Multi-Scale Protein Simulation Pipeline

SCN5A Cardiac Sodium Channel — WT vs. Pathogenic Mutant

0 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 $\Delta\Delta G = E_{\mathrm{mut}} - E_{\mathrm{WT}}$
Thresholds: $\Delta\Delta G > +1.0$ REU ⇒ destabilizing; $\Delta\Delta G < -1.0$ REU ⇒ stabilizing; $|\Delta\Delta G| < 1.0$ ⇒ neutral
Output: Mutated all-atom PDB + $\Delta\Delta G$ score

1 Stage 1 Coarse-Graining (AA → 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

2 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

3 Stage 3 CG Multi-Stage Equilibration

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

EM	NVT	NPT-1	NPT-2	NPT-3	NPT-4
Steep. desc. dt=0.02 ps	5 ns dt=0.01 ps POSRES	5 ns, dt=0.01 Berendsen τ_p=4 ps POSRES	10 ns, dt=0.02 Berendsen τ_p=4 ps POSRES	10 ns, dt=0.02 Berendsen τ_p=4 ps No restr.	10 ns, dt=0.02 Parr.-Rahm. τ_p=12 ps No restr.

Berendsen: damps wild pressure fluctuations — *P-R: correct ensemble*

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

Coarse-Grained (Martini 3)

4 Stage 4 Back-Mapping (CG → AA)

Tool: CG2AT **Target FF:** CHARMM36 (all-atom)
Method: Align atomistic fragments to CG bead centres of mass: $\mathbf{R}_i = \dfrac{\sum_{j \in I} m_j \mathbf{r}_j}{\sum_{j \in I} m_j}$
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^-

5 Stage 5 All-Atom Equilibration (CHARMM36)

Engine: GROMACS (GPU: `-nb gpu -pme gpu -bonded gpu -update gpu`) **FF:** CHARMM36
Electrostatics: PME ($r_{\mathrm{coulomb}} = 1.2$ 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:

EM	NVT	NPT	NPT
Steep. desc. 50,000 max	0.5 ns dt=2 fs POSRES	5 ns dt=2 fs Restrained	6 ns dt=2 fs Unrestrained

WT (back-mutation from mutant final frame):

EM	NVT	NPT	NPT	NPT
18 steps	100 ps dt=0.5 fs LINCS 2/6	50 ps, dt=0.5 fs Berendsen τ_p=20 ps	100 ps, dt=1 fs Berendsen τ_p=10 ps	1 ns, dt=1 fs C-rescale τ_p=5 ps

All-Atom (CHARMM36)

6 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

	Mutant	Wild-Type
Barostat	Parrinello-Rahman ($\tau_p = 5$ ps)	C-rescale ($\tau_p = 5$ ps)
Performance	45.4 ns/day	49.8 ns/day
Wall time	5 h 17 min	∼4 h 50 min

Compressibility: 4.5×10^{-5} bar^{-1} **Output:** xtc every 10 ps **Total atoms:** 743,194

7 Stage 7 Comparative Analysis (WT vs. Mutant)

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

Structural	Energetic	Membrane	H-Bond	PCA	Dihedral
RMSD, RMSF, R_g, DSSP	E_{pot}, T, P, ρ, E_{tot}	APL, SCD, density profile	Intra-protein H-bond count	PC1–PC3, scree plot	Ramachandran ϕ/ψ maps