Proteins in cell
modelling cell-like environments...
Proteins in silico

MD mainly confined to a single protein in solution or in membrane!

70's
- McCammon, Gelin, Karplus, Nature (1977)
- BPTI in vacuum
  - 9 ps

80's
- Levitt & Sharon, PNAS (1988)
- BPTI in water
  - 210 ps

90's
- Parallel SuperComputing
- DE Shaw Anton Computer
- PME / RESPA
- 10…100 ns

00's
- DE Shaw Anton Computer
- GPU
- μs-ms
Proteins in the Cell

Ellis, Trends in Biochemical Sci (2001)

... some keywords

heterogenous distributions
trafficking
functional cascades
network of interactions

... composition

water 70 % in mass
macromolecules ~30 %
ions < 1%

Goodsell, Scripps, California, US

model of E. coli
**Protein diffusion in cells**

**crowding effects on mobility**

- **geometric disorder**
- **distribution of times for jumping**
- **solvent mediated interactions**

---

**e.g. Neutron Scattering**

**timescale nanoseconds**

slowdown is due to solvent-mediated interactions

---

**Diffusion of BSE**

Roosen-Runge et al. PNAS (2011)
Stability under crowding

Stability of ubiquitin

Crowding by polymers
- Ficoll 100 g/L
- PVP 100 g/L

~2 kcal/mol

Crowding by proteins
- lysozyme 100 g/L
- BSA 100 g/L

~2 kcal/mol

different Cp

<\text{f}>/<\text{u}>

H/D exchange

Wang et al, JACS (2012)
Toward simulations of cell-like environments

All atom approach: too challenging
1000 proteins in solution at $\varphi \sim 30$
$\sim 90,000,000$ particles

Implicit solvent coarse-grained: good

but solvent mediated interactions?

Brownian Dynamics $+$ Hydrodynamics Interactions (Oseen/Rotne-Prager-Yamakawa)

poor scalability with size!

$+$
diffusion/folding
Ermak$+$McCammon JCP(1978)
Frembgen-Kesner$+$Elcock JCTC (2009)
Mikhailov$+$Kapral PNAS (2015)
Lipska et al JCP (2016)

molecular motor
Goldtvik et al JPC (2016)

aggregation
Ando$+$Skolnick BJ (2013)

MD $+$ Multiple Particle Collision

mobility/catalysis
Malevanets$+$Kapral, JCP(1999)
Schfield et al, JCP(2012)

MD $+$ Lattice Boltzmann accounting kinetics of solvent

colloid/polymers
Ahlrichs$+$Dunweg, JCP(1999)
Limbach, CPC(2006)

excellent scalability with size!
The coarse-grained model OPEP

Optimized Potential for Efficient protein structure Prediction

back-bone atomistic resolution Side-chain 1 bead

water and electrostatic free

cooperative term for HBs

ad hoc potential for ion-pairs

Techniques / Applications
Molecular Dynamics, Monte Carlo
REMD, Simulated Tempering, Metadynamics
Folding/Unfolding, Amyloid aggregation

Ion-Pair Potentials

Different stability for mesophilic/thermophilic proteins

Amyloid oligomer structures
A solute in fluid coupling MD and LB


**Fluid → Molecule**

\[
\mathbf{F}_{\text{drag},i} = -m\gamma (\mathbf{v}_i - \mathbf{u}_i)
\]

**Interpolation**

**Extrapolation on grid**

**Molecular Particle i**

**Molecule → fluid**

\[
S_p(x, t) = \omega_p \sum_i \left[ \mathbf{F}_{\text{drag},i} + \mathbf{F}_{r,i} \right] \cdot \mathbf{c}_p / c_s^2
\]

**Mesh**

**Random force**

**Lattice Boltzmann Particle**

**Discrete velocity (cp)**

\[
f_p(x, t) = \text{Velocity distribution along pth direction}
\]

\[
u(x, t) = \frac{1}{\rho} \sum_p f_p \mathbf{c}_p
\]
A solute in fluid coupling MD and LB

\[ f_p(x + c_p \Delta t, t + \Delta t) = f_p(x, t) - \omega \Delta t(f_p - f_p^{eq})(x, t) + S_p \Delta t \]

Relaxation Time \( \omega \sim 1/\nu \quad \nu = \text{Fluid Kinematic Viscosity} \)

Water 0.1 - 0.166 [Å² / fs]

Solute/Fluid coupling microscopic friction \( \gamma \) used as independent parameter

\[ F_D^D = -\gamma (v - u) \]
\[ \gamma = 6\pi \eta a \]

Macroscopic Stokes Law relates \( \gamma \) and \( \eta \)

Hynes, Kapral, Weinber, JCP(1978)
A solute in fluid

Multi-scaling

LB Time and MD Time

Grid Interpolation

Nearest Point

Multiple support

cheap

expensive

lattice

particle

Grid Resolution

High Res

Low Res

expensive

cheap

CPU Time $\sim N_g^3$
A solute in fluid toward the cell

**Diffusion/Stability**

[Diagram showing diffusion and stability metrics such as D/D0, volume fraction, msd, and ΔG over time and temperature.]

P. Derreumaux (LBT), experiments G. Pielak (Univ North Caroline)

**Aggregation Amyloid**

[Diagram illustrating aggregation and amyloid formation with proteins HSA, PrP, and Aβ.]

Problems: crowding and aggregation, coreceptor, membrane

CG model for lipids

P. Derreumaux (LBT), experiments U. Rezai (INSERM)

**Shear-Flow effect**

[vessel injuries and A2 shear unfolding with VWF (multimeric protein) sensing shear-flow (vessel injuries).]
Muphy/OPEP: Protein Stability

Same Stability in Langevin Dynamics and with Hydrodynamics when RMSD is high.

Arrangement of secondary structures, only minor instabilities.

Hydrodynamics:: longer living states

RMSD [Å]
- 1CLB 3.7
- 1E0L 3.4
- 1FCL 5.5
- 1SHG 5.3
- 2DA1 3.3

FS et al. JCTC (2015)
Protein mobility in “cell”
Massive simulation

Simulations
17576 Rat 1 yeast proteins (S. Pombe)
Rat 1 = 4013 CG particles
17756 GPU Titan Supercomputer (Oak Ridge)
for Gordon Bell Prize 2013 (SuperComputing)

Simulation time ~30 ns

Authors
Bernaschi / Bisson / Fatica / Melchionna

Crowding Φ=30%
Diffusion slowdown D/D₀~1/10

*Roosen-Runge et al. PNAS(2011)
Protein mobility in “cell”
Elastic Network for Proteins

Simulations
70 CI2 protein
CI2 = 331 CG particles
Elastic Network (EN) or protein

Box L=135, 145, 160, 180, 250 Å.

Simulation time 0.5 µs

D0~14 Å²/ns (*exp 15 Å²/ns)

EN cheap&good
possibility to modulate flexibility
time scale up to µs

€ affordable $ 
64 < cores < 512

*Wang, Li, Pielak JACS (2010)
Protein mobility in “cell”
Shake, sample, and back-map

LBMD:: sampling local packing
Enhanced Sampling :: stability curves
@CG level or all-atoms (back-map)
Protein mobility in “cell” lysozyme in powder + REST2

**Muphy/OPEP: Back-map…**

Katava et al in preparation

**Lysozyme in H2O**

**Lysozyme powder + H2O**
(h=0.3)

**Lysozyme powder + glycerol**
(h=0.3)

REST2 to sample f/u
thermal response via corresponding state principle
experimental trend reproduced

Stirnemann&FS, JCTC (2015)
Hydrodynamics speeds up peptides aggregation

Simulations
18 monomers $A\beta_{16-22}$
KLVFAE
CH3-CO and NH2 terminal
Cubic box 65 Å
c=100 mM
Two-step mechanism
Speed up of both collapses (45%)

Hydrodynamics

- Enhance diffusivity of proteins
  - Frembgen-Kesner&Elcock JCTC (2009)
- Enhance aggregation of lipids
  - Ando&Skolnick BJ (2013)
- Enhance folding kinetics
  - Cieplak&Niewieczerza JCP (2009)

$A\beta_{16-22} \sim 23 \text{Å}^2/\text{ns} \text{ (dilute solution)}$
Aβ aggregation toward big systems

Simulation
100 monomer Aβ(16-22)
all atoms equivalent 300k
L=150 Å
c~50 mM

Muphy/OPEP: amyloid aggregation
100 monomer Aβ(16-22)  
Chiricotto, et al JCP(2016)

HI speed up aggregation
Aβ aggregation toward big systems

HI enhance cluster formation and exchange

Muphy/OPEP: amyloid aggregation

100 monomer Aβ(16-22)

Chiricotto, et al JCP(2016)
Aβ aggregation toward big systems

extending simulation time
lower resolution LB

100 monomer Aβ(16-22)

Chiricotto, et al JCP(2016)

Aggregation Completed!

% β secondary structure

fusion of largest and second largest clusters

timescale ~300/400 ns
Aβ aggregation toward big systems

Simulation
1000 monomer Aβ(16-22) all atoms equivalent 2.4Mio L=300 Å c=55 mM

Intermediate sizes highly populated

at 100 ns only 6% in largest cluster

Comparing 100 Aβ and 1000 Aβ
Aβ aggregation toward big systems

extending simulation time
lower resolution LB

at 250 ns two leading aggregates (≈30% of the system)

branched structures (Lateral nucleation?)

inclusion of oligomers

14% β

1000 monomer Aβ(16-22)
Muphy/OPEP: amyloid aggregation

1000 monomer $\text{A}\beta(16-22)$

Fluid iso-kinetic surfaces

Local fluid streamlines
Protein unfolding in shear flow

Simulations

β-hairpin (GB1, fragment 41-56)
Box 50x50x50 Å
Shear Rate $10^{10}$ s$^{-1}$

**SHEAR**
a route to probe mechanical stability

**OPEN QUESTION**
do proteins unfold under shear?

Protein unfolds!
but at very high shear rate (*)

Protein unfolding in shear flow

Mechanical weakness

...where unfolding starts .... to be compare to .....T and Force unfolding

three regimes

forcing shear rate

kinetics of unfolding

$\beta$-hairpin

WW domain

Ca Binding

3 α helices

β sheets

β hairpin
Protein unfolding in shear flow

distribution of unfolding time for \textbf{\textit{\(\beta\)-hairpin}}

increasing shear rate

slow unfolding kinetics
Protein unfolding in shear flow

Reaction coordinate only sporadically aligned with shear gradient

- Force spike
- HB disruption

Solvent drag force

\[ f_{\text{drag}} = \text{force on HB}_{\text{donor}} - \text{HB}_{\text{acceptor}} \]
projected on HB direction

HBs break at different times as effect of different spikes
Protein unfolding in shear flow

test on large domain **A2 VWF**

<table>
<thead>
<tr>
<th>Time</th>
<th>Image Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>time = 0</td>
<td><img src="image1.png" alt="Image 1" /></td>
</tr>
<tr>
<td>time = 0.8 ns</td>
<td><img src="image2.png" alt="Image 2" /></td>
</tr>
<tr>
<td>time = 2.3 ns</td>
<td><img src="image3.png" alt="Image 3" /></td>
</tr>
<tr>
<td>time = 7.0 ns</td>
<td><img src="image4.png" alt="Image 4" /></td>
</tr>
</tbody>
</table>

unfolding steps:

1. separation peripheral domains
2. crack of hydrophobic cores (3 β-sheets)
Crowding in silico

Kesner&Elcock Biophys Rev (2013)

1996
Bicout&Field
ribosomes, tRNAs, proteins
spheres \{r\}
Langevin Dynamics
$N_p \approx 340$

2008
Ellison et al.
composition as in E.coli
spheres \{r\}
specialised RandomDynamics
$N_p \approx 1.7$ million

2010
McGuffee&Elcock
composition as in E.coli
all atoms
rigid body BD
$N_p \approx 1000$

Ando&Skolnick
composition as in E.coli
spheres
Stokesian Dynamics

Here we are
Feig&Sugita
all atoms
+ water
Hydrodynamics is included

Diffusion only mildly slowed down by excluded volume, only including Hydrodynamics there is agreement with experiments
Proteins in cell: a new frontier for Computational Biology

Effect of crowding on diffusion, stability and aggregation is a major challenge for both experiments and simulations.

Simplified models are necessary but is key to keep solvent effects.

Brownian Dynamics with Hydrodynamics is a standard way.
Lattice Boltzmann coupled to MD is a valuable alternative.

Friends & “Money”

- M. Chiricotto (IBPC, Paris)
- P. Derreumaux (IBPC, Paris)
- S. Melchionna (CNR, Rome)

http://x-proteins.blogspot.fr
Amyloid in shear flow

**SHEAR**
A model for understanding agitation processes and steps of fibril growth.

**OPEN QUESTION**
do amyloids aggregate faster under shear?

Same overall kinetics but different peptide organisation

Hamilton-Brown et al, JPCB (2008), Dunstan et al, ProteinEngDesSel (2009), Lee et al, JBC (2012)
Rules

- each site hosts up to 6 particles
- particles move along 6 discrete velocities
- 2 particles sitting on same site NOT move along same directions

Occupation site \( n_i(x,t) = 1,0 \)

Lattice State for \( N \) site = \( 2^{6N} \)

for site \( i \) where particles go \( |n_1 n_2 n_3 n_4 n_5 n_6> \) i.e. |001010>

Streaming

\[
n_i(\vec{x} + \vec{e}_i, t + 1) = n_i(\vec{x}, t)
\]

Collision

Operator altering the vector state \( n (+1,-1,0) \)

\[
n_i' - n_i = \Omega(\vec{n})
\]

LGCA crude BUT essential:

- conserve particle number, total momentum, if lattice is good invariant rotation!!!!!

Succi, The LBE for fluid Dynamics and Beyond (2001)
Lattice Boltzmann Method

Distribution function of displacement
discrete states $f(x,t)$

$\mathbf{f}_i = \langle n_i \rangle$

Fluid Density $\rho = \sum_{a=0}^{8} f_a$

9 velocity directions

Macroscopic velocity $\mathbf{u} = \frac{1}{\rho} \sum_{a=0}^{8} f_a \mathbf{e}_a$

Succi, The LBE for fluid Dynamics and Beyond (2001)
**Lattice Boltzmann Method**

Kinetic equation

\[
f_i(x + e_i \Delta x, t + 1) = f_i(x, t) + \Omega_i(f(x, t))
\]

Total Mass Conservation

\[
\sum_i \Omega_i = 0
\]

Total momentum conservation

\[
\sum_i \Omega_i e_i = 0
\]

**Problems**

Construct a functional form for collisions

Derive macroscopic hydrodynamic equation, Navier-Stokes

**Strategy**

Focus on long-wave-length and low-frequency

\[
\Delta x \sim \epsilon \quad \Delta t \sim \epsilon
\]

incremental length and time are small and of the same order \( \epsilon \)

Succi, The LBE for fluid Dynamics and Beyond (2001)
The collision term

Multi-scale separation

\[ f_i = f_i^{eq} + \epsilon f_i^{neq} \]

\[ f_i^{neq} = f_i^1 + \epsilon f_i^2 + O(\epsilon^2) \]

Taylor expansion of collision term

\[ \Omega_i(f) = \Omega_i(f^{eq} + \epsilon f^{neq}) \]

Equilibrium condition

\[ \Omega_i(f^{eq}) = 0 \]

Linearised collision

\[ \Omega_i(f) = \frac{\partial \Omega_i(f^{eq})}{\partial f_j}(f_j - f_j^{eq}) \]

if one single rate for relaxation to equilibrium

\[ \Omega_i(f) = -(f_i - f_i^{eq})/\tau \]

Succi, The LBE for fluid Dynamics and Beyond (2001)
Starting from Boltzmann Equation

Probability in phase-space $dx dp$ at time $t$  \[ f(\vec{x}, \vec{p}, t) \]

Core of Kinetic Theory

Evolution of one-body distribution
\[
D_t f = \left[ \partial_t + \frac{\vec{p}}{m} \cdot \partial_{\vec{x}} + \vec{F} \cdot \partial_{\vec{p}} \right] f(\vec{x}, \vec{p}, t) = C_{12}
\]

Evolution of one particle distribution
Collision

$C_{12}$ depends hierarchically on 2,3,4… n body

BGK for $C_{12}$

Assume Maxwell-Boltzmann
Expansion of MB

\[ -\frac{1}{\tau} (f - f^{eq}) \]

\[ f^{eq} \approx A \exp(-3/2\chi^2)[1 + 3(\chi \cdot u) + \frac{9}{2} (\chi \cdot u)^2 - \frac{3}{2} u^2] \]

$u$ fluid velocity  \hspace{1cm} $\chi$ particle velocity

Discrete $f \rightarrow f^D$
Obtain weight for lattice

\[ f^D_i = W_i f_i \]

LBM

\[ f_i(x + e_i \Delta x, t + 1) = f_i(x, t) + \Omega_i f(x, t) \]
A solute in fluid

Multi-scaling

time for 1000 MD step
solute 864 particle
Lx=Ly=Lz=65 Å
Intel Xeon 5660

t = \frac{A}{x^\alpha} + t_0

MD time + mapping

Synchronous \alpha \sim 3
Asynchronous \alpha \sim 2

t_0 \sim 12 s

\frac{\text{time}_{\text{NearestPoint}}}{\text{time}_{\text{MultipleSupport}}} \sim 0.7
A solute in fluid

Multi-scaling

Coarse-graining?
A well-known example: the kicked particle

- Coarse-graining would not consider the time evolution of coherent motion
- Hydrodynamics pervasive down to the micro scale
- Many-body motion would be increasingly hard to treat
- Handling systematic flows problematic

A solute in fluid

Multi-scaling

\[ \nu = \nu_0 \left( \frac{\Delta x_0}{\Delta x} \right)^\alpha \]

\[ [\nu] = [L^2]/[t] \]

expected \( \alpha = 2 \)

Questions:
- How molecularity affects dimensional scaling?
- How molecule length scale affects scaling at lower and lower resolution?
A solute in fluid

Multi-scaling

\[ \nu = c_s^2 \left( \frac{1}{\omega} - \frac{\Delta t}{2} \right) \]

Reference Relaxation
\( \tau_0 = 1 \text{ fs} \)
\( \nu_0 = 0.1666 \rightarrow 0.1666 \text{ A}^2/\text{fs} \)

Relaxation 1
\( \tau = 1.5 \text{ fs} \)
\( \nu = 0.1666 \left[ \Delta X^2/\Delta t \right] \)

Relaxation 2
\( \tau = 0.5 \text{ fs} \)
\( \nu = 0.1666 \left[ \Delta X^2/\Delta t \right] \)

Scaling
\[ \nu = \nu_0 \left( \frac{\tau}{\tau_0} \right) \]

Brownian Dynamics

The phase-space distribution function \( W(\{r\}, \{p\}, t) \)

The Fokker-Plank eq

\[
D_t W = \sum_{i,j} \frac{\partial}{\partial p_i} \gamma_{ij} \left( m_j^{-1} p_j W + kT \frac{\partial W}{\partial p_j} \right)
\]

friction tensor incorporating hydrodynamic interactions (2 body)

From the Fokker-Plank eq to the Diffusion eq, \( W(\{r\}, t) \)

\[
\frac{\partial W}{\partial t} = \sum_{i,j} \frac{\partial}{\partial r_i} D_{i,j} \left( \frac{\partial W}{\partial r_j} - \frac{1}{kT} F_j W \right)
\]

Diffusion tensor related to friction

inter-particles + external

\[
\text{Time}
\]

\[
t = 0 \quad \text{solution at first order of } W(r, t + \Delta t) \quad \text{extract } \{r\} \text{ from } W
\]

\[
\{r\}
\]

\[
< \Delta r_i(\Delta t) >= \sum_j \left( \frac{\partial D_{ij}}{\partial r_j} + \frac{D_{ij}}{kT} F_j \right) \Delta t
\]

\[
< \Delta r_i(\Delta t) \Delta r_j(\Delta t) >= 2D_{ij} \Delta t
\]

Ermak & McCammon JCP (1978)
Brownian Dynamics

Ermak-McCammon

Time evolution of particles

Langevin eq + Hydrodynamics

\[ M_i \dot{v}_i = -\sum_j \gamma_{ij} v_j + F_i + \sum_j \alpha_{ij} f_j \]

- friction tensor for hydrodynamic interactions (2 body)
- white noise

\[ r_i = r_i^0 + \sum_j \frac{\partial D_{ij}^0}{\partial r_j} \Delta t + \sum_j \frac{D_{ij}^0 F_{ij}^0}{kT} \Delta t + R_i(\Delta t) \]

Random Displacement (Depends on \((D_{ij})^{1/2}\))

**Problem**: Functional form for \(D_{ij}\)

Ermak & McCammon JCP (1978)
Brownian Dynamics

idea: sphere perturbs a fluid, this in turn acts on a probe sphere at distance $r$

expansion for $r \gg a$ (far field contribution)

**NB** a term for near field ($r < 2a$) can be added!

(Lubrication Forces)

### Oseen Tensor

\[
D_{ij} = \frac{kT}{6\pi \eta a} \delta_{ij}
\]

\[
D_{ij} = \frac{kT}{6\pi \eta r_{ij}} (I + \frac{r_{ij} r_{ij}}{r_{ij}^2})
\]

### Rotne-Prager Tensor

\[
D_{ij} = \frac{kT}{6\pi \eta a} \delta_{ij}
\]

\[
D_{ij} = \frac{kT}{8\pi \eta r_{ij}} [(I + \frac{r_{ij} r_{ij}}{r_{ij}^2}) + \frac{2a^2}{r_{ij}^2} (\frac{1}{3} I - \frac{r_{ij} r_{ij}}{r_{ij}^2})]
\]

\[
\sum_j \frac{\partial D_{ij}}{\partial r_j} = 0
\]
Brownian Dynamics

Random Displacement

Hydrodynamically Correlated Motion

\[ \langle R_i(\Delta t) \rangle = 0 \]
\[ \langle R_i(\Delta t)R_j(\Delta t) \rangle = 2D_{ij}\Delta t \]

\[ \text{Cost } N^3 \]

\[ D = BB^T \]
\[ \vec{R} = B\vec{X} \]

SQRT of D

X vector of 3N random numbers

Long Range Nature HI \( \sim 1/r \)

Strategies

Mean Field (from \( D_{ij} \rightarrow D_i \))
Screening (remove long range \( r_{ij} \))
Long range part as PME
Crowding in silico

Skolnick group

Cell Crowding

- macromolecules as spheres
- short range LJ interactions

Concentration 250/300/350 mg/mL
np 400 - 1200

Stokesian Dynamics
(BD + far + near + mb interactions)

\[ \frac{D}{D^0} \sim 0.1 \text{ for } c=300 \text{ mg/mL} \]

Lipid Aggregation

- lipid as dumbbell model

BD + Rotne-Prager-Yamakawa

HI speed up aggregation

Ando&Skolnick, PNAS (2010), BJ (2013)
Muphy/OPEP: Protein Relaxation

**Tuning the coupling**

\[ \mathbf{F}_{\text{drag},i} = -m \gamma (\mathbf{v}_i - \mathbf{u}_i) \]

**Hydrodynamics**
- Fluctuations around initial state
- Drift at weak coupling

**Langevin Dynamics**
- Drift for all coupling

Fluid pumps/dissipates energy and controls solute relaxation
in Cell 20-40 % of volume is occupied by macromolecules
the effect of excluded volume is non-linear with size of “probe”
Excluded Volume

a chemical perspective

chemical potential

\[ \mu_i = \mu_i^{ideal} + \mu_i^{nonideal} \]

effect of concentration

\[ \mu_i^{ideal} = \mu_i^0 + kT \ln c_i \]

effect of intermolecular interactions

\[ \mu_i^{nonideal} = kT \ln \gamma_i \]

macromolecules get closer under crowding

free energy of interactions between species i and the other macromolecules

Crowding enhances the effect of concentrations

\[ kT \ln \gamma_i c_i \]

thermodynamic activity

Excluded Volume

Crowding affects both thermodynamics and kinetics

free energy of confinement

\[ \Delta F^c = -kT \frac{Z^0}{Z^c} \]

partition function

\[ \int_{\Omega} dr^3N e^{-\beta U} \]

volume accessible

overall exclude volume

exclude volume of TS configurations

species A and B react ($k_s$)

\[ \frac{\partial C_B}{\partial t} = D \nabla^2 C_B - k_s C_B C_A \]

diffusive term

reactive term

A

Rc

reactive space

B

diffusion of B relative to A

Lg reaction rate constant

crowding

crowding enhances activity
crowding reduces diffusivity

Ellis, Trends in Biochemical Sci (2001)
Protein stability

crowding

stability under crowding reduces to estimate free energy of confinement for Folded and Unfolded States

Confining the folded state

Scaled Particle Theory

Free energy to transfer an ideal spherical particle of radius R in environment with occupied volume φ

\[
\Delta G^{Conf}_F = -kT \ln(1 - \phi) + \sum_{i=1}^{3} A_i Q^i
\]

\[
Q = \frac{\phi}{1 - \phi}
\]

\[
A_i = f(R)
\]

**PROBLEM**: unfolded state NOT globular

**STRATEGIES**

- Random Walk in crowded space
- Generate representative Unfolded states from $P(R_g)$ and try to insert in the crowded space or compute Excluded Volume