



Sanford-Burnham
Medical Research Institute
(La Jolla, CA)

HTRF®-powered detection of ubiquitin-like protein signaling and interactions

CONRAD PREBYS
Center for Chemical Genomics
Medical Research Institute

Eduard Sergienko, Ph.D.
Director, Assay Development

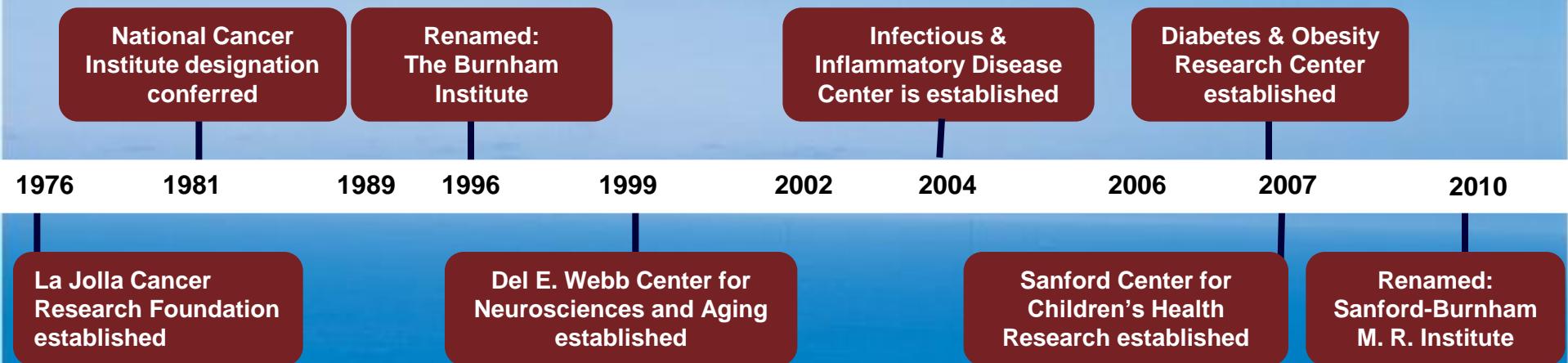
Topics

- SBMRI and CPCCG
- NIH Roadmap Initiative Screening Network
- UBL signaling cascades and related projects within CPCCG
- Acknowledgements

Development of Sanford-Burnham Medical Research Institute



Development of Sanford-Burnham Medical Research Institute



From Research, the Power to Cure

La Jolla
Est. 1976

- 2 Sites of operation
- 1200 People (over 500 Ph.D. scientists)
- 89+ Faculty
- \$175 MM annual operating budget

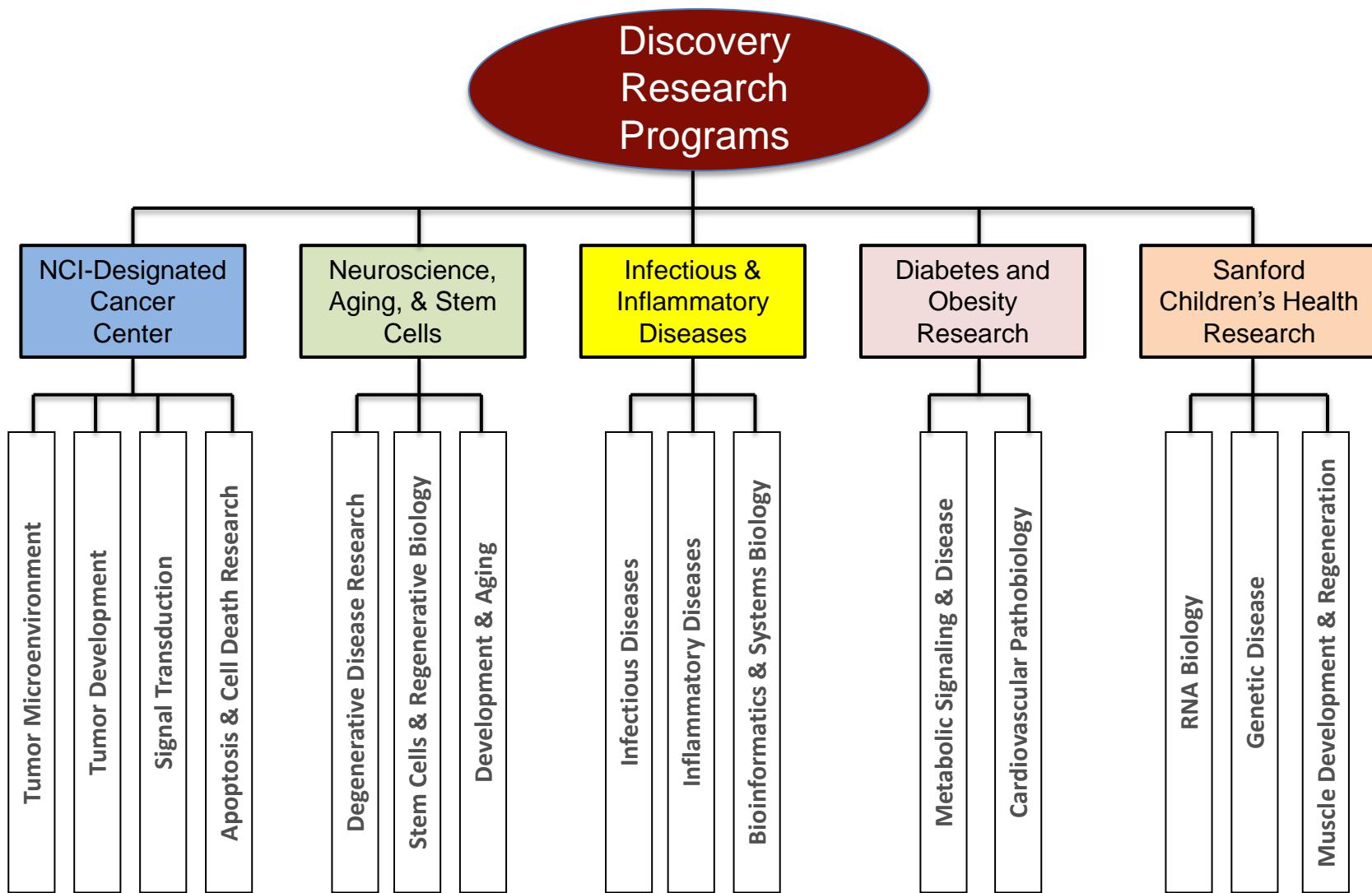
Orlando
Est. 2007

Sanford-Burnham Medical Research Institute (Florida site)

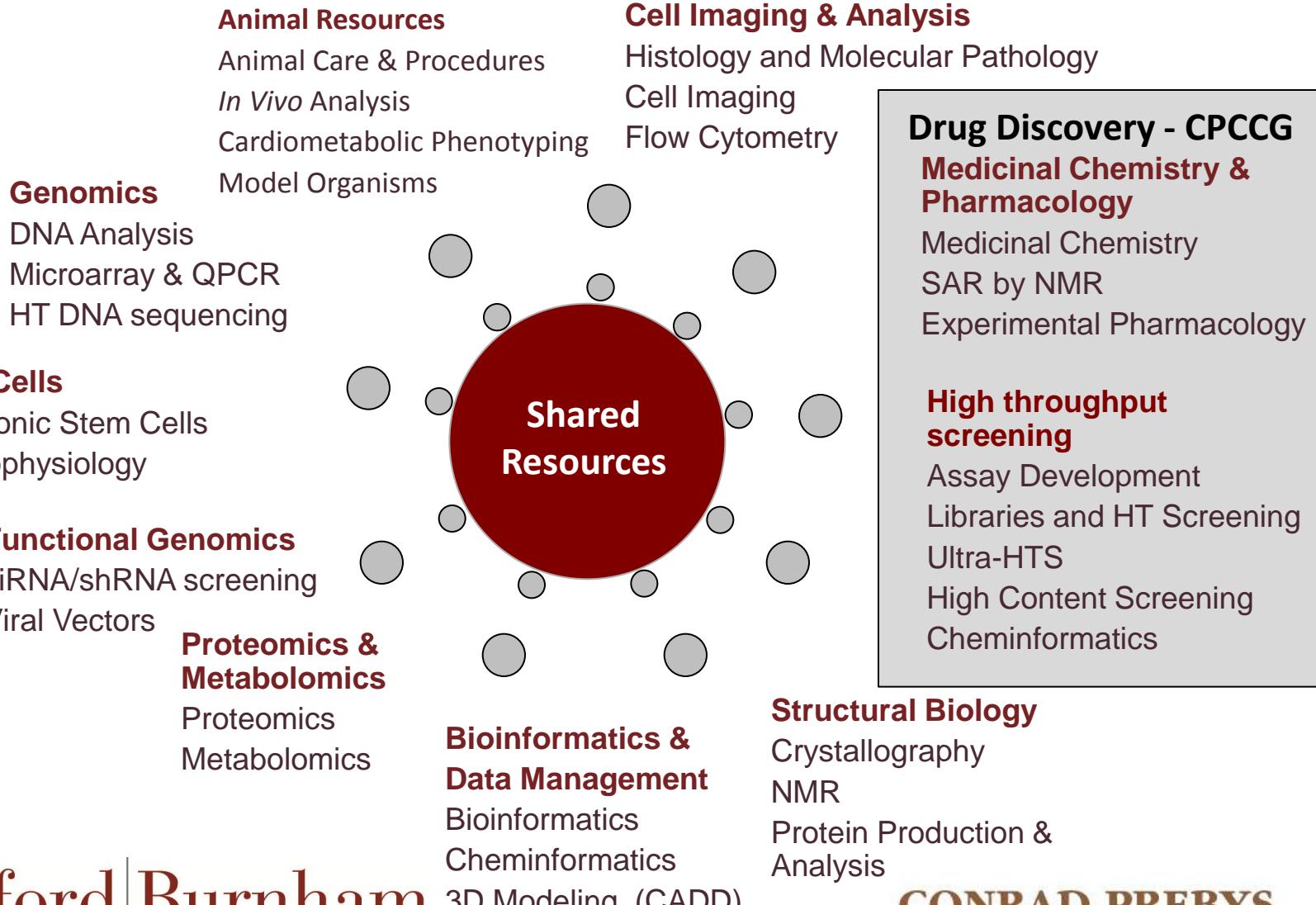


- **Occupied: April 2009**
- **175,000 sq ft facility**
- **Part of Lake Nona Medical City**
- **14,000 sq ft AAALAC-certified vivarium (rats & mice)**
- **Largest GOLD LEED independent scientific facility in Florida**
- **~30 Principal Investigators; ~300 employees (Phase I capacity)**

Sanford-Burnham's Discovery Research Programs

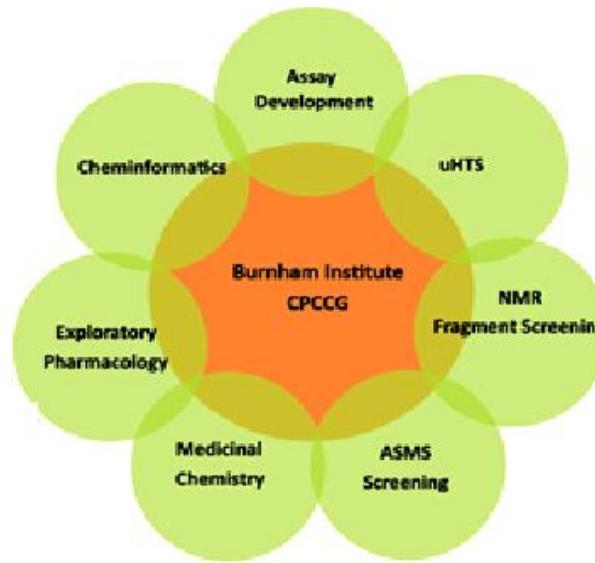


Superior Technology Infrastructure empowers world-class science



Conrad Prebys Center for Chemical Genomics

- Bicoastal operations (San Diego, CA and Orlando, FL)



- 1 of 4 comprehensive NIH MLPCN centers for the past 7 years
- 370K MLSMR library + 320K SBMRI collection; focused libraries
- Performed ~135 HTS-based projects, developed >300 secondary assays, generated >50 chemical probes
- Most of assays development and screened in 1536-well plates

S|B World Class Discovery Capabilities

>\$30 MM capital investment

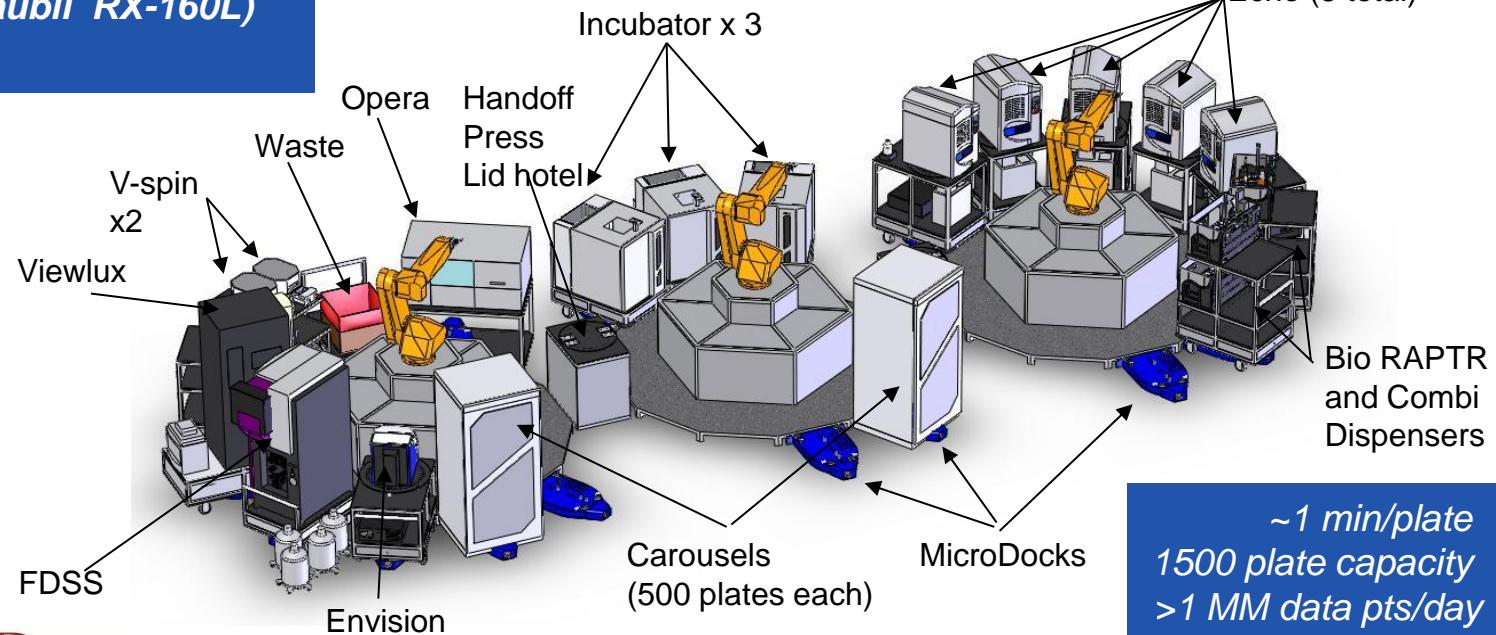
- **5 Fully Integrated Robotic Liquid Handling Systems**
(HRE unipod and tripod /1536 format), acoustic liquid dispensing, multiple plate readers, tip box & plate hotels, CO₂ Incubators, etc.
- **4 Fully integrated HCS Systems**
 - Robotics arms, tip wash & cell dispense, plate hotel & incubator
 - Live cell and 3D imaging
- **Off-line Instruments & Workstation**
 - 6 Plate readers, 2 Plate washers, 3 Bulk reagent dispensers
 - Plate Workstation: Plate sealer, barcode labeler, 384/1536 liquid handler
 - Liquid Handler Workstation 96/384/1536
 - High Content Microscope
 - GPCRs – 2 Hamamatsu FDSS-7000



Sanford-Burnham uHTS Facility (Orlando)

- Robot runs several screens simultaneously, permitting complex scheduling
- Swap out device “on the fly” & dock “off-line”
- Acoustic compound transfer ($x2.5\text{ nL}$) for 1° HTS & cherry-picking
- Perkin Elmer Opera HCS + Acapella software

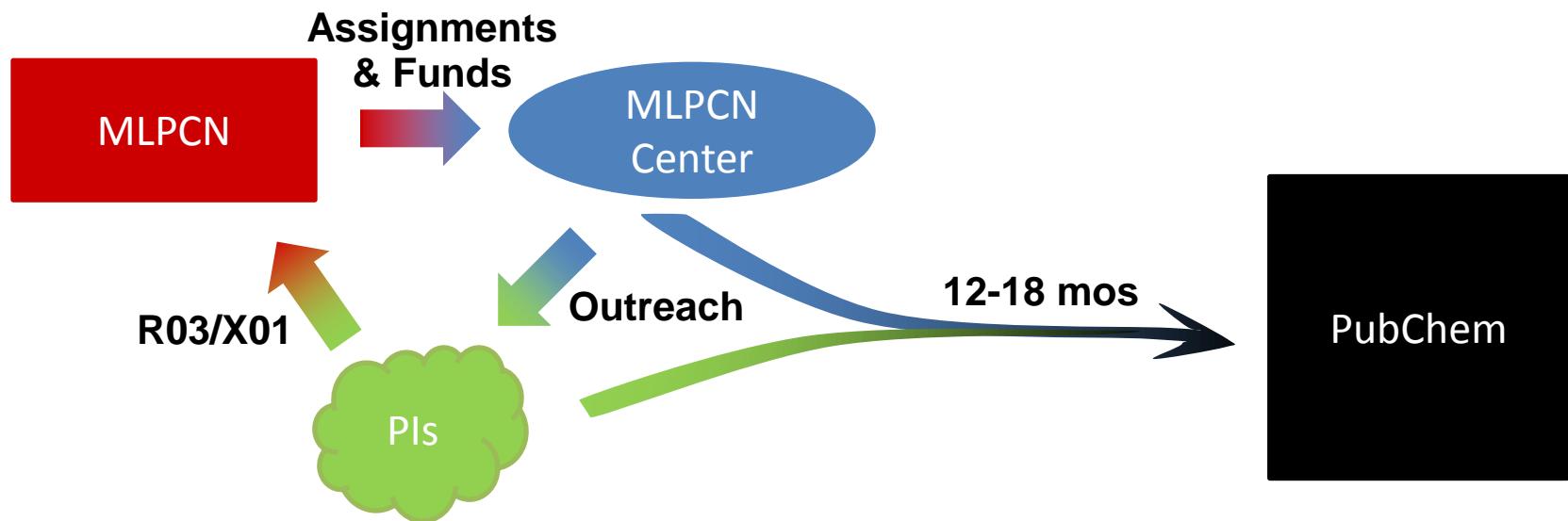
**5-Echos w/ On-Board library
3-Arms (Staubli RX-160L)
5 Readers**



- Molecular Libraries Probe Production Center Network (MLPCN)
 - Molecular Libraries Small Molecule Repository (MLSMR) maintains a collection that is shared by all centers in the network
 - HTS and SAR data released into PubChem database
 - ✓ Counter screen information
 - Generate knowledge relevant to human health
 - ✓ Target validation
 - ✓ Tool compounds for untargeted proteins and classes
 - ✓ Starting points for drug discovery

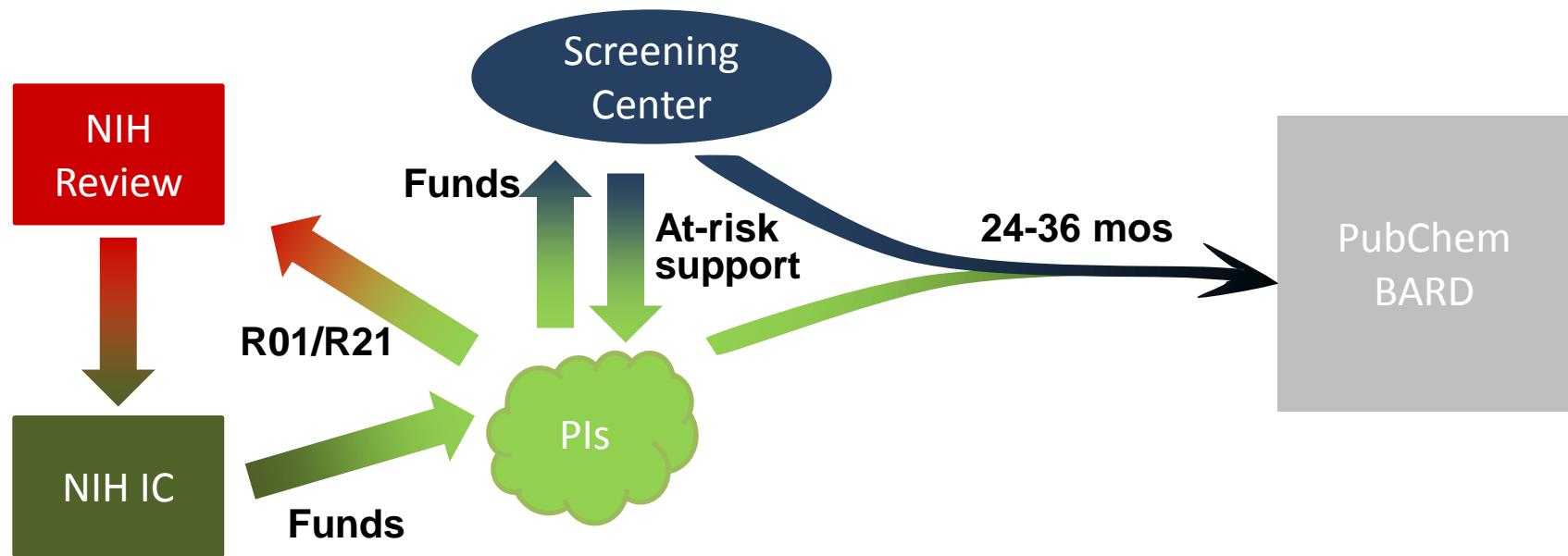
Specifics of MLPCN projects

- MLPCN centers funded by NIH Common Fund
- PIs access the network through MLPCN grants; the applications require working primary assays
- Part of Center's budget allocated to outreach

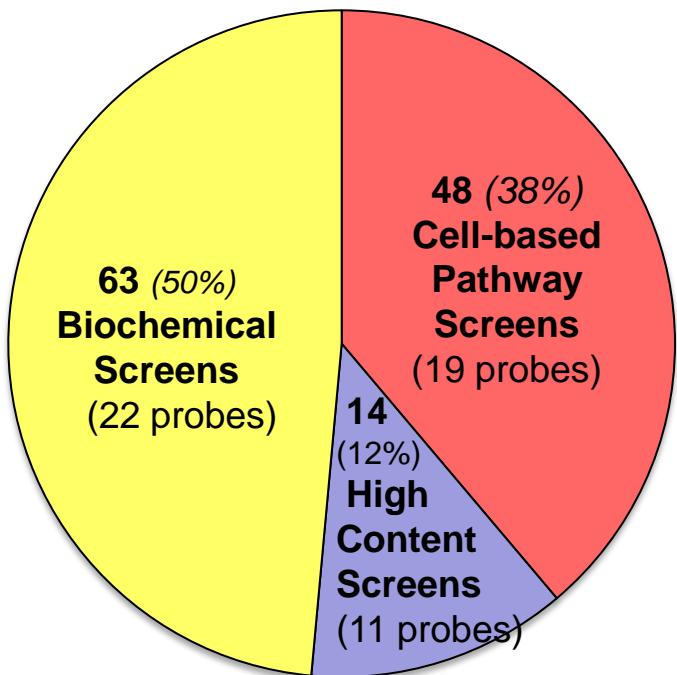


The next page in academic screening

- A novel mechanism for funding the academic screening is currently being tested with PAR-12-058 (HTS R01), -059 (HTS R21) and -060 (MedChem R01)
- Grants require working assays and testing funnels



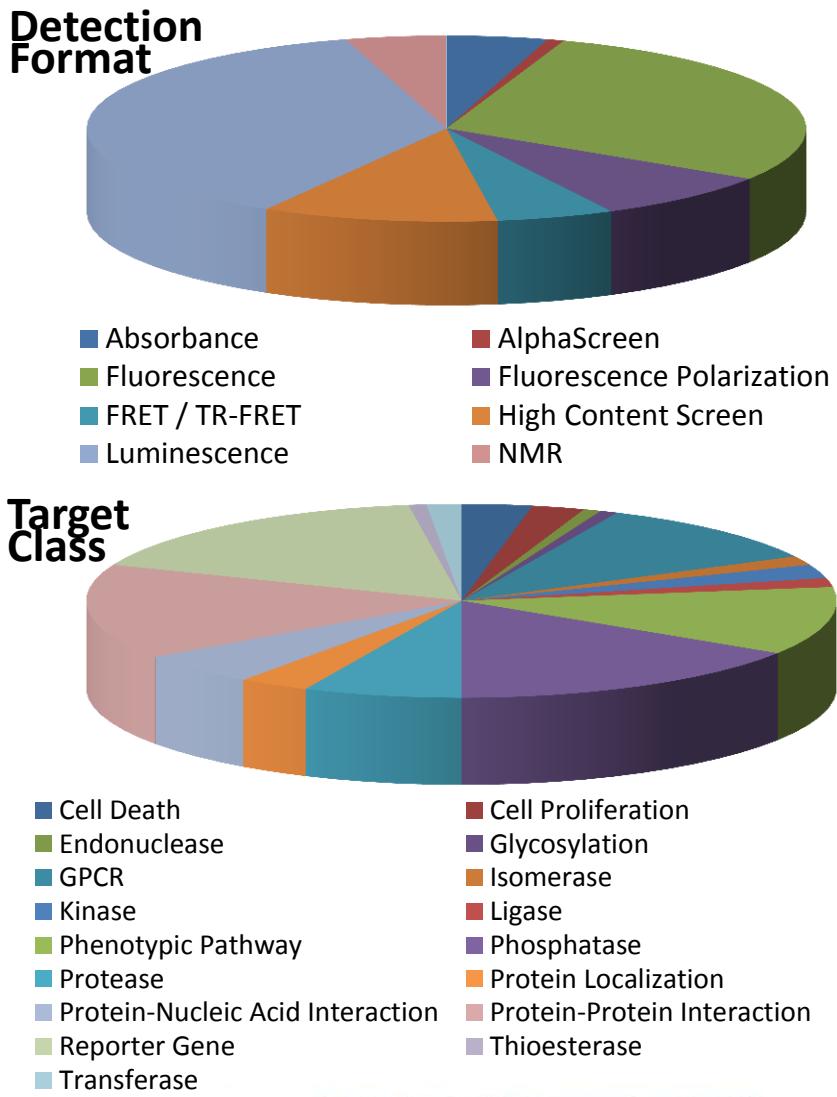
MLPCN screening at CPCCG



2005-2013

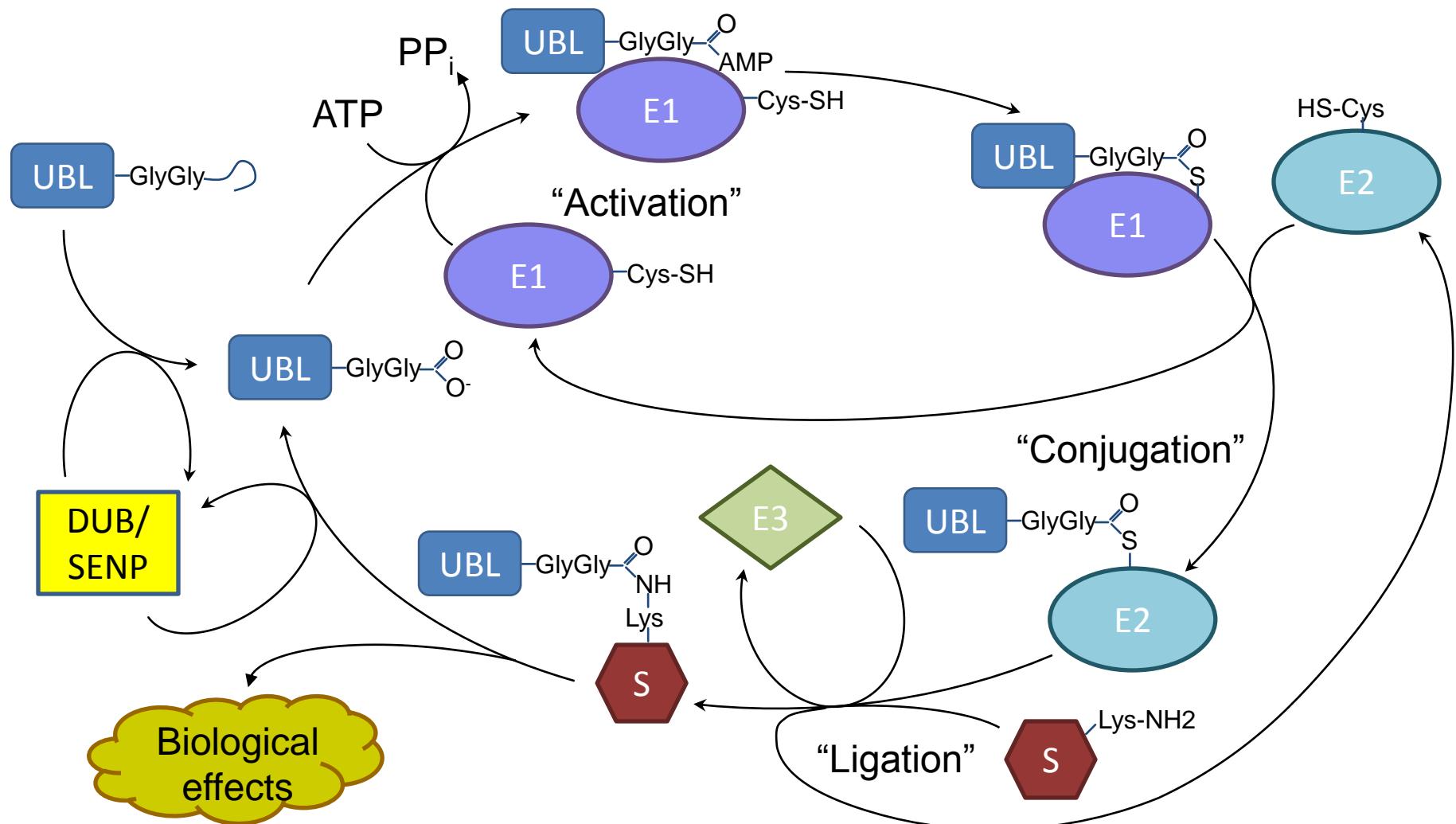
125 Library Screens
52 Probes Identified

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UBL Cycle



MLPCN UBL projects performed at CPCCG

Projects

Polyubiquitination

✓ SUMOylation

✓ Neddylation

✓ SUMO-PPI

Senp1/CFP-SUMO1-YFP

Senp6/Z-RLRGG-AL

Senp7/Z-RLRGG-AL

Senp8/Z-RLRGG-AL

Senp8/Nedd8-AMC

Rpn11

Csn5

PIs

Dr. John Reed (SBMRI, La Jolla, CA)

Dr. Yuan Chen (City of Hope, Duarte, CA)

Dr. Matt Petroski (SBMRI, La Jolla, CA)

Dr. Yuan Chen (City of Hope, Duarte, CA)

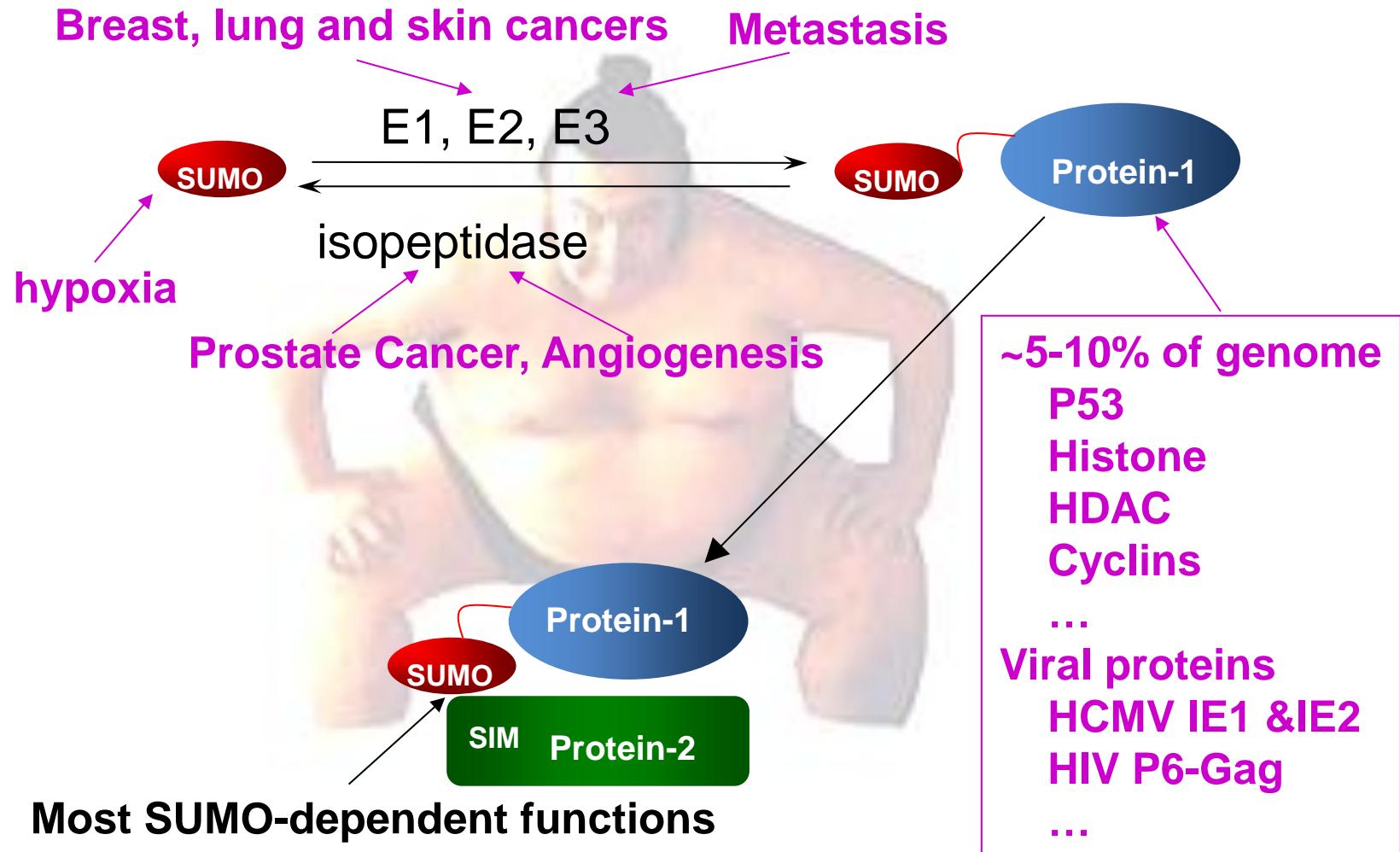
Dr. Jorge A. Iniguez-Lluhi (U. Michigan, Ann Arbor, MI)

Dr. Guy Salvesen (SBMRI, La Jolla, CA)

Dr. Ray Deschaines (CalTech, Los Angeles, CA)

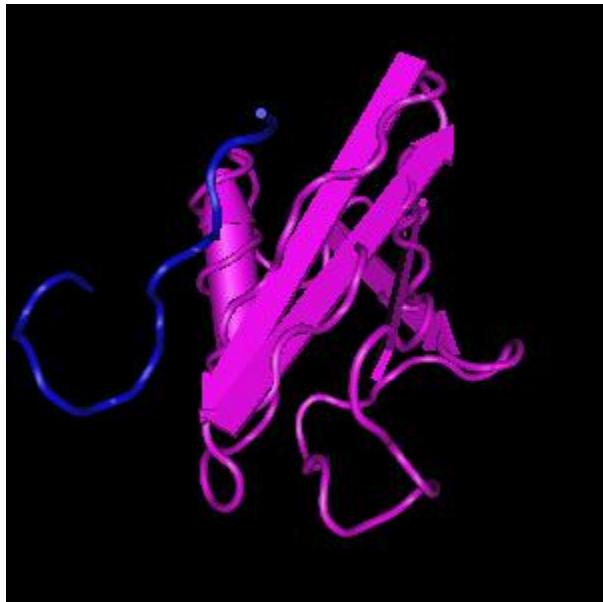
Dr. Ray Deschaines (CalTech, Los Angeles, CA)

SUMO and human health

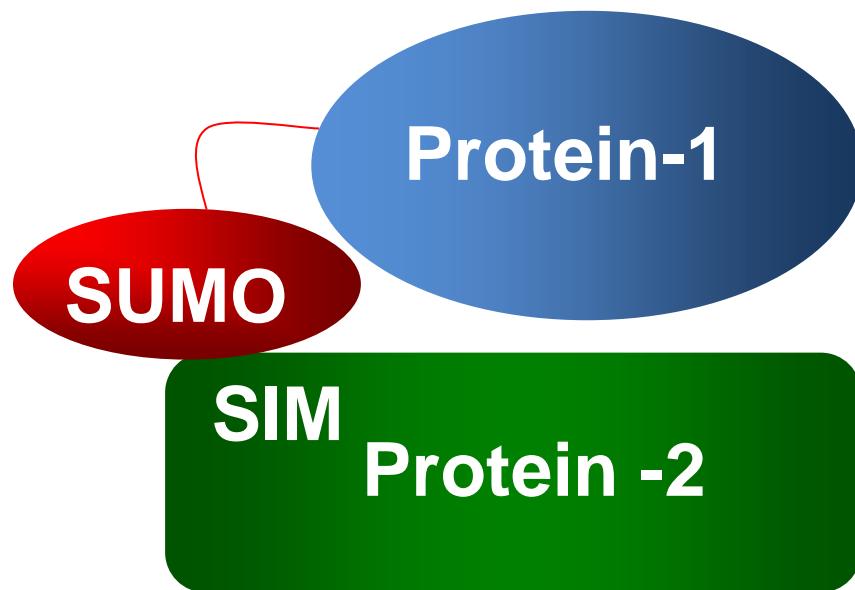


SUMO PPI project

- PI: Yuan Chen, PhD (City of Hope, Duarte, CA)

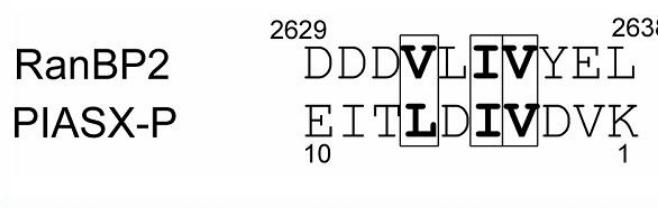
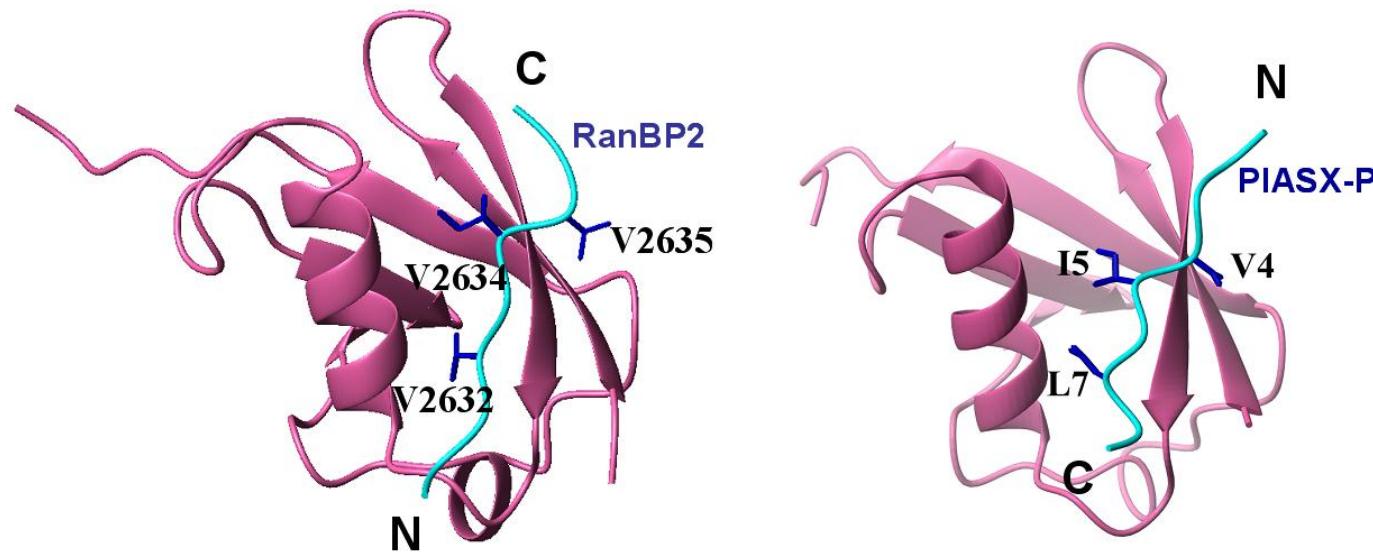


SUMO1-PIAS2 peptide complex
Song J, Zhang Z, Hu W, Chen Y,
J Biol Chem (2005) **280**: 40122



SUMO PPI project

- PI: Yuan Chen, PhD (City of Hope, Duarte, CA)
- MLPCN grant R21 NS066498-01



Song et al, PNAS, 2004
Song et al, JBC, 2005

Assay design for SUMO PPI project

Biological reagents:

- SUMO1-GST
- SUMO2-GST
- SUMO3-GST
- FITC-S1 peptide (F-S1)
- S1-FITC peptide (S1-F)
- FITC-S2 peptide (F-S2)

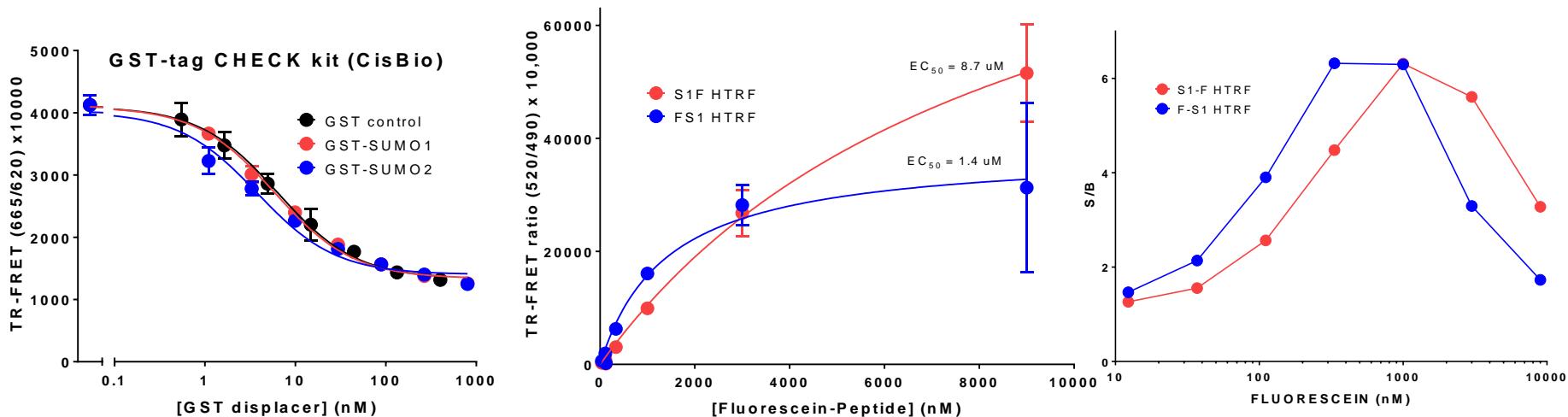
Detection reagents:

- Lumi4® Tb anti-GST (CisBio;
cat# 61GSTTLA)

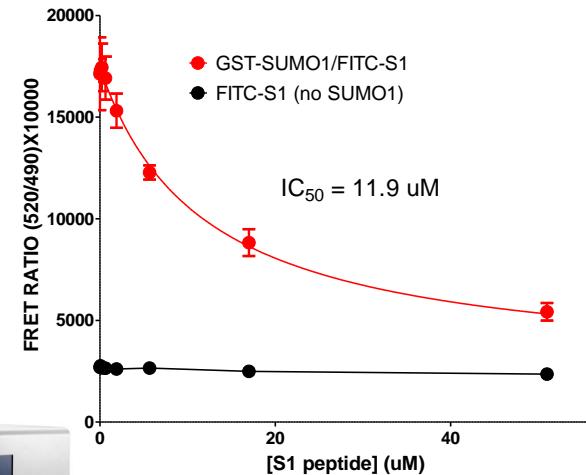
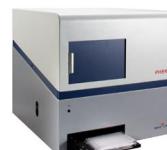
Tested a matrix of two SIM peptides and three SUMO paralogues in three assay formats

Established two potential pairs for further assay development: SUMO1/S1 and SUMO2/S1

Development and validation of HTS assay



- Binding assay for GST-SUMO1/FITC-S1 pair developed with HTRF Lumi4®-Tb anti-GST antibodies for primary HTS
- Developed counter screen assays
- Pilot HTS was performed on three assays in 1536-well plates using PHERAstars
- The project submitted into MLPCN



SUMO-PPI MLPCN screening project

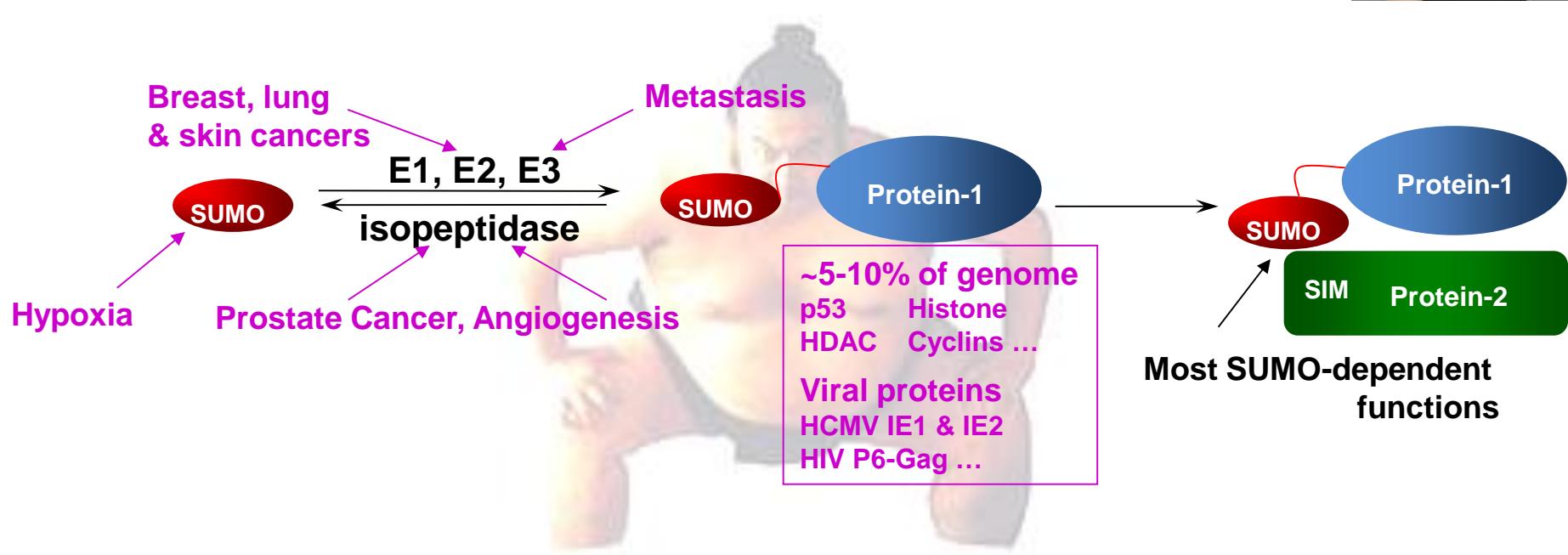
- Primary HTS (PubChem AID 602429)
 - TR-FRET assay using GST-SUMO1 and FITC-S1 peptide and Lumi4®-Tb anti-GST (1536-well plates)
 - 364K-compound MLSMR collection
- Hits profiled against PubChem data to eliminate artifacts
- Secondary assays:
 - FP assay SUMO1 and FITC-S1 (PubChem AID 624382)
 - TR-FRET SUMO2 and FITC-S1 (PubChem AID 624384)
 - FP assay SUMO2 and FITC-S1 (PubChem AID 624385)
- PubChem Summary AID 602467
- PI lab is currently characterizing the compounds identified in the screening

Danielle Key, Ada Kane, Siobhan Malany, PhD

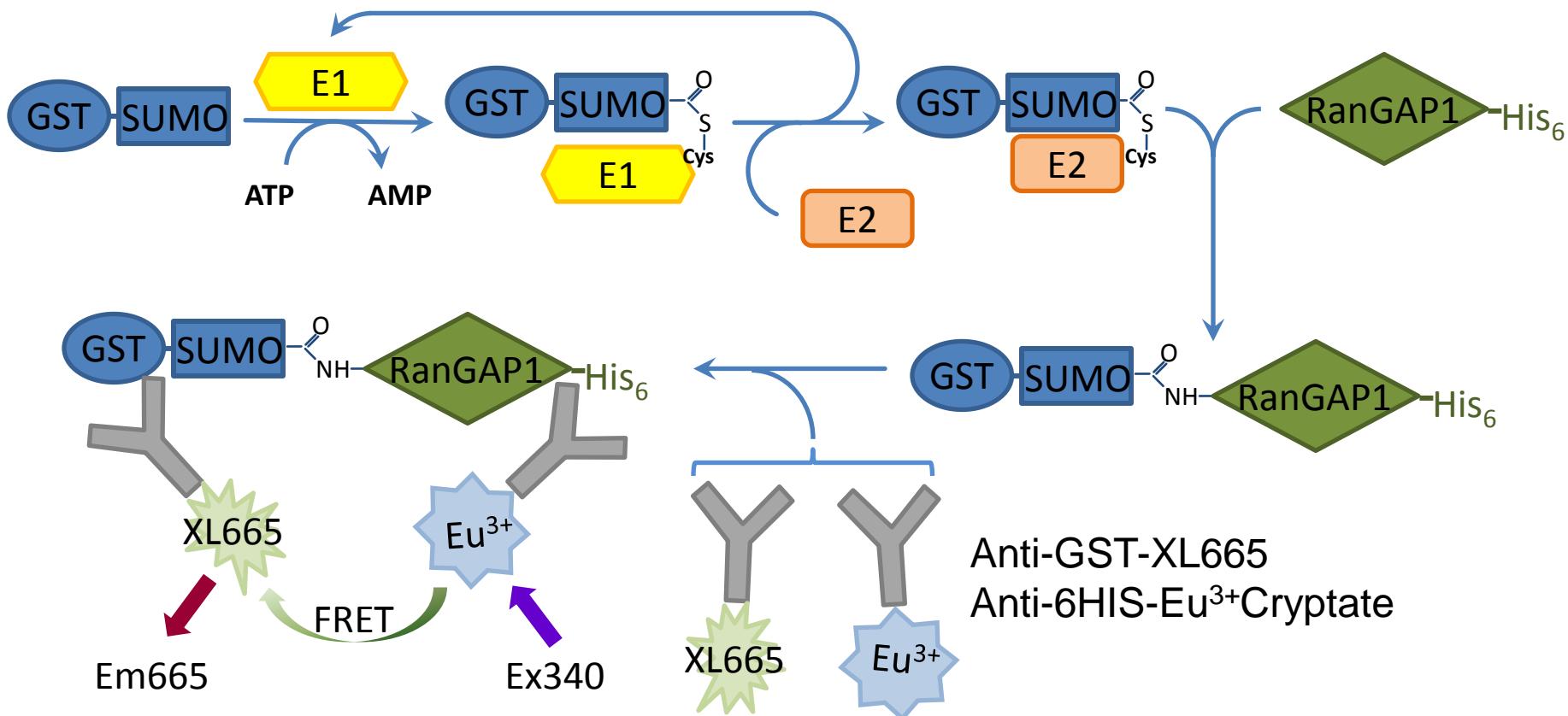
Eliot Sugarman, Kevin Nguyen, Eigo Suyama, PhD, Steve Vasile, PhD

SUMOylation project

- PI: Yuan Chen, PhD (City of Hope)
- MLPCN screening grant R03 MH084862-01



SUMOylation project: HTRF Assay



Enzymes and substrates (PI lab)

- E1 and E2
- GST-SUMO and His6-RanGAP1

Detection reagents (CisBio)

- Anti-GST-XL665 (61GSTXLB)
- Anti-6HIS-Eu³⁺Cryptate (61HISKLB)

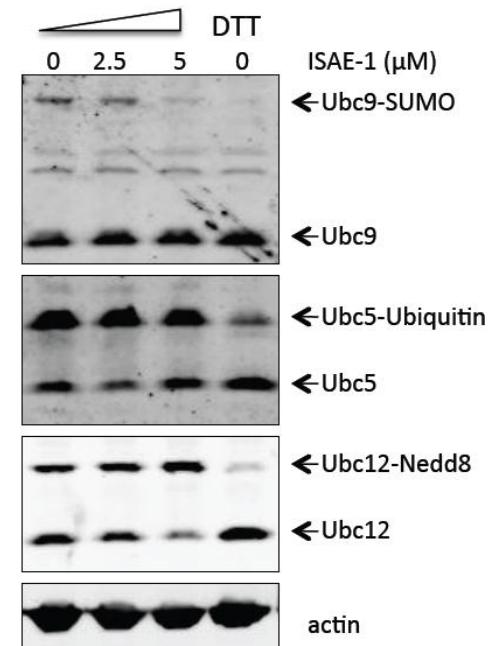
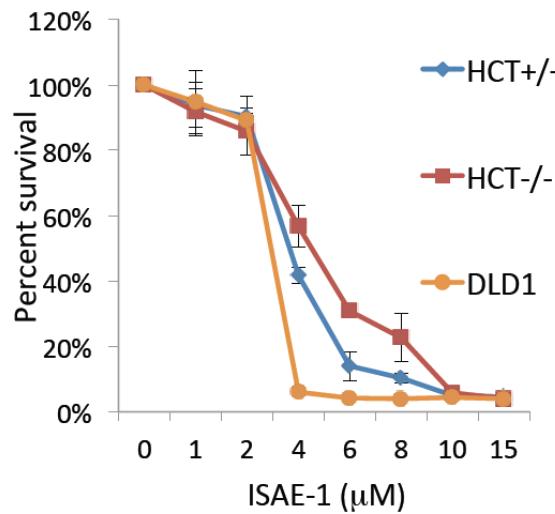
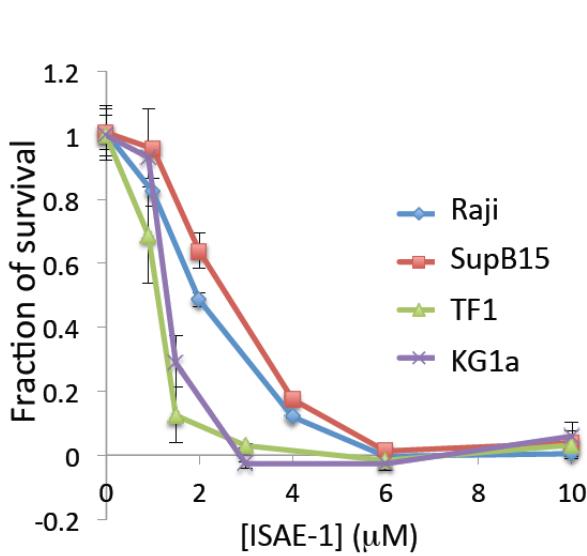
SUMOylation project: HTS

- Primary HTS (PubChem AID 2006)
 - 291K-compound MLSMR collection
- Hits profiled against PubChem data
- Secondary assays:
 - HTRF interference assay (PubChem AID 2069)
 - ALPHAscreen SUMOylation (PubChem AID 2018)
 - TR-FRET Ubiquitination (PubChem AID 2658)
- PubChem Summary AID 2011

*Sharon Colayco, Ekaterina Bobkova, PhD
Justin Rascon, Carlton Gasior, Fu-Yue Zeng, PhD*

SUMOylation project: Probe compound

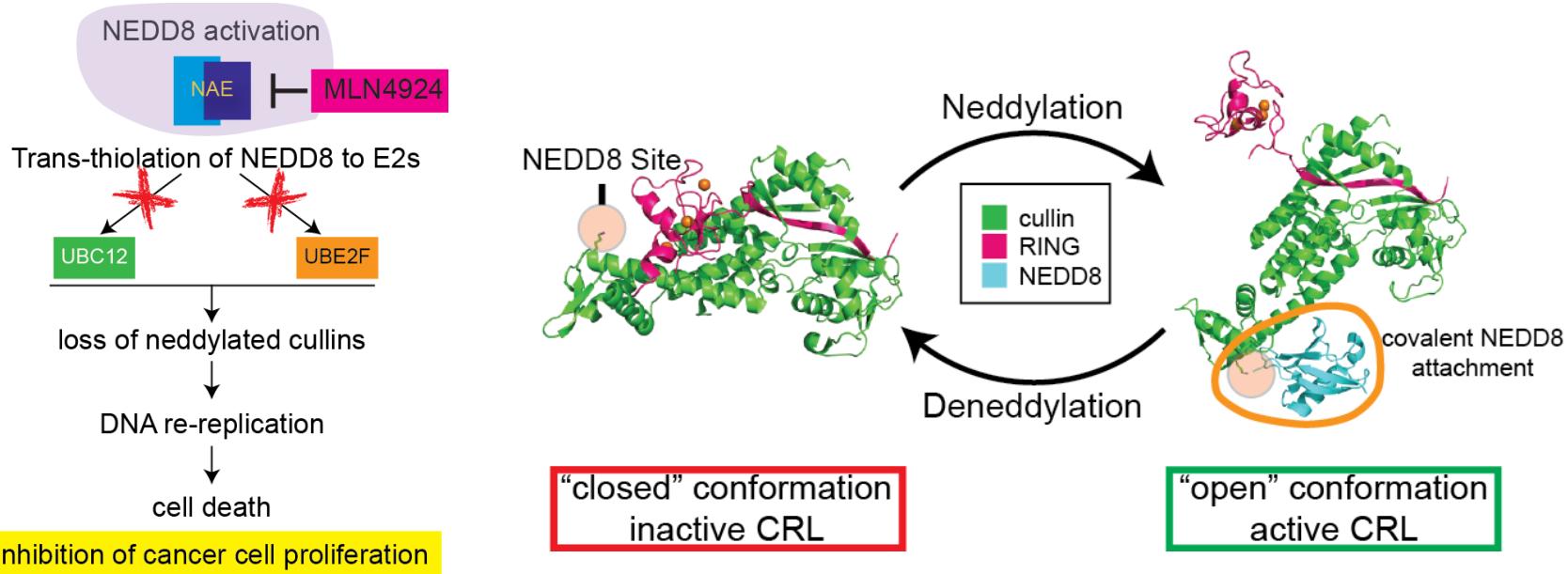
- The probe compound kills cancer cells
- The probe specifically inhibits SAE in the cells



Baozong Li, PhD, Yi-Jia Li, PhD, Yuan Chen, PhD

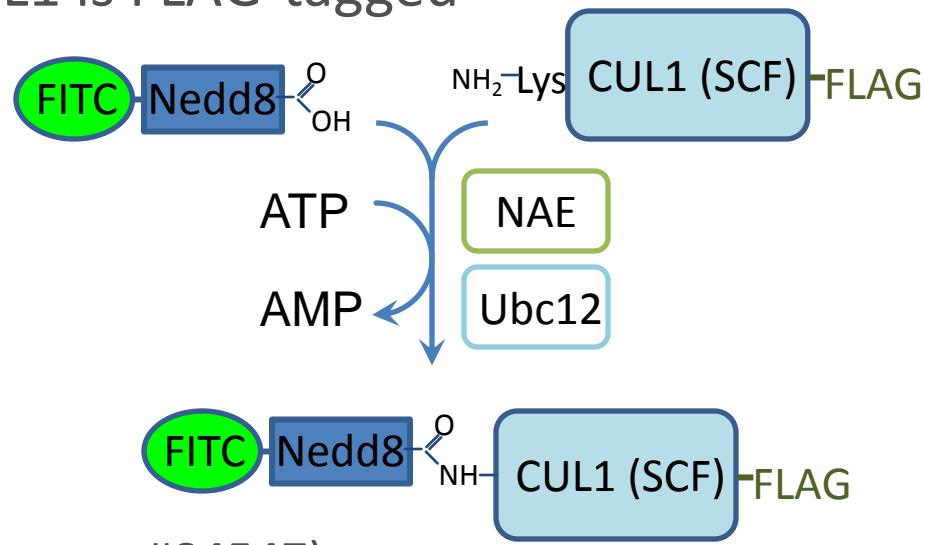
CUL1 Neddylation Project

- PI: Matt Petroski (SBMRI)
- The assay targeting NAE and Ubc12 designed and developed in collaboration with PI



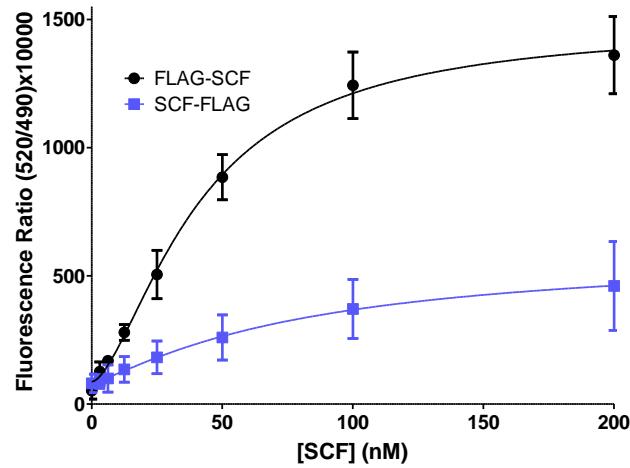
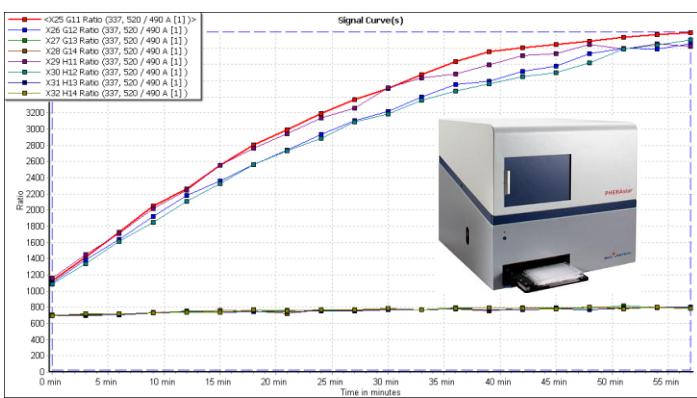
Assay captures CUL1 neddylation

- Assay Reagents:
 - NAE (His6-tag) and Ubc12 (His6-tag)
 - SCF (Skp1-CUL1-F-box) – CUL1 is FLAG-tagged
 - Nedd8
 - ✓ Fluorescein-Nedd8
 - ✓ Biotin-Nedd8
 - Anti-FLAG antibodies
 - ✓ Eu-anti-FLAG (Life Tech)
 - ✓ Lumi4®-Tb anti-FLAG (CisBio)
 - ✓ Streptavidin DyLight650 (Pierce; cat #84547)
 - ✓ Streptavidin DyLight488 (Pierce; cat #21832)

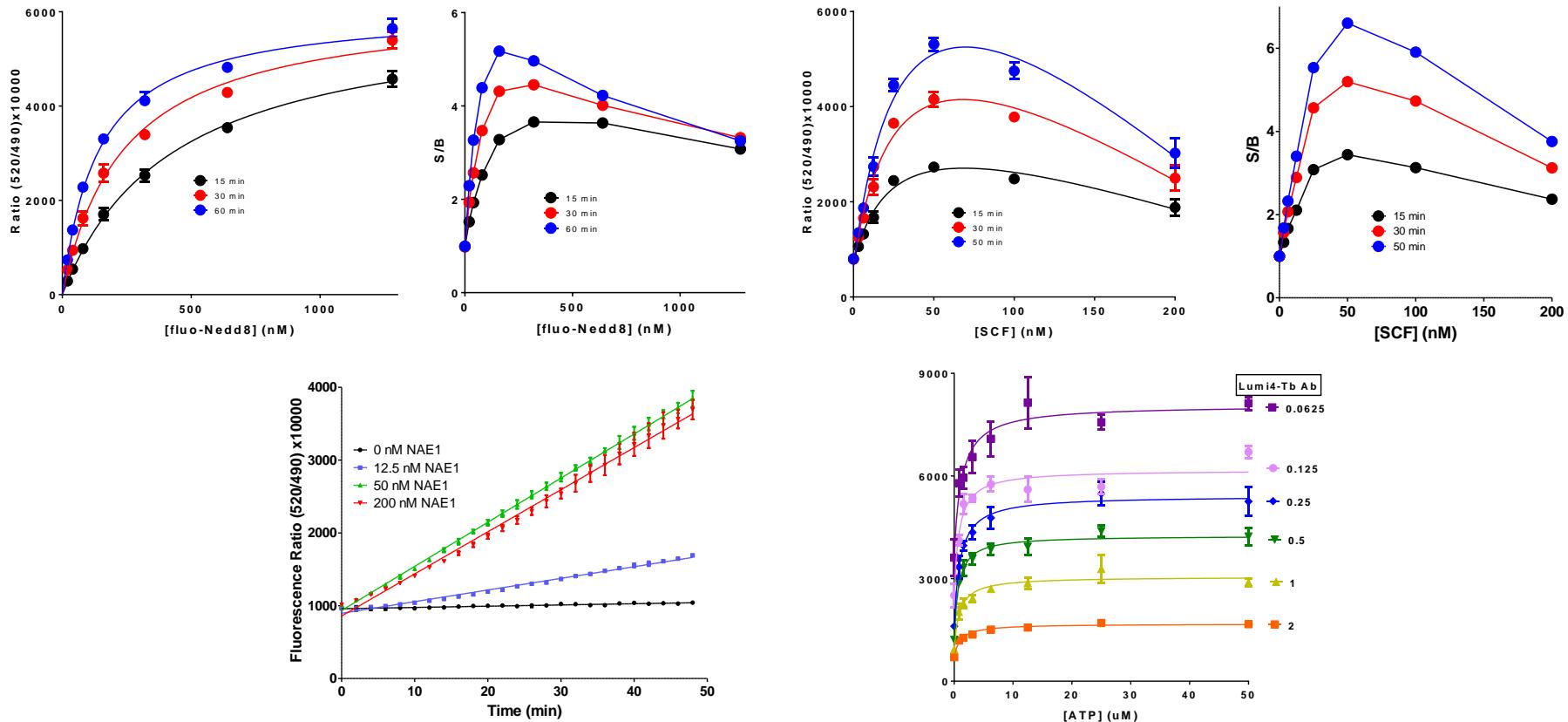


Nedd8 and CUL1 reagents

- Biotin-Nedd8 assay demonstrated low S/B and required end-point assay mode
- FITC-Nedd8 is consistent with kinetic mode
 - Lumi4®-Tb anti-FLAG Ab has no effect on the reaction
- CUL1 with N-terminal FLAG tag provides higher S/B in the assay than C-terminal FLAG tag

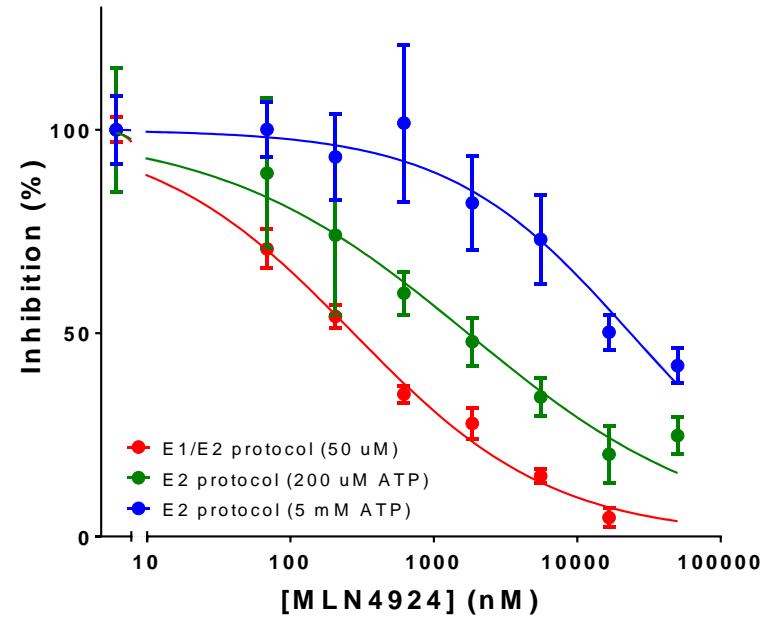
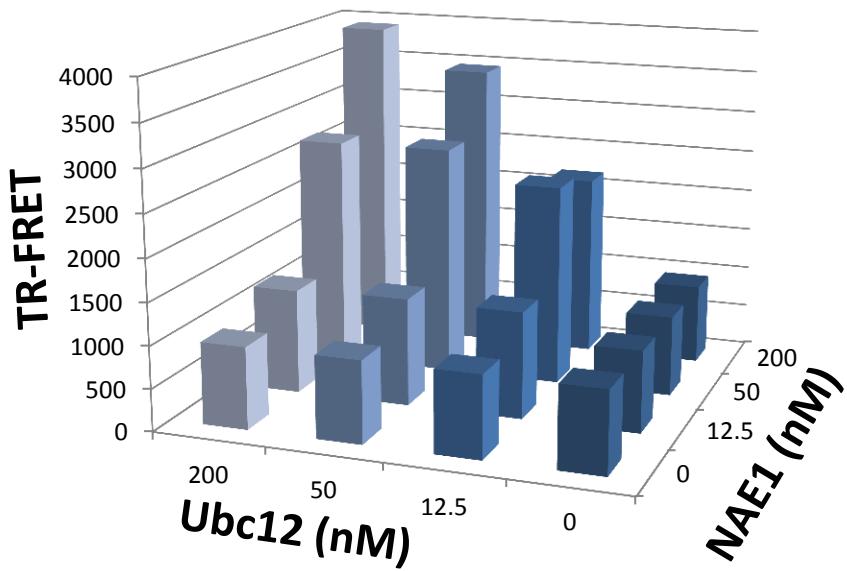


CUL1 neddylation assay development



- All assay parameters were optimized
- Many reagents tested in “matrix” experiments

CUL1 neddylation assay modalities



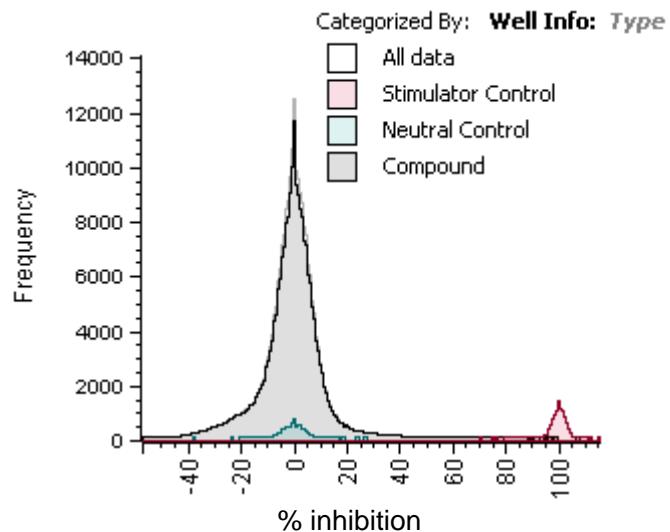
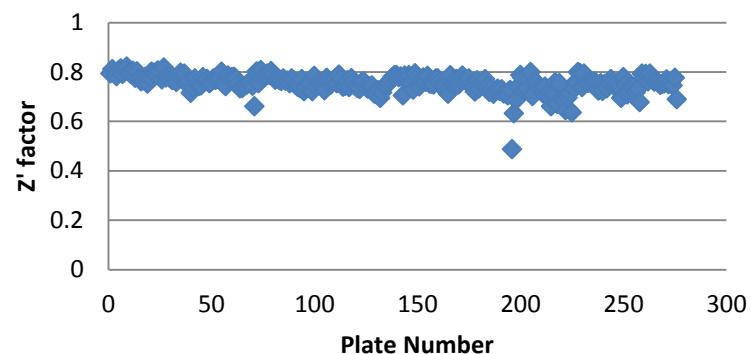
- Fine-tuned enzyme and substrate concentrations and the order of reagent addition to establish two assay modalities (sensitive to inhibition of either E1&E2 or E2)
- Submitted to MLPCN (R03 grant DA034599-01)

CUL1 neddylation project summary

- 364K compounds screened in 1536-well plates (PubChem AID 651699)
- Hits profiled vs other HTS data
- 27 hit reconfirmed in dose response mode (PubChem AID 652247)
- All compounds appear to inhibit NAE

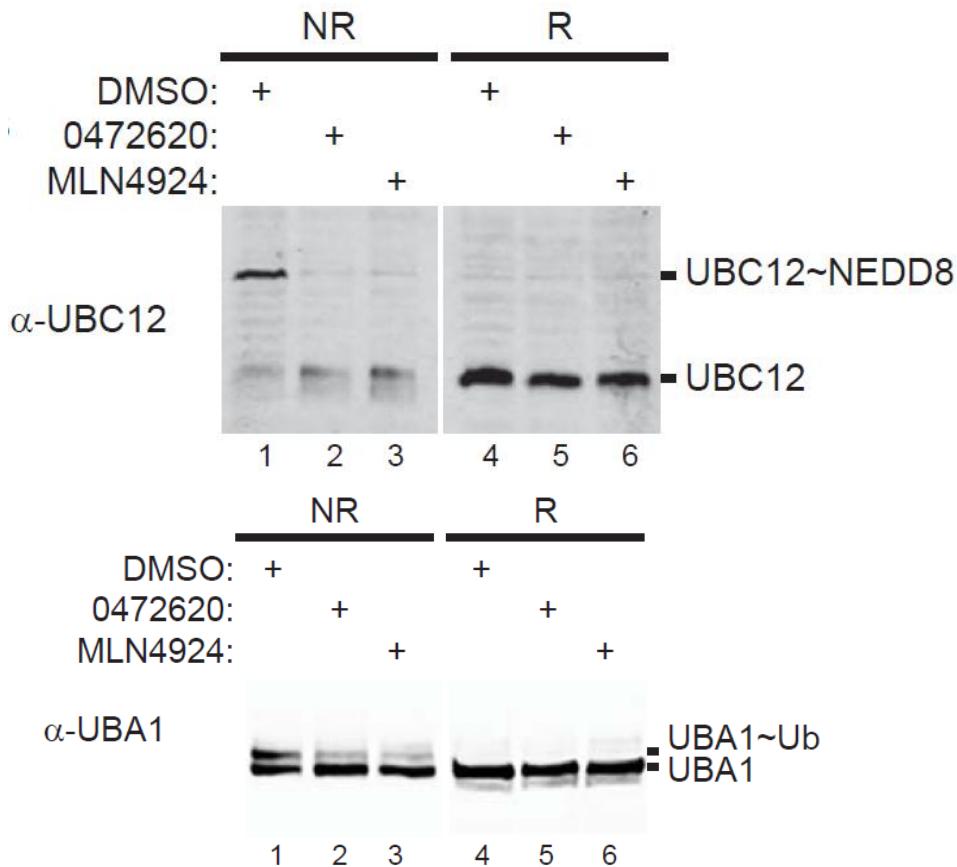
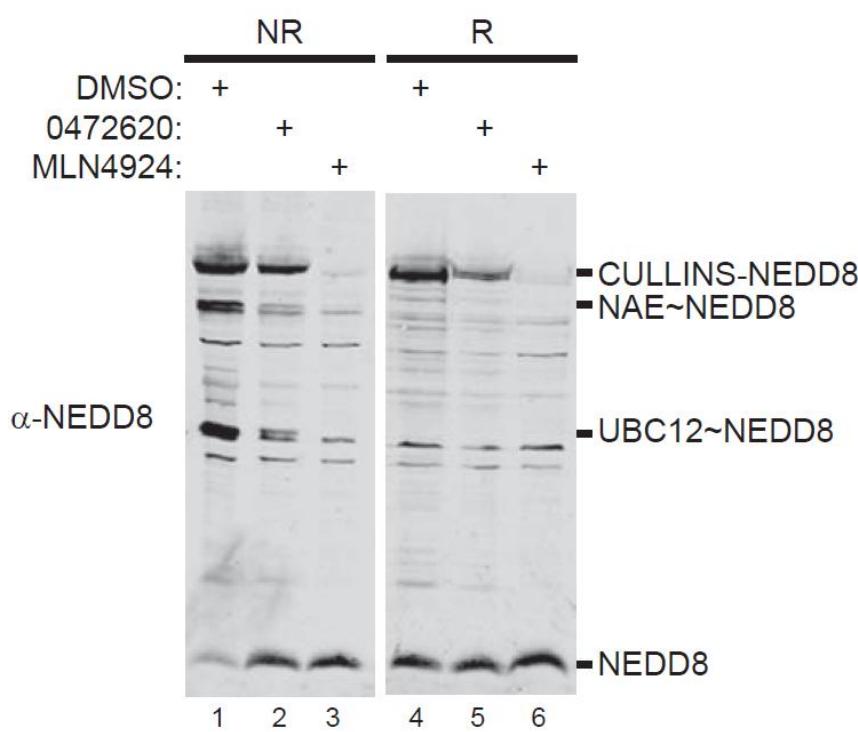


CV (positive control)	4.60%
CV (negative control)	6.50%
S/B	3.3 + 0.4
S/N	36.2 + 6.7
Signal window	13.1 + 3.6
Z'	0.75 + 0.04
hits*	2123
ordered hits	1922

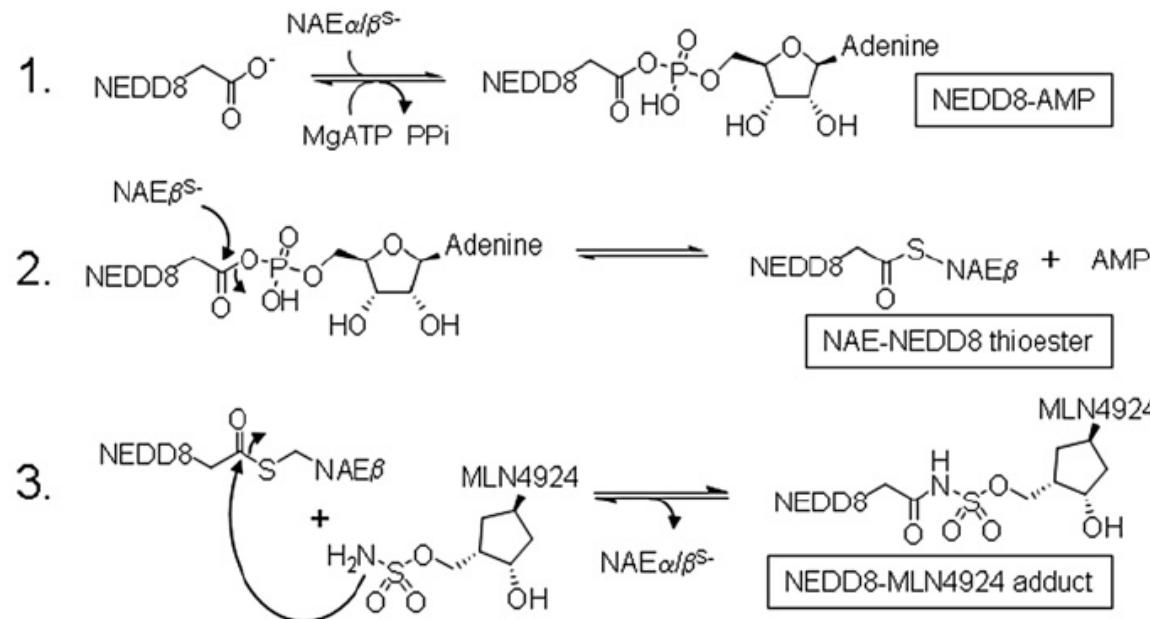
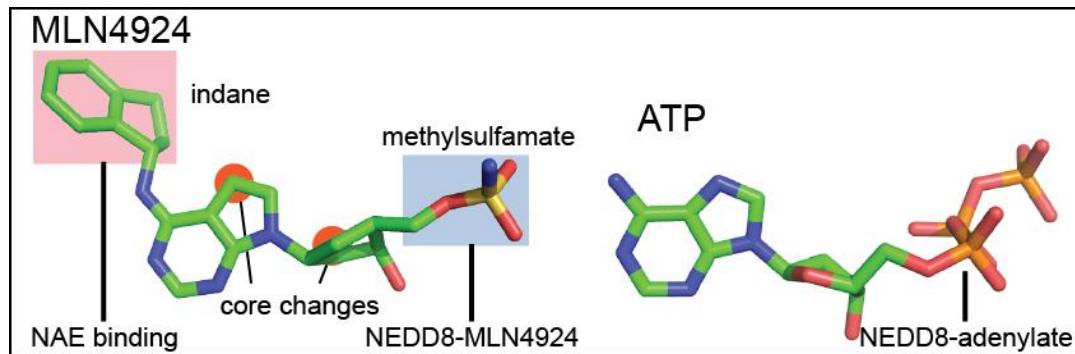


Probe compound is active in the cells

One NAE probe scaffold is active in cell-based
cullin neddylation assay



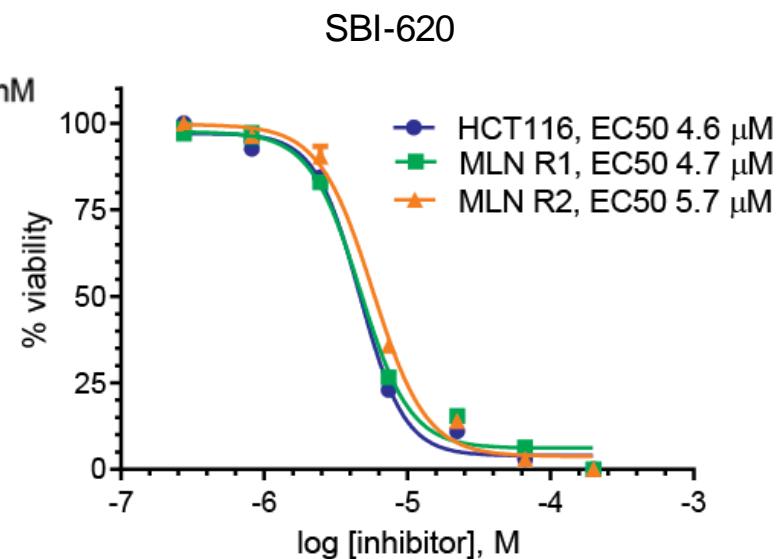
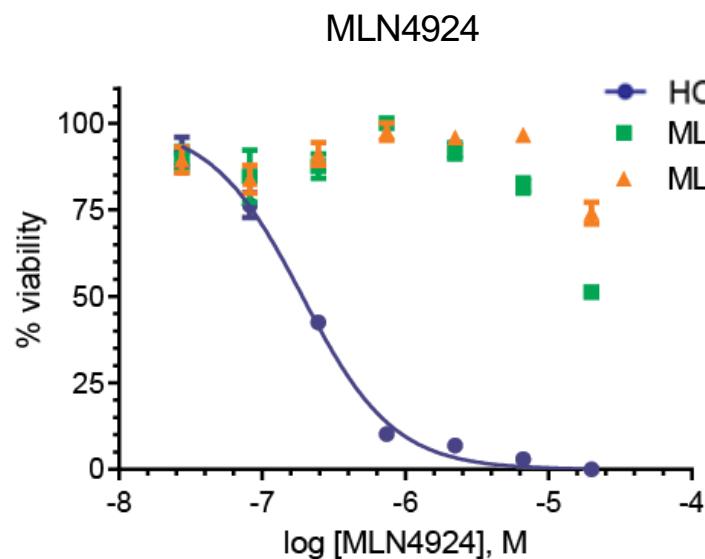
MLN4924 has a complex mechanism of action



Brownell et al, 2010,
Mol cell 37:102-111

Overcoming MLN4924 resistance

- PI's lab demonstrated that cancer cells could become resistant to MLN4924 through a single mutation in ATP or Nedd8 binding sites (Cell Rep. 2012, 1(4):309-16)
- The probe compound identified in our project kills MLN4924-resistant cells

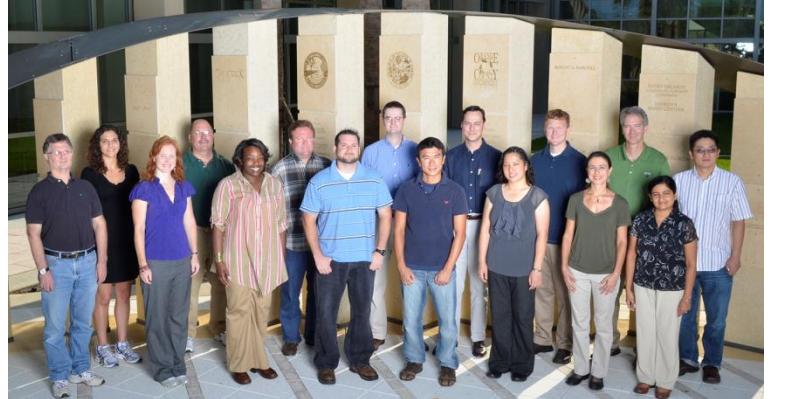


Concluding remarks

- HTRF approach worked well for projects involving UBL signaling and interactions performed at CPCCG
- Several first-in-class chemical probes with biological activities in SUMOylation or neddylation systems were identified with the help of these assays
- Access to HTS data of diverse assays, e.g. with similar targets or similar detection, allows early identification of assay artifacts and promiscuous compounds
- Utilization of a reagent toolbox for diverse detection approaches helps with rapid hit validation and profiling

Acknowledgements

- Chen-Ting Ma
- Sharon Colayco
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- Siobhan Malany
- Sylvia Kim
- Carlton Gasior
- Fu-Yue Zeng
- Thomas Chung
- Yuan Chen (City of Hope)
- Larry Tong (COH)
- Aileen Alontaga (COH)
- Matt Petroski (SBMRI)
- Julia Toth (SBMRI)
- Michael Jackson
- Kristiina Vuori
- John Reed



Lake Nona, Orlando, FL ↑

← La Jolla, San Diego, CA

NIH Roadmap grants:

- ✓ 5U54HG005033
- ✓ 5U54HG003916
- ✓ R03 MH084862
- ✓ R21 NS066498
- ✓ R03 DA034599