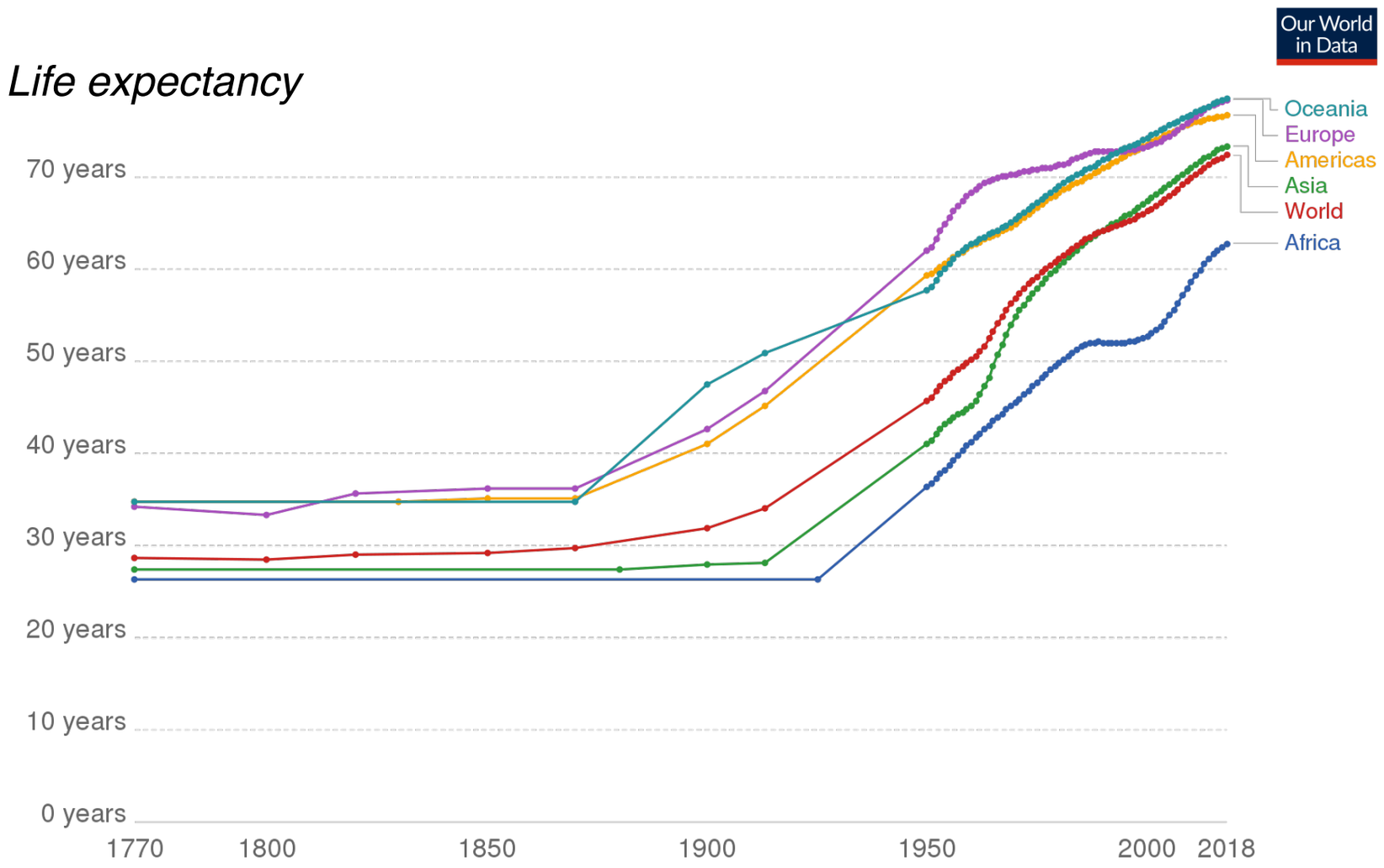
Protein Misfolding and Degenerative Diseases

Aaron Trowbridge

Literature Review
27th January 2021

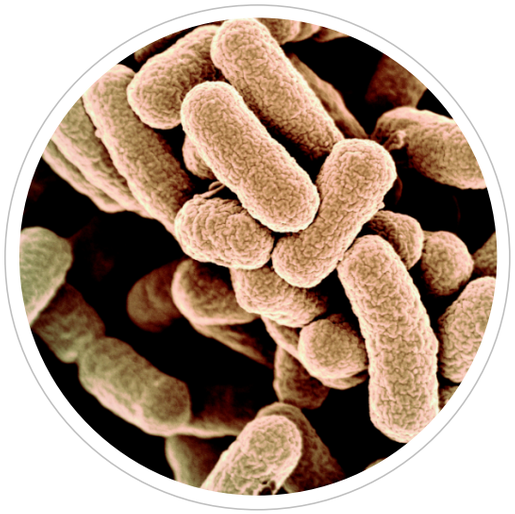
Global mortality linked to disease



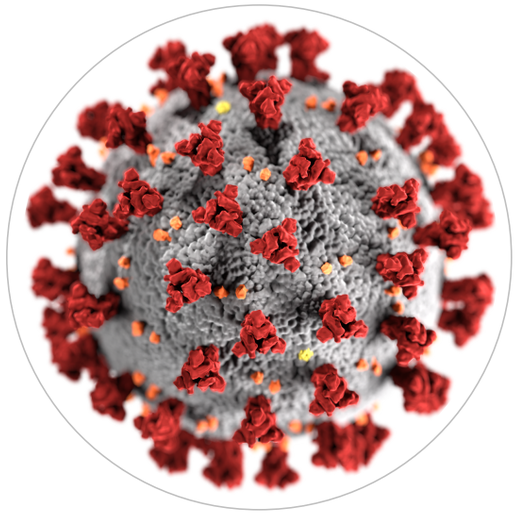
Source: Riley (2005), Clio Infra (2015), and UN Population Division (2019)
Note: Shown is period life expectancy at birth, the average number of years a newborn would live if the pattern of mortality in the given year were to stay the same throughout its life.

The major causes of global deaths have changed profoundly over the last 200 years

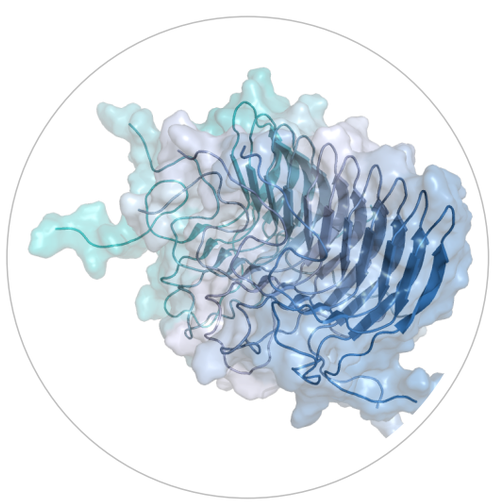
Global mortality linked to disease



Bacterial infections
(e.g. tuberculosis)



Viral infections
(e.g. influenza)

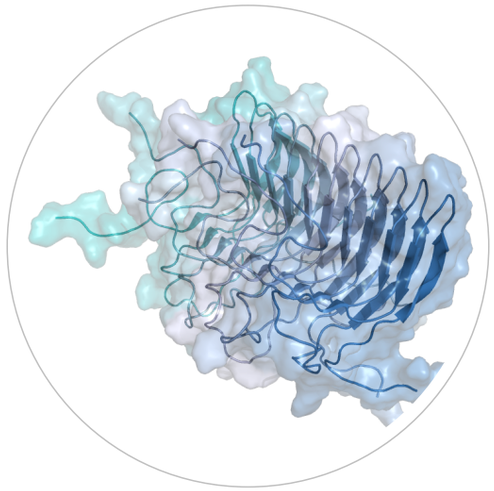


Proteopathic diseases
(e.g. AD, Parkinson's)

*Approximately **40%** of human diseases may be attributable to protein misfolding*

Leidenheimer, N. J., Ryder, K. G. Pharmacological chaperoning: a primer on mechanism and pharmacology, *Pharmacy. Res.*, **83**, 10–19, (2014)

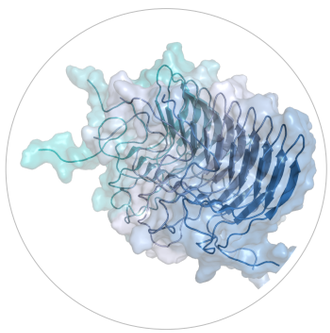
Global mortality linked to disease



Proteins participate in virtually every process within the cell

Functional impairment of proteins can be devastating

What are the genetic and molecular causes for incorrectly formed proteins?

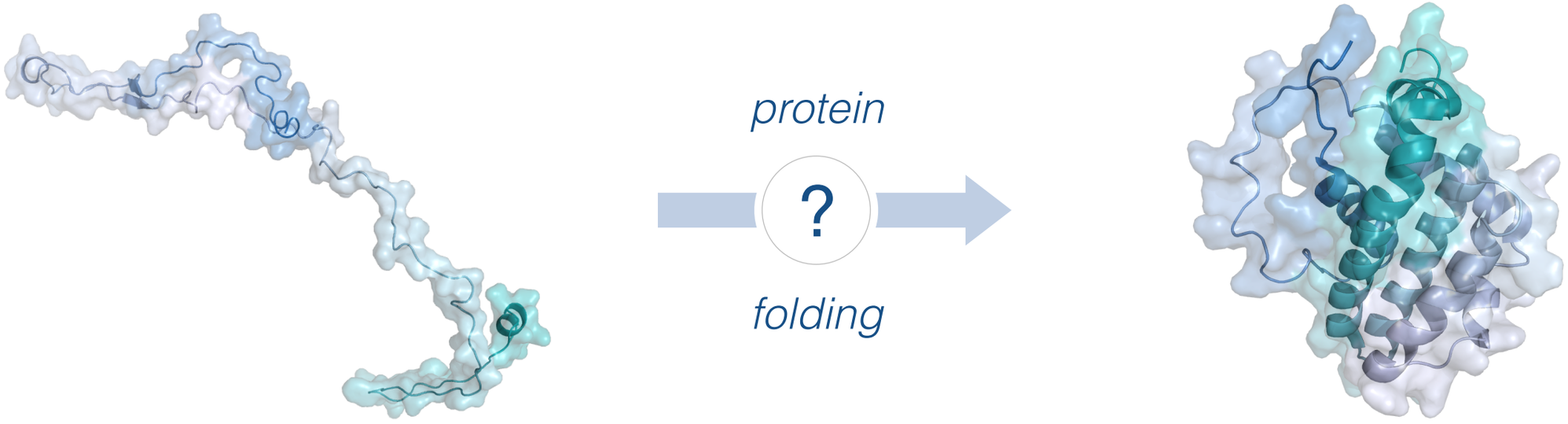


Protein Misfolding and Degenerative Diseases

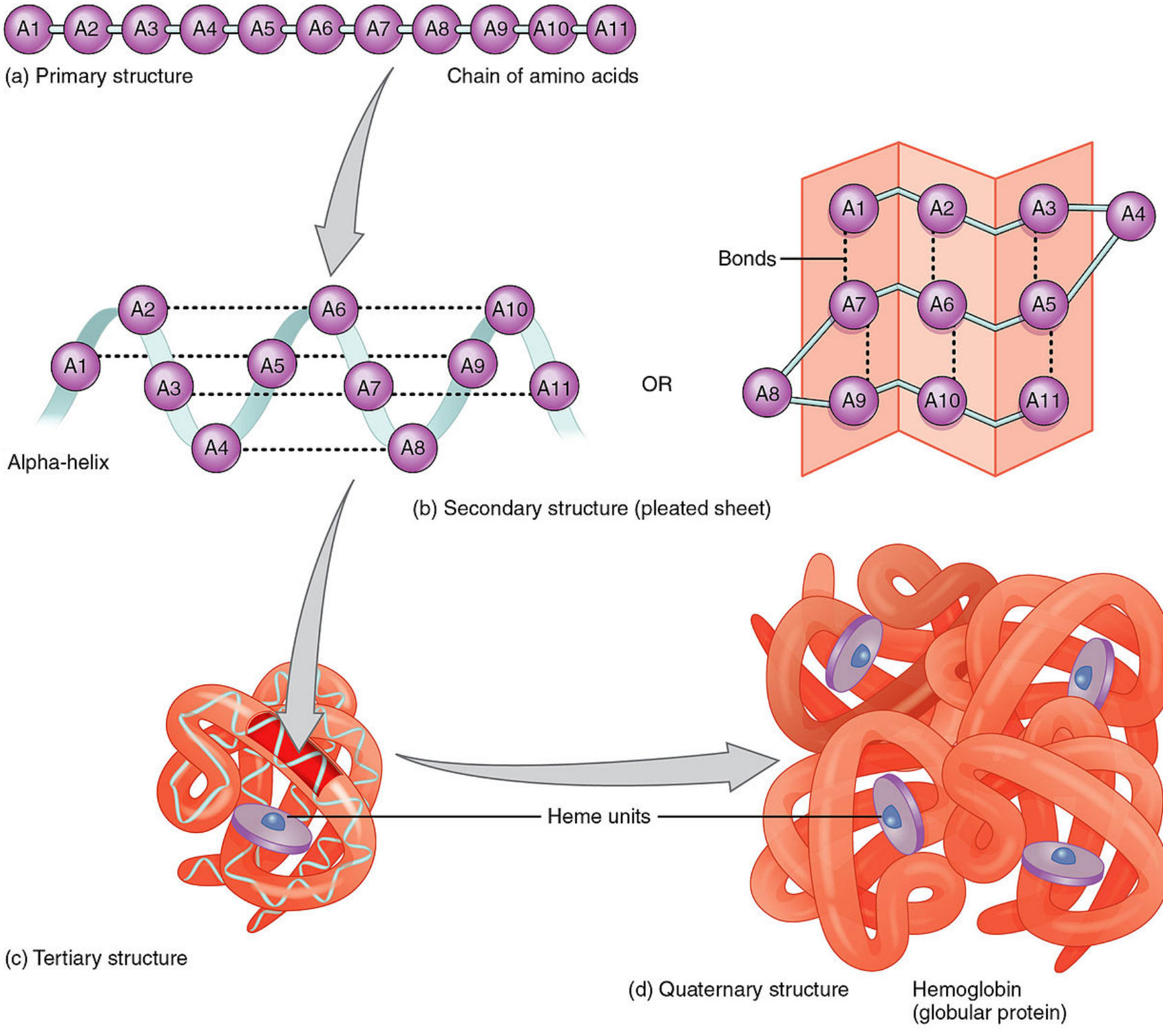
- Protein function and three-dimensional structure
- The energetic funnel
- Chaperones
- Misfolding in neurological diseases
- Misfolding in other diseases
- Looking forward and treatments
- DeepMind

Protein Function and Three-Dimensional Structure

In 1917, the German chemist Hermann Staudinger proposed that organic molecules such as proteins were organised into polymers, for which he received the Nobel prize in 1953.



Protein Function and Three-Dimensional Structure



Protein folding is driven by a series of interactions

Protein folding is fast…but should it be?

Levinthal’s paradox

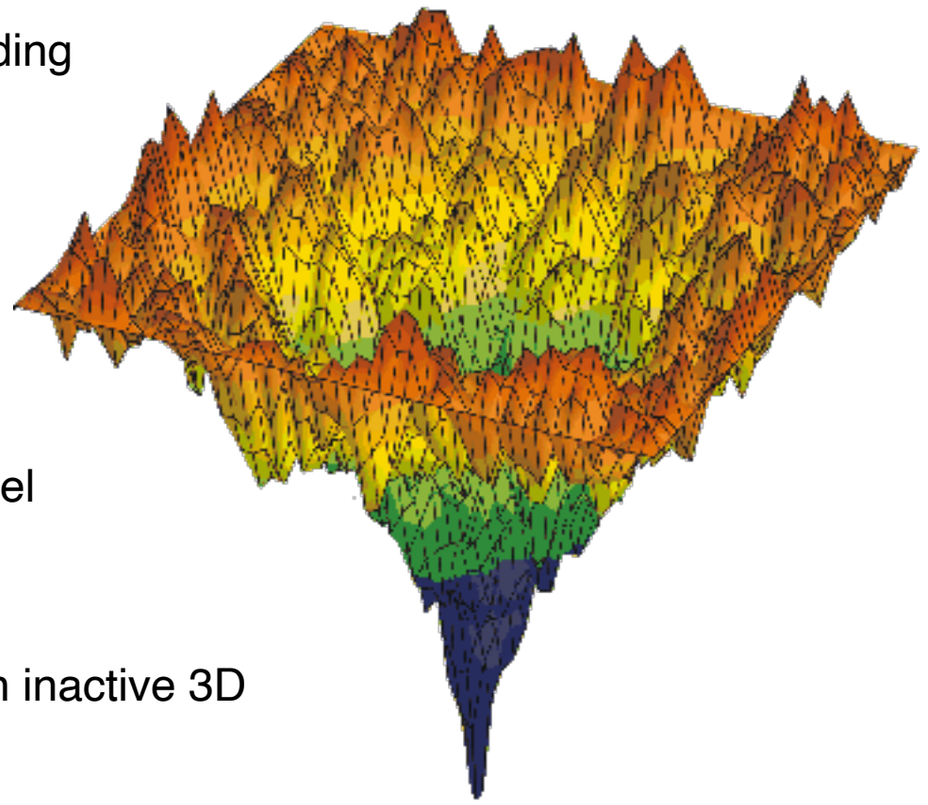
A protein with *N* amino acids should have 10^{2N} degrees of freedom. Thus a small protein (e.g. 150 amino acids) would have 10^{300} degrees of freedom. There is not enough time in the universe to try each of these combinations, and yet the protein is **folded within a second**.

Melkikh, A. V., Meijer, D. K. F., On a generalized Levinthal's paradox: The role of long- and short range interactions in complex bio-molecular reactions, including protein and DNA folding, *Progress in Biophysics and Molecular Biology*, **132**, 57–79, (2018)

Protein Function and Three-Dimensional Structure

Potential Energy Funnel

- 3D landscape representing the barriers towards folding
- Top rim represents high energy unfolded protein
- Minimum energy structure at the bottom of the funnel
- Numerous local minima that can trap a protein in an inactive 3D conformation



*This surface represents the '**dry state**' where protein folding is slow and challenging*

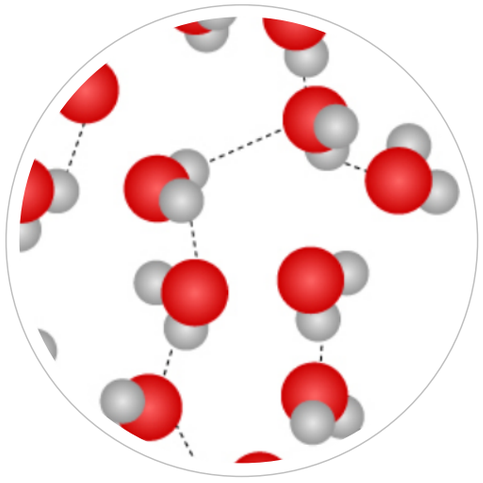
Riback, J. A., Bowman, M. A., Zmyslowski, A. M., Knoverek, C. R., Jumper, J. M., Hinshaw, J. R., Kaye, E. B., Freed, K. F., Clark, P. L., Sonic, T. R., Innovative scattering analysis shows that hydrophobic disordered proteins are expanded in water, *Science,* **358**, 238–241, (2017)

Protein Function and Three-Dimensional Structure

Protein folding is governed by many factors
*including **pH**, **ionic strength**, **temperature**, and **cofactors***

Water

The rearrangement of water around unfolded protein chains releases energy



- Protein folding is mainly driven by the '**hydrophobic effect**' through the burial of non-polar side chains

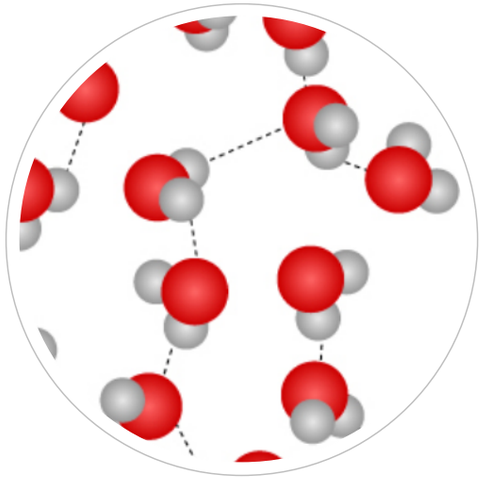
- Water adjacent to a hydrophobic group loses a hydrogen bond, therefore has **increased enthalpy**

- To overcome this the network expands to form lower-density water with a **lower entropy**

Dill, K. A. Dominent forces in protein folding, *Biochemistry,* **29**, 7133–7155, (1990);
Harano, Y., Kinoshita, M. Translational-Entropy Gain of Solvent upon Protein Folding, *Biophys. J.,* **89**, 2701–2710, (2005)

…an alternative view

- Protein conformation tends to minimise the disruption of the water matrix

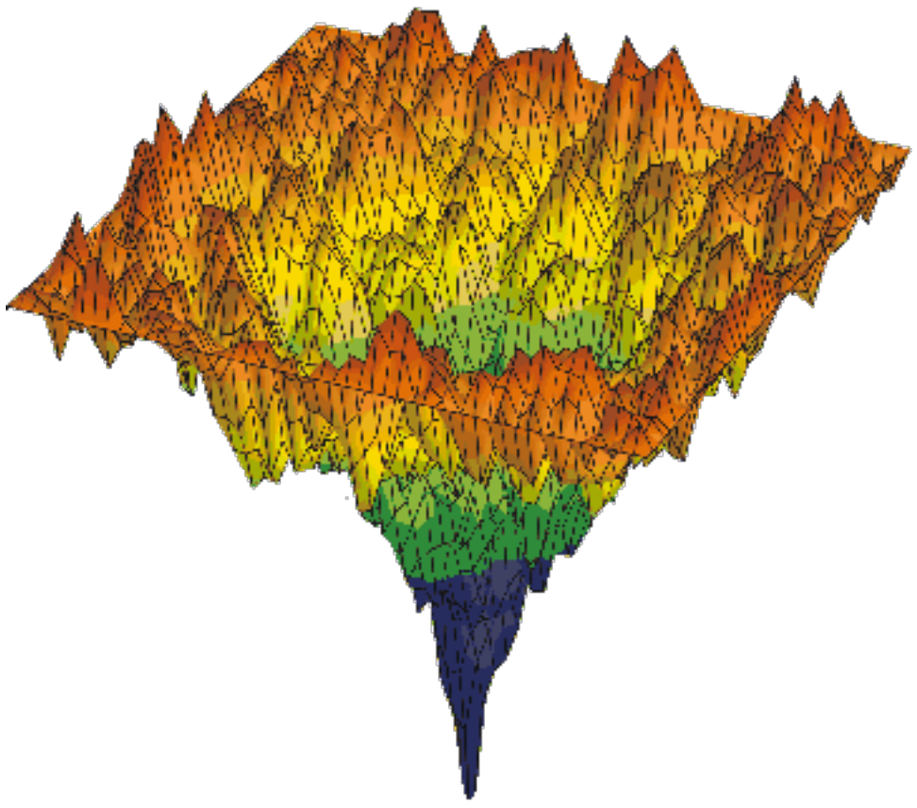


- Collapse of water around hydrophobic residues is accompanied by structural H-bond formation and the formation of strong ion-paired salt links

- A driving force for this is the release of water to be available for the hydration of other solutes, hence **maximising entropy**

Fernández, A., The principle of minimal epistemic distortion of the water matrix and its steering role in protein folding, *J. Chem. Phys.*, **139**, 085101, (2013); Fernández, A., Kardos, J., Goto, Y., Protein folding: could hydrophobic collapse be coupled with hydrogen-bond formation? *FEBS Lett.*, **536**, 187–192, (2003); Chen. S., Itoh, Y., Masada, T., Shimizu, S., Zhao, J., Ma, J., Nakamura, S., Okuro, H., Noguchi, H., Uosaki, K., Aida, T., Subnanoscale hydrophobic modulation of salt bridges in aqueous media, *Science*, **348**, 555–559, (2015); Harano, Y., Roth, R., Kinoshita, M., On the energetic of protein folding in aqueous solution, *Chem. Phys. Lett.*, **432**, 275–280, (2006)

Protein Function and Three-Dimensional Structure



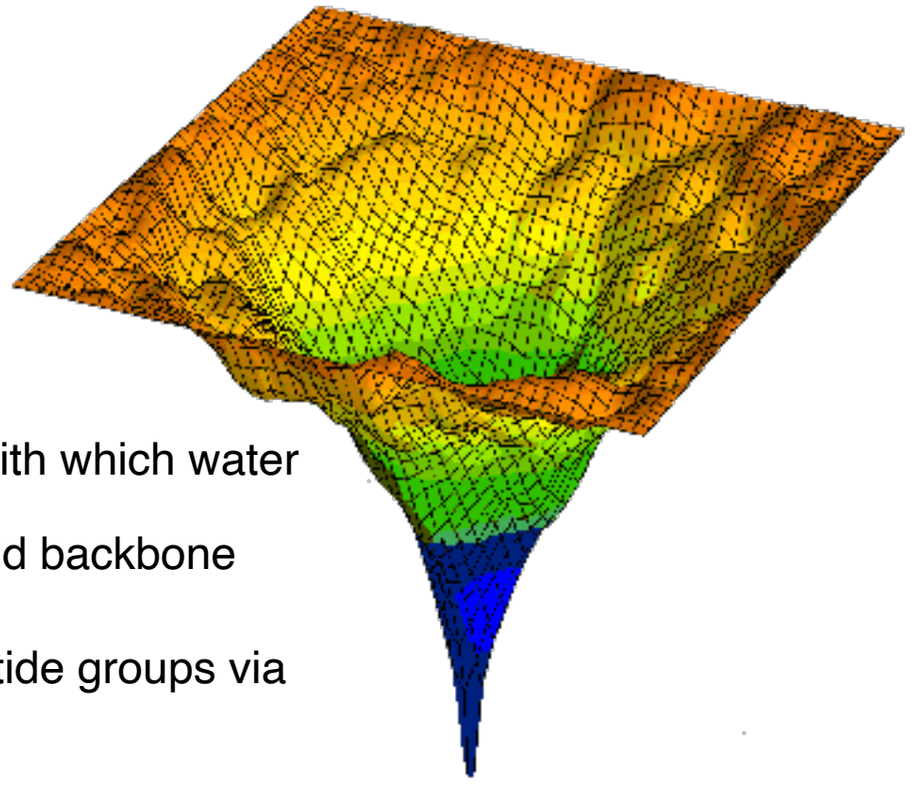
Dry state

Riback, J. A., Bowman, M. A., Zmyslowski, A. M., Knoverek, C. R., Jumper, J. M., Hinshaw, J. R., Kaye, E. B., Freed, K. F., Clark, P. L., Sonic, T. R., Innovative scattering analysis shows that hydrophobic disordered proteins are expanded in water, *Science*, **358**, 238–241, (2017)

Protein Function and Three-Dimensional Structure

Potential Energy Funnel

- When hydrated, the potential energy landscape is considerably smoother
- Minimum energy conformation can be attained rapidly
- Potential energy barriers lowered due to the ease with which water molecules can lubricate the movement of the amino acid backbone
- Amino acid side chains reduce the hydration of peptide groups via **shielding**, promoting folding



…a case study

Sieradzan, A. A., Lipska, A. G., Lubecka, E. A., Shielding effect in protein folding, *J. Mol. Graph. Model.* **79**, 118–132, (2018)

Case study:

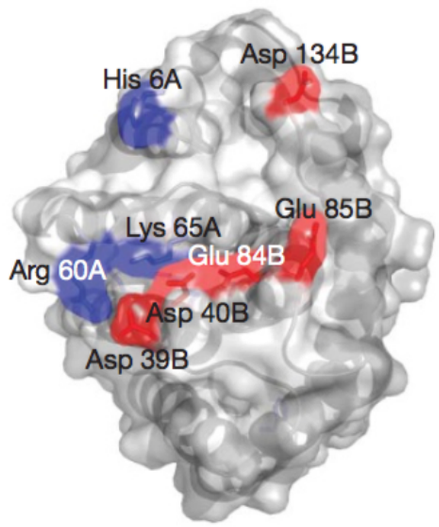
How do spiders avoid premature aggregation of silk (spidroin)?

Protein Function and Three-Dimensional Structure

Euprosthenops australis

Spidroins are stored at remarkably high concentrations (30–50% w/w) in the spider silk gland

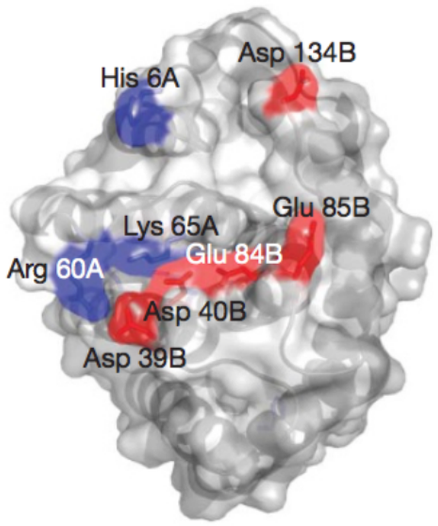
Spidroin is amphiphilic



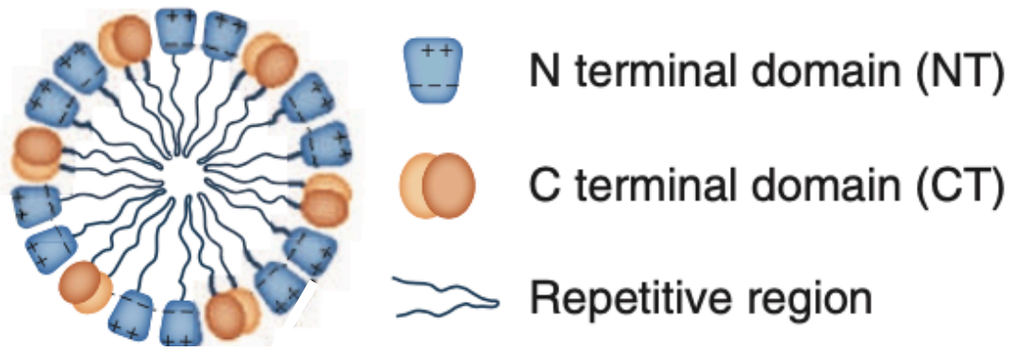
*Most of the charged residues are exposed in the dimer; **basic** residues at the positive pole and **acidic** residues at the negative poles*

Askarieh, G., Hedhammar, M., Nordling, K., Saenz, A., Casals, C., Rising, A., Johansson, J., Knight, S. D., Self-assembly of spider silk proteins is controlled by a pH-sensitive relay, *Nature*, **465**, 236–239

Spidroin is amphiphilic



Clustering of acidic residues increases the pK_a of the side chain residues by several pH units



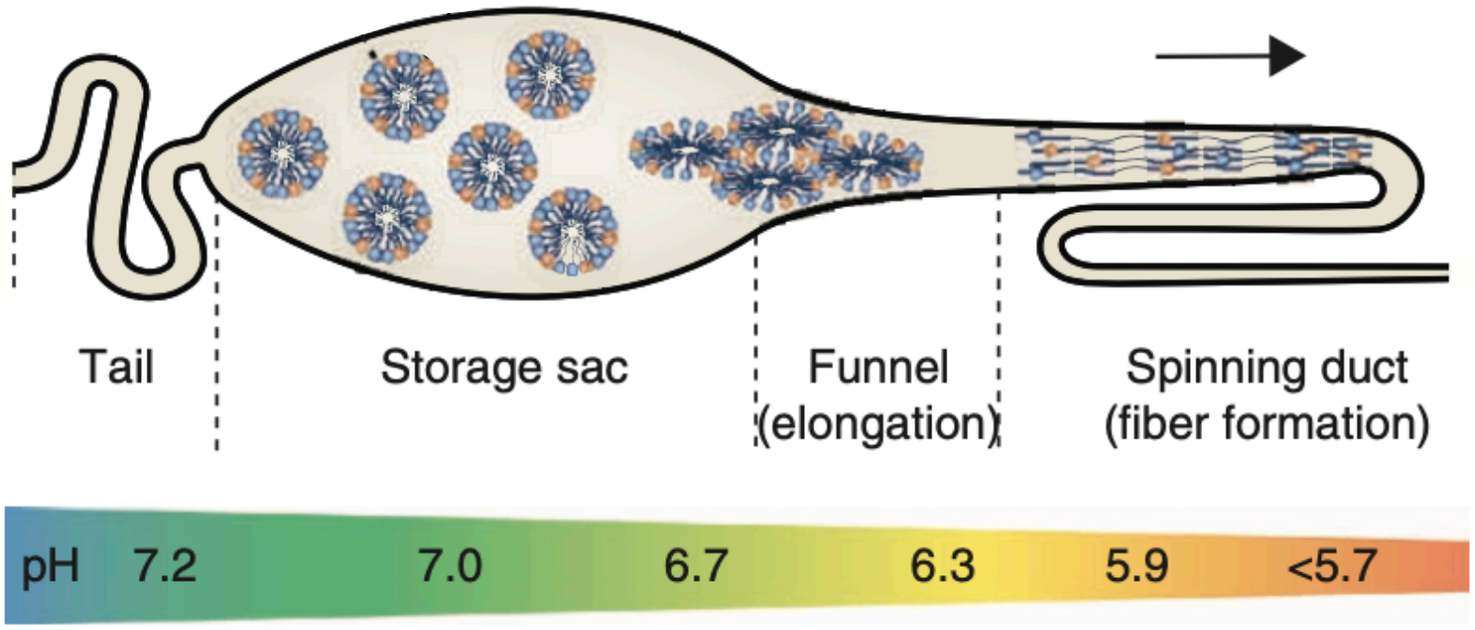
*Spidroins form **micelles**, where the hydrophilic NT and CT domains shield hydrophobic regions*

Askarieh, G., Hedhammar, M., Nordling, K., Saenz, A., Casals, C., Rising, A., Johansson, J., Knight, S. D., Self-assembly of spider silk proteins is controlled by a pH-sensitive relay, *Nature*, **465**, 236–239

Protein Function and Three-Dimensional Structure

The charge distribution provides a handle that can be precisely controlled by pH

This allows the intrinsic pH gradient of spider silk glands to regulate silk formation



pH = 7
self-assembly is slow (~2 h)

pH = 6
self-assembly is rapid (<5 min)

Askarieh, G., Hedhammar, M., Nordling, K., Saenz, A., Casals, C., Rising, A., Johansson, J., Knight, S. D., Self-assembly of spider silk proteins is controlled by a pH-sensitive relay, *Nature*, **465**, 236–239

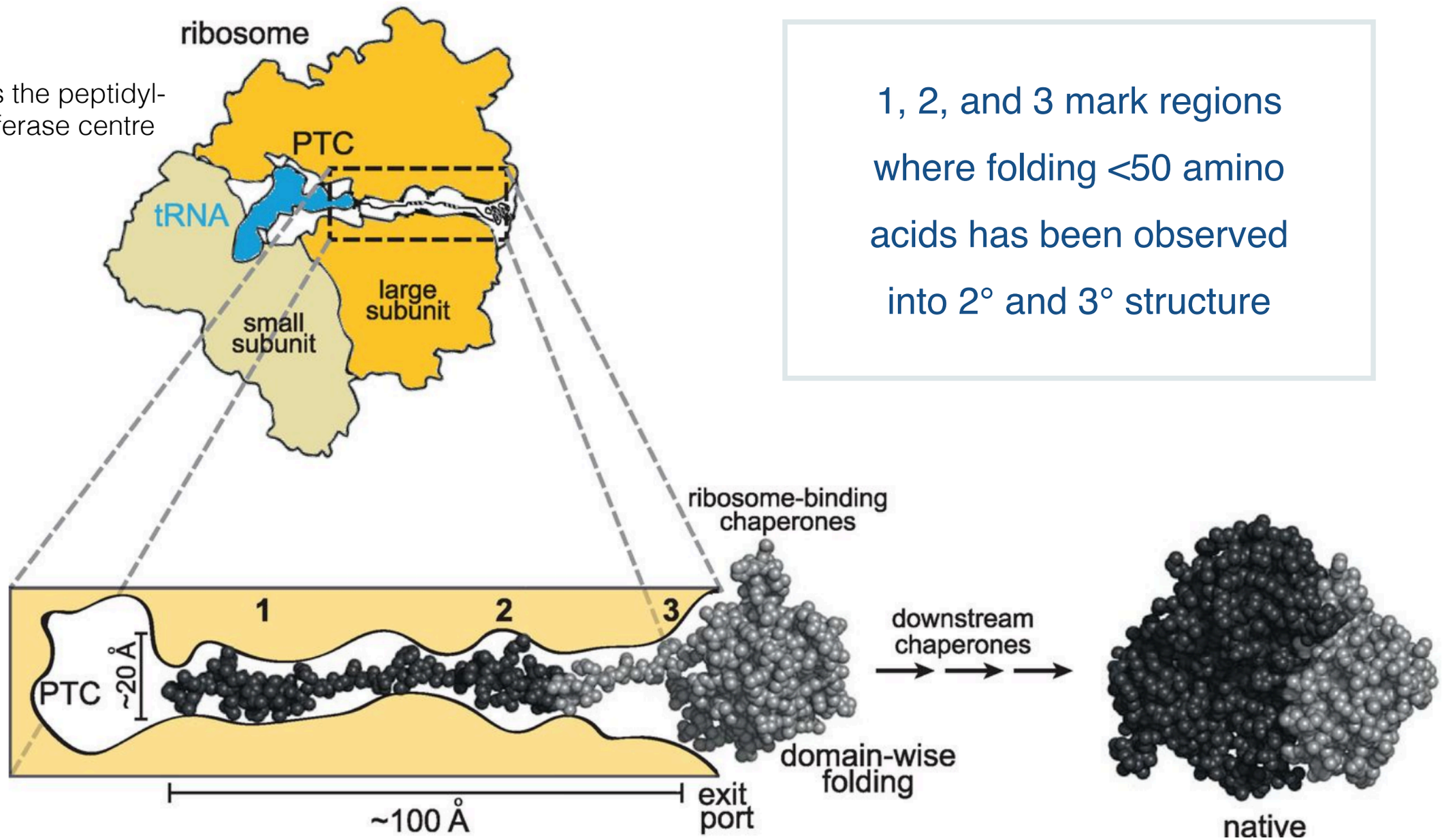
How does protein folding take place in cells?

Translation is slow (100aa ~ 25 s) compared to folding and so occurs co-translationally

Protein Function and Three-Dimensional Structure

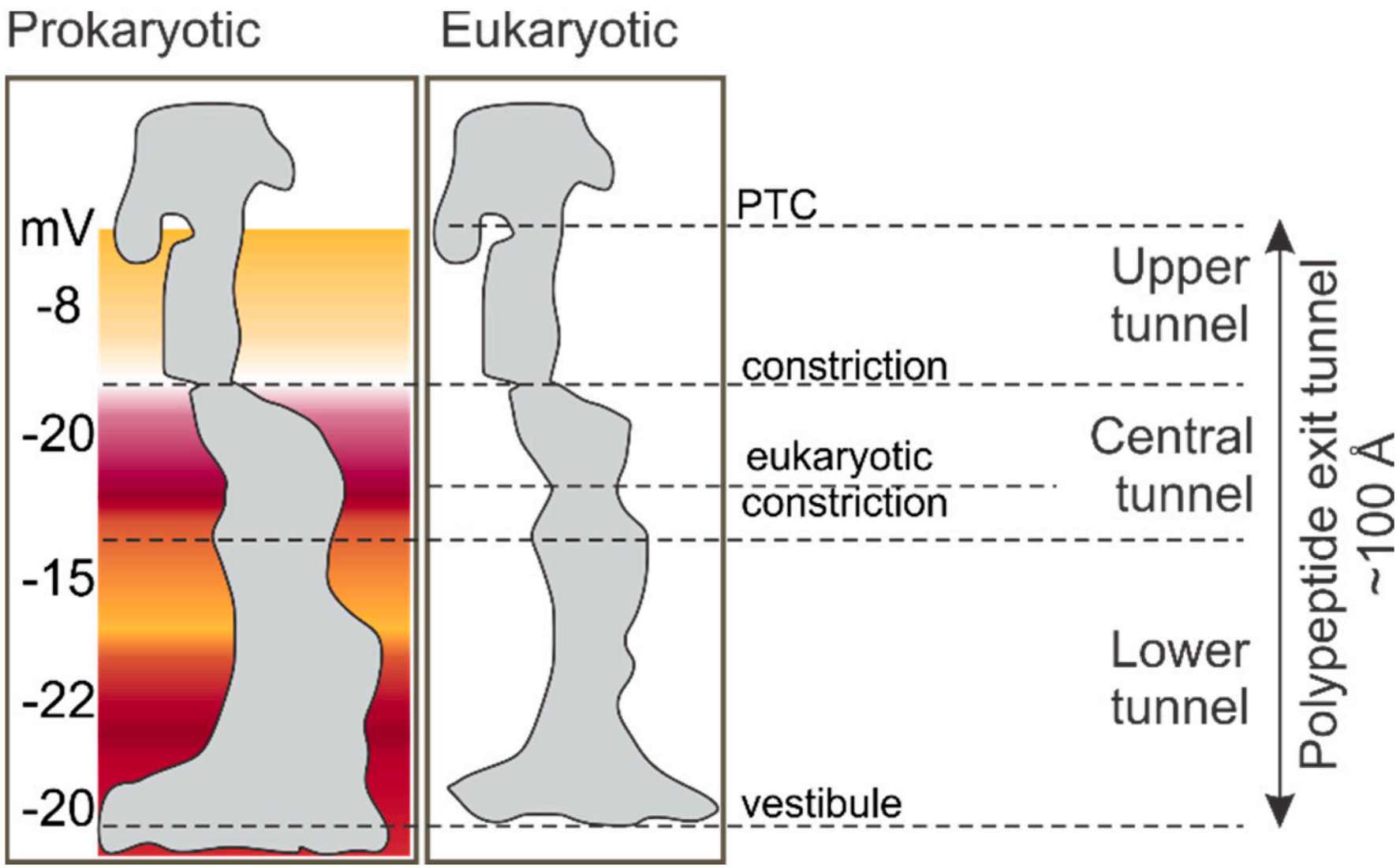
Protein folding starts in the ribosome tunnel

PTC is the peptidyl-transferase centre



1, 2, and 3 mark regions where folding <50 amino acids has been observed into 2° and 3° structure

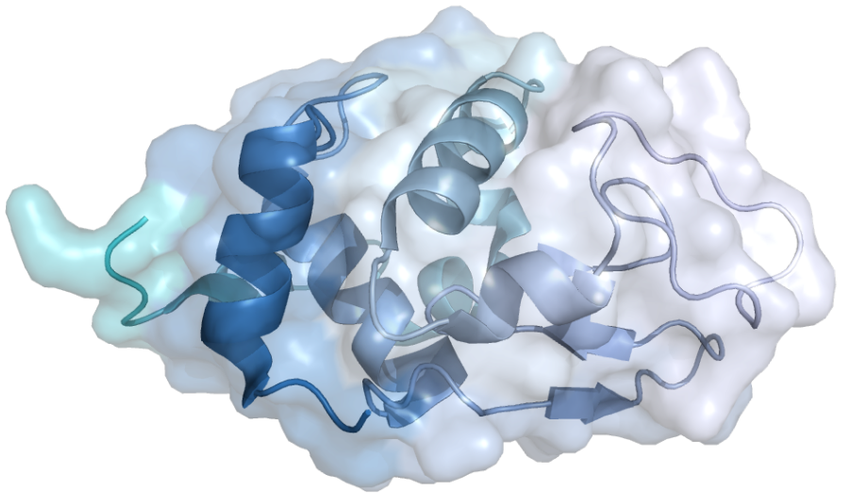
Wilson, D. N., Beckmann, R., The ribosomal tunnel as a functional environment for nascent polypeptide folding and translational stalling, *Curr. Opin. Struct. Biol.* **21**, 274–282, (2011)



Uneven tunnel geometry and electrostatics speed up and slow down folding

Protein folding does not occur by one path

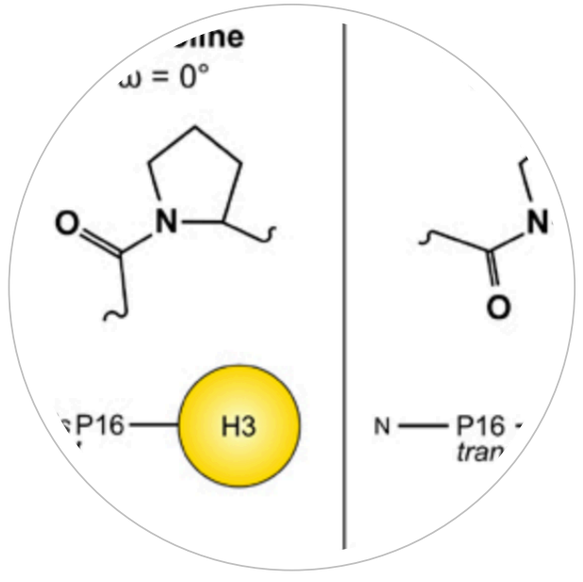
Protein Function and Three-Dimensional Structure



Hen lysozyme (14.3 kDa)

As proteins increase in size, the barriers to reorganisation in the collapsed state are ***larger*** *- resulting in multiple 'pathways' to the native state*

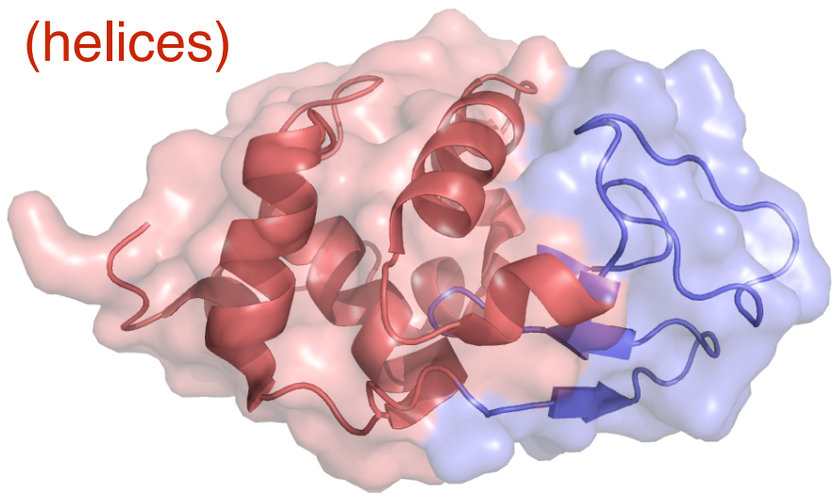
Real-life kinetics differ significantly from single exponential behaviour, and distinct folding populations are created by the accumulation of long-lived intermediates along particular pathways



Kim, P. S., Baldwin, R. L., Intermediates in the folding reactions of small proteins, *Annu. Rev. Biochem.*, **59**, 631–660, (1990)

Protein Function and Three-Dimensional Structure

α-domain
(helices)



β-domain
(sheets)

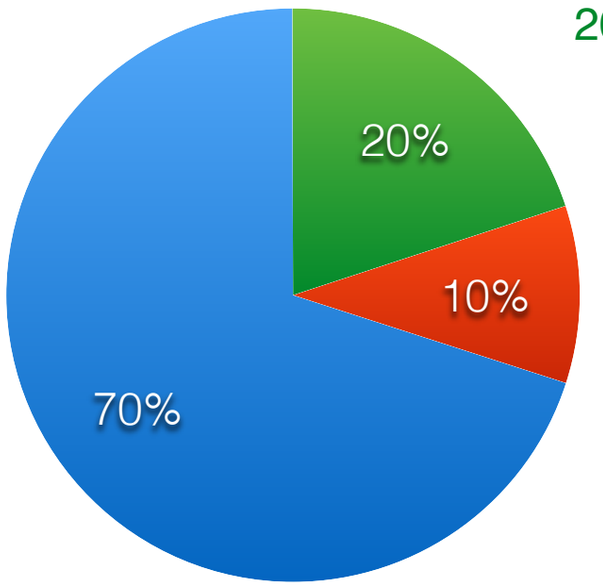
Significantly populated intermediates that

have both native and non-native interactions

Resulting in extreme heterogeneity

20% molecules fold to the native state <100 ms

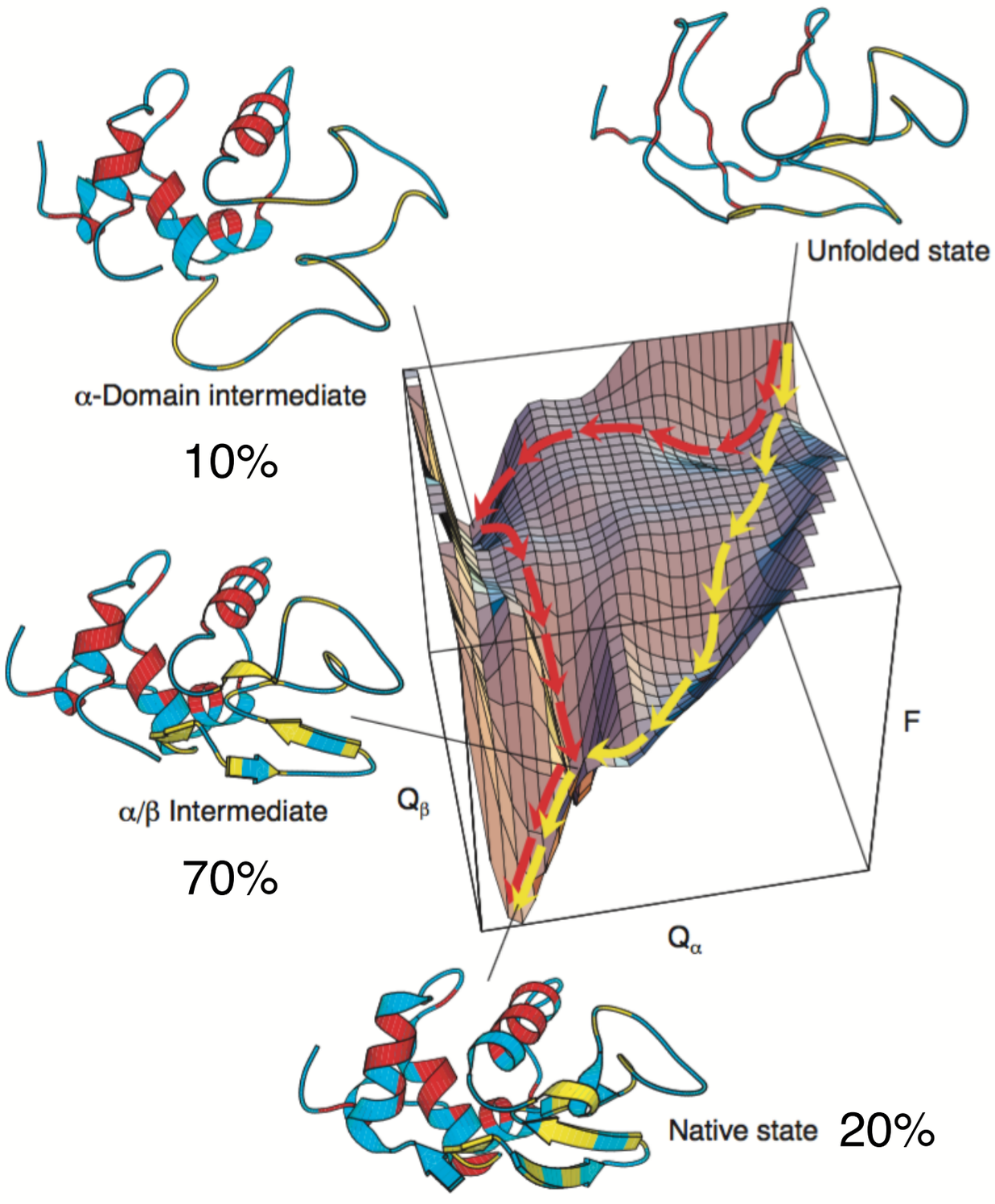
70% molecules populate an intermediate with persistent structure in the α-domain, followed by folding to the native state within 400 ms



10% molecules fold extremely slowly
(limited by proline isomerization)

Dinner, A. R., Šali, A., Smith L. J., Dobson, C. M., Karplus, M. Understanding protein folding via free-energy surfaces from theory to experiment, *Trends in Biochem. Sci*. **25**, 331–339, (2000)

Protein Function and Three-Dimensional Structure



Yellow Trajectory:

The α and β domains form concurrently, and the free energy decreases almost monotonically towards the native state (the 'fast track')

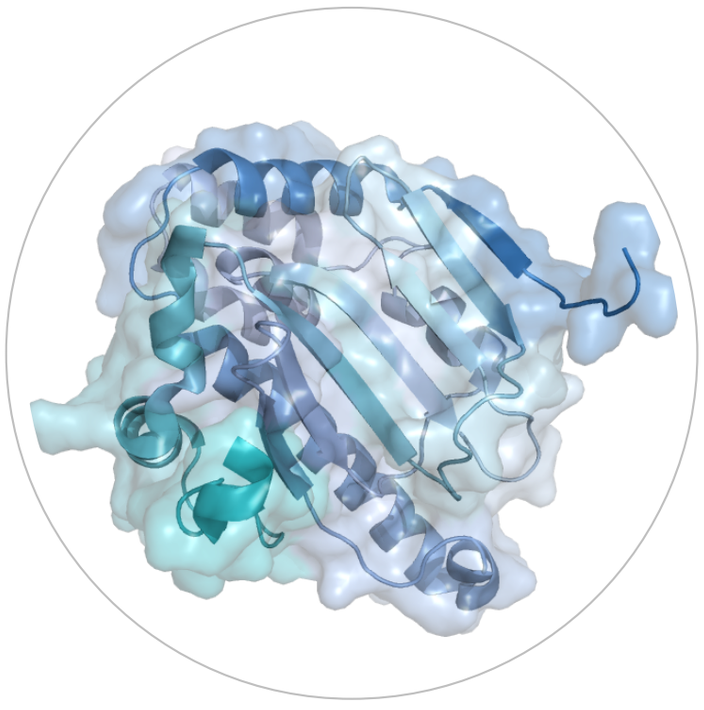
Red Trajectory:

Formation of the α domain precedes the β domain, and becomes trapped in a minimum. The α domain partially unfolds (reversal in the trajectory) to form the β domain.

Dinner, A. R., Šali, A., Smith L. J., Dobson, C. M., Karplus, M. Understanding protein folding via free-energy surfaces from theory to experiment, *Trends in Biochem. Sci.* **25**, 331–339, (2000)

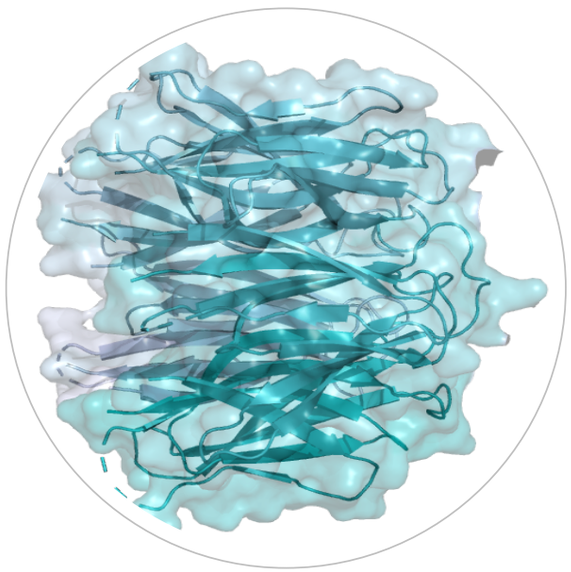
Protein Function and Three-Dimensional Structure

Bifurcating pathways can lead to proteins becoming stuck in energetically minimal structures that aren't the native conformation



Specialized proteins called ***molecular chaperones*** *help complicated or unstable proteins find their native conformation*

- Molecular chaperones were first mentioned in 1978 by Ron Laskey, who found that nucleoplasmin (a protein found in the nucleus of the cell) is able to bind to histones

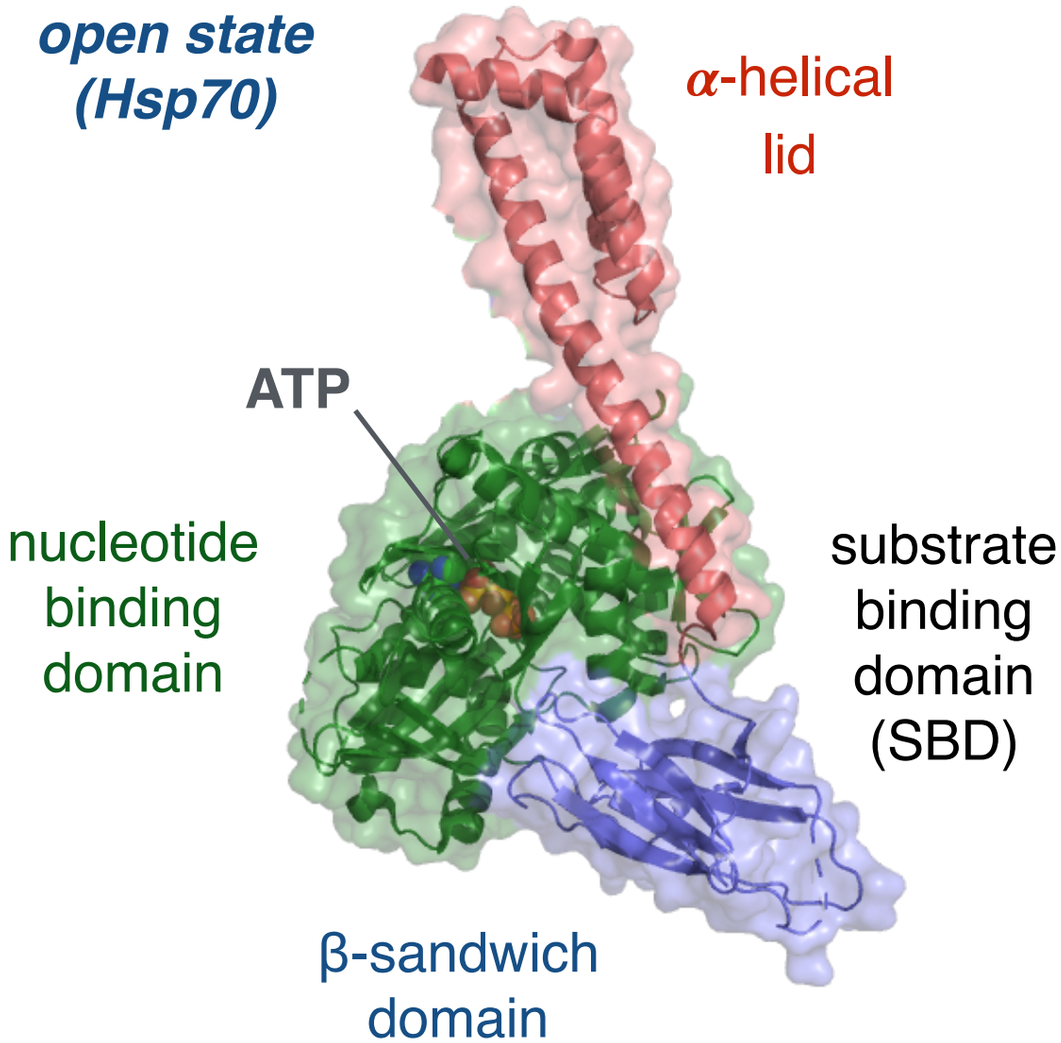


- Laskey observed that nucleoplasmin acted like a chaperone, preventing the aggregation of folded histone proteins in DNA during nucleosome assembly

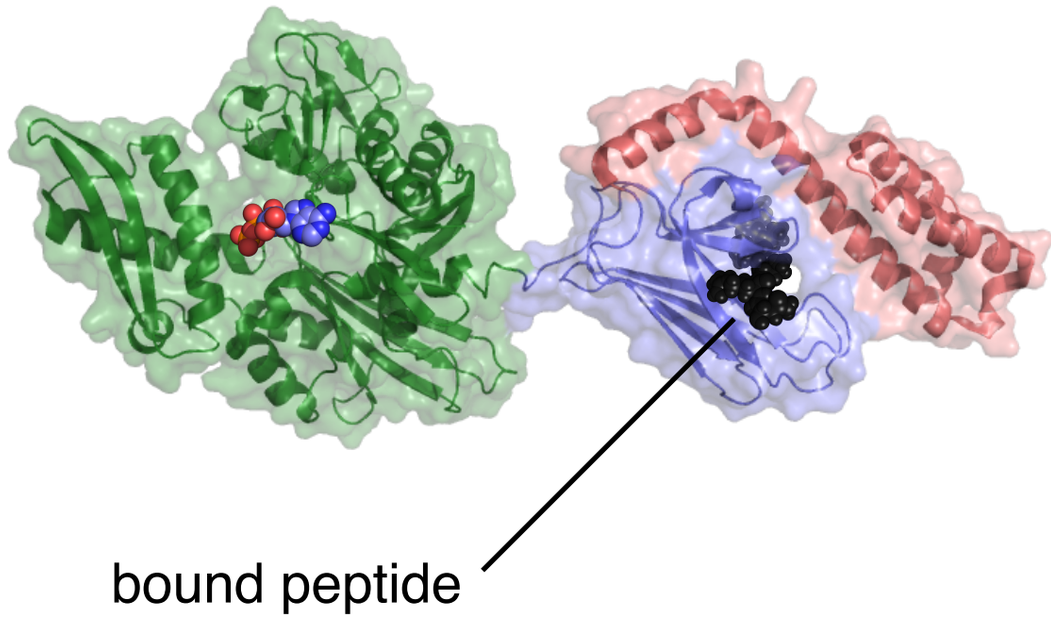
Laskey, R., Honda, B., Mills, A., Finch, J. T., Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA, *Nature*, **275**, 416–420, (1978)

HSPs regulate targeting and degradation of misfiled proteins at the lumen of the ER

open state (Hsp70)



closed state (Hsp70)



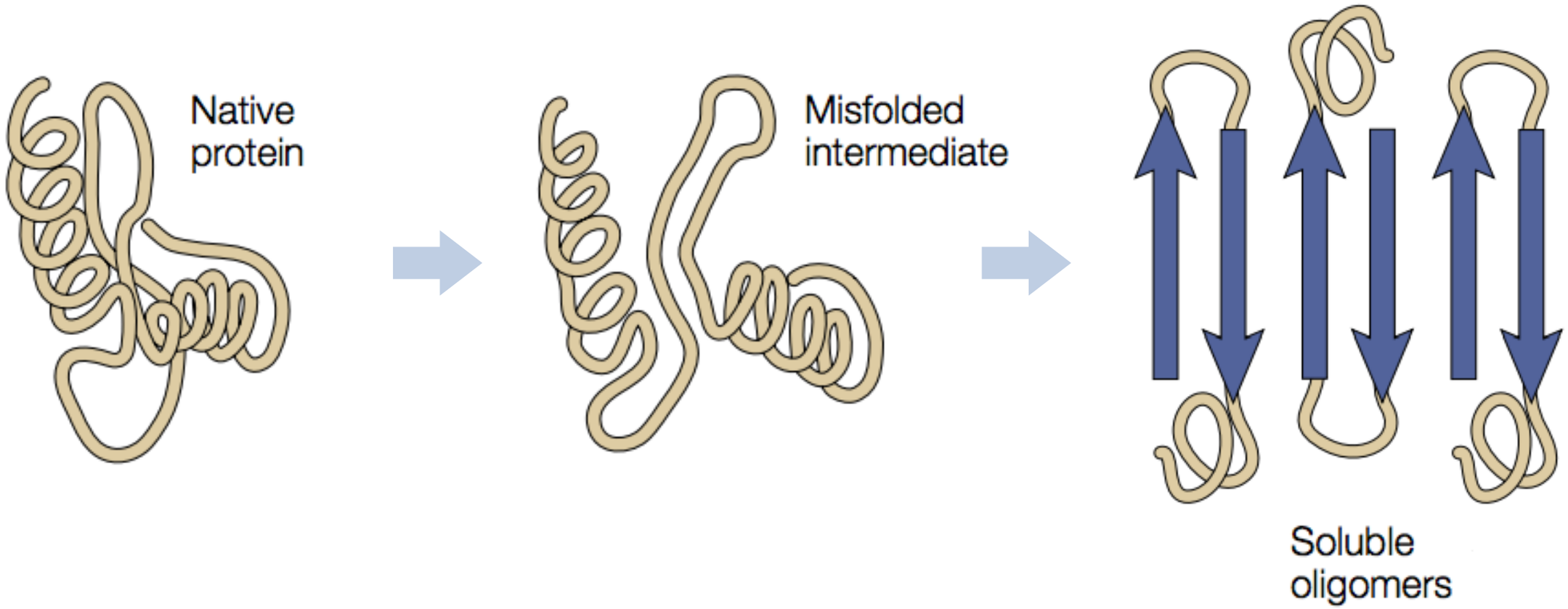
Rosenzweig, R., Nillegoda, N. B., Mayer, M. P., Bukau, B. The Hsp70 chaperone network, *Nat. Rev. Mol. Cell Biol.* **20**, 665–680

Some proteins can evade the ER 'quality control system'

Balchin, D., Hayer-Hartl, M., Hartl, F. U., In vivo aspects of protein folding and quality control, *Science*, **353**, aac4354, (2016)

Protein Function and Three-Dimensional Structure

As millions of copies of each protein are made during our lifetimes, sometimes a random event occurs leading to a misfolded protein (also called toxic conformations)



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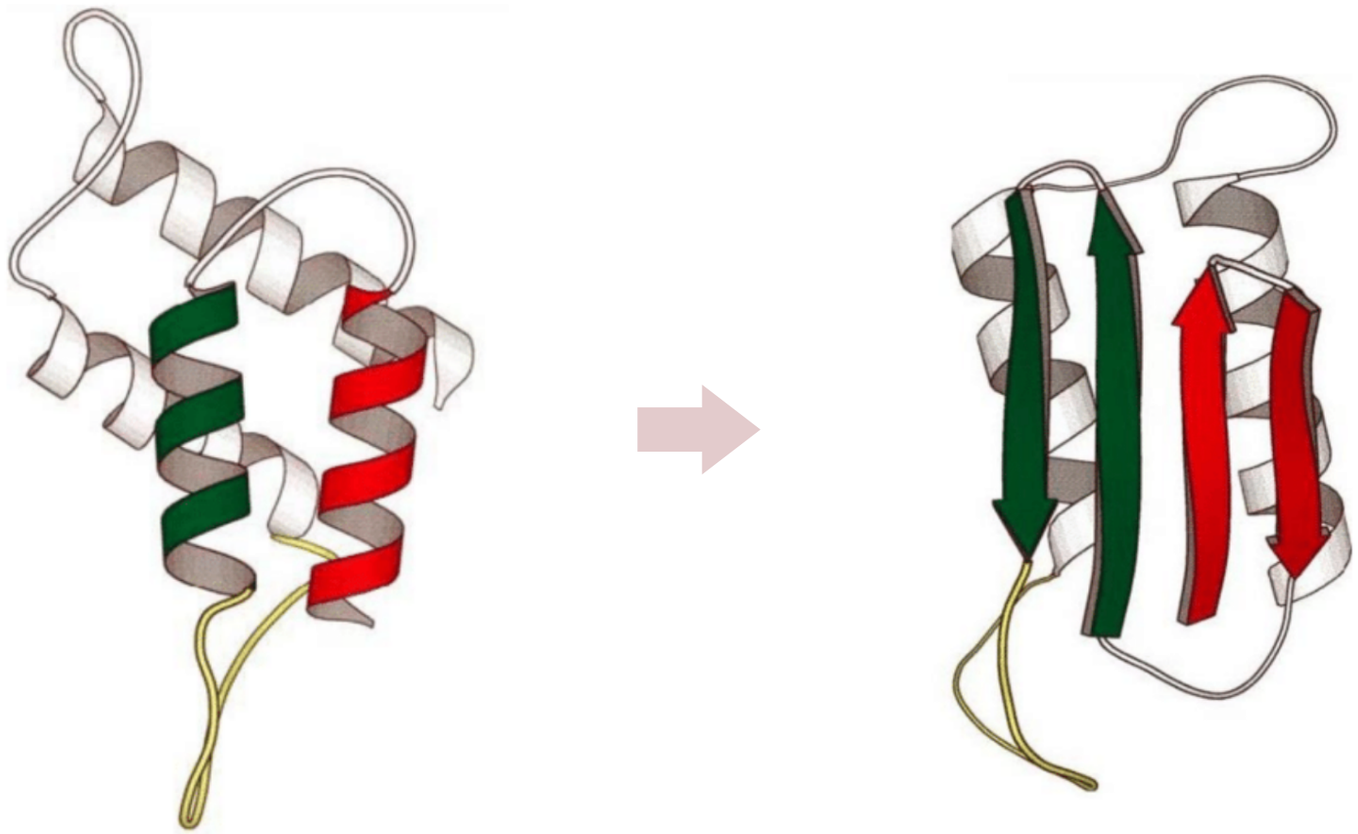
Protein Function and Three-Dimensional Structure

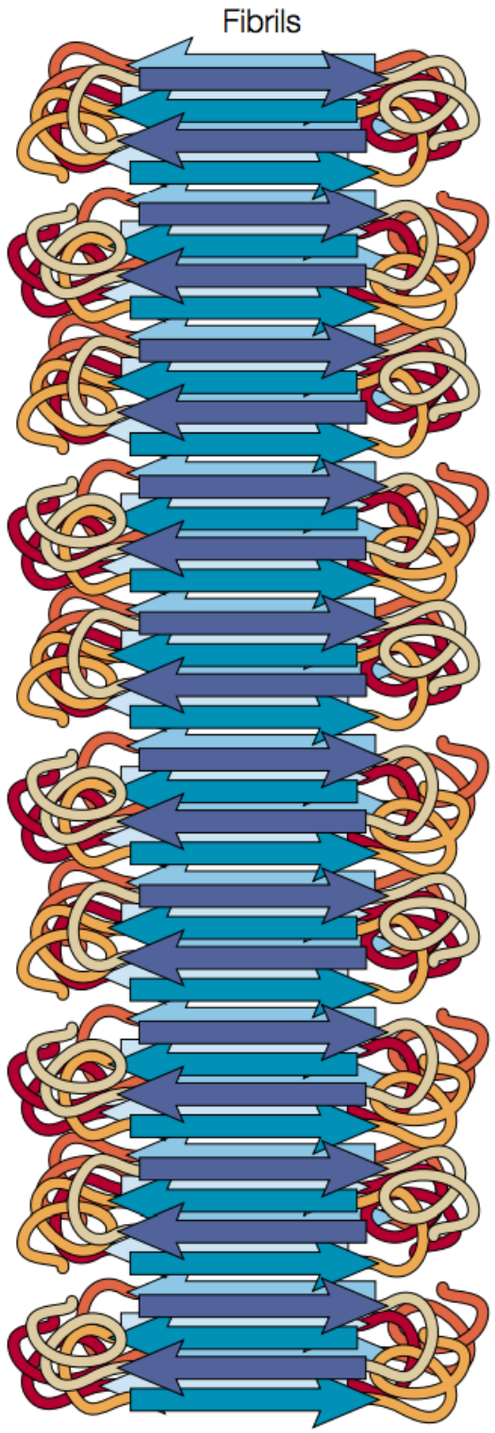


α-helix

β-sheet

When a protein becomes toxic, an extensive conformational change occurs and it acquires a motif known as the beta sheet

Protein Function and Three-Dimensional Structure

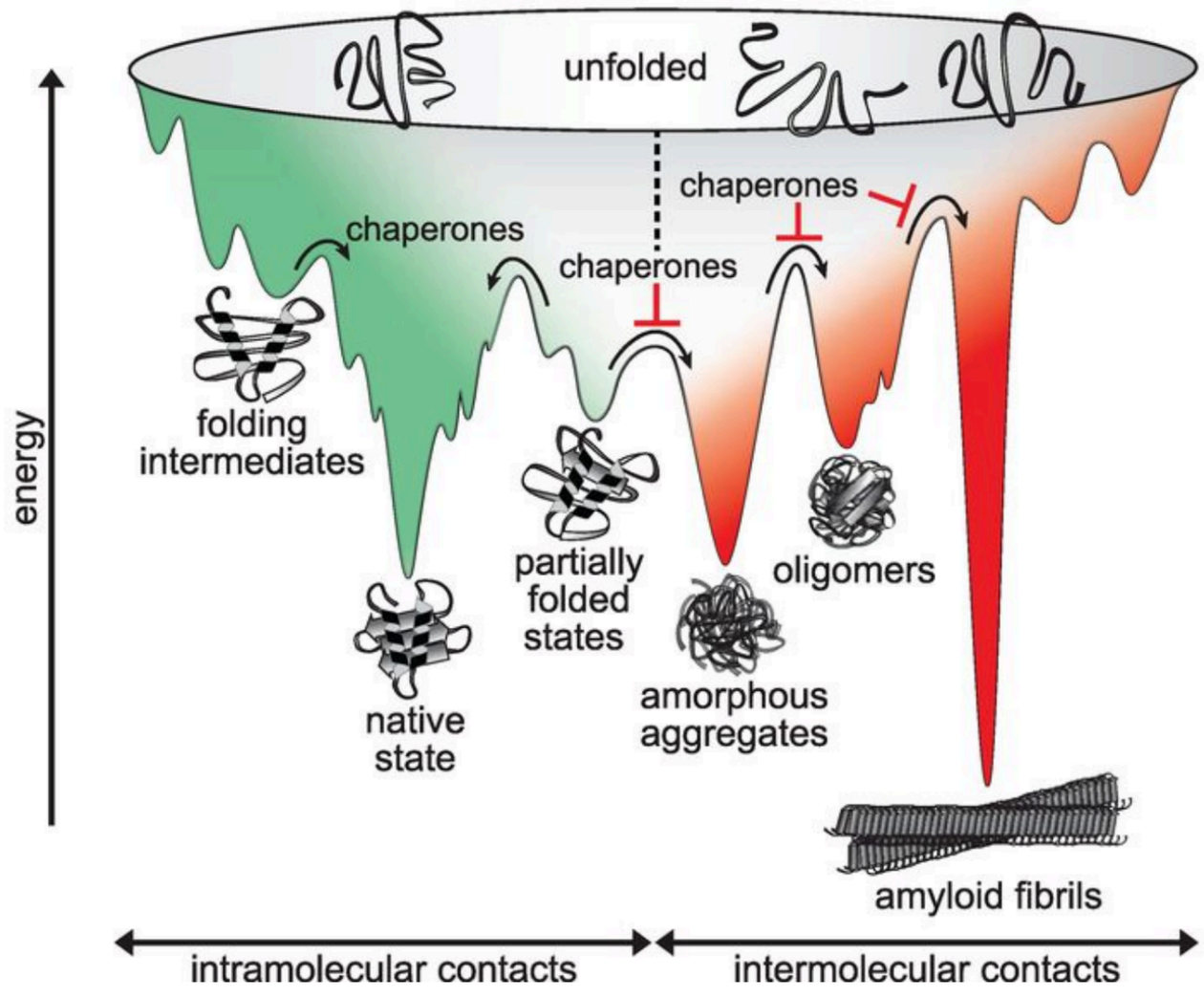


The conformational transition from alpha helix to beta sheet exposes hydrophobic amino acid residues and promotes protein aggregation

β-amyloid fibrils

Balchin, D., Hayer-Hartl, M., Hartl, F. U., In vivo aspects of protein folding and quality control, *Science*, **353**, aac4354, (2016)

Protein Function and Three-Dimensional Structure



The misfolded proteins attach to other healthy proteins, building a template that rapidly grows, resembling a crystallisation process

Balchin, D., Hayer-Hartl, M., Hartl, F. U., In vivo aspects of protein folding and quality control, *Science*, **353**, aac4354, (2016)

This continual cycle occurs until the cell dies and disperses the infectious protein to surrounding cells, which ultimately leads to damage

1997 Nobel prize for medicine

*The term **prion** (**pr**oteinaceous infect**ion**) was coined by Stanley Prusiner in 1982 to explain small mutated proteins responsible for various unusual brain diseases. He was not believed for nearly two decades.*

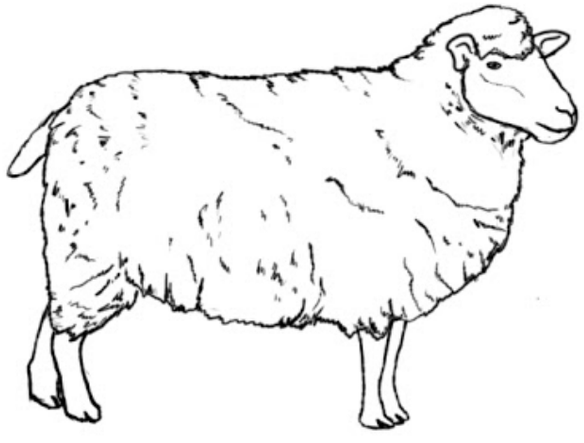
Prusiner, S. B., Novel proteinaceous infectious particles cause scrapie, *Science*, 136–144, (1982)

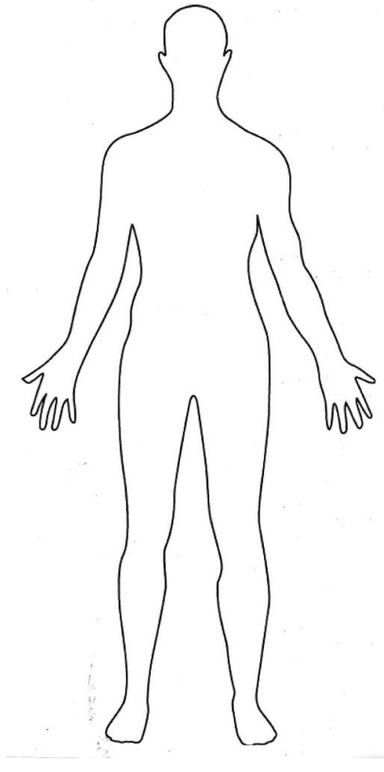
Scrapie *is a fatal degenerative disease found in sheep and goats. The name derives from the compulsive scraping off of the animals fleeces.*

There is no cure

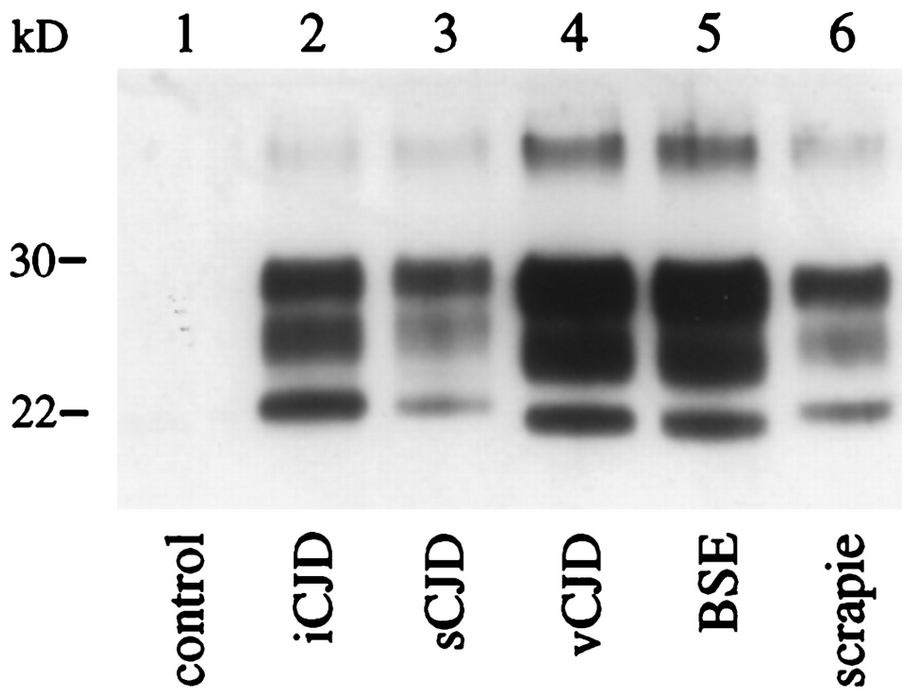
The cause of scrapie is unknown and a matter of debate

The agent is very difficult to destroy with heat, radiation and disinfectants, it can remain in the soil for >10 years, does not evoke any detectable immune response, and has a long incubation period of between 18 months and 5 years

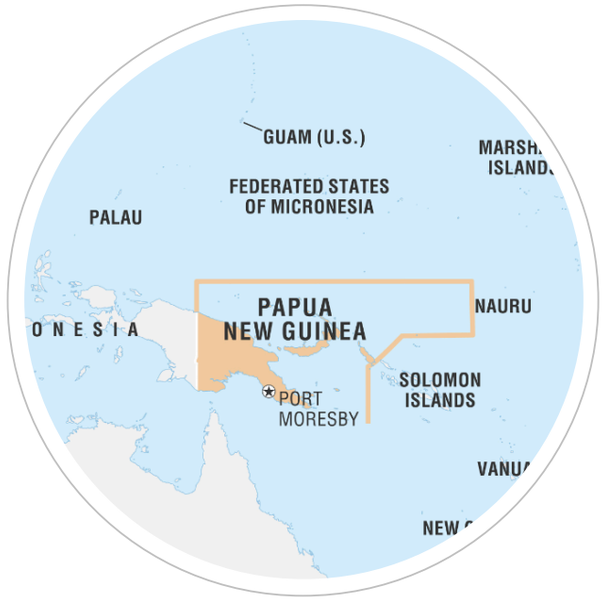




Alper, T., Cramp, W. A., Haig, D. A., Clarke, M. C., Does the agent of scrapie replicate without nucleic acid, *Nature*, **214**, 764–766, (1967);
Griffith, J. S., Nature of the scrapie agent: self-relocation and scrapie, *Nature*, **215**, 1043–1044, (1967)



*Prions are also responsible for BSE (mad cow disease), whose infective form can cause Creutzfeldt-Jakob disease in humans; and **kuru**, the only epidemic human prion disease known*

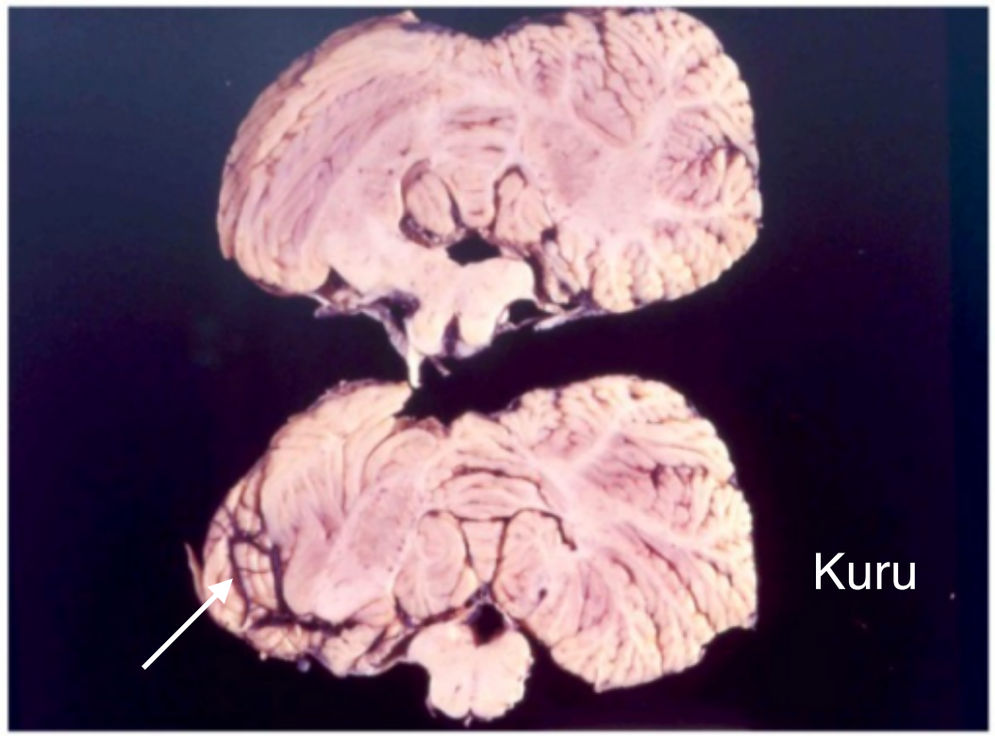


Lasmézas, C. I., Fournier, J.-G., Nouvel, V., Boe, H., Marcé, D., Lamoury, F., Kopp, N., Hauw, J.-J., Ironside, J., Bruce, M., Dormont, D., Deslys, J.-P., Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health, *Proc. Nat. Acad. Sci.* **98**, 4142–4247, (2001)

Kuru was discovered in the late 1950's among populations of the Fore tribe of the eastern highland of Papua New Guinea

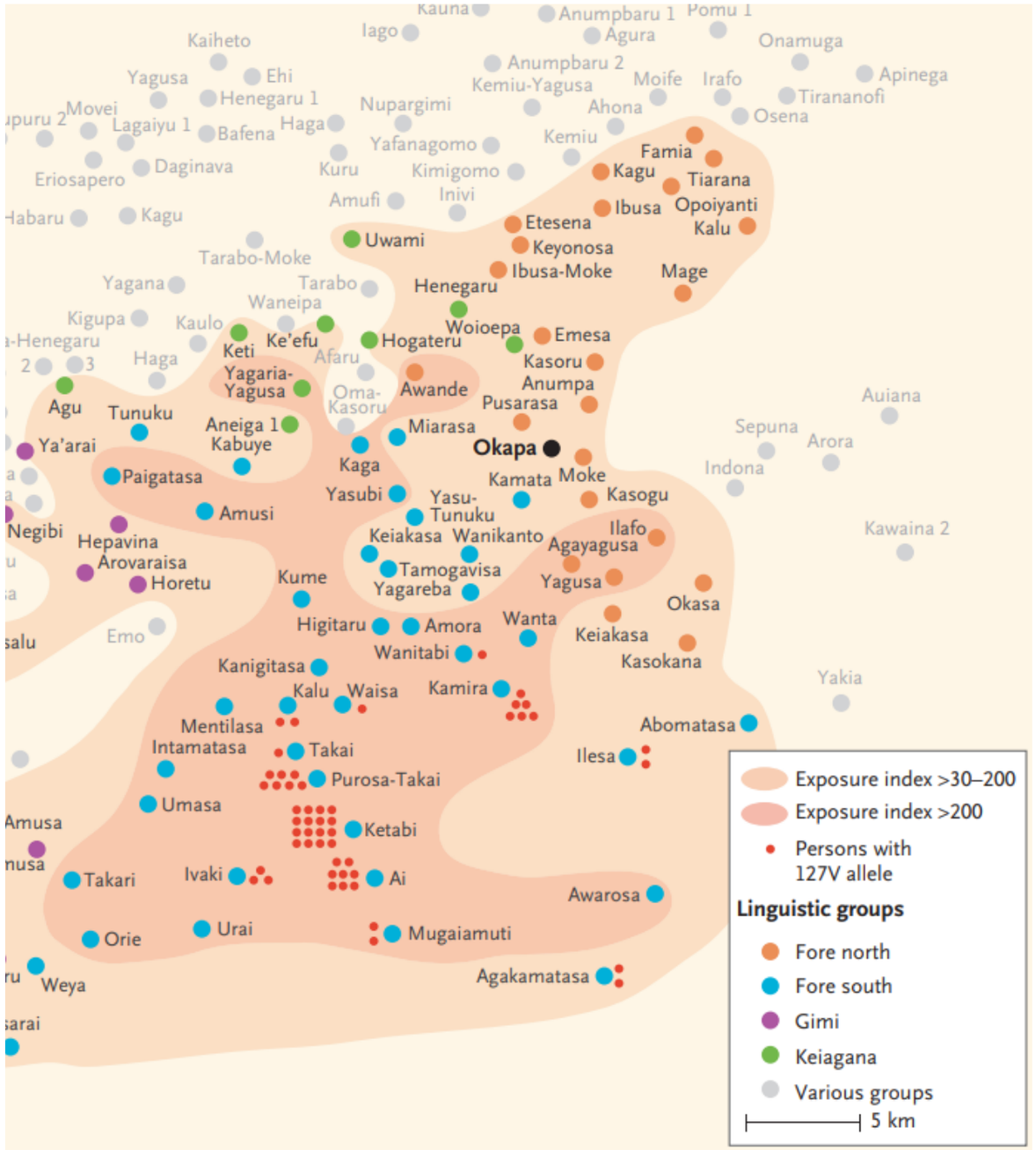
Kuru was transmitted by funerary cannibalism, where the deceased were traditionally cooked and eaten.

Women and children, who usually ate the brain, were the most severely effected.



Collinge, J. C., Whitefield, J., McKintosh, E., Beck, J., Mead, S., Thomas, D. J., Alpers, M. P., Kuru in the 21st century - an acquired human prion disease with very long incubation periods, *Lancet*, **367**, 2068–2074 (2006)

Protein Function and Three-Dimensional Structure



sCJD = natural (most common); vCJD = consuming meat (rare); iCJD = medical contamination (rare)

In 2009, a naturally occurring variant of the prion protein was found (in areas where Kuru was most widespread) that conferred strong resistance

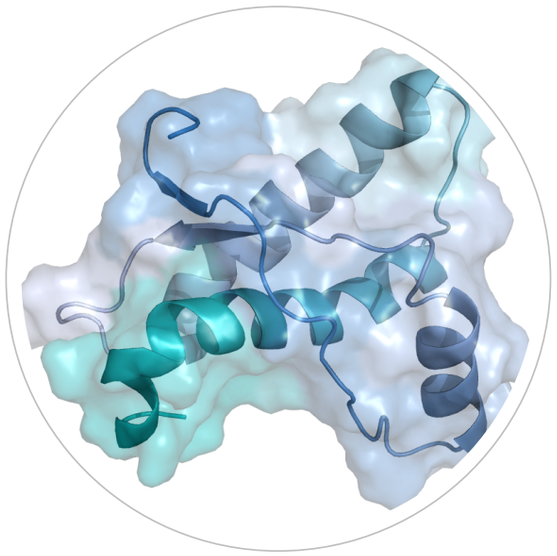
Heterozygous polymorphism in *PRNP* (coding either M or V) is protective against the development of CJD and Kuru. (All known cases of variant CJD have been MM)

Mead, S., Whitfield, J., Poulter, M., Shah, P., Uphill, J., Campbell, T., Al-Dujaily, H., Hummerich, H., Beck, J., Mein, C. A., Verzilli, C., Whittaker, J., Alpers, M. P., Collinge, J., A novel protective prion protein variant that colocalizes with Kuru exposure, *N. Engl. J. Med.* **361**, 2056–2065, (2009)

Protein Function and Three-Dimensional Structure

What does the prion protein (PrP) do?

Protein Function and Three-Dimensional Structure



PrP^{C} is a 208 residue GPI-anchored protein present on the cell surface

The exact biological function of PrP^{C} is unknown. It has been implicated in signal transduction, Cu-binding, synaptic transmission, and induction of apoptosis, but is not essential

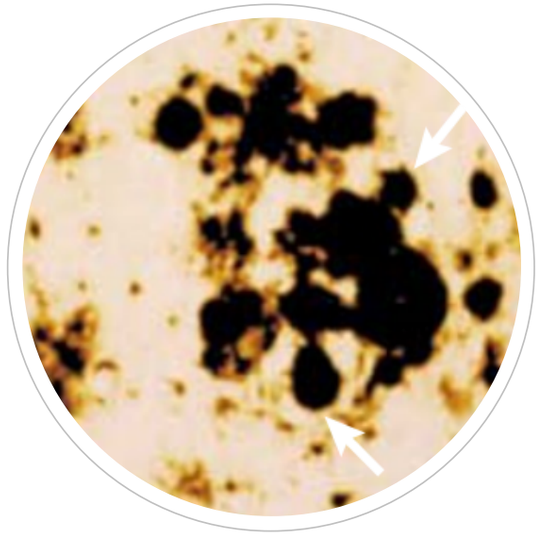
The exact composition of the protein form causing the prion diseases remains to be fully understood

Singh, J., Udgaonkar, J. B., Molecular mechanism of the misfiling and oligomerization of the prion protein: current understanding and its implications, *Biochem.*, **54**, 4431–4442, (2015)

Protein Function and Three-Dimensional Structure

Post-mortem studies show a poor correlation between the load of amyloid like deposits in the brain and the severity of clinical symptoms

Alternative forms of PrP have been reported to have important roles in prion-mediated neurodegeneration



Prion disease

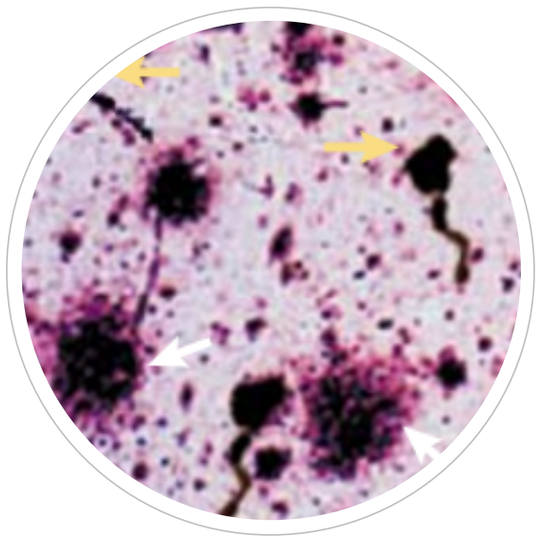
Several lines of evidence also implicate misfolded oligomers as playing a role in prion disease

Singh, J., Udgaonkar, J. B., Molecular mechanism of the misfiling and oligomerization of the prion protein: current understanding and its implications, *Biochem.*, **54**, 4431–4442, (2015)

Protein Function and Three-Dimensional Structure

Protein aggregates have different roles in distinct diseases

Protein aggregates have different roles in distinct diseases



Alzheimer's

Mode of transmission: *sporadic (95%), inherited (5%)*

Clinical features: *progressive dementia*

Affected regions: *hippocampus, cerebral cortex*

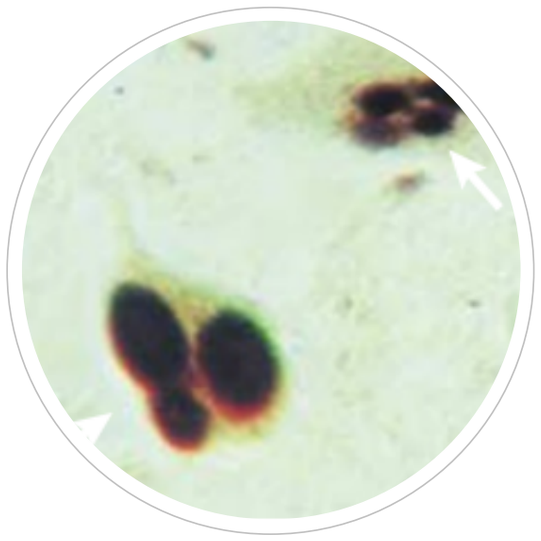
Proteins involved: *amyloid-β, tau proteins*

Cellular location: *extracellular, cytoplasmic*

Extracellular amyloid plaques (white arrows) deposited around the cerebral vessel walls are comprised of Aβ. Intracytoplasmic neurofibrillary tangles (yellow arrows) are comprised of hyperphosphorylated tau protein.

Glenner, G. G., Wong, C. W., Alzheimer's disease: initial report of the purification and characterisation of a novel cerebrovascular amyloid protein, *Biochem. Biophys. Res. Common.* **120**, 885–890, (1984); Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y. C., Zaidi, M. S., Wisniewski, H. M., Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J. Biol. Chem.* **261**, 6084–6089, (1986)

Protein aggregates have different roles in distinct diseases



Parkinson's

Mode of transmission: *Mostly sporadic*

Clinical features: *movement disorder*

Affected regions: *substantia nigra, hypothalamus*

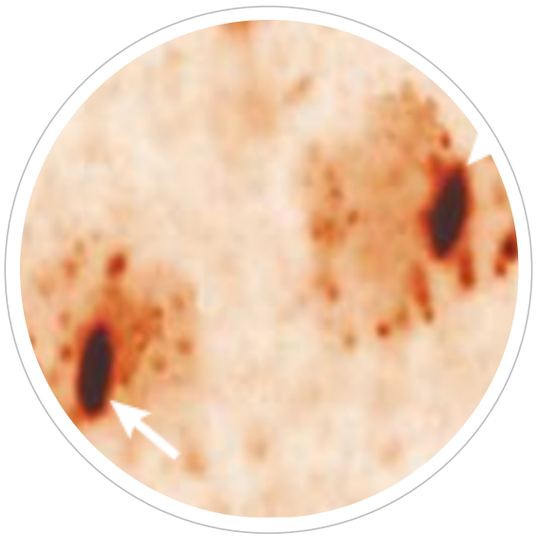
Proteins involved: *α-synuclein*

Cellular location: *cytoplasmic*

Cytoplasm of neurons from the substantia nigra contain aggregates called Lewy bodies (white arrows). The major constituent of these aggregates are fragments of a protein named α-synuclein.

Forno, L. S., Neuropathology of Parkinson disease, *J. Neuropathol. Exp. Neurol.* **55**, 259–272, (1996); Spillantini, M. G., Schmidt, M. L., Lee, V. M.-Y., Trojanowki, J. Q., Jakes, R., Goedert, M., α-Synuclein in Lewy bodies, *Nature*, **388**, 839–840, (1997)

Protein aggregates have different roles in distinct diseases



Huntington's

Mode of transmission: *Inherited (autosomal dominant)*

Clinical features: *dementia, motor, and psychiatric*

Affected regions: *striatum, cerebral cortex*

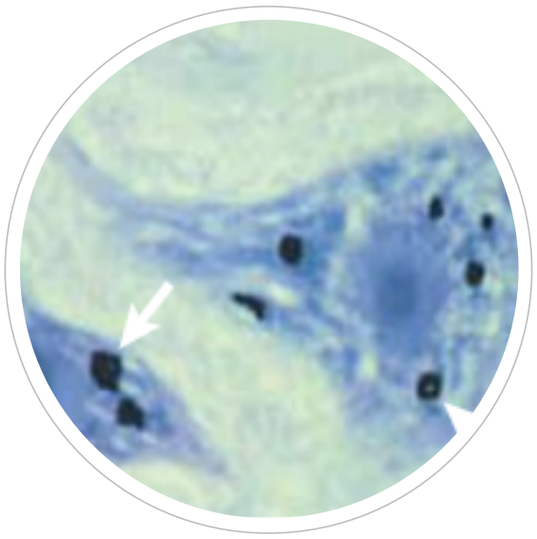
Proteins involved: *huntingtin*

Cellular location: *nuclear*

Intranuclear deposits of a polyglutamine-rich version of huntingtin protein (white arrows) are a typical feature of brains from patients with HD.

DiFiglia M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., Aronin, N., Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain, *Science*, **277**, 1990–1993, (1997)

Protein aggregates have different roles in distinct diseases



Amyotrophic lateral sclerosis (ALS)

Mode of transmission: *sporadic (90%), inherited (10%)*

Clinical features: *movement disorder*

Affected regions: *motor cortex, brainstem*

Proteins involved: *superoxide dismutase*

Cellular location: *cytoplasmic*

In ALS, patients have aggregates, mainly composed of superoxide dismutase (SOD1), in the cell bodies and axons of motor neurons.

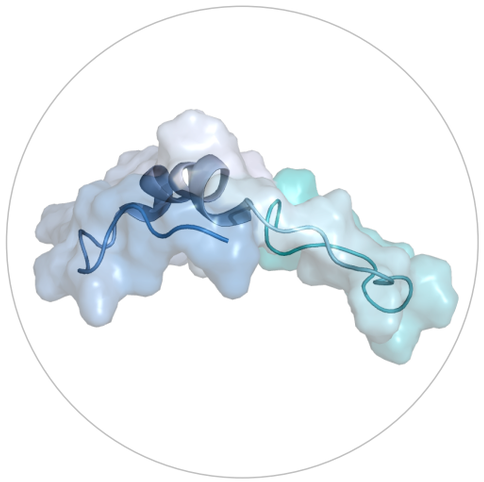
Bruin, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., Reaume, A. G., Scott, R. W., Cleveland, D. W., Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1, *Science*, **281**, 1851–1854, (1998)

*No other protein conformational disorders (aside from prion disease) have been convincingly shown to be transmissible**

*Some animal models have shown that AD pathology can be **accelerated** by the injection of enriched protein homogenate:

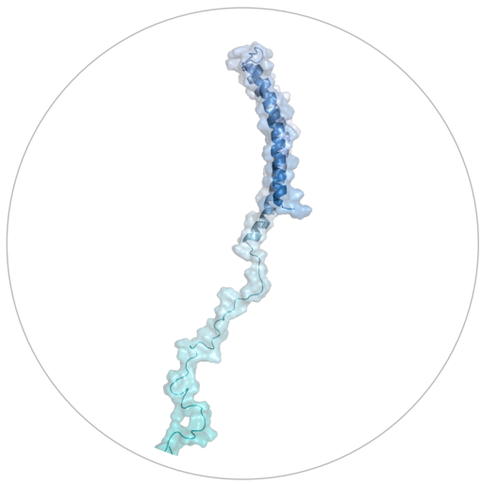
Lundmark, K., Westermark, G. T., Nyström, S., Murphy, C. L., Solomon. A., Westermark, P., Transmissibility of systemic amyloidosis by a prion-like mechanism, *Proc. Natl. Acad. Sci.* **14**, 6979–6984, (2002)

What causes misfolding amongst other proteins?



Internal hydrophobic region (AAs 17–21; LVFF) is implicated in the early steps of Aβ misfolding, with aggregation at the C-terminus

α-Synuclein fibrillogenesis, although less-well studied, stems from the central hydrophobic region (AAs 61–95)



Hilbich, C., Kisters-Woike, B., Reed, J., Masters, C. L., Beyreuther, K., Substitutions of hydrophobic amino acids reduce the amylidogenicity of Alzheimer's disease βA4 peptides, *J. Mol. Biol.* **228**, 460–473, (1992); Jarrett, J. T., Berger, E. P., Lansbury, P. T. Jr., The C-terminus of the β protein is critical in amyloidogenesis, *Ann. NY Acad. Sci.* **695**, 114–148, (1993); Stefanis, L. ***α***-Synuclein in Parkinson's disease, *Cold Spring Harb. Perspect. Med.* **2**, a009399, (2012)

Protein Function and Three-Dimensional Structure

In Huntington's and ALS, both disease and protein aggregation are associated with an inherited expansion of CAG (glutamine) codon repeats

Protein Function and Three-Dimensional Structure

Trinucleotide repeat disorders are genetic disorders caused by mutation in which repeats of three nucleotides increase in copy number until they become unstable

Huntington's

10 to 35 CAG repeats: ***Unaffected***

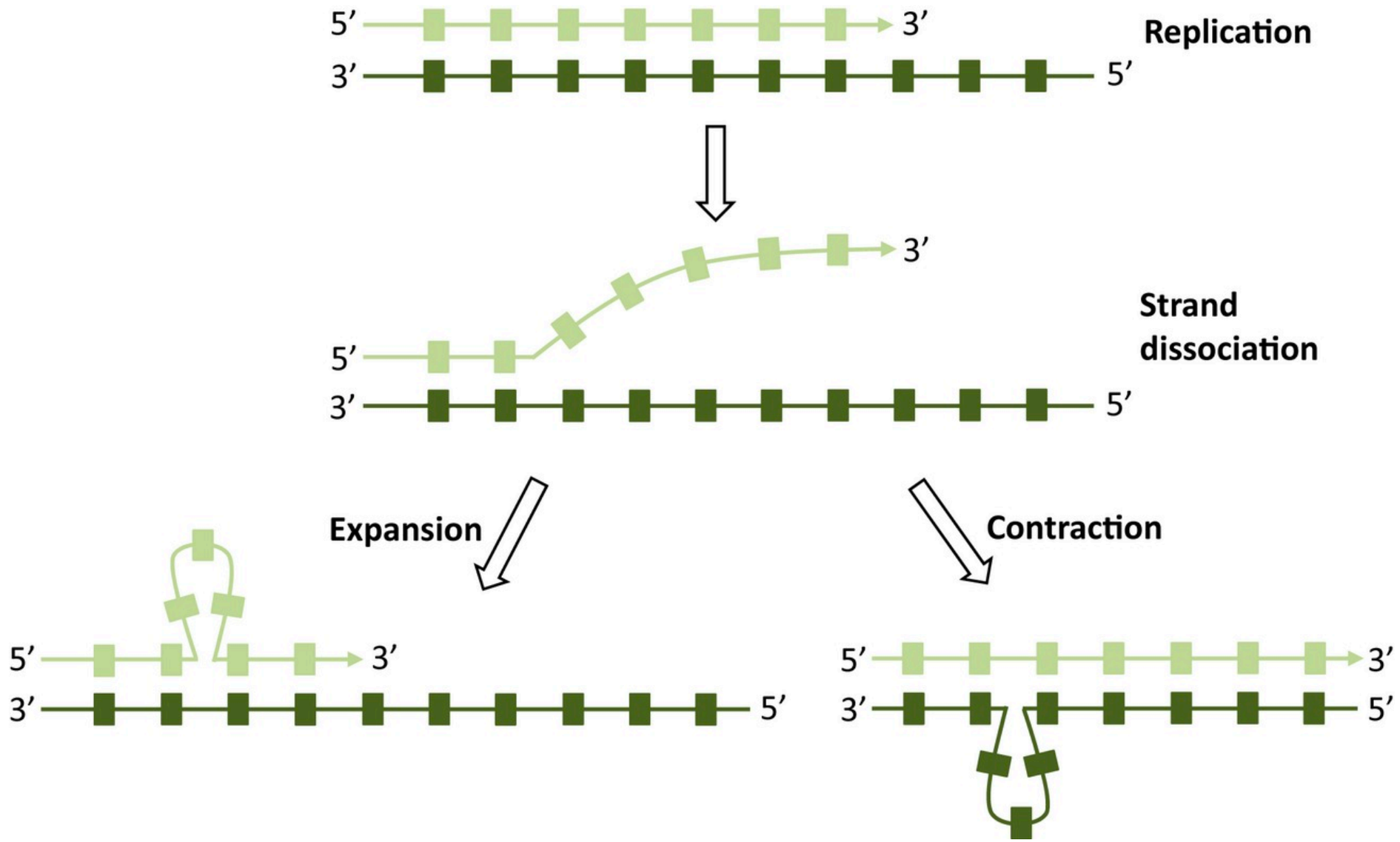
36 to 39 CAG repeats: ***At risk***

>40 CAG repeats: ***Affected***

Trinucleotide repeat disorders generally show genetic anticipation: their severity increases with each successive generation that inherits them

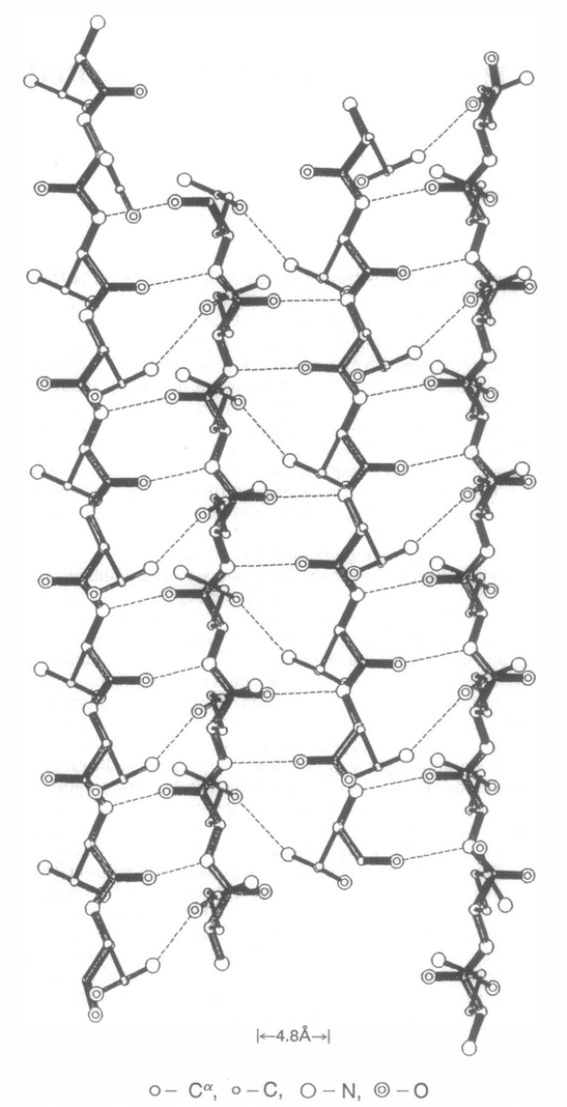
Orr, H. T., Zoghbi, H. Y. Trinucleotide Repeat Disorders, *Ann. Rev. Neurosci.* **30**, 575–621, (2007)

DNA slippage occurs during DNA synthesis due to misalignment in hybridisation



Mirkin, S. M. Expandable DNA repeats and human disease, *Nature,* **447**, 932–940, (2007)

Aggregation of huntingtin in vitro depends on the length of the polyglutamine repeat



Polar zipper model

β-sheets are formed and stabilized by the collective strength of cooperative hydrogen bonding involving the amide group of the glutamine residue

Perutz, M. F., Johnson, T., Mavashi, S., Finch, J. T., Glutamine repeats as polar zippers: Their possible role in inherited neurodegenerative diseases, *Proc. Natl. Acad. Sci.* **91**, 5355–5358, (1994)

Protein Function and Three-Dimensional Structure

What is the mechanism of neuronal death induced by protein misfolding?

Three main hypotheses have been put forward to explain neuronal cell death

1) Loss-of-function hypothesis

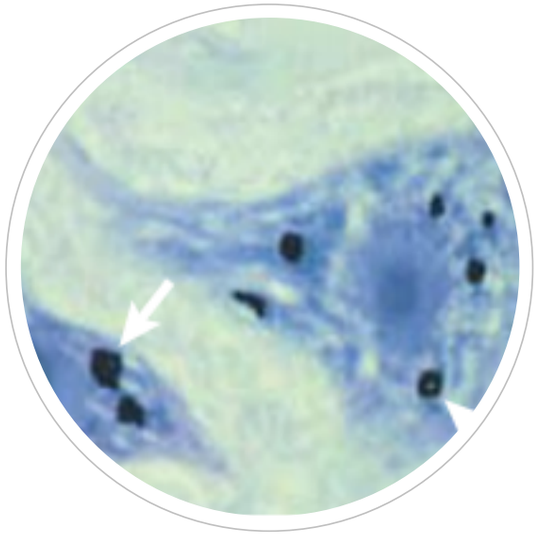
2) Gain-of-toxicity hypothesis

3) Inflammation hypothesis

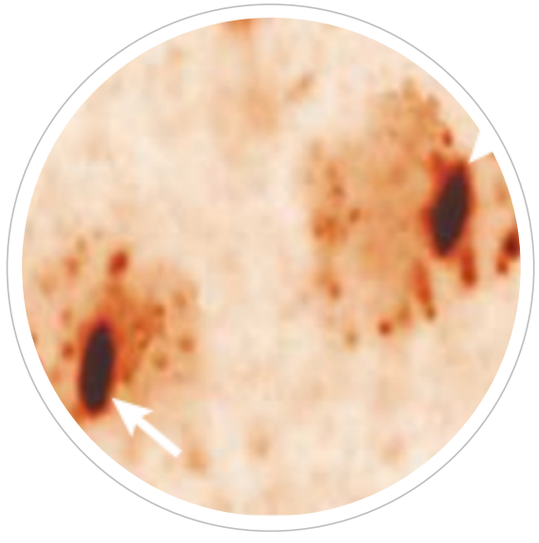
Protein Function and Three-Dimensional Structure

Loss-of-function hypothesis

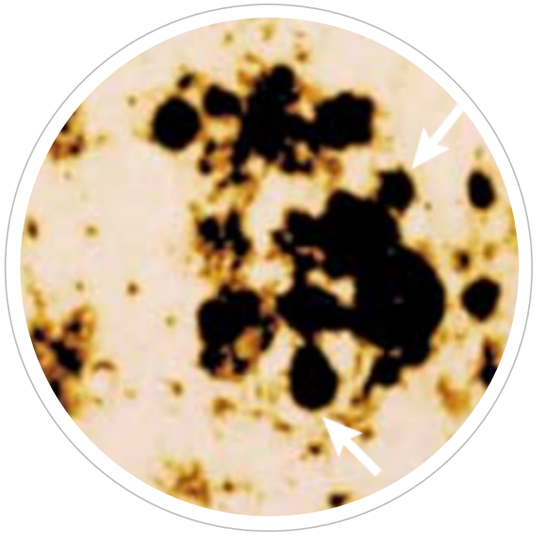
Native protein —*misfolding*→ Depletion of normal protein → Lack of biological activity → Cellular malfunction



Amyotrophic lateral sclerosis (ALS)

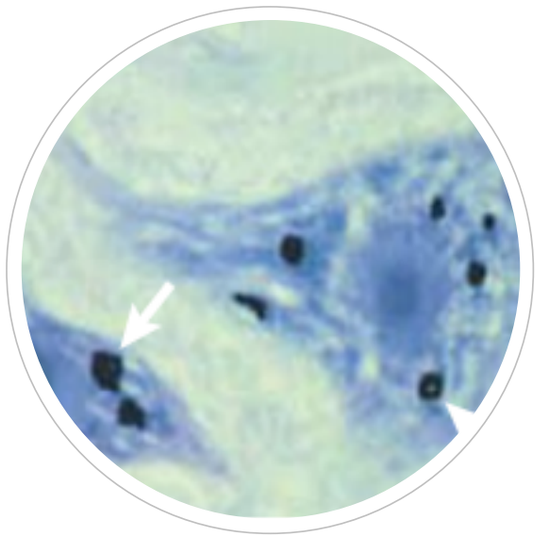


Huntington's



Prion disease

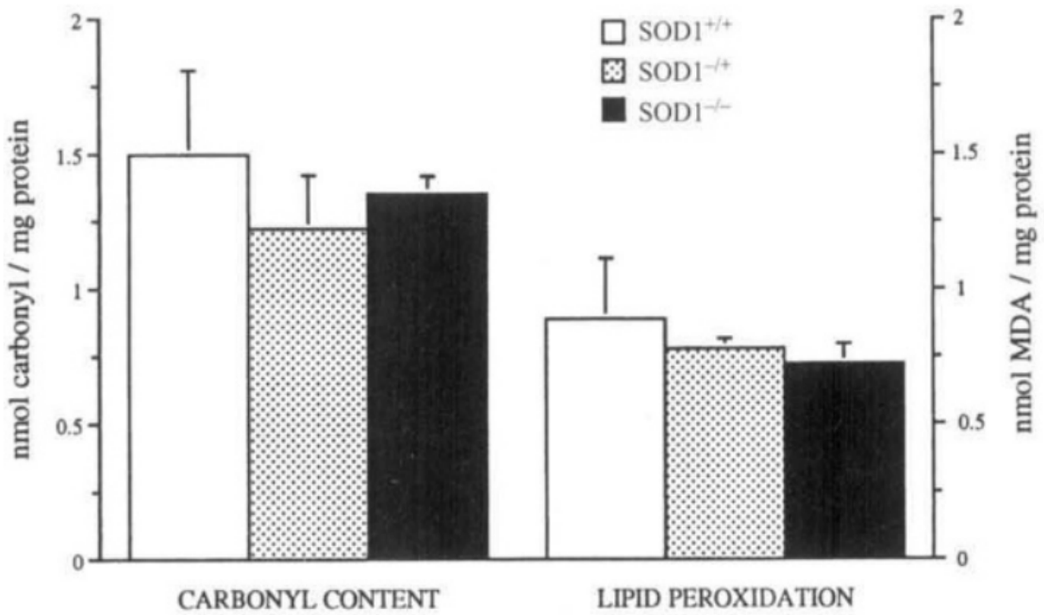
Protein Function and Three-Dimensional Structure



Amyotrophic lateral sclerosis (ALS)

- In ALS, the protein (that becomes misfolded) **SOD1** catalyses the conversion of superoxide to hydrogen peroxide

Depletion of SOD1 by misfolding and aggregation could lead to accumulation of superoxide radicals



- SOD1 knockout mice show no degeneration of motor neurons or oxidative damage

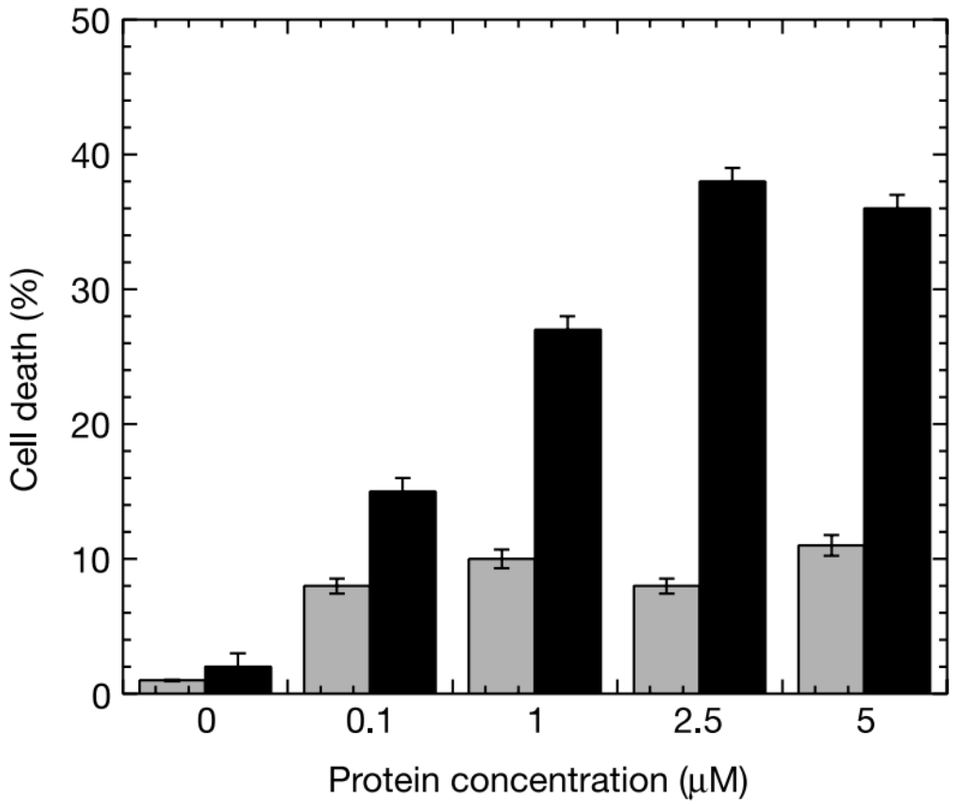
- SOD1 mutants with different activity do not correlate to age of onset or severity of disease

Reaume, A. G., Elliott, J. L., Hoffman, E. K., Kowall, N. W., Ferrante, R. J., Siwek, D. R., Wilcox, H. M., Flood, D. G., Beal, M. F., Brown Jr., R. H., Scott, R. W., Snider, W. D., Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury, *Nat. Genet.*, **13**, 43–47, (1996); Borchelt, D. R., Guarnieri , M., Wong, P. C., Lee, M. K., Slunt, H. S., Xu, Z.-S., Sisodia, S. S., Price, D. L., Cleveland, D. W., Superoxide dismutase 1 subunits with mutations linked to familial amyotrophic lateral sclerosis do not affect wild-type subunit function, *J. Biol. Chem.* **270**, 3234–3238, (1995)

Gain-of-toxic-activity hypothesis

Native protein —*misfolding*→ Misfolded proteins and aggregates → Protein deposits → Neurotoxicity

Percentage of cell deaths induced by 48-h-aged HypF-N aggregates (solid) and control protein (grey)



β-sheet oligomerization of non disease-related proteins is cytotoxic, indicating that misfolding and aggregation of any protein results in inherent toxicity

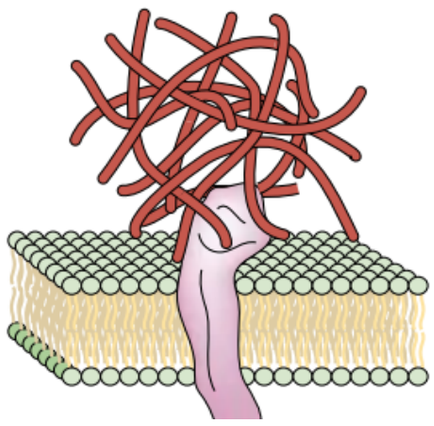
Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M., Steffani, M., Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases, *Nature* **416**, 507–511, (2002)

Protein Function and Three-Dimensional Structure

Gain-of-toxic-activity hypothesis

Native protein —*misfolding*→ Misfolded proteins and aggregates → Protein deposits → Neurotoxicity

Activation of a signalling pathway



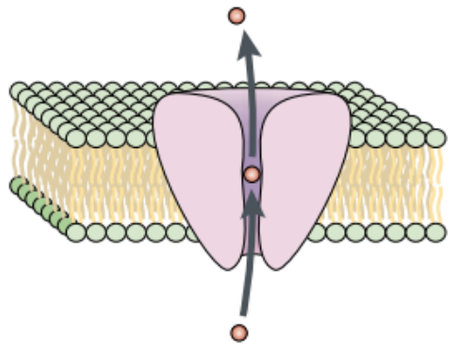
Oxidative stress

Protein and lipid oxidation

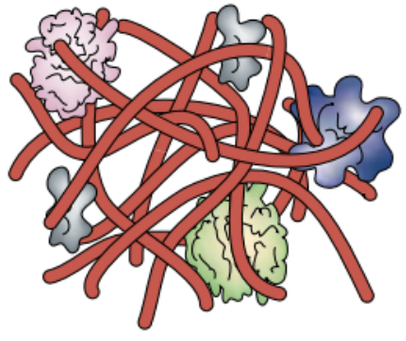
↑

$O_2^{-\bullet}$, H_2O_2, $OH^{\bullet}$, $CNOO^{-\bullet}$

Formation of ion channels

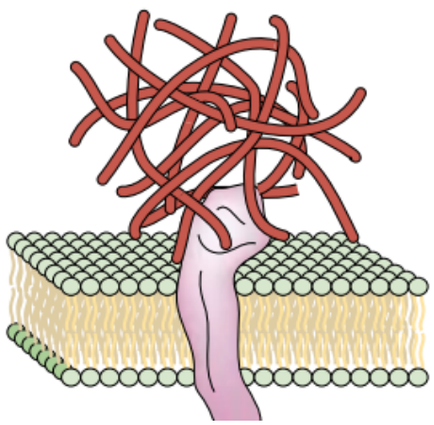


Recruitment of cellular factors



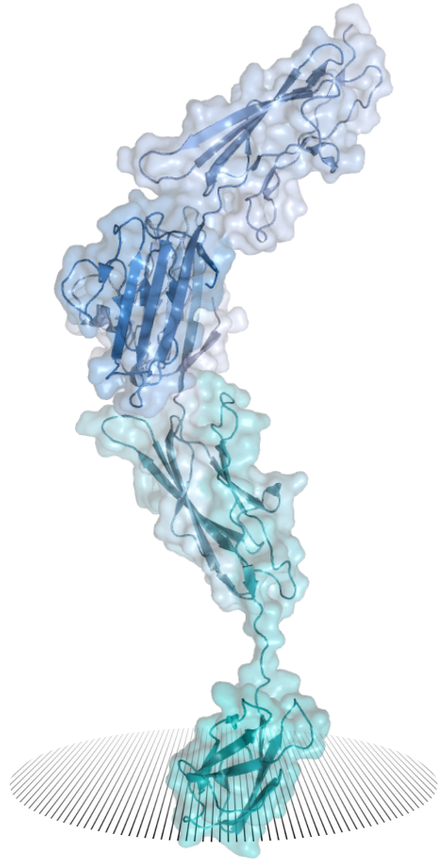
Soto, C., Unfolding the role of protein misfolding in neurodegenerative diseases, *Nat. Rev. Neurosci.* **4**, 49–60, (2003)

Activation of a signalling pathway



Extracellular aggregates might activate a signal transduction pathway that leads to apoptosis by interacting with specific cellular receptors

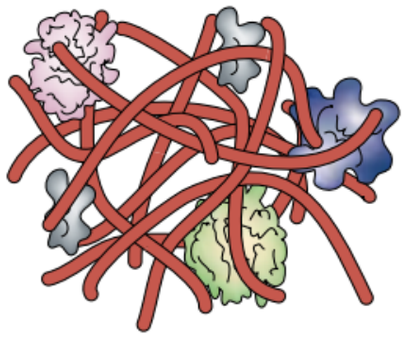
- RAGE (receptor for advanced glycation end products) is a transmembrane receptor of the immunoglobulin superfamily
  - Upon binding of a ligand (Aβ), RAGE signals activation of NF-κB9, which causes an inflammatory response
- In AD, where lots of Aβ is present, this generates a positive feedback loop leading to chronic inflammation and cell death



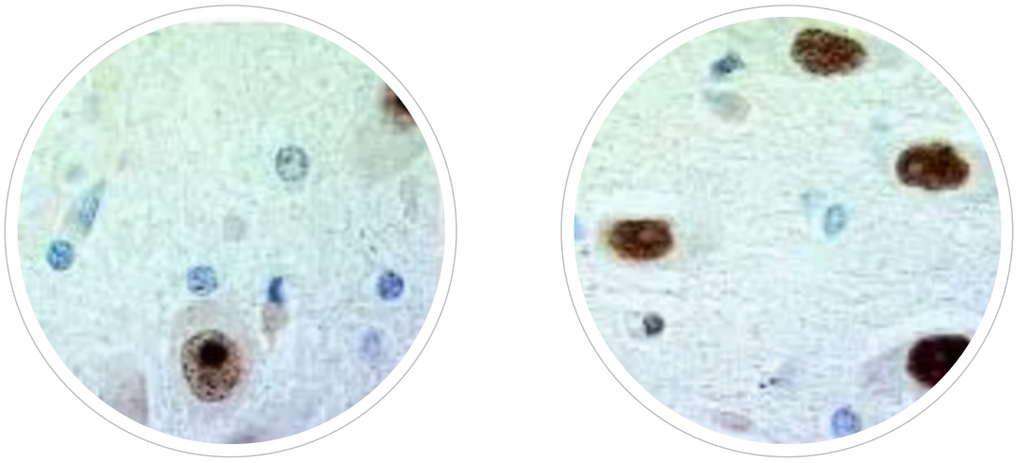
Yan, S. D., Zhu, H., Zhu, A., Golabek, A., Du, H., Roher, A., Yu, J., Soto, C., Schmidt, A. M., Stern, D., Kindy, M., Receptor-dependent cell stress and amyloid accumulation in systemic amyloidosis, *Nat. Med.* **6**, 643–651, (2000)

Protein Function and Three-Dimensional Structure

Recruitment of cellular factors



Intracellular aggregates might damage cells by recruiting factors that are essential for cell viability into the fibrillar aggregates



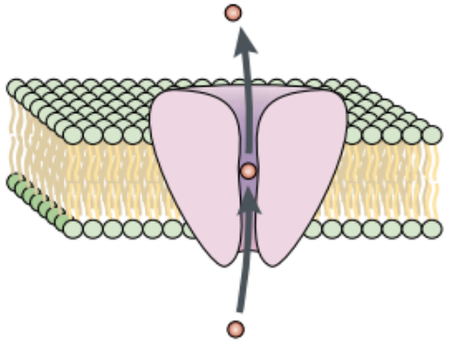
Distribution of the proteasome in neurons of the nucleus pontis centralis from an SCA1 patient (L) and control (R). Note the redistribution of the proteasome to the sites of ataxin-1 aggregation in the patient.

- Components of the proteasome, chaperone proteins, cytoskeletal proteins and transcription factors have been found in huntingtin and α-synuclein aggregates

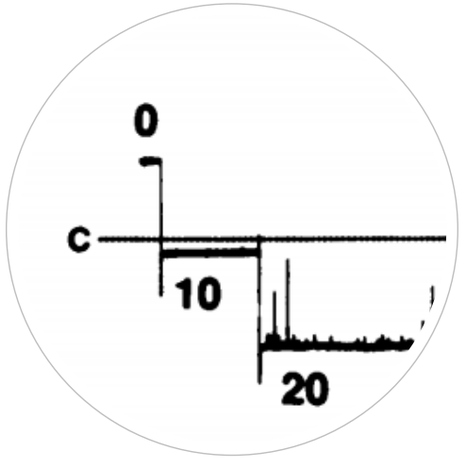
Cummnings, C. J., Mancini, M. A., Antalffy, B., DeFranco, D. B., Orr, H. T., Zoghbi, H. Y., Chaperone suppression of aggregation and altered sub cellular proteasome localisation imply protein misfolding in SCA1, *Nat. Gen.* ***19***, 148–154, (1998); Li, K., Ito, H., Tanaka, K., Hirano, A., Immunocytochemical co-localization of the proteasome in ubiquitinated structures in neurodegenerative diseases and the elderly, *J. Neuropathol. Exp. Neurol.* **56**, 125–131, (1997)

Protein Function and Three-Dimensional Structure

Formation of ion channels



Membrane disruption and depolarisation mediated by ion-channel formation, resulting in alteration of ion homeostasis and cell death



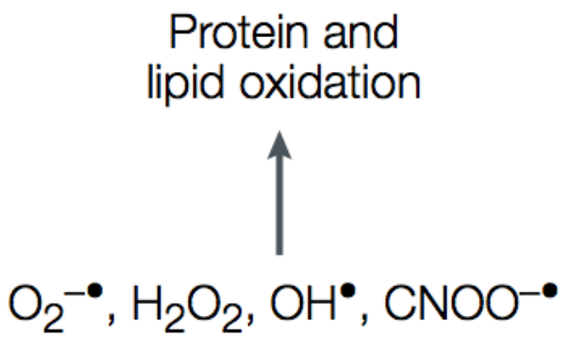
At zero membrane potential, a net negative current corresponding to the movement of K+ is shown

- Synthetic AβP forms cation-selective channels across planar lipid bilayers
- Mediation of cell death via discharge of cellular membrane potential

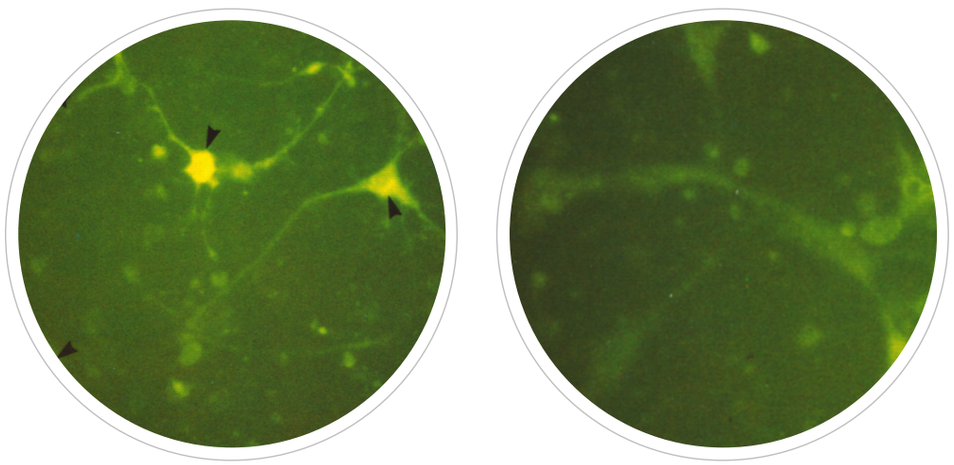
Arispe, N., Rojas, E., Pollard, H. B., Alzheimer disease amyloid β-protein forms calcium channels in bilayer membranes: Blockade by tromethamine and aluminium, *Proc. Natl. Acad. Sci.* **90**, 567–571, (1993); Lin M. C., Mirzabekov, T., Kagan B. L., Channel formation by neurotoxic prion protein fragment, *J Biol. Chem.*, **272**, 44–47, (1997)

Protein Function and Three-Dimensional Structure

Oxidative stress



The production of free radical species results in protein and lipid oxidation, elevating levels of intracellular calcium and mitochondrial disfunction



Primary cells exposed to Aβ for 23h (L) and control (R). Staining with peroxide active dye.

- Aβ causes increased levels of hydrogen peroxide and lipid peroxides in cells
- Breakdown of peroxide into hydroxyl radical results in cellular necrosis

Behl, C., Davis, J. B., Lesley, R., Schubert, D., Hydrogen peroxide mediates amyloid β-toxicity, *Cell*, **77**, 817–827, (1994); Hsu, L. J., Sagara, Y., Arroyo, A., Rockenstein, E., Sisk, A., Mallory, M., Wong, J., Takenouchi, T., Hashimoto, M., Masliah, E., α-synuclein promotes mitochondrial deficit and oxidative stress, *Am. J. Pathol.*, **157**, 401–410, (2000); *For review, see:* Lin, M. T., Flint Beal, M., Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases, *Nature*, **443**, 787–795, (2006)

The hypothesis that aggregates are toxic has been challenged by recent studies

- In some animal models of AD, TSE, HD and ataxias, cerebral damage and clinical symptoms have been detected before protein aggregates

- Neuropathological analysis of AD and PD patients show Lewy bodies and tangles that are healthier than neighbouring cells

- Amyloid-like plaques and Lewy bodies are found in people without evident neuronal loss or clinical signs of AD or PD

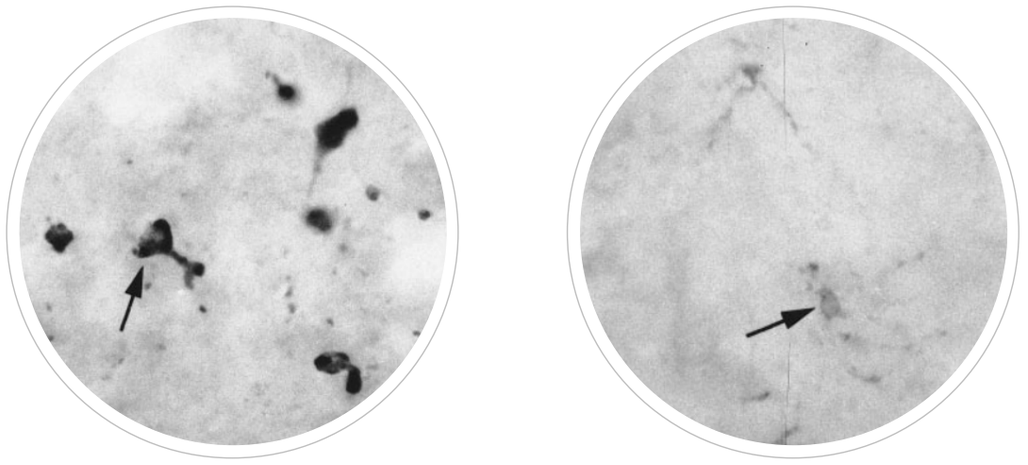
*The process of misfolding and **early stages** of oligomerization, rather than deposition of aggregates, may be the real culprits in neurodegeneration*

Hartley, D. M., Walsh, D. M., Ye, C. P., Diehl, T., Vasquez, S., Vassilev, P. M., Teplow, D. B., Selkoe, D. J. Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons, *J. Neurosci*. **19**, 8876–8884, (1999); Goldberg, M. S., Lansbury, P. T. Jr., Is there a cause-and-effect relationship between α-synuclein fibrillation and Parkinson's disease, *Nat. Cell Biol*. **2**, E115–E119, (2000)

Brain inflammation hypothesis

Native protein —*misfolding*→ Misfolded proteins and aggregates → Brain inflammation → Neuronal loss

Abnormal protein aggregates act as irritants and cause a chronic inflammatory reaction in the brain that leads to neuronal death and synaptic changes



HD cerebral caudate (L) and control (R) showing enlarged microglia and enhanced immnoreactivity

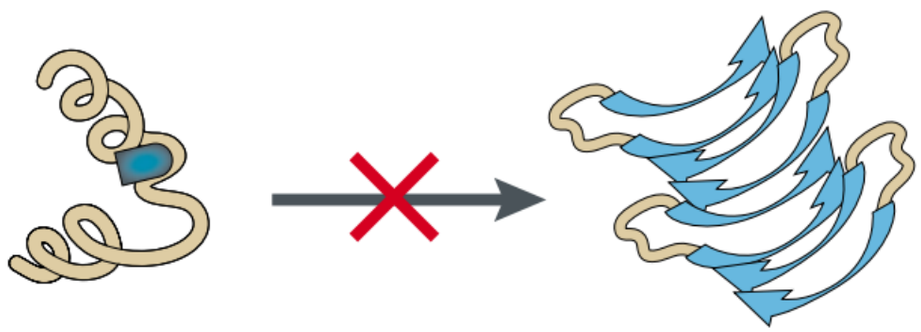
Recent studies show both positive and negative effects of inflammation

Wyss-Coray, T., Muckle, L., Inflammation in neurodegenerative diseases - a double-edged sword, *Neuron* **35**, 419–432, (2002)

What strategies exist to combat conformational diseases?

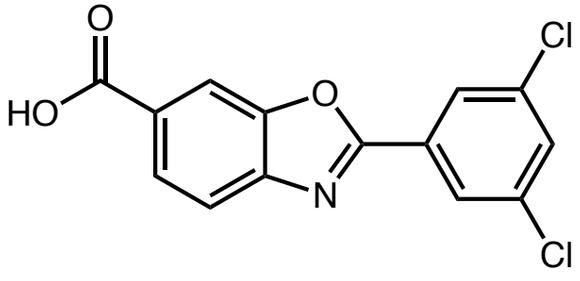
Despite impressive progress in understanding the pathogenesis of neurodegenerative diseases, ***none*** *of these disorders can be successfully treated*

At least four approaches have been proposed to attack protein misfolding and aggregation



Stabilisers of protein folding

- Chemical chaperones known to stabilise native protein conformations

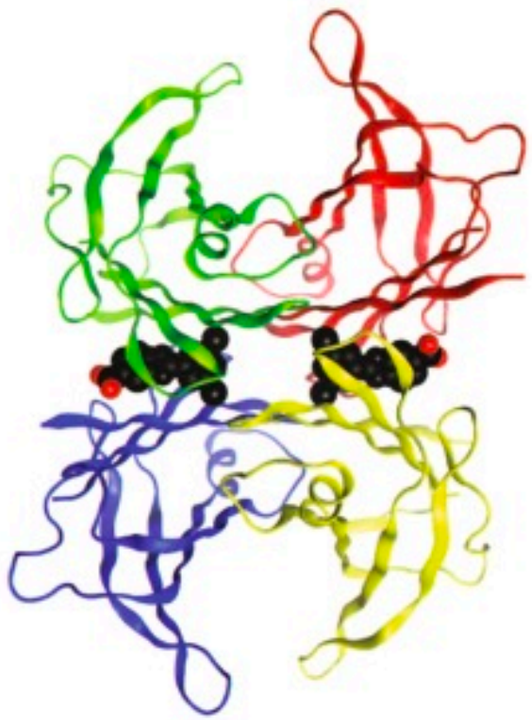


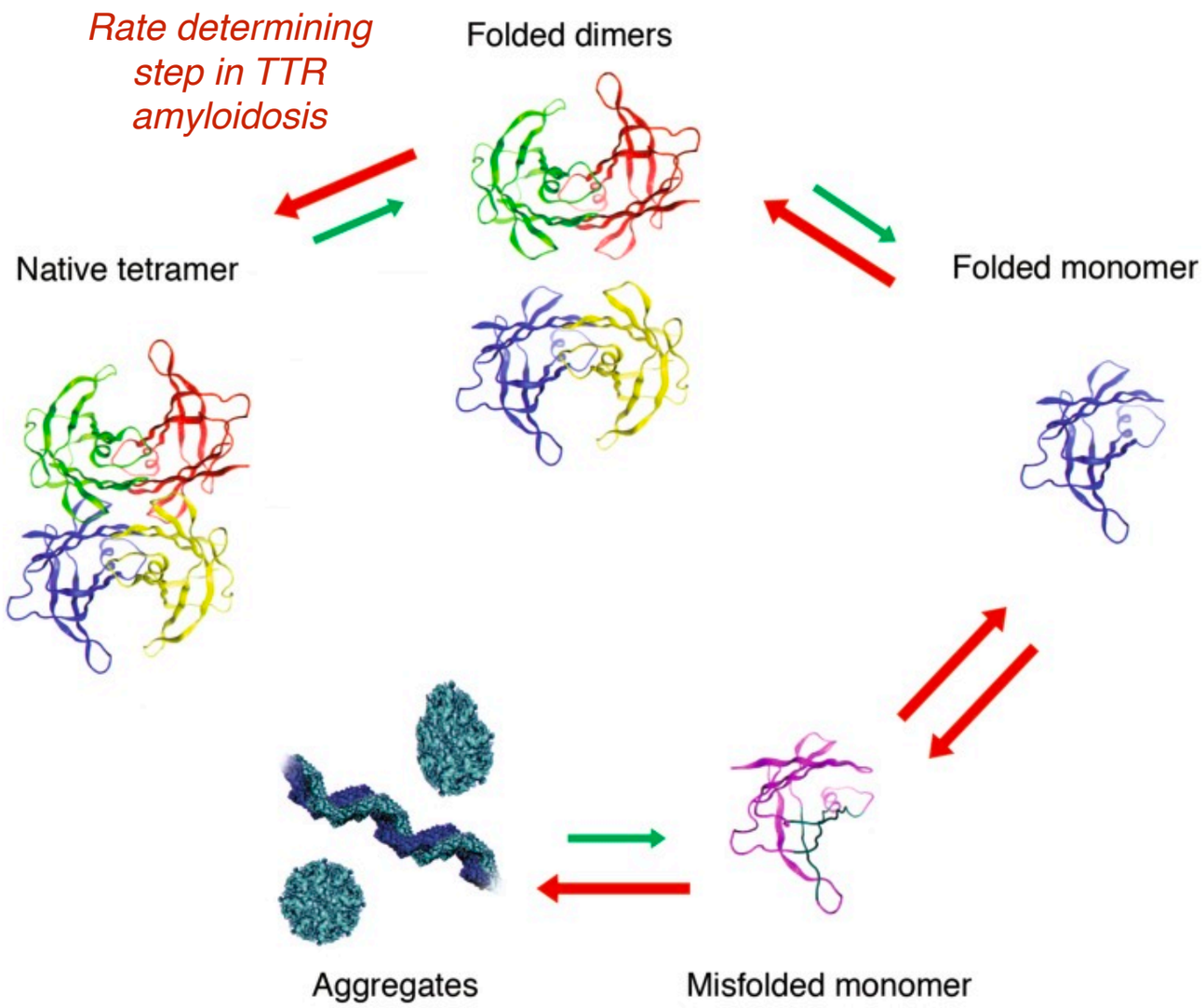
Tafamidis
Vyndaquel (Pfizer)

Received FDA approval in 2019 for the treatment of cardiomyopathy via the stabilisation of the protein ***transthyretin***

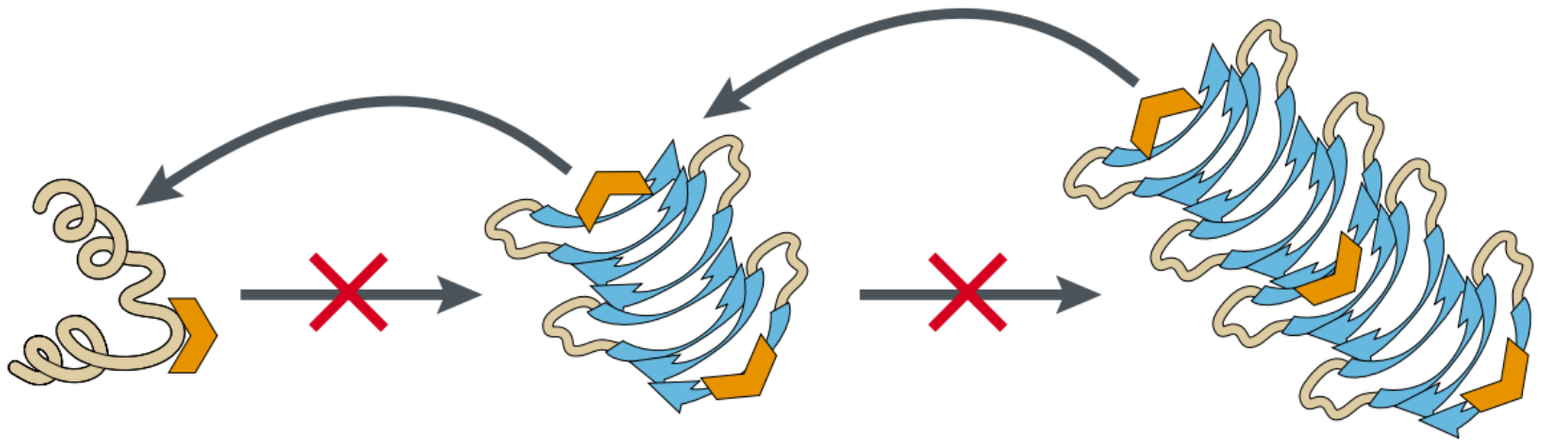
At least four approaches have been proposed to attack protein misfolding and aggregation

Tafamidis stabilises the β-sheet assembly in tetrameric species





At least four approaches have been proposed to attack protein misfolding and aggregation



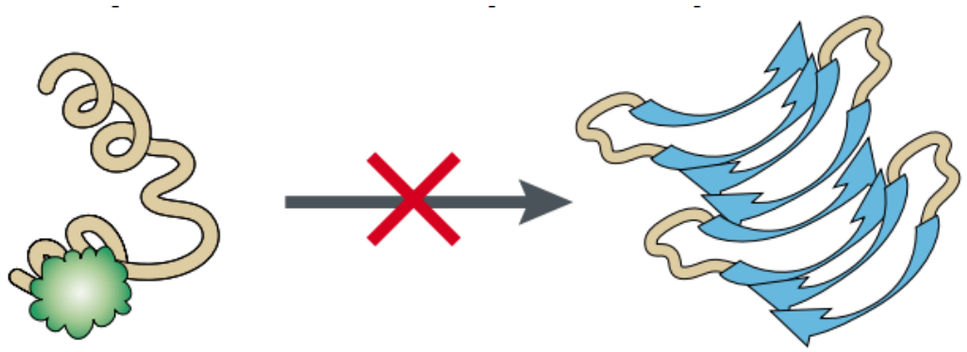
β-sheet breakers

- Short synthetic peptides

designed to arrest the folding of the polypeptide in the β-sheet

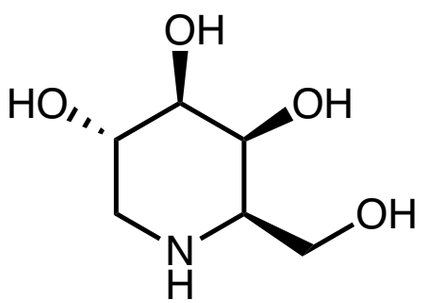
Typically unstable in vivo (therapeutic limitations)

At least four approaches have been proposed to attack protein misfolding and aggregation



Competitive inhibitors (monomers and oligomers)

- Compounds that block the interaction between monomers and molecules that tack at the edge of β-sheet aggregates



Migalastat
Galafold (Amicus)

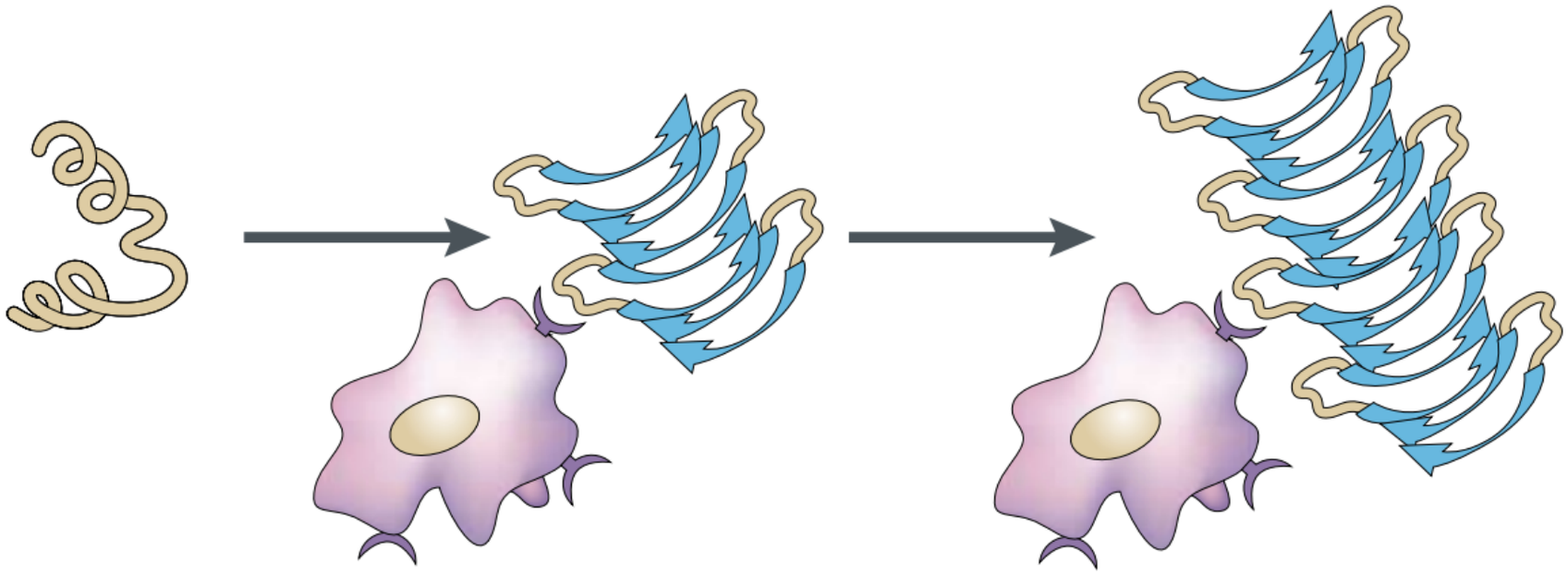
Fabry disease is a rare genetic disorder caused by mutations to the enzyme α-GalA , leading to ***misfolding***

Binding of migalastat to α-GalA shifts the folding behaviour towards the proper conformation, resulting in a functional enzyme

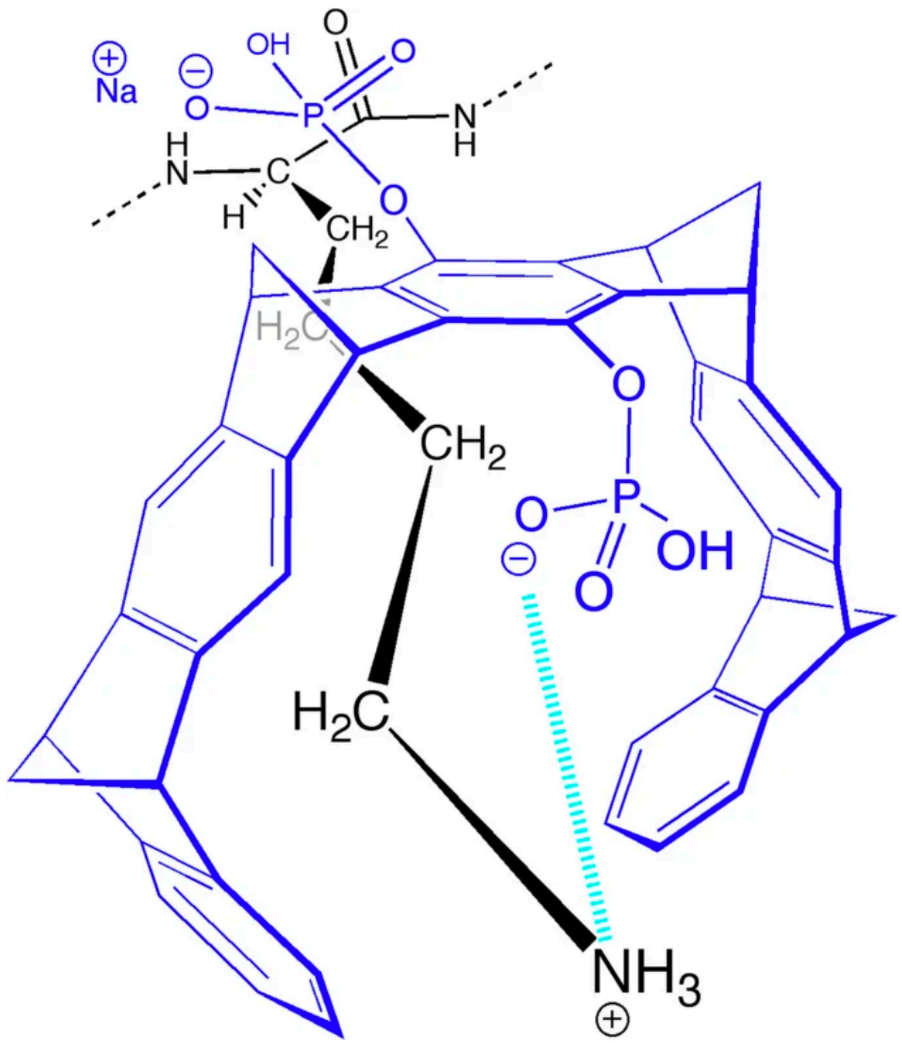
At least four approaches have been proposed to attack protein misfolding and aggregation

Clearance enhancers

- Aggregates of synthetic misfolded protein can be used as antigens to induce immune response, producing antibodies to clear them



At least four approaches have been proposed to attack protein misfolding and aggregation



Prof. Gal Bitan
(UCLA)

"CLR01 acts by decreasing synuclein in the brain by wrapping around lysine chains and remodelling the assembly into non-toxic structures"

Protein Function and Three-Dimensional Structure

Alzheimer's disease therapy: a critical appraisal

Protein Function and Three-Dimensional Structure

6th
most common cause of death

17%
occurence ages 75-84 years

$236b
annual cost to economy

100%
failure rate for new therapeutics

$204b
money spent in finding therapeutics

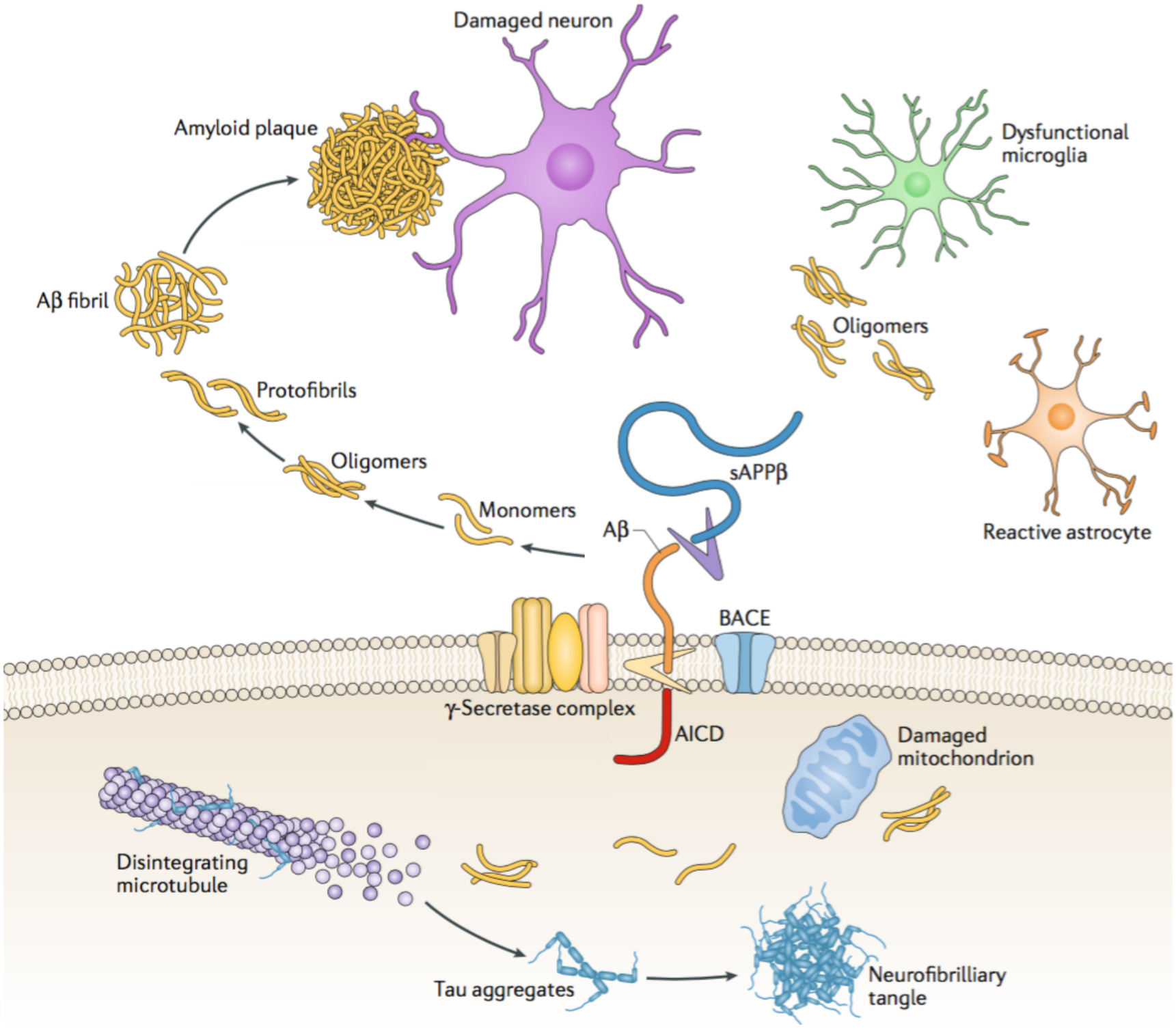
402 years
cumulative time spent finding therapeutics

Cummings, J., Reiber, C., Kumar, P., The price of progress: Funding and financing Alzheimer's disease drug development. *Alzheimers Dement. (NY)* **4**, 330–343 (2017)

Protein Function and Three-Dimensional Structure

Alzheimer's disease therapy is based on the amyloid cascade hypothesis

Amyloid cascade hypothesis



- Amyloid precursor protein (APP) is cleaved by **β-secretase 1** (BACE1) generating a soluble extracellular fragment (sAPPβ) and membrane bound fragment (C99)

- C99 is cleaved by an enzymatic complex of four proteins, collectively termed **γ-secretase** releasing Aβ and an intracellular peptide known as amyloid intracellular domain (AICD)

- Aβ aggregates to ultimately form plaques (hallmarks of AD)

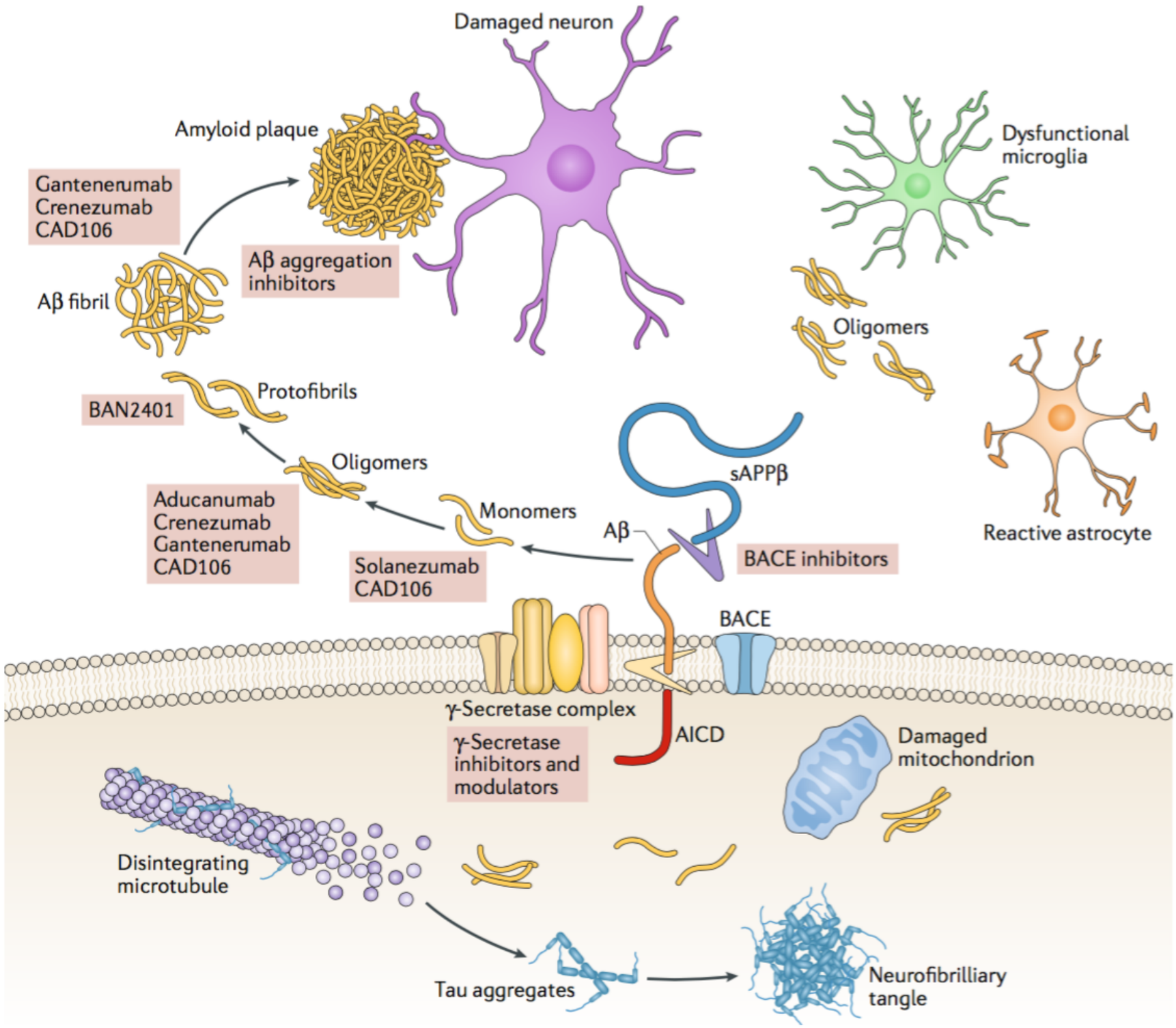
- Intracellular deposition of hyperphosphorylated tau protein in neurofibrillary tangles (NFTs) leads to progressive cytoskeletal changes and disrupts axonal transport

Karran, E., Mercken, M., De Strooper, B., The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Review Drug Discov.* **10**, 698–712 (2011)

Amyloid cascade hypothesis

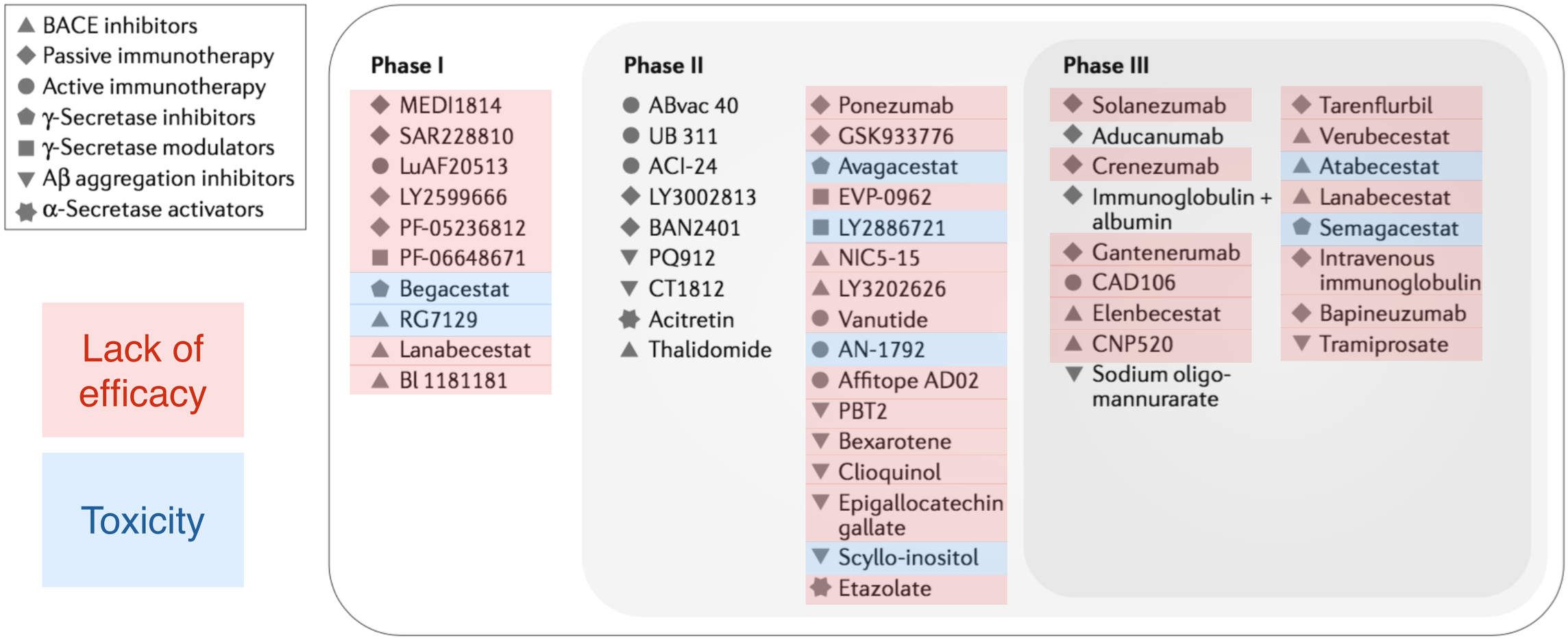
Many opportunities for therapeutics?

Putative disease modifying therapies



Karran, E., Mercken, M., De Strooper, B., The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Review Drug Discov.* **10**, 698–712 (2011)

Putative disease modifying therapies



Lack of efficacy

Toxicity

Almost all drugs that decrease the production of Aβ or increase Aβ brain clearance have failed to improve cognitive outcomes despite reducing brain Aβ

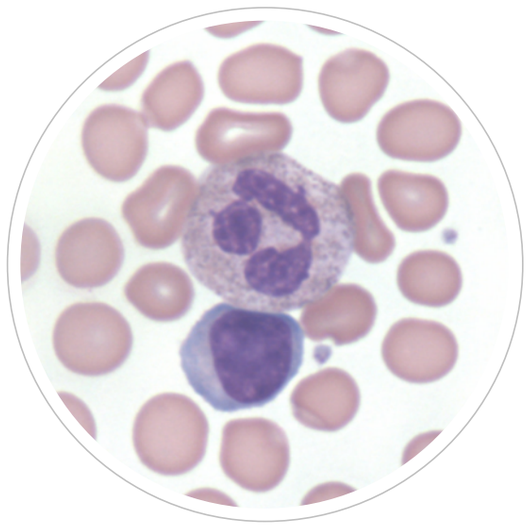
Karran, E., Mercken, M., De Strooper, B., The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Review Drug Discov.* **10**, 698–712 (2011)

Putative disease modifying therapies

Is it time to rethink the amyloid cascade hypothesis?

The sepsis parallel

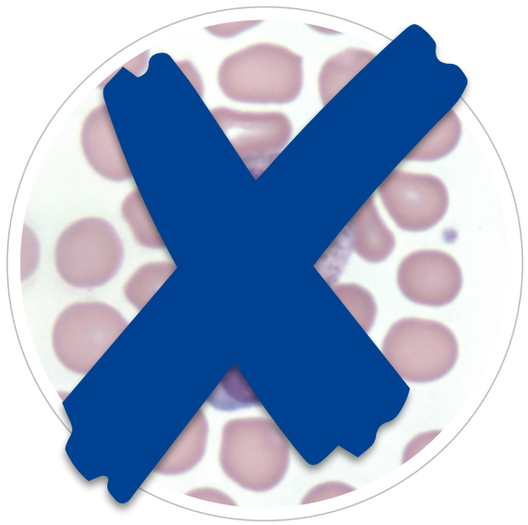
Sepsis is a complex disorder that develops as a dysregulated host response to an infection and is associated with high risk of death

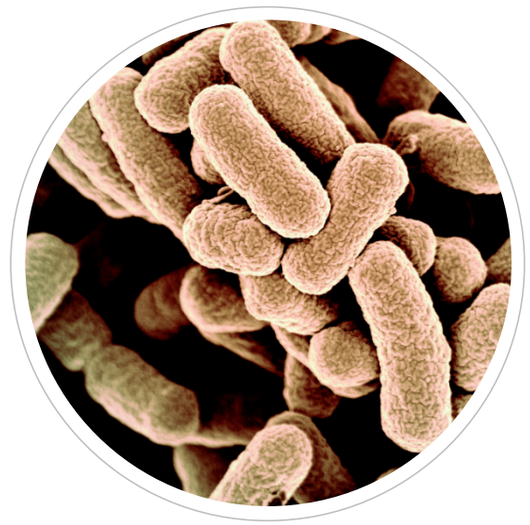


Leukocytosis is typical **finding**

The sepsis parallel

Sepsis is a complex disorder that develops as a dysregulated host response to an infection and is associated with high risk of death

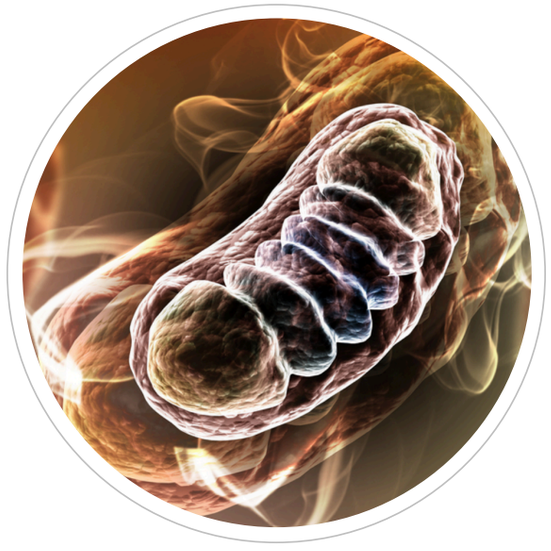




Leukocytosis is typical **finding**

Treatment is aimed at **underlying infection**

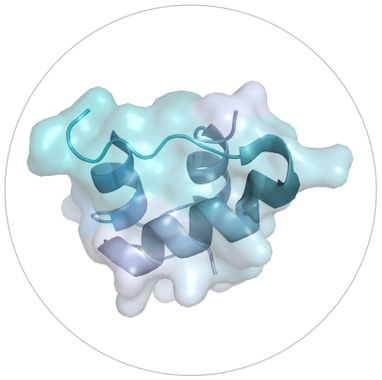
The sepsis parallel



*Aβ accumulation might be a reaction of the brain to **neuronal damage** (e.g. mitochondrial dysregulation)*

An alternative option for AD therapeutics is to address the modifiable risk factors for developing AD

Promising new avenues for AD therapy



Neurons are dependent on the

neurotrophic properties of insulin

Resistance to the effects of insulin make neurons vulnerable

to stress ***resulting in impairments in function***

In the aged brain, insulin might ***lose its effectiveness*** *as a growth factor.*

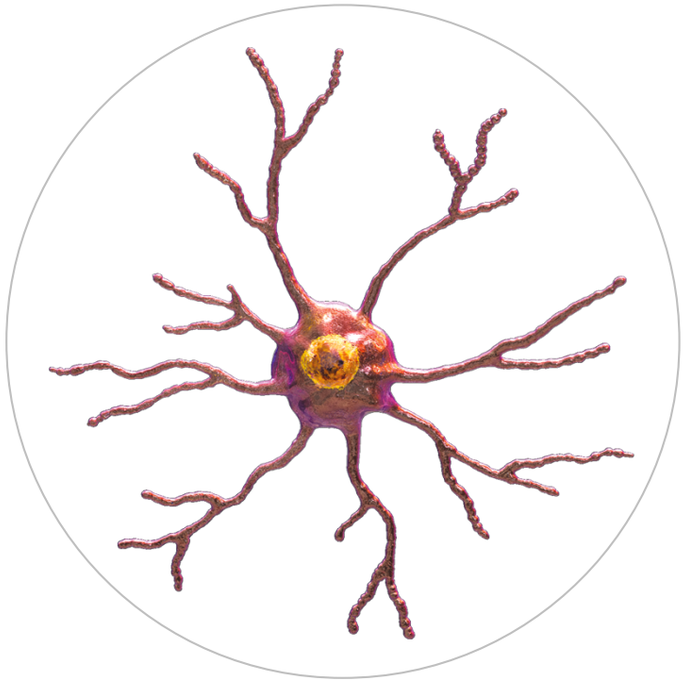
Therefore, ***anti-diabetes drugs*** *might be an effective approach for treating AD*

Benedict, C., Grillo, C. A., Insulin resistance as a therapeutic target in the treatment of Alzheimer's disease: a state-of-the-art. *Front. Neurosci.* **12**, 215 (2018)

Promising new avenues for AD therapy

*Several studies have identified a link between the brain's immune system and AD, of which **microglia** have a central role*

Microglia might be activated by peripheral inflammation and/or **gut microbiota**



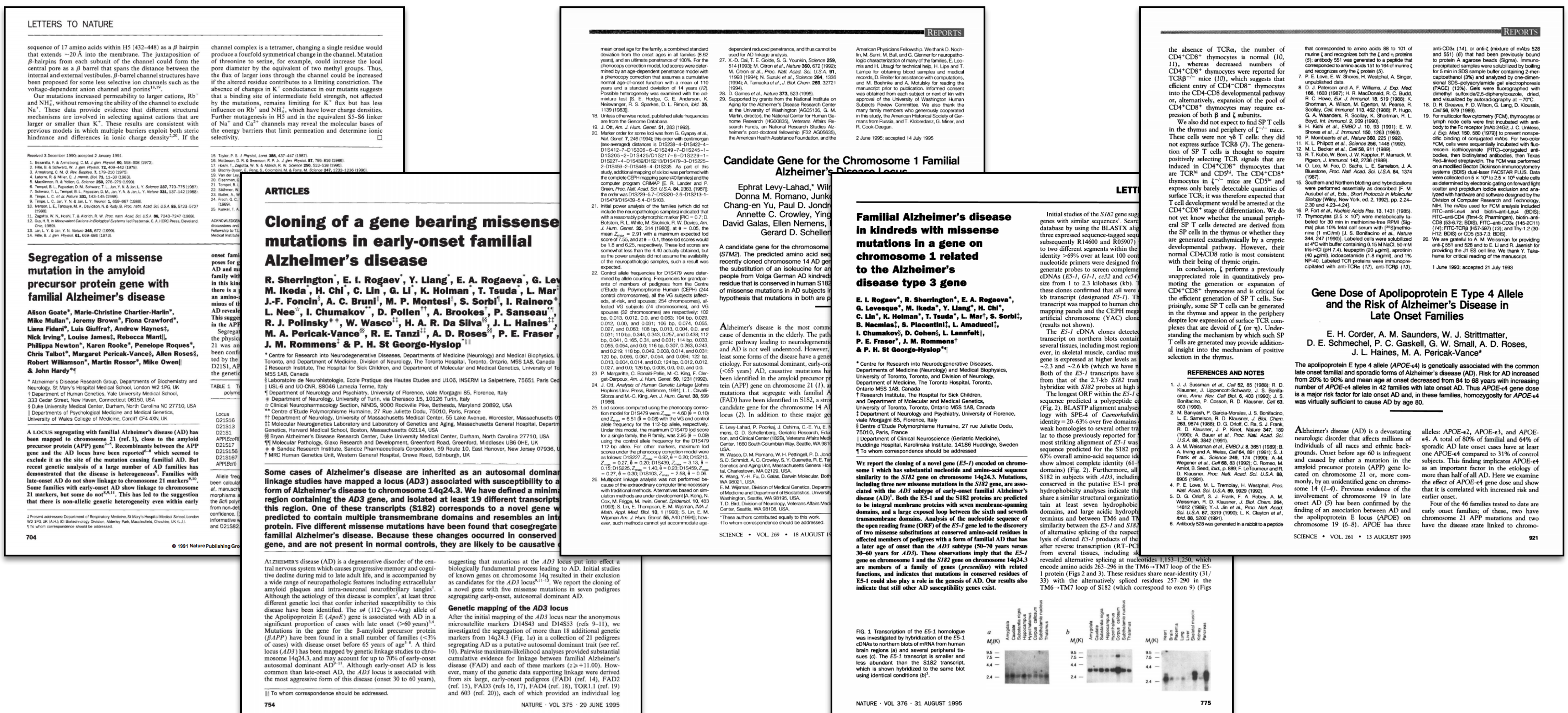
Salter, M. W., Stevens, B., Microglia emerge as central players in brain disease. *Nat. Med.* **23**, 1018–1027 (2017); Marsh, S. E., The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function, *Proc. Natl. Acad. Sci.* **113**, E1316–E1325, (2016)

Promising new avenues for AD therapy

The future of gene modifying therapies

Promising new avenues for AD therapy

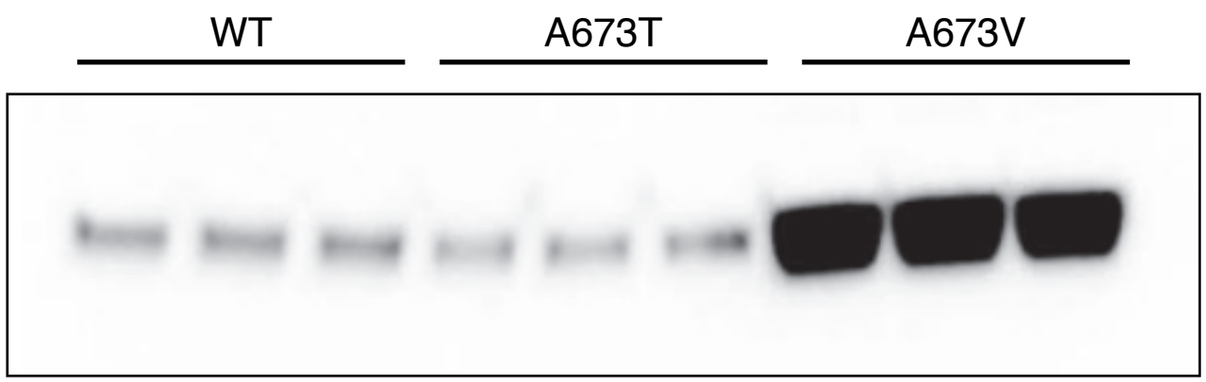
Autosomal dominant mutations were found in the genes coding for APP, PSEN1, and PSEN2. Transgenic mice that expressed these mutations developed brain Aβ plaques and memory deficits (1990s)

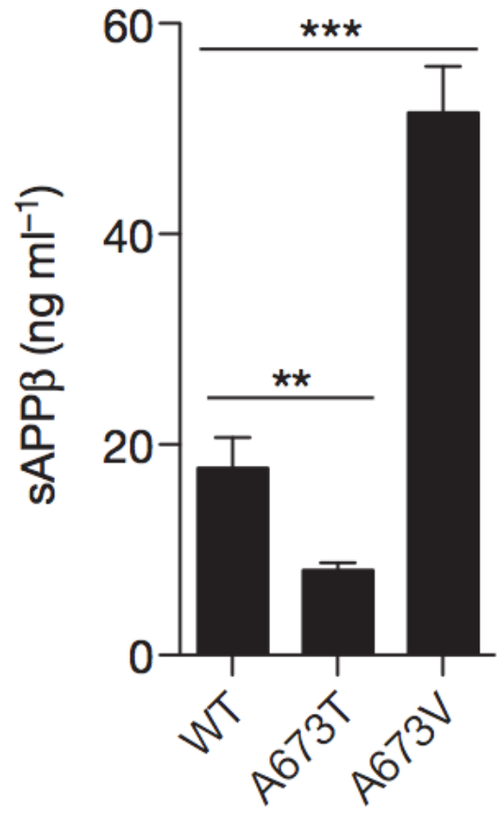


Karran, E., Mercken, M., De Strooper, B., The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Review Drug Discov.* **10**, 698–712 (2011)

Promising new avenues for AD therapy

APP amino acid substitution A673T was found to protect against AD onset and cognitive decline in cognitively healthy elderly individuals





The mutant amino acid is adjacent to the BACE cleavage site, and its presence resulted in an ~40% reduction in the formation of Aβ peptides in vitro

A673V is a pathogenic variant know to increase amyloid deposits

Jonsson, T., *et al*., A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* **488**, 96–99 (2012)

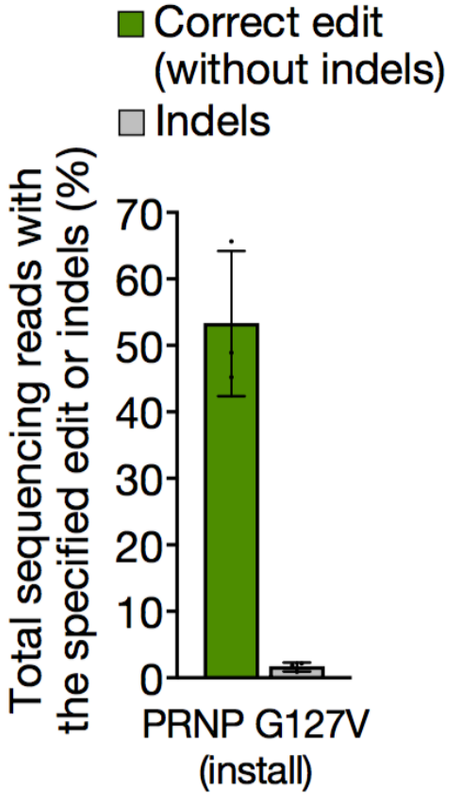
Promising new avenues for AD therapy

Prime editing enables insertions, deletions, and all possible base-to-base conversions without double-strand breaks

PRNP (prion) prime editing

Protection can be conferred by a G•C to T•A transversion

Prime editing: 53% installation of G127V with 1.7% indels



Exciting future directions in interfacing genome editing strategies with in vivo delivery for tackling disease

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., Liu, D. R., Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. **576**, 149–157 (2019)

Solving the protein folding problem

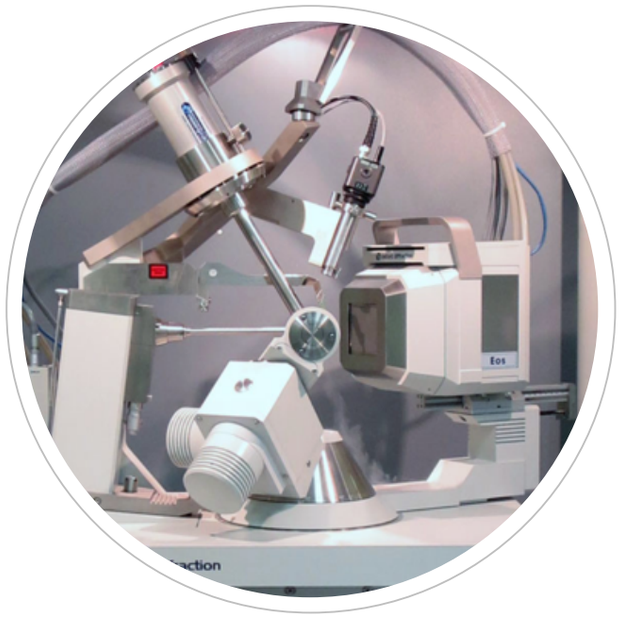
There may be many more misfolded proteins that contribute toward disease

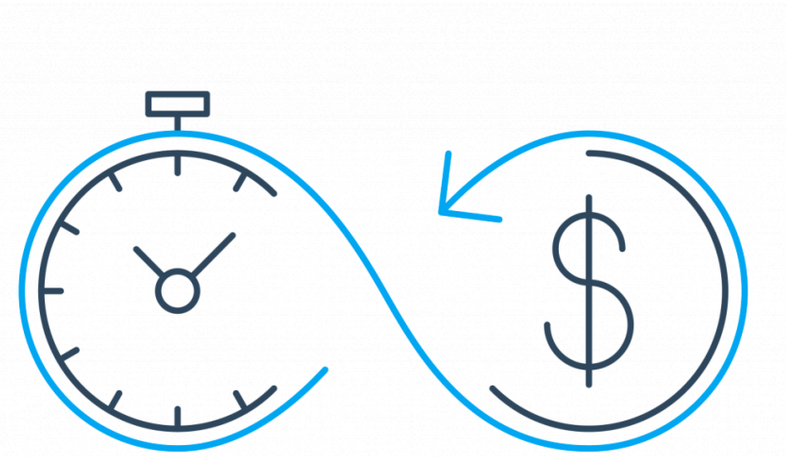
*UniProt contains **180 million** unique protein sequences;*

*to date **170,000** structures have been elucidated*

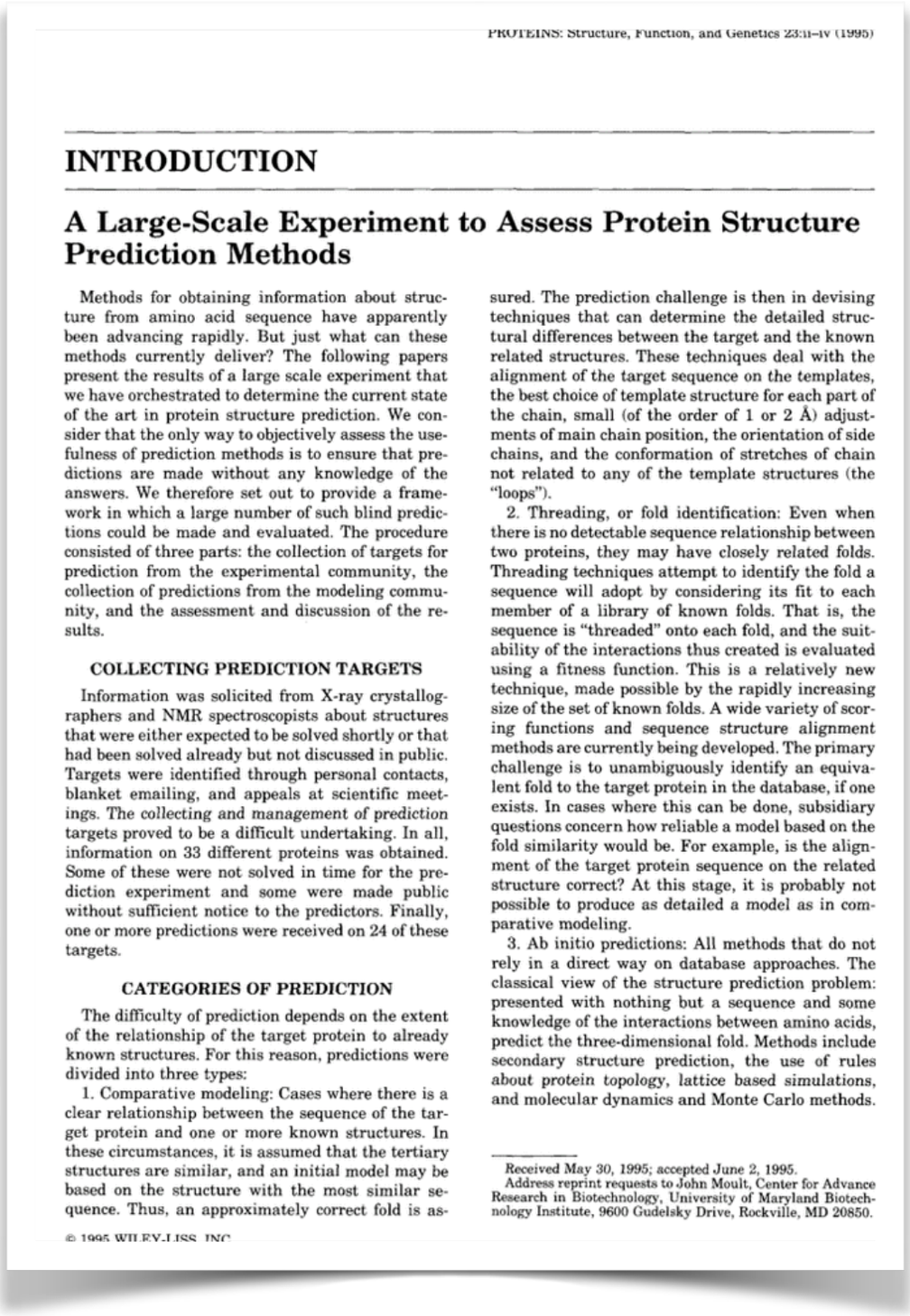
Solving the protein folding problem

There may be many more misfolded proteins that contribute toward disease





CASP: the protein solving challenge



PROTEINS: Structure, Function, and Genetics 23:ii–iv (1995)

INTRODUCTION

A Large-Scale Experiment to Assess Protein Structure Prediction Methods

Methods for obtaining information about structure from amino acid sequence have apparently been advancing rapidly. But just what can these methods currently deliver? The following papers present the results of a large scale experiment that we have orchestrated to determine the current state of the art in protein structure prediction. We consider that the only way to objectively assess the usefulness of prediction methods is to ensure that predictions are made without any knowledge of the answers. We therefore set out to provide a framework in which a large number of such blind predictions could be made and evaluated. The procedure consisted of three parts: the collection of targets for prediction from the experimental community, the collection of predictions from the modeling community, and the assessment and discussion of the results.

COLLECTING PREDICTION TARGETS

Information was solicited from X-ray crystallographers and NMR spectroscopists about structures that were either expected to be solved shortly or that had been solved already but not discussed in public. Targets were identified through personal contacts, blanket emailing, and appeals at scientific meetings. The collecting and management of prediction targets proved to be a difficult undertaking. In all, information on 33 different proteins was obtained. Some of these were not solved in time for the prediction experiment and some were made public without sufficient notice to the predictors. Finally, one or more predictions were received on 24 of these targets.

CATEGORIES OF PREDICTION

The difficulty of prediction depends on the extent of the relationship of the target protein to already known structures. For this reason, predictions were divided into three types:

1. Comparative modeling: Cases where there is a clear relationship between the sequence of the target protein and one or more known structures. In these circumstances, it is assumed that the tertiary structures are similar, and an initial model may be based on the structure with the most similar sequence. Thus, an approximately correct fold is assured. The prediction challenge is then in devising techniques that can determine the detailed structural differences between the target and the known related structures. These techniques deal with the alignment of the target sequence on the templates, the best choice of template structure for each part of the chain, small (of the order of 1 or 2 Å) adjustments of main chain position, the orientation of side chains, and the conformation of stretches of chain not related to any of the template structures (the "loops").

2. Threading, or fold identification: Even when there is no detectable sequence relationship between two proteins, they may have closely related folds. Threading techniques attempt to identify the fold a sequence will adopt by considering its fit to each member of a library of known folds. That is, the sequence is "threaded" onto each fold, and the suitability of the interactions thus created is evaluated using a fitness function. This is a relatively new technique, made possible by the rapidly increasing size of the set of known folds. A wide variety of scoring functions and sequence structure alignment methods are currently being developed. The primary challenge is to unambiguously identify an equivalent fold to the target protein in the database, if one exists. In cases where this can be done, subsidiary questions concern how reliable a model based on the fold similarity would be. For example, is the alignment of the target protein sequence on the related structure correct? At this stage, it is probably not possible to produce as detailed a model as in comparative modeling.

3. Ab initio predictions: All methods that do not rely in a direct way on database approaches. The classical view of the structure prediction problem: presented with nothing but a sequence and some knowledge of the interactions between amino acids, predict the three-dimensional fold. Methods include secondary structure prediction, the use of rules about protein topology, lattice based simulations, and molecular dynamics and Monte Carlo methods.

Received May 30, 1995; accepted June 2, 1995.
Address reprint requests to John Moult, Center for Advance Research in Biotechnology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, MD 20850.

*In 1994, Prof. John Moult and Prof. Krzysztof Fidelis founded **CASP** as a biennial assessment for blind protein structure prediction*

Global distance test (GDT)

Ranges from 0 to 100

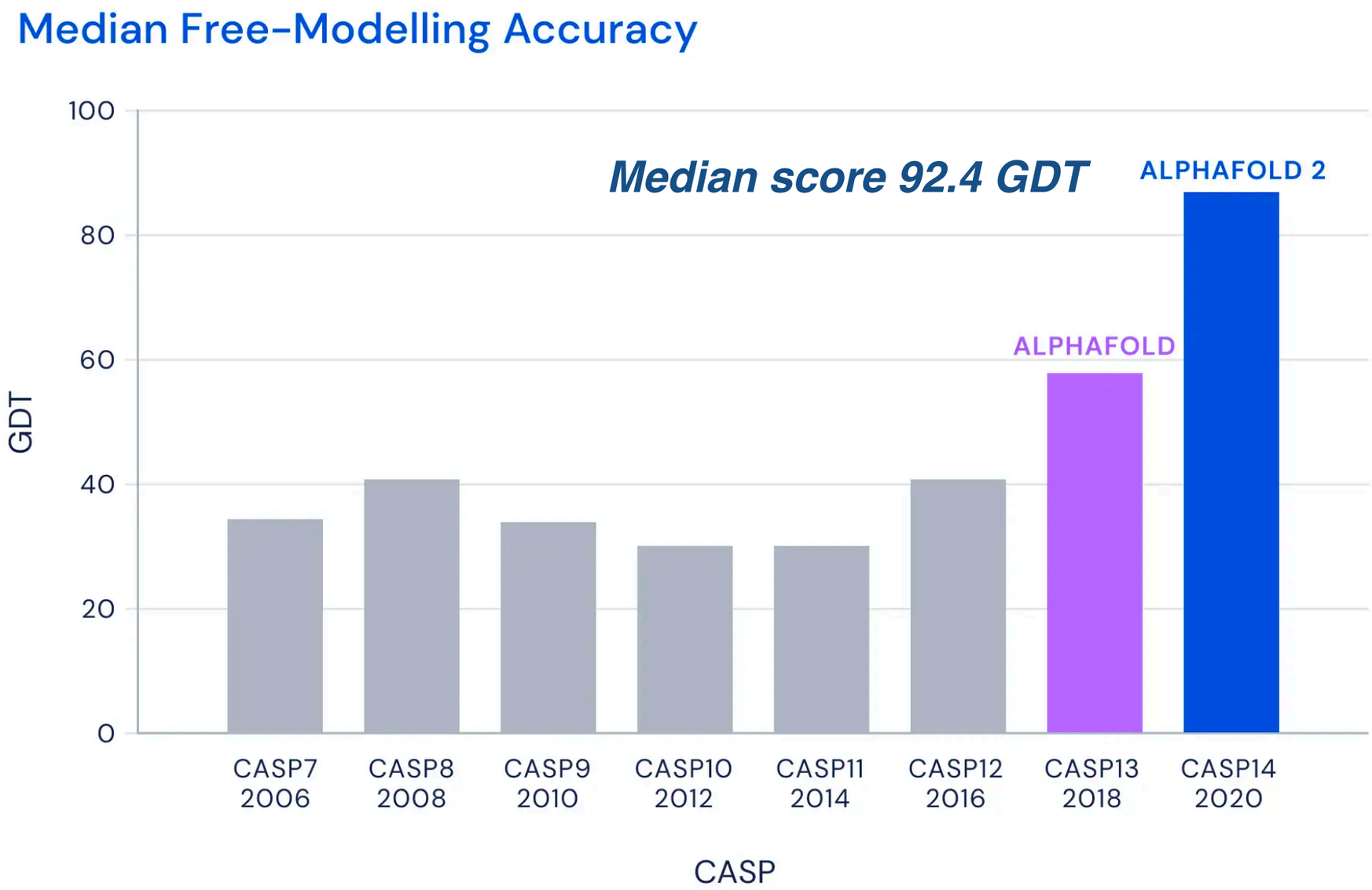
% proteins within a certain distance from their correct position

***>90 GDT** is competitive*

DeepMind

CASP: the protein solving challenge

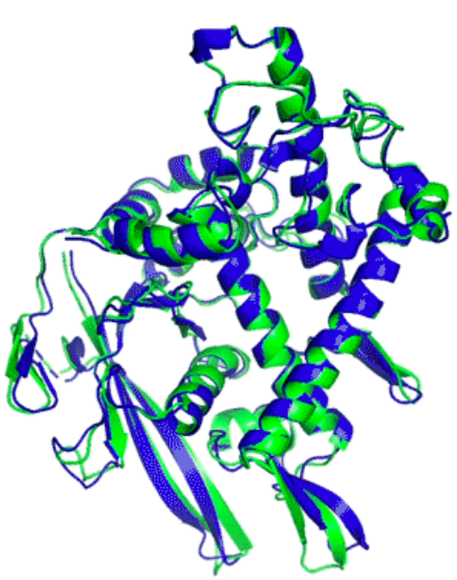
Google's UK-based lab and research company has solved the problem



https://deepmind.com/blog/article/alphafold-a-solution-to-a-50-year-old-grand-challenge-in-biology

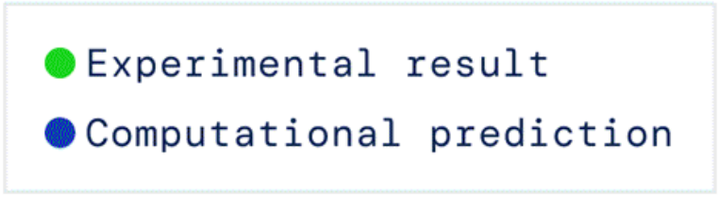
DeepMind

CASP: the protein solving challenge



T1037 / 6vr4
90.7 GDT
(RNA polymerase domain)

T1049 / 6y4f
93.3 GDT
(adhesin tip)



Requires very modest computing power: ~100-200 GPUs run over a few weeks

https://deepmind.com/blog/article/alphafold-a-solution-to-a-50-year-old-grand-challenge-in-biology

DeepMind

CASP: the protein solving challenge

Hopefully many more protein structures at risk of misfolding can be identified

https://deepmind.com/blog/article/alphafold-a-solution-to-a-50-year-old-grand-challenge-in-biology