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Lessons from computer simulations of Ras proteins in solution and in membrane

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Abstract

Background—A great deal has been learned over the last several decades about the function of Ras proteins in solution and membrane environments. While much of this knowledge has been derived from a plethora of experimental techniques, computer simulations have also played a substantial role.

Scope of Review—Our goal here is to summarize the contribution of molecular simulations to our current understanding of normal and aberrant Ras function. We focus on lessons from molecular dynamics simulations in aqueous and membrane environments.

Major Conclusions—The central message is that a close interaction between theory and simulation on the one hand and cell-biological, spectroscopic and other experimental approaches on the other has played, and will likely continue to play, a vital role in Ras research.

General Significance—Atomistic insights emerging from detailed simulations of Ras in solution and in bilayers may be the key to unlock the secret that to date prevented development of selective anti-Ras inhibitors for cancer therapy.

Keywords

Molecular dynamics; advanced simulations; protein motion; membrane binding; clustering; oncogenic Ras

Introduction

Ras (Rat Sarcoma) protein was discovered more than four decades ago as the first oncogene product [1, 2]. Subsequent discoveries of many other related genes gave rise to the Ras family of proteins, a group of lipid-modified and membrane-associated intracellular switches that regulate cell growth, proliferation and differentiation [3, 4]. The switching function of Ras involves cycling between a GDP-bound `off' and GTP-bound `on' conformational states [5–8]. However, this binary on/off picture is being challenged by the discovery of other (intermediate) conformational states in recent years [9–14]. Efficient cycling between the on/off states of Ras requires GDP release and GTP hydrolysis facilitated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) [7, 15], respectively. For instance, GAP increases the very slow intrinsic ability of Ras to hydrolyze

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GTP (k_{cat} = 0.028 min^{-1} [16]) by about 10^5-fold [17]. Therefore, interaction with GAPs is crucial for turning off signal transmission. Somatic or germline Ras mutations that interfere with its intrinsic and/or GAP-assisted ability to hydrolyze GTP can result in uncontrolled cell growth or cancer [18]. In fact, Ras is found mutated in about 15% of all human tumors and in up to 90% of cases in specific tumor types [19], as well as in a number of developmental disorders [20, 21]. Therefore, a staggering number of biochemical (e.g., [22–24]), structural [25–34], spectroscopic [14, 35–38] and theoretical [39–56] studies have been devoted to investigating the mechanistic aspects of the Ras GTPase function.

Our goal here is to summarize the contribution of molecular simulations to our current understanding of normal and aberrant Ras function. We focus on lessons from molecular mechanical simulations in aqueous and membrane environments. Though *ab initio* simulations with various flavors of quantum mechanics continue to play a central role in studying the catalytic process within the active site of Ras [57–70], they are beyond the scope of the current review. The review is organized as follows. As a background, we first provide a general overview of the accumulated knowledge on Ras. We then turn to simulations of the soluble catalytic domain in aqueous media, followed by the isolated lipid anchor and the full-length protein in lipid bilayers. Given the large number of reports in the field, we could not cite them all and apologize to those authors whose work was left out due to space limitation.

Overview of Ras biology, biochemistry and structure

There are three major Ras isoforms in humans: N-, H- and K-Ras. These isoforms share a nearly identical water-soluble catalytic domain [7, 71] comprising the N-terminal residues 1–166. The catalytic domain can be subdivided into two lobes [42]. Lobel (residues 1–86) is strictly conserved across the Ras family and contains the functionally critical switch regions (switch 1: residues 25–40 and switch 2: residues 57–75), as well as the phosphate binding P-loop (residues 10–17) (Fig 1, also see Fig 2). Lobe2 (residues 87–166) is also structurally conserved among the Ras isoforms despite sequence variations at several positions (Fig 1), and is attached to the C-terminal hypervariable region (HVR). The HVR contains the linker segment (residues 167–179) followed by a lipid-modified membrane-binding motif (or the lipid anchor, residues 180–185/6). The HVR is believed to be responsible for the distinct sub-cellular and plasma membrane distribution and therefore diverse biological activity of Ras isoforms [39, 72–78].

To date, over fifty-five H- and eleven K-Ras crystal structures have been deposited in the Protein Data Bank (PDB). Most of these structures were solved in the presence of GTP analogues or GDP and typically encompass residues of the catalytic domain only, with the exception of the recently reported ligand-bound K-Ras structures [79] that include the linker. Since the isolated catalytic domain of different Ras isoforms exhibits similar *in vitro* biochemical activity, much of the earlier structural work has focused only on H-Ras [27, 28, 31, 80–83]. Even with the recent addition of K-Ras structures, however, the non-redundant [84–87] biological activity of Ras proteins could not be fully explained in structural terms. Recent reports on the mechanism by which the HVR might modulate function has begun to shed light on this issue [72, 76–78].

Membrane binding is required for the biological function of Ras, and there is evidence that signaling specificity among Ras isoforms may be due to their distinct membrane localization and therefore differential accessibility to effectors and modulators [88–91]. A combined analysis of data from molecular dynamics (MD) simulations. Fluorescence Resonance Energy Transfer (FRET), Fluorescent Lifetime Imaging (FLIM) and functional assays has shown that GTP-H-Ras undergoes a global conformational change so that the catalytic

domain directly contacts the membrane surface [43, 92, 93]. Moreover, a fraction of membrane-bound Ras proteins form dynamic clusters that are cholesterol-dependent (GDP-H-Ras and GTP-N-Ras) or -independent (GTP-H-Ras, GDP-N-Ras and K-Ras); i.e., different Ras isoforms form distinct, non-overlapping nanoclusters in an activation state-dependent manner [73, 94–96]. This differential membrane-organization explains, at least in part, the functional diversity among Ras proteins [93, 97]. However, our understanding of the molecular/atomic interactions underlying membrane binding and clustering of Ras is limited. To fill some of this gap, molecular simulations of various resolutions have been used to probe the dynamics, allosteric character and role of each structural component of Ras for isoform-specific membrane binding and assembly.

Classical MD simulations of Ras in solution

The earliest unbiased MD simulations of Ras [98–102] were very short (typically 100–500 ps) by current standards but they were able to elucidate the flexible nature of the nucleotide-binding loops (for illustration, see Fig 2 from more recent simulations). These initial studies thus helped explain why the conformation of these loops, if observed at all, differ between X-ray structures of Ras solved with GDP and GTP analogues [25, 27, 82, 83]. Another slightly longer (1 ns) simulation suggested that the active site of the oncogenic G12V mutant of H-Ras is more dynamic than the wild type [103]. Displacement of the catalytic water molecule during the simulation of another oncogenic mutant (G12D) was suggested to be one of the reasons that this variant is unable to hydrolyze GTP [104]. Comparison of multiple 10–20ns long MD trajectories on homology models of the N- and K-Ras catalytic domain with that of H-Ras suggested that the three isoforms, as well as mutant and wild type H-Ras, may differ in dynamics [42]. For instance, the amplitude of fluctuations in the atomic position of residues at the canonical switch loops, helix3 and loop7 of K-Ras were found to be larger and more similar to A59G H-Ras than wild type H-Ras [105]. Interestingly, crystallographic studies have also suggested that the flexible loop7 is part of an allosteric site [33].

Based on principal component analysis of all the crystal structures available at the time, Gorfe *et al* [42] has shown that the structure of G12V-H-Ras lies outside (and roughly in-between) two major groupings that are populated by structures of GDP- and GTP (or analogue)-bound Ras. X-ray structures of other Ras variants harboring mutation at the switch loops were also found to be intermediate between the canonical active and inactive states [9, 13], consistent with other observations by spectroscopic methods [12, 14, 38]. It is tempting to speculate that the energy barrier between the active and inactive states of some oncogenic variants of Ras may be lower than that in the wild type.

The simulations in ref. [42] also predicted an isoform-specific communication pathway connecting the nucleotide-binding lobe1 with the C-terminal lobe2. Subsequent simulations by another group found similar pathways and showed that these inter-lobe communications vanish in the absence of a nucleotide [53–55]. The same group also showed that transition between the inactive and active states (or vice versa) is facilitated by the higher flexibility of an intermediate state, and that this phenomenon is conserved in the entire Ras superfamily [54]. An evolutionarily conserved intrinsic flexibility of lobe1, and the nucleotide-binding site in particular, was suggested to be important for function conservation. In contrast, inter-lobe communication (i.e. lobe1-lobe2 crosstalk) was associated with functional specialization in members of a GTPase family [54]. In general, inter-lobe dynamics is a function of the bound nucleotide, with the inactive GDP-Ras being more flexible than the active GTP-Ras. This suggests that enhanced flexibility may facilitate GEF-binding and/or transition between the active and inactive states or *vice versa* [55].

In addition to backbone conformational changes in switches 1 and 2, transition from the active to the inactive state of Ras also involves loss of interaction between the backbone amide nitrogen of Gly60 and the y-phosphate of GTP, as well as the side chain hydroxyl of Thr35 and the Mg^{2+} ion [106–108]. GTP-bound Ras itself can also adopt two distinct forms: state1 and state2 [12, 106–110]. State1 differs from state2 primarily by the lack of coordination between Mg^{2+} and Thr35 [106–108]. Side chains of Tyr32 and Tyr64 also adopt a somewhat intermediate orientation in state1 GTP-Ras relative to their orientation in GDP-Ras and state2 GTP-Ras. Due likely to these reorganizations and the flexibility of the effector-binding loop, state1 Ras has weak affinity for effectors [12]. In fact, state1 may be an intermediate between the inactive GDP-bound and the active GTP-bound state2 [106].

Tightly bound water molecules appear to play an important role in the relative stability of the active, inactive and intermediate states of Ras. This was the conclusion of a recent study based on a series of simulations on Q61H K-Ras, with and without selected structural water molecules [52]. Analysis of the trajectories using two previously uncharacterized reaction coordinates (the distance between the C_a atoms of Gly60 and Gly10 and the N-C_a-C-O dihedral of Gly60 (see Fig 2)) revealed that a few strategically placed water molecules act as allosteric ligands that stabilize the state2 conformation of GTP-bound K-Ras [52]. The absence of some of these water molecules, particularly a single water molecule that bridges a hydrogen bond network between residues at the two lobes, induces a shift toward an inactive conformation [52].

A number of simulations have also been carried out on effector- [111–113] and GAP-bound [114, 115] Ras. For example, it was suggested that GAP-induced structural reorganization within the active site of Ras enhances GTP hydrolysis [114], even though the simulations were too short (~1ns) to draw an unambiguous conclusion. Furthermore, a combined electron spin-echo envelope modulation (ESEEM) spectroscopy and a short (~50ps) MD simulation showed movement of Gly residues, particularly Gly13 and Gly60, towards Mg^{2+} in the presence of GAP [116]. Taken together, these results underscore the key role of dynamics in the GTPase function of Ras. Similarly, protein motion plays an important role in the interaction of Ras with effectors [113].

Enhanced and biased simulations of Ras in solution

The atomically detailed classical MD (cMD) simulations described in the previous section provided invaluable insights into the dynamics of wild type and mutant Ras. However, computational cost rarely allowed for running cMD simulations long enough to sample large timescale global motions. Such motions have been probed by enhanced or biased simulation approaches such as accelerated MD (aMD) [117] and targeted MD (tMD) [118, 119]. For instance, nucleotide-dependent spontaneous transition between the active and inactive states of wild-type Ras has been achieved by aMD [117], since aMD allows for crossing large energy-barriers inaccessible to cMD [120]. Analysis of the aMD trajectories enabled the identification of several intermediate conformations that differ from the canonical GDP- and GTP-bound structures [117]. Moreover, aMD simulations of nucleotide-free Ras sampled a variety of conformational states including the canonical active and inactive states [121]. Based on this and other observations, it was proposed that conformational selection and population shift, and therefore allostery, might play an important role in the GTPase function of Ras and related G-proteins [121]. Though further study is required to fully establish the relative role of conformational selection and induced-fit [122], the allosteric nature of Ras is now fairly well established [33, 34, 123–125].

More than a decade ago, two groups used tMD to probe the transition path between the active and inactive states of H-Ras [118, 119]. A number of important predictions, some of

which subsequently validated by experiments [9, 11], were made. These include the substantial reorientation of Tyr32, Arg68 and Tyr71 [119], and the involvement of the P-loop, the P2-P3 turn (or loop 3) and the helix3-loop7 region in state-to-state transitions [118]. These studies thus suggested that collective motions in the nucleotide-binding region could be felt by distal regions on lobe2, in agreement with subsequent findings by cMD and aMD [42, 117] as well as experiments [126, 127]. Most importantly, the tMD studies revealed that state-to-state transition involves multiple energy-barriers and therefore more than one transition state. Coarse-grain simulation approaches, such as Normal Modes Analysis (NMA), have also been used to study large-scale collective motions in Ras [128]. Among other things, NMA has shown that GTP-Ras is more rigid than GDP-Ras and that phosphate release is coupled with helix3 motion.

Atomistic simulations of Ras in membranes

In addition to characterizing the dynamics of the Ras catalytic domain in solution, cMD has played a central role in providing structural insights into bilayer-bound Ras [40, 41, 43–45, 47, 129, 130]. The simulation results were generally consistent with the available, albeit limited, experimental data from solid state NMR and other spectroscopic techniques [130–133]. One of the important observations from simulations of N- [45, 129, 130], H- [40, 41] and K-Ras [47] lipid anchors was that there is a delicate balance between hydrophobic and hydrophilic interactions that govern the positioning of the peptides on bilayer surfaces (Fig 3). In the case of the N-Ras lipid anchor, the backbone interacts with the lipid head group while the hydrophobic palmitoyl/farnesyl chains as well as side chains of Met182 and Leu184 interact with the hydrophobic interior of the bilayer [45, 129–133] (Fig 3A). This organization appears to be independent of the bilayer thickness [134]. Similarly, the polar residues of the H-Ras lipid anchor, Ser183 and Lys185, interact with the head group whereas the acyl chains of the lipid modified moieties and Met182 insert deep into the hydrophobic core of the bilayer [40, 41]. Note that the second palmitoyl in H-Ras is replaced by a Leu in N-Ras, so that the total number of the hydrophobic side chains in the two lipid anchors is the same. It is possible that this distribution of polar and apolar side chains ensures a parallel alignment of the backbone onto the membrane interface. Simulations of the K-Ras lipid anchor in a bilayer of zwitterionic POPC and negatively charged POPG lipids showed that the lysine residues immediately preceding the farnesylated cystein electrostatically interact with the POPG lipids while the farnesyl tail inserts into the hydrophobic core [47]. As a result, POPG lipids clustered around the peptides, as evidenced by analyzing time-dependent peptide-POPG hydrogen bonding and vdW interactions [47]. Insertion of the K-Ras lipid anchor brought about local changes in the bilayer structure, with the bilayer thickness near the peptides being shorter than in the rest of the bilayer or a peptide-free bilayer. A similar local bilayer perturbation was observed during MD simulations of N- and H-Ras lipid anchors in a DMPC bilayer [41, 45]. Unlike N- and H-Ras lipid anchors, however, the backbone of the K-Ras lipid anchor adopted a somewhat extended conformation with a pseudo-helical turn in the middle, and lies at an angle from the bilayer surface [47].

Potential of mean force (PMF) calculations for the transfer of the H-Ras lipid anchor from water to bilayer yielded a large free energy gain of up to –30 kcal/mol [40, 44]. As expected, most of the contribution to the free energy of transfer came from vdW interactions between the acyl tails of the lipid anchor and those of the bilayer lipids. In fact, insertion of about half of the acyl tails of the lipid anchor into the hydrophobic core of the bilayer appears to suffice for crossing the barrier at the lipid-water interface [40, 41]. However, the interaction of polar side chains and the backbone with the head group of the bilayer lipids also contributes to binding [41]. Decomposition of the insertion free energy into enthalpic and entropic contributions led to a surprising conclusion: an enthalpy-dominated hydrophobic

effect may underlie membrane binding of Ras proteins [135]. The hydrophobic effect is often associated with entropy though there are a few examples, discussed in the same paper, in which enthalpy plays a demonstrate role. Moreover, a similar insertion free energy was estimated from PMF calculations on monoand dually-palmitoylated H-Ras anchors [44], suggesting a non-additive contribution of the two palmitoyls for membrane binding. The implication of these and other PMF calculations on variants of the H-Ras lipid anchor, in which individual lipidations were systematically removed [44], was that palmitoylation at Cys184 may not be needed for affinity but rather for lateral segregation, as previously suggested [136].

Whilst the simulations on the isolated lipid anchors yielded useful insights into specific peptide-lipid atomic interactions, cMD simulations on full-length H-Ras in a DMPC bilayer indicated that the catalytic domain and the linker also play a role in membrane binding [43]. Membrane insertion and the overall organization of the anchor were similar among the isolated lipid anchor, the HVR and the full-length protein [43]. However, there were some structural differences. One of these was that the linker did not attain any specific conformation in the absence of the catalytic domain, but it adopted a coil-bend-coil geometry in the full-length protein [43]. Secondly, the linker interacts with the DMPC head group during simulations of GDP-H-Ras but it wrapped around the catalytic domain during GTP-H-Ras simulations. As a result, the backbone atoms of the lipid anchor were able to insert deeper in the former than in the latter [43]. Perhaps more significantly, the simulations of GDP-H-Ras and GTP-H-Ras in DMPC yielded two major modes of bilayer interaction (Fig 3). In one case, the catalytic domain directly interacts with and is roughly parallel to the bilayer surface. In the other case, the catalytic domain is oriented approximately perpendicular to the bilayer surface. In terms of population, GTP-bound H-Ras preferred the parallel orientation while perpendicular orientation was dominant in GDP-bound H-Ras (Fig 3B&C). Helices 4 and 5 are near the DMPC phosphates in the former whereas the N-terminal part of the linker (residues 167–172) and the β2-β3 turn make contact with the bilayer in the latter. Of special note may be the direct interaction of Arg128 and Arg135 with the phosphate head group in GTP-H-Ras; a similar interaction involving Arg169 and Lys170 exists in GDP-H-Ras (Fig 3C). These two pairs of positively charged residues thus seemed to be the key determinants of the nucleotide-dependent membrane binding of H-Ras [43]. This was found to be the case in subsequent FRET, FLIM/FRET and functional assays in cells expressing mutant Ras in which these pair of residues were separately mutated to Ala [92, 137, 138]. Recent spectroscopic studies of N-Ras in a synthetic lipid membrane [139] and the Ras-related Rheb in lipid nanodiscs [140] have observed similar nucleotide dependent membrane reorientation. Together, these results provided crucial insights into how the catalytic domain of Ras and related GTPases modulates function and how dynamics plays a critical role in the functional diversity of Ras isoforms. However, there is much more to learn about the (possibly many) different ways in which Ras proteins may interact with membrane lipids. A case in point is a recent report based on a combined cMD and spectroscopic analyses that proposed dimer formation by bilayer-bound N-Ras [141].

Coarse-grained simulations of Ras in membrane

Experiments in intact plasma membrane sheets [75] and synthetic bilayers [142, 143] have shown that Ras proteins assemble into dynamic clusters on membrane surfaces [72, 74, 75]. These nano-sized subdomains, or nanoclusters, are small (6–20nm radius) and contain about 7 proteins per cluster [72]. Different Ras isoforms form distinct and non-overlapping nanoclusters [94], with clusters of active H-Ras and K-Ras, as well as the isolated lipid anchor of K-Ras (tK), being localized at disordered membrane domains whereas clusters of inactive H-Ras and the lipid anchor of H-Ras (tH) segregate to raft-like membrane domains [142, 143]. That tK and tH form nancolusters with different domain preferences suggests the

key role of the lipid-modified moiety in the formation and localization of Ras nanoclusters. However, the physical driving forces underlying Ras clustering are difficult to access by current experimental techniques or atomistic simulations. This is because nanoclusters are too dynamic to be captured by high-resolution experimental techniques and their assembly/disassembly span large spatiotemporal scales that are hard to sample by atomistic simulations. One alternative is to use coarse-grained MD (CGMD), which has been proven useful to study lipid domain formation at near atomic resolution (e.g. [144]).

CGMD has been applied on tH embedded in a phase-separating lipid mixture of DPPC, DLiPC and cholesterol (CHOL) [48]. This mixture spontaneously forms CHOL/DPPC-enriched liquid ordered (L_o) and DLiPC-enriched liquid disordered (L_d) domains that capture some of the key features of raft and non-raft-like domains, respectively [144–146]. A key result from these simulations was that, for a tH/lipid ratio of about 0.01 or higher, ~40% of the tH molecules form dynamic clusters of size 4–11 molecules [48, 50], which agrees remarkably well with experimental results [75]. The clusters accumulated at the L_o/L_d domain boundary, and their stability was found to be a function of the extent of lipid phase separation and hence domain stability [48]. As a result, decreasing the simulation temperature or increasing the cholesterol content of the bilayer led to slightly larger but significantly more stable clusters [48, 50]. Simulations in which individual lipid-modifications were systematically removed demonstrated that the segregation of the clusters to the domain boundary is a consequence of the opposite preference of the palmitoyl and farnesyl tails for the L_o and L_d domains, respectively [48] (Fig 4). Clusters of tH variants with only farnesyl modification segregated to the L_d domain whereas those with only palmitoyl modification preferred the L_o domain. It was also noted that the organization of tH within clusters allows for maximum inter-tH and tH-lipid interactions [48, 50].

The effect of tH binding and clustering on the mechanical properties of the host bilayer was evaluated by computing line tension, lipid tilt and pressure profiles in the tH-bound and tH-free bilayers [48]. It was found that the line tension in the leaflet containing tH clusters was reduced relative to the corresponding monolayer of a tH-free bilayer [48]. Moreover, the asymmetric tH binding and interfacial localization caused a significant tilting of the boundary lipids and an asymmetric pressure profile, suggesting bilayer deformation.

The domain-preference of full-length H- and N-Ras proteins has also been studied by CGMD [147], in which GTP-H-Ras, GTP-N-Ras and depalmitoylated GTP-H-Ras were simulated in a bilayer made up of a similar lipid composition as that used in the tH simulations [48]. The conclusion was that GTP-H-Ras partitions into the L_o phase whereas GTP-N-Ras prefers the domain boundary. The latter is consistent with experiments [142] and simulations of tH variants containing a single palmitoyl and a farensyl [48]. It would be interesting to see if the domain preference of GDP-bound Ras would be different from that of GTP-Ras, as suggested by earlier experiments [148,149].

Perspective: Simulations can aid in anti-Ras inhibitor design

Decades of efforts by academia and industry have failed to yield selective Ras inhibitors. Complicating factors to directly targeting Ras include the conservation of the active site in a large number of small G-proteins and the high concentration and affinity of cellular GTP for Ras. Another reason could be lack of molecular-level insight into the protein-lipid interactions underlying the distinct spatiotemporal membrane-organization of different Ras proteins. The ultimate goal of the simulation approaches discussed in this review is to achieve a better understanding of Ras dynamics in solution and membrane environments, which is crucial for identifying and targeting novel pockets that transiently open during protein motion. In particular, conformers from MD trajectories can be used to find ligand

binding hotspots that have distinct features in ensembles of active, inactive and intermediate states of Ras. Along this line, analysis of representative structures from MD simulations and screening of drug libraries against these conformers identified four putative ligand binding sites: two on either side of helix-2, one behind the flexible effector-binding loop and another site distal from the nucleotide-binding region [150]. Some of the small molecule ligands predicted to be targeting these sites were found to inhibit Ras signaling in cells expressing oncogenic mutant Ras [150]. While the ability of these ligands to bind Ras at the predicted sites has yet to be confirmed, a number of NMR and X-ray structures of Ras bound to several different small molecules or molecular fragments were recently reported [79, 151–153]. In some of these studies, initial hits were derived from screening of ligand libraries against less populated Ras conformations [152, 153]. Moreover, a common feature of all of the reported ligand-bound Ras structures is that a significant backbone and side chain reorganization underpins ligand binding [79, 151–153]. The key question is whether the structural changes were induced by the ligands or if the ligands selectively target specific preexisting conformations with transiently open allosteric sites. The majority of the simulation results discussed in this review and a recent report from our laboratory [46] support the latter. We are of the opinion that simulations have more to offer in future efforts to anti-cancer drug discovery. One example would be utilizing MD-derived structural ensembles of membrane-bound Ras as an initial step toward the development of isoform-selective Ras inhibitors. The inhibitory potential of ligands that interfere with the proper localization of Ras to the plasma membrane has been demonstrated in several recent reports [154–156].

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Highlights

- Contribution of molecular simulations to the study of Ras GTPases
- Multi-scale molecular dynamics simulations of Ras in solution and in membrane
- Dynamics plays an important role in the biological activity of Ras proteins
- Implications of simulations and protein motion for anti-cancer Ras inhibitors

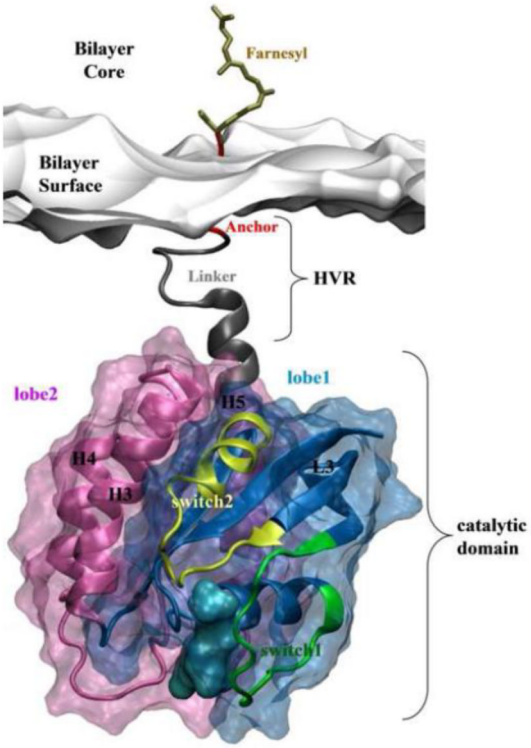

Figure 1.

Structure of Ras. Lobe1 (residues 1–86) and lobe2 (residues 87–166) of the catalytic domain as well as the surface of a model monolayer to which Ras is bound are shown in blue, pink and grey surfaces, respectively. Different regions of Ras are labeled and colored in green (switch1 residues 25–40), yellow (switch2 residues 57–75), grey (linker residues 167–179) and red (lipid anchor residues 180–185). The linker and lipid anchor together constitute the hypervariable region (HVR). The structure shown here is K-Ras, which is posttranslationally modified by a single farnesyl lipid (ochre). N-Ras and H-Ras share a nearly identical catalytic domain with K-Ras, but differ in sequence at the HVR and are further lipid-modified by one and two palmitoyls, respectively. Also, their lipid anchor is longer by 1 amino acid. The GTP nucleotide is shown in a cyan surface representation.

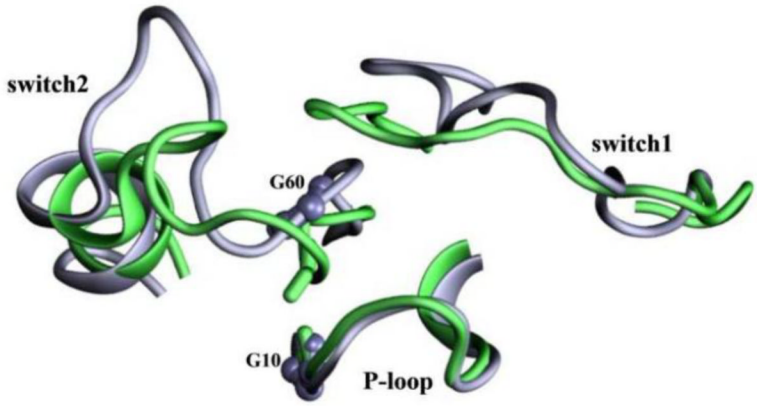

Figure 2.

An example of large conformational changes at the switch regions of K-Ras sampled during classical molecular dynamics simulations. Also highlighted are residues Gly10 and Gly60 used to define two reaction coordinates (the N-C_α-C-O dihedral of Gly60 and distance between Gly10-Gly60 C_α atoms) that helped classify Ras structures into active, inactive, intermediate and nucleotide-free [see ref. 52 for details].

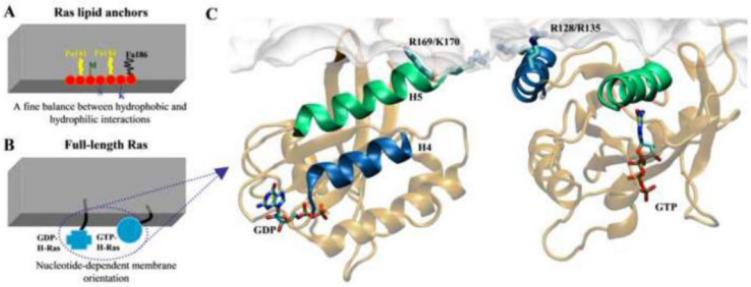

Figure 3.

Schematic summary of the main results from atomistic MD simulations of Ras lipid anchors (A) and the full-length H-Ras (B & C). Fig A highlights the different organization of the backbone and side chains in the H-Ras lipid anchor (which has two palmitoyl (Pa) and a farnesyl (Fa) modifications). The schematic in Fig B summarizes the two different membrane-orientations of the catalytic domain observed during the simulations. Fig C shows the molecular details of GDP- and GTP-bound H-ras in a DMPC bilayer, highlighting the close contact of residues Arg169 and Lys170 of the linker and Arg128 and Arg135 of helix-4 with the bilayer, respectively. Note the different orientation of helix-4 (dark blue) and helix-5 (green) with respect to the membrane plane. Selected side chains and the nucleotide are shown in licorice with nitrogen in blue, oxygen in red, carbon in cyan and phosphate in ochre. A hypothetical bilayer surface (A and B) and a monolayer from simulations (C) are shown in grey.

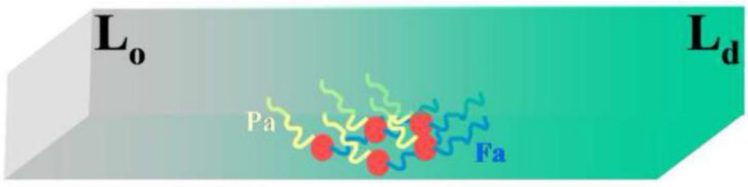

Figure 4.
Schematic summary of microsecond scale CGMD showing clustering and localization of H-Ras lipid anchors between lipid domains. A hypothetical two-domain bilayer is shown in a gradient color with the greyish region representing the liquid order (L_o) domain and the greenish region representing the liquid disordered (L_d) domain. The H-ras lipid anchor is shown in red circle with a single palmitoyl tail in yellow and farnesyl in blue.