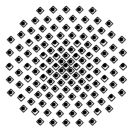


Thermal membrane fluctuations: the impact on lateral protein diffusion and specific adhesion

Ellen Reister



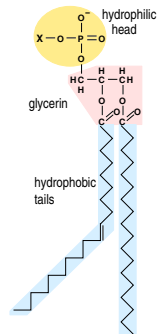
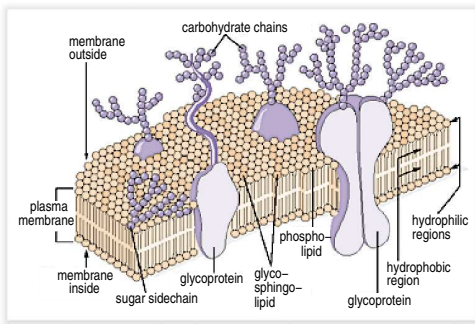
II. Institut für Theoretische Physik
Universität Stuttgart
Germany

11th May 2009

outline

- 1 motivation
 - system: cell membrane
- 2 lateral protein diffusion
 - free diffusion
 - curvature coupled diffusion
- 3 specific adhesion of a fluctuating membrane
 - model
 - simulations
 - results
- 4 conclusions

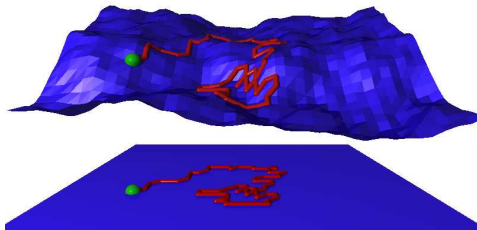
cell membrane



- flexible “plane”: shape fluctuations
- lipid bilayer: two-dimensional fluid
- several lipid species: possible domain formation
- proteins: lateral diffusion along membrane

lateral protein diffusion in a fluctuating membrane

usually neglected in analysis of lateral diffusion

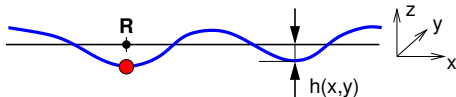


- measurement uses projected path
- fluctuating membrane
 - projected path \neq actual path
 - measured diffusion coeff. function of membrane fluctuations
 - fluctuations depend on temperature, osmotic pressure, ...

What's difference between intramembrane and projected diff.?

freely diffusing particle

- membrane shape: Monge gauge: $\mathbf{r} = (x, y, h(x, y))$



- fluctuations: $h(x, y, t)$ is time dependent

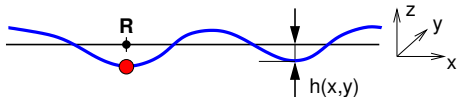
diffusion on a flat surface

- Smolouchovski equation = eq. of motion for particle probability distribution $P(x, y, t)$

$$\partial_t P(x, y, t) = D \Delta P(x, y, t)$$

freely diffusing particle

- membrane shape: Monge gauge: $\mathbf{r} = (x, y, h(x, y))$



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diffusion on a curved surface

- Smolouchovski equation = eq. of motion for particle probability distribution $P(x, y, t)$

$$\partial_t P(x, y, t) = D \square P(x, y, t)$$

- curved surface: replace Laplace op. Δ with Laplace-Beltrami op. \square
- $\square = \square[h_x, h_y, h_{xx}, h_{xy}, h_{yy}]$
with $h_x \equiv \partial h / \partial x$, etc.

preaveraging approximation

Both $P(x, y, t)$ and h_x, h_y , etc. time dependent!

estimate time scales of membrane fluctuations and diffusion

membrane fluctuations “faster” than diffusion

protein “feels” average membrane shape

preaveraging approximation

replace prefactors containing h_x, h_y , etc. with thermal averages

$$\square[h_x, h_y, h_{xx}, h_{xy}, h_{yy}] \longrightarrow \langle \square[h_x, h_y, h_{xx}, h_{xy}, h_{yy}] \rangle$$

- result: Smolouchovski equation for planar diffusion

$$\partial_t P(x, y, t) = D_{\text{proj}} \Delta P(x, y, t)$$

effective diffusion constant D_{proj}

projection causes rescaling of diffusion coefficient

$$D_{\text{proj}}/D = \frac{1}{2} \left(1 + \left\langle \frac{1}{g} \right\rangle \right)$$

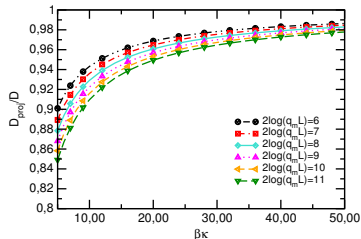
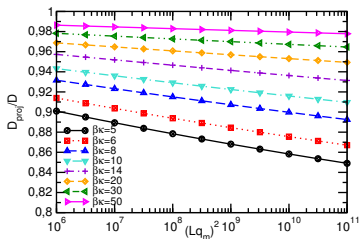
with metric $g \equiv 1 + h_x^2 + h_y^2$

- calculate $\langle 1/g \rangle$ using Helfrich Hamiltonian:

$$\mathcal{H}[h(\mathbf{r}, t)] = \int_A d^2\mathbf{r} \left[\frac{\kappa}{2} (\nabla^2 h)^2 + \frac{\sigma}{2} (\nabla h)^2 \right]$$

 κ bending rigidity σ surface tension

D_{proj}/D for $\sigma = 0$



- strongest effect for *low rigidity* κ , *large systems* L , and *low tension* σ

free diffusion

- D_{proj} up to 15% smaller than free D
- The stronger the fluctuations the stronger the reduction!
 - Fluctuations increase path of protein.

E.R. and U. Seifert, EPL 71:859 (2005)

stochastic simulations

free diffusion

- membrane dynamics

$$\partial h(\mathbf{r}, t)/\partial t = - \int_A d^2 \mathbf{r}' \Lambda(\mathbf{r}', \mathbf{r}) \delta \mathcal{H} / \delta h(\mathbf{r}') + \xi(\mathbf{r})$$

$\Lambda(\mathbf{r}', \mathbf{r})$ Onsager coefficient: expresses membrane dynamics caused by surrounding fluid

$\xi(\mathbf{r}, t)$ obeys fluctuation-dissipation theorem

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- free protein diffusion on curved surface

$$\frac{\partial R_i(t)}{\partial t} = \frac{D}{\sqrt{g}} \frac{\partial}{\partial R_j} (\sqrt{g} g^{ij}) + \zeta_i$$

$$\text{FDT: } \langle \zeta_i(t) \rangle = 0 \quad \langle \zeta_i(t) \zeta_j(t') \rangle = 2Dg^{ij} \delta(t - t')$$

$$\text{metric: } g \equiv 1 + h_x^2 + h_y^2 \quad \text{inv. metric tensor: } g^{ij} \equiv \frac{1}{g} \begin{pmatrix} 1 + h_y^2 & -h_x h_y \\ -h_x h_y & 1 + h_x^2 \end{pmatrix}$$

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apparent force on particle caused by curvature

simulation scheme

simulation: integration of coupled stochastic equations

- 1 evolve membrane shape (\mathbf{q} -space on lattice):

$$h(\mathbf{q}; t) = h(\mathbf{q}; t - \Delta t) - \Lambda(q) [\kappa q^4 + \sigma q^2] \Delta t + \sqrt{2\Lambda(q)\Delta t} r$$

- 2 Fourier-transform: $h(\mathbf{q}; t) \rightarrow h(\mathbf{r}; t)$
- 3 calculate h_x , h_y , g , etc. at particle position $\mathbf{R} = (X, Y)$
- 4 move particle in real space (off-lattice):

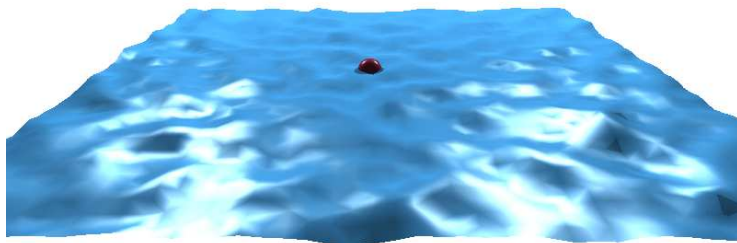
$$R_i(t + \Delta t) = R_i(t) + \Delta t \frac{D}{\sqrt{g}} \left[\frac{\partial}{\partial R_j} (\sqrt{g} g^{ij}) \right] + \sqrt{2D\Delta t} G^{ij} r_j$$

$$G^{ik} G^{kj} = g^{ij}$$

$r, r_j \dots$ Gaussian random numbers: $\langle r \rangle = 0$, $\langle r^2 \rangle = 1$

- 5 continue with 1.

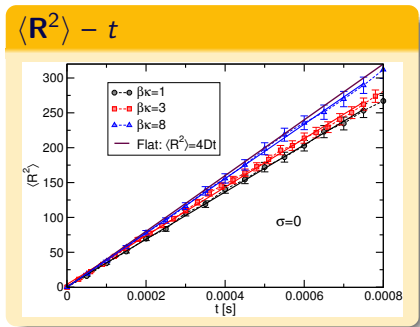
visualisation of free membrane bound diffusion



projected/measured diffusion constant D_{proj}

preaveraging regime

- simulation: $D_{\text{proj}}/D = \langle \mathbf{R}^2(t) \rangle / 4Dt$



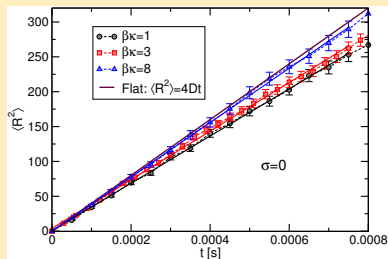
average over 600 runs; 50×50 lattice; 10^6 timesteps; ~ 12 min. per run

projected/measured diffusion constant D_{proj}

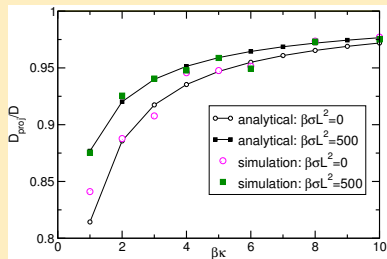
preaveraging regime

- simulation: $D_{\text{proj}}/D = \langle \mathbf{R}^2(t) \rangle / 4Dt$
- analytical ($\tau_M \ll \tau_D$): $D_{\text{proj}}/D = (1 + \langle 1/g \rangle) / 2$

$\langle \mathbf{R}^2 \rangle - t$



$D_{\text{proj}}/D - \beta\kappa$



average over 600 runs; 50×50 lattice; 10^6 timesteps; ~ 12 min. per run

good agreement

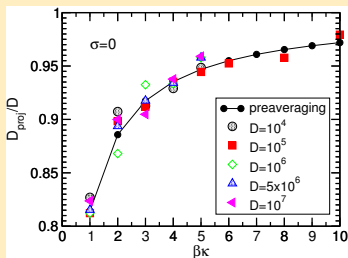
simulations beyond the preaveraging approximation

- simulations for various intramembrane diffusion coefficients D
- for largest regarded D : $\tau_M \simeq 2\tau_D$

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$$D_{\text{proj}}/D - \beta\kappa$$



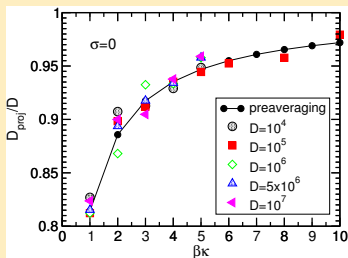
preaveraging is applicable for all physical membranes!

E.R., S.M. Leitenberger, and U. Seifert PRE 75:011908 (2007)

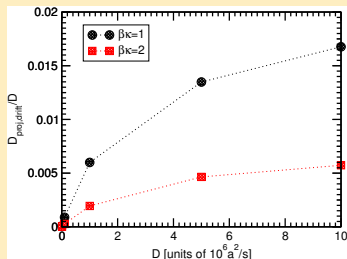
simulations beyond the preaveraging approximation

- simulations for various intramembrane diffusion coefficients D
- for largest regarded D : $\tau_M \simeq 2\tau_D$

$$D_{\text{proj}}/D - \beta\kappa$$



$$D_{\text{proj,drift}}/D - \beta\kappa$$

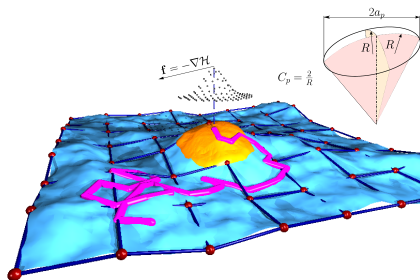


preaveraging is applicable for all physical membranes!

E.R., S.M. Leitenberger, and U. Seifert PRE 75:011908 (2007)

curvature coupled diffusion

- protein interacts with membrane
- here:** coupling to local membrane curvature
protein is attracted to position with certain curvature



- protein energy

$$\mathcal{H}_{\text{prot}}[h, \mathbf{R}] = \int_A d^2\mathbf{r} g(\mathbf{r} - \mathbf{R}) \left[\frac{m}{2} (\nabla_{\mathbf{r}}^2 h(\mathbf{r}) - C_p)^2 - \frac{\kappa}{2} (\nabla_{\mathbf{r}}^2 h(\mathbf{r}))^2 \right]$$

 C_p

spontaneous curvature

 m

bending rigidity of the protein

 $g(\mathbf{r}) = \exp(-\mathbf{r}^2/a_p^2)$

weighting function for protein extension

equations of motion

(total energy $\mathcal{H}_{\text{tot}} = \mathcal{H} + \mathcal{H}_{\text{protein}}$)

- membrane dynamics

$$\partial h(\mathbf{r}, t) / \partial t = - \int_A d^2 \mathbf{r}' \Lambda(\mathbf{r}', \mathbf{r}) \delta \mathcal{H}_{\text{tot}} / \delta h(\mathbf{r}') + \xi(\mathbf{r})$$

- protein diffusion (preaveraging)

$$\partial \mathbf{R} / \partial t = -\mu \nabla_{\mathbf{R}} \mathcal{H}_{\text{protein}}[h] + \zeta$$

with $\langle \zeta(t) \rangle = 0$ and $\langle \zeta_i(t) \zeta_j(t') \rangle = 2D_{\text{proj}} \delta_{ij} \delta(t - t')$

$\mu \dots$ mobility

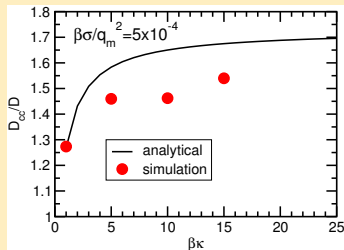
Einstein relation: $\mu = D_{\text{proj}} / k_B T$

curvature-coupled diffusion coefficient D_{cc}

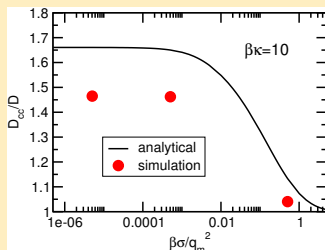
free membrane fluctuations

assumption: membrane fluctuations not influenced by protein

$$D_{cc}/D - \beta\kappa$$



$$D_{cc}/D - \beta\sigma/q_m^2$$



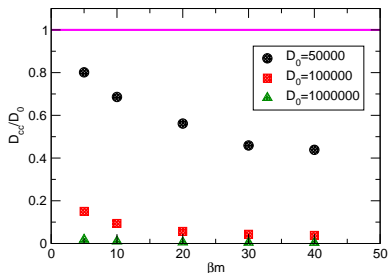
- curvature coupling enhances diffusion
- qualitative difference compared to free diffusion
- diffusion in simulations is less enhanced
 - particle will try to follow an energy minimum

S.M. Leitenberger, E.R.-G., U. Seifert, Langmuir 24:1259 (2008)

curvature-coupled diffusion coefficient D_{CC}

diffusion in a periodic potential

- simulations: membrane feels protein influence

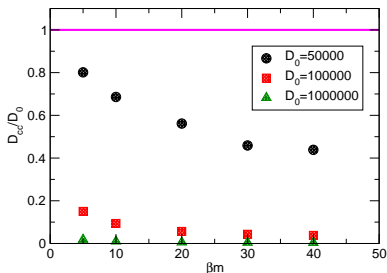


diffusion is reduced!

curvature-coupled diffusion coefficient D_{cc}

diffusion in a periodic potential

- simulations: membrane feels protein influence



diffusion is reduced!

- protein feels (time-dependent) periodic potential
- diffusion is always reduced (in equilibrium)

$$D_{cc} \leq D_0$$

- previous approximation effectively drives the system
 - possible relevance for active processes in membrane

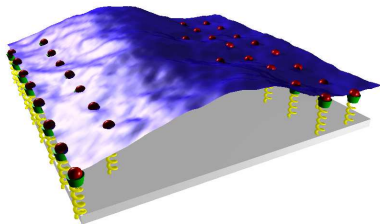
specific adhesion of a fluctuating membrane

specific membrane adhesion

- biological membranes are typically not free
 - confined by surrounding membranes
 - adhere to other membranes
- model systems that mimic biological membranes
 - lipid bilayers deposited on solid or polymer substrates

specific membrane adhesion

- biological membranes are typically not free
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 - lipid bilayers deposited on solid or polymer substrates
- **membrane adhesion via specific receptor-ligand pairs**

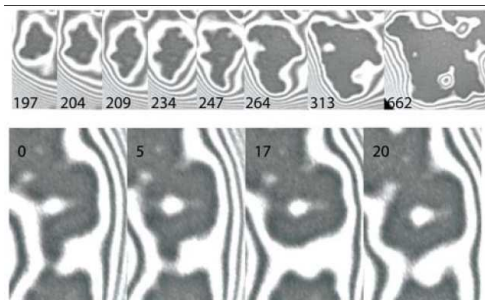


- membrane subject to thermal fluctuations
- reaction rate is height dependent

Goal: understanding of influence of shape fluctuations

motivation

- recent experiments with weak binding

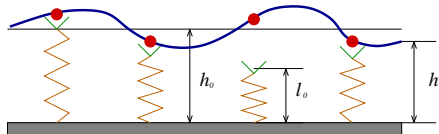


black regions = bound

weak binding → unbinding becomes important

energy

$$\mathcal{H} = \mathcal{H}_0 + \mathcal{H}_{\text{ns}} + \mathcal{H}_s$$



- Helfrich energy \mathcal{H}_0 (bending rigidity κ)

$$\mathcal{H}_0[h(\mathbf{r}, t)] = \int_A d^2\mathbf{r} \frac{\kappa}{2} (\nabla^2 h)^2$$

- non-specific potential \mathcal{H}_{ns} (strength γ)

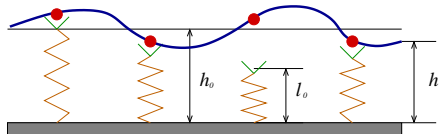
$$\mathcal{H}_{\text{ns}}[h(\mathbf{r}, t)] = \int_A d^2\mathbf{r} \frac{\gamma}{2} (h - h_0)^2$$

- energy of springs \mathcal{H}_s (binding energy ϵ_b ; tether stiffness K)

$$\mathcal{H}_s[h(\mathbf{r}, t)] = \sum_{i=1}^N b_i \left[\frac{K}{2} (h(\mathbf{r}_i) - l_0)^2 - \epsilon_b \right] \text{ with } b_i = \begin{cases} 1 & i\text{-th bound} \\ 0 & i\text{-th not bound} \end{cases}$$

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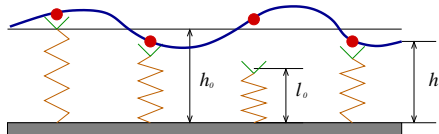
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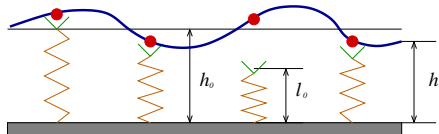
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dynamics

membrane fluctuations

- fluctuating membrane: $h(x, y, t)$ is time dependent
- equation of motion (Fourier space)

$$\frac{\partial h(\mathbf{k}, t)}{\partial t} = -\Lambda(\mathbf{k}) \left\{ [\kappa k^4 + \gamma] h(\mathbf{k}, t) + \sum_{i=1}^{N_t} b_i \left[K(h(\mathbf{r}_i, t) - l_0) e^{-i\mathbf{k} \cdot \mathbf{r}_i} \right] \right\} + \xi(\mathbf{k}, t)$$

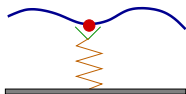
$\Lambda(\mathbf{k})$ Onsager coefficient

$\xi(\mathbf{k}, t)$. . . obeys fluctuation-dissipation theorem

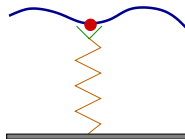
dynamics

receptor-ligand reaction

- receptors and ligands at fixed sites
- intuition



unbinding less likely



unbinding more likely

height-dependent reaction rates

- unbinding rate: Kramers' rate theory

$$k_{\text{off}} = k_0 \exp[\beta K \alpha (h(\mathbf{r}_i, t) - l_0)]$$

- binding rate: detailed balance

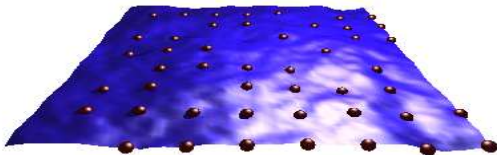
$$k_{\text{on}}/k_{\text{off}} = e^{\beta \epsilon_b} \exp \left[-\frac{1}{2} \beta K (h(\mathbf{r}_i, t) - l_0)^2 \right]$$

simulations

simulation: integration of coupled stochastic equations

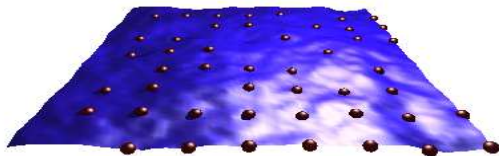
- membrane: 64×64 lattice with lattice spacing $a \simeq 10\text{nm}$
- $N = 64$ binding sites on 8×8 lattice with spacing $8a$
- initial distance membrane–substrate: $h_0 = 12a$
- rest length of springs: $l_0 = 8a$
- starting configuration: equilibrated membrane without bonds

visualization of adhesion process



$$\beta K = 1.25; \beta \kappa = 10; \epsilon_b = 3.91$$

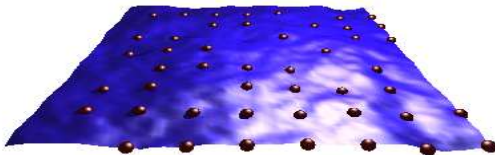
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unbinding also observed

visualization of adhesion process



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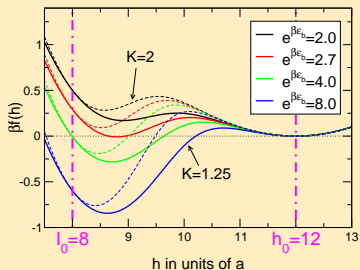
questions

- equilibrium for stiff and fluctuating membrane
- role of membrane fluctuations on adhesion dynamics
- connection to experimental results

Phase diagram for stiff membrane ($\kappa \rightarrow \infty$)

infinite system

stiff membrane — effective free energy per bond site

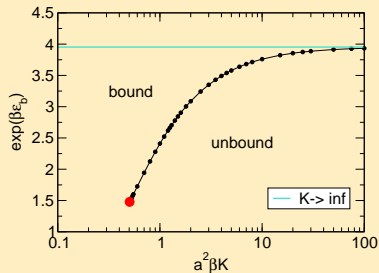
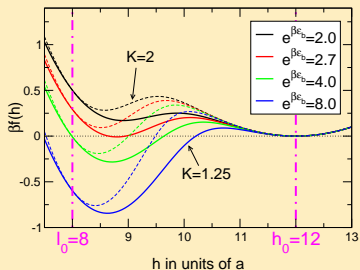


- two minima
- competition of bonds and non-specific potential

Phase diagram for stiff membrane ($\kappa \rightarrow \infty$)

infinite system

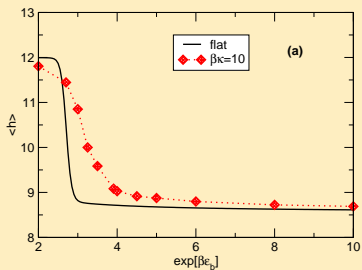
stiff membrane — phase diagram



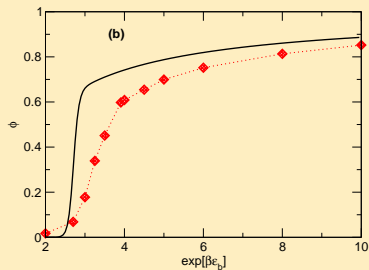
- first order transition from bound to free membrane
- for very stiff tethers saturation of coexistence line

influence of fluctuations and binding energy ϵ_b

height $\langle h \rangle$ — ϵ_b



bond density $\langle \phi \rangle$ — ϵ_b

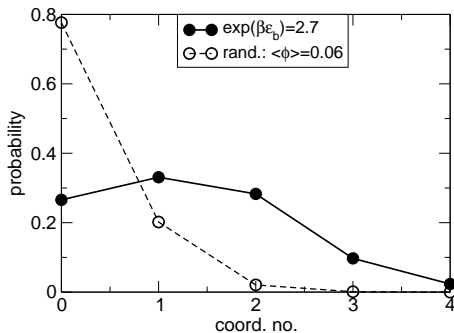


- fluctuations make transition more “continuous”
- higher binding energy necessary for comparable adhesion with fluctuations

fluctuations cause additional entropy contribution in free energy

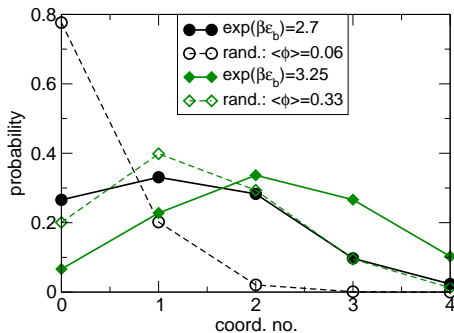
coordination number of bonds

compare coordination number of bonds for fluctuating membrane with random binding of same bond density ϕ



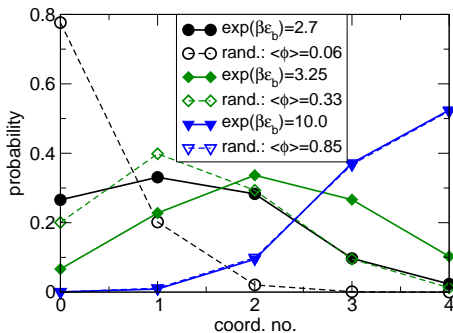
coordination number of bonds

compare coordination number of bonds for fluctuating membrane with random binding of same bond density ϕ



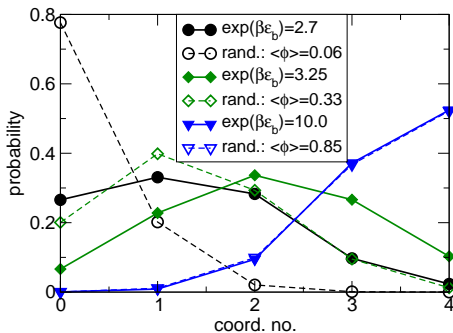
coordination number of bonds

compare coordination number of bonds for fluctuating membrane with random binding of same bond density ϕ



coordination number of bonds

compare coordination number of bonds for fluctuating membrane with random binding of same bond density ϕ

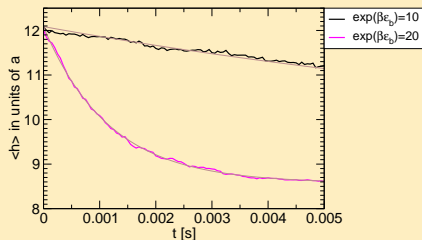


- membrane mediates interaction between bonds

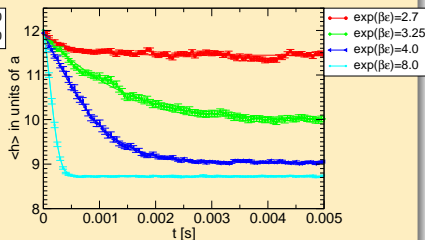
global adhesion dynamics

average height $\langle h(t) \rangle$

stiff membrane



fluctuating membrane

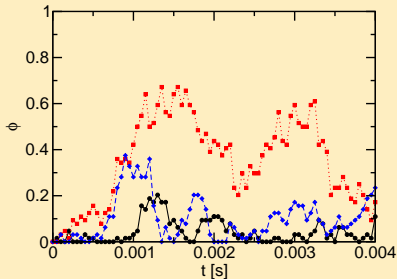


- adhesion takes place much faster with fluctuations

bond formation encouraged through chance encounters of membrane with substrate

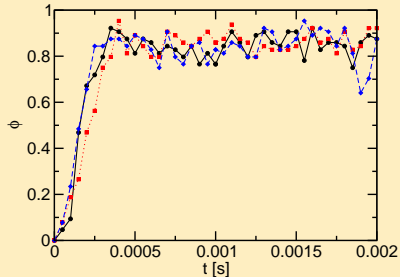
adhesion dynamics

single systems



weak binding: $e^{\beta\epsilon_b} = 3$

- much noise for weak binding
- competition between membrane dynamics and binding



“strong” binding: $e^{\beta\epsilon_b} = 10$

conclusions

- free lateral protein diffusion
 - projection causes apparent reduction of diffusion coefficient
- curvature-coupled protein diffusion
 - interaction of protein with membrane reduces effective diffusion coefficient
- specific membrane adhesion
 - fluctuations make higher binding energy necessary for adhesion
 - fluctuations speed up adhesion
 - membrane fluctuations induce effective interaction between bonds
- outlook
 - combination of both techniques: include lateral diffusion of ligands to specific adhesion simulations

acknowledgements

- Stefan M. Leitenberger
- Ana-Sunčana Smith
- Kheya Sengupta (CINaM/CNRS, Marseille)
- Udo Seifert