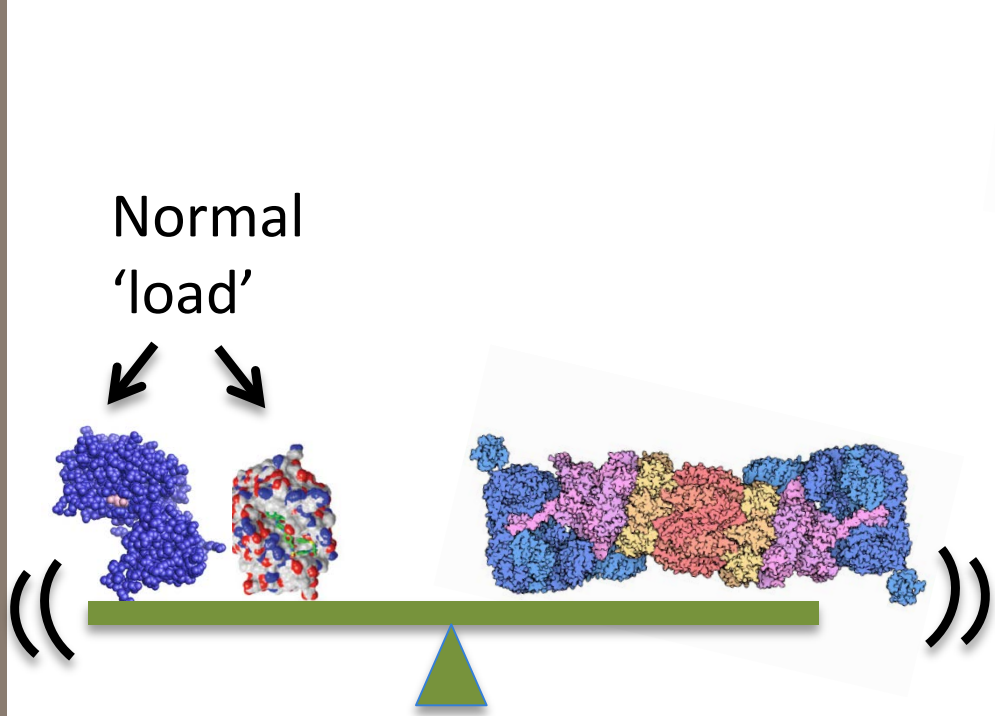
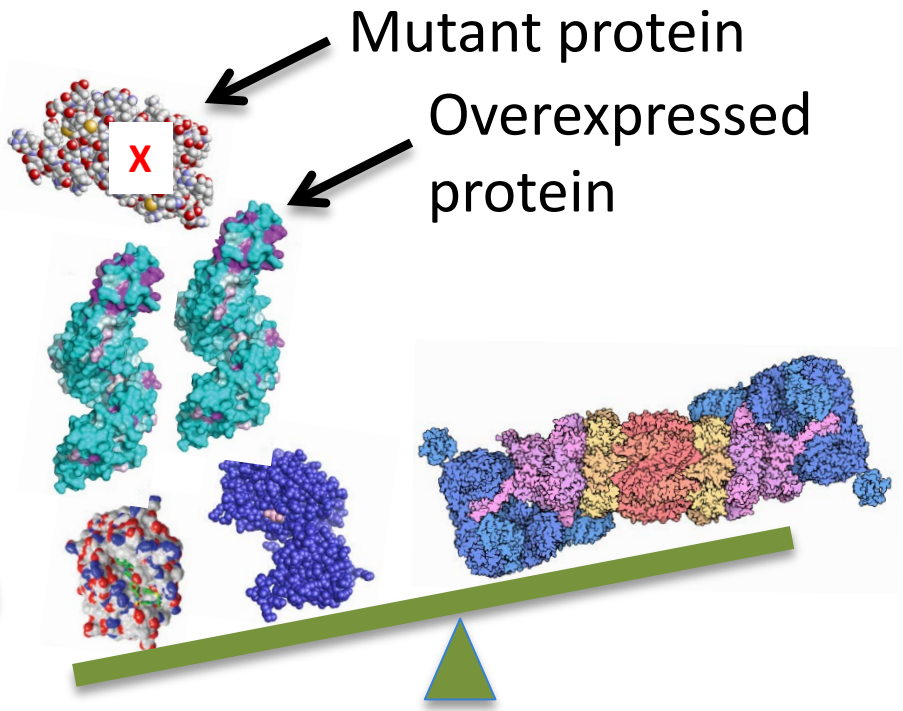
Development of allosteric inhibitors of p97

Applicant PI	Ray Deshaies, Caltech
NCI Project Leader	Barbara Mroczkowski
University of Pittsburgh	Donna Huryn, Peter Wipf
UCSF	Michelle Arkin, Jeff Neitz
UCLA	Tsui-Fen Chou
AMRI	Mark Wolf, Bill Paquette
Xtal Biostructures	Robert Suto
Pharmaron	Liang Qu, Tao Wang
MGH	Cyril Benes
NCI	Sriram Subramaniam, Joe Covey
Leidos	Neal Green, Gordon Stott, Apurva Srivastava
Project Management	John Giraldes

Cancer Cells Have a High Burden of UPS Stress Arising from a Mutation-Riddled Genome

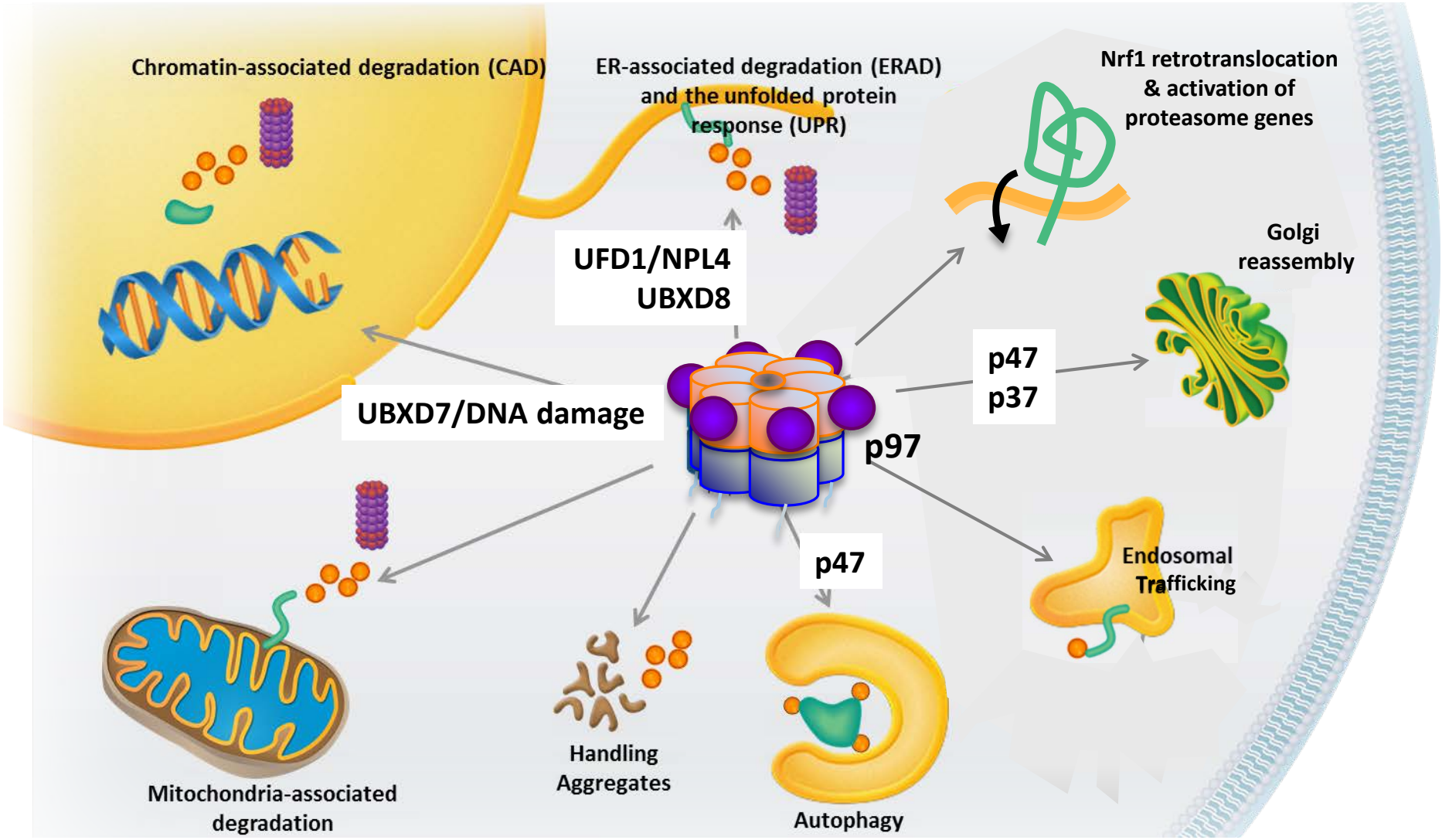


A. Normal cell



B. Cancer cell

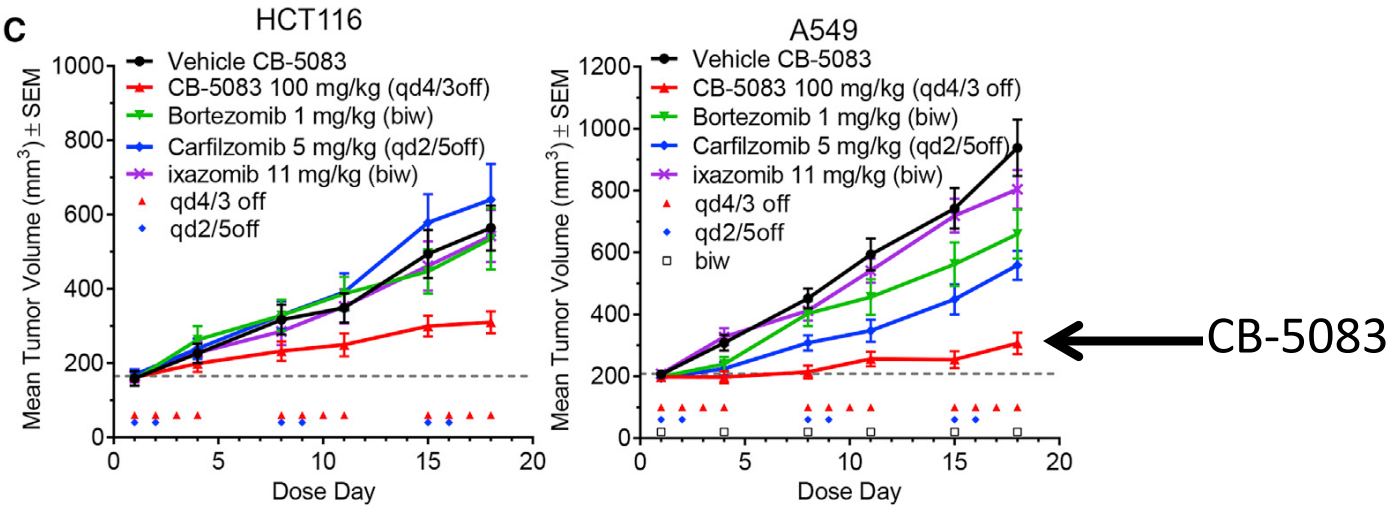
p97 is a Master Regulator of Protein Homeostasis



Adapted by Cleave Biosciences from Meyer et al., Nature Cell Biology (2012)

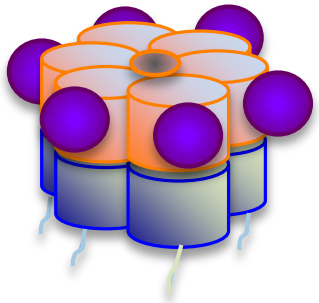
Opportunity to develop first-in class drug

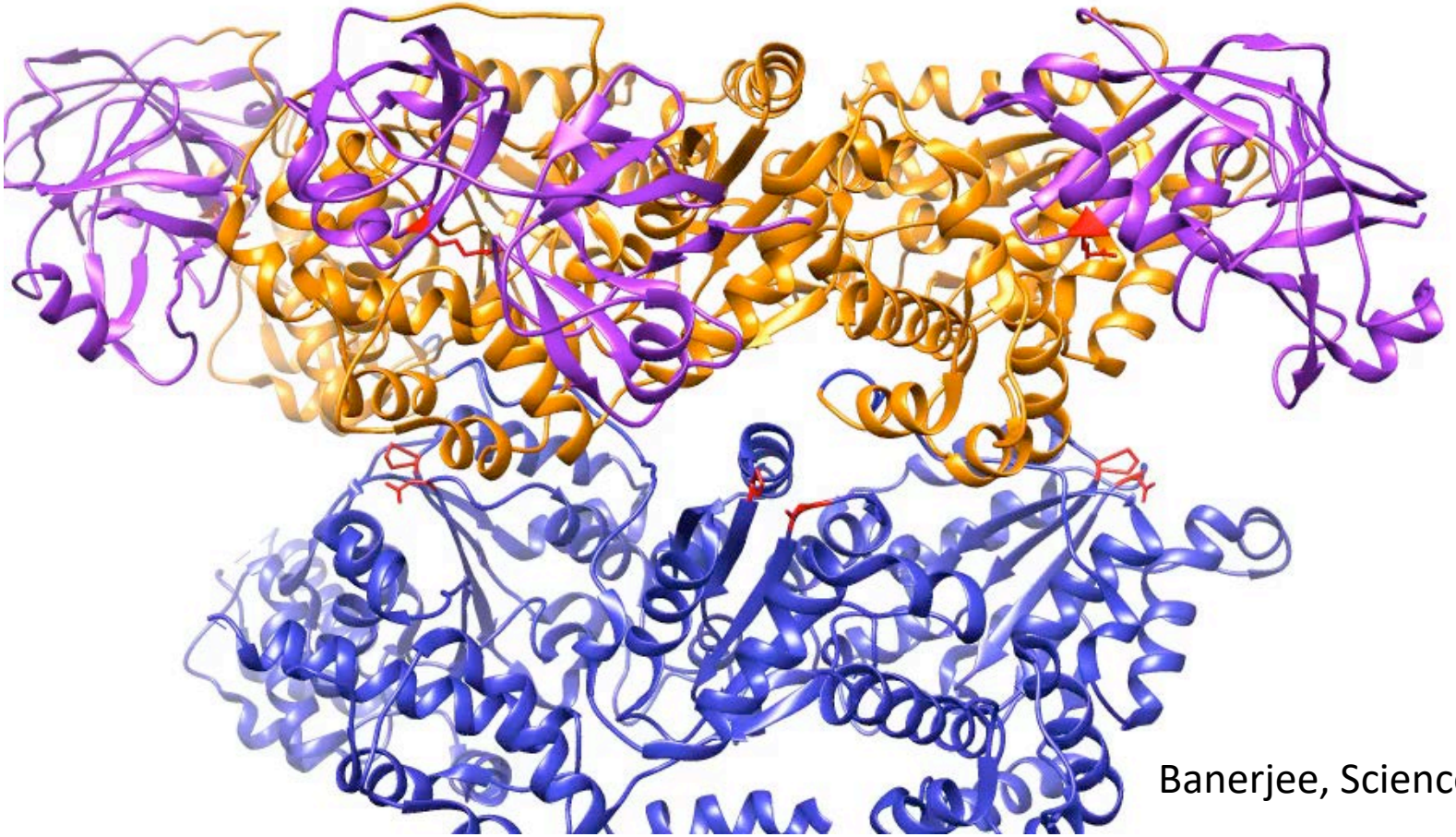
- **Clinical validation of proteasome inhibitors for hematological tumors** –
  - Is there an opportunity for UPS inhibitors in solid tumors?
- **In vivo efficacy for an ATP-competitive p97 inhibitor (Cleave Biosciences)**
  - More effective in solid tumors than proteasome inhibitors (*Cancer Cell*, **2015**, *28*, 653)



- **CB-5083 Phase I trials halted due to an off-target effect in the retina**
  - *Opportunity to develop first-in-class drug*

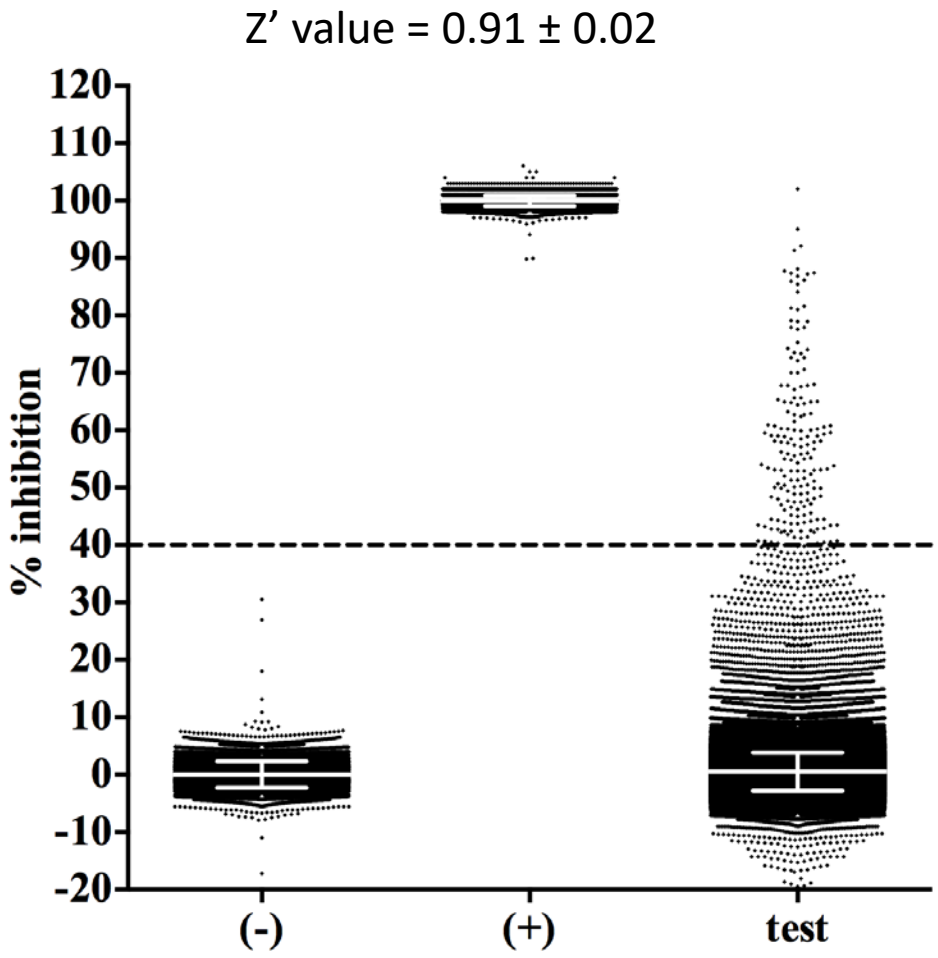
Conformational coupling of ATPase and PPI domains





Banerjee, Science 2016

HIGH THROUGHPUT SCREENING YIELDS ATPASE INHIBITORS



246,445 compounds screened

180 hits (> 40% inhibition)

113 inhibited WT and C522A mutant

44 repurchased

4 series with validated binding and inhibition mechanism

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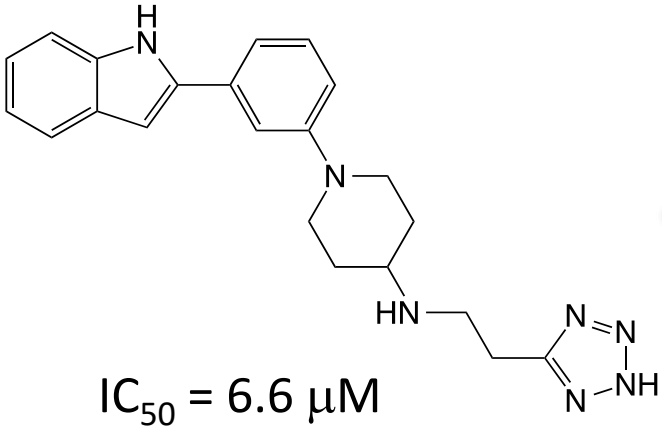
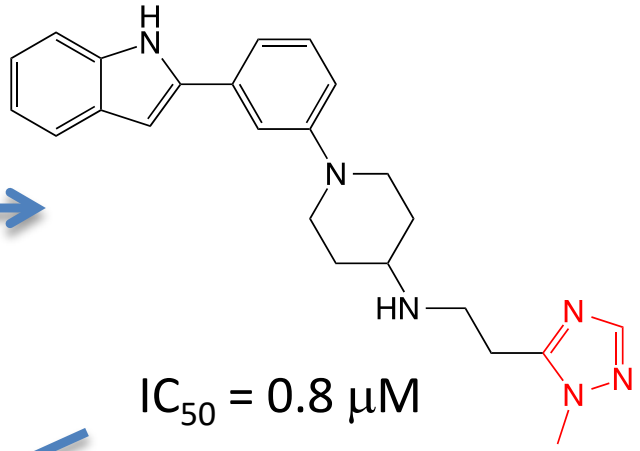
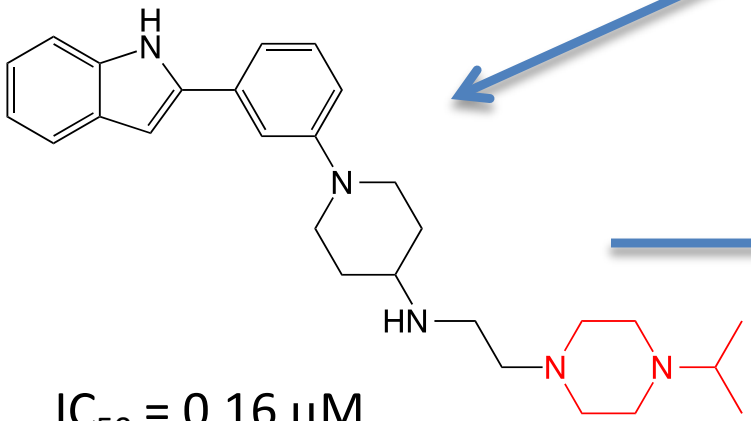
Development of potent inhibitor from HTS



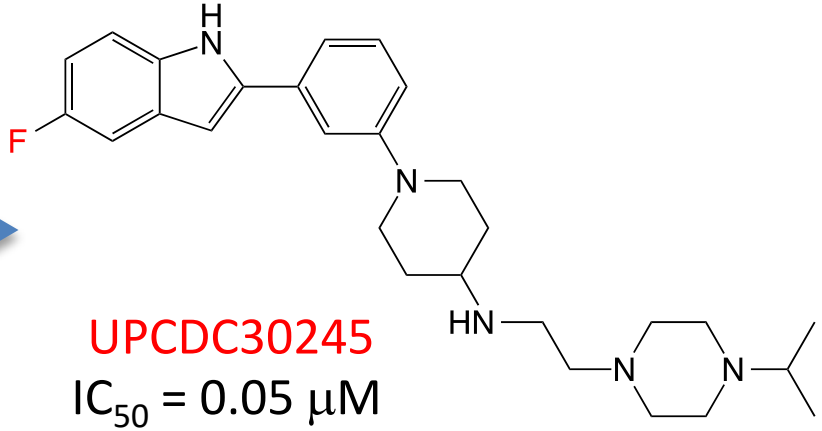
IC_{50} = 6.6 μM



IC_{50} = 0.8 μM



IC_{50} = 0.16 μM



UPCDC30245

IC_{50} = 0.05 μM

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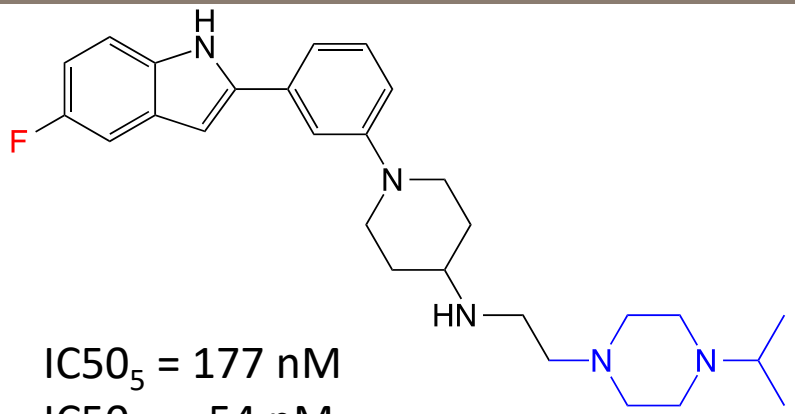
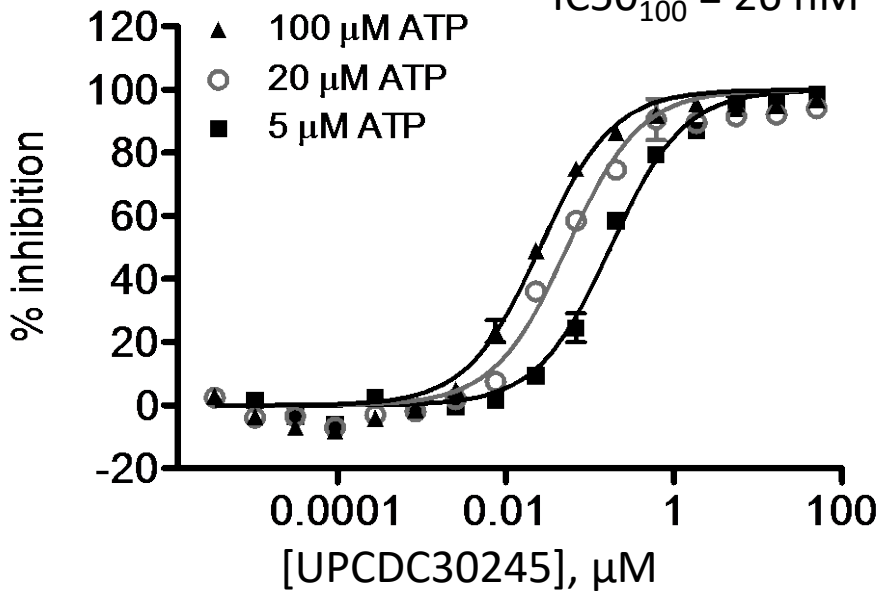
PHENYL INDOLES ARE UNCOMPETITIVE INHIBITORS OF P97

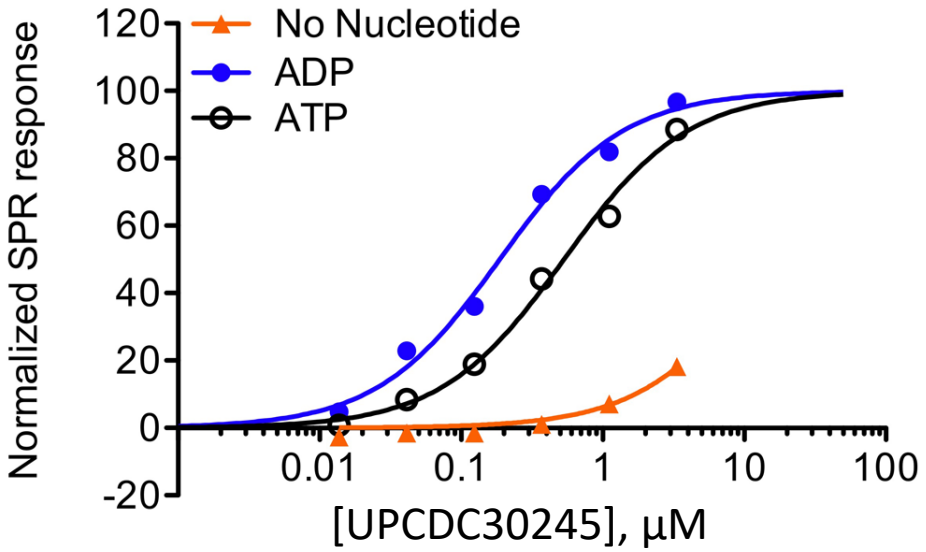


$IC50_5$ = 177 nM
$IC50_{20}$ = 54 nM
$IC50_{100}$ = 26 nM

Enzymology



SPR



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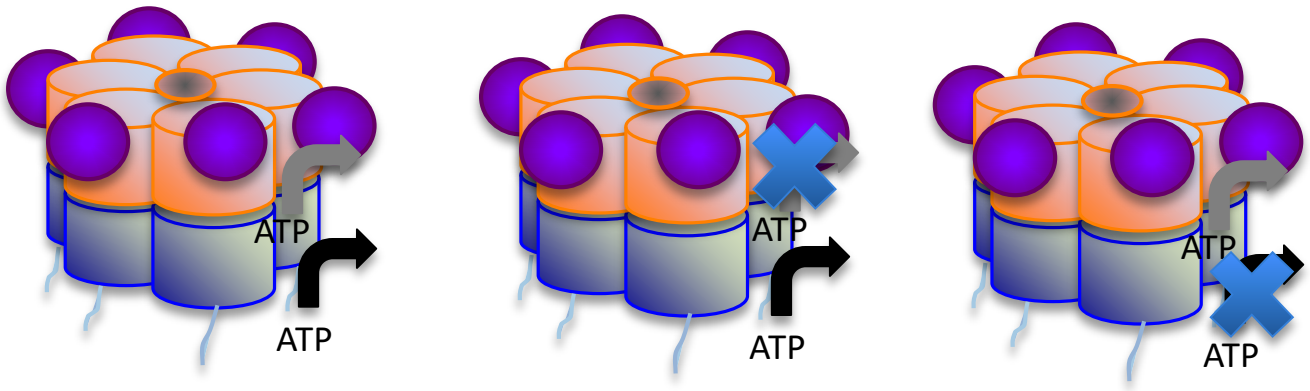
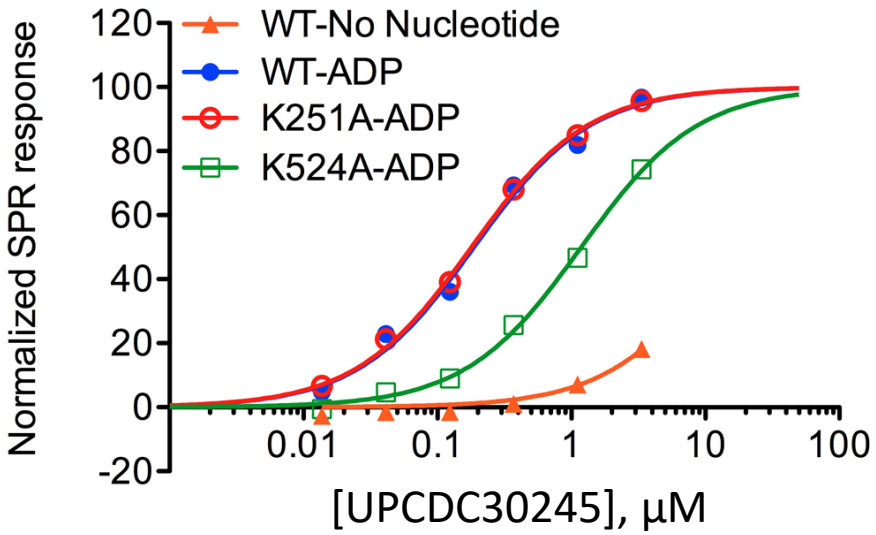
MUTAGENESIS: COMPOUND INTERACTS WITH D2 DOMAIN



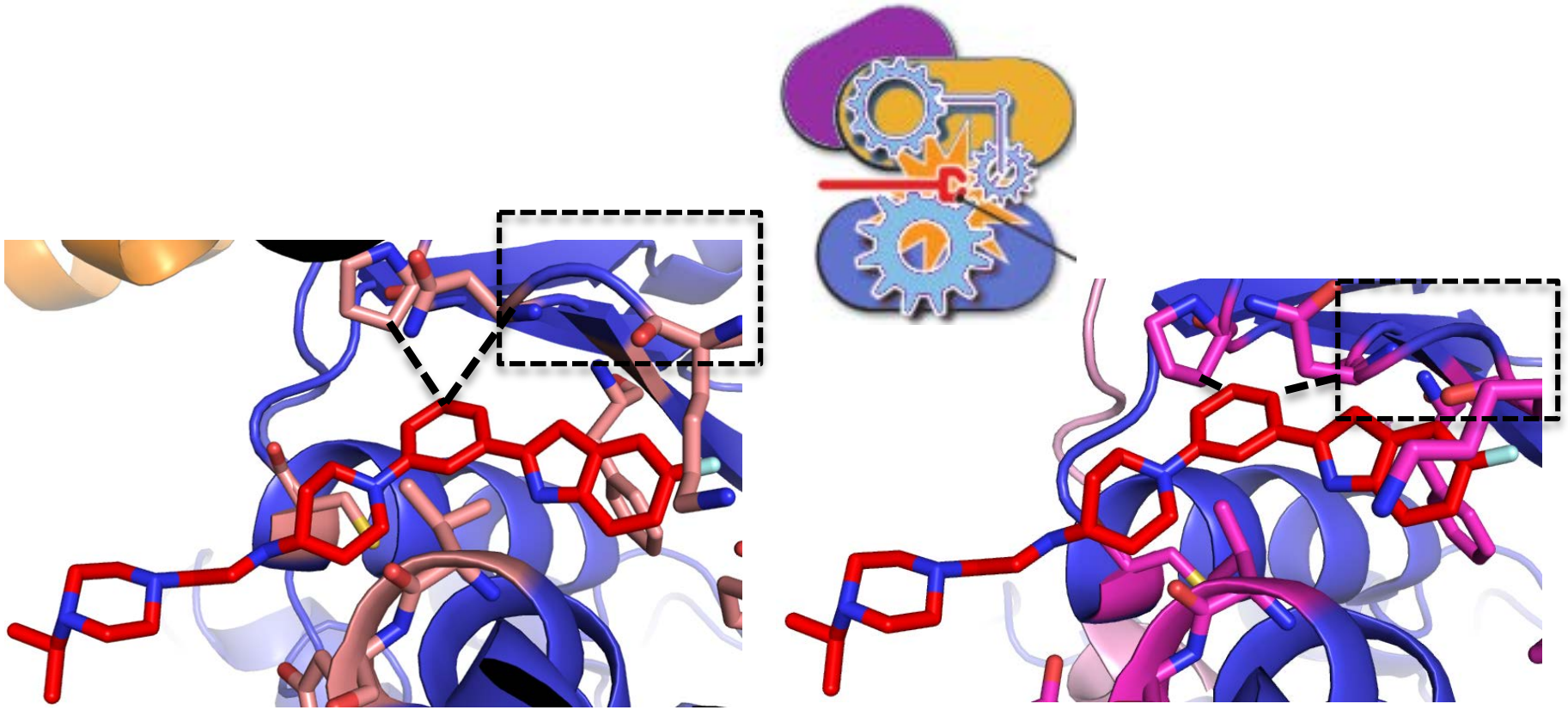
In the presence of ADP

1. WT
2. K251A
3. K524A



- '245 recognizes D2 domain
  - D2 binding also demonstrated by NMR
- D1 domain still 'organizes' allosteric site

‘245 CONFORMATION INCOMPATIBLE WITH ATP BINDING

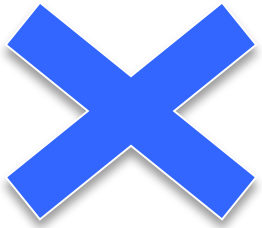


Binding of ‘245 prevents conformational changes in p97; thus inhibiting the enzyme

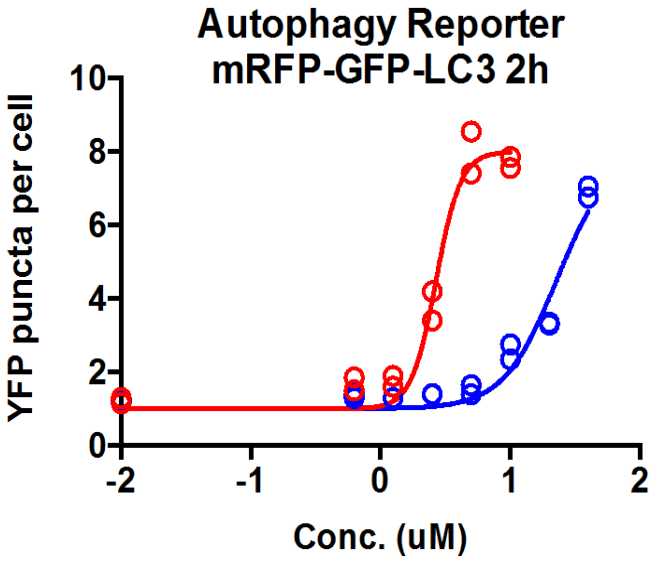
UNEXPECTED CELLULAR ACTIVITY → TRANSITION TO 2ND SERIES!

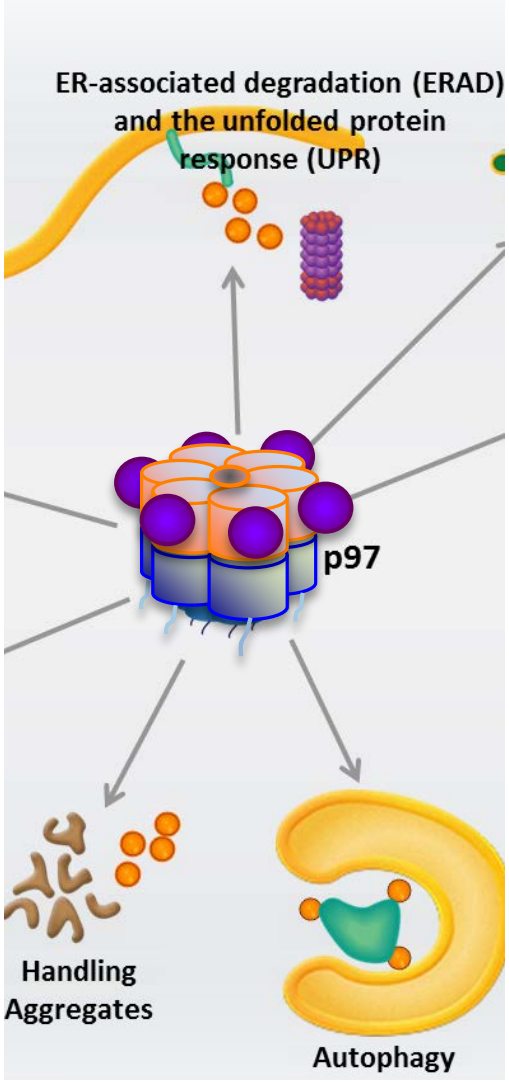
Series 1: uncompetitive

Proteasomal inhibition



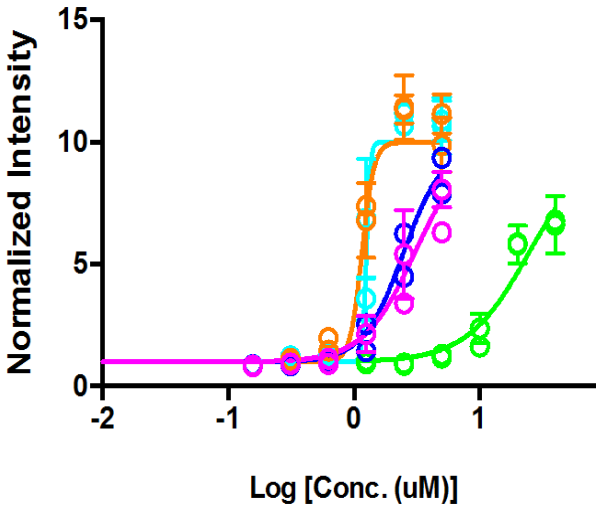
Autophagy inhibition

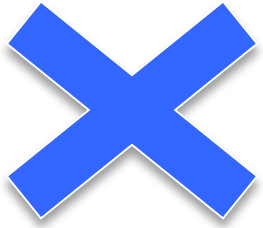




Series 2: ~noncompetitive

Proteasomal inhibition





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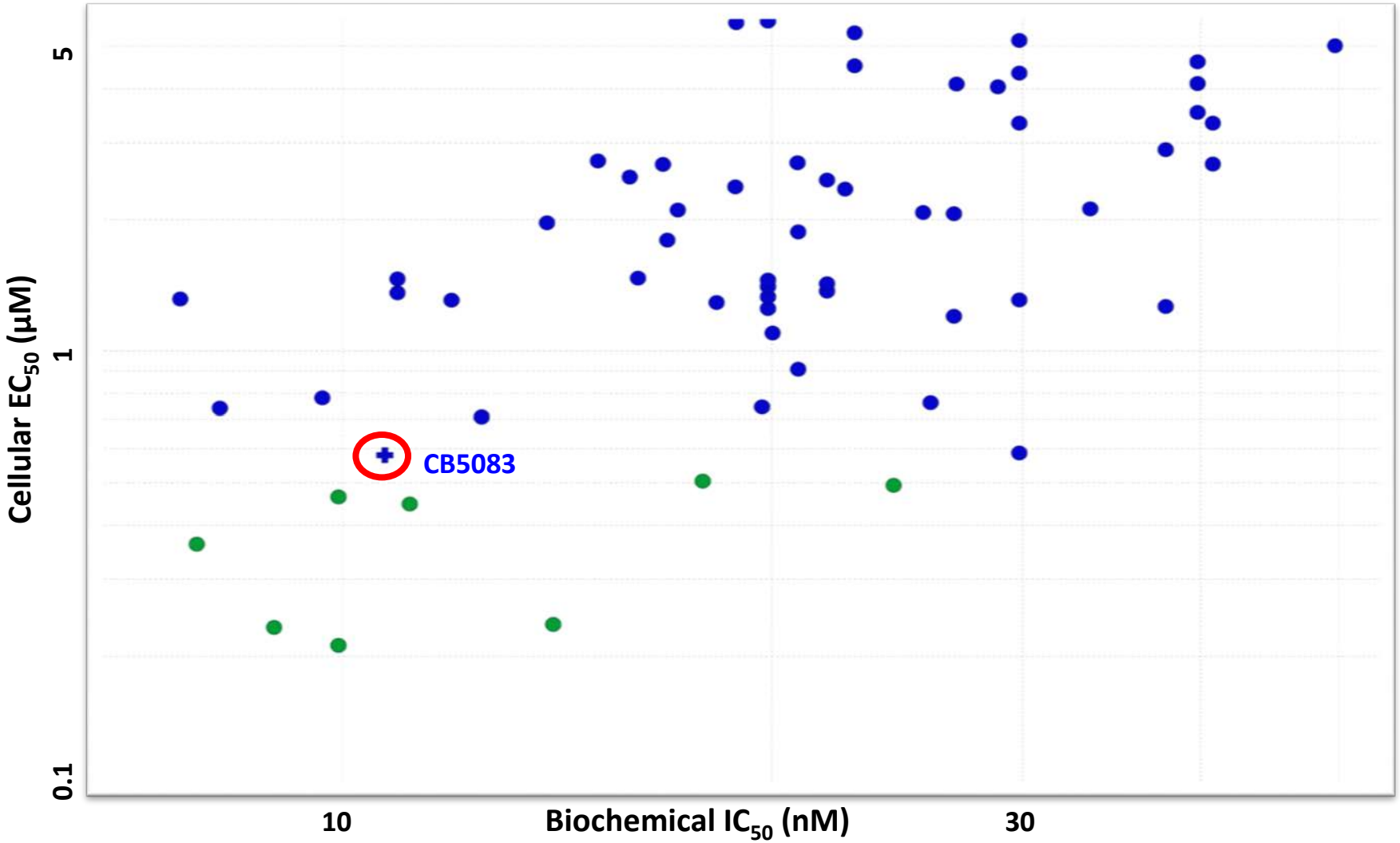
MULTIPLE ANALOGS WITH POTENCY GREATER THAN CB-5083



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KEY ASSAYS IN PLACE: BIOCHEMISTRY → IN VIVO EFFICACY

Primary Assays

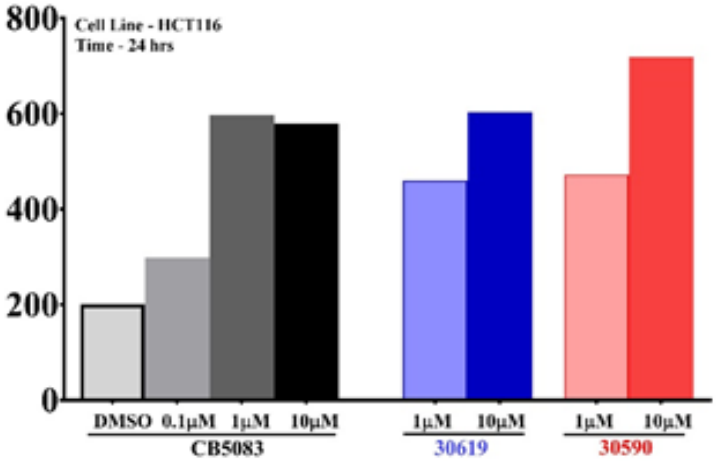
Biochemical assay:
p97 ATPase inhibition

Cell-based assay:
Ub-G76V Ubiquitin-protein accumulation

Cell-proliferation in HCT116 and RPMI8226

Additional Assays

Cellular PD assay:
P97 biomarkers K48, CHOP, cleaved Caspase3

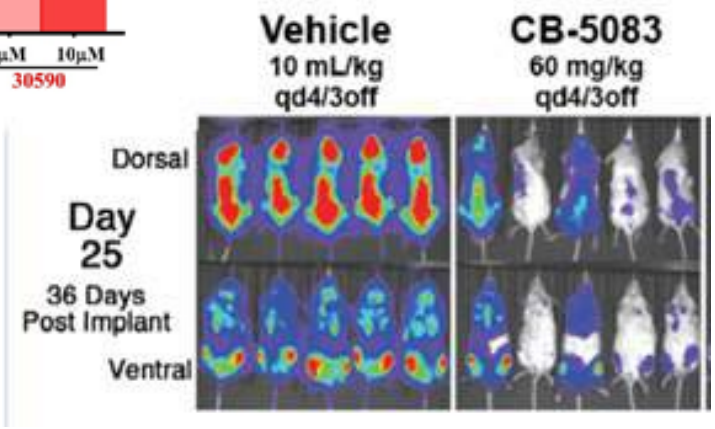


Cell line panels:
NCI-60
MGH 1000

In vivo Assays

In vivo PD assay:
P97 biomarkers K48, CHOP, cleaved Caspase3

In vivo efficacy:
solid tumor and disseminated multiple myeloma models



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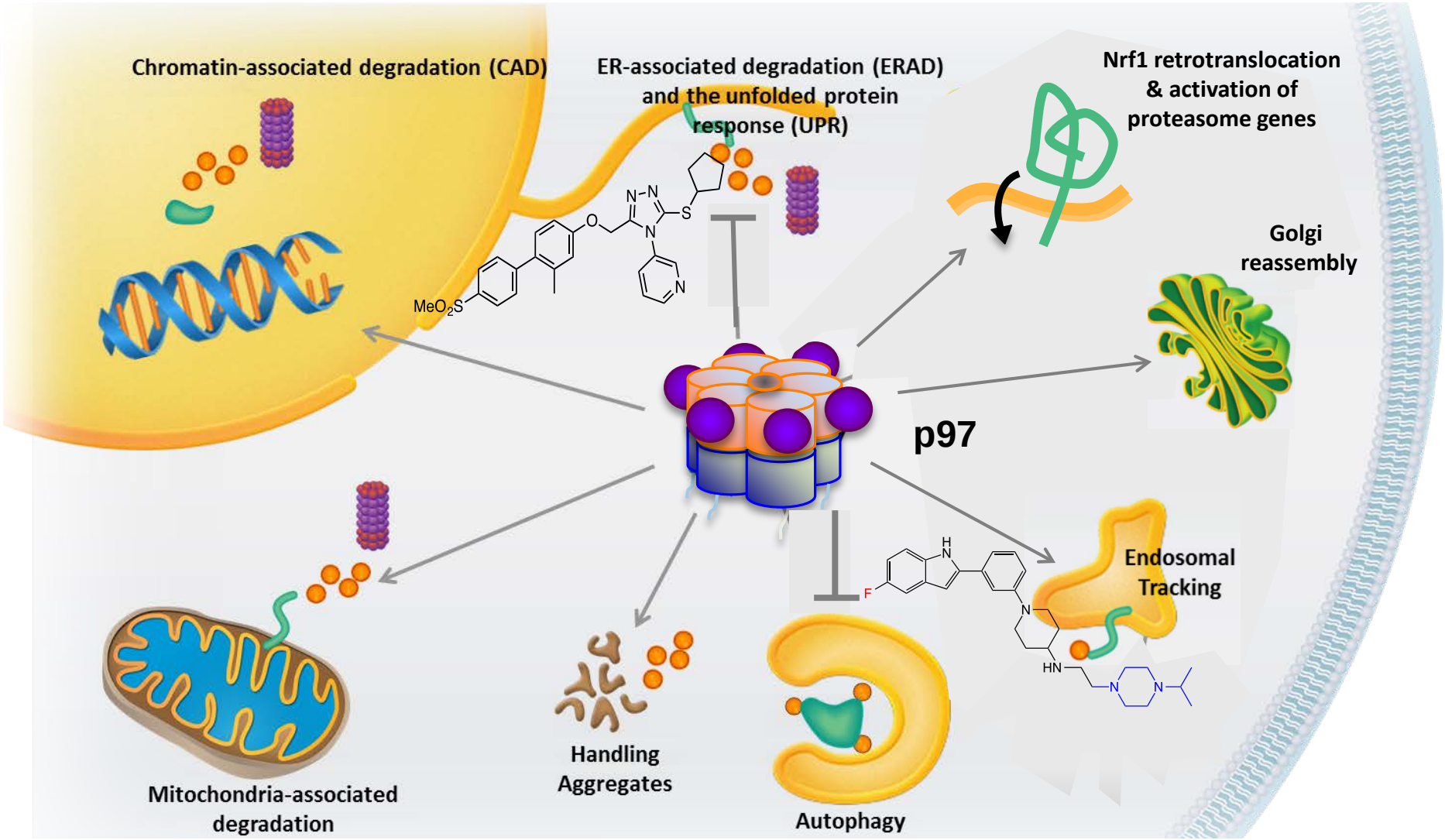
PROJECT PROGRESS & PLANS

- **2016-2017 Progress:**
  - Compounds with superior potency compared to **CB-5083**
  - Resistant cell lines confirm target engagement
  - ***In vivo Proof-of-Concept study underway***

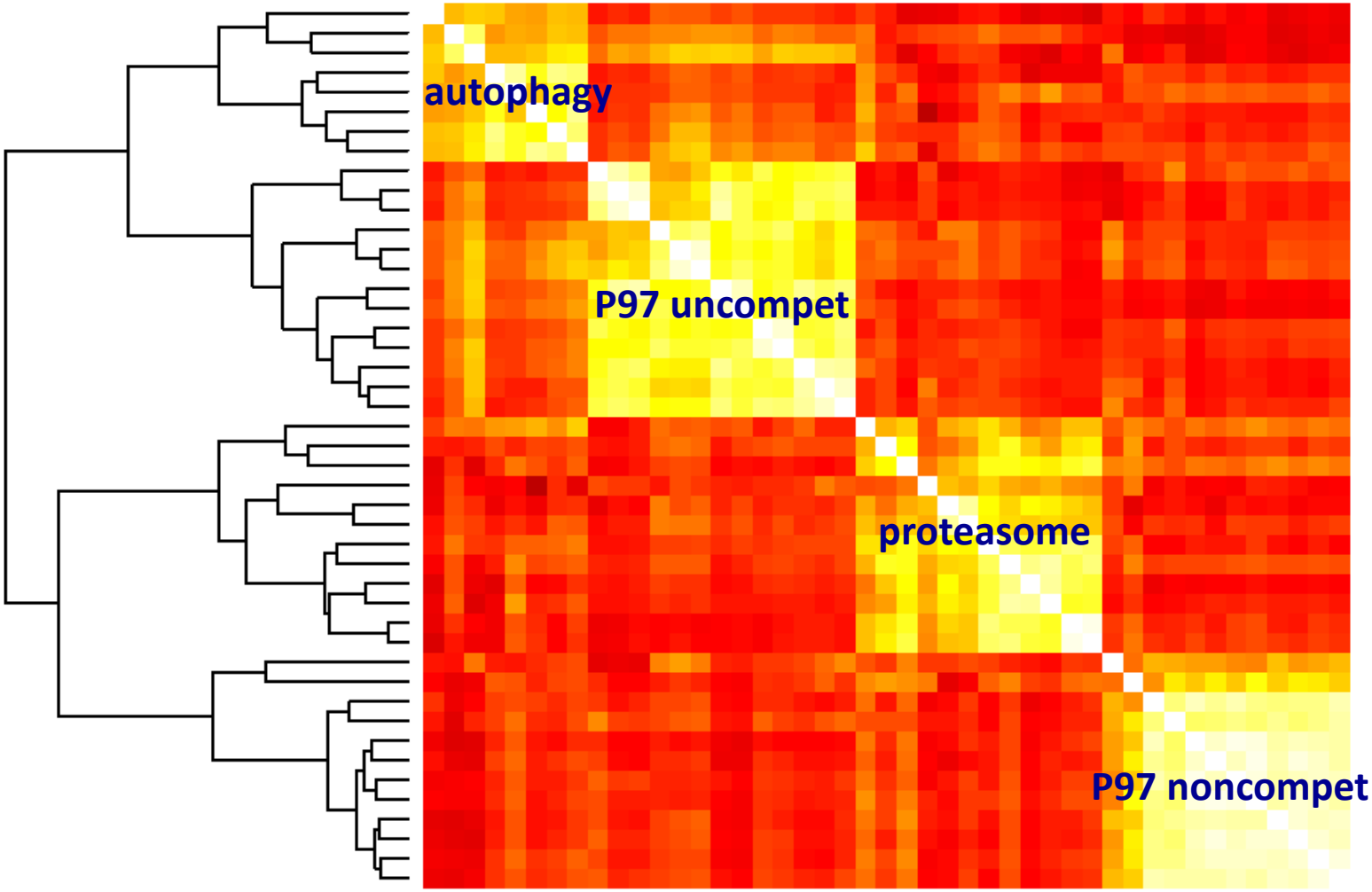
- **12 month Goal:** ***Identification of a Predevelopment Candidate***
  - Optimize for properties, potency and therapeutic index
  - Identify most sensitive cell lines from 1000 cell-line panel
    - In vivo models, target and patient selection

TALE OF TWO SERIES: WHY THE DIFFERENT CELLULAR ACTIVITY?

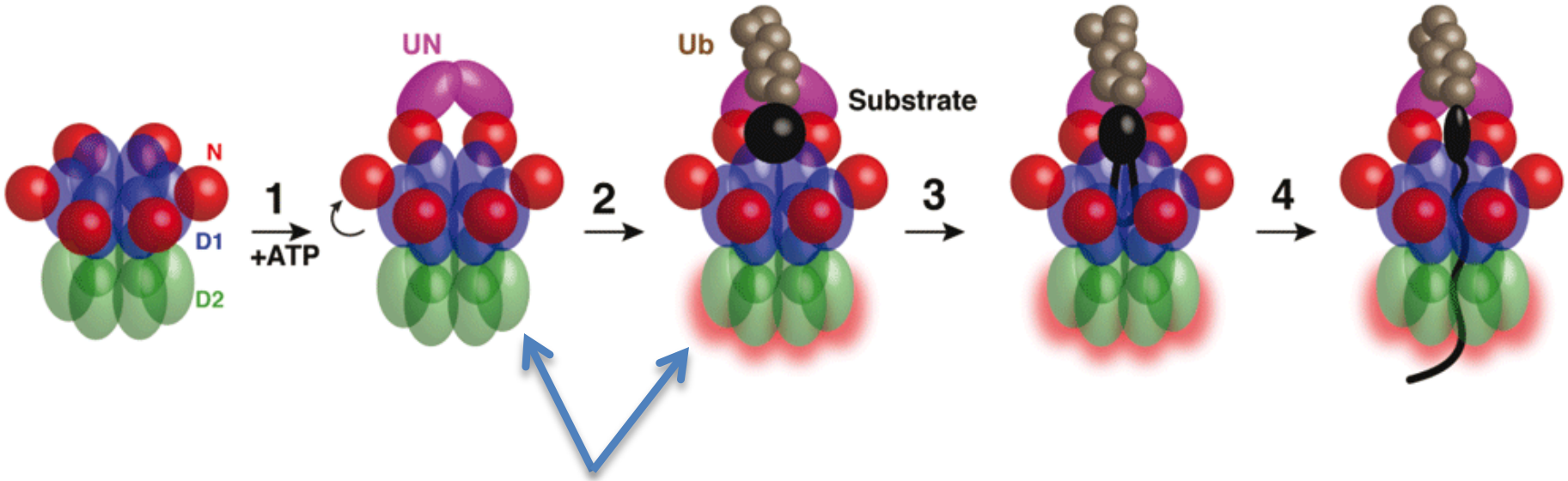


Adapted by Cleave Biosciences from Meyer et al., Nature Cell Biology (2012)

CELL-LINE SENSITIVITIES VARY BETWEEN MOA CLASSES



TRAPPING CONFORMATIONS DURING UNFOLDASE ACTIVITY



UPS-active compounds
can inhibit this transition

Bodnar N and Rapoport T 2017
F1000Research 2017, 6:1318

CBC p97 PROGRAM HAS SPAWNED BASIC RESEARCH PROJECTS

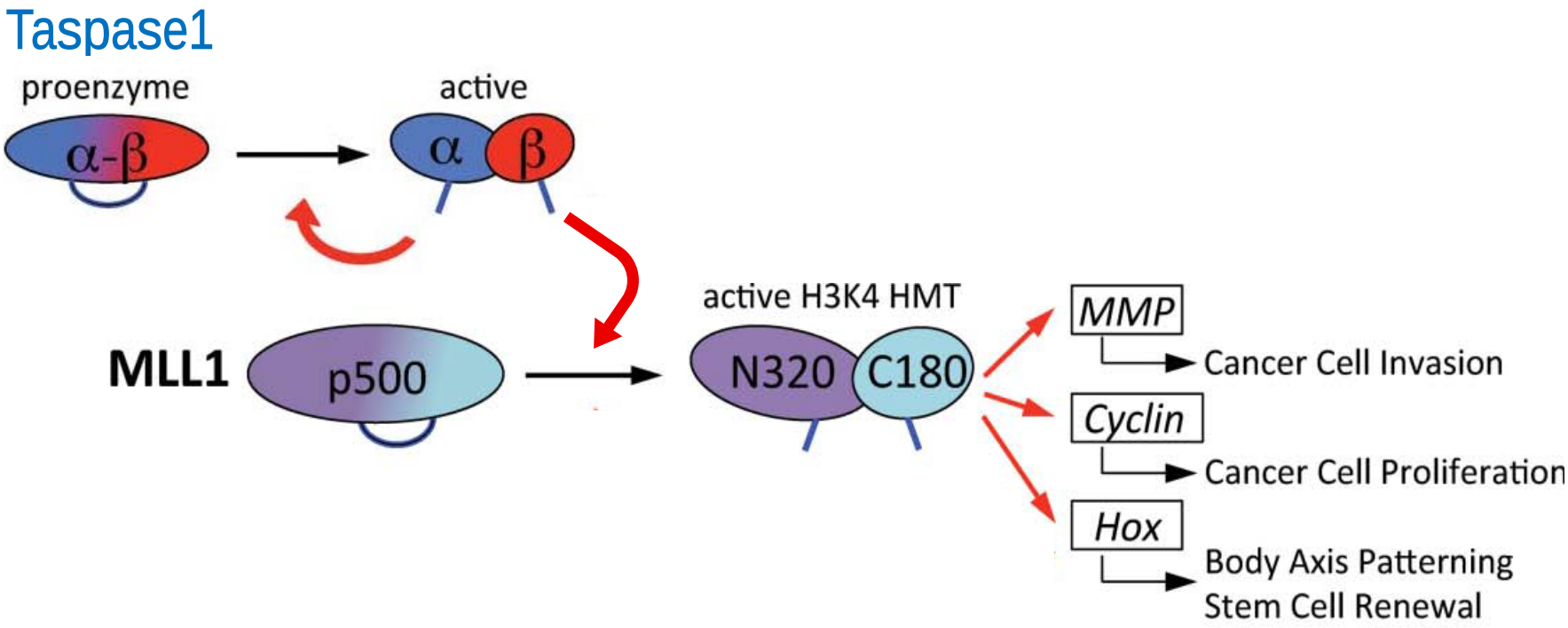
- **What functions of p97 are critical for different diseases?**
  - Design function-specific modulators
  - Modulate PPI networks

- **How do the conformations of p97 alter its functions?**
  - High-definition conformational analysis
  - Design new conformational locks

- **Goal: context-specific modulators of p97 function**

TASPASE1 IS A NOVEL CANCER TARGET

Threonine protease; drives cancer cell proliferation, EMT, invasion & metastasis



No known competition in drug discovery arena

TASPASE1 IS ALSO A CHALLENGING TARGET!

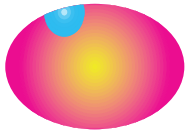
- Unique threonine protease
- Initial HTS failed to deliver tractable chemical matter
- Turn to alternative technologies based on

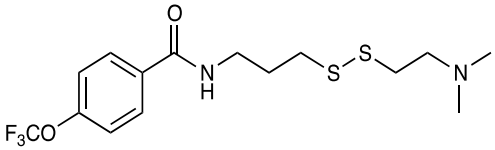
    a) binding to non-catalytic cysteine in substrate binding site

    b) very large libraries using split-and-pool synthesis

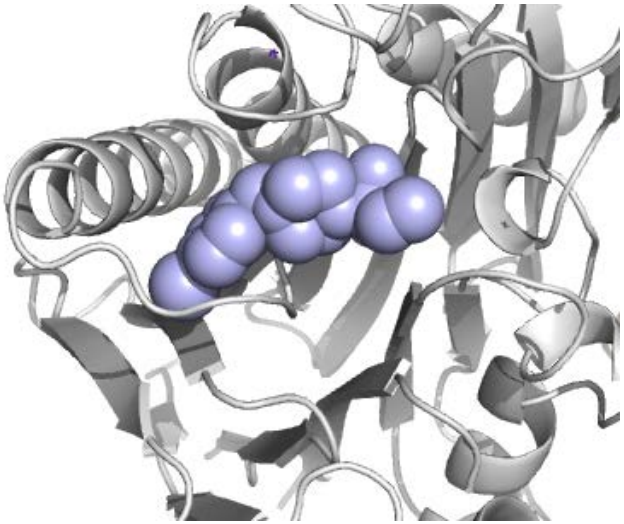
TARGETING CYS293 YIELDS FIRST NANOMOLAR INHIBITORS

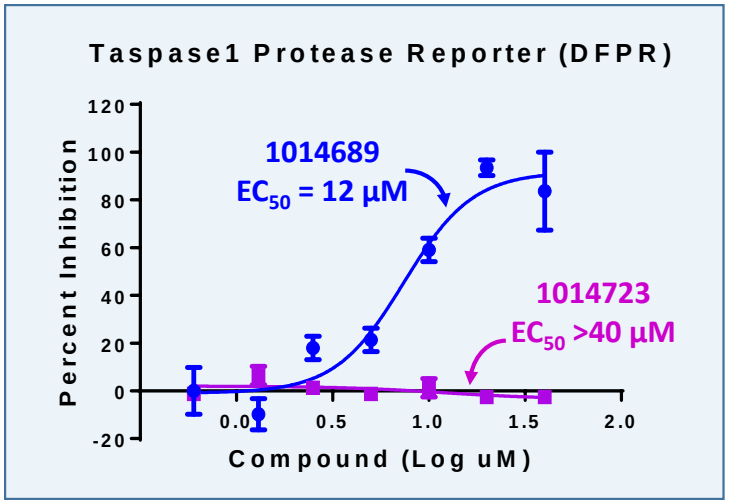
Screen approach: MS detection of cysteine-disulfide fragments





SMDC917671-L2
Biochem = 4.7µM





SMDC	IC_{50} (µM)	Cell IC_{50} (µM)	Fold selectivity vs Caspase-6	MDCK P_{app} ($x10^{-6}$ cm/s)	Cl_{int} (µL/min/mg) Mouse/human	K_i (noncovalent)
1014883	0.04	9	1300	40.1	46/7.5	20-40µM

HIGHLY DIVERSE, POOLED LIBRARIES → NONCOVALENT HITS

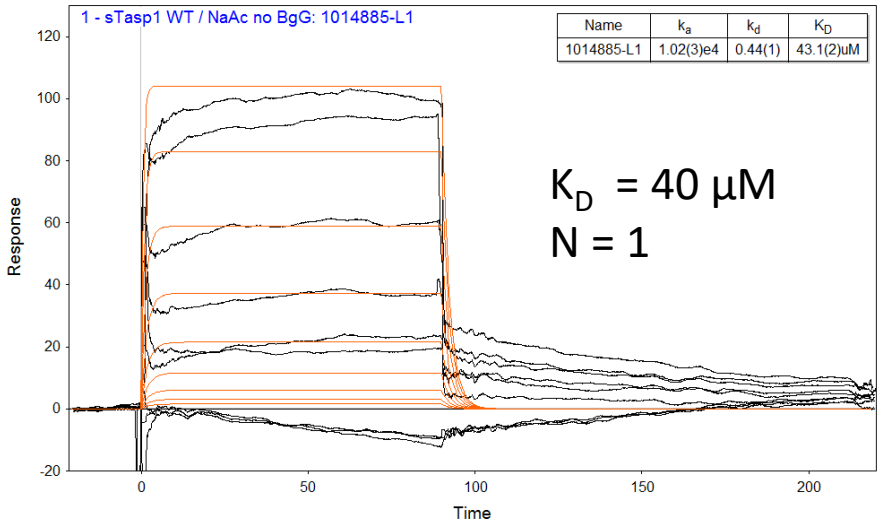
Scaffold Ranking Library
~85 samples
>30 million compounds

Positional Scanning Libraries
100-200 samples / library

Individual Compounds
20-50/series

Status: 2 chemical series

- IC_{50} ~ 2-5 µM
- 1:1 binding to Taspase1



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TASPASE1 PROGRAM IS RAPIDLY PROGRESSING

1 year ago

- First crystal structures
- First < 100 nM inhibitor

Now

- **9 ligand-bound** co-crystal structures
- **14** compounds with on-mechanism **cellular activity**
- *in vitro* ADME data supports viability of lead series
- **2 series** of non-covalent molecules with **<5 µM enzyme potency**

Near future

- Leads with potent cellular activity and demonstrated selectivity
- Mechanistic biology and PD to validate compounds, target, and biomarkers

THE CBC IS A UNIQUE PROGRAM

- Collaboration with top biology PIs and innovative drug-discovery technology

- Tackling some of the most difficult target classes
  - molecular machines
  - proteases
  - protein-protein interactions

- Focus on important and high-risk problems
  - Enables new technology development
  - Leads to fundamental science discoveries

- Innovative experimental medicines are sure to follow

SMALL MOLECULE INHIBITORS OF TASPASE1

Applicant PI	**James Hsieh, Wash. U., St. Louis**
NCI Project Leader	**Joel Schneider**
Vanderbilt	**Alex Waterson, Gary Sulikowski**
UCSF	**Michelle Arkin, Jeff Neitz**
SRI	**Lidia Sambucetti, Claire Repellin**
Beryllium	**Silvia Delker, Tom Edwards**
Columbia University	**Liang Tong**
Arizona State	**Petra Fromme, Mark Holl**
TPIMS	**Greg Welmaker, Richard Houghten**
SPMs	**Andrew Flint** (UCSF, Beryllium, ASU, Columbia) **Bill Moore** (Vanderbilt, TPIMS) **Gordon Stott** (SRI)
Project Manager	**Sidra Iqbal, John Giraldes**

P97 PROJECT TEAM

Chemical Biology Consortium

Initiating PI: **Ray Deshaies** (Caltech)

Biochemistry, screening, x-ray (UCSF)
- **Stacie Bulfer**
- **Kenny Ang**

Modeling, NMR
Matt Jacobson (UCSF)
Mark Kelly (UCSF)
- **Michael Chimenti**

Cell biology (UCLA)
Tsui-Fen Chou
- Shan Li
- Xiaoyi Zhang
- Lin Gui

Chemistry
Donna Huryn, Peter Wipf
(U Pittsburgh)
- Matt LaPorte
- Mary Liang
- Taber Lewis
- Marina Kovaliov
- Yongzhao Yan
- Celeste Alverez
- Lalith Samankumara
- Zhizhou Yue
- Raffaele Colombo
- Feng Zhang
- Chaemin Lim
- Alex Chatterley
- Catherine McAdams
- Mike Houghton

Jeff Neitz (UCSF)

Cryo-EM
Sriram Subramaniam (NCI)
- **Soojay Banerjee**
- Alberto Bartesaghi
- Alan Merk
- Prashant Rao
- Jacqueline Milne

NCI, Leidos
Barbara Mroczkowski
Bill Moore
Neal Green
Gordon Stott
Andrew Flint

NExT NCI Experimental Therapeutics Program

Funded by NCI, NIH CBC Contract No HHSN261200800001E

First look at full-length Taspase1

- All previous structures use truncations which lower activity
- Current best resolution: 3.5 Å
- Next step: send into space on NASA mission!