

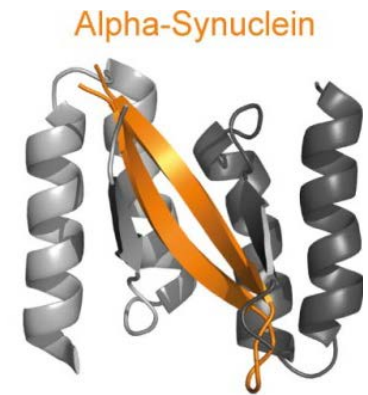
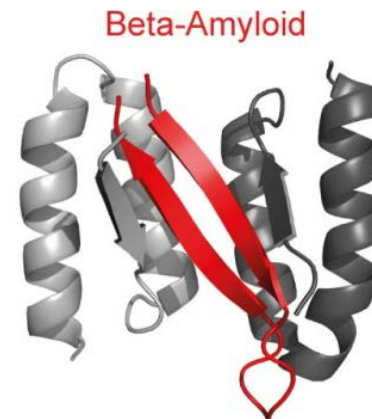
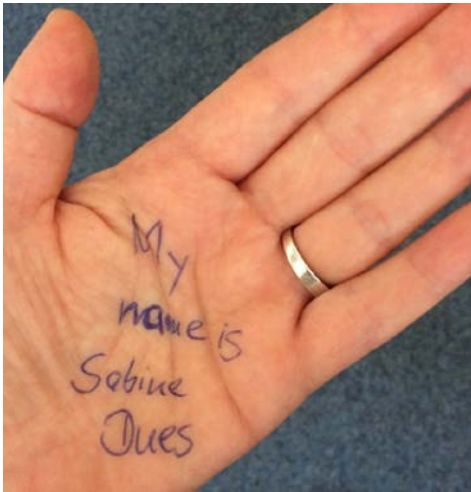
# INSTITUTE OF COMPLEX SYSTEMS STRUCTURAL BIOCHEMISTRY (ICS-6) PROF. DR. DIETER WILLBOLD

20. AUGUST 2018 | SABINE DUES

# STRUCTURAL BIOCHEMISTRY (ICS-6)

Overall Goal

Solutions to diseases

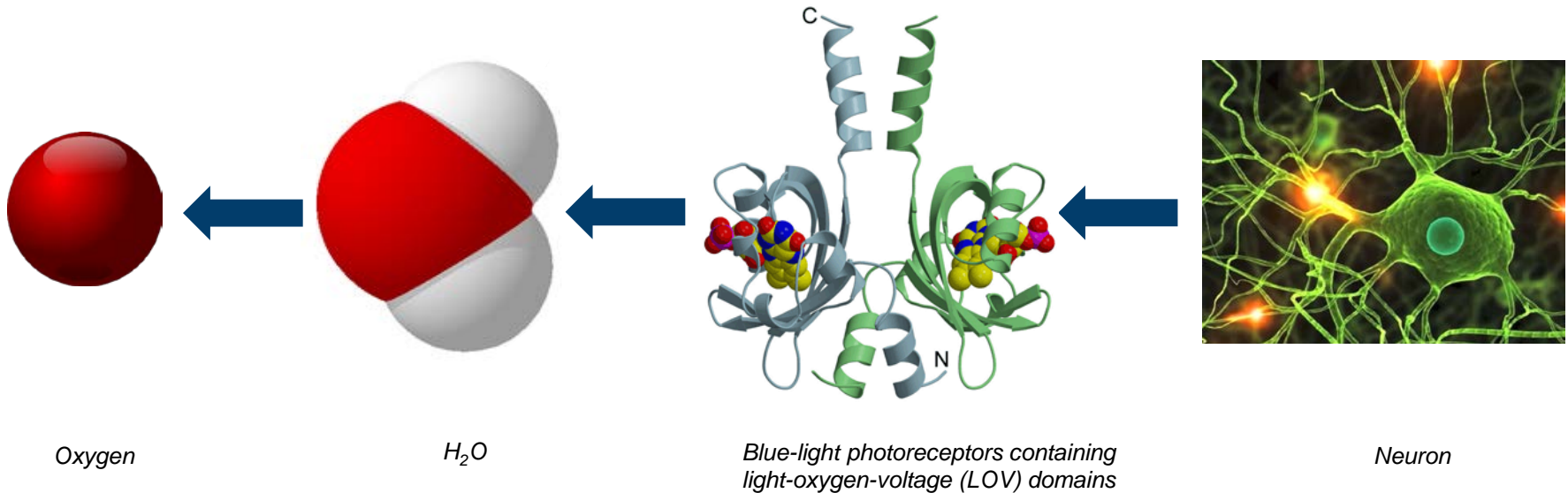


Protein structures and interactions

# INSTITUTE OF COMPLEX SYSTEMS

## Structural Biochemistry

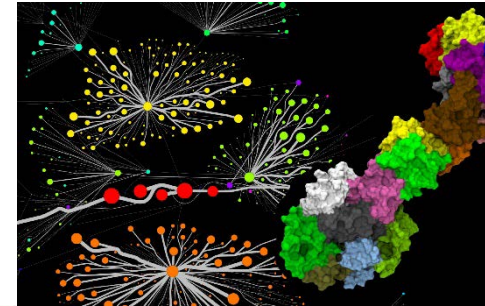
Atoms → Molecules → Complex Systems



To understand the structure of complex systems it is necessary to elucidate processes at atomic resolution level.

# OUTLINE

- Neurodegenerative Disease
  - Example: Alzheimer's Disease
- Methods in Structural Biochemistry



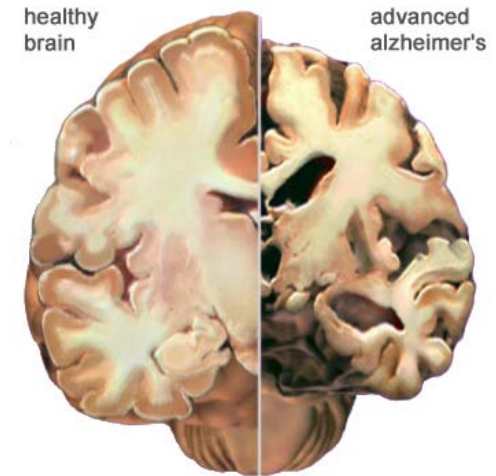
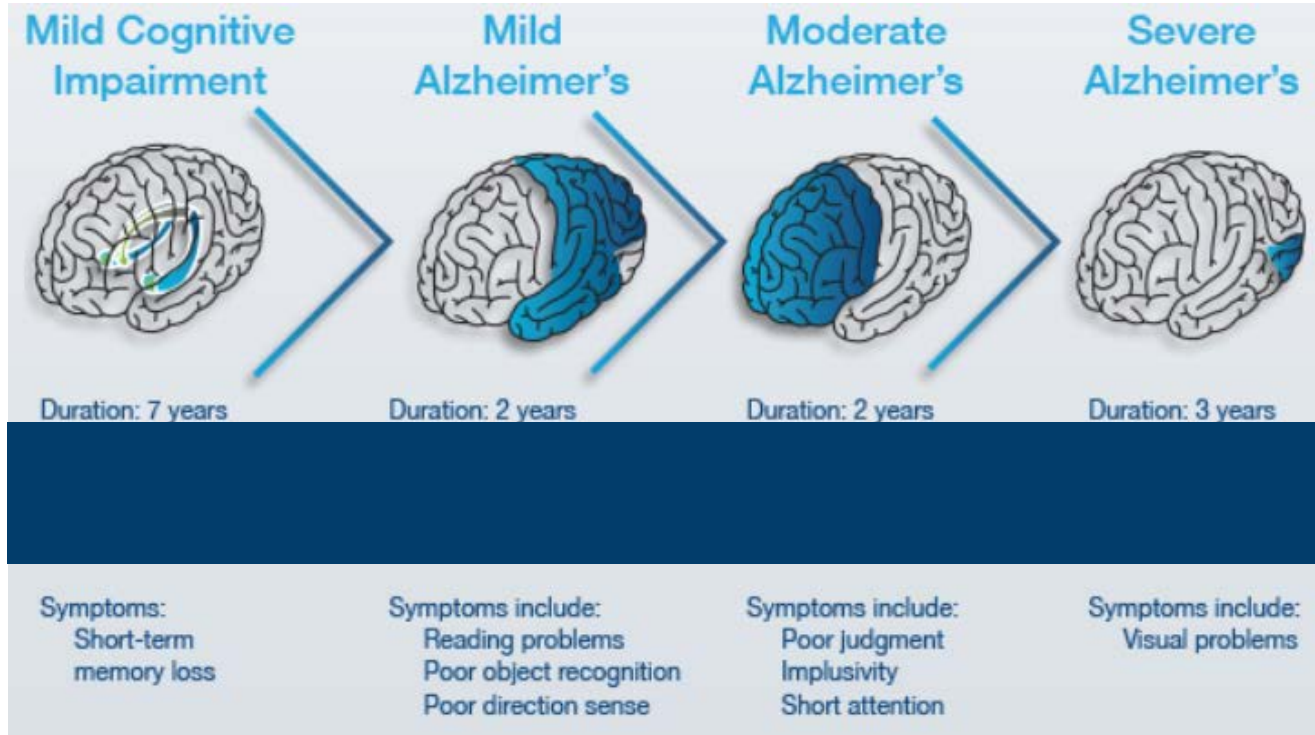
# ALZHEIMER'S DISEASE

Approx. 24 Mio people are affected worldwide number being doubled by 2030

- Neurodegenerative disorder
- Most common type of dementia
- Risk factor age
- First diagnosis based on symptoms
- No early, non-invasive and inexpensive diagnosis
- Cerebrospinal fluid (CSF) analysis or imaging techniques (MRI)
- No casual therapy, no drugs

# ALZHEIMER'S DISEASE

## How does Alzheimer's Disease progress?

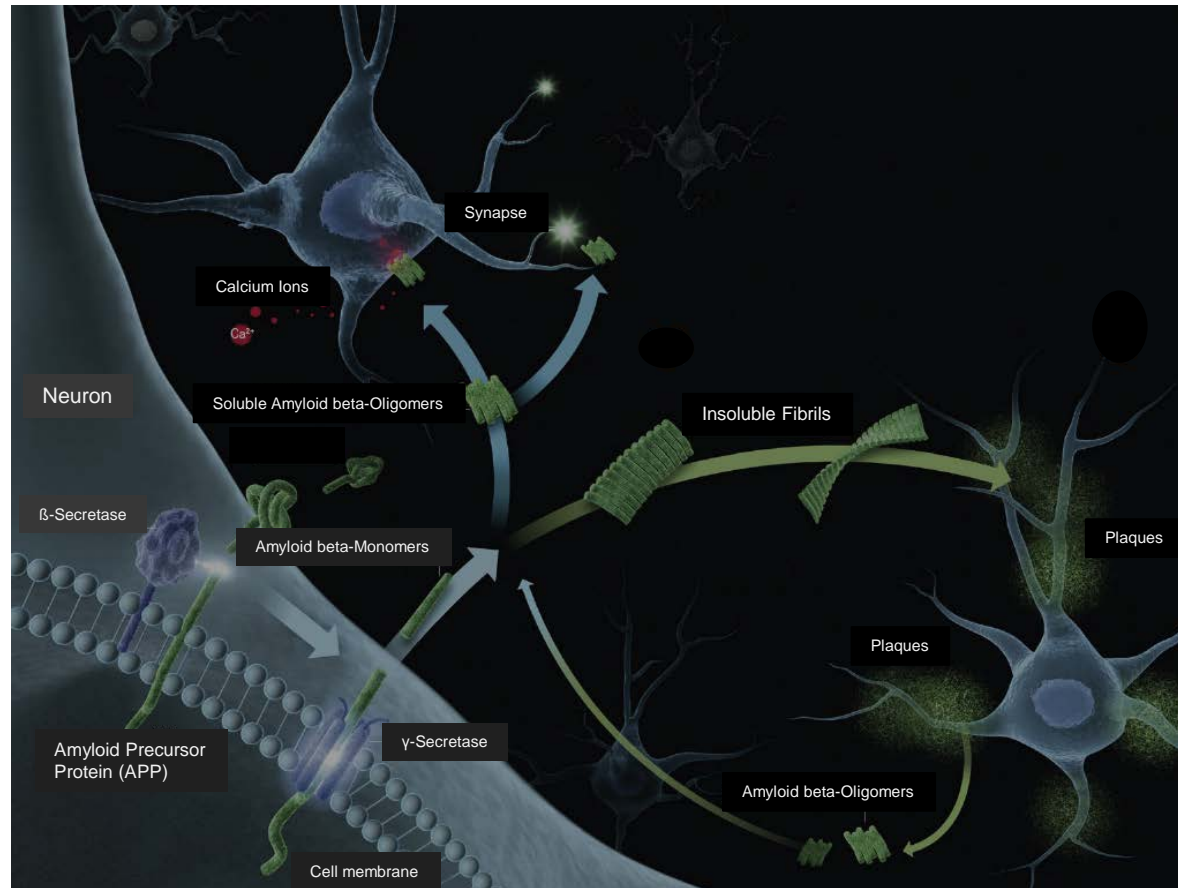


Info: Medical Care Corporation, [www.mccare.com](http://www.mccare.com)



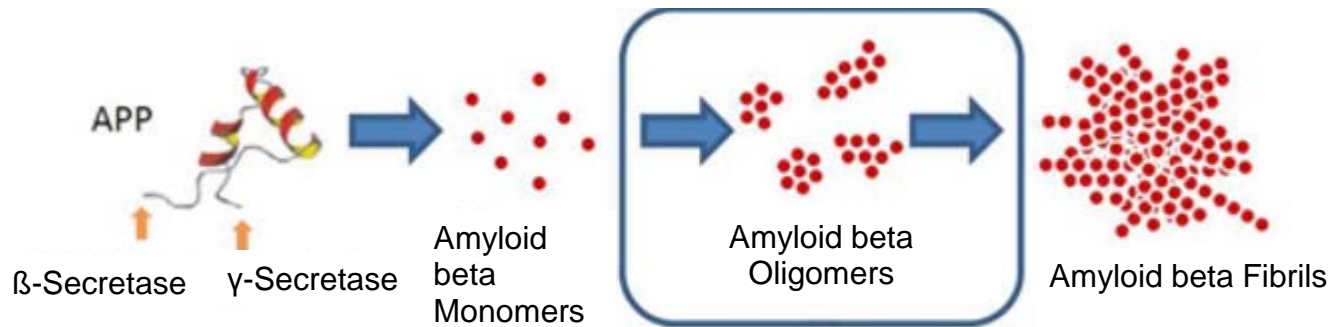
# ALZHEIMER'S DISEASE

What happens in the brain, in the neurons?



# ALZHEIMER'S DISEASE

## How to stop the Amyloid beta (A $\beta$ ) Oligomers?



- Looked for a specific agent
- Screened a billion of molecules
- Identified one candidate (called D3, a D-peptide)

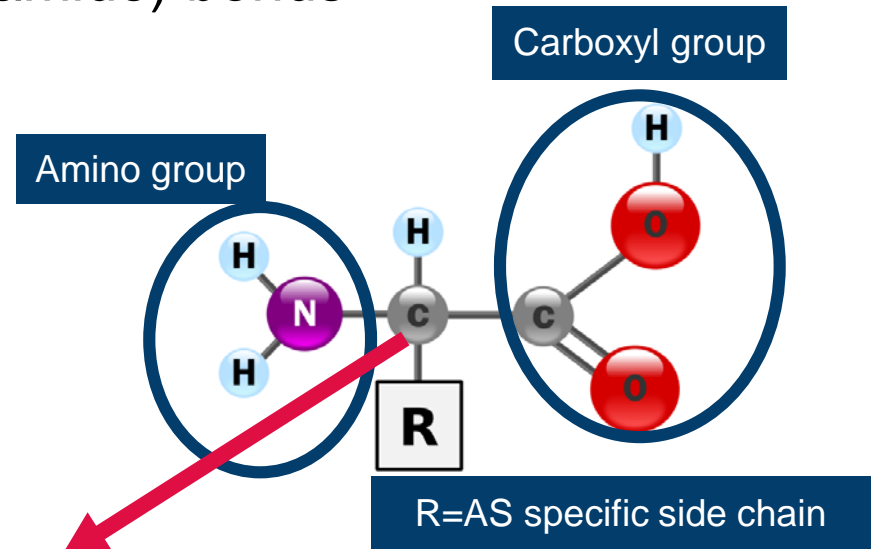
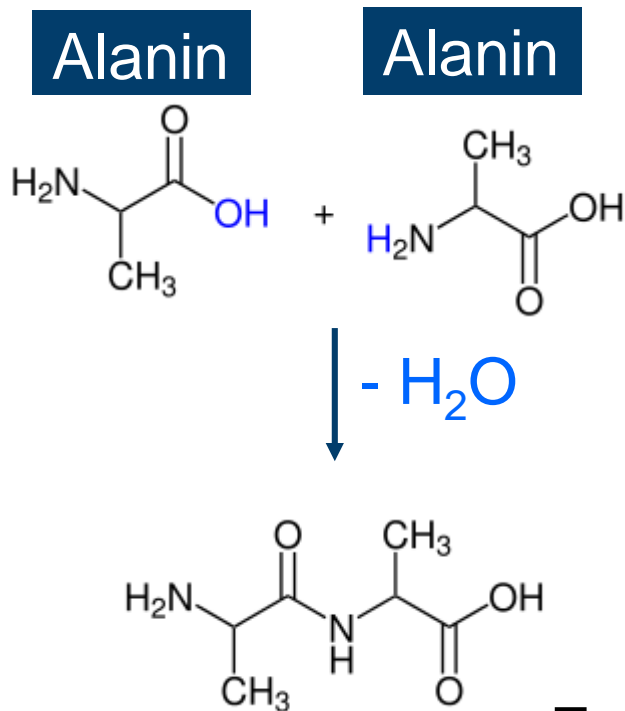
Why D-peptides?



# PEPTIDES

Made of building blocks called amino acids

- Short chains of <50 amino acids (AS)
- Amino acids linked via peptide (amide) bonds



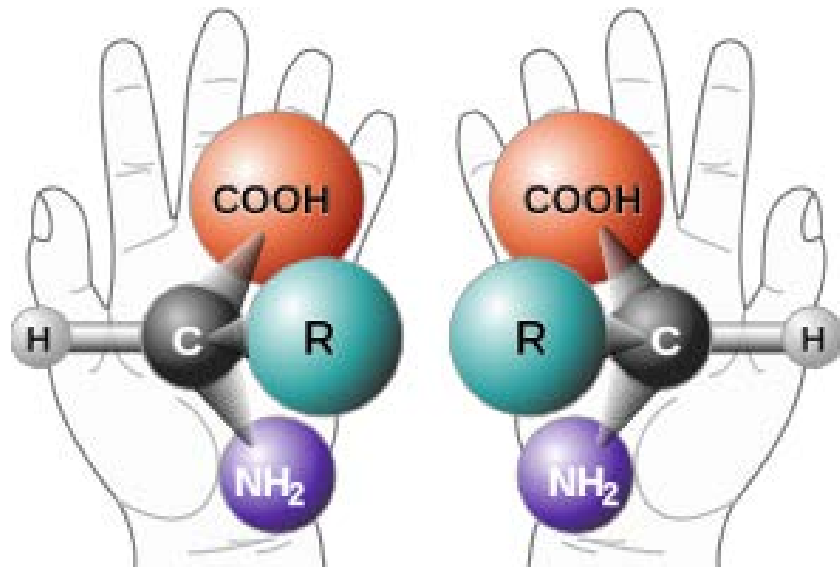
Isomeric forms

Configurational isomers

Enantiomers (mirror images)

# AMINO ACIDS

## L- and D-Amino acids



Molecules are not congruent

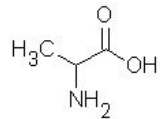
Enantiomers are chiral

- Molecular formula
- Density
- Refractive index
- Boiling and freezing point
- Spectra

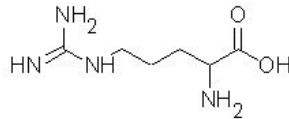
.....all the same, nevertheless different effects in the body.

# BUILDING BLOCKS OF PROTEINS

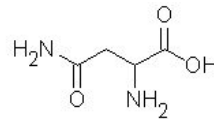
## 20 amino acids coded in our genome



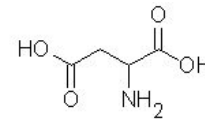
**Alanin (Ala)**



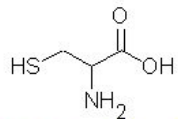
**Arginin (Arg)**



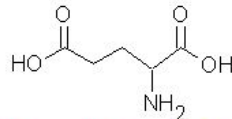
**Asparagin (Asn)**



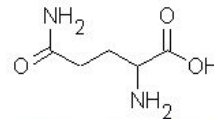
**Asparaginsäure (Asp)**



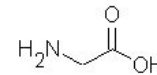
**Cystein (Cys)**



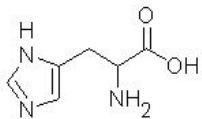
**Glutaminsäure (Glu)**



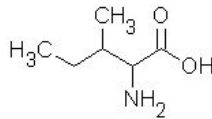
**Glutamin (Gln)**



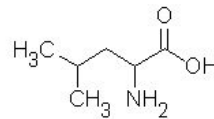
**Glycin (Gly)**



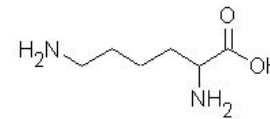
**Histidin (His)**



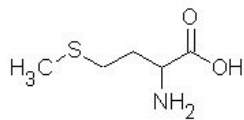
**Isoleucin (Ile)**



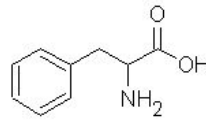
**Leucin (Leu)**



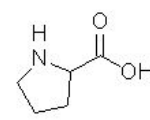
**Lysin (Lys)**



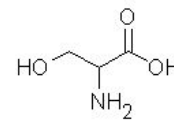
**Methionin (Met)**



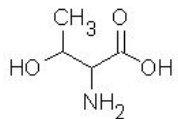
**Phenylalanin (Phe)**



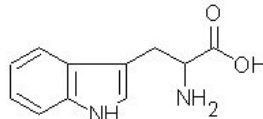
**Prolin (Pro)**



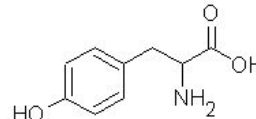
**Serin (Ser)**



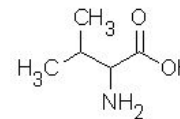
**Threonin (Thr)**



**Tryptophan (Trp)**



**Tyrosin (Tyr)**



**Valin (Val)**

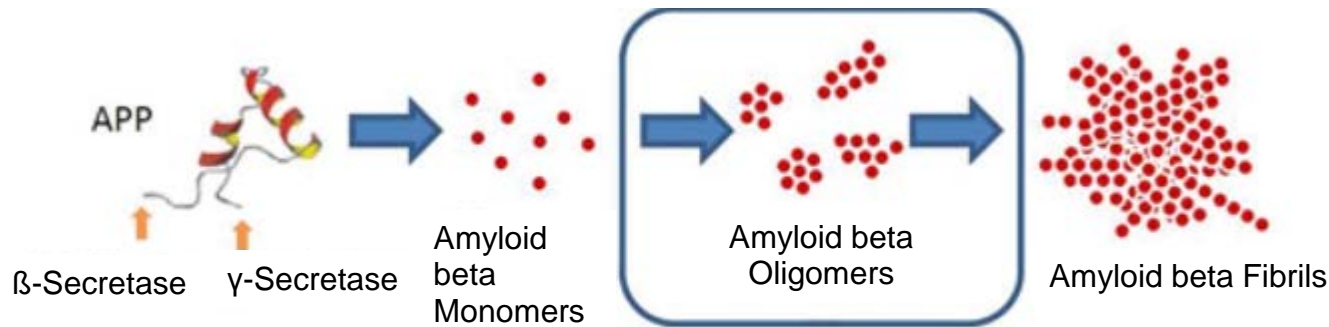
L-Amino acids

D-Amino acids

<https://commons.wikimedia.org/w/index.php?curid=543391>

# ALZHEIMER'S DISEASE

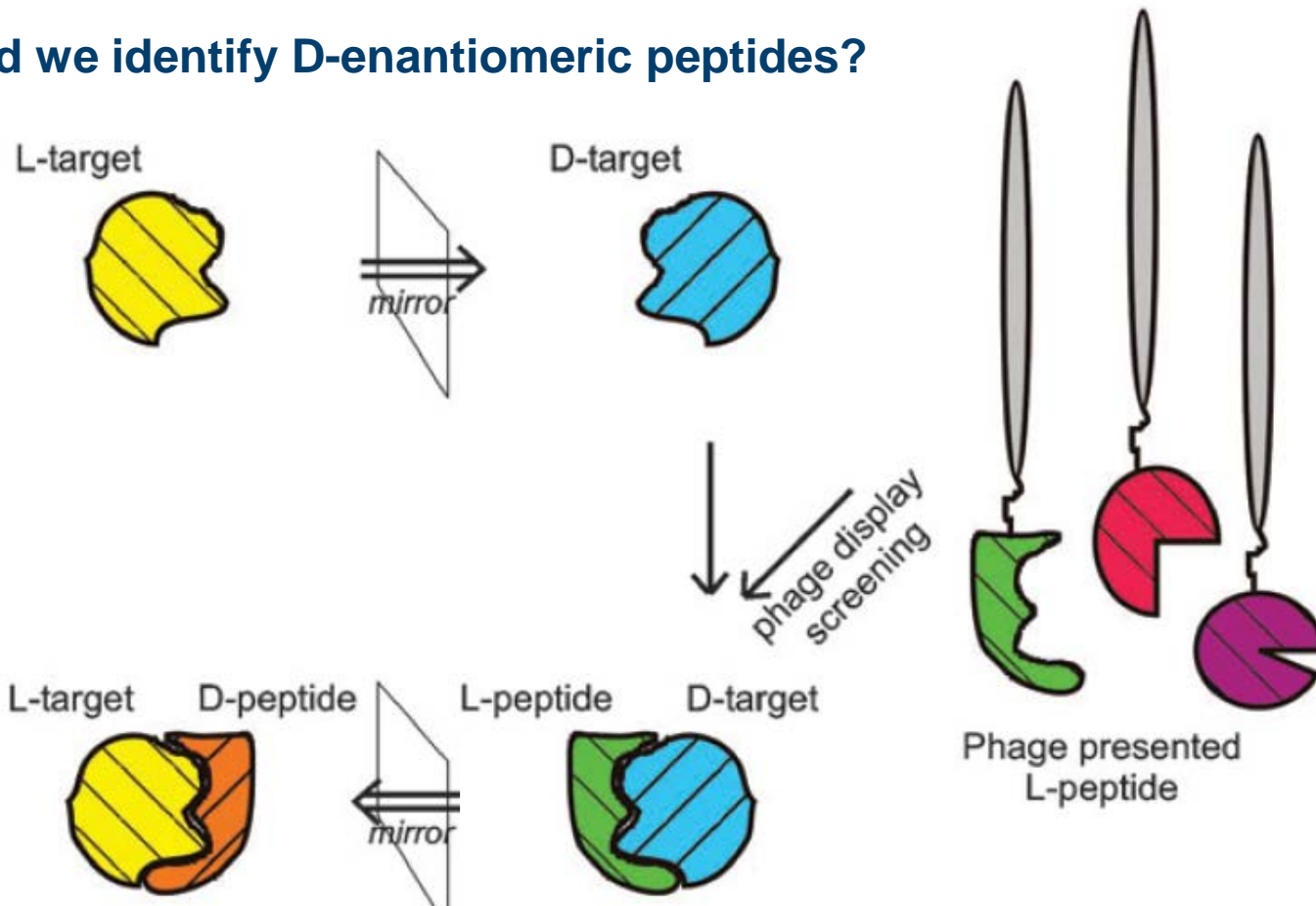
## Why an enantiomeric peptide?



- ➔ Protease resistant
- ➔ Show less immunogenicity
- ➔ Less effects on Amyloid  $\beta$ -Monomer concentration
- ➔ Products non-toxic
- ➔ Do not support prions like propagation

# ALZHEIMER'S DISEASE

How did we identify D-enantiomeric peptides?



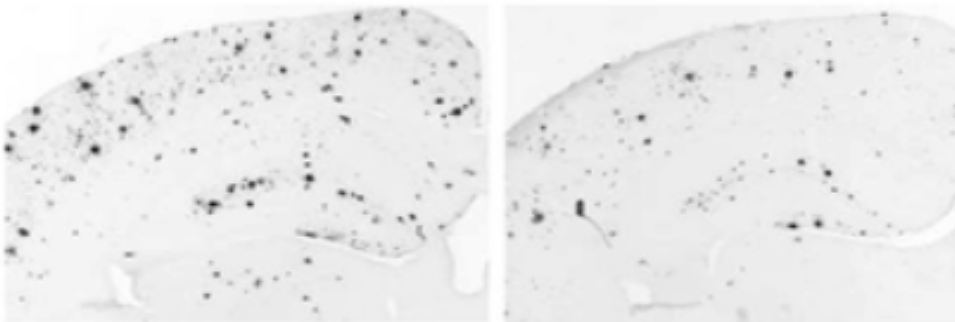
## Mirror-Image Phage Display

# ALZHEIMER'S DISEASE

## Efficacy of D3

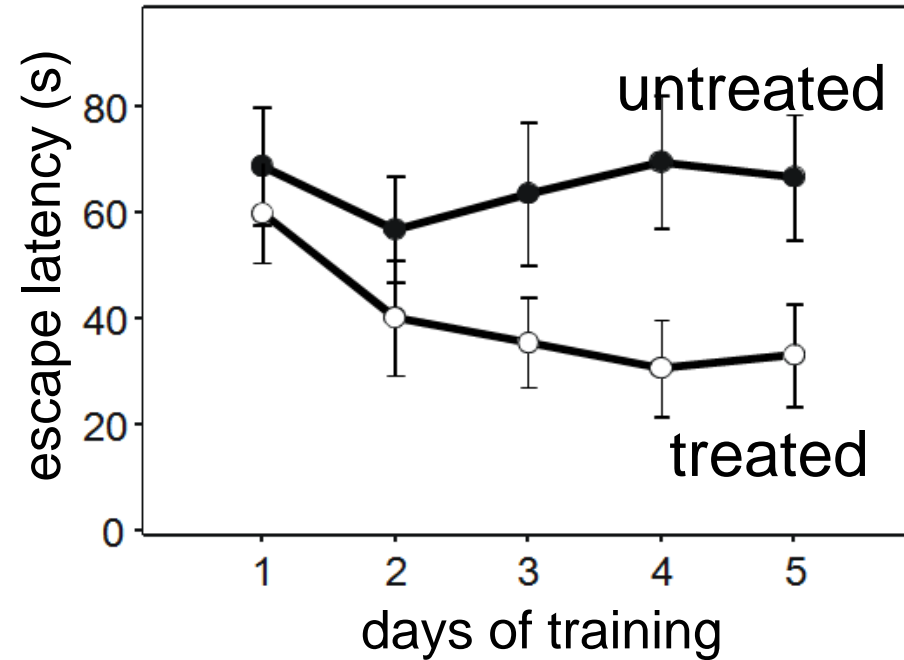
control

after D3 treatment

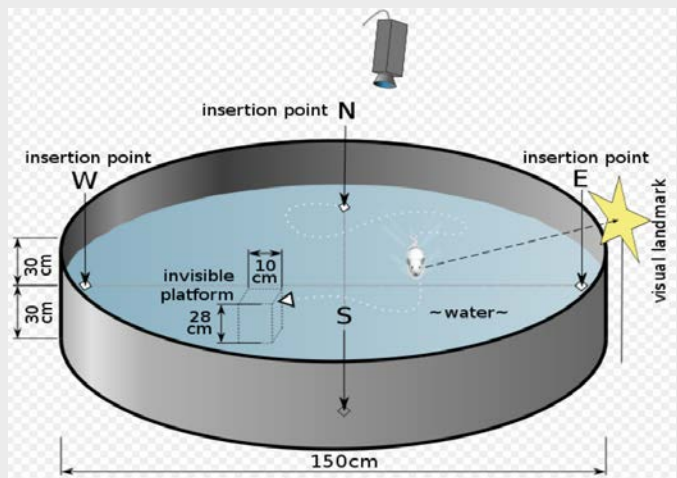


D3 reduced the quantity of amyloid

## Cognitive test



## Morris water maze test



Learning effect was significantly higher at D3 treated mice

# ALZHEIMER'S DISEASE

## Commercialization



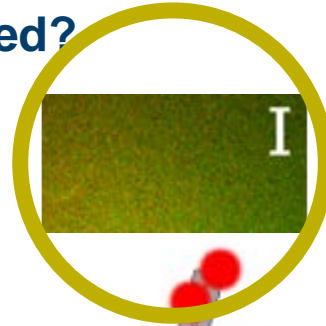
Founded September 2017

Disease	Target	Compound	Design/ Screening	Proof of Concept	Clinical Trial I	Clinical Trial II
Alzheimer's Disease	A $\beta$ Oligomers	D3 (PRI-002)	→			
Amyotrophic lateral sclerosis	Inflammation	PRI-003	→			
Amyotrophic lateral sclerosis	Mutant Superoxid Dismutase 1	In progress	→			
Chorea Huntington	Poly Glutamine tract (PolyQ)	In progress	→			
Parkinson's Disease	$\alpha$ -Synuclein	In progress	→			
Taupathies	Tau	In progress	→			



# ALZHEIMER'S DISEASE

How can the disease be diagnosed?



# amyloid

Detection Antibodies

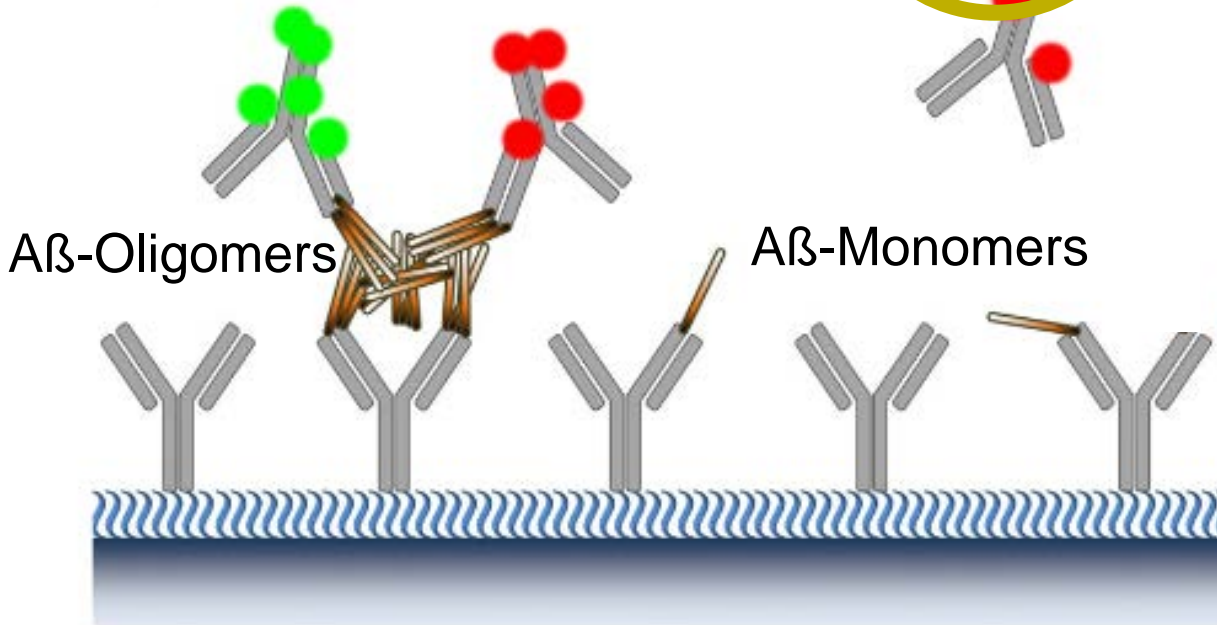
Fluorescent dye 1

Fluorescent dye 2

Cerebrospinal fluid or blood

Capture Antibody

Functionalized glass surface



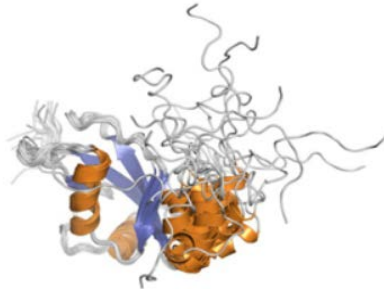
sFIDA: Surface-based Fluorescence Intensity Distribution Analysis

# DEGRADATION OF CELL COMPONENTS

## Autophagy

### Essential for:

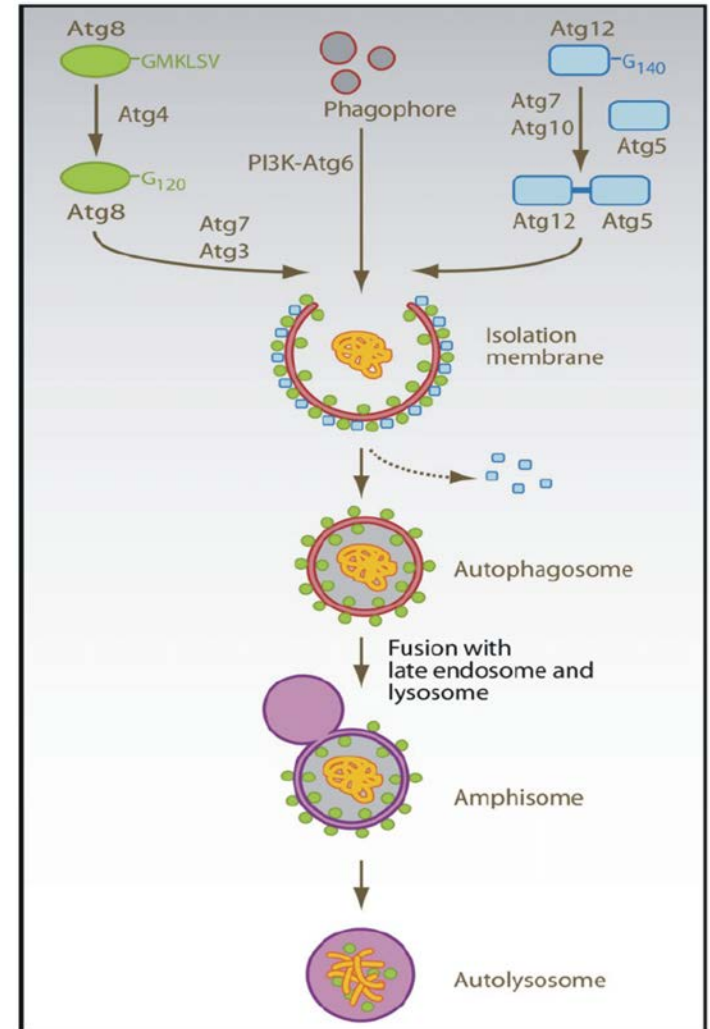
- Protein homeostasis
- Innate immune system
- Degradation of defective mitochondria
- Degradation of protein aggregates



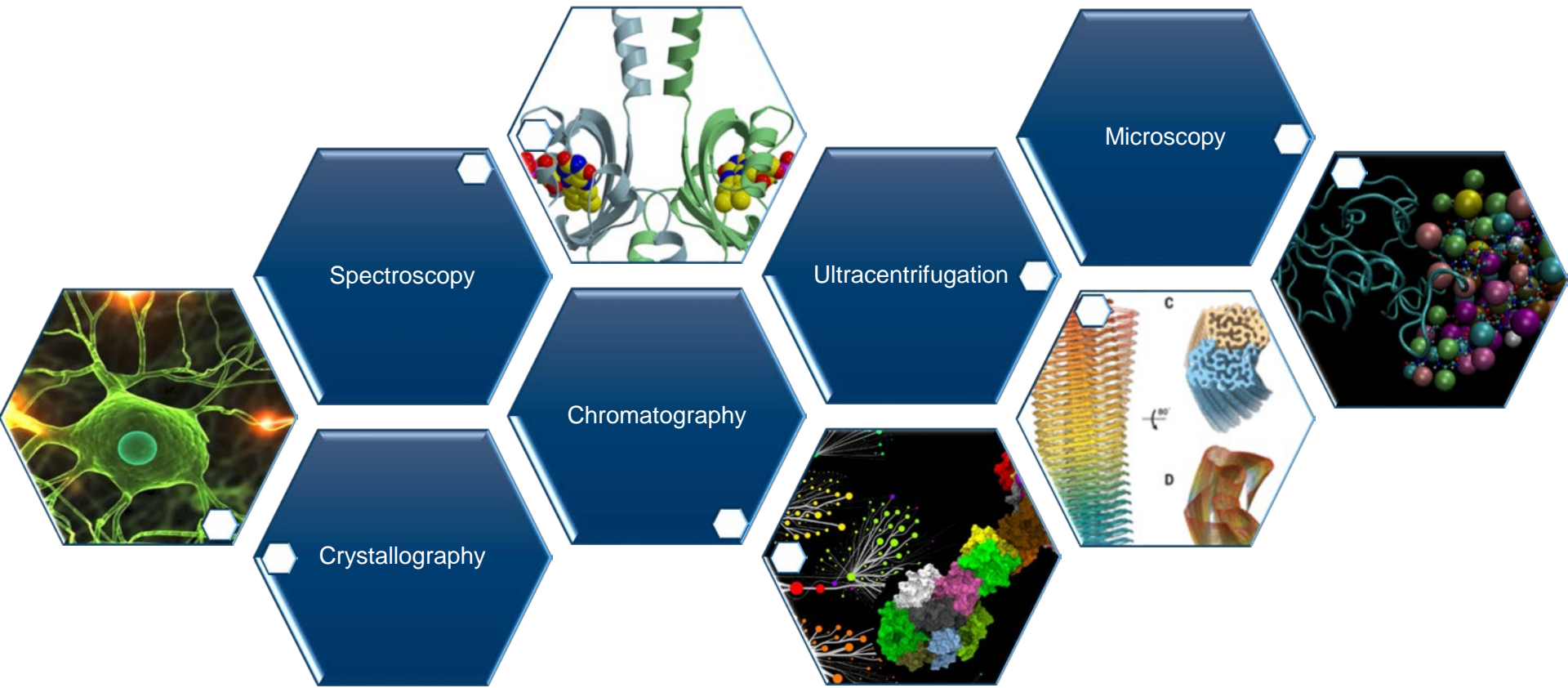
Autophagy-related protein 8 (Atg 8)

### Deficits lead to:

- Cancer
- Neurodegenerative diseases
- Diabetes Mellitus
- Viruses use or bypass the autophagy system
- ...



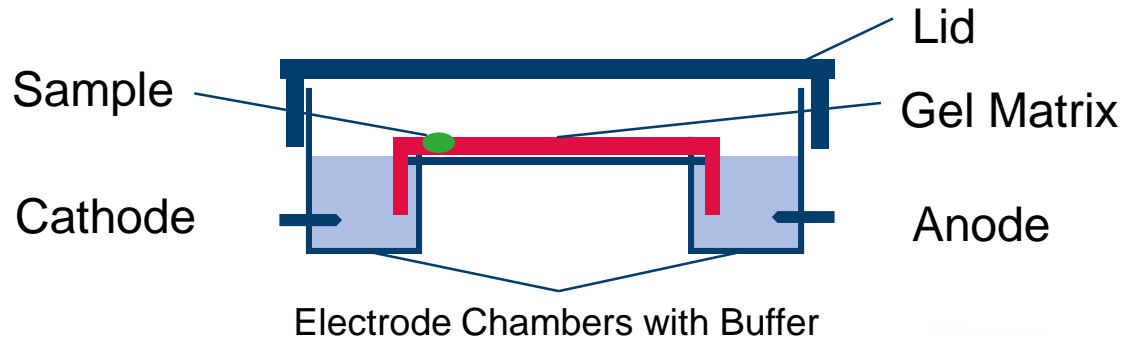
# METHODS USED IN STRUCTURAL BIOLOGY



# METHODS IN STRUCTURAL BIOLOGY

## Gel electrophoresis

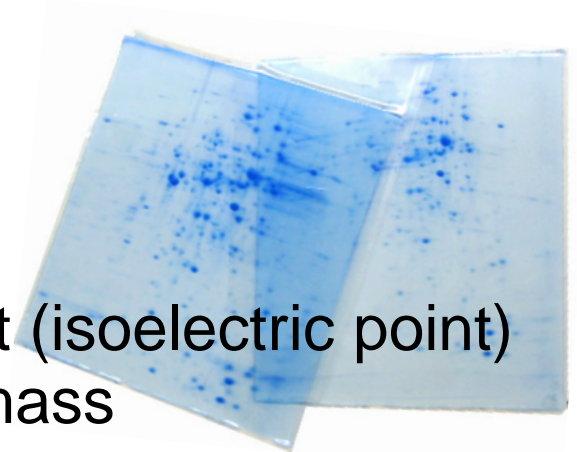
### Mobility of ions in an electric field



1D Gel:  
Molecular mass

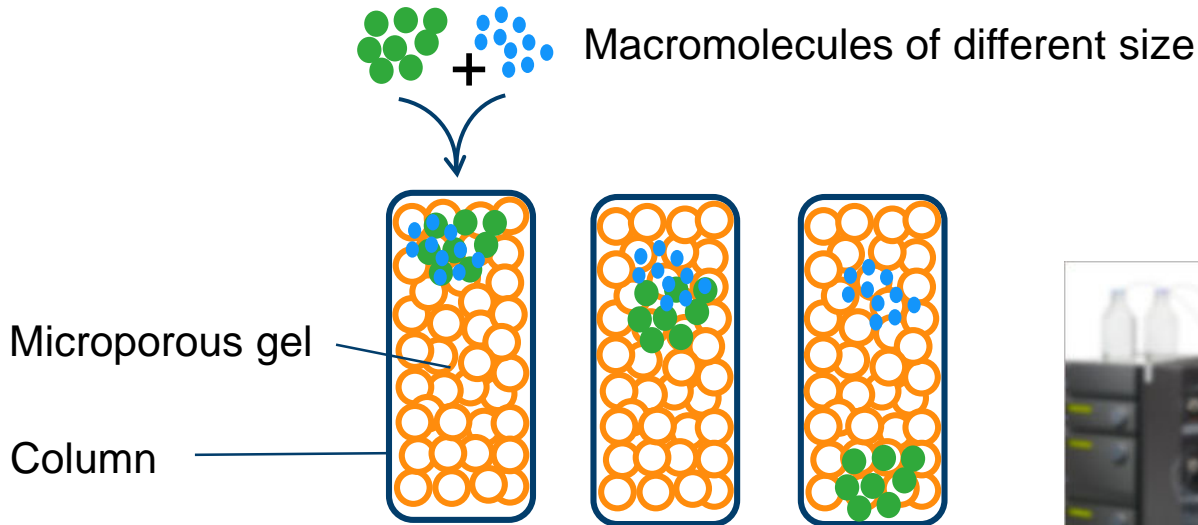


2D Gel:  
1) pH Gradient (isoelectric point)  
2) Molecular mass



# METHODS IN STRUCTURAL BIOLOGY

## Chromatography

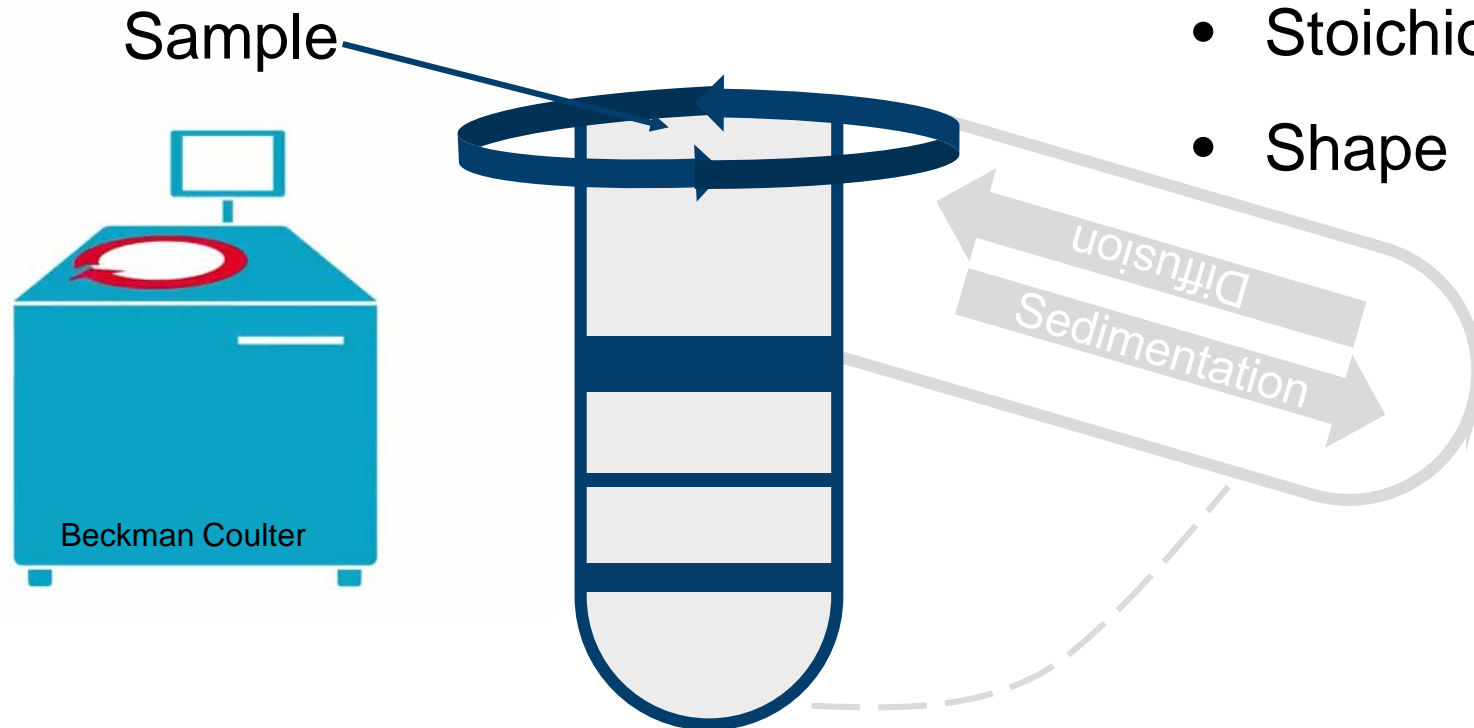


- Separation of a (protein) mixture
- Passing it in solution or suspension through a medium
- Components move at different rate

# METHODS IN STRUCTURAL BIOLOGY

## Analytical Ultracentrifugation

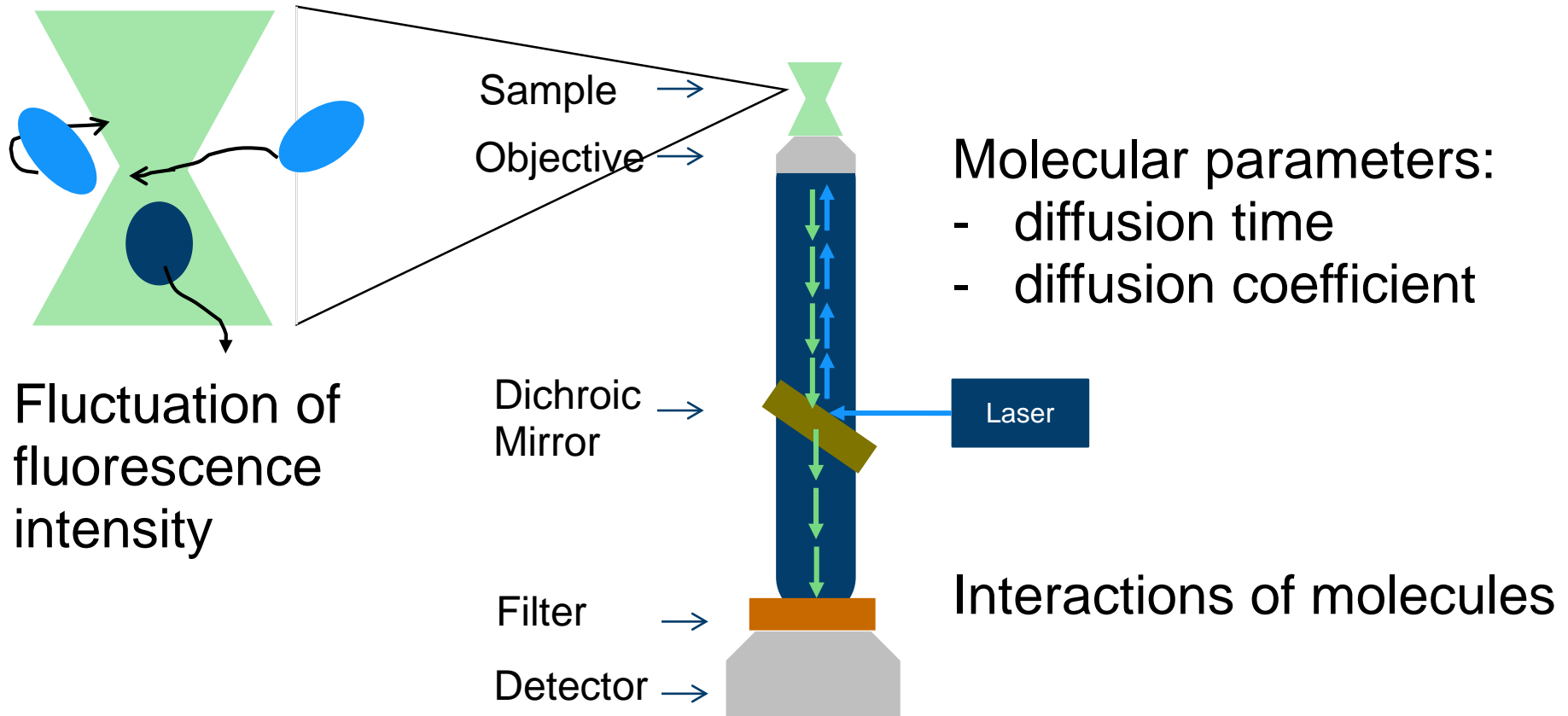
- Molecular weight
- Stoichiometry
- Shape



Physiological conditions

# METHODS IN STRUCTURAL BIOLOGY

## Fluorescence correlation spectroscopy (FCS)



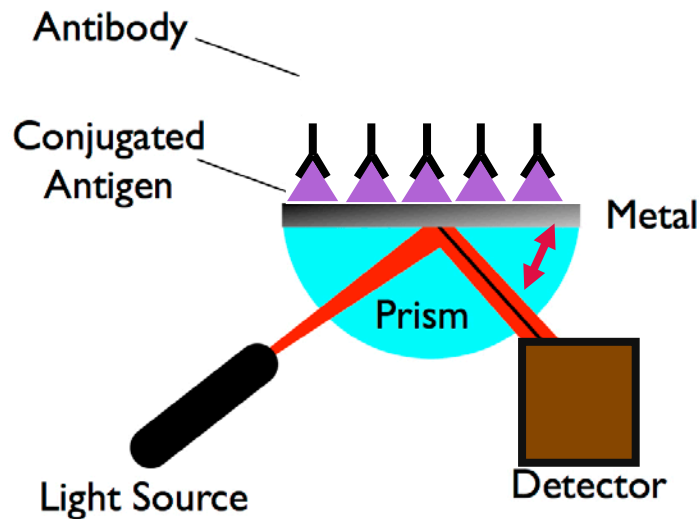


# METHODS IN STRUCTURAL BIOLOGY

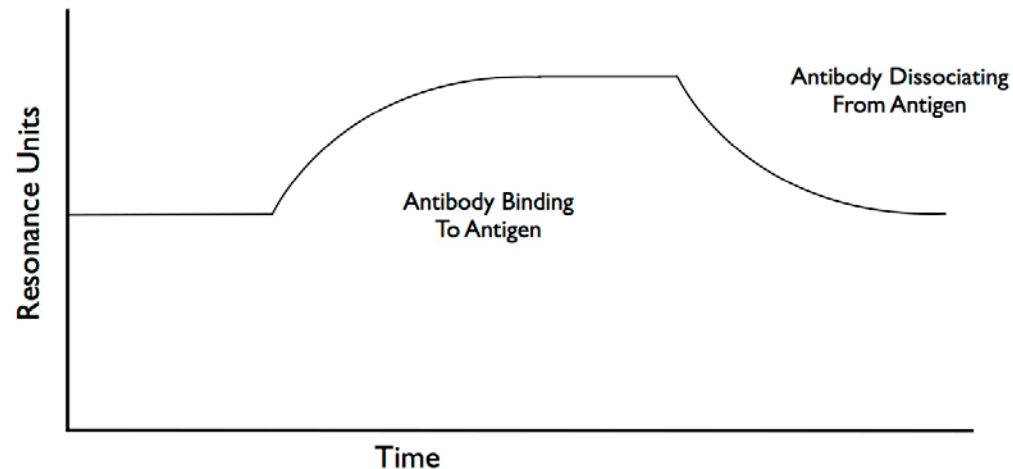
## Surface Plasmon Resonance

- Light is focused onto a metal film (gold, silver) through a glass prism
- Reflection is detected
- No labelled molecules

➔ Collective oscillations of free electrons (surface plasmons)



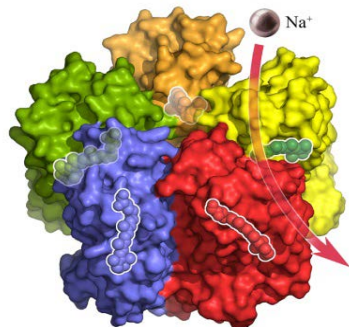
## Immunoassays



# METHODS IN STRUCTURAL BIOLOGY

## Nuclear Magnetic Resonance (NMR)

- Spectroscopic technique
- Local magnetic fields around atomic nuclei
- As higher the field strength as better the resolution
- Liquid and solid state NMR



KR2 Sodium Ion Pump  
Gushchin *et al.*, Science (2017)



900 MHz



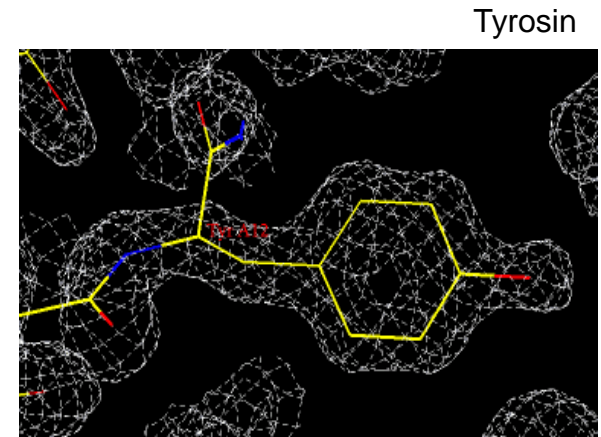
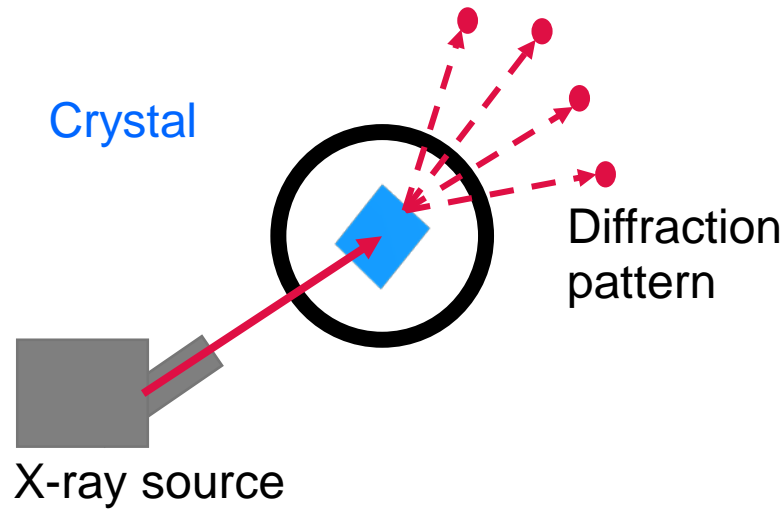
800 MHz



700 MHz

# METHODS IN STRUCTURAL BIOLOGY

## X-ray Crystallography



Picture: wikipedia

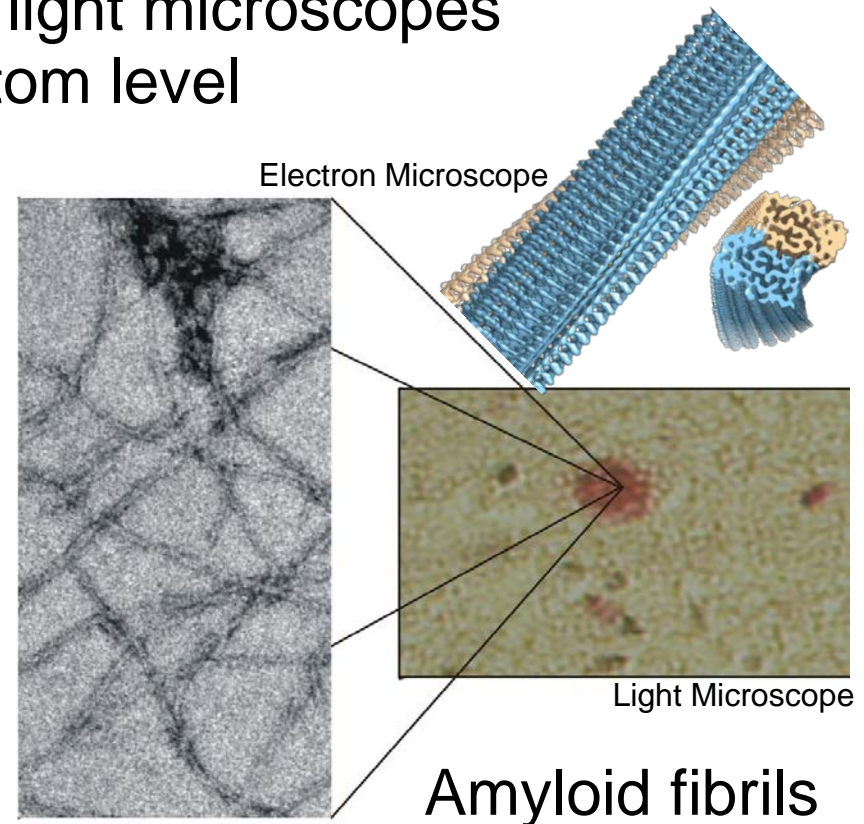
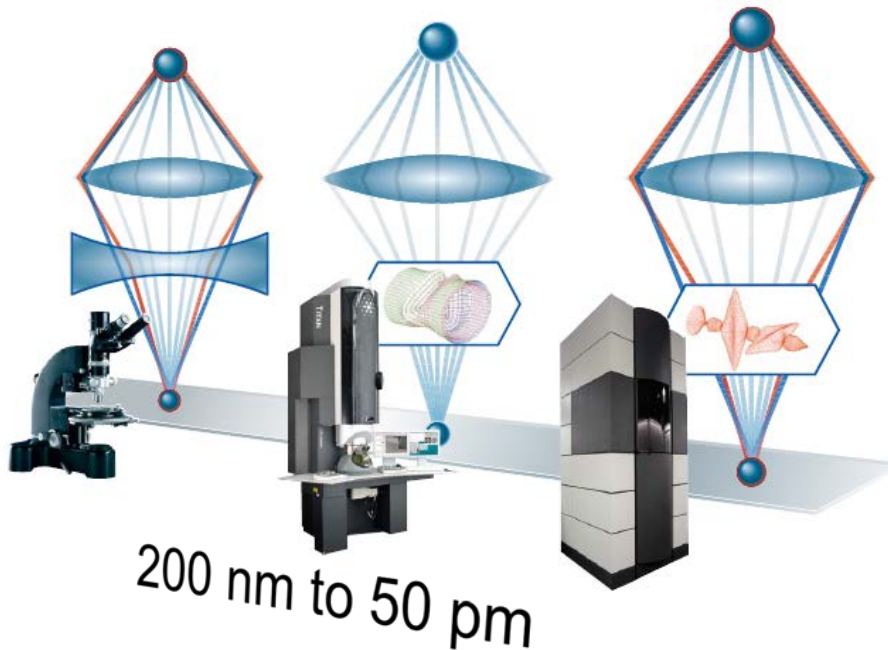


Atomic and molecular structure of crystals

# METHODS IN STRUCTURAL BIOLOGY

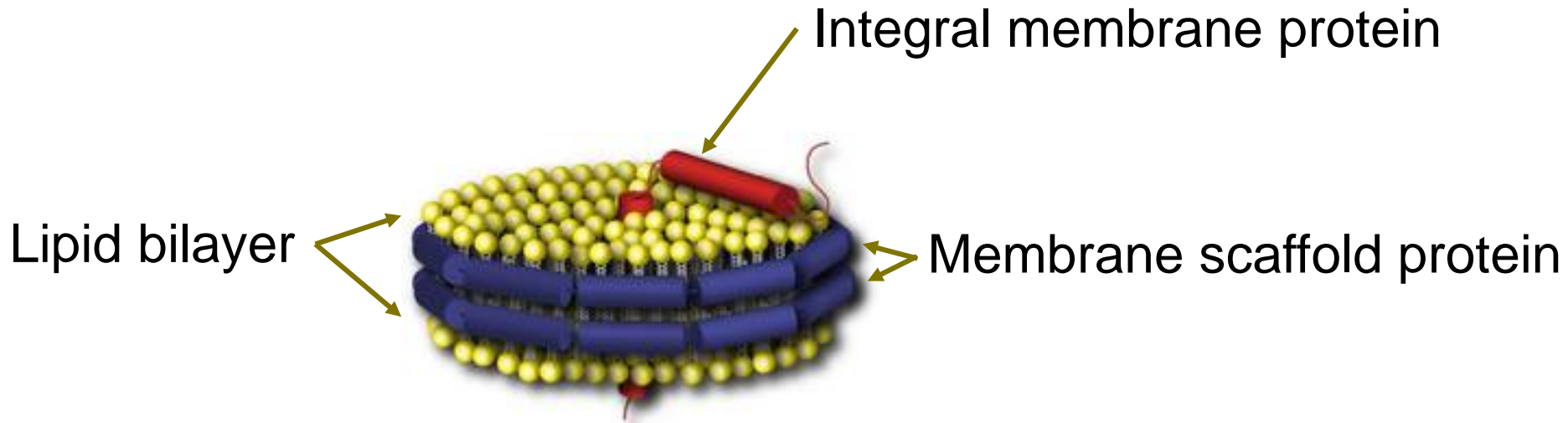
## Transmission Electron Microscopy (TEM)

- Electron wavelength up to 100,000 x shorter than visible light photons
  - ➔ Higher resolving power than light microscopes
  - ➔ Reveal structures down to atom level



# METHODS IN STRUCTURAL BIOLOGY

## Nanodiscs – Tool for membrane protein studies



Membrane proteins can be studied by high-resolution liquid state NMR

# CONCLUSION

## Structural Biochemistry: ICS-6

- Neurodegenerative diseases, focus on Alzheimer
- Parkinson, Chorea Huntington, Amyotrophic Lateral Scleroses, Taupathies, as well as diseases like AIDS and SARS
- Autophagy – disorders can lead to neurodegenerative diseases
- Using multiple methods for structural and functional analysis of proteins and protein complexes as well as computational methods
- (Photo receptors/Optogenetics)



# TAKE AWAY MESSAGES

## ICS-6

Determines the three-dimensional structure of the protein complexes involved in decisive cellular processes - if possible, in atomic resolution

## ICS-6

Develops novel methods for the early diagnosis and treatment of neurodegenerative diseases, with a strong focus on Alzheimer's dementia







Thank you for your attention