



## Welcome to Biophysics (BT 301)

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Ack: Few images are |

HDMI 2 / MHL



1080p 60Hz



## RECAP

### REFRESHER ON PROTEIN STRUCTURE



# REFRESHER ON PROTEIN STRUCTURE

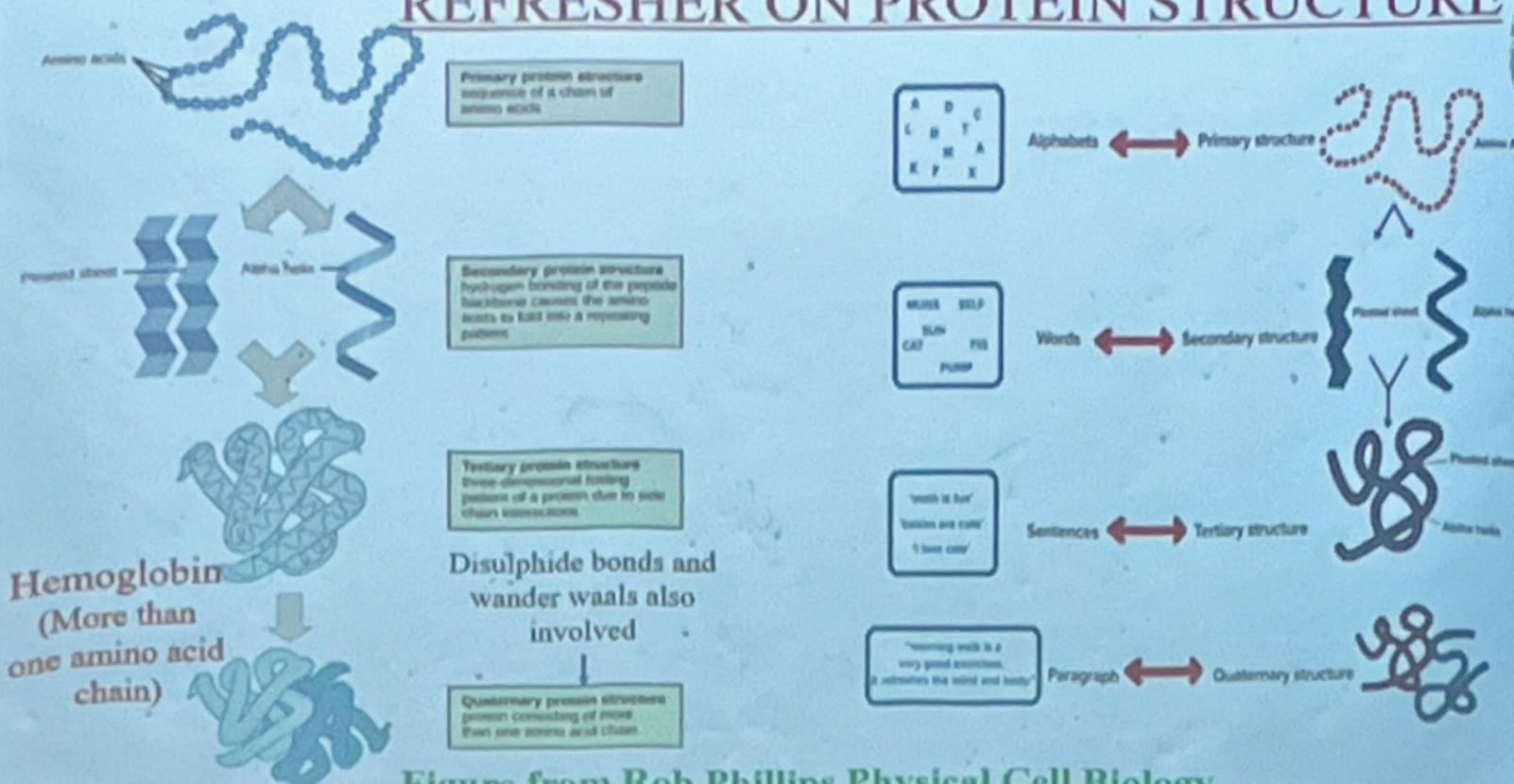


Figure from Rob Phillips Physical Cell Biology

## RECAP



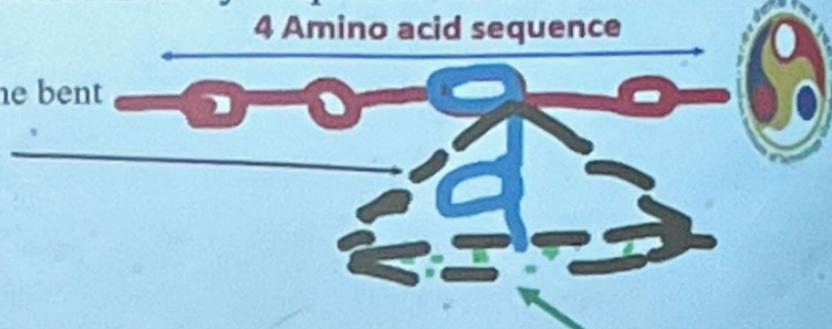
Why is determining Protein Folding a difficult problem  
Computationally??

## RECAP

### Why is determining Protein Folding a difficult problem??

- Folded structure of sequence determined by sequence of successive solid bend angles.

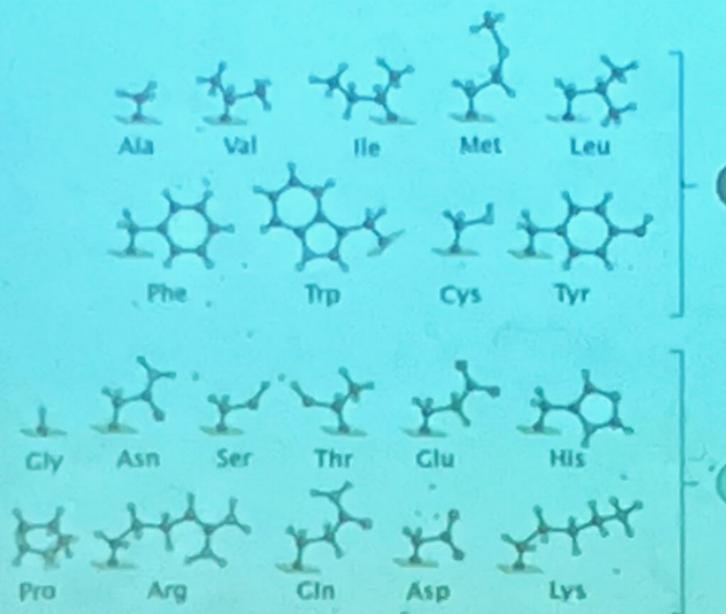
Cone is the range around which the bent sequence can wriggle



- We can limit the chain orientations in cone (say discretize to seven solid angles in cone (In green)). So we have search space of  $7^4$
- Hence determining the shape is the greatest computational problem.

## RECAP

## HP Model for Amino Acids



HYDROPHOBIC

HP- Amino acids classified based on similar properties

POLAR

Figure from Rob Phillips Physical Cell Biology

Figure 8.28: Mapping of the amino acids onto an HP alphabet. The 20 amino acids are coarsely separated into two categories, namely, hydrophobic (H) or polar (P).

## Schematic of Protein Folding using HP-Model RECAP

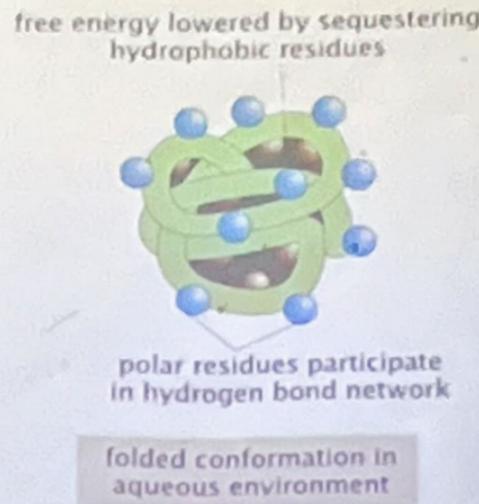
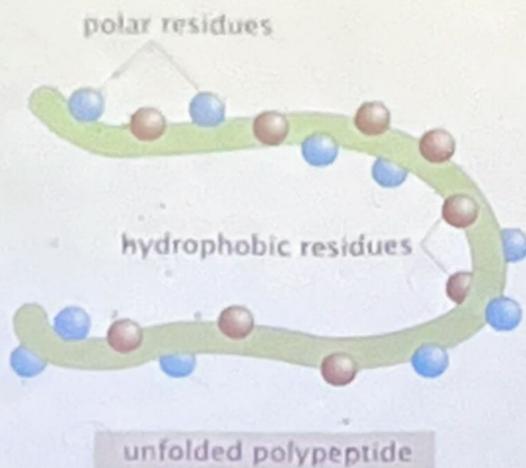


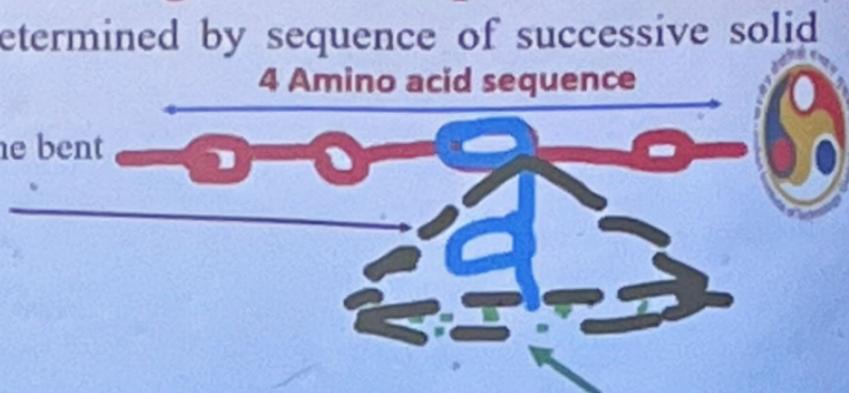
Figure from Rob Phillips Physical Cell Biology

## RECAP

### Why is determining Protein Folding a difficult problem??

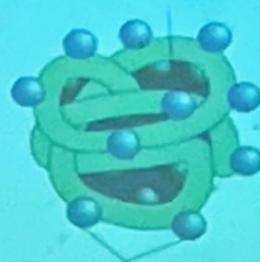
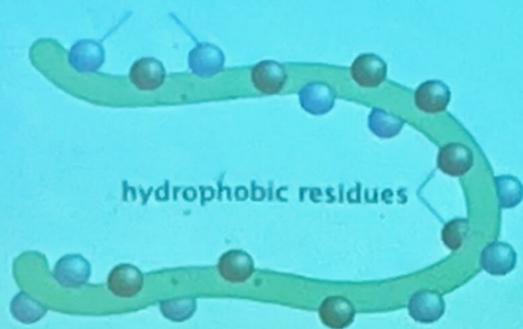
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- We can limit the chain orientations in cone (say discretize to seven solid angles in cone (In green)). So we have search space of  $7^4$
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## RECAP



## RECAP

## HP Model for Amino Acids

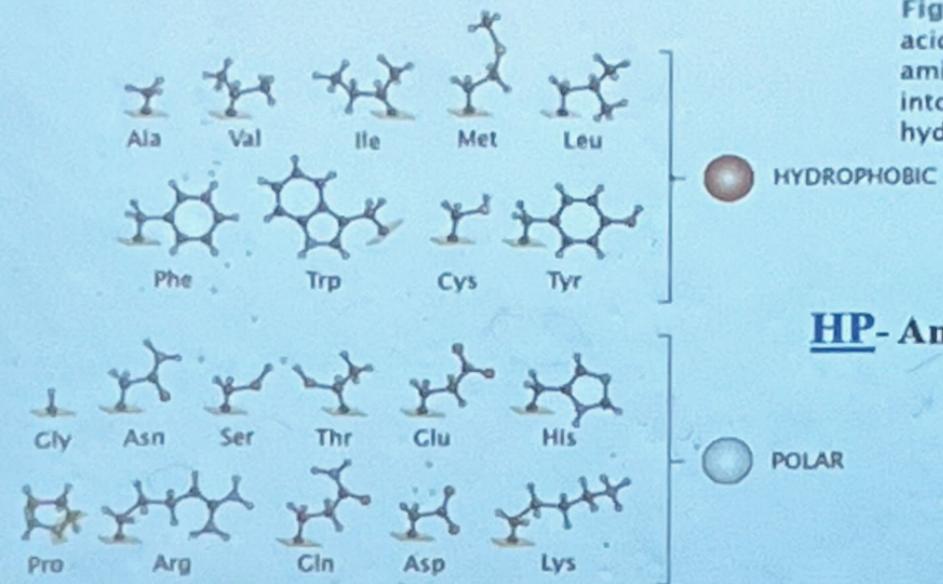


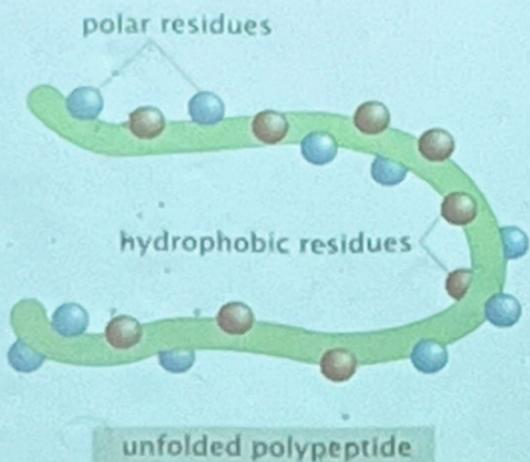
Figure 8.28: Mapping of the amino acids onto an HP alphabet. The 20 amino acids are coarsely separated into two categories, namely, hydrophobic (H) or polar (P).

**HP-** Amino acids classified based on similar properties

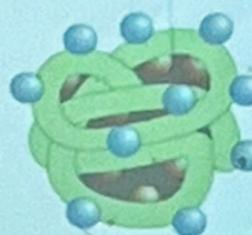
Figure from Rob Phillips Physical Cell Biology

## Schematic of Protein Folding using HP-Model

### RECAP



free energy lowered by sequestering hydrophobic residues



polar residues participate in hydrogen bond network

folded conformation in aqueous environment



Figure from Rob Phillips Physical Cell Biology



## Protein Folding RECAP

**insight review articles**

Cited more than 5000 times

# Protein folding and misfolding

Christopher M. Dobson

*University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK (e-mail: cmd44@cam.ac.uk)*

The manner in which a newly synthesized chain of amino acids transforms itself into a perfectly folded protein depends both on the intrinsic properties of the amino-acid sequence and on multiple contributing influences from the crowded cellular milieu.

**Depends On- Free Energy**

# The nature of protein folding pathways



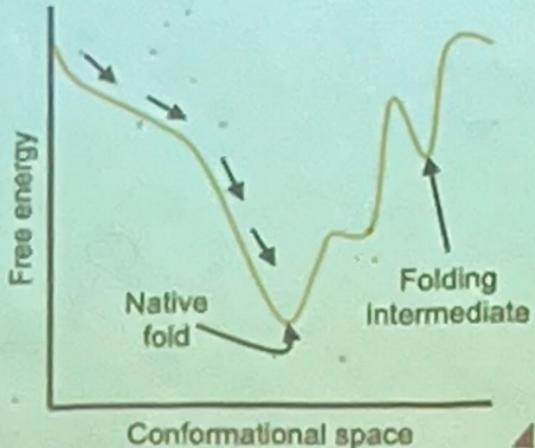
S. Walter Englander<sup>1</sup> and Leland Mayne

Johnson Research Foundation, Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania,  
Philadelphia, PA 19104

Edited by Alan R. Fersht, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom, and approved September 23, 2014 (received for review June 24, 2014)

## RECAP

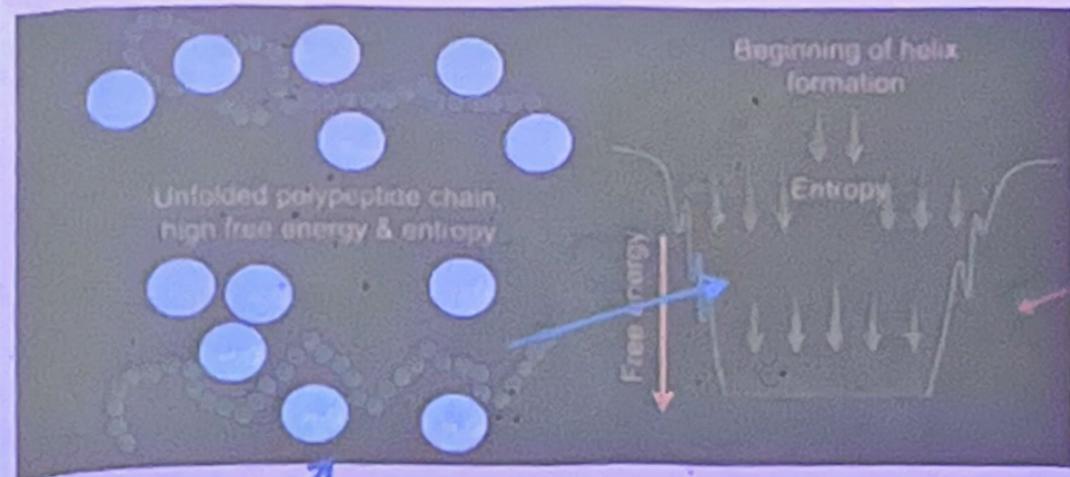
(B) the new view of multiple routes through a funneled landscape.



### How Does a Protein Reach its Ordered Folded State from Its Unfolded Ensemble?

What is the nature of the ordering in a folded native protein, and how does that ordering arise from its highly disordered denatured state? The basic ideas are expressed through statistical mechanics. The relative stabilities of states depend on their free energies. At equilibrium, the probability of occupying a state depends on its Boltzmann's weight,  $\exp(-\Delta G/k_B T)$ , where  $\Delta G$  is the difference in free energies of the states, native and unfolded in this case.  $k_B$  is Boltzmann's constant and  $T$  is temperature.<sup>44-49</sup> Small proteins typically fold cooperatively, i.e. through relatively sharp transitions between the disordered and ordered states.<sup>50,51</sup>

## Middle of Protein Folding Pathway or Protein Folding Funnel (During Folding process)



Hydrogen bonds interact with polypeptide chain ← Middle of the Funnel

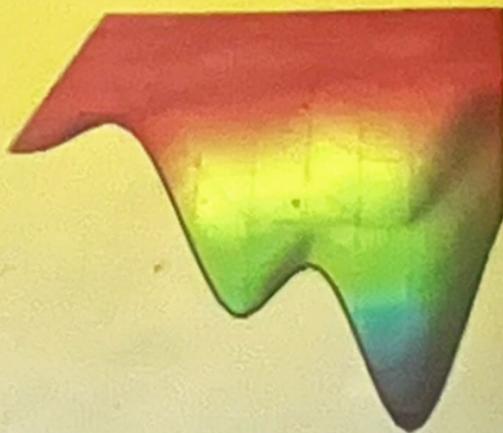
### RECAP

Entropy of the amino acid chain decreases in Middle of funnel

Entropy of surrounding water molecules increases down the funnel

Nice Summary video from Prof.Ken Dill (2012)

RECAP



## **TODAYS LECTURE**

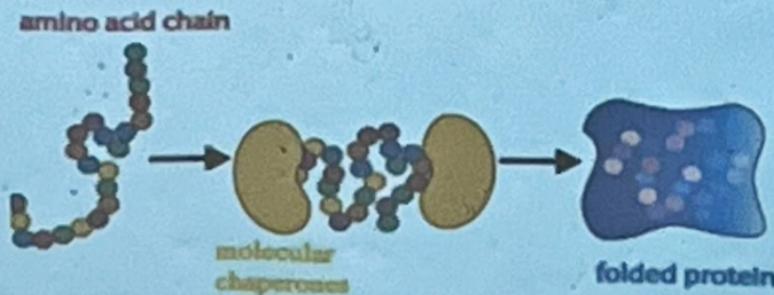
- A. Molecular Chaperones**
- B. Protein Aggregates**
- C. Flow Cytometry**
- D. Parkinson's and Alzheimer's**

**Acknowledgment: Aggregated Lecture from lot of resources  
(Textbook/Journal Papers/Youtube lectures)**

## Chaperones for Protein Folding

Most large proteins (i.e from large amino acid chains) can get stuck in KINETIC TRAPS. Chaperones are proteins with abilities of helping other proteins to **FOLD** without errors.

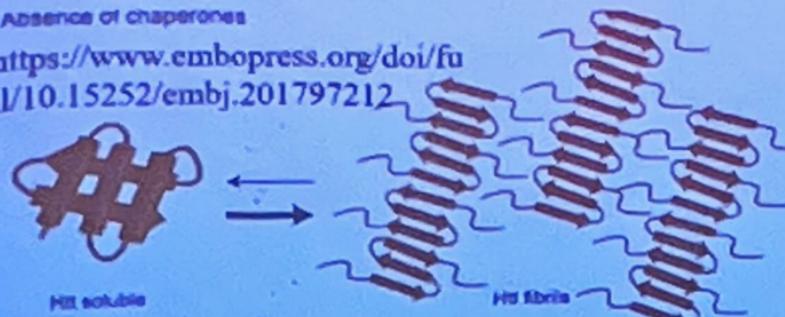
Most Chaperones are heat inducible protein which provide kinetic assistance during protein folding by ATP hydrolysis.



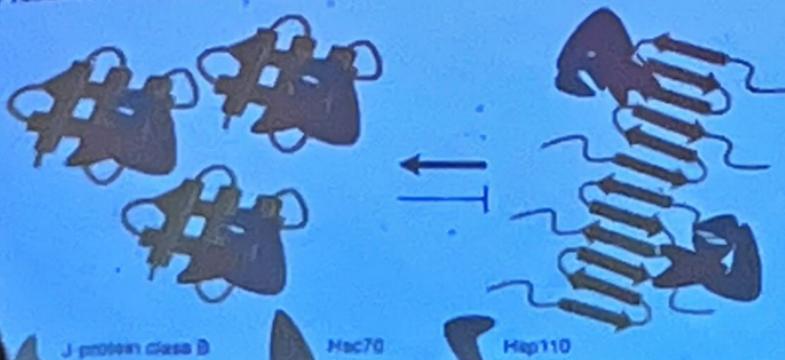
# Chaperones for Protein Folding (Huntington disease)-IN VITRO

Absence of chaperones

<https://www.embopress.org/doi/full/10.15252/embj.201797212>



Presence of chaperones



Complete suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex

Annika Scior, Alexander Buntru, Kristin Arnsburg, Anne Ast, Manuel Ihburg, Katrin Juenemann, Maria Lucia Pigazzini, Barbara Mlody, Dmytro Puchkov, Josef Priller, Erich E Wanker, Alessandro Prigione, Janine Kirstein

## Author Affiliations

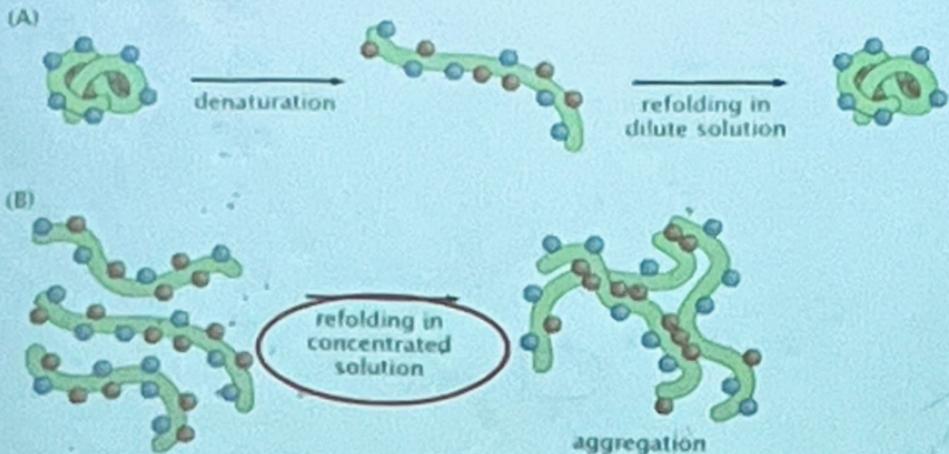
DOI 10.15252/embj.201797212 | Published online 06.12.2017  
The EMBO Journal (2018) 37, 282-299

Chaperones suppressed the build-up of pathogenic amyloid (Htt) fibrils and disaggregates them

Huntington's is an inherited disease that causes the progressive breakdown (degeneration) of nerve cells in brain

What is Protein Aggregation

# Protein Aggregation



Several amino acid chains in concentrated solutions folding simultaneously results in Aggregation

Figure 14.22: Protein folding and aggregation. A protein folded in its native state sequesters hydrophobic domains on the inside to hide the hydrophobic core. Denaturation disrupts the native structure, exposing these hydrophobic patches. (A) When the protein is allowed to refold in very dilute solution, the hydrophobic patches within a single molecule self-associate to reform the native hydrophobic core. (B) At high concentration, the hydrophobic patch of one protein molecule may associate with the hydrophobic patch of another, triggering protein aggregation rather than native refolding. Hydrophobic residues are shown in red, while hydrophilic residues are shown in blue.

Figure/Content from Rob Phillips "Physical Cell Biology"

## Protein Aggregation

- Aggregation occurs when many amino chains expose their hydrophobic residues and interact.
- Aggregation is very hard to reverse
- Aggregation mechanism depend on protein sequence/environment  
*(Different for various disorders)*
- Aggregates are risk for immune response.



**Combined look of  
Folded Protein Vs Aggregate  
(In context of Chaperones)**

# Aggregate Versus Folded Protein (Energy Funnel)

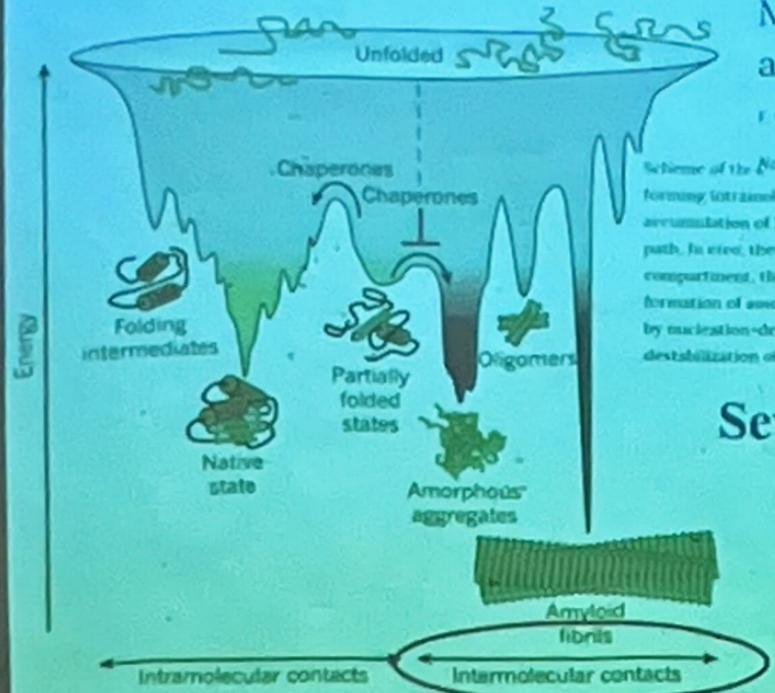
Review Article · Published: 20 July 2011



## Molecular chaperones in protein folding and proteostasis

F. Ulrich Hartl ■ Andreas Brügger & Manajit Hayen-Hartl

Scheme of the *Nature* 475, 324–332 (21 July 2011) · Download Citation & image (green) by forming intermolecular contacts (modified from refs 10 and 35). The ruggedness of the free-energy landscape results in the accumulation of kinetically trapped conformations that need to traverse free-energy barriers to reach a favourable downhill path. In fact, these steps may be accelerated by chaperones<sup>36,37,47</sup>. When several molecules fold simultaneously in the same compartment, the free-energy surface of folding may overlap with that of intermolecular aggregation, resulting in the formation of amorphous aggregates, toxic oligomers or ordered amyloid fibrils (red). Fibrillar aggregation typically occurs by nucleation-dependent polymerization. It may initiate from intermediates populated during *de novo* folding or after destabilization of the native state (partially folded states) and is normally prevented by molecular chaperones.



Several amino acids molecules fold simultaneously nearby



## LIFE CYCLE OF PROTEINS

# LIFE CYCLE OF PROTEINS

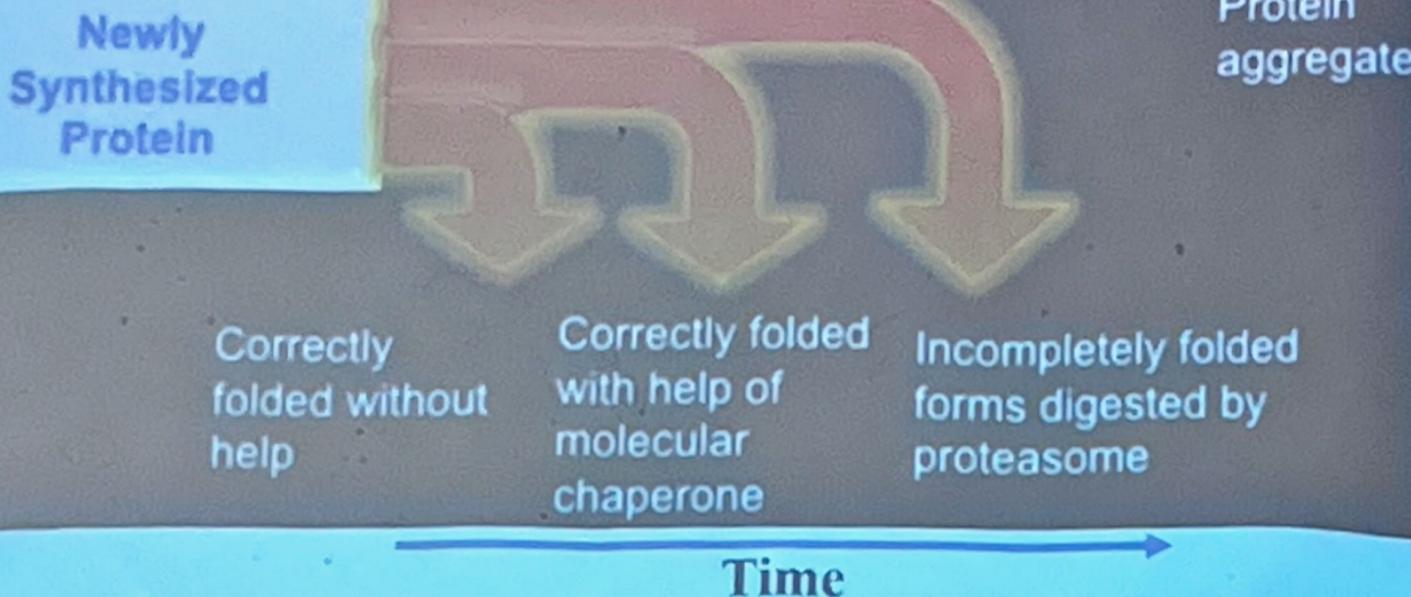


Figure from NPTEL Lecture (Dr.Sanjeeva Srivastava, IIT Bombay)

What is Parkinson's?? Any Idea

## What happens in Parkinson's Disease?



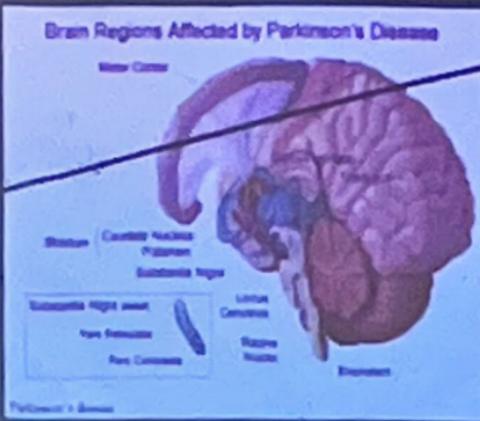
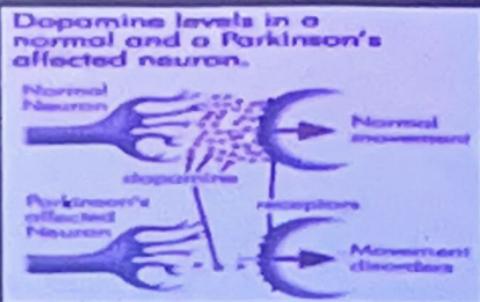
Parkinson disease

Degeneration of dopa-energic neurons that produce dopamine.

Loss of the melanin containing neurons produce characteristic changes in depigmentation.

Formation of LEWY BODIES

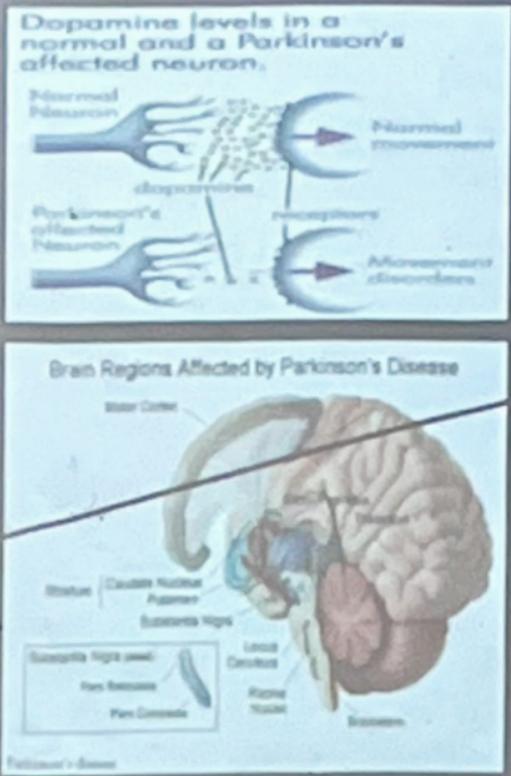
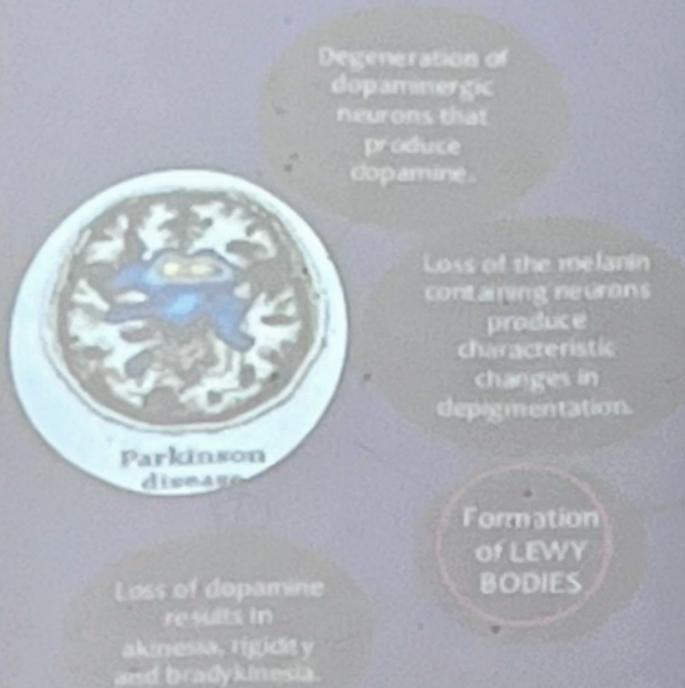
Loss of dopamine results in akinesia, rigidity and bradykinesia.



Slide prepared by my  
Phd student  
Ms.Tanmayee

Alpha  
synuclein  
protein  
aggregates

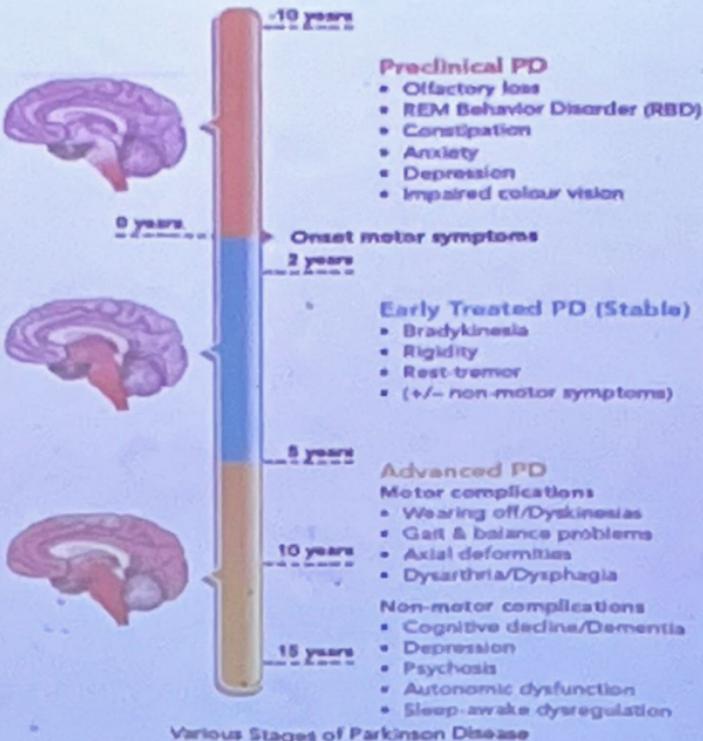
## What happens in Parkinson's Disease?



Slide prepared by my  
Phd student  
Ms. Tanmayee

Alpha synuclein protein aggregates

## Parkinson's (Stages)



<https://www.singhealth.com.sg/DoctorsAndHealthcareProfessionals/Medical-News/2014/Pages/Parkinson-Disease-Likely-Treatment-Options.aspx>

# Parkinson's (In Pictures)

Tremor



Tremor of one hand is an early manifestation of Parkinsonism



Tremor often improves or disappears with purposeful function

Bradykinesia

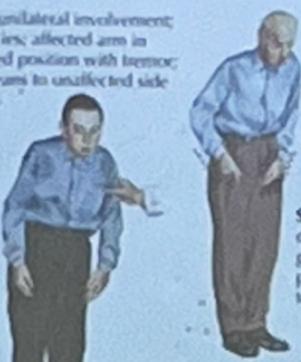


Difficulty in performing simple manual functions may be initial symptom

Rigidity and Gait Disorders



Stage 1: unilateral involvement; blank facies; affected arm in semi-flexed position with tremor; patient leans to unaffected side

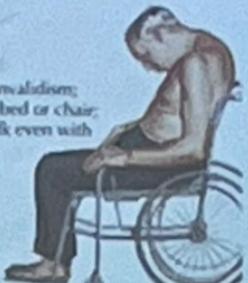


Stage 2: bilateral involvement with early postural changes; slow shuffling gait with decreased excursion of legs

Late Stage Disabilities



Stage 4: significant disability; limited ambulation with assistance



Stage 5: complete invalidism; patient confined to bed or chair; cannot stand or walk even with assistance

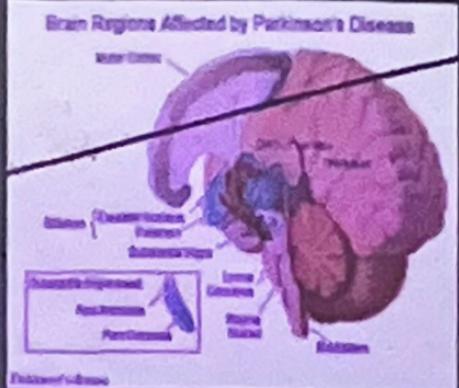
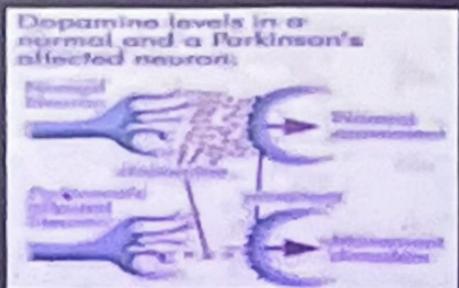
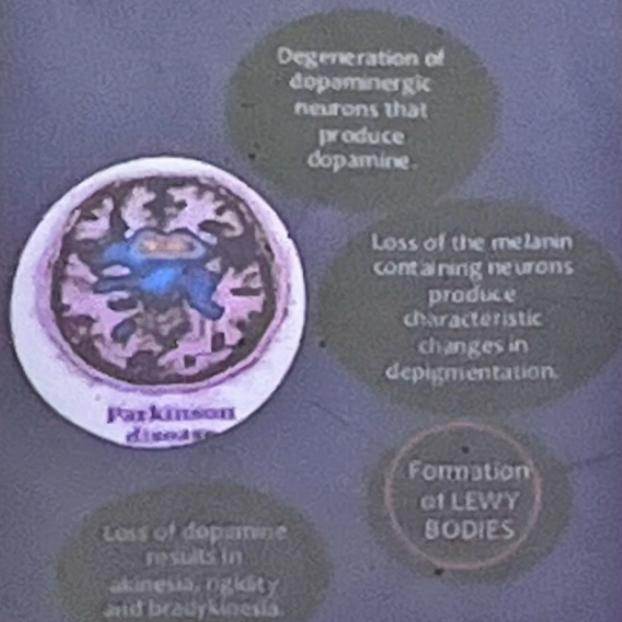
# Magnetic Resonance Imaging Scanner



MR scanner consists of electromagnet with 1.5 to 7 T

Earths Magnetic field is 0.00005 Tesla. 3T is 60,000 times stronger than earths magnetic field

## What happens in Parkinson's Disease?



Slide prepared by my  
Phd student  
Ms. Tanmayee

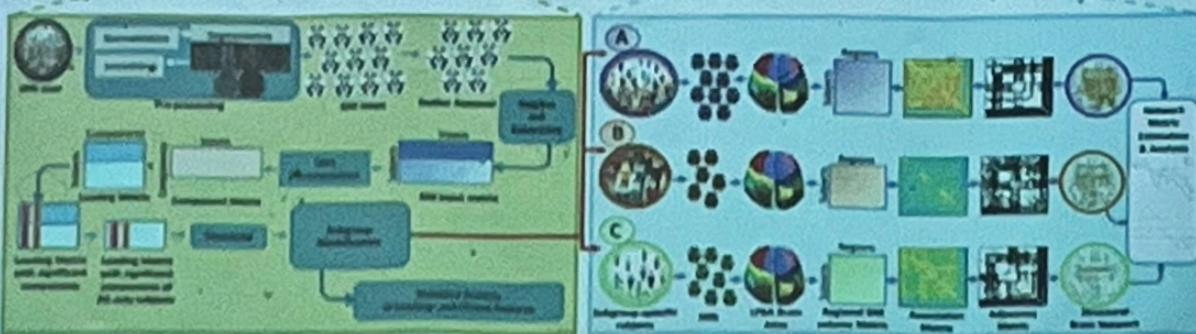
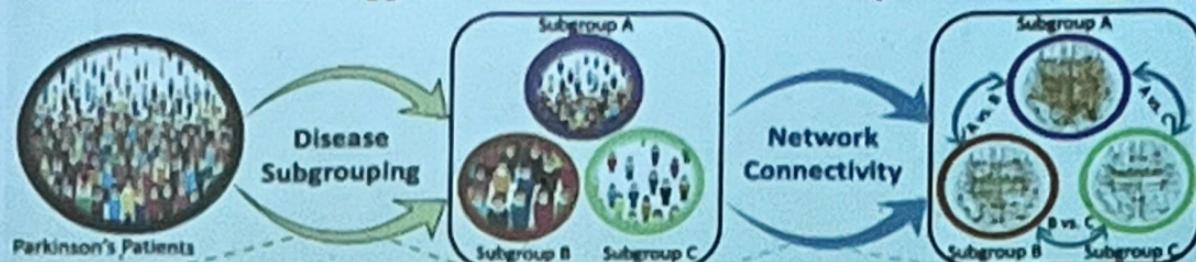
Alpha  
synuclein  
protein  
aggregates

## What Magnetic Resonance Imaging measures



- Body consist of multiple tissues which contain varying hydrogen (translates to differing signal intensity)
- Elements (H) with differing proton and neutrons in nucleus exhibit Non zero spin (Quantum mechanics)
- Different types of images can be generated for different tissue types (say gray, white and CSF in brain)
- No ionizing radiations used and is Non invasive.
- Looking at other nuclei is possible (Sodium in brain tumor).

# Subtying Parkinsons Individuals using Brain Connectivity

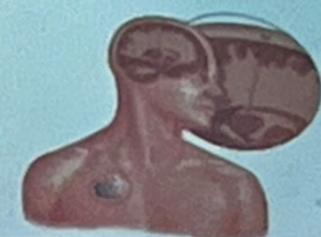


T.Samantaray, J.Saini, S.Pal and C.N.Gupta, "Brain Connectivity for Subtypes of Parkinson's Disease using structural MRI" *Under Review with Journal*

PARKINSONS CURRENT MEDICATIONS TREATMENT ??

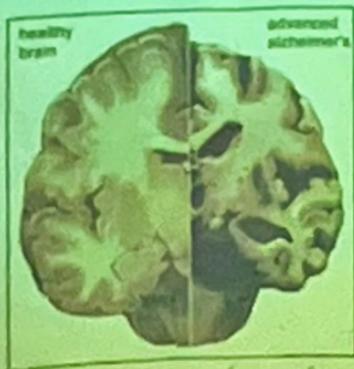
## PARKINSONS CURRENT TREATMENT (Mayoclinic, USA)

- Parkinson's disease can't be cured, but medications can help control symptoms, often dramatically
- Levodopa, the most effective Parkinson's disease medication, is a natural chemical that passes into your brain and is converted to dopamine
- Deep brain stimulation (DBS), surgeons implant electrodes to stimulate specific part of patients brain.
- Green Tea seems to reduce parkinson occurrence



What is Alzheimer's?? Any Idea

# Alzheimer's (Introduction)



Symptoms  
similar to  
ageing

Chronic neurodegenerative disease

Protein Aggregation,  
Age, multilingualism,  
Genetics, Brain Gray Matter etc.

Loss in memory, loss in attention,  
Confusion about places, Trouble  
completing tasks etc.

No Medication

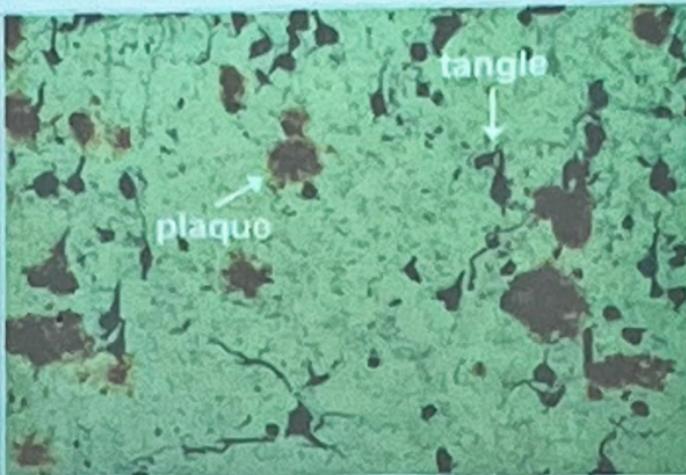
Multiple  
Factors

Life  
expectancy  
2-9 years

Content Integrated from various sources/articles

Proteins causing Alzheimer's??

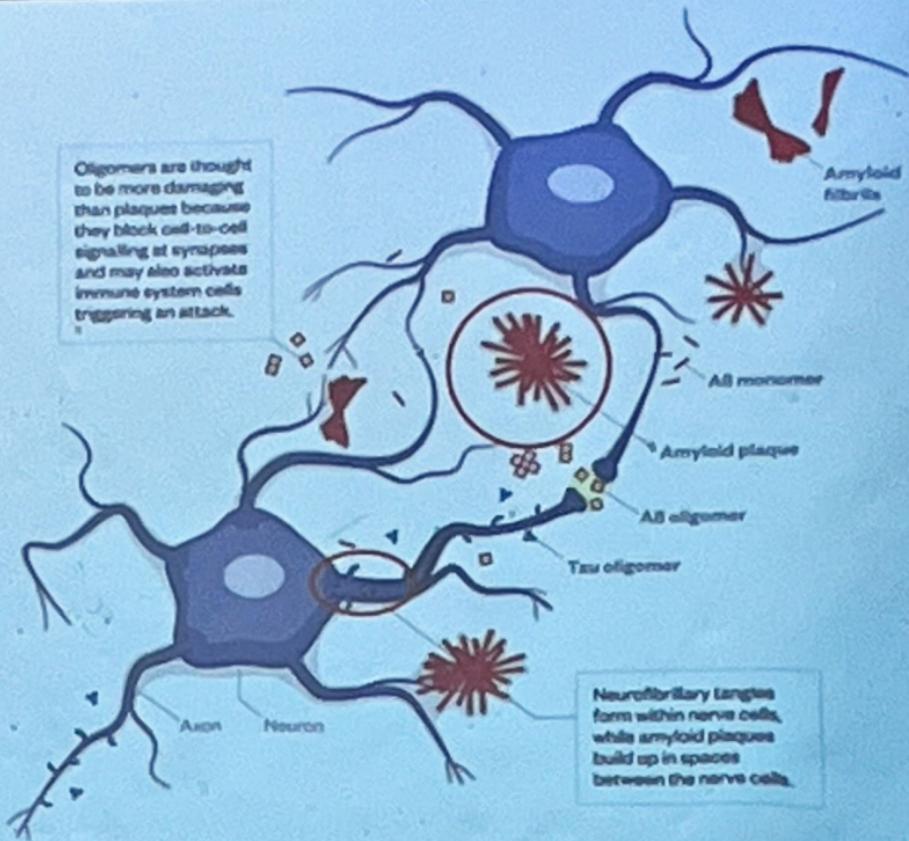
# Alzheimer's (Proteins Involved)



Histopathology of brain  
showing amyloid plaques  
and neurofibrillary tangles (Post mortem)

- Amyloid plaques  
(Alpha beta proteins)
- Neurofibrillary tangles  
(Tau proteins)

## Effect of Protein Aggregates (circled) on neural synapse in Alzheimer's



ALZHEIMERS CURRENT  
MEDICATIONS/TREATMENT ??

## ALZHEIMERS CURRENT TREATMENT (Mayoclinic, USA)

- Alzheimer's disease can't be cured as of today, but medications can help control your symptoms (unfortunately with side effects)
- Memantine (Namenda): This drug works in another brain cell communication network and slows the progression of symptoms with moderate to severe Alzheimer's disease.
- Inhibitors include donepezil (Aricept), galantamine (Razadyne)

**BIOPHYSICAL METHOD TO STUDY  
PROTEIN AGGREGATES??**

Cyto



Greek = Kytos  
"hollow basket"  
meaning CELL

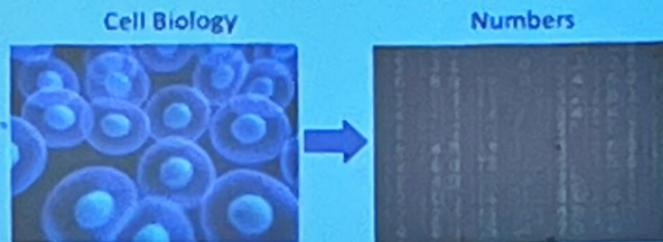
metry



Greek = Metria  
"process of  
measuring"

## WHAT IS CYTOMETRY

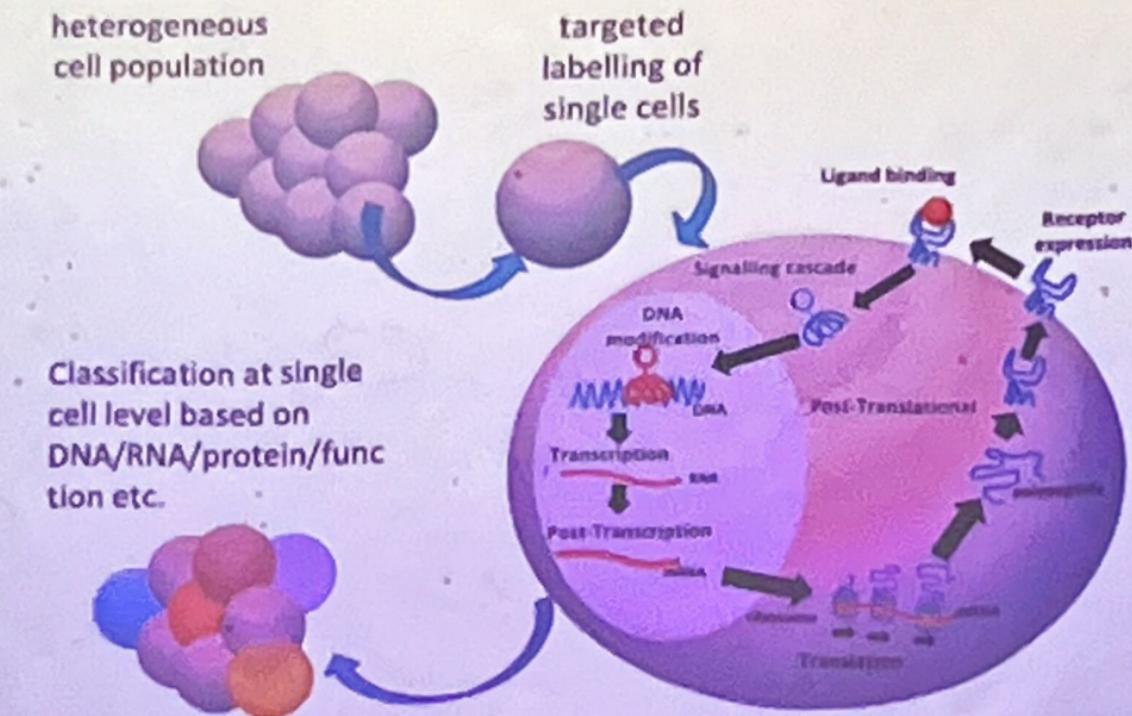
- The measurement of phenotype at a single cell level
- Conducted on a population of cells
- To understand heterogeneity in all systems



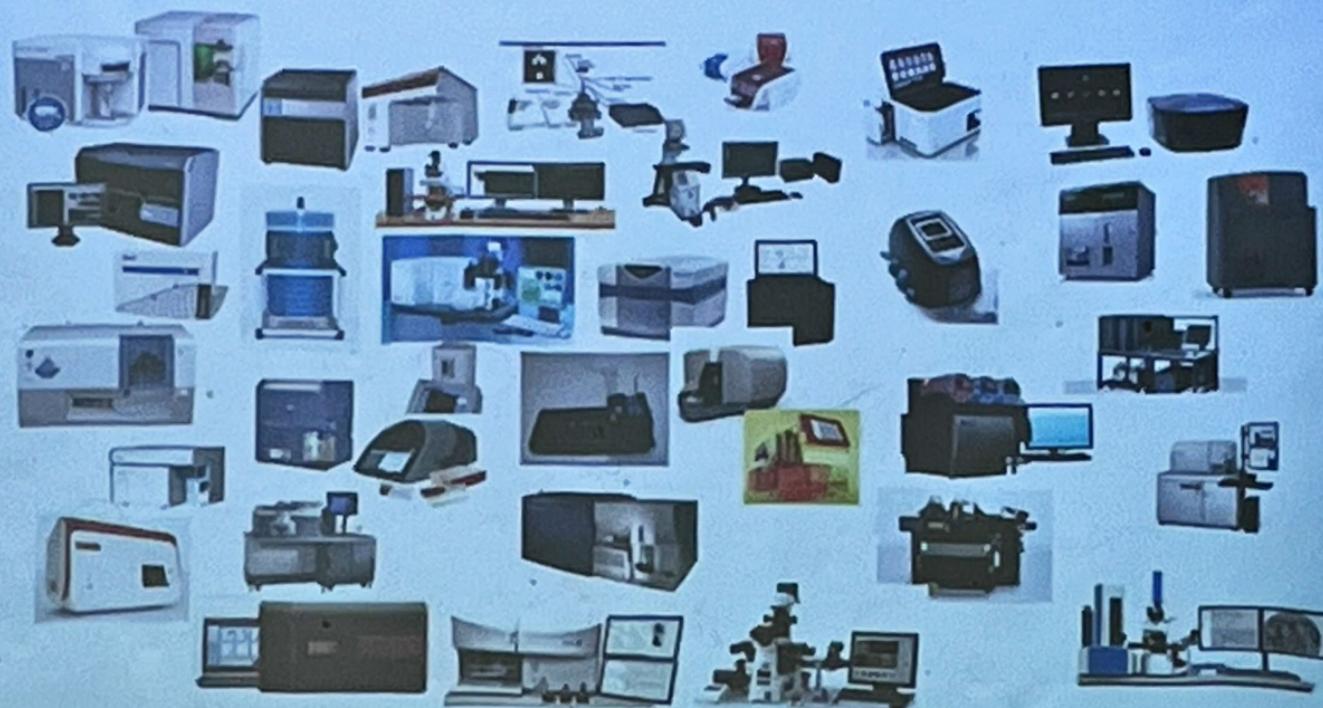
Questions we want to answer with these numbers:

- What types of cells are there and how many?
- What do they do?
- What role do they have in development/disease?

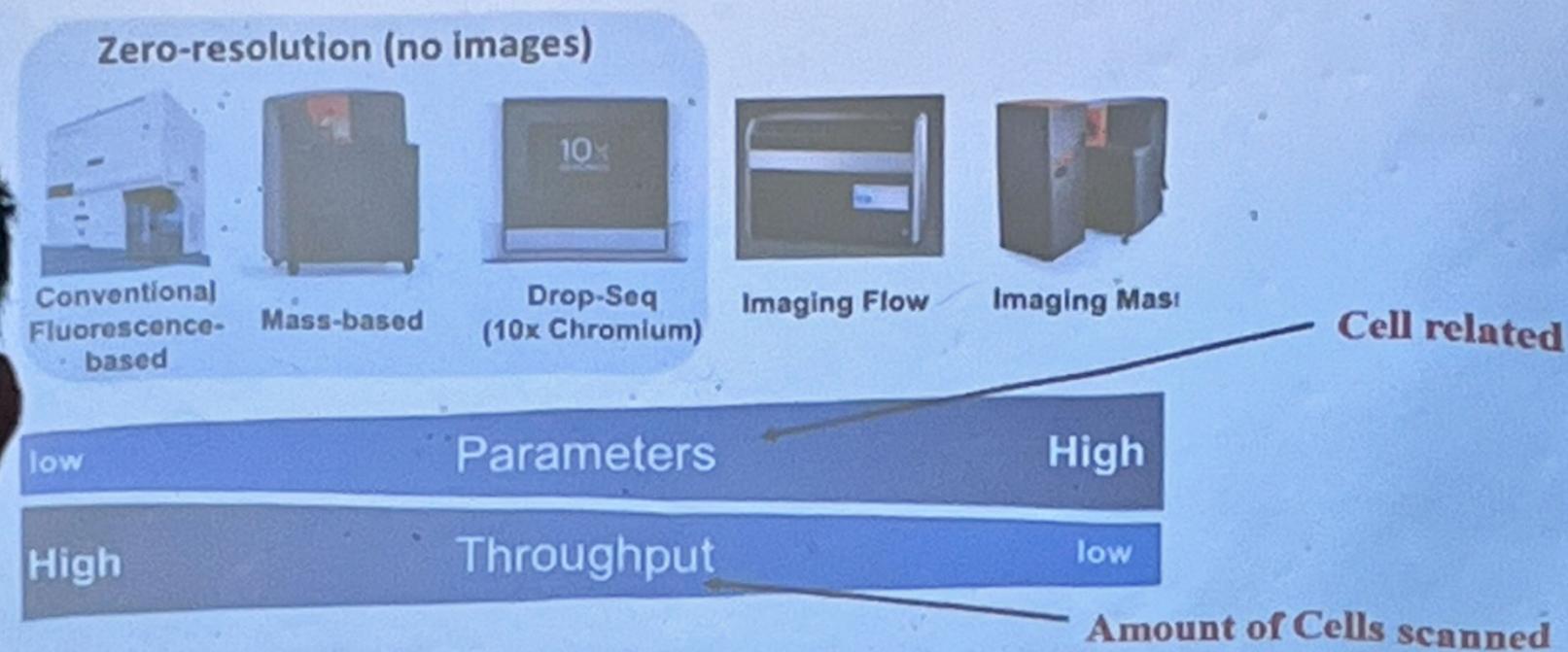
# Cytometry is the study of **EVERYTHING** single cell



## So many cytometers and ways to do cytometry



# Cytometers: The machines we use to measure the cells



**LETS STUDY FLOW CYTOMETRY**

# **FLOW Cytometry**



To move along  
in a stream

We make all cell/particle  
measurements in liquid  
**SUSPENSION**

Easy for samples  
derived from liquid  
biopsies(blood)

## The underlying principles of Flow Cytometry

### Fluidics

- Any Flow Cytometer is built around the unique principle of carrying particles of interest to a point of interrogation by means of a stable stream of fluid.
- Fluids are very difficult to compress and provide excellent stability of motion!



Flickr: Grand Canyon National Park

Apply pressure to Fluid and keep pressure constant over time, then Flow is constant

Flow is volume per time (Happens in RIVERS)

## **HYDRODYNAMIC FOCUSING**

# Hydrodynamic Focussing

Constant Pressure over volume

SHEATH FLUID

Stage 1

Isotonic solutions

LAMINAR FLOW

Fluid system which drives our cells forward at discrete pace with constant speed

Any particle injected will align in the middle of the stream

Cells samples focussed in time.

Fluidics

Single Cell Suspension in Cytometer

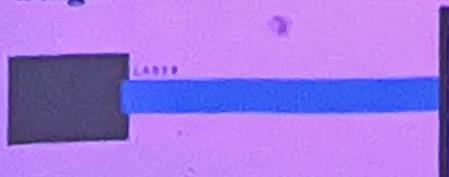
TAKING FLUIDICS TO REALITY

Stage 2

HYDRODYNAMIC FOCUSING

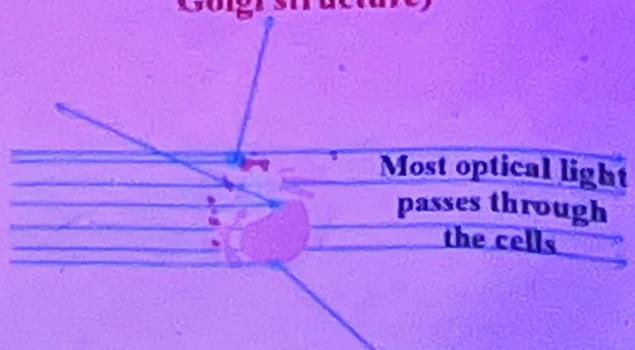
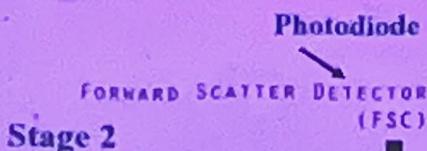
# Optics – Light Scattering

## Stage 1



Scattering is omnidirectional

Different optical densities in the cell (structures like nucleus, vesicles, Golgi structure)



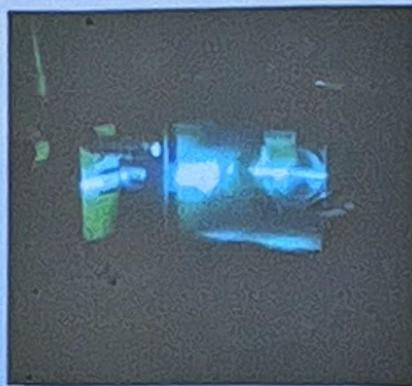
Most optical light passes through the cells

Obscuration bar is a horizontal piece of metal that blocks laser light but allows scattered light to pass over it and into the detector

## WHAT IS FLOW CYTOMETRY

### Optics – Signal generation and detection

- Flow Cytometers use lasers as coherent, focused and strong excitation light source to excite fluorescent dyes and provide light for particle scatter measurements
- Flow Cytometers convert fluorescent signals into scalable electric potentials which correlate in strength with the number of emitted fluorescent photons



## Hydrodynamic Focussing

Constant Pressure over volume

SHEATH TANK

Stage 1

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Fluid system which drives our cells forward at discrete pace with constant speed

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Cells samples focussed in time,

## TAKING FLUIDICS TO REALITY

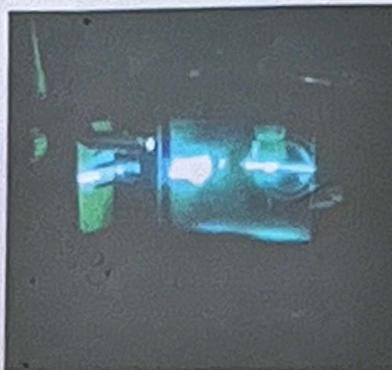
Stage 2

HYDRODYNAMIC FOCUSING

## WHAT IS FLOW CYTOMETRY

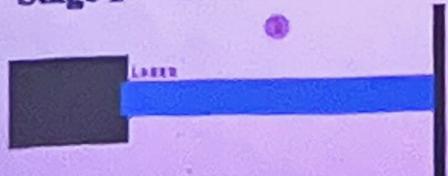
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# Optics – Light Scattering

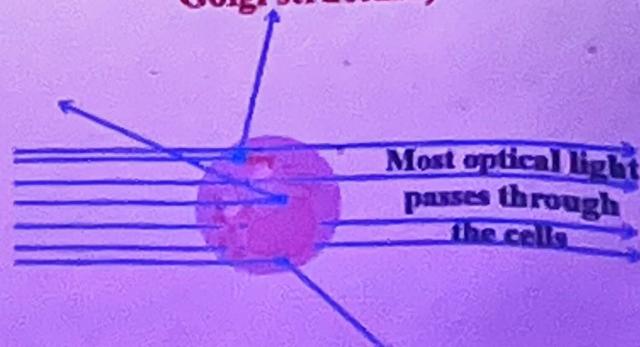
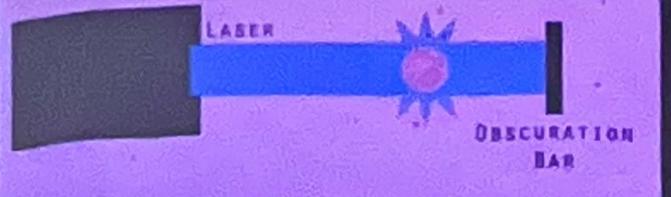
Stage 1



Scattering is omnidirectional

Different optical densities in the cell (structures like nucleus, vesicles, Golgi structure)

Stage 2



Obscuration bar is a horizontal piece of metal that blocks laser light but allows scattered light to pass over it and into the detector

## Flow Cytometric Analysis of Protein Aggregates

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### Abstract

**Background:** Misfolding of proteins often leads to aggregation. Accumulation of diverse protein aggregates in various cells, tissue and organs is the hallmark of many diseases, such as Alzheimer's disease and Parkinson's disease.

**Results:** Autofluorescence and scattering intensities could distinguish between amyloid and nonamyloid aggregates. Dot plots of both side scattering (SSC) and forward scattering (FSC) intensities also showed characteristic fingerprint of both the types of aggregates when compared with those of well known nanoparticles of oxides of Fe and Cu.

How does Protein Unfold

## Protein Unfolding (EGG)

- Increase Temperature (except primary structure all higher forms broken)
- Add Vinegar (disruption of ionic bonds- tertiary, quartenary broken).
- Chemicals (add alcohol) (except primary structure all higher forms broken)
- Enzymes (lets say we eat the egg then digestive system breaks it into amino acids for our body to absorb it).