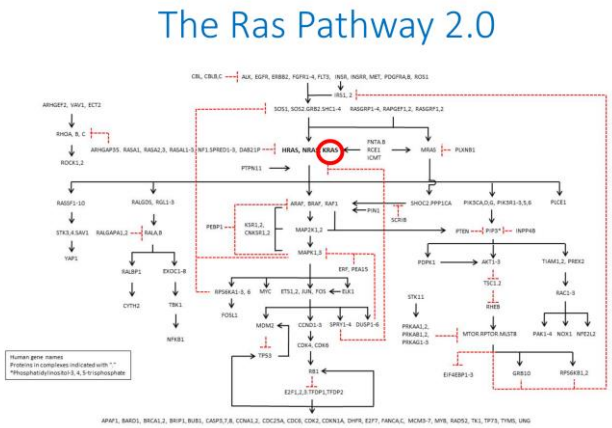
NCI Ras Initiative

- Identify compounds that bind directly to KRAS or prevent it activating its immediate effectors



- Molecular description of interaction between KRAS, RAF1 and the plasma membrane
  - Collaboration with the NCI, Dept of Energy, 4 National Labs

Uncovering a membrane-distal conformation of KRAS available to recruit RAF to the plasma membrane

Que N. Van[a], Cesar A. López[b], Marco Tonelli[c], Troy Taylor[a], Ben Niu[d,1], Christopher B. Stanley[e], Debsindhu Bhowmik[e], Timothy H. Tran[a], Peter H. Frank[a], Simon Messing[a], Patrick Alexander[a], Daniel Scott[f,2], Xiaoying Ye[a,2], Matt Drew[a], Oleg Chertov[a], Mathias Lösche[f,g,h], Arvind Ramanathan[i], Michael L. Gross[d], Nicolas W. Hengartner[b], William M. Westler[c], John L. Markley[c], Dhirendra K. Simanshu[a], Dwight V. Nissley[a], William K. Gillette[a], Dominic Esposito[a], Frank McCormick[a,3], S. Gnanakaran[b], Frank Heinrich[g,h], and Andrew G. Stephen[a,3]

[a]National Cancer Institute RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD 21702; [b]Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545; [c]National Magnetic Resonance Facility at Madison, Biochemistry Department, University of Wisconsin–Madison, Madison, WI 53706; [d]National Mass Spectrometry Resource, Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130; [e]Computational Sciences and Engineering Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; [f]Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213; [g]Department of Physics, Carnegie Mellon University, Pittsburgh, PA 15213; [h]Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD 20899; and [i]Data Science and Learning Division, Argonne National Laboratory, Lemont, IL 60439

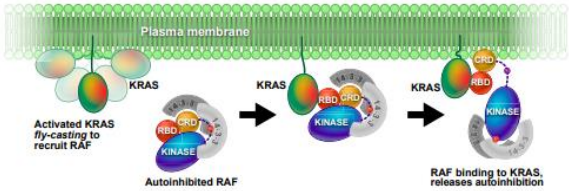


Fig. 5. Model of directional KRAS fly-casting, with the G-domain as bait for the RAF1-RBD, as a mechanism to recruit RAF to the membrane. Membrane-bound KRAS is dynamic, and both membrane-bound and membrane-tethered conformations coexist in fast dynamic exchange. Once freed from a transient, orientationally well-defined membrane-bound state, the KRAS G-domain, while firmly bound to the bilayer through its HVR, is dislodged from the surface under, at least approximate, conservation of the orientation defined by the membrane contact. This dynamic process facilitates the capture of the large effector protein which would not efficiently bind the small GTPase buried in the membrane. The autoinhibited RAF is modeled after a cryo-EM structure (10) in which the CRD is sequestered and the RBD, at the periphery of the RAF/14-3-3 complex, is available for interaction with KRAS.

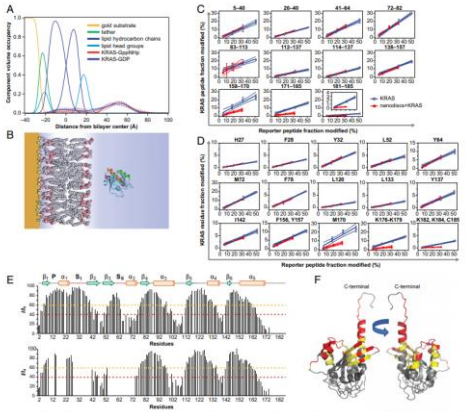


Fig. 1. NR, FPOP, and NMR data of membrane-bound KRAS. (*A*) NR-derived CVO profiles of KRAS-GDP and KRAS-GppNHp associated with 70:30 POPC:POPS stBLMs. The membrane structure shown is from the measurement of KRAS-GppNHp. The KRAS-GDP profile was scaled to match that of KRAS-GppNHp in surface coverage. (*B*) Cartoon of the stBLM-associated KRAS-GppNHp G-domain at a membrane distance in quantitative agreement with the NR results. The orientation of the rotationally symmetric G-domain cannot be determined from the NR data, and an exemplary orientation is shown. Helices α5 (orange ribbon), α4 (green ribbon), and α3 (red ribbon) are highlighted for reference. (*C*) FPOP dose–response curves for various regions of KRAS-GDP as represented by peptides from proteolysis of unbound (blue) and nanodisc-bound (red) protein. The scavenger concentration was varied to change the radical dose to the protein (or the time provided for reaction). Data for each region are plotted as the fraction modified of the representative peptide vs. the fraction modified of the reporter peptide at each histidine (scavenger) concentration. Ninety-five-percent confidence intervals for each curve are shown as shaded regions. (*D*) Residue-level FPOP response curves for nanodisc-bound and unbound KRAS as determined by MS/MS analyses of the peptides. (*E*) NMR-PRE ratios for KRAS-GDP (Top) KRAS-GppNHp (Bottom) on 70:30 POPC:POPS nanodiscs. Unassigned and excluded residues within the G-domain (2–170) are left blank, see *SI Appendix* for further information. Secondary structure elements are based on predictions from TALOS-N using backbone chemical shifts. (*F*) NMR-PRE ratios (I/I_0) from KRAS-GDP projected onto a model structure at 40% (red) and 60% (yellow) cutoffs.

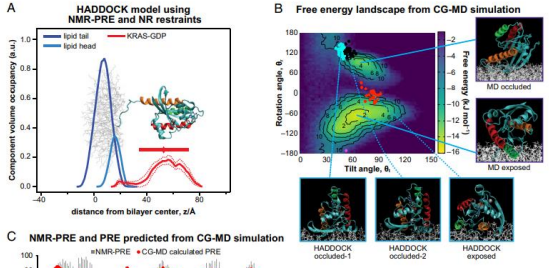


Fig. 2. Correspondence between experimental results and HADDOCK docking and CG MD simulation results of membrane-bound KRAS. (*A*) CVO profiles of the outer membrane monolayer leaflet and KRAS-GDP derived from HADDOCK with restraints and averaged over the 200 final structures. A representative structure is superimposed. A set of restraints favored G-domain orientations consistent with the NMR-PRE results and another, using the R_G (radius of gyration) feature in HADDOCK, approximated the distance of the G-domain from the membrane as determined by NR (indicated by the horizontal line with symbol for peak width and maximum, respectively). (*B*) Free energy landscape for membrane-bound KRAS that recapitulates the dynamics on a 70:30 POPC:POPS membrane obtained from MARTINI CG MD simulations. The tilt and rotation angles of KRAS with respect to the membrane (16) are based on 10 independent simulation replicas with a combined length of 1 ms. HADDOCK results (using only NMR-PRE restraints) in the same coordinate system are overlaid as red, black, magenta, and cyan dots. Insets here show averaged conformations for HADDOCK and CG MD-derived structures. α Helices are colored as described in the Fig. 1 legend. (*C*) Bar plot of measured NMR-PRE ratios for KRAS, overlaid with predictions (red dots) for the PRE effect from CG MD simulations for a mixture of 45% occluded and 55% exposed states and an offset distance of 0.45 nm.

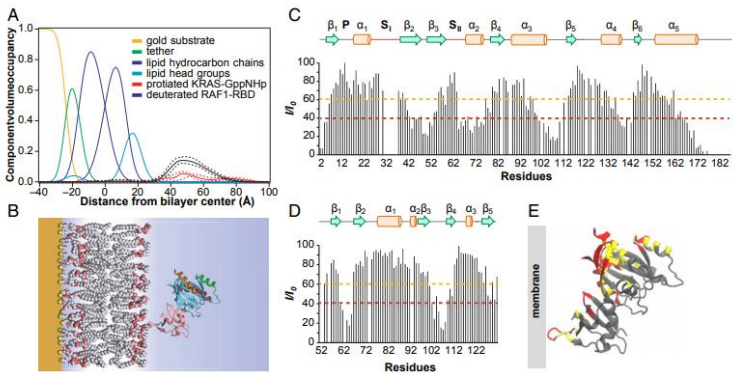


Fig. 3. NR and NMR data orient KRAS-GppNHp/RAF1-RBD on a membrane. (*A*) NR-derived CVO profiles of the KRAS-GppNHp/RAF1-RBD complex bound to an stBLM composed of 70:30 POPC:POPS. (*B*) Cartoon of the stBLM and the protein obtained by rigid-body modeling with the complex crystal structure (PDB 6VJJ). (*C* and *D*) NMR-PRE intensity ratios of ^{15}N-KRAS/RAF1-RBD and KRAS/^{15}N-RAF1-RBD in the presence of Gd^{3+}-labeled POPC:POPS nanodiscs, respectively. Unassigned and excluded residues within the G-domain (2–170) and RBD are left blank, see *SI Appendix* for further information. (*E*) NMR-PREs mapped onto the fitted structure in *B* using 40% (red) and 60% (yellow) cutoffs.

ARTICLE *Biophysics* *Computational Biology*

Machine Learning-driven Multiscale Modeling Reveals Lipid-Dependent Dynamics of RAS Signaling Proteins

Helgi Ingolfsson, Chris Neale, Timothy Carpenter, Rebika Shrestha, Cesar Lopez, Timothy Tran, Tomas Oppelstrup, Harsh Bhatia, Liam Stanton, Xiaohua Zhang, Shiv Sundram, Francesco Di Natale, Animesh Agarwal, Gautham Dharuman, Sara Kokkila Schumacher, Thomas Turbyville, Gulcin Gulten, Que Van, Debanjan Goswami, Frantz Jean-Francios, Constance Agamasu, De Chen, Jeevapani Hettige, Timothy Travers, Sumantra Sarkar, Michael Surh, Yue Yang, Adam Moody, Shusen Liu, Brian Van Essen, Arthur Voter, Arvind Ramanathan, Nicolas Hengartner, Dhirendra Simanshu, Andrew Stephen, Peer-Timo Bremer, S Gnanakaran, James Glosli, Felice Lightstone, Frank McCormick, Dwight Nissley, Frederick Streitz

NCI RAS Initiative - Resources and interactions

Frederick National Laboratory for Cancer Research

Reagents

552 Universities and NPOs, 42 states, 42 countries, 6 continents
10,067 plasmids & vectors (through Addgene)

- 1946 individual RAS and RAS pathway plasmids
- At least 1 individual request for each of the 180 genes
- 19 complete RAS pathway kits (360 plasmids each)
- 21 complete RAS mutant kits (61 plasmids each)

820 cell lines
RAS-dependent MEFs licensed to 9 companies
KRAS-FMe materials licensed to 6 companies

Collaborations

27 Academic Institutions
11 Strategic (National Labs, Advocacy, NIH)
10 Industry

Community Outreach and Engagement

2 RAS Symposia (3rd in 2021)
8 RAS Community Workshops
4 AACR/NCI Special Sessions Presentations
Participation in NCI Ras Synthetic Lethality Network
40 publications

cCRADAs

16 cCRADAs - universities, biotechs and pharma
$11.1 M in funds to FNLCR

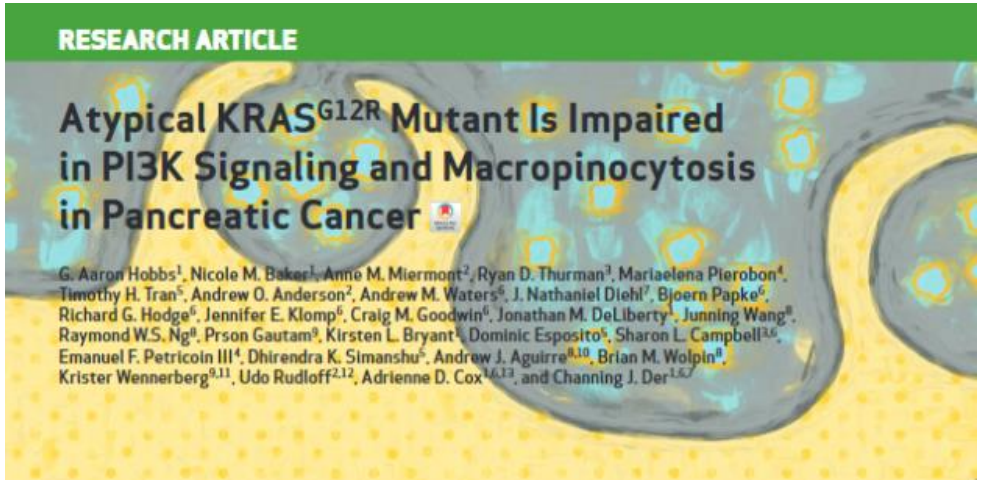
- 51 FTE years funded
- 28 in-kind FTE years from collaborator

Recent Publications

Frederick National Laboratory for Cancer Research

The small molecule BI-2852 induces a nonfunctional dimer of KRAS

Timothy H. Tran[a], Patrick Alexander[a], Srisathiyanarayanan Dharmaiah[a], Constance Agamasu[a], Dwight V. Nissley[a], Frank McCormick[a,b], Dominic Esposito[a], Dhirendra K. Simanshu[a], Andrew G. Stephen[a], and Trent E. Balius[a,1]



CANCER RESEARCH | METABOLISM AND CHEMICAL BIOLOGY

Undermining Glutaminolysis Bolsters Chemotherapy While NRF2 Promotes Chemoresistance in KRAS-Driven Pancreatic Cancers

Suman Mukhopadhyay[1,2], Debanjan Goswami[1,2], Pavan P. Adiseshaiah[2], William Burgan[1,2], Ming Yi[1,2], Theresa M. Guerin[3], Serguei V. Kozlov[3], Dwight V. Nissley[1,2], and Frank McCormick[1,2,4]

Cell Reports

CellPress OPEN ACCESS

Article

Structural Insights into the SPRED1-Neurofibromin-KRAS Complex and Disruption of SPRED1-Neurofibromin Interaction by Oncogenic EGFR

Wupeng Yan,[1,4] Evan Markegard,[2,4] Srisathiyanarayanan Dharmaiah,[1] Anatoly Urisman,[2] Matthew Drew,[1] Dominic Esposito,[1] Klaus Scheffzek,[3] Dwight V. Nissley,[1] Frank McCormick,[1,2,*] and Dhirendra K. Simanshu[1,5,*]
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[4]These authors contributed equally
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https://doi.org/10.1016/j.celrep.2020.107909

SCIENTIFIC REPORTS nature research

eLIFE elifesciences.org

RESEARCH ARTICLE

ssGSEA score-based Ras dependency indexes derived from gene expression data reveal potential Ras addiction mechanisms with possible clinical implications

Ming Yi[1✉], Dwight V. Nissley[1], Frank McCormick[1,2] & Robert M. Stephens[1✉]

Membrane interactions of the globular domain and the hypervariable region of KRAS4b define its unique diffusion behavior

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

[1]NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, United States; [2]Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, United States; [3]UCSF Helen Diller Family Comprehensive Cancer Center, School of Medicine, University of California, San Francisco, San Francisco, United States

KRAS G13D sensitivity to neurofibromin-mediated GTP hydrolysis

Dana Rabara[a,1], Timothy H. Tran[a,1], Srisathiyanarayanan Dharmaiah[a], Robert M. Stephens[a], Frank McCormick[a,b,2], Dhirendra K. Simanshu[a,2], and Matthew Holderfield[a,2,3]

[a]NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD 21701; and [b]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94158

NCI RAS Initiative – cCRADAs

Frederick National Laboratory for Cancer Research

- Beatson Institute
- Daiichi-Sankyo
- Eli Lilly and Company
- Evotec
- Genomic Institute of the Novartis Research Foundation
- KyRas Therapueutics
- Northeastern University
- Pharma Arava
- Sanofi
- TheRas, Inc
- TOSK, Inc.
- University of California at San Francisco
- University of Maryland Baltimore
- Weizmann Institute

CANCER RESEARCH UK | BEATSON INSTITUTE

Lilly

evotec

NOVARTIS

Daiichi-Sankyo

Northeastern University

Arava

KYRAS THERAPEUTICS

SANOFI

TheRas a bridgebio company

TOSK™

מכון ויצמן למדע WEIZMANN INSTITUTE OF SCIENCE

UNIVERSITY of MARYLAND BALTIMORE

UCSF University of California San Francisco

cCRADA with TheRas

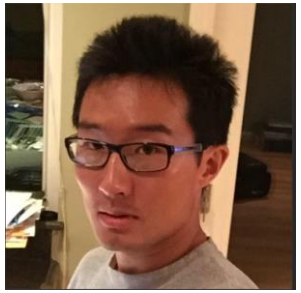
Frederick National Laboratory for Cancer Research

Anna Maciag

David Turner

Dhirendra Simanshu

Eli Wallace

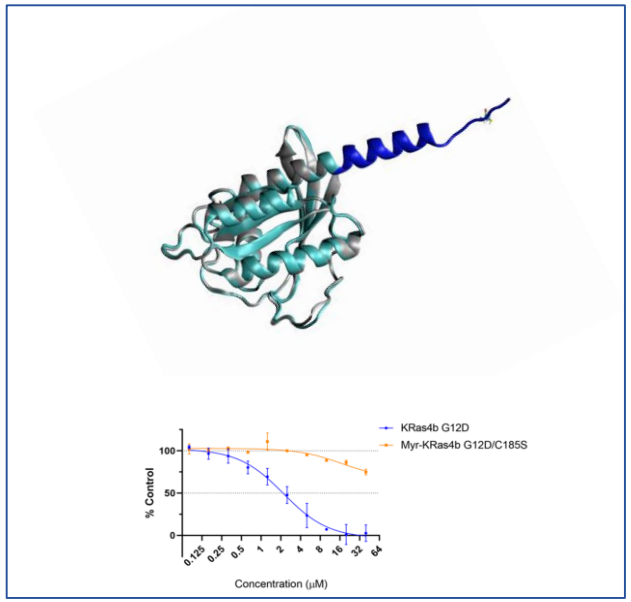


Yue Yang

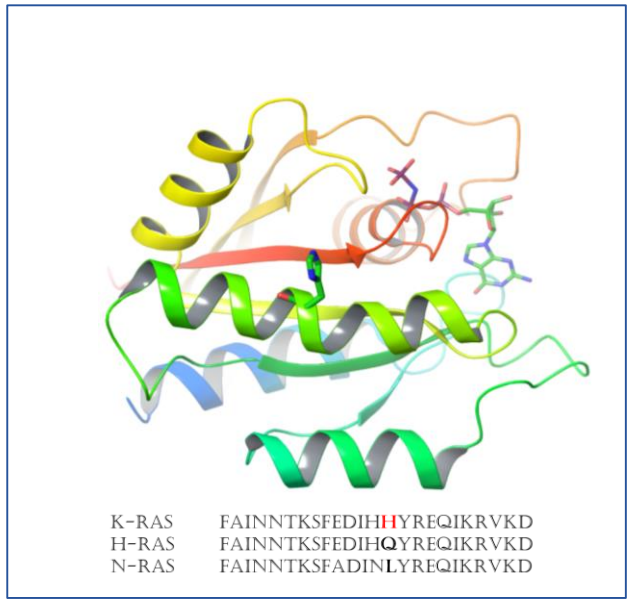
Felice Lightstone

Julian Downward

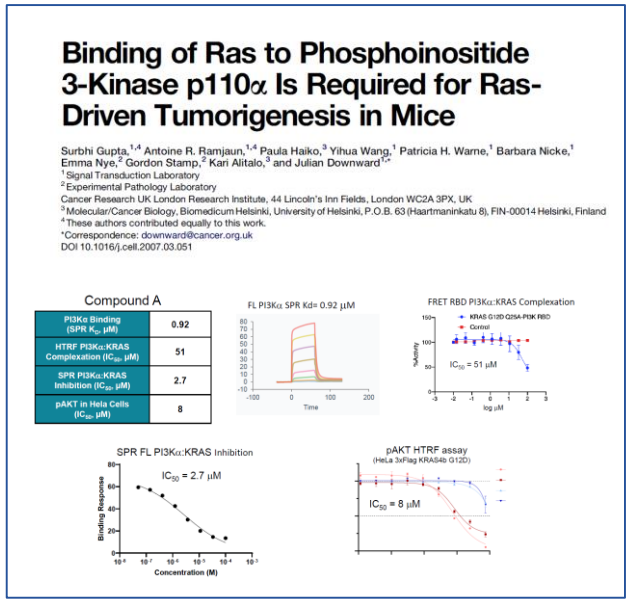
Preventing KRAS4B processing by blocking C185



Targeting H95: unique residue on KRAS



Blocking activation of PI3 kinase by KRAS



Binding of Ras to Phosphoinositide 3-Kinase p110α Is Required for Ras-Driven Tumorigenesis in Mice

Surbhi Gupta,[1,4] Antoine R. Ramjaun,[1,4] Paula Haiko,[3] Yihua Wang,[1] Patricia H. Warne,[1] Barbara Nicke,[1] Emma Nye,[2] Gordon Stamp,[2] Kari Alitalo,[3] and Julian Downward[1,*]
[1] Signal Transduction Laboratory
[2] Experimental Pathology Laboratory
Cancer Research UK London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, UK
[3] Molecular/Cancer Biology, Biomedicum Helsinki, University of Helsinki, P.O.B. 63 (Haartmaninkatu 8), FIN-00014 Helsinki, Finland
[4] These authors contributed equally to this work.
*Correspondence: downward@cancer.org.uk
DOI 10.1016/j.cell.2007.03.051

Compound A

PI3Kα Binding (SPR K_D, μM)	0.92
HTRF PI3Kα:KRAS Complexation (IC_{50}, μM)	51
SPR PI3Kα:KRAS Inhibition (IC_{50}, μM)	2.7
pAKT in Hela Cells (IC_{50}, μM)	8

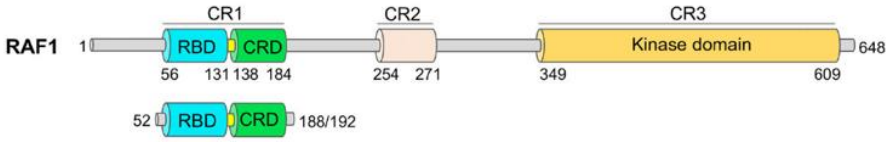
FL PI3Kα SPR Kd= 0.92 μM

FRET RBD PI3Kα:KRAS Complexation

SPR FL PI3Kα:KRAS Inhibition

pAKT HTRF assay

Targeting Ras-dependent Raf activation



MOLECULAR AND CELLULAR BIOLOGY, Nov. 1998, p. 6698–6710 Vol. 18, No. 11
0270-7306/98/$04.00+0

The RafC1 Cysteine-Rich Domain Contains Multiple Distinct Regulatory Epitopes Which Control Ras-Dependent Raf Activation

MARTINA DAUB,[1] JOHANNES JÖCKEL,[1] THOMAS QUACK,[1] CHRISTOPH K. WEBER,[2] FRANK SCHMITZ,[1] ULF R. RAPP,[2] ALFRED WITTINGHOFER,[1] AND CHRISTOPH BLOCK[1]*

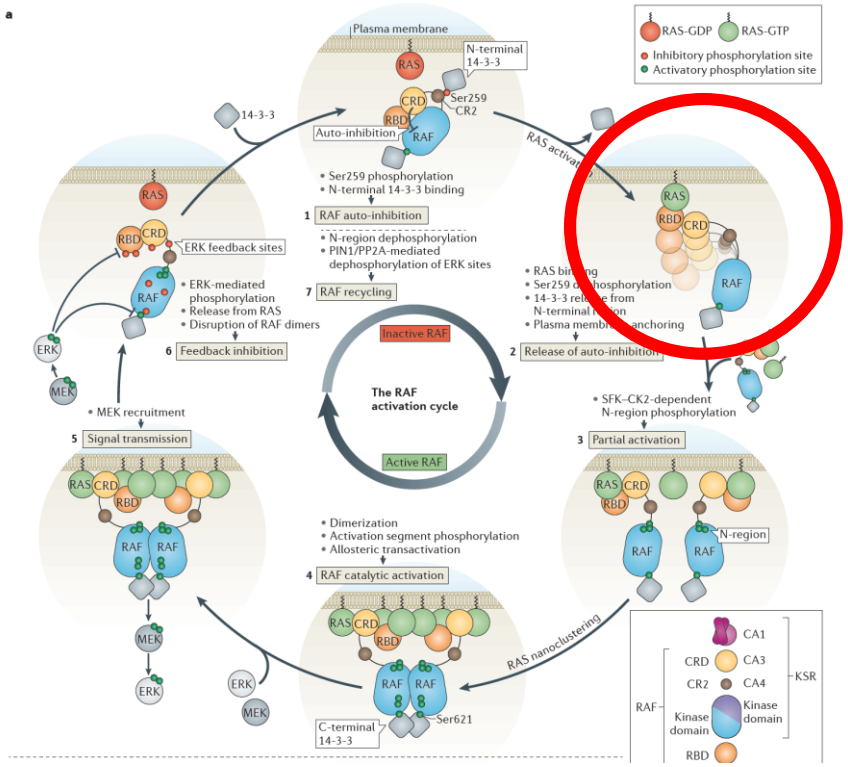
THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 273, No. 34, Issue of August 21, pp. 21578–21584, 1998
Printed in U.S.A.

Identification of Residues in the Cysteine-rich Domain of Raf-1 That Control Ras Binding and Raf-1 Activity*

(Received for publication, March 20, 1998, and in revised form, June 11, 1998)

David G. Winkler‡§, Richard E. Cutler, Jr.¶||, Jonelle K. Drugan‡‡§§, Sharon Campbell‡‡§§, Deborah K. Morrison¶**, and Jonathan A. Cooper‡¶¶**

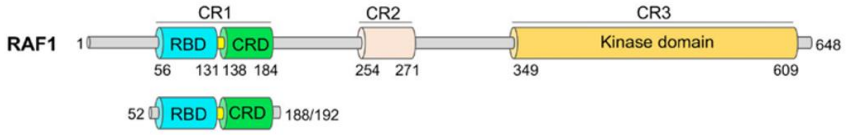
Mutation K144A R164A or K144A L160A did not interfere with Ras binding significantly but did inhibit Raf activation,

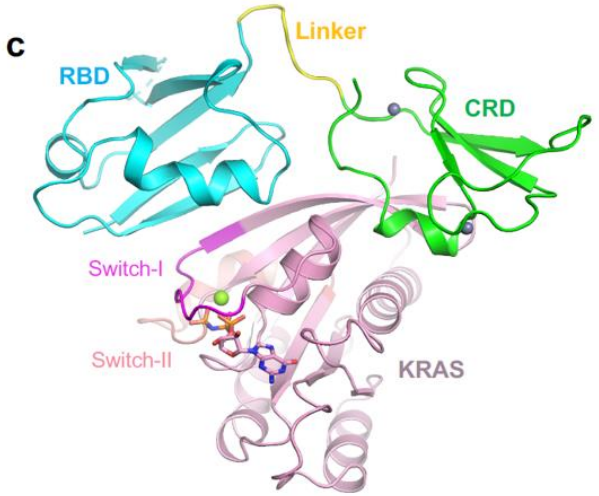


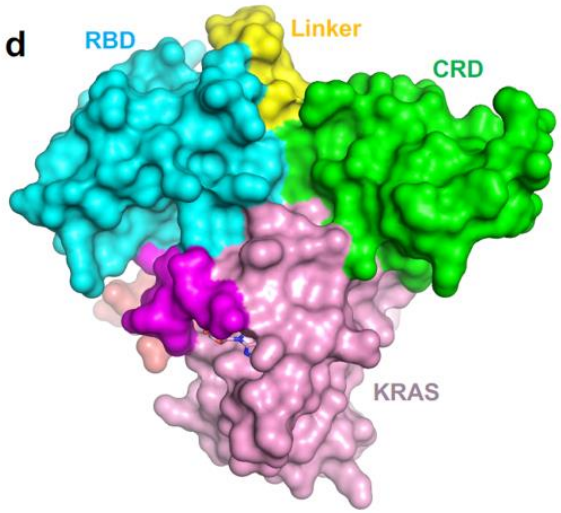
Regulation of RAF protein kinases in ERK signalling

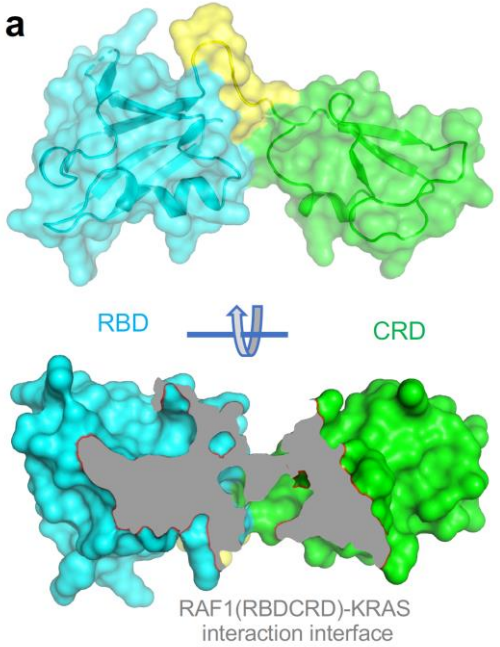
Hugo Lavoie[1] and Marc Therrien[1,2]

First crystal structure of the Raf1 RBD-CRD domain









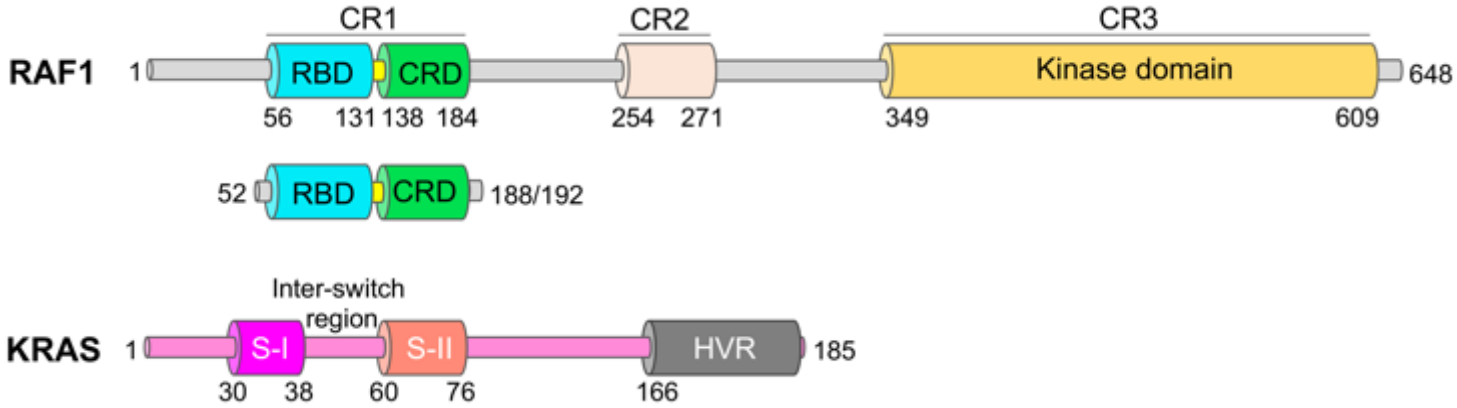
KRAS interaction with RAF1 RAS-binding domain and cysteine-rich domain provides insights into RAS-mediated RAF activation

Timothy H. Tran, Albert H. Chan, Lucy C. Young, Lakshman Bindu, Chris Neale, Simon Messing, Srisathiyanarayanan Dharmaiah, Troy Taylor, John-Paul Denson, Dominic Esposito, Dwight V. Nissley, Andrew G. Stephen, Frank McCormick, Dhirendra K. Simanshu

Simanshu

Tim Tran

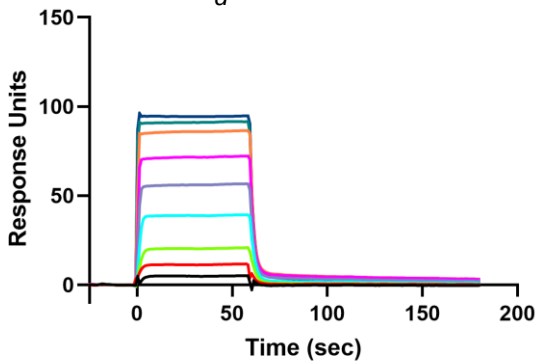
KRAS-RAF1(RBDCRD) complex and SPR analysis



SPR sensorgrams showing the binding of RAF1(RBD) and RAF1(RBDCRD) to KRAS

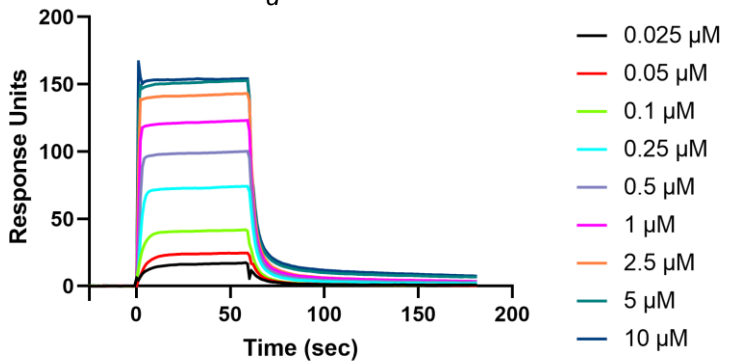
RAF1(RBD)

K_d = 371 ± 92 nM

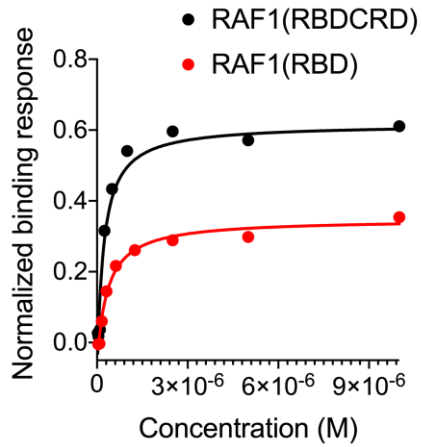


RAF1(RBDCRD)

K_d = 152 ± 45 nM



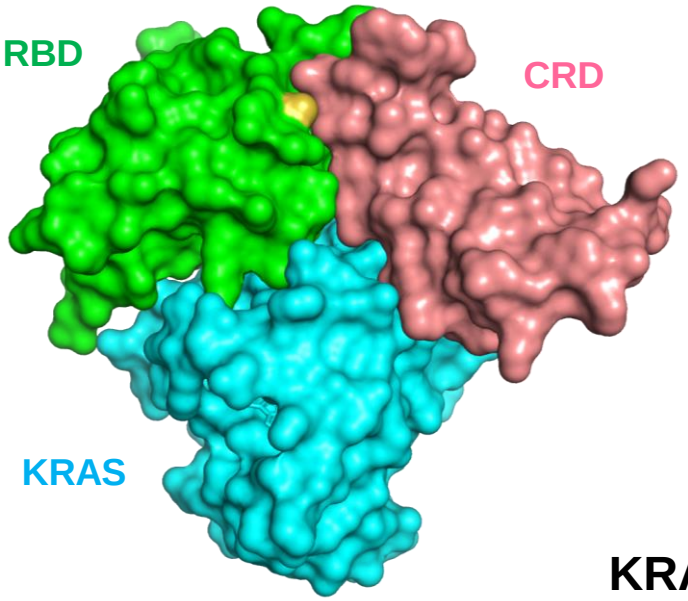
Steady-state binding isotherms derived from the SPR data



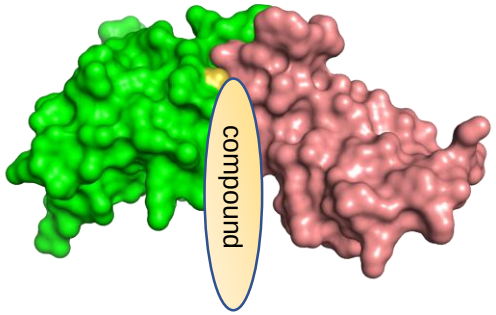
L. Bindu, Andy Stephen

Developing RAF1(RBD-CRD) inhibitors

Frederick National Laboratory for Cancer Research

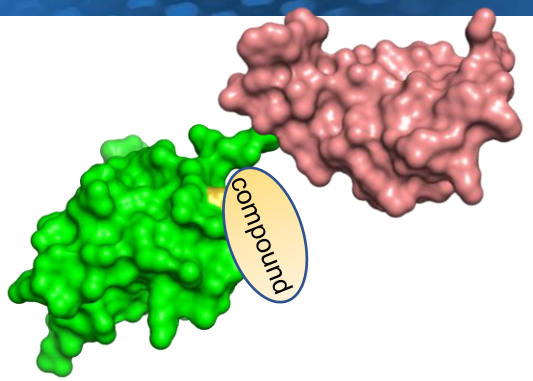


RBD-CRD binders



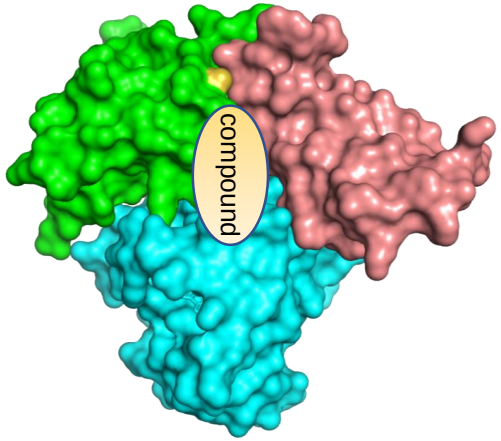
Compound clashes with KRAS

OR



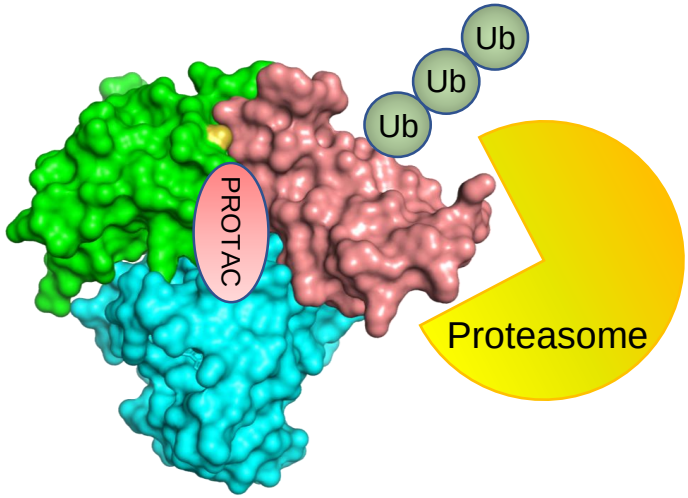
Compound alters RBD–CRD interaction or domain orientation

KRAS:RBD-CRD complex binders



Compound binds to the KRAS-RAF1 complex

Convert to PROTAC



KRAS-RAF1 complex tagged for degradation by proteasome

Sanofi cCRADA : Targeting KRAS-RAF1(RBDCRD) interaction

Frederick National Laboratory for Cancer Research

CRADA executed in April, 2020

- **Screening strategies**:
  - 19F NMR-fragment screening using Sanofi fragment library
  - In silico screening using Sanofi small-molecule library

  Screening completed.
  Compound synthesis/ hit validation.

  - DNA encoded library screening (covalent/non-covalent)
  - HTS screen using Sanofi small-molecule library
  - MTS SEC based Affinity Selection Mass Spectrometry screen

  Assay optimization.

FNL Team: D. Simanshu, Dom Esposito, Andy Stephen, Lick Lai, Maria Abreu Blanco

Model showing the crystal structure of KRAS-RBDCRD at the membrane

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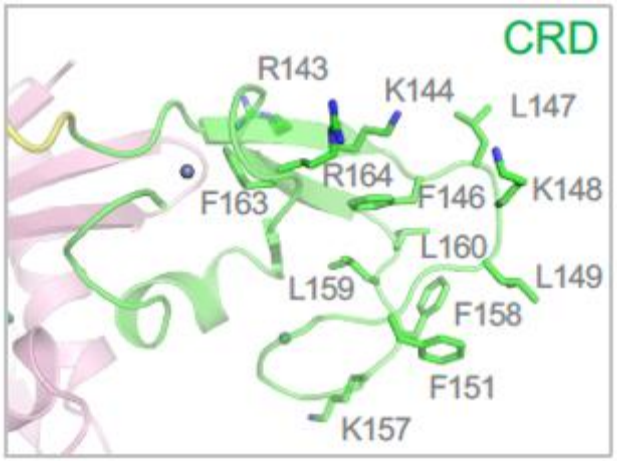
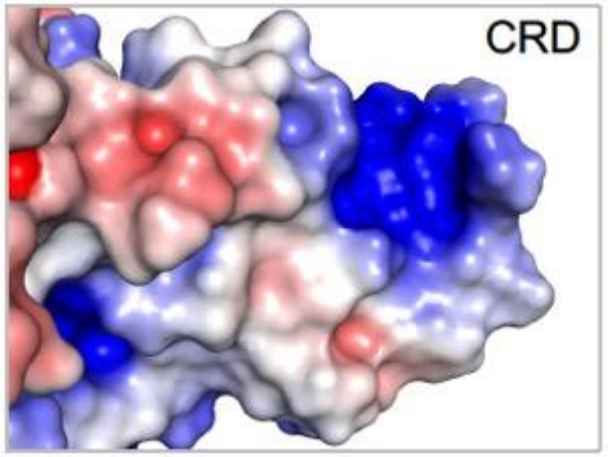
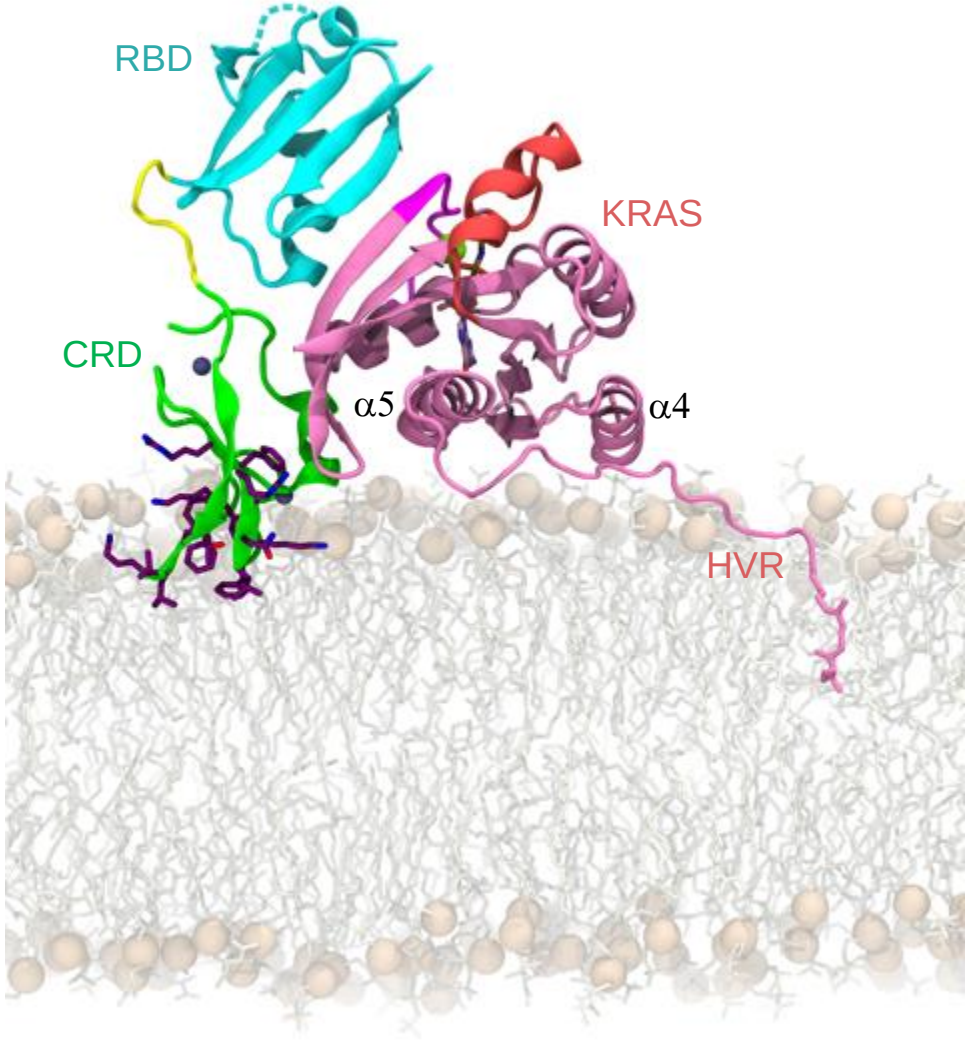
Membrane interacting residues in CRD







Chris Neale, LLNL

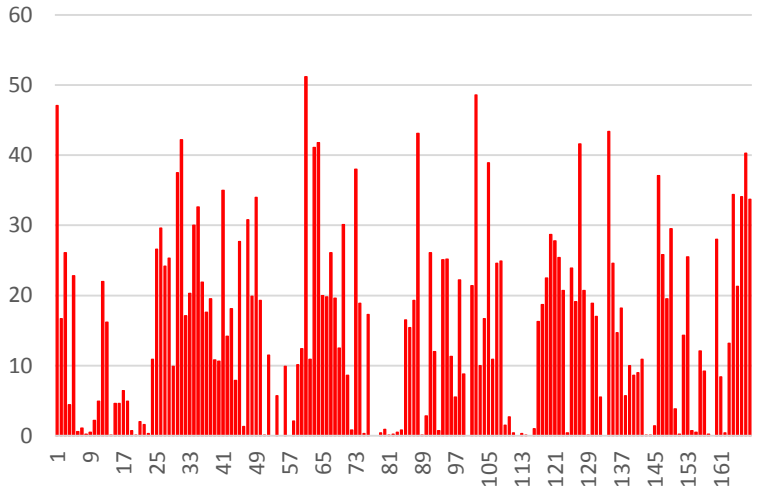
KRAS cysteine surface mutagenesis library

Frederick National Laboratory for Cancer Research

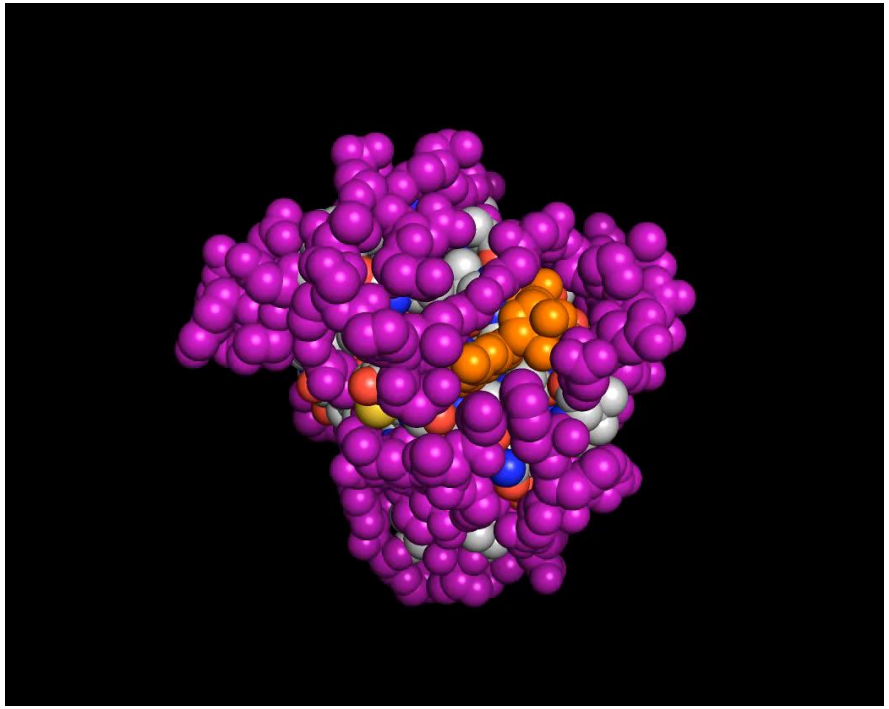
Dom Esposito

MTEYKLVVVGADGVGKSALTIQLIQNHFVDEYDP
TIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMR
DQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
VKDSEDVPMVLVGNKSDLPSRTVDTKQAQDLARS
YGIPFIETSAKTRQGVDDAFYTLVREIRKHKEK

Surface accessibility

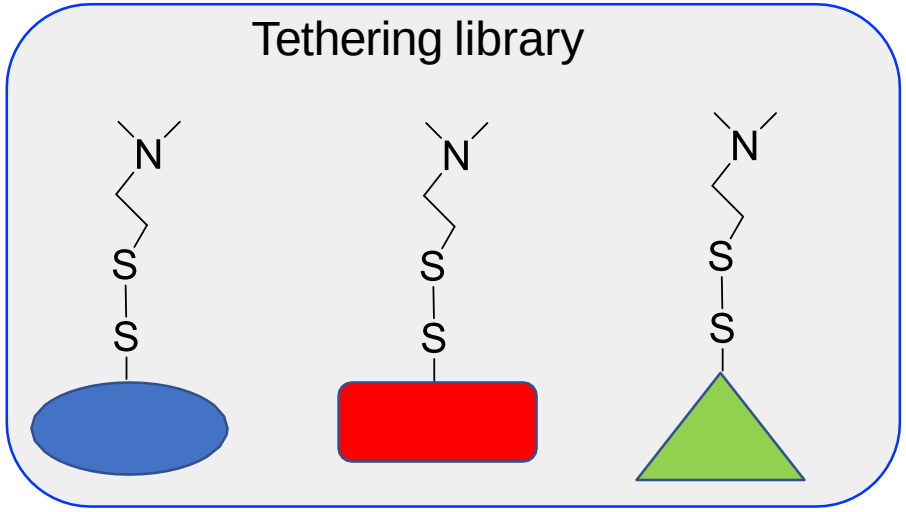


KRAS residue #



95 residues

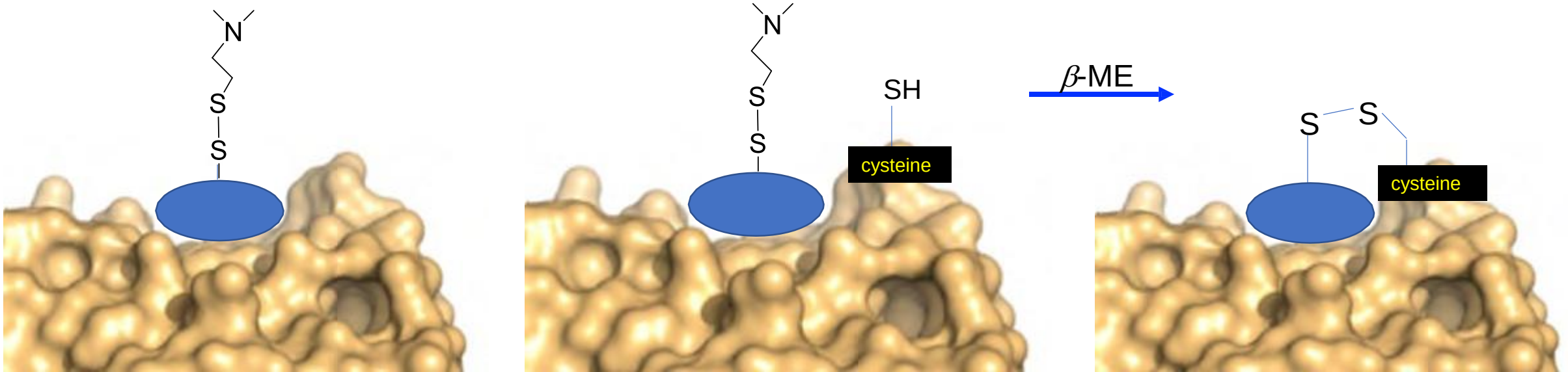
Tethering can identify fragment binding pockets adjacent to cysteines



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

Erlanson DA, Wells JA, Braisted AC. Tethering: fragment-based drug discovery. Annu Rev Biophys Biomol Struct. 2004;33:199-223.



FNL disulfide tethering library

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- >2000 high quality and chemically diverse fragments
- Good fragment-like properties
  - MW <300
  - ClogP ≤ 3
  - Number of hydrogen bond donors/acceptors ≤ 3
- Minimize overly complex molecules
- Minimize unnecessary stereochemistry
- Exclusion of PAINS / reactive groups
- Current library of 1163 compounds stored as 50 mM DMSO stocks

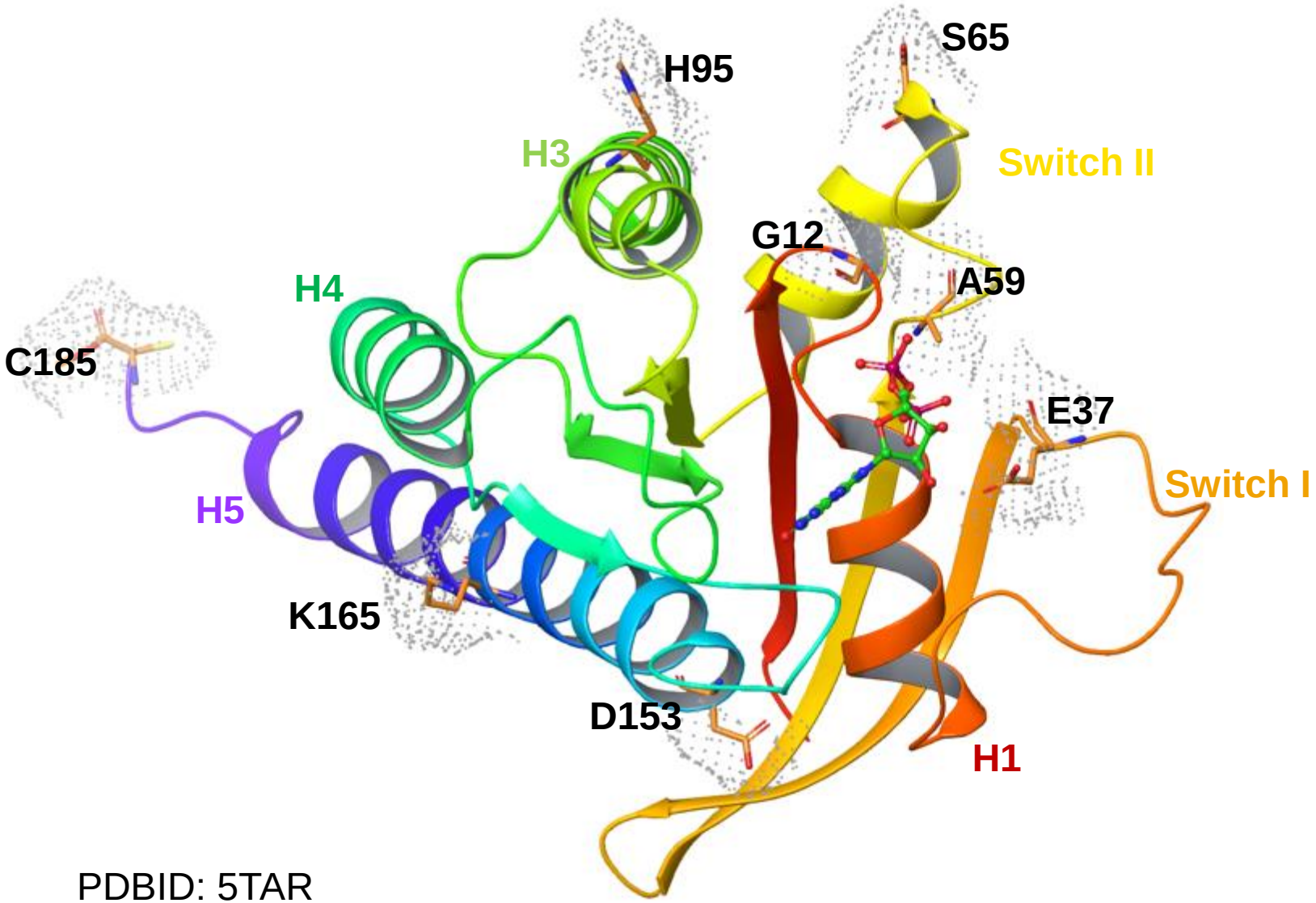
Parameter	Range
Molecular weight	80 - 343
Heavy atoms	5 - 21
Chiral centers	0 - 2
HB donors	0 - 2
HB acceptors	0 - 5
Rotatable bonds	0 - 4
Polar surface area	< 106 $Å^2$

Anna Maciag

David Turner

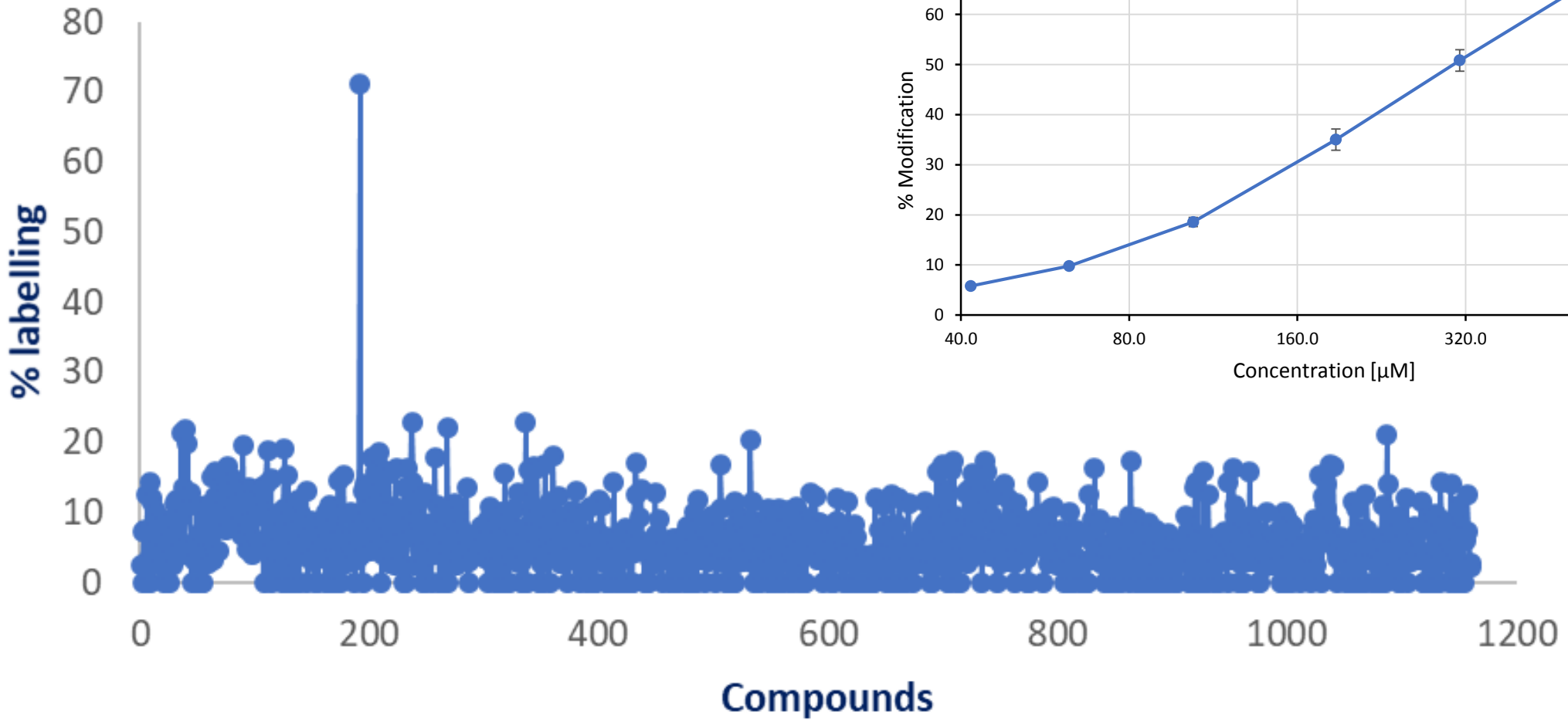
Vandana Kumari

Completed screens: residue mapping



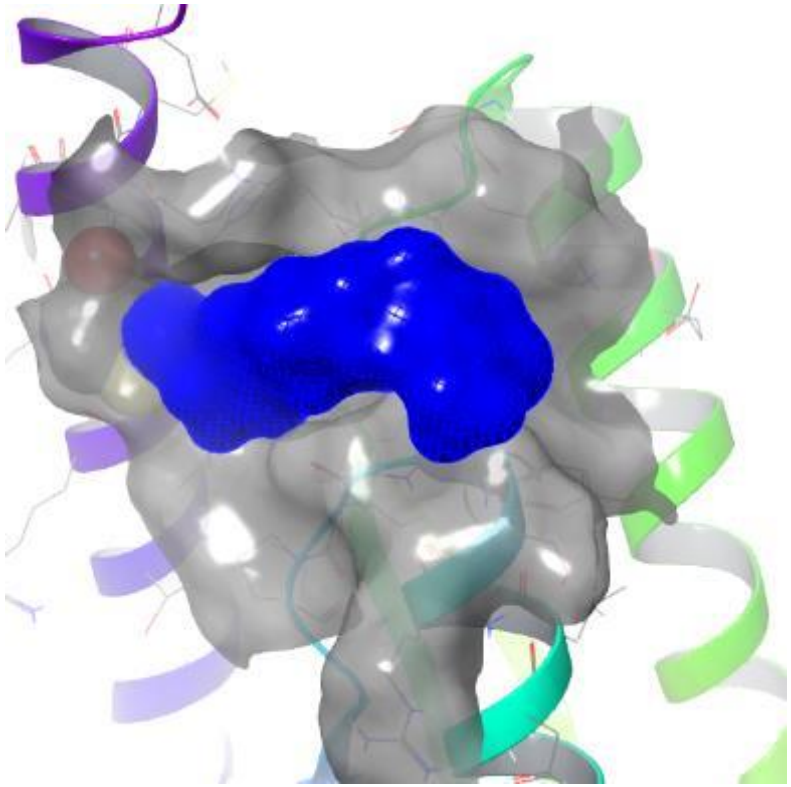
PDBID: 5TAR

Tethering screen: KRAS G12C/C118S (GppNHp)

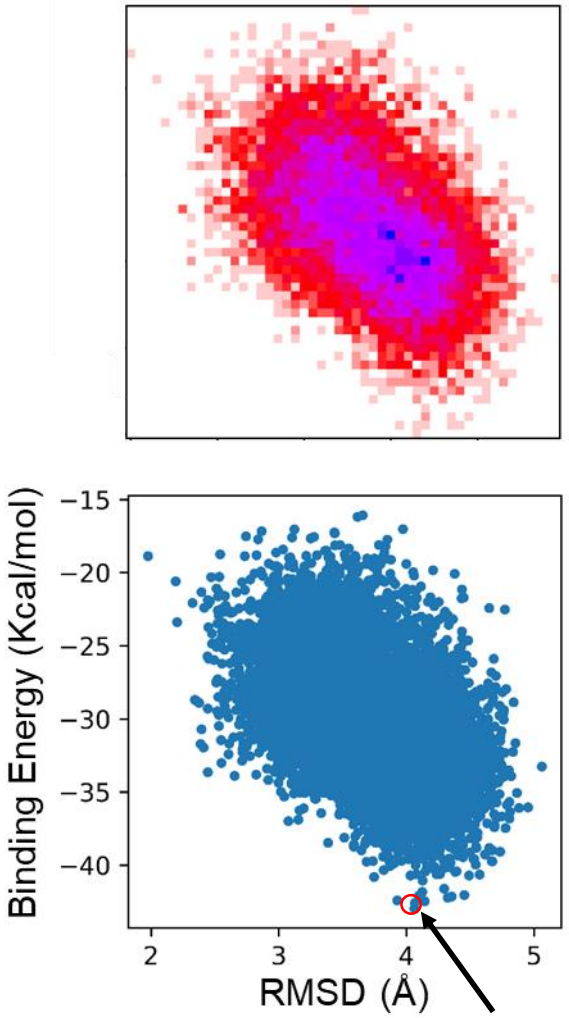


Resynthesis/dose response to confirm hit

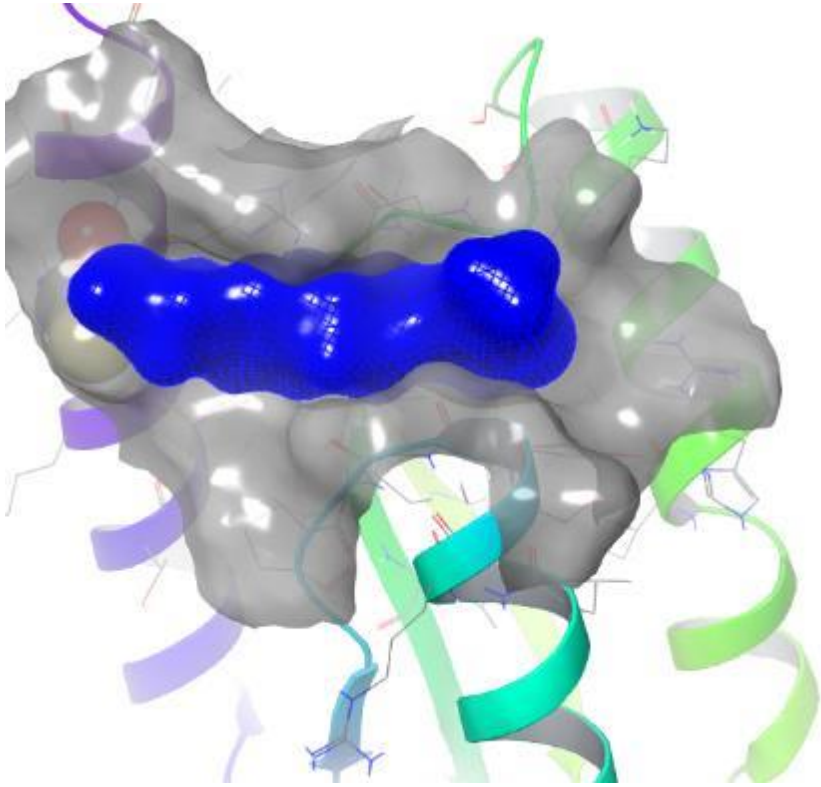
Best pose observed during MD simulations



Starting pose
(covalently docked pose to a crystal structure)



Best energy



Best pose

Best pose from all MD simulations
MM-GBSA binding energies : -42.94 kcal/mol

Goals and approaches

Frederick National Laboratory for Cancer Research

Goals:

- Hit optimizations with limited binding pocket/structural information
- Discovery of new pockets not detectable in crystal structures- identification of cryptic pockets

Approaches:

- Computer aided drug design
  - Molecular docking for pose prediction (starting point for simulations)
  - Molecular dynamics (MD) simulations for pose refinement
  - Enhanced MD for cryptic pocket identifications

- Thanks!