Frederick National Laboratory
for Cancer Research

Ras Initiative

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

http://www.cancer.gov/ras

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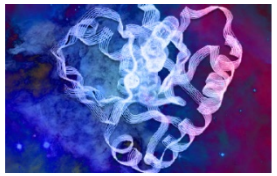


Structural Biology and Biochemistry

The structural and biochemical properties of KRAS and its most prevalent mutants will be characterized to look for ways to modulate their activity.

RAS Assays

New assays for RAS activity may be useful tools to screen for RAS pathway inhibitors.

Biology of Mutant KRAS Cell Lines

Commonalities in dozens of cell lines derived from human cancers that have mutant *KRAS* genes could reveal insights into selective vulnerabilities for treatment.

Pathways Analysis

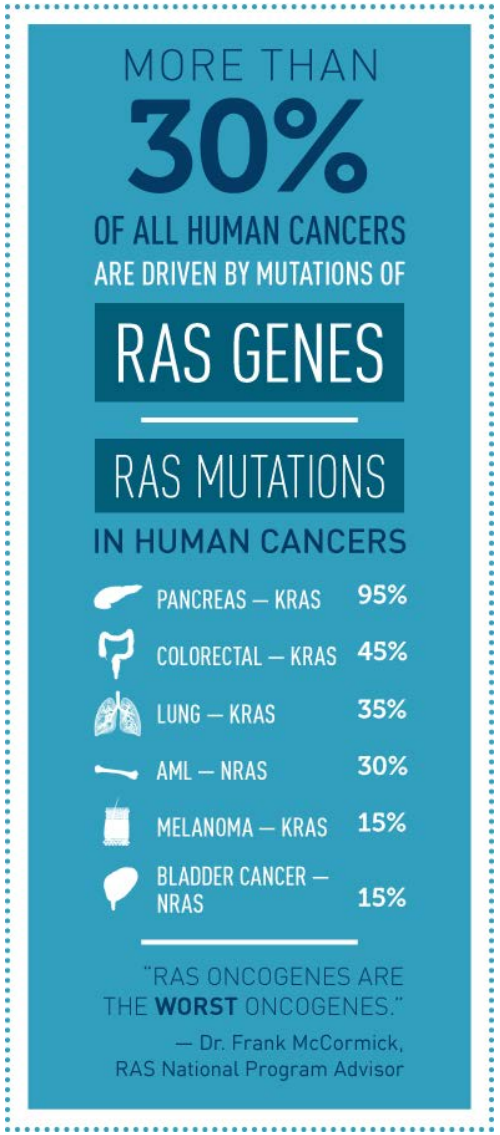
Surprising failures of new cancer treatments have made it clear that we do not know enough about how molecules in RAS signaling pathways interact with each other.

Cell Surface Analysis

Identifying cell surface features specific to mutant *KRAS* cancers could give us unique opportunities to develop treatments that target the cell surface.

RAS Reference Reagents

An important priority of the RAS Initiative is to distribute highly validated materials and methods to the world-wide community of RAS researchers.



Implementing the RAS Program

Hub, Spoke, and RAS Community model

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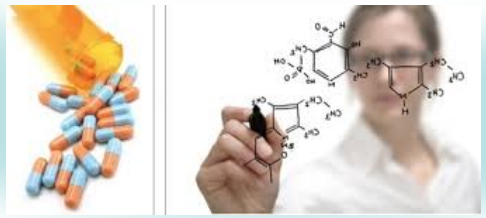
Intramural Labs

Extramural NCI-Supported Labs

FNLCR – The Hub

Biotechs



Pharma

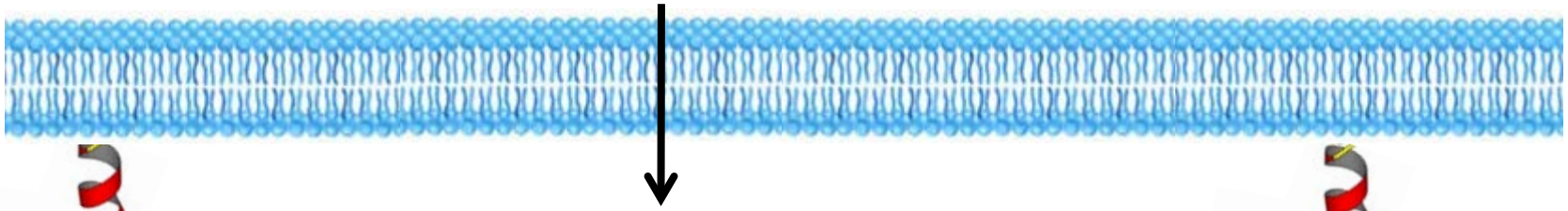
PANCREATIC CANCER ACTION NETWORK℠

PANCREATIC CANCER ACTION NETWORK℠

Advocacy

Parameters affecting normal Ras activity

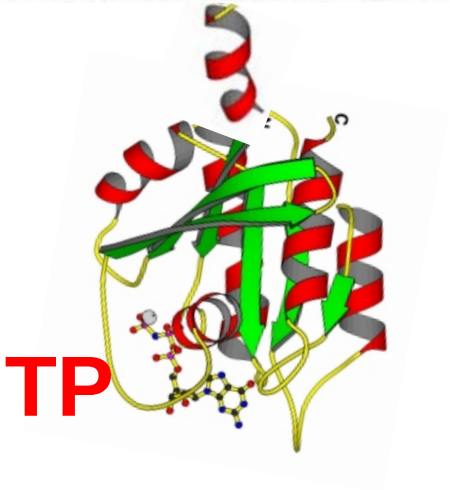
Growth Factors



GEF-mediated GDP dissociation

GAP-mediated GTP hydrolysis

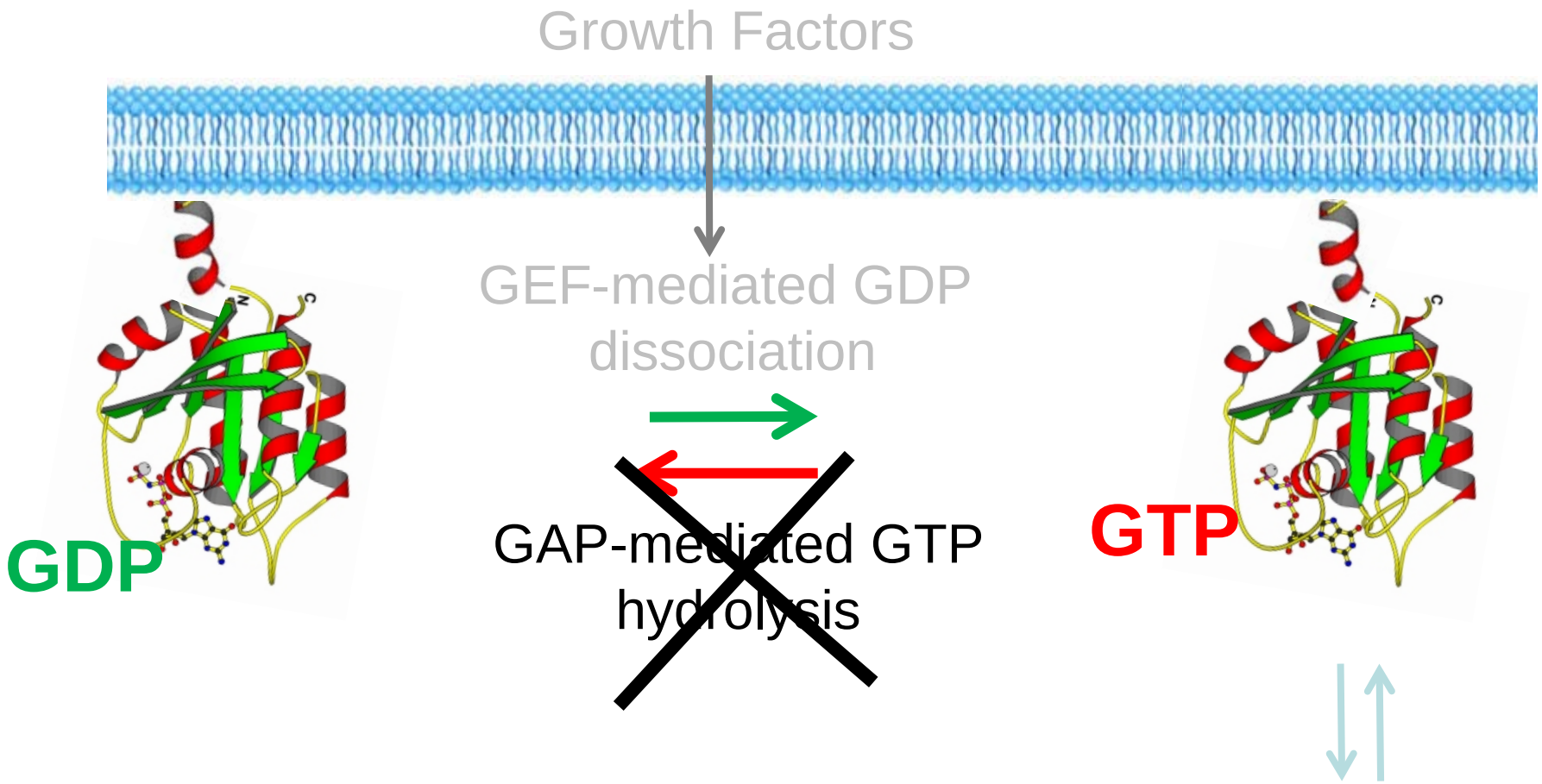
GDP



GTP

RalGDS | RAFs | PIK3CA

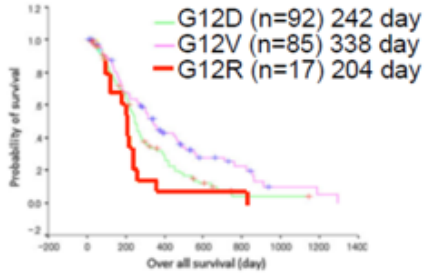
Parameters affecting oncogenic Ras activity



Distinct biological and clinical properties of KRAS alleles

-KRAS G12V, G12C: worse clinical outcome than G12D (lung cancer)
(Al-Mulla et al; Andreyev et al; Vega et al; Keohavong et al)

-KRAS G13D: respond to Cetuximab (colorectal cancer)
(de Roock et al, 2010)



T Ogura *et al. J Gastroenterol* (2013)
doi:10.1007/s00535-012-0664-2

Isogenic cell lines from RAS-less MEFs

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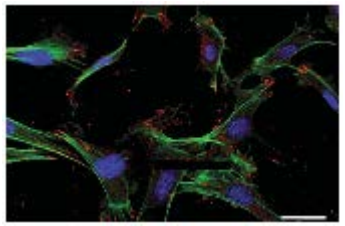

$HRAS^{-/-}$ $NRAS^{-/-}$ $KRAS^{lox/lox}$ MEFs

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

HRAS	NRAS	KRAS4A	KRAS4B
eGFP-WT	eGFP-WT	eGFP-WT	eGFP-WT
eGFP-G12V	WT	WT	WT
PAmCherry-WT	G12V	eGFP-mutant	eGFP-mutant
WT		G12V	G12V
G12V		G12D	G12D
		G12C	G12C
		G13D	G13D
		Q61L	Q61L
		Q61R	Q61R

RAS dependent MEFs

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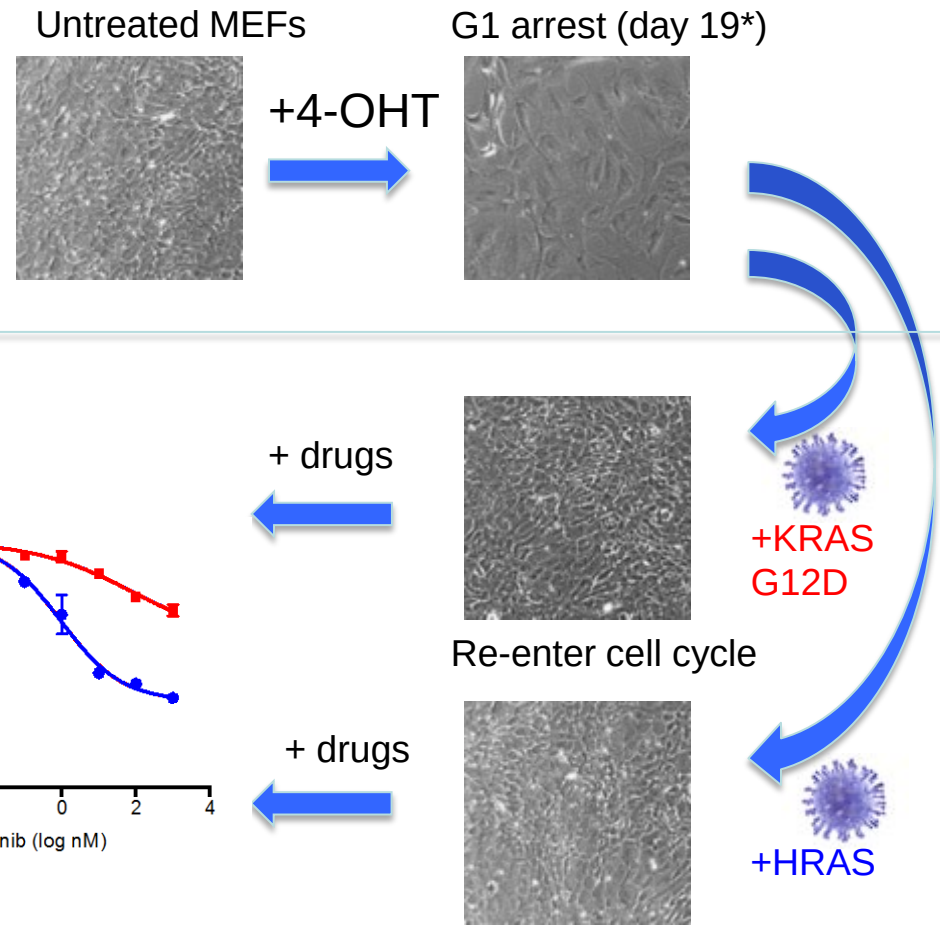
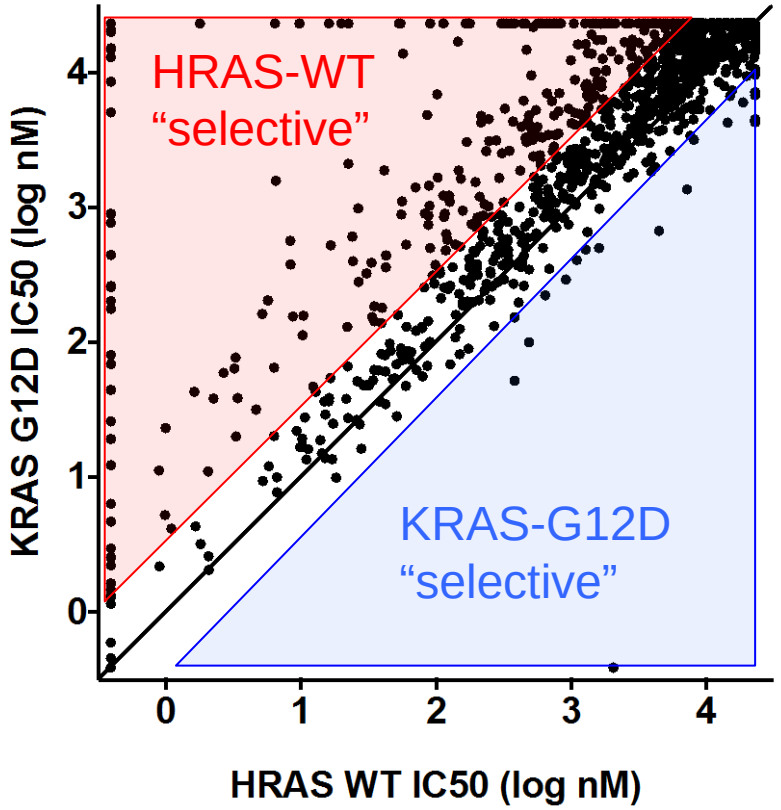
$HRAS^{-/-}$ $NRAS^{-/-}$ $KRAS^{lox/lox}$ MEFs

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



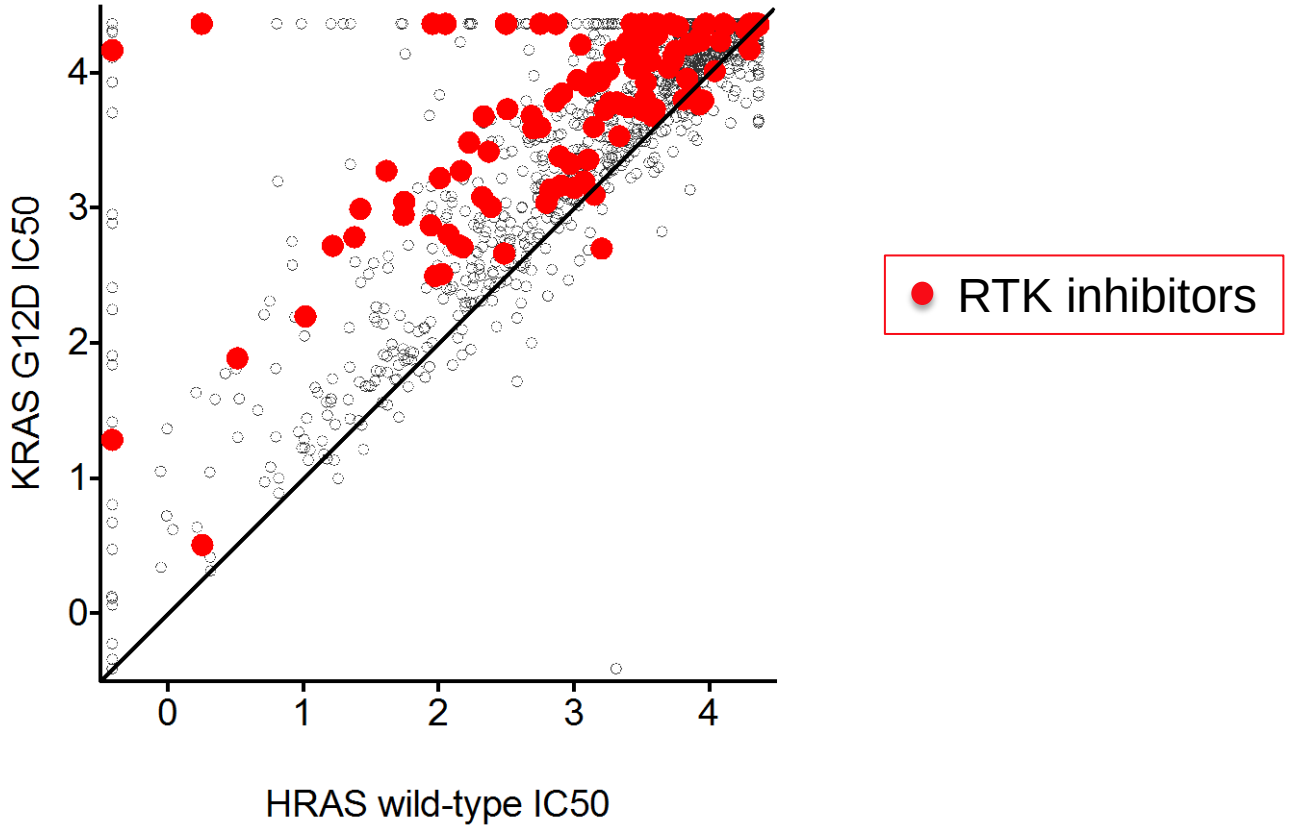
$HRAS^{WT}$ vs $KRAS^{G12D}$ MEF proliferation screen



- 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)

Receptor Tyrosine Kinase (RTK) inhibitors



Kanika Sharma (FNLCR), Kyle Brimacombe (NCATS)

Full-length KRAS in complex with GDP

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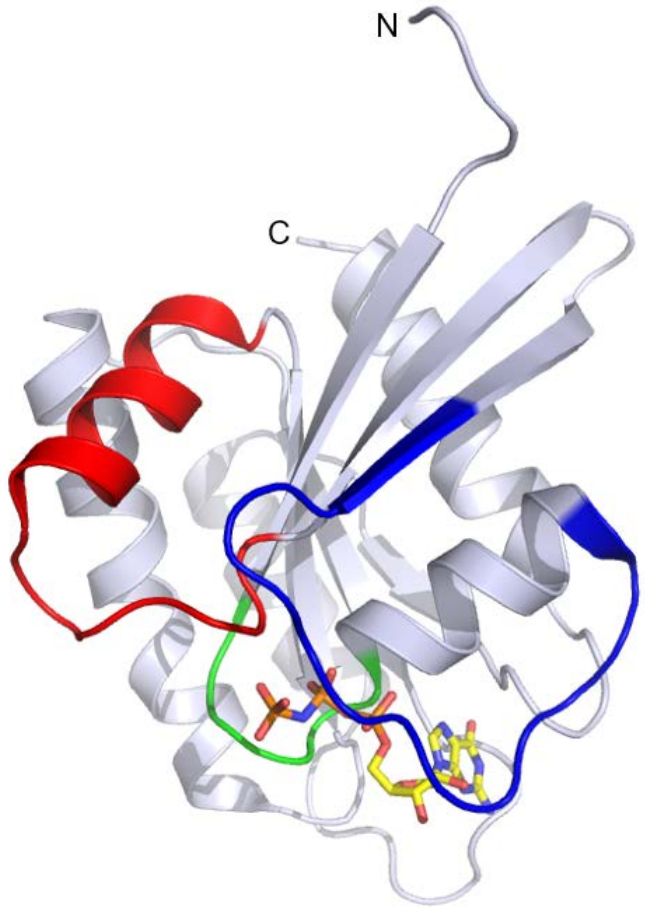
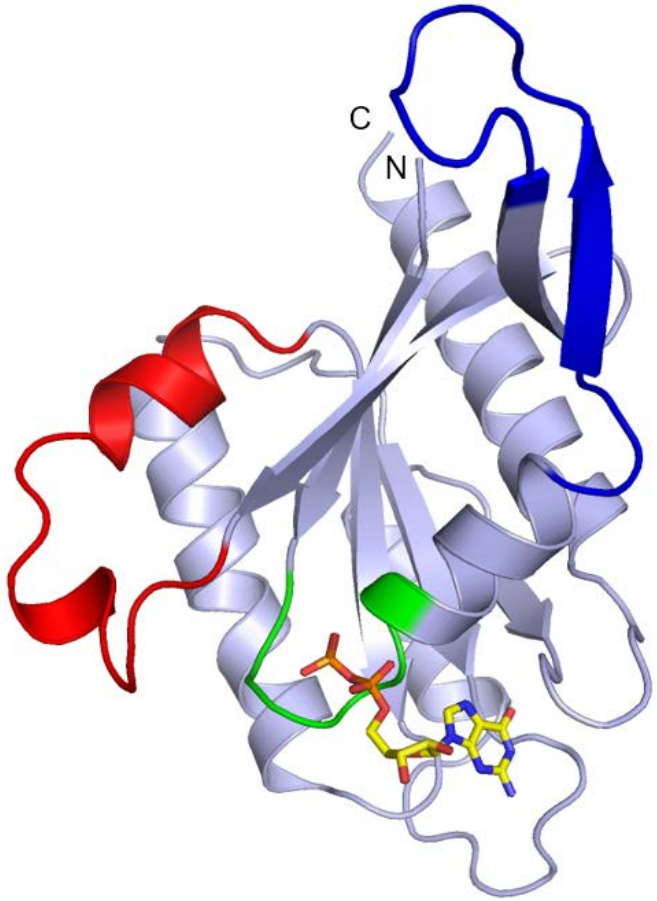
Wild-type KRAS(1-166)-GMPPNP complex at 1.35 Ang



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



Switch-I
Switch-II
P-loop

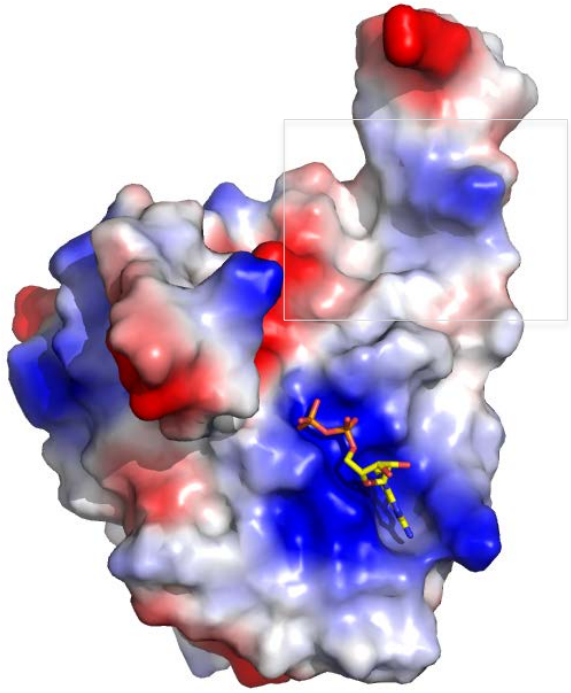
Extended switch-I conformation in KRAS

- **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.

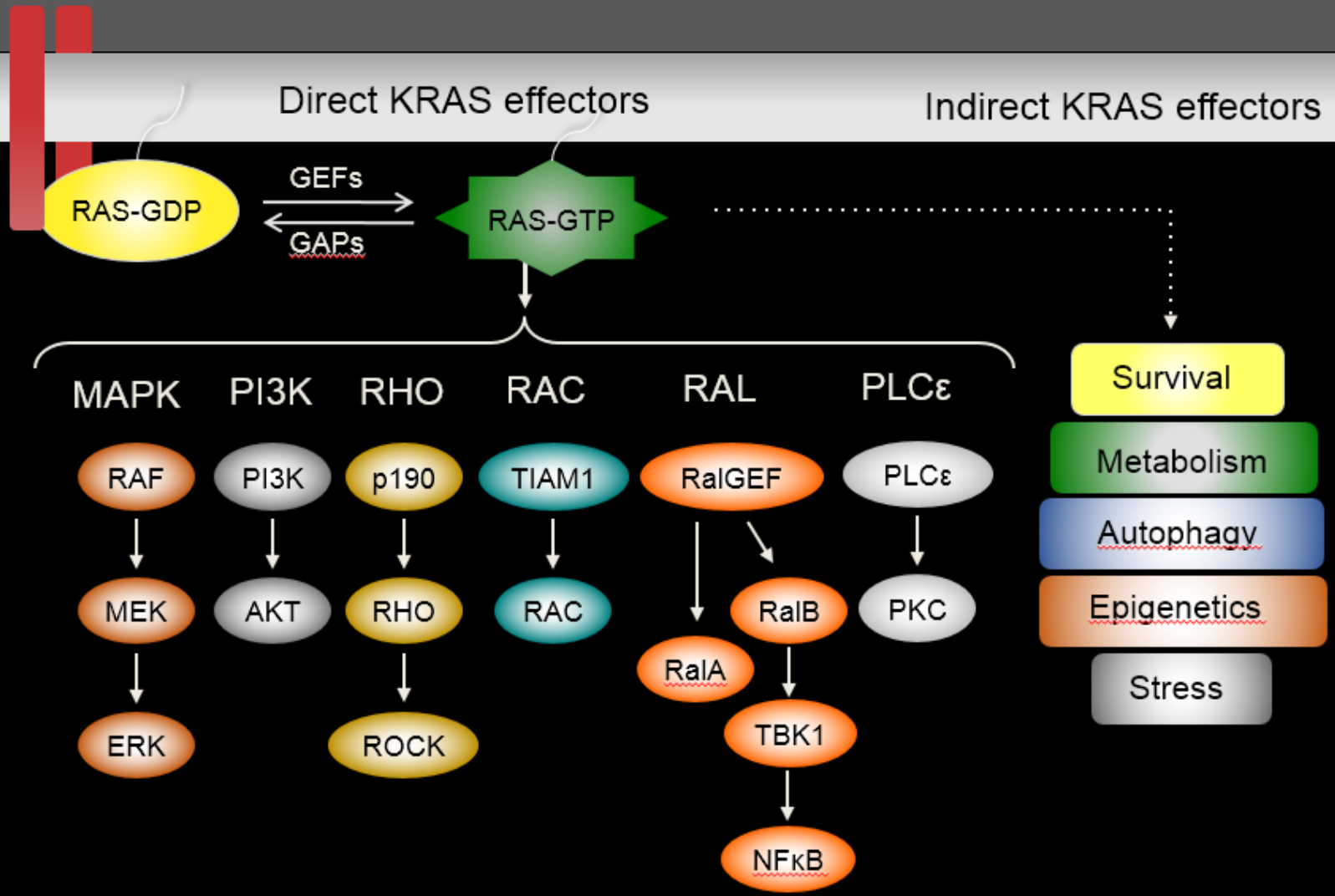
- **Virtual compound screening to target the groove present at the base of switch-I region**
  - in collaboration with Dr. Brian Shoichet's group at UCSF.

Electrostatic surface



Red - negative charge
White - neutral
Blue - positive charge

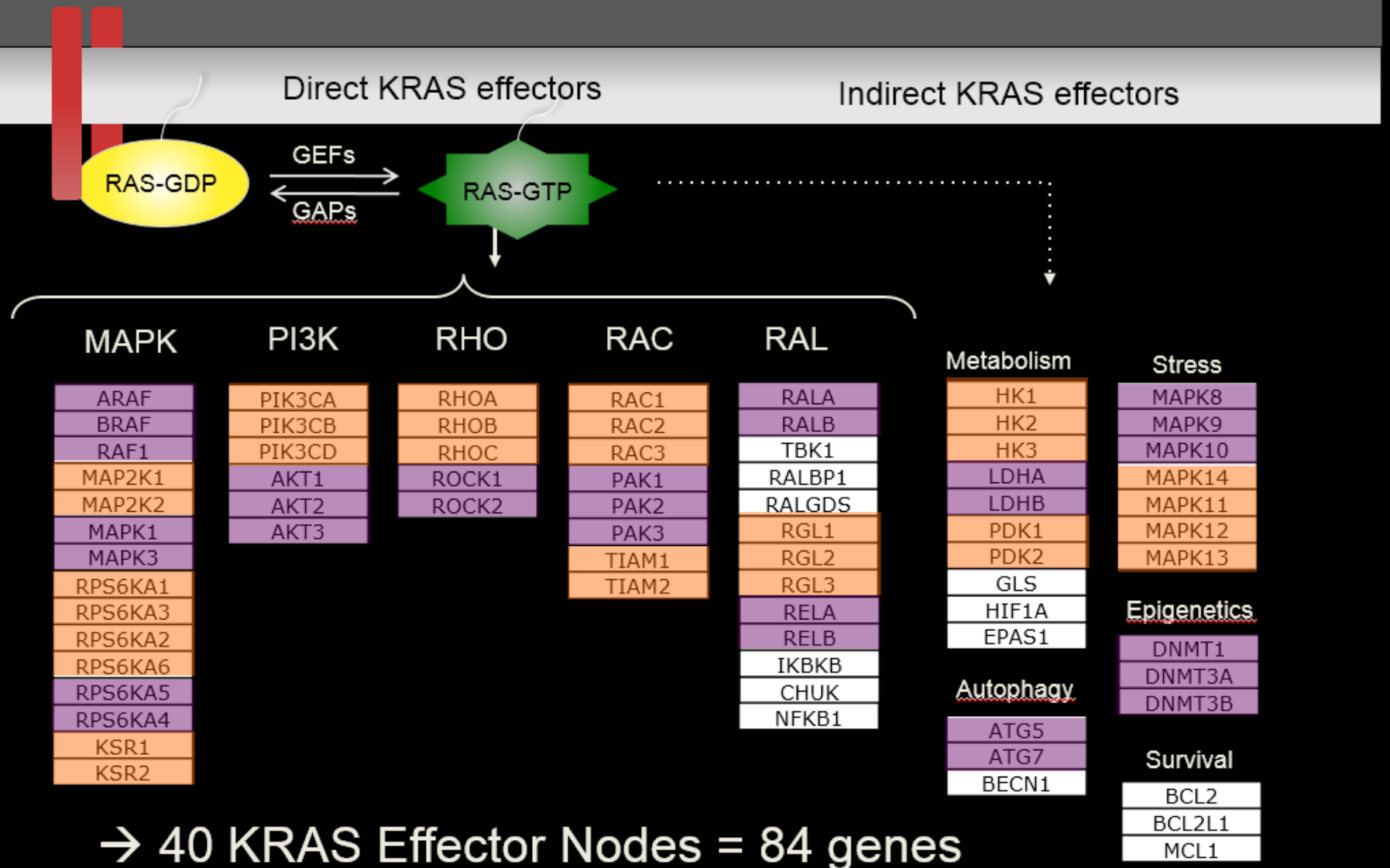
KRAS Effector Signaling: An extensive and complex network



→ 40 KRAS Effector Nodes

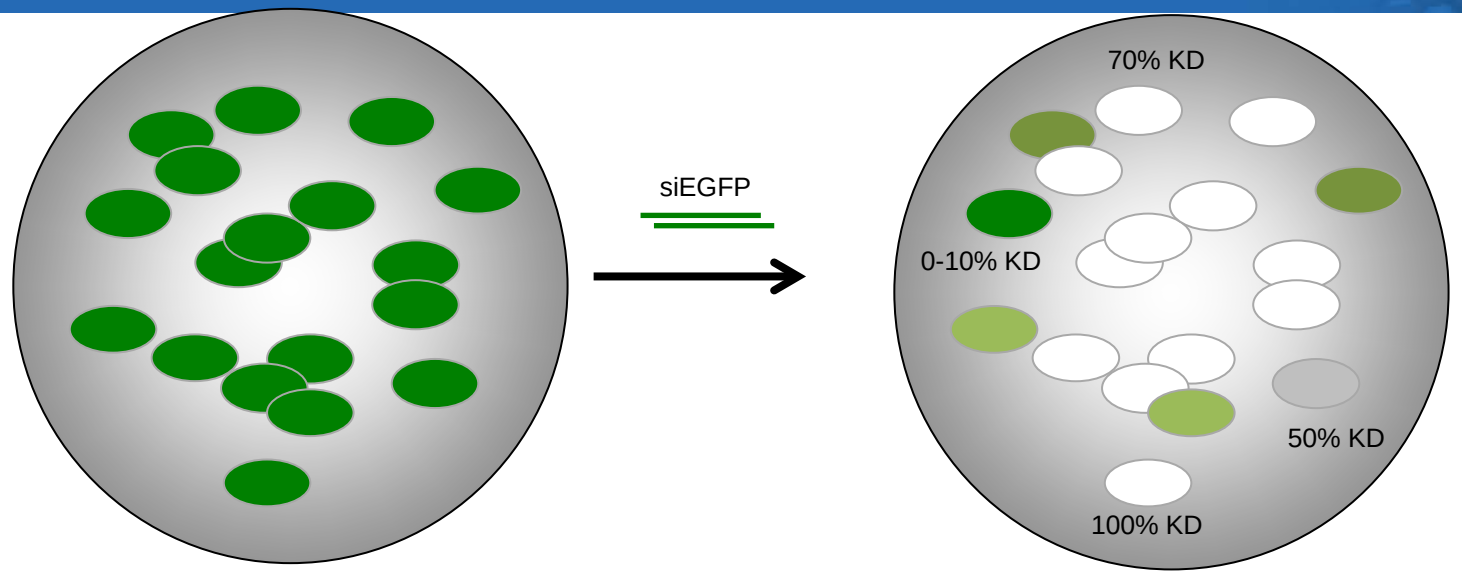
AACR
American Association for Cancer Research

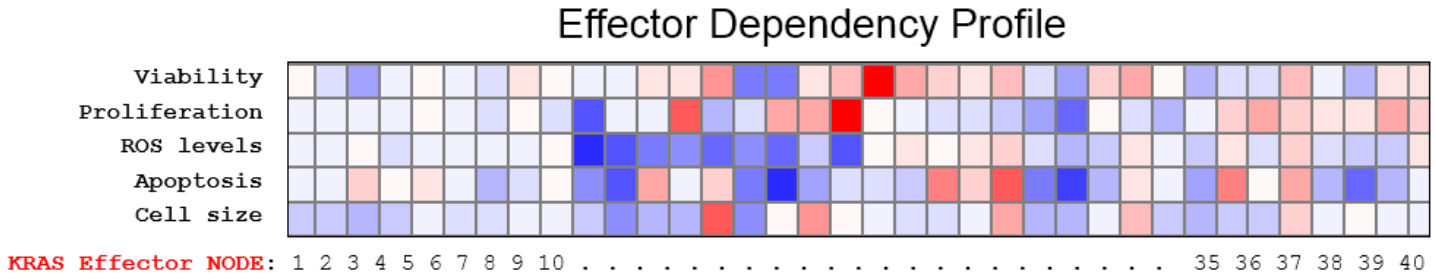
✧ Complete NODE knockdown: compensatory activation by redundant isoforms masks the importance of many nodes

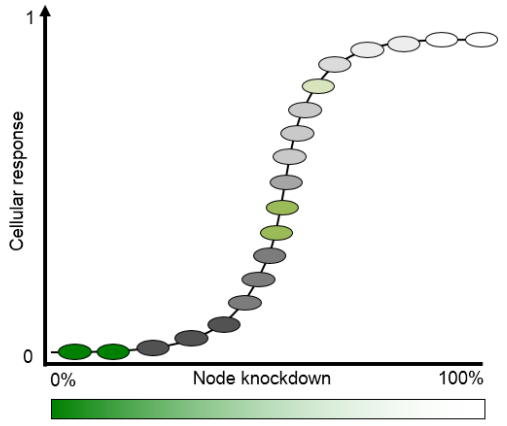


→ 40 KRAS Effector Nodes = 84 genes

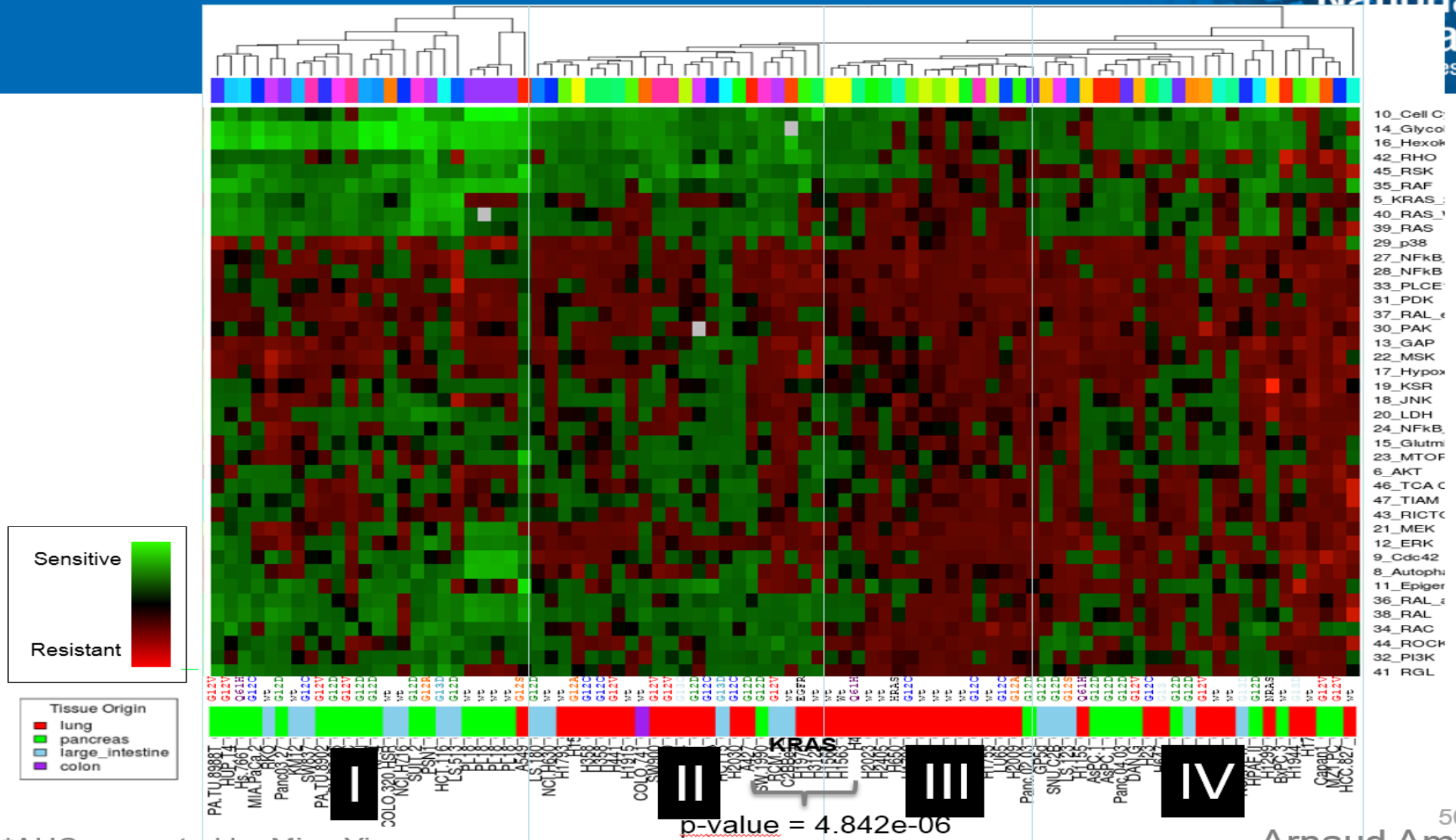
Christof Fellmann, Scott Lowe, Chih-Shia Lee, Ji Luo


siEGFP
70% KD
0-10% KD
50% KD
100% KD

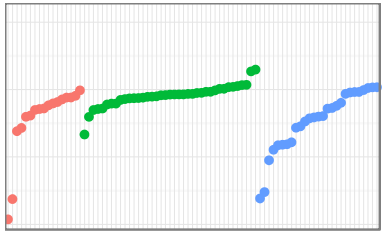

Effector Dependency Profile
Viability
Proliferation
ROS levels
Apoptosis
Cell size
KRAS Effector NODE: 1 2 3 4 5 6 7 8 9 10 . . . . . 35 36 37 38 39 40


1
0
Cellular response
0%
Node knockdown
100%

Global assessment of KRAS-effector dependency



*AUC computed by Ming Yi

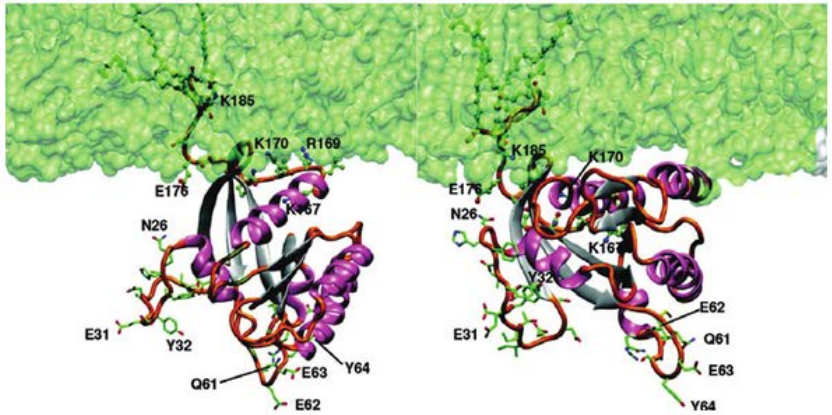


stine

Lung_NSCLC

Pancreas

Fully processed KRAS4b



A. Gorfe, U-Texas Houston

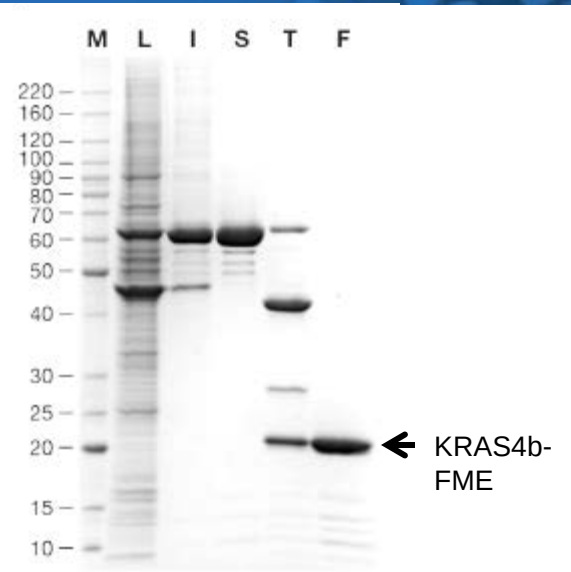
Engineering baculovirus for improved production of processed KRAS

- 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

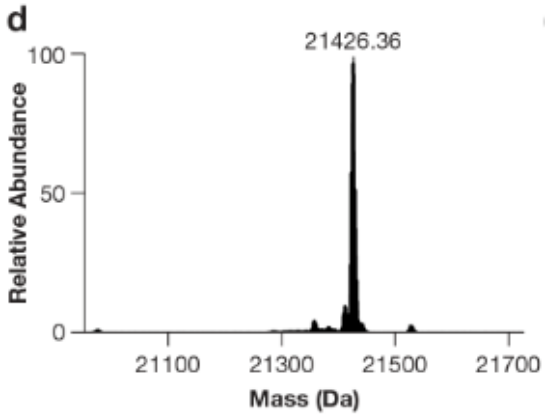
Carissa Grose, Dom Esposito, Bill Gillette

Processed KRAS4b characterization

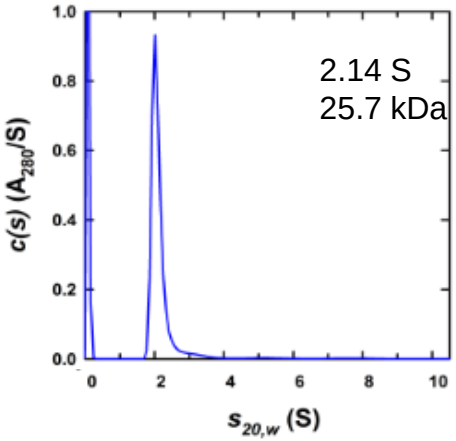
- **Extensive protein characterization**
  - Purified to homogeneity; yield >7mg/L
  - Intact mass
  - Predominantly monomeric
  - Secondary structure equivalent to non-processed KRAS4b
  - Lower thermal stability



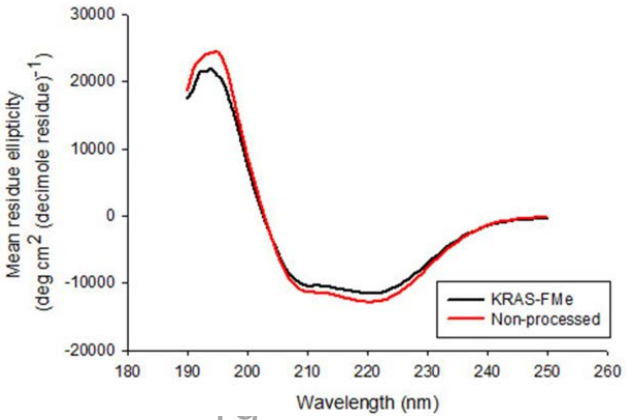
Intact mass analysis



Analytical ultracentrifugation



Secondary structure by CD



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

KRAS4b-FME binds to CRAF-RBD on Nanodiscs

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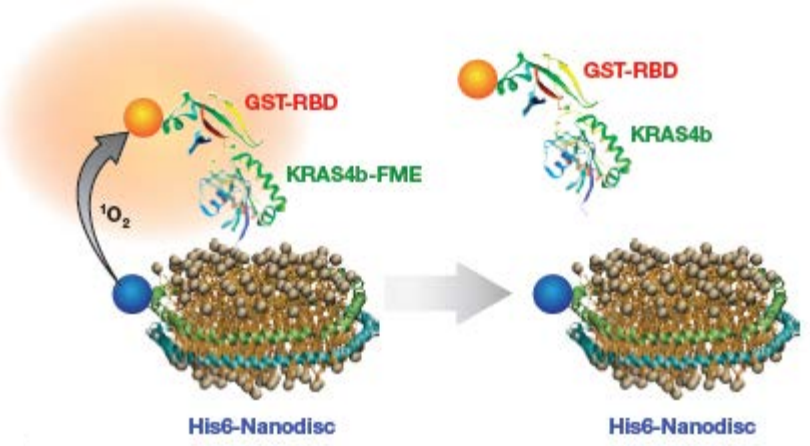
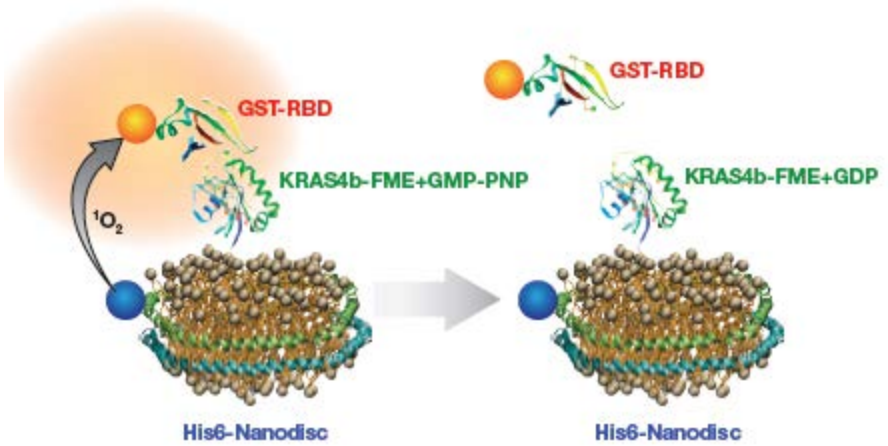
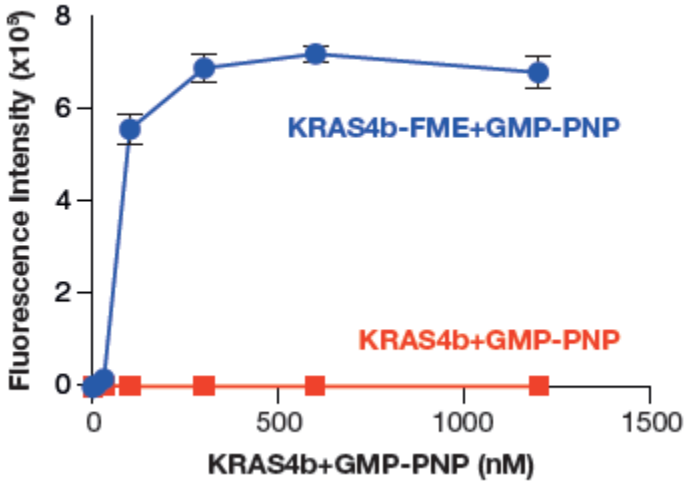
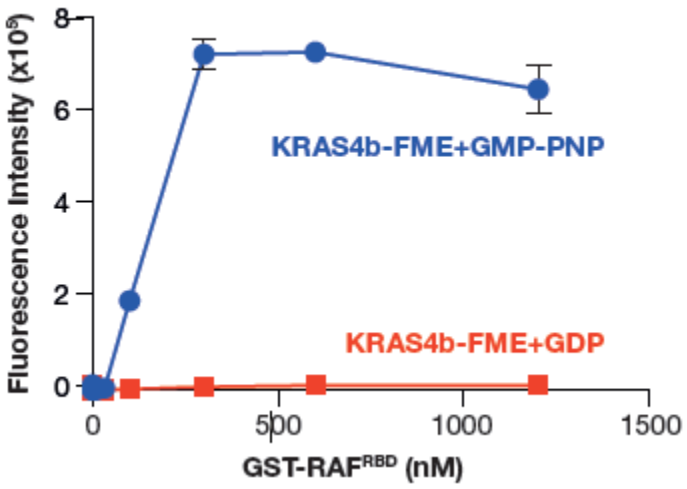


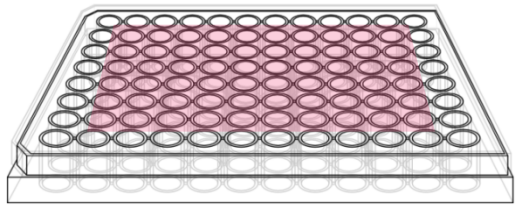


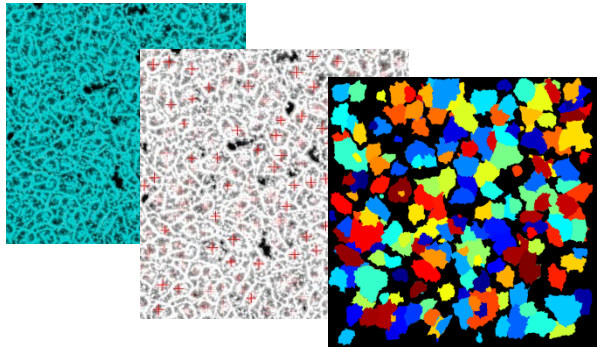


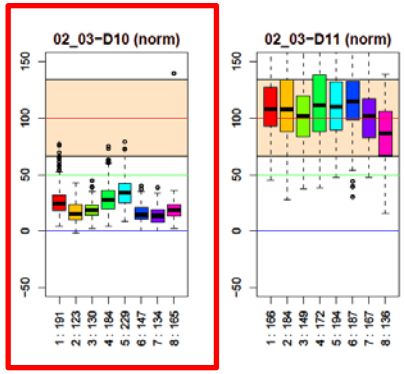


Matt Holderfield, Maria Abreu Blanco

RAS Localization Assay Overview







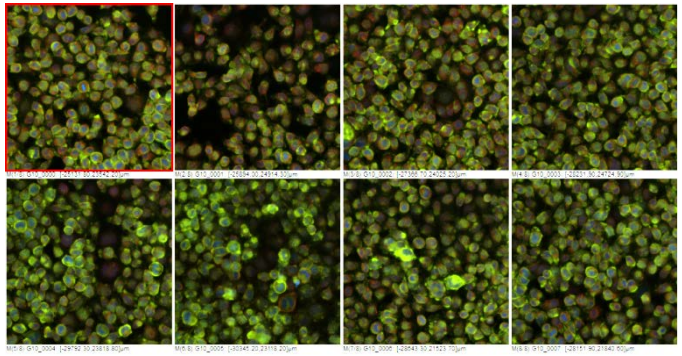
60 Wells

~125 Cells/location

Hit identification

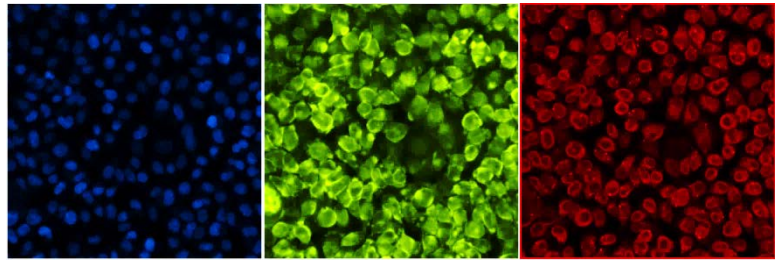
8 Locations/ well

Segmentation and Analysis



GFP-KRAS4b^{G12V}

Nucleus GFP Membrane

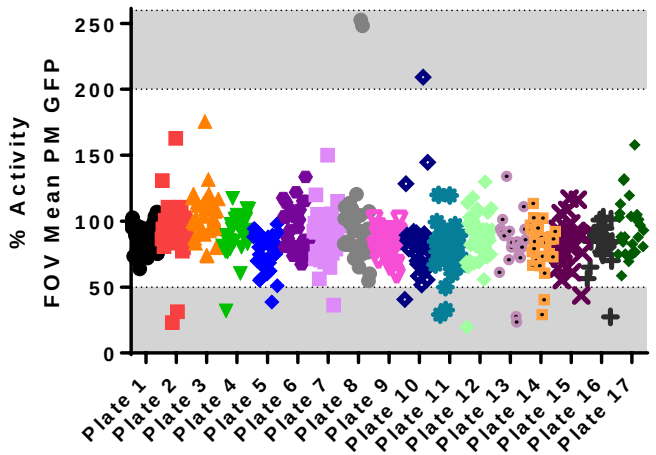


Alla Brafman

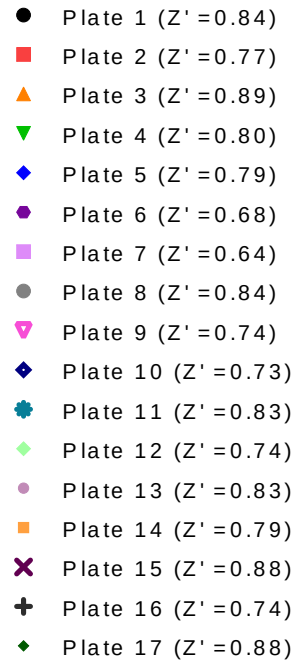
NCI Developmental Therapeutics Program screening set

Frederick National Laboratory for Cancer Research

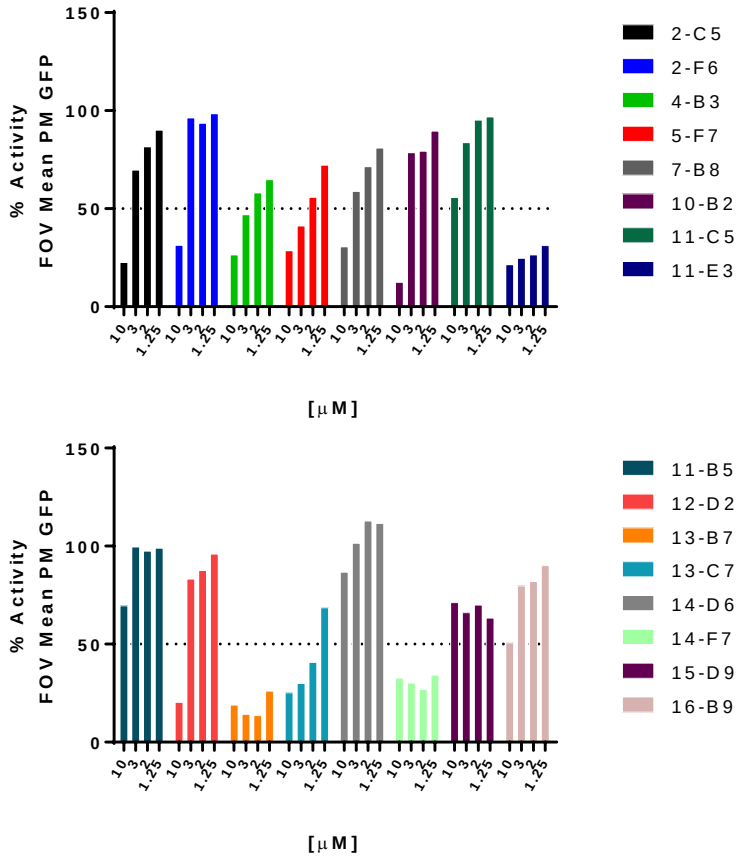
Primary assay:
GFP-KRAS^{G12V}



~800 small molecules with biological activity



Reconfirmed hits

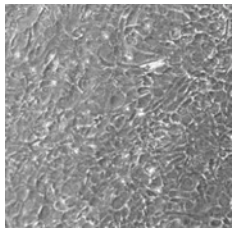


Alla Brafman

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

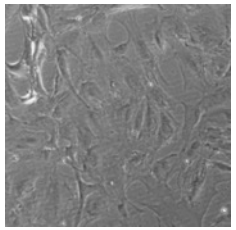
Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, **Barbacid M**. EMBO J. 2010

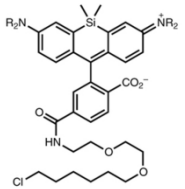
Untreated MEFs



+4-OHT

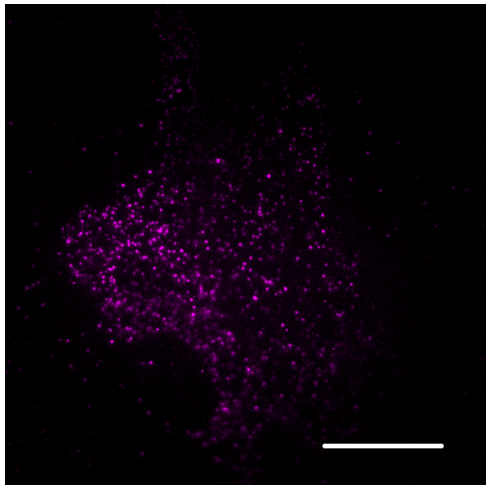
G1 arrest (day 19*)





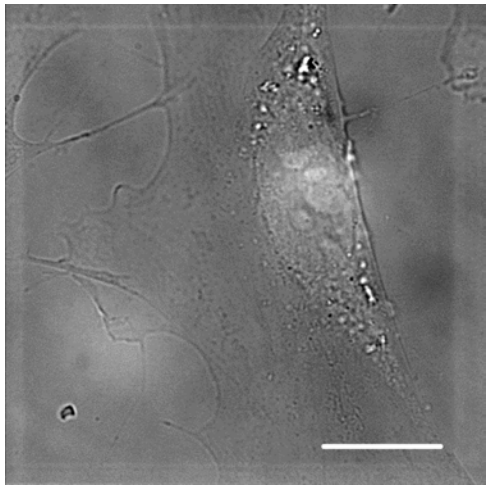
Cell permeant, super bright, fluorescent Halo ligand from Janelia Farms

HaloTag-KRAS4b can be imaged in live cells.



TIRF Image: membrane

HaloTag-KRAS4b rescues RASless MEF proliferation.



Transmitted light image

+HaloTagKRAS

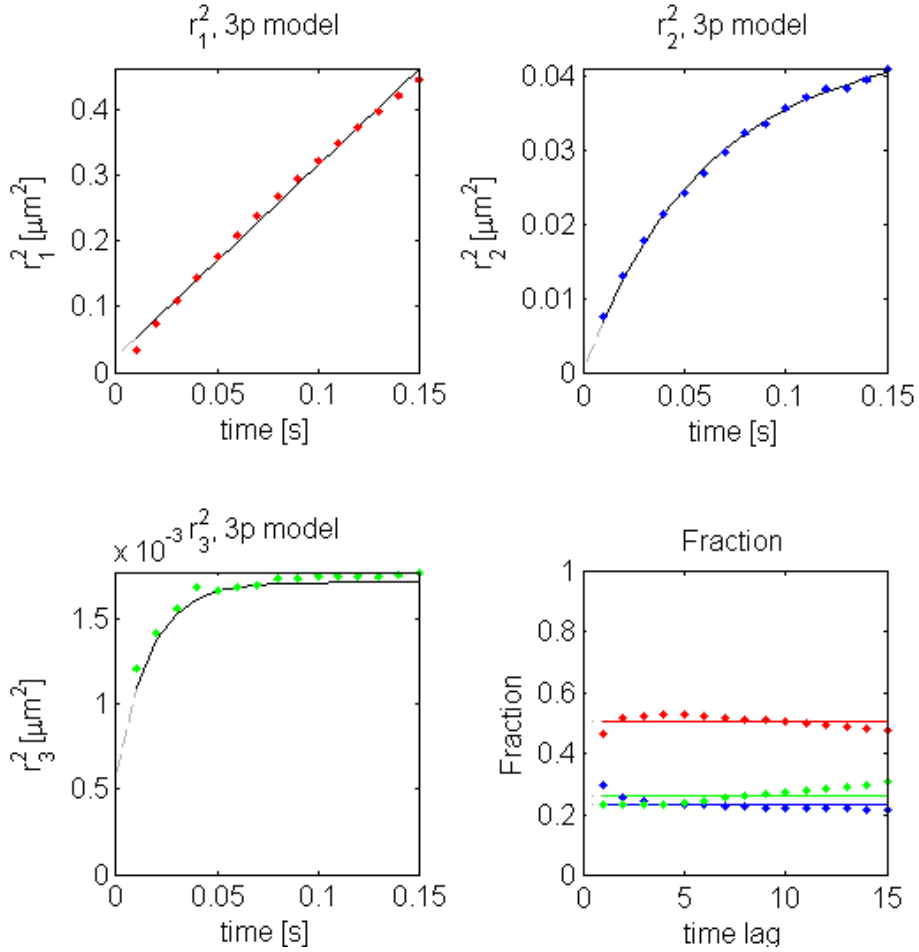
Scale bar 20 µm

Nikki Fer and De Chen

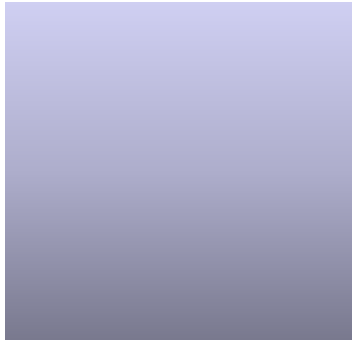
Characterization of RAS molecules in live cell membranes

Frederick National Laboratory for Cancer Research

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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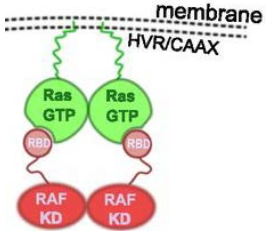
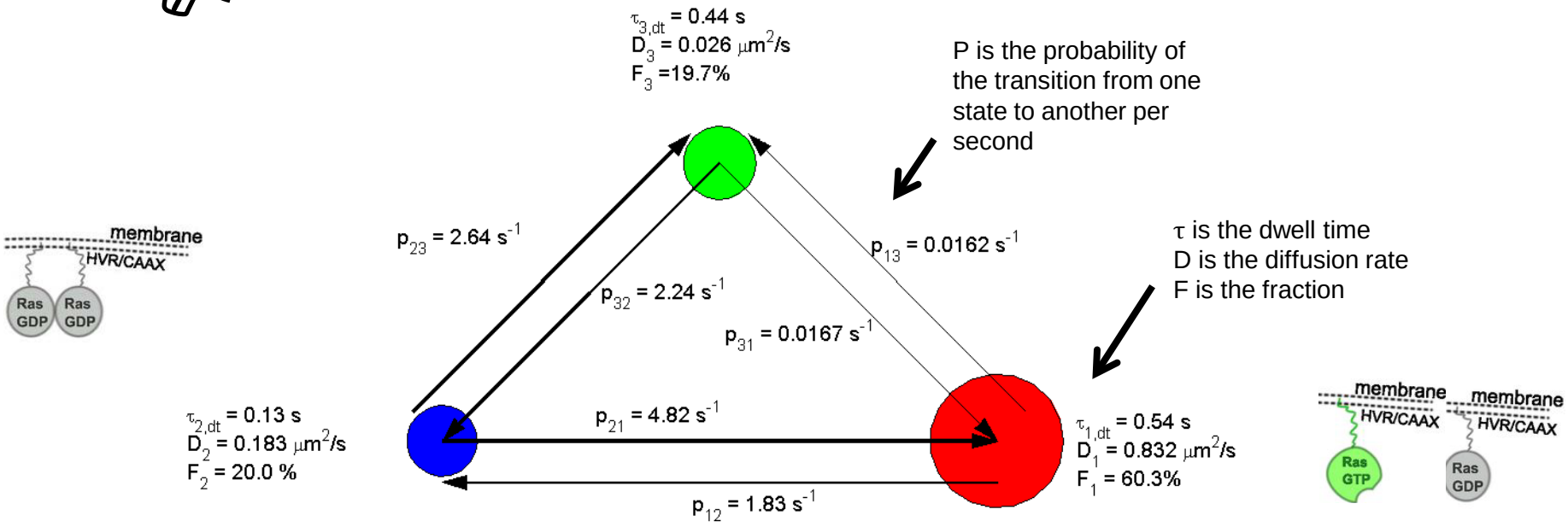
Information extracted from individual trajectories



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

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

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

Outside collaborators

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Jim Wells, USCF
Channing Der, UNC
Ken Westover, UTSW
Carla Mattos, Northeastern
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Jay Groves, Berkeley
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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

Collaboration with the RAS Community

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RAS Reference Reagents

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Tyler Jacks, MIT
Calvin Kuo, Stanford
Bill Hahn, Broad / Dana Farber
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