Targets, Tools, and Drugs: Advances in Molecular Discovery at BU

April 4, 2017

Boston University Office of the Vice President and Associate Provost for Research

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Adrian Whitty

Associate Professor
Chemistry
CAS

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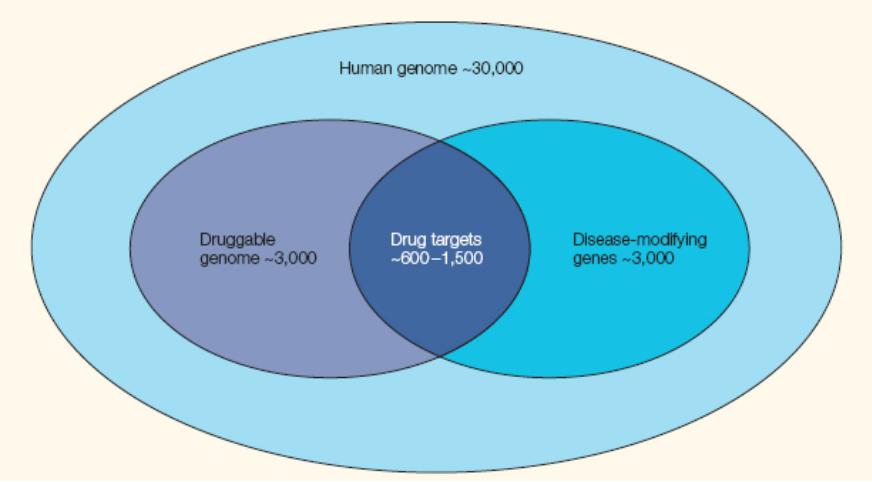
Drugs and probes for highly challenging protein-protein interaction (PPI) targets

Most disease modifying genes are not druggable by conventional means



Hopkins & Groom, (2002).

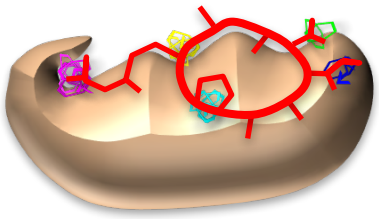
- **Topology** – lack suitable sized cleft or pocket
- **Polarity** – binding site too polar (or too hydrophobic)
- **Disorder** – additional energetic barrier to ligand binding; difficult to employ structure-based approaches

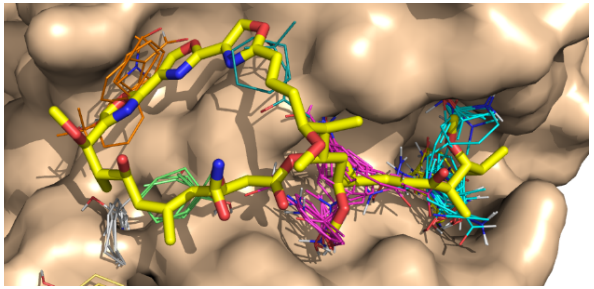
OUR APPROACH: Non-canonical drug chemotypes

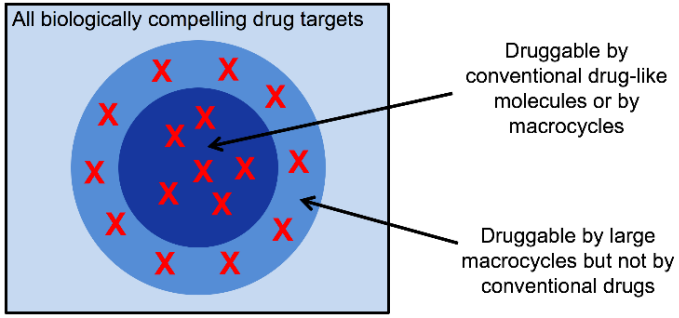
- **High MW synthetic macrocycles**
- **Targeted covalent inhibitors**

Team approach (with Porco, Allen, Vajda labs, Brown/BU-CMD, *et al.*)

EXAMPLE: Synthetic Macrocycles for PPI targets







Vajda
- Computational assessment of target "druggability"

Whitty
- Clone & express target, develop assay

Porco/ Brown/ CMD
- Design and make MC screening library
- Screen target, validate hits

Allen
- Obtain X-ray structures of bound hits
- Biological evaluation of hits

Gilmore
- Further optimization of compounds

Property [a]	Conventional drugs	Oral MC drugs
MW	≤500 [b]	600–1,200 [c]
clogP	≤5 [b]	−2 to 6 [c]
TPSA	≤140 A^2 [d]	≥0.23 x MW A^2 [e]
$PSA_{n.p.}$	≤140 A^2 [d]	≤140 A^2 [e]
HBD	≤5 [b]	≤12 [c]
HBA	≤10 [b]	12–16 [c]
Rotatable Bonds	≤10 [d]	≤15 [c]

From Villar et al., *Nat. Chem. Bio.* (2014)

OPPORTUNITY: We are keen to collaborate with BU investigators who seek to inhibit challenging PPI targets

Beyond HTS: BU-CMD Strategies for Focused Molecular Discovery

Lauren Brown

Assistant Director | Center For Molecular Discovery
Research Assistant Professor | Chemistry, CAS

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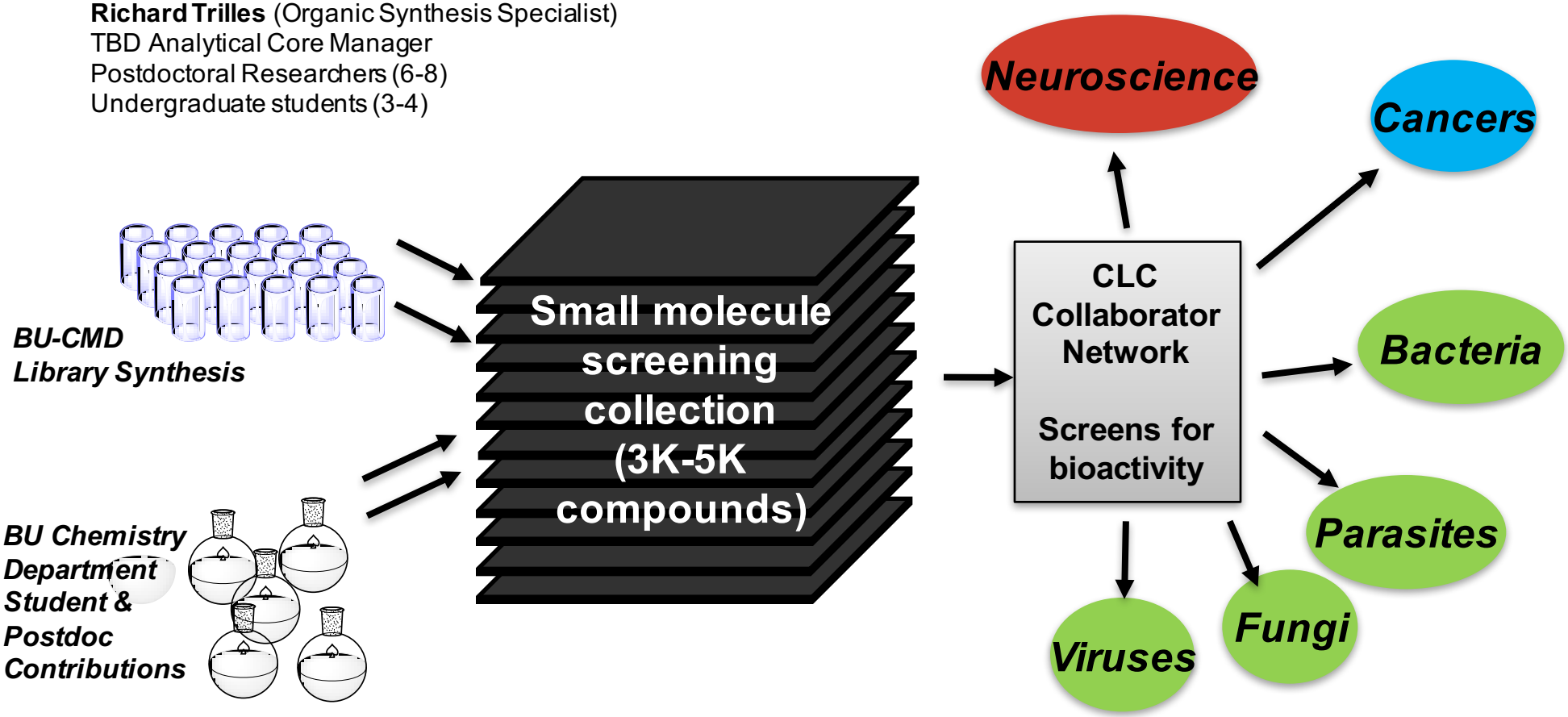
Center for Molecular Discovery: A Small Molecule Resource for Biomedical Research

Faculty:

John Porco (Director), **Lauren Brown** (Asst. Director), **Karen Allen, Aaron Beeler, Scott Schaus, Adrian Whitty, Sandor Vajda, Arturo Vegas**

Staff:

Lisa Holik (Center Administrator)
Richard Trilles (Organic Synthesis Specialist)
TBD Analytical Core Manager
Postdoctoral Researchers (6-8)
Undergraduate students (3-4)



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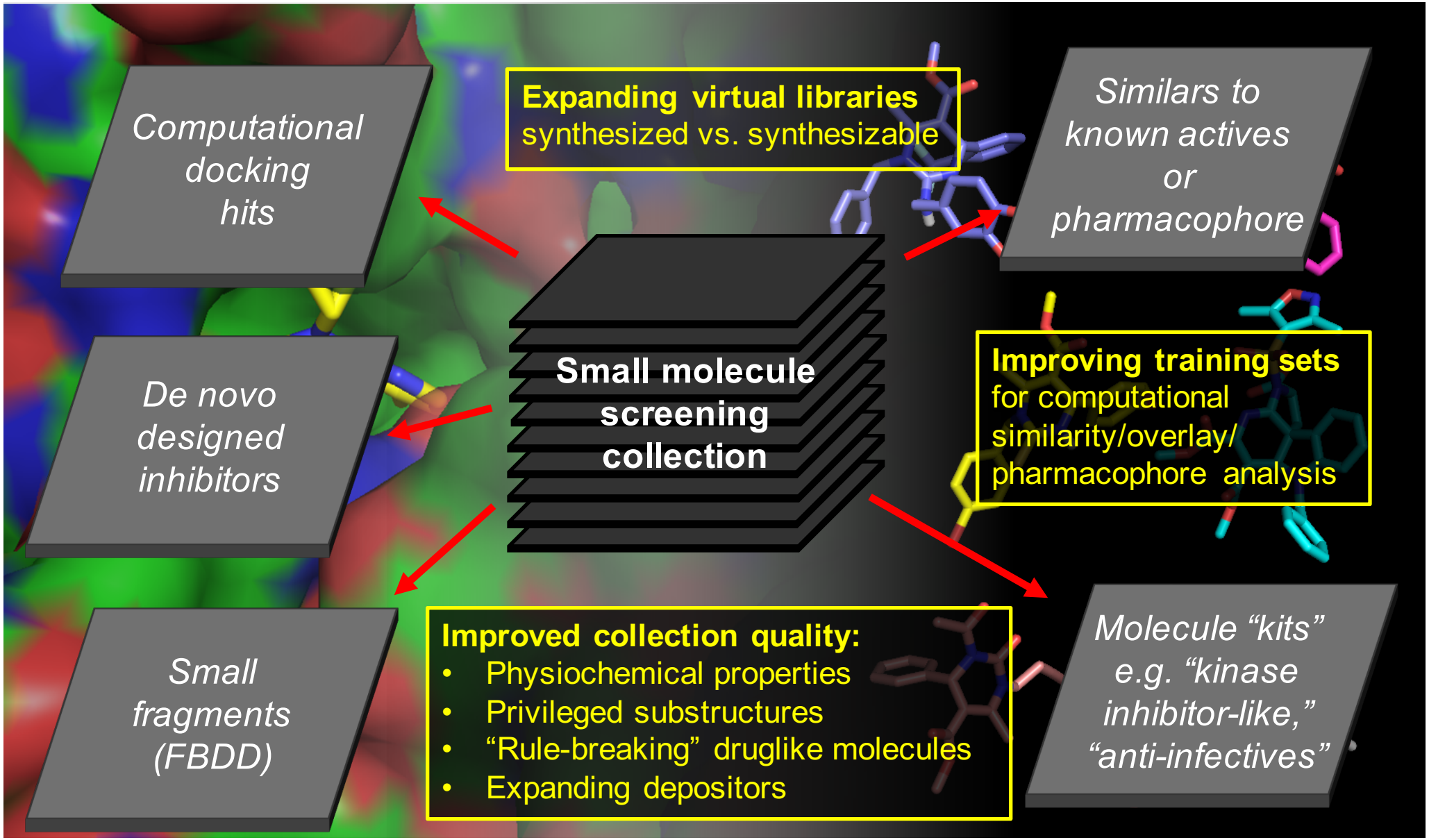
Strategies for Discovery Beyond High-Throughput Screening: Focused Subset Generation

Known target **vs.** **Known actives**



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Carmela Abraham

Professor
Biochemistry, Medicine, and Pharmacology & Experimental Therapeutics
MED

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Neuroprotection in Alzheimer's disease (1)

Reducing the levels of the neurotoxic Amyloid beta protein (Abeta)

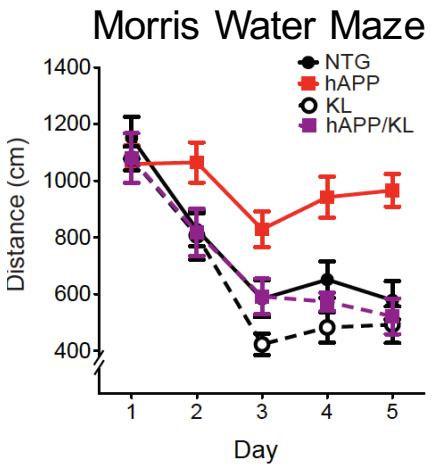
- The Abeta peptide is toxic to neurons and their synapses
- High throughput screen (HTS) to reduce Abeta identified compound Y
- Y analogs, such as Y10, inhibit the receptor tyrosine kinase cKit
- Inhibitors of a down stream effector also inhibit Abeta production
- Efforts are under way to optimize Abeta lowering compounds in close collaboration with Drs. Porco, Brown and Camara

Neuroprotection in Alzheimer's disease (2)

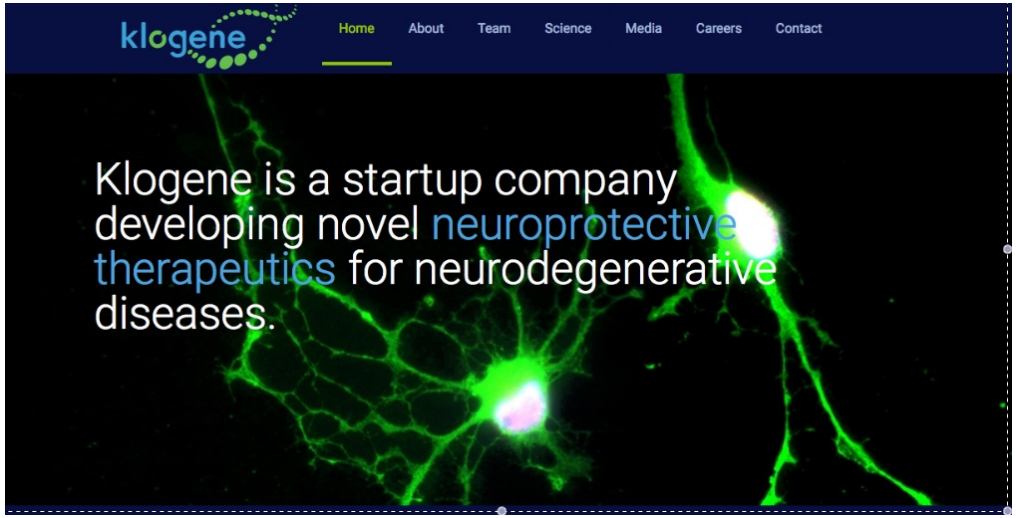
Increasing the levels of the neuroprotective and cognition enhancer protein Klotho

- Klotho is a large protein hormone that is essential for the function of most organs, including the brain
- Our group found that Klotho is low in the aged brain, is protective against Abeta and glutamate excitotoxicity *in vitro*, and Abeta *in vivo*
- Klotho also improves remyelination in a mouse model of MS
- Two HTS are being conducted to identify compounds that enhance Klotho expression using a novel coincidence reporter

Klotho overexpression improves cognitive deficits and ameliorates synaptic hippocampal dysfunction in the J20 model without affecting Abeta levels



A new company is born; Klogene



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Collaborative Research in the Beeler Lab

Aaron Beeler

Assistant Professor
Chemistry
CAS

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BEELER RESEARCH GROUP @ BU

Tweets by @BeelerGroupBU

Beeler Group BU Retweeted

Marius Lutz @ZurichChemist

Did the course last year and it was a great decision to do so.

EdX class in medicinal chemistry chemjobber.blogspot.com/2017/03/edx-cl… via @Chemjobber

09 Mar

Beeler Group BU Retweeted

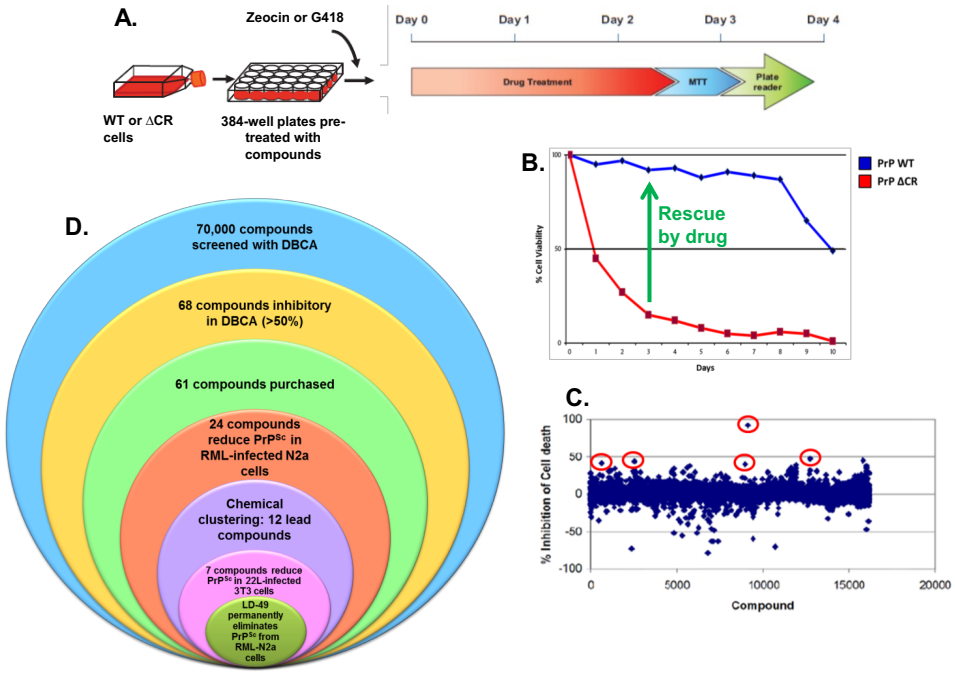
Angewandte Chemie @angew_chem

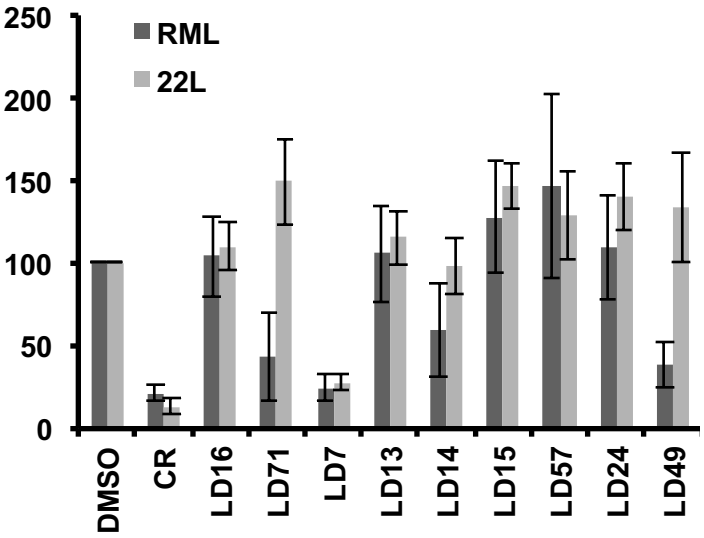
Sad to hear 1994 #chemnobel George A. Olah passed away yesterday chemistryviews.org/details/ezine/… @ChemistryViews @Wiley_Chemistry

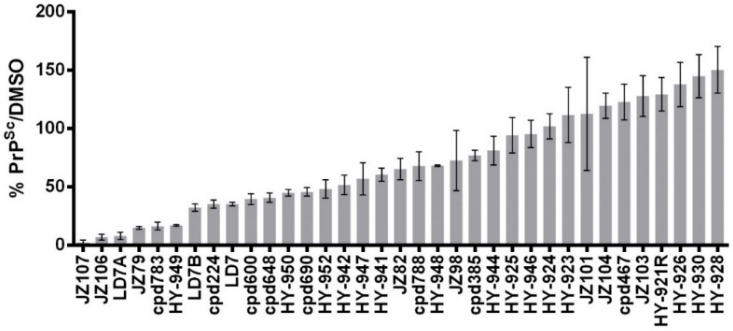
09 Mar

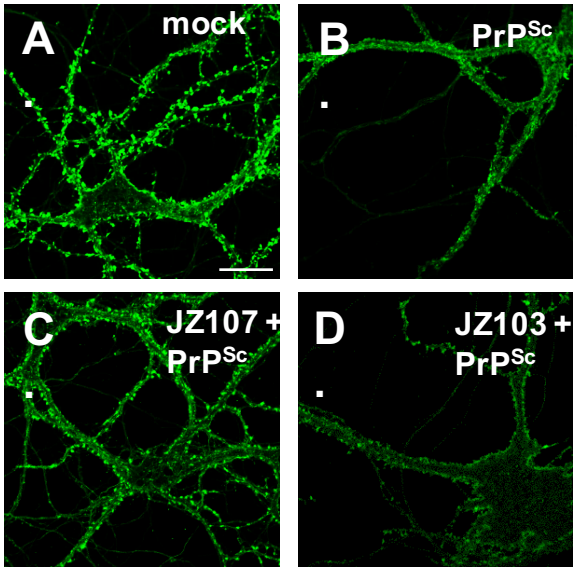
The Beeler Research Group is truly multidisciplinary, combining organic chemistry, engineering, and biology to solve problems in medicinal chemistry. All of these elements are combined and directed toward significant problems in human health. The Beeler Group is addressing focused disease areas (e.g., schizophrenia, Parkinson's, cystic fibrosis), as well as project areas with broader impact potential (e.g., new methods for discovery of small molecules with anti-cancer properties).

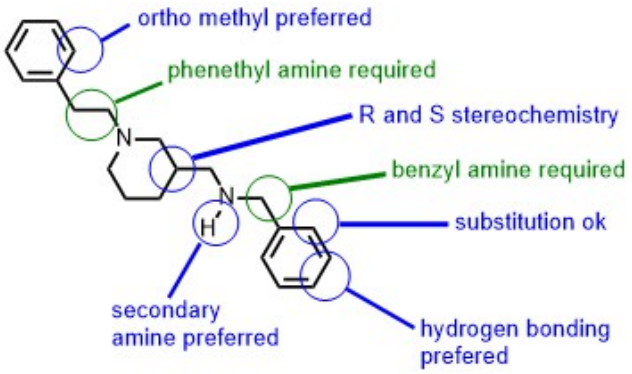
Collaboration with David Harris @ BUMC Department of Biochemistry





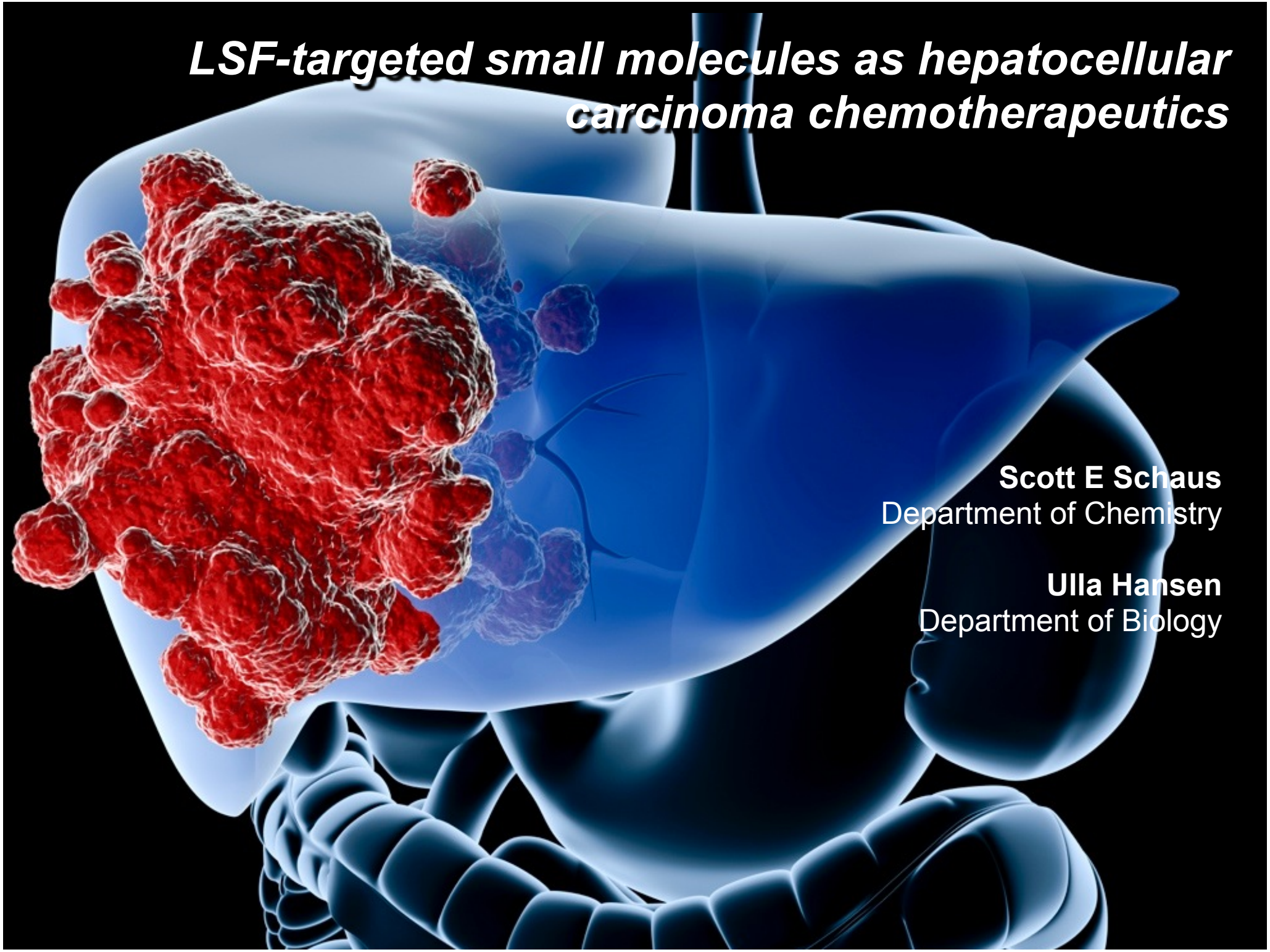






J. Bio. Chem. **2016,** *291,* 26164-26176

LSF-targeted small molecules as hepatocellular carcinoma chemotherapeutics

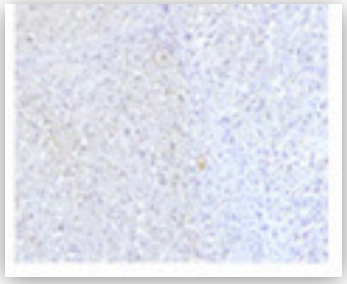


Scott E Schaus
Department of Chemistry

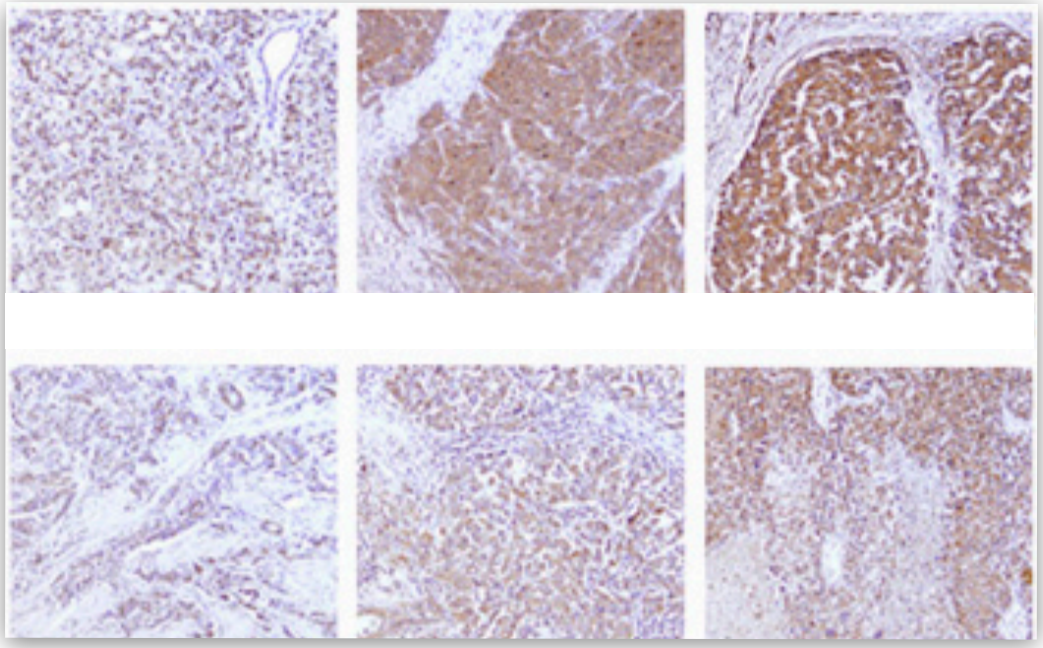
Ulla Hansen
Department of Biology

Clinical relevance of LSF in hepatocellular carcinoma

normal liver



Stage 1 Stage 2 Stage 3



poorly differentiated

moderately differentiated

Hansen & Sarkar, *PNAS* **2010** *107*, 8357.

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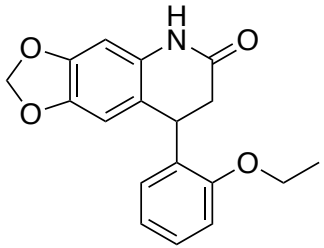
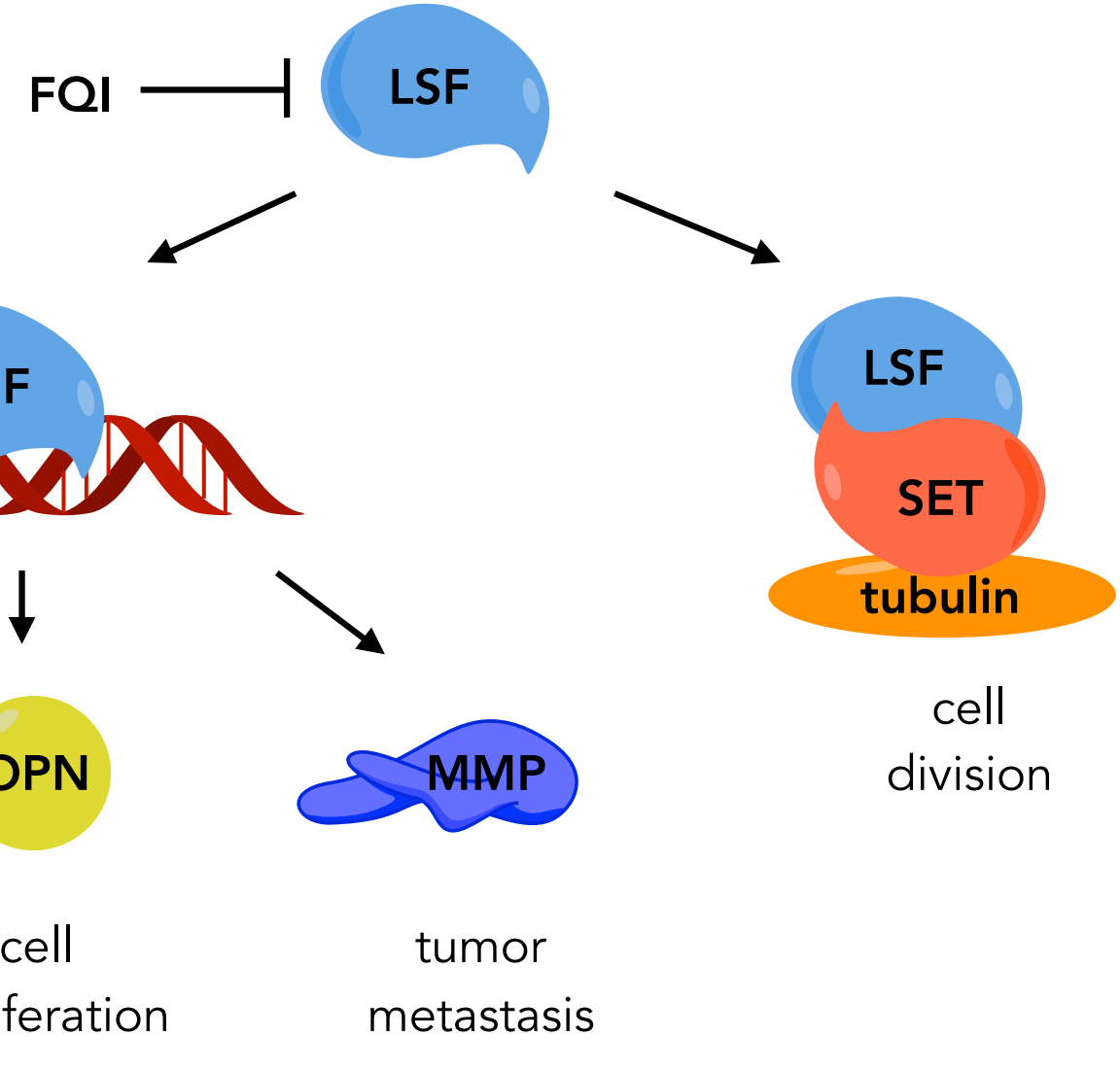
Late SV40 Factor (LSF)



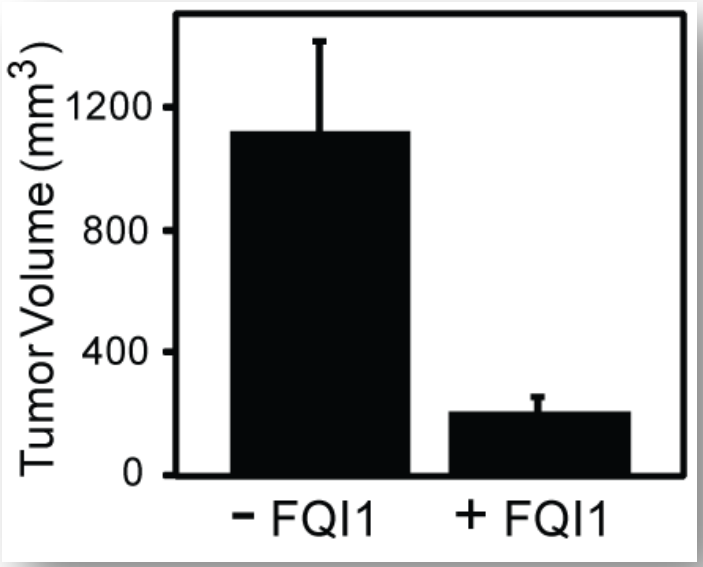
Factor Quinolinone Inhibitors



Schaus, Hansen & Sarkar. *J Am Cancer Res* **2012**, 269.

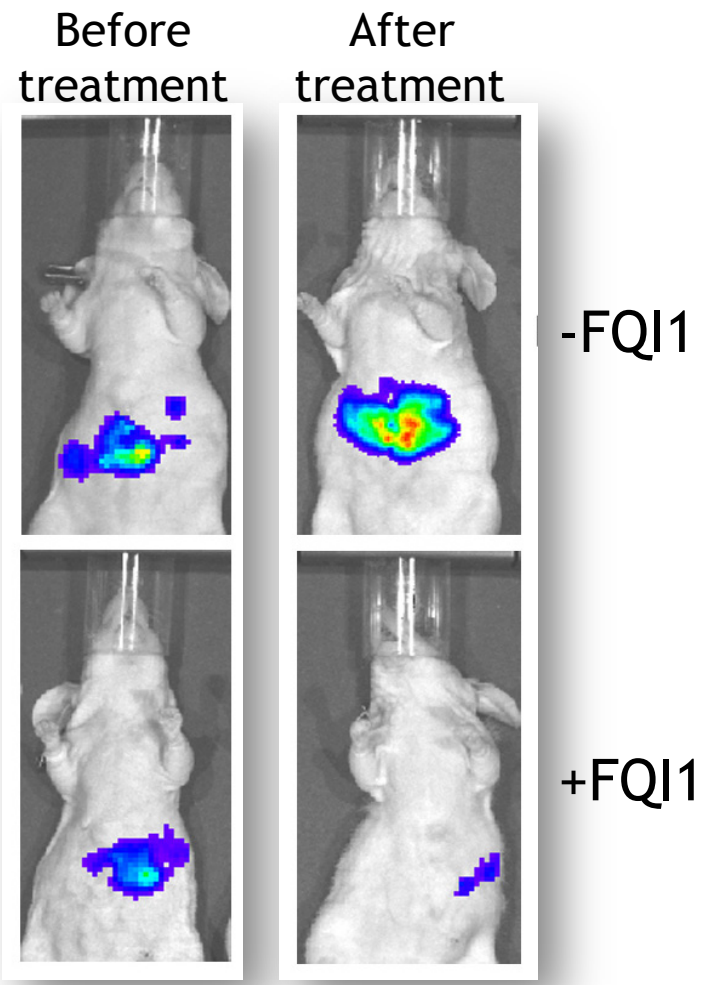
In vivo Tumor Reduction

xenograft model



2 mg/kg I.P.
2 weeks, treat on 3rd day
followed by 2 weeks no treatment

orthotopic



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Structure-Aided Inhibitor Design

Karen N. Allen

Professor
Chemistry
CAS

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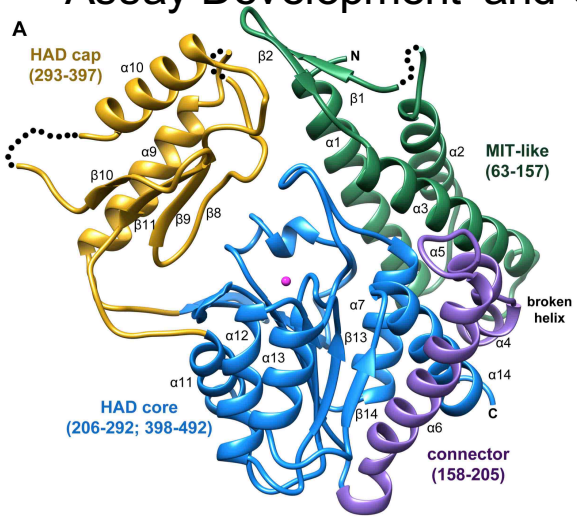
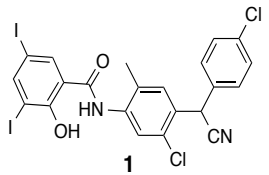
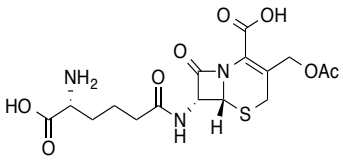
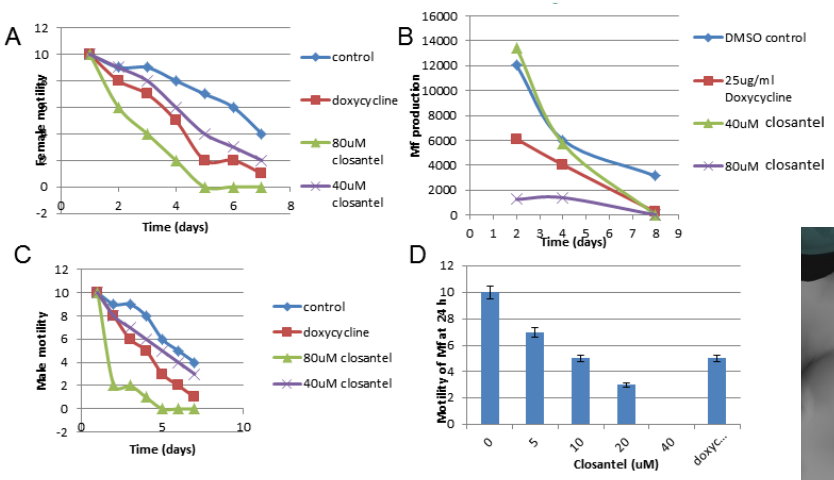
Assay Development and Screening

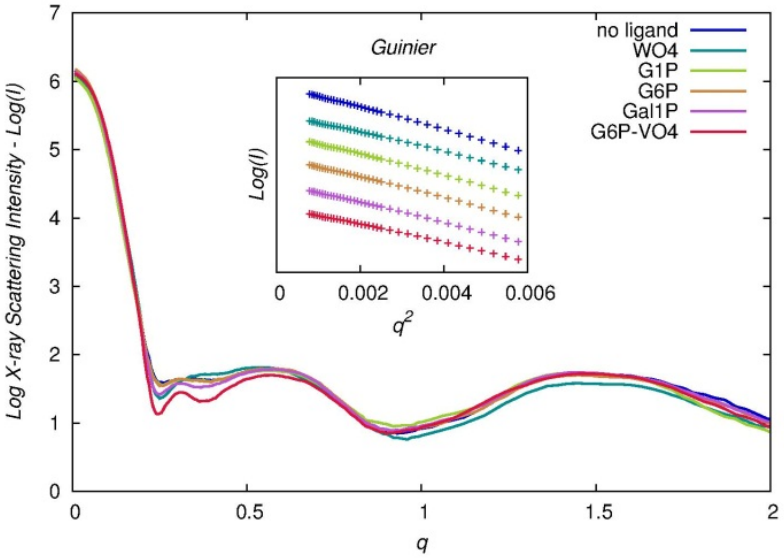


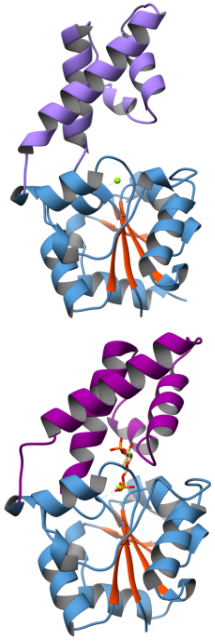




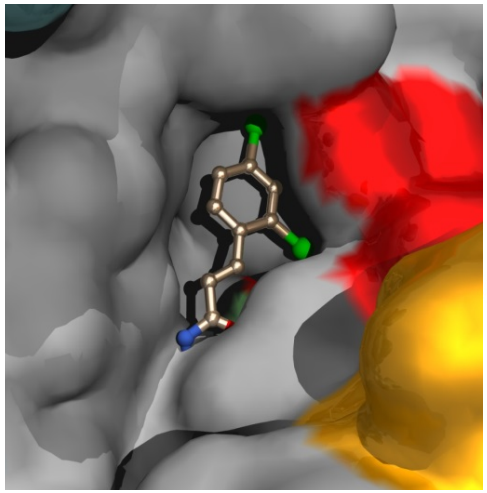


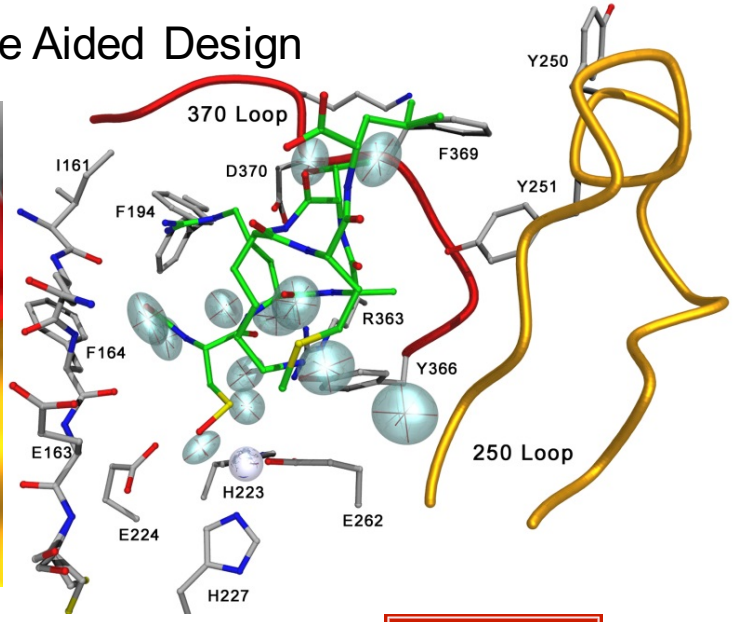
SAR by SAXS





Structure Aided Design





Targeting a Novel Signaling Interface in Metastasis

Mikel Garcia-Marcos

Assistant Professor
Biochemistry
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NORMAL CELLS

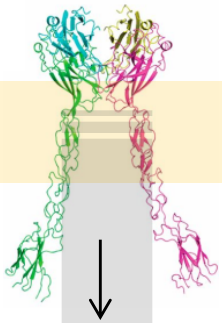
CANCER CELLS

Outside

GPCR

Inside

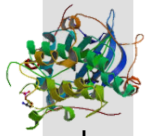
"Oncogenic" receptors



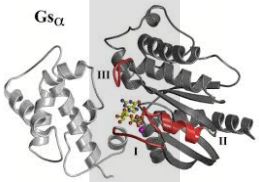
Overexpressed in most carcinomas

"GIV"

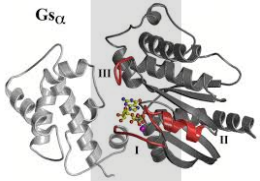
Non-GPCR G protein activators

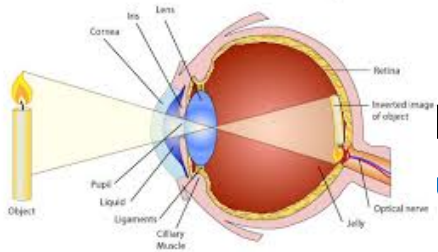


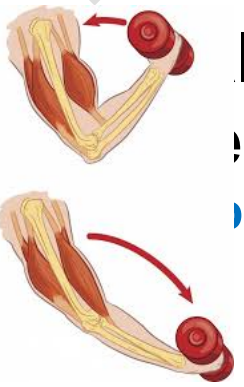
G protein



G protein





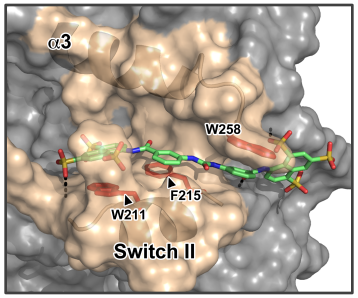


CANCER METASTASIS

"anything could happen"

High-throughput screen for “good” inhibitor molecules:

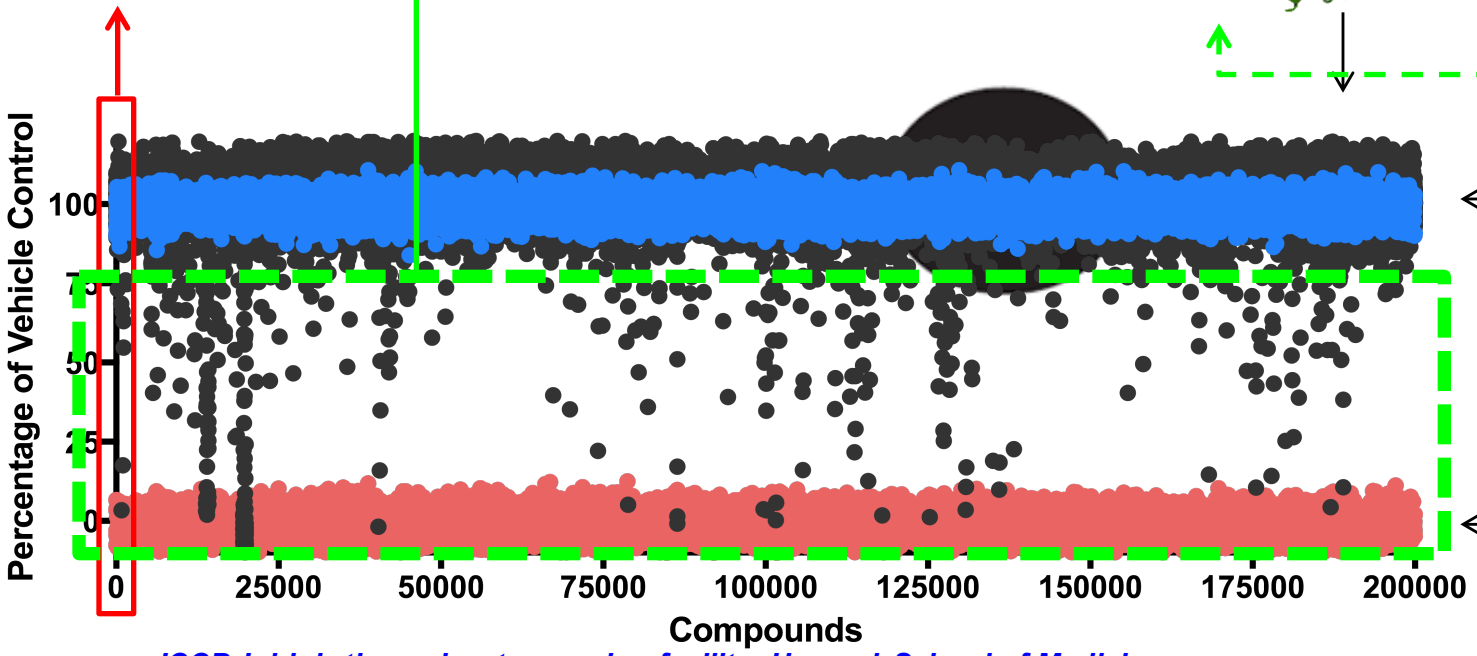
From 1,000 to 200,000 compounds



FILTER 1
Confirm in other assay formats

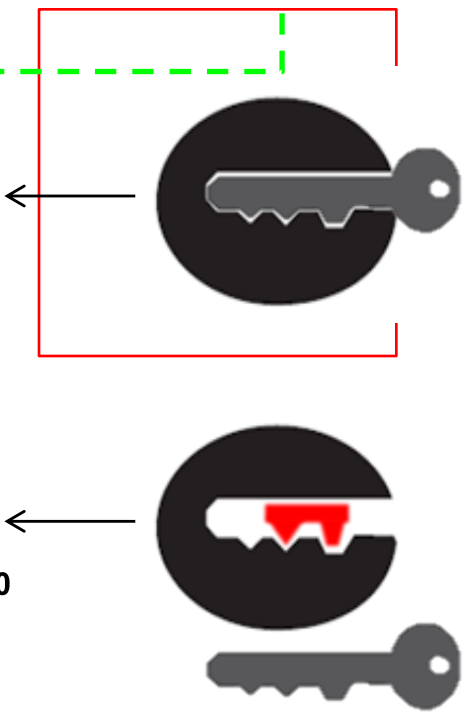
FILTER 2
Medicinal chemistry quality control and assessment

FILTER 3
Toxicity and tumor cell migration assays



ICCB-L high-throughput screening facility, Harvard School of Medicine

Experimental Vehicle (DMSO) Positive Inhibitior (AlF4-)



Hui Feng, MD, PhD

Assistant Professor of
Pharmacology and Medicine
Department of Pharmacology
and Experimental Therapeutics

Can Novel Cancer Therapeutics be Identified through Combined Genetic and Chemical Efforts?

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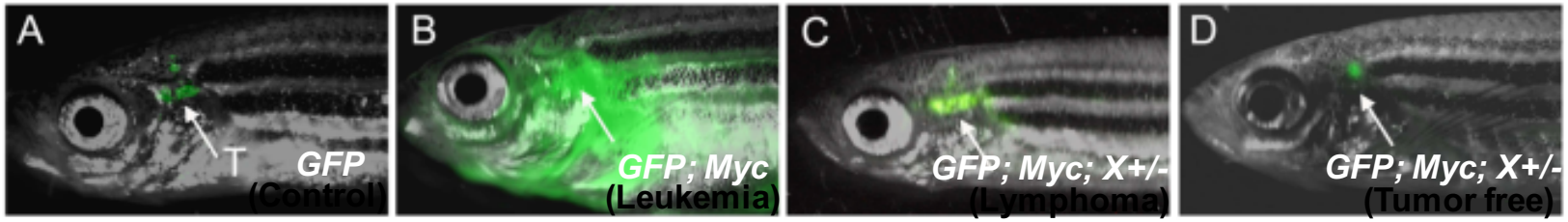
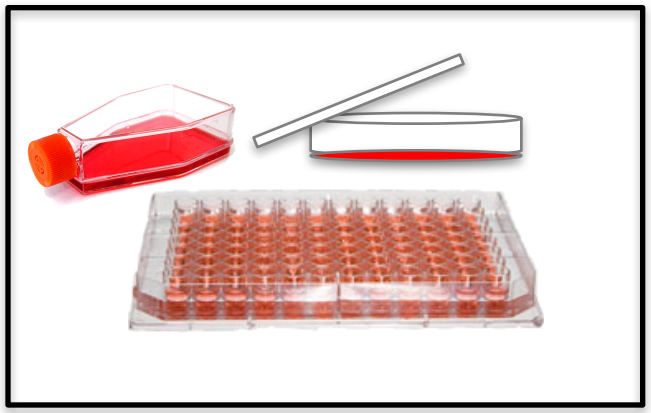
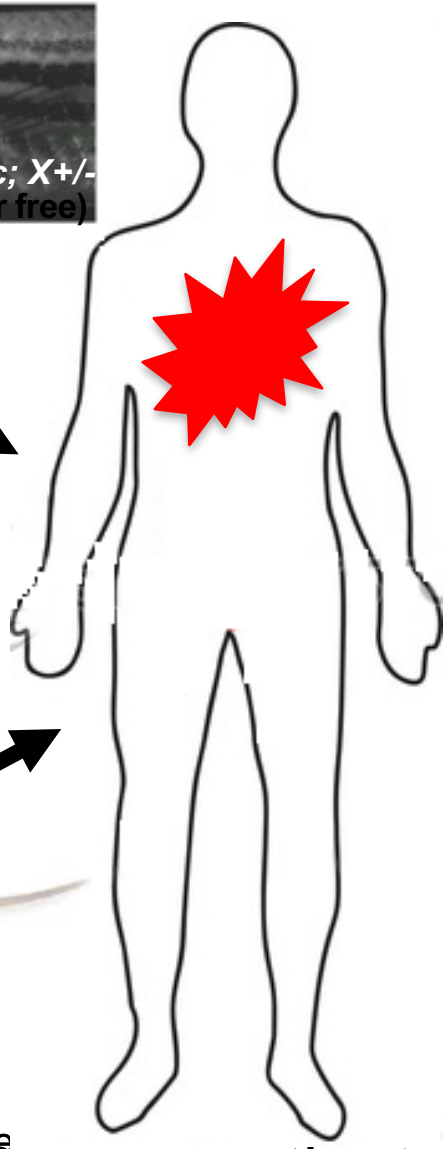
Our Strategies



zebrafish genetic studies

Collaboration with Chemists

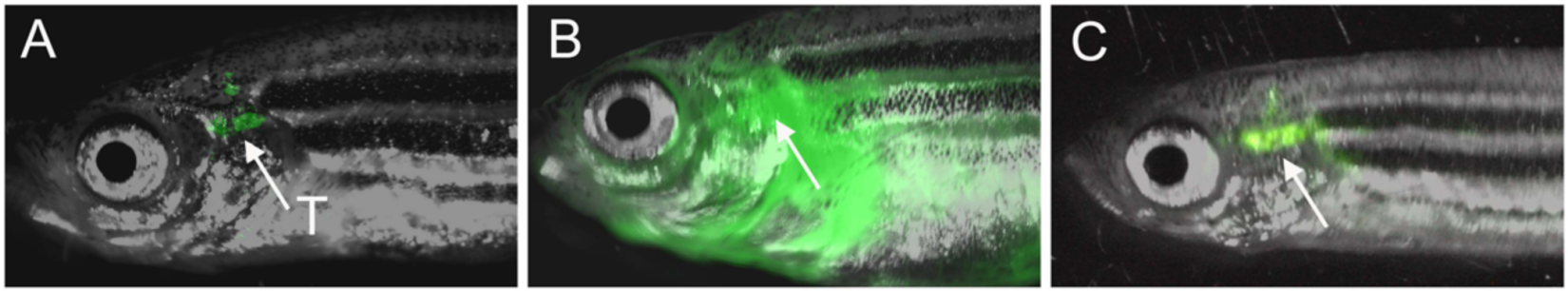




Human cell culture studies

Cancer patient

DLST inactivation impairs T-cell leukemogenesis



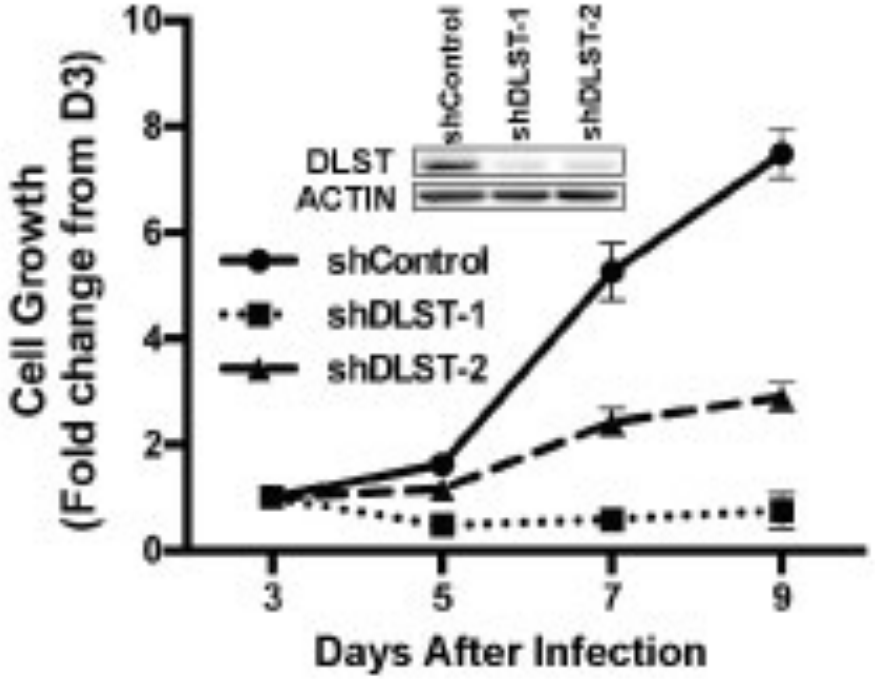
rag2-GFP

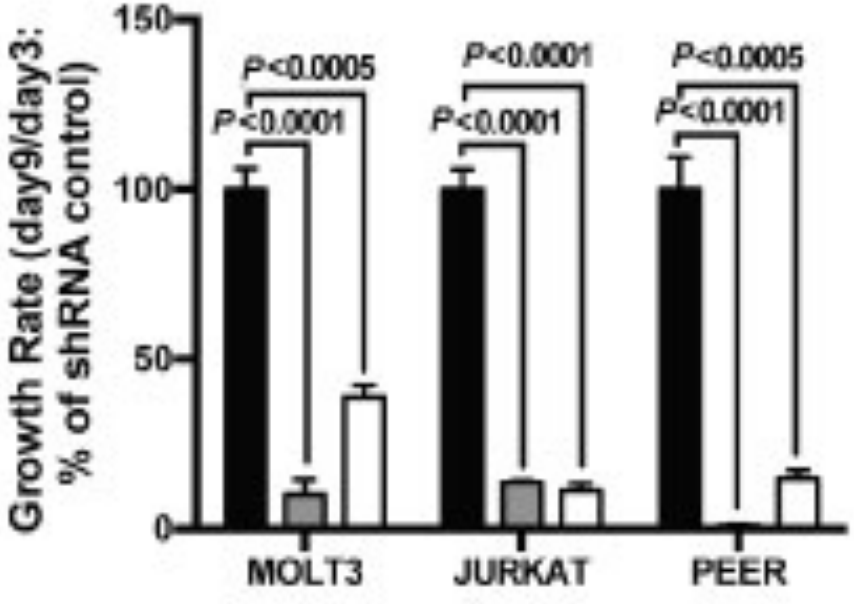
Leukemia

Myc;dlst +/+

early stage of tumor

Myc;dlst +/-





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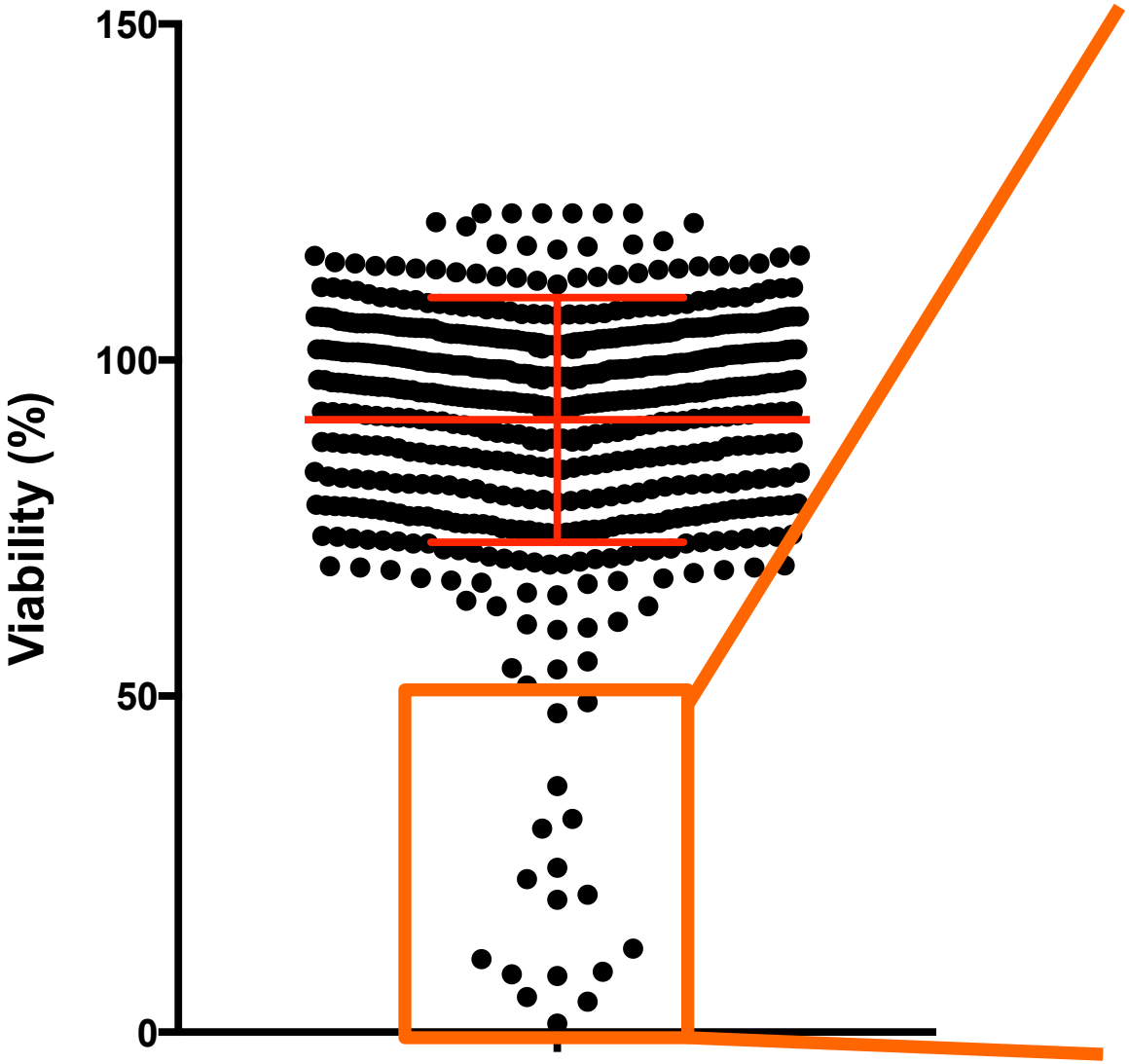
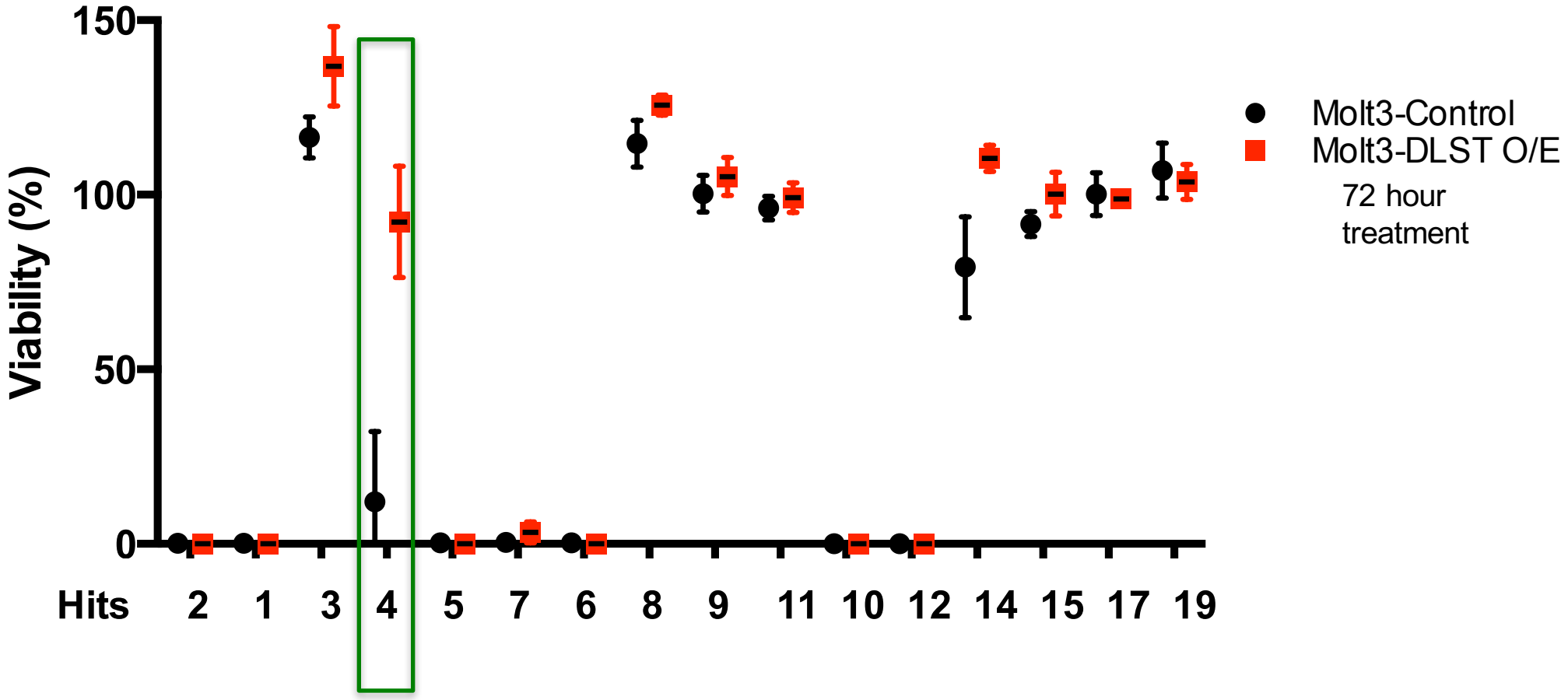
Searching for DLST inhibitors



Hit #	Viability
1	36.6
2	51.4
3	20.8
4	12.4
5	8.4
6	30.3
7	1.3
8	49.1
9	10.9
10	4.5
11	9
12	5.2
13	61
14	22.8
15	24.5
16	31.8
17	47.5
18	8.6
19	19.7

Searching for DLST inhibitors



Boston University Office of the Vice President and Associate Provost for Research

Collaboration with Dr. John Porco's group

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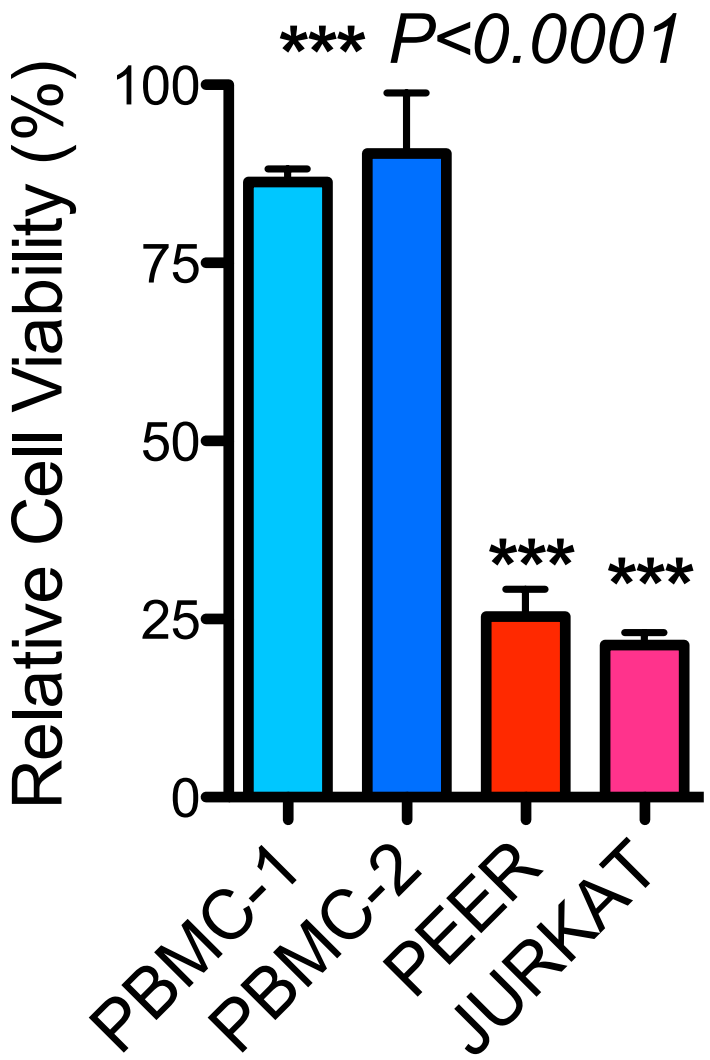
Searching for DLST inhibitors



Boston University Office of the Vice President and Associate Provost for Research

Collaboration with Dr. John Porco's group

BOSTON UNIVERSITY

Tsuneya Ikezu

Professor
Neurology and Pharmacology & Experimental Therapeutics
MED

Neurology Director | Laboratory of Molecular NeuroTherapeutics
Member | BU Alzheimer's Disease Center

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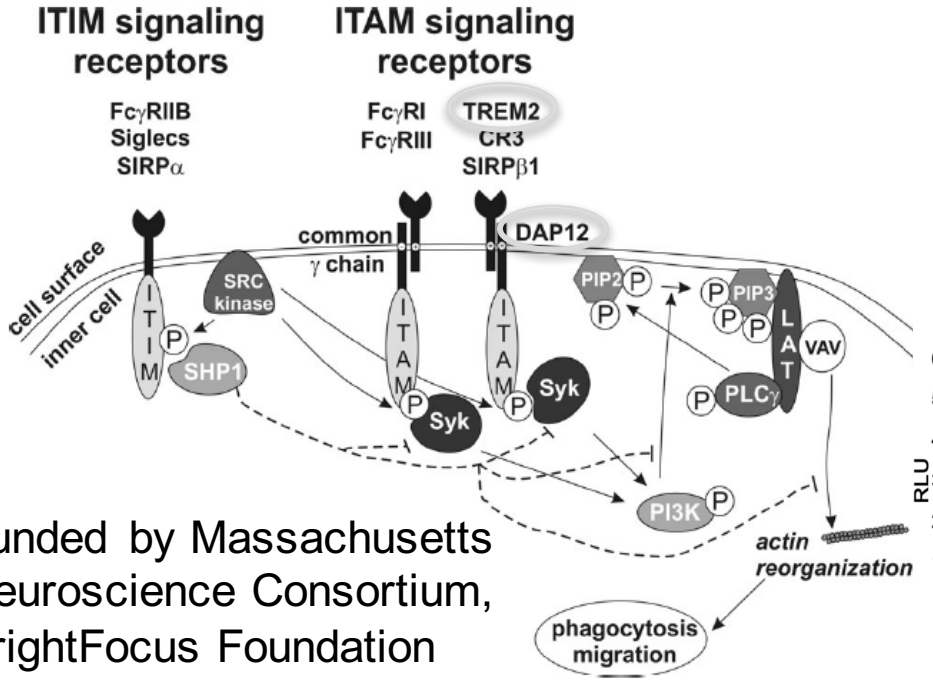
TREM2 activation reporter system

The NEW ENGLAND JOURNAL of MEDICINE

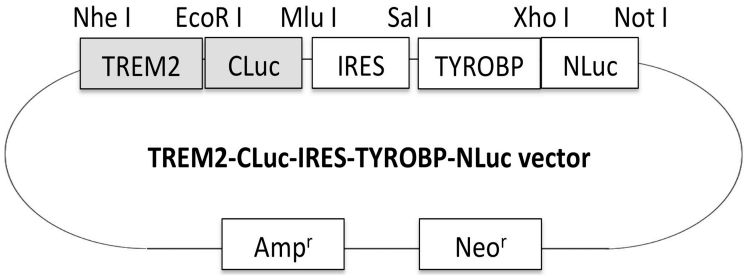
ESTABLISHED IN 1812 JANUARY 10, 2013 VOL. 368 NO. 2

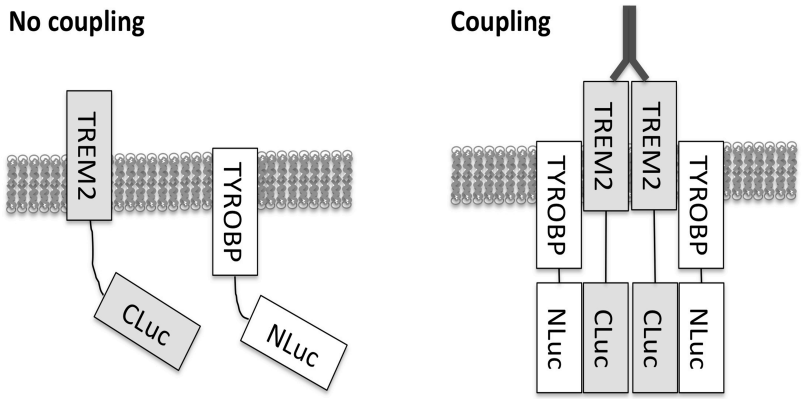
Variant of *TREM2* Associated with the Risk of Alzheimer's Disease

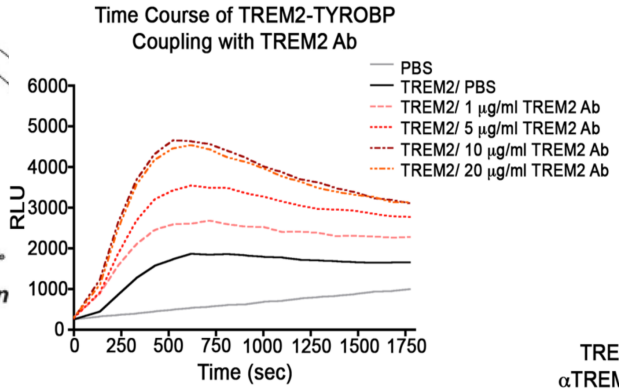
Thorlakur Jonsson, Ph.D., Hreinn Stefansson, Ph.D., Stacy Steinberg Ph.D., Ingileif Jonsdottir, Ph.D., Palmi V. Jonsson, M.D., Jon Snaedal, M.D., Sigurbjorn Bjornsson, M.D., Johanna Huttenlocher, B.S., Allan I. Levey, M.D., Ph.D., James J. Lah, M.D., Ph.D., Dan Rujescu, M.D., Harald Hampel, M.D., Ina Giegling, Ph.D., Ole A. Andreassen, M.D., Ph.D., Knut Engedal, M.D., Ph.D., Ingun Ulstein, M.D., Ph.D., Srdjan Djurovic, Ph.D., Carla Ibrahim-Verbaas, M.D., Albert Hofman, M.D., Ph.D., M. Arfan Ikram, M.D., Ph.D., Cornelia M van Duijn, Ph.D., Unnur Thorsteinsdottir, Ph.D., Augustine Kong, Ph.D., and Kari Stefansson, M.D., Ph.D.

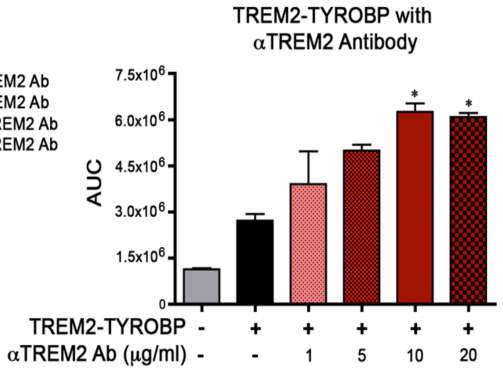


Funded by Massachusetts Neuroscience Consortium, BrightFocus Foundation









"M.tb has been studying us longer than we have been studying it"
Kyu Rhee

Igor Kramnik

Associate Professor

Medicine | MED

National Emerging Infectious Diseases Laboratory

Boston University National Emerging Infectious Diseases Laboratories

Boston University Office of the Vice President and Associate Provost for Research

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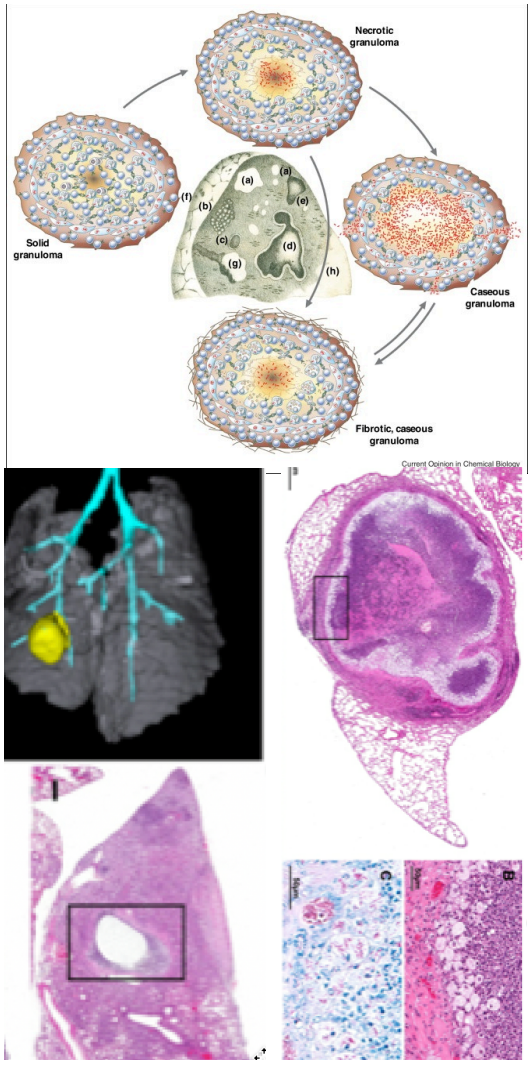
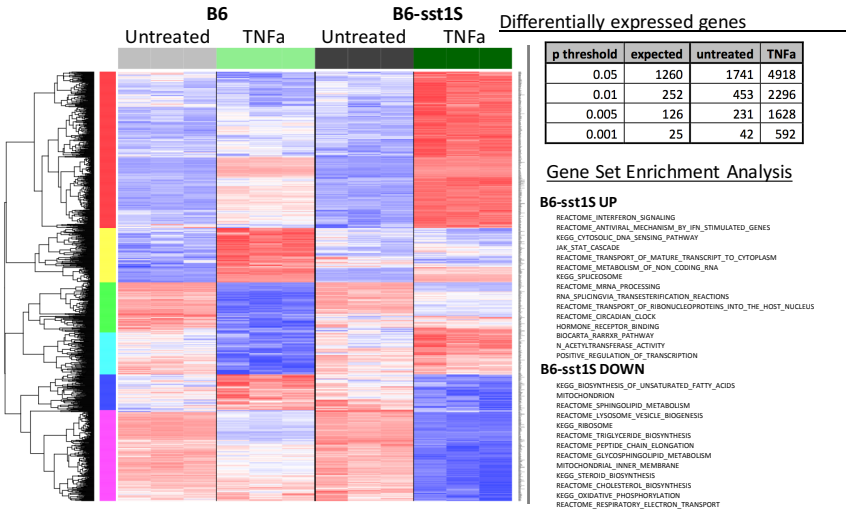
Genetic and Pharmacological Control of the Inflammatory Damage Caused by Tuberculosis and Other Infections





Differentially expressed genes

p threshold	expected	untreated	TNFa
0.05	1260	1741	4918
0.01	252	453	2296
0.005	126	231	1628
0.001	25	42	592

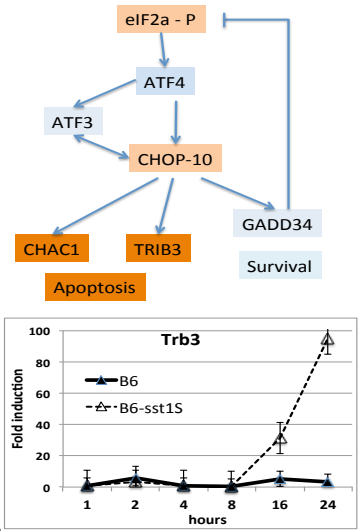
Gene Set Enrichment Analysis

B6-sst1S UP

REACTOME_INTERFERON_SIGNALING
REACTOME_ANTIVIRAL_MECHANISM_BY_IFN_STIMULATED_GENES
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY
JAK_STAT_CASCADE
REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM
REACTOME_METABOLISM_OF_NON_CODING_RNA
KEGG_SPLICEOSOME
REACTOME_MRNA_PROCESSING
RNA_SPLICINGVIA_TRANSESTERIFICATION_REACTIONS
REACTOME_TRANSPORT_OF_RIBONUCLEOPROTEINS_INTO_THE_HOST_NUCLEUS
REACTOME_CIRCADIAN_CLOCK
HORMONE_RECEPTOR_BINDING
BIOCARTA_RARRXR_PATHWAY
N_ACETYLTRANSFERASE_ACTIVITY
POSITIVE_REGULATION_OF_TRANSCRIPTION

B6-sst1S DOWN

KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS
MITOCHONDRION
REACTOME_SPHINGOLIPID_METABOLISM
REACTOME_LYSOSOME_VESICLE_BIOGENESIS
KEGG_RIBOSOME
REACTOME_TRIGLYCERIDE_BIOSYNTHESIS
REACTOME_PEPTIDE_CHAIN_ELONGATION
REACTOME_GLYCOSPHINGOLIPID_METABOLISM
MITOCHONDRIAL_INNER_MEMBRANE
KEGG_STEROID_BIOSYNTHESIS
REACTOME_CHOLESTEROL_BIOSYNTHESIS
KEGG_OXIDATIVE_PHOSPHORYLATION
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT



The sst1/Ipr1 pathway controls stress response in macrophages.
Unresolved stress leads to tissue necrosis in SUSCEPTIBLE hosts

Goals: to identify compounds that boost macrophage stress resilience to increase bacterial killing and mitigate the inflammatory damage;

Methods: novel assays based on gene expression patterns in relevant primary cells (macrophages) from susceptible individuals (mouse and humans)

Progress: in collaboration with the Porco and Beeler labs identified a novel rocaglate that acts in synergy with low doses of IFN-gamma to activate autophagy and suppress inflammation, but does not compromise host resistance to intracellular bacteria in vitro and in vivo;

Plans: to continue the development of assays for compounds that

1. synergize with IFN-gamma;
2. correct hyperinflammatory phenotype in SUSCEPTIBLE hosts;
3. Identify inflammatory diseases that benefit from those compounds.

James S. Panek

Samour Family Professor
in Organic Chemistry

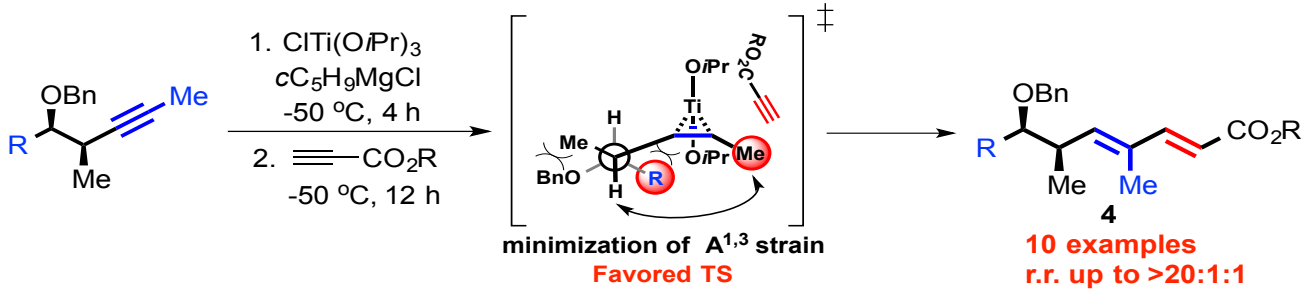
CAS

BOSTON UNIVERSITY

Reaction Development: Heteroatom Directed Reductive Coupling

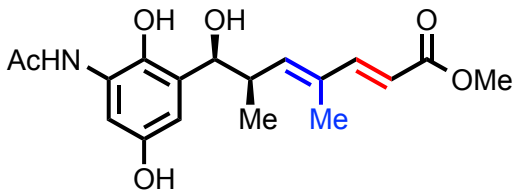
Bin Cai (BU), Professor Jie Wu (NUS) and Ryan Evans (Princeton)

Alkyne-alkyne reductive coupling with acetylenic esters



Target-Oriented Synthesis

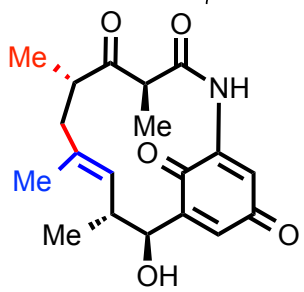
Alkyne-Alkyne coupling



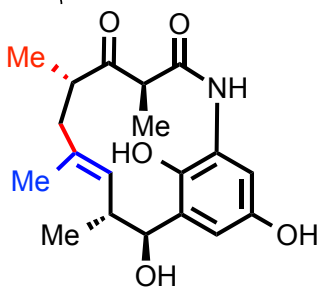
NFAT-68 an Immunosuppressant
Inhibits NFAT-depentent transcription factor

Organic Letters, **2016,** *18*, 4304

Alkyne-Alkene coupling



(-)-Q-1047H-A-A
Antioxidant



(-)-Q-1047H-R-A
Antioxidant

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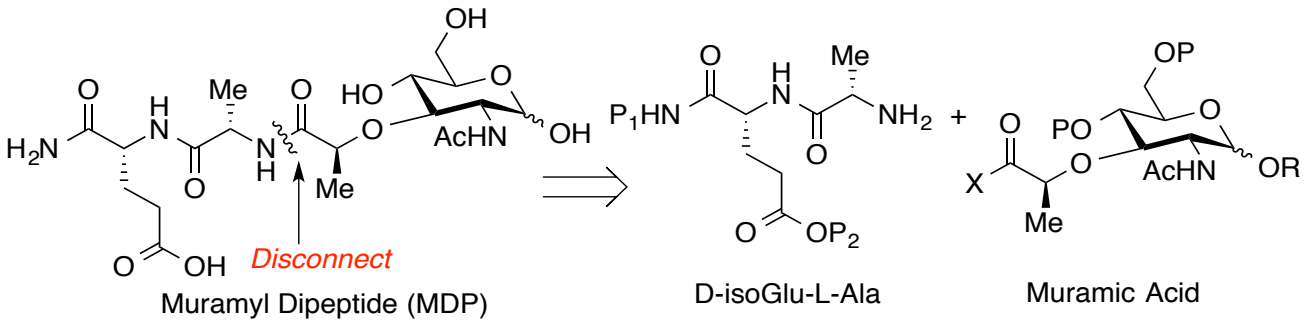
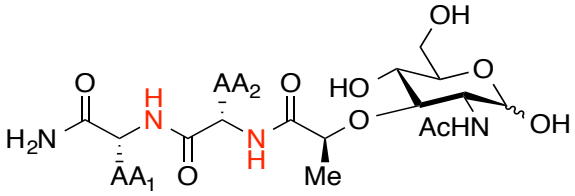
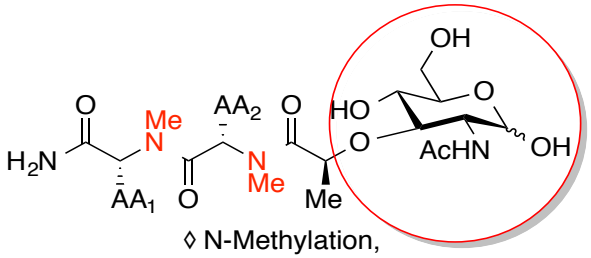
Convergent Synthesis of Novel Muramyl Dipeptide Analogs



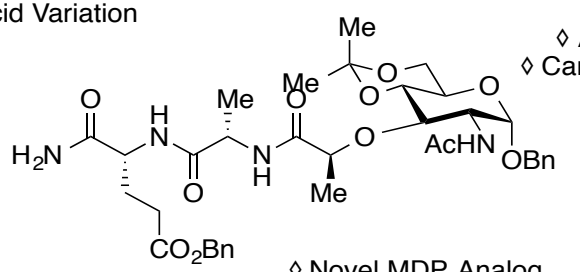
The effects of MDP are biphasic: at 10 µg/ml (MDP-low), MDP activates the inflammatory process, while a dose of 100 µg/ml or higher (MDP-high) dampens the process by inhibiting the NFkB-mediated cytokine response. Analogs of MDP were prepared through a convergent strategy involving the synthesis of two unique carbohydrate fragments, using the peptide coupling reagents, EDCI and HOAt. Analogs improved MDP function and P.g-induced activities. A new signaling pathway is proposed for TNF-α induction activated after exposing macrophages to both P.g and high concentrations of MDP.



◊ Amino Acid Variation



◊ N-Methylation,
◊ Amino Acid Variation
◊ Carbohydrate Modification



◊ Novel MDP Analog

◊ Created by Chemical Synthesis

Evidence highlighting a high dose MDP-dependent signaling pathway which activates JNKs, induces AP1, up-regulates A20 expression, restricts NOD2, inhibits NFκB, and consequently, reduces *P.g*-induced TNF-α production in mouse macrophages (inflammation).

B. Cai, J.S. Panek & S. Amar J. Med. Chem. 2016, 59(14), 6878. N.S Burres et. al. J, Antibiotics, 1995, 380.

John Connor

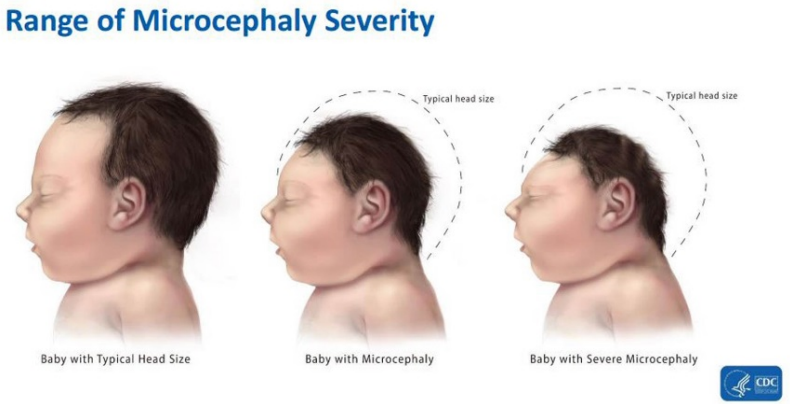
Associate Professor

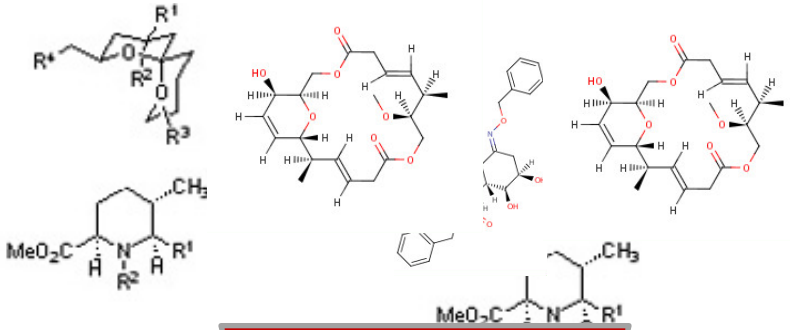
Microbiology | MED

Investigator | NEIDL

BOSTON UNIVERSITY

Small Molecule Probes of Virus Function





With Snyder Beeler, Porco

With Brown, Schaus, Porco

Tests To Find Molecules That Stop Viruses From Working

We Have Found Molecules That Keep Viruses From Making Copies of Themselves