From Matter to Life: Build Your Own Cell

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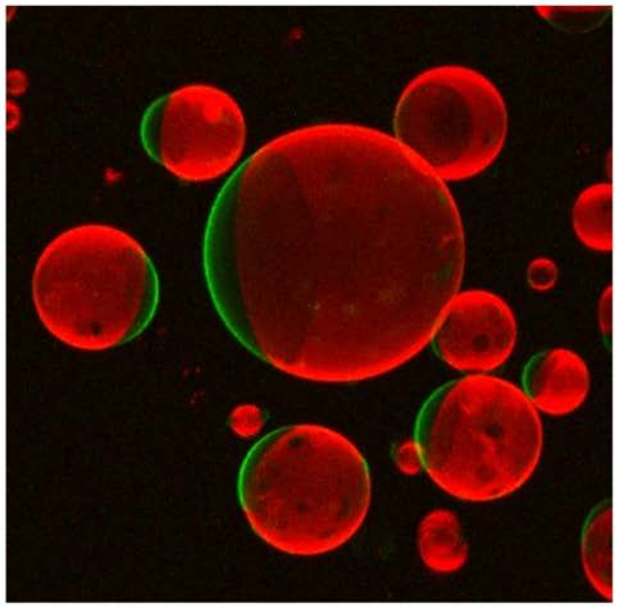
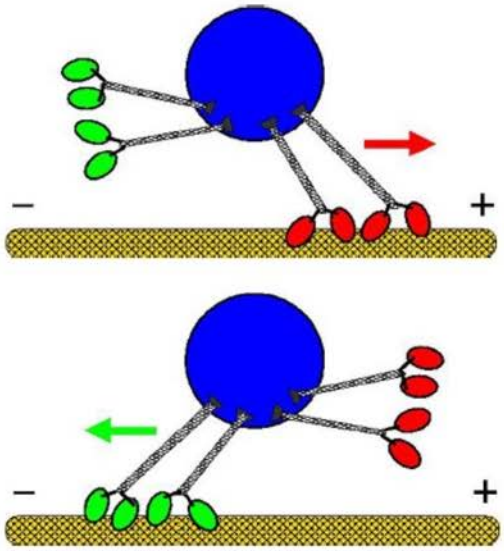
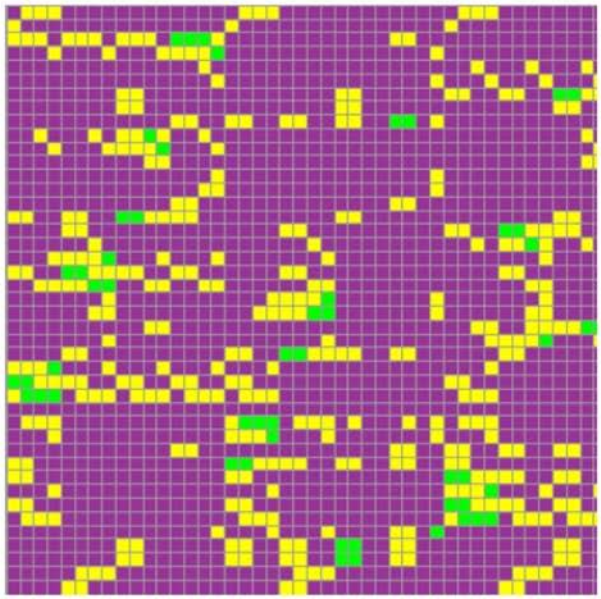
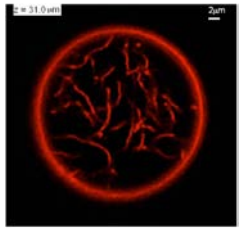


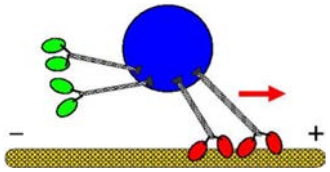


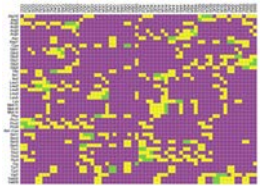


From Matter to Life: Overview

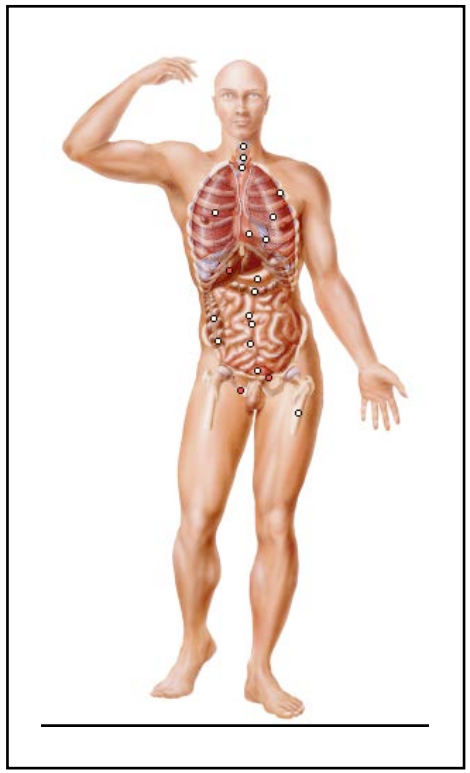
- Introduction to Biosystems
- Three Basic Modules:
  - ➢ Membrane Compartments
  - ➢ Molecular Motors
  - ➢ Protein Synthesis by Ribosomes
- Bottom-Up Assembly of Synthetic Cells
- Perspectives and Challenges



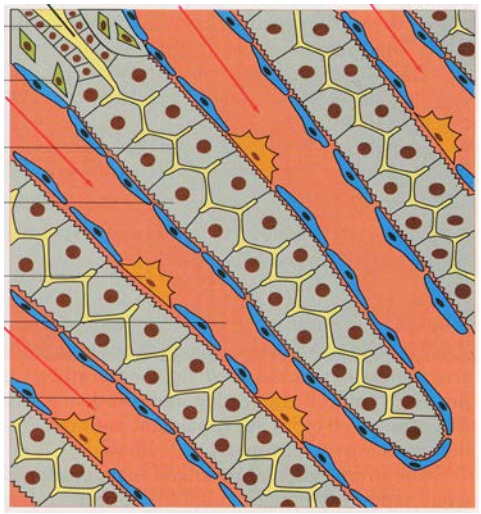




Biosystems: Top-Down

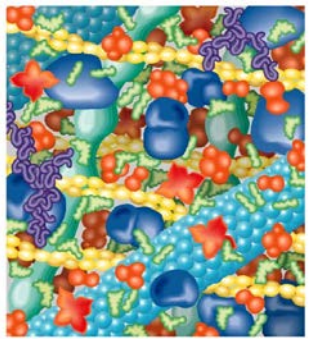


Human body
[m]



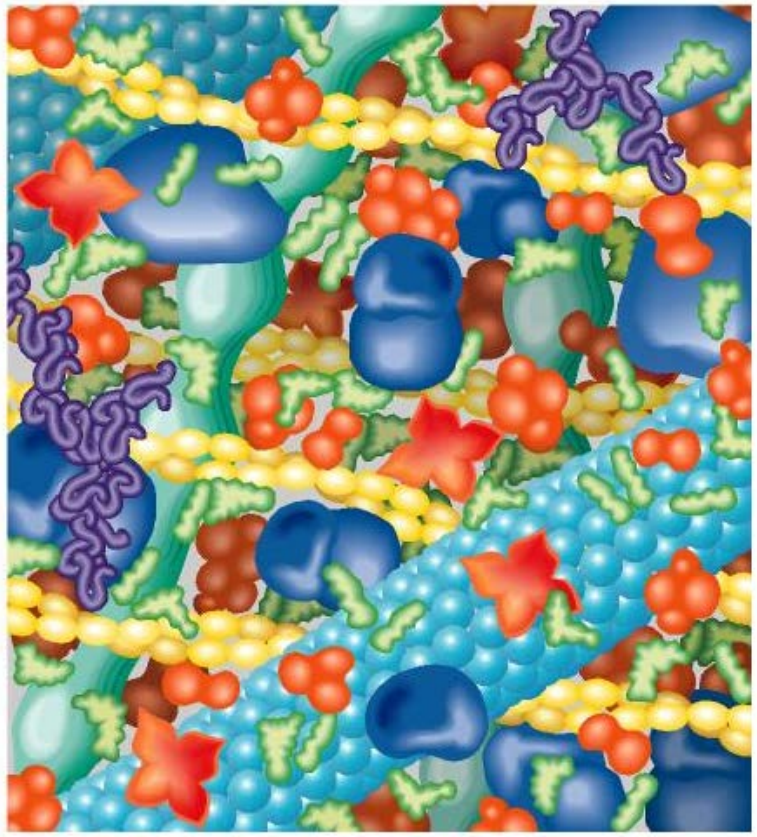
Tissues
[mm]

Cells
[μm]



Molecular
Assemblies
[nm]

Universal Nanostructures



25 nm

Bsp: Filament

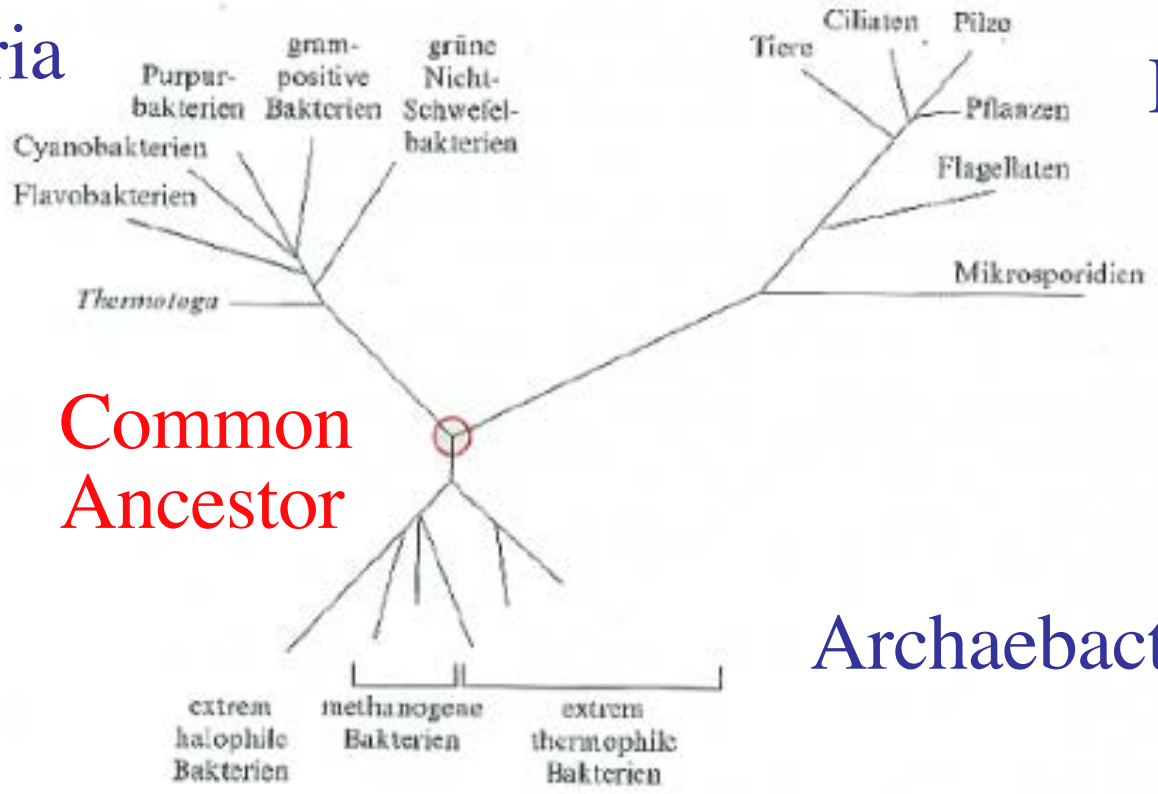
- Water and ions
- Small molecules (monomers) form macromolecules such as
  - Proteins
  - RNA, DNA
  - Lipids
  - Polysaccharides
- Macromolecules form molecular assemblies such as
  - Ribosomes
  - Filaments
  - Membranes ....

Universal Architecture of All Cells

- All present-day cells: same type of molecules, nanostructures,
- All present-day cells undergo the same type of processes

=> All created from a common ancestor by cell division or fusion

Eubacteria

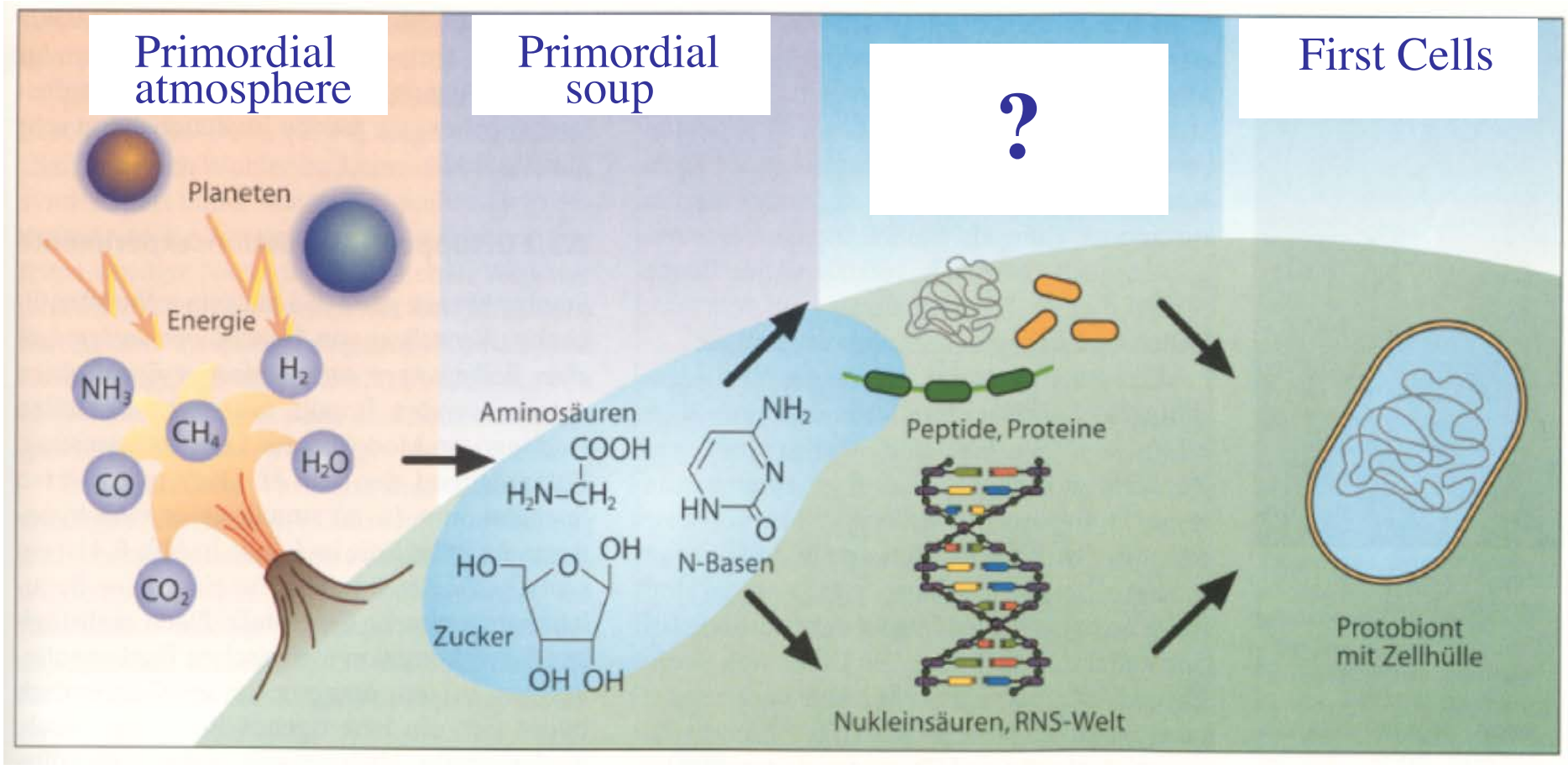


Common Ancestor

Eukaryotes

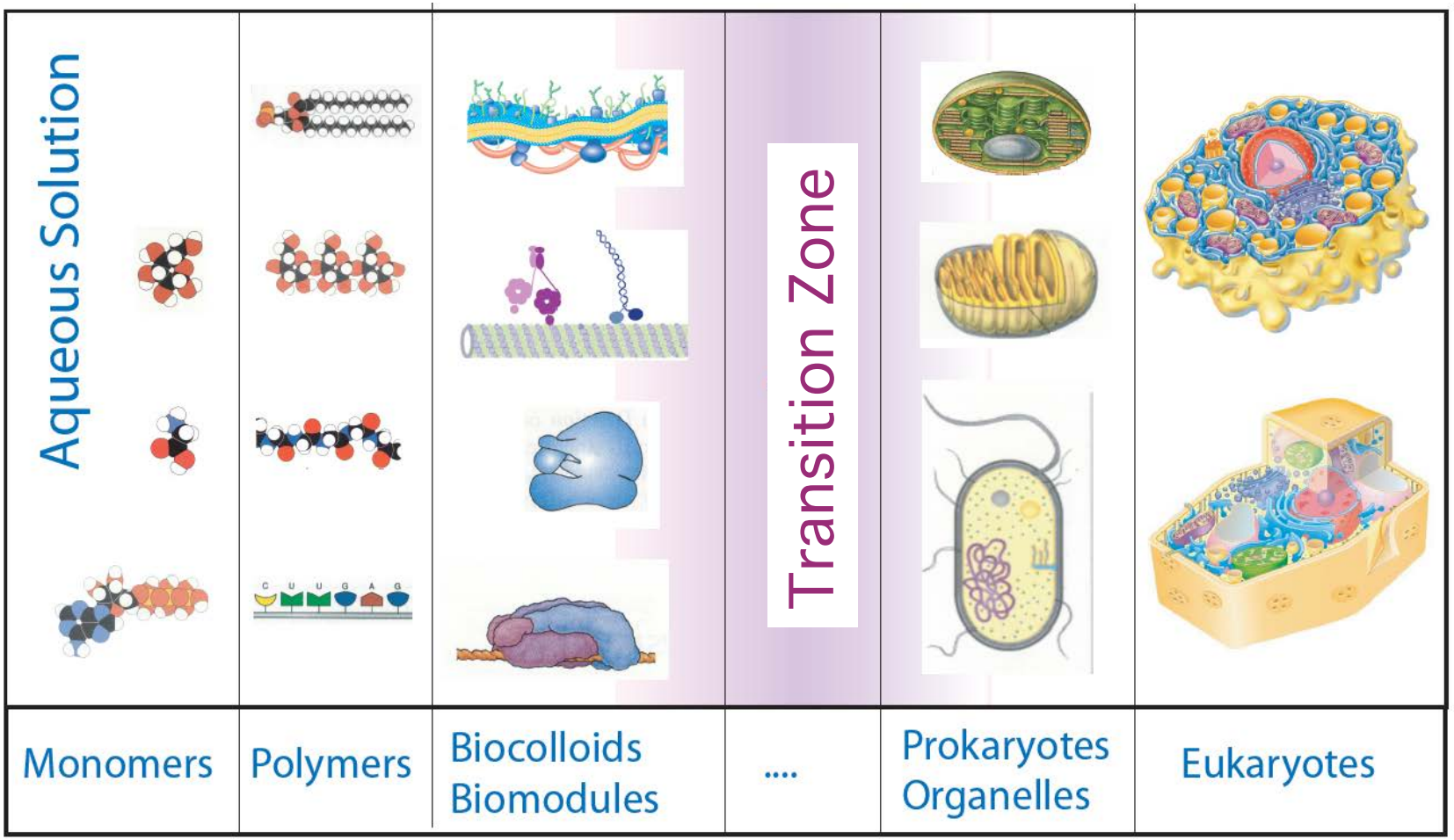
Archaebacteria

Origin of First Cells



- First cells were assembled bottom-up from smaller building blocks

From Matter to Life



Two Routes into Transition Zone

Bottom-Up: Synthetic Cells

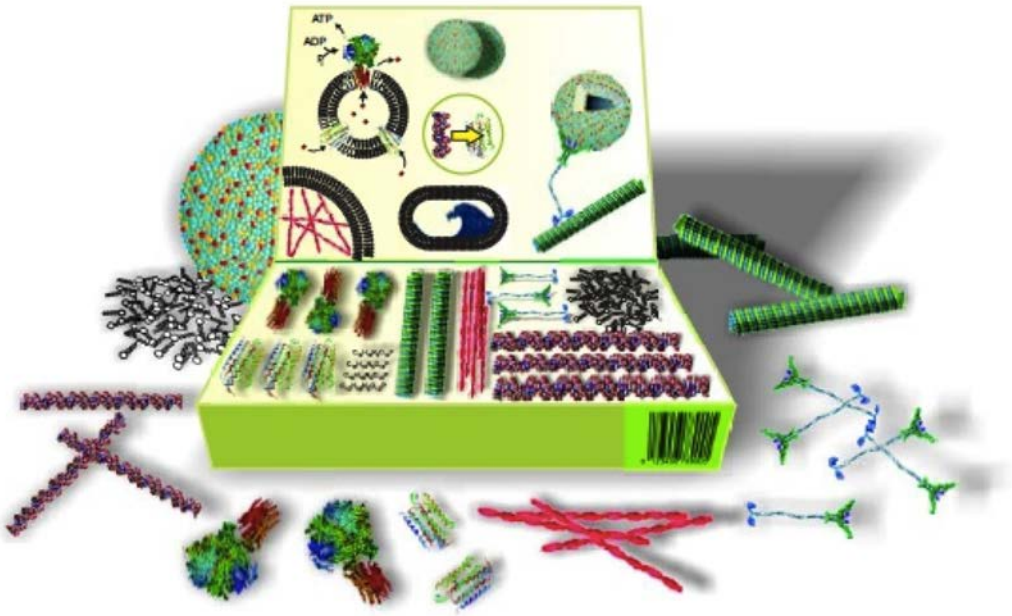
- Develop important building blocks or modules
- Assemble these modules into larger structures
- Integrate more and more modules ...

Top-Down: Minimal Cells

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

Synthetic Biology, Bottom-UP

MaxSynBio Consortium:
Create a toolbox of modules
to build a synthetic cell



9 Max Planck Institutes:

Motivation:
No understanding of minimal
cell from top-down approach

Stuttgart -> Heidelberg
Joachim Spatz, MPI-MF

Physical Understanding

„What I cannot create, I do not understand“

Richard Feynman

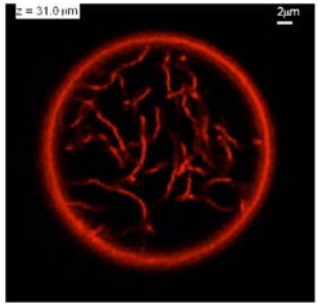
- Many creations by accidential discoveries (serendipities)
- However: "What I do not understand, I cannot develop"

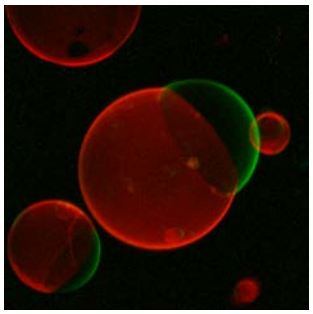
„Denken ohne Erfahrung ist leer,
Erfahrung ohne Denken ist blind“

Immanuel Kant

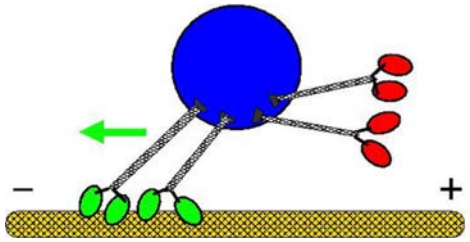
- Physical understanding from fruitful interplay between theory and experiment

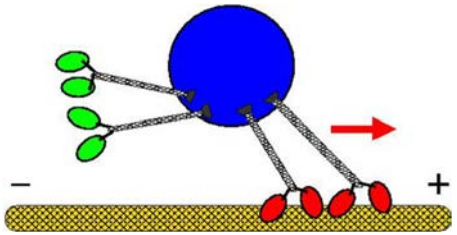
Basic Modules for Synthetic Cells



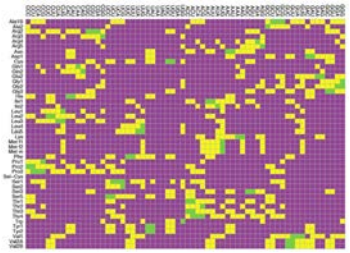


- Membrane and vesicles, fluid compartments, remodeling





- Directed transport by molecular motors, free energy transduction

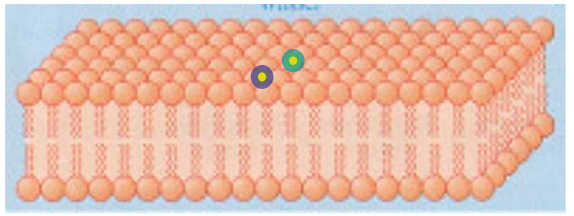


- Template-directed assembly, ribosomes, protein synthesis

Biomembranes are Fluid Bilayers

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

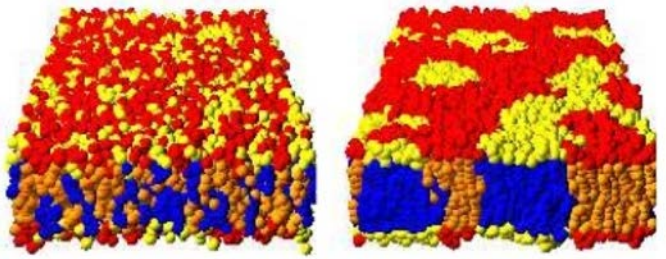
  Diffusion constant ~ $\mu m^2/s$



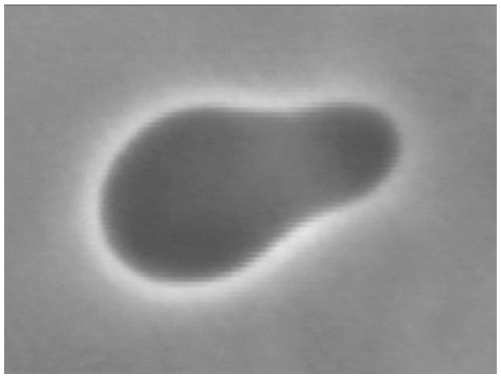
4 nm

lipid swapping ~ ns

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



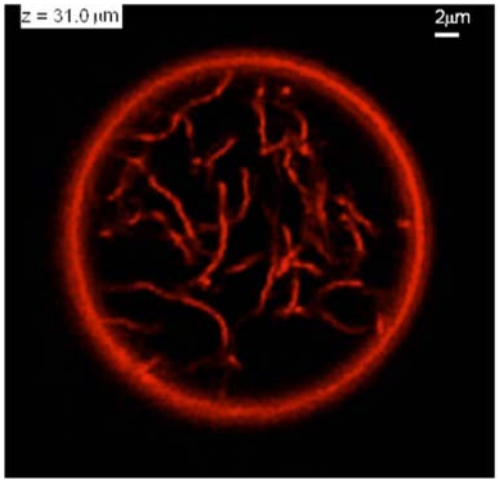
- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity



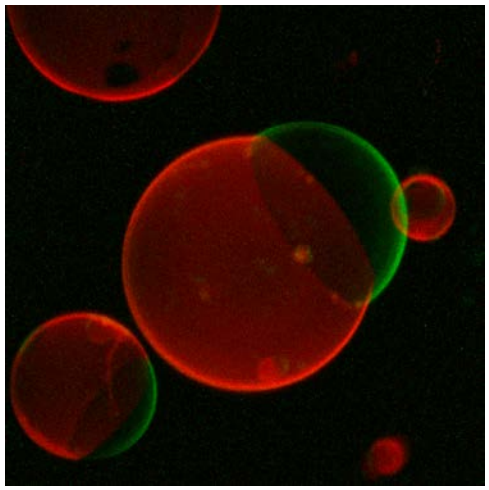
40 μm

Multiresponsive Behavior

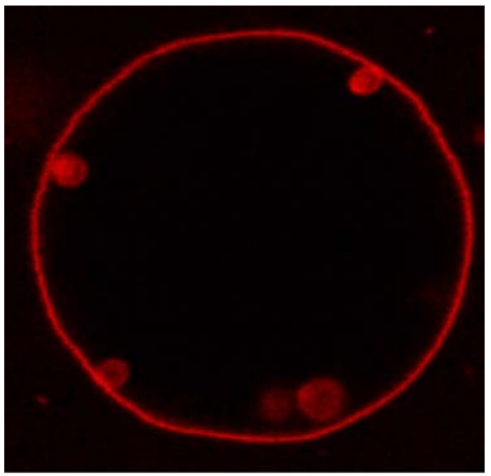
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



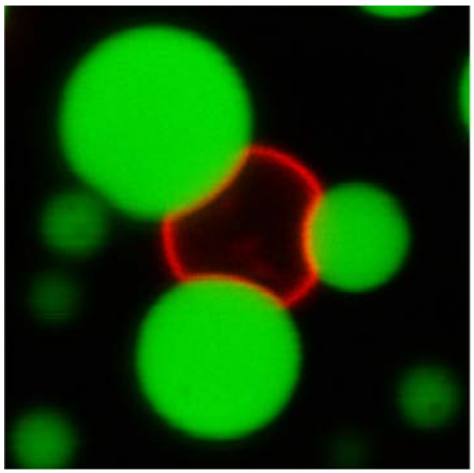
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



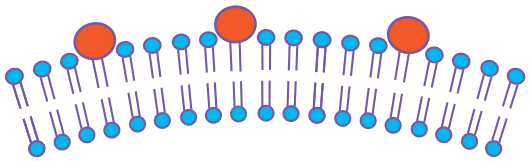
Small buds from adsorption of two ESCRT proteins



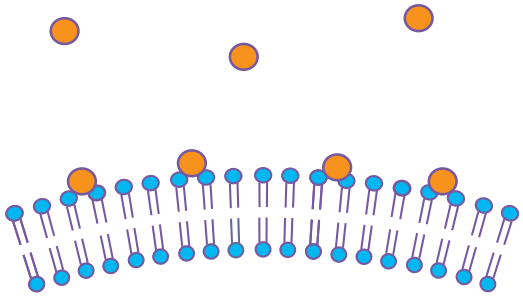
Shaping GUVs by membrane-less organelles, FUS

Spontaneous = Preferred Curvature

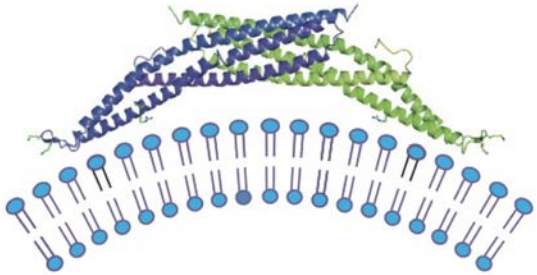
- Spontaneous or preferred curvature *m* describes bilayer asymmetry = asymmetry between two leaflets
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric composition, e.g., ganglioside



Asymmetric adsorption of small molecules

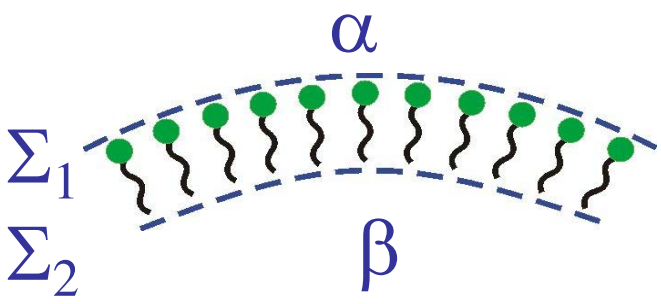


Asymmetric protein coats, e.g. BAR-domain

Concept of Spontaneous Curvature

- W. D. Bancroft (1913)

  'Theory of emulsification'



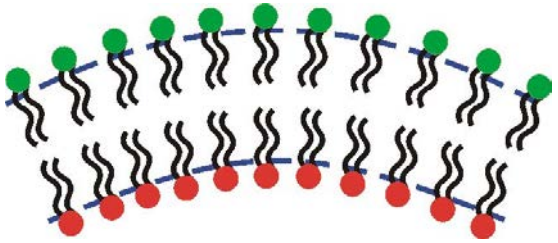
- F. C. Frank (1958)

  'On the theory of liquid crystals'

splay term from symmetry arguments

- W. Helfrich (1973)

  'Elastic properties of lipid bilayers'

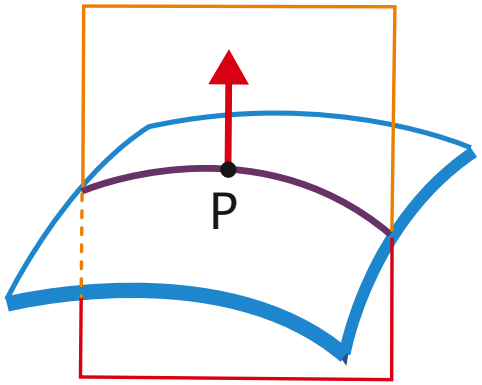


- Variants of curvature models:

  E. Evans, S. Svetina and B. Zeks, M. Wortis

Curvature Elasticity

- Mean curvature M tries to adapt to spontaneous (or preferred) curvature m



- Curvature or bending energy:

$$E_{cu} = \int dA \; 2 \; \kappa \, (M - m)^2$$

integral over membrane area A

- 2nd fluid-elastic parameter: Bending rigidity κ

  Dimensions of energy, $\kappa = 10^{-19}$ J = 20 k_B T

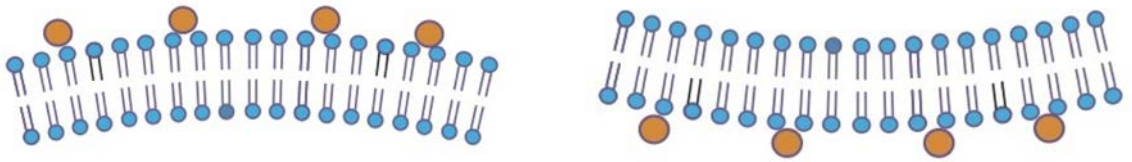
- Range of spontaneous curvatures m

  from 1/(20 nm) to 1/(20 μm)

Sign of (Spontaneous) Curvature

- Mean curvature M and spontaneous curvature m can be positive or negative
- Sign defined with respect to interior/exterior compartments = with respect to inner/outer leaflet

exterior compartment outer leaflet

$M > 0$  $M < 0$

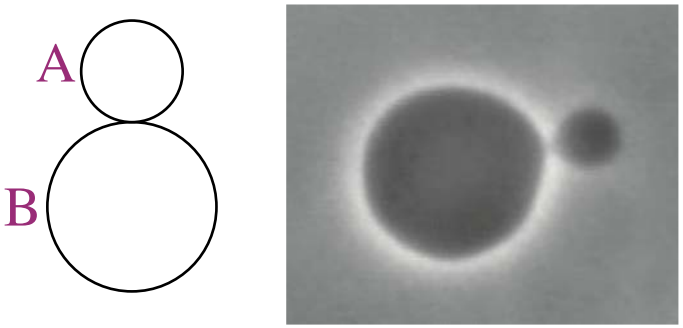
interior compartment inner leaflet

Mean curvature M is positive (negative) if membrane bulges towards exterior (interior) compartment

Membrane Buds and Necks

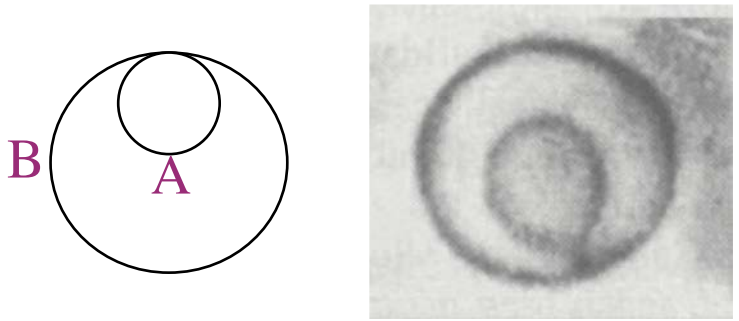
- For $m \neq 0$, curvature elasticity leads to spherical membrane segments connected by membrane necks

- Out-bud:



spont curv $m > \sqrt{2}/R_{ve}$

- In-bud:



spont curv $m < 0$

Seifert et al, *PRA* (1991)

- Closed neck is stable if:

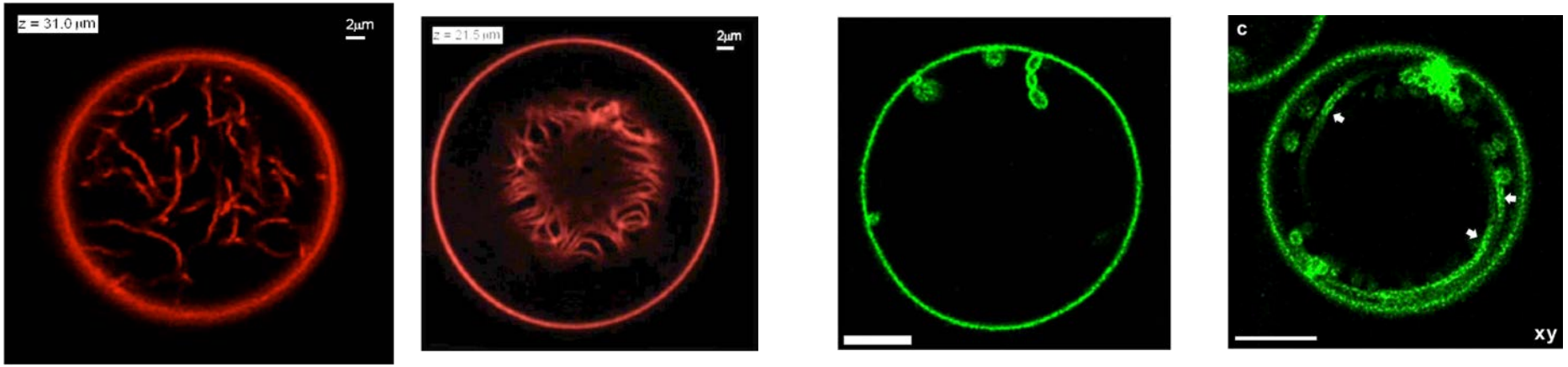
$$0 < M^A + M^B \leq 2m \qquad 2m \leq M^A + M^B < 0$$

- Relation between geometry and material parameter

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)



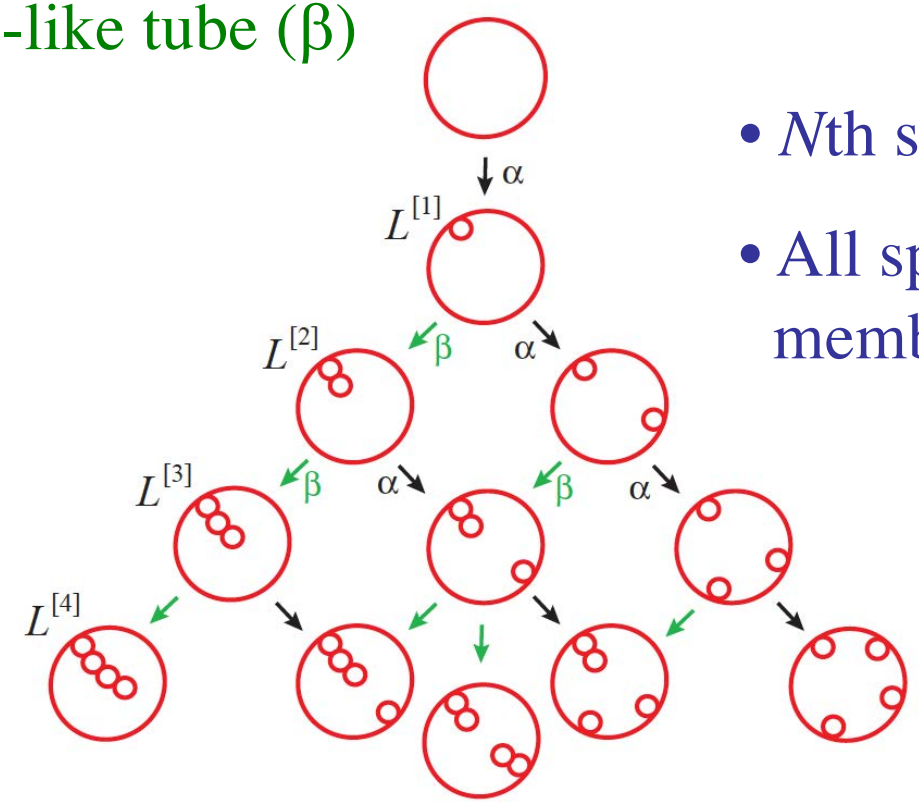
- Asymmetric environment, different PEG concentrations
- Deflation: Bud and tube formation without external forces
- Tubes can be necklace-like or cylindrical

Nucleation and Growth of Tubes

- Vesicle membrane with large spont curv *m*

Liu et al, *ACS Nano* (2016)

- Osmotic deflation of GUV in discrete steps
- At each step, nucleation of new bud (α) or extension of necklace-like tube (β)



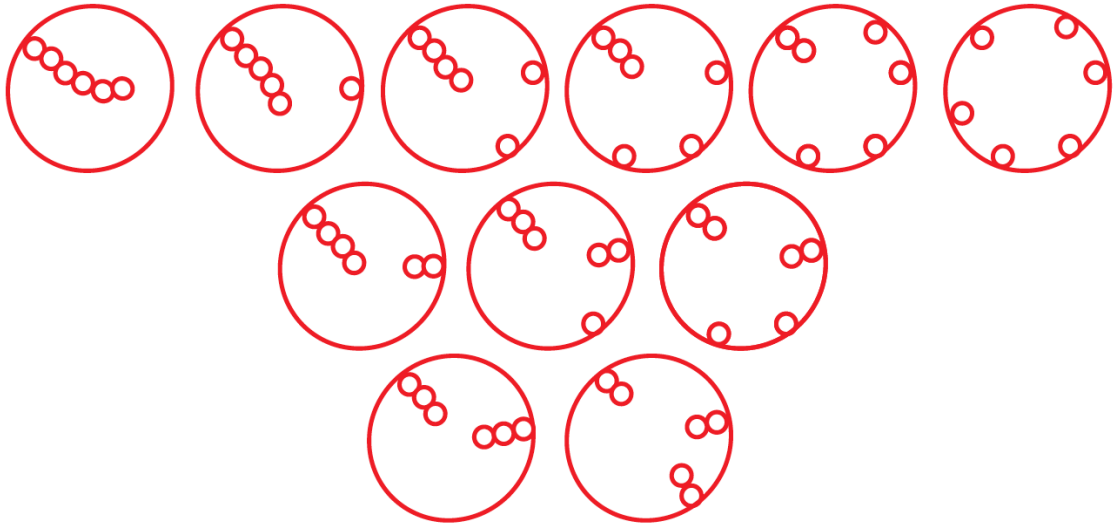
- *N*th step leads to *N* in-beads
- All spherules are connected by membrane necks (not visible)

=> Buds are nuclei for necklace-like tubes

Morphological Complexity

RL, *J. Phys. D* (in press)

- After 6th step, 11 morphologies with 6 spherules:



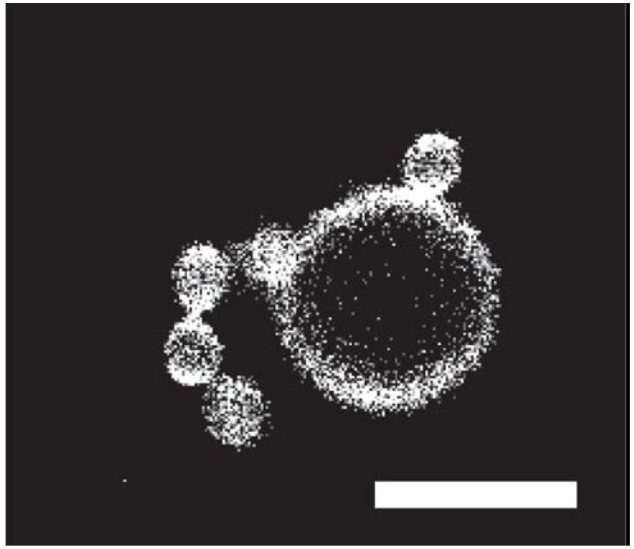
- All beads are connected by membrane necks
- All morphologies have the same area, volume, and curvature energy
- Rugged energy landscape contains 11 intersecting branches
- For large N, # of N-spherule morph grows as exp[c $\sqrt{N}$]

Morph Complexity: Experiment

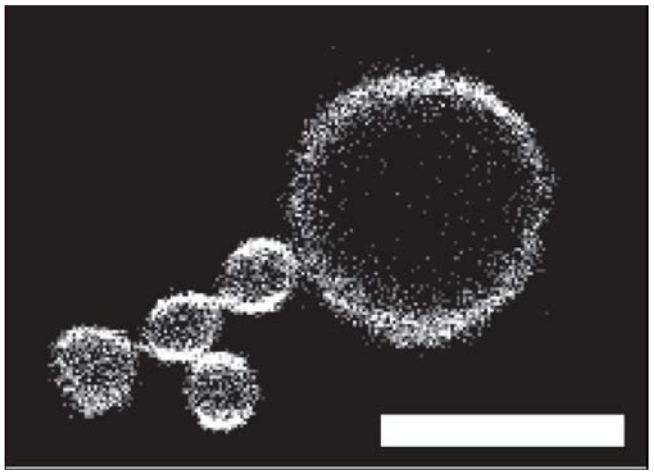
Tripta Bhatia

- Out-Necklaces

Linear

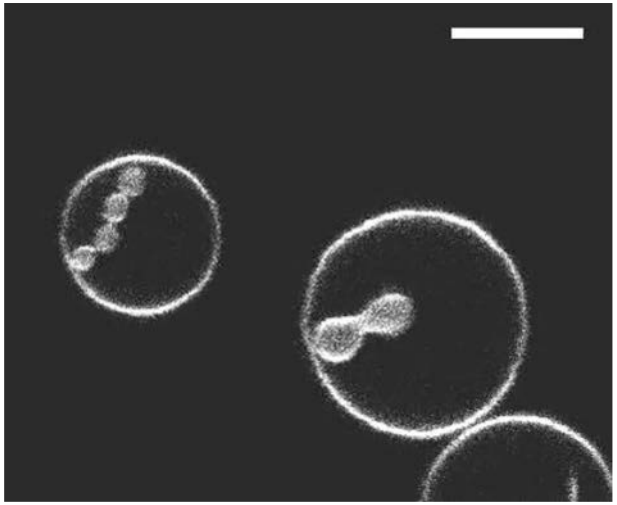


Branched



- In-Necklaces

Linear



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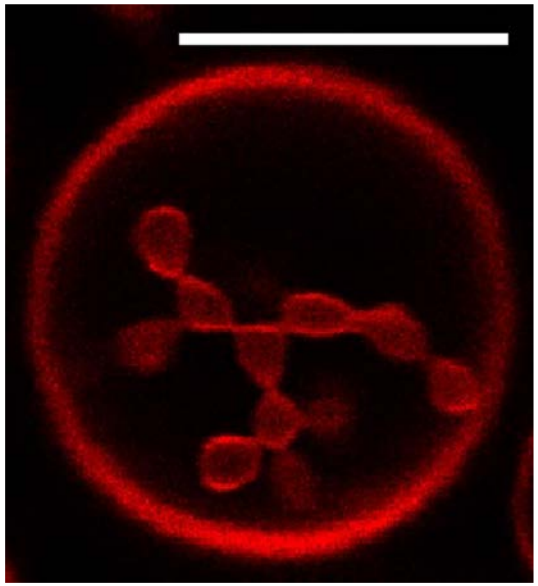
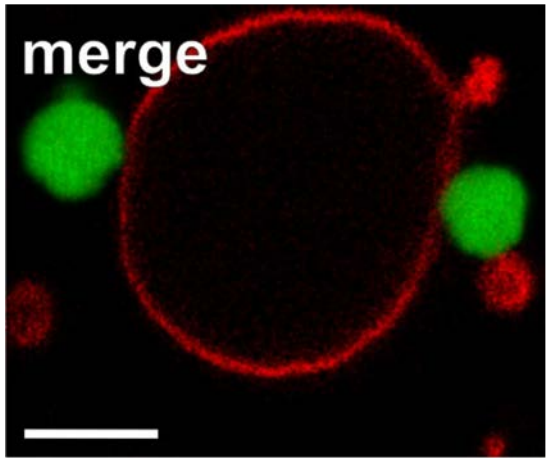


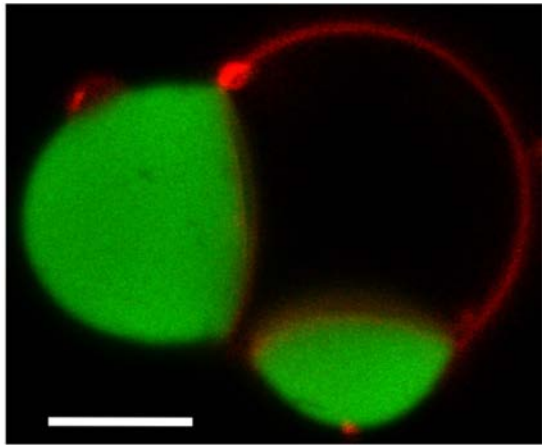
Membraneless Organelles

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

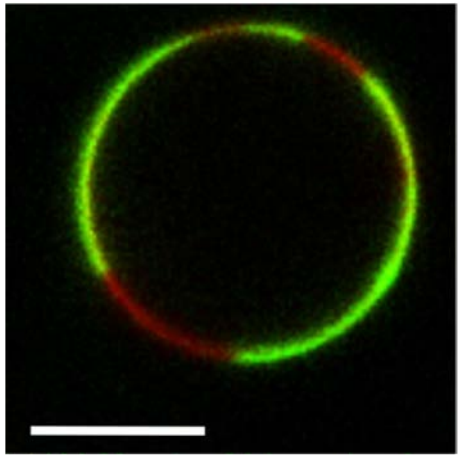
- Membrane-less organelles that behave like liquid droplets
- Enriched in intrinsically disordered proteins (IDPs)
- Example for IDP: RNA-binding protein FUS
- Interaction of FUS-droplets with GUVs,
  two subsequent wetting transitions:



dewetting for
high salt



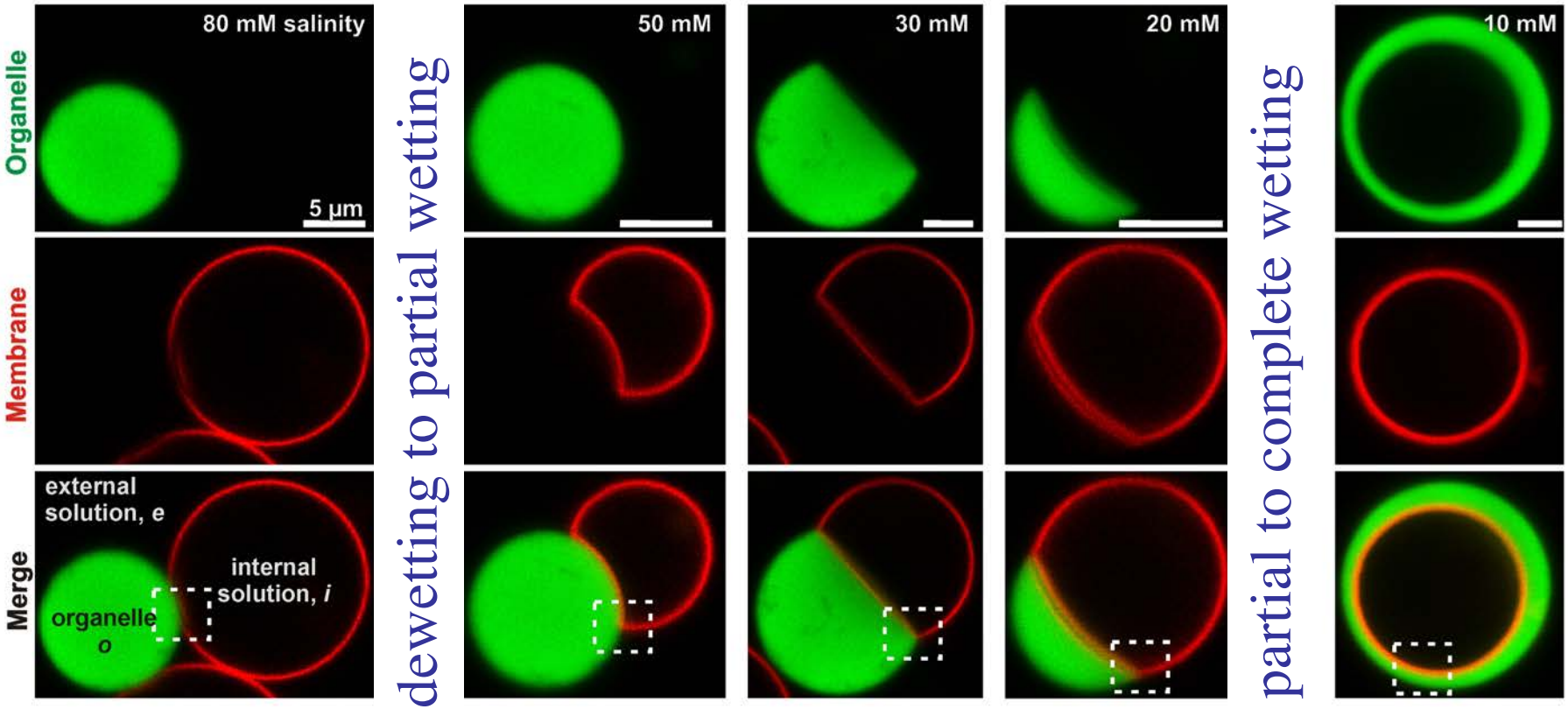
partial wetting for
intermediate salt



complete wetting
for low salt

Two Wetting Transitions

- GUV + FUS-rich organelle + salt



dewetting for high salt

partial wetting for intermediate salt

complete wetting for low salt

Membranes and Nanoparticles

Agudo-Canalejo, RL, *ACS Nano* (2015)
Nano Letters (2015)

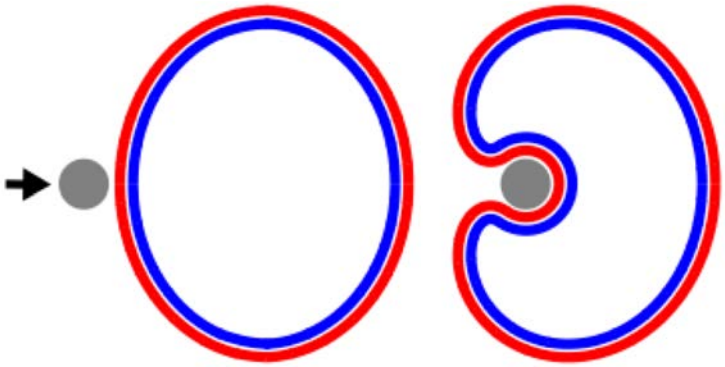
Jaime Agudo

- Nanoparticles interacting with membranes, vesicles and cells:

  biomedical imaging,

  drug delivery, nanotoxicity,

  virus infection ...



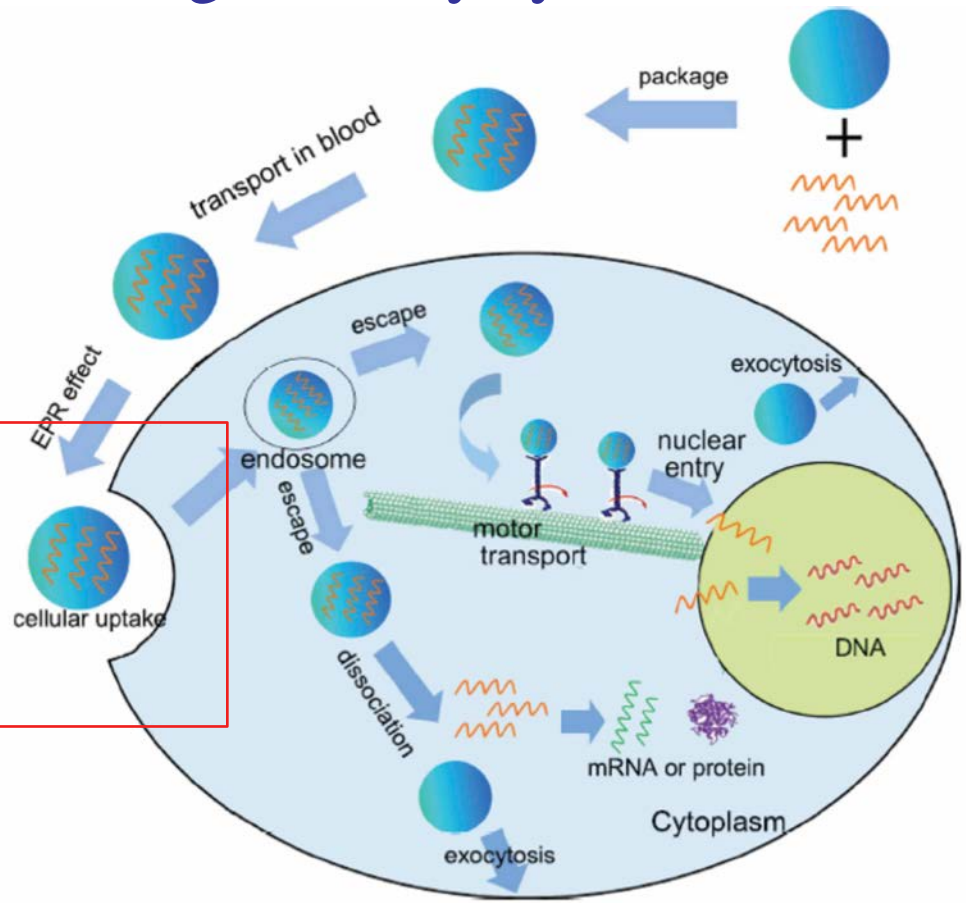
- Important control parameters:
  - Adhesive strength $W \sim$ surface chemistry
  - Particle size R_{pa}
  - Spontaneous curvature m

Targeting Nanoparticles to Cells

- Nanoparticles (NPs) as drug delivery systems:

Transport of NPs towards cells

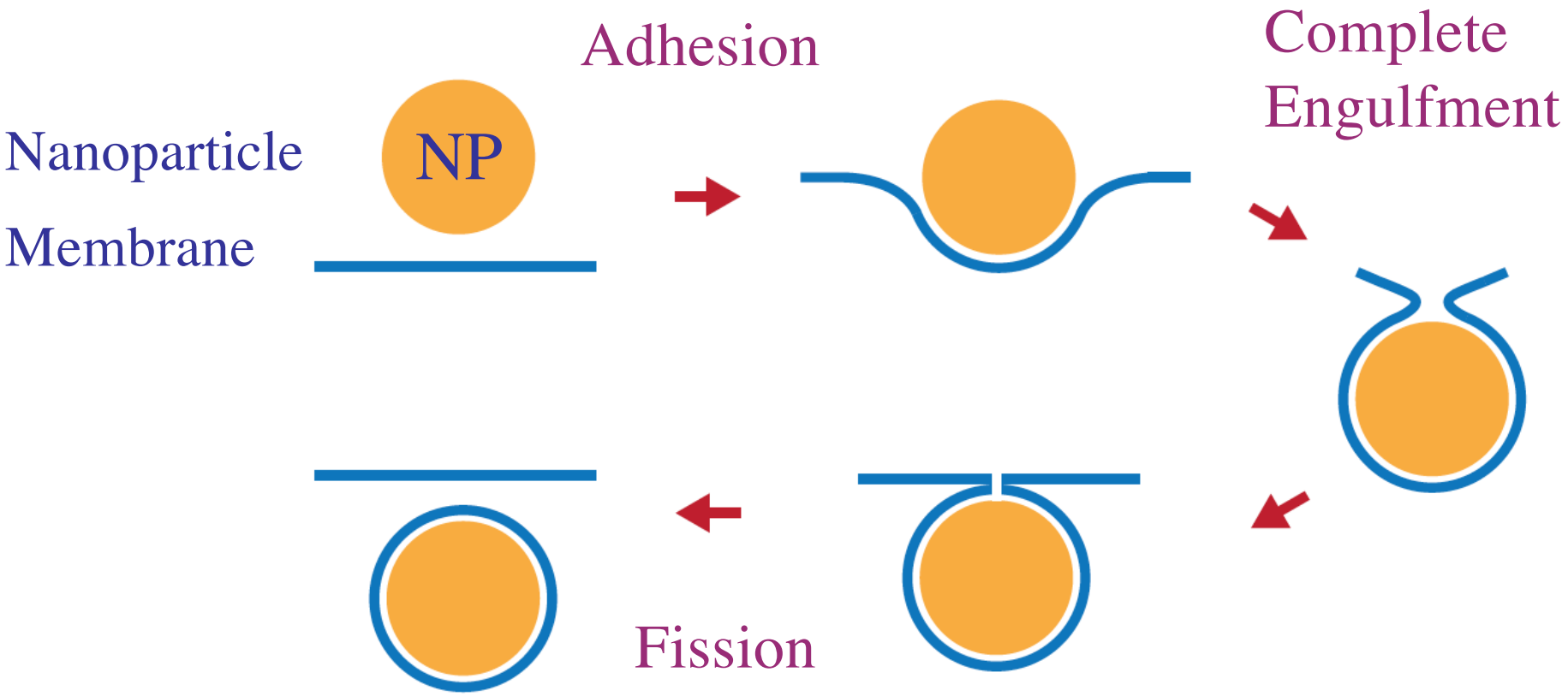
Transport across cell membrane by endocytosis



H.-M. Ding and Y.-Q. Ma, *Small* (2015)

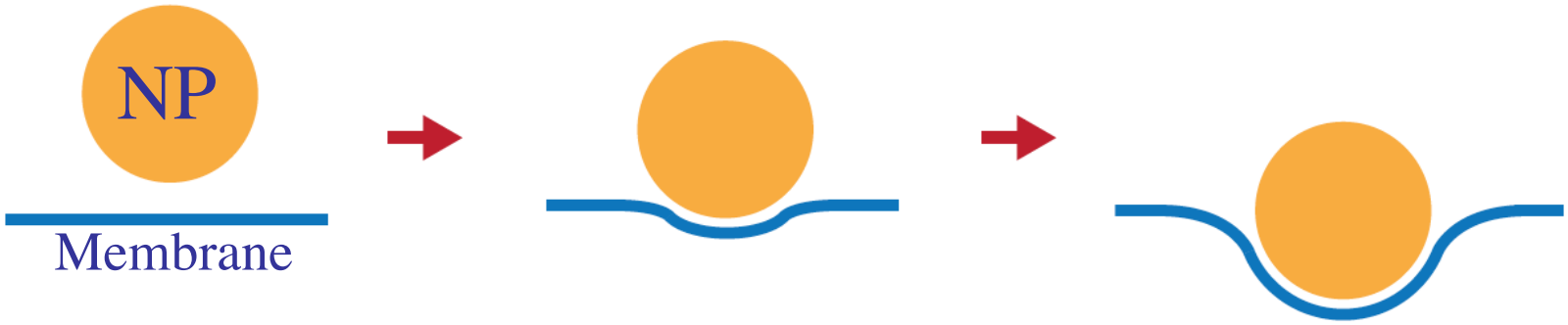
- Endocytic pathway also used by virusses, airborne ultrafine particles, ...

Endocytosis of Nanoparticles



- Dissecting endocytosis into three basic steps:
  Onset of Adhesion, Complete Engulfment, Fission

Adhesion: Basic Aspects



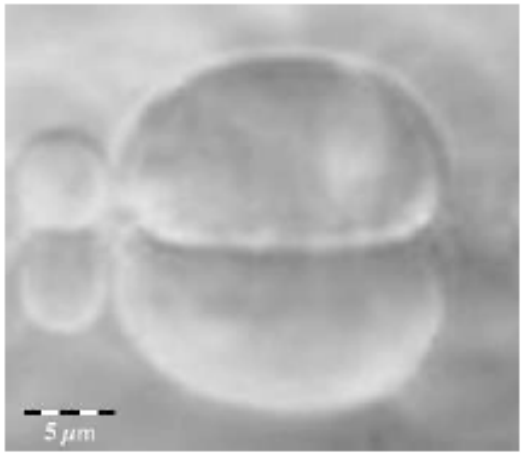
- Attractive interactions between NP and membrane
- Van der Waals, electrostatic, receptor-ligand
- Gain of adhesion free energy but
  increase of elastic membrane energy
- Competition between adhesion and bending
- Bending rigidity κ versus adhesive strength $|W|$

Adhesion Length

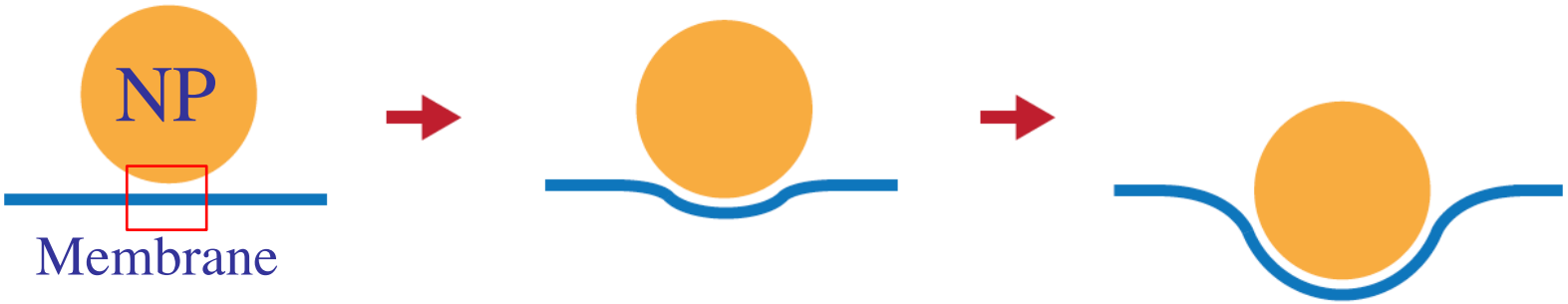
- Adhesive strength $|W|$ = adhesion free energy per area
- Bending rigidity κ and adhesive strength $|W|$ define adhesion length

$$R_W = (2\kappa/|W|)^{1/2}$$

- For specific NP-membane systems, R_W varies between 10 nm and 3 μm !
- Large R_W values can be measured via membrane curvature along contact line



Onset of Adhesion: Key Parameters

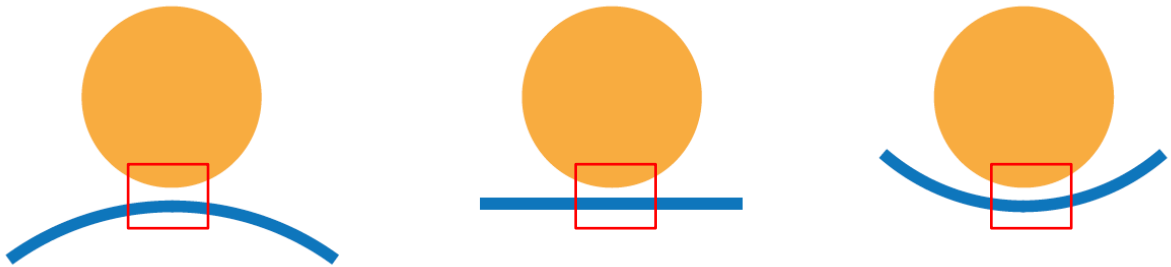


- Three key parameters for onset of adhesion:

  Adhesion length R_W , Particle size R_{pa} , and

  Membrane curvature M at point of contact

- Membrane curvature M can be positive or negative:



$M > 0$ $M = 0$ $M < 0$

Onset of Adhesion: Local Criterion

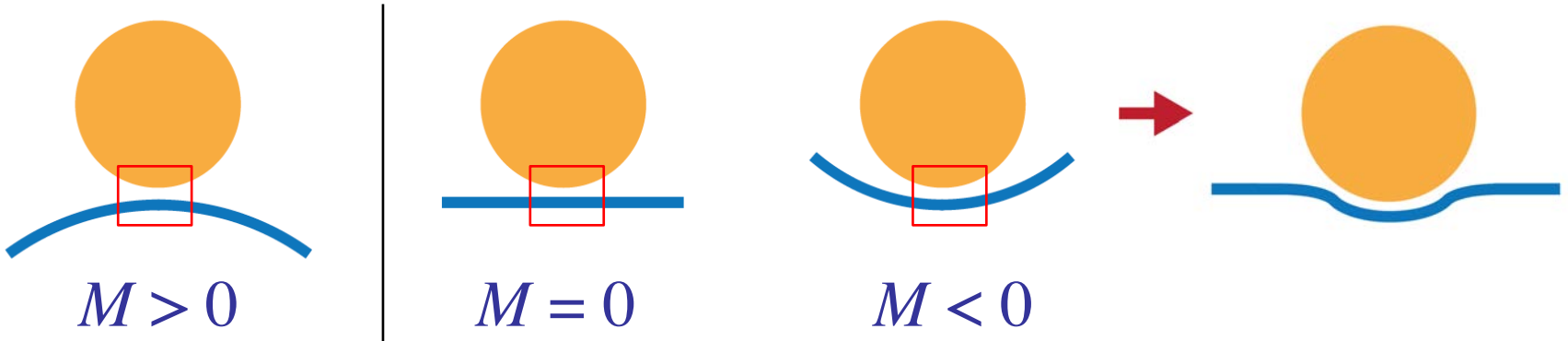
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Membrane starts to spread over particle if

$$M \leq 1/R_W - 1/R_{pa} =: M_{co}$$

contact curvature M_{co} is threshold value for M

- Example: $R_W = R_{pa}$ or $M_{co} = 0$

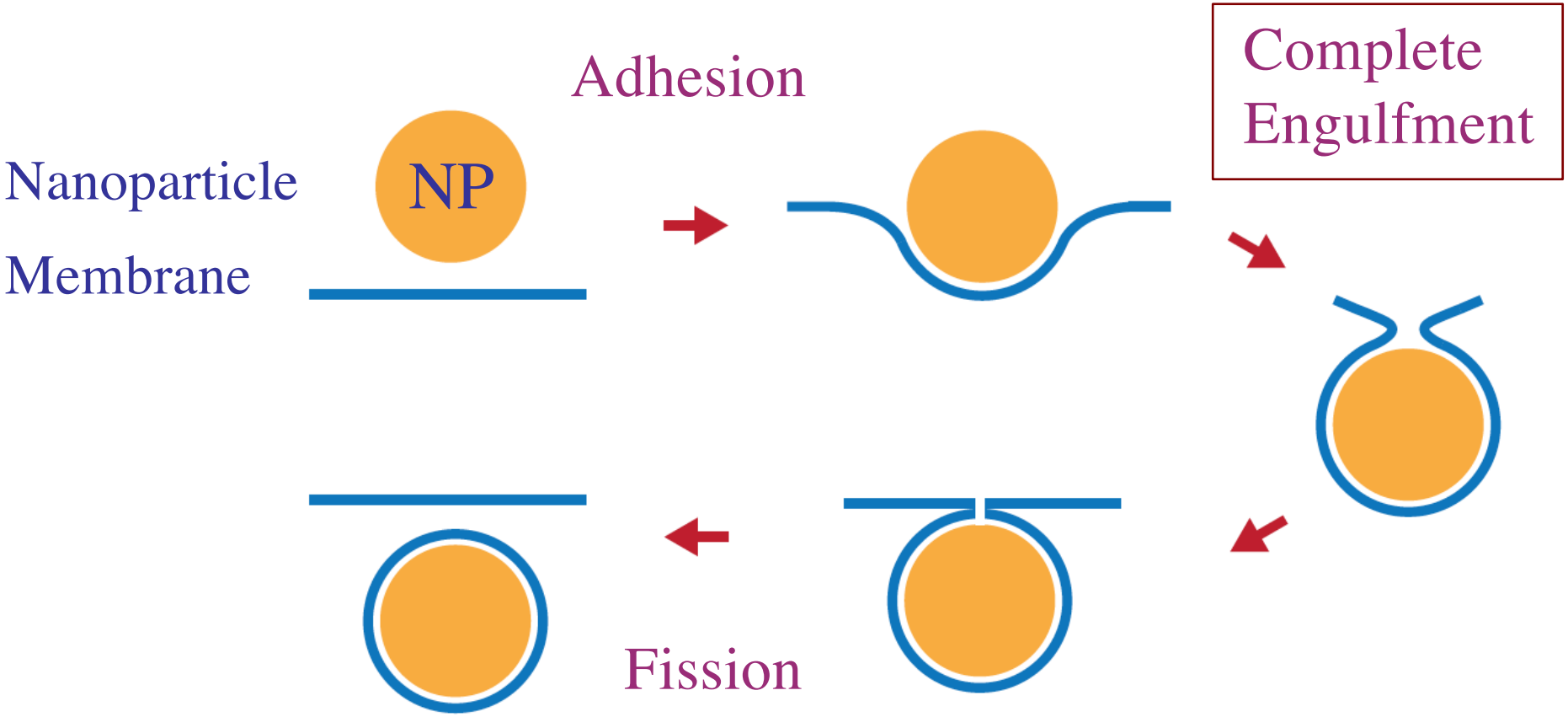


$M > 0$ $M = 0$ $M < 0$

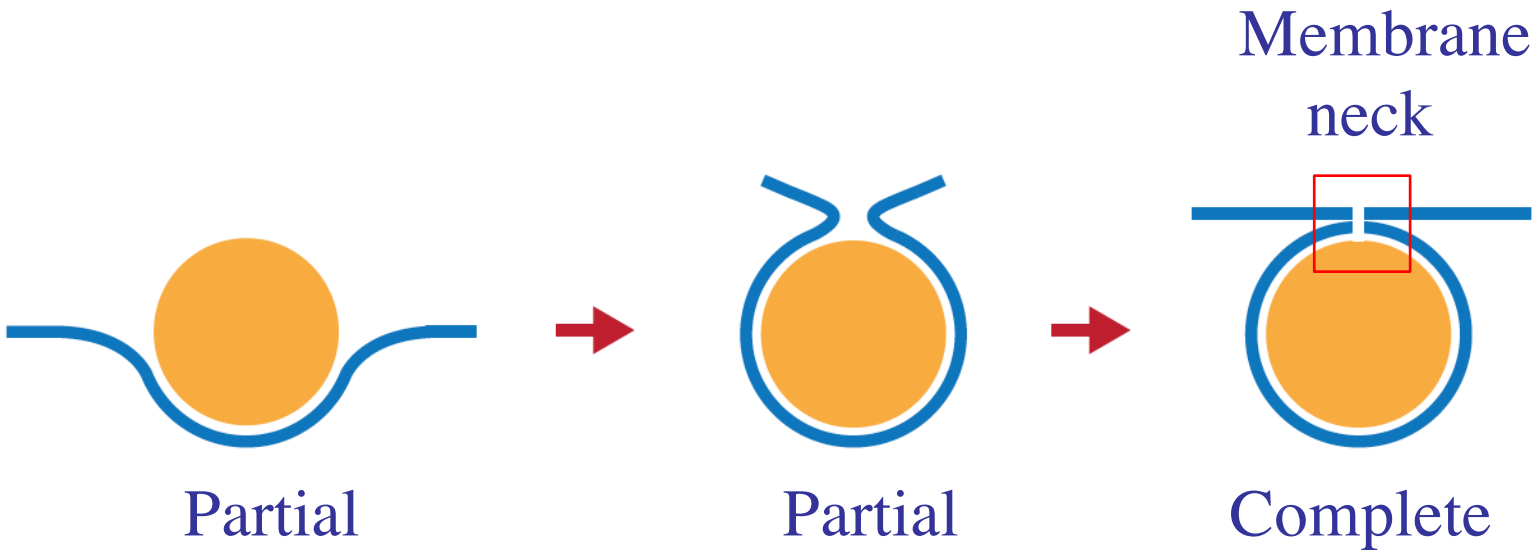
no adhesion adhesion and spreading

- Large M_{co} for small R_W or large $|W|$

Endocytosis: Complete Engulfment



Engulfment: Basic Aspects



- After onset of adhesion, membrane spreads over NP
- Membrane may engulf NP only partially or completely
- Complete engulfment involves closed membrane neck
- Necessary condition for complete engulfment:

  Closed membrane neck must be stable

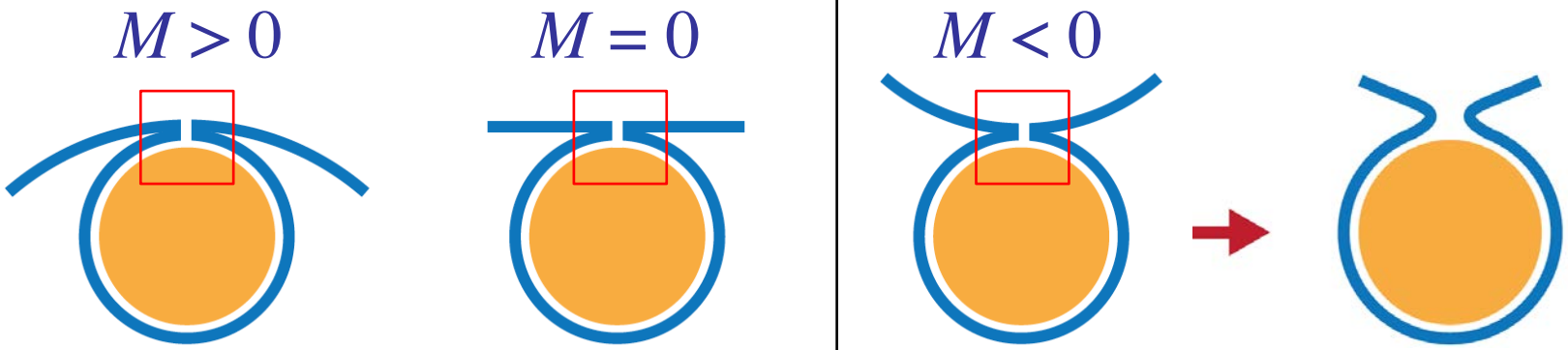
Neck Stability: Local Criterion

- Closed membrane neck is stable if membrane curvature

$$M \geq 2m + 1/R_{pa} - 1/R_W =: M_{ne}$$

2nd threshold value for M

- Example: $M_{ne} = 2m + 1/R_{pa} - 1/R_W = 0$

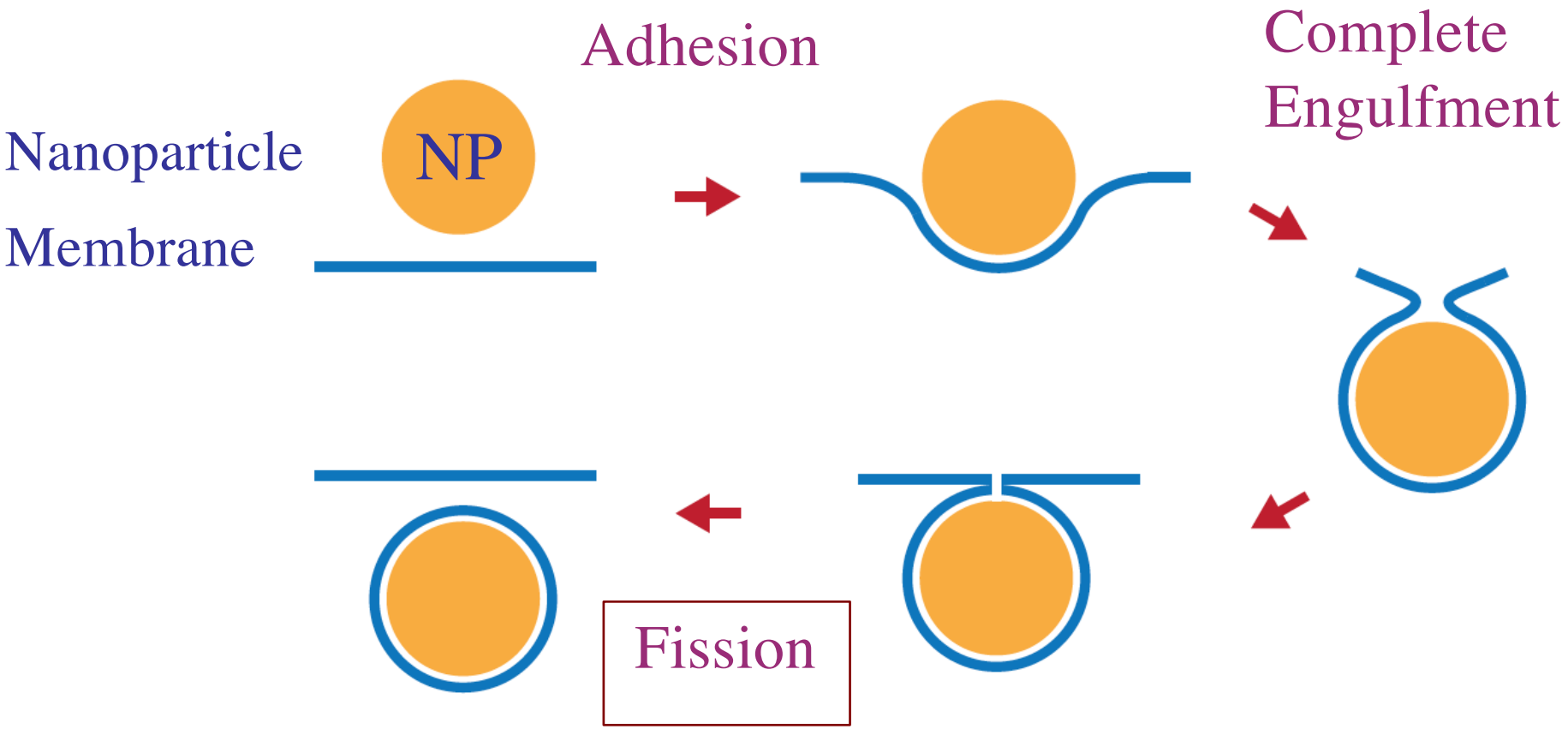


closed neck is stable

closed neck is unstable and opens up

Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

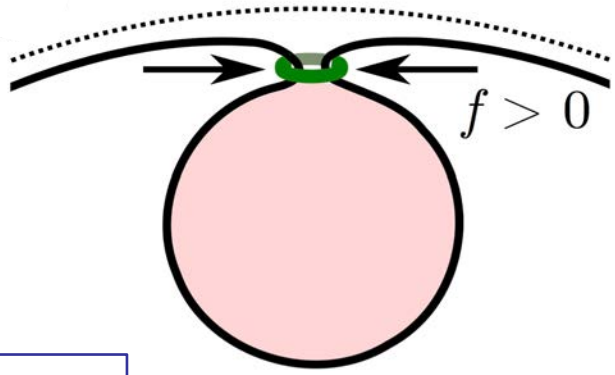
Endocytosis: Fission



Effective Constriction Forces

- General stability relation with force f:

$$M - M_{ne} + f\,(4\pi\kappa)^{-1} \geq 0$$



- Effective constriction force

$$f_{eff} = f + 8\,\pi\,\kappa\,|m| + 4\,\pi\,\kappa/R_W$$

- Spont curvature generates force $f_m = 8\,\pi\,\kappa\,|m|$
- Adhesion generates force $f_W = 4\,\pi\,\kappa/R_W$
- Example: $m = -1/(100\text{ nm})$ and $R_W = 20$ nm generate effective forces $f_m = 25$ pN and $f_W = 63$ pN

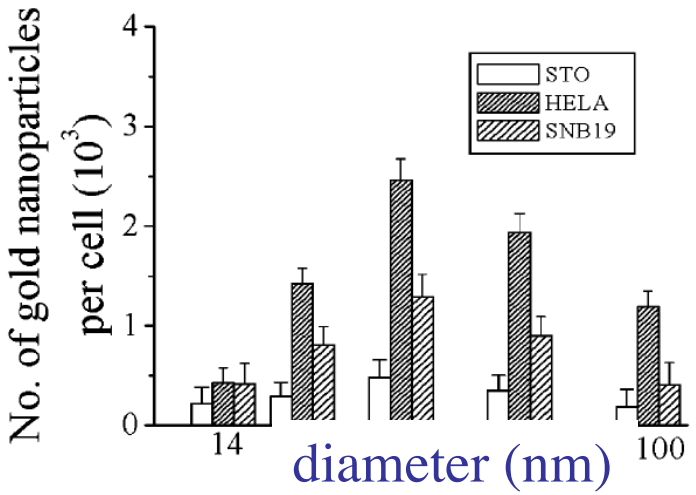
Agudo-Canalejo, RL, *Soft Matter* (2016)

Receptor-Mediated Endocytosis

Chithrani et al, *Nano Letters* (2007)

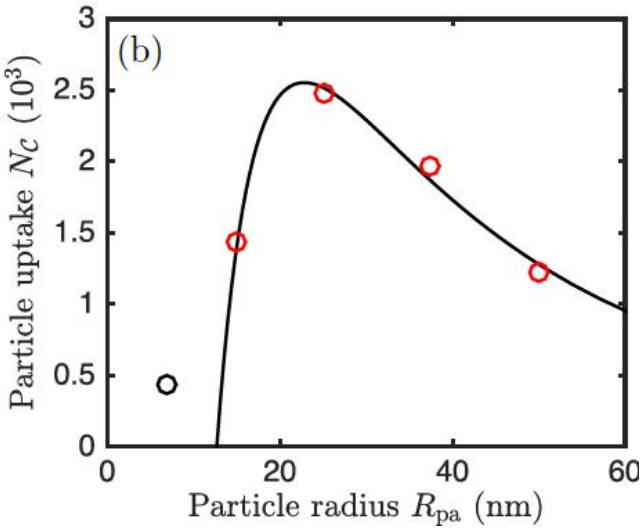
- Uptake of gold nanoparticles by cells
- Particles bind to transferrin receptors
- Assembly of clathrin-coated vesicles

Non-monotonic size-dependence !

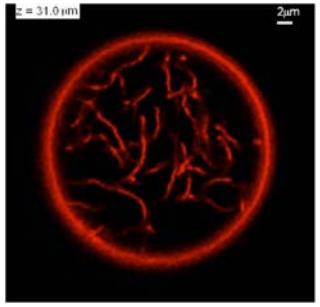


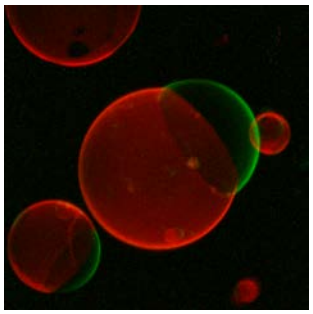
- Cell membrane with two types of segments, bound and unbound
- Bound segment contains protein coat with spont curv m_{bo} = - 1/(40 nm)
- Good agreement with exp data:

Agudo-Canalejo, RL: *ACS Nano* (2015)

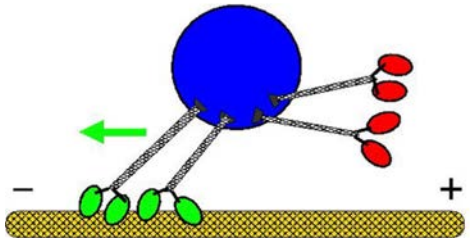


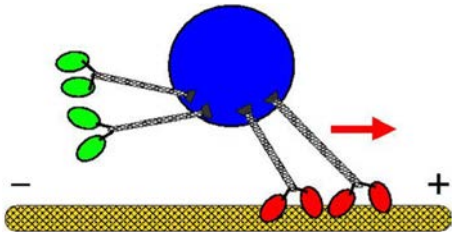
Basic Modules for Synthetic Cells



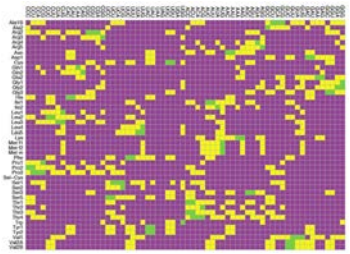


- Membrane and vesicles, fluid compartments, remodeling



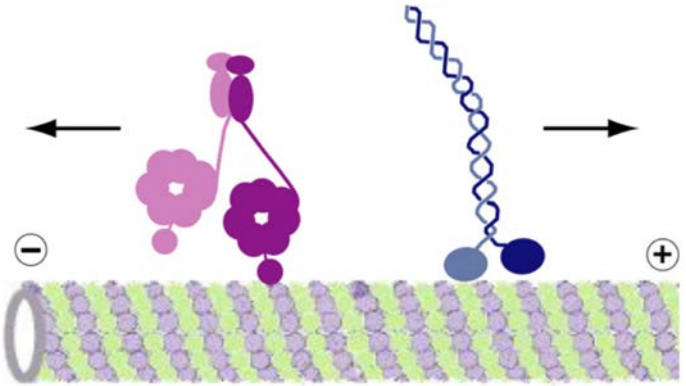


- Directed transport by molecular motors, free energy transduction

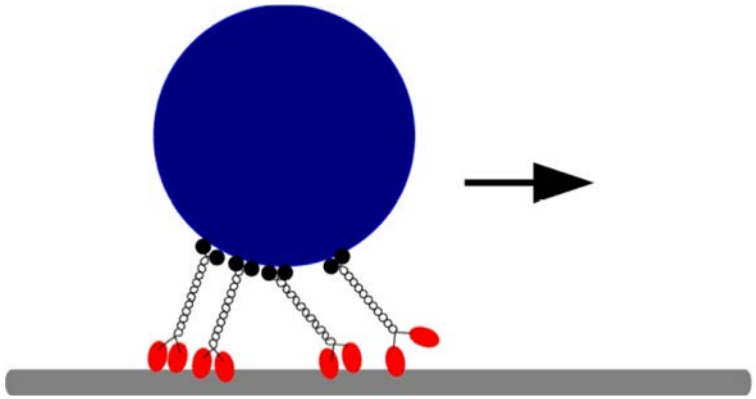


- Template-directed assembly, ribosomes, protein synthesis

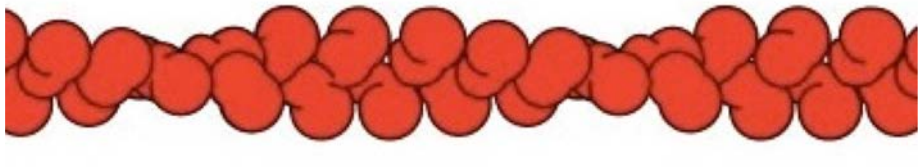
Biomolecular Machines



• Intro: Stepping motors



• Transport: Motor teams

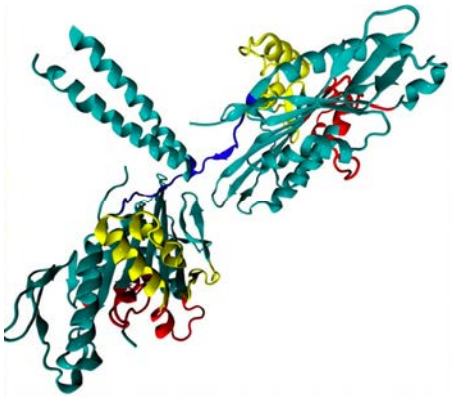


• Structural remodelling:
Actin filaments

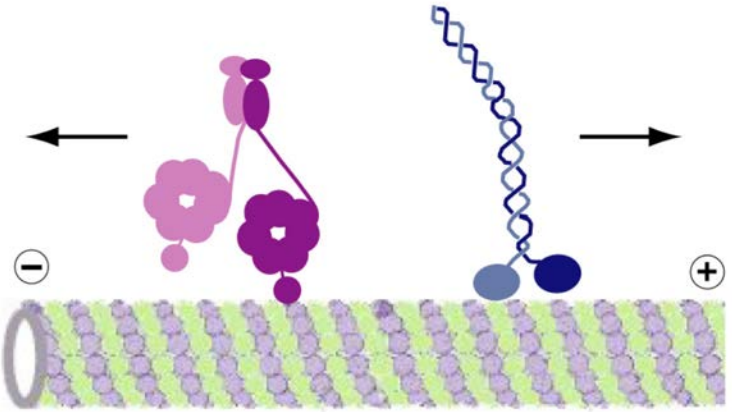
• Information processing:
Ribosomes

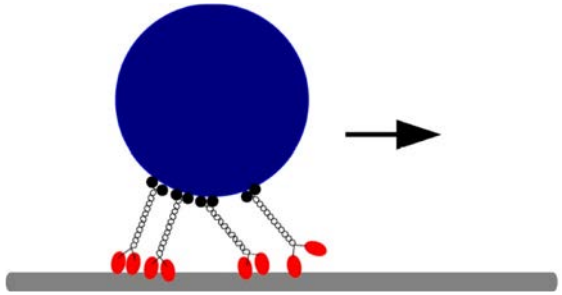
Multiscale Aspects of Mol Motors

- ATP hydrolysis ~ 1 nm

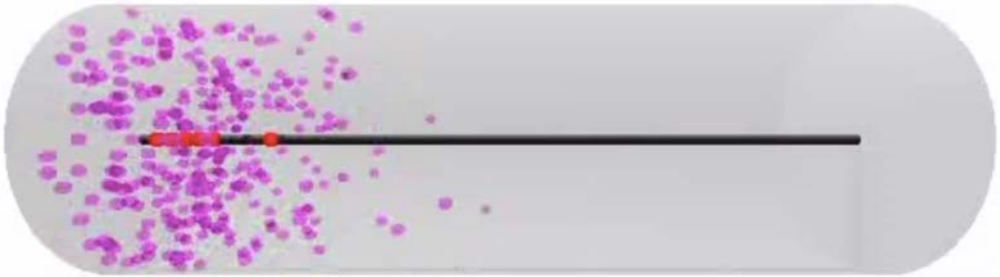


- Mechanical steps ~ 10 nm





- Cargo transport by motor teams ~ 100 μm



- Traffic of many motors/cargos and phase transitions

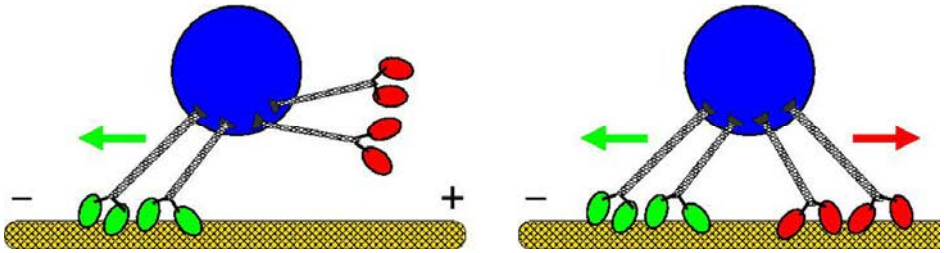
Cargo Transport by Motor Teams

- Transport by N identical motors

Klumpp and RL, *PNAS* (2005)

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

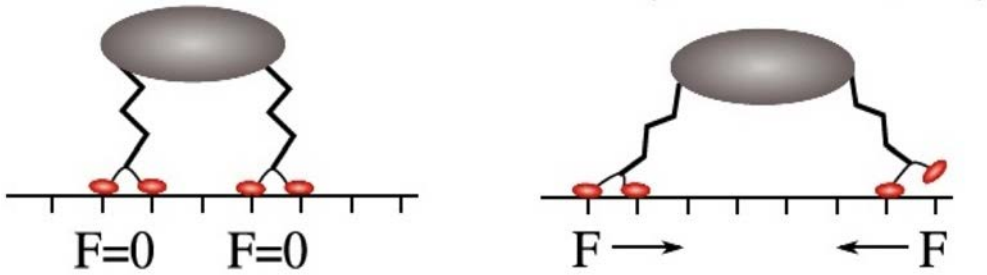
M. Müller et al, *PNAS* (2008)



- Elastic linkers between motors and cargo

Berger et al, *PRL* (2012)

Ucar, RL, *Soft Matter* (2017)



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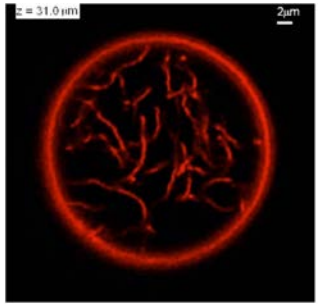

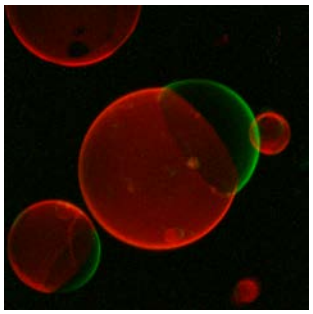

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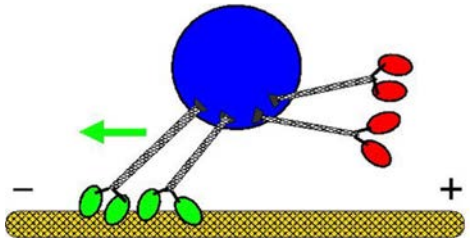

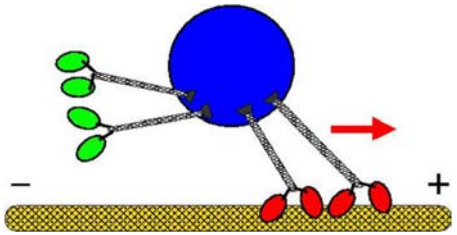

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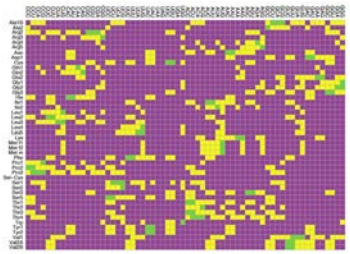
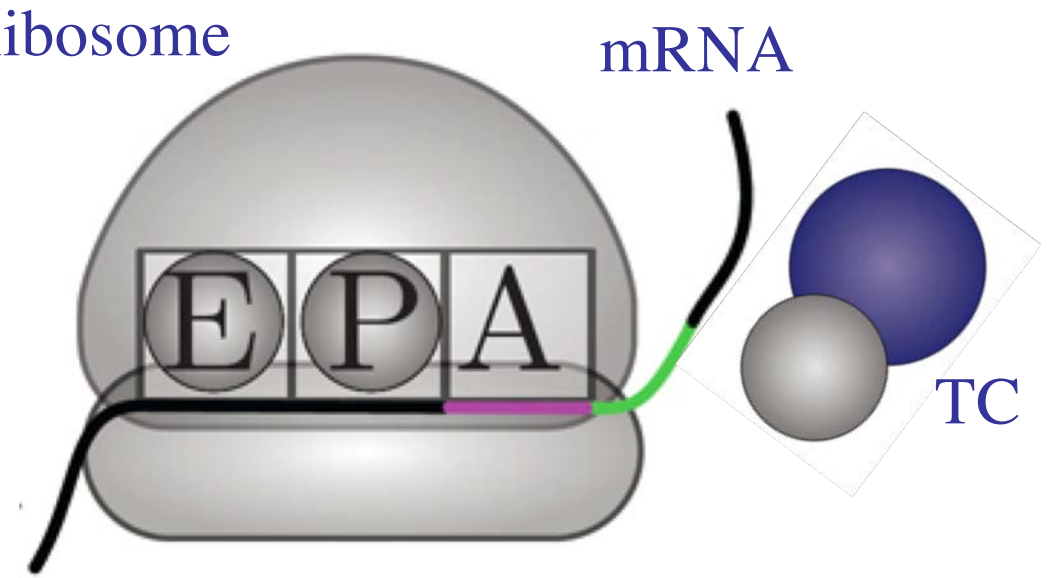


- Template-directed assembly, ribosomes, protein synthesis

Protein Synthesis by Ribosomes



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

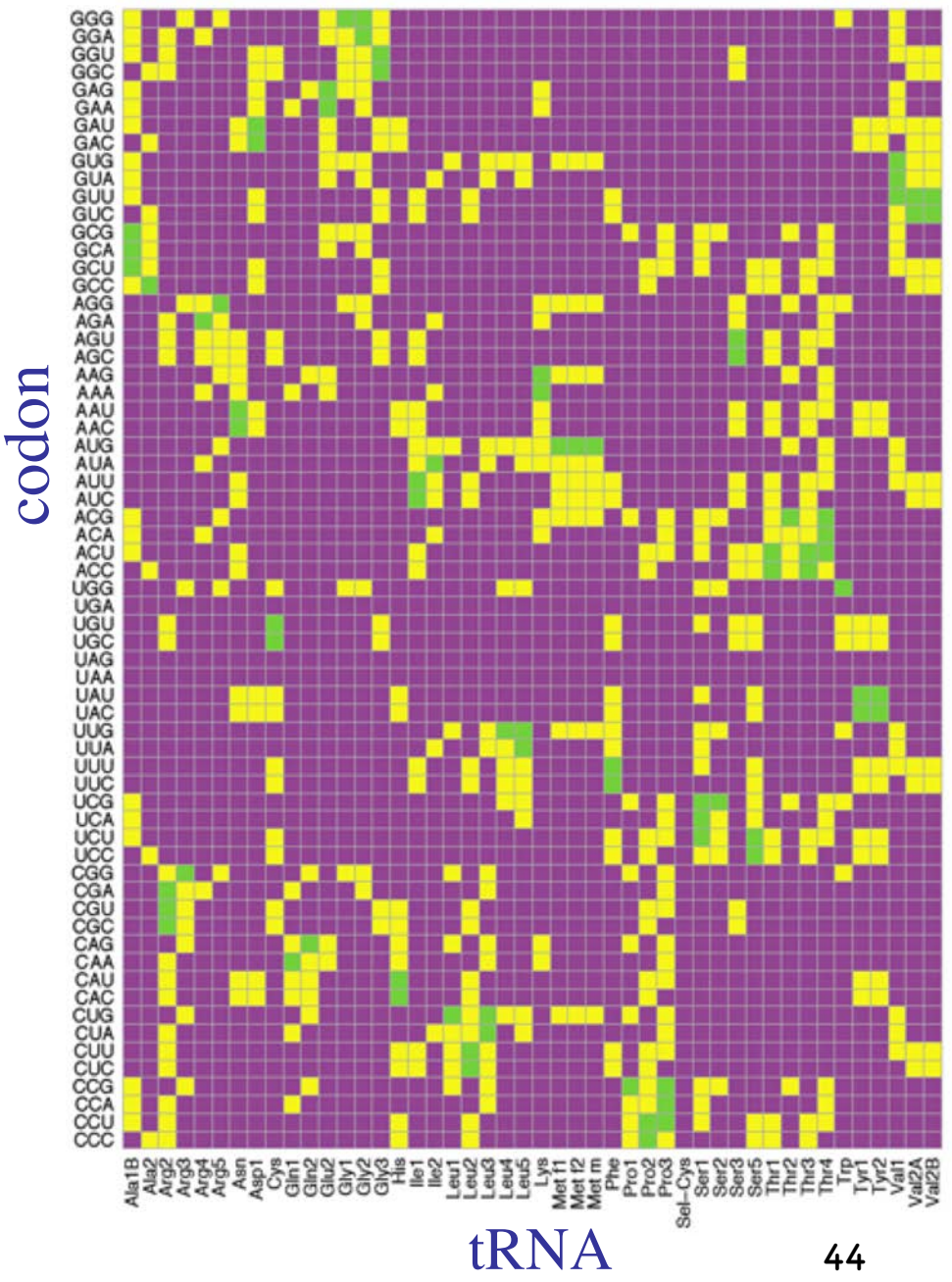
  Translocation of mRNA together with two tRNAs

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

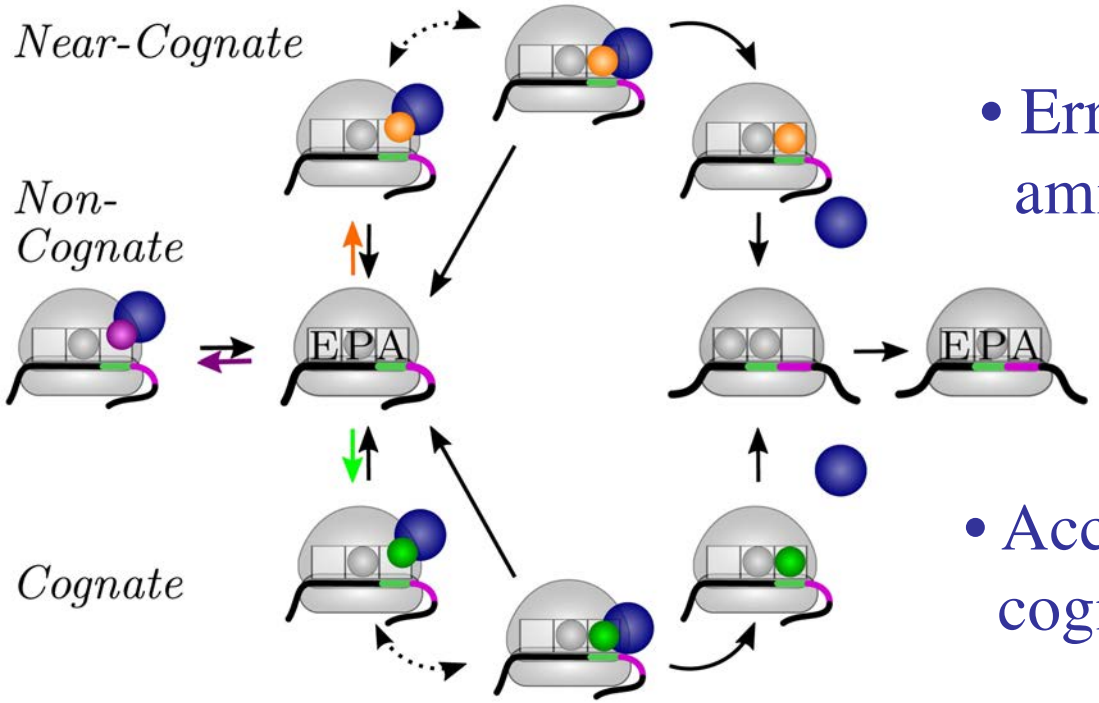


Single Elongation Cycle

Sophia Rudorf

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

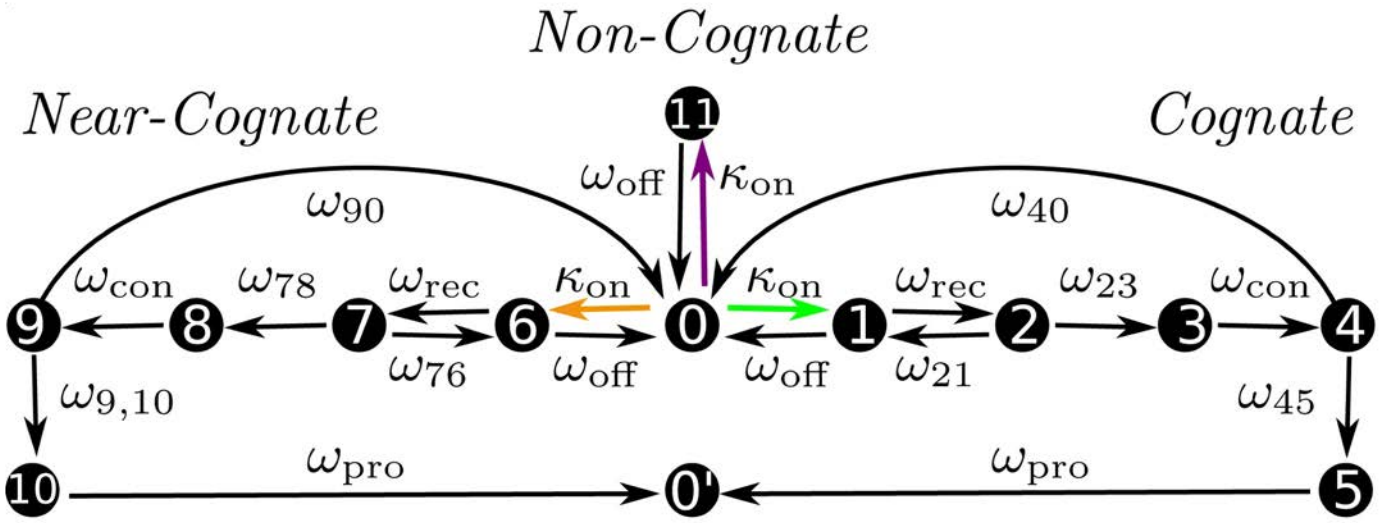
- Three branches for tRNA binding:



- Error: wrong amino acid
- Accommodation of cognate tRNA
- Competition between cognate, near-cognate, and non-cognate tRNAs

Markov Process

- Map cartoon of multistep process onto Markov chain:



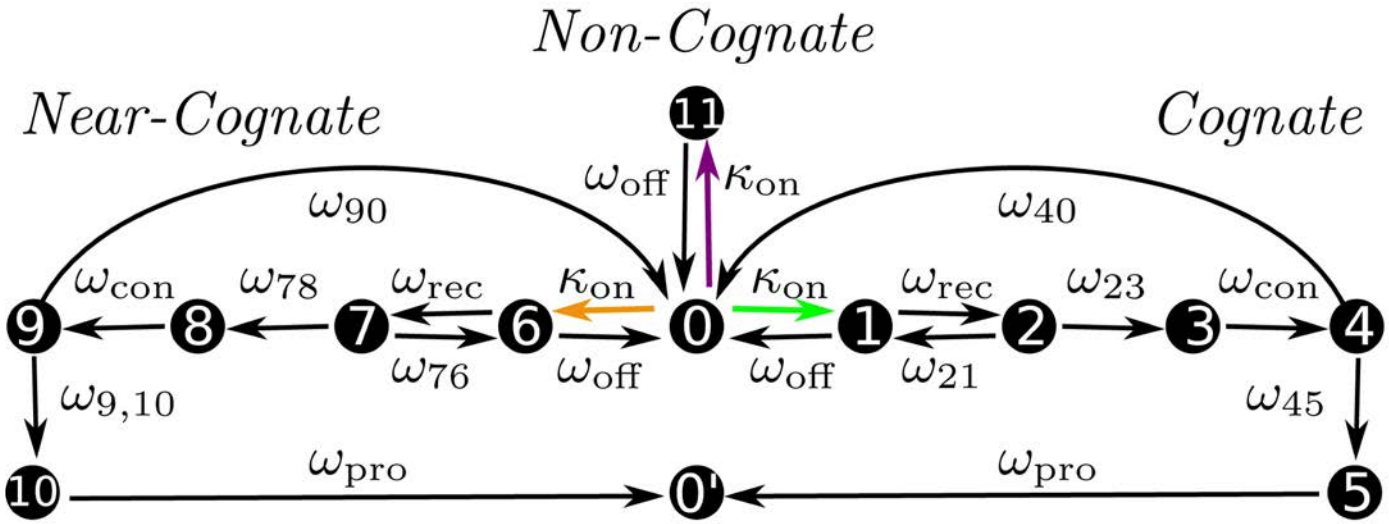
- Individual transitions:

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

- All transition rates ω_{ij} have been measured in vitro
- Individual rates not known in vivo

‘Similarity’ of In Vitro and In Vivo ?

- Multistep process with many individual transitions



- Set of in-vitro rates ω_{ij} ⇔ Set of in-vivo rates ω_{ij}^{*}
- How ‘similar’ or ‘close’ are the in-vivo to the in-vitro rates ?
- Quantitative measure for such a ‘similarity’ ?

Single Steps and Barrier Shifts

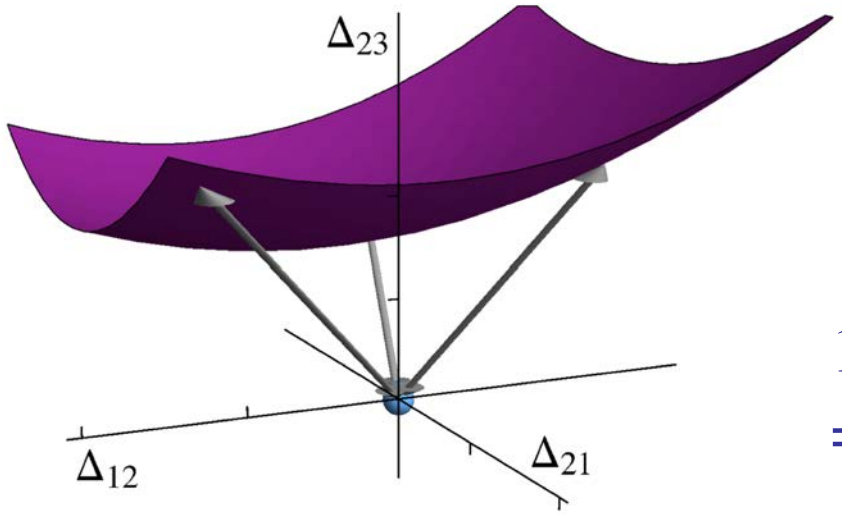
Rudorf et al, *PLoS Comp Biol* (2014)

- Set of in-vitro rates ω_{ij} , set of in-vivo rates ω_{ij}^*
- For each individual transition ij, define the barrier shift

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

in vivo | in vitro

- Multi-dimensional space with coordinates Δ_{ij}



3-dimensional subspace corresponding to three individual rates

12 individual transition rates
=> 12-dimensional Δ space

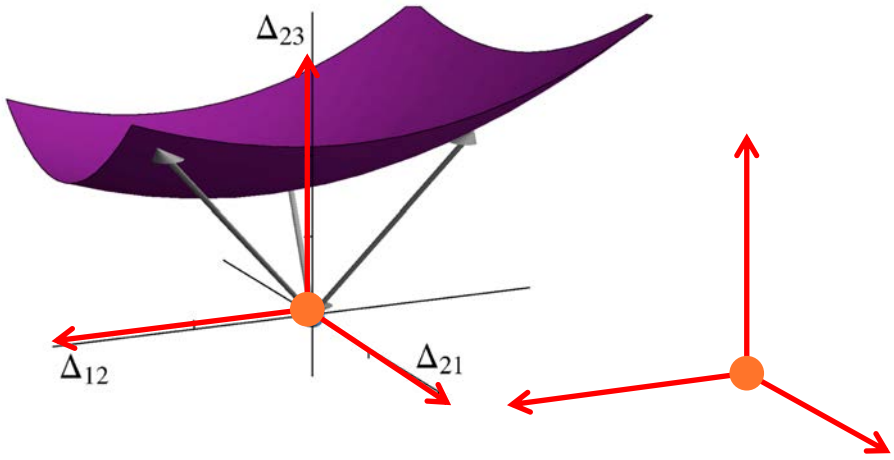
Kinetic Distance: Multistep Process

- Kinetic distance = Euclidean distance in Δ_{ij} –space:

$$\mathcal{D} \equiv \sqrt{\sum \Delta_{ij}^2} = \sqrt{\sum \left[\ln(\omega_{ij}^{\star}/\omega_{ij})\right]^2}$$

- What about ‚weight factors'? Δ_{ij} replaced by $u_{ij}\ \Delta_{ij}$
- Limit of single transition => all $u_{ij} = 1$

- Two different assays, A1 and A2
- Change from A1 to A2 leads to simple coordinate transformation = shift of origin

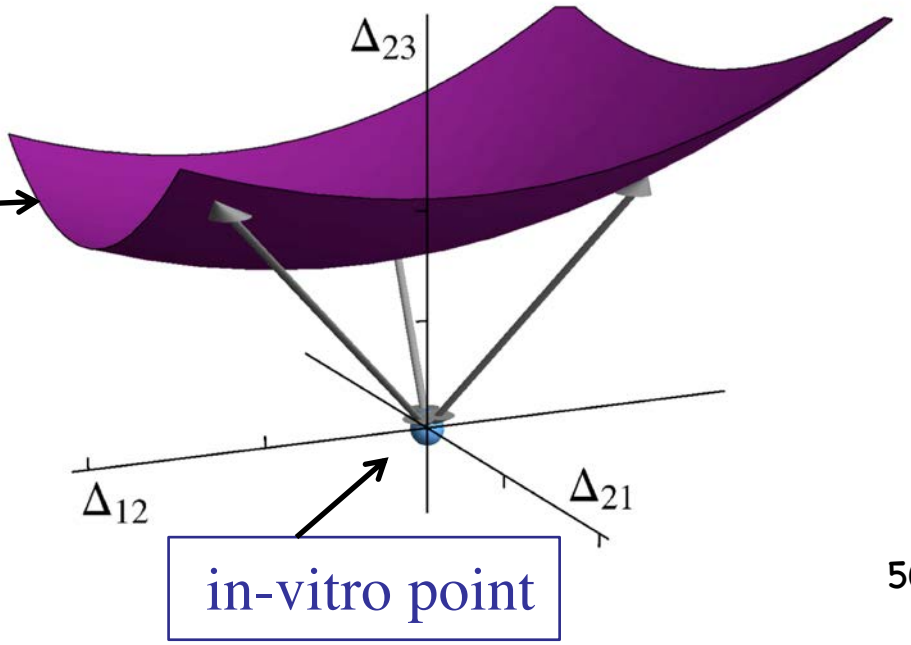


Minimization of Kinetic Distance

- Individual transition rates are not known *in vivo* but overall *in-vivo* speed is known (for different conditions)
- Minimize kinetic distance between known *in-vitro* rates and unknown *in-vivo* rates under overall constraint

- Multi-dimensional Δ_{ij} - space:

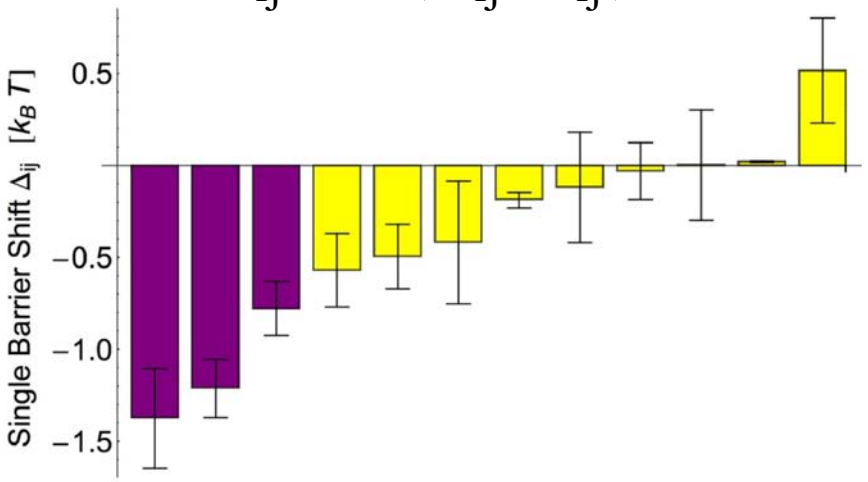
constraint => hypersurface with possible in-vivo points



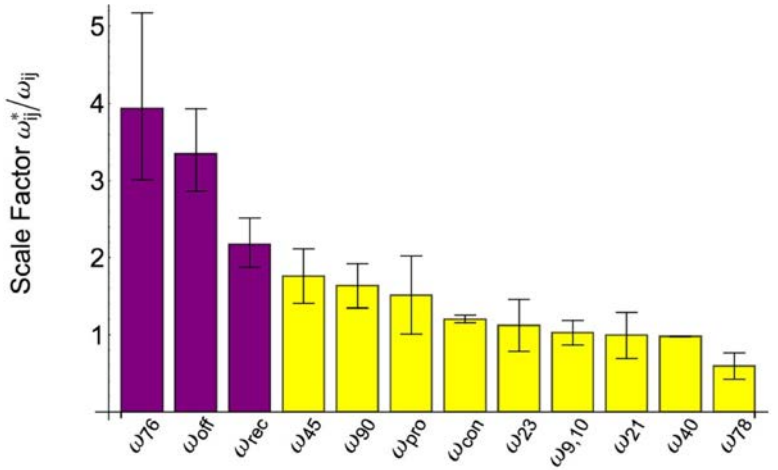
From In-Vitro to In-Vivo Rates

- Single barrier shifts

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



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



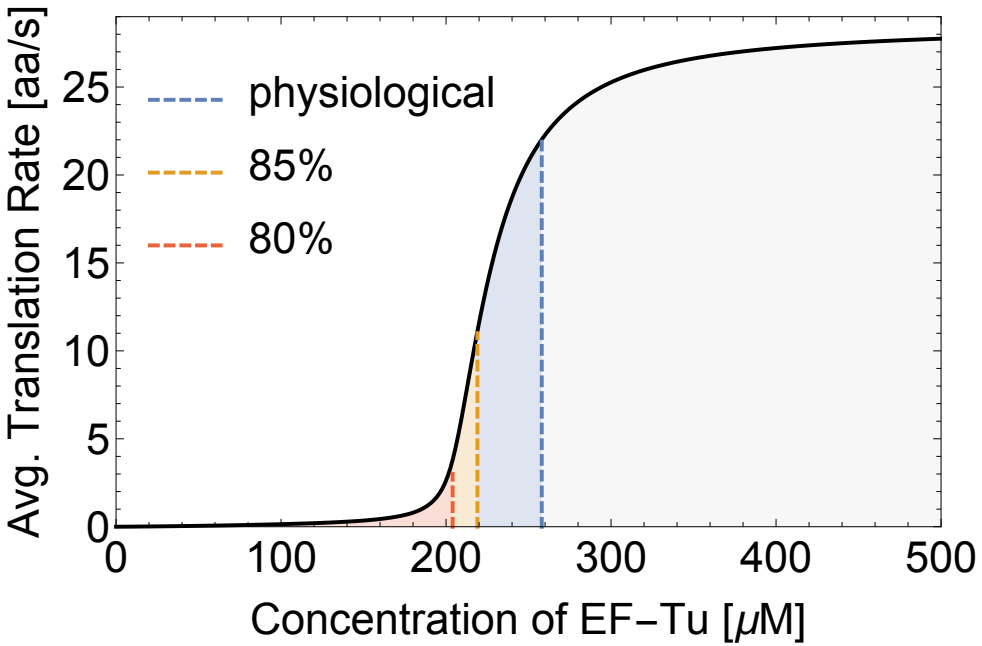
- Three in-vivo rates (purple) are significantly increased
- But not by orders of magnitude
- Confirmed by three in vivo data sets from the literature
- Recently confirmed by a new in vivo experiment

Mustafi, Weisshaar, *mBio* (2018)

Rudorf et al, *PLoS Comp Biol* (2014)

EF-Tu Concentration as a Switch

Sophia Rudorf



Threshold concentration:

$$\mathcal{E}^* = \mathrm{X}_2\left(\frac{\mathrm{X}_1}{\mathrm{X}_2} - \frac{\mathrm{p}_1}{\mathrm{p}_2}\right) = \mathrm{X}_1 + \mathrm{X}_2 - \frac{\mathrm{X}_2}{\mathrm{p}_2}$$

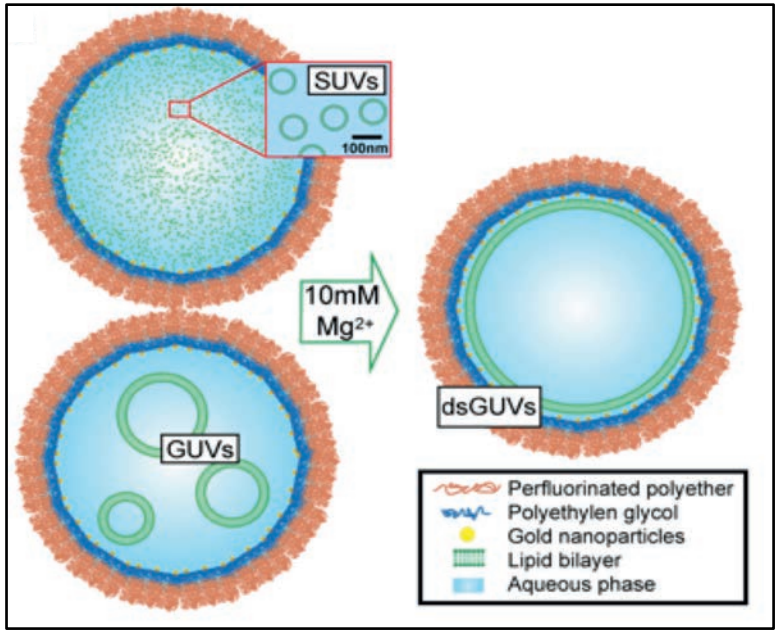
$$\text{for } \frac{\mathrm{X}_1}{\mathrm{X}_2} \geq \frac{\mathrm{p}_1}{\mathrm{p}_2} \text{ and } \mathrm{p}_1 + \mathrm{p}_2 = 1$$

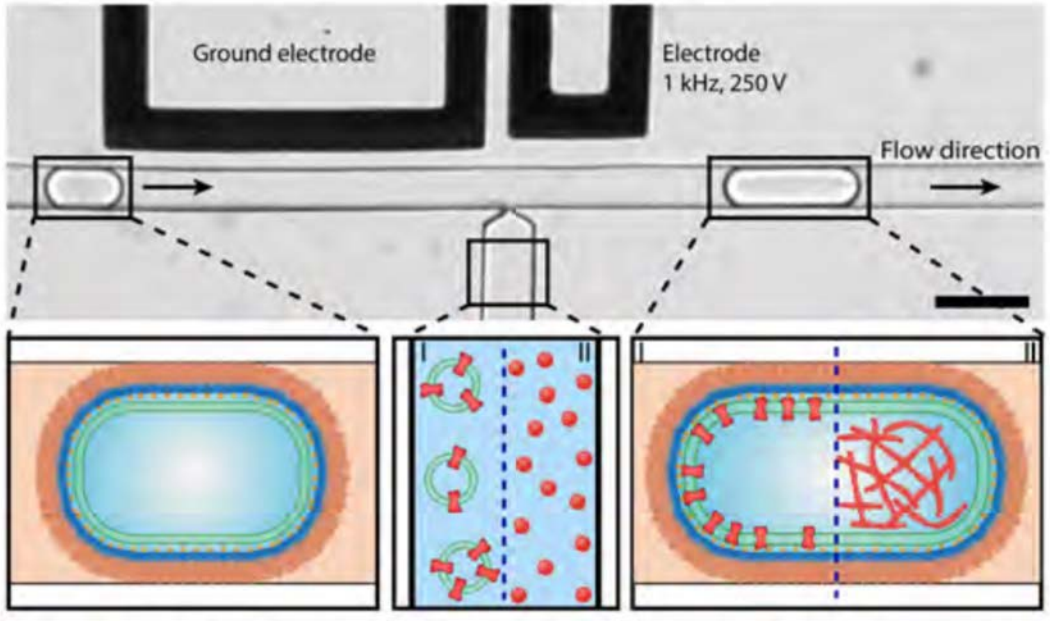
- Ultrasensitive dependence on EF-Tu concentration
- Threshold from imbalance between codons and tRNA

Sequential Bottom-Up Assembly

Weiss et al, *Nature Materials* (2018)

- Four MPIs within MaxSynBio, leading PI: Joachim Spatz



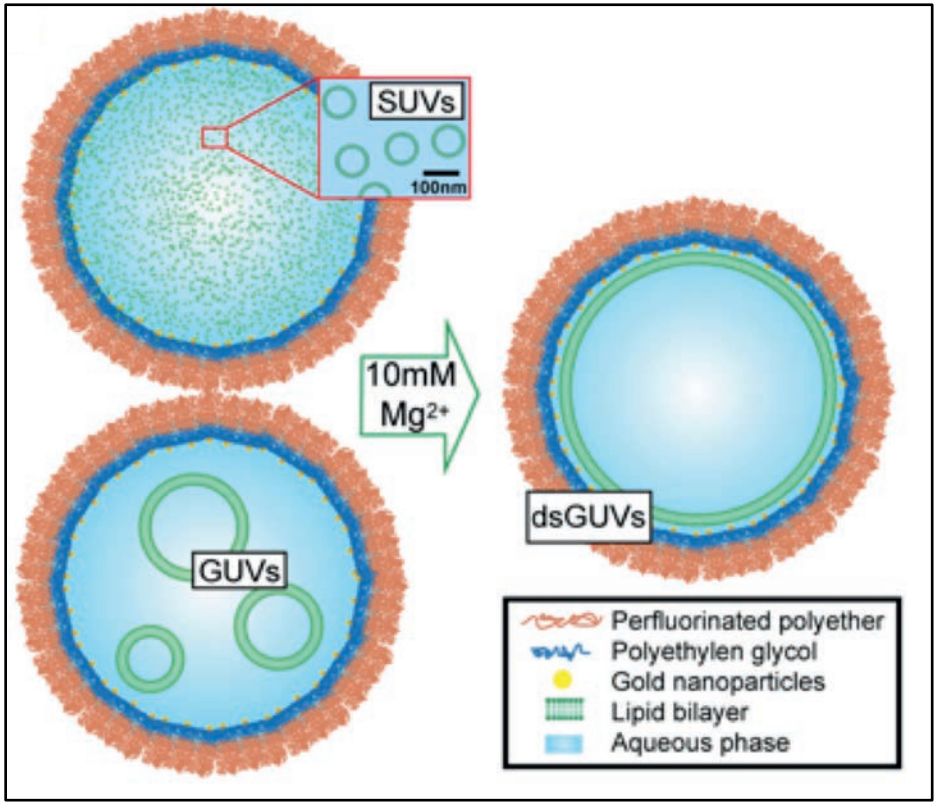


- Water-in-Oil emulsion droplets
- Formation of GUV supported by the droplet surface
- Additional components by pico-injection
- Example: ATP synthase

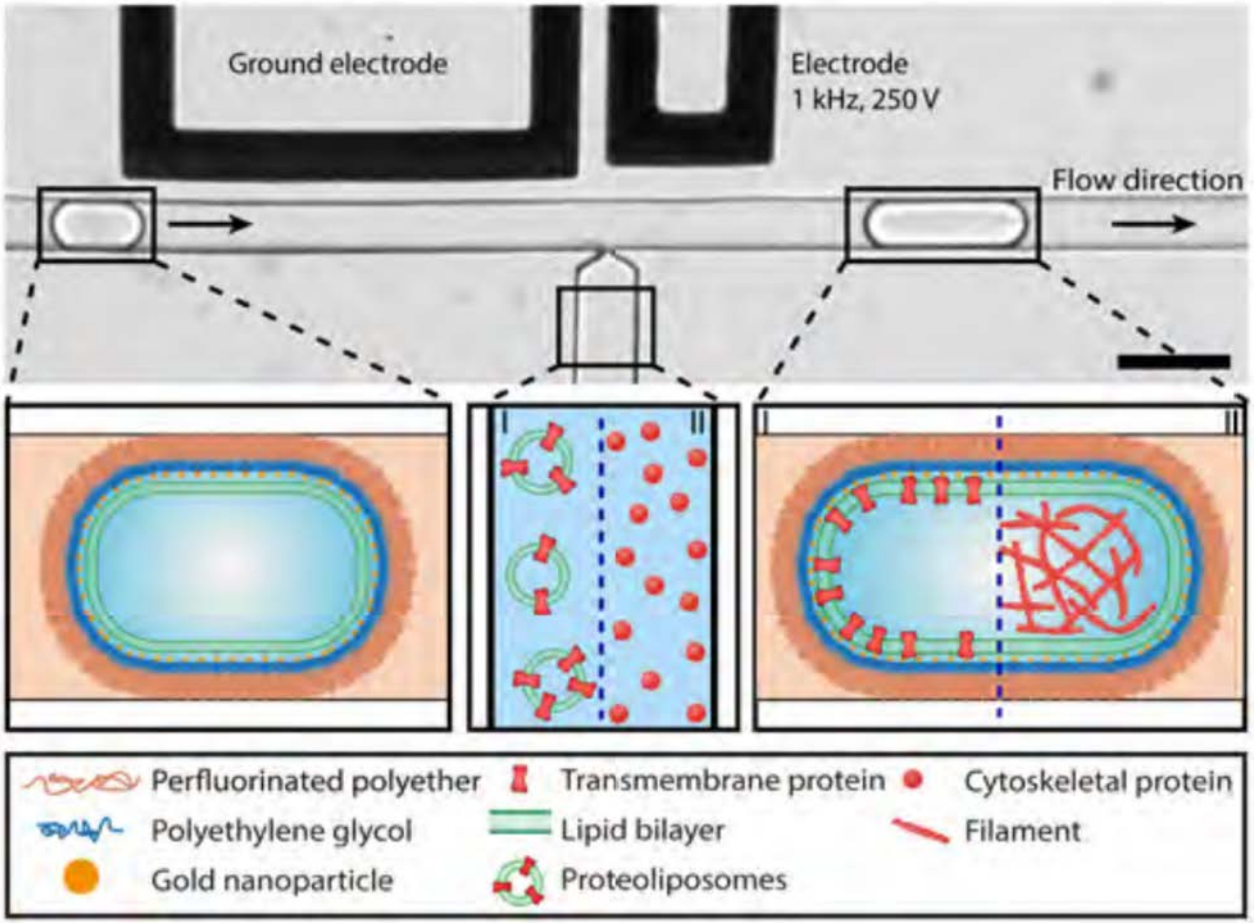
GUVs within W/O Emulsion Droplets

- 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



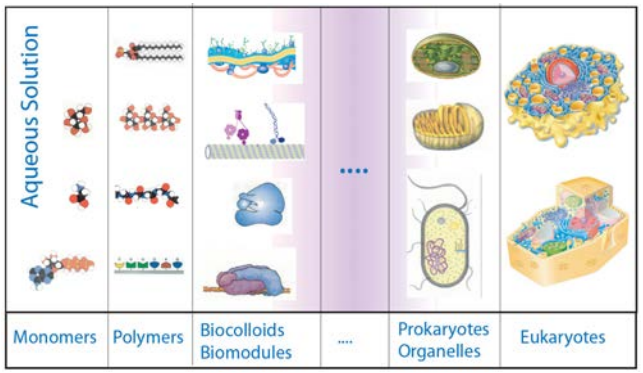
Sequential Pico-Injections



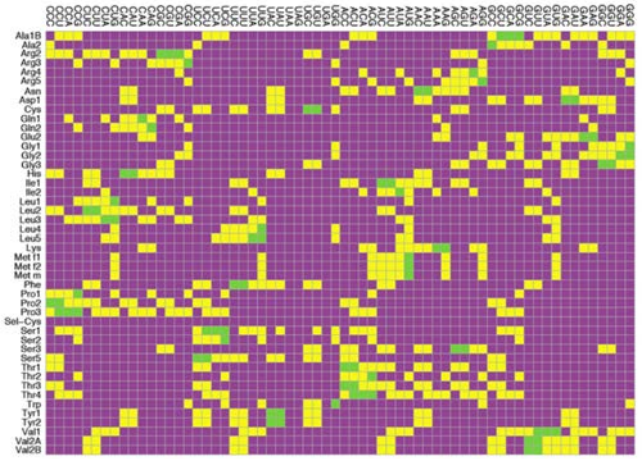
- Pico-injection of membrane and cytoskeletal proteins
- Incorporation of functional ATP Synthase

Perspectives and Challenges

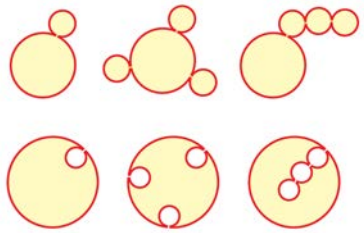
- Further steps of sequential assembly:
  Compartments + ATP synthase + filaments + motors + ...
- Importance of ionic conditions
- Unwanted interactions, complexes
- Alternative assembly pathways



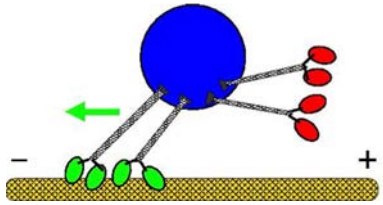
- Evolution via selection (failures)
- Evolution as a learning process
- Ancestor cells after ~ 10^8 years
- Synthetic cells after ??? years
- Persistent complexity gap ? Crisis ?



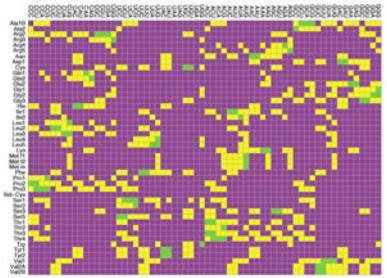
Summary



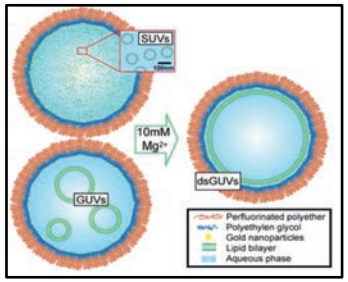
- Membrane compartments,
  Morphological complexity



- Molecular motors,
  Processes far from equilibrium



- Protein synthesis,
  Kinetic distance for multi-step processes



- Droplet-stabilized GUVs,
  new platform for sequential assembly

- Membranes
  - Rumiana Dimova
  - Tom Robinson
  - Jaime Agudo-Canalejo
  - Tripta Bhatia
  - Yunuen Avalos Padillo
  - Jan Steinkühler
- Motors + Ribosomes
  - Stefan Klumpp
  - Sophia Rudorf
  - Mehmet Ucar
  - Stefanie Foerste
  - Nadin Haase
  - Simon Christ
- Collaborations
  - Marina Rodnina
  - Joachim Spatz
  - Tony Hyman
  - Titus Franzmann
  - Günther Kramer
  - Roy Bar-Ziv