

Supporting Information

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SI Text

Simulation Setup

Each solvated system was prepared using the following steps: (1) a steepest descent energy minimization; (2) a density equilibration with a 500 ps dynamics at 300 K and constant pressure, coupling the systems to a Parrinello-Rahman barostat (1); (3) a 500-ps thermalization at constant volume and 300 K with Berendsen thermostat (2) and finally (4) another 2 ns dynamics at 300 K with the velocity-rescale thermostat (3) at constant volume.

A preliminary parallel tempering metadynamics (PT-metaD) with 5 replicas at increasing temperatures (295, 310, 325, 341, and 358 K) has been performed for each system biasing only the potential energy to increase its fluctuations and to reach a target exchange rate between neighbor replicas (30% in our case). This technique allows the sampling of the Well-Tempered Ensemble which has approximately the same average energy as the canonical ensemble but much larger fluctuations (4). Once this goal is reached the simulations are interrupted and the deposited energy bias is saved and used as a constant bias during the actual production runs. For the production runs the PT-metaD scheme is used with a sixth replica at $T = 300$ K with no energy bias to avoid any reweighting step during the analysis. Because the neighboring replicas ($T = 295$ K and $T = 310$ K) already exchange well thanks to the energy bias, a good exchange rate is expected for this “white” replica as well. The exchanges are attempted every 2 ps.

The set of contacts Γ defining the contact map distance for CV2 and CV3 are determined as follows: after equilibration, an energy minimization in vacuum is performed for both the active and inactive WT-EGFR catalytic domains. Then, among all of the pairs of CA, CB or backbone O atoms within 5 \AA in the active or in the inactive conformation, we keep only those pairs that specifically discriminate between the two structures, i.e., that appear either in one or the other discarding those pairs common to both. To this set of 293 regular contacts, we add 11 salt-bridge contacts involving residues of the A-loop and formed either in the active or in the inactive conformations weighting them 3 times more than a regular contact.

The distance between residues T790 and M766 in the inactive WT-EGFR is taken between the carbon C_ϵ of T790 and the sulfur atom of M766 and averaged over 10,000 structures uniformly taken in basin 1. Similarly, the distance between residues M790 and M766 in the active T790M mutant is taken between the sulfur atoms of the respective residues and averaged over 10,000 structures uniformly taken in the basin 1.

To be noted that in the clustering steps and in calculating the average distances we made the approximation that all of the structures in a basin are equiprobable picking them uniformly even though this is not strictly correct. We should have weighted them by their bias. Because the bias is similar in the minimum, we are confident the qualitative results are unaffected.

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3. Bussi G, Donadio D, Parrinello M (2007) Canonical sampling through velocity rescaling. *J Chem Phys* 126(1):014101.
4. Bonomi M, Parrinello M (2010) Enhanced sampling in the well-tempered ensemble. *Phys Rev Lett* 104(19):190601–190604.

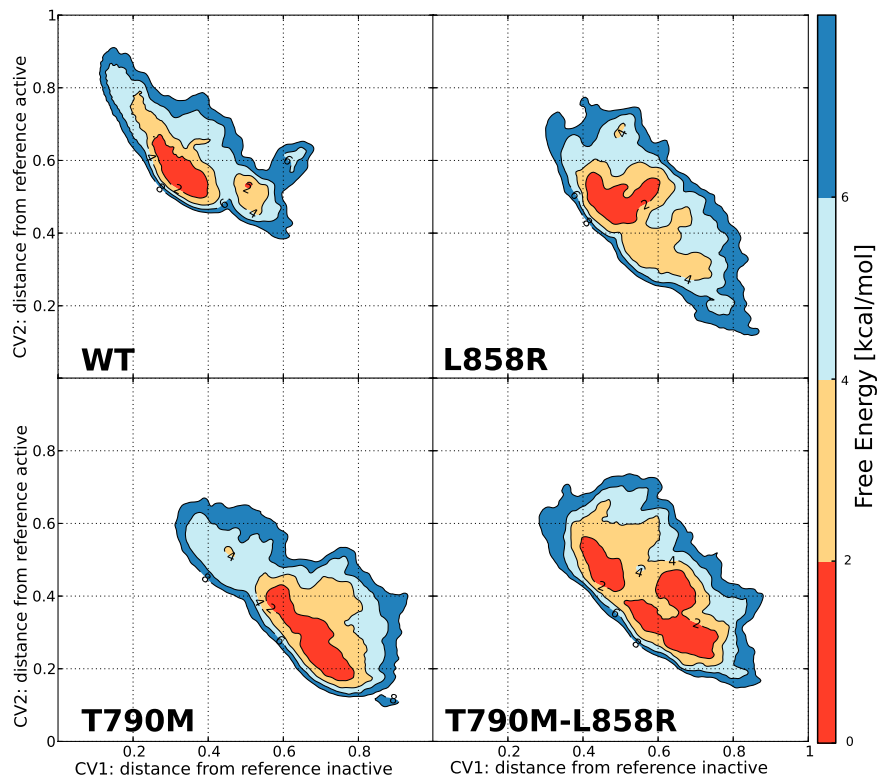


Fig. S1. Free energy surface of wild-type EGFR and the three mutants as a function of CV2 (x axis) and CV3 (y axis) in arbitrary units. The contour lines are drawn every 2 kcal/mol.

