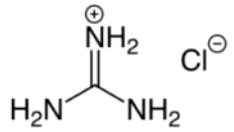
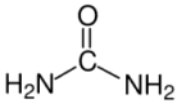
L1

Montag, 23. September 2019 12:44

Skript
Protein F...

- **Anifinsens Principle:** Unique 3D Protein structure completely encoded in AA- sequence
- Unfolded State U --> native State N
  - Spontaneous: Thermodinamicaly favored (native state is most stable state in phys. Conditions)
  - reversible

Denaturents • Shift equilibrium towards unfolded state ○ Non reversible after long incubation • GdmCl better (no covalent binding to protein) ○ Stronger	 Guanidinium chloride (GdmCl)  Urea

Refolding reaction: convert all proteins into unfolded state by incubating with high conc of denaturent. Then dilute, so that native statie is again favored

- Levinthal Paradox: random search for active confirmations implausable
  - Cooperativity: Cluster of native contacts are mantained and expand during search. Reduces searchsapce
    - Native contacts favour formation of neighbouring NCs
    - All or nothing reaction: no partially structured intermediates (Two-state-mechanism)
      - Mixture of both with $K_{eq} = \frac{[N]}{[U]} = \frac{k_{Fold}}{k_{Unfold}}$
    - multiple folding pathway
    - May form structured intermediate (or "all or nothing reaction")

Spectroscopic techniques

Only aromatic AAs absorb around 230

Absorption spec

- Cuvette with pathlength d is excited with monochromatic light with the intensity I0, and the intensity of the light that passes the sample, I, is detected
  - $A = \frac{I_0}{I} = \epsilon * c * d$
  - ϵ: extinction coeff. (1M in 1 cm cuvette)
  - $\epsilon_{native} = \epsilon * \frac{A_N}{A_U}$
- Peptide binds absorb in far UV spectrum (205 nm, good at very low conc.)
- Absorbance around 280 nm from Tyrosine + Tryptophan (aromatic) and S-S bonds
  - $\varepsilon_{280\,nm,calculated} = (n_{Tyr} \cdot 1490 + n_{Trp} \cdot 5500 + n_{SS} \cdot 125)\ M^{-1}cm^{-1}$
  - Tryptophan only one that absorbs > 300 nm

    - Phe absobas at 260
- Can accurately calculate protein conc.
    - The more absorbance, the higher the conc.
- unfolded vs. Native have very similar absorbtion, bur can still monitor folding process

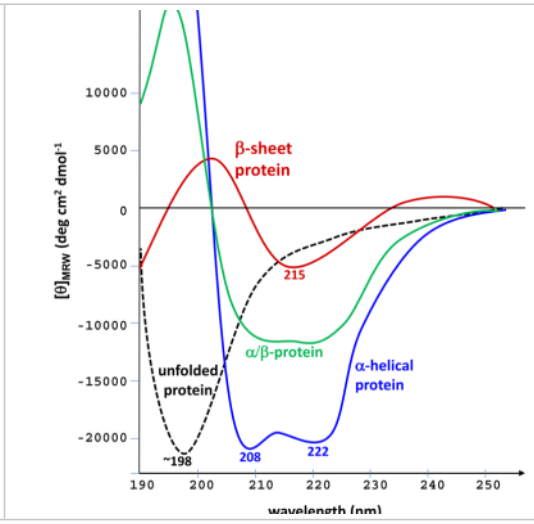
Fluerescence Spec

- Only Tyrosine + Tryptophan are fluorescent
    - Trp high absorbance -> better fluorophor
    - Excitation at 280 nm
    - Fluorescence Trp: 320-335 (native) -> 355 (unfolded)
- Spectra change upon folding, though intensity can increase or decrease
    - Trp downshifted when folded, no shift for Tyr
- Mixture of unfolded and native prots, measure fluorescence at wavelength of largest difference betweene U and N
    - Native may be more or less fluorescent
- Determine fraction of folded molecules by fluorescence of mixture

Circular dichroism spectroscopy

- Right and left circularly polarised light produce linearly polarised light when both have same amplitude
- Proteins absorb left and right pol.light at different extincrion coeffs (since they are asymmetric)-> no longer linear, but <u>eliptical</u>
    - Elipticity measured at different wavelengths

- $\theta = 33 * \Delta A = 3'300 * \Delta\epsilon = \frac{[\theta]cd}{100}$

Far UV- CD spectra provide info about secondary structure (α-heix & β-sheets) Unfolded prots have almsost no near UV extinction	

L2

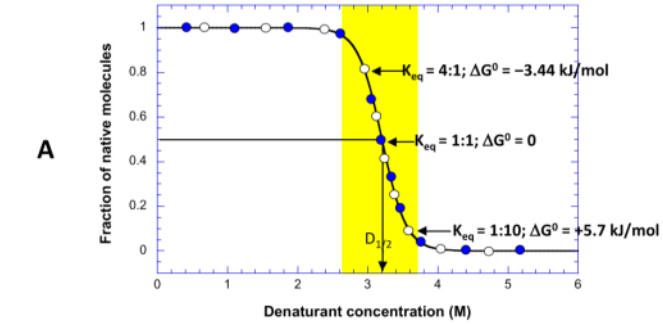
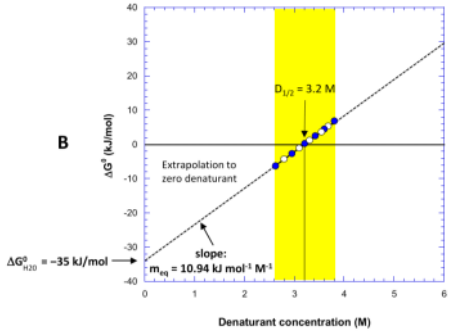
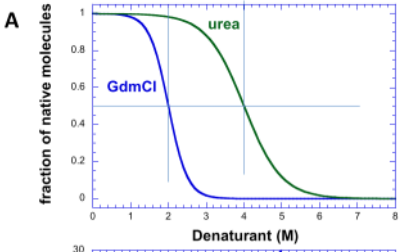
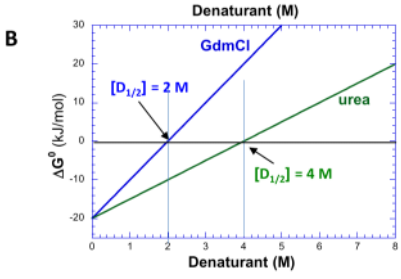
Montag, 30. September 2019 12:55

- Ca. 50 AA's minimum length for defied 3d structure
  - Less: most molecules stay unfolded

Determining free energy • Free energy by folding proportional to realtive freq. Of native proteins	$\Delta G^0 = -R \cdot T \cdot \ln K_{eq} = -R \cdot T \cdot \ln \frac{[N]}{[U]} = -R \cdot T \cdot \ln \frac{k_F}{k_U}$

- ΔG typically -10 - -80 kJmol^-1
  - If k_eq = 10/1 -> -5.7 kJmol^-1
  - Need to know unfolded concentration, but Detection error ca. 1%
    - Perturb equilibrium towards unfolded state (detergents from L1)

Unfolding Experiment

Search conc. Of denaturant for which 50 % are denatured/unfolded	 	
m_eq: Same Δ G0 but (2x) shallower slope fo Urea	 	

- Δ G depends linearly on denaturant conc. [D]
  -> can accurately determine physiological Δ G by extrapolating to 0
  - $\Delta G^0 = \Delta G^0_{H2O} + m_{eq} \cdot [D]$
    - m_eq ~ cooperativity of folding
- Unfolded protein much easier to degrade -> unfolded state removed from equilibrium
  -> proteins with highk_eq -> slower degredation (?)

Refolding Experiment

- Reverse experiment: incubate at high [D], then dilute it back to low [D]
- If equilibrium **is** obtained: then curves sould overlap
    - Otherwise activation energy for (un)folding is too high-> cannot calculate Δ G

Δ ASA: difference in accesible surface Area (between U&N)

- Higher Δ ASA, the more Denaturent Sensitice becomes the folding equilibrium
- Δ ASA ~ mass of AA-chian ~ m_eq
    - Meaning m_eq is predictable

Two state asumption no longer holds for large Proteins with multiple independently folding domains

- Underestimate m_eq value

Heat Capacity

$$\Delta G = \Delta H - T\Delta S$$

- U has higher c_p than N
    - Hidrogen bonds in water shells around aromatic AAs need more energy
      -> ΔASA ~ Δc_p
- .....

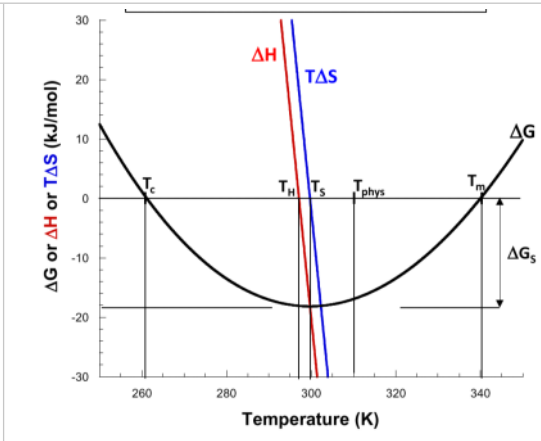
Protein stability is tempeature-dependent because of Δ H and Δ S

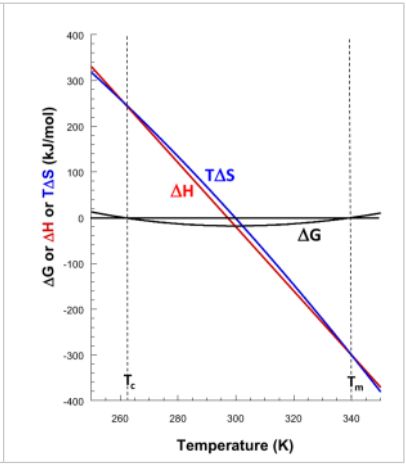
$$\Delta H(T) = \Delta H_m + \Delta c_P \cdot (T - T_m)$$

$$\Delta S(T) = \Delta S_m + \Delta c_P \cdot \ln\left(\frac{T}{T_m}\right)$$

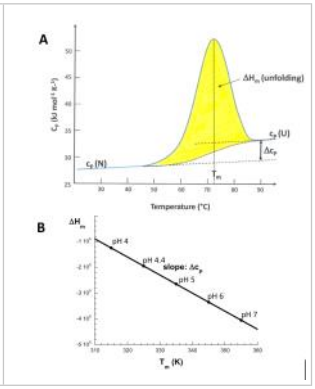
Δ H/S one order of magnitude larger than Δ G

Entropy-Enthalpy compensation (?)
ΔH approx one order of magnitude larger than ΔG at phys. Temps



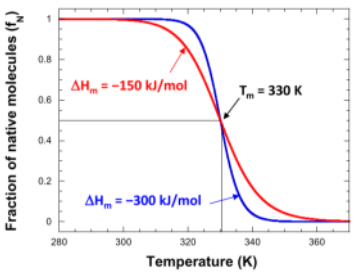


Differential scanning calorometry measures heat capacity of protein:
Δ Cp:
Δ Hm: yellow area
Tm: Median(?) of yellow area
linear dependence on pH



Thermal unfolding similar to denaturant unfolding

Midpoint is melting temp Tm



Vant Hoff Eq.

$$\frac{d(\ln K_{eq})}{dT} = \frac{\Delta H(T)}{RT^2}$$

Integrate by Tm:

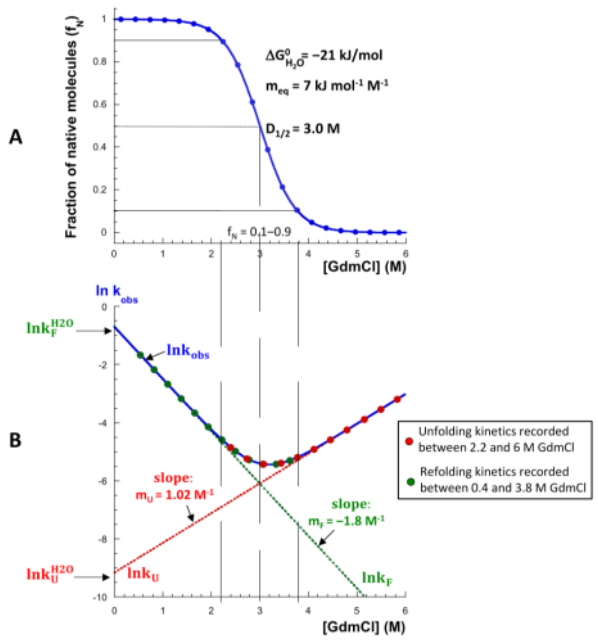
	$f_N = \frac{exp\left[\left(\frac{T}{T_m} - 1\right) \cdot \Delta H_m / RT\right]}{1 + exp\left[\left(\frac{T}{T_m} - 1\right) \cdot \Delta H_m / RT\right]}$

L3 Kinetics of Protein folding

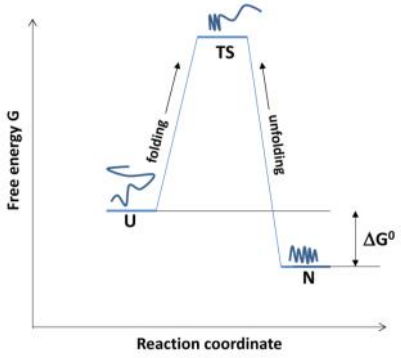
Montag, 7. Oktober 2019 12:56

…

- Cannot observe K of folding/ unfolding directly (only K_obs)
- But at very high/low denaturent conc. One of the two can be ignored (very small)
- Blue line (bottom): observed K; at transition state, k_obs is higher than ku/kf
- Slopes mu/mf:
  - $m_{eq} = (|m_u| + |m_F|)RT$

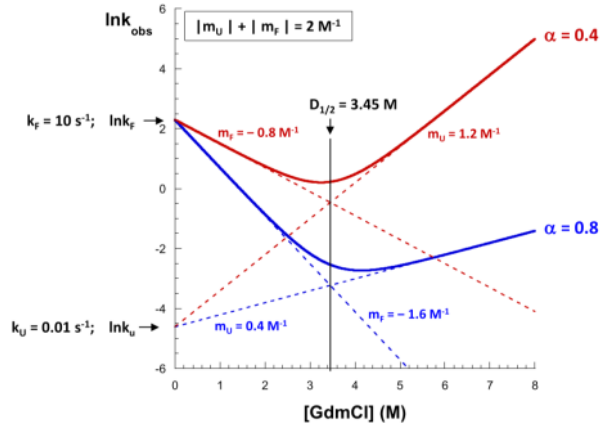


TS: is partially folded and its ASA is more similar to M than to U



$$\alpha = \frac{|m_F|}{|m_F|+|m_U|}$$

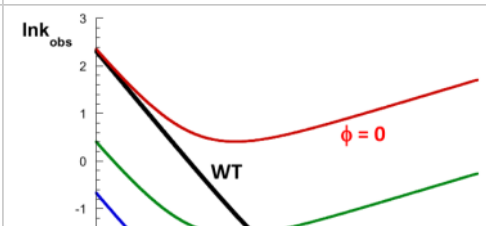
α:ASA_TS more similar to ASA_N
Transition state often already compact

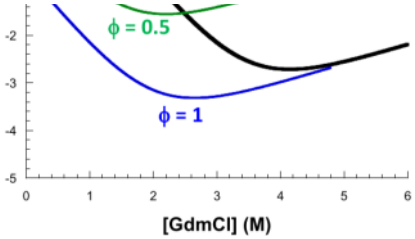


Phi-value analysis for characterization of TS

- $\phi = \frac{\Delta G_{TS}}{\Delta G_N}$

0.5: protein less stable. RC of folding



smaller. RC of unfolding higher	

L4

Montag, 14. Oktober 2019 12:45

Contact order: avg. Dist. Between pairs of residues which make contact

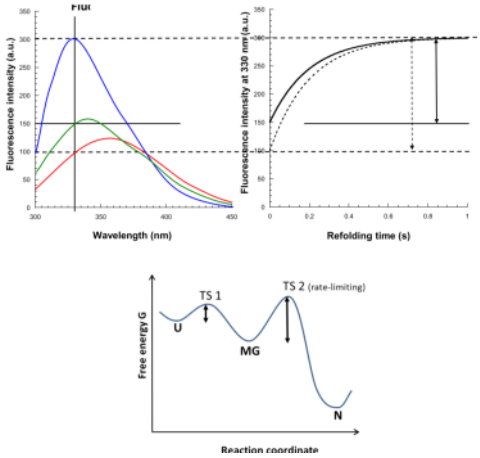
- Example: contct between AA20 and AA40 (20% of total length) -> 0.2
- Avg. Contact order of entire structure
  - Eg. alpha-helix very small (10%) contacts with direct neighbours); β-sheets very large (20%)

- Naturally Very slow folding proteins(ca 1 h, very high contact order) usually catalized

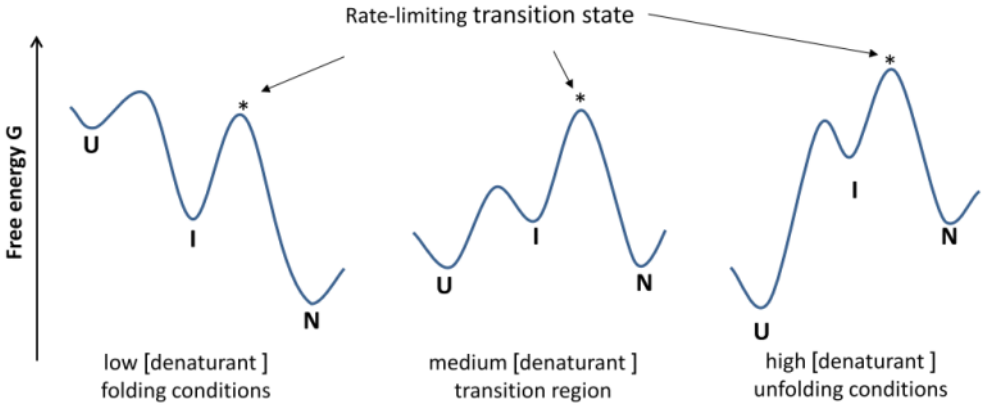
- Molten globule (MG)-like intermediates that lack tertiary structure but nevertheless are already relatively compact and already contain secondary structure elements..
  - Happens very quickly, rapid colapse into globule
- Next state is a structured intermediate
  - The more structured (the more stable) an intermediate is, the more it slows the folding reactio

How to detect molten globule state (very fast

- Can detect spectroscopic changes
  - Trasistion away from Molten globule is rate limiting



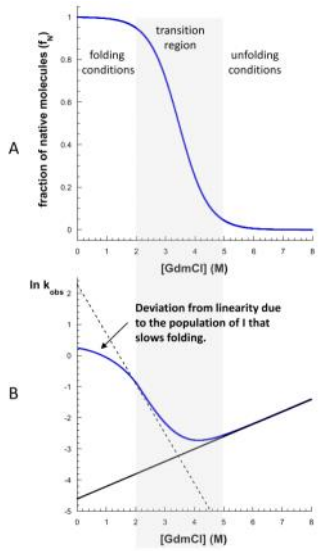
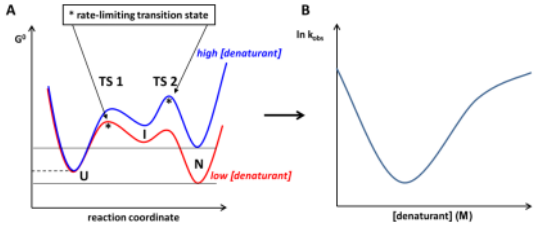
At transition midpoint, I has higher energy as U and N -> not observed



Problem: consecutive first order reactions

- Can inferr rate constants k1/k2 but cannot correctly assign them to first or second reaction
- Once difference between k 1/k2 is very large, one can no longer measure a lag phase in production of C (in a->b->c)
  - This is the case in protein folding

- Model with intermediate is always slower than simple two state model

Reaction is slower than direct u-Y N reaction In B: left part of curve will be lower than expected under 2 state model	
High energy intermediates I that are less stable than U and N under all conditions can also lead to deviations from the ideal V-plot when the rate-limiting transition state changes with increasing denaturant concentrations.	

Triangular Folding model where there exists also a direct folding patho from U -> N

- For Lysozyme, 14 % fold directly

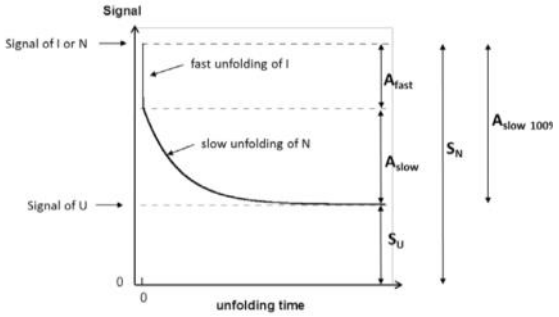
L5

Montag, 21. Oktober 2019 13:06

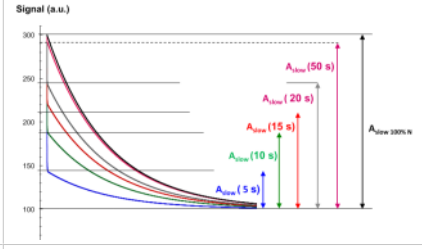
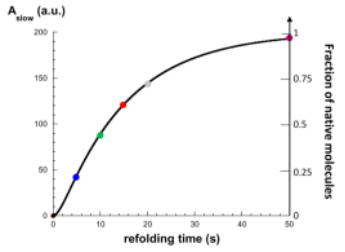
Detecting folding if I&N cannot be distinguished spectroscopically

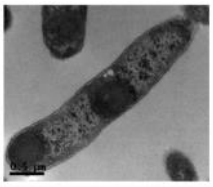
N-Test: determines fraction of Native mol, independent of spectroscopy

- Interupted refolding reaction
- Start with unfolded proteins, let refold for timet t -> mix of U/I/N
- Add high conc. GonCl
  - Activ. Energy for Unfolding **I** lower -> unfold rapidly
  - Choose GonCl-conc from Vplot, such that observation of Native unfolding is possible
  - Native molecules unfold slower (Biphasic Reactions)

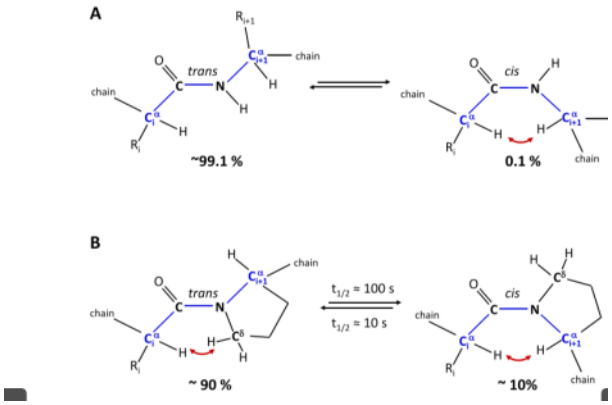


$$S(t) = S_U + A_{I\to U}\cdot e^{-k_{I\to U}\cdot t} + A_{N\to U}\cdot e^{-k_{N\to U}\cdot t}$$

Depending on when folding is interupted, amplitude A_slow will be shallower (shallower if low %N)	
Now plot A_(N->U) vs. Refolsing time	

Unspecific aggregation (xU, "inclusion bodies") competes with native folding Yield optimization • Low temp • Low ions • Low prot conc.	

Proline cis/trans isomerization as the rate-limiting step in protein folding



- Less favorable cis bond is still preset on some proteins (can be compensated)
- Cis-/trans Equilibrium in unfolded state (in vitro artifact, not in vivo)
    - Some fraciton of slow folding proteins (0.9^5 = ca. 0,5 for 5 prolines)

Glutathion buffer for oxidative refolding of disulfide bonds n vitro

- Native bonds are stable. Even high conc of reduced glutathion cannot brake them again.

L6

Montag, 28. Oktober 2019 12:54

MolecularC
haperone...

Definition:

1. It must assist the noncovalent assembly/disassembly of another protein-containing structure. The mechanism by which it does this is irrelevant.
2. It must not be a component of these structures when they carry out their biological function in the cell.

Heat shock proteins

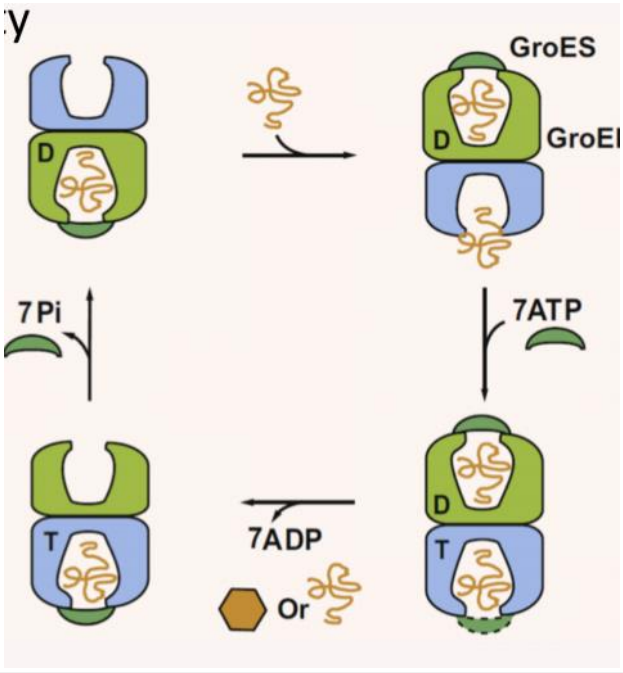
- HSP??:Heat schock protein + mol. weight
- Most are chaperones

HSP70, HSP40, (GrpE-like proteins) - Chaperone System

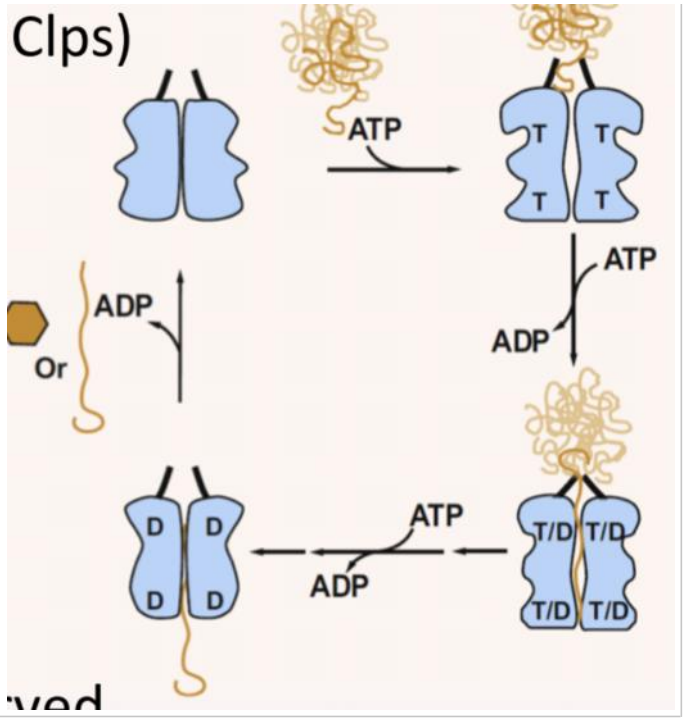
- Sometimes cotranslational
-
- Under stress
  - Prevention of aggrgates
  - Dissagregation (with other factors)

HSP90

- Recognizes finisched but inactive signaling peptides (is the final activation step)

Chaperonins or HSP60/10-Chaperone System	

HSP100 Proteins



Role of Chaperones

1. During the process of translation the synthesized polypeptide chains emerge from the ribosome in an extended non-native conformation.
2. During the process of translocation across organell membranes proteins have to be unfolded and then have to fold again in the new compartment.
3. After the synthesis of individual components of multisubunit assemblies, it can happen that the destined binding partner is not yet available and hydrophobic patches that are later burried in the protein/protein interface are still exposed.
4. When the cell is under stress, for example heat stress, native structures can get damaged. In addition, aggregation is a bigger problem at higher temperatures.

- Many side reactions compete with productive protein folding
- Chaperones do not provide steric information required for proteins to fold correctly, but prevent or reverse missfolding reactions

Cotranslational interaction

Chaperones that can interact with newly synthesized chains:

1. Chaperones bound at the exit site on the ribosome. example: trigger factor
2. Chaperones binding to nascent chain itself while it is attached to the ribosome: cotranslational interaction example: Hsp70/40/GrpE

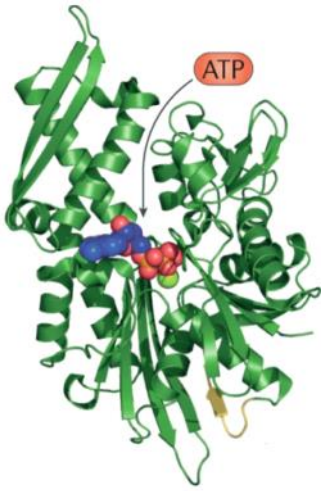
3. Chaperones binding to polypeptide chain **after release** from the ribosome: posttranslational interaction
   examples: GroEL/ES and also Hsp70/40/GrpE

Potential Function:

1. to physically separate non-native polypeptide from surface of ribosome
2. to postpone folding until complete sequence of autonomously folding domain has emerged
3. prevention of aggregation

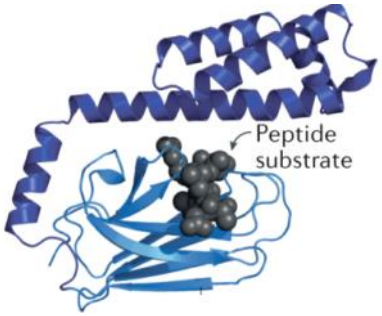
HSP70

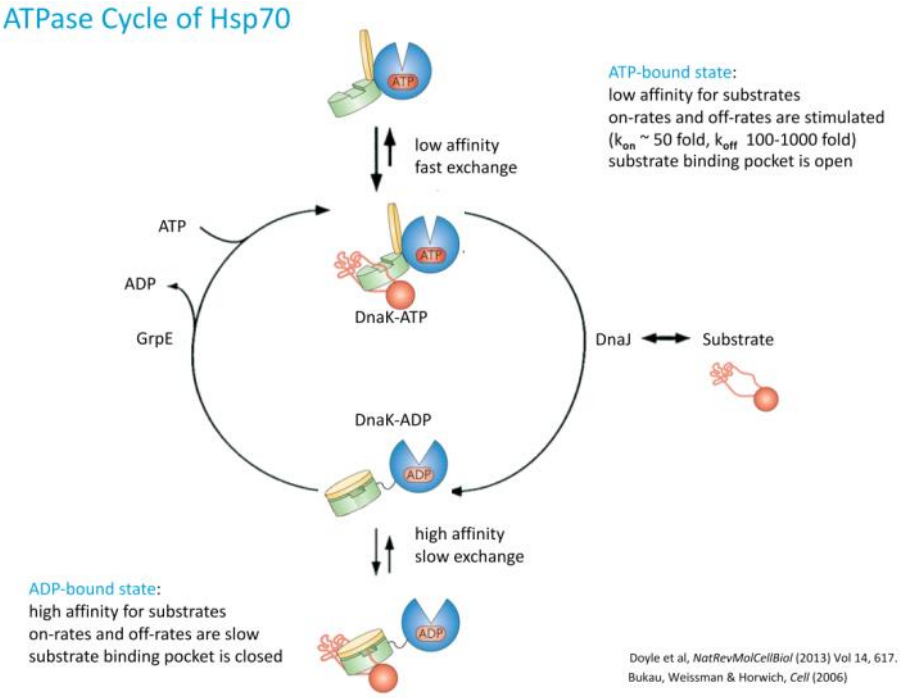
Ntemrinal Domain:



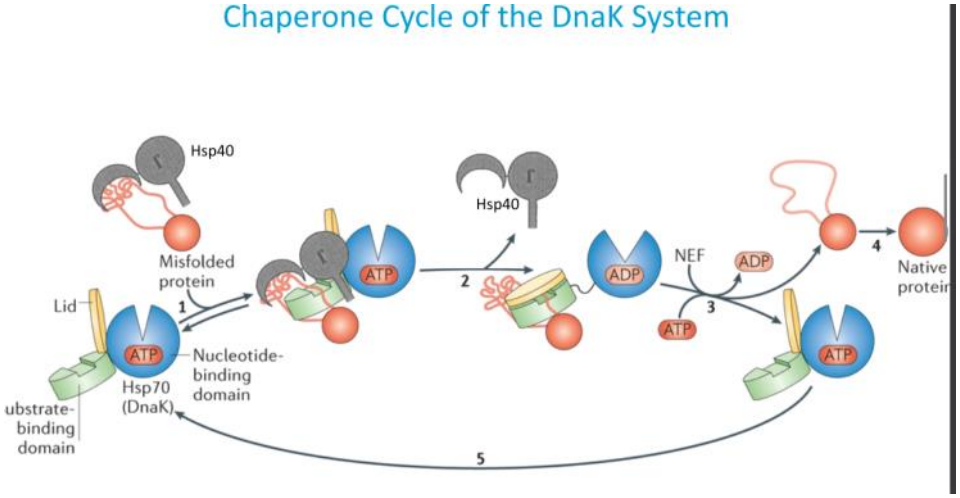
Structure changes when ATP -> ADP

Cterminal domain:





- Hsp40 has the role to stimulate the ATPase activity of Hsp70.
- After hydrolysis of ATP, the affinity of Hsp70 for Hsp40 is reduced and it is released again.
- Nucleotide Exchange Factors: GrpE-like chaperones trigger the release of ADP by opening up the ATP-binding cleft.

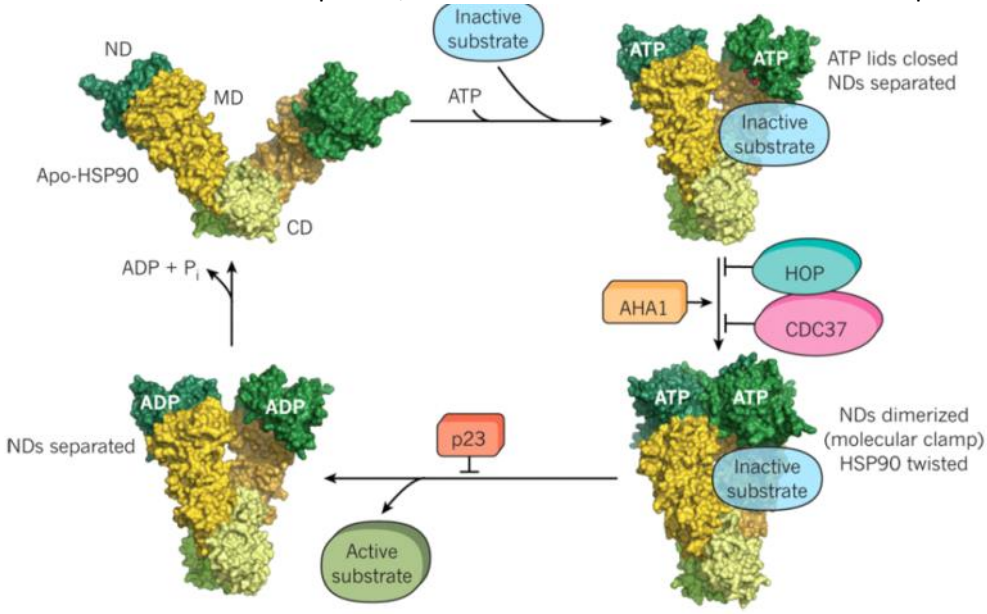


Hsp 90 Chaperone

exists in open and closed conformation

open: ATP can bind, exposed hydrophobic patches for substrate binding

closed: lid is on ATP pocket, N- and middle domains dimerize and clamp down on substrate



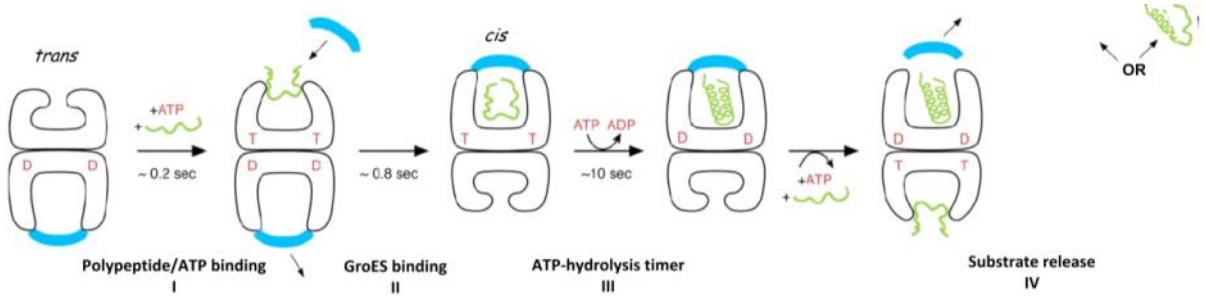
GroEL and GroES

- assists the folding of a variety of proteins in the E. coli cytosol. It accomplishes this by cyclic binding and release of substrate polypeptides.

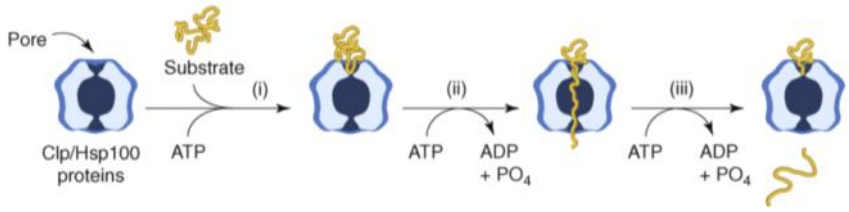
….

Opon binding of ATP

1. 25° downward rotation brings intermediate domain down on equatorial domain.->
   ATP gets locked in
2. dramatic 60° upward rotation of apical domain about the upper hinge
3. 90° twist of the apical domain. **The hydrophobic patches are buried in the binding interface with GroES and with other apical domains.**



Hsp100/Clp Proteins



- Refolder or disasemblers /feed to dissasemblers

They have (similar to many other chaperones):

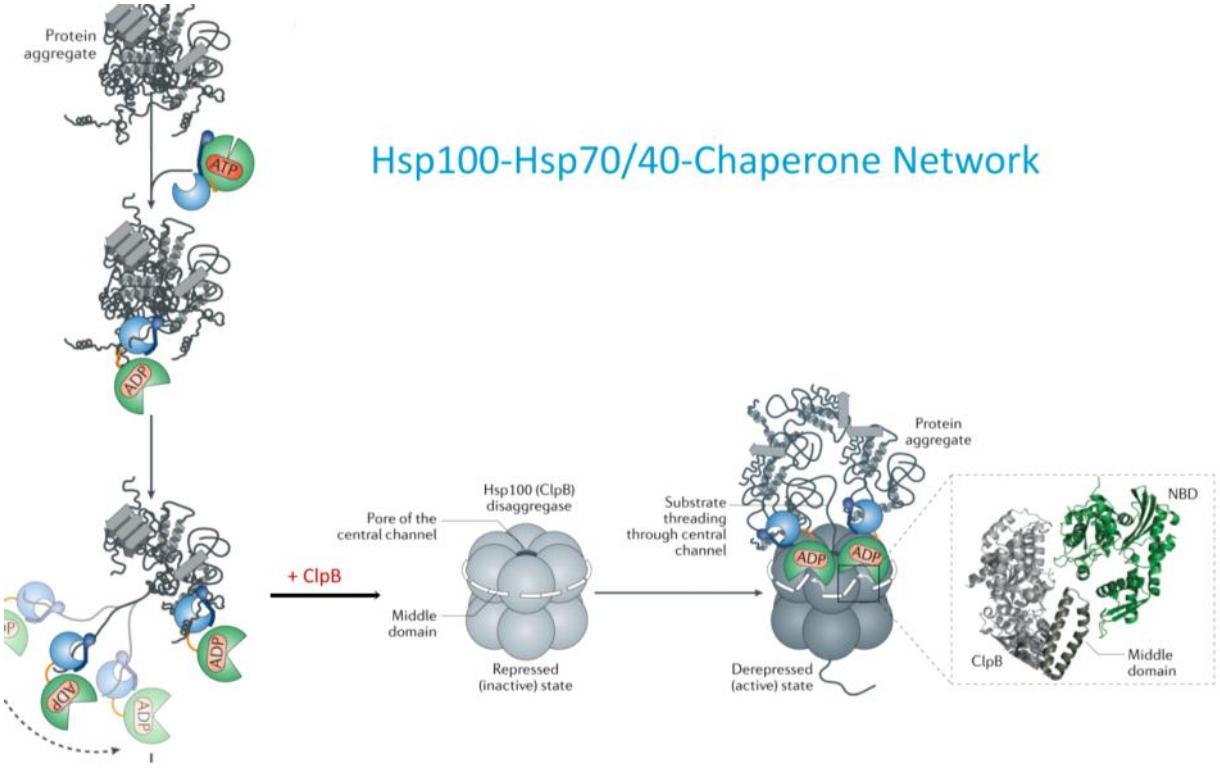
1. Ability to change the conformational state of their substrates.
2. Consumption of ATP to support this activity.
3. Induction during stress conditions.
4. Universality and high degree of conservation.
5. High concentration in the cell.
6. Multiple forms or subfamilies within the same cell.

ClpB/Hsp104 Chaperones: P of the Hsp100 protein family, large linker between the two nucleotide binding domains.

- disaggregate protein aggregates.
- do not interact with proteases.

Hsp100-Hsp70/40-Chaperone Network: M domain of hsp100 binds hsp70 by interacting with ATP bidingg domain. This stimulatesthe transport(?)

Hsp100-Hsp70/40-Chaperone Network



L7

Montag, 4. November 2019 12:59

ProteinDeg
radation_...
Degradation PAthways

- Ubiquitin protwasome
  - Compertmalisation compartmenatlised in protein
- Autophagy lysosome system
  - compartmentalised in organel

Catalysis Principles

- covalent catalysis attack by enzyme residue as nuleophile
- non-covalent catalysis attack by water (activated by acidic residue)
-

AAA proteins: ATPases Associated with various cellular Activities

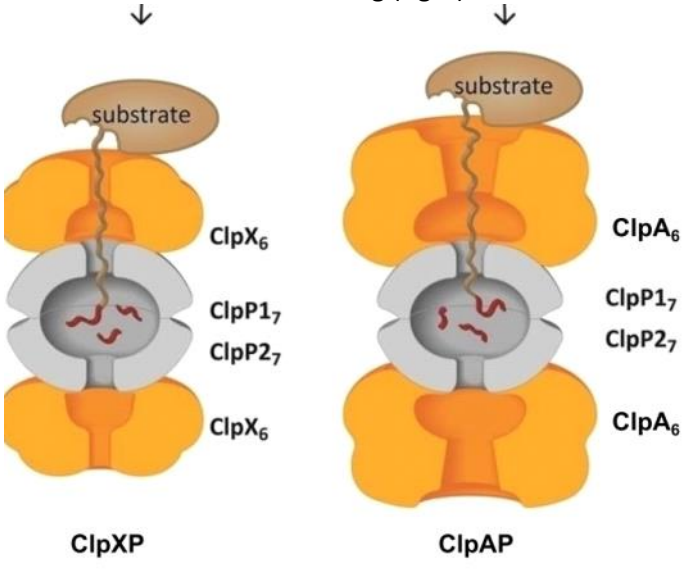
- coupling of ATP hydrolysis and conformational changes to thread DNA or protein substrates through central pore:
- WalkerA & WalkerB jointly bind MG2+
- Glutamate nucleophilic attack on phosphate
- Arrginine finger stabelises phosphate
- loops pointing into the translocation channel, featuring aromatic residue
  - Switch from up to down position: let go of substrate and back to up

Compartmentalization provides the following features:

- - Separation of substrate from bulk cytosol
- - Selection via folding-state à narrow entrance pores
- - Processivity by encapsulation of the protein à substrate is cleaved down to peptide size
- ring-stacking principle allows association with ring-shaped partners ligning up the pores as a conduit into the proteolytic core

Bacterial Caseiolytic Protease Clp

- Core ring 2x7 membered ring
- Can have double AAA ring (right)



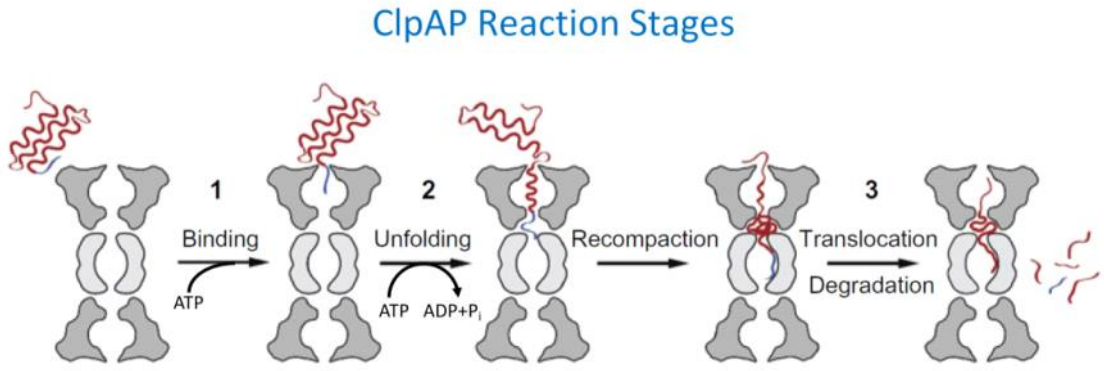
ClpAP: Caseino-lytic protease with ATPase and Protease Rings

ssrA taged substrates: rescuing "cloged" ribosomes
tmRNA (t and m RNA parts

- Codes for ssrA tag

N-end rule Substrates

- CLipAPS recognises destabilising N-end AA's



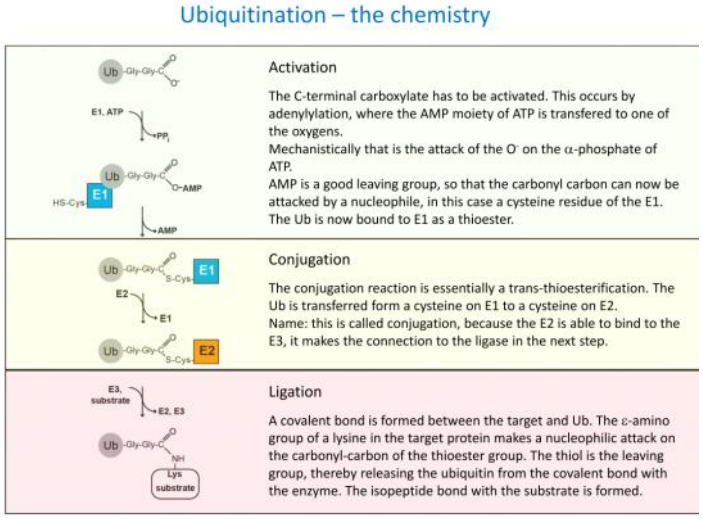
L8

Montag, 11. November 2019 13:12

ProteinDegradation_...

Ubiquitin

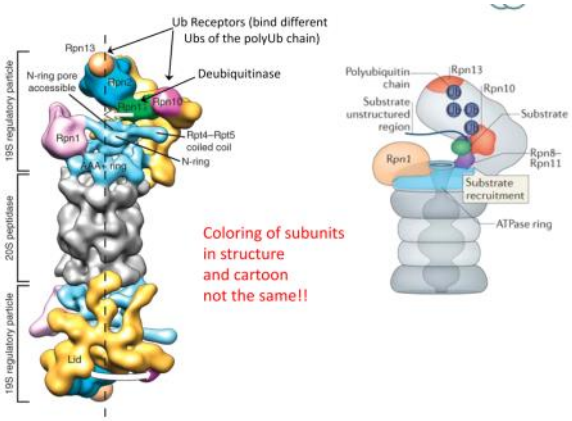
- Very conserved
- C terminus atteches to other proteins
  - Covalent postttranslationak midification
  - GG- C-terminus attached to K(Lysine) sidechain or N-terminus α-peptide
  - Isopeptide bonf: sidechain petide group
- Multiple ubiquitins can be chained (again attached ad Lys)
  - Different tags depending on which Lys is elongated
    - Homotypic and polytypic chaines
- **Lys48:** makes closed, globular chains
- Open chains: more like beads on a string
- Ile44-patch: hydrophobic region. important binding region



Specific K48 Polyubiquitination leads to degradation

- Protease Reaction: nterminal trheonine as catalytic core
  - Methyl group necessary for internal actiavation

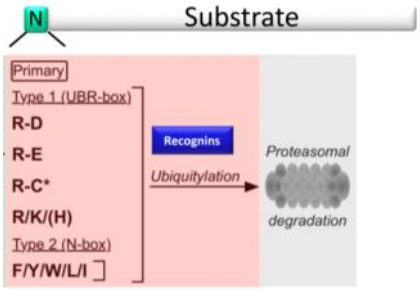
- Spacing between Rpn 13 /RPN10 requires Minimum 4 Ub necesary for efficient degradation
- Rpn11 cleaves ubiquitin of degradated peptides

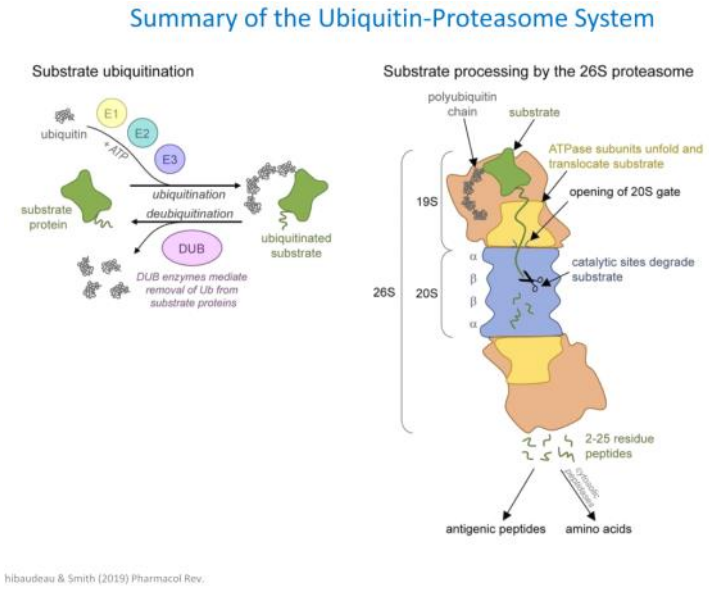
- 

- Unstructured C-terminal tail necesary for degradation

- Coordinated ATP-Hydrolysis and Substrate-Translocation Cycles
  - Not all Rtpts are in the same state at a time, cycle trough each step one after the other

- N-end rule in eukaryotes
  - E3 ligases important forselection process, influencing which proteins are marked with Ub and which are not. possess a specific binding site for a certain protein substrate





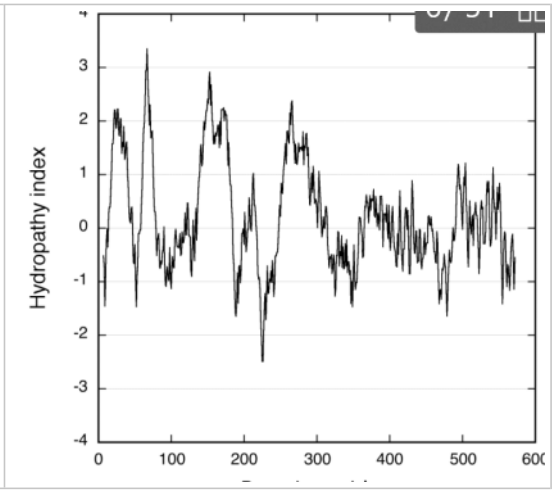
L9 Transporters

Montag, 18. November 2019 13:01

MSB1_201
9_Lecture...

- Passive v. active
- Second. v. primary
- Transporters expressed trough Sec61 Transöocon in ER membrane
- Hydrophobic residues often burried in membrane

- Kyte-Doolittle-Plot: sliding hydrophobcity window plot
  - Would predict TM domain drom 0-300. soluble Domain from 300-600



- Positive inside Rule
  - Arg & Lyc (positive charge) determnie orientation of Loop/End-Terminus inside Cytoplasm
    - Adding positive charges to outside reverses Protein
  - Poor prediction of two Helicises with very short loops vs. Long transmembrane helix
    - Short loops can be masked

Environments for membrane transport / channel protein studies
Full cell: plasma membrane protein

1. Full cell: plasma membrane protein
2. Native membrane vesicles (liposomes)
3. Artificial membrane vesicles (LUV), “proteoliposomes
   - Controll proteins and lipids in membrane
4. Mixed micelles
   - Nanodiscs allow selextion of lipids
   - SMALP based extrection allows membrane protein extraction without detergent

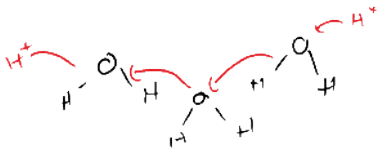
Rsat: saturation in lipid bilayer before dissolution ("softening liposumes up")
Add membrane Protein in detergent
Can spontaniously insert itself
Remove detergent while leaving proteoliposomes intact

Aquaporins

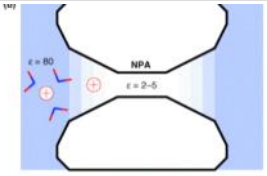
- Quick, specific Watertransport trouth cells (water diffuses only very slowly)
- Must not allow H+ diffusion

- Single file Watermolecule
- H bonds between H20s and asparagines in intermembrane motive
- Relatively hydrophobic, very narrow channel
    - Quick water transport, and nothing else can pass

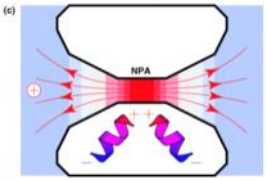
Why no protons, 3 hypothesies

1. Aquaporin prevents optimal placement of grotthus wire • Not actually prohibited enough in simulation	

2. Low dielectric region forming barrier for charged species



3. Electrostatic barrier from short helices forming macrodipoles



uct. Biol. 15: 176-183 (2005)

3. Electric dipole pointed at center of porin

Hypothesis 3 is the only one confirmed by simulations

L10

Montag, 25. November 2019 13:09

MSB1_2019
Lecture...

- Potential E (Volt)
- Current I (Ampere)
- Conductance g (Siemens)
- Ohms Law; I=gE
  - Linear dependence

- In > out -> neg. potential (only regarding this ion)

- Mixed potential for multiple ions
- Special cas neatively charged ions
  - $E_{Cl} = \frac{RT}{F} \ln \frac{[Cl]_i}{[Cl]_o}$
  - Negative charge -> flip log ratio

- Measurement: Cells restingpotential -60
  - Conclusion: potassium (K+) channel contribution more important/ generally open

The modified current-voltage law (Ohm's) then becomes

$$I_K = g_K(E - E_K)$$

The "electromotive force" in the pore is EK and the net driving force on K+ ions is now E - EK and not E. This modification was introduced by Hodgkin and Huxley.

① Δ Conc $\Delta G_{chem} = RT \ln \frac{[K^+]_{out}}{[K^+]_{in}}$

② Work against E $\Delta G_{Elec} = z \cdot F \cdot E_{K^+}$ (charge / Particle; el. Potential at Time t)

at equilibrium: $\Delta G = 0$

① = −②

$RT \ln \frac{K^+_{out}}{K^+_{in}} = -zF E_{K^+}$

Possive (Channel) Transport $\Rightarrow E_{K^+} = \frac{RT}{-zF} \ln \frac{K^+_{out}}{K_{in}}$ — Variable Part

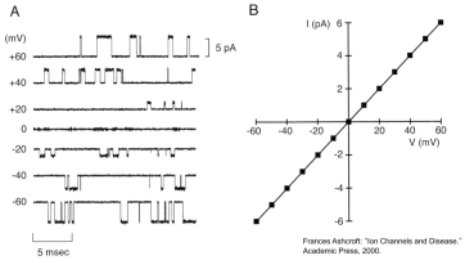
ΔG must be < 0 per Definition for spontaneous

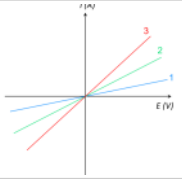
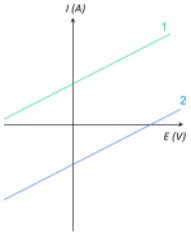
$[out] < [in] \Rightarrow \ln \ominus \Rightarrow \Delta G \oplus$ → correct with −z

K ⟷ K^+

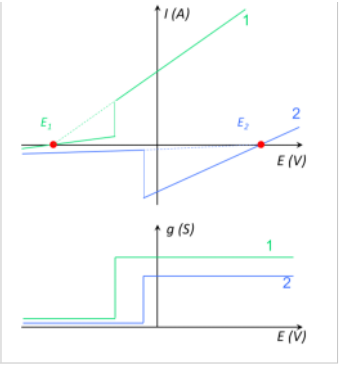
out In

- Black lipid bilayer
- Two-electrode cell clamping
- Patch clamping
  - Cell atached
  - Whole cell
  - Outside-out
  - Inside-out

- Ensemble measurements: measure multiple chanels simultaniously
- Upper limit: ca. 5 pA (10^7 - 10^8 charges/s)
  - Can calculate conductance with ohms equation
  - Range: 0.1 - 100 pS
- Can use singlemchannel recordings to reconstruct IV plot
  - 

1. Nonselective channel or equal concentration in and out Wiht different conductances	
2. Must have ion gradient • Switched ditectionality • Reversial potential: net ion flux is 0 • When is nernst equation ewual to reversial potential (exam question) • Same conductance	

3. Higher conductivity -> steeper slope



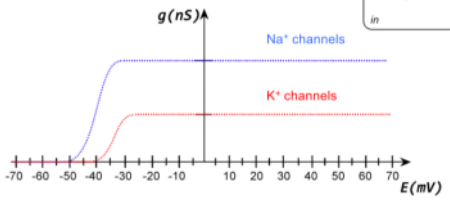
Channels and *IV* curves problem

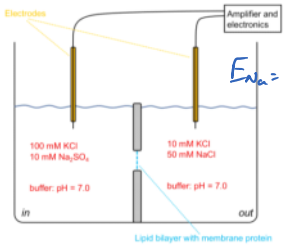
A. The figure to the right shows the setup of a black lipid bilayer experiment (ensemble measurements), conducted under standard conditions and using Hepes as a buffer.

Draw an *IV* diagram and indicate the expected data points at equilibrium assuming the lipid bilayer contains:

1. Permanently open K^+ channels
2. Permanently open Na^+ channels.

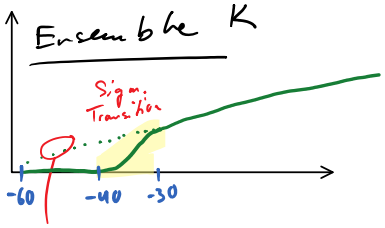
B. Consider the *g–E* graph below. Assuming the conditions shown on the right, draw the expected *IV* curves.



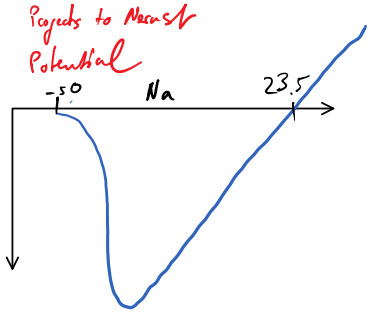


$E_K = \frac{K_{out}}{K_{in}} = 0.1$ Nernst Transform $\Rightarrow -59\,mV$ (<1 neg.)

$E_{Na} = \frac{Na_o}{Na_i} = 2.5 \Rightarrow 23.5\,mV$



Projects to Nernst Potential



L11 channels

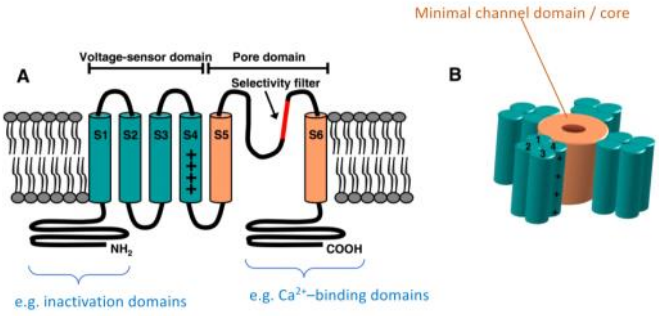
Montag, 2. Dezember 2019 12:34

MSB1_201
9_Lecture...

K+ channel

Topology of K+

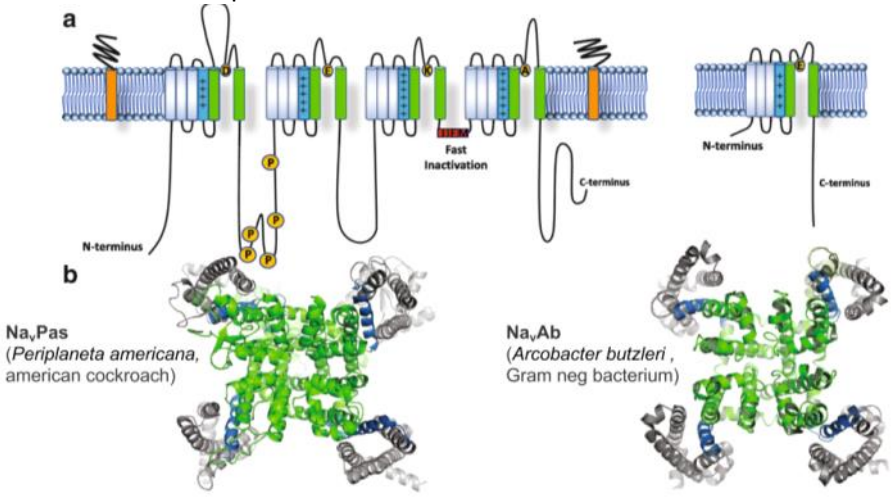
- Core architecture, but not selectivity mechanism, is simmilar to Na channels



- 4 homodimers
- Positive charges in S4 Helix sense voltage (changes?)
- Innactivation domains
- Inverted tippy
  - dehydration chamber strips water molecule, then
  - Negatively charged c termini form pore for Na's
  - In selectivity fiulter, probably Nas and water alternate
    - This makes it fast, as it dampens electrostatic repulsion between potassiums
- Selectivity
  - Desolvation of Na is slower than K+ (all but ine stripped away in in K channels, not in NA channels)
  - 1000x selective

Na+ channel

- All in one fusionprotein



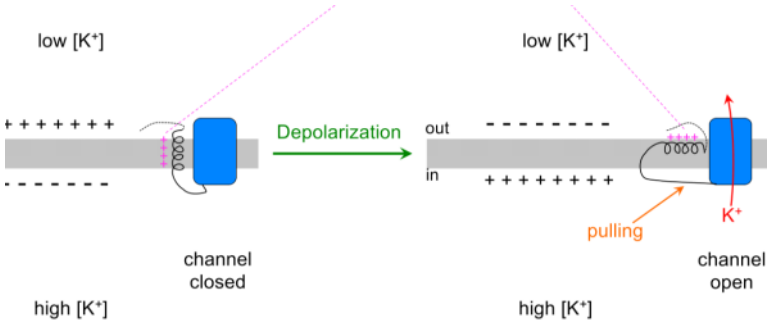
- Different intracellular loops
- Can use bacterial homotetramer version to study

- Selectivity filter directly at outside, just by shape electrostaticity
- Must not be as dehydrated to pass trough
    - ((But K+ has more water?)
    - Less selective (10x)
    - No strong selectivity needed, due to other ion concentrations

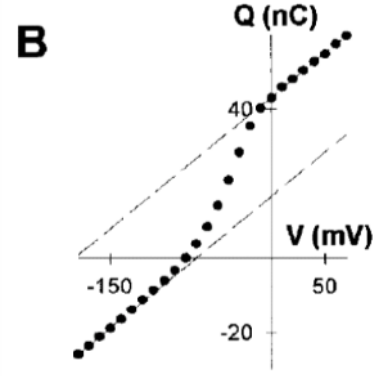
- Automatic innactivation after activation by innactivation domain
- Very simmilar to Ca channels

Gating (K+ channel)

- Helicies to dehydration center TM5/6 are opend/closed, selective pore does not change
- TM4 (s4) is connected, pull at TM4 --> TM5 -->opens TM6



Capacitive (Gating) currents: movements of charges by moving channel domains



L12 ClC channels

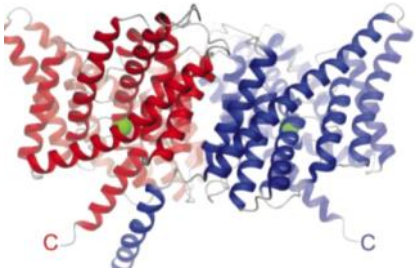
Montag, 9. Dezember 2019 12:47

MSB1_201
9_Lecture...

- “Chloride channels” Essential for chloride homeostasis in eukaryotic cells.
- dimers with two functionally independent (non-communicating, noncooperative) subunits. Each monomer contains a single chloride conduction pathway

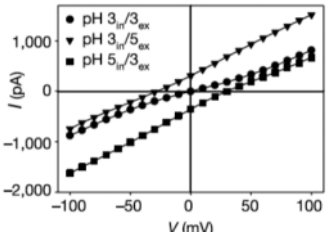
protein structure

- "gating" glutamate side chain is in the immediate vicinity of a bound Cl– ion.
  - No direct channel opening



Double channel

Channel exchanges Cl for H+:



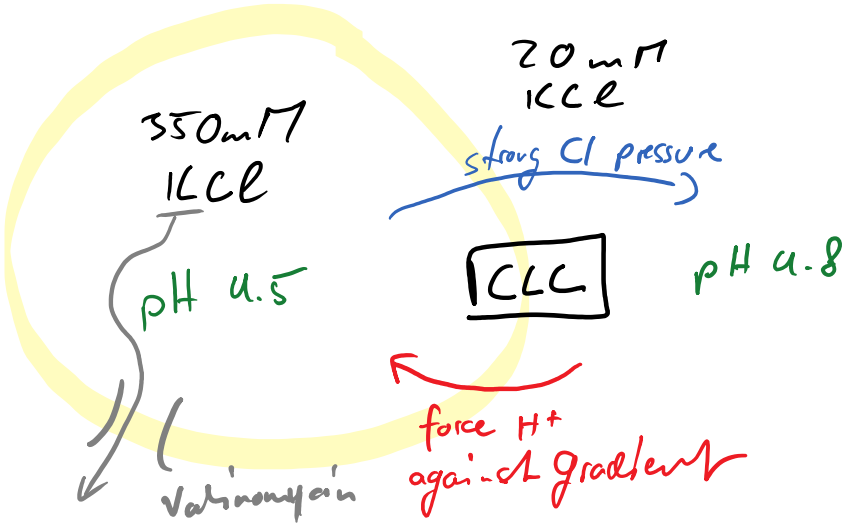
--> Channel, not a transporter

What kind of transport is it:
Symport? Would expect flat line, since HCL has no charge
Antiport: 2 charges moved
2/3 (?)

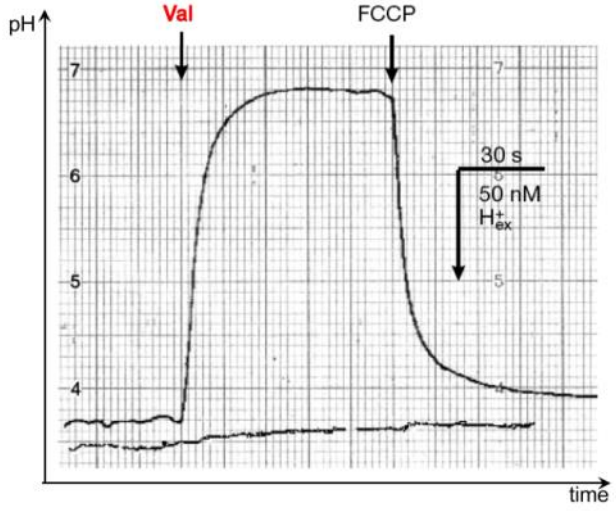
Solution: 1H+ <-> 2Cl-

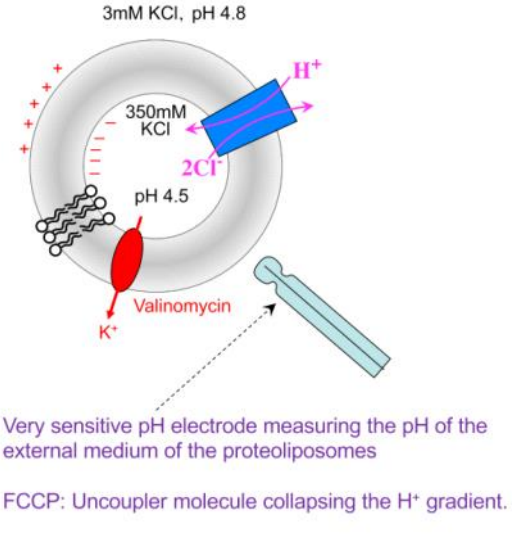
$$E_{comb} = \frac{1}{1 + r}\left(E_{Cl^-} + r\,E_{H^+}\right)$$

$$\text{with } r = \frac{m}{n}$$



E. *coli* ClC: Chloride-driven proton transport





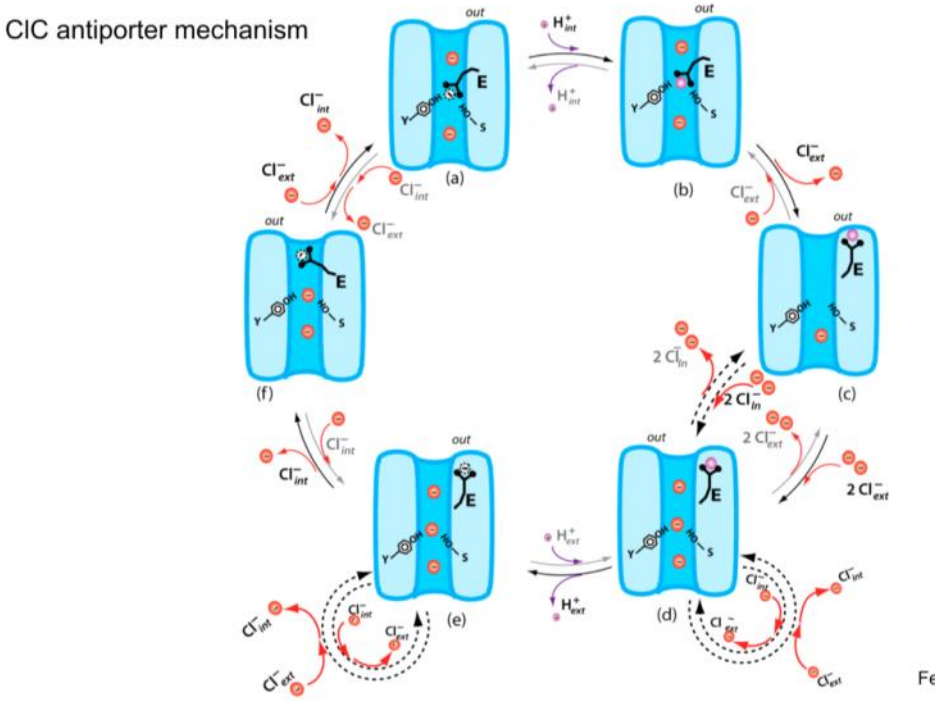
Very sensitive pH electrode measuring the pH of the external medium of the proteoliposomes

FCCP: Uncoupler molecule collapsing the H^+ gradient.

FCCP colpases proton gradient -> meaning a proton gradient was built up
Reversed experiment works similarly

Human CLC proteins are 50/50 antiporters and voltage gated channels

- Antiporters always in endosomes & lysosomes
    - Equilibrate depending on demad the concentration of Cl and H+



Clockwise exports Protons
Counterclockwise is physiological (cloride export, H+ import)

- 3 Cl position
  - Bottom has highest affinity
- Gating glutamate
  - Can reach outer and middle position, not lowest
- When protonated, the Gluc cannot stay in these posotion
  - Either passes proton to inside position or moves away
- Glu shifts clorides by moving from one to the other position
  => always have either Cl or deprotonated Glu in all 3 pockets

- ((Protins go trough protein core))