Frederick National Laboratory
for Cancer Research

Ras Initiative Update

Frank McCormick and Levi Garraway

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

RAS Initiative Accomplishments:

Evaluating Ras dependency

SiREN assay for Ras dependency

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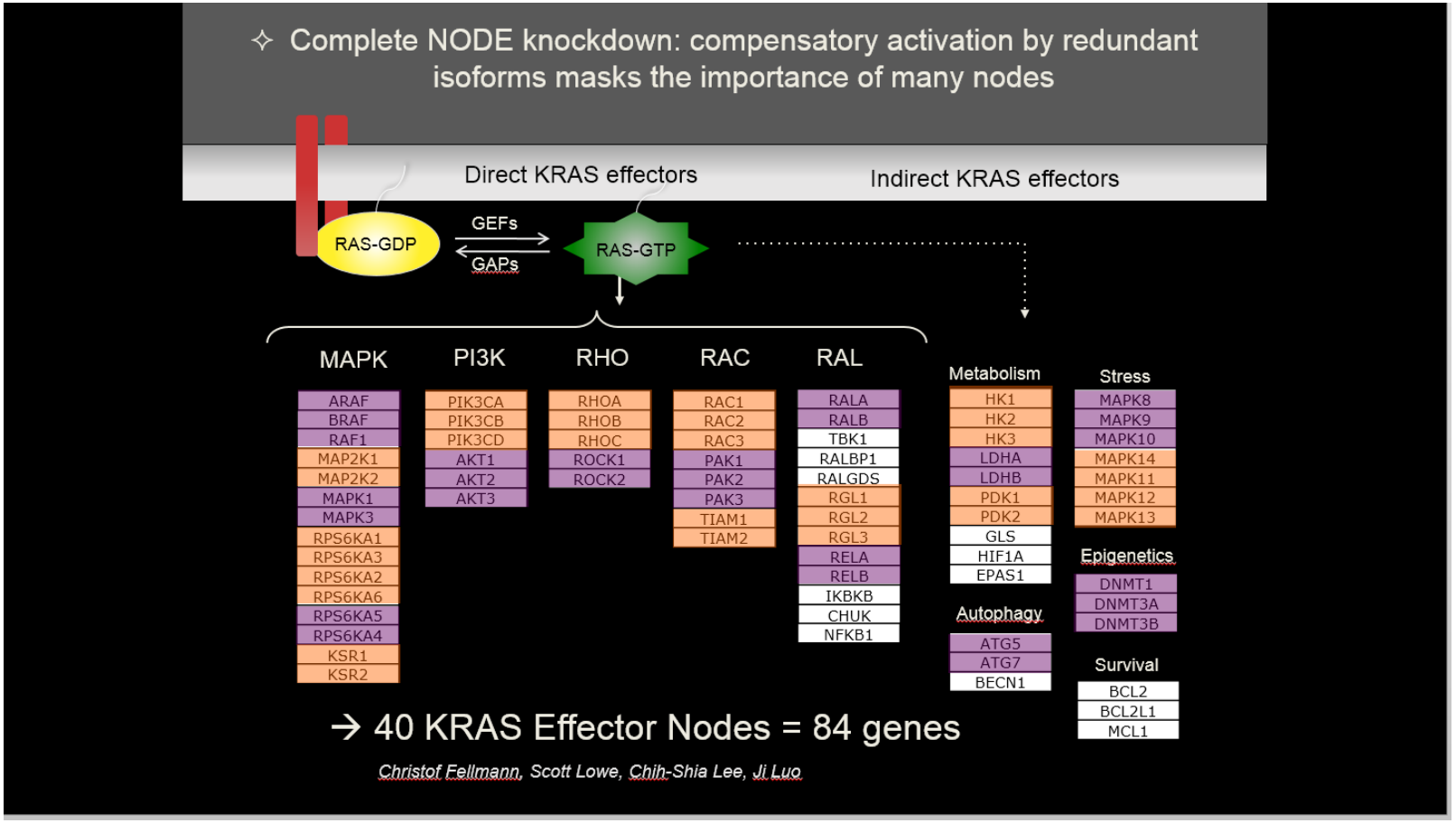

SiREN assay for Ras dependency

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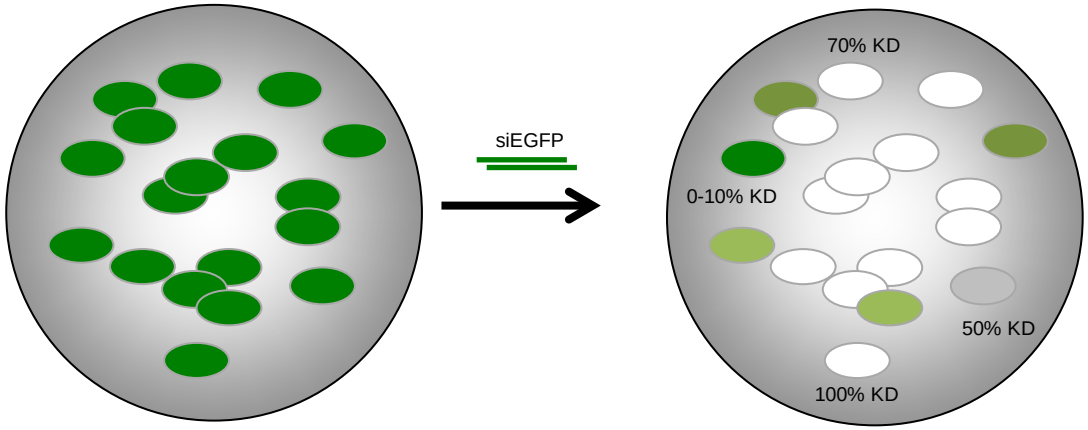
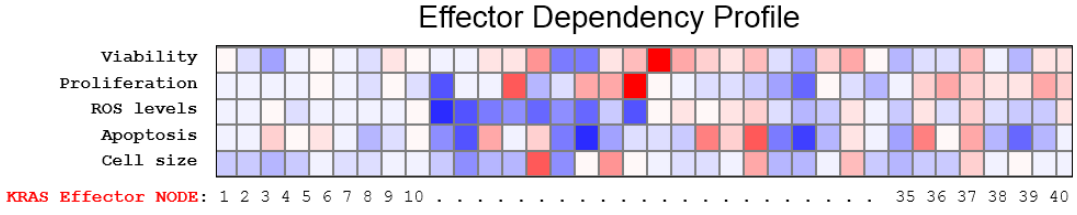
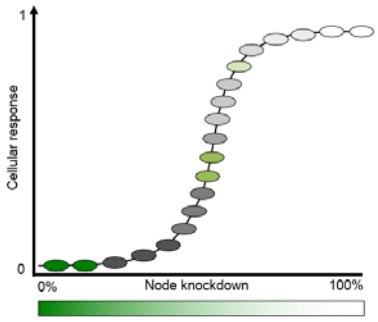
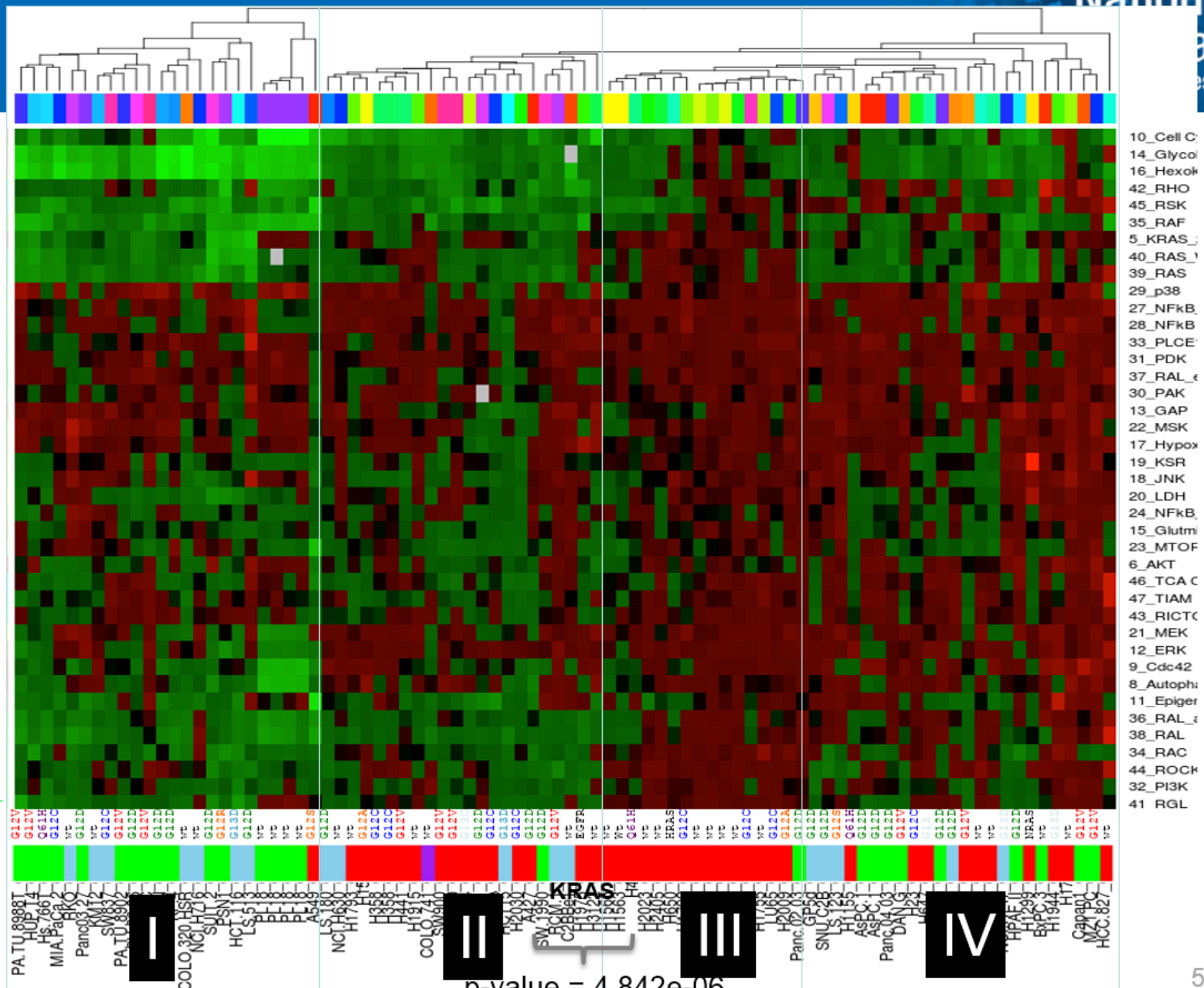






Global assessment of KRAS-effector dependency



*AUC computed by Ming Yi

SiREN assay for Ras dependency

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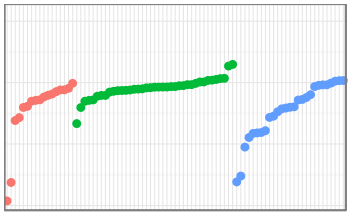


stine

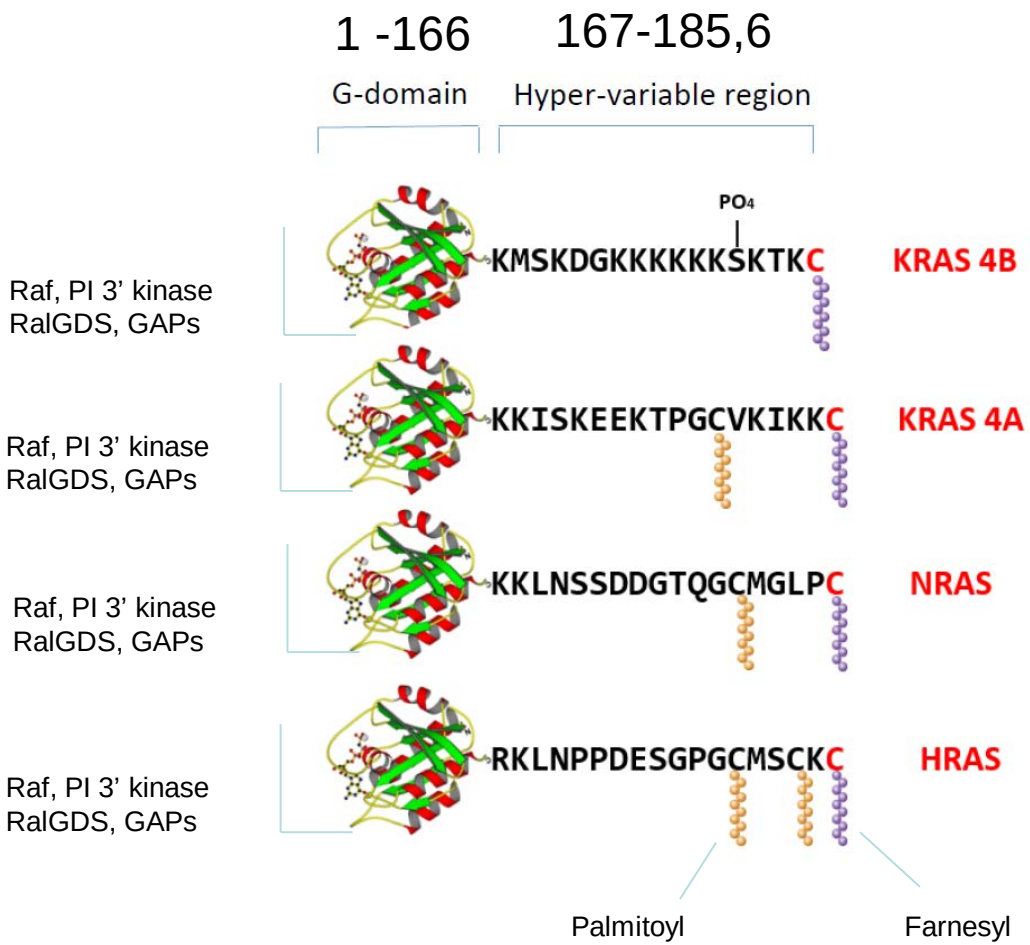
Lung_NSCLC

Pancreas

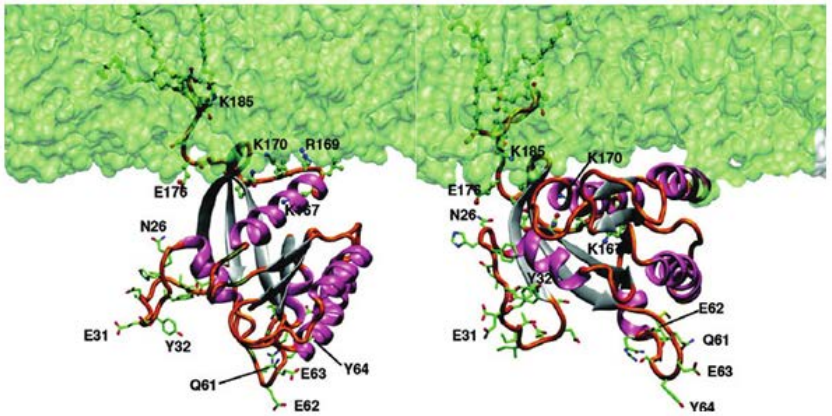
RAS Initiative Accomplishments:

Biophysical and structural analysis

Ras proteins



Fully processed KRAS4b



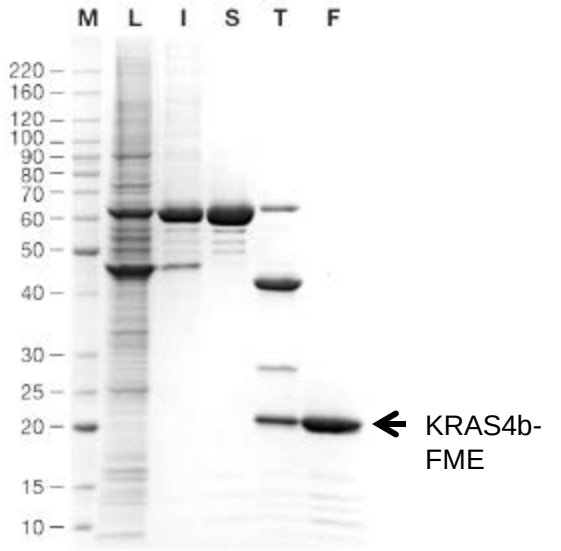
A. Gorfe, U-Texas Houston

Engineering baculovirus for improved production of processed KRAS

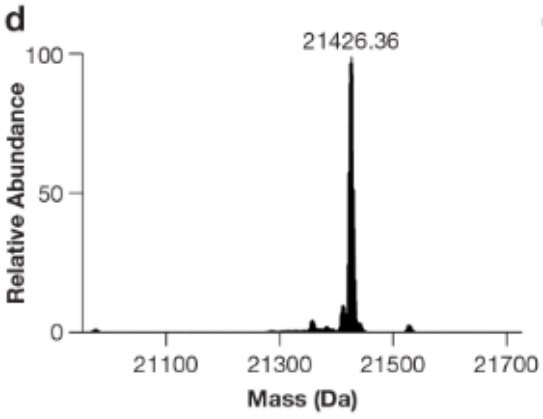
- recombineering used to insert FNTA/FNTB genes into the baculovirus genome
- eliminated issues with coinfection of multiple viruses
- maltose-binding protein (MBP) fusion for greater yield and solubility
- *Trichoplusia ni* (Hi5) insect cells for increased yield

Processed KRAS4b characterization

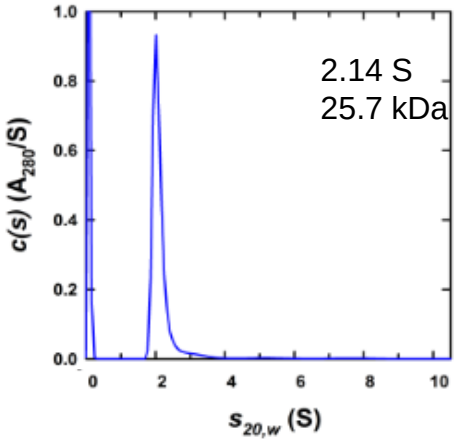
- **Extensive protein characterization**
  - Purified to homogeneity; yield >7mg/L
  - Intact mass
  - Predominantly monomeric
  - Secondary structure equivalent to non-processed KRAS4b
  - Lower thermal stability



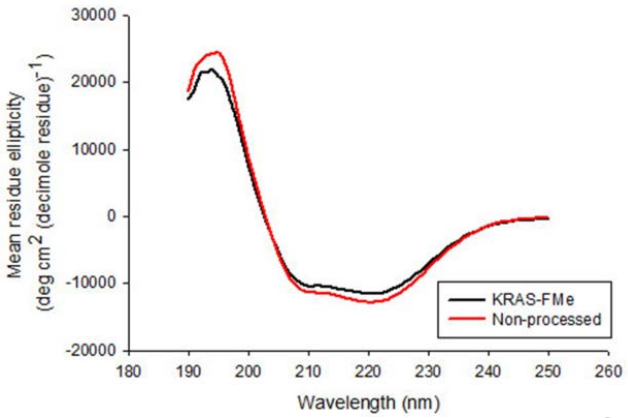
Intact mass analysis



Analytical ultracentrifugation

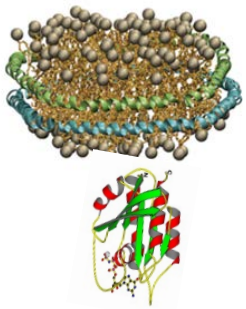


Secondary structure by CD

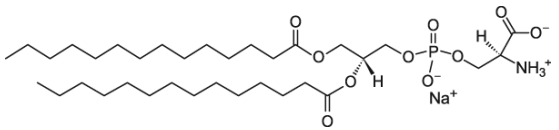


Bill Gillette, Zhaojing Meng, Shelley Perkins, Peter Frank, Pat Alexander, Rodolfo Ghirlando

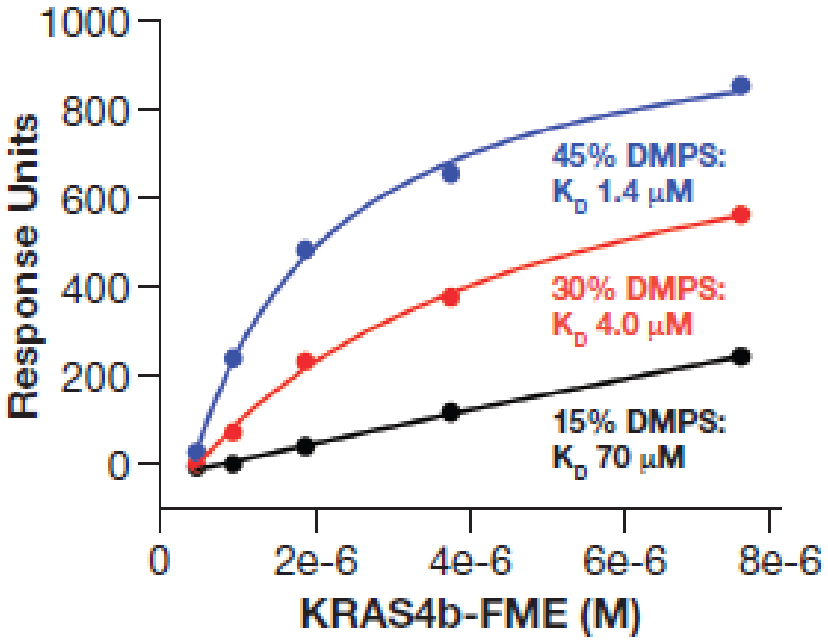
Processed KRAS4b binds to Nanodiscs in a phosphotidylserine-dependent manner



Nanodiscs containing DMPS



1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine



Lakshman Bindu, Que Van FNL; Steve Sligar and Mike Gregory UI-UC

Processed KRAS enables assays, screens and structural analysis in the context of membrane

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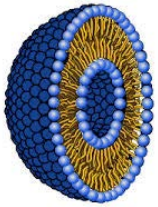
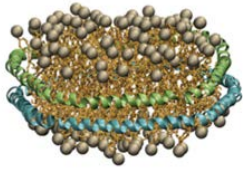
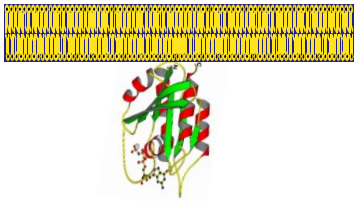


Liposomes and tethered bilayers



Lipid Nanodiscs



Processed KRAS

Characterize reagents

Assays and Screens

Structural analysis

Structures of KRAS and effectors on membranes

Protein QC
Biophysical properties
Membrane interactions

Sligar lab (U-Illinois)
Groves lab (UC-Berkeley)
Heinrich Lab (NIST)

Intrinsic GTP hydrolysis
GAP-stimulated hydrolysis
Effector binding
RBD-KRAS Alpha assays

NMR (NMRFAM)
Hi-res Cryo-EM (NCI)
Crystallography (FNL)

Processed KRAS4b workshop – collaborative opportunities

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- **Frank Heinrich** (Neutron reflectivity of protein/membranes; Carnegie Mellon University)
  - Determine orientation of KRAS4b-FME GDP/GMP-PNP on membrane
  - Impact of Calmodulin on HVR
- **Alemayehu Gorfe** (Modeling of RAS on membranes; University of Texas Health Science Center)
  - Interest in testing predicted dimer interactions
  - Molecular dynamics analysis of FNL extended switch 1 KRAS4b-GDP structure
- **Mitsuhiko Ikura** (NMR of KRAS on membranes; University of Toronto)
  - Analysis of processed KRAS4b by NMR
- **Jeff Perry** (Small angle X-ray scattering; University of California Riverside)
  - Screening of crystallography conditions of challenging targets
- **Vadim Cherezov** (Crystallography of membrane proteins; University of Southern California)
  - Attachment of transmembrane helix for anchoring of processed KRAS4b for crystallography
- **Jay Groves** (RAS on tethered bilayers; University of California at Berkley)
  - Effector interactions
  - Development of screenable assays on tethered bilayers

Full-length KRAS in complex with GDP

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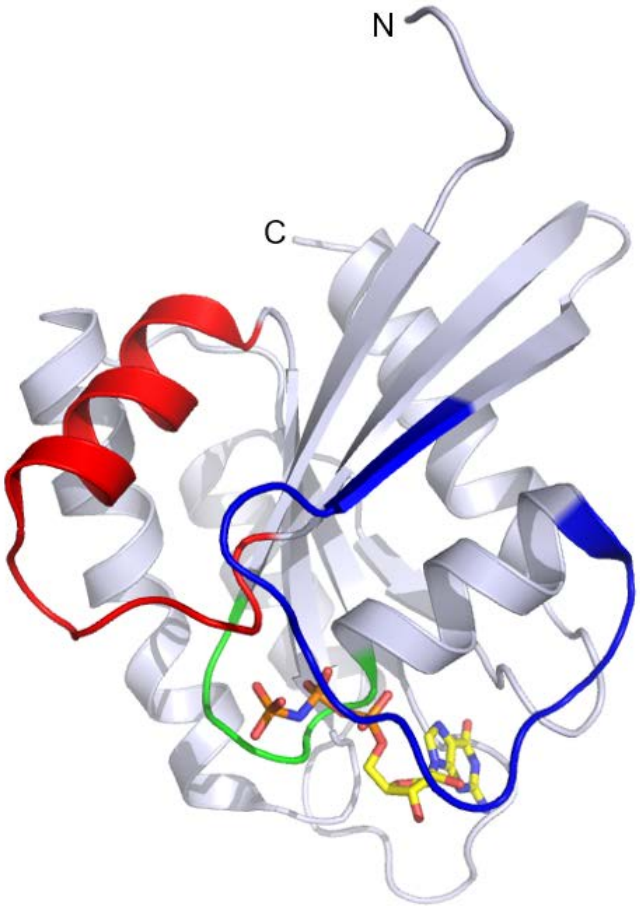
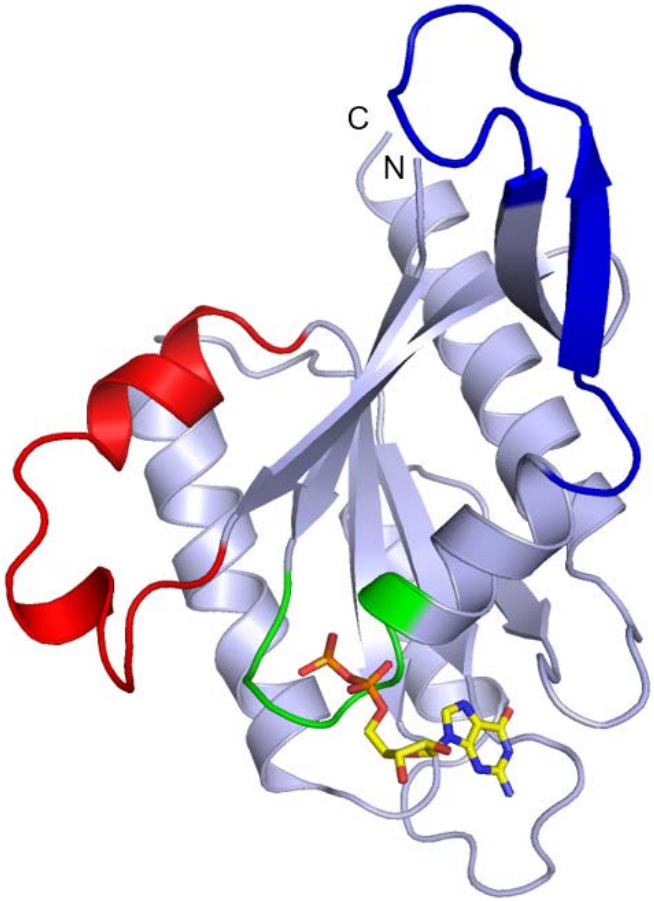
Wild-type KRAS(1-166)-GMPPNP complex at 1.35 Ang



Full-length Wild-type KRAS-GDP complex at 1.6 Ang

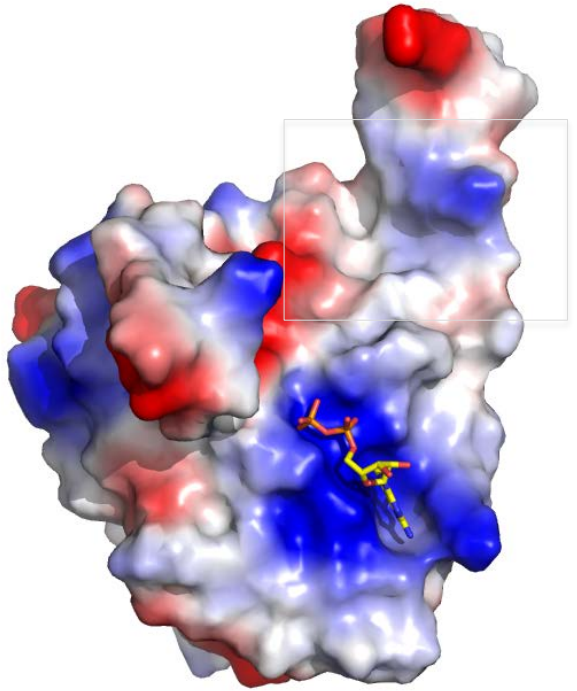


Switch-I
Switch-II
P-loop

Extended switch-I conformation in KRAS

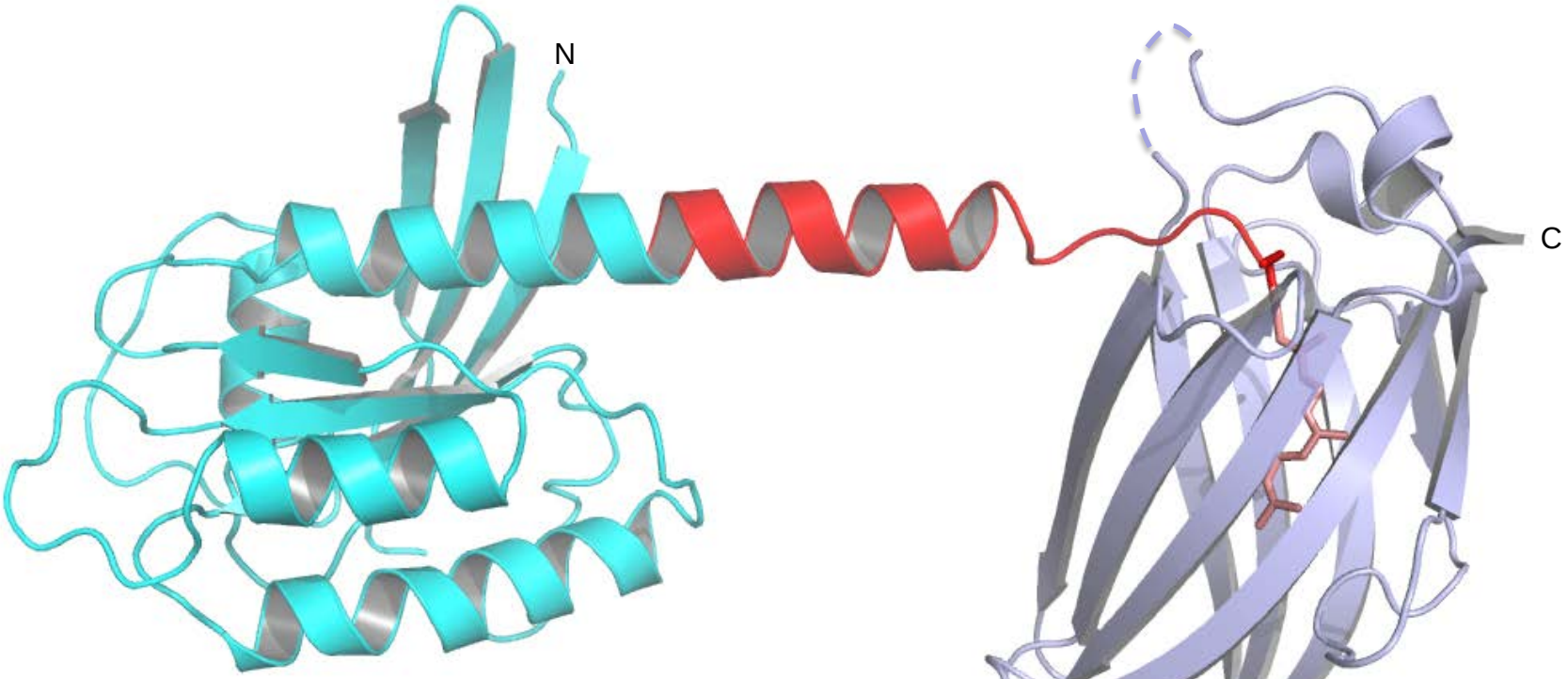
- **Validate presence of extended switch-I conformation in solution by NMR.**
  - Dynamic studies in collaboration with National Magnetic Resonance Facility at Madison.
    Que Van at FNLCR
  - High-pressure NMR studies in collaboration with Dr. Kalbitzer, University of Regensburg, Germany.
- **Virtual compound screening to target the groove present at the base of switch-I region**
  - in collaboration with Dr. Brian Shoichet's group at UCSF.

Electrostatic surface



Red - negative charge
White - neutral
Blue - positive charge

Processed KRAS in complex with PDEδ



KRAS

PDEδ

Resolution: 2.0 Ang

RAS Initiative Accomplishments:

Inhibitor Screens and Assays

Isogenic Screen for RAS Selective Inhibitors

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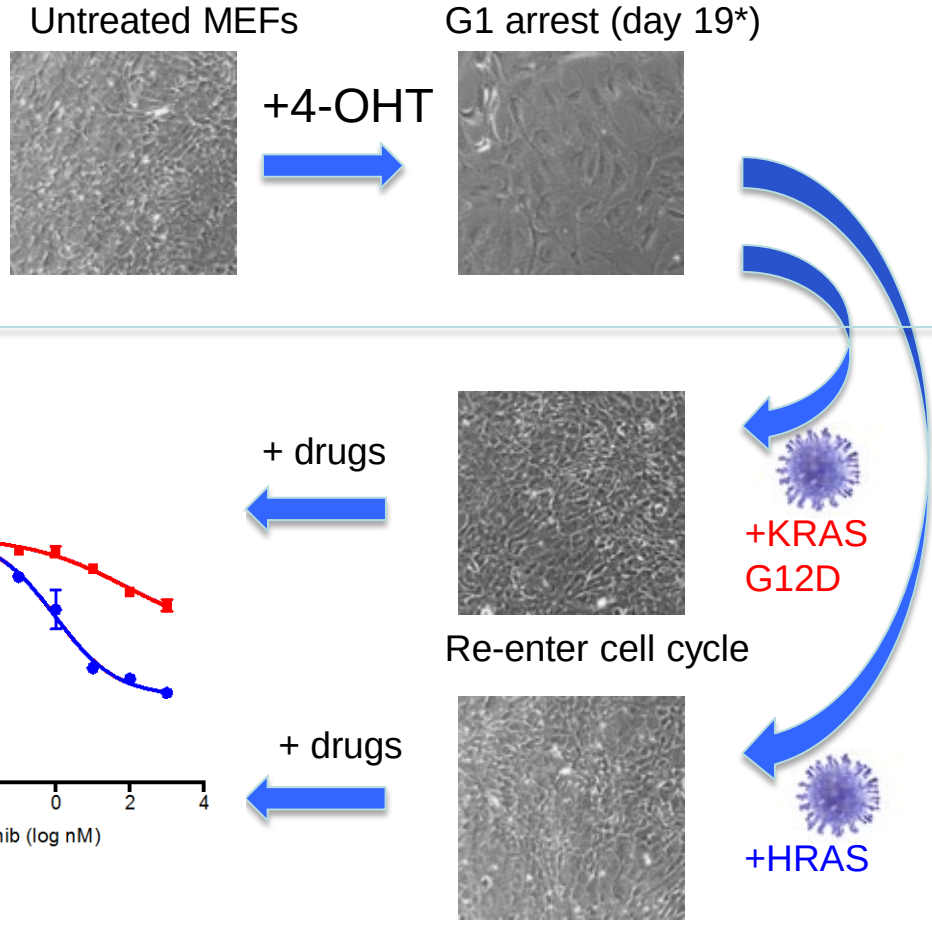
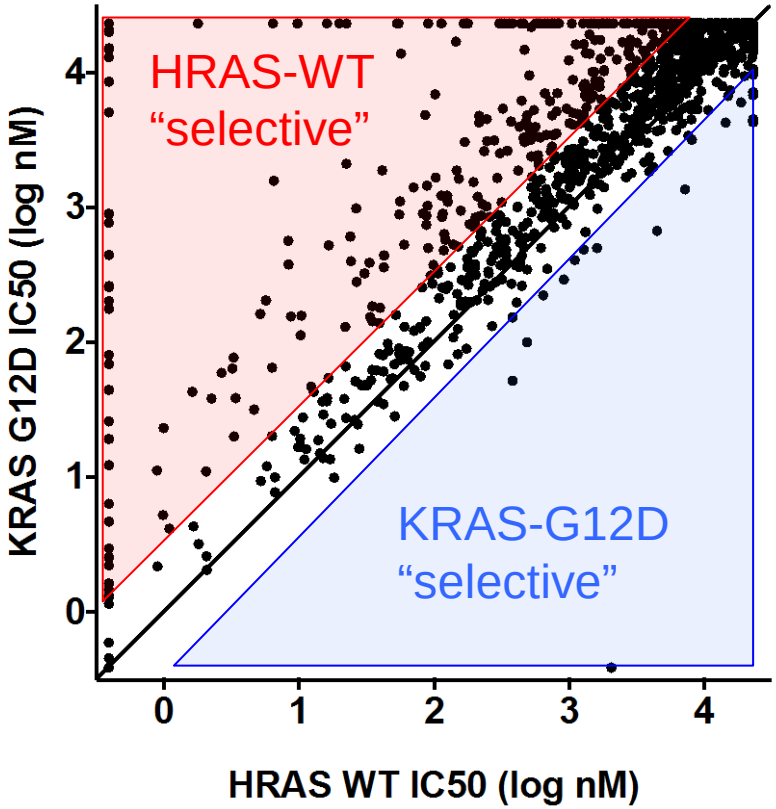
$HRAS^{-/-}$ $NRAS^{-/-}$ $KRAS^{lox/lox}$ MEFs

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



$HRAS^{WT}$ vs $KRAS^{G12D}$ Pilot Screen



- Compound library was provided by NCATS (National Center for the Advancement of Translational Sciences)
- The library is enriched for "tool" compounds, but also contains FDA approved drugs

Kanika Sharma (FNLCR), Kyle Brimacombe (NCATS)

KRAS-effector Inhibitor Screen
Daiichi-Sankyo Protein-Protein Interaction Library

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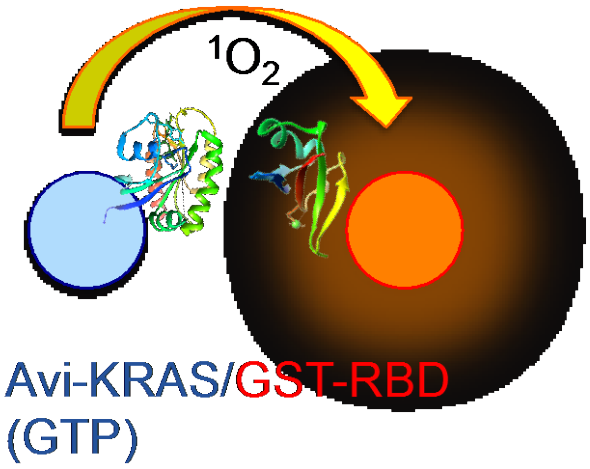
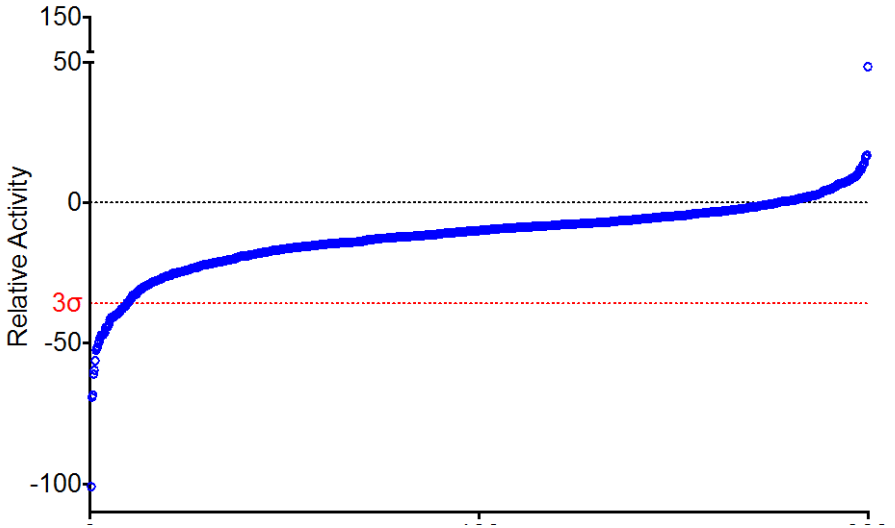
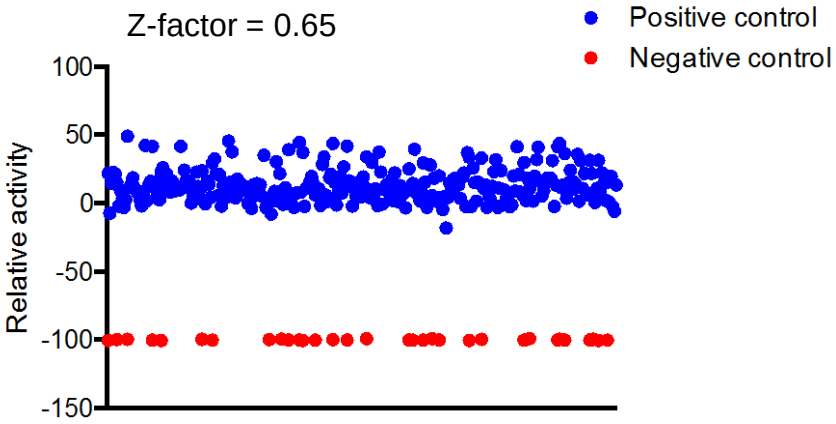
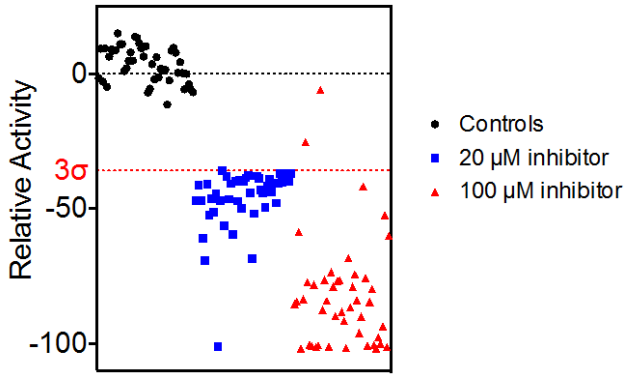
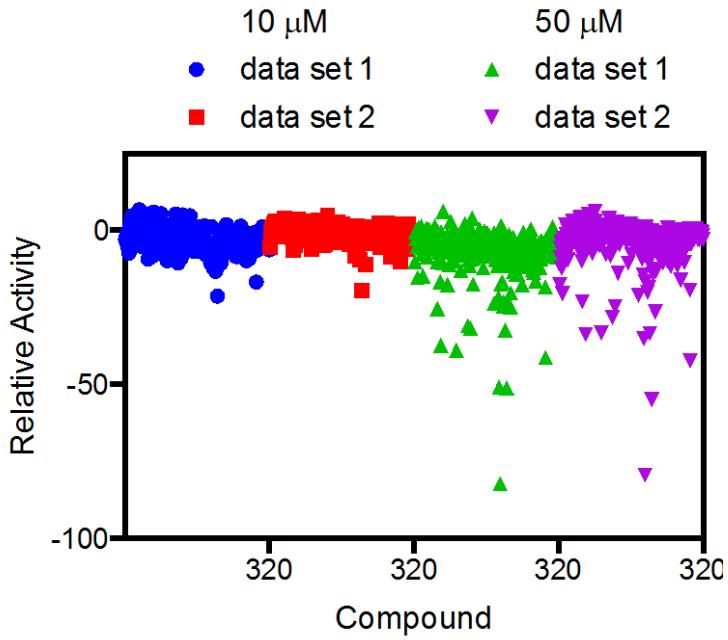


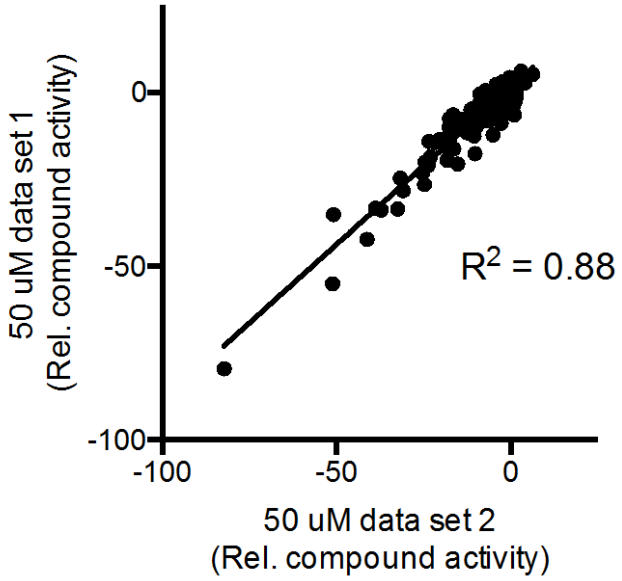






Reproducibility of KRAS-RBD screening assay





Assay is highly reproducible at 50 μM

The “hit” rate at 50 μM is approximately 4%

13/320 compounds inhibit the alpha signal >25%

KRAS4b-FME binds to CRAF-RBD on Nanodiscs

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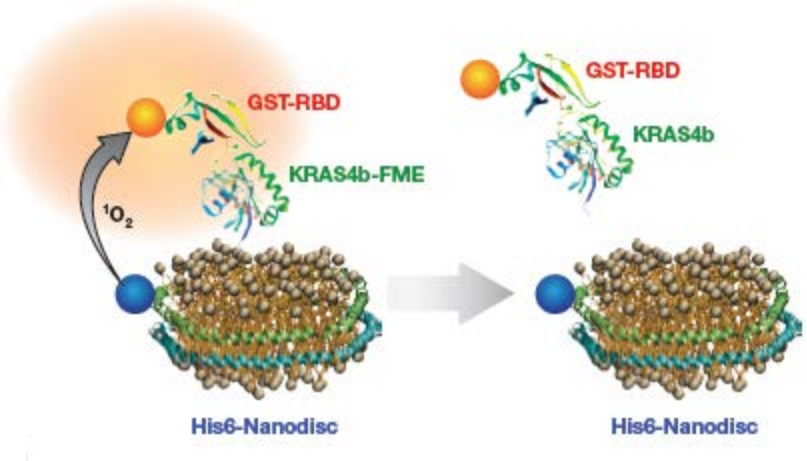

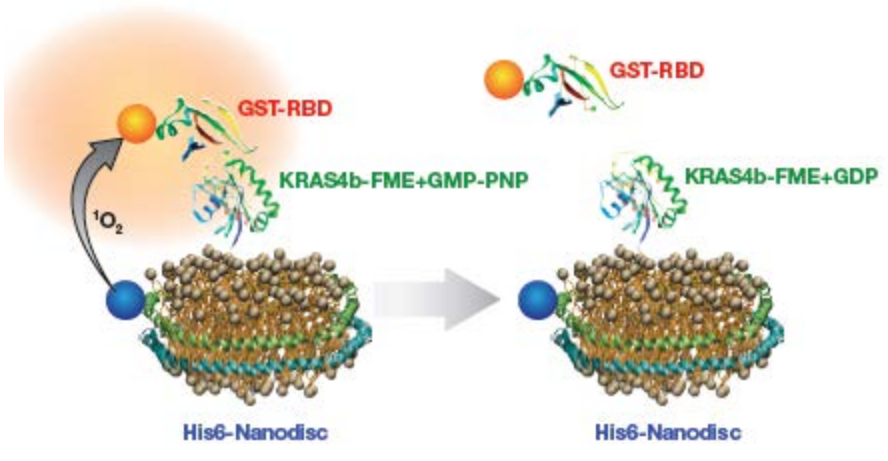

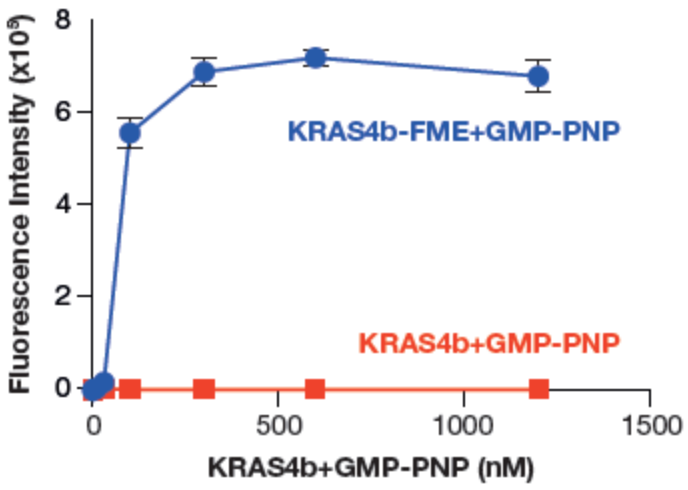

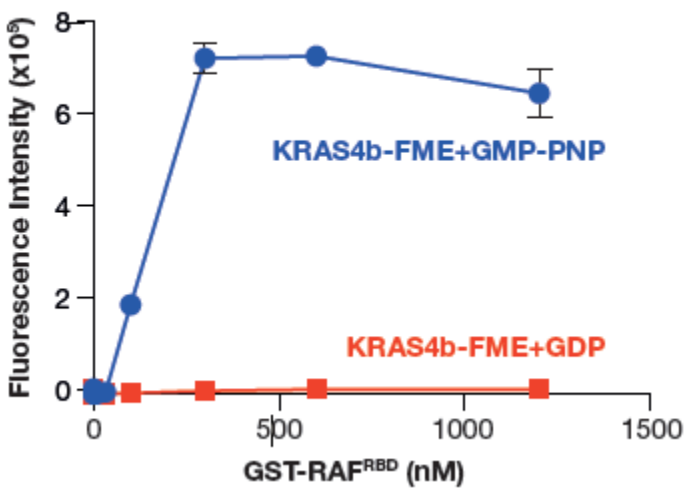

Matt Holderfield, Maria Abreu Blanco

RAS Localization Assay Overview

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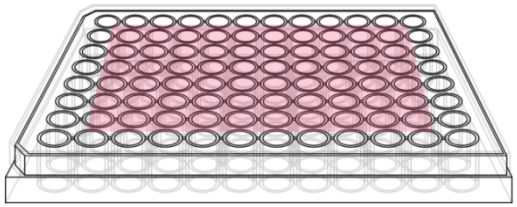
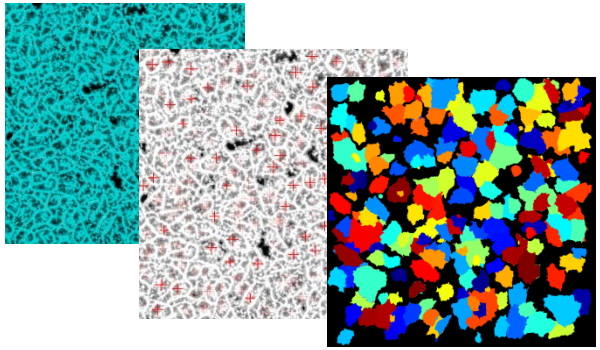
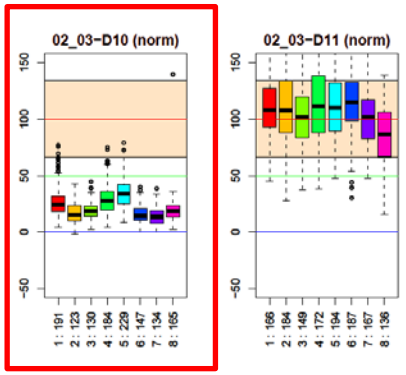






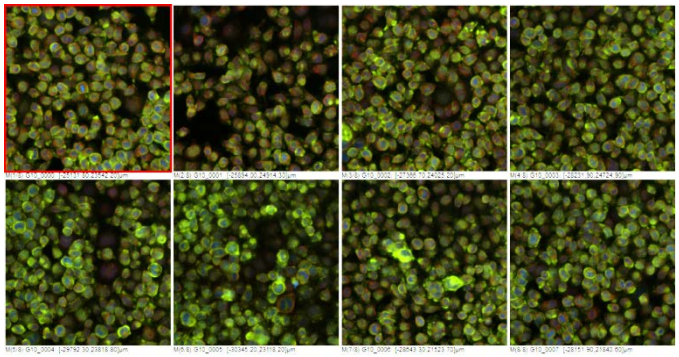
60 Wells

~125 Cells/location

Hit identification

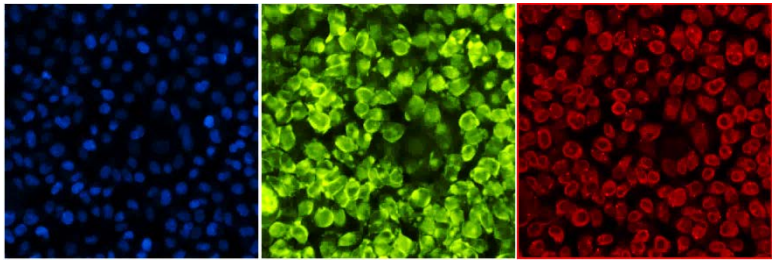
8 Locations/ well

Segmentation and Analysis



GFP-KRAS4b^{G12V}

Nucleus GFP Membrane



Alla Brafman

NCI Developmental Therapeutics Program screening set

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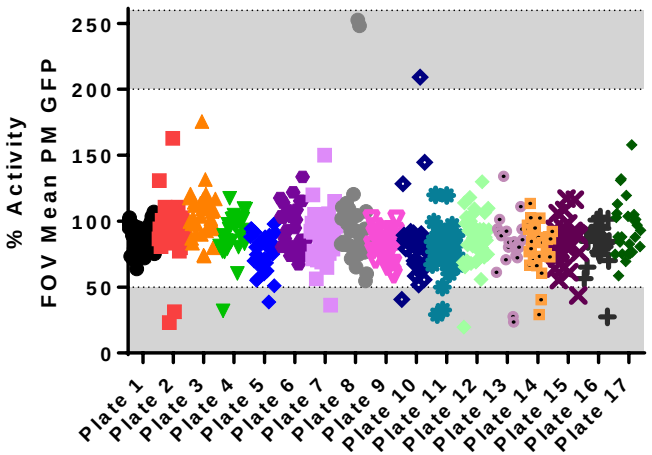
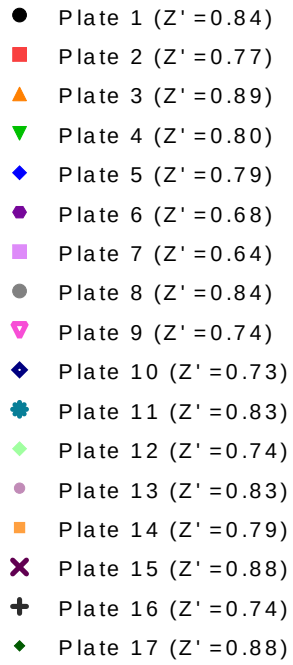
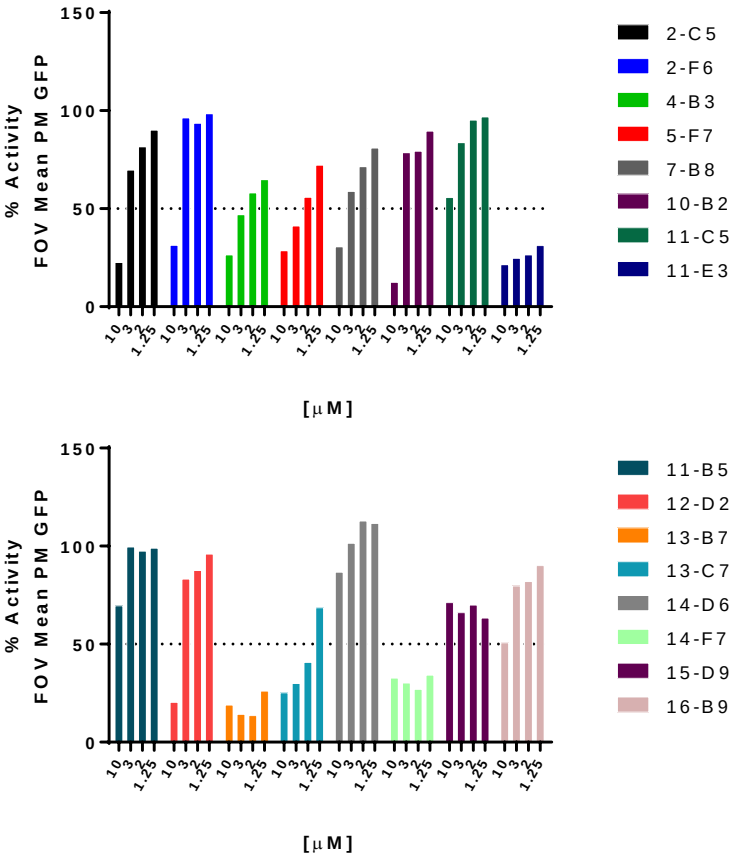
Primary assay:
GFP-KRAS^{G12V}



~800 small molecules with biological activity



Reconfirmed hits



Alla Brafman

HaloTag-KRAS^{WT} driven-MEFs Proliferate

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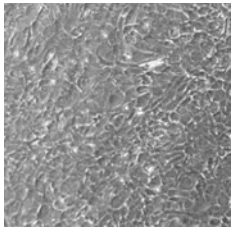
HRAS$^{-/-}$ NRAS$^{-/-}$ KRAS$^{lox/lox}$ MEFs

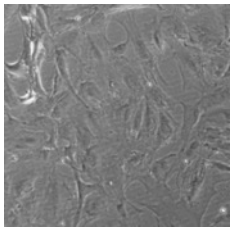
Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, **Barbacid M**. EMBO J. 2010

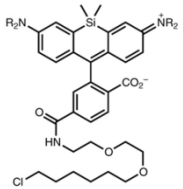
Untreated MEFs



+4-OHT

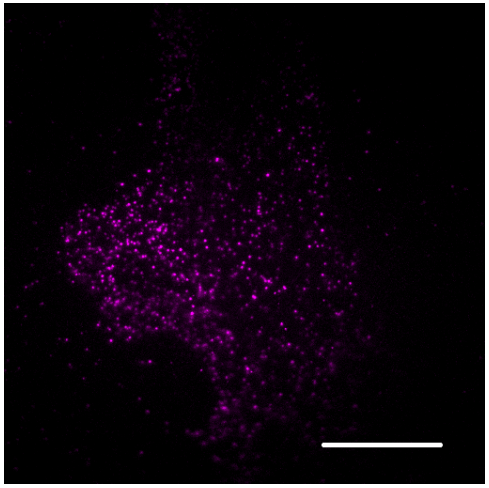
G1 arrest (day 19*)





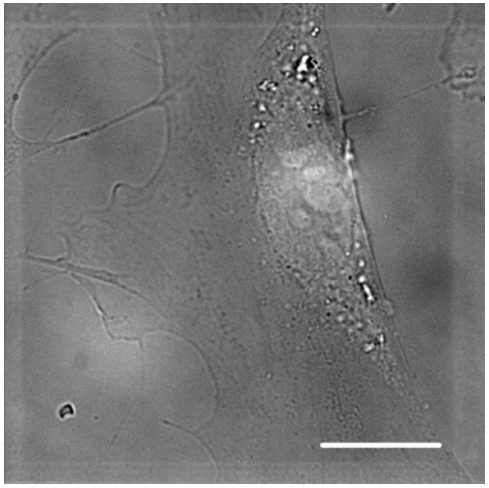
Cell permeant, super bright, fluorescent Halo ligand from Janelia Farms

HaloTag-KRAS4b can be imaged in live cells.



TIRF Image: membrane

HaloTag-KRAS4b rescues RASless MEF proliferation.



Transmitted light image

+HaloTagKRAS

Scale bar 20 µm

Nikki Fer and De Chen

Characterization of RAS molecules in live cell membranes

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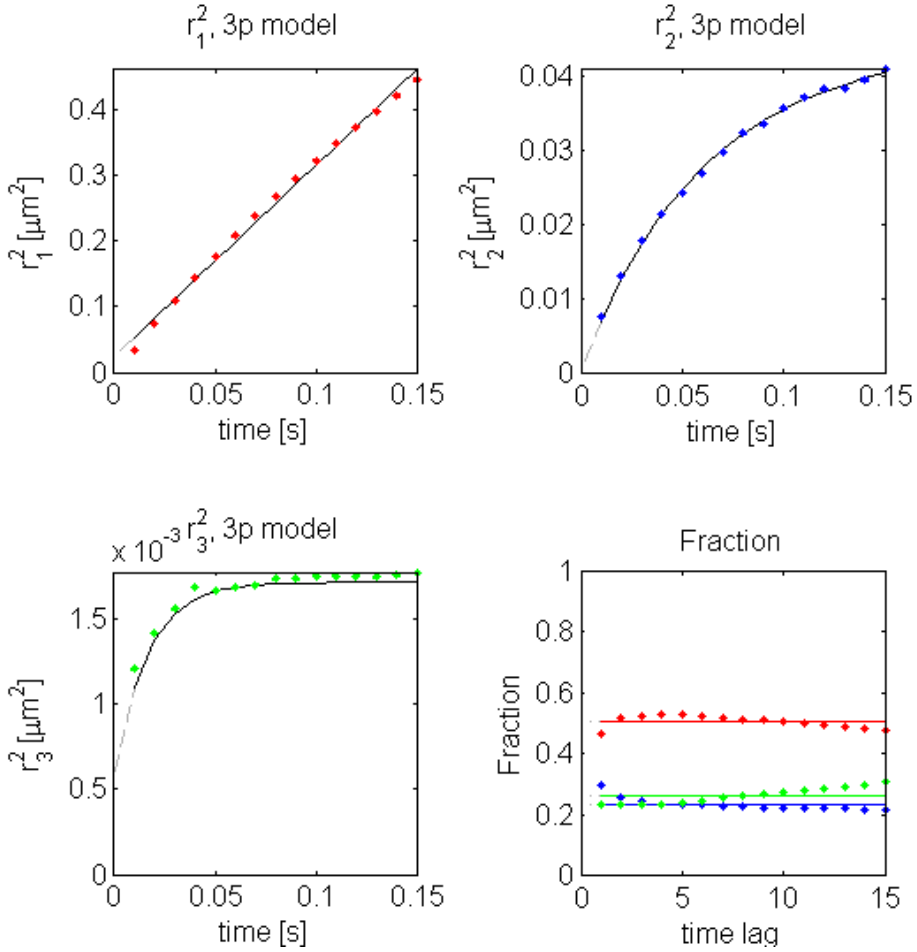
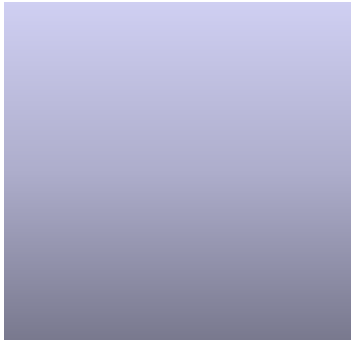
Jump squared displacement analysis



HaloTag-KRAS^{WT} driven-MEFs



Three components

Model	Diffusion (um²/s)	Fraction Mean (SDev)	Const. Rad. R_c (nm)
1 → Normal	0.73	0.505 (0.0193)	-
2 → Constrained	0.1805	0.233 (0.021)	44.2
3 → Constrained	0.0178	0.2624 (0.026)	1.2

De Chen and Prabhakar Gudla

Single molecule tracking analysis suggests three RAS states in live cell membranes.

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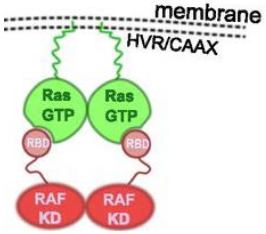
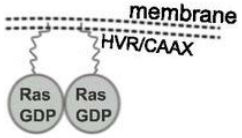
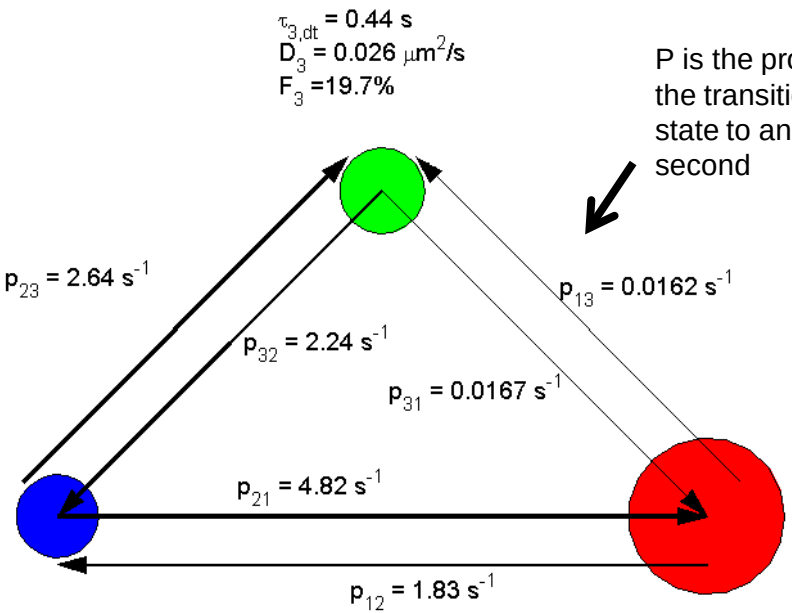
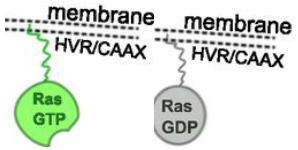
Information extracted from individual trajectories



Hypothesis: states represent different complexes in membrane.







RASless-MEFs, HaloTag-wtKRAS4b [JF646]=50pM, Serum Starved, 37°C, 22,325 trajectories and average trajectory length 12 frames.

De Chen and Prabhakar Gudla

RAS Initiative Accomplishments:

Towards a "RAS Interactome"

Cancer.gov/ras

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NIH NATIONAL CANCER INSTITUTE

1-800-4-CANCER Live Chat Dictionary

ABOUT CANCER | CANCER TYPES | RESEARCH | GRANTS & TRAINING | NEWS & EVENTS | ABOUT NCI | search

Home > Research > Key Initiatives > The RAS Initiative

THE RAS INITIATIVE

- The Problem
- A Collaborative Solution
- RAS Initiative at FNLCR
- RAS Projects at FNLCR +
- RAS Laboratory Groups +
- Oversight
- Spokes/Funding
- RAS Central +

A Collaborative Solution

During a series of NCI-led workshops in 2013, researchers presented data suggesting that it may now be feasible to target mutant RAS proteins directly or target other unique features of RAS-driven tumors.

Subsequently, NCI received unanimous endorsement from both the National Cancer Advisory Board and the NCI Board of Scientific Advisors to launch the RAS Initiative. Dr. Frank McCormick, a renowned expert in the field of RAS biology, joined the team at the Frederick National Laboratory for Cancer Research (FNLCR) as a consultant to lead the initiative.

Former NCI Director Harold Varmus, M.D., and National RAS Initiative Advisor Frank McCormick, Ph.D., explain the rationale for and the discussions that led to the formation of the RAS Initiative.

The Hub & Spoke Model

NCI hopes to attack mutant RAS-driven cancers through an integrated initiative that enlists collaborators from all sectors of the research community. This approach is called a "hub and spoke" model. The RAS Hub at the FNLCR and the larger community of RAS researchers around the world are now working together to find new ways to approach the RAS enigma.

The Hub – FNLCR

The FNLCR serves as the research hub that connects to research collaborators nationally and internationally. FNLCR scientists carry out a number of interlinked projects that employ the extensive infrastructure established by NCI in the areas of protein chemistry and biophysics, imaging, and genetics and genomics.

Spokes – Opportunities for Collaboration to Attack RAS

The RAS Initiative seeks to facilitate connections between and among researchers, bringing new ideas and technologies to bear on RAS. A guiding principle for these collaborations is close coordination with the activities at the FNLCR Hub, so that new efforts can leverage each other.

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RAS Spokes/Funding

The RAS Initiative seeks to facilitate connections between and among researchers, bringing new ideas and technologies to bear on RAS. A guiding principle for these collaborations is close coordination with the activities at the Frederick National Lab for Cancer Research (FNLCR) Hub, so that new efforts can have a maximum amount of leverage on each other.

Funding

RAS Initiative Postdoc Available

The Frederick National Laboratory for Cancer Research is currently seeking Postdoctoral Fellow candidates to join the RAS Initiative team. Please share this information with your trainees or other interested individuals.

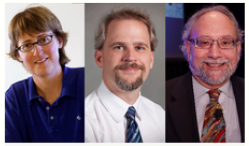
NCI Fellowships Available

The NCI requests post-doctoral fellowship applications related to the goal of building effective therapeutic approaches for KRAS-driven tumors. Applicants should apply through the Parent Program Announcement (F32); due dates are April 8, August 8, and December 8, 2015. For more information and how to apply, see the published Notice.

Spokes

Contract Awardees for RAS Pathway Assays

The NCI Clinical Proteomic Tumor Analysis Consortium has awarded contracts to the Fred Hutchinson Cancer Research Center (Dr. Amanda Paulovich), the Moffitt Cancer Center (Dr. John Koomen), and the Broad Institute (Dr. Steven Carr). They will develop quantitative immuno-multiple reaction monitoring (MRM) assays to measure important peptides and phospho-peptides in the RAS pathway. Data from these assays will provide a valuable link between phenotypes and genotypes in cancers. For more details please see the blog post in RAS Central.



From left to right: Dr. Amanda Paulovich, Dr. John Koomen, Dr. Steve Carr

KRAS Post-doctoral Fellowships

In

Dr. Lynn McGregor, University of California, San Francisco and Dr. John Hunter, University of Texas Southwestern Medical Center

cooperation with the Frederick National Laboratory for Cancer Research (FNLCR), The Pancreatic Cancer Action Network (PanCAN) has established fellowships to support talented post-doctoral researchers engaged in KRAS research. PanCan recently announced two outstanding fellowship awardees: John Hunter, Ph.D. who

RAS Lab (Basecamp)

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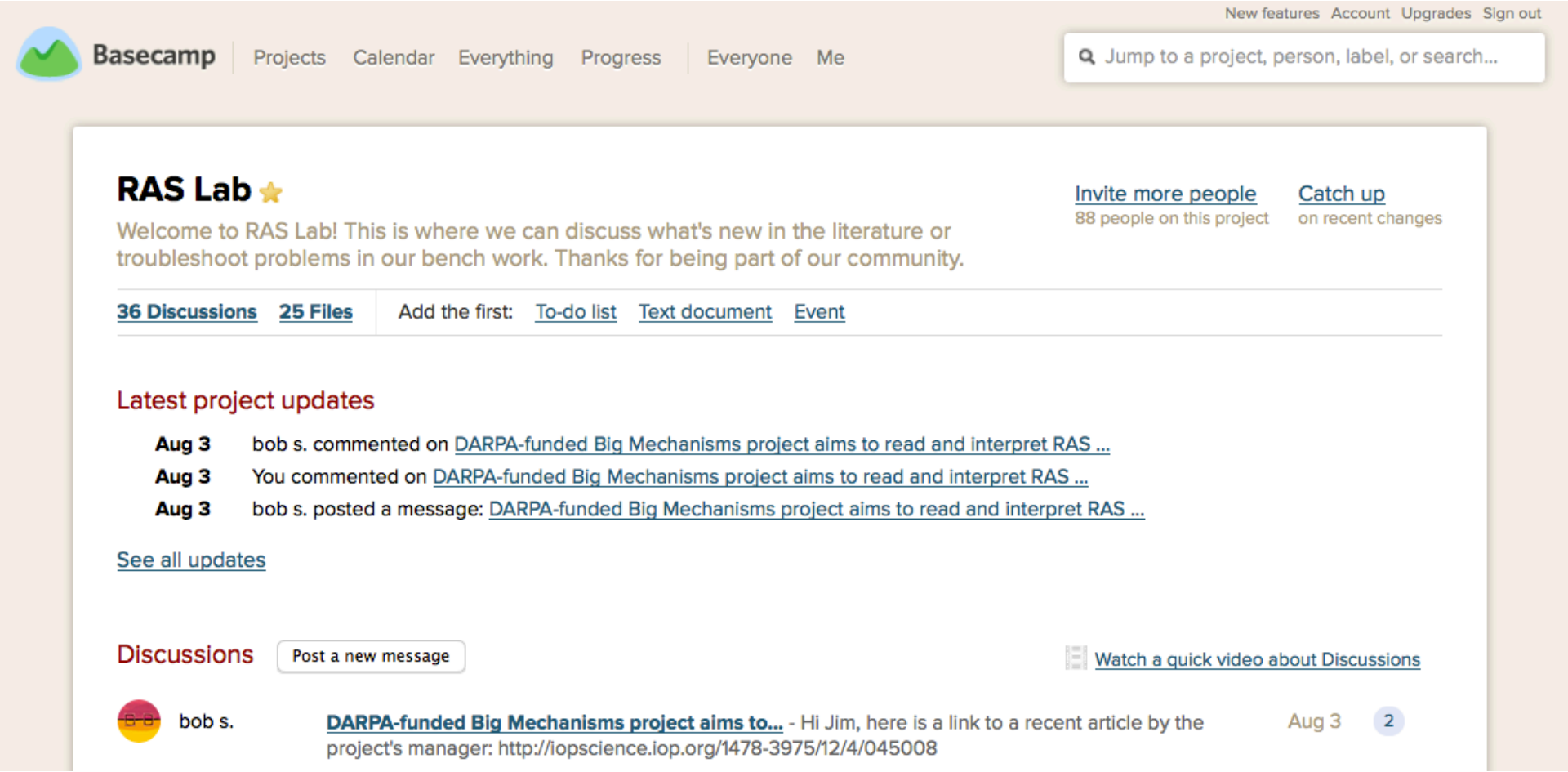
- Private, by invitation only
- All posts and comments publish immediately
- Supports uploads of documents, 1:1 interactions



RAS Reagents *Pathway 2.0 clone collection*

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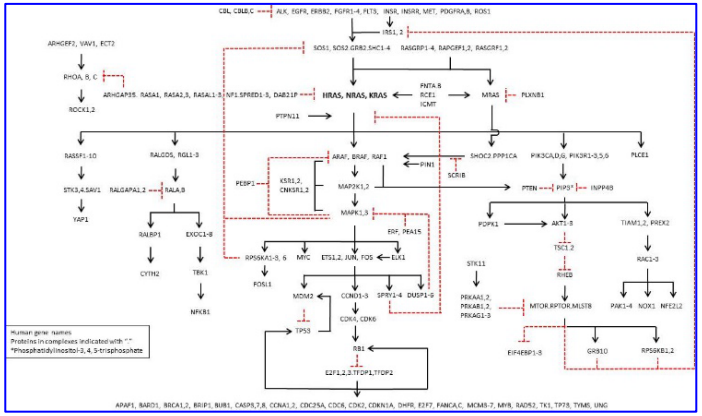

- Generate stop and nostop Gateway Entry clones for all genes
- Isoforms chosen by bioinformatic analysis (R. Stephens)
  - most common transcript observed across different cell lines
  - many are NOT “isoform 1” or “longest isoform”
- 180 genes - 360 total constructs (stop/nostop)
  - 17 not available commercially in correct isoform
  - 32 additional not available without mutations
- 98% completion

Vanessa Wall
Jen Mehalko

Collaboration with the RAS Community

Frederick National Laboratory for Cancer Research

RAS events

Synthetic Lethality Workshop, January 6-7 2014
RAS Pathways Workshop, June 11, 2014
Cell Surfaces Workshop, July 23, 2014
AACR Annual Meeting, April 21, 2015
RAS Structures Workshop, July 21-22, 2015

RAS Immunotherapy Workshop, November 3, 2015

RAS Symposium, December 15-16, 2015

RAS Symposium Confirmed Speakers I

Harold Varmus, Weill Cornell Medical Col
Kevan Shokat, University of California, Sa
Allan Balmain, University of California, Sa
Mariano Barbacid, Spanish National Can
James Bradner, Dana Farber, Harvard Ur
Karen Cichowski, Brigham and Women's Hospital, Harvard University
Channing Der, University of North Carolina, Chapel Hill
Stephen Fesik, Vanderbilt University
Jay Groves, University of California, Berkley
John Hancock, University of Texas, Houston
Frank McCormick, University of California, San Francisco and the RAS Initiative
Deborah Morrison, National Cancer Institute
Mark Philips, New York University
David Sabatini, Whitehead Institute, Massachusetts Institute of Technology
Kevin Shannon, University of California, San Francisco
David Tuveson, Cold Spring Harbor Laboratory
Michael White, University of Texas, Southwestern
Matthew Vander Heiden, Koch Institute, Massachusetts Institute of Technology

Seminars at FNLCR

Channing Der, UNC
Ken Westover, UTSW
Carla Mattos, Northeastern
Mark Philips, NYU
Vadim Gaponenko, U-Chicago
Josh Salafsky, Biodesy, Inc.
Calvin Kuo, Stanford
Kris Wood, Duke
Mariano Barbacid, CNIO, Madrid
Cyril Benes, Mass General
Carolyn Buser, GlaxoSmithKline
Jay Groves, UC-Berkeley
Stephen Sligar, UI-Champagne Urbana
Raffit Hassan, NCI
Renata Grifantini, Externautics Spa, Siena
Renata Pasqualini, U-New Mexico
Andrew Bradbury, Los Alamos
Kent Rossman, UNC
Shiva Malek, Genentech

Collaboration with the RAS Community

Frederick National Laboratory for Cancer Research

RAS Reference Reagents

Chris Kemp, Fred Hutch
Eric Chang, Baylor
Silvia Thöne, Munich
Peter Jackson, Stanford University
Tyler Jacks, MIT
Calvin Kuo, Stanford
Bill Hahn, Broad / Dana Farber
Karla Satchell, Northwestern
Julian Downward, Cancer Research UK
Daniel Abankwas, University of Turku
Said Sebti, Moffitt Cancer Center
Ian Prior, Liverpool
Muller Fabbri, Children's Hospital LA
Faraz Bishehsari, Rush
Amy Lee, USC
Yosef Yarden, Wiezmann
Richard Klemke, UCSD
Saidul Chowdhury, U-Texas Arlington
Christian Gocke, JHMI
Tobias Baumgart, U-Penn
Emil Lou, U-Minnesota
Ron Bose, Wash U
Neil Kelleher, Northwestern
Sourav Bandyopadhyay, UCSF
Robert Chapkin, Texas A&M

NIH collaborators

Ji Luo, NCI
Anton Simeonov, NCATS
Debbie Morrison, NCI
Rajat Varma, NIAID
Udo Rudloff, NCI
Sriram Subramaniam, NCI

Outside collaborators

Steve Almo, Einstein
Jim Wells, USCF
Channing Der, UNC
Ken Westover, UTSW
Carla Mattos, Northeastern
Steve Sligar, U- Ill
Jay Groves, Berkeley
Hirsch Nanda, Susan Kreuger, NIST
John Markley, NMRFAM, UW-Madison
Paul Cohen, DARPA
Kris Wood, Duke
David Weber, U-Maryland
Tina Yuan, Broad
Cameron Pitt, UCSF
Krishna Kota, USAMRIID
Sotirios Koutsopoulos, MIT
Fred Wittinghofer, Dortmund University
Lynn McGregor, UCSF (PanCan postdoc)
John Hunter, UTSW (PanCan postdoc)
Saori Sato, Daiichi-Sankyo
Walter Englaro, Sanofi-Aventis
Kirk Staschke, Lilly
Gad Getz, Mass Gen /Broad
Matt Meyerson, Dana Farber
Immuno-MRM of RAS pathway

- Amanda Paulovich, Fred Hutch
- Steve Carr, Broad Institute
- John Koomen, Moffit Cancer Center

Andreas Gosberg, Lilly

FNLAC RAS Working Group Recommendations

Science

Frederick National Laboratory for Cancer Research

- **Pursue a detailed understanding of Processed KRAS4b**
  - Unique reagent that shows promise as a new tool compound and thus illustrates the potential of the RAS Initiative
  - Biophysical and structural analysis of RAS on membranes
  - Dedicate staff to make processed KRAS4b for research community
  - In-house workshops for reagent generation
- **Biochemistry and structural biology (e.g., Cryo-EM) of RAS complexes and RAS:membrane interactions is a priority**
  - Collaborative effort with CCR and Sriram Subramaniam
- **The overall set of structures and reagents should represent a concrete and useful set of deliverables from the RAS effort**
- **Other efforts were more exploratory and open-ended; some are being scaled back or phased out**

Strategy

Frederick National Laboratory for Cancer Research

- **Develop a plan to augment industry partnership (see next slide)**
- **Implement a framework for tool/lead compound development**
  - Need path to validation and optimization not dependent on pharma
  - Define and plan for medicinal chemistry needs
- **De-convolution assays might emerge as an area of specialty**
  - Synergize biophysics/biochemistry with assays and screens
  - Develop an assay cascade for validation of hits/leads
- **Step-up awareness and dissemination efforts**
  - Package reagents, assays and capabilities for presentation to academia and pharma
    - Publicity/marketing
  - Develop additional next-generation assays
- **Develop plan for renewal phase**
  - Present to FNLAC as part of renewal

Interactions with Pharma

Frederick National Laboratory for Cancer Research

- **Pharma participation is a big plus**
  - Pharma brings credibility and resources
  - Roadshows and marketing to increase participation
- **Be creative when thinking about partnering possibilities**
  - Preferred partner(s)
  - Separate company that holds IP?
  - Venture philanthropy?
  - Develop strategy for prosecuting IP
- **Blueprint for bringing all projects to successful completion**
  - Define metrics for success up-front
  - Framework for division of labor during follow-up phase

Outreach & Resources

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- **Websites**
  - "Interactome" that engages the community may have valueProvide additional information
    - Protocols ; cell line mutation and RAS dependence
- **Compound collection and reagent distribution**
  - Resource for internal efforts and extended RAS community
  - Level of effort and source of compounds
  - Validate compounds with "assay cascade"
  - Track distribution and the experience/coaching needs of the recipients
- **Possibilities**
  - *Manuscript on test compounds with assay cascade?*
  - *De-bunk inaccurate claims?*
  - *RAS pathway proteomic studies?*