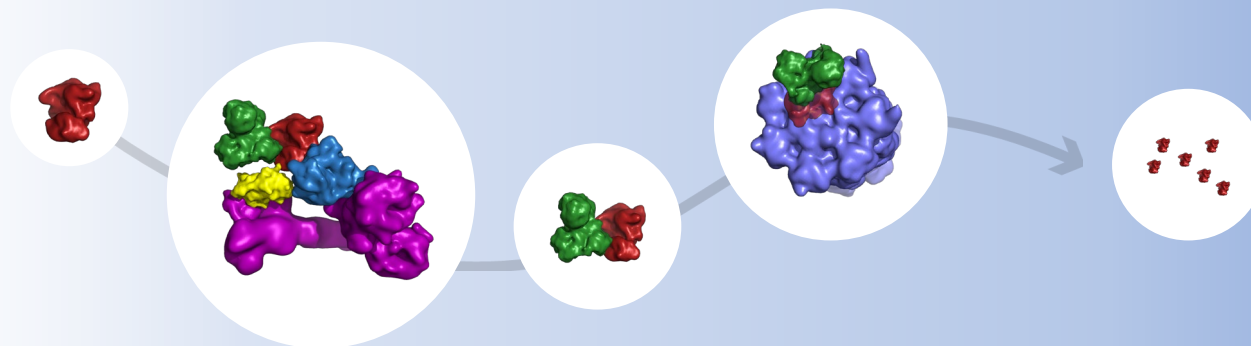


PIONEERING TRANSFORMATIVE **PROTEIN DEGRADATION** THERAPIES



A new class of small-molecule protein degraders harnessing powerful and universal cellular biology



C4Therapeutics

Destroying
~~Targeting~~ disease-causing
proteins to deliver hope

**Computational Methods for Degradation Ternary
Complex Prediction using HDX Data**

PSDI November 2020

Forward-looking Statements and Intellectual Property

Forward-looking Statements

The following presentation contains forward-looking statements. All statements other than statements of historical fact are forward-looking statements, which are often indicated by terms such as “anticipate,” “believe,” “could,” “estimate,” “expect,” “goal,” “intend,” “look forward to,” “may,” “plan,” “potential,” “predict,” “project,” “should,” “will,” “would” and similar expressions. These forward-looking statements include, but are not limited to, statements regarding the therapeutic potential of C4 Therapeutics, Inc.’s technology and products. These forward-looking statements are not promises or guarantees and involve substantial risks and uncertainties. Among the factors that could cause actual results to differ materially from those described or projected herein include uncertainties associated generally with research and development, clinical trials and related regulatory reviews and approvals, and that our product candidates that we are developing or may develop may not demonstrate success in clinical trials. Prospective investors are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date hereof. C4 Therapeutics, Inc. undertakes no obligation to update or revise the information contained in this presentation, whether as a result of new information, future events or circumstances or otherwise.

Intellectual Property

C4 Therapeutics, Inc. owns various registered and unregistered trademarks in the U.S. and overseas, including, without limitation, C4 THERAPEUTICS, TORPEDO, BIDAC and MONODAC. All trademarks or trade names referred to in this presentation that we do not own are the property of their respective owners. Solely for convenience, the trademarks and trade names in this prospectus are referred to without the symbols ® and ™, but such those references should not be construed as any indicator that their respective owners will not assert, to the fullest extent under applicable law, their rights thereto.

1

Introduction

Dissecting targeted protein degradation as a modality

2

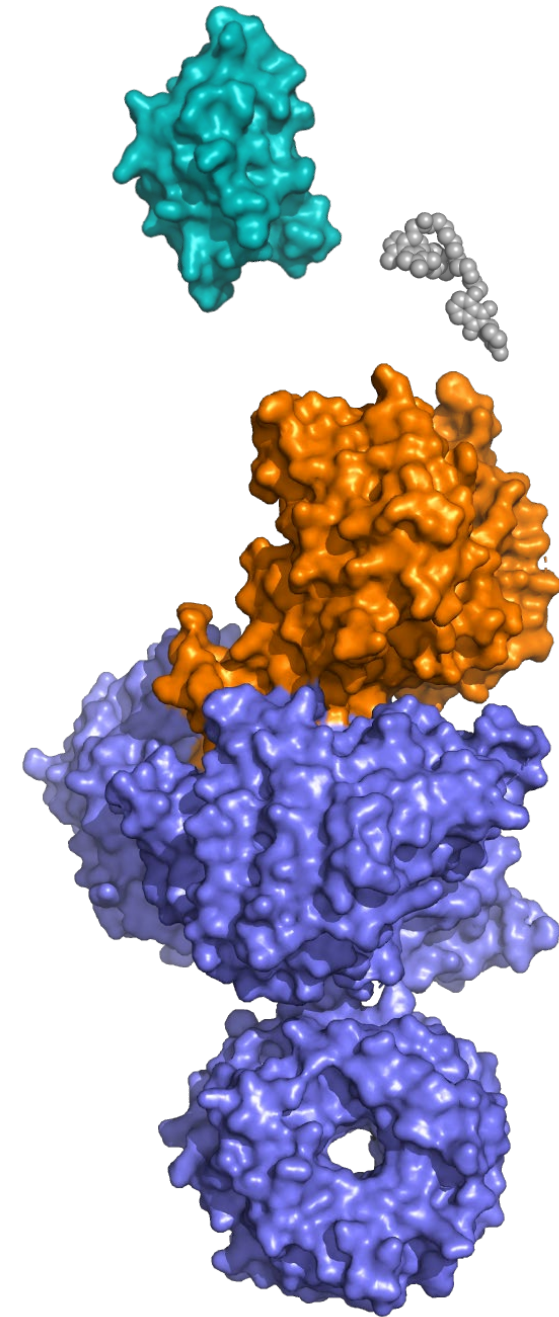
Ternary Complexes

How does the field study and visualize this crucial intermediary step?

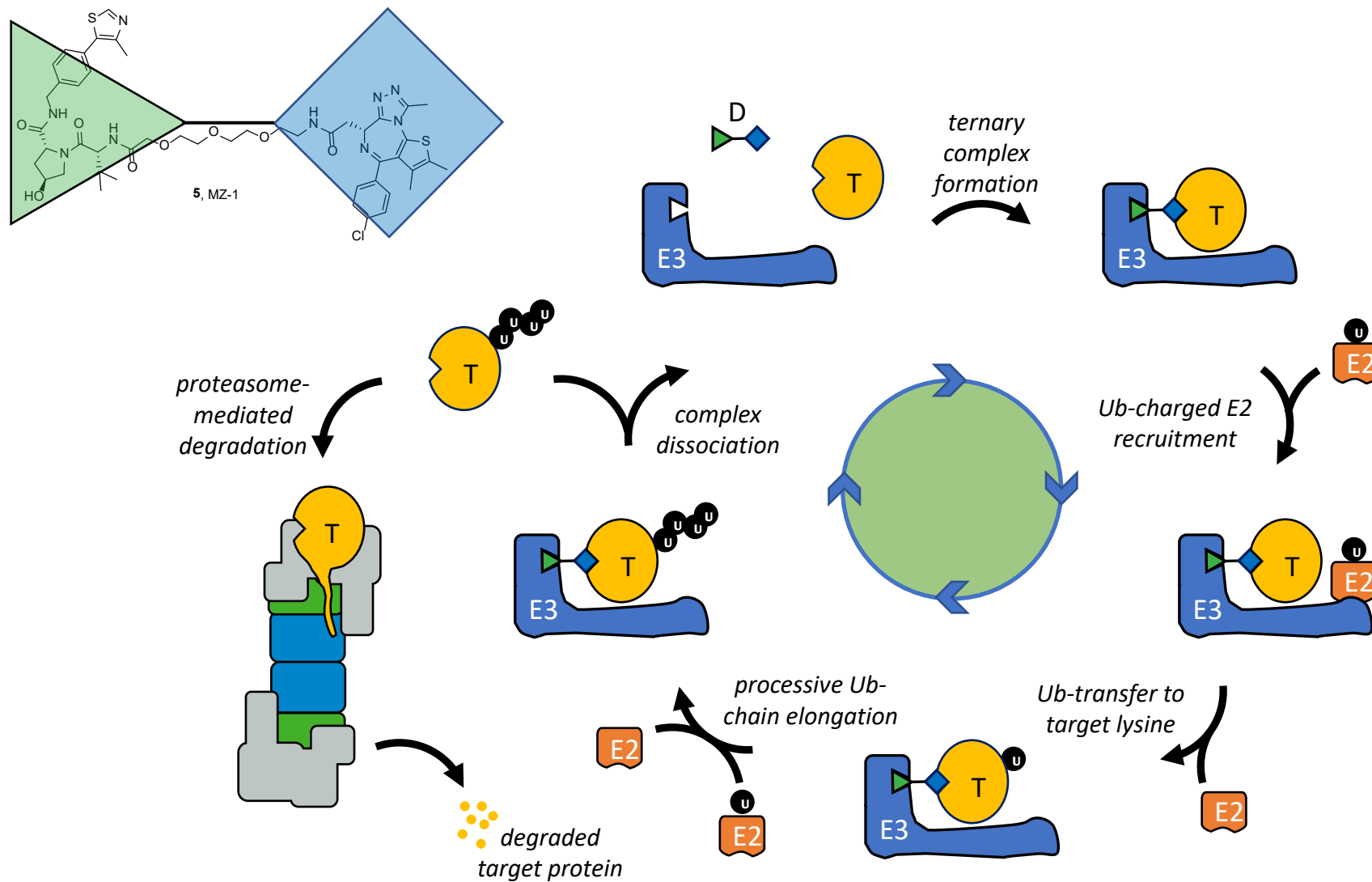
3

C4T's approach

Case study: using HDX-MS to interrogate degrader-induced ternary complexes

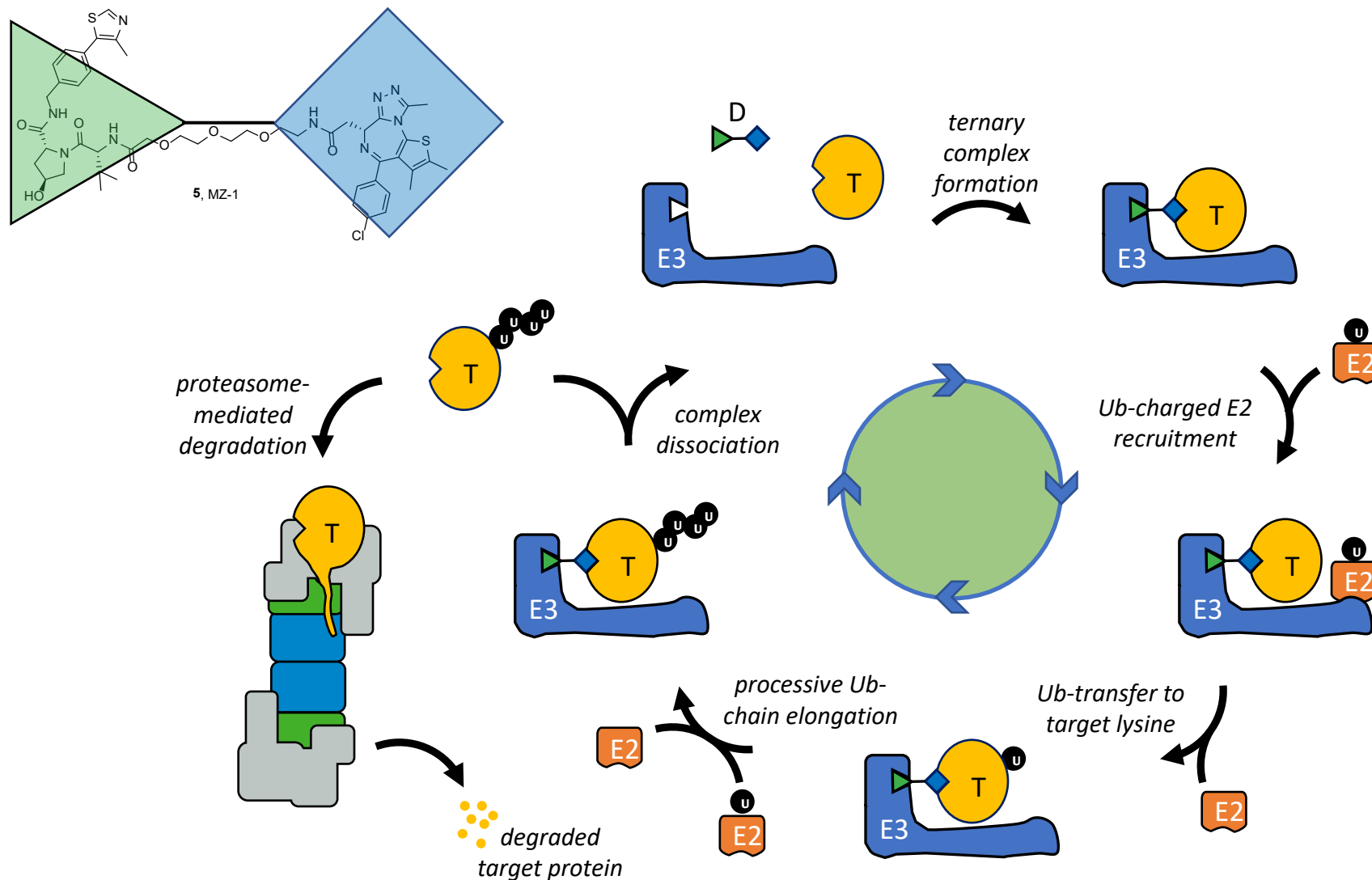


The Catalytic Cycle of a Degrador – Importance of the Ternary Complex



Fisher and Phillips, *Curr Opin Chem Biol.* 2018, 44: 47-55

The Catalytic Cycle of a Degradator – Importance of the Ternary Complex

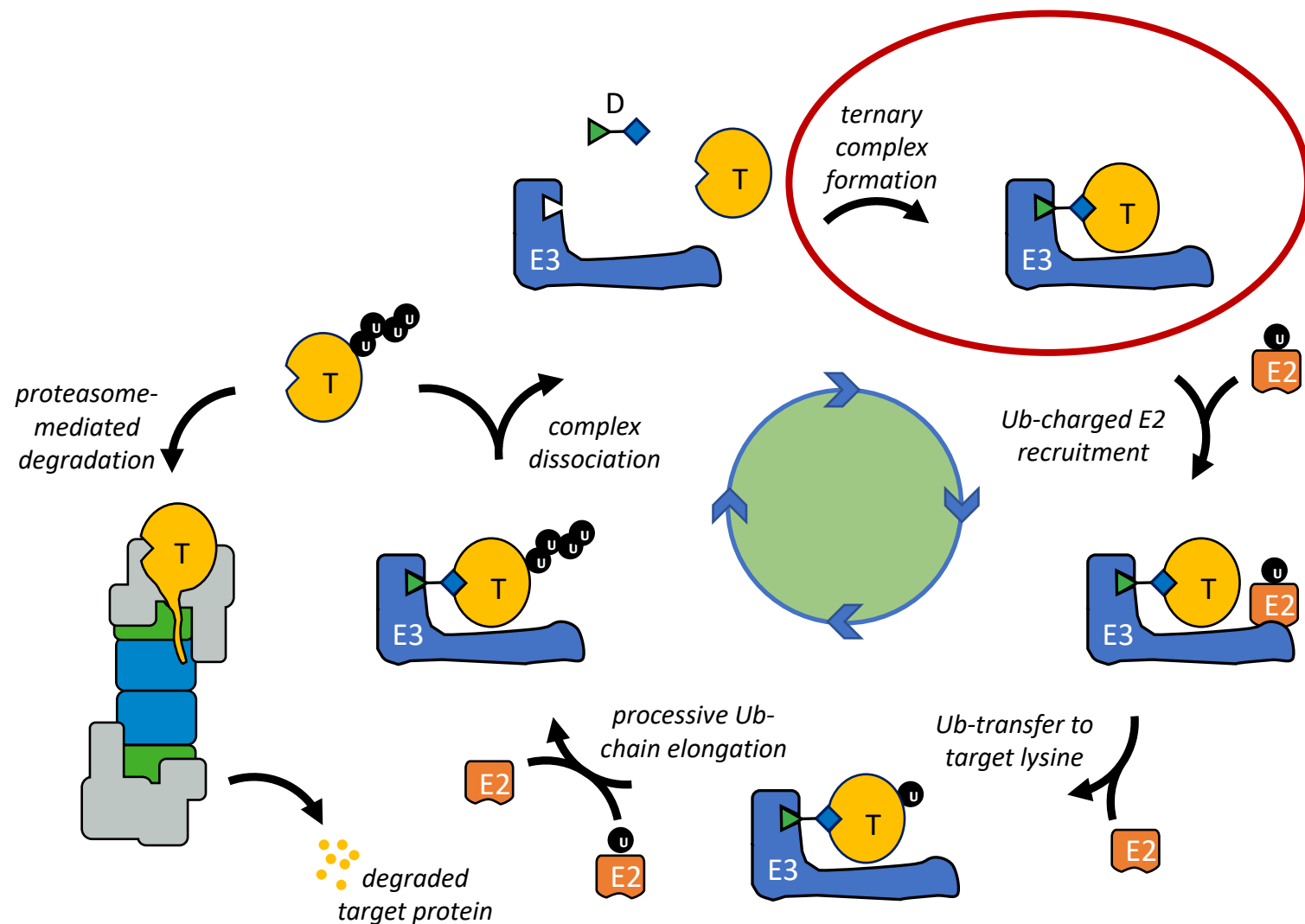


Advantages of degradation

- Substoichiometric catalytic nature
- Can target the “not-yet-drugged”
- Selectivity opportunities

Fisher and Phillips, *Curr Opin Chem Biol.* 2018, 44: 47-55

The Catalytic Cycle of a Degrador – Importance of the Ternary Complex



Understanding ternary complexes is essential for optimization of degraders

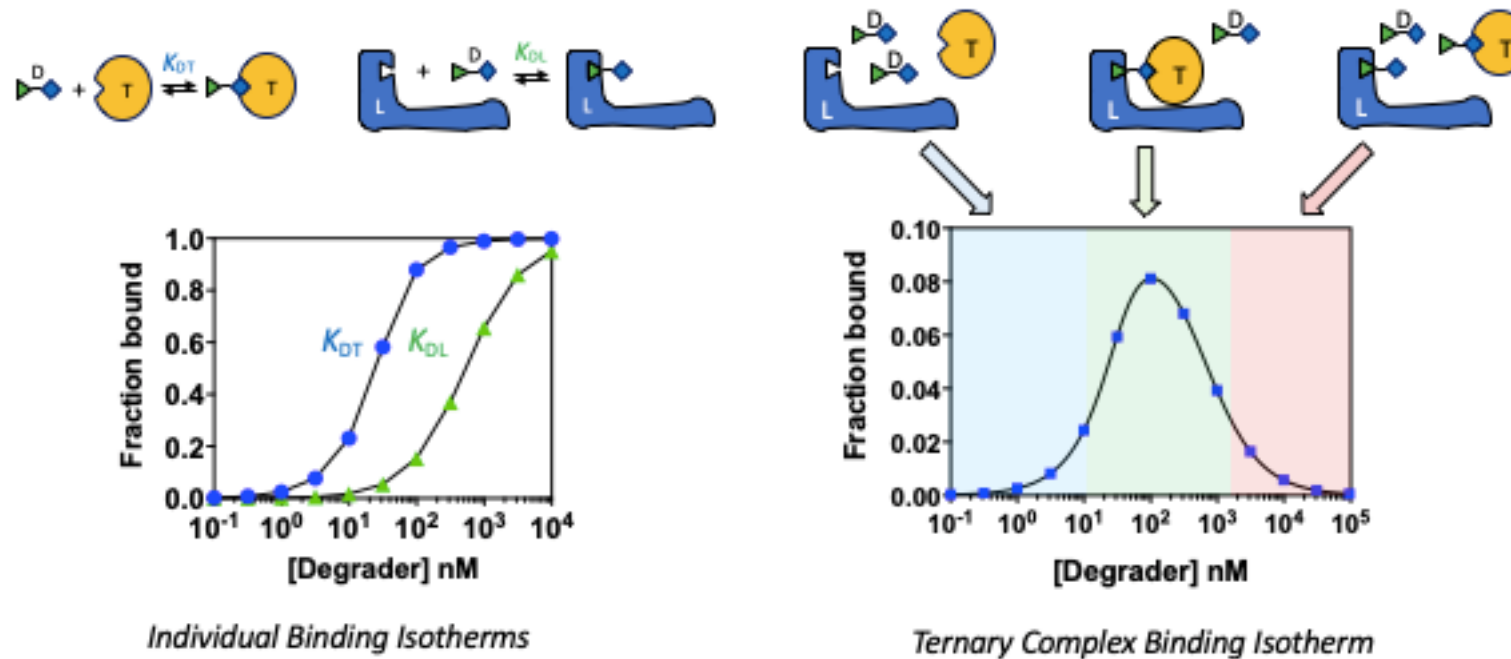
Guides strategy

- Rational degrader design
- Selectivity
- Catalysis
- Linkerology

Fisher and Phillips, *Curr Opin Chem Biol.* 2018, 44: 47-55

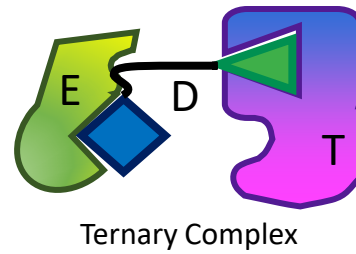
The Ternary Complex Framework

Ternary complex formation is governed by the thermodynamic features of ligand binding



Measured and computed data can be used to guide degrader optimization

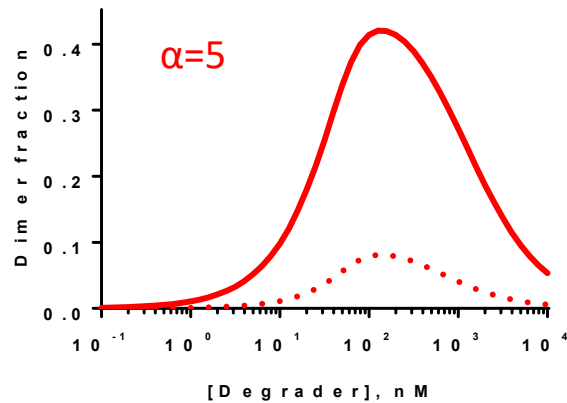
Cooperativity & Protein Dimerization



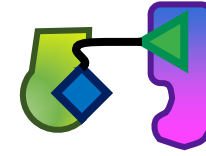
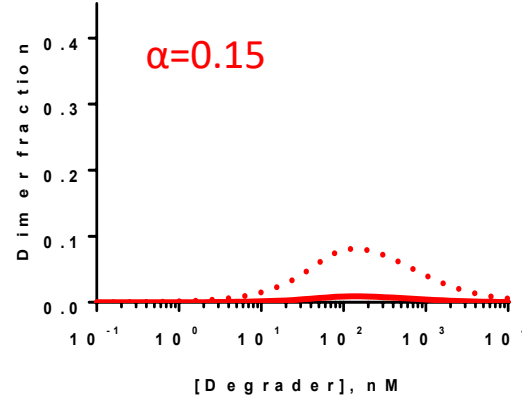
Most proteins are not optimized to interact with each other. How does this influence dimerization?



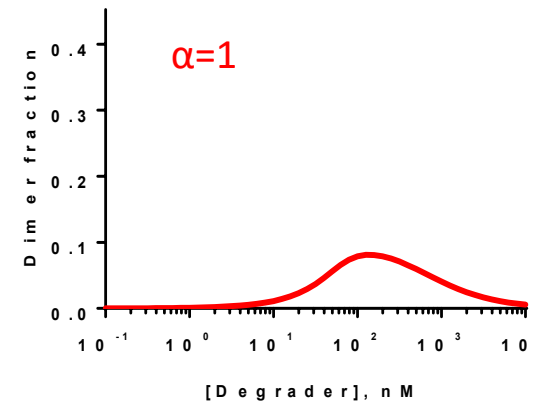
POSTIVE COOPERATIVITY
(synergistic)



NEGATIVE COOPERATIVITY
(antagonistic)



NON-COOPERATIVE
(neutral)



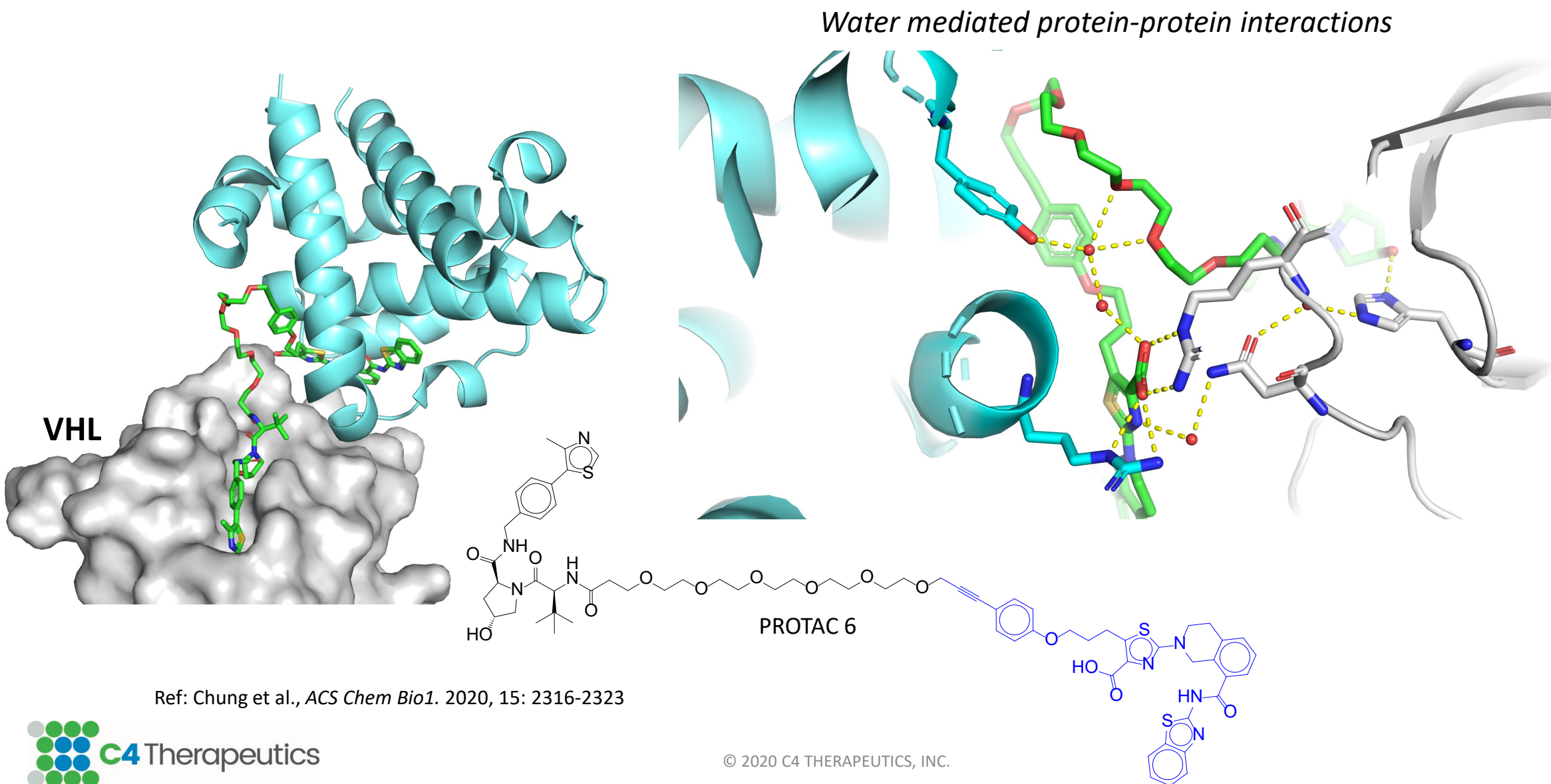
— Experimental
- - - Predicted/computed

$$\alpha = K_{\text{exp}} / K_{\text{pred}}$$

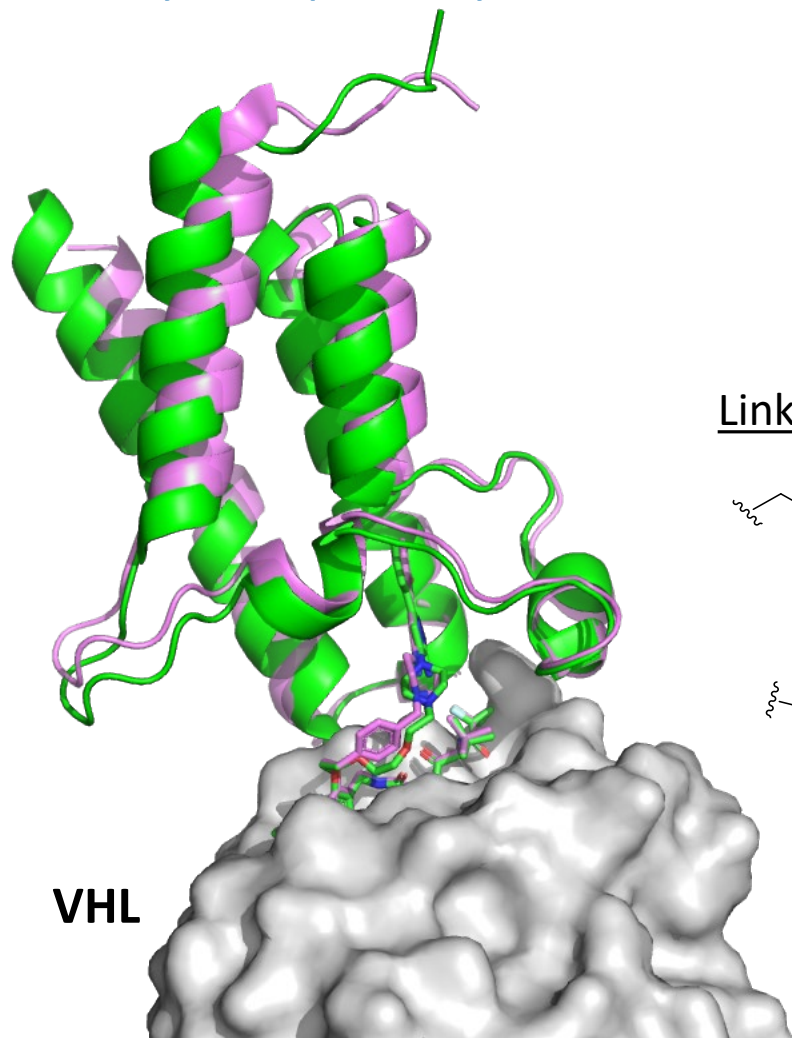
Positive cooperativity ($\alpha > 1$) enhances dimerization, negative cooperativity ($\alpha < 1$) decreases dimerization

Examples from the Literature: Structural Investigation of Ternary Complexes

Ternary Complex Crystals with VHL-PROTAC 6-Bcl-xL shows Conformational Collapse



Ternary Complex Crystals with VHL-PROTAC-SMARCA2 show Similar Geometry with two PROTACS



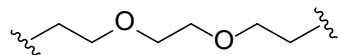
Green – SM2 + PROTAC1 (6hay)

Purple – SM2 + PROTAC2 (6hax)

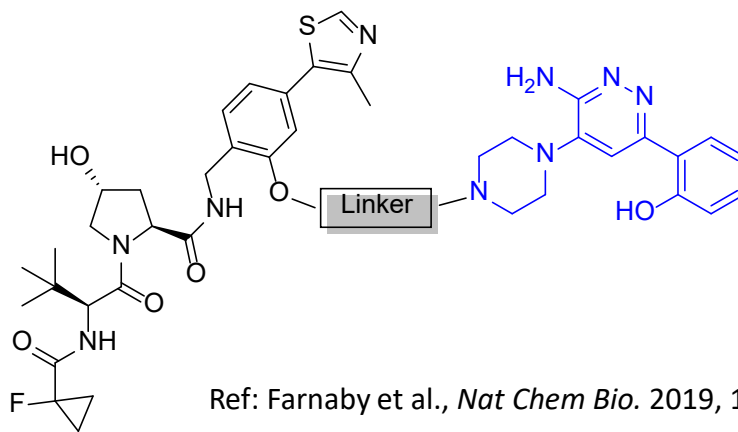
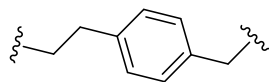


Linker

PROTAC1

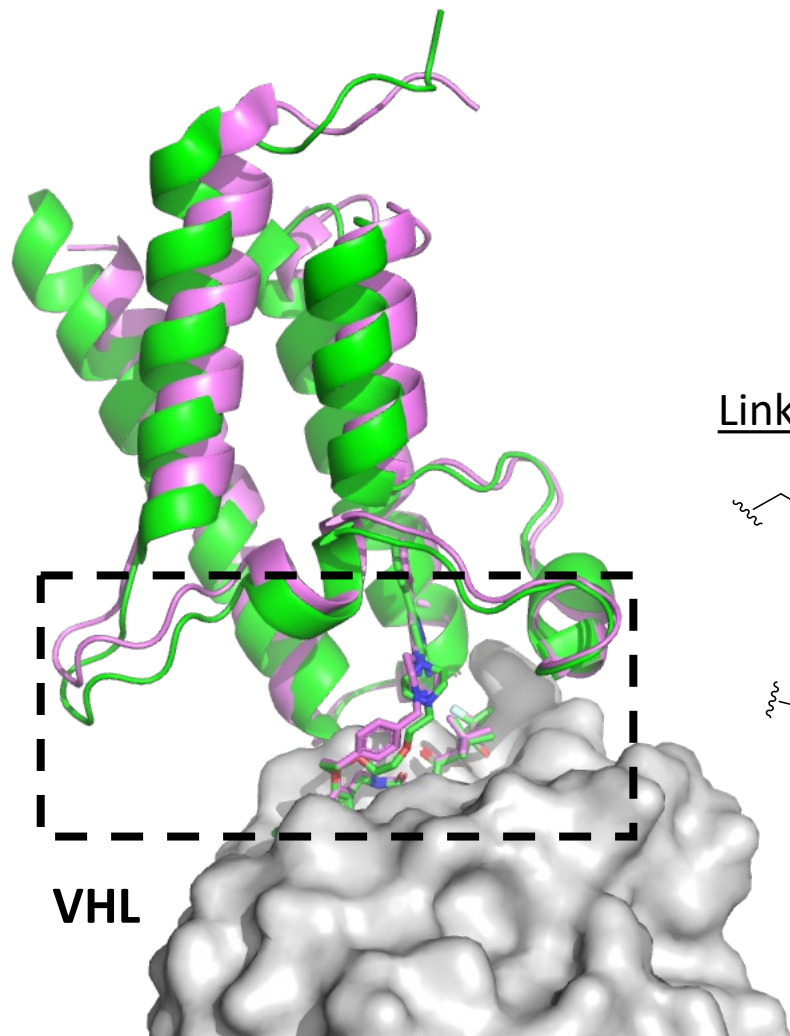


PROTAC2



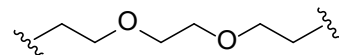
Ref: Farnaby et al., *Nat Chem Bio.* 2019, 15: 672-680

SBDD Focused on the Linker helps Improve Degradator while maintaining ternary complex

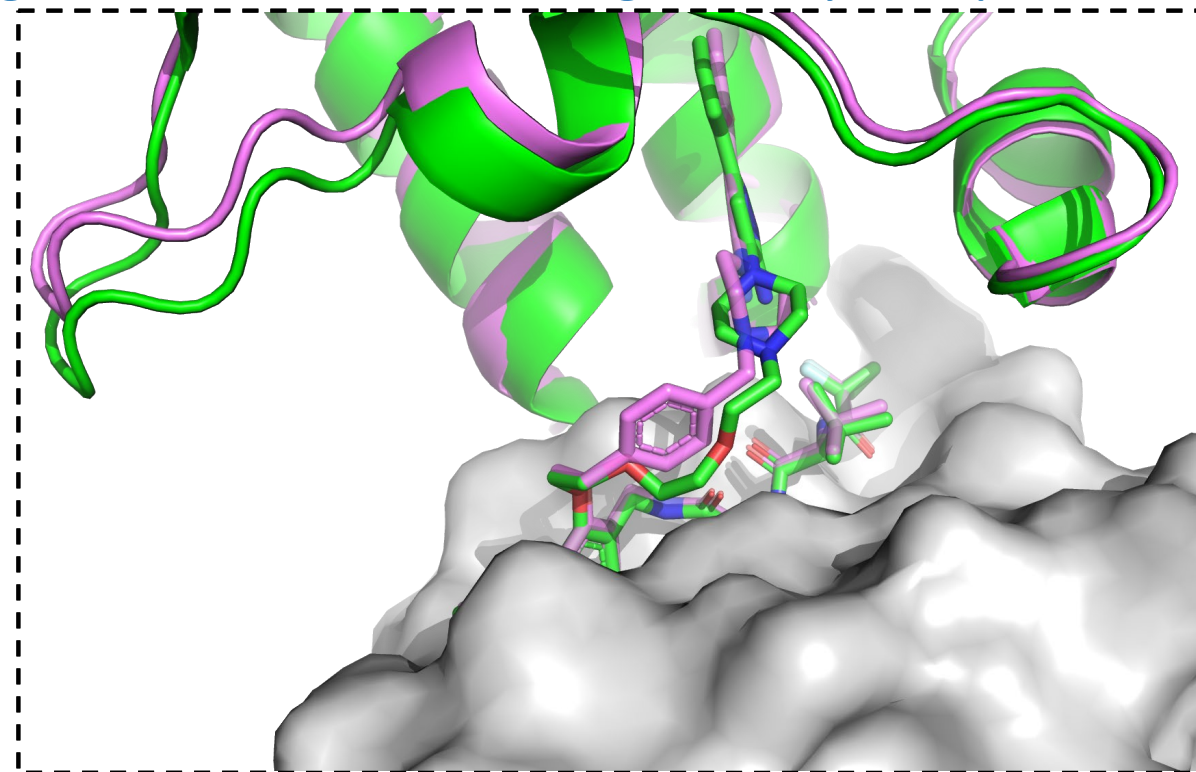
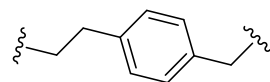


Linker

PROTAC 1

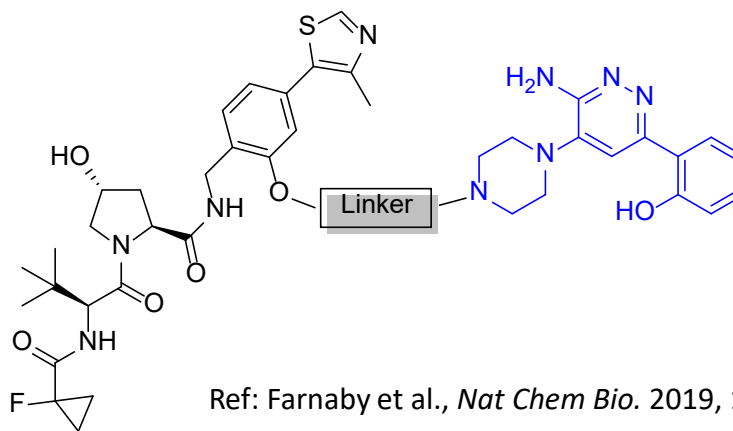


PROTAC 2



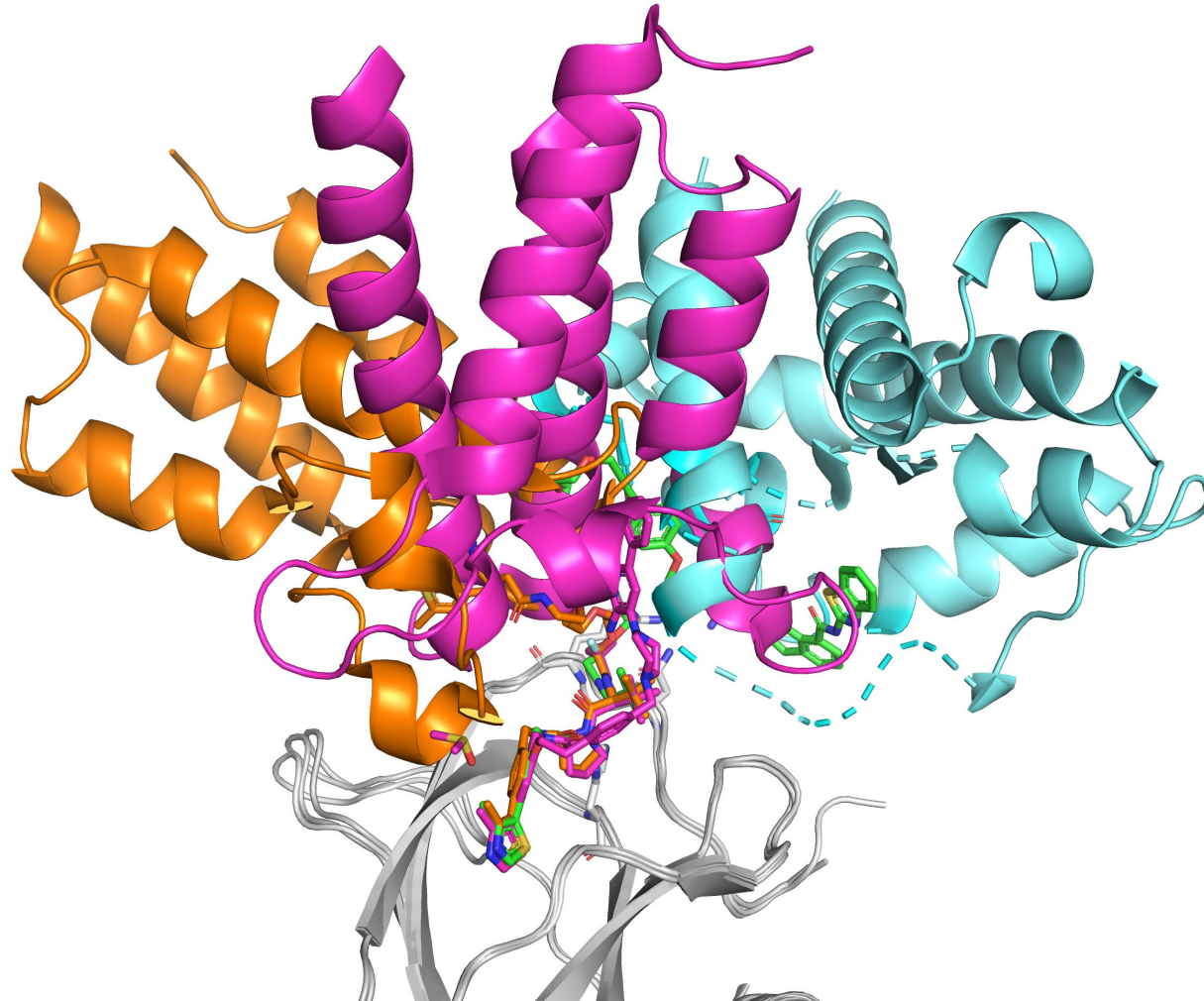
Green – SM2 + PROTAC1 (6hay)

Purple – SM2 + PROTAC2 (6hax)



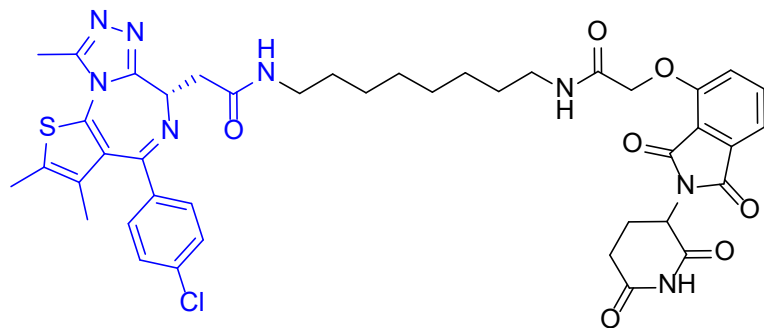
Ref: Farnaby et al., *Nat Chem Bio.* 2019, 15: 672-680

VHL Demonstrates Versatility of Ligases to Form Noncognate Complexes

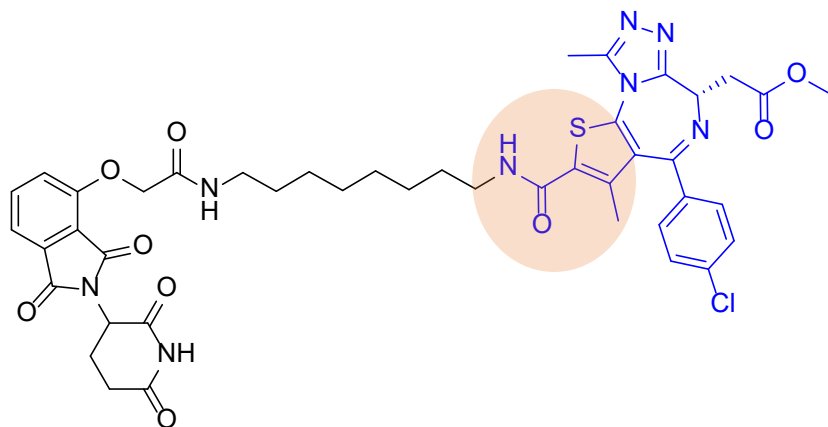


Bcl-xL (6zhc) BRD4_BD2 (5t35) SMARCA2 (6hr2)

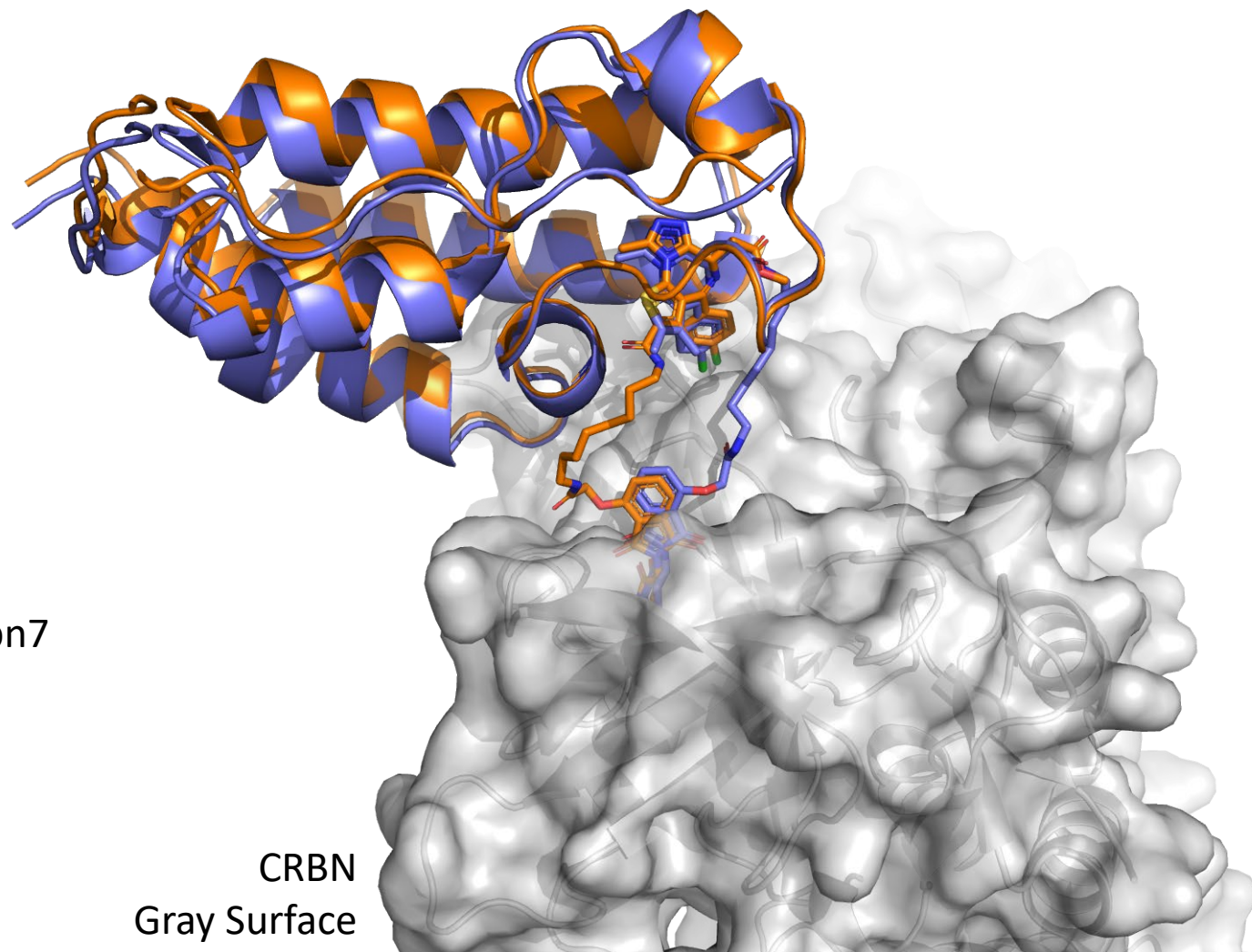
Ternary Complex Crystal Structures with CRBN-degrader-BRD4(BD1)



dBET6
Blue - 6boy



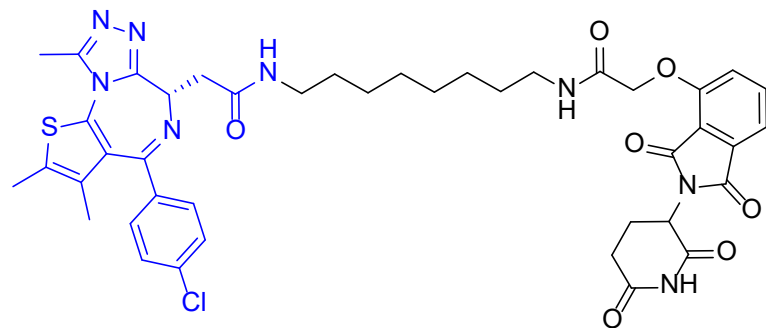
dBET23
Orange - 6bn7



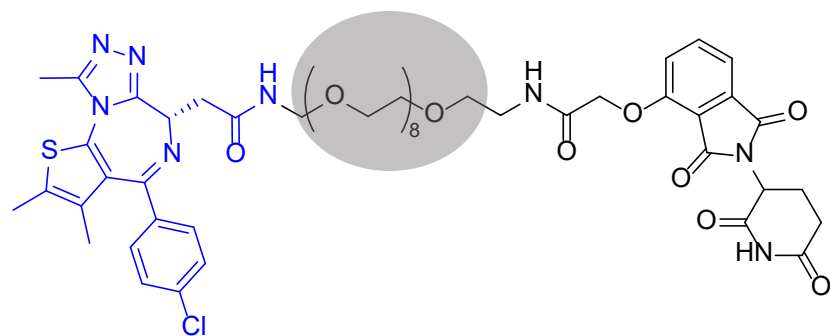
CRBN
Gray Surface

Ref: Nowak et al., *Nat Chem Bio.* 2018, 14:706-714

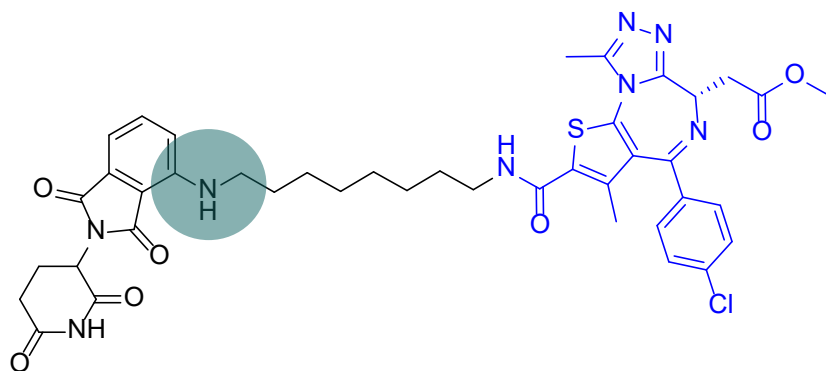
CRBN-degrader-BRD4(BD1) Crystals Maintain Ternary Complex Despite Changes to Degradator Chemistry



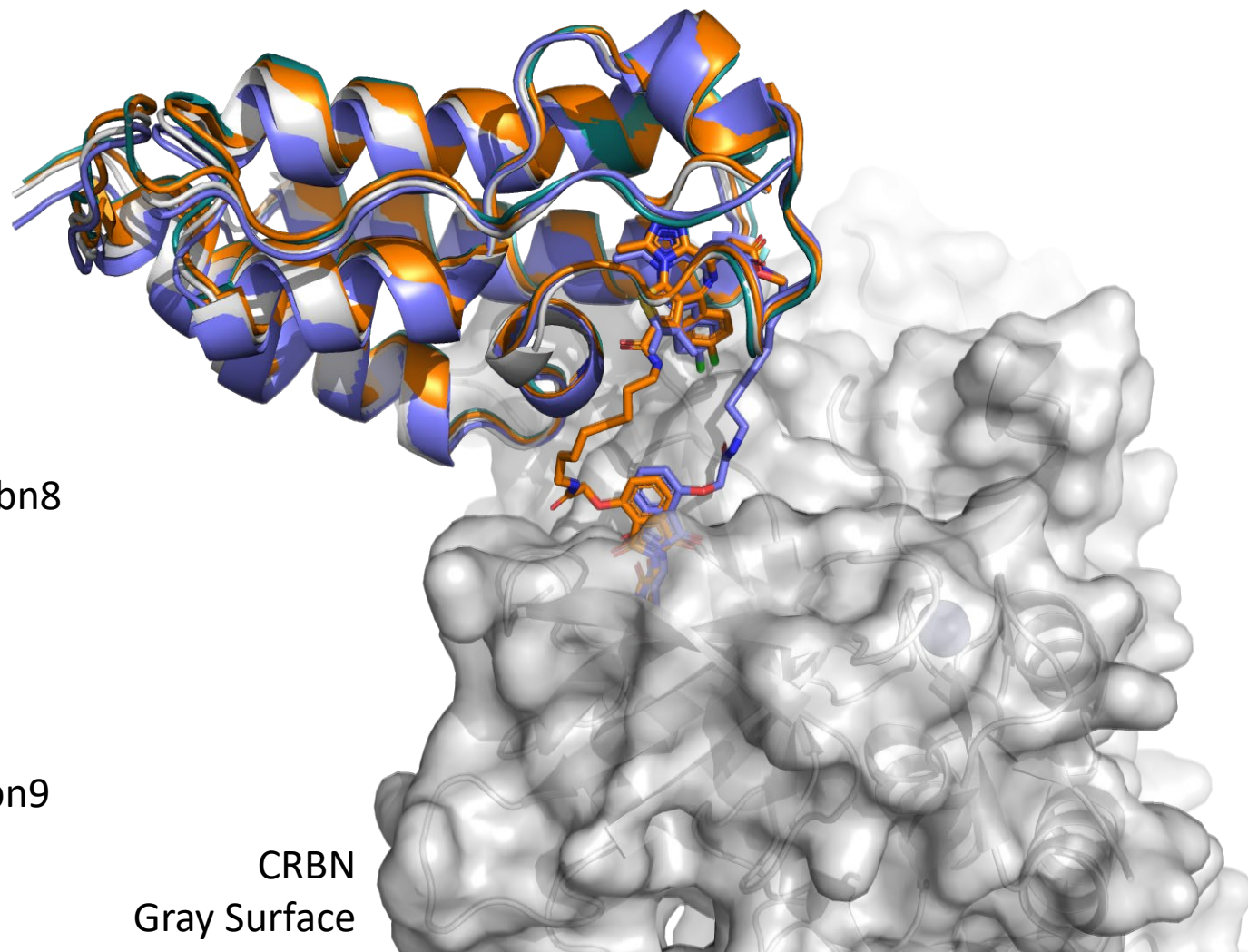
dBET6
Blue - 6boy



dBET55
Gray - 6bn8



dBET70
Teal - 6bn9



CRBN
Gray Surface

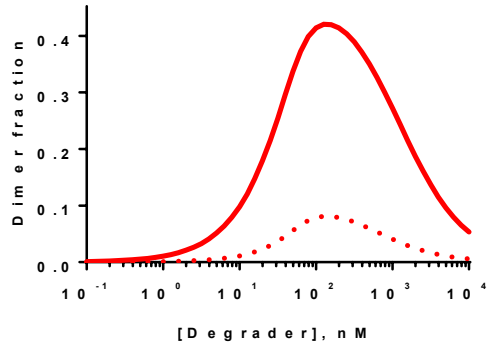
Ref: Nowak et al., *Nat Chem Bio.* 2018, 14:706-714

How Does C4T Interrogate Degradar-induced Ternary Complexes?

In addition to crystallography...

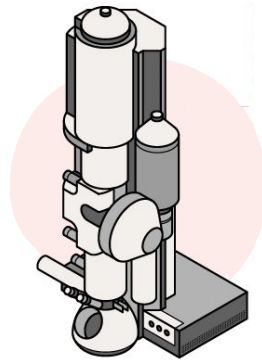
Biochemistry

- AlphaLISA

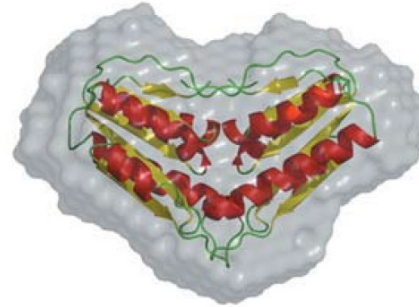


Structural Biology

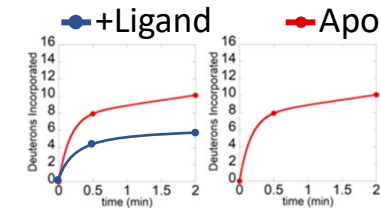
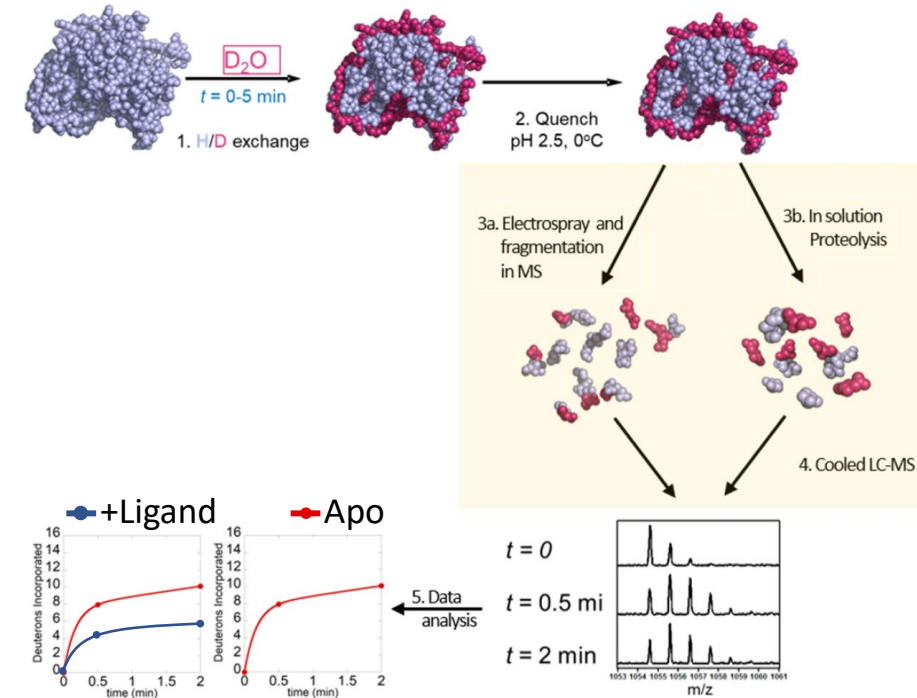
- CryoEM



- SAXS



- HDX-MS

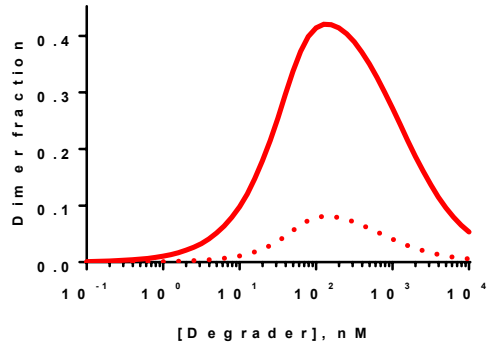


How Does C4T Interrogate Degradar-induced Ternary Complexes?

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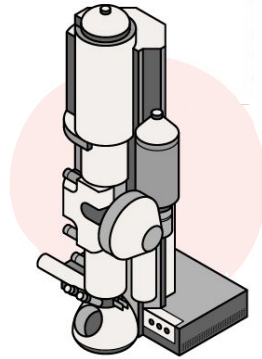
Biochemistry

- AlphaLISA

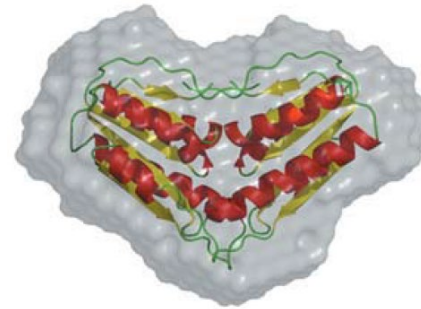


Structural Biology

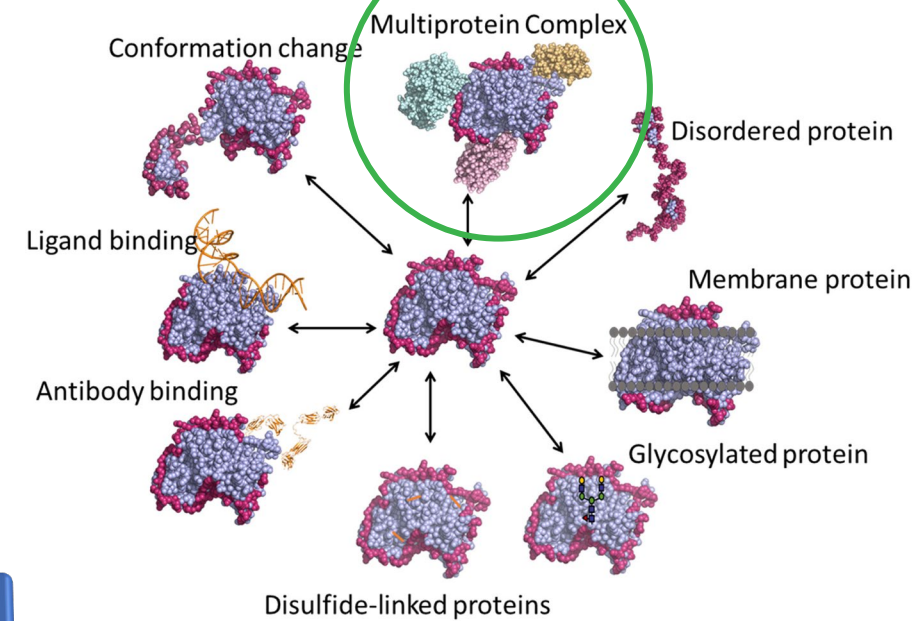
- CryoEM



- SAXS



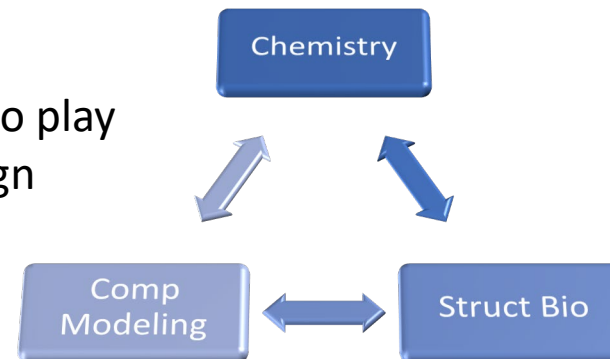
- HDX-MS (Applications)



Protein-protein interfaces
Dimerization interfaces
Ab/antigen

Heavy focus on chemistry

- Then structural biology comes into play
- Iterative rounds of degrader design



Grant et al., *Biopolymers*. 2011, 95: 517-530

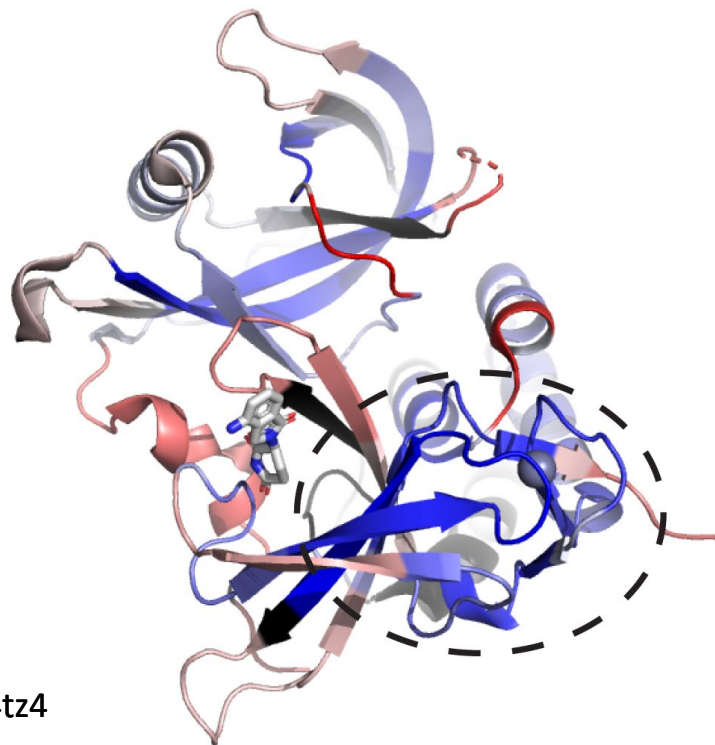
UnISR.it/en/news/2020/6

Narang, Lento, Wilson., *Biomedicines*. 2020, 8: 224-244

Utilizing HDX-MS to Interrogate Degradar-induced Protein-protein Interfaces

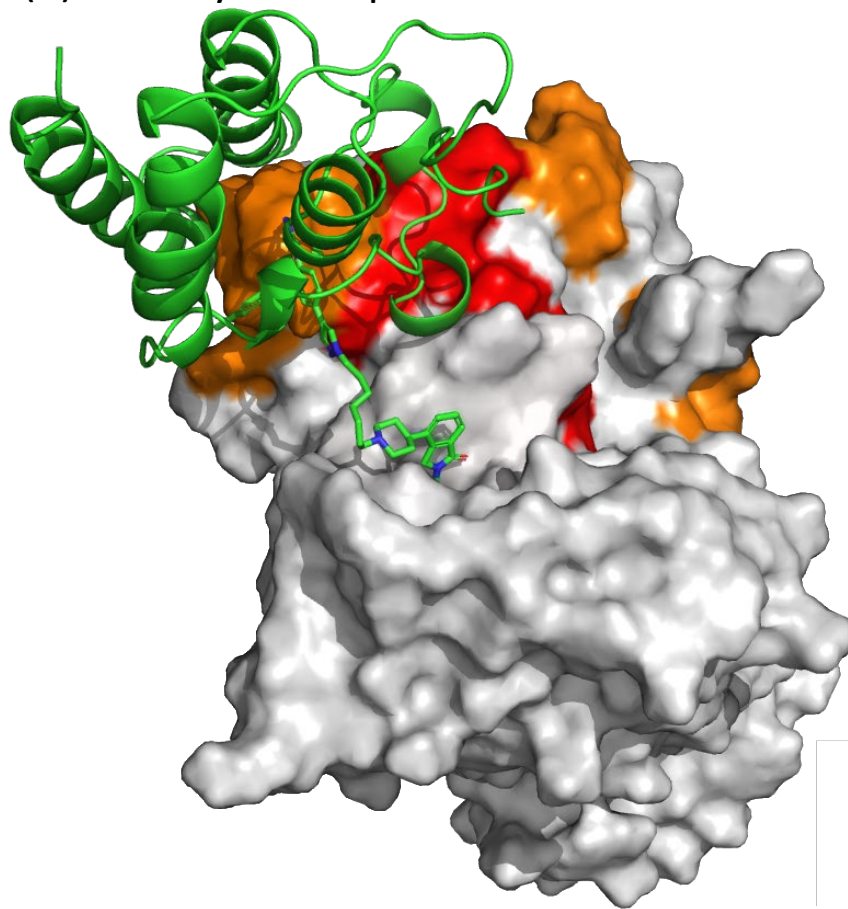
HDX Provides an Enormous Amount of Information

(1) DMSO – Conformational Dynamics

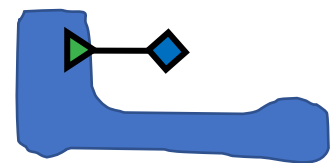


(2) Binary – Compound Shielding

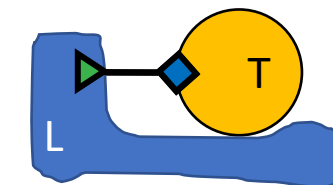
(3) Ternary – Complex Interface



Binary

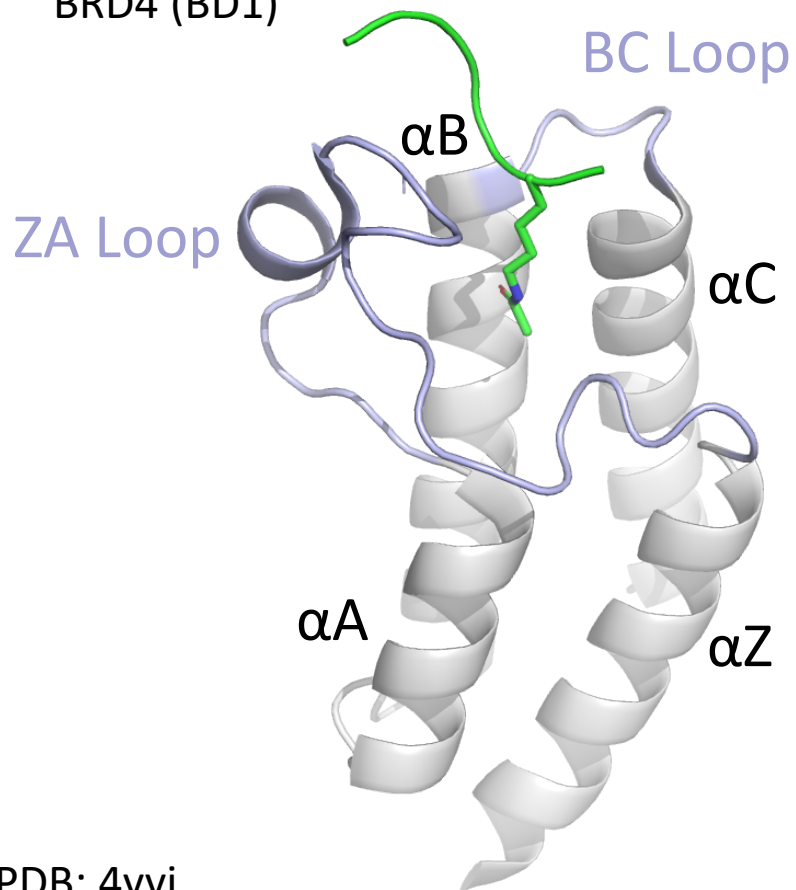


Ternary



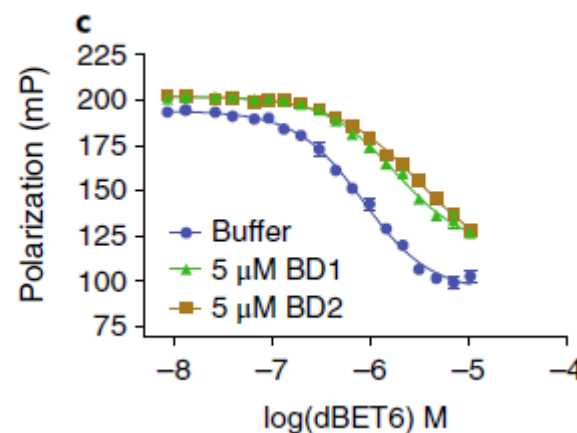
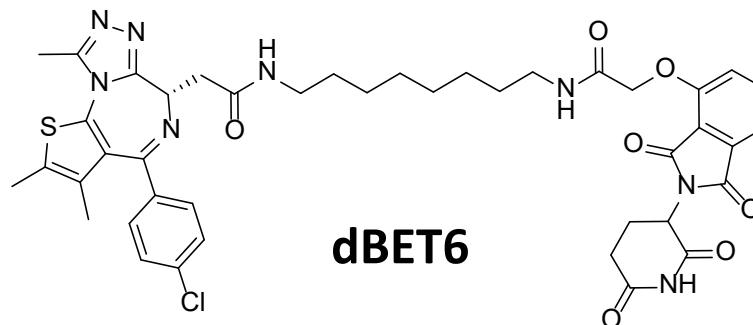
CFT1297 HDX Story – A Tale of Two (similar) Degradors

BRD4 (BD1)



PDB: 4yyi

Green – Acetylated Lys Substrate



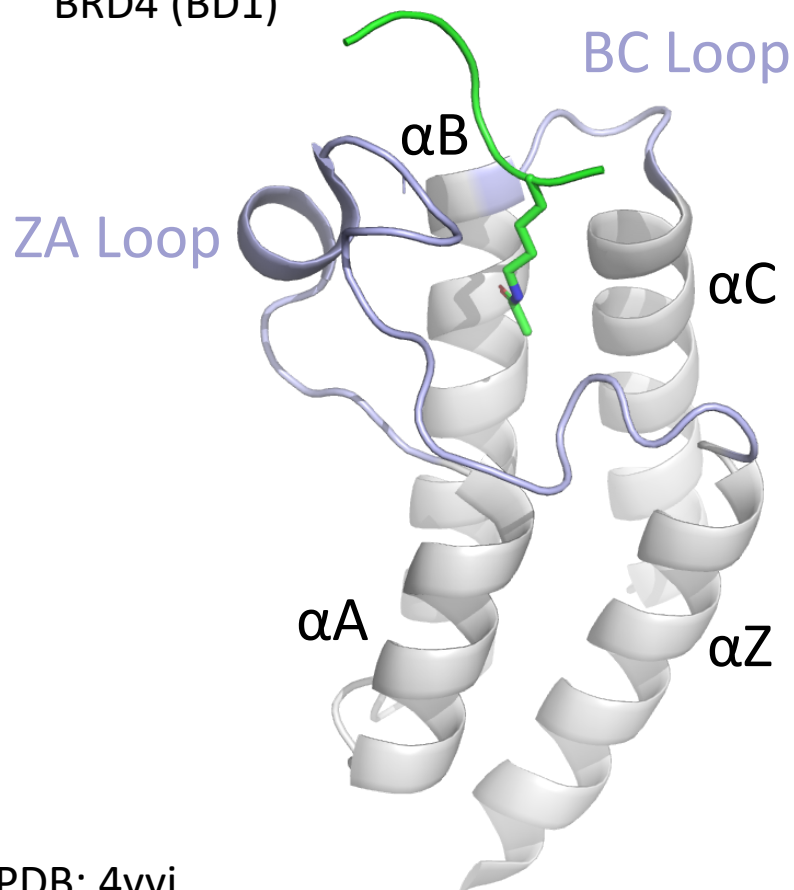
Ref: Nowak et al., *Nat Chem Bio* (2018)

dBET6 is well characterized

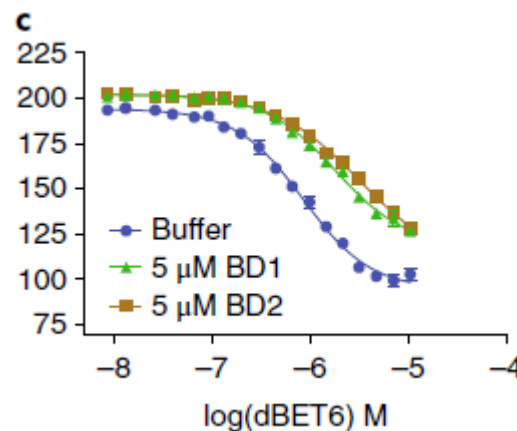
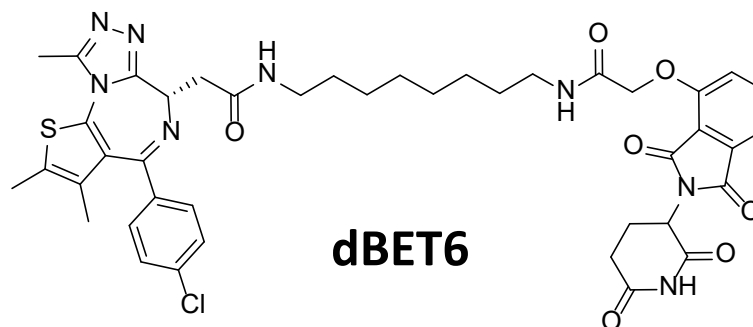
- Potent BRD4 degrader
- Crystal of ternary complex
- Biochemical data
- Exhibits negative cooperativity

CFT1297 HDX Story – A Tale of Two (similar) Degradors

BRD4 (BD1)



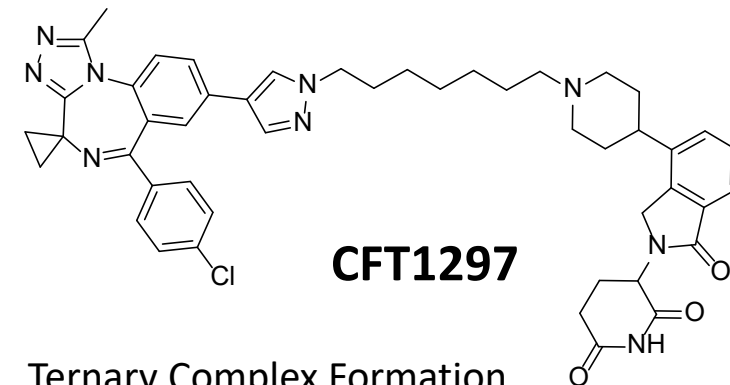
PDB: 4yyi
Green – Acetylated Lys Substrate



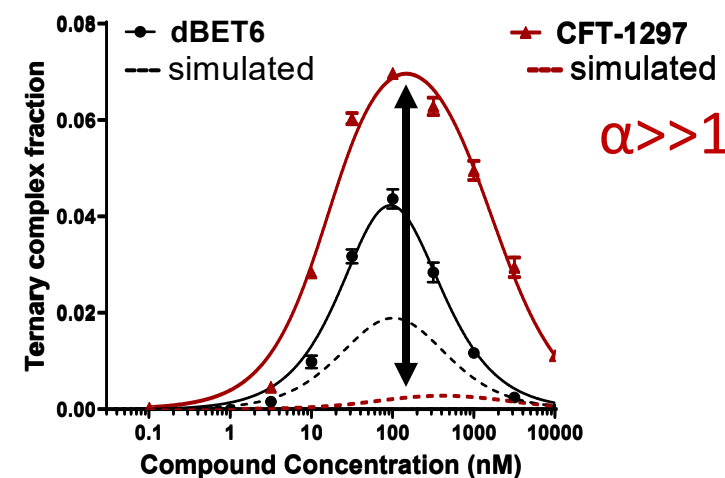
Ref: Nowak et al., *Nat Chem Bio* (2018)

dBET6 is well characterized

- Potent BRD4 degrader
- Crystal of ternary complex
- Biochemical data
- Exhibits negative cooperativity



Ternary Complex Formation

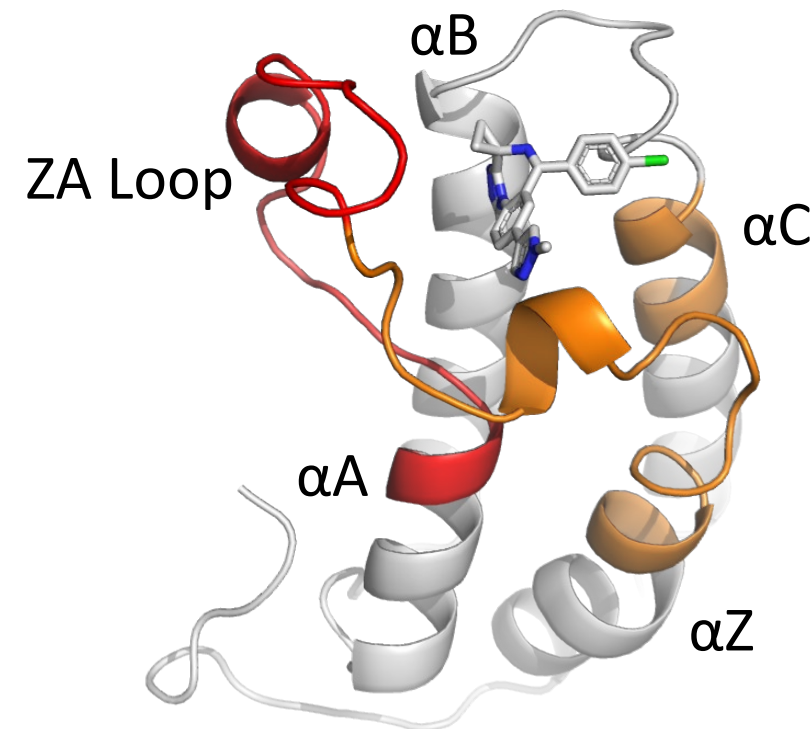
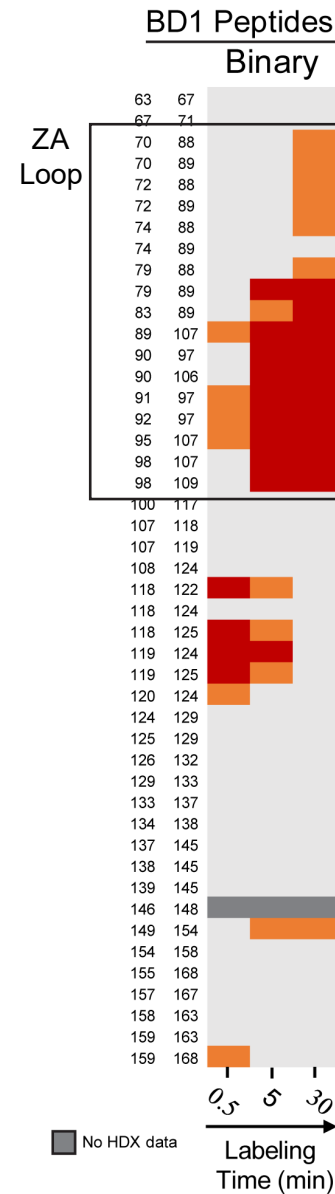
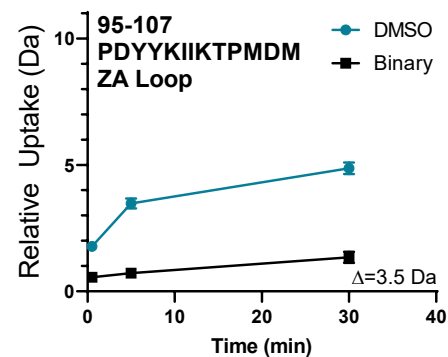
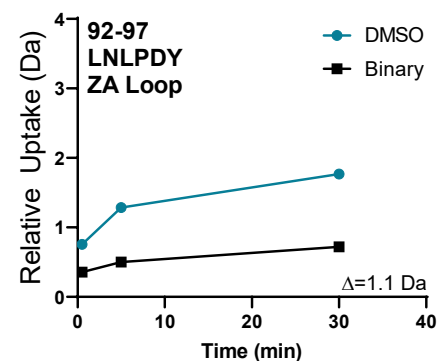
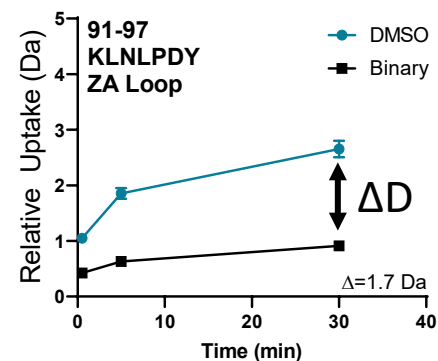
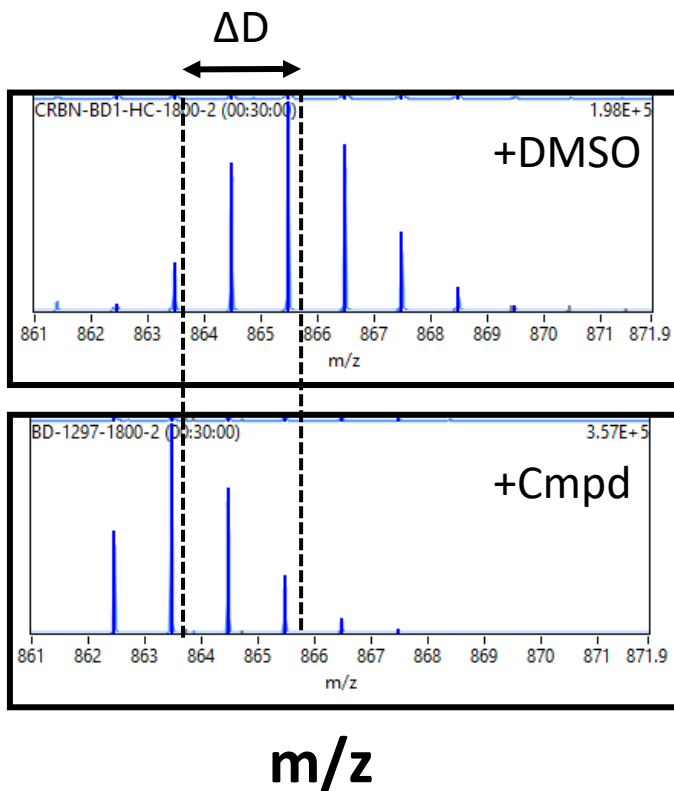
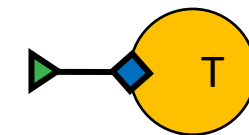


CFT1297 rivals other BRD4 degraders

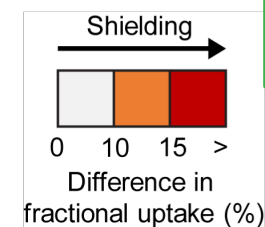
- Potent BRD4 degrader
- Exhibits positive cooperativity

Observed Shielding Effect in the Presence of Degradar CFT1297 (binary)

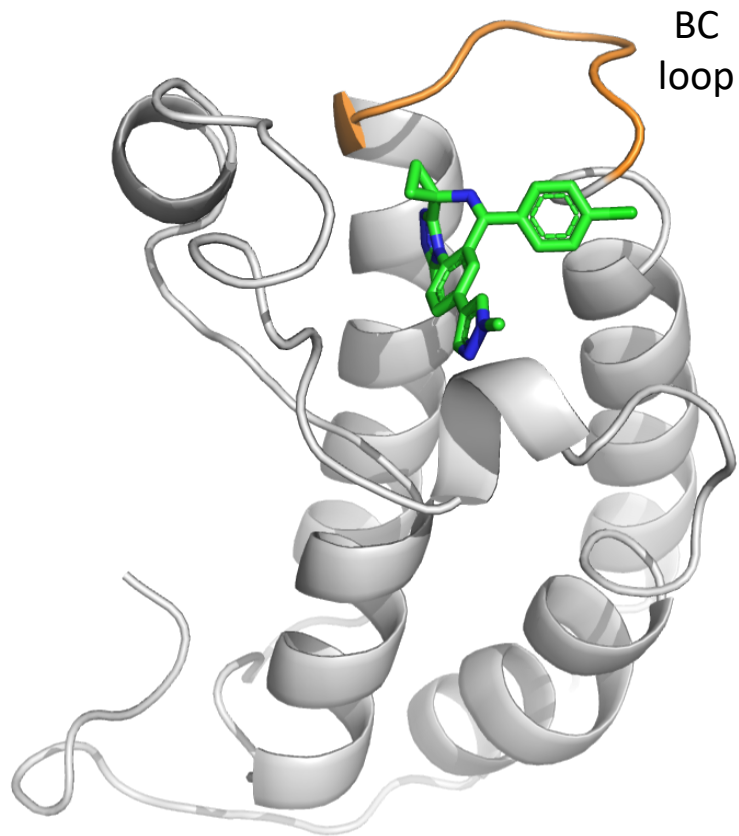
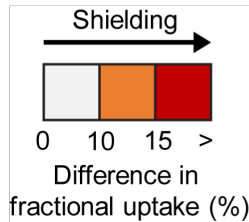
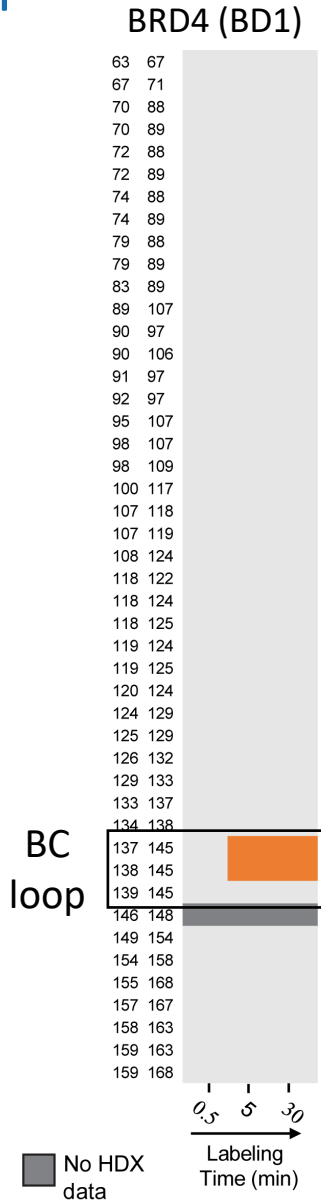
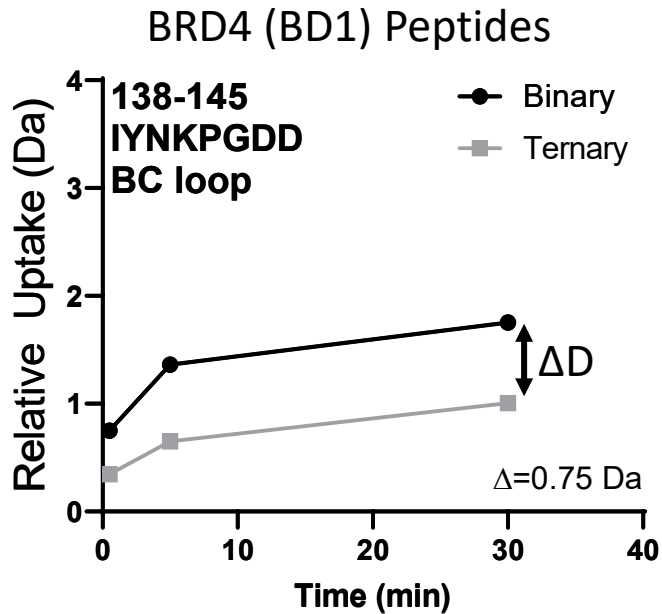
Binary



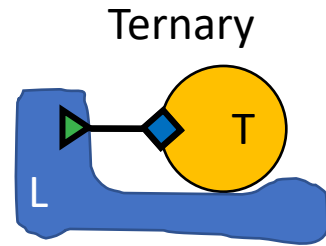
Observed change in deuterium uptake profile +degrader (binary system)



CFT1297 HDX Ternary Complex Reveals Shielding on BRD4 (BD1)

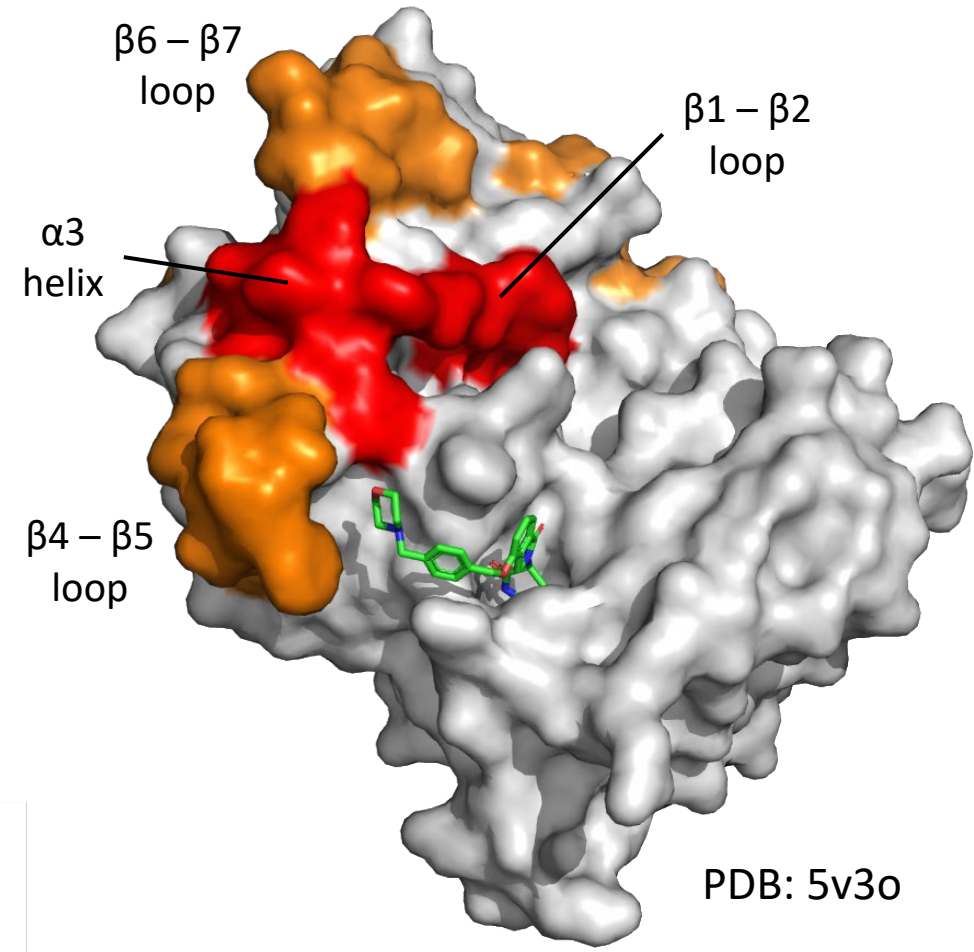
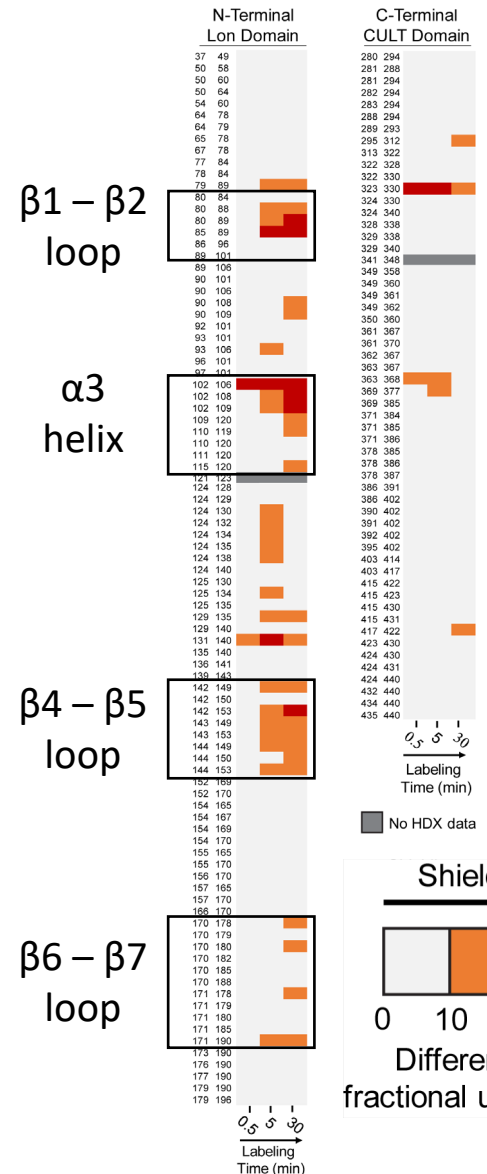
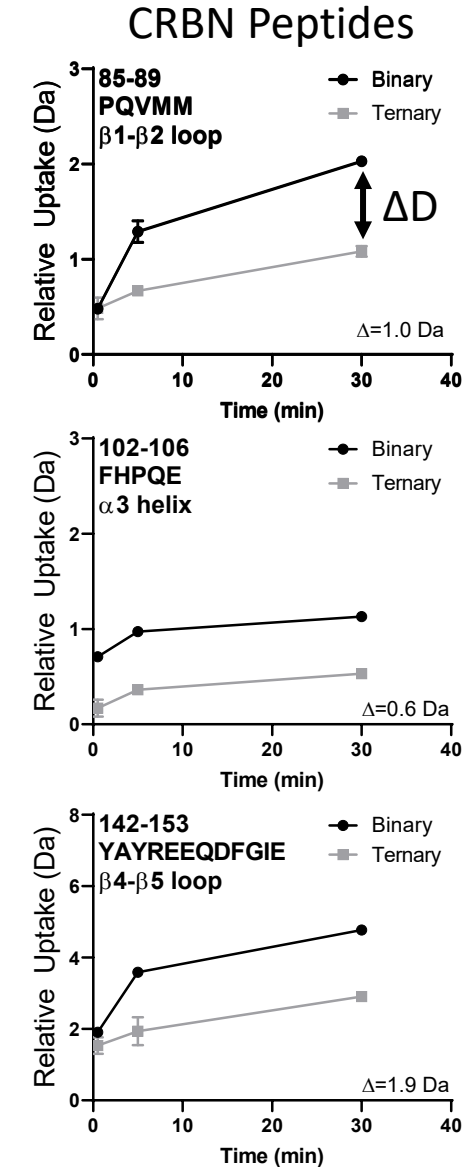
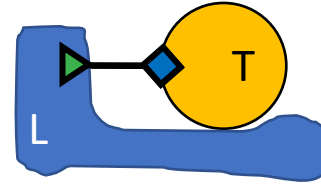


CFT1297 ternary complex experiment shows degrader-induced shielding on the BC loop of BRD4 (BD1)



CFT1297 HDX Ternary Complex Reveals Protein-protein Interface on CRBN

Ternary



PDB: 5v3o

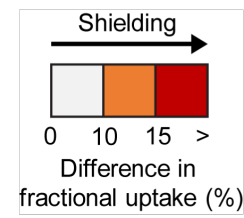
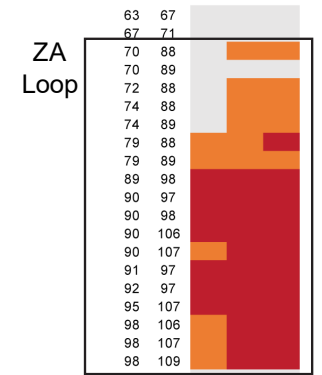
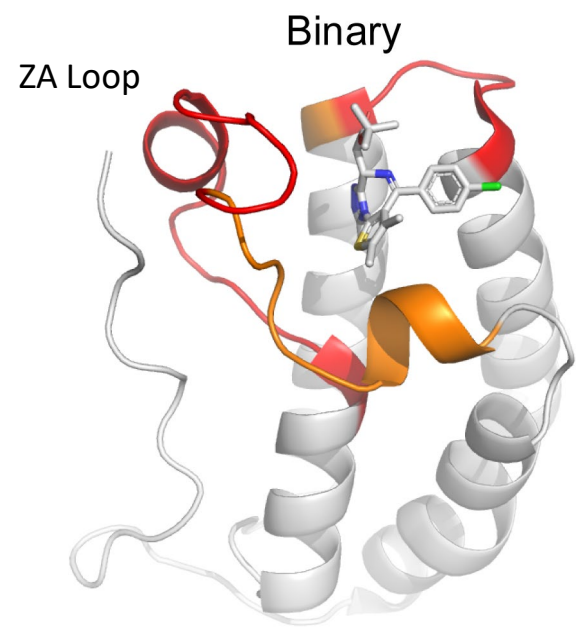
CFT1297 ternary complex experiment shows degrader-induced shielding and identifies the protein-protein interface



dBET6 Shows no Additional Shielding due to the Ternary Complex

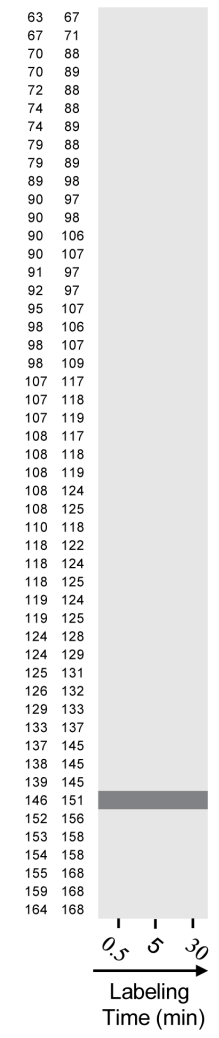


BD1 + dBET6

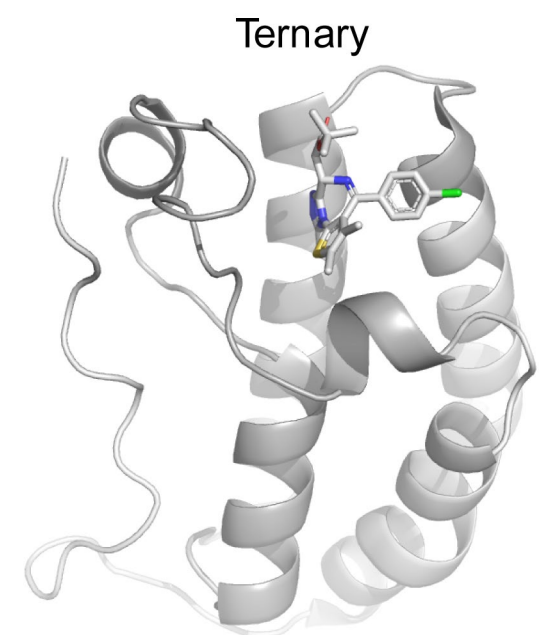


No HDX data

Labeling Time (min)

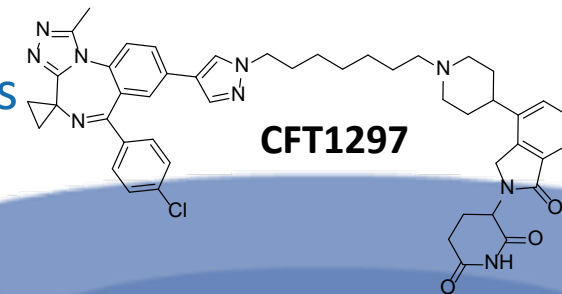


Labeling Time (min)

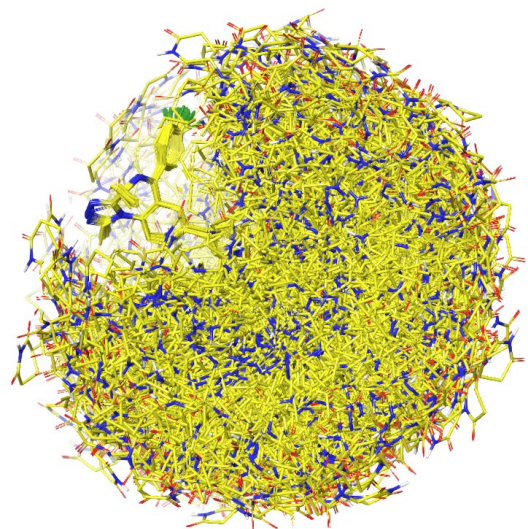


No additional shielding observed due to dBET6 degrader-induced ternary complex
No changes to BD1 or CRBN peptides

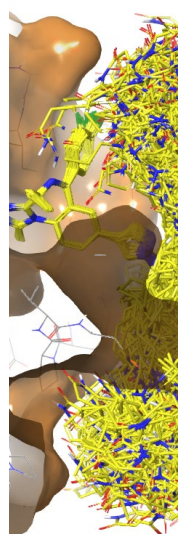
Ternary Complex Modeling for CFT1297 – Funnel to Triage Results



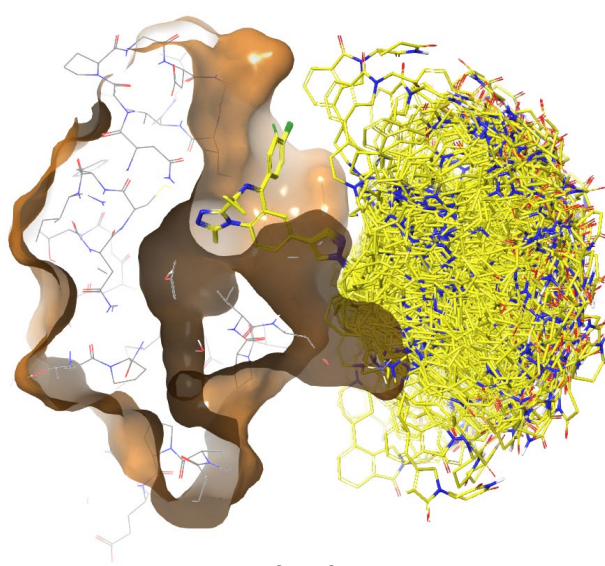
- (1) Conformational search of target degrader
 - Target ligand binding mode constrained
 - 10,000 conformations
- (2) Steric clash check of each conformation against target protein
 - 3,249 conformations
- (3) Superimpose CRBN onto glutarimide moiety
 - Remove molecules with predefined clash count
 - 439 conformations



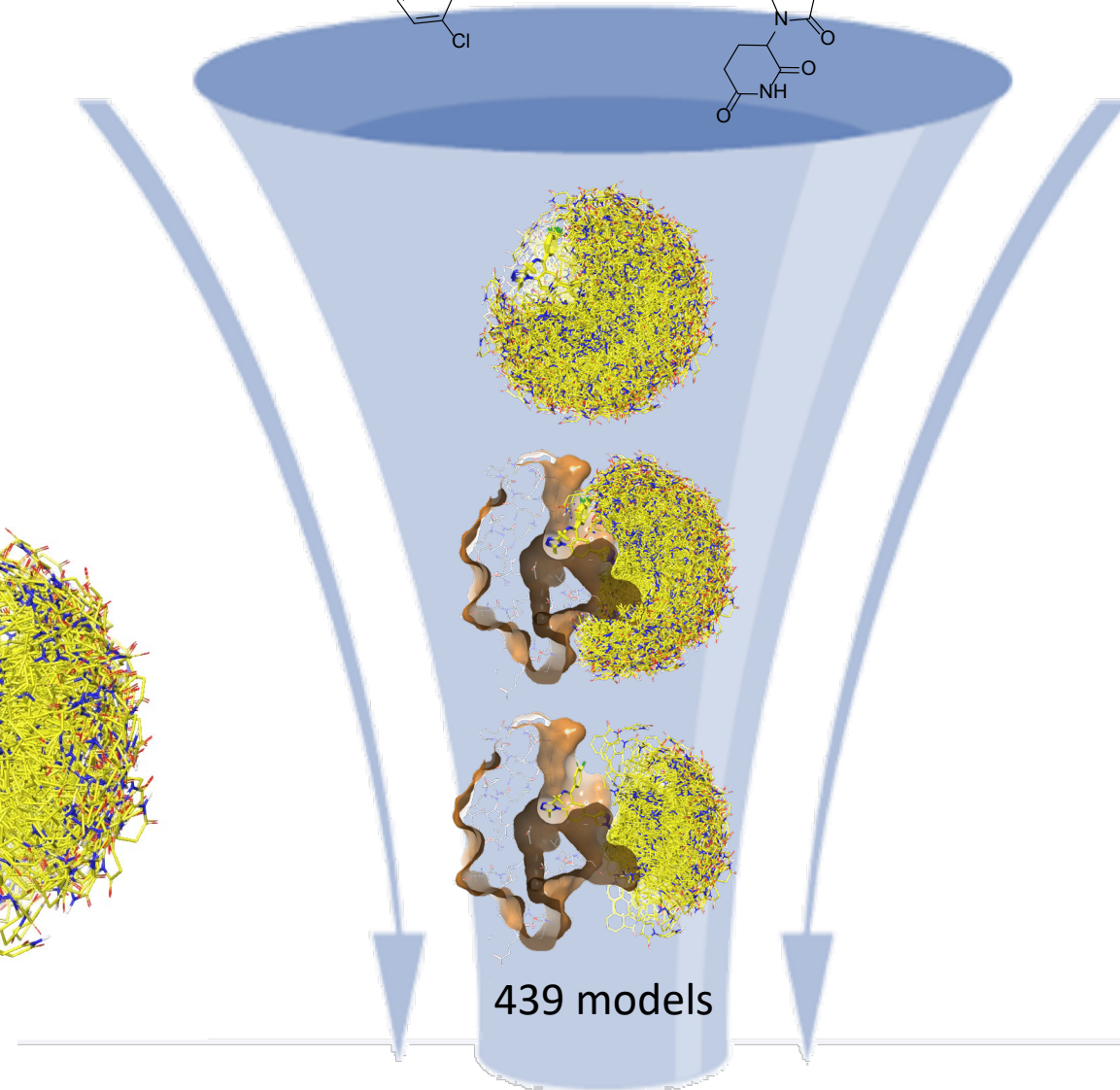
(1)



(2)

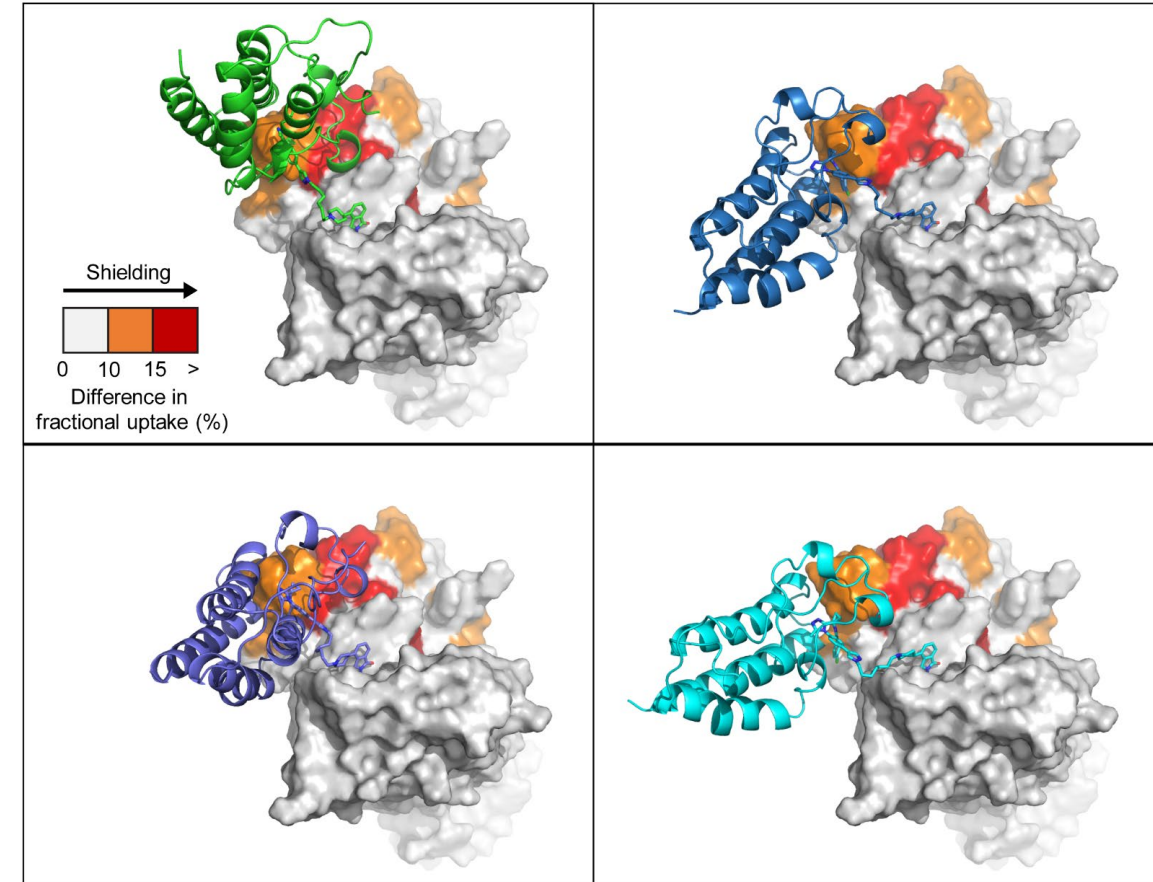
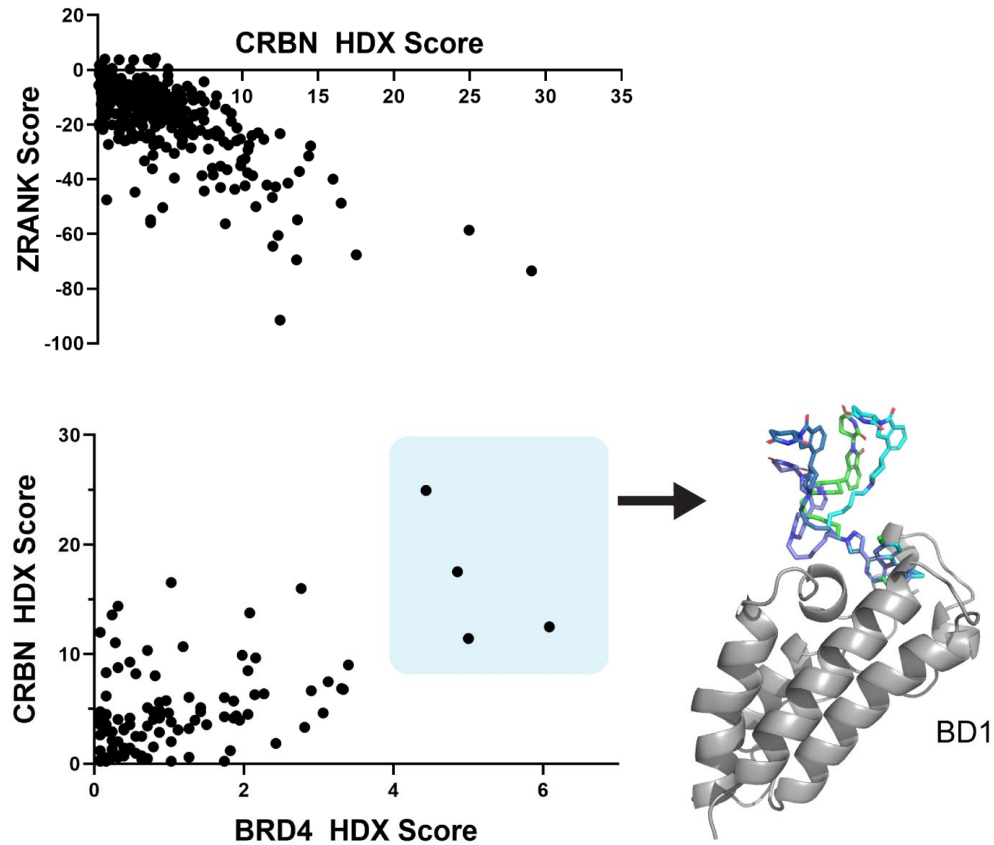


(3)



Ternary Complex Modeling – Degradator Docking & Scoring

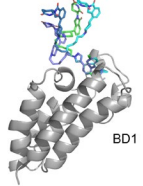
- For each passing ternary complex score interface using P-P docking scoring functions pre / post refinement
 - Rdock / Zdock / Piper



Computational Modeling & HDX Profiles Explain Selectivity

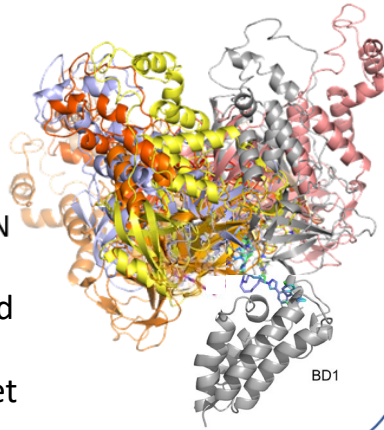
> 10,000 conformations
CFT-000XXXXX

4,000 conformations
Cmpd
+
Target

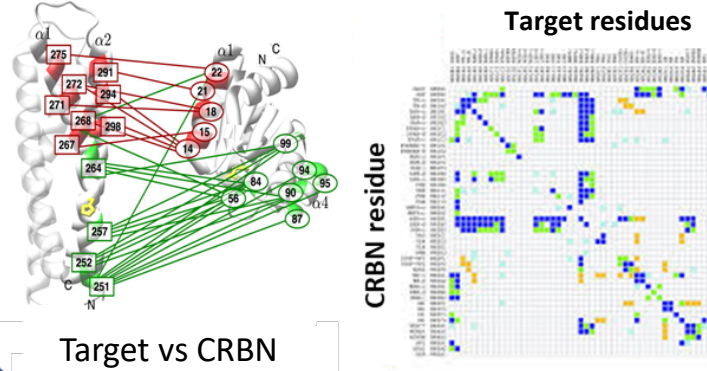


571 conformations

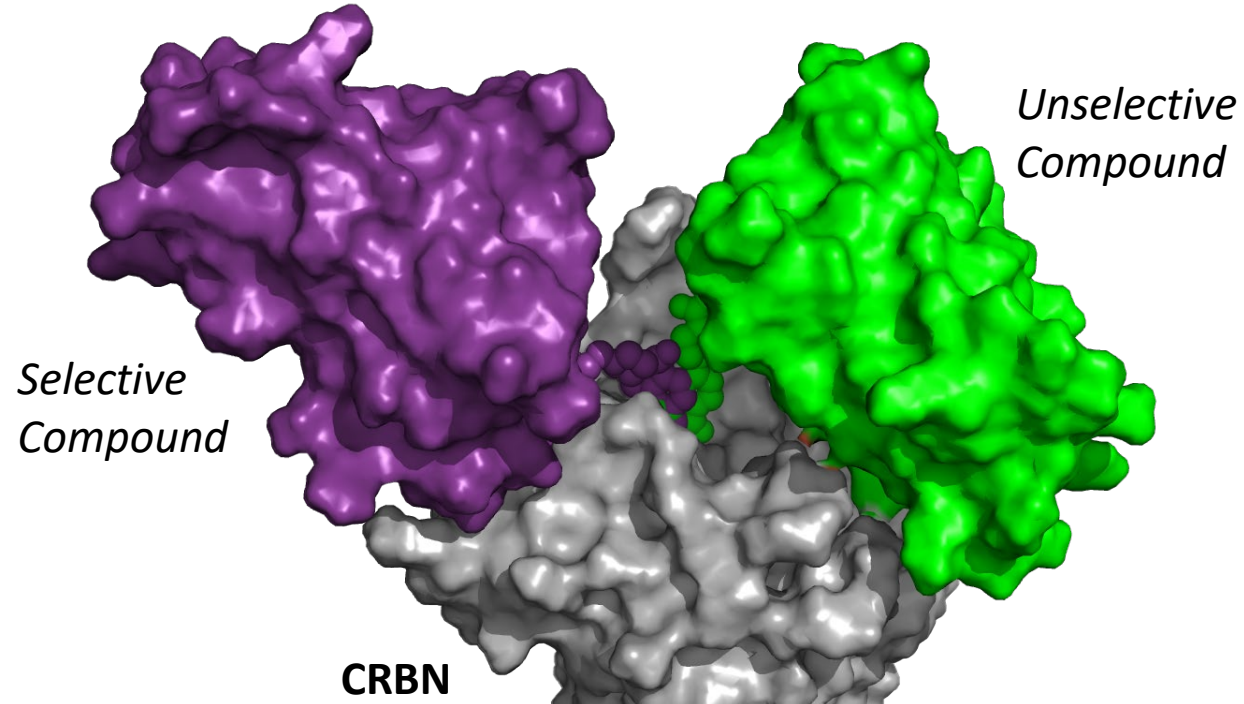
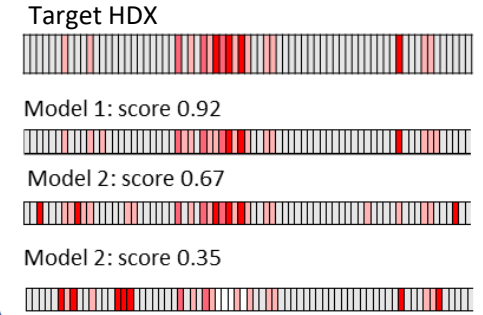
CRBN
+
cmpd
+
Target



Generating protein-protein interface (PPI)
matrices for 571 complexes

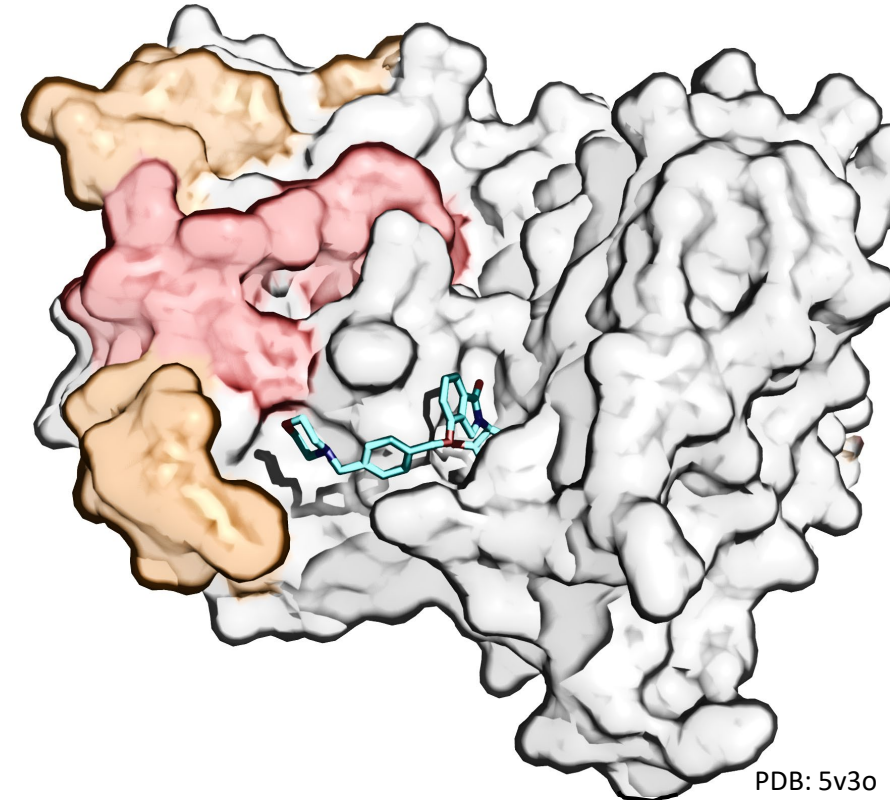


C4T in-house scoring system
Matching PPI matrices vs HDX matrix



Summary

- Degradation is a powerful strategy exhibiting numerous favorable characteristics as a therapeutic modality
- Ternary complex formation is a crucial step during targeted protein degradation
- Understanding ternary complexes is essential for optimization of degraders and critical for guiding future designs
- Can visualize ternary complexes through various techniques
 - Crystallography, HDX-MS, CryoEM, & SAXS
- C4T has successfully used HDX-MS to identify degrader-induced protein-protein interfaces
- Computational modeling plus HDX-MS has powerful synergy
- Applied HDX-MS approach to identify selective vs unselective degraders



The C4 Therapeutics Team

