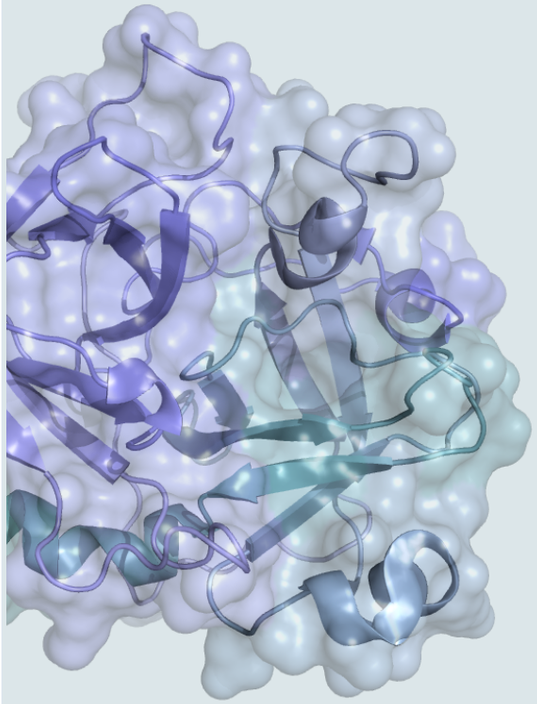
The "Undruggable" Targets of Medicinal Chemistry

Modern Therapeutic Strategies

Literature Group Meeting

May 2nd, 2025

Katherine Burton
MacMillan Lab
Princeton University



Modification Strategies for Undruggable Targets

Targeted Interactors

Part 1

Part 2

Designer Peptidomimetics and Synthetic Biologics

Exploiting structure-function properties to access novel therapeutics

Part 3

Prions and Multi drug resistance: The Looming Plagues

Investigating Druggability

What defines a target's druggability?

Swenson, C. S., Mandava, G., Thomas, D. M., Moellering, R. E. *Chem. Rev.* **2024**. *124*, 13020–13093

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What defines a target's druggability?

"An ideal small-molecule drug target is ***disease modifying****,*

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What defines a target's druggability?

"An ideal small-molecule drug target is ***disease modifying****, capable of binding a* ***selective, orally bioavailable molecule*** *at a site that elicits a functional effect, has no* ***on-target toxicity*** *and is* ***expressed in disease-relevant tissue"***

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Druggable Targets – the Human Proteome

***Identification* of disease-causal targets in the human proteome**

Human proteome (~20,000)

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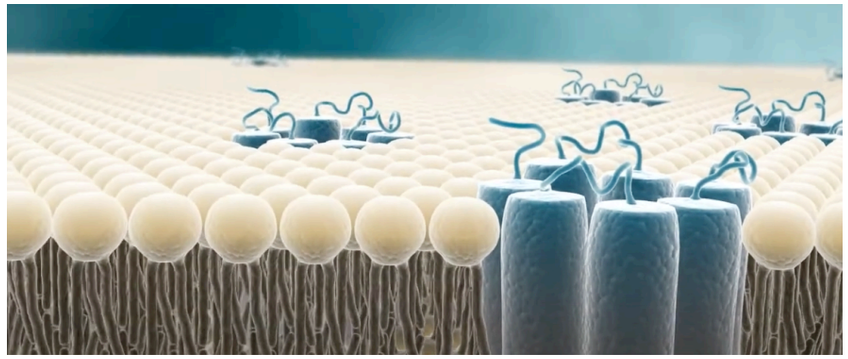
Identification of disease-causal targets in the human proteome

Conventionally 'druggable'

- ***Ion channels + GPCRs***



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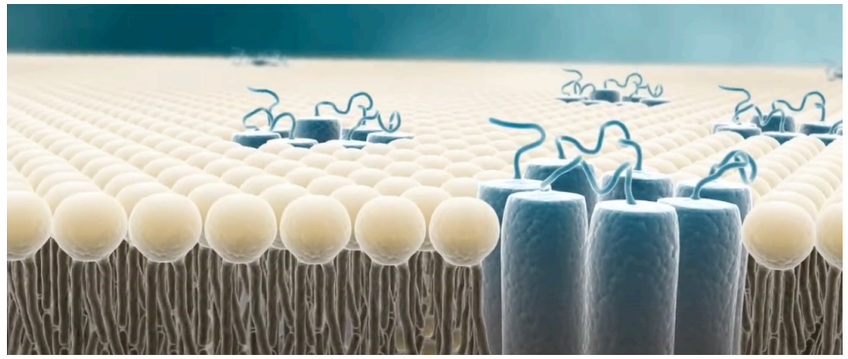

~30% of all drugs on the market act by binding GPCRs

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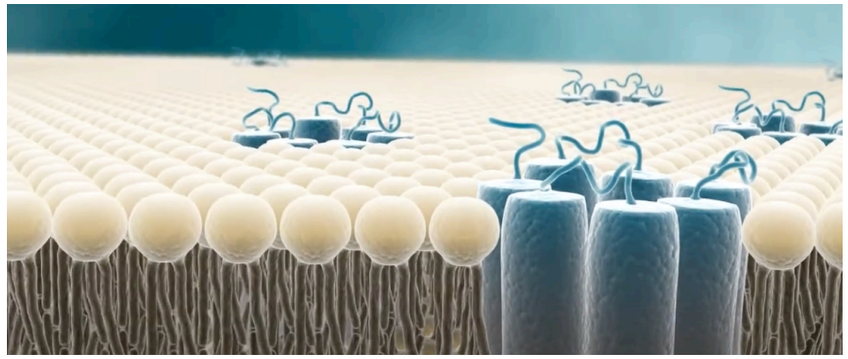
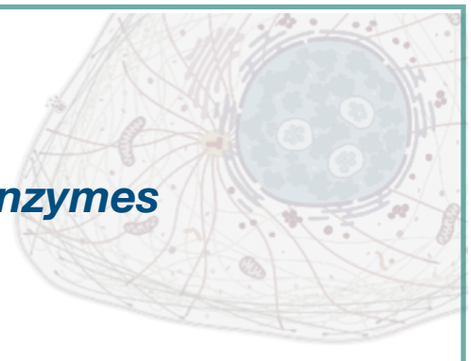


- ***Intracellular enzymes***



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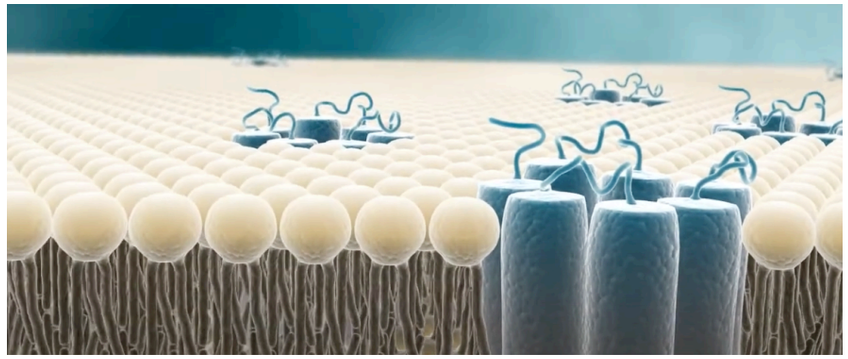

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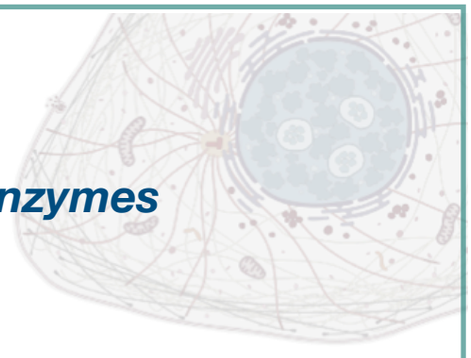

- *ligand binding sites*
- *other hydrophobic pockets*

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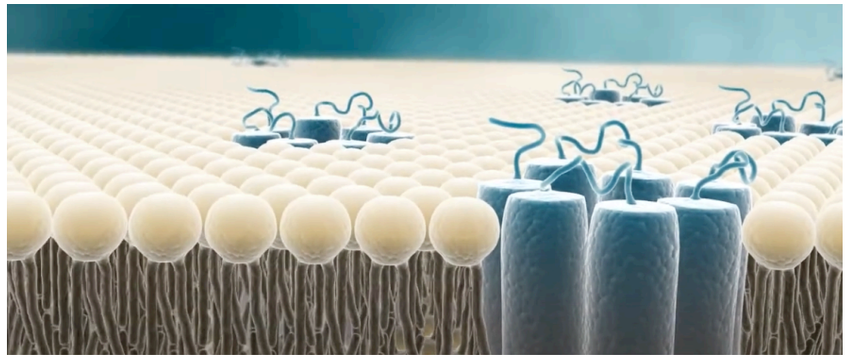

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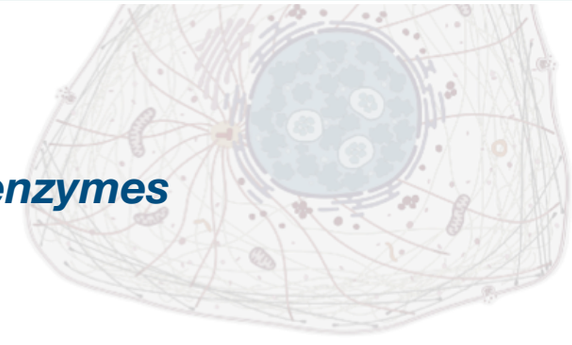

- *ligand binding sites*
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- ***Transcription factors***

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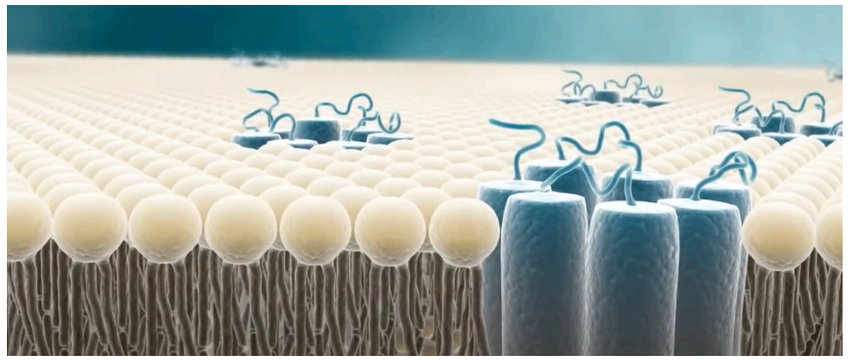

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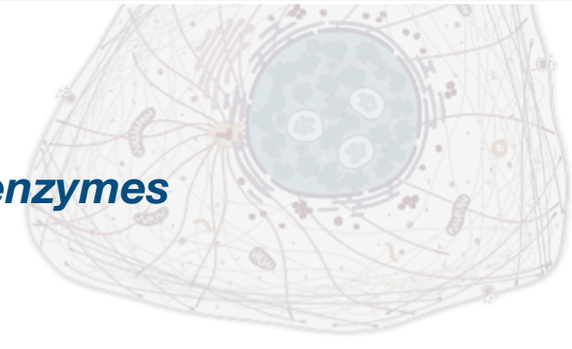

- *ligand binding sites*
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p53

Myc

Estrogen receptor (ER)

Androgen receptor (AR)

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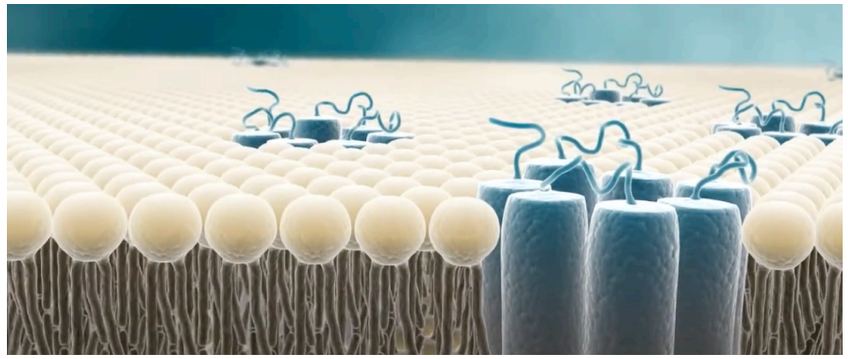

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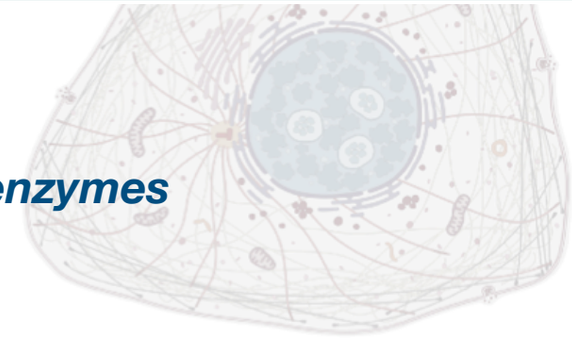

- *ligand binding sites*
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- ***Transcription factors***

- *Structural heterogeneity*
- *few tractable binding sites*

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Druggable Targets – the Human Proteome

*The **majority** of disease drivers **lack structural features** conventionally targeted by small molecules or antibodies*

Crazing, L. et al. *ACS. Med. Chem. Lett.* **2025**. *11. ASAP.*

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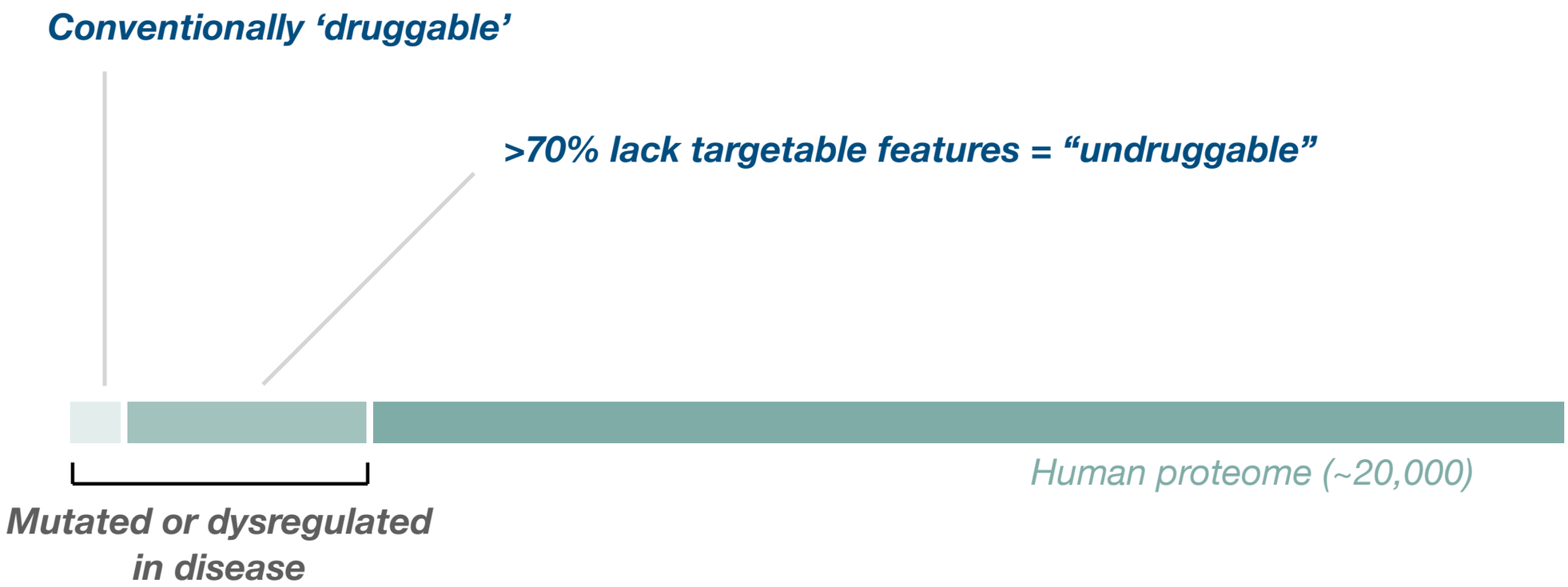

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Investigating Druggability

3 key factors to "Undruggabilty":

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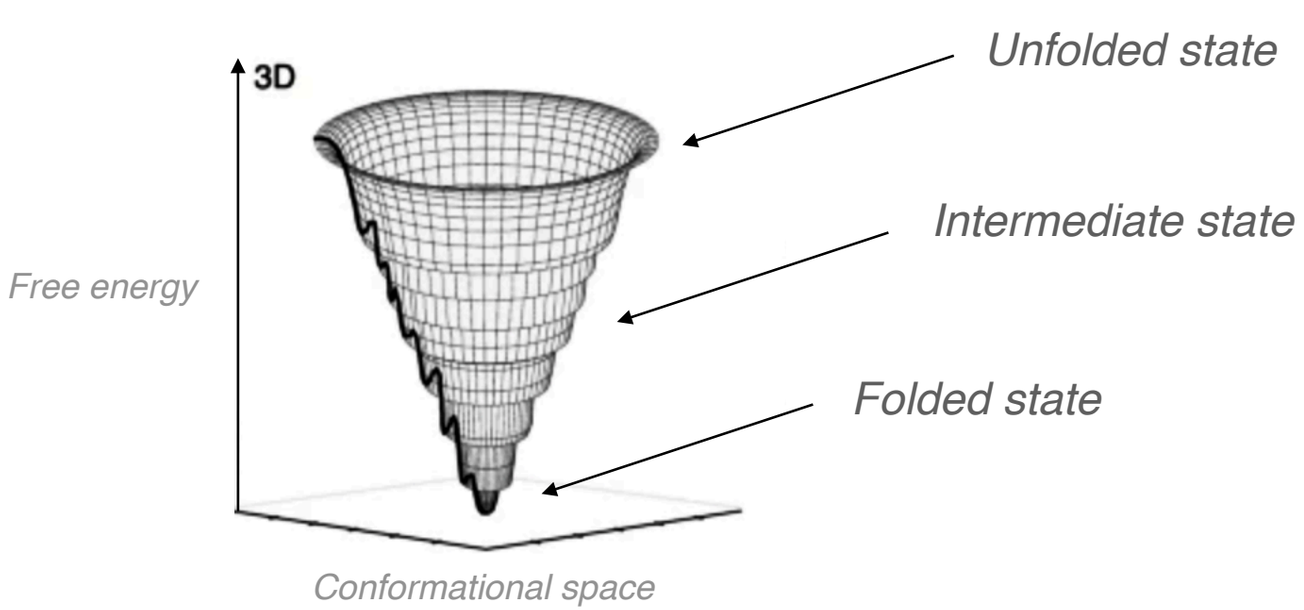
Folding funnel diagram – energy landscape



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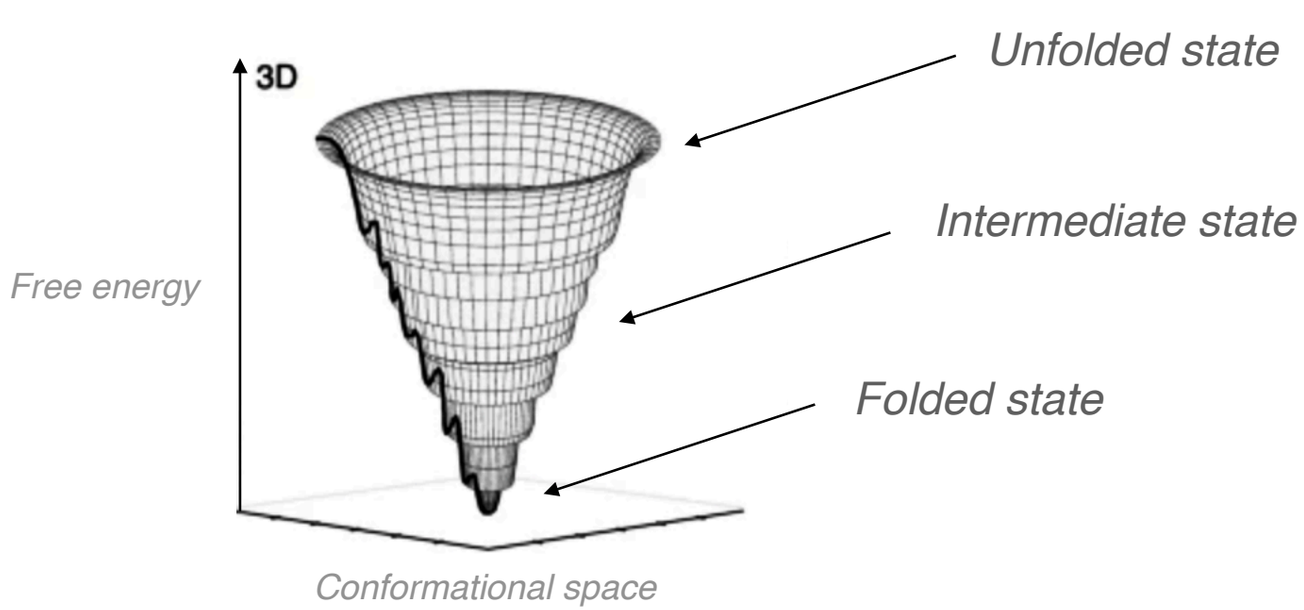

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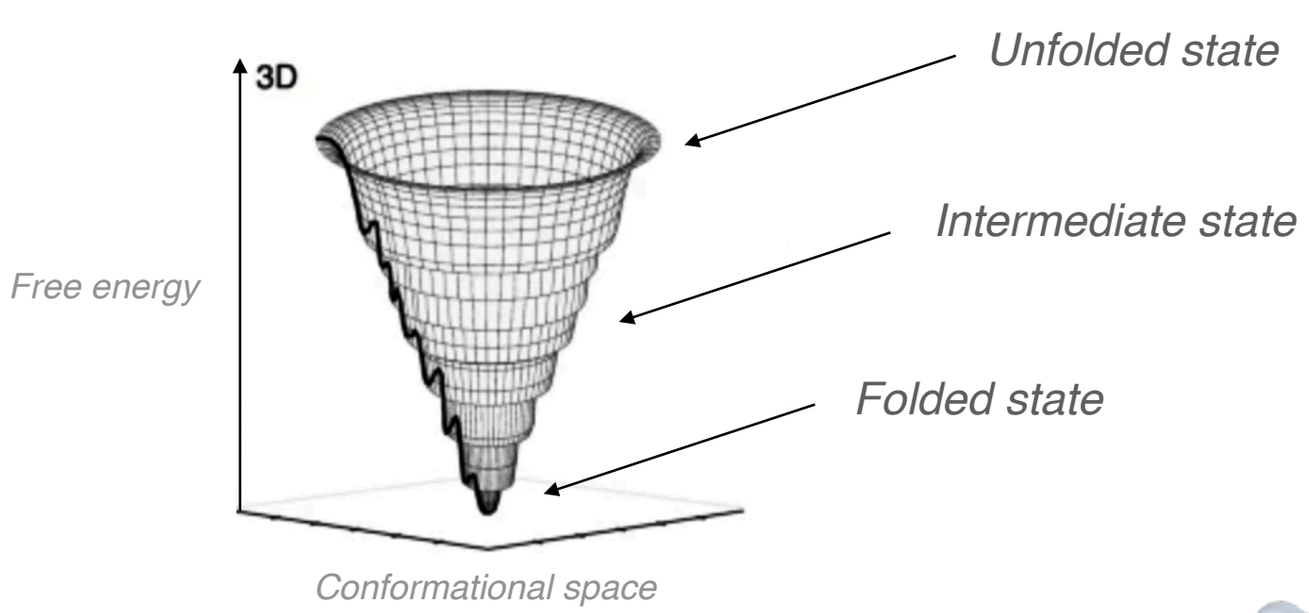
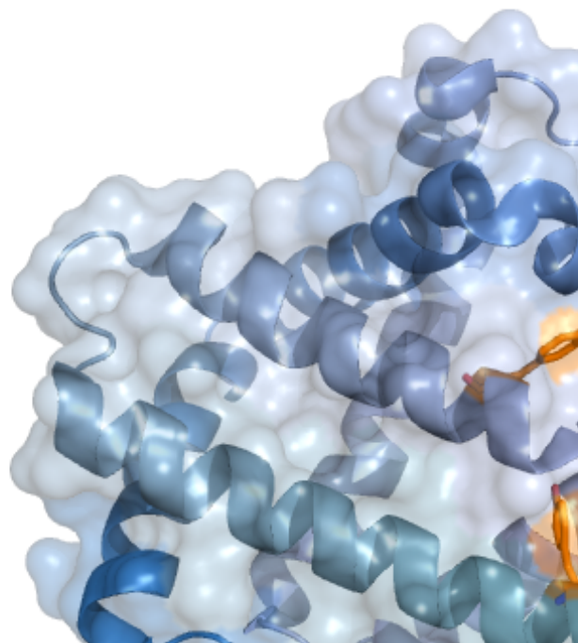


Folded proteins exhibit a deep minimum



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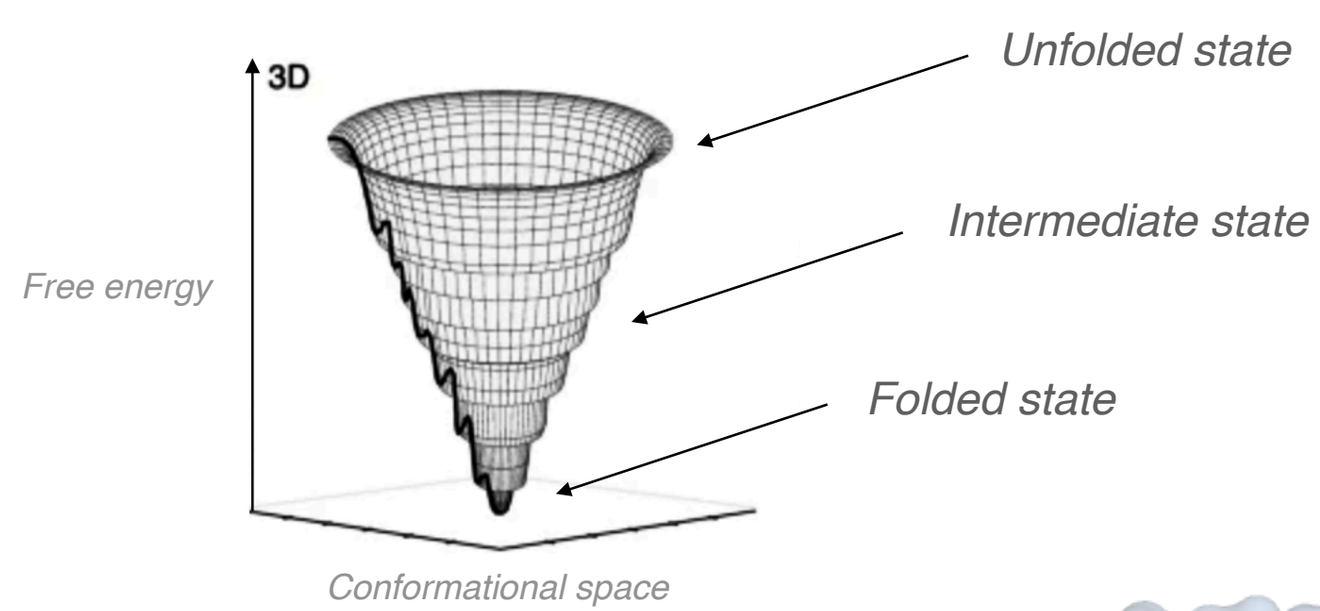
Intrinsically disordered proteins (IDPs)

- *Lack well-defined structure*

Folding funnel diagram



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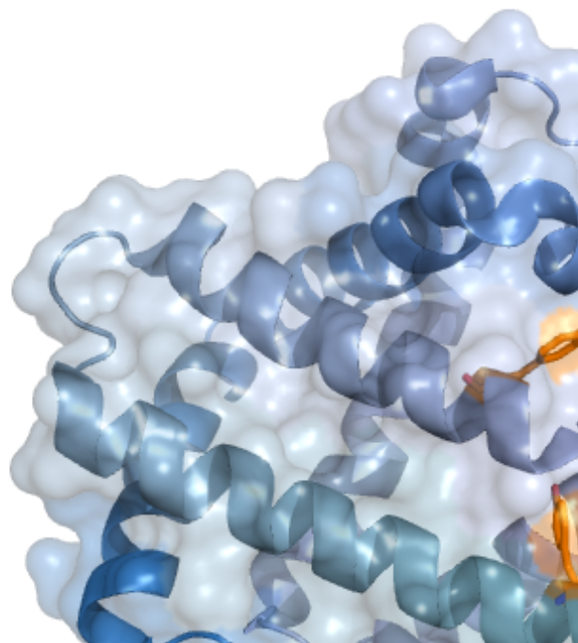

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- *Lack well-defined structure*
- ***Large entropic penalty of drug–target binding***

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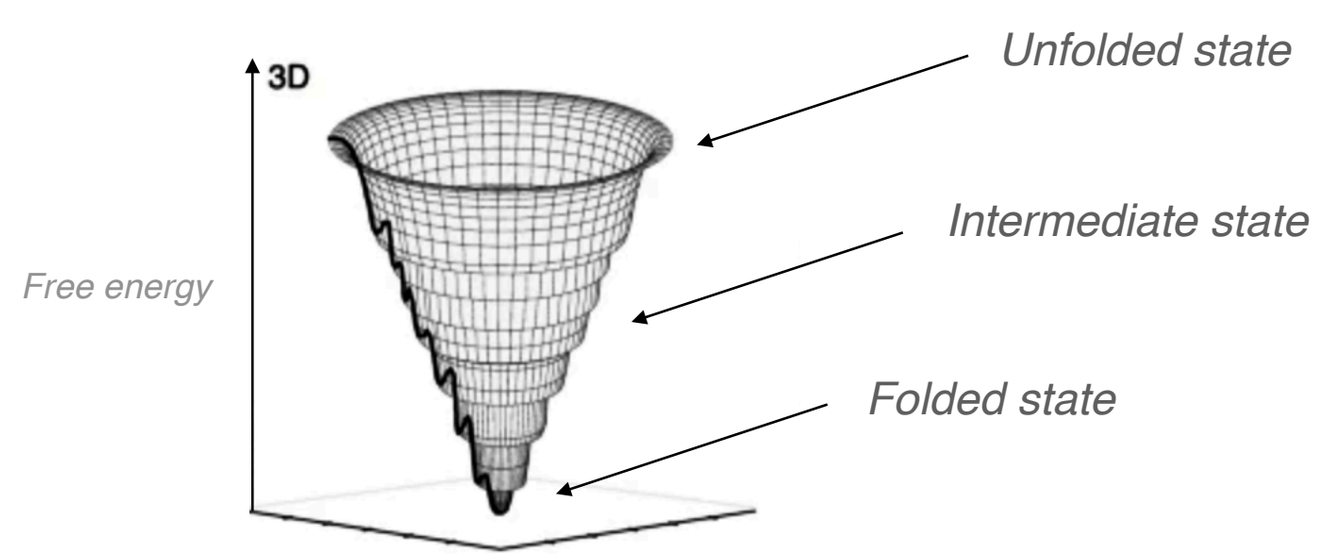

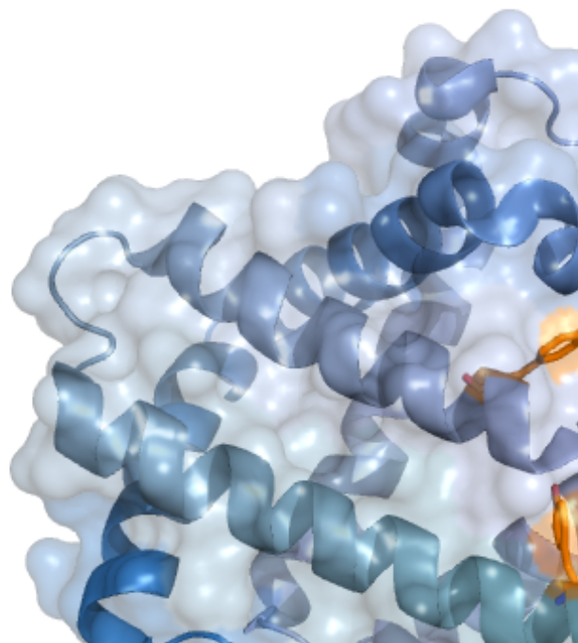

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Hard to develop small molecule drugs

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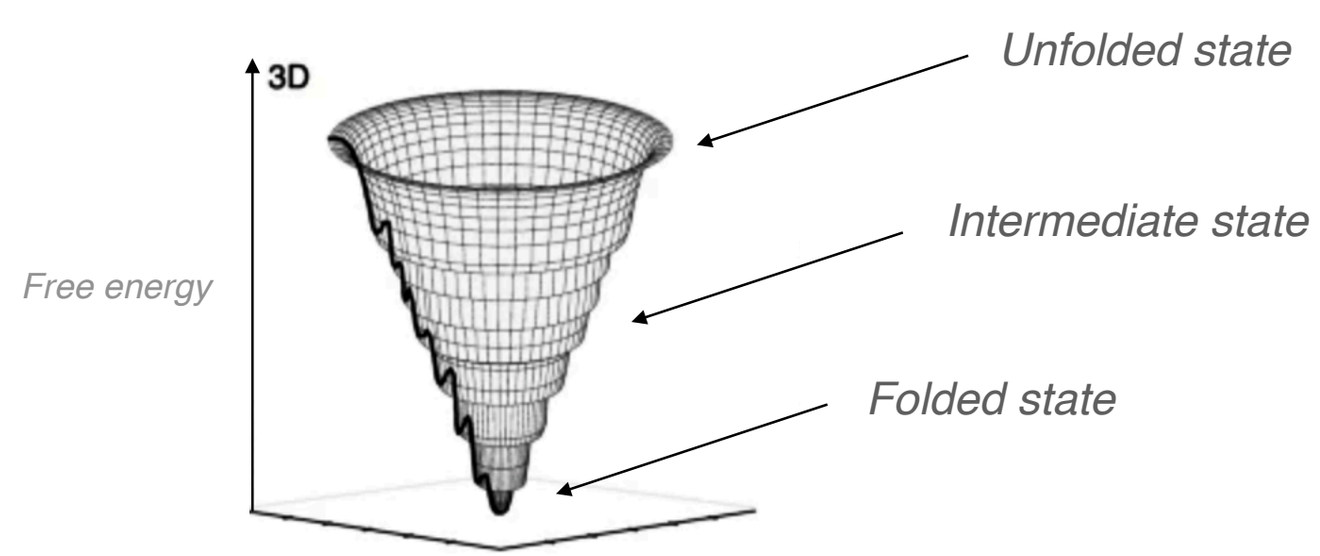

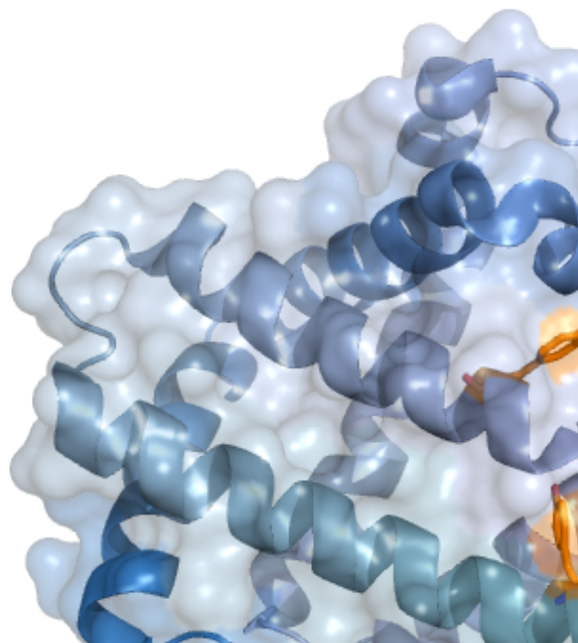

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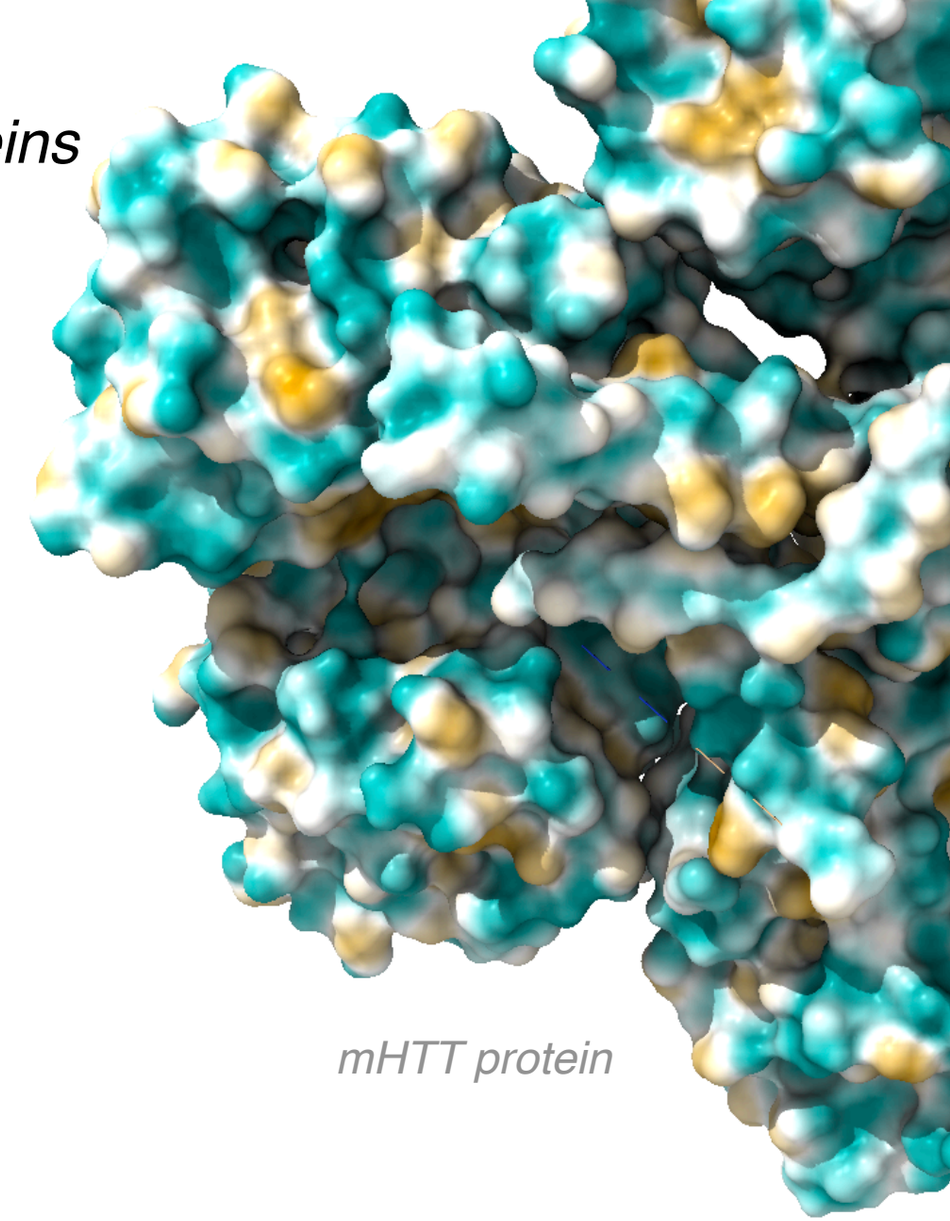
Intrinsically disordered proteins

Mutant ***Huntingtin protein (mHTT) = intrinsically disordered***



mHTT protein

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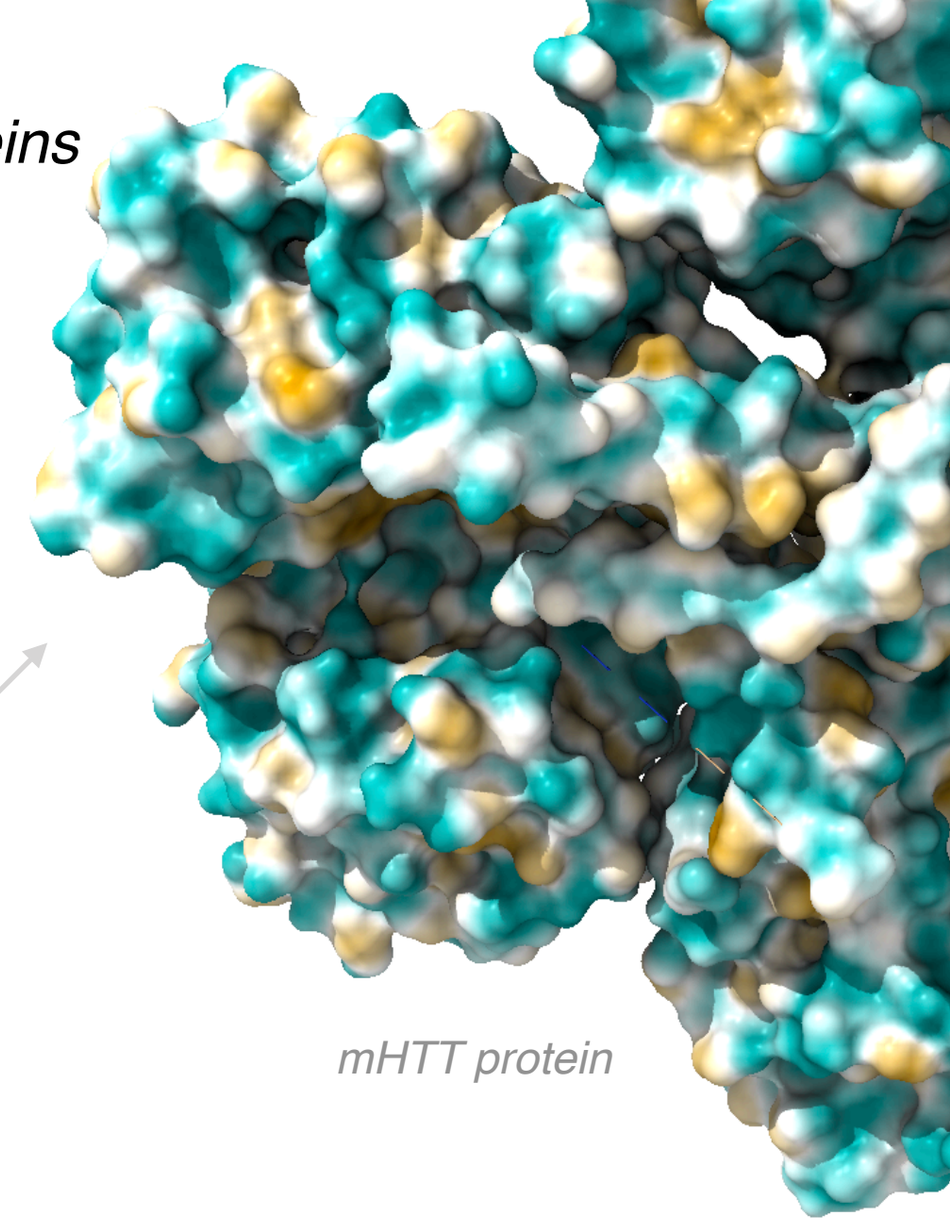
Multiple *interconverting conformational states*



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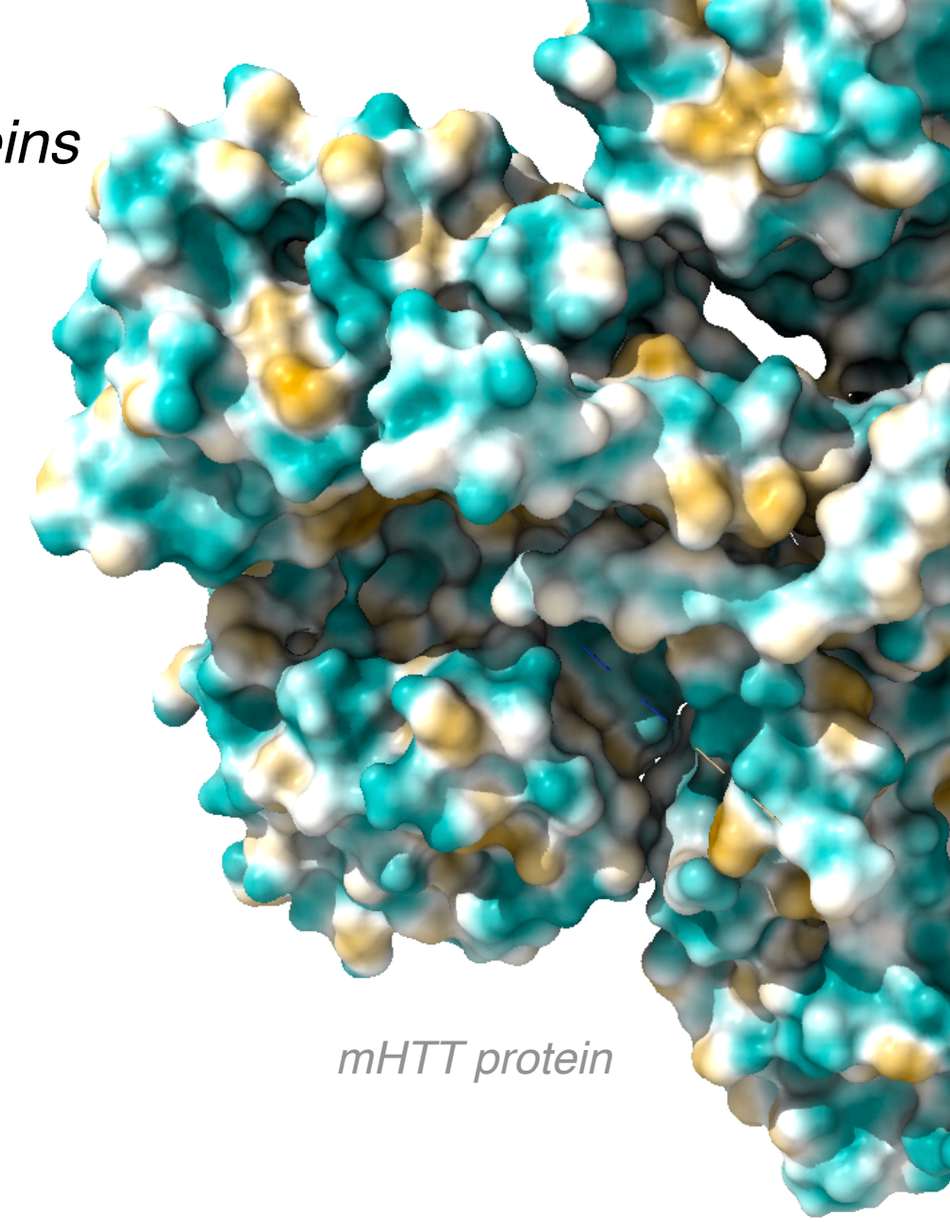
CAG triplet repeat expansion disease

CAG	***CAG***	***CAG***	***CAG***	***CAG***

- ***DNA-repeat expansion occurs throughout neuronal life***



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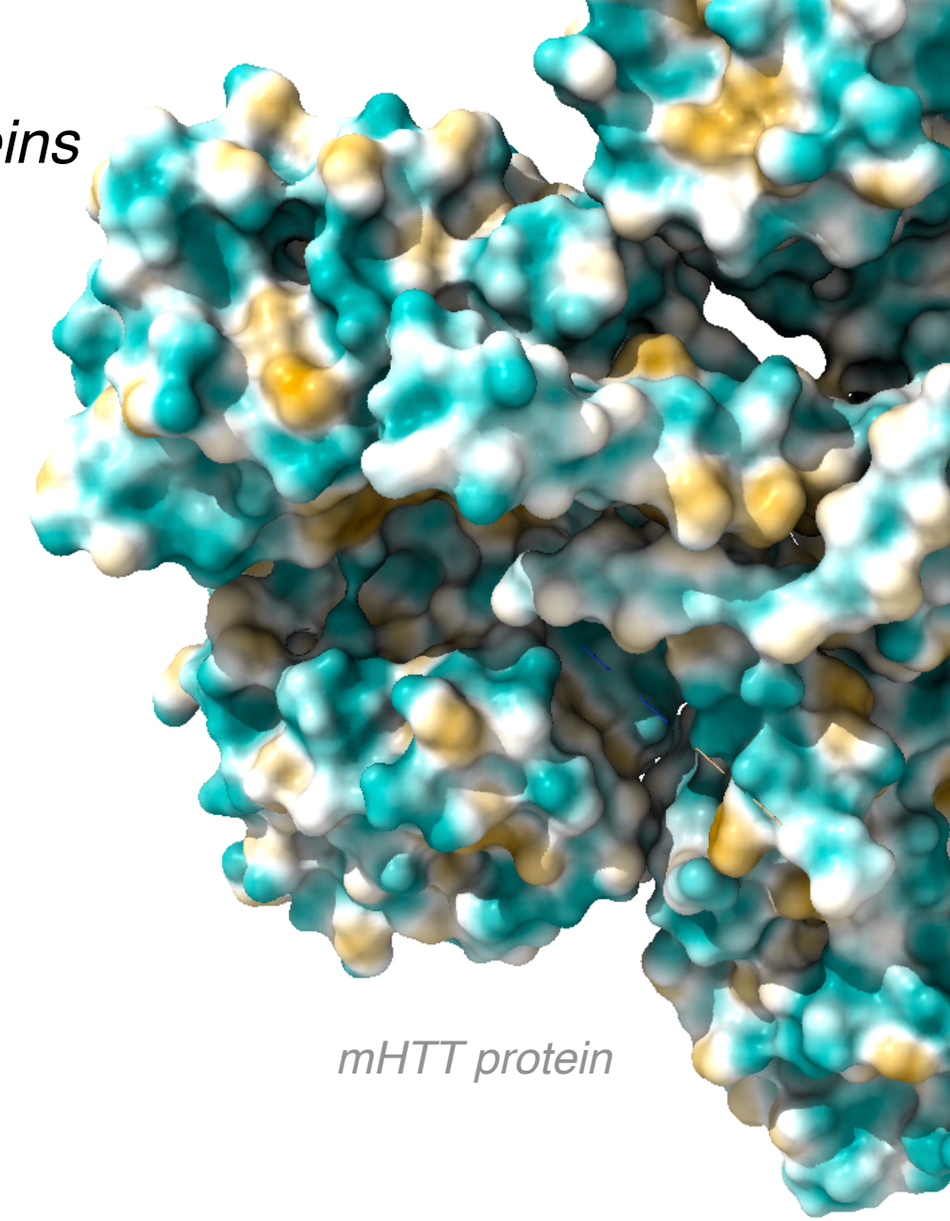

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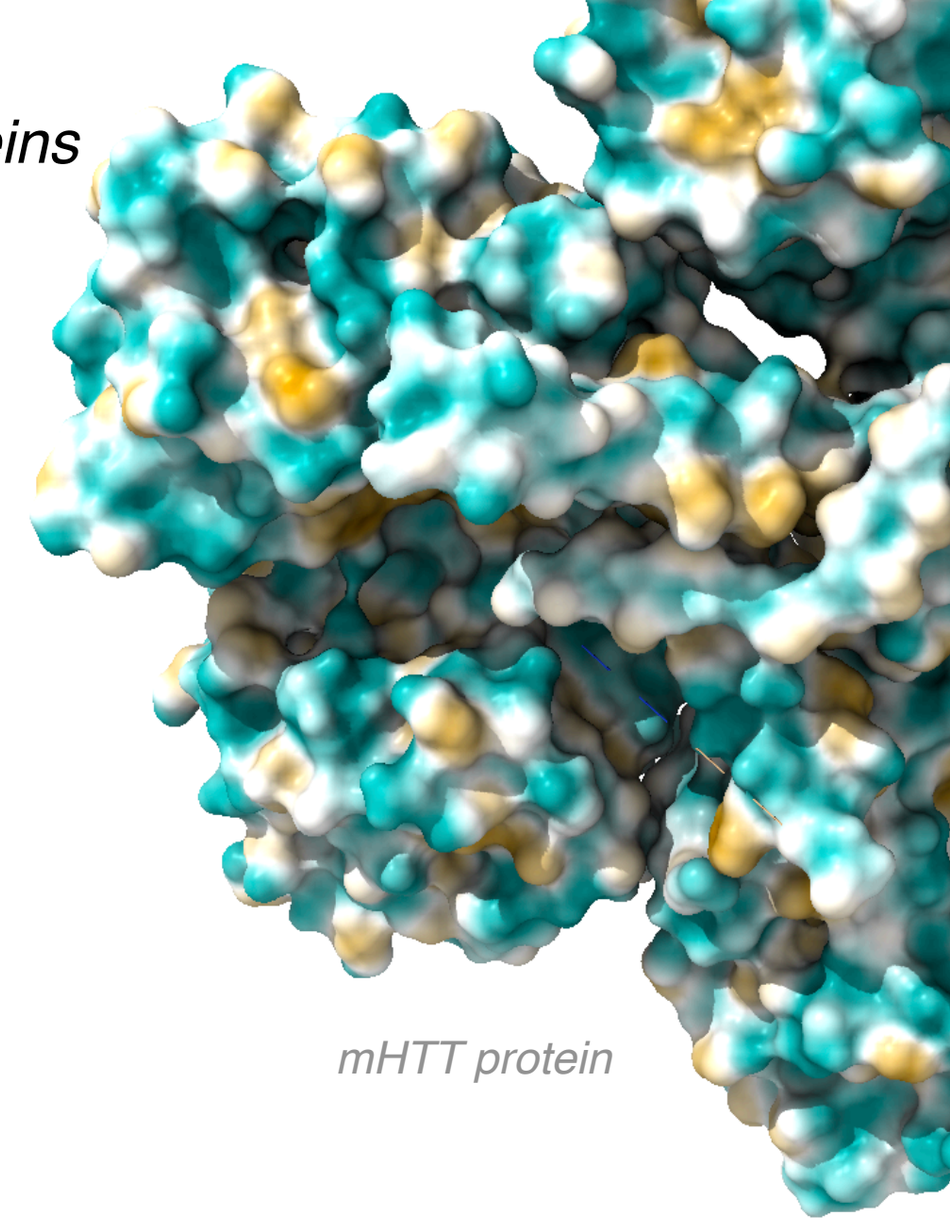

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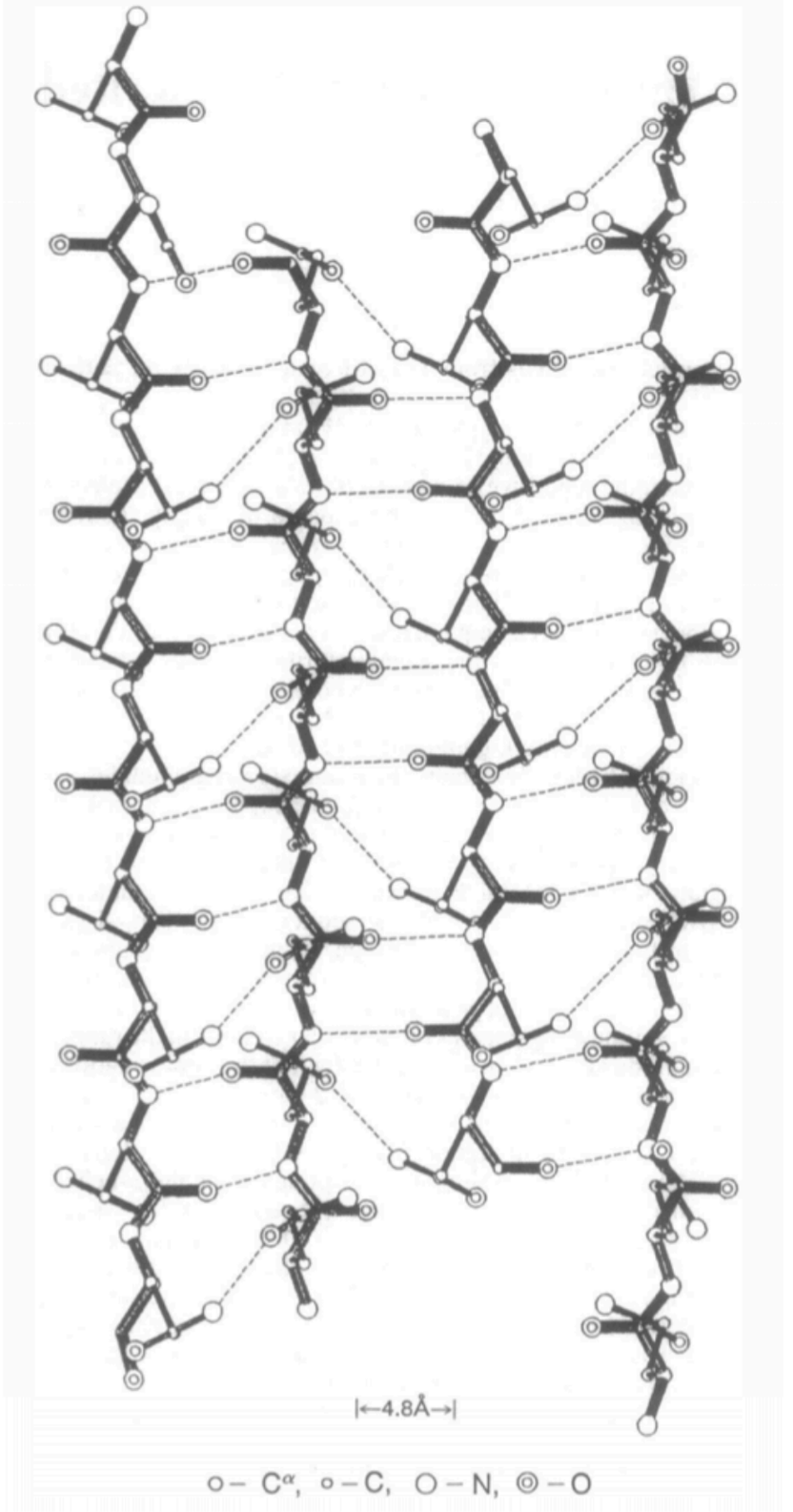
Depends on length of polyglutamine stretch!



Huntingtin polyglutamine repeat: β–sheets

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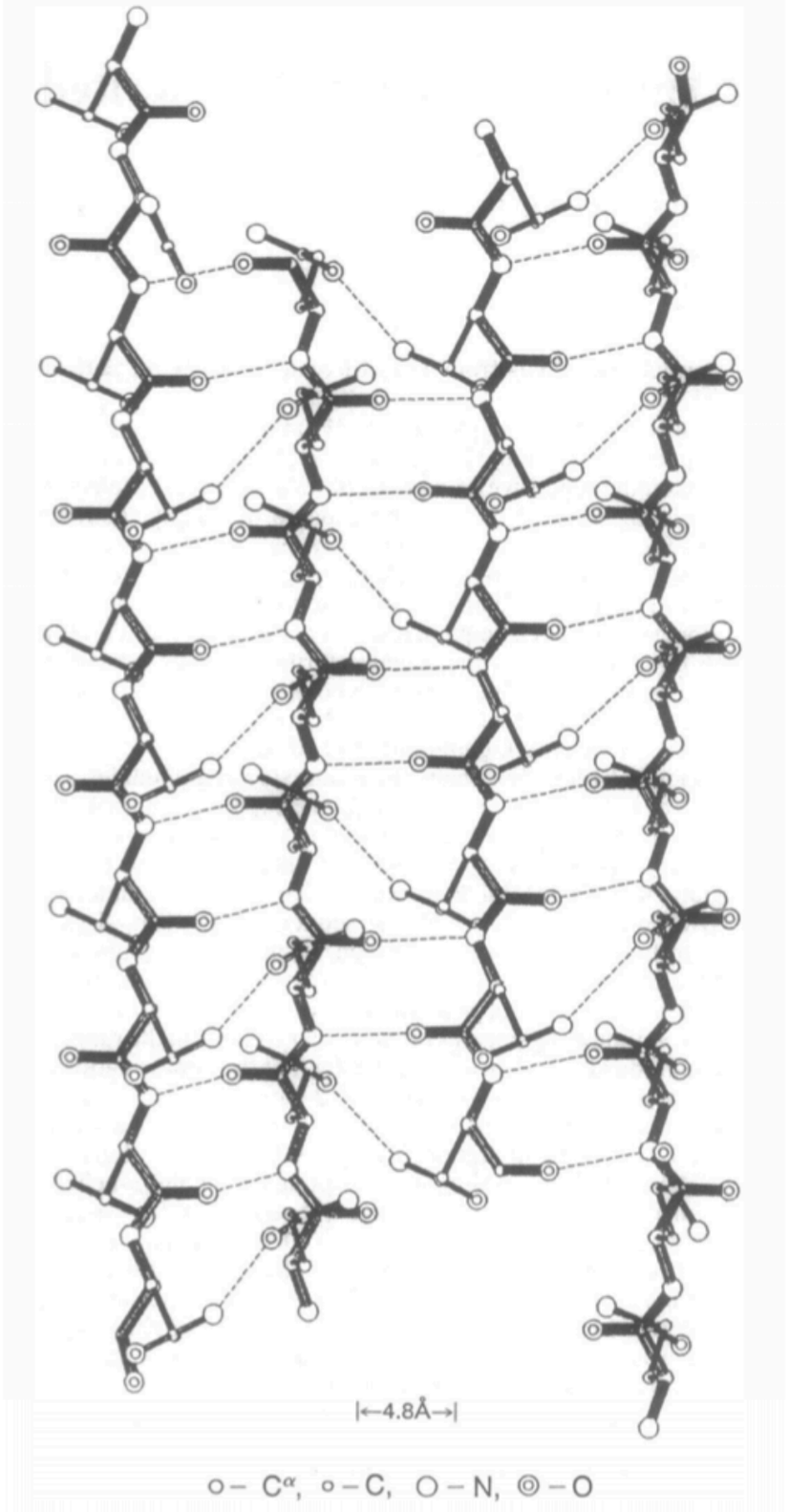

Stabilized by hydrogen bonding networks

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intrinsically disordered proteins

Irreversible aggregates

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Intrinsically disordered proteins

intrinsically disordered proteins

Irreversible aggregates

Not all intrinsically disordered proteins are pathological

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~130,000–650,000 *types of PPIs in the human interactome*

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Disruption of PPIs can promote disease states

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3 key factors to "Undruggabilty":

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- *Dynamic surfaces*
- *Undefined binding pockets*

Vasta, J. D., Peacock, D. M., Zheng, Q., Walker, J. A., Zhang, Z., Zimprich, C. A., Thomas, M. R., Beck, M. T., Binkowski, B. F., Corona, C. R., Robers, M. B., Shokat, K. M. *Nat. Chem. Bio.* **2022**. *18*, 596–604

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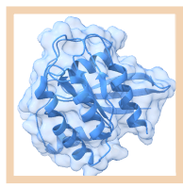
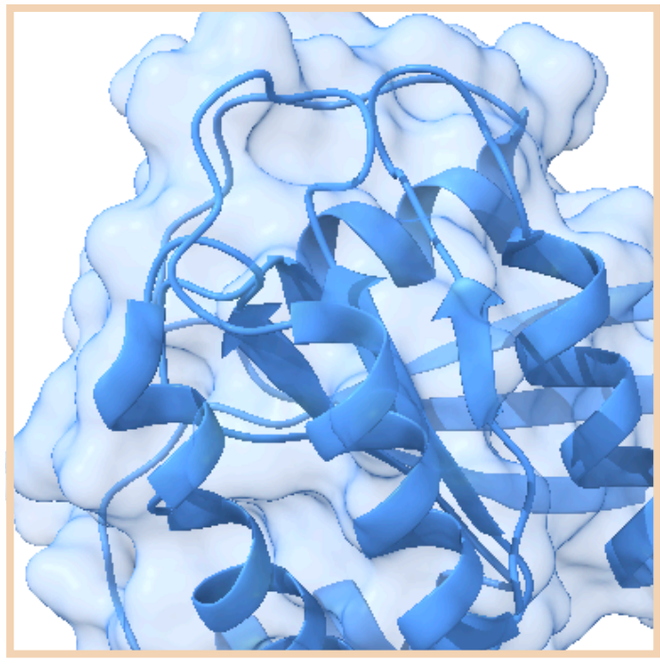
Undefined binding pockets

- ***Flat surfaces***
- ***Lack grooves or binding sites***





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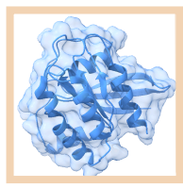

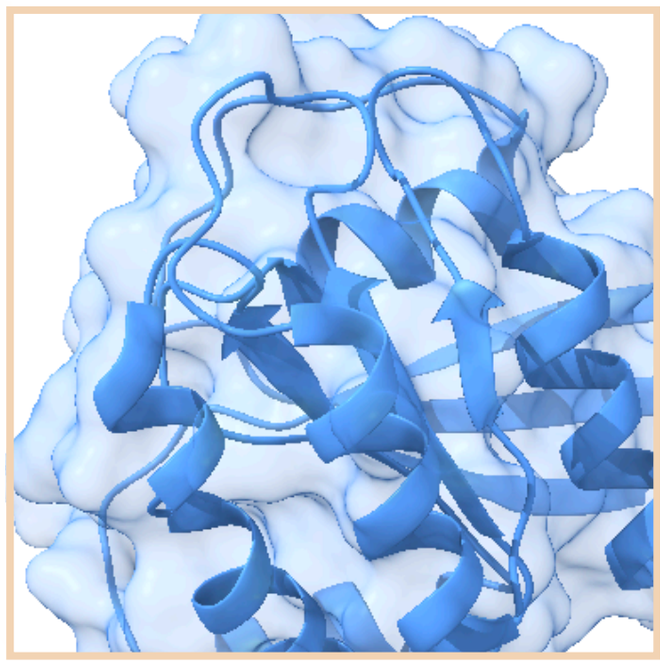

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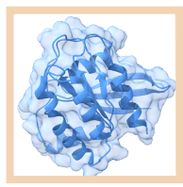

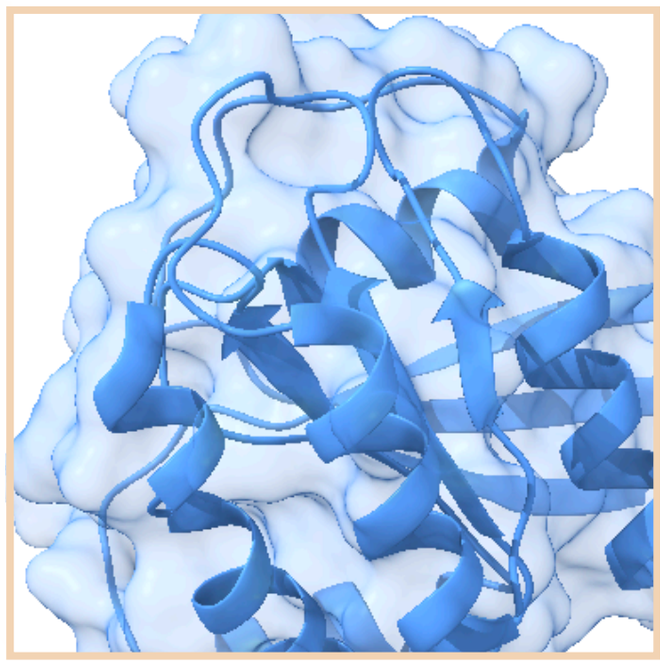

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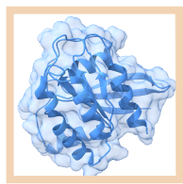

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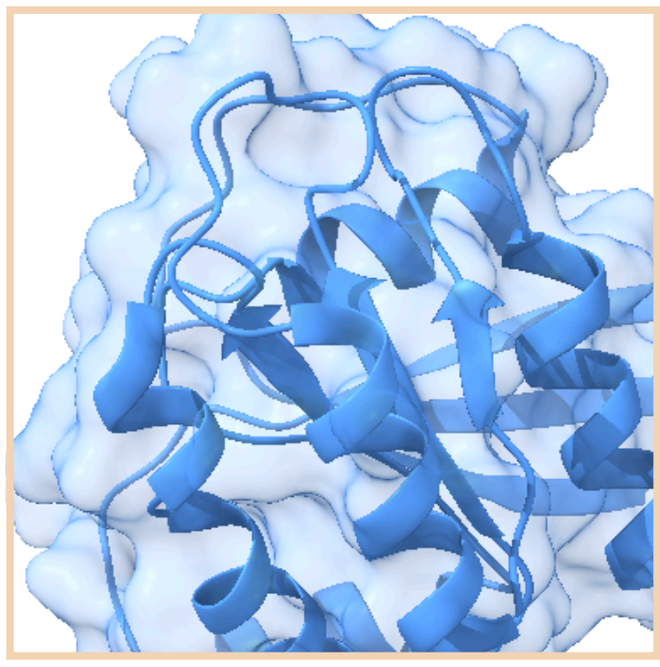

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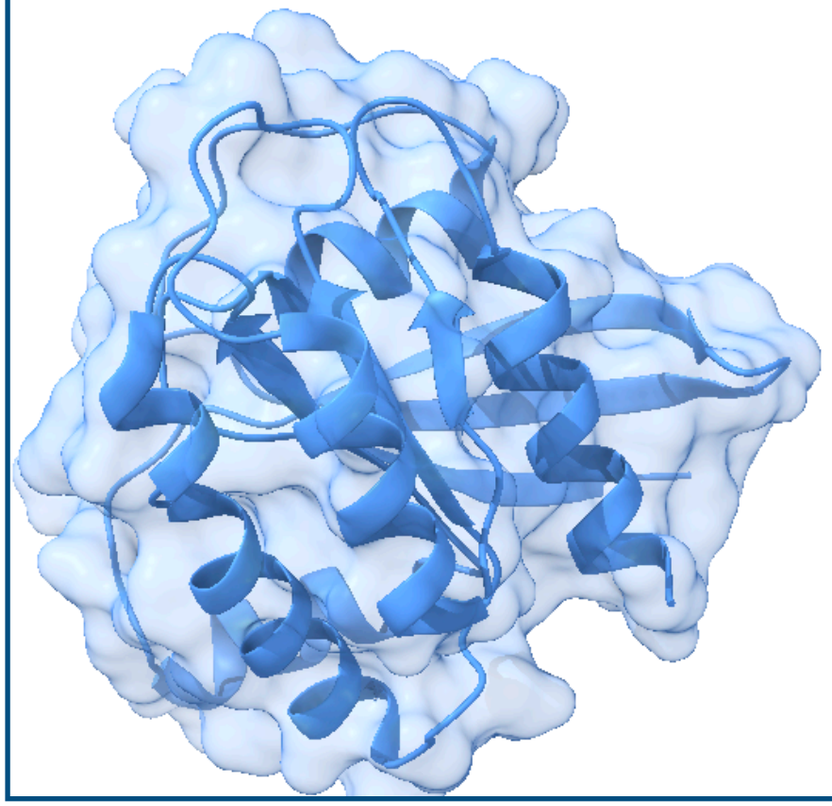
NIH NATIONAL CANCER INSTITUTE

The Ras Initiative

Lu, S., Jang, H., Muratcioglu, S., Gursoy, A., Keskin, O., Nussinov, R., Zhang, J. *Nat. Chem. Bio.* **2022**. *18*, 596–604



Killing Undruggable Targets

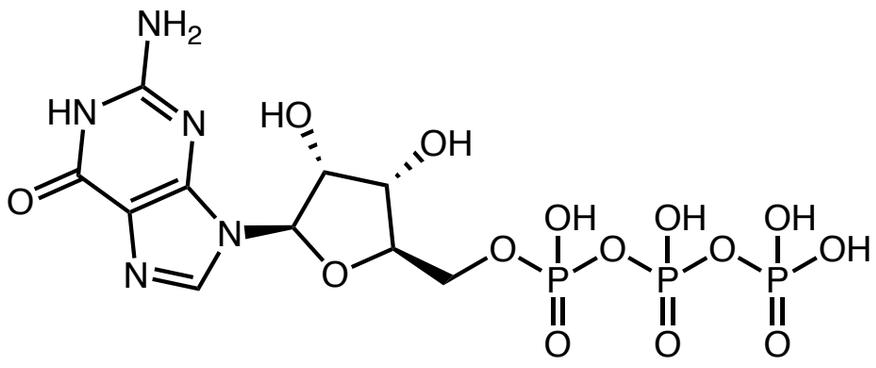
- ***Covalent inhibitors***

Part 1

Investigating Druggability

KRAS: to kill the undruggable

Ras proteins: ***Crucial in signal transduction (GTPase)***



GTP-bound

ACTIVE

Molecular switches to signaling networks

Vasta, J. D., Peacock, D. M., Zheng, Q., Walker, J. A., Zhang, Z., Zimprich, C. A., Thomas, M. R., Beck, M. T., Binkowski, B. F., Corona, C. R., Robers, M. B., Shokat, K. M. *Nat. Chem. Bio.* **2022**. *18*, 596–604

Investigating Druggability

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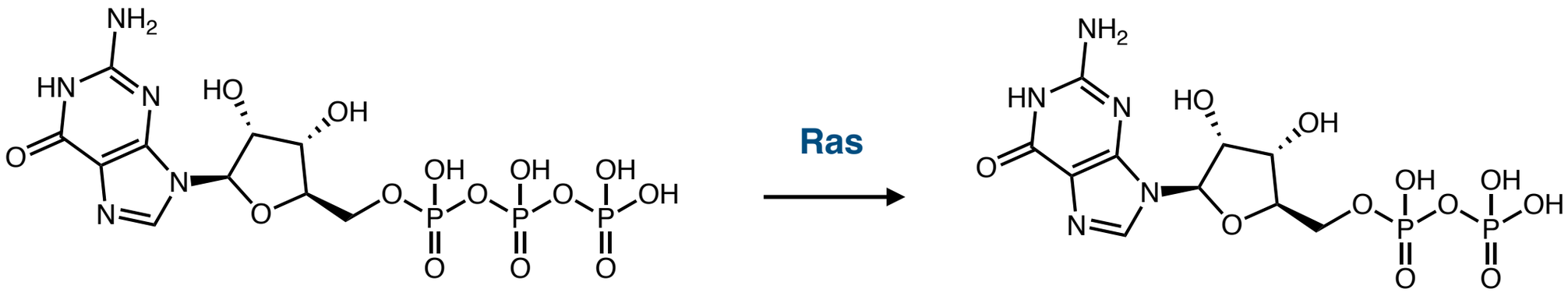

GTP-bound

ACTIVE

GDP-bound

RESTING

Molecular switches to signaling networks

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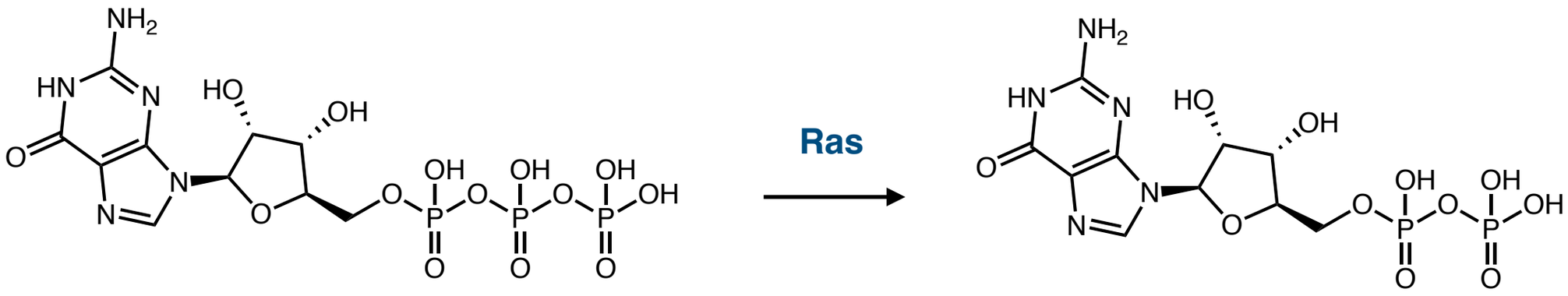

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↑[ACTIVE]

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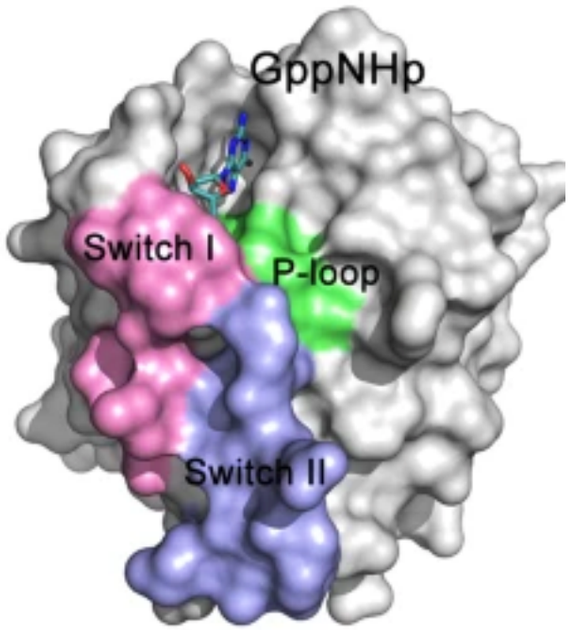
Stabilizing Surface Conformation

- ***Surface representations of protein states:***

Active



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Milburn, M. V., Tong, L., Devos, A. M., Brünger, A., Yamaizumi, Z., Nishimura, S. *Science.* **1990**. *247*, 939–945.

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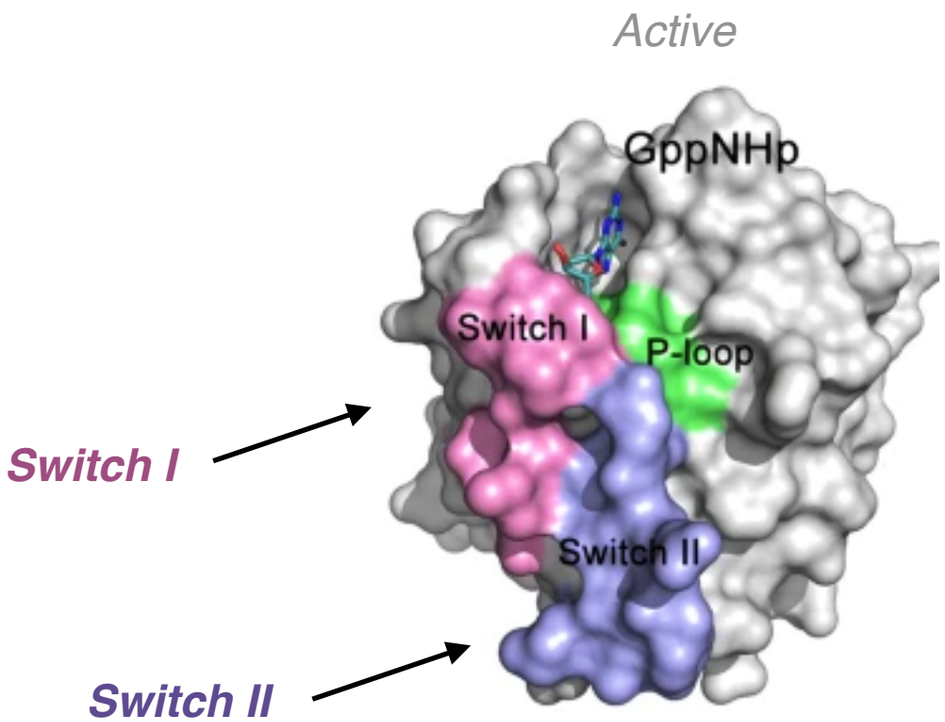

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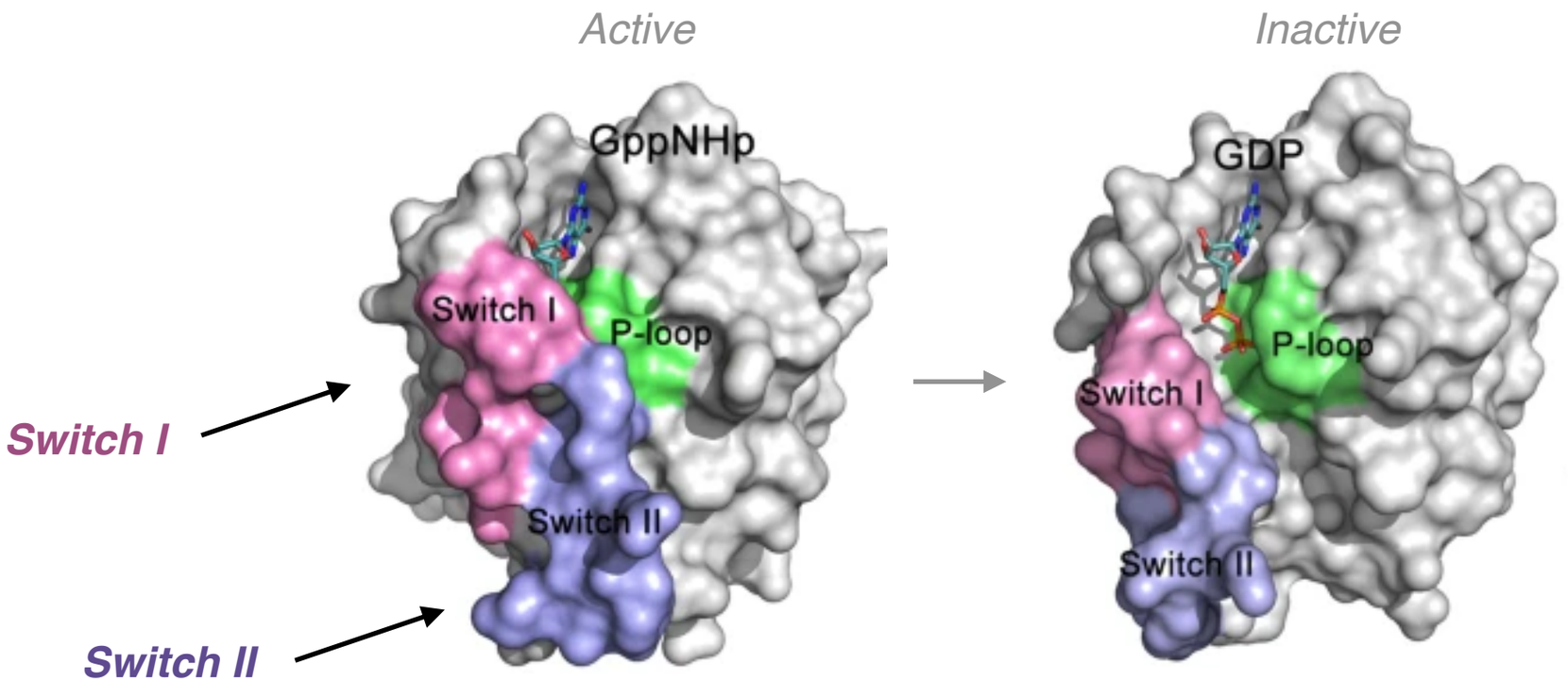

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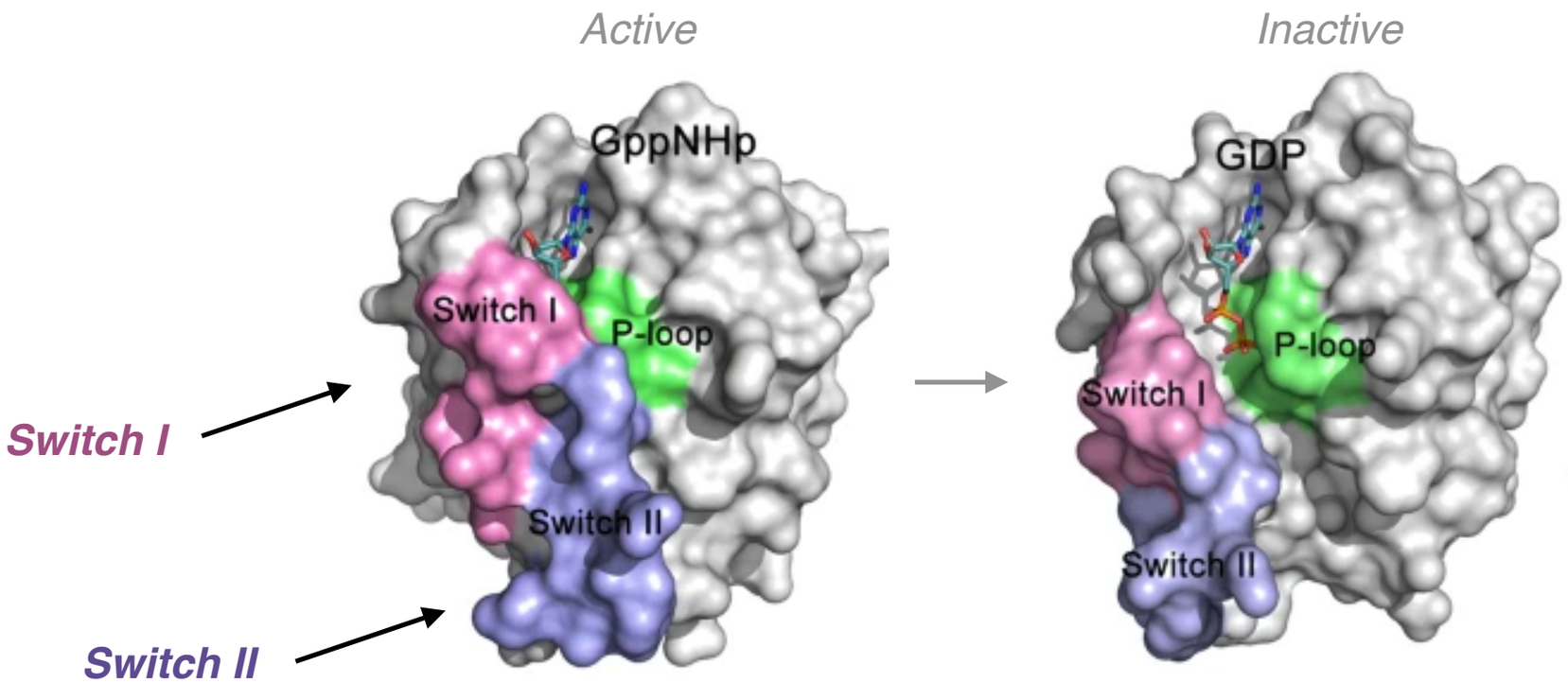

*exposing **nucleophilic** cysteine*

- ***Switch II binding pocket changes in $G_{12}C$ mutant***

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Investigating Druggability

KRAS: to kill the undruggable

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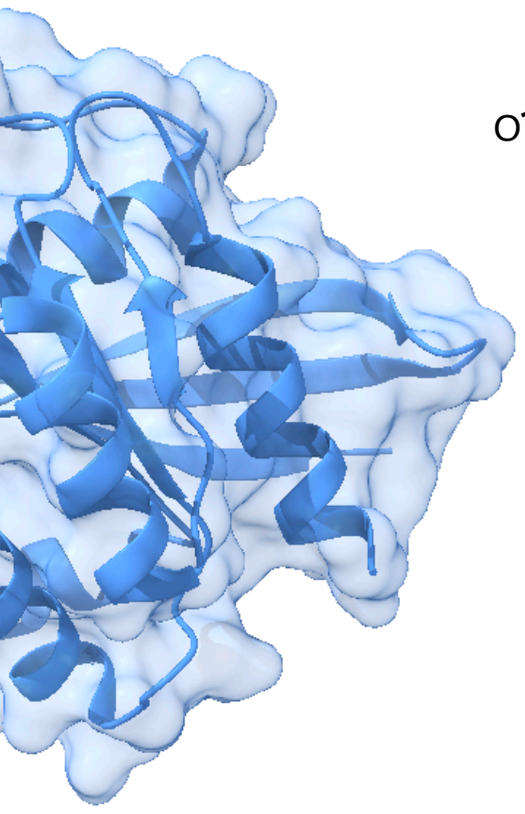

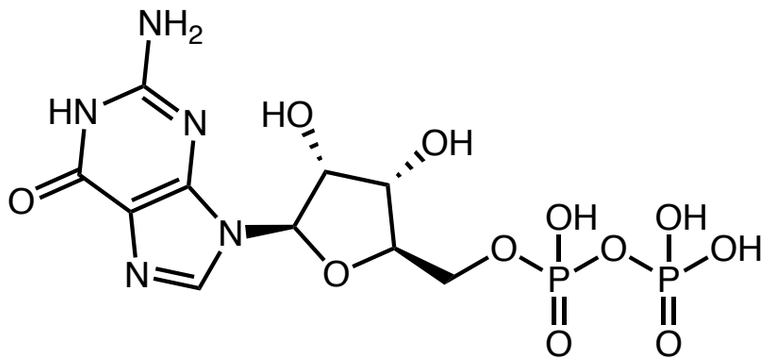

GDP-bound

RESTING

Qunaj, L., May, M. S., Neugut, A. I., Herzberg, B. O., *Front. Oncol.* **2023**. *13.*

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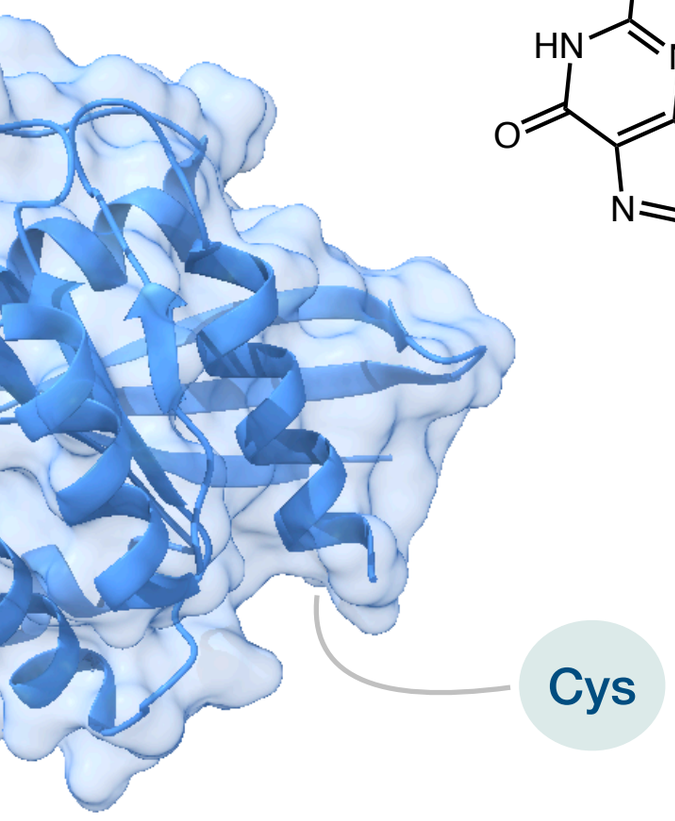

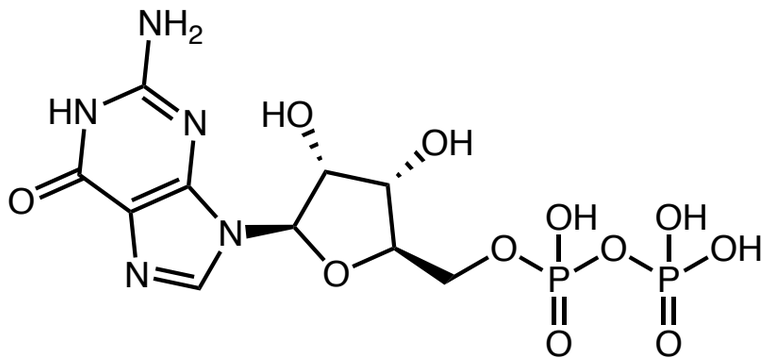

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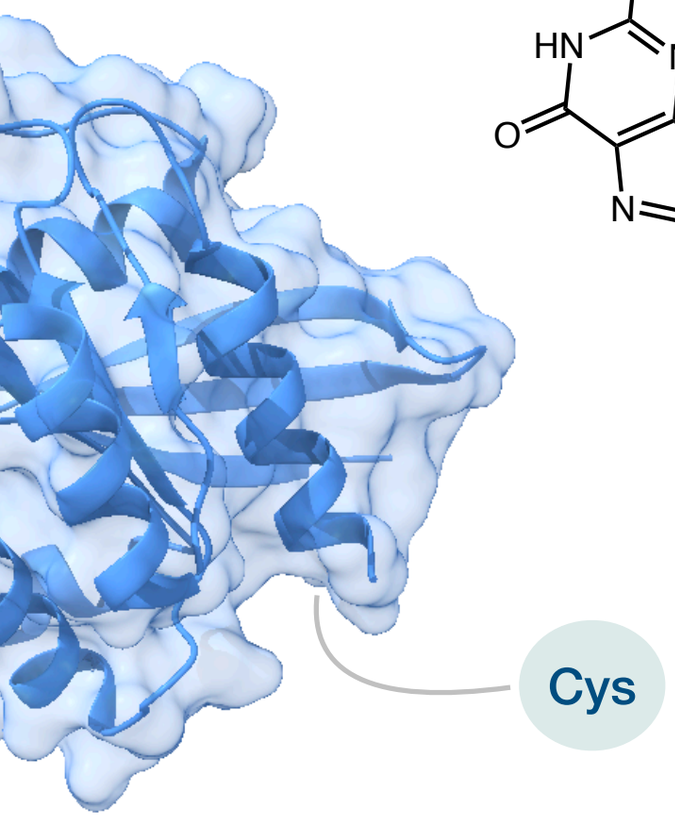

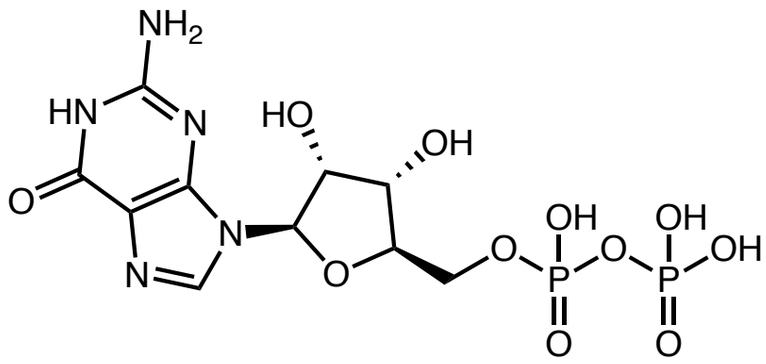

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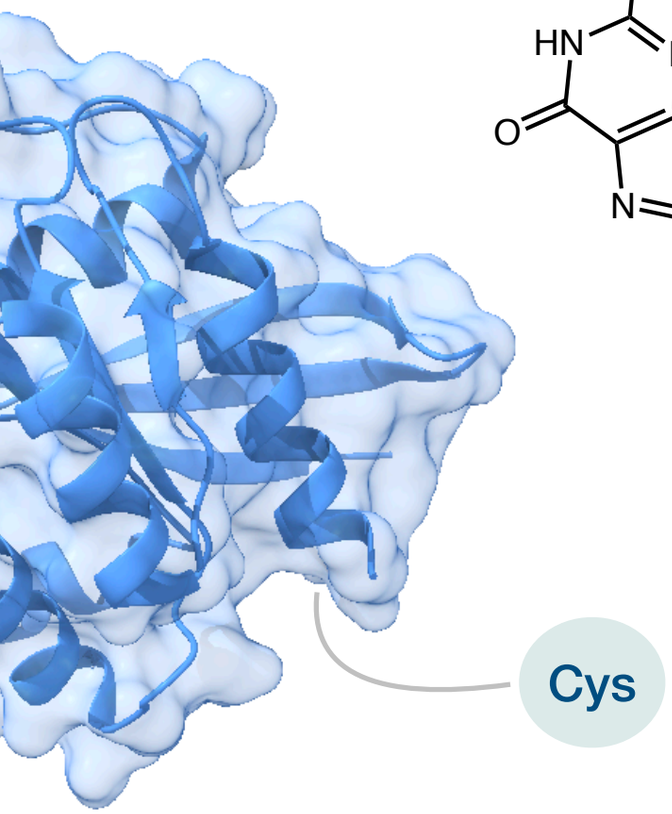

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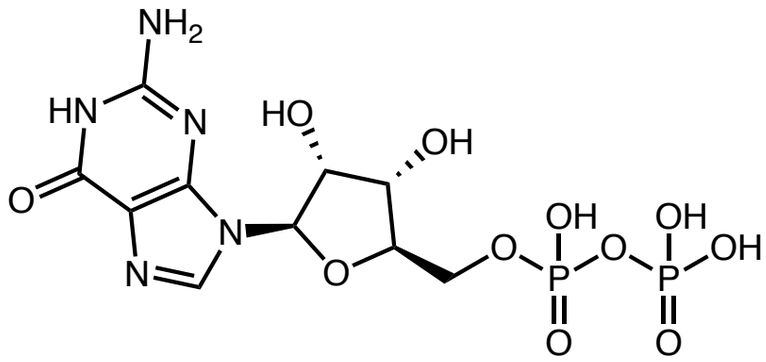

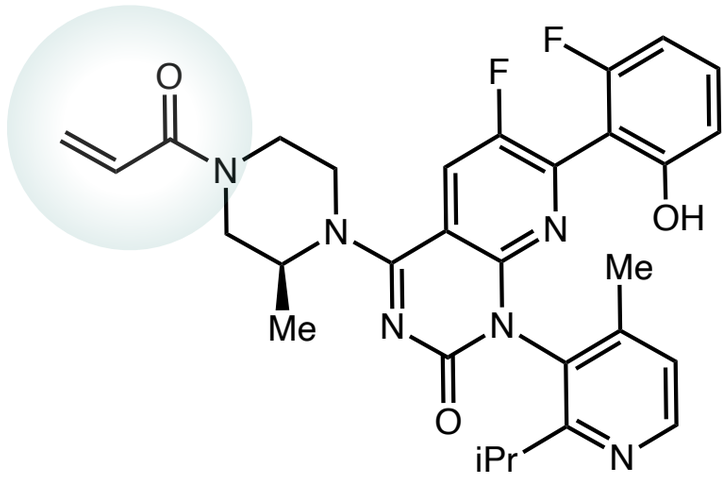

Sotorasib

LUMAKRAS®

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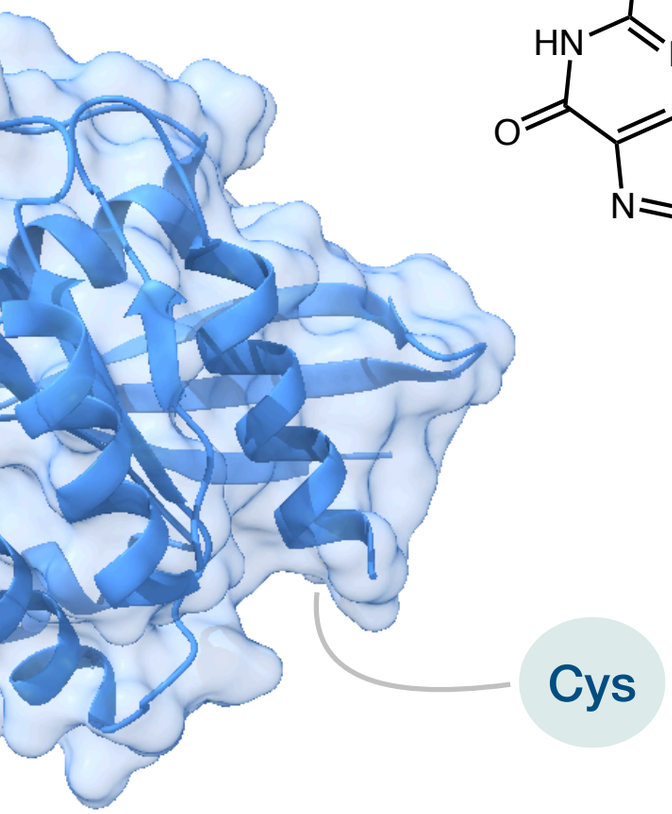

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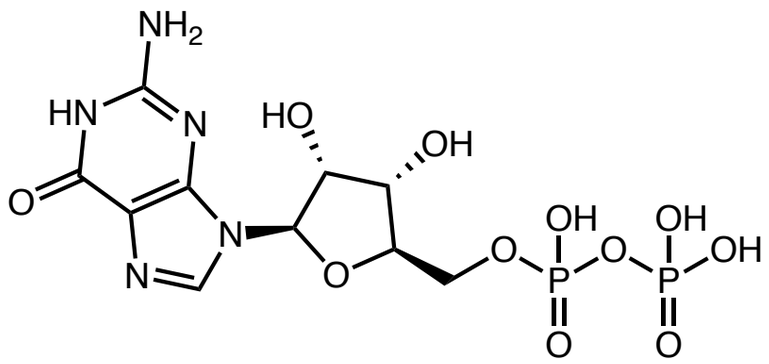

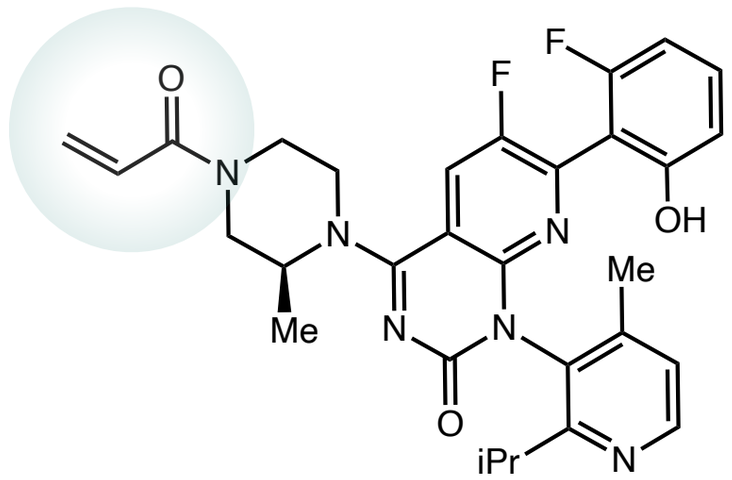

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LUMAKRAS®

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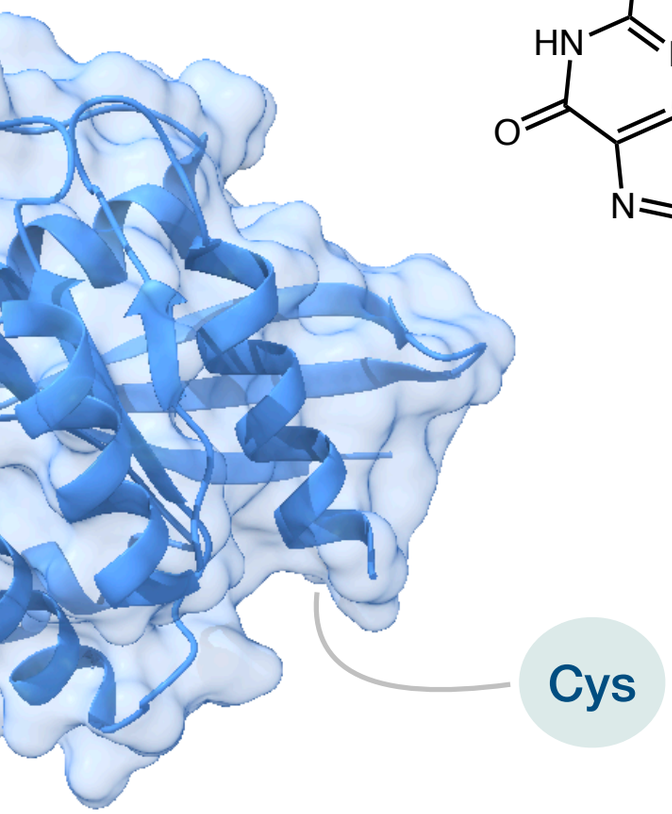

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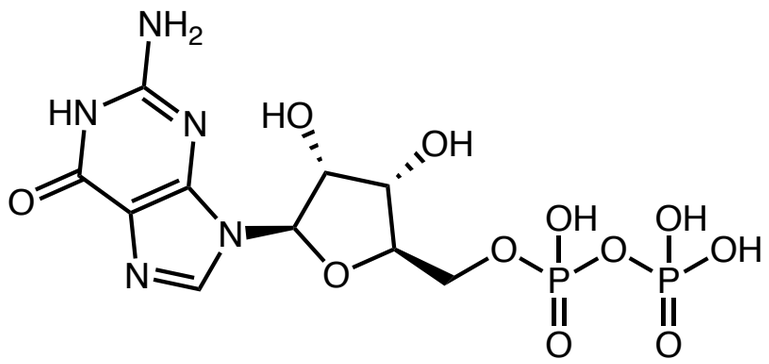

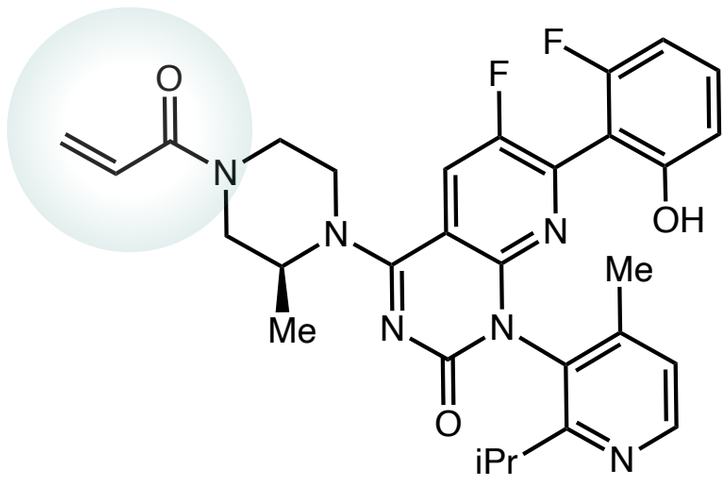

Sotorasib

LUMAKRAS®

Avoids pan RAS inhibition

Qunaj, L., May, M. S., Neugut, A. I., Herzberg, B. O., *Front. Oncol.* **2023**. *13.*

Covalent binding

Many immunomodulatory drugs covalently bind cysteine

- ***Electrophilic probes are a key component of T cell research***

Shindo, N., Ojida, A. *Bioorg. Med. Chem.* **2021**. *47*, 1–12

**in 2024*

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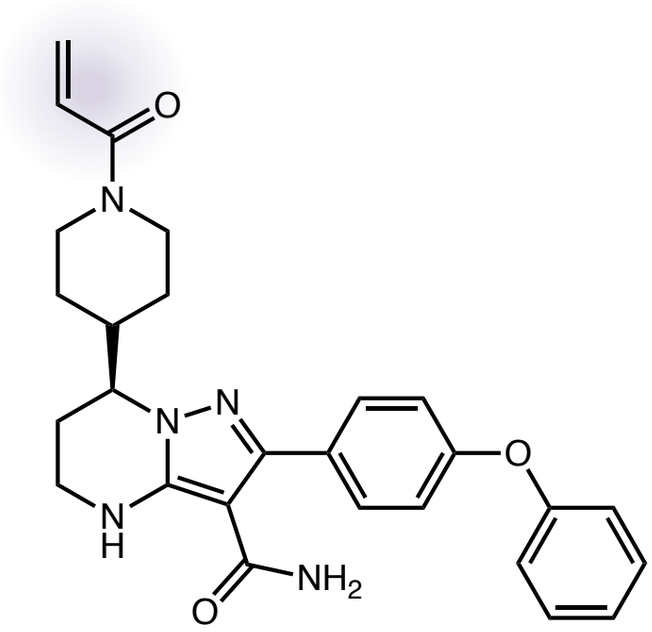

Brukinsa®

*$1.3 billion (USD)**

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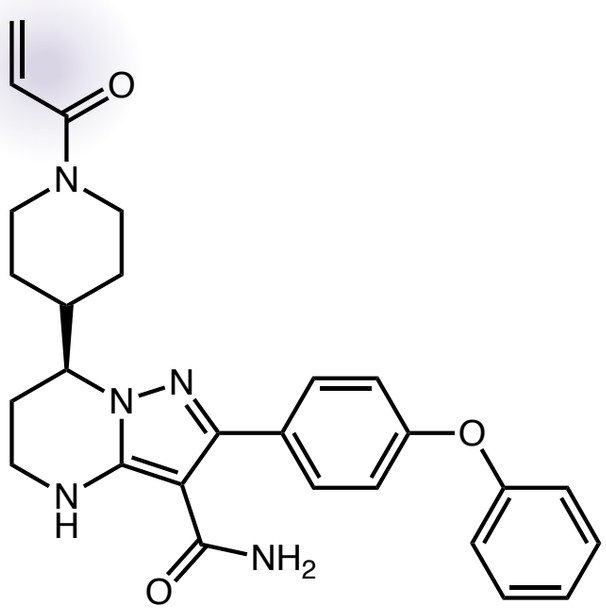

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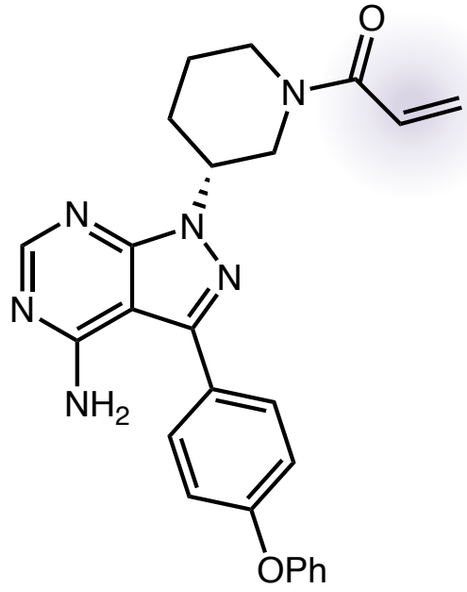

Ibruvica® (Ibrutinib)

*$4.9 billion (USD)**

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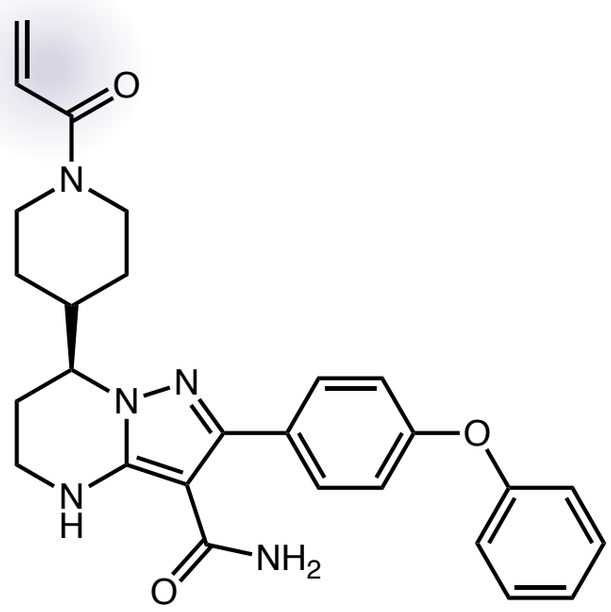

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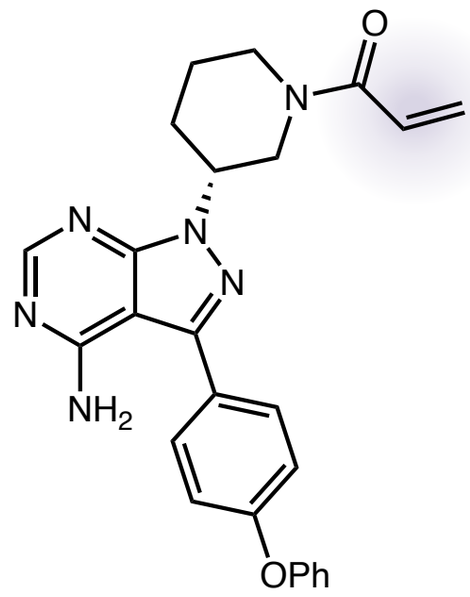

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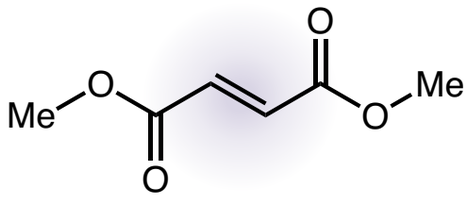

Tecfidera®

*$4.2 billion (USD)**

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Shindo, N., Ojida, A. *Bioorg. Med. Chem.* **2021**. *47*, 1–12

**in 2024*

Covalent binding

- ***How do you identify electrophilic probes?***

Activity Based Protein Profiling

Discovering drug–amino acid interactions

Activity Based Protein Profiling

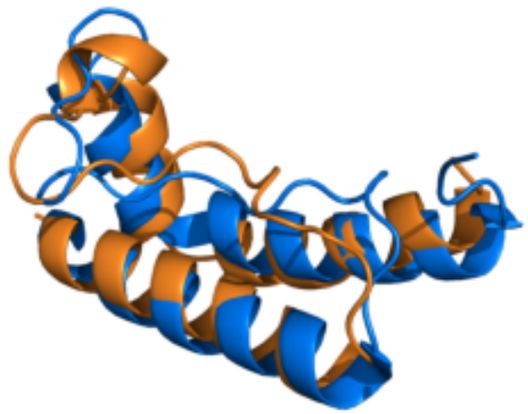
Porta, E. O. J., Steel, P. G. *Curr Res Pharmacol Drug Discov.* **2023**. *5.* 100164–100171.

Activity Based Protein Profiling

Discovering drug–amino acid interactions

Genome sequencing

Predicted proteins



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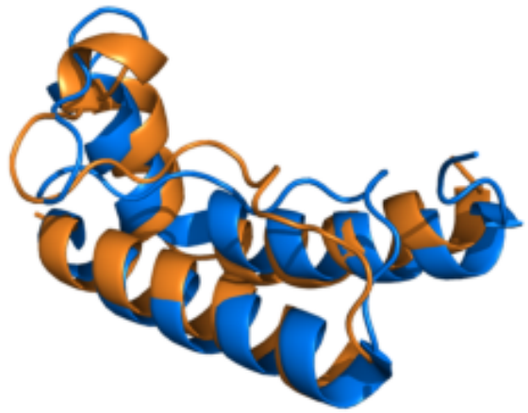

- ***Assignment of protein function remains a challenge***

Porta, E. O. J., Steel, P. G. *Curr Res Pharmacol Drug Discov.* **2023**. *5.* 100164–100171.

Activity Based Protein Profiling

Discovering drug–amino acid interactions

- ***Activity-based protein profiling (ABPP)*** *to probe small molecule–protein interactions*

Porta, E. O. J., Steel, P. G. *Curr Res Pharmacol Drug Discov.* **2023**. *5.* 100164–100171.

Activity Based Protein Profiling

Discovering drug–amino acid interactions

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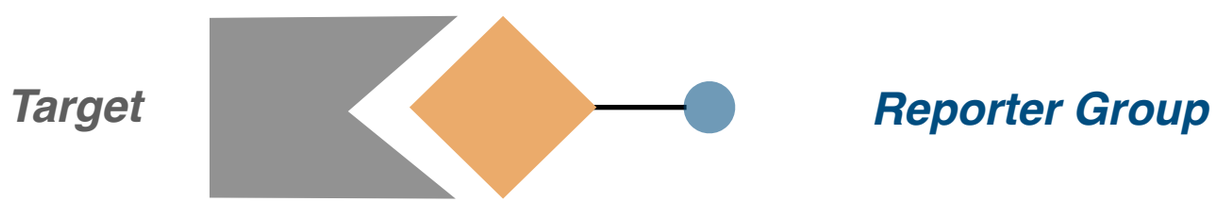
Recognition group "warhead"



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Discovering drug–amino acid interactions

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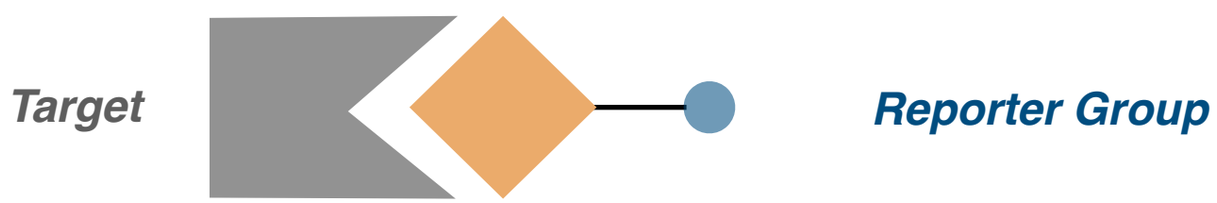


ABPP probes can be tuned towards:

- *Cellular potency*
- *Stereochemical selectivity*
- *Site-specificity*

Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795

Porta, E. O. J., Steel, P. G. *Curr Res Pharmacol Drug Discov.* **2023**. *5.* 100164–100171.

Activity Based Protein Profiling

Discovering reactivity profiles of interactors

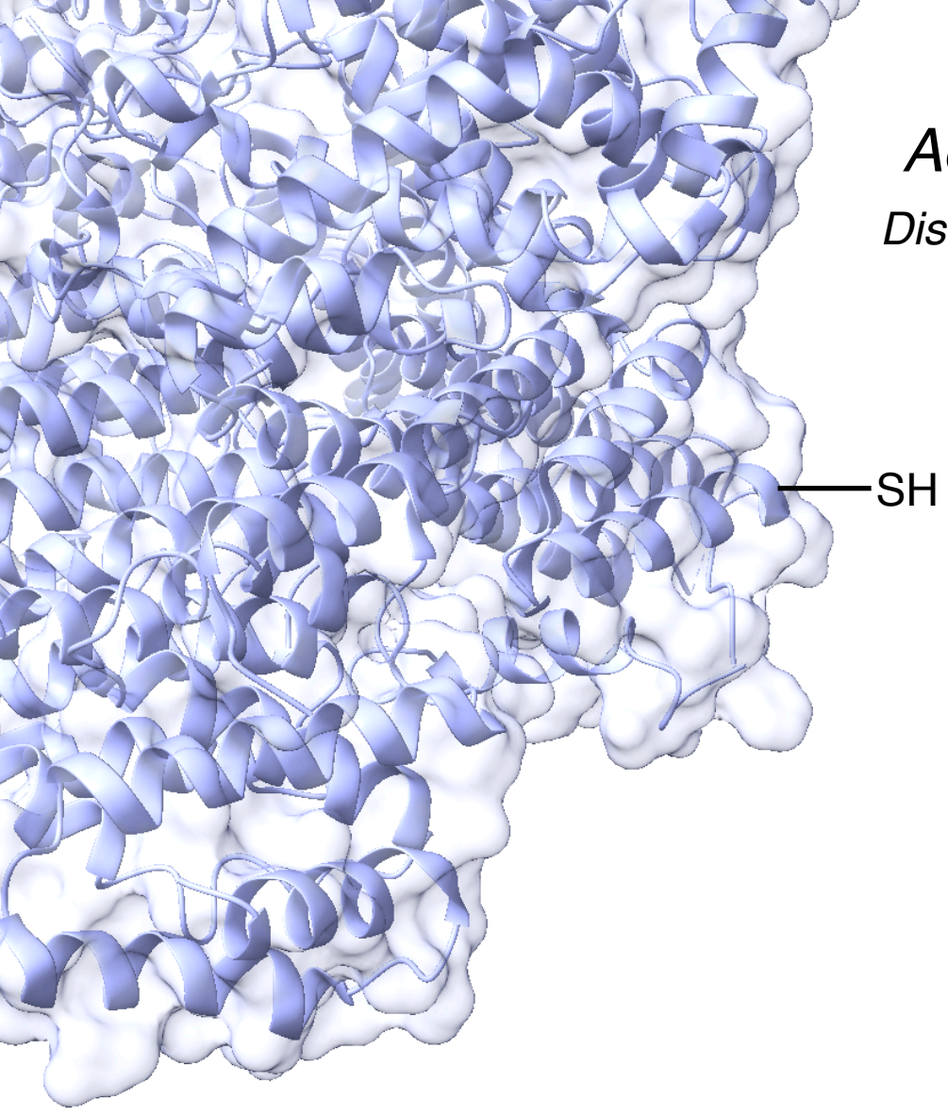
Article | Published: 17 November 2010

Quantitative reactivity profiling predicts functional cysteines in proteomes

Eranthie Weerapana, Chu Wang, Gabriel M. Simon, Florian Richter, Sagar Khare, Myles B. D. Dillon, Daniel A. Bachovchin, Kerri Mowen, David Baker & Benjamin F. Cravatt

Nature **468**, 790–795 (2010) | Cite this article

Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795

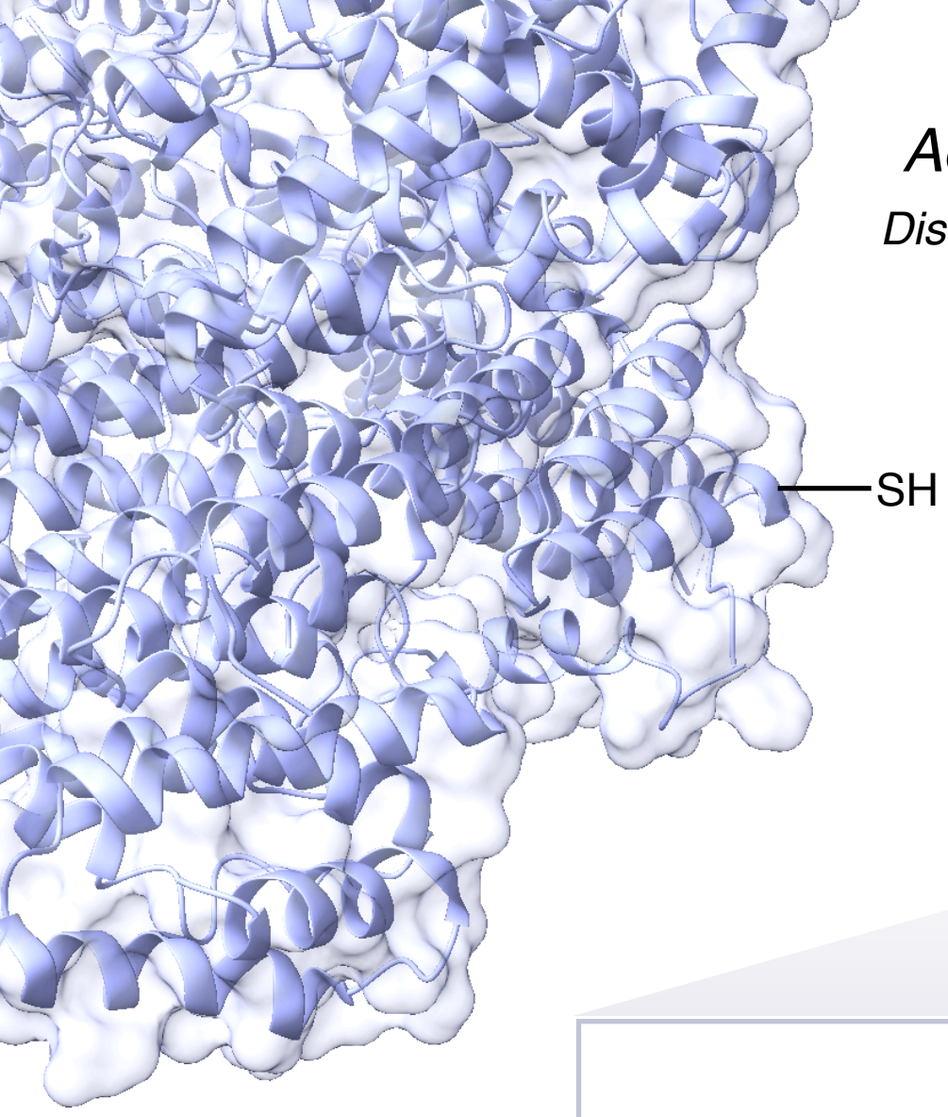


Activity Based Protein Profiling

Discovering reactivity profiles of interactors

- ***Cys is a nucleophilic amino acid***

Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795

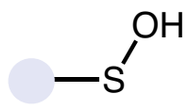


Activity Based Protein Profiling

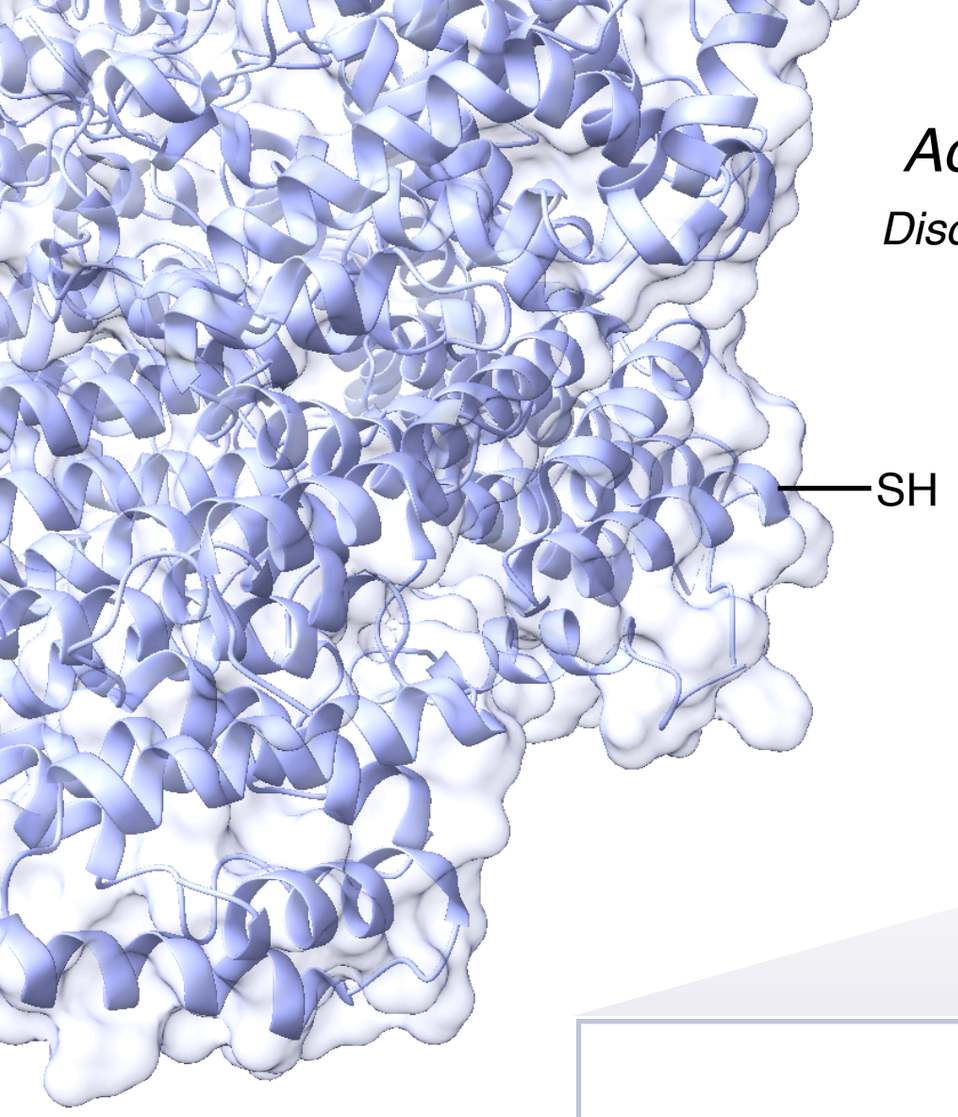
Discovering reactivity profiles of interactors

- ***Cys is a nucleophilic amino acid***

- ***Sulphenation***



Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795



Activity Based Protein Profiling

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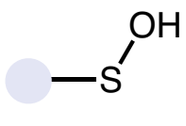
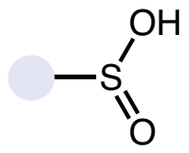
- ***Sulphenation***



- ***Sulphination***



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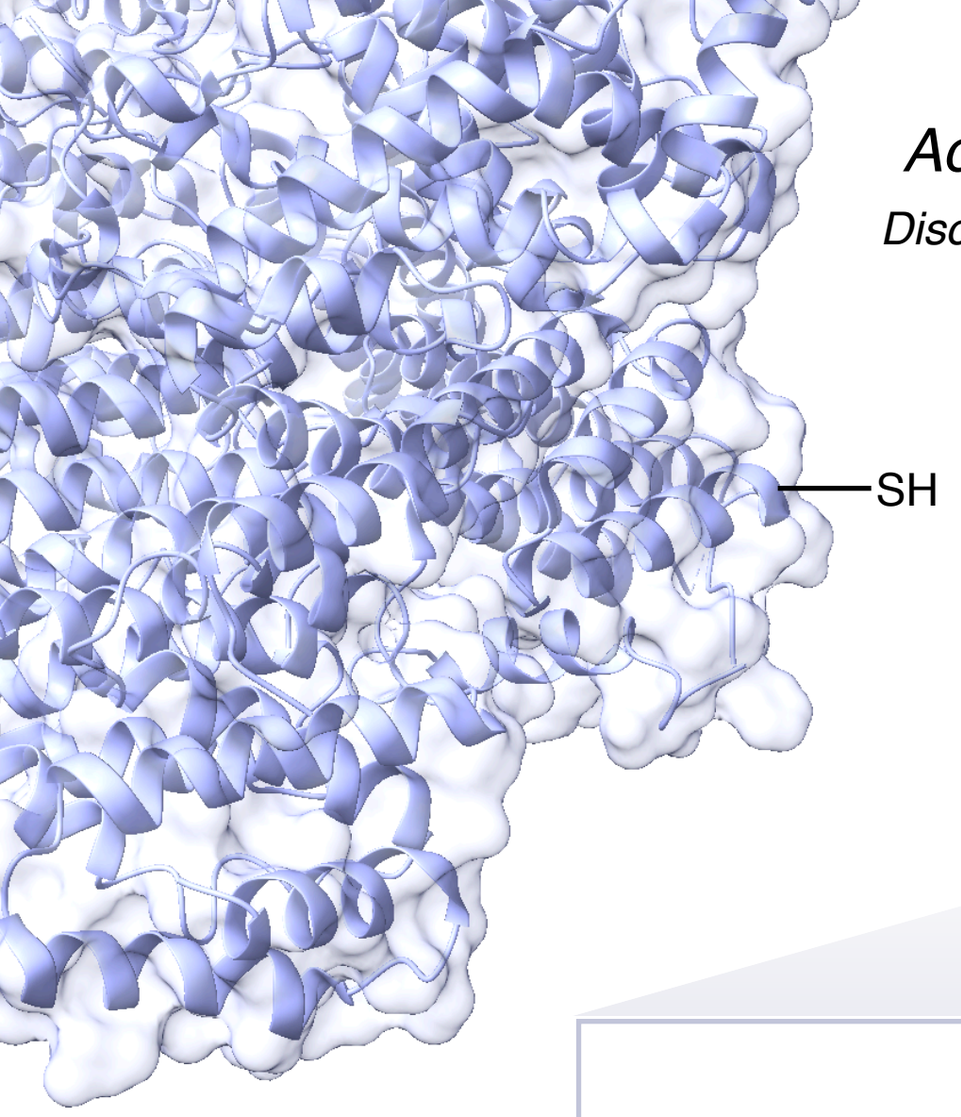

Activity Based Protein Profiling

Discovering reactivity profiles of interactors

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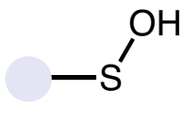
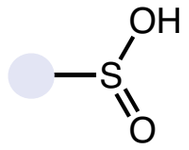
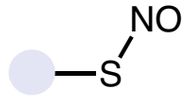
- ***Sulphenation***



- ***Sulphination***



- ***Nitrosylation***



- ***Oxidative PTMs enable regulatory behavior***

Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795

Activity Based Protein Profiling

Discovering reactivity profiles of interactors

Isotopic Tandem Orthogonal Proteolysis-Activity-Based Protein Profiling (isoTOP-ABPP)

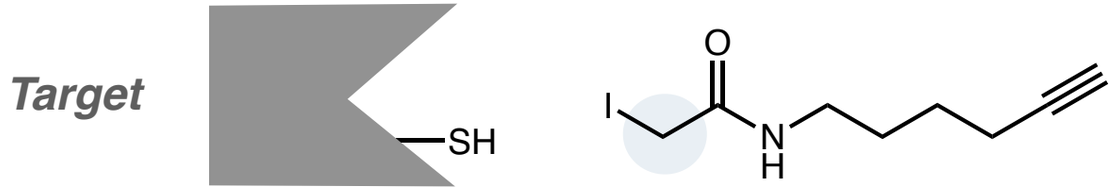
Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795

Activity Based Protein Profiling

Discovering reactivity profiles of interactors

Isotopic Tandem Orthogonal Proteolysis-Activity-Based Protein Profiling (isoTOP-ABPP)

1. Labelling



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Activity Based Protein Profiling

Discovering reactivity profiles of interactors

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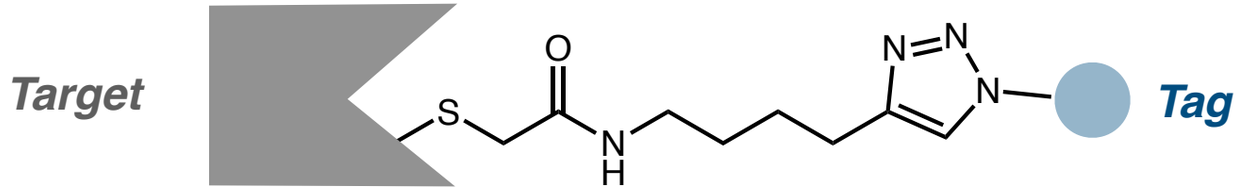
2. Conjugation



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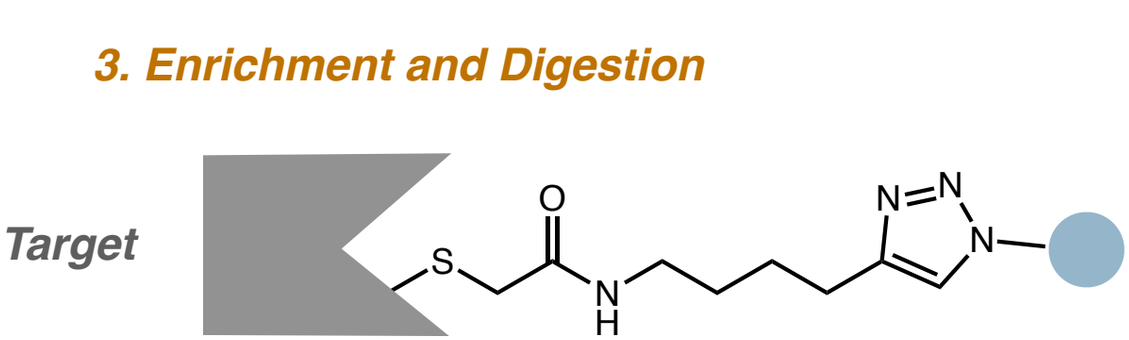
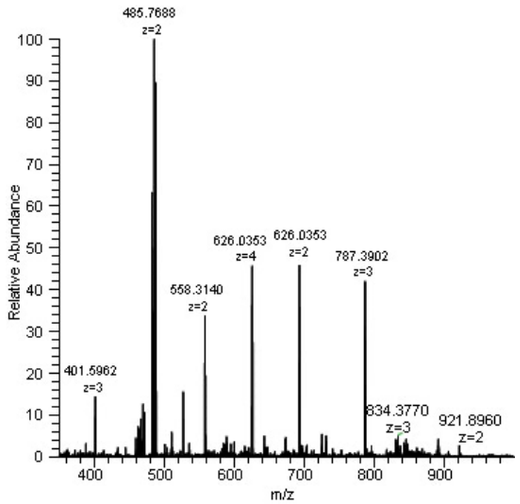
Activity Based Protein Profiling

Discovering reactivity profiles of interactors

Isotopic Tandem Orthogonal Proteolysis-Activity-Based Protein Profiling (isoTOP-ABPP)

3. Enrichment and Digestion





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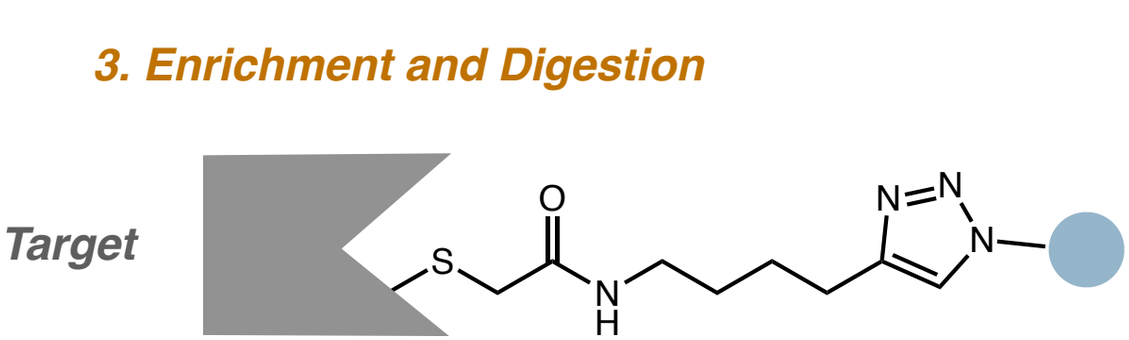
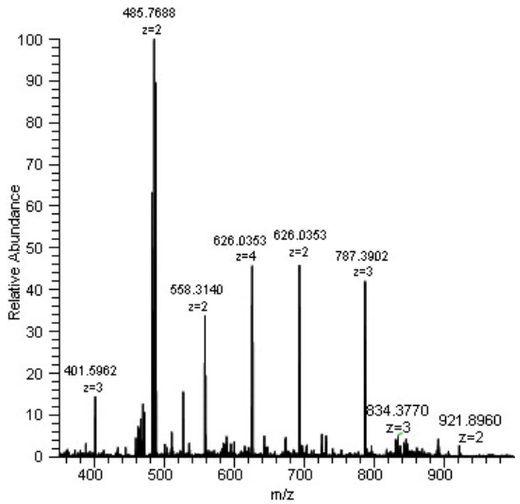


1. Avidin enrichment

2. Trypsin digest

3. LC/MS analysis



isotopic ratios of the peptides enable quantification in Cys reactivity

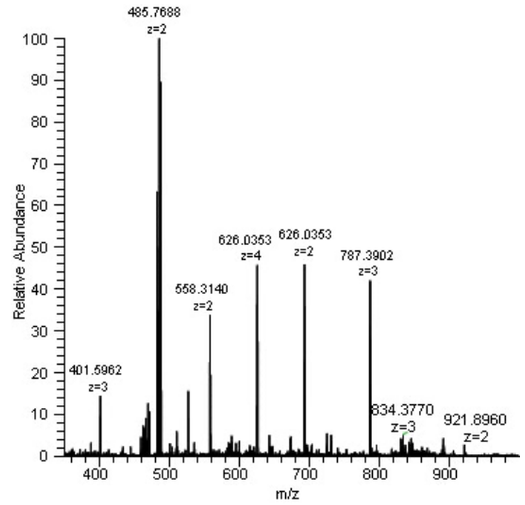
Weerapana, E., Wang, C., Simon, G. M., Richter, F., Khare, S., Dillon, M. B. D., Bachovin, D. A., Mowen, K., Baker, D., Cravatt, B. F. *Nature.* **2010**. *468*, 790–795

Activity Based Protein Profiling

Discovering reactivity profiles of interactors

- ***Activity-Based Protein Profiling (ABPP)***

MS Quantifies extent of Cys labelling



LC/MS analysis

Cravatt, B. F. *Isr. J. Chem.* **2023**. *63*, 3–4

Activity Based Protein Profiling

Discovering reactivity profiles of interactors

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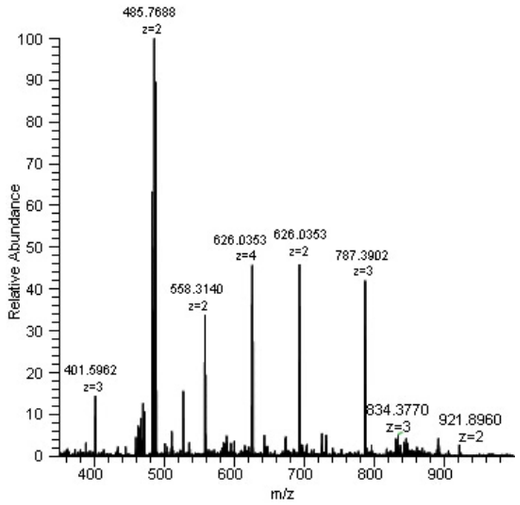


LC/MS analysis

Enables Target ID and SAR

Cravatt, B. F. *Isr. J. Chem.* **2023**. *63*, 3–4

Activity Based Protein Profiling

Discovering interactors

- *ABPP in drug discovery*

FRONTIER MEDICINES

abbvie

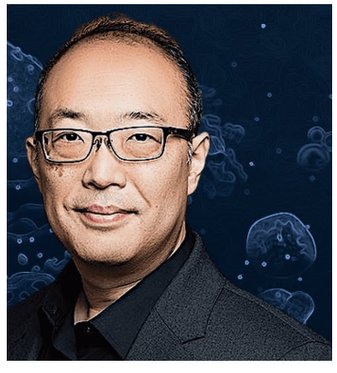
Founded in 2018

Prof. Dan Nomura

Activity Based Protein Profiling

Discovering interactors

- *Reactive binding site*
- *Library hit*



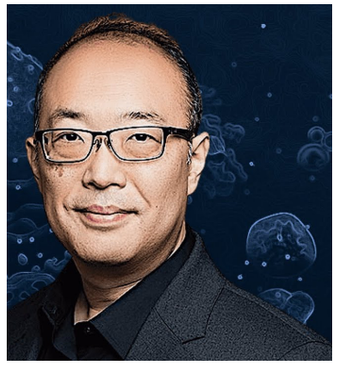
Prof. Dan Nomura

Activity Based Protein Profiling

Discovering interactors

- *Reactive binding site*
- *Library hit*

- ***Activation score enables a rank-prioritization across the proteome***



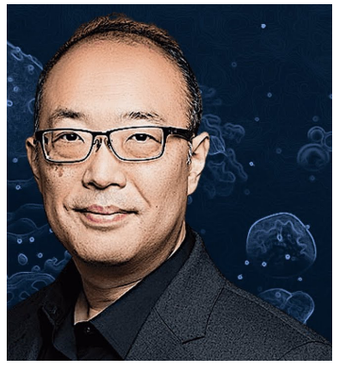
Prof. Dan Nomura

Activity Based Protein Profiling

Discovering interactors

- *Reactive binding site*
- *Library hit*

- ***Activation score enables a rank-prioritization across the proteome***
- ***Proposal: Informs drug discovery***

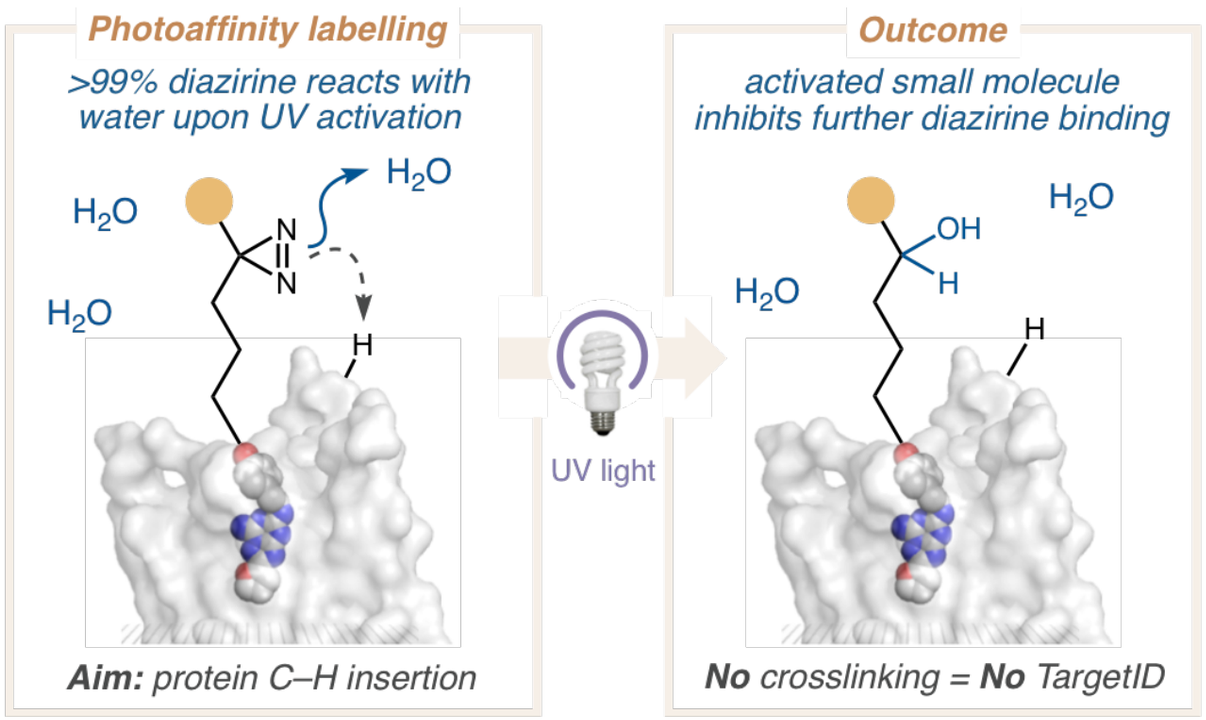


Prof. Dan Nomura

Small molecule photocatalysis enables drug target identification

Trowbridge, Aaron D.; Seath, Ciaran P.; Rodriguez,-Rivera, Frances P. et al. *Proc. Natl. Acad. Sci.* **2022,** 119 (34).

Small molecule photocatalysis enables drug target identification

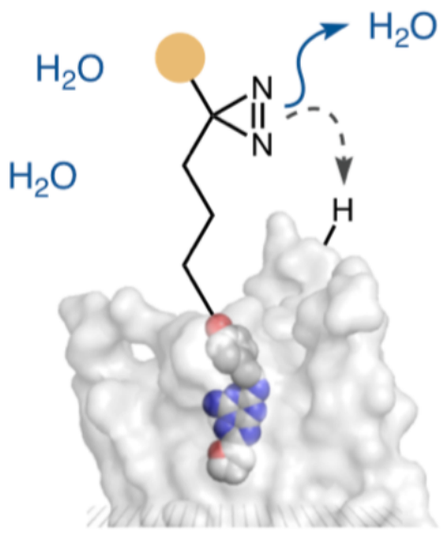


Trowbridge, Aaron D.; Seath, Ciaran P.; Rodriguez,-Rivera, Frances P. et al. *Proc. Natl. Acad. Sci.* **2022,** 119 (34).

Investigating Interactors

Modern Target ID methods:

1. PAL



Directly tag the target

Investigating Interactors

Modern Target ID methods:

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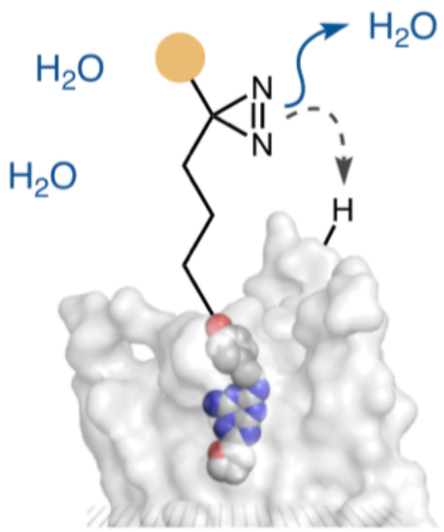
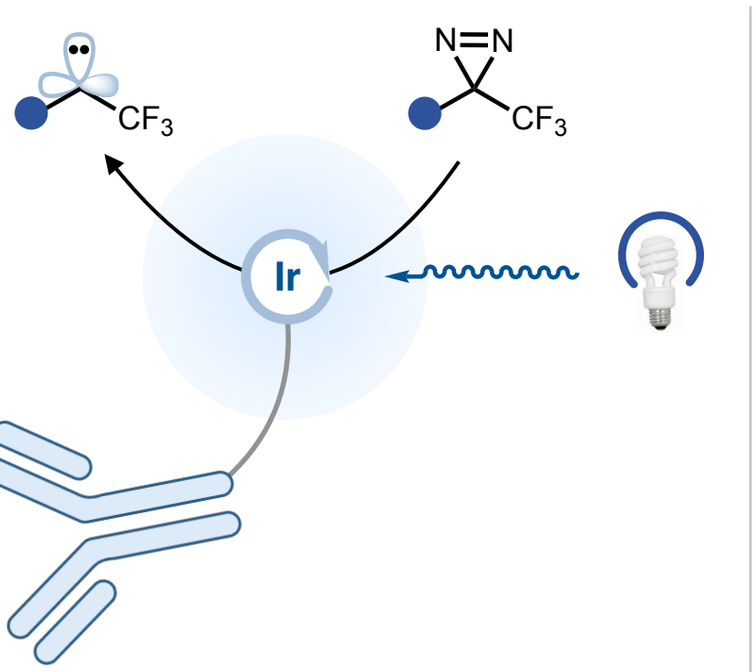


Directly tag the target

2. Photoreactive probes



μmap

Investigating Interactors

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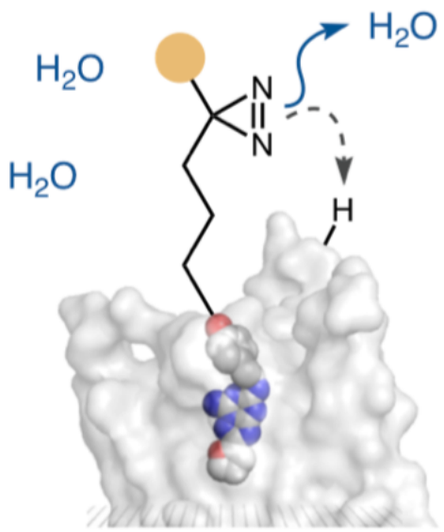

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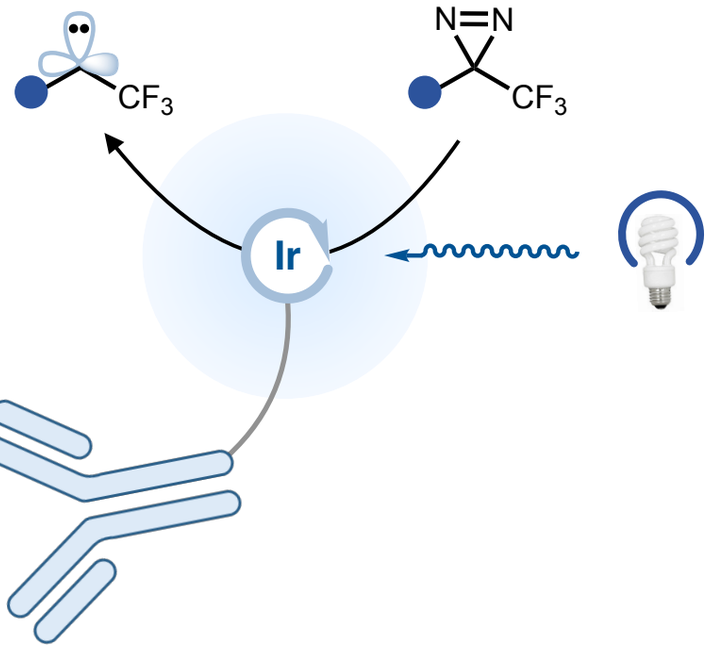
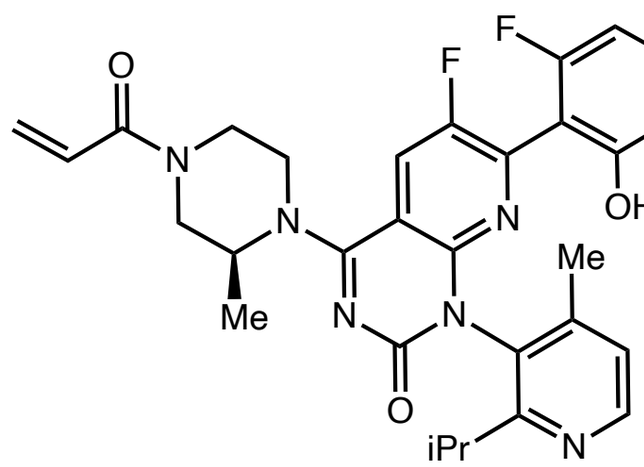


µmap

3. Activity Based Protein Profiling



No target modification

Investigating Interactors

Modern Target ID methods:

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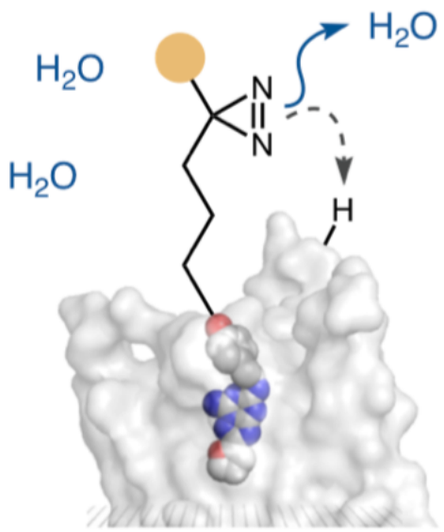

Directly tag the target

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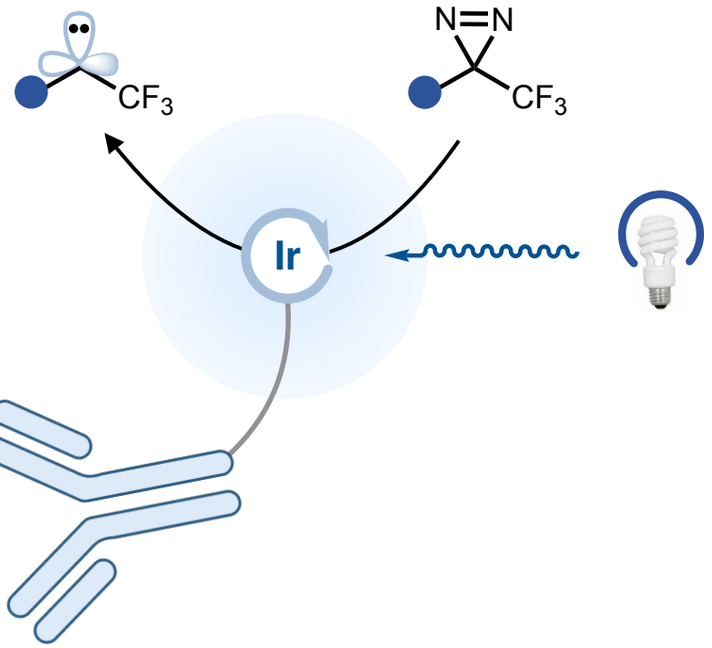

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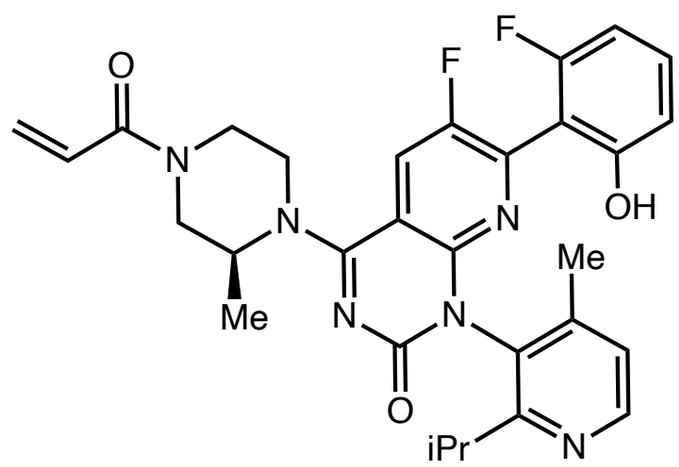
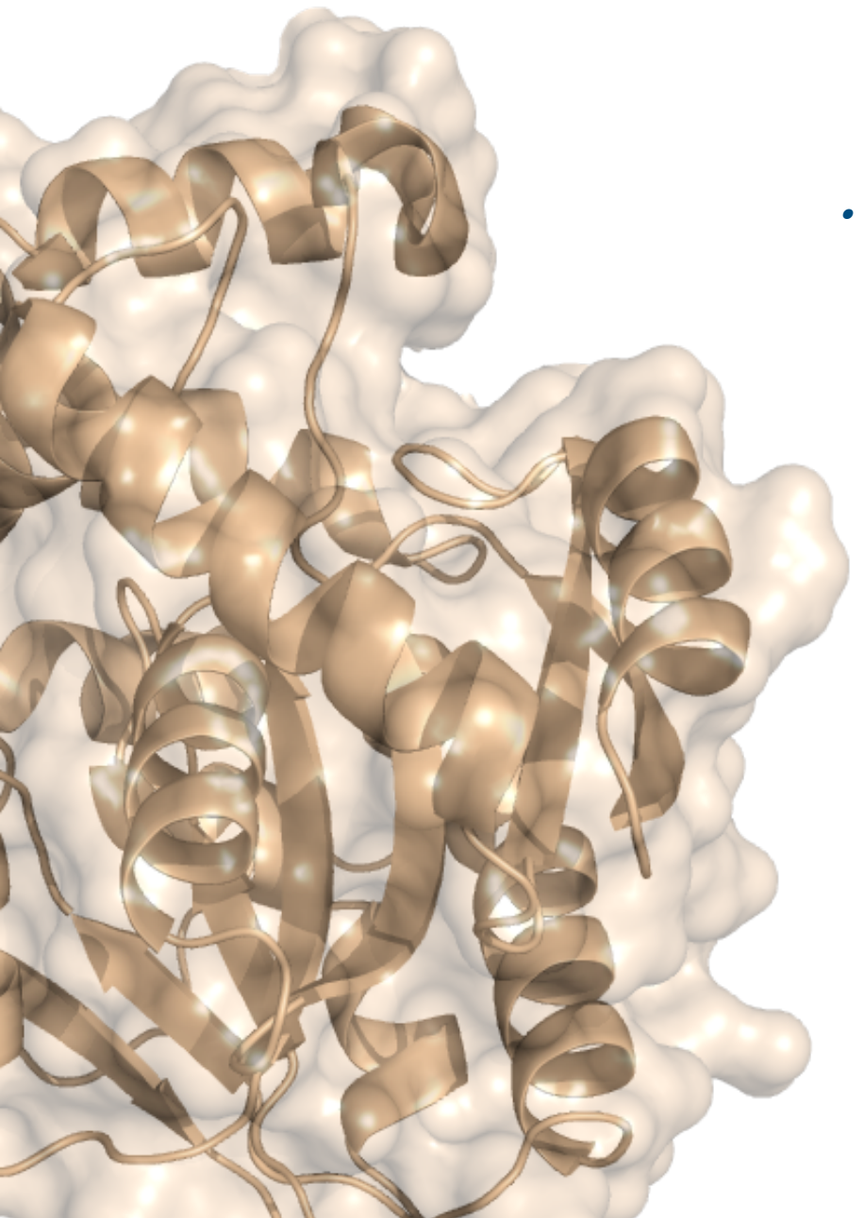


No target modification

Highly limited to Cys residues

Ligand Discovery

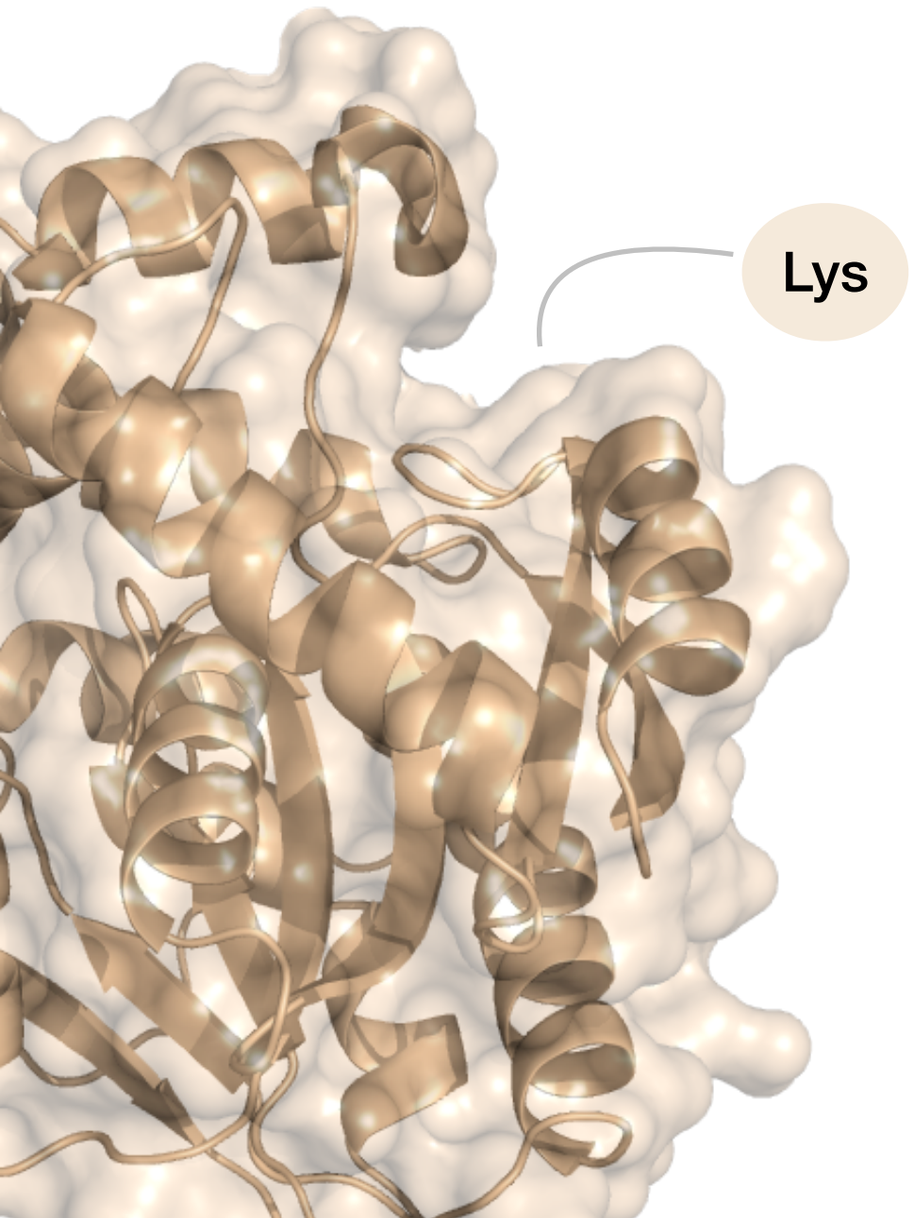
Expanding methods to drug nucleophilic residues



- ***The human proteome contains druggable pockets with no proximal cysteines***

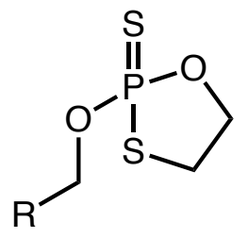
Ligand Discovery

Expanding methods to drug nucleophilic residues



- ***How do you drug other nucleophilic amino acids?***

Ligand Discovery

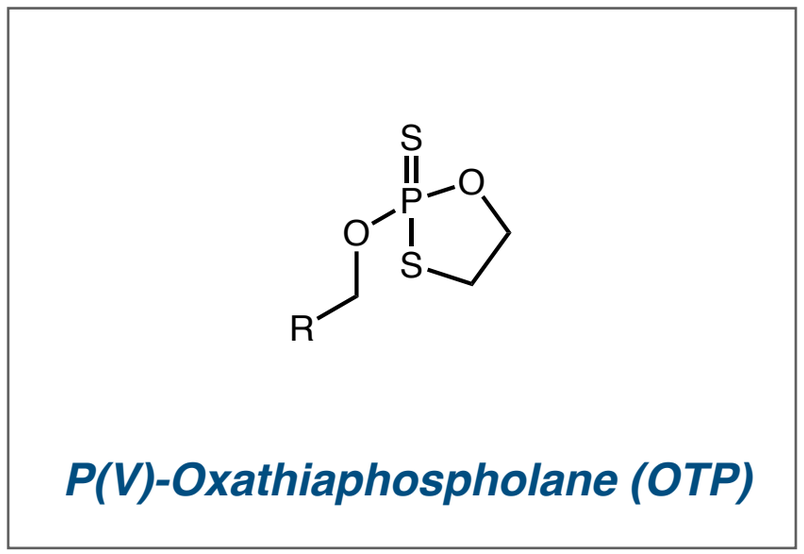


P(V)-Oxathiaphospholane (OTP)

Knouse, K. W. et al. *Science.* **2018**. *361*, 1234–1238

Bristol Myers Squibb®

Ligand Discovery



P(V)-Oxathiaphospholane (OTP)

selective bioconjugation

Knouse, K. W. et al. *Science.* **2018**. *361*, 1234–1238

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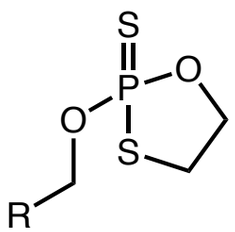
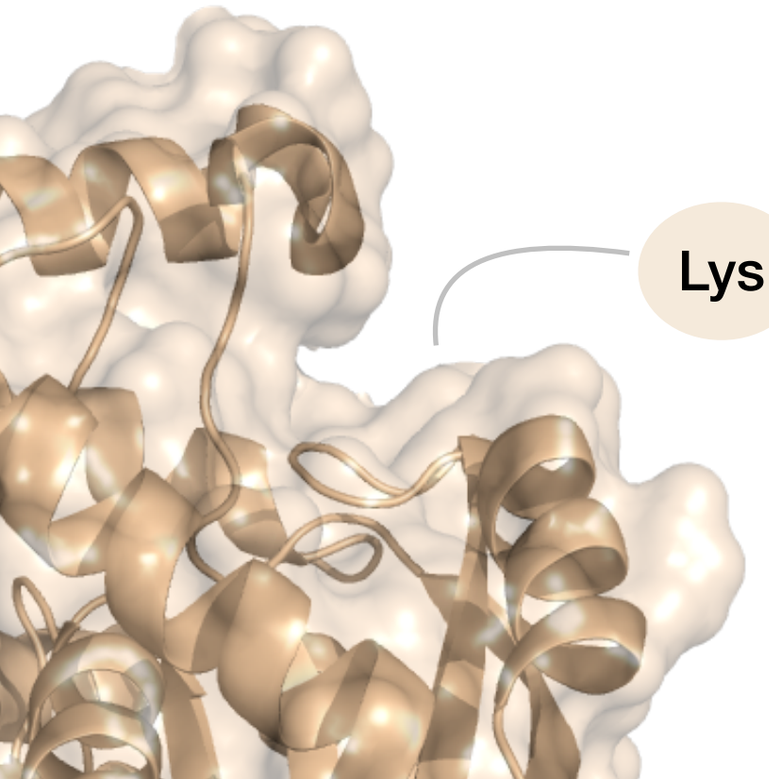
Ligand Discovery



P(V)-Oxathiaphospholane (OTP)



selective bioconjugation

Knouse, K. W. et al. *Science.* **2018**. *361*, 1234–1238

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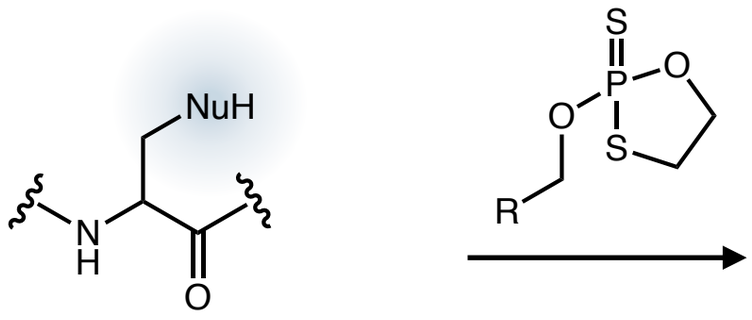
Ligand Discovery

Expanding methods to drug nucleophilic residues

- ***OTP probe for covalent targeting***



Sharma, H. A., Bielecki, M., Holm, M. A., Thompson, T. M., Yin, Y., Cravatt, J. B., Ware, T. B., Reed, A., Nassir, M., El-Hayek Ewing, T., Melillo, B., Bazan, J. F., Baran, P. S., Cravatt, B. F. *J. Am. Chem. Soc.* **2025**. *ASAP*

Ligand Discovery

Expanding methods to drug nucleophilic residues

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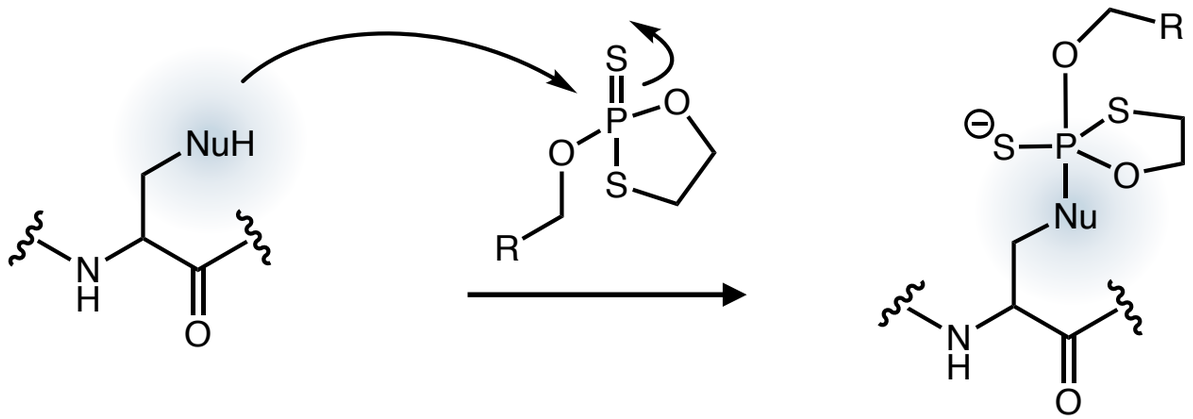

Anionic, pentavalent intermediate

Sharma, H. A., Bielecki, M., Holm, M. A., Thompson, T. M., Yin, Y., Cravatt, J. B., Ware, T. B., Reed, A., Nassir, M., El-Hayek Ewing, T., Melillo, B., Bazan, J. F., Baran, P. S., Cravatt, B. F. *J. Am. Chem. Soc.* **2025**. *ASAP*

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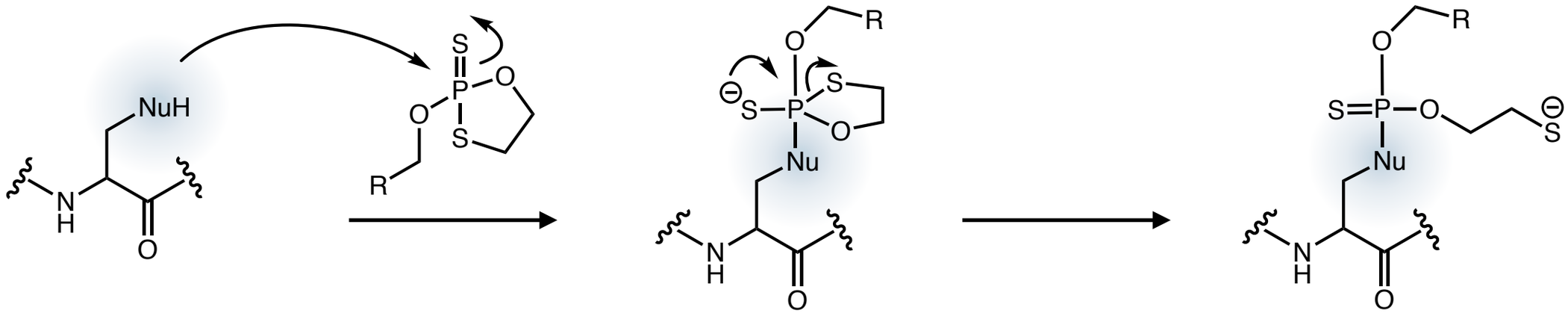

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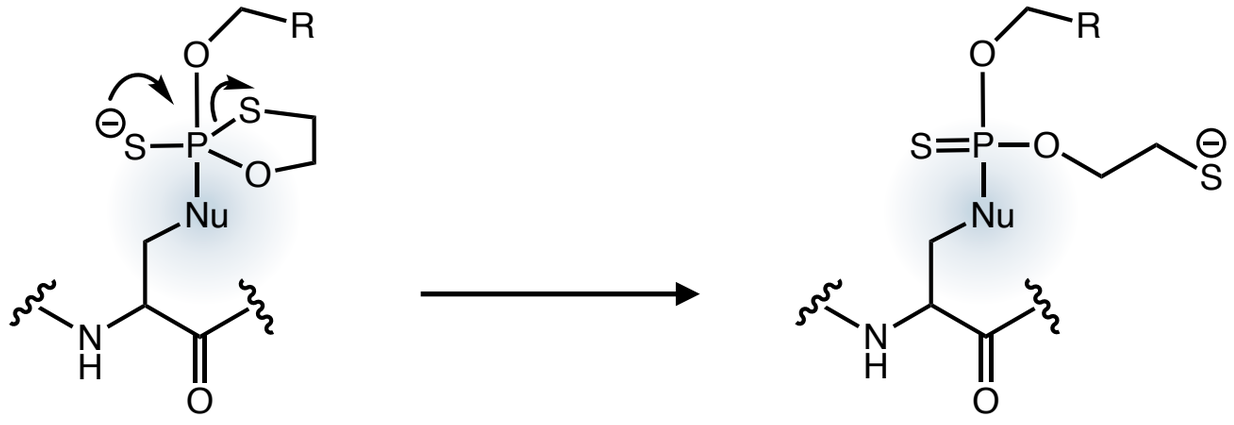

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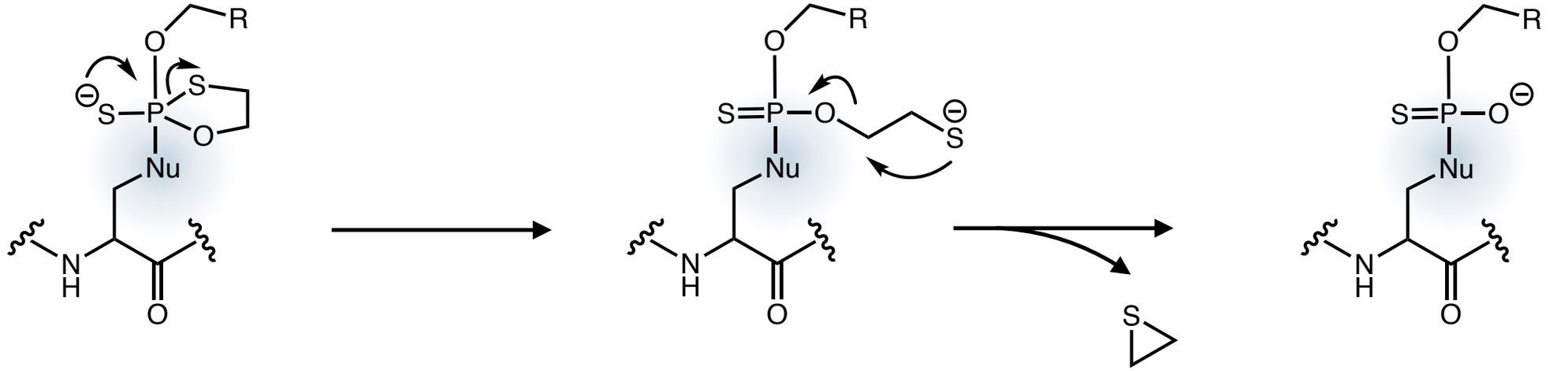

P(V)-amino acid

Modified P(V)-amino acid residue

Sharma, H. A., Bielecki, M., Holm, M. A., Thompson, T. M., Yin, Y., Cravatt, J. B., Ware, T. B., Reed, A., Nassir, M., El-Hayek Ewing, T., Melillo, B., Bazan, J. F., Baran, P. S., Cravatt, B. F. *J. Am. Chem. Soc.* **2025**. *ASAP*

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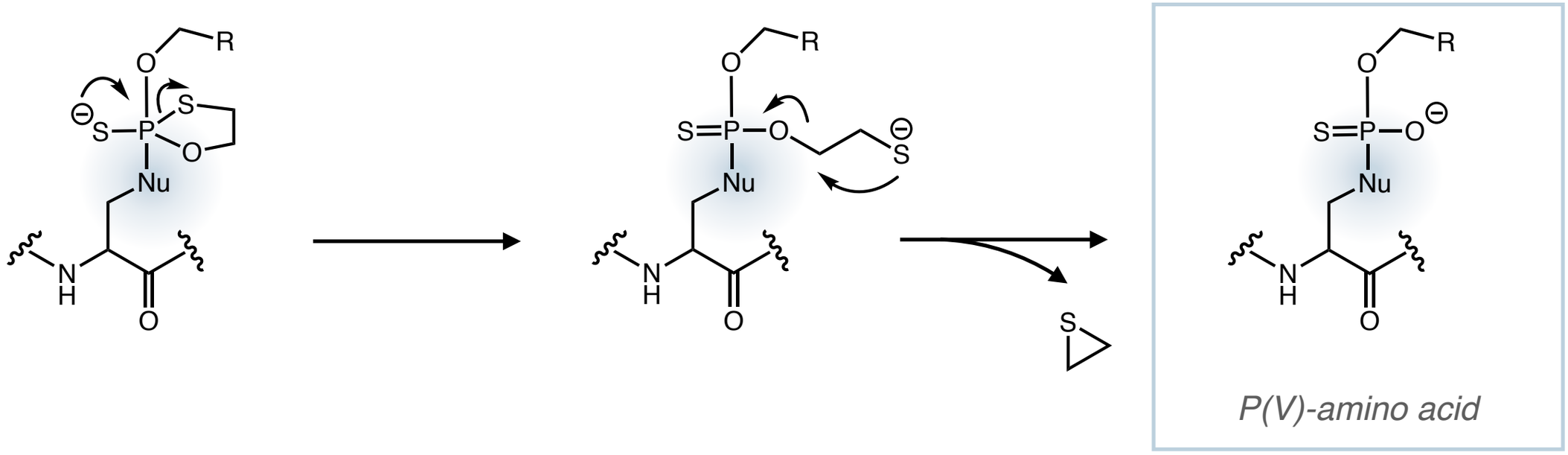

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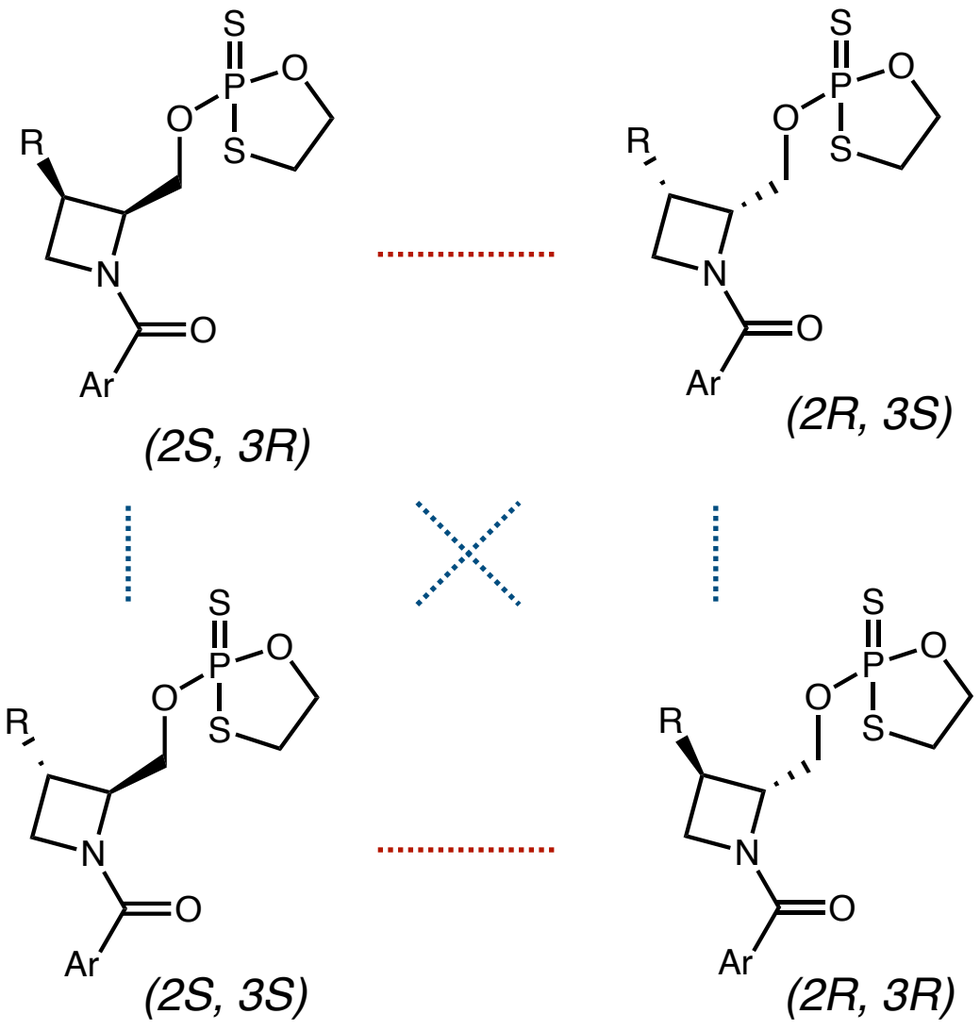

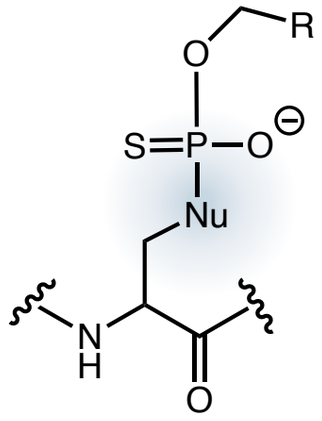

P(V)-amino acid

- *P(V) stereoprobes are stereosiomerically defined*

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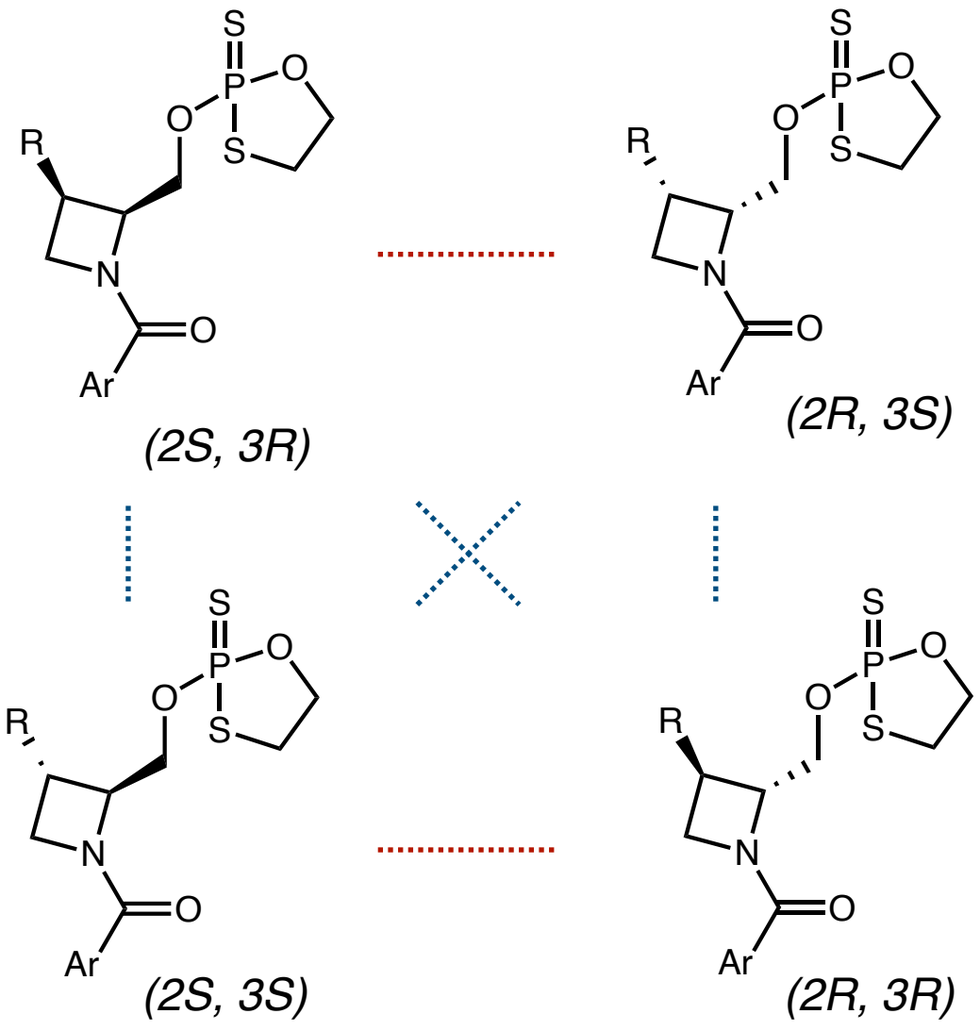
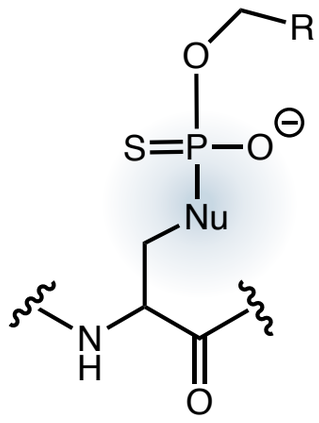




P(V)-amino acid

- *P(V) stereoprobes are stereosiomerically defined*
- *Enables study of stereoselective protein-ligand interactions*

Sharma, H. A., Bielecki, M., Holm, M. A., Thompson, T. M., Yin, Y., Cravatt, J. B., Ware, T. B., Reed, A., Nassir, M., El-Hayek Ewing, T., Melillo, B., Bazan, J. F., Baran, P. S., Cravatt, B. F. *J. Am. Chem. Soc.* **2025**. *ASAP*

Part 1

Covalent inhibitors

Part 2

Biologics

Non-covalent interactors

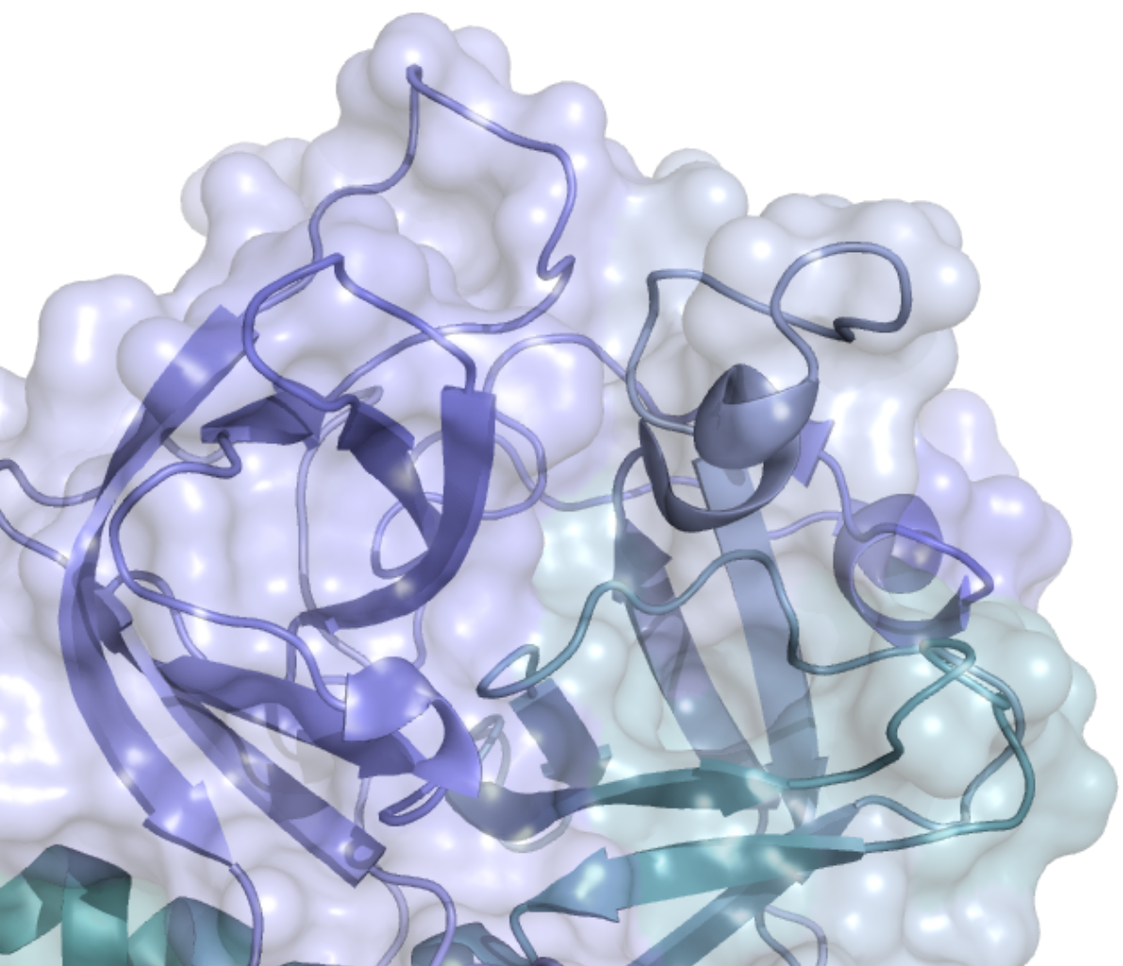
Part 3

Prions and Multi drug resistance: The Looming Plagues

Current Therapeutics

*Targeted Protein Degradation (**TPD**) technology*

A major limitation** to targeted protein degradation is reliance on **small molecule drugs

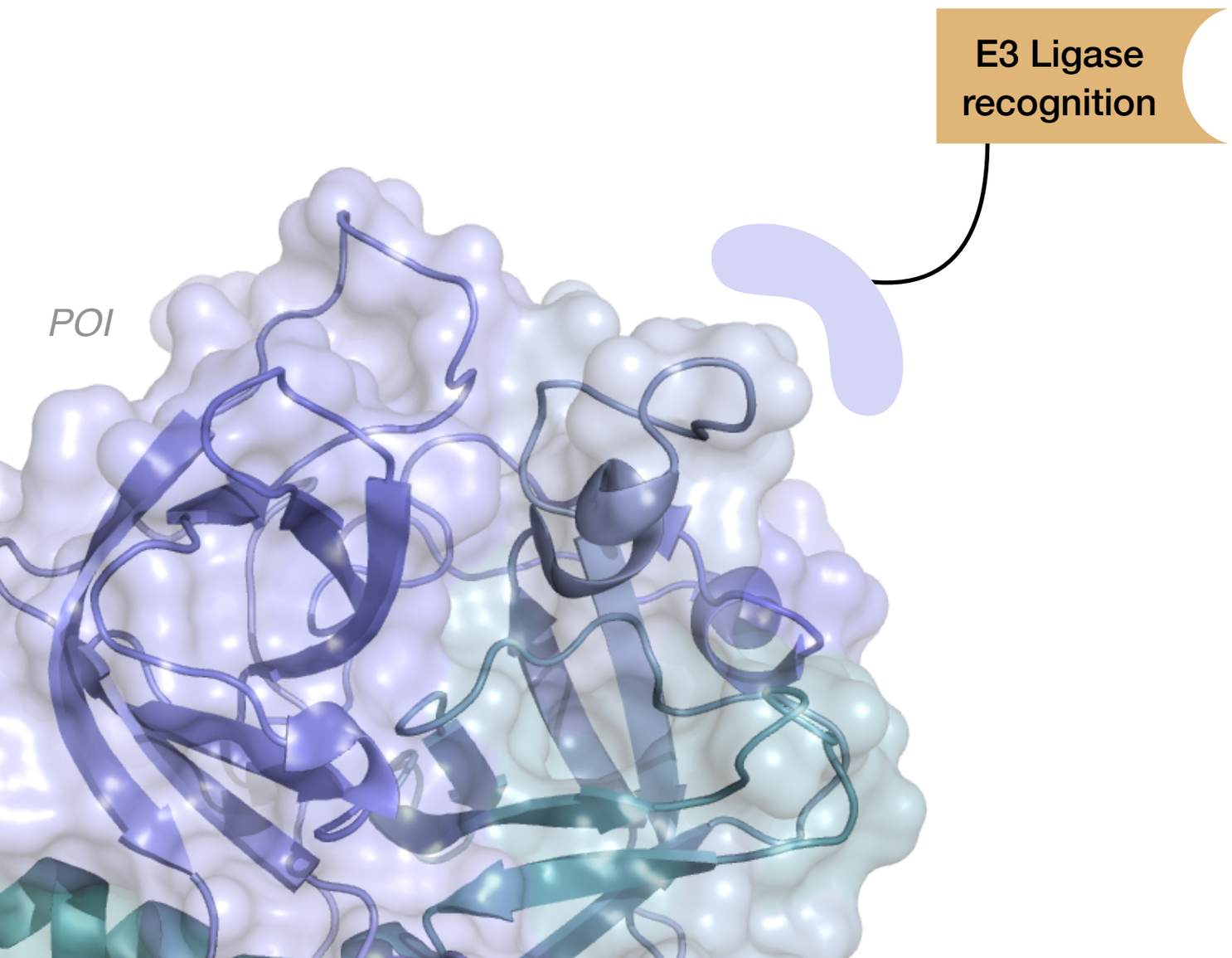


Current Therapeutics

Targeted Protein Degradation (TPD) technology

- ***2 major classes of small molecules for TPD:***

1. PROTACs



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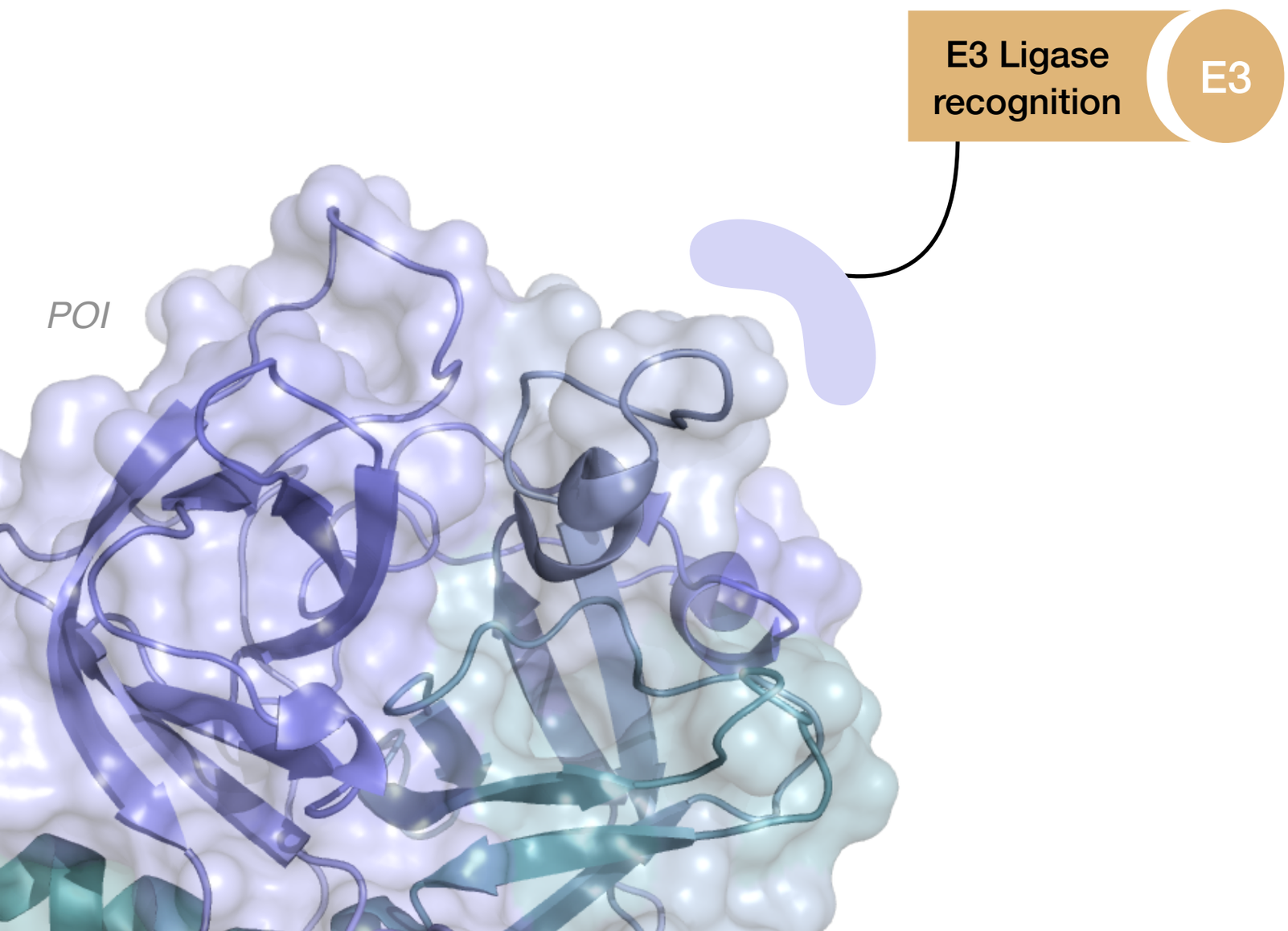


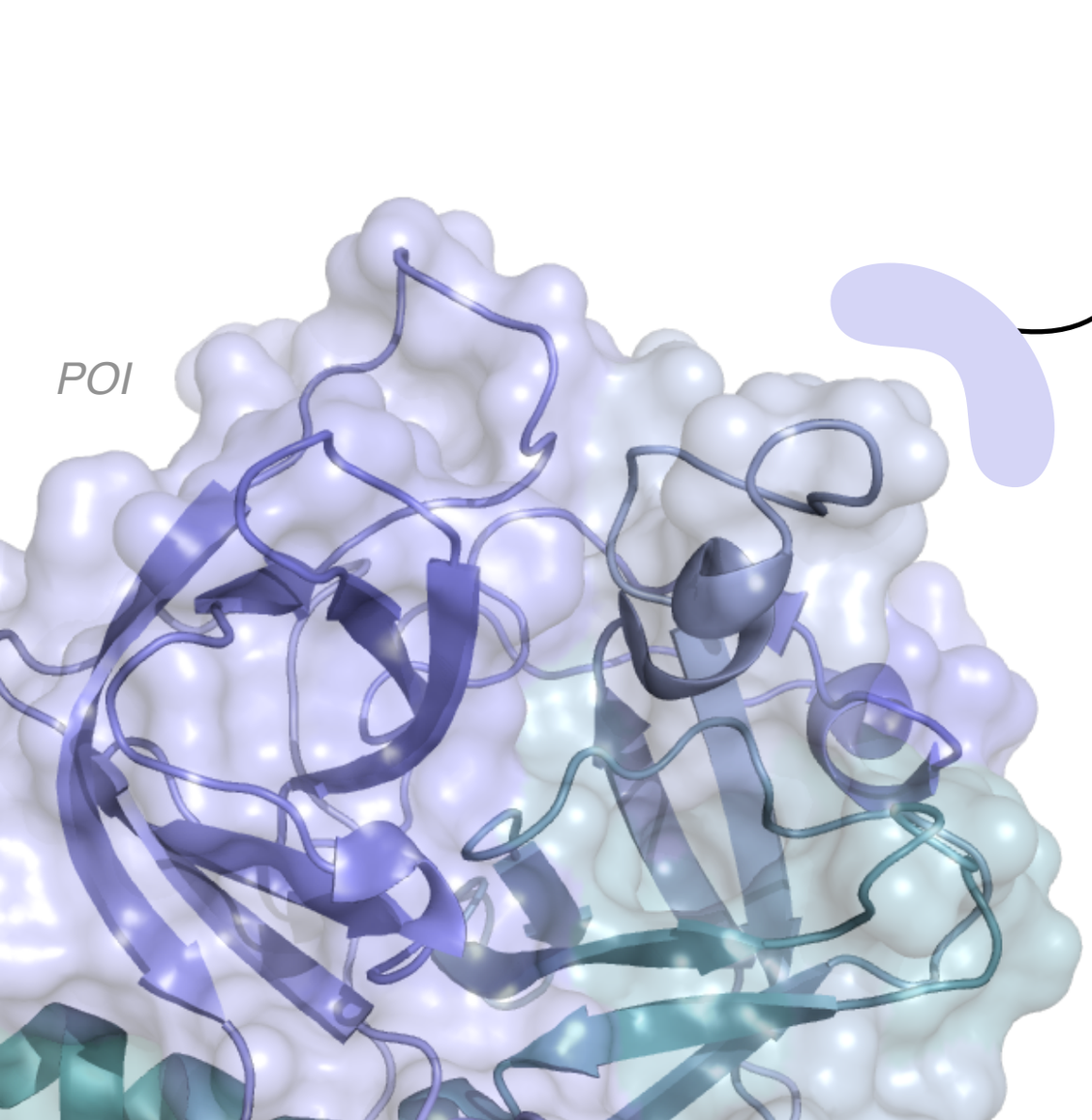
Current Therapeutics

Targeted Protein Degradation (TPD) technology

- ***2 major classes of small molecules for TPD:***

1. PROTACs

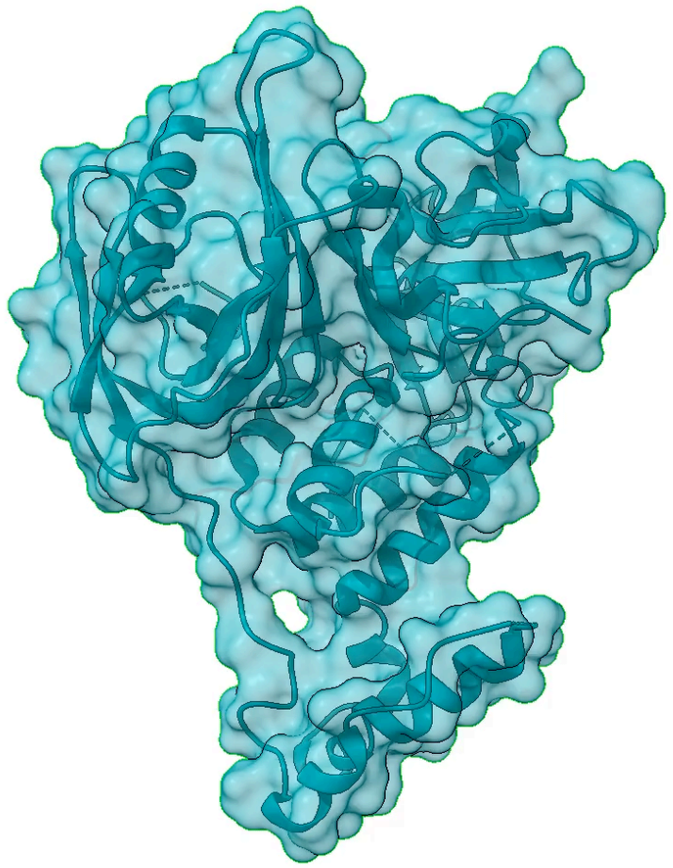
2. Molecular glues



Protein Targeting

Heterobifunctionals

Cereblon



IMiDs bind the E3 ligase protein, Cereblon

Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H. *Science* **2010**, *327*, 1345.

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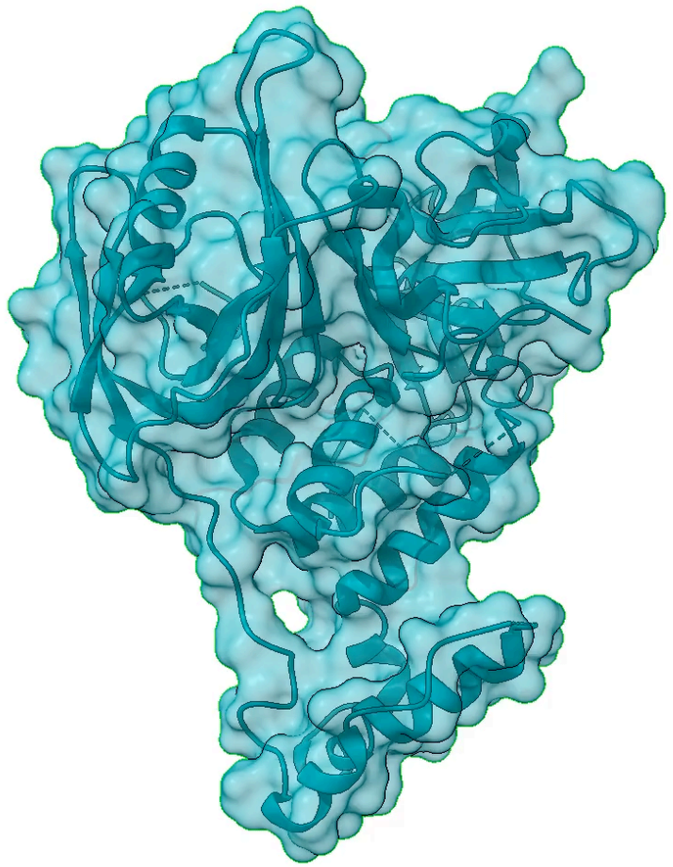

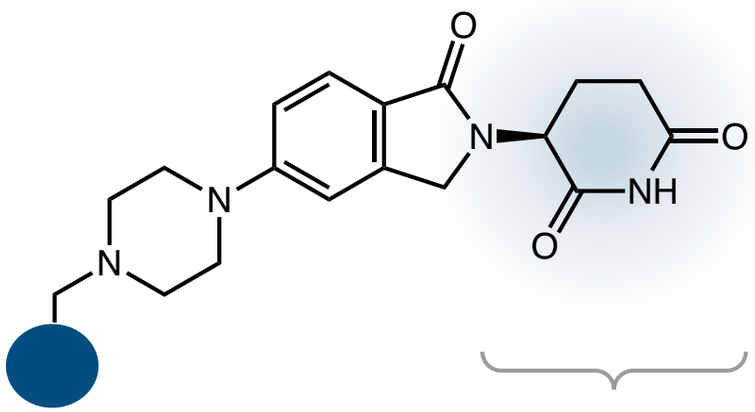

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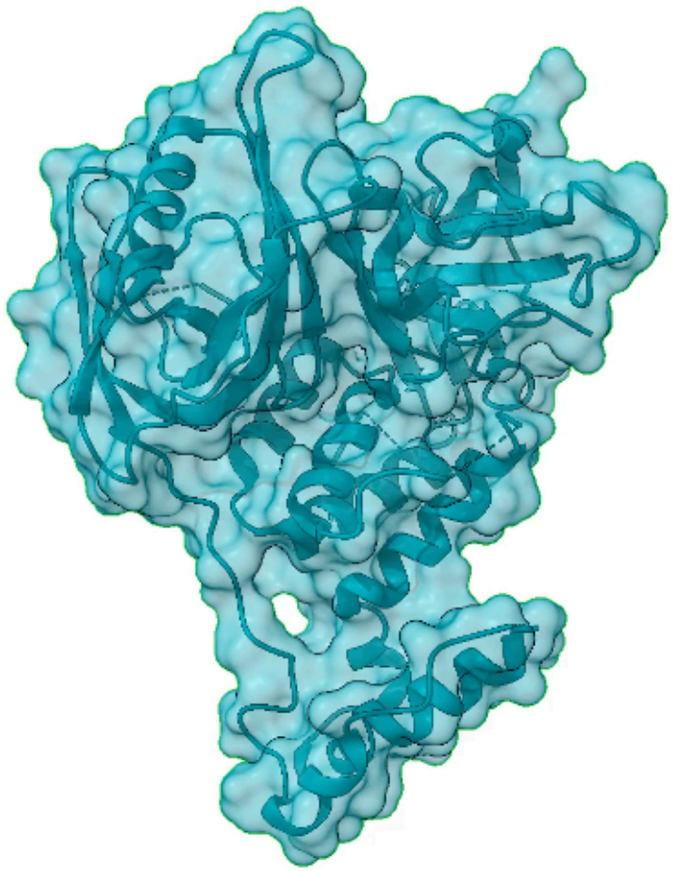
Protein Targeting

Induced proximity

Cereblon



Benefits to Heterobifunctionals:

1. Target non-enzymatic pockets

2. Catalytic degraders

3. Degradation removes entire protein

4. PROTAC warheads don’t require high binding affinity

Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H. *Science* **2010**, *327*, 1345.

Current Strategies

PROTACs in the clinic

- ***PROTACs***

Prof. Craig Crews

Current Strategies

PROTACs in the clinic

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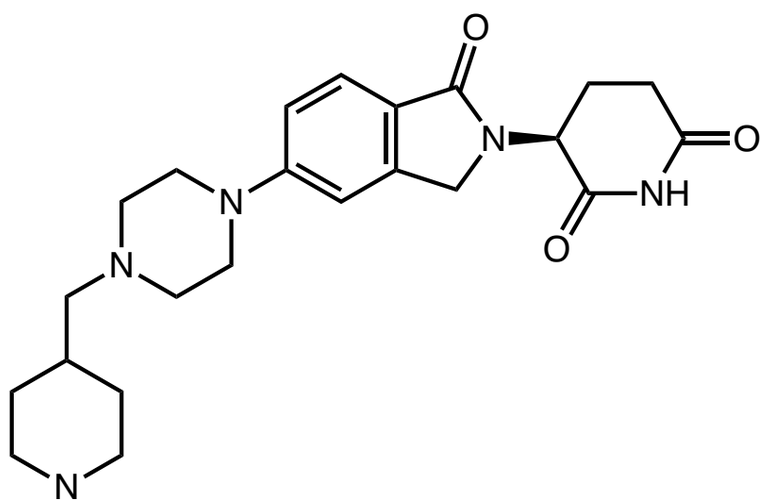


Vepdegestrant

Pfizer

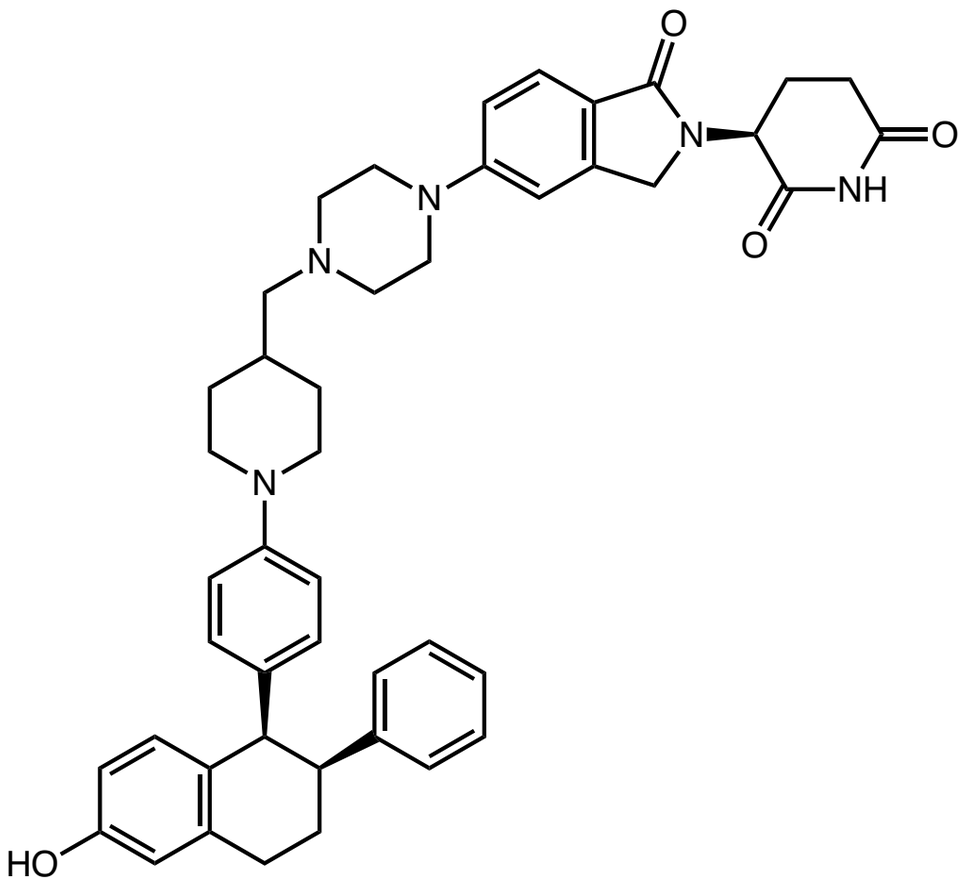
ARVINAS

Prof. Craig Crews

Current Strategies

PROTACs in the clinic

- ***PROTACs***



Vepdegestrant

- ***PROTAC protein degrader***
- ***ER+, HER2- breast cancer***
- ***Targets estrogen receptor (ER)***

Pfizer

ARVINAS

Prof. Craig Crews

Current Strategies

PROTACs in the clinic

Press Release Details

Arvinas and Pfizer Announce Positive Topline Results from Phase 3 VERITAC-2 Clinical Trial

March 11, 2025

– VERITAC-2 achieved its primary endpoint in the estrogen receptor 1-mutant population, demonstrating statistically significant and clinically meaningful improvement in progression-free survival –

– Vepdegestrant is the first PROTAC degrader to demonstrate clinical benefit in a Phase 3 trial –

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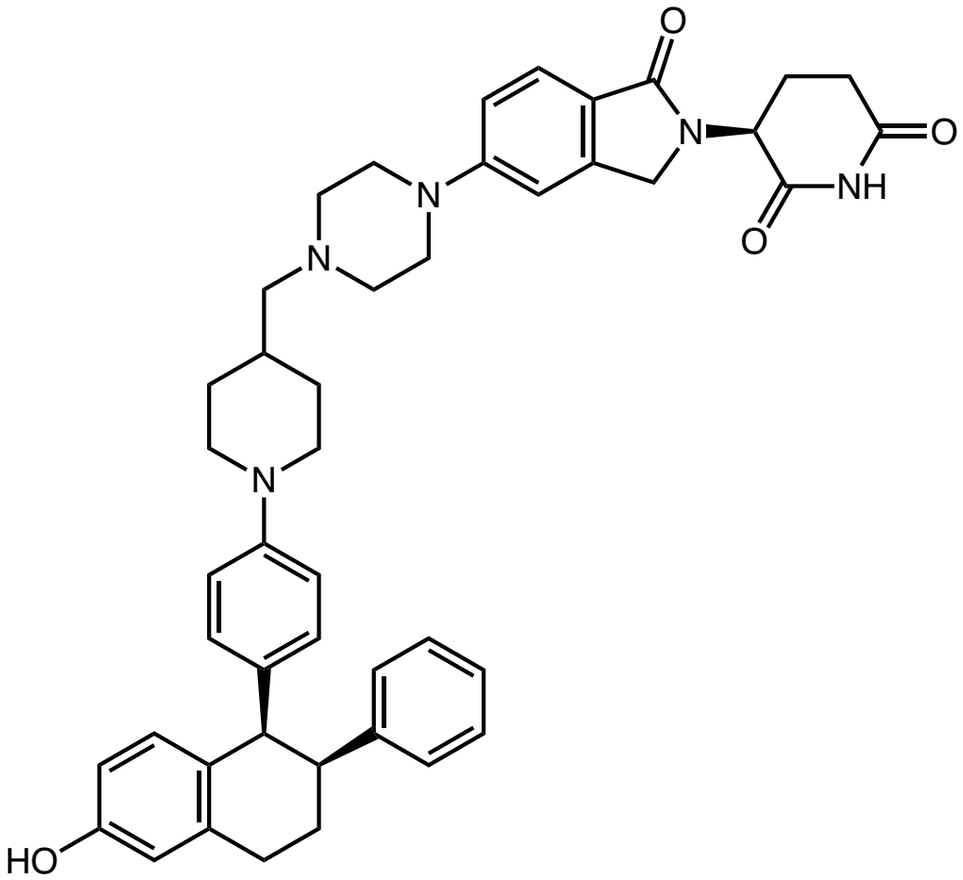
- ***1st PROTAC degrader to show clinical benefit in Phase III trial***

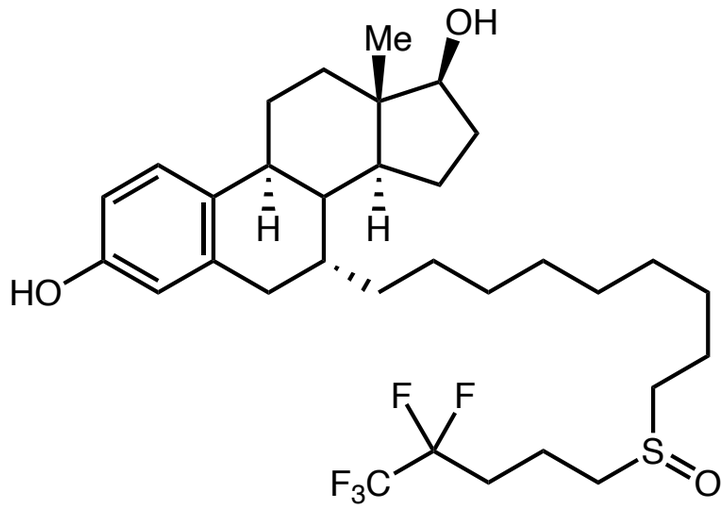
Current Strategies

Vepdegestrant PROTAC in the clinic



Vepdegestrant

versus



Fulvestrant

Arvinas Pfizer joint press release

Current Strategies

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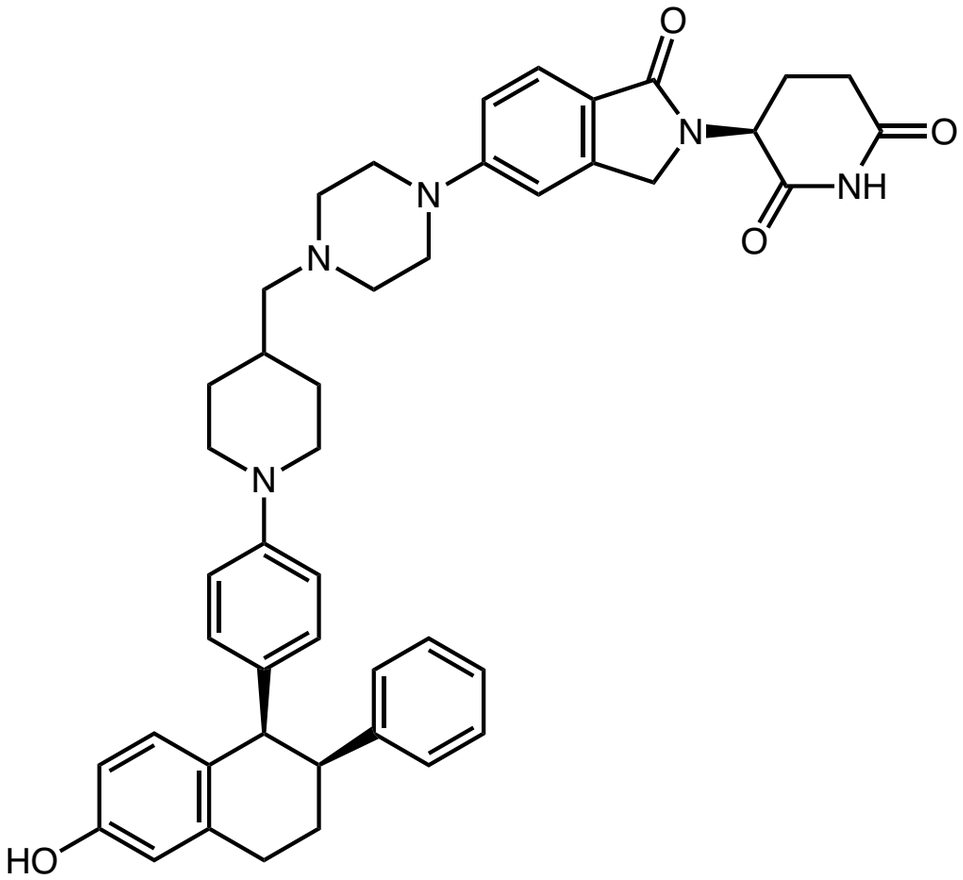

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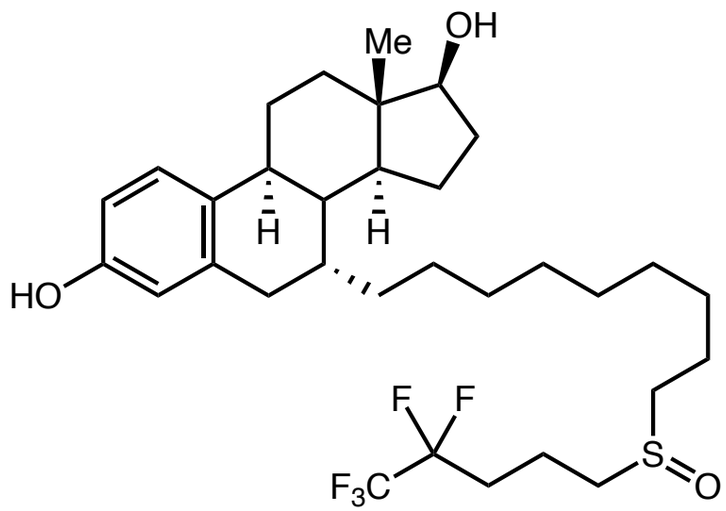
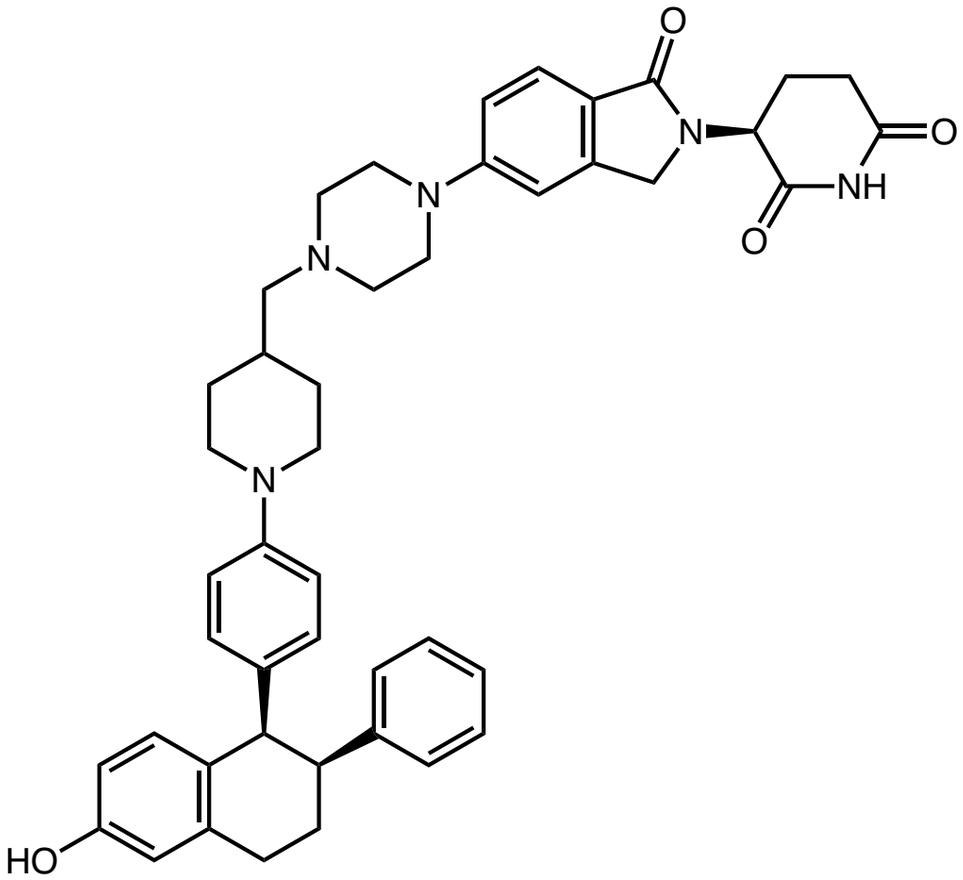


Fulvestrant

- ***624 patients with ER+/HER2- breast cancer***

Current Strategies

Vepdegestrant PROTAC in the clinic



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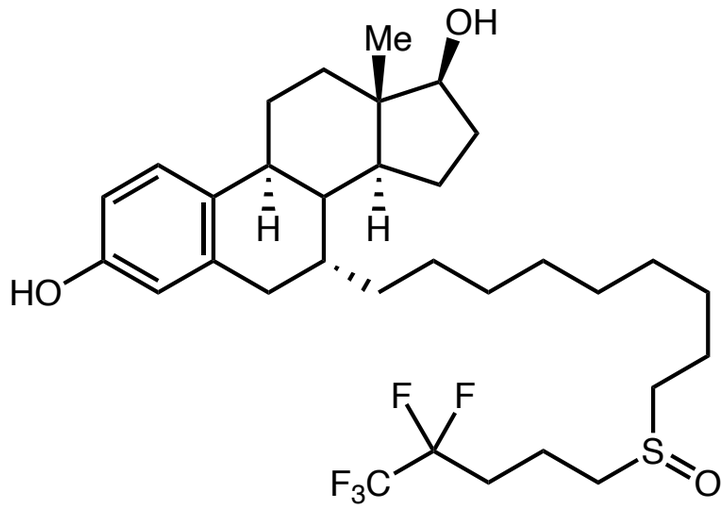

Fulvestrant

- ***<u>Vepdegestrant failed to meet key clinical study endpoints</u>***

Strategies to Access Undruggable Targets

What other strategies can be used to target the undruggable proteome?

Current Strategies

peptidomimetics

Pharmacophores *designed to* ***mimic a natural peptide***

Vagner, J., Qu, H., Hruby, V. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292–296.

Current Strategies

peptidomimetics

Pharmacophores *designed to* ***mimic a natural peptide***

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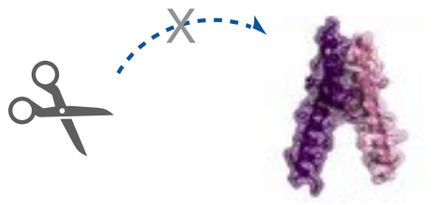

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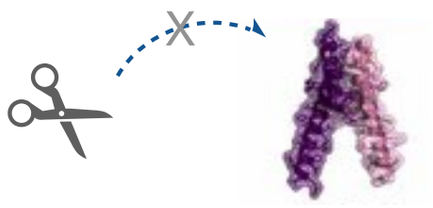
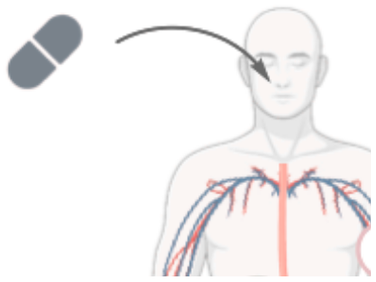


2. Increased oral bioavailability



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Investigating Druggability

3 key factors to "Undruggabilty":

- *Disordered Structures*
- *Dynamic surfaces*
- *Undefined binding pockets*

- ***Protein–protein interactions (PPIs)***
- ***Protein–nucleic acid interactions***

~130,000–650,000 *types of PPIs in the human interactome*

Disruption of PPIs can promote disease states

Lu, H., Zhou, Q., He, J. *et al. Sig Transduct Target Ther.* **2020***, 5*, 213–220.
Greenblatt, J. F., Alberts, B. M., Krogan, N. J. *Cell.* **2024***,187*, 6501–6517.

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peptidomimetics

- ***exhibit specific interactions with PPI and protein–nucleic acids***

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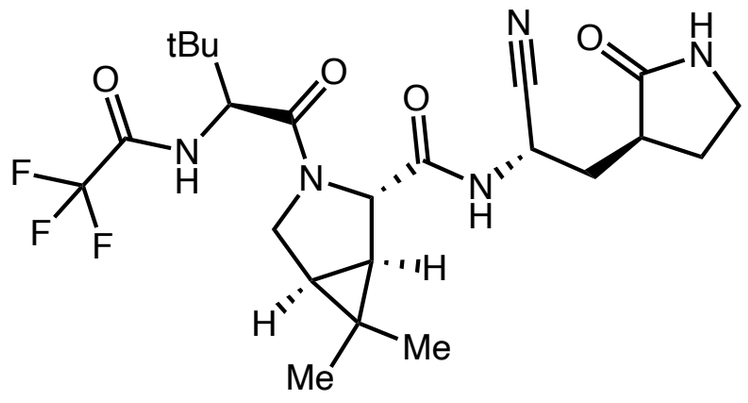
Greenblatt, J. F., Alberts, B. M., Krogan, N. J. *Cell.* **2024***,187*, 6501–6517.

Peptidomimetic inhibitors

- *Peptidomimetic inhibitors are ubiquitous*

Shindo, N., Ojida, A. *Bioorg. Med. Chem.* **2021**. *47*, 1–12

Peptidomimetic inhibitors



Nirmatrelvir

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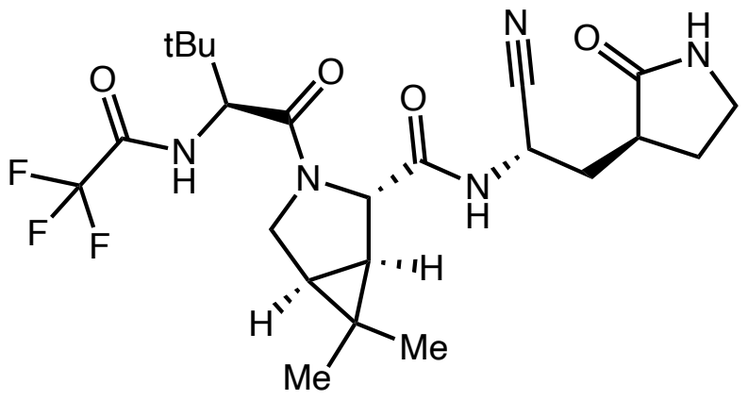
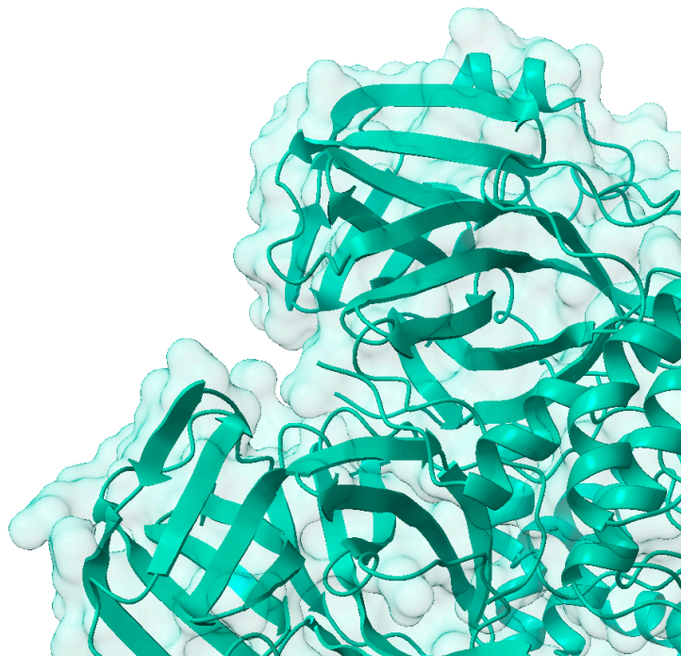


Nirmatrelvir

- *Peptidomimetic inhibitors are ubiquitous*
  - *MPro SARS-CoV-2 main protease*



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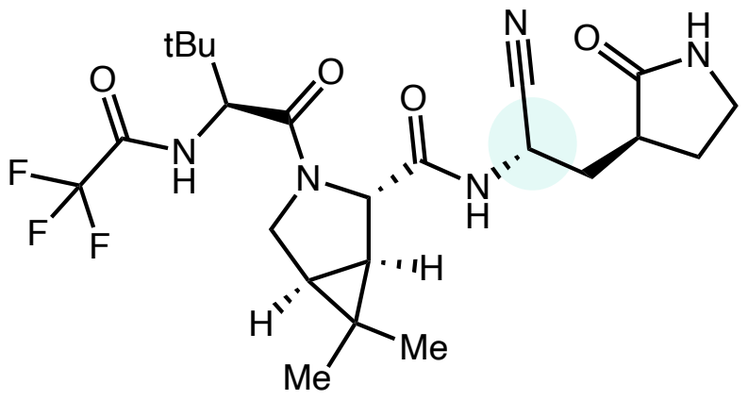

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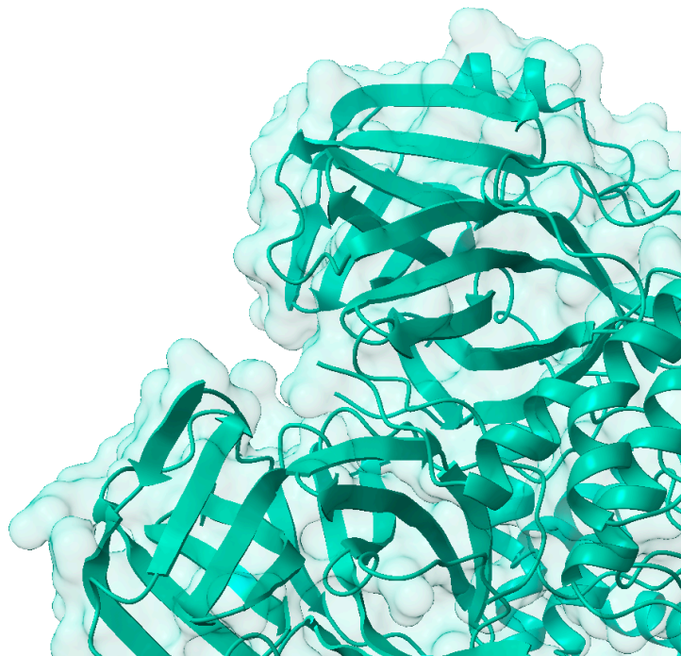

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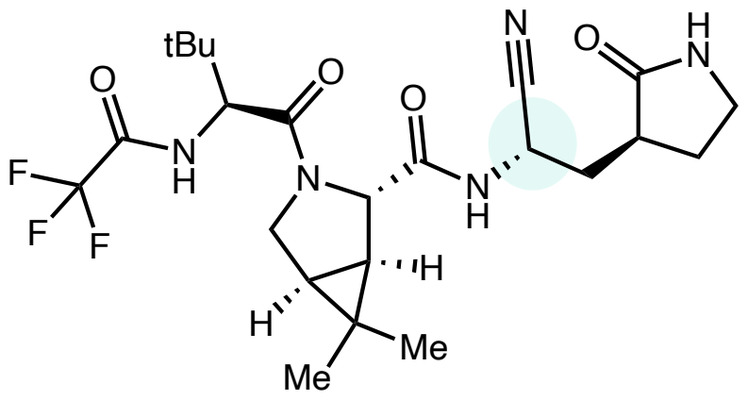

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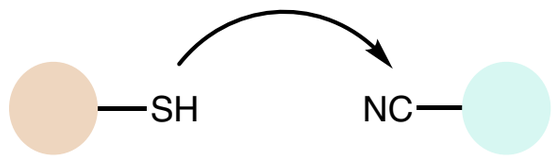
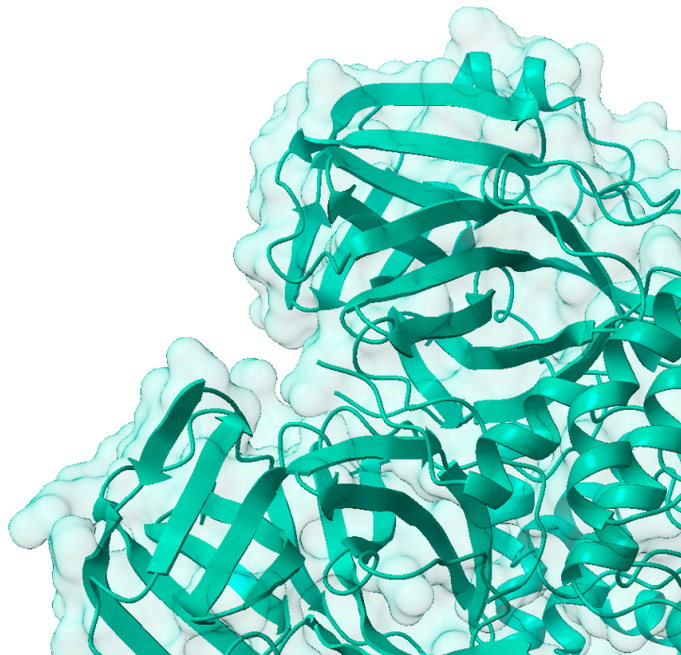
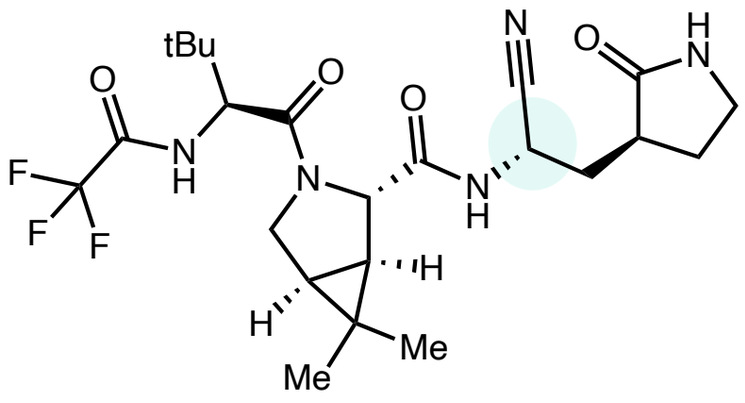


Cys145 M^{pro}



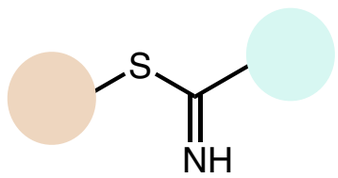
Shindo, N., Ojida, A. *Bioorg. Med. Chem.* **2021**. *47*, 1–12

Peptidomimetic inhibitors

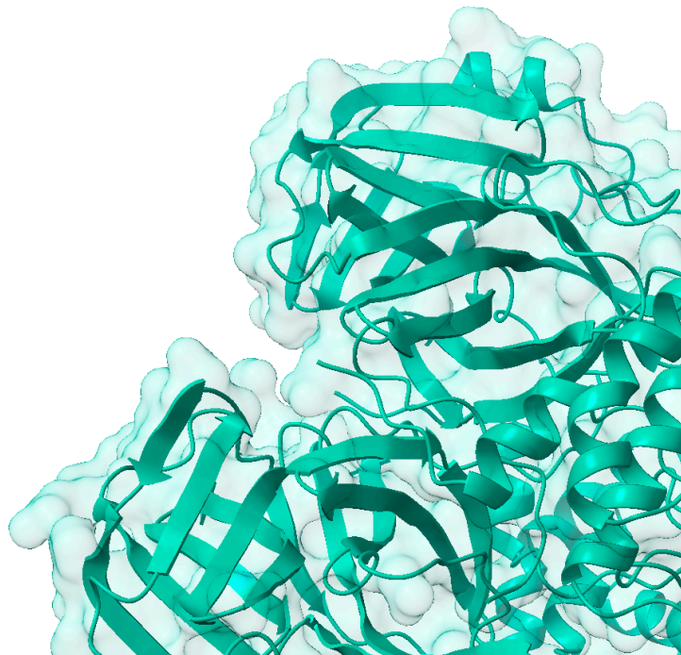


Nirmatrelvir

- *Peptidomimetic inhibitors are ubiquitous*
  - *MPro SARS-CoV-2 main protease*



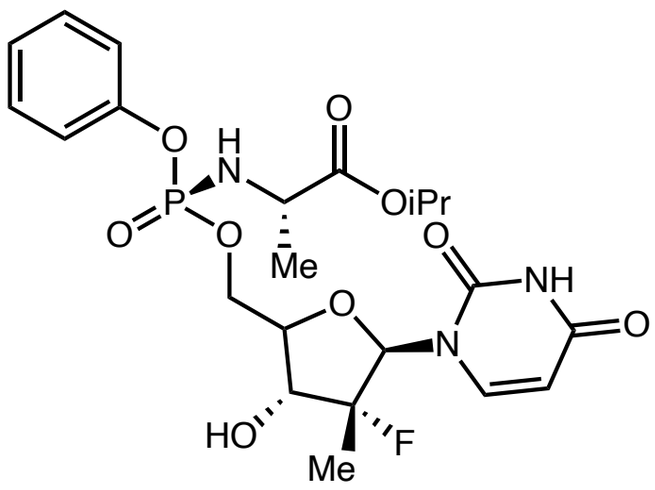
Thioimidate



Shindo, N., Ojida, A. *Bioorg. Med. Chem.* **2021**. *47*, 1–12

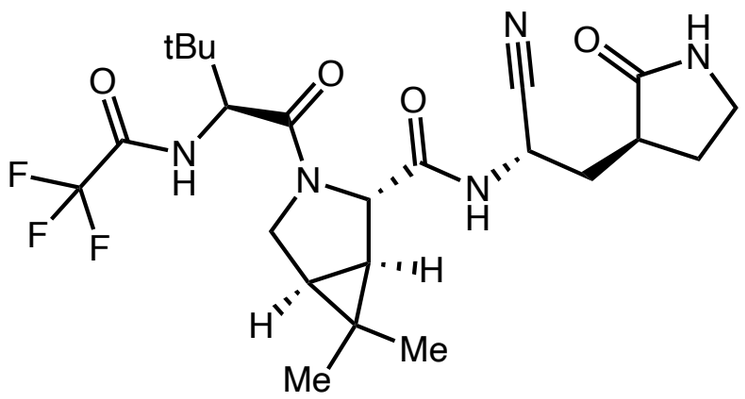
Peptidomimetic inhibitors

To name just a few…



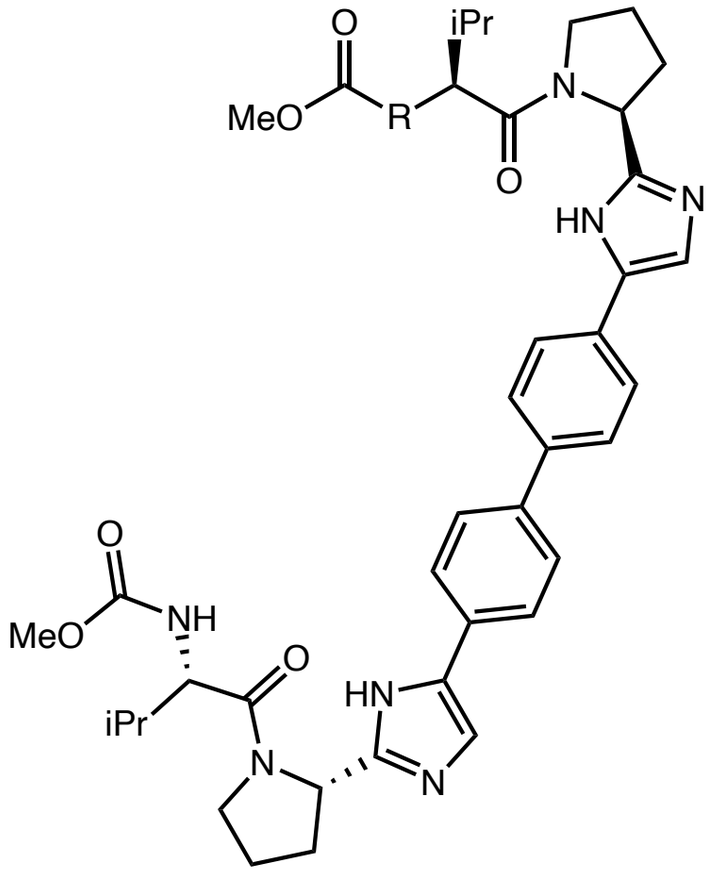
Sofosbuvir

Hep C



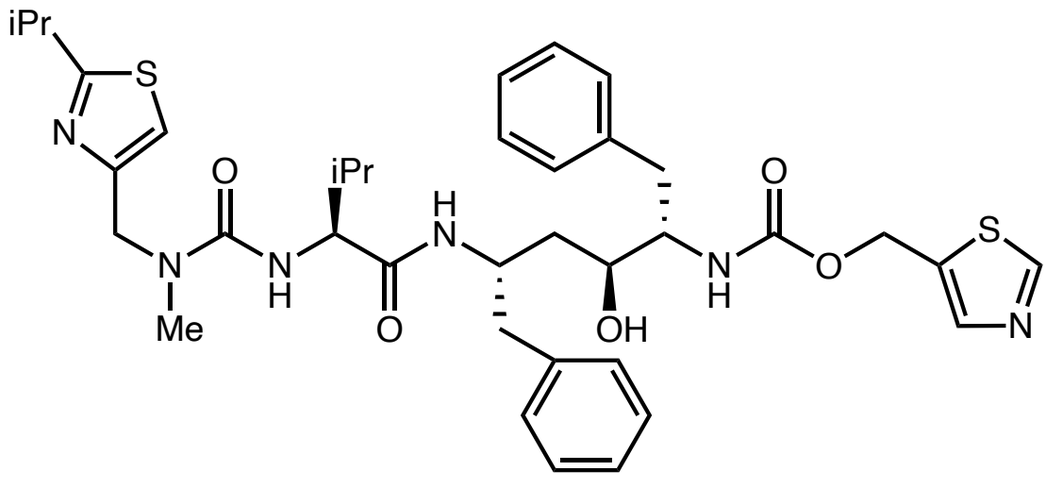
Nirmatrelvir

SARS CoV-2



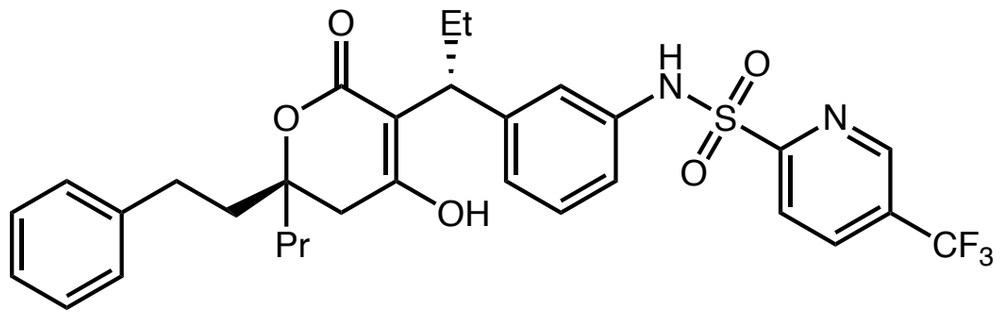
Daclatasvir

Hep C



Ritonavir

HIV



Tipranavir

HIV

Shindo, N., Ojida, A. *Bioorg. Med. Chem.* **2021**. *47*, 1–12

Strategies to Access Undruggable Targets

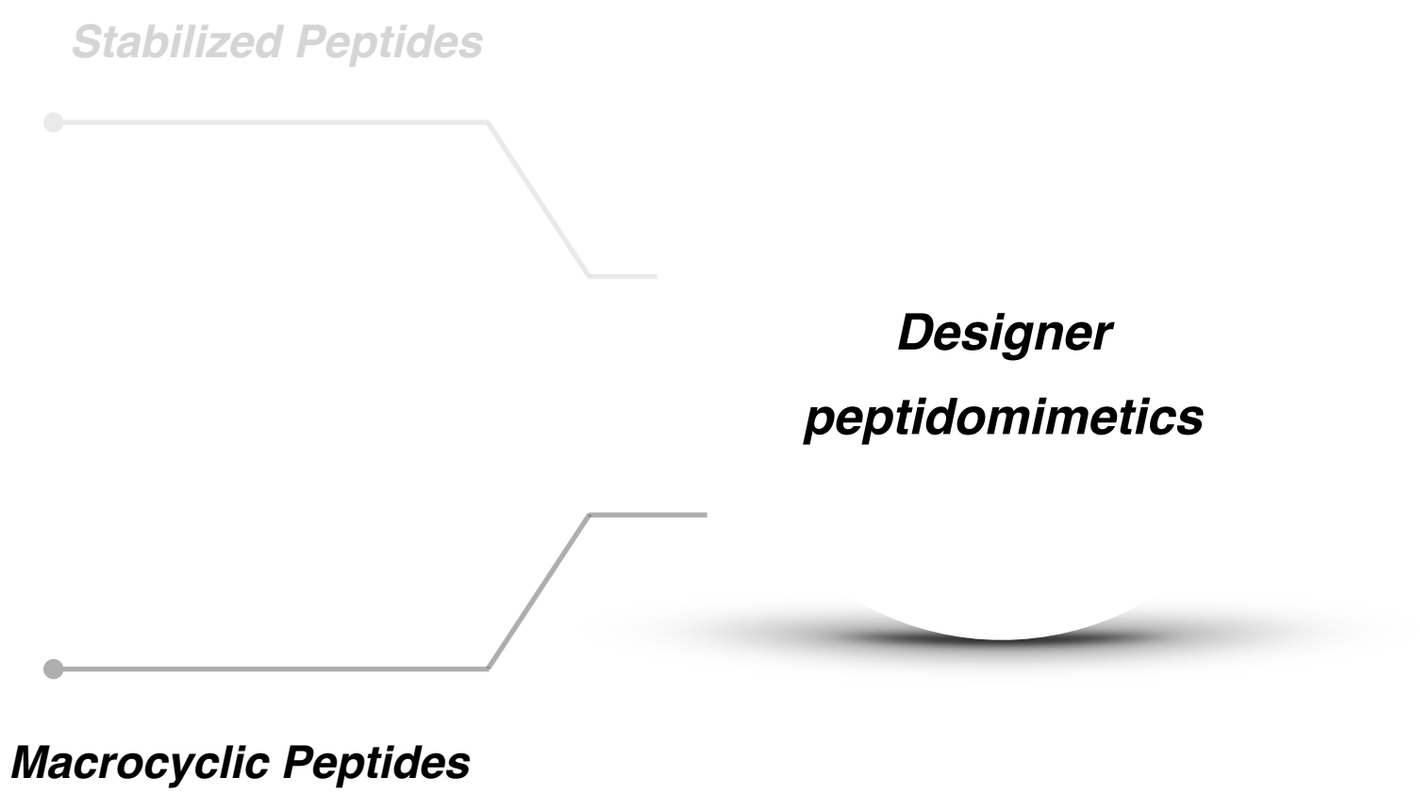
Stabilized Peptides

Designer peptidomimetics

Macrocyclic Peptides

Swenson, C. S., Mandava, G., Thomas, D. M., Moellering, R. E. *Chem. Rev.* **2024**. *124*, 13020–13093

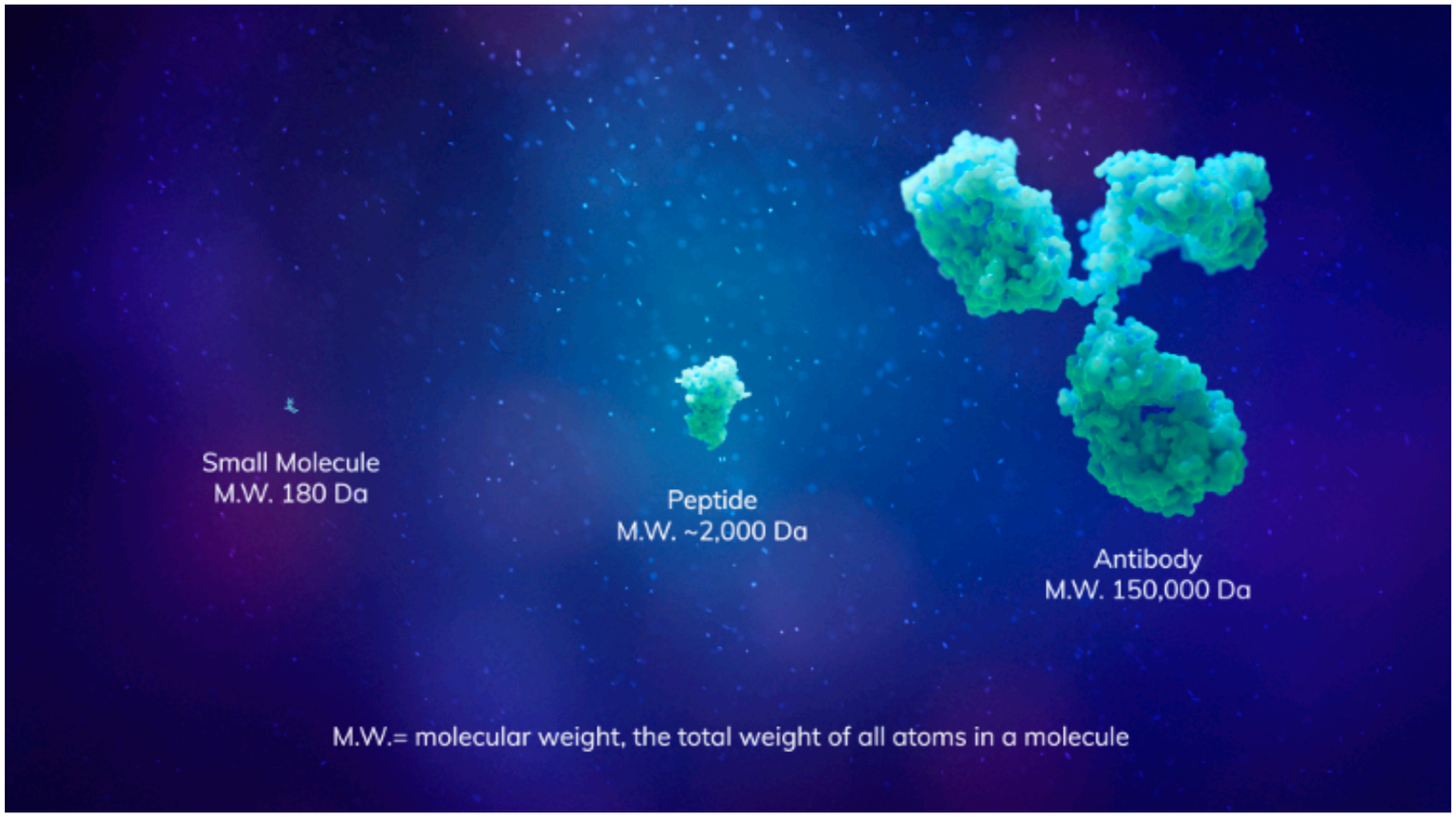
Strategies to Access Undruggable Targets



Swenson, C. S., Mandava, G., Thomas, D. M., Moellering, R. E. *Chem. Rev.* **2024**. *124*, 13020–13093

Macrocyclic Peptides

***MCPs** = The "goldilocks" chemical modality*



Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Macrocyclic Peptides

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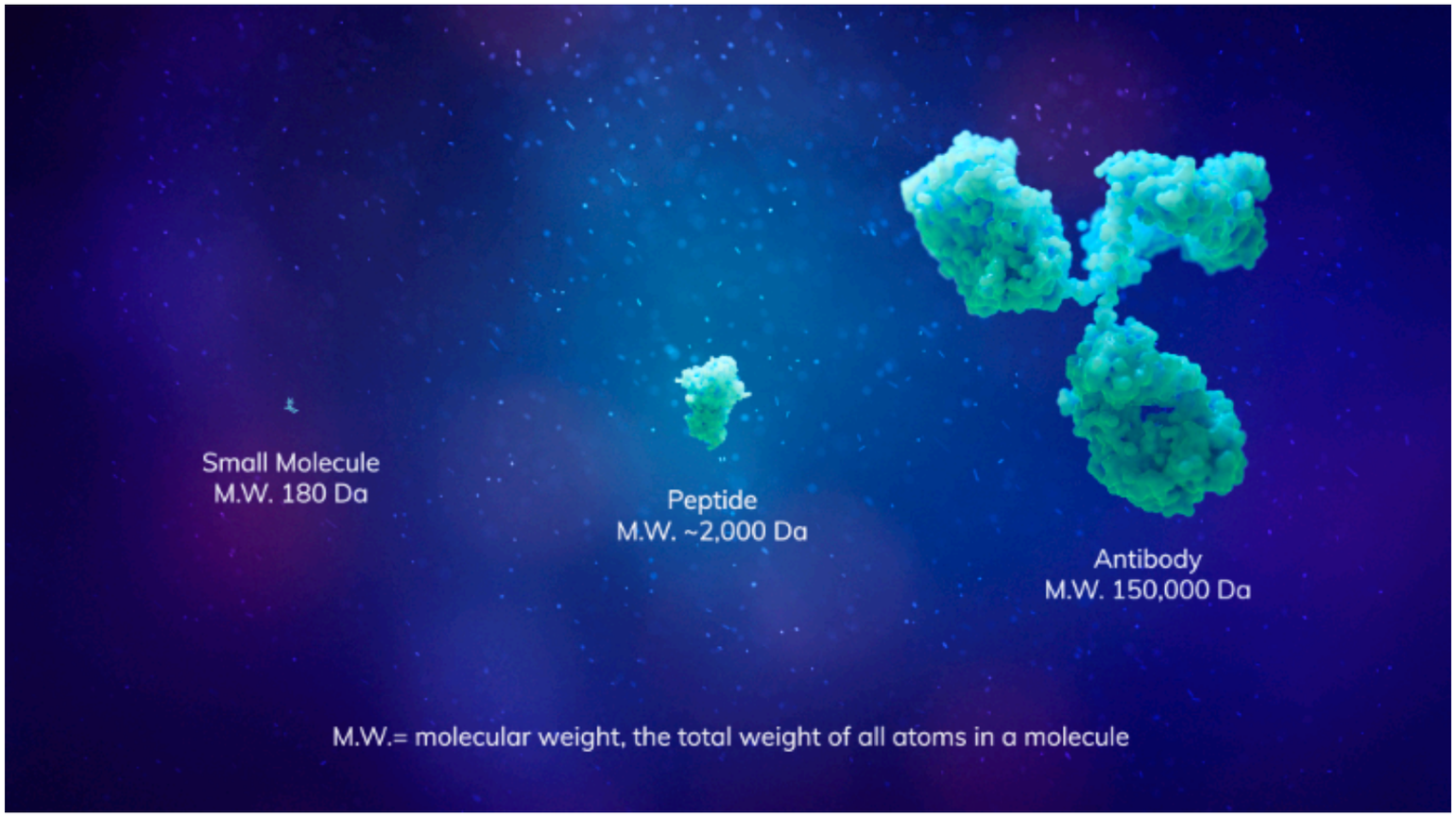

greater surface area: useful to disrupting protein–protein interactions

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Macrocyclic Peptides

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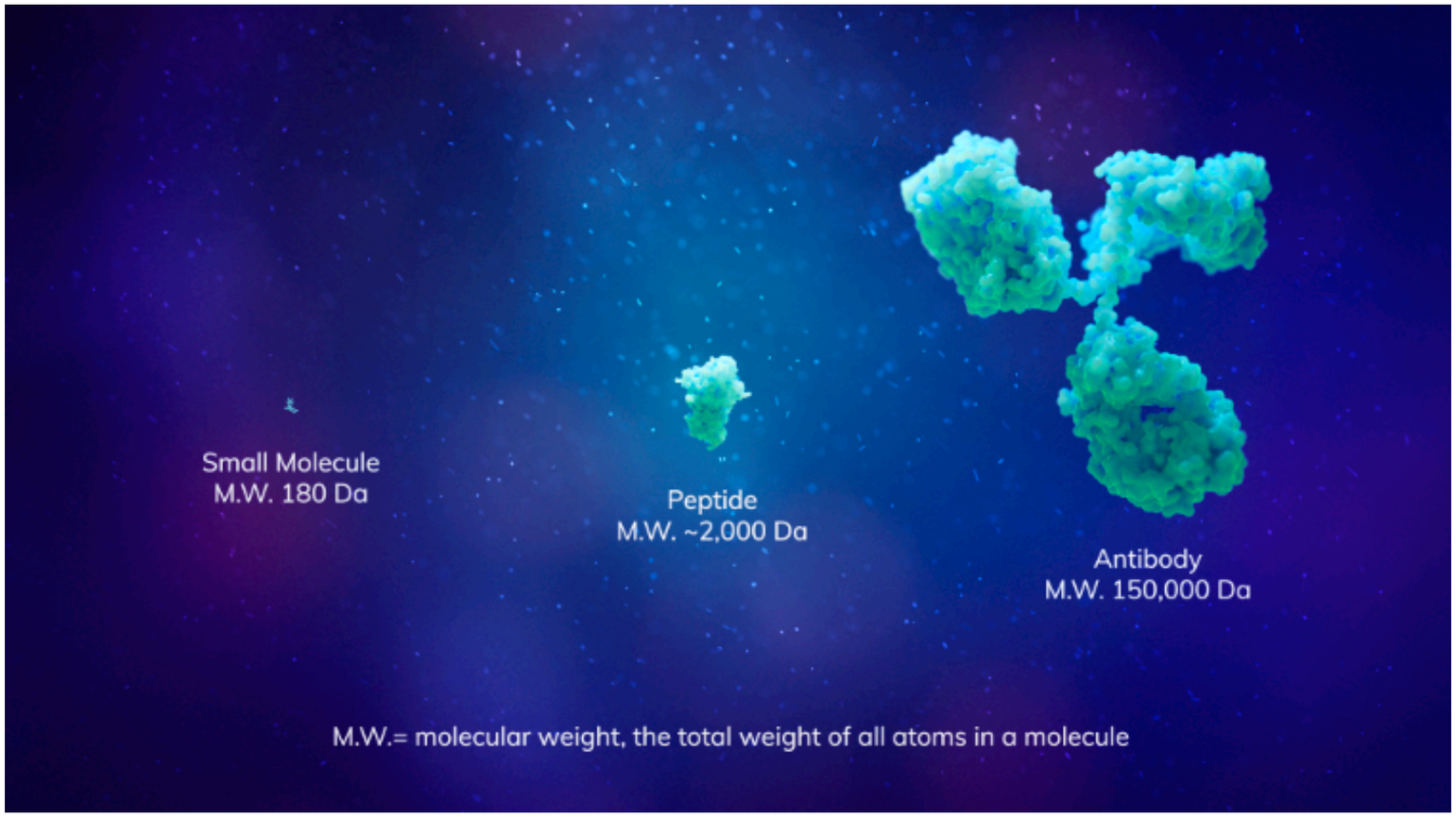

MCPs for Undruggable targets:

- ***Large <u>compound library screening</u> a powerful approach for the de novo discovery of MCPs***

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Macrocyclic Peptides (MCPs)

- *MCPs in drug discovery:*

  - ***MCPs exhibit constrained conformations and chemical stability***

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Macrocyclic Peptides (MCPs)

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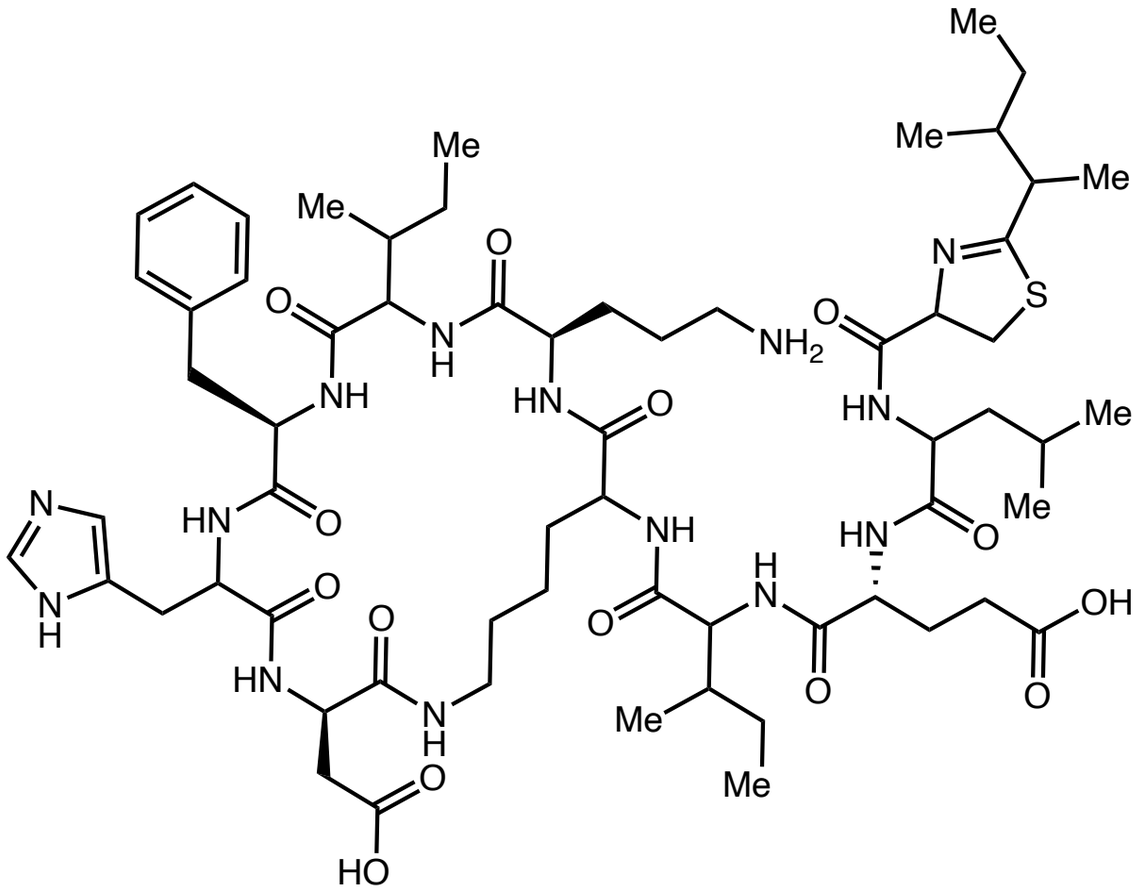

Bacitracin A

- ***Many clinical antibiotics are MCPs: polymyxins, bacitracin, daptomycin etc.***

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Macrocyclic Peptides (MCPs)

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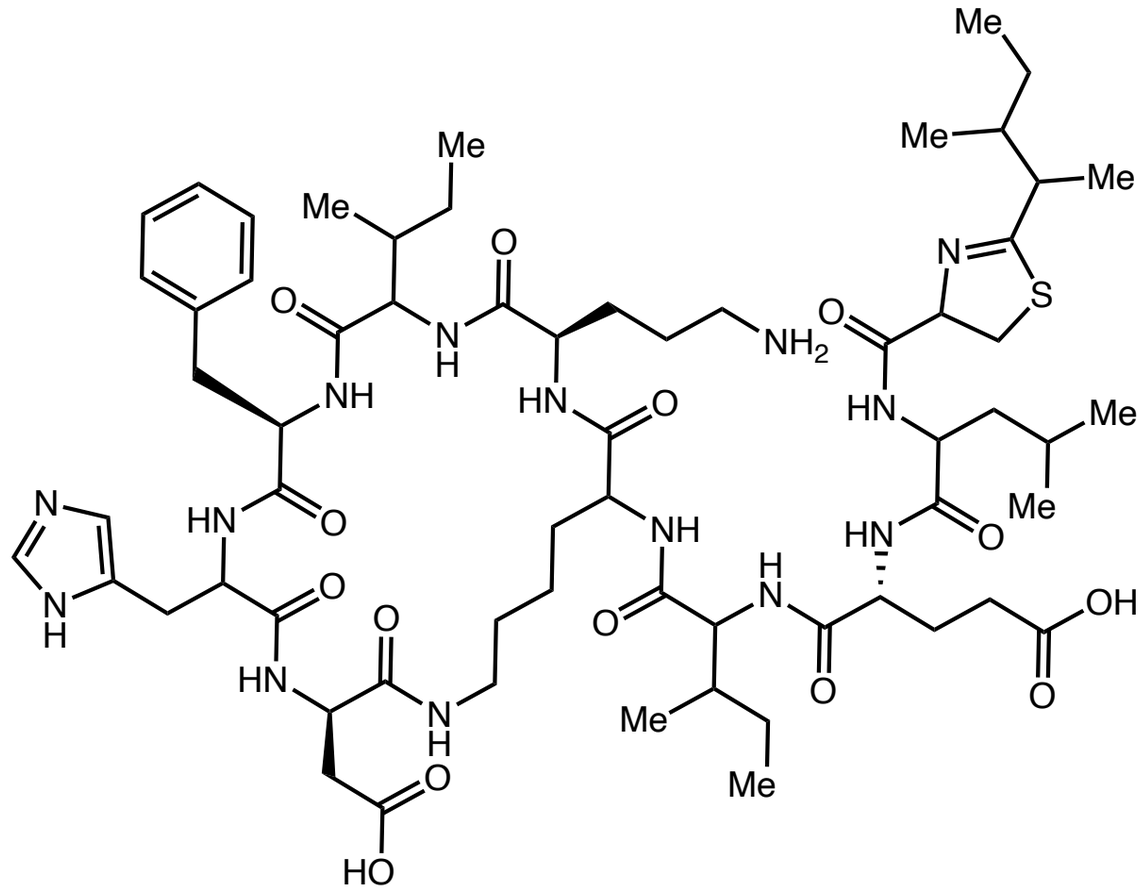


Bacitracin A

- ***MCPs are important sources for antibiotic discovery due to their constrained conformations and chemical stability***

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance

- *MCPs in drug discovery:*

drug–resistant bacterial infections:

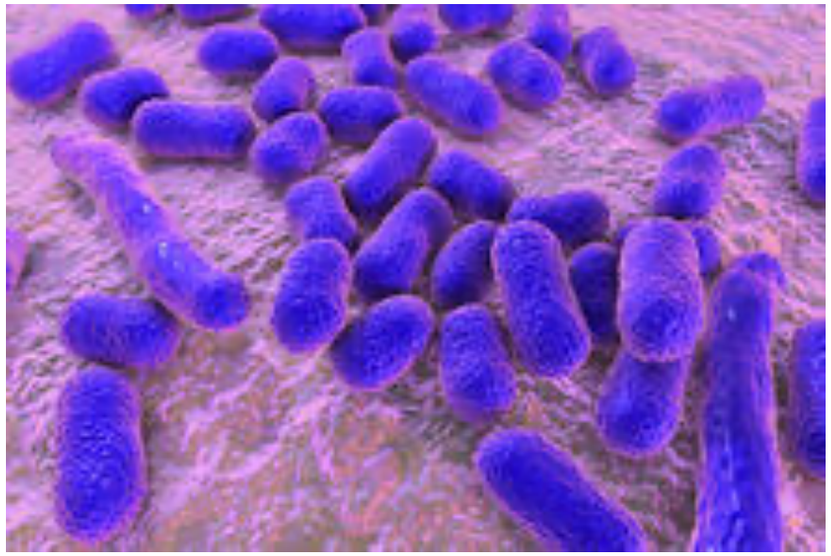
Undruggable targets that were previously treatable

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance

- *MCPs in drug discovery:*



Carbapenem-resistant Acinetobacter baumannii (CRAB)

- ***Gained multi-drug resistance 50 years ago***
- ***Resistant to β-lactam and carbapenem antibiotics***

Roche

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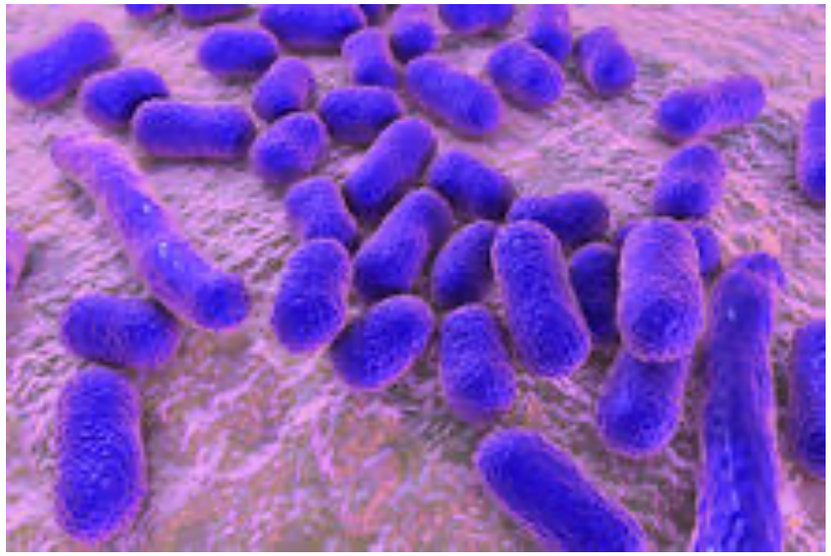

Priority 1 Critical WHO Pathogen

World Health Organization

Carbapenem-resistant Acinetobacter baumannii (CRAB)

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- ***Resistant to β-lactam and carbapenem antibiotics***

Centers for Disease Control and Prevention. 2019 AR Threats Report. *CDC* (2019)

Macrocyclic Peptides (MCPs)

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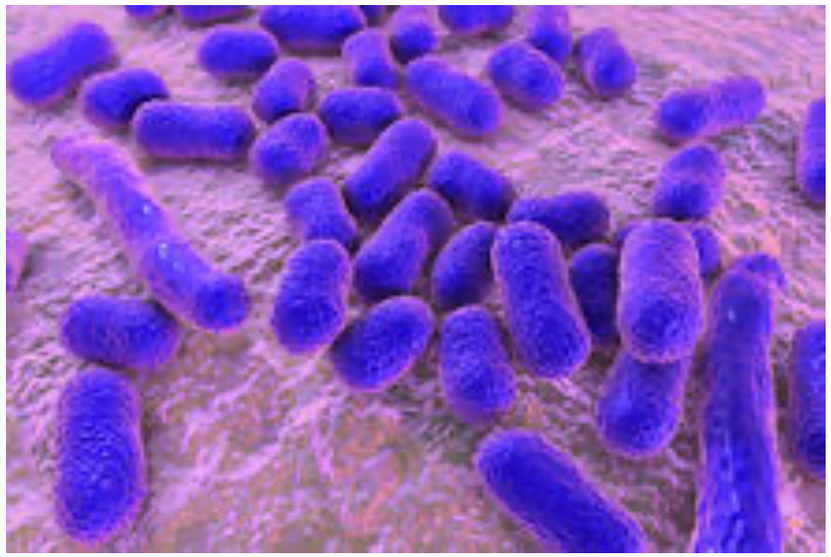

Carbapenem-resistant Acinetobacter baumannii (CRAB)

⟝ *Macrocyclic peptide drug*

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance

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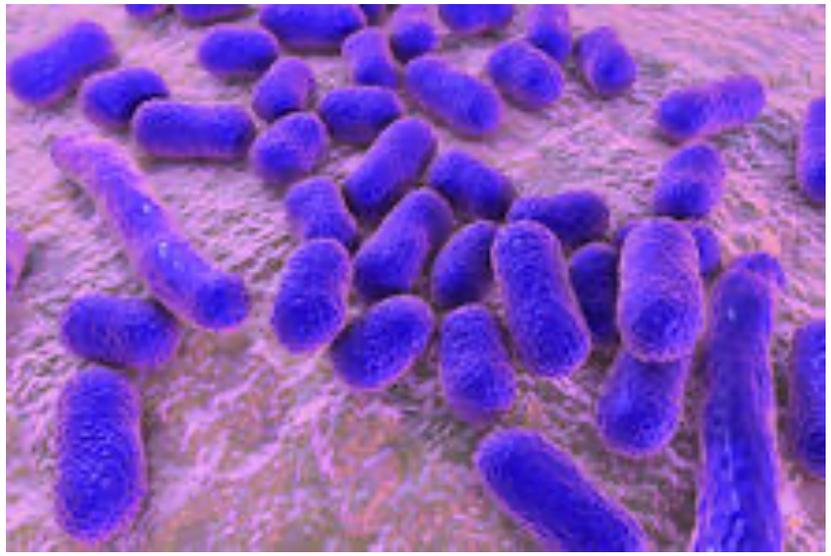

Carbapenem-resistant Acinetobacter baumannii (CRAB)

Macrocyclic peptide drug

- ***Large compound library screening a powerful approach for the de novo discovery of interacting MCPs***

Roche

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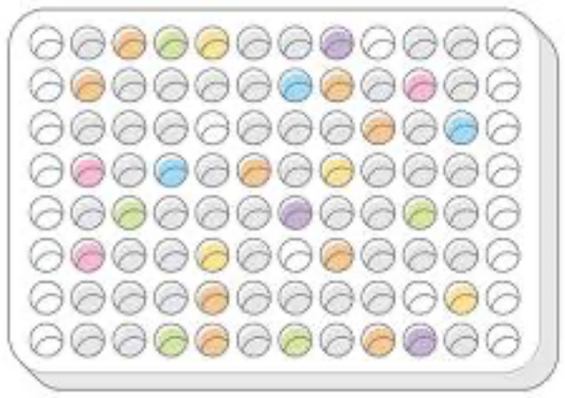
Macrocyclic Peptides (MCPs)

Targeting drug–resistance



Screened > 45,000 MCPs

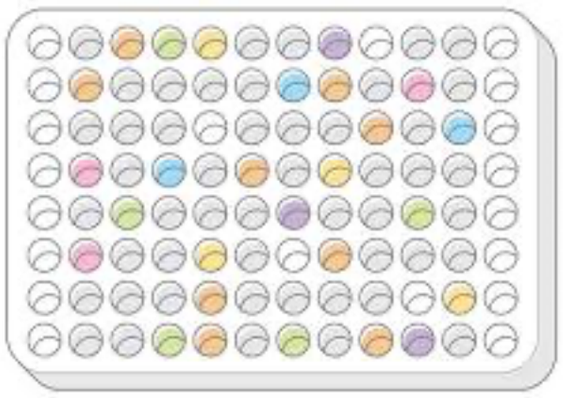
1 MCP selectively kills A. baumannii

Roche

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance



Screened > 45,000 MCPs

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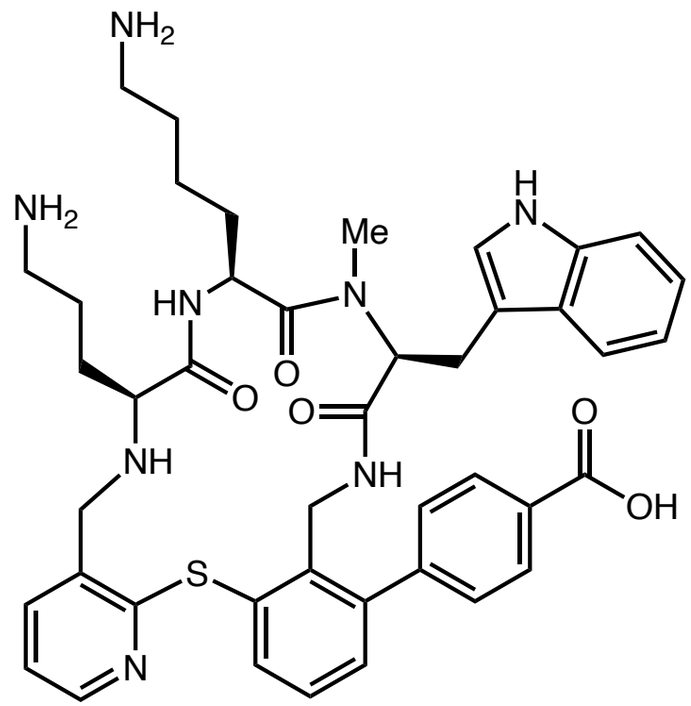
- ***Further optimization performed for efficacy and blood–plasma compatibility***

Roche

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance



Zosurabalpin®

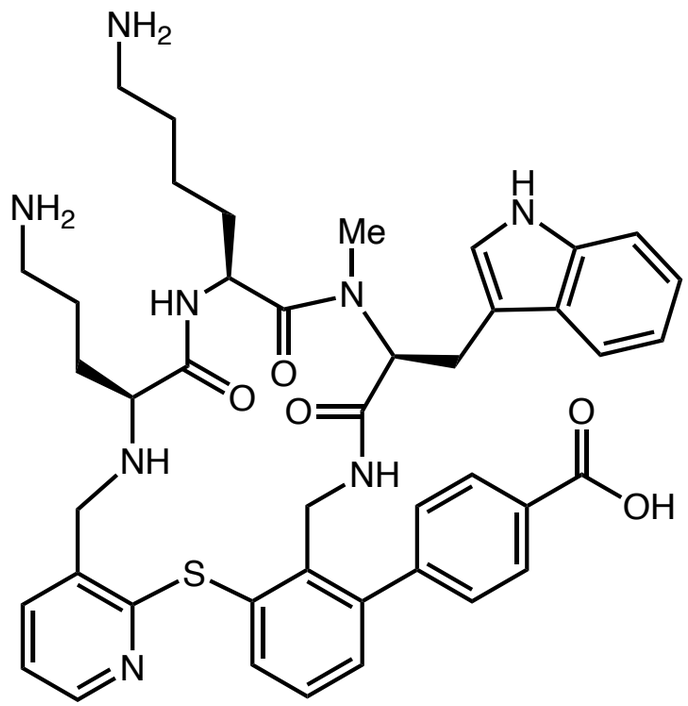
1 MCP selectively kills A. baumannii

Roche

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance



Zosurabalpin®

1 MCP selectively kills A. baumannii

Operative via novel mode of action

Roche

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance

- *MCPs in drug discovery:*

- ***Zosurabalpin inhibits transport of the molecule lipopolysaccharide (LPS)***

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

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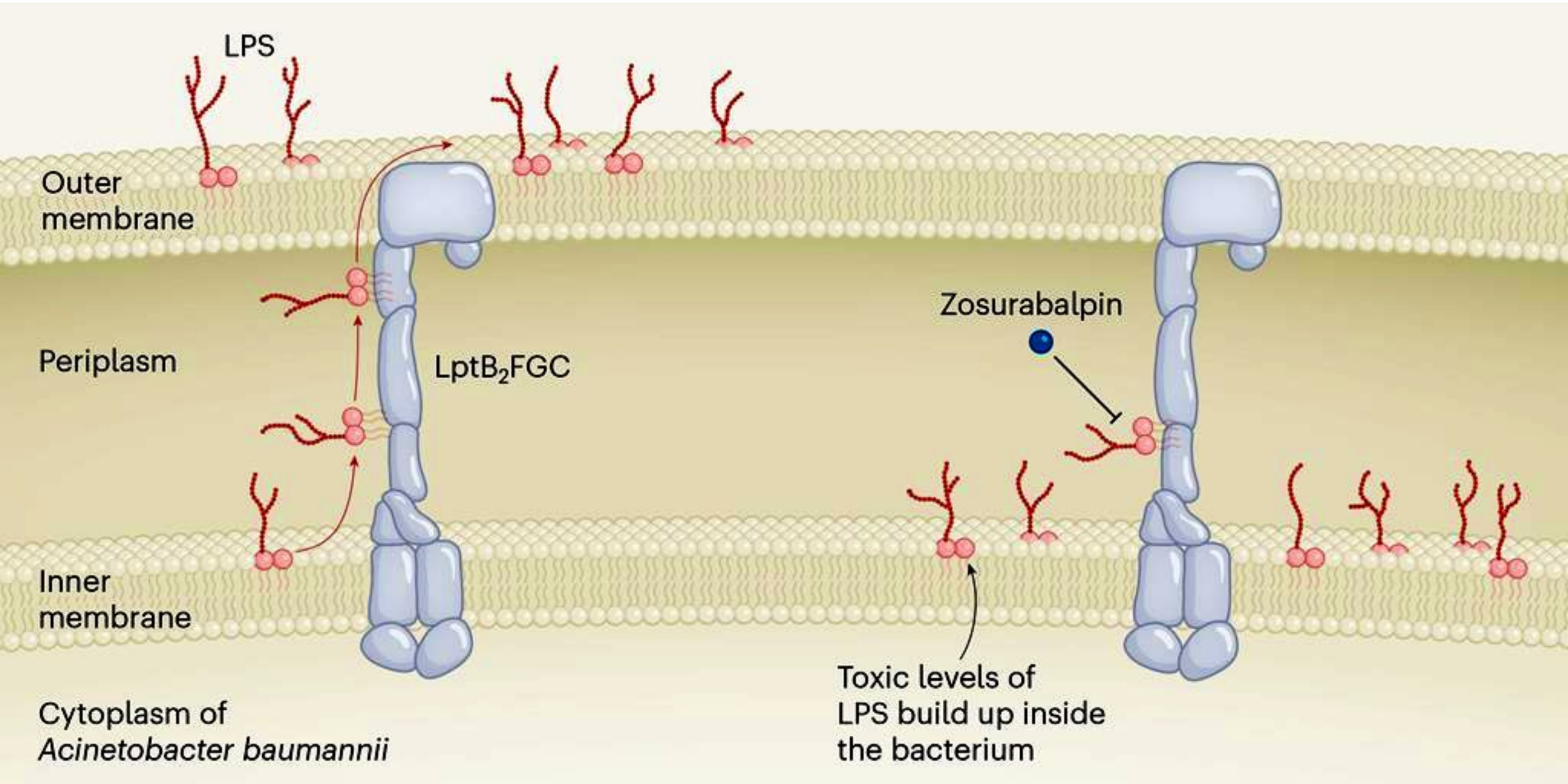

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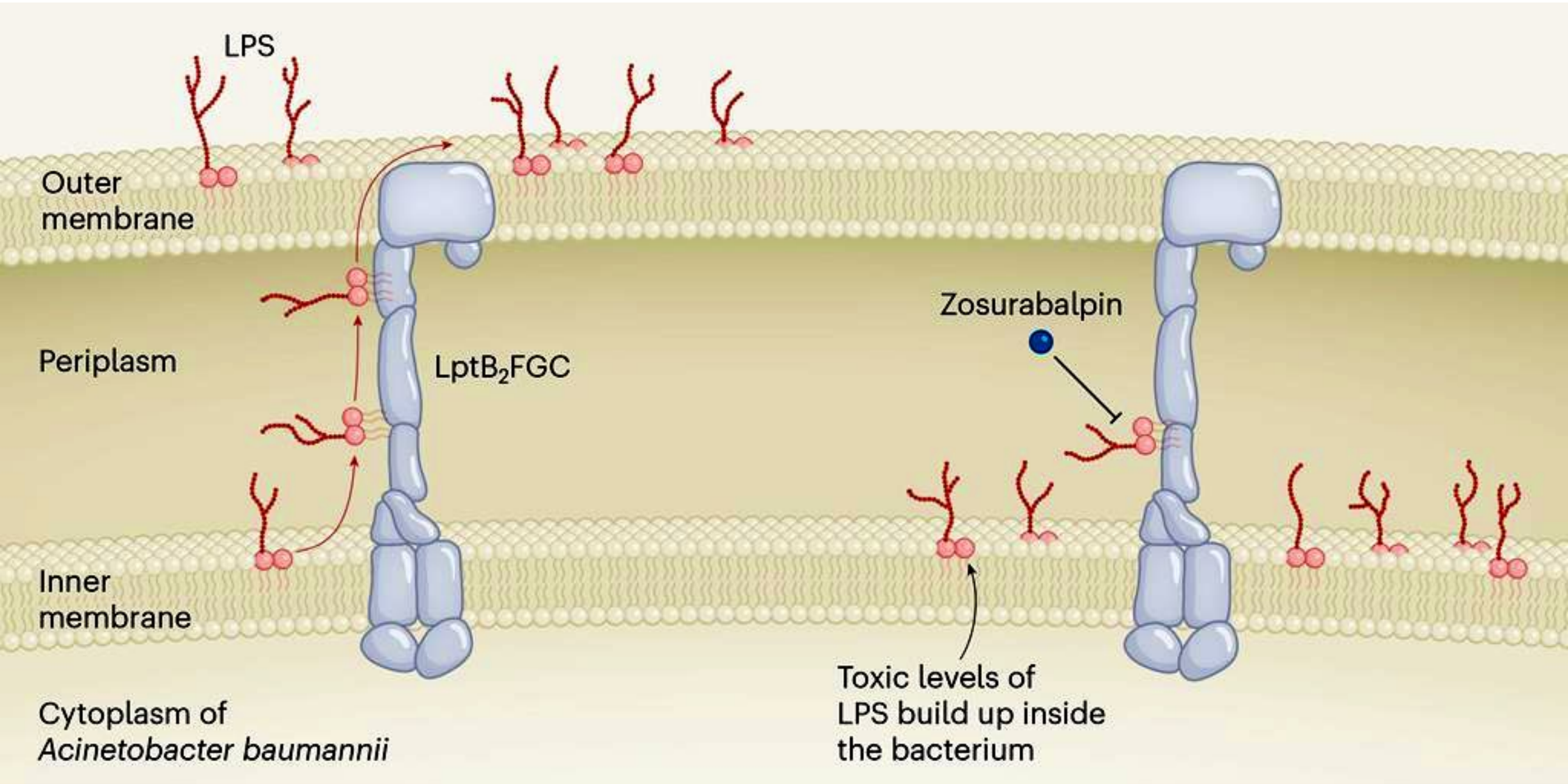
Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance

- *MCPs in drug discovery: inhibits LptB2FGC complex*



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Roche

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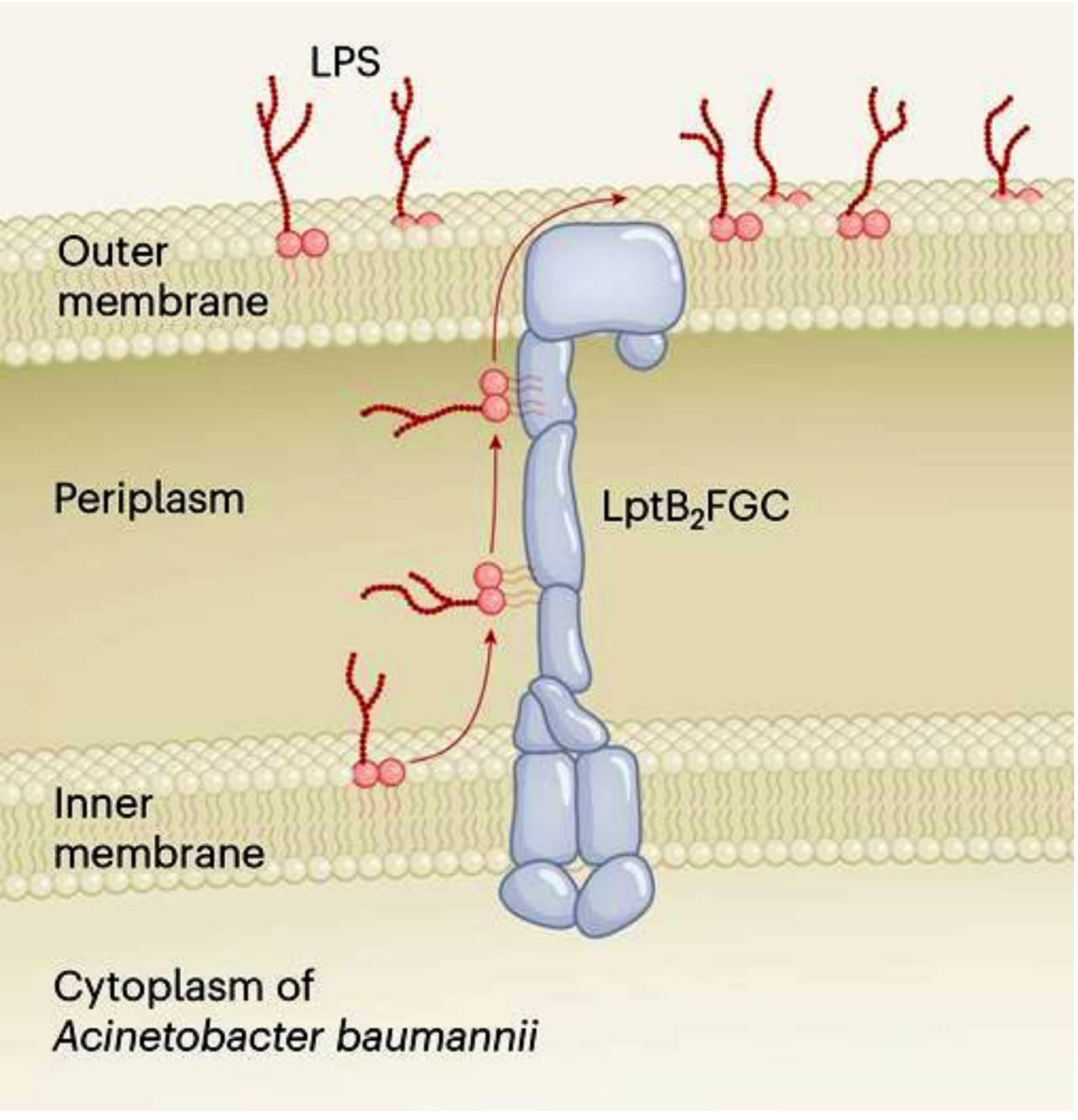
Macrocyclic Peptides (MCPs)

Targeting drug–resistance

- *MCPs in drug discovery: reaching novel targets*



- ***Antibiotics have focused on reaching cytoplasmic targets***

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

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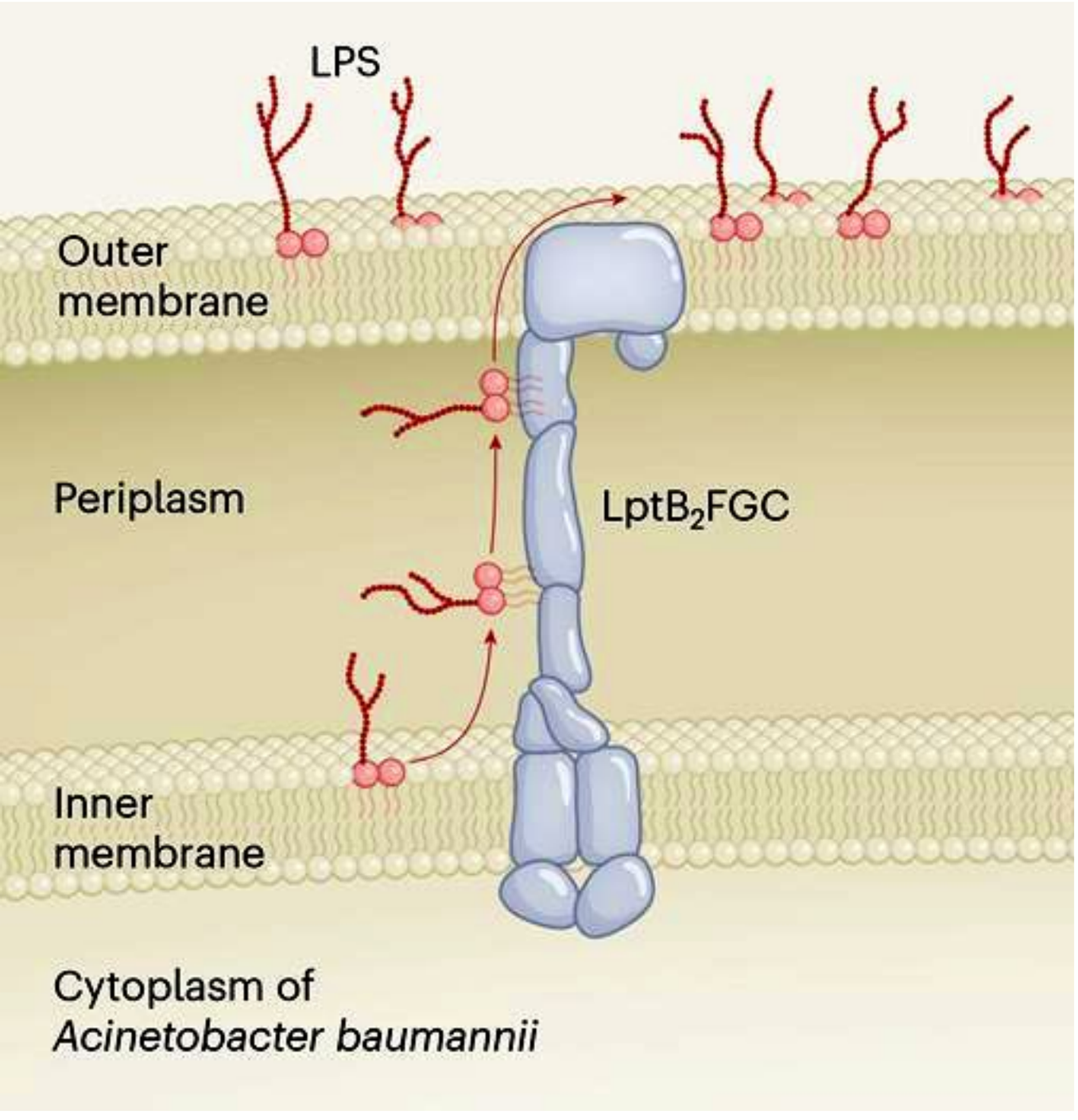


- ***Antibiotics have focused on reaching cytoplasmic targets***

- ***This limits prospective targets to cell surface***

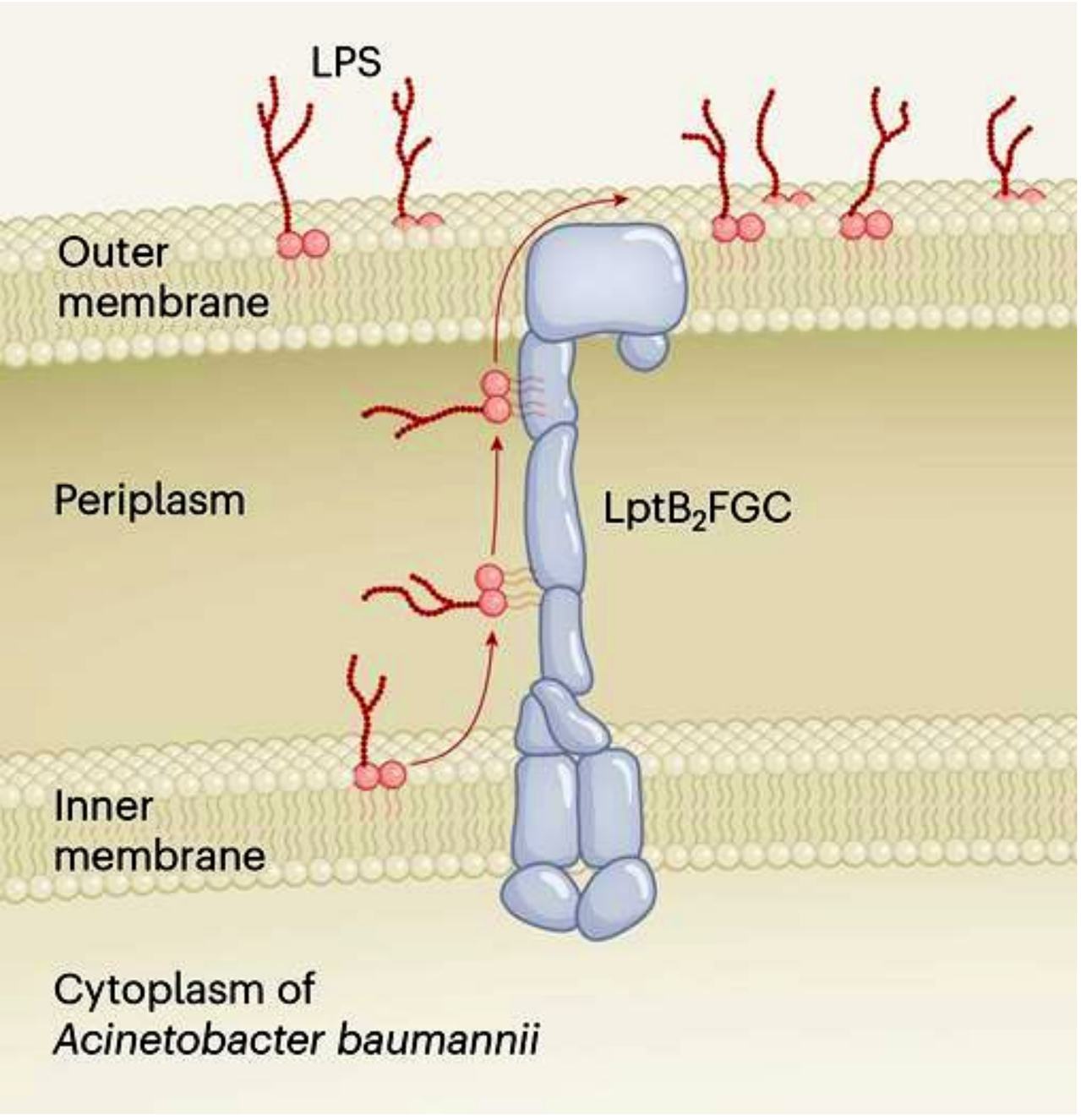
Roche

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Macrocyclic Peptides (MCPs)

Targeting drug–resistance

- *MCPs in drug discovery: reaching novel targets*

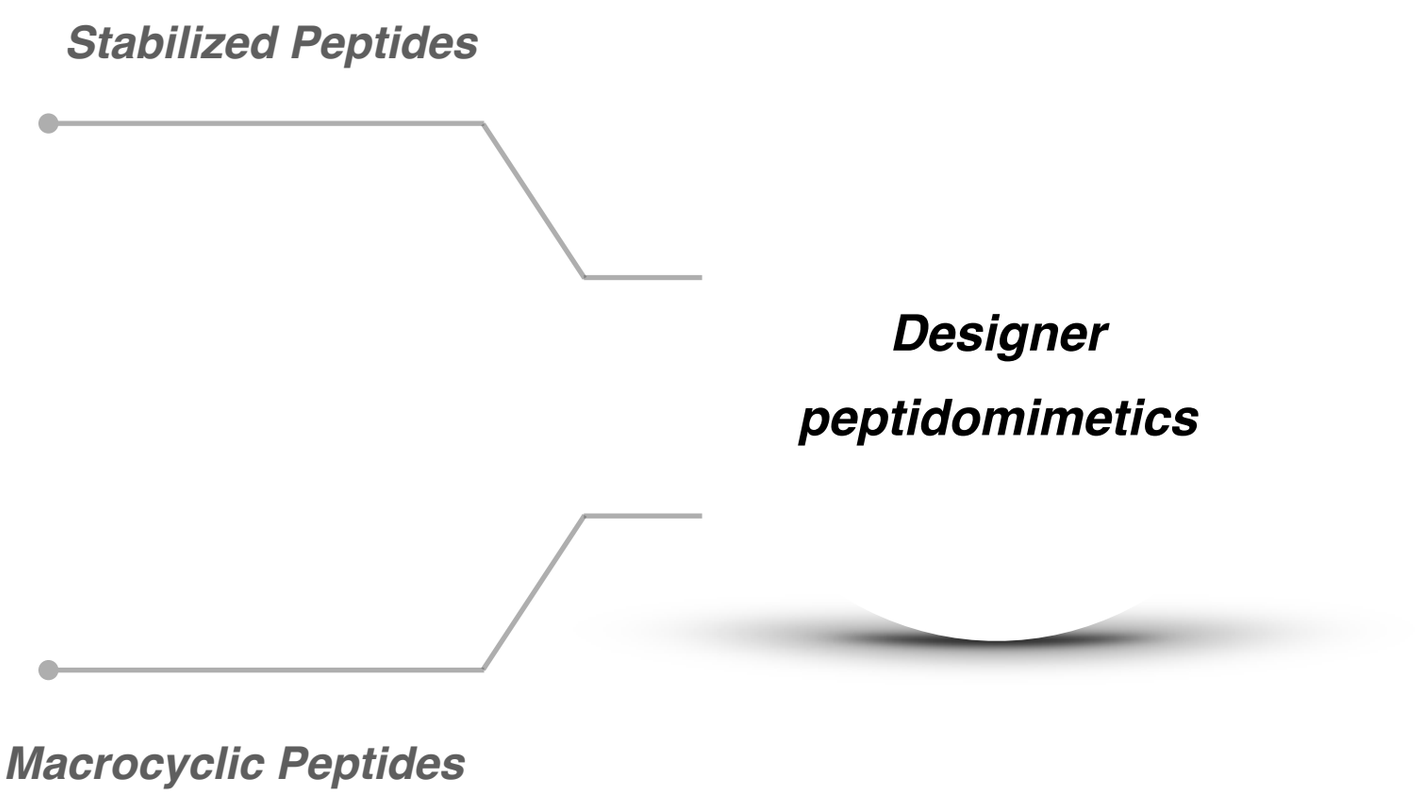


- ***Antibiotics have focused on reaching cytoplasmic targets***

- ***LptB2FGC resides in the periplasm, enabling Zosurabalpin delivery***

Zampaloni, C. Et al. *Nature.* **2024**, *625*, 566–571.

Strategies to Access Undruggable Targets



Swenson, C. S., Mandava, G., Thomas, D. M., Moellering, R. E. *Chem. Rev.* **2024**. *124*, 13020–13093

Strategies to Access Undruggable Targets

Stabilized Peptides

Designer peptidomimetics

Macrocyclic Peptides

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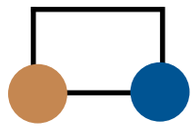
Strategies to Access Undruggable Targets

Stabilized Peptides

- *synthetic modifications to* ***stabilize local and global structural motifs***



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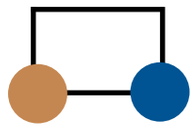


Confers enhanced affinity and specificity

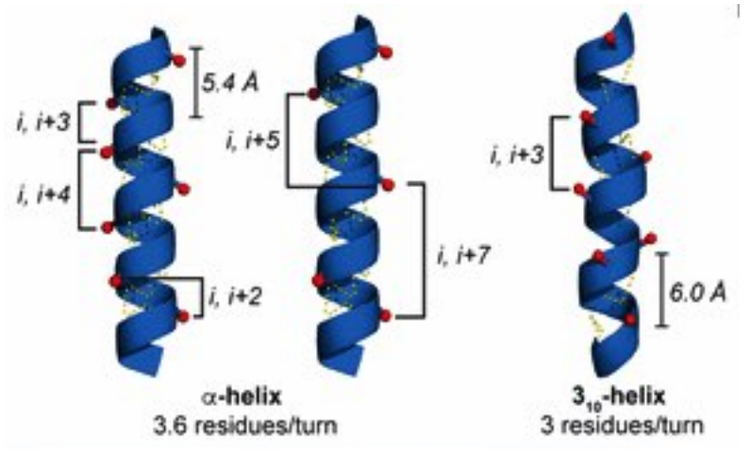
Swenson, C. S., Mandava, G., Thomas, D. M., Moellering, R. E. *Chem. Rev.* **2024**. *124*, 13020–13093

Stabilized Peptides

- *Imposing structure*

Naturally occurring motifs:

Helix motifs



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Stabilized Peptides

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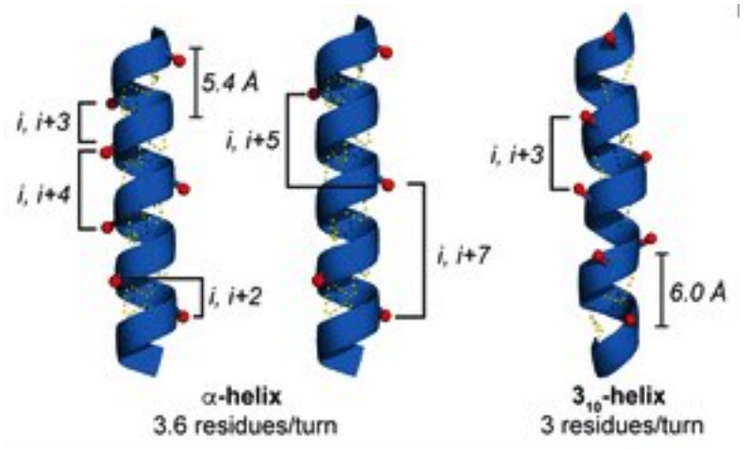

- ***The majority of interactions reported in PDB involve α-helices***

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Stabilized Peptides

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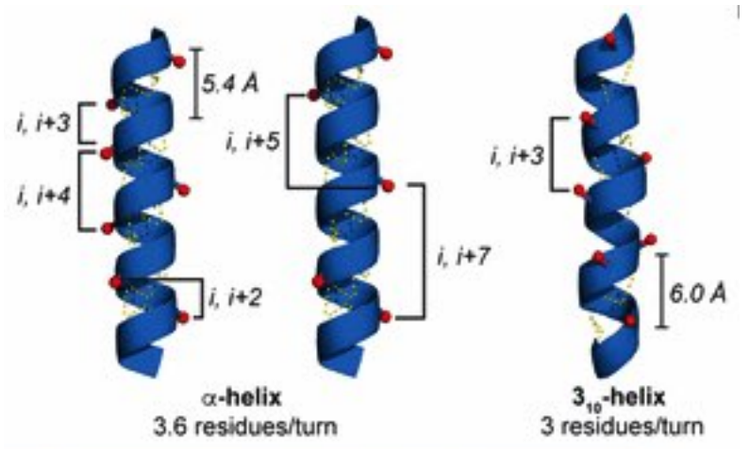
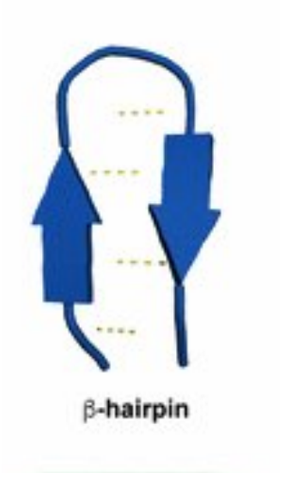


Hairpin motifs

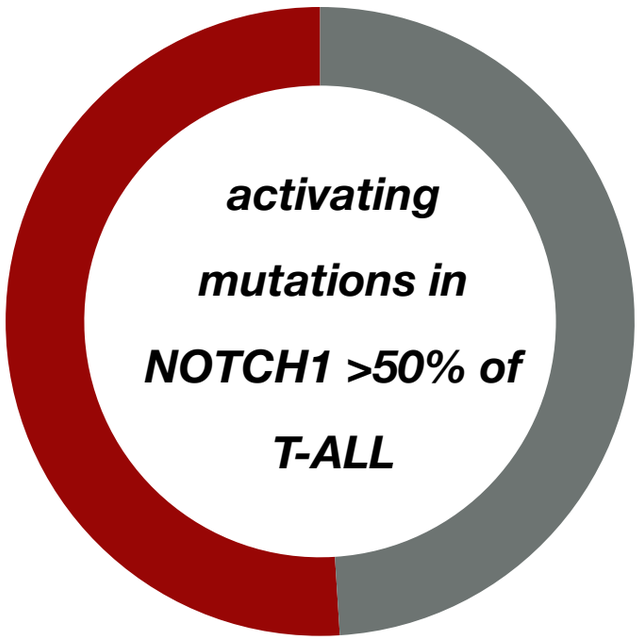


- ***Both exhibit internal hydrogen bonding***
- ***stabilizing mutations locks the peptide into desired conformation***

Muttenthaler, M., King, G. F., Adams, D. J., Alewood, P. F., *Nat. Rev.* **2021**, 309–325.

Stabilized Peptides

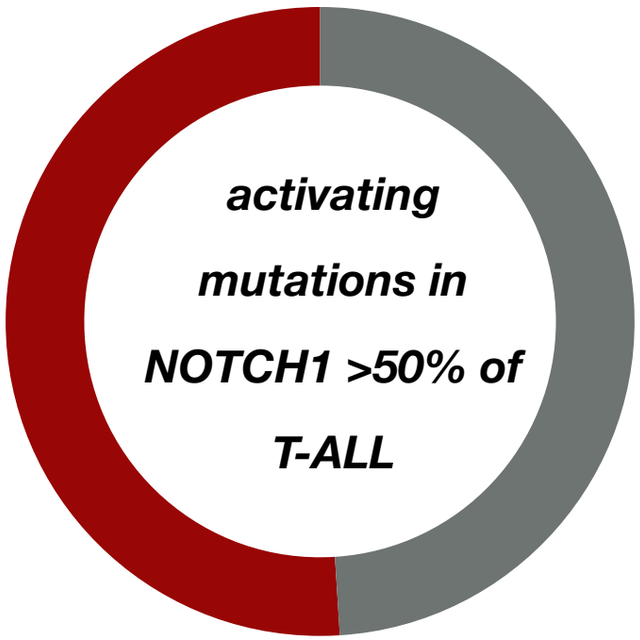
Inhibition of NOTCH



Zheng, R., Li, M., Wang, S. *et al. Exp Hematol Oncol*, **2020**, 9, 31–40

Stabilized Peptides

Inhibition of NOTCH



Inappropriate NOTCH activation is directly implicated in T-cell Acute Lymphoblastic Leukaemia (T-ALL)

Zheng, R., Li, M., Wang, S. *et al. Exp Hematol Oncol*, **2020**, 9, 31–40

Stabilized Peptides

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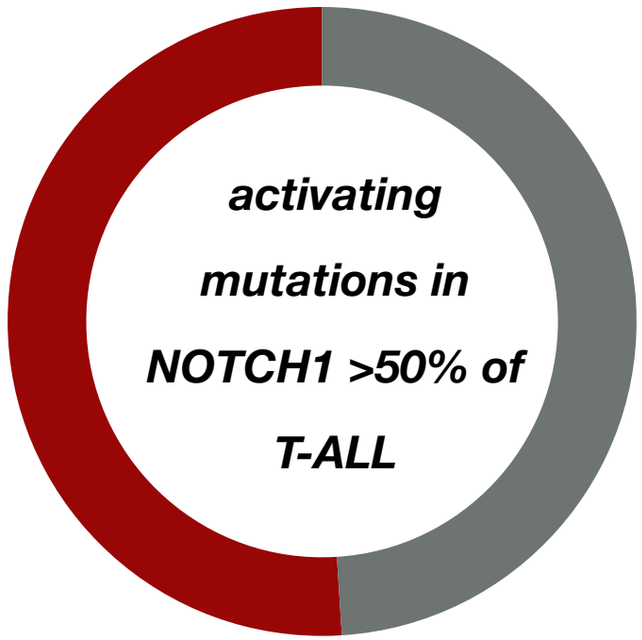

NOTCH1 = transcription factor

- ***transcription factors are challenging to target***

Zheng, R., Li, M., Wang, S. *et al. Exp Hematol Oncol*, **2020**, 9, 31–40

Stabilized Peptides

Inhibition of NOTCH

- *Imposing structure:*

NOTCH1 ternary complex

BROAD INSTITUTE

Moellering, R. E., Cornejo, M., Davis, T. N., Del Bianco, C., Aster, J. C., Blacklow, S. C., Kung, A., L., Gilliland, D. G., Verdine, G. L., Bradner, J. E. *Nature.* **2009**, 182–188.

Stabilized Peptides

Inhibition of NOTCH

- *Imposing structure:*

Binding by extracellular ligands induces signaling

NOTCH1 ternary complex

BROAD INSTITUTE

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Stabilized Peptides

Inhibition of NOTCH

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BROAD INSTITUTE

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Stabilized Peptides

Inhibition of NOTCH

- *Imposing structure:*

dnMAML1 Cofactor

Recruits transcription machinery

NOTCH1 ternary complex

Assembly promotes signalling

BROAD INSTITUTE

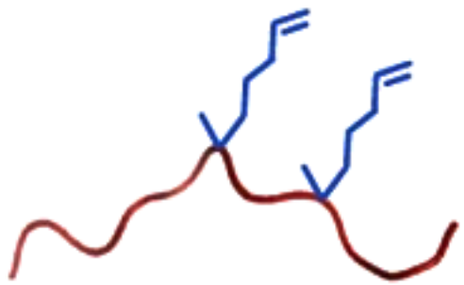
Moellering, R. E., Cornejo, M., Davis, T. N., Del Bianco, C., Aster, J. C., Blacklow, S. C., Kung, A., L., Gilliland, D. G., Verdine, G. L., Bradner, J. E. *Nature.* **2009**, 182–188.

Stabilized Peptides

Inhibition of NOTCH

- *Imposing structure:*

NOTCH1 ternary complex



ICN1 <u>normally</u> binds MAML1

Derived from MAML1 binding domain!

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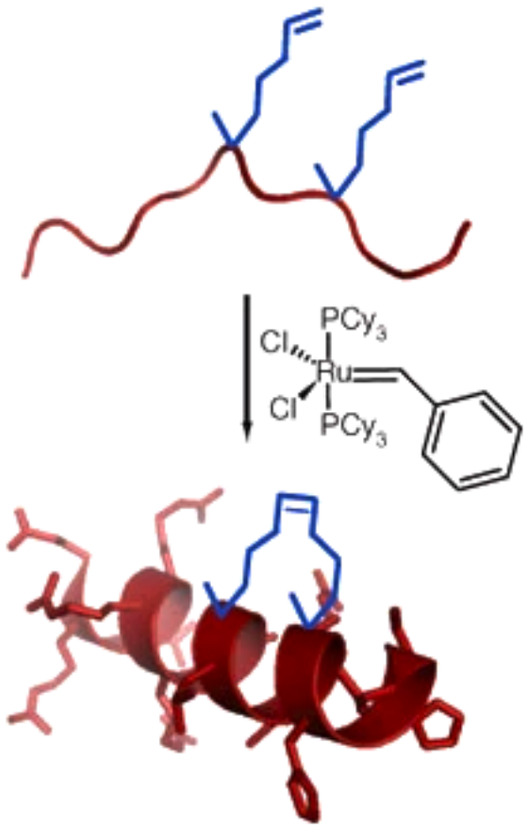

stapled α-helical peptides

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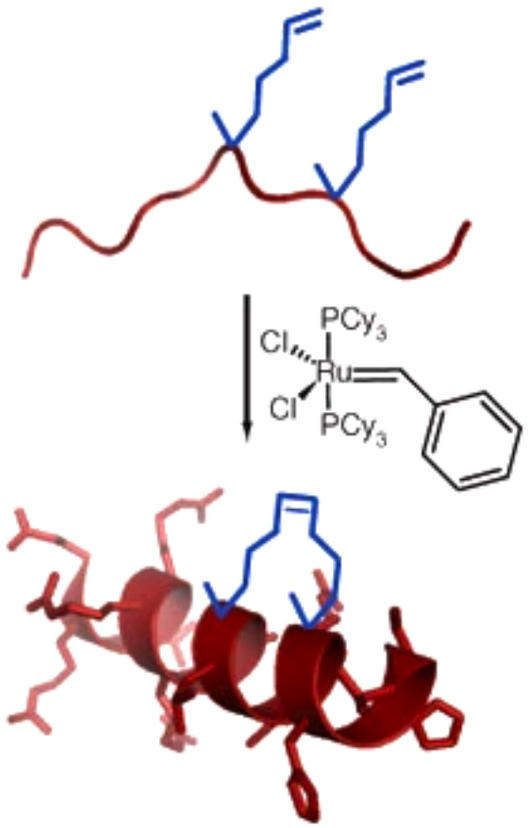

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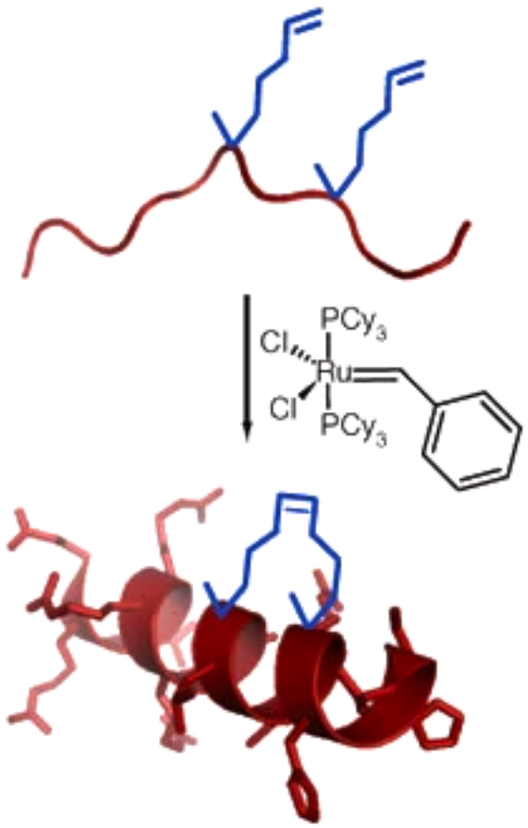

Forced as a helix (despite being short) due to stapling

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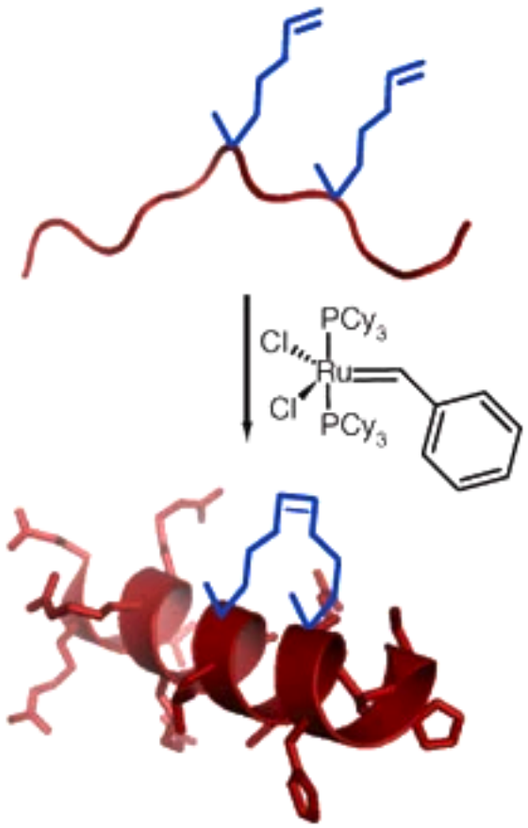

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stapled α-helical peptides

(No binding)

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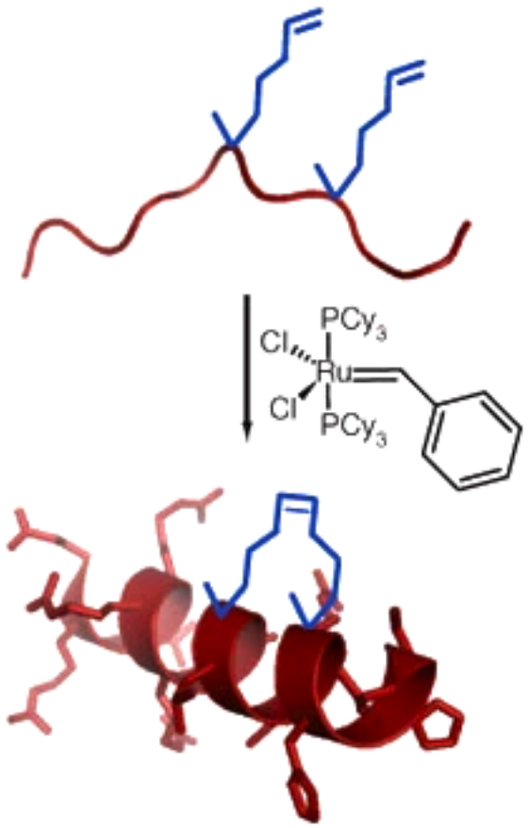
Stabilized Peptides

Inhibition of NOTCH

- *Imposing structure: Previous peptide stapling had never targeted a complex*

NOTCH1 ternary complex



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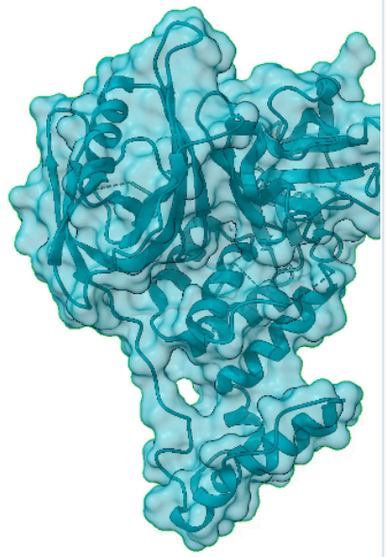
Modification Strategies for Undruggable Targets: Target ID and druggability

Part 2

Non-covalent interactors, peptidomimetics, etc.

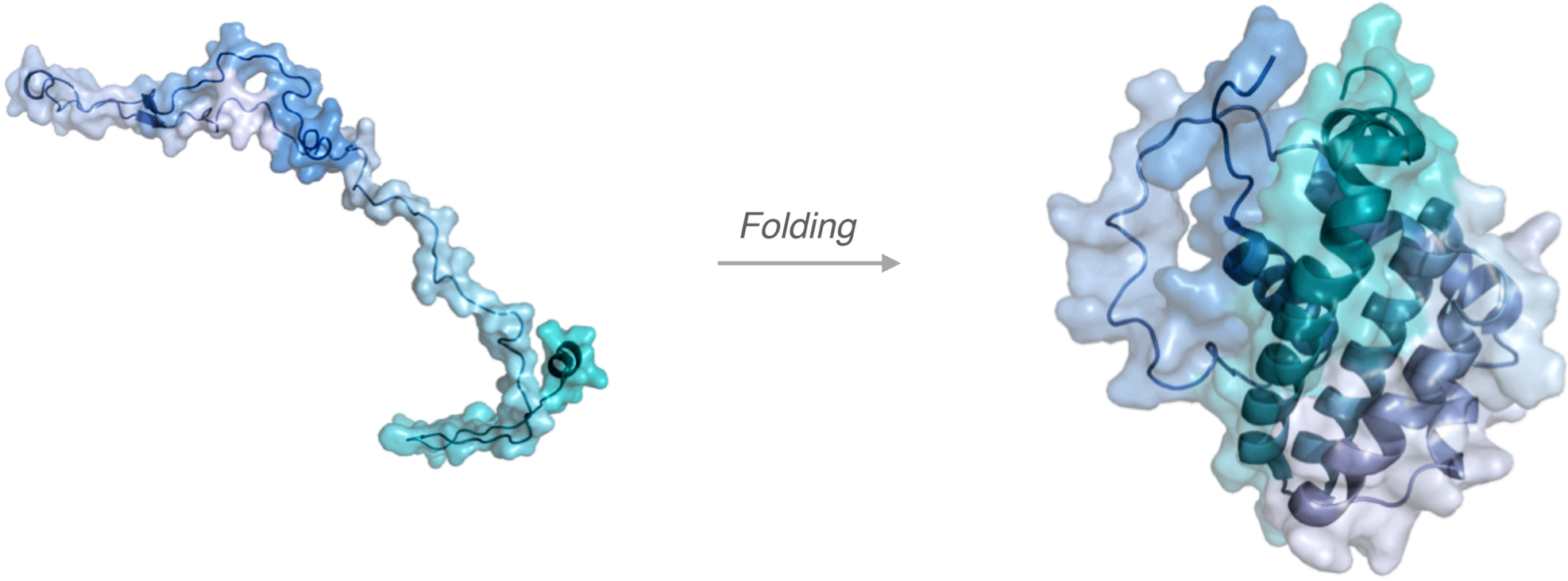
- *Exploiting structure–function to investigate therapeutics*



Part 3

Prions: Protein Misfolding

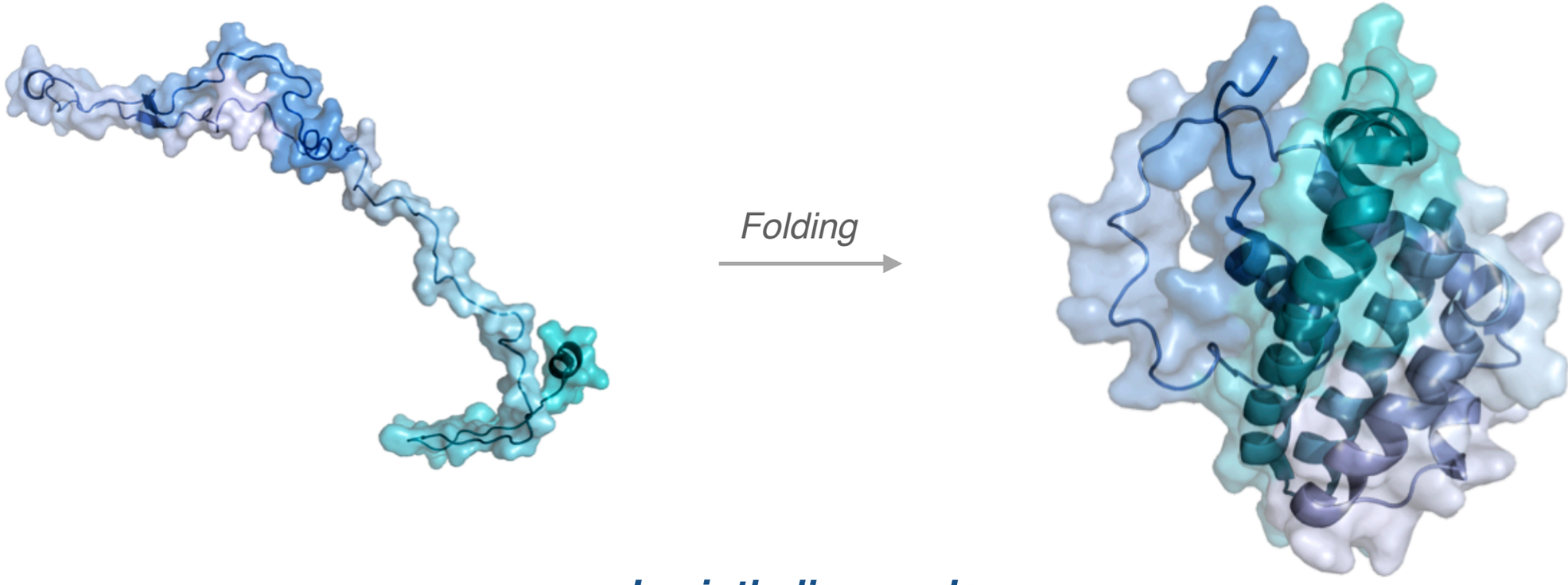
Intrinsically disordered proteins



Tang, C., Feigin A., *Neurodegenerative Dis Manag*. **2012,** 2, 421–435.

Intrinsically disordered proteins

Prion disease = proteopathic disease



Levinthal's paradox

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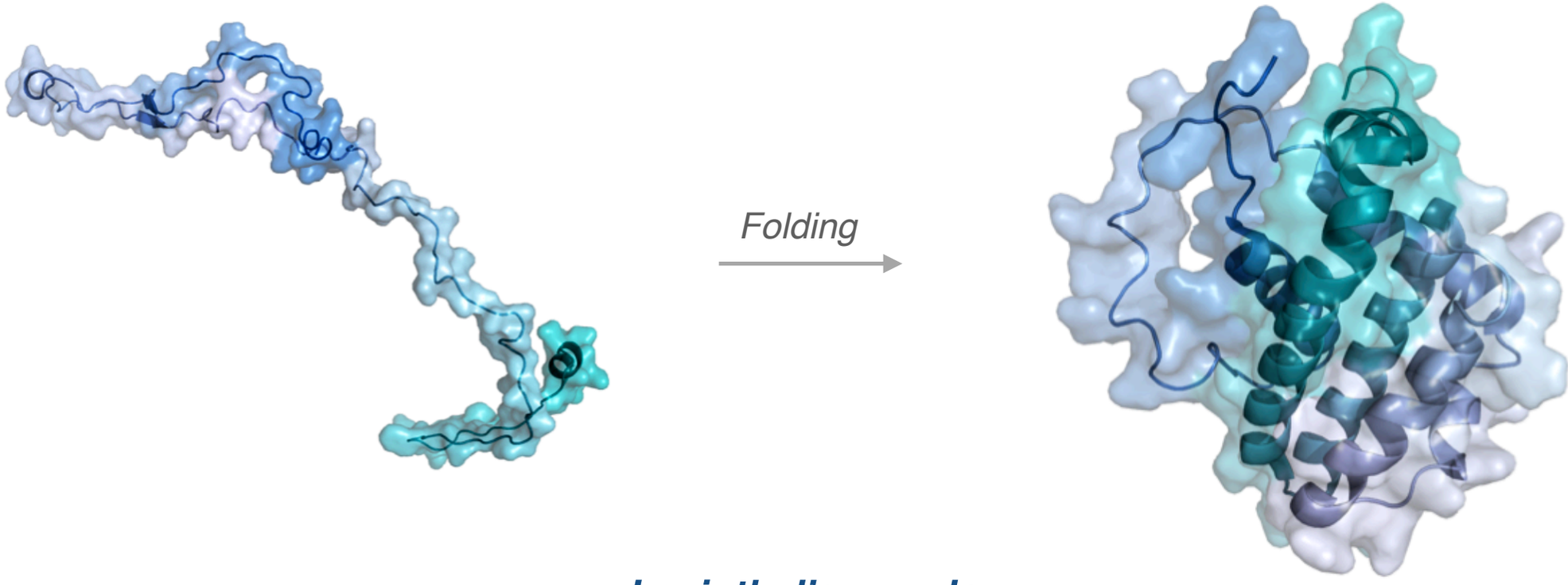

Levinthal's paradox

"A protein with N amino acids should have 10^{2N} degrees of freedom. Thus a small protein (e.g. 150 amino acids) would have 10^{300} degrees of freedom. There is not enough time in the universe to try each of these combinations, and yet the protein is ***folded within a second****."*

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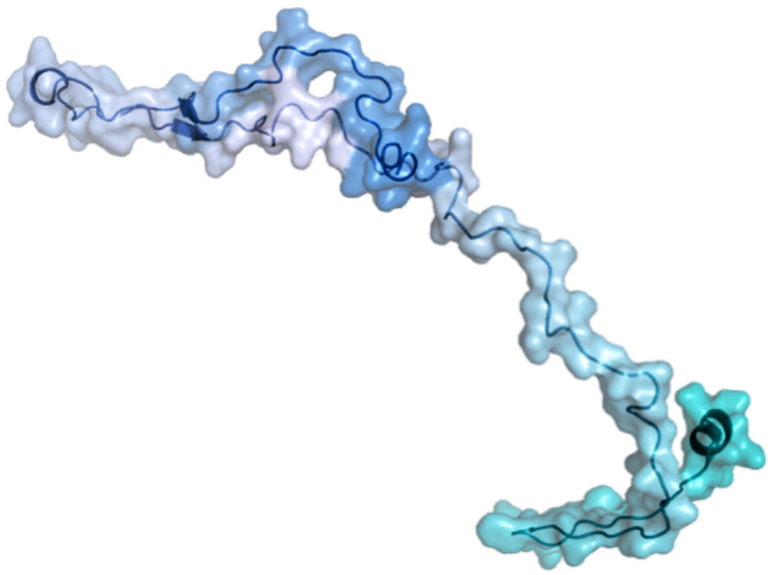
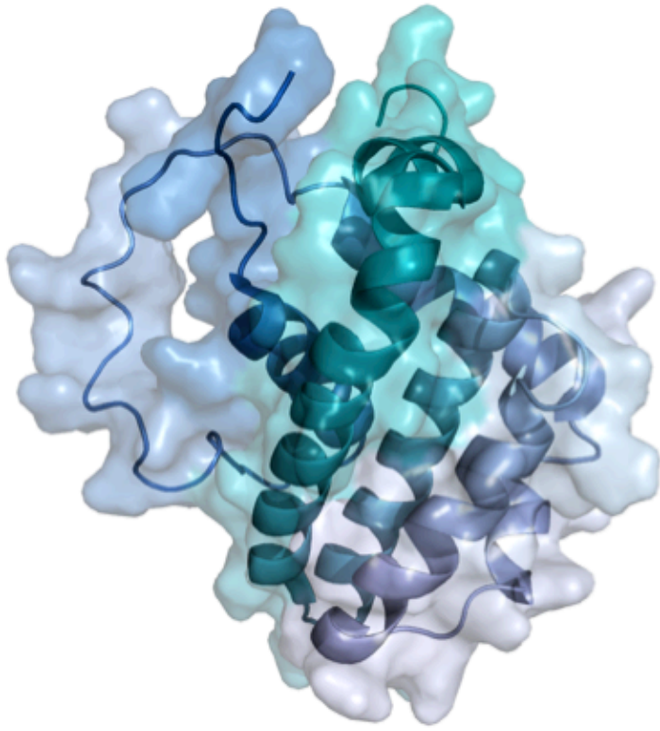


Folding



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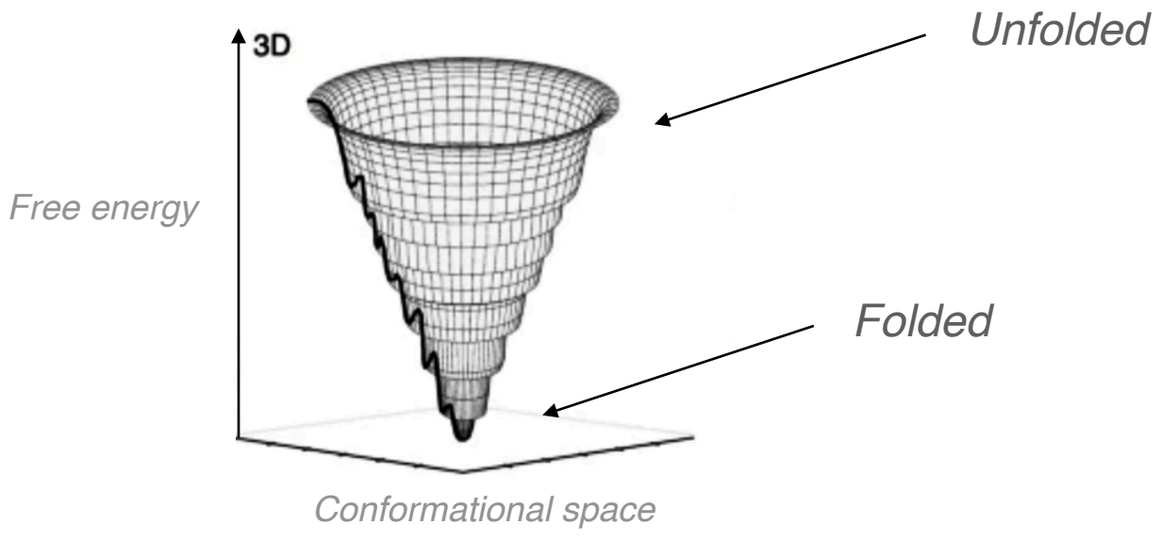

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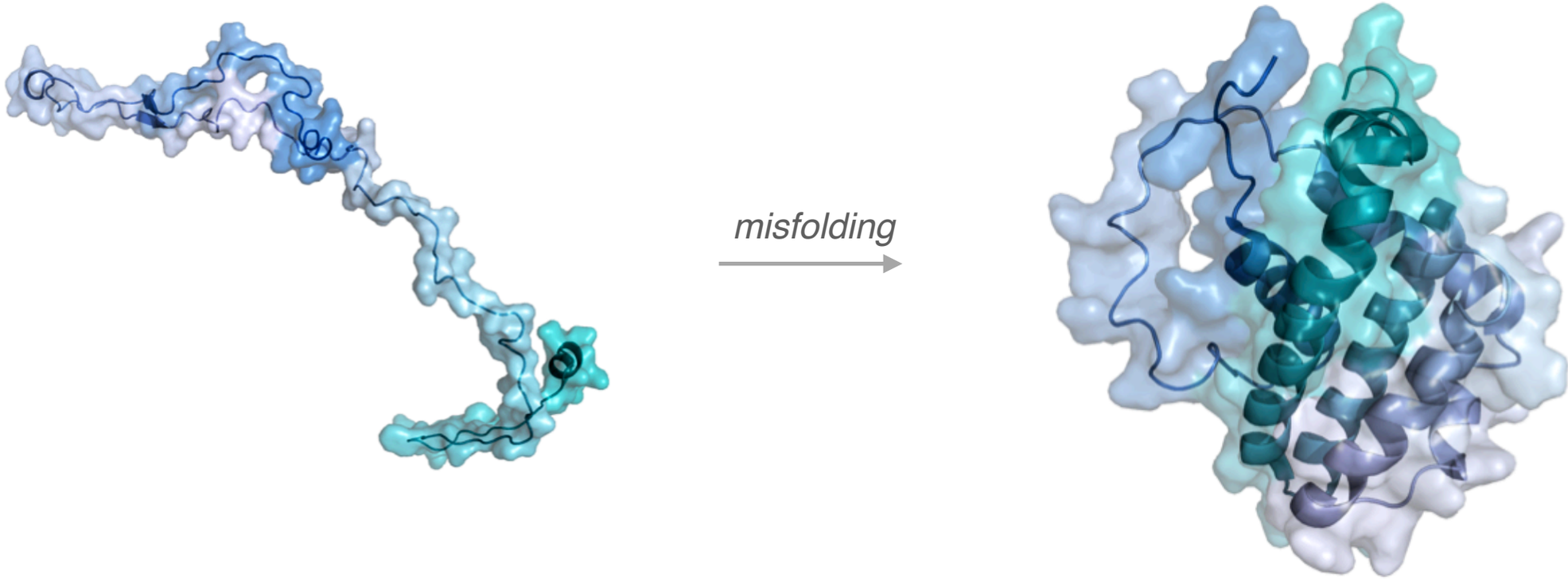

"Proteinaceous infection" = Prion

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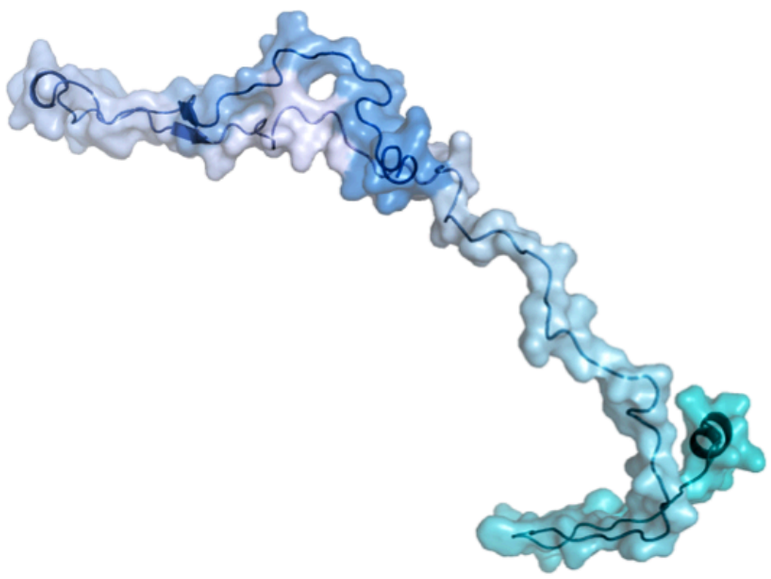
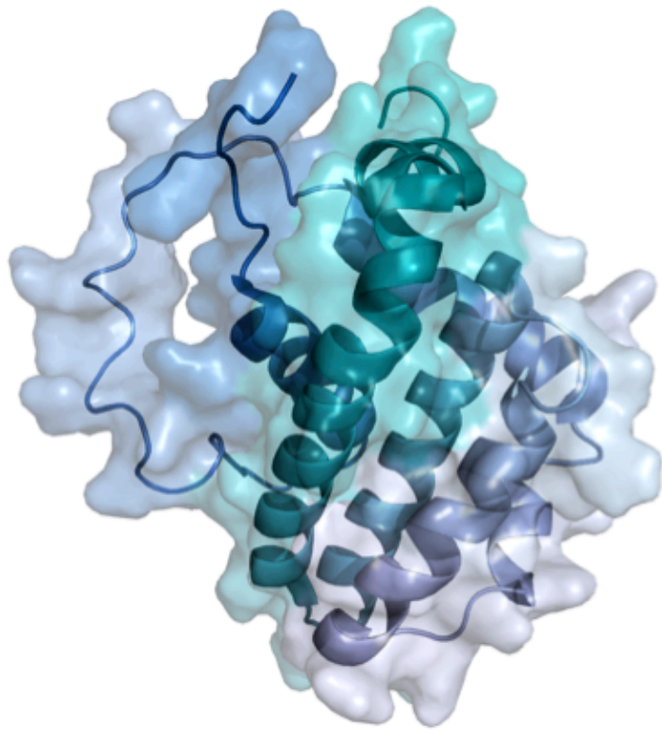
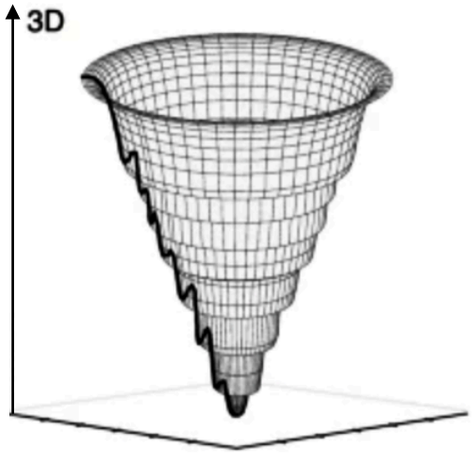


misfolding





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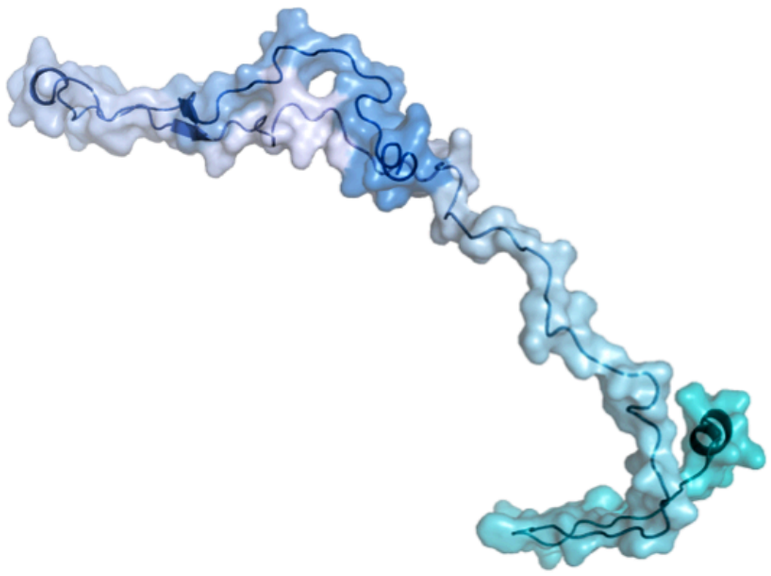

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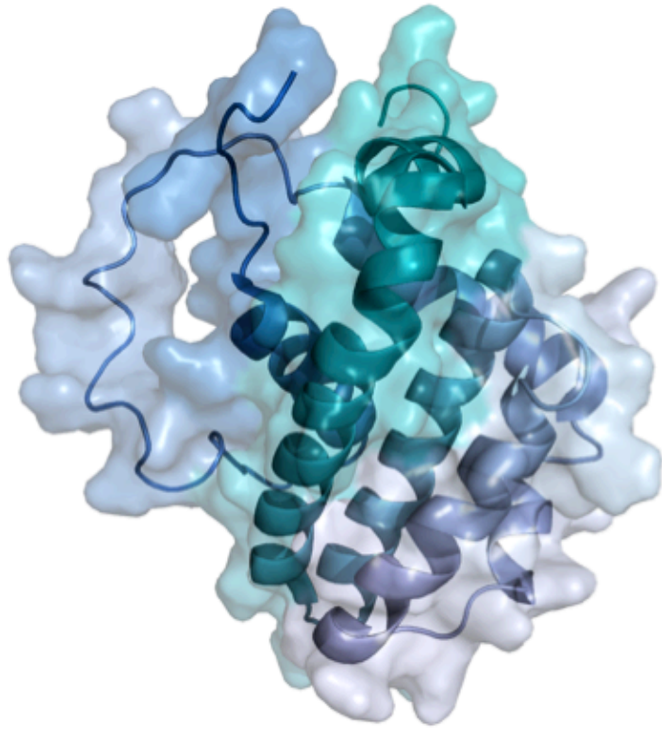

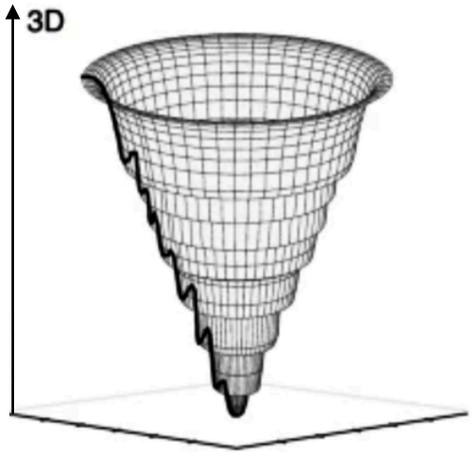

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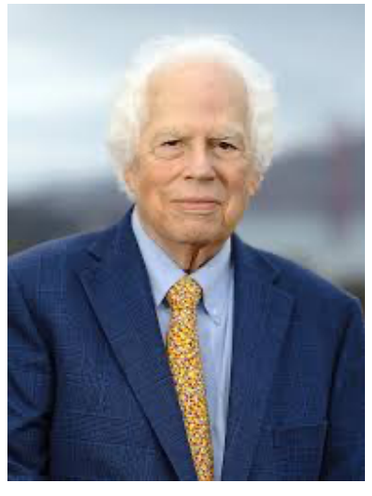

Dr. Stanley Prusiner

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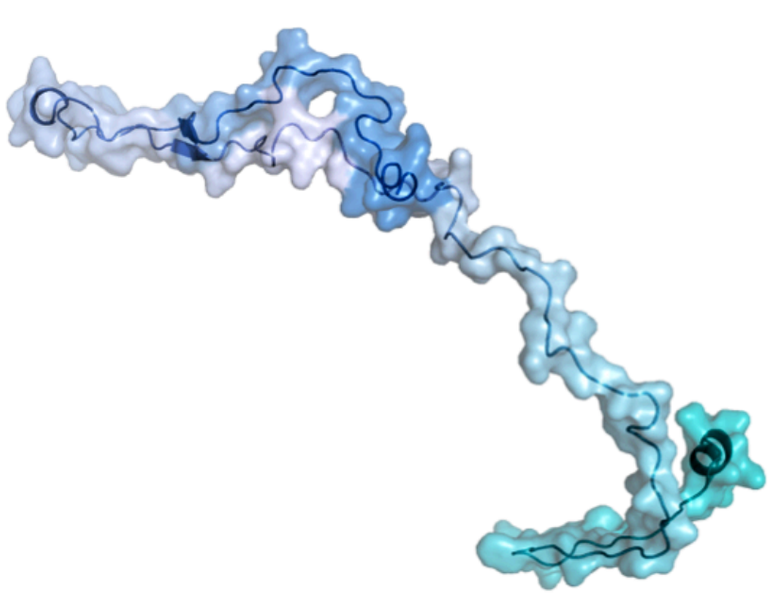
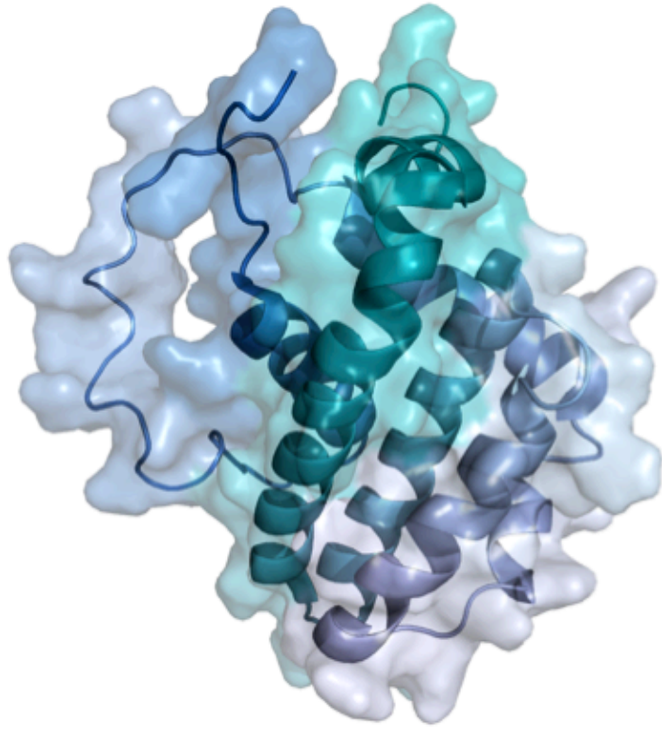
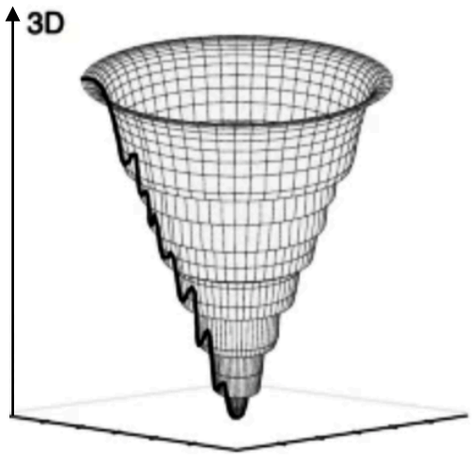
Intrinsically disordered proteins



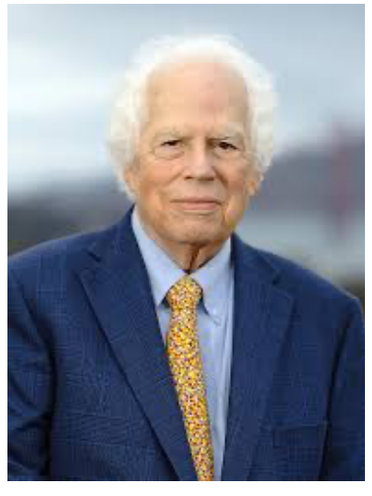
misfolding





"Proteinaceous infection" = Prion

Prions are exceedingly hard to sterilize/inactivate



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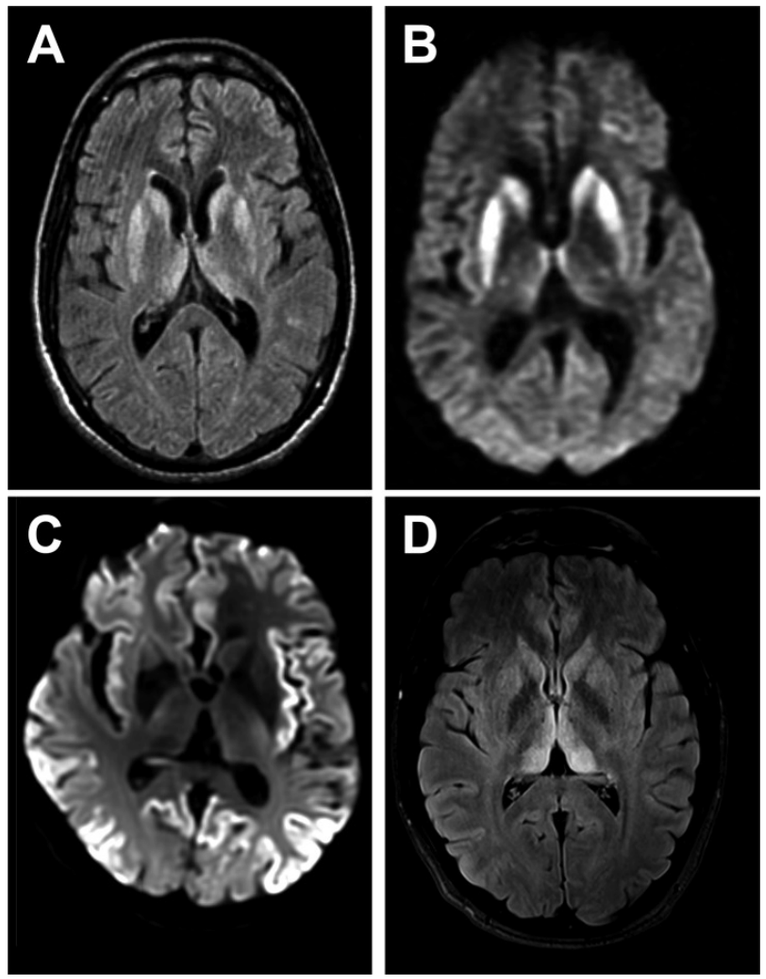

"Proteinaceous infection" = Prion

Jakob-Creutzfeldt disease, or Kuru

- *No known cure*

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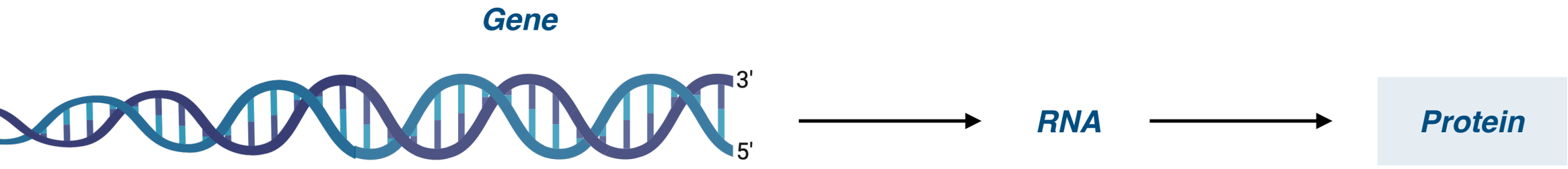
Intrinsically disordered proteins

Strategies to target the undruggable



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Intrinsically disordered proteins

Strategies to target the undruggable

In vivo base editing extends lifespan of a humanized mouse model of prion disease

Meirui An, Jessie R. Davis, Jonathan M. Levy, Fiona E. Serack, John W. Harvey, Pamela P. Brauer, Catherine P. Pirtle, Kiara N. Berríos, Gregory A. Newby, Wei-Hsi Yeh, Nikita Kamath, Meredith Mortberg, Yuan Lian, Michael Howard, Kendrick DeSouza-Lenz, Kenia Guzman, Aaron Thai, Samantha Graffam, Vanessa Laversenne, Alissa A. Coffey, Jeannine Frei, Sarah E. Pierce, Jiri G. Safar, Benjamin E. Deverman, ... David R. Liu + Show authors

Nature Medicine **31**, 1319–1328 (2025) | Cite this article

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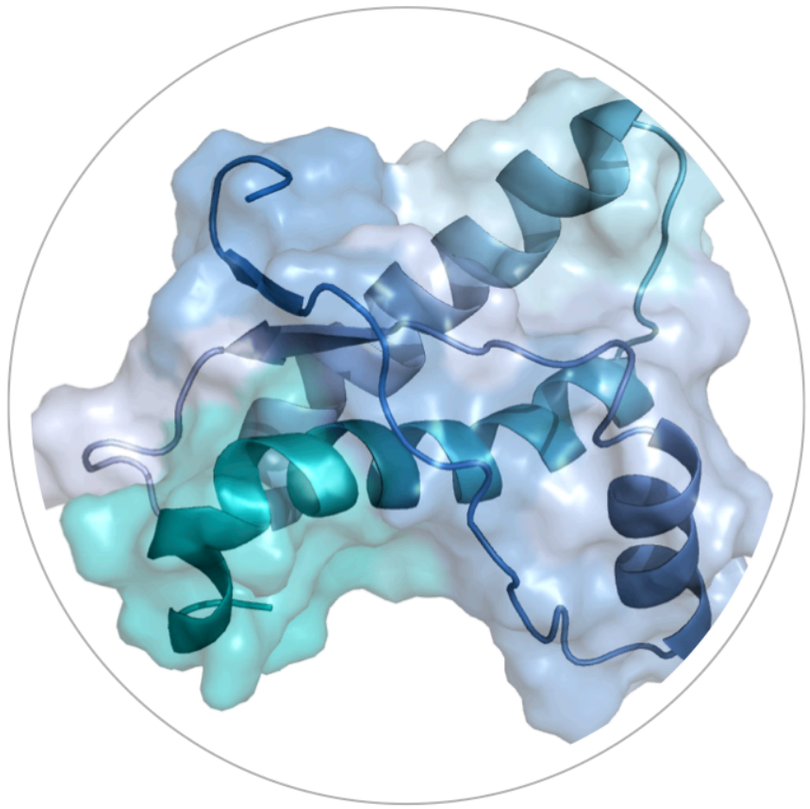
An, M. Et al. *Nat. Med.* **2025,** *31*, 1319–1328.

Intrinsically disordered proteins

Strategies to target the undruggable

PRNP Gene

PRP protein misfold



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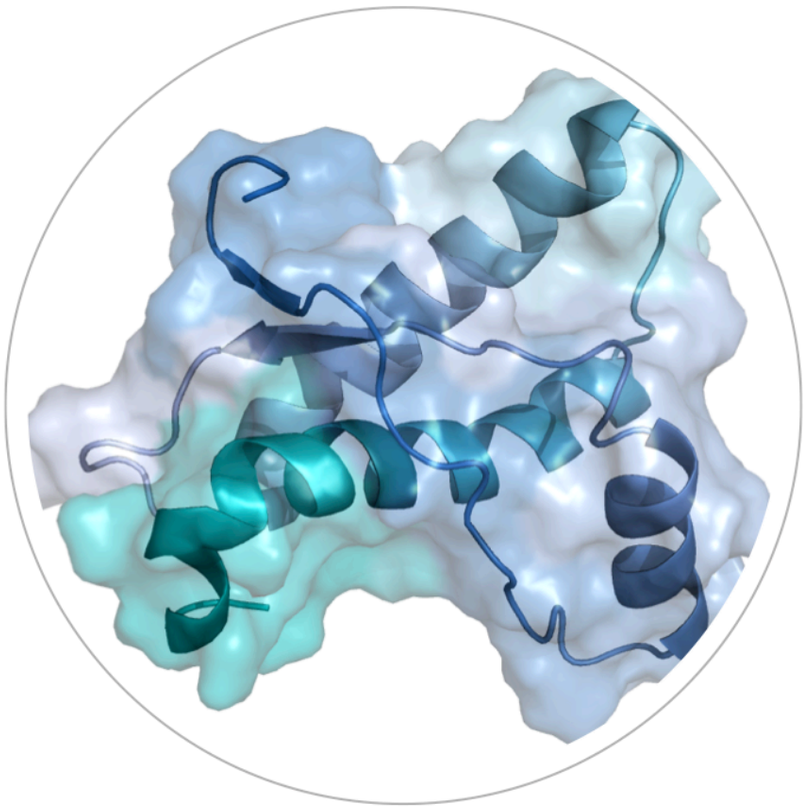

- ***strategy: gene therapy***

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Intrinsically disordered proteins

Strategies to target the undruggable

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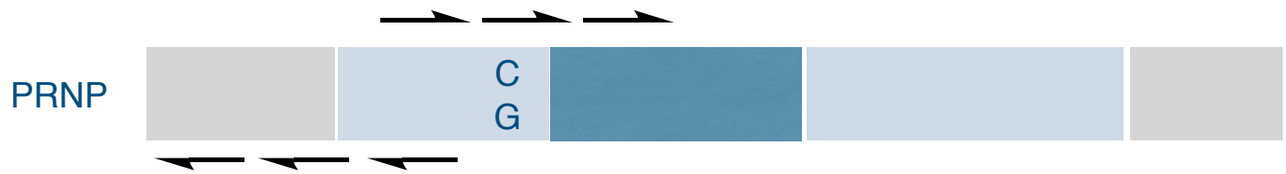

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Intrinsically disordered proteins

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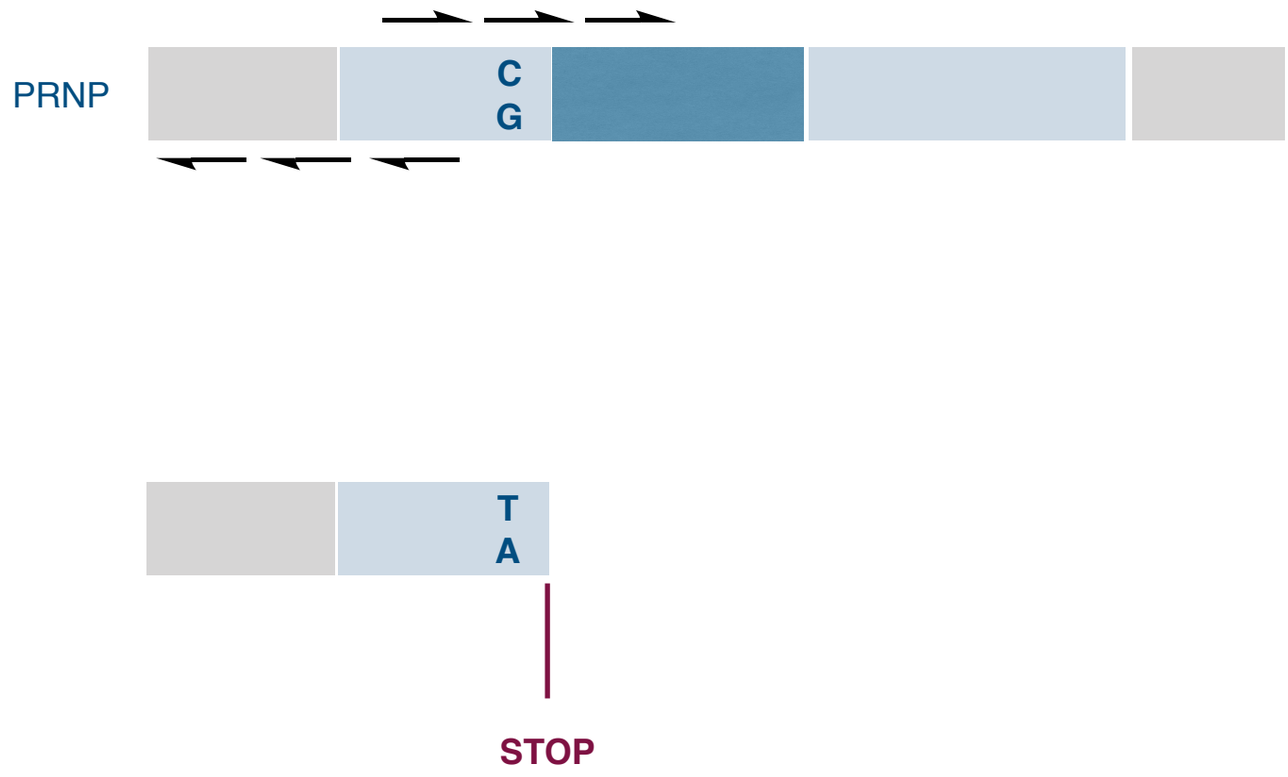

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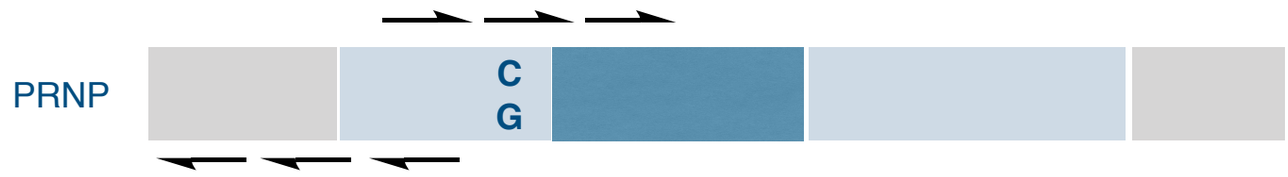

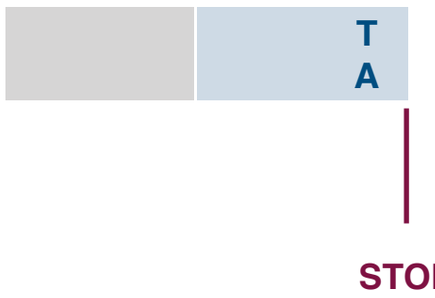

Key point: Arg, Gln, Trp codons → stop codons

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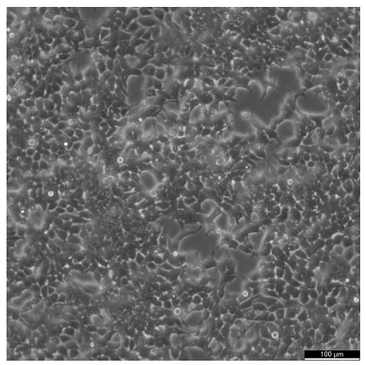

HEK293T cells

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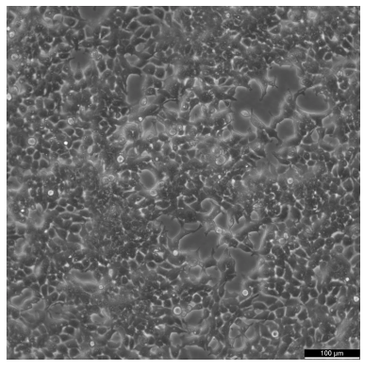

HEK293T cells

Transfected with plasmid encoding base editing tools

Base editing targeting Arg37 exhibited high editing efficiency (54%!)

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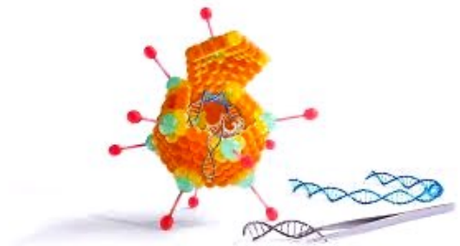

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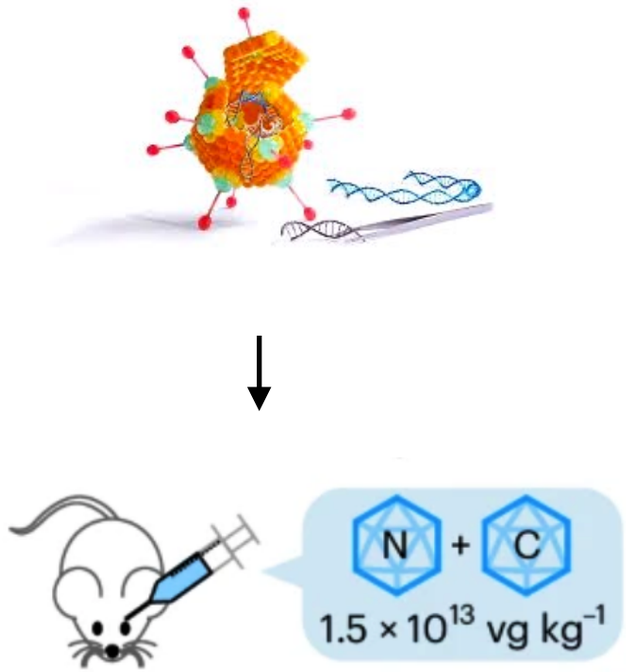

Tg25109 mice

(Humanized prion mice)

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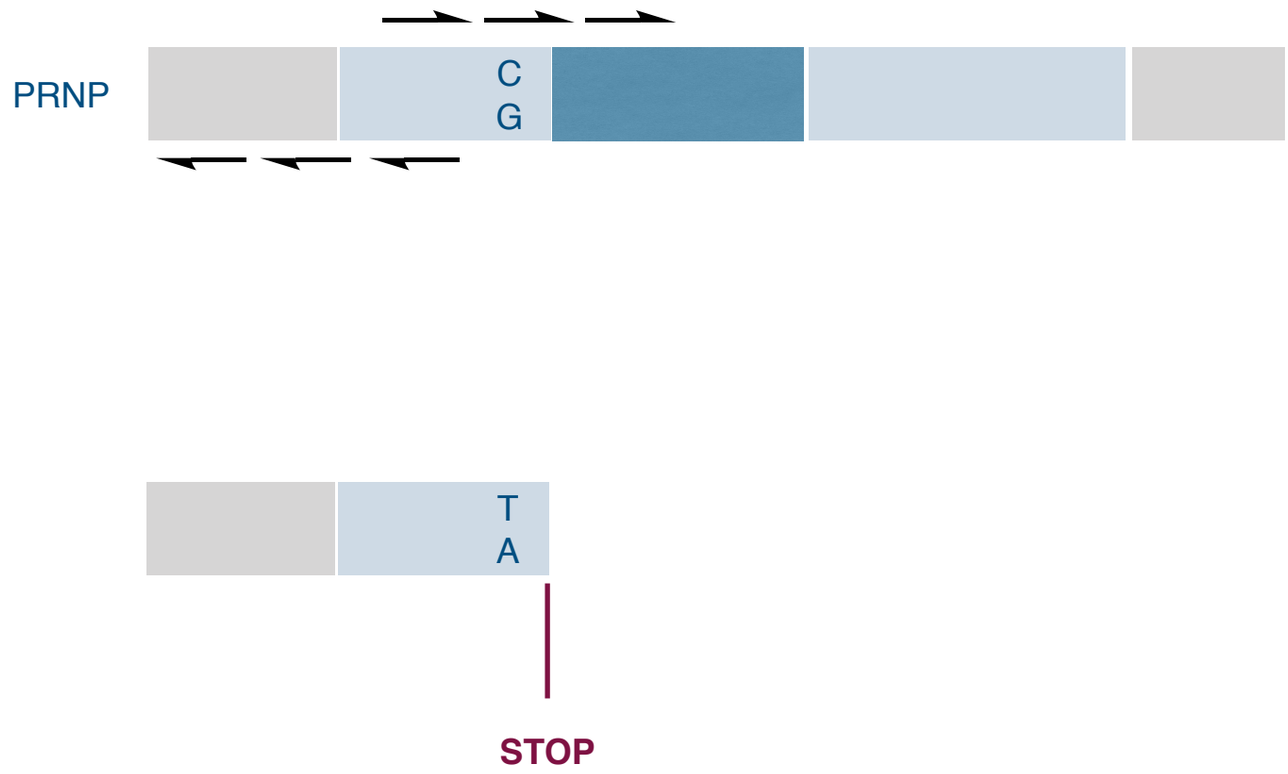

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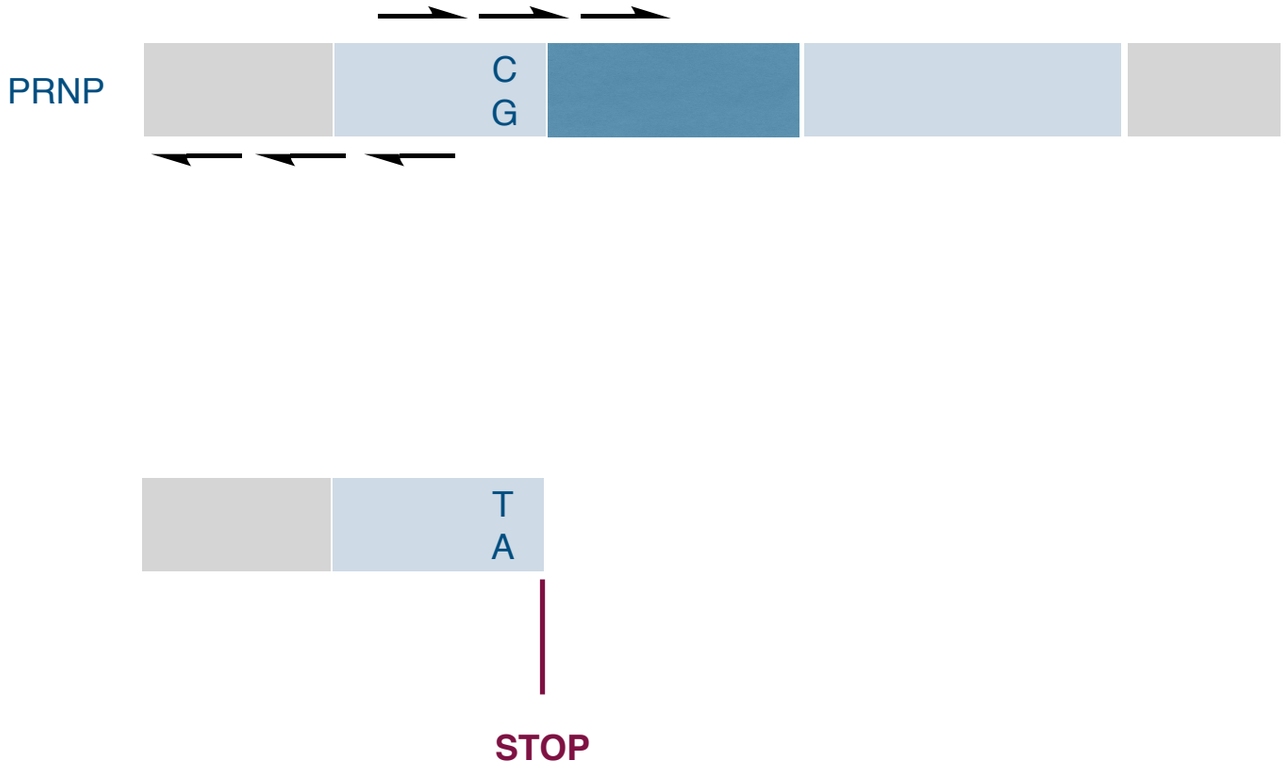

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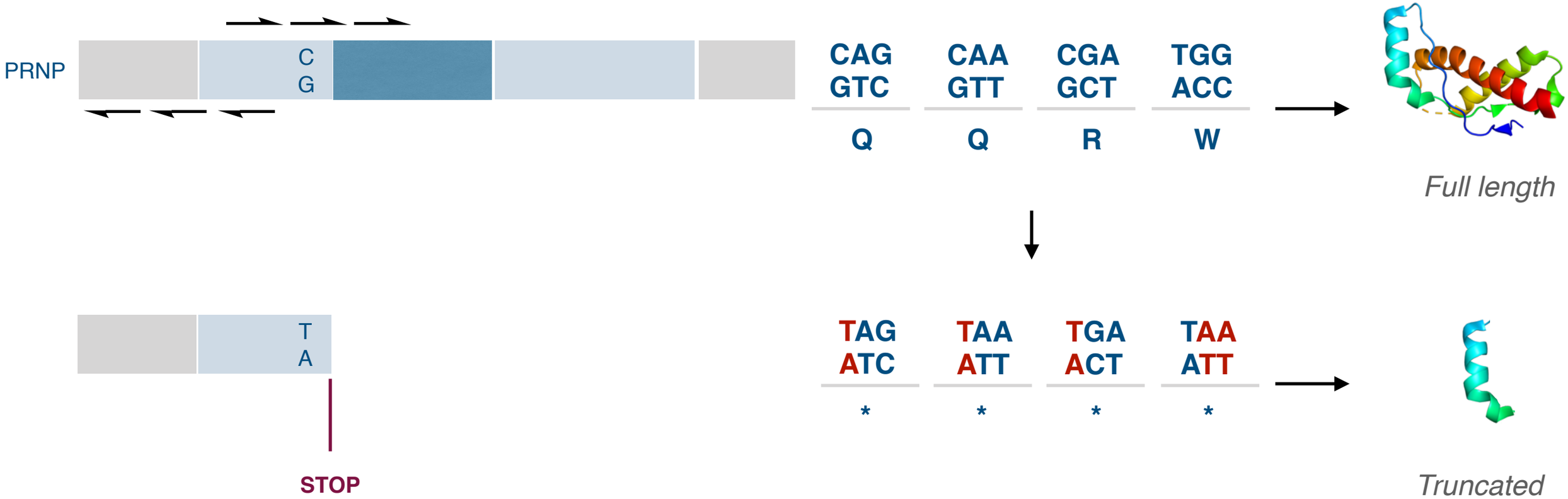

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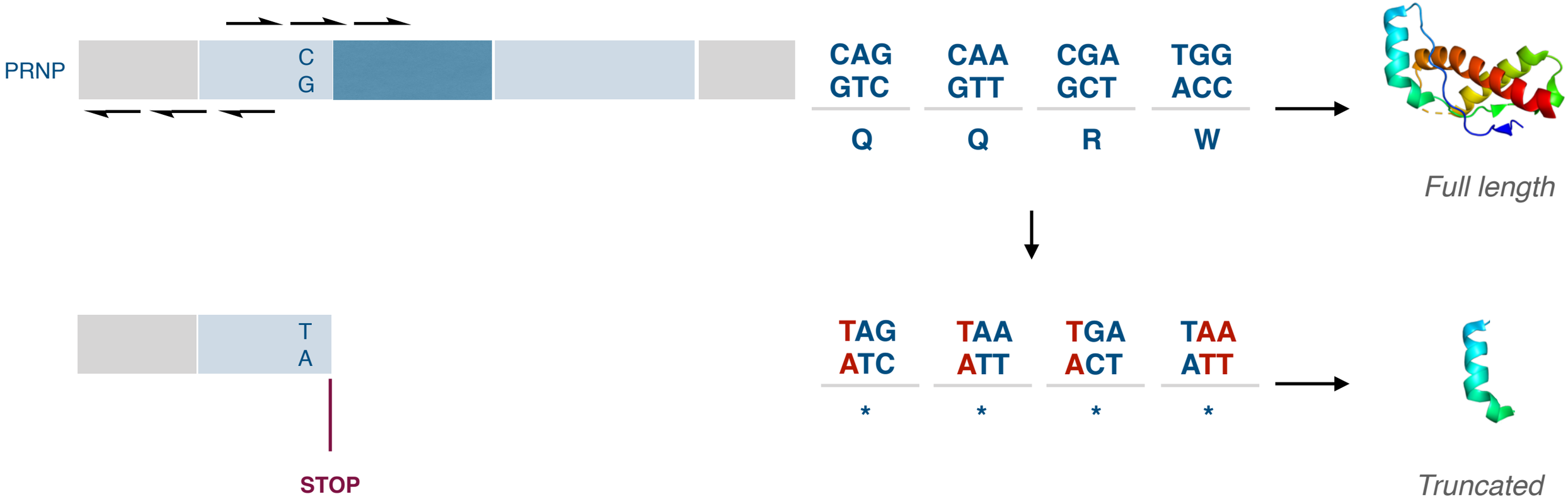

- **50% increase in lifespan in a humanized mouse model of prion disease!**

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Questions?

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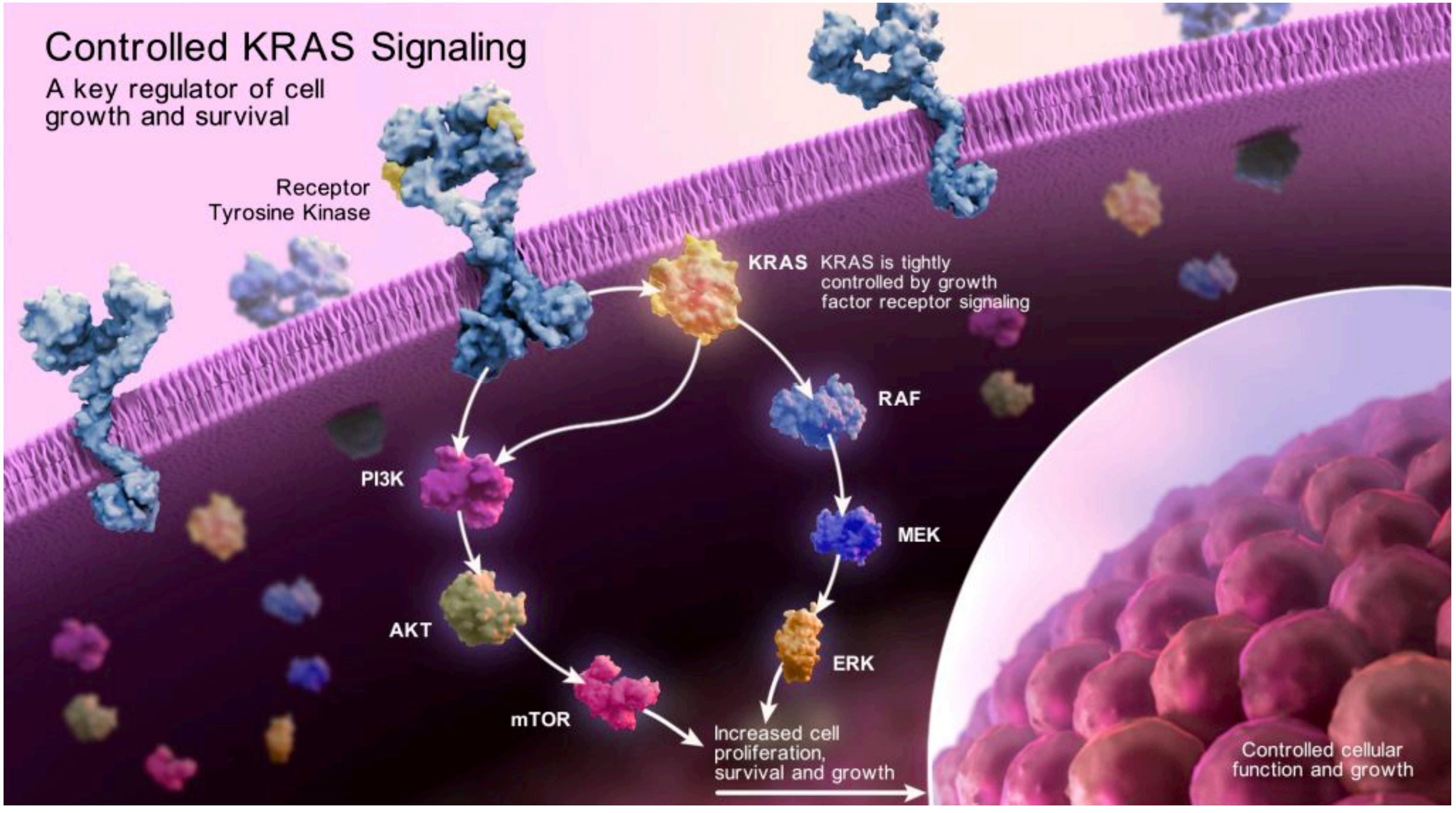
KRAS signalling

Controls cell proliferation and growth

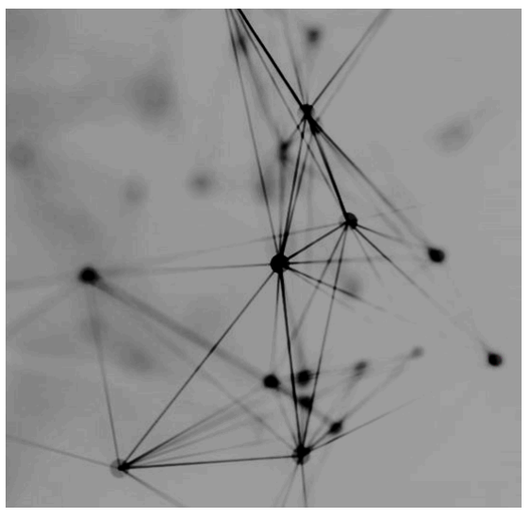


Langley, D.R.; Crews, C.M. *Nat. Rev. Drug Discov.* ***2022***, *21*, 181.

Druggable Targets

<u>Identification</u> of disease-causal targets has been driven by:

1. *Data-driven* ***"-omics"***
   - ***gives expression level data***



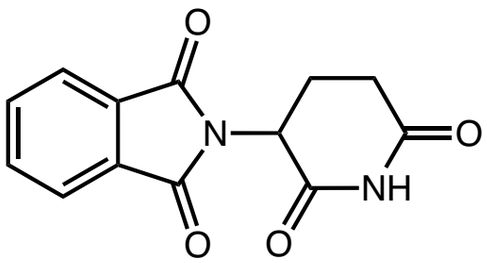
2. ***protein*** *and* ***nucleic acid*** *ID*

Swenson, C. S., Mandava, G., Thomas, D. M., Moellering, R. E. *Chem. Rev.* **2024**. *124*, 13020–13093

Molecular Glues

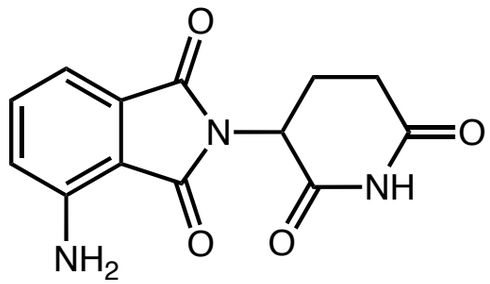
Immunomodulatory drugs

- Molecular glue mechanism of IMiDs influenced PROTAC design

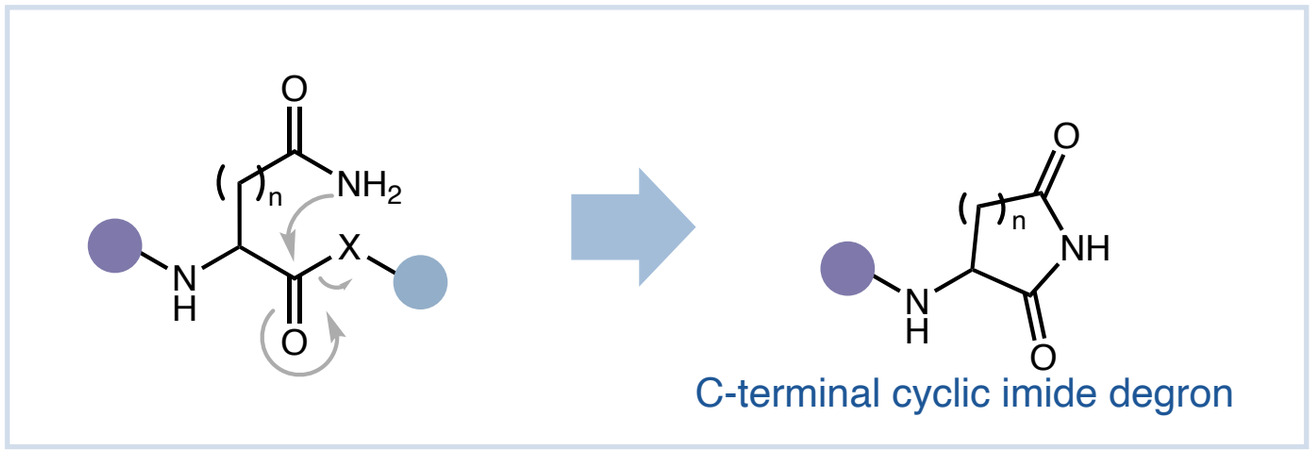


Thalidomide

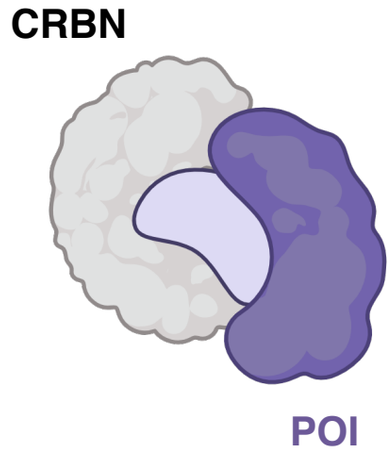
induces protein degradation



Lenalidomide

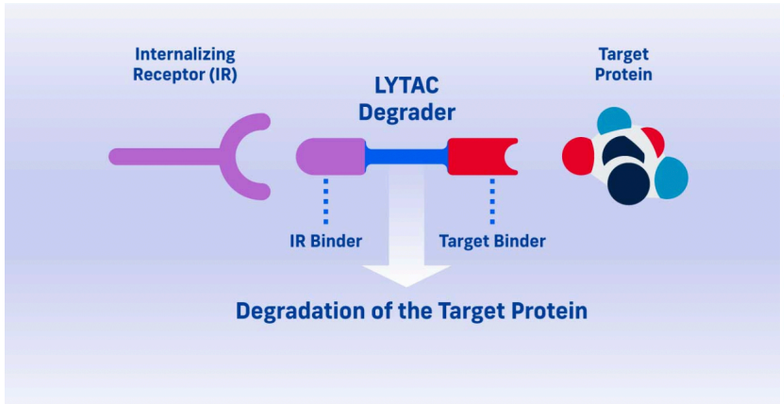


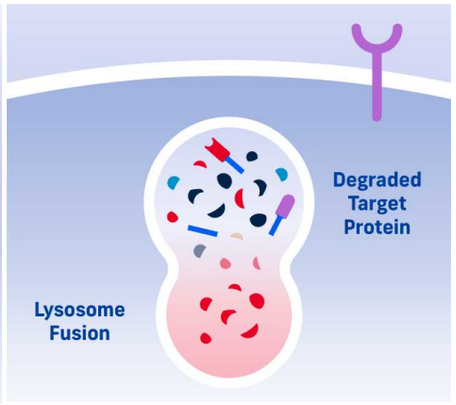
IMiDs mimic a PTM that recruits degradation machinery



Langley, D.R.; Crews, C.M. *Nat. Rev. Drug Discov.* ***2022***, *21*, 181.

LYTAC: Lysosome Targeting Chimeras

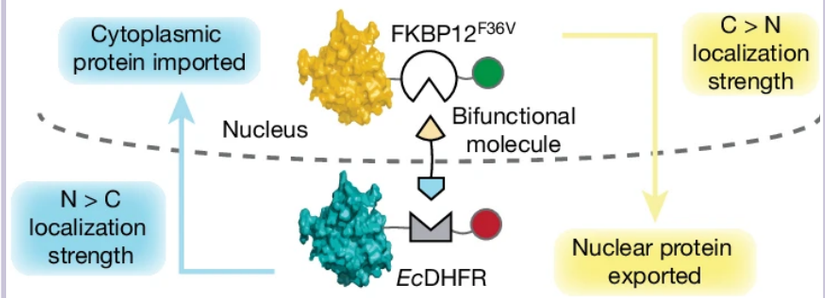




nature

LYCIA THERAPEUTICS

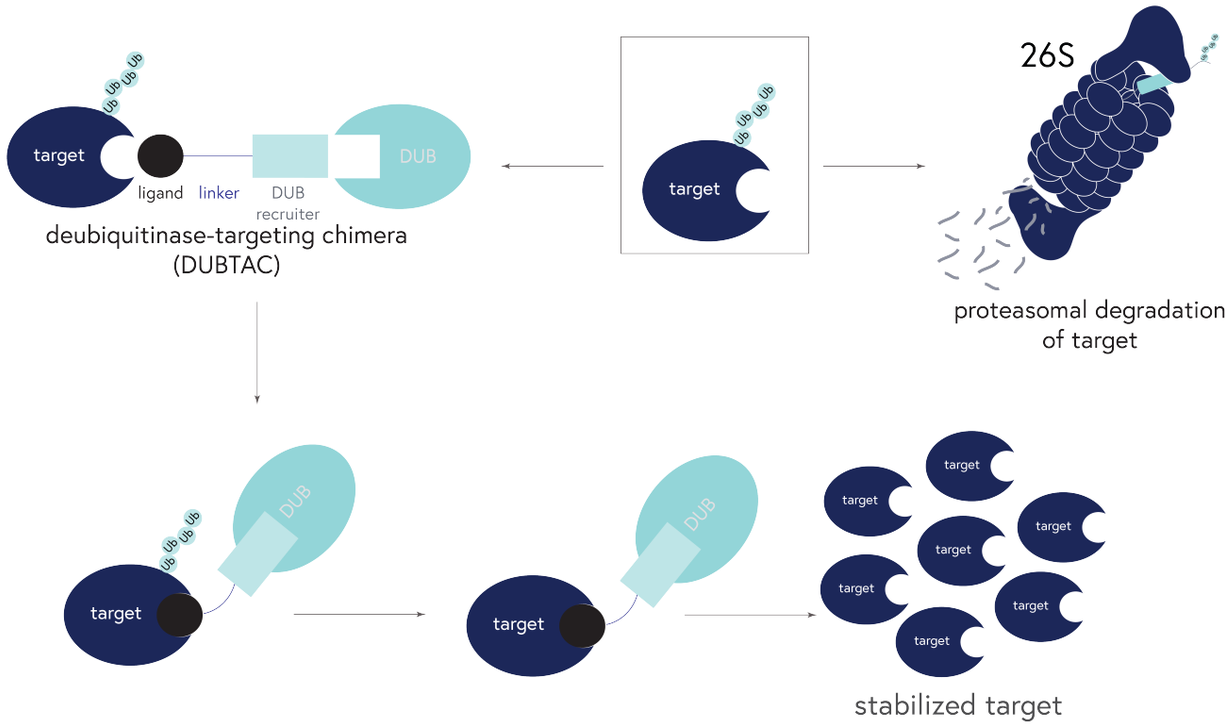
TRAM: Targeted Relocalization Activating Molecules



nature

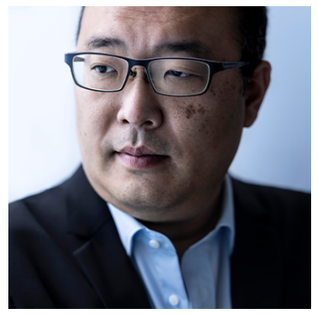
Steven Banik, Stanford

DUBTAC: Deubiquitinase Targeting Chimeras



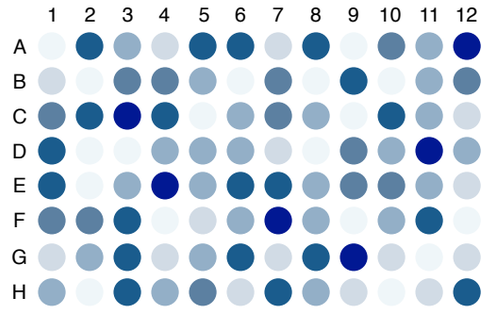
VICINITAS THERAPEUTICS

nature chem bio



Novel Chemical Reactivity Through AI-Augmented Experimentation

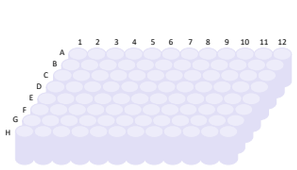
HTE instrument



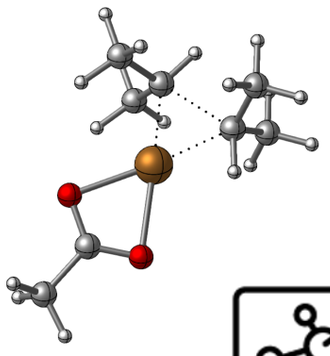
low yields → *high yields*

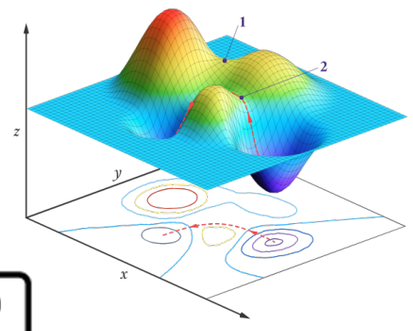
HT reaction outcomes

Chemspeed robot

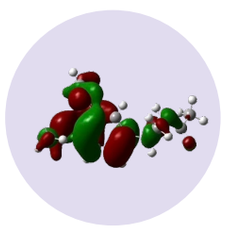


96-Well Plates

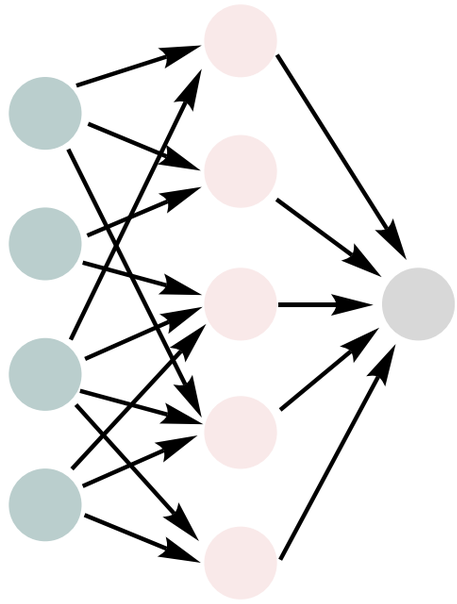




automated computational modeling

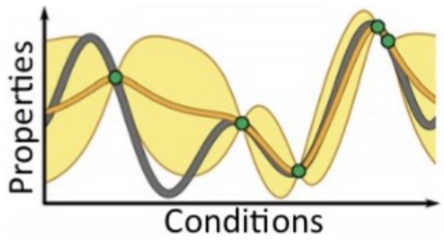


automated experimental feedback



AI & Machine learning

feedback helps develop new reactions



improved conditions and informative experiments for novel reactivity