Supporting Information for

Lanosterol disrupts aggregation of human γD-crystallin by binding to the hydrophobic dimerization interface

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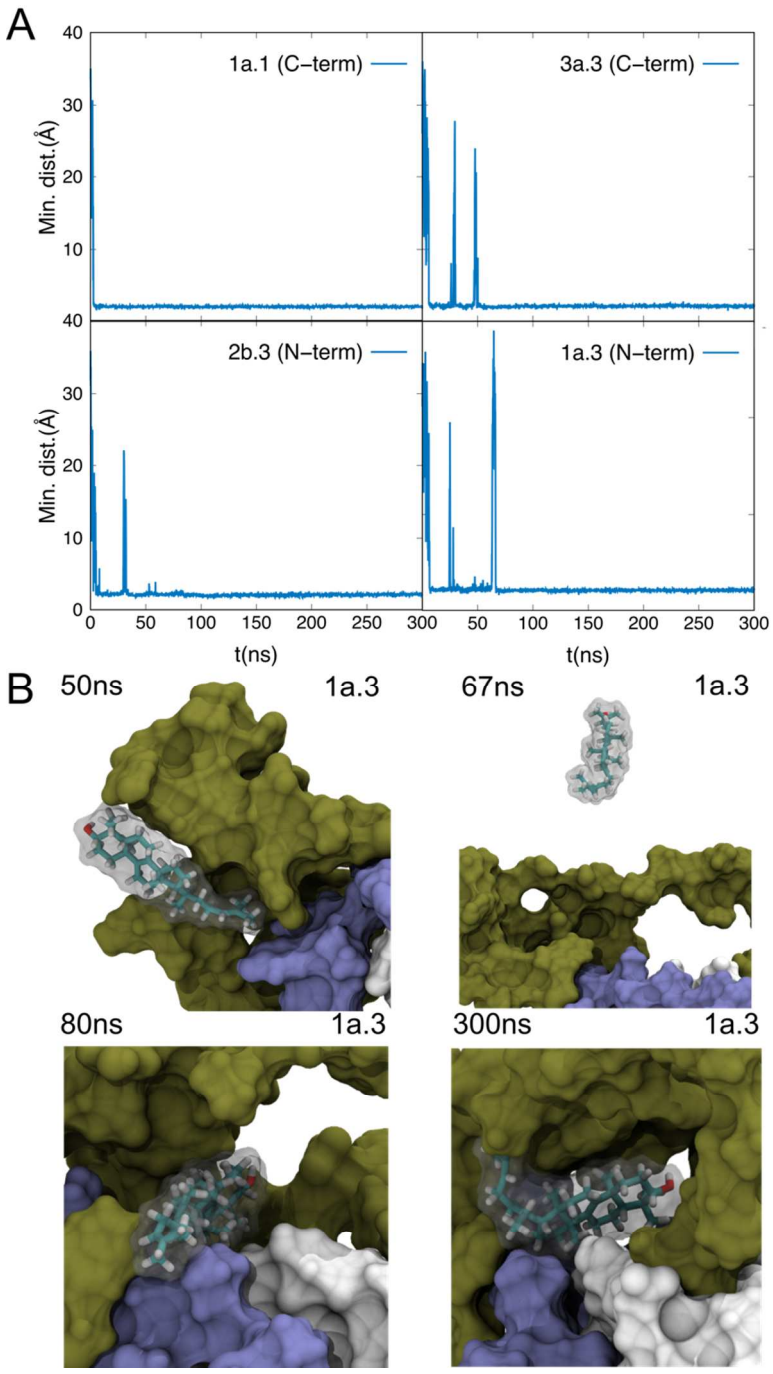


Figure S1 (A) Minimum distance from any atom in the aromatic rings of a lanosterol to HγD crystallin for the C-terminal (upper panel) and N-terminal binding modes (lower). The plots on the left side (1a.1 and 2b.3) display persistent bindings since first binding occur at early time (~30ns) whereas 3a.3 and 1a.3 demonstrate several unbinding and rebinding events up to 60ns. These indicate the existence of transient bindings in short-time simulations.(B) Unbinding-rebinding of the lanosterol in 1a.3. The ligand bound to N-terminal of the crystallin around 30ns and detached at 65ns. Finally, it rebound to C-terminal interface and remained stable for 235ns.

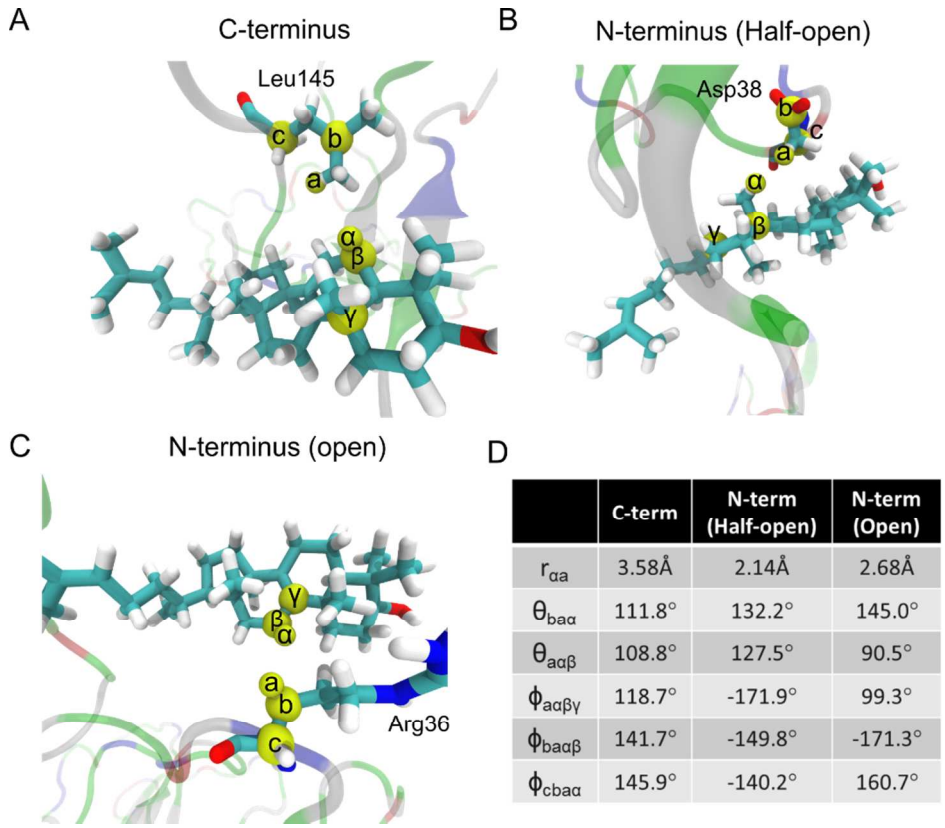
Unbiased MD simulations with extended simulation time for chosen binding poses

The stability of the four selected binding poses was tested by extended MD simulations. We chose two C-terminal binding poses labeled by 1a.1 and 3a.3 and another two N-terminal ones, 1a.3 and 2b.3. Here, 1A.1 means the trajectory number 1 for the conformation 1A in Fig. 1A, and so on. We extended the MD simulations for another 270ns. Since the simulation time for our previous runs was 30ns, the total simulation time for each binding pose was 300ns. Minimum distance from any atom in the aromatic rings of a lanosterol to the HγD crystallin was calculated as a guide for the stability of the binding mode (Fig. S1). The contacts in 1A.1 and 2B.3 look quite stable for the entire simulation times, whereas 1A.3 and 3A.3 display fluctuations during the extended simulation times, indicating the relative instability of the protein-ligand contacts.

Free Energy Perturbation (FEP) calculation details

The absolute binding free energy, ΔG, is estimated by the free energy perturbation method as described in the literature [1]. The singularity near the end of the calculation where excluded volume interaction is almost turned off is circumvented by introducing a soft-core potential [2-3]. In addition, to avoid numerical instability attributed to a wandering ligand, we restrained the relative position of lanosterol by harmonic forces acting on the distances, angles and dihedrals. The configurations of harmonic restraints used in our simulations are described in Figure S2.

Free energy perturbation was carried out by slowly switching on and off interactions between the ligand and protein. Our FEP calculation procedure comprised three phases. In phase 1, we gradually turn on harmonic restraints to fix the relative position of the ligands to the protein while other interactions remain constant. Then, in phase 2, electrostatic interaction is reduced to zero with unperturbed vdW interaction. In phase 3, vdW interaction is finally switched off. $\Delta G_{\mathrm{decouple}}$ for decoupling under harmonic restraints is calculated by summing up the change of energy at each step. The harmonic constraints were analytically lifted by adding $\Delta G_{\mathrm{unrest}}$ as described in [1]. Ligand desolvation free energy was also assessed by the same procedure without harmonic constraints. Concurrently, we obtained $\Delta G_{\mathrm{desolv}}$ for the ligand by FEP for ligand and water only system. Absolute binding affinity, ΔG, for ligand-protein binding is finally estimated by $\Delta G = -\Delta G_{\mathrm{decouple}} + \Delta G_{\mathrm{unrest}} + \Delta G_{\mathrm{desolv}}$. Binding free energy difference between lanosterol and cholesterol was calculated in similar way without constraints (phase 1).



	C-term	N-term (Half-open)	N-term (Open)
$r_{\alpha a}$	3.58Å	2.14Å	2.68Å
$\theta_{ba\alpha}$	111.8°	132.2°	145.0°
$\theta_{a\alpha\beta}$	108.8°	127.5°	90.5°
$\phi_{a\alpha\beta\gamma}$	118.7°	-171.9°	99.3°
$\phi_{ba\alpha\beta}$	141.7°	-149.8°	-171.3°
$\phi_{cba\alpha}$	145.9°	-140.2°	160.7°

Figure S2 Initial configurations of FEP calculations. Molecular geometry of lanosterol and proteins for C-terminus, N-terminus half-open and N-terminus open binding are illustrated in (A), (B) and (C), respectively. Harmonic constraints are imposed on one radial distance, two angles and three dihedrals. Detail fixed values for each degree of freedom are presented in (D) where r_{xy} stands for a distance between atom x and y, θ_{xyz} is an angle between vectors from atom y to x and from atom y to z, and ϕ_{xyzw} is a dihedral between vectors from y to x and from z to w. Each degree of freedom is constrained by harmonic force whose strength is 41.84 kJ/mol/nm .

During FEP calculation, temperature and pressure were set to 310K and 1atm using velocity scaling with a stochastic term and Parrinello-Rahman algorithm, respectively. Damping constants for the thermostat and the barostat, τ_t and τ_t, were 1ps^{-1} and 2ps^{-1}, respectively. We used a 2fs time step. The cut-off distance for electrostatic and vdW interactions was 12Å. Coulomb interaction was integrated with Particle Mesh Ewald (PME) scheme. All bonds with hydrogens are constrained by 4th order LINCS algorithm with a single iteration. Simulation time for each λ was 500ps and energy of the system was recorded at every 100 time steps. Total number of λ is 29 for lanosterol-protein binding affinity calculation and 33 for transformation of lanosterol to cholesterol. Free energy at each λ and its error were computed using Bennett Acceptance Ratio (BAR) method. The detailed FEP results are summarized in Table S1 and S2.

Table S1. Binding free energy values for absolute binding FEP simulations. Units of kcal/mol			
λ	C-term ΔG	Half-open ΔG	Open ΔG
1	0.32	0.14	0.40
2	0.66	0.32	0.53
3	1.15	0.54	0.57
4	0.87	0.53	0.45
5	0.67	0.48	0.37
6	1.87	0.89	1.05
7	1.38	0.30	0.94
8	0.53	0.25	0.59
9	0.47	0.36	0.83
10	0.33	0.27	0.76
$\Delta G_{\mathrm{restr}}$	8.26	4.07	6.48
11	1.80	1.77	1.90
12	1.10	1.05	1.12
13	0.67	0.58	0.60
14	0.34	0.21	0.22
ΔG_{coul}	3.91	3.62	3.84
15	1.45	1.61	1.52
16	1.42	1.58	1.54
17	2.79	3.08	2.99
18	2.67	2.97	2.85
19	2.39	2.80	2.63
20	1.91	2.47	1.99
21	1.24	1.98	1.00
22	0.30	0.68	0.01
23	0.07	0.29	-0.57
24	-0.40	-0.33	-1.58
25	-1.82	-2.05	-3.33
26	-3.02	-4.20	-4.54
27	-2.90	-3.51	-3.84
28	-1.96	-2.03	-2.47
29	-0.62	-0.64	-0.99
ΔG_{vdw}	3.51	4.69	-2.79
ΔG_{ann}	15.69	12.38	7.53
$\Delta G^{\mathrm{lan}}_{\mathrm{desolv}}$	-1.95	-1.95	-1.95
$\Delta G^{\mathrm{lan}}_{\mathrm{res-off}}$	-6.50	-7.27	-7.04
$\boldsymbol{\Delta G_{\mathrm{bind}}}$	**-10.31±0.81**	**-6.15±0.81**	**-1.59±0.58**

Table S2. Binding free energy values for transforming lanosterol to cholesterol FEP simulations. Units of kcal/mol			
λ	C-term	Half-open	Open
1	-0.02	-0.06	-0.03
2	-0.06	-0.14	-0.10
3	-0.06	-0.22	-0.19
4	-0.03	-0.16	-0.17
5	0.01	-0.12	-0.21
6	0.01	-0.53	-1.75
7	0.04	-0.28	-4.01
8	0.17	0.07	-0.92
9	0.37	0.22	-1.18
10	0.14	0.12	-0.53
11	-0.02	-0.16	-0.25
12	-0.03	-0.24	-0.06
13	0.19	-0.22	0.16
14	0.39	0.10	0.46
$\Delta\Delta G_{\mathrm{bond}}$	1.10	-1.62	-8.79
15	-0.09	-0.14	-0.12
16	-0.01	-0.09	-0.04
17	0.05	-0.08	0.02
18	0.06	-0.07	0.06
$\Delta\Delta G_{\mathrm{coul}}$	-0.22	-0.38	-0.08
19	0.02	-0.01	0.12
20	0.01	0.03	0.17
21	0.12	0.03	0.47
22	0.29	-0.04	0.52
23	0.40	0.00	0.58
24	0.37	0.01	0.53
25	0.16	0.01	0.50
26	0.17	0.03	0.28
27	0.20	0.03	0.31
28	0.16	0.02	0.32
29	-0.02	0.04	0.33
30	-0.12	0.03	0.35
31	-0.05	0.01	0.35
32	0.01	0.02	0.32
33	0.02	0.05	0.24
$\Delta\Delta G_{\mathrm{vdw}}$	1.72	0.26	5.26
$\boldsymbol{\Delta\Delta G_{\mathrm{bind}}}$	**2.61**	**-3.60**	**-1.74**
$\boldsymbol{\Delta G_{\mathrm{bind}}}$	**-7.70±0.57**	**-9.75±0.81**	**-3.33±0.62**

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2. Zacharias, M.; Straatsma, T. P.; McCammon, J. A., Separation-shifted scaling, a new scaling method for Lennard-Jones interactions in thermodynamic integration. *J. Chem. Phys.* **1994,** *100*, 9025-9031.
3. Beutler, T. C.; Mark, A. E.; van Schaik, R. C.; Gerber, P. R.; van Gunsteren, W. F., Avoiding singularities and numerical instabilities in free energy calculations based on molecular simulations. *Chem. Phys. Lett.* **1994,** *222*, 529-539.