Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Update for FNLAC June 2019 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

New recruits

Frederick National Laboratory for Cancer Research

Caroline DeHart ·

Research Assistant Professor, Department of Molecular Biosciences, Northwestern Univer

Precise characterization of KRAS4b proteoforms in human colorectal cells and tumors reveals mutation/modification cross-talk

Ioanna Ntai[a,b,1,2], Luca Fornelli[a,b,2], Caroline J. DeHart[a,b,2], Josiah E. Hutton[a,b], Peter F. Doubleday[a,b], Richard D. LeDuc[a,b], Alexandra J. van Nispen[a,b], Ryan T. Fellers[a,b], Gordon Whiteley[c], Emily S. Boja[d], Henry Rodriguez[d], and Neil L. Kelleher[a,b,3]

[a]Department of Chemistry, Proteomics Center of Excellence, Northwestern University, Evanston, IL 60208; [b]Department of Molecular Biosciences, Proteomics Center of Excellence, Northwestern University, Evanston, IL 60208; [c]Frederick National Laboratory for Cancer Research, Leidos Biomedical Research Inc., Frederick, MD 21701; and [d]Office of Cancer Clinical Proteomics Research, National Cancer Institute, Bethesda, MD 20892

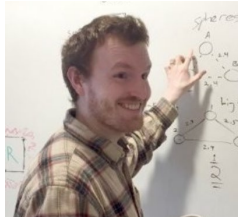
Dr Alok Sharma
ex Warpdrive
NMR

Biomolecular NMR Assignments (2019) 13:227–231
https://doi.org/10.1007/s12104-019-09882-1

ARTICLE

NMR ^{1}H,^{13}C, ^{15}N resonance assignment of the G12C mutant of human K-Ras bound to GppNHp

Alok K. Sharma[1] · Seung-Joo Lee[1] · Minyun Zhou[1] · Alan C. Rigby[1] · Sharon A. Townson[1]



Dr Trent Balius
ex Shoichet group, UCSF
Computational drug design

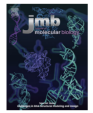
Testing inhomogeneous solvation theory in structure-based ligand discovery

Trent E. Balius[a,1], Marcus Fischer[a,1,2], Reed M. Stein[b], Thomas B. Adler[a], Crystal N. Nguyen[c], Anthony Cruz[d,e], Michael K. Gilson[c], Tom Kurtzman[d,e,f], and Brian K. Shoichet[a,3]

[a]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158; [b]Graduate Program in Pharmaceutical Sciences and Pharmacogenomics, University of California, San Francisco, CA 94158; [c]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093; [d]Department of Chemistry, Lehman College, Bronx, NY 10468; [e]Ph.D. Program in Chemistry, The Graduate Center, City University of New York, New York, NY 10016; and [f]Ph.D. Program in Biochemistry, The Graduate Center, City University of New York, New York, NY 10016

Gabriel Cornilescu

Researcher at National Magnetic Resonance Facility at Madison (UW-Biochemistry)



Structural Analysis of Multi-Helical RNAs by NMR–SAXS/WAXS: Application to the U4/U6 di-snRNA

Gabriel Cornilescu[†], Allison L. Didychuk[†], Margaret L. Rodgers, Lauren A. Michael, Jordan E. Burke, Eric J. Montemayor, Aaron A. Hoskins and Samuel E. Butcher

Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA

New machine…

Bruker 700 Mhz

Frederick National Laboratory for Cancer Research

Biophysical Journal
Article

Biophysical Society

KRAS Prenylation Is Required for Bivalent Binding with Calmodulin in a Nucleotide-Independent Manner

Constance Agamasu,[1] Rodolfo Ghirlando,[2] Troy Taylor,[1] Simon Messing,[1] Timothy H. Tran,[1] Lakshman Bindu,[1] Marco Tonelli,[3] Dwight V. Nissley,[1] Frank McCormick,[4] and Andrew G. Stephen[1,*]

[1]NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland; [2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland; [3]National Magnetic Resource Facility at Madison, Biochemistry Department, University of Wisconsin-Madison, Madison, Wisconsin; and [4]Diller Family Comprehensive Cancer Center, University of California, San Francisco, California

Confidential

JBC ARTICLE

Quantitative biophysical analysis defines key components modulating recruitment of the GTPase KRAS to the plasma membrane

Received for publication, August 31, 2018, and in revised form, November 28, 2018 Published, Papers in Press, December 17, 2018, DOI 10.1074/jbc.RA118.005669

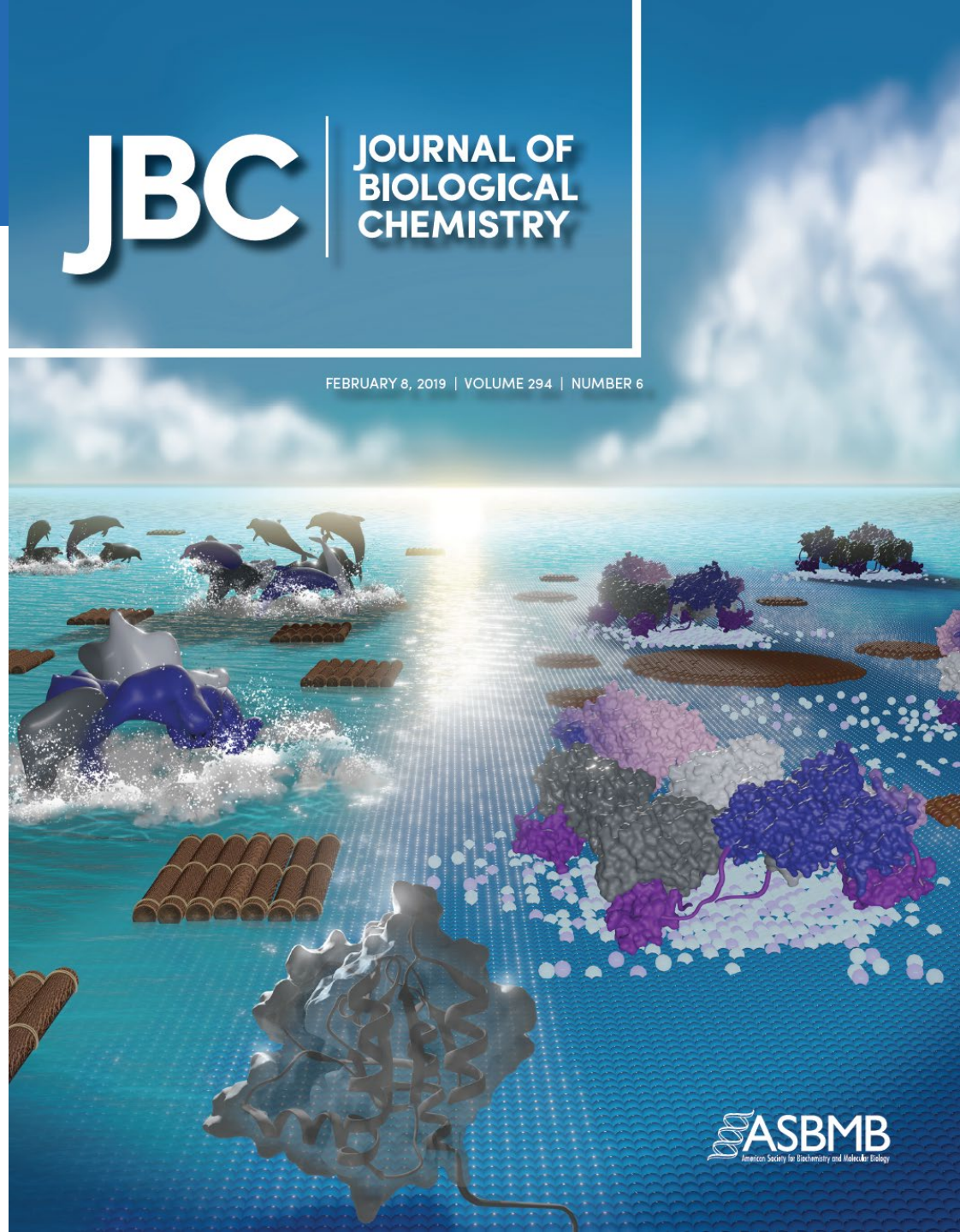
Bindu Lakshman[‡], Simon Messing[‡], Eva M. Schmid[§], Jeffrey D. Clogston[¶], William K. Gillette[‡], Dominic Esposito[‡], Bailey Kessing[‡], Daniel A. Fletcher[§‖**], Dwight V. Nissley[‡], Frank McCormick[‡ ‡‡], Andrew G. Stephen[‡], and Frantz L. Jean-Francois[‡1]

*From the [‡]NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland 21702, [§]Department of Bioengineering, University of California Berkeley, Berkeley, California 94720, [¶]Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, 21702, [‖]Division of Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, California 94720, **Chan Zuckerberg Biohub, San Francisco, California 94158, [‡‡]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, California 94158*

Edited by Karen G. Fleming

The gene encoding the GTPase KRAS is frequently mutated in pancreatic, lung, and colorectal cancers. The KRAS fraction in the plasma membrane (PM) correlates with activation of the mitogen-activated protein kinase (MAPK) pathway and subsequent cellular proliferation. Understanding KRAS's interaction with the PM is challenging given the complexity of the cellular environment. To gain insight into key components necessary for KRAS signal transduction at the PM, we used synthetic membranes such as liposomes and giant unilamellar vesicles. Using surface plasmon resonance (SPR) spectroscopy, we demonstrated that KRAS and Raf-1 proto-oncogene Ser/Thr kinase (RAF1) domains interact with these efforts to disrupt this critical interaction in diseases such as cancer.

The GTPase KRAS is frequently mutated in pancreatic (98%), lung (31%), and colorectal cancer (45%) (1). RAS GTPases are implicated in cellular proliferation through activation of the MAPK[2] pathway. They are primarily anchored at the membrane through lipidated hypervariable regions (HVR) which are responsible for localization to specific microdomains (2, 3). A RAS anchor consists of two components: a C-terminal *S*-farnesyl cysteine carboxymethyl ester, common to all iso-



Membrane interactions of the G-domain and the HVR of KRAS4b define its unique diffusion behavior

Debanjan Goswami[1†], De Chen[1†], Yue Yang[2], Prabhakar Gudla[1], John Columbus[1], Karen Worthy[1], Megan Rigby[1], Katie Powell[1], William Burgan[1], Vanessa Wall[1], Dominic Esposito[1], Dhirendra Simanshu[1], Felice Lightstone[2], Dwight V. Nissley[1], Frank McCormick[3], Thomas J. Turbyville[1]

***For correspondence:**

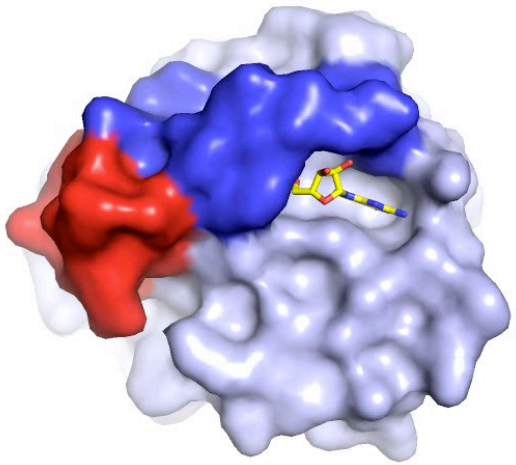
[†]These authors contributed equally to this work

[1]Frederick National Laboratory for Cancer Research, Frederick, Maryland, United States of America; [2]Lawrence Livermore National Laboratory, Livermore, California, United States of America; [3]UCSF Helen Diller Family Comprehensive Cancer Center, School of Medicine, University of California, San Francisco, San Francisco, California, United States of America

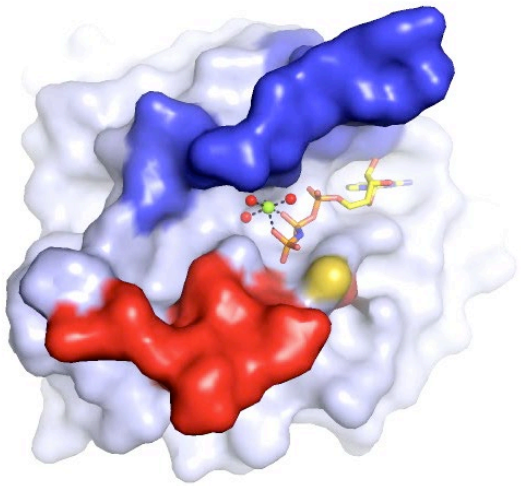
New pockets in oncogenic KRAS mutants

Frederick National Laboratory for Cancer Research

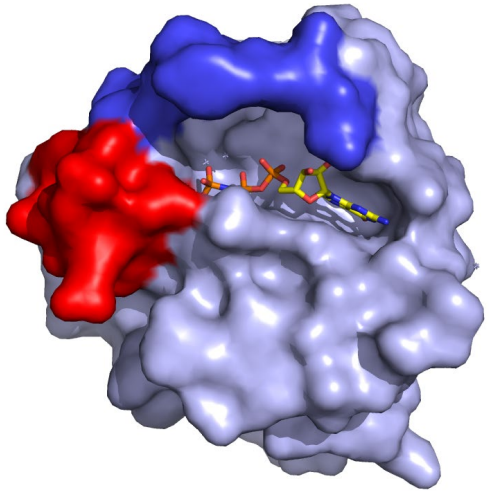
Switch-1
Switch-2



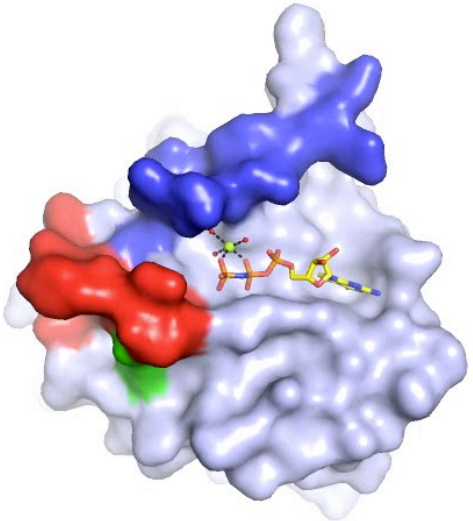
WT-KRAS bound to GMPPNP and Mg



G12C in complex with GMPPNP and Mg



G13D in complex with GMPPNP and Mg



Q61L in complex with GMPPNP and Mg

Simanshu Dhirendra

Albert Chan, Sathiya Dharmaiah, Tim Tran and Wupeng Yan

Dom Esposito, Bill Gillette and members of RAS Reagents Core

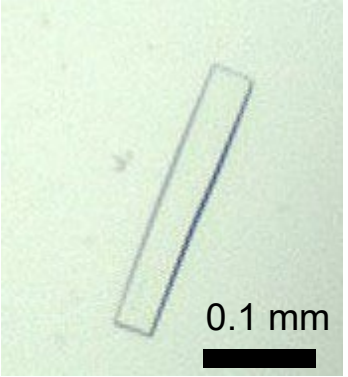
G13D KRAS-GMPPNP crystals
1.2 -1.4 Å

Fragment Screening for compounds that bind to G13D-KRAS in state 1 conformation

Frederick National Laboratory for Cancer Research

Diamond Light Source (UK)

- Screen >1000 fragments per week
- Includes soaking, harvesting, data collection, data analysis



G13D KRAS-GMPPNP
1.2 – 1.4 Å

Proposal submitted (Sept 2018)

↓

Proposal awarded (Jan 2019)

↓

Fragment Screening (April 2019)

Reproduce crystals

↓

Soak compounds to crystals

↓

Collect data at synchrotron

↓

Structure determination

↓

Fragment search

Albert Chan

Crystal structure of N-acetylated KRAS-GDP

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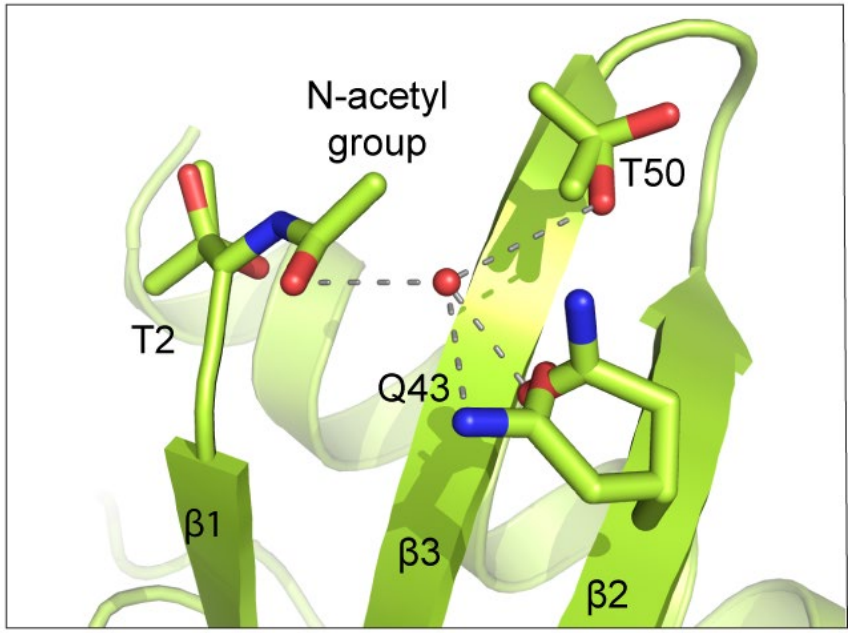
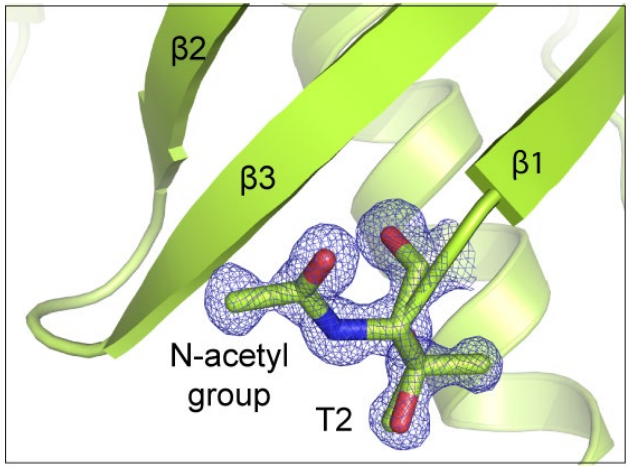
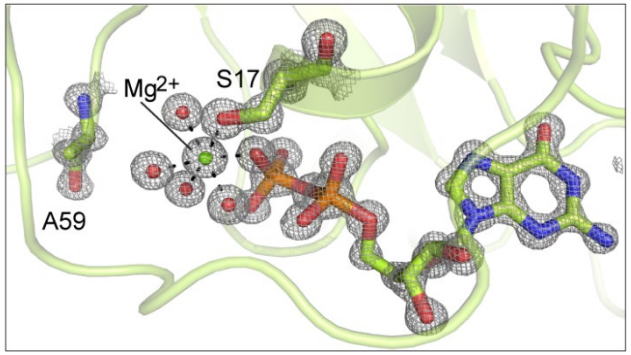
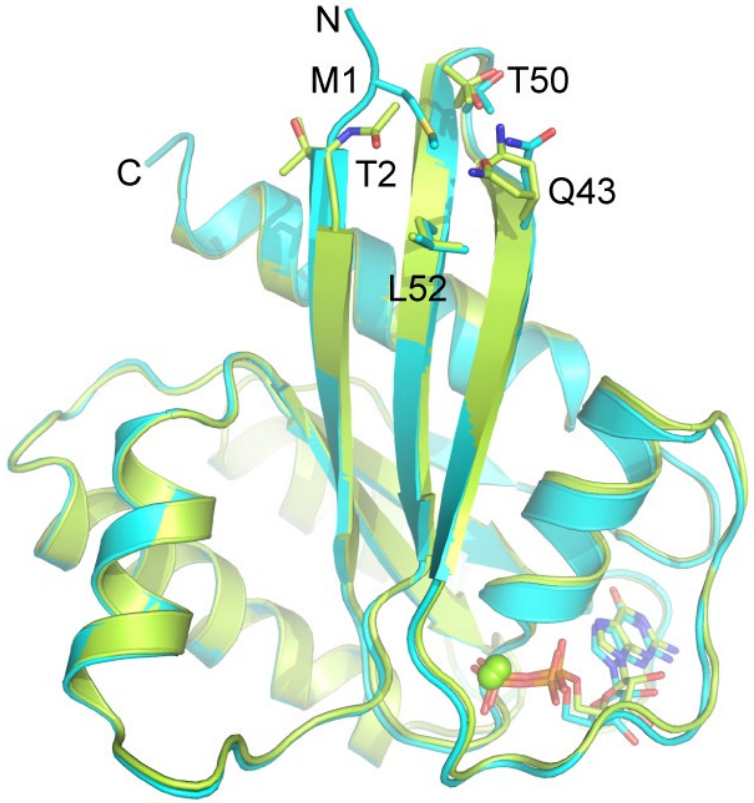






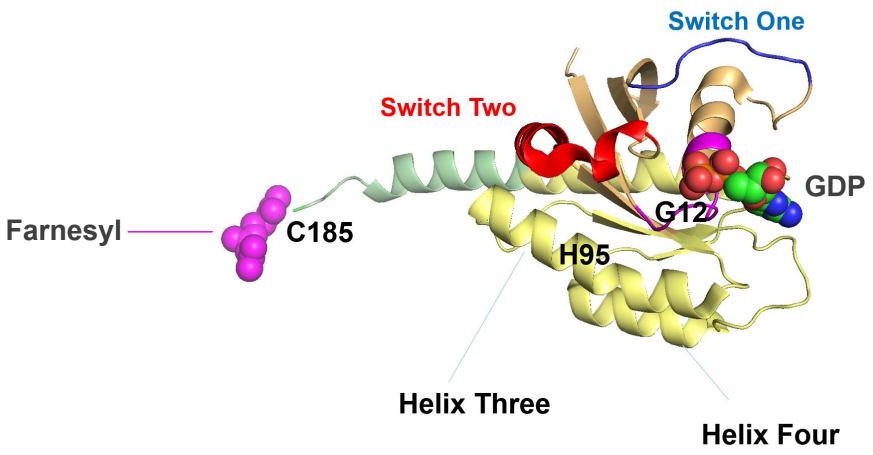


Acetylated-KRAS(2-169)-GDP
KRAS(1-169)-GDP

Resolution: 1 Ang

Dom Esposito, Simanshu, Bill Gillette, Sathiya Dharmaiah

Targeting C185 (site of KRAS processing)

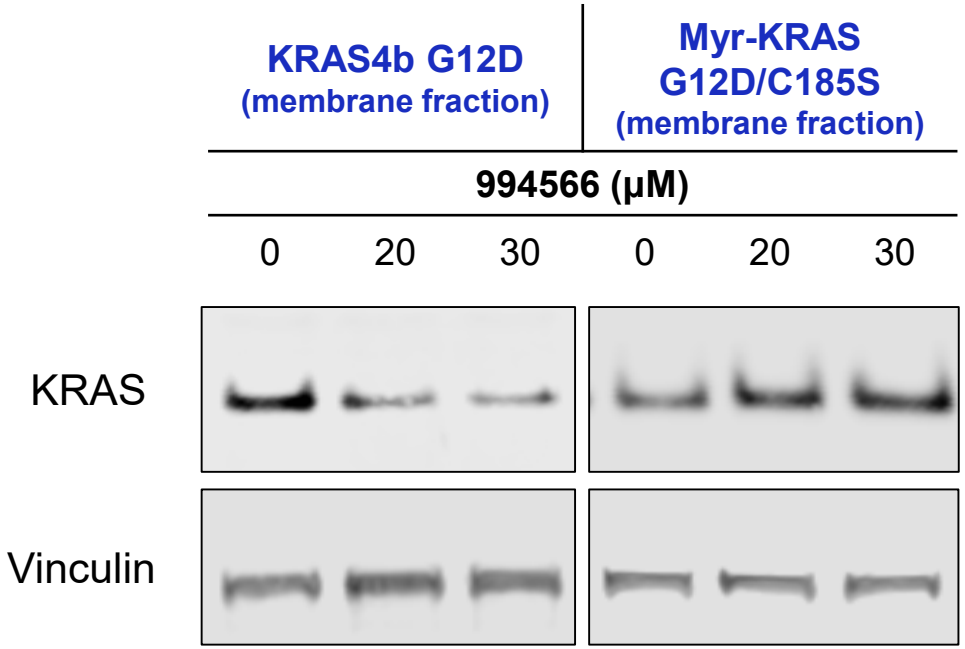


Anna Maciag

David Turner
Marcin Dyba
Vandana Kumari
Brian Smith
Alok Sharma

Blocking processing of newly synthesized KRAS

Frederick National Laboratory for Cancer Research



Targeting C185: target engagement in cells

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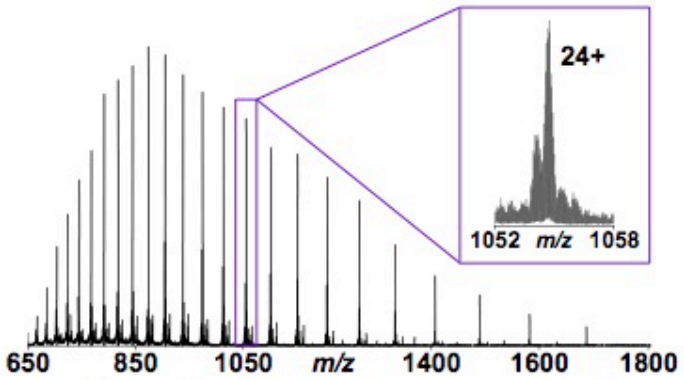
HEK 293 – tet-on 3xFLAG KRAS4b G12D +/- FNL-0175

Observed Mass: 25289.62 Da
Theoretical Mass: 25289.66 Da
Error: 0.04 Da, 2 ppm



N-terminal acetylated, C185 farnesylated, C-terminal methylation

```
N  G D Y K D H D G D Y K D H D I D Y K D D D D K G S   25
26 T T L Y K K V G T M T E Y K L V V V G A D G V G K   50
51 S A L T I Q L I Q N H F V D E Y D P T I E D S Y R   75
76 K Q V V I D G E T C L L D I L D T A G Q E E Y S A  100
101 M R D Q Y M R T G E G F L C V F A I N N T K S F E  125
126 D I H H Y R E Q I K R V K D S E D V P M V L V G N  150
151 K C D L P S R T V D T K Q A Q D L A R S Y G I P F  175
176 I E T S A K T R Q G V D D A F Y T L V R E I R K H  200
201 K E K M S K D G K K K K K K S K T K C C
```

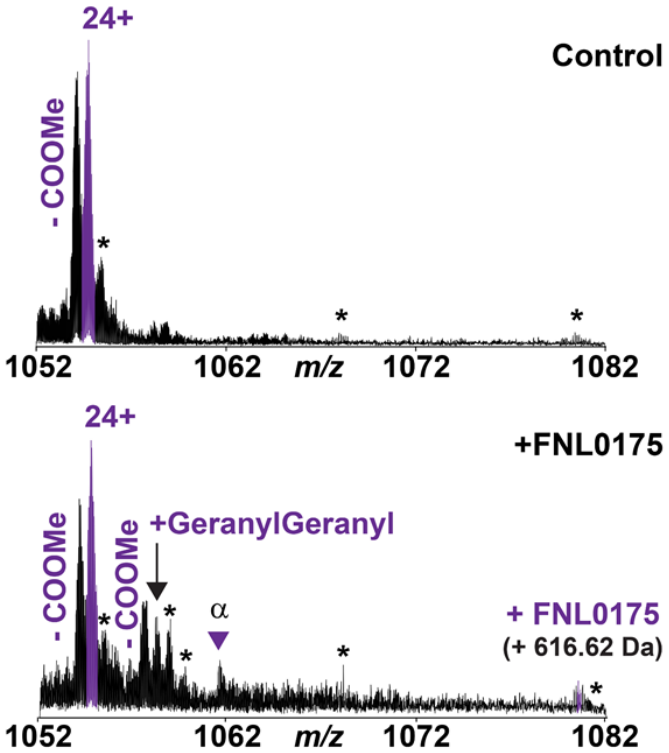
P-score: 7e-36

FLAG-KRAS4B-G12D plus FNL0175 (+ 491.17 Da)

```
N  G D Y K D H D G D Y K D H D I D Y K D D D D K G S   25
26 T T L Y K K V G T M T E Y K L V V V G A D G V G K   50
51 S A L T I Q L I Q N H F V D E Y D P T I E D S Y R   75
76 K Q V V I D G E T C L L D I L D T A G Q E E Y S A  100
101 M R D Q Y M R T G E G F L C V F A I N N T K S F E  125
126 D I H H Y R E Q I K R V K D S E D V P M V L V G N  150
151 K C D L P S R T V D T K Q A Q D L A R S Y G I P F  175
176 I E T S A K T R Q G V D D A F Y T L V R E I R K H  200
201 K E K M S K D G K K K K K K S K T K C V I M C
```

C + FNL0175 (+ 491.17 Da)

Theoretical Mass: 25905.82 Da
D Mass: + 616.16 Da



Caroline DeHart,
Neil Kelleher

On-target vs off-target

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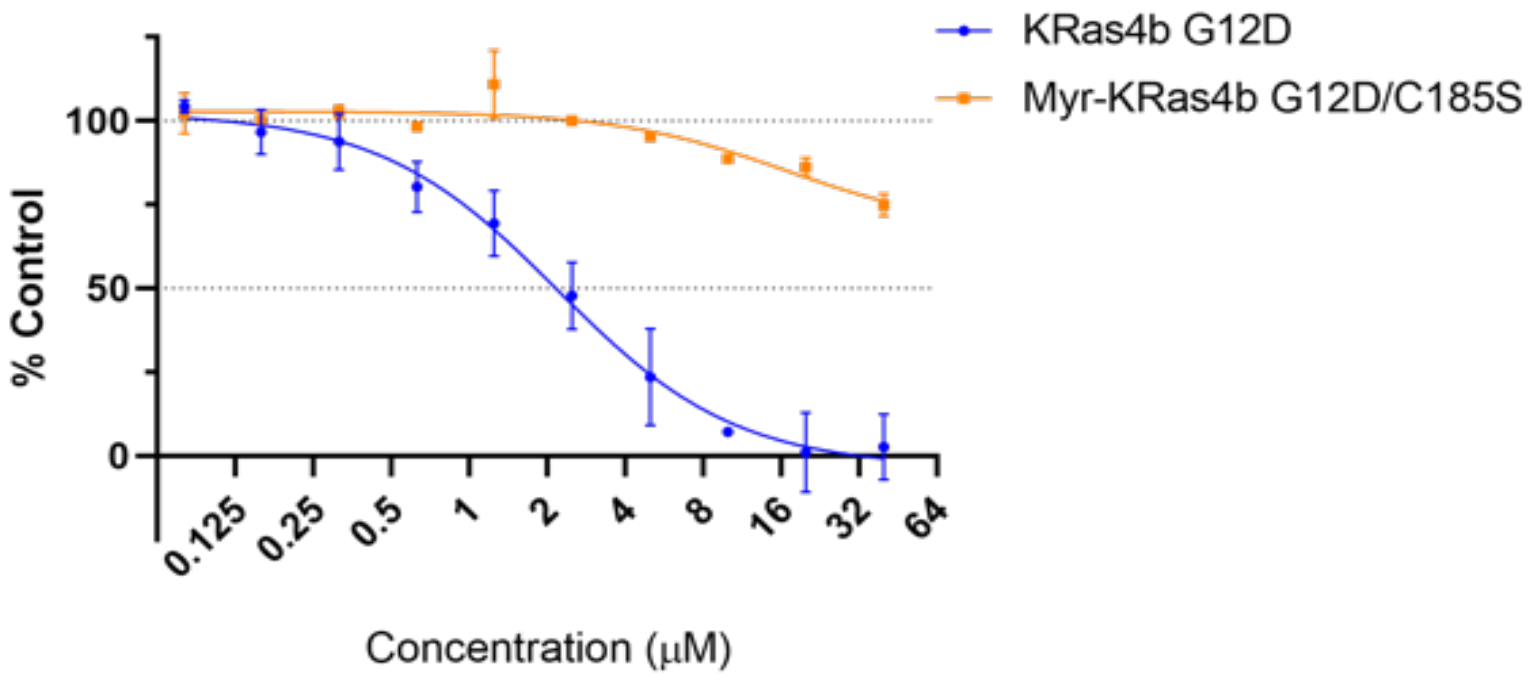


Anna Maciag

Nikki Fer
Brian Smith

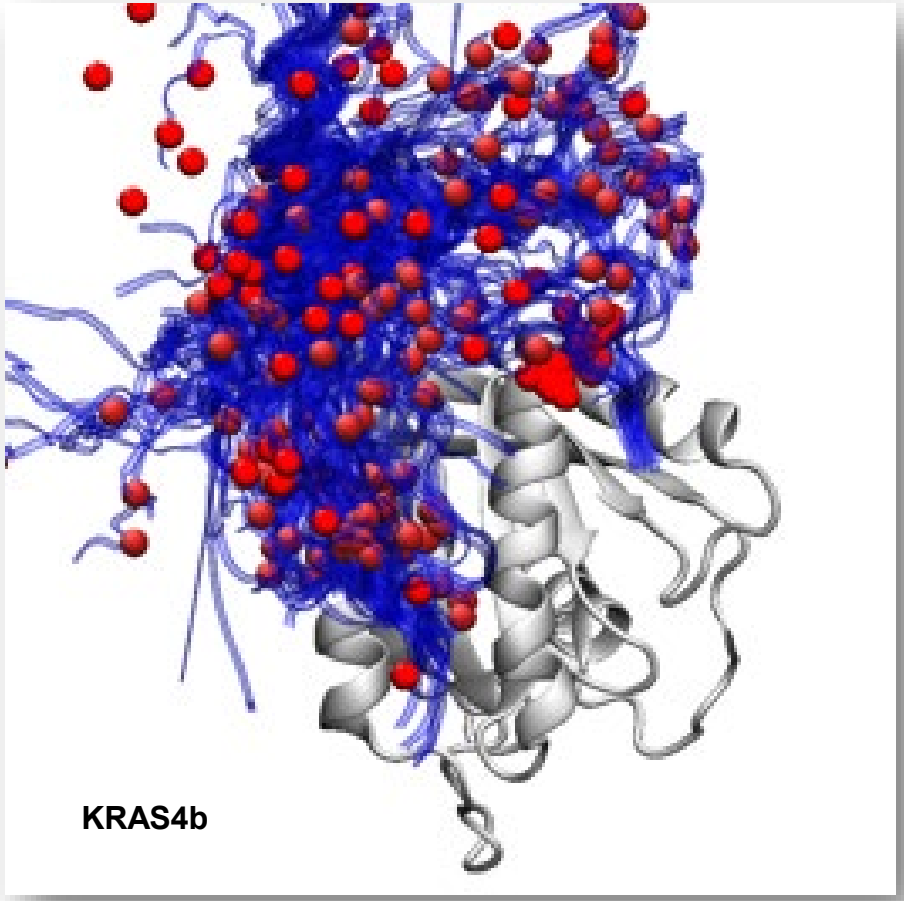
Can the C-terminus interact with the G-domain?

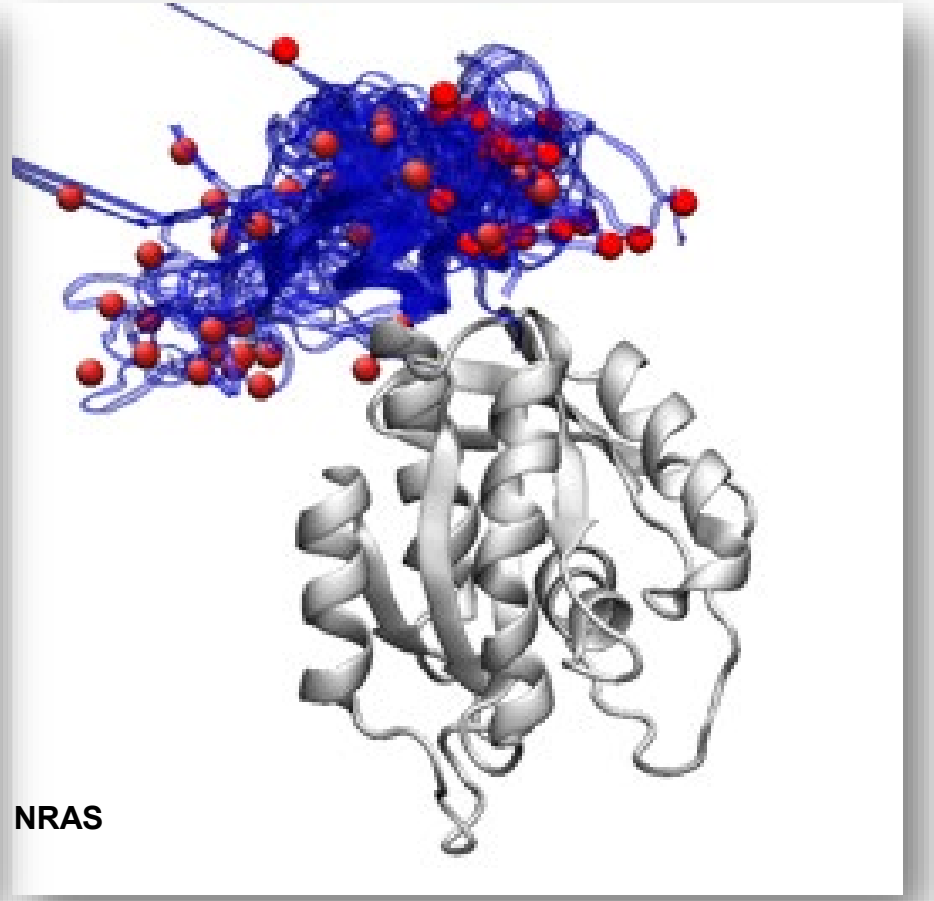
Frederick National Laboratory for Cancer Research

H95

Yue Yang
Felice Lightstone (LLNL)

KRAS4b vs NRAS





KRAS4b - NRAS comparison

	H3/H4 sampled	H3/H4 sampled 100+ns
KRAS4b	21/26, ~35μs	18/26
NRAS	0/10 ~11μs	0/10

SAXS – ligand stabilizes HVR/G-domain binding

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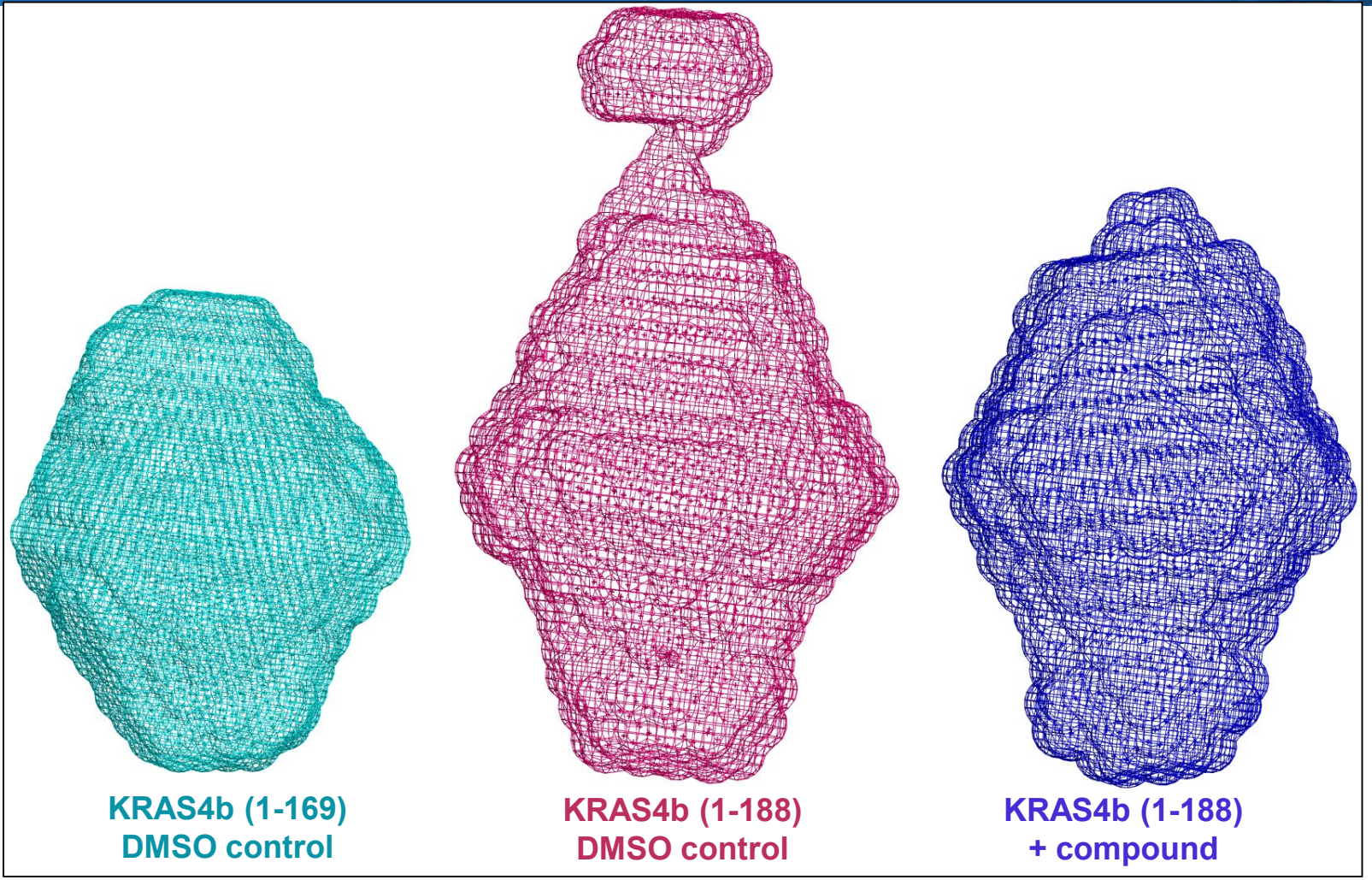

Lixin Fan, The Small-Angle X-ray Scattering Core Facility, NCI

HVR interacting with the G-domain of KRAS4b

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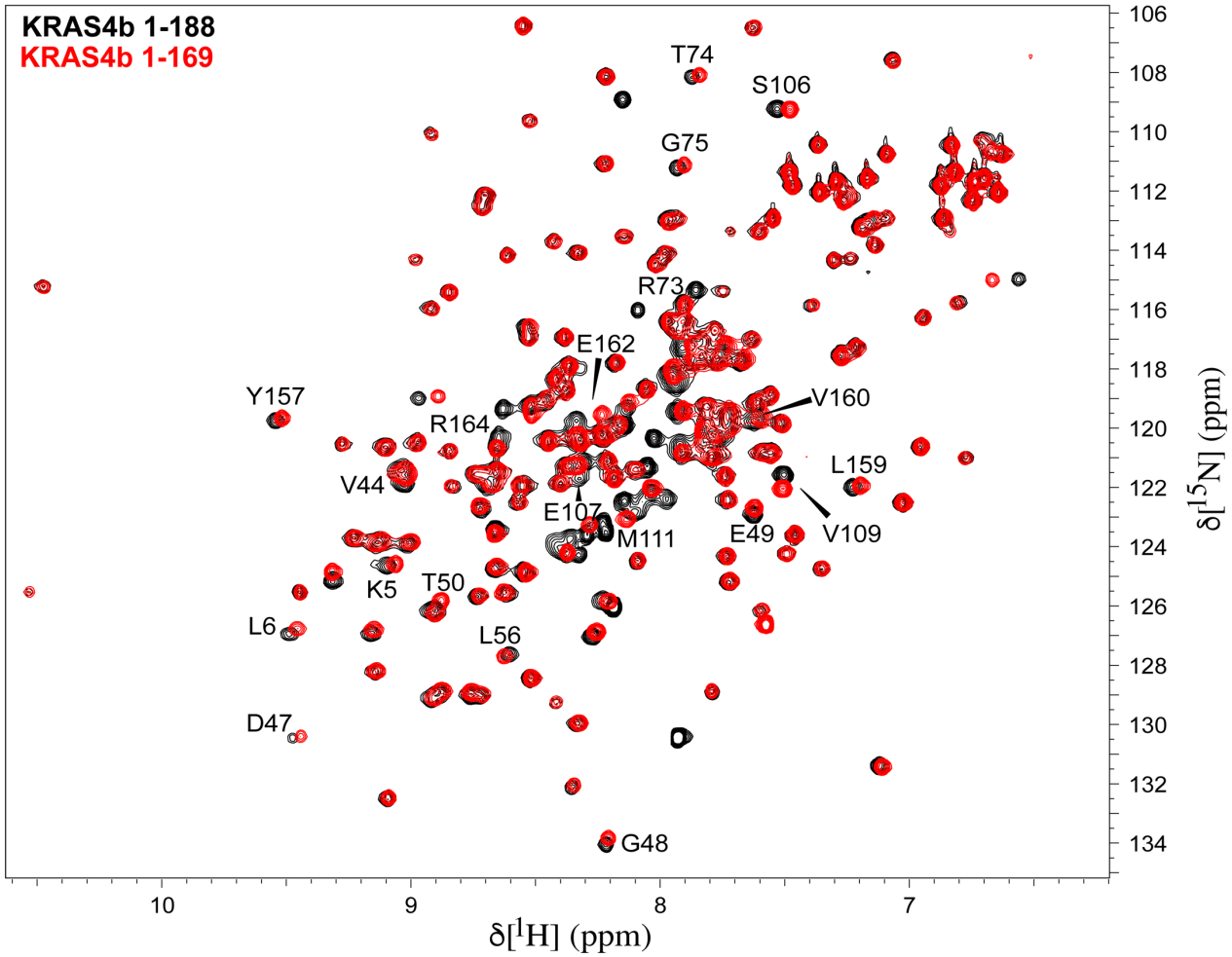
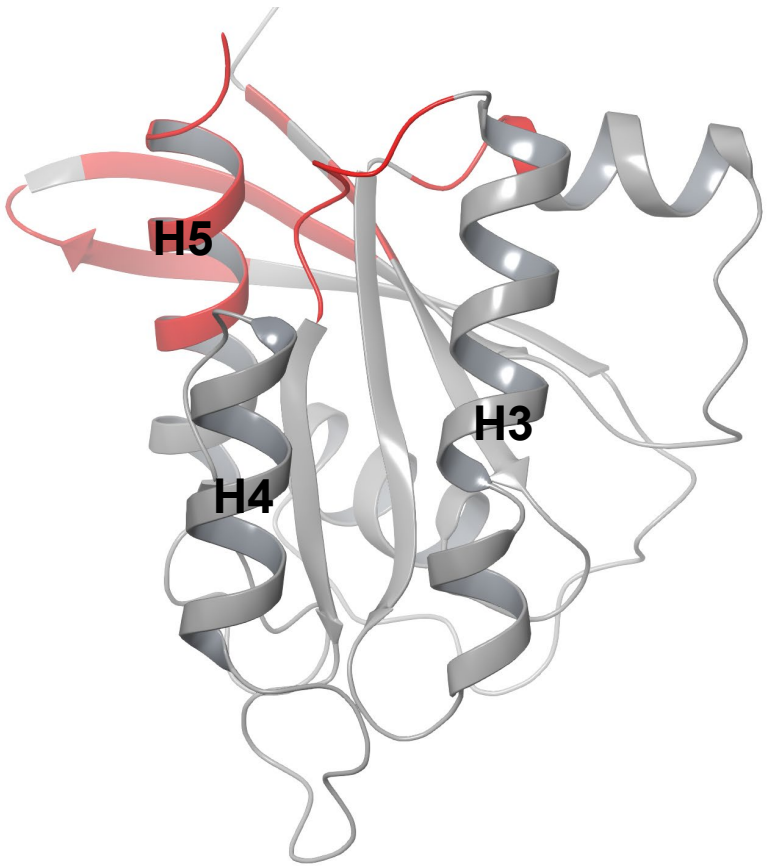




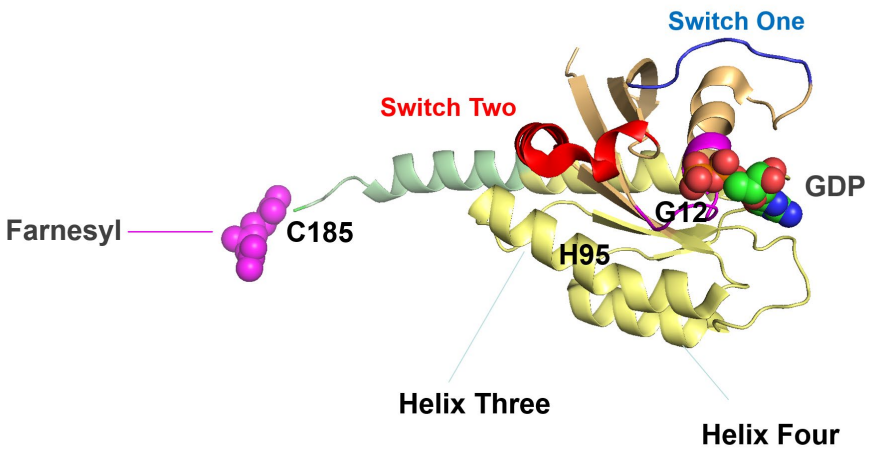
Constance Agamasu

Frederick National Laboratory for Cancer Research

Targeting H95 (unique residue in KRAS)

Anna Maciag

David Turner
Vandana Kumari
Brian Smith
Alok Sharma
Albert Chan
Simanshu Dhirendra
Chris Brassard
Marcin Dyba



H95-switch 2 pocket

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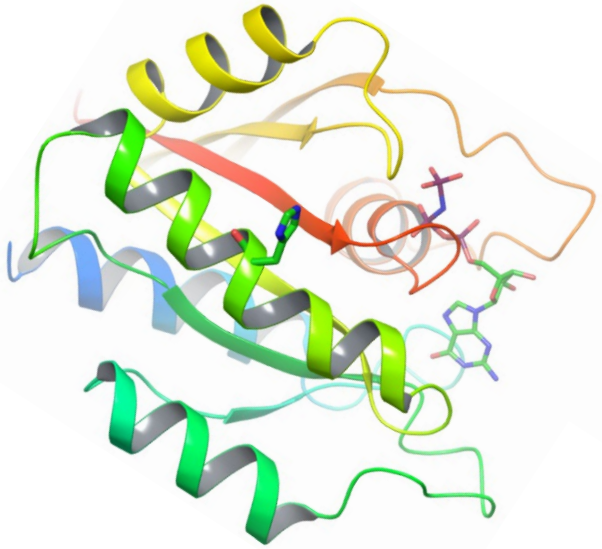
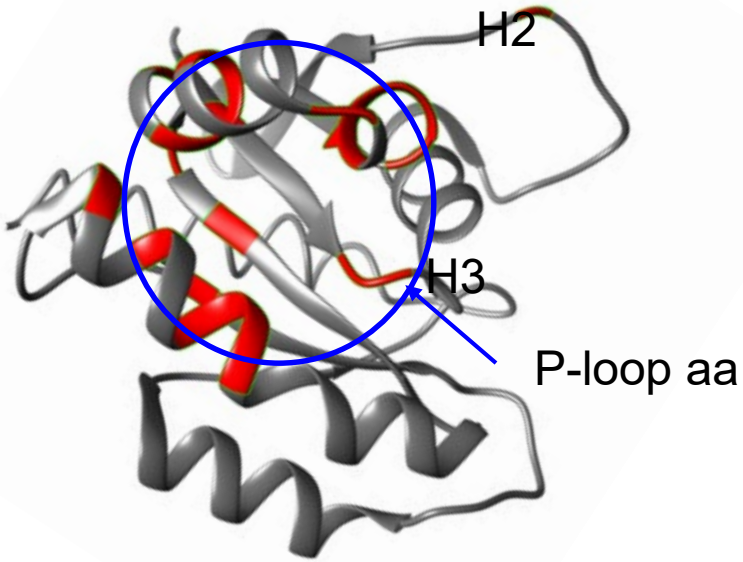
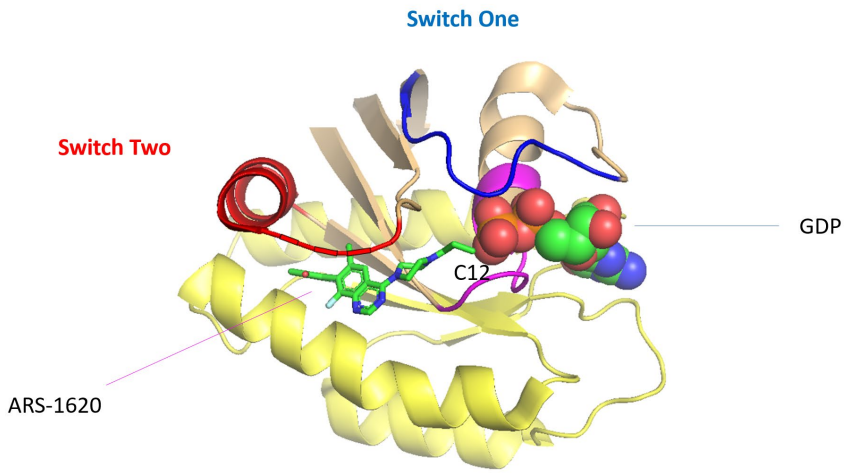
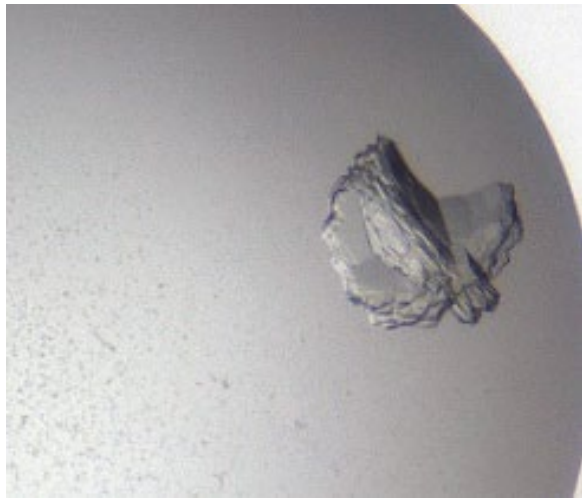






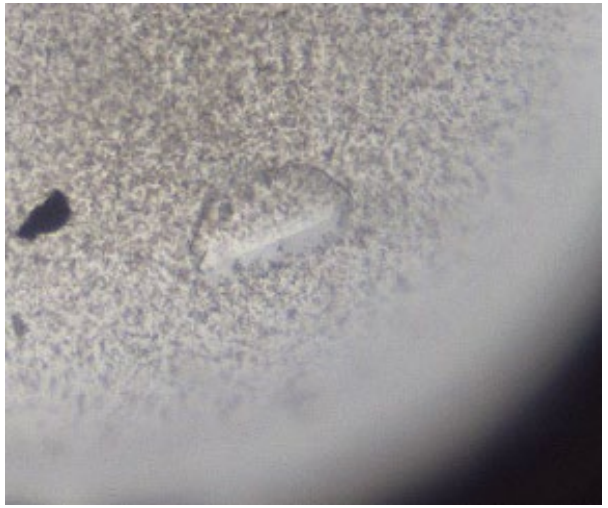
H95 Project: Crystals

Frederick National Laboratory for Cancer Research



Initial crystal, Jan 2019
20% PEG 3350, 0.2 M Ca acetate
~3.5 Å. Heavily mosaic

optimization



Optimized crystal, Mar 2019
19.8% PEG 3350, 0.36 M Ca acetate, 3% 1,6-hexanediol
1.9 Å

Albert Chan
Dhirendra Simanshu

Frederick National Laboratory for Cancer Research

Disulfide Tethering Library

David Turner

Vandana Kumari
Marcin Dyba

TETHERING: Fragment-Based Drug Discovery

Daniel A. Erlanson, James A. Wells,
and Andrew C. Braisted
Sunesis Pharmaceuticals, Inc., 341 Oyster Point Boulevard, South San Francisco, California 94080; email: erlanson@sunesis.com; jaw@sunesis.com

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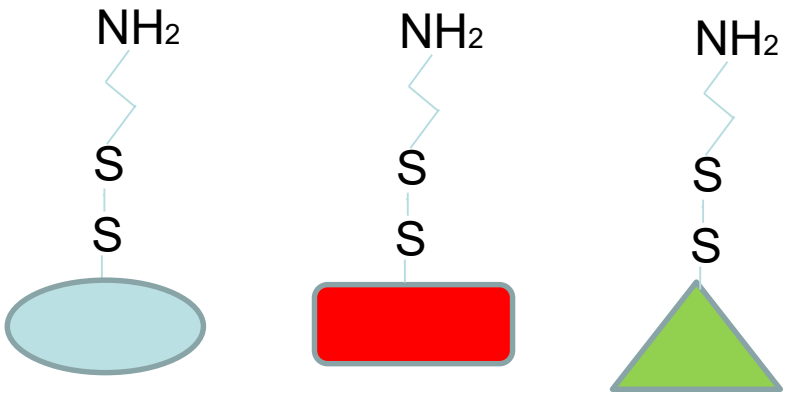
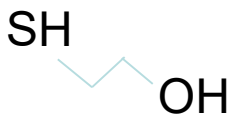
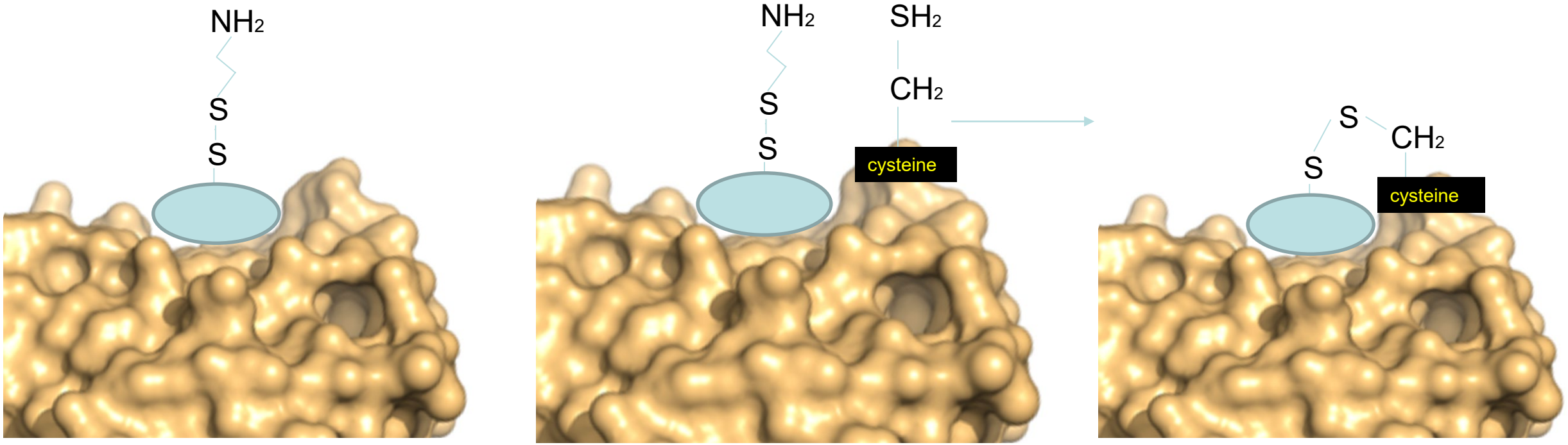




100 uM β-ME



FNL Disulfide Tethering Library

Frederick National Laboratory for Cancer Research

- 1500-2000 high quality and chemically diverse fragments
- Good fragment-like properties (MW <300, ClogP ≤ 3, $_n$(H-bond donors/acceptors) ≤3 etc.)
- Minimize overly complex molecules
- Minimize pointless stereochemistry (e.g racemizable groups)
- Exclusion of PAINS / reactive groups
- Removal of chemically unappealing fragments

Disulfide Tethering Library

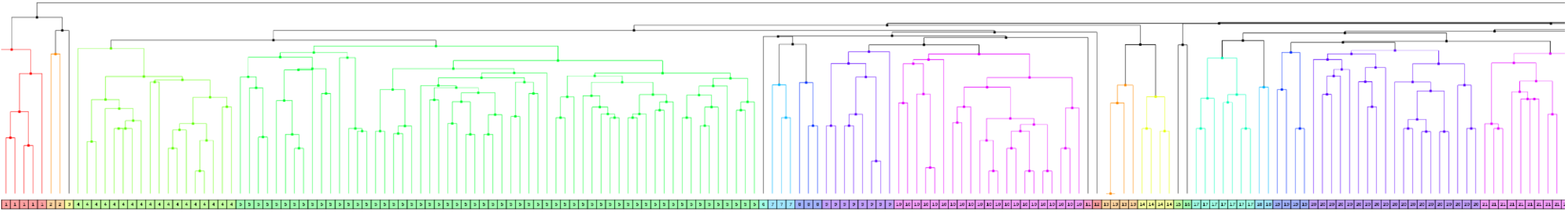
Frederick National Laboratory for Cancer Research

- CRO Library of 13,000 carboxylic acid building blocks
- Trialed synthesis of 100 structurally diverse analogues
- 1200 compounds selected (1000+200 contingency) through computational analysis
- Expected synthesis time (8-12 weeks)
- 5-Year Exclusivity
- Further analogues to be prepared through alternative CRO's and CREST student (summer 2019)

Enamine tethering library development (In-house)

Frederick National Laboratory for Cancer Research

- **Clustering based on R-group diversity**
  - Kmean clustering (Lloyd's algorithm)
  - Hierarchical clustering (Tanimoto similarity metric)
  - Diversity-based selection (Soergel distance metric)
    - Both 2D linear and 3D pharmacophore based fingerprints were used for all clustering methods



Hierarchical clustering example (different clusters represented by color)

Vandana Kumari

Distributions: Molecular properties of the tethering library

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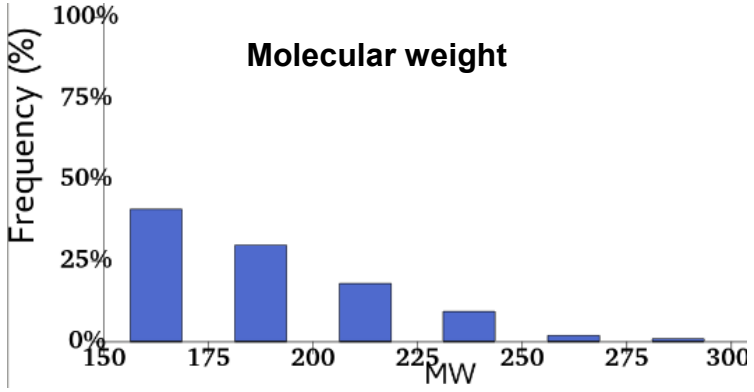

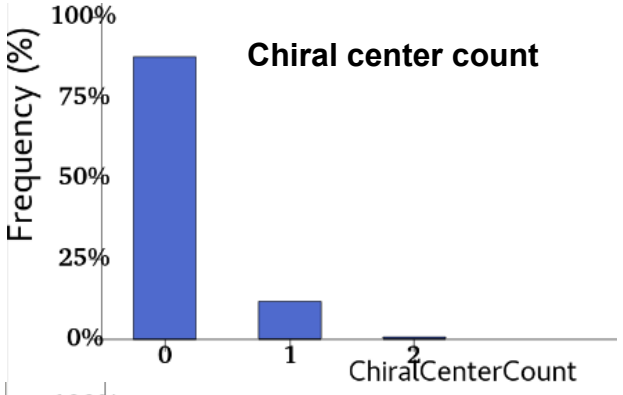

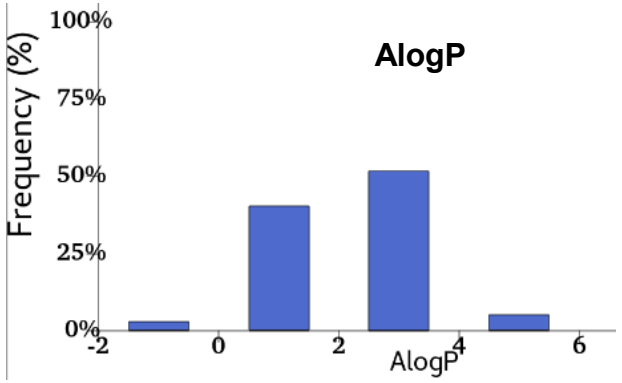

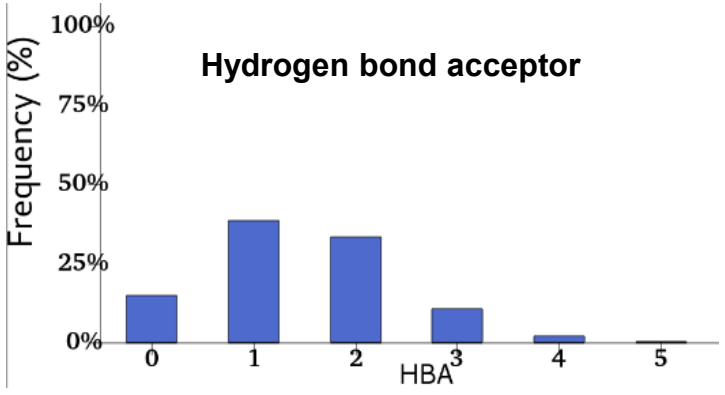

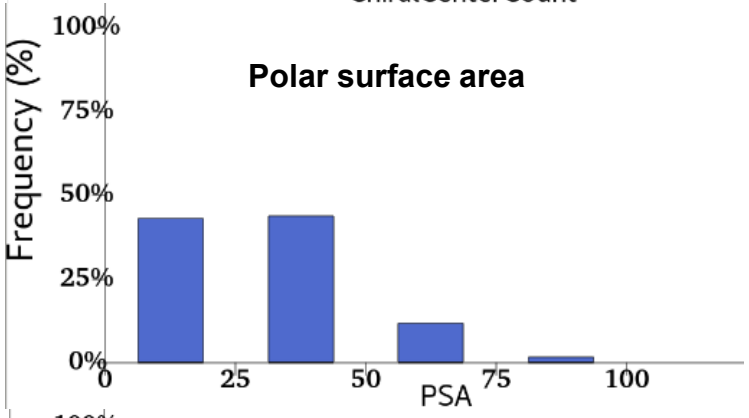

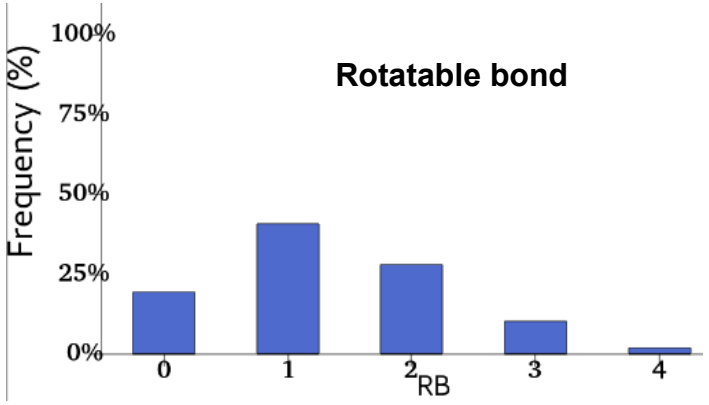

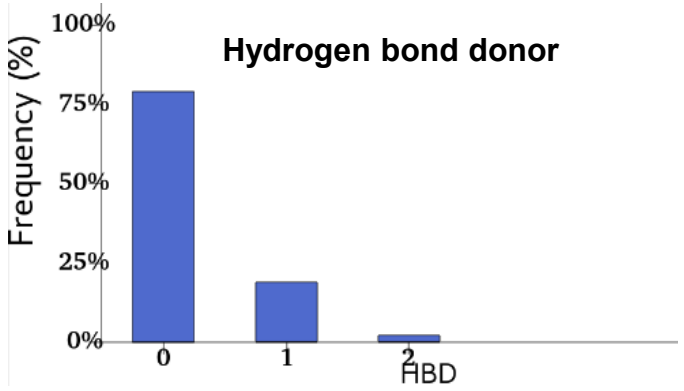

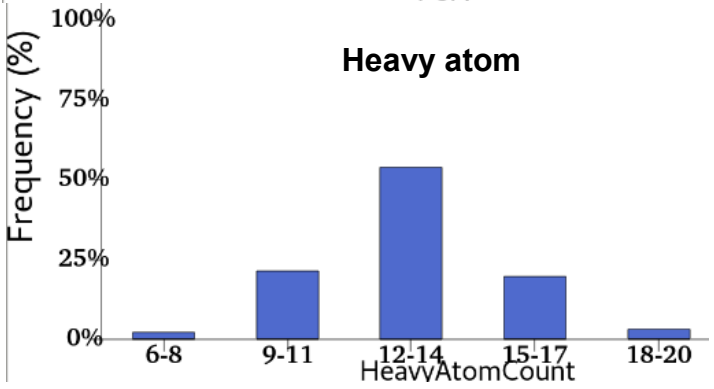

Vandana Kumari

Frederick National Laboratory for Cancer Research

KRAS CSSM library (cysteine surface saturation mutagenesis)

Emily Alberico
Dom Esposito

Stephanie Widmeyer (purification)
Vanessa Wall (cloning)
Matt Smith (expression)

Project Goals

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- **Generate library of Kras proteins that contain single point mutations to Cys**
- **Validate functionality of purified mutants (stability, folding, activity)**
- **Utilize mutants for drug discovery and understanding RAS biology**
  - Tethering screens using FNL library to identify new pockets
  - SHG (Second Harmonic Generation) screening for conformational changes
  - EPR/DEER NMR experiments (distance measurements)
  - Specific labeling for single molecule experiments (AFM, optical microscopy)

Defining the library

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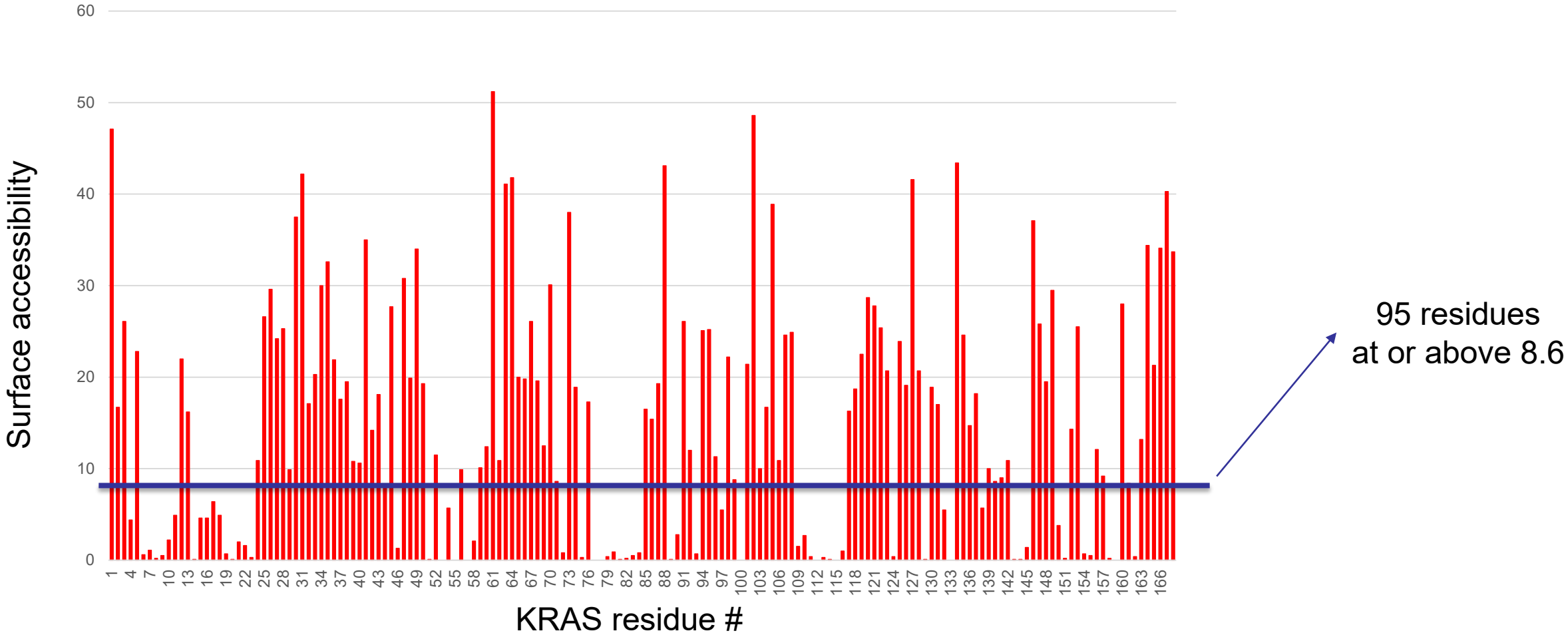
- **Parental protein: KRAS4b (1-169) G12D/C118S**
- **Utilized 2 methods for determining surface residues (Simanshu/Messing)**



Cysteine mutations in KRAS4b (1-169) G12D/C118S

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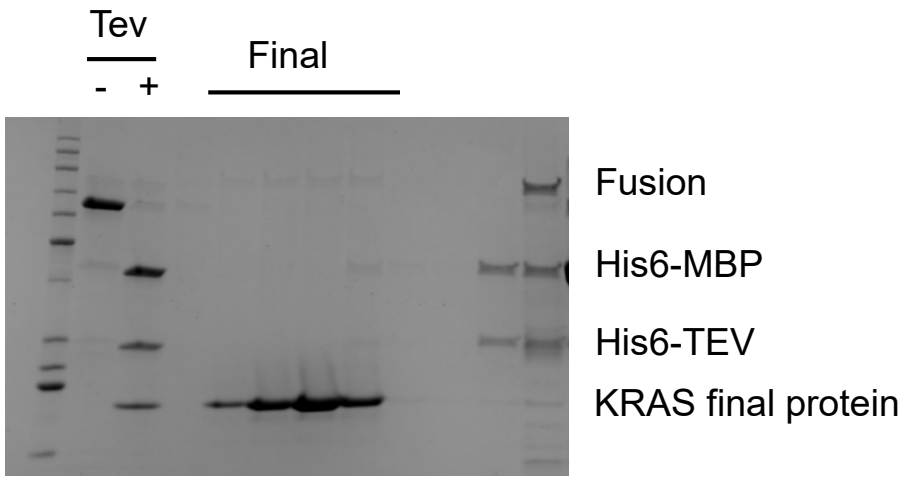
```
  1: MTEYKLVVVGADGVGKSALT
 21: IQLIQNHFVDEYDPTIEDSY
 41: RKQVVIDGETCLLDILDTAG
 61: QEEYSAMRDQYMRTGEGFLC
 81: VFAINNTKSFEDIHHYREQI
101: KRVKDSEDVPMVLVGNKSDL
121: PSRTVDTKQAQDLARSYGIP
141: FIETSAKTRQGVDDAFYTLV
161: REIRKHKEK
```

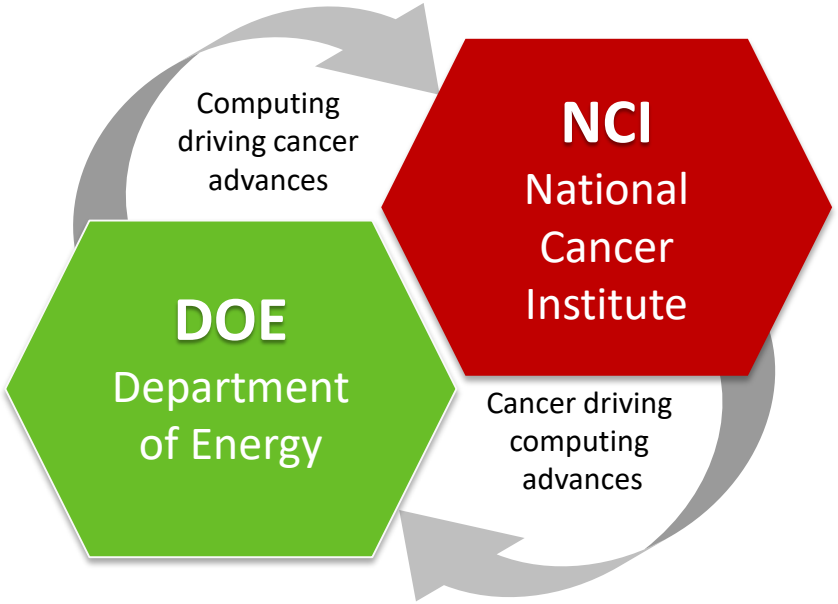
- **Synthetic library of DNA clones obtained for all 95 mutants**
- **E. coli expression carried out at small-scale for all mutants**



IMAC2

Advanced Computation to better Understand RAS Biology

DOE-NCI partnership to advance exascale development through cancer research



High Resolution KRAS:membrane Simulations

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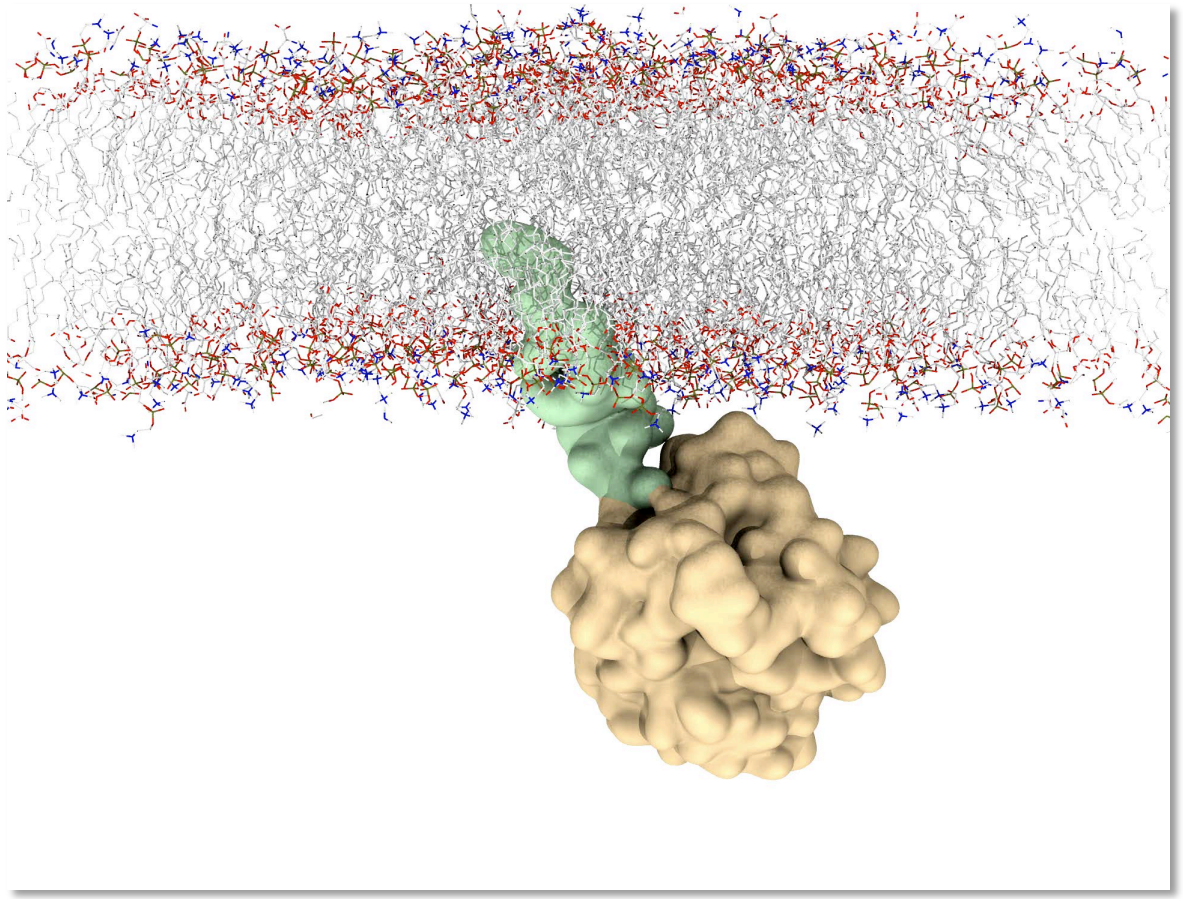

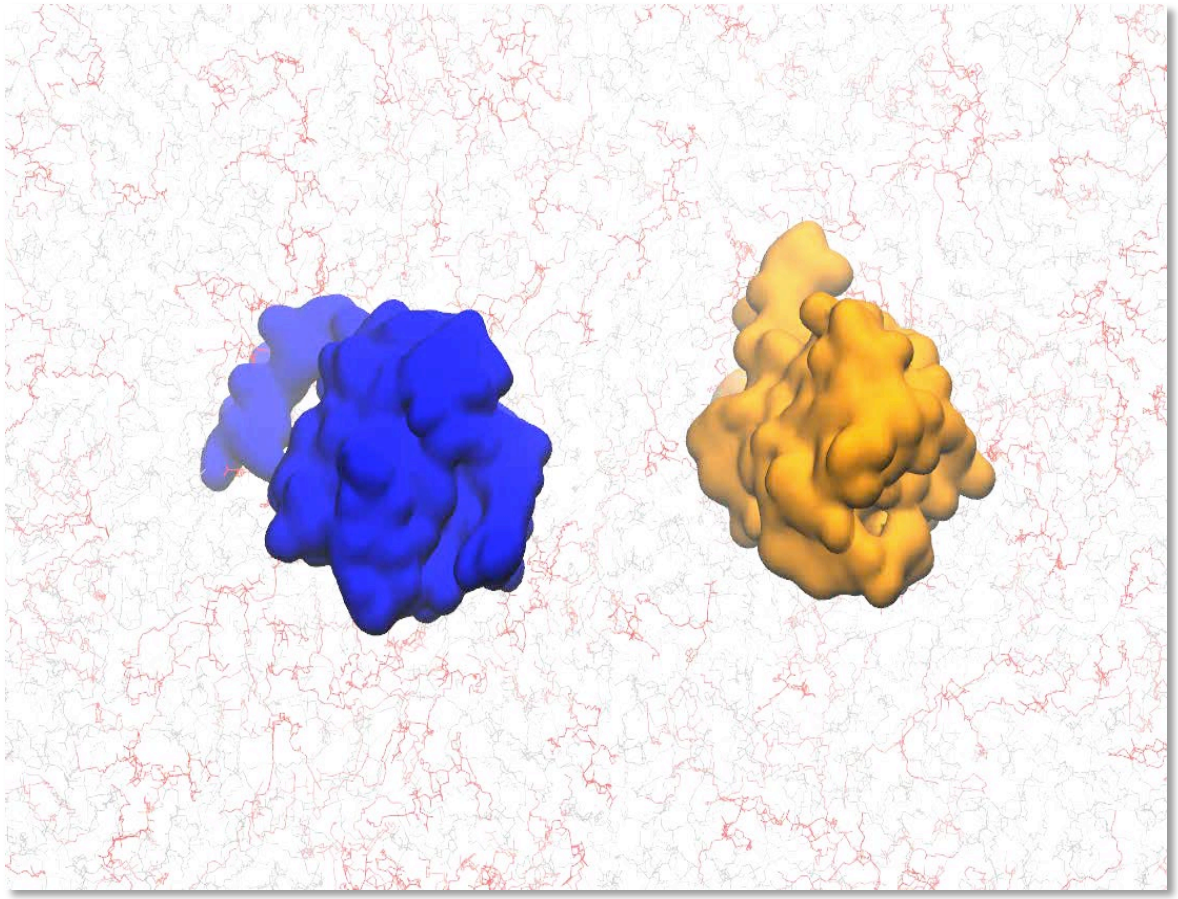

Chris Neale and Angel Garcia, LANL

Experimental investigation into KRAS membrane orientation

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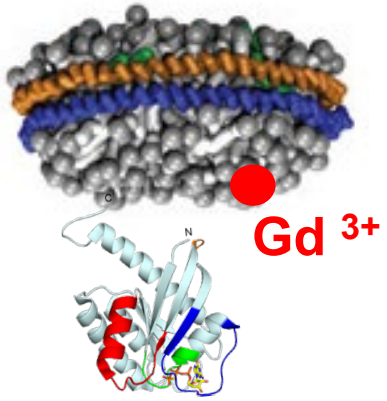
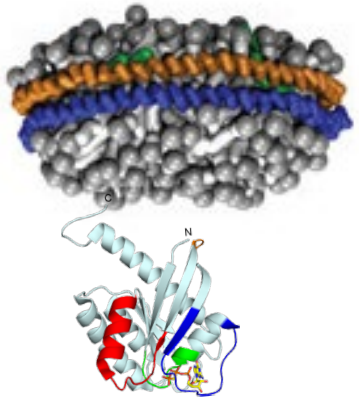
- Neutron reflectivity indicates the KRAS center of mass is ~30 Å from the lipid headgroups
- Protein foot printing identified several residues within the HVR that directly contact the membrane, but not within the G-domain
- No chemical shift perturbations are observed between free or KRAS bound to lipid nanodiscs in 2D NMR experiments.
- Paramagnetic relaxation enhancement NMR to identify membrane bound states.



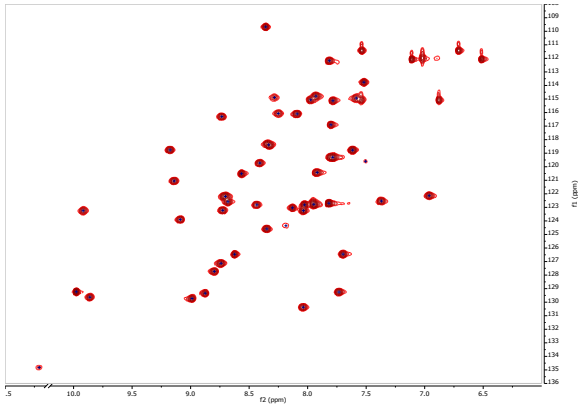
^{15}N-KRAS-FMe

and



^{15}N-KRAS-FMe

N-TROSY data



PRE-NMR identifies membrane bound states

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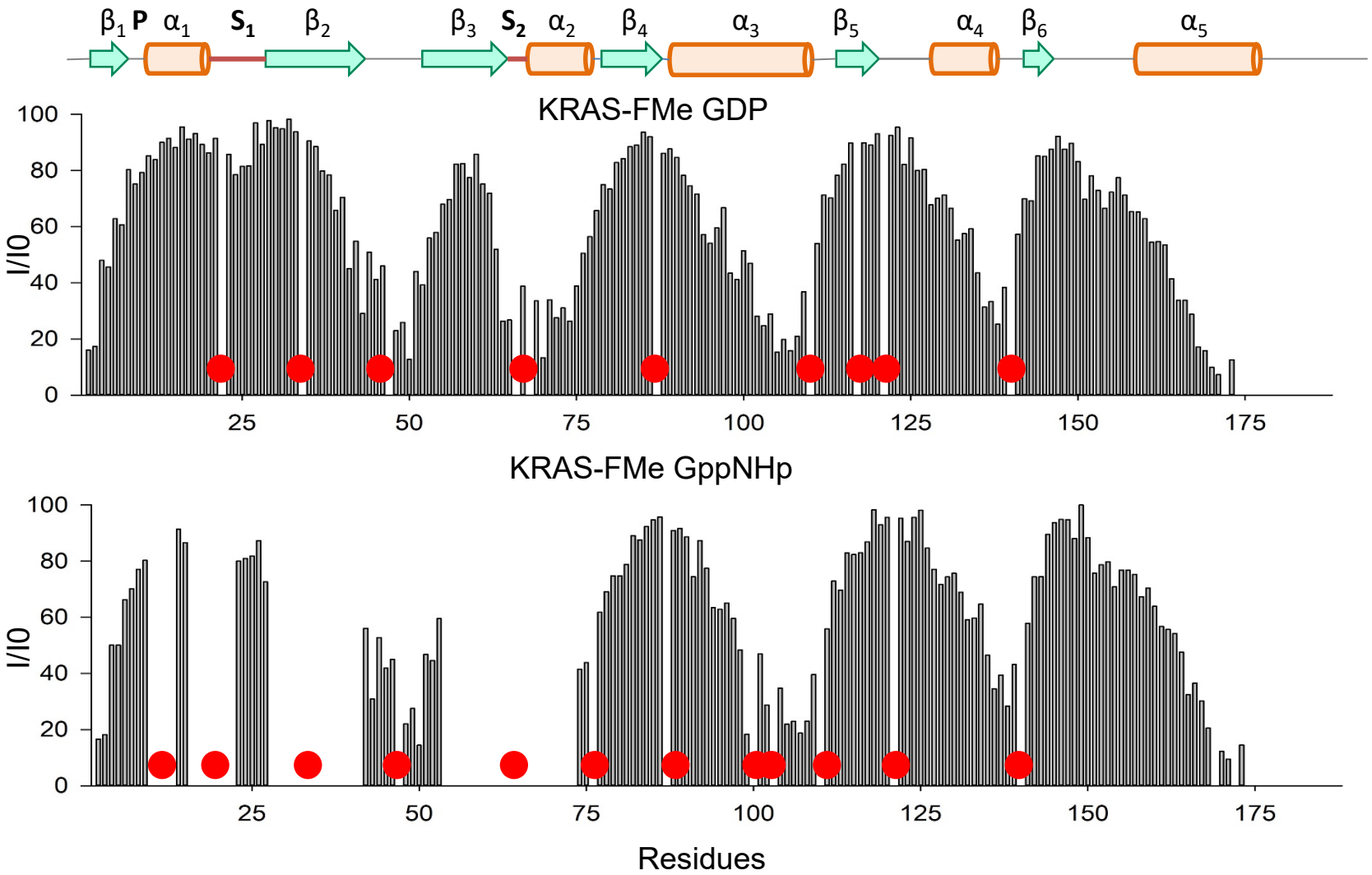
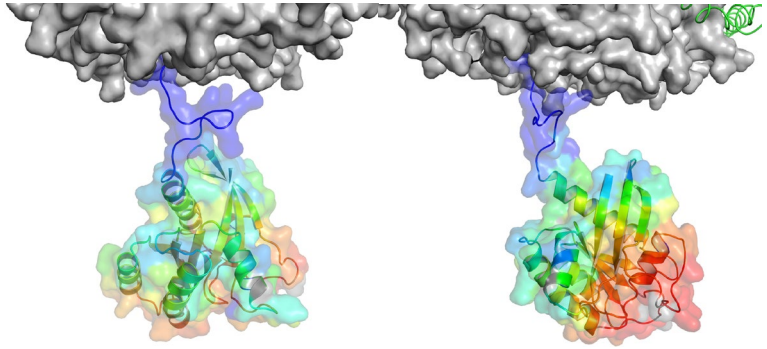
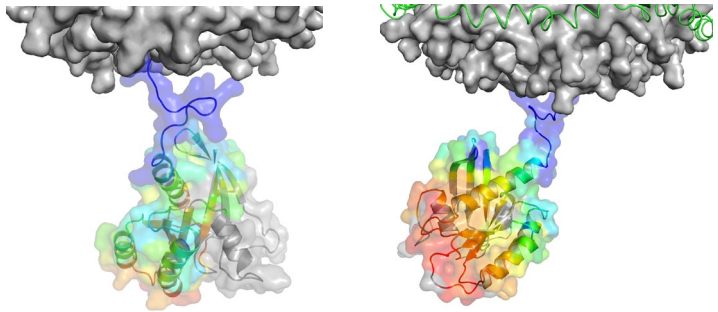


KRAS-FMe GDP



KRAS-FMe GppNHp



Que Van, FNL; Marco Tonelli, NMRFAM

Macro model captures the influence of membrane on RAS

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Experimental data drives computational modeling

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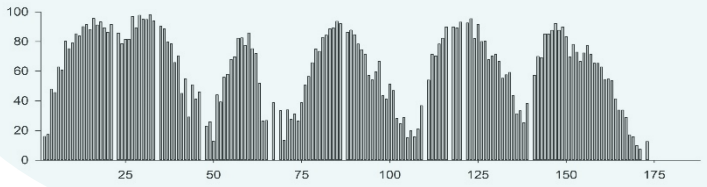
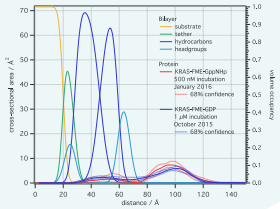
Experimental data

PRE-NMR



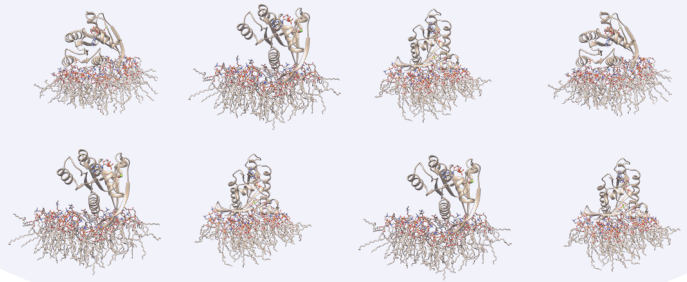
Neutron reflectivity



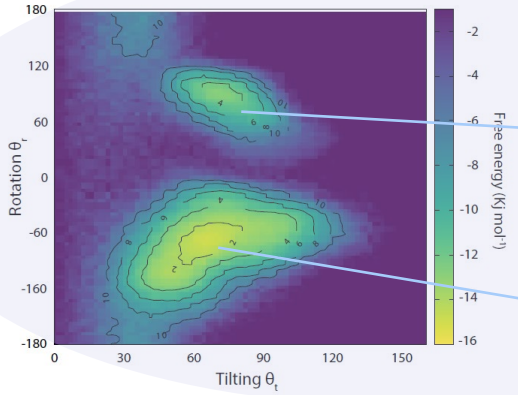
Experimental restraints drive modeling

Fitting MD models to experimental data

Haddock docking



Coarse Grained MD simulations



Focusing on Raf CRD

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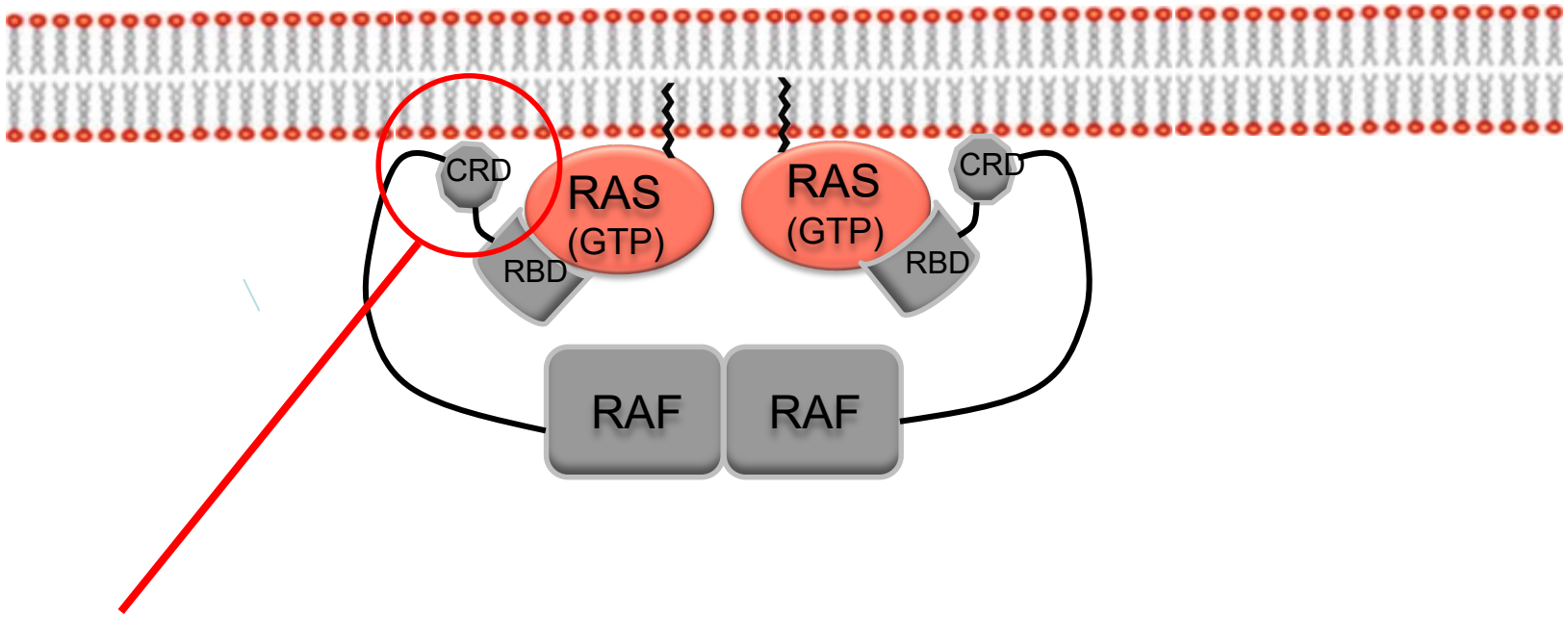

The Raf Cysteine Rich Domain: Interacts with the plasma membrane, essential for Ras-dependent Activation of Raf kinase

NMR structure of RAF1 CRD on membrane mimetic

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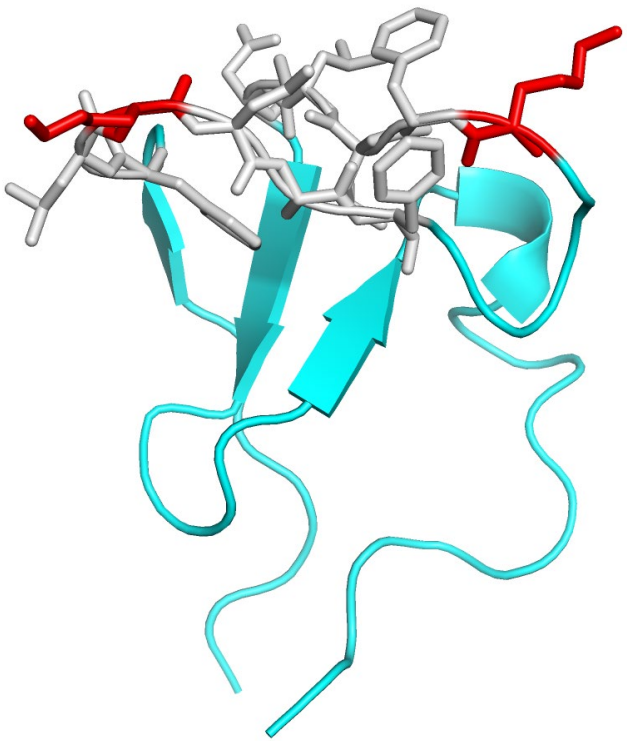
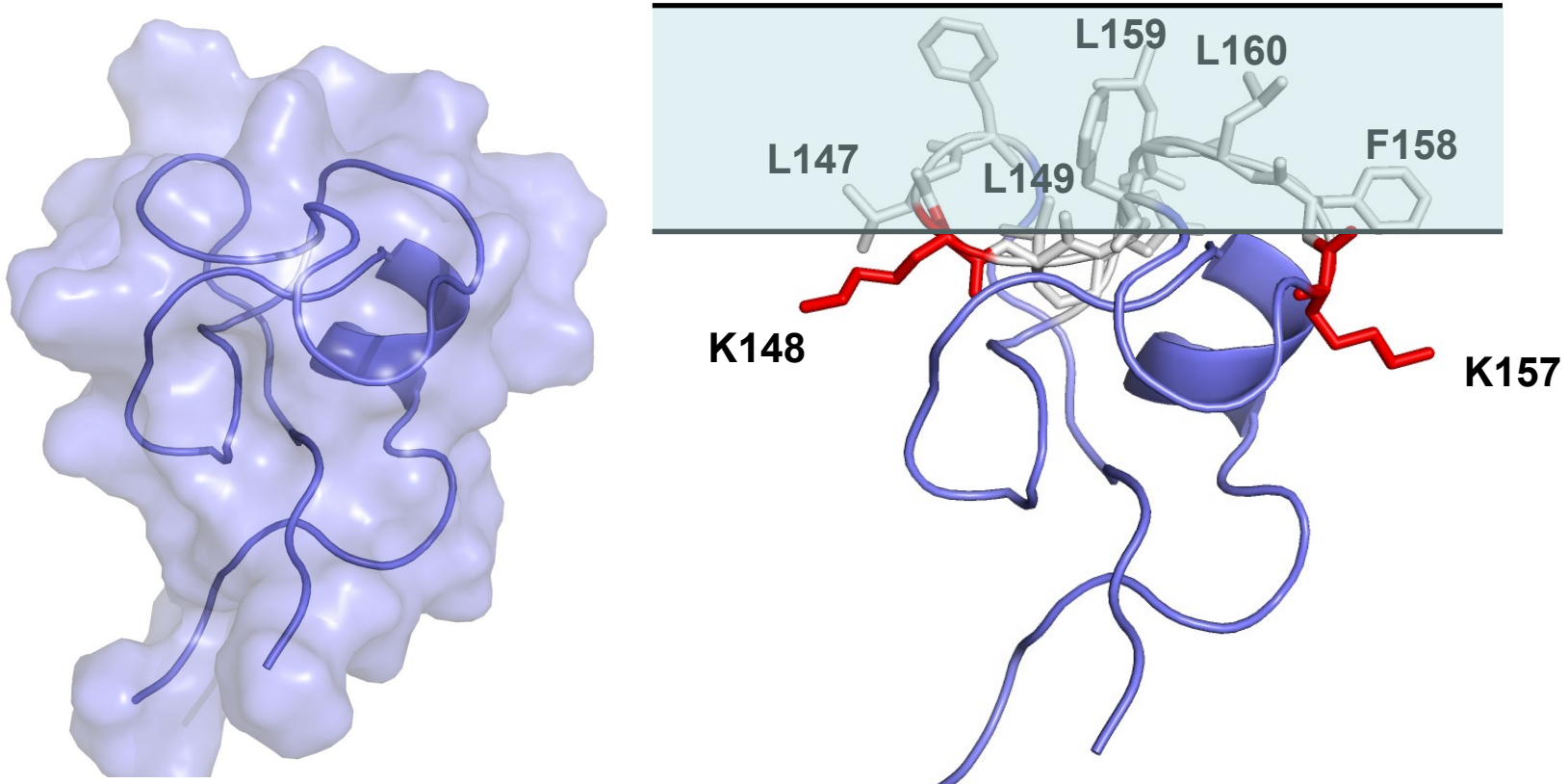


Solution RAF1-CRD
PDB:1FAR



Membrane bound RAF1-CRD

- CRD hydrophobic resides are pointed up towards the membrane (inserted)
- K148 is pointing towards the membrane
- Other basic residues are close to the membrane but are solvent exposed

Constance Agamasu
Andy Stephen

CRD residues L149, F158 and L160 deeply insert into the bilayer

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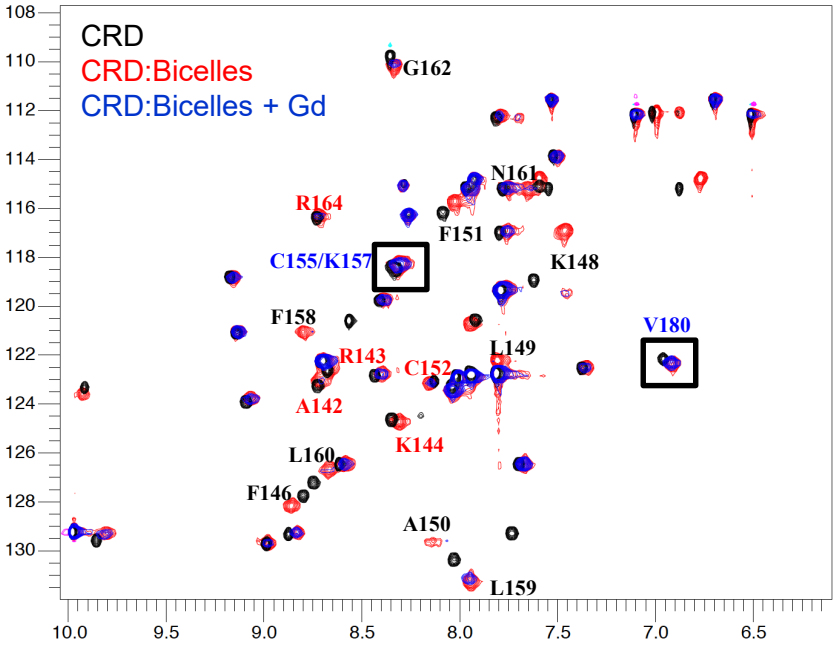
Gd^{3+}

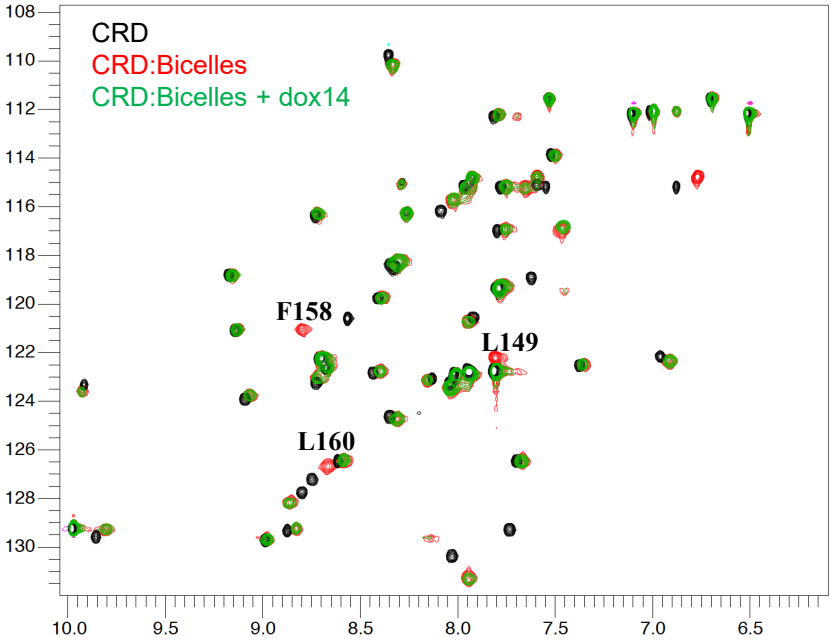
Distance from Surface = ~2 A
Distance from Center = ~18A



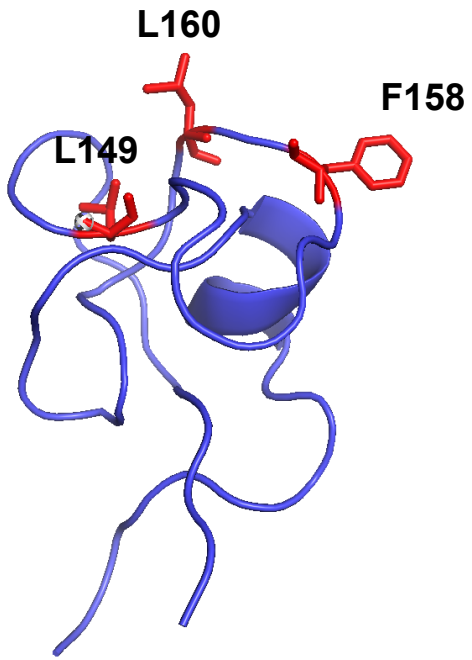
R143, K144,C152,R164- closer/weak contacts with the membrane

doxyl-14

Distance from Surface = ~18 A
Distance from Center = ~3A



CRD is inserted deeply into the membrane via L149 (loop 1) and F158 and L160 (loop2)



RBD-CRD binds tighter to membrane than CRD alone

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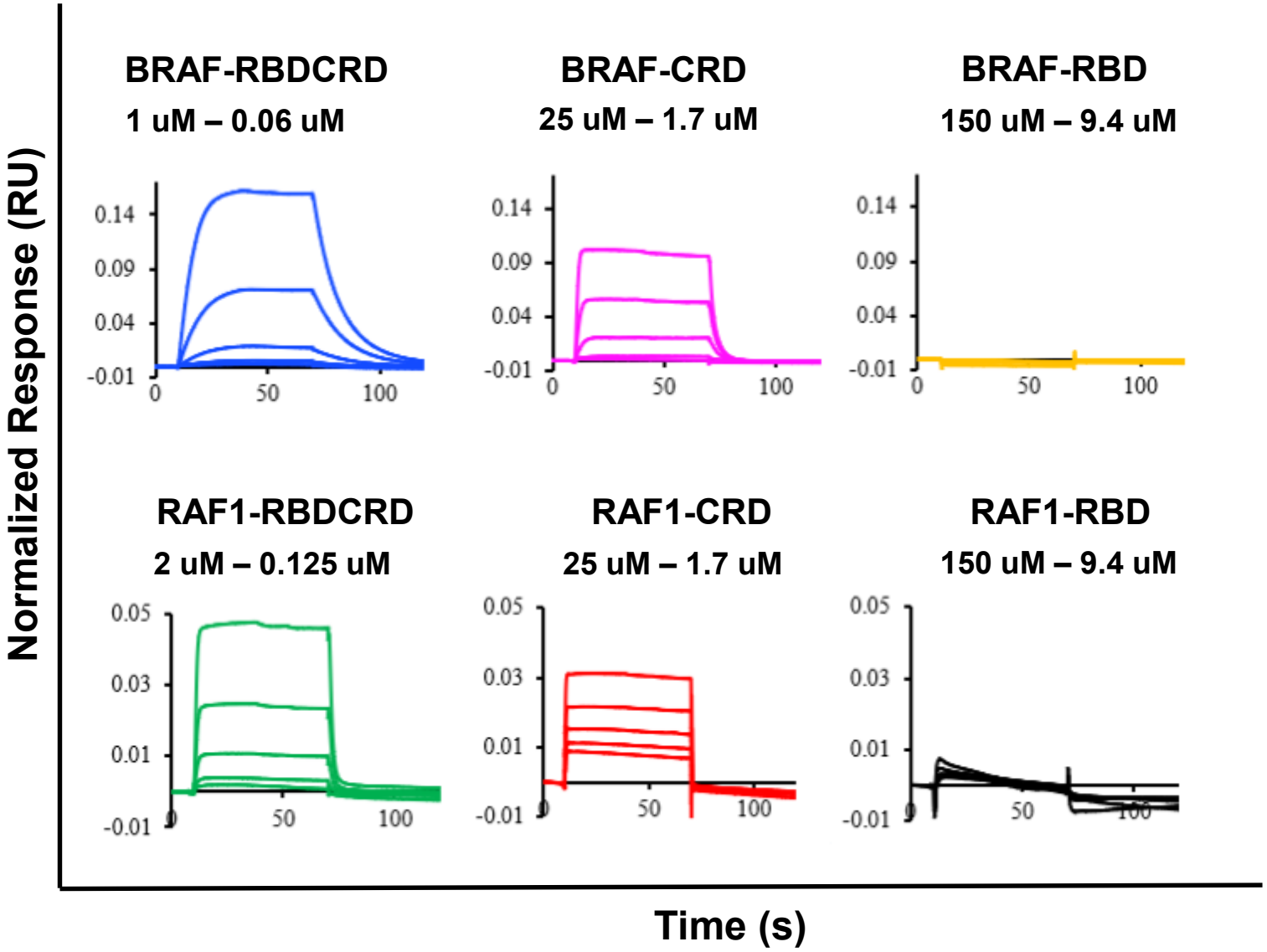

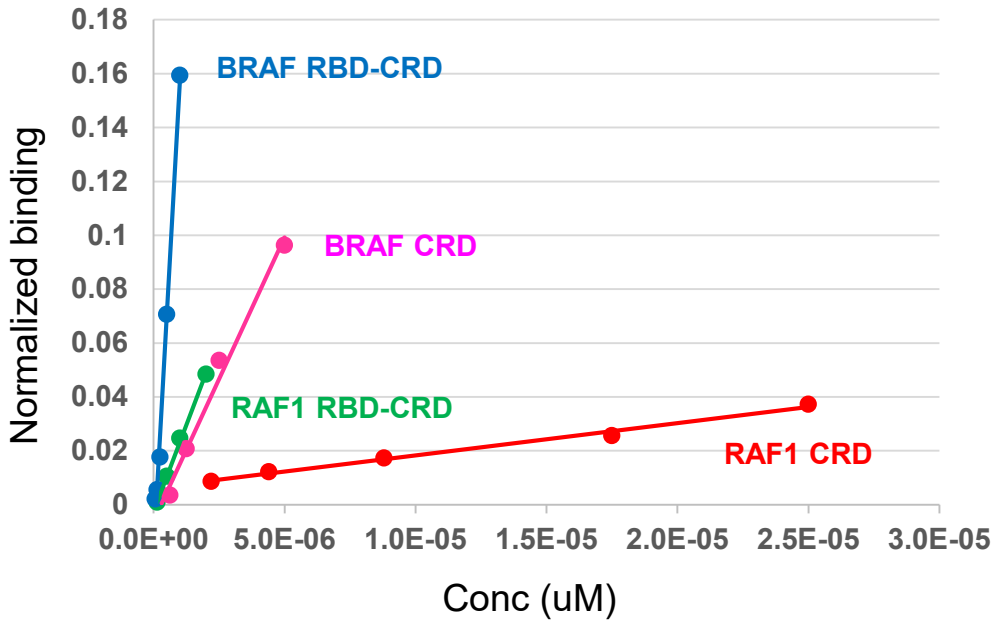

- BRAF binds better to membrane than RAF1

Summary and next steps

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- **BRAF RBD-CRD binds tighter to membrane mimetics than RAF1**
- **RBD-CRD binds tighter than CRD alone to membrane mimetics**
- **RBD-CRD inserts deeper into the membrane than CRD alone**

- **Next steps**
  - Compare NMR data with MD simulations
  - Complete structural determination of RBD-CRD bound to bicelles
  - Perform ^{13}C double filtered NOE experiments to validate residues inserted into membrane mimetics

  - ^{15}N ^{13}C labeled RBD-CRD with KRAS-FMe on bicelles
  - $^{15}N/^{13}C$ methyl labeled KRAS tethered to nanodisc + RBD-CRD
  - ^{13}C methyl labeled KRAS-FMe + MTSL spin label KRAS-FMe