

# タンパク質結晶構造解析を用いたペプチドヒット化合物の構造生物学的研究

曾我部 智 (Axcelead Drug Discovery Partners 株式会社)

## Abstract

創薬ターゲットとなりうるタンパク質のなかで、タンパク質間相互作用に関与するタンパク質や明確なリガンド結合ポケットが少ないタンパク質は、一般的に“Undruggable”なタンパク質と呼ばれている。

ターゲットタンパク質の構造学的知見を得ることは、スクリーニング後の“Hit to Lead”的段階でリガンド化合物を設計する際に大変有用である。特にヒット化合物がペプチドの場合、コンフォメーションがフレキシブルなため、結合様式や結合サイトを計算化学的手法などで推定することが難しく、ターゲットタンパク質との複合体の構造情報はヒット化合物の構造活性相関を理解する上で大変重要である。今回、昨年度に論文発表したKEAP1<sup>1</sup>, BCL6<sup>2</sup>, GPX4<sup>3</sup>, KRAS<sup>4</sup>とそのペプチドヒット化合物に関する結晶構造解析の成果を報告する。

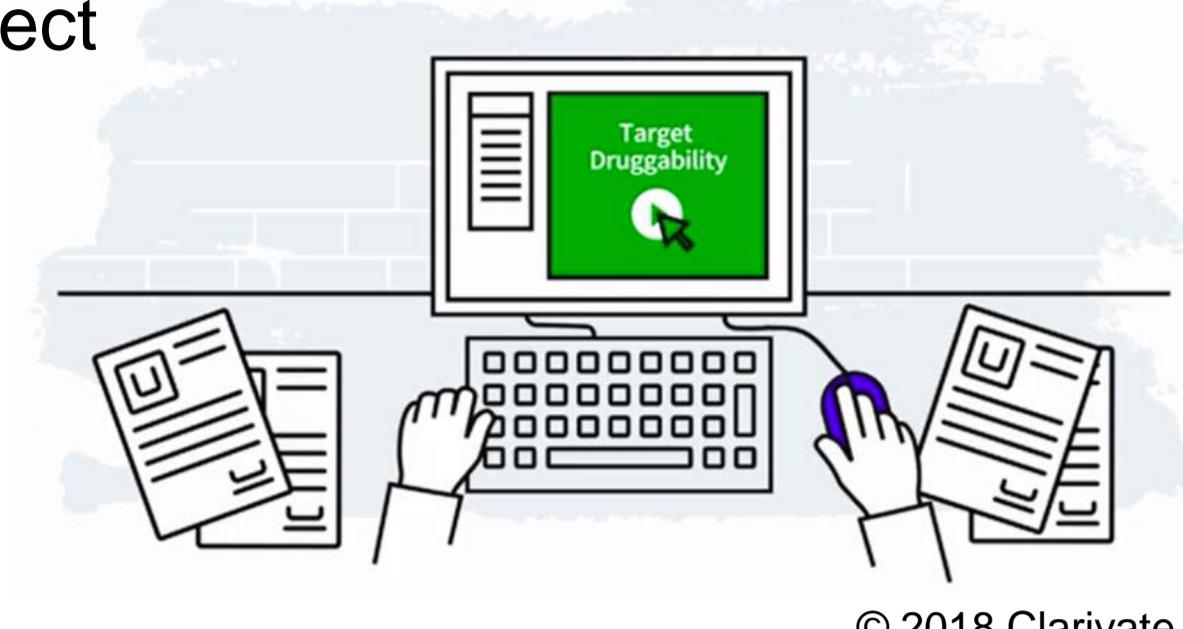
## Introduction

### Druggability prediction

- A key role in the first step of drug discovery project

### Difficult drug targets

- Protein-protein interaction targets
- Small globular targets
  - ✓ Broad interaction areas
  - ✓ Lack of well-defined pockets
  - ✓ Flexibility of residues on each interface



© 2018 Clarivate

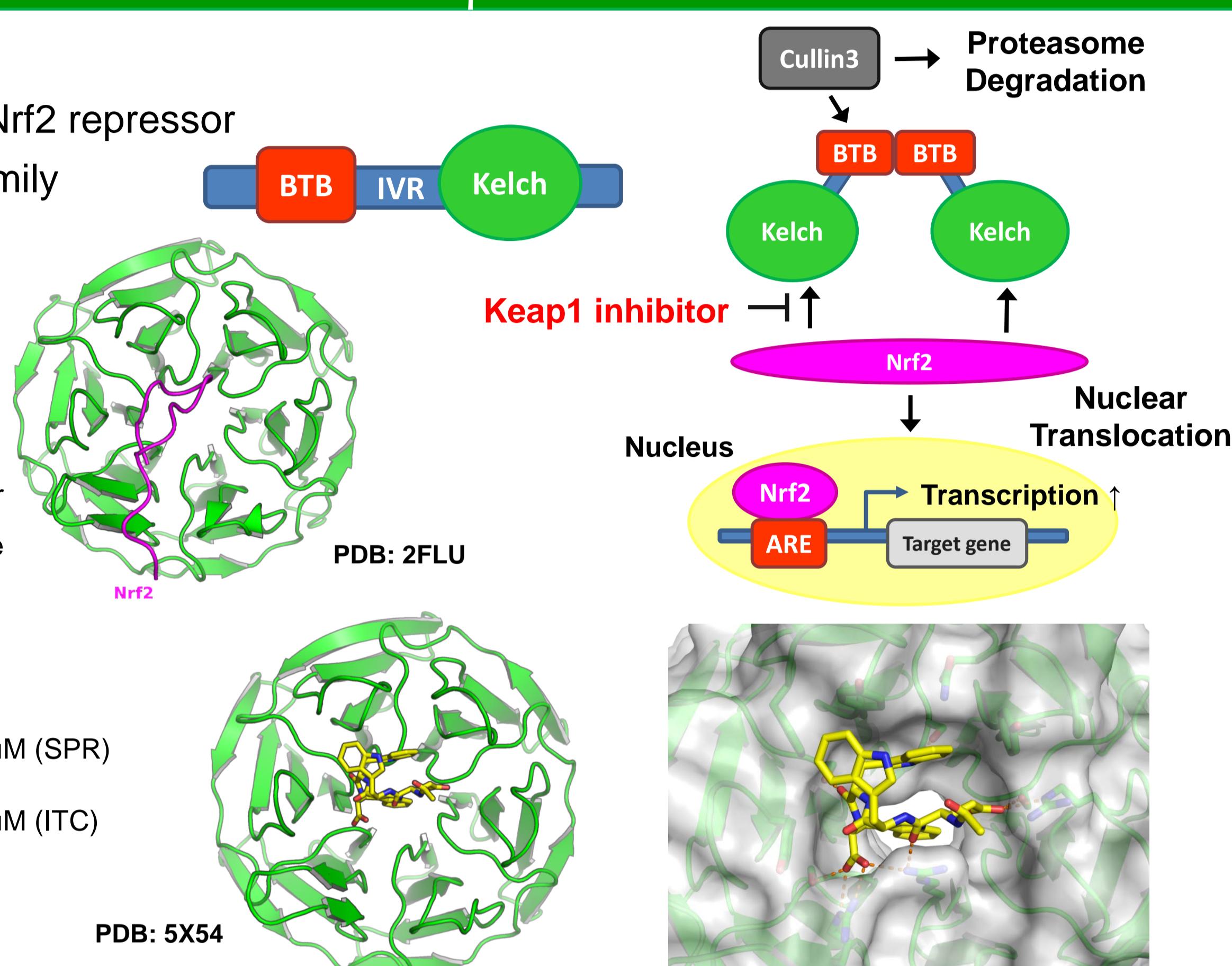
3D structures provide valuable information for further investigation.

We determined the structure of target complexed with its binding peptide.

## Keap1<sup>1</sup>

### Function

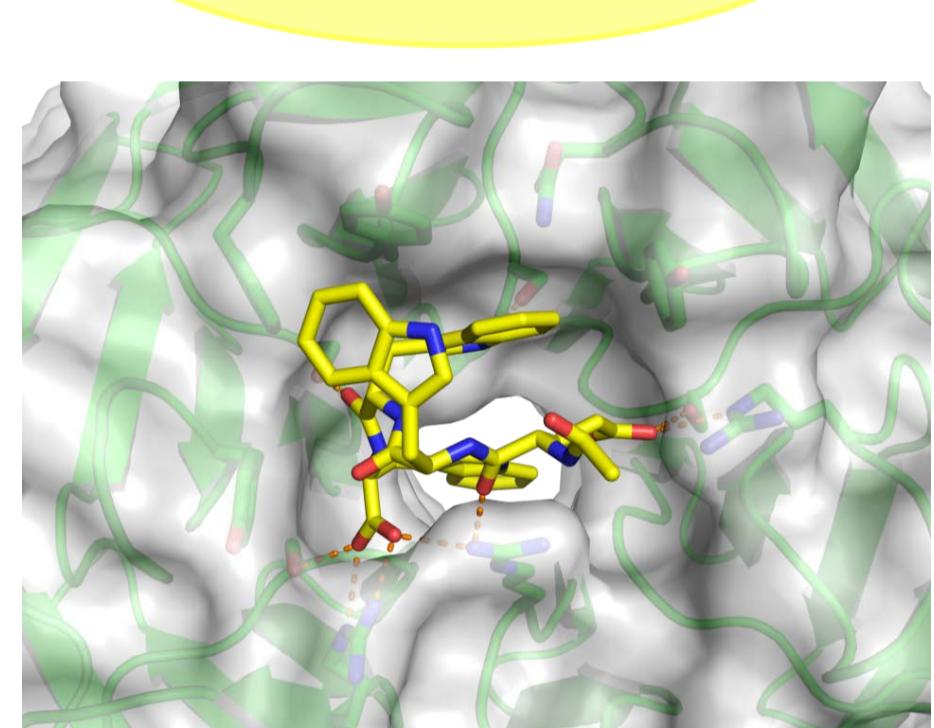
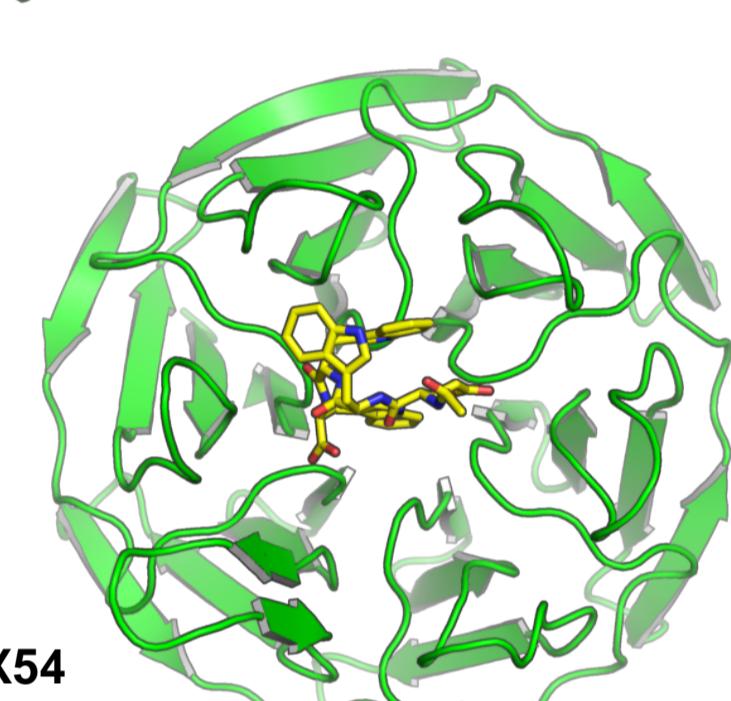
- Cytoplasmic Nrf2 repressor
- BTB-Kelch family



### Peptide

- EWWW

$K_D = 77 \mu\text{M}$  (SPR)  
 $K_D = 10 \mu\text{M}$  (ITC)

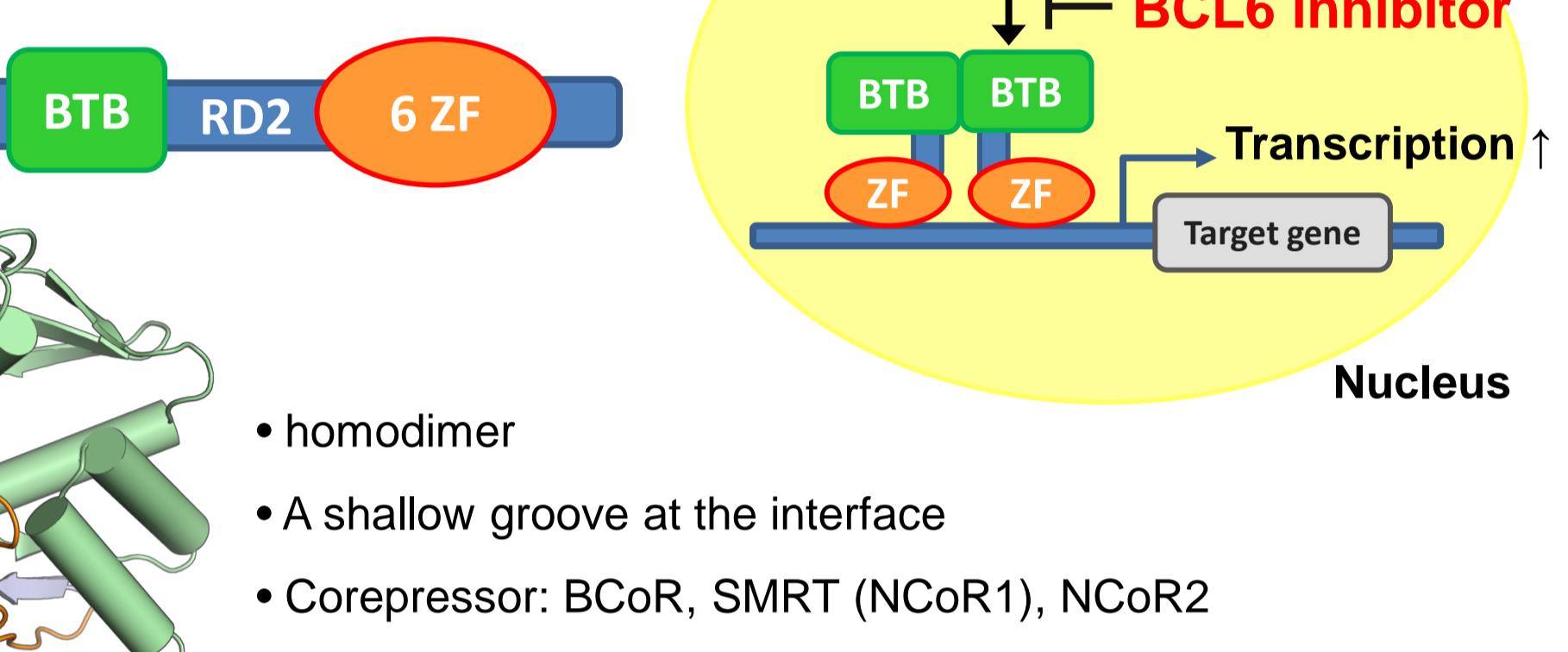


- The peptide occupies the central cavity with ligand-induced structural rearrangement.
- Binding conformation of the peptide is different from that of the Nrf2 peptide.

## BCL6<sup>2</sup>

### Function

- A transcriptional repressor of germinal centers
- BTB-Zinc finger family

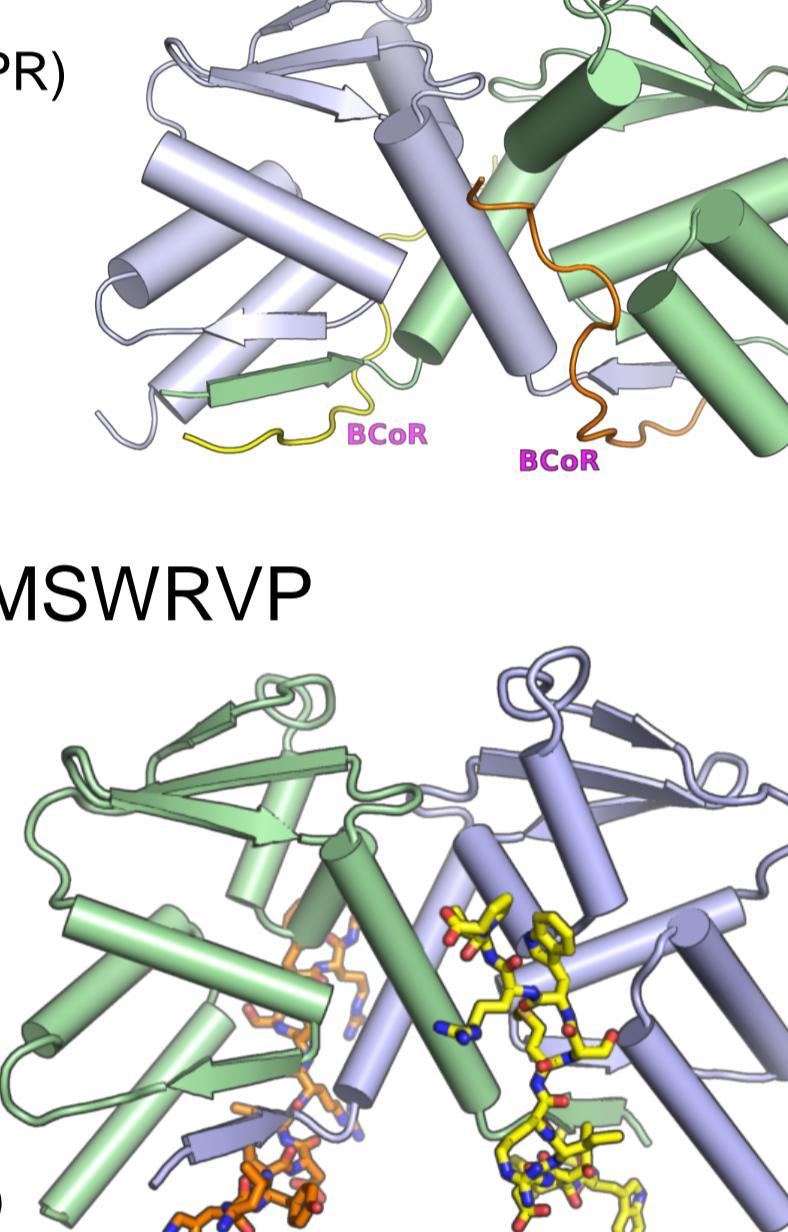


### Peptide

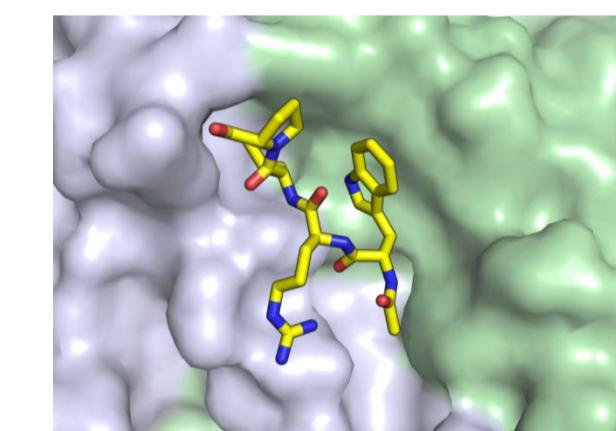
- LWYTDI RMSWRVP

PDB: 5H7G

$K_D = 0.57 \text{nM}$  (SPR)



- WRVP



PDB: 5H7H

$K_D \sim 100 \mu\text{M}$  (SPR)

- The peptide adopts a similar conformation to that of the BCoR peptide.
- Fragmentation of the peptide suggested the “hot-spot” within the binding pocket.

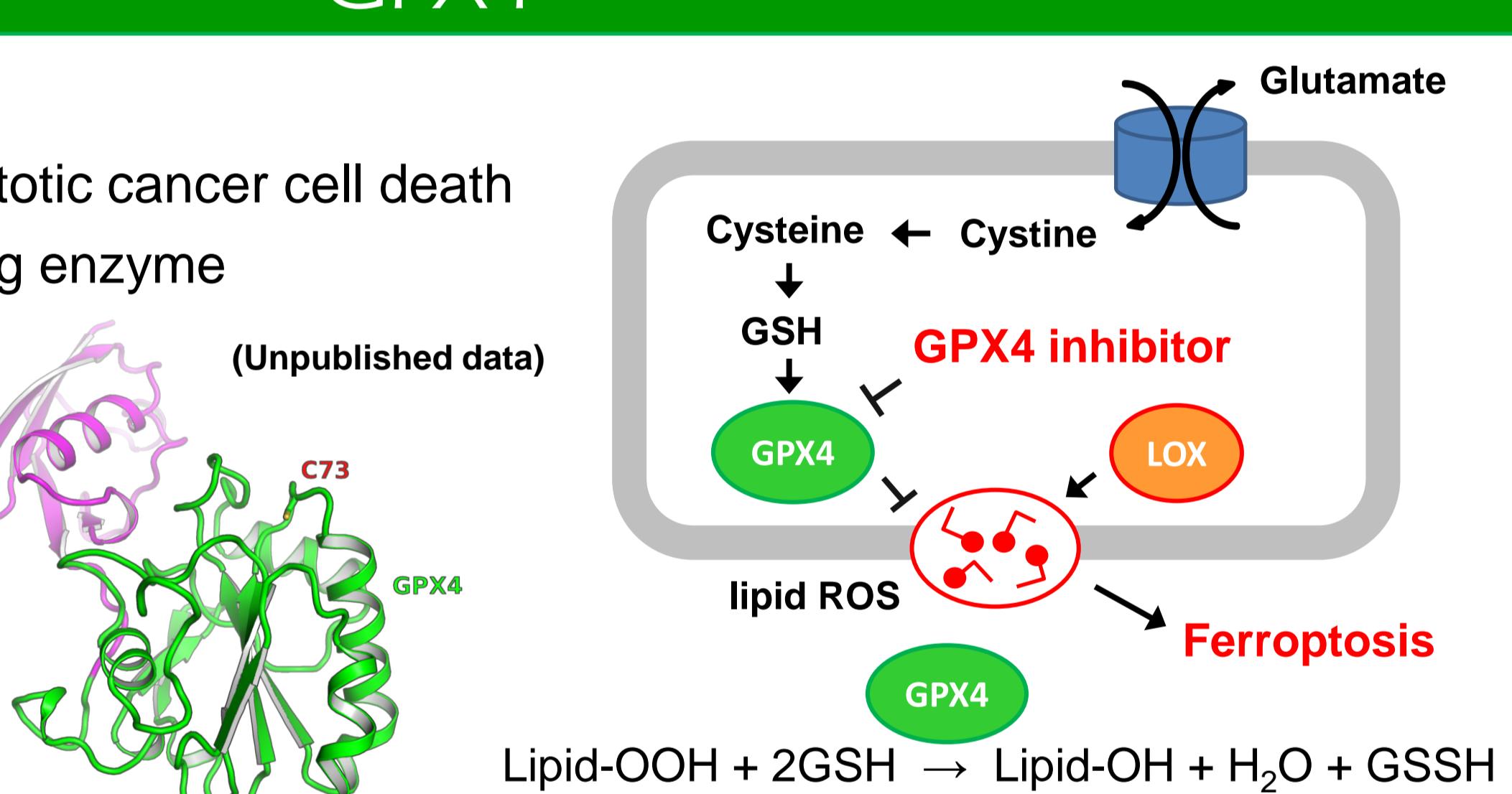
## GPX4<sup>3</sup>

### Function

- Regulator of ferroptotic cancer cell death
- selenium-containing enzyme

(Unpublished data)

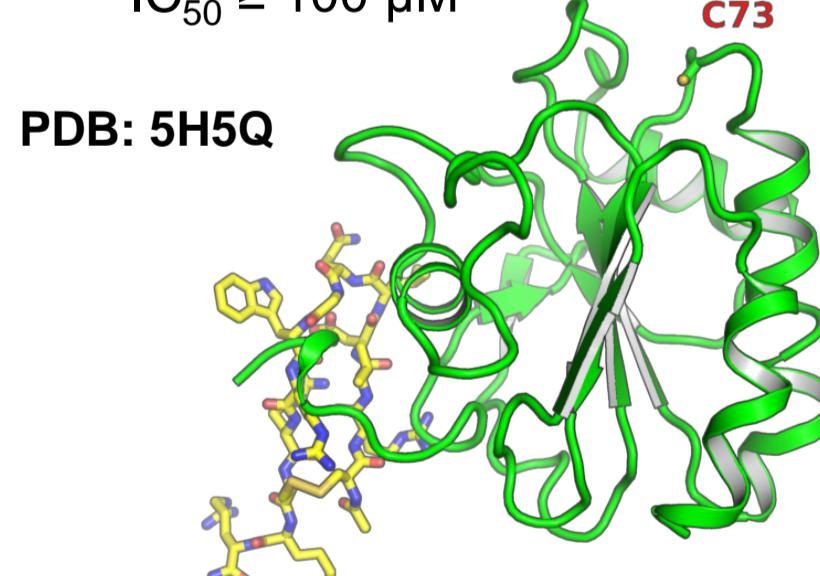
- Active site: Sec73 mutated to Cys
- No structure of the complex with ligand in PDB



### Peptide

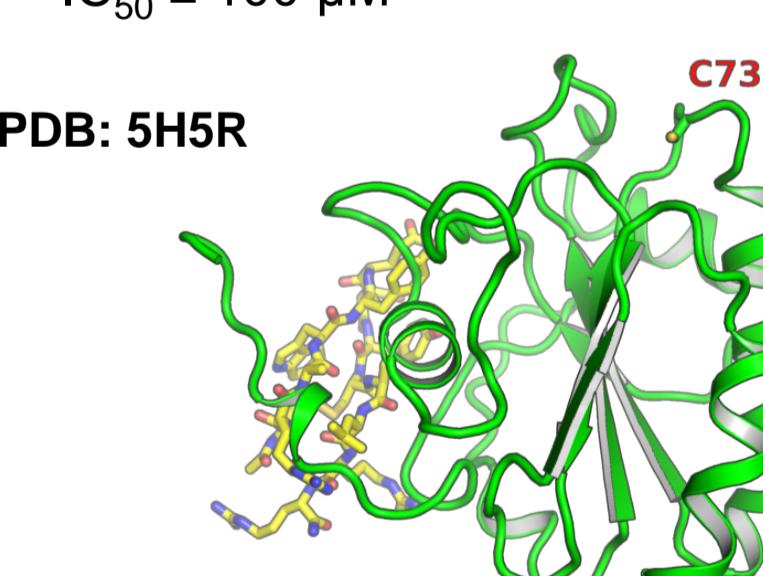
- CRVLDLQGWRRCCR

$K_D = 1.8 \mu\text{M}$  (SPR)  
 $IC_{50} \geq 100 \mu\text{M}$



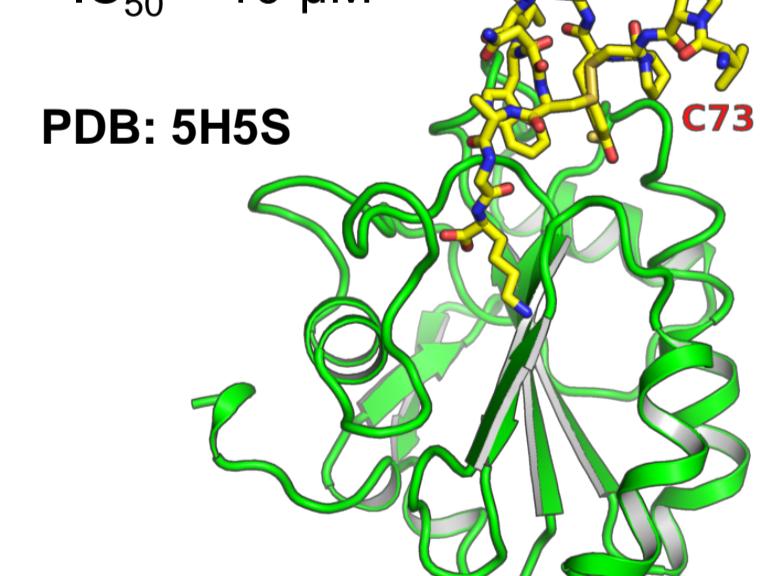
- CRAWYQNYCALRR

$K_D = 0.61 \mu\text{M}$  (SPR)  
 $IC_{50} \geq 100 \mu\text{M}$



- VPCPYLPLWNCGAK

$K_D = 8.2 \mu\text{M}$  (SPR)  
 $IC_{50} = 10 \mu\text{M}$



- One peptide binds to the catalytic site and two other peptides bind to the distinct site.
- All cyclic peptides occupy large surface area with different conformations.

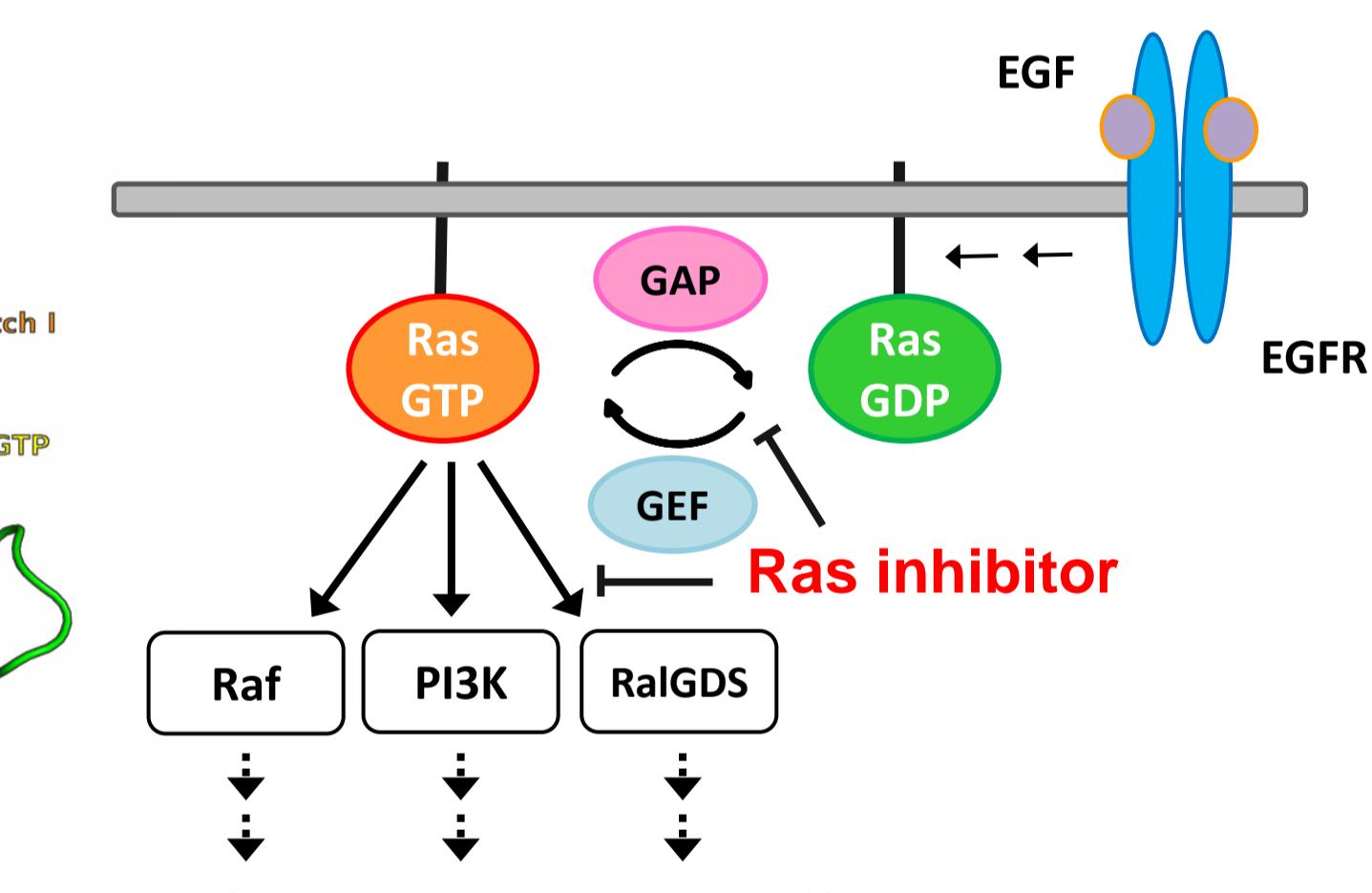
## KRAS<sup>4</sup>

### Function

- A molecular switch in cell signaling
- Small GTPase

(PDB: 3GFT)  
Switch I (residues 30–38)  
Switch II (residues 60–76)

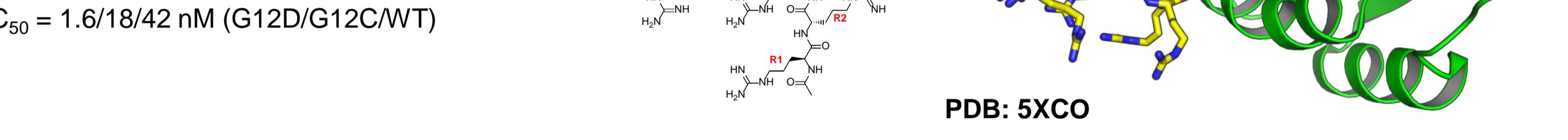
• Inactive (switch off; the GDP state)  
• Active (switch on; the GTP state)



### Peptide

- RRRRCPLYISYDPVCRRRR

$K_D = 8.9/35/58 \text{nM}$  (GDP; G12D/G12C/WT)  
 $K_D = 11/250/200 \text{nM}$  (GTP; G12D/G12C/WT)  
 $IC_{50} = 1.6/18/42 \text{nM}$  (G12D/G12C/WT)



- The peptide binds in the vicinity of Switch II, inducing structural rearrangements.
- The peptide allosterically inhibits interaction with GEF (SOS) and/or Ras effectors (Raf).

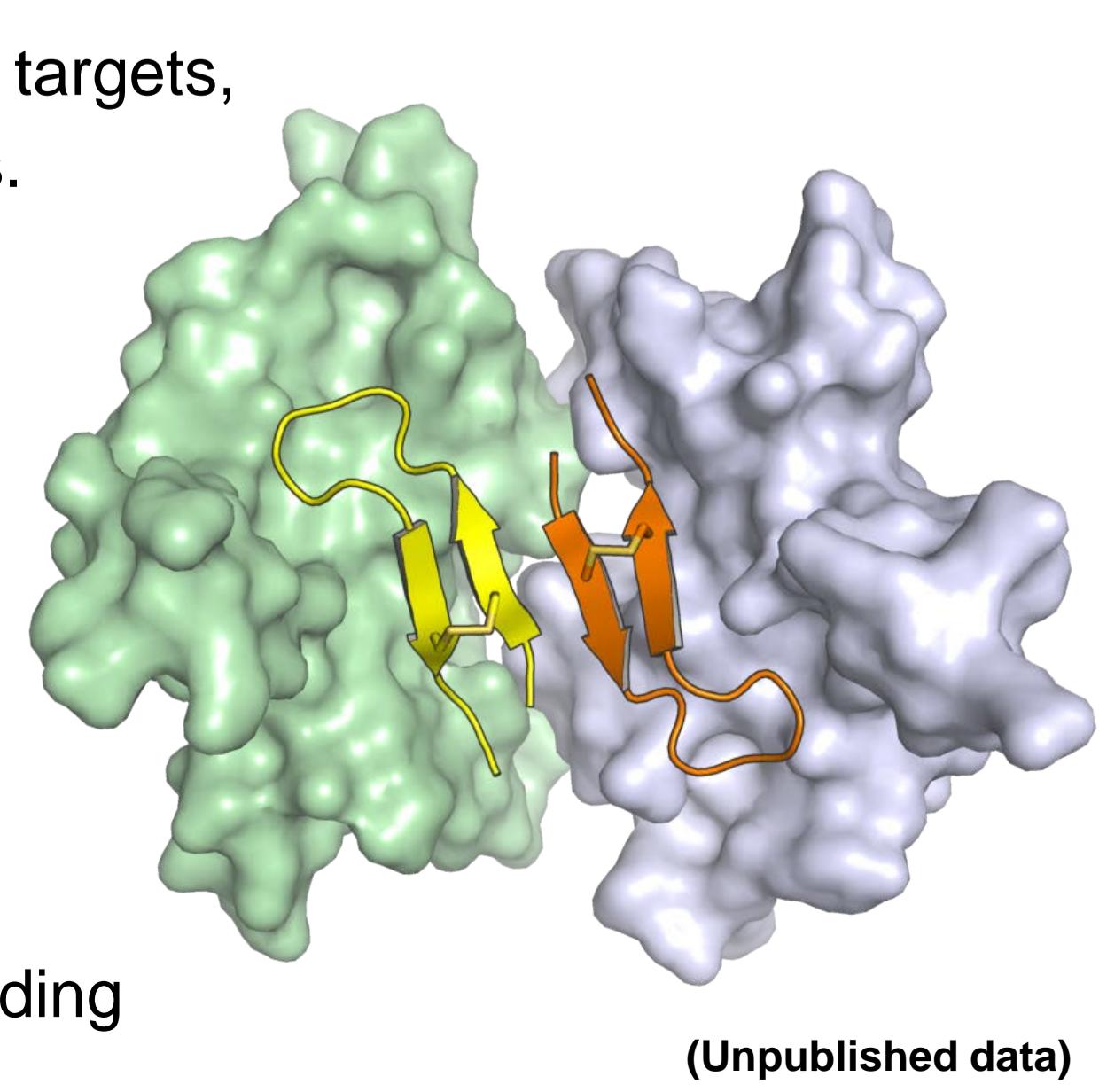
## Conclusion

### Peptide phage display screening

- The binding peptides were identified for difficult targets, such as PPI targets and small globular proteins.

### X-ray crystallography

- The binding conformation of peptides indicated “hot spot” of the targets as well as fragment and HTS hits.
- The binding site of peptides showed unique binding regions to estimate the druggability of difficult targets.
- Crystal structures provide valuable clues to better understand the probabilities of ligand binding sites for drug discovery projects.



## Acknowledgements

結晶解析 : Gyorgy Snell, Lane Weston

ペプチドスクリーニング : 坂元 孝太郎

SPR : 鎌田 祐輔

タンパク質調製 : 三輪 真敬、井田 孝、角谷 亮人、吉松 美恵

BCL6 : 坂井 望、沖 英幸、飯田 基雄、麻野 耕平、今枝 泰宏、坂本 潤一、松井 純二

Keap1 : 福田 保則、坂本 潤一 GPX4 : 松本 真一、坂本 潤一、谷 昭義

KRAS : 新居田 歩、鮫島 知哉、竜浦 政宏、米森 和子、佐々木 茂和、坂本 潤一

## References

1. Sogabe, S. et al., Biochem. Biophys. Res. Commun. 486, 620–625 (2017)
2. Sakamoto, K. et al., Biochem. Biophys. Res. Commun. 482, 310–316 (2017)
3. Sakamoto, K. et al., Biochem. Biophys. Res. Commun. 482, 195–201 (2017)
4. Sogabe, S. et al., ACS Med. Chem. Lett. 8, 732–736 (2017)

無断転載禁止