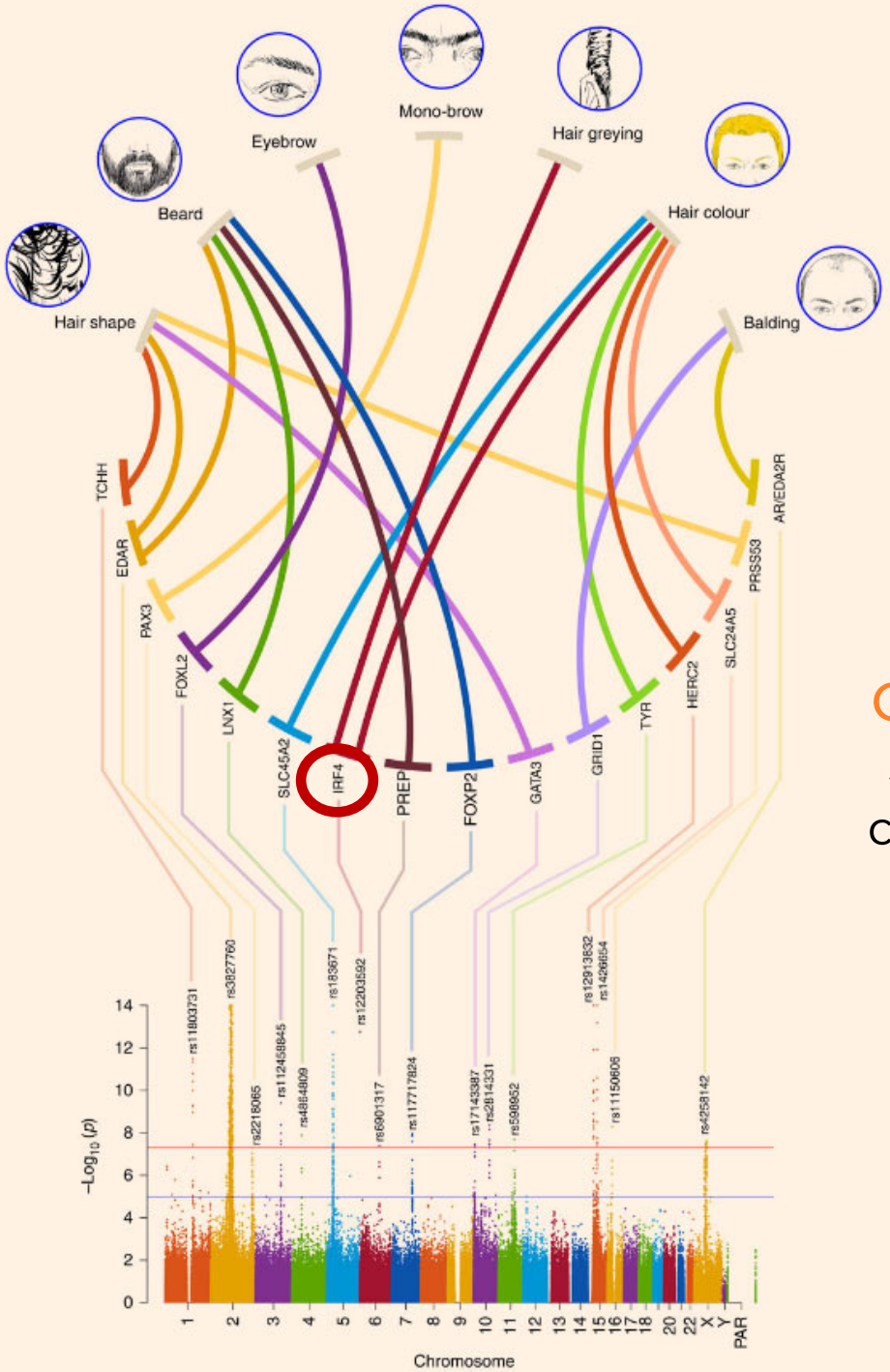
Which process or biomolecule would you study with a chemical probe if you had one in hand?

Genome Wide Association Study:

Hair-related phenotypes



A genome-wide association scan in admixed Latin Americans identifies loci influencing facial and scalp hair features

Kaustubh Adhikari et al. Nature Communications, **2016**

doi: 10.1038/ncomms10815.

GWAS: correlating single-nucleotide changes across the genome with specific traits

depmap

depmap.org

Look up dependencies

Enter a gene, cell line, lineage or compound

Use this portal to:

UNDERSTAND Dependency profiles at genome-scale across more than 500 human cell lines

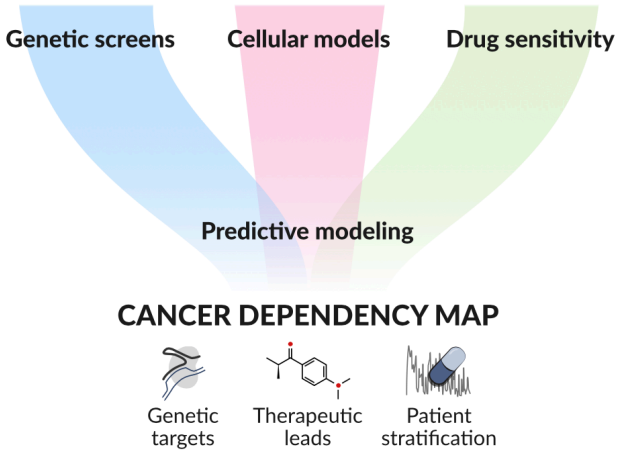
FIND Detailed genetic and pharmacologic characterization of over 1000 cell lines

IDENTIFY Genetic drivers that have functional importance as potential drug targets

SEARCH For cell line models that best represent your research interests

EXPORT Presentation-quality figures

The goal of the Cancer Dependency Map is to create a comprehensive preclinical reference map connecting tumor features with tumor dependencies to accelerate the development of precision treatments. Our strategy is to systematically characterize cellular models of cancers and to test those models for sensitivity to genetic and small-molecule perturbations. By integrating data beyond those collected at the Broad, DepMap hopes to develop a complete understanding of the vulnerabilities of cancer, identify targets for therapeutic development, and design strategies to optimize patient responses to those therapies.

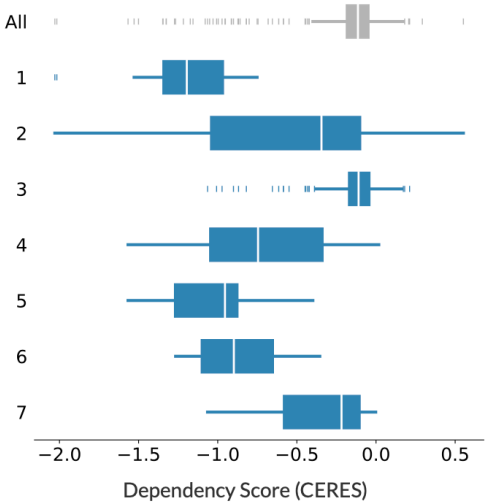


To date DepMap has profiled more than 500 cell lines. Over the next several years we will greatly expand the diversity of cell lines profiled for genetic vulnerabilities with quarterly data release. Additionally, limited drug sensitivity data are available.

IRF4

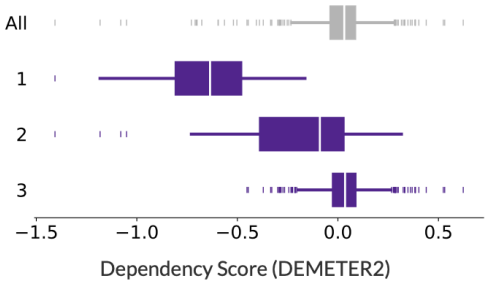
Enriched Lineages

CRISPR



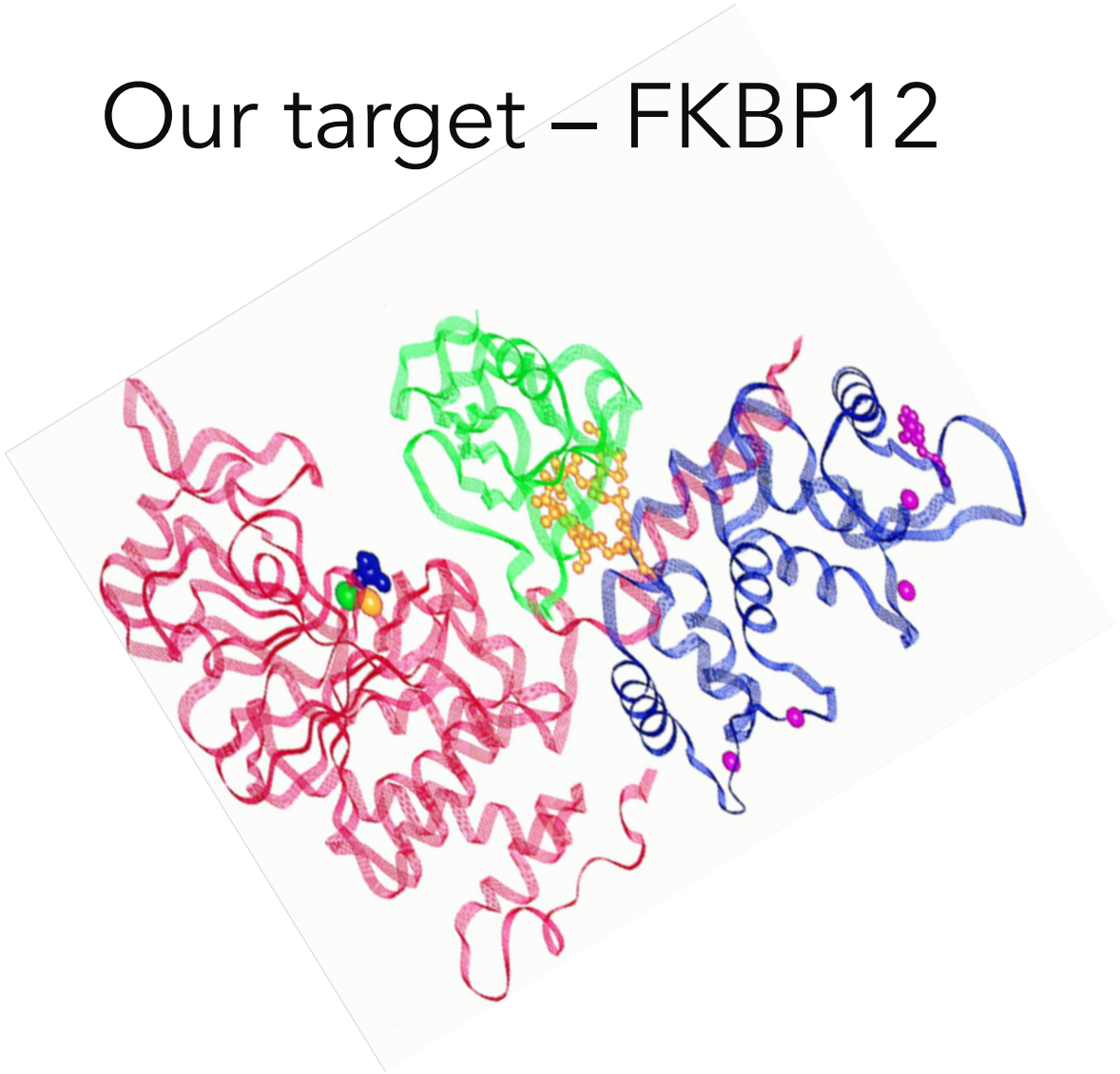
1. Multiple Myeloma (4.4e-79)
2. Haematopoietic And Lymphoid (3.4e-39)
3. Solid (4.5e-38)
4. Lymphoma (4.0e-15)
5. T-cell lymphoma Other (9.5e-12)
6. ALCL (9.9e-07)
7. Melanoma (1.3e-04)

RNAi



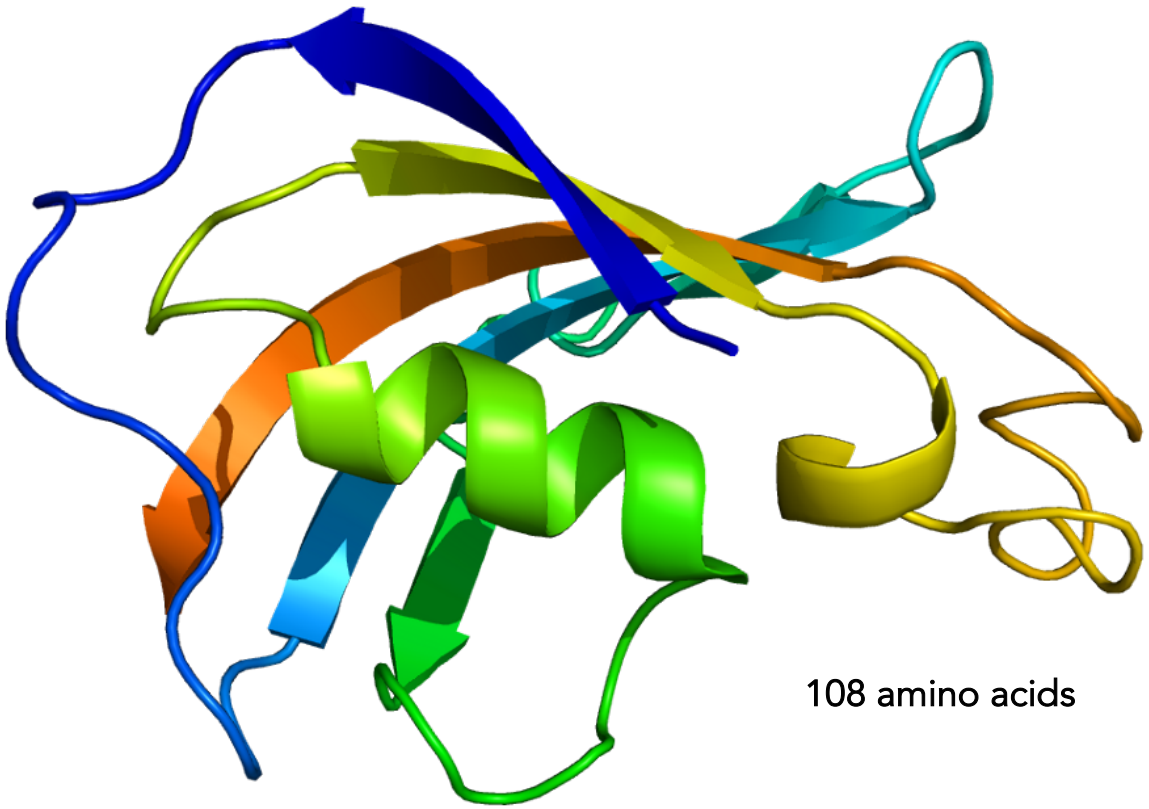
1. Multiple Myeloma (1.3e-72)
2. Haematopoietic And Lymphoid (3.3e-28)
3. Solid (6.5e-21)

Our target – FKBP12



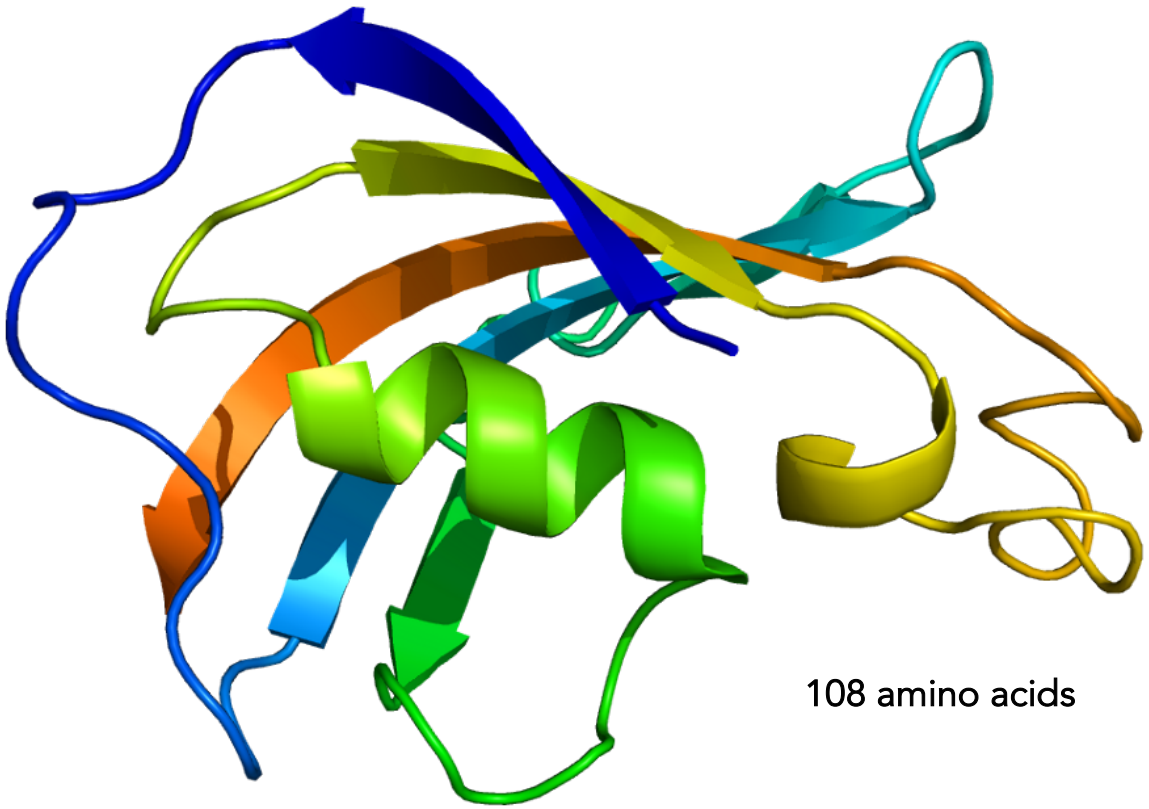
December 3, 2019

FKBP12



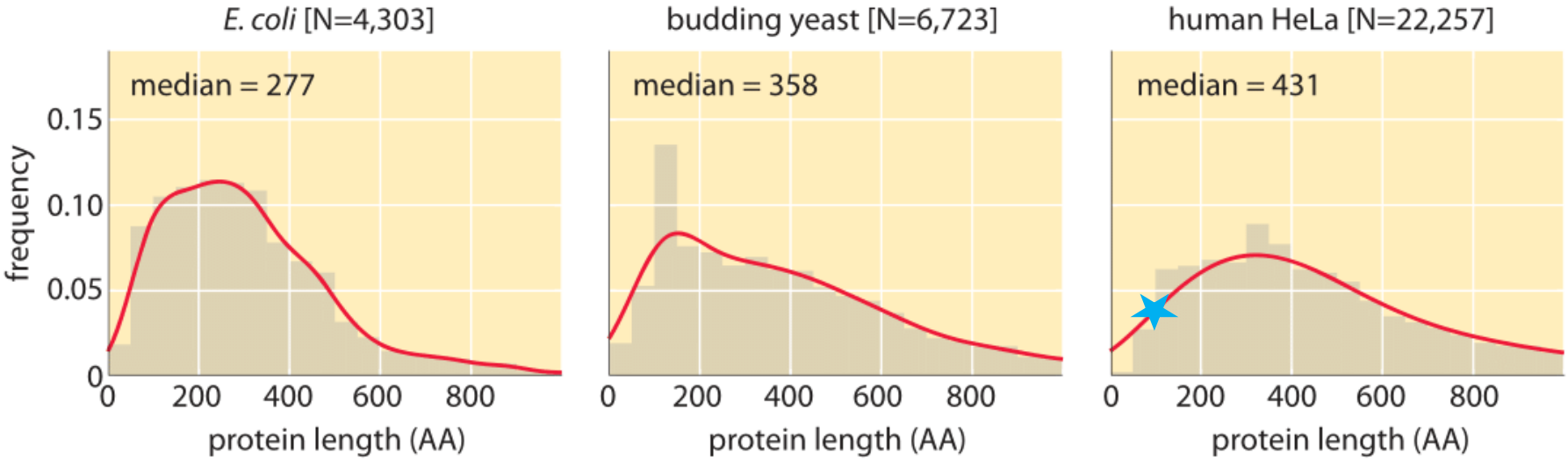
FK-506 Binding Protein that is 12 kilodaltons

FKBP12



FK-506 Binding Protein that is 12 kilodaltons

How big is the typical protein?



Predicted Genomic Lengths, *Trends in Genetics* (2001)

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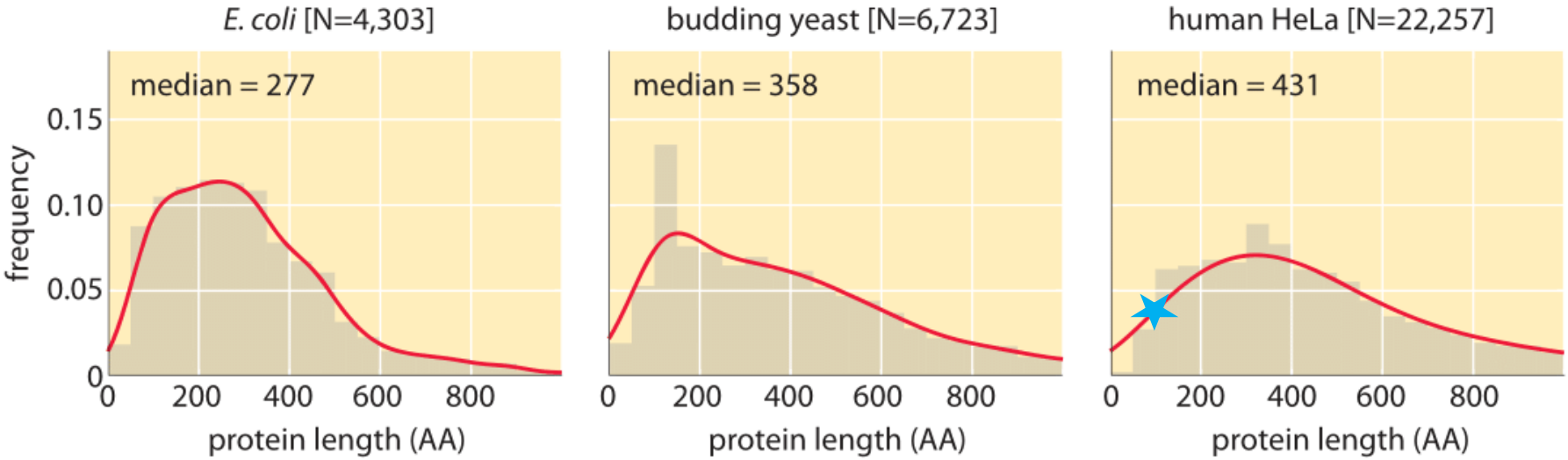
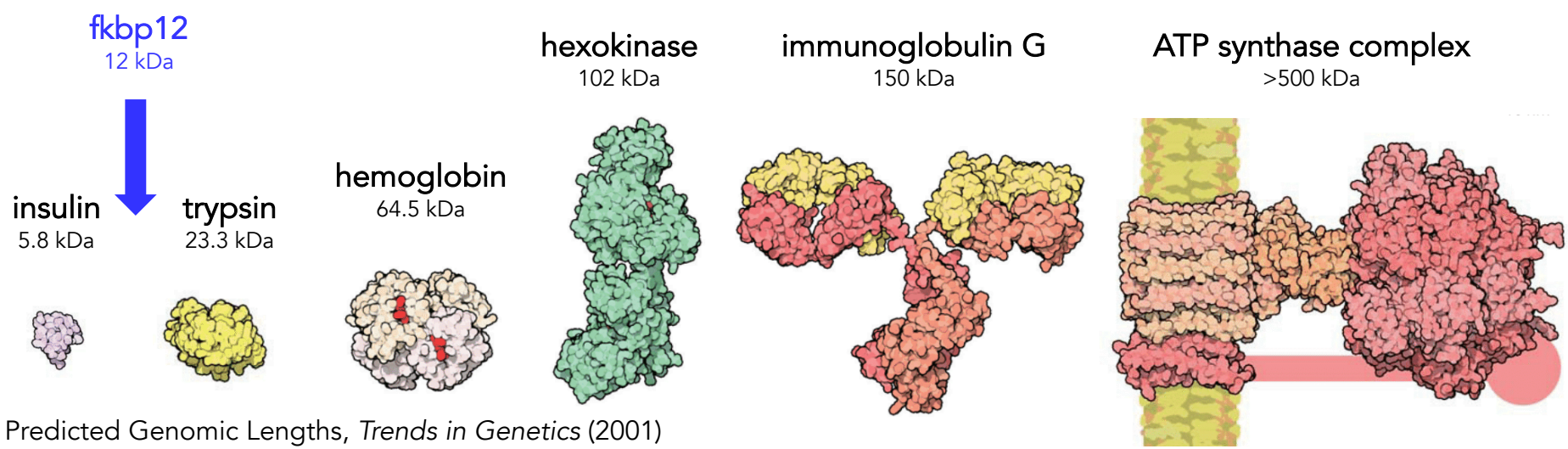
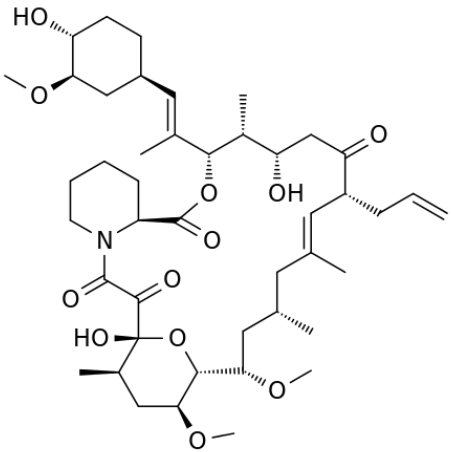


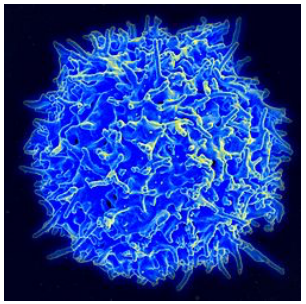


Predicted Genomic Lengths, *Trends in Genetics* (2001)

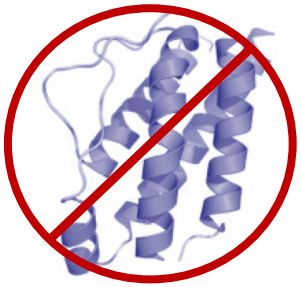
FK-506 is an immunosuppressant drug



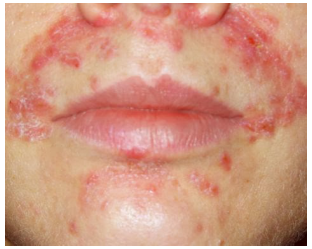
FK-506 (Tacrolimus)
inhibits the development of
T-cells for immune responses



blocks T-cell proliferation



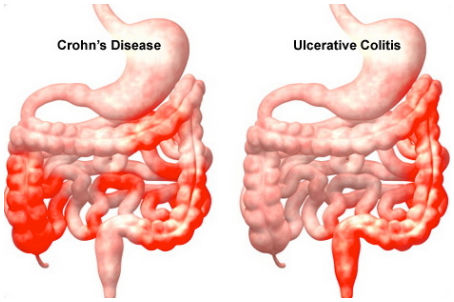
inhibits secretion of
interleukin-2



eczema and other
skin conditions

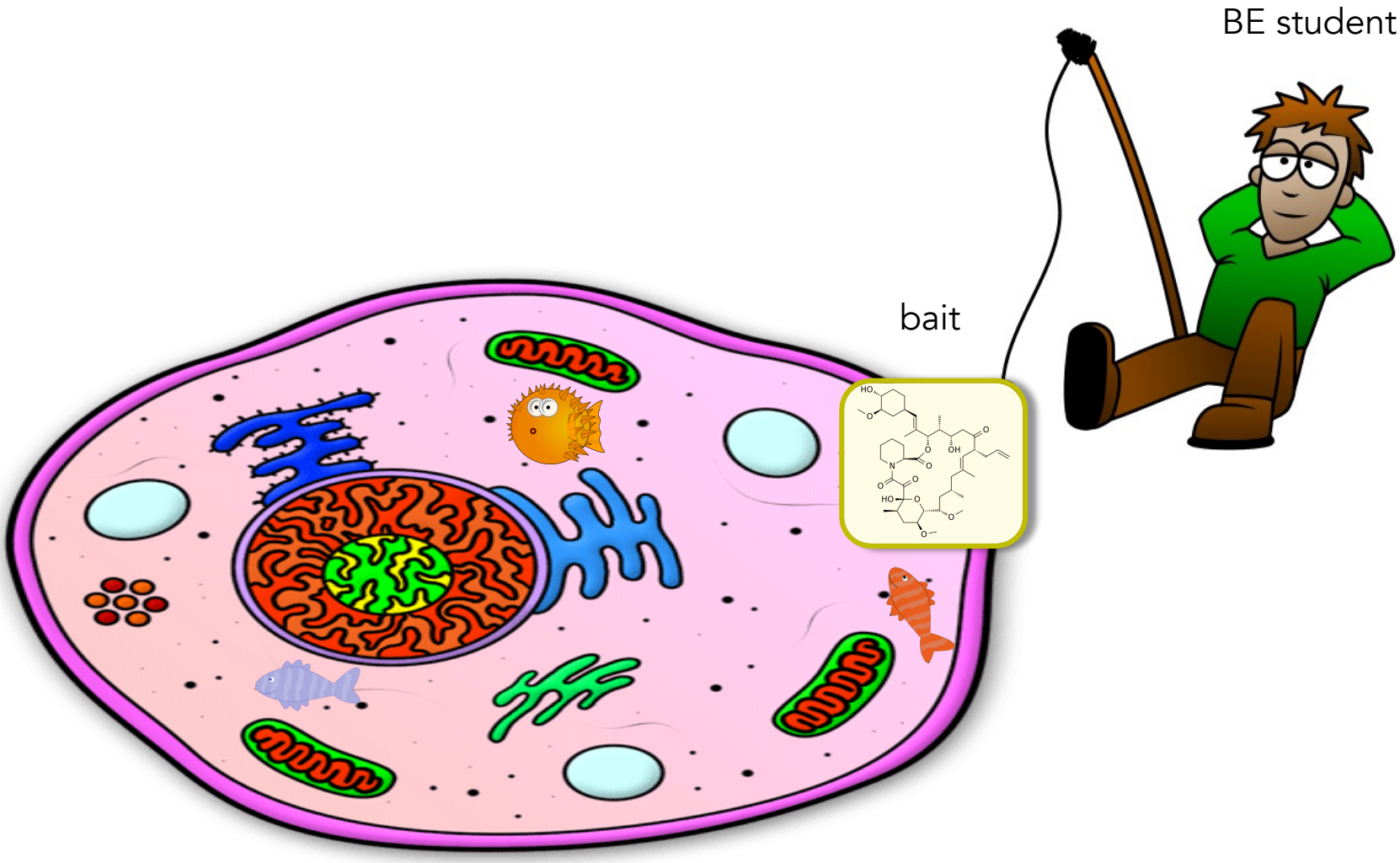
transplant rejection

fermentation broth of Japanese soil sample
Streptomyces tsukubaensis



inflammatory bowel disorders

Fishing for the target of FK-506

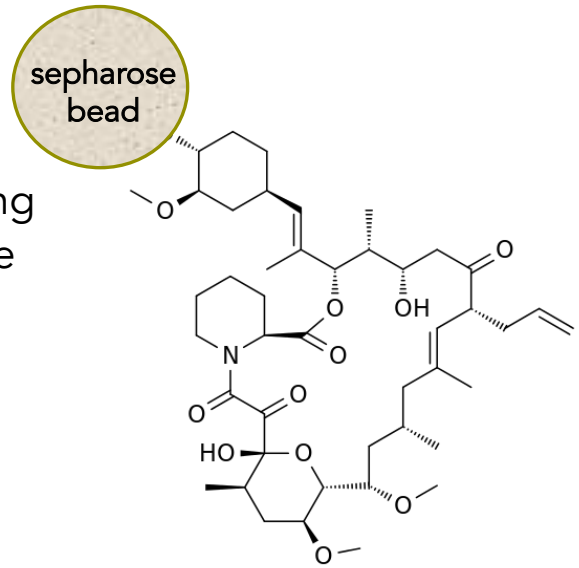


cellular pond

Fishing for the target of FK506

affinity chromatography

couple 'bait' to matrix



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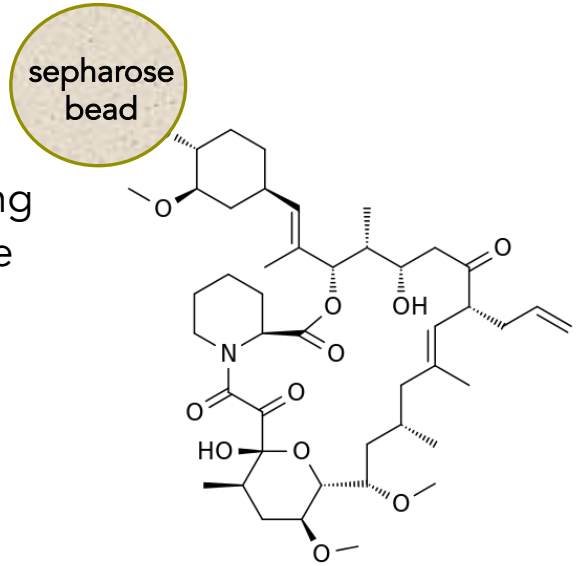
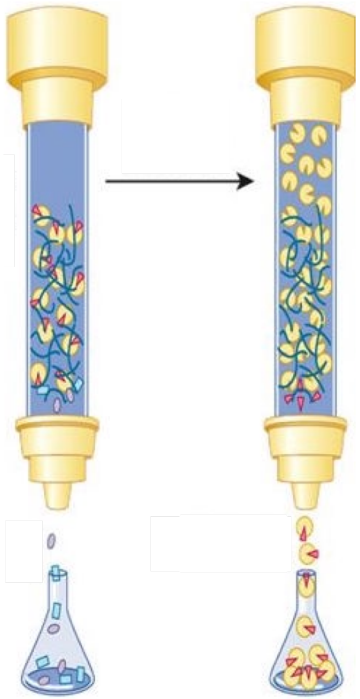


add tissue homogenate

elute



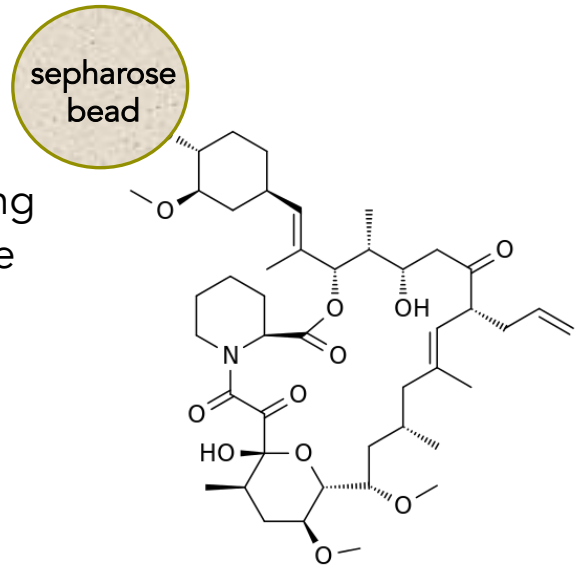
wash column with free FK506

bovine thymus &
human spleen tissue

Fishing for the target of FK506

affinity chromatography

couple 'bait' to matrix



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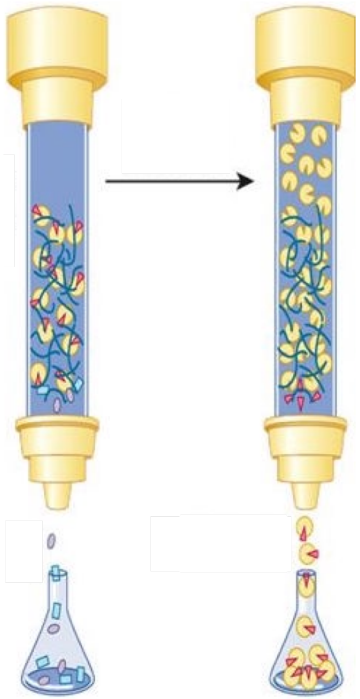
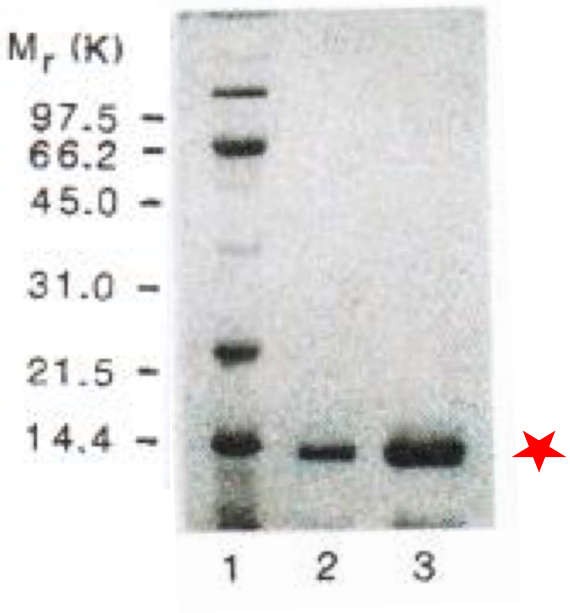


SDS-PAGE



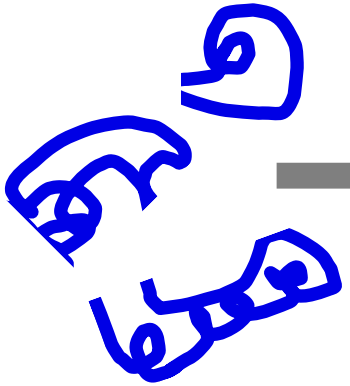
Harding *et al*. Nature 341, 758-760 (1989)
Siekierka *et al*. Nature 341, 755-757 (1989)

Fishing for the target of FK506

determining the protein sequence

purified protein
from calf thymus
(the ~14kDa band)

Edman degradation:
proteolytic fragments



7.05 and 5.07!!!

N-terminal sequencing
methods

VALENTYN

IHARTMIT

MITRCKS

Fishing for the target of FK506

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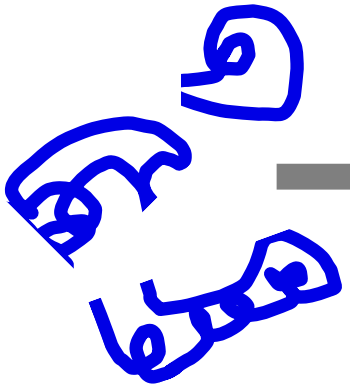
from calf thymus
(the ~14kDa band)

Edman degradation:
proteolytic fragments



computational analysis of
overlapping 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)

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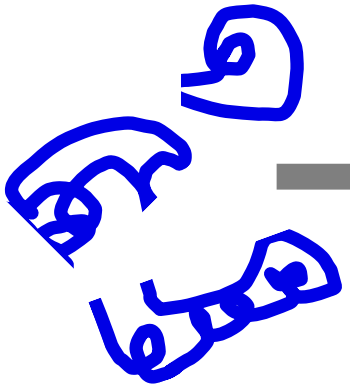

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DYAYGATGHPGIIP
PNATLIFDVELLKLE

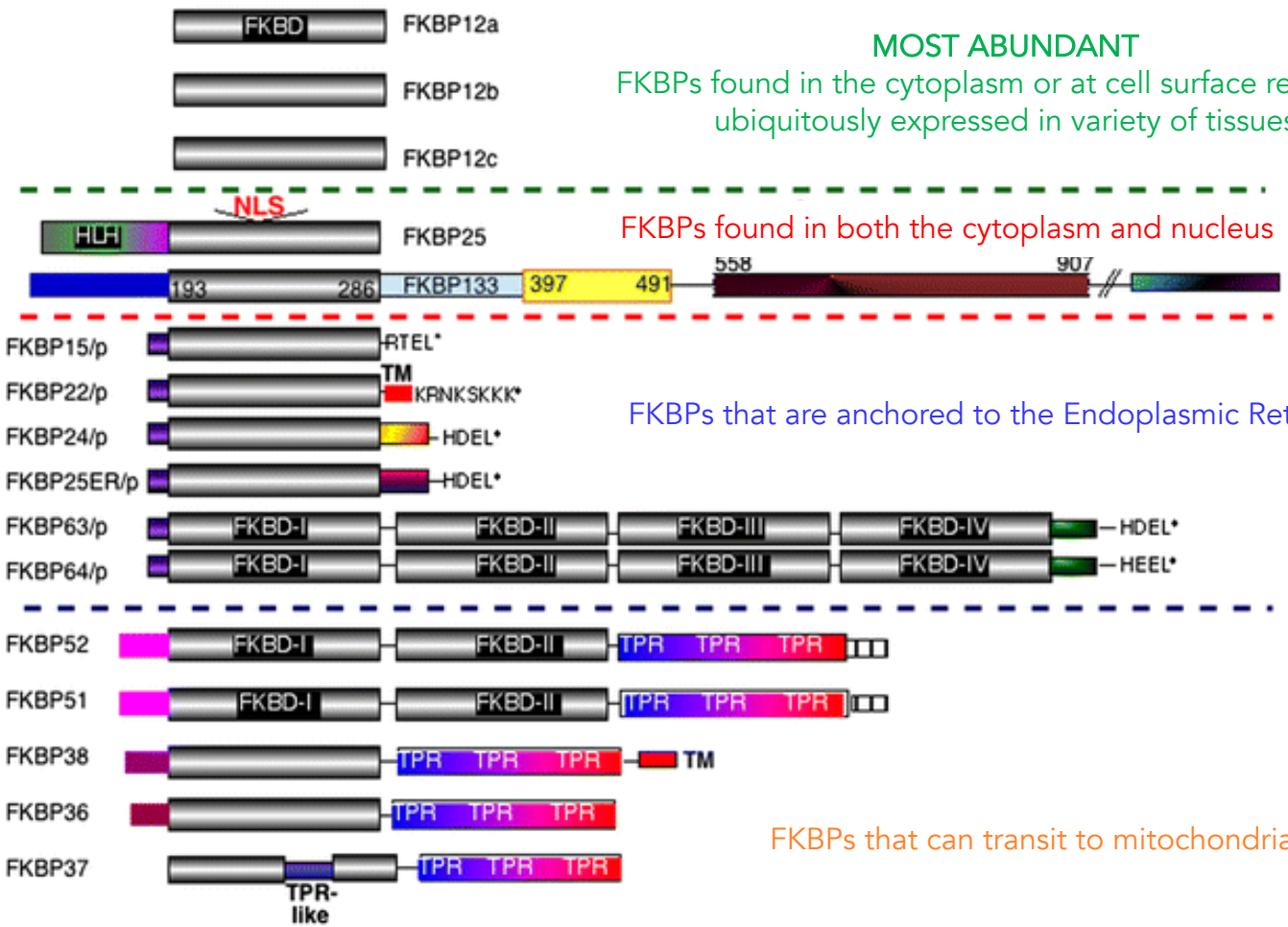
electrospray mass spectrometry to measure the full mass
of purified protein

11.8 kDa

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 – domain architectures



MOST ABUNDANT

FKBPs found in the cytoplasm or at cell surface receptors
ubiquitously expressed in variety of tissues

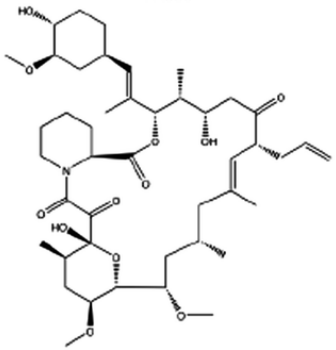
FKBPs found in both the cytoplasm and nucleus

FKBPs that are anchored to the Endoplasmic Reticulum

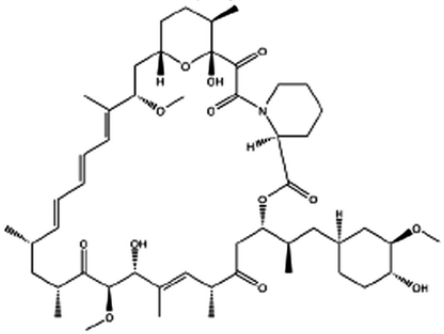
FKBPs that can transit to mitochondria and other organelles

Several drugs bind to FKBP12 with high affinity

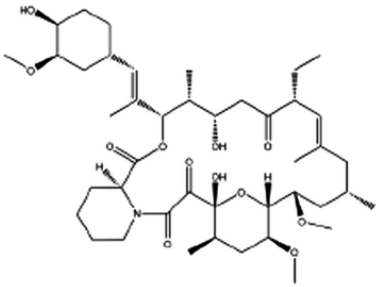
FK506



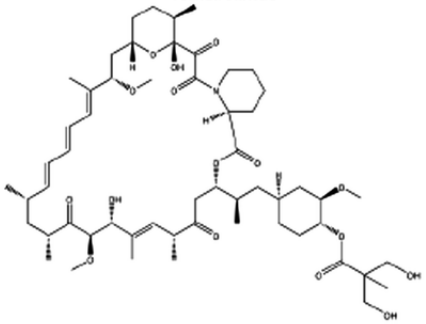
Rapamycin



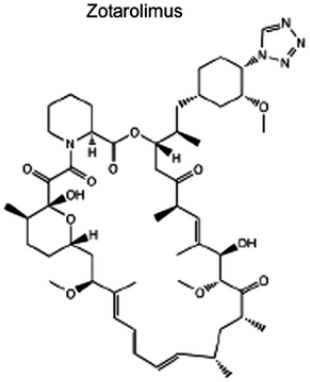
Ascomycin



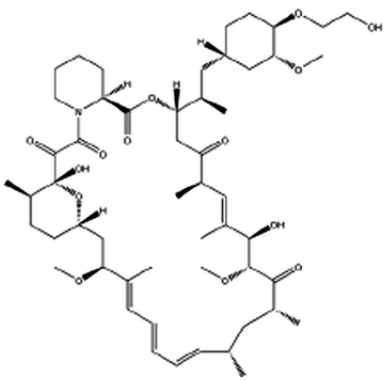
Temsirolimus



Zotarolimus



Everolimus



potent
i.e. 1-10 nM

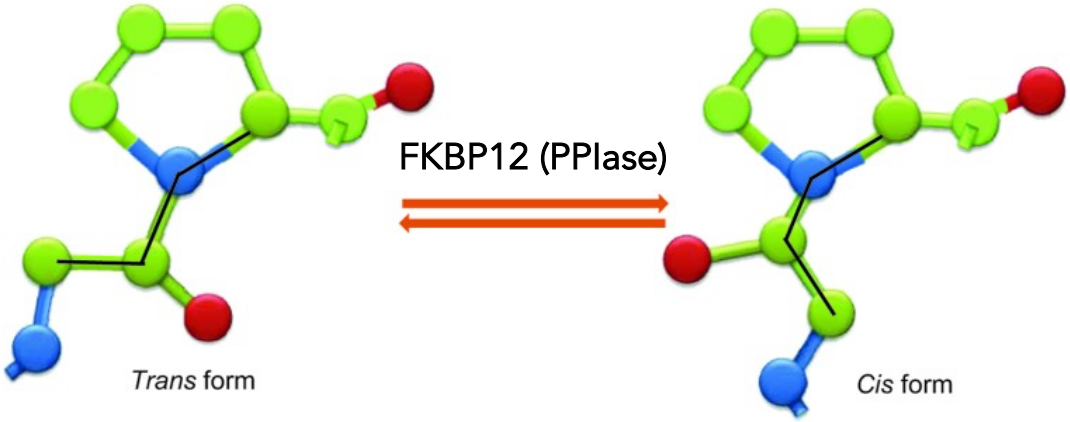
anti-immune effects
anti-tumor effects

FKBP12 is a peptidyl-prolyl isomerase enzyme

'PPIase'

Proline is
highly constrained
-

Side chain is bonded
to its secondary amine
Nitrogen



80%
preferred due to steric hindrance

20%

7.05 and 5.07!!!
20.320!!

FKBP12 is a peptidyl-prolyl isomerase enzyme

'PPIase'

Proline is
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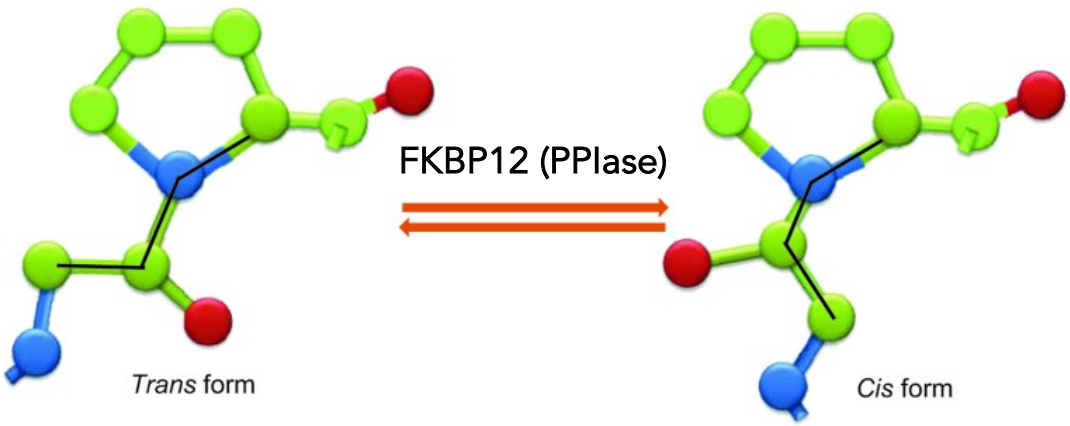

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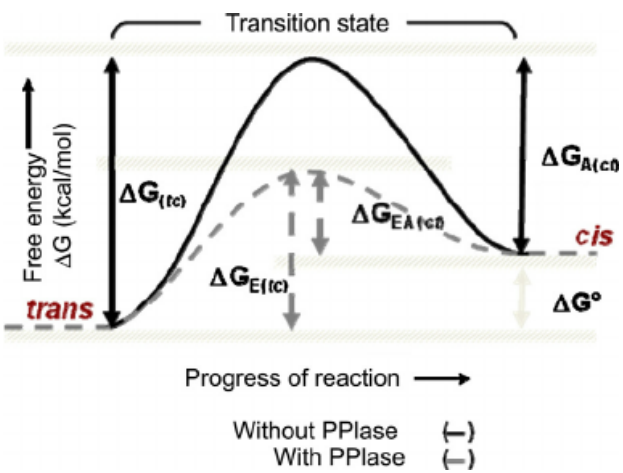
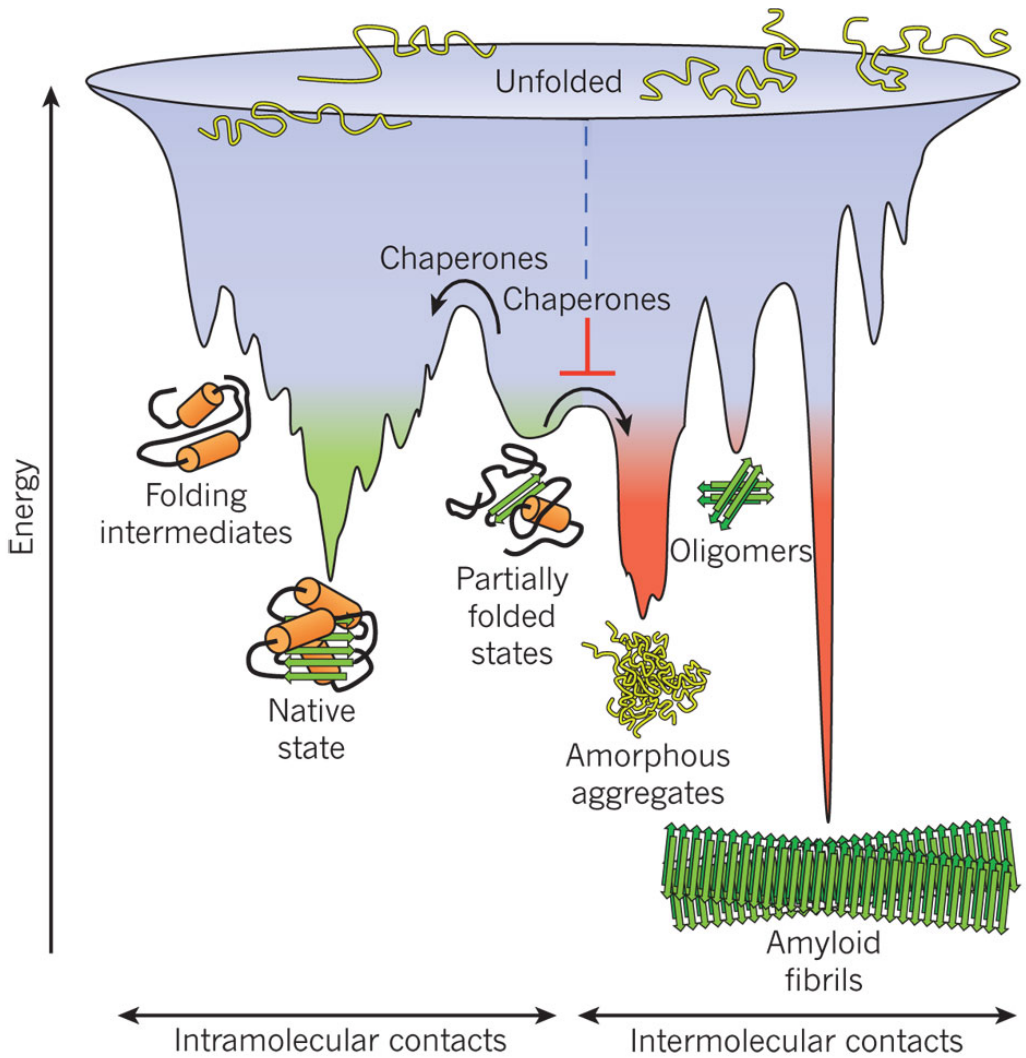


overall free energy difference between cis/trans is small but…

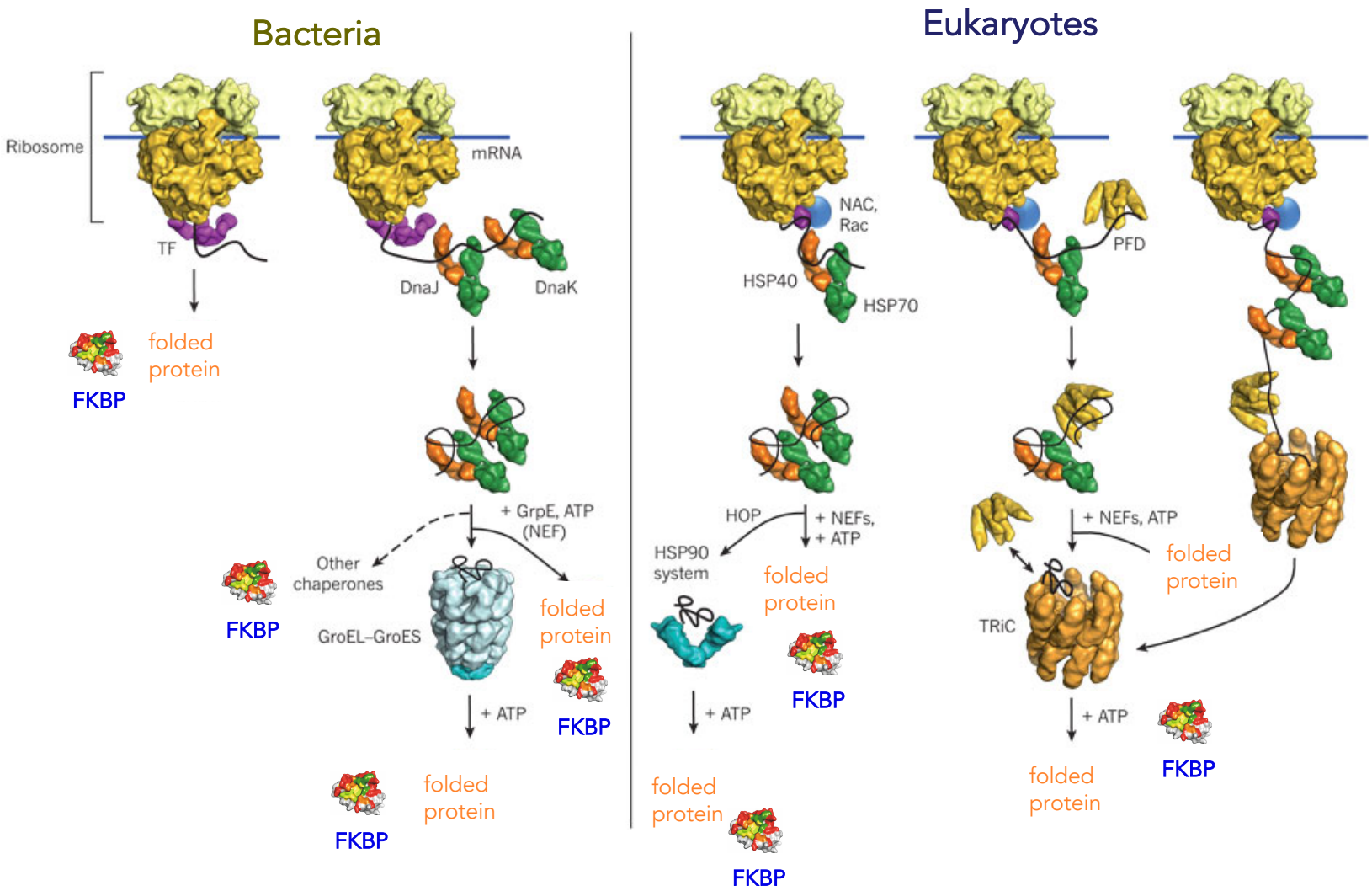
E_a ~ 20 kcal/mol **HIGH!!**
(v. 0.1 kcal/mol for typical pep bond)
rate limiting!!

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

FKBP12 is a molecular chaperone: enabler of proper protein folding

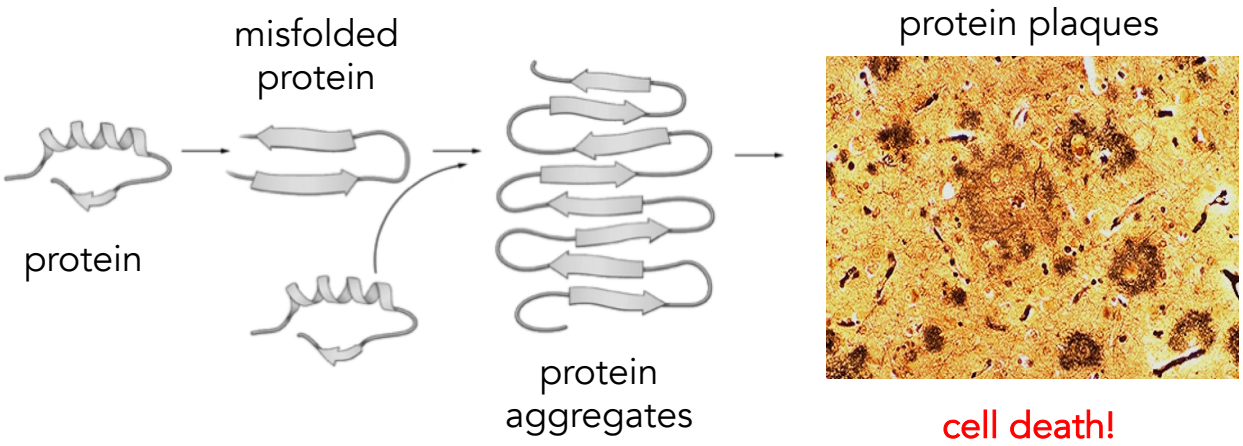


FKBPs are ‘downstream’ chaperones

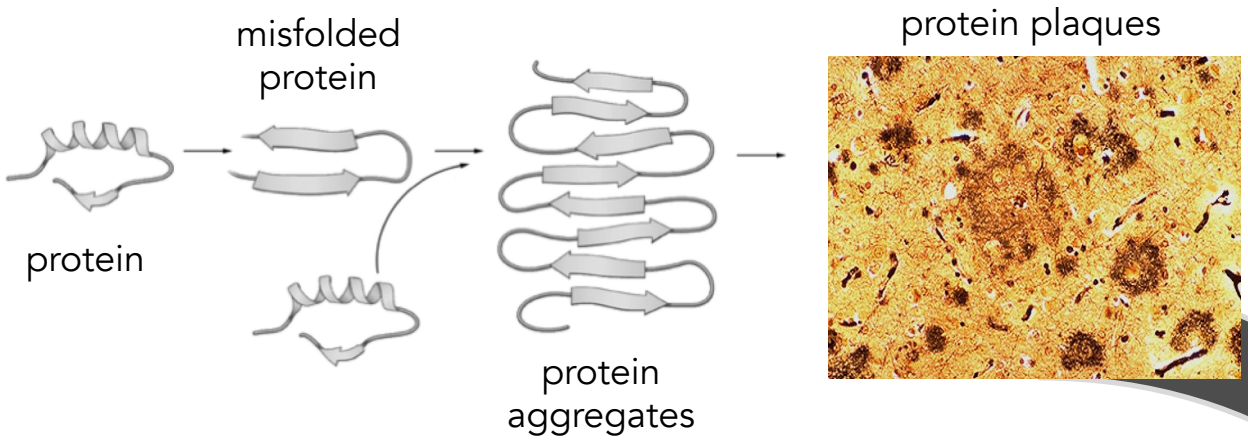


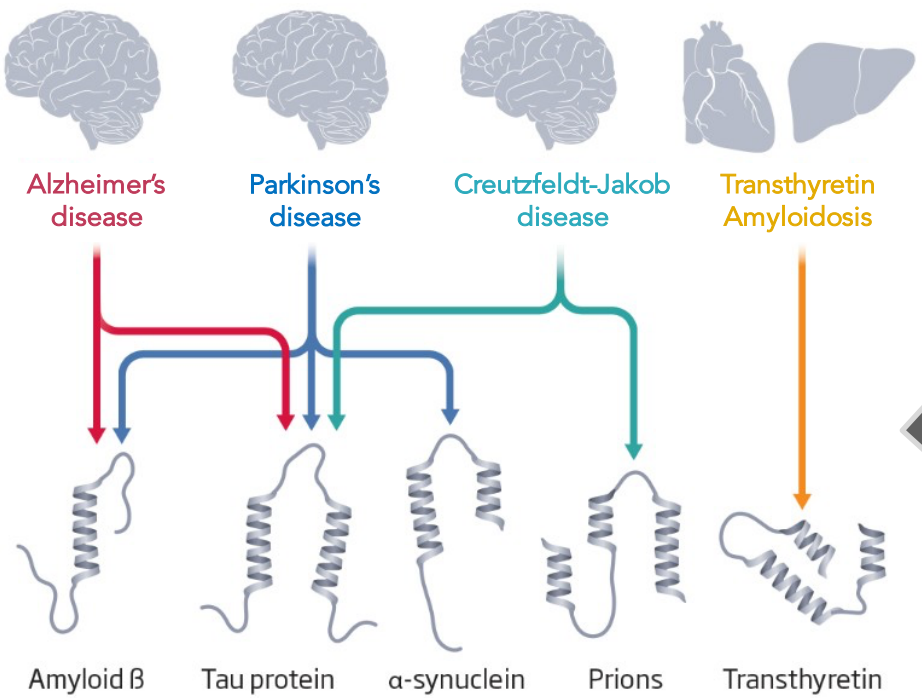
acts at the final stages of protein folding

FKBPs in diseases of protein folding



FKBPs in diseases of protein folding

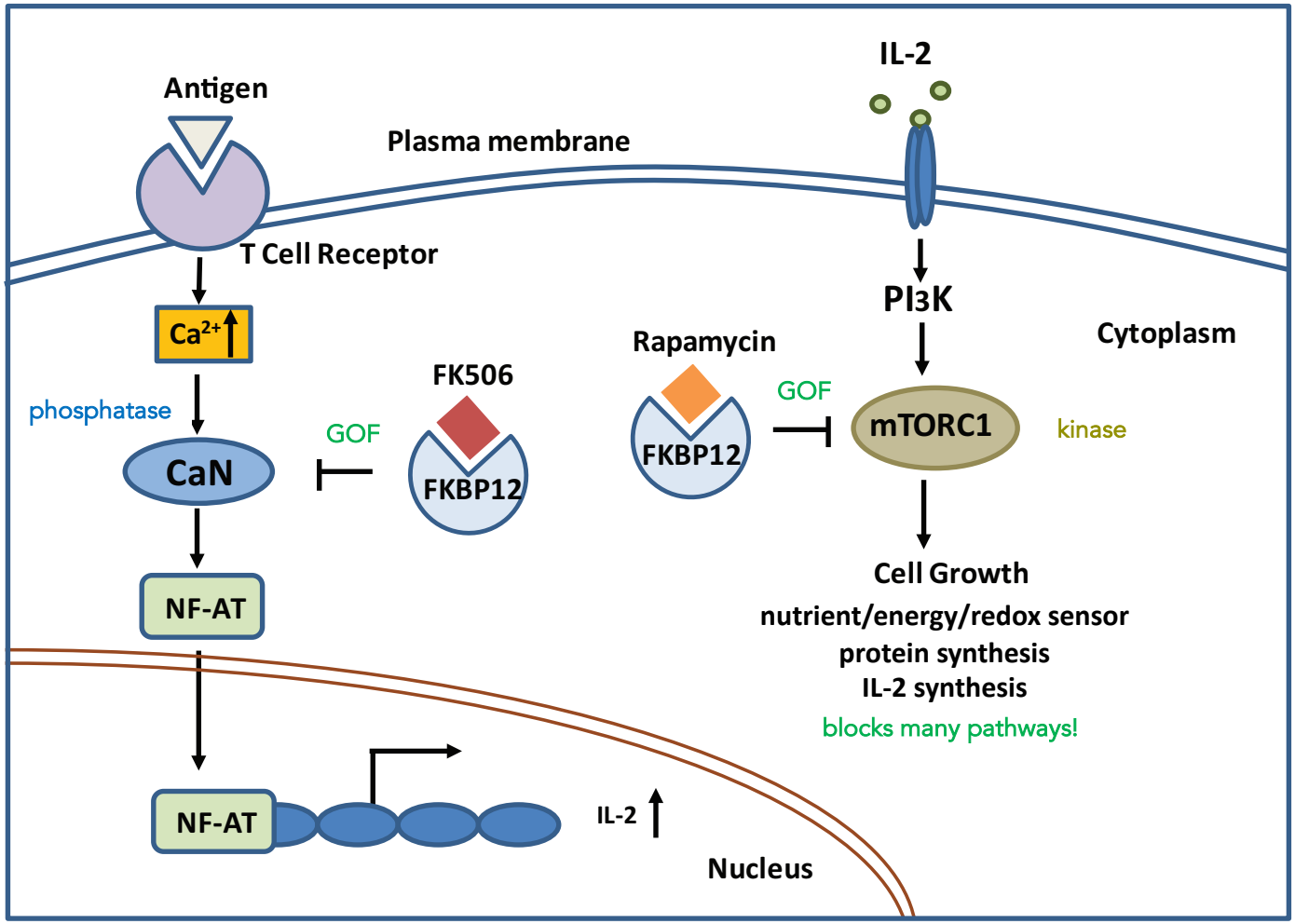




higher FKBP52
=
lower Tau aggregation

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

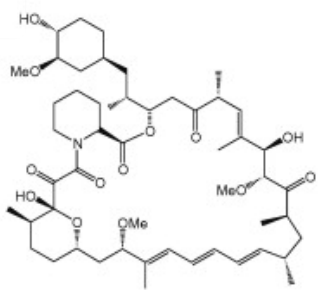
immunosuppressive activities are unrelated to PPIase activity



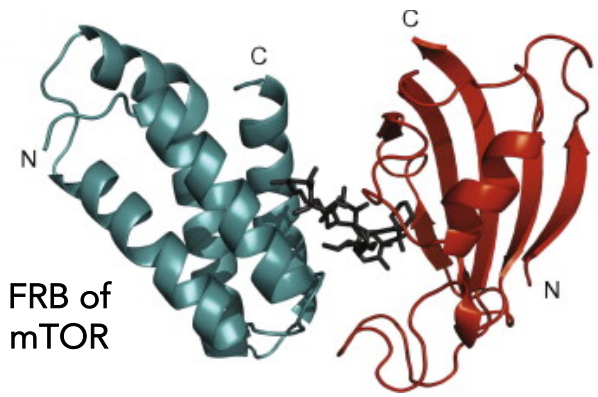
blocks IL-2 production!

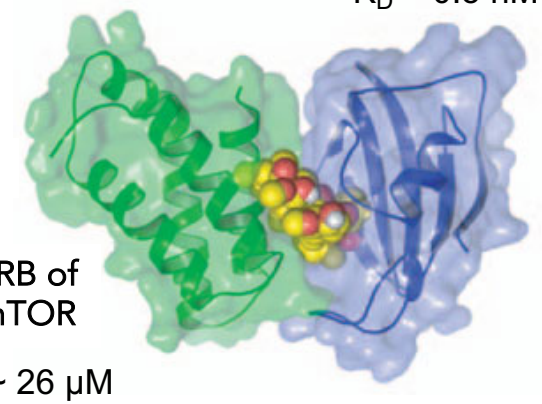
FKBP12 'ternary' complexes

Rapamycin and mTOR



rapamycin





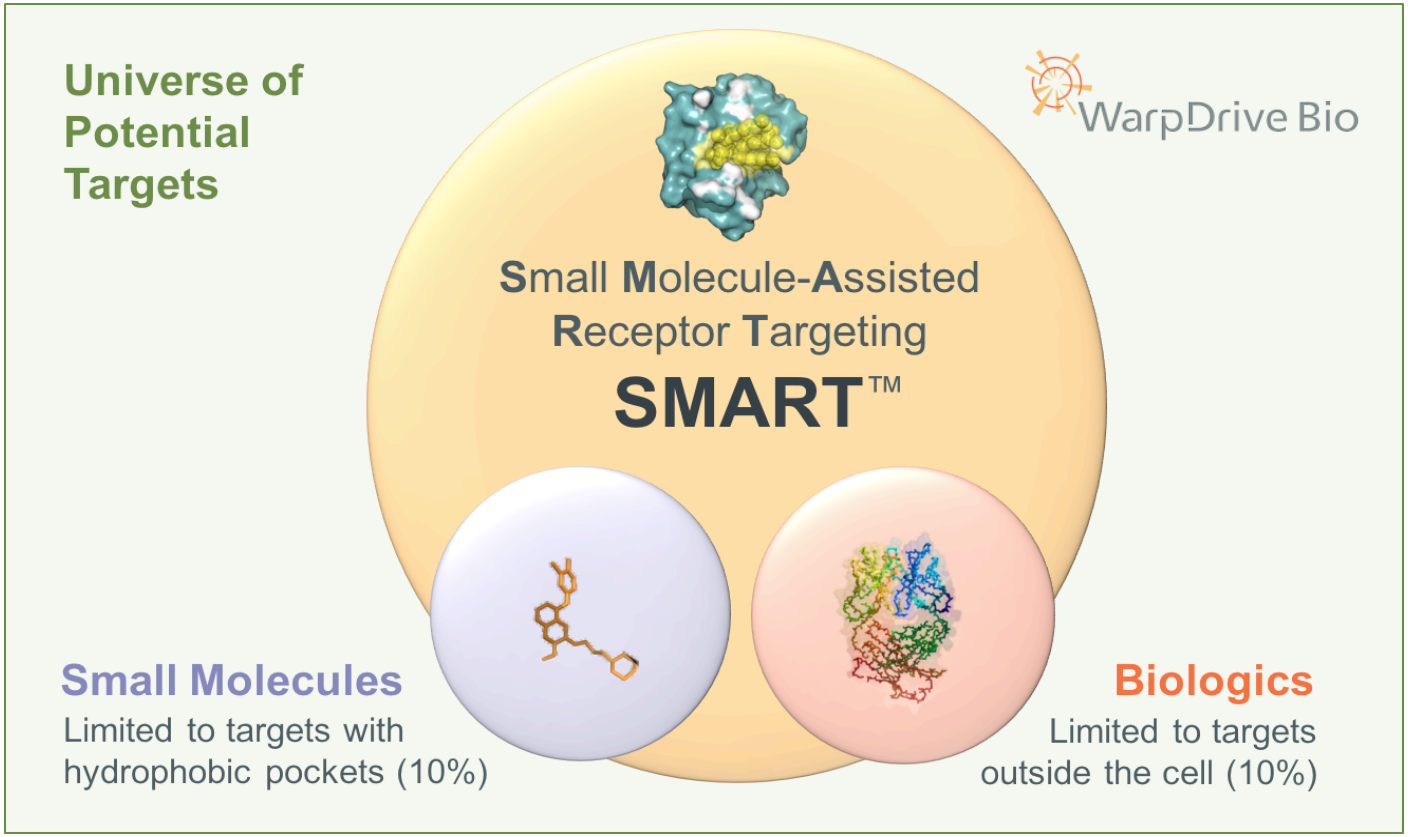
K_D ~ 26 μM

K_D ~ 12 nM vs. >50 μM

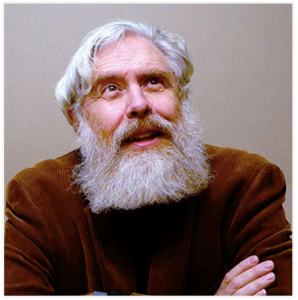
Choi *et al*. Science, 273, 239-42 (1996)
Banaszynski, Liu, and Wandless, J. Am. Chem Soc., 127, 4815-21 (2005)

PDB entry:
1NSG

Drugging the ‘undruggable’ through gain of function



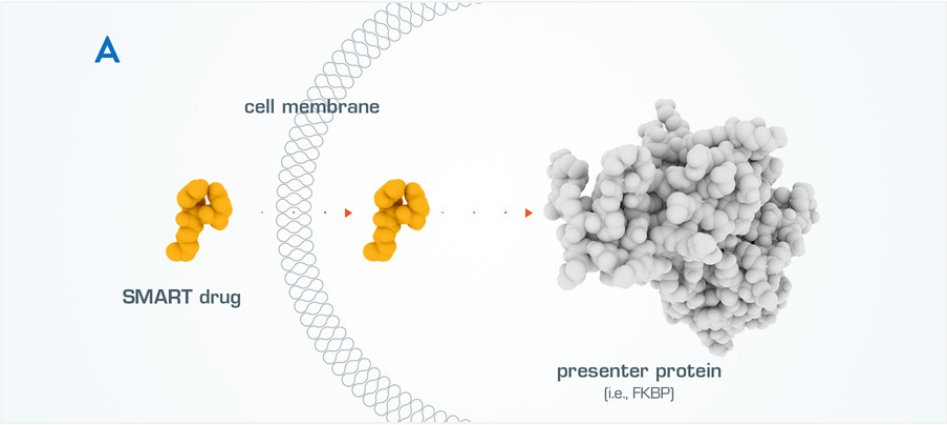

Greg Verdine, Harvard


George Church, Harvard


Jim Wells, UCSF

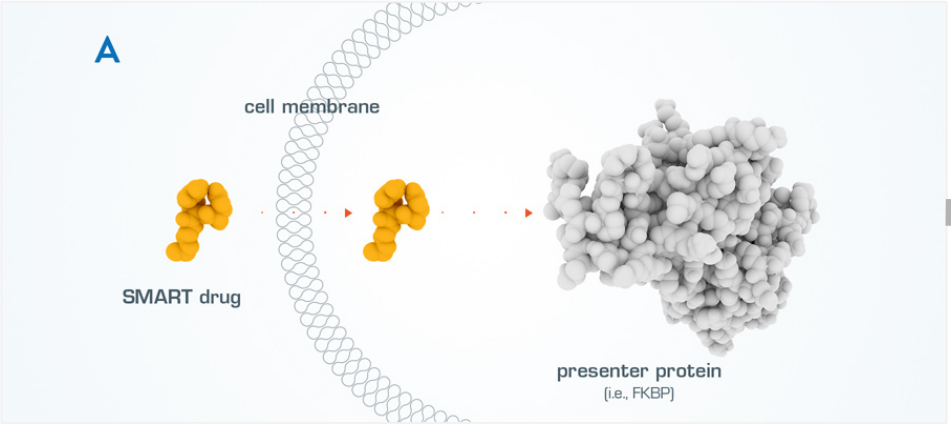
REVOLUTION MEDICINES

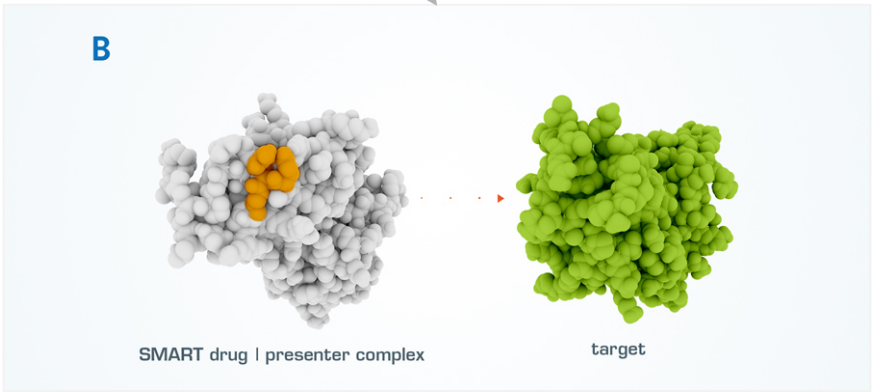
SMART™ – small molecule-assisted receptor targeting



REVOLUTION MEDICINES

SMART™ – small molecule-assisted receptor targeting





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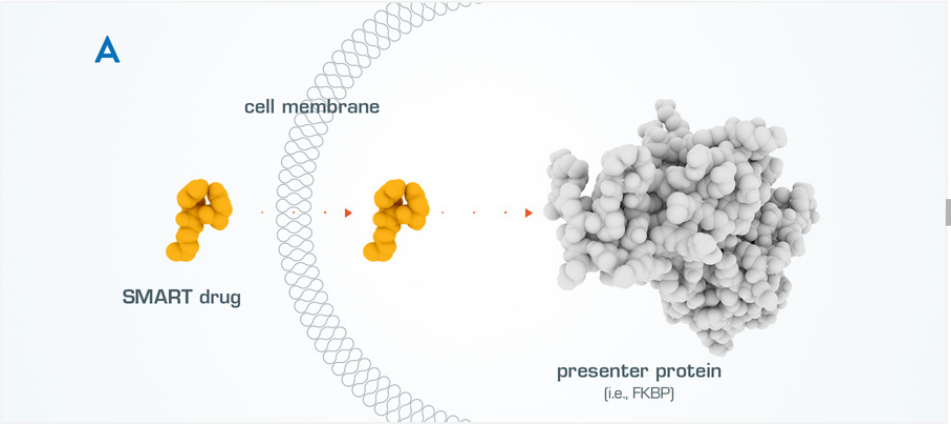

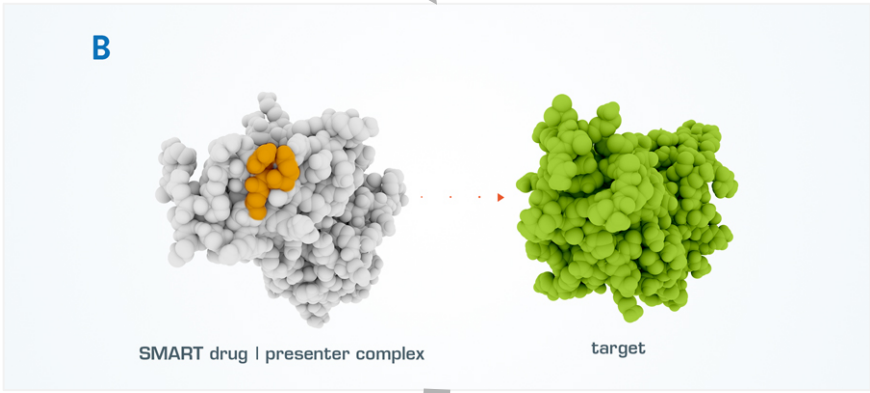

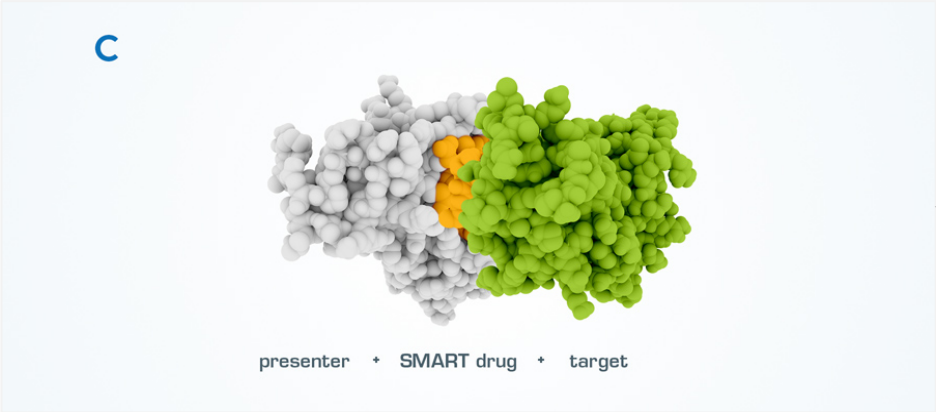

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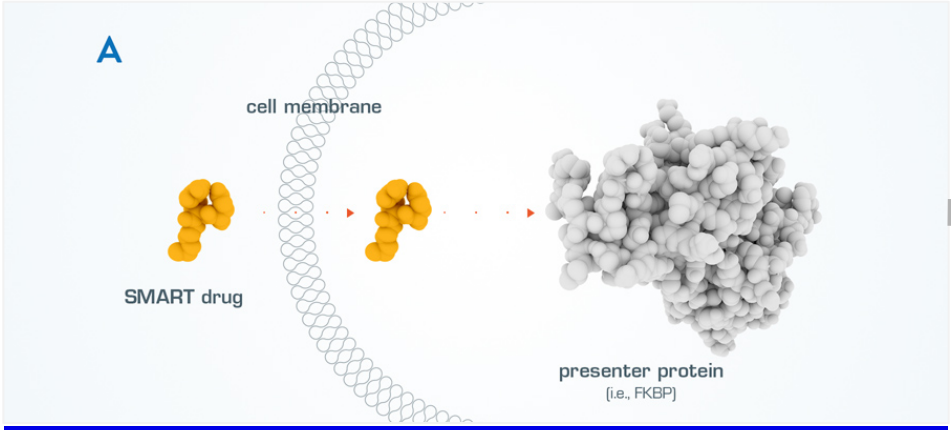
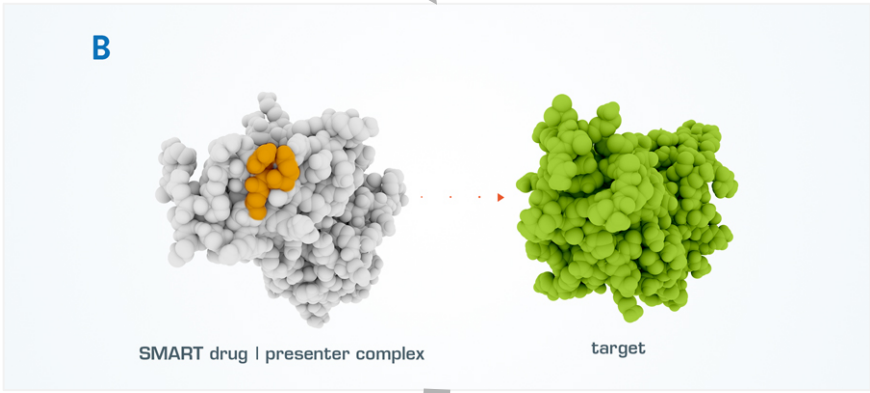
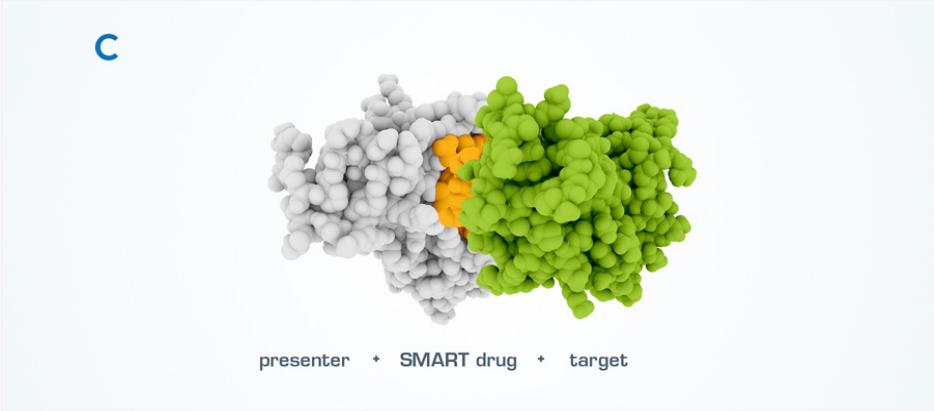


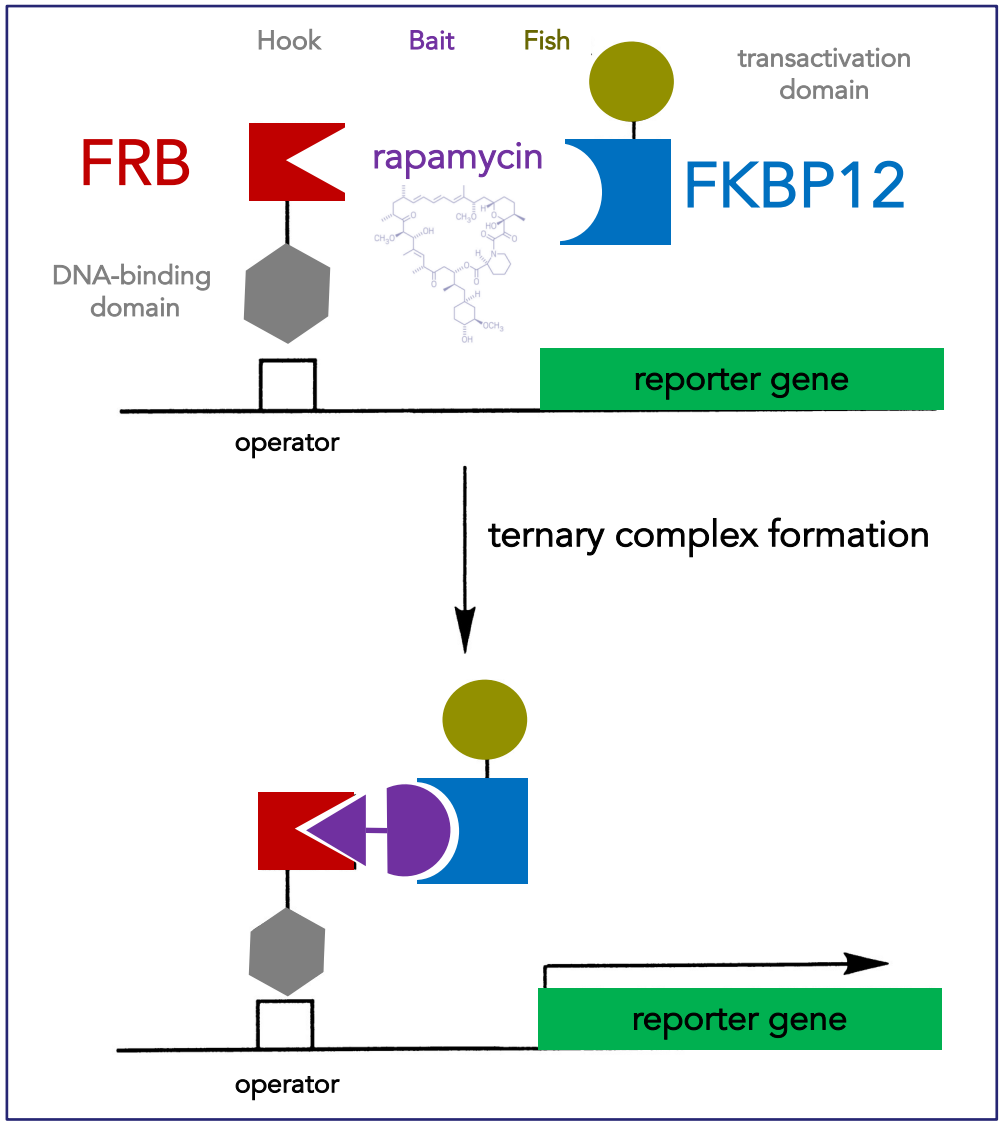
Novel molecules that you find may serve as new starting points for this concept, providing new molecular interfaces with FKBP12 that can be used to engage a new proteins through design or screening





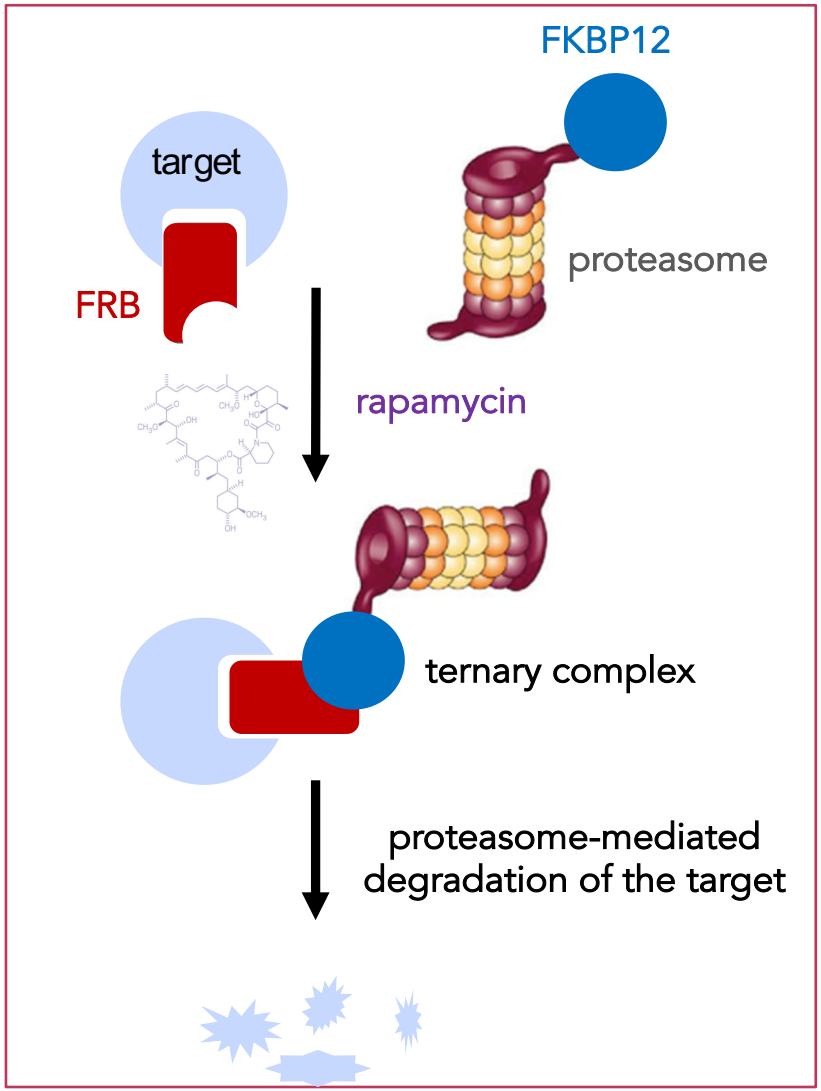
REVOLUTION MEDICINES

FKBP12 as a tool for biological engineering



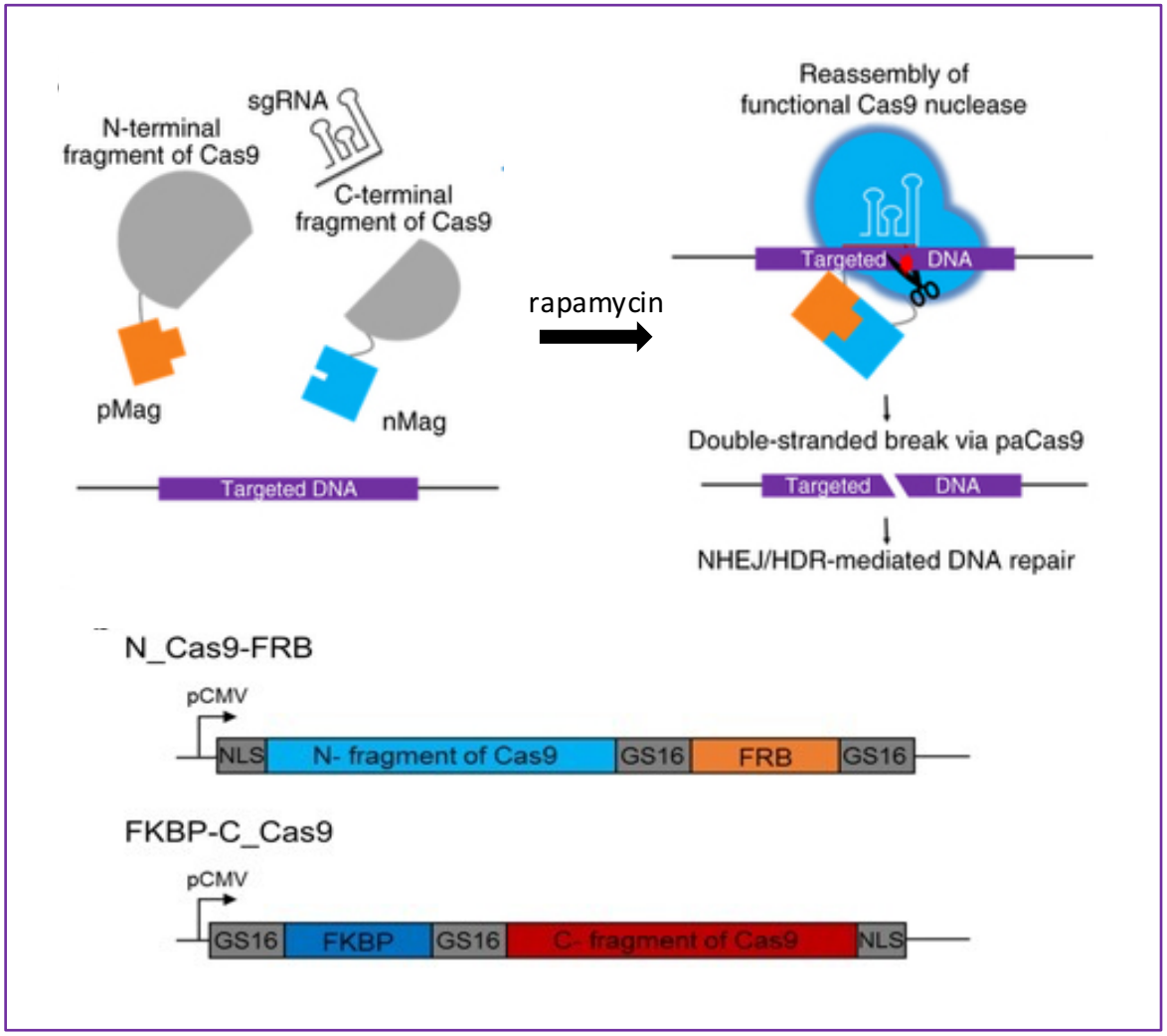
controlling transcription

FKBP12 as a tool for biological engineering



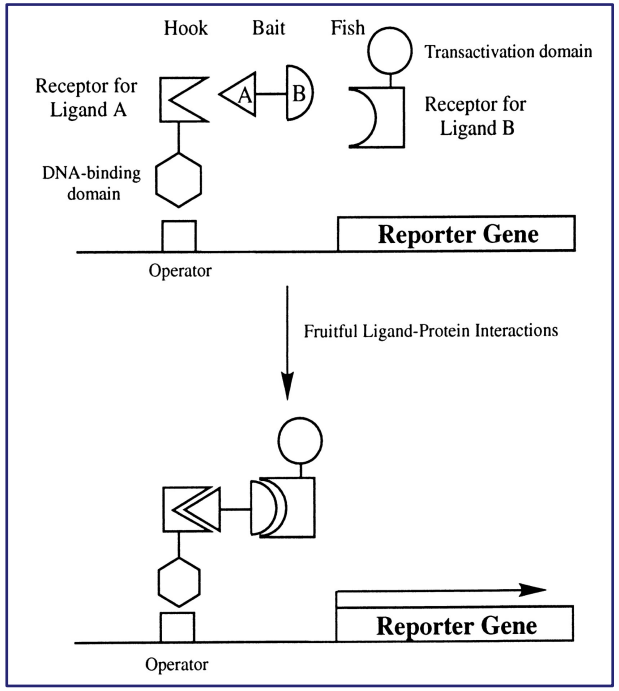
inducing protein degradation

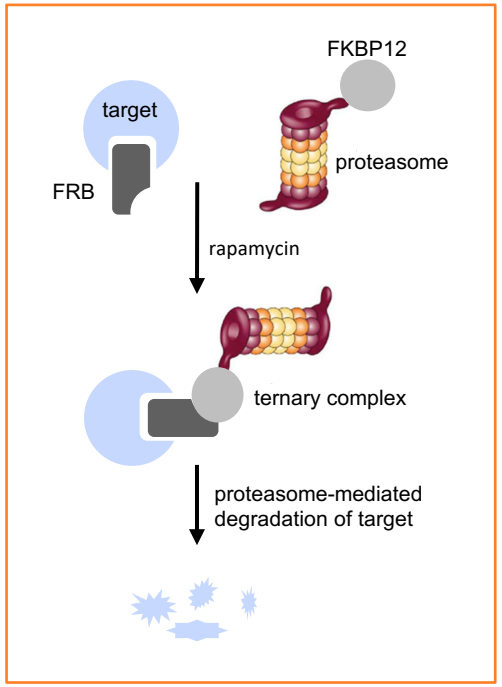
FKBP12 as a tool for biological engineering

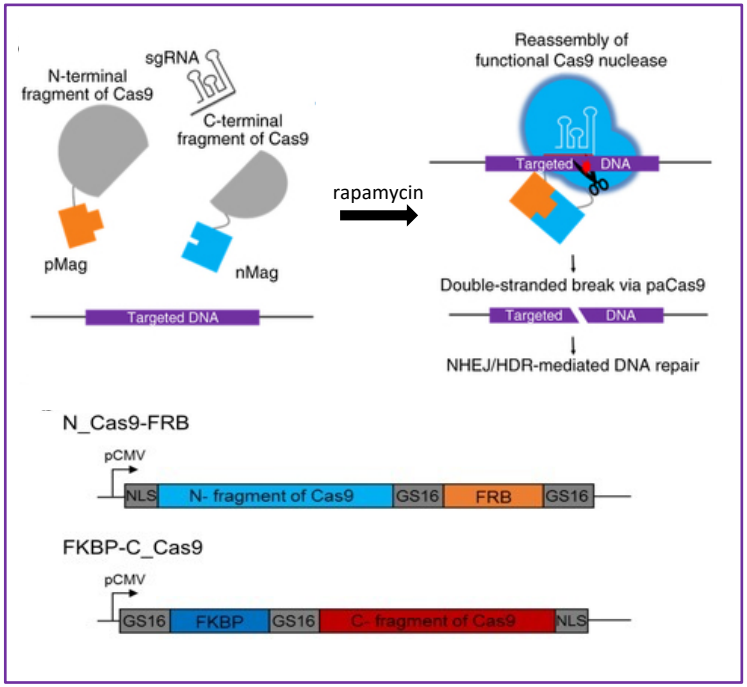


induced genome editing

FKBP12 as a tool for biological engineering







controlling transcription

inducing protein degradation

induced genome editing

proximity induction strategies