FNLAC Pilot 2 Discussion

Frank McCormick
Parameters affecting Ras activity

GEF-mediated GDP dissociation

GAP-mediated GTP hydrolysis

GDP

GTP

RaLGDS

RAFs

PIK3CA
Ras GEFs and GAPs
Ras-dependent Raf activation

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

1. Relief of autoinhibition
   - Ras (or other G-protein) binds to Raf, relieving the autoinhibitory state.
   - Catalytic site becomes accessible, allowing Raf to dephosphorylate itself.

2. Dimerization
   - Ras activated GTPase binds to Raf, inducing dimerization and activation.
   - Raf-Raf interaction stabilizes the dimer, promoting activation.

3. Transphosphorylation
   - Activated Raf phosphorylates itself (autophosphorylation), leading to activation.
   - Phosphorylated Raf dimerizes and transphosphorylates KSR1, preparing for MAPK cascade activation.

4. MAPK cascade activation
   - Phosphorylated Raf activates MAPK kinases (MKKs) which in turn activate MEKs, leading to MAPK activation.

Termination of signaling
- MEKs activate ERKs, which phosphorylate target substrates.
- Feedback inhibition occurs as ERKs phosphorylate Raf, deactivating it.

Phosphorylation of effector proteins
- ERKs phosphorylate various proteins, including transcription factors and other signaling molecules.
- MAPKs activate other effectors, such as MKKs, to further propagate the signal.

KSR can recruit ERK1 and ERK2 to inhibit excess Raf activity, serving as a negative regulator.

In the absence of a cellular stimulus, most Raf molecules are found in the cytoplasm, in an inactive state. Upon activation, Raf translocates to the membrane, where it becomes active and phosphorylates other proteins, leading to downstream signaling events.

Additional details:
- KSR is a negative regulator of Raf, inhibiting its activation by binding to Raf in a phosphorylation-dependent manner.
- ERKs are key effectors of Raf, mediating the downstream consequences of Raf activation.
- MAPKs (MKKs) are integral to the MAPK cascade, linking the Raf pathway to the activation of transcription factors and other signaling proteins.

This simplified overview highlights the key steps in the activation of Raf protein kinases, illustrating the dynamic interplay between these molecules in cellular signaling pathways.
Structural and functional analysis of KRAS on a membrane

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

Molecular dynamics
Gorfe et al., 2007

NMR analysis with $^{13}$C-Ile labeling
Mazhab-Jafari et al., 2015
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 44nm
  – Radius of KRAS4b ~1.8nm
  – Area of KRAS4b ~10nm
Collaborations

- **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 Nan¹, Tanja M. Tarngüney², Eric A. Collins³, Li-Jung Lin³, Cameron Pitt³, Jacqueline Galeas³, Sophia Lewis³, Joe W. Gray²,³, Frank McCormick¹,², and Steven Chu³

¹California Institute for Bioscience (CIBS), University of California, Berkeley, CA 94720; ²Life Sciences, St. Jude Children’s Research Hospital, Memphis, TN 38105; ³School of Medicine, University of California, San Francisco, CA 94143.

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Single molecule measurements in the membrane of live cells

Cell permeant, super bright, fluorescent Halo ligand

Trajectories of single molecules identified.
Three states in the plasma membrane

Information extracted from individual trajectories

22,325 trajectories and average trajectory length 12 frames.
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.

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

- 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

Molecular Dynamics Simulation Modeling

RAS biology ID targets New inhibitors