Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

RAS Initiative: Progress and Working Group Update

Dwight Nissley, Director, Cancer Research Technology Program, FNLCR

October 29, 2018

DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute
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Ras Initiative: Major Goals

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- **Discover small molecules that bind to RAS directly or disrupt RAS/effector interactions**
  - RAS activity Assays
    - Cell-based / biochemical
  - Biophysical screens
    - Tethering / covalent inhibitors
  - *In silico* screens
  - Partner through cCRADAs

- **Molecular description of RAS/RAF signaling complexes in membranes**
  - Biochemistry, biophysics and structural biology
  - RAS/RAF dynamics in cells
  - Molecular dynamic (MD) simulations with DOE National Labs

RAS Initiative Collaborations: Hub and Spokes

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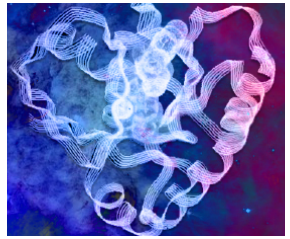
RAS Initiative



"The Hub"

cCRADAs

Strategic Collaborations

Industry Collaborations

Academic Collaborations

RAS Resources

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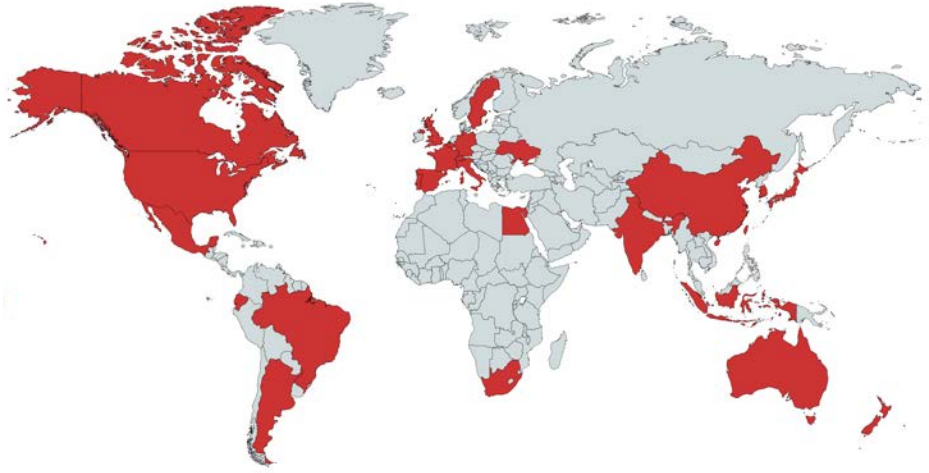
In FY2018:

- 1,121 clones generated and sequence verified
- 647 large-scale protein productions (24 grams of protein produced)
- 412 small-scale protein production scouting experiments performed
- 427 liters of insect cell protein expression culture grown, 100 liters of baculovirus produced
- 1,750 liters *E. coli* protein expression culture grown
- 230 cell lines generated

Overall since 2014:

- 7,057 plasmids distributed through Addgene
  - 314 universities and NPOs, 38 states, 36 countries, 6 continents
- 119 MTAs for direct distribution (323 plasmids, 476 cell lines)
- RAS-dependent MEFs licensed to 5 companies
- KRAS-FMe materials licensed to 4 companies



Dom Esposito and RAS Reagents Core

Structural Biology Reveals Potential Therapeutic Opportunities

Pockets in oncogenic KRAS mutants

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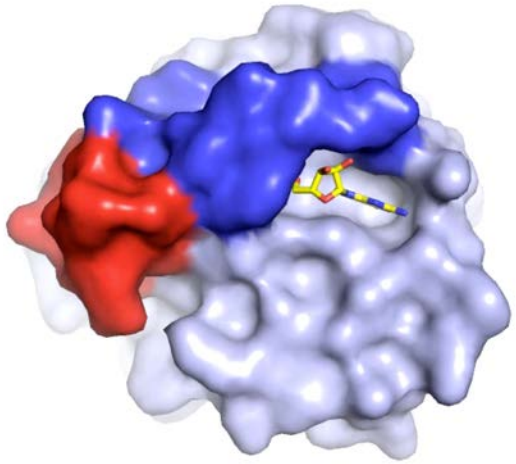
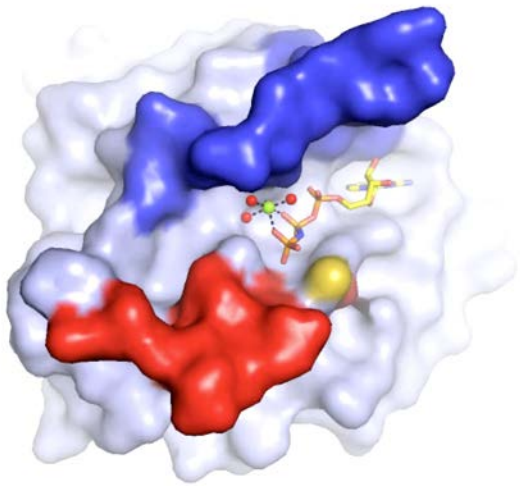
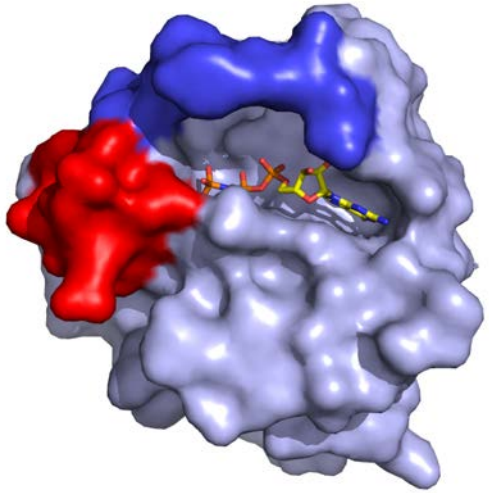
Switch-1
Switch-2



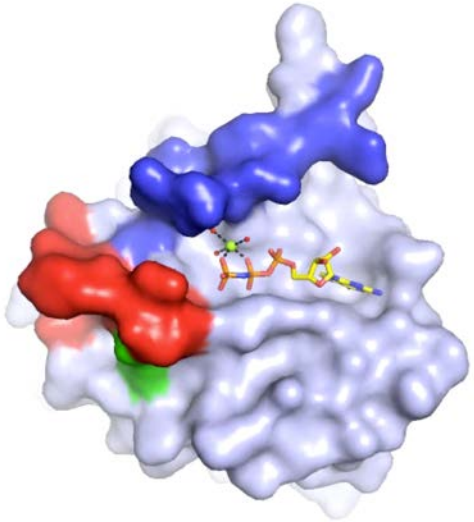
WT-KRAS bound to GMPPNP and Mg



G12C in complex with GMPPNP and Mg



G13D in complex with GMPPNP and Mg



Q61L in complex with GMPPNP and Mg

Dhirendra Simanshu

In silico screening

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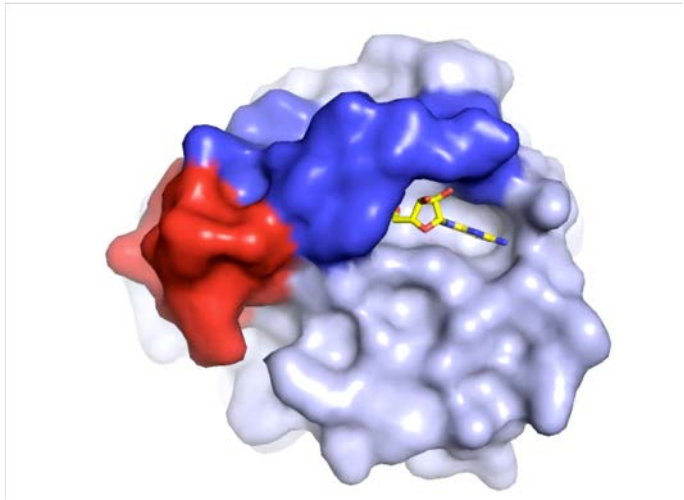
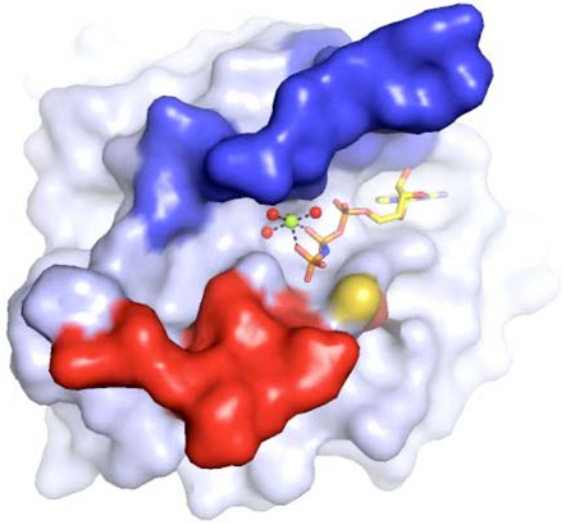
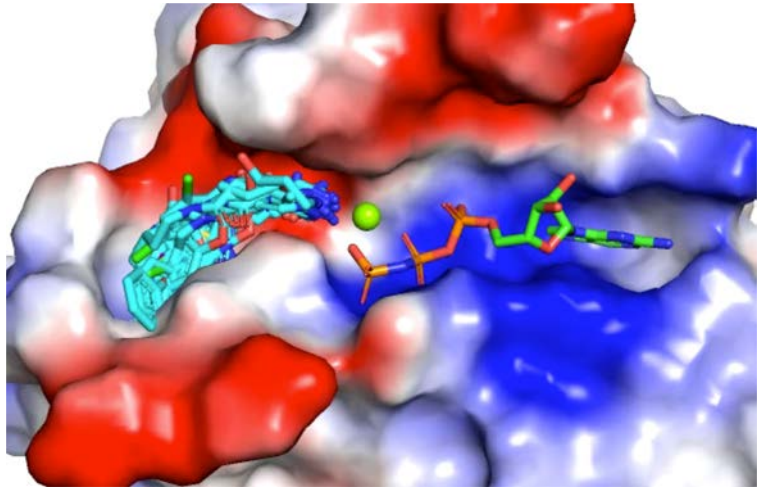
- In silico screening - in collaboration with **Nir London**, Weizmann Institute of Science, Israel.
- Hits validated using SPR and NMR (**Andy Stephen**, RAS Biophysics)

G12C in complex with GMPPNP and Mg







- 6 out of 14 compounds showed weak binding to G12C mutant by SPR
- Test these 6 compounds for binding by NMR
- Testing additional 44 analogs for SPR binding

Nir London (Weizmann)
L. Bindu, Andy Stephen (FNLCR)

cCRADAs Amplify RAS Initiative Efforts

RAS cCRADAs

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Contractor Cooperative Research and Development Agreement (cCRADA)

Agreement between FNLCR and a private company or university to work together on research and development.

Provides IP protections to the collaborator.

Sanofi

All efforts are in-kind, Chemistry supported by Sanofi, Biology shared between Sanofi and FNLCR

Project team: 8 FNLCR, 24 Sanofi

Proposal to carry out fragment screen against Oncogenic KRAS allele under negotiation

Kyras

Funds to support 1 FTE equivalent over 2 years

Project team: 4 FNLCR, 5 Kyras

Beatson

Funds to support 5 FTE equivalents over 2 years

Hired 3 new employees, additional funds are to support existing FNLCR staff

PharmaArava

Funds to support 2 FTE equivalents over 2 years

Chemistry supported by PharmaAarava, Biology supported by FNLCR

TheRas

Expanded to support 6 FTE equivalents over 2 years (chemistry and in silico modeling and design)

Chemistry shared between TheRas and FNLCR, Biology supported by FNLCR

Theras engaged CROs for additional chemistry and LLNL for *CADD*

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In–house discovery efforts transition to cCRADA with Theras

Anna Maciag
David Turner
Vandana Kumari
Marcin Dyba
Chris Brassard
Joseph Saavedra
Brian Smith

Small Molecules that prevent Kras4b membrane localization

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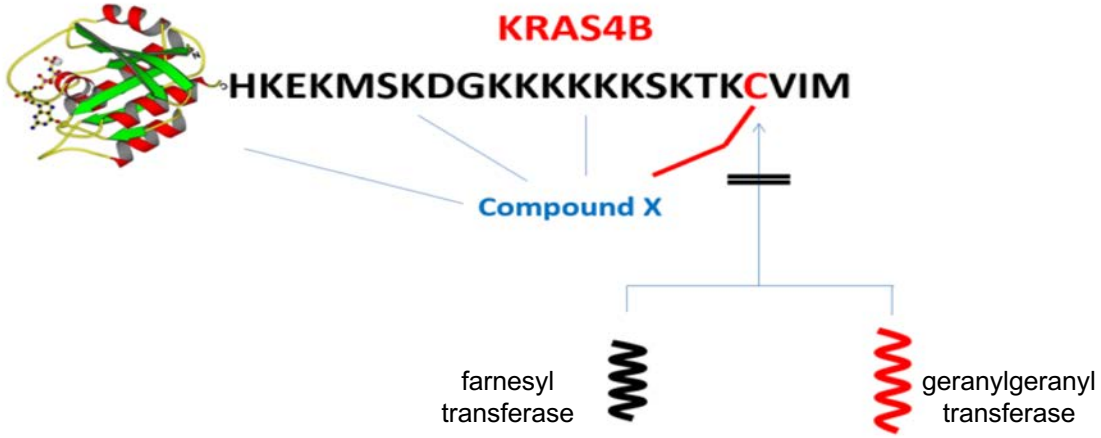
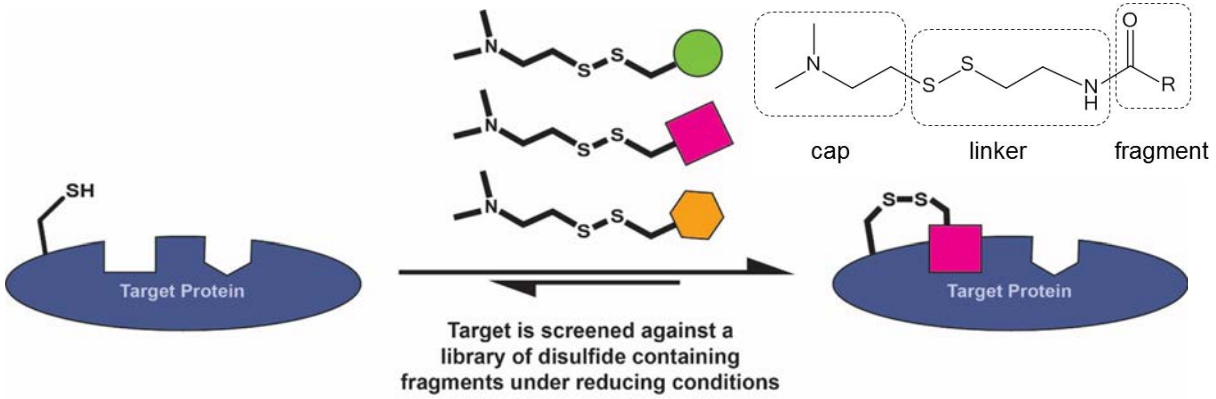
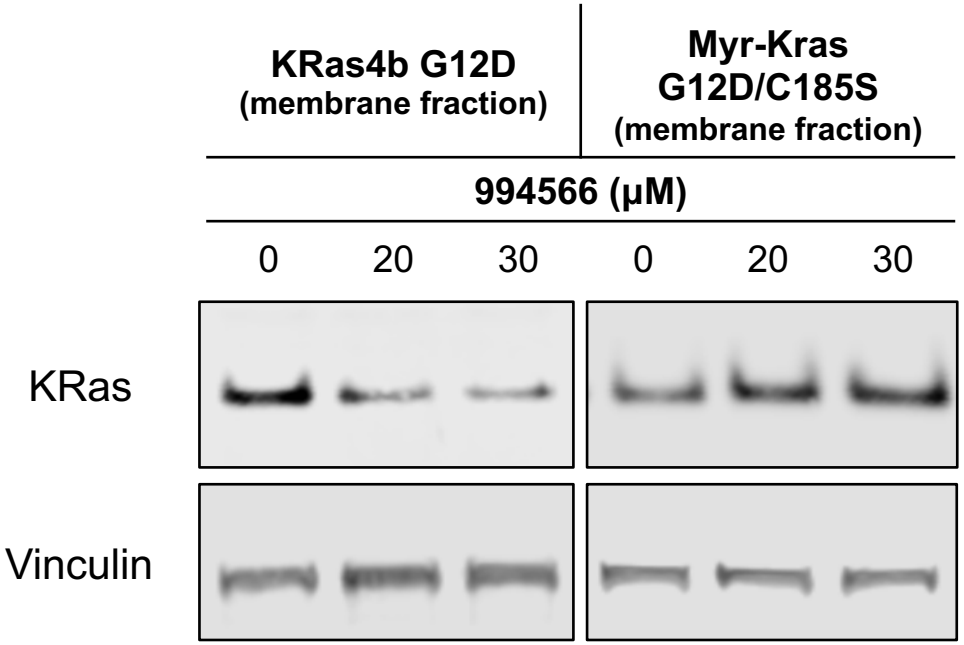
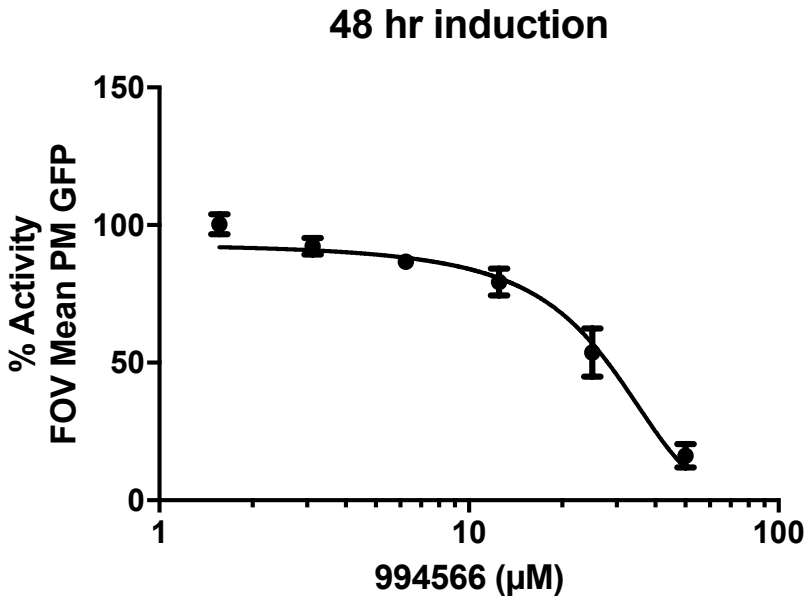
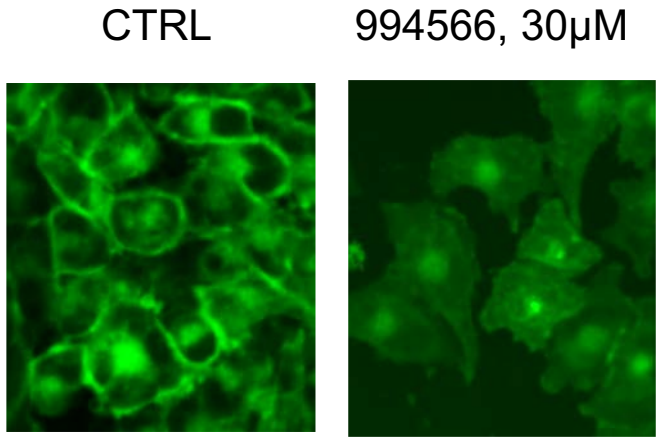


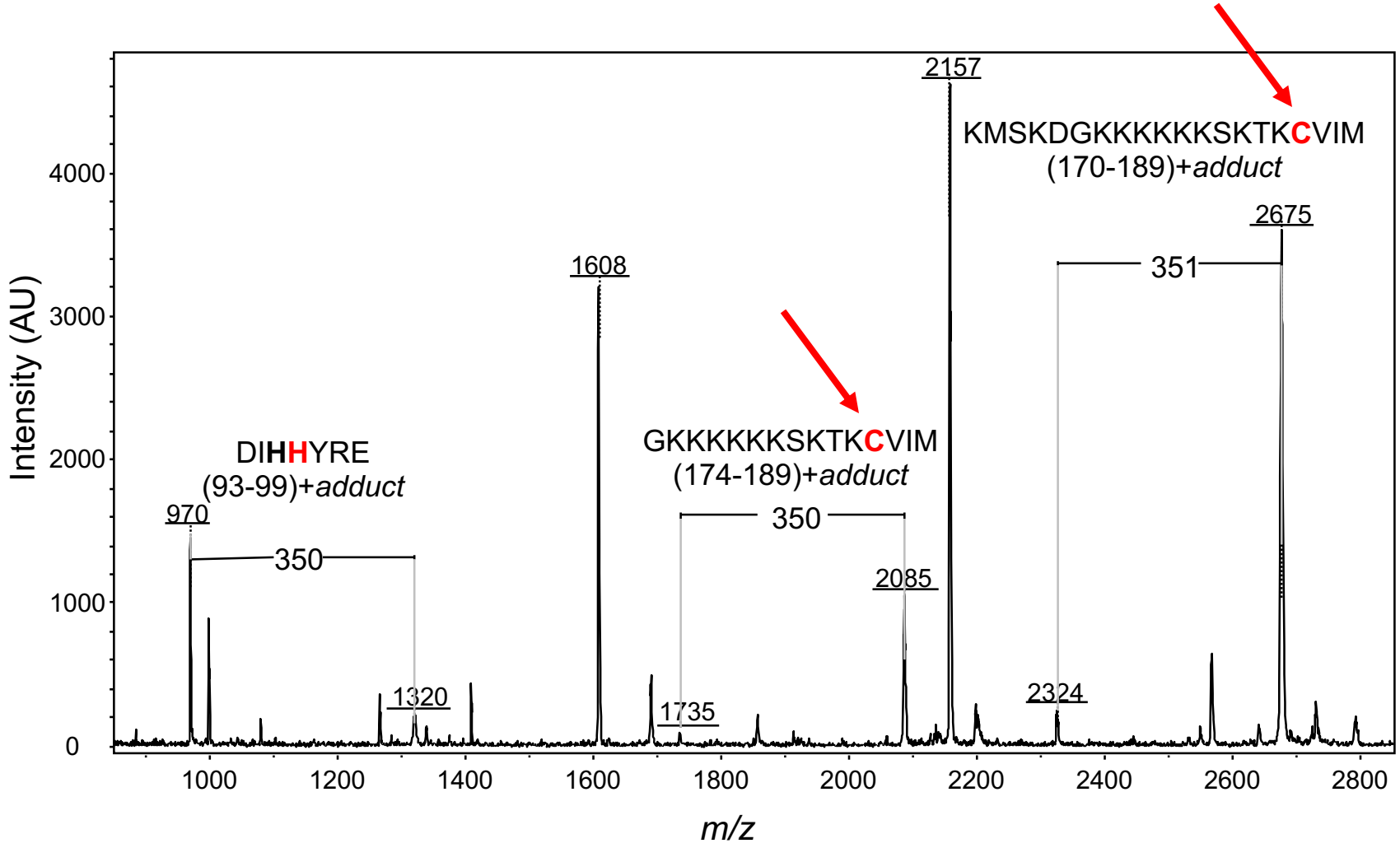








Covalent derivatives of tethering hits modify C185



MD simulation of HVR dynamics in KRAS4b

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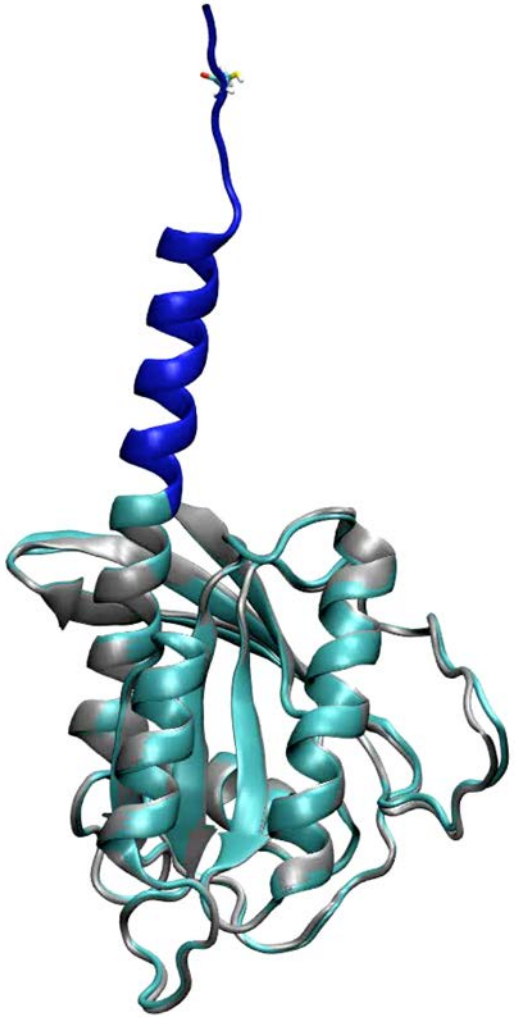

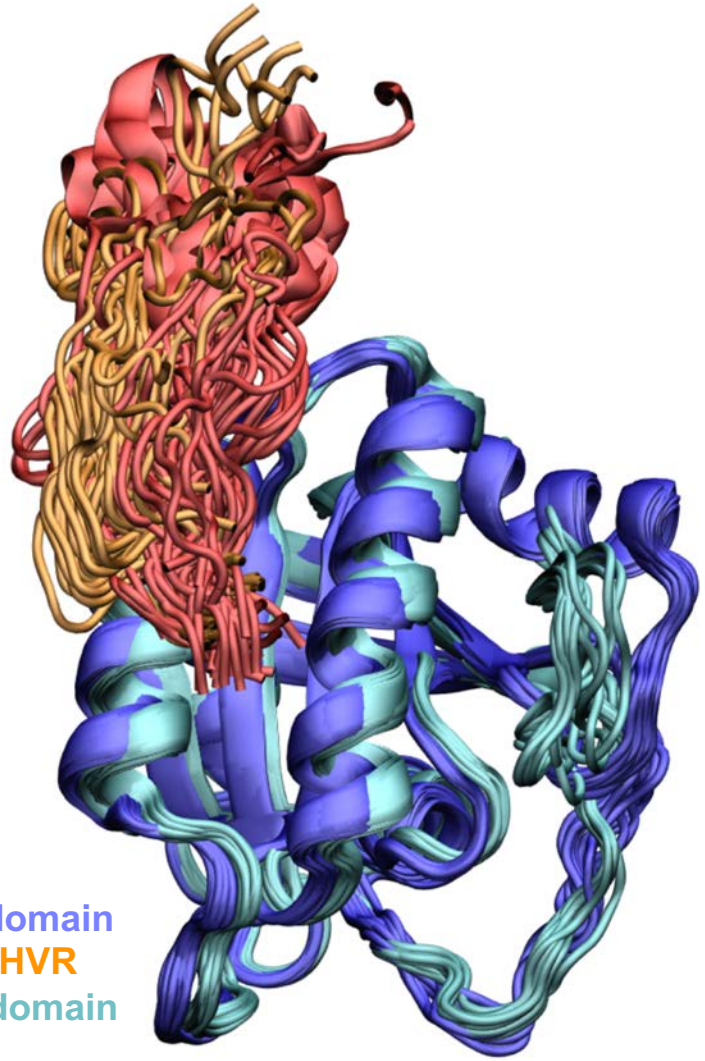

blue – GDP-loaded KRAS G-domain
orange – GDP- loaded KRAS HVR
cyan – GTP-loaded KRAS G-domain
red – GTP-loaded KRAS HVR

Yue Yang and Felice Lightstone, LLNL

HVR interaction with G-domain may generate a binding pocket

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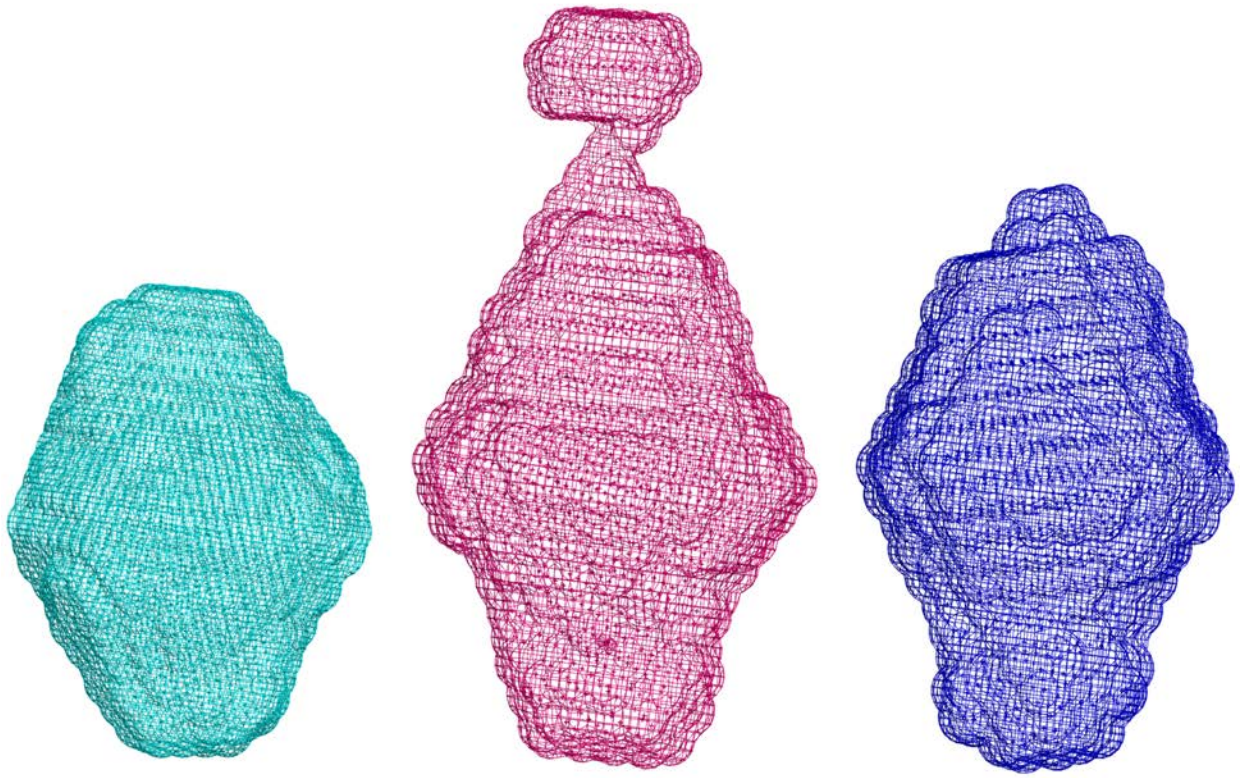

KRAS4b (1-169)
DMSO control

KRAS4b (1-188)
DMSO control

KRAS4b (1-188) + C185
tethering compound

Lixin Fan, The Small-Angle X-ray Scattering Core Facility, NCI

Targeting C185 - Status

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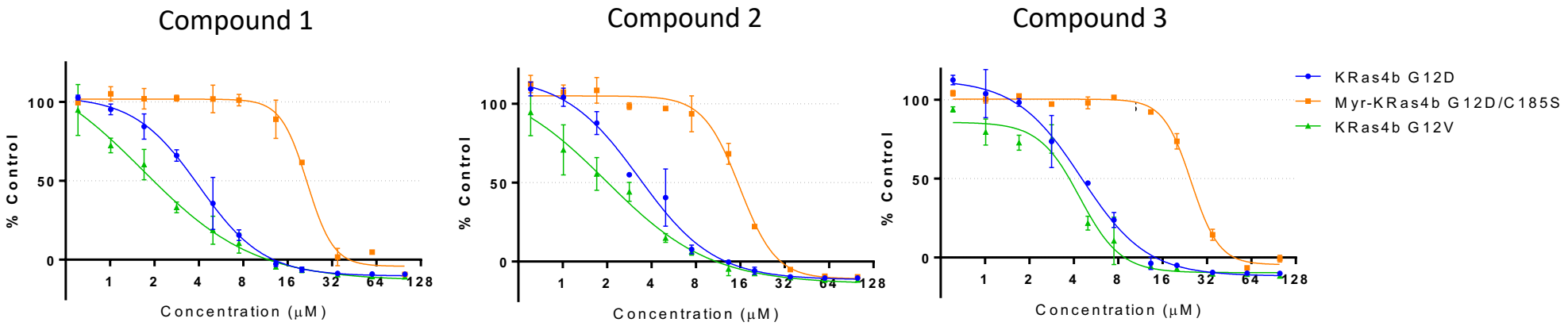
- C185 covalent binders are KRAS4b specific
- Prevent prenylation at C185 and membrane localization
- Inhibit proliferation of KRAS4b-dependent cell lines
- MD simulations suggest that the HVR interacts with the G-domain to form a pocket
- Potency/selectivity increased by CADD/Med Chem
- Design and synthesis of analogues in progress



Covalent derivatives of tethering hits also modify H95

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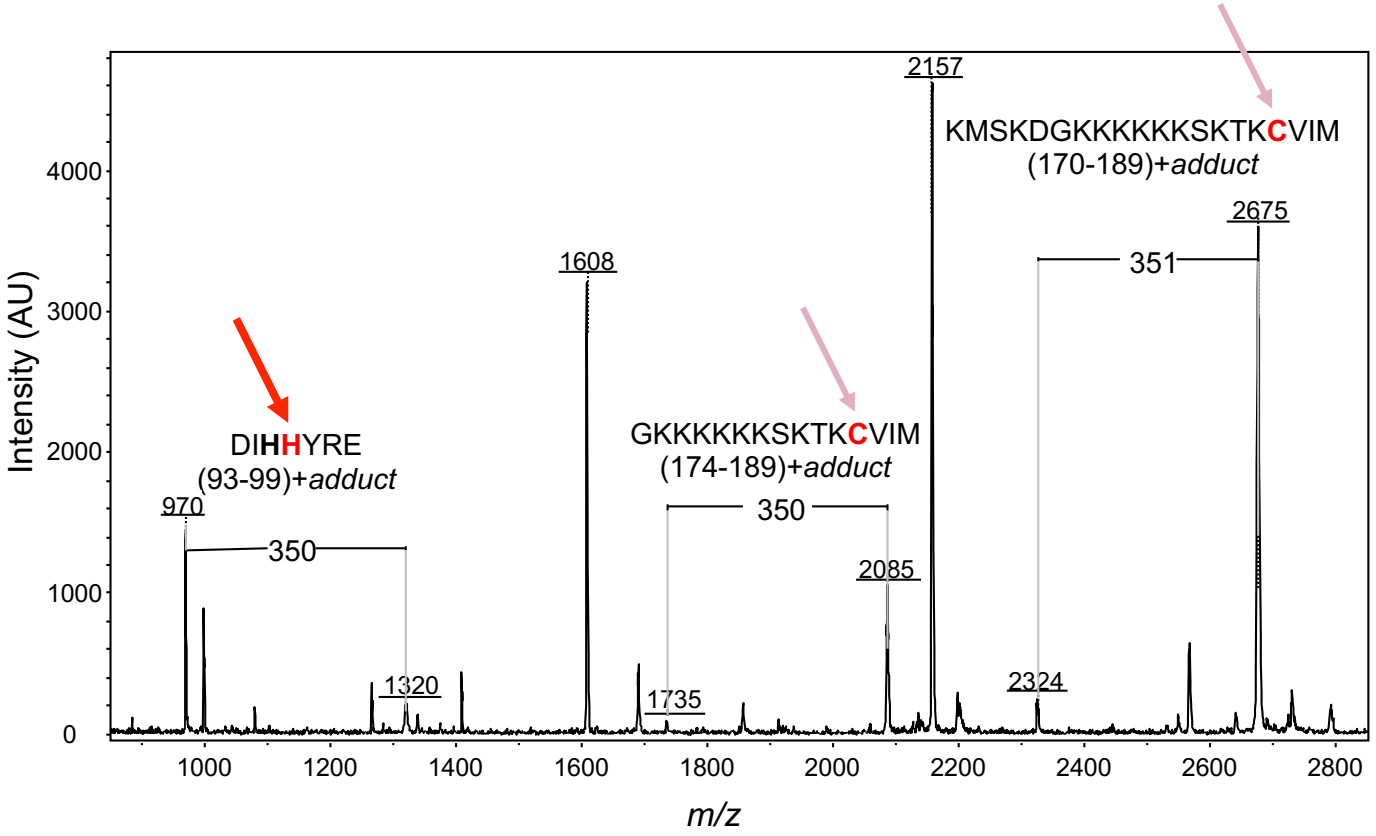
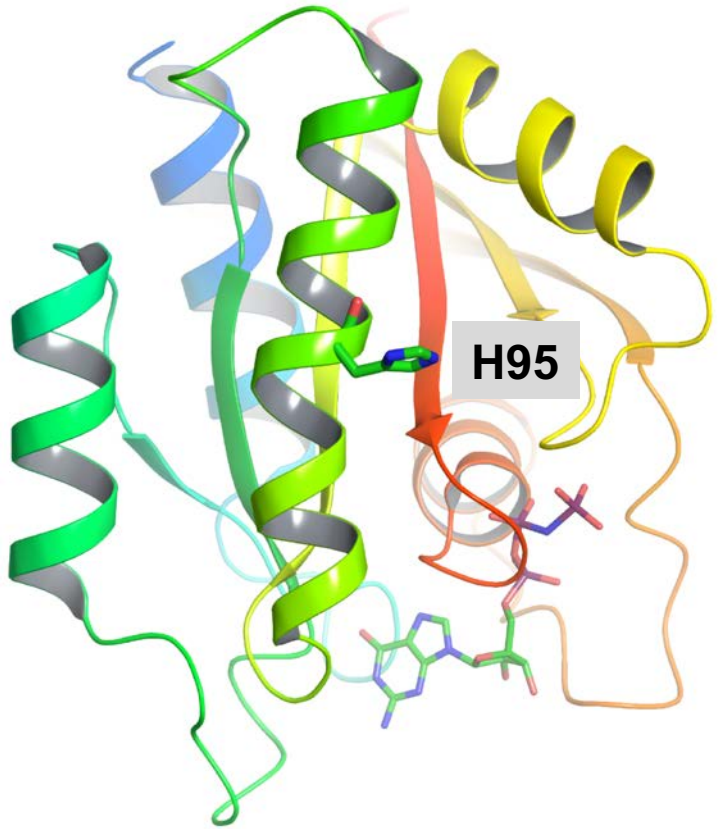




KRAS FAINNTKSFEDIHHYREQIKRVKD

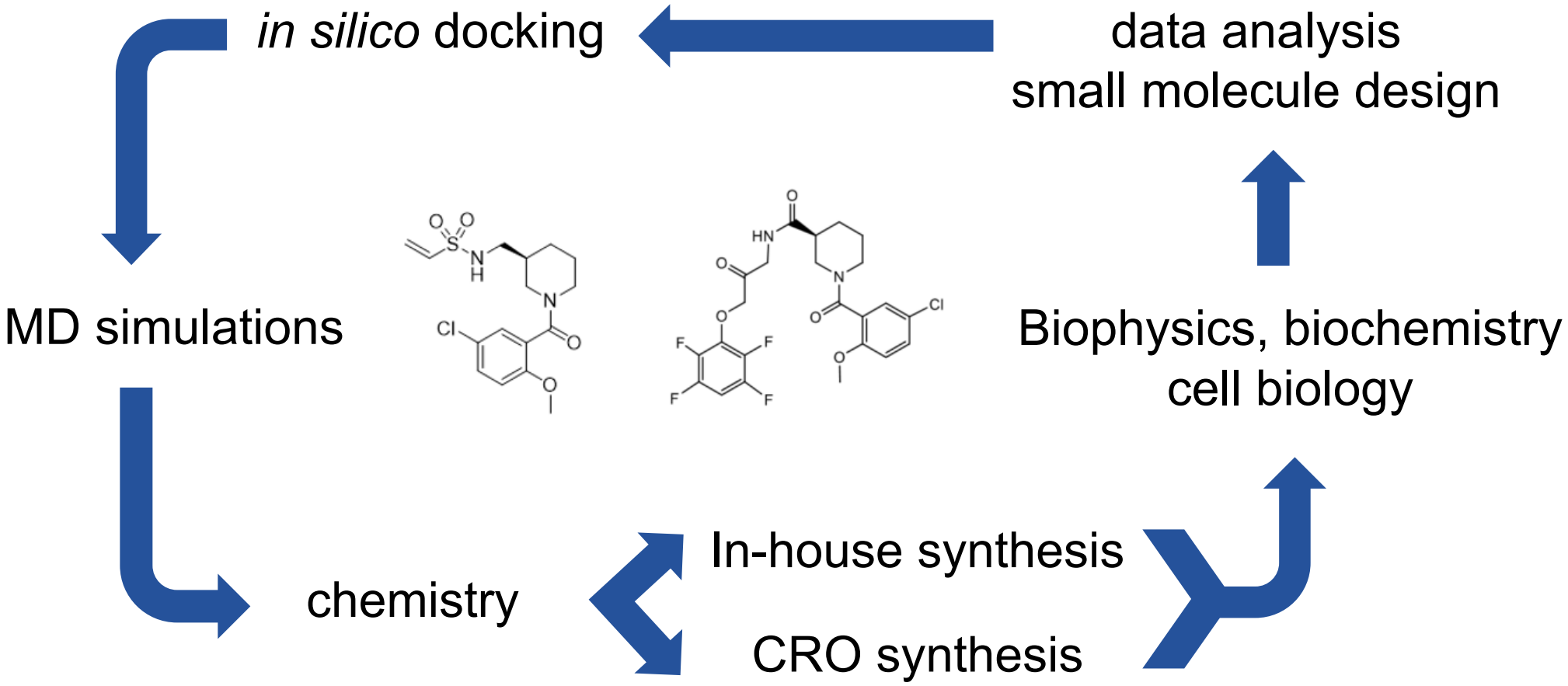
HRAS FAINNTKSFEDIHQYREQIKRVKD

NRAS FAINNTKSFADINLYREQIKRVKD

Targeting H95 - status

- H95 binders are KRAS specific
- Docking/MD/NMR suggest a pocket between Helix3/Switch2
- Bind to KRAS, not RhoA (by SPR)
- Design and synthesis of analogues in progress

RAS Inhibitor development



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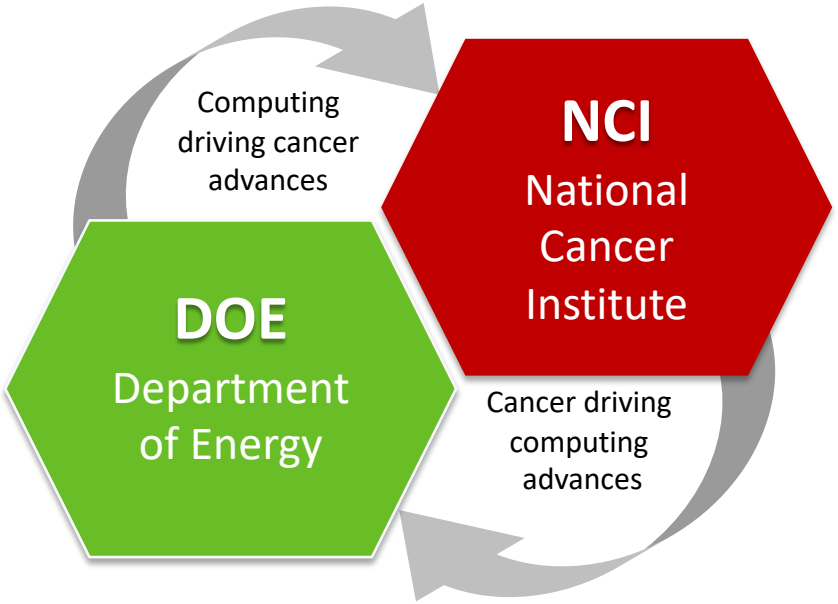
Advanced Computation to better Understand RAS Biology

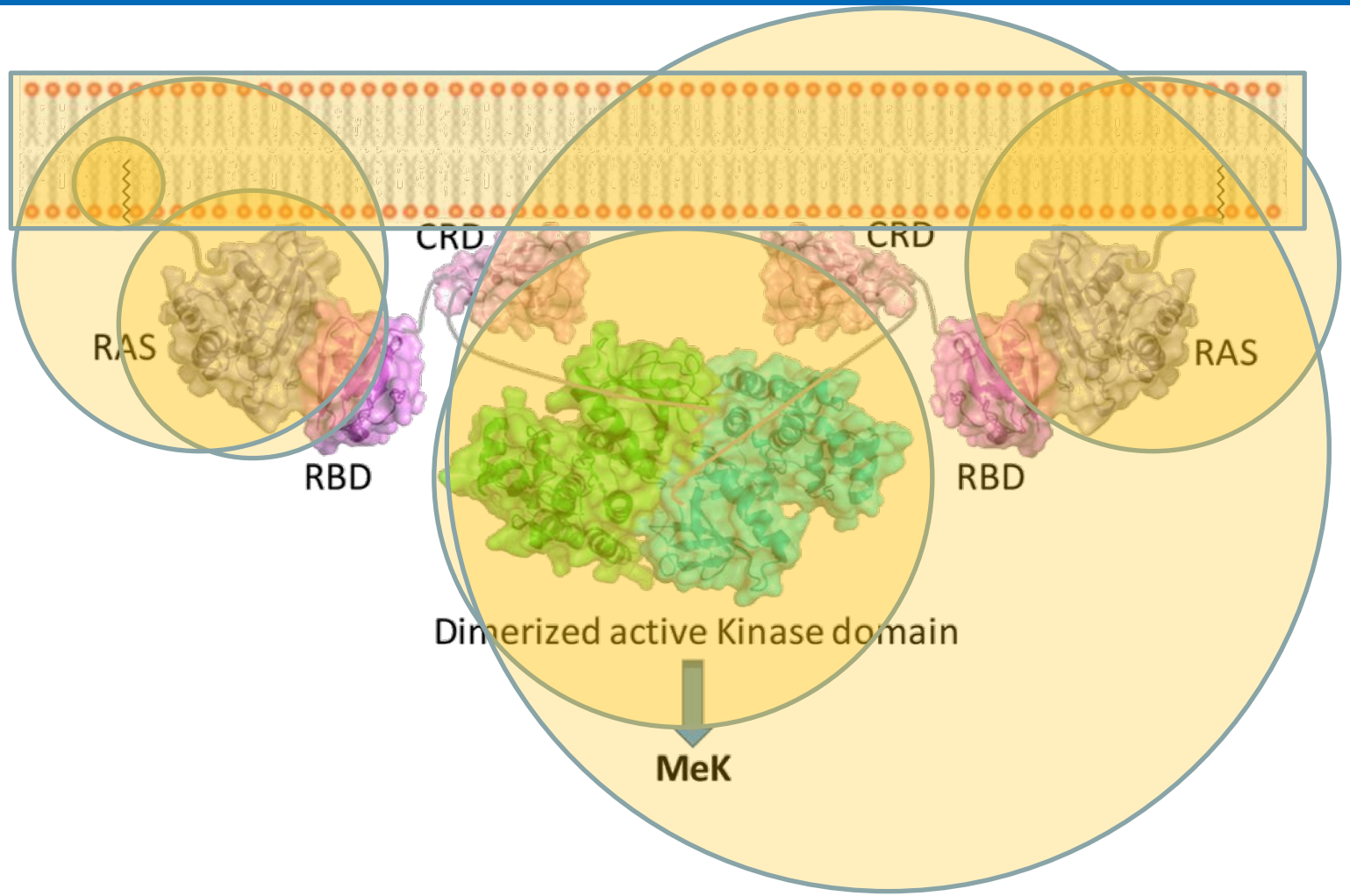
Fred Streitz, Director HPC, LLNL

DOE Lead for Pilot 2

DOE-NCI partnership to advance exascale development through cancer research



Experimental Gaps – Activation of RAF



- RAS structure in context of membrane
- RAS dynamics
  - states
- RAS monomer/dimer/multimer
  - Interfaces
- RAF structure
- RAS:RAF engagement
  - Order of addition
  - Activation/conformational dynamics
- Local lipid environment

RAS Initiative studies used as input to parameterize MD simulations: KRAS4b

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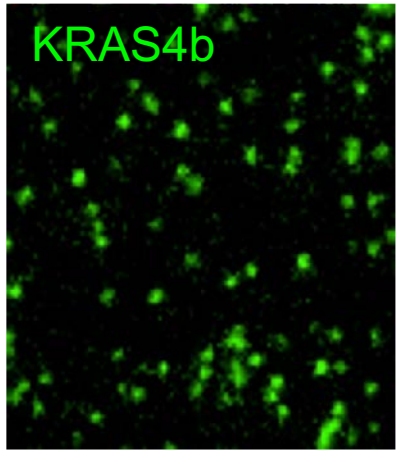
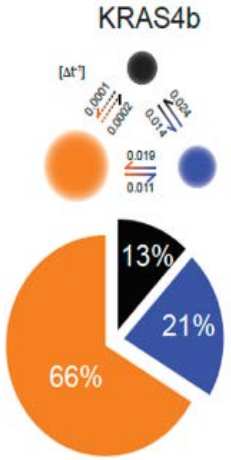
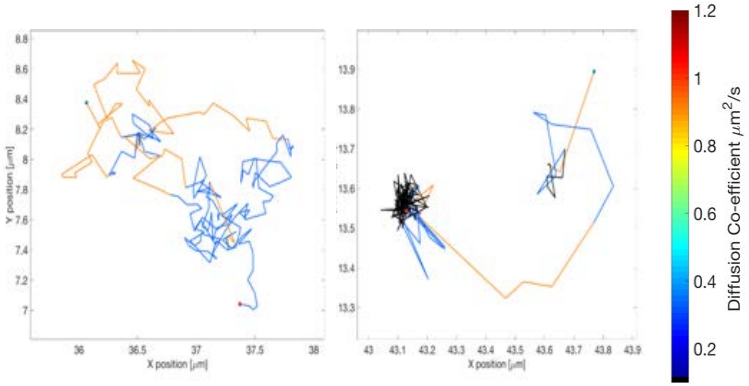
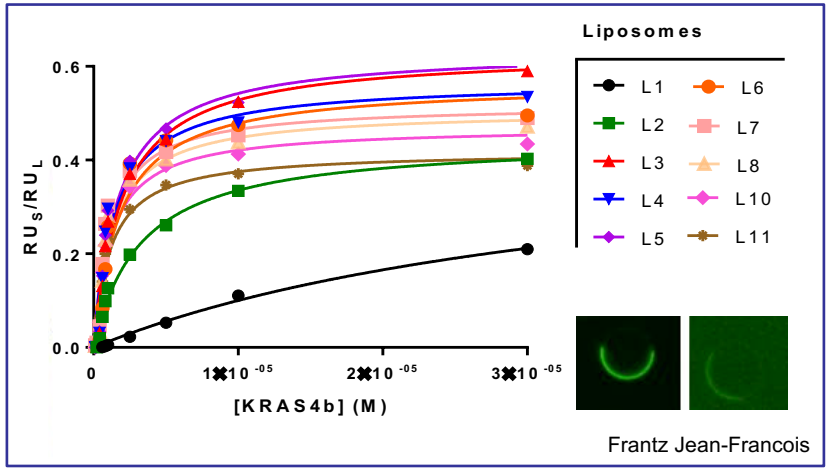


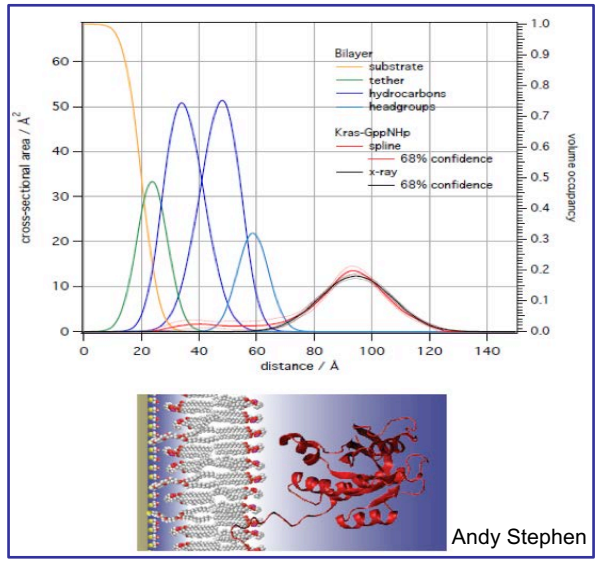




Debanjan Goswami, De Chen,Tommy Turbyville

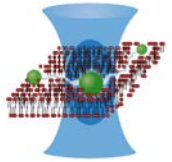


Frantz Jean-Francois

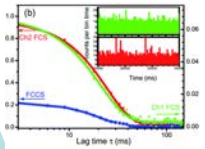


Andy Stephen

1. Diffusion

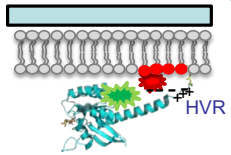


2. Lipid-protein interaction



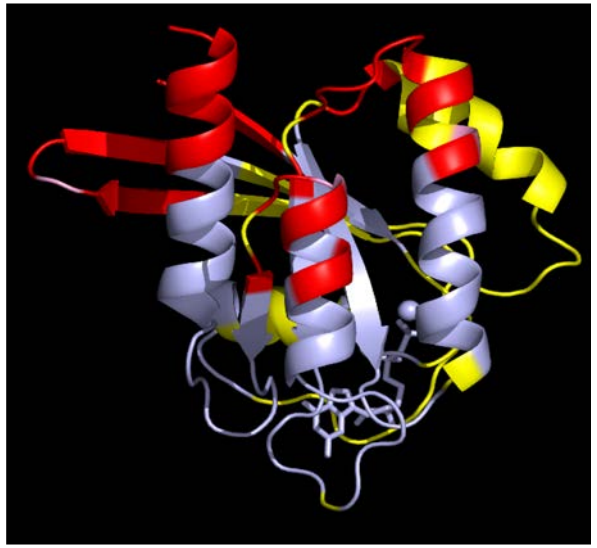
Fluorescence: FCS, FLCCS, FRET, Aniso-tropy

3. Orientation



4. Rotational diffusion of KRAS4b on lipid bilayer

Rebika Shresta,Tommy Turbyville



Andy Stephen, Que Van

Initial KRAS:membrane Simulations

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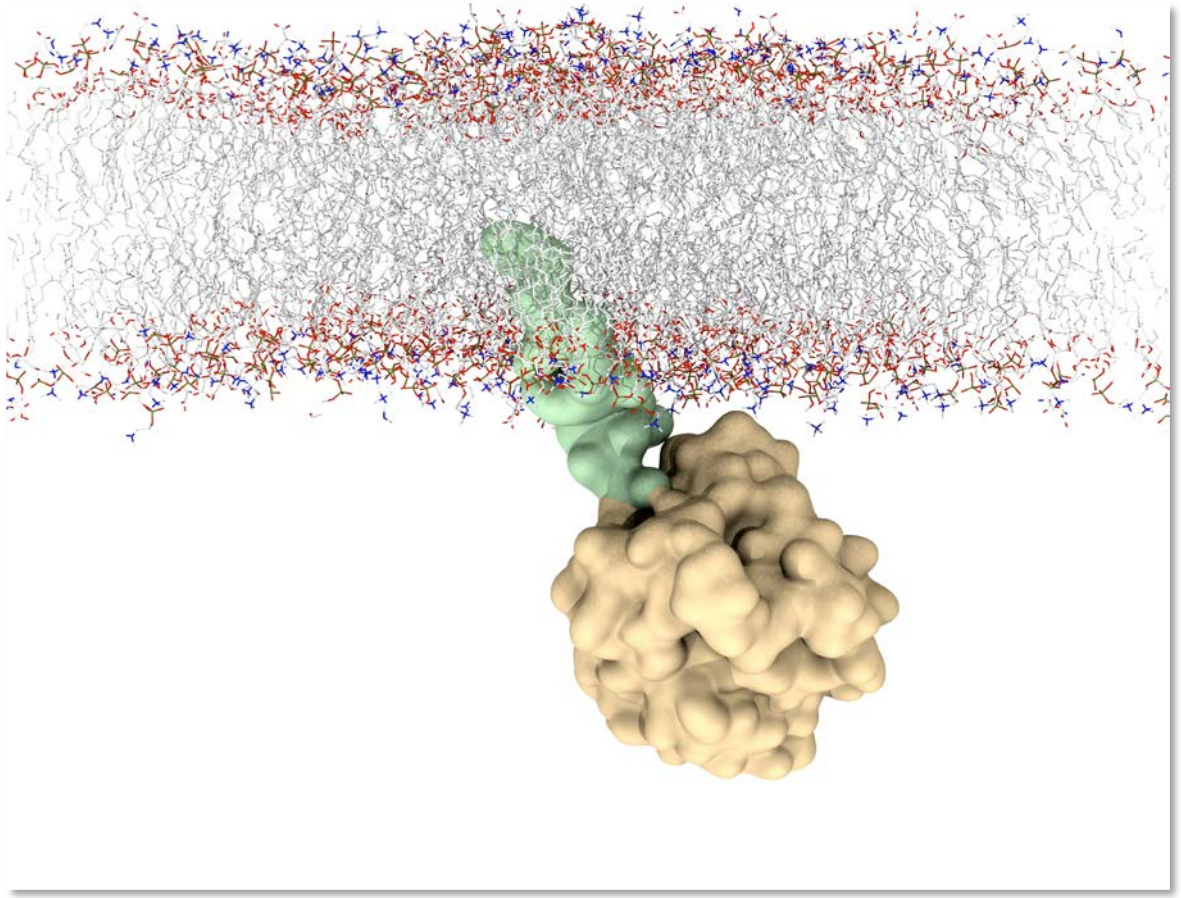

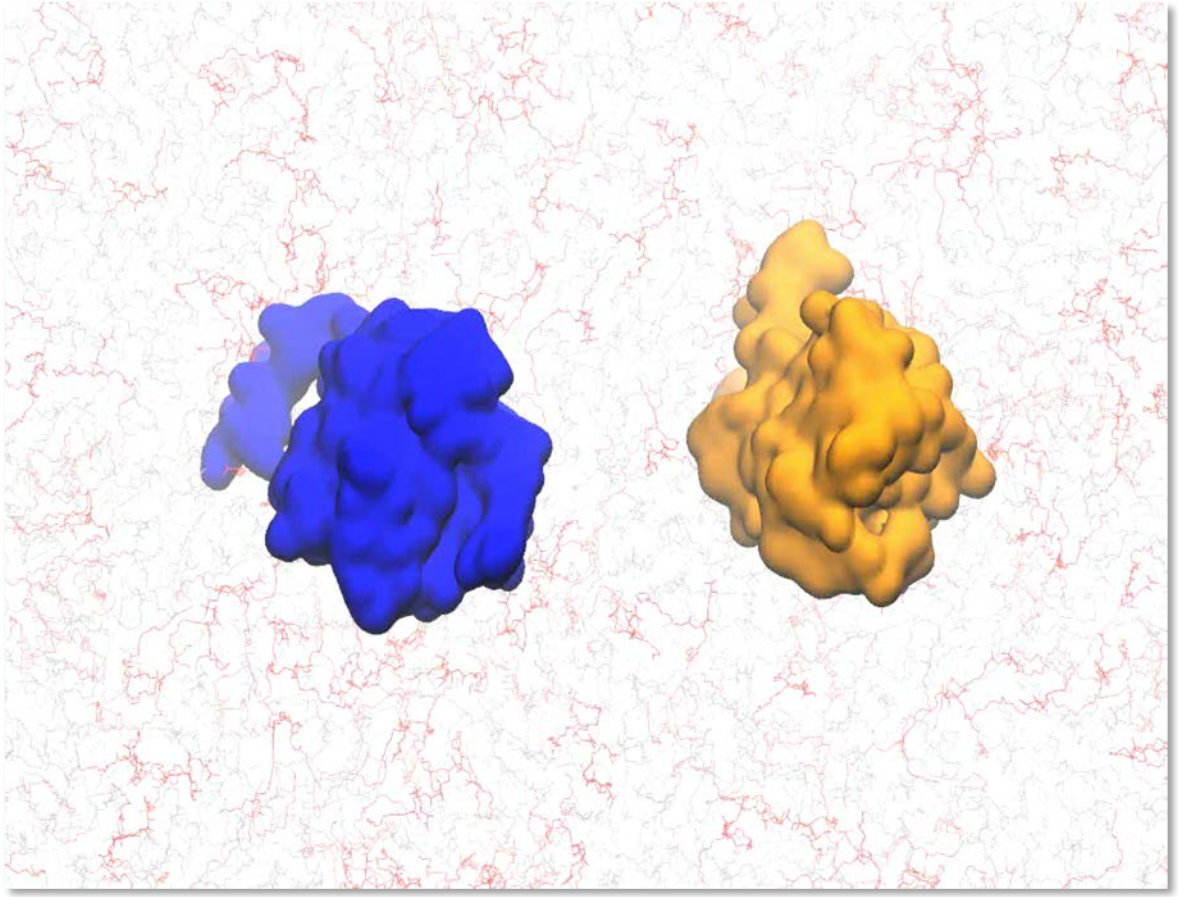

Chris Neale and Angel Garcia, LANL

A framework to couple macro and micro scale simulations

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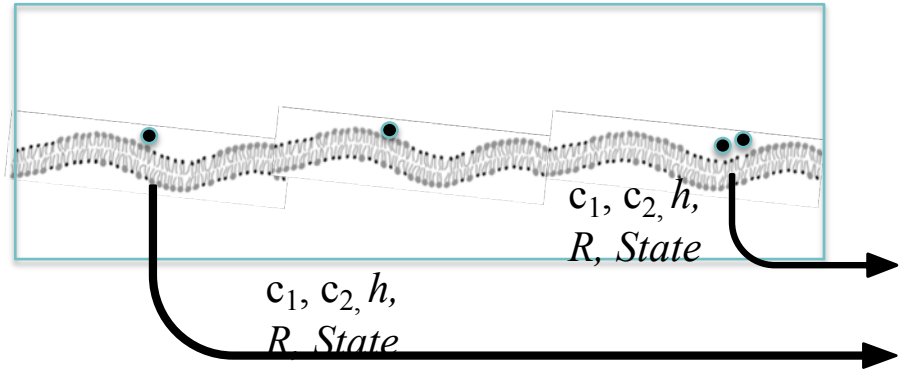
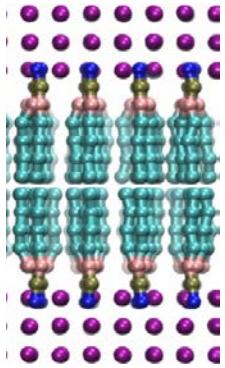
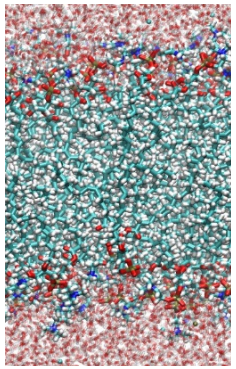
DDFT +HyCoP simulations

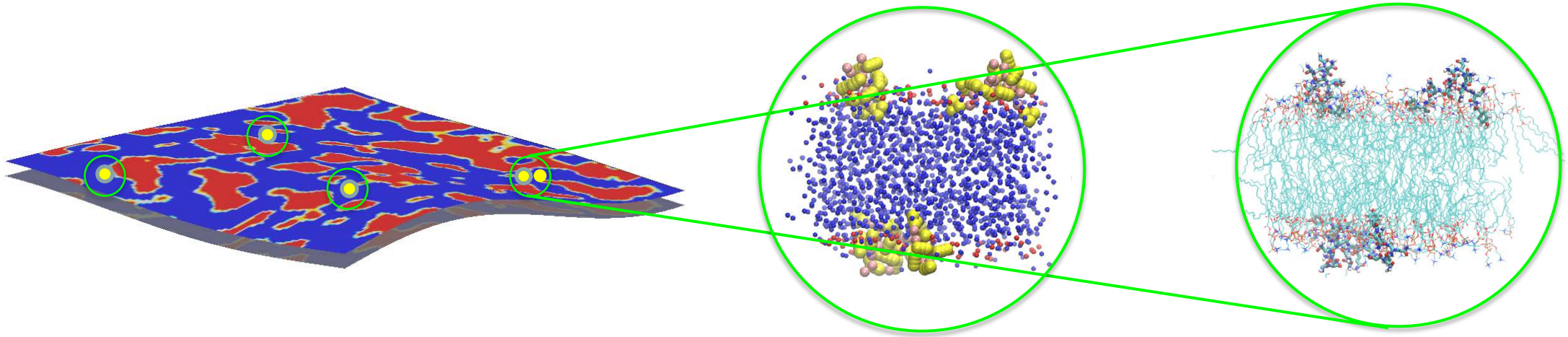


MARTINI (coarse grain)



CHARMM (all atom)





New framework simulation of longer time and length scales

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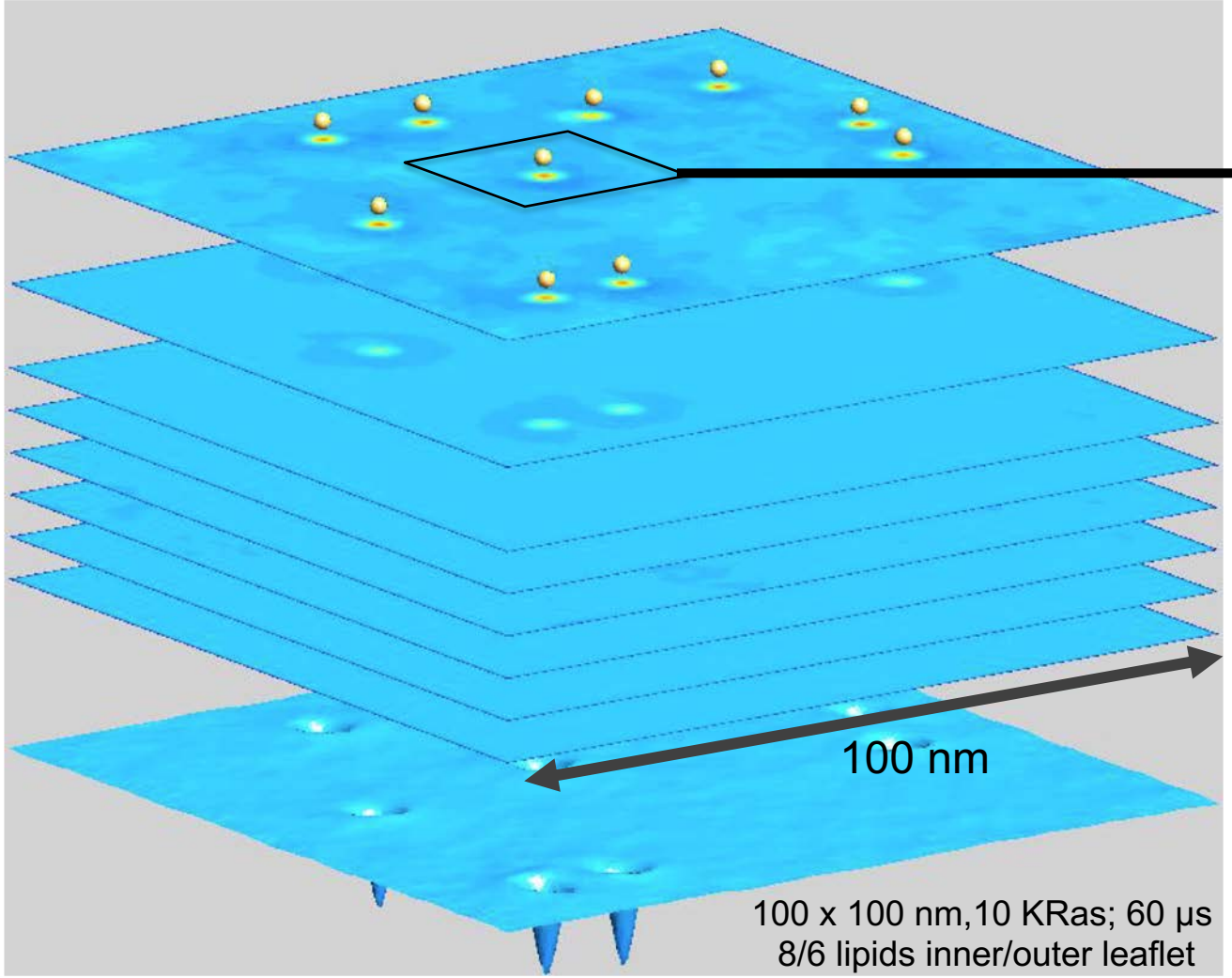
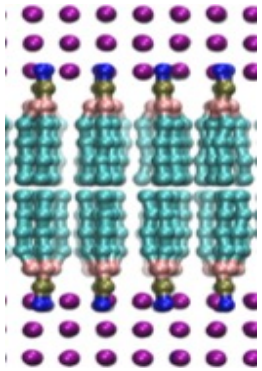
Marco Scale - Continuum Phase Field Model

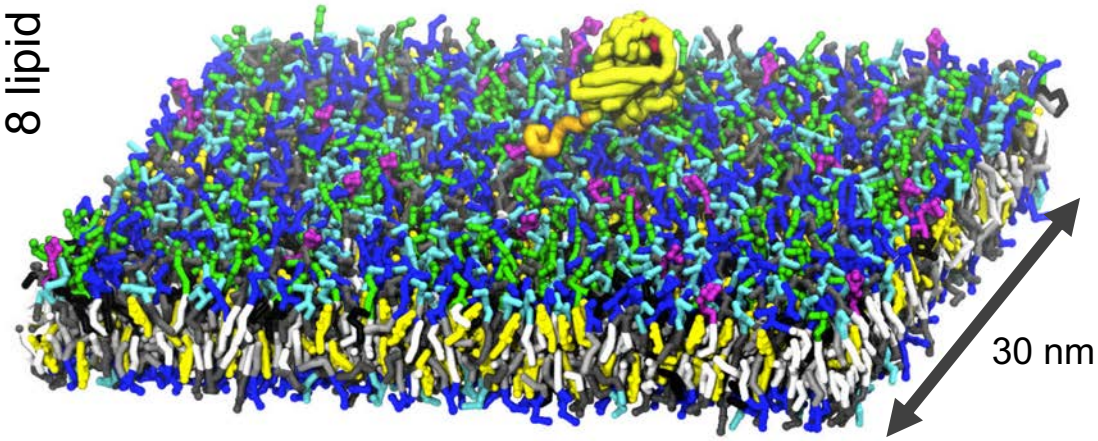
Micro Scale - Coarse Grain Bead Martini Model



Back-mapping



8 lipid types



1 μs into production simulation

First Run on Sierra: RAS Dynamics

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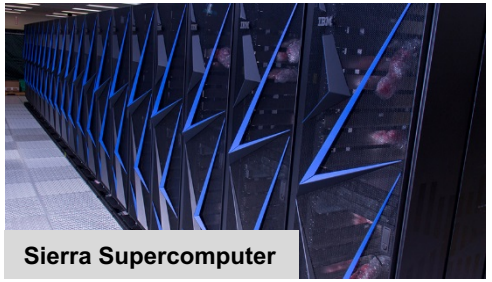
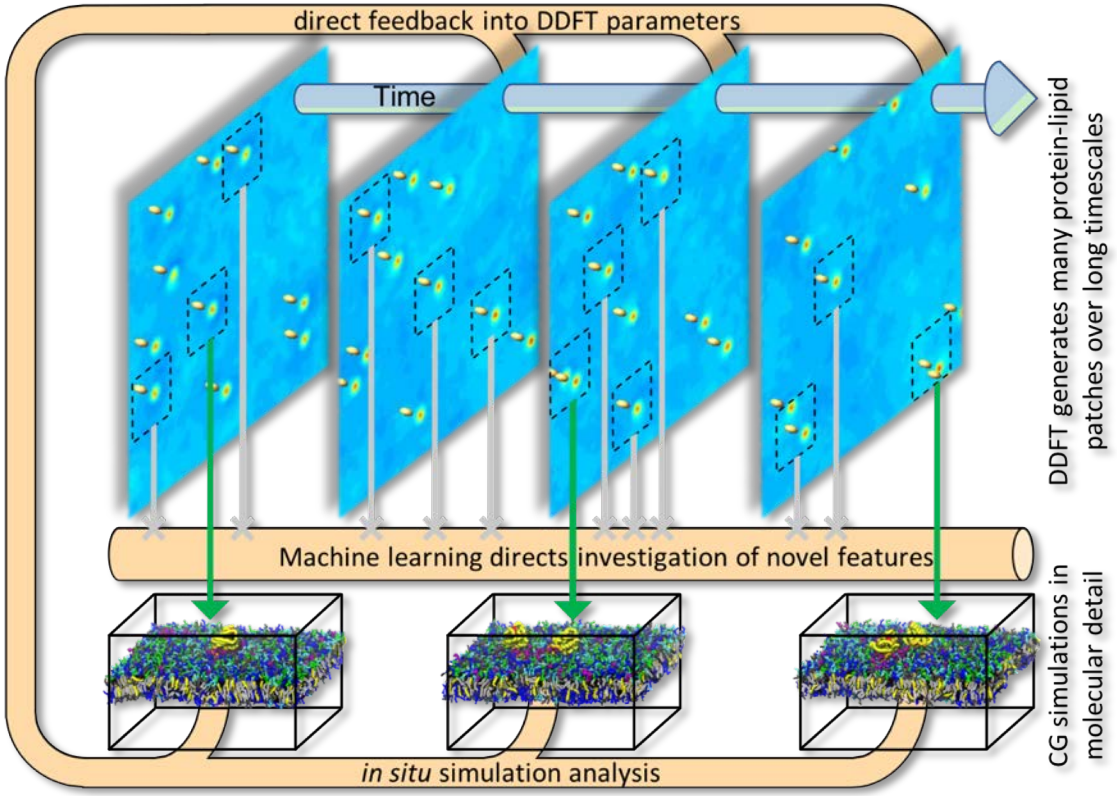
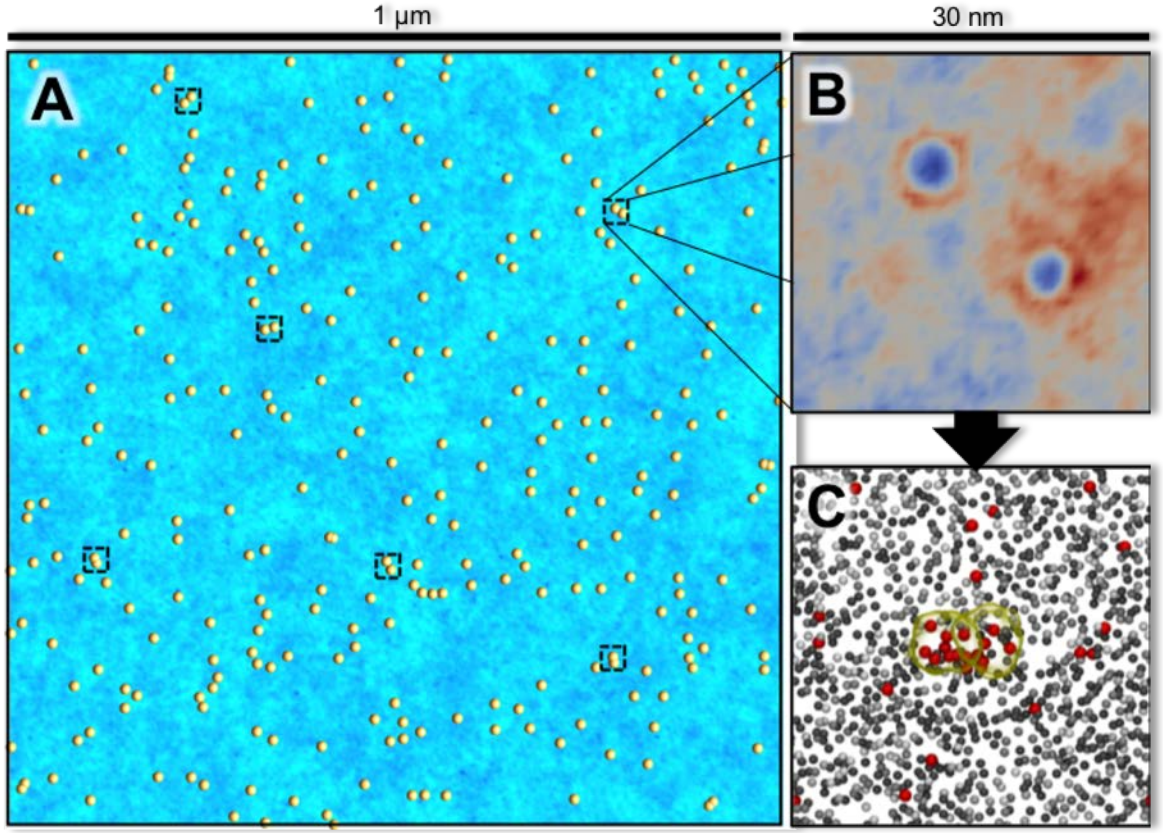
First-of-a-kind simulations will explore:

- Dependence of KRAS mobility and and dynamics as a function of membrane environment
- Aggregation of KRAS in context of realistic membrane
- Effect of KRAS concentration on local membrane composition







On-the-fly feedback from the macro simulations improve micro model parameters

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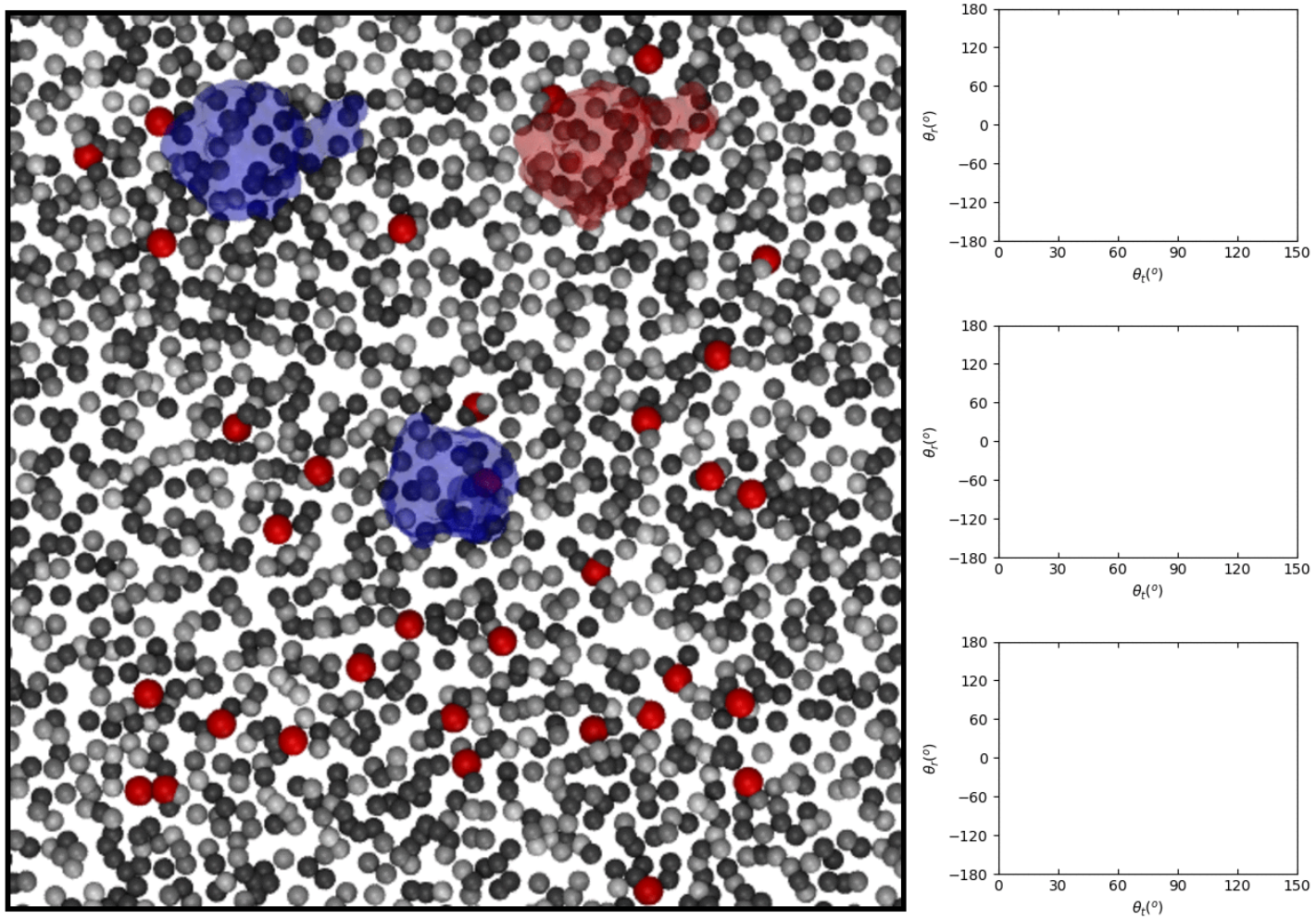
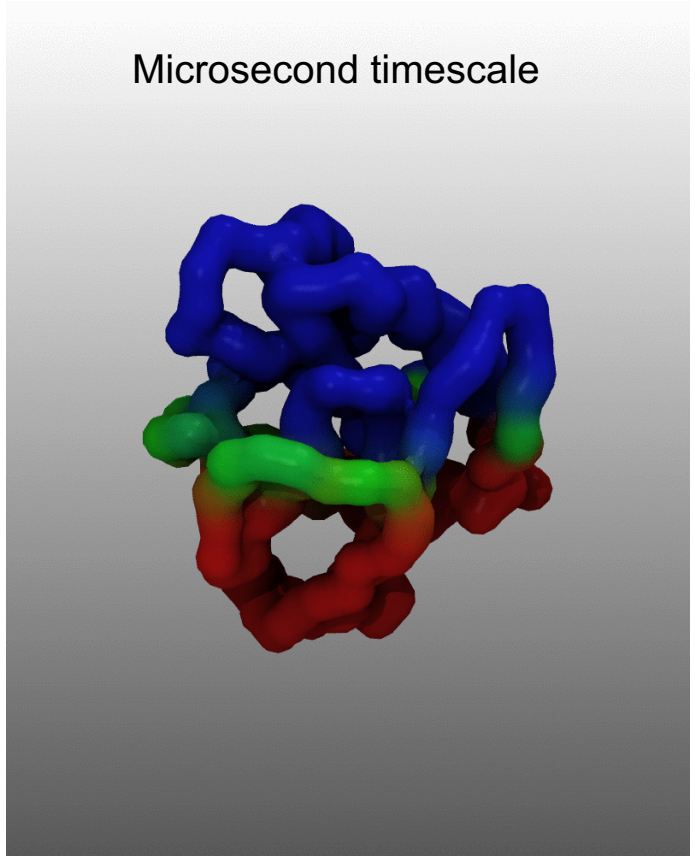
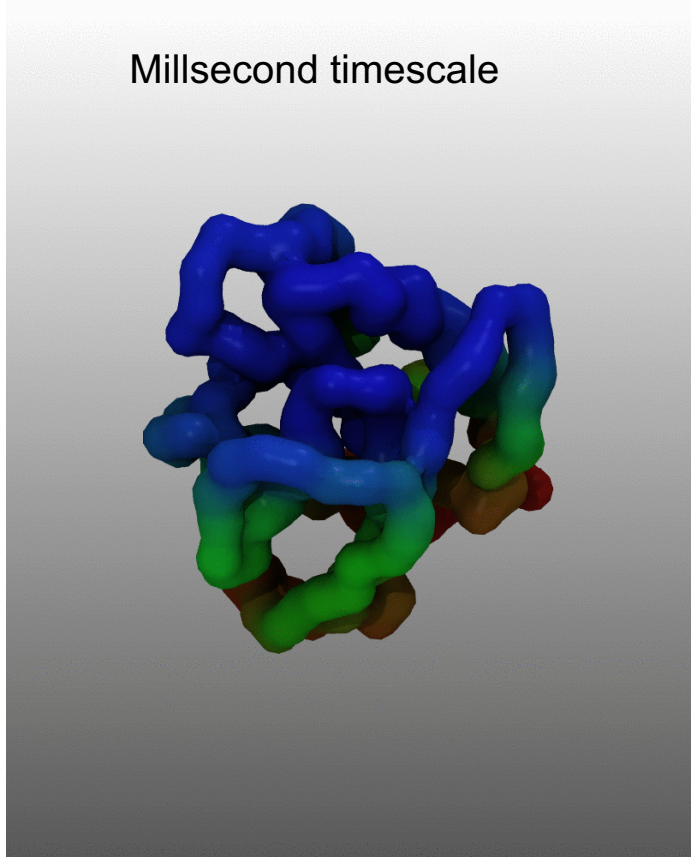


- Online analysis of state dependent RAS lipid interactions
- Micro model parameters are updated after unbiasing through the ML framework

Tim Carpenter, LLNL

Simulation predicted RAS-Lipid contacts





Tim Carpenter, LLNL

Summary

Frederick National Laboratory for Cancer Research

- RAS Initiative has consolidated to two primary focus areas
  - Directly Targeting KRAS
  - Understanding the biology of KRAS in the context of the plasma membrane
- Identified novel classes of compounds that specifically target KRAS
- Multiple screens to identify leads are ongoing
- Working with DOE National Labs to bridge experimental gaps using computation
- Partnering with Biotech, Pharma and NCI to develop leads and push towards clinic