Project no: LSHG-CT-2004-512052

Project acronym: UPMAN

Project title: Understanding Protein Misfolding and Aggregation by NMR

Instrument: Specific Targeted Research or Innovation Project

Thematic Priority 1: Life Sciences, Genomics and Biotechnology for Health

Final Report

Period covered: from 01.11.2004 to 30.04-2008

Date of preparation: 25.10.2008

Start date of project: 01.11.2004

Duration: 42 months

Project Co-ordinator: Prof. Harald Schwalbe

Project Co-ordinator Organisation Name: Johann-Wolfgang Goethe Universität, Frankfurt/Main

Section 1 – Final Activity Report

1 Publishable Final Activity Report

1.1 Introduction

The failure of proteins to fold into their functional forms leads occasionally to "misfolding" or "conformational" diseases. Many among the most common and debilitating of these diseases are associated with the formation of protein amyloid, an insoluble material that is deposited as fibrils or plaques in different body tissues and organs. Amyloid formation is known to be accelerated by a variety of cellular factors, including metal ions, such as copper and zinc, and interactions with other species, such as lipids and RNA.

Aberrant folding can lead to protein aggregates deposited inside or outside cells, and is the cause of several neurological and systemic diseases (e.g. Alzheimer's disease and the transmissible prion disorders) compromising the quality of life and the health resources of society.

The number of diseases now known to be associated with misfolding is large and increasing all the time (Table 1). Understanding the processes responsible for the failure to achieve or maintain the normal functional structures of proteins is crucial for developing strategies to protect or enhance human health.

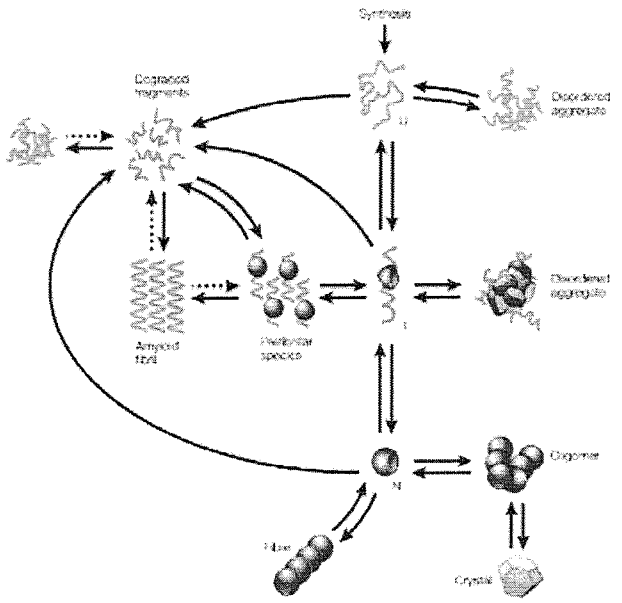

Figure 1: States accessible to a protein molecule

Detailed knowledge of structure and dynamics of misfolded or conformationally heterogeneous proteins is still largely missing. Proteins in many of these states are constantly changing shape, such that they are better described as ensembles of conformations rather than in terms of well-defined structures, as is normally the case for native states. Methods in which molecular simulations are combined with experimental measurements are emerging as a powerful route to the accurate determination of the conformational properties of these states of proteins.

Table 1: Selected members of the family of amyloid diseases

Clinical Syndrome	Fibril Component
Alzheimer's disease	Aβ peptide
Spongiform encephalopathies	Prion
Senile systemic amyloidosis	Wild-type transthyretin and fragments
Type II diabetes	Fragment of islet-associated polypeptide
Medullary carcinoma of the thyroid	Fragments of calcitonin
Atrial amyloidosis	Atrial natriuretic factor or full-length insulin

1.1.1 The Partnership

Taken from the UPMAN WEB-site: http://schwalbe.org.chemie.uni-frankfurt.de/upman/participants/

The consortium consisted of eight partners stemming from academia and one partner as an industrial collaborator (Partner 9).

Partner 1:
Prof. Harald Schwalbe - Coordinator
Johan Wolfgang Goethe Universität Frankfurt, Center for Biomolecular Magnetic Resonance (BMRZ), Inst. for Organic Chemistry and Chemical Biology, Frankfurt am Main, DE

Partner 2:
Prof. Lucia Banci
Consorzione Interuniversitario Risonanze Magnetiche di Metalloproteine, Sesto Fiorentino (Florence), IT

Partner 3:
Prof. Rolf Boelens
Utrecht University, Bijvoet Center for Biomolecular Research, Utrecht, NL

Partner 4:
Prof. Christopher Martin Dobson
Department of Chemistry, University of Cambridge, Cambridge, UK

Partner 5:
Prof. Astrid Gräslund
Stockholms Universitet, Department of Biochemistry and Biophysics, Stockholm, SE

Partner 6:
Prof. Flemming Martin Poulsen
Structural biology and NMR laboratory at Institute of Molecular Biology, University of Copenhagen, Copenhagen, DK

Partner 7:
Dr. Ago Samoson
National Institute of Chemical Physics and Biophysics, Tallinn, EE

Partner 8:
Prof. Kurt Wüthrich
Eidgenössische Technische Hochschule Zürich, Institute of Molecular Biology and Biophysics, Zürich, CH

Partner 9:
Dr. Jesus Zurdo
Zyentia Limited, Babraham Research Campus, Cambridge, UK

1.1.2 Project objectives to address these requirements

During the period of the project, complementary NMR approaches were developed. These approaches included a variety of NMR techniques (Workpackage 1-3, see Figure below) and enabled us to couple them with novel computational approaches to define the disorganised ensembles characteristic of disordered protein species involved in protein folding diseases. These techniques were applied to representative examples of the various types of proteins that are associated with misfolding diseases (Workpackage 4 and 5). These ranged from native unfolded species (such as α-synuclein associated with Parkinson's disease) and partially unfolded intermediates (such as forms of superoxide dismutase associated with motor neuron disease), to the precursors of aggregation prone fragments (such as the Alzheimer precursor protein) and the prion proteins, which are uniquely associated with transmissible conditions. One of the major aims of this project was to provide a novel view of the conformational behaviour of protein molecules, which had a broad significance for understanding important aspects of functional genomics, including the fundamental links between genetic mutations and disease, and the mechanisms by which normally soluble proteins can sporadically misfold, giving rise to a wide range of disorders associated with diet, medical and agricultural practices and ageing.

NMR spectroscopy is here the observation method of choice because it provides both dynamic and structural information about proteins in a variety of different states at atomic resolution. It has the potential for probing residual structure, the size of aggregating molecules and variation in the internal dynamical properties based on diffusion-weighted NMR spectroscopy, heteronuclear relaxation measurements, paramagnetic enhancement of relaxation induced by paramagnetic spin labels, and residual dipolar couplings.

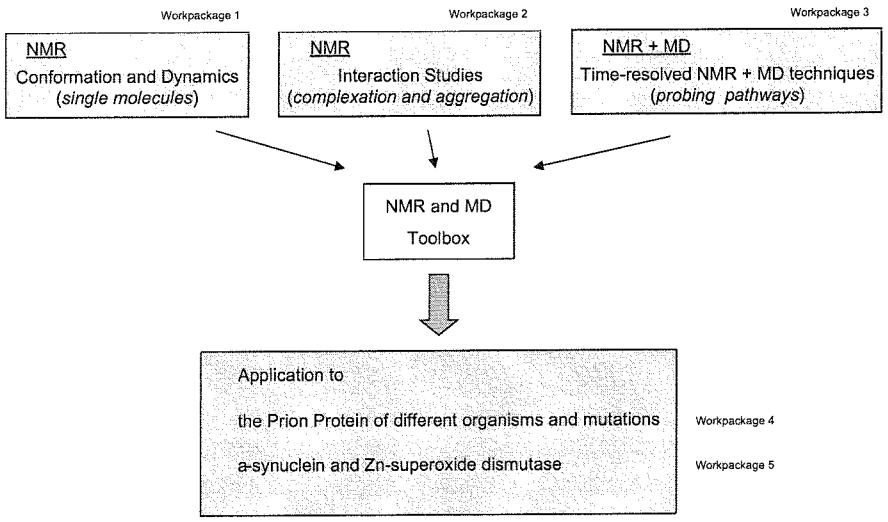

Figure 2: Graphical presentation of the workflow as stipulated in Annex I.

1.2 *Project Results and Achievements*

1.2.1 What was learned about disordered proteins?

1.2.1.1 Quantitative techniques for the structural characterization of disordered states

Studies of disordered states are complicated by the heterogeneity and rapid interconversion of conformers, leading to methodological challenges. In addition, although intrinsically disordered proteins, by definition, are highly populated disordered states, unfolded states are typically only weakly populated under non-denaturing conditions. The methods employed to destabilize folded proteins to enable studies of denatured states are extremes of temperature, addition of denaturants (such as guanidinium chloride, urea or acid), truncation or mutation, respectively.

NMR spectroscopy, which provides atomic-resolution information on secondary structure, tertiary contacts, solvent exposure, overall molecular dimensions and dynamic properties was the dominant observation technique for the studies of disordered proteins. The following box summarises the various NMR methods which were developed and validated in Workpackages 1 - 3.

Box 1:	NMR Tool box to study intrinsically disordered proteins as part of
Secondary chemical shifts	• NMR resonance assignments are required for the site-specific interpretation of NMR data and also contain inherent structural information. The deviations of experimental chemical shifts from their expected random-coil values, termed secondary chemical shifts, exhibit residue specific **propensity for β-sheet formation or α-helical properties**.
Hydrodynamic methods	• NMR diffusion experiments yield the translational diffusion coefficient, which can be used to calculate the **hydrodynamic radius** (R_h), to distinguish between **monomeric states and multimeric states** of the protein. • Hydrogen exchange rates give information on **protection from solvent exposure** for labile nitrogen-bound protons. • Fast-Field Cycling Relaxation Measurements of Unfolded Proteins at Low Magnetic Fields gives information on **residual order in unfolded proteins**.
Nuclear Overhauser enhancements (NOE)	• **Short and medium range distances between two protons** used for **structure determination** that can be far away in primary sequence and up to 10 Å away in space
Paramagnetic relaxation enhancement (PRE)	• **Long-range distances** are revealed from a spin label to amide protons in the protein up to 20–25 Å away. Spin probes are introduced either by Cysteine-coupled spin labels or by paramagnetic metal ions or small organic compounds dissolved in the buffer, respectively.
Relaxation dispersion spectroscopy	• Characterization of **protein millisecond timescale motions** or **sparsely populated, excited state conformations** which may exist only transiently.
Residual dipolar couplings (RDC)	• Ensemble averaged orientations of internuclear vectors with respect to the alignment frame. Differences between experimental and calculated RDCs can used to confirm **secondary and even tertiary structural features** e.g. to provide evidence of the **long-range orientation** between the N- and C-terminal regions of a given protein.
Time-resolved NMR experiments	• Protein folding can be initiated by e.g. pulsed laser excitation and **folding kinetics at atomic resolution** can be observed. The transient formation of possible **folding intermediates** can be exhibited.

Moreover, the developed NMR methodology was extensively combined with light detecting methods, stopped-flow Circular Dichroism and UV-fluorescence to give further input by complementary data or to validate experimental findings.

1.2.1.2 Computational methods for the generation of representative structure ensembles of disordered proteins

As significant progress was made on the experimental side, more accurate data could be offered to the *in-silico* approach (Workpackage 3) as input or to validate the theoretical models, respectively. Folding models were developed from all-atom MD simulations that quantitatively predict protein folding rates and mechanisms of protein folding. The theoretical algorithms, simulation methods, potentials and other factors were improved in order to be able to make quantitative predictions at rising precision.

Methods of structure determination that had been developed for native states were extended to highly heterogeneous states. This heterogeneity of many of the states populated by proteins made it necessary to represent them as ensembles of conformations. Such ensembles provide an effective way to represent the fluctuations around an average structure.

In this approach, the experimental information is used to construct structural restraints to be used in molecular simulations. The sampling is biased to take place in regions of conformational space that are consistent with the available experimental information. Interproton distances derived from nuclear Overhauser effects (NOEs), residual dipolar couplings or given information on protection from solvent exposure can be used to define the structures of unfolded states. In a very prominent approach, chemical shift information was used to restrain molecular simulations to obtain structural models of the molten globule states or protein folding intermediates.

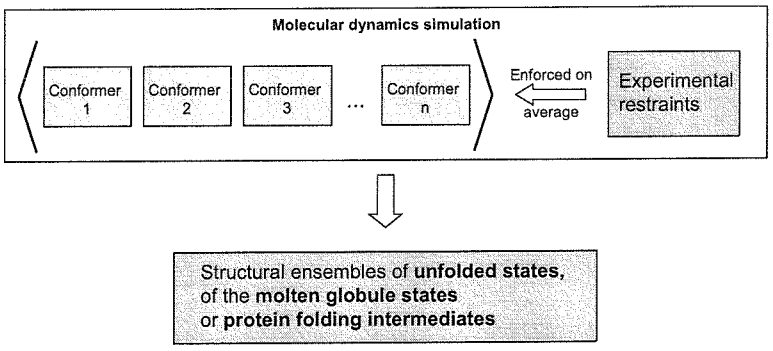

Figure 3: NMR restrained molecular dynamics (MD) simulations of disordered states.

It proofed very useful to cross-validate properties of the calculated ensembles with experimental parameters that were not used as restraints. Here, NMR relaxation experiments played an important role in the validation process because they highlighted clusters of residues with restricted motion in disordered proteins. Additionally, residual dipolar couplings constituted a source of information on the transient structure of the disordered states and they are very helpful in confirming that the ensemble is representative of the disordered state.

1.2.2 Characterisation of monomers and their oligomers – a starting point for nucleation and elongation

Although our focus in this project is mainly on structural and precursor/product relationships within the universe of disordered protein states, further information about the mechanisms that lead to the amyloid fibril deposits was obtained. Amyloid growth almost invariably showed a lag phase, which is nearly always interpreted as sufficient proof of a nucleated growth mechanism. It can be hypothesised that the rate limitation to overall fibril growth may be due to the existence of a short lived state — the nucleus — limited either by the kinetics of elongation reaction or by its thermodynamic instability.

1.2.2.1 The Prion protein

The prion protein (PrP) was one of the main focuses of attention in the UPMAN project. It has attracted a lot of interest because of its relation to transmissible spongiform encephalopathies (TSEs), which are a group of invariably fatal neurological diseases. Very promising results were discovered by our Nobel laureate, Prof. Kurt Wüthrich, as transgenic mice with mutations between the loop region of amino acids 165-175 exhibited spontaneous transmissible spongiform encephalopathy, which is hereditary to the offspring. Upon these very important findings, several PrP^C structures from mammalian and non-mammalian species (man, cattle, mouse, Syrian hamster, bank vole, horse and elk) were solved by NMR spectroscopy and compared. It could be shown that the most evident structural variation concerns a loop which was found to be critical for the hereditary transmission of the disease. Transgenic mice carrying two specific amino acid substitutions at the aforementioned loop developed a spontaneous transmissible spongiform encephalopathy which could be transmitted to transgenic mice overexpressing wild-type prion protein. The presented data suggests that the local structure of the loop β2–α2 as well as medium-range and long-range sequence effects are responsible for the finding.

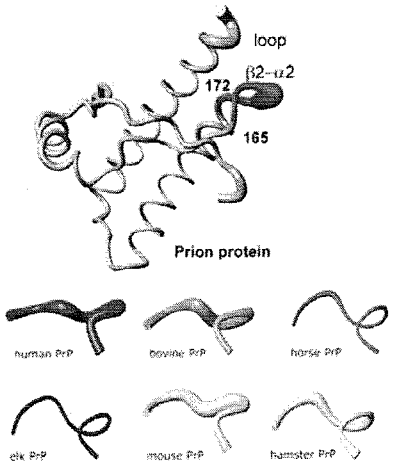

Figure 4. **Three-dimensional structure of Prion Proteins** and structural variability in the loop β2-α2 of different mammalian PrPs

Additionally, conformational Prion protein isoforms (^{Ctm}PrP and ^{Ntm}PrP) were investigated by introducing mutations adjacent to or within this transmembrane region that favour the expression of the ^{Ctm}PrP isoform. It is known that transgenic mice that favor ^{Ctm}PrP expression develop spontaneous neurodegeneration with evidence of apoptotic cell death. It was possible to develop a membrane mimetic model system on the basis of phospholipids and with solution conditions that affect the conformation of the transmembrane region of the variant prion proteins, but not of wt PrP. It is proposed that during the pathological processes, conformational polymorphism displayed by membrane-inserted prion protein may play a role at perturbing the general architecture of the membrane lipid bilayer and inducing protein-protein aggregation at membrane surfaces.

1.2.2.2 *The beta-amyloid (Aβ) peptide*

The beta-amyloid (Aβ) peptide, a major component of senile plaques in Alzheimer's disease brain, was the second major focus of the UPMAN endeavour. NMR Relaxation data recorded of the amyloid β-peptide ($A\beta_{1-40}$) showed that the N-terminus becomes more structured upon zinc binding. Moreover, relaxation and translational diffusion NMR data showed that there are no soluble small oligomers induced by zinc binding to Aβ. The N-terminus of the free Aβ peptide in aqueous solution has an extended conformation which may help to keep the peptide soluble. When the N-terminus binds zinc (or copper) its solubility properties are compromised and this may explain the increased aggregation propensity brought about by the metal ions. Upon metal binding the extended N-terminus folds and promotes aggregation.

The rapid kinetics of Aβ peptide aggregation was studied by time-resolved spectroscopy. The aggregation of the $A\beta_{1-28}$ peptide was induced by a rapid, sub-millisecond pH jump and monitored on the millisecond to second time-scale. The pH jump generated in the experimental setup is used to model the Aβ peptide structural conversions that may occur in the acidic endosomal/lysosomal cell compartment system. The proton release was induced by laser-flash photolysis of a caged acidic compound. The aggregation of the peptide induced by the pH jump yields an antiparallel β-sheet structure. The kinetics of the structural transition is biphasic. An initial rapid phase with a transition from random coil to an oligomeric β-sheet form with approximately 5-10 strands at 3.6 s is followed by a second slower transition, which yields larger aggregates during 48.0 s.

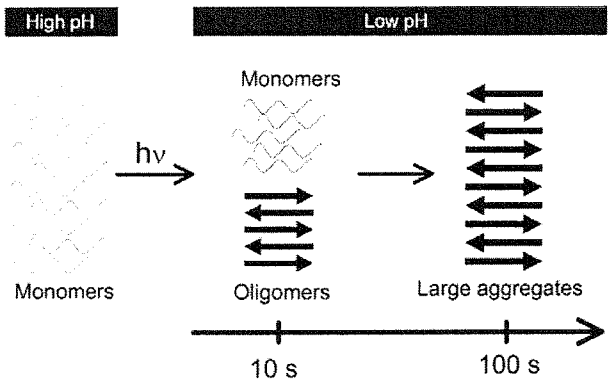

Figure 5: Kinetic model of beta-amyloid ($A\beta_{1-28}$) peptide aggregation

It has been shown that fibril formation in vitro is explained by a nucleation-elongation-fibrillization mechanism. The results indicate a first rapid formation of β-sheet oligomers, which is followed by a slower formation of larger aggregates. The large aggregates are formed by adding unstructured peptides to the oligomeric β-sheet template.

1.2.2.3 *The superoxide dismutase 1*

As the cytosolic superoxide dismutase 1 (SOD1) is linked with familial Amyotrophic Lateral Sclerosis (fALS, a form of motor neuron disease), the protein was also of high interest for the UPMAN consortium. It is well documented that fALS produces protein aggregates in the motor neurons of fALS patients, which have been found to be associated to mitochondria. There are about 100 single point mutations of the copper and zinc binding superoxide dismutase 1 (SOD1) which are reported to be disease-related. These mutations are spread all over the protein. Eleven SOD1 mutants were selected, most of them reported as pathological, and characterized them by NMR spectroscopy. Besides, their propensity to

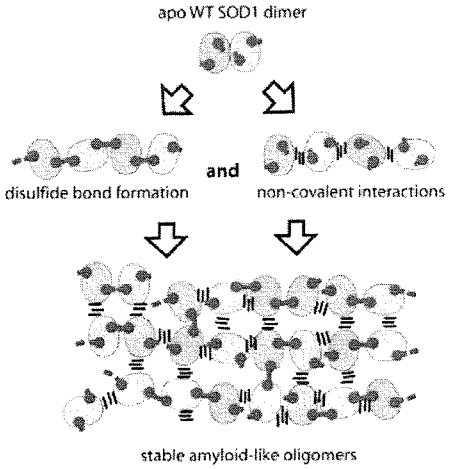

Figure 6: Possible mechanism for in vivo formation of amyloid deposits

aggregation was investigated using different techniques, from circular dichroism spectra to ThT-binding fluorescence, size-exclusion chromatography and light scattering spectroscopy. It can be shown that these eleven SOD1 mutants, only when they are in the metal-free form, undergo the same general mechanism of oligomerization as found for the WT metal-free protein.

It has been further shown that disulfide bonds are formed during the process of oligomerization. The formation of the soluble oligomers can be monitored by their ability to enhance the fluorescence of thioflavin T, a benzothiazole dye that increases in fluorescence intensity upon binding to amyloid fibers, and by disruption of this binding upon addition of the chaotropic agent guanidinium hydrochloride.

These results suggest a general, unifying picture of the process of SOD1 aggregation that can be operative when SOD1 lacks its metal ions. Even if we cannot exclude that other mechanisms are operative for SOD1 aggregation in SOD1-linked fALS, the one here proposed has the strength of rationalizing a common event, i.e., protein oligomerization, which occurs in a quite large and diverse set of SOD1 mutant proteins.

1.2.3 A structural model of a mature amyloid fibril

A new ß-helical model was developed that explains species barrier and strain variation in transmissible spongiform encephalopathies (TSE). The left-handed ß-helix serves as a structural model that can explain the seeded growth characteristics of ß-sheet structure in Prion Protein^{Sc} fibrils. MD simulations demonstrate that the left-handed ß-helix is structurally more stable than the right-handed ß-helix, with a higher β-sheet content during the simulation and a better distributed network of inter-strand backbone-backbone hydrogen bonds between parallel ß-strands of different rungs. Multiple sequence alignments and homology modelling of prion sequences with different rungs of left-handed ß-helices illustrate that the PrP region with the highest ß-helical propensity (residues 105-143) can fold in just 2 rungs of a left-handed ß-helix. Even if no other flanking sequences participate in the ß-helix, the two rungs of a ß-helix can give the growing fibril enough elevation to accommodate the rest of the PrP protein in a tight packing at the periphery of a trimeric ß-helix.

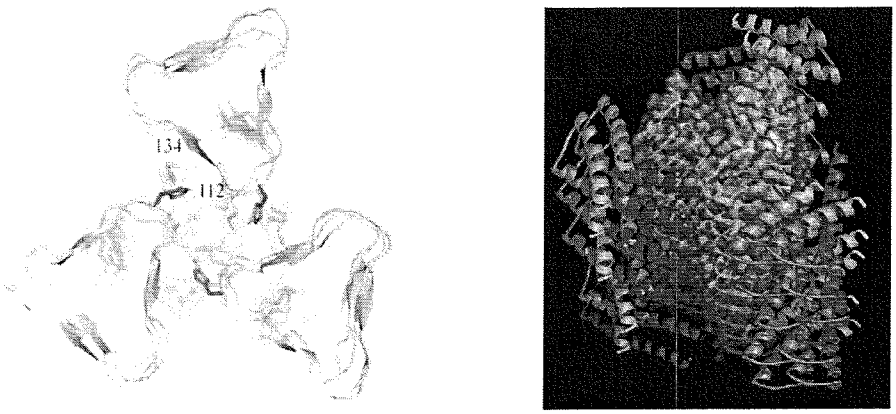

Figure 7: ß-helical model for the Prion protein fibrils. Trimers of ß-helical rungs are formed which stack on top of each other to form the fibril. (Left side: view along the fibril axis; right side: view sidewards)

The folding of ß-helices is driven by backbone-backbone hydrogen bonding and stacking of side chains in adjacent rungs. The sequence and structure of the last rung at the fibril end with

unprotected ß-sheet edges selects the sequence of a complementary rung and dictates the folding of the new rung with optimal backbone hydrogen bonding and side chain stacking. An important side chain stack that facilitates the ß-helical folding is between methionine residues 109 and 129, which explains their importance in the species barrier of prions. Because the PrP sequence is not evolutionary optimised to fold in a ß-helix and because the ß-helical fold shows very little sequence preference, alternative alignments are possible that result in a different rung able to select for an alternative complementary rung. A different top rung results in a new strain with different growth characteristics. Hence, in the present model, sequence variation and alternative alignments clarify the basis of the species barrier and strain specificity in PrP-based diseases.

1.2.4 Small-molecule inhibitors targeting the aggregation process

Our industrial collaborator, Zyentia Limited, developed tools to model the interaction of designed aggregation inhibitors with target proteins. This approach has been successfully used in the design of more potent and specific aggregation inhibitors. The main benefit from this approach is to reduce the pharmacological dose for these compounds, which has important implications in the development of drugs against protein depositional disorders.

This approach has been exemplified with the development of a new generation of aggregation inhibitors for Alzheimer's disease (inhibitors of Aβ42 aggregation) and Parkinson's disease (inhibitors of α-synuclein).

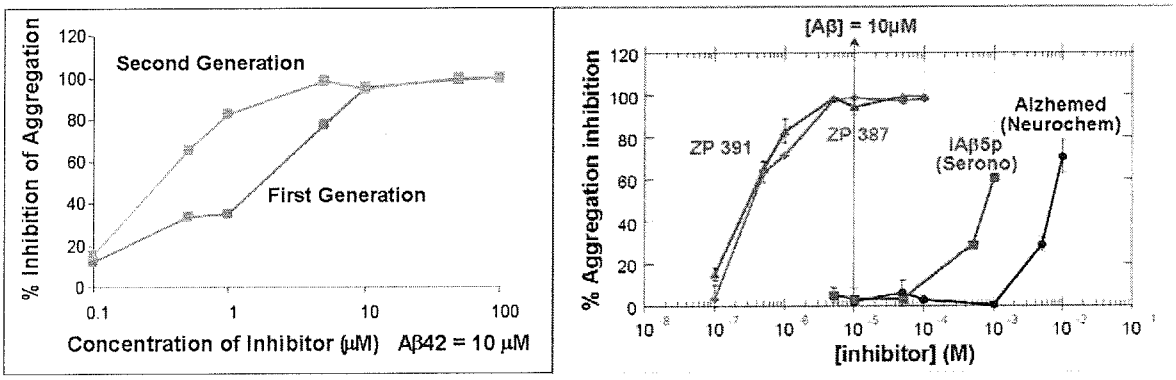

Figure 8: *In vitro* evolution of more potent Aβ42 aggregation inhibitors

The panels above show the increase in potency (about 10 fold) between the 1st generation and 2nd generation compounds. When these compounds are compared to drugs in clinical development by other corporations (iAβ5p and Alzhemed) they show a more than 2,000 and 20,000 fold higher potency. One implication of this work is that it supports the hypothesis that accumulation of oligomeric aggregates of misfolded proteins is an important mechanism underlying neurodegenerative disease. This concept necessarily predicts that attempts to block the formation of toxic oligomeric aggregates should also block the disease phenotype, as was shown here.

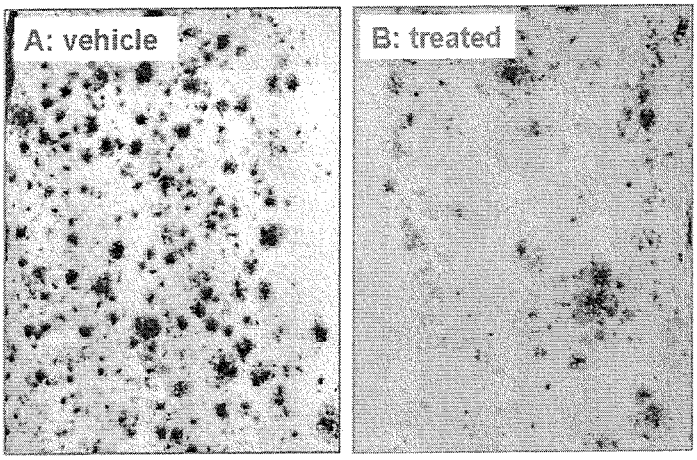

Figure 9: Brain sections exhibiting amyloid plaques

These inhibitors have shown to diminish substantially the toxicity of Aβ42 to cells in culture, but also they have shown to be able to remove amyloid plaque in brain in a transgenic mouse model. Aged animals (13 months) were treated for 2 weeks and the morphology of the brains were analysed afterwards. The experiment shows that inhibitor compounds are able to clear amyloid deposits from the brain.

Various small-molecule inhibitors of amyloid formation were discovered in attempts to develop anti-amyloid therapeutics. Interestingly, a surprisingly large number of these compounds seem to act by altering the course of aggregation, rather than by blocking it completely. In some cases, compounds promote the formation of an aggregate morphology that is distinct from those observed during the normal time course of amyloid formation. Many of these compounds produce their effect through non-covalent interactions, but some seem to require covalent cross-linking between the amyloidogenic peptide and the small molecule. Usually, these compounds stimulate very rapid alternative aggregate formation and then stabilize the aggregated product against further morphology changes to neurotoxic species.

1.2.5 Conclusions

We have been amply able to show that the methodological challenges due to the heterogeneity and rapid interconversion of conformers of disordered proteins can be overcome. Appropriate use of NMR spectroscopic techniques together with a much improved computational approach has enabled us to tackle several aspects of the underlying basics which lead to misfolding diseases. Based on the work we undertook during the project and the assessment of our work by the referees of the highly ranked journals, we are able to conclude with confidence that NMR spectroscopy in combination with other biophysical techniques is a scientifically viable technology in the field of disordered or misfolded proteins. Further evidence is given as many invited talks were held in front of the structural biology community which helped to disseminate the results of our project in Europe as well as world-wide. Our results are published and communicated to a wide and relevant audience.

A large fraction of the achievements of this project have set the state-of-the-art, this can be seen from the high impact factor journals many of the results have been published in. In terms of lasting impact, it is clear that this project has helped act as a major spur to others. During the course of the UPMAN project, a strong, clear message was provided that European NMR scientists play a leading role in this field.

1.3 *Final plan for using and disseminating the knowledge*

1.3.1 Exploitable knowledge and its Use

The results achieved thus far are scientifically relevant but do not foresee the generation of IP-related results. The major components of an NMR toolbox were provided to obtain NMR data for solving structures or revealing properties of proteins and their complexes both in solution and in the solid state. The investigated proteins were relevant in diseases like Alzheimer, ALS, MS and prion diseases. Though these results are new and were published in peer reviewed scientific journals, they constitute no major scientific breakthrough or new methods. Thus they are not suitable for formal protection of IPR at this stage.

Partner 09, Zyentia Limited, was declared insolvent during the course of the third UPMAN period. Generally, Zyentia dealt with research and development of novel treatments for the project related protein folding diseases and was responsible for competitive biopharmaceuticals or biomaterials, respectively. Their exit out of the consortium affected the commercially exploitable results which could not be pursued any more. The drug discovery process to design aggregation inhibitors came to an halt. Thus, the consortium will not invoke the relevant articles on intellectual property rights in the consortium agreement for UPMAN and in the contract with the European Commission.

1.3.2 Dissemination of knowledge

The following dissemination activities were undertaken during the course of the project:

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
2004-2008	***Publications:*** 36 papers in peer-reviewed scientific journals, seven more are in preparation	*Research*	*World*		*All*
Since Nov. 2006	***Project web-site:*** http://schwalbe.org.chemie.uni-frankfurt.de/upman/	*Research, general public*	*World*		*1*
2004-2008	**Conferences:** Several (>50) invited talks/poster presentations at various scientific symposia and meetings	*Research*		*20-600 each*	*All*

Some examples may be given:

- 13th ICBIC (Wien, July 15-20, 2007)
- 16th ISMAR conference, Kenting, Taiwan, October 2007.
- 28th Discussion Meeting of the Magnetic Resonance Spectroscopy Division of the GDCh, Tübingen, 28.9.2006.
- 2nd FEBS Special Meeting ABC, Innsbruck, Österreich, 01.03. – 08.03.2008
- 48th Experimental NMR Conference, Daytona Beach (Florida, USA), April 22-27, 2007.

- 49th ENC Conference Asilomar, Californien, 09.03. – 14.03.2008
- 5th International Workshop on Structural Characterization of Proteins by NMR, X-ray Diffraction and Computational Methods (San Vito di Cadore, Italy, June 15-18, 2006)
- 7. Igler NMR Tage Obergurgl (Austria), 12 - 15 February 2006.
- 7th CCPN Conference, entitled NMR of problematic biological systems, July 31 - August 2, 2007, at St Martin's College, Ambleside, The Lake District, UK.
- 9ème Réunion de travail RMN du solide d'Eveux, La Baume, France, November 2006.
- Bruker BioSpin User Meetings in Germany, Belgium, and England, November 2007.
- CCPN meeting, Ambleside (U.K.), 28-30 July 2006.
- Chianti Workshop (11th) on Magnetic Resonance, with a focus on relaxation phenomena, June 3-8, 2007, Vallombrosa (Florence), Italy.
- EISAI Symposium "Structural Biology and Disease". London, England, 5th-6th May 2007
- ENC - 47st Experimental Nuclear Magnetic Resonance Conference, Asilomar conference center, Pacific Grove, CA (USA), 23-28 April 2006.
- ESF LESC/PESC Explatory Workshop: Experimental and computational aspects of high-throughput protein NMR. Göteborg, Sweden, 17th-20th June 2006
- ESF workshop on experimental and computational aspects of high-throughput NMR, Göteboorg (Sweden), 17–20 June 2006.
- ESF-EC Conference on Structural Genomics: Structure, Dynamics and Interactions of Biomolecules, 20-25 August 2005, Höör (near Malmö), Sweden,
- EUROMAR, 3 - 8 July 2005, Veldhoven, The Netherlands
- EUROMAR, Taragona (Spain), 1-5 July 2007.
- EUROMAR, York, England, July 2006.
- European Biophysics Congress 2007, London, 14.07. – 18.07.2007
- Gordon Conference Computational Aspects of Biomolecular NMR. Aussois, France, 24-29 September, 2006
- Gordon Research Conference 'Computational aspects of biomolecular NMR', Aussois, France, September 2006
- Groupe d'Etudes de Résonance Magnétique (GERM), Blankenberge, Belgium, March 2006.
- International Conference on Multidimensional Spectroscopy, Rigi Kulm, Lucerne, Switzerland, May 2006 (2 talks).
- Journée Chimie-Biologie de l'université Paul Sabatier, Toulouse, France, May 2007.
- Magnetic Resonance, June 5-10, 2005, Connecticut College, New London
- NMR in drug discovery, Barcelona (Spain), 19-21 October 2006.
- Protein misfolding and aggregation in ageing and disease. Conference Jacques-Monod, Roscoff, France, 11th-15th April 2007
- RMN VIII NMR: a tool for biology, Paris, Frankreich, 28.01. – 30.01.2008

- SFB 625 - International Discussion Meeting on the Molecular and Structural Basis of Functional
- Swedish NMR Meeting, Goteborg (Suede), September 4-5, 2007.
- Symposium on "New NMR in Structural Biology". Frankfurt, Germany, 16^{th}-17^{th} November 2007
- Symposium on Future Perspectives of Biomolecular NMR, Munich (Germany, January 16, 2008).
- Systems, Mainz, Germany, September 2007.
- University of Verona, 2007
- XI Chianti Workshop on Magnetic Resonance, Vallombrosa, Italy, June 2007.
- XXème Congrès du GERM, Alenya (France), March 25-30, 2007.
- XXIInd ICMRBS, Göttingen (Germany), 20-25 August 2006.

1.3.3 Publications relevant to the project

1. Skora, L.; Cho, M. K.; Kim, H. Y.; Becker, S.; Fernandez, C. O.; Blackledge, M.; Zweckstetter, M., Charge-induced molecular alignment of intrinsically disordered proteins. **Angew Chem Int Ed Engl** 45, (42), 7012-5, **2006**.

2. Banci, L.; Bertini, I.; D'Amelio, N.; Libralesso, E.; Turano, P.; Valentine, J. S., Metalation of the amyotrophic lateral sclerosis mutant glycine 37 to arginine superoxide dismutase (SOD1) apoprotein restores its structural and dynamical properties in solution to those of metalated wild-type SOD1. **Biochemistry** 46, (35), 9953-62, **2007**.

3. Fischer, D.; Mukrasch, M. D.; von Bergen, M.; Klos-Witkowska, A.; Biernat, J.; Griesinger, C.; Mandelkow, E.; Zweckstetter, M., Structural and microtubule binding properties of tau mutants of frontotemporal dementias. **Biochemistry** 46, (10), 2574-82, **2007**.

4. Oglecka, K.; Lundberg, P.; Magzoub, M.; Goran Eriksson, L. E.; Langel, U.; Graslund, A., Relevance of the N-terminal NLS-like sequence of the prion protein for membrane perturbation effects. **Biochim Biophys Acta** 1778, (1), 206-13, **2008**.

5. Collins, E. S.; Wirmer, J.; Hirai, K.; Tachibana, H.; Segawa, S.; Dobson, C. M.; Schwalbe, H., Characterisation of disulfide-bond dynamics in non-native states of lysozyme and its disulfide deletion mutants by NMR. **Chembiochem** 6, (9), 1619-27, **2005**.

6. Bertini, I.; Felli, I. C.; Luchinat, C.; Parigi, G.; Pierattelli, R., Towards a protocol for solution structure determination of copper(II) proteins: the case of Cu(II)Zn(II) superoxide dismutase. **Chembiochem** 8, (12), 1422-9, **2007**.

7. Schroeder, C.; Werner, K.; Otten, H.; Kratzig, S.; Schwalbe, H.; Essen, L. O., Influence of a joining helix on the BLUF domain of the YcgF photoreceptor from Escherichia coli. **Chembiochem** 9, (15), 2463-73, **2008**.

8. Lofgren, K.; Wahlstrom, A.; Lundberg, P.; Langel, U.; Graslund, A.; Bedecs, K., Antiprion properties of prion protein-derived cell-penetrating peptides. **Faseb J** 22, (7), 2177-84, **2008**.

9. Danielsson, J.; Jarvet, J.; Damberg, P.; Graslund, A., The Alzheimer beta-peptide shows temperature-dependent transitions between left-handed 3-helix, beta-strand and random coil secondary structures. **Febs J** 272, (15), 3938-49, **2005**.

10. Danielsson, J.; Pierattelli, R.; Banci, L.; Graslund, A., High-resolution NMR studies of the zinc-binding site of the Alzheimer's amyloid beta-peptide. **Febs J** 274, (1), 46-59, **2007**.

11. Christen, B.; Wuthrich, K.; Hornemann, S., Putative prion protein from Fugu (Takifugu rubripes). **Febs J** 275, (2), 263-70, **2008**.

12. Wahlstrom, A.; Hugonin, L.; Peralvarez-Marin, A.; Jarvet, J.; Graslund, A., Secondary structure conversions of Alzheimer's Abeta(1-40) peptide induced by membrane-mimicking detergents. **Febs J**, **2008**.

13. Binolfi, A.; Rasia, R. M.; Bertoncini, C. W.; Ceolin, M.; Zweckstetter, M.; Griesinger, C.; Jovin, T. M.; Fernandez, C. O., Interaction of alpha-synuclein with divalent metal ions reveals key differences: a link between structure, binding specificity and fibrillation enhancement. **J Am Chem Soc** 128, (30), 9893-901, **2006**.

14. Bertini, I.; Gupta, Y. K.; Luchinat, C.; Parigi, G.; Peana, M.; Sgheri, L.; Yuan, J., Paramagnetism-based NMR restraints provide maximum allowed probabilities for the different conformations of partially independent protein domains. **J Am Chem Soc** 129, (42), 12786-94, **2007**.

15. Banci, L.; Bertini, I.; D'Amelio, N.; Gaggelli, E.; Libralesso, E.; Matecko, I.; Turano, P.; Valentine, J. S., Fully metallated S134N Cu,Zn-superoxide dismutase displays abnormal mobility and intermolecular contacts in solution. **J Biol Chem** 280, (43), 35815-21, **2005**.

16. Banci, L.; Bertini, I.; Cantini, F.; D'Amelio, N.; Gaggelli, E., Human SOD1 before harboring the catalytic metal: solution structure of copper-depleted, disulfide-reduced form. **J Biol Chem** 281, (4), 2333-7, **2006**.

17. Banci, L.; Bertini, I.; Cantini, F.; DellaMalva, N.; Herrmann, T.; Rosato, A.; Wuthrich, K., Solution structure and intermolecular interactions of the third metal-binding domain of ATP7A, the Menkes disease protein. **J Biol Chem** 281, (39), 29141-7, **2006**.

18. Mukrasch, M. D.; von Bergen, M.; Biernat, J.; Fischer, D.; Griesinger, C.; Mandelkow, E.; Zweckstetter, M., The "jaws" of the tau-microtubule interaction. **J Biol Chem** 282, (16), 12230-9, **2007**.

19. Schlorb, C.; Ackermann, K.; Richter, C.; Wirmer, J.; Schwalbe, H., Heterologous expression of hen egg white lysozyme and resonance assignment of tryptophan side chains in its non-native states. **J Biomol NMR** 33, (2), 95-104, **2005**.

20. Wirmer, J.; Peti, W.; Schwalbe, H., Motional properties of unfolded ubiquitin: a model for a random coil protein. **J Biomol NMR** 35, (3), 175-86, **2006**.

21. Jarvet, J.; Danielsson, J.; Damberg, P.; Oleszczuk, M.; Graslund, A., Positioning of the Alzheimer Abeta(1-40) peptide in SDS micelles using NMR and paramagnetic probes. **J Biomol NMR** 39, (1), 63-72, **2007**.

22. Obolensky, O. I.; Schlepckow, K.; Schwalbe, H.; Solov'yov, A. V., Theoretical framework for NMR residual dipolar couplings in unfolded proteins. **J Biomol NMR** 39, (1), 1-16, **2007**.

23. Richter, B.; Gsponer, J.; Varnai, P.; Salvatella, X.; Vendruscolo, M., The MUMO (minimal under-restraining minimal over-restraining) method for the determination of native state ensembles of proteins. **J Biomol NMR** 37, (2), 117-35, **2007**.

24. Christen, B.; Peréz, D.; Hornemann, S.; Wuthrich, K.; , NMR Structure of the Bank Vole Prion Protein at 20 degrees C Contains a Structured Loop of Residues 165-171. **J Mol Biol** ahead of print, **2008**.

25. Peralvarez-Marin, A.; Barth, A.; Graslund, A., Time-resolved infrared spectroscopy of pH-induced aggregation of the Alzheimer Abeta(1-28) peptide. **J Mol Biol** 379, (3), 589-96, **2008**.

26. Schlepckow, K.; Wirmer, J.; Bachmann, A.; Kiefhaber, T.; Schwalbe, H., Conserved folding pathways of alpha-lactalbumin and lysozyme revealed by kinetic CD, fluorescence, NMR, and interrupted refolding experiments. **J Mol Biol** 378, (3), 686-98, **2008**.

27. Wong, A.; Pike, K. J.; Jenkins, R.; Clarkson, G. J.; Anupold, T.; Howes, A. P.; Crout, D. H.; Samoson, A.; Dupree, R.; Smith, M. E., Experimental and theoretical 17O NMR study of the influence of hydrogen-bonding on C=O and O-H oxygens in carboxylic solids. **J Phys Chem A** 110, (5), 1824-35, **2006**.

28. Danielsson, J.; Andersson, A.; Jarvet, J.; Graslund, A., 15N relaxation study of the amyloid beta-peptide: structural propensities and persistence length. **Magn Reson Chem** 44 Spec No, S114-21, **2006**.

29. Sigurdson, C. J.; Nilsson, K. P.; Hornemann, S.; Manco, G.; Polymenidou, M.; Schwarz, P.; Leclerc, M.; Hammarstrom, P.; Wuthrich, K.; Aguzzi, A., Prion strain discrimination using luminescent conjugated polymers. **Nat Methods** 4, (12), 1023-30, **2007**.

30. Banci, L.; Bertini, I.; Boca, M.; Girotto, S.; Martinelli, M.; Valentine, J. S.; Vieru, M., SOD1 and amyotrophic lateral sclerosis: mutations and oligomerization. **PLoS ONE** 3, (2), e1677, **2008**.

31. Salvatella, X.; Dobson, C. M.; Fersht, A. R.; Vendruscolo, M., Determination of the folding transition states of barnase by using PhiI-value-restrained simulations validated by double mutant PhiIJ-values. **Proc Natl Acad Sci U S A** 102, (35), 12389-94, **2005**.

32. Gsponer, J.; Hopearuoho, H.; Whittaker, S. B.; Spence, G. R.; Moore, G. R.; Paci, E.; Radford, S. E.; Vendruscolo, M., Determination of an ensemble of structures representing the intermediate state of the bacterial immunity protein Im7. **Proc Natl Acad Sci U S A** 103, (1), 99-104, **2006**.

33. Banci, L.; Bertini, I.; Durazo, A.; Girotto, S.; Gralla, E. B.; Martinelli, M.; Valentine, J. S.; Vieru, M.; Whitelegge, J. P., Metal-free superoxide dismutase forms soluble oligomers under physiological conditions: a possible general mechanism for familial ALS. **Proc Natl Acad Sci U S A** 104, (27), 11263-7, **2007**.

34. Cavalli, A.; Salvatella, X.; Dobson, C. M.; Vendruscolo, M., Protein structure determination from NMR chemical shifts. **Proc Natl Acad Sci U S A** 104, (23), 9615-20, **2007**.

35. Wirmer, J.; Berk, H.; Ugolini, R.; Redfield, C.; Schwalbe, H., Characterization of the unfolded state of bovine alpha-lactalbumin and comparison with unfolded states of homologous proteins. **Protein Sci** 15, (6), 1397-407, **2006**.

36. Best, R. B.; Vendruscolo, M., Structural interpretation of hydrogen exchange protection factors in proteins: characterization of the native state fluctuations of CI2. **Structure** 14, (1), 97-106, **2006**.

Publications submitted or in preparation:

1. Banci, L., Bertini, I., Boca, M., Calderone, V., Cantini, F., Girotto, S. and Vieru, M.: **SOD1 and its mutants: structural and kinetic aspects related to oligomarization.** *In preparation.*

2. Christen B, Hornemann S, Damberger, FF & Wüthrich K: **NMR structure of a mouse prion protein variant reveals long-range sequence effects on the loop β2–α2.** *In preparation.*

3. Perez DR, Damberger, FF & Wüthrich K: **NMR structure and dynamics of the horse prion protein domain(121-231) and related mouse variant prion proteins.** *In preparation.*

4. Roodveldt, C., Bertoncini, C.W., Andersson, A., Fernández-Montesinos, R., Hsu, S.-T.D., Pozo, D., Christodoulou, J., Dobson, C.M.: **Mechanistic insights into the Hsp70/a-synuclein complex: nucleotide-dependent anti-amyloid activity and modulation by Hip.** *In preparation.*

5. Sigurdson CJ, Nilsson KPR, Hornemann S, Heikenwälder M, Manco G, Schwarz P, Ott D, Rülicke T, Liberski P, Julius C, Falsig J, Stitz L, Wüthrich K & Aguzzi A: **De novo generation of a transmissible spongiform encephalopathy by mouse transgenesis.** *Submitted.*

6. Silvers R, Wirmer-Bartoschek, J & Schwalbe H: **Disulfide dynamics in intrinsically unstructured proteins.** In preparation

7. Wirmer-Bartoschek, J, Schlörb, C., Richter, C, Schwalbe, H. **On the origin of elevated transverse relaxation rates in intrinsically unstructured proteins.** In preparation