

Uncovering the intricacies in amyloid illness

Focused on the amyloid self-assembly processes that cause neurodegenerative illnesses, **Professor Ludmilla A Morozova-Roche** discusses her project's recent achievements



Could you begin with an overview of your wide-ranging amyloid research?

We study the molecular and cellular mechanisms of amyloid formation and inhibition as a generic phenomenon with particular focus on the amyloid role in neurodegenerative Alzheimer's and Parkinson's diseases as well as in some other human amyloidoses. We also explore the application of amyloids in medical diagnostics and nanobiotechnology. Our research is highly diversified and interdisciplinary, bridging together specialised biophysical studies with medical sciences.

What led you to pursue studies in the area of neurodegenerative and amyloid diseases?

Amyloid-related ailments are on the rise in modern society due to growing elderly populations. In Sweden, 150,000 people suffer from dementia and primarily from Alzheimer's disease, which involves treatment and maintenance costs of SEK 50 billion per year, and this number is predicted to rise sharply in coming years. Parkinson's disease, which is the second most common neurodegenerative disorder after Alzheimer's, is characterised by resting tremor, akinesia and rigidity, and significantly affects the life of the sufferers. Its prevalence progressively increases from 0.6 to 3.5 per cent during ageing. A recent study, conducted in Northern Sweden, demonstrated that the incidence of

Parkinson's disease reaches around 3 per cent among the elderly population.

Corpora amylacea inclusions, which we have found to be calcified amyloids, begin to develop in the prostate of middle-aged men and contribute to the process of age-dependent prostate tissue remodelling, representing a significant risk factor for prostate cancer.

Amyloids as the cause of disease are a generic form of often unnecessary and potentially highly hazardous proteinaceous products accumulated in the body. We can anticipate that more amyloid deposits and related diseases will be identified in the human body in the coming years as we become more aware about this property of polypeptide chain and are able to analyse it more closely. The type and progression of these amyloids will determine how significantly they impair our life functions. These diseases affect our parents and elderly relatives and they will affect our children and our children's children, especially if they live longer. All these factors and thoughts represent a background of our biomedical crusade on these diseases.

How did your discovery of endogenous antibodies to the amyloid producing protein in Parkinson's disease come about, and what is its significance?

That was a result of long and systematic studies. We have investigated the autoimmune responses to amyloid oligomers and fibrils as well as to amyloid precursor proteins in neurodegenerative Alzheimer's and Parkinson's diseases over the last decade. Based on our findings, we suggested that the autoimmunity may play a protective role in neurodegenerative disease development, targeting and eliminating unwanted proteins and their amyloid assemblies. This protective mechanism is particularly important in the early stages. During disease progression, the protective immune responses become exhausted. Consequently, their role subsides and the overall decrease of the level of protective antibodies is observed. Thus, peripheral autoimmune responses can be sensitive indicators of underlying pathophysiology and also have a significant

diagnostic potential especially at the early stages of amyloid disease development.

This is how we have determined significantly increased levels of autoantibodies to protein α -synuclein, forming amyloid inclusions known as Lewy bodies in the *substantia nigra* of the Parkinson's brain, especially at the early stages of disease, which is very important for early disease diagnostics and earlier intervention with modifying therapeutic strategies. The measurement of the levels of endogenous antibodies in patients' blood serum is easy and requires no intervention other than a blood test.

Do some people have a genetic predisposition towards developing a degenerative amyloid disease later in life? If so, would you advocate genetic testing, given its likely psychosocial impact (eg. revealing a risk to family members or encouraging prenatal testing)?

In short: no. I am not an advocate of genetic testing.

Amyloid diseases, in their majority, are not genetic disorders. Parkinson's disease is 85 to 90 per cent sporadic. There are some familial mutations in Parkinson's and Alzheimer's diseases and, if the family members carry them, they will have a higher risk of developing disease at an earlier age. Mutations which enhance the propensity of polypeptide chains to aggregate will promote amyloid formation of amyloid precursor proteins or peptides and ultimately lead to disease. Nevertheless, in their vast majority, amyloid diseases are age-dependent rather than related to genetic variants.

The underlying mechanism of amyloid formation is an intrinsic in nature: a generic property of polypeptide chain to form β -sheet stacking stabilised by a network of hydrogen bonds, which can grow indefinitely if there are no effective clearance mechanisms in the body. The clearance mechanisms become naturally weaker with ageing, including the clearance by autoimmune antibodies, which we study. Consequently, amyloid oligomers and fibrils will grow in size and quantity and lead to cell death and tissue damage.

Amyloid causes

Neurodegenerative illnesses, such as Parkinson's and Alzheimer's diseases, have long been a serious issue for elderly generations. Now, through a programme of innovative research into protein amyloid self-assembly, scientists at **Umeå University** are making breakthroughs into the structural basis of this process

AS ELDERLY POPULATIONS around the globe increase, so too do the cases of age-dependent degenerative diseases. Today, about 40 million people over 60 are diagnosed with Alzheimer's disease worldwide, while around 1-2 per cent of all men over 65 develop Parkinson's disease. Cases of diabetes have also risen and now affect millions of sufferers.

Recent research has found a number of neurodegenerative illnesses to share links with age-dependent amyloid formations in the body. Amyloid-related diseases, such as Alzheimer's, Parkinson's, Huntington's, and prion diseases, arise from a generic accumulation in the body of unnecessary and potentially hazardous proteinaceous amyloid products. Appearing in various organs and tissues, these aggregated deposits can eventually lead to physiological dysfunction with the degree of damage depending on many factors, including protein origin, location and individual characteristics. This means that any person can develop amyloidoses and become susceptible to age-related disease.

Over the last two decades alone, intensive research has been conducted on amyloids. However, after countless studies, experts still lack a full understanding of what triggers the amyloid formations in the body and, consequently, degenerative disease. The key to these complex causes lies in the integrated molecular and cellular level studies by using high resolution and high sensitivity biophysical and biochemical techniques.

FURTHER INVESTIGATIONS

Based at the Department of Medical Biochemistry and Biophysics in Umeå University, Sweden, Professor Ludmilla A Morozova-Roche is conducting a project to further address amyloids and the processes involved in their development. Her team focuses on several aspects of amyloids, including the structural and functional mechanisms of their formation, inhibition and removal. Morozova-Roche hopes that their work will lead to strategies on how to slow, and eventually reverse, the effects of amyloid formation, improving the diagnosis and treatment of amyloid-related diseases.

The project concentrates on the structural characterisation of amyloid assembly with particular focus on the early events of the fibrillation process. For this, atomic force microscopy (AFM) and a range of biophysical and biochemical techniques are employed. The Morozova-Roche lab has developed a powerful AFM platform incorporating 3 AFM microscopes as a part of the Biochemical Imaging Center, Umeå. The group is also working to identify toxic amyloid species and determine how they cause cell damage and death, alongside the analysis of the apoptotic activity of proteinaceous complexes towards tumour cells.

NEW DISCOVERIES

Since beginning their research, Morozova-Roche and her colleagues have made great leaps in progress. They recently found that calcified

corpora amylacea formations in ageing prostates are brought on by the amyloids of inflammatory proteins S100A8 and S100A9. These deposits can easily grow in size, exacerbate damage of the prostate tissues and ultimately lead to cancer. The discovery of this particular amyloidosis supports strategies that aim to reduce inflammation and prevent prostate cancer as well as inflammation-dependent amyloid growth in other organs and tissues.

The team discovered that autoimmune responses to A β oligomers reflected mild to moderate phases of Alzheimer's disease dementia, while responses to S100B and dopamine closely matched moderate to severe dementia progression. Based on these findings, Morozova-Roche has since expressed the importance of profiling the autoimmune responses to the stages of Alzheimer's disease before selecting the specific clinical strategy relevant to the illness' progression.

A significant part of the project's research has been the evaluation of autoimmune reactions to biomarkers of disease pathology in Parkinson's disease patients including endocrine (insulin), astrocytocal (S100B) and amyloid (α -synuclein) biomarkers. These reactions were found to reflect the neurodegenerative damage that occurs in Parkinson's disease. Of particular note was the discovery of increased levels of autoimmune antibodies to α -synuclein during the early stages of disease, which can significantly help clinical staff in giving accurate diagnosis. Many Parkinson's disease patients only visit a clinic once the symptoms,

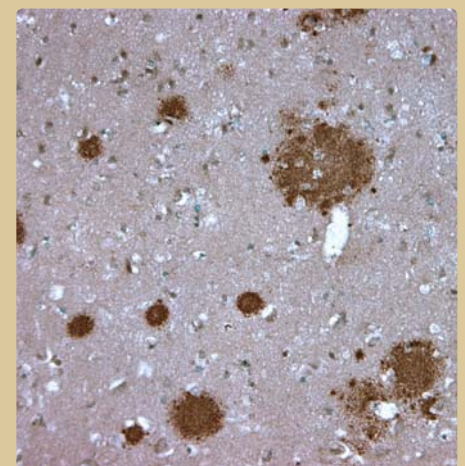
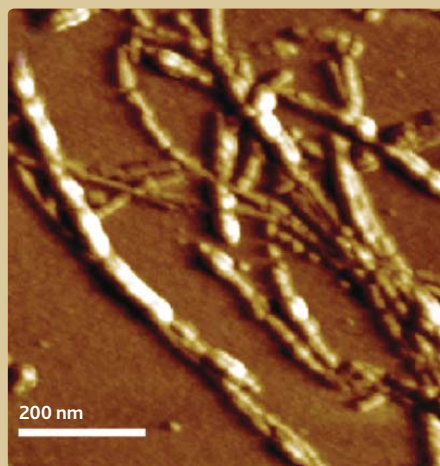
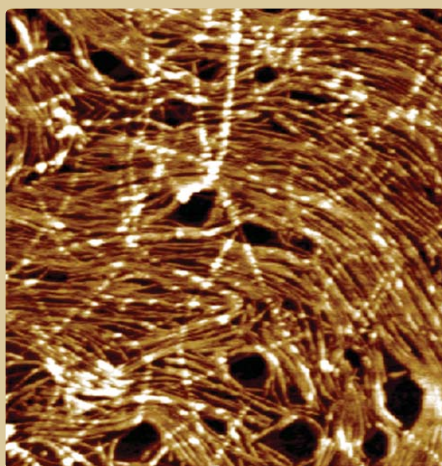


FIGURE 1. Amyloid plaque formation: AFM images of amyloid fibrils (**left and centre**), which clump together to form plaques in Alzheimer's brain tissues (immunostaining, 20x magnification, **right**).

INTELLIGENCE

MIND-BRAIN-BODY INTERACTIONS IN NEURODEGENERATIVE DISORDERS

OBJECTIVES

To study the molecular and cellular mechanisms of amyloid formation and inhibition as a generic phenomenon, with particular focus on the amyloid role in neurodegenerative Alzheimer's and Parkinson's diseases as well as in some other human amyloidoses; and also to explore the applications of amyloids in medical diagnostics and nanobiotechnology.

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PROFESSOR LUDMILLA MOROZOVA-ROCHE

completed her PhD in Biophysics in the Institute of Biophysics, Russia in 1984. In the 1990s she worked as a guest scientist at the Max-Planck Institute of Biochemistry, Martinsried, Germany; Leuven University, Belgium and as the Wellcome Trust fellow at the University of Oxford, UK. Since 2001 she has held various professorial positions at Umeå University, where she is presently Professor in Medical Biophysics at the Department of Medical Biochemistry and Biophysics.

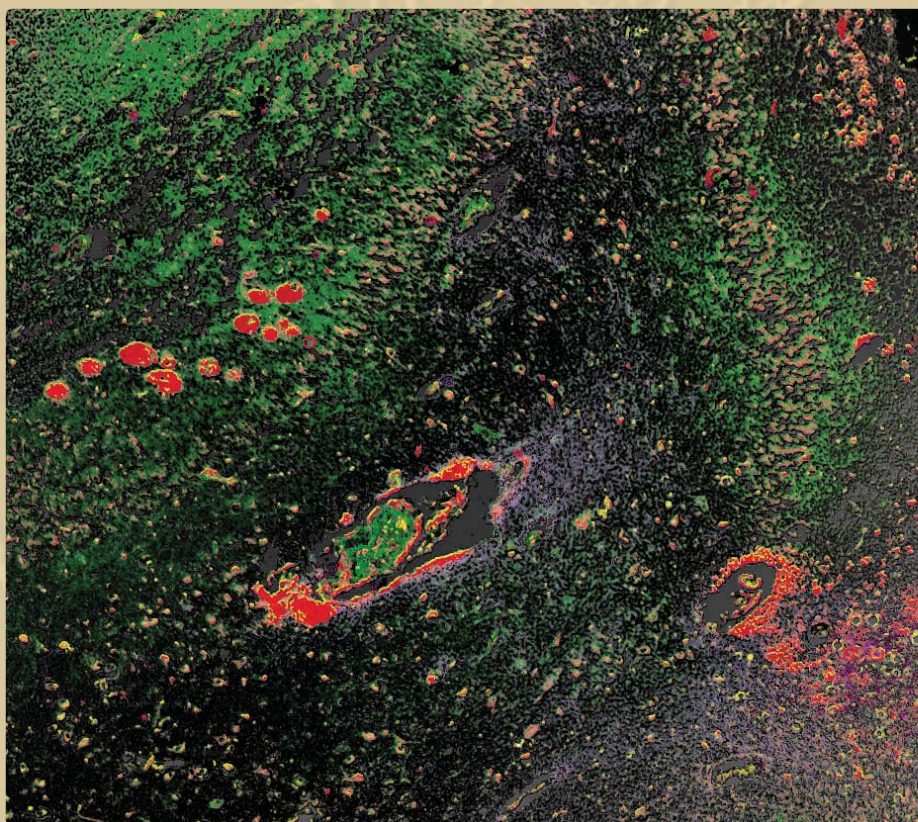


FIGURE 2. Immunohistochemical staining of Alzheimer's brain tissues show the amyloid plaques (red) are surrounded by inflammatory lesions (green).

such as tremors or akenisia, become apparent. However, by this time, 60 to 80 per cent of their neurons in *substantia nigra* are already dead. Due to this, it is imperative that medical practitioners be able to diagnose as early as possible.

BRANCHING OUT

Research into amyloids is not only in the interest of patients and practitioners working with age-related degenerative diseases; since almost any protein under destabilised conditions can assemble into amyloids and lose its native functions, with safety in mind, it is also greatly important to the biopharmaceutical and food industries that heavily rely on proteinaceous products.

While the project's main focus has been on stopping the effects of amyloid formations, other properties, such as the protein's remarkable strength, can be utilised for positive means. Morozova-Roche's team recently designed and patented a method of producing silver nanowire by using lysozyme amyloid fibrils as a supporting template. The thinnest nanowire to date through use of biotemplating (two nm diameter), this discovery alone demonstrates the various ways in which amyloids can prove beneficial if properly studied.

This ongoing work is of a multidisciplinary nature, involving communication between structural biology, protein sciences, cell biology, immunology and neurobiological sciences.

This exchange of expertise significantly helps the group's success rate, as Morozova-Roche affirms: "Being situated within the Medical Faculty at Umeå University, we have the privilege of working in close connection with clinics and so can directly relate our biophysical research to clinical needs. Our belief is that in modern medicine the understanding of molecular basis will ultimately lead to better treatment".

The wide array of local, national and international partnerships that the team has managed to foster over the years has assisted greatly in the project's achievements. However, it can be a double-edged sword. Aims and goals, for instance, can differ from discipline to discipline and, as such, slow processes more so than any boundary created by national border or language. However, by the same token, Morozova-Roche accepts that without interaction between varied disciplines, many of the project's discoveries would not have been possible.

Following all of the project's research and discovery, it now appears that amyloid formation and the associated effects on tissues and health is a complex process of multiple phases, mechanisms and regulatory interactions. In order to continue on their path to success, the biophysicists have now begun to focus on physiology and clinical practice with the aim of developing *in vitro* and *in vivo* models and interventions alongside their practical observations.

