Supporting Information for "Atomic-level mechanisms of abnormal activation in NRAS oncogenes from two dimensional free energy landscapes"

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1 The characteristics of systems and convergence of well-tempered metadynamics simulations

The five classes of NRAS proteins that have been considered in this work are GTP-bound wild-type NRAS, NRAS-Q61R, NRAS-Q61K, NRAS-Q61L and GDP-bound wild-type NRAS. The initial setups of these systems are the same as in a previous work[1]. The initial structure of NRAS-WT were obtained from Protein Data Bank (structures 6zio.pdb and 5uhv.pdb, respectively). The energy minimization and equilibration of the simulation systems have been reached under the same conditions as in a previous work[1]. Afterwards, five long 2D well-tempered metadynamics simulations of 3 μs each and two long 1D well-tempered metadynamics simulations of 1 μs each have been simulated in order to explore the free-energy surface of NRAS and its mutants.

Concerning the reliability of the simulations reported in the present work, we can use several criteria. Three of the most efficient and reliable are: (1) the time evolution of the fluctuations of the hills of the biased potential along the full trajectory. Usually the size of the hills of the Gaussian kernels deposited along the simulation is monitorized. As the simulation progresses and the added bias grows, the Gaussian height would be progressively reduced, eventually including low-height spikes.[2] (2) the time cumulative average of 1D free energy profiles as defined in main text (Section 3.4) and (3) considering the so-called block analysis of the average error along free-energy profiles as a function of the block length. This method consists on dividing the data (N values) in n chunks or blocks of equal size s ($n * s = N$). Then, the method will compute the mean value for each block, the mean of these block means, and the standard error for the latter (the mean of means).[3–5] Here, for convenience, we mainly report the time evolution of the fluctuations of the hills (Fig. 1 for 2D WTM simulations and Fig. 2 for 1D WTM simulations) and the block analysis of the average error along free-energy profiles as a function of the block length (Fig. 3 for 2D WTM simulations and Fig. 4 for 1D WTM simulations).

The evolution of the hills is presented in Fig. 1 and Fig. 2. We observe an overall decreasing behavior, eventually including low-height spikes. In all cases, the height of the biased potential decreased accordingly along the simulation runs. In all cases, a quasi-flat profile is already reached. This is a clear indication of the convergence of the WTM runs. Moreover, from the results of Fig. 3 and Fig. 4, where we report the size of the average error in the free-energy profiles calculated from different sets of well-tempered metadynamics simulations as a function of the block size. The full length of the metadynamics trajectories were taken into account. As expected, the errors increase with the block length until they reach a plateau in all cases, ensuring we fully reached the converge of all the metadynamics calculations. In order to obtain the errors for block sizes, scripts provided by the PLUMED project[4,5] have been employed.

2 The data of 2D free-energy landscapes minima, CVs (d, ϕ).

The detailed coordinates of minima and the corresponding free energy values are reported in Table 1 to Table 5. The representative full NRAS minima conformations are listed in Fig. 5. In order to directly compute the height of free-energy barriers, the absolute minima are set equal to zero.

Table 1 The data of 2D free-energy landscapes minima: GTP-bound wild-type NRAS

Basins	d [nm]	ϕ [rad]	free energy [kJ/mol]
A	0.7	2.1	0.0
B	0.7	1.7	1.4
C	0.9	2.1	1.6
D	0.7	0.5	2.4
E	0.5	1.8	2.7
F	0.9	0.5	3.0
G	1.2	1.7	6.3
H	1.7	0.7	11.6
I	0.5	0.7	13.6

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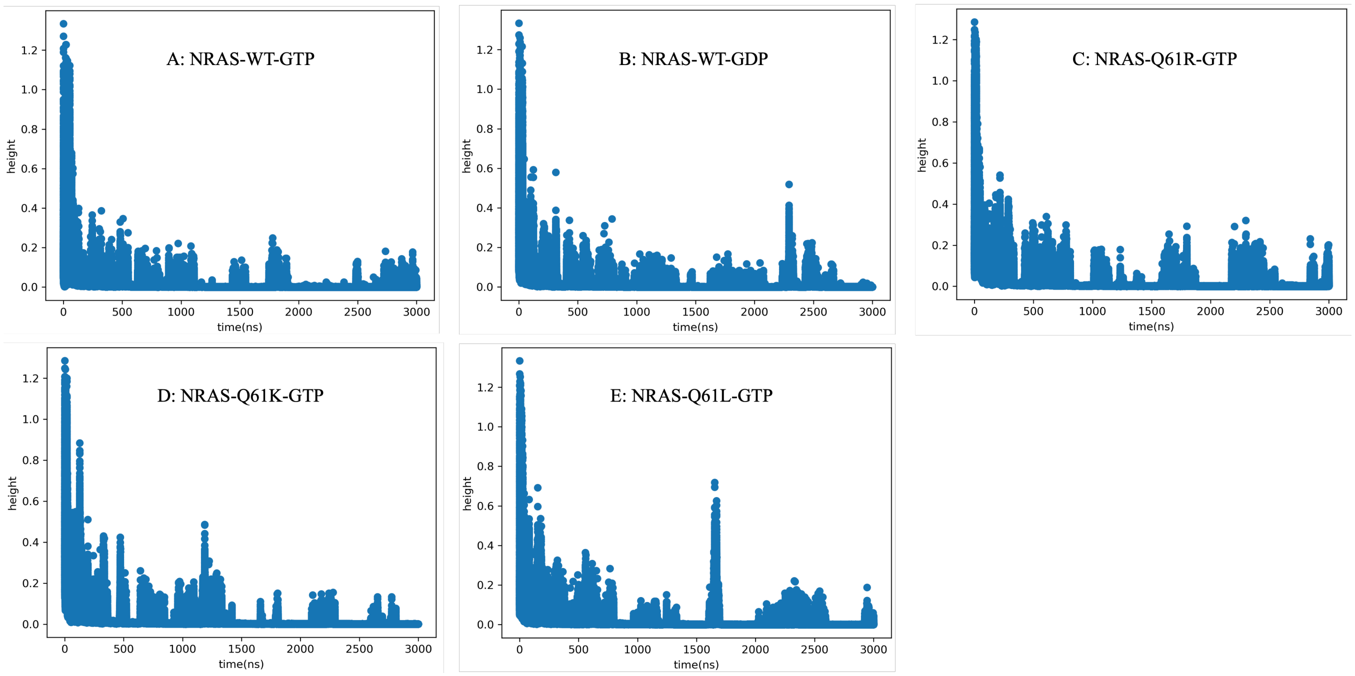

Fig. 1 Height of the hills of the Gaussian-biased potential as a function of time. All profiles correspond to the 2D WTM simulations (CVs d, ϕ) of each NRAS systems.

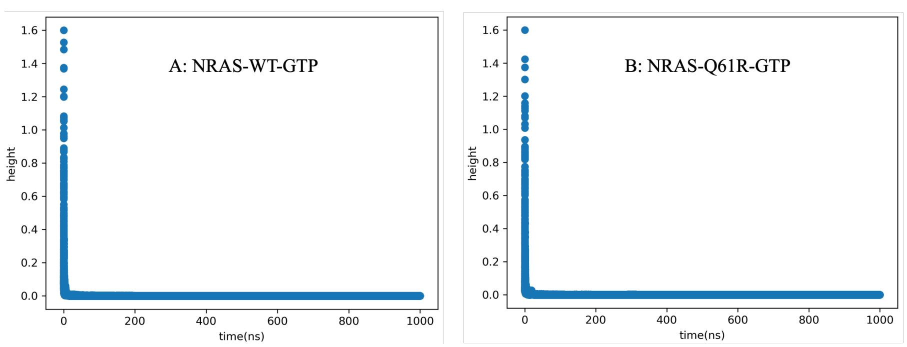

Fig. 2 Height of the hills of the Gaussian-biased potential as a function of time. All profiles correspond to the 1D WTM simulations (CV ψ) of each NRAS systems.

Table 2 The data of 2D free-energy landscapes minima: GDP-bound wild-type NRAS

Basins	d [nm]	ϕ [rad]	free energy [kJ/mol]
A	1.2	2.3	0.0
B	0.7	2.3	0.0
C	0.5	2.3	12.5
D	1.0	0.4	14.2
E	0.7	0.5	15.6
G	0.5	0.4	18.6

Table 3 The data of 2D free-energy landscapes minima: GTP-bound NRAS-Q61R

Basins	d [nm]	ϕ [rad]	free energy [kJ/mol]
A	0.4	1.1	0.0
B	0.4	0.4	2.7
C	1.4	0.5	3.1
D	0.4	1.6	5.4

Table 4 The data of 2D free-energy landscapes minima: GTP-bound NRAS-Q61K

Basins	d [nm]	ϕ [rad]	free energy [kJ/mol]
A	0.4	0.5	0.0
B	1.4	0.7	3.5
C	1.4	2.1	7.7
D	0.4	2.2	8.0
E	0.4	0.9	8.4
F	0.4	1.3	9.4
G	0.6	0.8	22.6

Table 5 The data of 2D free-energy landscapes minima: GTP-bound NRAS-Q61L

Basins	d [nm]	ϕ [rad]	free energy [kJ/mol]
A	0.3	0.8	0.0
B	1.5	1.1	9.1
C	1.0	1.1	12.9

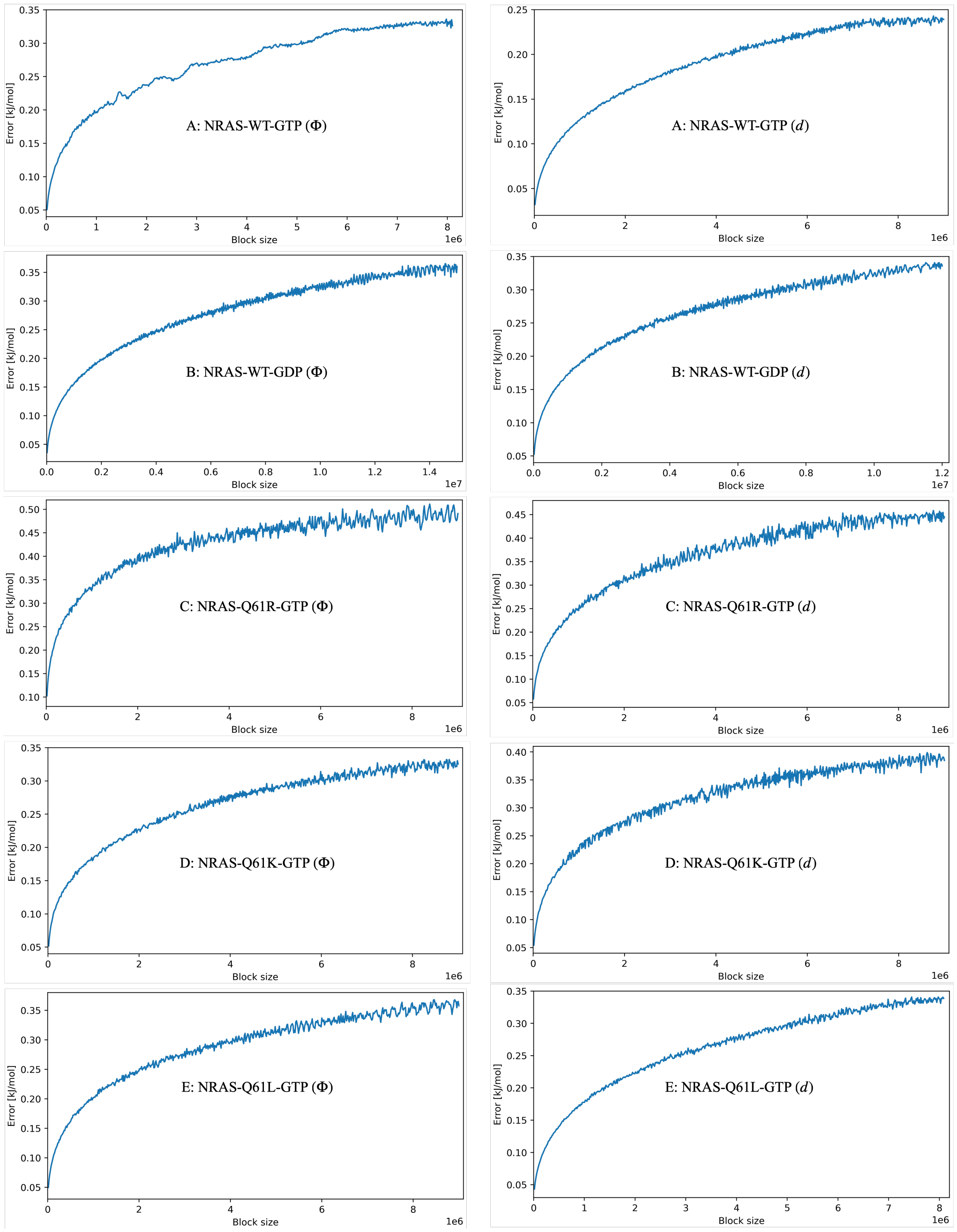

Fig. 3 Block analysis of the average errors along the free-energy profiles for CVs as a function of the block length. (A) NRAS-WT-GTP case angle ϕ: the block analysis with block sizes from 5000 to 8200000 (every 5000); distance d: the block analysis with block sizes from 5000 to 9000000 (every 5000). (B) NRAS-WT-GDP case angle ϕ: the block analysis with block sizes from 5000 to 15000000 (every 5000); distance d: the block analysis with block sizes from 5000 to 12000000 (every 5000). (C) NRAS-Q61R-GTP case angle ϕ: the block analysis with block sizes from 5000 to 9000000 (every 5000); distance d: the block analysis with block sizes from 5000 to 9000000 (every 5000). (D) NRAS-Q61K-GTP case angle ϕ: the block analysis with block sizes from 5000 to 9000000 (every 5000); distance d: the block analysis with block sizes from 5000 to 9000000 (every 5000) and (E) NRAS-Q61L-GTP case angle ϕ: the block analysis with block sizes from 5000 to 9000000 (every 5000); distance d: the block analysis with block sizes from 5000 to 8100000 (every 5000). For these analysis the entire metadynamics simulation (3.0 μs for each system) is taken into account.

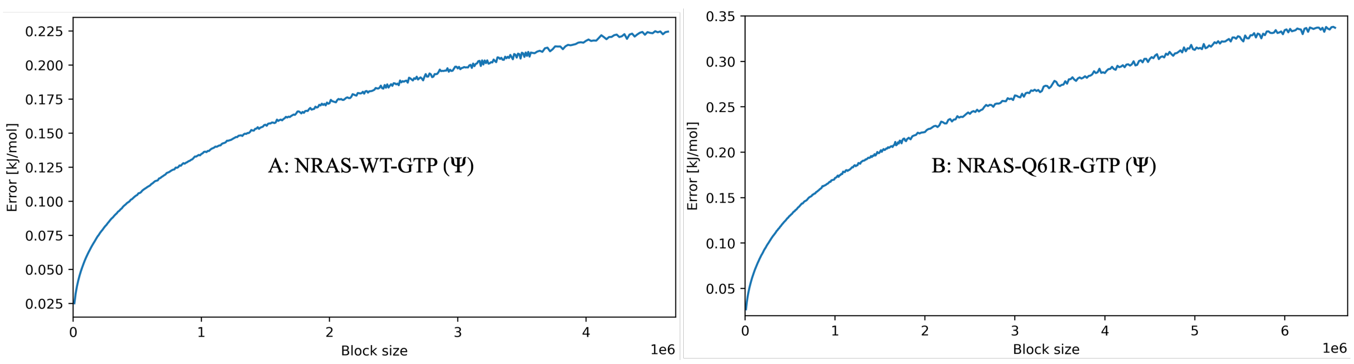

Fig. 4 Block analysis of the average errors along the free-energy profiles for CV ψ as a function of the block length. (A) NRAS-WT-GTP case torsion angle ϕ: the block analysis with block sizes from 5000 to 4640000 (every 5000) (B) NRAS-Q61R-GTP case angle ϕ: the block analysis with block sizes from 5000 to 6560000 (every 5000). The entire metadynamics simulation (1.0 μs for each system) is taken into account.

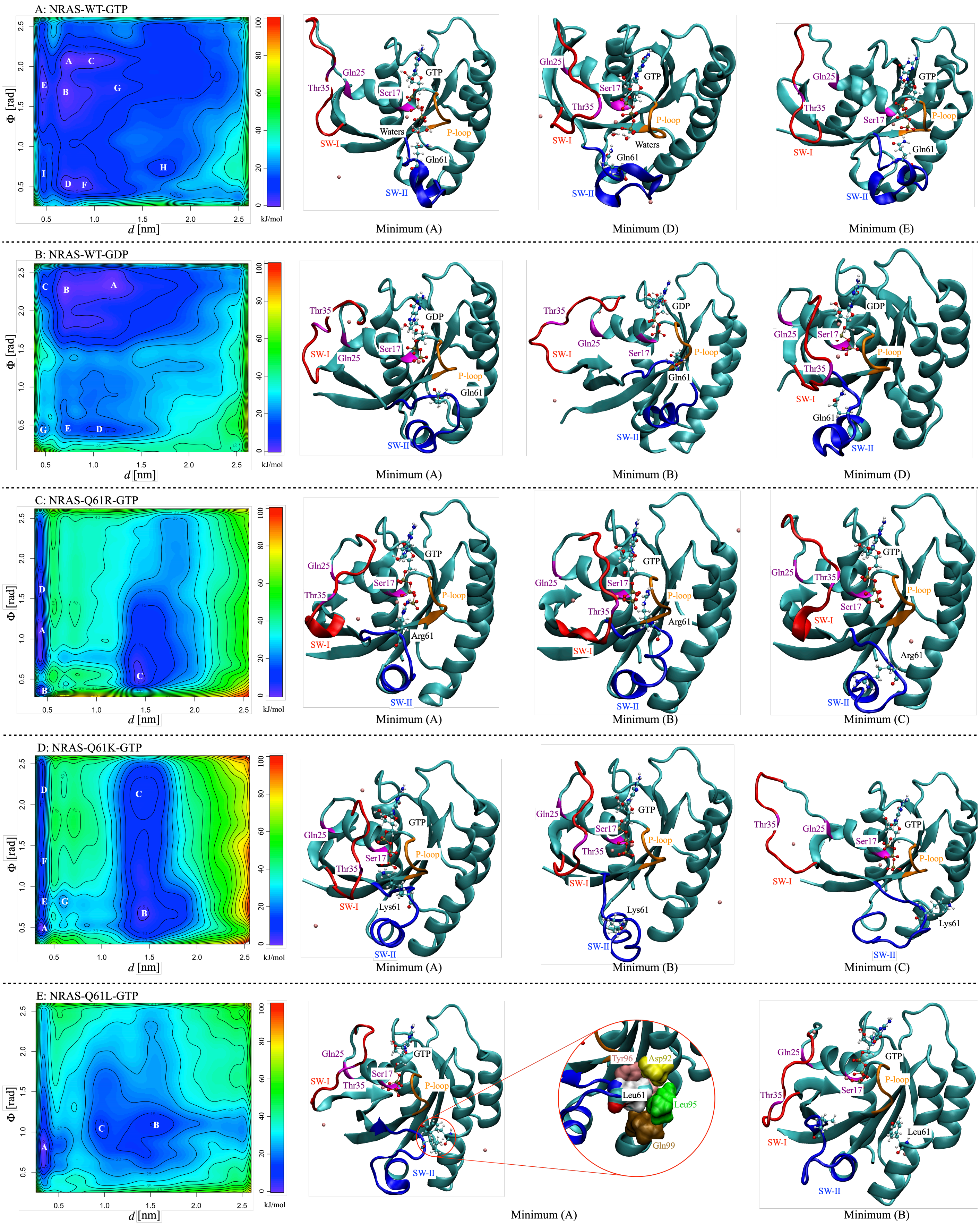

Fig. 5 The 2D free energy landscape F(d, ϕ) (kJ/mol) for the wild-type NRAS and its Q61 mutations. Basins "A", "B", "C", "D", "E", "F", "G", "H" and "I" are the stable states (minima) and are depicted in white. The conformations corresponding to the representative basins are listed on the right side of the 2D free energy landscape.

Notes and references

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