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Supplemental Information

Dynamics of Membrane-Bound G12V-KRAS from Simulations and Single-Molecule FRET in Native Nanodiscs

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1) **Reaction coordinates:** Figure S1 (top) shows that the distribution of the distance between Cα atoms of residues 132 and 183 (ζ) sampled during the 20 μs MD run could be fit to three Gaussians (blue, green and red). We used these fits to split the trajectory into three different pools of conformations: ζ < 24 Å, 24 ≤ ζ ≤ 43 and ζ > 43 Å, representing 31.3%, 44.4% and 24.3% of the 200,000 conformations that we have saved during the simulation. Another reaction coordinate, the angle between a vector from residue 5 to residue 9 Cα atoms and the membrane normal (Θ), was also able to similarly group the sample into three pools (Figure S1, bottom), with the two best-delineated groups being Θ > 95° and Θ = 90.

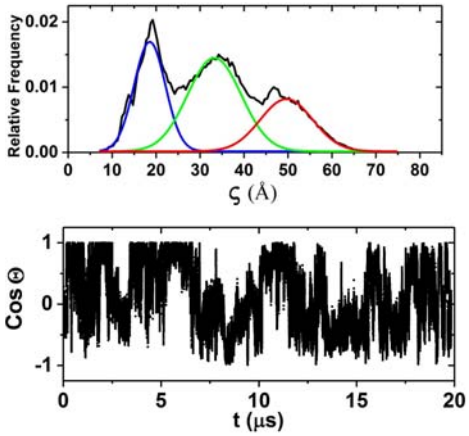

Figure S1: (**Top**) Normalized density distribution of ζ fitted to three Gaussian with R^2 = 0.98. Distributions centered at peak values of 18.56 ± 0.10 (blue), 33.27 ± 0.23 (green), and 49.67 ± 0.48 (red) correspond to OS_1, OS_0 and OS_2 orientation states (see main text). (**Bottom**) Time evolution of the cosine of the angle between a vector along β1 (residues 5-9) and the membrane normal (Θ).

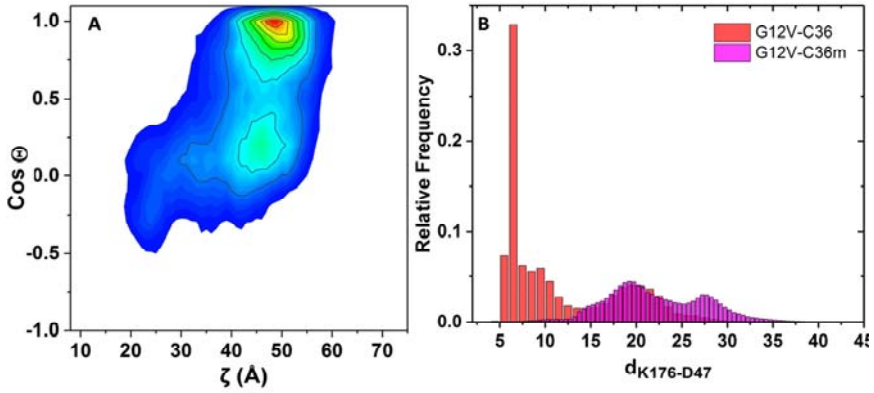

Figure S2: (A) Normalized number density distribution of conformations from the CHARMM36 simulation projected on to a plane defined by the reaction coordinates ζ and CosΘ. **(B)** Histogram of distance between the Cα atoms of an HVR residue K176 and D47 from the β2-β3 loop region of lobe1 from simulations of G12V-KRAS using the CHARMM36 (C36) and CHARMM36m (C36m) force fields.

2) **Flow chamber preparation:** Glass coverslips (22 × 22 mm No. 1) were plasma cleaned and aminosilanized using Vectabond (Vectabond in acetone 2% vol/vol; Vector Laboratories). A section of the coverslip was isolated using silicone templates (Grace bio-Labs) and treated with a PEG solution containing 5 kDa biotin-terminated PEG (2.5% w/w in molecular biology grade (MB) water, NOF Corp.), and 5 kDa mPEG succinimidyl carbonate (25% w/w in MB water, Laysan Bio Inc.) in 0.1M sodium bicarbonate (Sigma-Aldrich) overnight. The day of the experiment, the coverslips were treated with short chain 333 Da NHS-ester PEG (Thermo Scientific) and incubated for 2–3 h. The excess PEG was then washed off and coverslips were dried with nitrogen gas. A flow chamber was then constructed using custom hybriwell chambers (Grace bio-Labs) with dual silicon press-fit tubing connectors (Grace bio-Labs).

3) **Protein preparation and attachment to coverslips:** Streptavidin in PBS was injected into the flow chamber and incubated for 10 min. 10nM biotinylated rabbit Anti-6XHis antibody (Rockland Inc., cat. no. 600-406-382) in PBS was then flowed into the chamber and incubated for 20 min. Intact, solubilized KRAS protein was then attached to a glass slide for FRET data acquisition using in situ immunoprecipitation[1] by passing the solubilized protein through the chamber in three 60 μL injections and incubating for 20 min before flushing the chamber with a reactive oxygen scavenging solution ROXS (1 mM methyl viologen, 1 mM ascorbic acid, 0.01% w/w glucose oxidase, 0.001% w/v catalase, 3.3% w/w glucose; all from Sigma-Aldrich).

Supporting Reference

1. Jain, A.; Liu, R.; Ramani, B.; Arauz, E.; Ishitsuka, Y.; Ragunathan, K.; Park, J.; Chen, J.; Xiang, Y. K.; Ha, T., Probing cellular protein complexes using single-molecule pull-down. *Nature* **2011,** *473* (7348), 484-8