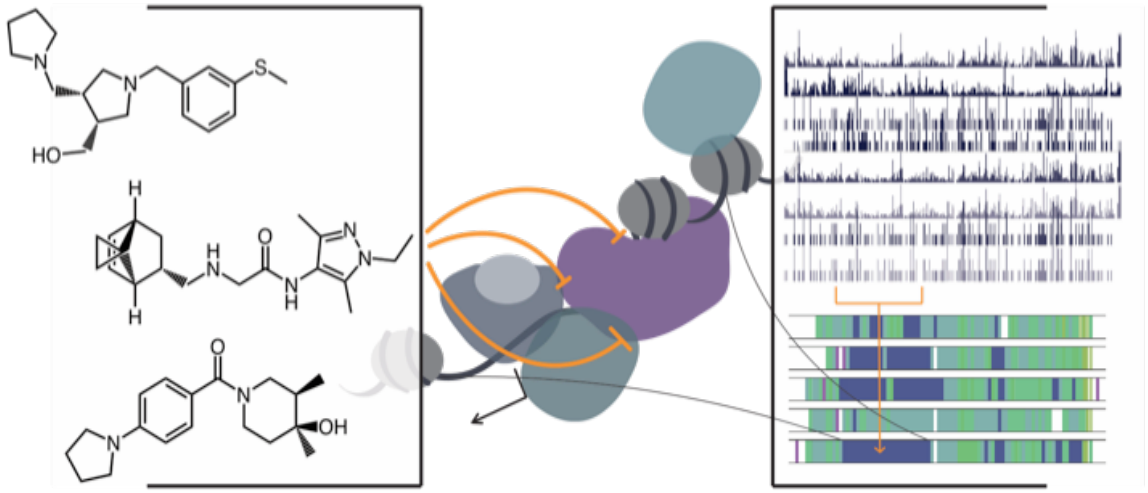
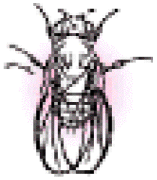


L6 – Engineering Transcriptional Responses with a Chemical Probe

November 19, 2019



D. melanogaster

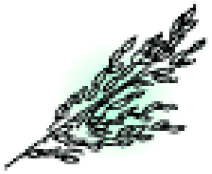
13,600

C. elegans

19,500

Homo sapiens

21,000



Oryza sativa

45,000

Zea mays

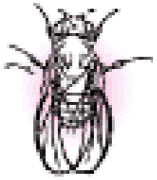
50,000

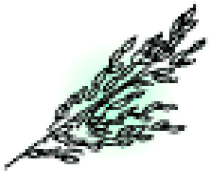
alternative splicing

post-translational processing

>100,000 proteins of unknown structure or function

How do these parts give rise to organismal complexity?




D. melanogaster	*C. elegans*	*Homo sapiens*	*Oryza sativa*	*Zea mays*
13,600	**19,500**	**21,000**	**45,000**	**50,000**
450	750	2,600	2,000	760

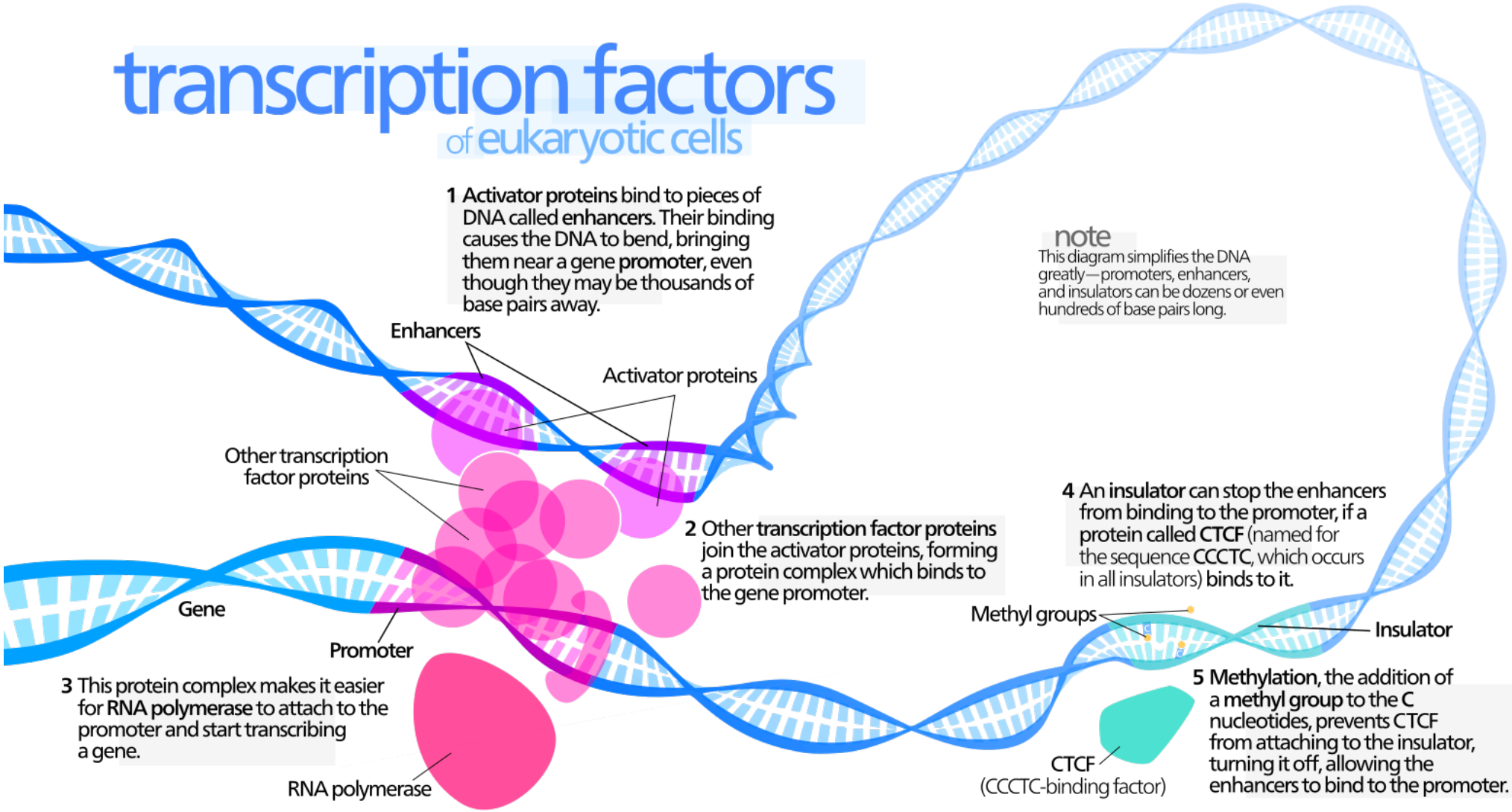
alternative splicing

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



Transcriptional protein complex 1

Individual factors

Transcriptional protein complex 2

Transcriptional protein complex 3

Transcriptional
protein complex 1

Transcriptional program 1

Individual factors

Transcriptional program 4

Transcriptional
protein complex 2

Transcriptional program 2

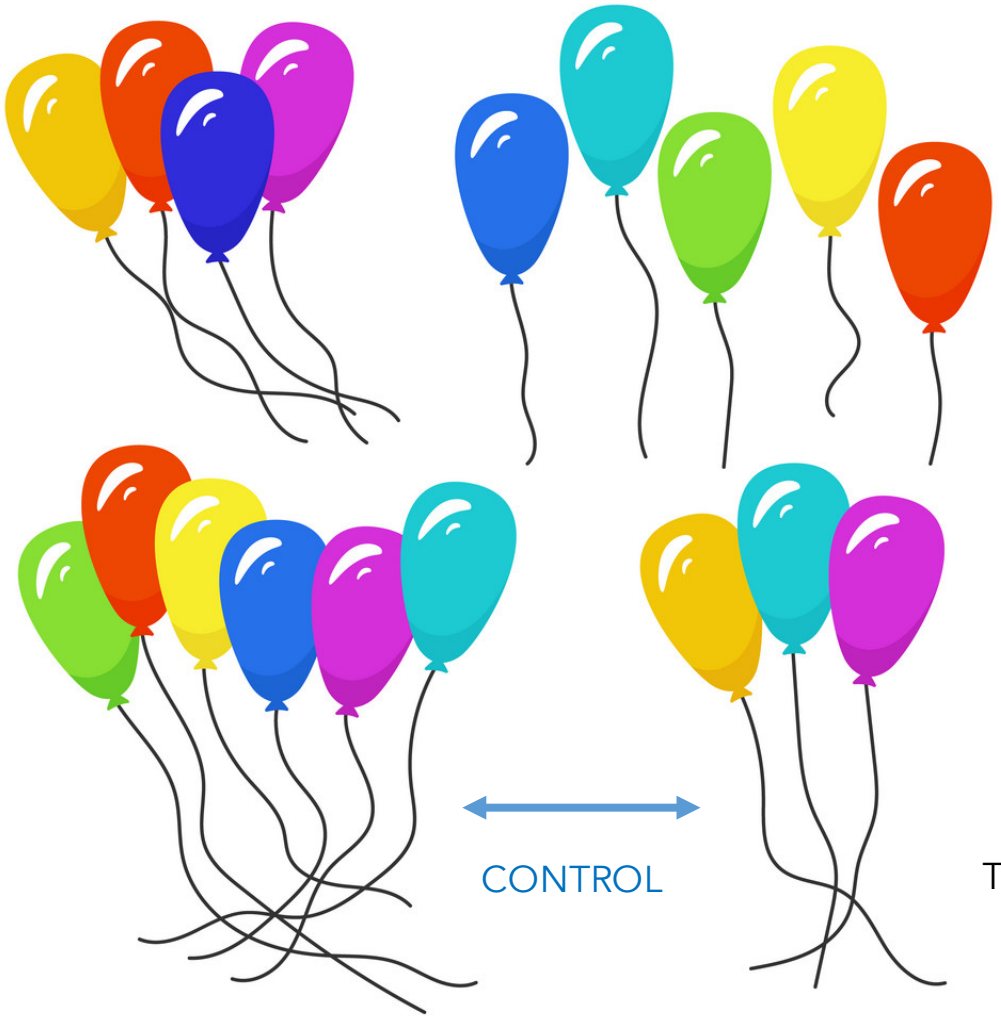
Transcriptional
protein complex 3

Transcriptional program 3

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

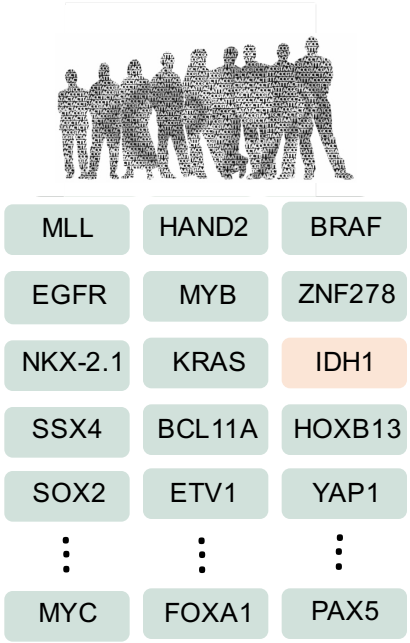
Transcriptional
protein complex 3

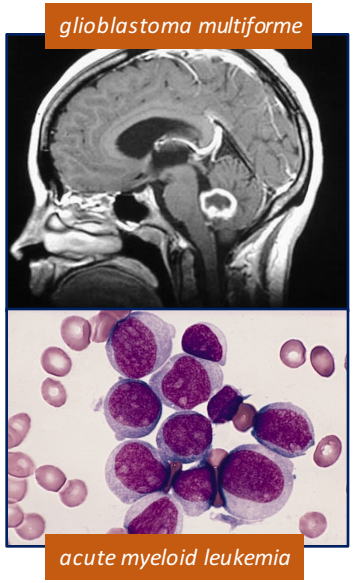
Transcriptional program 3

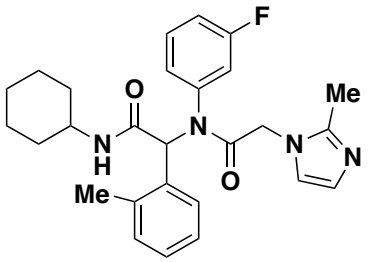
Cell state 3

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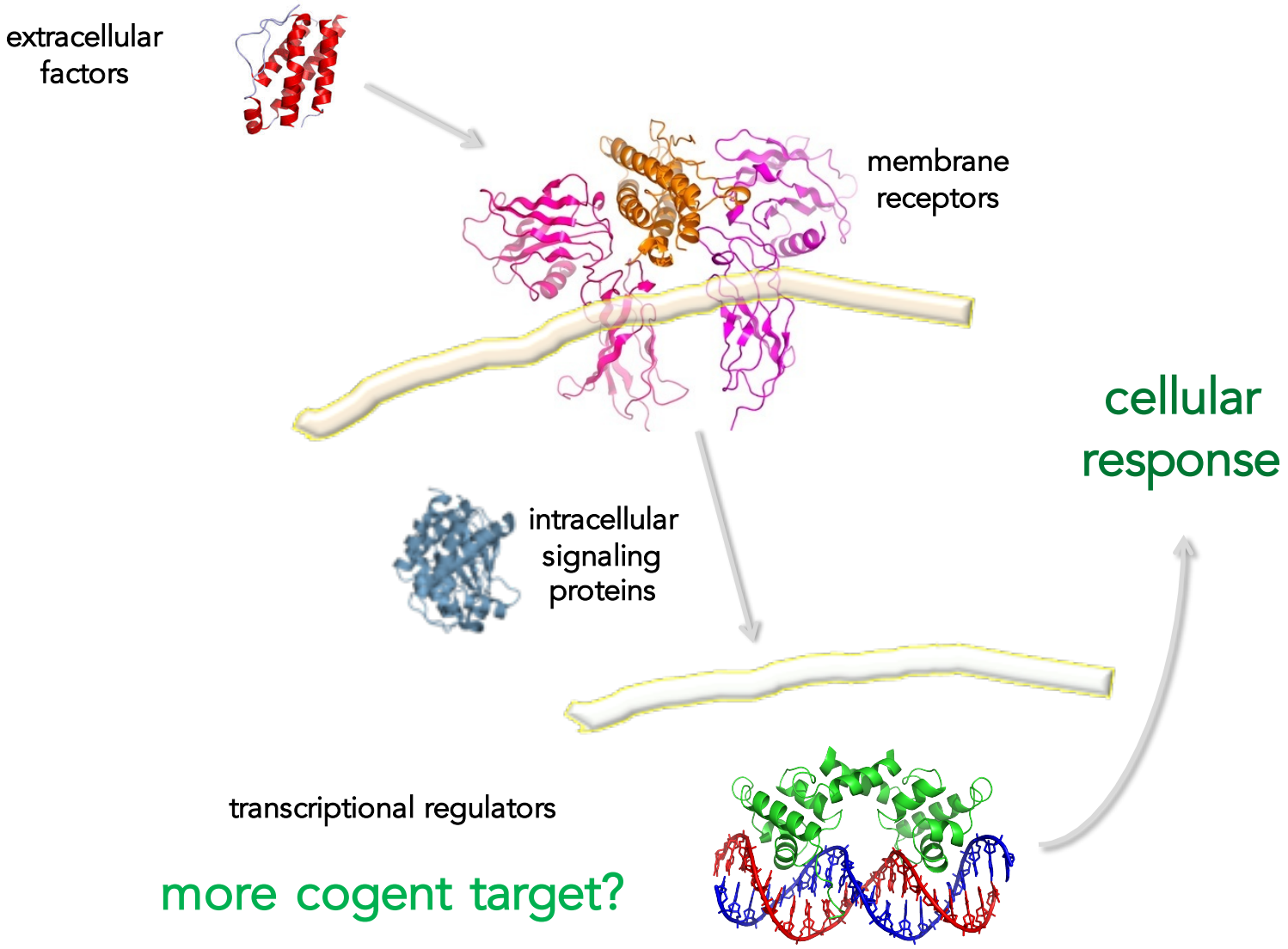
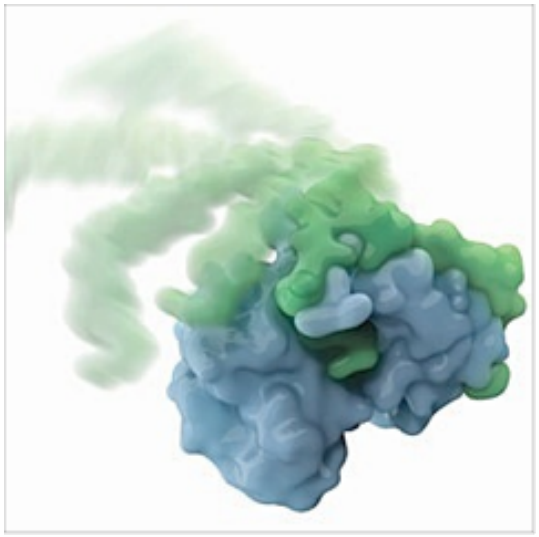

extracellular
factors
membrane
receptors
intracellular
signaling
proteins
cellular
response
transcriptional regulators
more cogent target?

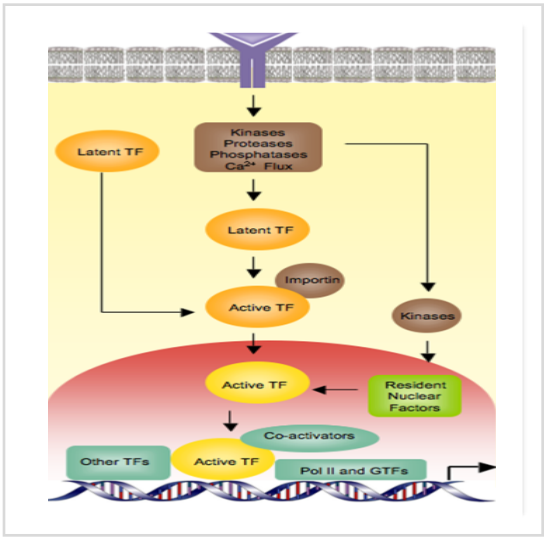
A complex task?

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

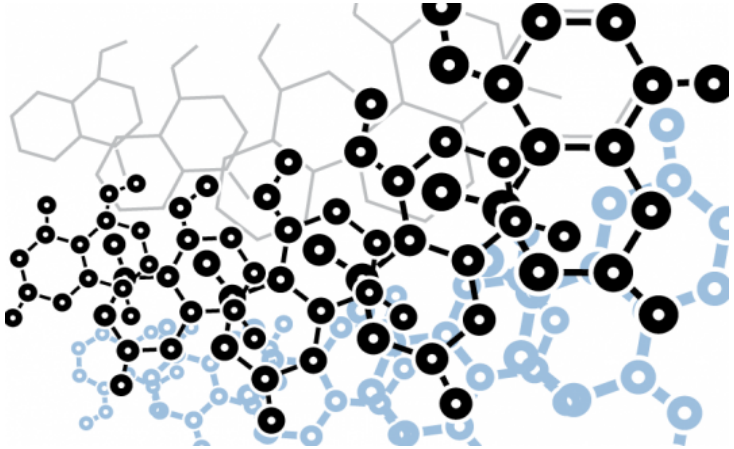


disordered when isolated from binding partners

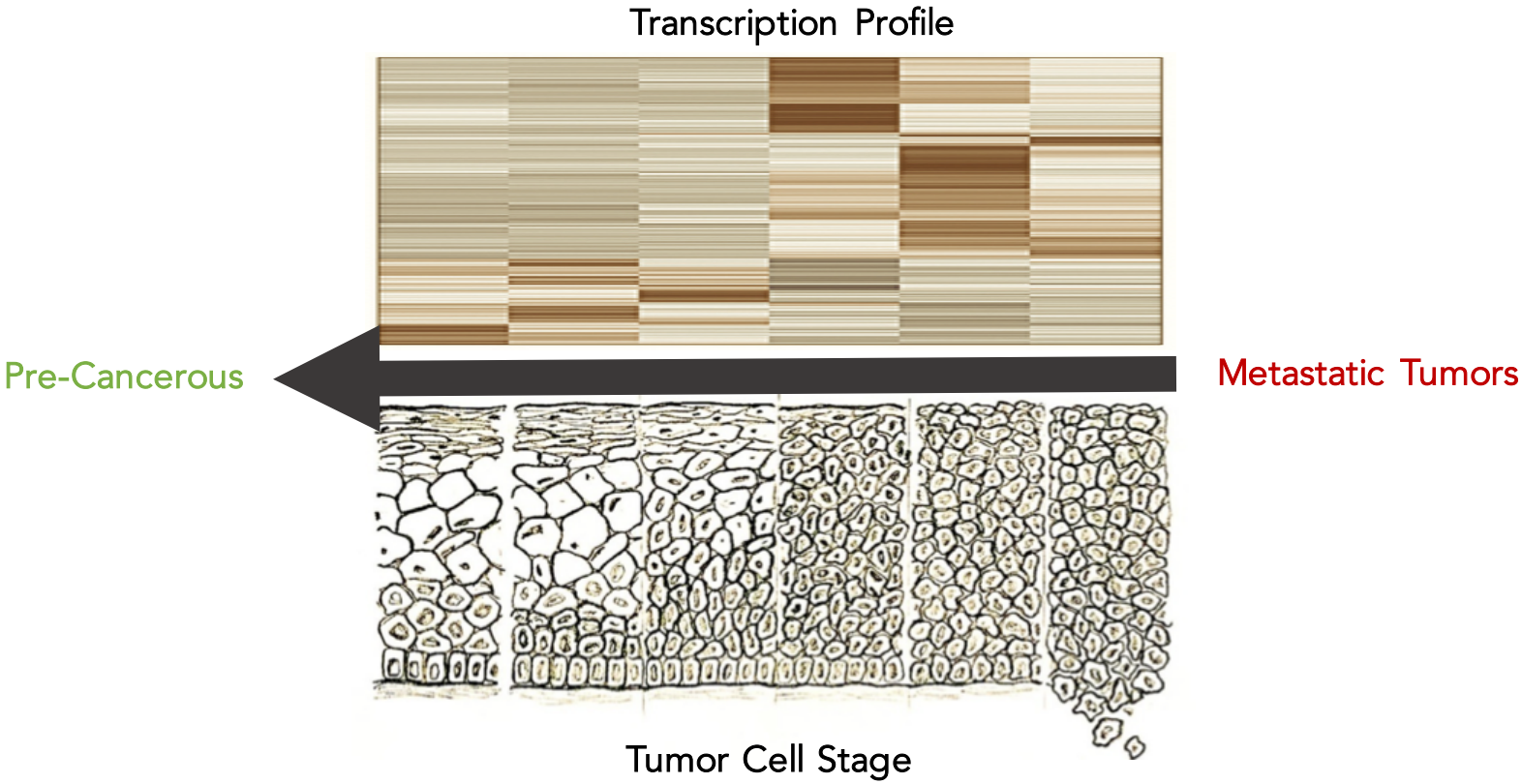
DNA-binding domains lack obvious pockets



transit to reach resident nuclear factors



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



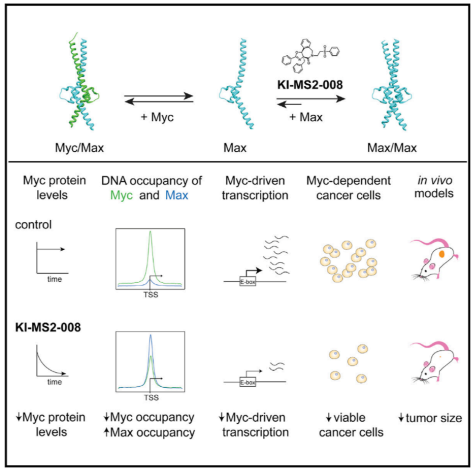
Can we tune dysregulated gene expression programs and impact cell state?

Article

Cell Chemical Biology

Stabilization of the Max Homodimer with a Small Molecule Attenuates Myc-Driven Transcription

Graphical Abstract



Highlights

- KI-MS2-008 is a Max-binding small molecule that attenuates Myc-driven transcription
- The compound stabilizes the Max homodimer
- Effects on DNA occupancy and the transcriptome resemble loss of Myc
- Treatment with KI-MS2-008 exhibits efficacy in cellular and murine cancer models

Authors

Nicholas B. Struntz, Andrew Chen, Anja Deutzmann, ..., Charles Y. Lin, Dean W. Felsher, Angela N. Koehler

Correspondence

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In Brief

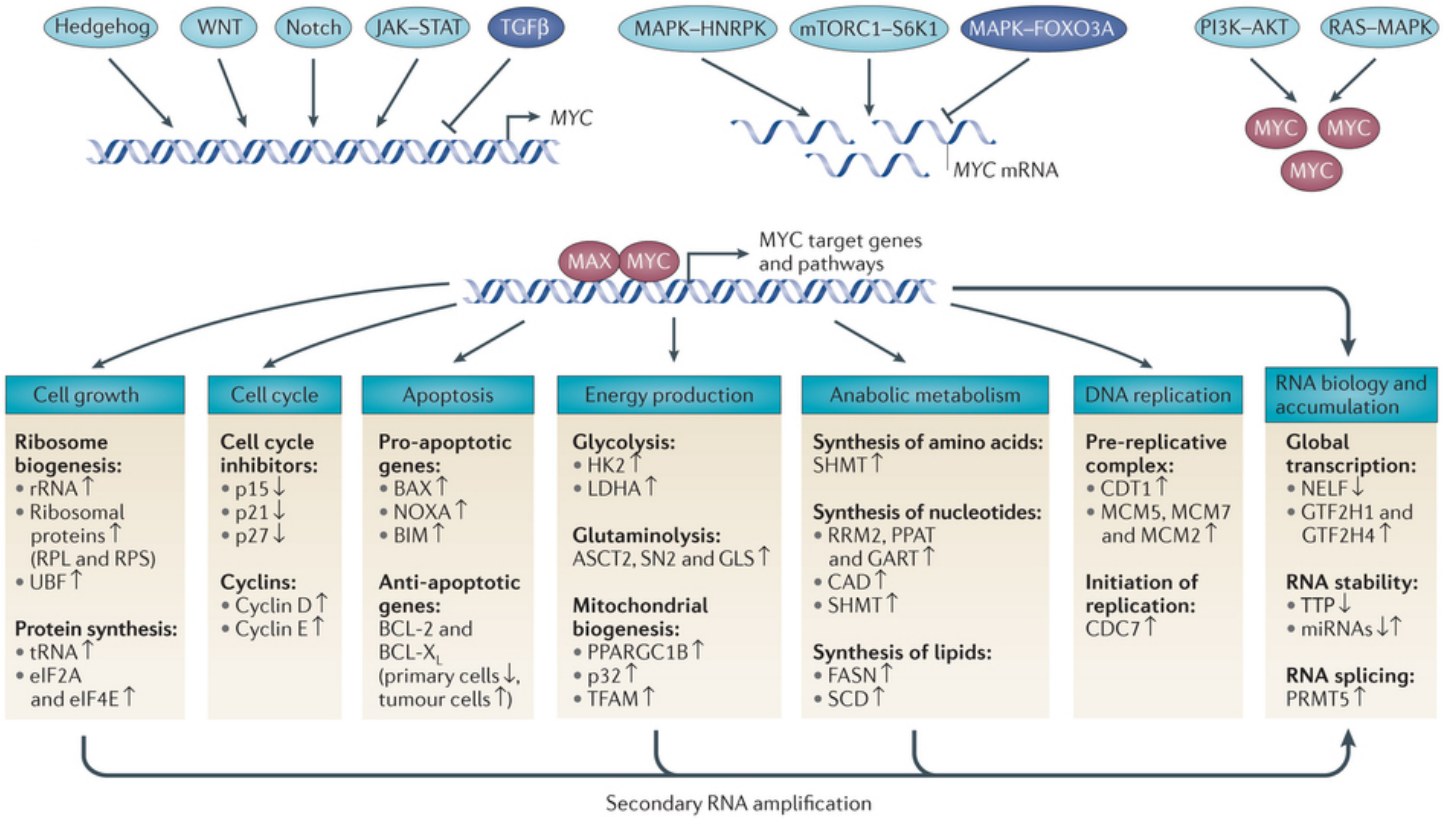
Myc/Max-mediated transcription is deregulated in most of human cancers. Struntz et al. discovered a small molecule that stabilizes the Max homodimer and attenuates Myc-driven transcription with efficacy in cellular and murine cancer models. This discovery reinforces an alternative Myc-targeting strategy and could inform development of compounds to treat Myc-dependent cancers.

Struntz et al., 2019, Cell Chemical Biology *26*, 1–13
May 16, 2019
https://doi.org/10.1016/j.chembiol.2019.02.009

CellPress

MYC family of transcription factors

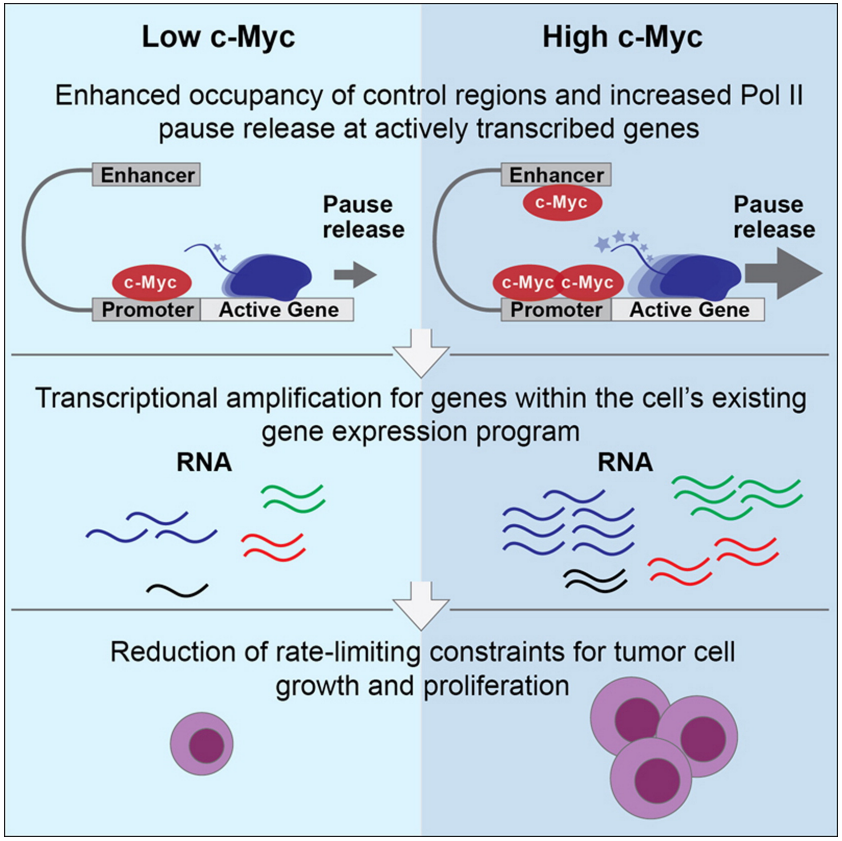
master regulators of broad cellular processes



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,[3] Bin Feng,[4] Xiaxi Li,[5] Thomas Serre,[5] Wenbo Qi,[2] Holly Van Remmen,[2] Richard A. Miller,[6] Kevin G. Bath,[5] Rafael de Cabo,[3] Haiyan Xu,[4] Nicola Neretti,[1] and John M. Sedivy[1,*]

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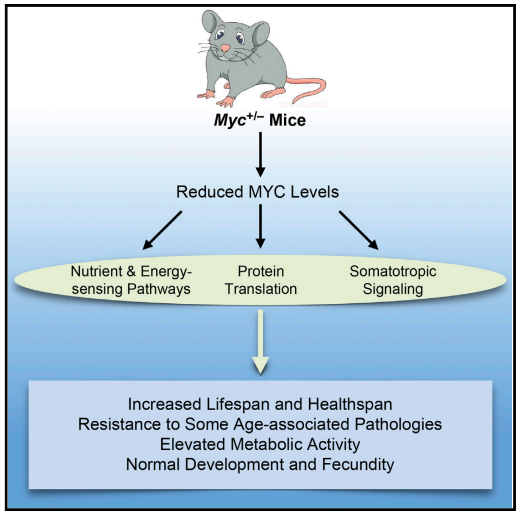
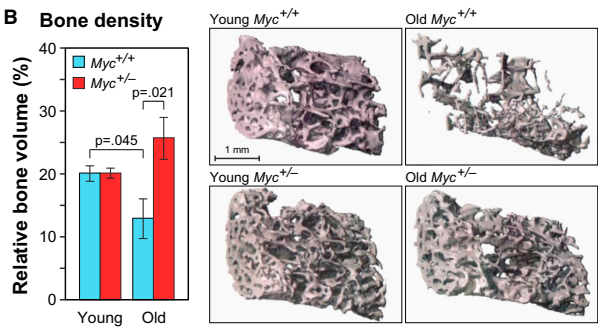
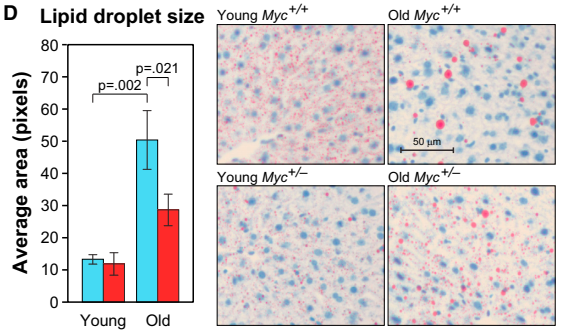
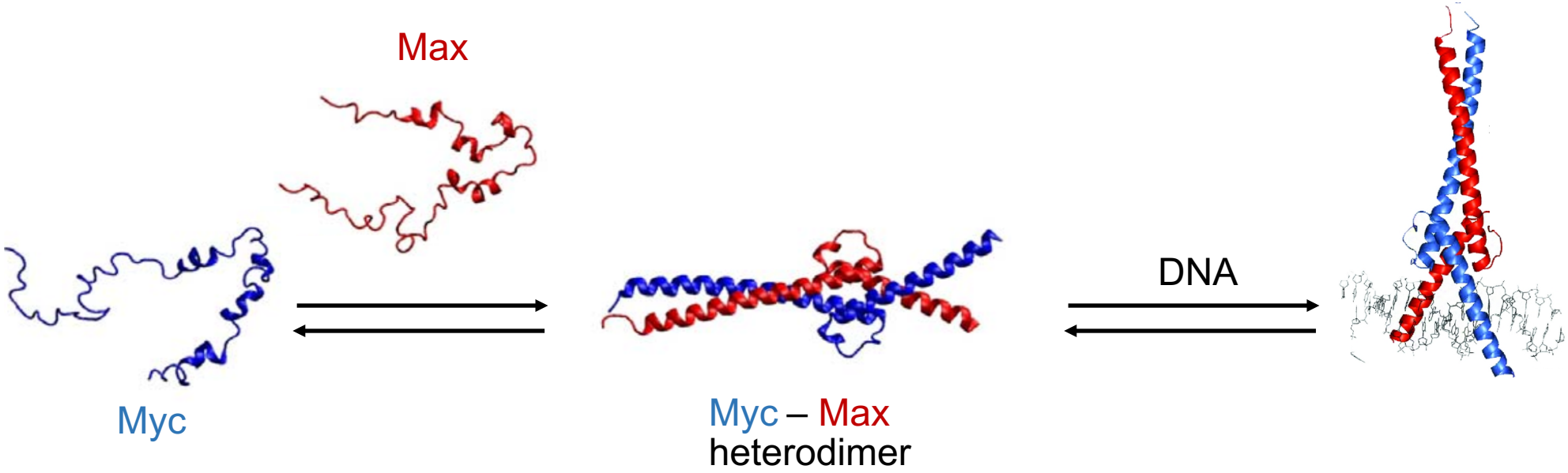
[6]Department of Pathology and Geriatrics Center, University of Michigan, Ann Arbor, MI 48109, USA
[7]Co-first author
*Correspondence: john_sedivy@brown.edu
http://dx.doi.org/10.1016/j.cell.2014.12.016





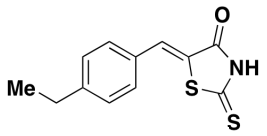


Myc and Max form a heterodimer to bind DNA and drive transcription

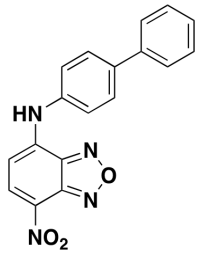


Michel, J. & Cuchillo, R., *PLoS One* **7**, e41070, 2012.

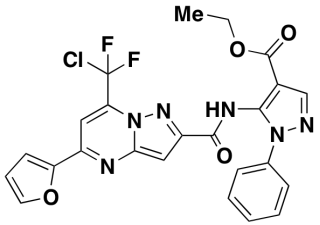
Current Myc modulators lack potency and selectivity



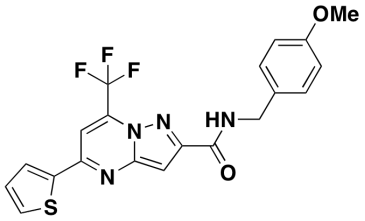
10058-F4



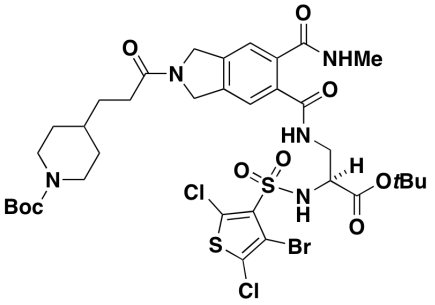
10074-G5



Mycro3



Mycro1



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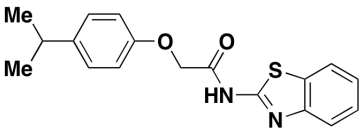

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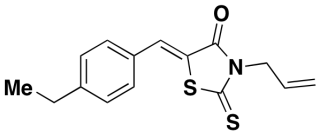

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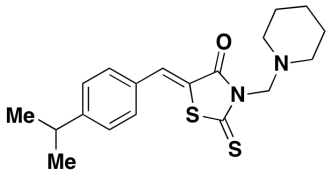

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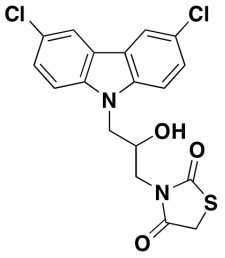

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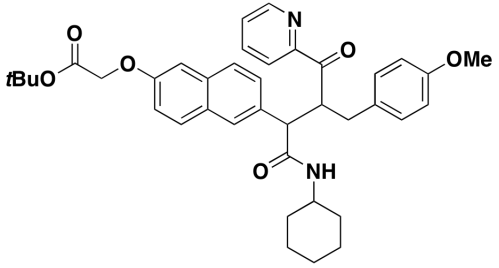
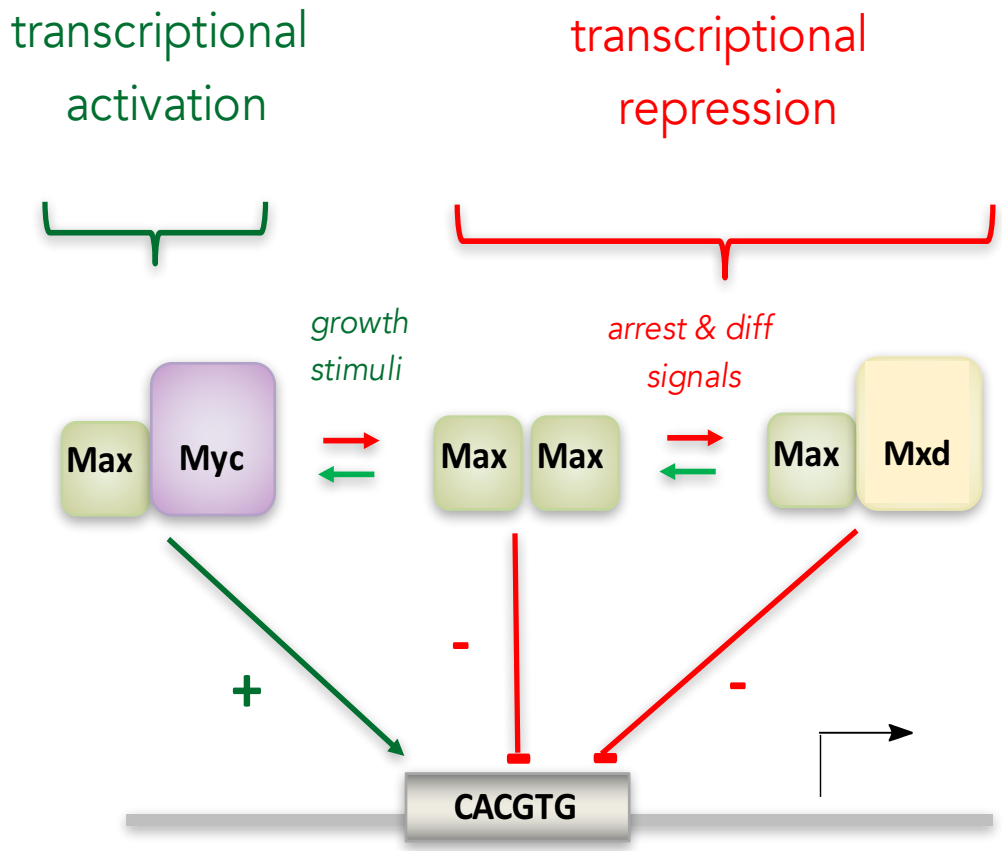


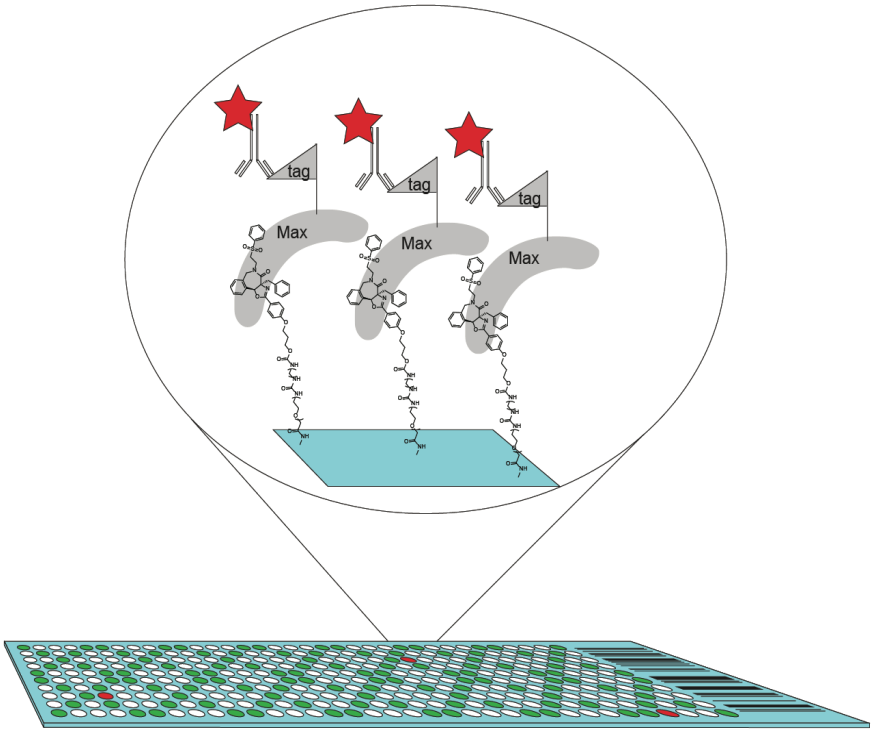
NY2267

Berg, T. *Curr. Top Microbiol Immunol.* **2011**, 348, 139-149.

Max as a target: heterodimer/homodimer dynamics

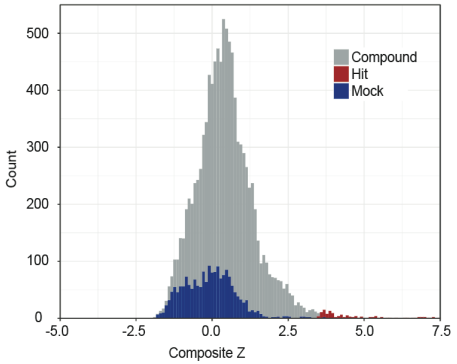


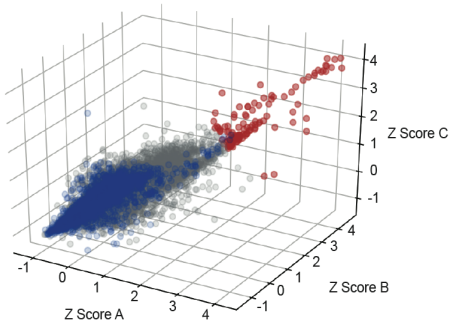
SMM screens: purified Max transcription factor



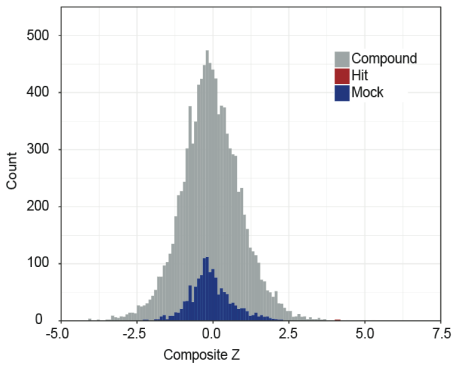
>21k compounds screened

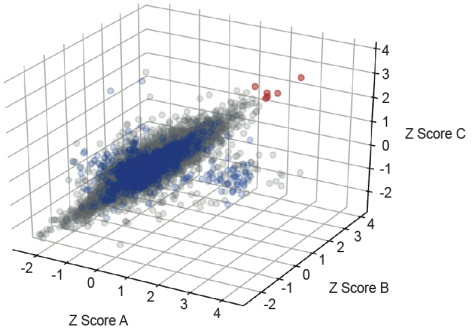
synthetic compound collection





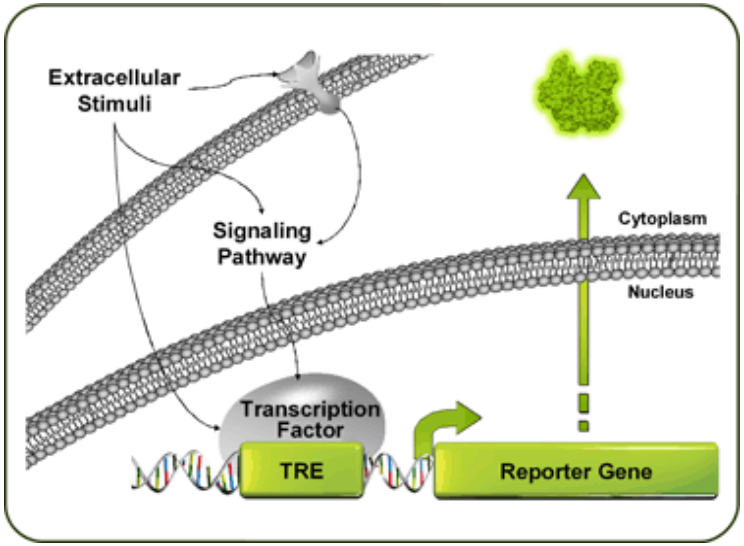
natural products

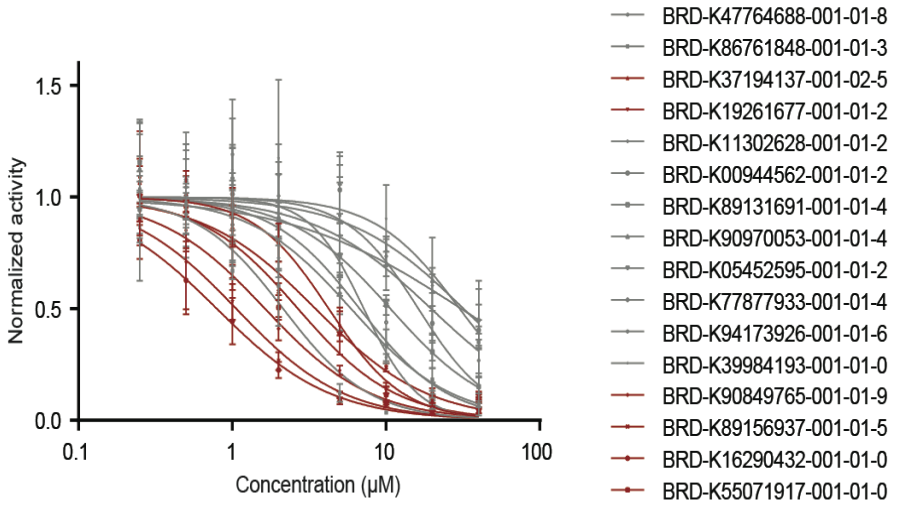




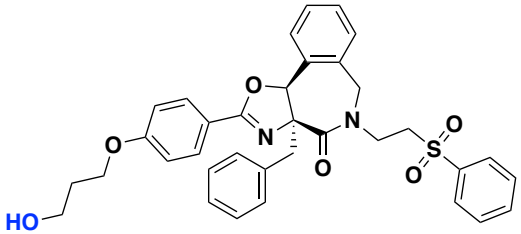
117 assay positives

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





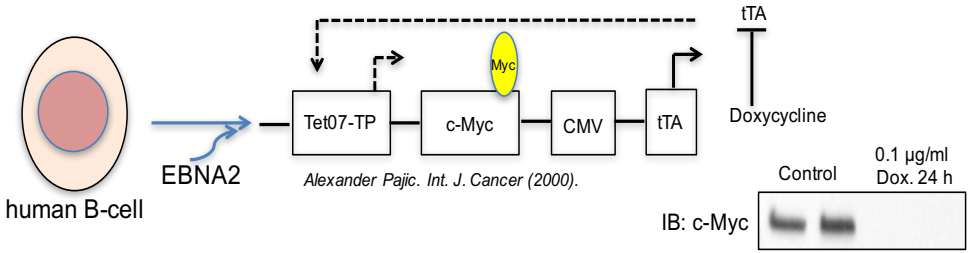
'KI-MS2'



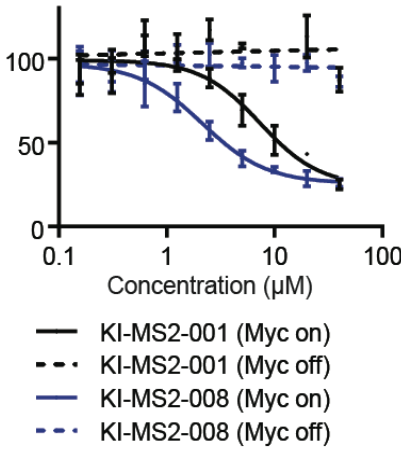
IC_{50} = 1.06 μM
MW = 610.73
cLogP = 5.15

Cell viability assays: Are Myc or Max required?

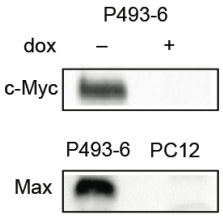
P493-6 Dox-repressible cells for MYC 'on/off' studies



P493-6



	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



Cell viability assays: Are Myc or Max required?

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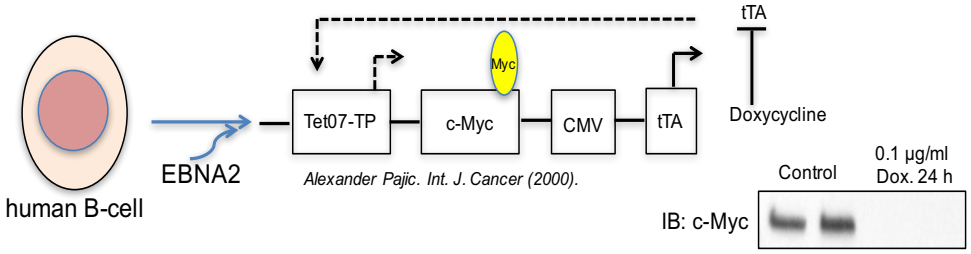
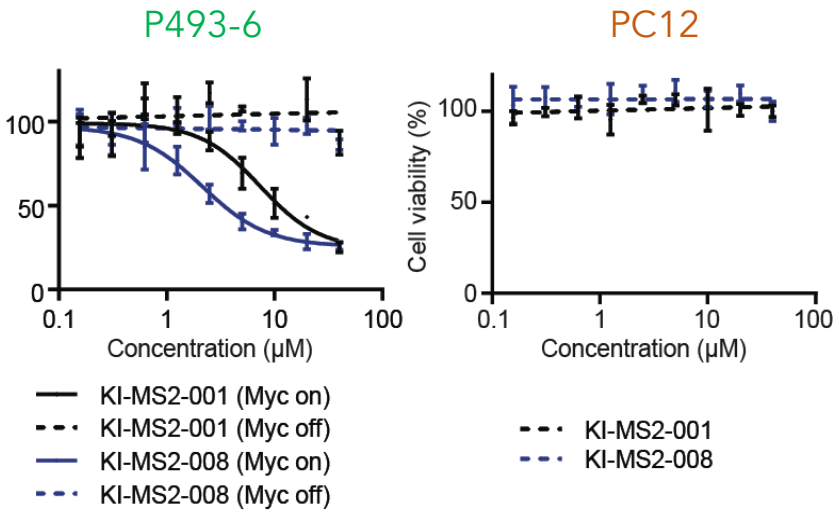
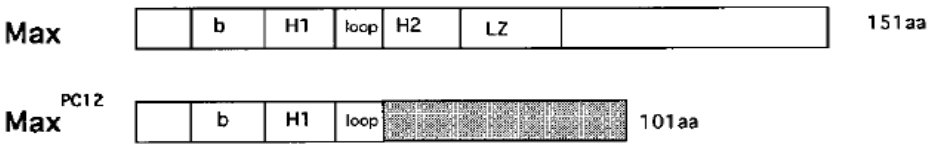
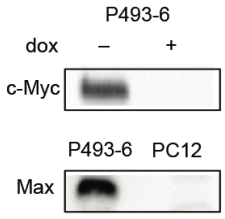




Max-deficient PC12 pheochromocytoma cells



	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

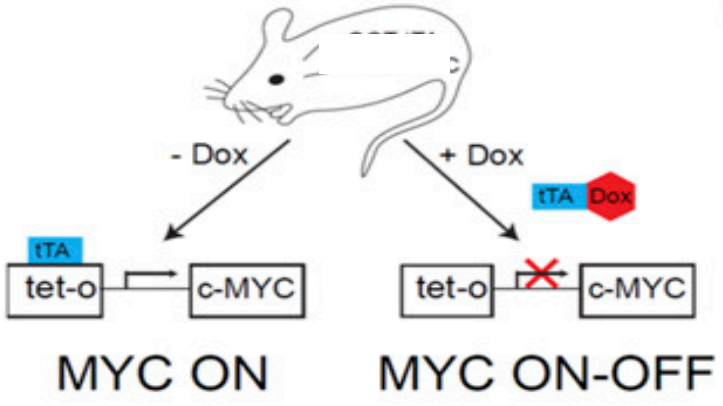
Myc 'on/off' mouse models:

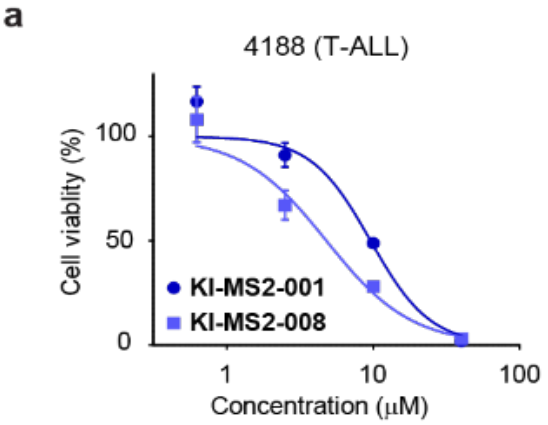
lymphoma

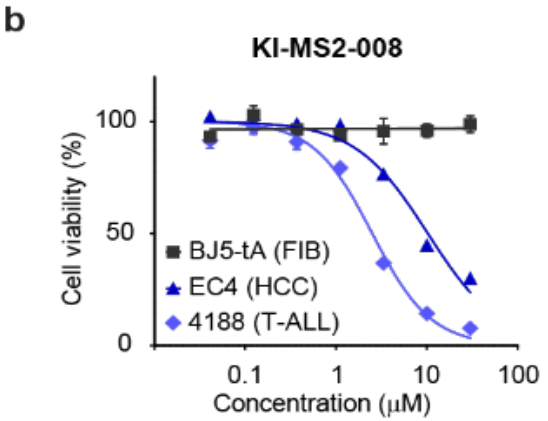
HCC

RCC

osteosarcoma



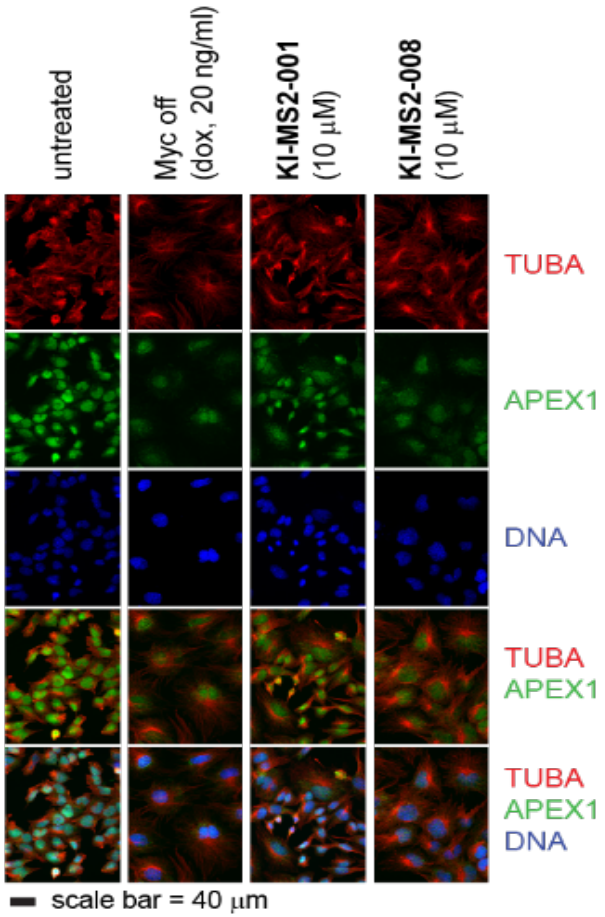




Anja Deutzmann, Felsher Lab Stanford

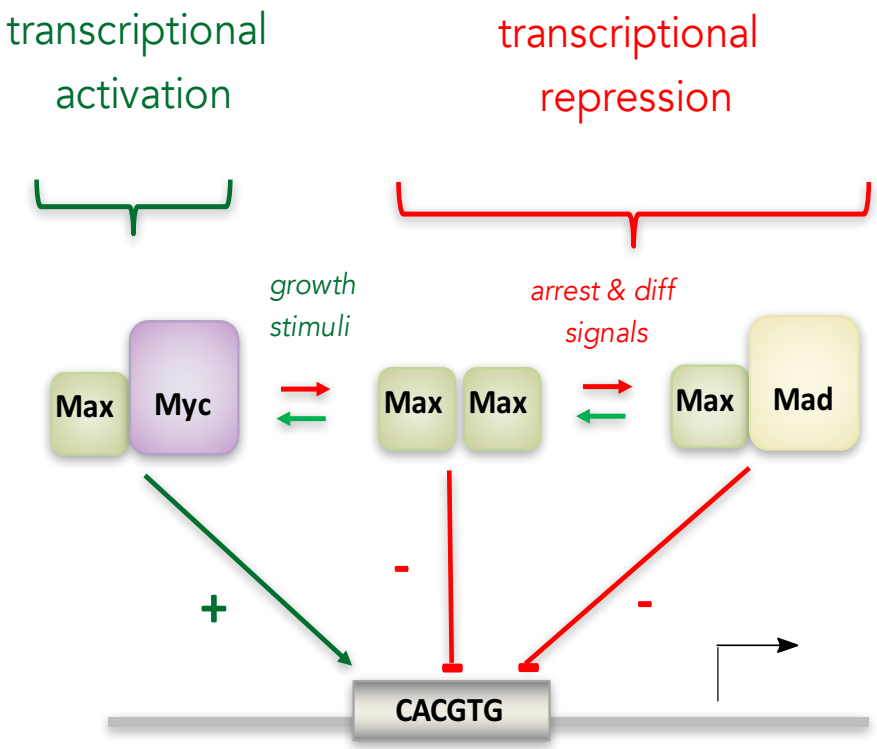
Imaging of biomarkers: conditional vs. chemical modulation

modulating Myc in an engineered osteosarcoma model

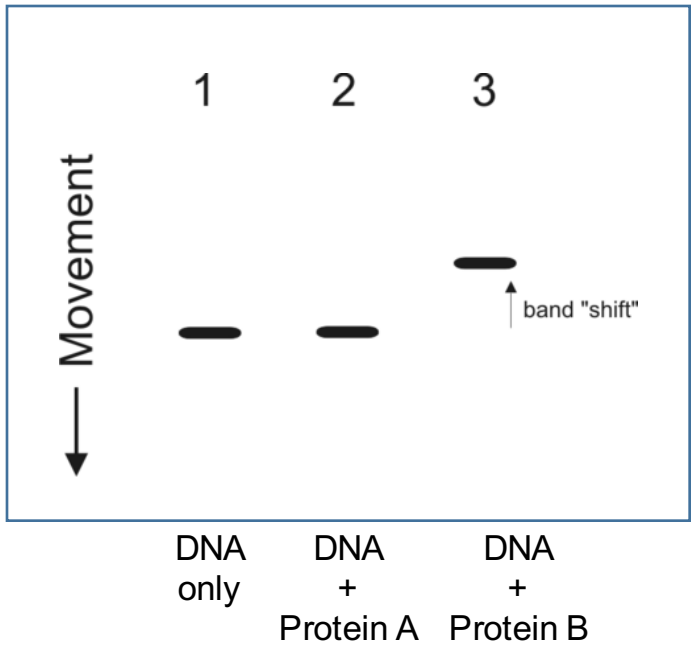


Anja Deutzmann, Felsher Lab Stanford

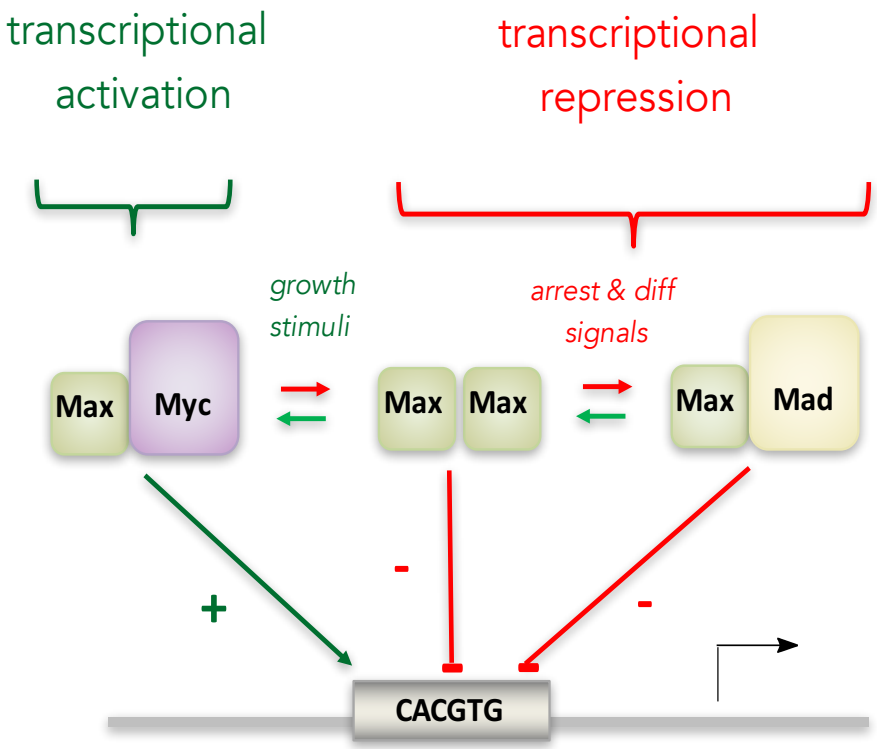
Does the probe antagonize the Myc/Max heterodimer?

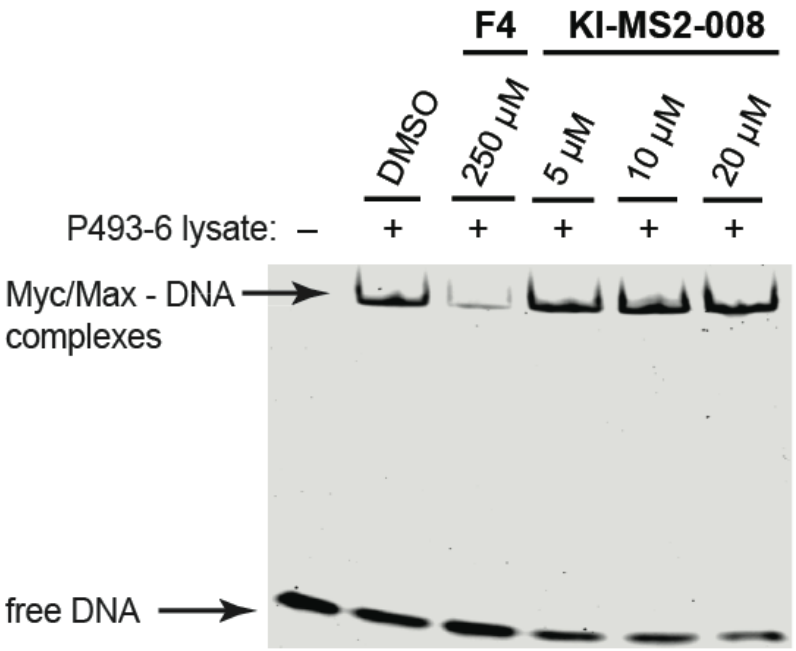


Electrophoretic Mobility Shift Assay (EMSA)
aka Gel Shift Assay



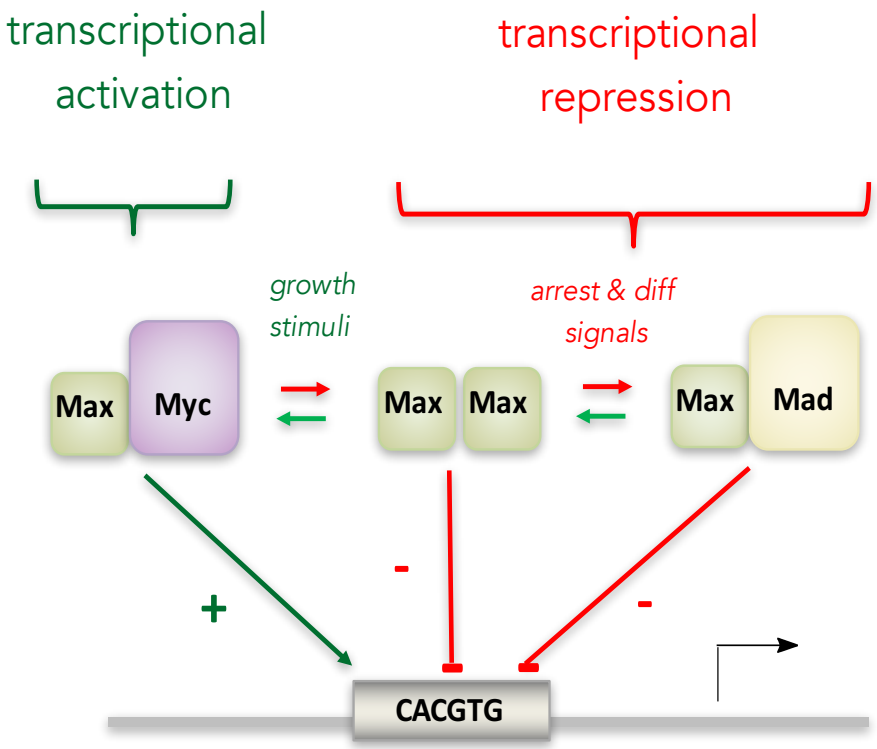
Does the probe antagonize the Myc/Max heterodimer?

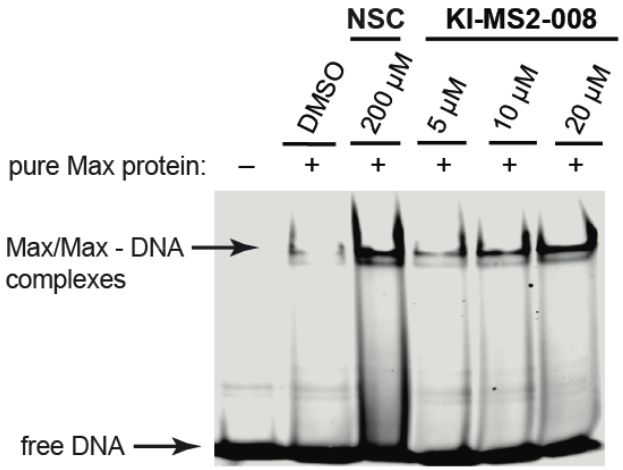


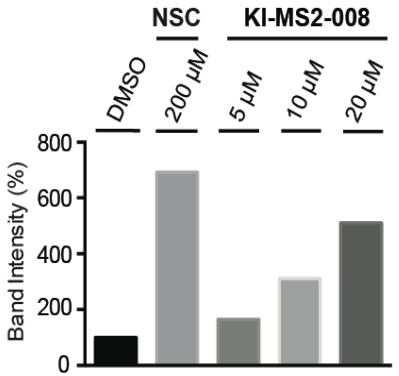


EMSA

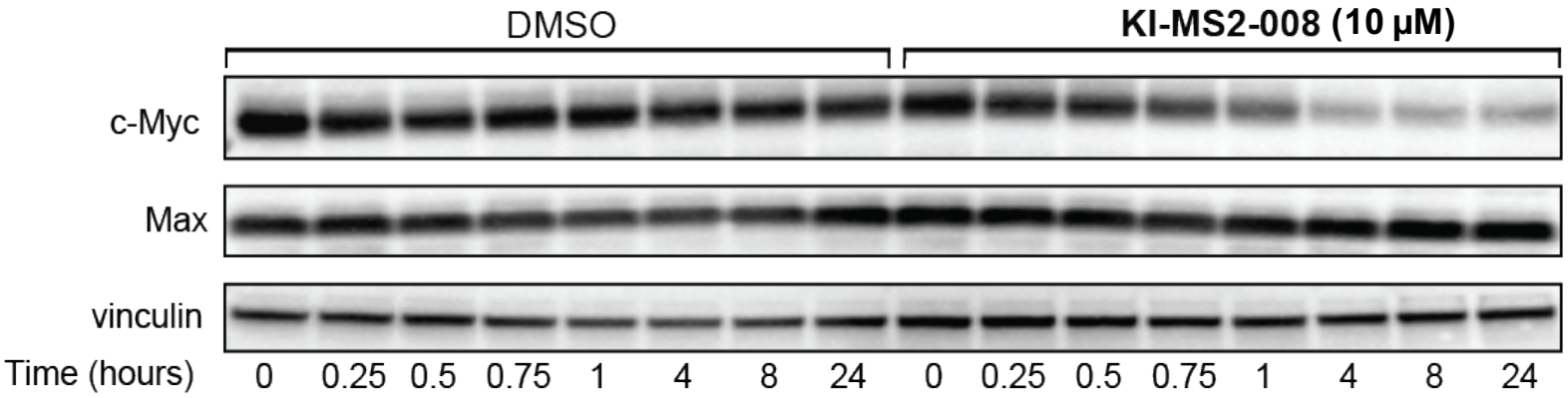
Does the probe stabilize the Max/Max homodimer?

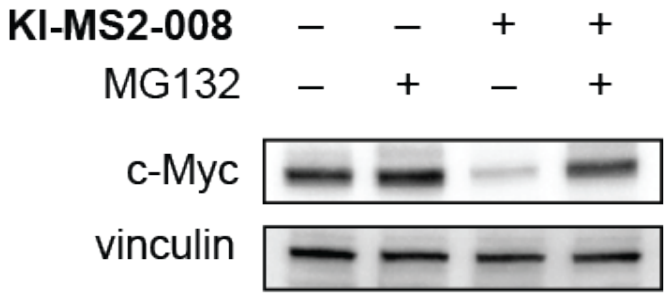






Western blots: KI-MS2-008 alters Myc protein levels

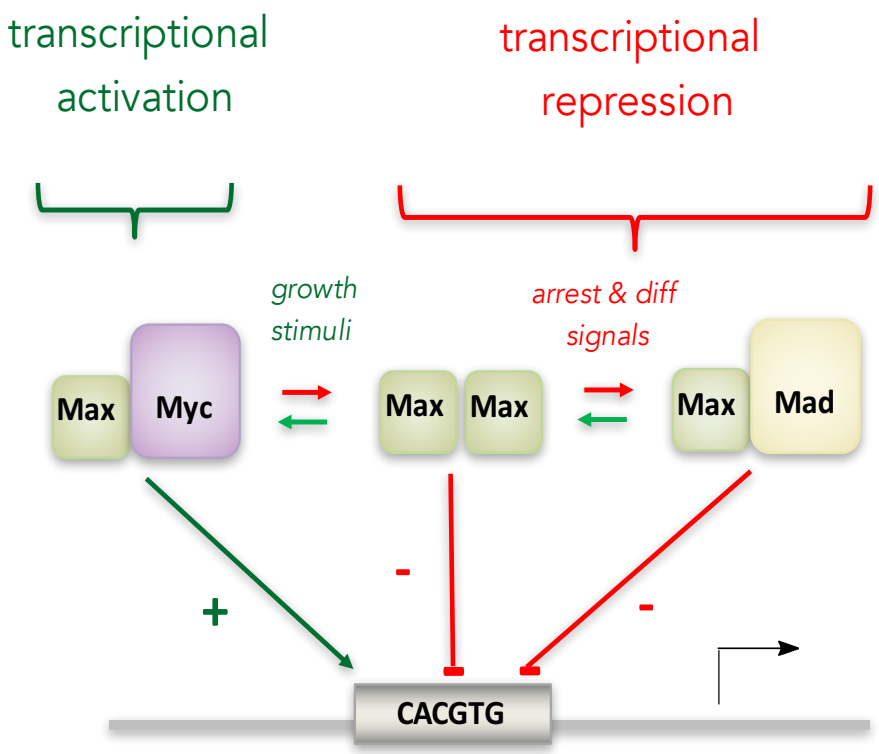




rescue experiment with
10 μM proteasome inhibitor
MG132

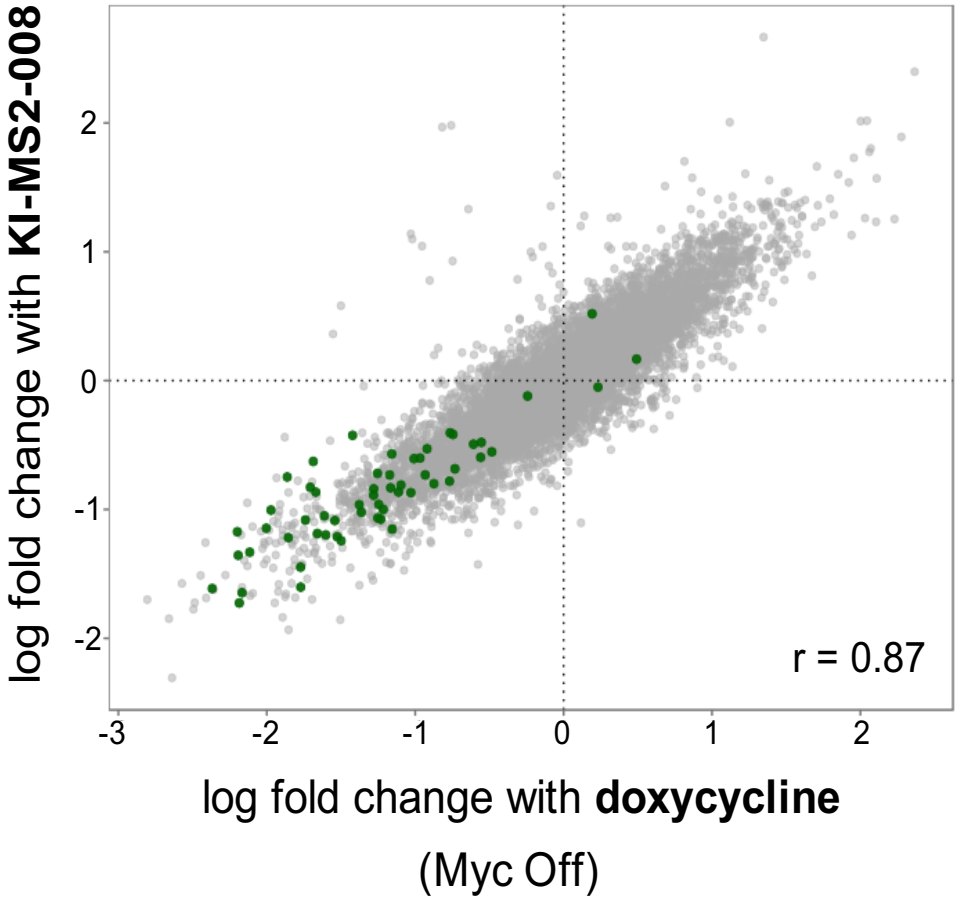
KI-MS2-008

mixed mechanism inhibitor?



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

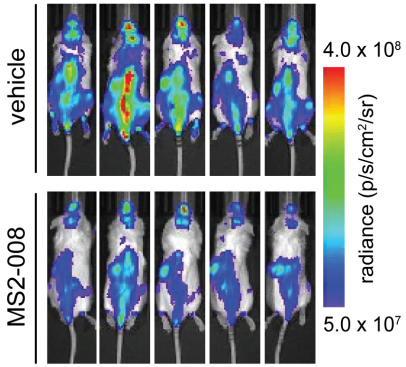
Gene Set Enrichment Analysis reveals an enrichment of Myc target genes

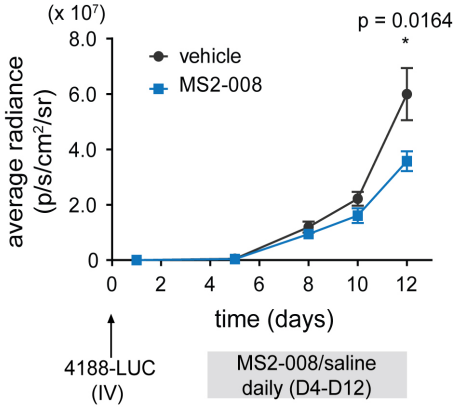


Hallmark V2 MYC-dependent genes in green

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

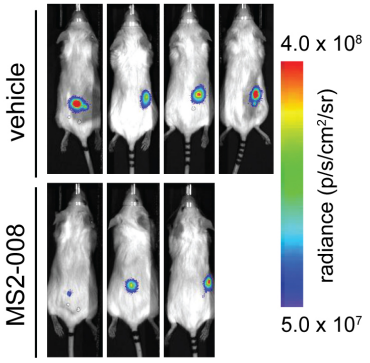
T-cell acute lymphoblastic leukemia
blood cancer

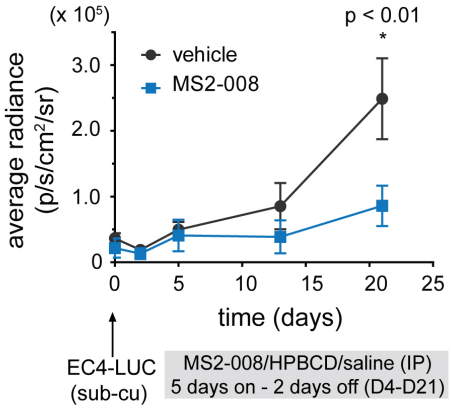




0.06 mg/kg
daily IV administration

hepatocellular carcinoma
solid tumor

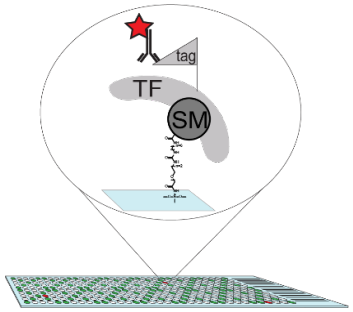




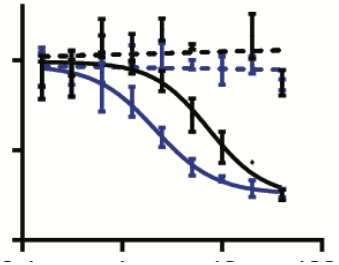
0.24 mg/kg
subcutaneous administration
5d on/2d off cycles

Anja Deutzmann, Felsher Lab Stanford

Summary

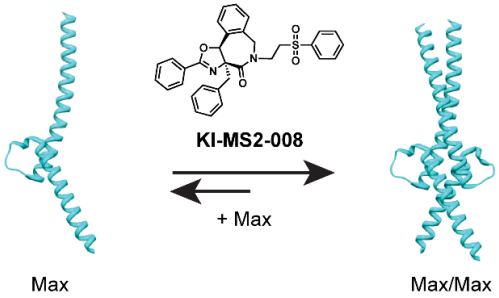


- Identified KI-MS2-001 as a putative Max binder that modulates Myc transcriptional activity.

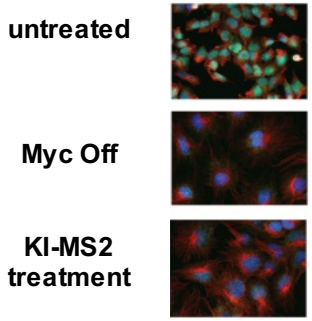


- KI-MS2-001 and KI-MS2-008 inhibited viable cell levels of P493-6 in a Myc-dependent manner.

Summary



- KI-MS2-008 stabilizes Max homodimers.



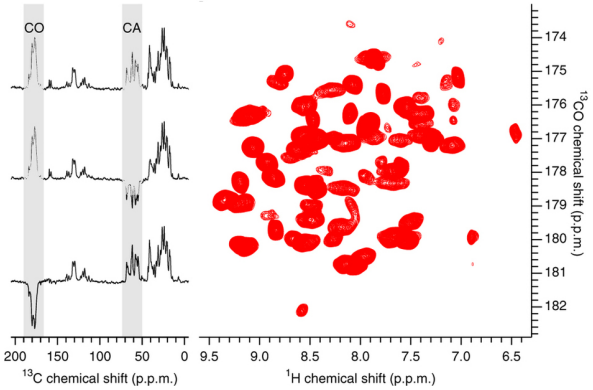
- KI-MS2-008 decreased Myc protein levels and mimicked Myc inactivation in cell morphology and the transcriptome.

- KI-MS2-008 treatment suppressed the growth of T-ALL and HCC *in vivo*.

Current directions

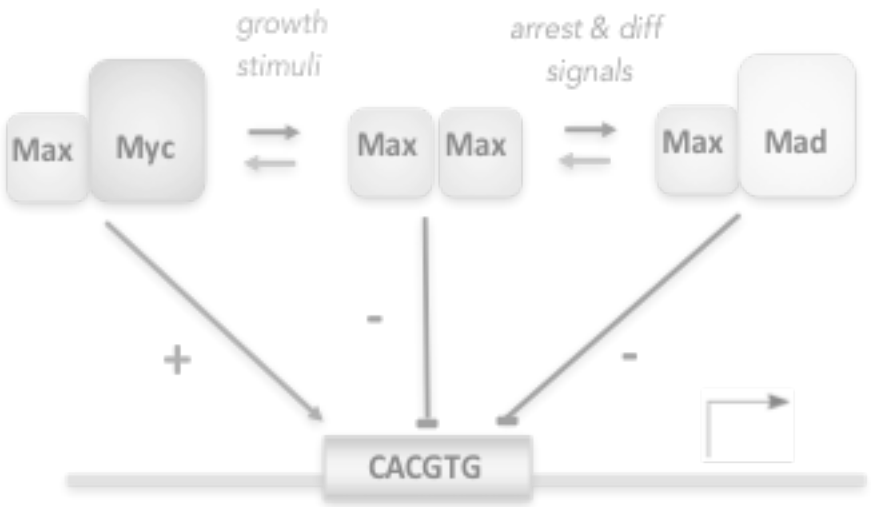
optimize potency and solubility, PK/PD-guided medicinal chemistry

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

11/7/19	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
11/12/19		No lecture, Monday schedule
11/14/19	Lecture 2	Low-tech ligand discovery using microarrays
11/19/19	Lecture 3	Guest Lecture – Nick Struntz, Ph.D. Engineering transcriptional responses with a small molecule
11/21/19	Lecture 4	Quantitative evaluation of protein-ligand interactions
11/26/19		Student Pitches
12/3/19	Lecture 5	The story of FKBP12 – our protein target