

# Frederick National Laboratory for Cancer Research



## *NCI RAS Initiative Update*

**Dwight Nissley**, Director Cancer Research Technology Program (CRTP), FNLCR

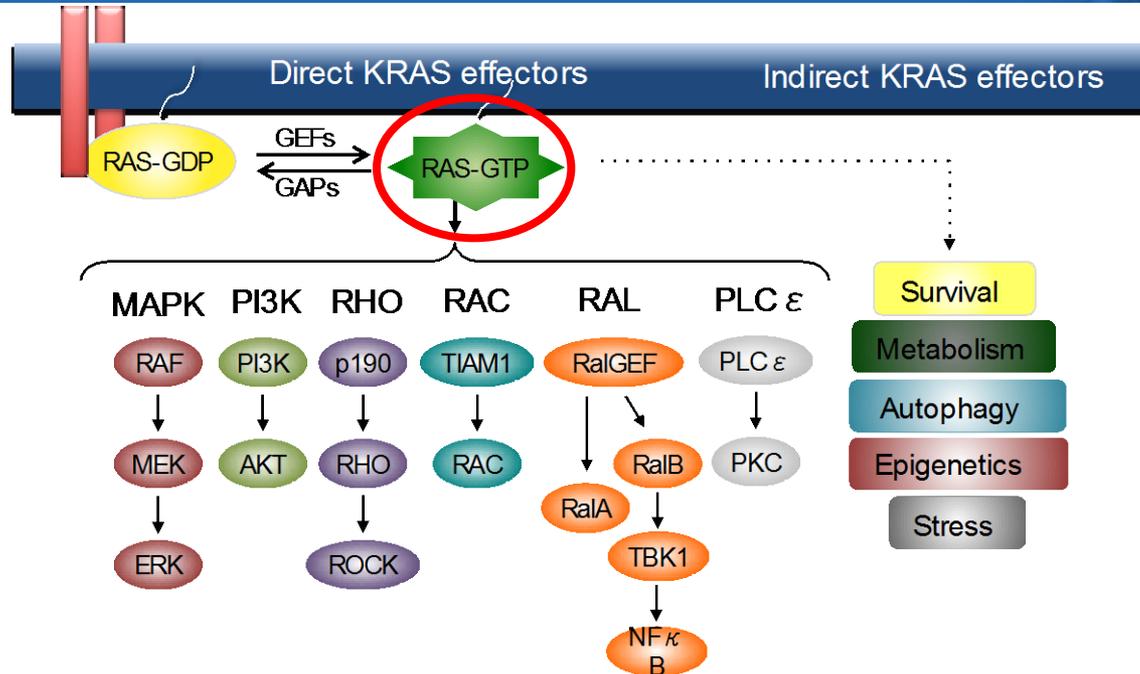
**Dhirendra Simanshu**, RAS Initiative Structural Biology Lead, CRTP, FNLCR

February 3<sup>rd</sup>, 2015

- **Introduction**
- **Structural Biology and Biophysics**
- **Targets and Assays**
  - Biochemical screens
  - Cell-based screens
  - Multimerization and localization
  - Cell surface
- **RAS Community**
- **Oversight and Feedback**

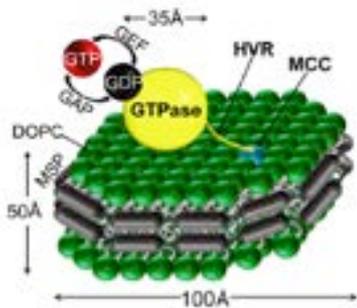
# The NCI RAS Initiative

*What is RAS, and why is it so important?*



Cancer	KRAS Mutation	US - new KRAS cases/yr	5 yr survival
Colorectal	45 %	60,000	45 %
Lung	35 %	45,600	17 %
Pancreas	95 %	32,200	6 %
		<b>137,800</b>	

## Protein Biology/Biophysical Characterization



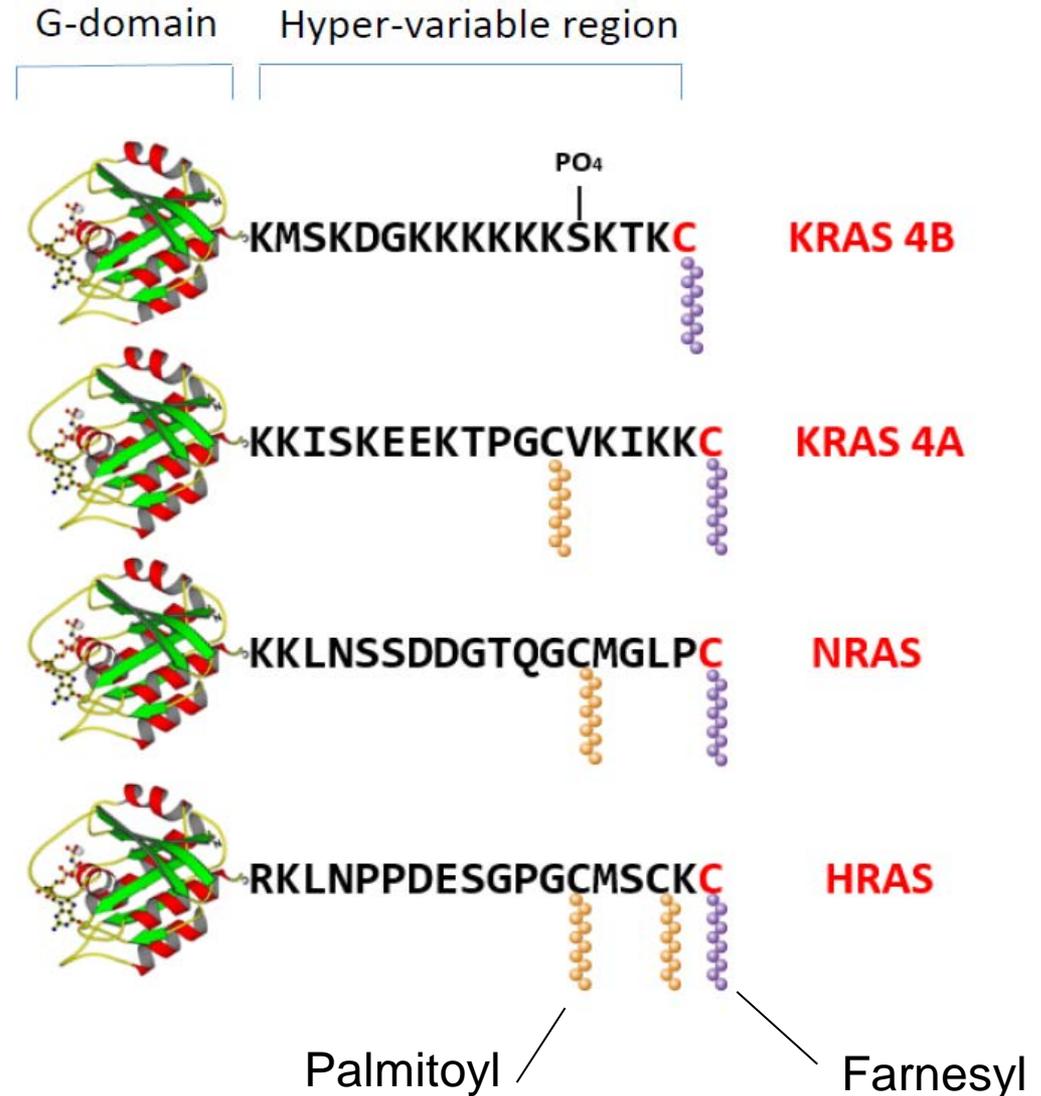
**Andy Stephen**



**Dom Esposito**

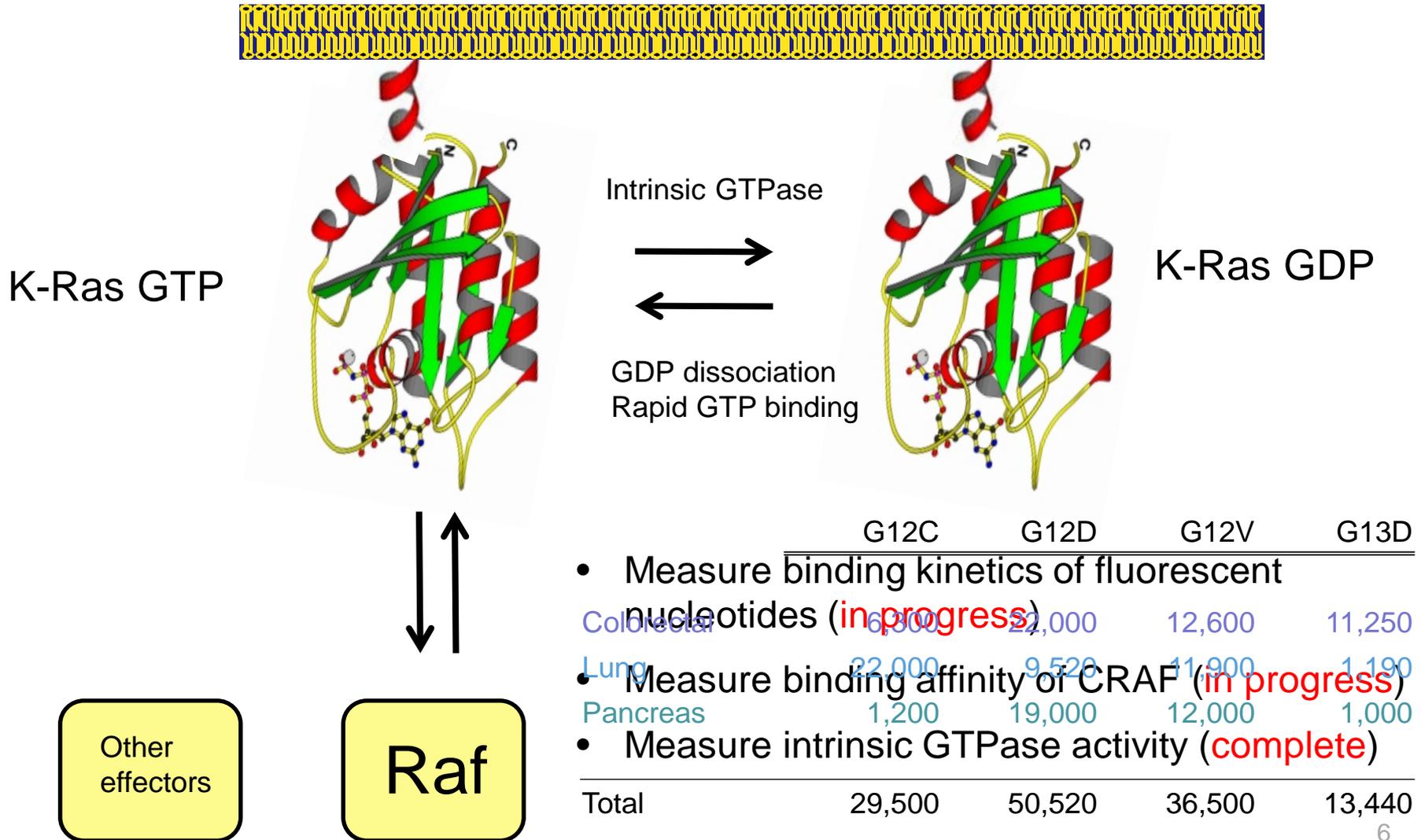
# The RAS family of small GTPases

- 21 kDa small GTPases
- high homology in first 164 aa
- More than 100 members
- post-translational modifications
- membrane association

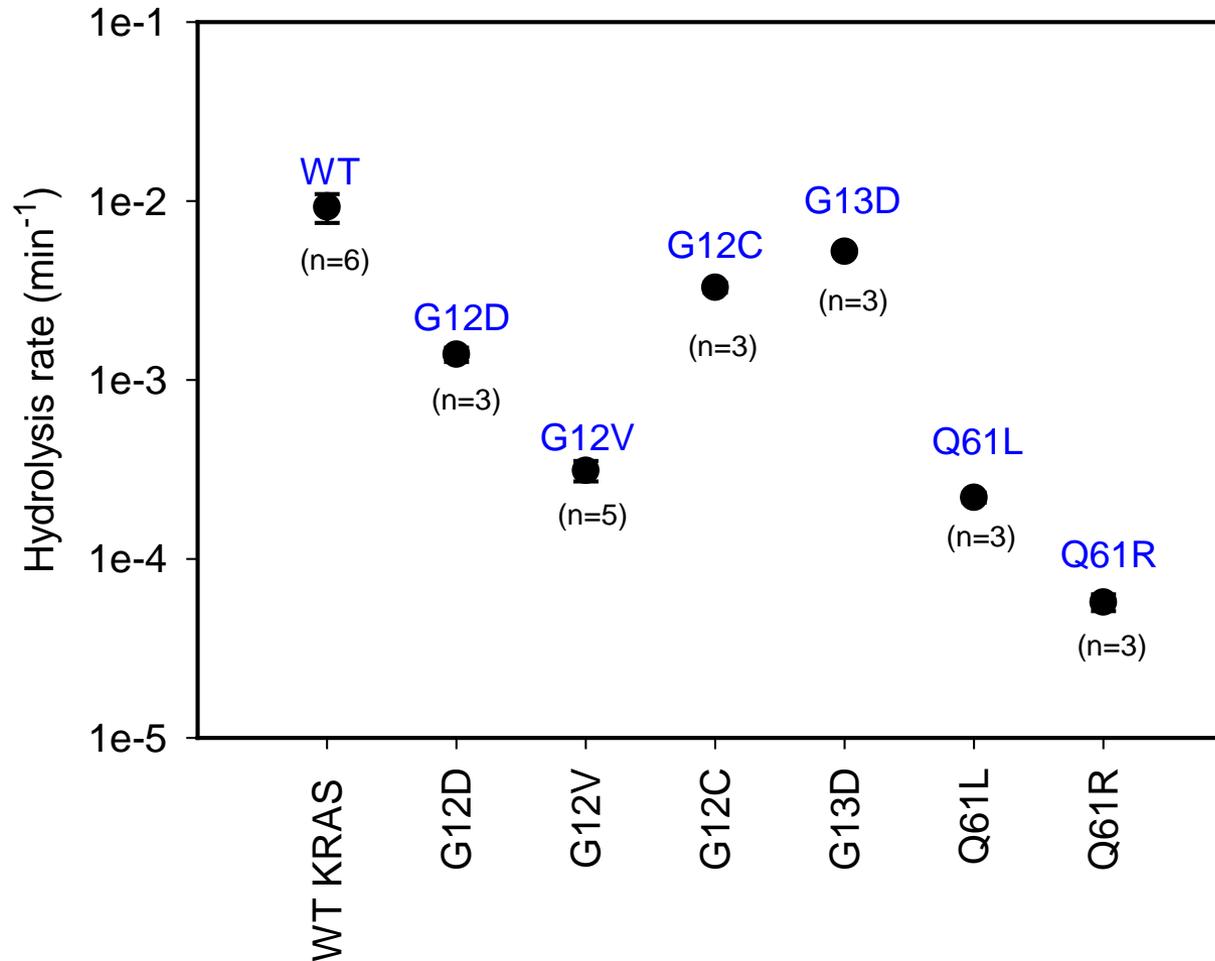


# Characterization of WT KRAS and mutants –

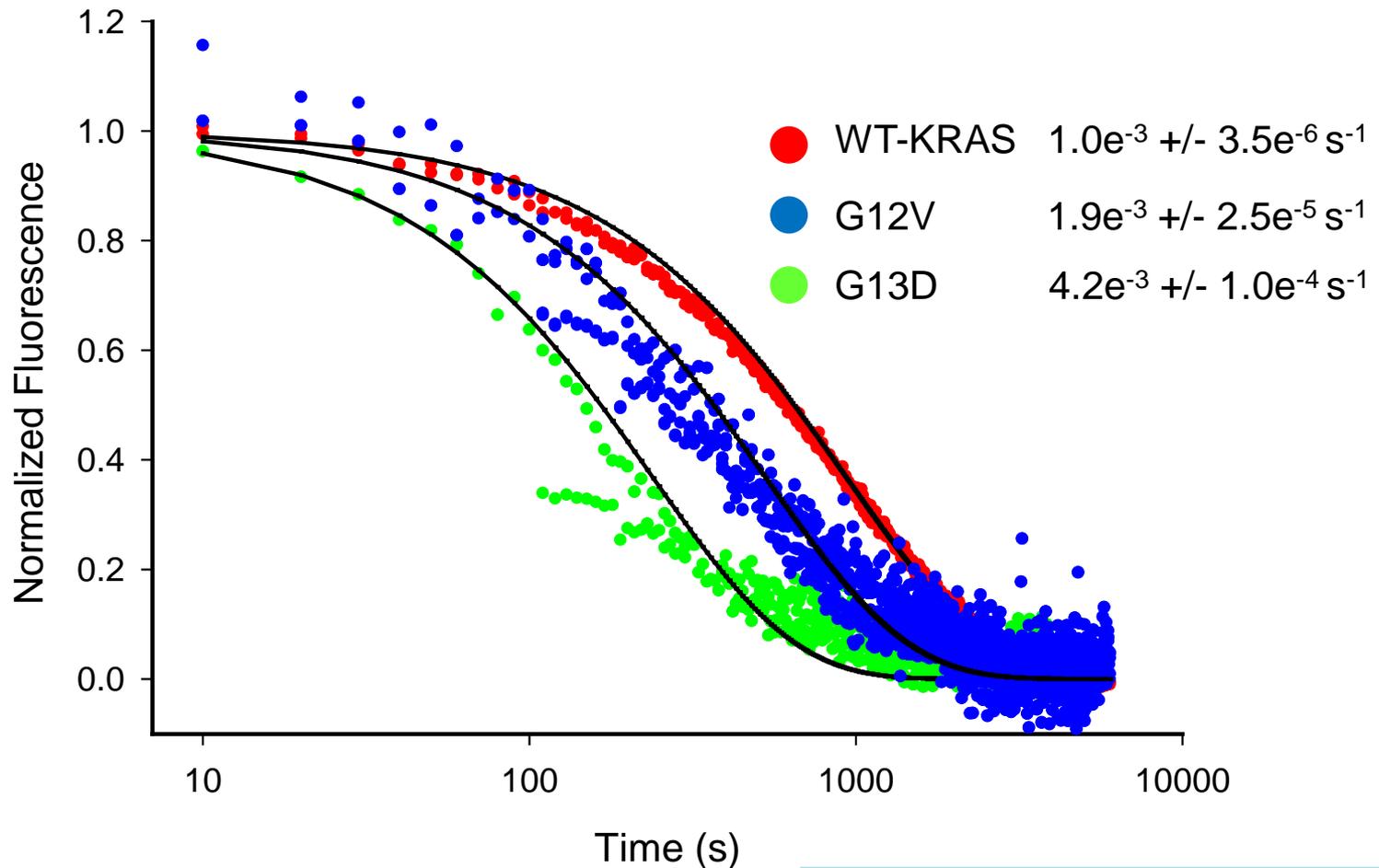
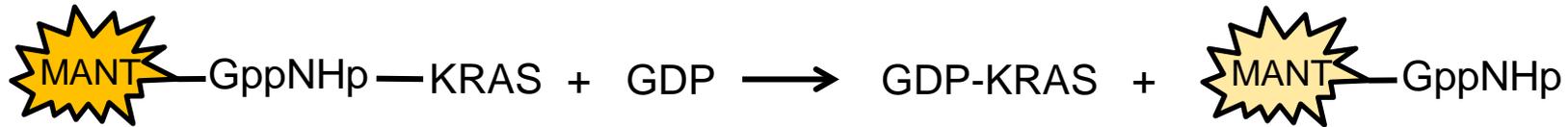
## Nucleotide binding kinetics determine KRAS signaling



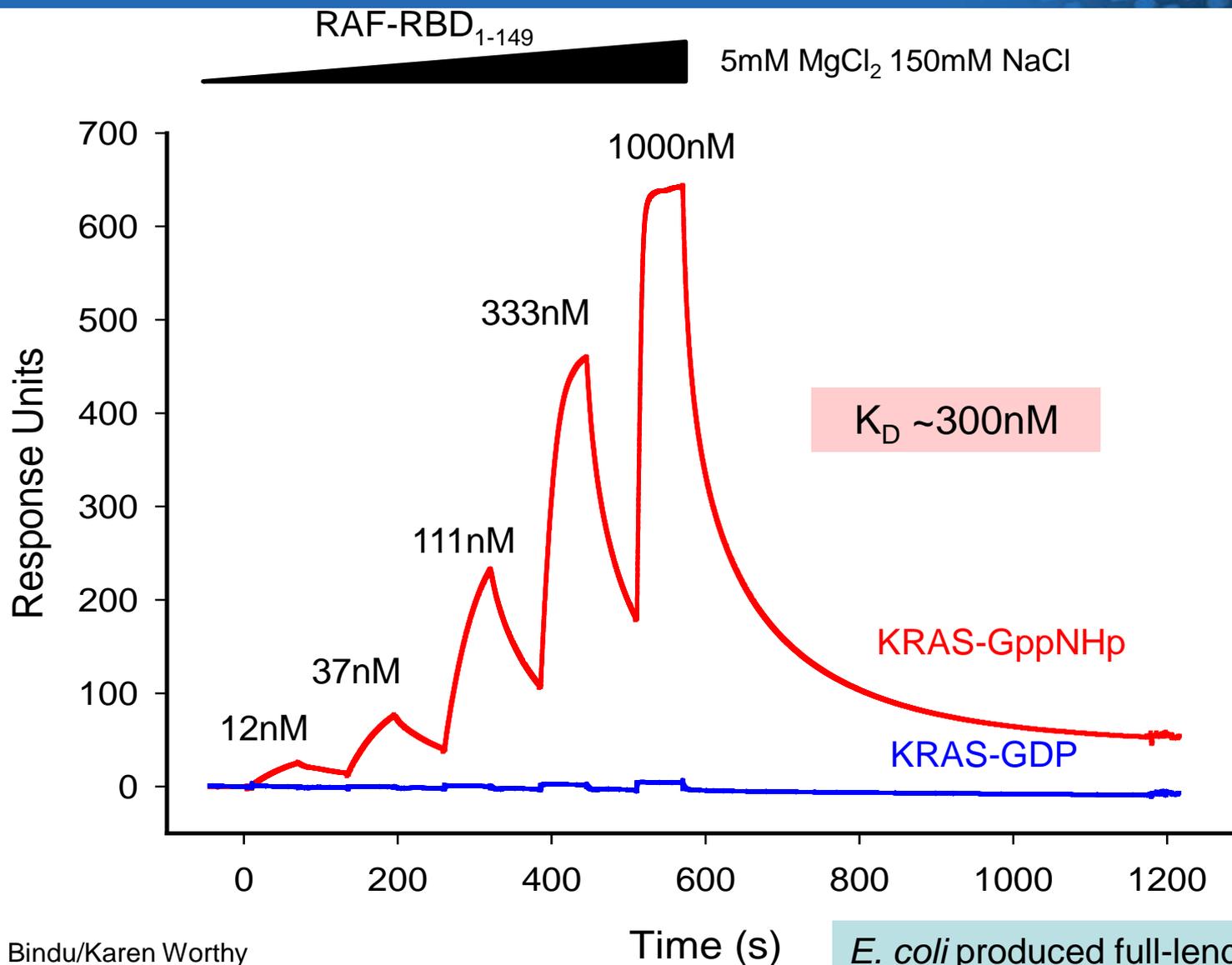
# KRAS characterization: Intrinsic GTPase activity



# MANT-GppNHpNon-hydroly dissociates 4 times faster from G13D compared with WT KRAS



# Binding of RAF Ras Binding Domain (RBD)<sub>1-149</sub> to WT KRAS

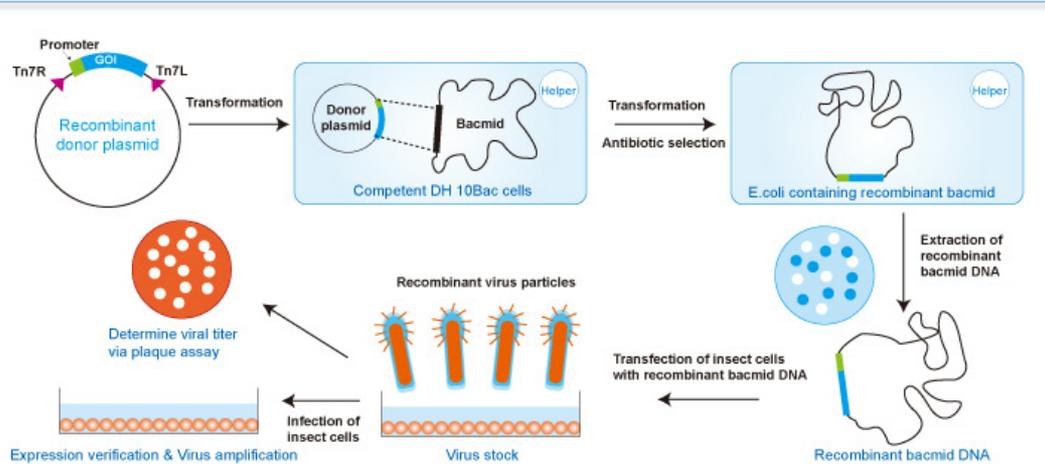


## Ongoing characterization

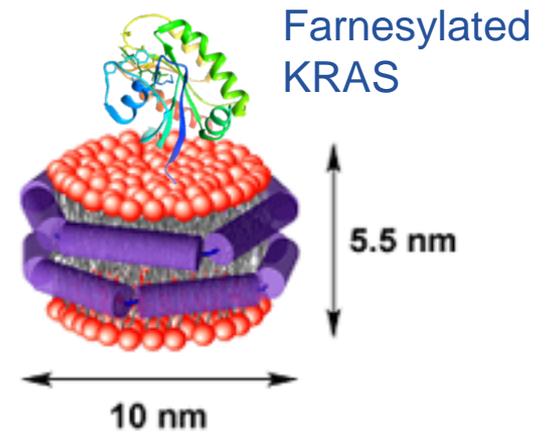
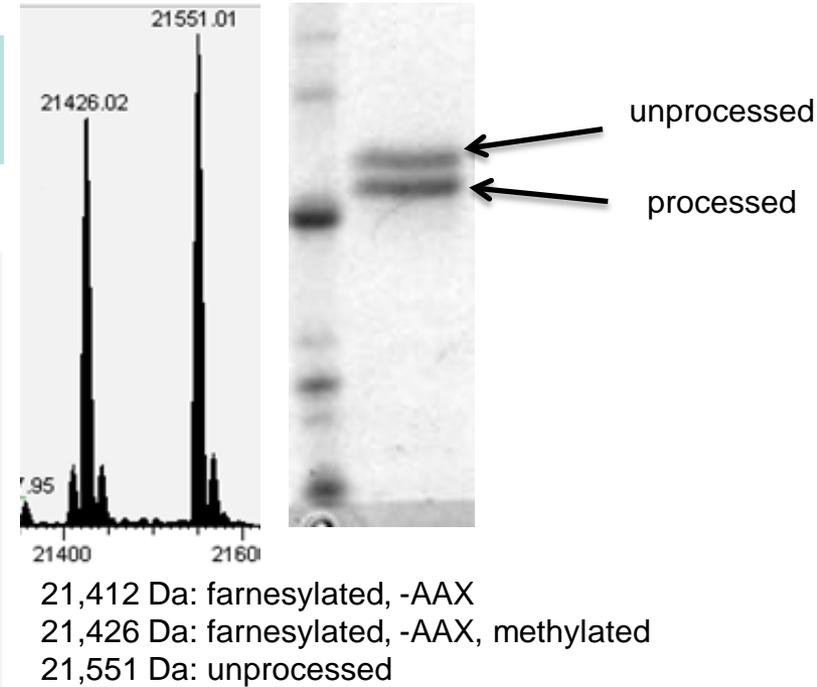
- **Analysis of WT, G12C, G12D, G12V, G13D, Q61H, Q61L**
  - GTP hydrolysis rate of truncated protein
  - nucleotide off-rate
  - RAF-RBD binding
- **Limited analysis of “rare” KRAS oncogenic mutants:  
G12A, G12H, G12S, G12R, G13C, Q61H, R68S, K117N, A146T, A146V**
  - Intrinsic GTP hydrolysis
  - GppNHp off-rate for subset

# Fully Processed Recombinant KRAS

RAS only works when in the membrane  
- Drugs that affect membrane dependent signaling?



- KRAS expressed in insect cells
- Human farnesyltransferase (FNTA/FNTB), ICMT cloned
- Triple infection of FNTA/FNTB, KRAS, and ICMT



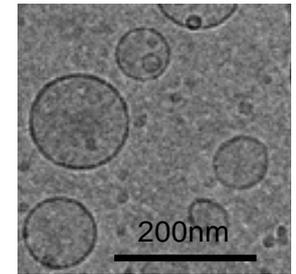
# Processed KRAS and lipid interactions – liposomes and nanodiscs

**Literature suggests poly-basic region at KRAS C-terminus interact with negatively charged lipids in the plasma membrane.**

## Processed KRAS – lipid interactions

### Liposomes

- SPR to evaluate optimal lipid composition

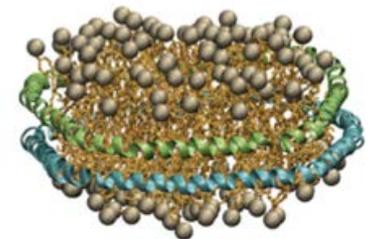


### Tethered bilayers

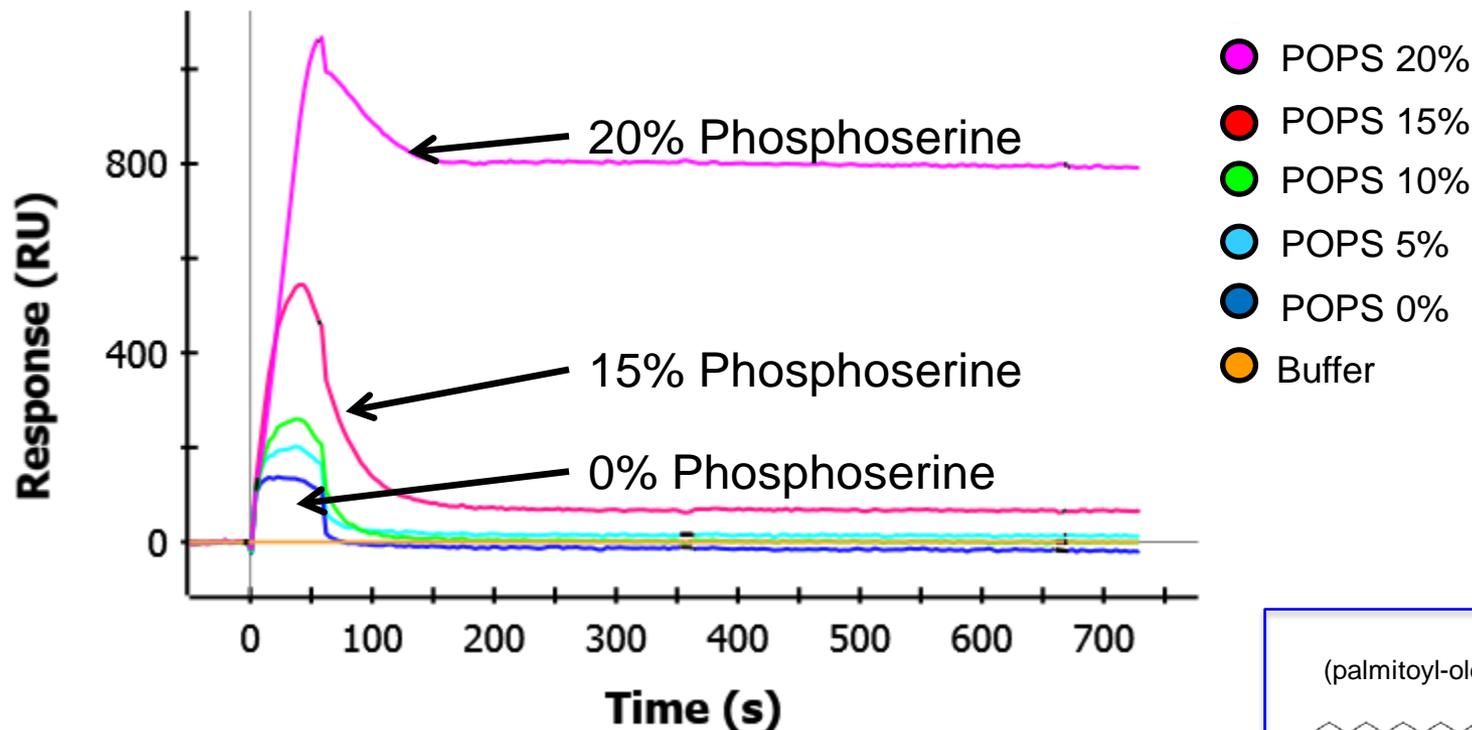
- Fluorescence fluctuation spectroscopy (Jay Groves, UC Berkeley)
- Neutron scattering (NIST)

### Nanodiscs

- Collaboration with Steve Sligar (UI Champaign-Urbana)
- Structural biology by cryo-EM and NMR
- Next-generation HTS assays

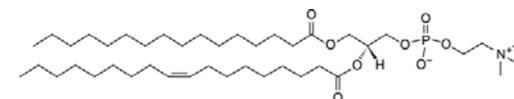


# Binding of processed KRAS to liposomes is dependent on the phosphoserine content

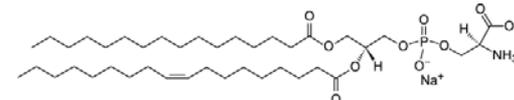


5 μM processed KRAS binding to liposomes with variable phosphoserine content

**POPC**  
(palmitoyl-oleoyl phosphatidylcholine)



**POPS**  
(palmitoyl-oleoyl phosphatidylserine)

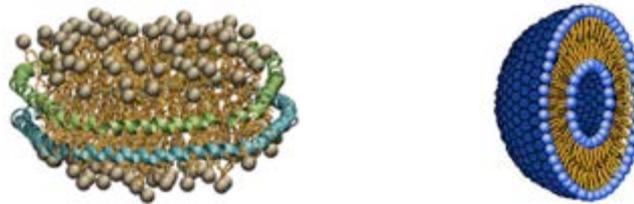


# Plans for structural and biophysical analysis of fully processed KRAS

KRAS-lipid  
interactions

Prepare and QC  
nanodiscs

Prepare and QC  
liposomes



Crystallography

Hi-Res Cryo-EM  
structure (NCI)

NMR structure  
KRAS-nanodisc  
(NMR-FAM)

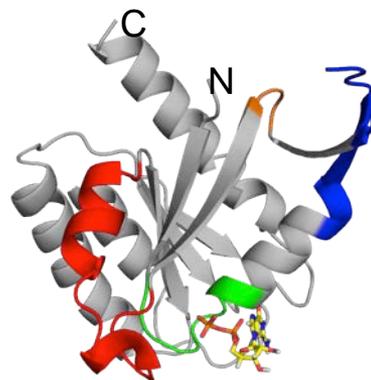
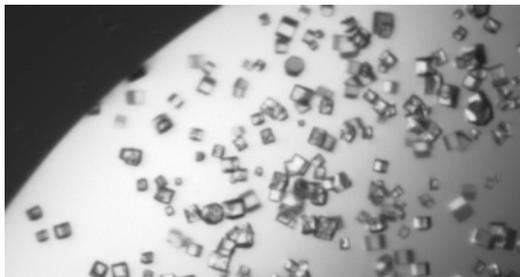
Quantitate KRAS-  
liposome interactions  
by SPR or FCS  
(Sligar/Groves)

KRAS-membrane  
orientation by neutron  
reflectivity (NIST)

Intrinsic/GAP GTP hydrolysis  
RAF-RBD binding  
Next generation screening assays

## Structural Biology

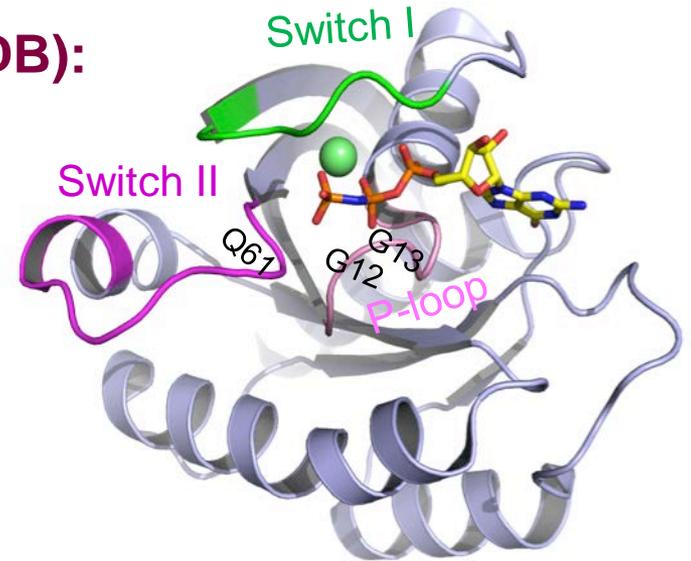
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**Dhirendra Simanshu**

## Structures available in Protein Data Bank (PDB):

- HRAS: 120 structures
- KRAS: 36 structures
- NRAS: 1 structure



KRAS bound to GTP analog  
PDB code: 3GFT

## Challenges to targeting RAS cancers

- No structure of KRAS mutants with any effector or regulator.
- No structural insights about how RAS activates Raf kinase.
- No structural information on full-length processed RAS.
- No structural information on full-length Raf – free or in complex with RAS.

# Structural Biology Goals

- **Determine structures of wild-type KRAS and oncogenic mutants in inactive (GDP-bound) and active (GTP/GMPPNP) states**

G12C    G12D    G12V    G13D    Q61H    Q61L

- **Determine structures of KRAS complexes with various effectors and regulatory proteins to aid structure-based drug design**

- Calmodulin
- GAPs :                    RASA1,    NF1
- Effectors :                Raf (RBD and Kinase domain, full-length),    PI3-Kinase
- Farnesyl binding :      PDE6 $\delta$ ,    smgGDS

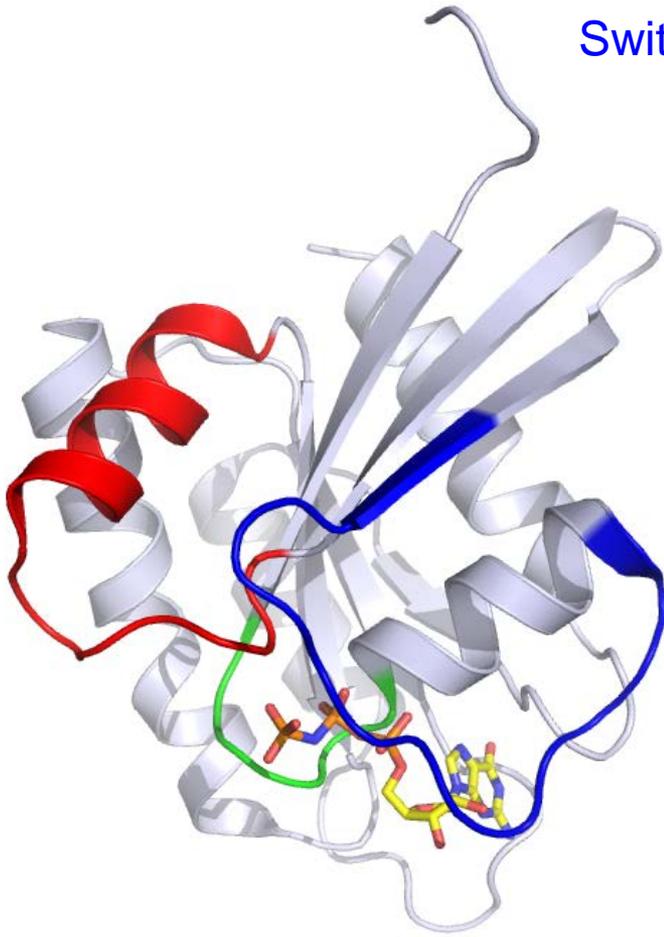
- **KRAS4a structure – Comparison with KRAS4b**

- **NMR efforts: processed full-length KRAS bound to nanodisc.**

*Que Van at FNLCR*

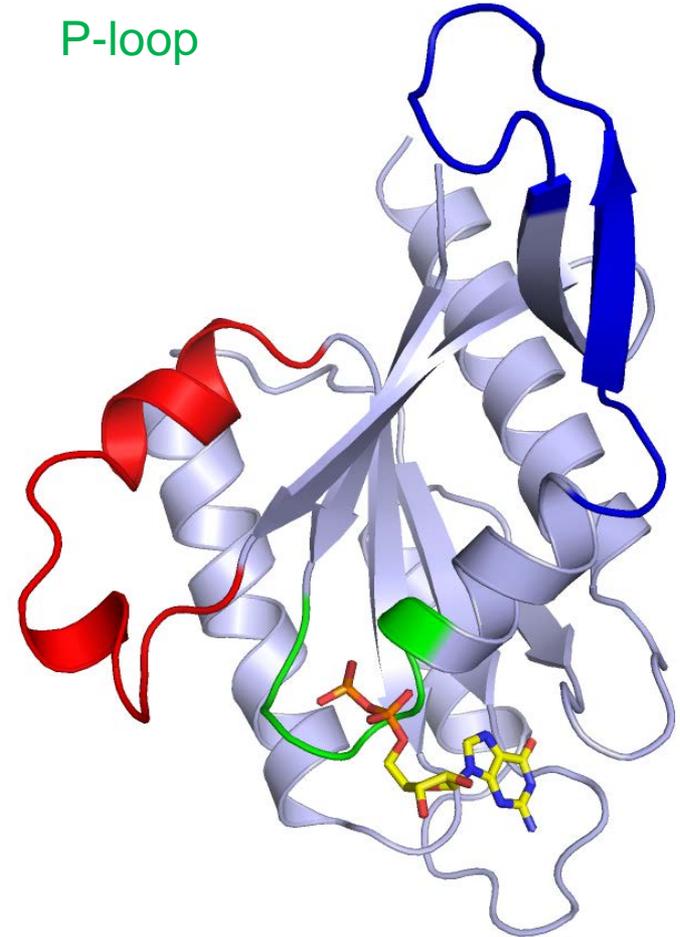
# Crystal structures of KRAS in complex with GDP and GMPPNP (non-hydrolysable GTP analog)

Switch-I   Switch-II   P-loop



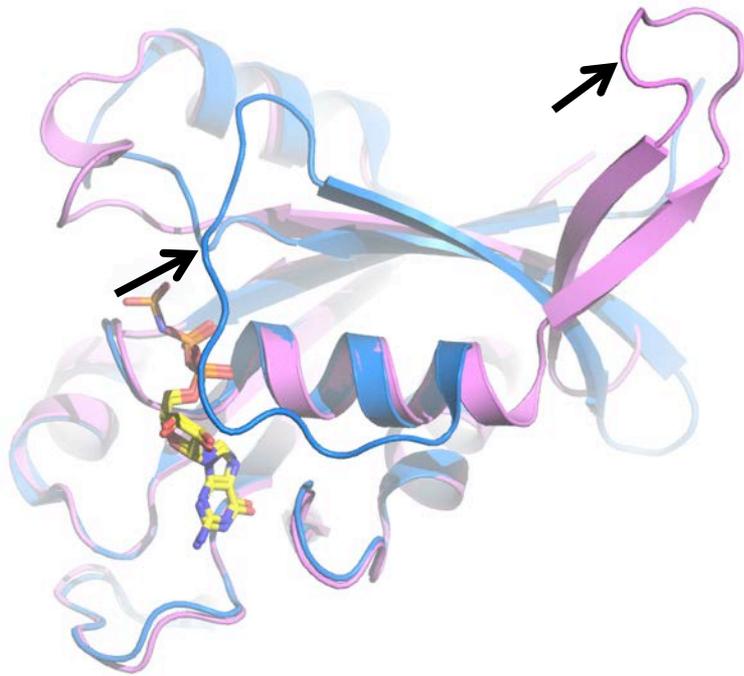
KRAS(1-166)-GMPPNP complex  
at 1.35 Ang

*Beryllium CRO*

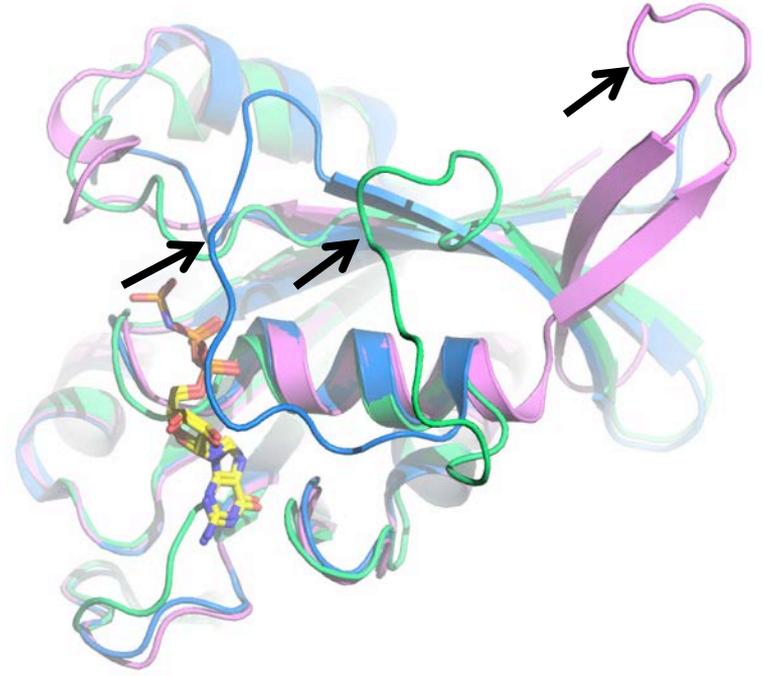


Full-length-KRAS-GDP complex  
at 1.6 Ang

# Comparison of Switch-I conformations suggests large inherent flexibility



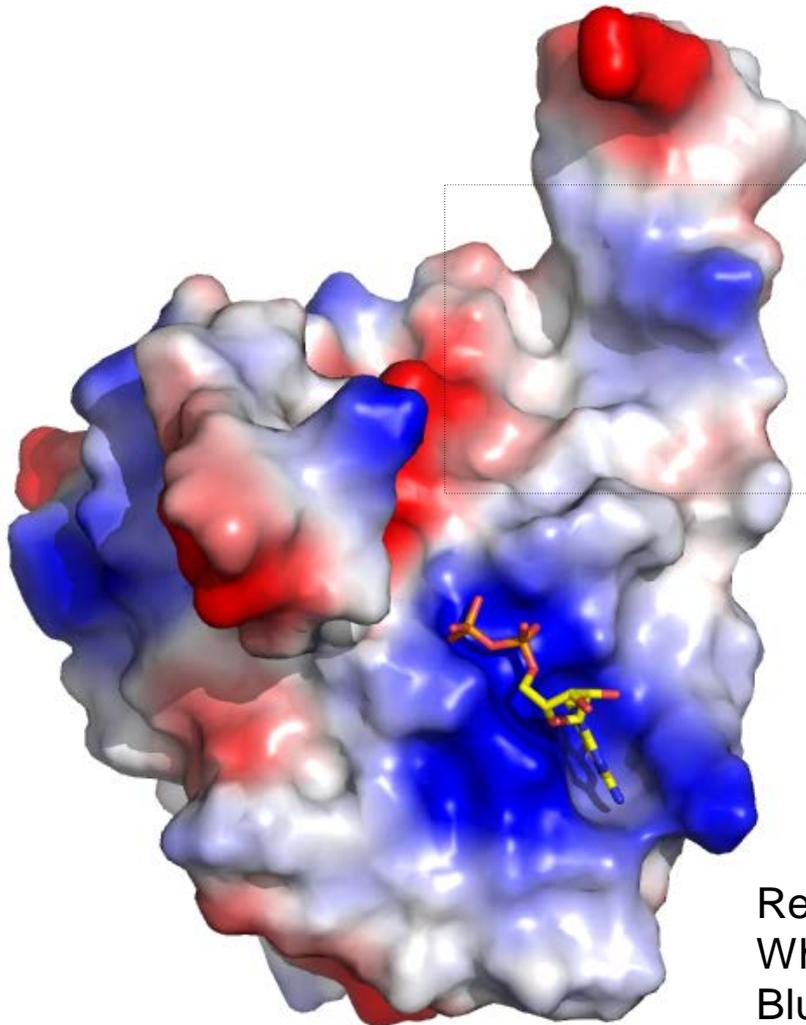
KRAS-GDP complex  
KRAS-GMPPNP complex



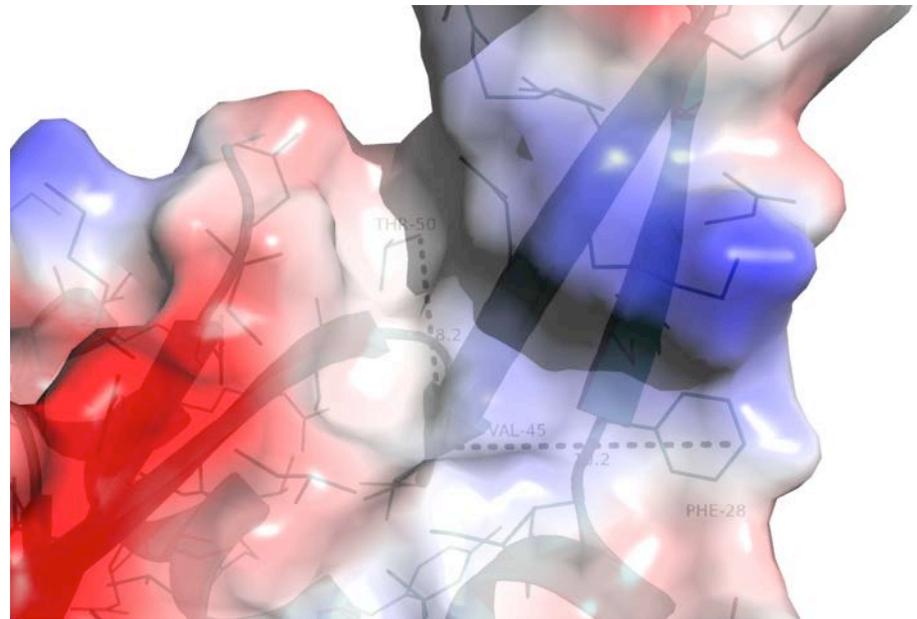
KRAS-GDP complex  
KRAS-GMPPNP complex  
Allosteric HRAS-SOS complex

# Structural analysis of KRAS-GDP complex

## Electrostatic surface representation of KRAS-GDP complex



Red - negative charge  
White - neutral  
Blue - positive charge



Enlarged view of the hinge region

## **Agni Ghosh and Steve Almo (Albert Einstein College of Medicine)**

- KRAS<sup>G12D</sup>-GAP complex (RASA1 and NF1)

## **Carla Mattos (Northeastern University)**

- Calmodulin-KRAS complex

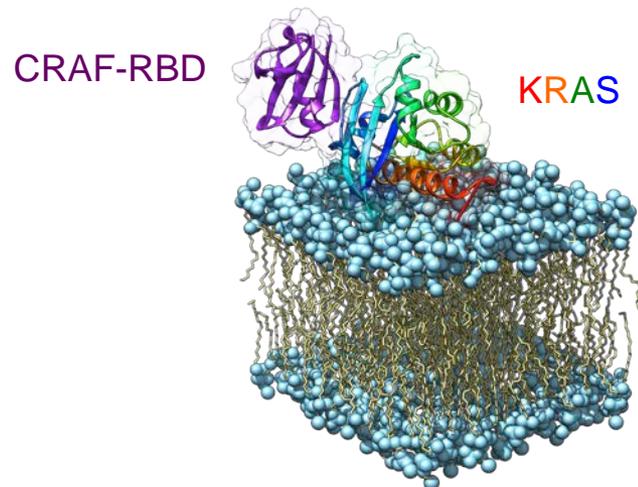
## **Ken Westover (UT-Southwestern)**

- GDP bound structures of KRAS oncogenic mutants

## **National Magnetic Resonance Facility at Madison (NMRFAM)**

- NMR structure of processed full-length KRAS bound to nanodisc

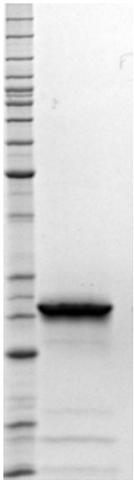
## Targets & Assays



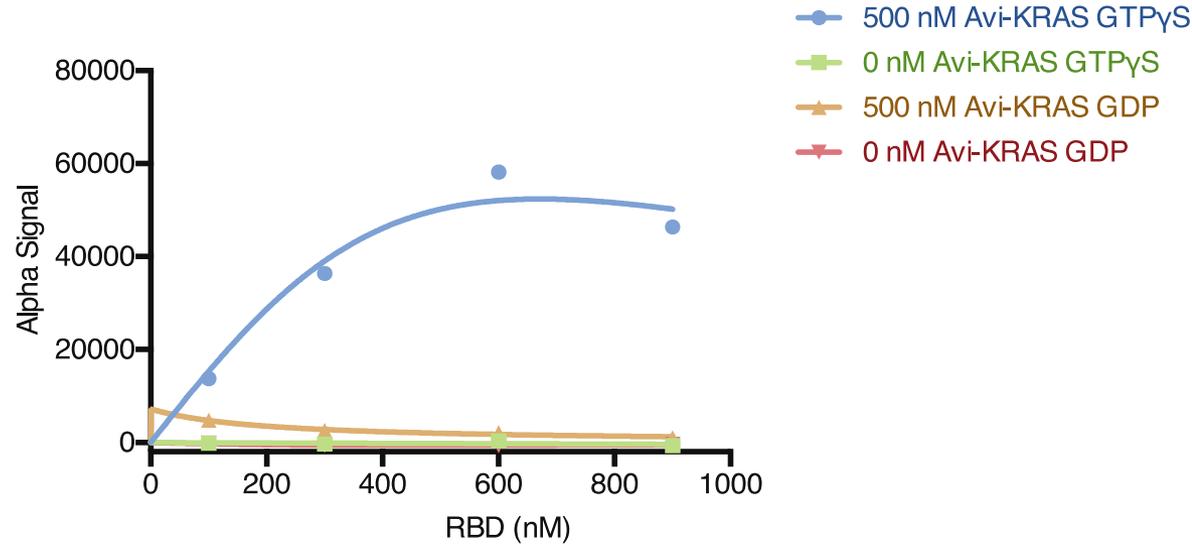
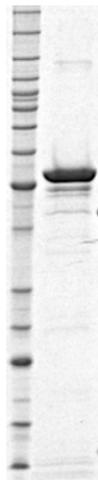
**Matt Holderfield**

# KRAS-effector binding AlphaScreen assay

Avi-KRAS



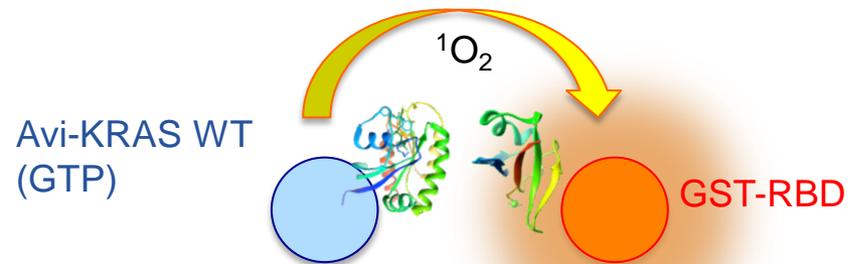
GST-CRAF-RBD



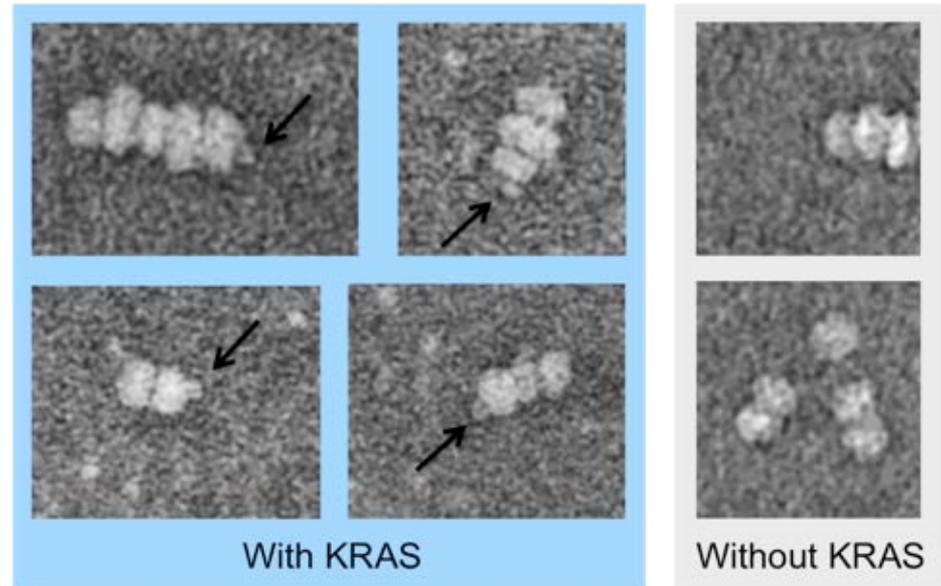
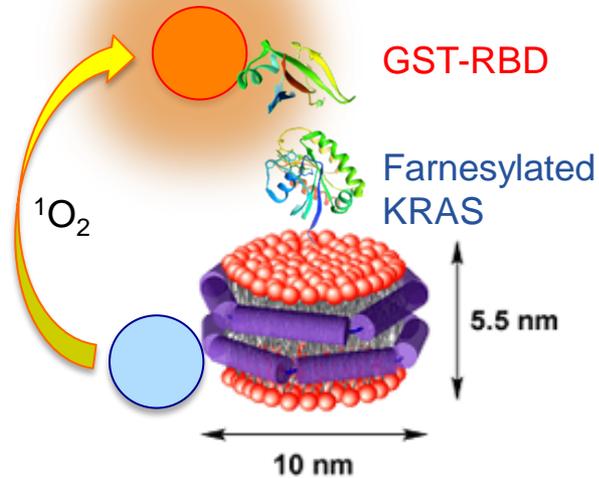
Purified, recombinant protein used for KRAS:CRAF-RBD binding assay in vitro using AlphaScreen technology

Binding is highly GTP dependent

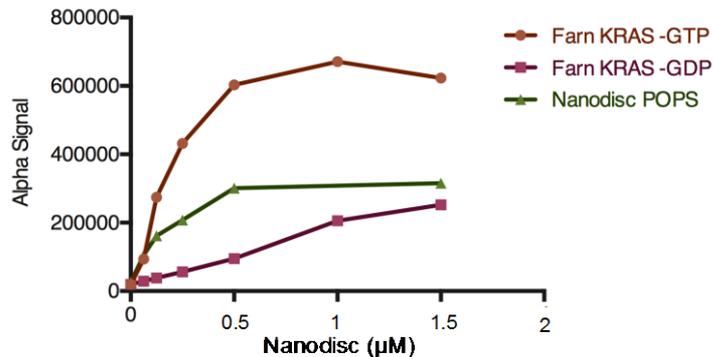
Assay is ready for pilot screening



# KRAS Nanodisc complex



Ulrich Baxa and Que Van



Belt protein and lipids self-assemble into bilayer disk structures

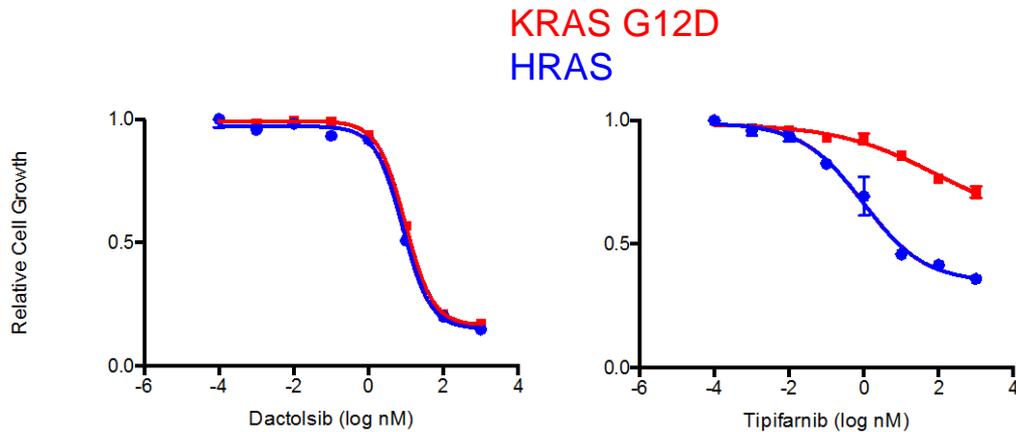
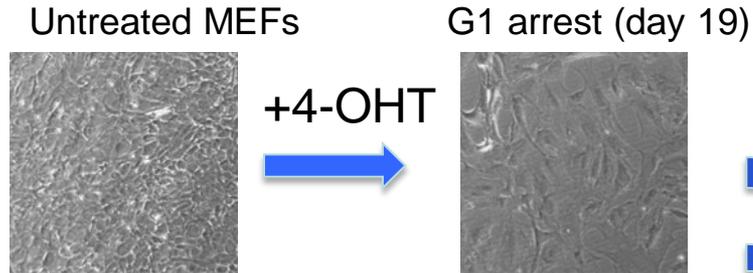
Farnesylated KRAS self-associates with the lipid surface of nanodiscs

His6 tag on the belt protein is available for tag-based binding assays such as AlphaScreen

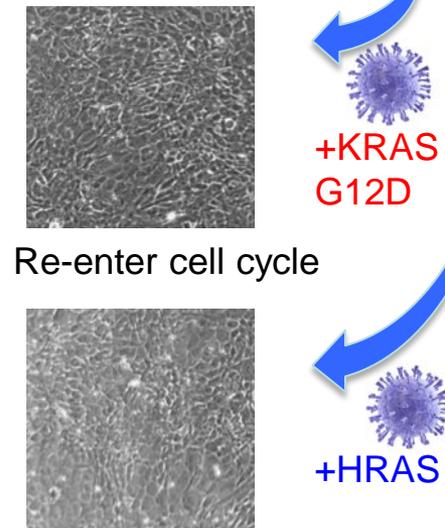
# RAS-Dependent MEFs

## HRAS<sup>-/-</sup> NRAS<sup>-/-</sup> KRAS<sup>lox/lox</sup> MEFs

Drosten M, Dhawahir A, Sum EY, Urosevic J,  
Lechuga CG, Esteban LM, Castellano E,  
Guerra C, Santos E, **Barbacid M.**  
EMBO J. 2010



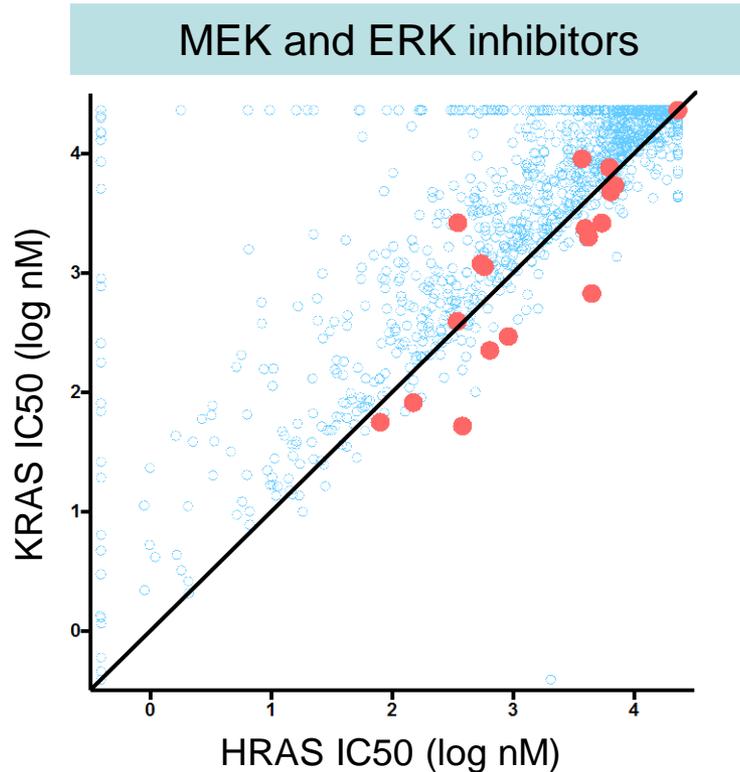
drug  
sensitivity



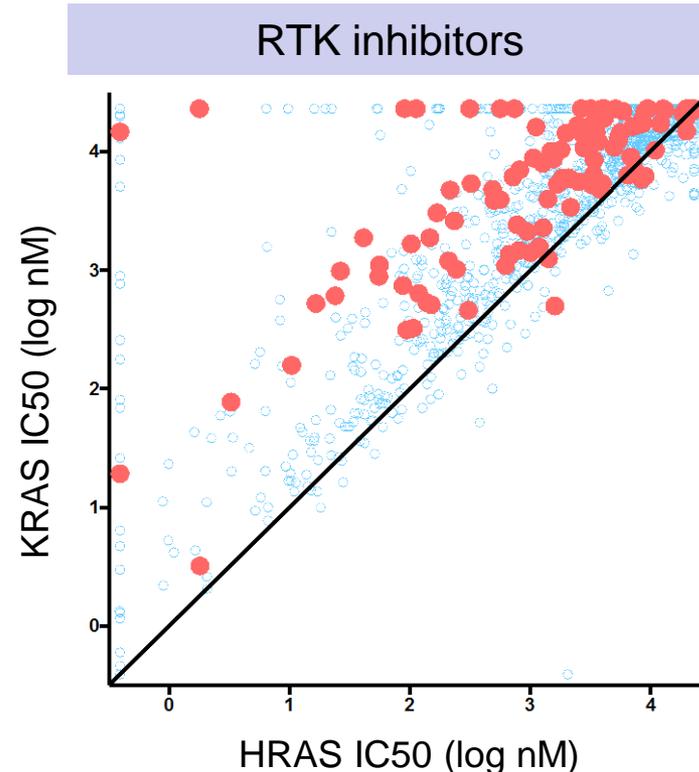
In the queue:

KRAS G12V	KRAS G12A	KRAS Q61R	RB <sup>-/-</sup>
KRAS G12C	KRAS WT	BRAF V600E	P53 <sup>-/-</sup>

# RAS-dependent MEF Pilot screening results

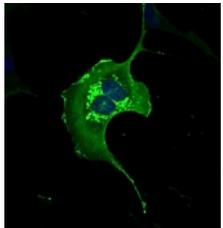


MEK/ERK inhibitors are equipotent in HRAS-WT and KRAS G12D

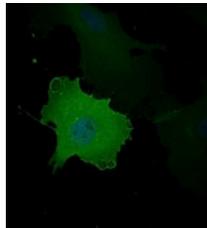


Receptor tyrosine kinase inhibitors preferentially inhibit HRAS-WT but not KRAS-G12D

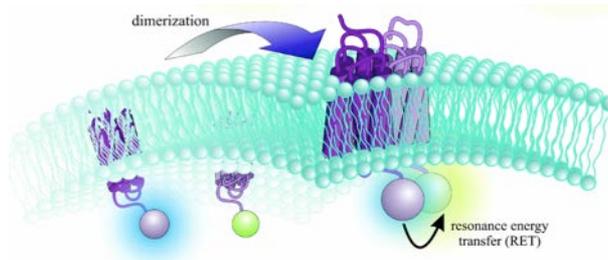
## Multimerization and Localization Assays



Ras-GFP

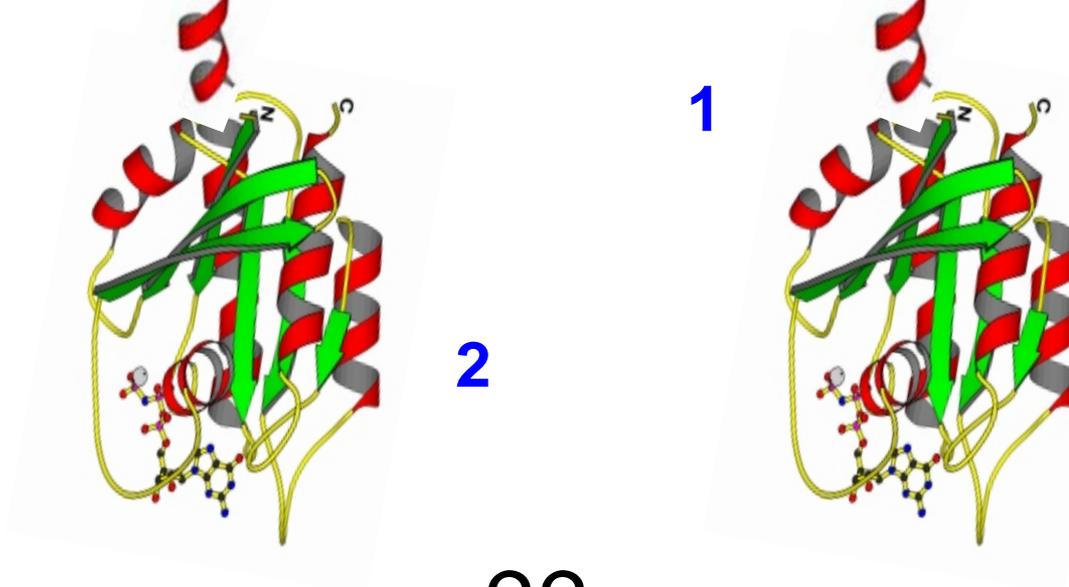


+ FTI



Tommy Turbyville

# Assays for Compounds that Disrupt KRAS Signaling



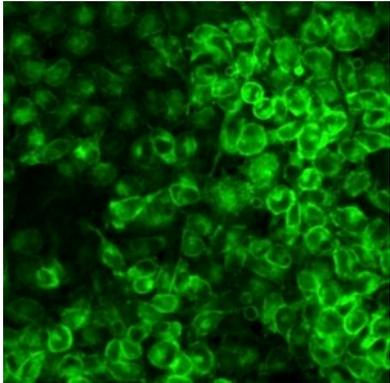
??

- 1) Membrane localization
- 2) Multimerization assay

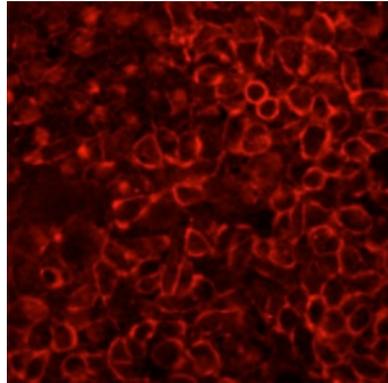
Signal

# Localization Assay

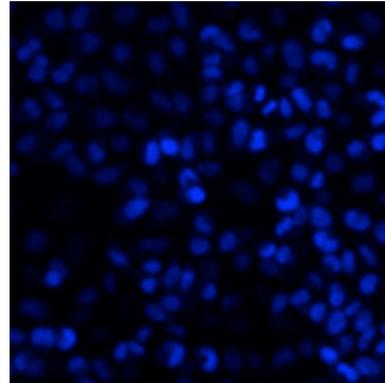
GFP-KRAS-G12V



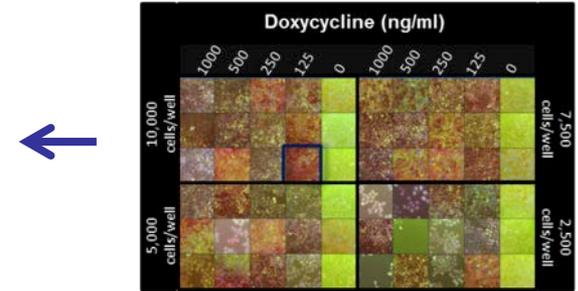
Membrane



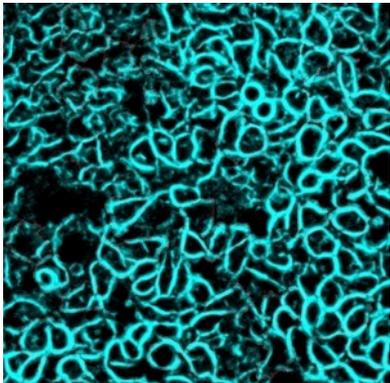
Nuclei



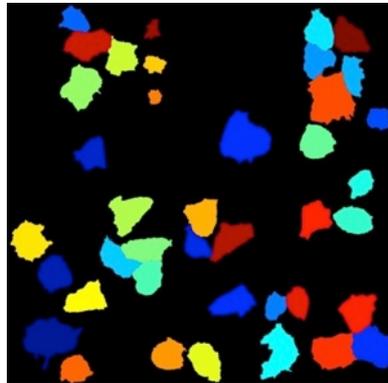
## Multiwell confocal imaging



## Segmentation and Data Analysis →



Probability Map

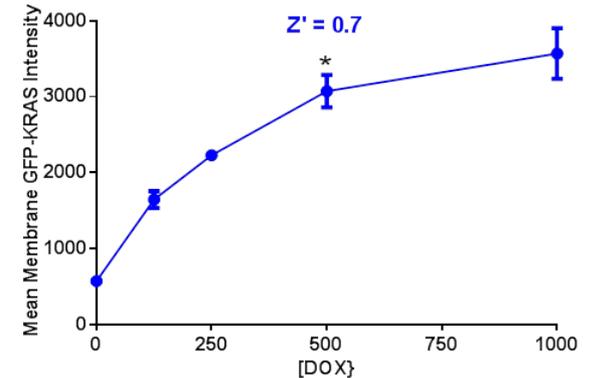


Mask

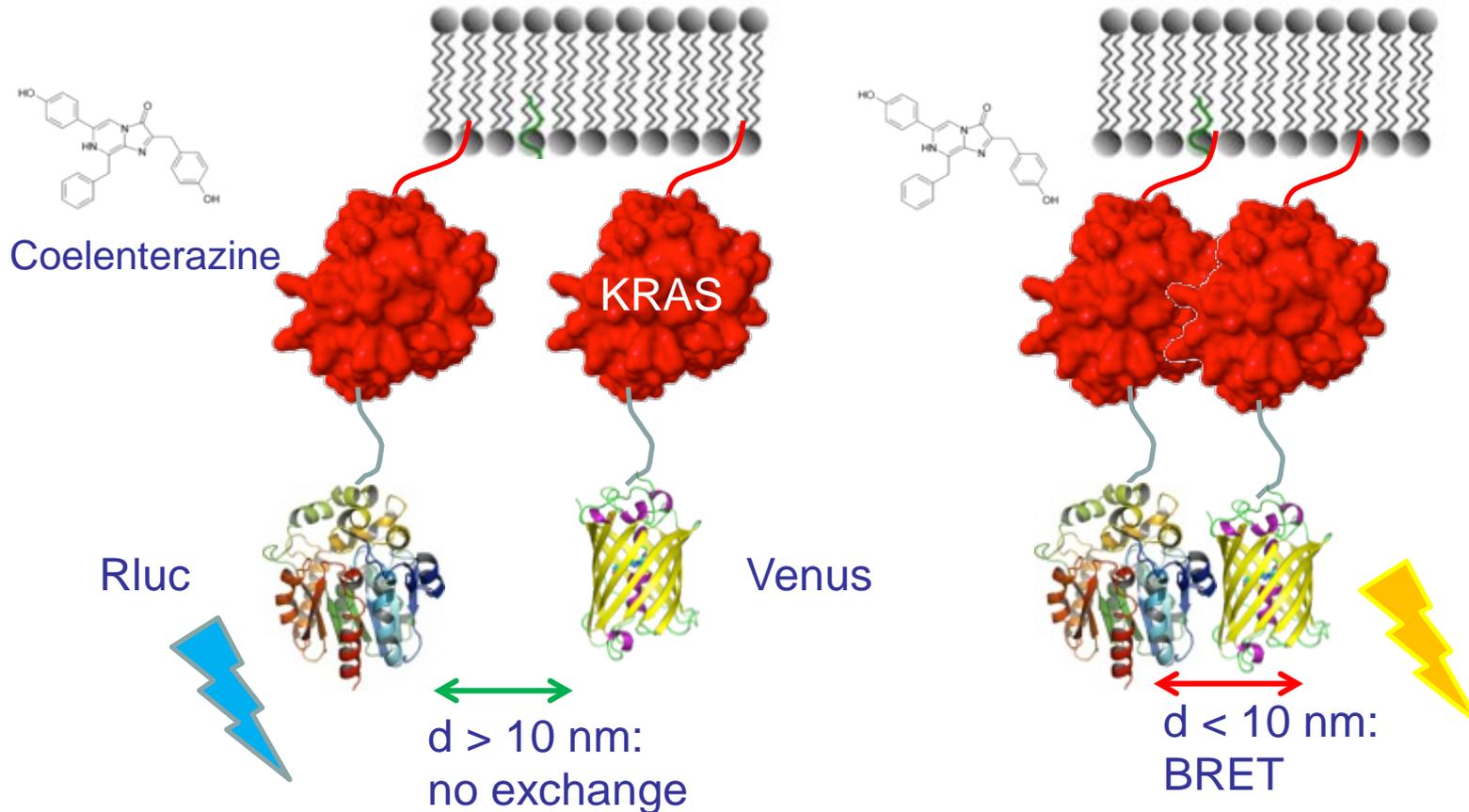


Segmented Boundary

## Membrane Localized GFP Signal



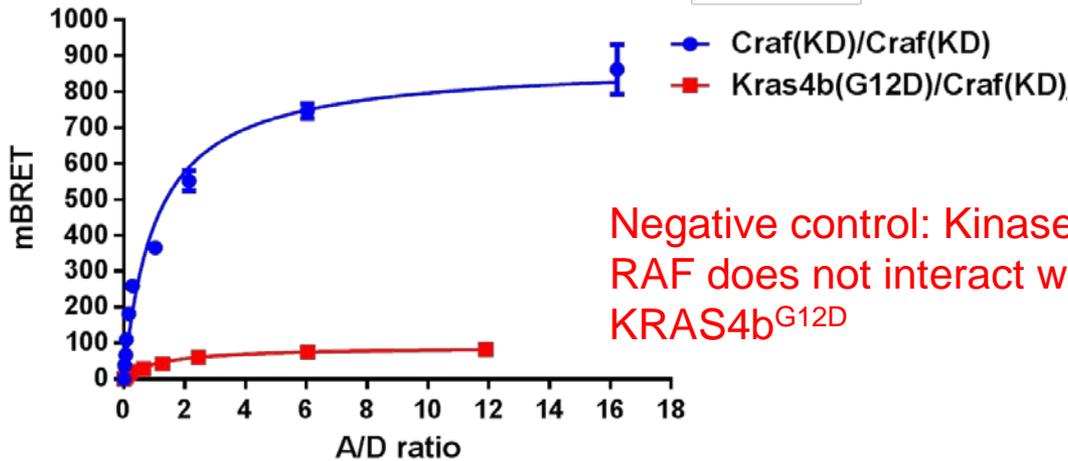
# Bioluminescence resonance energy transfer (BRET)



Goal is to develop a primary assay to screen for inhibitors of KRAS multimerization.

# BRET Control Saturation Curves

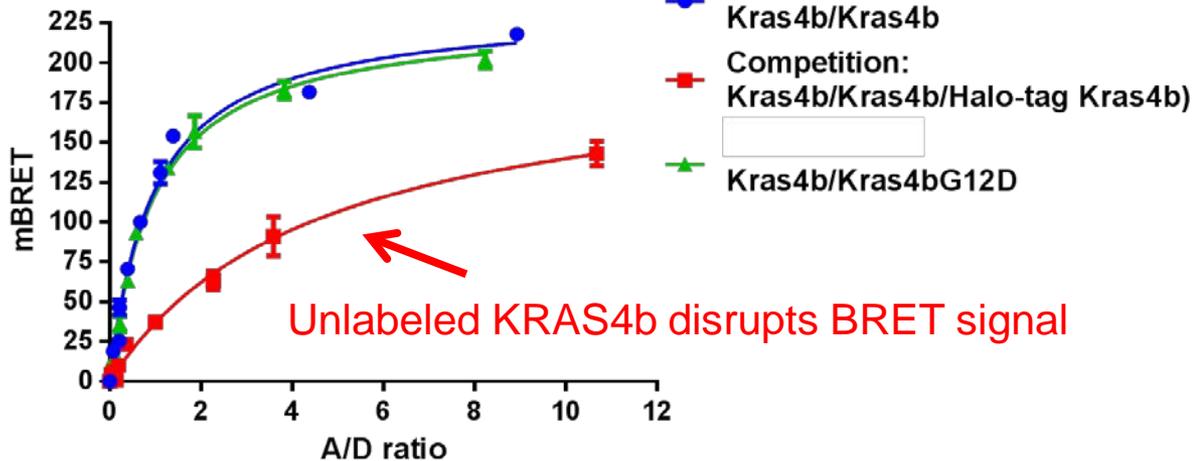
Craf(KD)/Craf(KD) vs. Kras4b(G12D)/Craf(KD)



Kinase domain of RAF serves as a positive control

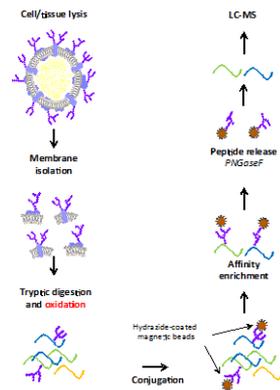
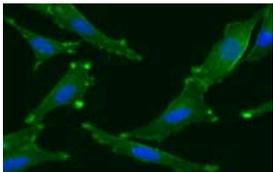
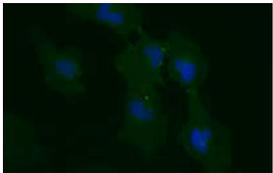
Negative control: Kinase domain of RAF does not interact with KRAS4b<sup>G12D</sup>

Saturation Curves



Unlabeled KRAS4b disrupts BRET signal

## RAS Cell Surface

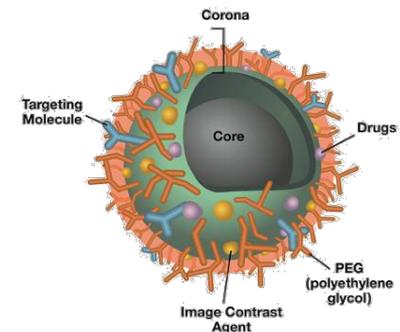
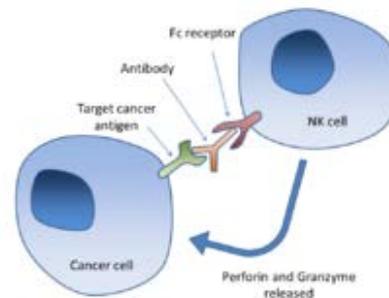
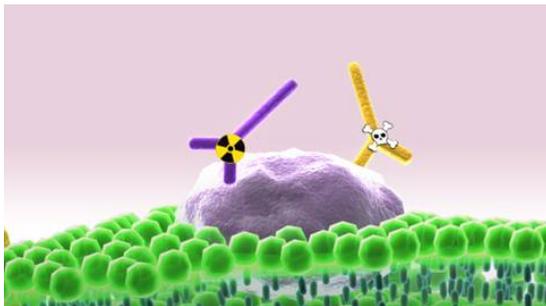


**Gordon Whiteley**

# Mapping the surface of KRAS cancer cells

- **Objectives and rationale**

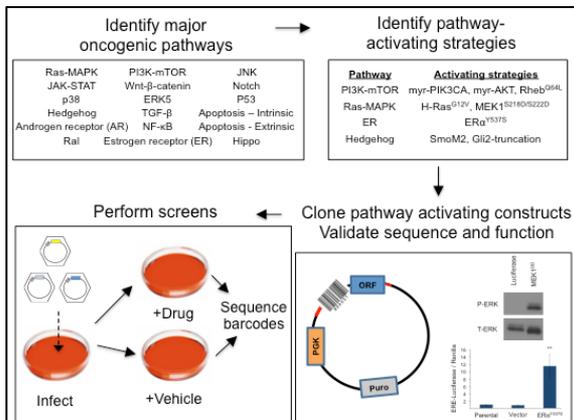
- Survey the surface of KRAS-driven cells to generate a list of proteins differentially associated with KRAS phenotype
- These KRAS associated cell surface determinants could represent new targets for
  - antibody-mediated attack
  - immune based therapy
  - nanoparticle delivery



## Cell Surface Strategy

- **Adapt cell surface protein labeling for mass spec proteomic analysis of KRAS cells *in vitro* and *in vivo***
  - In house development of “tumor-surface proteomics”
- **Collaborate to use selective panning technologies (phage display) to survey the KRAS cell surface**
  - Robert Rottapel and Sachdev Sidhu, Univ Toronto
  - Renata Pasqualini, Univ New Mexico (December visit to FNL)
- **Use RNA seq and ER-polysome profiling to predict protein complement on KRAS cell surface**
  - Martin McIntosh, FHCRC
- **Bioinformatic Approaches**
  - Renata Grifantini, Externautics, Italy (December visit to FNL)
- **Immunotherapy Workshop**
  - Elizabeth Jaffe and Bob Schrieber to help organize (at 2015 AACR)

# Enabling the Community: RAS Reference Reagents and Cancer.gov/RAS



Home > Research & Funding > NCI Research Priorities and Budget > The RAS Initiative

**RAS Central**

One way we can help solve the 30-year challenge of how to treat RAS-driven cancers is to build an open model of collaboration among government, academic, and industry researchers that will re-energize efforts to develop RAS therapeutics. Whether you are a dedicated RAS expert or curious researcher, we encourage you to help us advance our research by being part of the RAS community.

**RAS Dialogue**  
Post comments, ask questions, and share information with RAS experts.

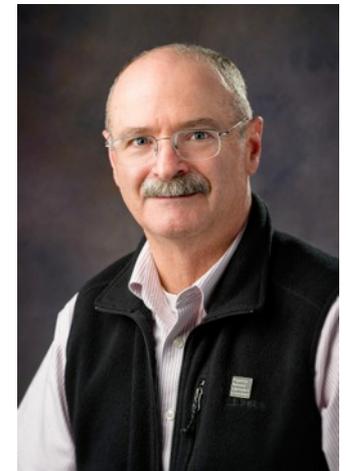
**RAS Events & Videos**  
Explore information for upcoming and past RAS-related workshops, seminars, and conferences.

**RAS Pathways & Reagents**  
Learn about and purchase the latest RAS pathways and reagents.

**RAS Tools & Resources**  
Find everything from the RAS Collaboration Assessment and Visualization Tools to interactomes and



Dom Esposito



Jim Hartley

# RAS Reference Reagents

## External reagent requests (since July 2014)

- 15 requests for RRR reagents (clone sets and other items)
- 8 approved TSAs, 2 approved MTAs, 5 others in progress
- Fred Hutch, Baylor, Munich, Stanford, MIT, Broad, Northwestern, CRUK

## RAS Initiative support—344 constructs generated since July 2014

- 212 constructs for protein expression (TBU-C/Project 1)
- 79 constructs for Project 3
- 53 constructs for TBU-Z

## RAS Pathway clone set underway (181 clones)

## Kris Wood collaboration underway (200+ cancer toolkit clones)

In English | [En español](#)

## National Cancer Institute

at the National Institutes of Health

We Can Answer Your Questions  
1-800-4-CANCER

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### The RAS Initiative



#### The RAS Initiative

- ▶ [The Problem](#)
- ▶ [A Collaborative Solution](#)
- ▶ [RAS Projects at FNLCR](#)
- ▶ [RAS Laboratory Groups](#)
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### Development of the RAS Initiative at the Frederick National Laboratory for Cancer Research (FNLCR)

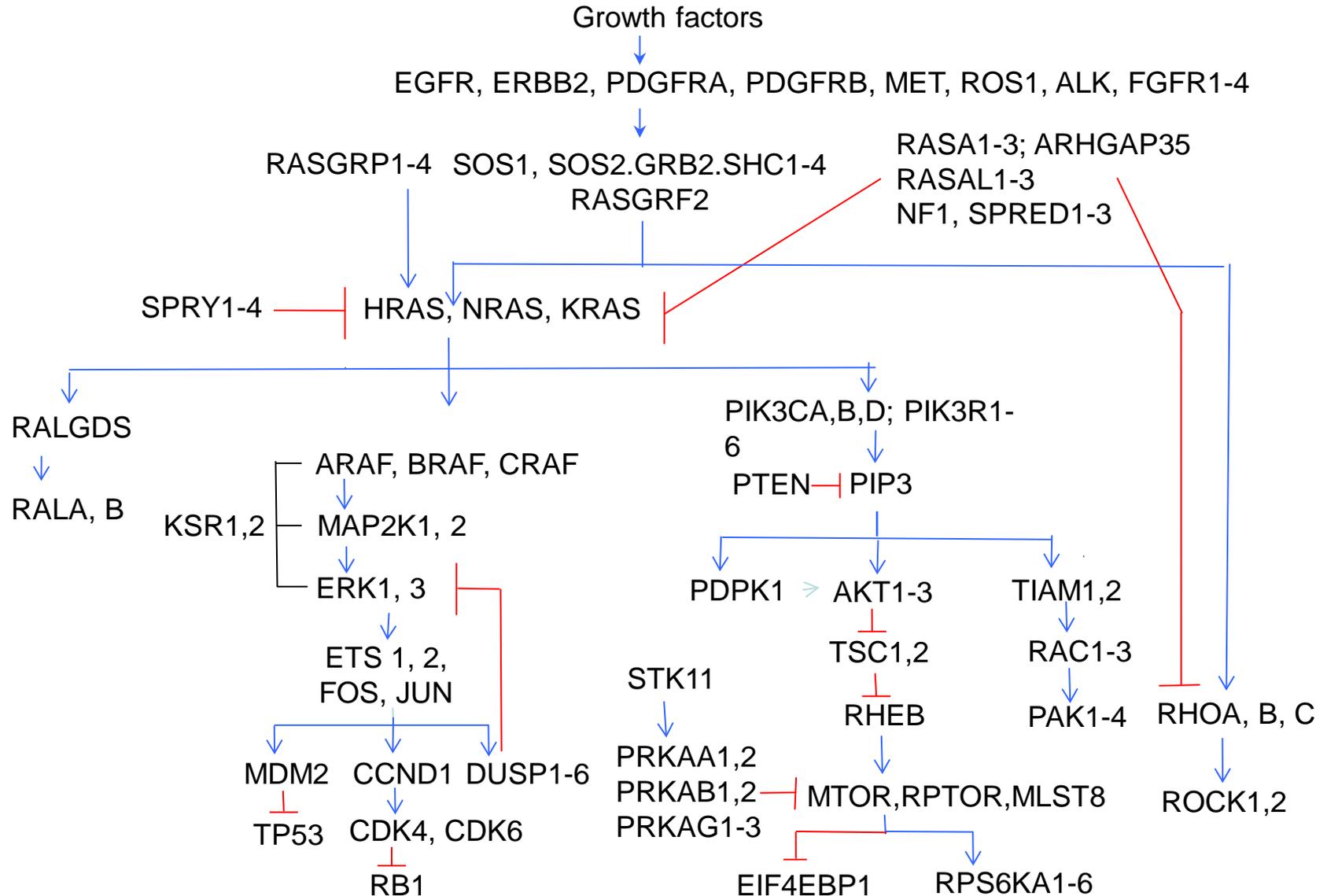
Since the early 1970s, the National Cancer Institute (NCI) has been responsible for a contract that supports the only Federally Funded Research and Development Center (FFRDC) devoted principally to biomedical research. Located on a government campus in Frederick, MD, the FFRDC has provided a variety of laboratory services to the scientific community, performed research in response to national needs, and supervised subcontracts for the NCI for over 40 years.

In 2011, following a suggestion by the NCI's National Cancer Advisory Board, NCI Director Harold Varmus named the operational laboratory arm of the FFRDC Frederick National Laboratory for Cancer Research (FNLCR) and established an advisory committee (now called the



[Message from NCI  
Director Harold Varmus](#)

# A RAS Initiative view of RAS signaling: An invitation for discussion at [Cancer.gov/RAS](http://Cancer.gov/RAS)



# RAS Community feedback on RAS signaling model:

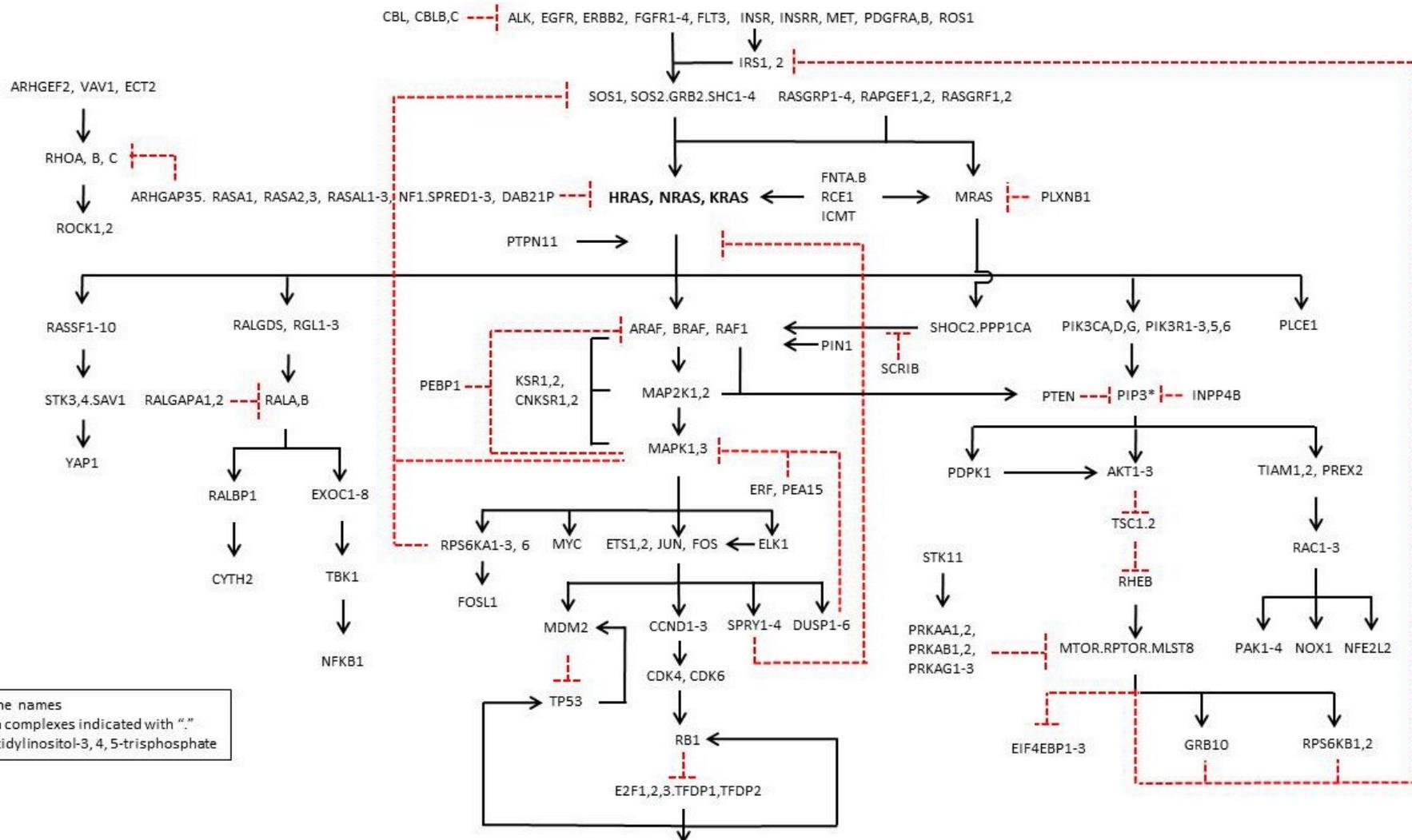
## RAS Pathway v2.0

January 13, 2015 by Frank McCormick

Our original pathway diagram (posted on October 22, 2014) has been altered incorporating suggestions from the following investigators:

- Phil Stork (stork@ohsu.edu) suggested adding RASGRP1, but we left out PDZGEF1 which (I think) is a RAP1 GEF, not a RAS GEF. Any input on this would be welcome.
- Julie Irving (julie.irving@ncl.ac.uk) suggested adding FLT3 (also suggested by Kevin Shannon shannon@peds.ucsf.edu), PTPN11 (also suggested by Ben Braun, UC San Francisco) and CBL. On Ben's advice, PTPN11 was kept vague with a positive arrow to RAS activation of RAF, without a clear target.
- Manuela Baccharini (manuela.baccharini@univie.ac.at) noted that MAP2K1 activates PTEN by sending it to the plasma membrane. RAF1 inhibits ROCK2, but was not included in our updated diagram because the drawing was getting too complicated. This may be fixed in a subsequent version.
- Mike Nickerson (nickersonml@mail.nih.gov) included negative feedback to IRS1 and 2 from the PI3 kinase pathway.
- Anne Goriely (anne.goriely@imm.ox.ac.uk) suggested adding the MRAS (RRAS3) –SHOC2.PPP1CA pathways that activate RAF kinase and ERF, an ETS-family protein that inhibits ERK1 (aka MAPK3) and 2 (MAPK1).
- Eric Collisson (eric.collison@ucsf.edu) suggested adding the RHO GEF ARHGEF2, aka GEF-H1. Rob Rottopel has recently shown this also acts on the MAPK pathway via interaction with KSR1, but I couldn't find a way of representing that in this version.
- Jim DeCaprio (james.decaprio@dfci.harvard.edu) suggested adding cyclin D2 (CCND2) and 3 (CCND3) in addition to Cyclin D1 (CCND1) as MAPK targets. Ping Lu (klu@bidmc.harvard.edu) suggested adding PIN1 as an activator of RAF1 kinase (aka CRAF). It could have been added at many other sites (see Cell Res 24, 1033, 2014 for a review by Zhimin Lu and Tony Hunter).
- Michael Tainsky (mat@wayne.edu) noted that AP2 is essential for RAS transformation, but I couldn't find a direct link to the RAS pathway. Any suggestions are welcome.
- Maria Zajac-Kaye (mzajackaye@ufl.edu) suggested adding E2F target genes, including thymidilate synthase (TYMS). Target genes that appear on other parts of the pathway were not listed, to keep it simple.
- Ramon Parsons (ramon.parsons@mssm.edu) suggested adding the RAC GEF, PREX2.
- Shyam Biswal (sbiswal@jhu.edu) and Carola Neumann (neumannc@upmc.edu) suggested adding NFE2L2 (aka NRF2), and NOX1.
- Eric O'Neill (eric.oneill@oncology.ox.ac.uk) suggested adding a mini-version of the Hippo pathway (RASSF, MST [STK3 and 4], SAV1, YAP1).
- Joe Ramos (joeramos@hawaii.edu) and Naoto Ueno (nueno@mdanderson.org) suggested adding PEA15.
- Larry Feig (larry.feig@tufts.edu) suggested adding RASGRF1 and 2.
- Howard Crawford (crawford.howard@mayo.edu) proposed adding VAV1, ECT2 and TIAM1. Howard also suggested DOCK10, but I wasn't sure where to put it. Suggestions welcome!
- Mike White (michael.white@utsouthwestern.edu) suggested adding EXOC1-8 connected to the RALA, B node and CNKSR1, CNKSR2, SHOC2 connected to the RAF node.
- Karen Cichowski (kcichowski@rics.bwh.harvard.edu) suggested adding DAB21P and INPP4B.
- Andrew Sharrocks (a.d.sharrocks@manchester.ac.uk) suggested adding ELK1.
- Mark Philips (philim01@nyu.edu) suggested adding FNTA and B, RCE1 and ICMT1.
- Debbie Morrison (morrisond@mail.nih.gov) suggested adding a feedback arrow from MAPK1,3 to the RAFs.
- Channing Der (cjder@med.unc.edu) suggested adding MYC as a MAPK1/3 substrate. He also included RGL, RGL2 and RGL3 with RALGDS as another effector that links RAS with RAL. Additionally, the RALGAPs, RALGAPA1 and RALGAPA2, and PLCE1 are included as RAS effectors.
- Philippe Roux (philippe.roux@umontreal.ca) pointed out errors in the pathway as originally drawn: "The pathway should indicate that RPS6KB1 and RPS6KB2 are targets of mTOR (they are the p70 S6Ks). The related p90 S6Ks (RPS6KA1, RPS6KA2, RPS6KA3, RPS6KA6) are actually targets of MAPK1/3, and should be transferred to that branch of the pathway." Steen Hansen (steen.hansen@childrens.harvard.edu) also pointed out these errors and suggested we add FOSL1 downstream of RPS6KA1,2,3 and 6. Additionally, there are two more isoforms of EIF4EBPs, and thus the pathway should indicate EIF4EBP1-3.
- Julian Downward (julian.downward@cancer.org.uk) also noted some errors, "You should have PIK3CA, PIK3CD and PIK3CG here, but not PIK3CB as p110beta does not interact with RAS. Also, for regulatory subunits, there should be PIK3R 1, 2, 3, 5, and 6, but not 4 – this is VPS15, the regulatory partner of VPS34. PIK3R 5 and 6 are the regulatory subunits of p110gamma, so are fine to have here."

# A RAS Initiative view of RAS signaling: v 2.0 at [Cancer.gov/RAS](http://Cancer.gov/RAS)



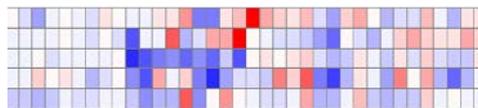
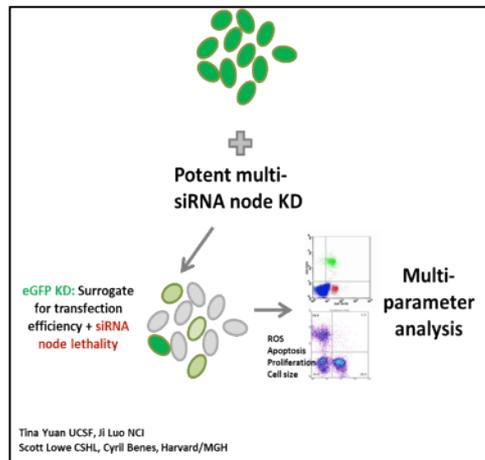
## **RAS Initiative Oversight Follow-up**

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- **Publish protocols for production of fully processed KRAS protein and associated structural studies and assays.**
  - Manuscript to be submitted by end of February 2015
- **Establish process for collaboration and providing reagents to community**
  - Processed KRAS will be provided to collaborators
  - Protocols and reagents provided to others
- **Optimize RAS-less MEF screen: understand and eliminate sources of variation**
  - Use validation inhibitor panels at NCATS
  - Evaluate conditional oncogenic KRAS MEFs (Tuveson)
- **Node-knockdown-based (SiREN) approach will produce a large data set that will be discussed at the next Working Group meeting.**
  - Experiments completed, data analysis ongoing

# RAS Ad Hoc Working Group Meeting (October 31, 2014)

## – Action Items



Rachel Bagni



Bob Stephens

- **Node-knockdown-based (SiREN) approach will produce a large data set that will be discussed at the next Working Group meeting.**
  - Experiments completed, data analysis ongoing

# RAS Initiative Postdoctoral Fellowships

- **Postdoc Program**
  - *Pancreatic Action Network/FNL Fellows*
    - *Lynn McGregor (Shokat lab)*
    - *John Hunter (Westover lab)*

## THE PANCREATIC CANCER ACTION NETWORK AND THE NATIONAL CANCER INSTITUTE'S FREDERICK NATIONAL LABORATORY FOR CANCER RESEARCH COLLABORATE ON TWO 2015 FELLOWSHIPS TO ADVANCE KRAS RESEARCH

A critical area of continuing research aimed at better understanding pancreatic cancer and developing new, more effective ways to treat it is focused on a genetic mutation found in most pancreatic tumors: KRAS.

And now, the Pancreatic Cancer Action Network has formed a unique partnership with the National Cancer Institute's (NCI's) Frederick National Laboratory for Cancer Research (FNLCR) to advance KRAS research. FNLCR is a government-owned, contractor-operated facility devoted exclusively to biomedical research and development.

Our organization and FNLCR have awarded year-long Fellowships that commenced on January 1, 2015, to John Hunter, Ph.D., and Lynn McGregor, Ph.D. Dr. Hunter is working in the laboratory of Kenneth Westover, M.D., Ph.D., at the University of Texas Southwestern Medical Center. His Fellowship is being funded by Ambassador Cynthia Stroum, the Pancreatic Cancer Action Network's Founding Board Chair Emeritus, in memory of her father, Samuel Stroum. Dr. McGregor is conducting her postdoctoral work under the mentorship of Kevan Shokat, Ph.D., at the University of California, San Francisco (UCSF).

# Interactions with the RAS Community

## NCI RAS Initiative at FNL

### Seminars at FNL

Channing Der, UNC  
Ken Westover, UTSW  
Carla Mattos, Northeastern  
Mark Philips, NYU  
Vadim Gaponenko, U Chicago  
Josh Salafsky, Biodesy  
Calvin Kuo, Stanford  
Kris Wood, Duke  
Mariano Barbacid, CNIO Madrid  
Cyril Benes, MGH  
Carolyn Buser, GSK  
Stephen Sligar, U Illinois  
Raffit Hassan, NCI  
Renata Grifantini, Externautics Siena  
Renata Pasqualini, U New Mexico  
Andrew Bradbury, Los Alamos  
Kent Rossman, U North Carolina

### Recipients of RAS Reference Reagents

Chris Kemp, Fred Hutch  
Eric Chang, Baylor  
Silvia Thone, Munich  
Peter Jackson, Stanford  
Tyler Jacks, MIT  
Calvin Kuo, Stanford  
Bill Hahn, Broad/DFCI  
Karla Satchell, Northwestern  
Julian Downward, CRUK

### NIH collaborators

Ji Luo, NCI  
Anton Simeonov, NCATS  
Debbie Morrison, NCI  
Rajat Varma, NIAID

### RAS workshops

Synthetic Lethality, January 6-7 2014  
Pathways, June 11, 2014  
Cell Surfaces, July 23, 2014  
2015 AACR Annual Mtg, agenda pending

### Outside collaborators

Steve Almo, AECOM  
Jim Wells, UCSF  
Channing Der, UNC  
Ken Westover, UT Southwestern  
Carla Mattos, Northeastern  
Steve Sligar, U Illinois  
Jay Groves, UC Berkeley  
Hirsch Nanda, Susan Kreuger, NIST  
John Markley, National Magnetic Resonance Facility at Madison (NMRFAM)  
Kris Wood, UNC  
Immuno-MRM of RAS pathway  
Mandy Paulovich, Fred Hutch  
Steve Carr, Broad  
John Koomen, Moffit  
Tina Yuan, Cameron Pitt, UCSF  
Dave Tuveson, CSH

### Information exchanges

David Weber, U Maryland  
Hirsch Nanda, Susan Kreuger, NIST  
Amanda Altieri, U Maryland  
David Barford, ICR UK  
Bill Sellers, Novartis  
Kurt Auger, GSK  
Paul Cohen, DARPA  
Ian Prior, U Liverpool  
Said Sebti, Moffitt