Frederick National Laboratory
for Cancer Research

Implementation of the RAS Program

David C. Heimbrook, Ph.D.
CEO, SAIC-Frederick (soon to be Leidos Biomedical Research)

Presentation to NFAC Sept 24, 2013

The Frederick National Laboratory is a federally funded research and development center operated by SAIC-Frederick, Inc., for the National Cancer Institute
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

Implementing the RAS Program
Agenda

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- **Introduction** **David Heimbrook**
- **Ras Projects** **Frank McCormick**
- **Implementation of the Ras Hub** **Atsuo Kuki**

Frederick National Laboratory Missions
What is RAS, and why is it so important?

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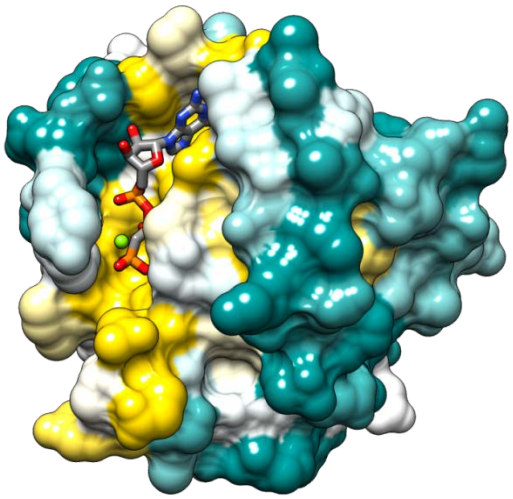
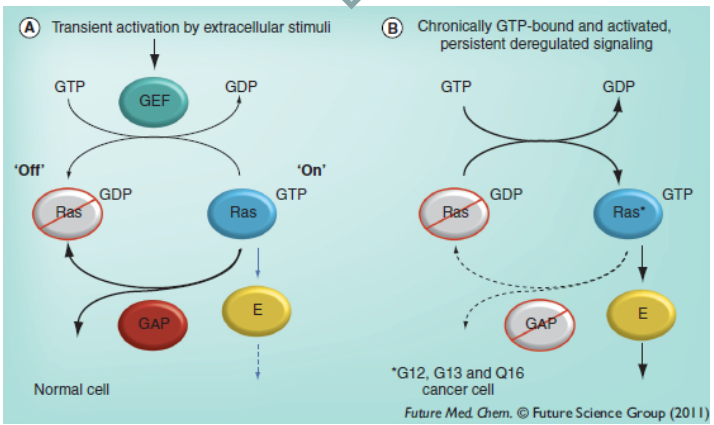
RAS is a key regulator of signal transduction in normal and cancerous cells

- Four flavors : Harvey, Kirsten (A & B), and Neuroblastoma



HRAS
(Wikimedia commons)

Growth factor receptors



Cell growth, survival, stability

Mutated RAS is found in ~33% of human cancers, is currently undruggable, and enables resistance to many existing cancer therapies

Etiology of the RAS Program at FNLCR

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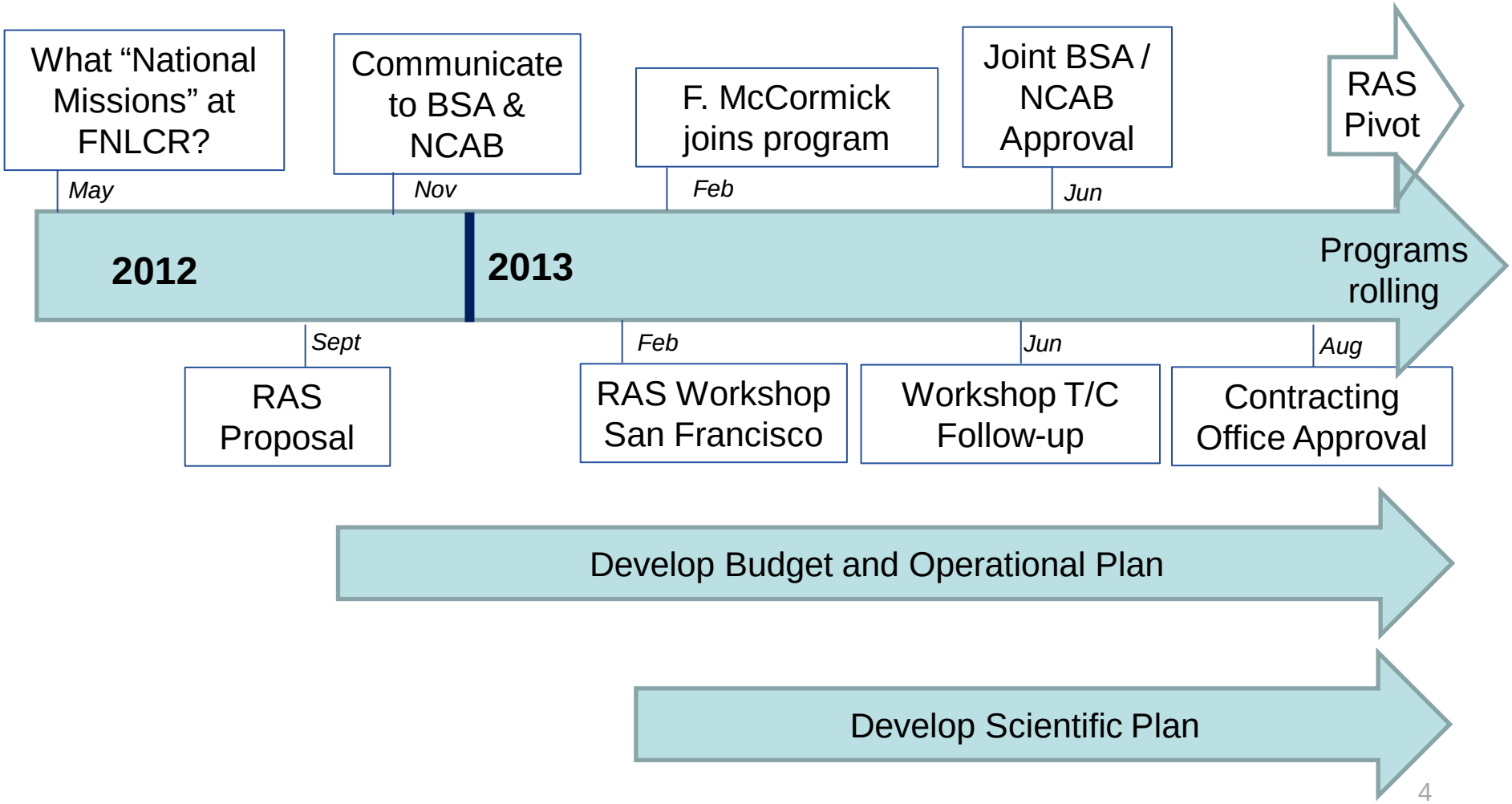

Implementing the RAS Program
Hub, Spoke, and RAS Community model

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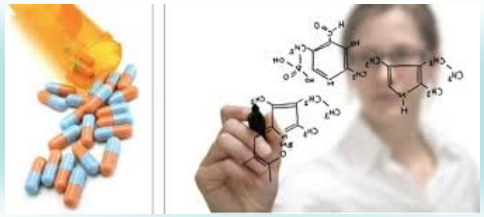
Intramural Labs

Extramural NCI-Supported Labs

FNLCR – The Hub

Biotechs



Pharma

Contract Research

The RAS Program

A FNLCR mission and a new way of doing things

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- **Approved plan "recommends operating practices that, while within the terms of the existing OTS contract, are generally different (from) the current practices"**
  - SAIC-F maintains obligation of transparency and accountability
  - NCI staff provides additional latitude for SAIC-F / Leidos to accomplish program goals

- **Examples :**
  - Program leader (Dr. McCormick) is a SAIC-F / Leidos consultant to better enable direct interaction with technical staff
  - Management of RAS Program space at ATRF
  - Implementation of approved research plans

Result : Enhanced "Pride of ownership" of Program's success

The RAS Program
Strategic Oversight and Governance

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- **Strategic Oversight**
  - An NCI-Frederick Advisory Committee (NFAC) subgroup has been named
    - Chaired by Dr. Levi Garraway
- **Research Program Prioritization and Oversight**
  - Monthly review meeting with Drs. Varmus, Lowy, Harlow, McCormick, and Heimbrook
- **Research Program Implementation – Budgetary and Scientific**
  - Dr. D, Lowy – Project Officer (NCI)
  - Dr. S. Hook – Contracting Officer's Representative (NCI)
  - Dr. E. Harlow – Consultant w/ focus on Spokes and RAS Community (NCI)
  - Dr. F. McCormick – Consultant, RAS guru and Program Leader (SAIC-F / Leidos)
  - Drs. A. Kuki and D. Nissley – Project implementation and oversight "on the ground" (SAIC-F / Leidos)

The RAS Program
Funding Model

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- **Funding for FNLCR RAS Hub**
  - Approximately $10 M / yr from re-prioritization of ongoing activities within the existing FFRDC contract - *No new money*
    - The Advanced Technology Program "Pivot" re-orients a primarily technology and shared service-based intramural effort towards driving the RAS Hub
    - Additional "one-time" funds from within the existing contract facilitate start-up activities
  - This funding supports ongoing research activities within the Hub, as well as initial phase of subcontracts between the Hub and external laboratories

- **Funding for RAS Spokes**
  - Contract Research Organization – subcontracts from FNLCR RAS Hub
  - Other Government Labs – To be determined
  - Pharma, Biotech – Self-funded
  - Academic : Some subcontracts from FNLCR RAS Hub
    - Anticipate Program Announcement

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for Cancer Research

Implementation of the RAS Hub

Frank McCormick, Ph.D.
RAS National Program at FNLCR

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RAS mutations in human cancer

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Pancreas	95%	KRAS
Colorectal	45%	KRAS
Lung	35%	KRAS
AML	30%	NRAS
Melanoma	15%	NRAS
Bladder Cancer	5%	HRAS

Project Zero: Validate mutant KRAS as a target for tumor maintenance

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Background:

Ablating KRAS (G12D) in pancreas cancer model destroys tumor

Wide range of KRAS dependency in mutant KRAS cell lines

Differing dependencies in 2D vs 3D

Re-wiring upstream following mutant KRAS knock-down in cell culture

Mechanisms of resistance unknown

Project Zero: Validate mutant KRAS as target for tumor maintenance

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Goals:

Determine the profile of mutant KRAS-driven tumors which respond to ablation of mutant KRAS

Determine pathways of acquired resistance to mutant KRAS ablation

Develop panel of cells to support drug discovery

Project Zero: short-term goals

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- **Characterization of mutant KRAS cell lines**
  - Cell lines with G12C, G12D, G12V, G13D mutations from lung, colon and pancreas primary and metastatic tumors
  - Bioinformatics approach to inform relevancy of cell lines based on clinical data
  - Generation of inducible shRNA (mutant specific) stable cell lines
- Cell-based assays to assess the effect of knock-down
  - KRAS addiction
  - Cancer hallmarks
- Cell culture support for Projects 1 -5
  - Cell lines from validation project
  - Ras-less MEFs: 4A only vs 4B only cells
  - Human Ras-less cells (to be developed in-house)

Project Zero: Bioinformatics analysis to down-select cell line candidates

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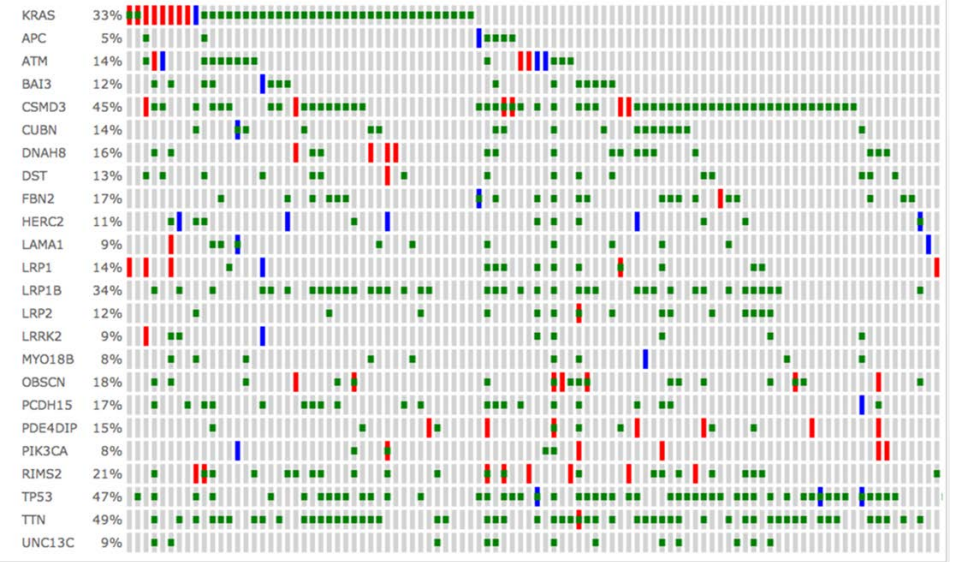
Predominant KRAS mutations in Cancer Cell Line Encyclopedia database

Mutation	count
p.G12D	42
p.G12V	30
p.G12C	20
p.G13D	10
p.G12A	9
intronic	6
p.G12R	6
p.Q61H	5
p.A146T	4
p.G13C	4
p.G12S	3
p.A59T	2
p.Q61K	2



Lung adenocarcinoma OncoPrint

Co-mutations present in >20 cell lines:

- **APC***
- **ATM***
- BAI3
- CSMD3
- CUBN
- DNAH8
- DST
- FBN2
- HERC2
- **KRAS***
- LAMA1
- LRP1
- LRP1B
- LRP2
- LRRK2
- **MLL3***
- MYO18B
- OBSCN
- PCDH15
- **PDE4DIP***
- **PIK3CA***
- RIMS2
- **TP53***
- TTN
- UNC13C

*** Included in Cancer Gene Census (COSMIC)**

Project Zero: isogenic cell lines for drug screens

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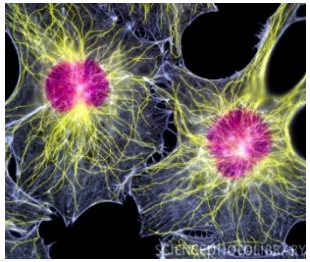
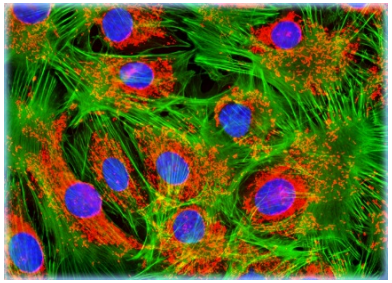
1. Rescue proliferation with normal Ras isoforms

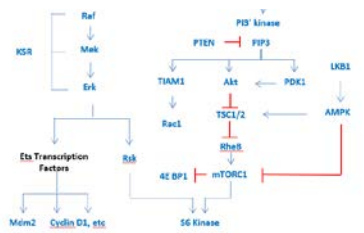


Rasless MEFs

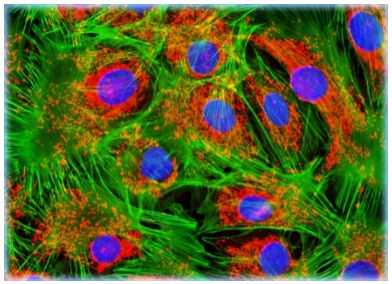
Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, **Barbacid M**. EMBO J. 2010

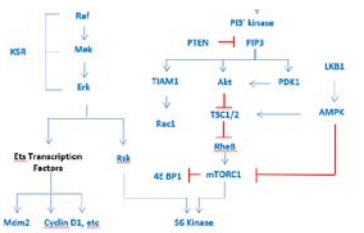
HRAS



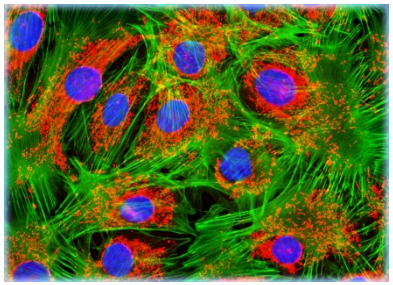


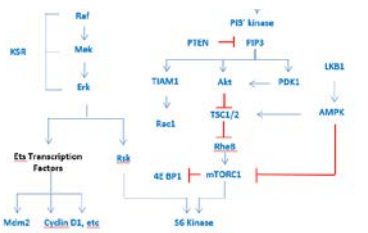
NRAS



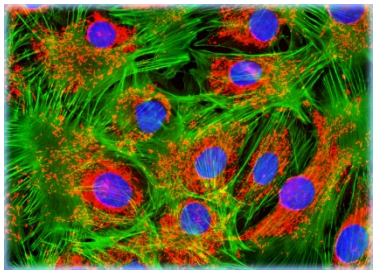


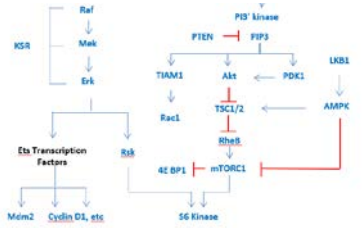
KRAS4A





KRAS4B





Project Zero: isogenic cell lines for drug screens

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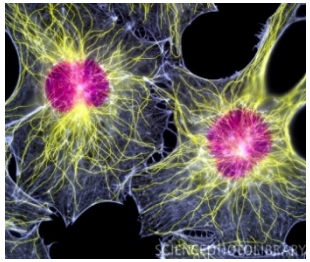
2. Rescue proliferation with mutant KRAS alleles



Rasless MEFs

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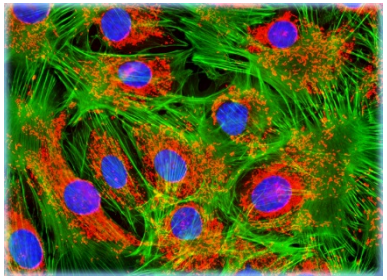
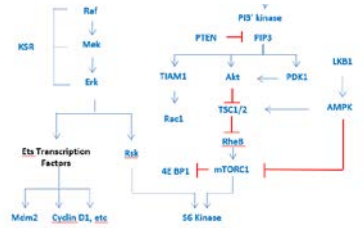
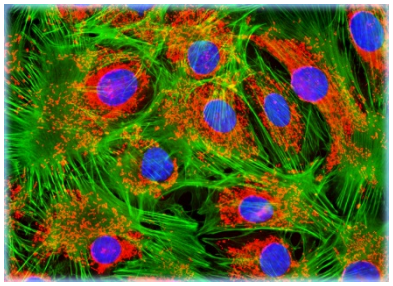
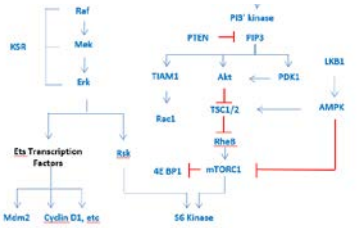
KRAS G12V



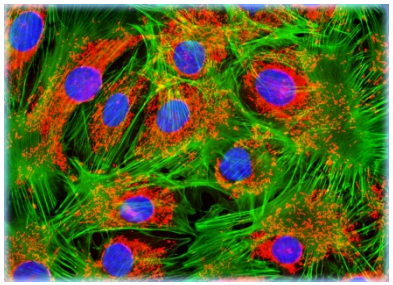


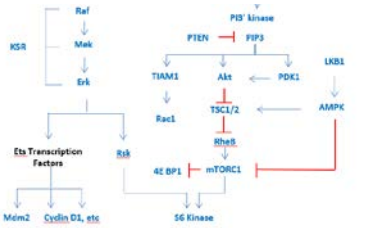
KRAS G12C



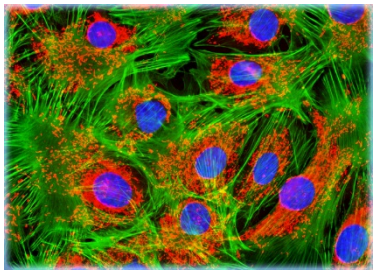


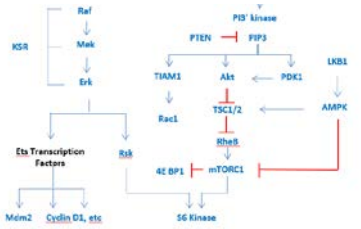
KRAS G12D





KRAS G13D





Project 1: Identify Allele Specific Compounds

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Background:

Four alleles of mutant KRAS account for most RAS cancers

Few structures of KRAS proteins are available

No co-structures of KRAS with effectors or regulators have been solved

No structures have been solved for any Ras protein complexed with full-length Raf

Project 1: Identify Allele Specific Compounds

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Goals

Generate new structures for mutant KRAS alleles and complexes

Identify allele specific complexes/interfaces that present new therapeutic opportunities

Short-term (6 months)

- Develop complete protein and activity analyses of KRAS prior to structural analysis
- Bring on-line pilot crystallization for KRAS variants (WT KRAS, G12D, G12V, G12C and G13D)
- Biophysical analysis of KRAS-Calmodulin interaction to inform HTS assay development
- Develop conditions to isolate KRAS allele complexes from cells via IP and characterize by mass spec

Access from the outside

- Consultation with Alfred Wittinghofer (RAS crystallography)
- Crystallization through collaboration/CRO

KRAS mutations in 3 diseases

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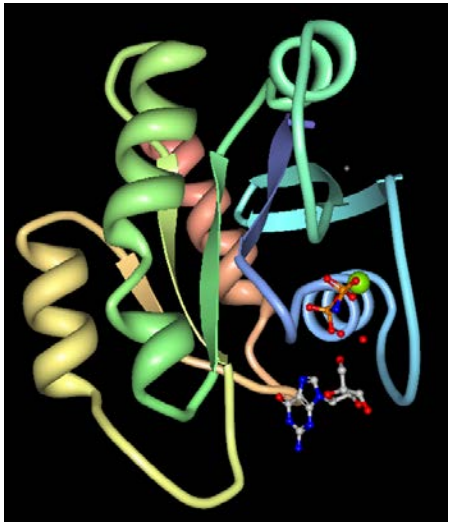
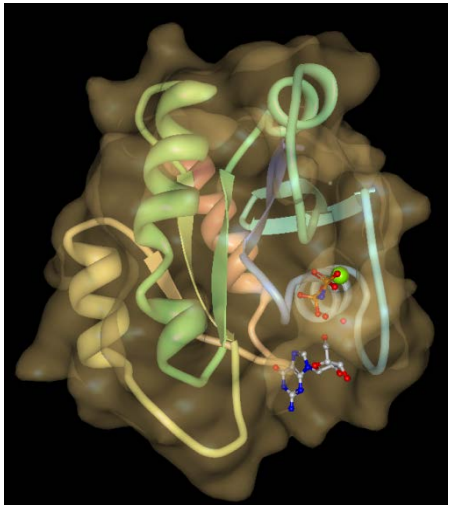
	G12C	G12D	G12V	G13D
Colorectal	6,300	22,000	12,600	11,250
Lung	22,000	9,520	11,900	1,190
Pancreas	1,200	19,000	12,000	1,000
Total	29,500	50,520	36,500	13,440

Available RAS structures

A search for "RAS" in the PDB database results in 132 structures:

	Human	Rat	Mouse	Yeast	Total
KRAS	11	2	0	0	13
HRAS	113	2	1	1	113
NRAS	2	0	0	0	2





PDB = 3GFT

KRAS structures:

- **3GFT a human wild type KRAS + GTP analog, residues 1-169, expressed in E.coli, X-ray diff at 2.27A, R-Free 0.267**

- **Ten structures, with human mutant KRAS bound to:**
  - **fragment, NMR, affects SOS interaction (Maurer T, Genentech)**
  - **small compound, affects SOS (Sun Q, Vanderbilt)**

- **Two structures, co crystal between Rat Farnesyltransferase and KRAS 4B peptide with FPP analog**

RAS – “Raf” structures

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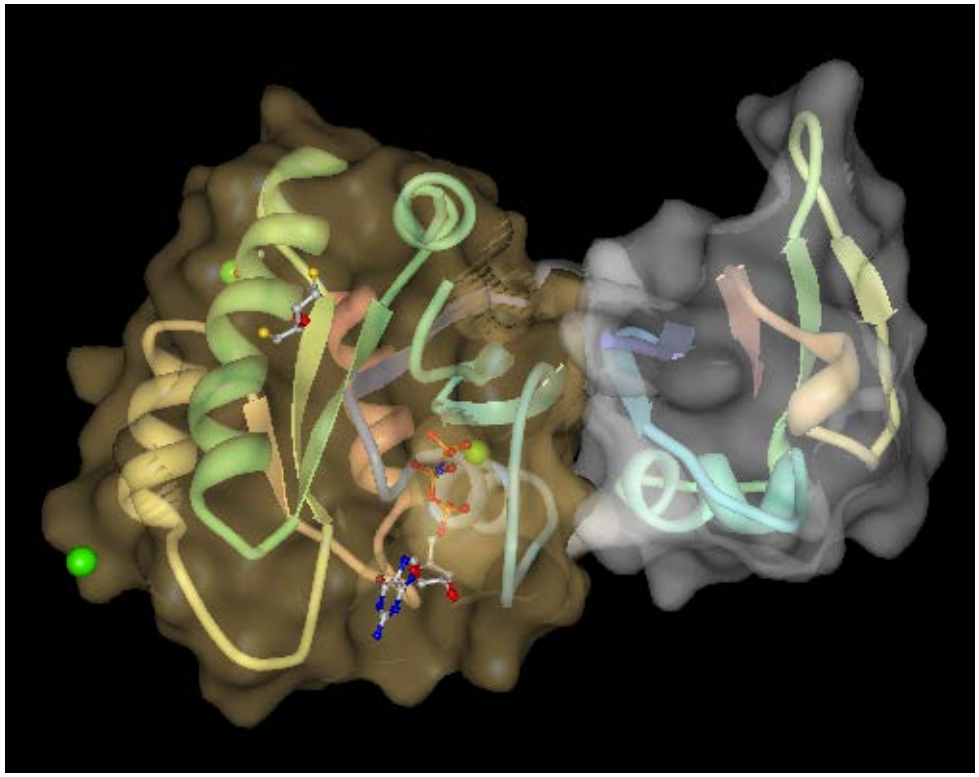
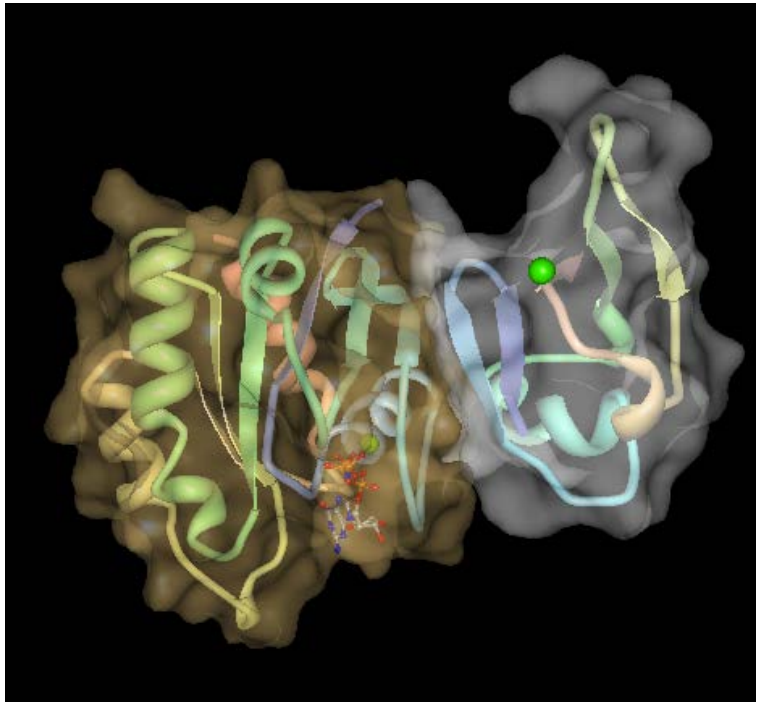


HRAS aa 1-166, Raf 54 – 131, expression system E.coli
PDB = 4G0N



1GUA: Cocrystal between Rap and Raf-RBD, were the Switch I region on Rap has been changed to the RAS switch I by mutating the E30D and K31K.
Best available model for HRAS-”Raf” molecular interaction (Ruth Nussinov).

Project One: Priorities

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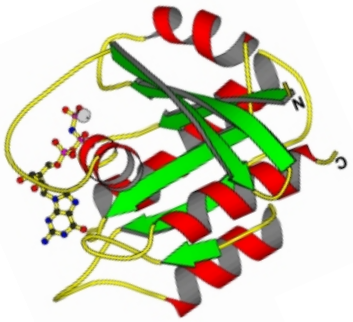
KRAS 4B

Wild type, G12C, G12V, G12D G13D and KRas4A (1-188)

KRAS 4B G12D

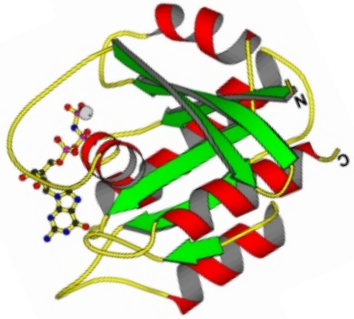
Complexed with GAP, Raf RBD, RalGDS RBD, calmodulin

KRas4B G12D with full length RAF



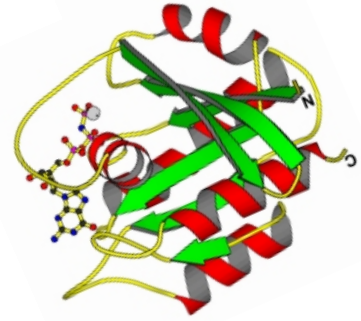
KRAS 4B

KEKMSKDGKKKKKKSKTKC



NRAS

KKLNSSDDGTQGCMGLPC



HRAS

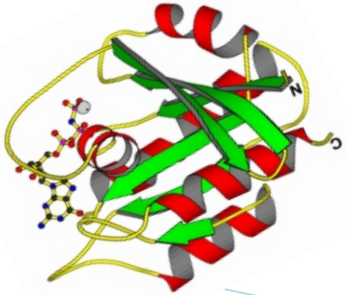
RKLNPPDESGPGCMSCKC

Farnesylation

Palmitoylation

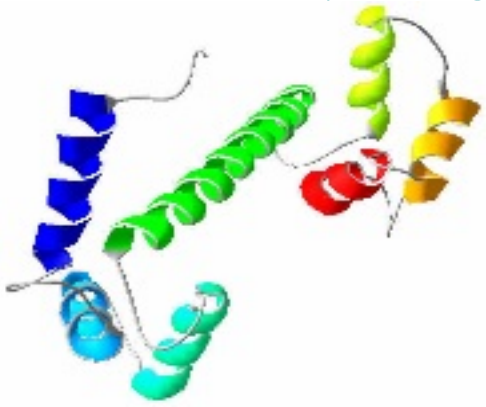
Calmodulin Binds to K-Ras, but Not to H- or N-Ras, and Modulates Its Downstream Signaling

PRIAM VILLALONGA,[1] CRISTINA LÓPEZ-ALCALÁ,[1] MARTA BOSCH,[2] ANTONIO CHILOECHES,[2] NATIVITAT ROCAMORA,[3] JOAN GIL,[4] RICHARD MARAIS,[2] CHRISTOPHER J. MARSHALL,[2] ORIOL BACHS,[1] AND NEUS AGELL[1]*



KRAS 4B

KEKMSKDGKKKKKKSKTKC



Project 2: KRAS Selective Binding Compounds

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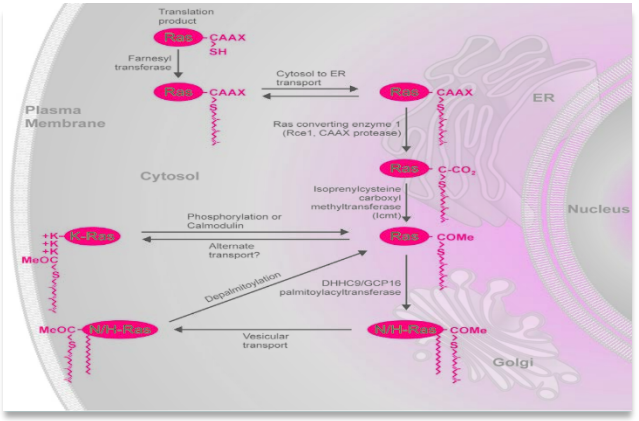
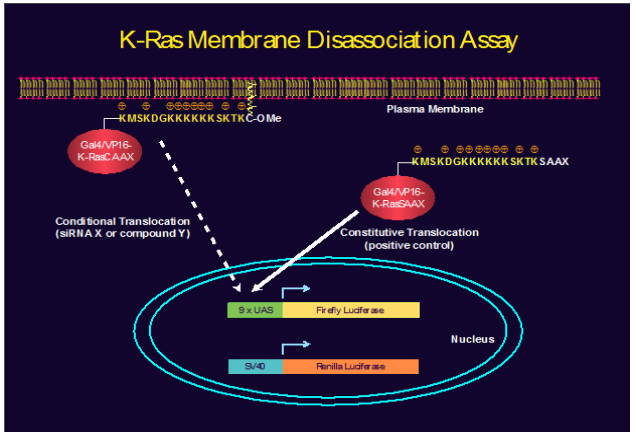
Identify compounds that inactivate KRAS independent of mutation status

- disrupt membrane localization
- selective inhibition of processing
- prevent calmodulin binding
- modulate Kras expression



Short-term

- Acquire and qualify assay for KRAS membrane association
- Develop an Intracellular Calmodulin Assay



M. Philips

Access from outside

- Philips Assay/collaborate to improve
- Engage NCATS to collaborate on reporters and compounds

Project 3: Disrupting KRAS Complexes

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Characterize and disrupt KRAS protein complexes in cells and probe the nature of KRAS dimerization

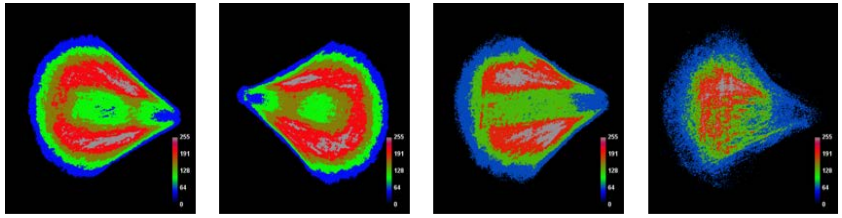
- PALM imaging
- single-molecule fluctuation techniques (FRET-FCCS/FLIM/Polarization)

Short-term

- Configure and install the appropriate super-resolution and other optical microscopes and instrumentation at the ATRF
- Develop and test the appropriate fluorescent protein constructs for conducting super-resolution and single-molecule fluctuation experiments.
- Design and test the feasibility of a primary high content screening (HCS) assay for identifying small molecules that disrupt KRAS localization and/or signaling.

Access from outside

- HCS capabilities
- Small molecule libraries



Disrupting KRAS dimers in the plasma membrane

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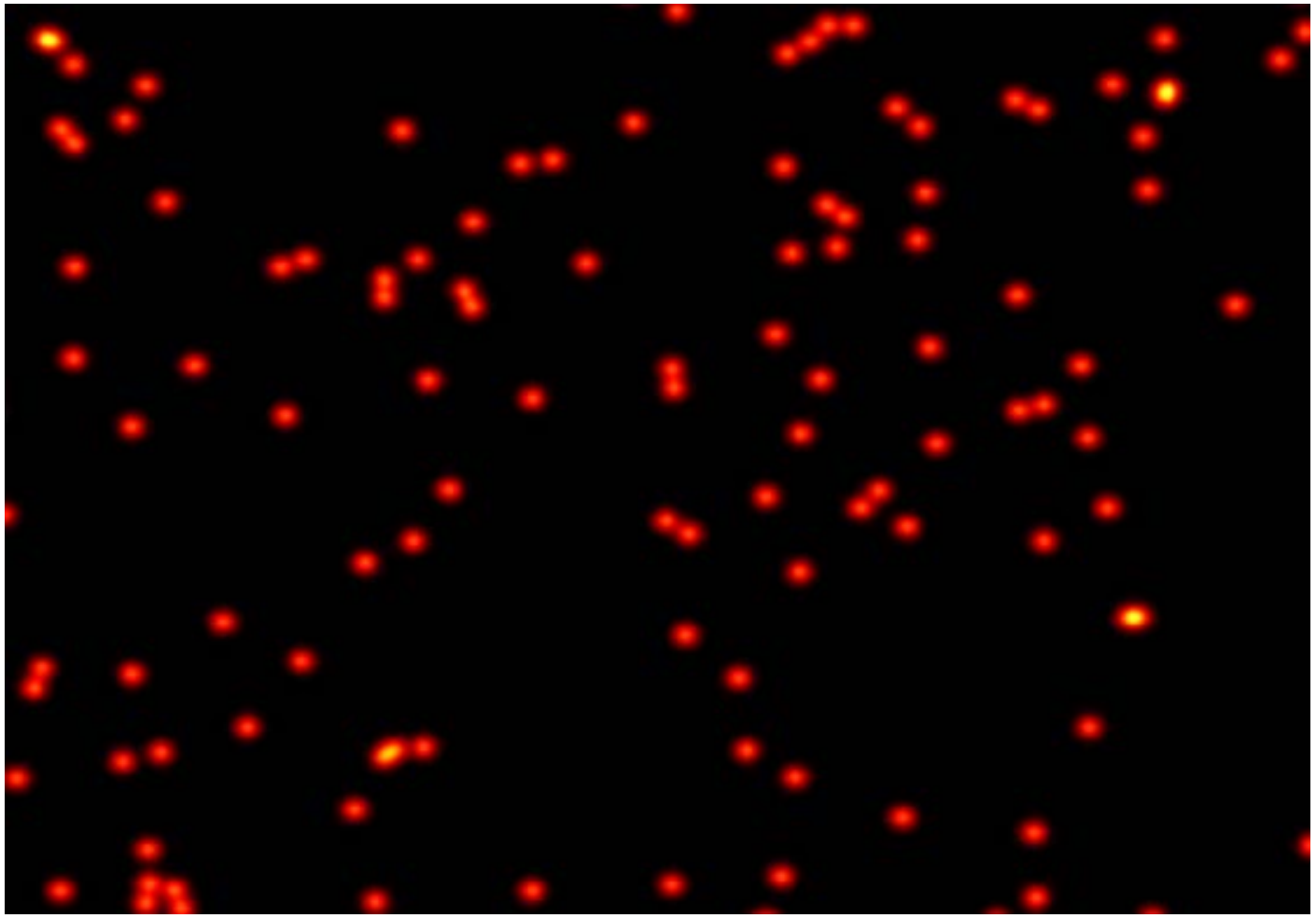
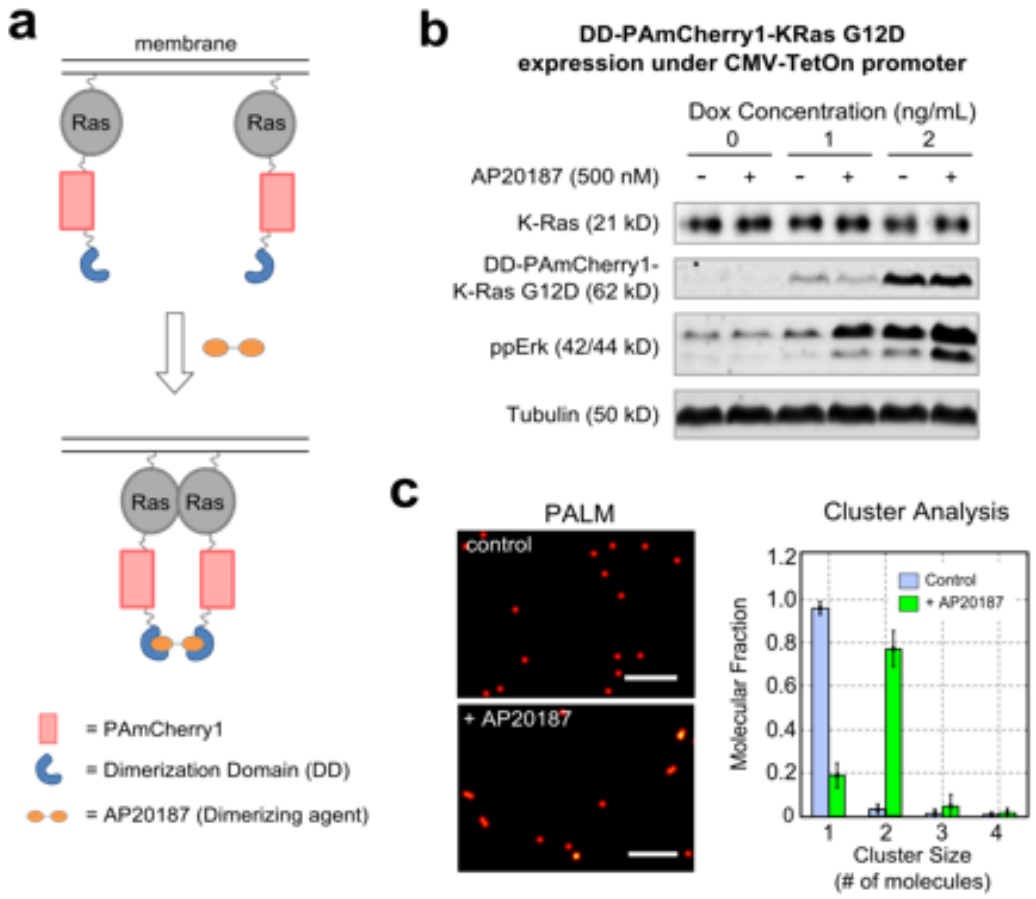
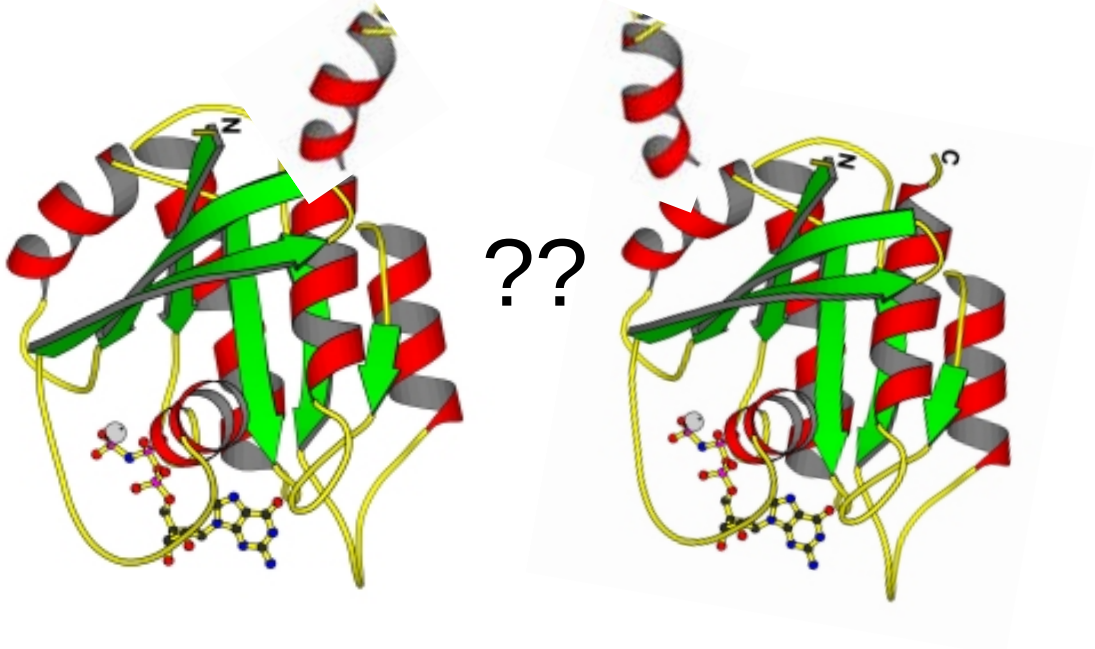



a
membrane
Ras
Ras
Ras
Ras
= PAmCherry1
= Dimerization Domain (DD)
= AP20187 (Dimerizing agent)
b
DD-PAmCherry1-KRas G12D
expression under CMV-TetOn promoter
Dox Concentration (ng/mL)
0
1
2
AP20187 (500 nM)
- + - + - +
K-Ras (21 kD)
DD-PAmCherry1-
K-Ras G12D (62 kD)
ppErk (42/44 kD)
Tubulin (50 kD)
c
PALM
control
+ AP20187
Cluster Analysis
Control
+ AP20187
Molecular Fraction
1.2
1.0
0.8
0.6
0.4
0.2
0
1
2
3
4
Cluster Size
(# of molecules)



Signal

Project 4: Cell Surface Mapping

Frederick National Laboratory for Cancer Research

Molecular description of KRAS cancer cell surface

- Identify new targets for nano-particle/antibody-mediated attack

Short-term

- Mass spec mapping of MCF10 cell surface
  - Compare perturbed vs non-perturbed cells (drugs, radiation)
  - Quantitate differences
  - Protein content (PTM's or newly expressed proteins)
- Compare Mass Spec discovery with phage display approach (J. Wells, UCSF)
- Identify/develop relevant cell and tumor models

Access from outside

- Collaboration with UCSF phage display project.

Identify and validate KRAS synthetic lethal targets

Short-term

- Current synthetic lethal screening technology needs further development
- Do not initiate synthetic lethal screen(s) in year one
- Focus on parallel development of assays (Project Zero and others) that may be appropriate for screens
- Convene meeting / workshop of spoke and rim experts to design improved screens

Access from outside

- Collaborative expertise

Project 6: Production and validation of reference reagents

Frederick National Laboratory for Cancer Research

Support internal FNL/CRTP RAS projects with qualified and standardized reagents

Generate high-quality reference reagents for the Ras extramural community

- Reagents will include DNA clones, cell lines, viruses, antibodies, and proteins

Short-term (six months)

- Develop production and QC protocols for generation of materials essential for initiation of the FNL/CRTP Ras Mission projects
- Identify and develop mechanisms for vetting extramural reagent requests and delivering materials to the extramural community

What we need from outside

- Advisory group for prioritization and vetting of requests for reference reagents (possibly linked to the Ras Interactome)
- Depending on the level of demand for reagents, external support for repository and distribution services may be required

Examples of Discussions and Collaborations with Hub (first wave) for critical path RAS Hub Program Priorities

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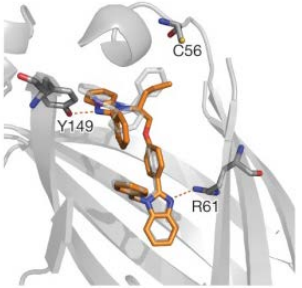
Project 1: Identify Allele Specific Compounds

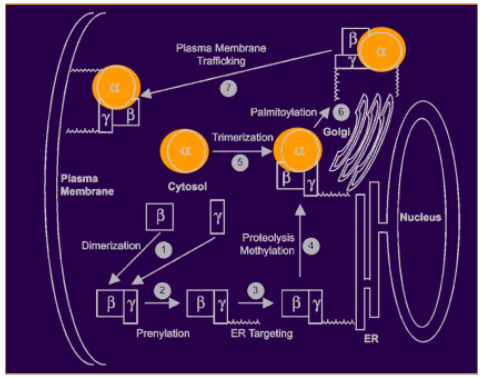
- Alfred Wittinghofer (Max Planck Institute) conducted seminal work in RAS crystallography and structure-based development of inhibitors.
- Project 1 team is consulting with Wittinghofer lab on modifications to promote crystallization, conditions to generate most relevant structures and complexes, and assays to evaluate activity.



G Zimmermann et al. *Nature*, e-pub 1-5 (2013)

Project 2: KRAS Selective Ablation

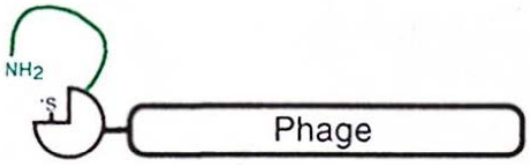
- Unique processing and trafficking of KRAS to the plasma membrane = Opportunity for intervention.
- Mark Philips (NYU) has developed assays for probing KRAS CAAX processing and membrane localization.
- Project 2 team establishing collaboration for further R&D.



D Michaelson et al. *Mol. Biol. Cell*;13:3294-3302 (2002)

Project 4: Cell Surface Mapping (mass spectrometry)

- Collaboration with Jim Wells (UCSF) initiated for cross-comparison with his phage display results and to inform mapping. Currently preparing to conduct inter-laboratory comparison on MCF10a cell lines to be grown by the same protocol as the Jim Wells' lab.



Atwell et al. *PNAS*; 96(17): 9497–9502 (1999)

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for Cancer Research

Implementation of the RAS Hub

Atsuo Kuki, Ph.D.
Chief Technology Officer, SAIC-Frederick (soon to be Leidos Biomedical Research)

Presentation to NFAC Sept 24, 2013

The Frederick National Laboratory is a federally funded research and development center operated by SAIC-Frederick, Inc., for the National Cancer Institute
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

Advanced Technology Research Facility : Redeploying tech labs to form the RAS hub

Frederick National Laboratory for Cancer Research

Pivoting strengths within the NCI FNL contract into a new mode of resource deployment and to drive RAS projects: the RAS Pivot

Progress in Launching the RAS hub

- **FNL: Advanced Technology laboratories and capabilities**
- **Mapping and pivot plan to form new directorate, to anchor RAS Hub**
- **New CRTP Directorate in the FNL (Dwight Nissley, director)**
- **Produce, validate, distribute "RAS-enabling" Biological Reagents** (*Hub core labs out to Spoke*)
- **Transition Year FY14 (balancing intramural / FNL / extramural roles)**
- **Collaborations** (first wave, on specific Hub projects, Hub ⇔Spoke)
- **Drug Discovery and Cross-sector Partnerships for RAS Tx/Dx** (timely opportunity to leverage cCRADA approach)

Technology integration and Applied Science in the FNL

Advanced Technology Program Circa 2012

Frederick National Laboratory for Cancer Research

Protein Expression Lab

Protein Chemistry Lab

Proteomics and AnalyticalTech

Molecular Technology

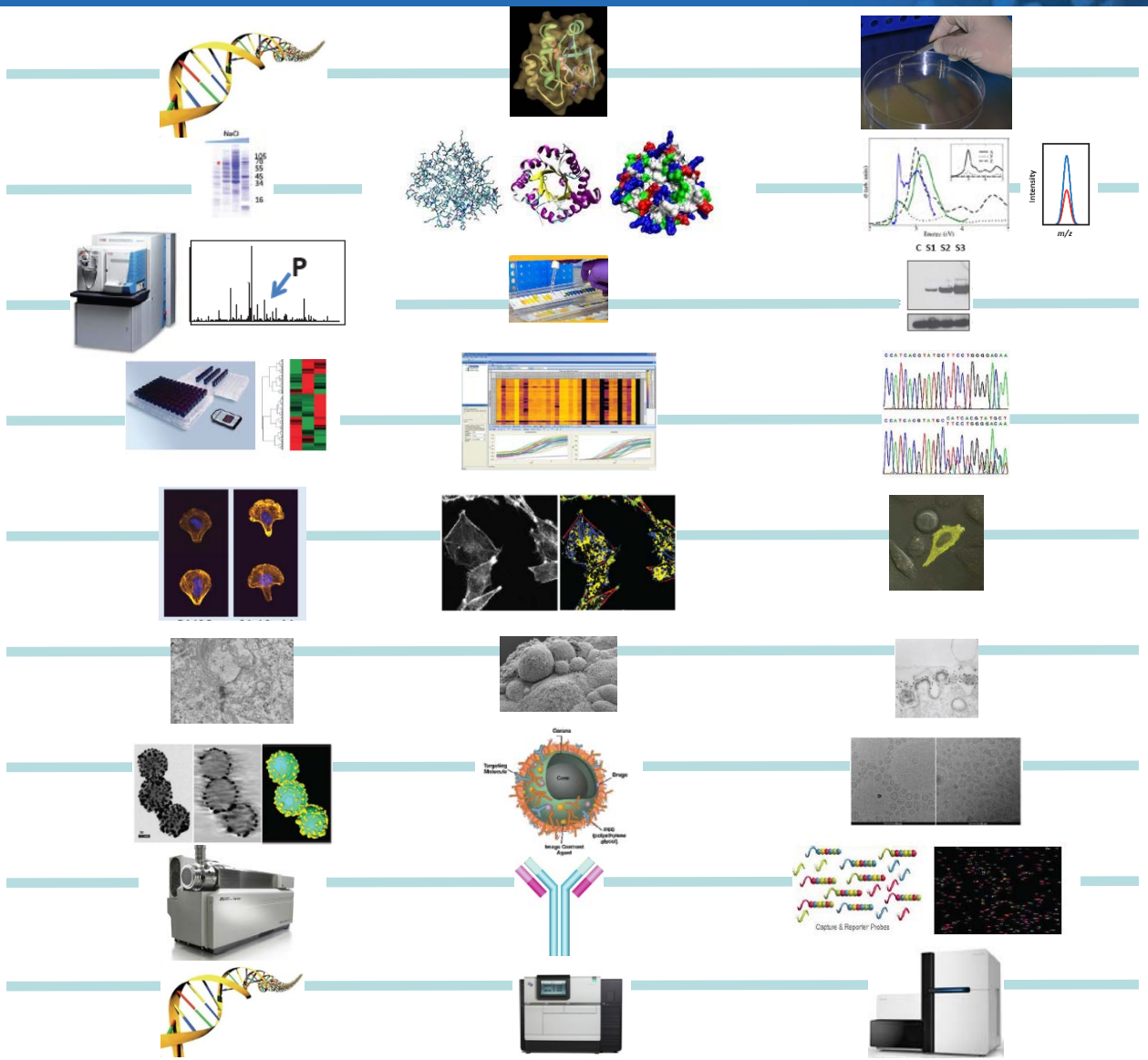
Optical Microscopy and Analysis Lab

Electron Microscopy Lab

Nanotechnology Characterization Lab

Antibody Characterization Lab

CCR Sequencing Facility



Advanced Technology Program (ATP)
Modes of Resource Deployment Circa 2012

Frederick National Laboratory for Cancer Research

- **Protein Expression** Lab
- **Protein Chemistry** Lab
- **Proteomics** and MassSpec/NMR
- **Molecular Technology**
- **Optical Microscopy** and Analysis Lab
- **Electron Microscopy** Lab
- **Nanotechnology** Characterization Lab
- **Antibody** Characterization Lab
- **CCR Sequencing** Facility

Four shared core labs provide advanced tech capabilities to NCI, to other NIH institutes, and other federal agencies

(also, dedicated sub-units with assured bandwidth within the large core labs)

Division of Canger Epidemiology and Genetics ⇔ dedicated Hormone function (within MassSpec core)

Imaging Labs (7 FTE each) in blended mode

Half-dedicated (CCR, NCL) assures bandwidth, Half as shared core lab

Dedicated Labs: Specific contract funding

1. NCI/OD/OCNR ⇔ NCL ⇔ world
2. NCI/OD/OCCPR ⇔ ACL ⇔ world
3. NCI/CCR ⇔ Sequencing Facility

Office of Cancer Nanotechnology Research

Office of Cancer Clinical Proteomic Research

Center for Cancer Research

May 2013: Resource Map for the RAS Program

Frederick National Laboratory for Cancer Research

- Working closely with Frank McCormick, we completed the Map of RAS Program needs to Hub capabilities || gaps analysis
- Protein production, biophysics of protein complexes, and novel cellular assays are priority
- Establish structural biology capability (pilot crystallization)
- Refocus expression analysis and NextGen Sequencing for cancer models and clinical studies
- Leverage multi-laboratory team-oriented talent

	RAS Program Resources: Available or	RAS Program Resources: Gaps
Cells, Ce lines, Vectors, DNA		

	RAS Program Resources: Available or can be developed at FNL	RAS Program Resources: Gaps
Optical Imaging Electron Microscopy	• Multiple color PALM imaging • Photo-switchable fluorescent tags for PALM • Super resolution PALM • TIRF microscopy • FRET biosensors for studying conformational changes of RAS. • Imaging screens for compounds that affect KRAS localization	• Resources for cloning existing technologies at ATRF (i.e. TIRF/PALM), and for personnel. • High content imaging, multi-well assays, automated detection of phenotypic changes in cells • High-content imaging for

RAS Hub projects: Matrix Approach in FNL Cancer Research Technology (CRTP)

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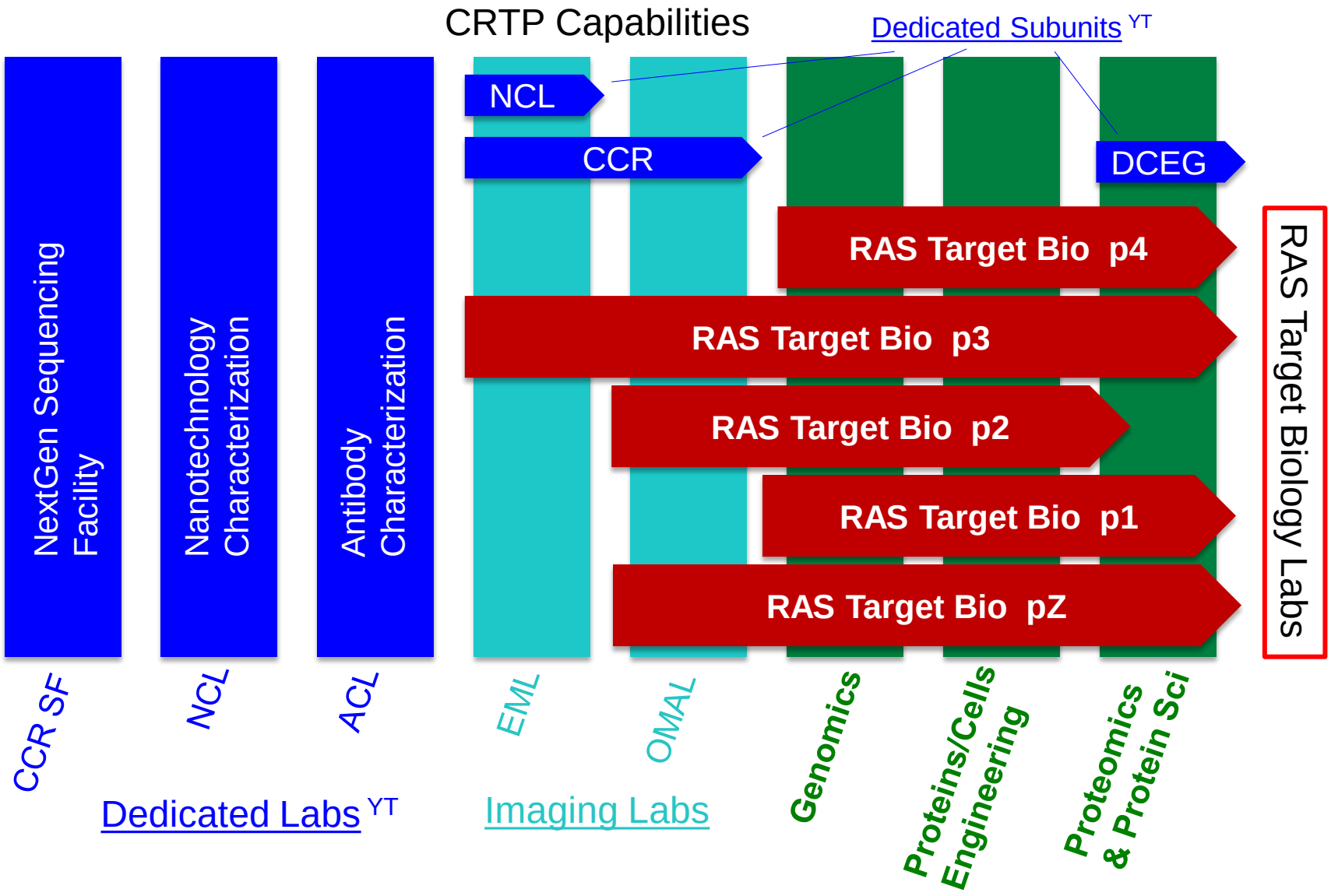

Matrix Allocation of Resources from core labs to RAS Hub priority projects

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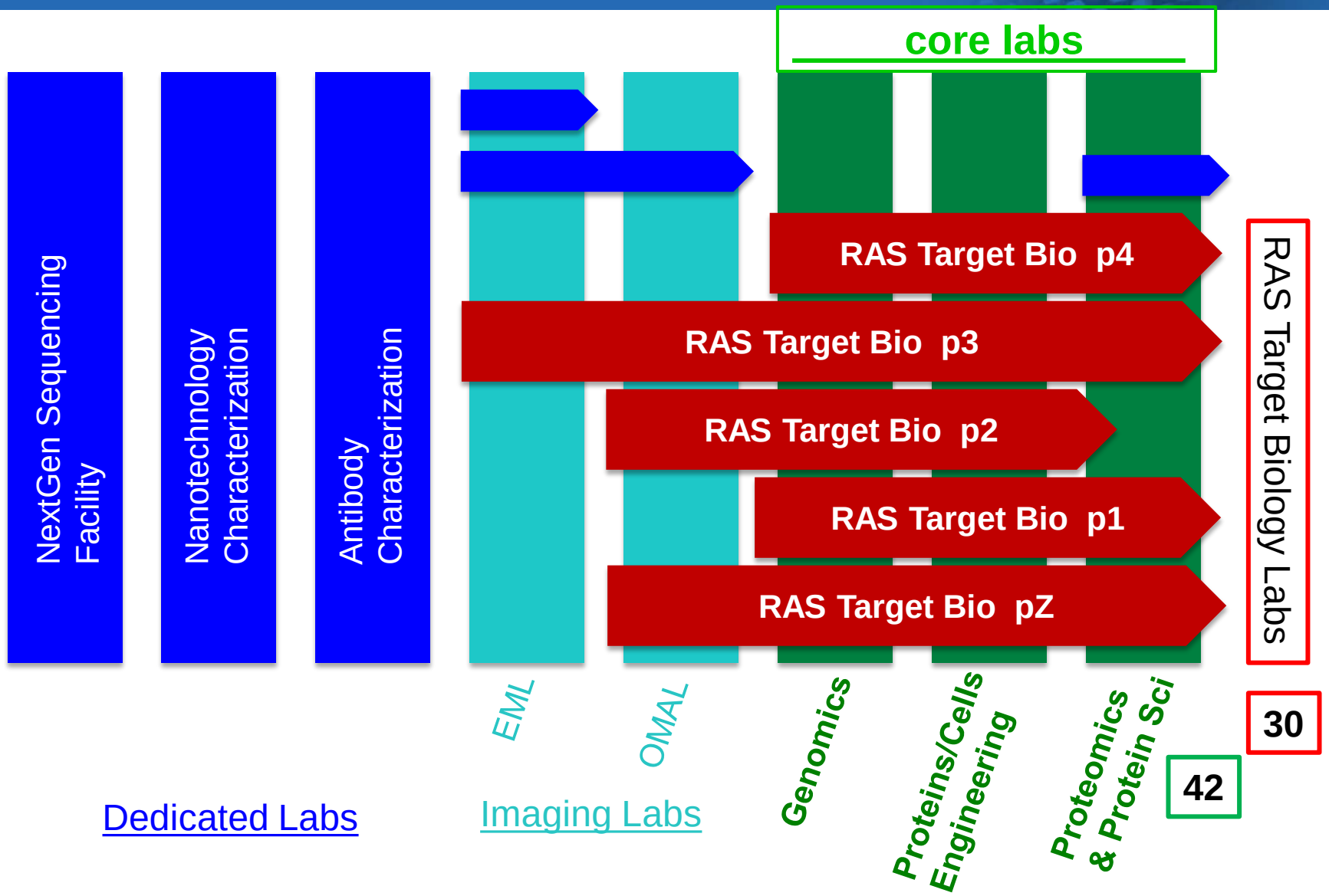

Principles of the RAS Program Hub

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RAS Target Biology and CORE labs managed synergistically in RAS Program hub

Teams launched, intense prep for new mode of resource deployment

CORE

- Retain depth of shared core lab technical expertise, leveraged and reshaped to support RAS
- Maintain breadth of capabilities to enable RAS and NCI cancer research efforts
- Matrixed approach provides flexibility to adapt to rapidly changing RAS project needs
- Resources can swiftly be reallocated or repurposed to meet changing needs
- RAS teams will draw from core labs to greatly amplify their output

RAS Target Biology

- RAS project teams: unencumbered focus on national program objectives
- Reach into CORE to obtain prioritized technology and research support
- RAS project team leaders: critical FNL function for this mission

Essential Transitions Moving Forward

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Strategic drawdown in intramural core services

- Establish alignment of core services with RAS Project needs
- Develop list of RAS-Aligned services that will be available as capacity exists
- Work with customers to develop new mechanisms and sources for support
- Phased Reduction in Intramural Services

Staffing RAS Hub – Increase talent and broaden expertise

- Identify key RAS Program roles that require recruitment
- Develop recruitment strategy and initiate national searches
- Work with RAS community and NCI leadership to find team-oriented game-changers
- Integrate new talent into FNL RAS Program

Coordination for spokes and also for hub ⇔ spoke collaborations

- Identify RAS Program objectives where spoke synergy and collaboration will accelerate
- Host RAS planning and brainstorming meetings at ATRF
- Recruit collaborators and establish mechanisms for FNL participation
- Develop RAS Interactome and foster greater RAS spoke network

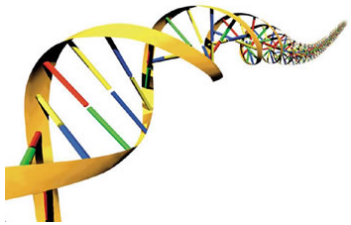
Cultural ecosystem transition – Building intellectual critical mass

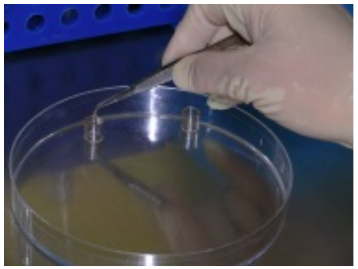
- Transform technology powerhouse *espirit de corps* into RAS Hub team culture
- Nucleate intellectual hub through seminar series, symposia and in-resident visiting researchers
- Develop RAS Initiative postdoctoral fellowship program
- Leverage partnership development to co-locate technology and expertise

Production, Validation and Distribution of Reagents for Hub projects, and for Spoke projects

Frederick National Laboratory for Cancer Research

- Support internal FNL/CRTP RAS projects with qualified and standardized reagents
- Provide differentiated contributions to accelerate extramural RAS research and, over time, a range of external entrepreneurial and pharmaceutical efforts
- How? FNL contract **Technical Service Agreements** (TSA) will enable wide distribution of reference quality reagents to the RAS extramural R&D community





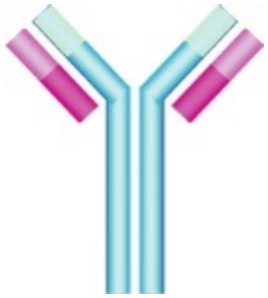
DNA clones

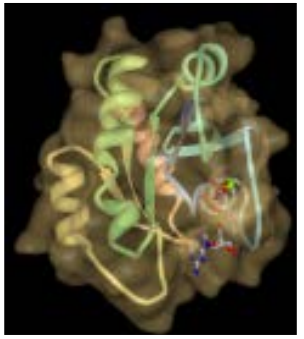
Cell lines

Viruses

Proteins

Antibodies





Transition Year FY2014

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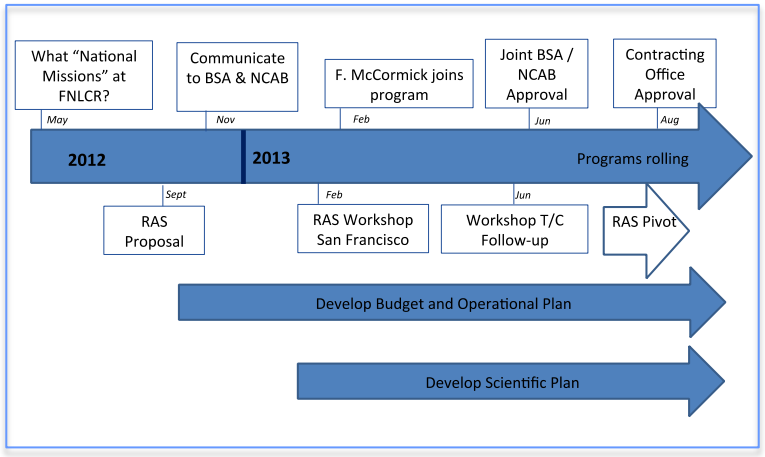

What is next?

Oct **Jan 2014** **Apr** **Jul**

RAS

RAS Project Teams pZ, p1, p2, p3, p4 **up and running** (with *ad interim* leaders)

Begin to recruit RAS Project Team leaders

Initiate formal reporting on progress

Host RAS Program Meetings at ATRF (crystallography, synthetic lethal, Spoke collaborations)

Establish CRTP, Work with COR, Pivot resources to RAS Program requirements

Start to provide reagents to spokes

Identify and develop synergistic RAS Program Collaborations

Assist intramural labs to determine the cost effectiveness of utilizing core labs and outside services

Services

A perspective on likely National Laboratory roles in enabling Drug Discovery

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Technology integration and Applied Science in the FNL

- Biomarkers and Dx: YES, very natural roles for FNL
  - technology strength in reference quality measurements
- RAS (or future) Tx mode of action: YES, and incl. GEMMs
  - pharmacodynamics, dependency on original drivers, and resistance
- New cell-based assays: YES
  - thus enabling the broad R&D community (hub and/or spoke)
- National Program emphasis and open FNL resources drive new FNL partnership mode (cCRADA) for: ***Screening, Hit Triage, Lead Family optimization, ADMET and Preclinical Development***
  - Potential for co-located partnerships in the ATRF
  - Potential for NCI-based clinical trials
- *Applied Science*

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- **Downstream Information Follows**

Application Phase: Lead finding and optimization

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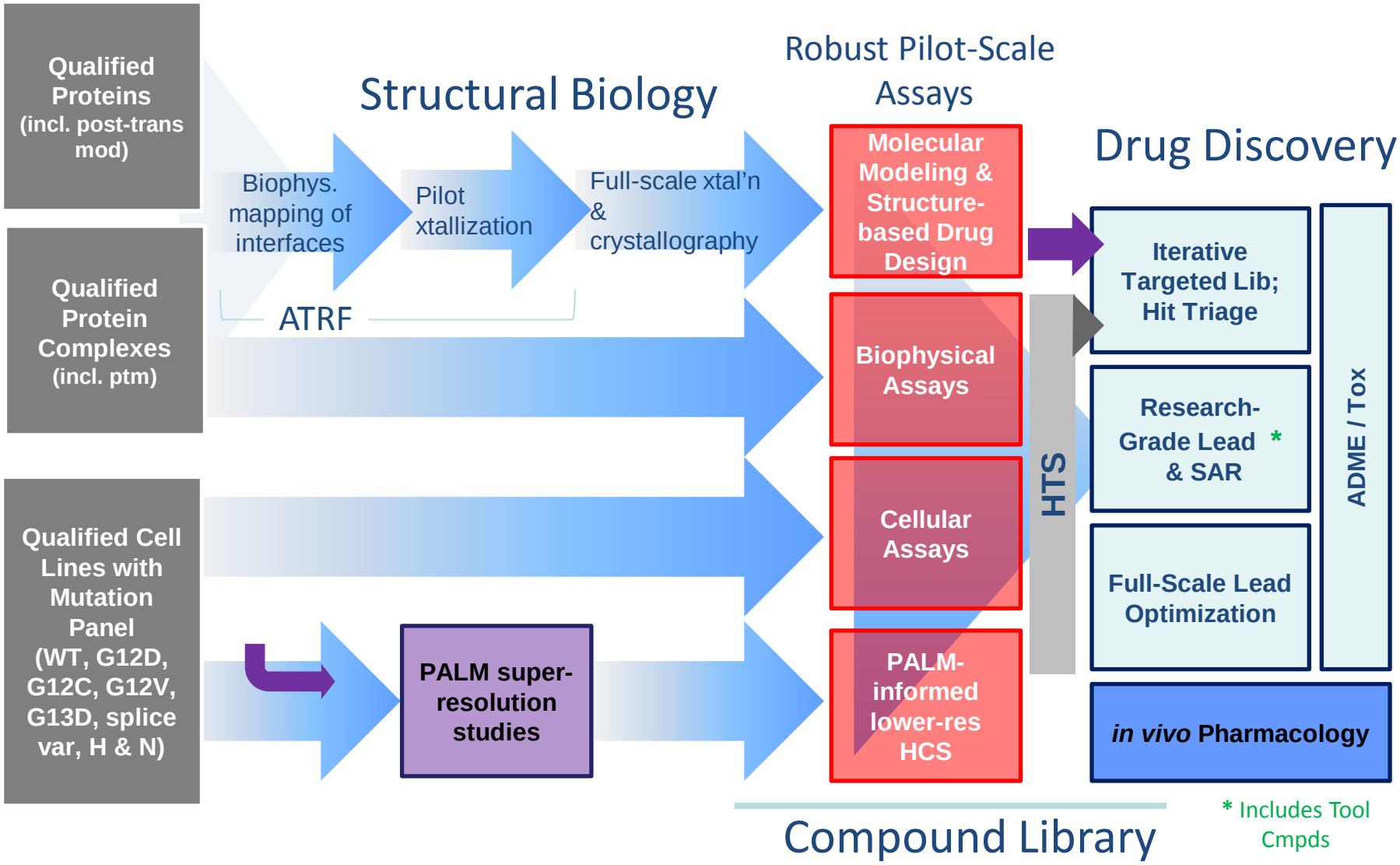

Drug Discovery and Cross-sector Partnerships for RAS: timely opportunity to leverage cCRADA

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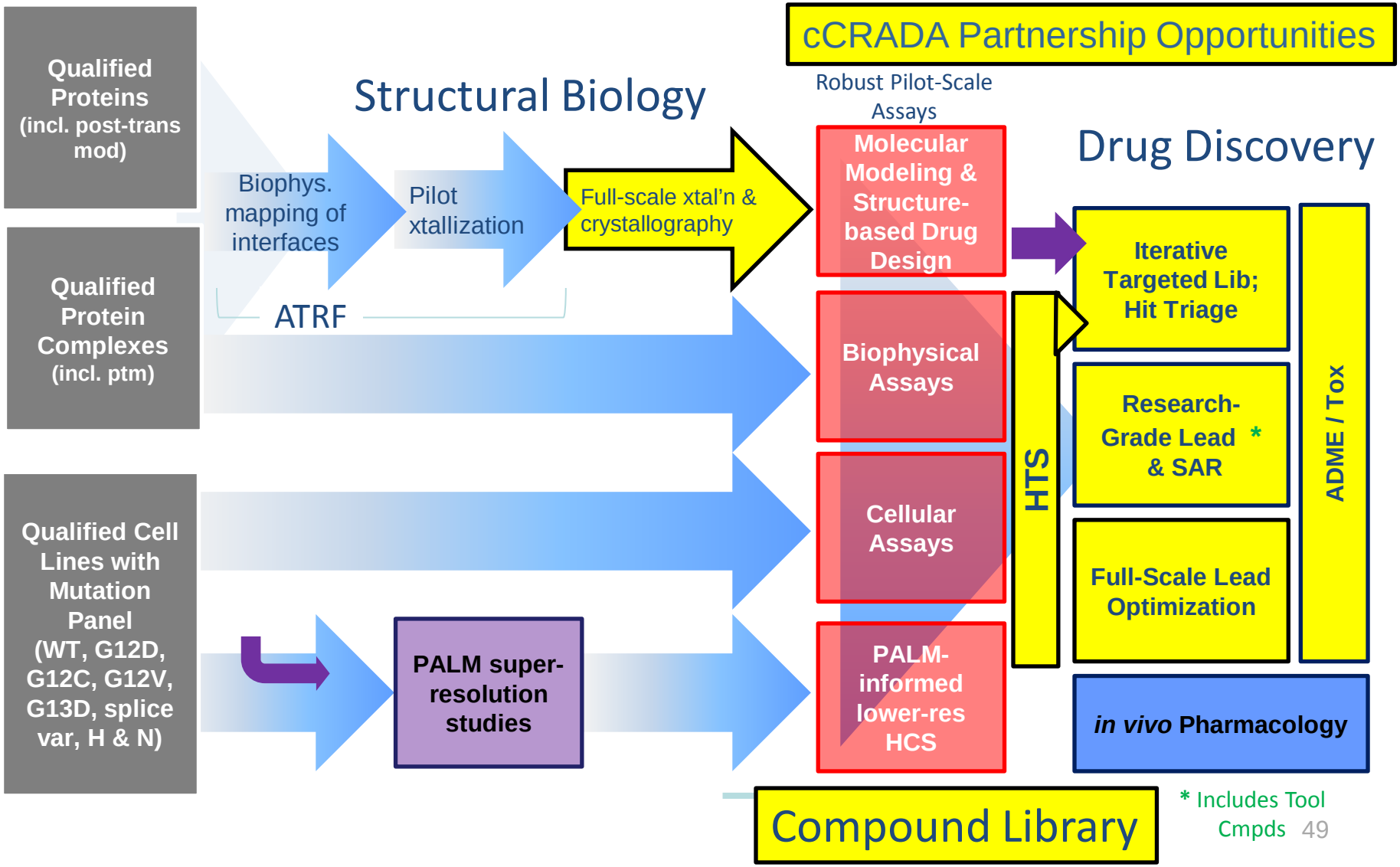

Custom Cell Lines and Cell-based assays

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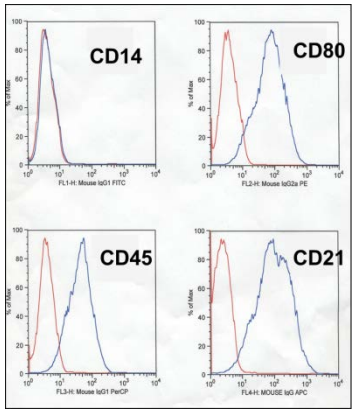
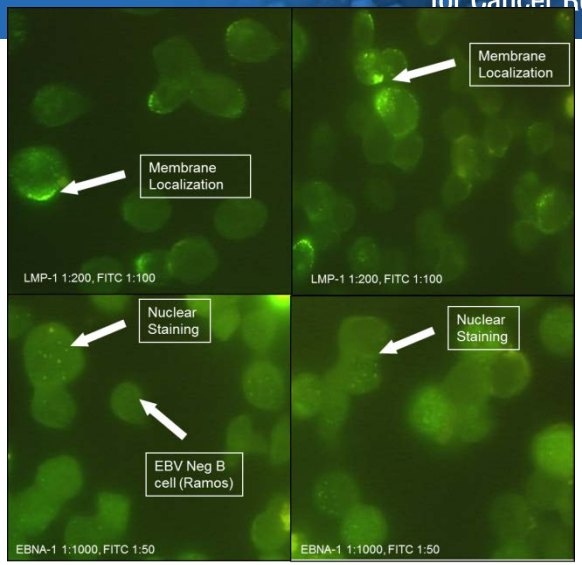
Marker	Cell Type	HCL-B Passage 21,24 +/-	HCL-P Passage 3,10 +/-
CD45	Leukocytes	+	+
CD19	B-lymphocyte	+	+
CD20	B-lymphocyte	+	+
CD21	B-lymphocyte	+	+
CD80	B-lymphocyte*	NT	+
HLA-DR	Antigen Presenting	+	+
CD3	T-lymphocyte	-	-
CD14	Monocyte	-	-
CD25	T&B-lymphocytes	-	-
CD40	B-lymphocyte	-	-
CD56	NK	-	-

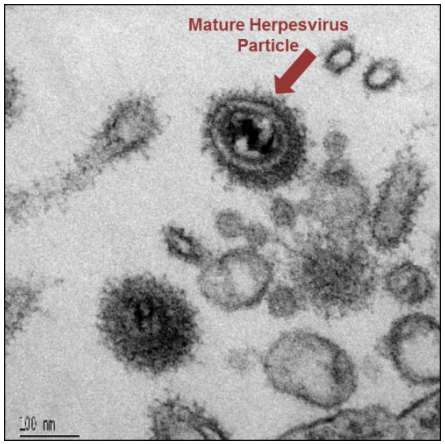
NT = Not Tested



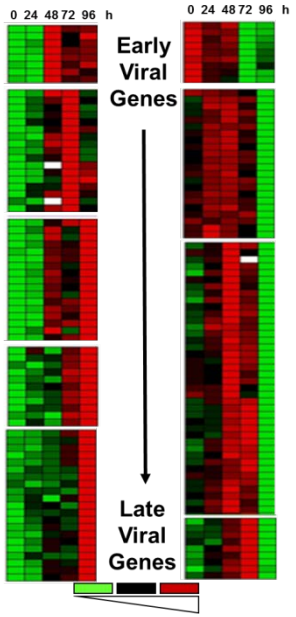
Cell surface profiling



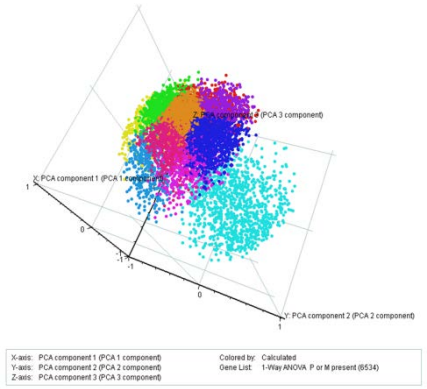
Immunofluoresence – viral genes



EM analysis



Viral gene expression



Cellular gene expression

Proteomic Characterization of Mutant RAS Activation

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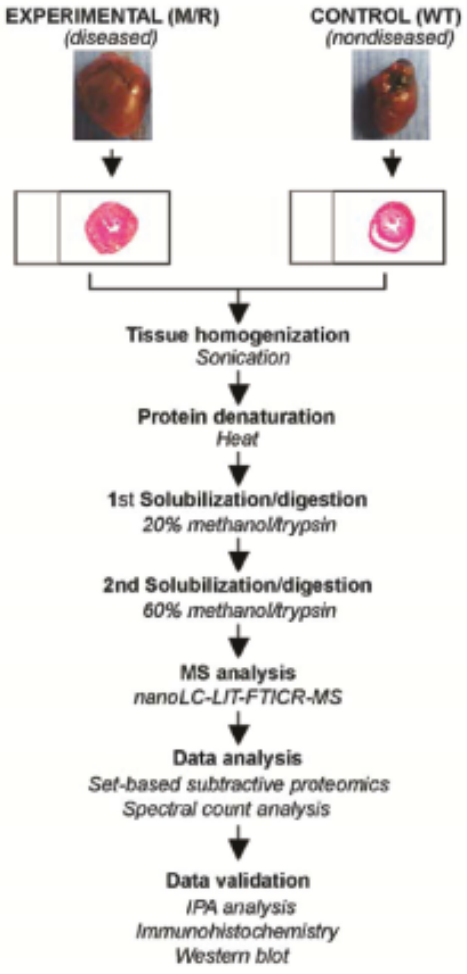
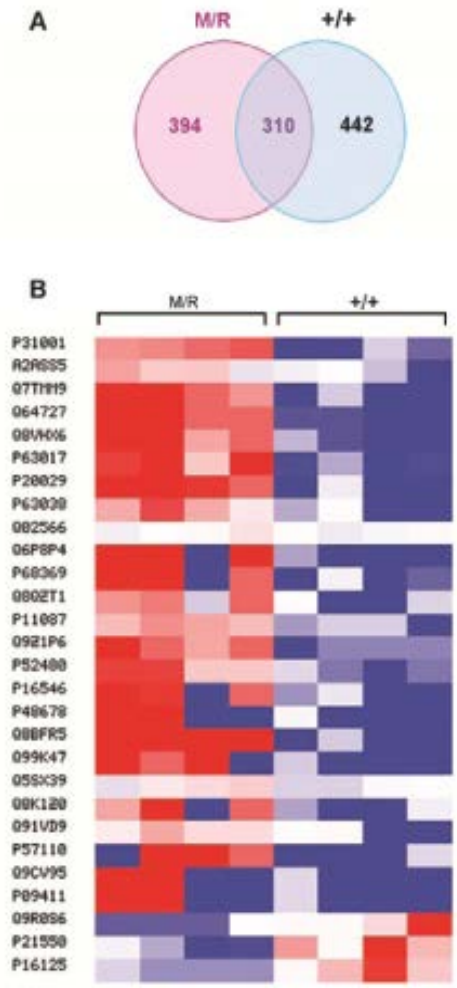
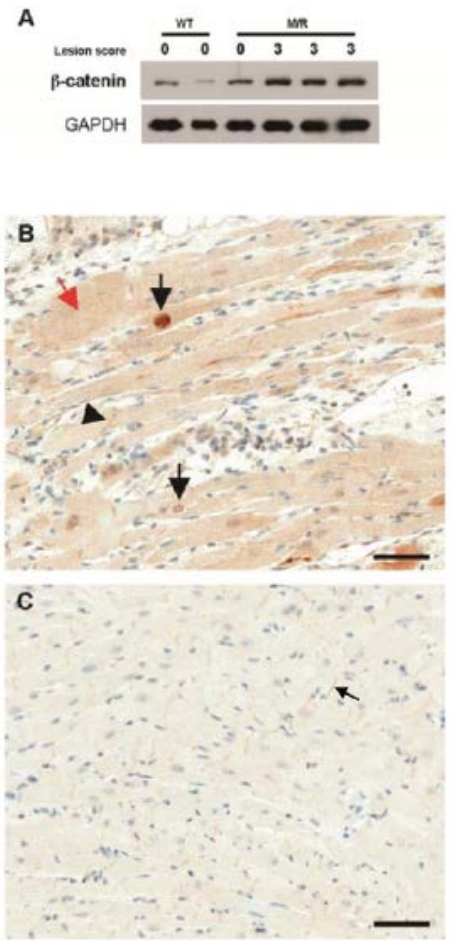
Controlled HRAS Expression in Transgenic Mice



LC-MS-based Proteomics of Thin Histologic Fresh Frozen Tissue Sections



Activation of Canonical Wnt/β-catenin Pathway Secondary to Constitutive HRAS Activation



MALDI Imaging – Nanoformulated Drug Distribution

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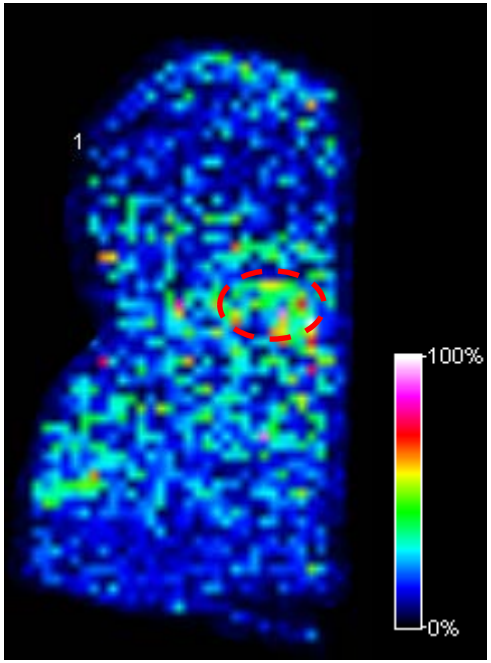
Liver tissue from mouse sacrificed 1h after drug administration

Stain/C Stain H&E

Molecular Image m/z 788.25



- MALDI imaging was able to verify that the intact drug (taxol in a nanoformulation) reached the target organ 1 h after the drug administration
- The drug is concentrated in the main vessel (central vein) and starts to diffuse into the rest of the organ
- More peripheral regions of the tissue show lower intensity of signal, consistently with a gradual diffusion path from the main vessel