

Multi-Scale Protein Simulation Pipeline

SCN5A Cardiac Sodium Channel — WT vs. Pathogenic Mutant

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Stage 0 Atomistic Mutation & $\Delta\Delta G$ Scoring

Input: AlphaFold-predicted PDB (scn5a_af.pdb) **Tool:** PyRosetta (ref2015 score function)
Method: Local repacking within 8 Å of mutation site (Kellogg *et al.* 2011) — 3-round averaged $\Delta\Delta G = E_{\text{mut}} - E_{\text{WT}}$
Thresholds: $\Delta\Delta G > +1.0$ REU \Rightarrow destabilizing; $\Delta\Delta G < -1.0$ REU \Rightarrow stabilizing; $|\Delta\Delta G| < 1.0 \Rightarrow$ neutral
Output: Mutated all-atom PDB + $\Delta\Delta G$ score

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Stage 1 Coarse-Graining (AA \rightarrow CG)

Tool: martinize2 **Force Field:** Martini 3.0.0.1 **Source FF:** CHARMM
Parameters: Elastic network (enabled) | Disulfide bonds auto-detected (0.24 nm) | DSSP secondary structure | Ignore H (-ignh)
Output: CG PDB + topology (topol.top) + protein ITP (molecule_0.itp) — ~4,699 CG beads

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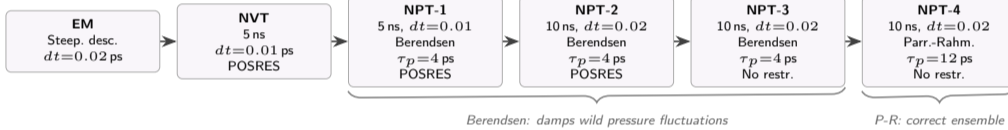
Stage 2 Asymmetric Membrane Construction

Tool: INSANE (INSert membrANE) **Box:** $18.2 \times 18.2 \times 25.0$ nm
Upper leaflet: POPC:POPE:CHOL = 6:2:4 (182:60:121 lipids)
Lower leaflet: POPC:POPE:POPS:CHOL = 4:3:1:4 (115:86:28:115 lipids)
Solvent: 52,594 CG water (W) **Ions:** 615 Na⁺ / 544 Cl⁻ (0.15 M NaCl, charge auto)
Total: ~750,000 CG particles

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Stage 3 CG Multi-Stage Equilibration

Engine: GROMACS **FF:** Martini 3 **Electrostatics:** Reaction-field ($\epsilon_r = 15$) $r_{\text{coulomb}} = r_{\text{vdw}} = 1.1$ nm
Thermostat: V-rescale, $T = 310$ K **Groups:** Solute / Solvent



Hardware
CG stages:
Apple M4 (10-core, 32 GB)
Apple M2 (12-core, 16 GB)
AA production:
H100 GPU, 16 CPUs, 200 GB
Small proteins:
RTX 3060, 8 CPUs, 40 GB

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Stage 4 Back-Mapping (CG \rightarrow AA)

Tool: CG2AT **Target FF:** CHARMM36 (all-atom)
Method: Align atomistic fragments to CG bead centres of mass: $\mathbf{R}_i = \frac{\sum_{j \in I} m_j \mathbf{r}_j}{\sum_{j \in I} m_j}$
Outputs: All-atom PDB + CHARMM36 topology (per-molecule ITPs, position restraints, distance restraints)
System: 743,194 atoms — PROTEIN, POPC, POPE, POPS, CHOL, TIP3P (210,376), 615 Na⁺, 544 Cl⁻

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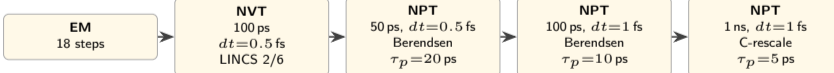
Stage 5 All-Atom Equilibration (CHARMM36)

Engine: GROMACS (GPU: -nb gpu -pme gpu -bonded gpu -update gpu) **FF:** CHARMM36
Electrostatics: PME ($r_{\text{coulomb}} = 1.2$ nm, pme_order = 4, spacing = 0.12) **vdW:** Force-switch at 1.0–1.2 nm
Constraints: h-bonds (LINCS) **Thermostat:** V-rescale, $T = 310$ K

Mutant Equilibration:



WT (back-mutation from mutant final frame):



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Stage 6 Production MD (10 ns)

Both systems: $dt = 2$ fs, 5×10^6 steps, $T = 310$ K (V-rescale, $\tau = 1$ ps), semiisotropic barostat
Barostat: Mutant Parrinello-Rahman ($\tau_p = 5$ ps) Wild-Type C-rescale ($\tau_p = 5$ ps)
Performance: Mutant 45.4 ns/day Wild-Type 49.8 ns/day
Wall time: Mutant 5 h 17 min Wild-Type ~4 h 50 min
Compressibility: 4.5×10^{-5} bar⁻¹ **Output:** xtc every 10 ps **Total atoms:** 743,194

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Stage 7 Comparative Analysis (WT vs. Mutant)

Tools: GROMACS analysis, MDAnalysis, scikit-learn, matplotlib

Structural
RMSD, RMSF,
 R_g , DSSP

Energetic
 E_{pot} , T , P ,
 ρ , E_{tot}

Membrane
APL, SCD,
density profile

H-Bond
Intra-protein
H-bond count

PCA
PC1–PC3,
scree plot

Dihedral
Ramachandran
 ϕ/ψ maps

Coarse-Grained (Martini3)

All-Atom (CHARMM36)