Pseudoatom-driven solvent accessibility refinement (PaDSAR) Method

This suite of NAMD input files is to support the application of the PaDSAR method for membrane protein refinement based on EPR data. This method is used for incorporating solvent accessibility data from electron paramagnetic resonance experiments in the structural refinement of membrane proteins through restrained molecular dynamics simulations. The restraints have been parameterized from oxygen (O(2)) and nickel-ethylenediaminediacetic acid (NiEdda) collision frequencies, as indicators of lipid or aqueous exposed spin-label sites. These are enforced through interactions between a pseudoatom representation of the covalently attached Nitroxide spin-label and virtual "solvent" particles corresponding to O(2) and NiEdda in the surrounding environment. Interactions were computed using an empirical potential function, where the parameters have been optimized to account for the different accessibilities of the spin-label pseudoatoms to the surrounding environment

The Files are:

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PaDSAR.namd         - NAMD input file
NIC-120-120-80.dx   - spatial restraints map for the NiEdda
OXY-120-120-80.dx   - spatial restraints map for the O2
epr-tabulated.par   - command for tabulated potentials
epr-tabulated.dat   - potentials for all pseudo atom types
toph19_epr_VMD.inp - CHARMM topology file
param19-epr.inp     - CHARMM parameter file
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Patching and solvating the full-length models with pseudoatoms

Two categories of pseudoatoms are introduced in the system, i.e., spin-label pseudoatoms and environment pseudoatoms [1], as summarized in Table 1. The spin-label pseudoatoms are further classified into five different types, denoted EP1, EP2, EP3, EP4, and EP5, according to electron paramagnetic resonance (EPR) spectroscopy solvent accessibility data. Briefly, EP1 is buried within the protein with low O_2-accessibility (PO_2) and NiEdda-accessibility (PNiEdda) values. EP2 is water exposed with high PNiEdda but low PO_2 values. EP3 is lipid exposed with high PO_2 but low PNiEdda values. EP4 and EP5 correspond to sites having significant changes in the PO_2 values in the isolated domains and in the full-length protein. They are considered to be at the interface of the two isolated domains. EP4 is residing in the PD (domain1) and VSD (domain2) exposed, while EP5 is residing in the exposed part of the domains, see Fig. 1. Spin-label pseudoatom is covalently patched to the C_α atom of the labeled residues (Table 2), and in the plane of the C_α, N, and C atoms using VMD [2]. The topology parameters for the spin-label pseudoatoms are summarized in Table 2 and Fig. 2. The environment pseudoatoms are the contrasting particles interacting with the spin-label pseudoatoms. They include PROT, OXY and NIC, representing amino acid residues,

molecular oxygen and NiEdda, respectively. PROT is attached at each C_α atom of the protein. The topology and parameters for the protein were taken from the CHARMM 19 force field. In addition, the charges of the ionizable residues, i.e., Arg^{+1}, Lys^{+1}, Glu^{-1}, and Asp^{-1}, and the patched $Nter^{+1}$ and $Cter^{-1}$ were offset by adding a countercharge to each of the nonbackbone atoms and patched atoms, respectively. A pre-equilibrated box of OXY is used to build a 100 × 100 × 24 Å membrane, into which the prepared model with patched pseudoatoms is embedded using VMD. The normal axis of the membrane and the pore of the protein are both aligned in the *Z*-direction. Then, a slab of NIC is added on each side of the membrane to mimic water solution with VMD.

Table 1. Descriptions of the spin-label and environment pseudoatoms.

Name	Type	Descriptions
EP1	Spin-label	Buried
EP2	Spin-label	Water exposed
EP3	Spin-label	Lipid exposed
EP4	Spin-label	VSD exposed
EP5	Spin-label	PD exposed
PROT	Environment	Amino acid residue
OXY	Environment	Molecular oxygen
NIC	Environment	NiEdda complex

Table 2. Force field parameters for the spin-label pseudoatoms.

Type		$K_f(\varepsilon)$	I_0 (R_{min})
Bonds	CA-EPR*	100.0 (kcal mol^{-1} $Å^{-2}$)	6.0 (Å)
	CA-PROT	100.0 (kcal mol^{-1} $Å^{-2}$)	0.0 (Å)
Angles	N-CA-EPR*	50.0 (kcal mol^{-1} rad^{-2})	120.0 (º)
Improper torsions	CA-N-C-EPR*	55.0 (kcal mol^{-1} rad^{-2})	0.0 (º)
Van der Waals	EP1-PROT†	0.05 (kcal mol^{-1})	7.0 (Å)
	EP4, EP5-PROT†‡	1 (kcal mol^{-1})	7.0 (Å)
	EP2-NIC, EP3-OXY	2.0 (kcal mol^{-1})	2.0 (Å)
	EP1, EP4, EP5-OXY, NIC EP2-OXY, PROT EP3-NIC, PROT	0.05 (kcal mol^{-1})	6.0 (Å)
	OXY-NIC	0.0 (kcal mol^{-1})	6.5 (Å)
	OXY-OXY	0.1 (kcal mol^{-1})	5.0 (Å)
	NIC-NIC	0.1 (kcal mol^{-1})	8.0 (Å)

*EPR: EP1, EP2, EP3, EP4, EP5.

†Modified LJ interaction.

‡ EP4 (EP5) only interacts with PROT particles of the domain1 (domain2) of the adjacent subunit.

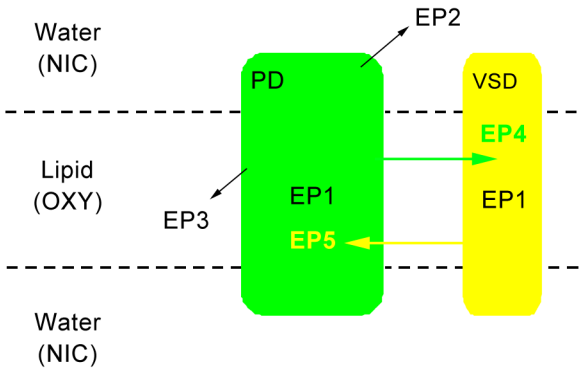

Fig. 1. Cartoon illustration of the definition of spin-label and environment pseudoatoms.

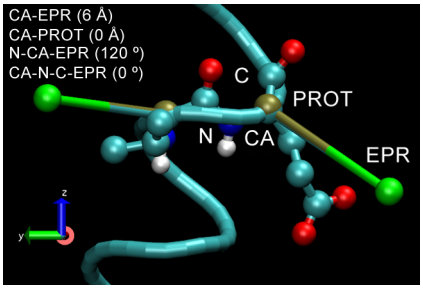

Fig. 2. Topology of the spin-label pseudoatoms patched to the protein backbone.

Force field for pseudoatoms

In the PaDASR method, an empirical molecular mechanics (MM) potential function is employed to calculate the restraint energy of spin-label pseudoatoms, including bond stretching, angle bending, improper torsion, and Lennad-Jones (LJ) type van der Waals (VDW) interactions. The spin-environment VDW interacting pairs can be divided into two types, i.e., matching (EP1-PROT, EP2-NIC, EP3-OXY, EP4-PROT, and EP5-PROT) and mismatching (EP1-OXY, EP1-NIC, EP2-OXY, EP2-PROT, EP3-NIC, EP3-PROT, EP4-OXY, EP4-NIC, EP5-OXY, and EP5-NIC) pairs according to the definition of the pseudoatoms. For domain-domain interactions, it should be noted that EP4 should only interact with PROT particles in domain1, and EP5 should only interact with PROT particles in the domain2. In addition, a modified LJ potential function, $E_m = E_{LJ}$ (if $r > R_{min}$) and $E_m = E_{min}$ (if $r \leq R_{min}$), is used to describe the EP1-PROT, EP4-PROT, and EP5-PROT interactions (Fig. 6), to allow the buried EP1, EP4 and EP5 particles to overlap with PROT particles without a dramatic increase on the LJ energy. Furthermore, spin-label and PROT pseudoatoms see neither the protein atoms nor the pseudoatoms of the same category.

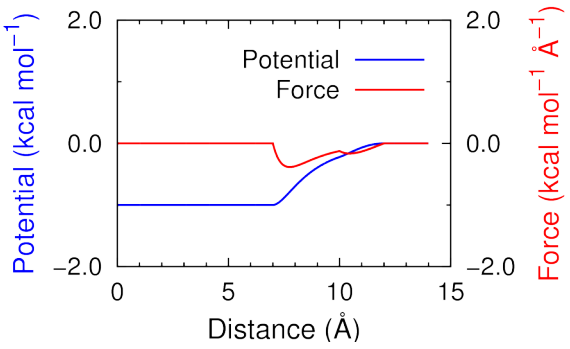

Fig. 3. Modified LJ interaction for specific EPR-PROT pairs. A switching function is applied between 10 Å and 12 Å.

All PaDSAR simulations can be performed with the program NAMD [3] and the CHARMM united-atom force field PARAM19. The VDW and electrostatic potentials are calculated with a switching function between 10 Å and 12 Å. The Langevin dynamics with a friction coefficient of 5 ps^{-1} was used to control the system temperature at 310 K or 400 K. All simulations were carried out with a time step of 2 fs. Tabulated energies were used for the modified LJ potentials for specified spin-environment pseudoatom pairs.

```
# Tabulated nonbonded interaction parameters
Parameters                ./epr-tabulated.par
# Tabulated parameters
tabulatedEnergies         on
tabulatedEnergiesFile     ./epr-tabulated.dat
tableInterpType           cubic
```

Planar harmonic restraints with a force constant of 1 kcal·mol·$Å^{-2}$ were applied on OXY and NIC particles to distribute in the inner and outer membrane compartments, respectively, using the grid forces in NAMD, as shown in Fig. 4.

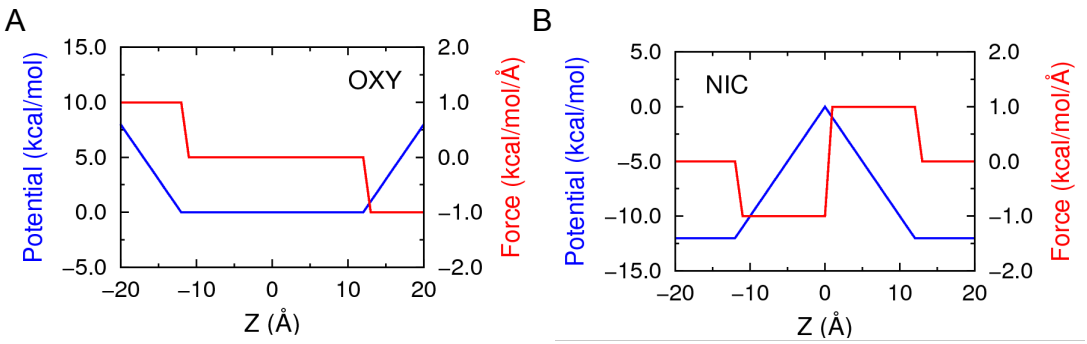

Fig. 4. The grid-based potentials and forces for OXY (A) and NIC (B) particles.

```
# Grid forces
set scale              1
mgridforce             on
mgridforcefile         OXY     ${MySystem}-OXY.pdb
mgridforcecol          OXY     B
mgridforcechargecol    OXY     O
mgridforcepotfile      OXY     ${MySystem}-OXY.dx
mgridforcescale        OXY     $scale $scale $scale
mgridforcecont1        OXY     yes
mgridforcecont2        OXY     yes
mgridforcecont3        OXY     yes
mgridforcefile         NIC     ${MySystem}-NIC.pdb
mgridforcecol          NIC     B
mgridforcechargecol    NIC     O
mgridforcepotfile      NIC     ${MySystem}-NIC.dx
mgridforcescale        NIC     $scale $scale $scale
mgridforcecont1        NIC     yes
mgridforcecont2        NIC     yes
mgridforcecont3        NIC     yes
```

Reference

[1] Sompornpisut, P., Roux, B. & Perozo, E. (2008). Structural refinement of membrane proteins by restrained molecular dynamics and solvent accessibility data. *Biophys. J.,* **95**, 5349–5361.

[2] Humphrey, W., Dalke, A. & Schulten, K. (1996). VMD: visual molecular dynamics. *J. Mol. Graph.,* **14**, 33–38.

[3] Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R. D., Kale, L. & Schulten, K. (2005). Scalable molecular dynamics with NAMD. *J. Comput. Chem.*, **26**, 1781–1802.