0 **Atomistic Mutation & $\Delta\Delta G$** — PyRosetta

AlphaFold PDB → local repack (8 Å) → $\Delta\Delta G = E_{\mathrm{mut}} - E_{\mathrm{WT}}$ | ref2015

CG

1 **Coarse-Graining (AA → CG)** — martinize2

Martini 3.0.0.1 | elastic net | DSSP | ~4,699 CG beads

2 **Membrane Assembly** — INSANE

$18.2 \times 18.2 \times 25$ nm | Upper: POPC:POPE:CHOL = 6:2:4 | Lower: +POPS = 4:3:1:4 | 0.15 M NaCl
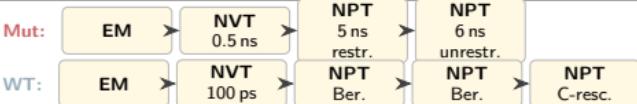
3 **CG Equilibration** — GROMACS / Martini 3

EM → NVT 5 ns → NPT 5 ns Ber. → NPT 10 ns Ber. → NPT 10 ns Ber. → NPT 10 ns P-R

AA

4 **Back-Mapping (CG → AA)** — CG2AT

CHARMM36 | 743,194 atoms | TIP3P, POPC, POPE, POPS, CHOL

5 **AA Equilibration** — GROMACS / CHARMM36 / GPU

Mut: EM → NVT 0.5 ns → NPT 5 ns restr. → NPT 6 ns unrestr.

WT: EM → NVT 100 ps → NPT Ber. → NPT Ber. → NPT C-resc.

6 **Production MD (10 ns)** — GROMACS / H100 GPU

$dt = 2$ fs | $T = 310$ K | P-R / C-rescale | 45–50 ns/day | 743k atoms

7 **Comparative Analysis (WT vs. Mut)** — GROMACS / MDAnalysis

RMSD RMSF, R_g | Energetics E, T, P, ρ | Membrane APL, SCD | H-bonds intra-prot. | PCA ϕ/ψ