О влиянии наночастиц на структурную стабильность биологических макромолекул

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Outline

Introduction

- Synthetic complex of magnetoferritin
- Amyloids and Amyloidosis
- Impact of nanoparticles on amyloids structure

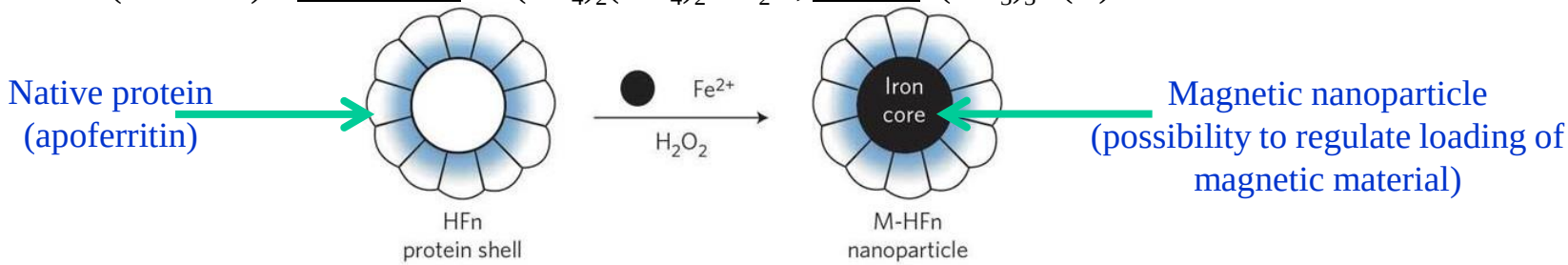
- Structure and stability of magnetoferritin solutions
  - Impact of loading factor on structure and stability of magnetoferritin complexes
- Structure characterization of amyloids fibrils solutions
  - Structure parameters of amyloids aggregates in aqueous solutions
- Interaction of nanoparticles with amyloids fibrils
  - Impact of magnetoferritin on amyloids structure
  - Interaction of magnetic nanoparticles with amyloids

Conclusions

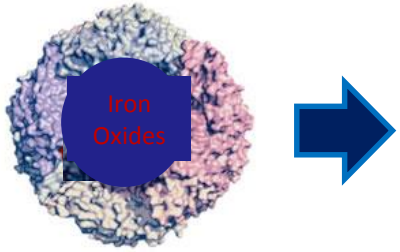
Magnetoferritin

Synthetic bio-complex of protein shell (apoferritin) with various amount of magnetic material inside

Conditions – aqueous, anaerobic (N_2), alkaline (pH 8.6), temperature 65°C
Reactants (solutions) – Fe^{2+} source: $Fe(SO_4)_2(NH_4)_2.6H_2O$, oxidant: $(CH_3)_3N(O)$



Magnetoferritin

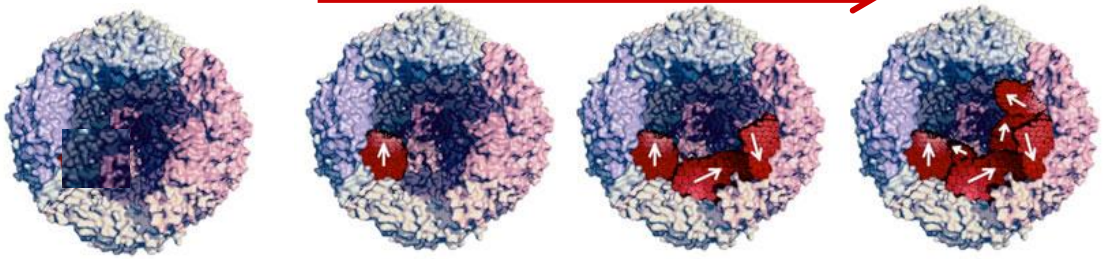


apoferritin

$\varnothing_{out}$ 12 nm
$\varnothing_{in}$ 8 nm

- **Native MNP stabilization (by protein shell)**
- **Limitation on maximal size of MNP**

Loading factor (LF)

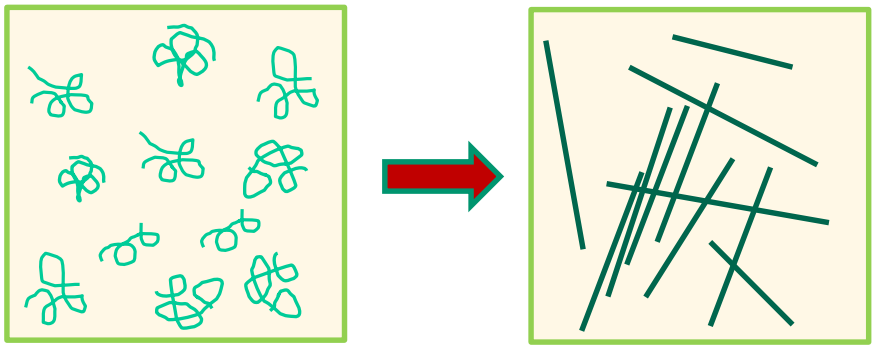


Magnetoferritin – *in vitro* model system with high potential for biomedical applications (e.g. in targeted transport, MRI, nanocatalytic chemistry)

Amyloids aggregates

Protofilaments – amyloid primary fibrils

Specific self organization (aggregation) of soluble peptides or proteins into insoluble amyloids fibrils



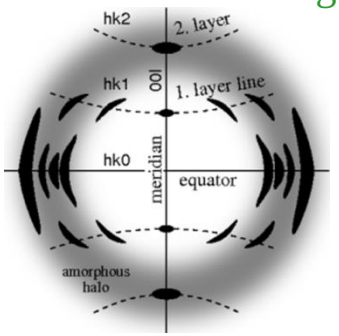
Proteins monomers (soluble form)

Specific protein aggregation

Helical-type/cylindrical structure. Width *d*~10 nm, length *L*~300 nm (up to micron)

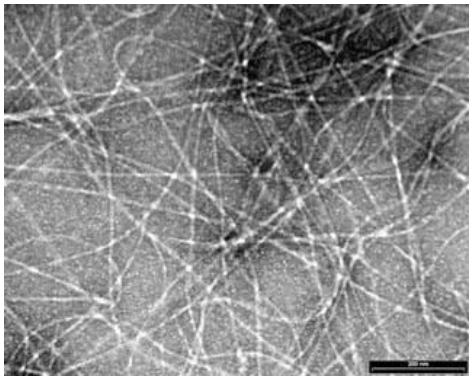
Structures of amyloids fibrils deposited at substrates

high-resolution X-ray diffraction

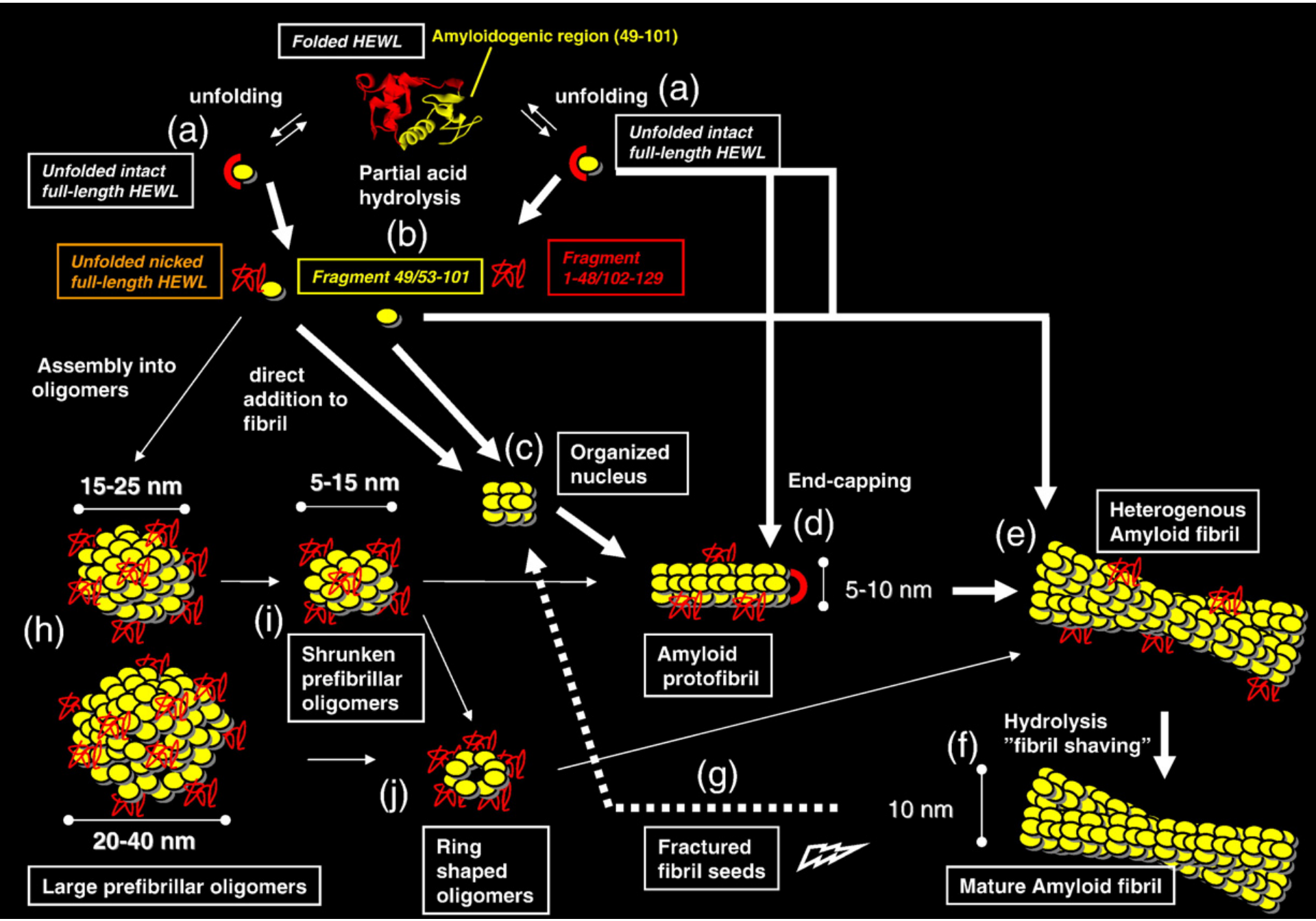


11.5 nm

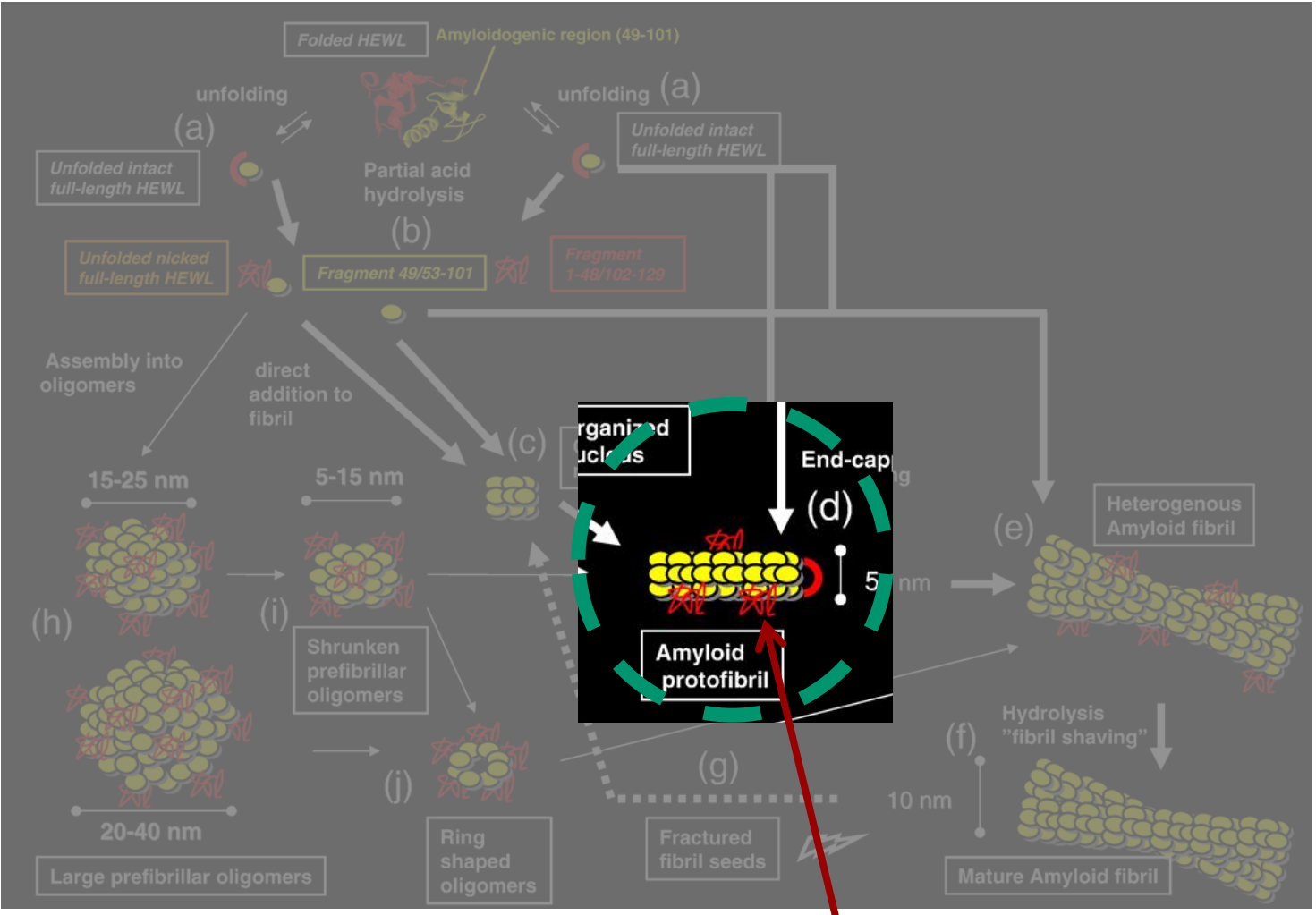
Microscopy



Such structure is a common structure of amyloids formed by the proteins of quite different nature



R.Mishra et al. *J. Mol. Biol.* 366 (2007) 1029–1044



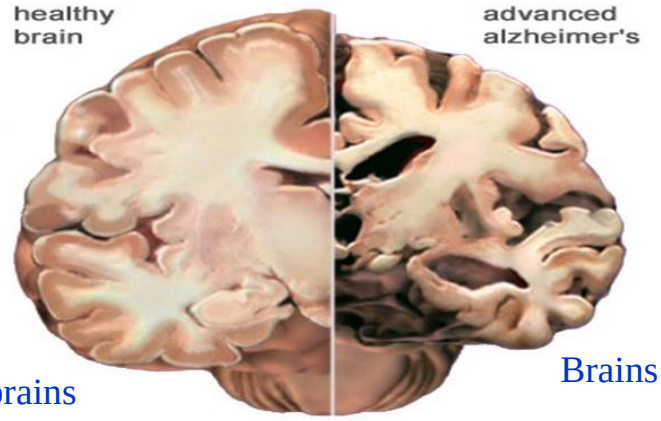
Preparation of HEWL amyloids protofilaments:

HEWL amyloid aggregates were prepared by dissolving the protein in water at the acidic environment. The solution was incubated at 65 °C and stirred for 8 h in thermomixer.

Amyloids and Amyloidosis

In medicine **Amyloidosis** refers to a variety of conditions wherein normally soluble proteins become insoluble and deposit in various organs.
Symptoms vary widely depending upon where in the body amyloid deposits accumulate and which kind of protein is participated in aggregation.

Amyloids are associated with the pathology of more than 20 serious human diseases in that, abnormal accumulation of amyloid fibrils in organs may lead to amyloidosis, and may play a role in various neurodegenerative disorders



Normal (healthy) brains

Brains at pathology

- Alzheimer's disease
- Parkinson's disease
- Huntington's disease
- Diabetes type 2
- Atherosclerosis
- Rheumatoid arthritis

…..

…..

Why should we study neurodegenerative diseases?

- **Older citizens** have very **high probability** to get neurodegenerative diseases.
- For example, in USA in 2000, 1.6% of the total population have AD,
- 1.6% the age group of 65-74 have AD,
- 19% of the age group of 75-84 have AD,
- 42% of the age group higher than 84 have AD.

Impact of nanoparticles on amyloids

protein amyloid aggregation + nanoparticles

– found impact of the protein amyloid aggregation is very controversially!!!

- **promote and enhance** the rate of amyloid fibril formation

copolymer, cerium oxide, TiO_2 nanoparticles, carbon nanotubes, quantum dots
(Linse S et al., Proc. Natl. Acad. Sci. 2007, Wu et al. Biochem. Biophys. Res. Commun, 2008)

- **no potential to induce** protein amyloid aggregation

negatively charged silica nanoparticles
(Wu et al. *Biochem. Biophys. Res. Commun, 2008)*

- the **significant inhibition** of amyloid polymerization

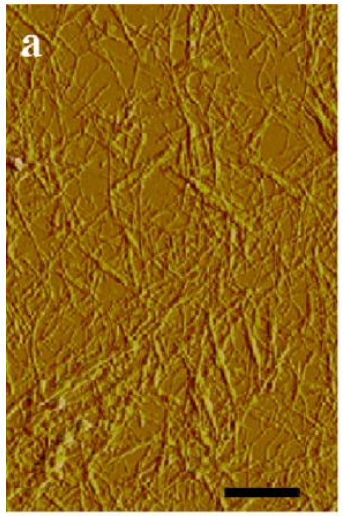
fluorinated and hydrophobic teflon nanoparticles
(Rocha et al., *Biophys. Chem, 2008)*

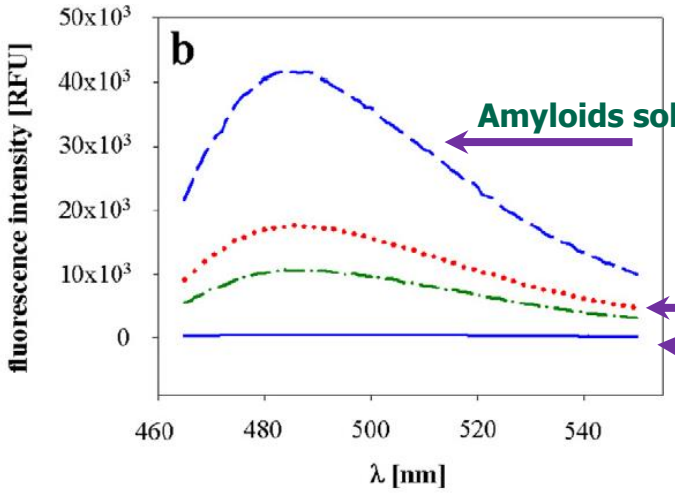
- **Destruction/depolymerization due to magnetic nanoparticles**, extraction of fibril/nanoparticle assemblies from the aqueous phase via a magnetic field

(Skaat H, et al. J. Biomed.Mater. Res. 2008, Bellova A., et al. Nanotechnology 2010 , Siposova K. , et al. Nanotechnology 2012)

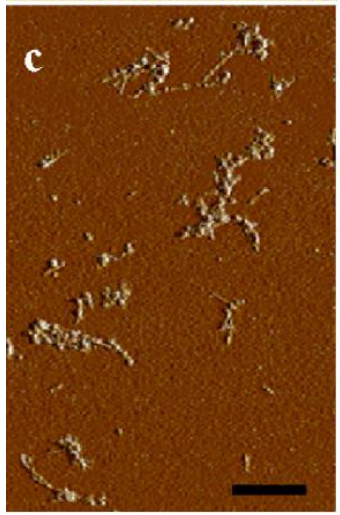
Destruction of amyloids by magnetic nanoparticles

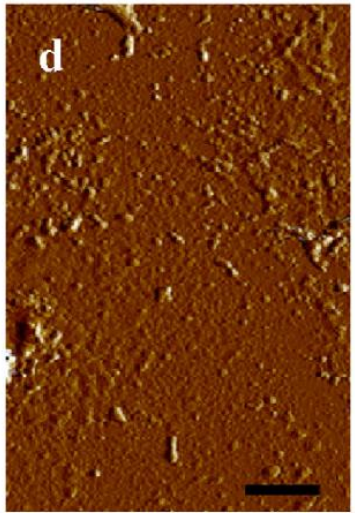
Amyloids solution
(a)





Ferrofluids
+
Amyloids solutions
(c,d)





Magnetic Fluid
(e)

1:1 2:1

Addition of magnetic nanoparticles affects protein amyloids aggregations

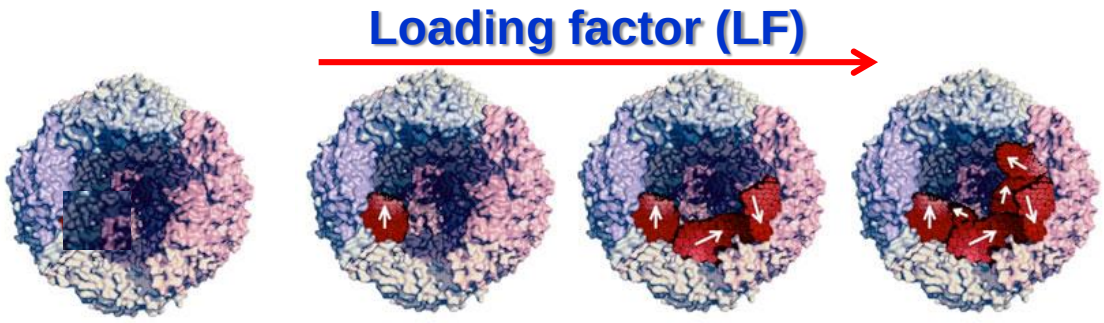
K. Siposova et al. *Nanotechnology* 23 (2012) 055101
A. Bellova et al. *Nanotechnology* 21 (2010) 065103

Aim of work

Structure characterization of complex solutions of magnetic nanoparticles and bio-macromolecules.

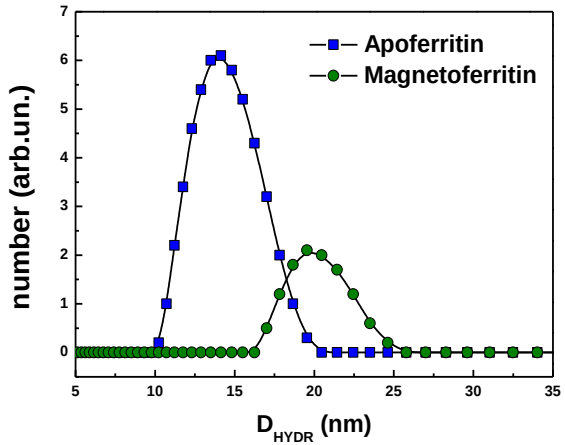
- **Impact of Loading Factor on the structure and stability of aqueous magnetoferritin solutions.**
- **Structure characterization of aqueous amyloid solutions of hen egg white lysozyme (HEWL).**
- **Interaction of magnetic nanoparticles with amyloids.**
- **Impact of nanoparticles on the amyloid structures.**

- Structure and stability of magnetoferritin solutions

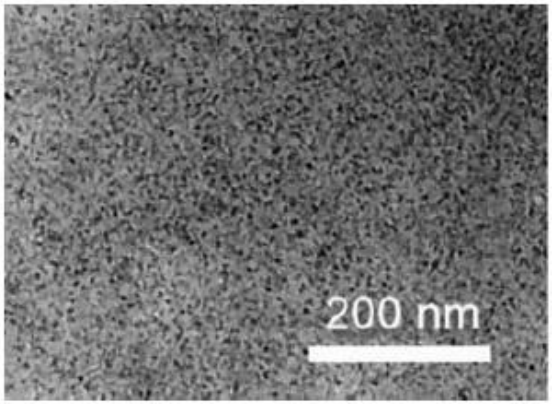


Structure characterization

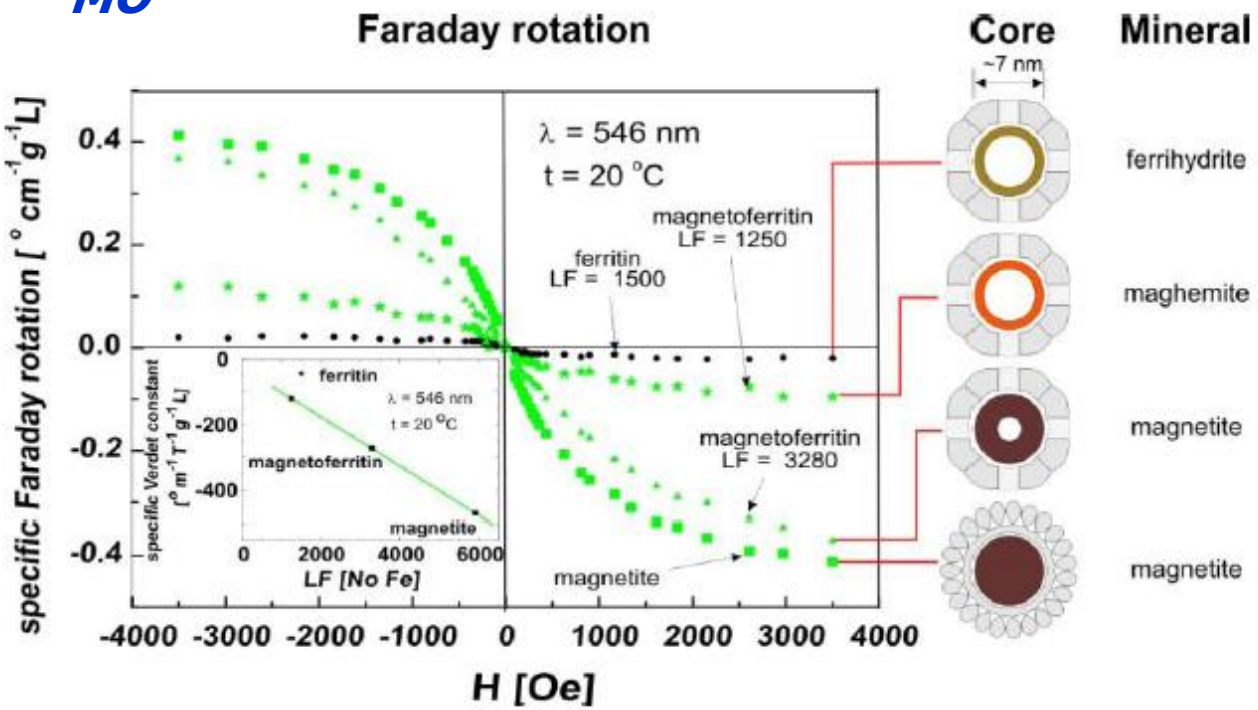
DLS



EM



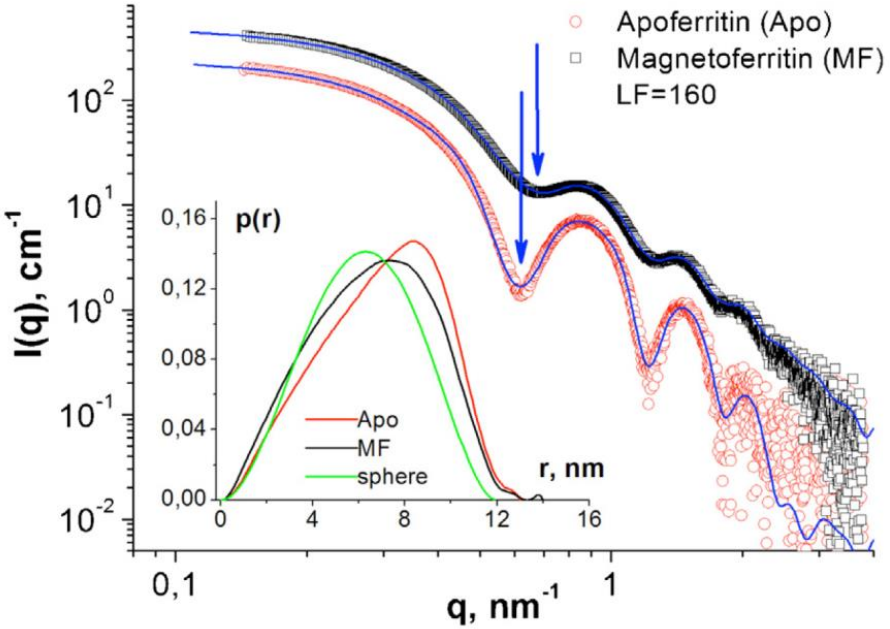
MO



M.Koralewski, M.Pochylski, Z.Mitroova, M.Timko, P.Kopcansky, L. Melnikowa, *JMMM* (2012).

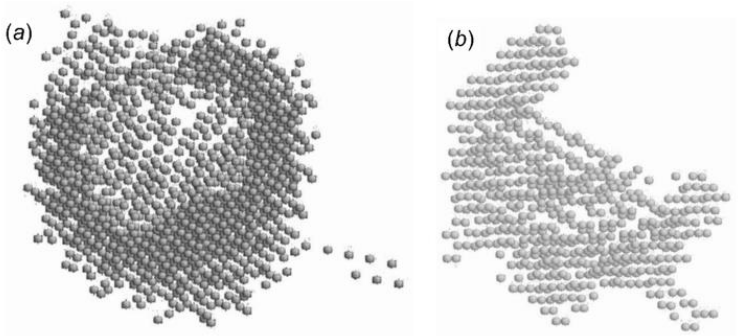
Instability of protein shell

(SAXS data)



Magnetoferritin with quite low loading factor (LF)

Apoferritin **Magnetoferritin**

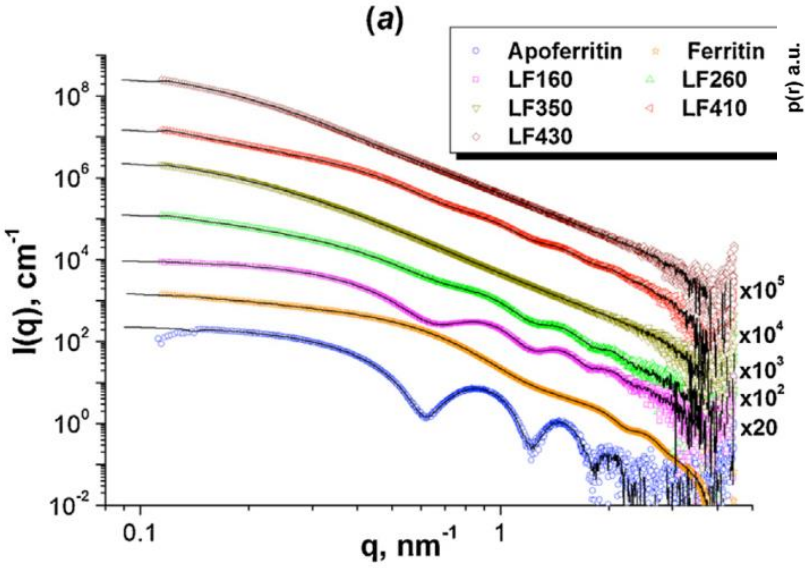


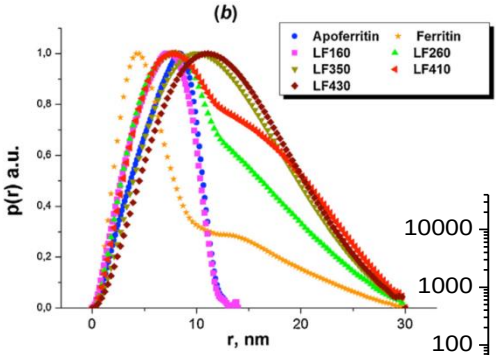
***Ab initio* modeling**

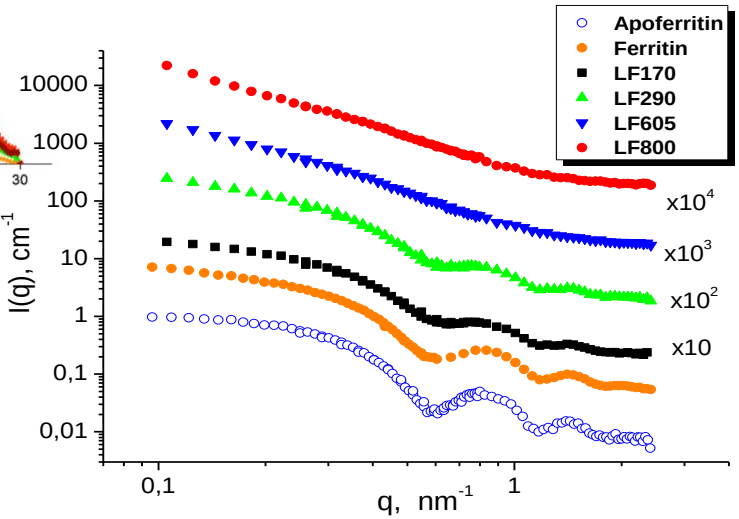
Partial destruction of MF shell due to presence of magnetic core

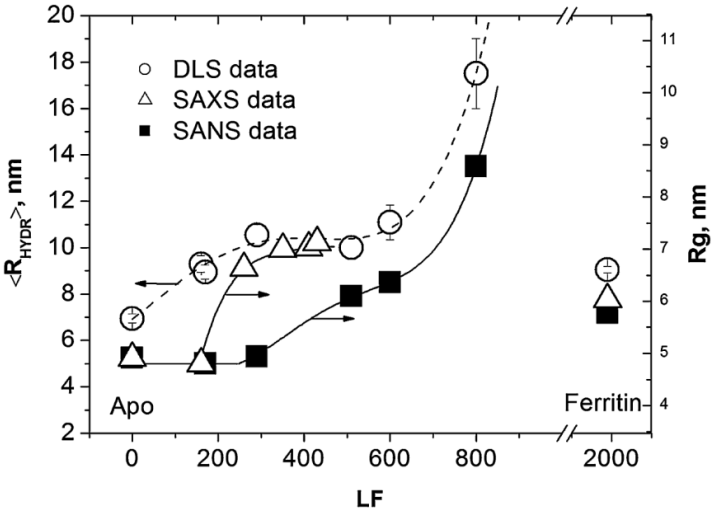
L.Melníková, V.I.Petrenko, M.V.Avdeev, et al. *Coll. Surf. B: Biointerfaces*. 123 (2014) 82-88

Aggregation of magnetoferritin complexes
(SAXS, SANS, DLS)







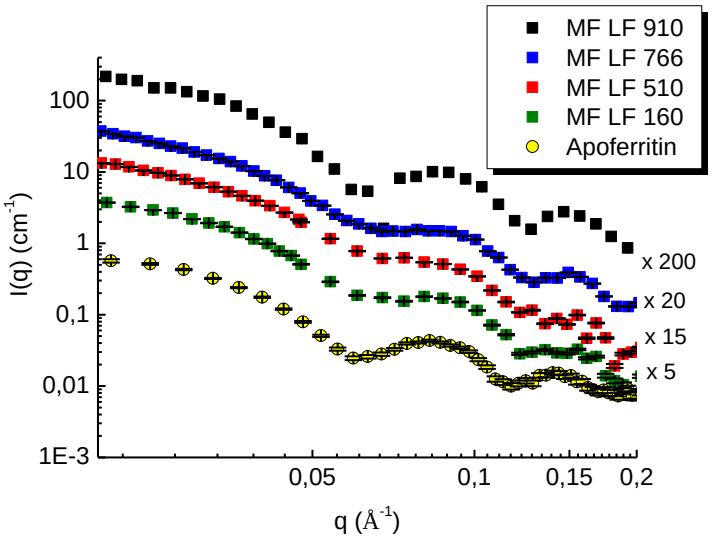


Increasing of aggregation with the loading factor growth:

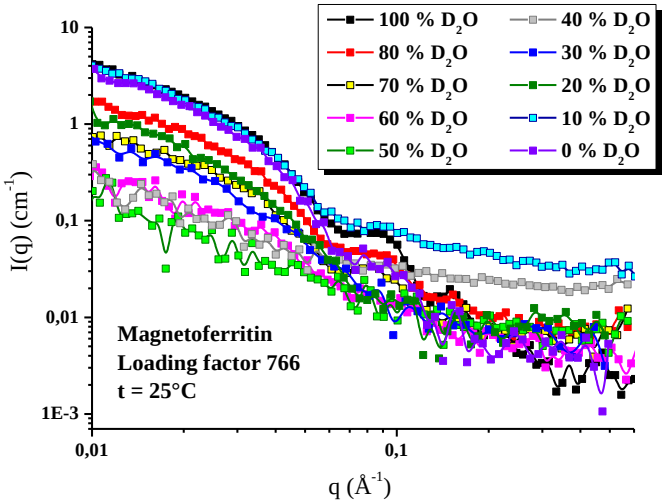
- ***Structural changes at the loading fators 160-510.***
- ***Intensive aggregation of MF with the loading factor above 600.***

Instability of protein shell

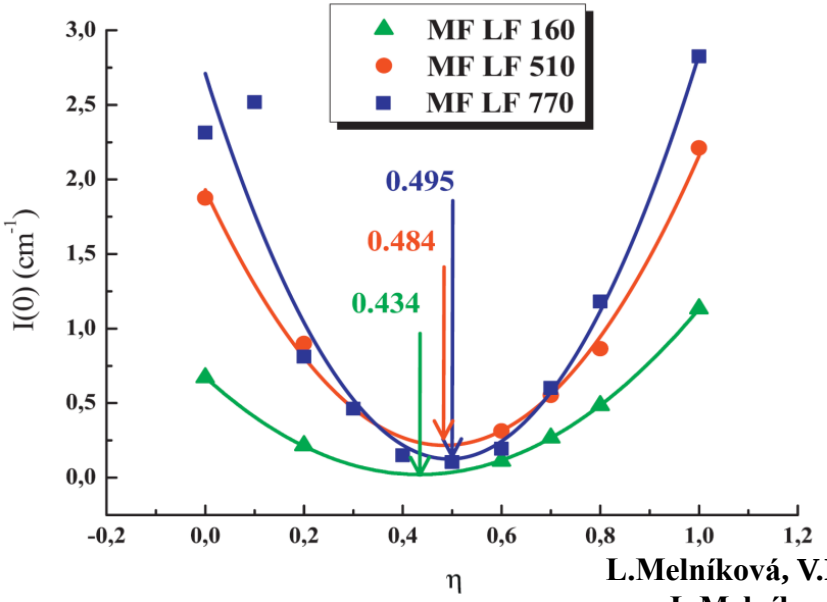
(SANS contrast variation)



Initial magnetoferritin solutions



SANS contrast variation at different amount of H2O/D2O in the solution



Precipitate at highest LF and SANS signal just from apoferritin shell in supernatant

Size and structural polydispersity with the loading factor growth

Partial destruction of MF shell (increase ratio of magnetic component:protein)

L.Melníková, V.I.Petrenko, M.V.Avdeev, et al. *Coll. Surf. B: Biointerfaces*. 123 (2014) 82-88

L.Melníková, V.I.Petrenko, M.V.Avdeev, et al. *J. Magn. Magn. Mater.* 377 (2015) 77-80

Structure and stability of magnetoferritin solutions

BRIEF SUMMARY

- Impact of magnetic loading on magnetoferritin was concluded:

  - Partial destruction of protein shell was found as well as aggregation of complexes with loading factor increase.

  - Dark precipitates were observed at LF=910. Just pure apoferritin shell was detected in supernatant solutions.

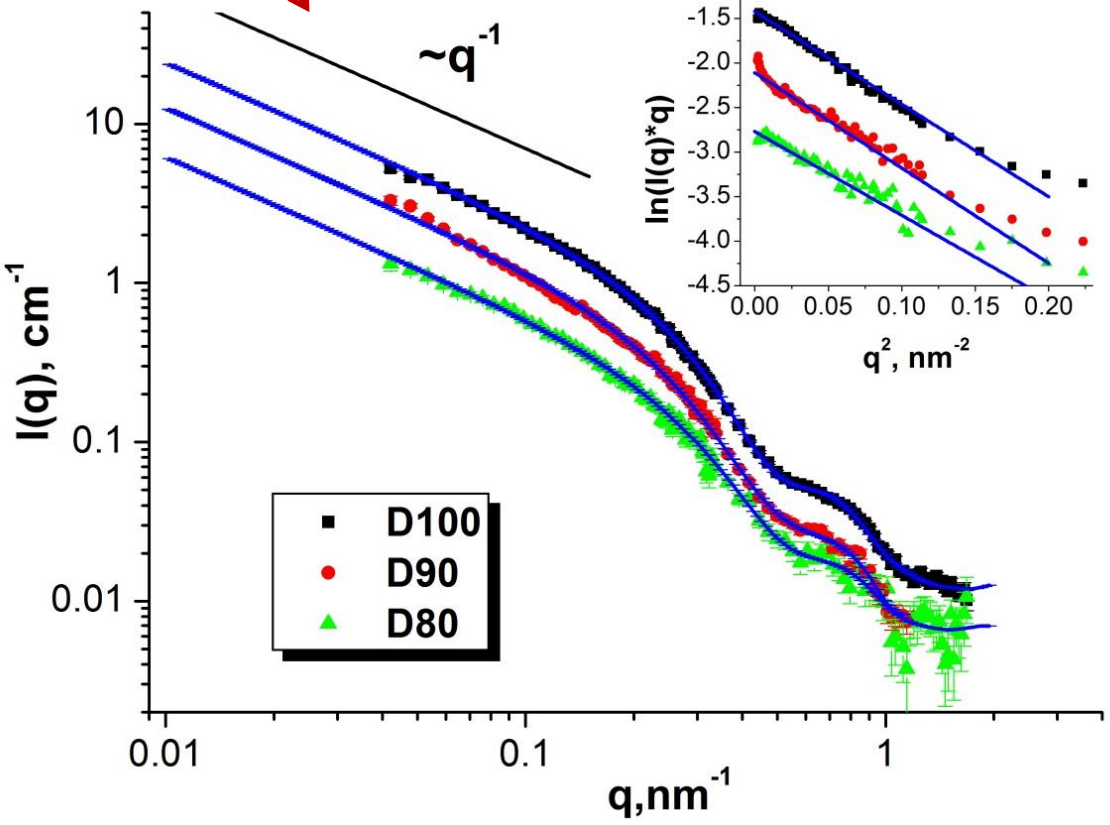
- Further improvement of the magnetoferritin synthesis procedure is necessary for practical application of magnetoferritin complexes: some kind of separation procedure is required with respect to 'good', 'destroyed' and 'empty' magnetoferritin .

- Structure characterization of amyloids fibrils

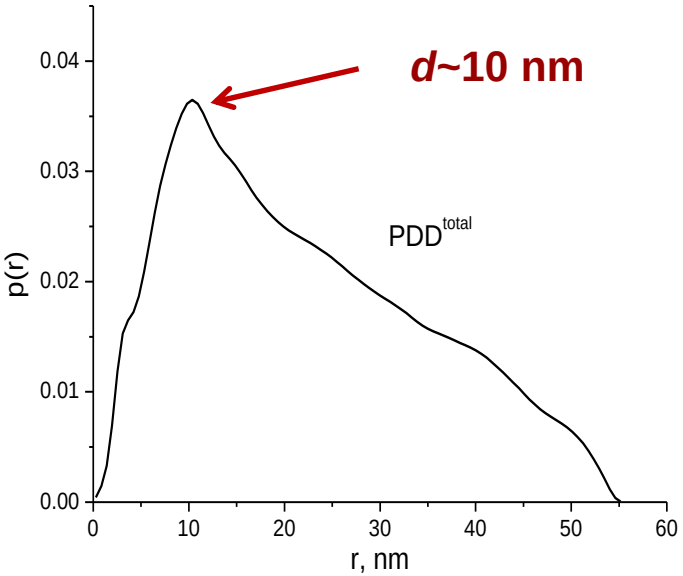
HEWL amyloids in heavy water

(SANS data)

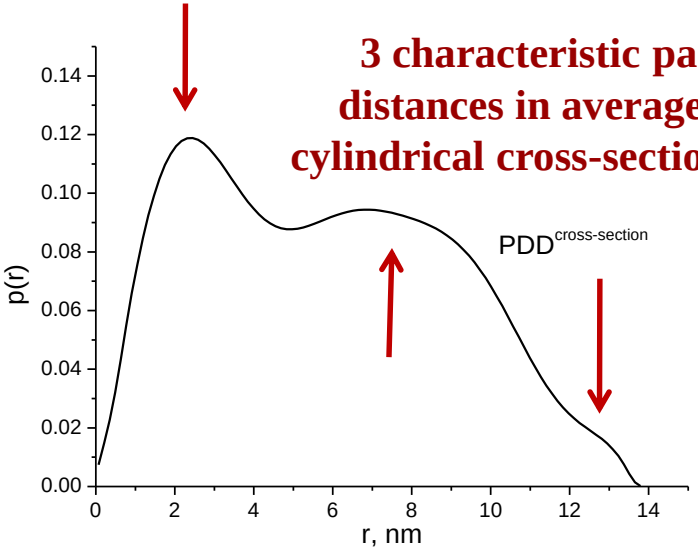
Power law – anisotropic elongated particles



Indirect Fourier Transformation (IFT)



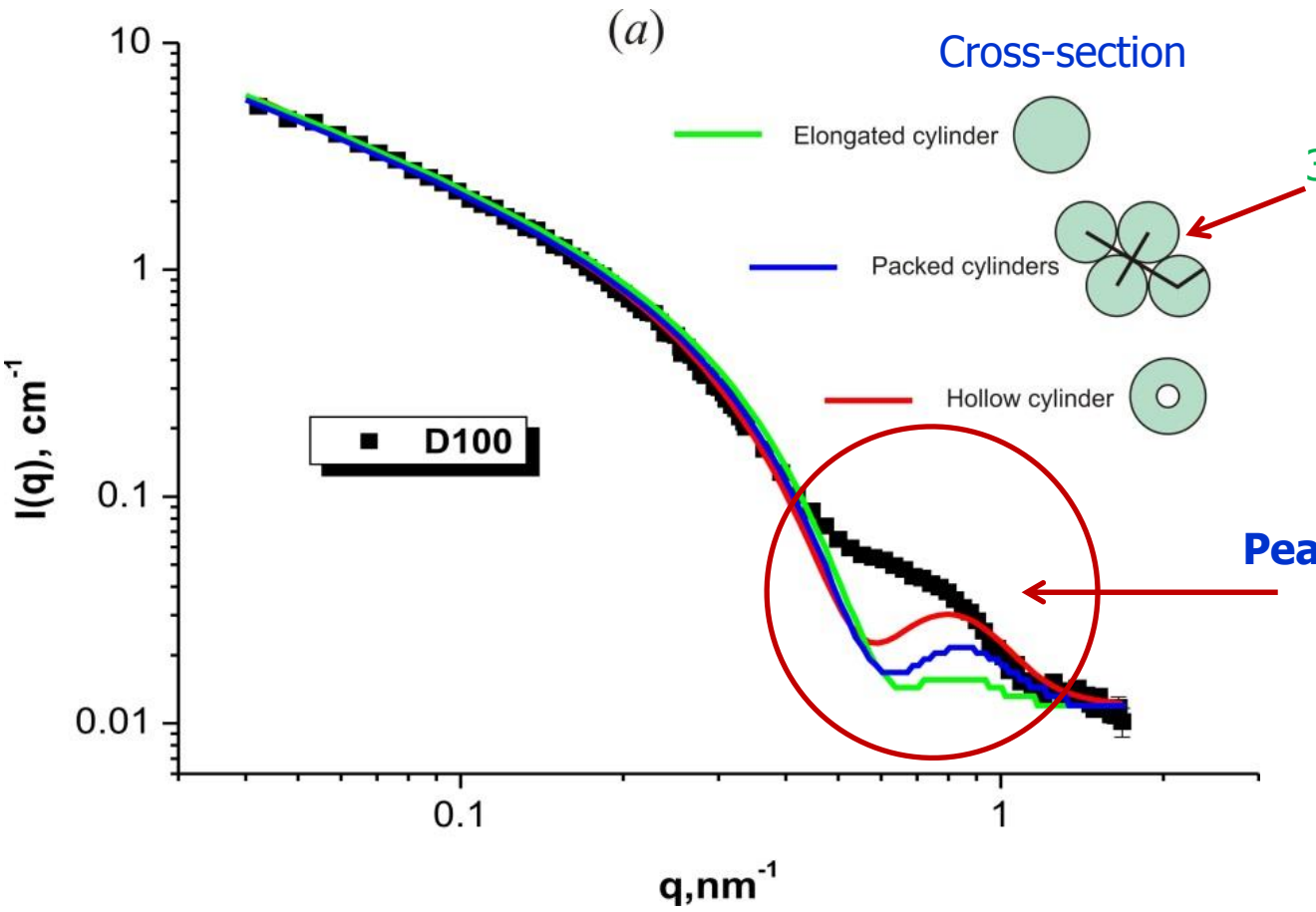
3 characteristic pair distances in averaged cylindrical cross-section



Pair Distance Distribution (PDD) functions

Cylindrical models of amyloids

Possible cylindrical models to describe the experimental SAS data from HEWL amyloids in water



Cross-section

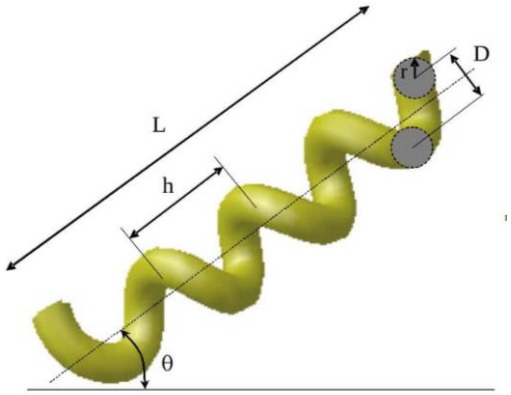
3 characteristic distances for model of 4-th cylinders

Peak is due to periodic helix-like structure???

Only initial part of the SAS curves can be satisfactory fitted by various cylindrical models

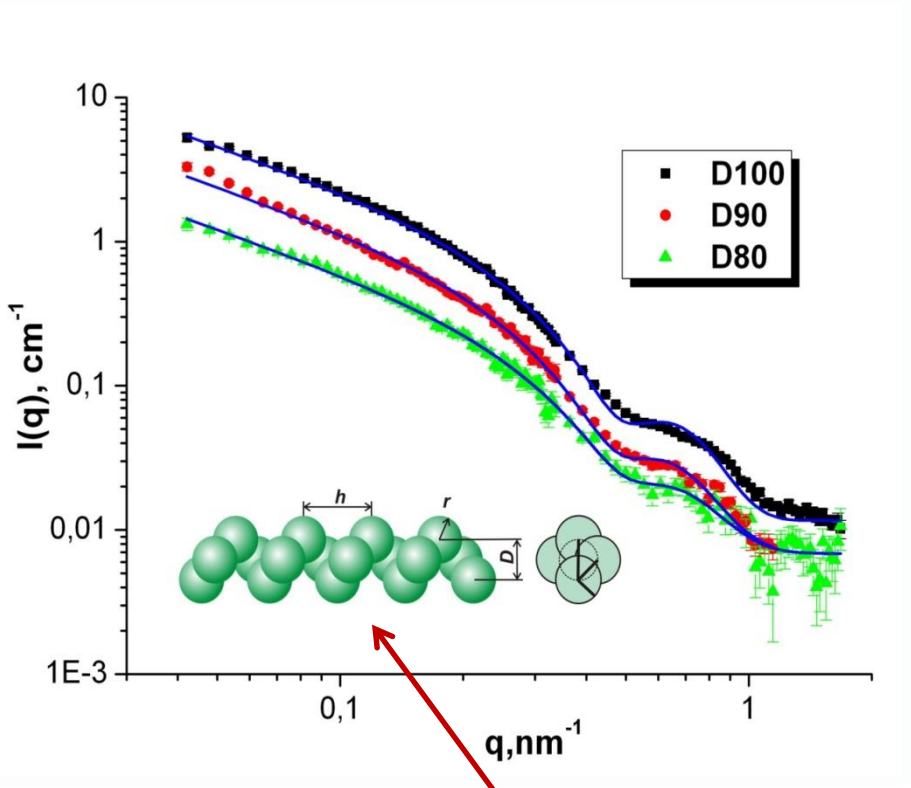
V.I.Petrenko, M.V.Avdeev, V.M.Garamus, et al. *Phys. Solid State* 56(1) (2014) 129-133

HEWL amyloids structure
(SANS data)



D – mean diameter of the helix
r – radius of the structural unit (sphere)
h – pitch (period of the structure along the helix axis)

D.V.Lebedev et al. *FEBS Lett*. 537 (2003) 182-186



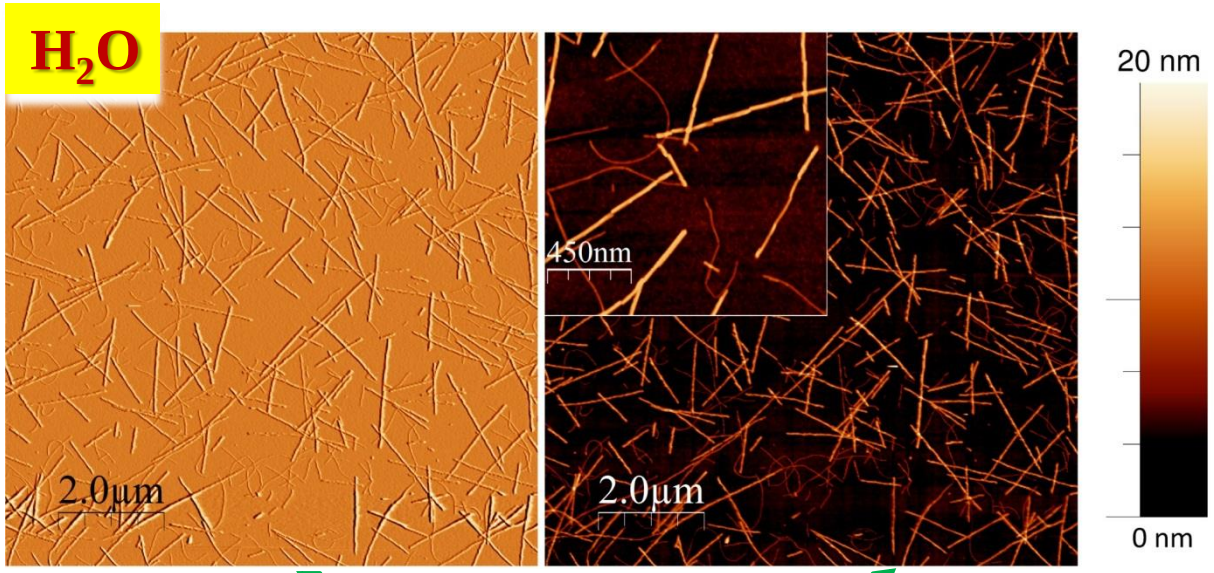
Obtained view of the helix and 3 characteristic distances in cross-section

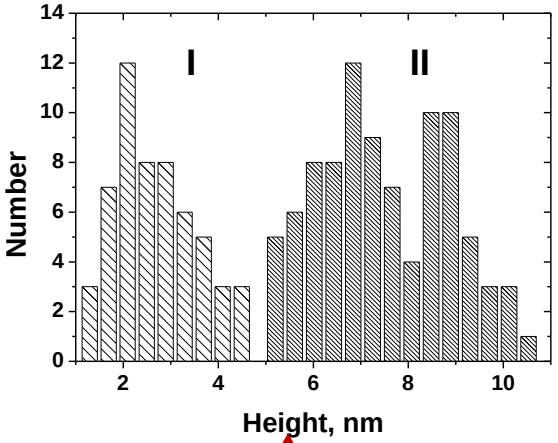
Samples	*r*, nm	*D*, nm	*h*, nm	R_{c1}, nm	R_{c2}, nm	R_{c3}, nm
100% D_2O	3.83(1)	7.63(2)	12.2(1)	4.83(2)	4.74(2)	4.56(8)
90% D_2O	3.94(4)	7.75(5)	12.5(1)	4.93(5)	5.0(1)	4.6(1)
80% D_2O	3.64(8)	7.06(1)	12.1(2)	4.52(4)	4.3(1)	4.3(1)

R_{c1}, R_{c2} and R_{c3} – cross-section radii of gyration from model, IFT and Guinier approxymation

M.V.Avdeev, V.L.Aksenov, Z.Gazova, et al. *J. Appl. Cryst.* 46 (2013) 224-233

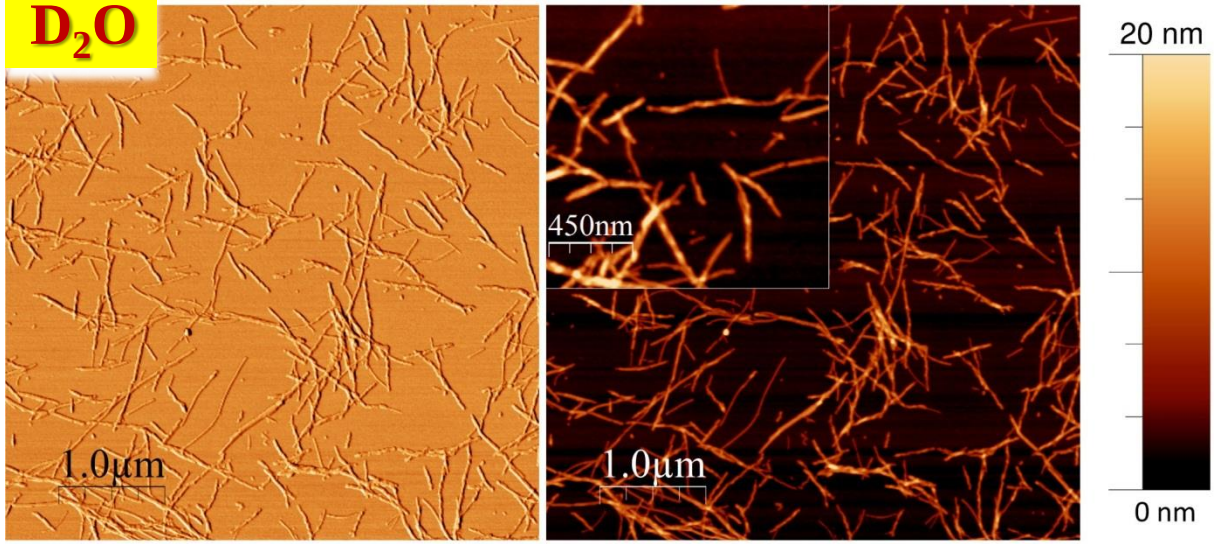
Atomic-force microscopy for HEWL amyloids solutions

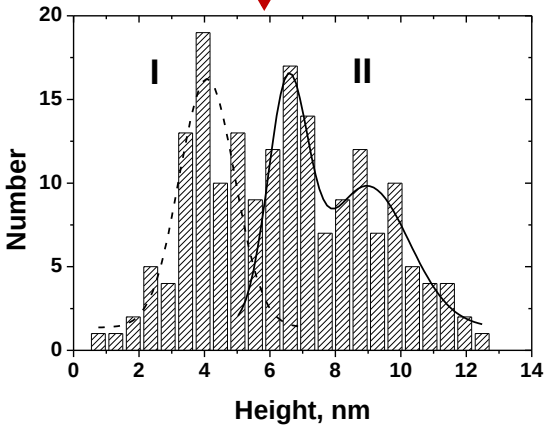




Phase and height AFM images

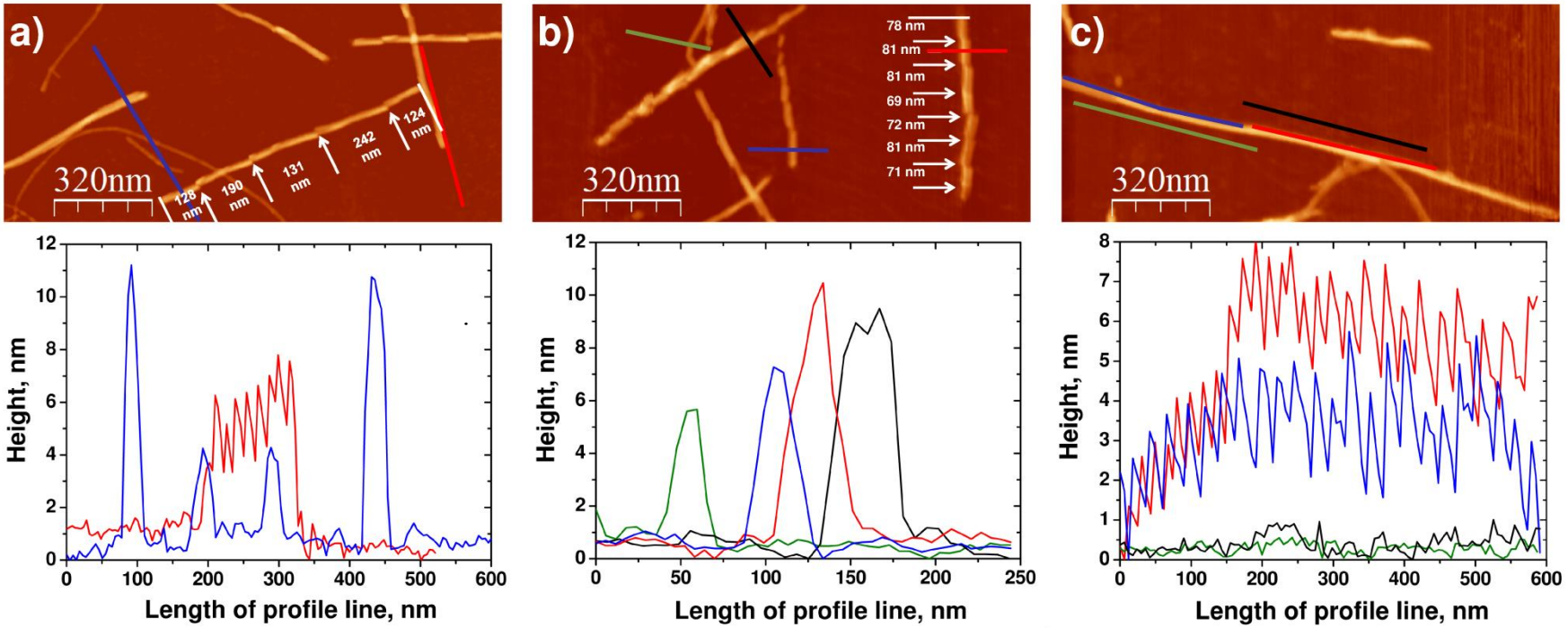
Height distributions from analysis of cross-section profiles





HEWL amyloids structure

(AFM data)



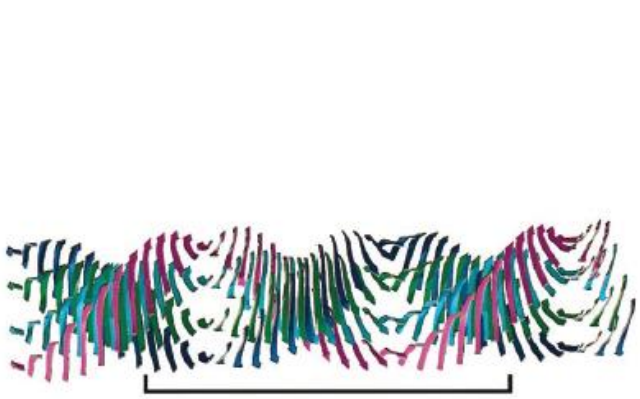
Sample	*Mean diameter*						*Pitch, nm*	
	population I			population II				
	D-interval, nm	<*D*>, nm	*RMSD*, nm	*D*-interval, nm	<*D*>, nm	*RMSD*, nm	pop. I	pop. II
H_2O	1.0 – 4.5	2.6	0.7	5.5 – 11.0	7.7	1.5	–	10.4±2.5
D_2O	1.0 – 7.0	4.1	1.1	5.0 – 12.5	8.0	1.7	–	17.8±5.4

Structure characterization of amyloids fibrils

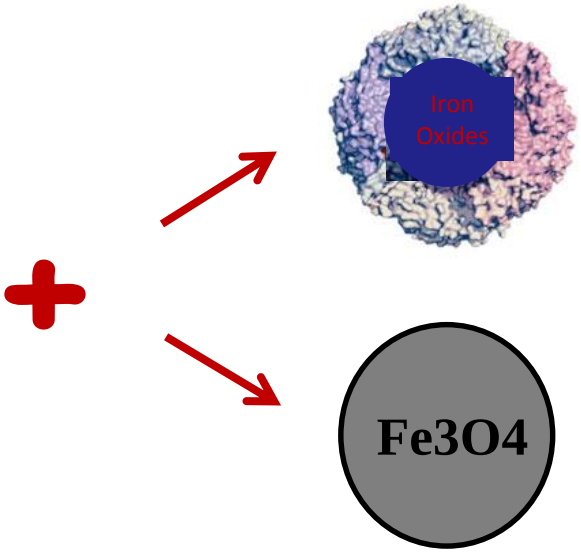
BRIEF SUMMARY

- The used helix model is quite good describe experimental SAS data for aqueous protofilaments lysozyme solutions. The main structural parameters of amyloids were obtained.
- SAS and AFM give reasonably consistent results for amyloids solutions.
- Determined helical structure of protofilaments is sensitive to the environmental conditions. The influence of the $H_2O\backslash D_2O$ content in the solution on the amyloids structure was confirmed by both SAS and AFM methods.

•Interaction of nanoparticles with amyloids fibrils

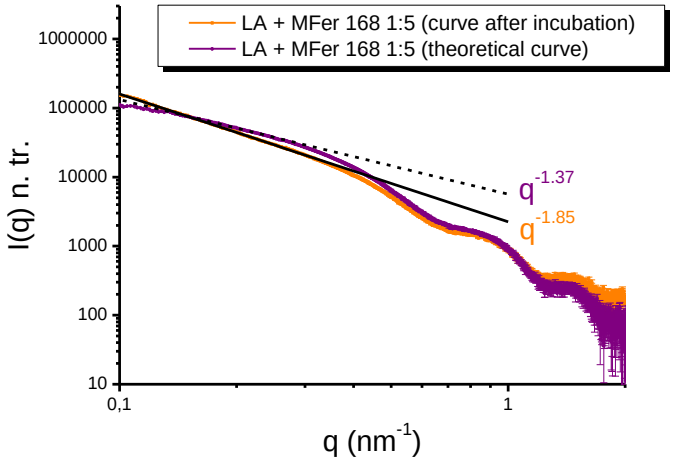


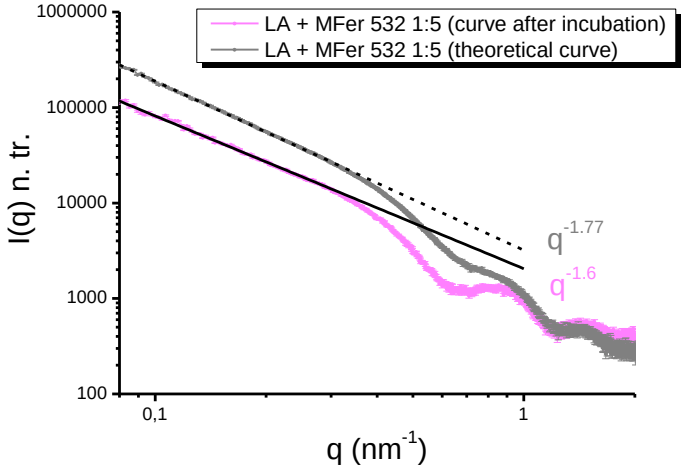
protein
amyloid aggregation

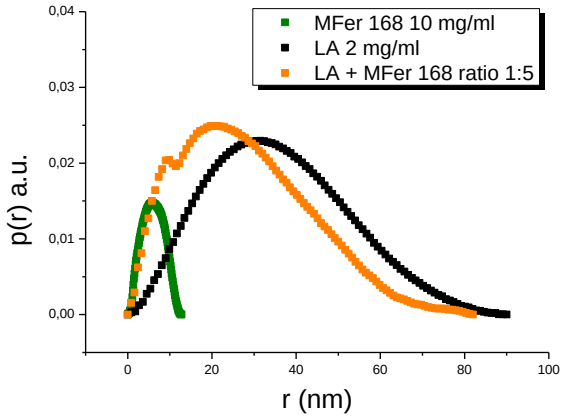


nanoparticles

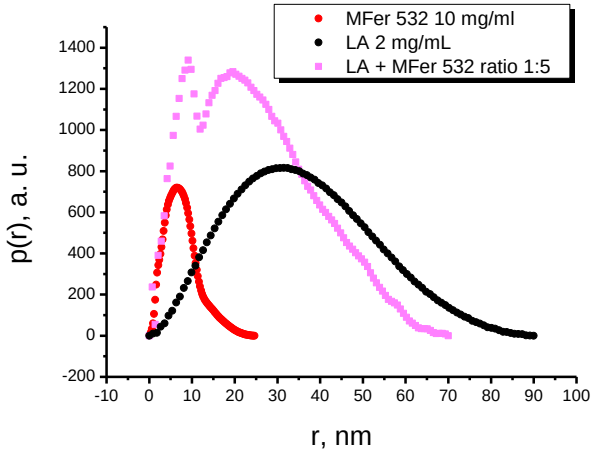
Destruction of amyloids due to magnetoferritin complexes







Pair Distance Distribution (PDD) functions



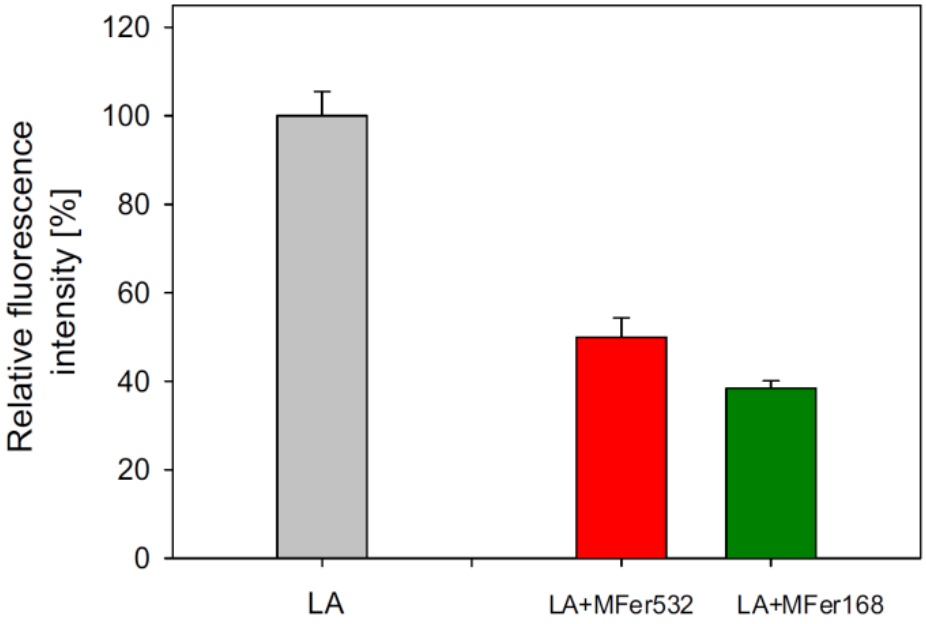
Destruction activity of magnetoferritin (MFer) with various loading factor on lysozyme amyloid fibrils (LA)

Decreasing of the size in mixtures amyloids+MFer in comparison with pure lysozyme amyloid fibrils

P.Kopcansky, K.Siposova, L.Melnikova, et al. *J. Magn. Magn. Mater.* 377 (2015) 267-271

Destruction of amyloids due to magnetoferritin complexes

Fluorescence spectrophotometry



ThT (tioflavin T) test for visualization of LA

Reduction of the amount of LA in mixtures with MFer – **destruction activity of MFer**

MFer with LF 168 and 532 affects the structure of LA

Magnetic core of MFer plays the key role (there is no any effects of pure apoferritin on amyloids)

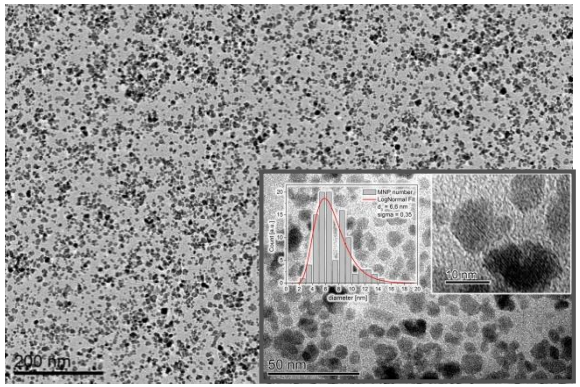
Mechanism is not clear!
The reason of LA destruction and interaction with MFer depend on...

- Concentration of magnetic nanoparticles, size, LF of MFer
- Surface: partial destructed MFer - uncovered redox surface of magnetic core
- Electrostatic interaction
- Distribution of surface charge on LA/ MFer
- Magnetism
- Other factors ….

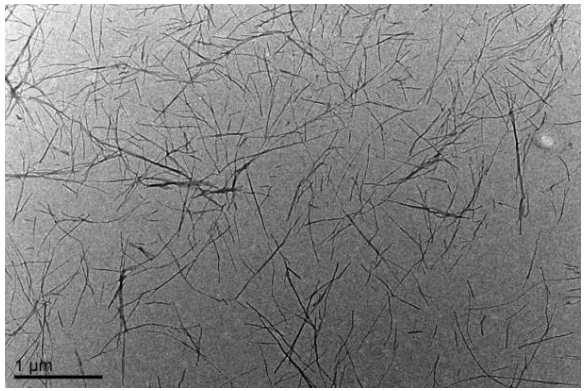
P.Kopcansky, K.Siposova, L.Melnikova, et al. *J. Magn. Magn. Mater.* 377 (2015) 267-271

Amyloids and magnetic nanoparticles

(Electron Microscopy)



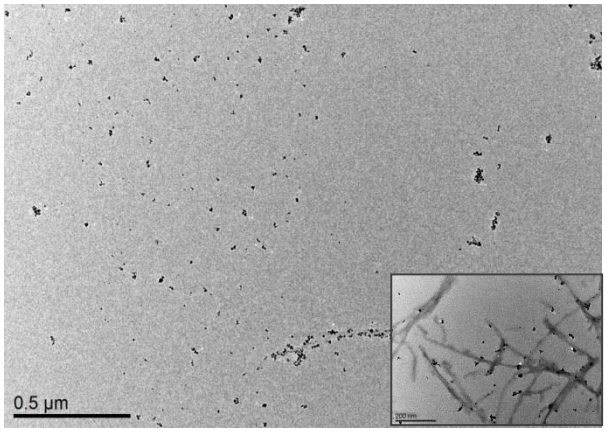
Magnetic Fluids



Amyloids

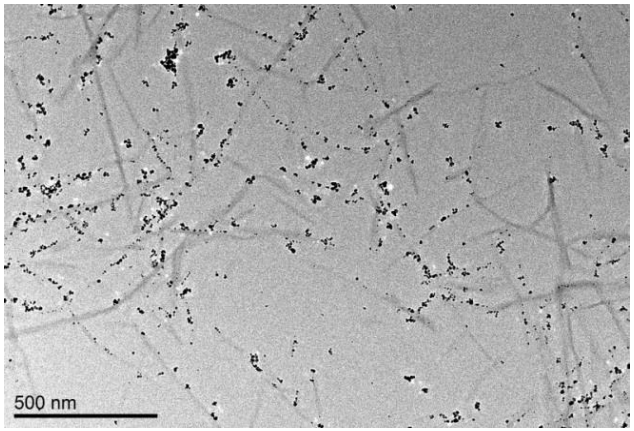
Mixtures of amyloids with various ammount of MNP`s

φ=1×10^{-4}



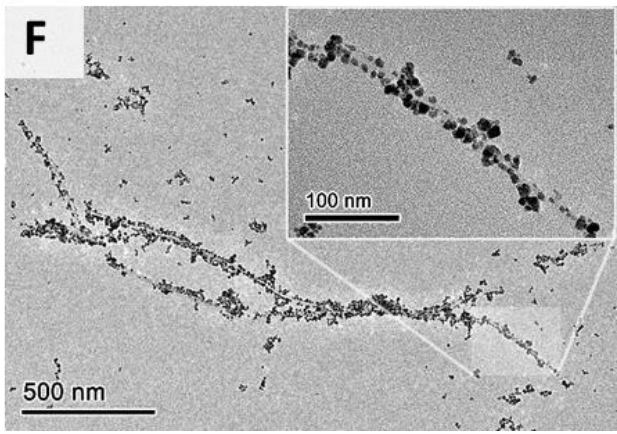
No big adsorption

φ=5×10^{-4}



Adsorption

φ=1×10^{-3}

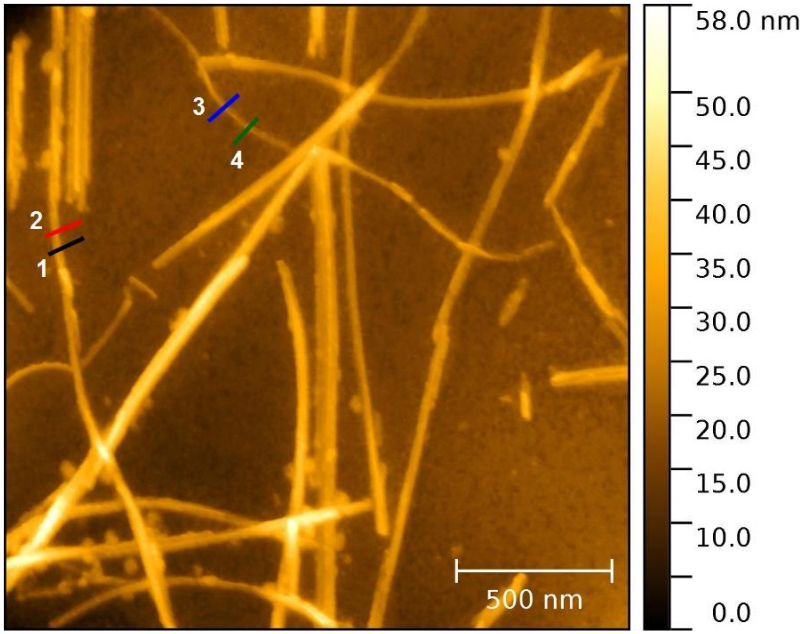


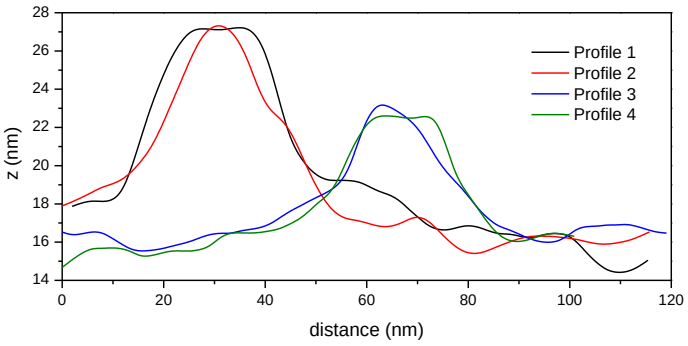
Considerable adsorption and penetration

Amyloids and magnetic nanoparticles

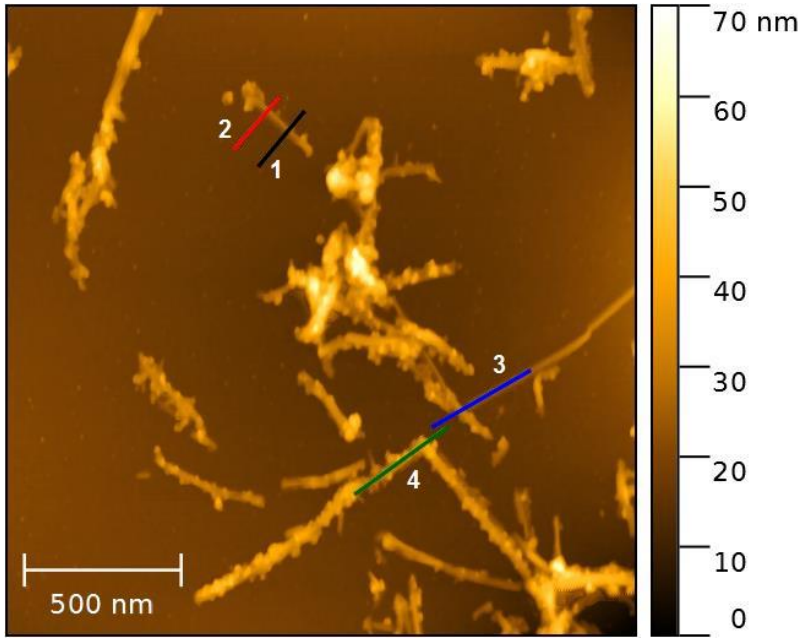
(Atomic Force Microscopy)

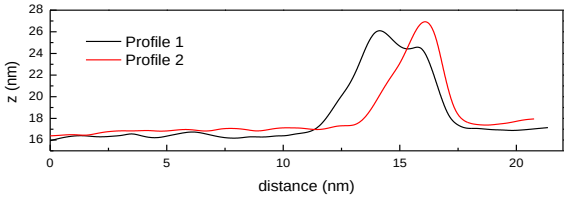
LAF

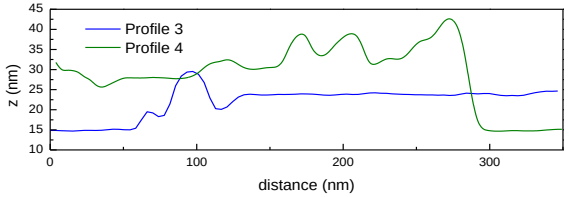




LAF/MF= 1/0.5





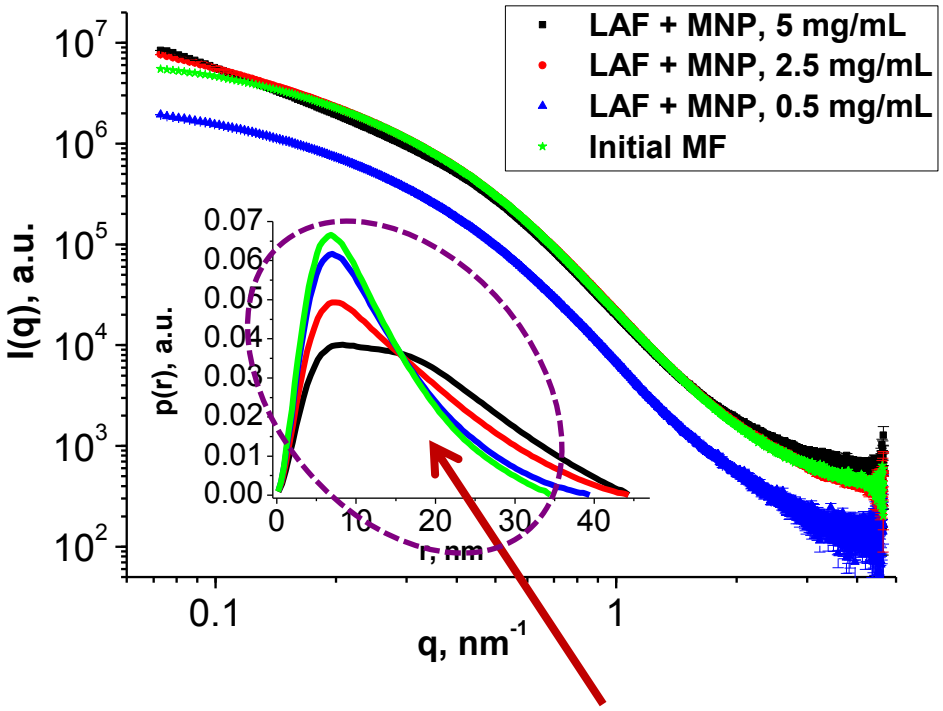


V.Gdovinova, N.Tomasovicova, I.Batko, et al. *JMMM* (2016) in press

Solutions of HEWL with magnetic nanoparticles

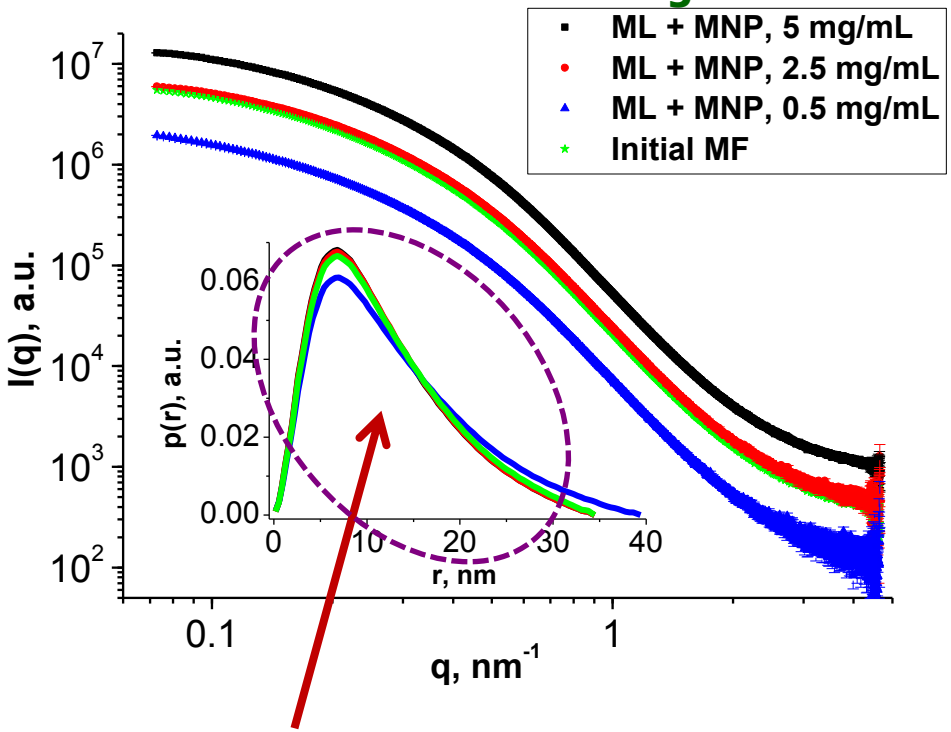
(SAXS)

HEWL Amyloids with Magnetic Fluids



MNP rod-like aggregation

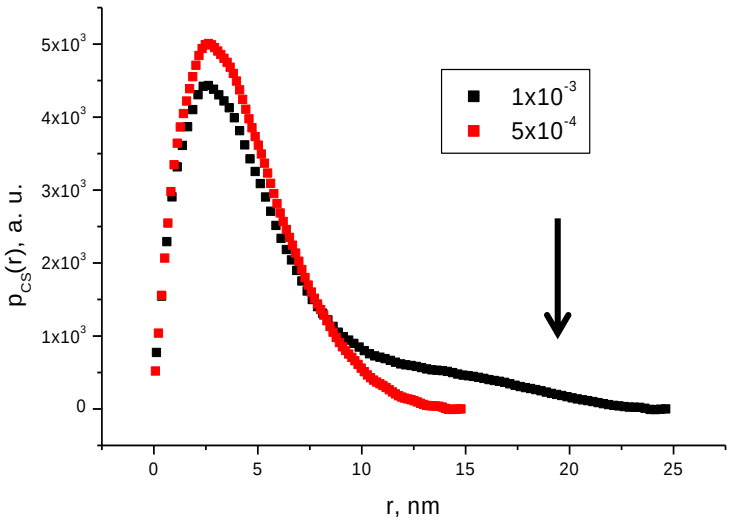
Monomer HEWL with magnetic fluids

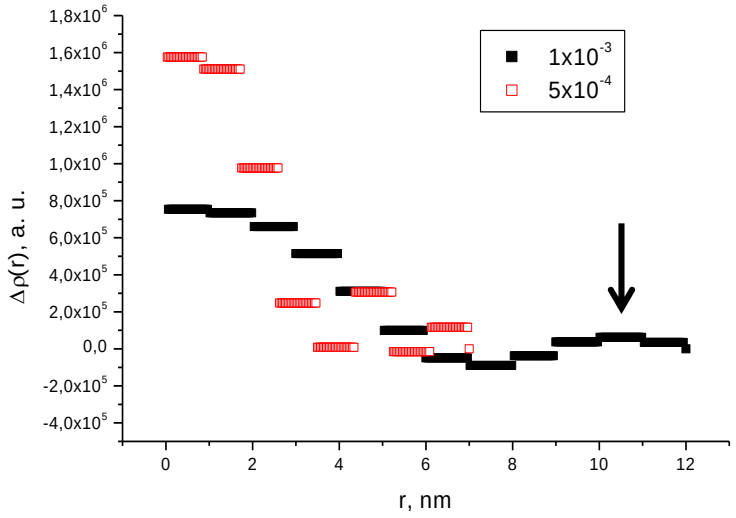


No any MNP aggregation

Single particle adsorption at lowest concentration of magnetic nanoparticles(10^{-4});
Formation of rod-like objects by adsorbed nanoparticles at middle concentration (5×10^{-4});
Some network of rod-like objects (10^{-3}).

Cross-section profile of adsorbed rod-like aggregate

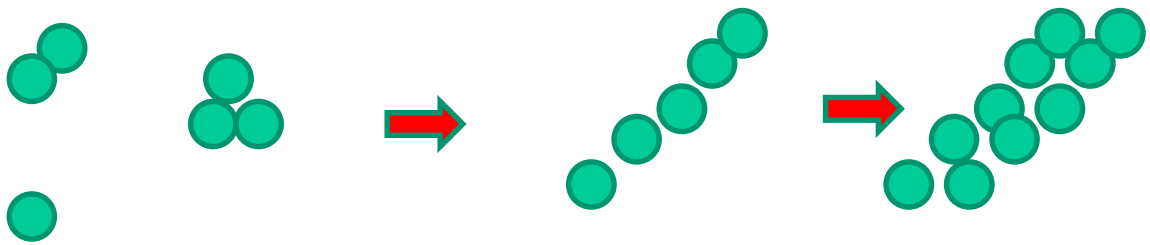




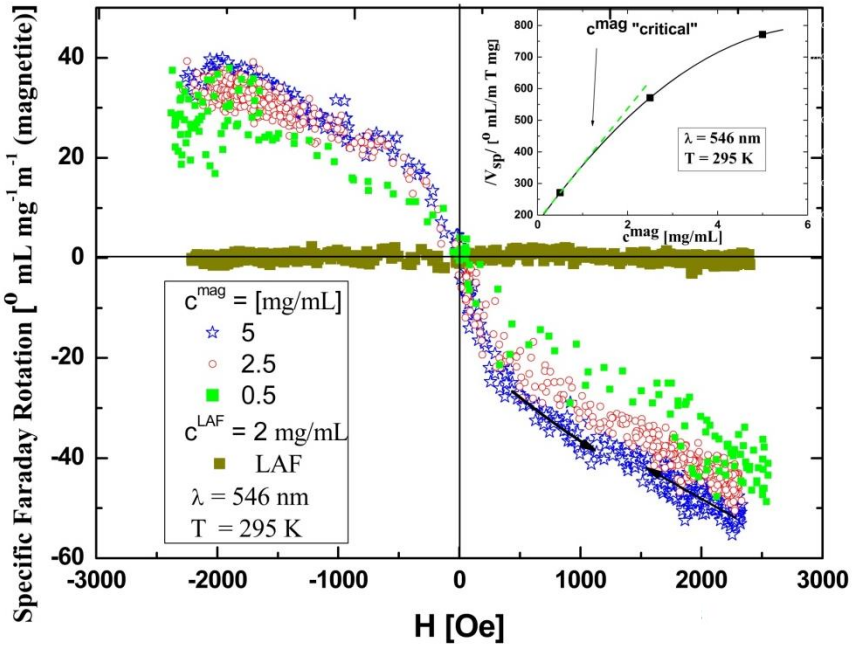
Cross-section pair distribution function

radial contrast of scattering length density profile

$$p_{CS}(r) = \frac{c}{2\pi M_L} \int r \Delta\rho(\mathbf{r}')\Delta\rho(\mathbf{r}+\mathbf{r}')d\mathbf{r}'$$



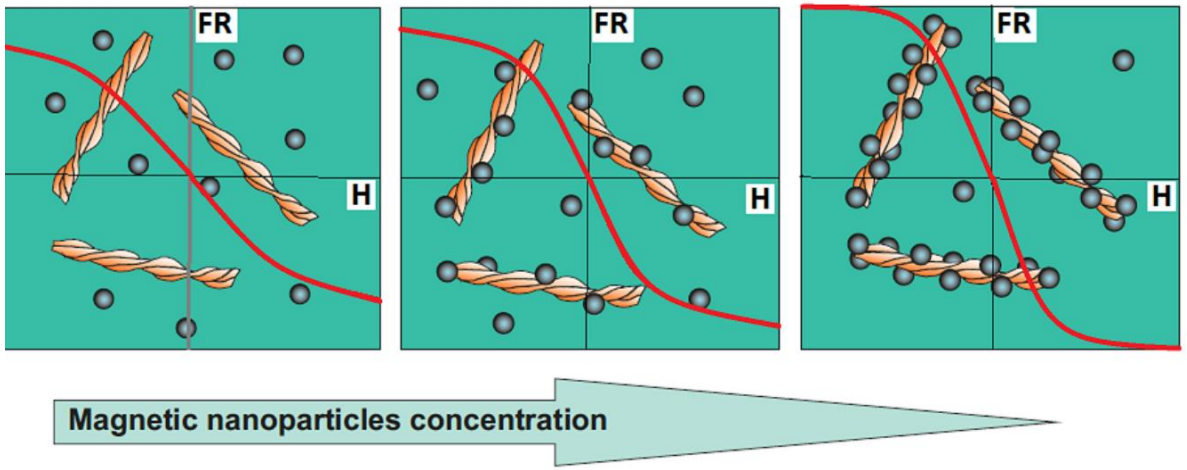
Solutions of Amyloids and Magnetic nanoparticles



Specific Faraday rotation angle for λ = 546 nm as a function of applied magnetic field for:
aqueous suspension of pure LAF and mixture of MF and LAF for different concentrations of magnetite

magneto-optical measurements

TEM, SAXS and Faraday rotation data



Adsorption of magnetic nanoparticles on amyloid fibrils follows the bulk particle concentration in mixed solutions and affects their magnetic properties

J.Majorosova, V.I.Petrenko, K.Siposova, et al. *Colloids Surf. B* 146 (2016) 794–800

Liquid Crystals (LC) doped with magnetic nanoparticles (Ferronematic)

Biological Liquid Crystals – under certain conditions they form liquid crystal phases

Corrigan et al. have shown that amyloid fibrils of hen lysozyme (LAF) can form liquid crystal phases

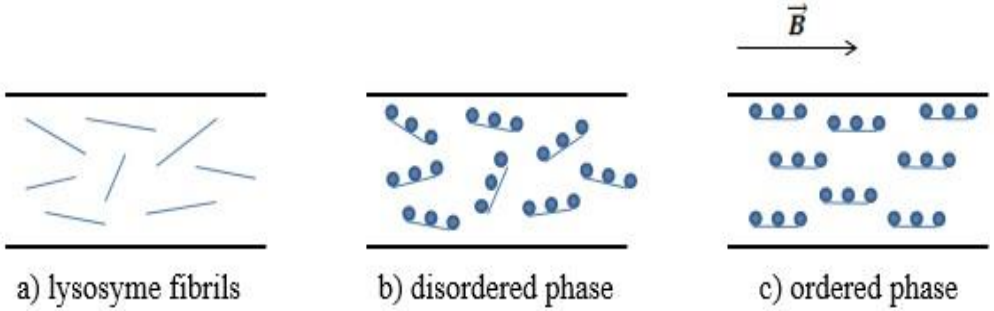
[Adam M. Corrigan, CH. Muller, M. R.H. Krebs, JACS Communications 10, 28, 2006].

J.J. Vallooran et al. demonstrated that usage of magnetic particles can facilitate liotropic LC alignment

[J.J. Vallooran, S. Bolisetty , and R. Mezzenga, Advanced Materials 23, 3932 (2011)].

M. Zaman et al. suggested that some minimal (critical) concentration (c_c) exists at which the amyloid fibrils order to pure biologic LC

[M. Zaman, et al., Nanoparticles in relation to peptide and protein aggregation, International Journal of Nanomedicine, 9 (2014), 899-912].



Effect of magnetic nanoparticles on the liquid crystalline ordering of amyloid fibrils which can be helpful in production of biological liquid crystals.

Interaction of nanoparticles with amyloids fibrils

BRIEF SUMMARY

- Destruction of amyloids due to magnetoferritin complexes was observed. Effect is increased with increase of magnetoferritin loading factor. It can be used for treatment of amyloidosis.

- Some "critical" concentration of magnetic nanoparticles (between $1 \cdot 10^{-4}$ and $5 \cdot 10^{-4}$) exists at which nanoparticles start to adsorb to the amyloids surface and form cylinder-like objects themselves. This observation opens prospects for manipulation of the Liquid Crystal transition by external magnetic field (ferronematics).

Conclusions

- Small-angle neutron scattering is quite informative method in respect to structure characterization of various complex multicomponent liquid systems.
- To follow the corresponding changes in big ensembles in solutions the 'averaging' methods such as scattering (DLS, SAXS, SANS) should be complementary applied.
- Impact of magnetic nanoparticles to the structural stability of bio-macromolecules (disaggregation of amyloids, amyloid-based ferronematics) were successfully studied by SAS.

Acknowledgements: This work was performed within framework of RFBR grants and also JINR-Slovakia cooperation program.