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Pioneering science delivers vital medicines™

Covalent Inhibitors for Challenging Targets: Covalent Library Construction, Screening, and Hit Optimization

Victor J. Cee

Amgen, Inc. Thousand Oaks, CA USA

2016 International School of Advanced Organic Chemistry

KRAS - The Most Challenging Target?



C&EN June 6, 2016: Credit: Yang H. Ku/C&EN/Shutterstock

Selected Events in the Discovery of KRAS

Morphologic Responses to a Murine Erythroblastosis Virus [1, 2]

[1] Received February 16, 1967.

W. H. KIRSTEN [3] *and* L. A. MAYER,[4] *Departments of Pathology and Pediatrics, Division of Biological Sciences, the University of Chicago, Chicago, Illinois 60637*

Discovery of a Transforming Retrovirus
Kirsten **M**urine **S**arcoma **V**irus: **Ki-MSV**



https://ncifrederick.cancer.gov/careers/student_programs/internships/sip/About.aspx

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THOMAS Y. SHIH[1], MAUREEN O. WEEKS, HOWARD A. YOUNG, AND EDWARD M. SCOLNICK

Laboratory of Tumor Virus Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Accepted February 27, 1979

Identification of 21 kDa Ki-MSV Protein



https://www.broadinstitute.org/scientific-community/science/programs/psychiatric-disease/stanley-center-psychiatric-research/edward-scolnick-md

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Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3637–3640, June 1982
Medical Sciences

Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses

(human tumors/transfection/retroviruses)

CHANNING J. DER, THEODORE G. KRONTIRIS, AND GEOFFREY M. COOPER

Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Communicated by Baruj Benacerraf, March 22, 1982

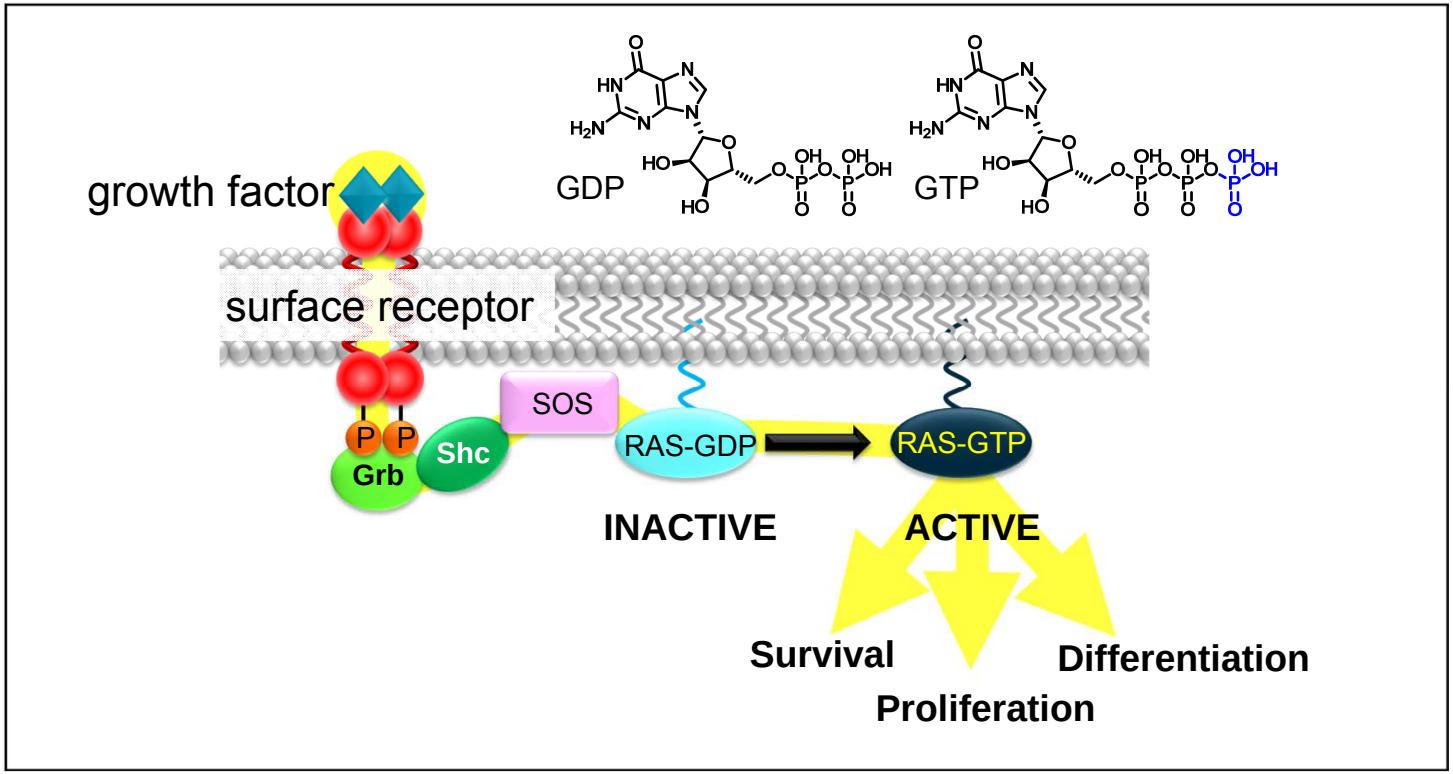
Identification of a Homologous Protein in Human Cancers
Kirsten **RA**t **S**arcoma Virus: **KRAS**



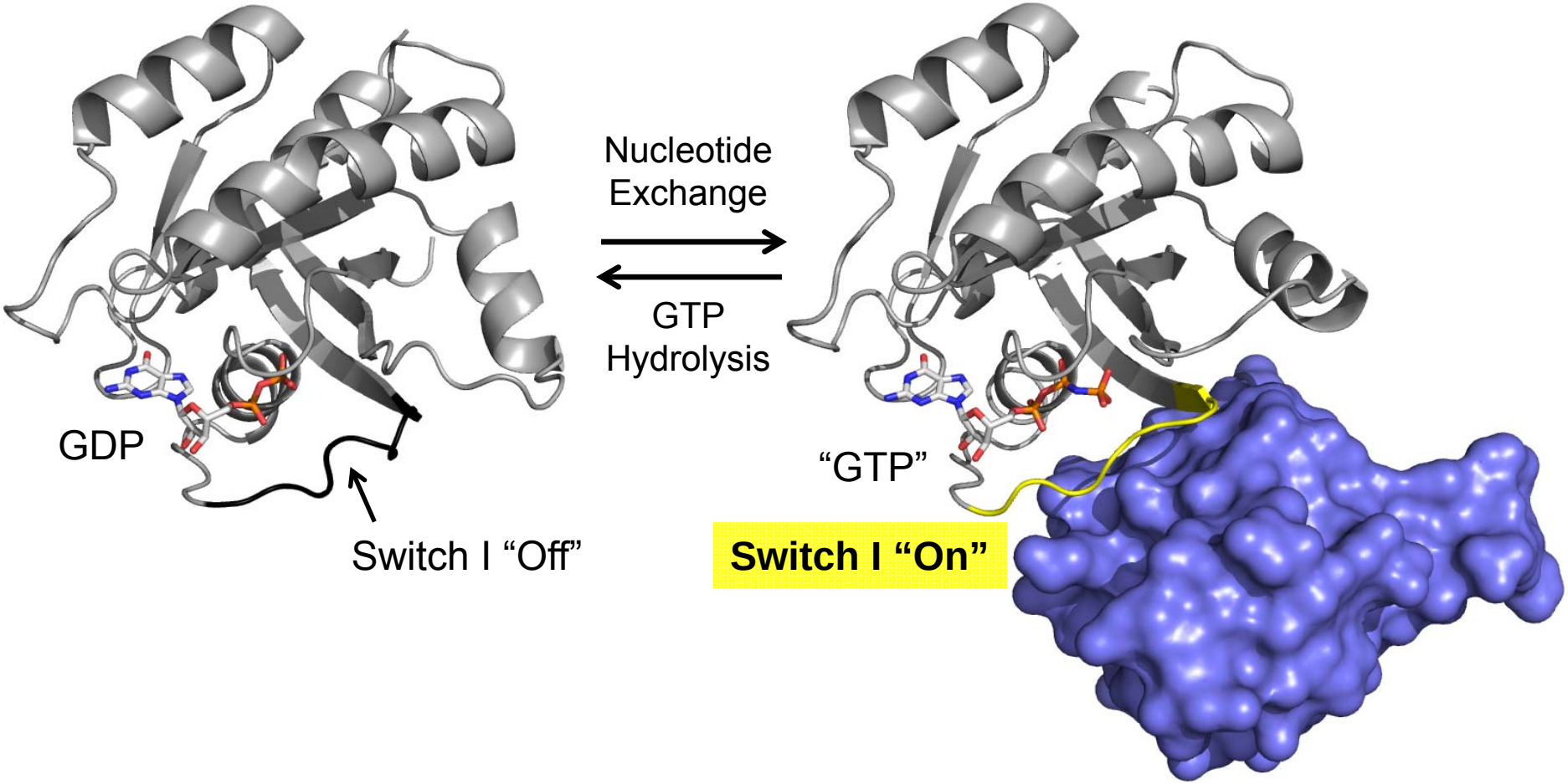
https://www.bu.edu/biology/people/profiles/geoffrey-m-cooper/

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KRAS is a Guanine Nucleotide Binding Protein That Functions as a Binary Molecular Switch



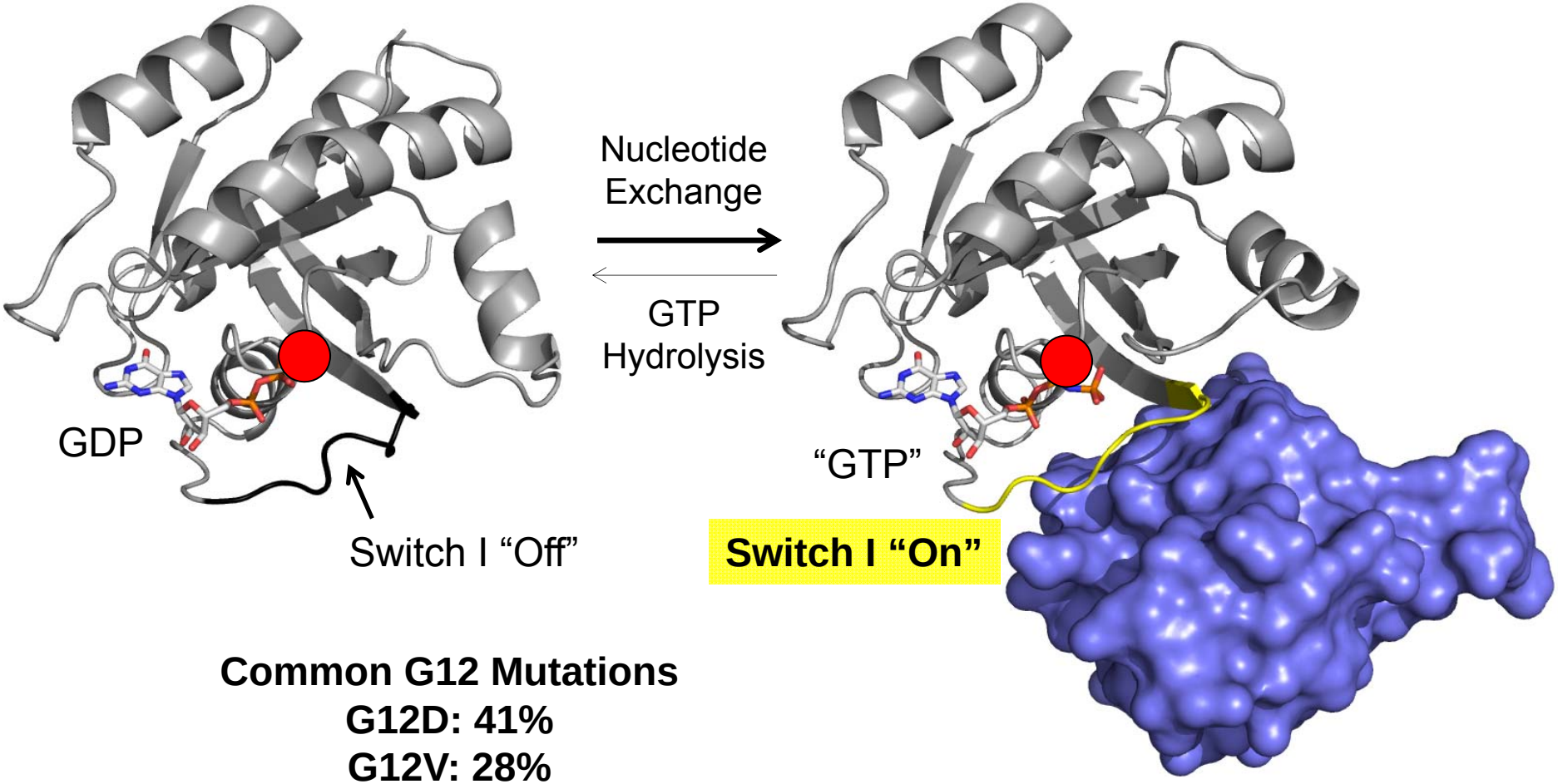
KRAS is a Guanine Nucleotide Binding Protein That Functions as a Binary Molecular Switch



Effector protein binding
↑Proliferation and survival

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Mutation of Glycine-12 ● Impairs the Regulated Hydrolysis of GTP and is Oncogenic

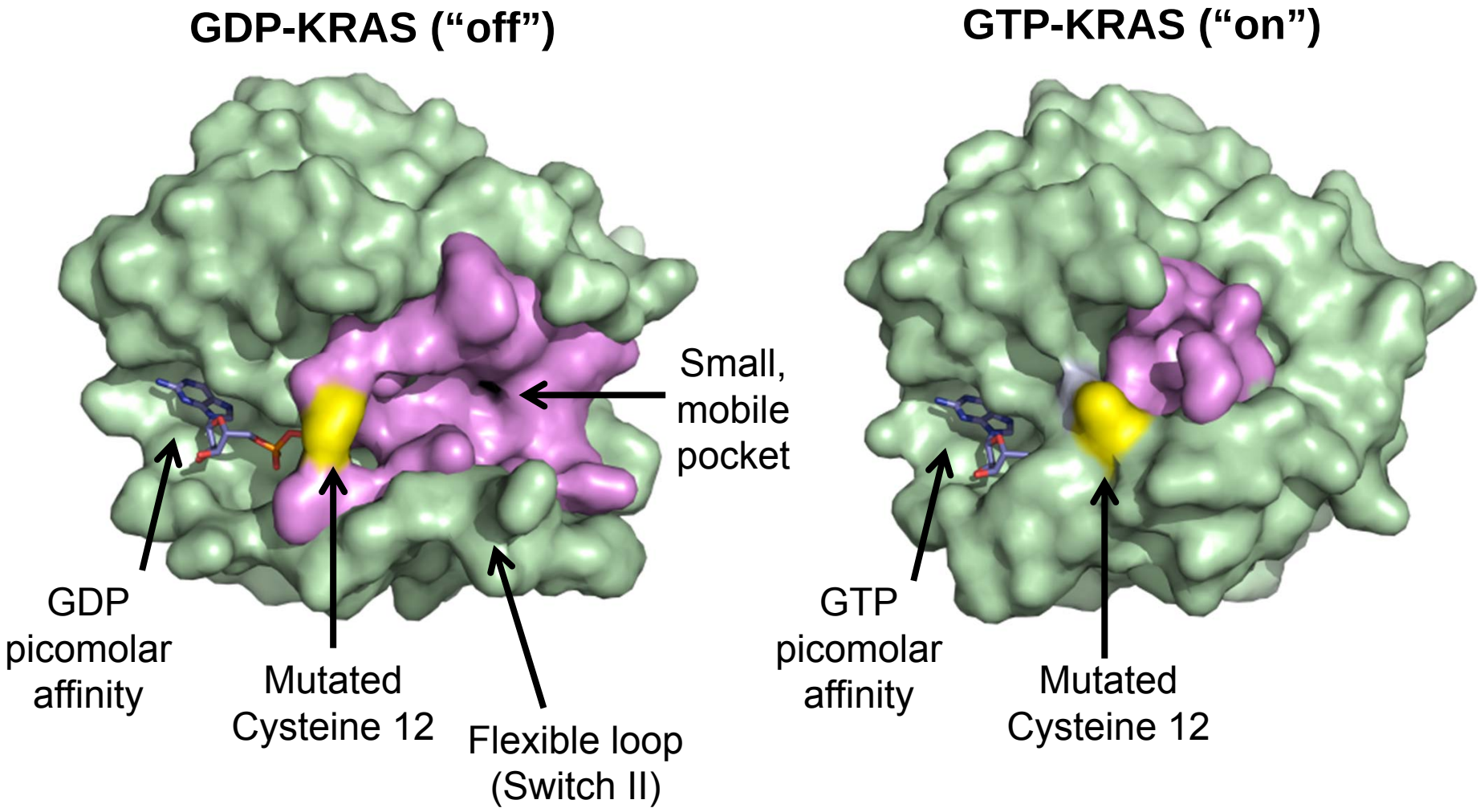


Common G12 Mutations
G12D: 41%
G12V: 28%
G12C: 14%

Effector protein binding
↑↑↑↑ Proliferation and survival

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X-Ray Crystal Structures of KRAS

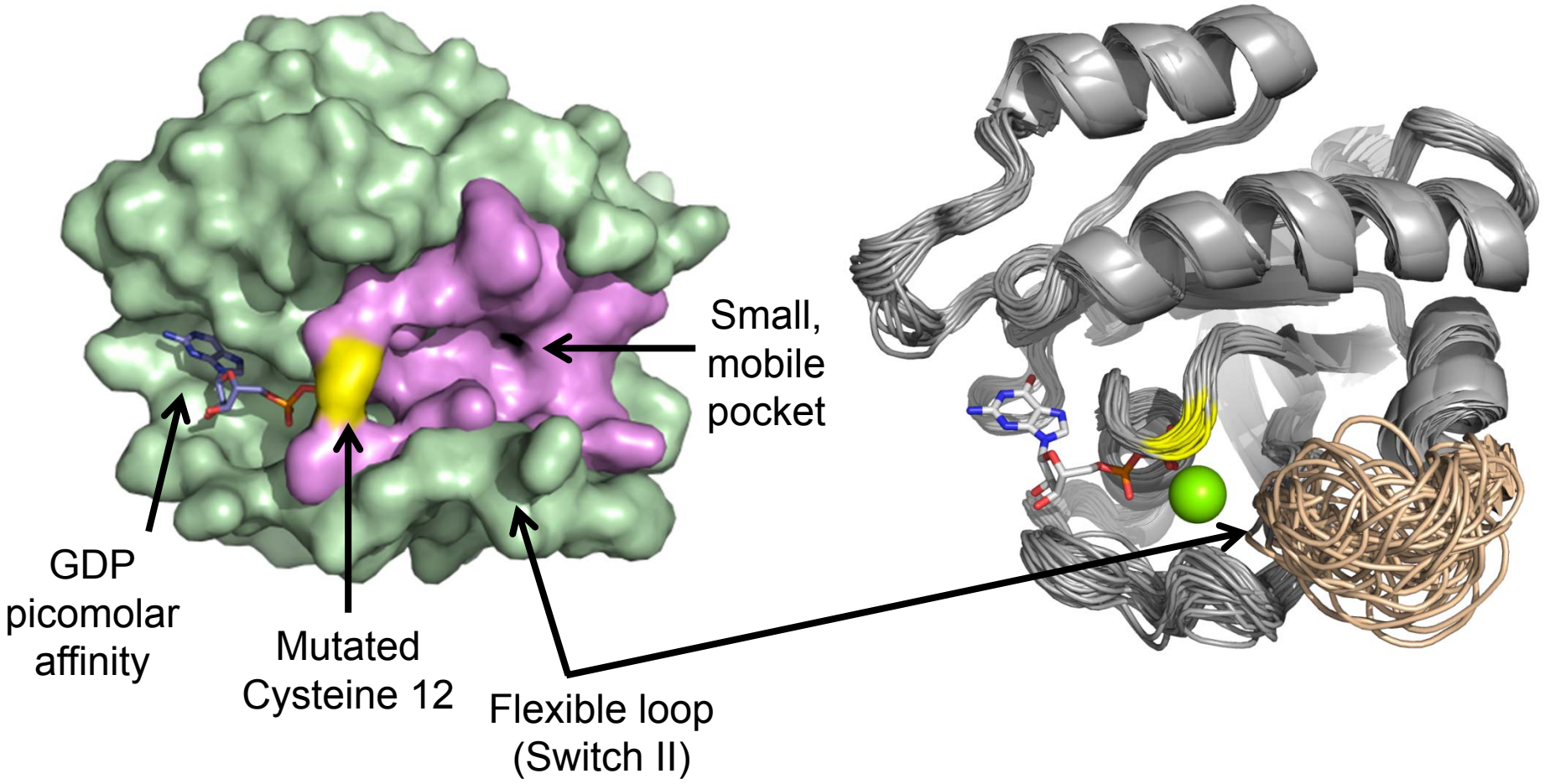


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Crystal and Solution Structure of GDP-KRAS

GDP-KRAS (“off”)

1CRP: NMR Structure of GDP-RAS



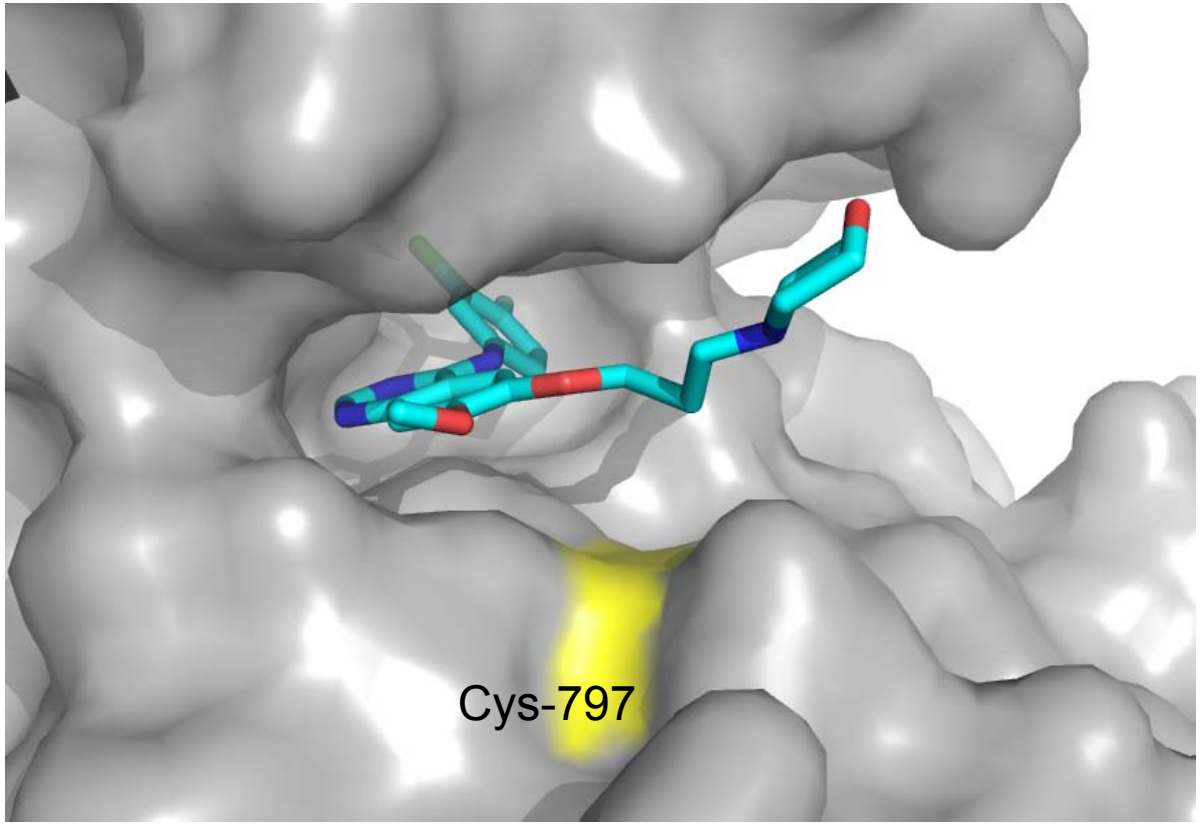
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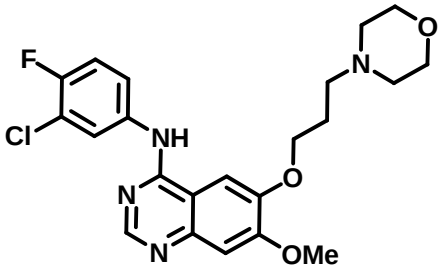
How does one generate covalent inhibitors?

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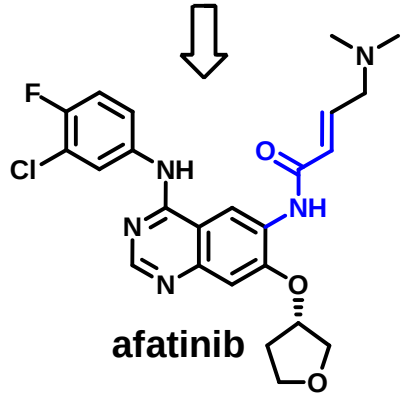
Noncovalent Binder to Covalent Inhibitor

Gefitinib/EGFR, PDB: 2ITY





gefitinib

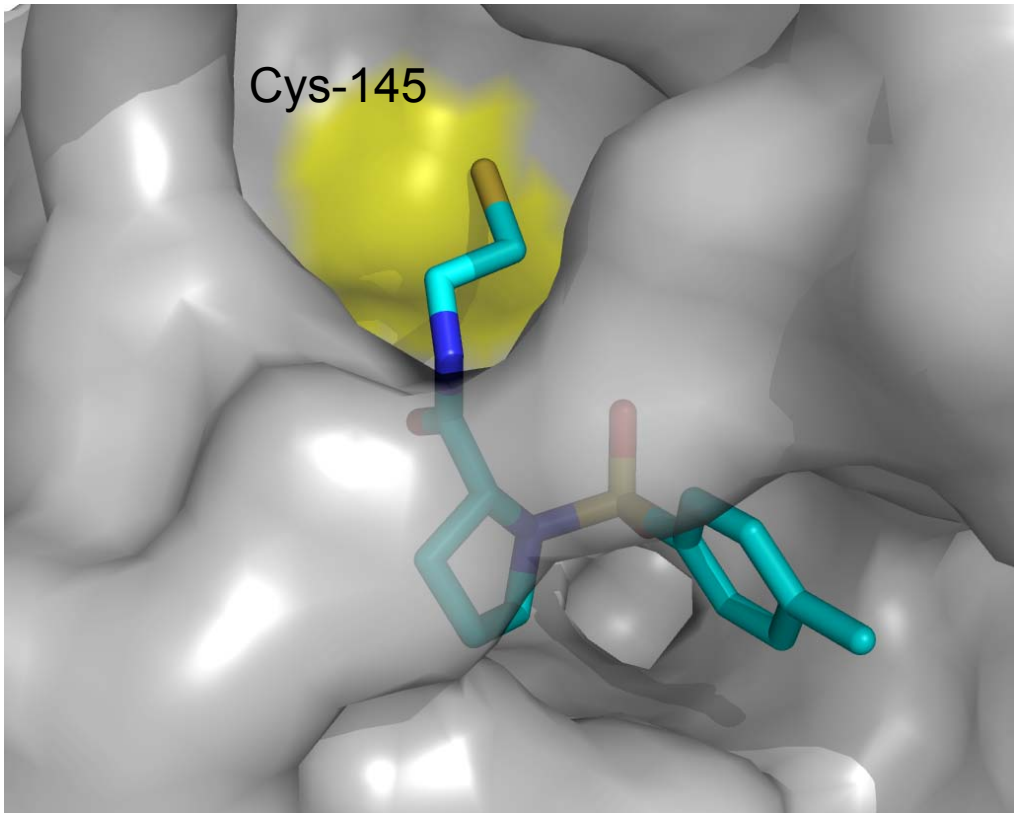


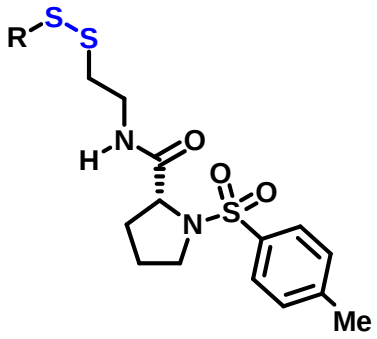
afatinib

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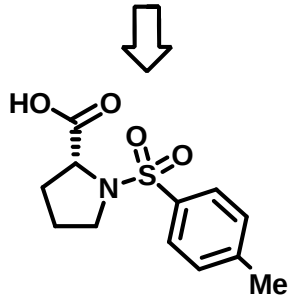
Electrophile Library Screening

Thymidylate Synthetase PDB:1F4C





Thymidylate Synthetase Hit
(n=1200, Mass Spectrometry)



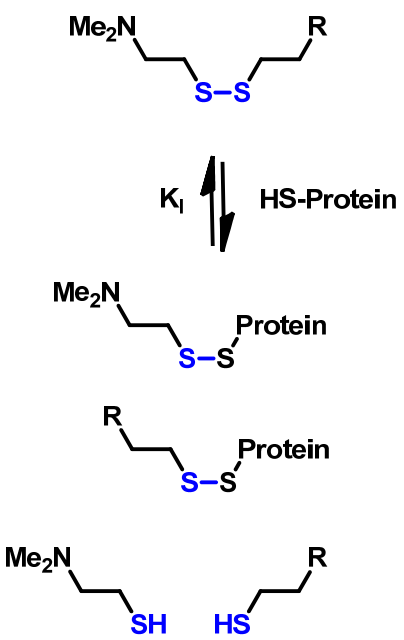
Noncovalent Fragment
TS K_i = 1.1 mM

Proc. Nat. Acad. Sci. **2000**, 9367–9372

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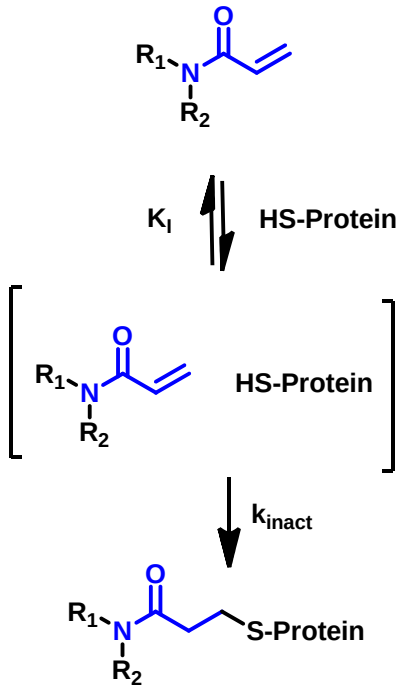
Electrophile Screening: Reversible or Irreversible

Disulfide – Reversible



- Thermodynamic conditions
- Hits dictated by K_I
- Counterscreening unnecessary
- Disulfide covalent drugs are rare

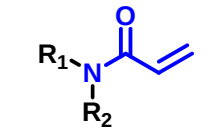
Acrylamides - Irreversible

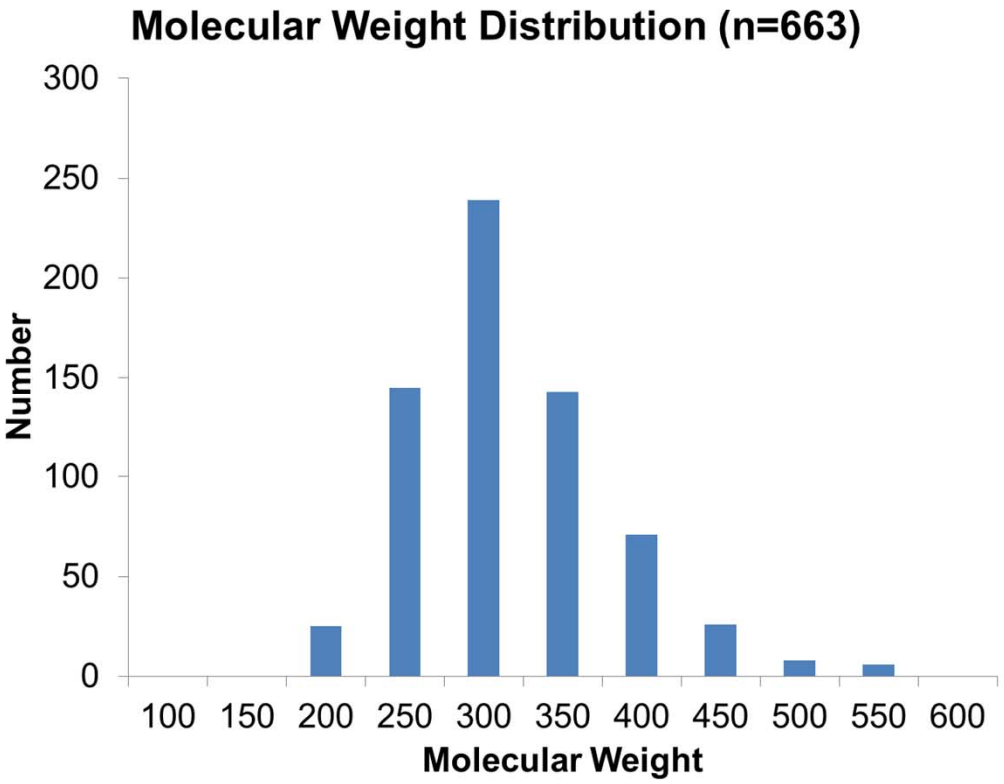


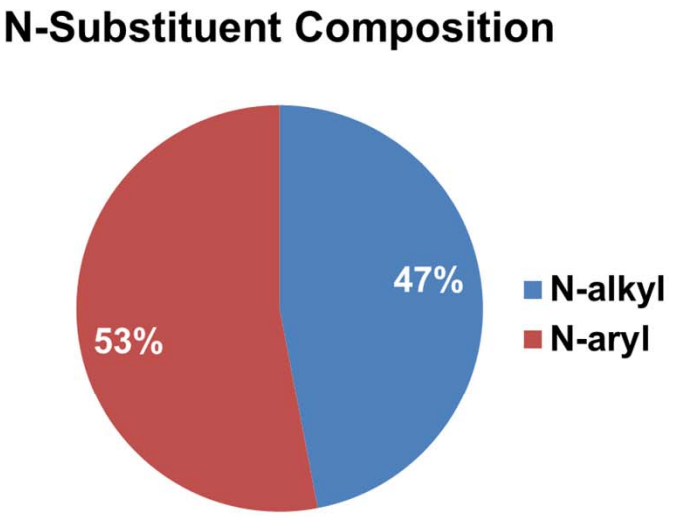
- Kinetic conditions
- Hits dictated by K_I *and* k_{inact}
- Counterscreening necessary
- Acrylamide drugs are common

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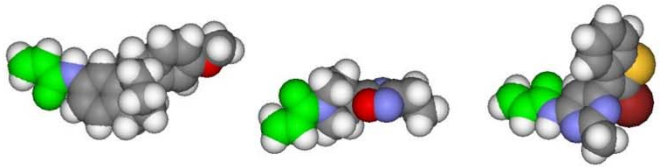
We Built a Small Screening Deck of Acrylamides (n=663)







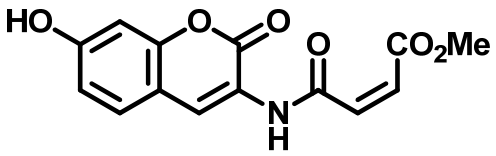
Examples



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Initial Screen of KRAS G12C Utilized a Reactive Fluorescent Probe (Thiolprobe IV)

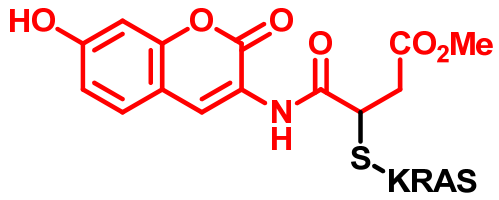
Detection: Thiolprobe IV to assess KRAS-SH



KRAS-SH

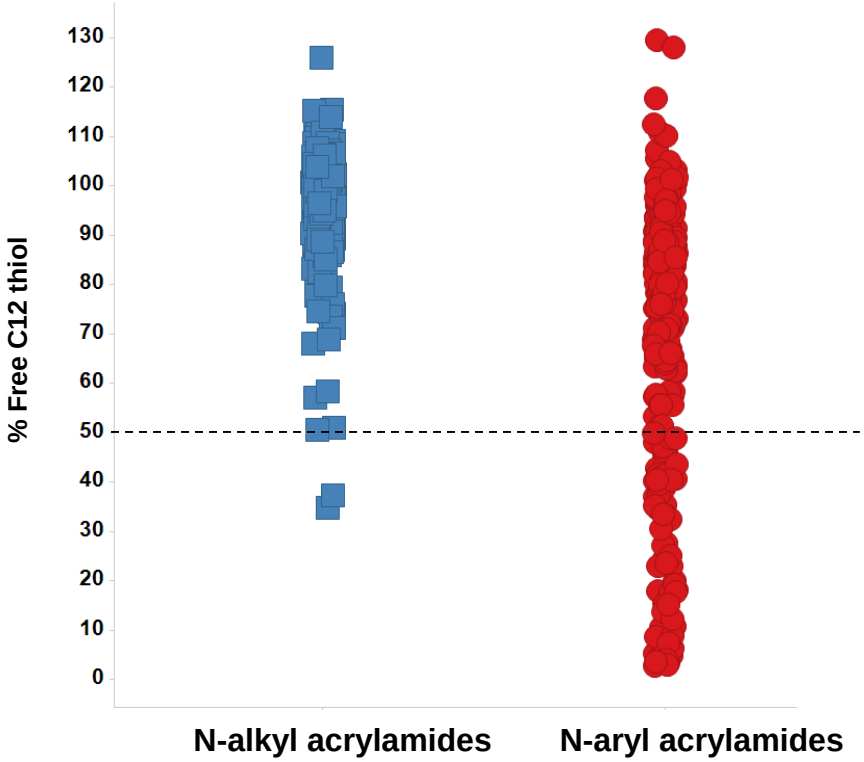
Thiolprobe IV (nonfluorescent)

KRAS-SH



Covalent Adduct: Ex 400 nm, Em 465 nm

Screen: 20 h pre-incubation, 25 μM (n=663)

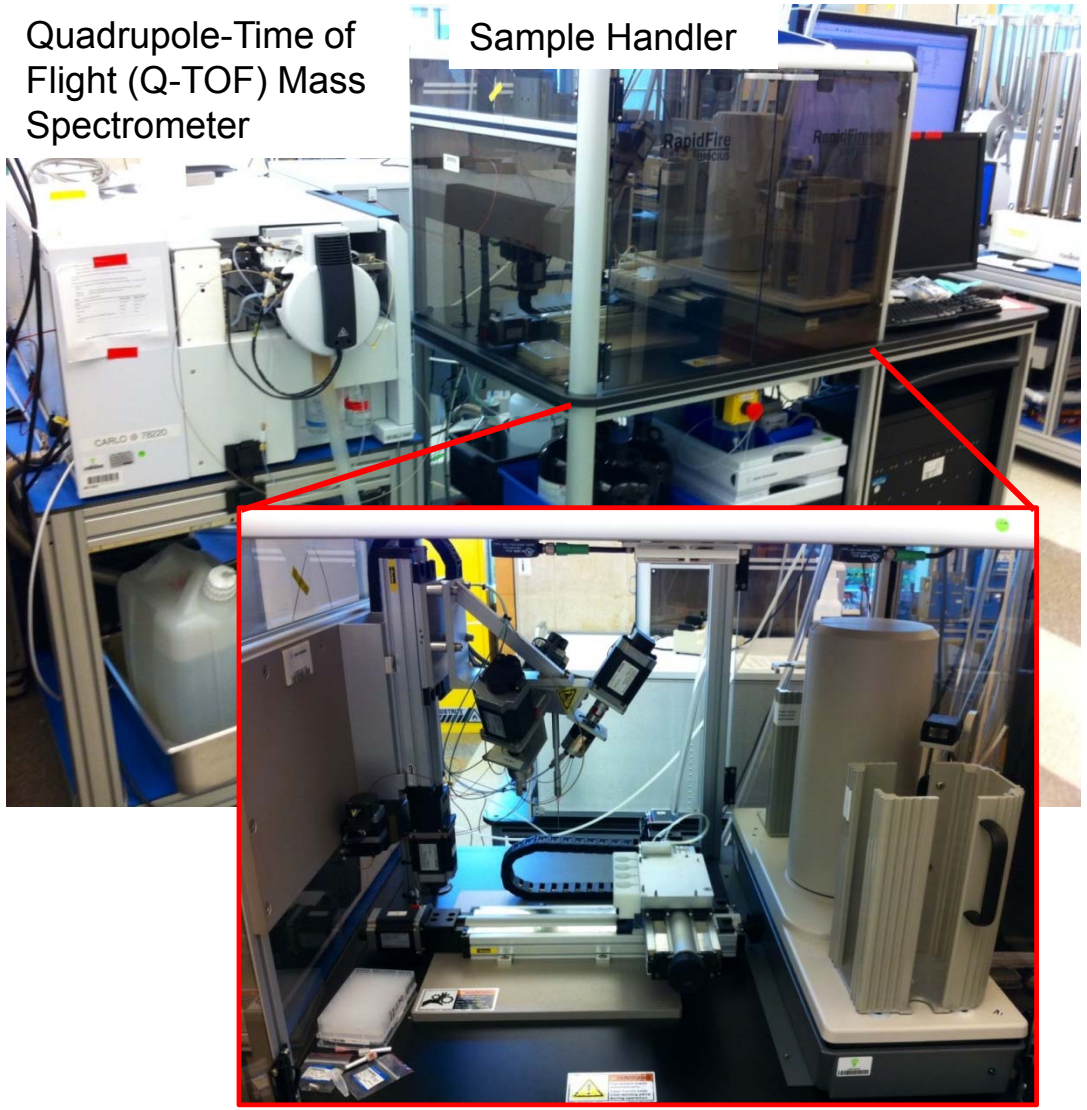


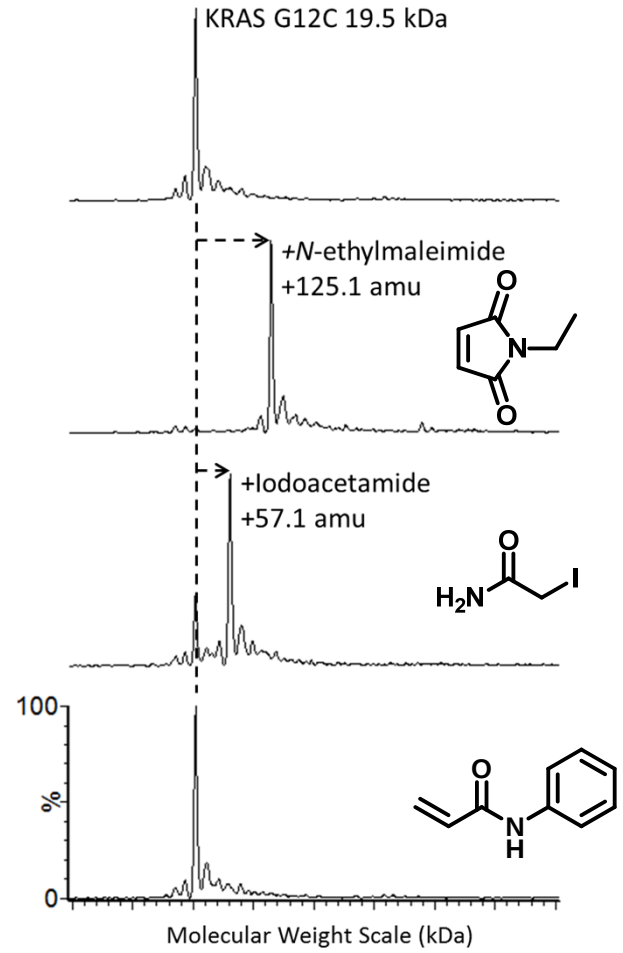
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Enabling Tool for Hit Assessment: Protein Mass Spectrometry with a RapidFire System

Quadrupole-Time of Flight (Q-TOF) Mass Spectrometer

Sample Handler

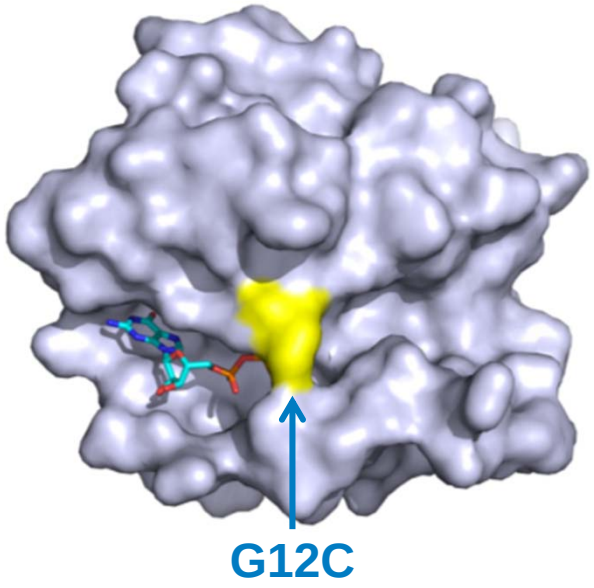




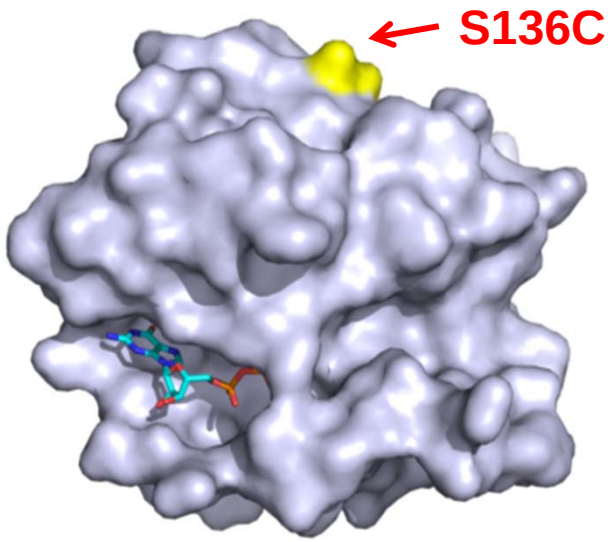
Campuzano, I., *et al*. *J. Biomol. Screen.*, **2016**, 136–144

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Hit Assessment: Engineered Surface Cys Mutant



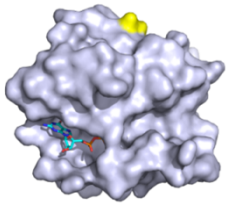
GDP-KRAS G12C



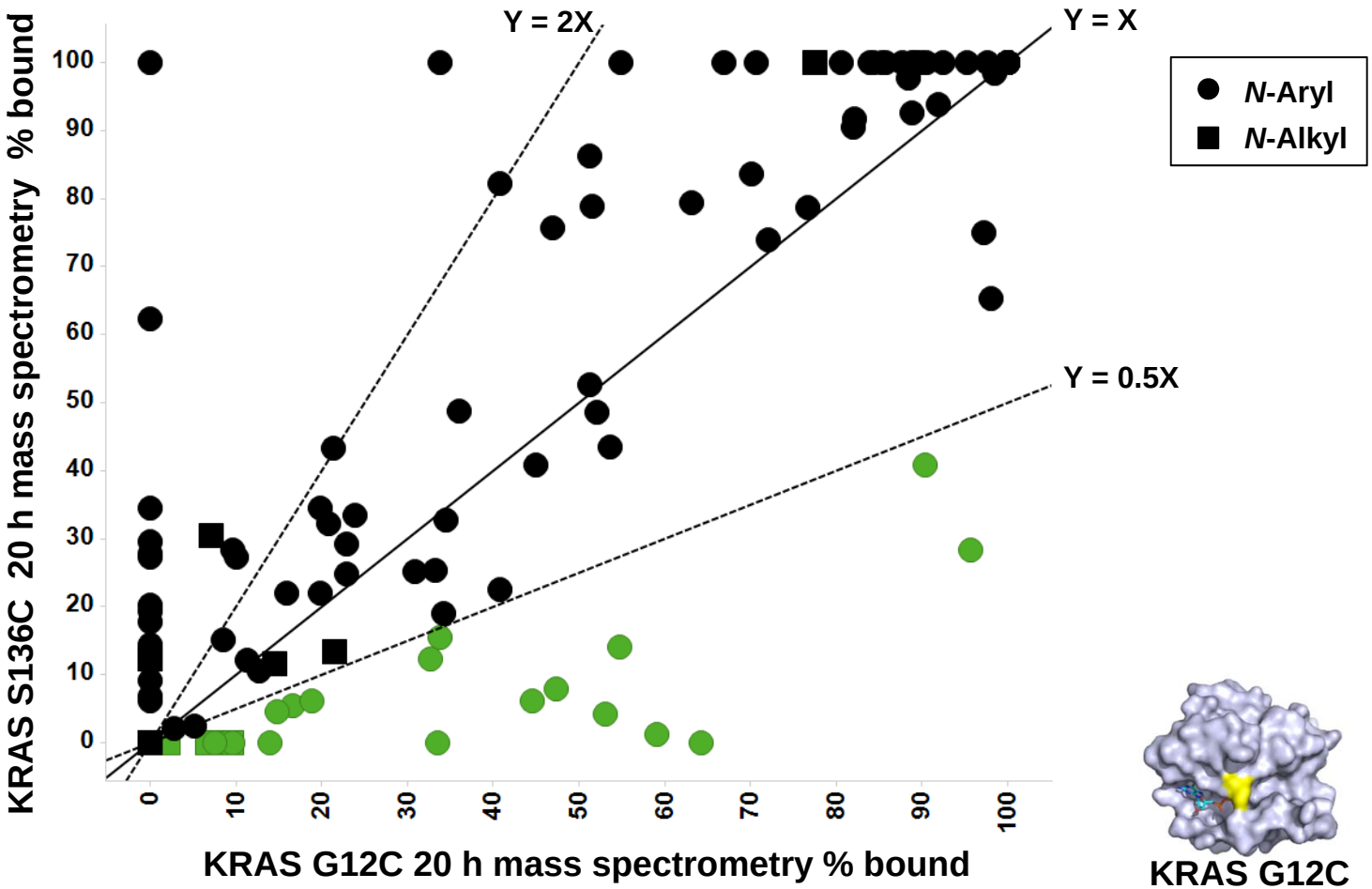
GDP-KRAS S136C

Final Assessment by Mass Spectrometry

20 h Incubation at 100 μM Compound, Mass Spectrometry (n=91)



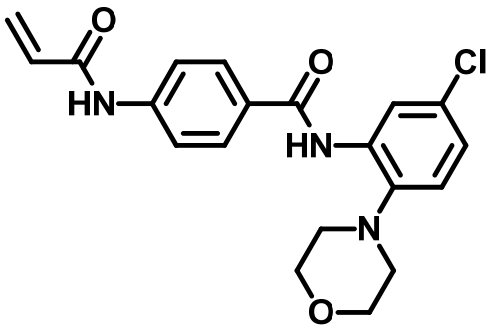
KRAS S136C

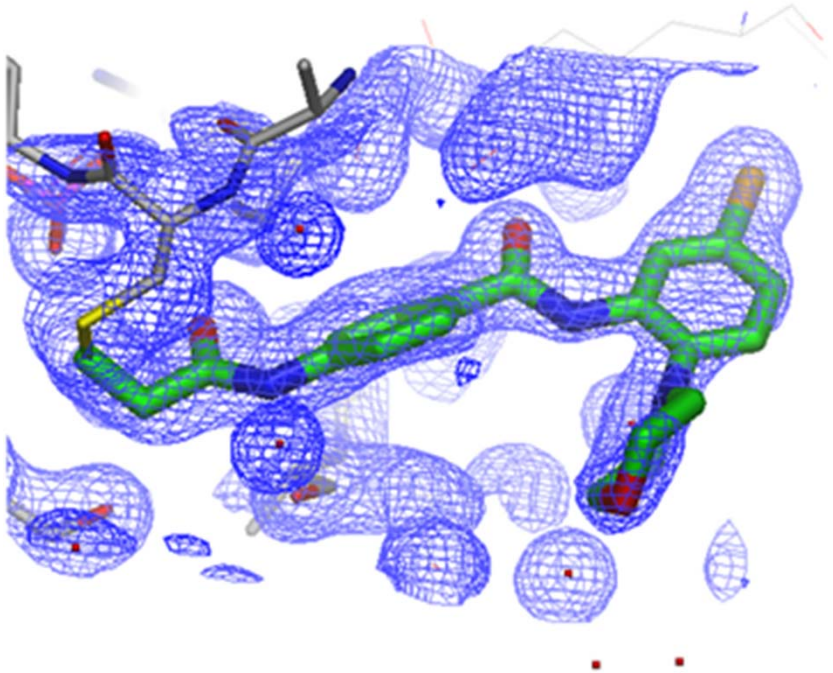


KRAS G12C

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First Covalent X-Ray Cocrystal Structure

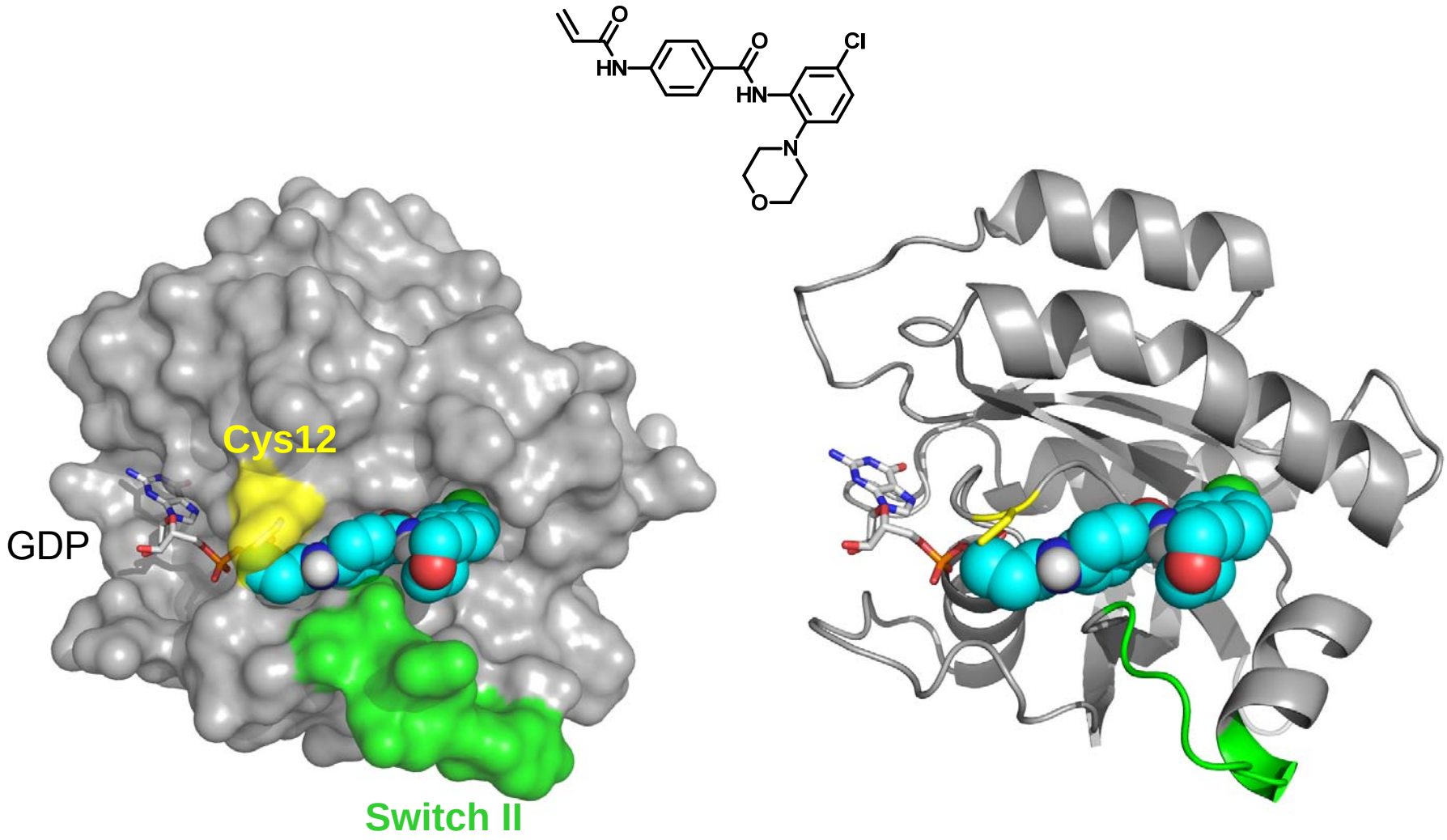




G12C Mass Spec: 41% bound (20 h, 100 μM)
Est. $k_{obs}/[I] = 0.007\ M^{-1}s^{-1}$

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First Covalent X-Ray Cocrystal Structure



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