Targeting GPCR-signaling via allosteric control of regulatory proteins

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Abstract

Regulators of G protein signaling (RGS) proteins modulate GPCR signaling by binding to Gα-subunits of heterotrimeric G proteins and accelerating hydrolysis of GTP. Therefore, RGS proteins are becoming increasingly important therapeutic targets to be directly or allosterically inhibited from binding to Gα-subunits. While structures of several known RGS proteins are highly similar and largely contain α-helical motifs, some *thiadiazolidinone* (TDZD) compounds that target cysteine residues have shown different levels of specificities and potencies for closely related proteins thereby suggesting intrinsic differences in dynamics of these proteins. In this work, we have studied the dynamics of three different RGS proteins (apo-RGS4, apo-RGS8, and apo-RGS19) using microsecond-scale classical molecular dynamics (MD) simulations with CHARMM and AMBER force-fields. Analyses of these trajectories reveal high fluctuations in α5 and α6 helices and the loops connecting them. These fluctuations lead to perturbations in residues in the RGS-Gα interface and in the vicinity of cysteines that are targets of allosteric inhibitors. These findings have significant implications for understanding differences in potencies and specificities of inhibitory small-molecules.

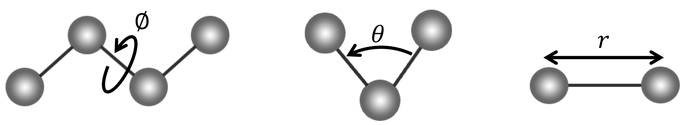
Methods and Software

NAMD (**NA**noscale **M**olecular **D**ynamics) is a parallel molecular dynamics code designed for high performance simulation of large systems of particles based on Newtonian equation of motion:

$$m_i\vec{\ddot{r}}_i = -\frac{\partial}{\partial \vec{r}} U_{total} \quad i = 1, 2, 3, \dots, N$$

$$U_{total} = U_{bond} + U_{angle} + U_{dihedral} + U_{vdw} + U_{coulomb}$$

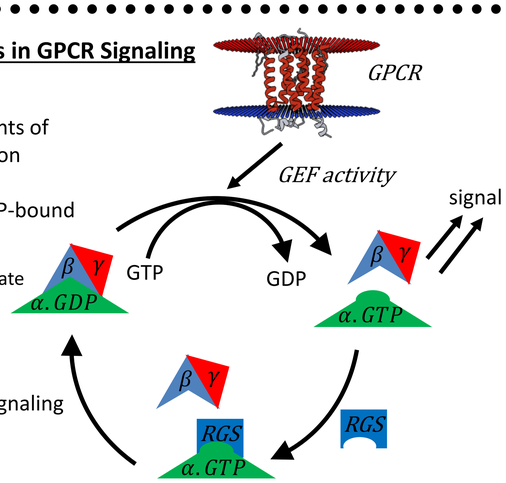
$$U_{Coulomb} = \frac{q_1 q_2}{4\pi\varepsilon_0 r_{12}} \qquad U_{vdw} = 4\varepsilon_{12}\left[\left(\frac{\sigma_{12}}{r_{12}}\right)^{12} - \left(\frac{\sigma_{12}}{r_{12}}\right)^{6}\right]$$



$$U_{dihedral} = k_d(1 + \cos(n\emptyset - \gamma)) \qquad U_{angle} = k_a(\theta - \theta_0)^2 \qquad U_{bond} = k_b(r - r_0)^2$$

VMD (**V**isual **M**olecular **D**ynamics) is a molecular visualization software for displaying and analyzing MD simulations.

Role of RGS Proteins in GPCR Signaling



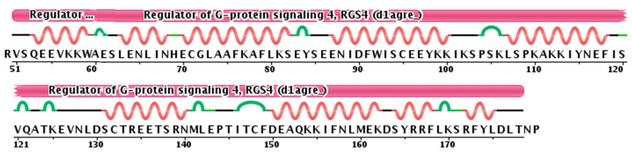
As being key components of GPCR signal transduction

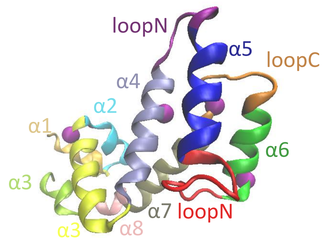
Bind directly to the GTP-bound Gα subunit

Markedly accelerate the rate of GTP hydrolysis

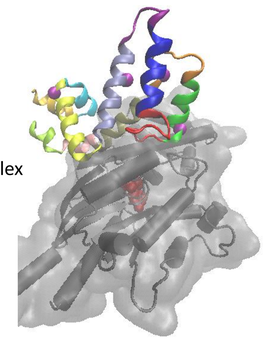
Accelerate the rate of inactivation of GPCR signaling

Structure and Sequence of RGS Protein





Cartoon representations of the box domain of RGS4 (1AGR)



RGS4-Gi.α1 complex

MD Simulations of RGS Proteins (RGS4, RGS8, RGS19)

Equilibration simulation parameters:

- ✓RGS immersed in water box (~70 Å x ~65 Å x ~65 Å)
- ✓Neutralized in saline water (0.05 mol/L KCl)
- ✓Periodic boundary conditions
- ✓ Number of atoms: ~29000 atoms

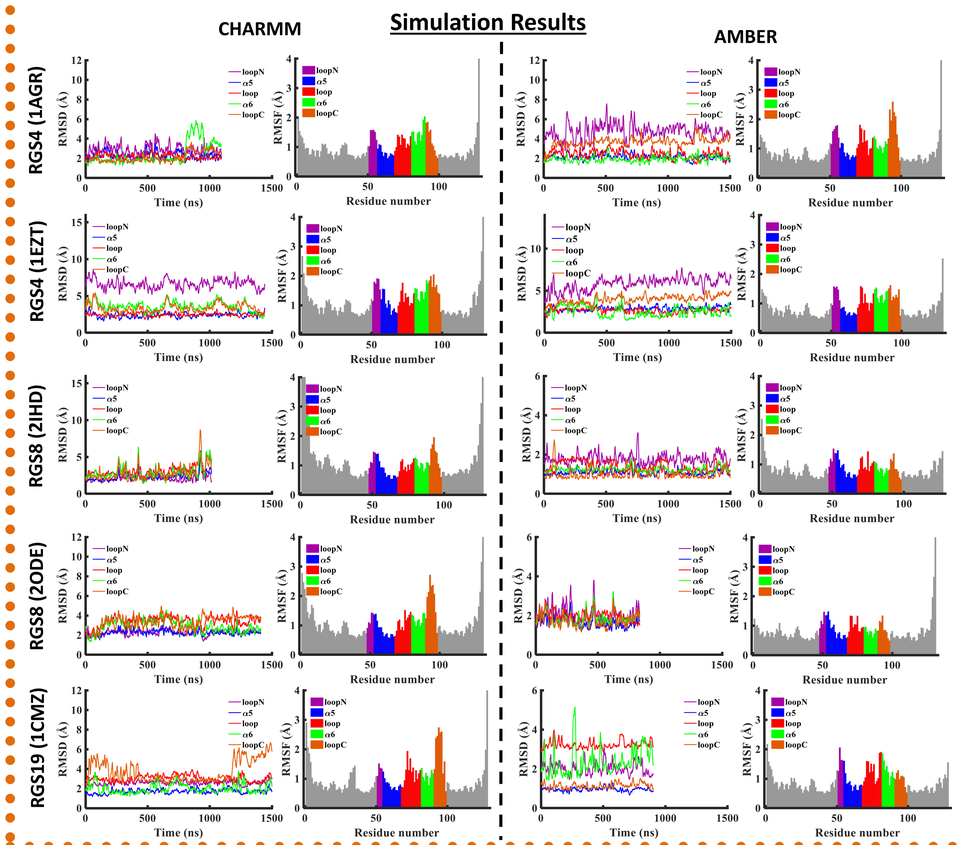
Equilibration steps:

1- 0.4 ns in NPT ensemble
2- ~ 1.5 μs in NVT ensemble

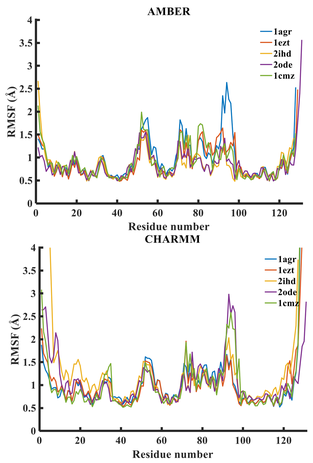
Force fields:

- ✓CHARMM force filed (CHARMM36, tip3p)
- ✓AMBER force filed (ff14SB, tip3p)

Simulation Results



Simulation Results



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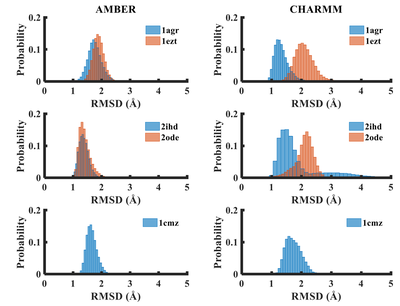
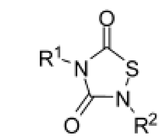


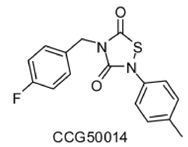
Distribution of RGS conformations based on RMSD of the whole protein. AMBER has narrower distributions in comparison with CHARMM. Also, AMBER has generated similar conformational distributions for RGSs in the same family.

Small Molecule Inhibitors of RGS



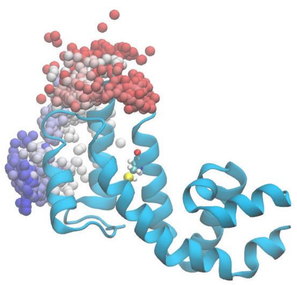
Thiadiazolidinone (TDZD) Analogues [5]

Inhibitor of RGS proteins that blocks a protein–protein interaction (RGS-Gα) in screening assay.

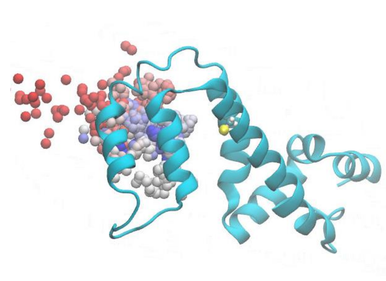


CCG50014
RGS4 IC_{50} 30.1 nM
RGS8 IC_{50} 11.0 μM

Selective RGS4 inhibitor [5]



Wild type protein conformation



Open protein conformation

Simulations of RGS4 interaction with the selective inhibitor (CCG50014). Only the sulphur atoms of the drug are shown. The drug mostly interacts with α5 and α6 helices and the connecting loops.

References and Acknowledgements

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