

Frederick National Laboratory for Cancer Research



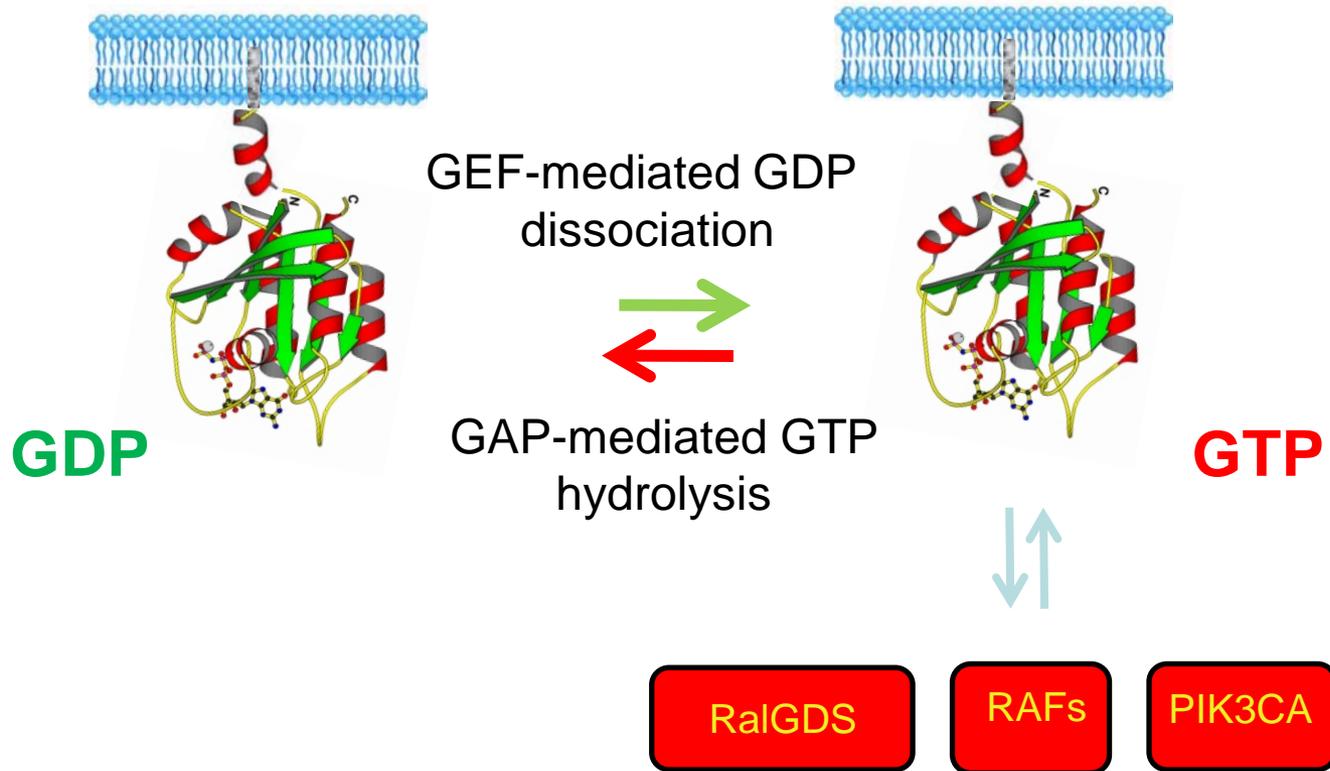
FNLAC Pilot 2 Discussion

Frank McCormick

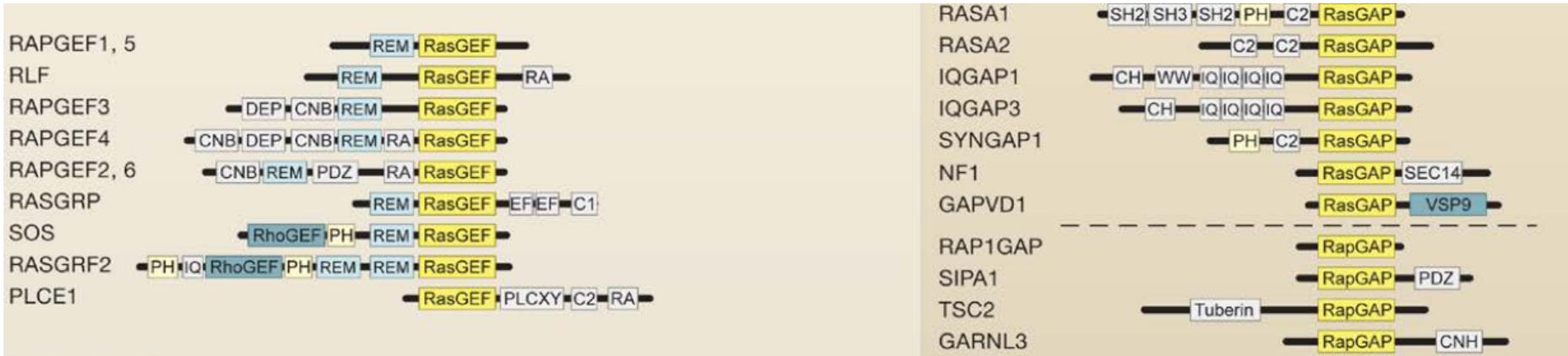
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

The Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

Parameters affecting Ras activity

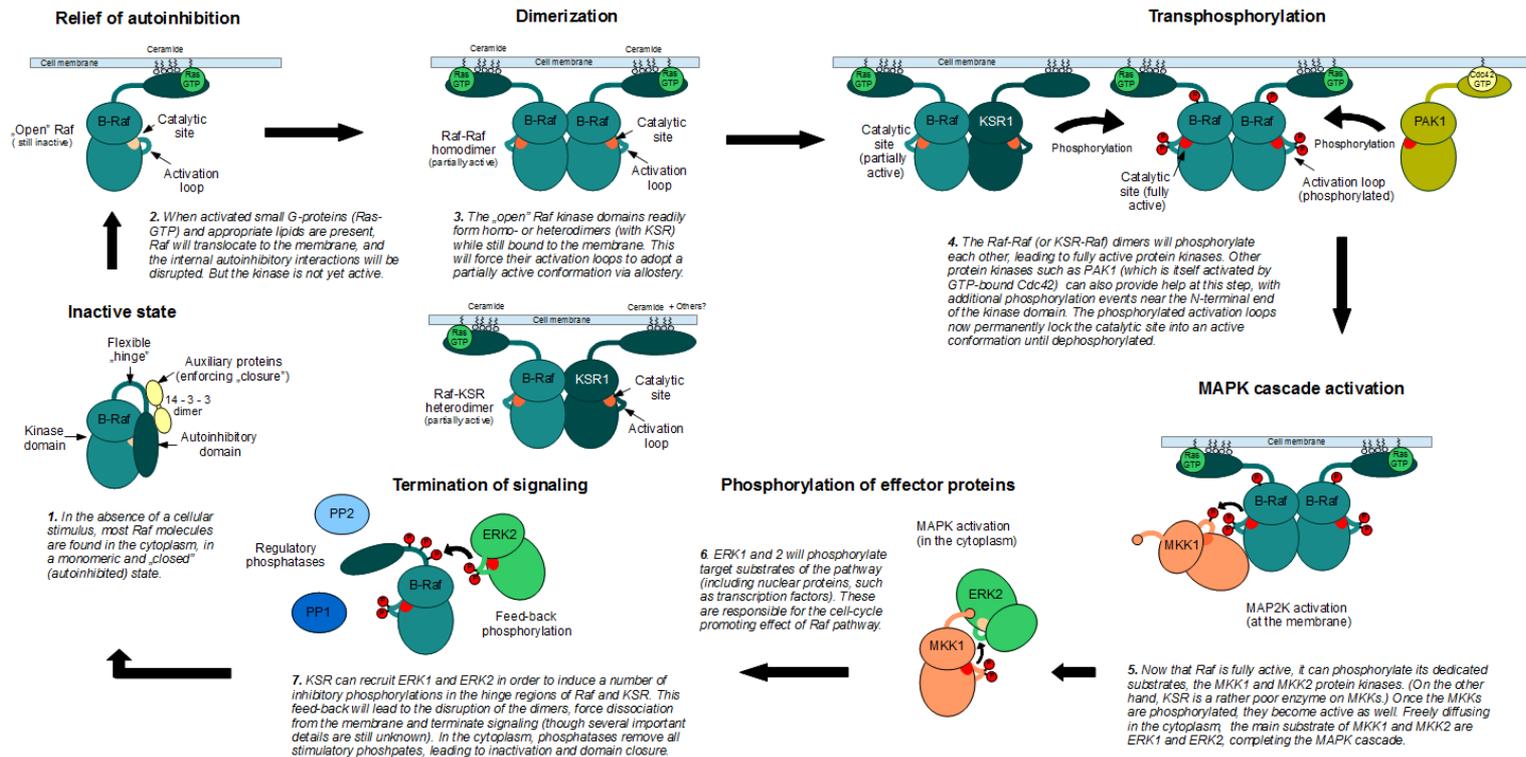


Ras GEFs and GAPs

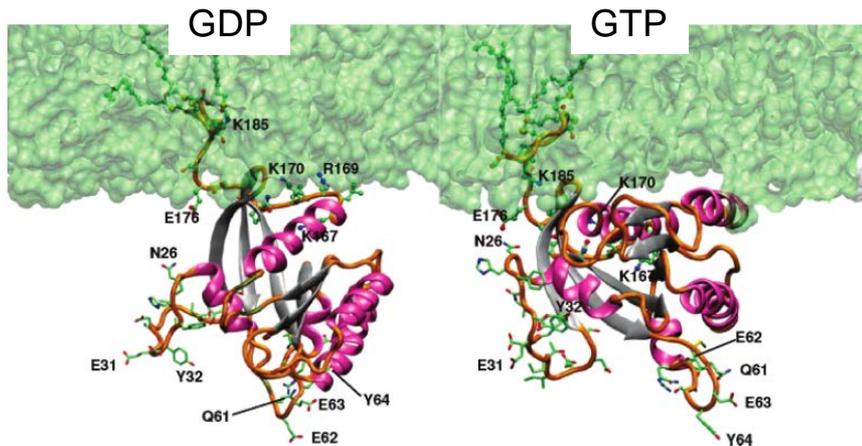


Ras-dependent Raf activation

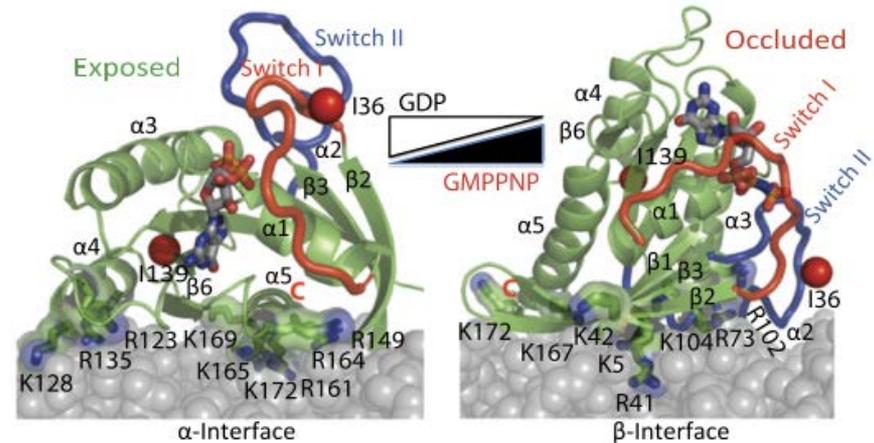
The activation cycle of mammalian Raf protein kinases (simplified overview)



Structural and functional analysis of KRAS on a membrane



Molecular dynamics
Gorfe et al., 2007

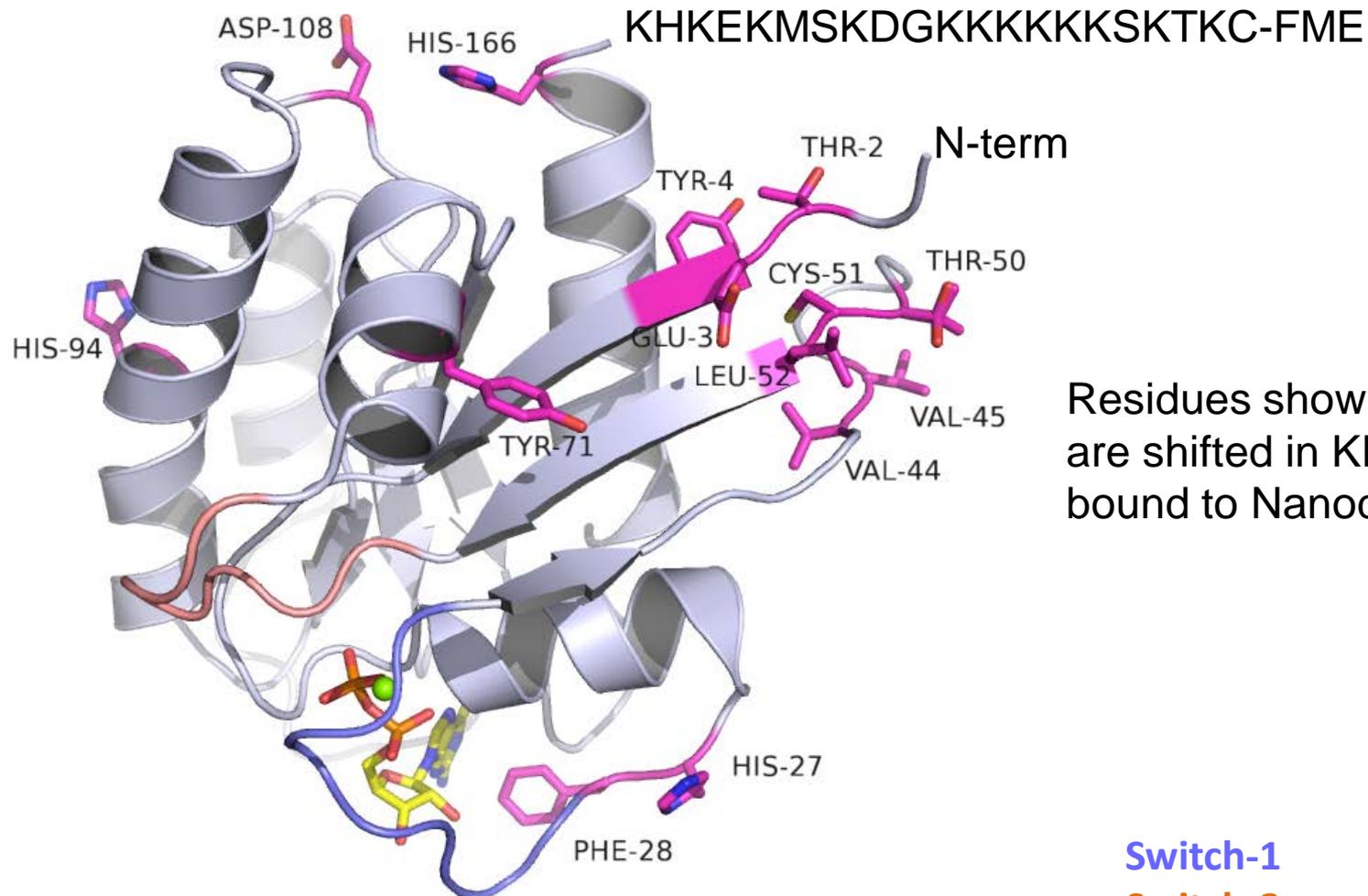


NMR analysis with ^{13}C -Ile labeling
Mazhab-Jafari et al., 2015

- **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

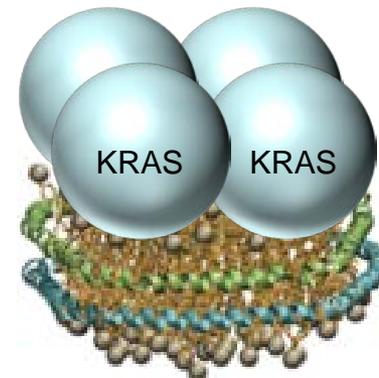


Residues shown in purple are shifted in KRAS when bound to Nanodiscs

Switch-1
Switch-2

Determine the stoichiometry of KRAS-FME on Nanodiscs

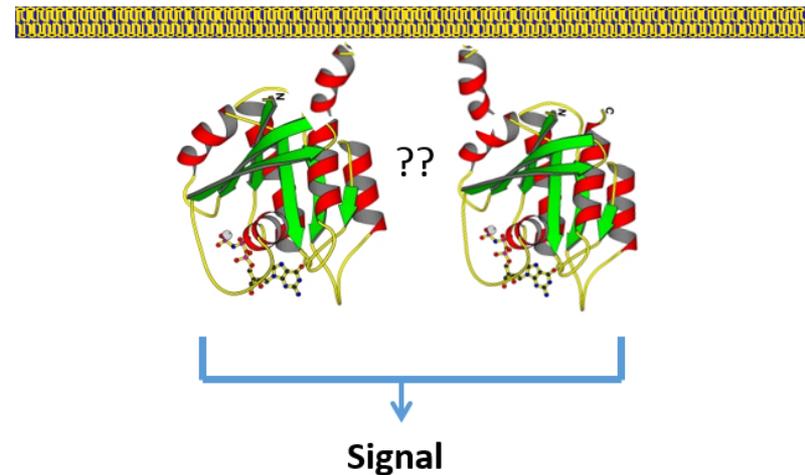
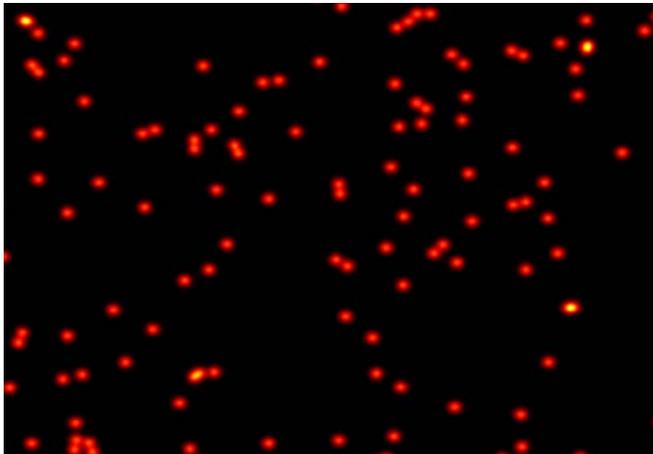
- **Use Analytical Ultracentrifugation to determine maximal number of KRAS molecules that can fit on one face of a Nanodisc**
 - Investigate lipid requirements for KRAS –KRAS interactions on a Nanodiscs
 - Application for KRAS-effector stoichiometry measurement on Nanodiscs
- **Maximum stoichiometry predicted to be 4 KRAS molecules per face.**
 - Radius of a Nanodisc is 3.75nm
 - Area of Nanodisc is 44nm²
 - Radius of KRAS4b ~1.8nm
 - Area of KRAS4b ~10nm²



- **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

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



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

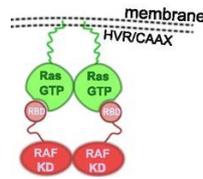
Xiaolin Nan^{a,b,c,1}, Tanja M. Tamgüney^{d,2}, Eric A. Collisson^{b,d,2}, Li-Jung Lin^{c,2}, Cameron Pitt^d, Jacqueline Galeas^d, Sophia Lewis^b, Joe W. Gray^{b,c,d,1}, Frank McCormick^{d,1}, and Steven Chu^{a,1}

^aCalifornia Institute for Quantitative Biosciences (QB3), University of California, Berkeley, CA 94720; ^bLife Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; ^cDepartment of Biomedical Engineering, Knight Cancer Institute, and OHSU Center for Spatial Systems Biomedicine (OCSB), Oregon Health and Science University, Portland, OR 97239; ^dHelen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94158; and ^eDepartments of Physics and Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305

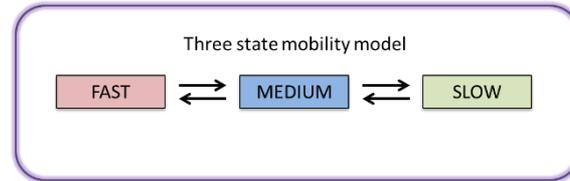
Contributed by Steven Chu, May 16, 2015 (sent for review September 3, 2014; reviewed by Guowei Fang, Tyler Jacks, Mark Phillips, and Neal Rosen)

Three states in the plasma membrane

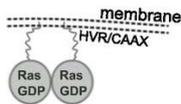
Information extracted from individual trajectories



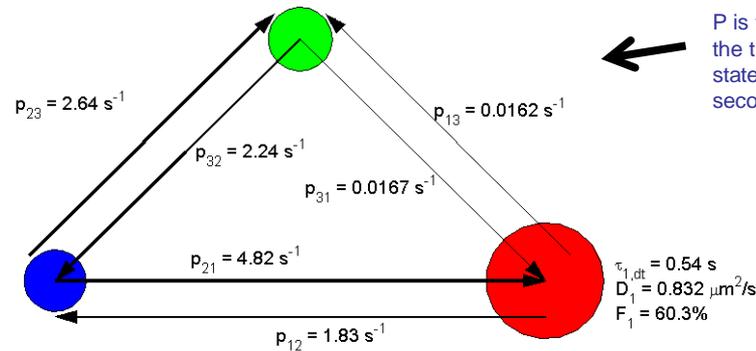
$\tau_{3,dt} = 0.44$ s
 $D_3 = 0.026$ $\mu\text{m}^2/\text{s}$
 $F_3 = 19.7\%$



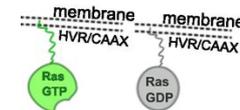
Probability of direct transition from slow to fast is low.



$\tau_{2,dt} = 0.13$ s
 $D_2 = 0.183$ $\mu\text{m}^2/\text{s}$
 $F_2 = 20.0\%$



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



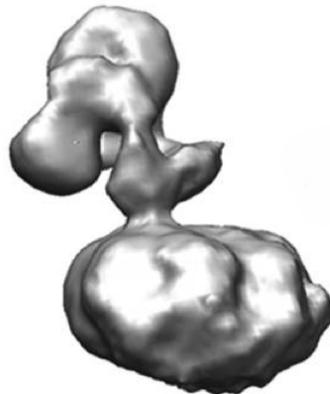
$\tau_{1,dt} = 0.54$ s
 $D_1 = 0.832$ $\mu\text{m}^2/\text{s}$
 $F_1 = 60.3\%$

22,325 trajectories and average trajectory length 12 frames.

cryo-EM of RAS on nanodiscs

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

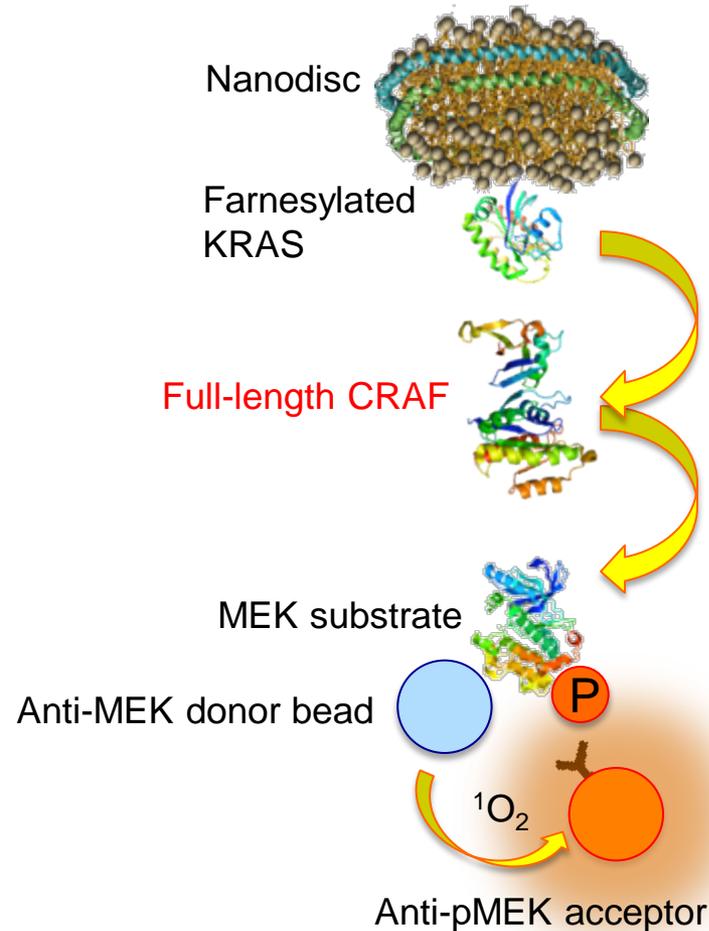
- KRAS is too small to be targeted by cryo-EM directly.
- Create a large enough complex with relevant RAS binding proteins and/or Fab fragments and bind to a nanodisc.
- Generated several mAb against KRAS and are characterizing them with regards to electron microscopy
- Working on creating stable KRAS 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, Collier BS (2013) Blood 122:4165-4171

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



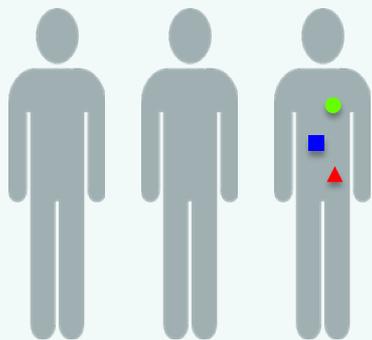
- **Information:**

- 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

30% of cancers have mutated RAS

~1M deaths/year



Current therapies ineffective against
RAS-driven cancer

Facilitate discovery and development
of novel therapeutics

