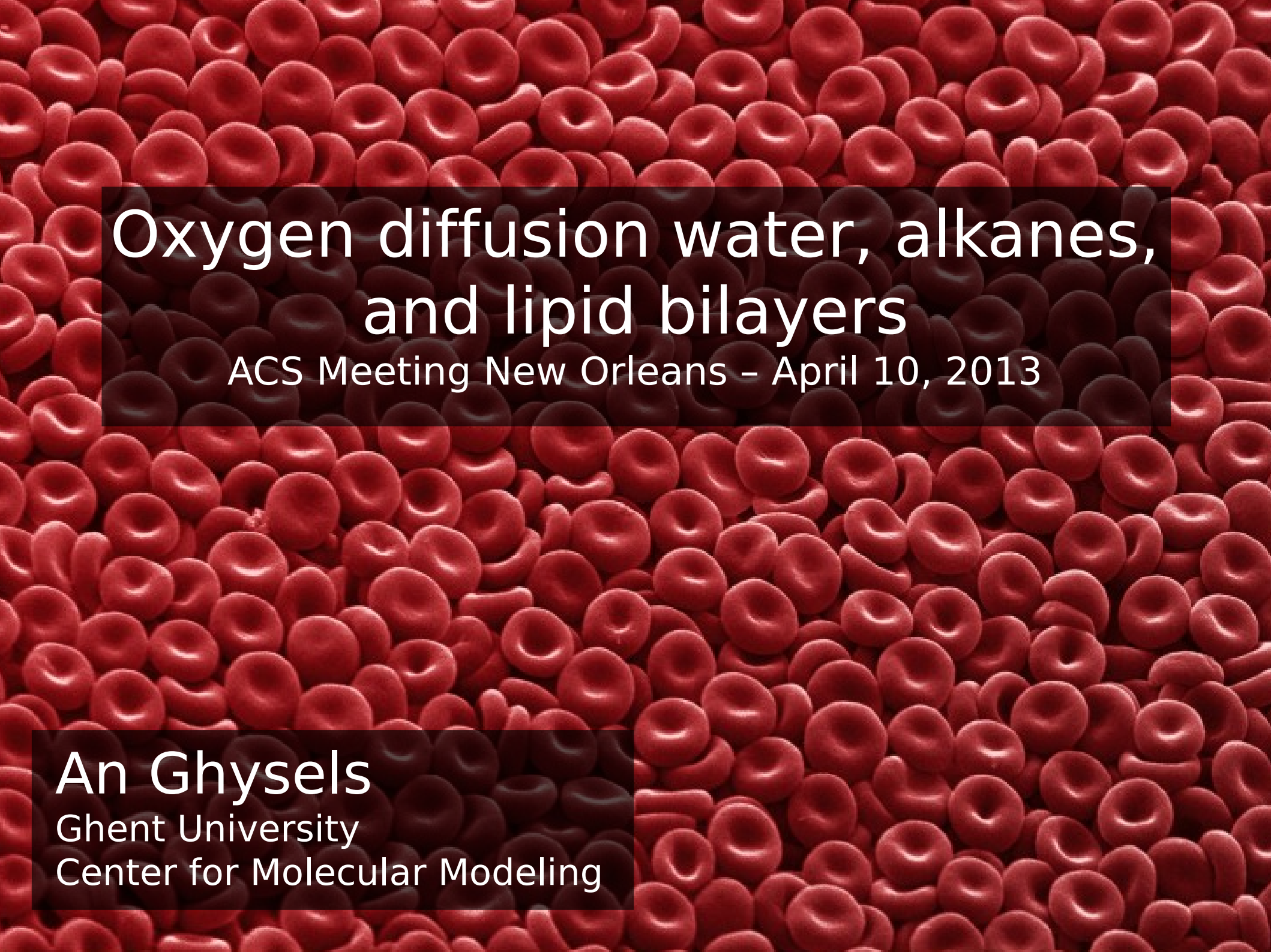


Oxygen diffusion water, alkanes, and lipid bilayers

ACS Meeting New Orleans – April 10, 2013

An Ghysels

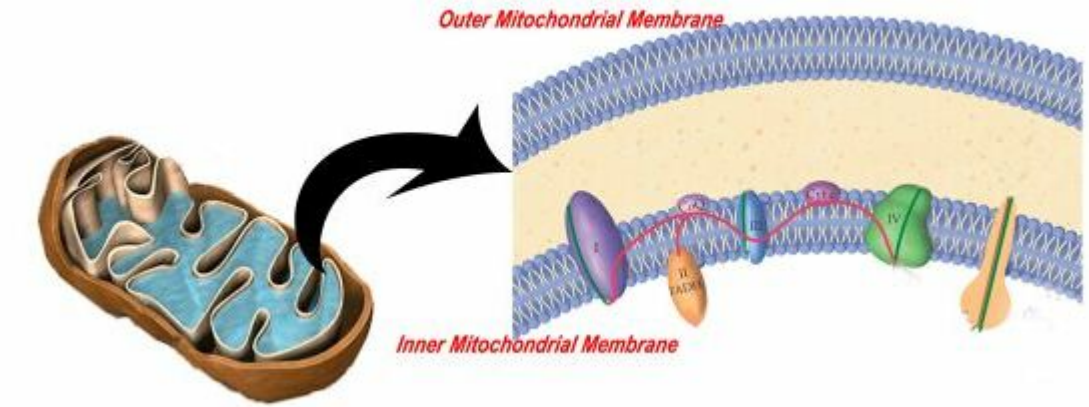
Ghent University

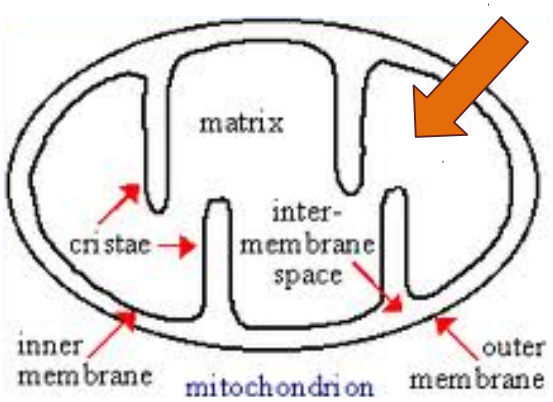
Center for Molecular Modeling

Oxygen transport to the “fuel cells” of living organisms

Oxygen is consumed in the mitochondria.

How does the very last step of the transport occur?

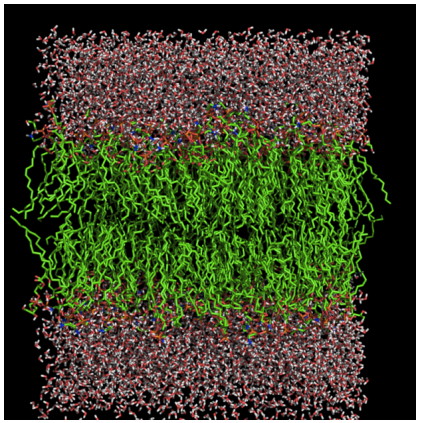


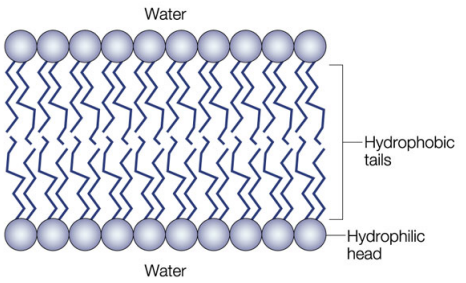


Mitochondria have double membranes: inner and outer.

Inner membrane is a phospholipid bilayer
– curved (large surface)
– contains cardiolipin
– O_2 has to diffuse through (or uses oxygen pumps)

We study this last step with molecular simulations





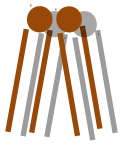
O_2 transport through mitochondrial membranes

Where is O_2 motion slow, where fast?

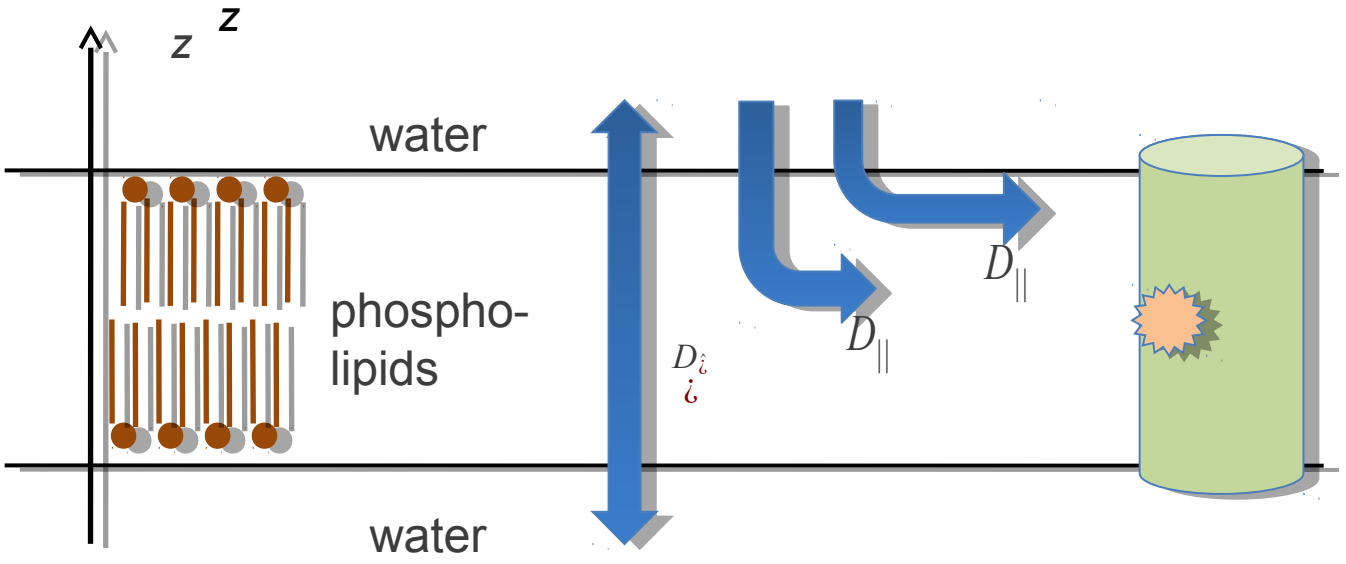
Is there a build-up of O_2?

Has cardiolipin an effect?

Optimal route to the middle of the layer?



cardiolipin



cytochrome C oxidase
O_2 consuming protein
with entrance in
middle of bilayer

=> needs a position-dependent description

Key assumption: O_2 behavior is approximately diffusive.

i.e. the time scale is large enough that the motion of O_2 seems random,

i.e. O_2 looses its ‘memory’ quickly,

Position dependent diffusion ‘constant’

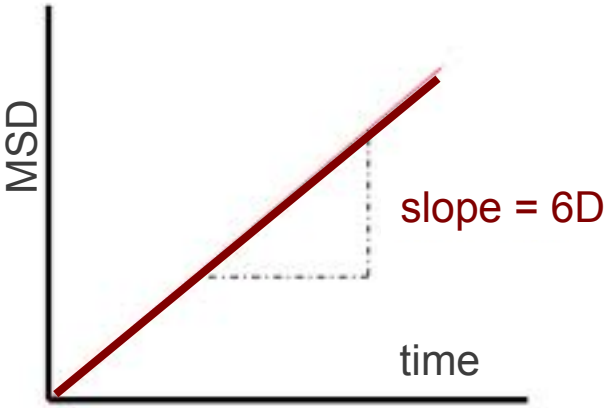
Difficult, because a lipid bilayer is inhomogeneous and anisotropic.

Standard method to measure *D*

In a *homogeneous* and *isotropic* medium,
mean square distance (MSD) grows linearly with time.

$$\mathrm{MSD} = \langle \Delta r^2(t) \rangle = 6Dt \quad \text{when} \;\; t \to \infty$$

$$\frac{\partial p(\bar{r},t)}{\partial t} = D\nabla^2 p(\bar{r},t)$$



In the lipid bilayer

- *D* is a **tensor**,
- *D* is **position-dependent**, and
- oxygen feels a positon-dependent **mean field potential *F***.

$$\frac{\partial p(\bar{r},t)}{\partial t} = \nabla . \left[\bar{\bar{D}}(\bar{r}) e^{-\beta F(\bar{r})} \nabla \left(e^{\beta F(\bar{r})} p(\bar{r},t) \right) \right]$$

Smoluchowski equation

$p(\bar{r},t)$ is the local oxygen concentration

Outline – Simulating oxygen diffusion through membranes

1. Diffusion in inhomogeneous and anisotropic media

2. Five test systems:
   - pure water, pure hexadecane, hexadecane layer
   - simple POPC bilayer
   - model for inner mitochondrial membrane (MITO)

3. Deriving diffusion profiles from statistics

4. Free energy profiles and diffusion profiles

Five studied systems

3 test systems for validation

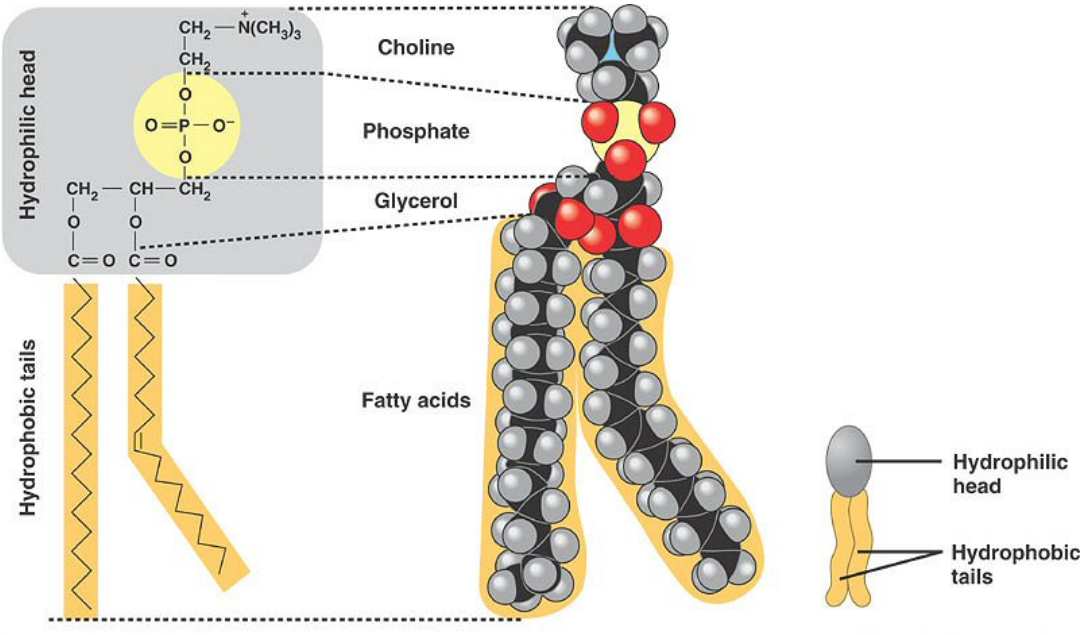
pure water

pure hexadecane

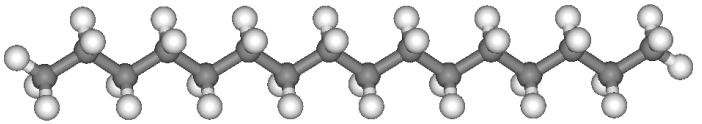
hexadecane layer
in water

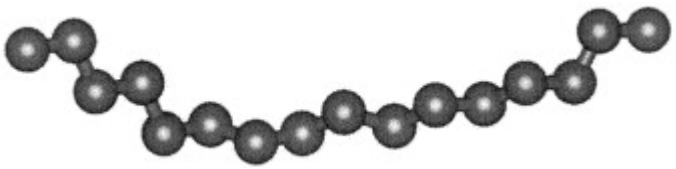
simple **POPC** bilayer
containing only one lipid type

inner mitochondrial membrane: **MITO**
modeled as bilayer with four lipid types



model: (C16) hexadecane $C_{16}H_{34}$
is less inhomogeneous,
resembles tails of lipids





Five studied systems

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pure water

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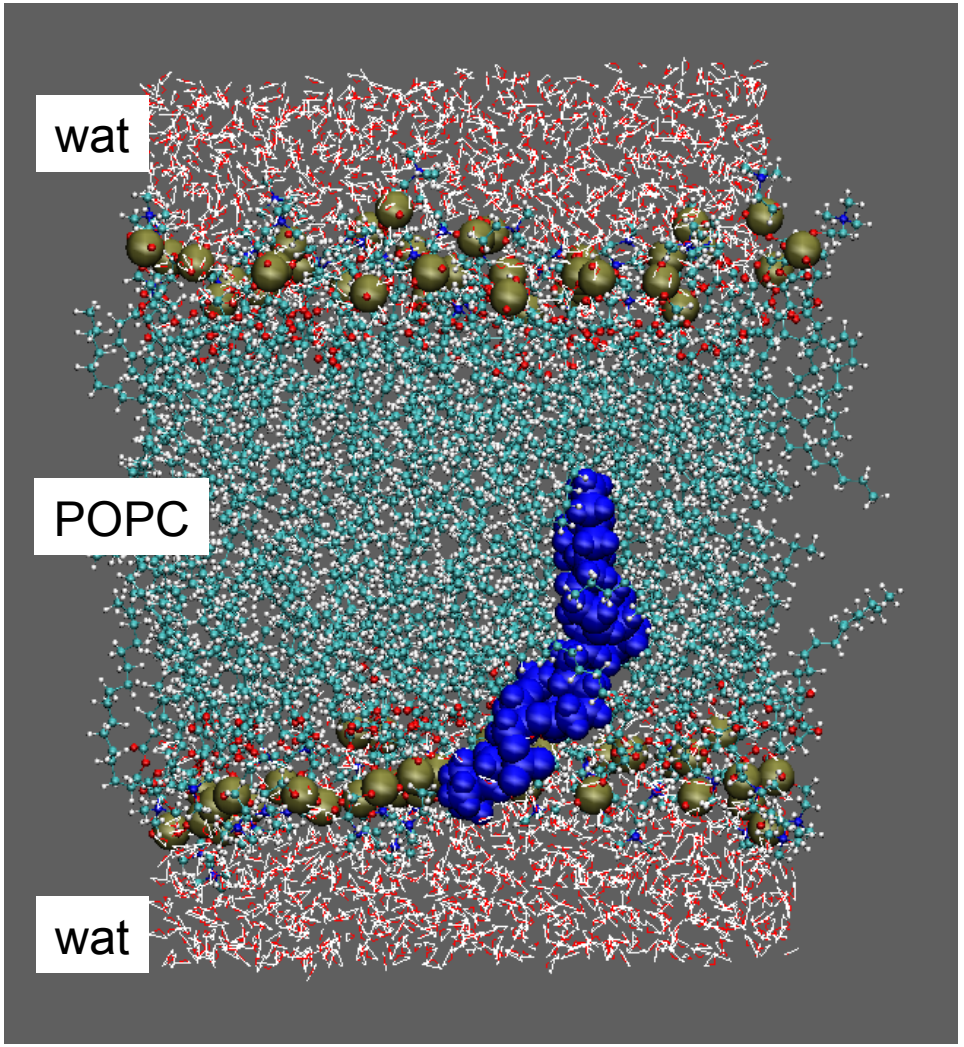


Simulation details

CHARMM 36 force field
MD with CHARMM program
coupled (Nosé-Hoover) to thermostat (constant *T*) and barostat (constant *P*)

10 oxygen molecules are inserted
(no quadrupole moment)

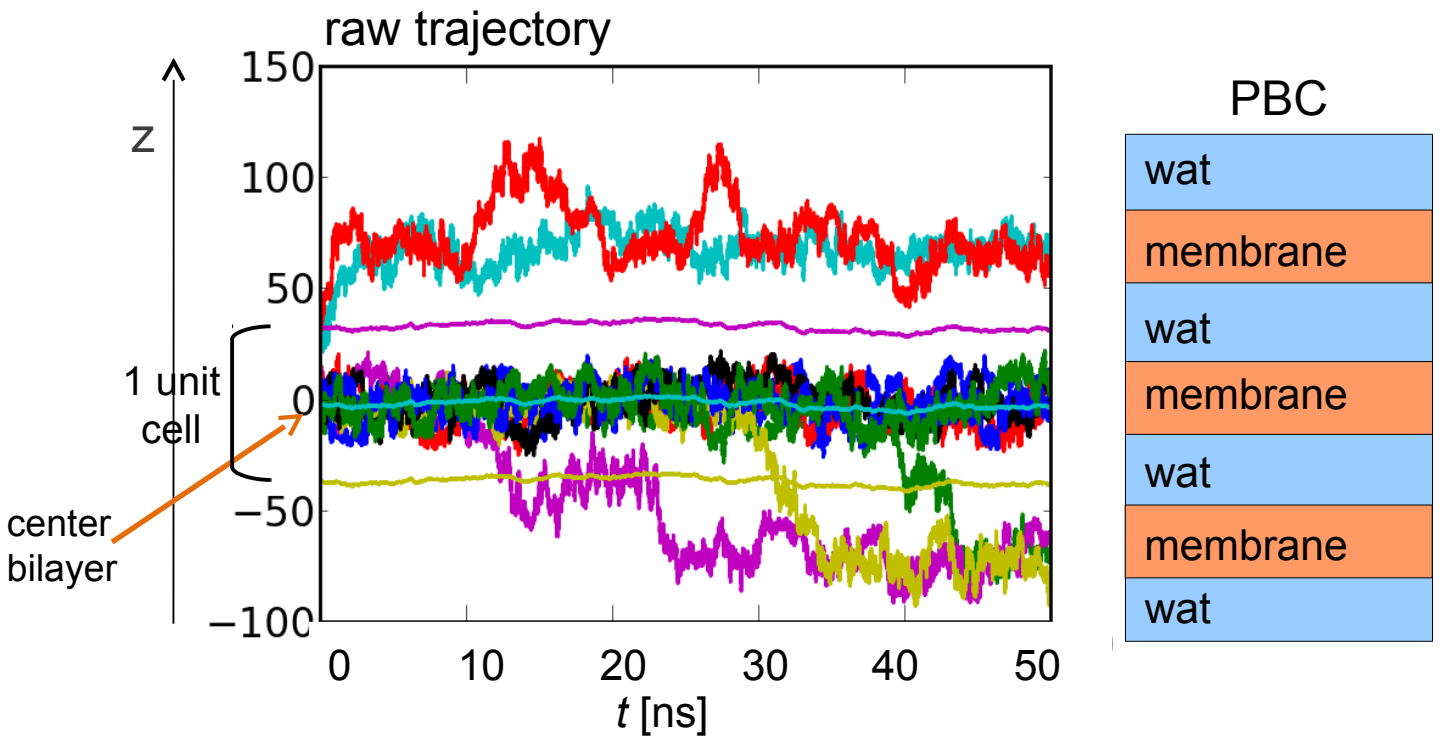
box: periodic boundary conditions (PBC)
width about 50 Angstrom in *x,y* direction
height between 52 and 70 Angstrom in *z* direction

POPC and MITO bilayer: 36 lipids on each side (72 total)
water layer is about 30 Angstrom thick

initial run: NPAT or NPT relaxation
a = *b,* but *c* fluctuates independently
T = 310 K: body temperature

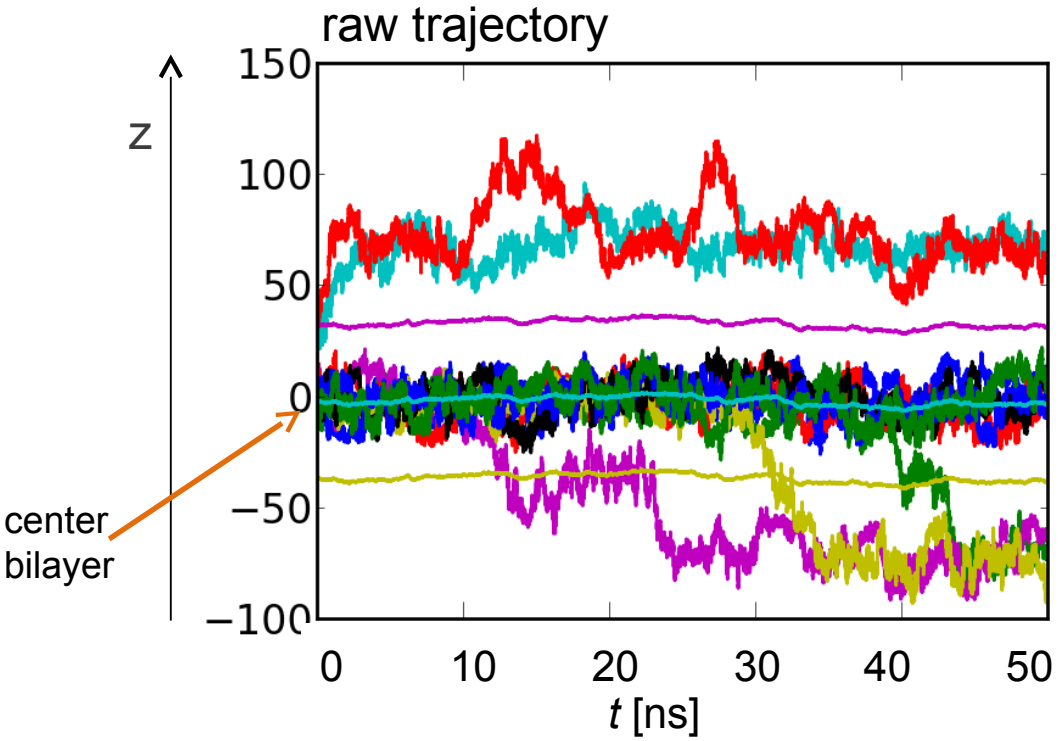
production run: NVT
time integration step 1 fs
sampling coordinates every 1 ps
50 ns for pure systems, 200 ns for layered systems

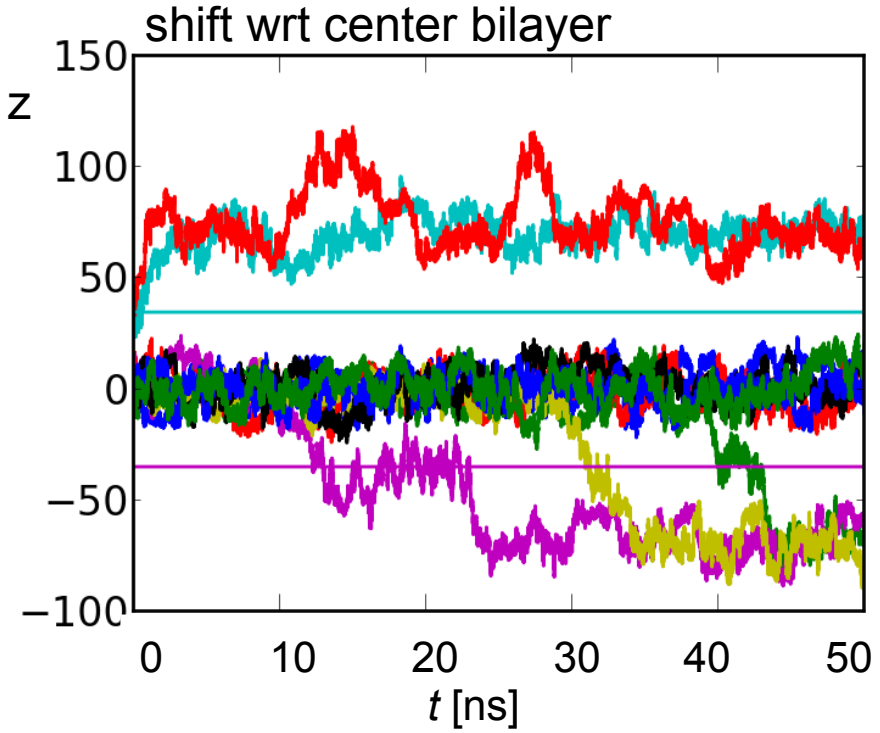
Example: MITO

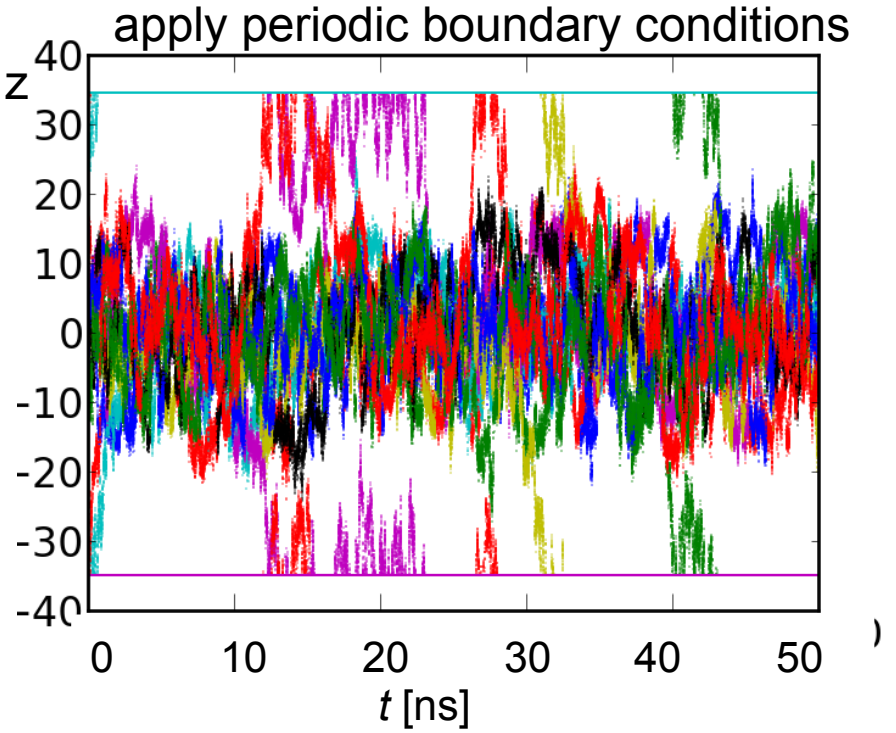


Define reaction coordinate: center of bilayer
=> trajectories should be corrected for small drift

Example: MITO







Solving the diffusion equation in a layered medium

general $$\frac{\partial p(\bar{r},t)}{\partial t} = \nabla . \left[\bar{\bar{D}}(\bar{r}) e^{-\beta F(\bar{r})} \nabla \left(e^{\beta F(\bar{r})} p(\bar{r},t) \right) \right]$$

translational symmetry in the x,y-plane simplifies

free energy $$F(\bar{r}) \rightarrow F(z)$$

diffusion tensor $$\bar{\bar{D}}(\bar{r}) \rightarrow \bar{\bar{D}}(z) = \begin{pmatrix} D_{||}(z) & 0 & 0 \\ 0 & D_{||}(z) & 0 \\ 0 & 0 & D_{\perp}(z) \end{pmatrix}$$

Solving the diffusion equation in a layered medium

general $$\frac{\partial p(\bar{r},t)}{\partial t} = \nabla . \left[\bar{\bar{D}}(\bar{r}) e^{-\beta F(\bar{r})} \nabla \left(e^{\beta F(\bar{r})} p(\bar{r},t) \right) \right]$$

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using **polar coordinates** ***z*** and $r = \sqrt{x^2 + y^2}$
and initial conditions $p(z,r,0) = \delta(z - z_0)\delta(r)$ $\qquad p(\bar{r},t) \rightarrow p(z,r,t) \rightarrow p(r,z,t|z_0)$

using **separation of variables** r and (z,t) separation constant α

(1) radial solution (textbook): Bessel functions of first type, zeroth order $J_0(\alpha r)$

(2) orthogonal solution: functions $Z_\alpha(z,t|z_0)$

=> the propagator: $$p(z,r,t|z_0) = \int d\alpha \; r J_0(\alpha r) Z_\alpha(z,t|z_0)$$

Solving the diffusion equation in a layered medium

propagator $$p(z, r, t|z_0) = \int d\alpha \, r J_0(\alpha r) Z_\alpha(z, t|z_0)$$

Methodology: search for *F,D* profiles such that the propagator corresponds to the propagation observed in simulated trajectories

F,D profiles appear in the **derived propagators *Q* and *Z***
so do in two steps

Solving the diffusion equation in a layered medium

propagator $$p(z,r,t|z_0) = \int d\alpha\, r J_0(\alpha r) Z_\alpha(z,t|z_0)$$

Methodology: search for *F,D* profiles such that the propagator corresponds to the propagation observed in simulated trajectories

F,D profiles appear in the **derived propagators *Q* and *Z***

(1) ***Q*** depends on $F(z), D_\perp(z)$

$$Q(z,t) = \int p(z,r,t)dr$$

propagation from plane to plane in *z*-direction

$$\frac{\partial Q(z,t)}{\partial t} = \frac{\partial}{\partial z}\left[D_\perp(z)e^{-\beta F(z)}\frac{\partial}{\partial z}\left(e^{\beta F(z)}Q(z,t)\right)\right]$$

$$Q(z,0) = \delta(z-z_0) \rightarrow Q(z,t|z_0)$$

(2) set of ***Z*** depends also on $D_{||}(z)$

particles diffuse also radially

sink term

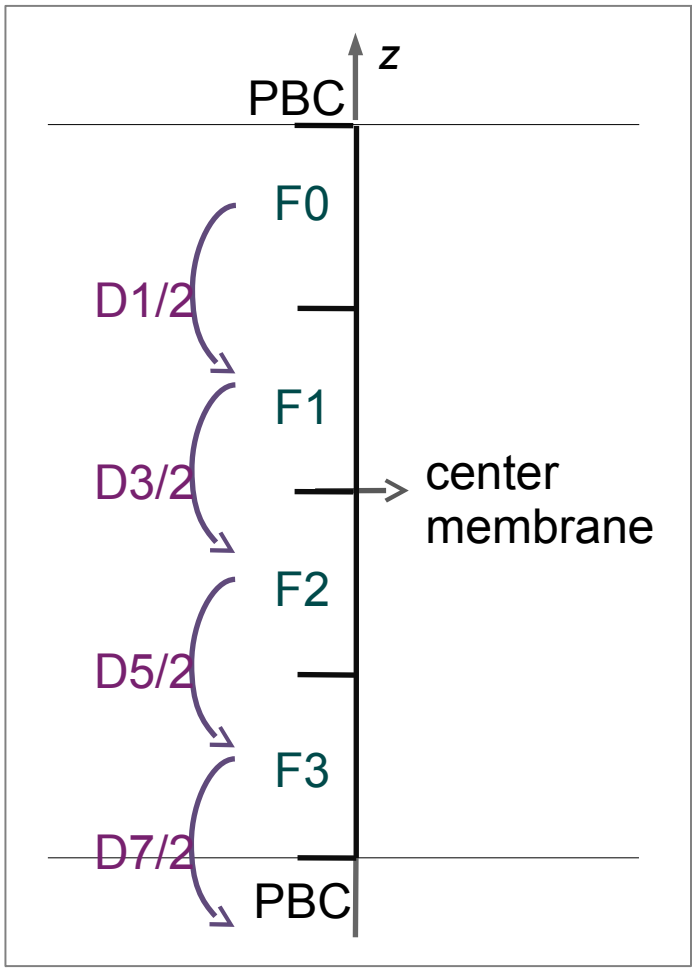
$$\frac{\partial Z_\alpha(z,t)}{\partial t} = \frac{\partial}{\partial z}\left[D_\perp(z)e^{-\beta F(z)}\frac{\partial}{\partial z}\left(e^{\beta F(z)}Z_\alpha(z,t)\right)\right] - \alpha^2 D_{||}(z) Z_\alpha(z,t)$$

$$Z_\alpha(z,0) = \alpha\delta(z-z_0) \rightarrow Z_\alpha(z,t|z_0)$$

Calculating the diffusion profile with Monte Carlo

Solving 1-D equation: **binning the *z*-axis**

discretized profiles F*i* and D*i+1/2*
PBC: periodic boundary conditions



Calculating the diffusion profile with Monte Carlo

Solving 1-D equation: **binning the *z*-axis**

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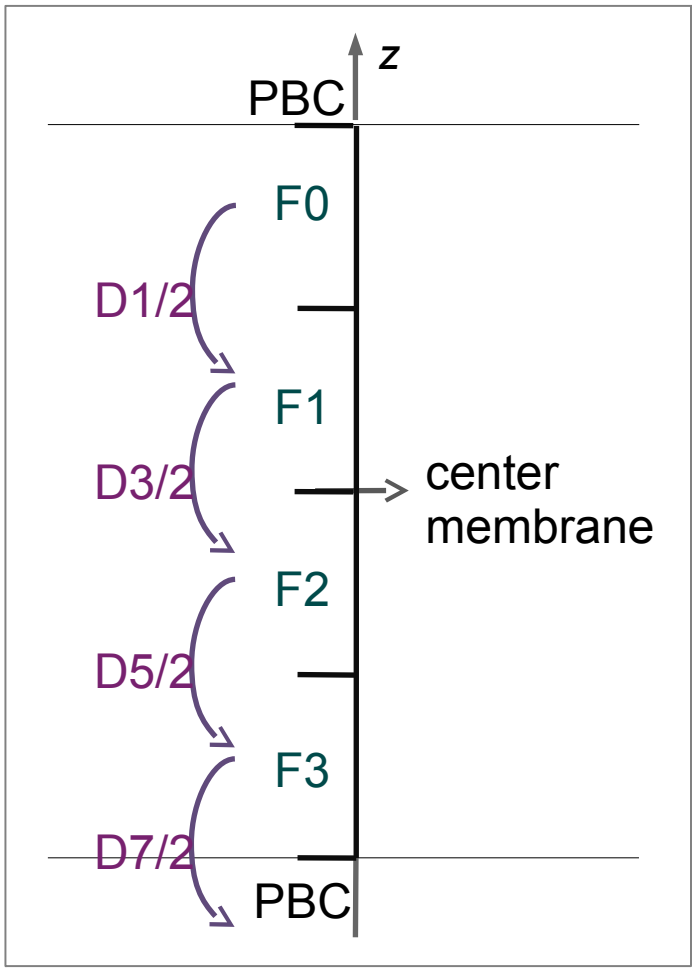


$$\frac{\partial Q(z,t)}{\partial t} = \frac{\partial}{\partial z}\left[D_{\perp}(z)e^{-\beta F(z)}\frac{\partial}{\partial z}\left(e^{\beta F(z)}Q(z,t)\right)\right]$$

rate equations: discretized *Q(z,t)* equation

$$\dot{Q}_i(t) = \sum_j R_{ij}Q_j(t)$$

Q_i is probability to be in bin *i*

with rate matrix ***R***, e.g.

$$R_{i+1,i} = D_{i+1/2}\exp\left[-\beta(F_{i+1} - F_i)\right]$$

given start bin *j*
what is probability to be in bin *i*
after a lag time *t*?

$$P(i,t|j) = \left[\exp(tR)\right]_{ij}$$

matrix exponential

discretized propagator

Calculating the diffusion profile with Monte Carlo

We want to determine the profile $F(z), D_{\perp}(z), D_{||}(z)$

This is done by comparing TRAJECTORIES (T) with the diffusion MODEL (M).

TRAJECTORIES (T)
from computer experiment
molecular dynamics

MODEL (M)
$F(z), D_{\perp}(z), D_{||}(z)$

The optimal model **M** has the maximum likelihood.

likelihood = L = P(T|M)
= probability that model **M** explains/produces available data **T**

Model parameters should be varied, until the model is found
that is most likely to produce the measured trajectories.

We want to find the maximum of the P(T|M) distribution.
=> **maximum-likelihood method**

Alternatively, the whole P(T|M) distribution is sampled,
by testing all possible models.
=> **Bayesian analysis**

Calculating the diffusion profile with Monte Carlo

TRAJECTORIES (T)
molecular dynamics

$z(t)$

↓ discretize

$i(t)$

⟺

MODEL (M)

$F(z), D_\perp(z)$

↓ discretize

$F_i, D_{\perp,i+1/2}$

count transitions
from bin *j* to bin *i*
when waiting for some time *t*
(*t* = lag time)

histogram $N_{ij}(t)$

construct rate matrix ***R***

propagator $P(i,t|j) = [\exp(tR)]_{ij}$

likelihood

$$L \sim P(T|M) = \prod_{\substack{\text{trans} \\ j \to i}} [\exp(tR)]_{ij}^{N_{ij}}$$

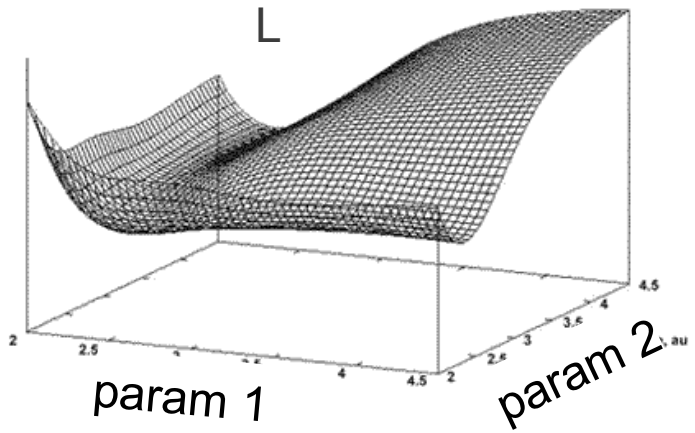
$$\ln L \sim \ln P(T|M) = \sum_{\substack{\text{trans} \\ j \to i}} N_{ij} \ln [\exp(tR)]_{ij}$$

in practice: use ln(L)

Calculating the diffusion profile with Monte Carlo

likelihood = L = P(T|M)

regard L as a surface in model parameter space
and use Monte Carlo to construct surface
in practice: ln(L) surface



Monte Carlo (MC) algorithm

– propose new parameters
– calculate L
– compare old L and new L
if L increases: accept new
if L decreases: accept new with probability $e^{\frac{\ln L_{\mathrm{new}} - \ln L_{\mathrm{old}}}{T}}$

T is parameter temperature

=> Maximum L: reduce parameter temperature gradually to “freeze” to a local extremum.
=> Bayesian analysis: *T=1*

Calculating the diffusion profile with Monte Carlo

Extension to 2-D

in practice, **apply a boundary condition**: far away, concentration is zero
=> condition ***p*(*r*=*s*)= 0** discretizes α

$$p(z,r,t|z_0) = \sum_{\alpha_k \; s=x_k} r\, J_0(\alpha_k r) Z_{\alpha_k}(z,t|z_0)$$

x_k zeros of Bessels

remember 1-D $\dot{Q}_i(t) = \sum_j R_{ij} Q_j(t)$

sink term

now we have $\dot{Z}_{\alpha,i}(t) = \sum_j \left(R_{ij} - \alpha^2 D_{||,i}\delta_{ij}\right) Z_{\alpha,j}(t)$

given start bin *j*
what is probability to be in z-bin *i* and r-bin *m*
after a lag time *t*?

sink term

$$P(i,m,t|j) = \sum_{\alpha_k \; s=x_k} r_m \frac{J_0(\alpha_k r_m)}{\pi J_1^2(\alpha_k s)} \left[\exp(tR - t\alpha_k^2 \mathcal{D}_{||})\right]_{ij}$$

sum over Bessels

discretized propagator

Calculating the diffusion profile with Monte Carlo

Extension to 2-D

TRAJECTORIES (T)
molecular dynamics
$x(t), y(t), z(t)$
↓ discretize z-bins, r-bins
$i(t), m(t)$

⟺

MODEL (M)
$F(z), D_{\perp}(z), D_{||}(z)$
↓ discretize
$F_i, D_{\perp,i+1/2}, D_{||,i}$

count transitions
from z-bin *j* to z-bin *i* and *r*-bin *m*
when waiting for some time *t*
(*t* = lag time)

histogram $N_{im,j}(t)$

construct rate matrix ***R***
and sink term

propagator $P(i,m,t|j)$

likelihood

$$\ln L = \sum_{\substack{\text{trans} \\ j \to i,m}} N_{im,j} \ln \left[P(i,m,t|j) \right]$$

Outline – Simulating oxygen diffusion through membranes

1. Diffusion in inhomogeneous and anisotropic media

2. Five test systems:
   - pure water, pure hexadecane, hexadecane layer in water
   - simple POPC bilayer
   - model for inner mitochondrial membrane

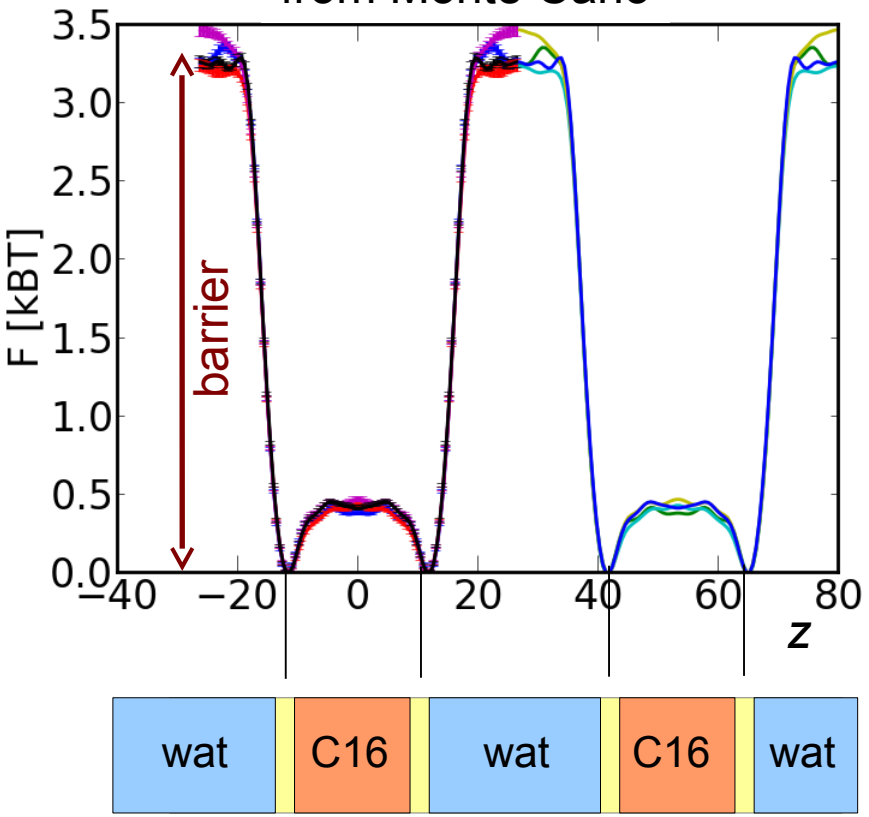
3. Deriving diffusion profiles from statistics

4. Free energy profiles and diffusion profiles

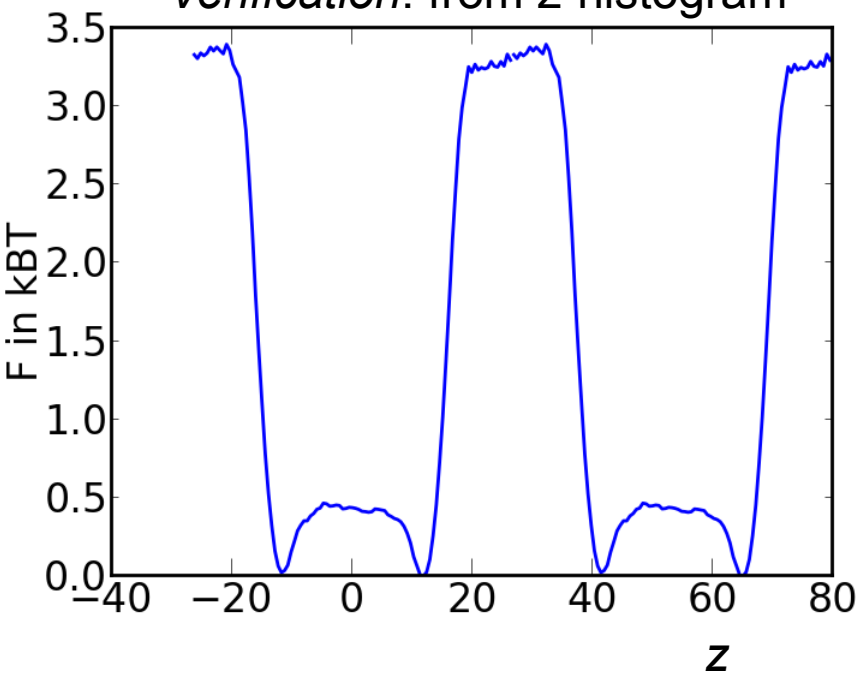
Free energy profile *F(z)* for oxygen in C16-layer/water

potential of mean force felt by O_2 molecule

from Monte Carlo



verification: from z-histogram



using equilibrium trajectories

barrier: 3.4 $k_B T$

preferential location: at interface C16/wat (grooves) or in C16

Free energy profile *F(z)* for oxygen in membranes

Improvements Monte Carlo routine:

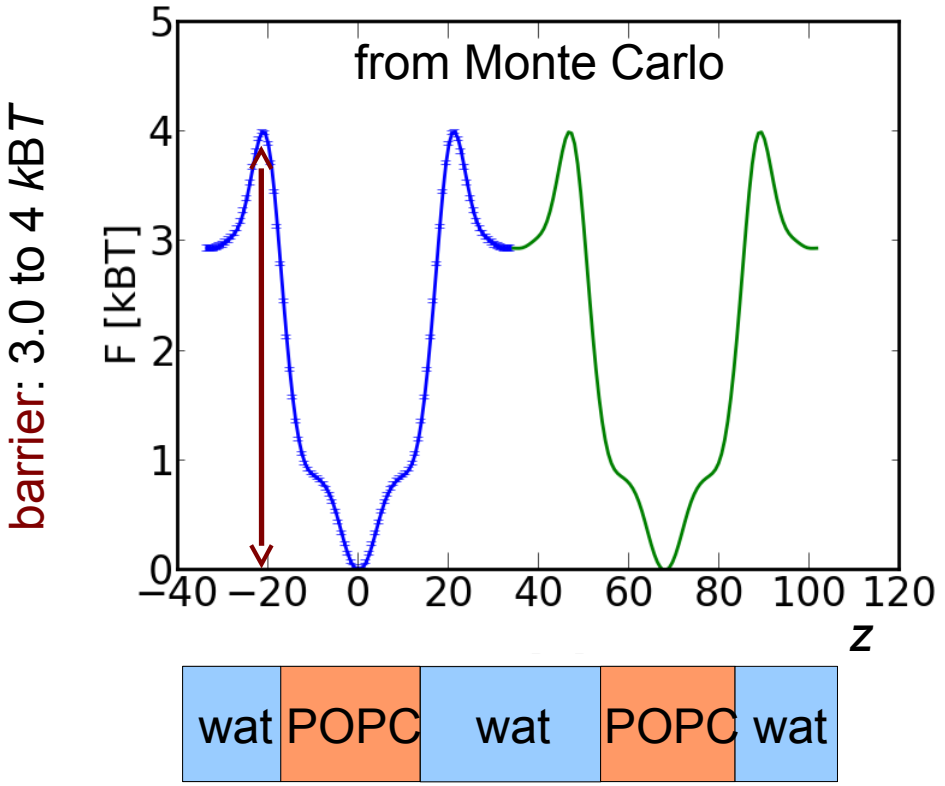
1) to improve smoothness: add penalty function $\sum_i (f_i - f_{i+1})^2$

2) to impose symmetry, improve smoothness: express F and D with basis functions

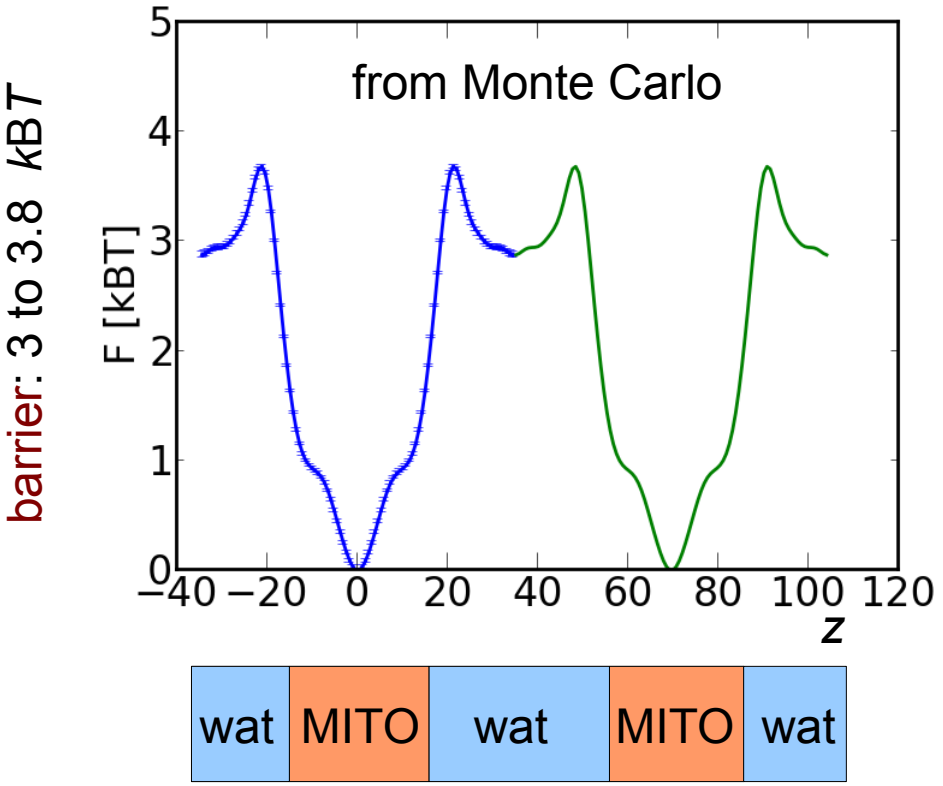
$$F(z) = \sum_n a_n \cos(\omega_n z)$$

$$\ln D(z) = \sum_n b_n \cos(\omega_n z)$$

POPC



MITO

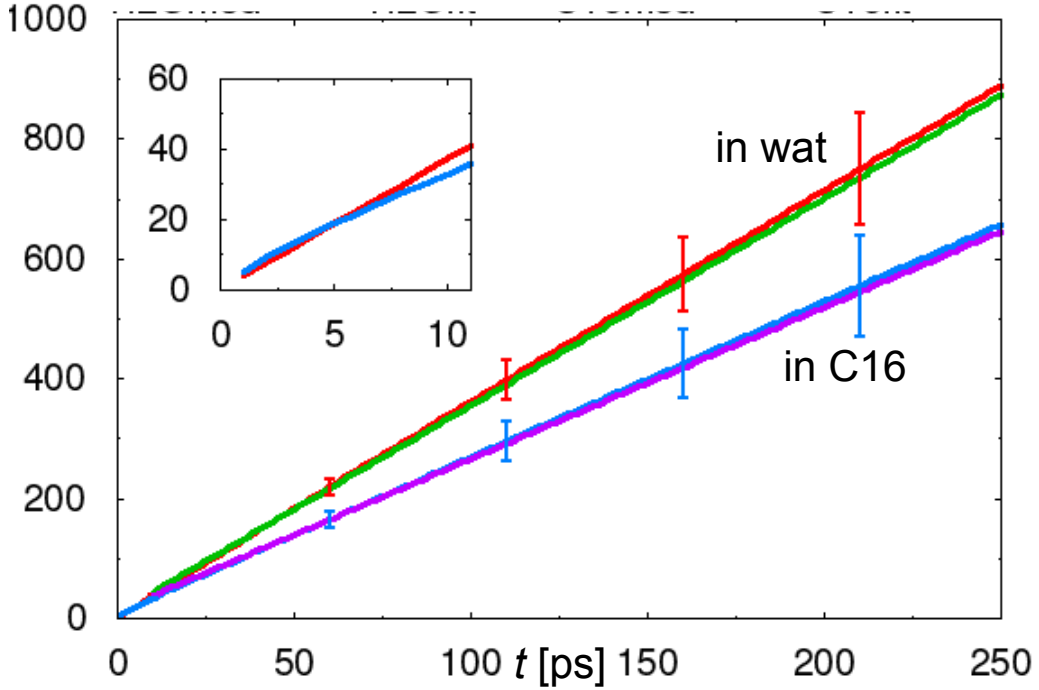


Oxygen diffusion $D_{\perp}(z)$: validation in pure phase

	t = 1 ps	t = 10 ps	t = 100 ps
pure wat			
from Monte Carlo	0.83	0.64	0.61
from MSD	0.85	0.63	0.60
pure C16			
from Monte Carlo	1.02	0.56	0.45
from MSD	1.03	0.56	0.46

all *D* values in A^2/ps
error: +- 0.02 A^2/ps

verification: MSD [A^2]



for bulk *D* (where *F* is flat):
Monte Carlo corresponds to MSD

Note on lag times:
choose $t > 5$ ps
to see long time behavior

Inner mitochondrial membrane composition

Three dominant head groups: PE, PC, CL

head	*%P*	*%lipid*
PE	37.9	49
PC	26.5	34
CL	25.4	16

are combined with saturated/unsaturated tails

	PE	PC	CL
14-0	1.7	2.4	1.4
15-0	1.8	5.6	0.0
16-0	9.1	27.6	9.0
17-0	2.0	0.8	1.6
18-0	19.7	9.4	6.3
16-br	3.2	1.0	1.7
18-br	2.6	1.0	0.0
16-1	3.8	1.6	2.4
18-1	8.6	16.4	11.5
18-2	22.1	29.0	65.2
18-3	2.3	1.3	0.0
20-1	2.1	0.0	0.0
20-4	20.5	1.8	0.0
% saturated	39.1	47.8	20.0
% unsaturated	59.4	52.1	79.1
ratio	66%	91%	25%

Comte, Maïsterrena, Gautheron
Biochim. Biophys. Acta 419, 271-284 (1976)

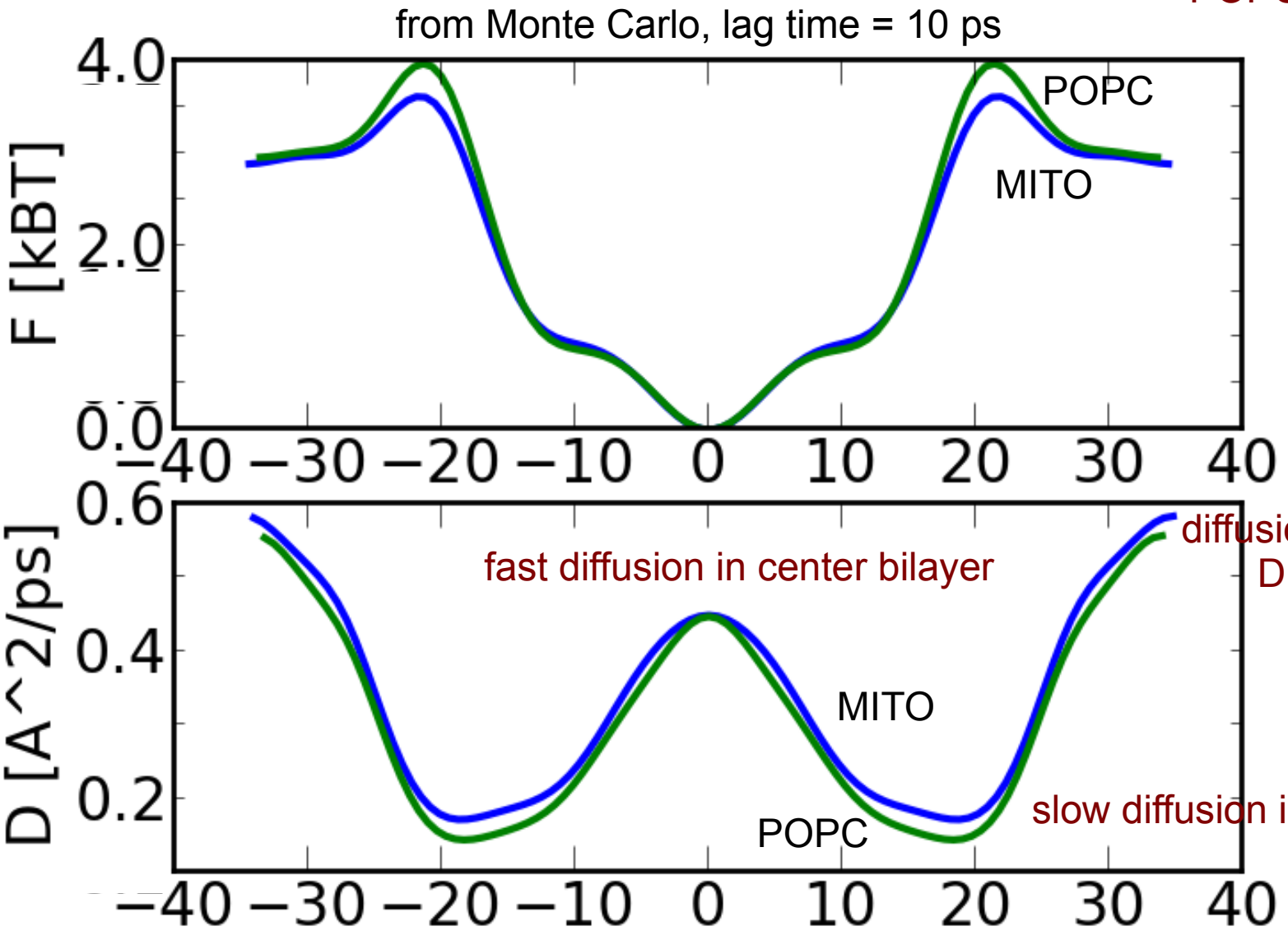
composition in simulations:

name		*head*	*sn1*	*sn2*	*sn3*	*sn4*
SAPE	9/side	PE	18-0	20-4		
SLPE	9/side	PE	18-0	18-2		
PLPC	12/side	PC	16-0	18-2		
CL	6/side	CL	16-0	18-2	18-2	18-2

and not yet speaking about plasmalogens, cholesterol, lipids, etc…

Oxygen diffusion $D_{\perp}(z)$ in membranes

little difference between POPC and MITO

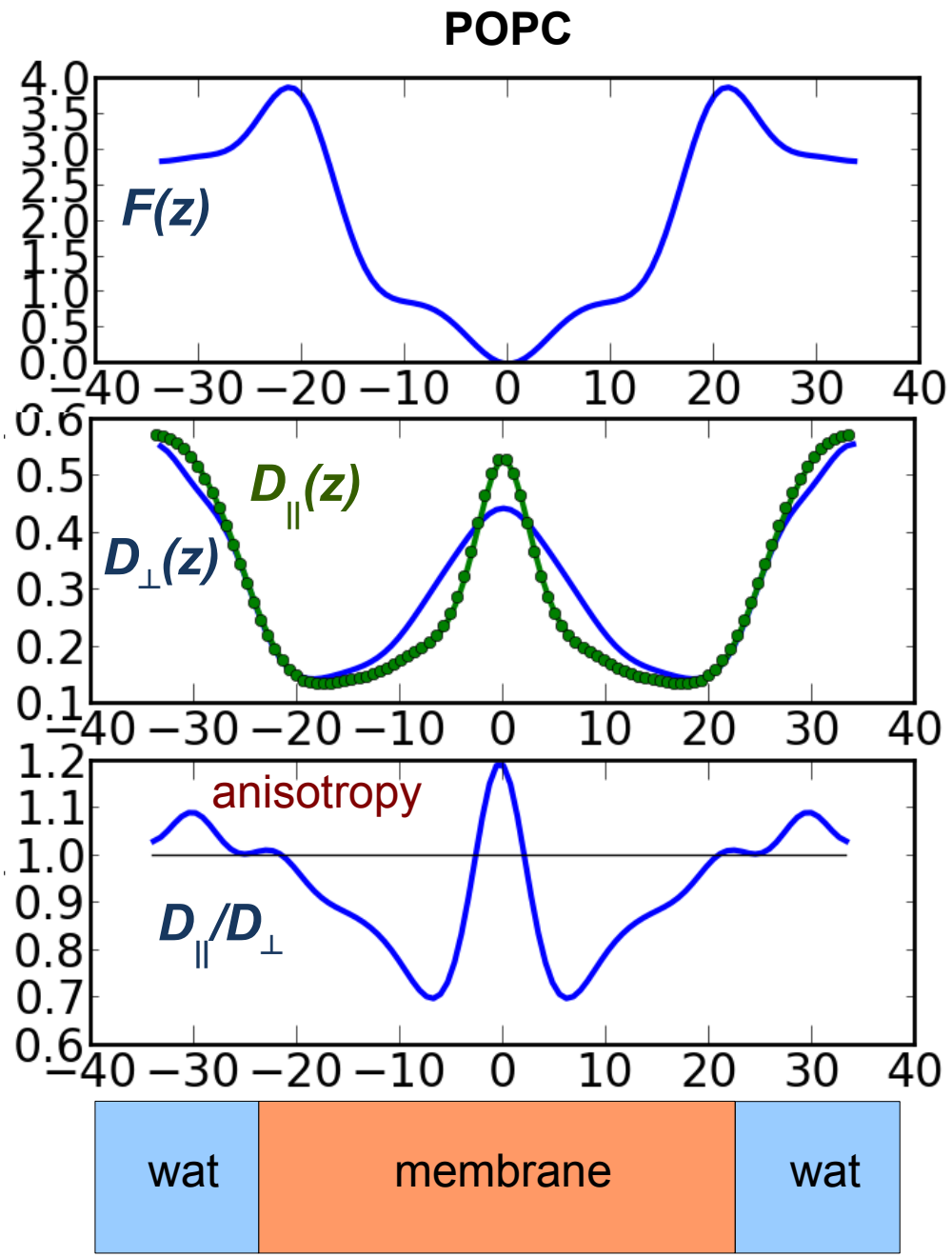


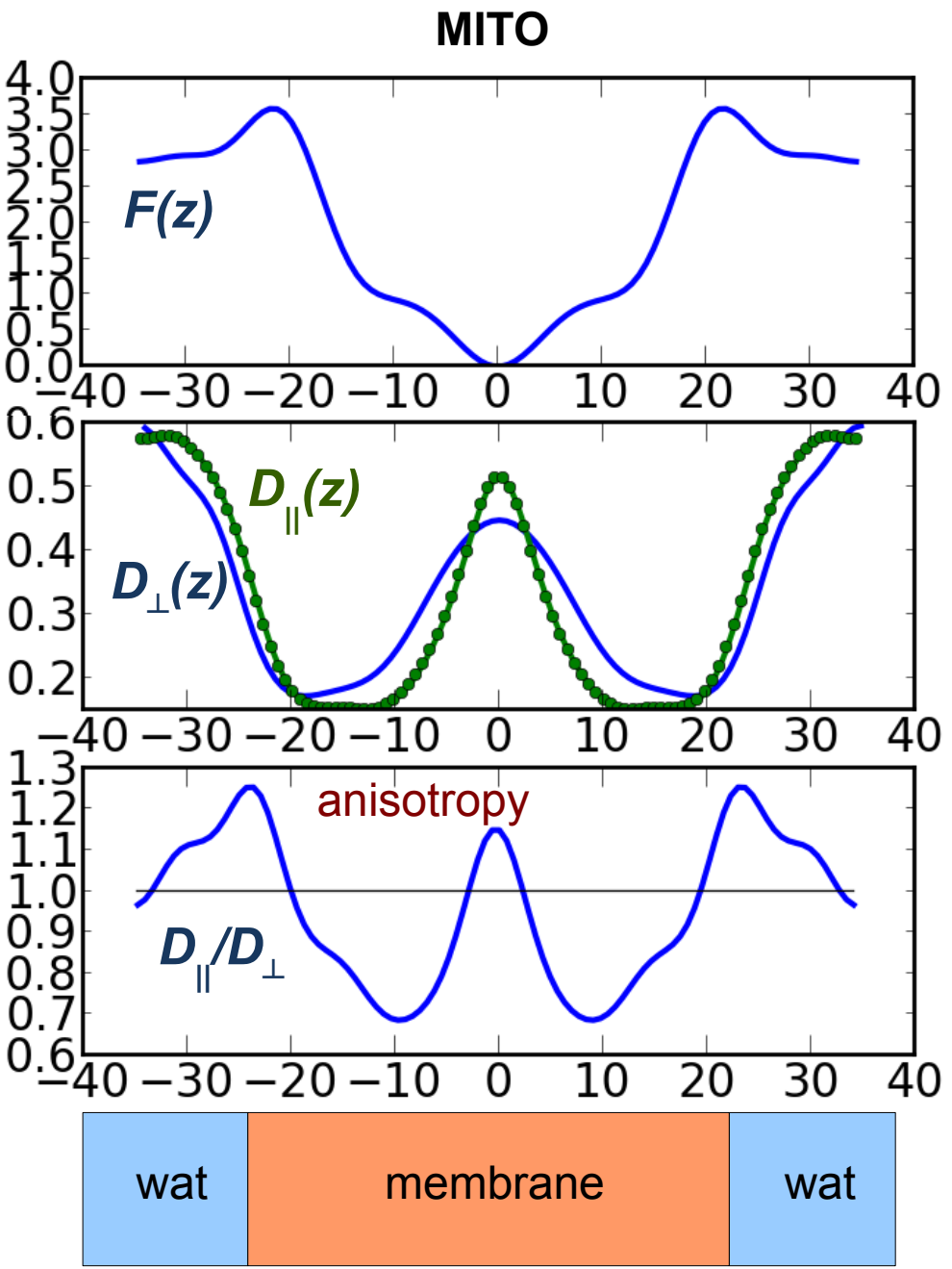
diffusion as in bulk water
D = 0.60 A^2/ps

slow diffusion in head group region

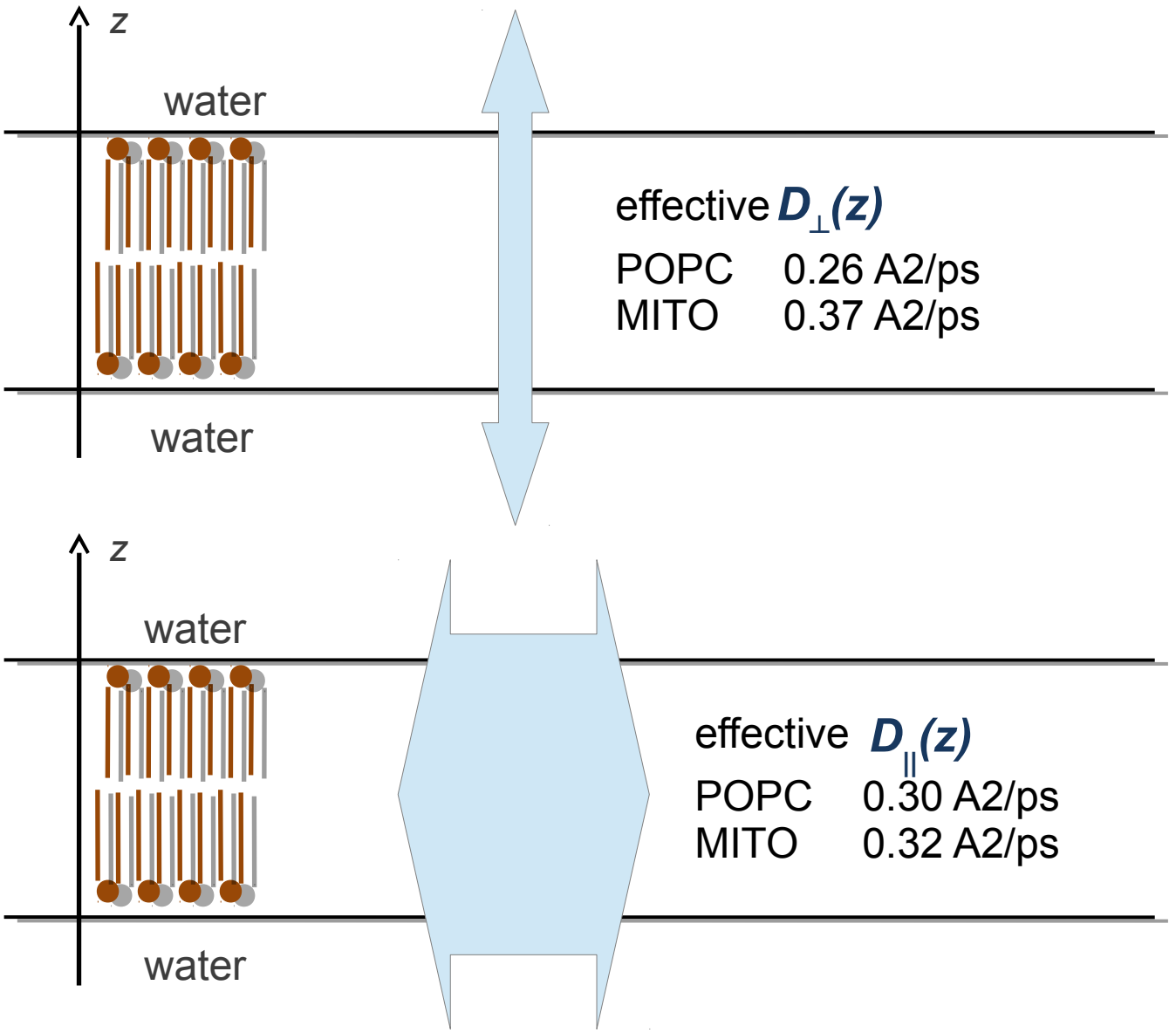
Oxygen diffusion $D_{||}(z)$ in membranes

little difference between POPC and MITO





Efficiency of oxygen diffusion: fluxes



effective $D_{\perp}(z)$

POPC	0.26 A2/ps
MITO	0.37 A2/ps

effective ***permeability***

POPC	0.0043 A/ps
MITO	0.0059 A/ps

effective $D_{||}(z)$

POPC	0.30 A2/ps
MITO	0.32 A2/ps

1. Membranes capture oxygen (slower than in bulk water D = 0.58 A^2/ps)
2. Diffusion in MITO is approx 30% more efficient

Conclusions

1. Methodology:
Membranes are inhomogeneous and anisotropic.
The standard MSD-versus-time method then requires short lag times.
We constructed profiles using Bayesian analysis (Monte Carlo),
allowing for longer lag times.

2. Profiles
Head-group region: diffusion is slow, free energy barrier
Center of bilayer: diffusion is fast, free energy is favorable

3. Anisotropy
Tail region: faster along the direction of the chains, than in radial direction
Center of bilayer: radially faster than in orthogonal direction.

4. Efficiency
Membranes capture oxygen (slower diffusion than in bulk water).
Little difference in POPC and MITO profiles.
Nevertheless, diffusion in MITO is approx 30% more efficient than in POPC.

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