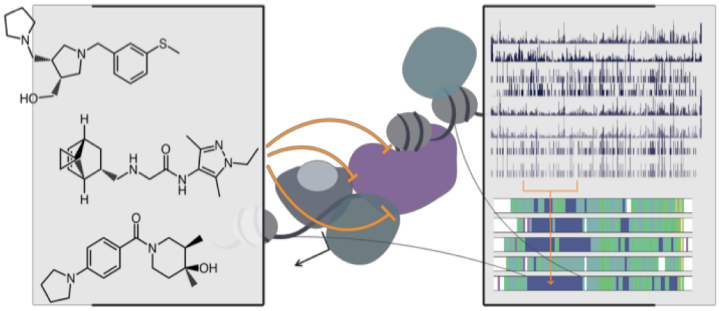


L6 – Engineering Transcriptional Responses with a Chemical Probe

February 28, 2019

D. melanogaster

13,600

C. elegans

19,500

Homo sapiens

21,000

Oryza sativa

45,000

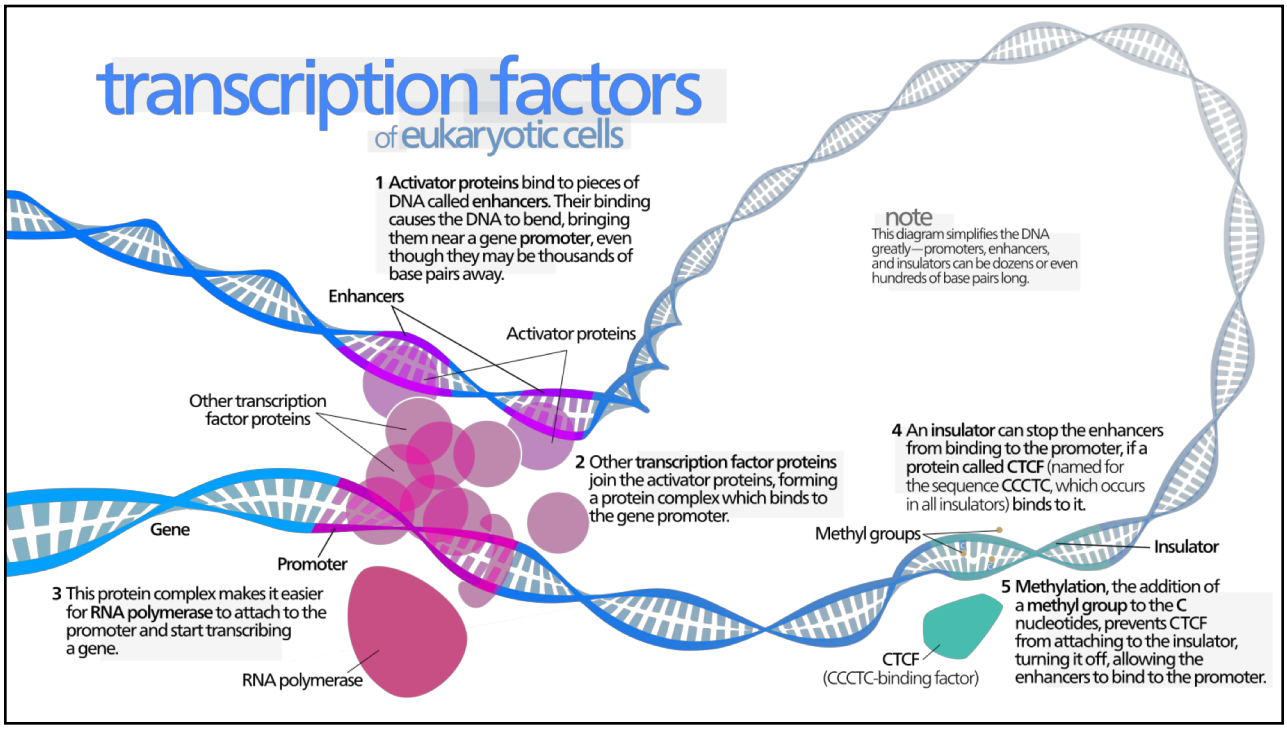
Zea mays

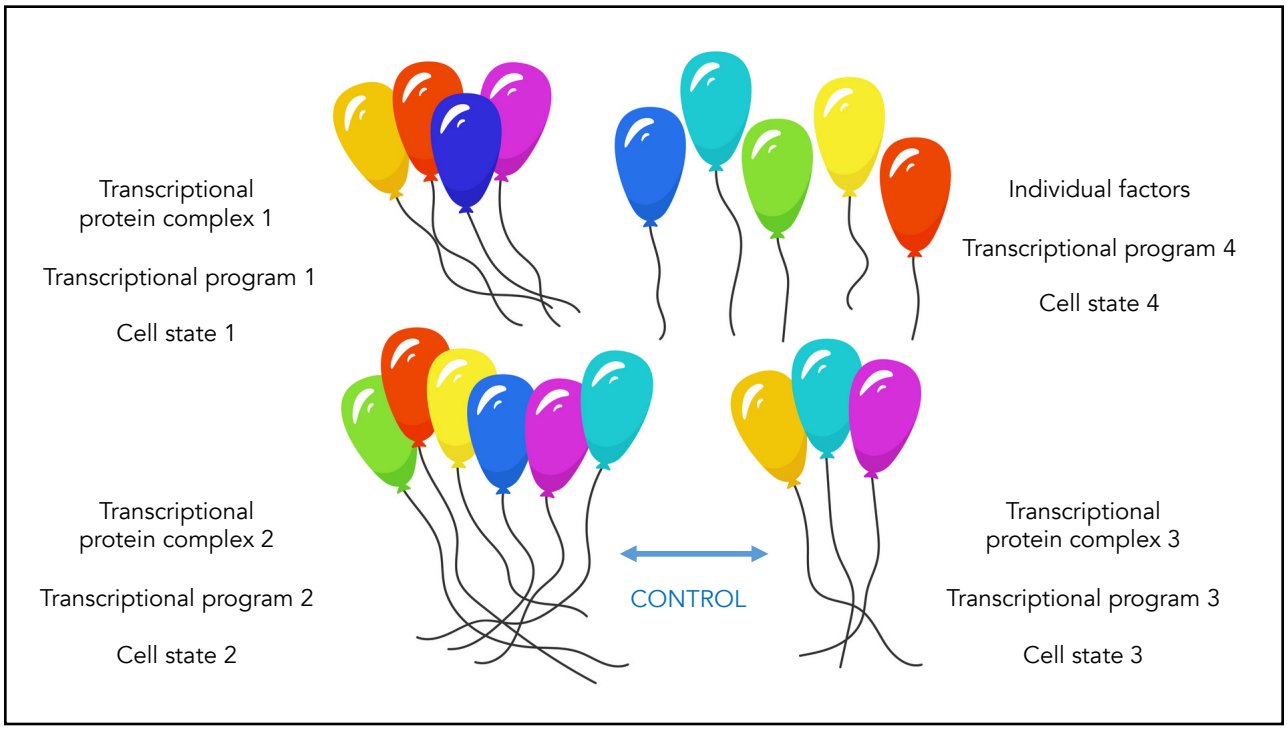
50,000

alternative splicing

post-translational processing

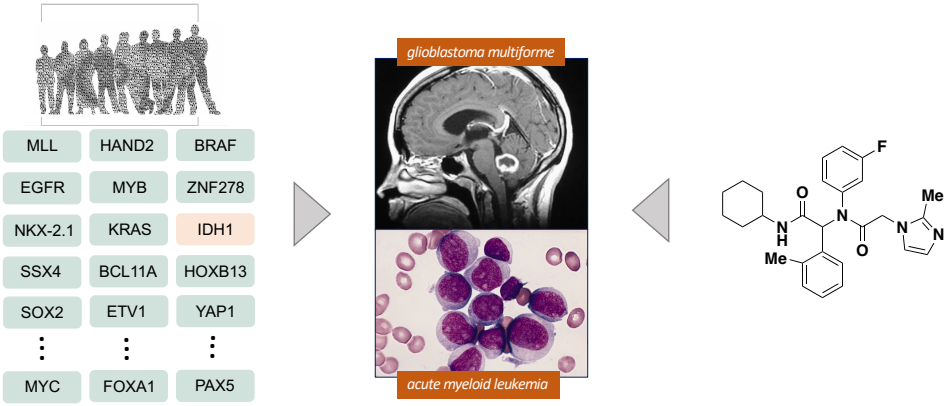
>100,000 proteins of unknown structure or function
How do these parts give rise to organismal complexity?


transcription factors
of eukaryotic cells
1 Activator proteins bind to pieces of DNA called enhancers. Their binding causes the DNA to bend, bringing them near a gene promoter, even though they may be thousands of base pairs away.
Enhancers
Activator proteins
Other transcription factor proteins
2 Other transcription factor proteins join the activator proteins, forming a protein complex which binds to the gene promoter.
Gene
Promoter
3 This protein complex makes it easier for RNA polymerase to attach to the promoter and start transcribing a gene.
RNA polymerase
note
This diagram simplifies the DNA greatly—promoters, enhancers, and insulators can be dozens or even hundreds of base pairs long.
4 An insulator can stop the enhancers from binding to the promoter, if a protein called CTCF (named for the sequence CCCTC, which occurs in all insulators) binds to it.
Methyl groups
Insulator
5 Methylation, the addition of a methyl group to the C nucleotides, prevents CTCF from attaching to the insulator, turning it off, allowing the enhancers to bind to the promoter.
CTCF
(CCCTC-binding factor)


Transcriptional protein complex 1
Transcriptional program 1
Cell state 1
Individual factors
Transcriptional program 4
Cell state 4
Transcriptional protein complex 2
Transcriptional program 2
Cell state 2
CONTROL
Transcriptional protein complex 3
Transcriptional program 3
Cell state 3

Therapeutically-driven probe discovery

target cause of disease revealed by human genetics



patient samples reveal list of disease genes

test impact of disease genes in a physiologic settings

discover molecules that reverse impact of disease genes

Transcription factors

implicated in a broad spectrum of disease

AVGR8	central corneal thickness
BCL11A	β-hemoglobin disorders
CAMTA1	episodic memory deficit
ELF1	systemic lupus erythematosus
ETS1	systemic lupus erythematosus
GATA3	periodontitis
GTF2H1	amyloidosis
HHEX-IDE	T2D
HIF2A	RCC
HNF1B	T2D
HPB1	osteoarthritis
IRF5	various AI disorders
IRF8	MS
LBXCOR1	restless leg syndrome
MAF	early-onset obesity
MECP2	autism
MEIS1	restless leg syndrome
MLXIPL	coronary artery disease
NFATC2	T1D
NOTCH2	T2D
PBX4	coronary artery disease
PPARG	T2D
RELA	rheumatoid arthritis
RFX4	Parkinson's disease
SP7	BMD
STAT3	various AI disorders and cancers
STAT4	systemic lupus erythematosus
TCF4	schizophrenia, corneal dystrophy
TCF7L2	T2D
THAP1	early-onset torsion dystonia
ZNF469	central corneal thickness
ZNF804A	schizophrenia

...

Transcription factors

misregulation in cancer

amplified TF cancer genes

JUN	sarcoma
LMO1	T-ALL, neuroblastoma
MITF	melanoma
MYC	various cancers
MYCL1	small cell lung
MYCN	neuroblastoma
NKX2-1	folicular lymphoma
REL	Hodgkin lymphoma
SOX2	NSCLC, esophageal SCC

germline mutated TF cancer genes

HNF1	HCC, hepatic adenoma
LMO1	neuroblastoma
PHOX2B	neuroblastoma
RB1	various cancers
SMAD4	gastrointestinal polyps
SMARCB1	malignant rhabdoid
SUFU	medulloblastoma
TP53	various cancers
WT1	Wilms tumor

TF cancer genes with frameshift mutations

ARID1A	clear cell ovarian carcinoma, RCC
ASXL1	MDS, CMML
ATRX	pancreatic neuroendocrine
CEBPA	AML, MDS
CREBBP	ALL, AML, DLBCL, B-NHL
DAXX	pancreatic neuroendocrine
EP300	various cancers
GATA1	megakaryoblastic leukemia
GATA3	breast
HNF1	HCC, hepatic adenoma
HRPT2	parathyroid adenoma
NOTCH2	marginal zone lymphoma, DLBCL
PBRM1	breast, clear cell renal carcinoma
PHOX2B	neuroblastoma
PRDM1	DLBCL
RB1	various cancers
SMAD4	gastrointestinal polyps
SMARCA4	NSCLC
SMARCB1	malignant rhabdoid
SUFU	medulloblastoma
TP53	various cancers
WT1	Wilms tumor

...

somatically mutated TF cancer genes

AFF4	ALL
ARNT	AML
ATF1	melanoma, AFH
BTG1	BCLL
CBFB	AML
CDX2	AML
CEBPA	AML, MDS
CIC	soft tissue sarcoma
CIITA	PMBL, Hodgkin lymphoma
CREB1	clear cell sarcoma
CREBBP	ALL, AML, DLBCL, B-NHL
CRTC3	salivary gland mucoepidermoid
DUX4	soft tissue sarcoma
EBF1	lipoma
ELF4	AML
ELK4	prostate
ELKS	papillary thyroid
EP300	various cancers
ERG	AML, Ewing sarcoma, prostate
ETV1	Ewing sarcoma, prostate
ETV4	Ewing sarcoma, prostate
ETV5	prostate
ETV6	various cancers
EVI1	AML, CML
EWSR1	Ewing sarcoma, ALL
FEV	Ewing sarcoma
FLI1	Ewing sarcoma
FOXL2	ovarian
FOXO1A	alveolar rhabdomyosarcomas
FOXO3A	AL
FOXP1	ALL
GATA1	megakaryoblastic leukemia
GATA2	AML
GATA3	breast
HLF	ALL
HLXB9	AML
HMGA1	various cancers
HMGA2	various cancers
HOXA11	CML
HOXA13	AML
HOXA9	AML
HOXC11	AML
HOXC13	AML
HOXD11	AML
HOXD13	AML
HNF1	HCC
HRPT2	parathyroid adenoma
IKZF1	ALL
IRF4	MM
JAZF1	endometrial stromal tumors
JUN	sarcoma
KLF6	prostate, glioma
LAF4	ALL
LMO1	T-ALL, neuroblastoma
LMO2	T-ALL
LPP	lipoma, leukemia
LYL1	T-ALL
MAFB	MM
MAML2	salivary gland
MDS1	MDS, AML
MDS2	MDS
MECT1	salivary gland
MHC2TA	head-neck squamous cell, renal
MITF	melanoma
MKL1	AML
MLF1	AML
MLLT1	ALL
MLLT10	ALL, colorectal
MLLT2	ALL, breast cancers
MLLT3	AML
MLLT4	AML
MLLT6	ALL
MLLT7	ALL
MYB	adenoid cystic sarcoma
MYC	various cancers
MYCL1	small cell lung
MYCN	neuroblastoma
NCOA1	alveolar rhabdomyosarcoma
NCOA2	AML
NCOA4	papillary thyroid
NFIB	lipoma, ACC
NFKB2	B-NHL
NKX2-1	NSCLC
NOTCH1	T-ALL
NOTCH2	DLBCL, marginal zone lymphoma
NR4F3	EMC
NRF2	NSCLC, HNSCC
OLIG2	T-ALL
PAX3	alveolar rhabdomyosarcoma
PAX5	NHL
PAX7	alveolar rhabdomyosarcoma
PAX8	follicular thyroid
PBX1	pre B-ALL
PHOX2B	neuroblastoma
PLAG1	salivary adenoma
PMX1	AML1
POU2AF1	NHL
POU5F1	sarcoma
PPARG	follicular thyroid
PRDM1	DLBCL
PRDM16	MDS, AML
RARA	APL
RB1	various cancers
REL	Hodgkin lymphoma
RUNX1	AML, pre B-ALL
RUNXBP2	AML
SMAD4	colorectal, pancreatic
SMARCA4	NSCLC
SMARCB1	malignant rhabdoid
SOX2	NSCLC, esophageal SCC
SS18	synovial sarcoma
SS18L1	synovial sarcoma
SSX1	synovial sarcoma
SSX2	synovial sarcoma
SSX4	synovial sarcoma
SUFU	medullablastoma
SUZ2	endometrial stromal tumors
TAF15	ALL, EMC
TAL1	lymphoblastic leukemia
TAL2	T-ALL
TCEA1	salivary adenoma
TCF12	EMC
TCF3	pre B-ALL
TFE3	renal, alveolar soft sarcoma
TFEB	renal (child epithelioid)
TFPT	pre B-ALL
THRAP3	aneurysmal bone cysts
TIF1	APL
TLX1	T-ALL
TLX3	T-ALL
TP53	various cancers
TRIM27	papillary thyroid
TRIM33	papillary thyroid
TSHR	toxic thyroid adenoma
WT1	Wilm tumor
ZNF145	APL
ZNF198	MPD, NHL
ZNF278	Ewing sarcoma
ZNF331	follicular thyroid adenoma
ZNF384	ALL
ZNF521	ALL
ZNF9	aneurysmal bone cysts
ZNFN1A1	\ALL, DLBCL

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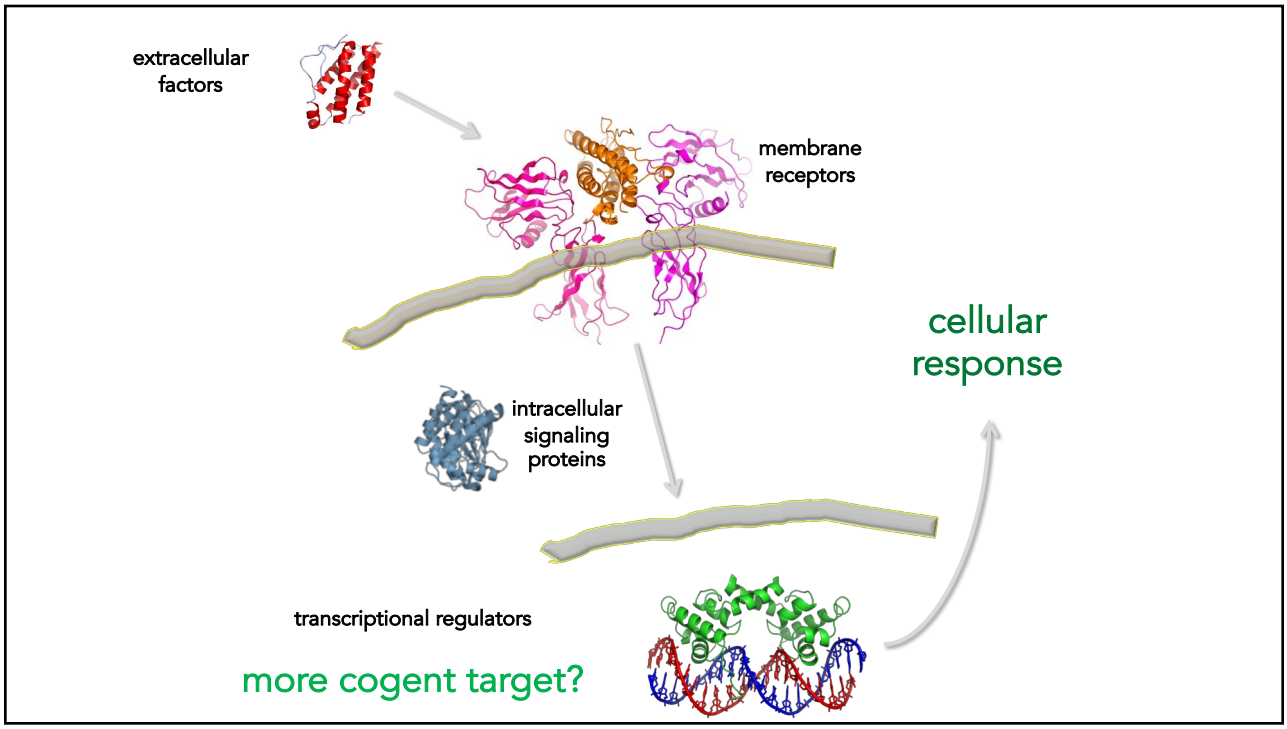
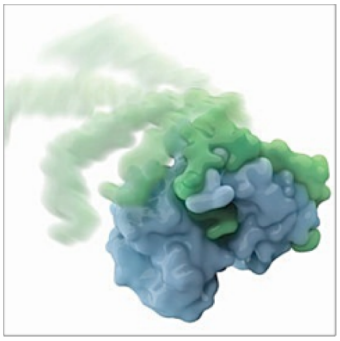
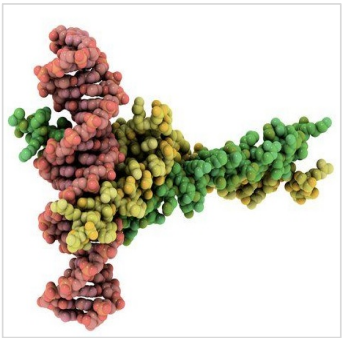


A complex task?

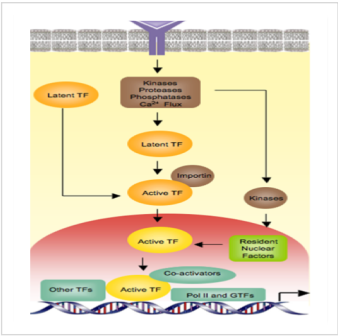
transcription factors are the prototype of an '*undruggable*' target



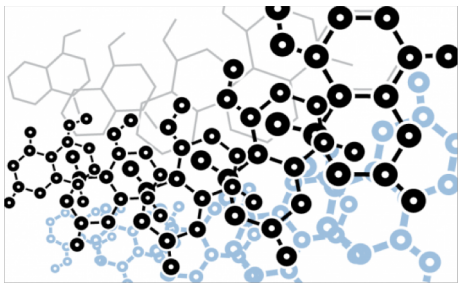
disordered when isolated from binding partners



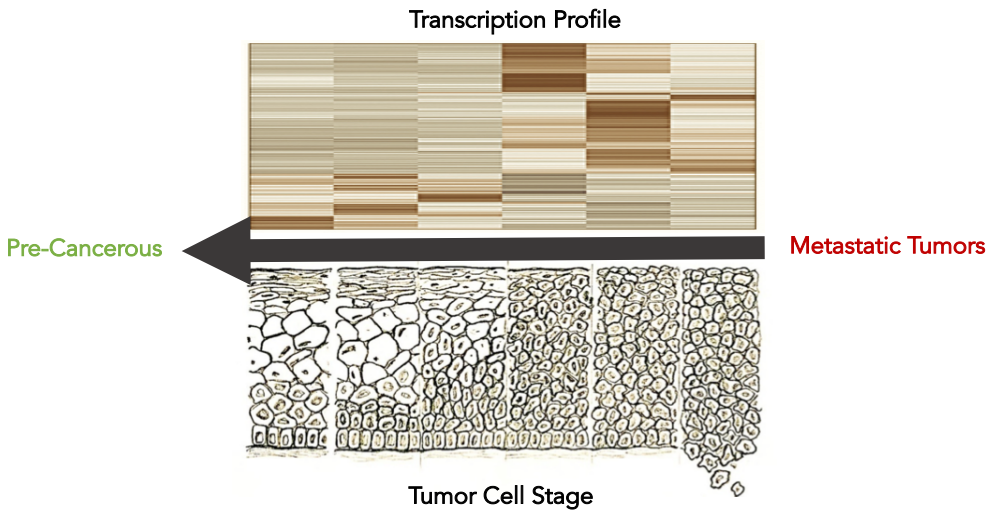
DNA-binding domains lack obvious pockets



transit to reach resident nuclear factors



Can we build general and systematic platforms for developing chemical probes for transcriptional regulators?



Can we tune dysregulated gene expression programs and impact cell state?

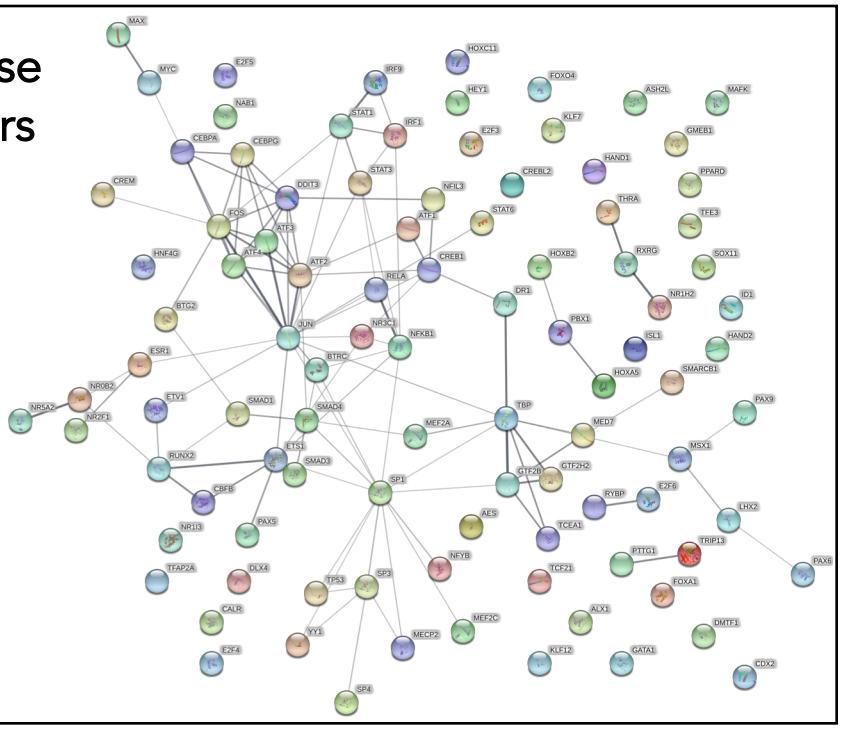
Profiling 100 diverse transcription factors

commercially available
purified, His-tagged

145 InterPro domains
e.g. bZip, Znf_C2H2, Fbox, Ets, etc.

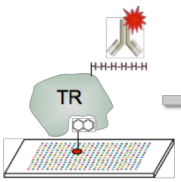
>500 GO terms
e.g. nuclear, chromatin remodeling, basal transcription, etc.

>100 KEGG pathways
e.g. Wnt signaling, chronic myeloid leukemia, circadian entrainment, etc.

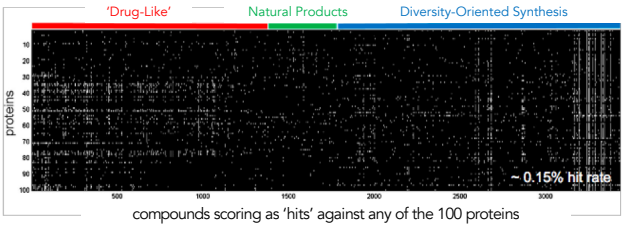


Clemons et al., PNAS 107, 18787-18792, 2010

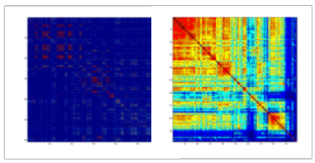
'100 Transcription Factor' SMM Screen



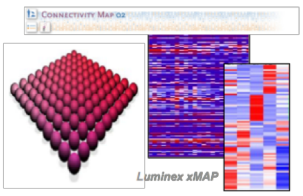
6.6M individual assays
2.2M theoretical interactions



compounds scoring as 'hits' against any of the 100 proteins



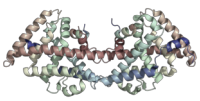
computational chemical biology

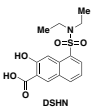


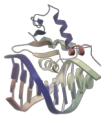
HT-gene expression studies

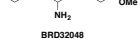
Clemons et al., PNAS 107, 18787-18792, 2010
Clemons et al., PNAS 108, 6817-6822, 2011

Chemical stabilizer of Small Heterodimer Partner Yang et al., Mol Cancer Ther, 15, 2294-2301, 2016









Chemical inhibitor of ETV1
Pop et al., Mol Cancer Ther 13, 1492-1502, 2014

characterize and optimize probes for individual TFs

100 diverse transcription factors

commercially available
purified, His-tagged

145 InterPro domains
e.g. bZip, Znf_C2H2, Fbox, Ets, etc.

>500 GO terms
e.g. nuclear, chromatin remodeling, basal transcription, etc.

>100 KEGG pathways
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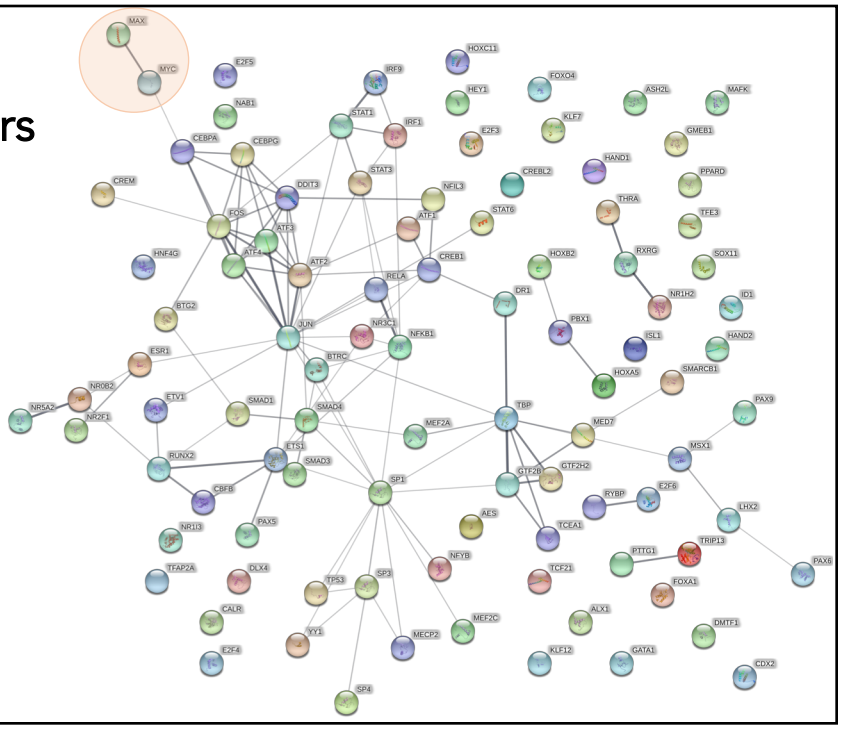
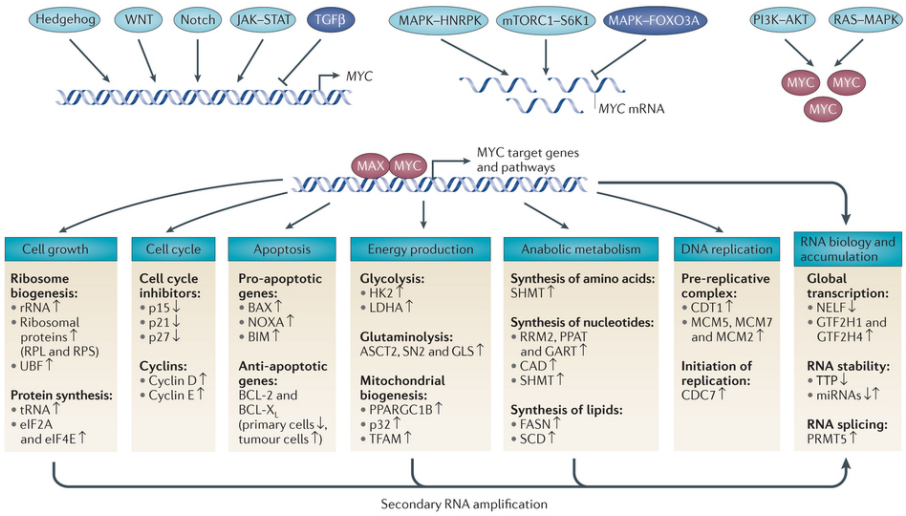


Clemens et al., PNAS 107, 18787-18792, 2010

MYC family of transcription factors

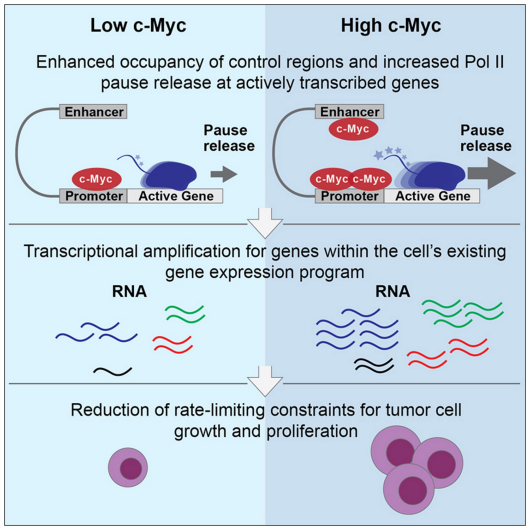
master regulators of broad cellular processes



Nature Reviews | Cancer

c-Myc

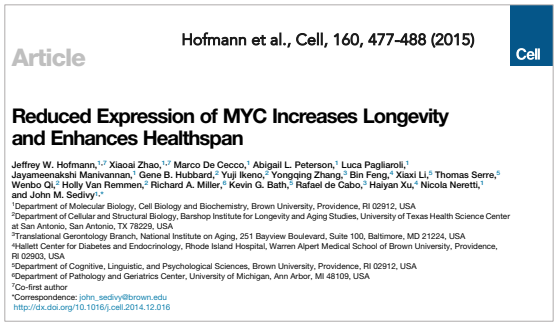
accumulates in promoter regions and amplifies transcription when overexpressed in cancer



Lin et al., Cell, 151, 56-67 (2012); Nie et al, Cell 151, 68-79 (2012)

MYC expression in haploinsufficient mice

amelioration of age-associated phenotypes



Article

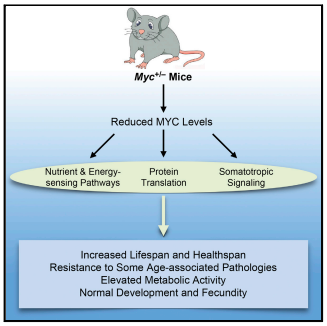
Hofmann et al., Cell, 160, 477-488 (2015)

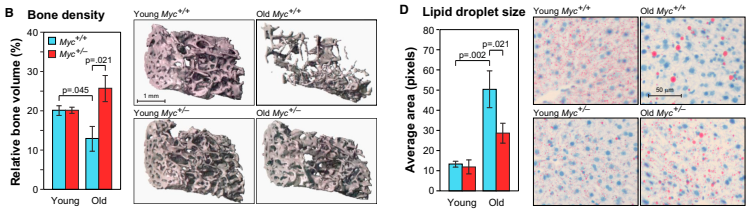
Cell

Reduced Expression of MYC Increases Longevity and Enhances Healthspan

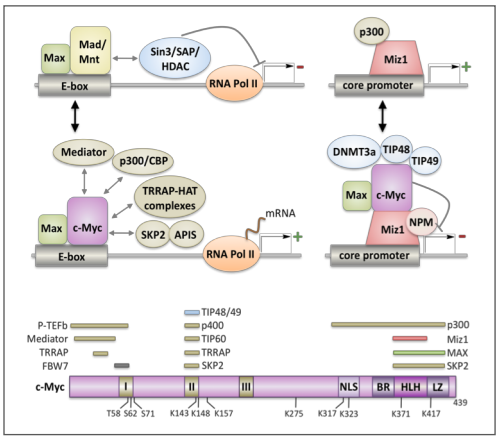
Jeffrey W. Hofmann,[1,7] Xiaoai Zhao,[1,7] Marco De Cecco,[1] Abigail L. Peterson,[1] Luca Pagliaroli,[1] Jayameenakshi Manivannan,[1] Gene B. Hubbard,[2] Yuji Ikeno,[2] Yongqing Zhang,[2] Bin Feng,[2] Xiaxi Li,[3] Thomas Serre,[3] Wenbo Qi,[2] Holly Van Remmen,[2] Richard A. Miller,[4] Kevin G. Bath,[5] Rafael de Cabo,[3] Haiyan Xu,[6] Nicola Neretti,[1] and John M. Sedivy[1,*]

[1]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02912, USA
[2]Department of Cellular and Structural Biology, Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
[3]Translational Gerontology Branch, National Institute on Aging, 251 Bayview Boulevard, Suite 100, Baltimore, MD 21224, USA
[4]Hellett Center for Diabetes and Endocrinology, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI 02903, USA
[5]Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, Providence, RI 02912, USA
[6]Department of Pathology and Geriatrics Center, University of Michigan, Ann Arbor, MI 48109, USA
[7]Co-first author
*Correspondence: john_sedivy@brown.edu
http://dx.doi.org/10.1016/j.cell.2014.12.016

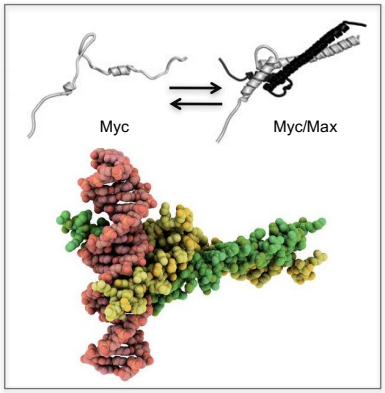




An obstinate therapeutic target

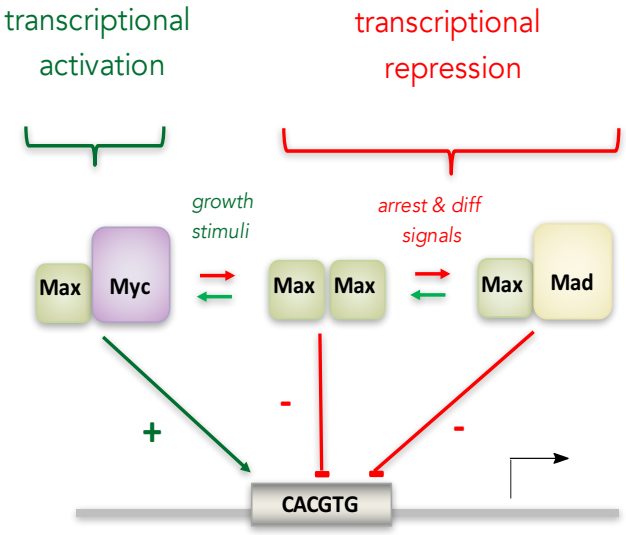


many protein-protein interactions

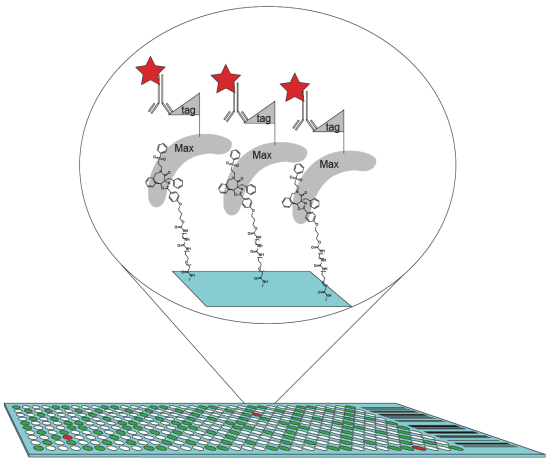


unstructured domains
no traditional binding pockets
large buried interface

Max as a target: heterodimer/homodimer dynamics

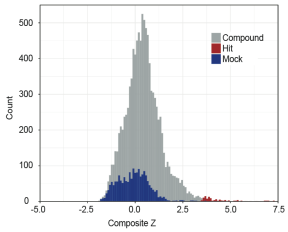


SMM screens: purified Max transcription factor

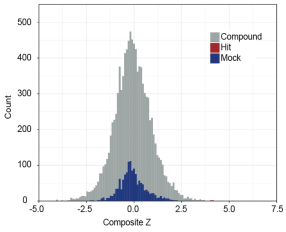


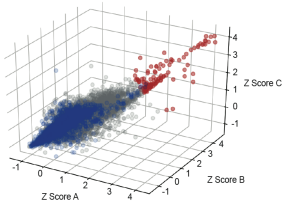
>45k compounds screened

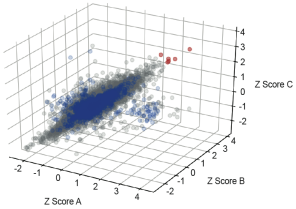
synthetic compound collection



natural products

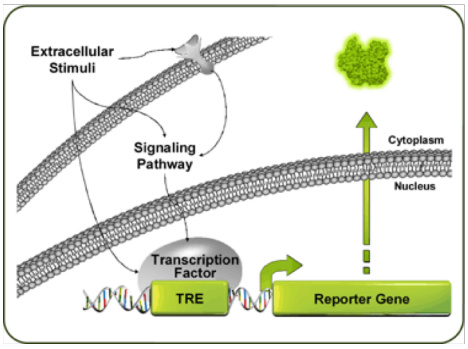


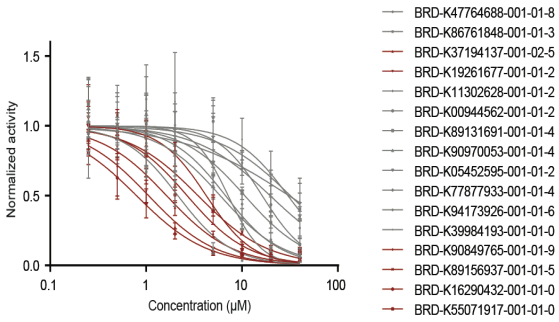




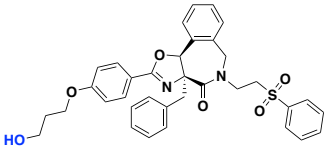
117 assay positives

Reporter gene assays: putative Max binders modulate Myc-driven transcription





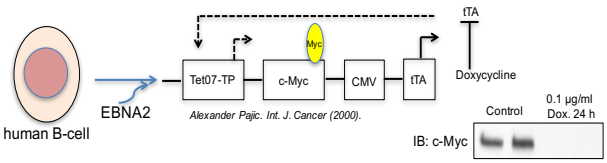
'KI-MS2'

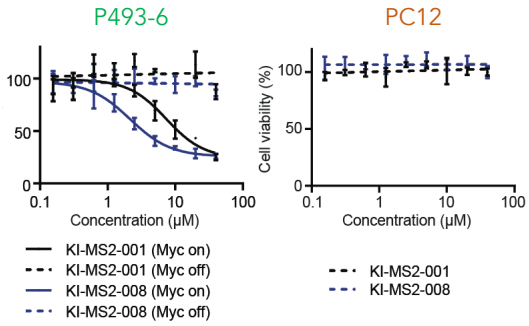


IC_{50} = 1.06 μM
MW = 610.73
cLogP = 5.15

Cell viability assays: Are Myc or Max required?

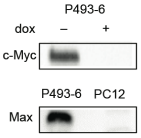
P493-6 Dox-repressible cells for MYC 'on/off' studies





Max-deficient PC12 pheochromocytoma cells

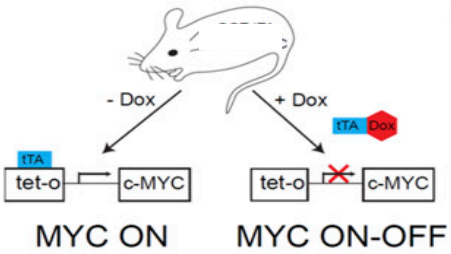
	KI-MS2-001	KI-MS2-008
Myc reporter	1.98 µM	1.28 µM
P493-6 Myc on	7.36 µM	2.15 µM
P493-6 Myc off	>50 µM	>50 µM
PC12	>50 µM	>50 µM

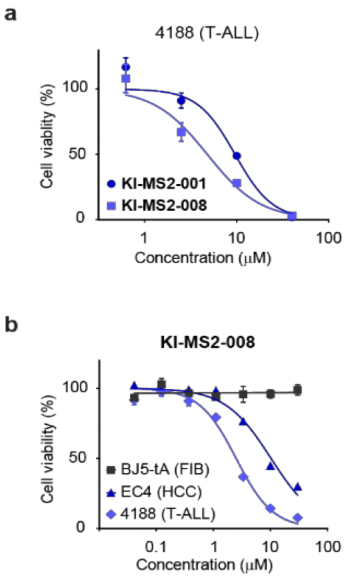


Conditional cellular models of *MYC* expression

Myc 'on/off' mouse models:

lymphoma
HCC
RCC
osteosarcoma

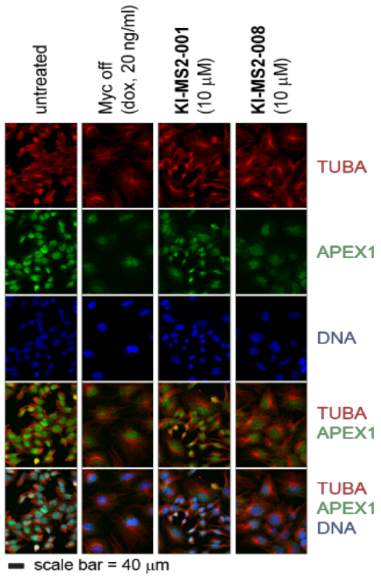




Anja Deutzmann, Felsher Lab Stanford

Imaging of biomarkers: conditional vs. chemical modulation

modulating Myc in an engineered osteosarcoma model



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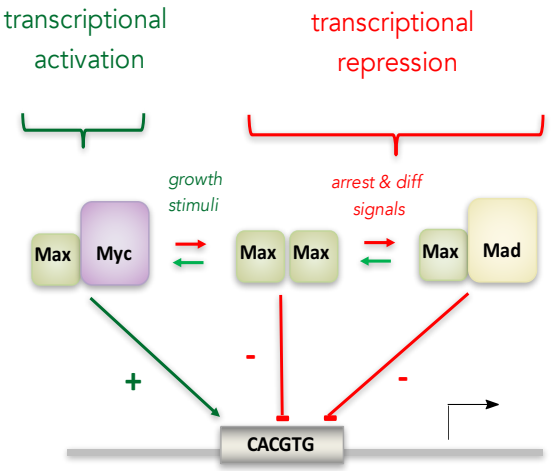
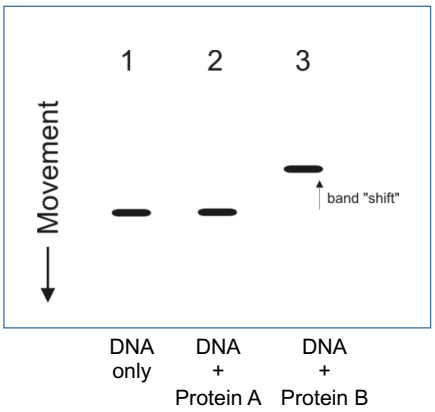
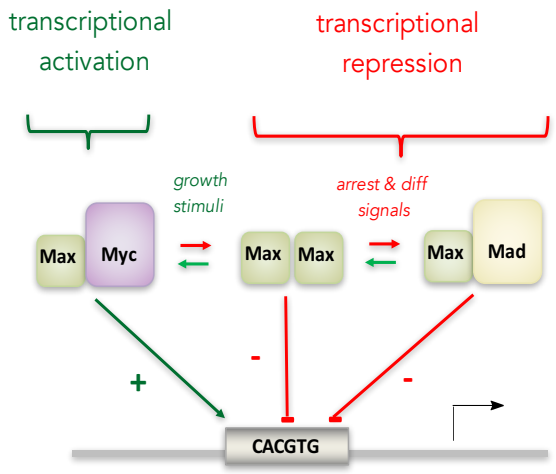
Does the probe antagonize the Myc/Max heterodimer?

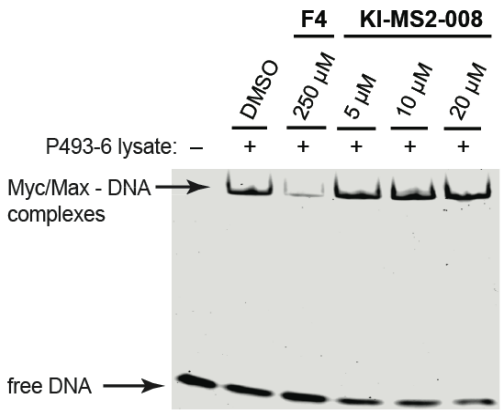


Electrophoretic Mobility Shift Assay (EMSA)
aka Gel Shift Assay



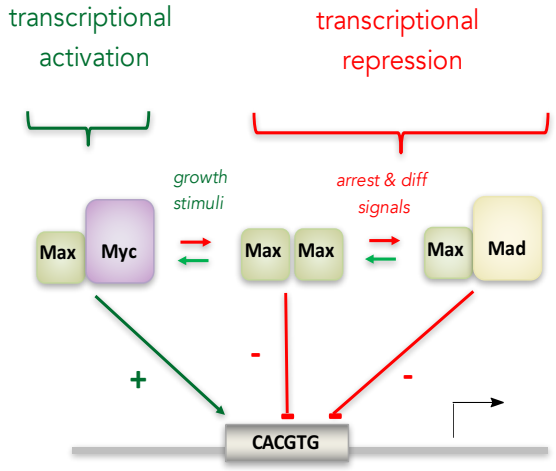
Does the probe antagonize the Myc/Max heterodimer?

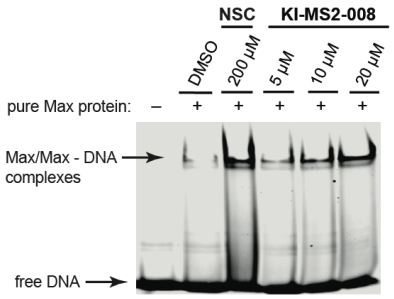


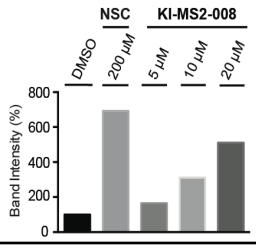


EMSA

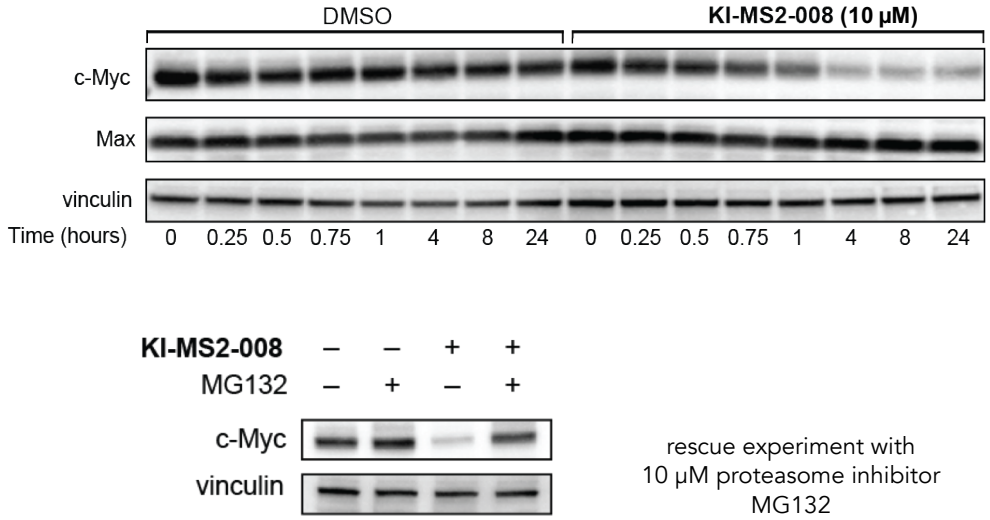
Does the probe stabilize the Max/Max homodimer?





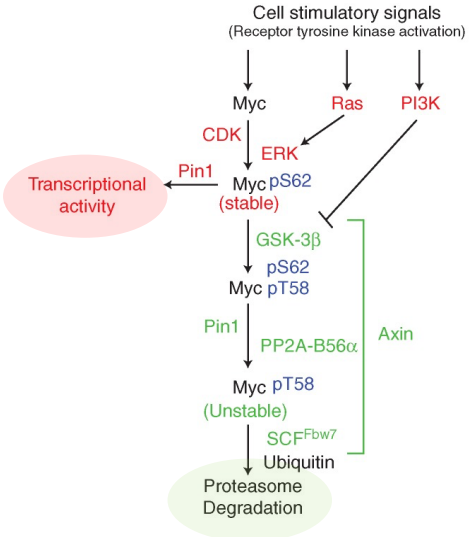


Western blots: KI-MS2-008 alters Myc protein levels



rescue experiment with
10 μM proteasome inhibitor
MG132

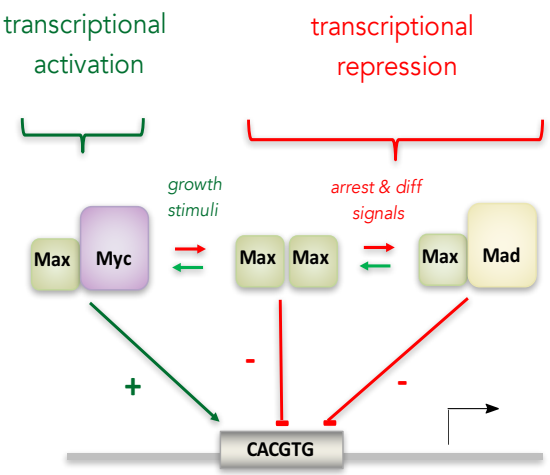
Myc protein stability is regulated by the ubiquitin-proteasome system



Farrell & Sears, Cold Spring Harb Perspect Med, 4 (2014)

KI-MS2-008

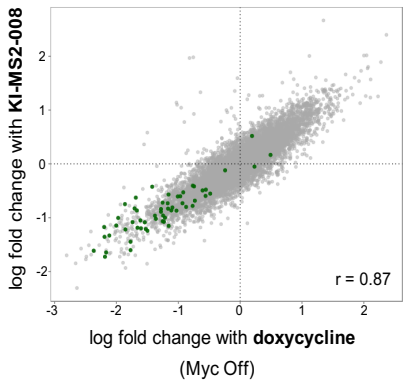
mixed mechanism inhibitor?

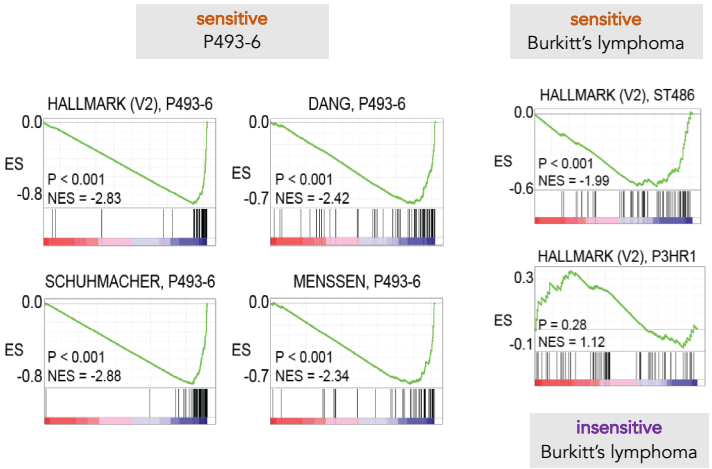


Gene expression profiling: KI-MS2-008 mimics *MYC* inactivation

Gene Set Enrichment Analysis reveals an enrichment of Myc target genes

Hallmark V2 MYC-dependent genes in green

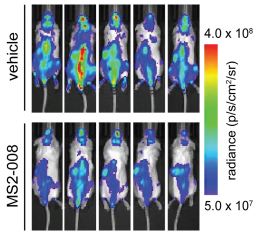


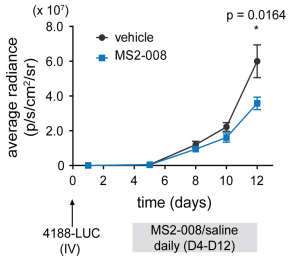


You will learn more about this method you will learn more about in module 2

In vivo studies: KI-MS2-008 modulates tumor volume in Myc-dependent mouse models of cancer

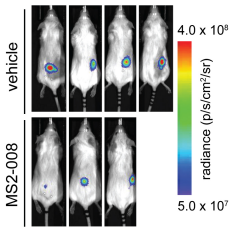
T-cell acute lymphoblastic leukemia
blood cancer

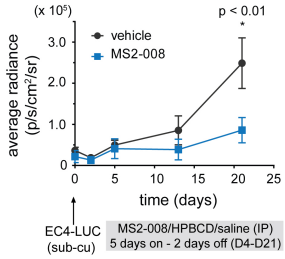




0.06 mg/kg
daily IV administration

hepatocellular carcinoma
solid tumor





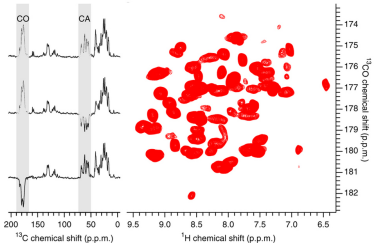
0.24 mg/kg
subcutaneous administration
5d on/2d off cycles

Anja Deutzmann, Felsher Lab Stanford

Current directions

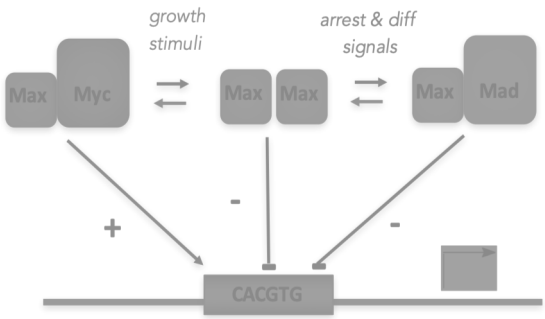
optimize potency and solubility, PK/PD-guided medicinal chemistry

structural biology



additional tumor models
same tumor models + new readouts

stabilizing repressive states vs. inhibiting activating states?



stabilizing vs. inhibiting PPIs?

Our path to evaluate ligands - lectures

2/9/19	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/12/19	Lecture 2	Small Molecule Microarrays
2/14/19	Lecture 3	For the love of proteins: FKBP12 and immunophilins
2/19/19	No Lecture	
2/21/19	Lecture 4	Quantitative evaluation of protein-ligand interactions
2/26/19	Lecture 5	A ligand discovery vignette: sonic hedgehog
2/28/19	Lecture 6	Engineering transcriptional responses with a small molecule
3/5/19	Lecture 7	Wrap up discussion: suggestions for how to report your findings