Frederick National Laboratory
for Cancer Research

FNLAC Pilot 2 Discussion

Frank McCormick

Parameters affecting Ras activity

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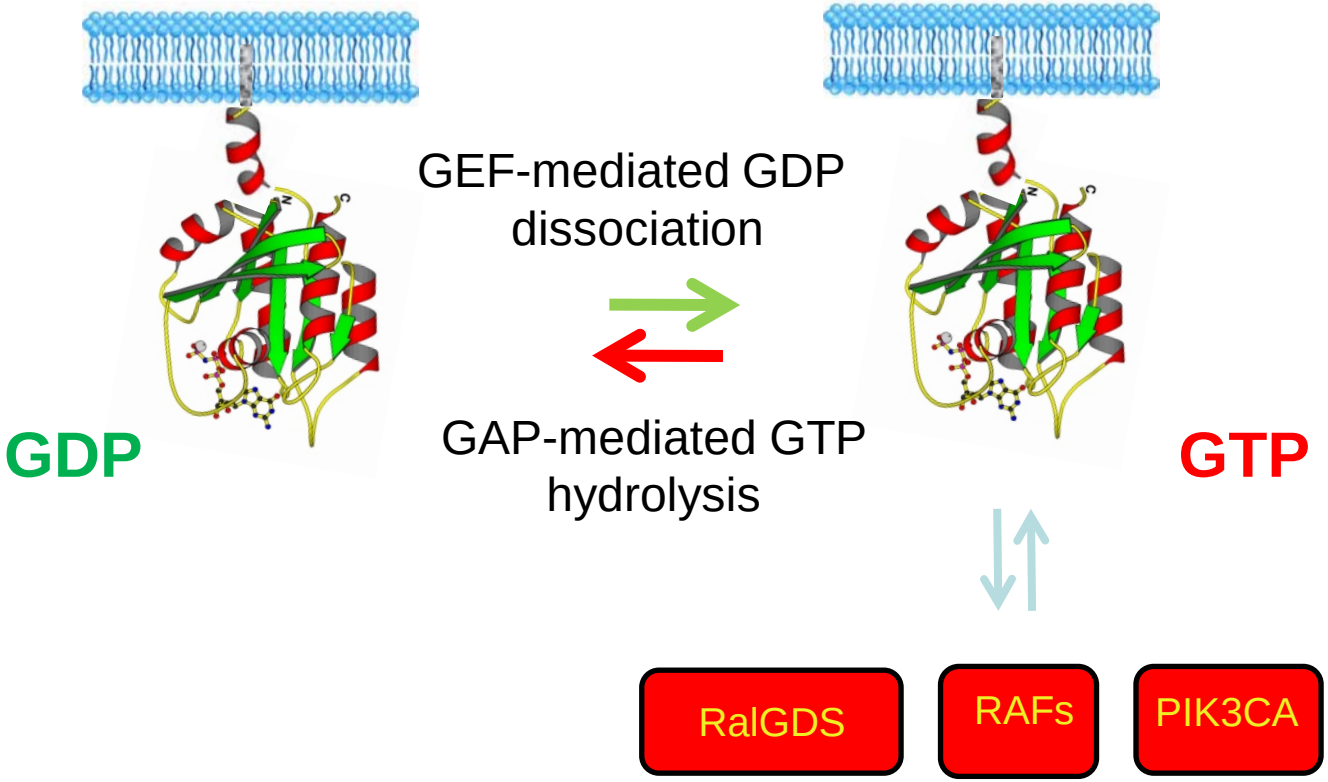

Ras GEFs and GAPs

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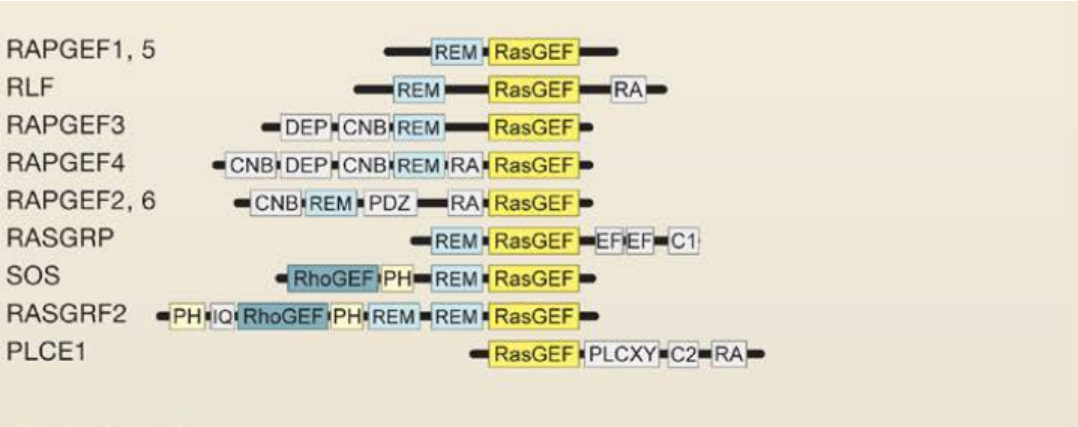

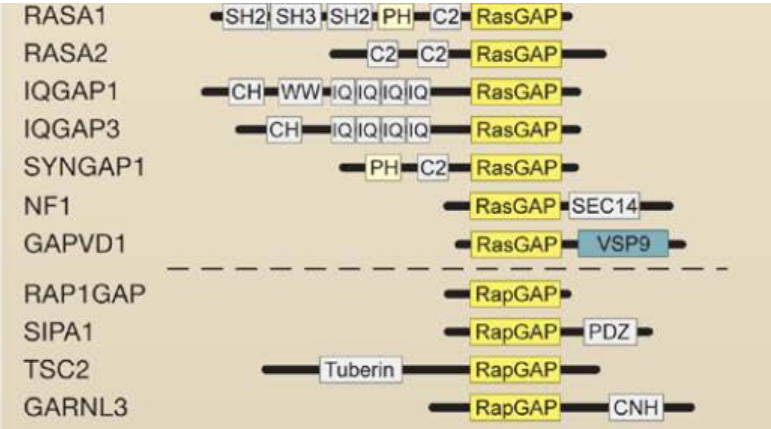

Ras-dependent Raf activation

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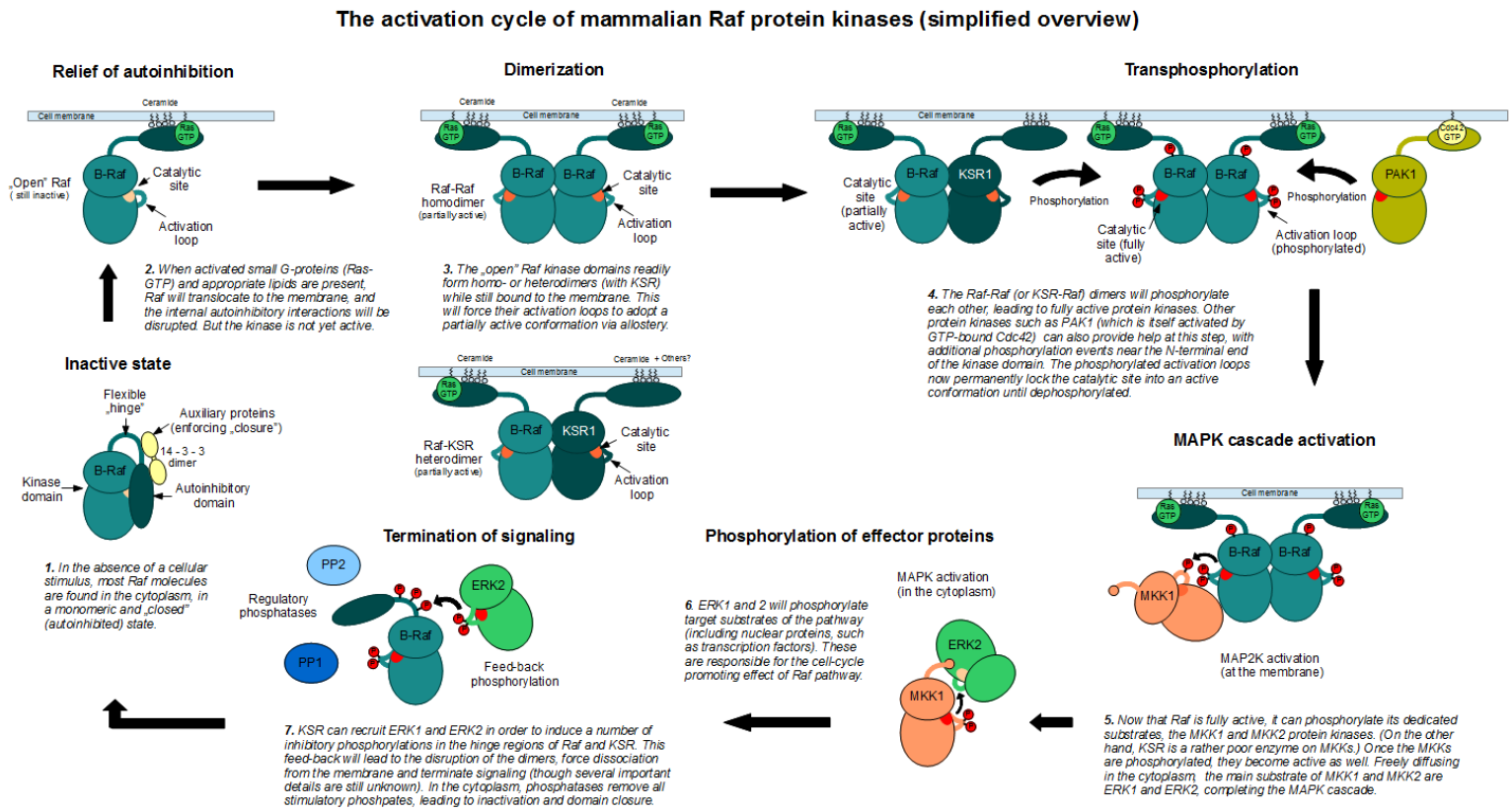

Structural and functional analysis of KRAS on a membrane

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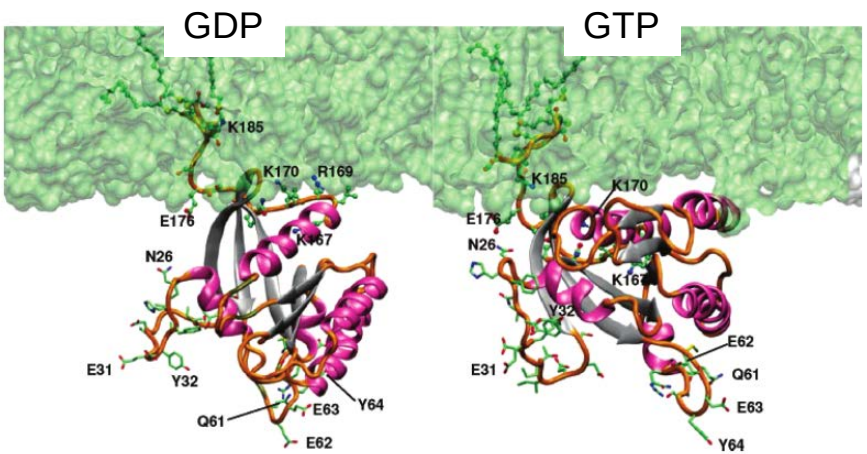
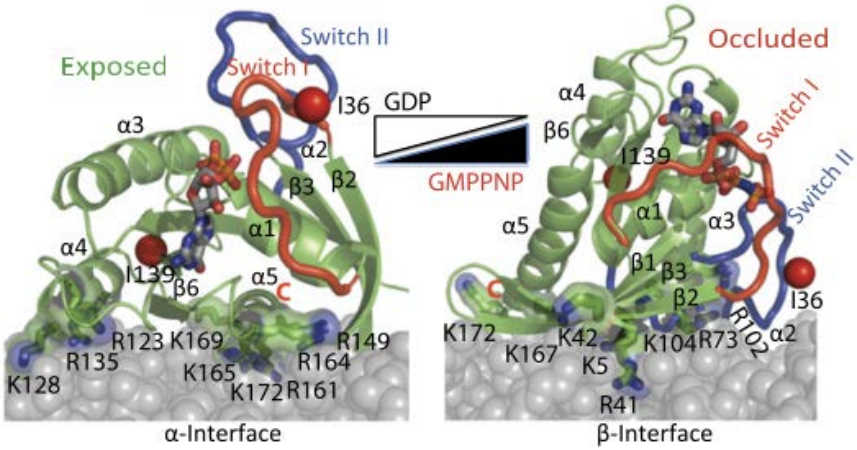


Molecular dynamics
Gorfe et al., 2007



NMR analysis with ^{13}C-Ile labeling
Mazhab-Jafari et al., 2015

- **Objectives:**
  - Determine the structural information of KRAS on a membrane (Nanodisc)
  - Evaluate the effect of nucleotide state, effector interaction and lipid composition on the structure of KRAS
  - Establish a functional assay of KRAS on the membrane by measuring RAF activation

KRAS residues with NMR shifts on binding to Nanodisc

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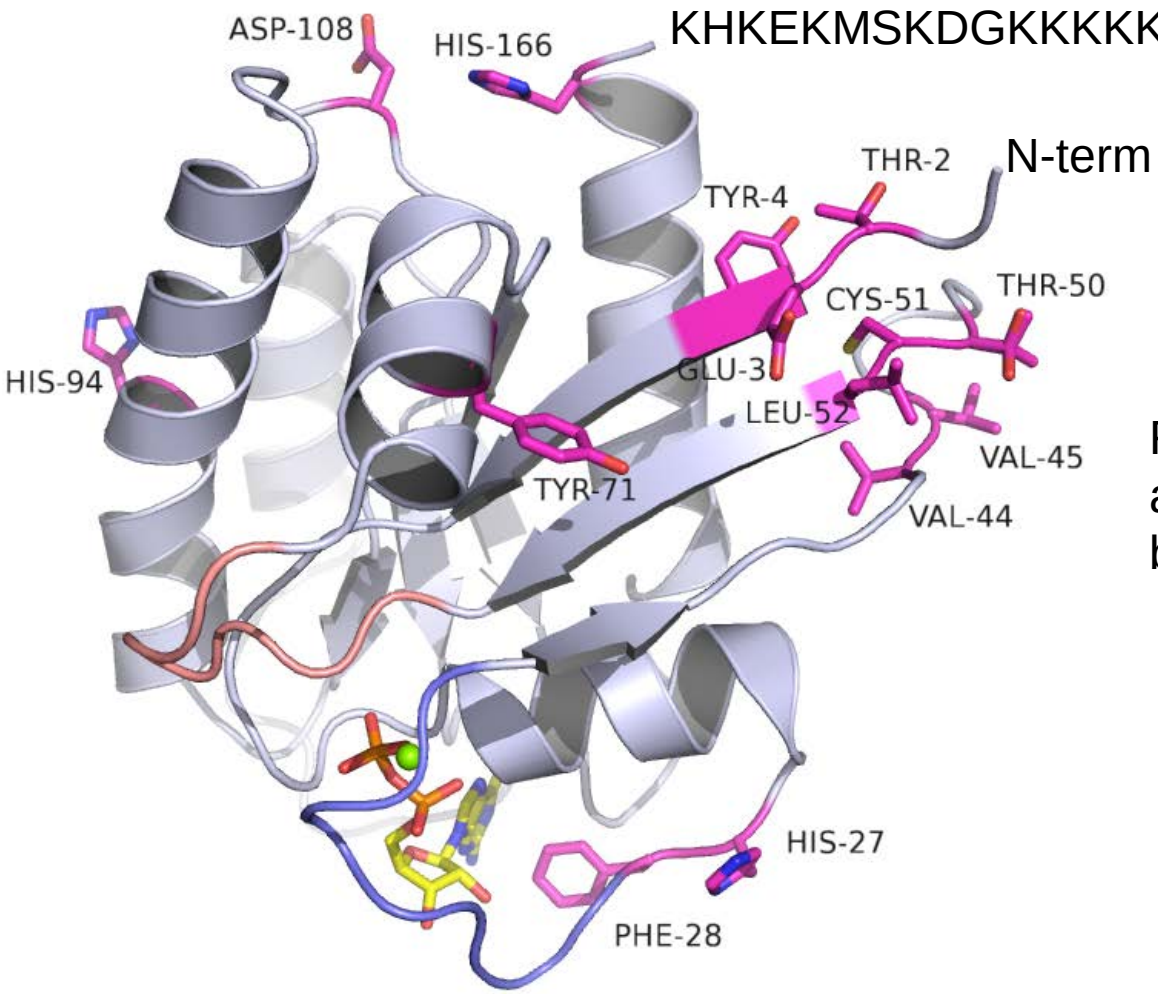

Residues shown in purple are shifted in KRAS when bound to Nanodiscs

Switch-1
Switch-2

Determine the stoichiometry of KRAS-FME on Nanodiscs

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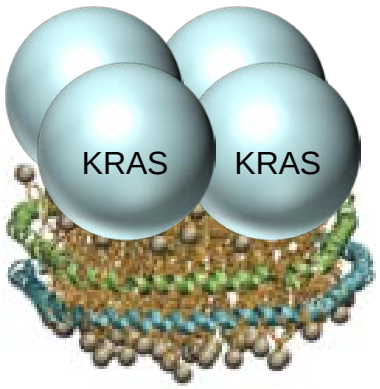
- **Use Analytical Ultracentrifugation to determine maximal number of KRAS molecules that can fit on one face of a Nanodisc**
  - Investigate lipid requirements for KRAS –KRAS interactions on a Nanodiscs
  - Application for KRAS-effector stoichiometry measurement on Nanodiscs
- **Maximum stoichiometry predicted to be 4 KRAS molecules per face.**
  - Radius of a Nanodisc is 3.75nm
  - Area of Nanodisc is 44nm
  - Radius of KRAS4b ~1.8nm
  - Area of KRAS4b ~10nm



Collaborations

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- **Sligar Lab – University of Illinois Urbana-Champaign**
  - Analysis of lipid dependence in KRAS-FME binding to Nanodiscs
- **Groves Lab – UC Berkeley**
  - PIP2 may be required for KRAS-FME dimerization
- **Mattos Lab – Northeastern University**
  - KRAS-FME-GppNHp for complex with CaM
- **UMB**
  - NMR analysis of CaM-KRAS complex and KRAS Cys185 tethering compounds
- **Oak Ridge National Laboratory**
  - Small angle neutron scattering of KRAS-FME on Nanodiscs
  - Molecular modeling of KRAS-FME on a membrane
- **DOE Pilot 2**
  - Preliminary discussions to support modeling data with structural/biophysical measurements of KRAS-FME on membrane

Disrupting KRAS complexes

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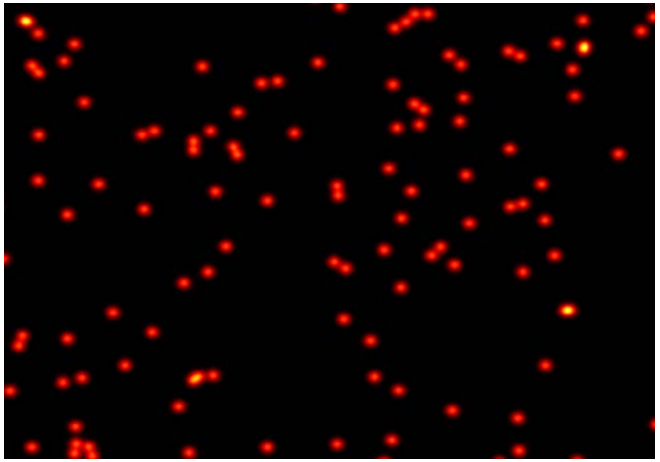
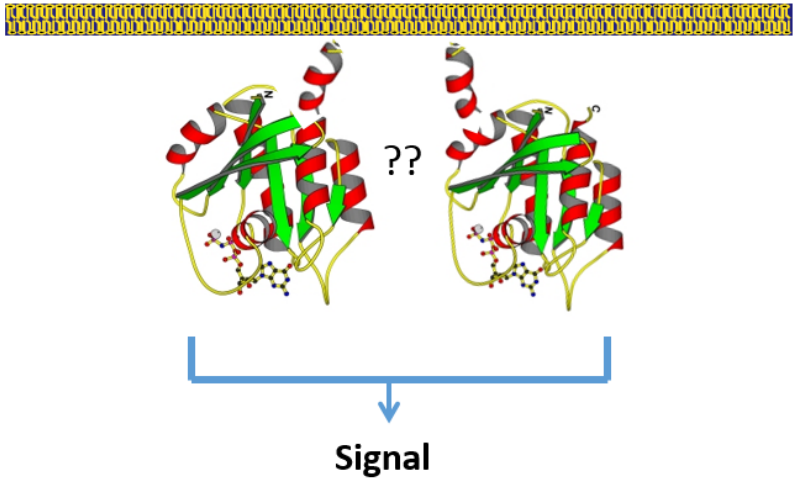
- Develop imaging methods to identify KRAS complexes in c
- Develop screens for disrupting complexes





Ras-GTP dimers activate the Mitogen-Activated Protein Kinase (MAPK) pathway

Xiaolin Nan[a,b,c,1], Tanja M. Tamgüney[d,2], Eric A. Collisson[b,d,2], Li-Jung Lin[c,2], Cameron Pitt[d], Jacqueline Galeas[d], Sophia Lewis[b], Joe W. Gray[b,c,d,1], Frank McCormick[d,1], and Steven Chu[e,1]

[a]California Institute for Quantitative Biosciences (QB3), University of California, Berkeley, CA 94720; [b]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [c]Department of Biomedical Engineering, Knight Cancer Institute, and OHSU Center for Spatial Systems Biomedicine (OCSSB), Oregon Health and Science University, Portland, OR 97239; [d]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94158; and [e]Departments of Physics and Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305

Contributed by Steven Chu, May 16, 2015 (sent for review September 3, 2014; reviewed by Guowei Fang, Tyler Jacks, Mark Phillips, and Neal Rosen)

Single molecule measurements in the membrane of live cells

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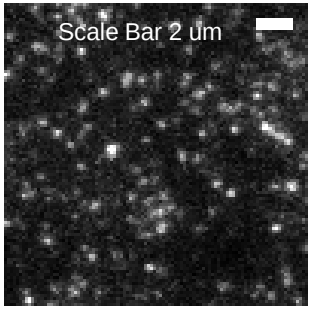
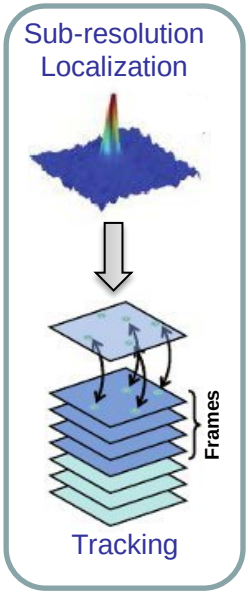
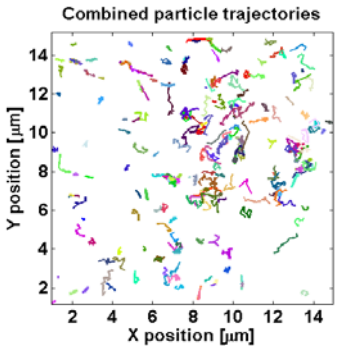
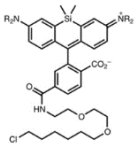






Cell permeant, super bright, fluorescent Halo ligand



Trajectories of single molecules identified.

Three states in the plasma membrane

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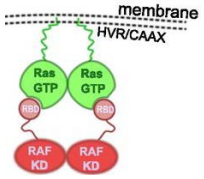
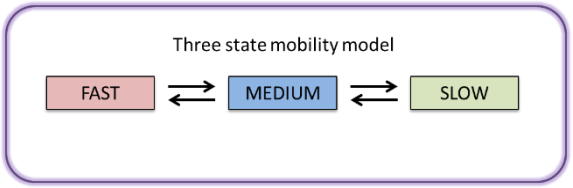
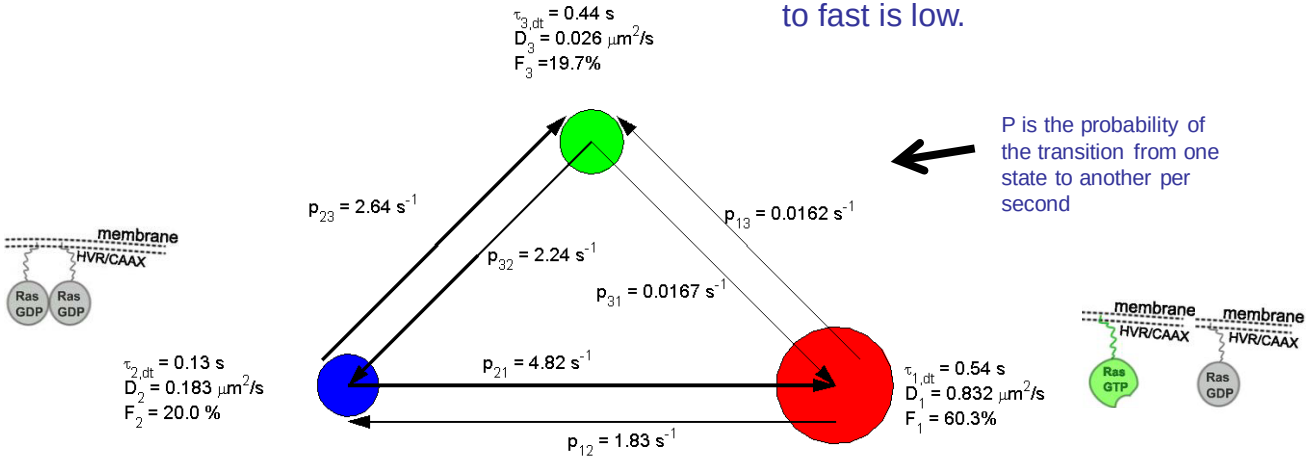
Information extracted from individual trajectories





Probability of direct transition from slow to fast is low.



P is the probability of the transition from one state to another per second

22,325 trajectories and average trajectory length 12 frames.

cryo-EM of RAS on nanodiscs

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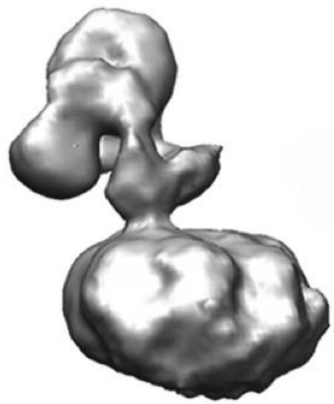
Goal: Image full-length KRAS in a native membrane-bound environment

- KRAS is too small to be targeted by cryo-EM directly.
- Create a large enough complex with relevant RAS binding proteins and/or Fab fragments and bind to a nanodisc.
- Generated several mAb against KRAS and are characterizing them with regards to electron microscopy
- Working on creating stable KRAS complexes with some of its binding partners



3D reconstruction of intact human integrin (200 kDa) in a nanodisc from negative stained data.

Choi WS1, Rice WJ, Stokes DL, Coller BS (2013) Blood 122:4165-4171

RAF Activation Assay - An example of a screening assay that could be used to inform modeling

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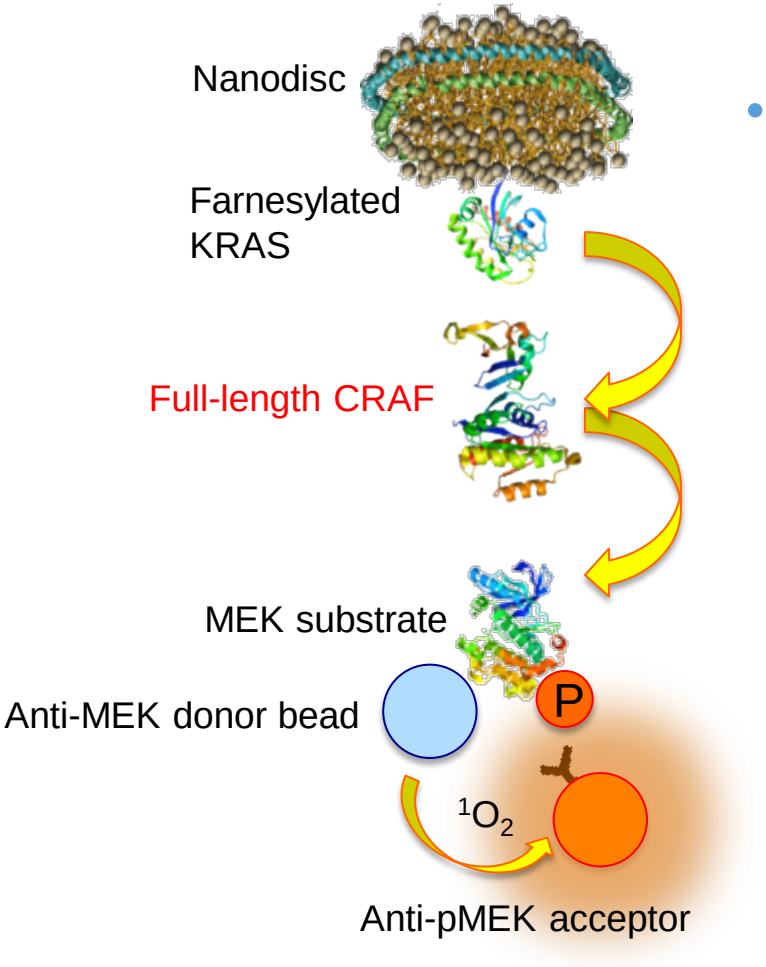

- **Information:**
  - Functional assay of KRAS on the membrane by measuring RAF activation (2016)
  - Determine the structure of KRAS on a membrane (nanodisc or alternate)
  - Evaluate the effect of nucleotide state, CRAF-RBD and CRAF-RBD-CRD interaction and lipid composition on signaling
  - Identify additional components
  - Model tool compounds that perturb activation to define protein-protein interaction

Pilot 2 – Dynamic multi-scale data
Predict novel therapeutic targets for RAS drug discovery

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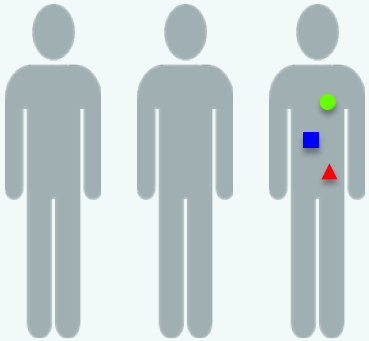
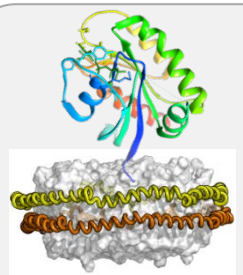
30% of cancers have mutated RAS

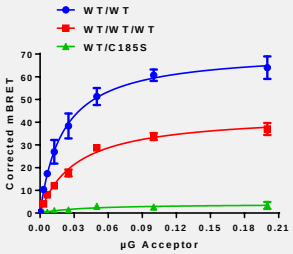
~1M deaths/year

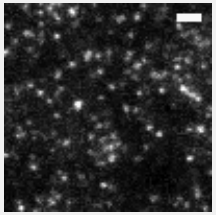


Current therapies ineffective against RAS-driven cancer

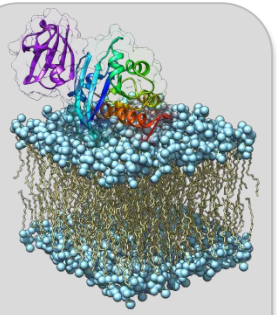
Facilitate discovery and development of novel therapeutics







Molecular Dynamics
Simulation
Modeling



RAS biology
ID targets
New inhibitors