Endocytosis of Nanoparticles

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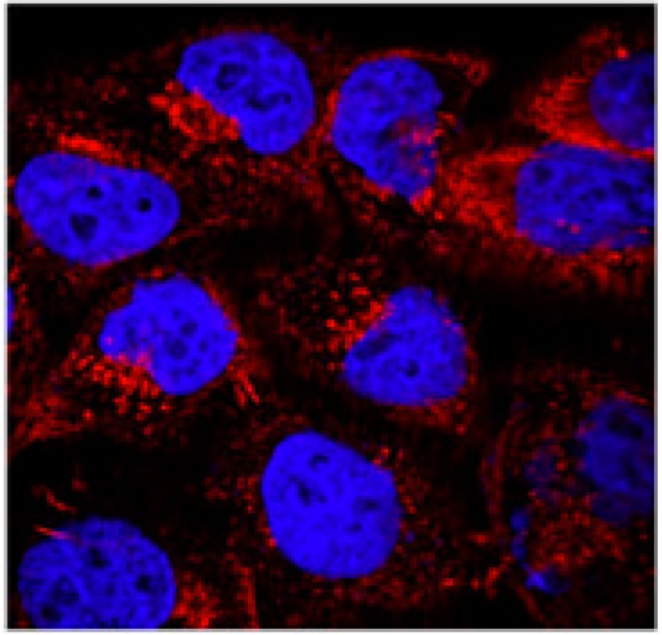
- Motivation: Cellular Uptake of NPs
- Dissecting Endocytosis into
  Adhesion + Engulfment + Fission
- Quantitative Relationships
- Outlook: Engulfment regimes and patterns, Receptor-mediated endocytosis ...

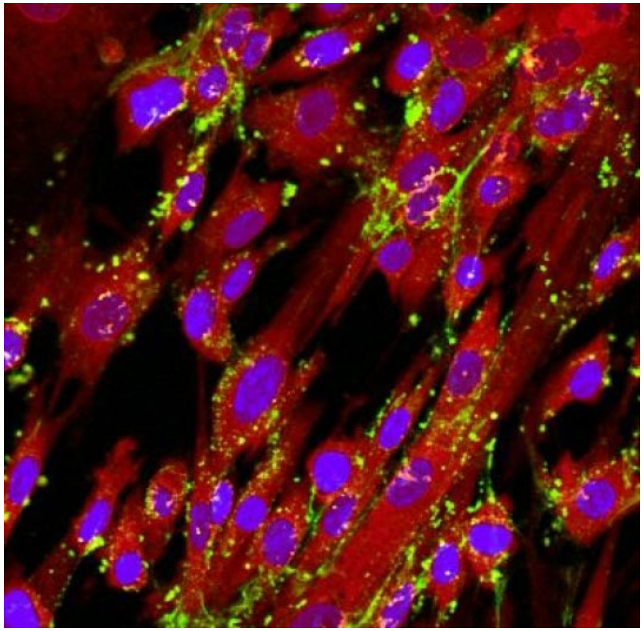
Nanoparticles in Cells: Examples

- Theranostic NPs for siRNA delivery



Liu et al, *PNAS* (2016)

- Gender-dependent cellular uptake of NPs



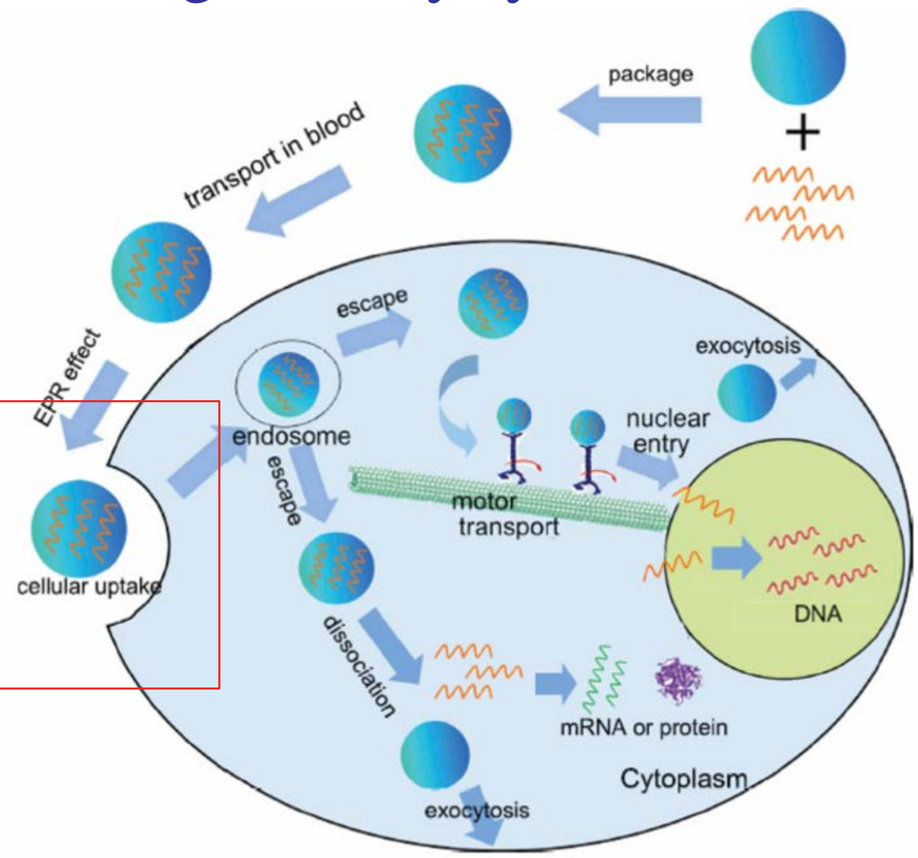
Serpooshan et al, *ACS Nano* (2018)

Targeting Nanoparticles to Cells

- Nanoparticles (NPs) as drug delivery systems:

Transport of NPs towards cells

Transport across cell membrane by endocytosis

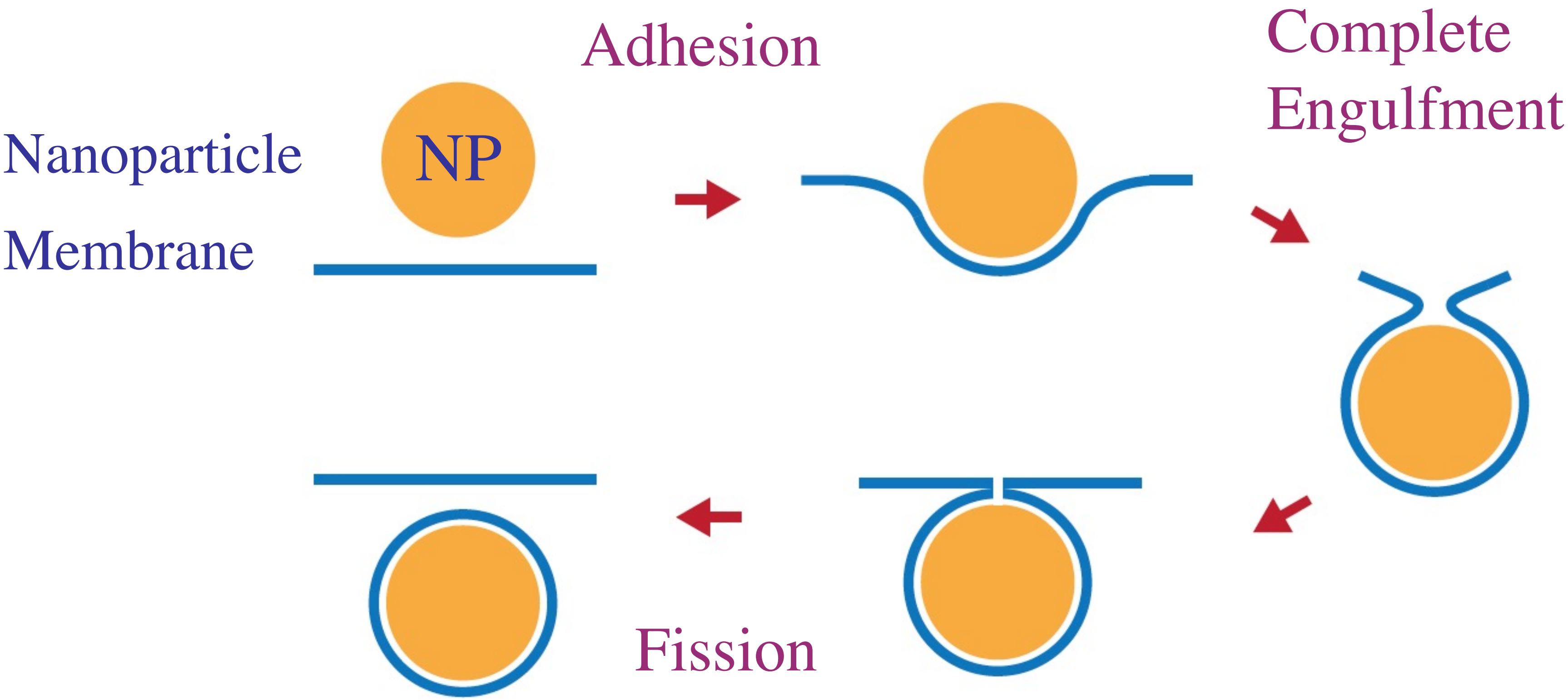


H.-M. Ding and Y.-Q. Ma, *Small* (2015)

- Endocytic pathway also used by virusses,

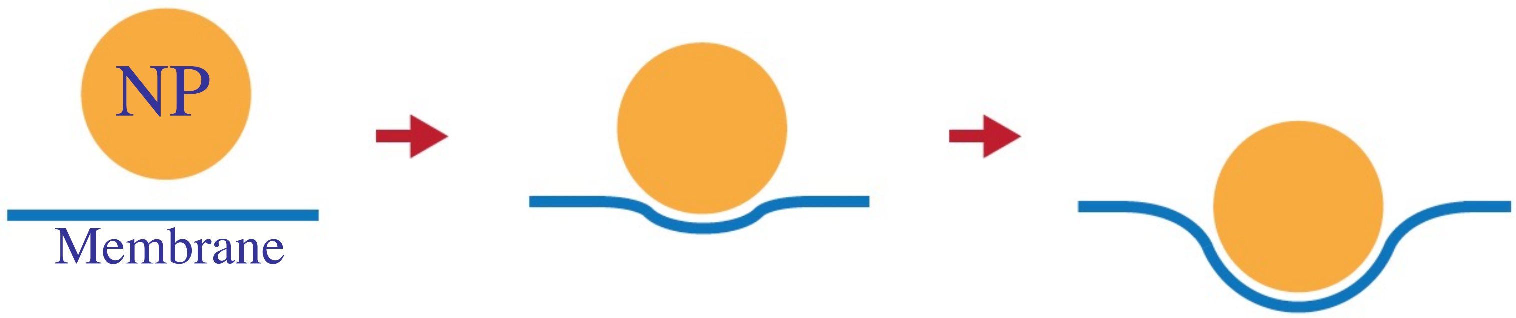
  airborne ultrafine particles, ...

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 $\varkappa$ versus adhesive strength $|W|$

Bending Rigidity

- Bending energy proportional to bending rigidity $\varkappa$
- Bending rigidity is of the order of 10^{-19} J = 20 k_B T
- Some values measured for lipid bilayers:

lipid bilayer	adhesive material	κ [10^{-19} J]	$\|W\|$ [mJ/m^2]	R_W [nm]
DMPC	silica	0.8	$0.5 - 1$	13 - 18
eggPC	glass	$\simeq 1$	0.15	26
DMPC	receptor-ligand	0.8	0.03	73
DOPC/DOPG	coated glass	0.4	3×10^{-4}	510
DOPC/DOPG	glass	0.4	10^{-5}	2800

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

Adhesive Strength

- Adhesion free energy proportional to contact area
- Adhesive strength |W| = adhesion free energy per area
- Adhesive strength $|W|$ reflects NP surface chemistry and membrane composition

lipid bilayer	adhesive material	κ $[10^{-19}$ J]	$\lvert W \rvert$ [mJ/m^2]	R_W [nm]
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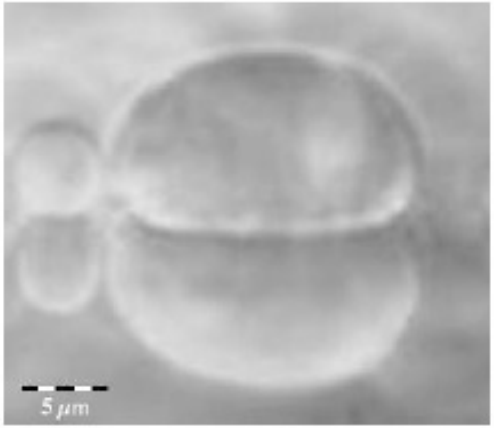
- Adh strength |W| varies over orders of magnitude

Adhesion Length

- Competition between κ and |*W*| :

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



- Experimentally accessible via membrane curvature along contact line

Gruhn et al, *Langmuir* (2007)

lipid bilayer	adhesive material	κ $[10^{-19}$ J $]$	$\|W\|$ $[\mathrm{mJ/m^2}]$	R_W [nm]
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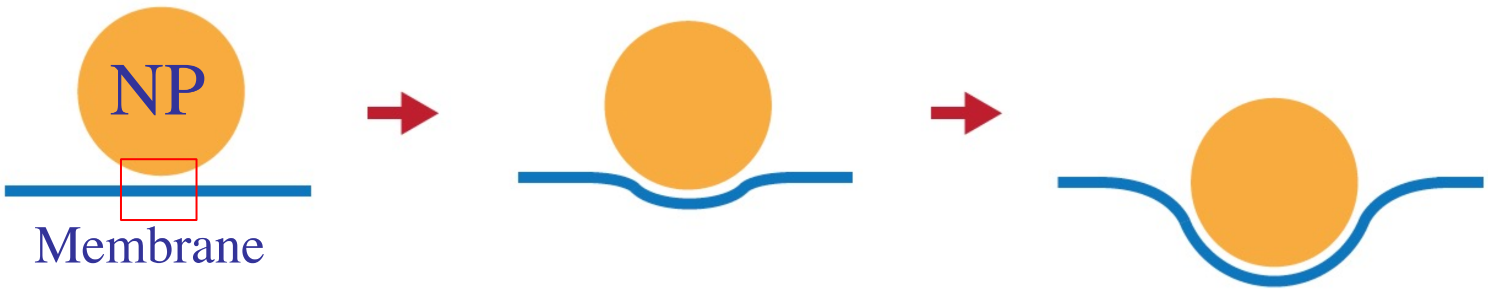
- Strong/weak adhesion = small/large adhesive length R_W

Onset of Adhesion: Key Parameters

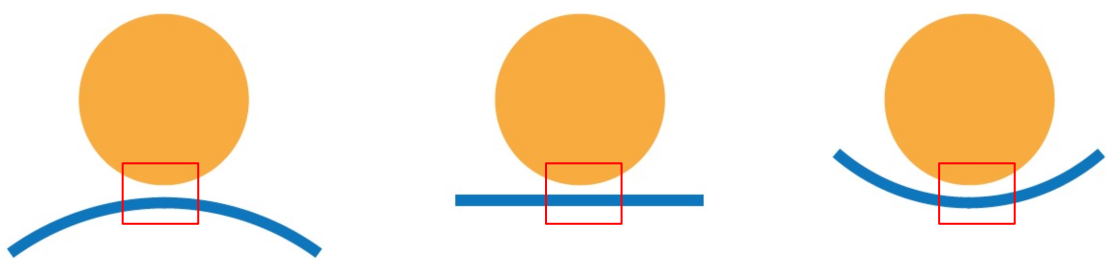


- Three key parameters for onset of adhesion:

  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

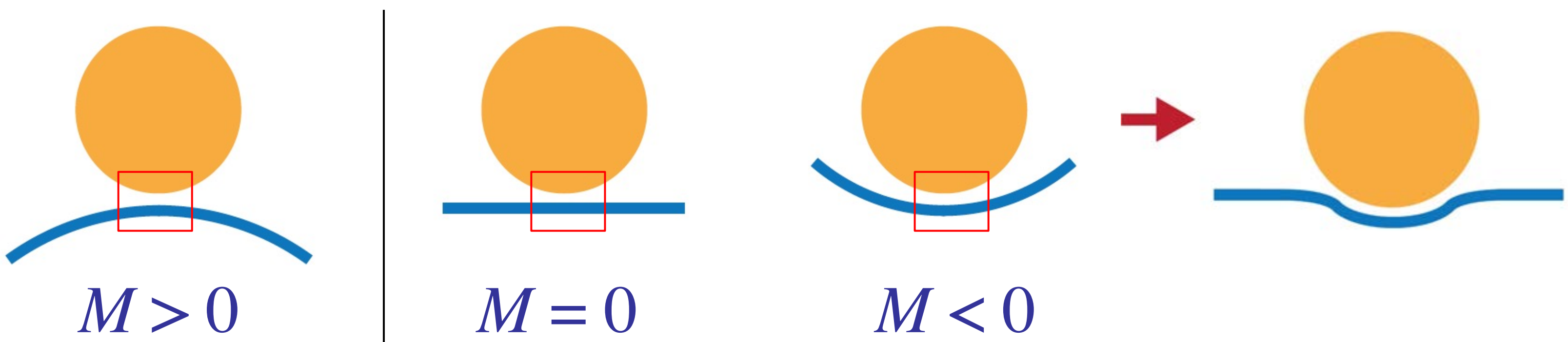
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Membrane starts to spread over particle if

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

contact curvature M_{co} is threshold value for M

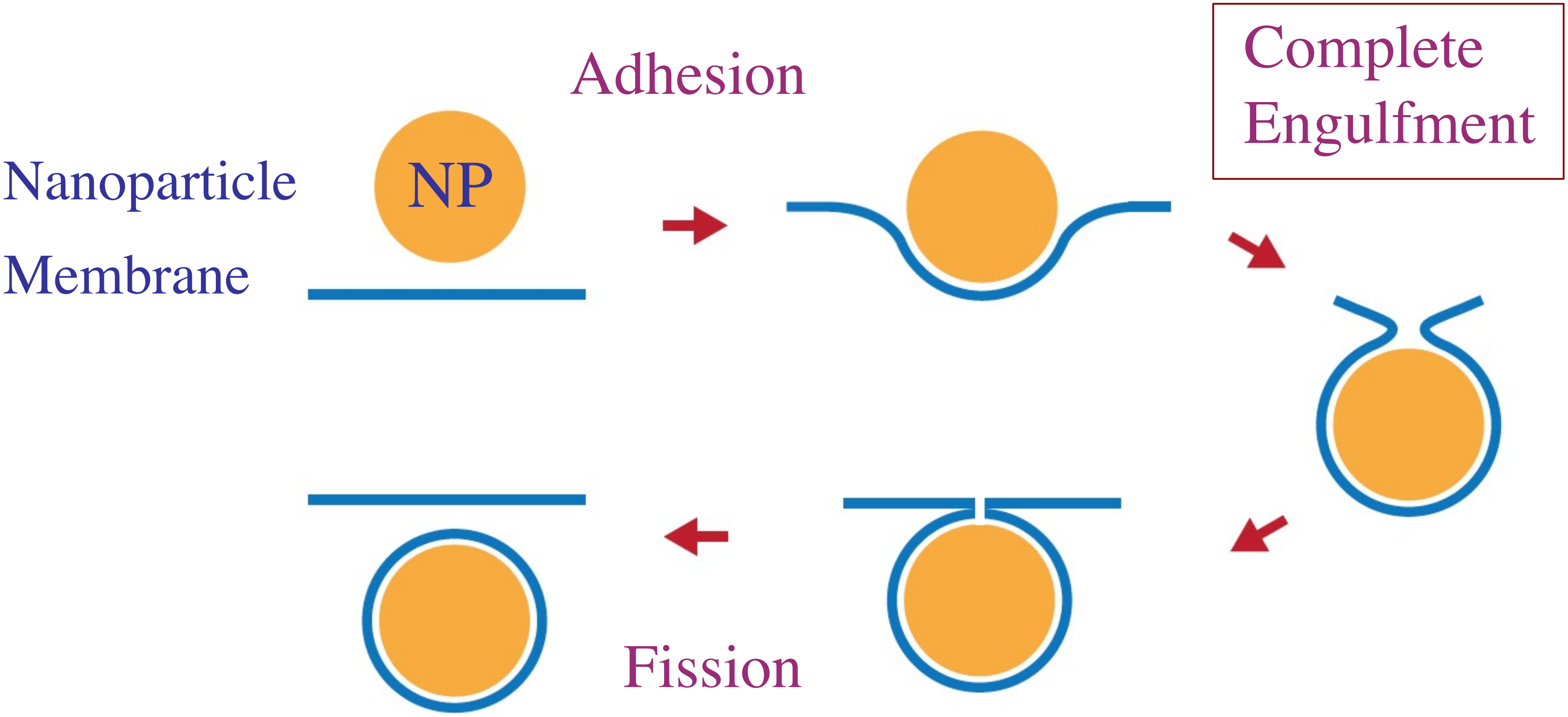
- Example: $R_{\mathrm{W}} = R_{\mathrm{pa}}$ or $M_{\mathrm{co}} = 0$



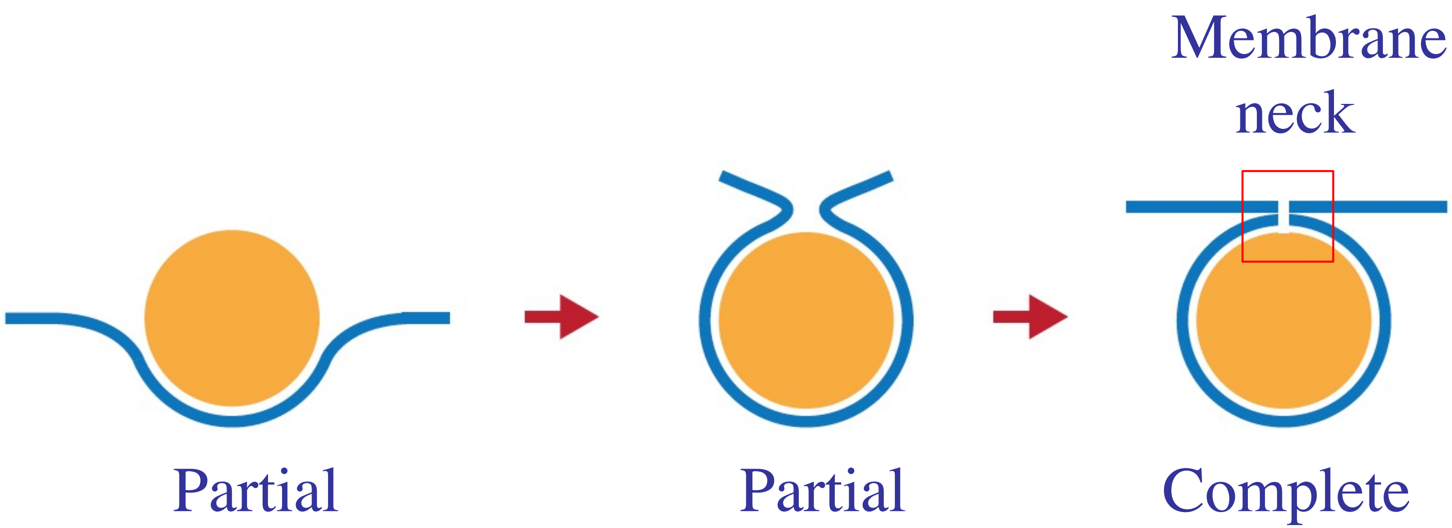
no adhesion | adhesion and spreading

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

Endocytosis: Complete Engulfment



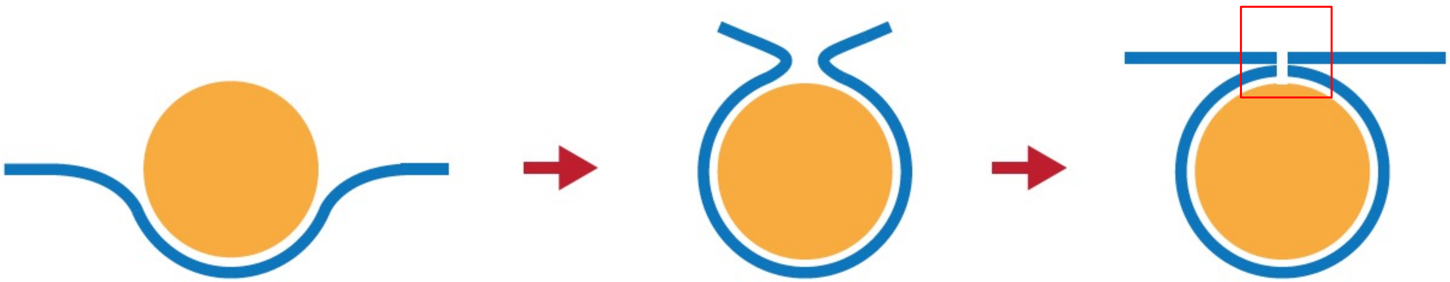
Engulfment: Basic Aspects



- 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: Key Parameters



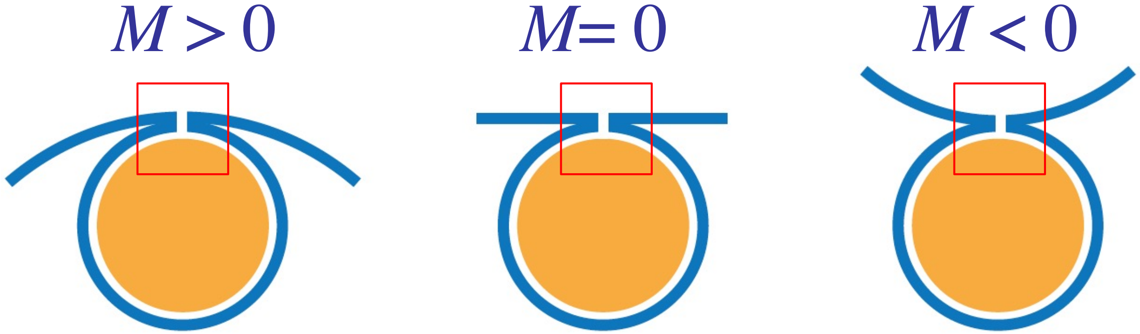
- Four key parameters for neck stability:

  Adhesion length R_W , Particle size R_{pa} ,

  Curvature M of unbound neck segment

  Spontaneous (or preferred) curvature m

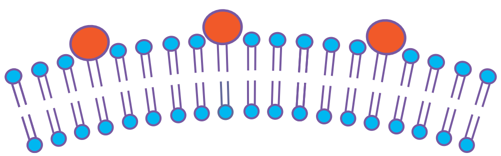
- Membrane curvature M can again be positive or negative:



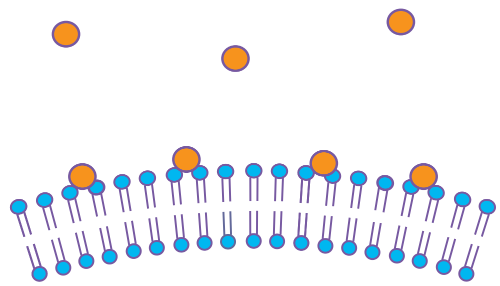
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

Spontaneous Curvature

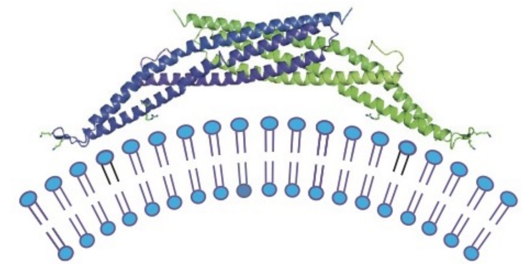
- Spontaneous curvature *m* describes bilayer asymmetry:



Asymmetric composition, e.g., ganglioside



Asymmetric adsorption of small molecules



Asymmetric protein coats, e.g. BAR-domain

- Membrane prefers to curve towards one side
- Large *m*-values lead to spontaneous tubulation
- Several methods to determine *m* from nanotubes

Liu et al, *ACS Nano* (2016); Bhatia et al, *ACS Nano* (2018)

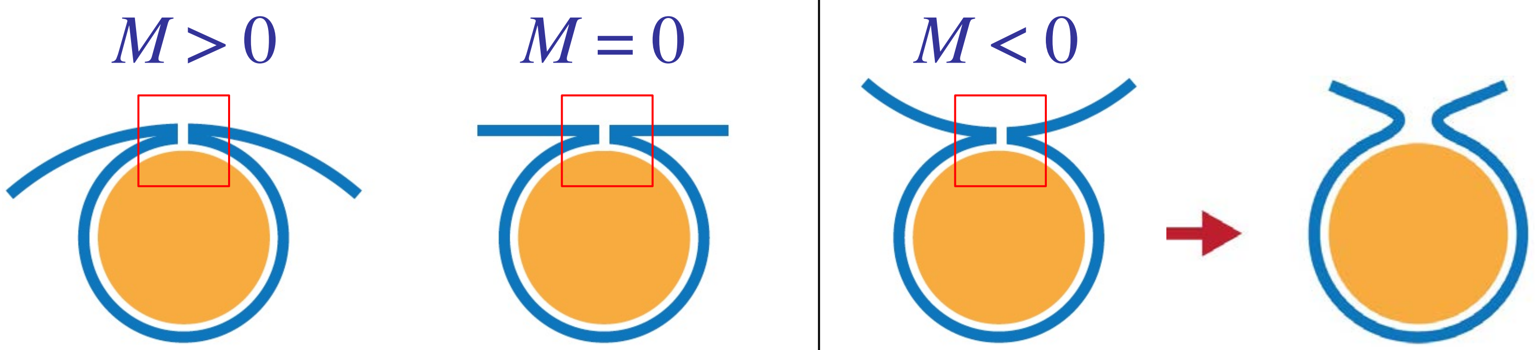
Neck Stability: Local Criterion

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

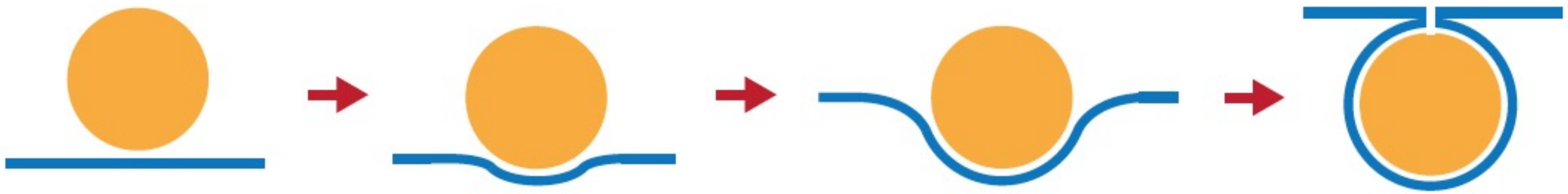


closed neck is stable

closed neck is unstable and opens up

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

From Adhesion to Engulfment



- Two local conditions for
  onset of adhesion and stability of closed neck

- Combination of both local conditions:

$$2m + 1/R_{pa} - 1/R_{W} \leq M \leq 1/R_{W} - 1/R_{pa}$$

- Technical point: Limit of small particle size R_{pa}

Agudo-Canalejo and RL, *Soft Matter* (2017)

Role of Spontaneous Curvature

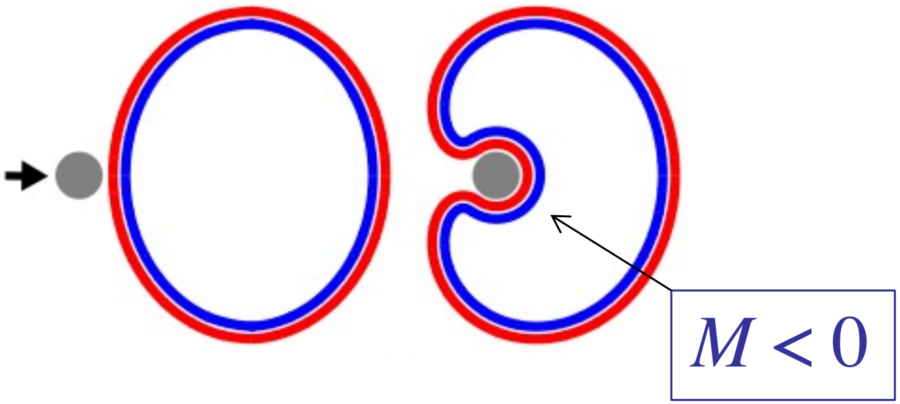
- Contact curvature $M_{co} = 1/R_W - 1/R_{pa}$
- Combined conditions:

$$2m - M_{co} \le M \le M_{co}$$

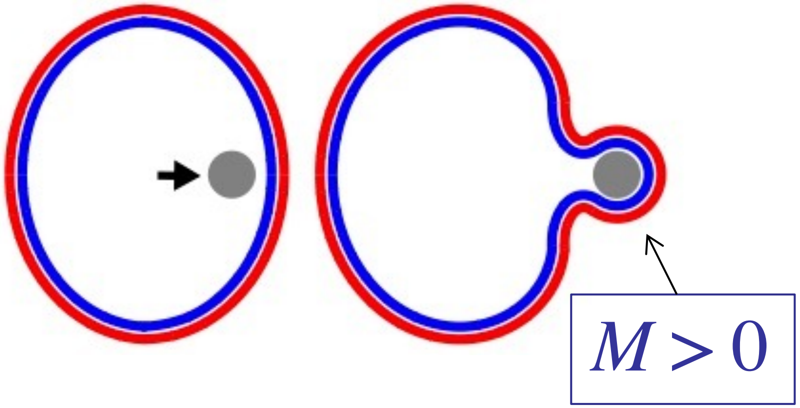
- Symmetric membrane with $m = 0$:
  complete engulfment for $M_{co} > 0$ or $R_{pa} > R_W$
- Positive spont curv $m > 0$ suppresses endocytosis
- Negative spont curv $m < 0$ enhances endocytosis

Endocytosis versus Exocytosis

- Endocytic engulfment:



- Exocytic engulfment:



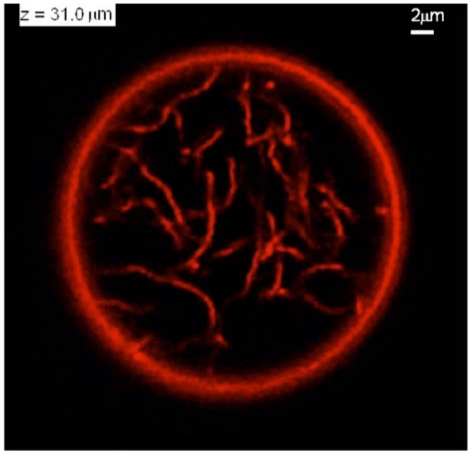
- Particles from exterior
- Negative curvature M of bound membrane segment
- Favored by $m < 0$

- Particles from interior
- Positive curvature M of bound membrane segment
- Favored by $m > 0$

Spont Curvature from Experiments

Significant spont curvature m leads to membrane nanotubes with width ~ $1/m$

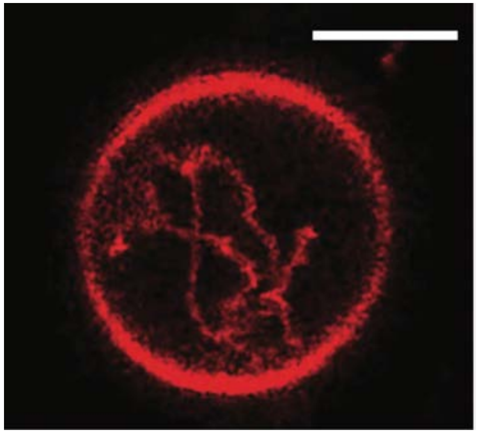
Liu et al, *ACS Nano* (2016)



- Ternary lipid membranes (DOPC, DPPC, Chol) and PEG-dextran solutions,

$m \approx - 1/(125 \text{ nm})$

Bhatia et al, *ACS Nano* (2018)



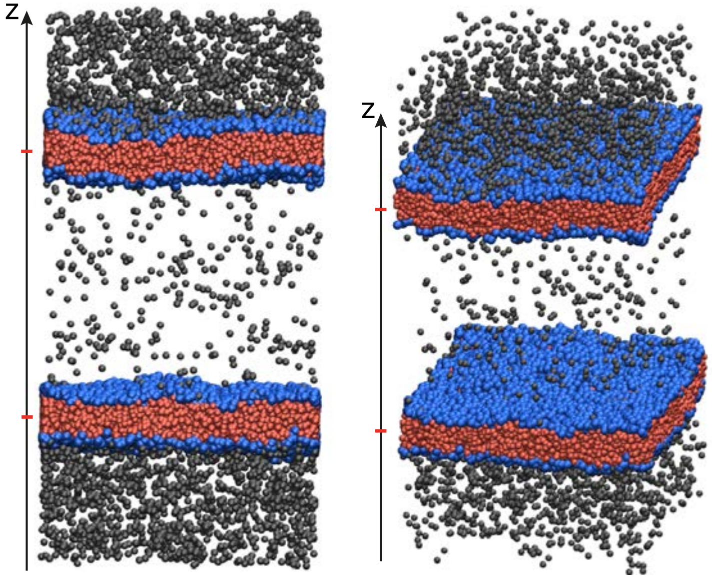
- POPC membranes doped with ganglioside GM1

$m \approx - 1/(95 \text{ nm})$

Spont Curv from MD Simulations

Rozycki and RL, *J. Chem. Phys.* (2015); *J. Chem. Phys.* (2016)

- Example: Adsorption and depletion layers:



Particle concentration X_{ex}

Bilayer 1

Particle concentration X_{in}

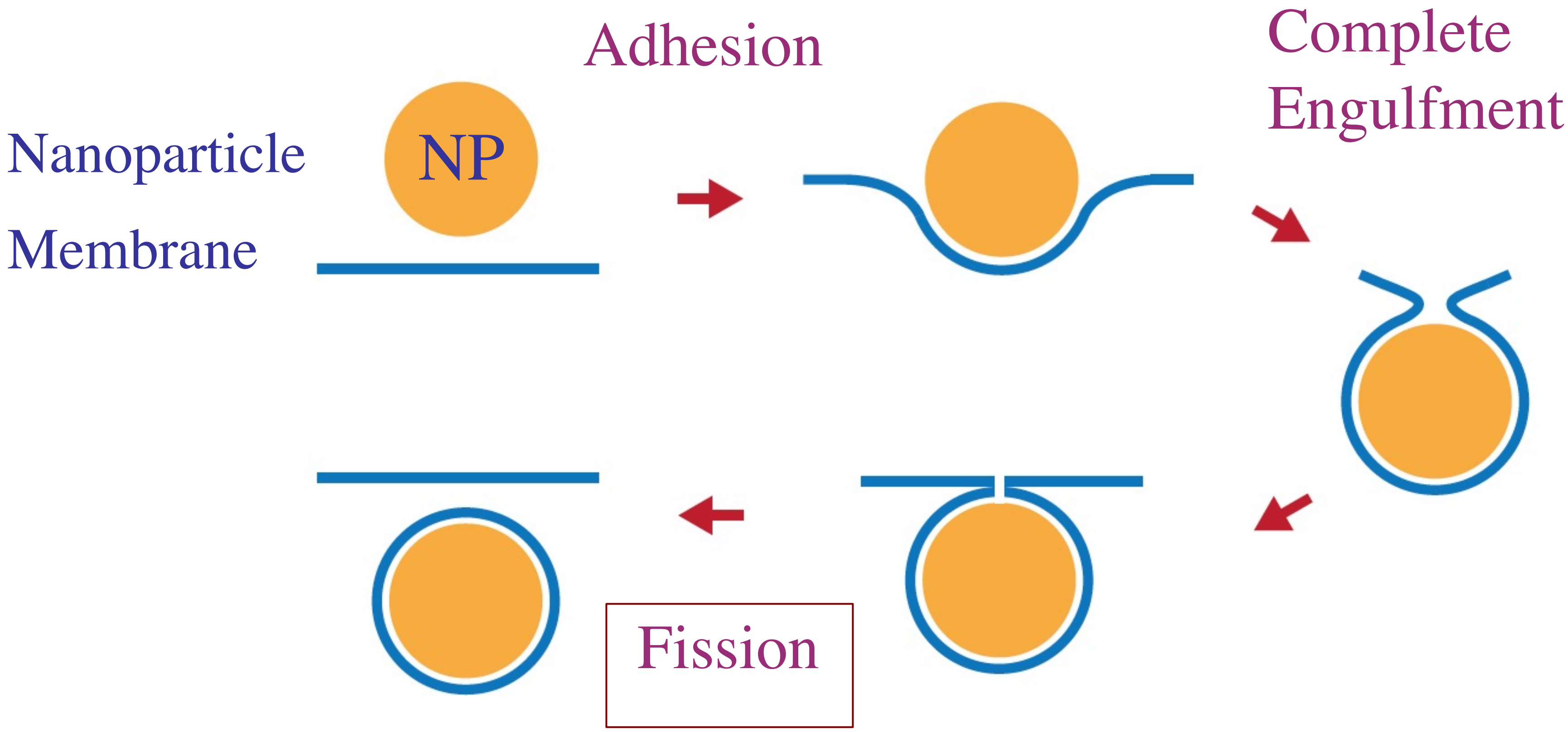
Bilayer 2

Particle concentration X_{ex}

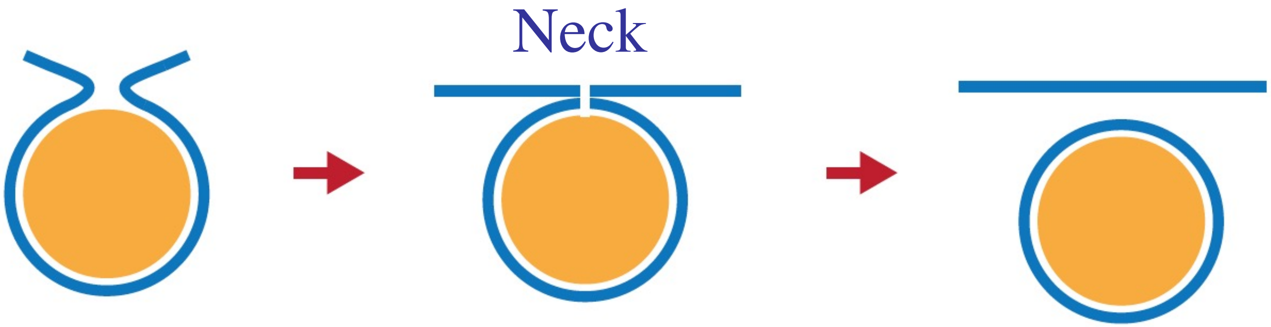
- Spont curv proportional to $\pm\,(X_{ex} - X_{in}) = \pm\,\Delta X$
- Example: 1 nm particles, $\Delta X = 100$ mM

Adsorption: $m = \pm 1/(77\text{ nm})$, Depletion: $m = \pm\, 1/(270\text{ nm})$

Endocytosis: Fission



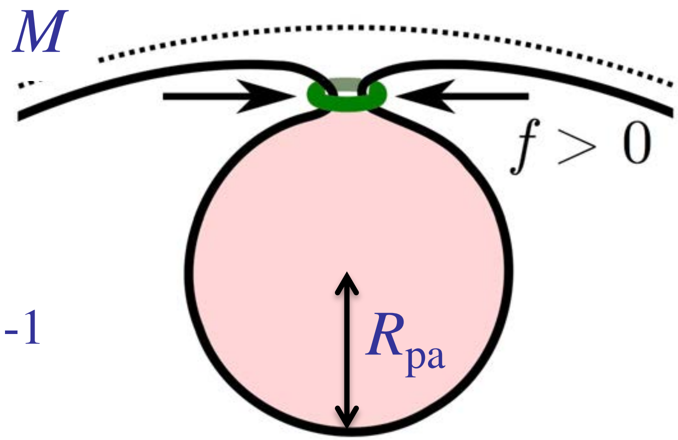
Fission: Basic Aspects



- Closure and cleavage of membrane neck
- Both steps are facilitated by constriction forces
- Biologists emphasize forces generated by proteins
- But both adhesion and spontaneous curvature generate effective constriction forces

Stability of Membrane Necks II

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



- Include constriction force f

- Generalized stability relation:

$$M \geq 2m + 1/R_{pa} - 1/R_W - f\,(4\pi\kappa)^{-1}$$

- Additional f-term stabilizes closed neck
- Three mechanisms for neck stabilization:

  - large, negative spontaneous curvature m
  - large adhesive strength $|W|$
  - large constriction force f

Linear superposition !

Effective Constriction Forces

- General stability relation for m < 0:

$$M + f\,(4\pi\kappa)^{-1} - 2\,m + 1/R_W - 1/R_{\text{pa}} \geq 0$$

- Effective constriction force

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

- 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

Summary: Local Relations

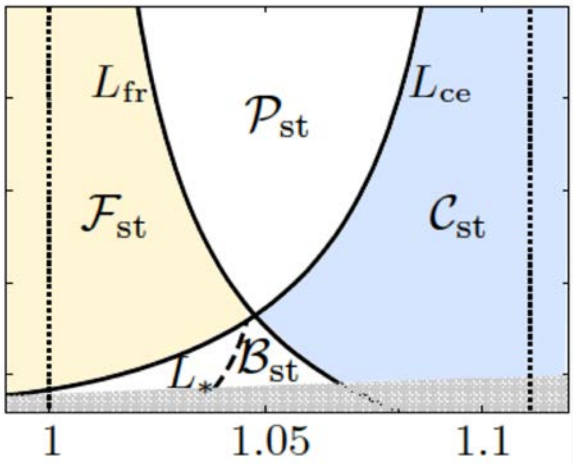
- Onset of adhesion: $M \leq 1/R_{\mathrm{W}} - 1/R_{\mathrm{pa}} = M_{\mathrm{co}}$
- Alternative interpretation: Instability of free NP state
- Stability of closed membrane neck: $M \geq 2m - M_{\mathrm{co}}$
- Combined relations: $2m - M_{\mathrm{co}} \leq M \leq M_{\mathrm{co}}$
- Defines regime C_{st} of complete engulfment with unstable free state and stable membrane necks
- Effective constriction force:

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

Consequences of Local Relations

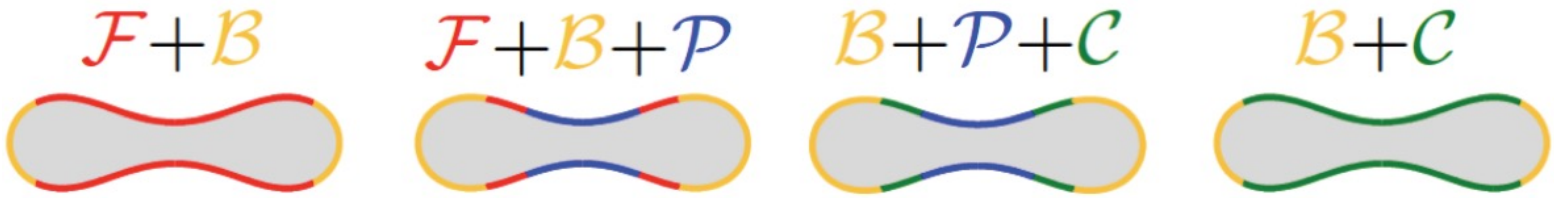
- Critical particle sizes for engulfment

  Combination of two stability relations for ***F*** and ***C*** states leads to four stability regimes $\boldsymbol{F}_{st}$, $\boldsymbol{B}_{st}$, $\boldsymbol{C}_{st}$ and $\boldsymbol{P}_{st}$



- Engulfment patterns from many particles

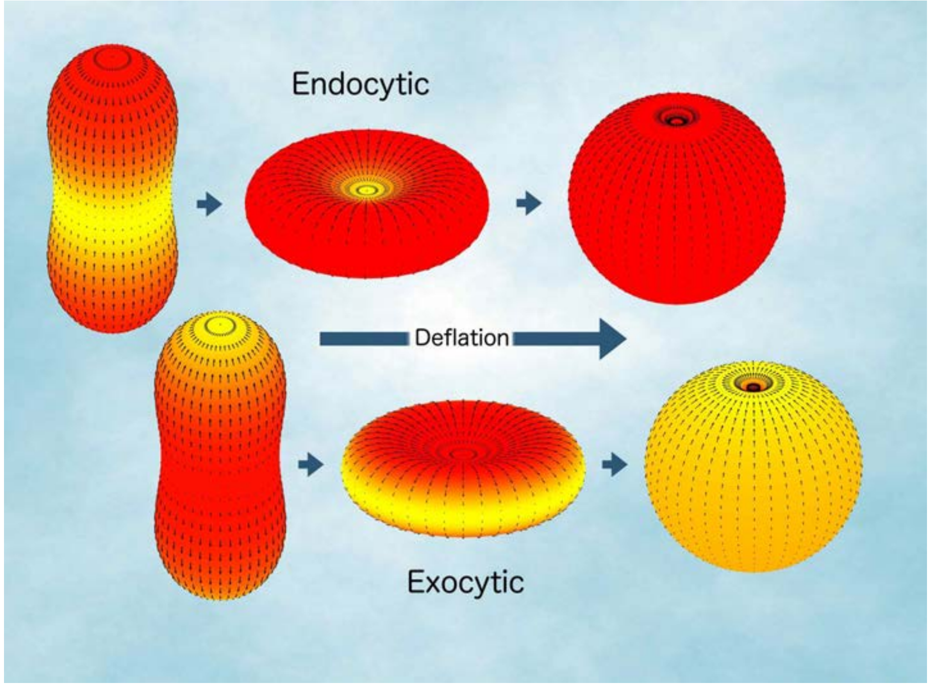
  Nonspherical shapes have variable segment curvature
  Different engulfment states coexist on the same vesicle



Curvature-Induced Forces

Agudo-Canalejo and RL, *Soft Matter* (2017)

- Vesicle shape with variable membrane curvature *M*



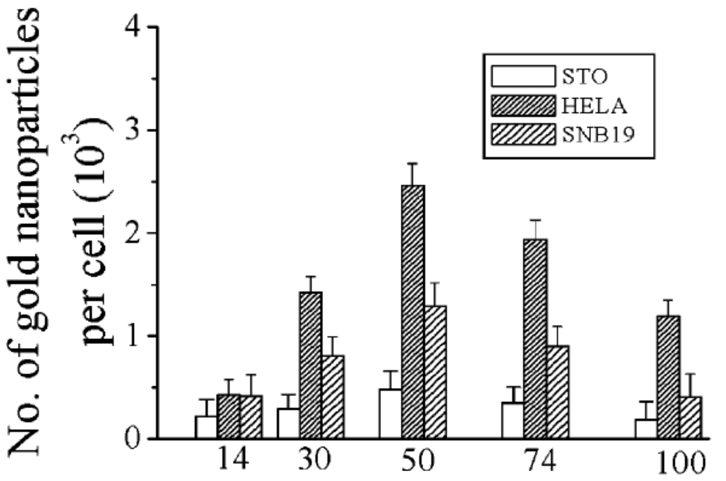
- Curvature gradients
- NPs follow gradients
- Endocytosis: NPs move to minima of curvature
- Exocytosis: NPs move to maxima of curvature

Receptor-Mediated Endocytosis

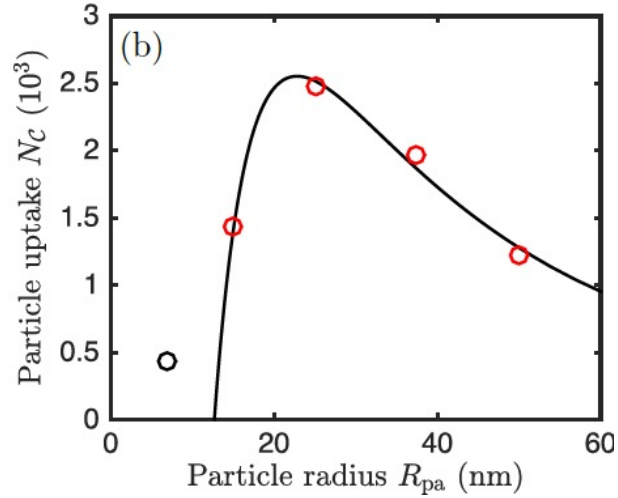
Chithrani et al, *Nano Letters* (2007)

- 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)

Coworkers

Jaime Agudo-C.

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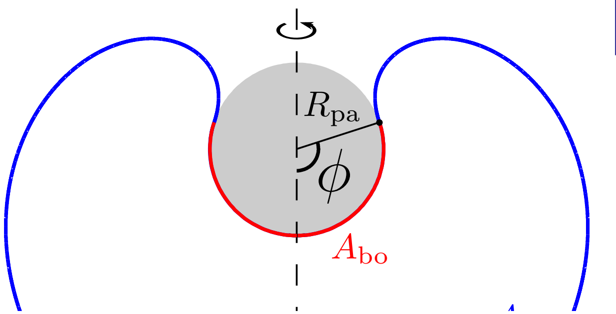
Shape Functional with Adhesion

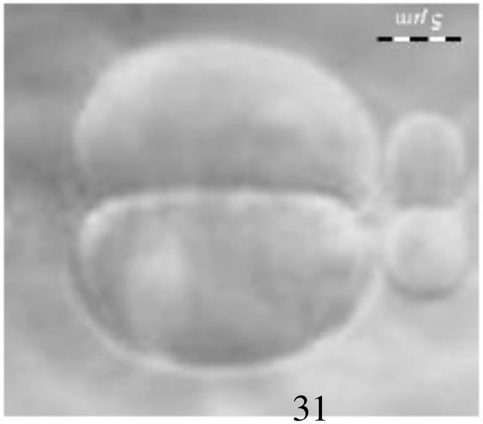
- Shape functional: $E = E_{cu} + \Sigma A - \Delta P\, V - W A_{bo}$

  Adhesive strength $W > 0$
  Area A_{bo} of bound membrane segment

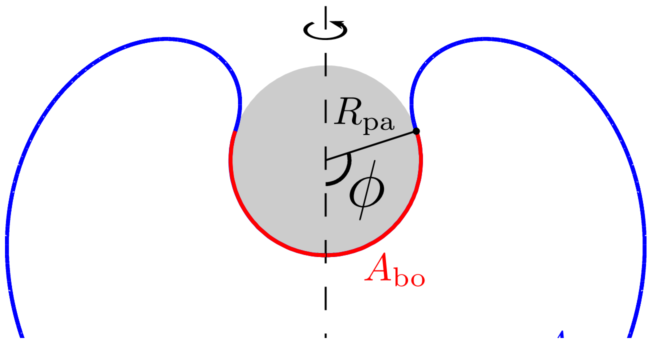


- Competition between adhesion and bending encoded in adhesion length $R_W = (2\kappa/W)^{1/2}$
- Contact mean curvature M_{co} for membrane adhesion to planar surface:

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



Contact Mean Curvature at Particle



- Membrane adhering to particle
- Bound membrane segments (red) follows particle surface
- Contact line provides boundary condition for unbound segment (blue)
- Principical curvatures along contact line:

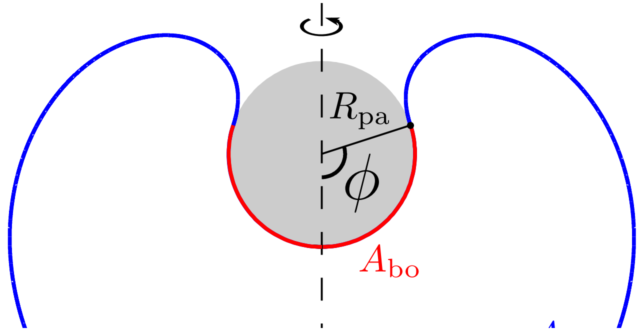
$$C_1 = (2W/\kappa)^{1/2} - 1/R_{pa} \quad \text{(along contour)}$$

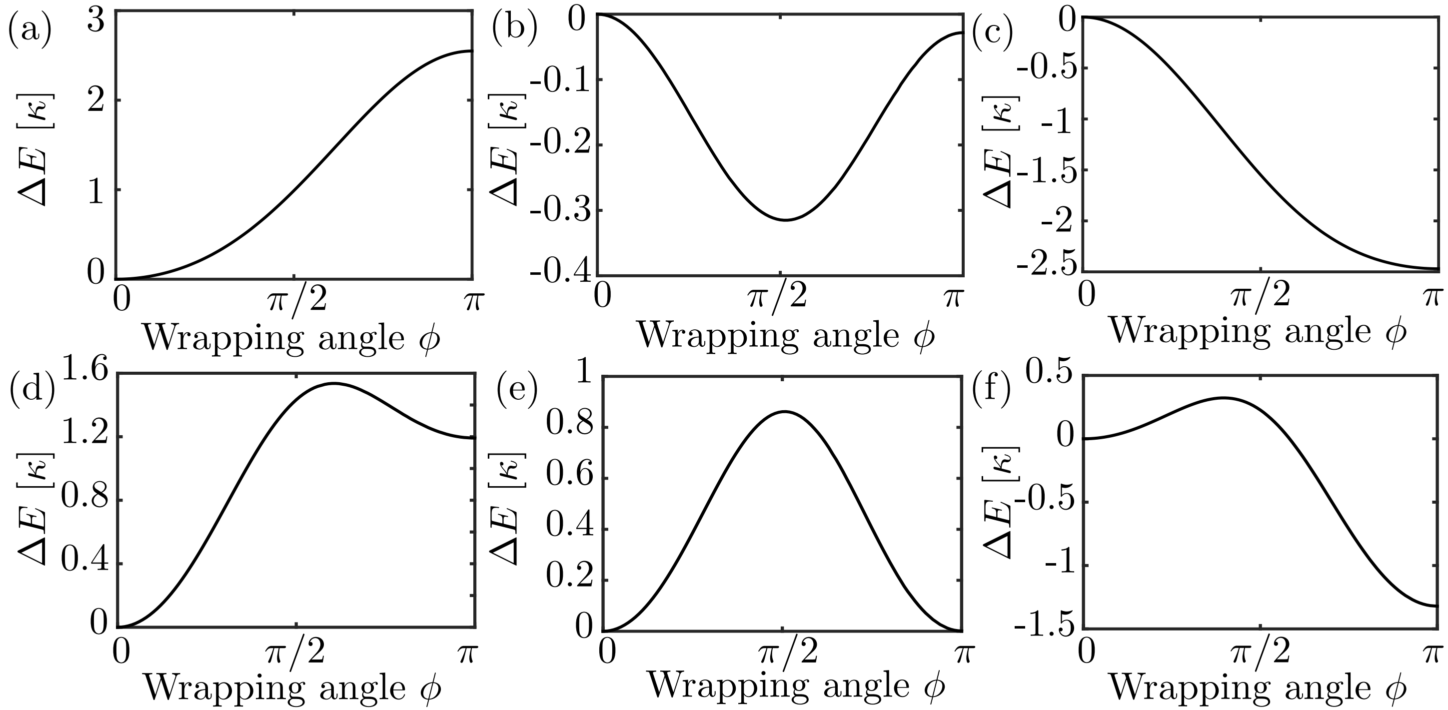
$$C_2 = -1/R_{pa} \quad \text{(perp to contour)}$$

- Contact mean curvature: $$\boxed{M_{co} = 1/R_W - 1/R_{pa}}$$
- Independent of spontaneous curvature *m* !

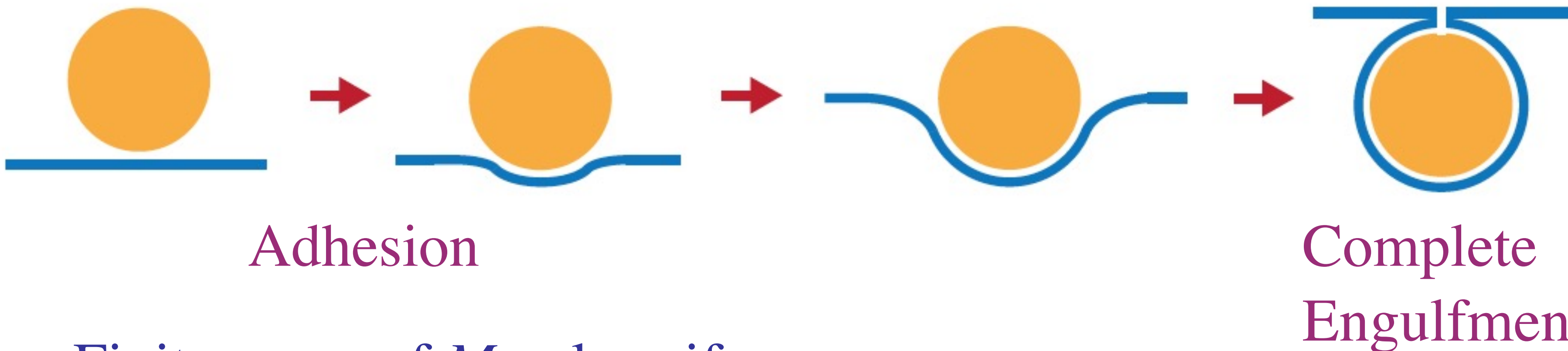
(In)Stabilities from Energy Landscapes

- Wrapping angle ϕ can vary from $\phi = 0$ to $\phi = \pi$ i.e., from state $\boldsymbol{F}$ to state $\boldsymbol{C}$





Condition on Material Parameters



- Finite range of *M*-values if

$$M_{cn} = 2m + 1/R_{pa} - 1/R_W < M_{ad} = 1/R_W - 1/R_{pa}$$

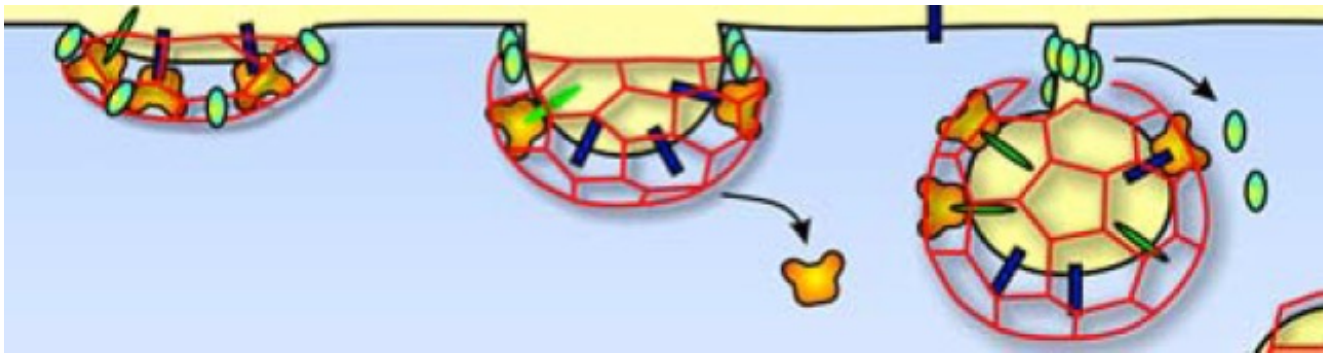
or

$$m < 1/R_W - 1/R_{pa}$$

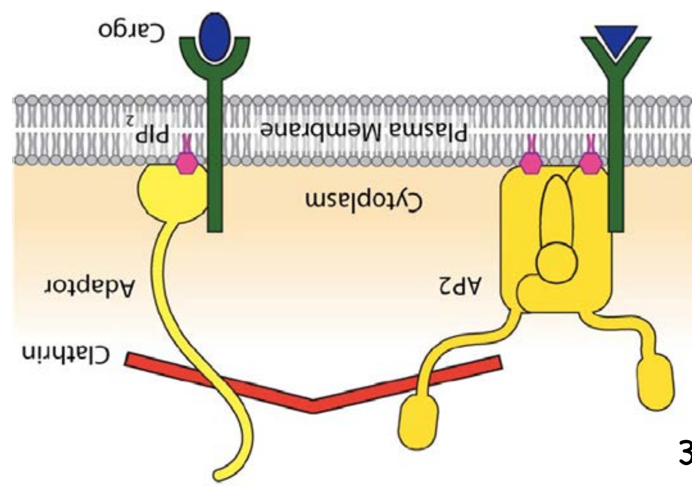
- Symmetric membrane with m = 0:
- Large range of *M*-values for large and negative spont curv *m*

Clathrin-Dependent Endocytosis

- Assembly of clathrin-coated pit, budding, fission



- In-bud implies negative spontaneous curvature
- Assembly of thick
  protein coat: AP2 ++

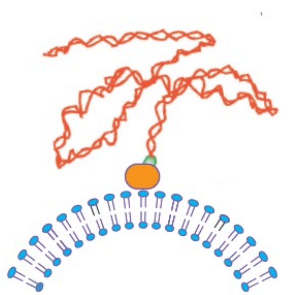


Local Curvature Generation

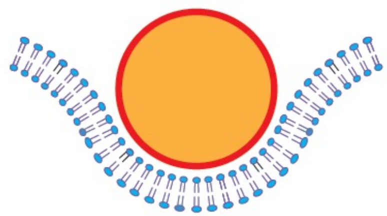
RL, *Europhys. Lett.* (1995)   RL, Doebereiner, *Europhys. Lett.* (1998)

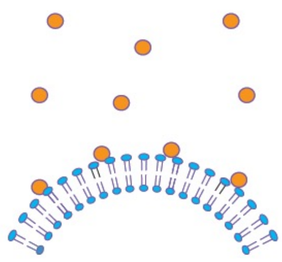
- Different molecular mechanisms:

Anchored polymer

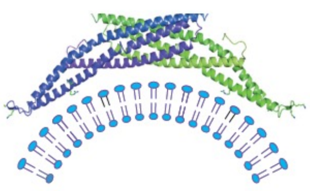


'Large' particle





'Small' particles



BAR-domain protein

Nonspherical Janus particles

Peter ... McMahan,
Science (2004)