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Supplementary information for: In-silico design of a lipid-like compound targeting KRAS4B-G12D through non-covalent bonds†

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Supplementary Notes

LIG1 is a chiral compound. In this work we have explored whether its isomer would exhibit any difference in biological activities, such as their influences on the dynamic of KRAS4B-G12D. We have run two independent MD simulation trajectories with a total simulation time of 2 μs for each isomer-related system, see Table 1.

In Fig. S1 A, B present the chemical structures of LIG1 and its isomer. We have conducted MD simulations with total length of 2 μs for each guanosine-bound KRAS4B-G12D. All simulations were started with the same initial configurations for KRAS4B as sys3 and sys4 (Fig. S1 C). From Fig. S1 D, the value of RMSD values for the backbone stays around 2 Å, indicating the well-equilibrated structure for the G12D mutant, and the probability of the corresponding orientation of KRAS4B-G12D is shown in Fig. S1 E. With isomer binding into SII, GTP-bound mutant stays mainly in its active and intermediate states. For GDP-bound KRAS4B, its orientation mainly focuses on the active orientation state compared to sys0 in which no ligand molecule is considered, exhibiting more oncogenic property. In Table 2 we report the binding free-energies between isomer and KRAS4B-G12D. By comparing with the binding affinities of LIG1 in Table 2 of the main text, we do not observe any significant difference of binding affinities between the GTP-bound KRAS4B-G12D while in its active orientation state with LIG1 (56.3 kcal/mol) or LIG1-isomer (58.3 kcal/mol), indicating that the chiral carbon atom has little influence on their binding energies for these two species with KRAS studied in this work. In conclusion, the isomer of LIG1 can not play the role as the KRAS4B-G12D inhibitor.

Recently reported warheads include α,β-unsaturated carbonyl moieties such as AMG510[1] and MRTX849[2], electrophilic warheads such as heteroaromatic motifs, sulfur(VI) motifs, terminal alkynes motifs, etc. see Fig. S2. Examples of chemical structures of drugs approved by US Food and Drug Administration using lipid-based formulations are listed in Fig. S3. Fig. S4 records the corresponding distance profiles of mass of center of interest for DBD and LIG1. In Fig. S4 B, we can observe that FAR binds into SII of KRAS4B in two independent trajectories of sys2 while for sys3 and sys4 FAR keeps anchoring into the PM during the whole simulation time, showing a great tendency of FAR serving as the binding hook of the newly designed drug reported here. And Fig. S4 D shows that LIG1 is able to bind with KRAS4B-G12D in both nucleotide states. In the same fashion, we have calculated the distance profiles of mass of center of isomer with backbone atoms of CD and SII along with the simulation time, shown in Fig. S5. We can observe that in both cases, the corresponding distances for GTP-bound KRAS4B-G12D are around 3 Å lower than those for GDP-bound systems, indicating much stronger interactions between isomer and SII, which holds the tendency for LIG1 in Fig. S4 D. Fig. S6 exhibits the two-dimensional dynamics cross-correlation map of the residues motions of switch regions, showing the influence of the presence of PM on the intramolecular correlation in motion. The initial configurations of KRAS4B-related systems are illustrated in Fig. S7.

Graphics and tables

Notes and references

1 T. Pantsar, *Scientific reports*, 2020, **10**, 11992.
2 J. Hallin, L. D. Engstrom, L. Hargis, A. Calinisan, R. Aranda, D. M. Briere, N. Sudhakar, V. Bowcut, B. R. Baer, J. A. Ballard *et al.*, *Cancer discovery*, 2020, **10**, 54–71.
3 G. J. Brighty, R. C. Botham, S. Li, L. Nelson, D. E. Mortenson, G. Li, C. Morisseau, H. Wang, B. D. Hammock, K. B. Sharpless

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† Electronic Supplementary Information (ESI) available. See DOI: 00.0000/00000000.

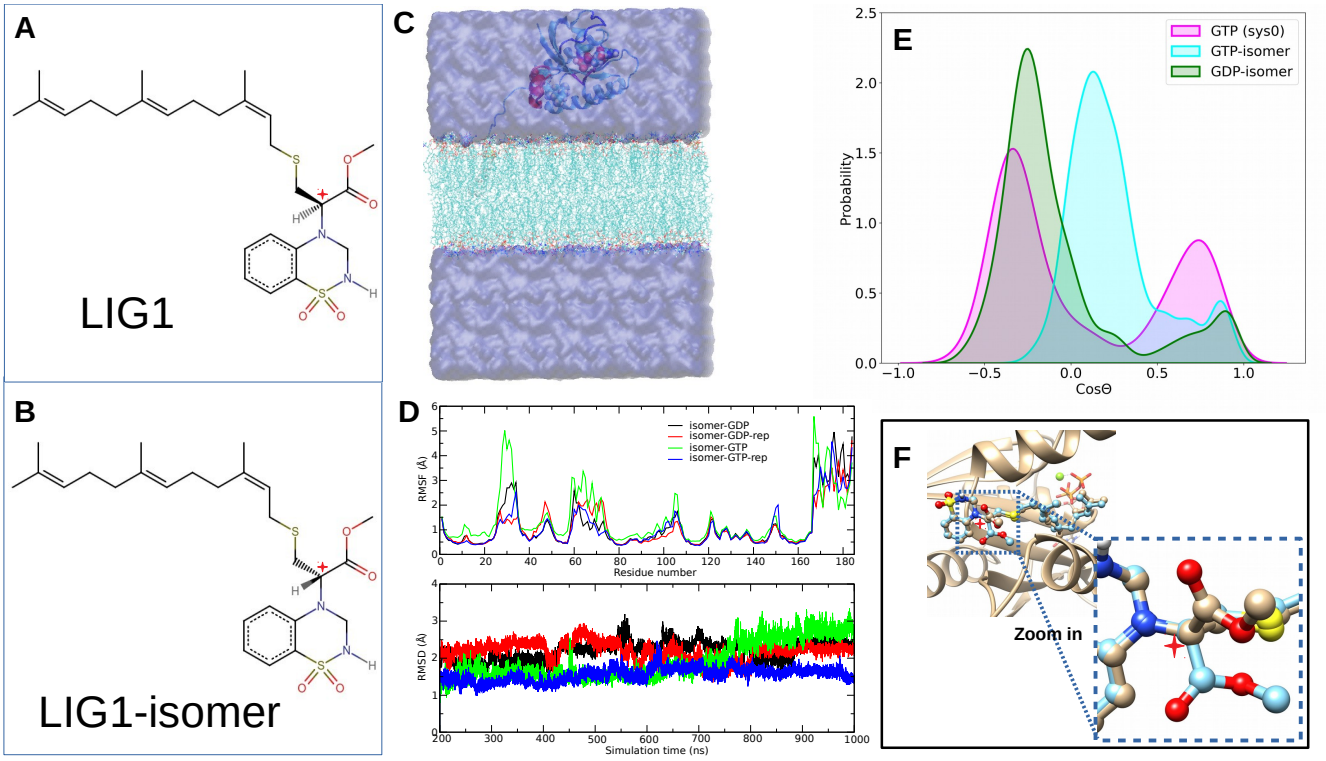

Fig. S1 A,B: Chemical structure of LIG1 and its isomer, the asymmetric carbon atom is indicated with a red star. C: A general initial configuration, water shown in blue and ions not shown here. D. RMSD and RMSF profiles of backbone atoms of KRAS4B-G12D. E: Distributions of the orientations of KRAS4B-G12D in the context of isomer inserting into the SII pocket during the whole simulation time. F. Comparison of LIG1 (ball and stick in tan colour) and isomer (ball and stick in colour blue) binding with GTP-bound KRAS4B in their initial configurations, interactions between SII pocket and the hydrophobic tail of LIG1/isomer were conserved.

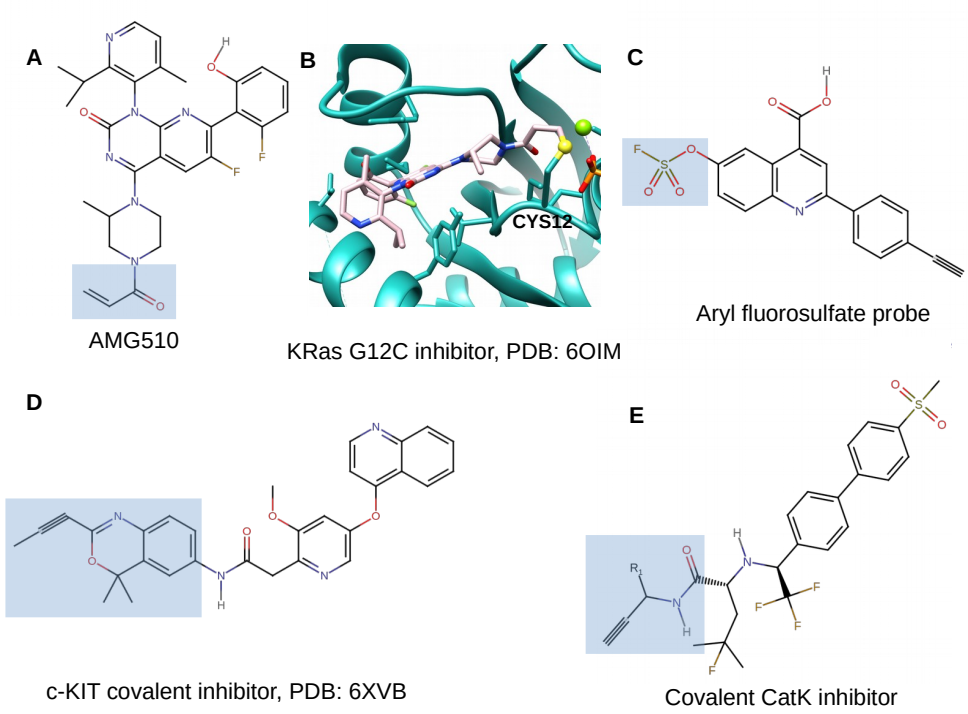

Fig. S2 Examples of different warheads marked inside blue boxes: A. α,β-unsaturated carbonyl warhead: Chemical structure of AMG510, B. X-ray crystal structure of AMG510 bound to CYS12 of KRAS-G12C, AMG510 shown in pink colour, C. Chemical structure of one sulfur(VI) warhead[3], D. Chemical structure of one heteroaromatic warhead[4], and E. Chemical structure of the terminal alkyne moiety-based CatK covalent inhibitor[5].

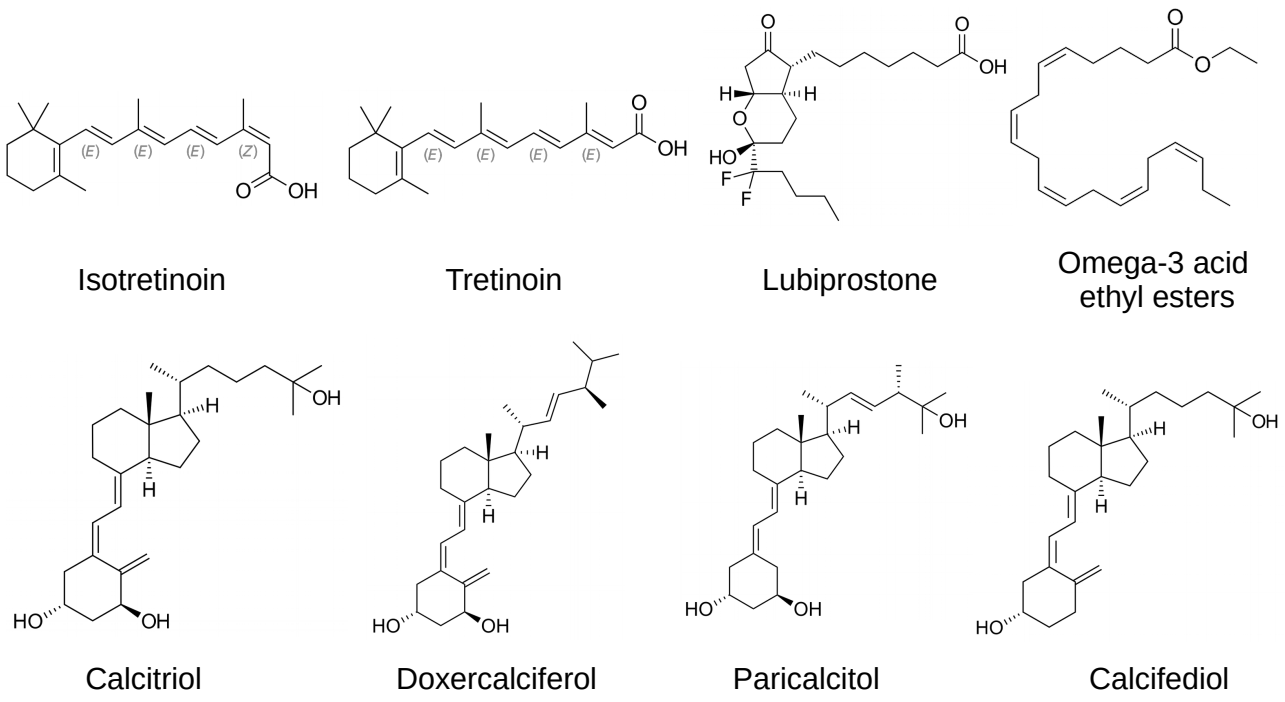

Fig. S3 Chemical structures of some FDA approved drugs using formulations containing lipid chains[6].

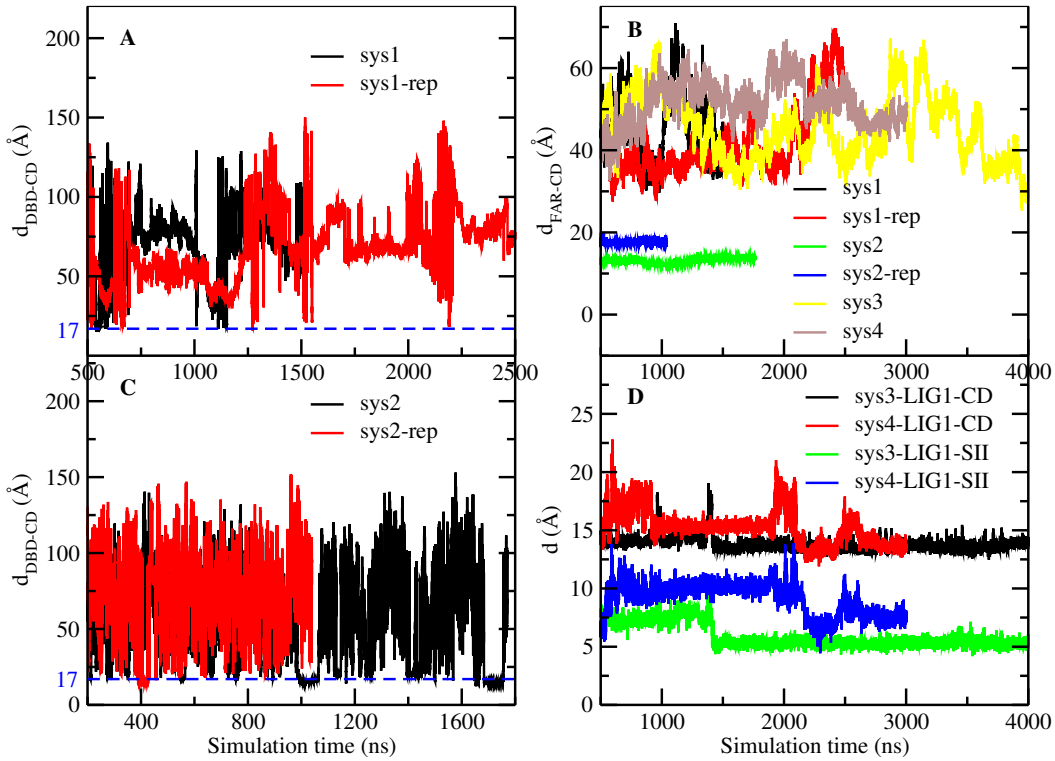

Fig. S4 Selected distance profiles of mass of center along with the simulation time.

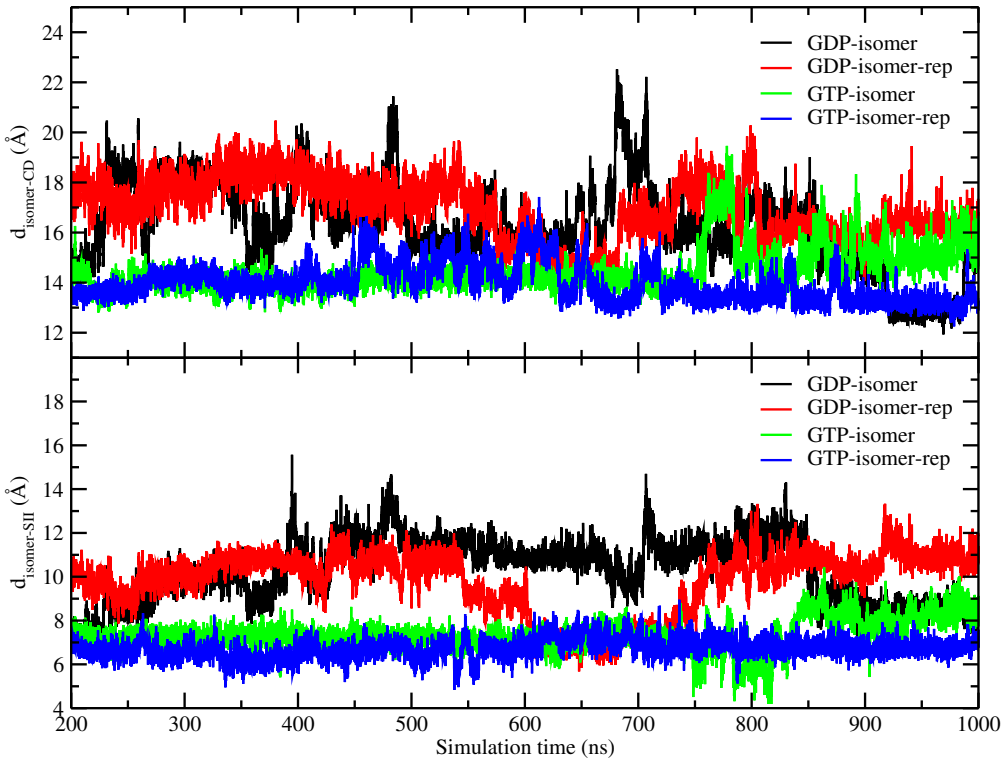

Fig. S5 Distance profiles of mass of center of isomer with backbone atoms of CD and SII along with the simulation time.

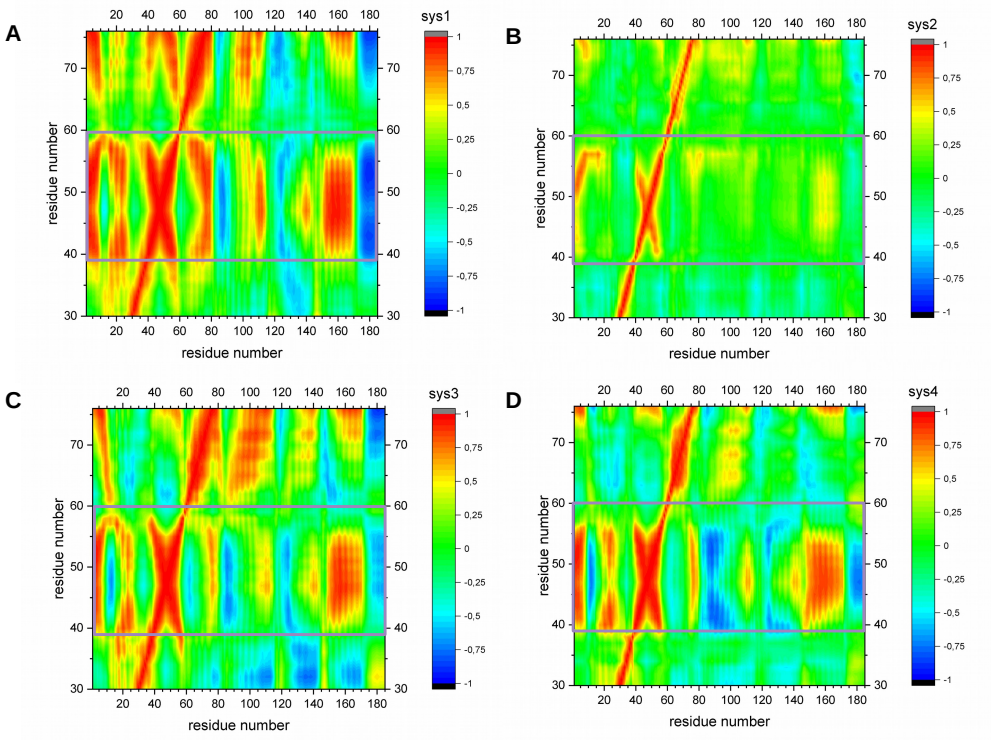

Fig. S6 Two-dimensional dynamics cross-correlation map of the residues motions of the switch regions for KRAS4B systems studied here.

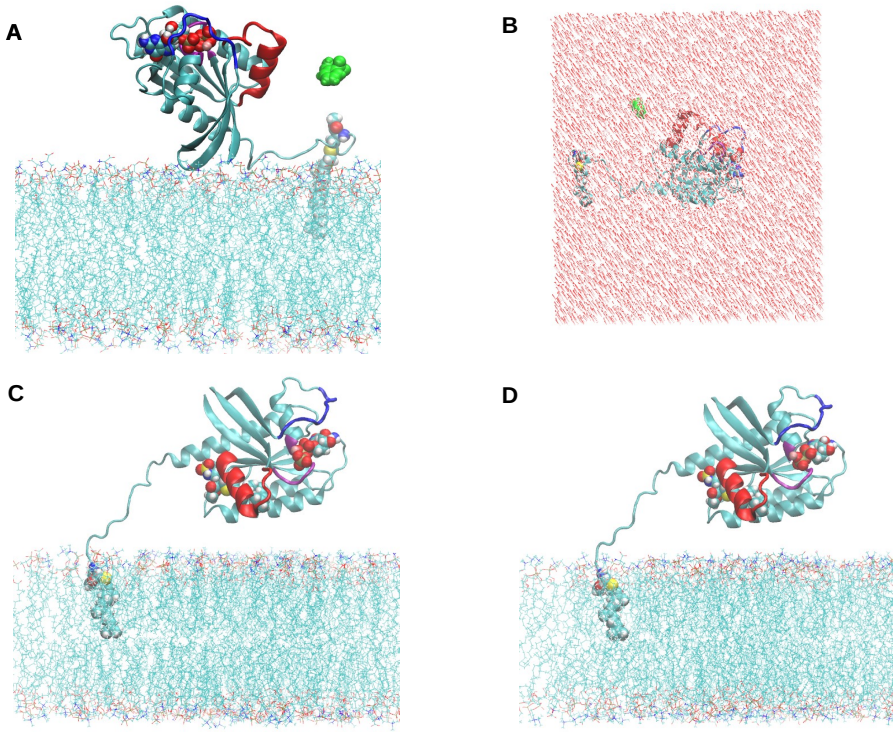

Fig. S7 Initial Configurations of KRAS4B systems studied in this work: A(sys1), B(sys2), C(sys3), and D(sys4). Water molecules and ions are not shown for systems in which the phospholipid bilayer is included for the sake of clarity. Herein, PM is shown in line, KRAS4B shown in NewCartoon, and DBD molecule (green), Mg ion (pink), GTP/GDP, and LIG1 are in depicted using VDW style. SI, SII, and P loop are shown in NewCartoon representation in colour blue, red, and purple, respectively.

Table 1 Detailed setups of extra simulation systems considered in Supporting Information.

Systems	ligand	Environment	No. of atoms	simulation time
isomer-GTP	isomer	PM	110820	1000 ns
isomer-GTP-rep	isomer	PM	110820	1000 ns
isomer-GDP	isomer	PM	110758	1000 ns
isomer-GDP-rep	isomer	PM	110758	1000 ns

Table 2 Binding affinities of isomer to SII of KRAS4B-G12D estimated by ABF calculations of the corresponding binding free-energy differences ΔG.

System	State (orientation on PM)	ΔG (kcal/mol)
isomer-GTP	active	58.3
isomer-GTP	intermediate	90.2
isomer-GDP	active	69.4

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