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Martini WorkshOp 2015

Martini Proteins

Alex de Vries

Experience is simply the name we give our mistakes

Oscar Wilde

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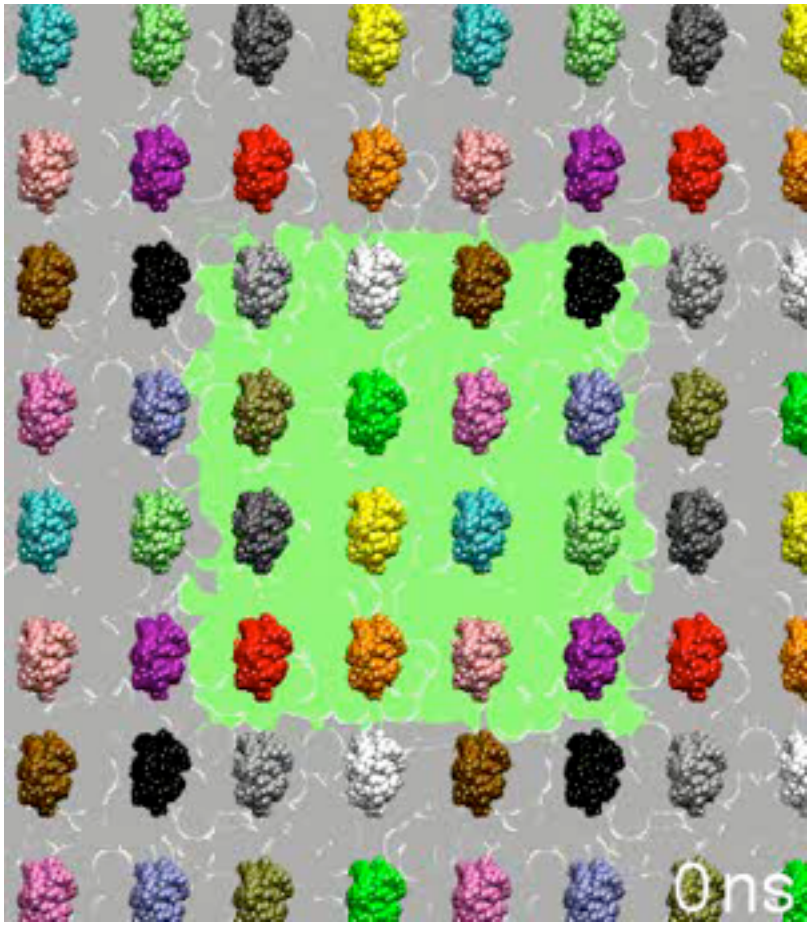
Early Martini protein work

› Formation of rhodopsin clusters in membranes of different thickness

- G-protein coupled receptor molecule visual rhodopsin in single-component membrane
- 16 independent membrane proteins in simulation cell
- clustering preference and dynamics depends on bilayer thickness
- neighboring proteins explore different binding interfaces



Periole et al. *J. Amer. Chem. Soc.* **129**, 10126 (2007)

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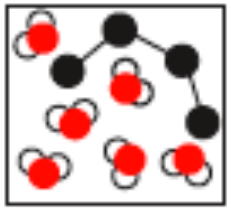
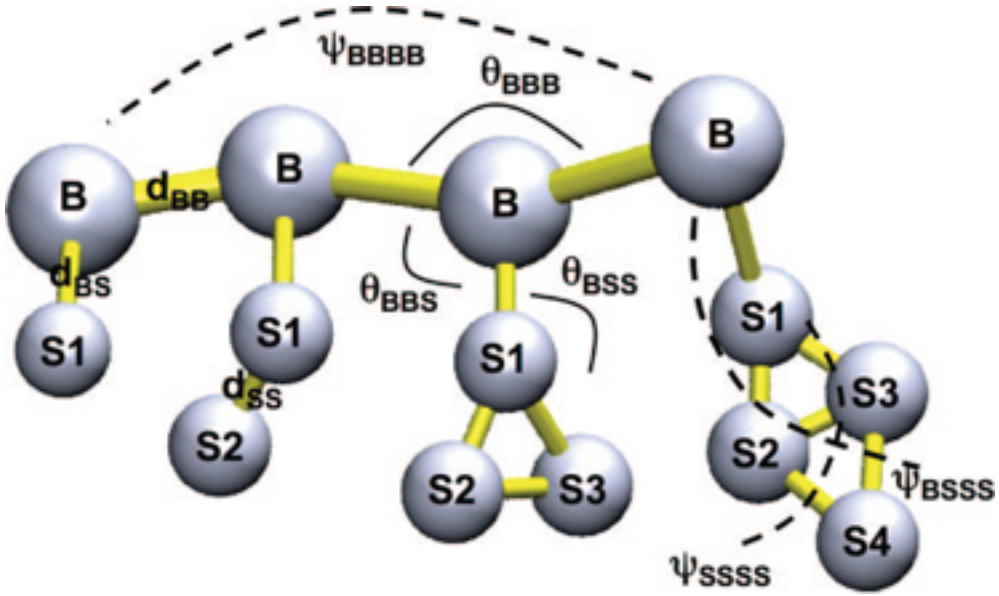
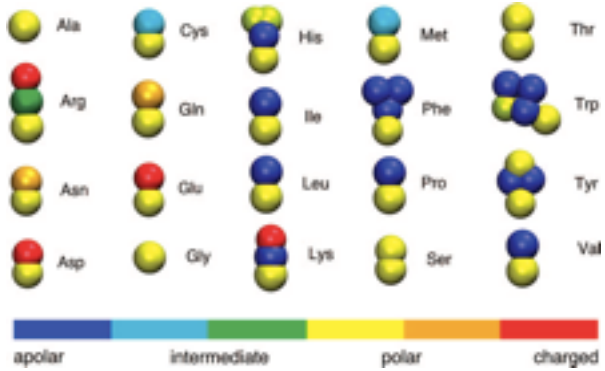
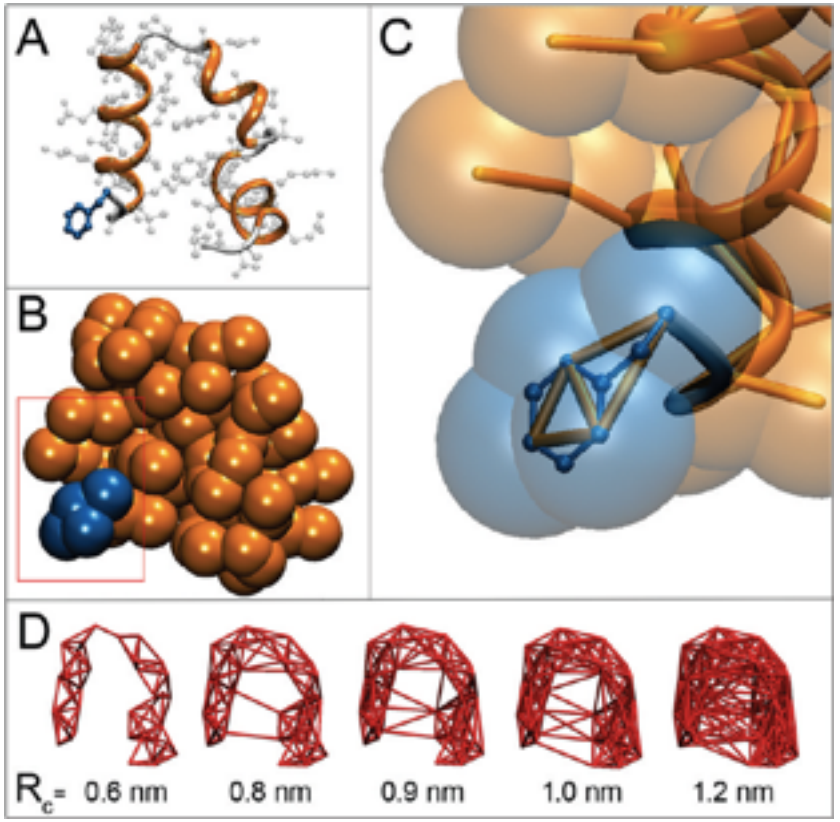
There are two Martini protein models!







- Original by Monticelli et al, 2008



- Integrated with elastic network by Periole et al, 2009

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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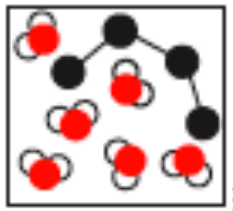
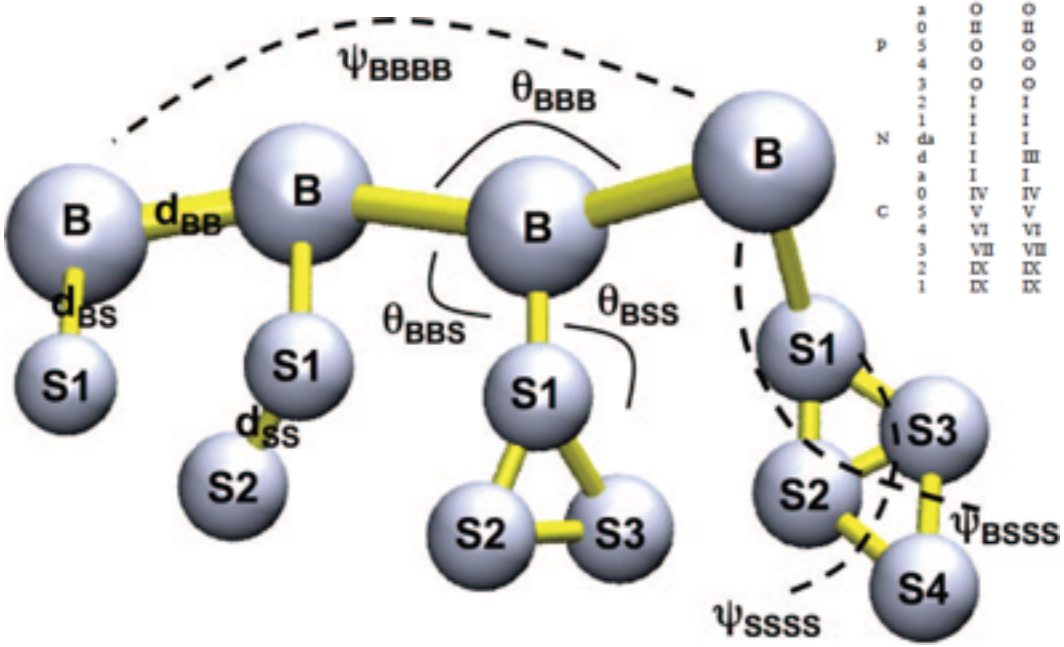


Standard Martini protein model

- Compatible with Martini model for lipids
- Uses the Martini interaction matrix for interactions
- Developed for membrane proteins: the study of protein-lipid and protein-protein interactions



	sub	Q da	Q d	Q a	Q 0	P 5	P 4	P 3	P 2	P 1	N da	N d	N a	N 0	C 5	C 4	C 3	C 2	C 1
Q	da	O	O	O	II	O	O	O	I	I	I	I	I	IV	V	VI	VII	IX	IX
	d	O	I	O	II	O	O	O	I	I	I	III	I	IV	V	VI	VII	IX	IX
	a	O	O	I	II	O	O	O	I	I	I	I	III	IV	V	VI	VII	IX	IX
	0	II	II	II	IV	I	O	I	II	III	III	III	III	IV	V	VI	VII	IX	IX
P	5	O	O	O	I	O	O	O	O	O	I	I	I	IV	V	VI	VI	VII	VIII
	4	O	O	O	O	O	I	I	II	II	III	III	III	IV	V	VI	VI	VII	VIII
	3	O	O	O	I	O	I	I	II	II	II	II	II	IV	IV	V	V	VI	VII
	2	I	I	I	II	O	II	II	II	II	II	II	II	III	IV	IV	V	VI	VII
	1	I	I	I	III	O	II	II	II	II	II	II	II	III	IV	IV	IV	V	VI
N	da	I	I	I	III	I	III	II	II	II	II	II	II	IV	IV	V	VI	VI	VI
	d	I	III	I	III	I	III	II	II	II	II	III	II	IV	IV	V	VI	VI	VI
	a	I	I	III	III	I	III	II	II	II	II	II	III	IV	IV	V	VI	VI	VI
	0	IV	IV	IV	IV	IV	IV	IV	III	III	IV	IV	IV	IV	IV	IV	IV	V	VI
C	5	V	V	V	V	V	V	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	V	V
	4	VI	VI	VI	VI	VI	VI	V	IV	IV	V	V	V	IV	IV	IV	IV	V	V
	3	VII	VII	VII	VII	VI	VI	V	V	IV	VI	VI	VI	IV	IV	IV	IV	IV	IV
	2	IX	IX	IX	IX	VII	VII	VI	VI	V	VI	VI	VI	V	V	V	IV	IV	IV
	1	IX	IX	IX	IX	VIII	VIII	VII	VII	VI	VI	VI	VI	VI	V	V	IV	IV	IV

- 4-to-1 mapping scheme *on centers of mass*
- uses extension for rings
- time step 25 fs

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
Marrink et al. *J. Phys. Chem. B* **111**, 7812 (2007)

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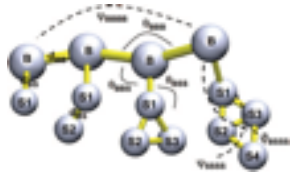
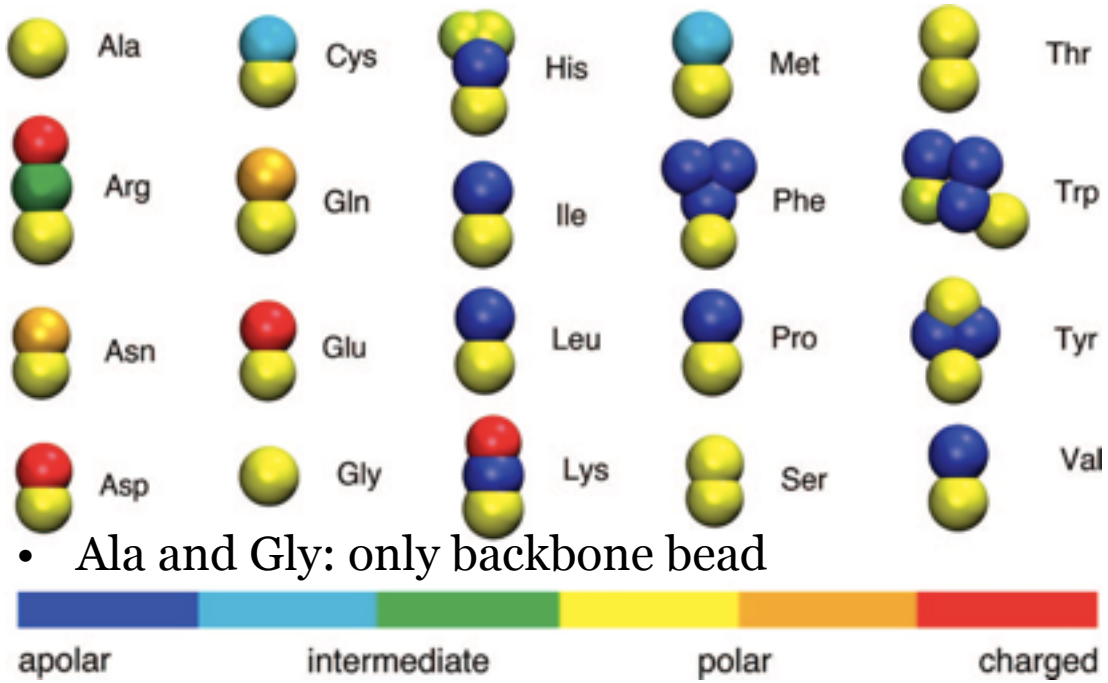


Standard protein model: side-chain beads

type	building block	examples
Q_{da}	$H_3N^+—C_2—OH$	ethanolamine (protonated)
Q_d	$H_3N^+—C_3$	1-propylamine (protonated)
	NA^+OH	sodium (hydrated)
Q_a	PO_4^-	phosphate
	CL^-HO	chloride (hydrated)
Q_0	C_3N^+	choline
P_5	$H_2N—C_2=O$	acetamide
P_4	HOH (× 4)	water
	$HO—C_2—OH$	ethanediol
P_3	$HO—C_2=O$	acetic acid
	$C—NH—C=O$	methylformamide
P_2	$C_2—OH$	ethanol
P_1	$C_3—OH$	1-propanol
		2-propanol
N_{da}	$C_4—OH$	1-butanol
N_d	$H_2N—C_3$	1-propylamine
N_a	$C_3=O$	2-propanone
	$C—NO_2$	nitromethane
	$C_3=N$	proprionitrile
	$C—O—C=O$	methylformate
	$C_2HC=O$	propanal
N_0	$C—O—C_2$	methoxyethane
C_5	$C_3—SH$	1-propanethiol
	$C—S—C_2$	methyl ethyl sulfide
C_4	$C_2=C_2$	2-butyne
	$C=C—C=C$	1,3-butadiene
	$C—X_4$	chloroform
C_3	$C_2=C_2$	2-butene
	$C_3—X$	1-chloropropane
		2-bromopropane
C_2	C_3	propane
C_1	C_4	butane
		isopropane



- Ala and Gly: only backbone bead

› Initial side-chain bead type assignments made according to Martini v2.1 (2007) scheme, i.e. based on oil-water partitioning

Marrink et al. *J. Phys. Chem. B* **111**, 7812 (2007);
Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008)

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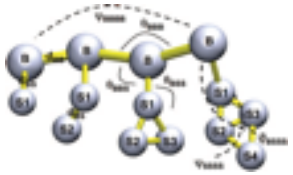
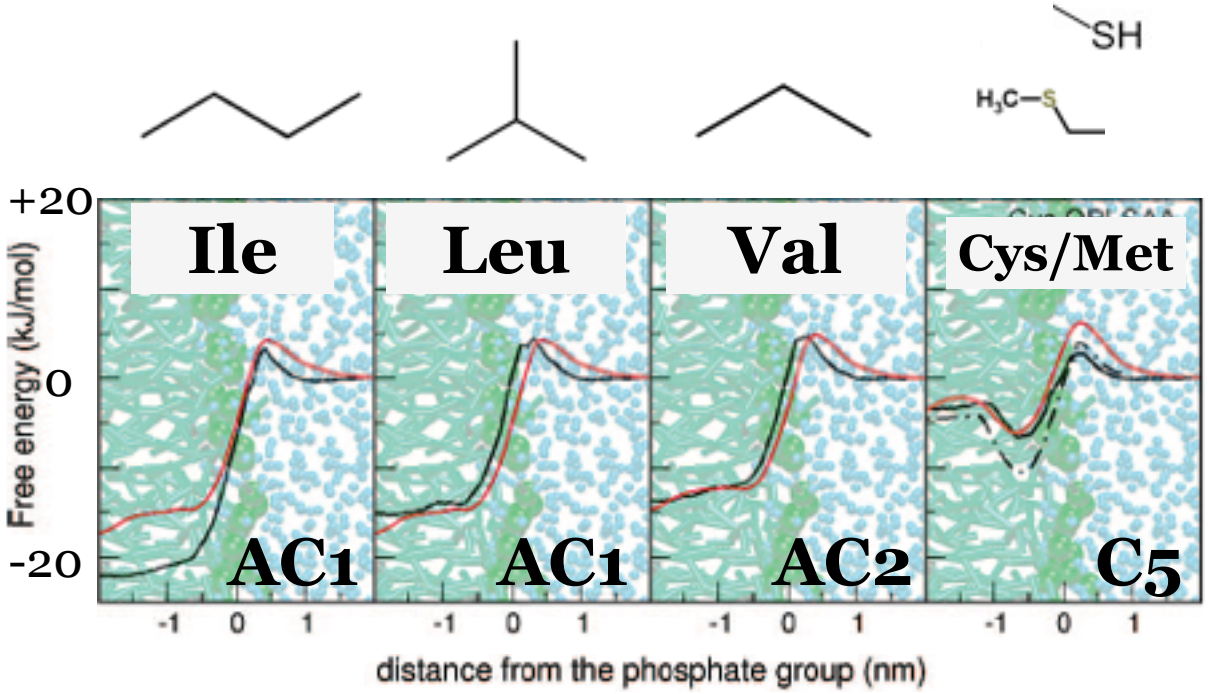


Standard protein model: side-chain beads

C_5	C_3—SH	1-propanethiol
	C—S—C_2	methyl ethyl sulfide
C_4	C_2—C_2	2-butyne
	C—C—C—C	1,3-butadiene
	C—X_4	chloroform
C_3	C_2—C_2	2-butene
	C_3—X	1-chloropropane
		2-bromopropane
C_2	C_3	propane
C_1	C_4	butane
		isopropane

side chain	CG representation	mapping scheme[a]	free energy (kJ/mol) CG	free energy (kJ/mol) exptl.
Leu	C1[b]		22	22
Ile	C1[b]		22	22
Val	C2[b]		20	17
Pro	C2[b]		20	
Met	C5		9	10
Cys	C5		9	5

$\Delta G_{water/oil}$



› [b] Note that interactions of Q-types with protein C1 and C2 use normal σ=0.47 nm instead of σ=0.62 nm; this is implemented by types AC1 and AC2

› PMF of side-chain analogues across membrane was studied and by comparing OPLS-AA (**black**) to Martini (**red**), refinements on side-chain bead assignments were made in some

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008); MacCallum et al. *Biophys. J.* **94**, 3393 (2008); Marrink et al. *J. Phys. Chem. B* **111**, 7812 (2007);

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Enhanced Sampling Opportunities

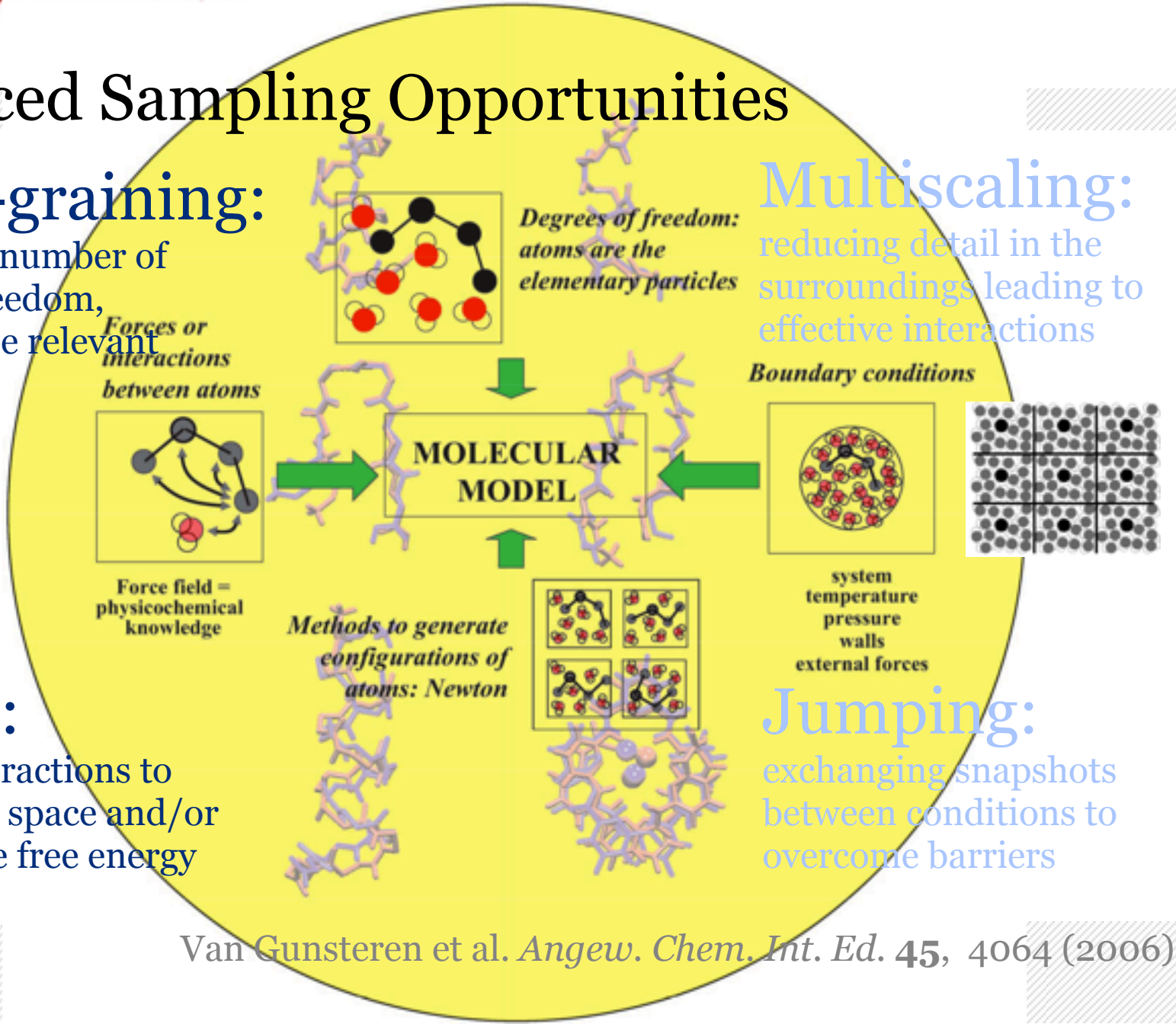
Coarse-graining:

reducing the number of degrees of freedom, preserving the relevant physics

+

Biasing:

adapting interactions to reduce phase space and/or smoothen the free energy landscape



Multiscaling:

reducing detail in the surroundings leading to effective interactions

Jumping:

exchanging snapshots between conditions to overcome barriers

Van Gunsteren et al. *Angew. Chem. Int. Ed.* **45**, 4064 (2006)

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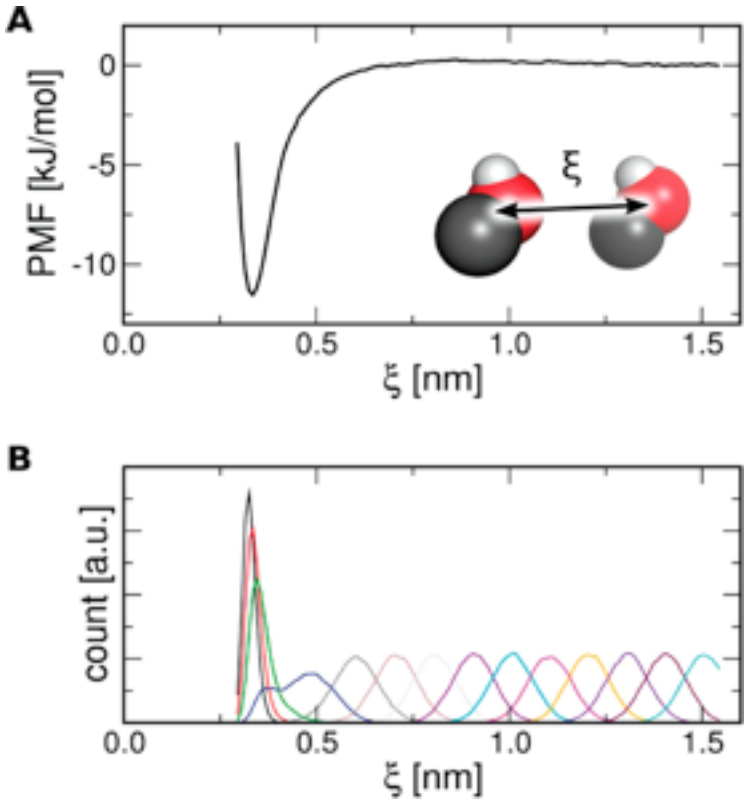
$$K_{12} = \frac{p_2^{eq}}{p_1^{eq}} = e^{-\Delta G_{12}^0/RT} \Rightarrow \Delta G_{12}^0 = -RT \ln \frac{p_2^{eq}}{p_1^{eq}}$$

Free energy differences from Simulations

› Weighted Histogram Analysis Method
  - Apply a restraining potential at different "points"



$$PMF(\xi) = -RT \ln \frac{P(\xi)}{P(\xi_0)}$$

› Methanol dimer PMF
  - Potential of Mean Force
  - Add harmonic restraining potential (bias) to the distance between centers-of-mass

$$\Delta U_R(\xi, d) = \frac{K}{2}(\xi - d)^2$$

› The original potential is obtained after correcting for the bias

`g_wham`

Hub et al. *J. Chem. Theor. Comput.* **6**, 3713 (2010)

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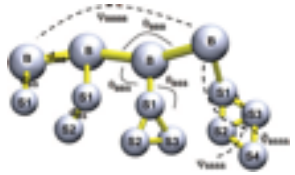
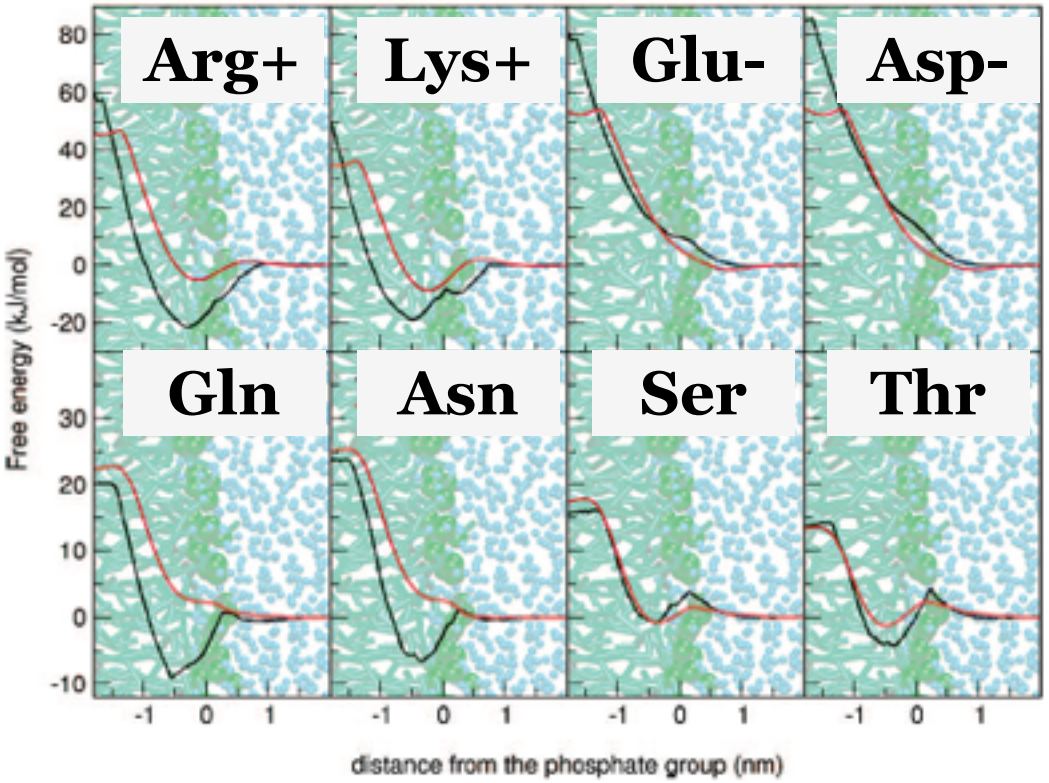


Standard protein model: side-chain beads

- Fine-tuning of side-chain bead type assignments based on water-membrane partitioning
- Comparing to OPLS-AA:
  - Profiles of charged side chains miss some subtleties and are generally too low in the middle of the membrane
  - Profiles of polar side chains miss interface minimum for Gln and Asn
- This is addressed in the updated version 2.2 (see below)



Comparison of PMF across membrane for AA side-chain analogues for OPLS-AA (black) and Martini (red)

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008); MacCallum et al. *Biophys. J.* **94**, 3393 (2008); Marrink et al. *J. Phys. Chem. B* **111**, 7812 (2007);

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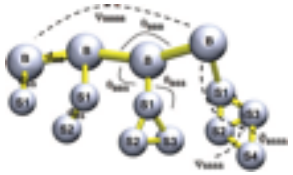
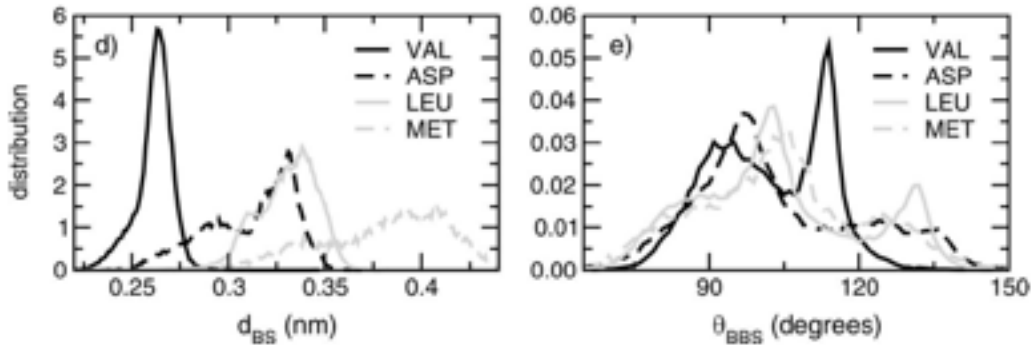


Standard protein model: bonded parameters

- Based on matching distributions from Protein Data Bank
- 2,000 protein structures forming representative set
  - Map structures to Martini model (4-to-1/2-to-1, center of mass mapping)
  - Try to reproduce target distributions using simple potentials
- NOTE: dihedral (torsion) potentials are used!



- Target distributions of backbone-side chain distances and angles after mapping to Martini model

Table 5. Equilibrium Angles, Improper Dihedral Angles and Force Constants for Side Chains

side chain	θ (deg)	K (kJ mol^{-1})
θ_{BBS} (all)	100	25
θ_{BSS} (Lys, Arg)	180	25
θ_{BSS} (His, Tyr, Phe)	150	50
θ_{BSS} (Trp)	90, 210	50, 50

side chain	ψ (deg)	K (kJ rad^{-2} mol^{-1})
ψ_{BSSS} (His, Tyr, Phe)	0	50
ψ_{BSSS} (Trp)	0, 0	50, 200

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008)

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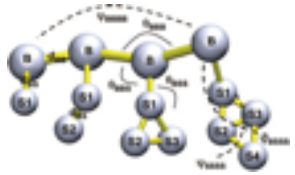

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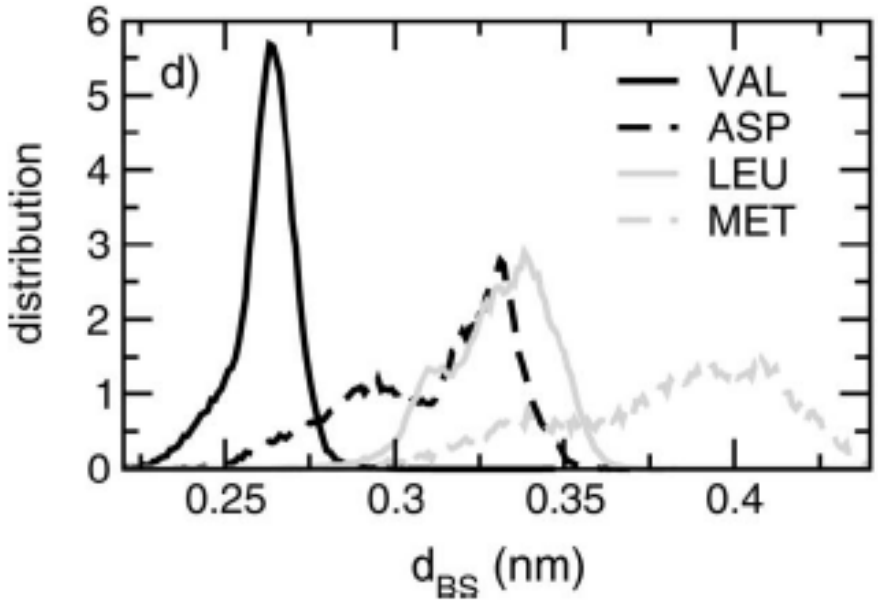

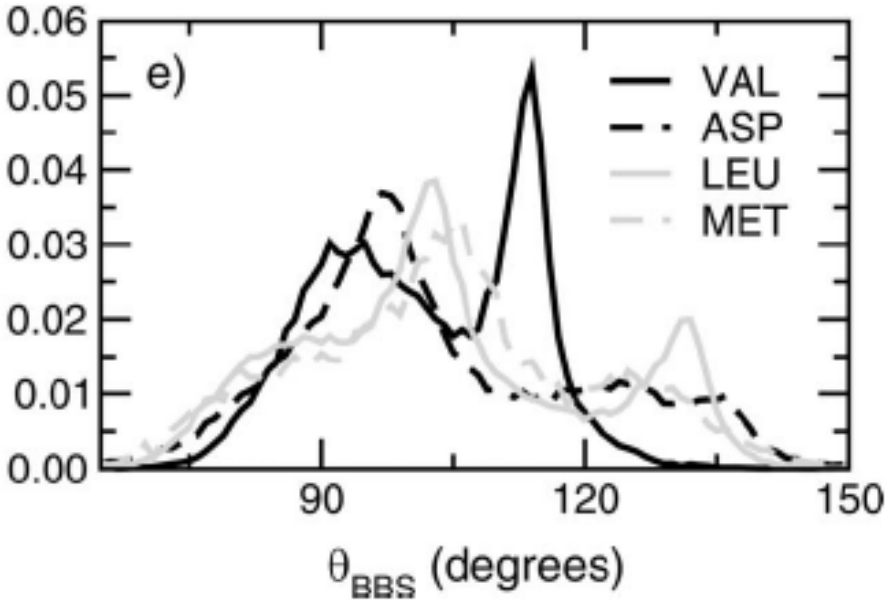

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side chain	ψ (deg)	K (kJ rad^{-2} mol^{-1})
ψ_{BSSS} (His, Tyr, Phe)	0	50
ψ_{BSSS} (Trp)	0, 0	50, 200

- Target distributions of backbone-side chain distances and angles after mapping to Martini model show how to distinguish between similar residues

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008)

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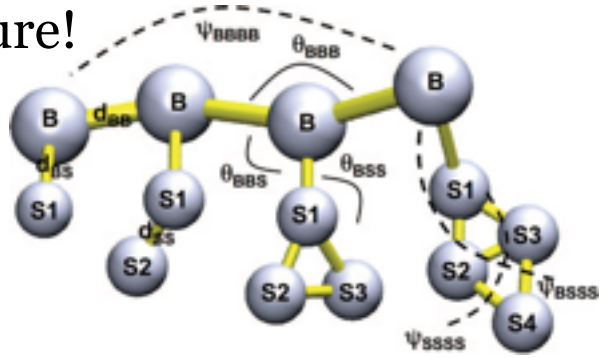
Standard protein model: bonded parameters

- Based on matching distributions from Protein Data Bank
- Backbone parameters depend on secondary structure!
  - need to impose secondary structure
  - model not suitable for folding!!!
  - model uses dihedral potentials
    - this is the main reason for using time step of 25 fs iso 40-50 fs



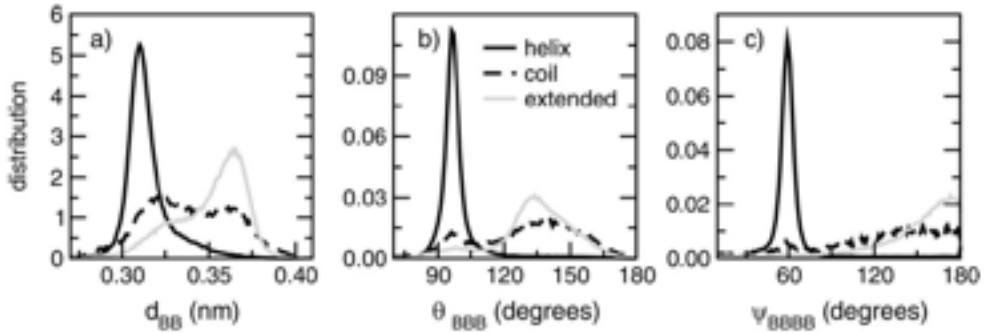


Table 3. Backbone Bonded Parameters

backbone	d_{BB} (nm)	K_{BB} (kJ nm^{-2} mol^{-1})	θ_{BBB} (deg)	K_{BBB} (kJ mol^{-1})	ψ_{BBBB} (deg)	K_{BBBB} (kJ mol^{-1})
helix	0.35	1250	96[a]	700	60	400
coil	0.35	200	127	25		
extended	0.35	1250	134	25	180	10
turn	0.35	500	100	25		
bend	0.35	400	130	25		

[a] $\theta_{BBB} = 98°$ when Proline is in the helix; $K_{BB} = 100$ kJ mol^{-1}.

- Target distributions of bonded parameters involving backbone beads

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008)

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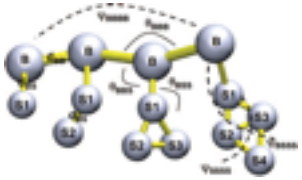
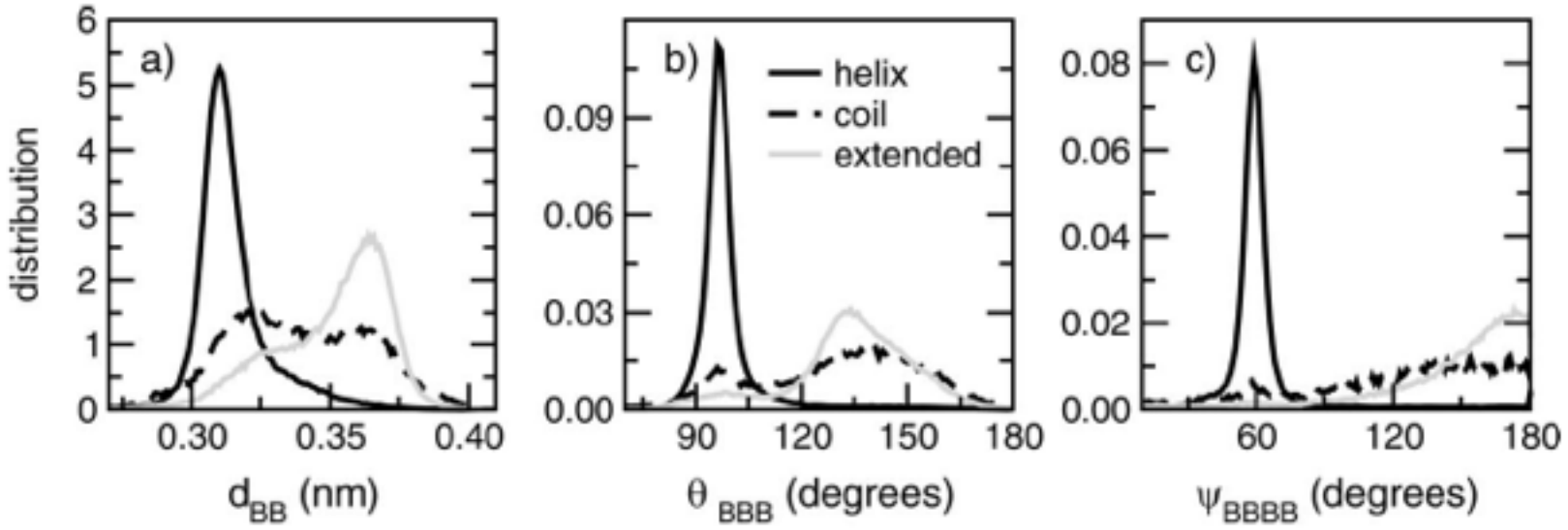


Standard protein model: bonded parameters



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[a] $\theta_{BBB} = 98°$ when Proline is in the helix; $K_{BB} = 100$ kJ mol^{-1}.

› Target distributions of bonded parameters involving backbone beads show that secondary structure influences bonded parameters

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008)

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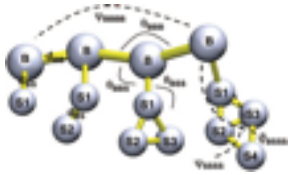
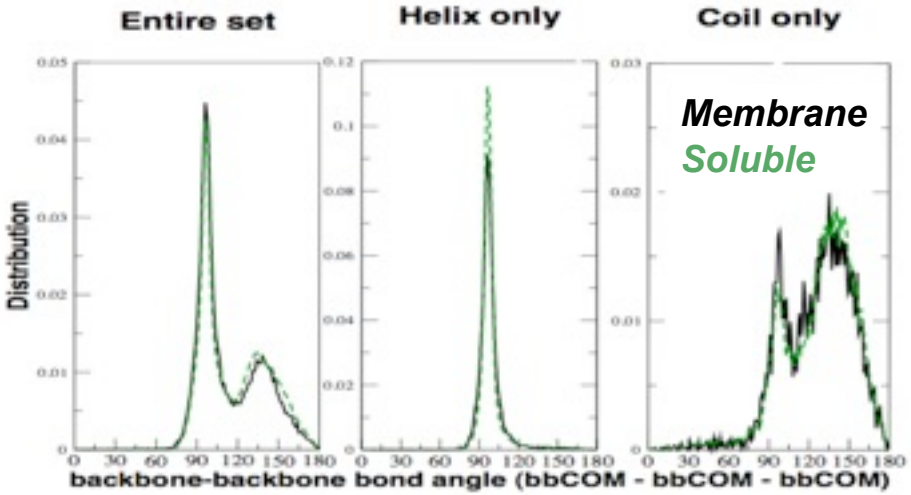
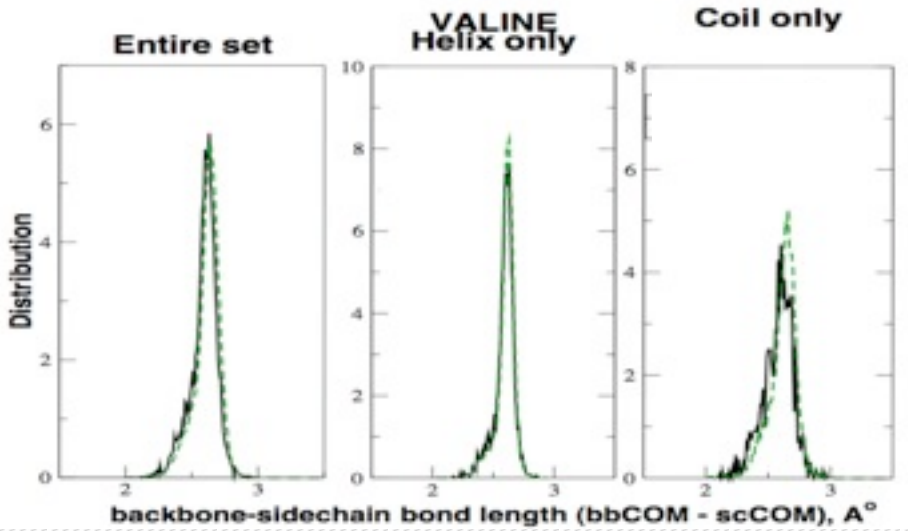


Standard protein model: bonded parameters

- Secondary structure affects angle distributions but not BB-SC distributions
- Similar distributions for membrane (200 out of 2,000) and soluble proteins
- Unimodal distributions for particular amino acid
  - Distinction between amino acids result of using different bonded parameters in addition to possibly different bead types





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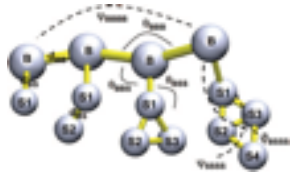

Standard protein model: backbone parameters

- Secondary structure also affects backbone bead type!
- Accessibility to water differs in different conformations and causes differences in backbone polarity and H-bond capability towards water
- `martinize.py` tool builds topology for you

Table 2. Backbone Particle Type in Different Kinds of Secondary Structure[a]

	coil		helix	β-strand
backbone	bend free	helix	(N-terminus/C-terminus)	turn
backbone	P5	N0	Nd/Na	Nda
Gly	P5	N0	Nd/Na	Nda
Ala	P4	C5	N0	N0
Pro	Na	C5	N0/Na	N0

[a] Both glycine and alanine have no side chain.

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013)

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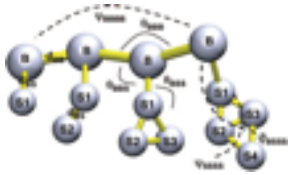
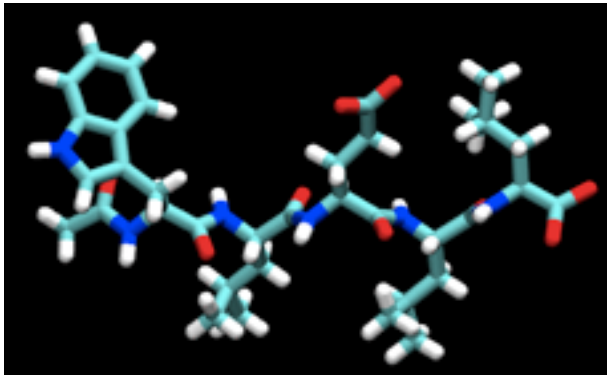
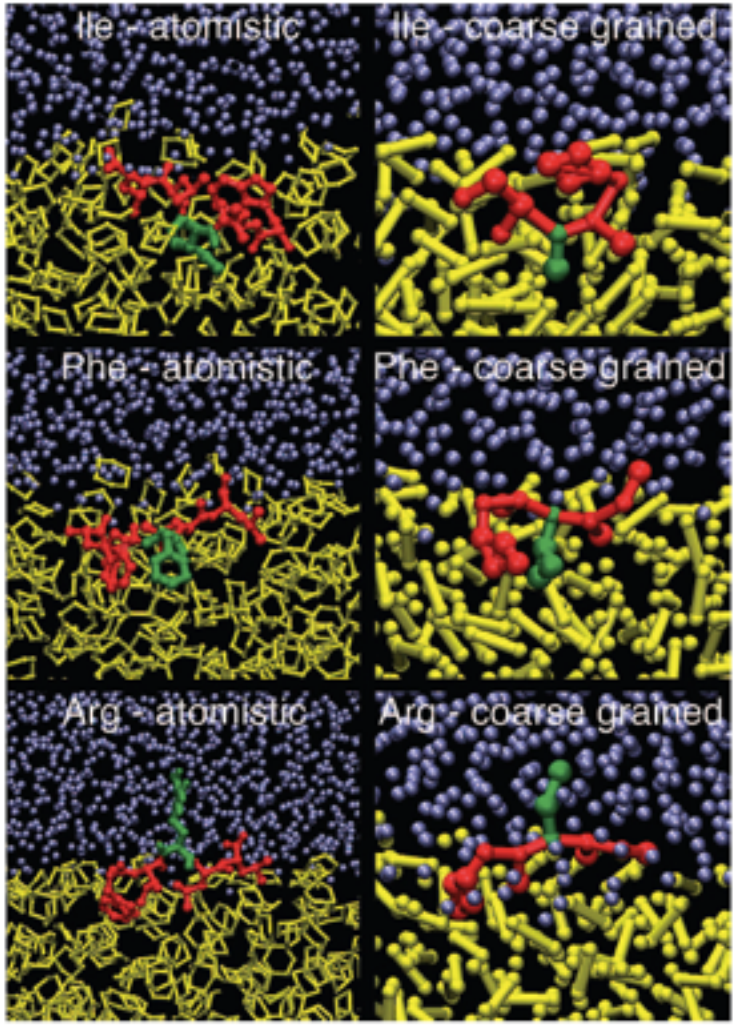


Standard protein model: validation (1)

- Partitioning of Wimley-White pentapeptides between water and oil (octanol in experiment, octane in CG model)
- Ace-WL-X-LL
  - here E



- Study position of W (Trp) and X with respect to the interface
- Validation based on comparison to atomistic results regarding position of residues (uses cyclohexane)



Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
Wimley et al. *Biochemistry* **35**, 5109 (1996)

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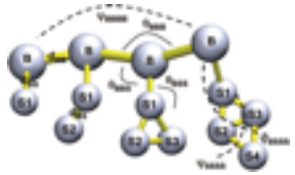
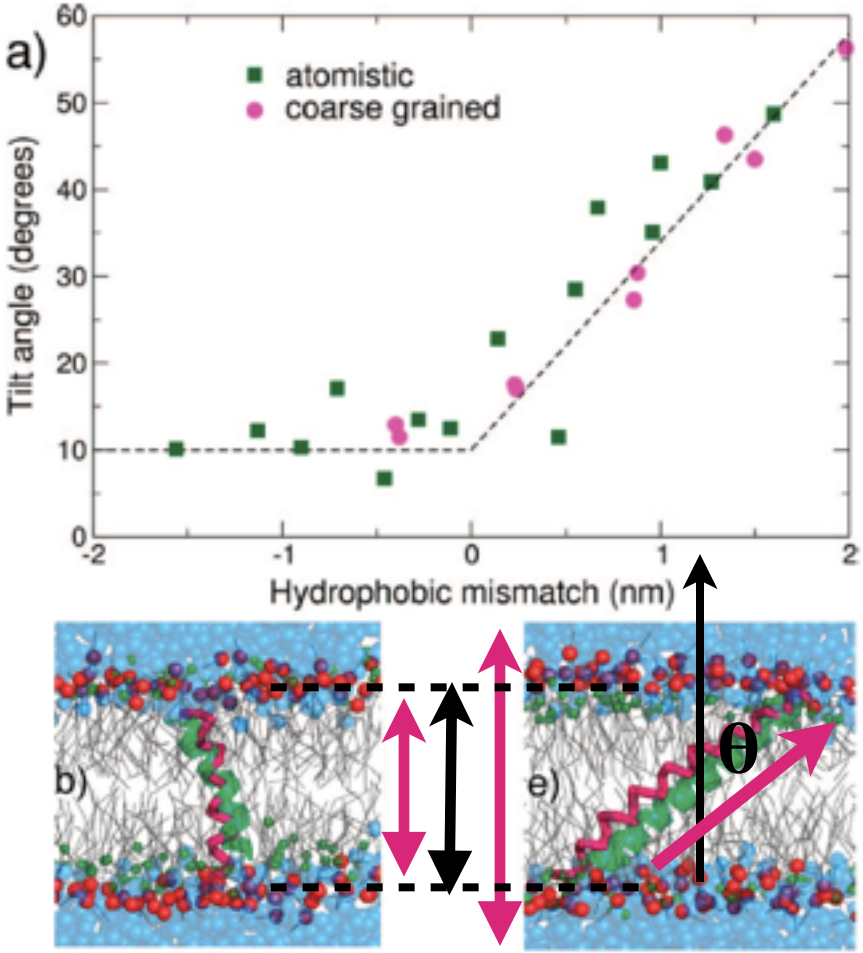


Standard protein model: validation (2)

- Multiple validation simulations
- Tilt of transmembrane (TM)helices: WALP and KALP in DOPC and DLPC as a function of hydrophobic mismatch
- Experimental data available
- At negative mismatch, lipids adapt around peptide
- $WW(AL)_nWW$, $KK(AL)_nKK$
  - anchors in interface
    - causes helical fold
      - determines helix length



Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
de Planque et al. *Biochemistry* **37**, 9333 (1998)

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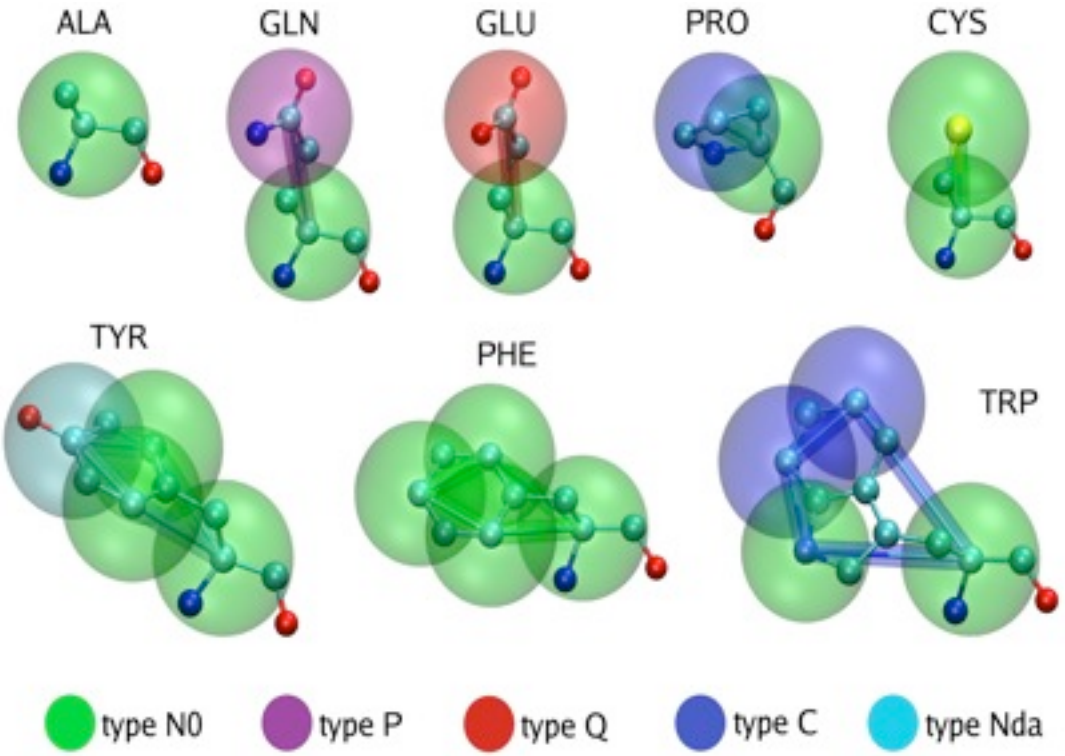
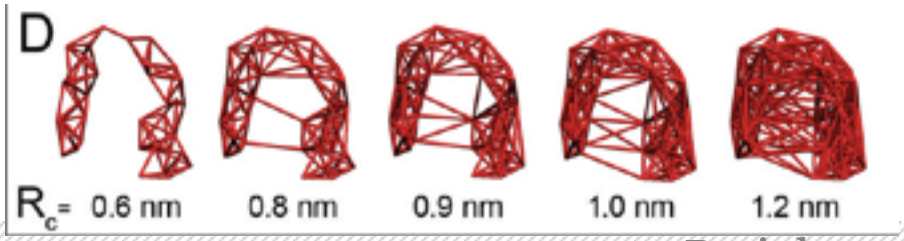
Alternative protein model: ElNeDyn

- Uses different mapping of backbone: to Cα instead of center of mass
- Applies *selected* elastic bonds inspired by elastic network protein models
- Martini bead types apply
- Called ElNeDin in the original publication





- mapping scheme uses atoms, not center of mass
- time step 20 fs
- (but use S-bead mass 72 iso 45)

Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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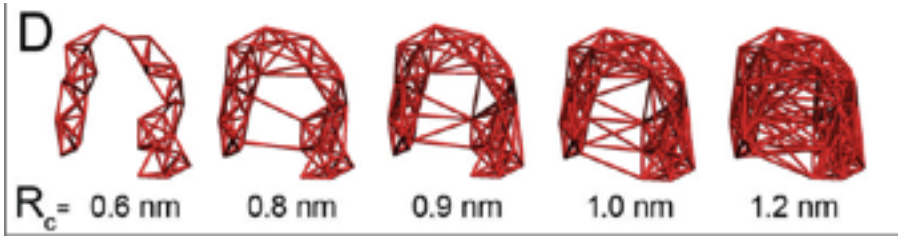
ElNeDyn model basics

- Only apply elastic bonds to backbone beads of residues *i* and *i+3* and further

$$V(d) = \frac{k_{SPRING}}{2}\left(d - d_0\right)^2$$

- Exclude other interactions (Lennard-Jones, dihedral) between the beads connected by an elastic bond



- Cut-off determines beads between which elastic bond network is applied
- Object of ElNeDyn is to quantitatively reproduce structural flexibility of protein native state

- BB-BB distances and BB-BB-BB angles from PDB structure
- BB-SC distances and BB-BB-SC angles and force constants from mapped atomistic simulations of Ala-X-Ala tripeptides in water

Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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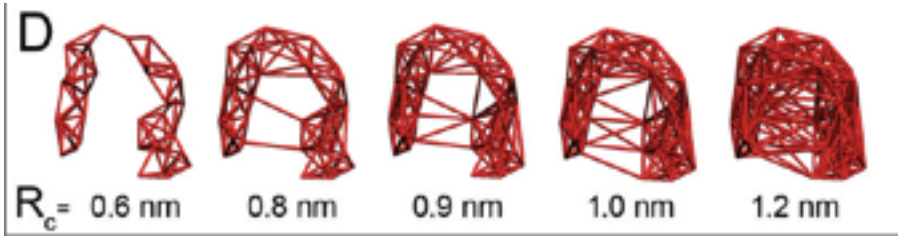
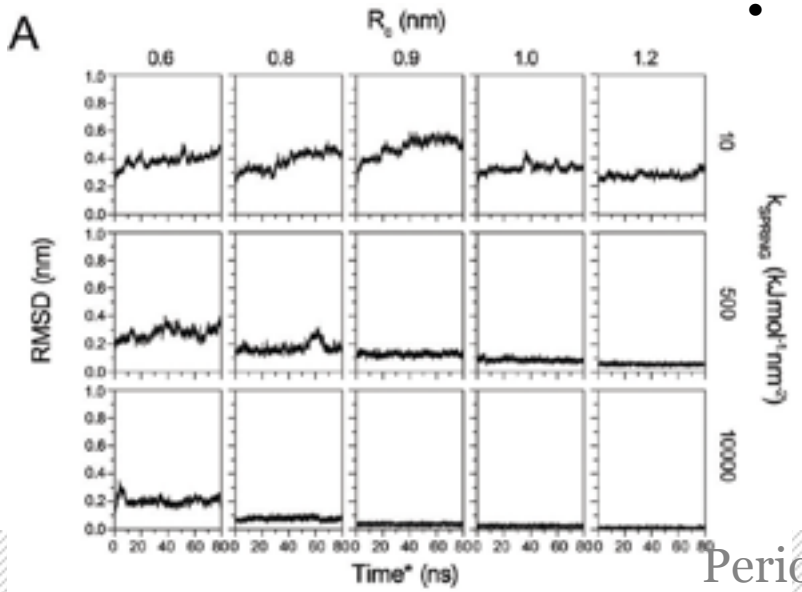
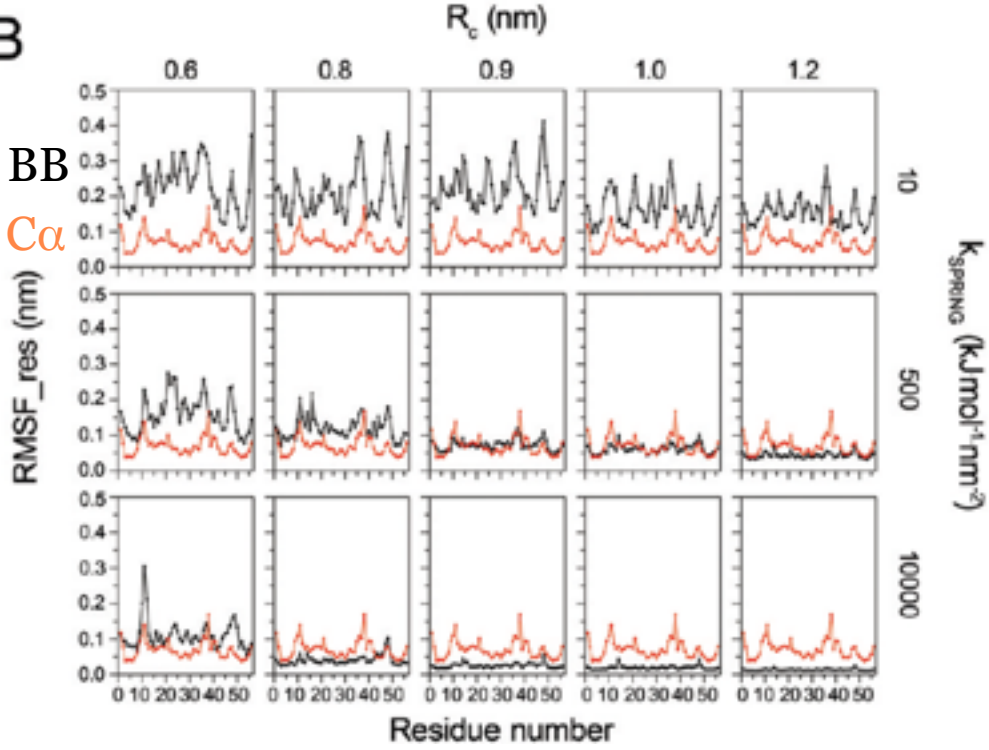
ElNeDyn parameterization (1)

- Scan combination of different cut-offs and force constants
- Monitor RMSD and RMSF (and other measures of structural similarity and flexibility)





- CG BB
- AA Cα



Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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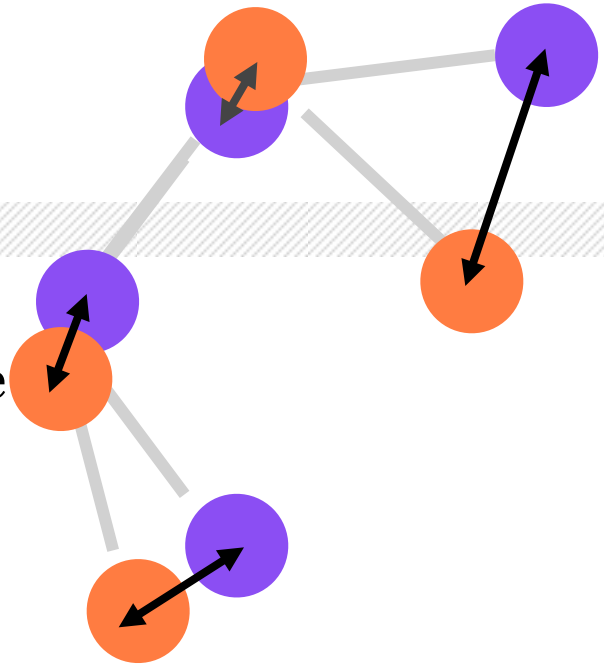
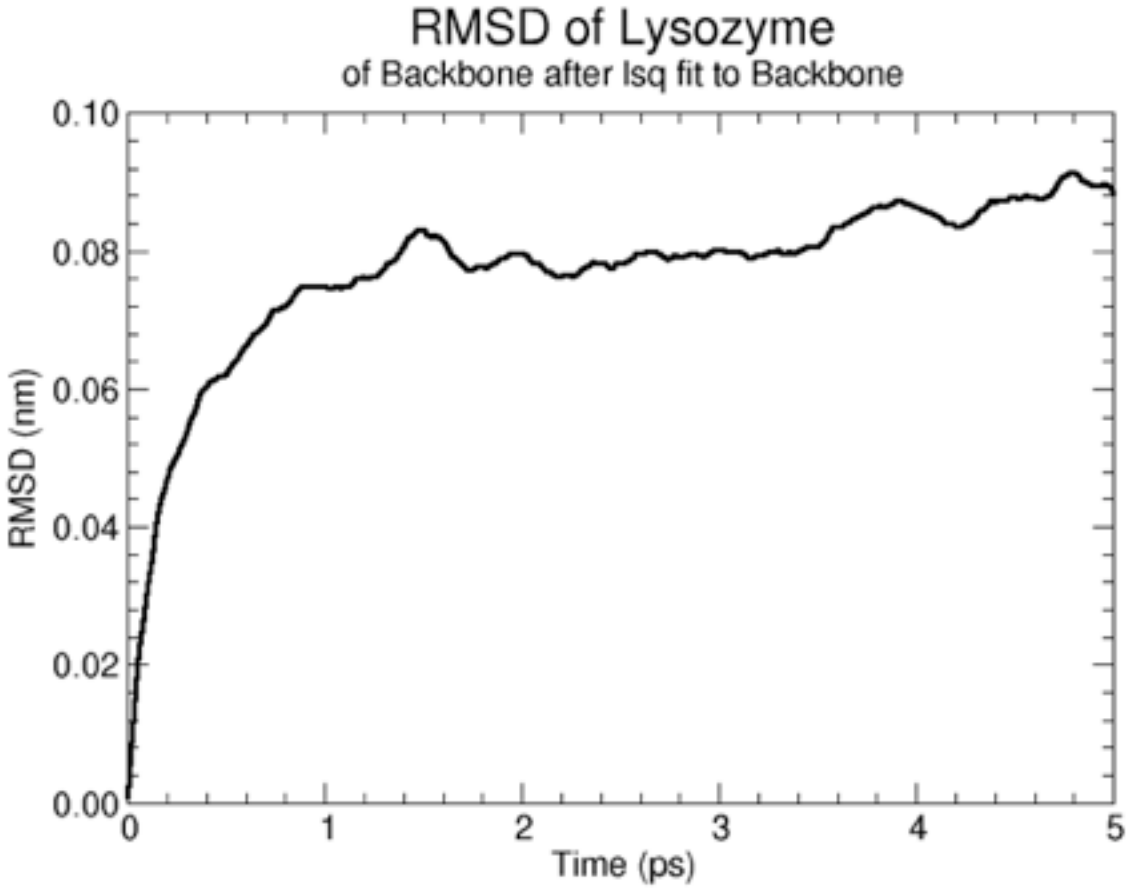
g_rms

RMSD: structural similarity



- Root-Mean-Square Deviation
  - average over all particles at one point in time
- Extensively used in Protein Modeling



$$\sqrt{\frac{1}{N_p}\sum_k \left(r_k(t) - r_k^{ref}\right)^2}$$

- Here, N_p is the number of particles (atoms/beads) in the molecule
- r_k^{ref} is the position of particle k in the reference structure
- $r_k(t)$ is the position of particle k at time t

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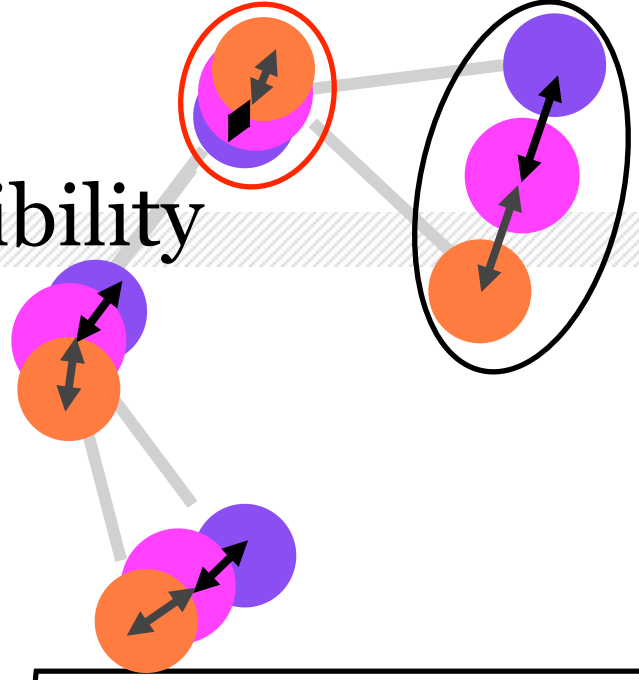
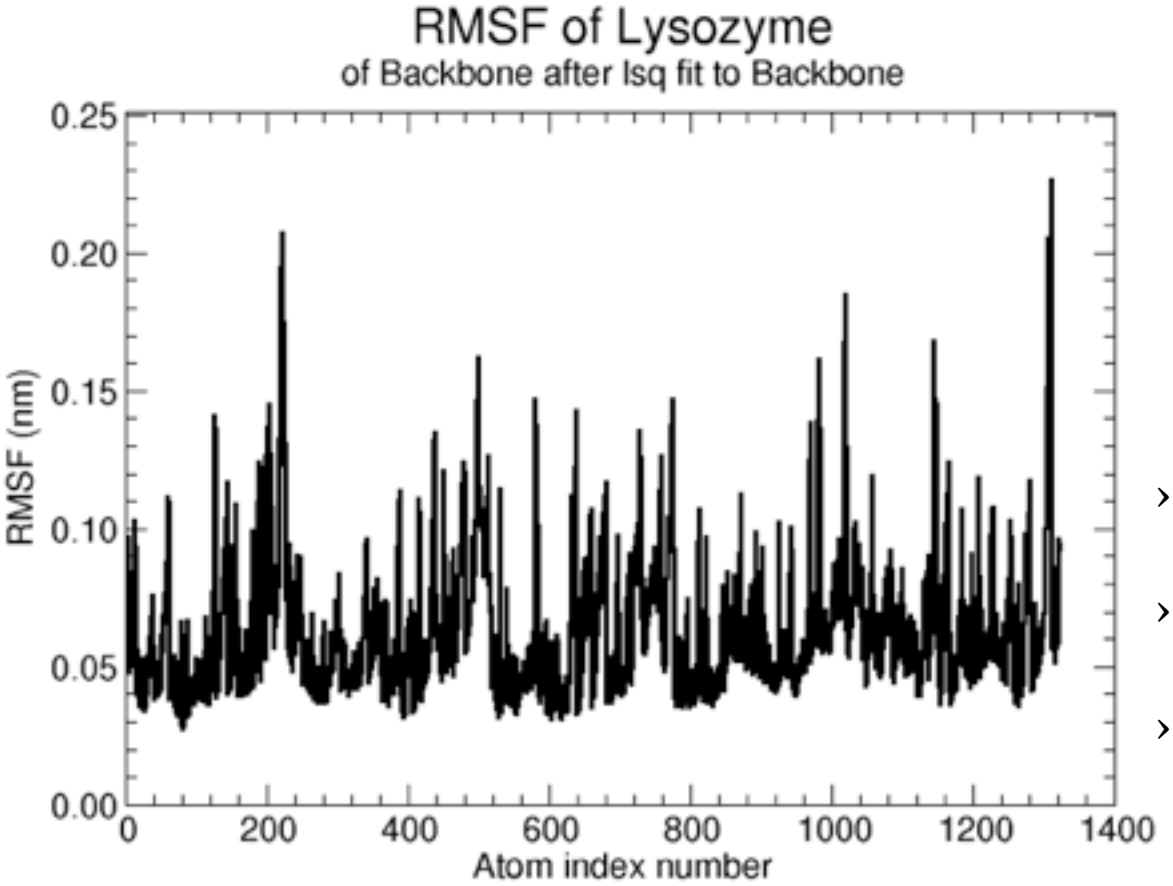
`g_rmsf`

RMSF: structural mobility/flexibility



- Root-Mean-Square Fluctuation
  - average over time for each atom (or residue)



$$\sqrt{\frac{1}{N_f}\sum_i \left(r_k^i - \langle r_k \rangle\right)^2}$$

- Here, N_f is the number of frames in the trajectory
- $\langle r_k \rangle$ is the average position of particle k in the simulation
- r_k^i is the position of particle k in frame i

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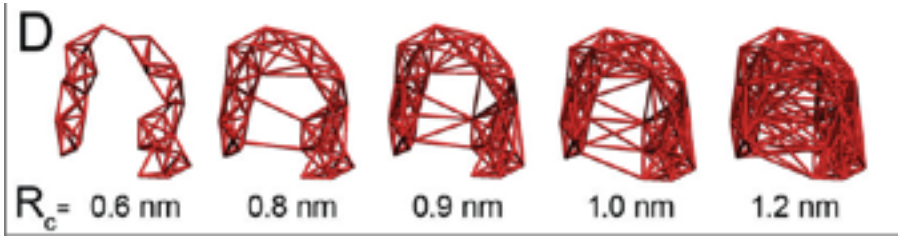
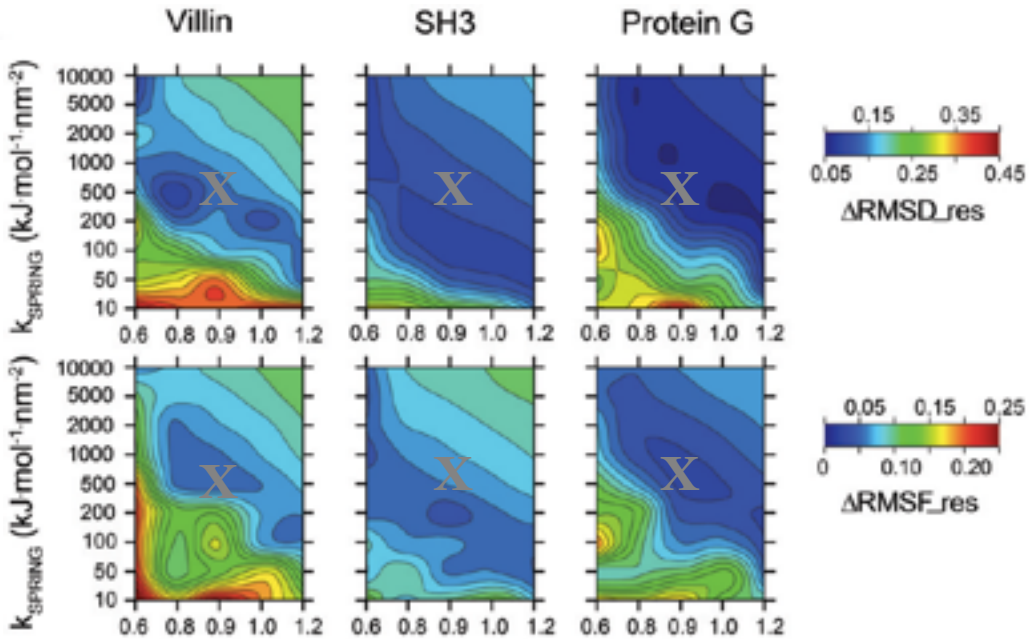
ElNeDyn parameterization (2)

- Use elastic network between backbone beads only and only within a secondary structure element



- Scan combination of different cut-offs and force constants
- Monitor RMSD and RMSF (and two other measures) for different types of proteins and find best overall combination



- Recommended values are R_C=0.9 nm and k_{SPRING}= 500 kJ·mol^{-1}·nm^{-2}

Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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Which protein model should I use?

- The Standard Martini protein model imposes only secondary structure, either based on DSSP or your own assignment; it allows tertiary structure changes and its force field parameters do not depend on the details of the starting structure, as long as the secondary structure assignment is the same

- ElNeDyn requires a structure from which to determine BB-BB bond lengths and BB-BB-BB angles - these are used as parameters for the elastic bonds

- Surveying the Groningen MD group literature, the general rule seems to be that single TM helices are done using the standard model, whereas multipass transmembrane proteins are done using ElNeDyn

- There is little published by Groningen MD group on soluble proteins

- In general, researchers feel free to apply simple or more complex elastic networks in combination with standard Martini or ElNeDyn to their own taste

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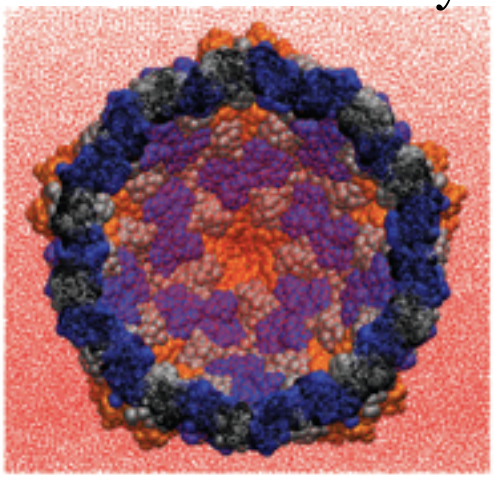
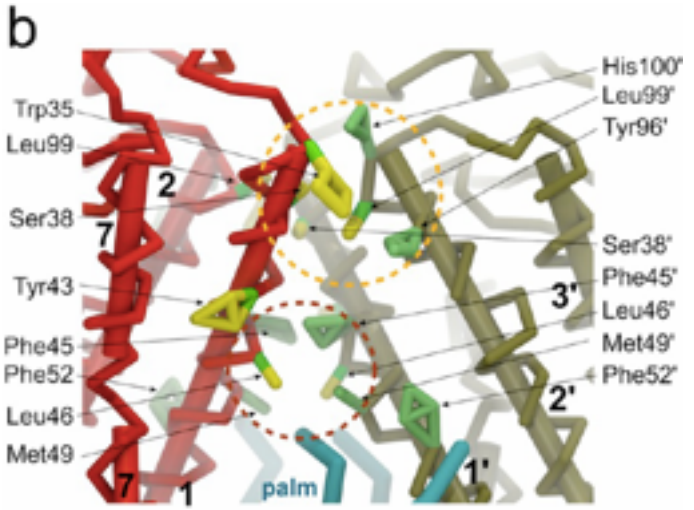
Two brief illustrations using ElNeDyn

- ElNeDyn is designed to reproduce protein flexibility of the native folded state of well-defined folded proteins and has limited (but finite) capability of altering tertiary structure compared to the standard model

- ElNeDyn models have been used successfully in simulation of large protein assemblies
  - Cowpea Mosaic virus (~270,000 CG beads, 400 ns* in 2009)



  - G-protein coupled receptor complexes (rhodopsin)



Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009);
Periole et al. *J. Amer. Chem. Soc.* **134**, 10595 (2012)

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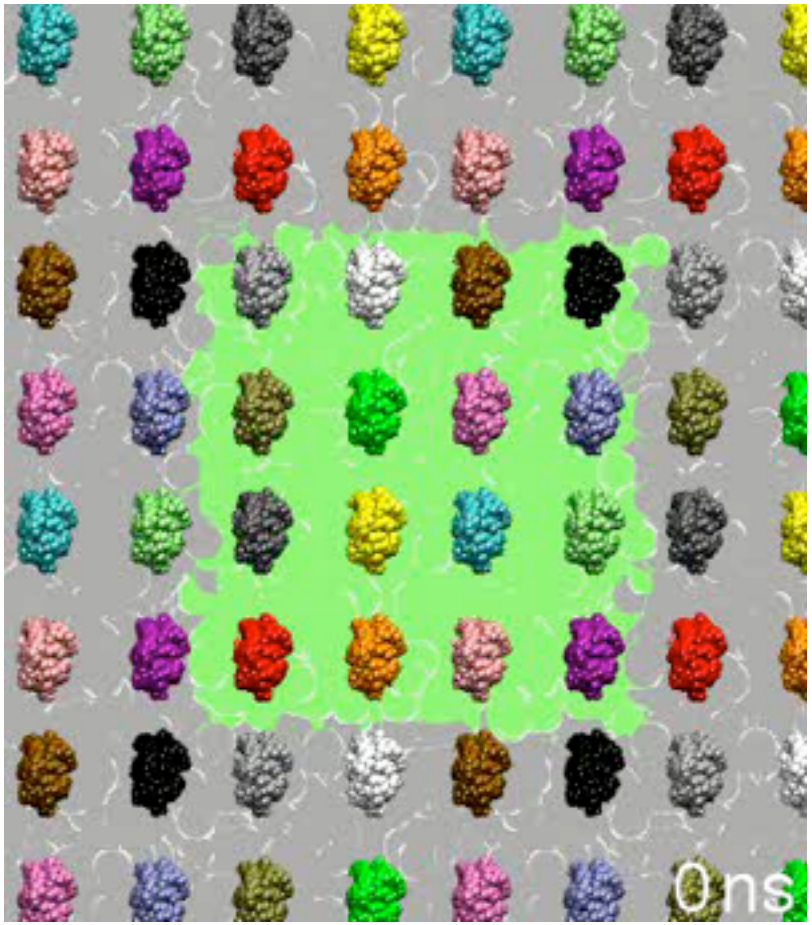
Early Martini protein work revisited

› Formation of rhodopsin clusters in membranes of different thickness

- G-protein coupled receptor molecule visual rhodopsin in single-component membrane
- 16 independent membrane proteins in simulation cell in 2007 paper, 64 in 2012
- clustering preference and dynamics depends on bilayer thickness
- neighboring proteins explore different binding interfaces



Periole et al. *J. Amer. Chem. Soc.* **129**, 10126 (2007) and **134**, 10595 (2012)

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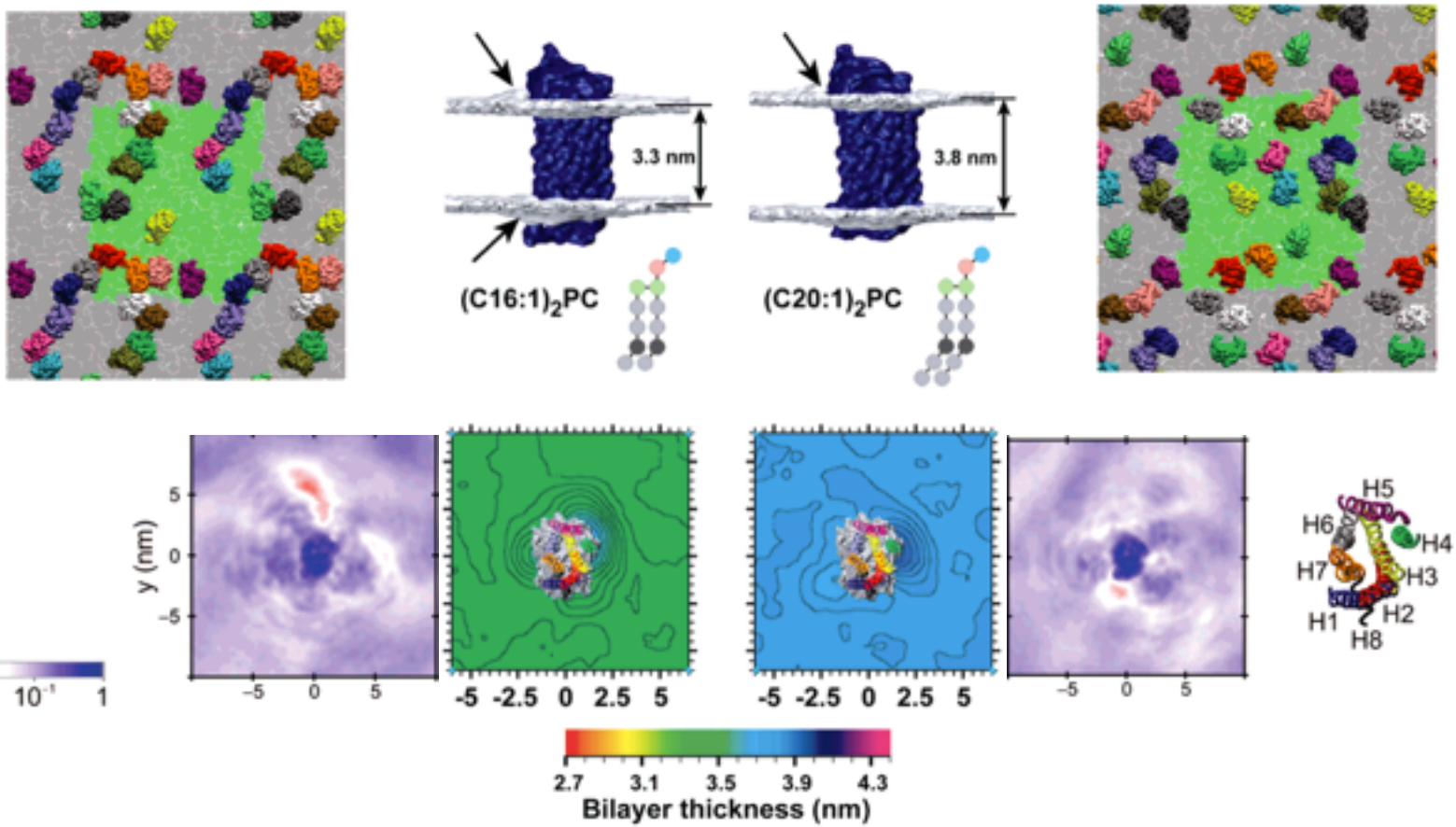
The power of simulation

› Toward realistic systems: aggregation of Rhodopsin in bilayers



› Large hydrophobic mismatch

› Small hydrophobic mismatch

Periole et al. *J. Amer. Chem. Soc.* **129**, 10126 (2007)

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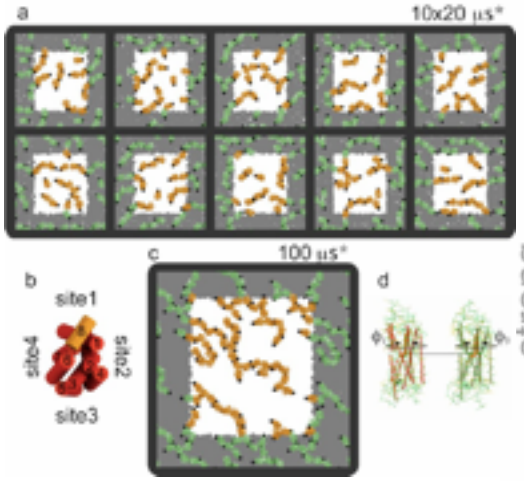
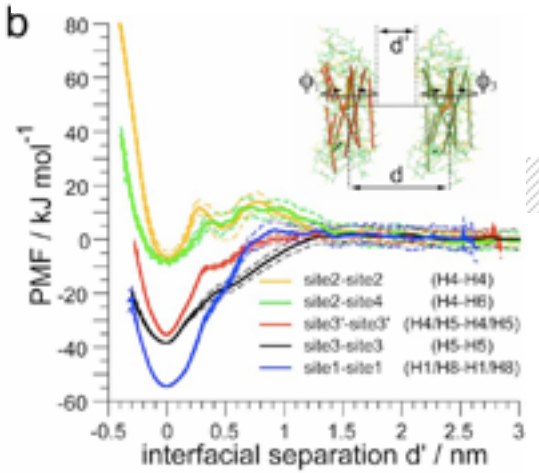
Beware convergence of sampling!

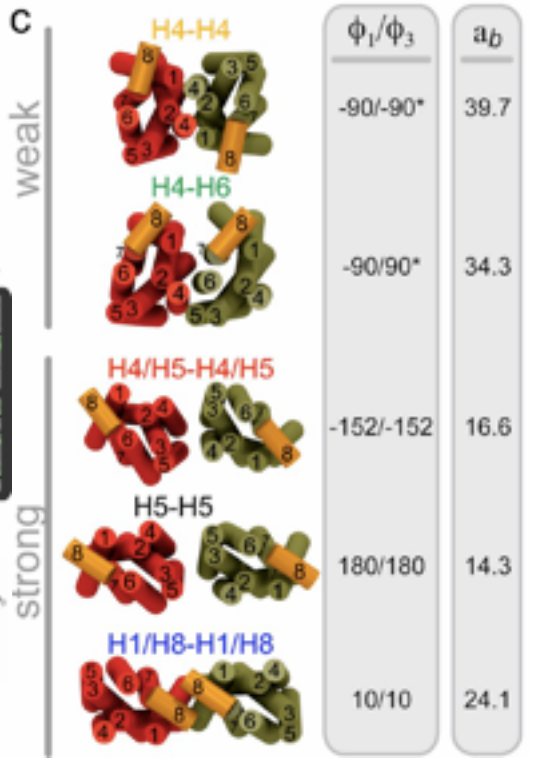
- A general observation from membrane protein association simulations is that proper sampling is a problem, even at coarse-grained level

- GPCR rhodopsin has several possible interfaces of different strengths, some of which have a barrier to association which are therefore less likely to be sampled in a self assembly simulation

- Combination of multi microsecond self-assembly simulation and PMF simulations at different fixed orientations reveal the relative stability of the different interfaces







Periole et al. *J. Amer. Chem. Soc.* **129**, 10126 (2007) and **134**, 10595 (2012)

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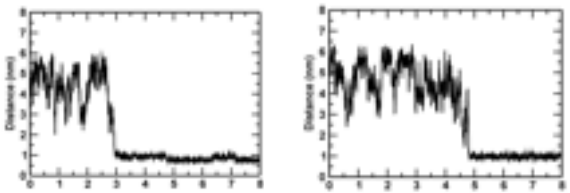
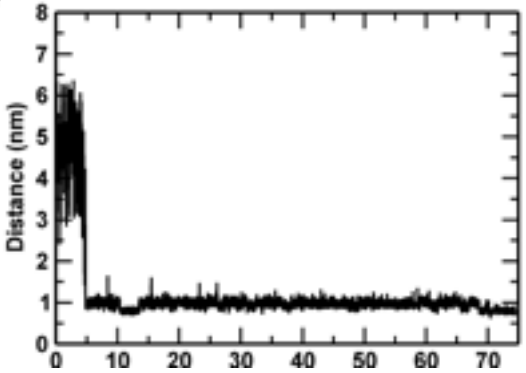


Beware convergence of sampling!

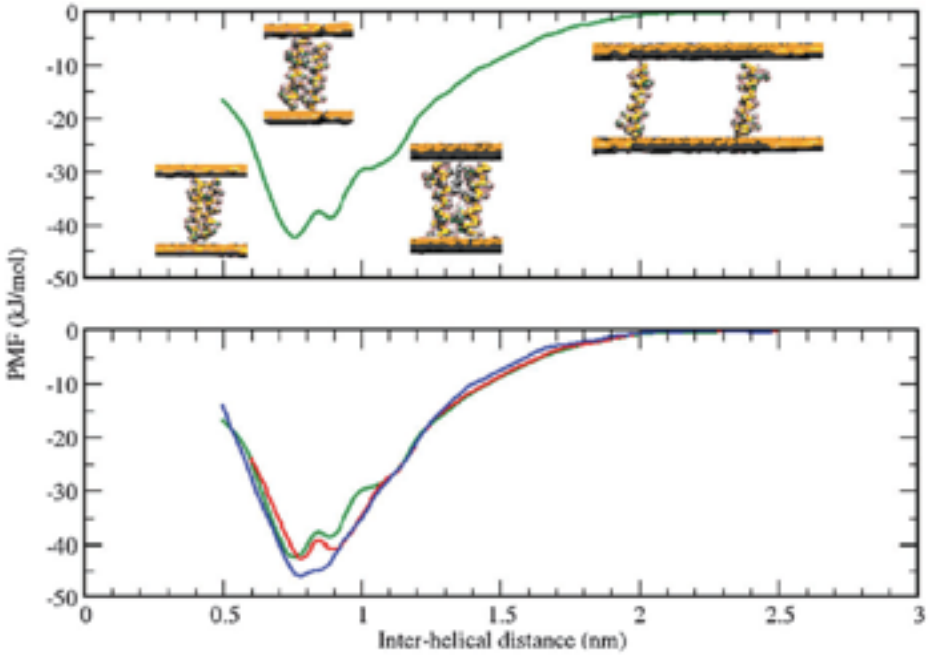
- A general observation from membrane protein association simulations is that proper sampling is a problem, even at coarse-grained level



- Glycophorin A complexes may get trapped in a particular type of binding interface

- PMFs reveal three different minima only when sampling a total of 8 μs (green line); shorter simulations show only one minimum (0.5 μs, blue) or two (4 μs, red) minima

- DAFT approach may spot these cases efficiently



Sengupta et al. *Phys. Chem. Chem. Phys.* **12**, 12987 (2010);
Wassenaar et al. *J. Chem. Theor. Comput.* **11**, 2144 (2015)

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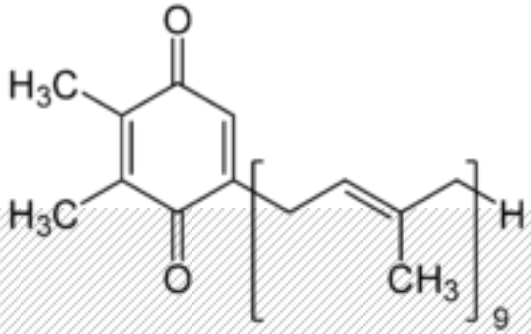
Protein-Ligand Interactions

- An important problem of Martini proteins for studying Protein-Ligand interactions is that in the apo-form, the binding site may collapse and not allow entry of substrate
  - HIV-1 protease is an early example (see protein tutorial)
  - May be remedied by applying elastic network on top of standard Martini or on top of ElNeDyn
  - You need to think about the relation of your model to your research question!

- Interactions between soluble proteins appear problematic, as well as protein-ligand interactions: improvement is an active field of research

- Recent success: observation of plastoquinone insertion in photosystem II



Picture: Wikipedia, 25-08-2015

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Martini WOrkshop 2015

Developments in Martini proteins

With special thanks to Djurre de Jong (now at Münster)

Version 2.2 and 2.2P

Beyond 2.2 for soluble protein

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Beyond standard Martini for proteins

- The standard and ElNeDyn Martini protein models appear to be quite successful for describing protein-lipid interactions and protein-protein interactions for membrane-bound proteins

- Interactions between soluble proteins appear problematic, as well as protein-ligand interactions: improvement is an active field of research
  - Are Martini proteins too “sticky”? In self-assembly simulations, we (and others) got the impression any protein will stick to any other protein, often forming kinetically trapped structures

- Systematic study into interaction between amino acids was undertaken to substantiate this impression

- More recently, soluble protein aggregation has been studied more in detail connecting to experimental data

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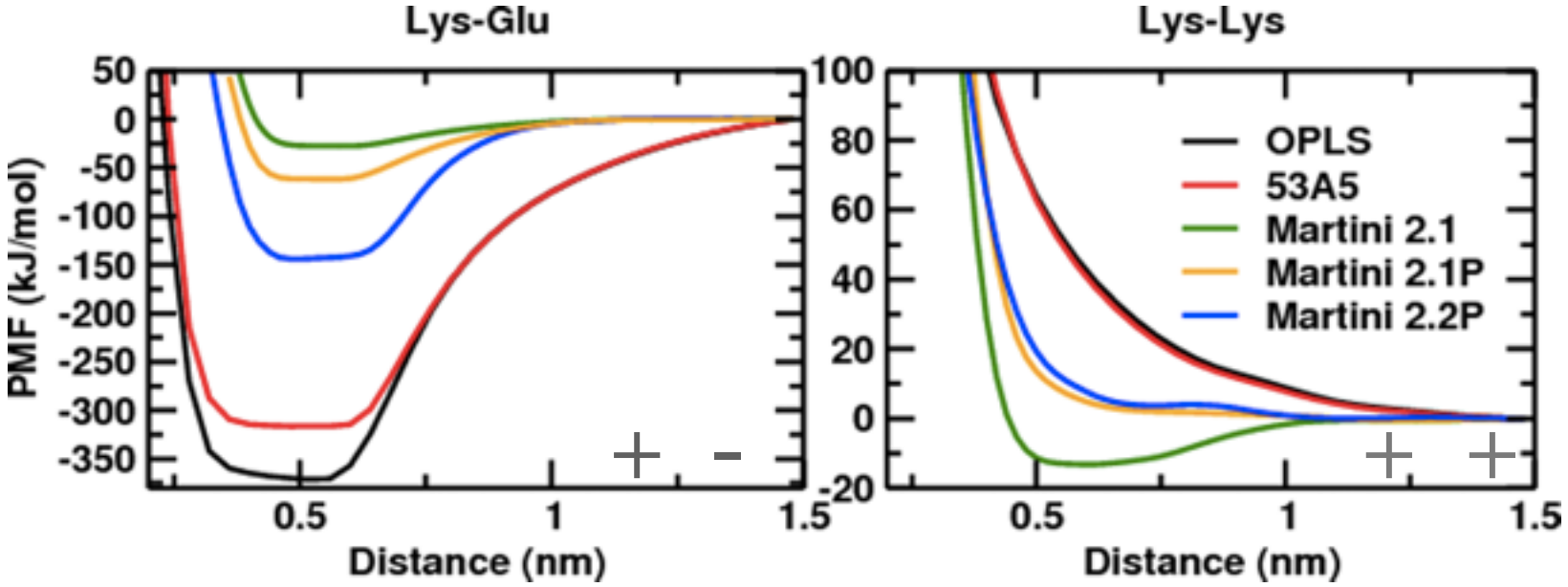
PMFs for amino acids side chain interactions

- The PMFs for dimerization of charged residues in oil (pure alkane) reveals a problem of the standard Martini protein model (v2.1)
  - Charge-charge interaction screened too much in non-polar environment: remember, in Martini we use a dielectric constant $\varepsilon_r = 15$ because our water model is a LJ particle
  - Switching to polarizable water model (v2.1P) helps, because $\varepsilon_r = 2.5$



de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013)

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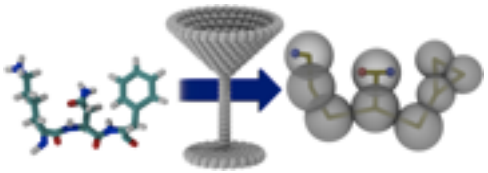
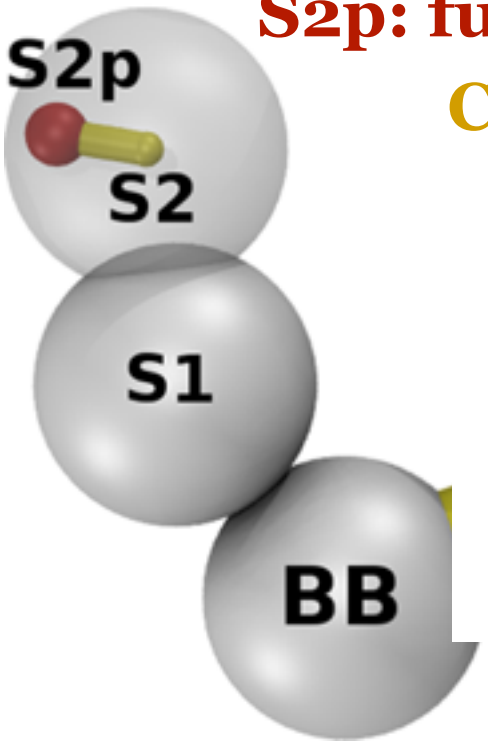


PMFs for amino acid side chain interactions

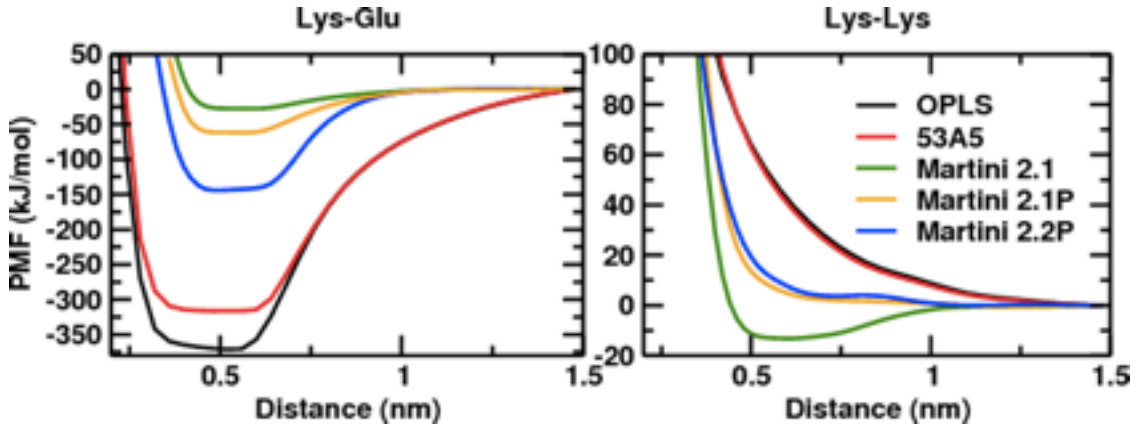
- Compared to atomistic description, unlike charges are too far apart
  - In 2.2P model, an off-center charge is added to the model, leading to a deeper minimum for unlike charge pairs



S2p: full charge ±1, no LJ interaction

Constraint length 0.11 nm

S2: LJ interaction only



de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013)

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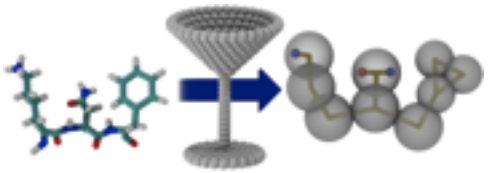


Polar amino acid side chains

- For polar residues, similar arguments as for charged residues apply
  - In 2.2P model, two off-center charges are added to the model, modeling the reorientation of a permanent dipole

S1p, S1n: partial charge ±q, no LJ interaction

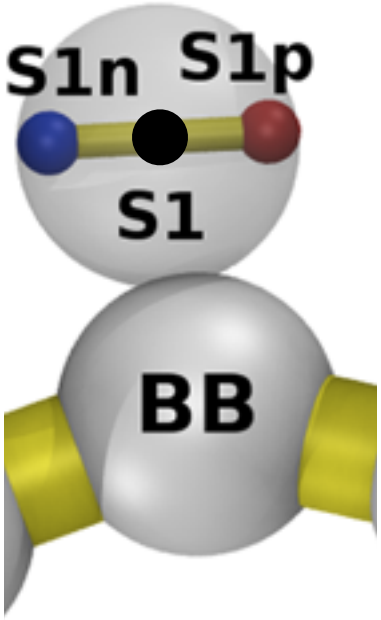
Partial charge

Ser: ±0.40

Thr: ±0.36

Asn:±0.46

Gln:±0.42



Constraint length S1n-S1p 0.28 nm

Constraint length S1-S1n,p 0.14 nm

S1: Virtual site (no mass)

LJ interaction only

NOTE: differs from polarizable water set-up!

de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013)

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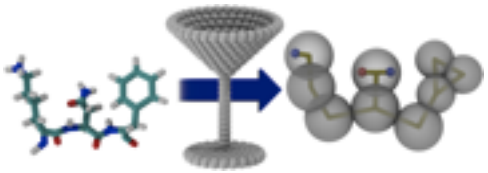
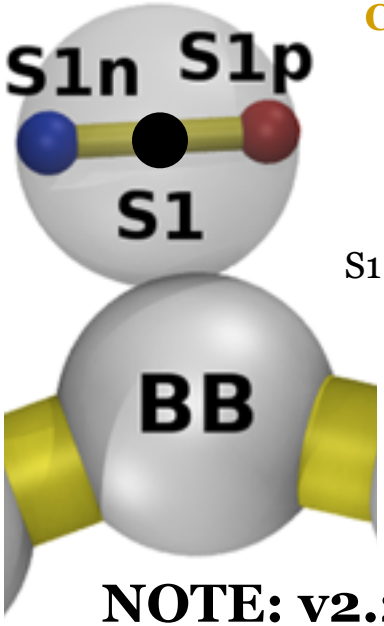


Polar versus polarizable

› In the 2.2(P) version, amino acid side chains have permanent dipole, whereas water has a varying dipole

S1p, S1n/WP,WM: partial charge ±q, no LJ interaction



Constraint lengths:

S1n-S1p 0.28 nm

S1-S1n,p 0.14 nm

S1: Virtual site (no mass)

LJ interaction only

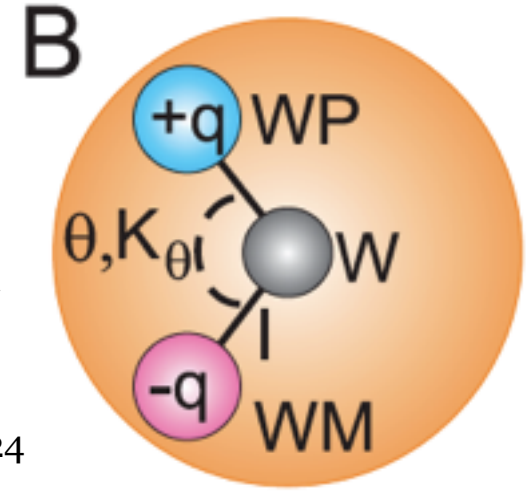
masses S1n, S1p: 36

Constraint lengths:

W-WP, W-WN 0.14 nm

W: LJ interaction only

masses W, WP, WN: 24



When using P version, set ε_r=2.5 iso 15

NOTE: v2.2(P) for AAs differs from polarizable water set-up because in water the angle between the particles is not fixed!

de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013);
Yesylevskyy et al. *PLoS. Comput. Biol.* **6**, e1000810 (2010)

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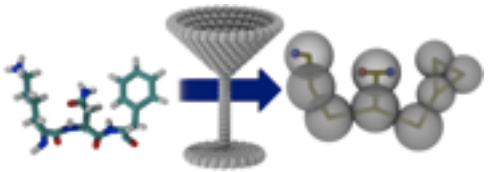


Reparameterization of polar amino acids

- Parameterized on oil/water partitioning and dimerization free energies in water and in oil
- Checked against partitioning of Wimley-White pentapeptides and PMF across lipid membrane
- Not all equally well reproduced but general improvement wrt v2.1

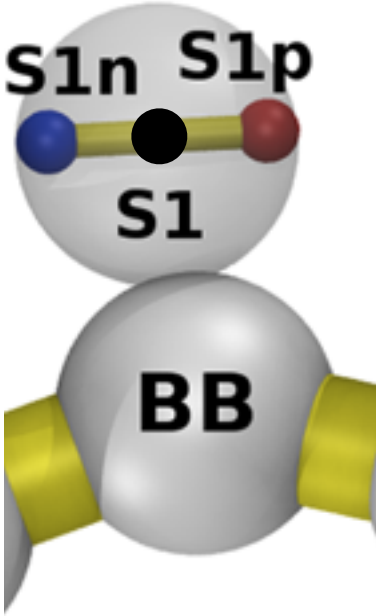
ref: Exp or atomistic MD

italic: v2.1

bold: v2.2P final model

Final parameters are those that reproduce PMF across lipid membrane best

SC		type (charge)[b]	$\Delta\Delta G^{WW}$ [c]	ΔG^{sat} [d]	ΔG^{dis}_{water} [e]	ΔG^{dis}_{oil} [e]
Thr	ref.		0.1 ± 0.4	−11	0.2	−5.8
	CG	*P1*	−1.9 ± 0.1	−12	0.0	−2.3
		N0 (0.36)	−0.3 ± 0.3	−12	−0.5	−4.0
		Nda (0.31)	2.3 ± 0.3	−13	−0.5	−4.2
Ser	ref.		0.2 ± 0.4	−14	1.6	−5.9
	CG	*P1*	−1.9 ± 0.1	−12	0.0	−2.3
		N0 (0.40)	−0.5 ± 0.3	−14	−0.2	−5.2
Asn	ref.		−1.0 ± 0.4	−28	−0.1	−17.3
	CG	*P5*	−2.7 ± 0.1	−31	0.3	−4.2
		Nda (0.51)	1.9 ± 0.7	−28	−0.2	−20.6
		Nda (0.46)	2.0 ± 0.4	−23	−0.4	−13.9
		N0 (0.54)	−1.3 ± 0.3	−27	−0.2	−18.1
Gln	ref.		−1.7 ± 0.4	−25	−1.2	−17.2
	CG	*P4*	−2.0 ± 0.1	−23	−0.1	−3.4
		Nda (0.42)	2.4 ± 0.2	−20	−0.2	−7.2
		N0 (0.51)	−1.1 ± 0.5	−24	−0.6	−14.6



de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013);
Wimley and White *Nat. Struct. Biol.* **3**, 842 (1996)

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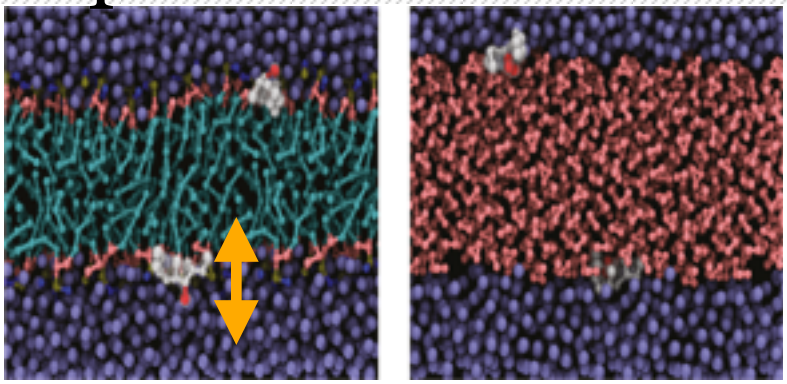
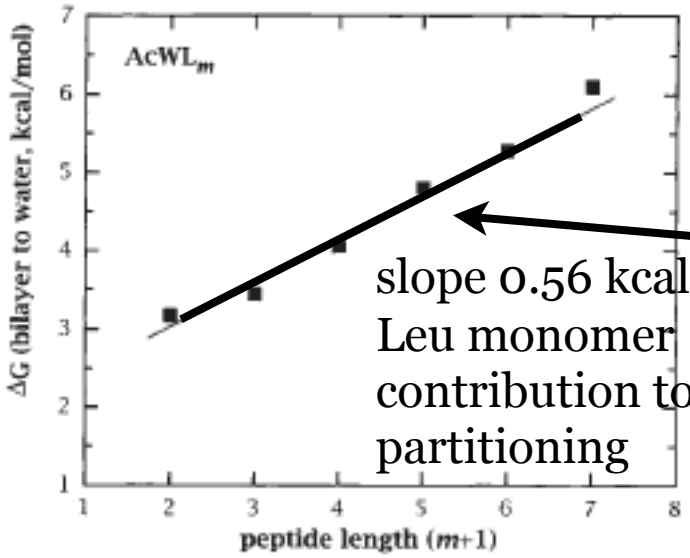
Wimley-White peptide hydrophobic scale

- Partitioning of Wimley-White peptides between water and POPC membrane

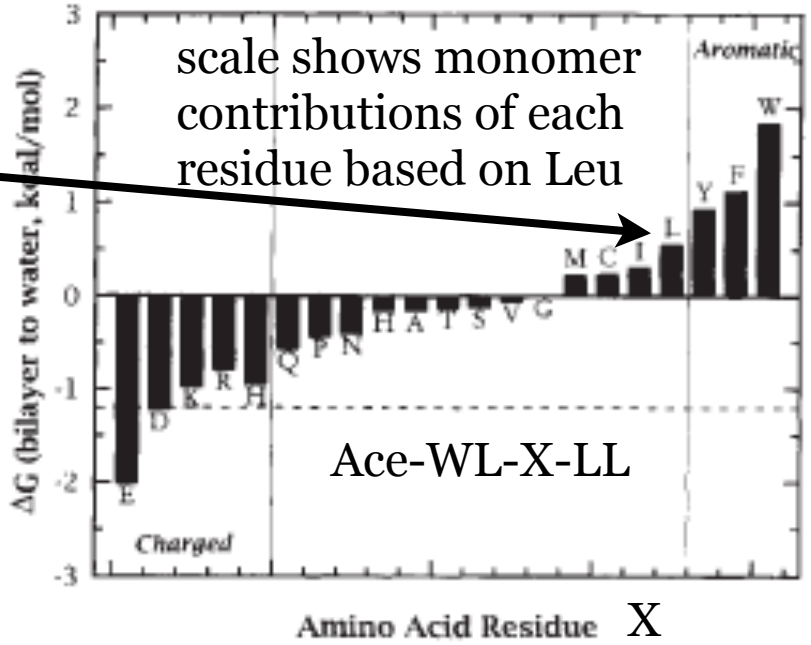


- Series Ace-WL_m, m = 1,6



slope 0.56 kcal·mol^{-1}: Leu monomer contribution to partitioning

slope to membrane about half of that to octanol - reflects more complex interface?!



Wimley and White *Nat. Struct. Biol.* **3**, 842 (1996);
Singh and Tieleman *J. Chem. Theor. Comput.* **7**, 2316 (2011)

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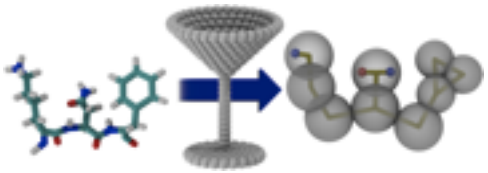


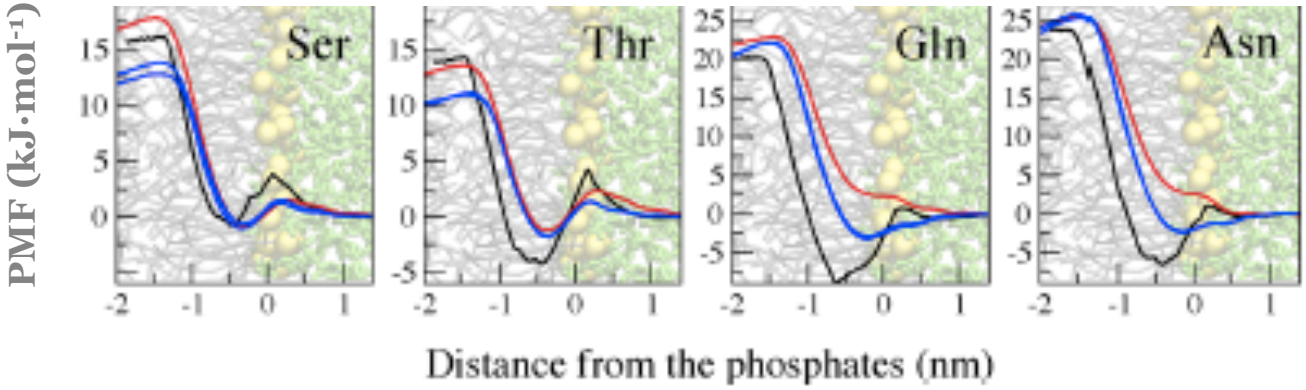
PMF of polar amino acids across bilayer

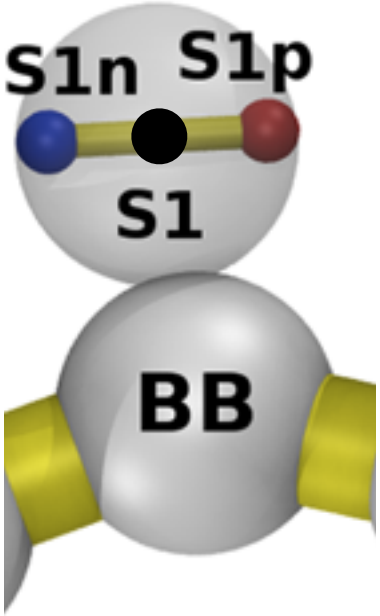
- Final parameters Martini v2.2(P) were decided by looking at PMF across lipid membrane
- Gln and Asn now show minimum in bilayer-water interface
  - Price: Wimley-White behavior can be better by choosing different particle type and partial charge

OPLS-AA

2.1

2.2





de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013)

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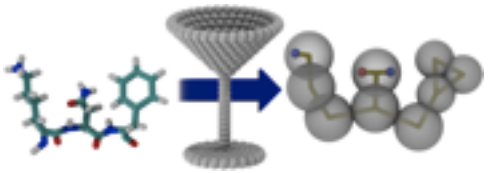


Further changes in Martini v2.2 and v2.2P

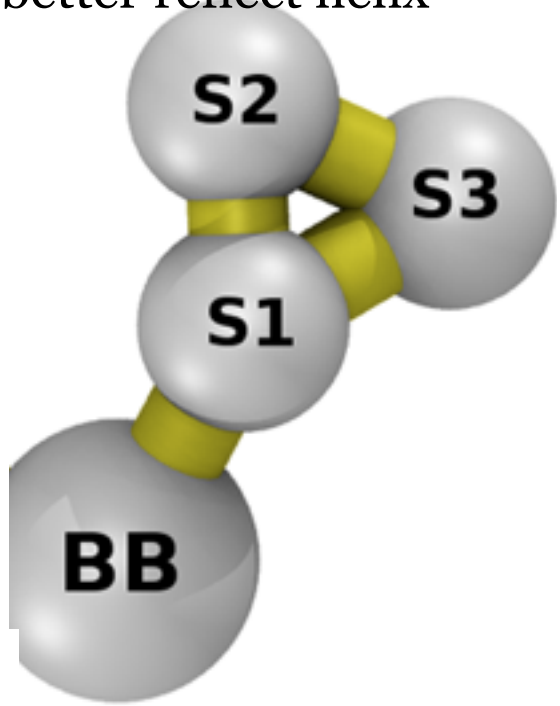
- Particle type of aromatic residues changed to better reflect oil-water partitioning
- His+ added; Pro bead types changed
- BB-BB distances in helical stretches shortened to better reflect helix length
- Recommend shorter S-S bond*

ref: Exp or atomistic MD

italic: v2.1

bold: v2.2(P) final model

SC		type (charge)[b]	$\Delta\Delta G^{ww}$ [c]	ΔG^{part} [d]	ΔG^{dis} water[e]	ΔG^{dis} oil[e]
Phe	ref.		5.4 ± 0.3	12	−1.6	−2.9
	CG	*SC4-SC4-SC4*	12.2 ± 0.1	21	−4.5	−1.3
		SC5-SC5-SC5	7.7 ± 0.1	10	−3.0	−1.7
Trp	ref.		8.5 ± 0.4	9	−3.3	−3.3
	CG	*SC4-SP1-SC4-SC4*	9.2 ± 0.1	10	−4.7	−3.0
		SC4-SNd-SC5-SC5	9.4 ± 0.1	8	−4.0	−2.7
Pro[f]	ref.		−1.2 ± 0.6			
	CG	*Na-AC2*	7.6 ± 0.1	20		
		P4-AC2	4.1 ± 0.1	20		
		P4–C3	1.9 ± 0.1	12		
His+	ref.				1.0	
	CG	SC4-SP1-SQd		−66	0.4	
		SC4-SP1-SQd (off-center)		−90	0.5	



* not published, but implemented in current `martinize.py` script for S-S bond, use constraint 0.24 nm iso 0.39, fc 5,000

de Jong et al. *J. Chem. Theor. Comput.* **9**, 687 (2013)

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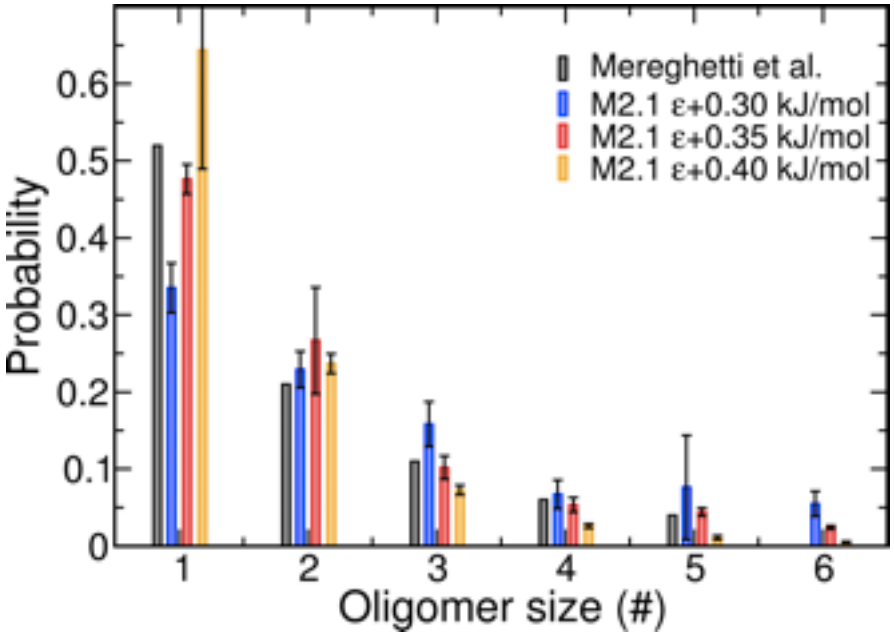
There is still more room for improvement...!

- Aggregation of soluble protein is still too pronounced
- Case study of BPTI (56 a.a., +6e) oligomer distributions shows that by changing the levels (ε values of LJ parameters) of all protein-water bead interactions the correct distribution can be obtained
- Straightforward Martini 2.1 simulation of 48 copies shows single large aggregate
- Interaction between water and protein is made stronger to help solvate the protein (ε values of LJ parameters are increased)
- $\varepsilon' = \varepsilon + 0.35$ kJ·mol^{-1} yields best overall result



de Jong et al. *in preparation* (2015);
Mereghetti et al. *Biophys. J.* **99**, 3782 (2010)

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Determination of oligomer distribution

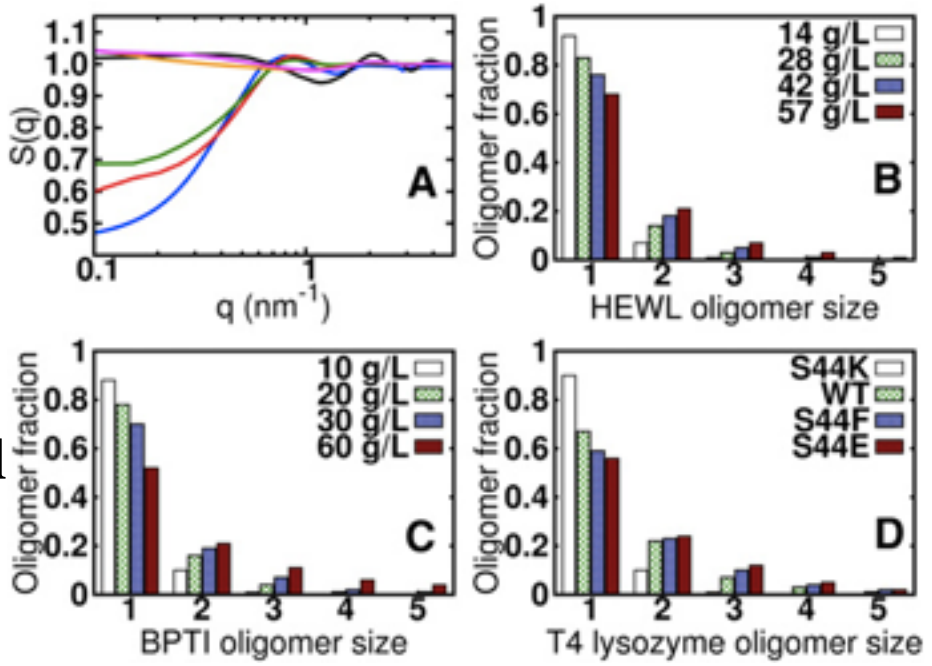
- Rather indirect
- Experiment measures structure factor, e.g. from Dynamic Light Scattering
- *S(q)* is the Fourier transform* of *g(r)*

$$S(q) = 1 + \frac{4\pi\rho}{q}\int_0^\infty r\left(g(r)-1\right)\sin(qr)\,dr$$

- *g(r)* is related to B_{22}, the osmotic second virial coefficient

$$B_{22} = -2\pi\int_0^\infty \left(g(r)-1\right)r^2\,dr$$

- *g(r)* was obtained by Mereghetti et al in an effective solvent (but all atom protein) Brownian dynamics simulation of 512 proteins, 10 μs at different ionic strengths



Mereghetti et al. *Biophys. J.* **99**, 3782 (2010)

* formula for isotropic liquid

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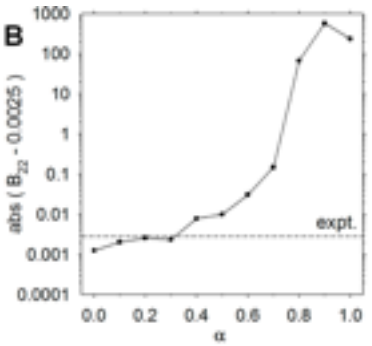
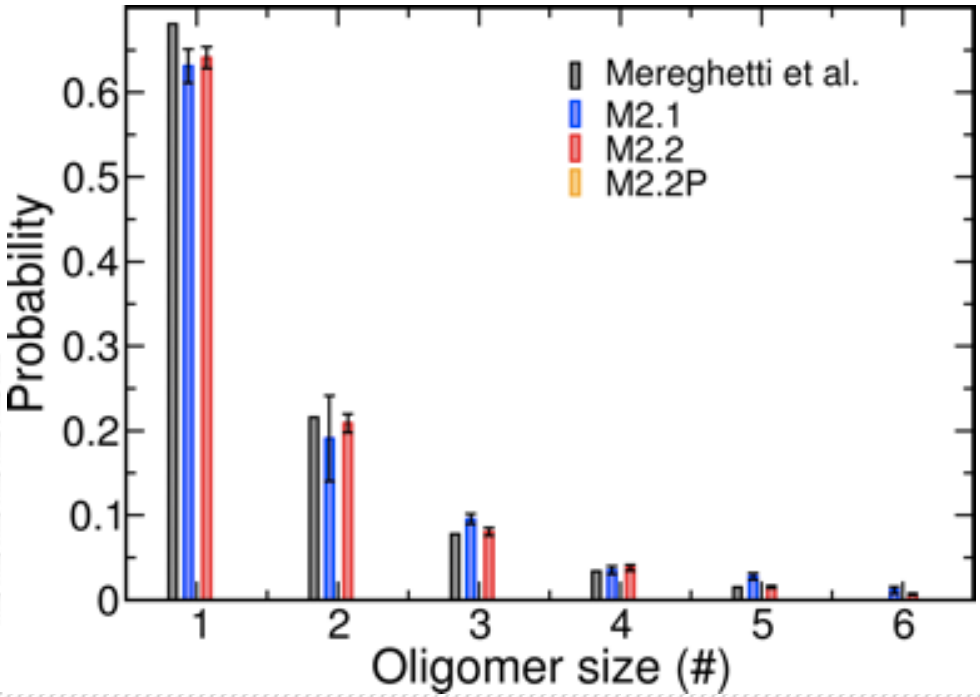
There is still more room for improvement...!

- Data for protein oligomerization is also available for HEWL (129 a.a., +8e)
- Using the BPTI result for Martini 2.1 shows that the finding is transferable to HEWL (64 copies) and that versions 2.1 and 2.2 give similar results
- Other strategies have been applied to Martini such as the uniform scaling down of all interactions, except to P4 (water bead) and Qa and Qd (ions) by Stark et al

$$\varepsilon_\alpha = \varepsilon_{VIII} + \alpha\left(\varepsilon_L - \varepsilon_{VIII}\right)$$

L designates the normal level





de Jong et al. *in preparation* (2015); Mereghetti et al. *Biophys. J.* **99**, 3782 (2010); Stark et al. *J. Chem. Theor. Comput.* **9**, 4176 (2013)

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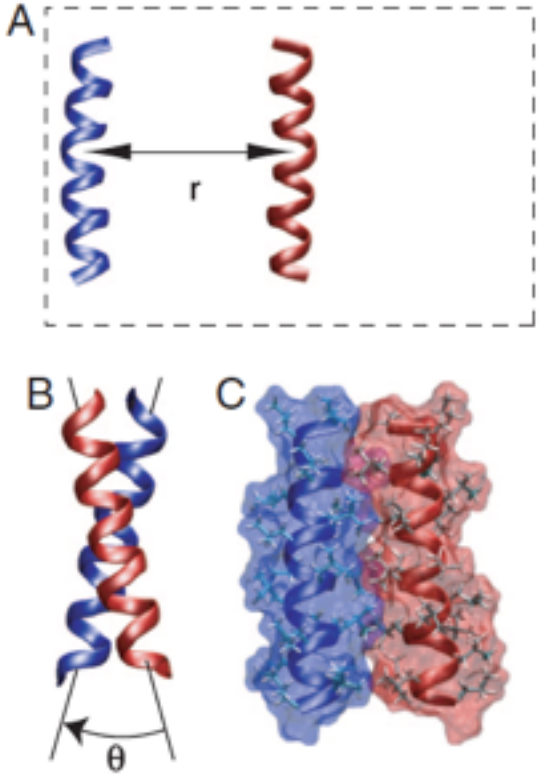
Soluble protein association

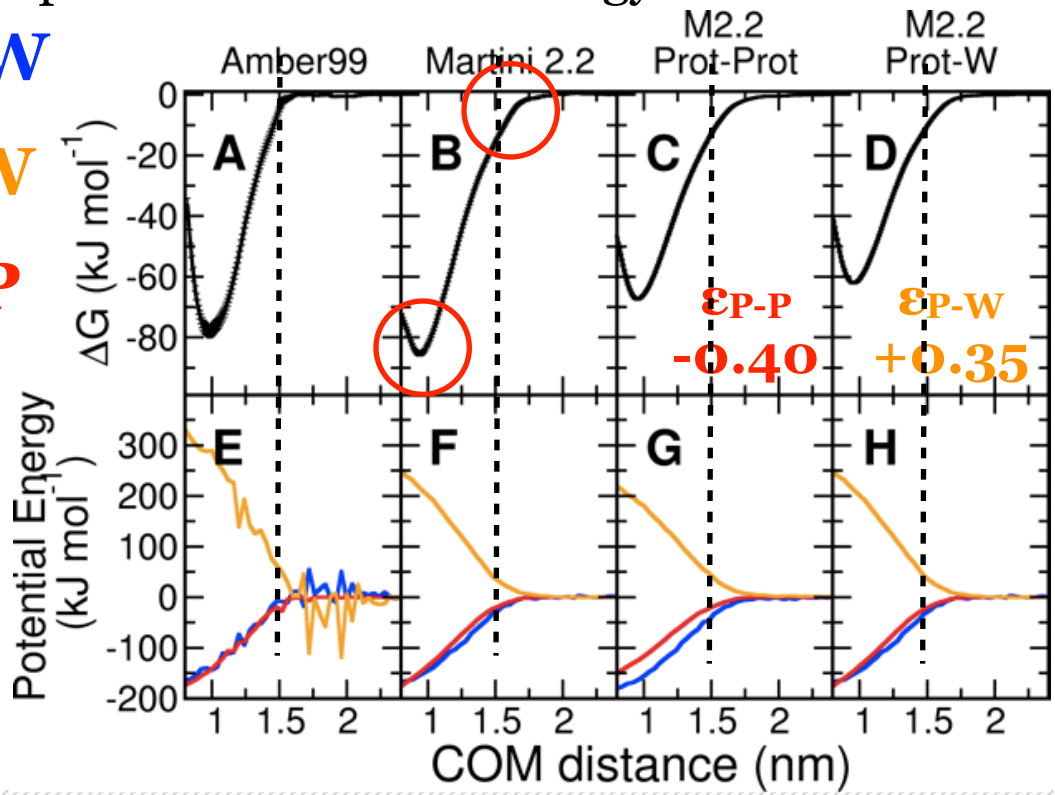
- PMF for association of hydrophobic helices (Leu_{20} helices) in water similar in Martini 2.2 to that in Amber99, but overall more attractive
- P-P or P-W interaction may be changed; both result in better overall behavior but P-W changes reproduces atomistic energy contributions best



W-W

P-W

P-P



PMF at fixed orientation!

de Jong et al. *in preparation* (2015); McCallum et al. *PNAS* **104**, 6206 (2007)

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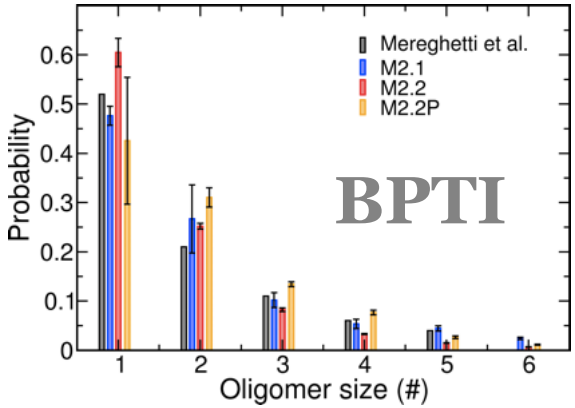
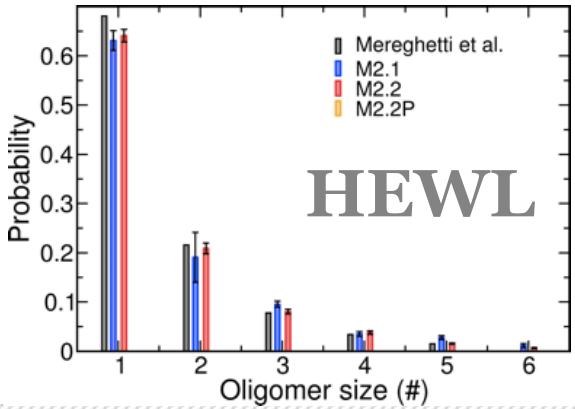
No free lunch…

› Even though oligomerization distributions look good, the shifting of the levels leads to overall worse partitioning behavior of amino acid side chains between water and oil as shown below for $\Delta G_{w/o}$ (kJ·mol^{-1})

AA	Exp	v2.2	v2.2 shift
Ile/Leu	+22	+20	+17
Val	+17	+18	+15
Cys/Met	+5/+10	+5.9 (2)	+2.4 (2)
Phe	+12	+11.7 (3)	+2.1 (3)
Trp	+9	+6.8 (5)	-4.2 (2)
Tyr	-2	+1.7 (4)	-7.6 (4)
Ser/Thr	-14/-11	-12.2 (2)	-15.9 (4)
His	-20	-18	-26
Gln	-25	-24	-28
Asn	-28	-31	-35





de Jong et al. *in preparation* (2015); Mereghetti et al. *Biophys. J.* **99**, 3782 (2010)

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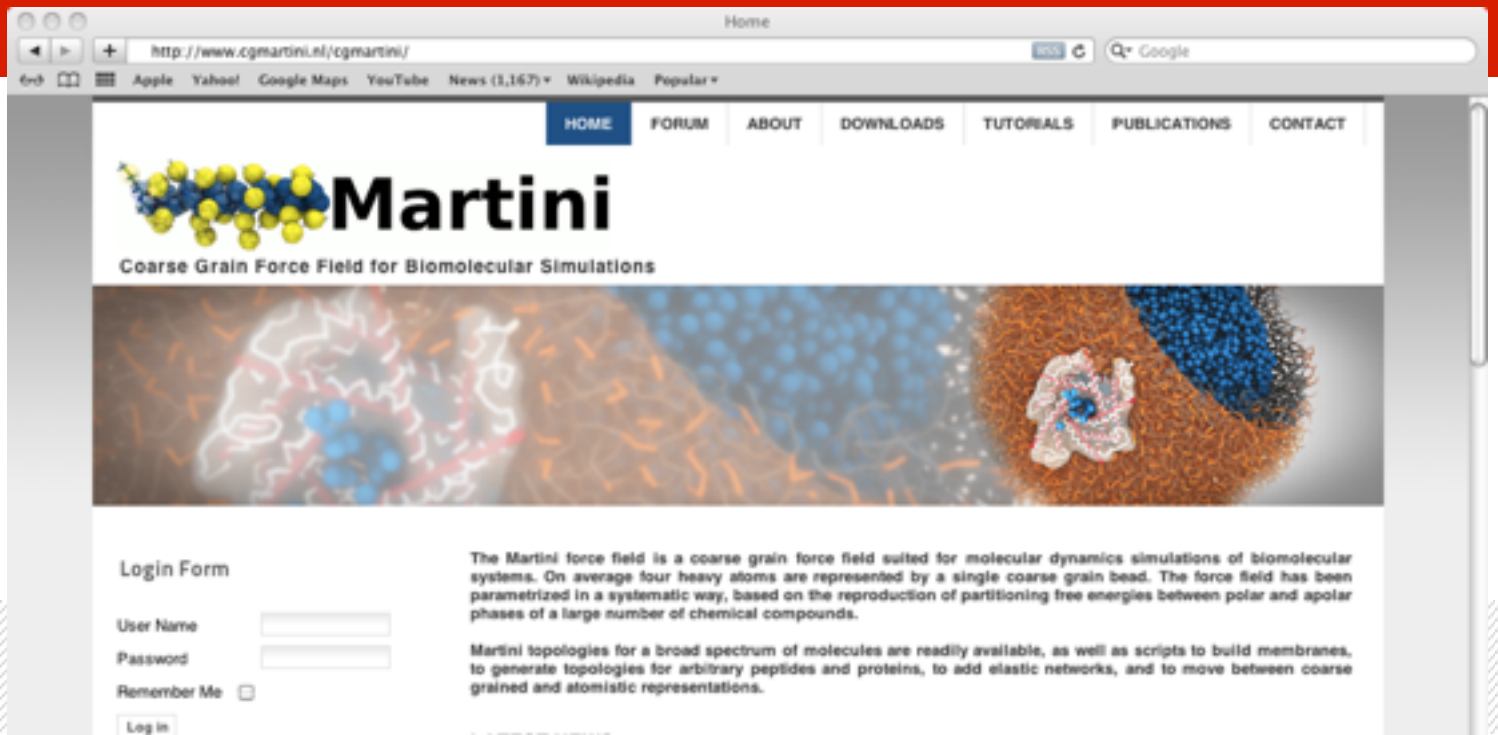
The Martini model is a semi-empirical force field and will be under continued development



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Brief Martini Protein Hands-on overview

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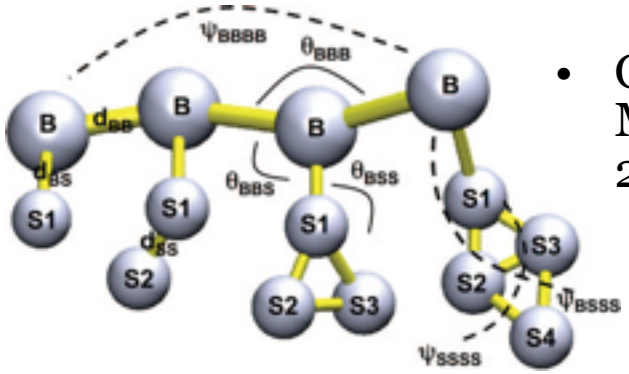
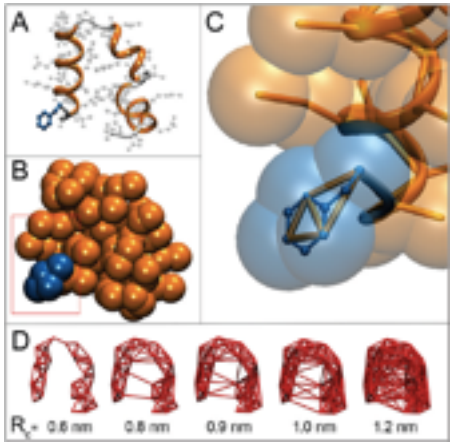
Basic

There are two Martini protein models!



- Original by Monticelli et al, 2008
- ElNeDyn by Periole et al, 2009



- Combined with elastic network

Standard tutorial takes you through setting up Martini simulations for a soluble protein (ubiquitin) starting from a PDB structure, using the tool `martinize.py` (more on that in tomorrow's lecture), in three versions and lets you compare some properties

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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Advanced

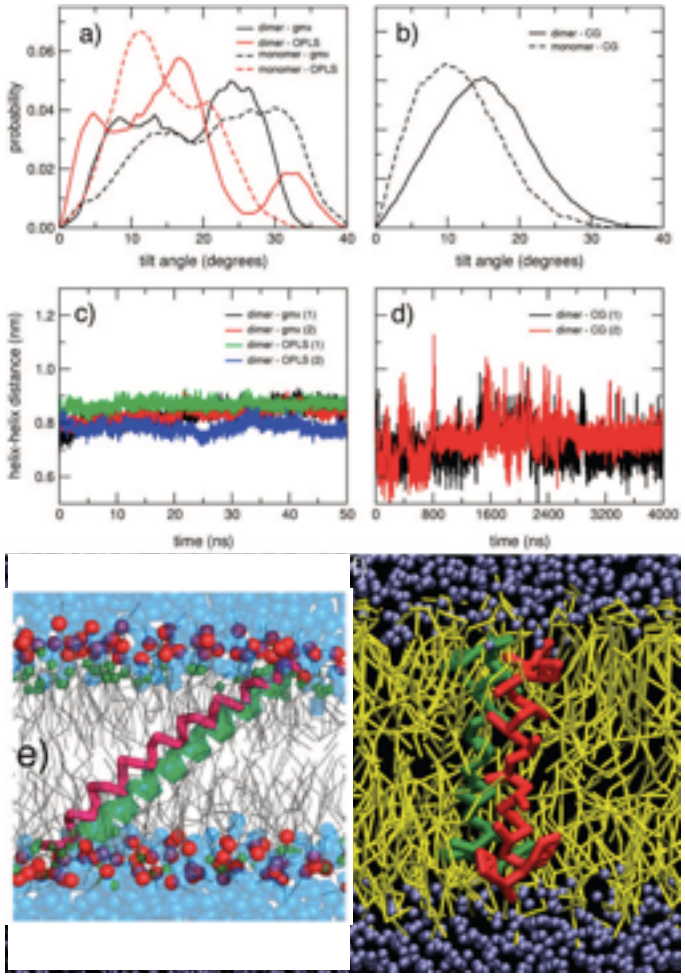
TM helices are basis for protein-lipid interactions

Advanced tutorial sets up membrane protein model (KALP), using the tool `insane.py` (more on that in tomorrow's lecture) and prompts you to study tilt and diffusion

Go on to study dimerization

Use external tutorial (see gromacs website) to set up calculation of PMF

And feel free to experiment with your own set-ups or try CHARMM-GUI!



Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008)

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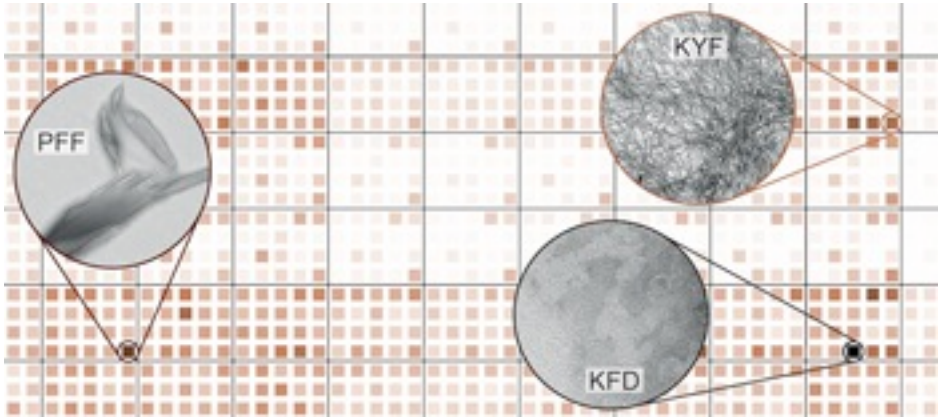
Advanced, brand new tutorial

High throughput screening of peptides

Advanced tutorial lets you automate a protocol for studying self-assembly of tripeptides. Here, Martini is used as an industrial tool for high-throughput library screening. It combines a number of tools used in the protocol, building topologies, creating random solution starting structures, equilibration and production runs, and analysis of the final assembly

Good for learning about scripting!

Challenge yourself and put atomistic details back into the CG assembly



Frederix et al. *Nat. Chem.* 7, 30 (2015)

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POSTER SESSION
17:00-19:00 hours in the canteen

PUT UP POSTERS JUST BEFORE THE START
and take them down at the end…

Drinks & snacks will be served!

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Supporting: Overview of Martini Protein publications MD group Groningen

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Overview of Martini Protein-Protein studies

Main author, Journal, Year	System	Model	Comments
Periole, JACS, 2007	16 rhodopsin (GPCR) in different membranes	Standard + EN bonds	Intermediate toward ELNEDYN, elastic bonds used to preserve tertiary structure
Yefimov, BJ, 2008; Louhivuori, PNAS, 2010; Ollila, BJ, 2011, Deplazes, PLOS 2012, Mukherjee, FASEB, 2014, Konijnenberg, PNAS, 2014	MscL in membrane	Standard	A number of these papers have combined simulation and experimental results
Treptow, JPCB, 2008	Kv1.2 channel in membrane	Standard	500 ns CGMD of closed state of the channel compared to short atomistic MD and experiment
Berntsson, EMBO J, 2009	OppA* - octapeptide	Standard	Dynamic shifts in register seen
Sengupta, MMB, 2009	ATPase C-subunit in membrane	Standard	C-subunit peptide interfaces in dimer and cyclic decamer
Lycklama, JBC, 2010	SecY channel in membrane	Standard	Dynamics of helix wrt complex in SecY machinery

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Overview of Martini Protein-Protein studies

Main author, Journal, Year	System	Model	Comments
Sengupta, PCCP, 2010	TM helix association	Standard	GpA and mutants
Schafer, PNAS, 2011	TM helix association	Standard	WALP helices of different length in mebrane
Sorensen, JPCL, 2011	protofibrillar assembly	ELNEDYN	Self-assembly of 27 amylin protofibrils, consisting of 20 peptides each
Wassenaar, JCTC, 2015	TM helix association	Standard	The DAFT approach
Arnarez, PhD Thesis, 2014	CIII-CIV respiratory chain subunits	ELNEDYN	Role of cardiolipin in protein interfaces

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Overview of Martini Protein-Lipid studies

Main author, Journal, Year	System	Model	Comments
Catte, BJ, 2008; Vuorela, PLOS , 2010	HDL	Standard	Lipid droplet including apoA-I protein envelop
Fuhrmans, JACS, 2009; Fuhrmans 2012	Fusion peptides in lipid-water system	Standard	Fusion peptides can induce or stabilize lipid diamond phase
Murtola, SM, 2011	LDL	ELNEDYN	Interaction between ApoB-100 and cholesterol (esters)
Domanski, BBAM, 2012	TM helices in membrane	Standard and ELNEDYN	TM helices can induce lipid domain formation
Arnarez, Sci Rep, 2013	CIV in mixed lipid bilayer	ELNEDYN	Cardiolipin explores different sites on cytochrome c oxidase
Arnarez, JACS, 2013	CIV in mixed lipid bilayer	ELNEDYN	Cardiolipin explores different sites on cytochrome bc_1 oxidase

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Supporting: comparison of Protein force fields

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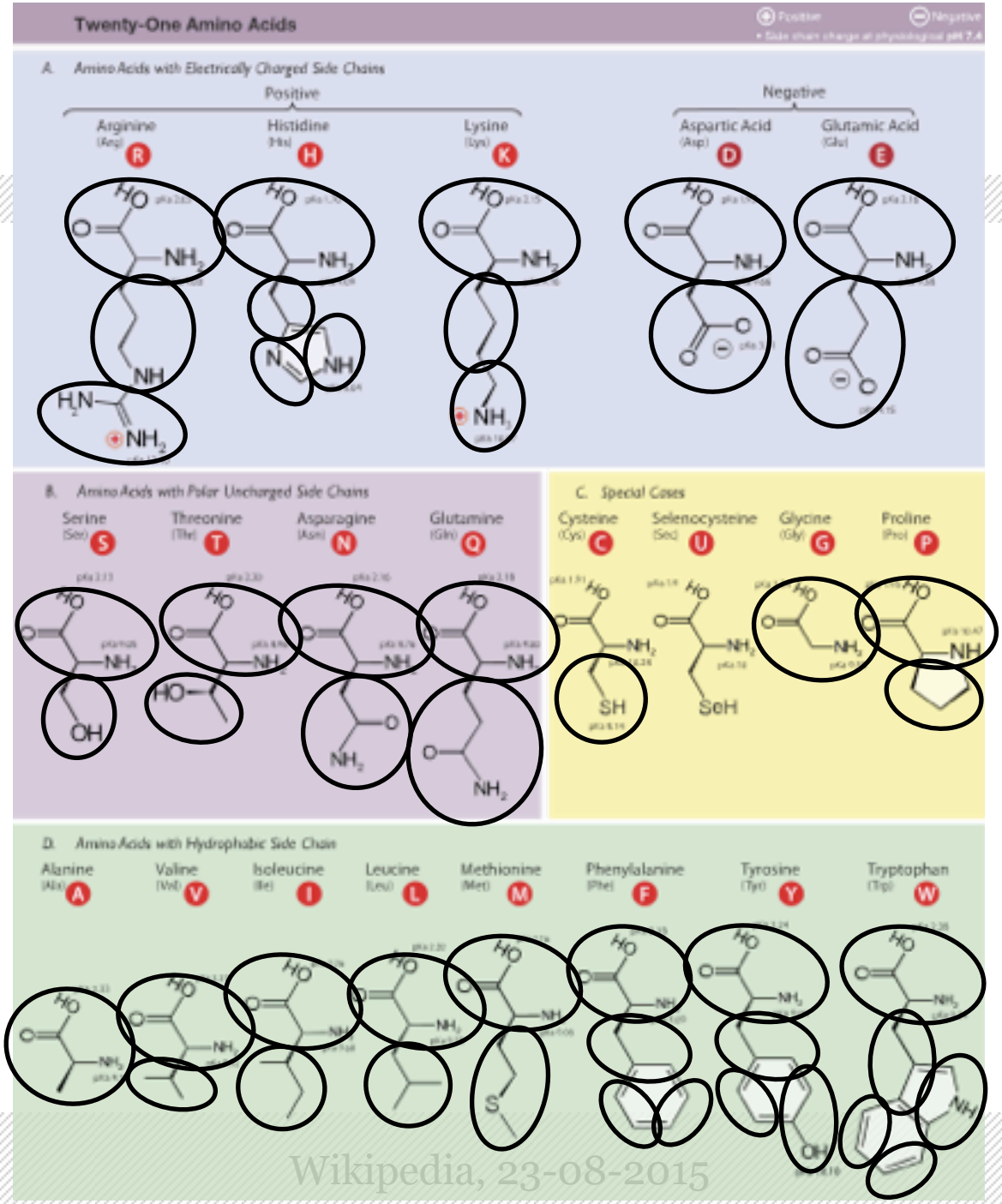
The amino acids

- and the Martini mapping in the standard model



Wikipedia, 23-08-2015

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$$V(d) = \frac{k}{2}\left(d - d_0\right)^2$$

Comparison of Standard Martini and ElNeDyn

› Backbone bonds (BB-BB)

	Standard		ElNeDyn	
Sec Struct	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**
helix	0.35	1,250	from PDB	150,000
coil	0.35	200	from PDB	150,000
extended	0.35	1,250	from PDB	150,000
turn	0.35	500	from PDB	150,000
bend	0.35	400	from PDB	150,000

Monticelli et al. *J. Chem. Theor. Comput.* **4**, 819 (2008);
Periole et al. *J. Chem. Theor. Comput.* **5**, 2531 (2009)

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$$V(\theta) = \frac{k_\theta}{2}\left(\cos\theta - \cos\theta_0\right)^2$$

Comparison of Standard Martini and ElNeDyn

› Backbone angle (BB-BB-BB)

	Standard		**ElNeDyn**	
Sec Struct	**θ_0 (deg)**	**k_θ (kJ·mol^{-1})**	**θ_0 (deg)**	**k_θ (kJ·mol^{-1})**
helix	96 (PRO: 98)	700 (100)	from PDB	40
coil	127	25	from PDB	40
extended	134	25	from PDB	40
turn	100	25	from PDB	40
bend	130	25	from PDB	40

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$$V(\varphi) = K_\varphi\left[1+\cos\left(\varphi-\varphi_0\right)\right];\quad V(\chi) = \frac{k_\chi}{2}\left(\chi-\chi_0\right)^2$$

Comparison of Standard Martini and ElNeDyn

› Backbone dihedral (BB-BB-BB-BB and BB-SC-SC-SC)

	Standard		**ElNeDyn**	
Sec Struct	**φ_0 (deg)***	**k_φ (kJ·mol^{-1})**	**φ_0 (deg)**	**k_φ (kJ·mol^{-1})**
helix	-120	400	-	-
coil	-	-	-	-
extended	0	10	-	-
turn	-		-	-
bend	-		-	-
Amino acid	**χ_0 (deg)***	**k_χ (kJ·mol^{-1}·rad^{-2})**		
His. Tyr, Phe	0	50	-	-
Trp	0/0	50/100	-	-

*** φ_0 not properly stated in 2007 paper!**

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$$V(d) = \frac{k}{2}\left(d - d_0\right)^2$$

Comparison of Standard Martini and ElNeDyn

› Backbone-side chain bonds (BB-SC)

	Standard		ElNeDyn	
Amino acid	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**
Leu (AC1)	0.33	7,500	0.265	81,500
Ile (AC1)	0.31	constr	0.225	13,500
Val (AC2)	0.265	constr	0.20	constr
Pro (AC2)	0.30	7,500	0.19	constr
Met (C5)	0.40	2,500	0.31	2,800
Cys (C5)	0.31	7,500	0.24	94,000
Ser (P1)	0.25	7,500	0.195	constr
Thr (P1)	0.26	constr	0.195	constr
Asn (P5)	0.32	5,000	0.25	61,000
Gln (P4)	0.40	5,000	0.30	2,400
Asp (Qa)	0.32	7,500	0.224	65,000
Glu (Qa)	0.40	5,000	0.31	2,500

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$$V(d) = \frac{k}{2}\left(d - d_0\right)^2$$

Comparison of Standard Martini and ElNeDyn

› Backbone-side chain bonds (BB-SC and SC-SC)

	Standard		ElNeDyn	
Amino acid	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2}**
Arg (BB-No)	0.33	5,000	0.25	12,500
Arg (No-Qd)	0.34	5,000	35	6,200
Lys (BB-C3)	0.33	5,000	0.25	12,500
Lys (C3-Qd)	0.28	5,000	0.30	9,700
His (BB-SC4)	0.32	7,500	0.195	constr
His (all sc-sc)	0.27	constr	0.193/0.216/0.295	constr
Phe (BB-SC4)	0.31	7,500	0.34/0.34	7,500/7,500
Phe (all sc-sc)	0.27	constr	0.24	constr
Tyr (BB-SC4)	0.32	5,000	0.335/0.335	6,000/6,000
Tyr (all sc-sc)	0.27	constr	0.24/0.31/0.31	constr
Trp (BB-SC4)	0.30	5,000	0.255	73,000
Trp (all sc-sc)	0.27	constr	0.22/0.25/0.28/0.255	constr

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$$V(\theta) = \frac{k_\theta}{2}\left(\cos\theta - \cos\theta_0\right)^2$$

Comparison of Standard Martini and ElNeDyn

› Backbone-side chain angles (BB-SC-SC)

	Standard		ElNeDyn	
Amino acid	**θ_0 (deg)**	**k_θ (kJ·mol^{-1})**	**θ_0 (deg)**	**k_θ (kJ·mol^{-1})**
Arg (BB-No-Od)	180	25	150	15
Lys (BB-C3-Qd)	180	25	150	20
His (BB-SC4-SP1)	150/150	50/50	135/115	100/50
Phe (BB-SC4-SC4)	150/150	50/50	70/125	100/100
Tyr (BB-SC4-SC4)	150	50	70	100
Tyr (BB-SC4-SP1)	150	50	130	50
Trp (BB-SC4-SP1)	90	50	142	30
Trp (BB-SC4-SC4)	210	50	143/104	20/50
BB-BB-SC	100	25	-	-

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$$V(d) = \frac{k}{2}\left(d - d_0\right)^2$$

Comparison of Standard and Martini 2.2(P)

› Backbone bonds (BB-BB)

	Standard		Martini 2.2(P)	
Sec Struct	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**	**d_0 (nm)**	**k_b (kJ·mol^{-1}·nm^{-2})**
helix	0.35	1,250	0.31	constr
coil	0.35	200	0.35	1,250
extended	0.35	1,250	0.35	1,250
turn	0.35	500	0.35	1,250
bend	0.35	400	0.35	1,250

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$$V(\theta) = \frac{k_\theta}{2}\left(\cos\theta - \cos\theta_0\right)^2$$

Comparison of Standard and Martini 2.2(P)

› Backbone angle (BB-BB-BB)

	Standard		Martini 2.2(P)	
Sec Struct	**θ_0 (deg)**	**k_θ (kJ·mol^{-1})**	**θ_0 (deg)**	**k_θ (kJ·mol^{-1})**
helix	96 (PRO: 98)	700 (100)	96 (PRO: 98)	700 (100)
coil	127	25	127	20
extended	134	25	134	25
turn	100	25	100	20
bend	130	25	130	20

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$$V(\varphi) = K_\varphi\left[1+\cos\left(\varphi-\varphi_0\right)\right];\quad V(\chi) = \frac{k_\chi}{2}\left(\chi-\chi_0\right)^2$$

Comparison of Standard and Martini 2.2(P)

› Backbone dihedral (BB-BB-BB-BB and BB-SC-SC-SC)

	Standard		**Martini 2.2(P)**	
Sec Struct	**φ_0 (deg)***	**k_φ (kJ·mol^{-1})**	**φ_0 (deg)***	**k_φ (kJ·mol^{-1})**
helix	-120	400	-120	400
coil	-	-	-	-
extended	0	10	0	10
turn	-		-	-
bend	-		-	-
Amino acid	**χ_0 (deg)***	**k_χ (kJ·mol^{-1}·rad^{-2})**	**χ_0 (deg)***	**k_χ (kJ·mol^{-1}·rad^{-2})**
His. Tyr, Phe	0	50	0	50
Trp	0/0	50/100	0/0	50/100

*** φ_0 not properly stated in 2007 paper!**

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