PNAS

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

Cryo–EM structure determination of small therapeutic protein targets at 3 Å–resolution using a rigid imaging scaffold

Published in 2023.07

Guo Jiayi

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA

Publisher name: NATL ACAD SCIENCES

Journal Impact Factor ™

9.4	10.8
2023	Five Year

JCR Category	Category Rank	Category Quartile
MULTIDISCIPLINARY SCIENCES *in SCIE edition*	13/134	Q1

Authors

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

Yeates, Todd O

UCLA

- Large proteins Assemblies
- Computational Genomics

Roger Castells-Graells

Post-Doc at UCLA

- Develop new imaging tools to study small proteins

Background

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

- The Cryo-EM technology allowed scientists to determine the **structure** of a specific protein.

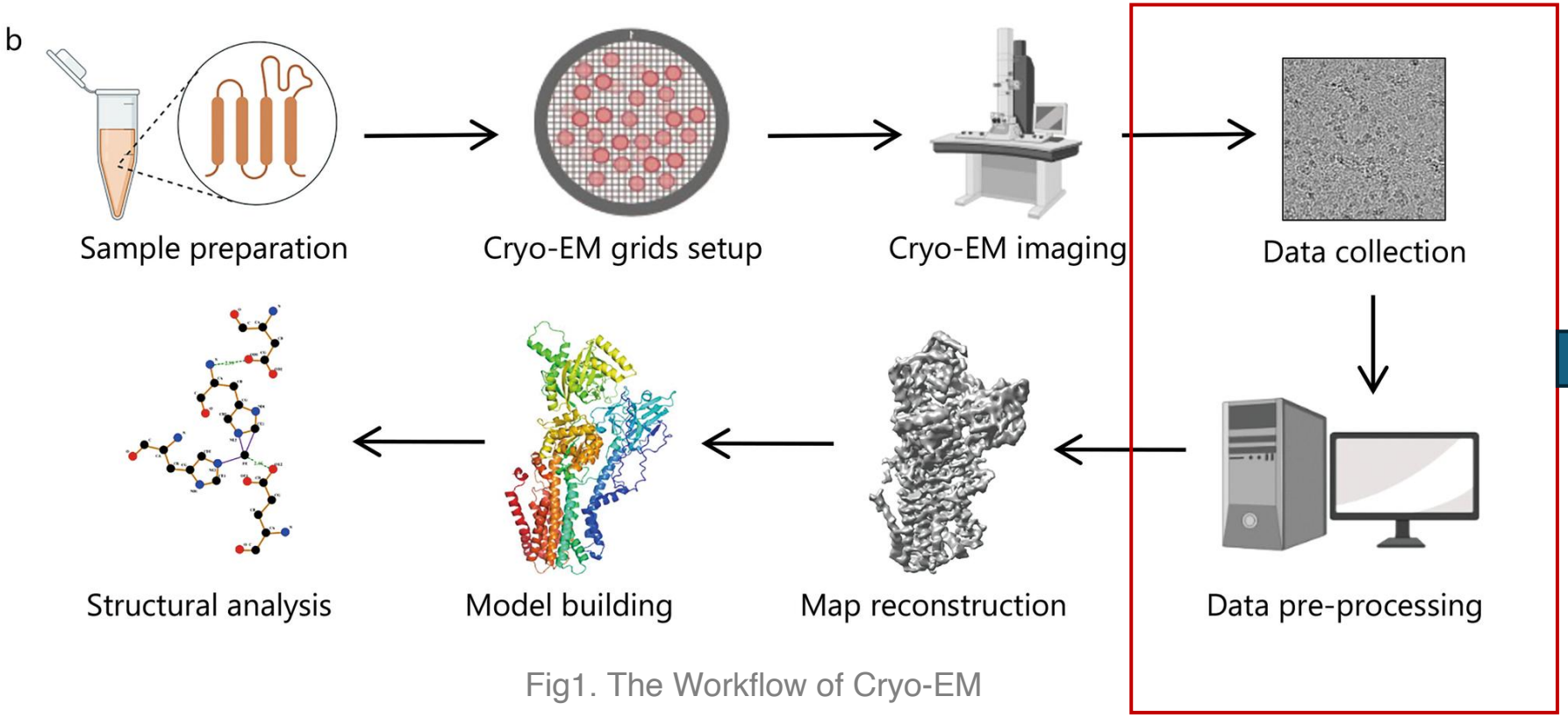


Fig1. The Workflow of Cryo-EM

- Low **signal to noise ratios**
- Poor **Contrast in images**

Background

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

- A previous cage-scaffold design reached a resolution of about 3.8Å for attached cargo protein

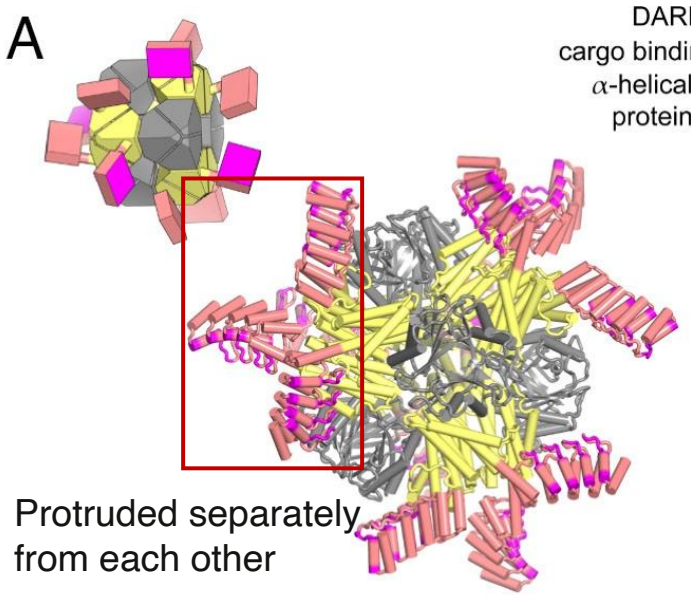


Fig1. A scheme for a previously described scaffold

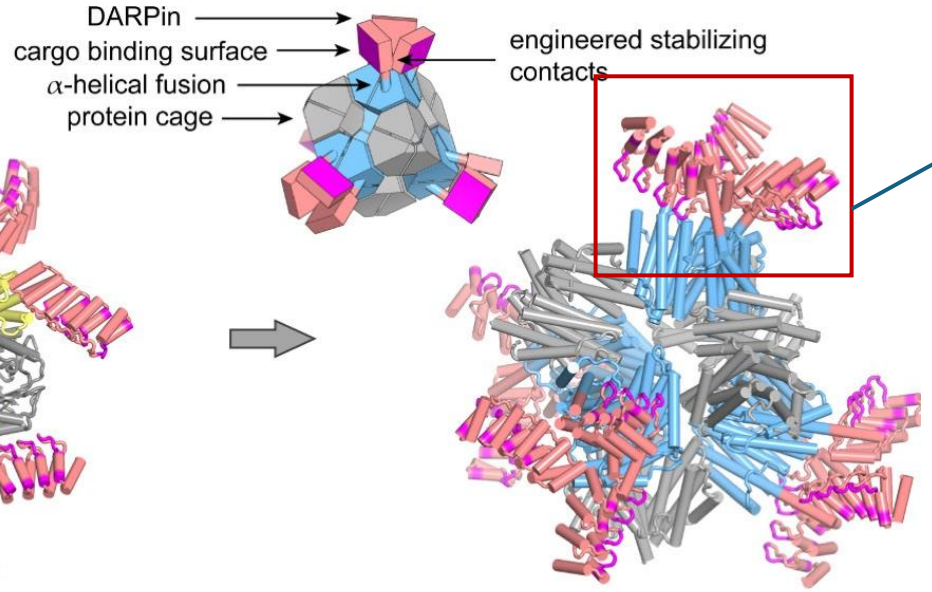


Fig2. Scheme for a redesigned scaffold

Make additional protein–protein contact
Stabilize the DARPin domain

Higher Resolution

But residual flexibility made it impossible to reach the higher resolution needed for reliable atomic interpretation

Testing of Rigidified scaffold

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

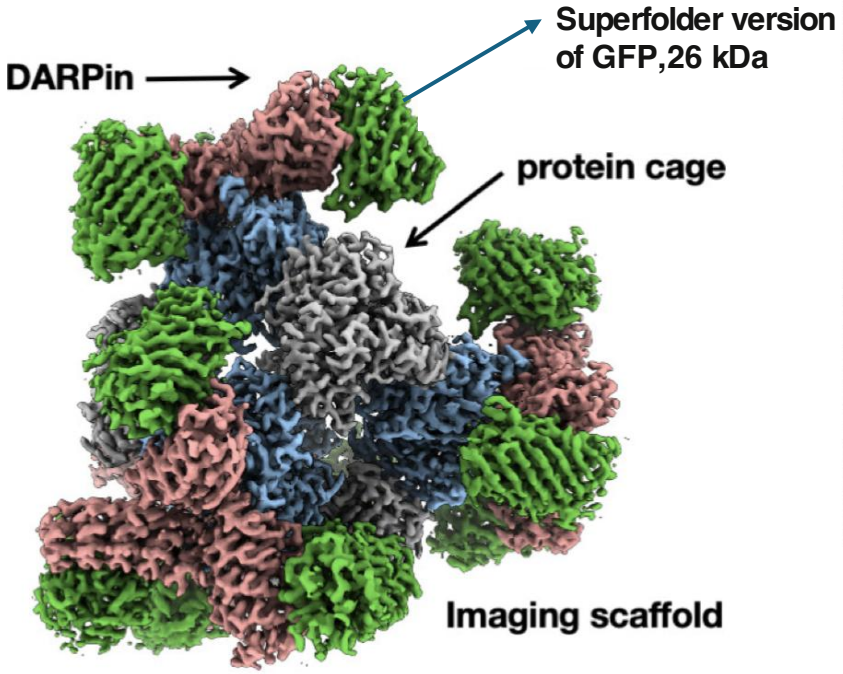


Fig1. Cryo-EM map after GFP bound to a rigidified imaging scaffold

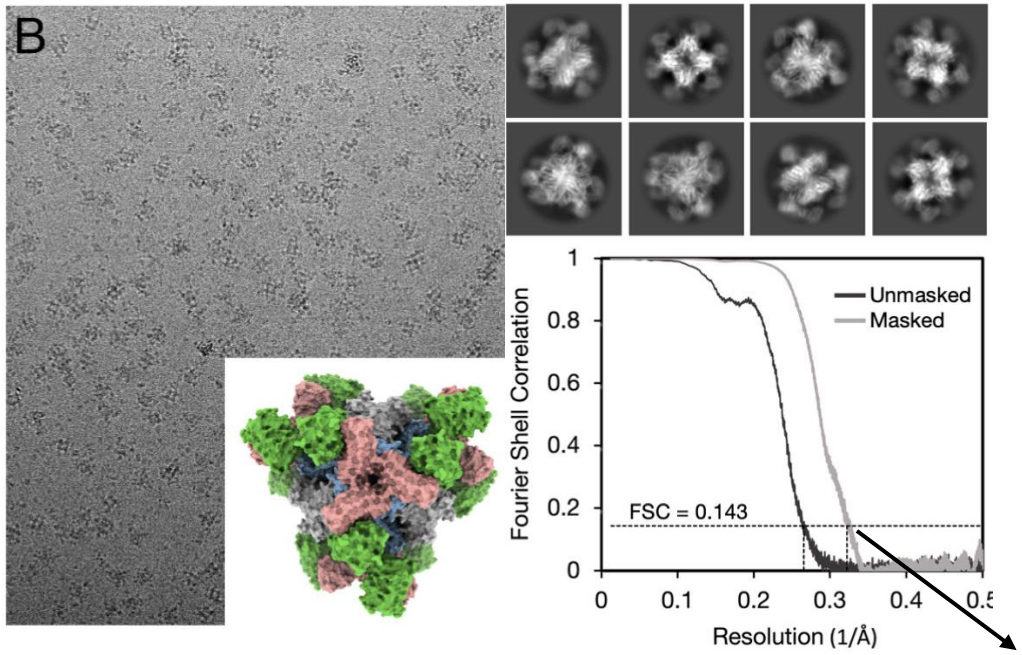


Fig2. Cryo-EM micrograph of the rigidified imaging scaffold bound to GFP

- Particles of the scaffold-GFP complex **distributed in the ice**
- The structure has been determined with a **good level of detail**

~3.0

Successfully obtained the Atomic detail of Cargo protein

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

- Obtained **density map** based on the Cryo-EM data.
- The rigidified DARPin **stabilized GFP** for detailed imaging

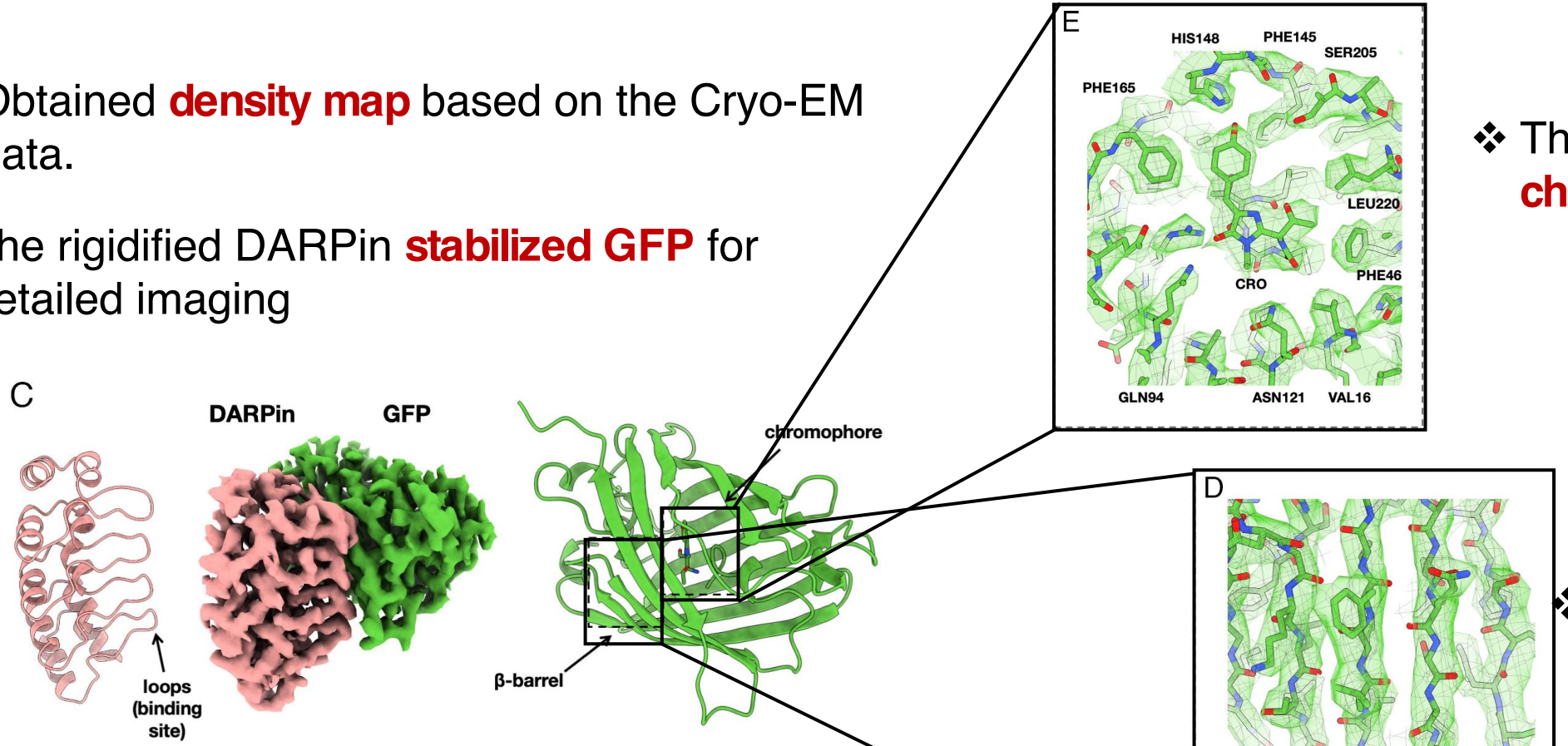


Fig1. The final density map covering the DARPin and its bound GFP protein

- The detailed view of **chromophore of GFP**
- Amplified view of the ***β*-barrel**

The Modification of scaffold can dramatically improve the stability

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

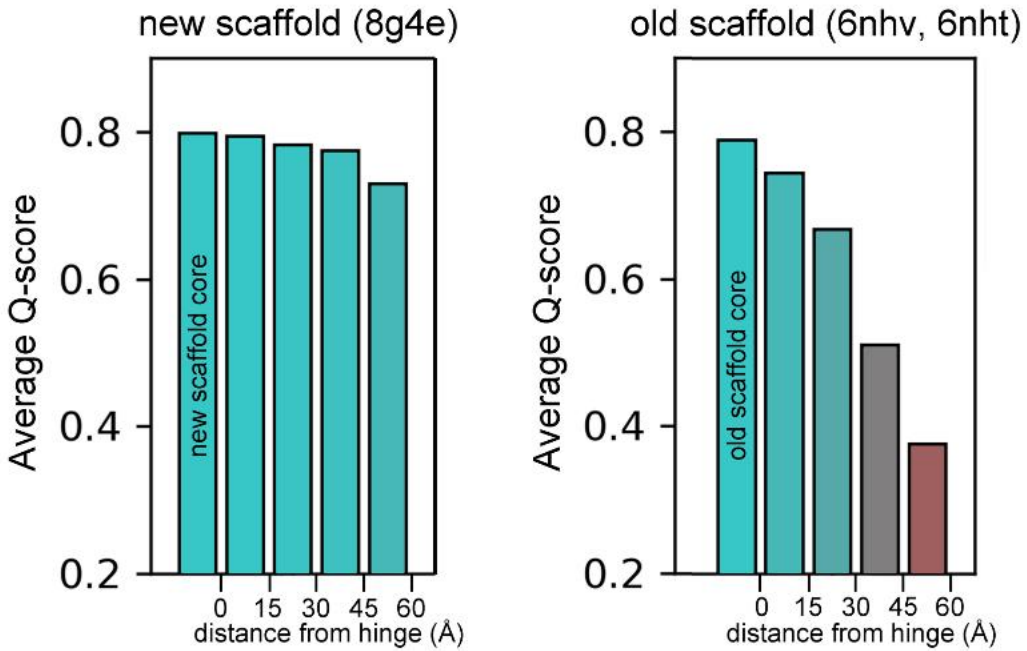


Fig1. Improvement in Q-score i.e. correspondence between the atomic model and the observed data

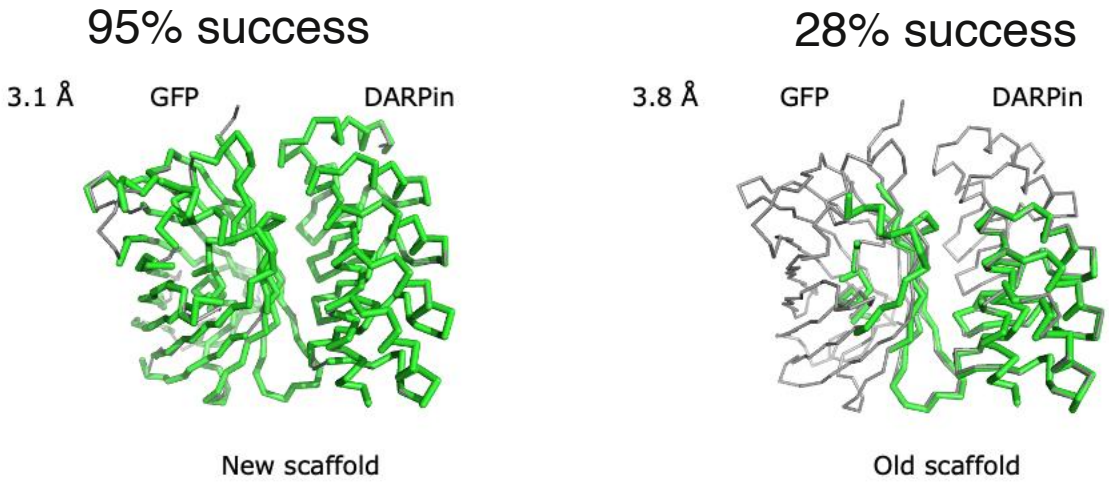


Fig2. Comparison of automatic atomic model-building using density maps from the two scaffold

- The new scaffold design effectively **reduces the flexibility** of the cargo attachment
- The prediction of Atomic model also **become more accurate** with redesigned scaffold

Cryo-EM structure of KRAS-GDP on a rigidified imaging scaffold.

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

- *"KRAS protein as a target of **high clinical importance**. KRAS is a **19-kDa GTPase** involved in signal transduction in cell proliferation pathways."*

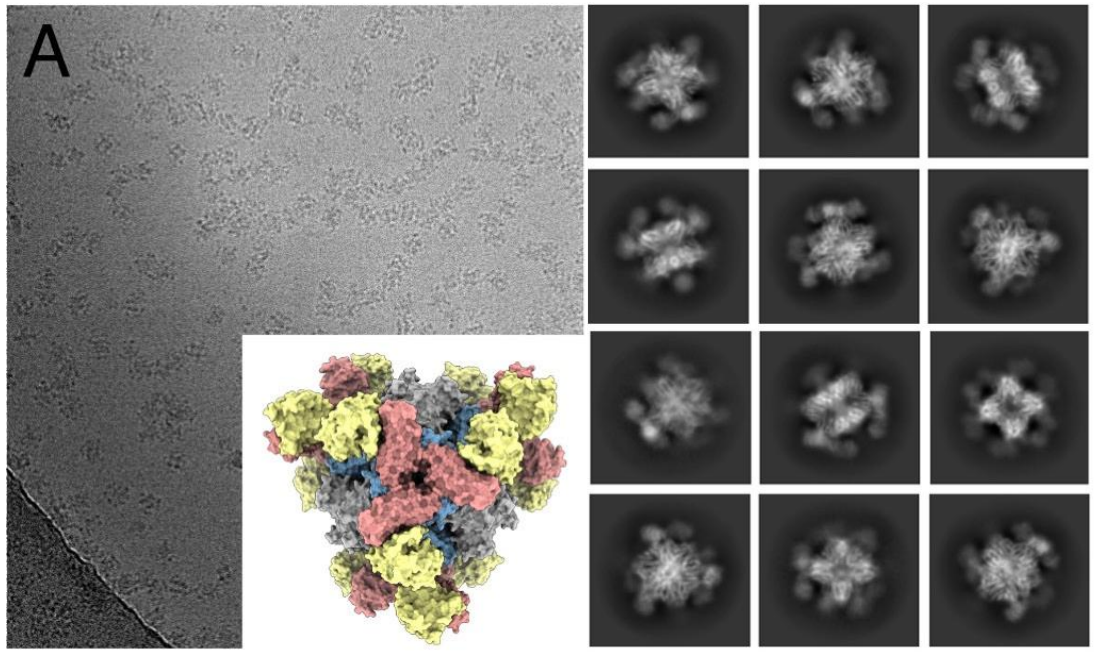


Fig1. Cryo-EM micrograph of the rigidified imaging scaffold bound to KRAS

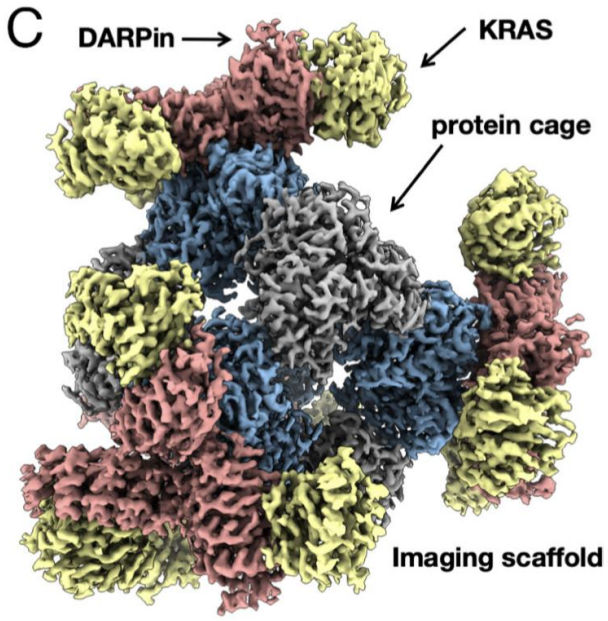


Fig2. Cryo-EM map after KRAS bound to a rigidified imaging scaffold

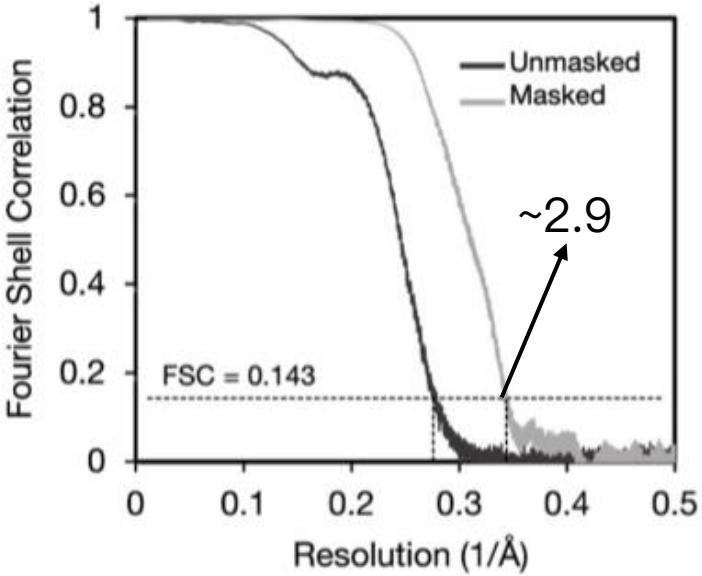


Fig3 An FSC plot illustrates agreement between independent half-maps.

- Particles of the scaffold-KRAS complex **distributed in the ice**
- The structure has been determined with a **good resolution**

The structural details of KRAS in different regions are well-resolved

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

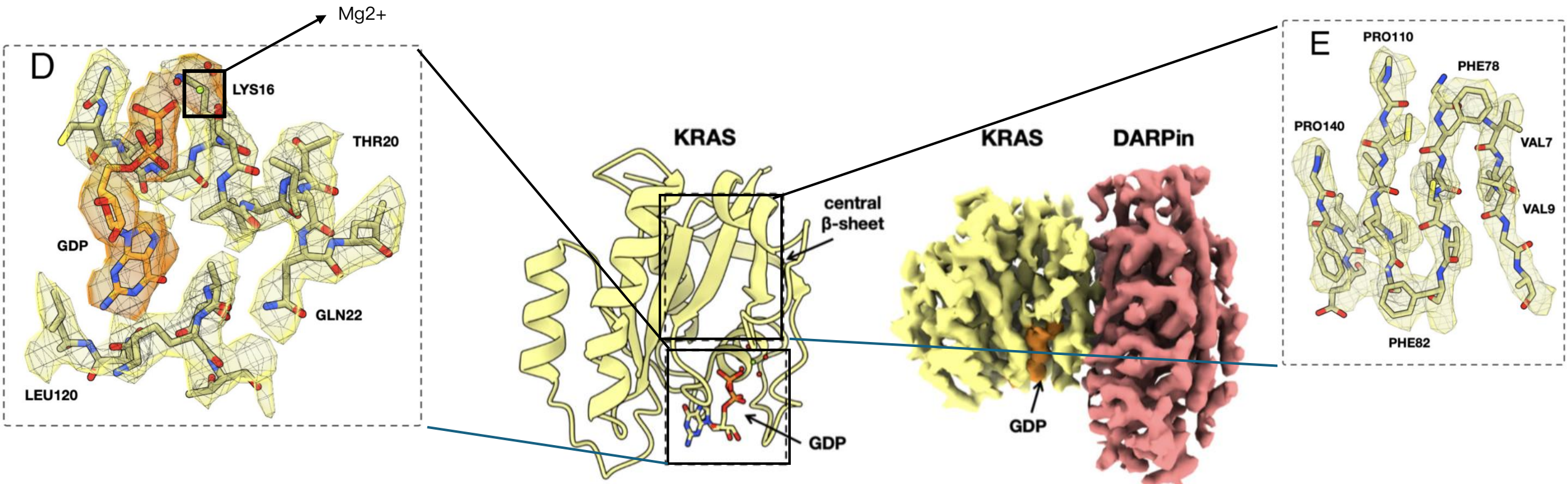


Fig1. Density map and atomic details of KRAS G13C binding with DARPin

- The scaffold reliably captures the **interaction details of KRAS with GDP**

Cryo-EM reliably captures the dynamic properties of the KRAS protein

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

- *"Our refined structure of the G13C mutant overlaps with a previous X-ray crystal structure with an rms deviation of only **0.5 Å over protein backbone atoms**."*

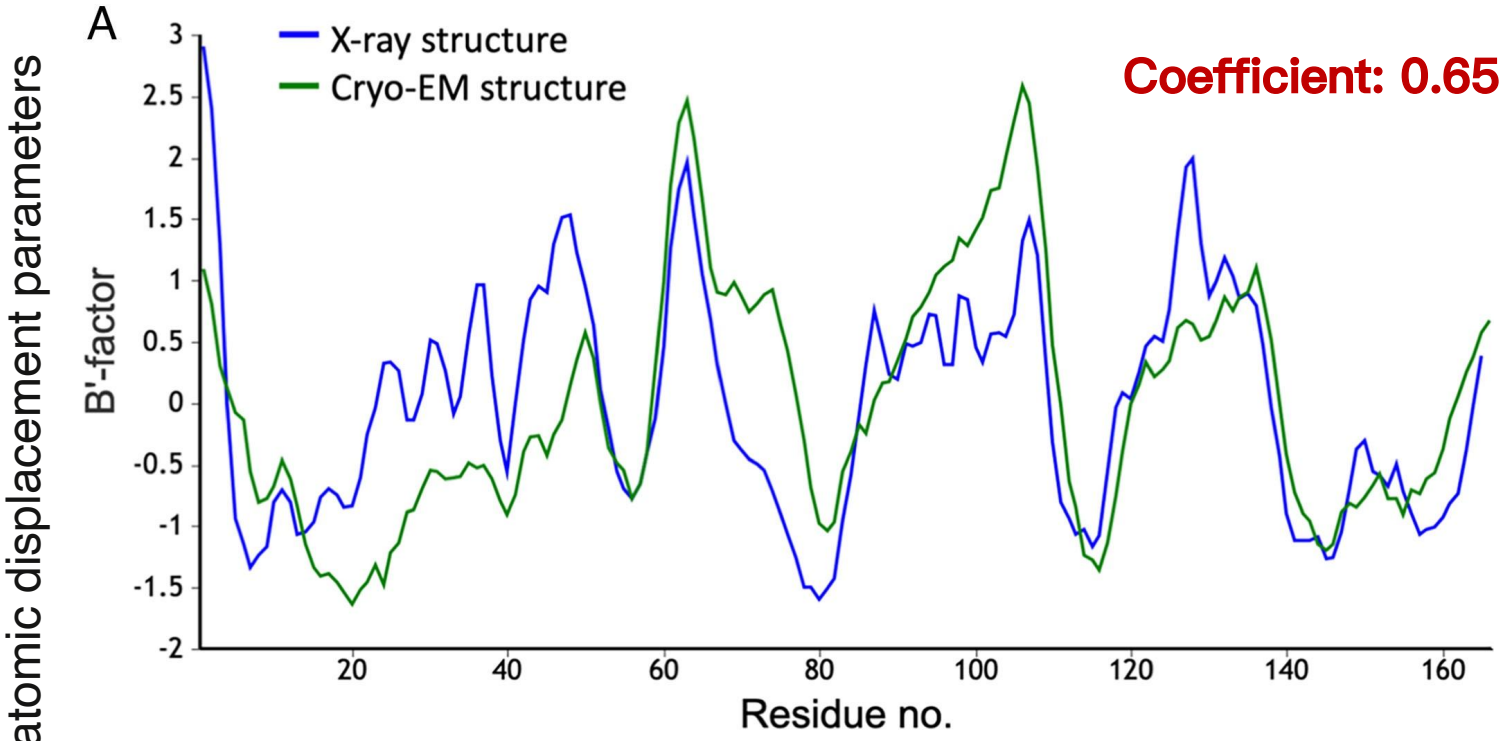


Fig1. Correlation between the flexibility profiles (as represented by B-factors) of the KRAS protein as determined by X-ray crystallography and cryo-EM.

- This rigidified scaffold **effectively restrict** the dynamics of KRAS protein, and without affecting too much the structure of KRAS

The rigidified scaffold can precisely capture the structure of different mutant

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

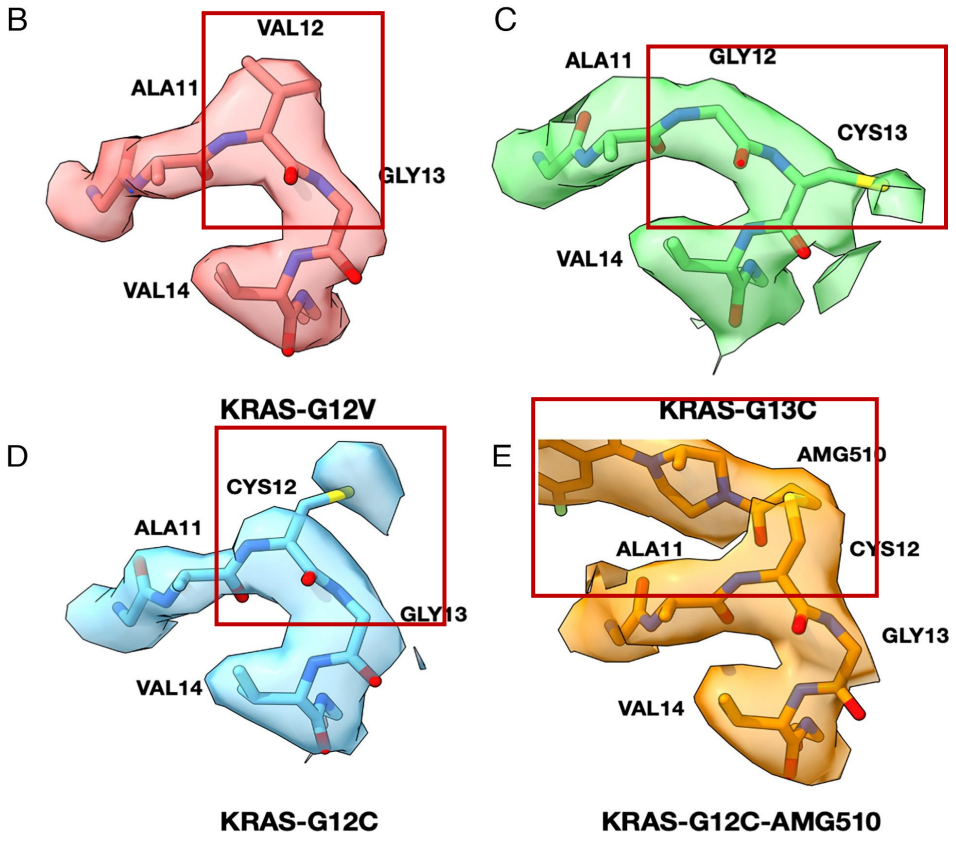


Fig1. Cryo-EM density map for different mutants of KRAS protein

- There's **significant differences** in the maps occurring only at the mutated **amino acid side chains**.

Table 1 Rmsd alpha-carbons (Å) values between X-ray structures and the cryo-EM maps

Target (pdb ID)	Moving	RMSD alpha-carbons (Å)
6oim	8G42-kras-g12c-gdp	1.08
6oim	8G47-kras-g12c-AMG510	0.73
6oim	8G4F-kras-g12v-gdp	0.99
6oim	8G4H-kras-g13c-gdp	1.07
6b9c	8G4E-gfp.pdb	0.59

- The rigidified scaffold Cryo-EM can **better capture conformational changes** than X-ray

Significant conformation changes in the KRAS G12C mutant protein upon binding the AMG510

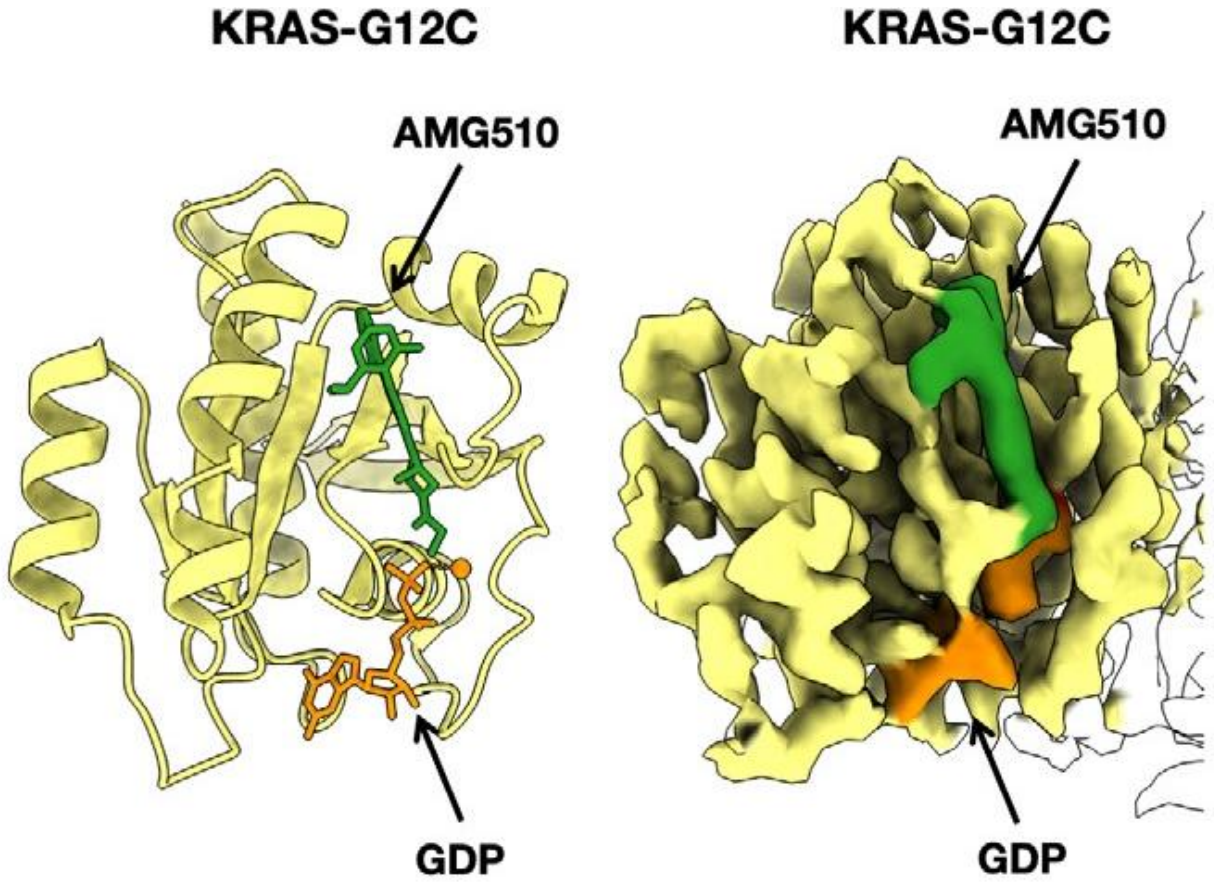


Fig1. Cryo-EM density map and atomic model for AMG510 binding with KRAS-GDP

	KRAS-G12C + imaging scaffold (RCG-1, RCG-33)	KRAS-G12C-AMG510 + imaging scaffold (RCG-1, RCG-33)
	Focused on KRAS-G12C + DARPin domain	Focused on KRAS-G12C-AMG510 + DARPin domain
Initial model used (PDB code)	5o2s	6oim
Non-hydrogen atoms	2498	2575
Protein residues	323	325
Residue range modelled	KRAS-G12C (1-165) + RCG-33 (163-320)	KRAS-G12C-AMG510 (1-168) + RCG-33 (163-319)
R.M.S. deviations		
Bond lengths (Å)	0.003	0.002
Bond angles (°)	0.46	0.44

❖ The cryo-EM result shows the structure **significantly changed** after binding to AMG510

Small chemical structural changes may also lead to significant changes structure of KRAS-drugs

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

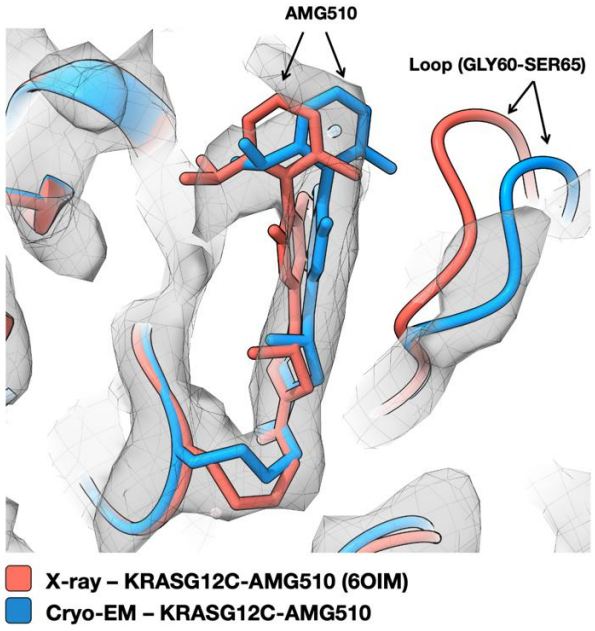


Fig1. Binding position of the AMG510 drug to KRAS G12C

Q–score for Cryo–EM: 0.59
Q–score for X–ray: 0.45

- Scaffold Cryo-EM model is a **better fit** compared to the conformation see in the X-ray crystal structure
- The **Conformation variation** of KRAS protein binding to drugs

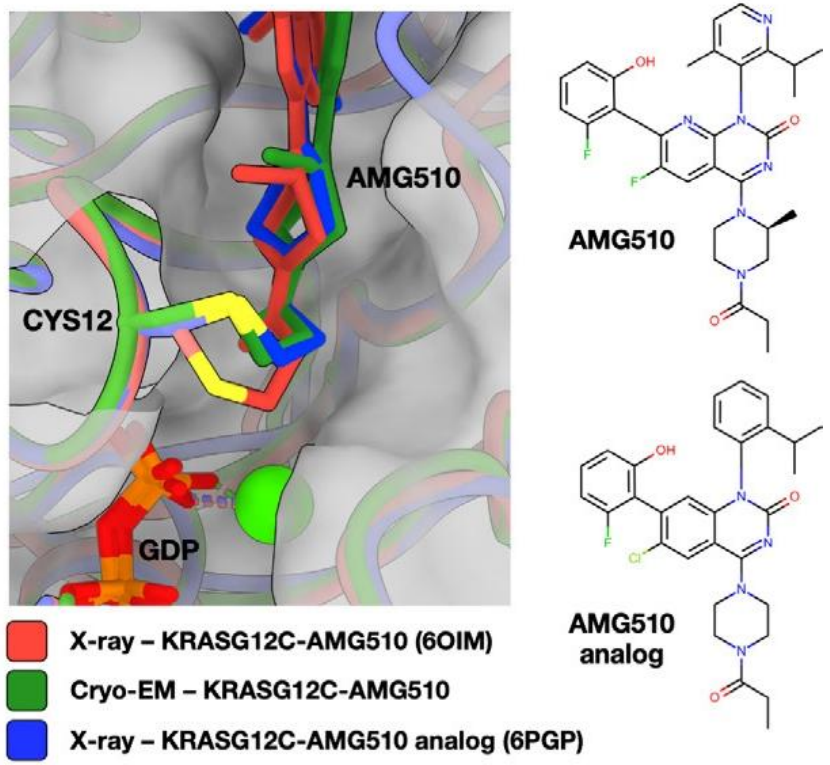
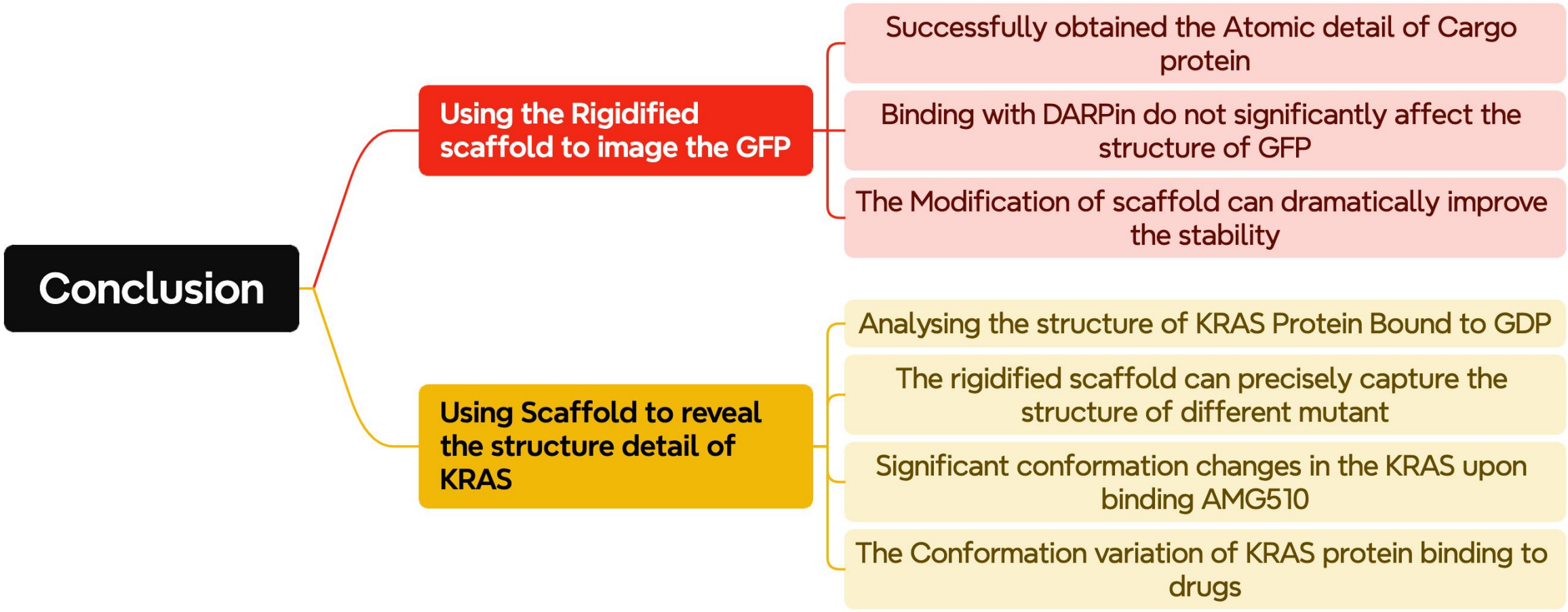


Fig2. Difference between the Conformation variation of AMG510 and its analog in Cryo-EM and X-ray

Conclusion

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY



Limitation

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

1. Did not talk about limits of this rigidified scaffold

2. The discussion about the how this rigidified scaffold work was missing

3. Did not show the structure of KRAS G12C without binding to AMG

4. Only test the default parameter of the Model Angelo while use this model to build complete atomic model

5. The drug can not bind to where DARPin binds

Questions

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

1. With this Rigidified scaffold, what kind of research can we make in the drug development?

   Conformation changes upon drug binding

2. Whether we can add more DARPin to make this scaffold more stabilized? Or Why author's test result shows the scaffold with 3 DARPin domain is the best?