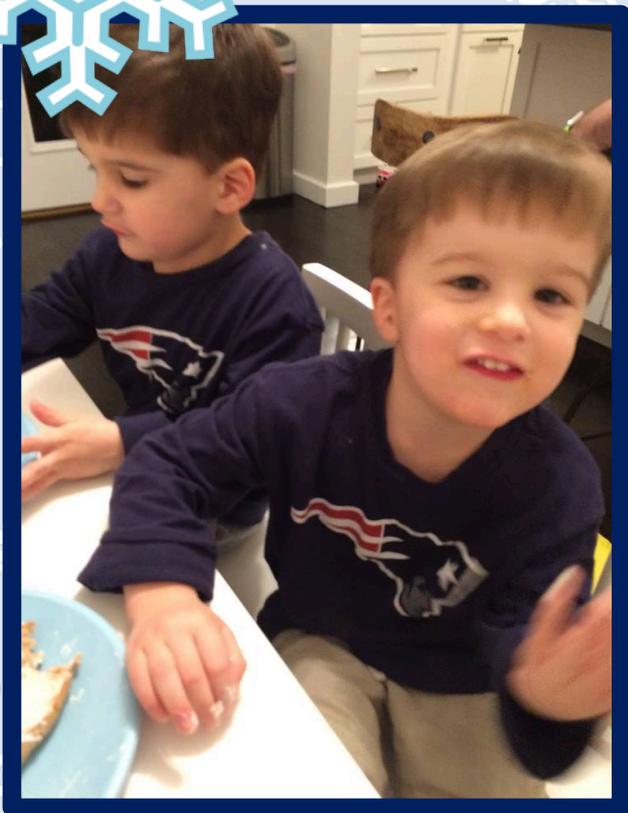


SNOW DAY!



Welcome to Module 1

High-Throughput Ligand Discovery



L1 Intro to chemical biology: small molecules, probes, and screens

February 14, 2017



Angela Koehler
koehler@mit.edu

Instructor
76-361c

Lectures 1-3, 5, 6



Shelby Doyle
spursley@mit.edu

BE Graduate Student
Comm Lab Fellow
Koehler Lab

Lecture 4



Rob Wilson
rwilson@mit.edu

BE Graduate Student
20.109 TA
Koehler Lab

Lab and Data Analysis

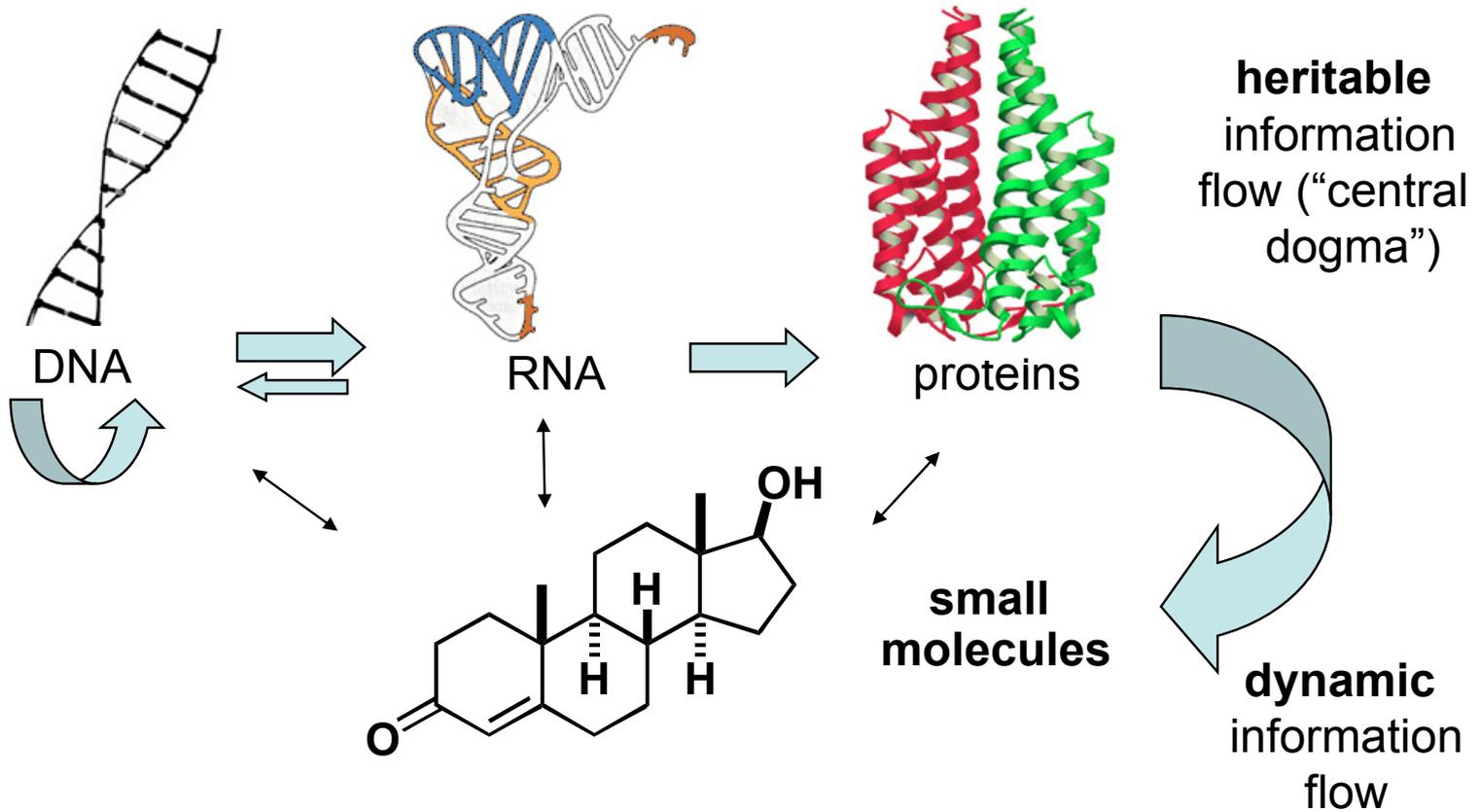


Becky Leifer
beleifer@mit.edu

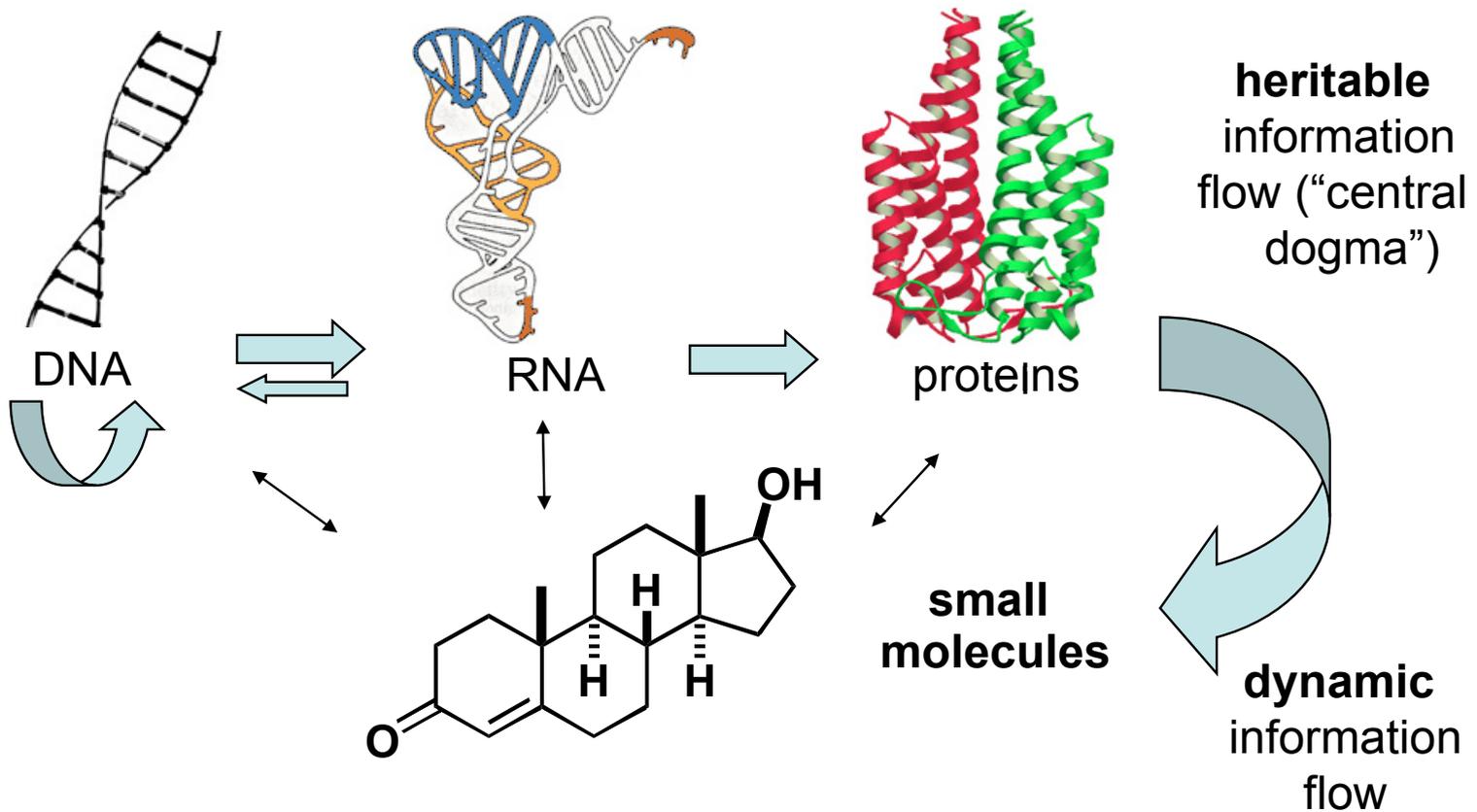
Research Associate
SMM Specialist
Koehler Lab

Data Analysis

The central dogma



The central dogma



cell signaling, cognition, metabolism, life's origins
chemical probes and drugs

Defining chemical biology

Chemical biology is a discipline that spans multiple fields and involves the application of chemical techniques, tools, and analyses to the study and **manipulation of biological systems**

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Chemical biologists attempt to use chemical approaches to **modulate systems** to either investigate underlying biology, typically using **quantitative measures**, and to **engineer new functions**

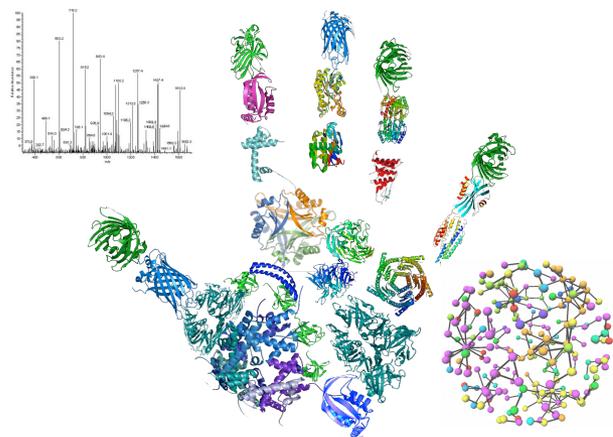
Defining chemical biology

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Chemical biologists attempt to use chemical approaches to **modulate systems** to either investigate underlying biology, typically using **quantitative measures**, and to **engineer new functions**

Research done by chemical biologists is often more closely related to cell or systems biology than biochemistry. Biochemists study the chemistry carried out by biomolecules and how metabolites function in pathways while chemical biologists apply novel chemical tools to biology, including basic, disease, and synthetic applications.

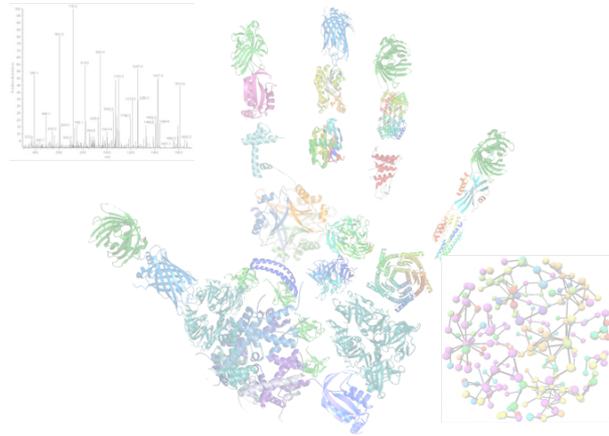
Systems of interest to chemical biologists



quantitative proteomics

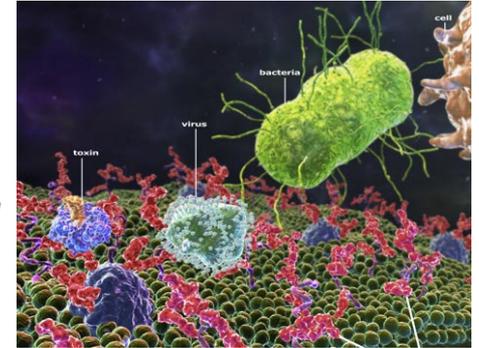
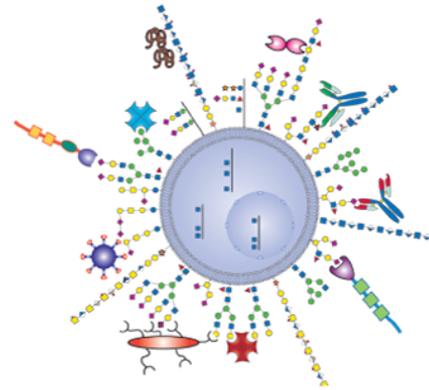
Pete Dedon, Ernest Fraenkel, Angela Koehler, Forest White

Systems of interest to chemical biologists



quantitative proteomics

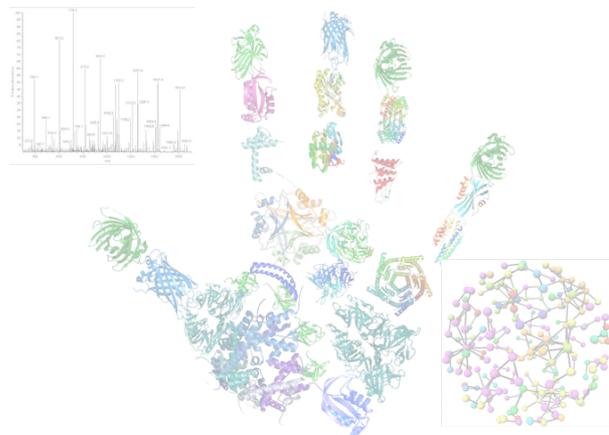
Pete Dedon, Ernest Fraenkel, Angela Koehler, Forest White



glycobiology

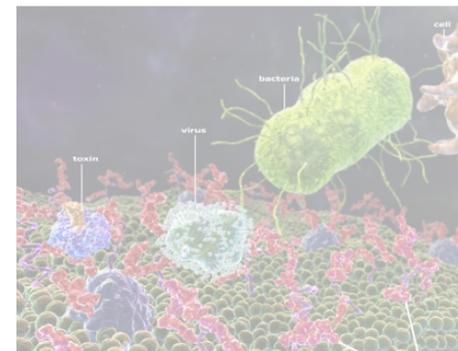
Barbara Imperiali, Katharina Ribbeck, Ram Sasisekharan

Systems of interest to chemical biologists



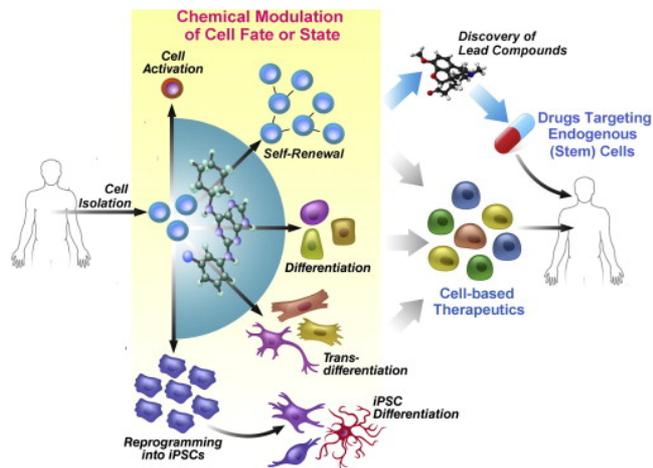
quantitative proteomics

Pete Dedon, Ernest Fraenkel, Angela Koehler, Forest White



glycobiology

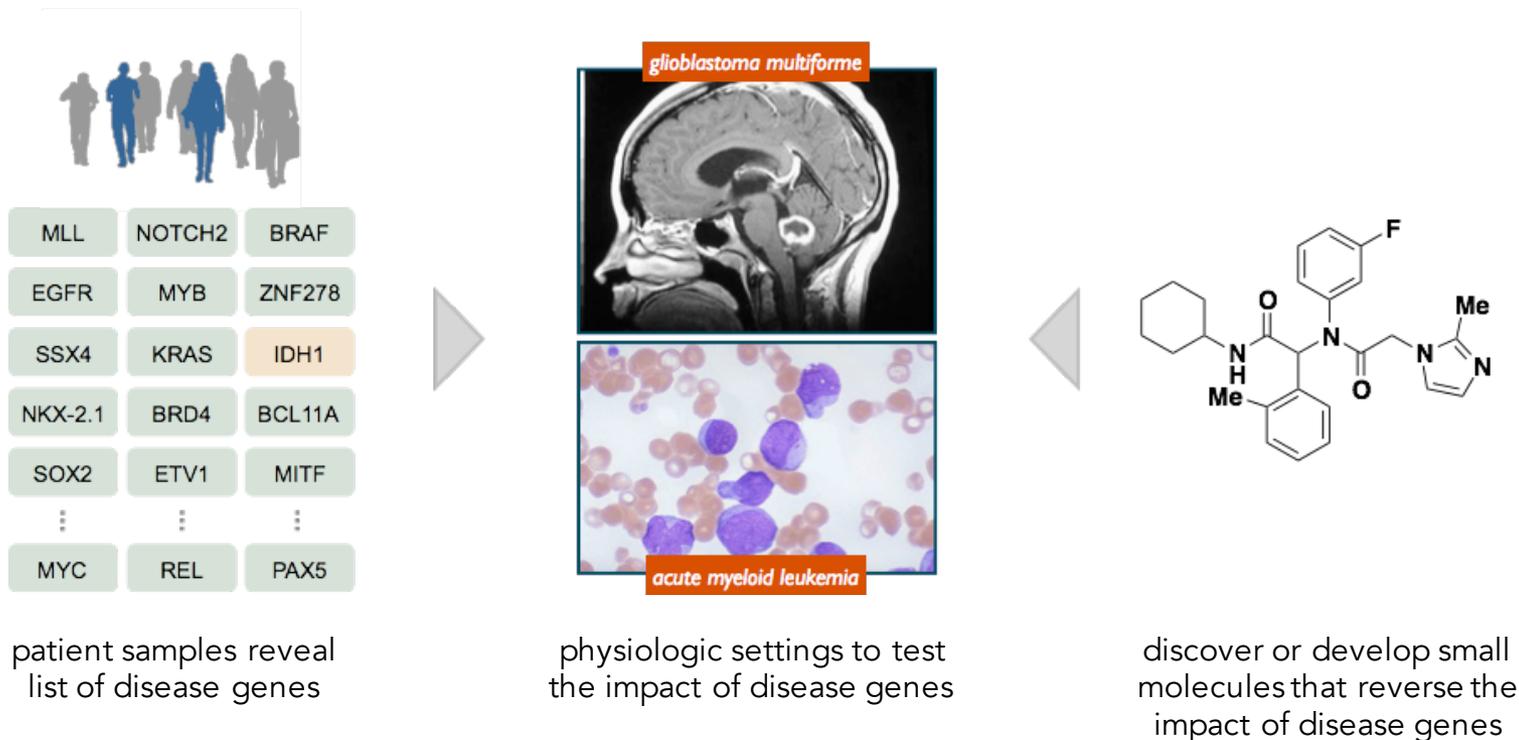
Barbara Imperiali, Katharina Ribbeck, Ram Sasisekharan



stem cell biology and programming cell fate

Laurie Boyer, Leonard Guarente, Piyush Gupta, Rudolf Jaenisch, Angela Koehler, Robert Weinberg, Rick Young

Chemical probes of disease biology



Approach: use small molecules to test emerging concepts in human disease in physiologically relevant settings

Output: validated small-molecule probe to facilitate human clinical development or diagnostic applications

Chemical biology courses at MIT

suitable for advanced undergraduates

20.554 Frontiers in Chemical Biology (F)

Matthew Shoulders

Introduction to current research at the interface of chemistry, biology, and bioengineering. Topics include [imaging of biological processes](#), [metabolic pathway engineering](#), [protein engineering](#), [mechanisms of DNA damage](#), RNA structure and function, macromolecular machines, protein misfolding and disease, metabolomics, and methods for analyzing signaling network dynamics.

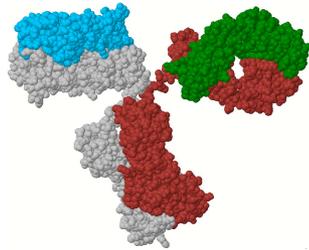
7.73 Principles of Chemical Biology (S)

Barbara Imperiali, Jing-Ke Weng

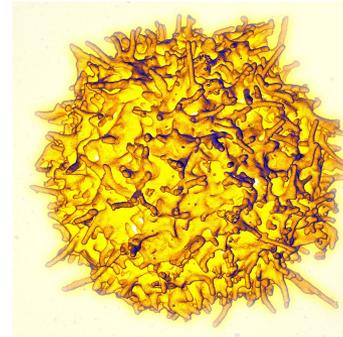
Spanning the fields of biology, chemistry and engineering, addresses the principles of chemical biology and its application of chemical and physical methods and reagents to the study and manipulation of biological systems. Topics include [activity-based protein profiling](#), [small molecule inhibitors and chemical genetics](#), [fluorescent probes for biological studies](#), [chemical biology approaches for studying dynamic post-translational modification reactions](#), [natural product biosynthesis](#), and [high-throughput drug screening](#).

What is a small molecule?

antibodies



T-cells

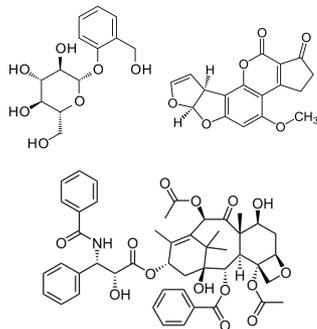


10^{-9} m

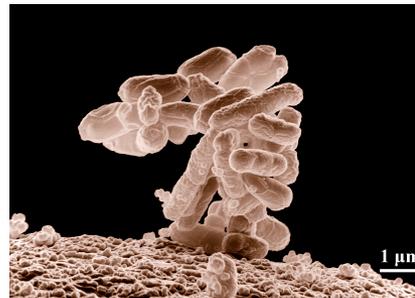
10^{-6} m

$>10^{-4}$ m

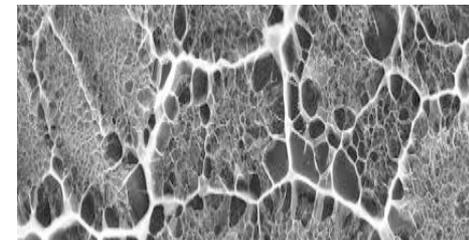
small molecules



microbes

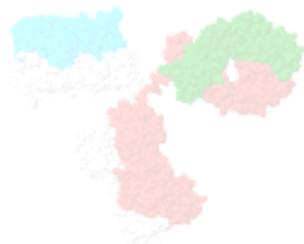


bio-materials

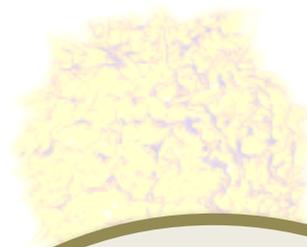


What is a small molecule?

antibodies



T-cells



<1000 Da

Typically C, N, O
(occasionally S, P, B, etc.)

natural or synthetic

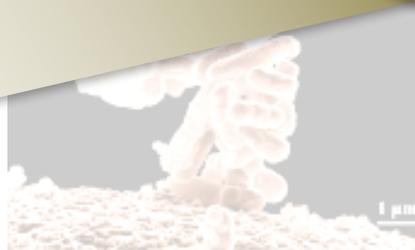
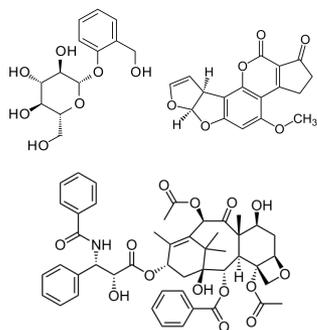
probes/therapeutics

10⁻⁹ m

10⁻⁶ m

10⁻⁴ m

small molecules

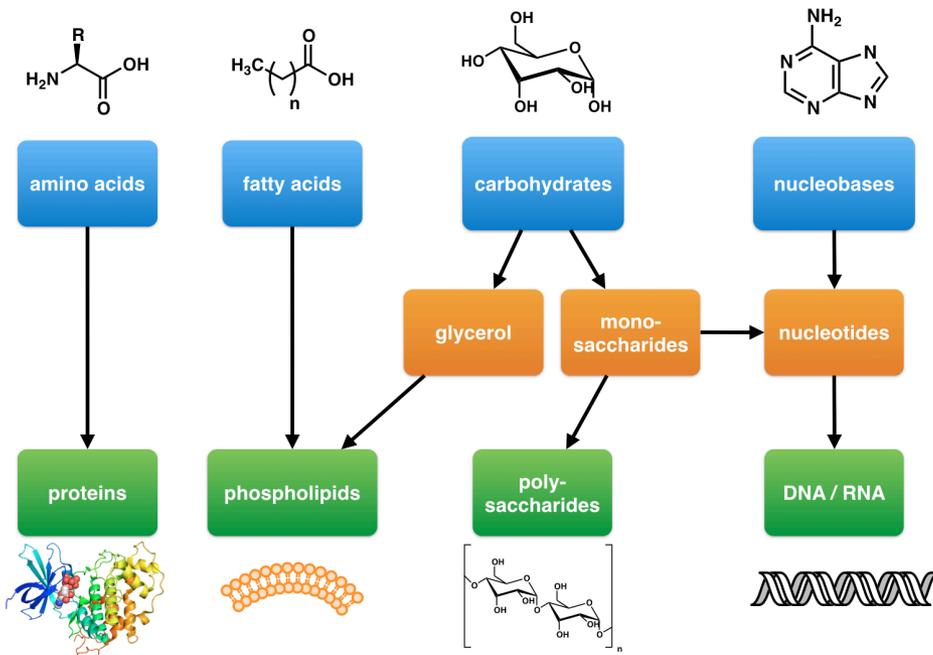


10⁻⁷ m

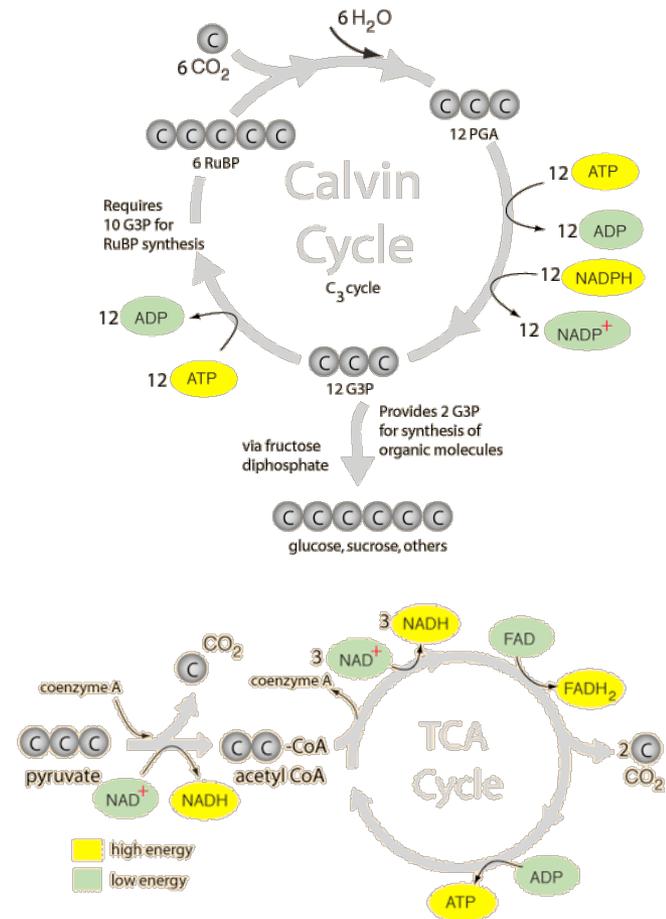


Small molecules of life

primary metabolites - intrinsic function is essential to survival of organism



first messengers – signaling molecules that control metabolism and cell differentiation (e.g. hormones, biogenic amines, etc.)



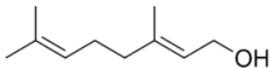
Small molecules of life

secondary metabolites – non-essential to organism, extrinsic function that affects other organisms; broad range of functions, narrow species distribution, increase competitiveness of an organism

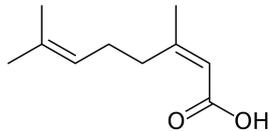
pheromones – social interactions

transporters and chelators

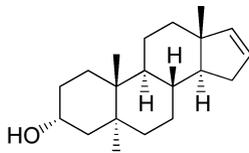
toxins – competitive weapons



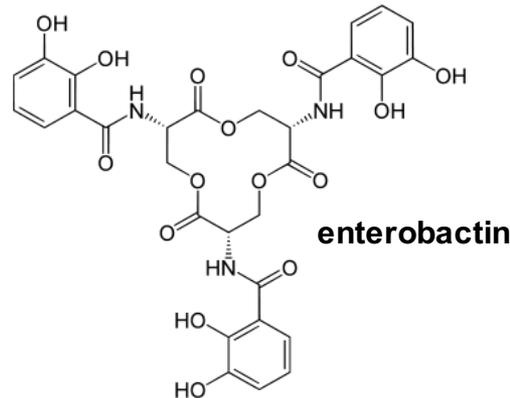
geraniol



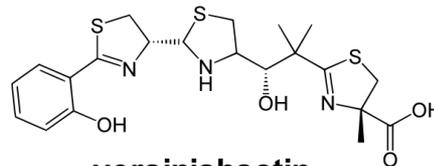
nerolic acid



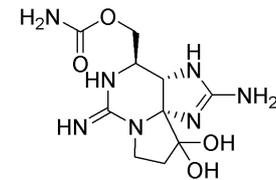
androstenol



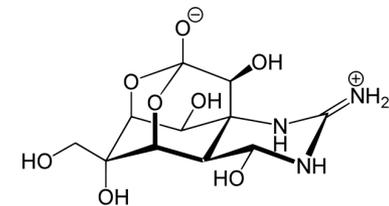
enterobactin



yersiniabactin



saxitoxin (TZ)

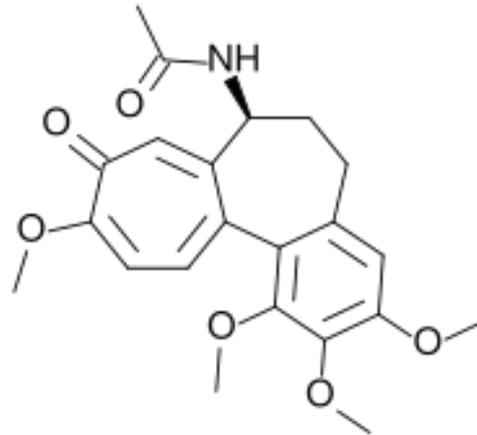


tetrodotoxin (TTX)

Significant interest in exploring bioactivity of these 'natural products' for biological probe and therapeutic applications

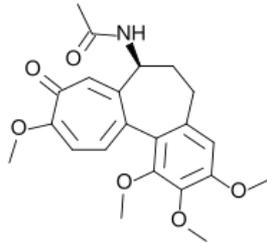
Small molecules and their partners

the compound that changed my life



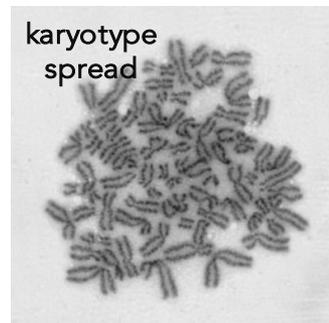
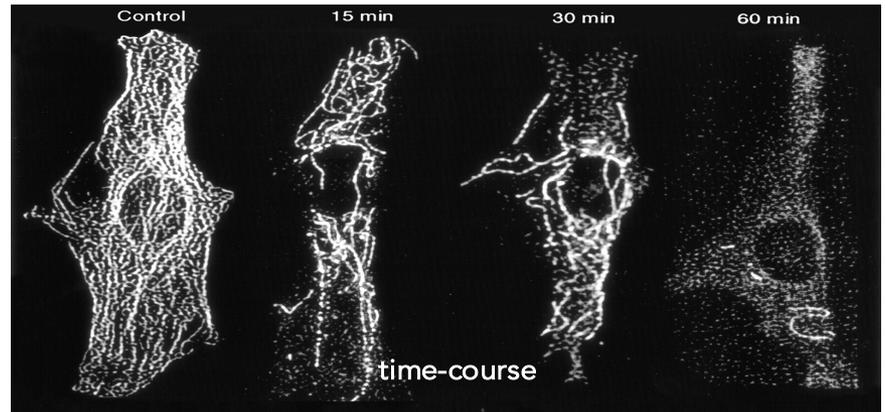
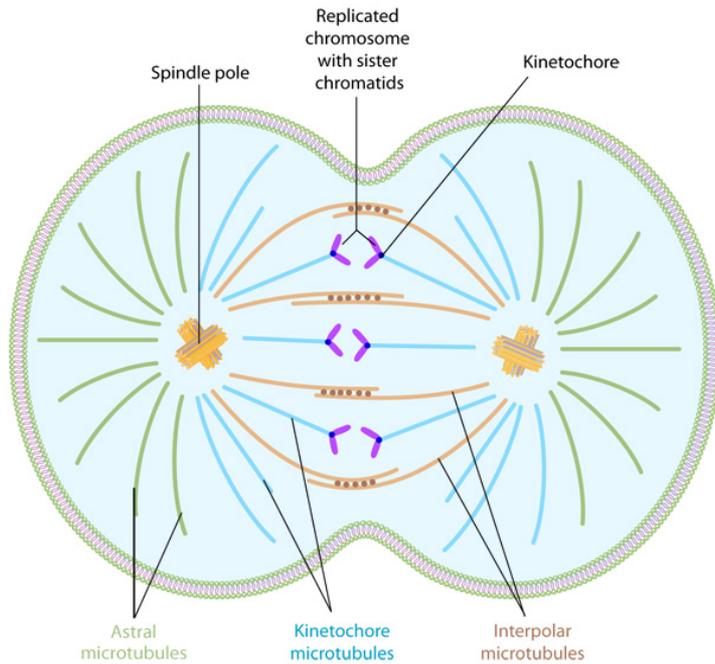
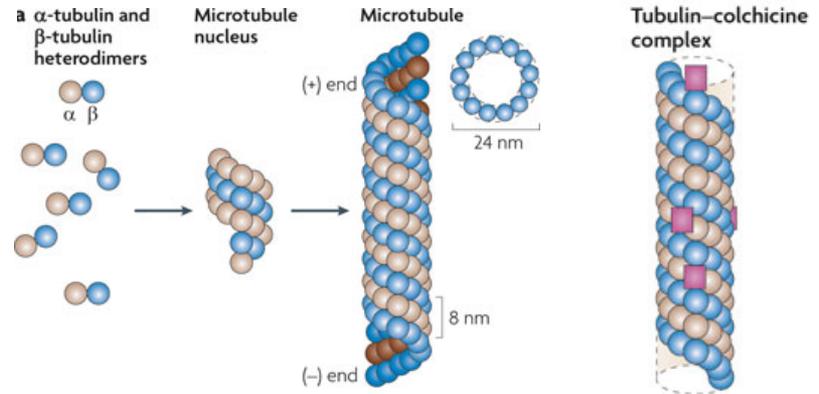
colchicine

Colchicine is a *mitotic spindle poison*



colchicine

binds to tubulin protein
blocks microtubule polymerization



colchicine prevents chromosome segregation and enables study chromosome count and physical characteristics

Colchicine informs therapeutic strategies

inflammatory diseases



gout



pericarditis

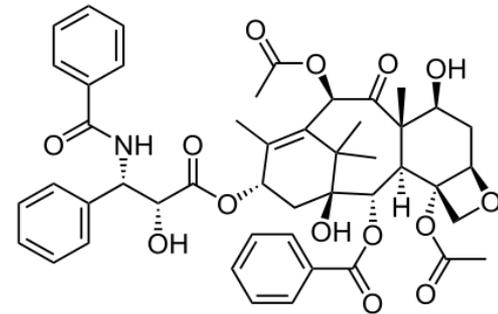


Behçet's disease

Egyptians
Ben Franklin

mitotic poisons for cancer therapy

Taxol
stabilizes MTs



Chemical genomic toolkit

How many specific probes do we need to study the entire 'expressed genome?'

Chemical genomic toolkit

How many specific probes do we need to study the entire 'expressed genome?'

92,000 expressed proteins

1 inhibitor of function

1 activator of function

184,000 unique chemical probes!

Chemical genomic toolkit

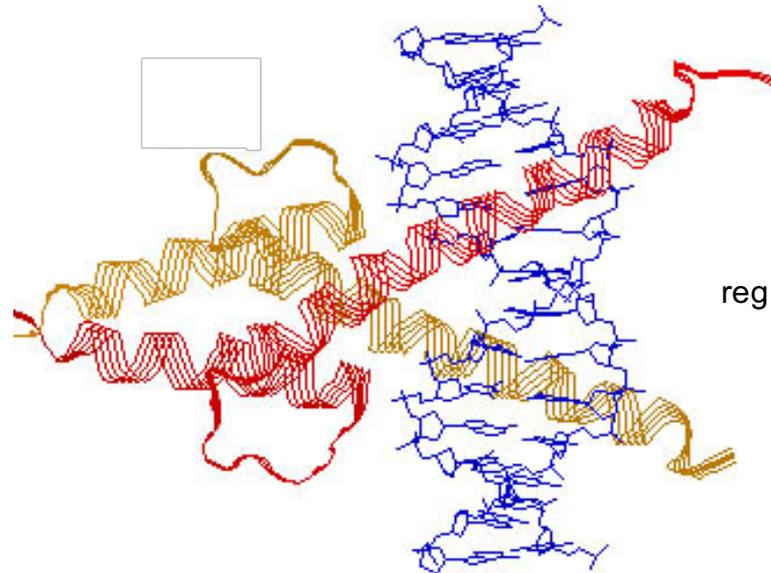
How many specific probes do we need to study the entire 'expressed genome'?

92,000 expressed proteins

1 **inhibitor** of function

1 **activator** of function

184,000 unique chemical probes?



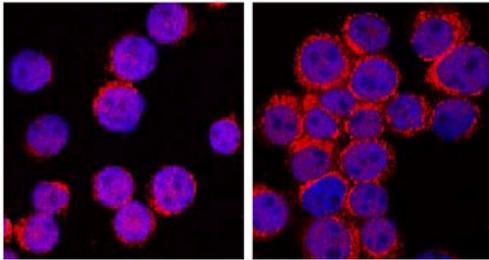
MyoD:

regulates smooth muscle differentiation
'exercise transcription factor'

Approaches to probe discovery

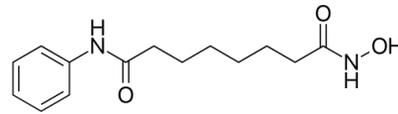
'forward' chemical genetics

screen for phenotype of interest



- small molecule

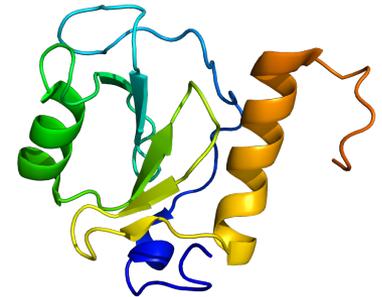
+ small molecule



assay positive



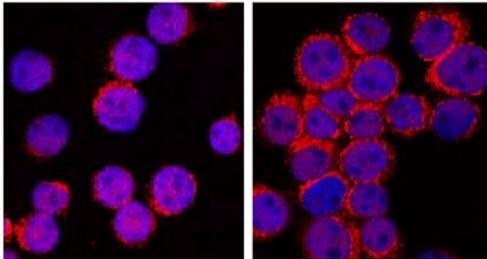
identify protein target



Approaches to probe discovery

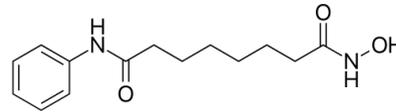
'forward' chemical genetics

screen for phenotype of interest



- small molecule

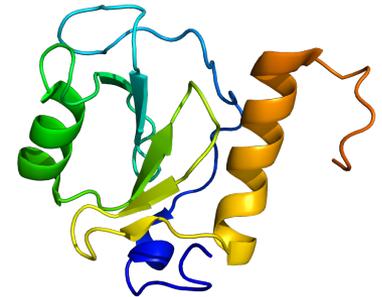
+ small molecule



assay positive



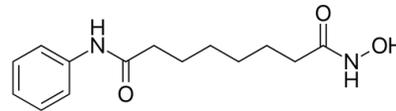
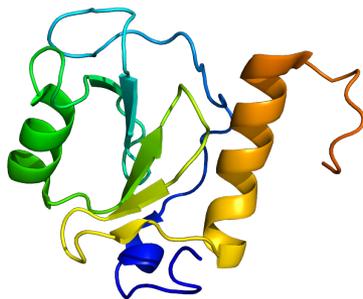
identify protein target



'reverse' chemical genetics

our approach for this class

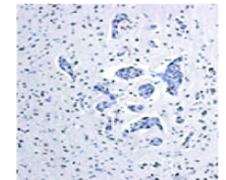
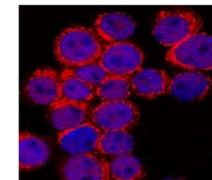
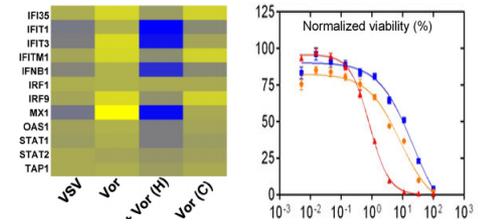
directly bind target of interest



assay positive



broad survey of phenotypic outcomes

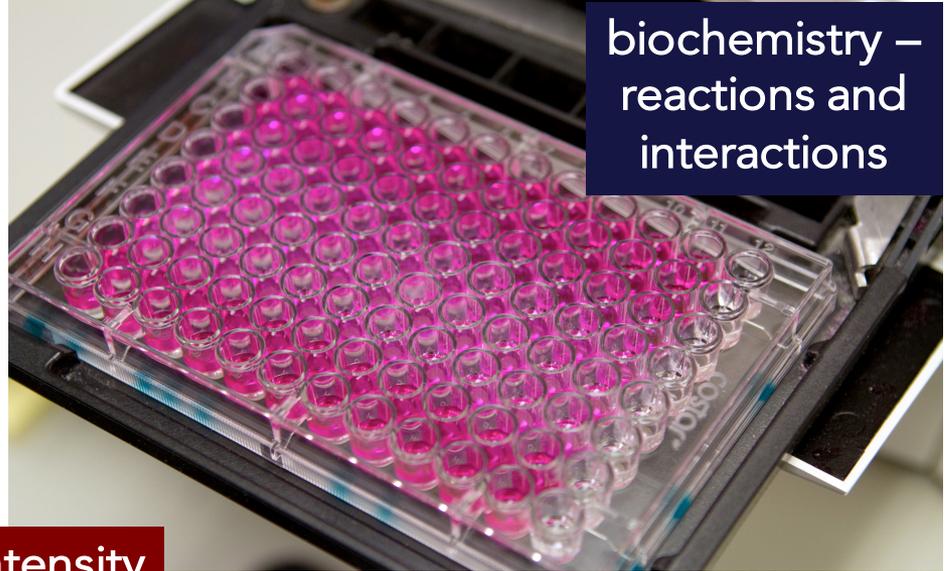


High-throughput assays

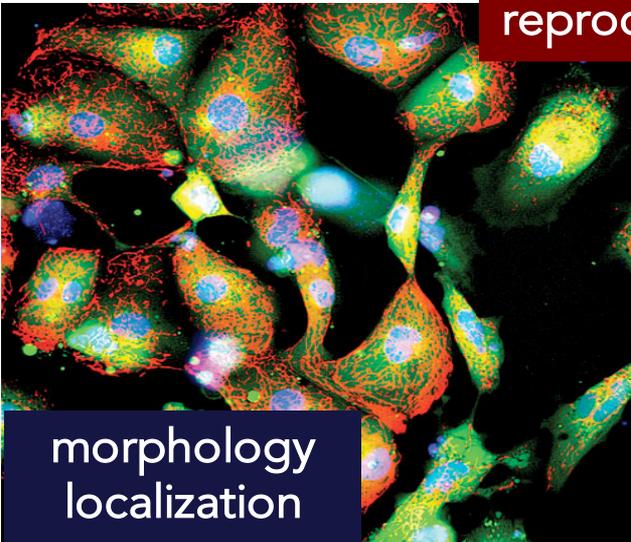
viability



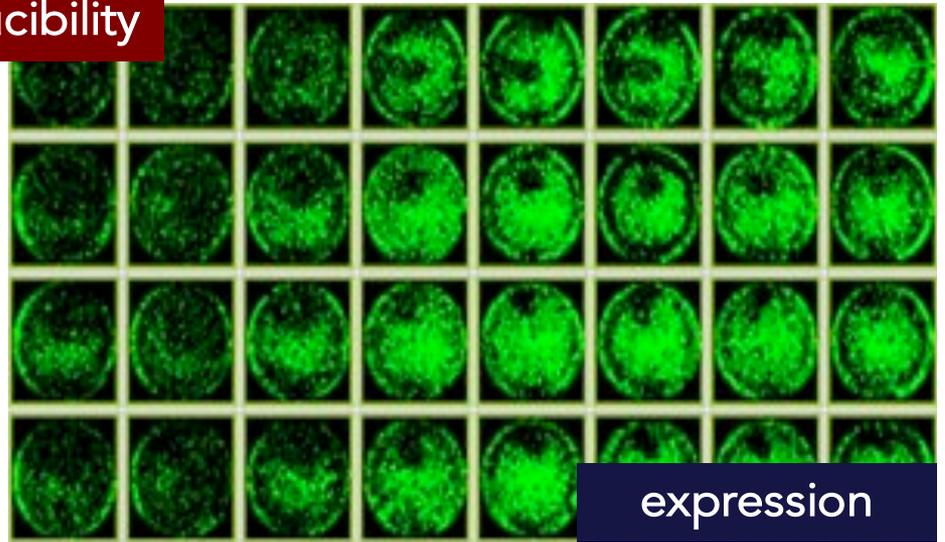
biochemistry –
reactions and
interactions



signal intensity
reproducibility

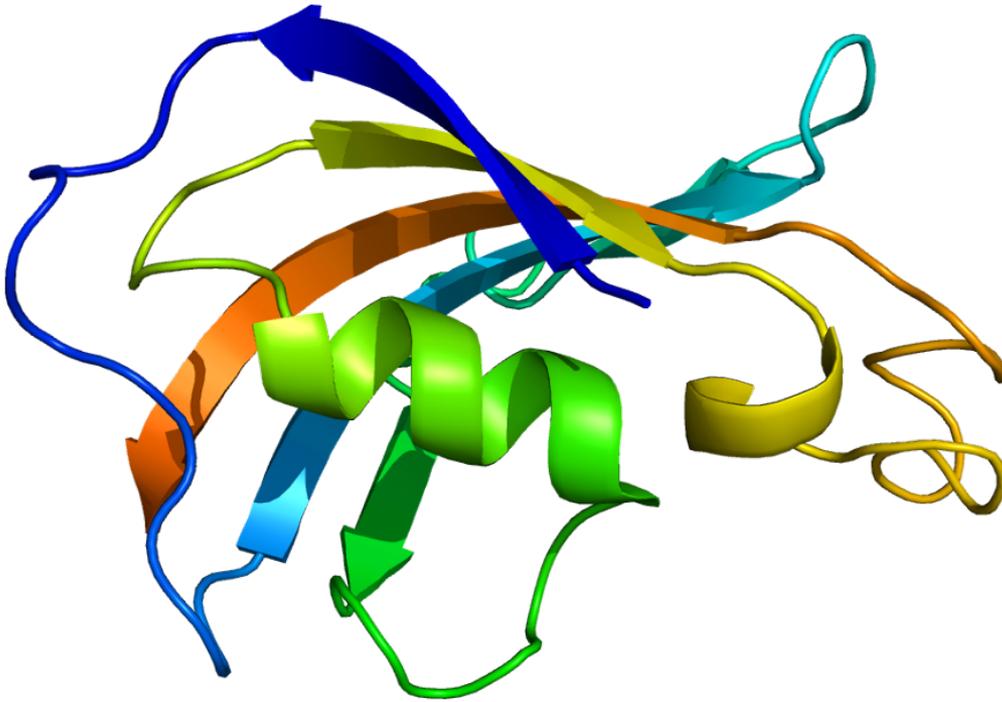


morphology
localization



expression

Our protein target: **FKBP12**



Cellular roles:

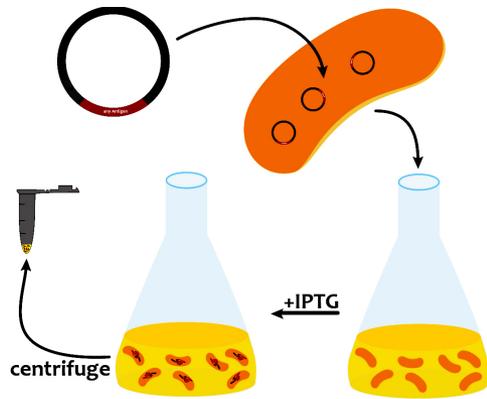
plays a role in immune regulation
regulates protein folding
regulates protein trafficking in the cell

Molecular functions:

peptidyl-prolyl cis-trans isomerase
binds several other signaling proteins
target of several immunosuppressants

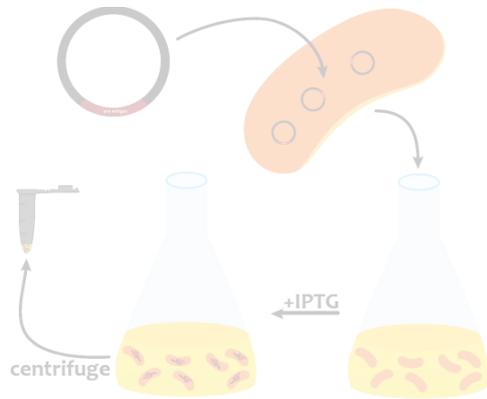
more details to come in our next lecture!

Our path to probe discovery



overexpress FKBP12
lab day 1

Our path to probe discovery

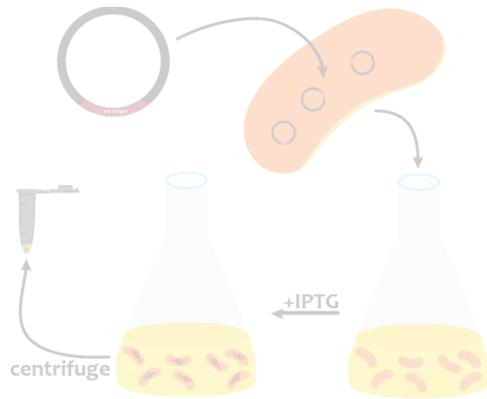


overexpress FKBP12
lab day 1

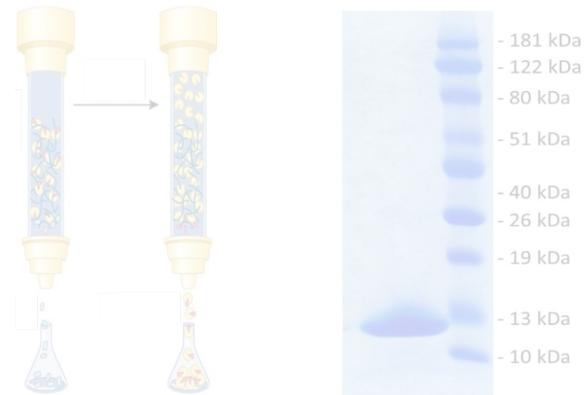


purify and characterize FKBP12
lab days 2 and 3

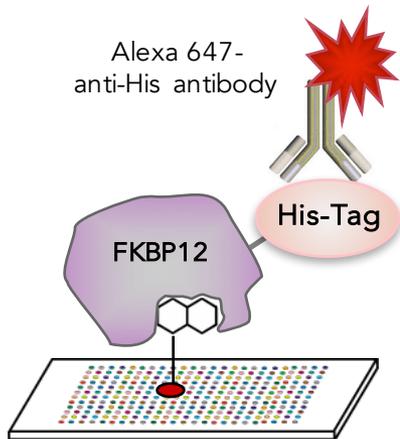
Our path to probe discovery



overexpress FKBP12
lab day 1

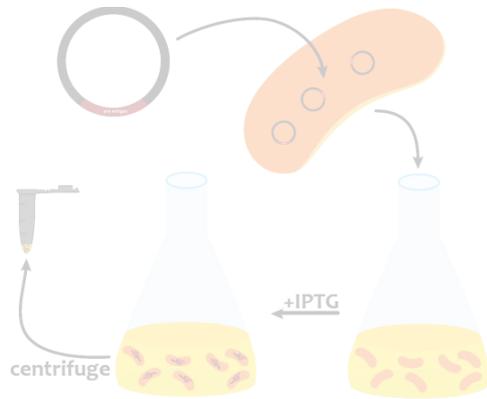


purify and characterize FKBP12
lab days 2 and 3

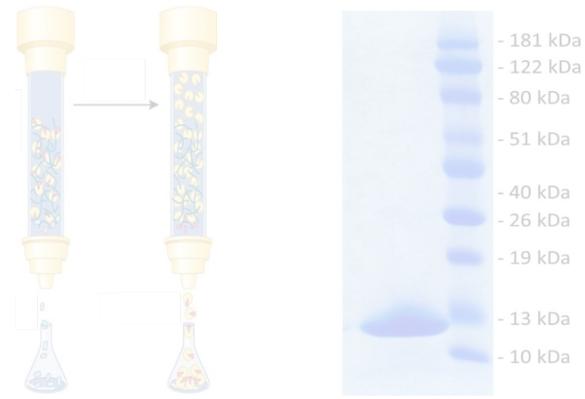


SMM screen
lab day 4

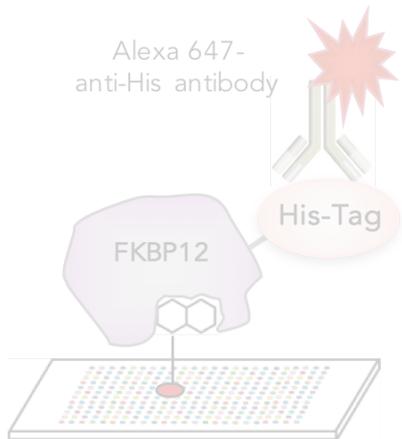
Our path to probe discovery



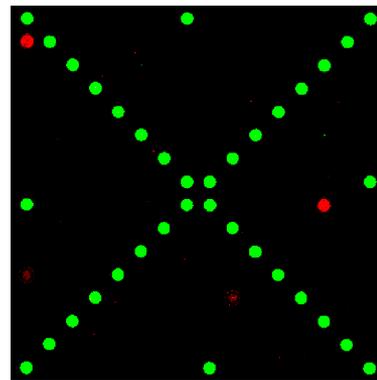
overexpress FKBP12
lab day 1



purify and characterize FKBP12
lab days 2 and 3

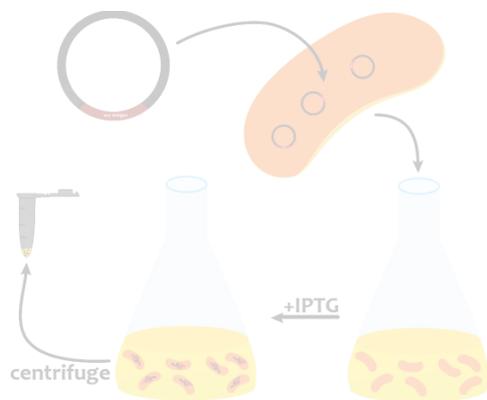


SMM screen
lab day 4

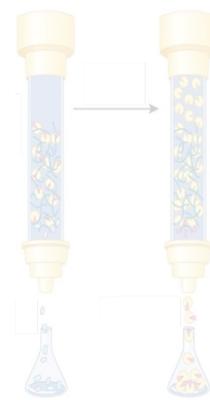


scan images and analyze data
lab days 5 and 6

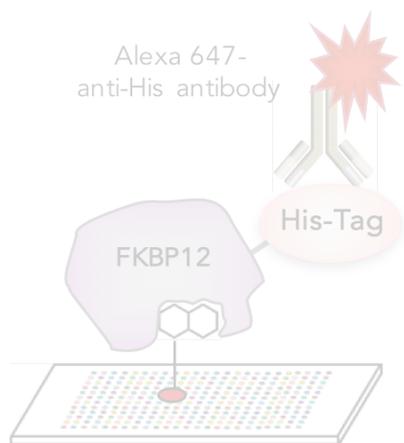
Our path to probe discovery



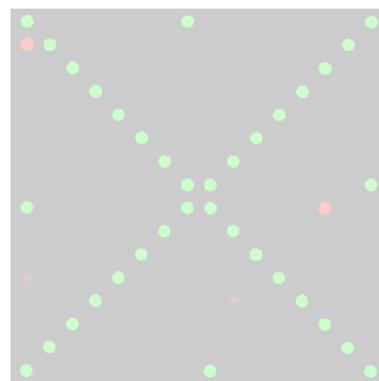
overexpress FKBP12
lab day 1



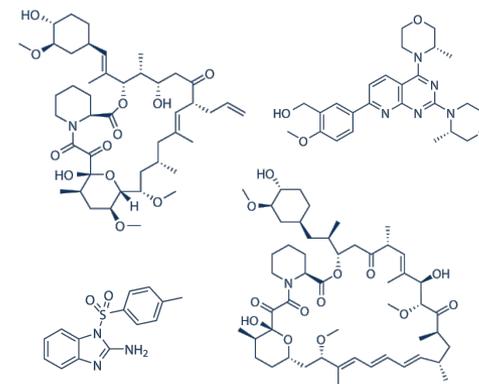
purify and characterize FKBP12
lab days 2 and 3



SMM screen
lab day 4



scan images and analyze data
lab days 5 and 6

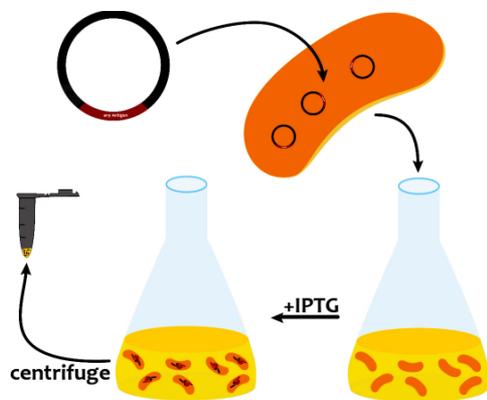


compare hit lists for teams
lab day 7

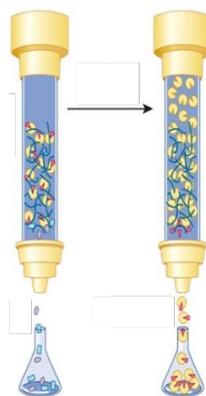


cheminformatics helps probe and drug finders make sense of the tidal wave of information coming from their screens

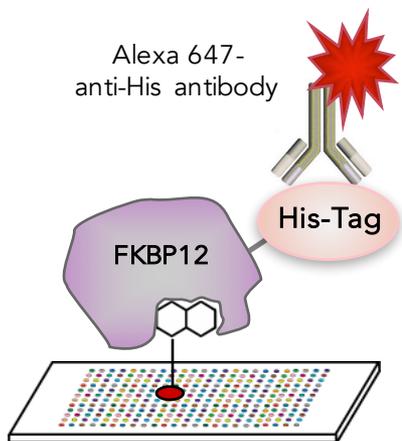
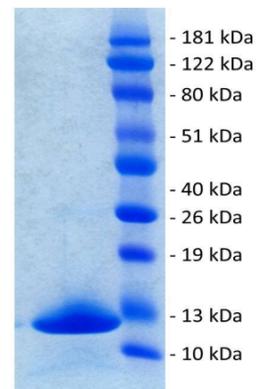
Our path to probe discovery



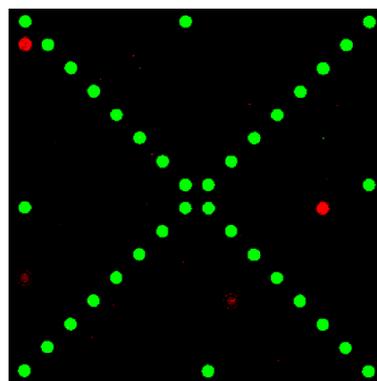
overexpress FKBP12
lab day 1



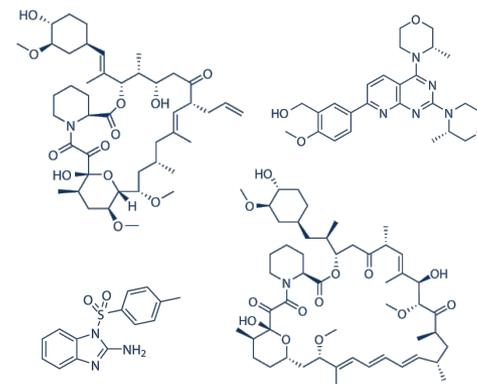
purify and characterize FKBP12
lab days 2 and 3



SMM screen
lab day 4



scan images and analyze data
lab days 5 and 6



compare hit lists for teams
lab day 7

Our path to probe discovery - lectures

2/9/17	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
 2/14/17	Lecture 2	<i>For the love of proteins: FKBP12 and immunophilins</i>
2/16/17	Lecture 3	Making proteins
2/21/17	No Lecture	
2/23/17	Lecture 4	Small-molecule microarrays
2/28/17	Lecture 5	Analyzing SMM data sets (Shelby Doyle)
3/2/17	Lecture 6	Small molecules as tools for engineering cells
3/7/17	Lecture 7	Wrap up discussion: suggestions for how to report your findings

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3/2/17	Lecture 5	Engineering fun with with proteins and small molecules
3/7/17	Lecture 6	Wrap up discussion: suggestions for how to report your findings