoglycosidases from bacteriophages. Structures of several family members have been determined by crystallography, their stability, their folding and assembly, and their interaction with complex carbohydrates have been analyzed using solution biophysical techniques. The talk focused on the origins of extreme kinetic stability and on the assembly of trimeric β-helix proteins.

Andrey KAJAVA *(Montpellier, France)* surveyed and categorized the structures of β-solenoids (β-rolls and β-helices) determined by X-ray and NMR studies. In particular, he described the recurrent conformations and sequence motifs of β-arcs (distinctive turns found in β-solenoids) and β-arches (strand-turn-strand motifs). He also demonstrated that amyloid fibrils with the coil and serpentine folds are likely to have β-arches stacked into β-arcades with parallel in-register β-structures similar to those found in β-solenoids. This analysis has direct implications for sequence-based detection, structural prediction, and *de novo* design of other β-solenoid proteins and can be used to localize likely β-arc positions in amyloid fibrils.

Petr LEIMAN *(Lausanne, Switzerland)* suggested that Type VI secretion apparatus and phage tails share a common evolutionary origin. Type VI secretion system (T6SS) is composed of 15–20 proteins. Using crystallographic, biochemical, and bioinformatic analyses, they identified 3 T6SS components, which are homologous to bacteriophage tail proteins. These include the tail tube protein; the membrane-penetrating β- helical needle, situated at the distal end of the tube; and another

protein associated with the needle and tube. This led to the conclusion that T6SS is a multicomponent structure whose extracellular part resembles a bacteriophage tail.
Andrea DESSEN *(Grenoble, France)* presented a series of works on sortase-mediated pilus fiber biogenesis in *Streptococcus pneumoniae.* The electron microscopy analyses of *in vitro* reconstituted RrgB fibers revealed that they structurally mimic the pneumococcal pilus backbone. They also showed that SrtC-1 sortase is the main pilus-polymerizing transpeptidase. Crystal structures of sortases SrtC-1 and SrtC-3 reveal active sites whose access is controlled by flexible lids, unlike in non-pilus sortases, and suggest that substrate specificity is dictated by surface recognition coupled to lid opening. These results can be exploited for the development of broad spectrum antibacterials.

<u>Session 2.</u> Folds related to infectious (prion) and non-infectious amyloid fibrils

Over the past decade, substantial progress has been made in understanding the structural arrangements in amyloid fibrils. Although these specimens remain refractory to the classical approach of X-ray crystallography, progress has stemmed largely from the application of new experimental techniques such as solid state NMR, scanning transmission electron microscopy mass measurements, cryo-electron microscopy, and electron paramagnetic resonance spectroscopy of spin-labelled derivatives, as well as longer-established approaches such as X-ray fiber diffraction, conventional electron microscopy, and optical spectroscopy. There is also strong current interest in the features that may distinguish infectious amyloids (prions) from non-infectious amyloids. The speakers in this session were scientists who have contributed to the recent progress in this field.
Sven SAUPE *(Bordeaux, France)* demonstrated that depending on pH, HET-s prion protein of the fungus *Podospora anserina* was found to be able to assemble into either infectious or non-infectious amyloids. They have also shown that the prion forming domain of the homolog of the HET-s protein in another fungus, *Fusarium graminearum* conserves the amyloid-forming ability and prion propagation properties described for the Podospora HET-s protein. The Fusarium HET-s amyloids however differ from the Podospora ones by a nucleation rate, stability and Thioflavine T binding. He reported breaching of the species barrier for prion propagation between the two proteins.
Beat MEIER *(Zurich, Switzerland)* surveyed the principles and prospects of NMR structure determination in amyloids. He also presented the recently determined 3D structure of the HET-s(218-289) prion-forming domain in its infectious form and compared it with the non-infectious form. The structure solved by solid-state NMR allows to explain the high level of structural order and the stability of HET-s(218-289) prion fibrils. His group found considerable differences between infectious amyloids (prions) obtained at pH 7 and non-infectious amyloids obtained at pH 3. In addition,

spectra of full-length HET-s were presented and an outlook for structure determination of the other yeast prions were given.

Ulrich BAXA *(Bethesda, USA)* reported a series of works on establishment of the architecture of Sup35p filaments by negative staining, cryo-EM, metal-shadowing and scanning transmission electron microscopy (STEM) of intact and protease-digested specimens. His study revealed that the filaments have a core fibril (~8nm) surrounded by a diffuse halo of globules, presumably C-domains, extending outwards as much as 30 nm and connected with the fibril core by the M-domain in highly extended conformations. STEM measurements of mass-per-unit-length on intact filaments gave ~1 subunit per 0.47 nm. He also compared the arrangement found for Sup35p filaments *in vitro* to ones observed *in vivo* in [*PSI*+] yeast cells after high pressure freezing and freeze substitution.

Anant PARAVASTU *(Tallahassee, USA)* reported study on amyloid fibrils of the 40-residue Alzheimer's β-amyloid peptide with a structure that is distinct from those of previously well-characterized Aβ fibrils. Using solid state NMR and electron microscopy, they have assessed the fibril dimensions and mass, secondary structure, structural order, and site-specific inter-atomic proximities for these new fibrils. These results are the experimental basis for a novel 3-fold symmetric Aβ fibril structural model that is distinct from the previous solid state NMR-derived 2-fold symmetric Aβ fibril model.

Markus FÄNDRICH *(Halle, Germany)* demonstrated that with cryo EM techniques they were able to reconstruct the structure of an Aβ amyloid fibril with a structural resolution of ~9 Å. Analysis of several fibril morphologies from Aβ(1-40) and Aβ(1-42) peptides suggests that two paired β-sheet motif could represent a fundamental structural unit of different Aβ amyloid fibrils. In the second part of the talk, he showed that both the reconstructed *in vitro* fibrils and Alzheimer amyloid plaques are recognized by a recently generated antibody fragment B10. *In vitro* aggregation reactions show that this antibody fragment possesses anti-amyloidotic activity.

Jens RADZIMANOWSKI *(Grenoble, France)* described the crystal structures of the Fe65-PTB1 domain and the Fe65-PTB2 domain in complex with the 50 residues long cytoplasmic domain of Amyloid Precursor Protein called AICD. The adaptor protein Fe65 is an important interaction partner, which is predominantly expressed in the brain, where it plays a critical role in neuronal development, APP translocation, processing and signaling. The structure of the Fe65-PTB2/AICD complex reveals a unique interface involving 28 AICD residues forming one β strand and two α helices.

Louise SERPELL *(Falmer, United Kingdom)* surveyed recent advances in the X-ray fiber diffraction of amyloid fibrils. They have used biophysical and diffraction techniques to follow assembly and to analyse crystal and fibre structures. She presented computer tool which allows to explore and to analyse many structural models of the amyloid fibrils that have been suggested for their ability to fit experimental X-ray diffraction data. This work enabled them to arrive at some criteria necessary for a model amyloid structure.

Sheena RADFORD *(Leeds, United Kingdom)* presented study on the mechanisms of protein folding and aggregation of the naturally amyloidogenic protein, beta-2-microglobulin. Using NMR the conformational properties of species that initiate the amyloid cascade have been identified and an array of mutants have been created to explore the role of individual residues in governing the rates of aggregation. She also discussed a challenge of the identification of early oligomeric species and their structural characterisation, since such species are aggregation-prone, shortlived and rapidly equilibrating.

Ruth NUSSINOV *(Frederick, USA)* reviewed current views of Alzheimer amyloid toxicity that results from calcium leakage into the cell. Her experimental and theoretical data favour the mechanism of the toxicity in which ion-specific permeable channels formed by small amyloid oligomers. The structural model of the channel obtained by combining experiment with detailed modelling was described. The modeling has been carried out using detailed atomistic molecular dynamic simulations and experimental data. Perspectives of modeling of the Alzheimer A-beta oligomers in solution and in the lipid bilayer were presented.

Stephen BOTTOMLEY *(Melbourne, Australia)* suggested a novel mechanism by which polyQ region of ataxin-3 aggregate. Ataxin-3 protein has a C-terminal polyQ tract which expansion causes the disease spinocerebellar ataxia type-3. He demonstrated that ataxin-3 aggregation involves not only the polyQ tract but other folded domains of the protein. They have identified regions of the protein which are involved in the early stages of the aggregation. Furthermore, they showed that some of the physiological binding partners of ataxin-3 play an important role in modulating the aggregation kinetics.

Human REZAEI *(Jouy-en-Josas, France)* used biophysical techniques, mutational analysis and molecular dynamics simulations to elucidate the mechanisms of unfolding and oligomerization of ovine prion protein PrP. Upon partial unfolding of the monomer three types of β-sheet-rich soluble oligomers were observed, which form in parallel rather than in a sequential process. Furthermore, they have identified the minimal region of PrP that leads to the same oligomerization profile as the full-length protein. The existence of distinct oligomerization pathways and the effect of mutations revealed the conformational diversity of PrP and a possible relationship with prion strain phenomena.

Salvador VENTURA *(Bellaterra, Spain)* investigated the degree of *in vivo* co-aggregation between two self-aggregating proteins, Abeta42 amyloid peptide and foot-and-mouth disease virus VP1 capsid protein, in prokaryotic cells. The data indicate that *in vivo* protein aggregation exhibits a remarkable specificity that depends on the establishment of selective interactions and results in the formation of oligomeric and fibrillar structures displaying amyloid-like properties. These features allow prokaryotic Abeta42 intracellular aggregates to act as effective seeds in the formation of Abeta42 amyloid fibrils.

Ralf LANGEN *(Los Angeles, USA)* reviewed studies of amyloid fibrils and interactions of amyloid proteins with membranes by site-directed spin labelling.

His group developed a pulsed EPR approach for determining long-range (up to ~50Å) distances between spin labeled sites in the same polypeptide chain. These distances allow them to test previously proposed models and to provide important structural constraints for the refinement of amyloid fibril structures. They also proposed a mechanism by which membranes mediate the aggregation and misfolding of IAPP and used pulsed EPR methods to determine the structure of α-synuclein in its physiologically relevant helical membrane bound form.

Sangeeta NATH *(Leuven, Belgium)* presented data on early aggregation steps in α-synuclein as measured by FRET and Fluorescence Correlation Spectroscopy. It was shown that the monomers of ~3.8 nm diameter disappeared rapidly leading to the formation of higher oligomers. A rod shaped model of dimer and early oligomers with a diameter of 3.8 nm and multiples of the diameter in length fits well the experimentally observed diffusion coefficients. The conformational change during formation of early oligomers was also registered by using FRET.

Hilal LASHUEL *(Lausanne, Switzerland)* reported recent progress in the development of chemical and biophysical approaches and novel tools to characterize and control protein misfolding and self-assembly of amyloidogenic peptides. More specifically, he discussed recent work on the development of new chemical switch elements to facilitate mechanistic studies aimed at elucidating the molecular basis of protein misfolding, aggregation and disassembly. He highlighted the current challenges and opportunities in this field and the potential impact of applying these tools to addressing many of the technical and experimental challenges in studying protein folding and self-assembly.

Stravos HAMODRAKAS *(Athens, Greece)* surveyed examples of natural protective amyloids. He focused on chorion proteins, the major component of silkmoth eggshell which protects the oocyte and the developing embryo from environmental hazards. Peptide-analogues of chorion proteins form amyloid fibrils. This observation led them to conclusion that the chorion is a natural protective amyloid and revealed an important role of the tandemly repeating hexapeptide motifs in the amyloid fibril formation.

In the last talk of the session, **Rosemary STANIFORTH** *(Sheffield, United Kingdom)* presented the crystal structure of a tetrameric form of cystatin which shows structural re-arrangement involving loop exchanges and proline trans-cis isomerisation. This work allowed to understand better the mechanism of cystatin amyloid formation. Hydrogen exchange and cross-linking experiments reveal that much of the native cystatin fold including the domain-swapping interface remains intact but that the alpha-helical component is displaced from the main core of the amyloid structure. Possible structural models were presented.

<u>Session 3.</u> Viral surface proteins.

This session covered a variety of pertinent topics, focussing mainly on viral capsid architecture and structural motifs that occur in capsid proteins and host cell recognition proteins.

Felix REY *(Paris, France)* presented the recently determined crystal structure of the RNA-coating nucleoprotein (N- Protein*)* of Respiratory Syncytial Virus (RSV), an important agent of pediatric respiratory tract disease, worldwide. He went on to compare its structure with those of the corresponding N protein complexes of vesicular stomatitis virus (Green et al, Science, 2006) and rabies virus (Albertini et al, Science, 2006). The RSV complex is a ring of 10 N-protein monomers with the RNA wrapping around the ring, such that its bases are solvent-accessible. This complex provides the first model for the organization of the template of a member of the *Mononegavirales*, and explains how the bases can be read by the viral polymerase during transcription and replication without dissociation of the complex.

Alasdair STEVEN *(Bethesda, USA)* discussed how the unusual polymorphism of retrovirus capsids correlates with the presence of a short but flexible linker peptide that connects the two domains of its capsid protein. The observed wide variety of capsid shapes can be explained in terms of irregular polyhedra formed from 12 variously distributed pentamers together with larger and variable numbers of hexamers. This model is called the "fullerene" conjecture. Data was presented in support of this idea in the form of the first quasi-atomic model of the pentamer which was obtained by cryo-electron microscopy. These results underline the importance of nucleation as a shape-determining factor, and suggest that the differential formation of pentamers and hexamers is controlled primarily by electrostatic screening.

Vladimir LORMAN *(Montpellier, France)* addressed the question of capsid architecture which since 1962 has been dominated by the quasi-equivalence theory of Caspar and Klug (CK). In the interim, a number of viral structures such as papillomavirus, reovirus, and adenovirus have been determined experimentally that do not comply with the tenets of quasi-equivalence, so that theory is in need of generalization. The speaker's approach has been to relate capsid protein density to phenomenological free energy of the self-assembly process, using the Landau density-wave theory, which has been used to understand problems in condensed matter physics, from superconductivity to melting. His approach was illustrated primarily as applied to the icosahedrally symmetric but non-equivalent lattice of the envelope glycoprotein of Dengue virus, considering both the self-assembly process and the pH-induced structure reorganization of the viral particle.

Joseph COCKBURN *(Paris, France)* summarized an investigation by X-ray crystallography of a monoclonal antibody specific for the dengue envelope protein that is capable of neutralizing all four serotypes of the virus. The presence of sub-neutralizing antibodies has been a major obstacle to vaccine development. This

antibody, raised against DENV 1, recognises an epitope on domain 3 of the E-protein. Its interaction with the domains 3 of all four serotypes were determined by X-ray crystallography. These epitopes share a common core, centred on a conserved hydrophobic patch which is suggested to be the key determinant for broad-ranging DENV specificity. Insights from these structures should contribute to the design of synthetic antigens capable of eliciting a broad antibody response against the virus.

Rob RUIGROK *(Grenoble, France*) reported on the interaction between the Sendai virus N-protein/RNA and its polymerase co-factor, phosphoprotein P. PX binds to the C-terminal tail of N-protein, NTAIL, which appears to be natively unfolded, as is the N-terminal half of PX. A variety of NMR measurements were performed, yielding data that showed the presence of an ensemble of helical elements in equilibrium with the unfolded state. Interactions between NTAIL and PX may be initiated via interactions outside the helical regions; such non-specific probing has been named "fly-casting". Dynamics measurements on PX showed that the unfolded part influences stability of the three-helix bundle. These data lead to the proposal that, when polymerase moves along its template, the unfolded part of PX pulls on helix 1 causing it to unfold. Subsequent rotation of helix 3 destroys the platform of binding for NTAIL, which then detaches. Such detaching by deformation of the binding surface is reminiscent of "Velcro".

Gilles TRAVE *(Illkirch, France)* focussed on the conformational properties of *t*he E6 and E7 oncoproteins of human papillomaviruses, which – of particular pertinence to this Meeting – both have substantial regions that are natively unfolded. Human papillomaviruses are the main cause for cervical cancer and their oncogenic properties are mainly due to E6 and E7. E7 has a natively unfolded region of 50-100 residues followed by a 50-residue zinc-binding domain. E6 has two zinc binding domains flanked by natively unfolded regions. Remarkably, the zinc-binding domains of both E6 and E7 have folds that are found only in papillomaviruses. Despite of their small size, E6 and E7 bind to many target proteins controlling a variety of processes. The NMR solution structure of the E6 C-terminal domain was described as was ongoing work on the complementary N-terminal domain. These observations were discussed in the context of the interactions of E6 with cellular targets such as E6AP, p53 and PDZ domain proteins.

Lucienne LETELLIER *(Paris, France)* delivered a presentation concerning molecular mechanisms employed in host cell entry by tailed bacteriophages, focussing on the coliphage T5 system.Typically for these phages, a protein located at the distal part of the tail first binds to its receptor, followed by conformational changes in the tail that are transmitted to the capsid, leading to transfer of the phage DNA into the host cell. T5 infection is accompanied by the insertion in the membranes of pb2, the straight tail fiber protein, an oligomer of 5-6 subunits. Bioinformatic analysis indicates that pb2 has three domains. A fragment of called Pb2-Cterm has features in common with fusogenic membrane polypeptides; inserted into liposomes, it triggers their fusion. Observations led to a model whereby receptor binding generate major changes in pb2 such that the C-terminal region penetrates the host envelope, allowing the local

degradation of the peptidoglycan and the formation of a pore by fusion of the outer and inner membranes.

<u>Session 4.</u> Naturally unfolded protein regions as precursors of disease-related folds.

There is growing evidence that many protein molecules or protein domains do not assume well defined three-dimensional structures under (some) physiological conditions but instead, remain intrinsically disordered. It appears that many of the fibrous beta-rich proteins and fibrils that were discussed in the previous sessions of the conference have precursor states in which they are natively unfolded. The goal of this session was to review computational methods designed to identify amino acid sequences with a propensity to be natively unfolded; experimental analysis of their conformation in that state; and mechanisms that lead to their folding.

Joel SUSSMAN *(Rehovot, Israel)* demonstrated a possible role of Intrinsically Disordered Proteins (IDP) in Nervous System Development, describing as an example their study of a family of neural cell adhesion proteins CLAMs. By using physicochemical and bioinformatics studies, they have shown that the cytoplasmic domains of these proteins are IDPs. '*In silico*' studies (by using their web-based tool, FoldIndex) were compared with their solution studies on CLAMs and their adhesion partners, as well as their studies on the life-time of IUPs *in vivo*. He also discussed application of the FoldIndex tool to aid in expression and crystallization of proteins.

Peter TOMPA *(Budapest, Hungary)* surveyed the basic observations pertaining to the identity of Intrinsically Disordered Proteins implicated in disease such as cancer and neurodegeneration, and the link between their structural state and involvement in disease. He discussed if their pattern of disease-causing mutations is compatible with the notion of disorder being causally involved in disease, and whether their physiological half-lives suggest their rapid removal when no longer needed. Their physiological structural state, involvement in complexes, and interactions with chaperones were also taken a closer look to assess whether structural disorder does represent a particular danger to the organism.

Anne POUPON *(Nouzilly, France)* described how protein disorder predictions can be used to predict genetic constructs with improved expression, solubility and stability. Her group developed and used Prelink software to detect unfolded segments. By testing proteins for which genetic constructs have been successfully produced and their structure determined experimentally, she showed that it is possible to predict, for a given protein, an ensemble of genetic constructs with better experimental behaviour than the full length protein.

By creating and testing databases of fibril forming and fibril non-forming peptides **Oxana GALZITSKAYA** *(Pushchino, Russia)* demonstrated that regions with high expected probability of formation hydrogen bonds and high packing density may be responsible for the formation of amyloid fibrils. Moreover, she demonstrated a correlation between locations of residues involved in the folding nuclei (calculating

Φ-values of proteins) and locations of predicted amyloidogenic regions (by using several methods).

Sonia LONGHI *(Marseille, France)* described implications of structurally disorder regions of the replicative complex of measles virus for its pathogenicity. They characterized the intrinsically disordered C-terminal domain of the viral MeV nucleoprotein (NTAIL), as well as the mechanisms governing its disorder-to-order transition upon binding to the C-terminal X domain of the viral phosphoprotein. The disordered NTAIL exposed at the surface of the viral nucleocapsid, allows interactions with various partners, leading to tethering of the polymerase complex, stimulation of viral transcription and replication, immunosuppression, stimulation of cytokine expression, and virus assembly.

Malene RINGKJOBING JENSEN *(Grenoble, France)* demonstrated that NMR is uniquely placed to study dynamic processes, resolving detailed site-specific information about motions spanning a vast range of time scales in both folded and unfolded proteins, and in both the liquid and the solid phase. She showed examples of how they use NMR spectroscopy, Small Angle Scattering and molecular simulation to to enhance their understanding of the role of flexibility in processes involving intrinsically disordered proteins, such as viral replication, the development of neurodegenerative diseases and cancer.

In the last presentation of the conference, **Alexey MURZIN** *(Cambridge, United Kingdom)* formulated a new concept of "metamorphic" proteins. He surveyed a growing number of examples of the "metamorphic" proteins which adopt different folded conformations for the same amino acid sequence in native conditions. Unlike prions, they undergo reversible conformational changes. The discoveries of a new metamorphic protein capable of independent interconversion and of an abrupt fold change in a protein lineage suggests that the generally accepted rule that natural proteins possess unique, evolutionarily conserved three-dimensional structures may be in need of revision.

Concluding remarks

The Conference generated a remarkable atmosphere, which was marked by high scientific level of presentations and by constructive, friendly and productive discussions. It was highly appreciated by participants who expressed the overwhelming support for organizing another meeting in 3 years time. Andrey Kajava (vice-president of this conference and president of the next) accepted to present an application for the next conference. Robert Seckler was elected unanimously on the last evening of the Conference as the next vice-president and he accepted the charge. He will assist Andrey Kajava for the organization of the next Conference.

Finally, we wish to thank the Centre National de la Recherche Scientifique (CNRS) for its decisive financial and administrative support. We are also grateful to VWR International (Fontenay-sous-Bois, FRANCE) from whom some financial support was also obtained. We want to point out that the successful organization of the Conference, from the registration and welcoming of the participants to the delicious food was largely due to the good practical skills of Mme Dominique Lidoreau, a secretary of the Conference, as well as the hospitality of the staff of the Center Paul-Langevin. Dominique Lidoreau was also of extremely valuable in the editing of the abstract book. We thank her warmly for her help and work in all the matters relating to the practical organization of the Conference.