

Therapeutic proteins

 Course	 Essential Protein Structure and Function
 Confidence	Not Confident
 Next Review	@April 30, 2024
 Last Edited	@May 3, 2024 9:46 AM

Principles of proteins as therapeutic targets

Essential function of proteins in living systems

Proteins at the molecular level

- 20,000 different types of proteins
- Each protein has a unique amino acid & sequence structure
- Tertiary structure needed for biological activity
 - Intrinsically disordered proteins that do not have persistent tertiary structure also have roles in biological function

Proteins at the cellular level

- Correctly folded proteins are involved in carrying out a range of cellular processes
 - Gene regulation
 - Transcription
 - Protein synthesis
 - Signaling
 - Proteolysis
 - Trafficking
 - Degradation
 - Apoptosis

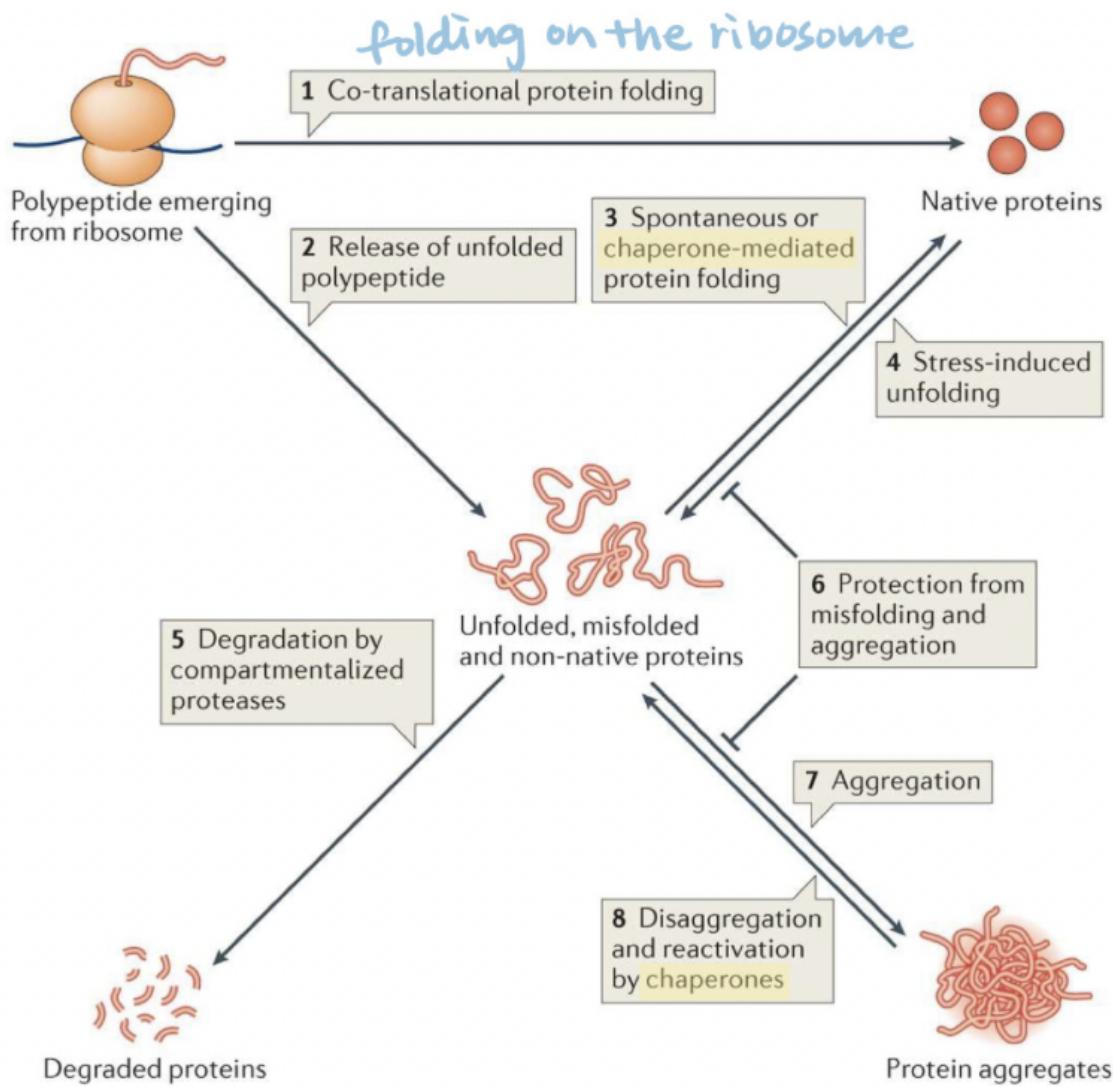
- Autophagy
- Any cellular process is underpinned by one or more protein

Protein acquire their 3D structures by folding

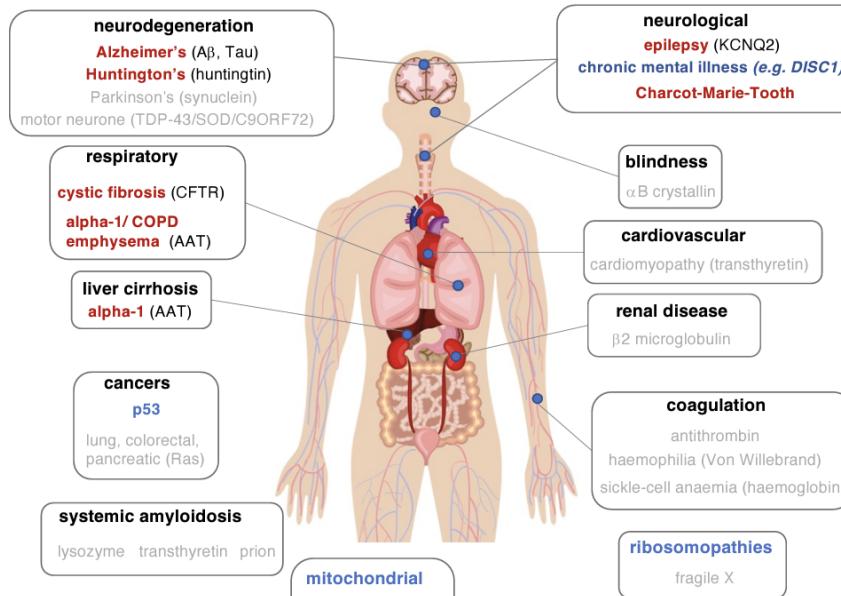
- Protein acquire 3D structure by “folding”, forming essential contacts within the polypeptide chain
 - In vitro: Polypeptide chain folds as a whole
 - In cells: co-translational
- Protein acquire structure as a downhill search on an energy landscape
- The completed 3D structure is the most energetically favorable state (lowest point in a landscape)
 - There are defined ways in which polypeptide chain must form contacts to acquire structure
 - Proteins have highly dynamic structures, which is useful

▼ Chaperone proteins deal with misfolded protein

- Most proteins fold spontaneously, driven by the hydrophobic effect.
- Misfolded proteins often have hydrophobic amino acids on their surfaces. These hydrophobic patches can interact, forming large aggregates.
 - If misfolded proteins are not mediated by chaperones; the rate of aggregation is faster than chaperone's activity.
 - Protein aggregation plays an important role in diseases such as Alzheimer's, Parkinson's, and mad cow disease.
- **Chaperones interact with misfolded proteins and assist in protein folding by binding to hydrophobic regions to prevent aggregation or by providing a microenvironment to promote folding.**
 - E.g. "heat shock proteins" (Hsps) and the bacterial chaperonin system GroEL/GroES.



- Misfolded proteins can also be targeted for destruction by the proteasome.
- When misfolded proteins cannot be dealt with normal cellular processes due to mutations, aging, etc., it causes diseases
 - There are 50 different proteins involved in **conformational diseases**
 - Unable to fold or stay folded causes aggregates, which is associated with AD and PD



Knowles TP et al. (2014) *Nat Rev Mol Cell Biol* 15: 384-396
Chiti F & Dobson CM, *Ann. Rev. Biochem.*, Vol 86. 2017

- Many (inherited) diseases are monogenic (single gene causing)
- Mutations can result in a malfunctioning protein
 - Not produced in sufficient quantities
 - Misfolded
 - Degraded easily
 - Low activity

disease	protein	genetic origin	protein	disease type
cystic fibrosis	cystic fibrosis transmembrane regulator	autosomal recessive	misfolded, degraded	respiratory
sickle-cell anaemia	beta haemoglobin	autosomal recessive	misfolded	red blood cell disorder
haemophilia A	Factor VIII	X-linked recessive	missing or defective protein	coagulation
muscular dystrophy	dystrophin	X-linked recessive	missing or defective protein	muscle-weakening
Huntington's disease	Huntington	autosomal dominant	Protein aggregation	neurological
phenylketonuria	phenylalanine hydroxylase	autosomal recessive	poor enzyme activity	metabolic
alpha-1-antitrypsin deficiency	antitrypsin	autosomal recessive	protein misfolding	emphysema liver cirrhosis
albinism	oculocutaneous albinism II	autosomal recessive	defective enzyme	pigmentation

mendelian genetics (inheritance pattern)

autosomal recessive
2 copies of defective gene (needed)

X-linked
Mutations on X chromosome

autosomal dominant
1 copy of defective gene (needed)

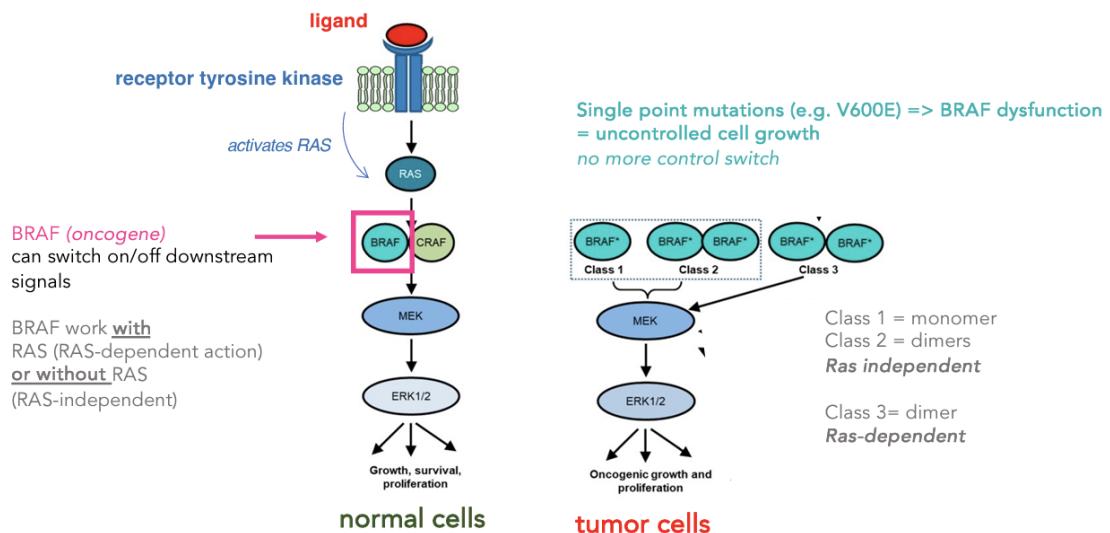
- Most diseases involve complex pathways & proteins involved in deep interaction networks
 - May involve sets of mutated genes

Proteins are involved in complex signalling pathways

- Mitogen-Activated Protein Kinase (MAPK) signalling pathway
- Essential signalling pathway in regulating many cellular processes:
 - Inflammation, cell response, cell differentiation, cell division, cell proliferation, metabolism, motility & apoptosis
- MAPK pathway implicated in cancer, immune-based disorders & neurodegenerative diseases

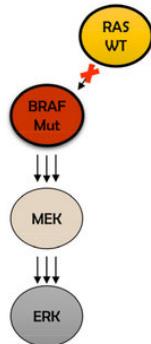
BRAF mutations - Example of an error in signalling pathway leading to disease

- Typically through the binding of growth factors to cell surface receptors such as receptor tyrosine kinases (RTKs), Ras gets activated, which activates different downstream proteins
- BRAF phosphorylates and activates downstream kinases in the MAPK signaling pathway.
 - BRAF switch on/off downstream signals
- When mutated, BRAF may work with Ras or without Ras
 - This leaves BRAF on all the time, causing oncogenic growth and proliferation
- Mutations have a complex effect on BRAF's physical properties
 - Point mutation dysfunctioning a useful cellular switch is involved in lots of cancer
 - One mutation can affect not only one protein, but lots of downstream processes
- BRAF is a clear target for therapeutic intervention (e.g. colorectal & melanoma)



Class I

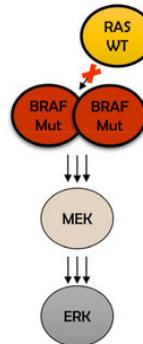
- V600 mutant
- Kinase activated
- RAS-independent
- BRAF monomers



V600E, V600K, V600D
V600R, V600M

Class II

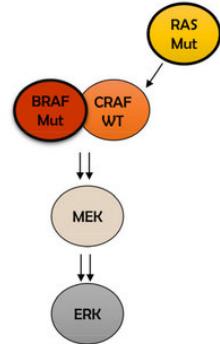
- Non-V600 mutant
- Kinase activated
- RAS-independent
- BRAF dimers



K601E, K601N, K601T
L597Q, L597V, G469A
G469V, G469R, G464V
G464E, Fusion Proteins

Class III

- Non-V600 mutant
- Kinase impaired
- RAS-dependent
- BRAF heterodimers



D287H, V459L, G466V
G466E, G466A, S467L
G469E, N581S, N581I
D594N, D594G, D594A
D594H, F595L, G596D
G596R

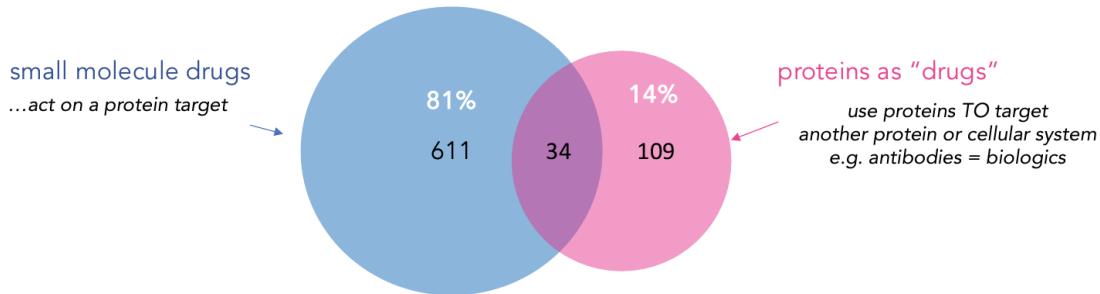
The human proteome is “druggable”

- 4 types of macromolecule targets
 - Proteins
 - Polysaccharides
 - Lipids
 - Nucleic acids
- Proteins make excellent workhorses, as they have instrumental roles in the cell

- Typically target protein >> Nucleic Acid because of their diversity and structural and dynamic properties of 3D structure
- We can study the physio/chemical, structural, kinetic, binding, catalytic & mechanistic properties of proteins and peptides
 - Proteins are involved deeply in mechanisms and tools to dissect these pathways
- We can study similarities between protein families: structural folds, amino acid sequence properties, biochemical properties, downstream pathways
 - Make predictions on functions based on their families and structures
- Design and produce proteins with improved or superior characteristics for use as biopharmaceuticals
- Make predictions of what could bind to a protein
- Almost all approved drugs act on or affect proteins in some way

What are the types of therapeutics?

- Therapeutics can fall into 2 main classes

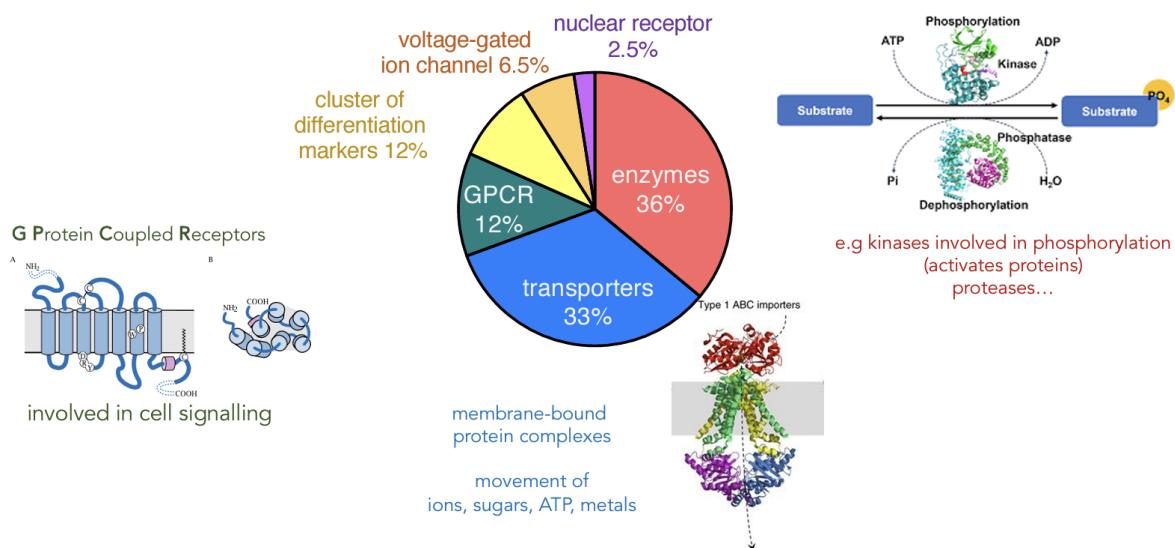


- Small molecule drugs
 - Act on a protein target
- proteins as "drugs"
 - Use proteins to target another protein or cellular system
 - E.g. antibodies = biologics
- ~4009 genes: experimental evidence for involvement in disease
 - E.g. cancer, neurological & cardiovascular diseases
 - Most disrupts some form of signal transduction in disease

- Thus research into develop therapeutics that modify or alter signal transduction issue

Target proteins

- 3 main classes
 - Enzymes
 - Transporters/ion channels
 - Receptors



Why proteins as therapeutic targets?

- Not all proteins (>20000) makes good targets.
 - Because a small molecule (or antibody) needs to bind with affinity and chemical properties at the same time within a disease
- Structure-function relationship needed (mechanism)
- Study the protein in isolation (in vitro) and in a physiological context (in vivo)

Characterising the therapeutic protein targets

- Develop models for a protein's biological role and its mode using a range of biochemical and biophysical approaches
- Produce recombinant protein in systems such as E.coli, yeast, human cell (ascending advanced systems)

- E.g. human cells involves post-translational modification
- Ideal: isolated protein behave close to real one in vivo

In vitro	Cell-based models	Animal models
<ul style="list-style-type: none"> • PAGE & Western blot • ELISA <ul style="list-style-type: none"> ◦ Quantify interactions (strength) such as determining Kd • Circular dichroism <ul style="list-style-type: none"> ◦ Understand secondary structure • Mass spectrometry • Fluorescence <ul style="list-style-type: none"> ◦ Interaction on time scale 	<ul style="list-style-type: none"> • Immunofluorescence <ul style="list-style-type: none"> ◦ Expression and location • Profiling <ul style="list-style-type: none"> ◦ How protein behave inside cells ◦ What other protein involved 	<ul style="list-style-type: none"> • Fruit flies, zebrafish, worms, mice, rats

High resolution structural techniques

- A knowledge of protein's (3D) structure can inform us its mechanism of function
 - Crystallography
 - Cryo electron microscopy
 - Larger proteins
 - NMR spectroscopy
 - Structure and dynamic properties

Computational biology: structural-based drug design

- Apply computational biology tools (*in silico*) to high-resolution 3D structures of proteins to develop new drug targets: "virtual screening" that

is validated experimentally

- Note that it is built upon the knowledge of high-resolution 3D structures
- OR: use an initial screen, and then use in silico tools to design better candidates
 - Predictions of binding to protein of interest
 - Understand whether a type of binding will take place

Importance of molecular recognition

- Drugs act by targeting proteins and affecting their activity
 - E.g. many protein targets involve receptors
- 2 main classes for targeting receptors
 - Agonists: occupy a binding site & activates the protein
 - Antagonists: occupy a binding site but do not activate them
 - Block activation by agonists by blocking binding sites
 - Allosteric modulations

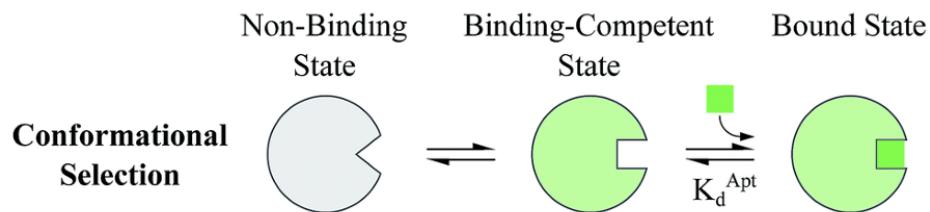
What about other types of protein-based interactions? Allosteric modulations?

- The binding of other molecules or ligands is key to the action of most proteins
 - Ligands: peptides, hormones, partner proteins, small molecules (ATP, GTP)
- Can introduce new or competing ligands to alter
 - The shape of a protein
 - The activity or function of a protein
 - How it affects communication to downstream partners
- Targeting involves molecular recognition of the ligand by the protein and this is usually very specific

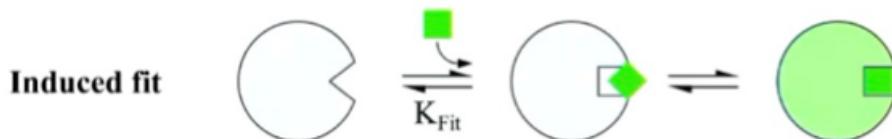
How do proteins interact with a ligand?

- Proteins have dynamic structures

- Enables a protein to rapidly alter its structure to adapt to a ligand and permit binding
- Rapid changes are often essential for protein function
- Recognition can typically be described as one of the three models
 - Lock and key
 - Ligand binds to a protein that has a structurally complementary binding site
 - Conformational selection
 - Protein may exist in different conformations, ligand binds to competent state and shift the equilibrium to the bound state



- Induced fit
 - Ligand forces to fit into the protein and shift equilibrium to bound state



What does molecular recognition involve?

Common characteristics of binding sites:

- Lined with key amino acids which interact specifically with the ligand
- Can be different shapes to accommodate different ligands
 - Macromolecules (large) need concave, convex, flat, or grooves
 - Small molecules bind to clefts, pockets, or cavities
- Catalytic active sites often occur

- At the interface between subunits or domains in proteins
- Generally have a higher than average amount of exposed hydrophobic groups
 - Allow small molecules to bind
- Interactions
 - **Weak interactions** between ligand and protein can favor **easy exchange of molecules**
 - The energy for driving binding events is often provided by the displacement of water molecules from the ligand binding site

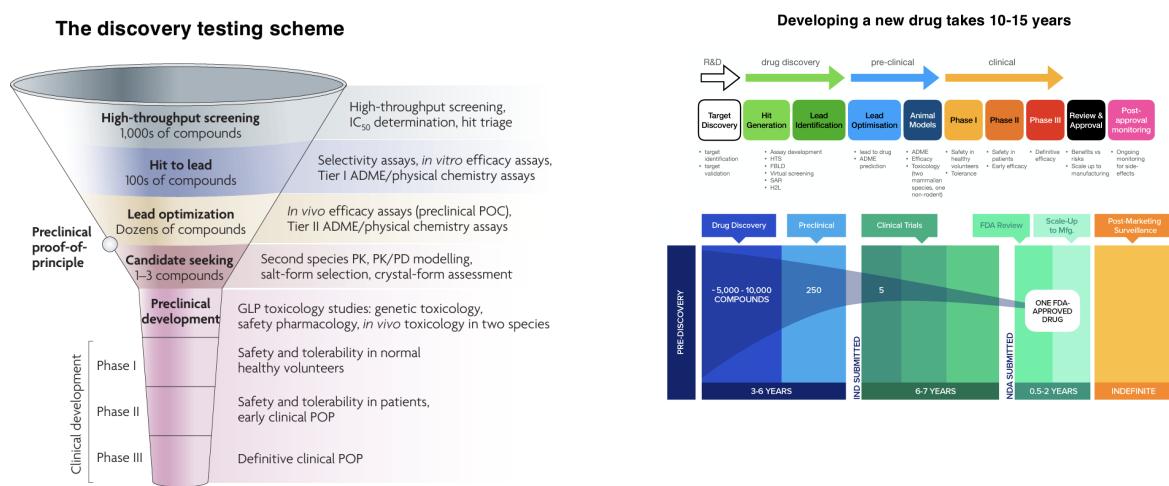
How do we design good therapeutic targets?

- The key to good drug design is working out and capturing the clinical spectrum of disease and the exact role a potential therapeutic target plays in the disease
 - Drugs will not act unless they are bound
- From a protein's perspective
 - A confirmed role in disease or is disease-modifying
 - Expression is not evenly distributed (i.e., it is in a local place)
 - To avoid side effects
 - A detailed knowledge of biochemical properties, structure & mechanism of action (including kinetics)
 - 3D structure available to assess "druggability"
- From a drug's perspective
 - Critical role in modifying a disease-state, but less significant role in important processes
 - Need structural/functional properties for drug specificity
 - Limit potential side effects
 - Need tissue-specific understanding of drug's action

What makes a good drug candidate?

- A good drug will have favorable pharmacokinetic and pharmacodynamic properties

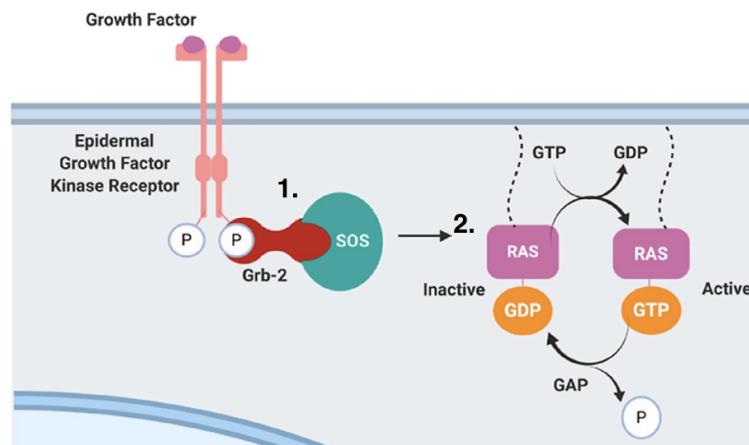
- Pharmacokinetics - Principle of ADME
- Pharmacodynamics - how biological processes respond to or are impacted by drugs
- Oral drugs need good in vivo absorption & permeability
- A set of 5 guidelines was developed "Lipinski's Rule of 5"
 - Candidate not fitting the guidelines likely to have poor absorption or bioavailability
 - Absorption: movement of drug into the GI tract
 - Bioavailability: fraction of the drug makes it into circulation or site of action
- Guidelines
 - Polar surface area: metric of permeability
 - Molecular weight: small
 - Lipophilicity: allow permeability through membranes
 - Number of H-bond donors (HBD) and H-bond acceptors (HDA)
 - Rotatable bonds - molecular flexibility
 - The compound should have fewer than 10 rotatable bonds
 - Compounds with fewer rotatable bonds tend to have greater structural rigidity, which can contribute to improved binding specificity and pharmacological activity.



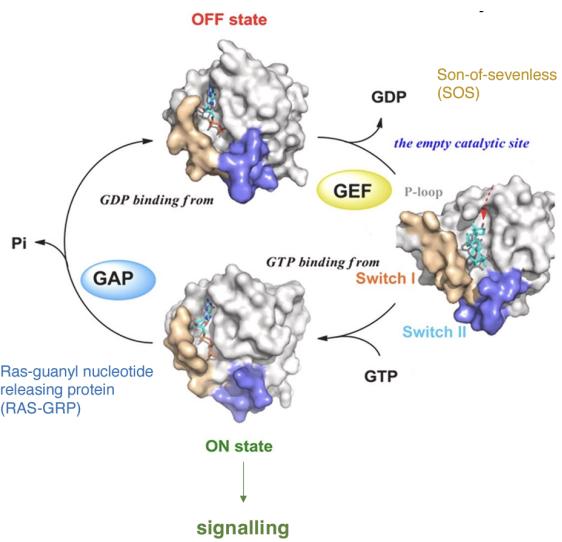
Ras

- Membrane-bound GTPase (one of the 3 Ras isoforms: H,K,N)
- Cell proliferation, differentiation, survival
- Regulate multiple signal transduction pathways = oncogene
- One of the most frequently mutated gene within cancer
 - Mutated in 17-25% of all cancers; 30-40% colorectal cancer

A binary switch



- Ras proteins act as binary switches, cycling between GTP-bound active and GDP-bound inactive states
- Upon stimulation at the epidermal growth factor receptor (EGF)
 - Grb-2 activates son of sevenless (SOS)
 - SOS binds to Ras to catalyse GDP-to-GTP exchange and activates Ras



Note the difference between Ras protein and RAS-GRP.

- GDP has a slow off-rate
 - Half-life = 6 min, $k_{off} = 2E-3$ /s
 - SOS is a catalyst
- GTP is in vast excess in the cell (10 times of GDP)
 - Thus GTP binds rapidly to an empty active site
- GTP hydrolysis is slow
 - Half-life = 16 min, $k_{off} = 6E-4$ /s
 - RAS-GRP (Ras-guanyl nucleotide releasing protein) is a catalyst
 - Thus RAS-GRP switches off the signalling

- When SOS binds

- Conformational change in the switch regions (I, II) & P-loop
 - weakens GDP affinity
 - GTP binds to active site

- 5 mutations (G12 D/V/C, G13D & Q61R) account for 70% of all RAS-mutant cancers
 - Lung, colorectal, pancreatic
 - Mutations alter the GTP/GDP switching process
- G12C prevents Arg finger of GAP (Ras-GRP) from entering Ras

- No Ras-GRP entry = no hydrolysis via Gln61 (Ras pocket) = no turnover of GTP (remains bound)

→ KRas never switches off

→ Downstream signalling remains switched on

Undruggable

- Kras 3D structure is relatively "smooth", i.e., no well-defined pockets to bind small molecules
- GTP binds instantly and with high affinity (picoM range), and high cellular [GTP] ($\sim 500 \mu\text{M}$)
 - Competition is thus difficult to achieve
- Allosteric pocket found beneath switch II region
 - Switch II pocket, SIIP
- Pocket induced or stabilised by modulating the mutated cysteine site (SIIP)
- A new dynamic "pocket" was identified using disulfide-linked fragments
- Screening for:
 - a. Available pocket recognised by ligand
 - b. Disulphide bridge formation



G12C inhibitors - AMG510 (sotorasib)

- Compounds bind to Ras in GDP-bound state
- Decreasing affinity for GTP and block SOS catalysis (GDP to GTP)
 - Thus the Ras remains in inactivated state longer

Drug discovery

- Drug discovery is a major area of translational research
 - Taking observations in a laboratory onto a larger scale
 - Finding effective disease-modifying compounds

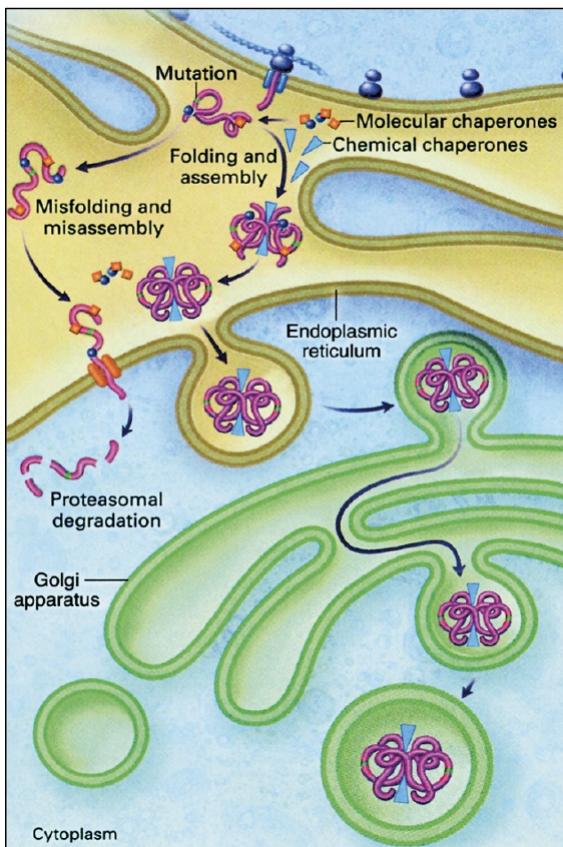
- Need to understand the biology of the disease
 - Molecular players & how they interact
- Compounds need to be extensively tested
 - In vitro characterisation → cells → animals → people

Summary

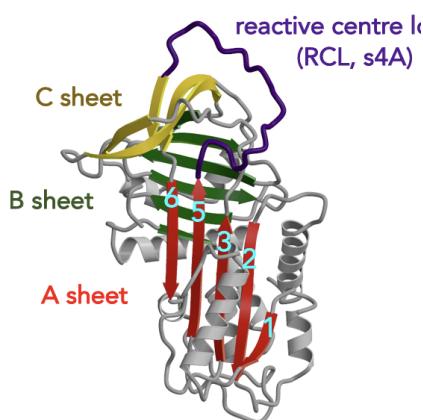
- Complexity in studying (& curing human disease)
 - Many diseases are now not considered single gene only
 - Proteins are involved in complex networks (e.g. cell signalling)
- The human proteome is “druggable”
 - Proteins are reliable workhorses - several classes are key targets
- Need to produce proteins and characterise structure, stability, interactions using biophysical and structural methods, as well as in cells
- Targeting depends on the availability of “pockets” which rely on the structural and dynamic properties of a protein
 - Example: Ras
- Targeting proteins effectively needs a knowledge of the protein’s role in disease AND an effective protein-modifying drug candidate

Proteins as therapeutics

α 1-antitrypsin (AAT)



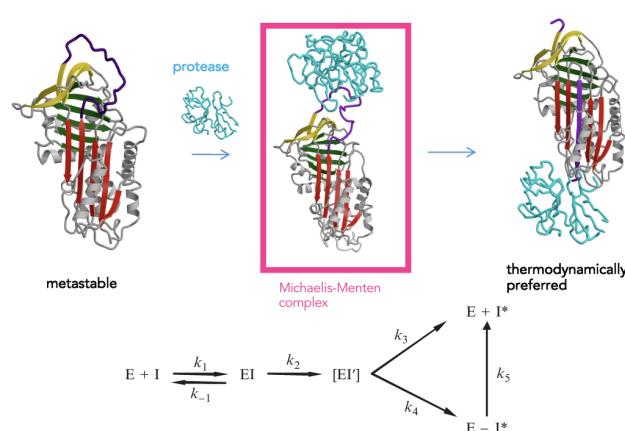
- AAT is a very abundant protein in human plasma (1.75g/L)
- About 70-80% of the protein synthesised in the endoplasmic reticulum of hepatocytes.
 - Note that the protein folds in the ER
 - It is glycosylated and secreted into the bloodstream & is transported to the lungs, where it functions
- Half-life = 5 days
- It is a serine proteinase inhibitor and inhibits elastase secreted from neutrophils (WBC)
 - Neutrophil elastase (NE) inhibitor
- NE is upregulated during inflammation, when neutrophil production increases
- Balance of NE in the lungs is important as it can degrade lung tissue
 - Thus, a sufficient AAT production is essential to maintain the balance
- AAT belongs to the serpin superfamily (a serine proteinase inhibitor)
 - The superfamily share 70% structural homology, yet <30% amino acid sequence identity
 - I.e, low sequence identity, sequence not conserved but structure is



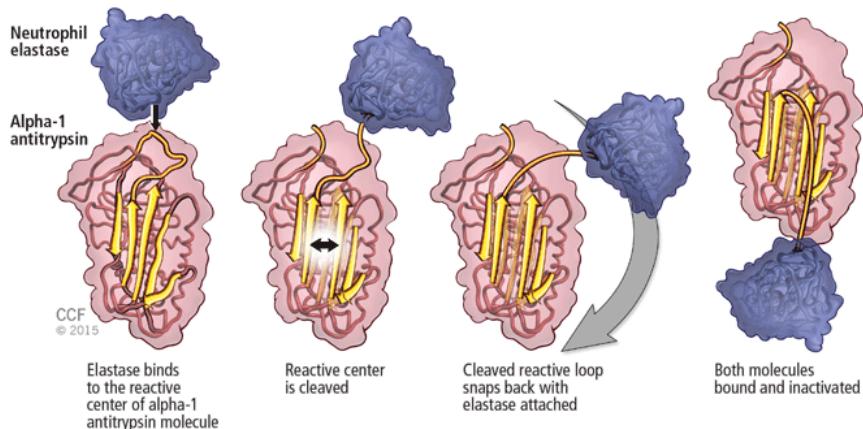
- 3 beta sheets (ABC) e.g. strand 1, sheet B = s1B
- 9 alpha helices (A to F) e.g. F helix = hF
- 1 dynamic loop (RCL)
- The reactive centre loop enables protease activity

AAT has a metastable native state is a highly dynamic molecule

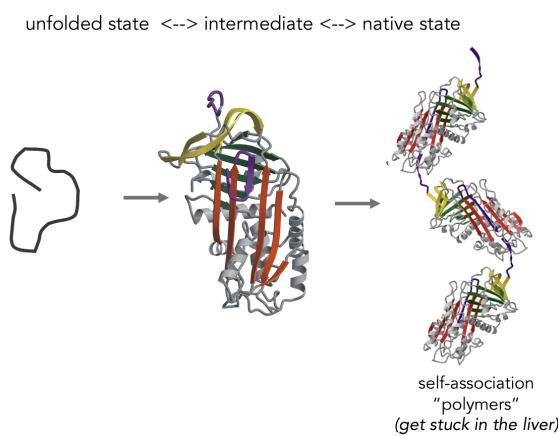
- Metastable - not the most energetically preferred state, which is unusual for protein
 - Tm (melting temperature) 55C, when protein begins to unfold
- Cleaved state is the most stable state for the protein
 - Tm (melting temperature) >100C
- Metastability helps AAT to be extremely dynamic



- AAT folding is described as a three-state pathway and is very slow
- Dynamic properties needed for rapid movements for inhibition
- Inhibitor (I) of NE (protease) using a suicide-substrate "mouse-trap" mechanism
- Inhibition follows Michaelis-Menten kinetics



AAT can misfold and self-associate



- The intermediate structure (native-like structure) unclear but is a crucial point in folding pathway, can lead to alternative pathway
 - Important for downstream processes to acquire native structure
 - This is also due to the dynamic intrinsic of the native state not being the most thermodynamically preferred state
- The intermediate state may deviate from the normal folding pathway and forms a series of self-associated polymers
 - The polymers get stuck in the liver and cannot be delivered to the lungs for its function

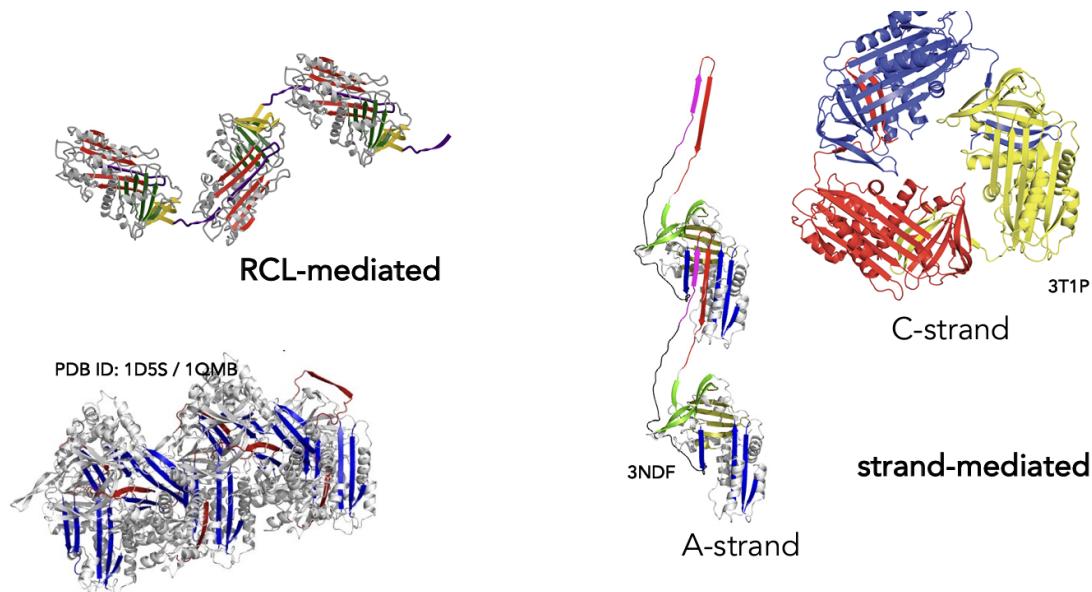
AAT deficiency

- 1:2500 people UK autosomal co-dominant disorder (monogenic disease)
 - More than 100+ associated point mutations
 - Most severe mutation: E342K "Z mutation"
 - "common disorder rarely diagnosed"

- Deficiency of AAT leads to lung tissue damage
 - ATT protects lung pro NE in healthy individuals
 - Protease in excess leads to proteolytic degradation
 - Causing early onset (30-50) of COPD
- Build up of abnormal aggregated alpha1 ATT trapped within liver cells (ER) leads to cirrhosis of the liver
 - Normally the liver releases ATT into the blood
 - No AAT exit liver, overproduction of polymers cannot be cleared and built up in the liver
- Cure: liver and/or lung transplant

AAT polymerisation models

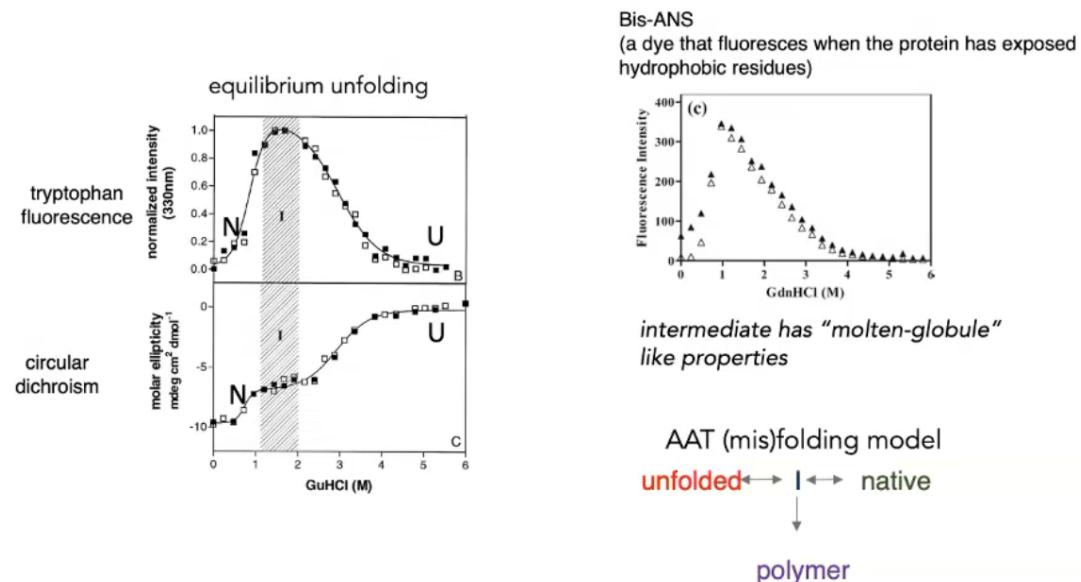
- Polymerisation involves self-association of many AAT molecules and can be induced by mutation, pH, temperature
- Different conditions within buffers give rise to different polymerisation models



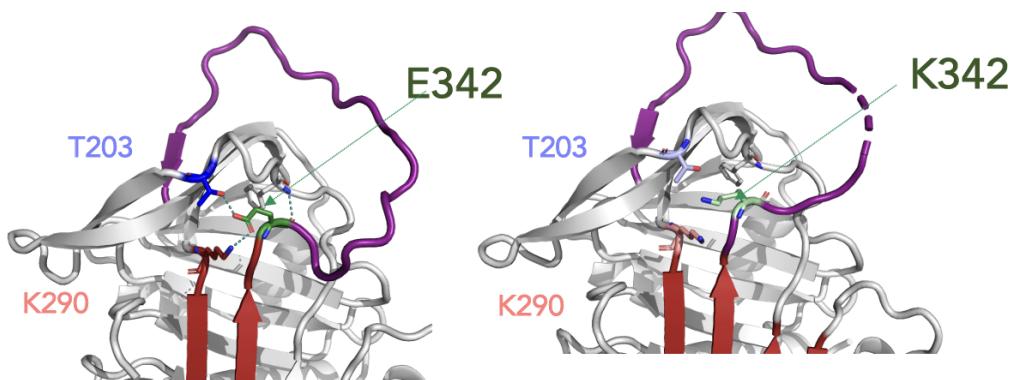
Note that all are proposed self-assembly models, no 3D structures of polymers exist

- RCL mediated: insertion of a reactive loop from one AAT (donor) into the beta sheet of another AAT
- AAT misfolding can begin during its biosynthesis, which is the earliest stages of polymerisation

- The growing polypeptide chain recruit released chains
- AAT folds and misfolding via a protein folding intermediate
- Biophysical studies of AAT to dissect its folding mechanism
- Equilibrium measurements by unfolding the protein in increasing concentrations of guanidine and measure the extent of folding using fluorescence or CD (circular dichroism)



Z AAT has a kinetic folding defect: molecular level



- Once folded, Z is functionally indistinguishable from WT
 - Z mutation
- Only 15% of Z is successfully secreted
- The E342K mutation causes destabilisation of A sheet - slight change, not affecting global destabilisation

- Loss of a salt bridge (K290)
- Loss of an H-bond (T203)

→ kinetically-trapped intermediate and promotes misfolding

- The mutation does not affect native state but increase duration of intermediate state and thus increase probability of misfolding (kinetic trap)
 - Note that its folding can often become trapped in both the wild-type protein and the Z variant as the folding is very slow

Current treatments &therapeutics for alpha-1 AAT deficiency

Replacement/augmentation therapy

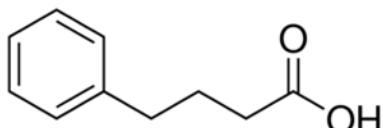
- AAT offered as infusions to patients to replace missing protein
- 0.49 g/L (compared to normal 1.75 g/L) is needed in bloodstream to maintain therapeutic level (administered at 60 mg/kg per week)
- Randomised trials on clinical efficacy remain inconclusive
- An FDA approved therapy, despite low biochemical efficacy (30%)
- Expensive
 - Cost per year USD50,000-130,000 per year
 - Each donation is 0.83L, about 0.3g alpha-1 recovered
 - Blood donor centres per annum collect about 5.5M litres of blood (1650 kg per year)

Development of 'disease-modifying' approaches

Can we target AAT instead?

- Drug discovery approaches aimed at either improving AAT's folding or stopping polymerisation

4-PBA



- 4-phenylbutyric acid (4-PBA)
- Has FDA approval
- Increases secretion of Z AAT

- Mechanism of action
 - Binding of 4-PBA to AAT as measured by fluorescence (measuring Kd)
 - Low affinity binding - predicted in the "breach region", which is destabilised in Z mutant
 - Phase 2 clinical trials completed (but didn't progress beyond this)
 - Problem: being a chemical chaperone → too broad spectrum
 - Helps protein folding but not specific to AAT

GSK 716 - new promising molecule?

- Found from screening DNA encoded library of small molecules
 - Selective binding to Z mutants better than WT
- Increase detection of monomer and decrease polymer
 - Increase in secretion measured
- A cryptic pocket is not evident in apo structure (infrastructure) of breach position (mutated site)
 - Only available once molecule is bound

Monoclonal antibodies in neurodegeneration

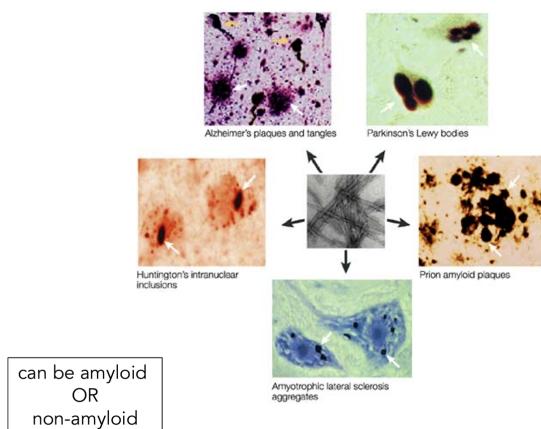
Conformational diseases

- Diseases often referred to as "conformational" or "misfolding" disease because these proteins readily alter their native structure and form aggregates
- >50 proteins implicated in recognised conformational diseases

Disease	Aggregating protein or peptide	Polypeptide length (number of residues)	Structure of protein or peptide
Neurodegenerative diseases			
Alzheimer's disease	Amyloid- β peptide	37–43	Intrinsically disordered
Spongiform encephalopathies	Prion protein or its fragments	230	Intrinsically disordered and α -helical
Parkinson's disease	α -synuclein	140	Intrinsically disordered
Amyotrophic lateral sclerosis	Superoxide dismutase 1	153	β -sheet and Ig-like
Huntington's disease	Huntingtin fragments	Variable	Mostly intrinsically disordered
Familial amyloidotic polyneuropathy	Transthyretin mutants	127	β -sheet
Non-neuropathic systemic amyloidosis			
Amyloid light chain (AL) amyloidosis	Immunoglobulin (Ig) light chains or its fragments	~90	β -sheet and Ig-like
Amyloid A (AA) amyloidosis	Serum amyloid A1 protein fragments	76–104	α -helical and unknown fold
Senile systemic amyloidosis	Wild-type transthyretin	127	β -sheet
Haemodialysis-related amyloidosis	β_2 -microglobulin	99	β -sheet and Ig-like
Lysozyme amyloidosis	Lysozyme mutants	130	α -helical and β -sheet
Non-neuropathic localized amyloidosis			
Apolipoprotein A1 (Apo A-1) amyloidosis	Apo A-1 fragments	80–93	Intrinsically disordered
Type II diabetes	Amylin	37	Intrinsically disordered
Injection-localized amyloidosis	Insulin	21 and 30	α -helical and insulin-like

*A selection of diseases associated with extracellular amyloid deposits or intracellular inclusions with amyloid-like characteristics. See REF. 5 for a more comprehensive list of the approximately 50 human protein misfolding diseases and their associated proteins.

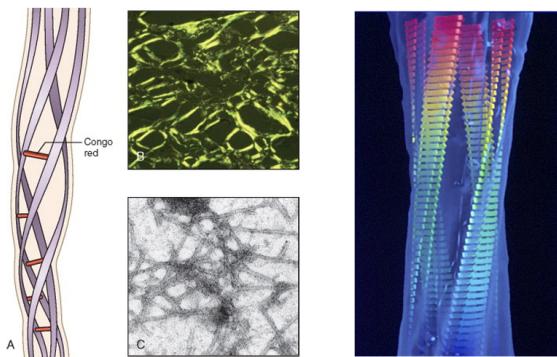
Proteinaceous inclusions are a common hallmark



- Cause negative impact on cell leads to cell death
- Amyloid = alternative form of protein
- Non-amyloid (amorphous): protein come together in a non-specific way

Inclusions are made up of amyloid

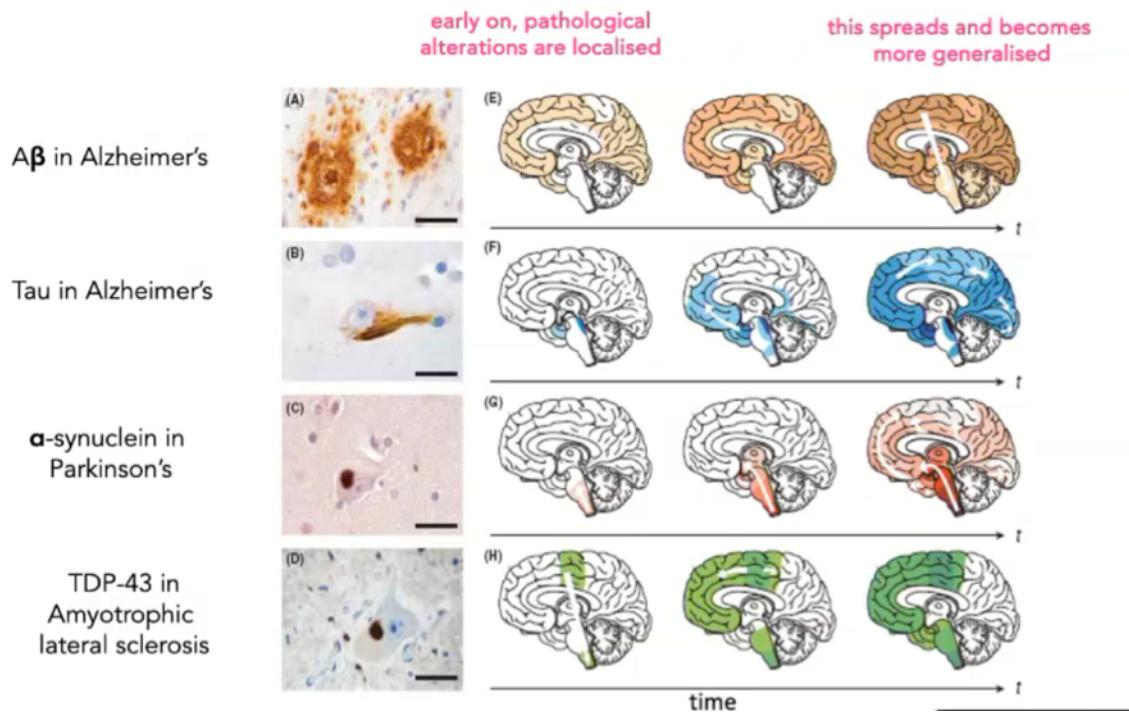
- Amyloid forms a fibrillar structure 10–100+ nm in length
- Composed of beta strands, self associate to form 'protofilaments'
- Protofilaments are twisted around each other in either compact or ribbon-like arrangements



- Fibrils are made up of different numbers of component protofilaments (here 4)
 - Insoluble
 - Heterogeneous beta-sheet
 - Twisted
 - Structure characterised by biophysics, NMR, cryoEM

Effects of protein deposits in the brain: Alzheimer's disease

- Post-mortem is the definitive way to diagnose some neurodegenerative diseases
 - AD shows severe atrophy of the brain due to accumulation of protein deposits
 - Disease progression involves spreading
 - Causing different effects associated with diseases, e.g. cognitive, memory, motion etc.

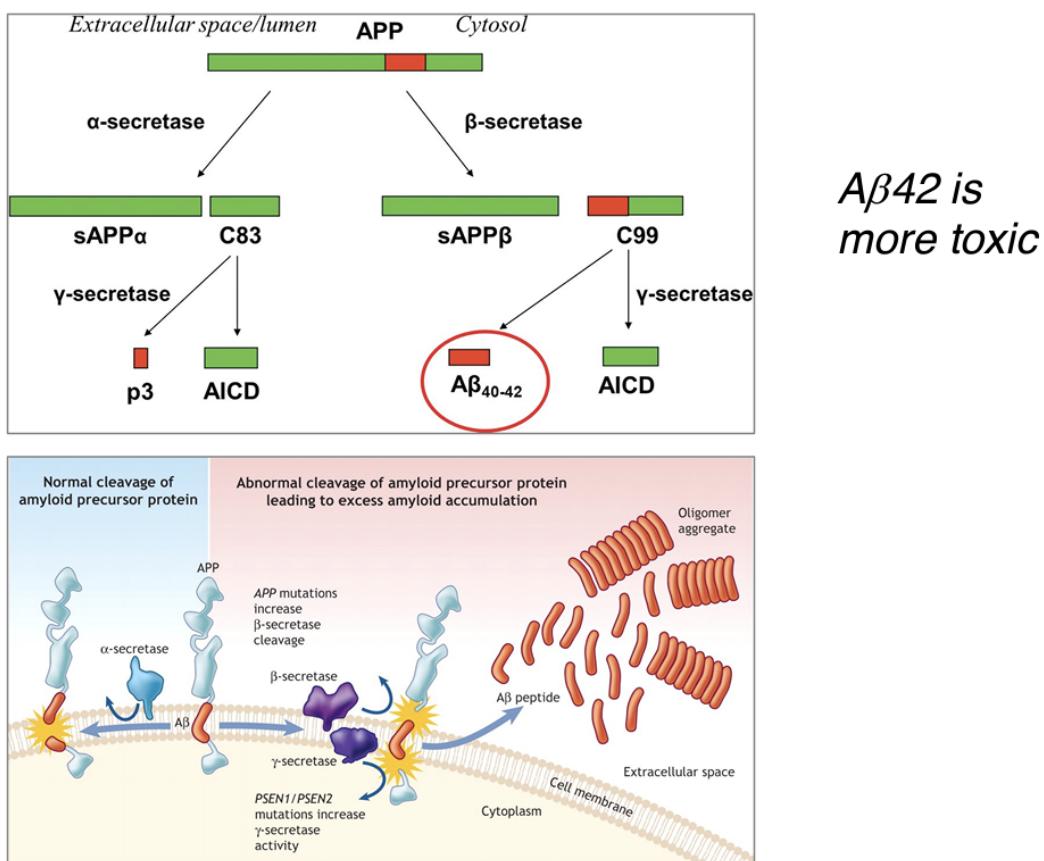


Statistics of AD

- Estimated that by 2025 the number of people suffering from dementia in the UK will double, and triple worldwide
- There are currently no treatments to address the cure of AD, treatments just manage the symptoms of the disease

How might we study molecular mechanisms of protein misfolding and aggregation?

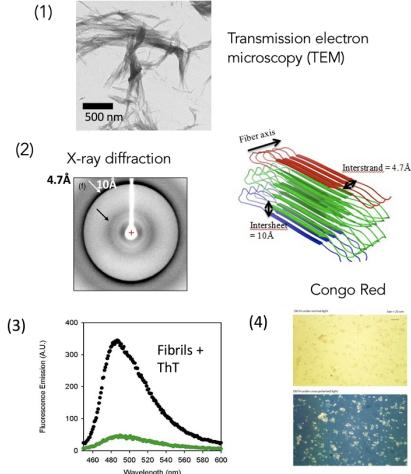
Amyloid precursor protein & Abeta (Amyloid cascade theory)



- Abnormal proteolytic cleavage of the Amyloid Precursor Protein (APP)
 - Produces A β fragment(s) 42 (amyloidogenic) or 40 (normal)
 - Abeta forms oligomer aggregate
 - Prevent formation of Abeta or stops its self-association
- Function of fragments unknown

Detection of amyloid

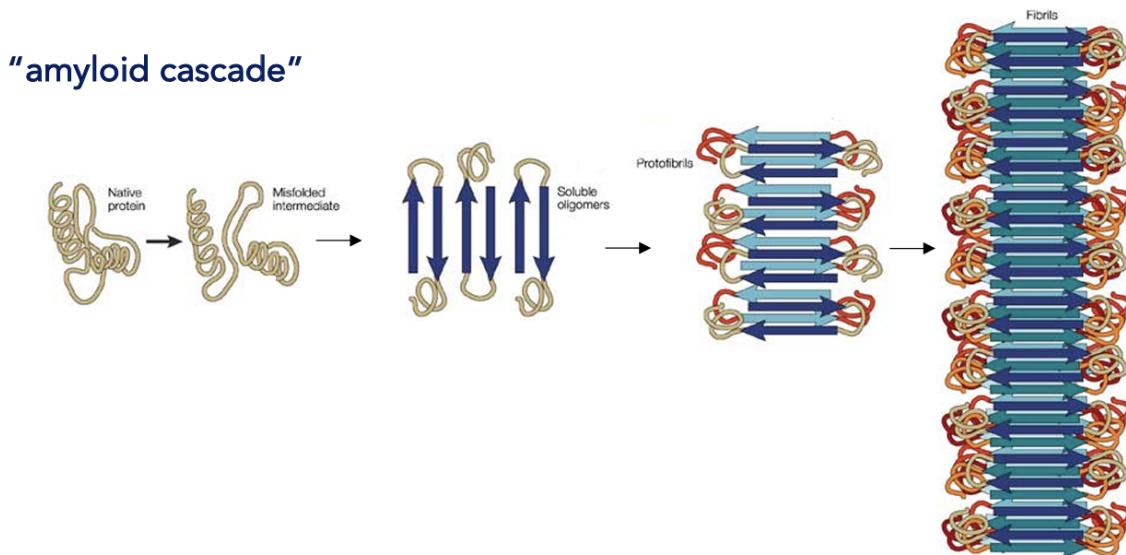
- We can study amyloid formation using biophysics



- Amyloid fibrils possess distinct biophysical characteristics
 1. Fibrillar morphology
 2. Cross-beta core structure
 3. Binds to dyes such as Thioflavin-T (indicative of abundant beta-sheet structure)
 4. Displays Congo Red birefringence

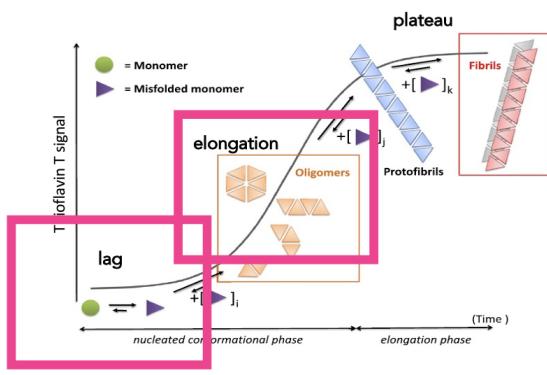
A kinetic pathway for misfolding and fibril formation - amyloid cascade

- nucleation, oligomerisation, protofibril formation, fibril formation



Misfolded peptide self-associate into oligomers.

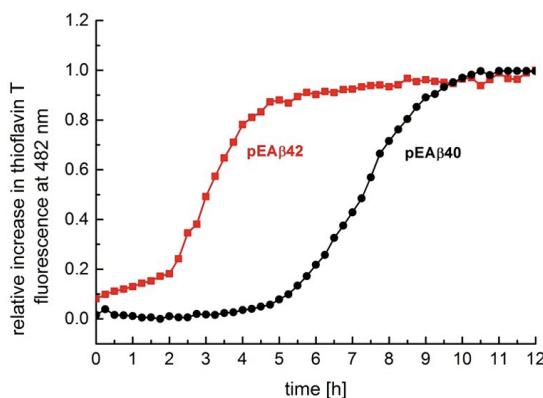
- Research has focussed on dissecting the details of the kinetic mechanism of fibril formation
- Very detailed mathematical modelling enables the amyloid mechanism to be dissected and see at what stage the amyloid mechanism can be inhibited most effectively



Thioflavin T binds to fibrils.

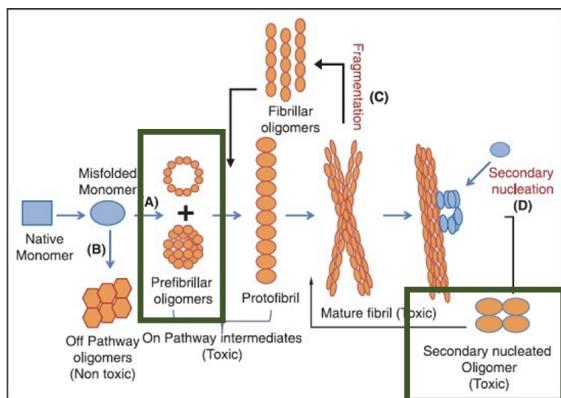
Fibril formation can be characterised by a sigmoidal curve containing a number of process:

- Lag phase (region of nucleation)
- Elongation (Rapid growth phase)
 - Peptide undergoing conformational change to form oligomers?
- Plateau (formation of mature fibrils)
- Mutations alter the rate and severity of amyloid formation
 - Due to difference in electrostatic interactions → affects fibril formation
 - Hydrophobic or steric interactions → affects nucleation



How do amyloid fibrils relate to pathology?

- There is a poor correlation between mature amyloid deposits and the progression of disease in AD patients (as well as other neurodegenerative diseases)
- A better correlation exists between soluble levels of Abeta & disease severity



- Mechanism of amyloid formation is made up of many steps
- Oligomers are the toxic species to cells as they puncture the cells

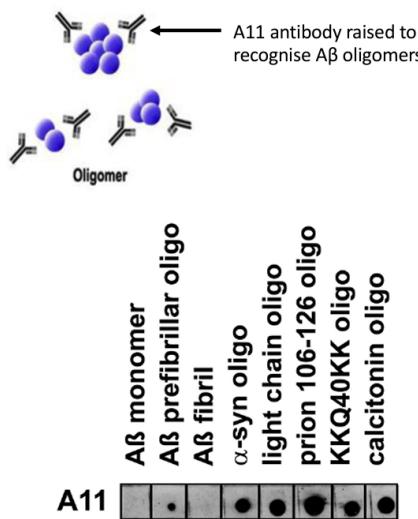
Soluble oligomer species

- Oligomers are transient structures present in the early (nucleation) stage of aggregation
- They have increased hydrophobic surfaces
 - Measured by ANS fluorescence
- Can appear as spherical, sometime pore-like
- Oligomers formed from different proteins and peptides *have common biophysical* attributes
 - Any protein may form amyloid in the right condition
 - Amyloid is a generic structure
- Thus therapeutics may prevent oligomer formation

Anti A β monoclonal antibodies

- Antibodies are proteins used by the immune system to identify and neutralise foreign objects (like bacteria and viruses)
- Monoclonal antibodies have monovalent affinity and bind to the same peptide
- Antibody based immunotherapeutic approaches are of great interest, with monoclonal antibodies that are reactive to aggregated A β (1-42) being developed and tested

Oligomers of different proteins share a common conformation



Note that all the oligomers are recognised by a particular Ab

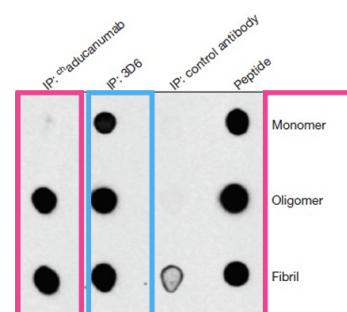
- A molecular mimic of soluble oligomers created, based on biophysical information of A β oligomers
- The "A11 antibody" recognised the oligomeric state of A β (and NOT the monomer or fibrils)
- A11 also recognises the oligomeric state of a number of different aggregated proteins/peptides



Like amyloid fibrils, distinct conformational attributes are shared by oligomeric species

Aducanumab reduces A β plaques in Alzheimer's disease

- Promise of antibody recognition has lead to the development of new antibodies
- Aducanumab selectively reacts with A β aggregates and recognises a conformational epitope present in soluble oligomers & insoluble fibrils
- In mice, an analog of aducanumab was shown to cross the BBB and reduce soluble and insoluble A β in a dose-dependent manner
- This was taken to phase 1b clinical trials
- Aducanumab binds between position 3-7 in the A β protein and recognises oligomers
- Patients with mild AD and A β -positive PET scans received 1 year of monthly IV infusions of aducanumab



Different conformations of A β_{1-42} peptide incubated with Aducanumab or 3D6 (which binds all conformers), and isolated by immunoprecipitation techniques.

Samples were blotted and probed to detect the presence of A β_{1-42} peptide

- Dose- & time-dependent changes were observed - Significant decrease of all plaque sizes
- In August 2015, Biogen launched two identical 18-month phase 3 studies
- Trials for Aducanumab were stopped in May 2019, but received accelerated FDA-approval in 2021
- Anti-A β antibodies - pros and cons
 - Aducanumab is not the first anti-A β antibody to spark interest
 - Many have failed to deliver, such as crossing BBB
 - Safety and tolerance of anti-A β antibodies has been acceptable and serious complications are rare
 - Efficacy - to date, no anti-A β antibody has demonstrated significant efficacy
 - Though aducanumab shows the most promising results
 - Anti-A β antibody treatment requires repeated administration and there are significant costs involved with production

One explanation for anti-A β antibody therapy failure is that these treatments are set too late in the disease process

- Due to diagnostic problem, by the time of start of treatment, it is too far into the disease to have massive effects

Lecanemab - a new player

- Antibody targeting Abeta protofibril
- Early AD = mild cognitive impairment & mild AD + confirmed amyloid pathology in brain
- Lecanemab treatment met the primary endpoint and reduced clinical decline on the global cognitive & functional scale compared with placebo at 18 months by 27%

Summary

Antitrypsin as an example of therapeutics

- Synthesis, structure, (mis)folding and function

- Development as a therapeutic - both as a replacement therapy to a druggable target

Monoclonal antibodies in the treatment of neurodegeneration

- Amyloids as a basis in some neurodegenerative diseases
- Amyloid cascade in Alzheimer's disease
- How amyloid formation can be targeted
- New ideas for amyloid-based kinetics measurements

Receptors & Enzymes as Therapeutics

Cystic Fibrosis & CFTR

Cystic Fibrosis (CF)

- Inherited monogenic disease, mutations in *CFTR* gene
- Autosomal recessive disorder
 - 1 in 25 of population is faulty CFTR gene carrier
- A respiratory disease: upper and lower areas of lungs
- Leads to a build up of mucus and widening of bronchial trees
 - Thus narrowing
- Mucus microenvironment for micro-organisms
 - Susceptibility to bacterial infections
 - Persistent chest infections, chronic cough, wheezing, airway obstruction
- It is a life-limiting condition: prognosis
 - CF tends to get worse over time
 - Can be fatal with serious infections or if the lungs stop working properly
 - Only 50% of CF patients live past late 30%
 - Average life expectancy is 38 years old (patients born later than 2017 has life expectancy to be 53 years old)
- 100,000 CF individuals world-wide, ca. 10,800 people with CF in the UK

CFTR biogenesis pathway

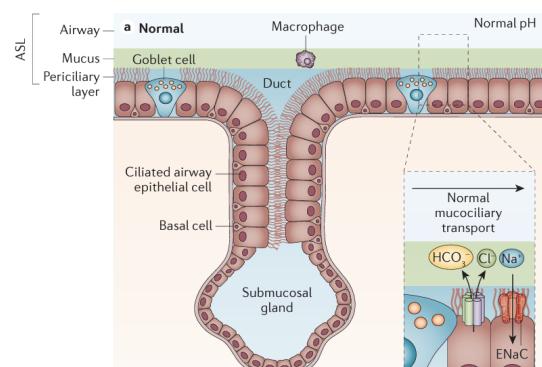
- CFTR mRNA = cystic fibrosis transmembrane conductance regulator protein
- Protein expressed primarily by epithelial cells: lung, pancreas, small intestine
- 170kDa glycosylated protein expressed on the plasma membrane of cells
 - Glycosylated protein transported from endoplasmic reticulum to trans-Golgi network (TGN) for further training (COPII-mediated transport)
 - Protein exits TGN in vesicles via exocytosis facilitated by myosin Vb (Myo5b) and Rab11
 - It is embedded within the vesicle and integrate into the apical membrane
- CFTR can be released and reinternalised in the cell by endocytic pathway

CFTRs are ion channels

- Ion channels are a portal between the intra and extracellular locations of the cell
- Formed by pore-forming membrane proteins
- Move ions in and out of the cell
 - Shape action or electrical potentials, control the flow of ions across cells, and regulate cell volume

Functions in the lung

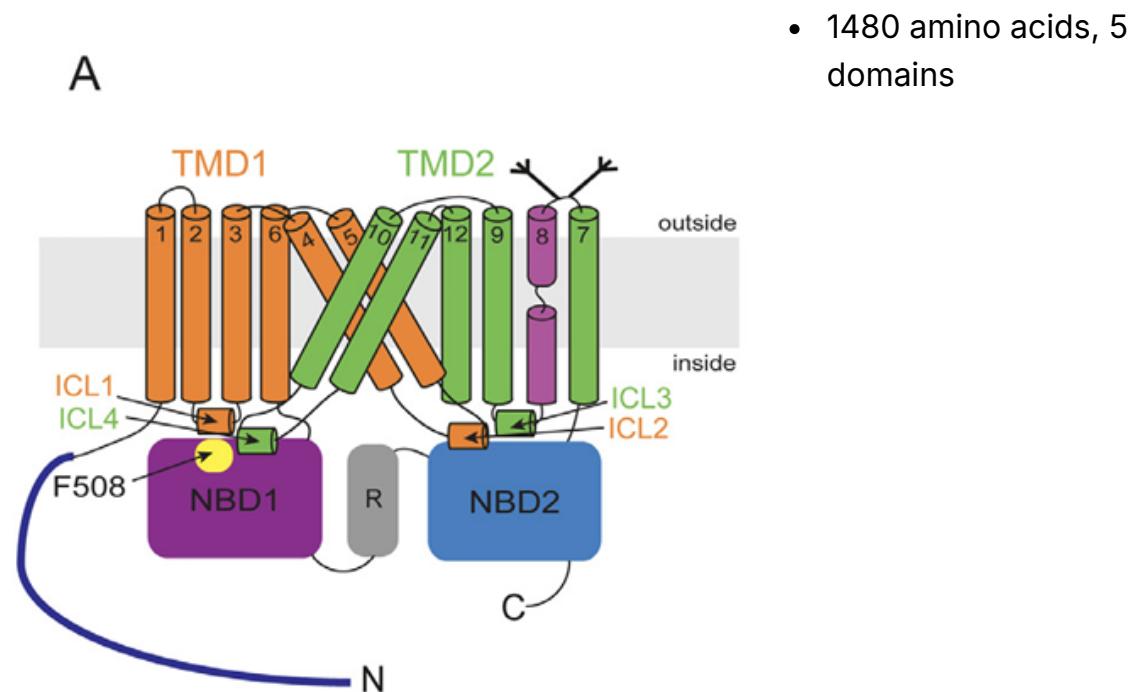
- Maintain airway surface homeostasis in lungs
- The lung contains a 2-layer gel (mucus) for clearance & airway defence needed
 - pH of the mucus (on the surface) need appropriate regulation by bicarbonate & Cl⁻ ions
 - CFTR transport bicarbonate and Cl⁻ ions



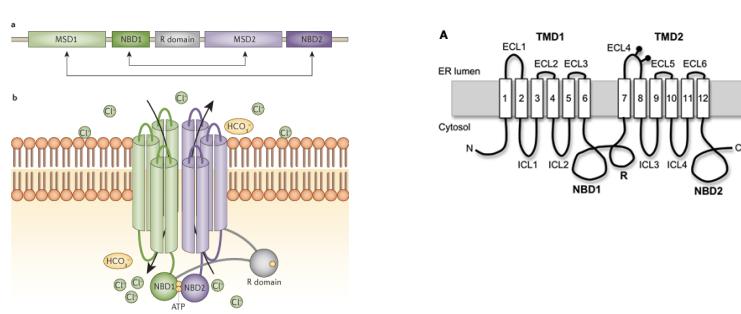
ASL = air surface layer
ENaC = epithelial sodium channel

- Ion transport through CFTR promotes effective production of mucus from airway surface goblet cells and submucosal glands
- CFTR with the ENaC work together to maintain ion balance

Structure



Structure-function relationship

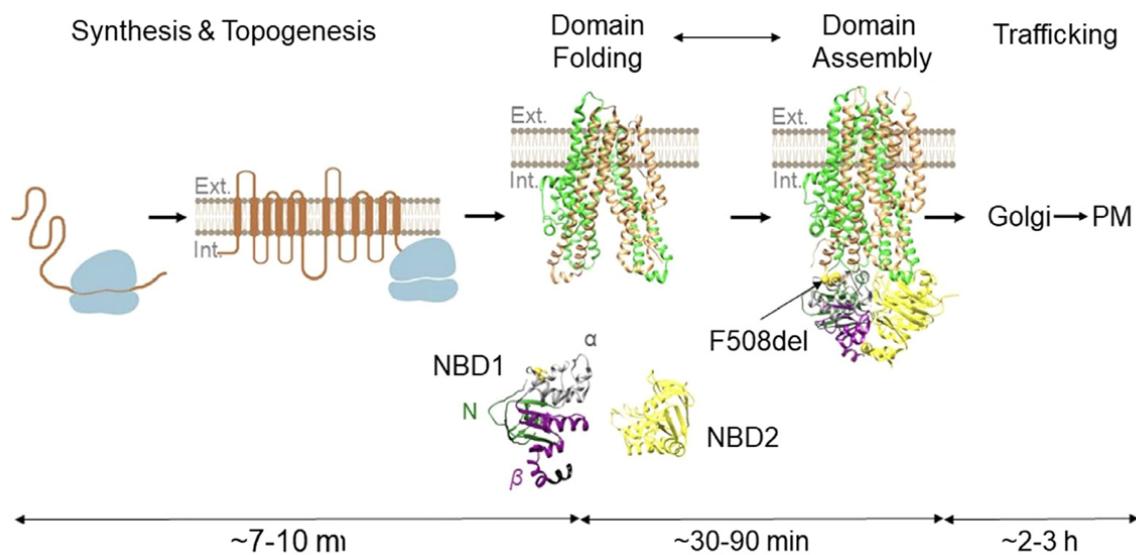


- ATP-binding cassette (ABC transporter) with a regulatory domain
- Membrane spanning domains that form the ion channel through the plasma membrane

Mode of action

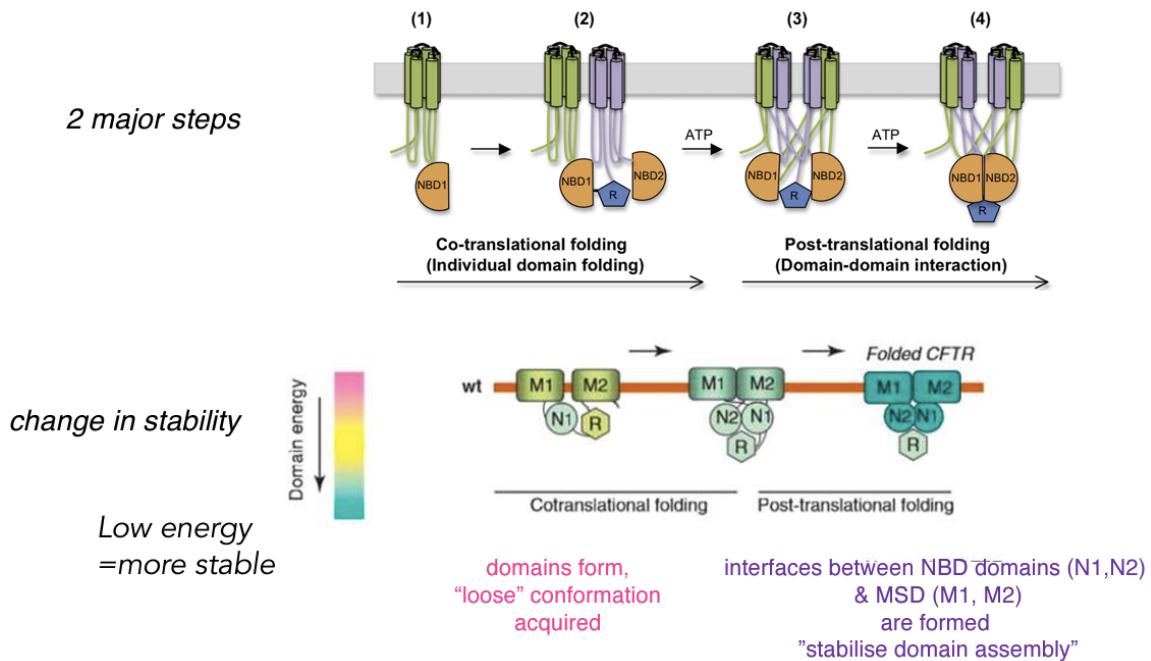
- R domain interacts with NBD1 causing a steric hindrance
- ATP-driven reaction
- R domain is phosphorylated by PKA
- R domain is released from “binding confirmation”
- NBD1 (nucleotide binding domain) and NBD2 can dimerise and open the channel (to allow passage of bicarbonate and Cl ions)

Folding



- Because timescale for folding is long, misfold is more likely to occur
- Folding is co-operative in a domain-by-domain manner
 - 5 domains, synthesised domain helps the folding of the next by interaction

2 major steps:

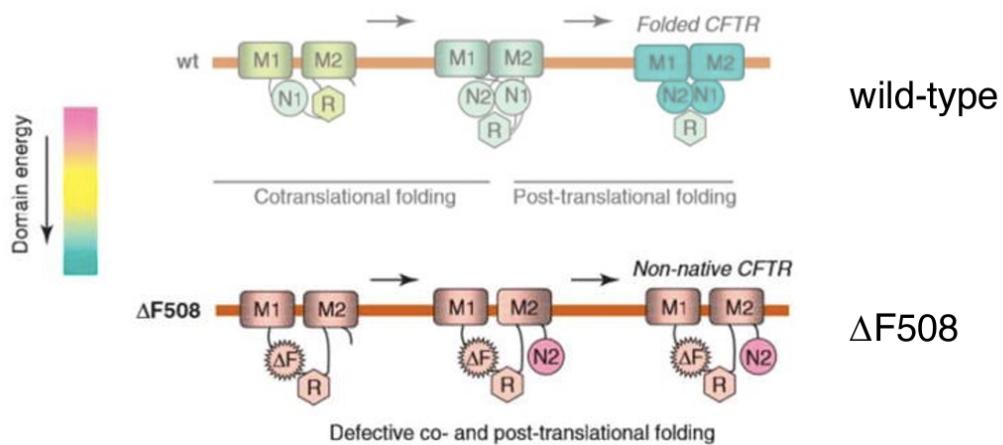


1. Co-translational folding: Protein integrates itself into ER and individual domain formed
 2. Post-translational folding: After synthesised protein released, domains associate with one another
 - a. Stabilise the domains after synthesis
- 20-40% newly synthesised CFTR reaches maturity (i.e. trafficked to the membrane)
 - Each domain is intrinsically unstable, slow folding kinetics
 - Inefficient synthesis, lots of unable to fold correctly

CFTR ΔF508 variant

- ΔF508 occurs in 90% of CF patients
- Mutation in NBD1
- Disrupts a critical interface: cytoplasmic loops 4 and 2 in MSD2 and MSD1

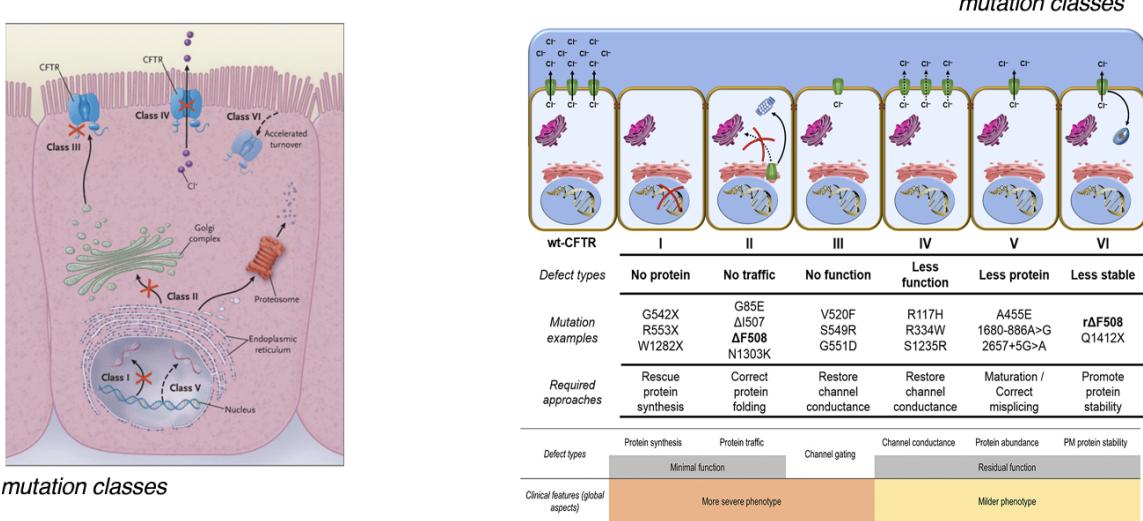
ΔF508 folding defect disrupts cotranslational folding and assembly



- Protease stability assays show proteolytic sensitivity of NBD1 and NBD2 increases nearly 2-5 fold & 60-fold respectively
 - As NBD1 is one of the first structures made by the ribosome
- I.e., mutation disrupts interface with MSD's, destabilising assembly (co-translational and post-translational) and reduces functional activity

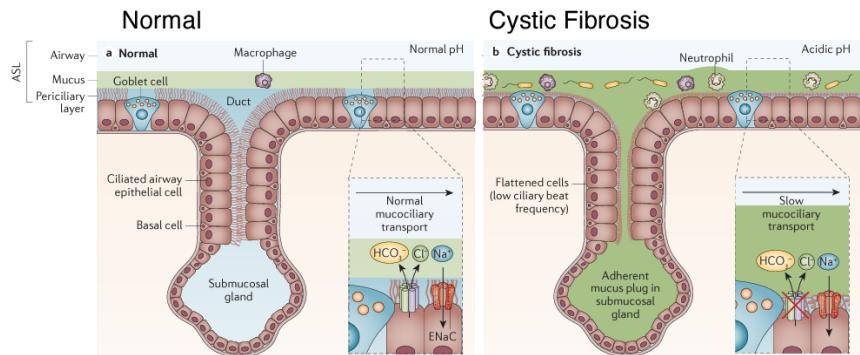
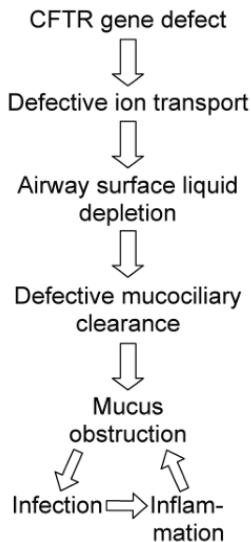
CFTR has classes of mutations

- 2000 CFTR variants identified
 - Variants may be pathogenic when in cis (more than one variant on a single allele) leading to variable clinical phenotypes
 - E.g. One mutation affects folding, one affects function
- Mutations = misfolded or unstable CFTR
- Severely misfolded CFTR is degraded by ER quality control (proteasome) - Type I&II
- Destabilised CFTR can still be integrated into the plasma membrane - Type III-VI



Pathophysiology

Cystic Fibrosis Pathophysiology



- Decreased bicarbonate transport
- Acidic pH on cell surface
- Abnormal mucus emerges & sticks
- Pro-inflammatory airway environment

Disease modification by folding correction

- Small molecules → pharmacological chaperones: improve protein folding, stability, function in a protein-specific manner

Kalydeco (Ivacaftor, VX-770)

- One of the first molecules to be developed and released (2012)
- Found to assist with G551D mutation (TMD) (mild-phenotype, Type III)
- Improve “gate opening”, improve Cl⁻ ion movement

- Role in “potentiation”

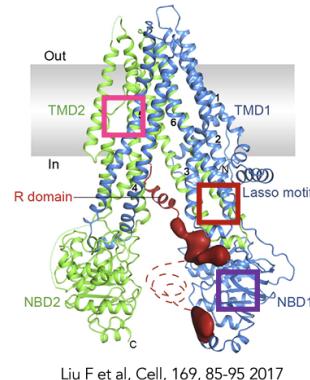
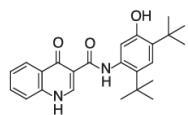
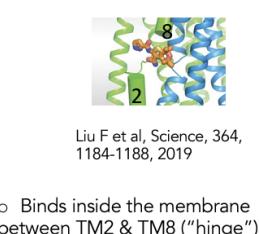
Treatment types available for managing CF

- Potentiators: increase the function of CFTR channels on the cell surface (Type III, IV, V, VI?)
- Correctors: improve the processing and delivery of functional CFTR protein to the cell surface (Type II)
 - This increases the amount of CFTR protein at the cell surface, resulting in enhanced ion transport
- Production correctors: or “read-through” agents, promote the read-through of premature termination codons in CFTR mRNA (Type I)
- Challenge is that disease classes are not ‘neat’, i.e., overlapping complex phenotypes of major cellular defects
 - One drug difficult to cure all

Combining drugs

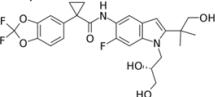
- Small molecules discovered through high-throughput screening and lead optimisation
- Previous CF drugs involved targeting a single “class”, but only a single mutation (or type)
- Combination drugs (dual/triple) are evolving to cover more CF patients
- Trikafta (US) or Kaftrio (UK): treats 90% of CF patients, homozygous / heterozygous ΔF508

Ivacaftor (VX-770): Potentiator



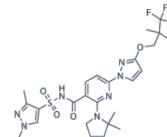
Tezacaftor (VX-661): Corrector

- increasing CFTR trafficked to surface
- binds to Membrane Spanning Domain I (MSD-1)



Elexacaftor (VX-445): "Next-gen folding corrector"

- Said to bind to NBD1 and works in an allosteric manner with VX-661



See Veit, G et al, JCI insight 2020

Tracing back co-translational folding?

- Studying cotranslational folding using Fluorescence Resonance Energy Transfer (FRET)
 - Changes in FRET signal = folding reporter
- Co-translational folding studies of NBD1
 - A455E missense mutation causes mild CFTR mutation interferes with gating
 - NBD1 adopts cotranslational folding intermediates and A455E disrupts co-translational intermediate formation

Receptors as therapeutics - G protein coupled receptors

Roles of GPCRs

- Essential communicators - take external stimuli and transduce signals within cells
- GPCRs are an enormous family of proteins which are central to mediating a range of cellular responses
 - Brain (mood): neurotransmitters e.g. serotonin, dopamine
 - Opsins, e.g. rhodopsin uses photoisomerisation to convert electromagnetic radiation into cellular signals
 - Receptors in olfactory epithelium bind odorants and pheromones

- Gustducin: in response to bitter/umami
- Chemokine receptors (e.g. histamine) regulate inflammation
- Nervous system (autonomic e.g. blood pressure, heart rate)
- Cell growth, metastasis

GPCRs share a common fold

- A series of 7 transmembrane helices (7-TM) that pass through a membrane
- Connected by 3 intracellular loops (IL-1,2,3) and 3 extracellular loops (EL-1,2,3)
- **EL-2** typically serves as a platform for **binding ligands**
 - Ligands: photons, odorants, hormones, neurotransmitters
- Tertiary structure is **barrel-shaped**. Forms a pore or cavity in a plasma membrane
- Related GPCRs are similar in structure

GPCR

- Central to signal transduction within cells
- They bind ligands produced by the cell & relay a signal from outside the cell to inside via G proteins which bind GDP & GTP
- Once activated, G proteins may activate different effector proteins in cascade mechanisms
- The result of GPCR signalling usually involves the modulation of effector proteins either by activating or inhibiting them (ligand binding: agonists vs antagonists).
- Essential components in cell signalling & two major signal transduction pathways cAMP signal pathway (e.g. muscle contraction, gene regulation) phosphatidylinositol signal pathway (cell growth, proliferation, intracellular trafficking etc)

Mode of action

- GPCR has an **N-terminal ligand binding region (extracellular)** and an associated G protein (intracellular, C-terminus)
- G protein has 3 domains: alpha (a), beta (b) & gamma (g) GDP is bound to the alpha domain

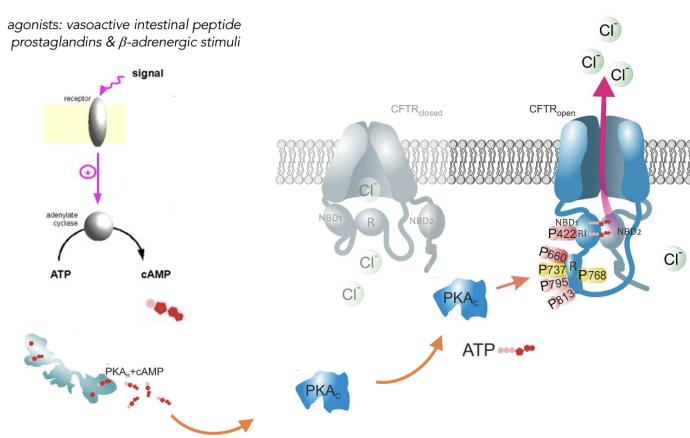
- Ligand binds to the GPCR & causes a conformational change
- Conformational change enables GPCR to activate an associated G protein
 - GDP is exchanged for GTP in the α domain
 - Causes dissociation of the β and γ domains
 - α domain(and/or beta and gamma domains) set off a cascade of downstream events

GPCR signalling is multi-component & complex

- A single GPCR may activate different G proteins and even non G proteins
- GPCRs tend to exhibit baseline activity and can be active when not binding their agonist
- A single GPCR may respond to different types of ligand with different outcomes for each. The following type of ligands can bind:
 - Agonists that elicit maximum receptor activity
 - Partial agonists that induce partial activity.
 - Antagonists that prevent other ligands from binding to the receptor.
 - Inverse agonists that **decrease the baseline activity** of the receptor
- GPCR activity may be affected by other factors such as oligomerisation, lipid composition of membrane or localisation to other membrane components

Example: $\beta 2$ -adrenergic receptor

- Binding of an agonist e.g. adrenaline activates a G protein, $G_{\alpha s}$ by binding GTP
- $G_{\alpha s}$ then stimulates adenylyl cyclase
- This leads to the accumulation of cyclic AMP (cAMP)
- cAMP activates cAMP dependent protein kinase A (PKA)
- PKA phosphorylates proteins e.g. to stimulate muscle contraction, vasodilation (blood vessels, arteries)



- Beta₂-adrenergic receptor regulates CFTR
- PKA phosphorylates R domain and activates CFTR

GPCR families

- 6 families on the basis of their sequence and structural similarity
 - TYPE 1 or A : Rhodopsin Class:amine, opsin, melatonin, prostaglandin, cannabinoid, adenosine some peptide hormones
 - TYPE 2 or B: Secretin Class: Glucagon, secretin, calcitonin, vasoactivepeptides, GLP 1, CRF, PTH
 - One of the larger classes
 - TYPE 3 or C: Glutamate Class: Metabotropicglutamate receptors, calcium sensing receptors, GABA receptors
 - Other minor classes include pheromone, embryonic development, taste receptors and odorant receptors.
- Despite the similarities, individual GPCRs have **unique combinations** of signal-transduction activities involving **multiple G-protein subtypes**, as well as **G-protein-independent signalling pathways** and complex regulatory processes

GPCR & peptide-based drugs

- It is clear that the peptide and hormone ligands or these type 2/B GPCR's modulate very important biochemical and physiological processes
- Much research activity has been directed towards the elucidation of the structural and binding characteristics of these peptides with their receptors with a view to the design of new agonists and antagonists as drugs to treat diseases associated with their important roles

- Major challenge: isolating GPCR (since they are membrane proteins)
- Development of peptides that act as agonist antagonists to regulate GPCR activity, which target Class Type B GPCRs.
 - Peptides are often isolated from unusual sources, but have enhanced agonist properties compared to the natural peptide

Drug development status of peptide and nonpeptide ligands targeting Class B G-protein-coupled receptors*							
Receptor	Indication	Agonist or antagonist	Name	Peptide	Company	Status	Refs
Peptide ligands							
Calcitonin	Osteoporosis	Agonist	Miacalcin	Salmon calcitonin	Novartis	On market	[3]
	Paget's disease	Agonist	Cibacalcin	Human calcitonin	Novartis	On market	
	Hypercalcemia	Agonist	Calcimar	Salmon calcitonin and Aventis	Rhone-Poulenc Rorer	On market	
PTH	Osteoporosis	Agonist	Forteo	PTH(1–34)	Lilly	On market	[5,82]
			PREOS	PTH(1–84)	NPS	Phase III	
GLP-1	Type II diabetes	Agonist	Exenatide	Exendin-4	Amylin and Lilly	Phase III	[6,83]
			Liraglutide	GLP-1 analogue	Novo Nordisk	Phase III	
			CJC-1131	GLP-1 analogue	ConjuChem	Phase I and II	
Secretin	Autism	Agonist	RG1068	Porcine secretin	RepliGen	Unknown	[84]
Nonpeptide ligands							
CGRP	Migraine	Antagonist	BIBN4096BS	NA	Boehringer Ingelheim	Phase II	[71]
CRF	Depression, anxiety, IBS	Antagonist	Numerous	NA	Numerous	Phase I/II	[9,62]
Glucagon	Type II diabetes	Antagonist	Numerous	NA	Numerous	Unknown	[74,85]

*Abbreviations: CGRP, calcitonin gene-related peptide; CRF, corticotropin-releasing factor; GLP, glucagon-like peptide; IBS, irritable bowel syndrome; NA, not applicable; PTH, parathyroid hormone.

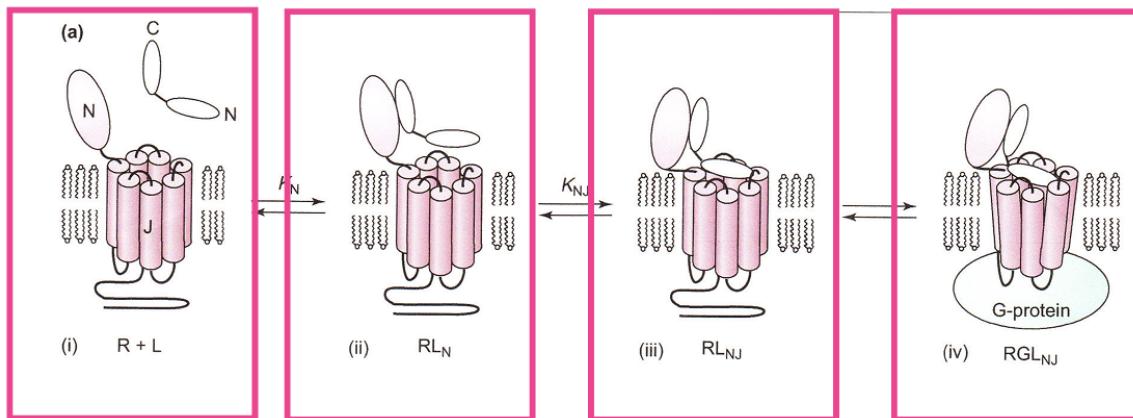
- Calcitonin is a hormone that is naturally produced in the thyroid
 - When given to patients, it helps to increase bone mass by activating calcium uptake

- Glucagon like protein 1 (GLP-1) increases insulin secretion from pancreas & inhibits glucagon.
 - Exenatide-4 is a peptide found in a venomous lizard

Binding mechanism of GPCR

- Peptide ligands are generally disordered in solution and often amphipathic
- Central sequence of the ligand tend to have turns and bends which assist in binding
- Molecular modelling (computational) NMR spectroscopy and cryoEM have shown that bound ligand to assume α-helical structures

A two-domain binding model



- The **C-terminal portion of the peptide** binds as an alpha helix to the **N-domain of the receptor**.
 - This interaction covers a broad surface on the N-domain and is mediated by electrostatic and hydrophobic interactions
- These interactions forms an “**affinity trap**” and increases the local concentration of the N-terminal portion of the peptide to the vicinity of the J-domain
 - This is typically a high affinity interaction ($K_d = 1-100 \text{ nM}$)
- The **N-terminal portion of the peptide** contacts the J-domain in an interaction that activates the receptor
- The activated J-domain mediates G-protein activation via conformational changes

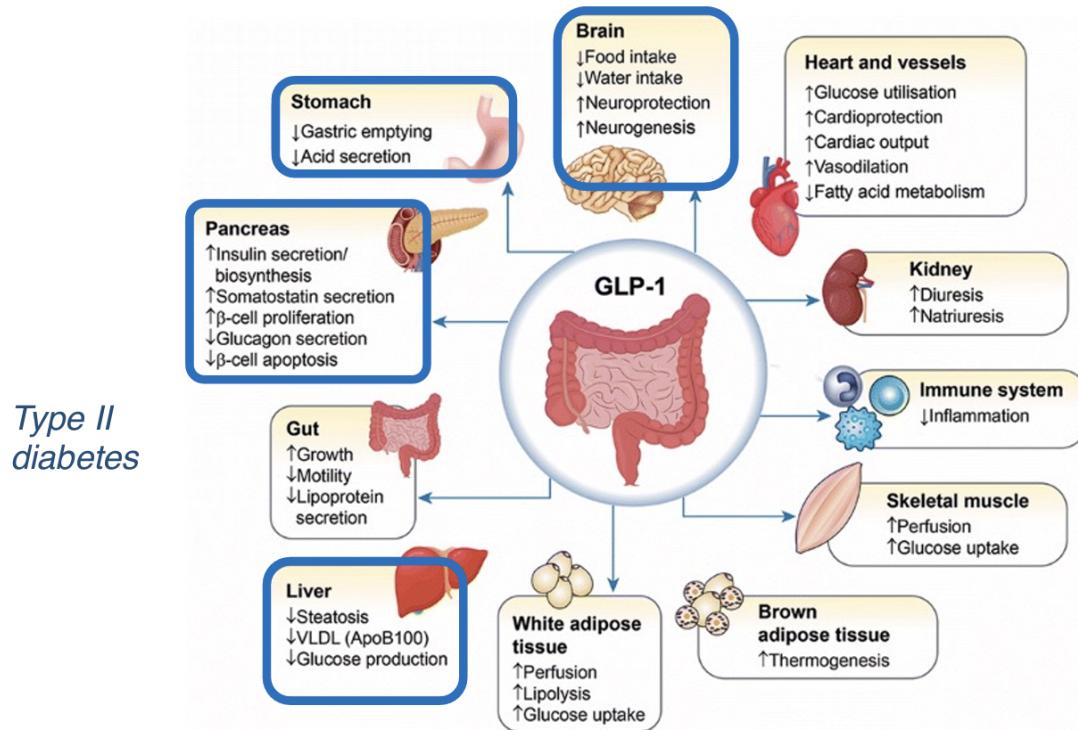
New drug development

A deeper understanding of the binding characteristics of the peptide and non-peptide ligands has informed the design of new peptide mimics

- Only the J-domain is needed for GPCR activation
 - New peptides have been designed that only interact with the J-domain
- Issues with low affinity and need modification to bind to J-domain and remain bioavailable
- A whole range of non-peptide antagonists have been designed or are in trials

Type B Class: GLP-1 receptor

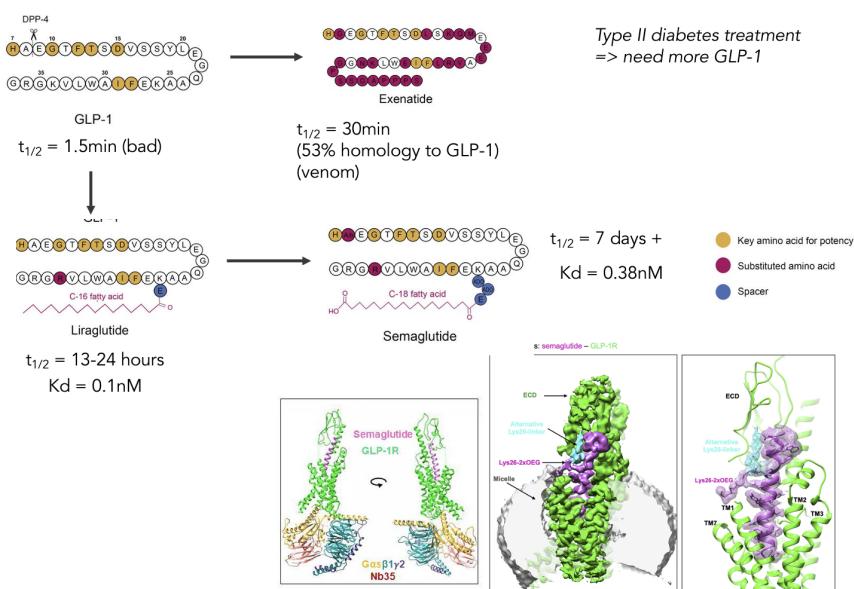
- GLP-1 receptor interactions involve multi-organ action
 - It can be targeted in treatment of Type II diabetes



*insulin production => aim to reduce blood sugar levels

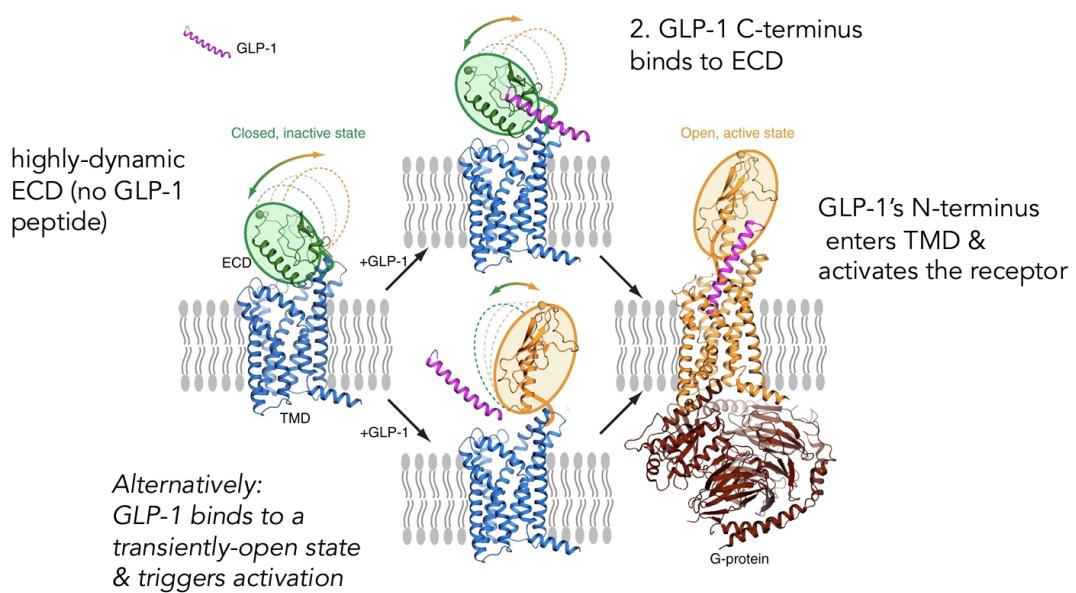
1. stimulates cellular glucose uptake
2. inhibit further glucose production (e.g., liver)

- Biochemical approaches to understand half-life and Kd with knowledge of conformations and interactions takes place between ligand and receptor leads to better drug design



- For Type II diabetes treatment, agonists were generated to activate GLP-1R

- Binding model of GLP-1
- GLP-1 may either:
 - Binds to closed extracellular domain cause a conformational change to active state
 - GLP-1 N terminus enters the TMD and activates the receptor
 - GLP-1 binds to a transiently-open state and directly triggers activation



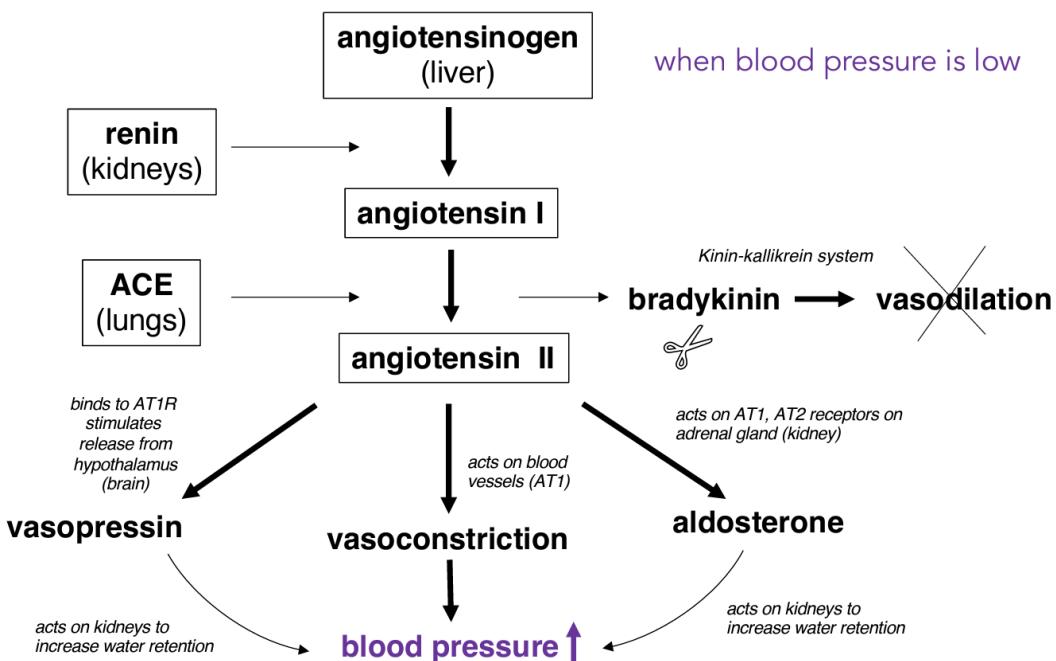
Wu F et al, Nat Comm, 11, 2020, 1272
ECD = extracellular domain = "N-terminal domain of receptor"
TMD = transmembrane domain = "J-domain"

Enzymes as therapeutics - Angiotensin Converting Enzyme (ACE) inhibitors

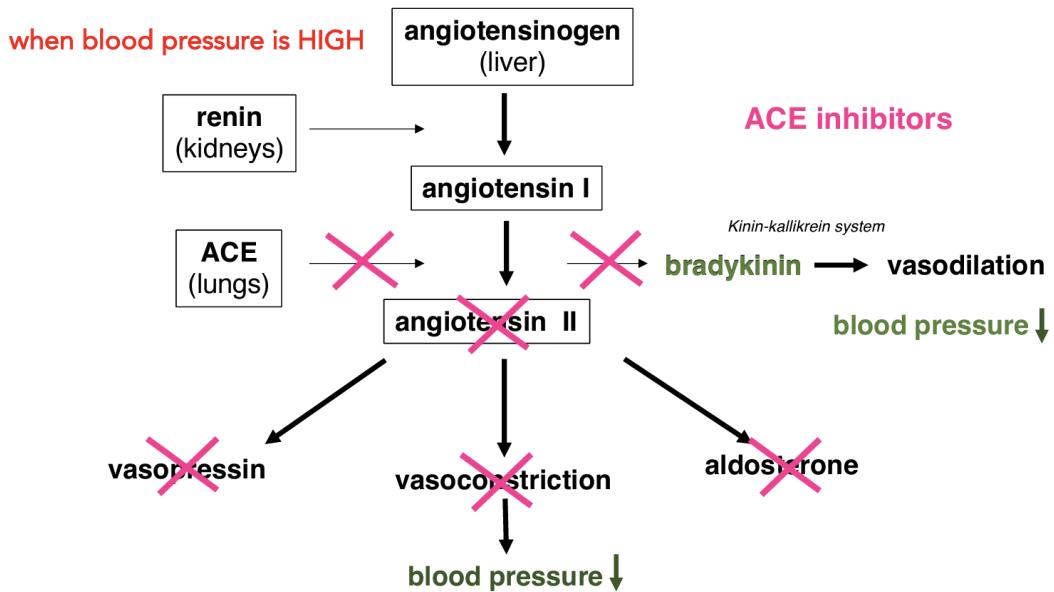
- Chronic hypertension affects 26% of the world's adult population
- Life threatening condition: high blood pressure can lead to cardiovascular complications kidney or heart failure and stroke
- A range of anti-hypertensive drugs developed including: Diuretics, β -blockers, calcium channel antagonists, angiotensin receptor blockers**, α -blockers** and ACE inhibitors**
 - **Act on the renin-angiotensin-aldosterone (RAA) system

RAA system

- The RAA system is an important pathway for regulating blood pressure

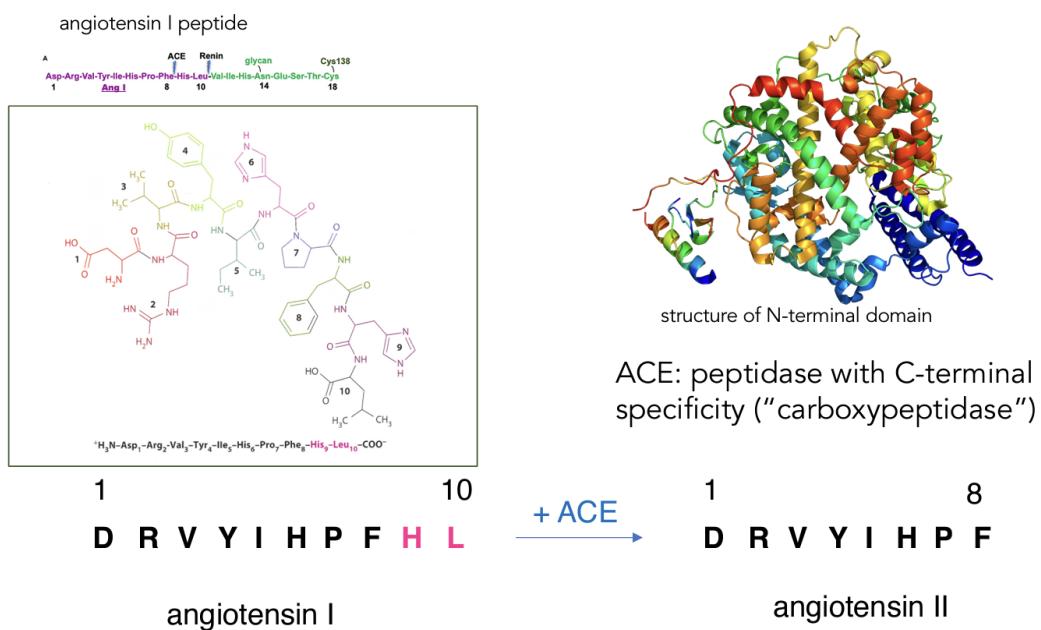


- When blood pressure is high, ACE inhibitors stop conversion of angiotensin I to its active form angiotensin II, thus inhibit vasoconstriction, and production of vasopressin and aldosterone



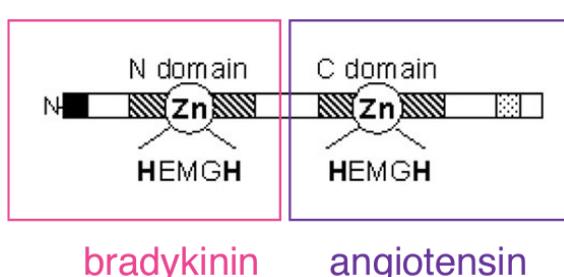
ACE as peptidase

- ACE is a key peptidase in the RAA system
- Protease with C-terminal specificity for 10aa angiotensin I peptide
 - Carboxypeptidase
 - Removes 2 amino acids from angiotensin I's C-terminus to form angiotensin II



- 1306 amino acid protein
- Two homologous domain:

- N terminal domain: 30-630 amino acids
- C terminal domain: 631-1232



- Each domain has a Zn-containing active sites
 - Zinc-dependent metalloprotease
 - Each Zn cation bound to conserved HEMGH amino acid motif

- A rare example of a single polypeptide chain containing two homologous active sites acting on 2 different substrates
 - 2 specificities

Example of rational drug design: ACE inhibitor

Early development of ACE inhibitors

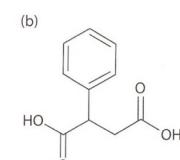
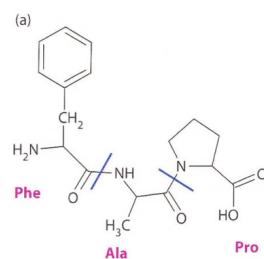
- 1970's Cushing and Ondetti discovered a group of ACE inhibiting peptides from the venom of the South American Pit Viper, Bothrops jararaca.
- The best inhibitor was isolated and found to be **Phe-Ala-Protripeptide**
- However, the naturally-sourced peptides were poor drugs for oral administration (digested in the stomach)

First generation ACE inhibitor: Captopril

- Designing non-peptide mimics of Phe-Ala-Pro
 - Basis of rational design: Known carboxypeptidase inhibitor benzylsuccinic acid

designing non-peptide mimics of Phe-Ala-Pro

Phe-Ala-Pro
(original)

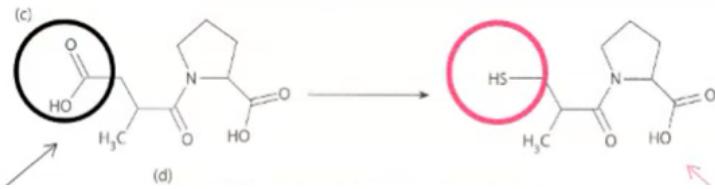


Known carboxypeptidase inhibitor benzylsuccinic acid

(basis for rational design)

- Understand where box

- Designed ACE inhibitors

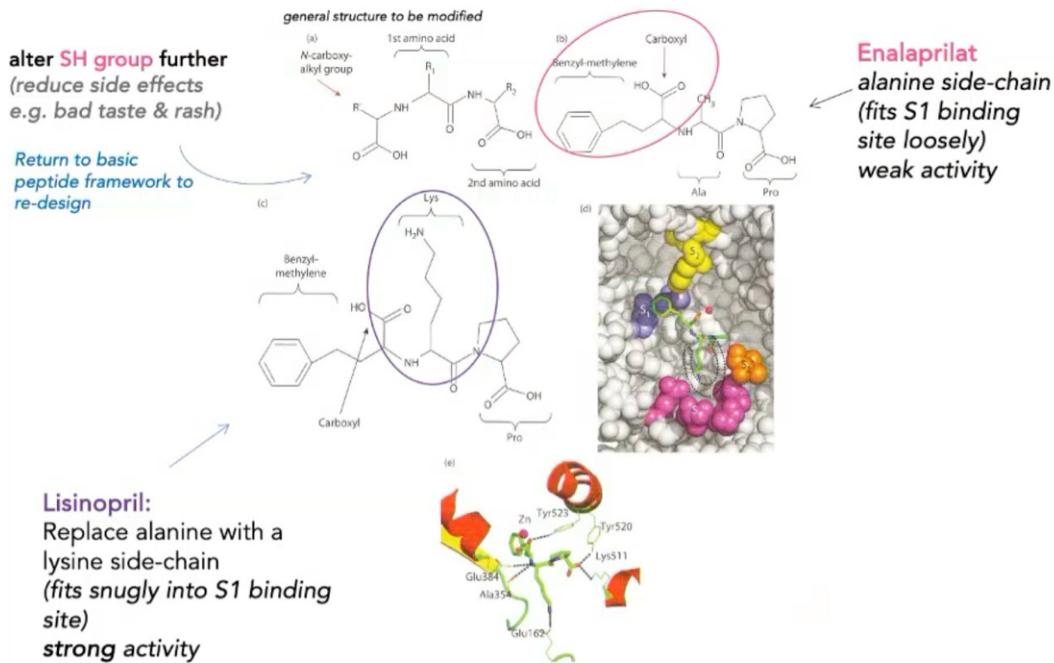


- Methyl-Succinyl-Pro: OH group binds zinc ($IC_50 = 20\mu M$)
- Captopril: Replacement of OH with SH, which binds zinc very tightly ($IC_50 = 20nM$)

Second generation of ACE inhibitor: Lisinopril

- Alter SH group of Catopril further to reduce side effects (e.g. bad taste & rash)
 - Return to basic peptide frame work to re-design
- Enapaprilat: Ala side-chain (fit S1 binding site loosely), weak activity
- Lisinopril: Replace alanine with lysine side-chain (fits singly into S1 binding site), strong activity

Second generation ACE inhibitor: Lisinopril



Summary

Cystic Fibrosis & CFTR

- Structure, function & druggability of CFTR
- Importance of folding - structure - function relationship

GPCRs

- Family, characteristics, structure & mode of action
- Examples include beta2AR & GLP-1

Renin-angiotensin-aldosterone system (regulating blood pressure)

- Role of ACE & ACE inhibitors
- Drug development through rational design

Proteostasis - A network of pathways used to maintain the correct concentration of proteins within cells