Bottom-Up Approach to Synthetic Cells

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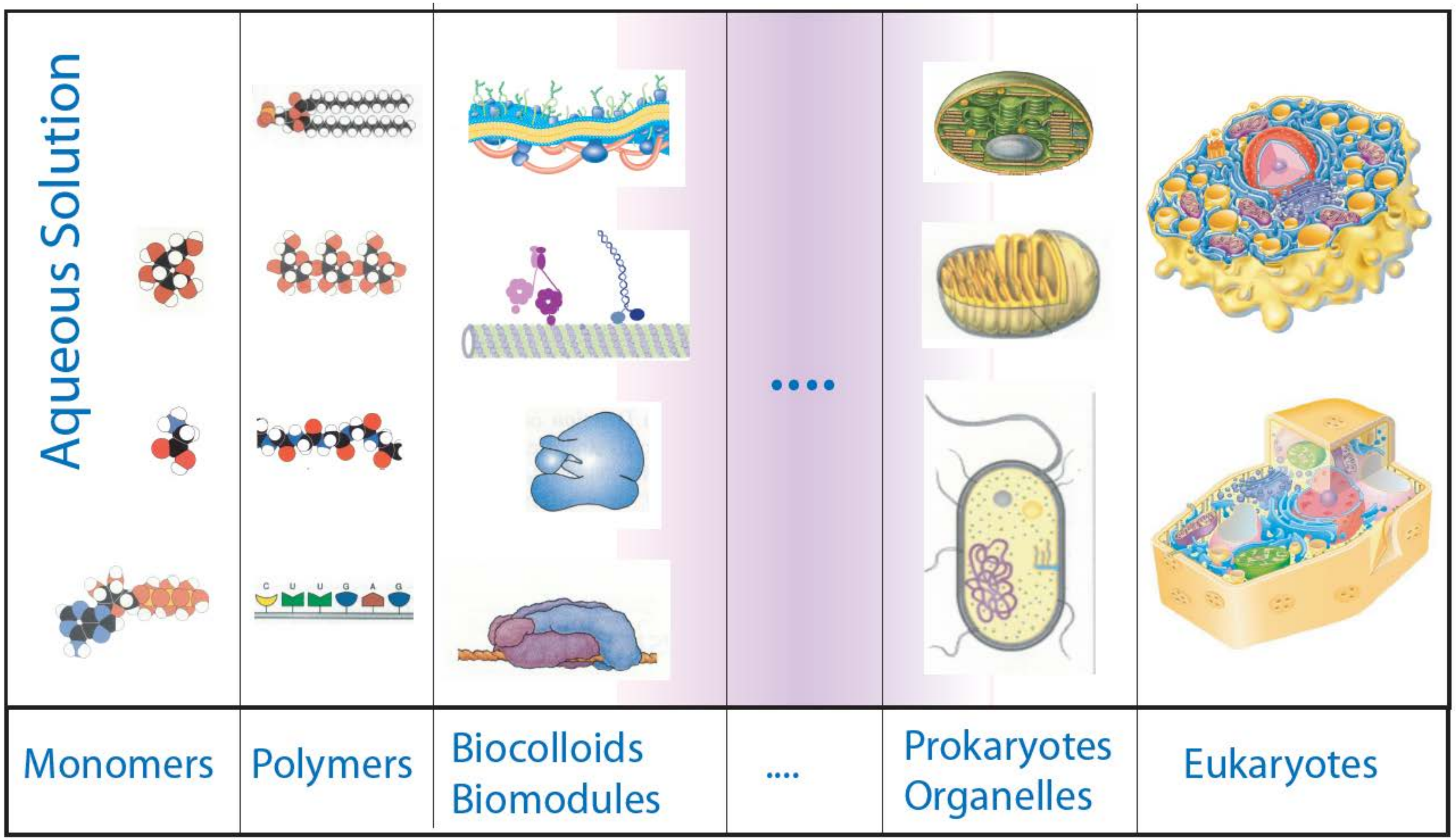
- From Matter to Life
- Three Basic Modules:
  Compartments, Motors, Assemblers
- New Platform for Bottom-Up Assembly
- Future Challenges

From Molecules to Cells

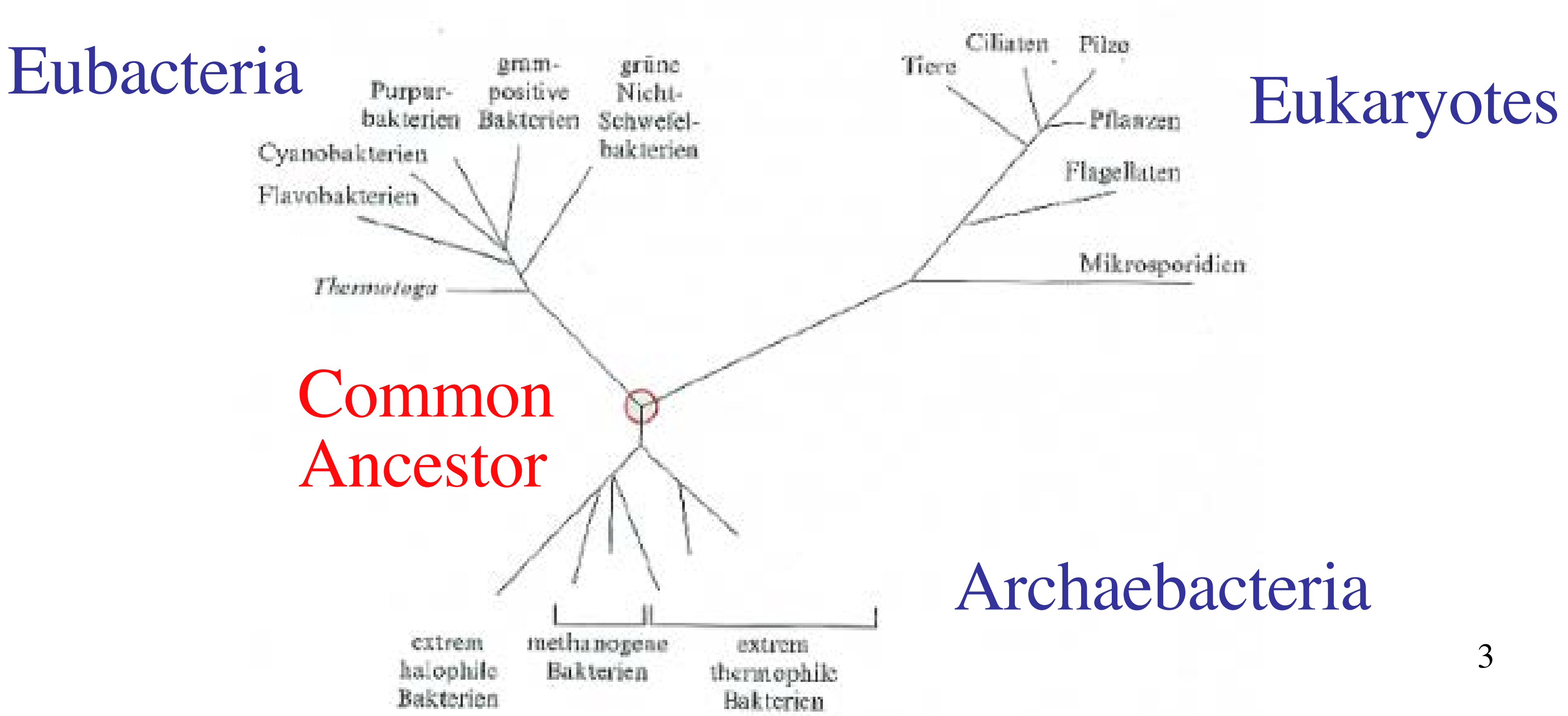
Transition Zone



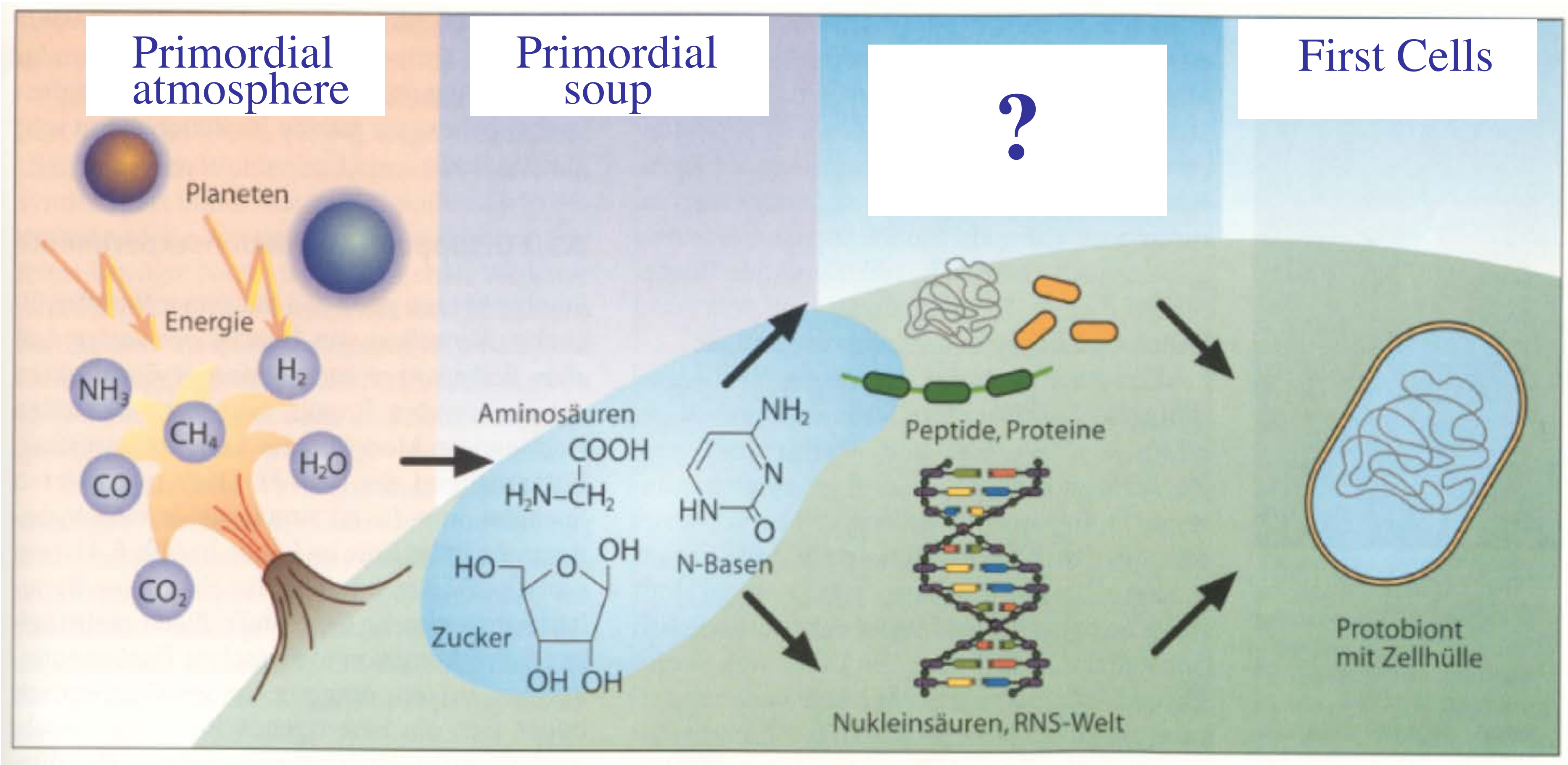
... Matter Life ...

Universal Architecture of All Cells

All present-day cells contain the same type of molecules and molecular processes ->
All cells arose from a common ancestor



Origin of Life



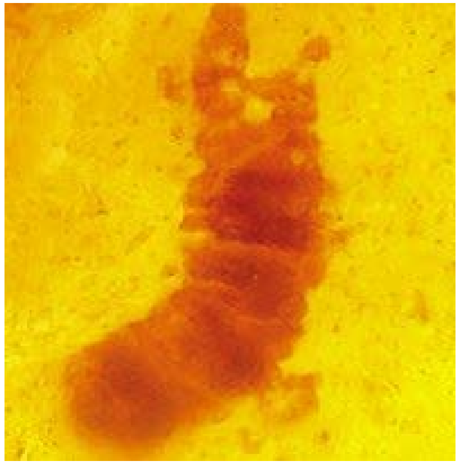
Rough Time Schedule

- 4.6 Ga Solid earth

- 4.0 Ga Large lunar craters

- 3.8 Ga Sedimentary rock -> Water

- 3.5 Ga First microfossils resembling
  cyanobacteria = procaryotes?



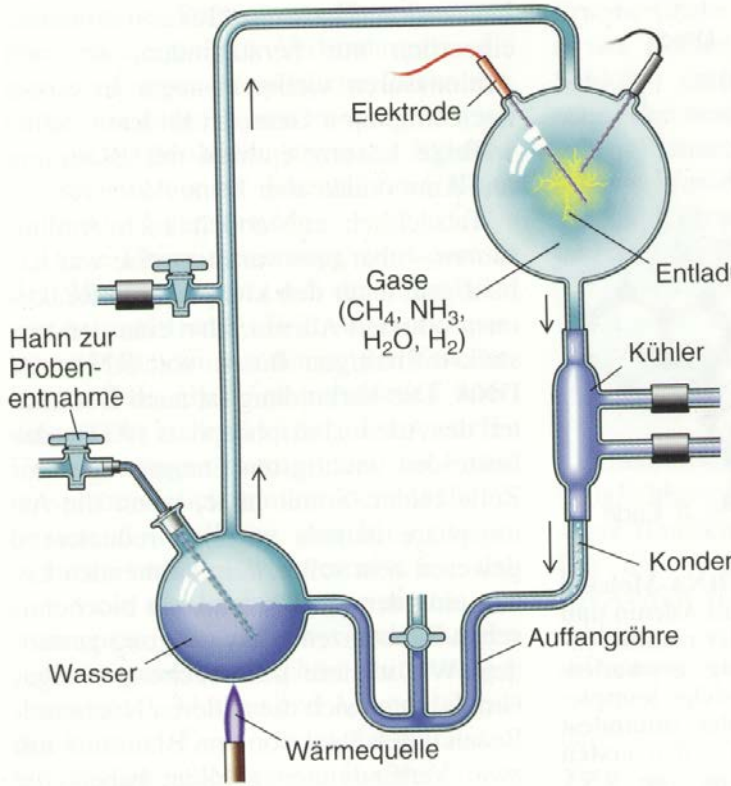
- 1.8 Ga First microfossils resembling
  red algae = eukaryotes?

- ?.? Ga Invention of meiosis and sex

- 0.6 Ga Multicellular organisms

Prebiotic Synthesis of Amino Acids

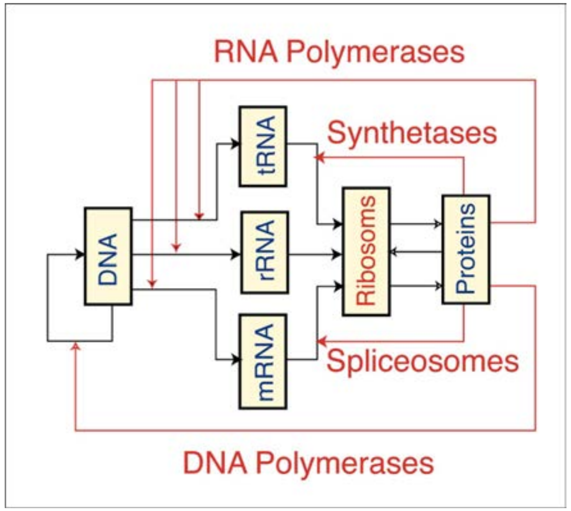
- Miller and Urey Experiment (1953)

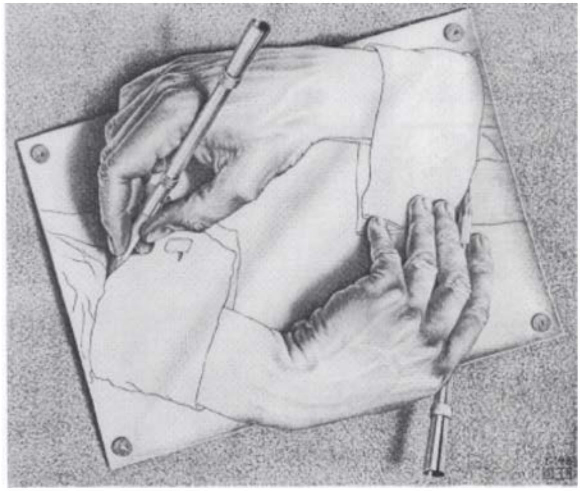


Aminosäure	Murchison-Meteorit	künstliche Ursuppe
Glycin	• • • •	• • • •
Alanin	• • • •	• • • •
α-Amino-n-Buttersäure	• • •	• • • •
α-Aminoisobuttersäure	• • • •	• •
Valin	• • •	• •
Norvalin	• • •	• • •
Isovalin	• •	• •
Prolin	• • •	•
Picolinsäure	•	◖
Asparaginsäure	• • •	• • •
Glutaminsäure	• • •	• •
β-Alanin	• •	• •
β-Amino-n-Buttersäure	•	•
β-Aminoisobuttersäure	•	•
γ-Aminobuttersäure	•	• •
Sarkosin	• •	• • •
n-Ethylglycin	• •	• • •
n-Methylalanin	• •	• •

Puzzle: Proteins + Nucleic Acids

- "Entanglement" of proteins and nucleic acids :



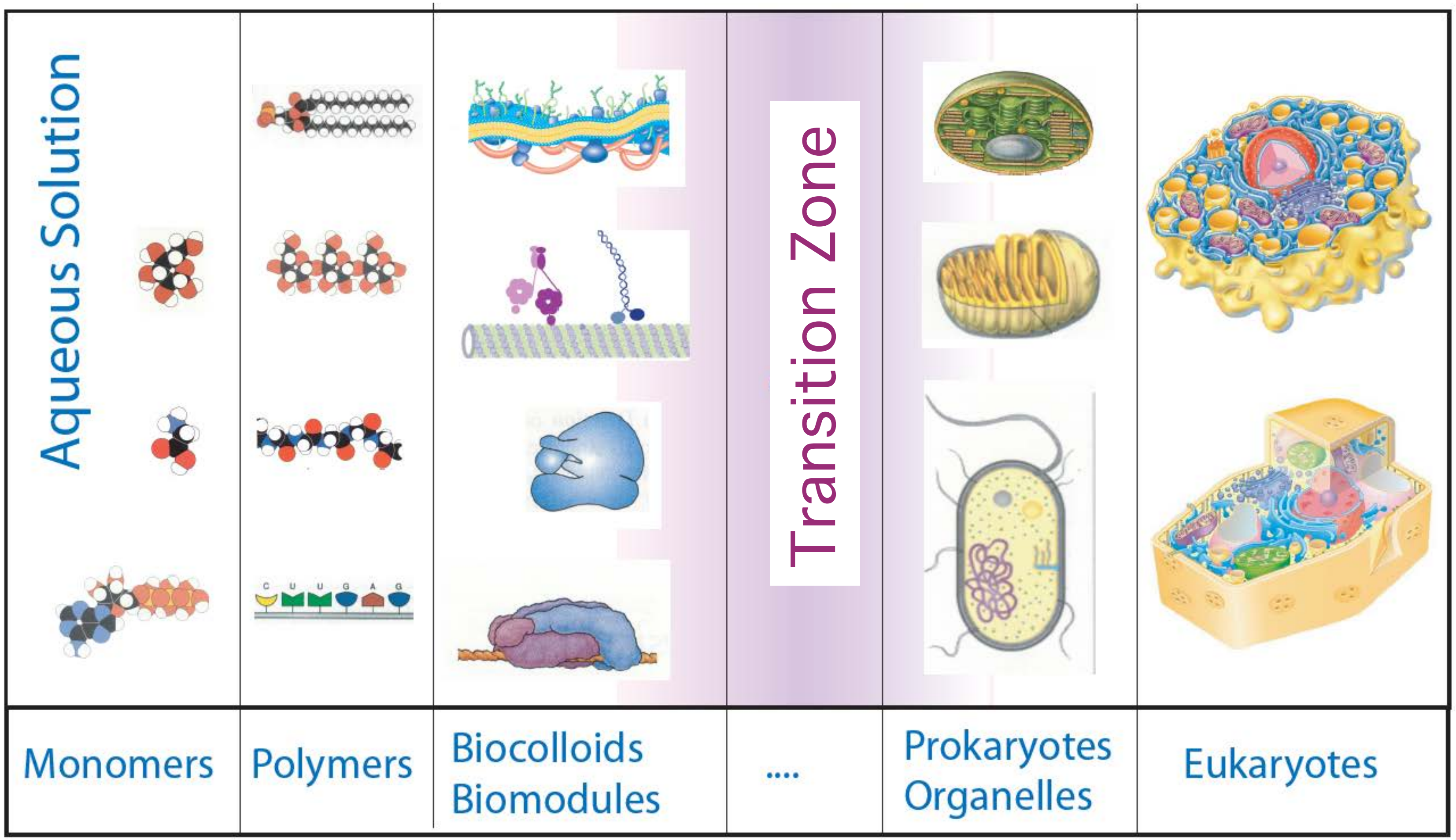


DNA contains blueprints of proteins
Proteins perform replication, transcription of DNA

- Egg and hen problem, Eigen's paradoxon
- Common ancestor arose from prebiotic evolution:
  RNA world, iron-sulphur world, clay world ... ?

Approaching the Transition Zone

Bottom-Up -> <- Top-Down



Bottom-Up versus Top-Down

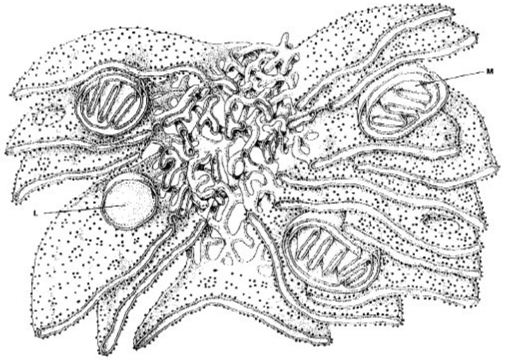
Bottom-Up: Synthetic Cells

- Develop important building blocks or modules
- Assemble these modules into larger structures
- Integrate more and more modules ...

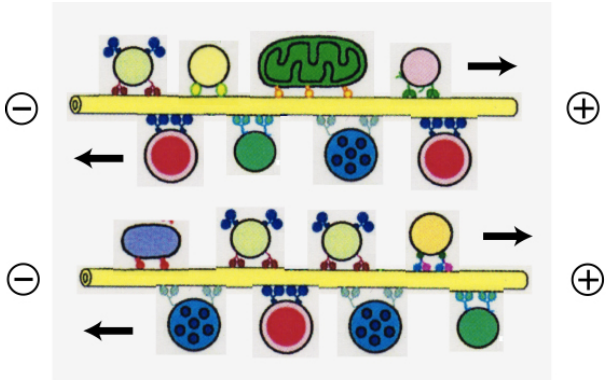
Top-Down: Minimal Cells

- Start with relatively simple cells
- Eliminate more and more components
- Problem: many remaining genes with unknown functions

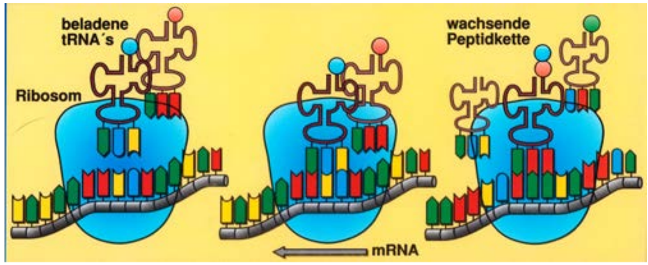
Three Basic Modules



- Membrane compartments, fluid architecture



- Molecular motors, free energy transduction

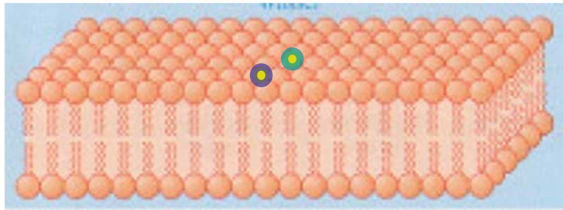


- Molecular assembly, ribosomes, protein synthesis

Membrane Fluidity

- Fluid membranes, i.e., fast lateral diffusion:

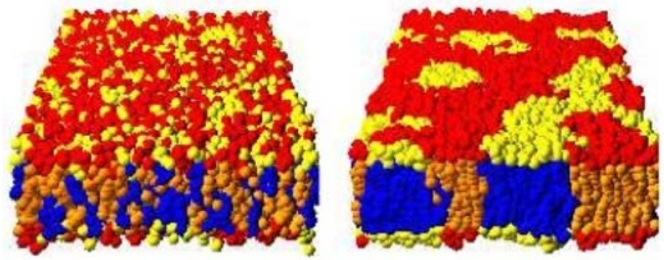
  Diffusion constant ~ μm^2/s



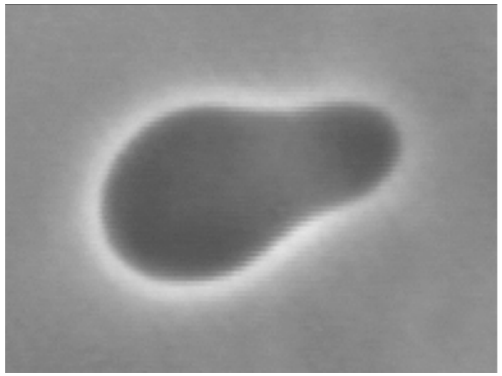
4 nm

lipid swapping ~ ns

- Lateral diffusion => Compositional responses, demixing, domain formation ...



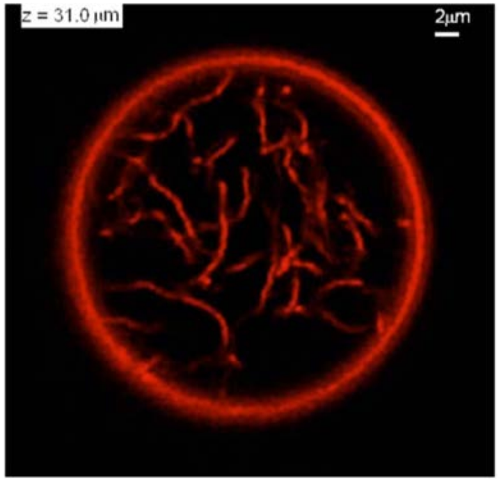
- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity



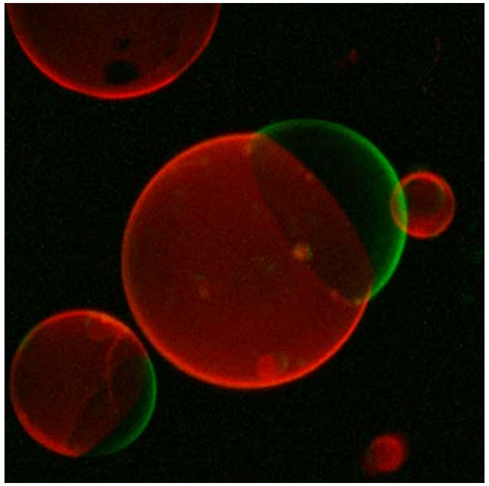
40 μm

Multiresponsive Behavior

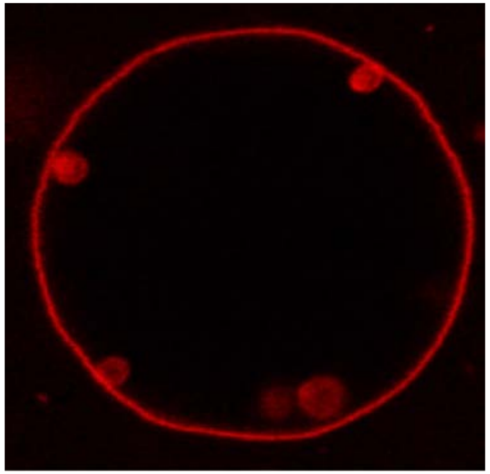
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



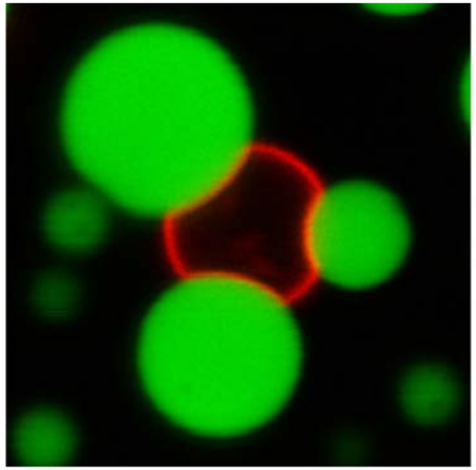
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



Small buds from protein adsorption, bud size ~ µm

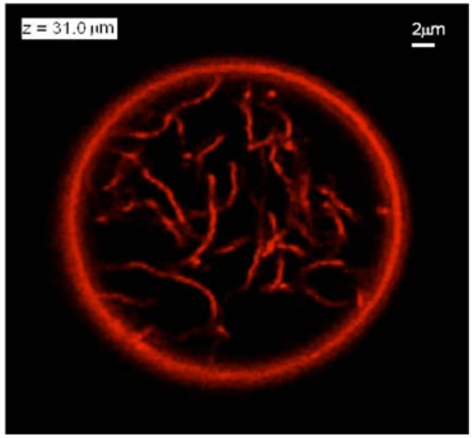


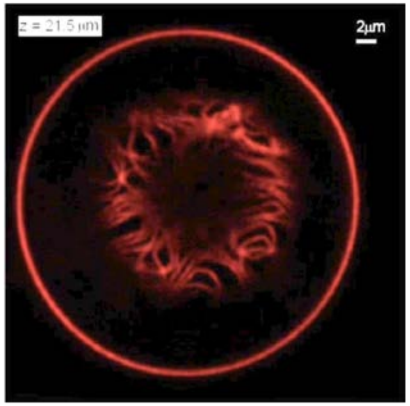
Remodelling by adhering or partially wetting droplets

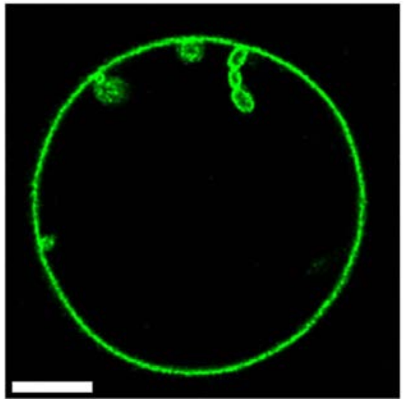
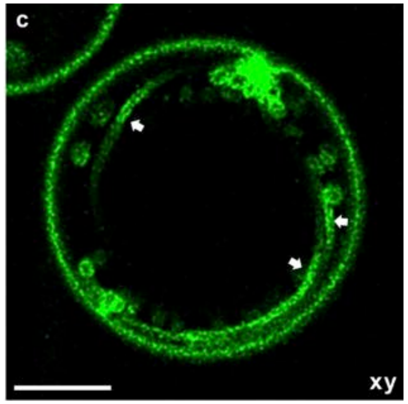
Buds and Nanotubes

Liu et al, *ACS Nano* (2016)

- Lipid mixture of DOPC, DPPC, cholesterol
- Membranes labeled by fluorescent dyes
- Liquid-disordered (red) and liquid-ordered phase (green)



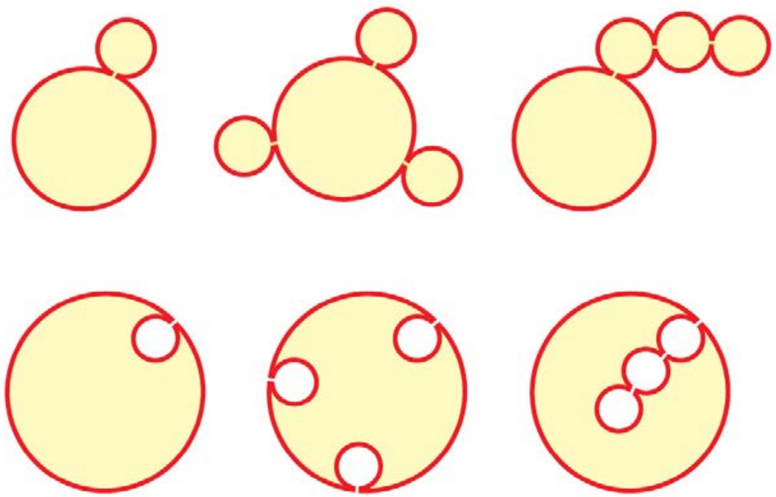


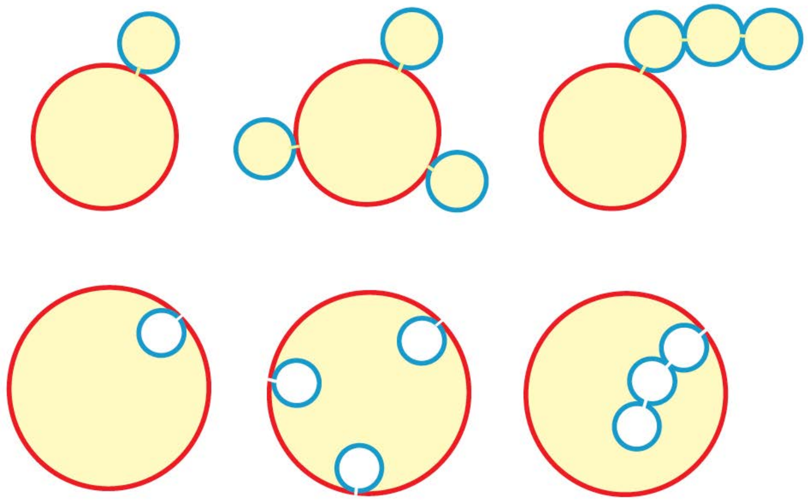
- Asymmetric environment, different PEG concentrations
- Deflation: Bud and tube formation without external forces
- Tubes can be necklace-like or cylindrical

Multi-Compartments from Curv Elasticity

- Buds and necklaces, uniform membranes:



- Buds and necklaces, multi-domain membranes:

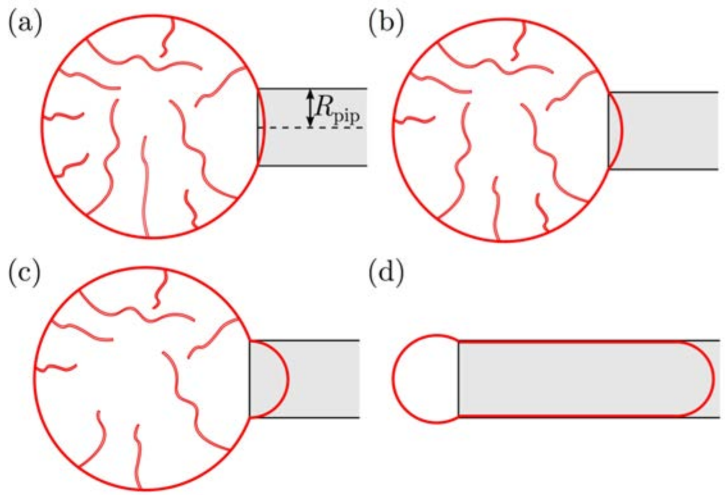


- Each shape = single membrane with membrane necks
- Each shape is stable for large parameter regime
- Key parameters: membrane area, vesicle volume, spontaneous (or preferred) curvature

Spontaneous Tension

RL, *Faraday Discuss*. (2013) Bhatia et al (under review)

- Spont curvature m generates spon tension $\sigma = 2\,\kappa\, m^2$
- Micropipette aspiration of tubulated vesicle:



Initial aspiration up to hemispherical tongue,

then vesicle starts to flow like a liquid droplet

- Vesicle behaves as liquid droplet with interfacial tension equal to spontaneous tension σ

ESCRT-Induced Budding

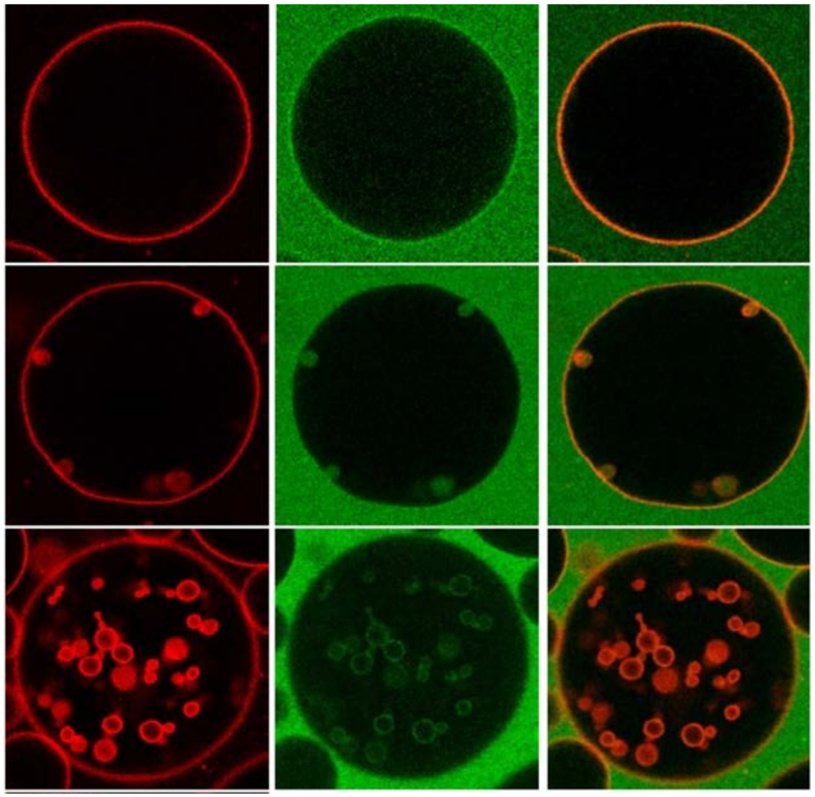
Avalos Padilla et al, unpublished

- Sequential addition of three ESCRT proteins to GUVs:

+ ESCRT 1 Vps20 — adsorption of Vps20

+ ESCRT 2 Vps32 — multiple buds + necks

+ ESCRT 3 Vps24 — fission of necks

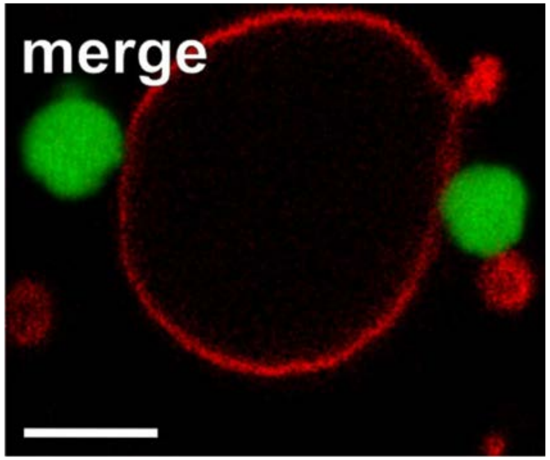


- Interpretation: Domain-induced budding

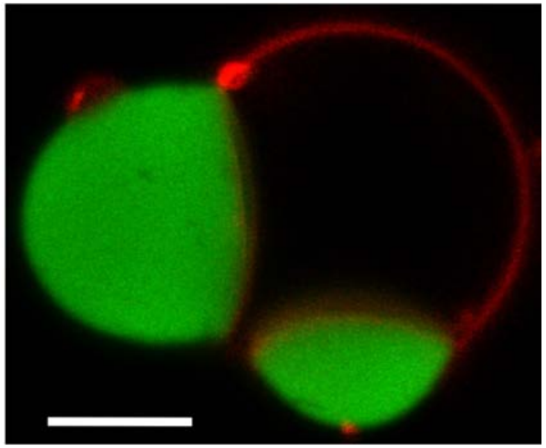
Protein-rich Droplets

Brangwynne ... Hyman, *Science* (2009)

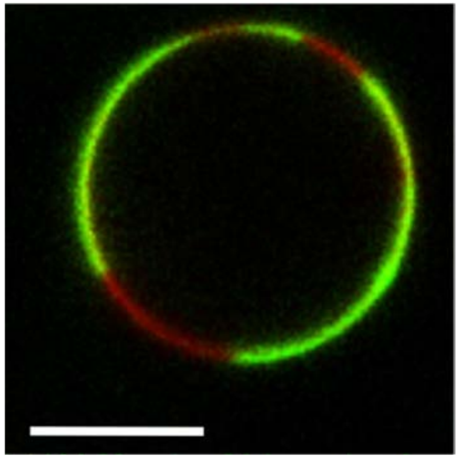
- Membrane-less organelles that behave like liquid droplets
- Enriched in intrinsically disordered proteins (IDPs)
- Example for IDP: RNA-binding protein FUS
- Interaction of FUS-droplets with membranes, two subsequent wetting transitions:



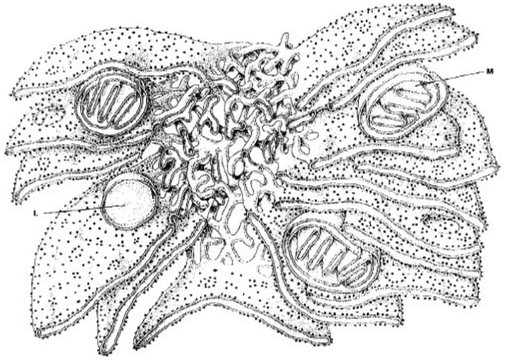
dewetting for high salt



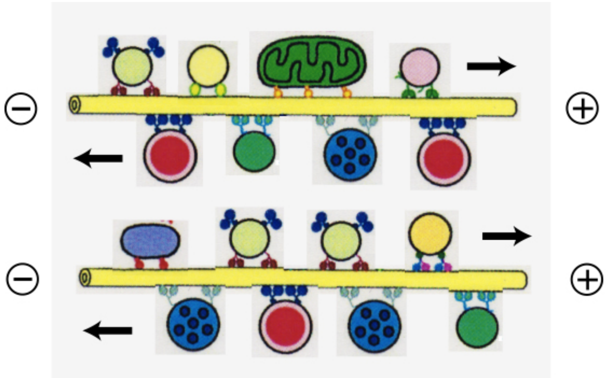
partial wetting for intermediate salt



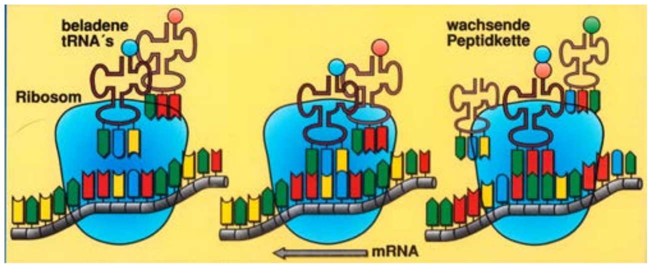
complete wetting for low salt



- Membrane compartments, fluid architecture

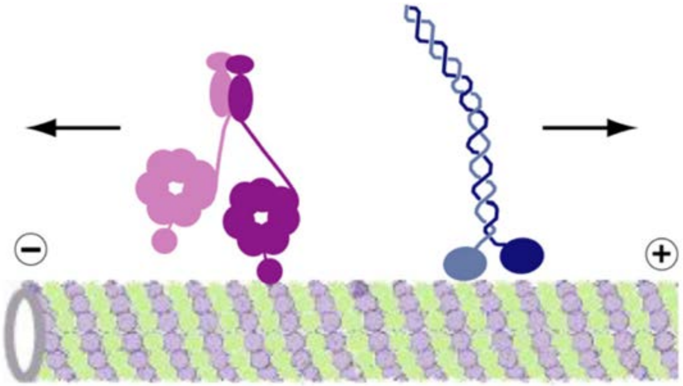


- Molecular motors, free energy transduction

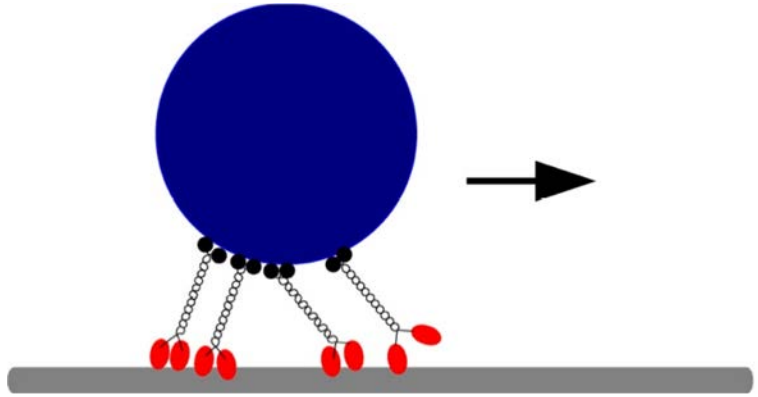


- Molecular assembly, ribosomes, protein synthesis

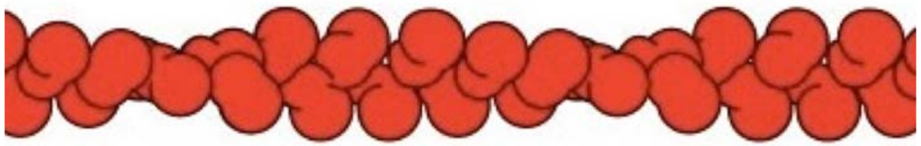
Biomolecular Machines



• Intro: Stepping motors



• Transport: Motor teams

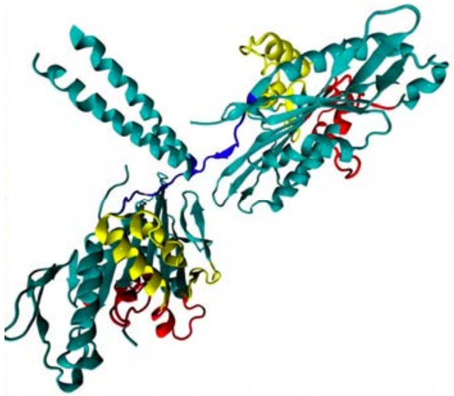


• Structural remodelling: Actin filaments

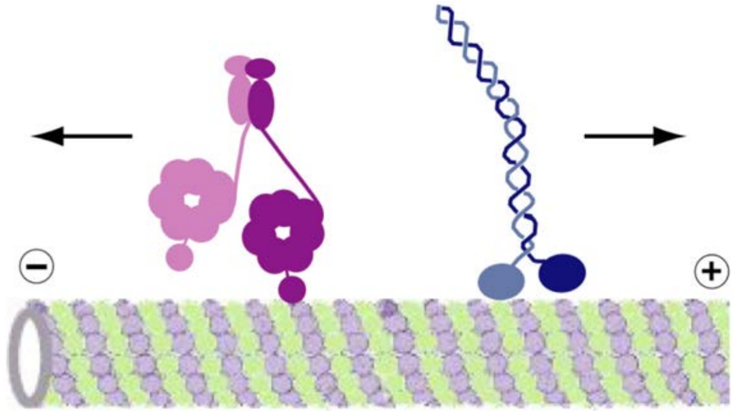
• Information processing: Ribosomes

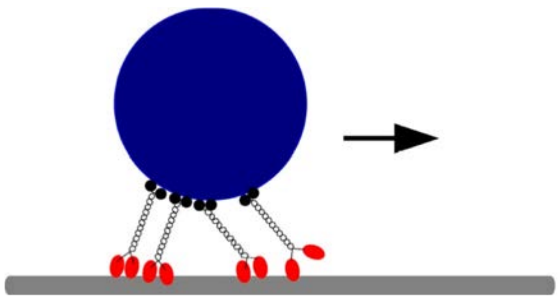
Multiscale Aspects of Mol Motors

• ATP hydrolysis ~ 1 nm



• Mechanical steps ~ 10 nm





• Cargo transport by motor teams ~ 100 μm

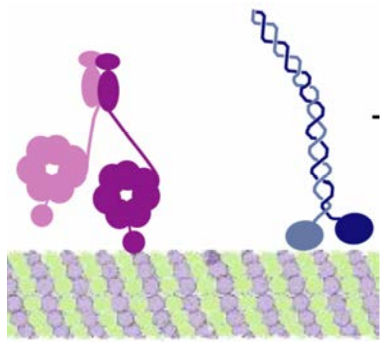
•Traffic of many motors/cargos and phase transitions

Nanoscale: Mechano-Enzymes

- Motor action based on ATP hydrolysis
- Motor = ATPase with several catalytic domains

  M = # catalytic domains ≤ # ATP binding sites

- Examples:



Kinesin: M = 2

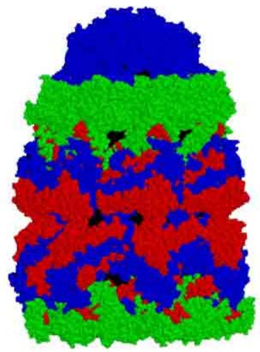
Myosin V: M = 2

Dynein: M = 2 - 4 ≤ 8

....

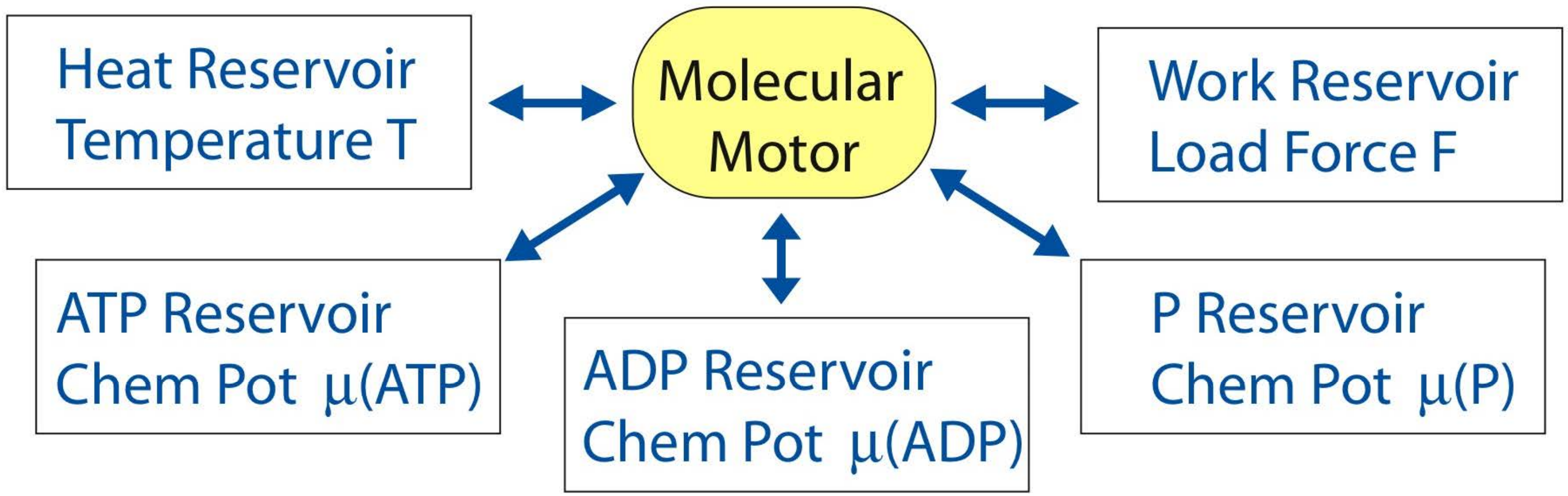
F1 ATPase: M = 3 < 6

GroEl : M = 7 < 14



Mesoscale: Thermodynamics

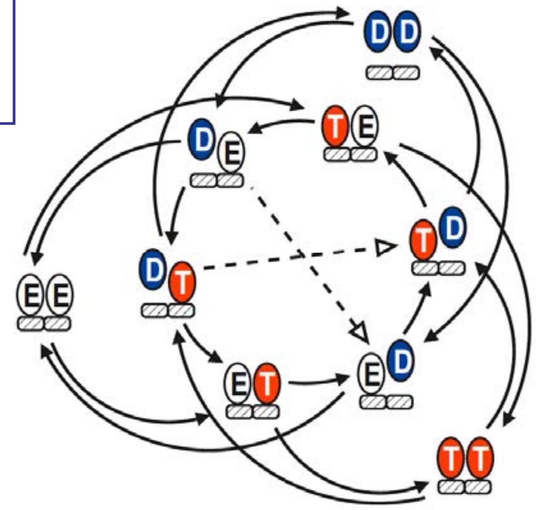
- Motor molecule coupled to several reservoirs:



- Isothermal motor activity at fixed temperature T
- Chemical energy change $\Delta\mu = \mu(ATP) - \mu(ADP) - \mu(P)$
- Mechanical work $W_{me} = \ell F$ during spatial displacement ℓ

State Space of Motor

RL et al: J. Stat. Phys 135 (2009)



- Motor states *i* and *j*
- Transition |ij> from *i* to *j* with rate ω_{ij}
- Energy change during |ij> arising from chemical potential difference $\Delta\mu_{ij}$ and mechanical work W_{ij} :

$$U_j - U_i = \Delta\mu_{ij} - Q_{ij} - W_{ij} \qquad \text{(first law of TD)}$$

- Free energy change from constrained equilibrium:

$$H_j - H_i = \Delta\mu_{ij} - W_{ij} - k_B T \ln (\omega_{ij} / \omega_{ji})$$

- Entropy change from thermodynamic relation:

$$S_j - S_i = k_B \ln (\omega_{ij} / \omega_{ji}) - Q_{ij} / T = \Phi_{ij} - Q_{ij} / T$$

Cyclic Balance Conditions

- Summation of transitions along a complete directed cycle C_ν^d, all state functions cancel

- Released heat: $Q(C_\nu^d) = \Sigma\ Q_{ij} = \Delta\mu(C_\nu^d) - W(C_\nu^d)$

- Produced entropy I: $T\,\Phi(C_\nu^d) = \Sigma\ T\,\Phi_{ij} = Q(C_\nu^d)$

- Produced entropy II: $T\,\Phi(C_\nu^d) = k_B T\ \ln(\Xi_\nu^d)$

with $\Xi_\nu^d = \prod_{|ij>}^{\nu,d} (\omega_{ij} / \omega_{ji})$

$$k_B T\ \ln(\Xi_\nu^d) = \mu(C_\nu^d) - W(C_\nu^d) = Q(C_\nu^d)$$

Relation between kinetics and thermodynamics, must be fulfilled for thermodynamic consistency

Classification of Cycles

- Balance condition for each directed cycle C_ν^d :

$$k_B T \ln(\Xi_\nu^d) = \mu(C_\nu^d) - W(C_\nu^d)$$

Classification of cycles:

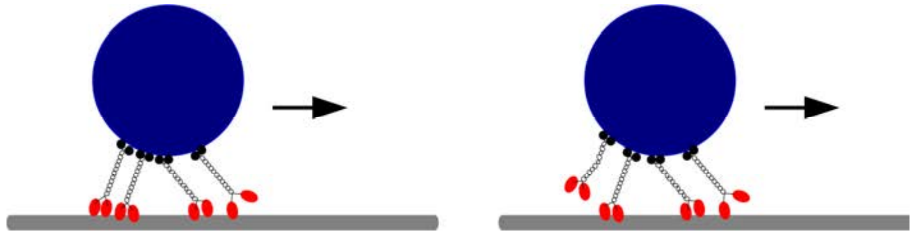
- Detailed balance: $\mu(C_\nu^d) = 0$ and $W(C_\nu^d) = 0$
- Mech nonequilibrium: $\mu(C_\nu^d) = 0$ and $W(C_\nu^d) \neq 0$
- Chem nonequilibium: $\mu(C_\nu^d) \neq 0$ and $W(C_\nu^d) = 0$
- Chemomech coupling: $\mu(C_\nu^d) \neq 0$ and $W(C_\nu^d) \neq 0$

RL, Liepelt: *J. Stat. Phys.* ***130*** (2008)

Cargo Transport by Motor Teams

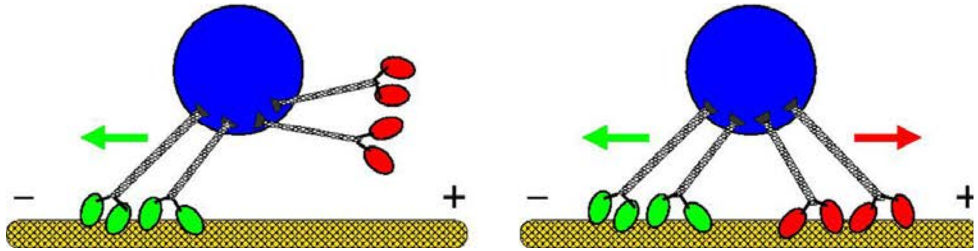
- Transport by N identical motors

  Klumpp and RL, *PNAS* (2005)



- Transport by two antagonistic motor teams, Stochastic tug-of-war

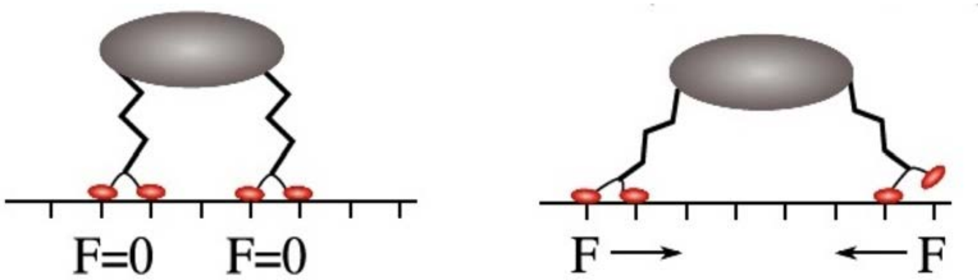
  M. Müller et al, *PNAS* (2008)



- Elastic linkers between motors and cargo

  Berger et al, *PRL* (2012)

  Ucar, RL, *Soft Matter* (2017)



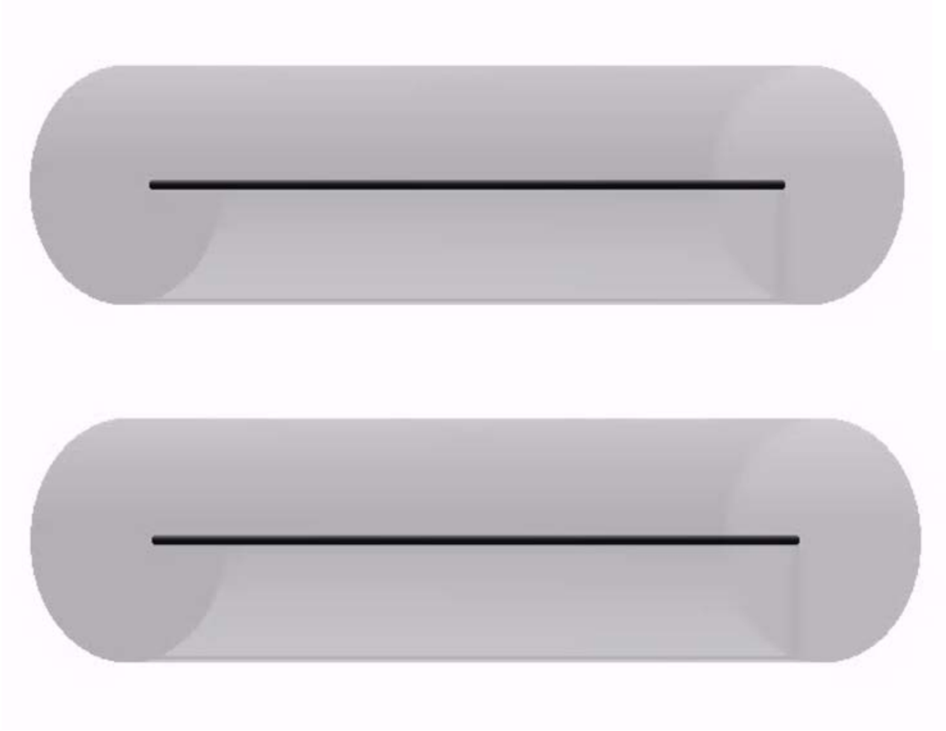
Concentration Gradients from Motors

M. Müller et al, *J. Phys. CM* 17 (2005)

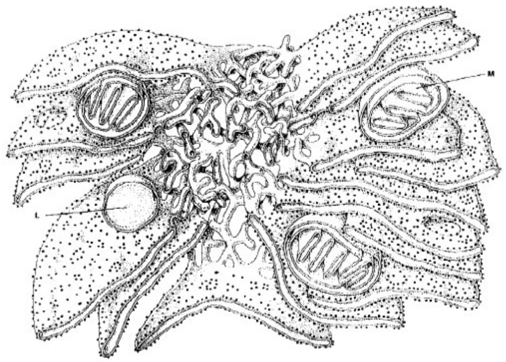
- Half open tube:
  left boundary open, reservoir of motors = 'cell body'
  right boundary closed = 'Synapse'

- (+) Motors (kinesins)
  moving to the right

- (-) Motors (dyneins)
  moving to the left



Concentration gradient created by motors



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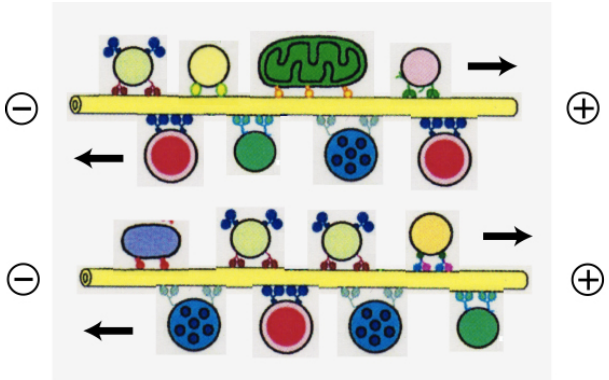

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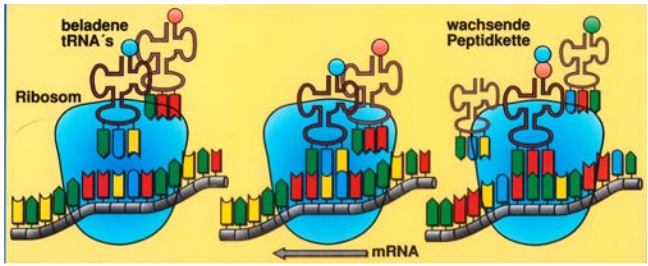
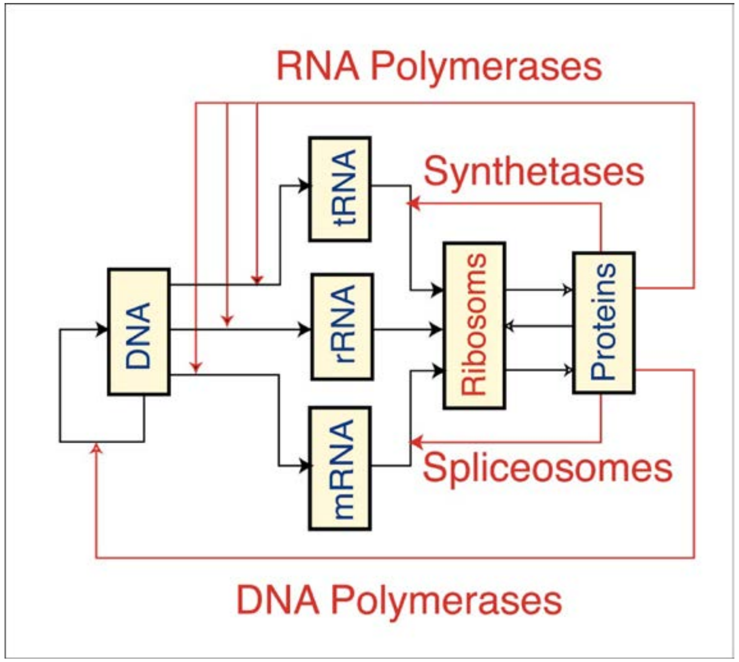
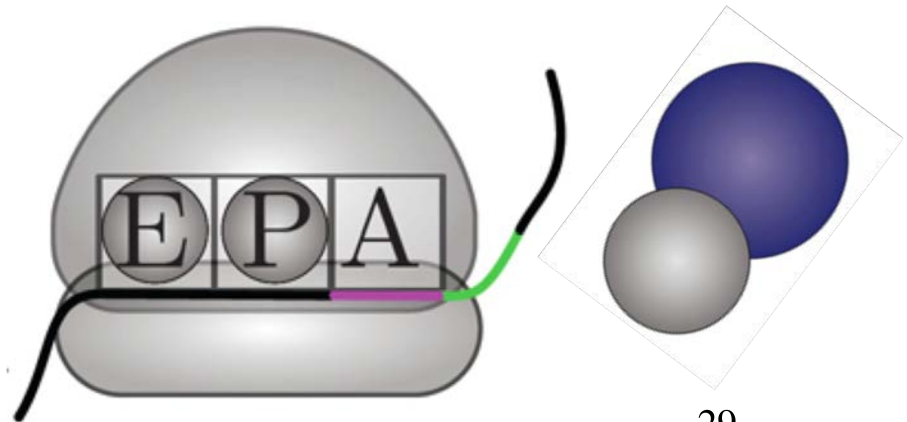


- Molecular assembly, ribosomes, protein synthesis

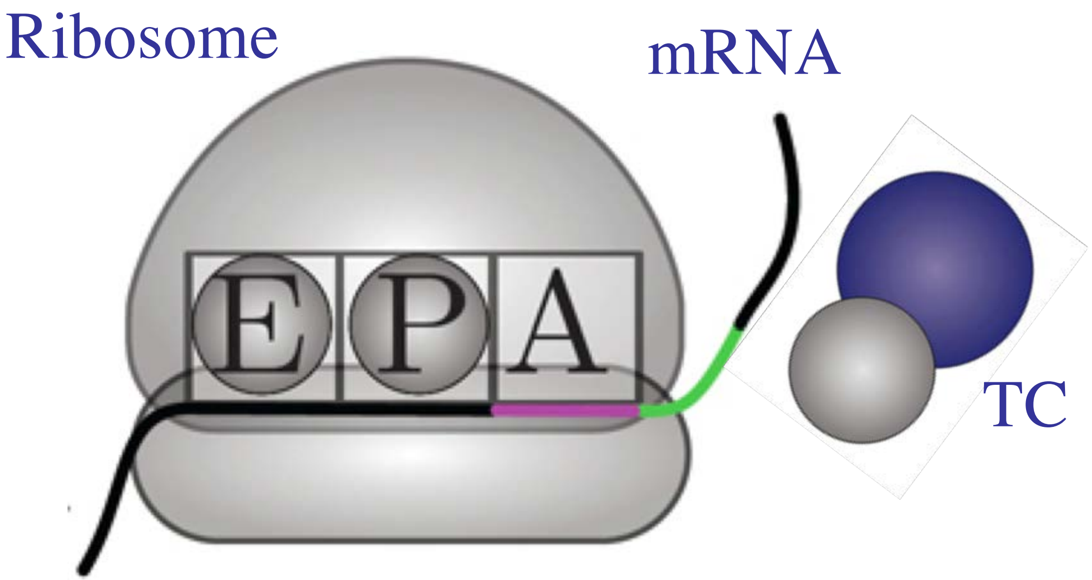
Protein Synthesis by Ribosomes

- Ribosomes are assemblies of rRNA and r-proteins
- Complex and hierarchical assembly process in vivo
- In vitro assembly from rRNA and r-proteins without additional components
- No assembler for ribosomes
- Protein synthesis requires many molecular players:





Ribosome + mRNA + tRNAs



TC = ternary complex = tRNA + EF-Tu + GTP

EF-Tu = most abundant protein

- Ribosome steps along codons of mRNA (purple -> green) consuming one ternary complex at each codon
- Elongation cycle during one step:

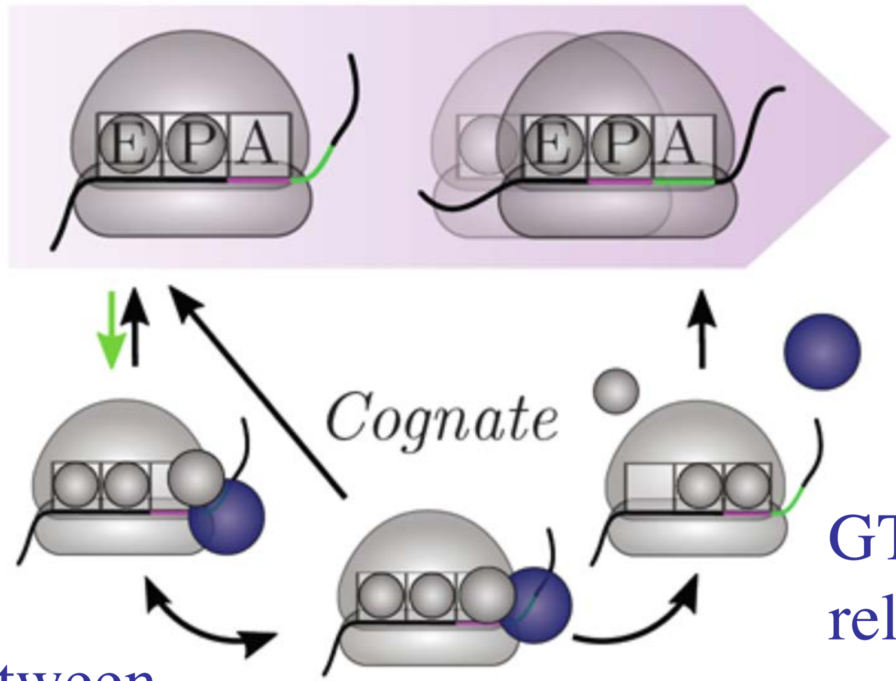
Decoding of codon by binding/accommodation of tRNA

Elongation of growing peptide chain by one amino acid

Translocation of mRNA together with two tRNAs

Single Elongation Cycle

Initial binding of ternary complex



Translocation

Recognition between codon and anti-codon

GTP hydrolysis, release of EF-Tu and E-site tRNA, peptide bond formation

- Complexity of decoding:

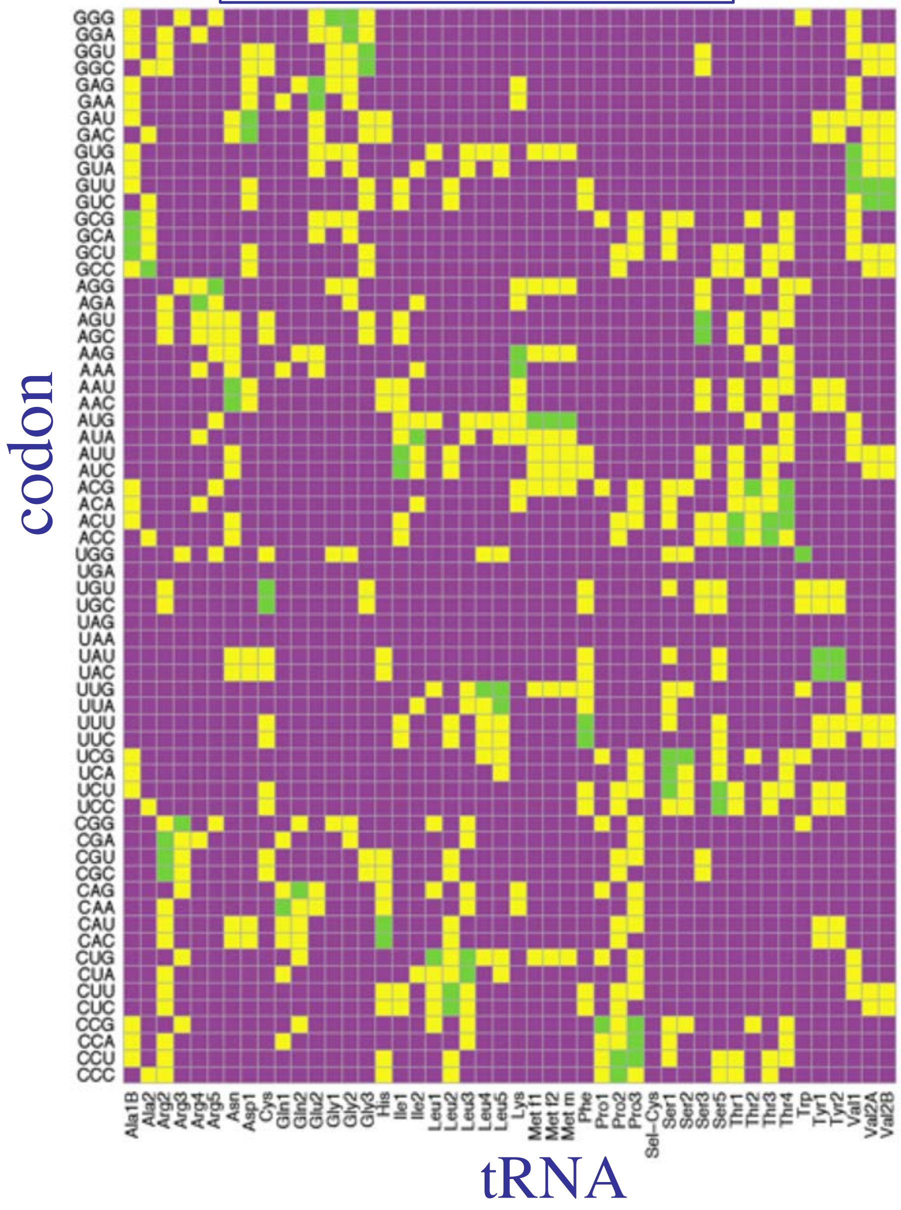
  61 sense codons and 43 elongator tRNA species (E. coli)

Codon-tRNA Relationships

- red/purple = non-cognate
  released after initial binding
- yellow = near-cognate
  decoding => wrong amino acid
- green = cognate
  decoding => correct amino acid

- ‚Ocean' of non-cognates
  with some near-cognates
  and a few cognates

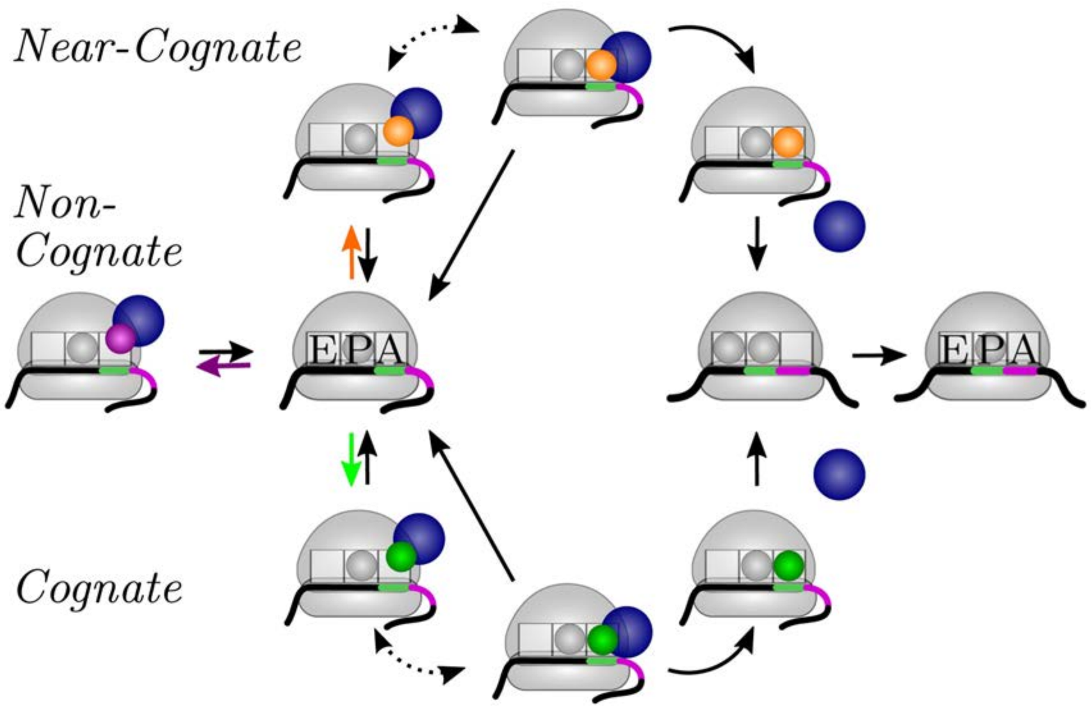
Decoding pattern



Single Elongation Cycle - Refined

Rudorf, Thommen, Rodnina, RL, *PLoS Comp Biol* (2014)

- Possible binding of cognate/near-cognate/non-cognate tRNAs:



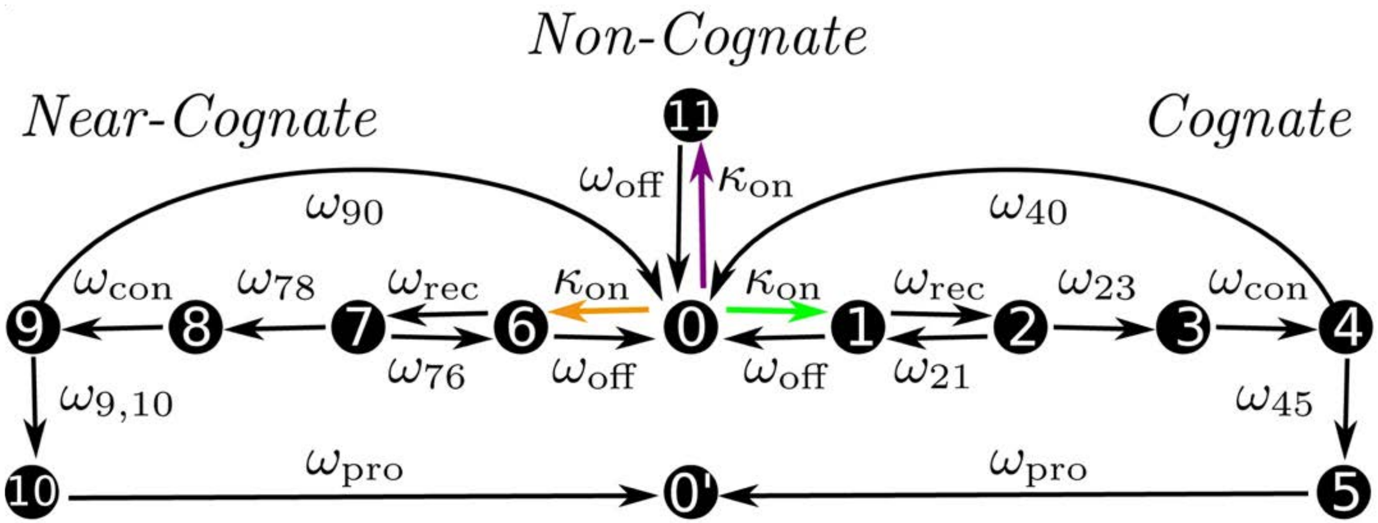
- Accommodation of near-cognate tRNA
  => error rate

- Accommodation of cognate tRNA

- Competition between cognate, near-cognate, and non-cognate tRNAs

Markov Process

- Map cartoon of multistep process onto Markov chain:



- Individual transitions:

  initial binding, recognition, initial selection, GTP hydrolysis, phosphate release, proof reading, full accommodation

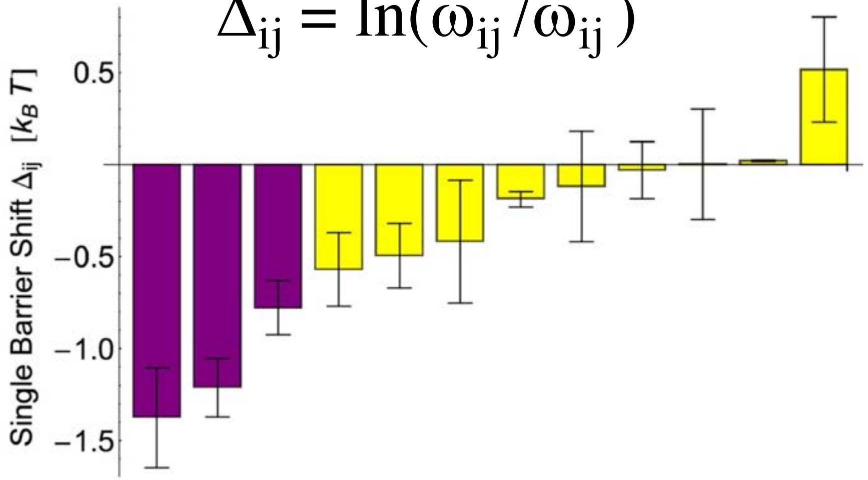
- All transition rates ω_{ij} have been measured in vitro
- Some rates identical for both cognates and near-cognates

From In-Vitro to In-Vivo Rates

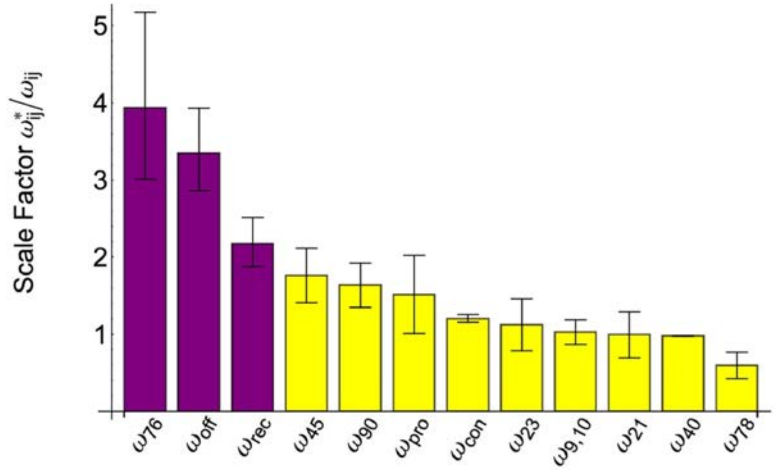
Rudorf, Thommen, Rodnina, RL, *PLoS Comp Biol* (2014)

- Single barrier shifts

$$\Delta_{ij} = \ln(\omega^*_{ij}/\omega_{ij})$$



- Scale factors $\omega^*_{ij}/\omega_{ij}$



- Three in-vivo rates (purple) are significantly increased:

  rejection rate ω_{76} for near cognates

  dissociation rate ω_{off} after initial binding

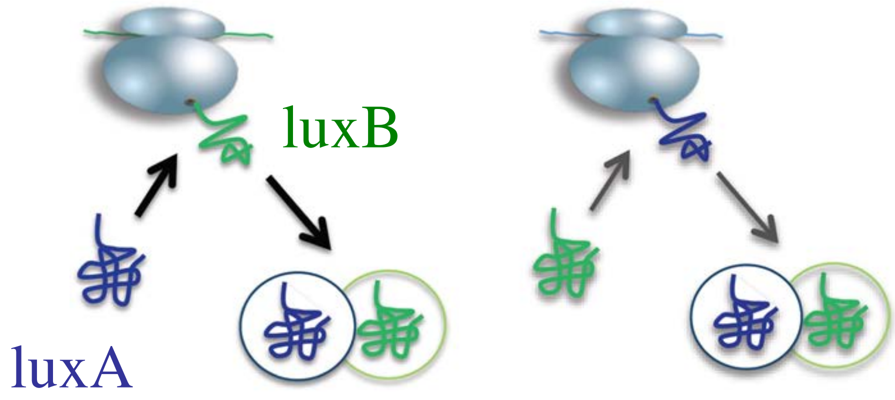
  recognition rate ω_{rec} for cognates and near-cognates

Assembly of Protein Complexes

Shieh ... Kramer, Bukau, *Science* (2015)

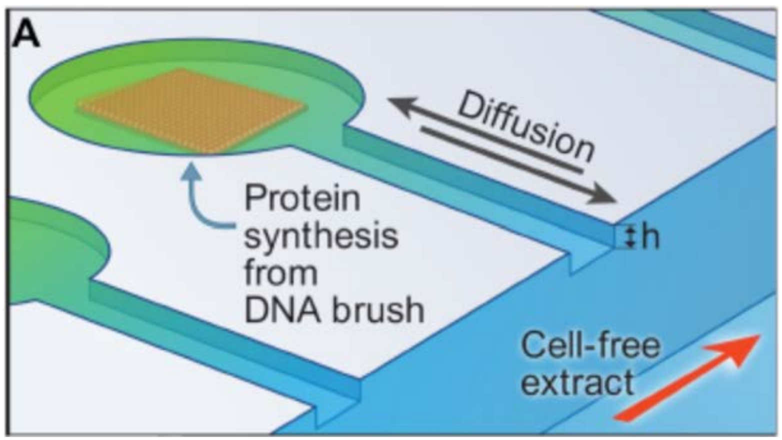
- Co-translational assembly:

  in vivo synthesis of two proteins,
  assembly during translation,
  luxA binds to emerging luxB



- Protein synthesis on a chip:

  different DNA compartments
  for different proteins,
  control of spatial separation
  between different compartments



Karzbrun ... Bar-Ziv, *Science* (2014)

New Platform for Bottom-Up Assembly

Weiss ... Spatz, Nature Materials (Nov. 2017) #

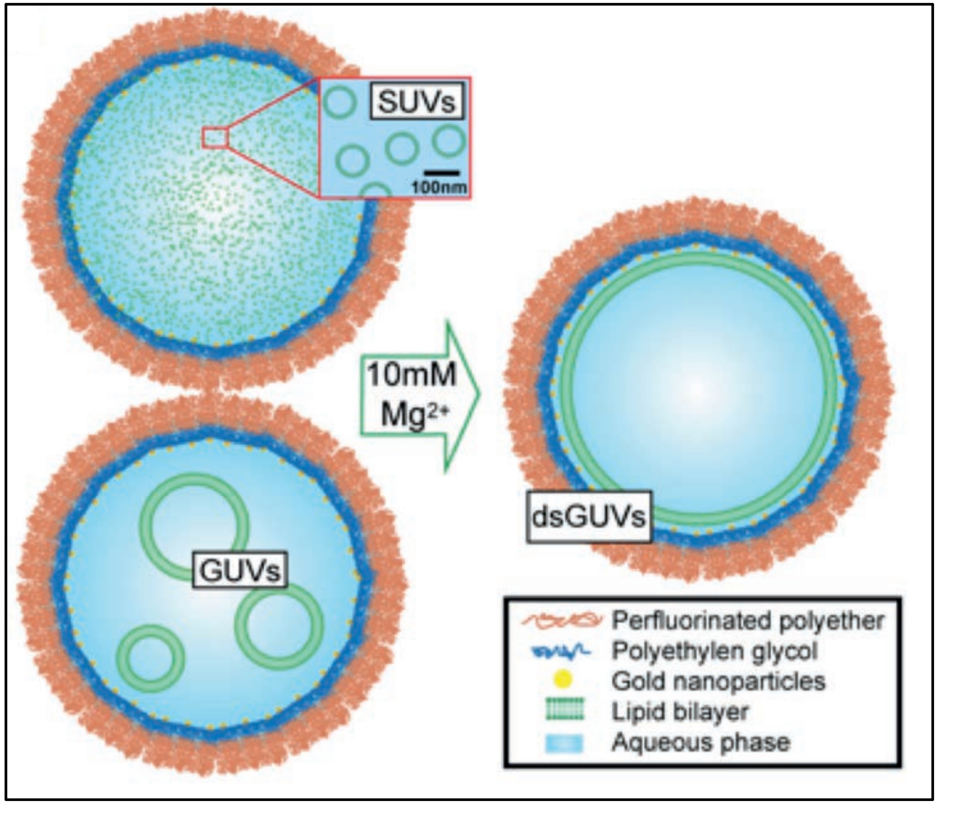
- Collaboration within MaxSynBio
- Project leader: Joachim Spatz, Heidelberg
- Collaboration between 4 MPIs and 3 Universities

- Water-in-oil emulsion droplets
- Generated by microfluidics, stabilized by surfactant

List of coauthors:
MarianWeiss, Johannes Patrick Frohnmayer, Lucia Theresa Benk,
Barbara Haller, Jan-Willi Janiesch, Thomas Heitkamp, Michael Börsch,
Rafael B. Lira, Rumiana Dimova, Reinhard Lipowsky,
Eberhard Bodenschatz, Jean-Christophe Baret, Tanja Vidakovic-Koch,
Kai Sundmacher, Ilia Platzman and Joachim P. Spatz

GUVs within W/O Emulsion Droplets

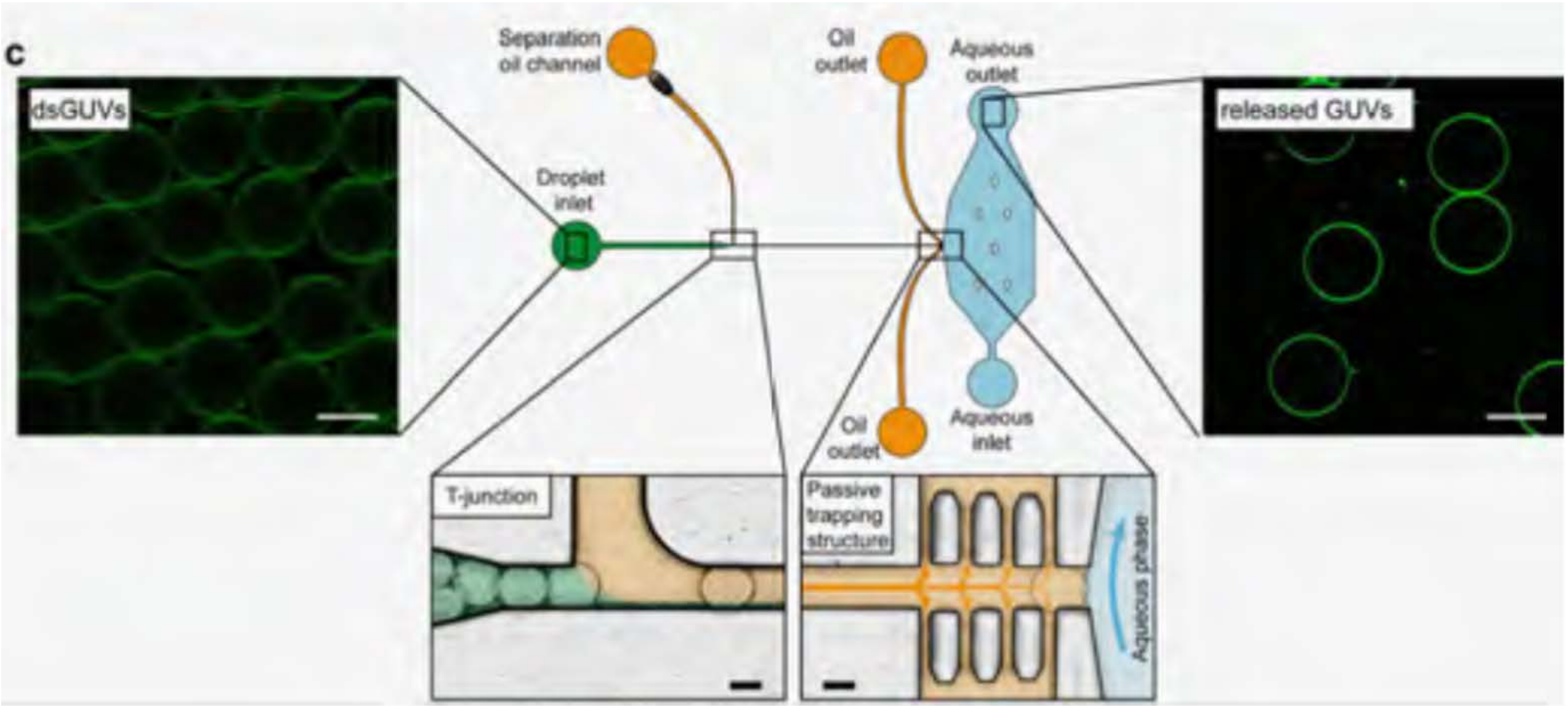
- Emulsion w/o droplet stabilized by surfactant
- Pico-Injection of small vesicles
- Pico-Injection of Mg^{++}
- Adhesion of vesicles to surfactant layer
- Rupture of vesicles
- Fusion of fragments
  => Formation of a GUV supported by surfactant layer



- Release of encaged GUV from droplet

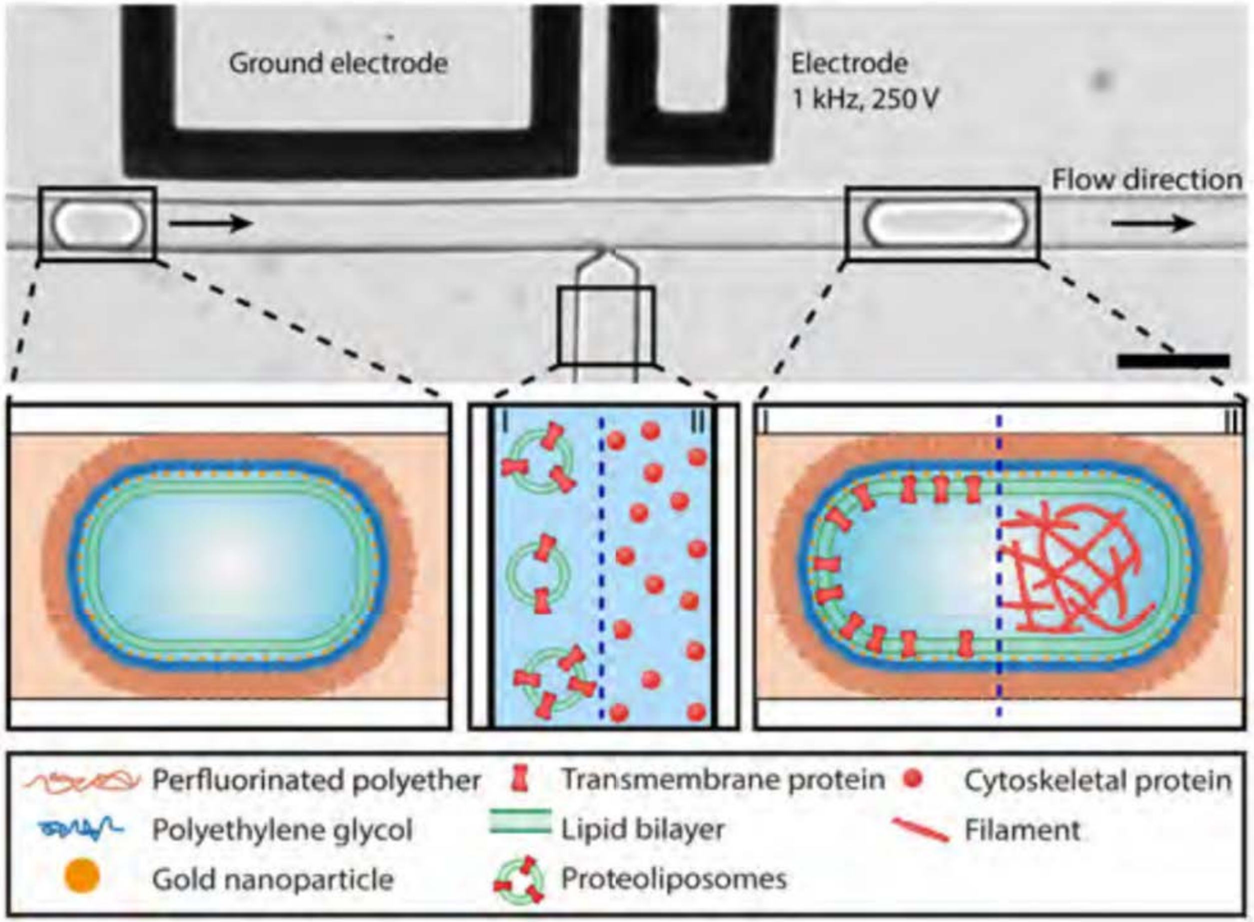
Release of GUVs from Droplets

Weiss ... Spatz, Nature Materials (Nov. 2017)



- General platform for subsequent bottom-up assembly

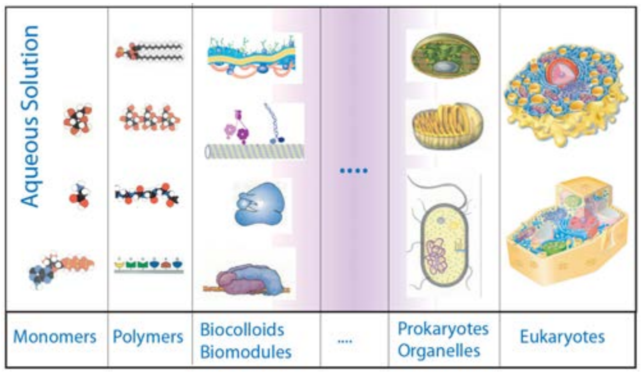
Sequential Bottom-Up Assembly



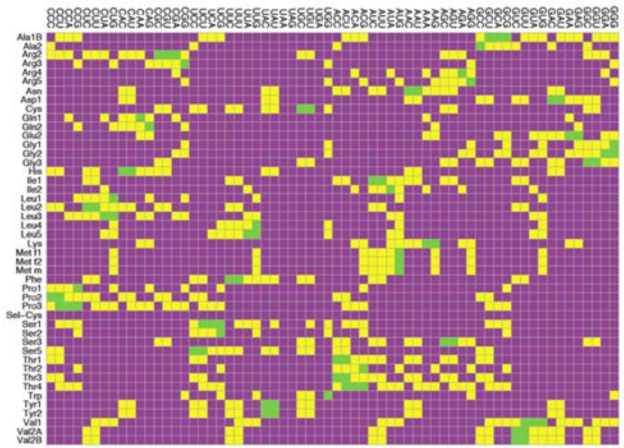
- Pico-injection of membrane and cytoskeletal proteins
- Incorporation of functional ATP Synthase

Perspectives and Challenges

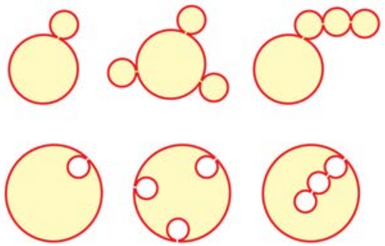
- Further steps of sequential assembly:
  Compartments + ATP Synthase + motors + ...
- Importance of ionic conditions
- ‚Broken hierarchy‘ of biolevels
- Wanted and unwanted interactions



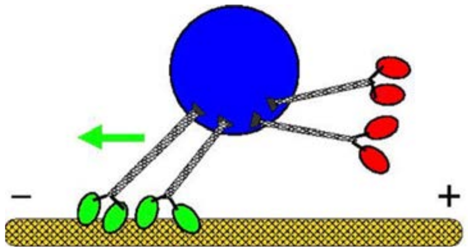
- Evolution via selection (failures)
- Evolution as a learning process
- Natural cells after 10^8 years ?
- Synthetic cells after xx years ?
- Can science replace evolution?



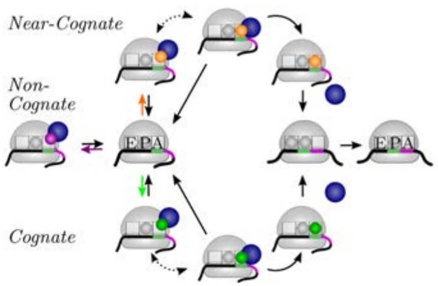
Summary



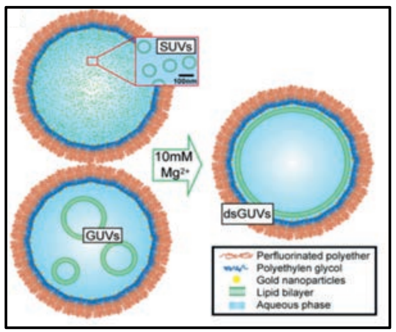
- Membrane compartments, multiresponsive, many architectures



- Molecular motors, cargo transport and concentr gradients



- Protein synthesis, comparison of in vivo and in vitro



- Droplet-stabilized GUVs, new platform for sequential assembly

Coworkers

- Membranes

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  Tom Robinson
  Jaime Agudo-C.
  Tripta Bhatia
  Yunuen Avalos Padillo
  Jan Steinkühler

- Motors + Ribosomes

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  Sophia Rudorf
  Mehmet Ucar
  Stefanie Foerste
  Nadin Haase
  Simon Christ

- Collaborations

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  Joachim Spatz
  Tony Hyman
  Titus Franzmann
  Günther Kramer
  Roy Bar-Ziv