Challenge accepted:
Finding and classifying cryptic pockets in K-Ras

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CUP 2023

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Outline

A challenge was born

A dead person quote

Background on K-Ras

Our MD simulations of K-Ras

Results of pocket finding in K-Ras

What kind of pocket did we find?

Conclusions and outlook

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The challenge issued at JCUP last year…

"That's cool, but if you can show that your floes work for K-Ras *I'll use them every day*." – Dave Lawson, Mirati - Tokyo 2022

"Challenge accepted" – me

A new hope for K-Ras G12D: MRTX1133

RETURN TO ISSUE | < PREV **FEATURED ARTICLE** NEXT >

Identification of MRTX1133, a Noncovalent, Potent, and Selective KRAS^{G12D} Inhibitor

Xiaolun Wang*, Shelley Allen, James F. Blake, Vickie Bowcut, David M. Briere, Andrew Calinisan, Joshua R. Dahlke, Jay B. Fell, John P. Fischer, Robin J. Gunn, Jill Hallin, Jade Laguer, J. David Lawson, James Medwid, Brad Newhouse, Phong Nguyen, Jacob M. O'Leary, Peter Olson, Spencer Pajk, Lisa Rahbaek, Mareli Rodriguez, Christopher R. Smith, Tony P. Tang, Nicole C. Thomas, Darin Vanderpool, Guy P. Vigers, James G. Christensen, and Matthew A. Marx*

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SUBJECTS: Assays, High-performance liquid chromatography, Inhibitors, Mixtures,

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Gertrude Stein: Describer of K-Ras cryptic pockets

***Everybody's Autobiography*, 1937**

"There is no there there."

Credit: wikipedia

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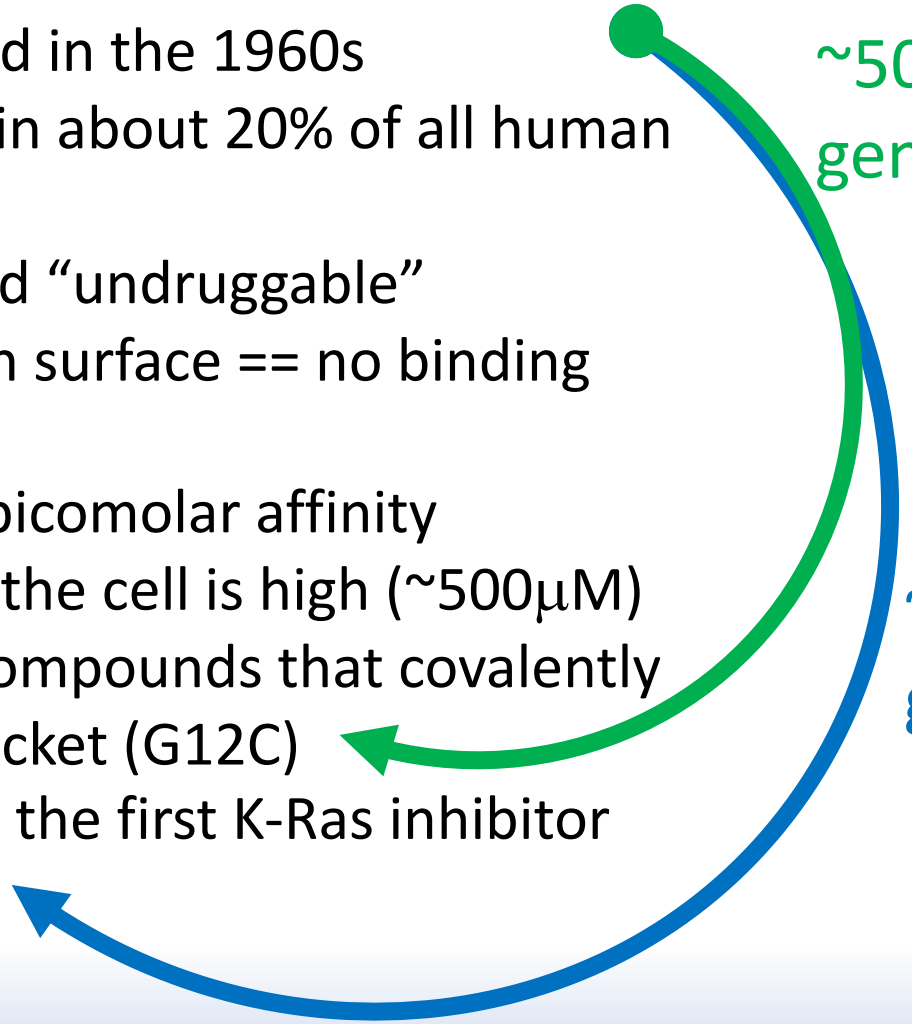
Finding druggable protein states is hard

The textbook example: K-Ras

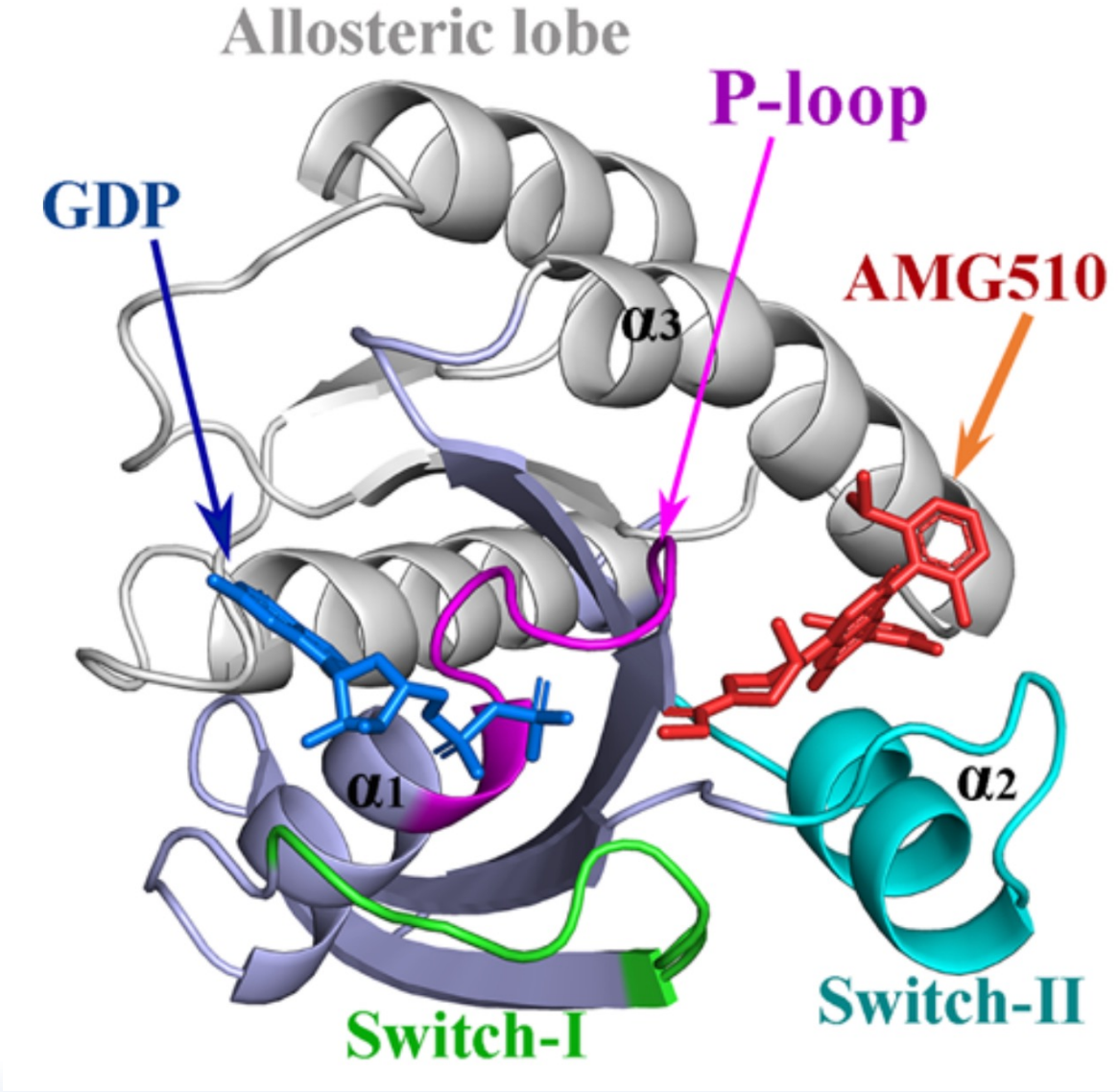
- KRAS gene first discovered in the 1960s
- K-Ras protein is involved in about 20% of all human cancers
- K-Ras was long considered “undruggable”
  - Small protein (smooth surface == no binding sites)
  - GTP/GDP binds with picomolar affinity
  - GTP concentration in the cell is high (~500μM)
- 2013: Shokat lab found compounds that covalently bound to the Switch-II Pocket (G12C)
- May 2021: FDA approved the first K-Ras inhibitor (sotorasib; Amgen)



~50 years from gene to pocket

~60 years from gene to drug

A visualization of the K-Ras structure



Adapted from: Zhao, Z., Bohidar, N., Bourne, P., JCIM, 2023

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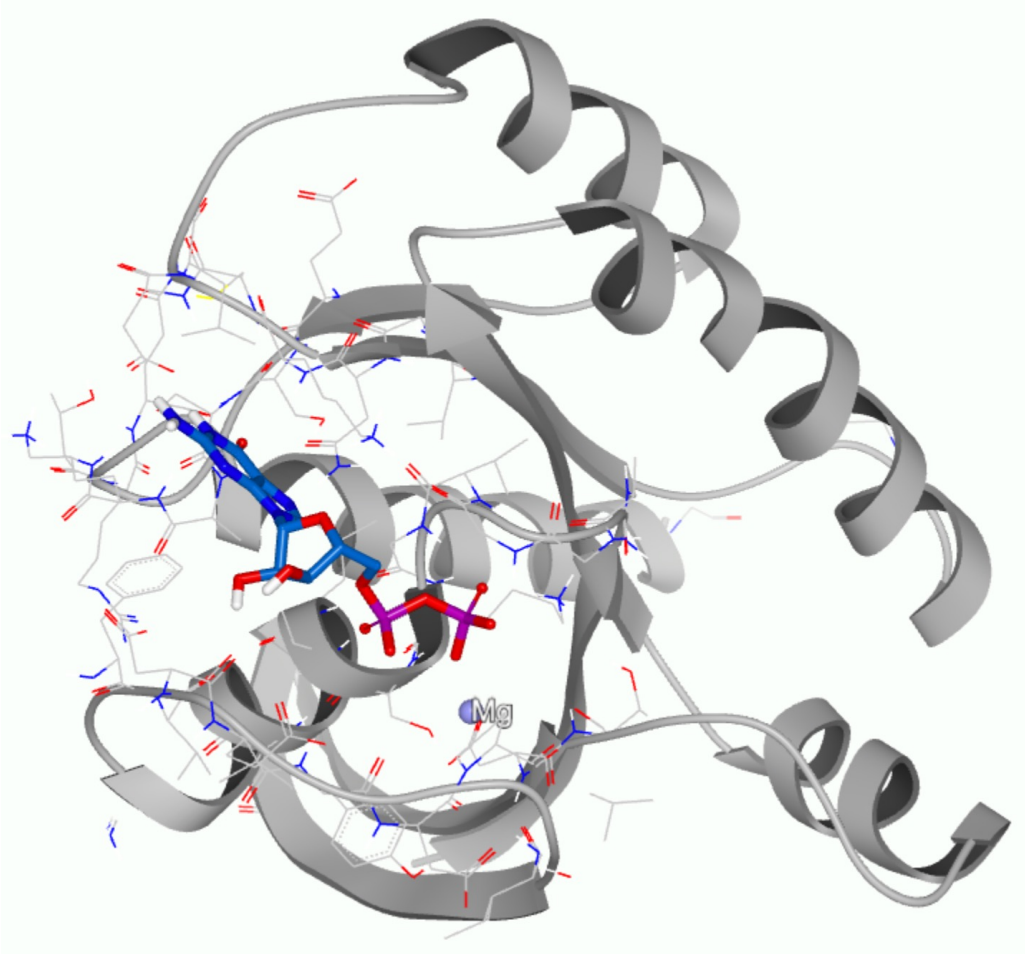
We set up and performed MD simulations on K-Ras

- First, we spruce prepped a high-quality WT K-Ras structure (4OBE; 1.2 Å)
- Then, we use Spruce to mutate G12 to D12
- Each of the WT and G12D structures were prepped for simulation using MDOrion
- Several simulations on WT and G12D were performed



SPRUCE prepped WT K-Ras (4OBE)

The varieties of K-Ras systems we simulated

The K-Ras simulations that were performed (300-500 iter. WE)

- WT, G12D (2D NMA)
- Xe, ethanol, and benzene cosolvents (2D NMA)
- MRTX-1133 binding started from random position (with 1D NMA)
  1. Ligand RMSD to Xray pose
  2. Ligand distance to Xray position
  3. Sitehopper Tanimoto of Xray pocket (7RPZ)

In total, ~60 μs of K-Ras simulation data was generated

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How did we search for pockets our simulations?

- We analyzed our data using the mutual information methods just Neha presented
  - Cooperative solvent exposure
  - Cooperative cosolvent binding
- Take our data and cluster on the MD feature of interest
- Make a MSM of the feature data
- Use the MSM to calculate mutual information

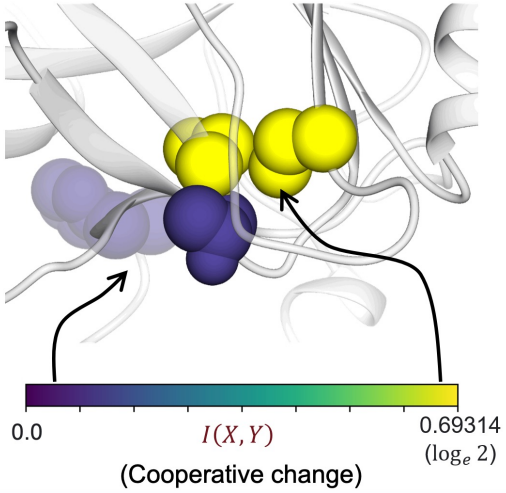
Measurement of correlated changes in cosolvent-binding

$$I(X,Y) = \sum_{x \in X} \sum_{y \in Y} p(x,y)\, log\left(\frac{p(x,y)}{p(x)p(y)}\right)$$

(X,Y) : *A pair of residues*

x : *xenon bound state of residue X*

y : *xenon bound state of residue Y*



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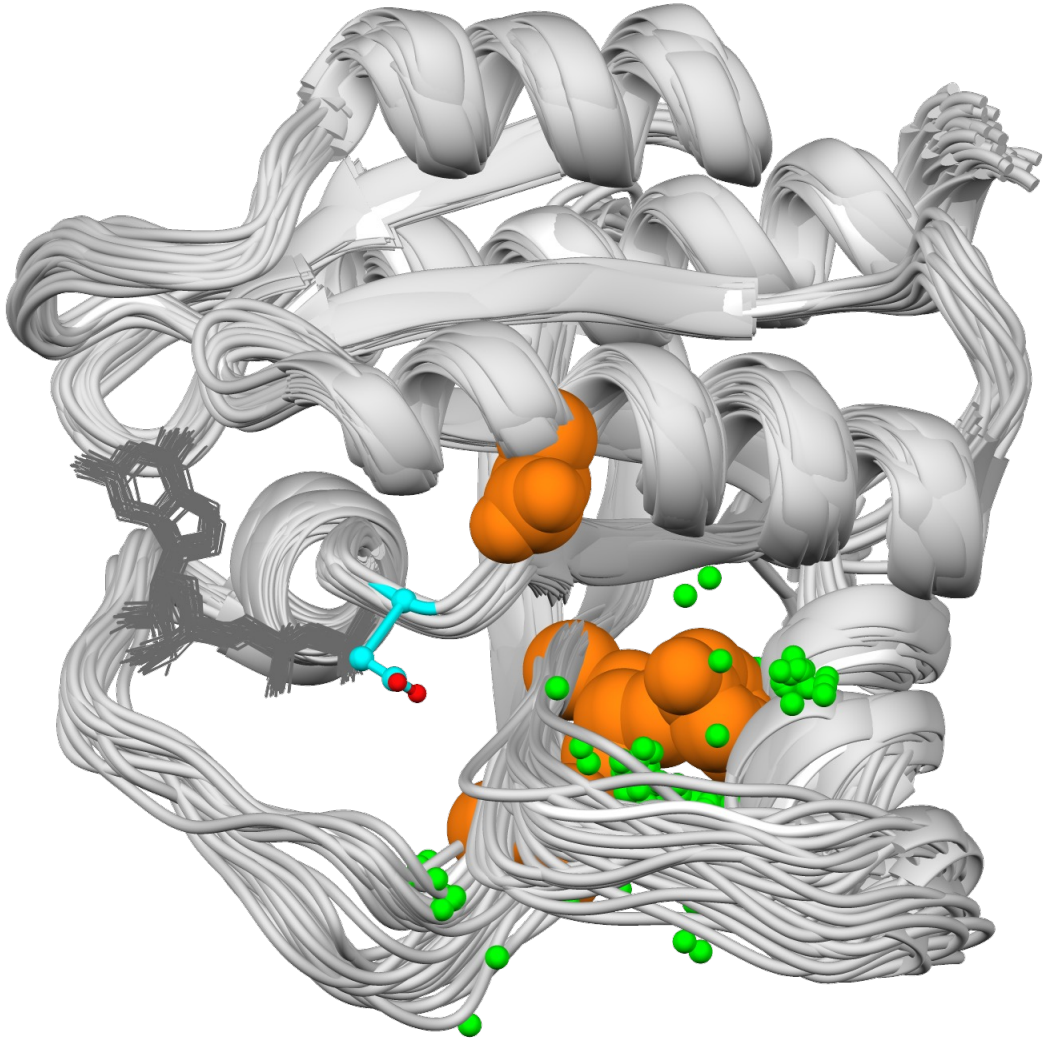
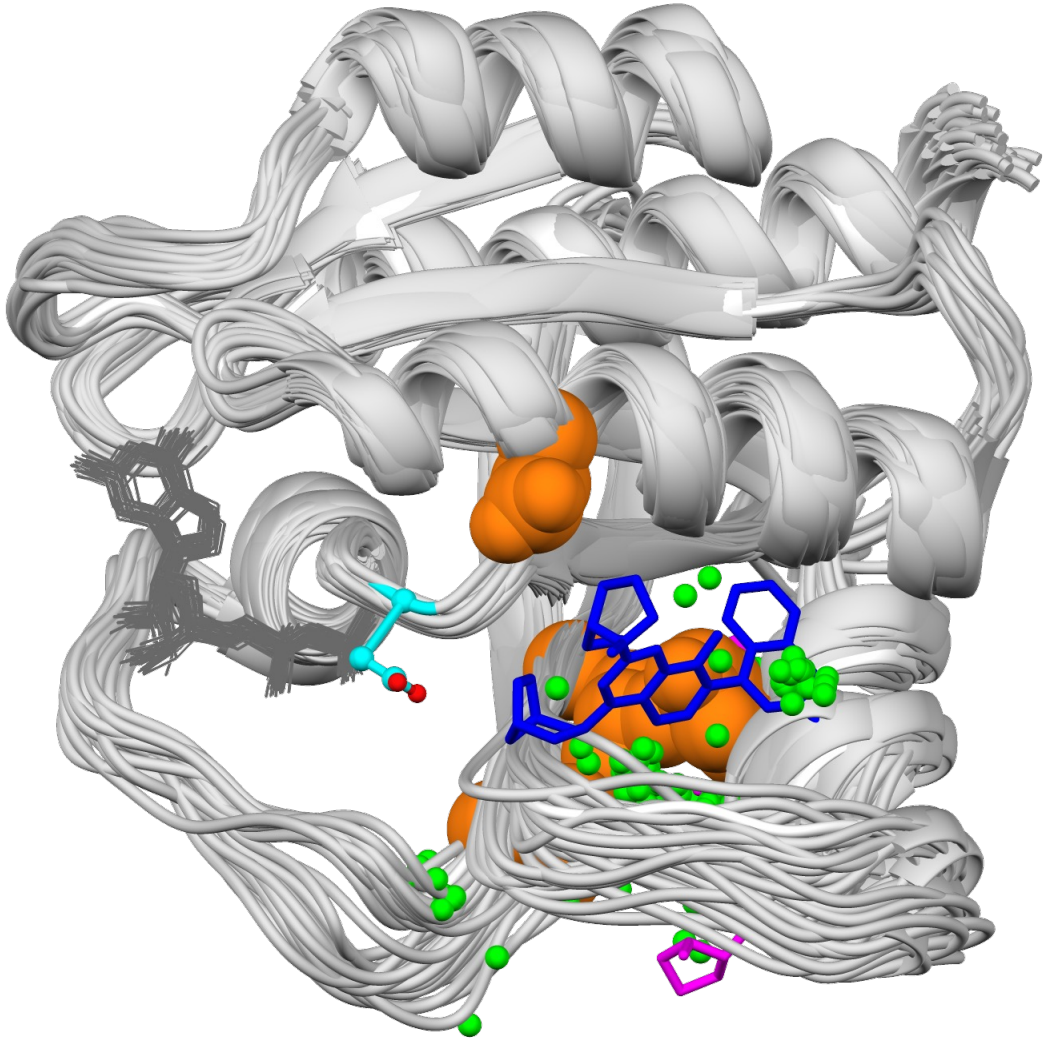
Xenon-binding site detection in K-Ras (G12D)



Xe binding overlaps with the MRTX1133 pocket



(PDB ID: 7RPZ)

Wang X. et al, J Med Chem. 2022; 65(4):3123

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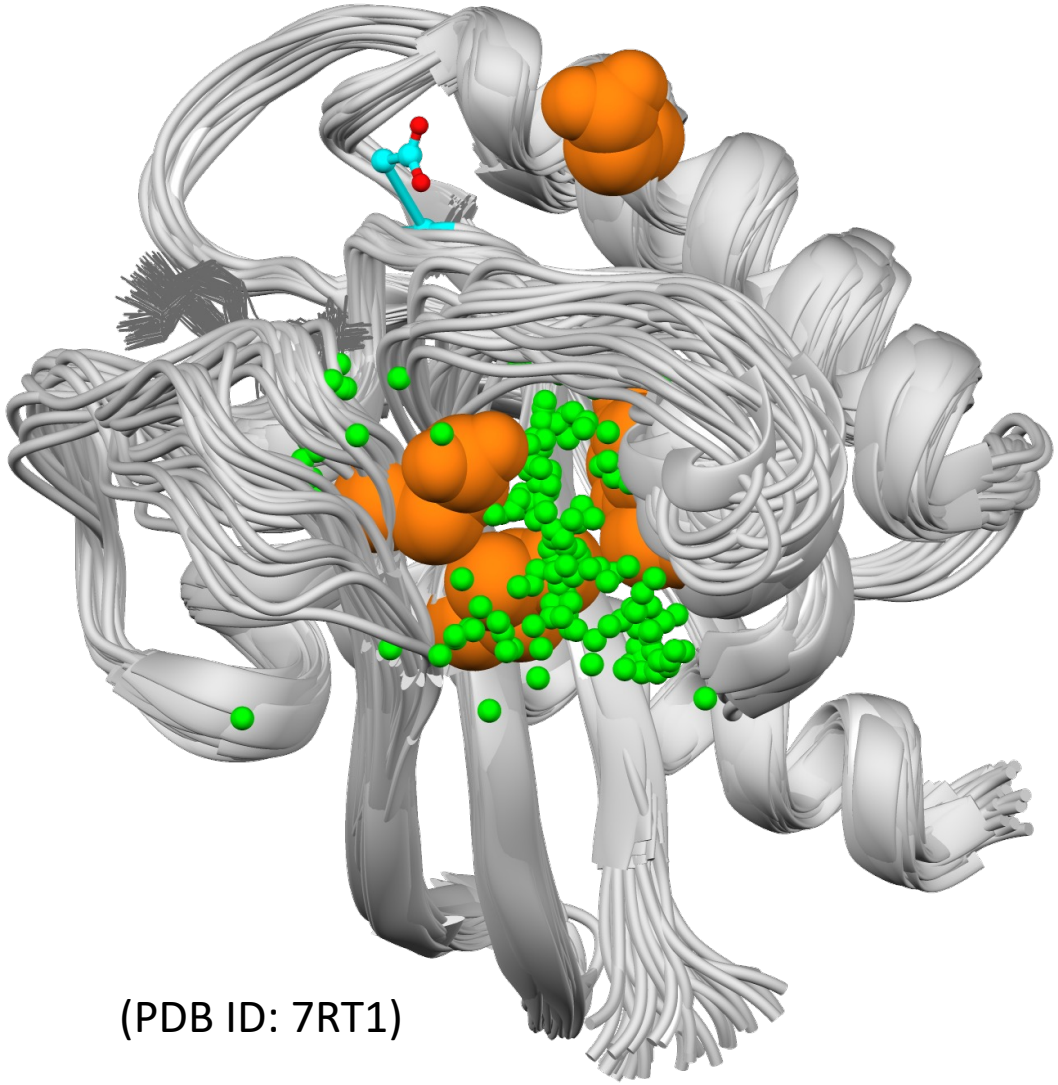
A second Xe site exists near the MRTX1133 pocket



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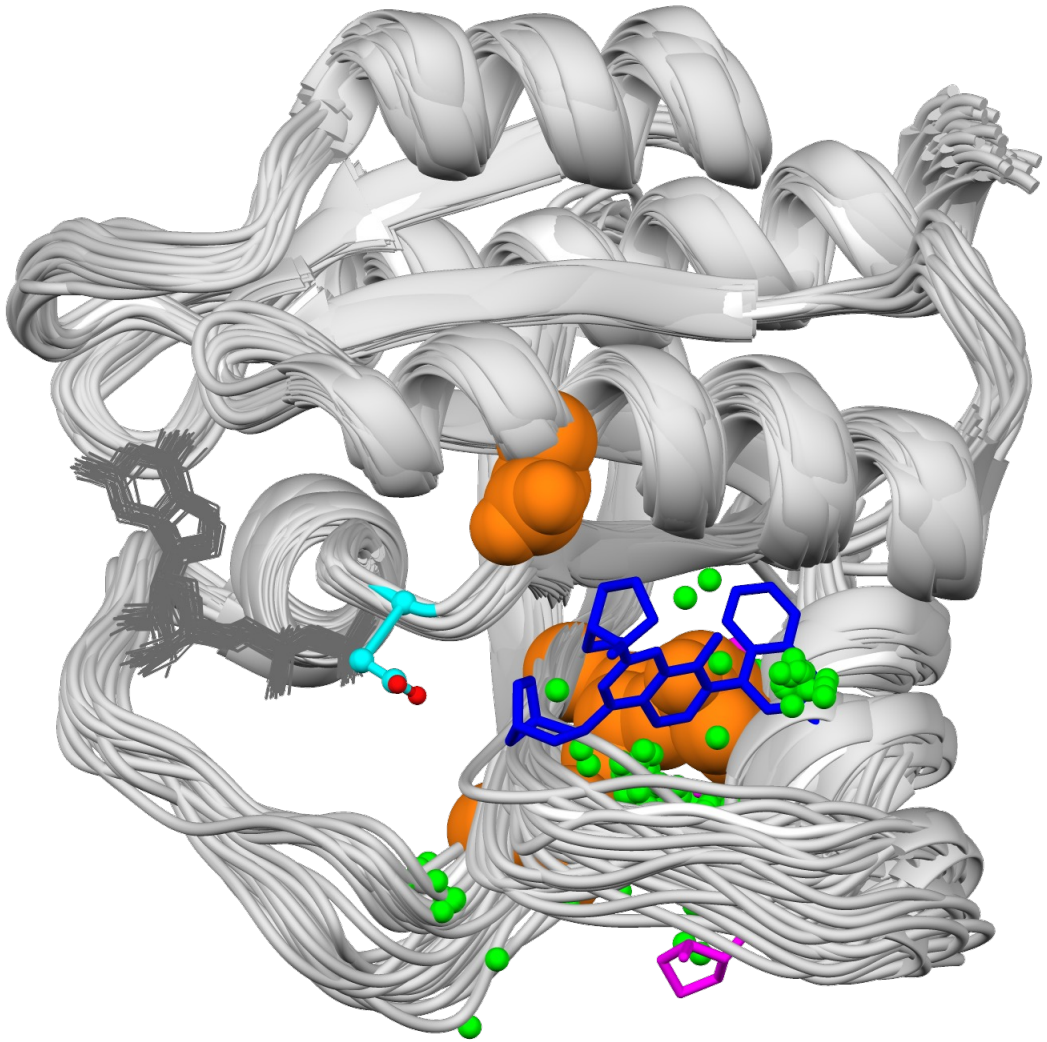

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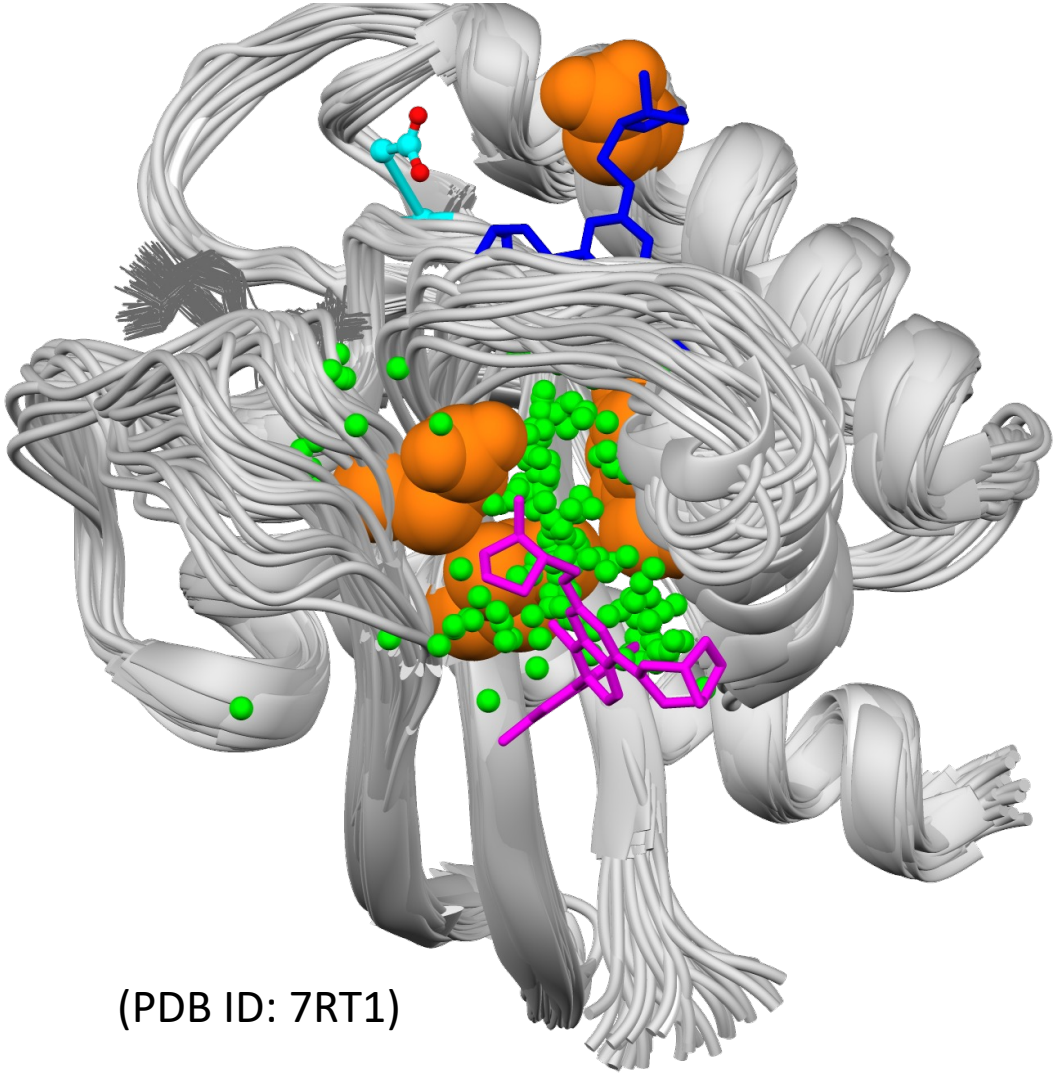
The second Xe site binds a lower potency inhibitor



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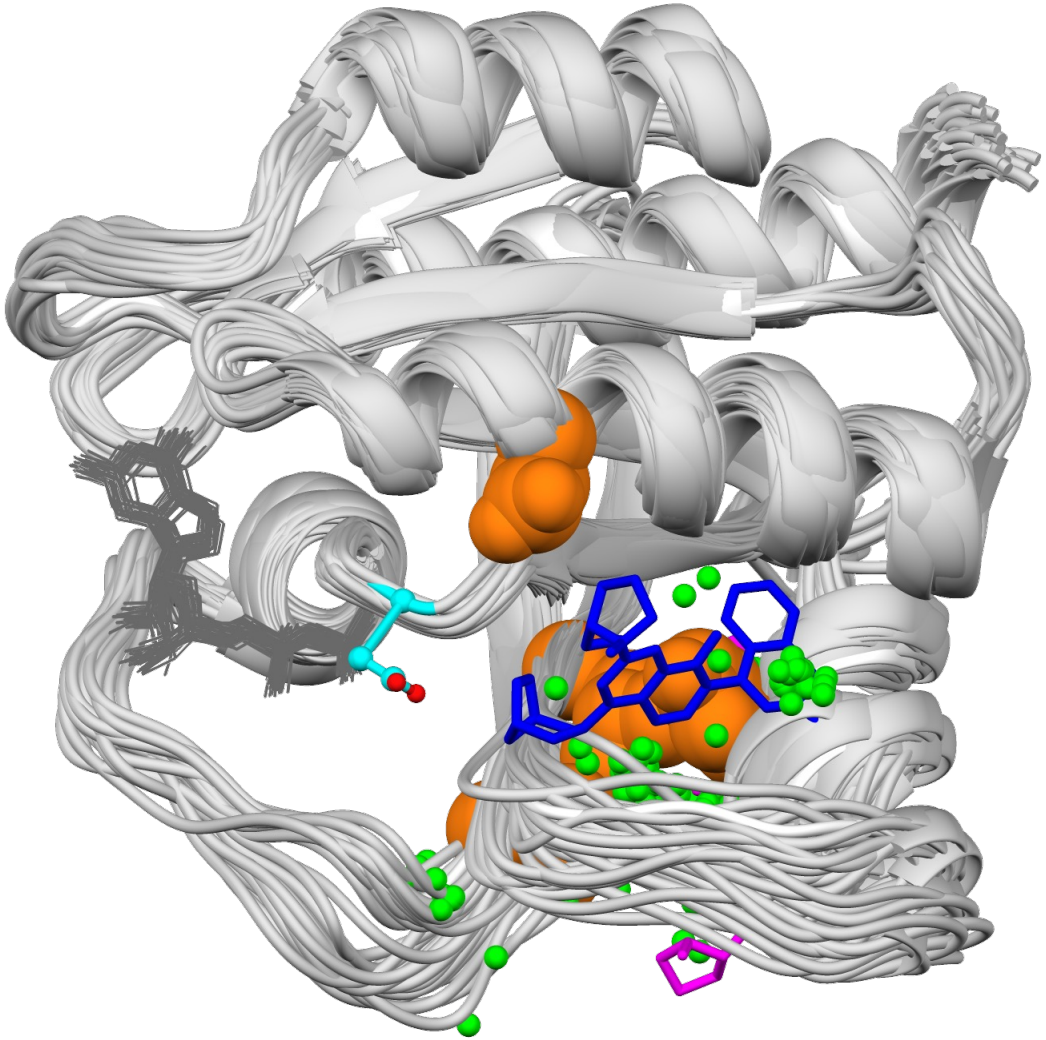

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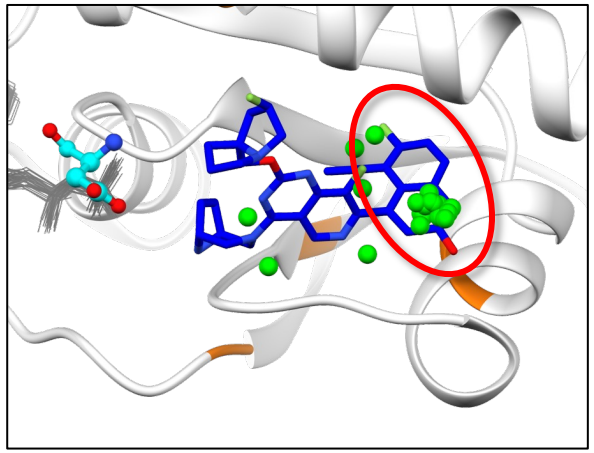
Xe favors the pyrido-pyrimidine subpocket in G12D



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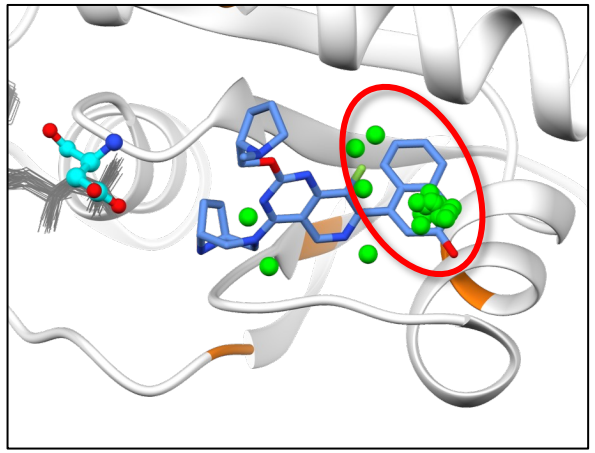

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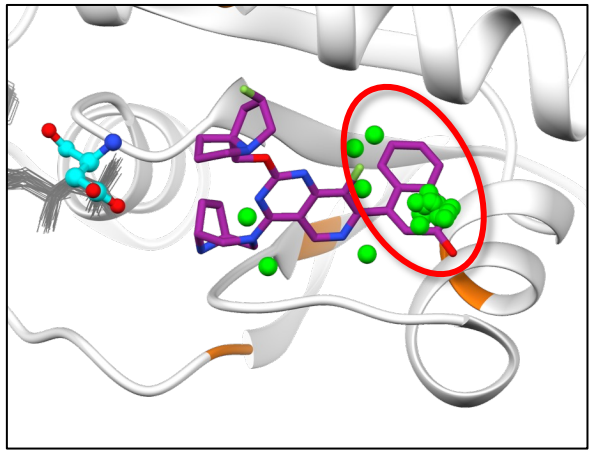

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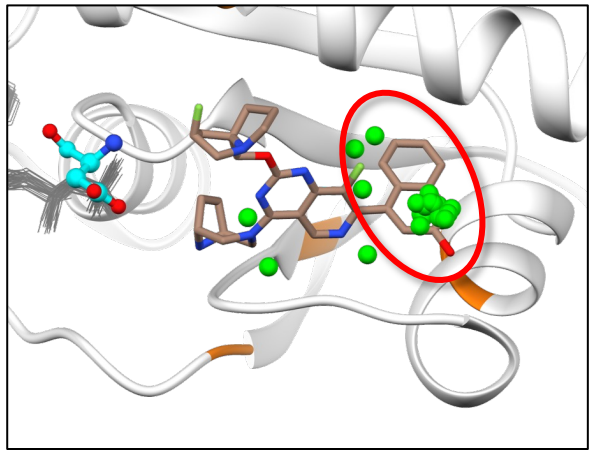

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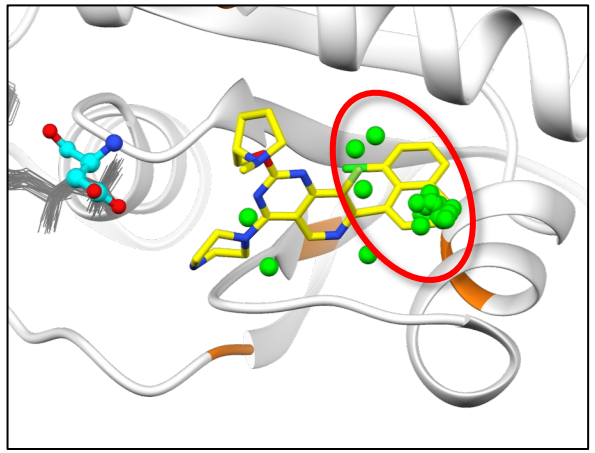

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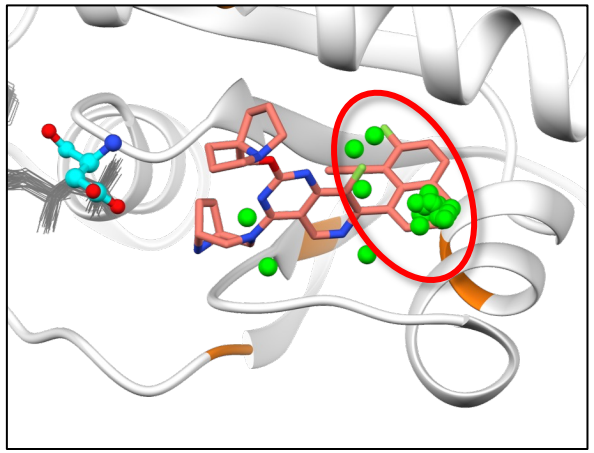

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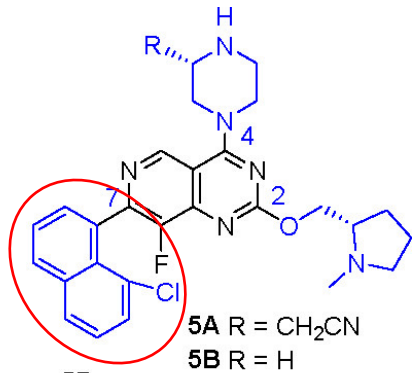

5A R = CH_2CN
5B R = H

5B
$KRAS^{G12D}$ SPR K_D 3.5 μM
$KRAS^{WT}$ SPR K_D 36 μM

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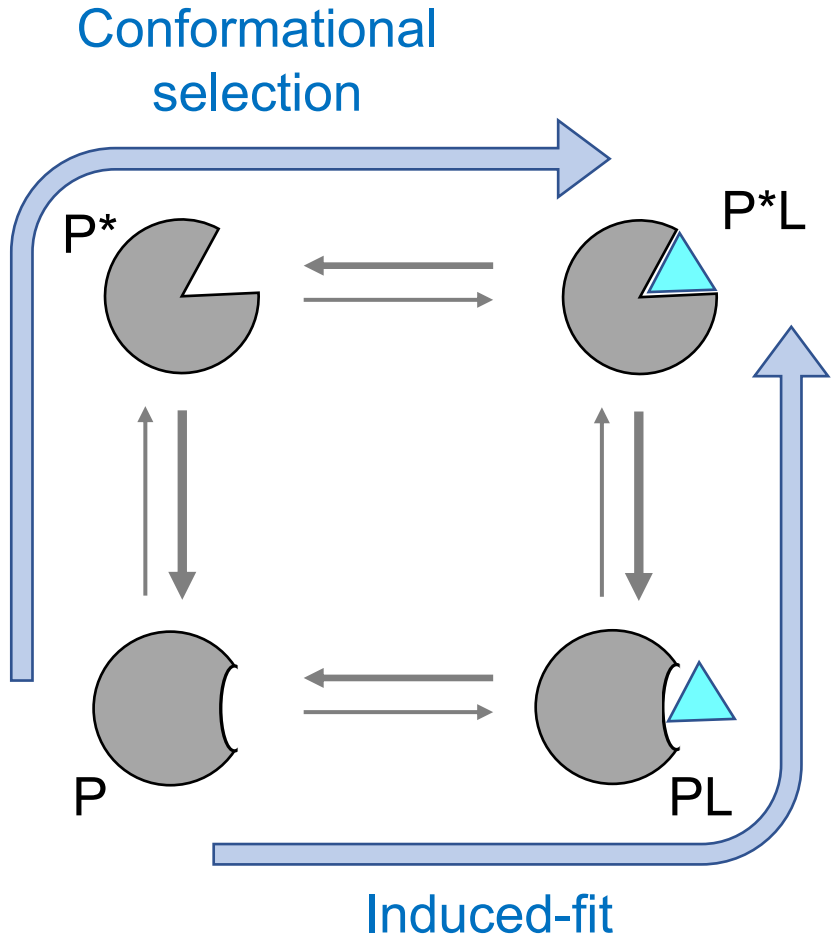
How does K-Ras form its cryptic pockets?

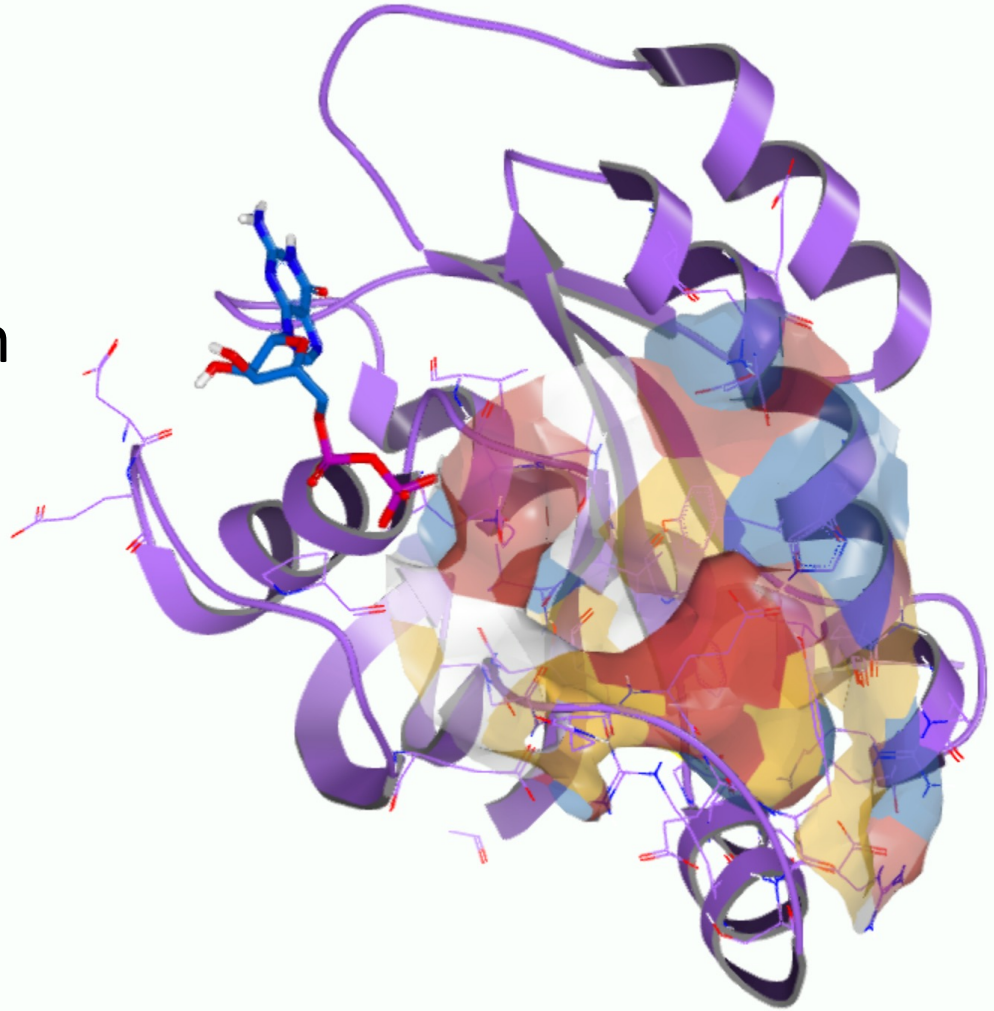
Can we classify the MRTX-1133 pocket as opening through either:

a. conformational selection or
b. induced fit mechanism?



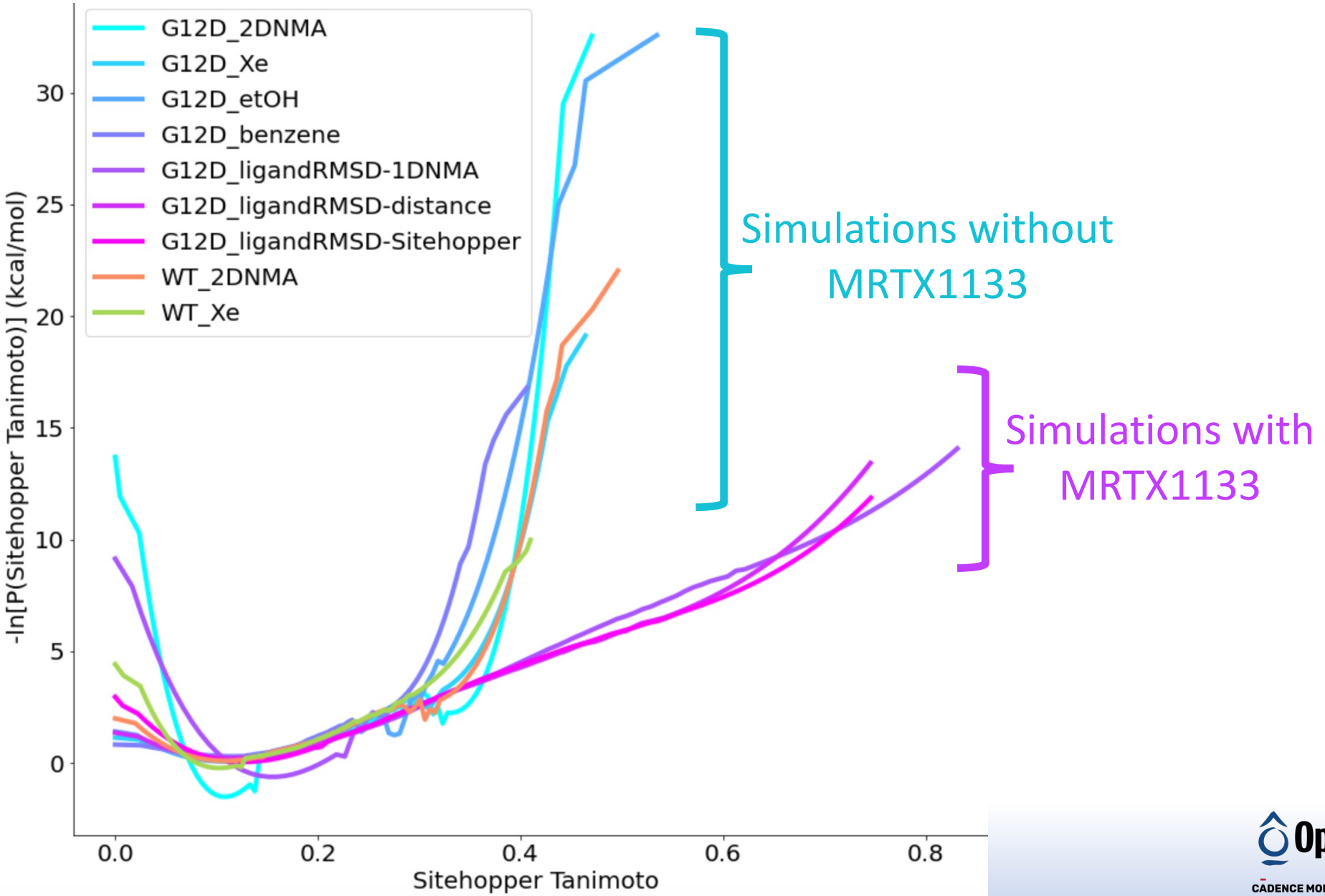
Conformational selection or induced fit for K-Ras?

- We will analyze our K-Ras data for the MRTX1133 pocket
- First, we will create a Sitehopper patch from the MRTX1133 pocket
- Then, we will take each trajectory frame, construct the same patch, and get a Sitehopper Tanimoto score.
- Compare where the Sitehopper Tanimoto score is higher – that will indicate how the pocket is formed



Sitehopper patch from K-Ras G12D MRTX1133 site (7RPZ)

Mechanism for the MRTX1133 pocket opening?



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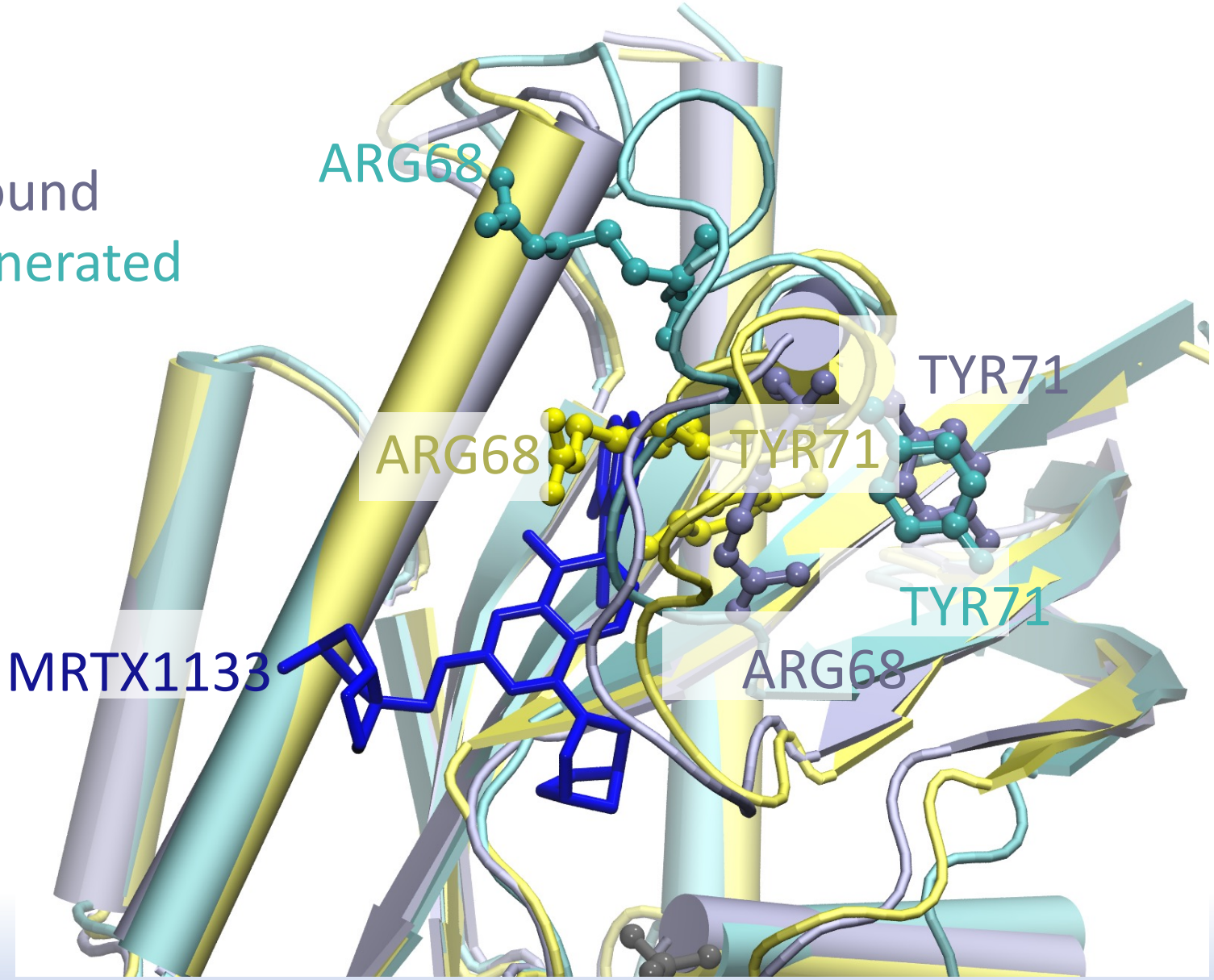
Pocket changes we see in K-Ras G12D

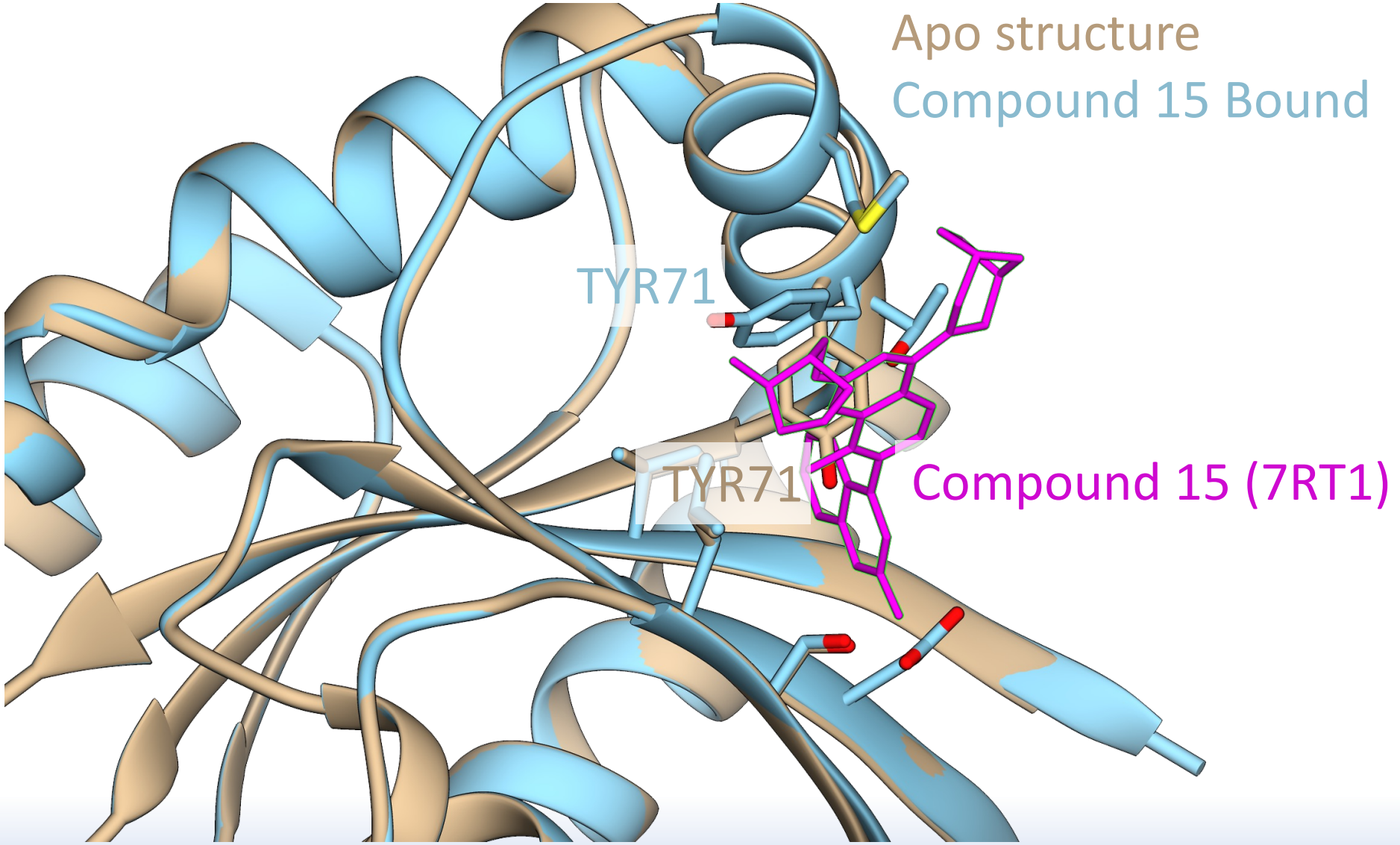
Apo structure

MRTX1133 Bound

Simulation generated



The less potent K-Ras pocket is easier to find



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Dave Lawson (Mirati) heard my talk at JCUP on normal mode analysis (NMA) sampling for cryptic pocket detection and challenged me to prove that it could work on K-Ras. This challenge was gleefully accepted.

As a test of our methodology, we used our standard Weighted Ensemble-based NMA sampling in pure water and in several cosolvents for both the WT and G12D mutant of K-Ras.

The closest pockets to D12 that were identified with our method were those where MRTX1333 and compound 15 from that series bind.

Our Sitehopper analysis suggests the MRTX133 pocket is mostly induced fit.

Acknowledgements

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Orion Backend developers

Orion Frontend developers

WESTPA developers


Thank You
The End
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