Which process or biomolecule would you study with a chemical probe if you had one in hand?

Genome Wide Association Study:
Hair-related phenotypes

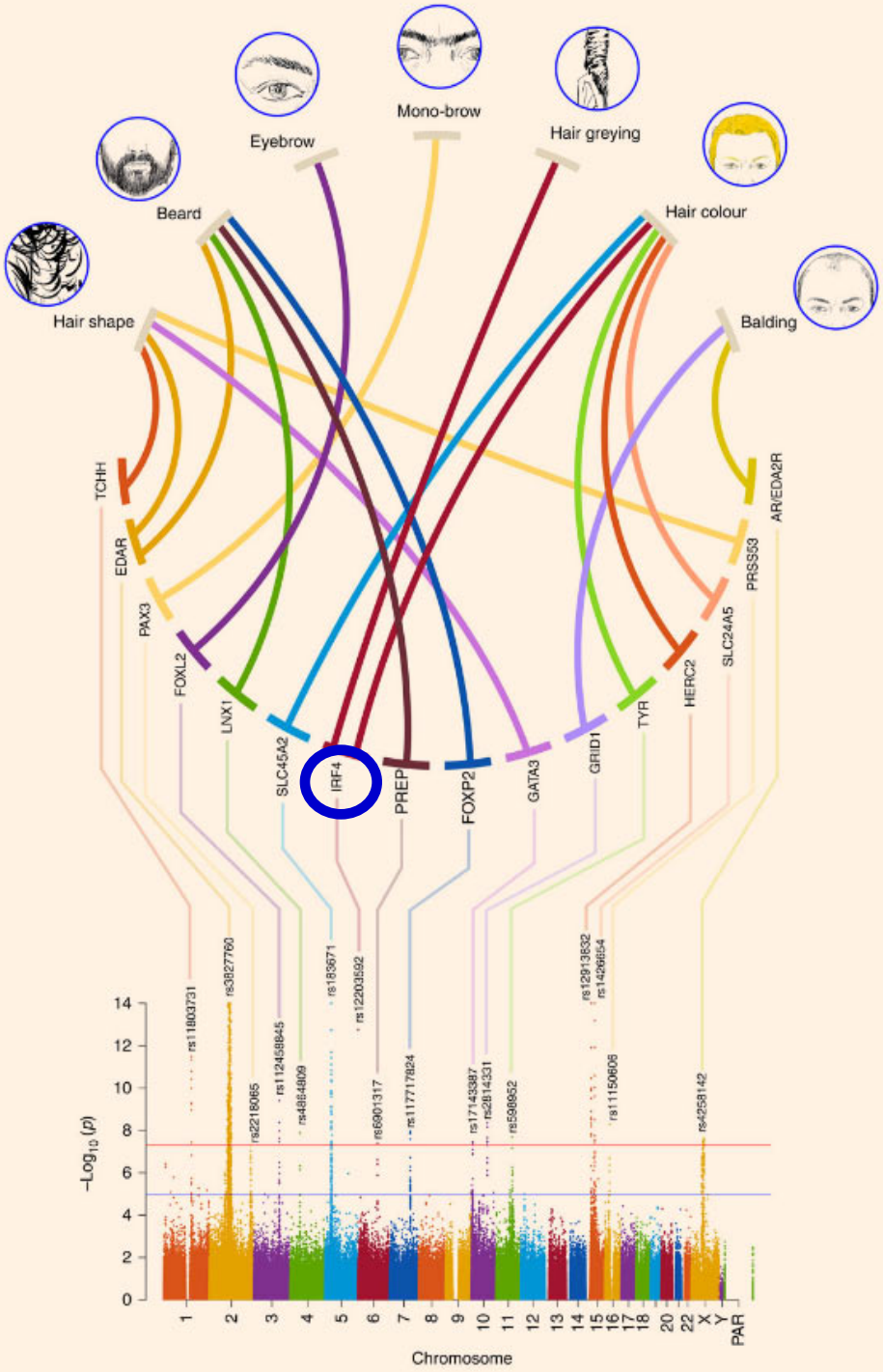
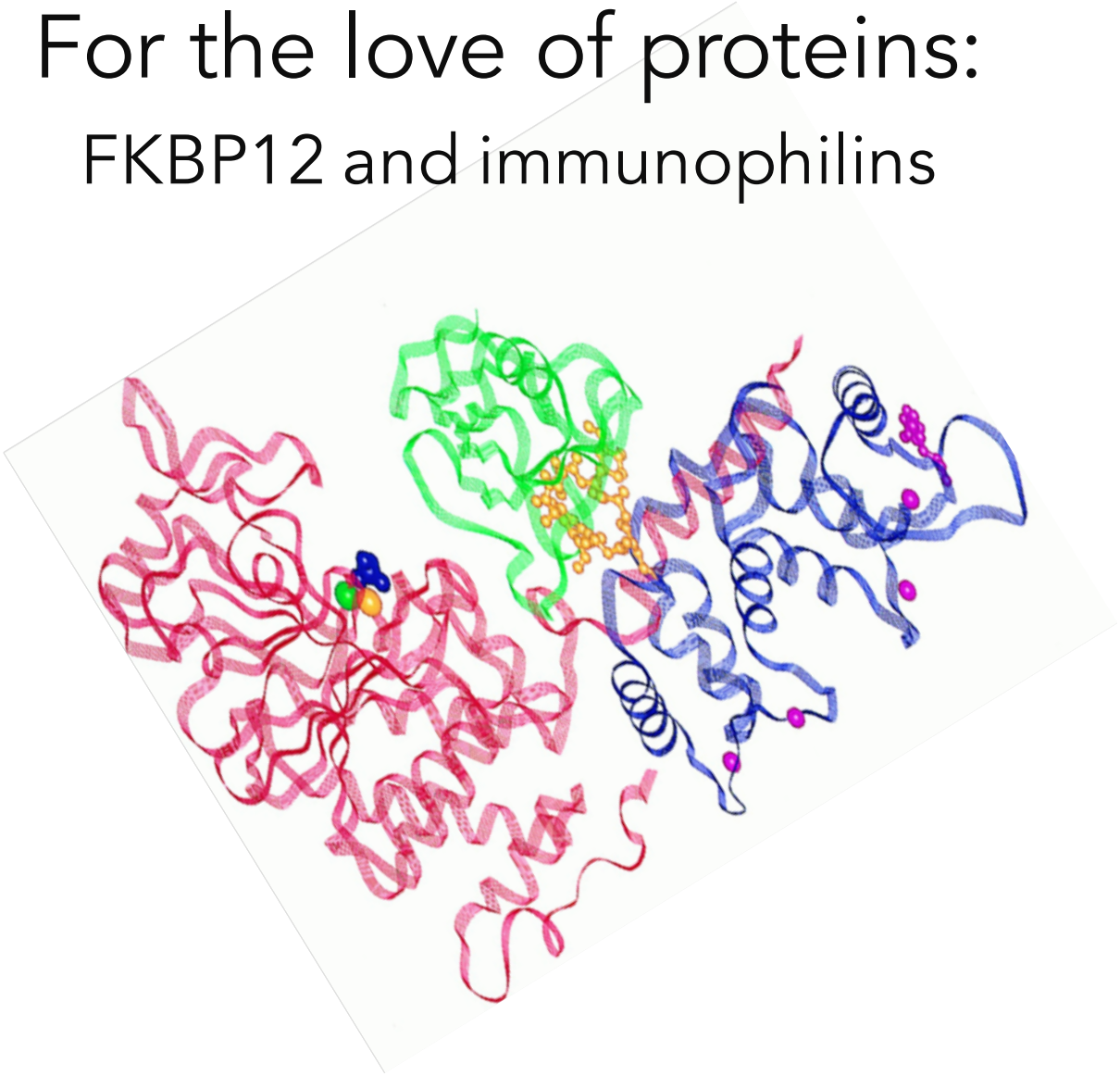


Image credit: Kaustubh Adhikari et al., **doi: 10.1038/ncomms10815**.

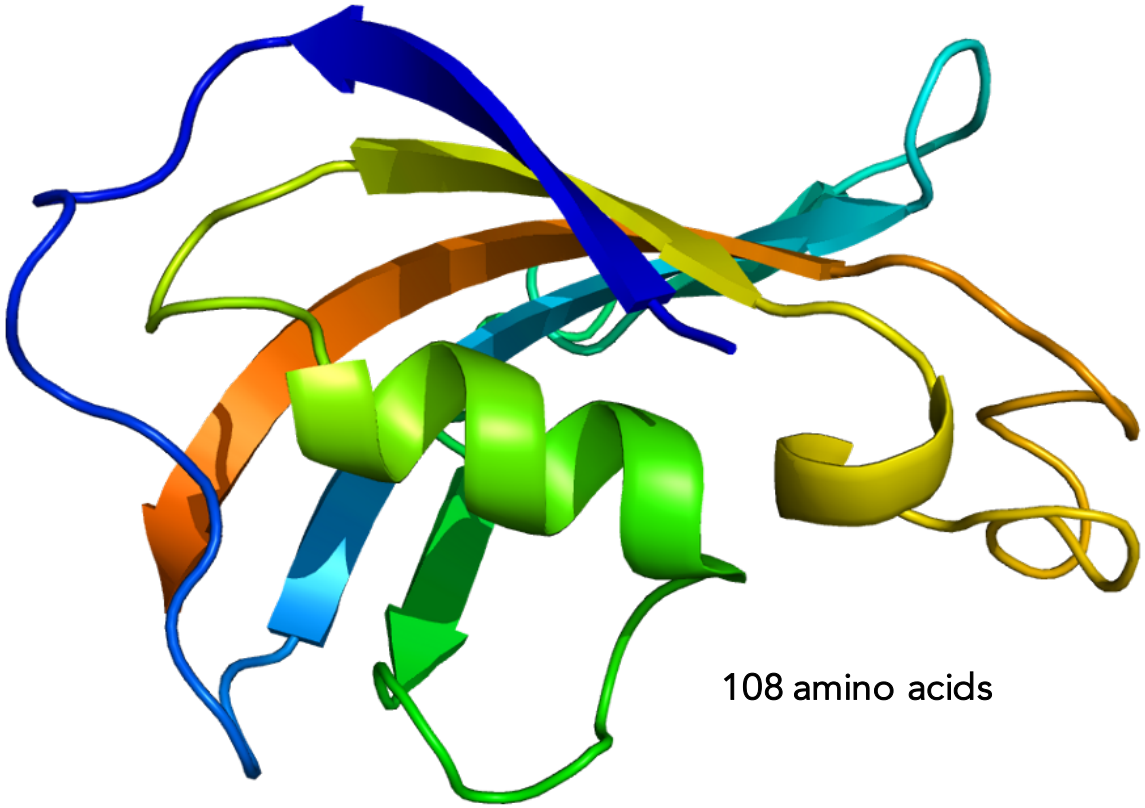
For the love of proteins:

FKBP12 and immunophilins



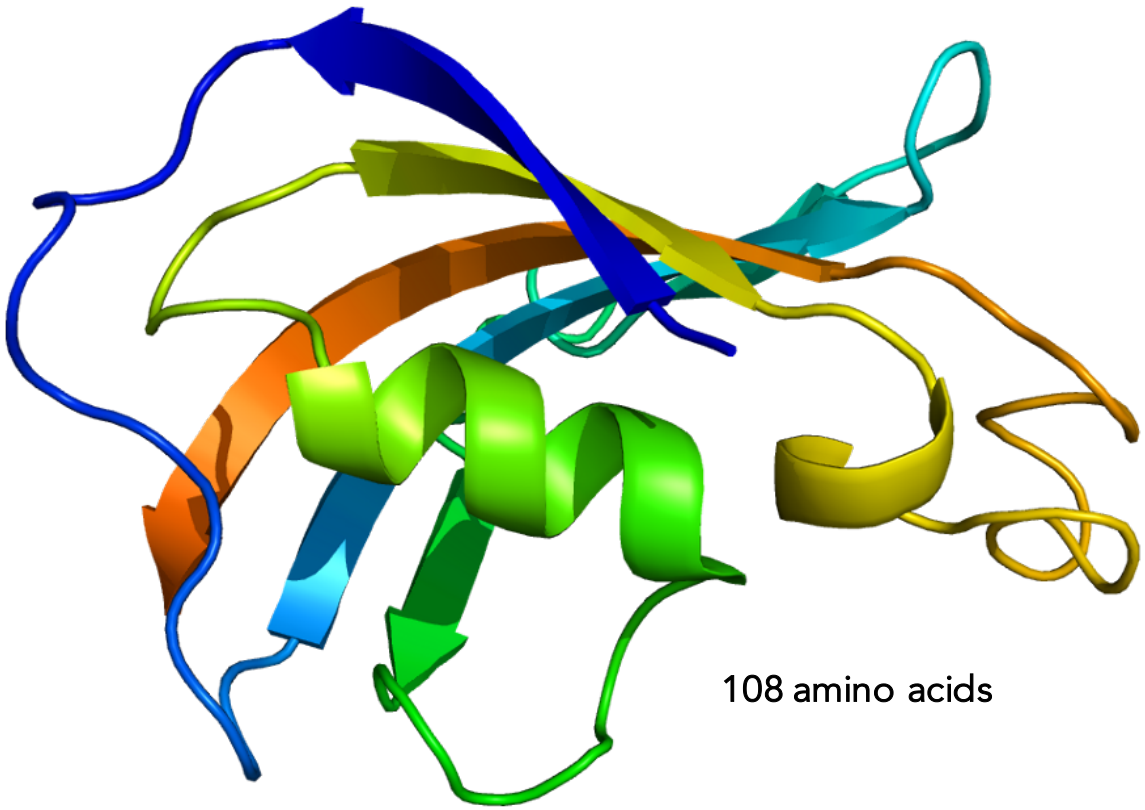
February 16, 2017

Our target protein: FKBP12



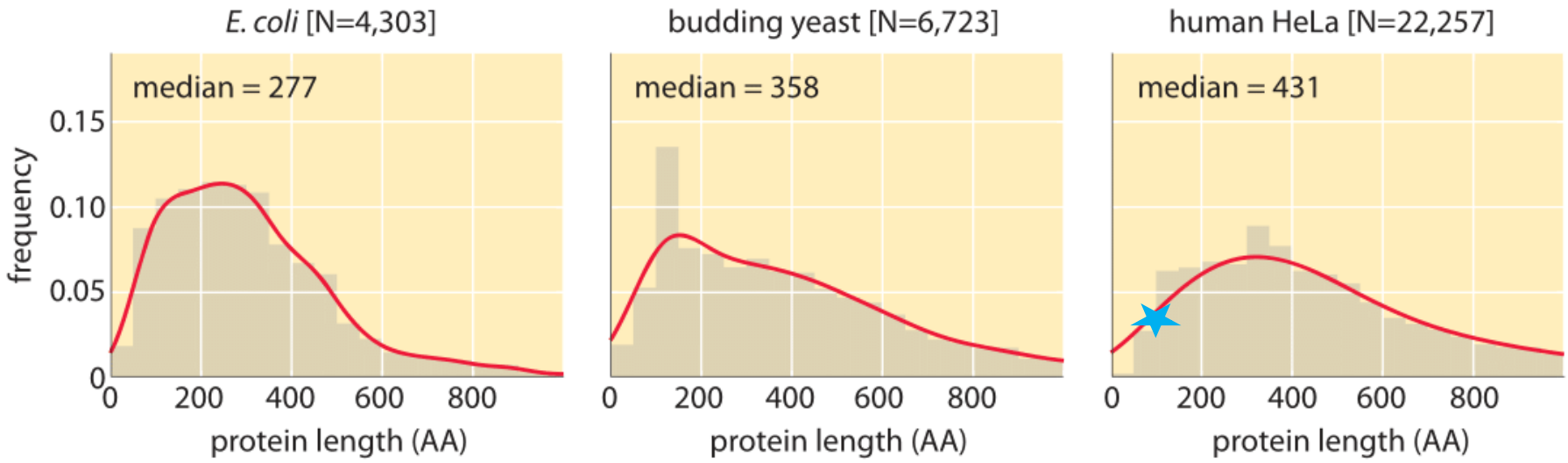
FK-506 Binding Protein that is 12 kilodaltons

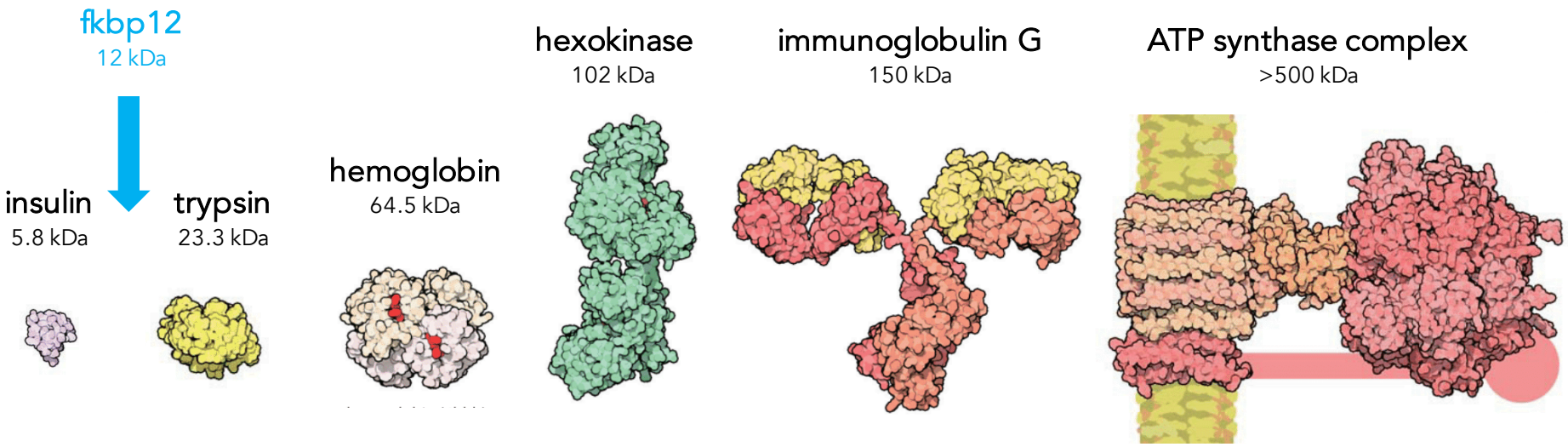
Our target protein: FKBP12



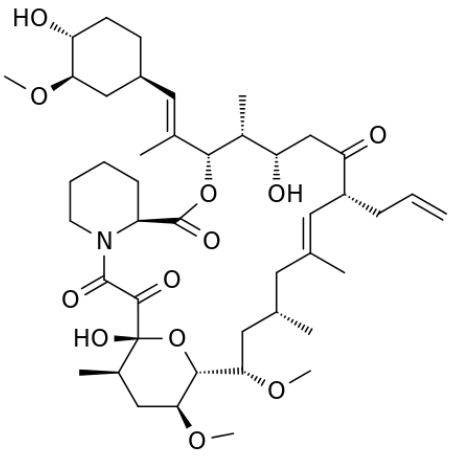
FK-506 Binding Protein that is 12 kilodaltons

How big is the typical protein?

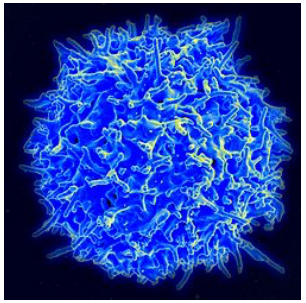




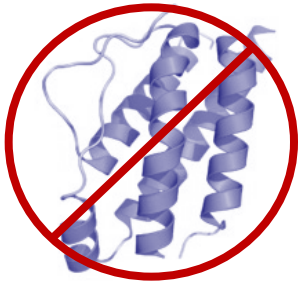
FK-506 is an immunosuppressant drug



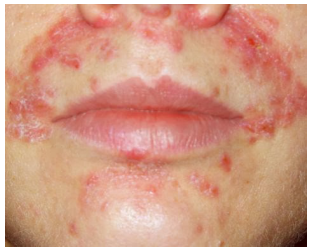
FK-506 (Tacrolimus)
inhibits the development of
T-cells for immune responses



blocks T-cell proliferation

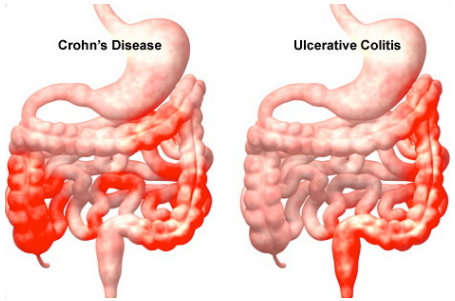


inhibits secretion of
interleukin-2



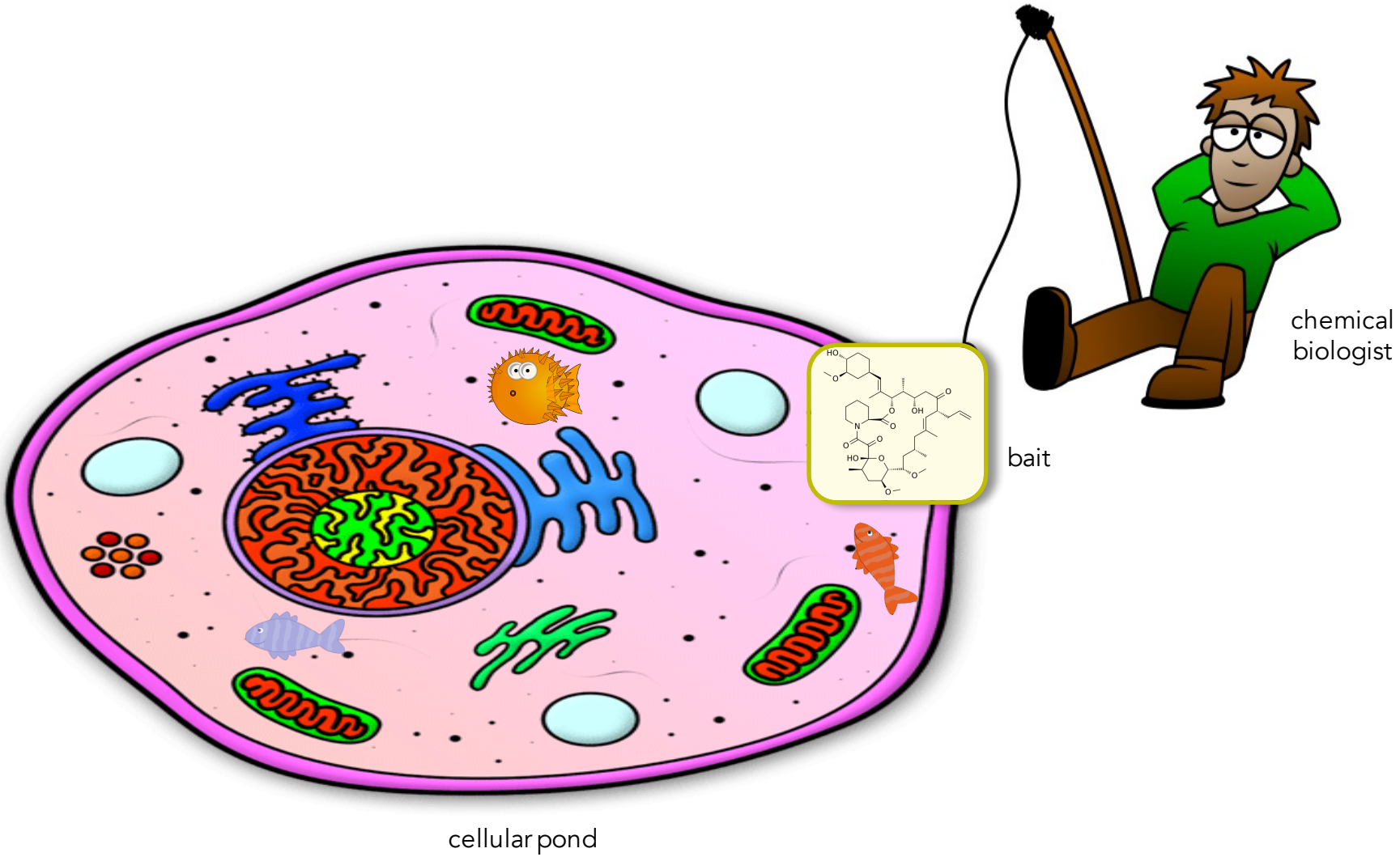
eczema and other
skin conditions

transplant rejection



inflammatory bowel disorders

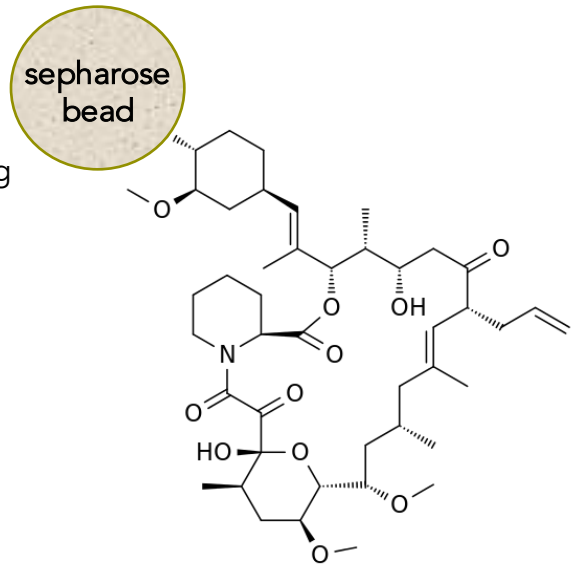
Fishing for the target of FK506



Fishing for the target of FK506

affinity chromatography

couple ‘bait’ to matrix



bait

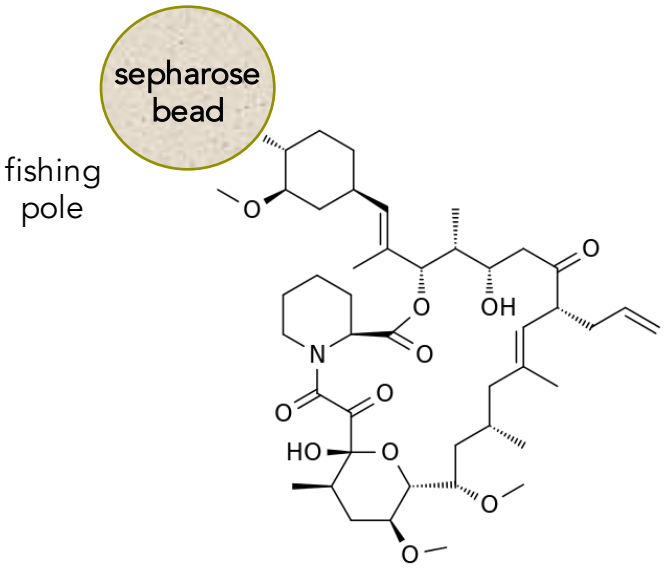
Fishing for the target of FK506

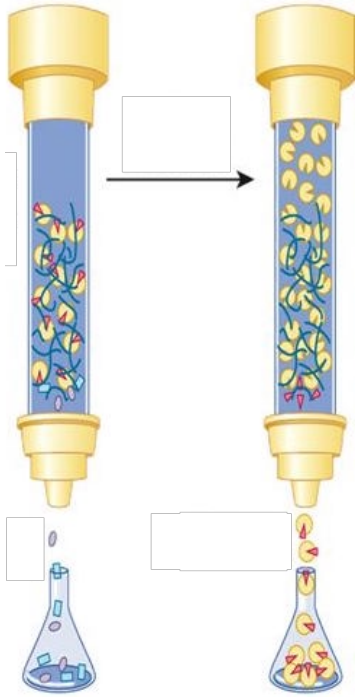
affinity chromatography

couple 'bait' to matrix

add tissue homogenate

elute

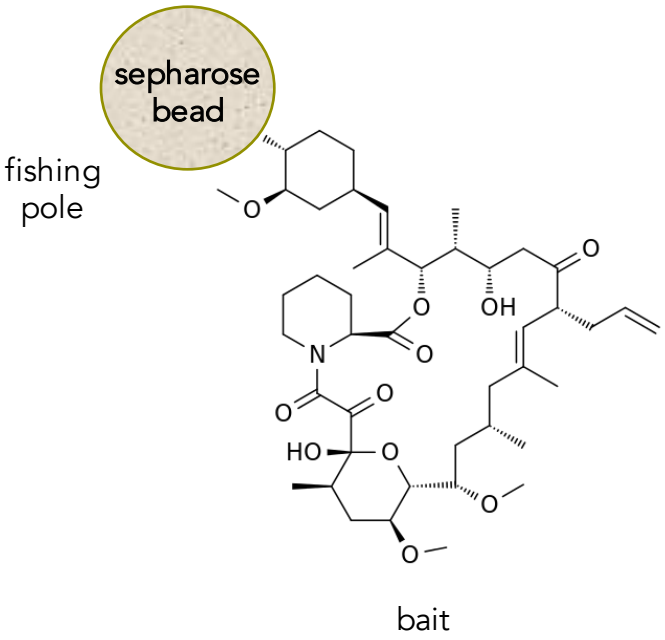




Fishing for the target of FK506

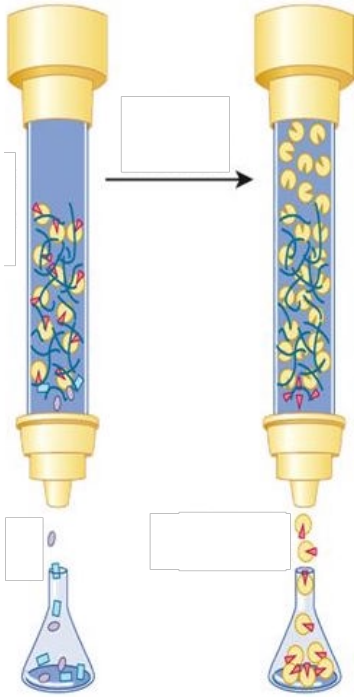
affinity chromatography

couple 'bait' to matrix

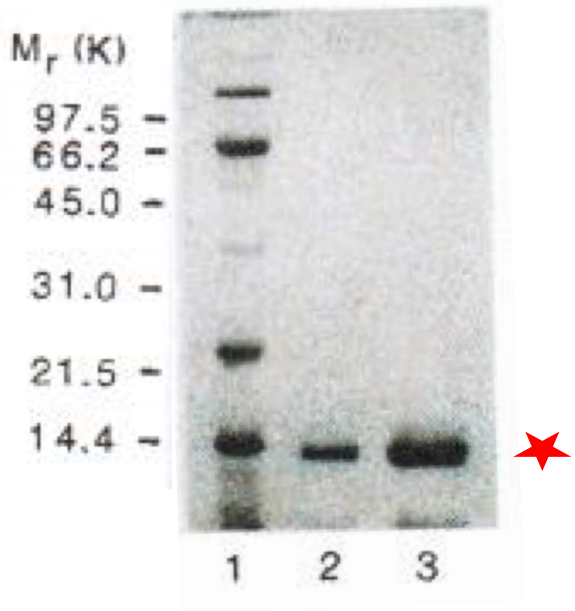


add tissue homogenate

elute



SDS-PAGE



Harding *et al*. Nature 341, 758-760 (1989)
Siekierka *et al*. Nature 341, 755-757 (1989)

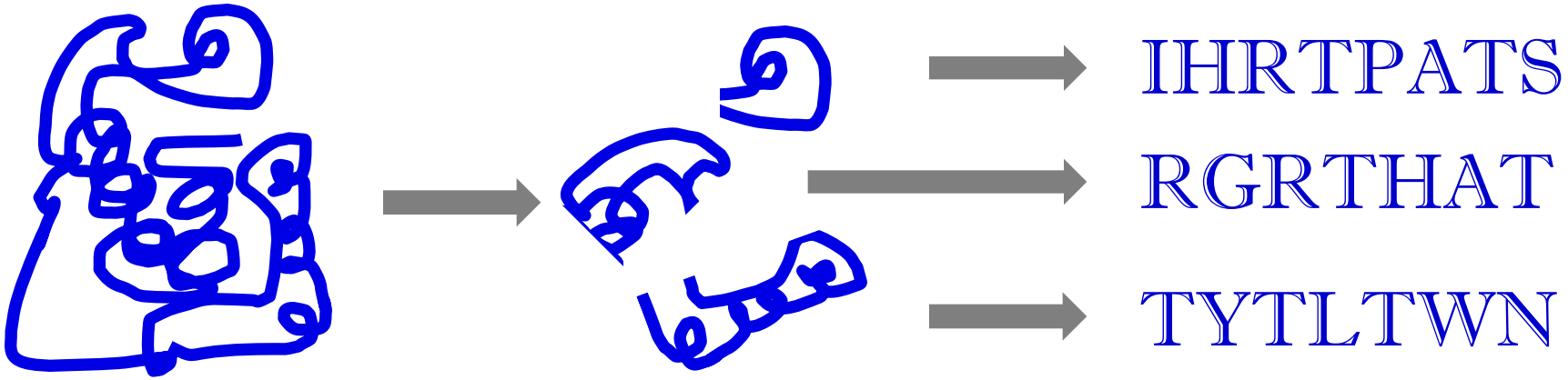
Fishing for the target of FK506

determining the protein sequence

purified FKBP protein

FKBP proteolytic fragments

N-terminal sequencing methods



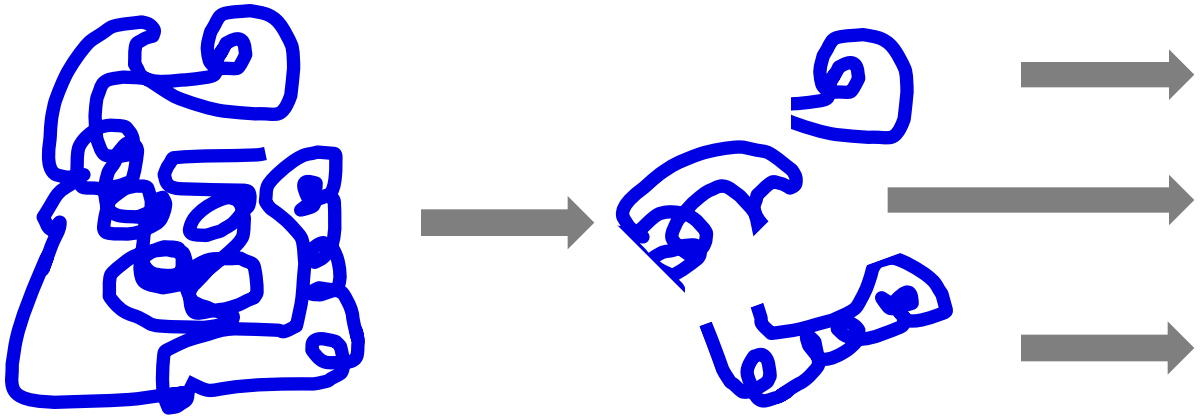
Fishing for the target of FK506

determining the protein sequence

purified FKBP protein

FKBP proteolytic fragments

108 amino acid peptide chain
(11.8 kilodaltons)

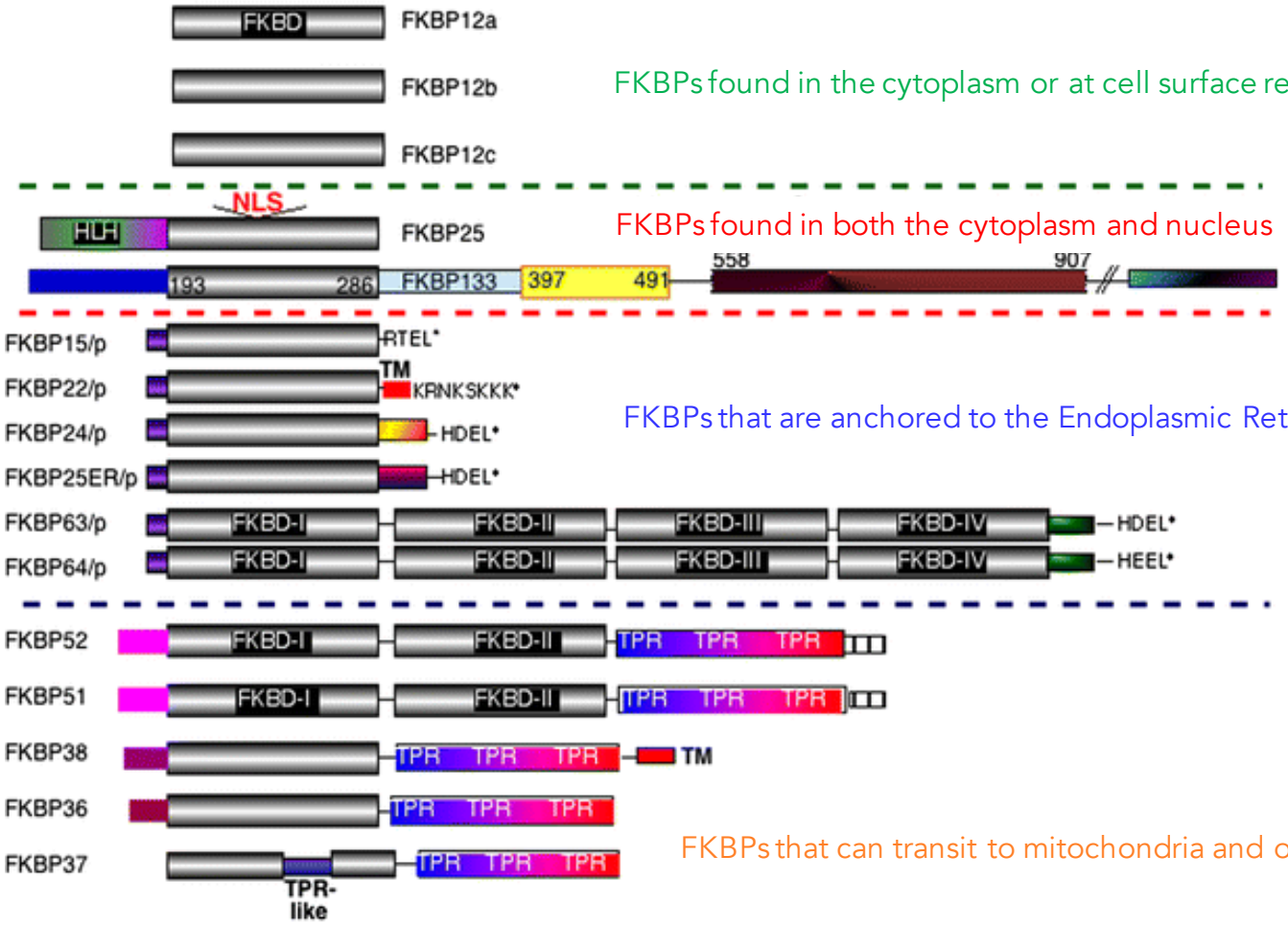


MGVQVETISPGDG
RTFPKRGQTCVVH
YTGMLEDGKKFDS
SRDRNKPFKFVLG
KQEVIRGWEEGVA
QMSVGQRAKLTISP
DYAYGATGHPGIIP
PNATLIFDVELLKLE

Standaert *et al*. Nature 346, 671-674 (1990)
Lane *et al*. Journal of Protein Science 10, 151-160 (1991)

FKBPs are everywhere!

FKBPs encoded in the human genome



FKBPs found in the cytoplasm or at cell surface receptors

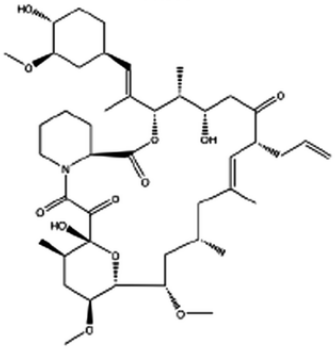
FKBPs found in both the cytoplasm and nucleus

FKBPs that are anchored to the Endoplasmic Reticulum

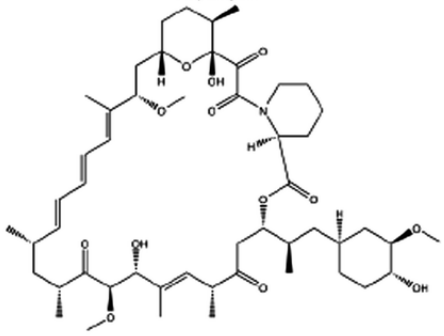
FKBPs that can transit to mitochondria and other organelles

Several drugs bind to FKBP12 with high affinity

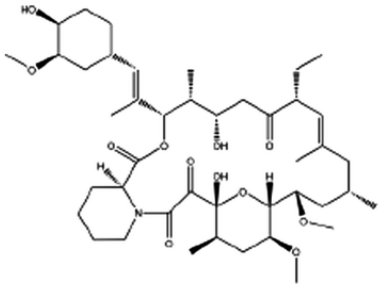
FK506



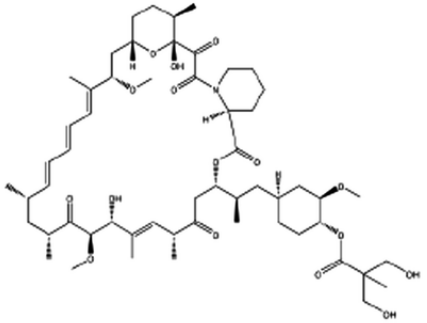
Rapamycin



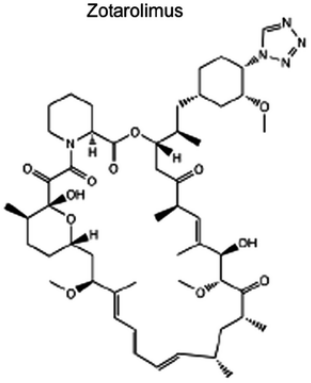
Ascomycin



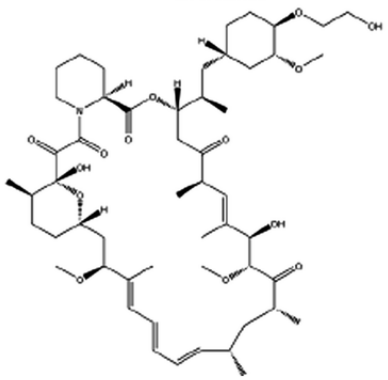
Temsirolimus



Zotarolimus



Everolimus



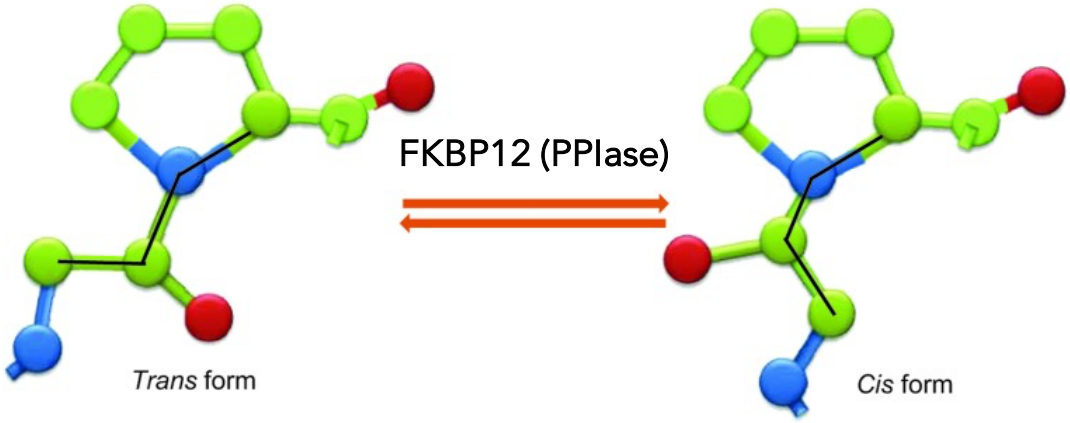
anti-immune effects
anti-tumor effects

FKBP12 is a peptidyl-prolyl isomerase

Proline is highly constrained

-

Side chain is bonded to its secondary amine Nitrogen

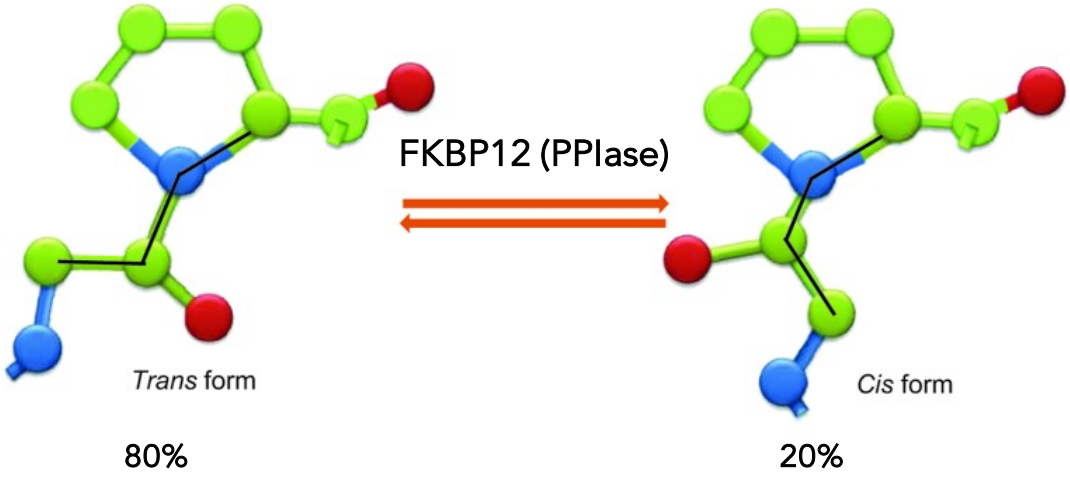


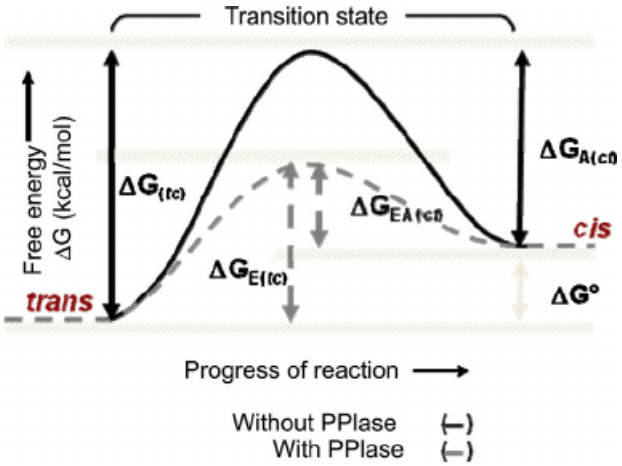
FKBP12 is a peptidyl-prolyl isomerase

Proline is highly constrained

-

Side chain is bonded to its secondary amine Nitrogen

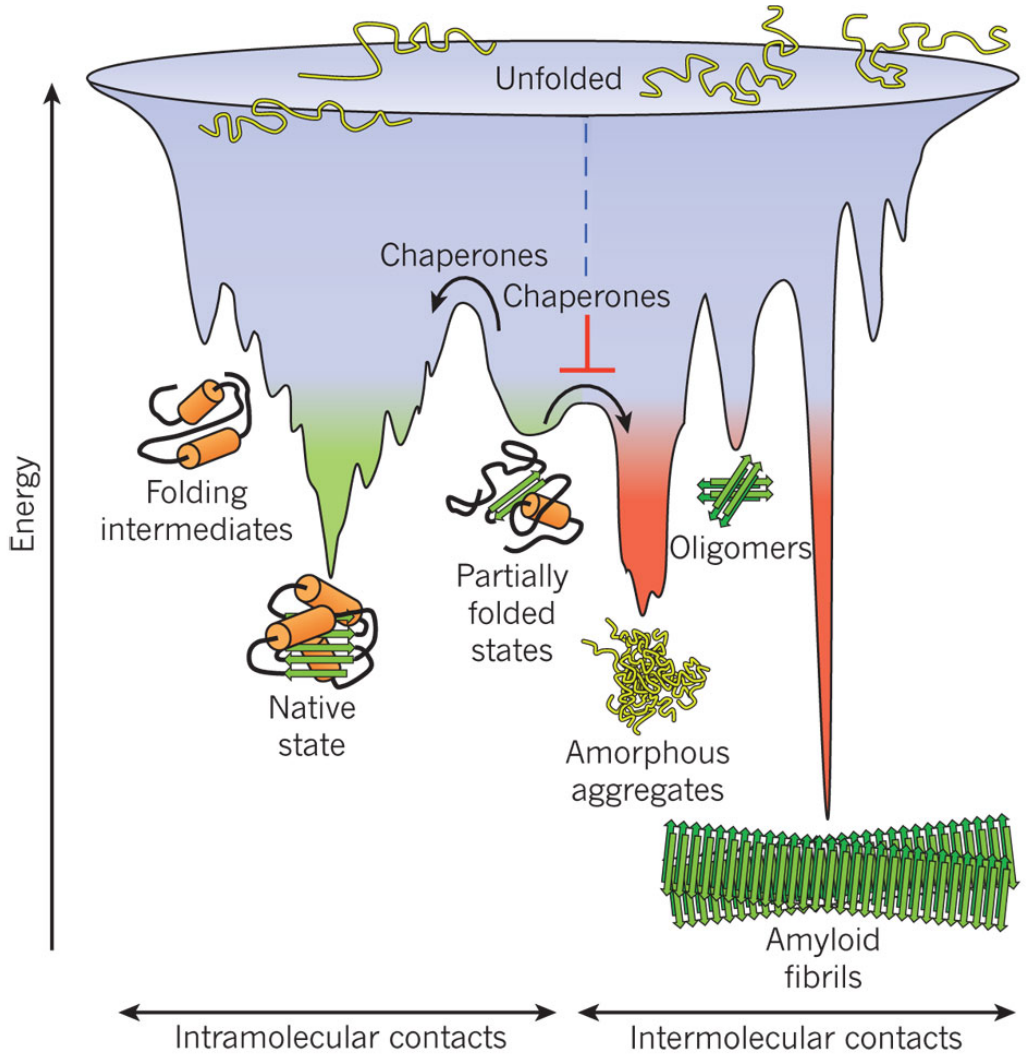




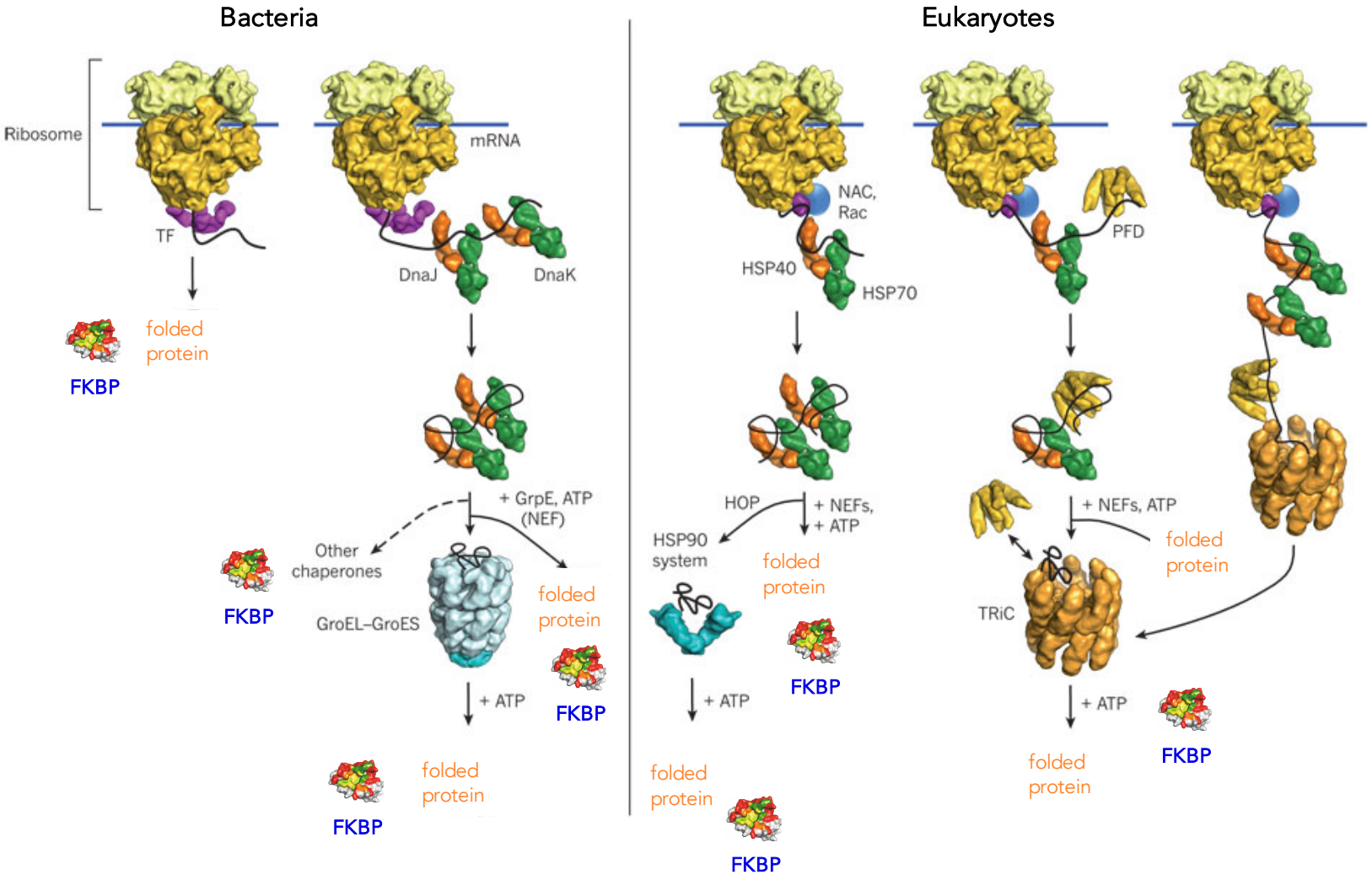
E_a ~ 20 kcal/mol

Enzyme catalyzes rate acceleration of 10^6 fold over non-enzymatic cis-trans isomerization

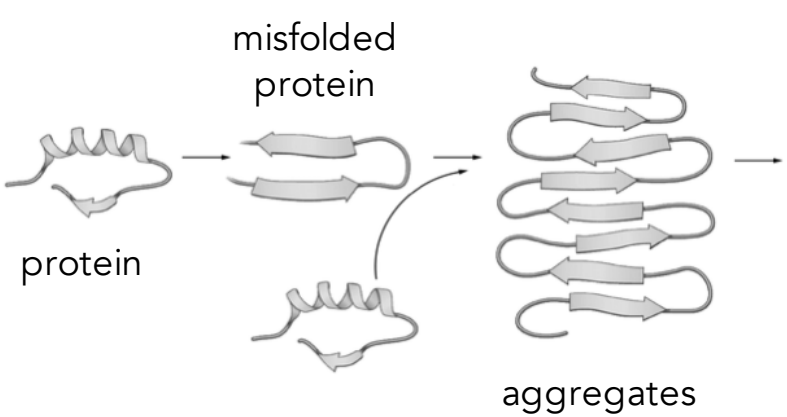
FKBP12 is a molecular chaperone



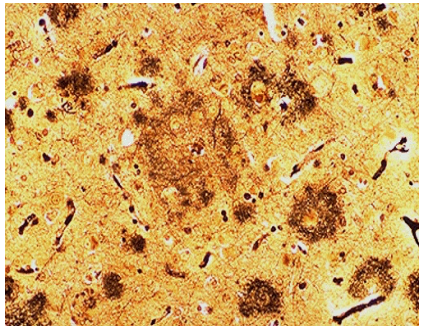
FKBPs are ‘downstream’ chaperones



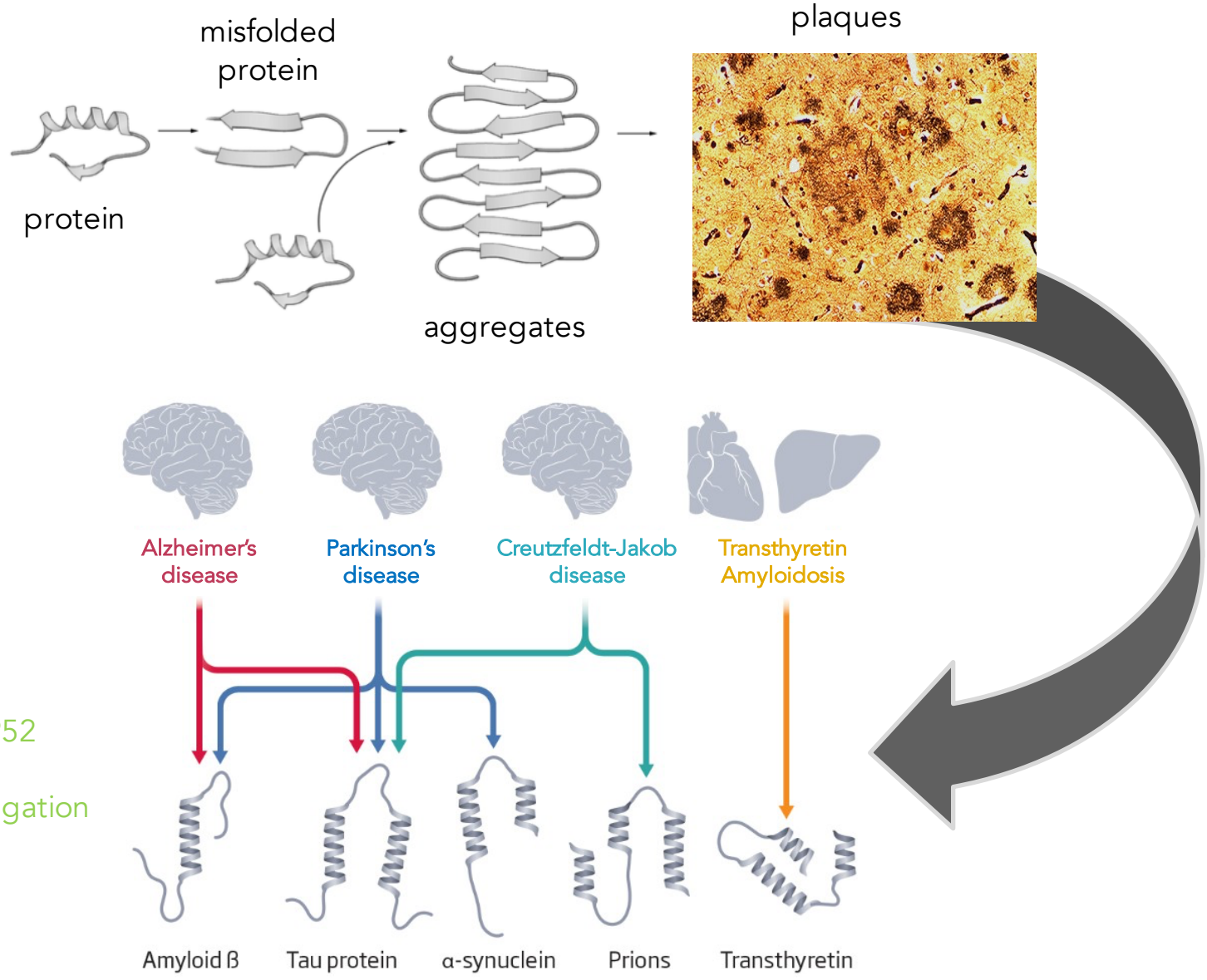
FKBPs in diseases of protein folding



plaques

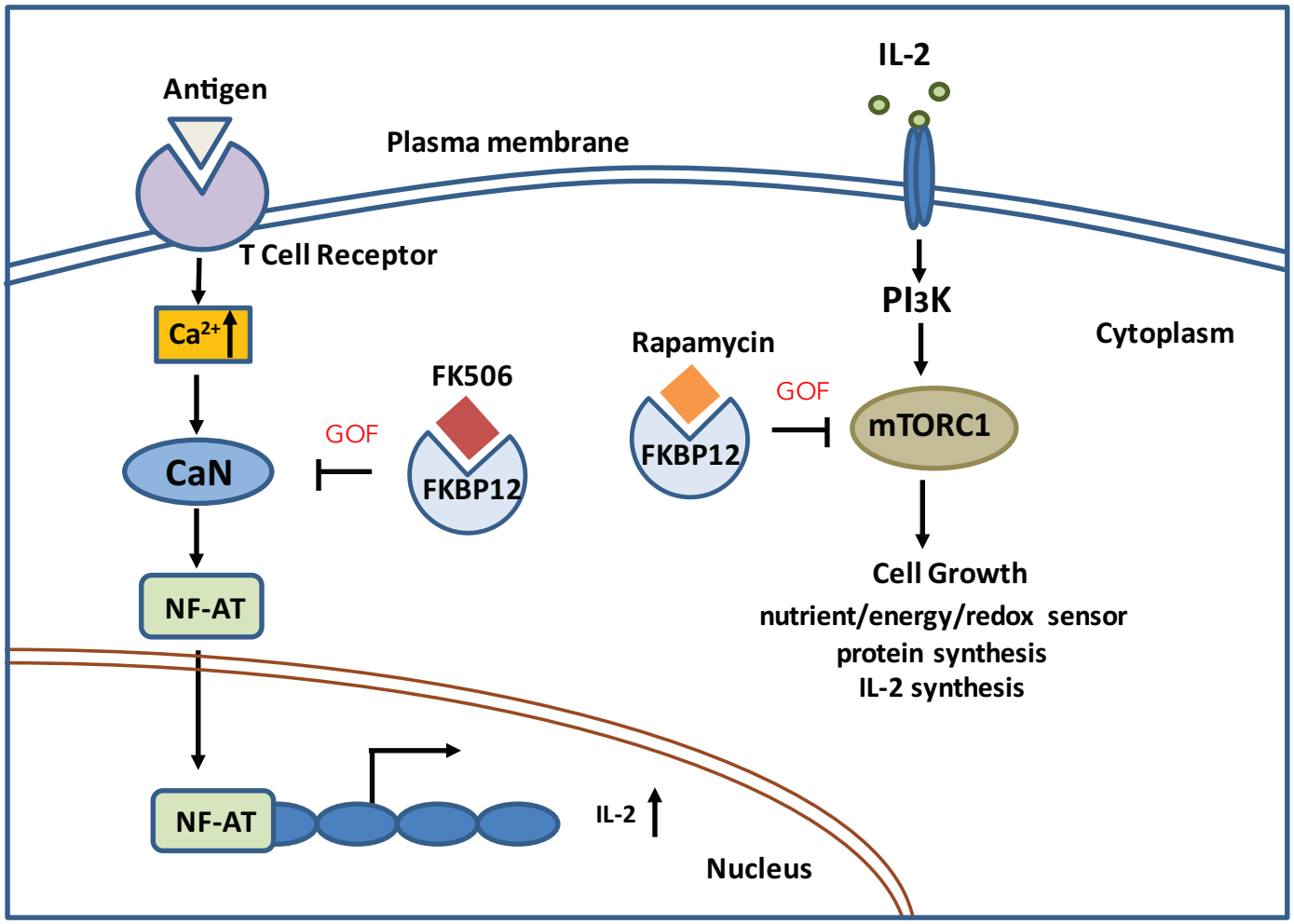


FKBPs in diseases of protein folding



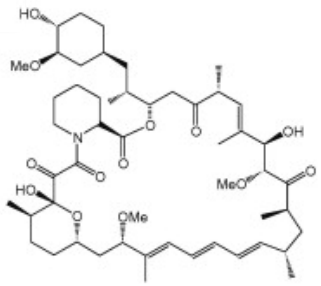
higher FKBP52
=
lower Tau aggregation

FKBP12 'gains a function' to inhibit T-cell activity

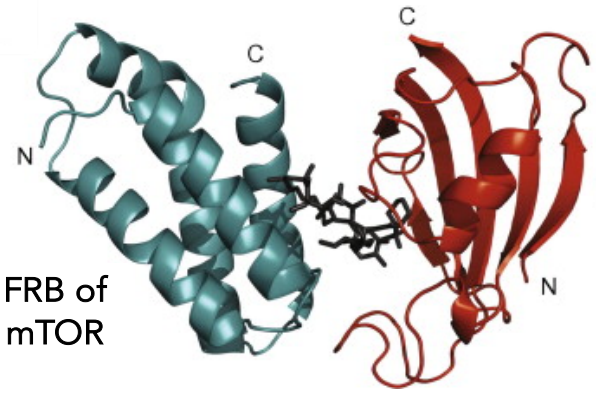


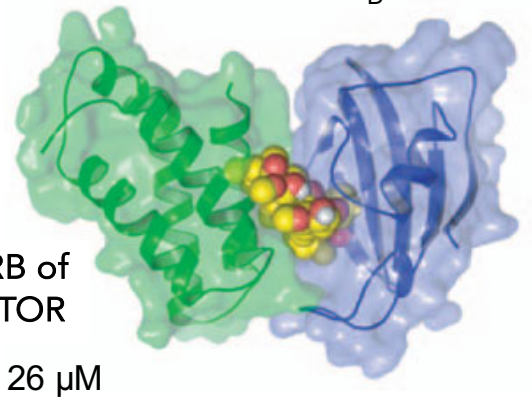
FKBP12 'ternary' complexes

Rapamycin and mTOR



rapamycin





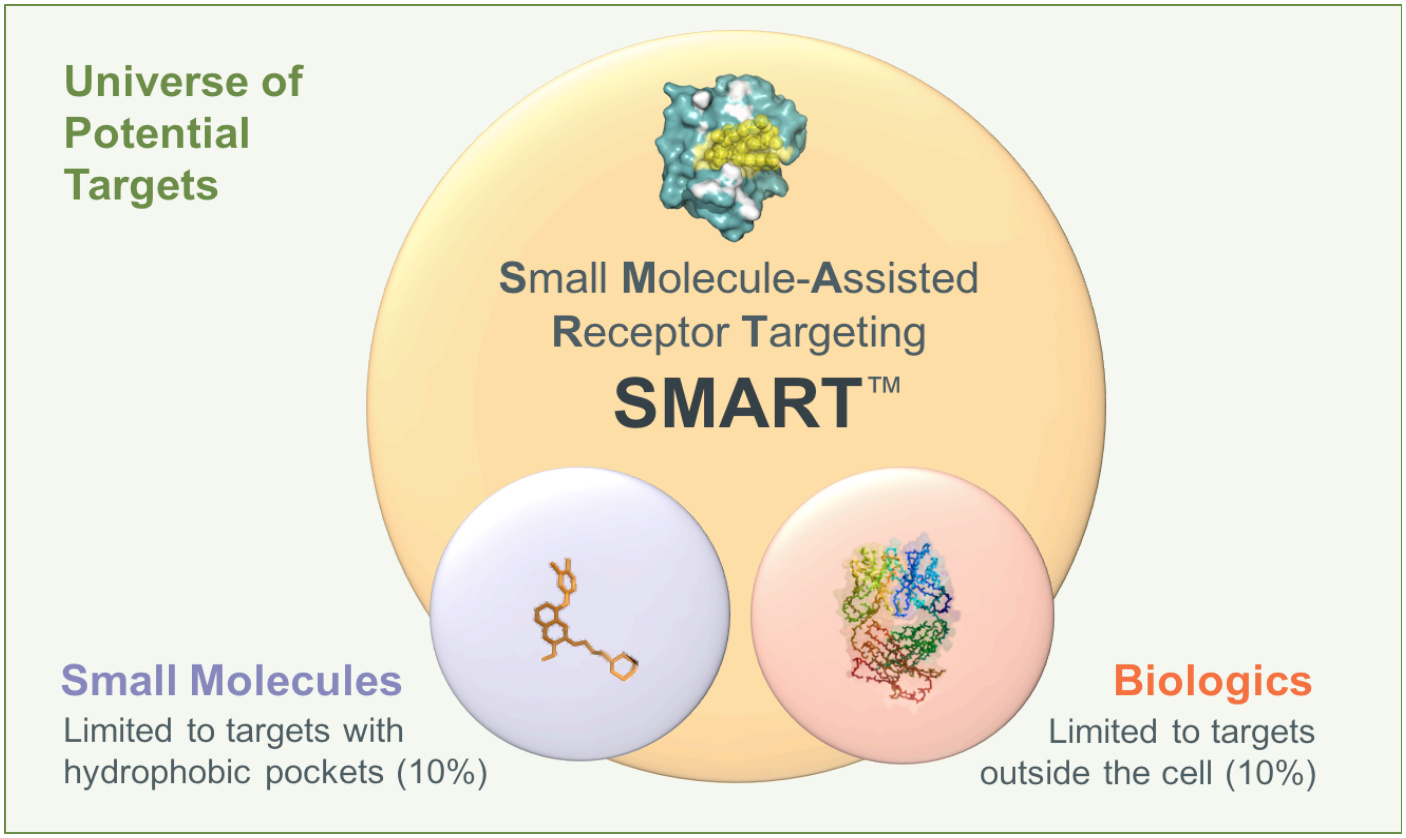
K_D ~ 26 μM

K_D ~ 12 nM

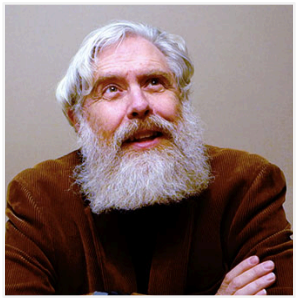
Choi *et al*. Science, 273, 239-42 (1996)
Banaszynski, Liu, and Wandless, J. Am. Chem Soc., 127, 4815-21 (2005)

PDB entry:
1NSG

Drugging the 'undruggable' through GOF



Greg Verdine, Harvard

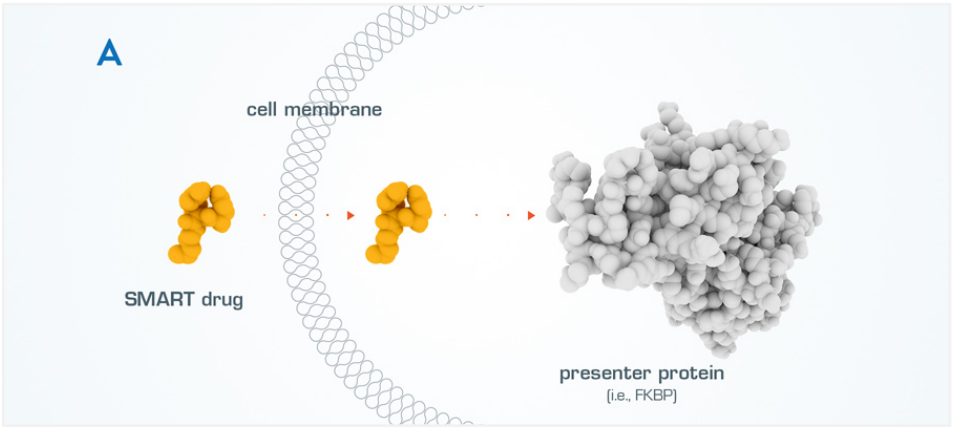


George Church, Harvard

Jim Wells, UCSF

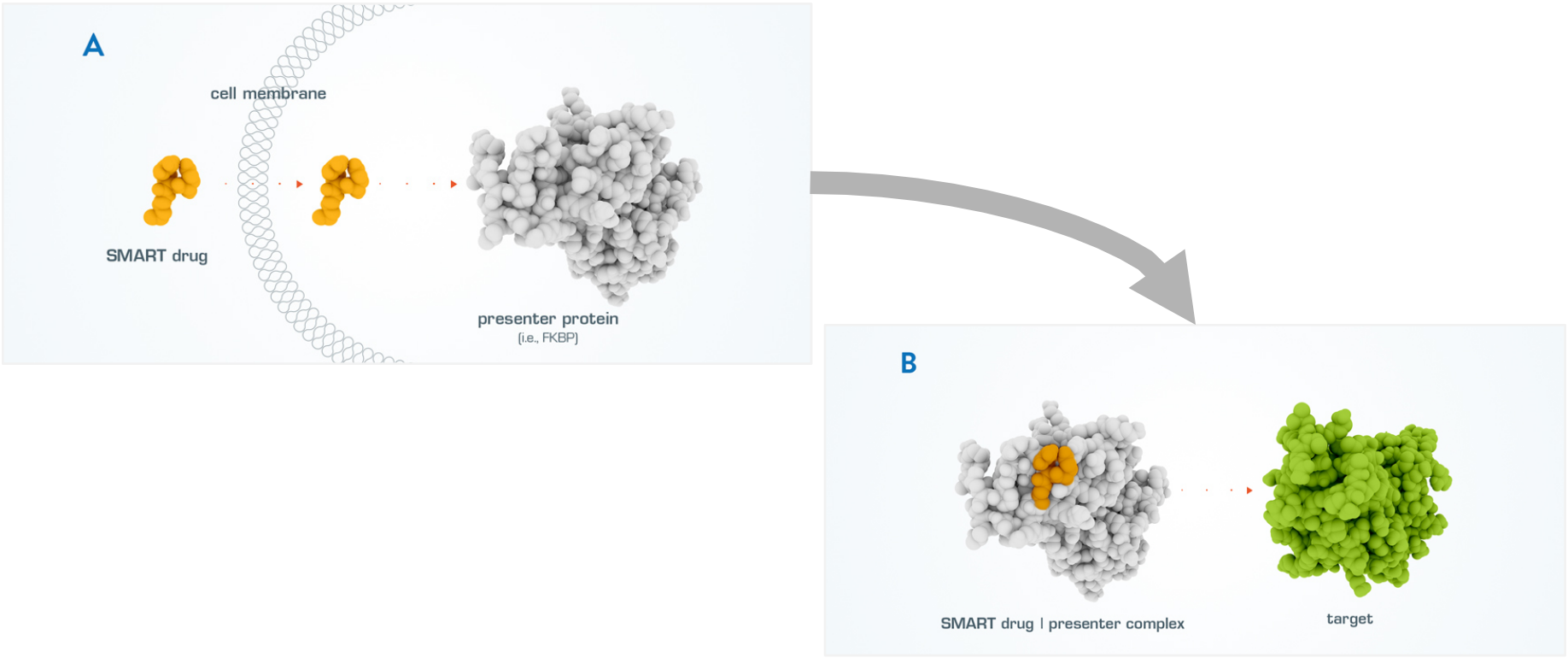
WarpDrive Bio

SMART™ – small molecule-assisted receptor targeting



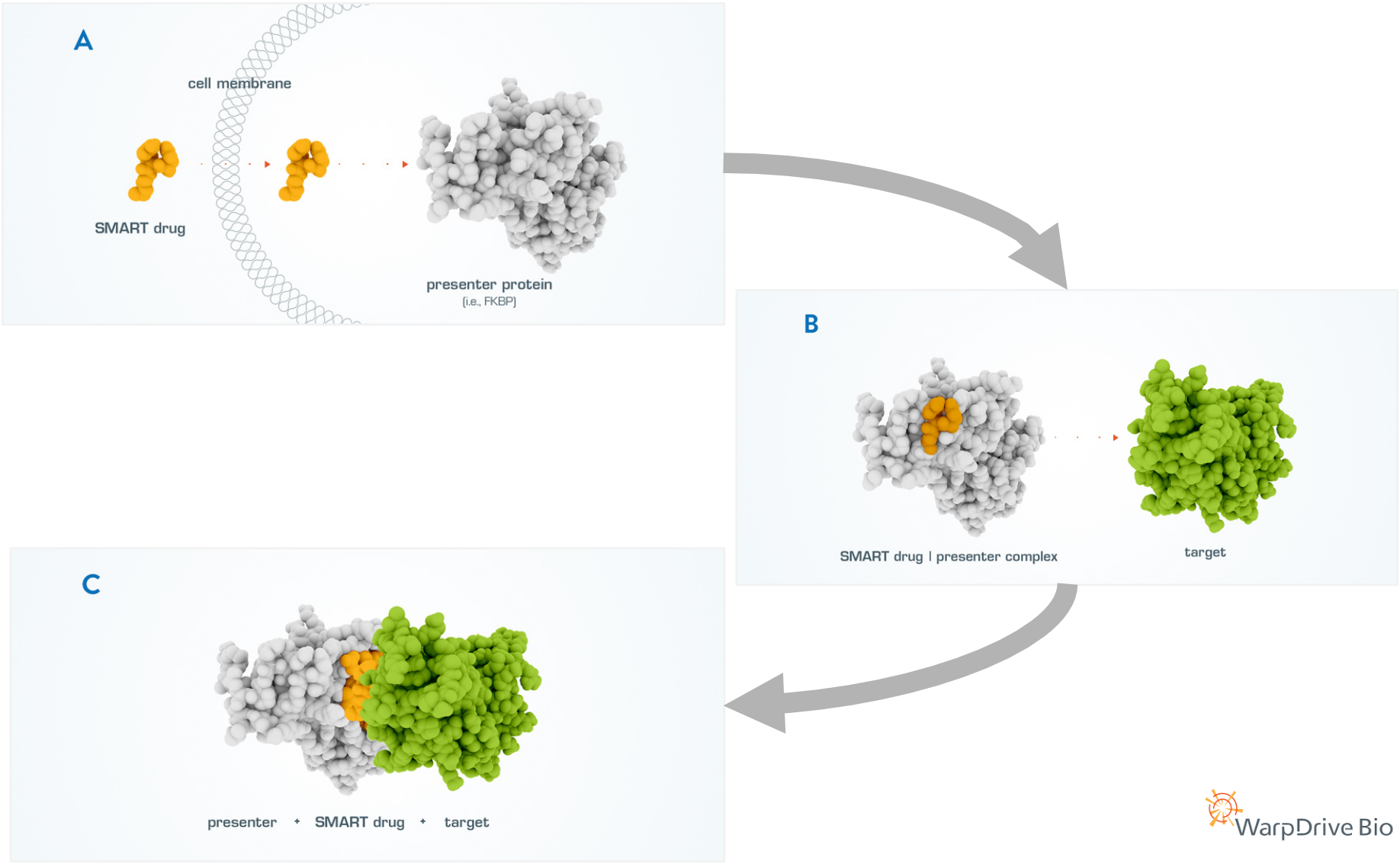
WarpDrive Bio

SMART™ – small molecule-assisted receptor targeting



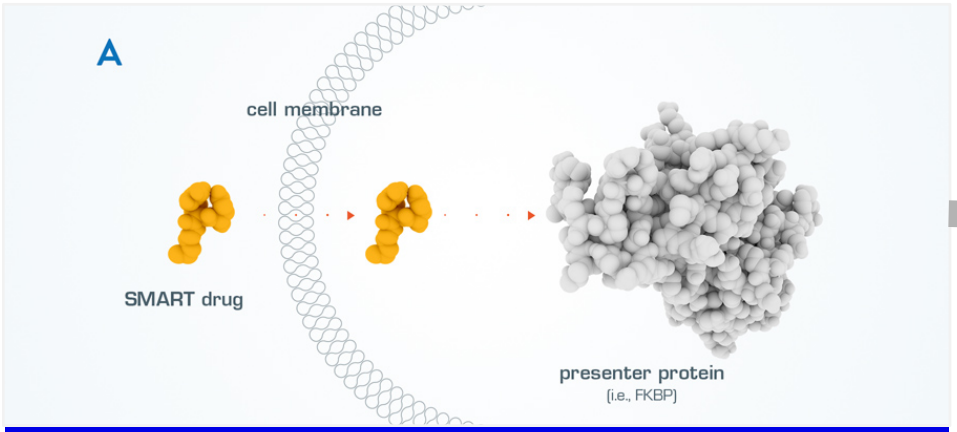
WarpDrive Bio

SMART™ – small molecule-assisted receptor targeting

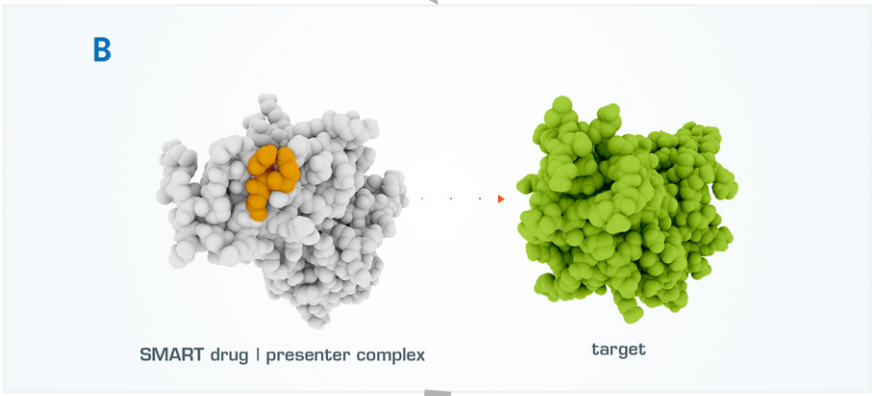


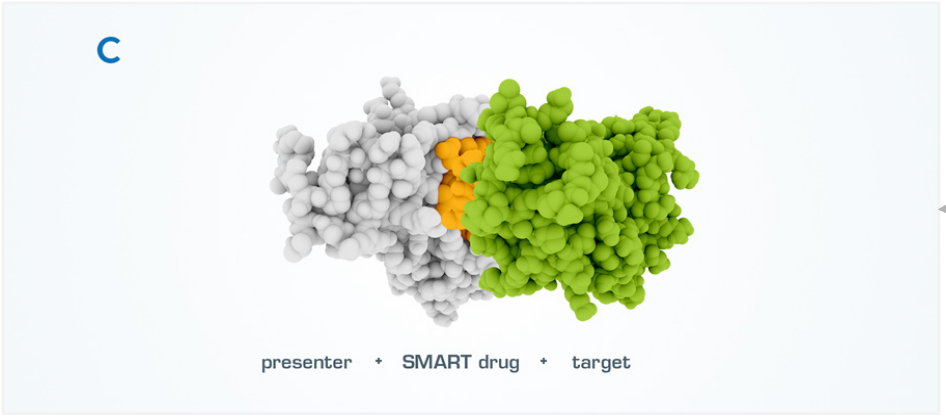
WarpDrive Bio

SMART™ – small molecule-assisted receptor targeting



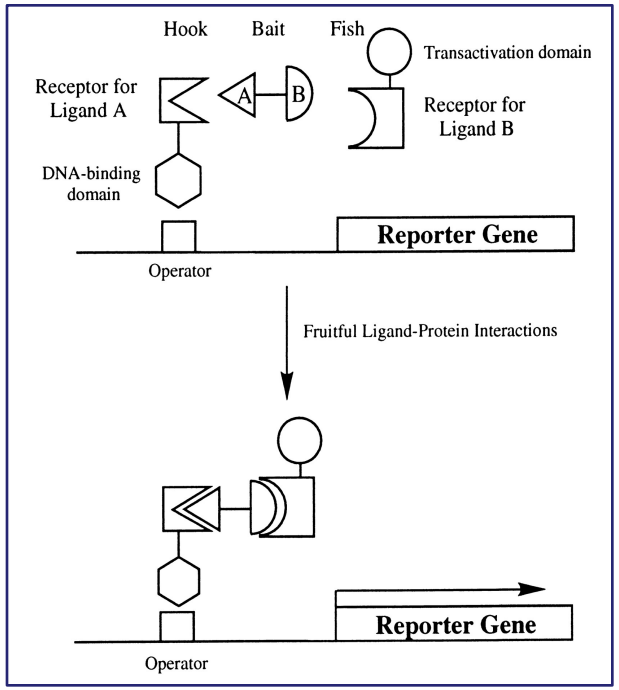
Novel molecules that you find during this class may serve as new starting points for this concept, providing new molecular interfaces with FKBP12 that can be used to engage a new proteins through design or screening





WarpDrive Bio

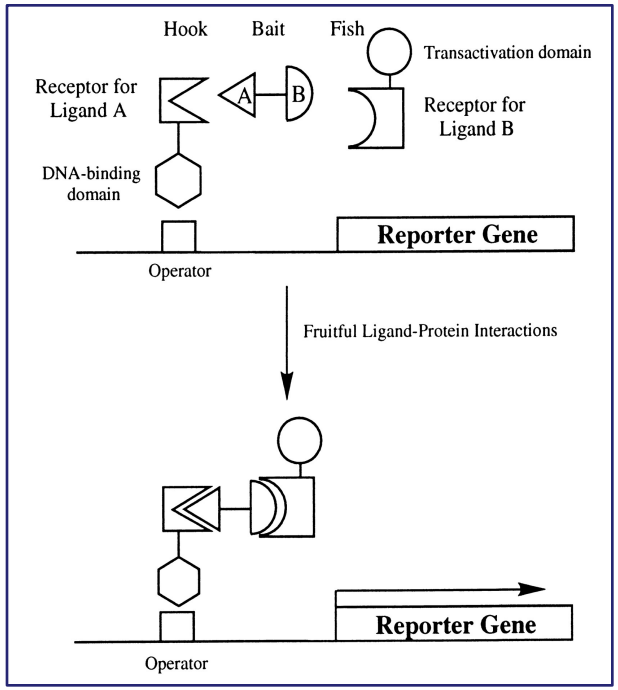
FKBP12 as a tool for biological engineering - preview

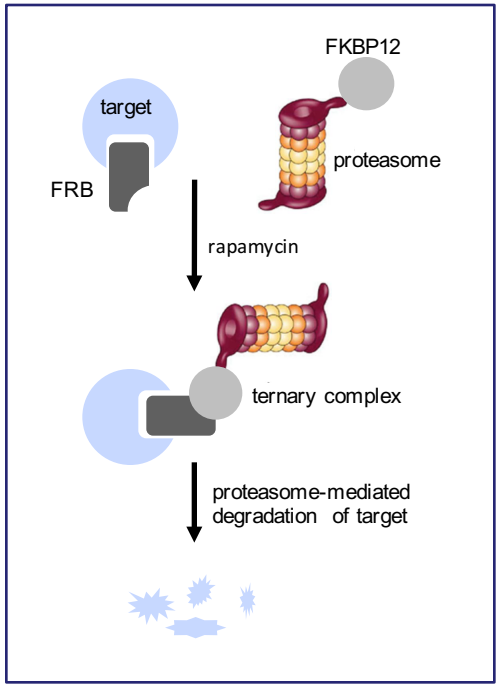


controlling transcription

proximity induction strategies

FKBP12 as a tool for biological engineering - preview



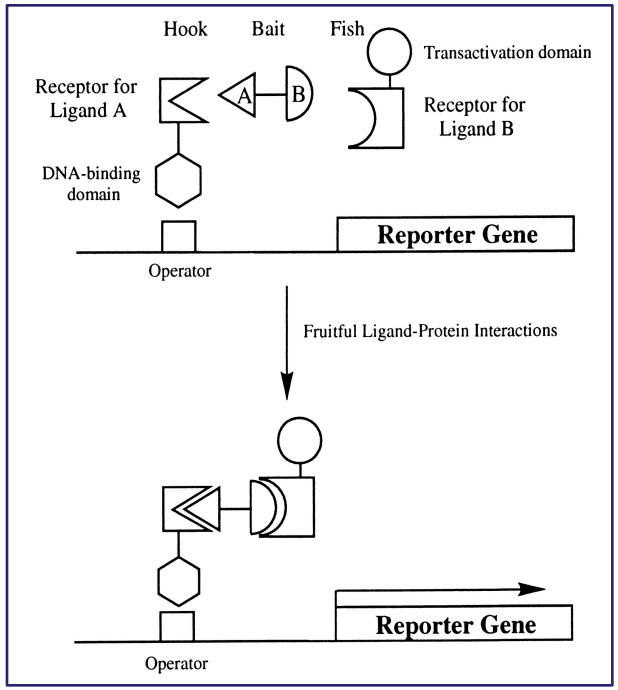


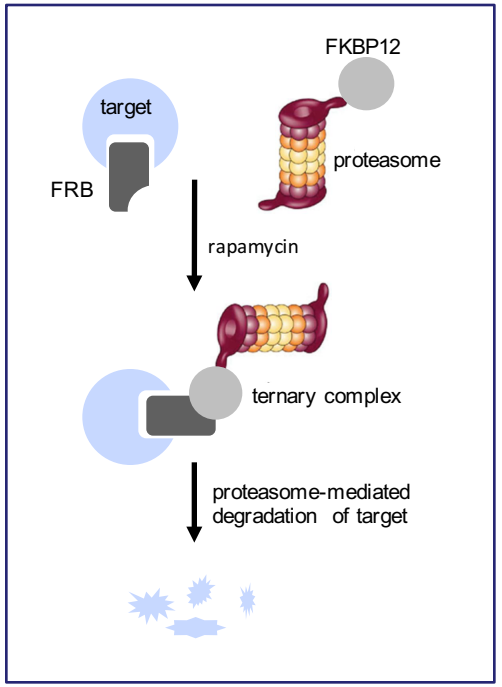
controlling transcription

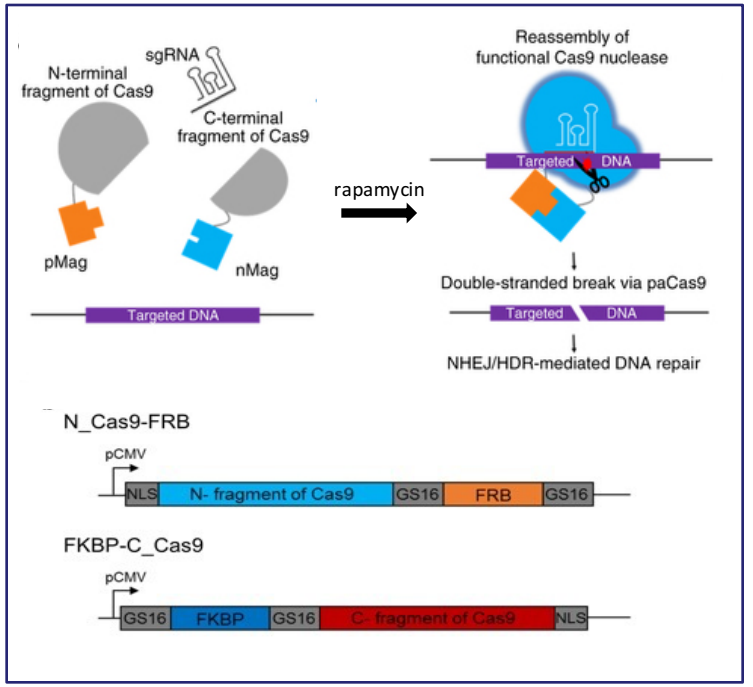
inducing protein degradation

proximity induction strategies

FKBP12 as a tool for biological engineering - preview







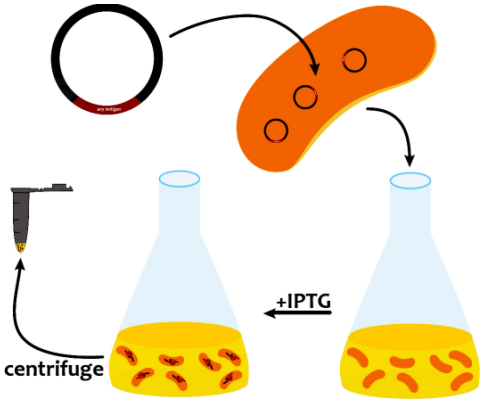
controlling transcription

inducing protein degradation

induced genome editing

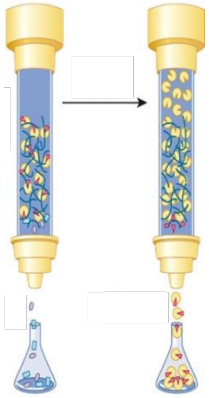
proximity induction strategies

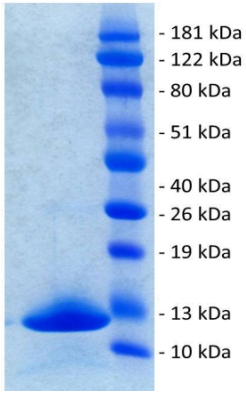
Reminder - our path to probe discovery



overexpress FKBP12

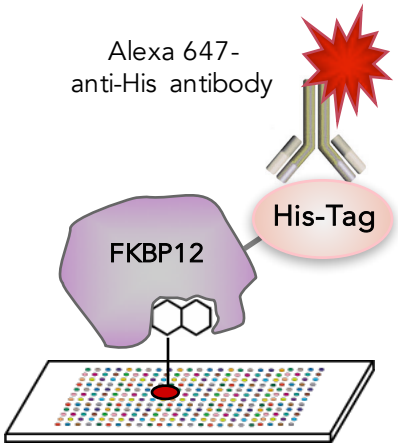
lab day 1





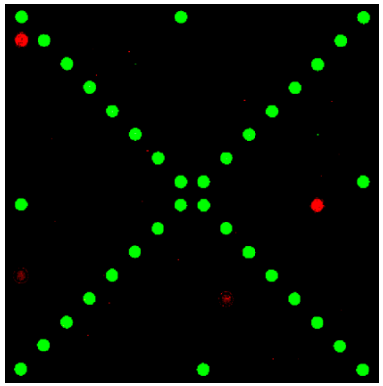
purify and characterize FKBP12

lab days 2 and 3



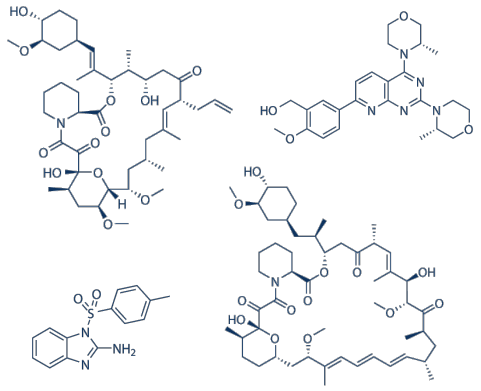
SMM screen

lab day 4



scan images and analyze data

lab days 5 and 6



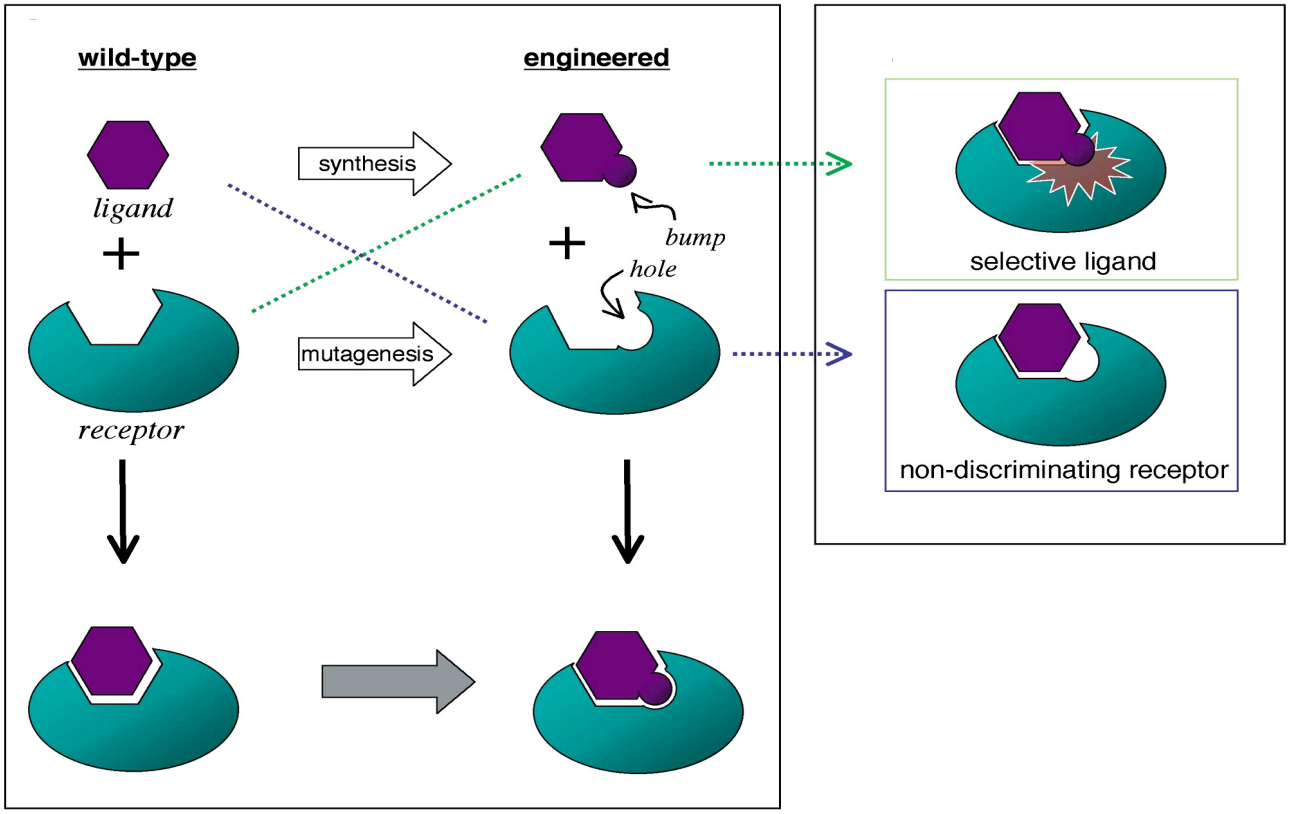
compare hit lists for teams

lab day 7

Our path to probe discovery - lectures

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

FKBP12 as a tool for biological engineering - preview



orthogonal receptor ligand pairing
aka 'bump-hole'