

# Protein engineering

Změna primární struktury proteinu s cílem získat nový protein s požadovanými vlastnostmi

Zvýšení odolnosti proteinů

teplota

pH

osmotický tlak

Zesílení interakce

terapeutické protilátky

Změna specificity interakci

fosfospecifické protilátky

Vytváření nových vlastností

fluorescenční proteiny

změna isotypu protilátek

Změna enzymatické aktivity

biodegradace

Fúzní proteiny

Editace genomu

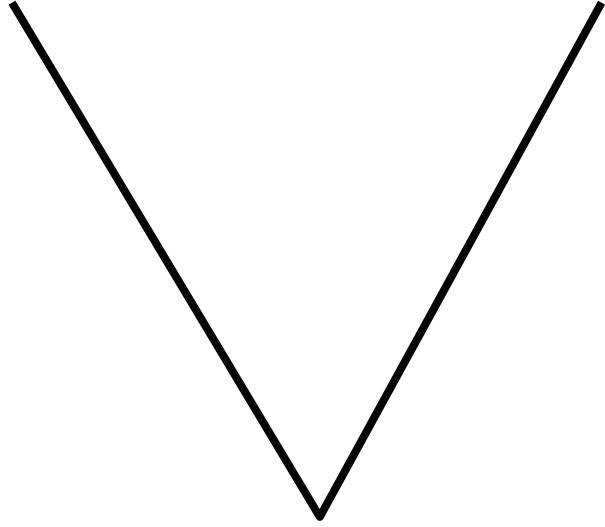
specifické nukleázy

**cílená změna  
design na základě znalosti struktury a vlastností**

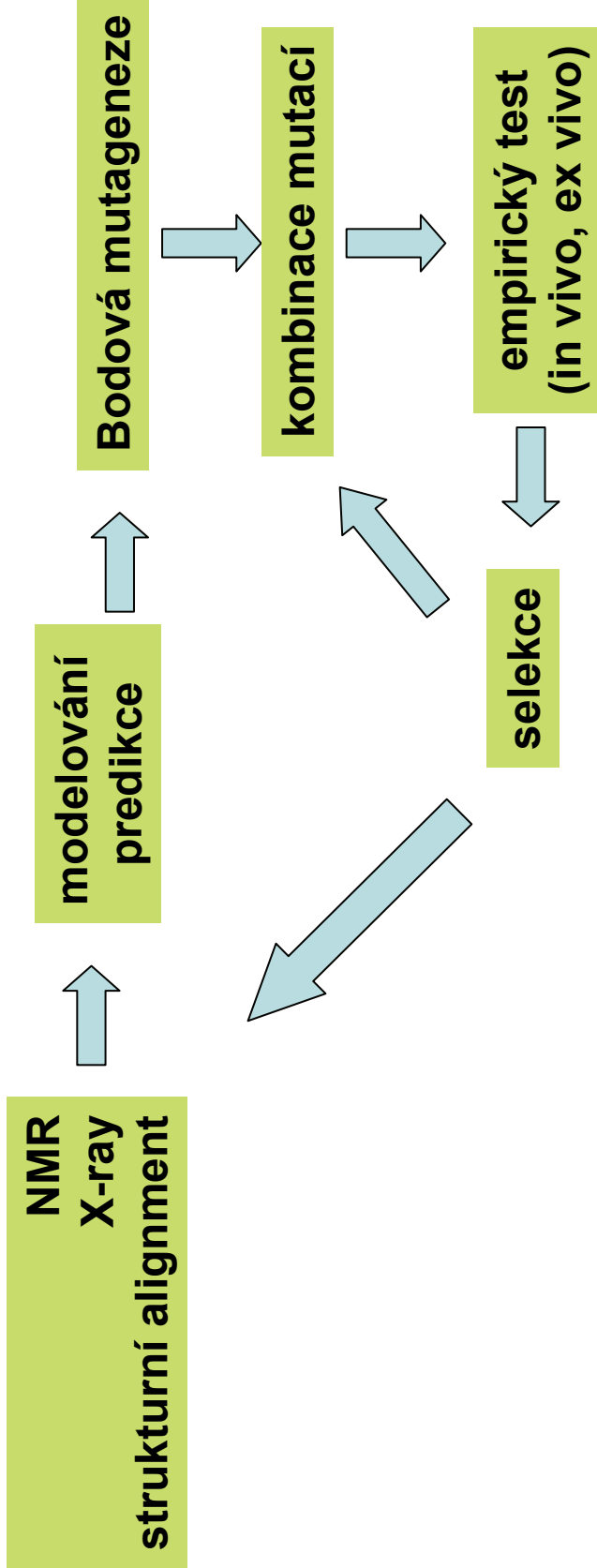


**mnohastupňový proces  
kombinace v obou přístupů**

**náhodná změna  
in vitro evoluce**



# Cílená změna





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# Enhancing the stability and solubility of TEV protease using in silico design

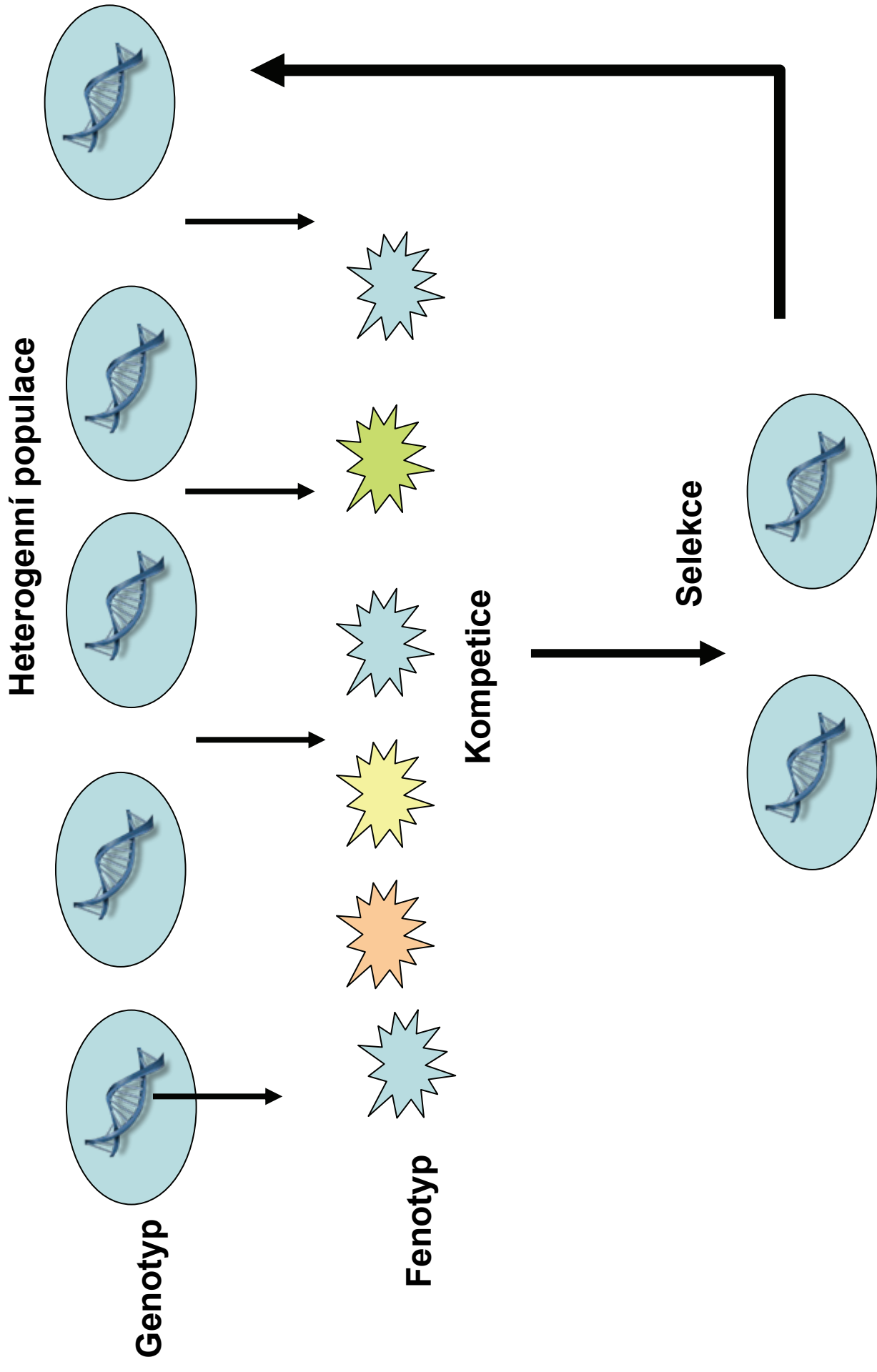
LISA D. CARRITA,<sup>1,3</sup> DIMITRI GILIS,<sup>2,3</sup> AMY L. ROBERTSON,<sup>1</sup> YVES DEHOUCK,<sup>2</sup> MARJANNE ROOMAN,<sup>2</sup> AND STEPHEN P. BOTTOMLEY<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton 3800, Australia

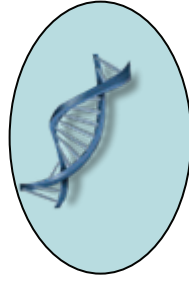
<sup>2</sup>Genomic and Structural Bioinformatics, CP 163961, Free University of Brussels, 1050 Brussels, Belgium

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# In vitro evolve



## Podmínky nutné pro in vitro evoluci:



- kompartmentace genotypu a fenotypu
- heterogenita genotypu-mutace
- schopnost amplifikace

## Design in vitro evoluce:

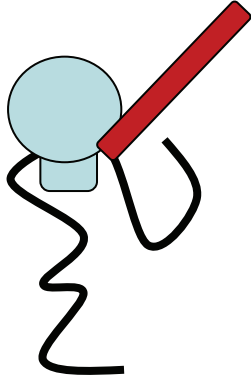
požadavky na fenotyp



způsob selekce

# Způsoby kompartmentace genotypu a fenotypu

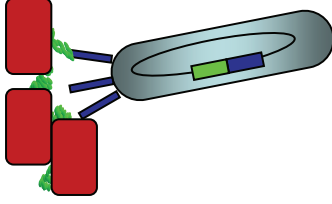
Ribosomal display



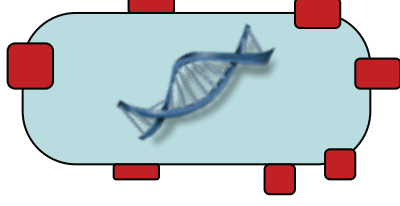
Emulsní systémy



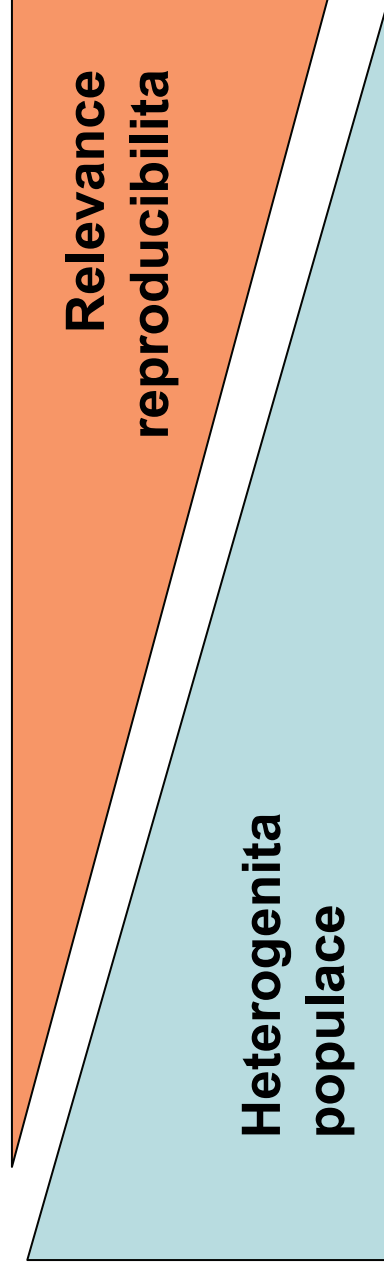
Phage display



Expresie na povrchu bakterií

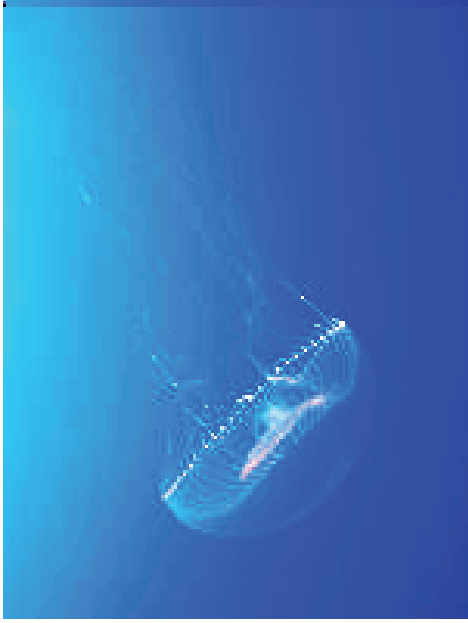


In vitro transkripce translace

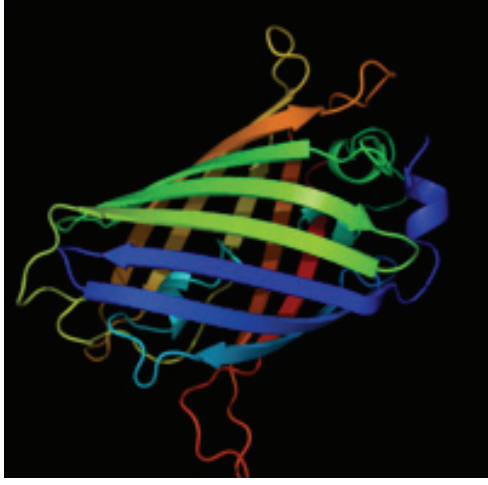




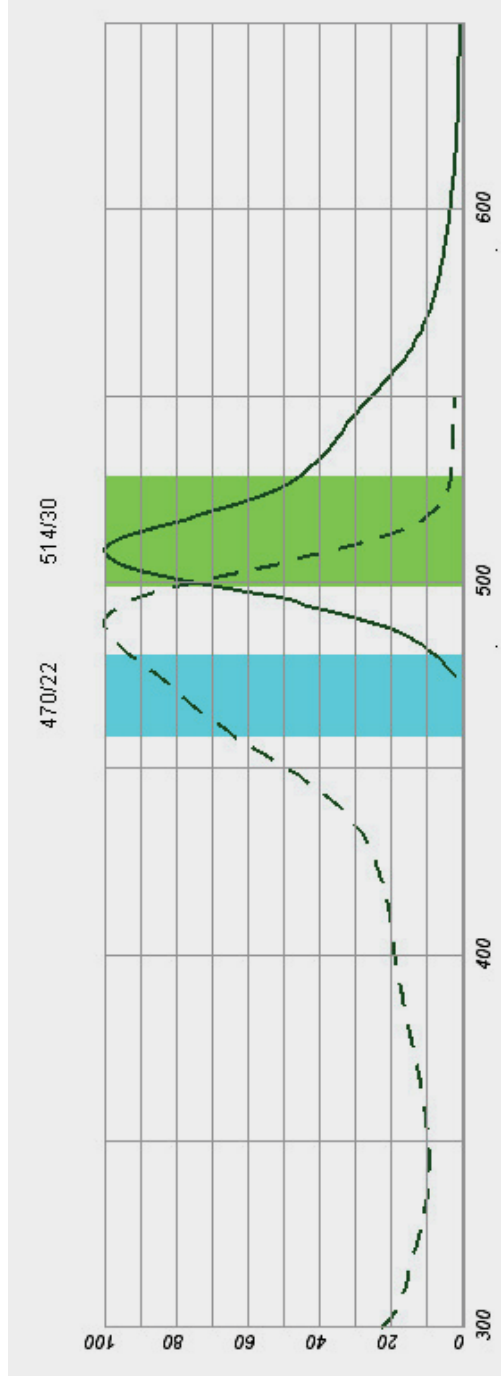
# GFP Green Fluorescent protein



*Aequorea victoria*

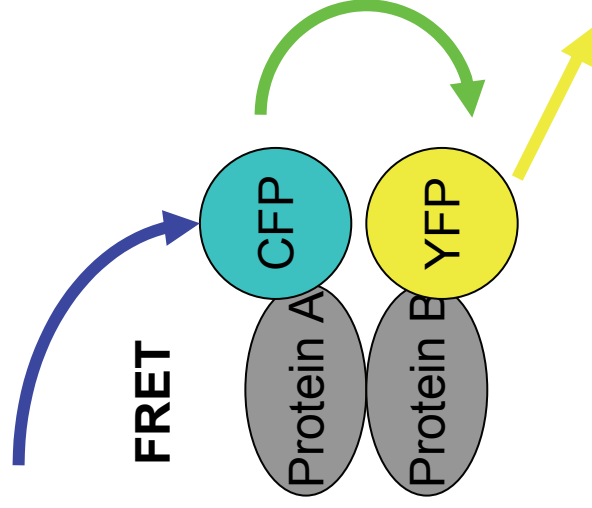


GFP structure

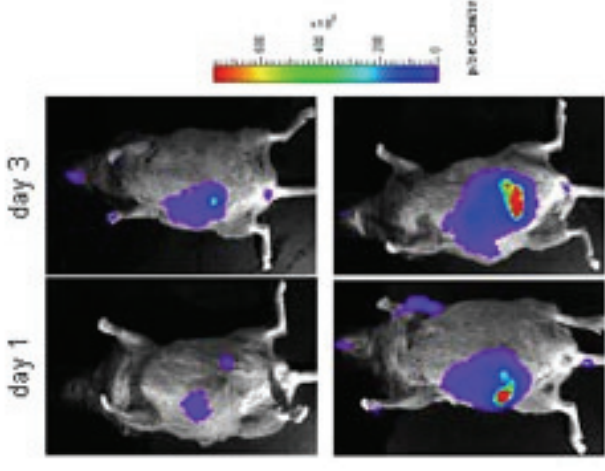


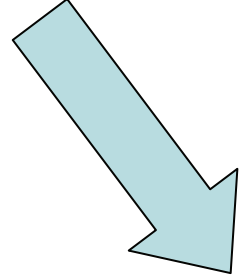
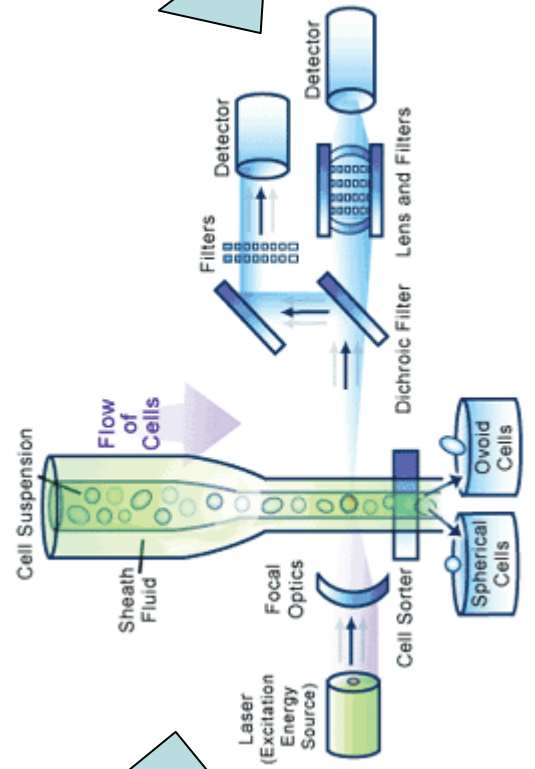
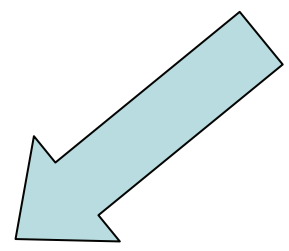
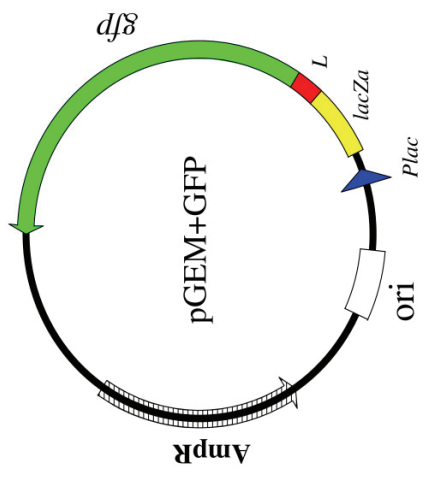
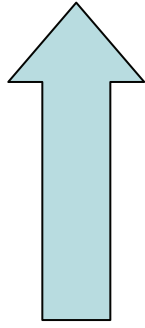
## Cíl změny GFP

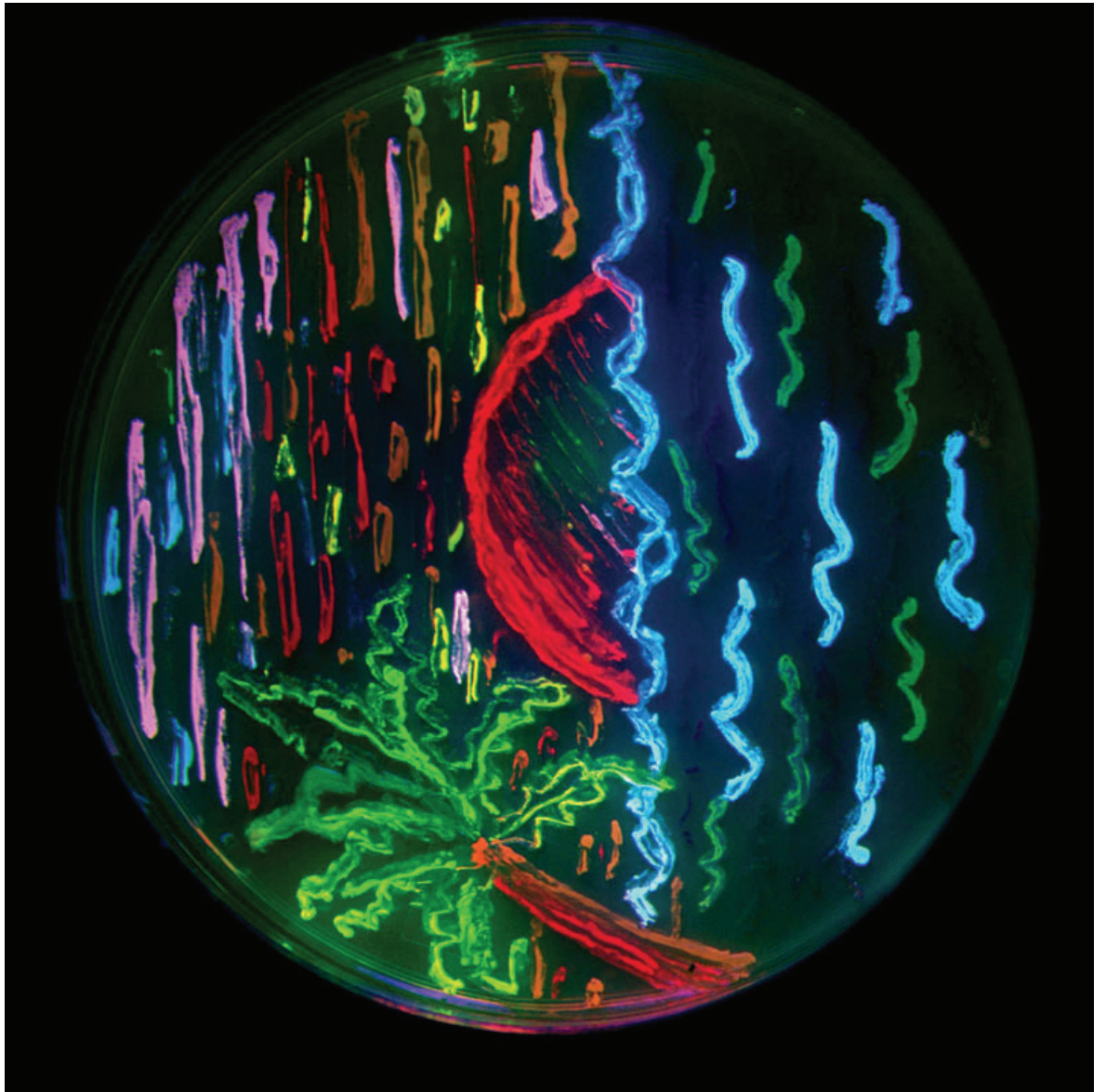
- Vyšší intenzita
- Změna vlnové délky
- Možnost fúze s ostatními proteiny
- Krátký nebo dlouhý poločas rozpadu
- Rozpustnost
- Monomer



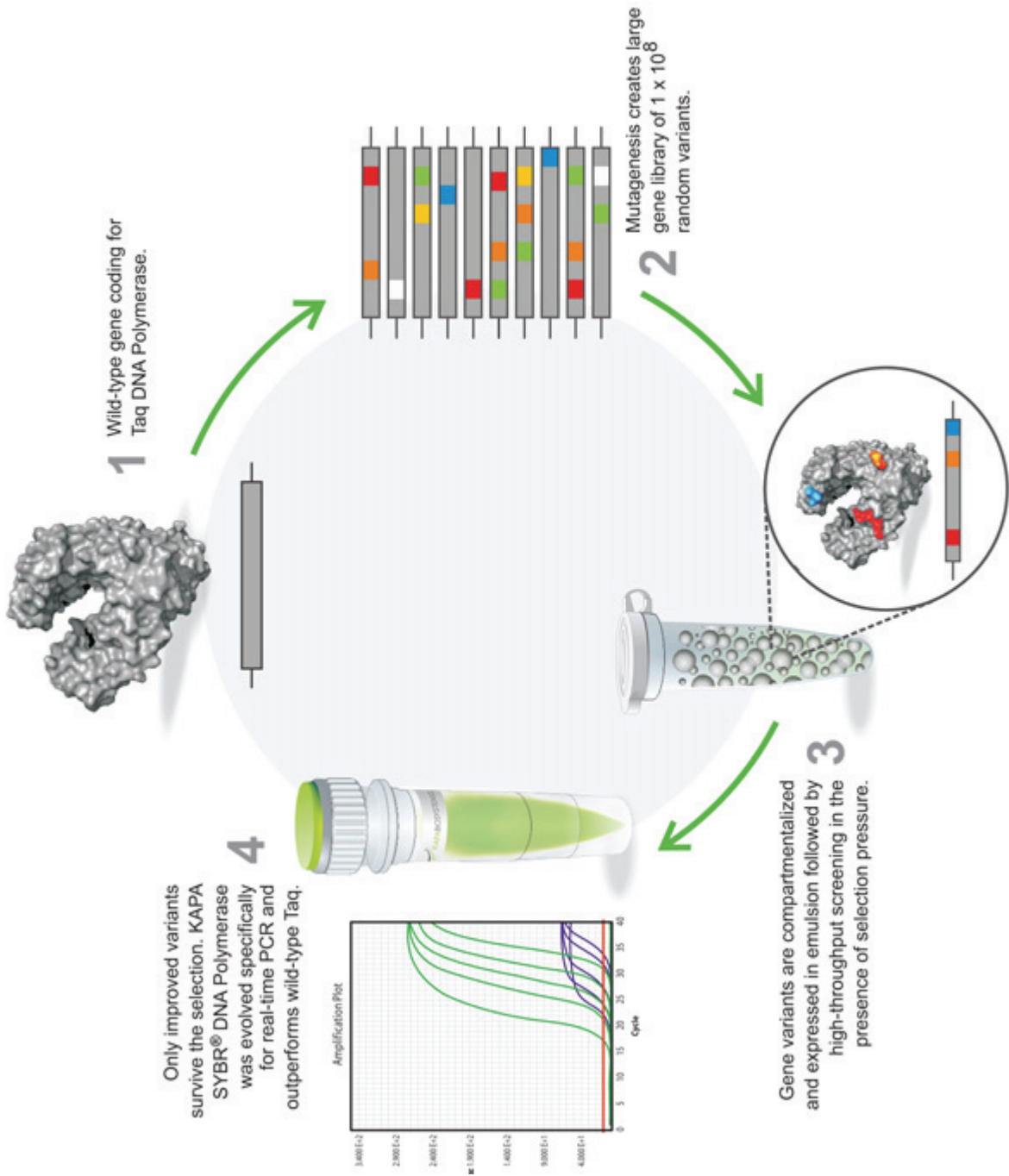
Fluorescence Resonance Energy Transfer





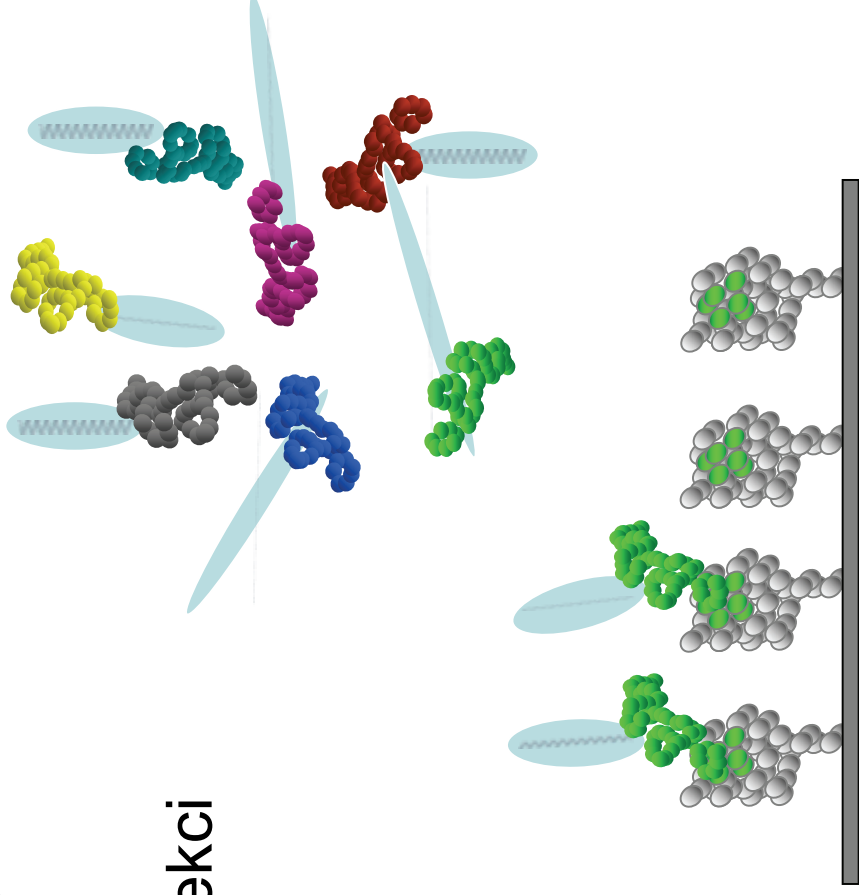


# Evolve PCR enzymü

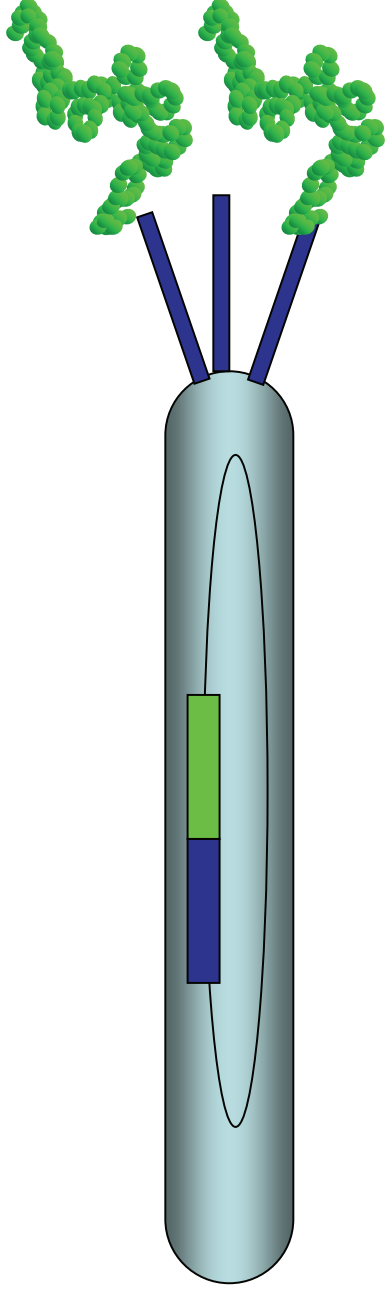


# Phage display

- Knihovny peptidů po produkci si zachovávají kódující sekvence
- Je nutné provést selekci na vašem cílovém proteinu



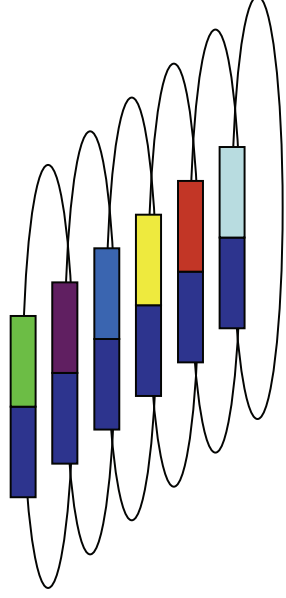
# „Phage display“



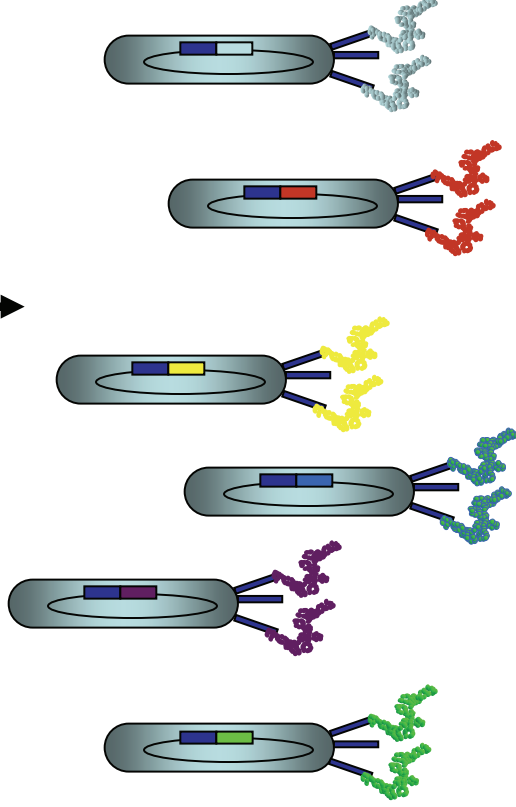
- Expresse peptidů na povrchu
- Obvykle se k expresi využívá gen 111
- 5 kopií

# Konstrukce peptidových knihoven

## knihoven



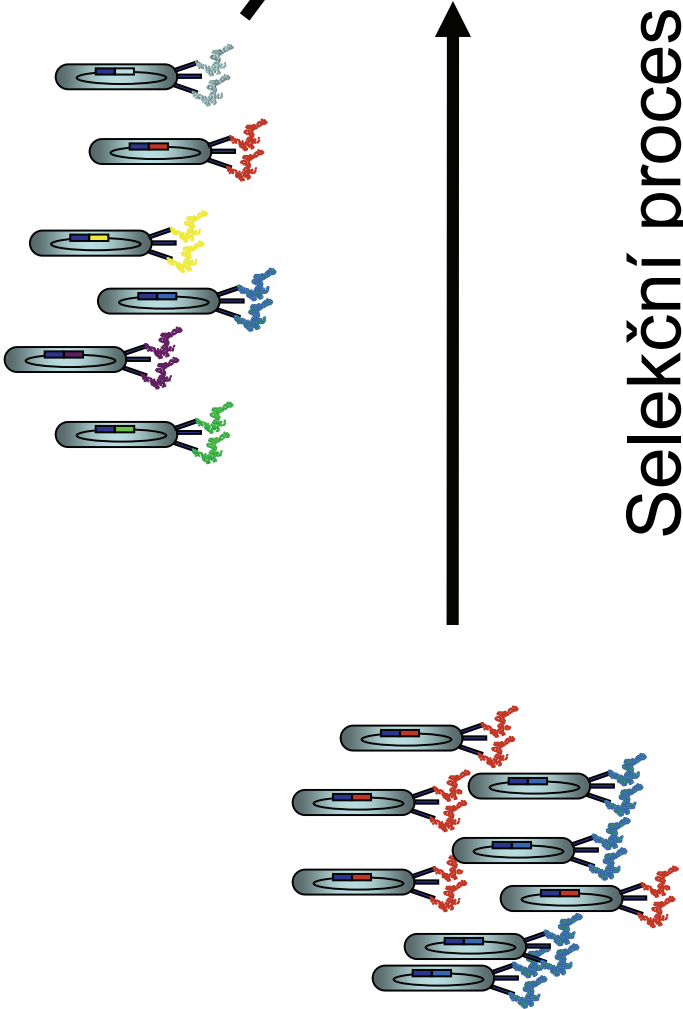
Transfekce do  
bakterií a produkce  
fágů



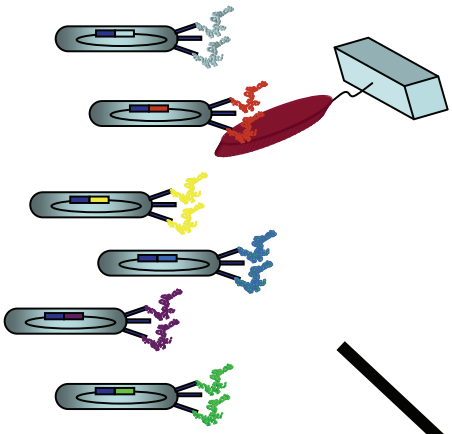
Knihovny mají  
velikost až do  
 $10^9$



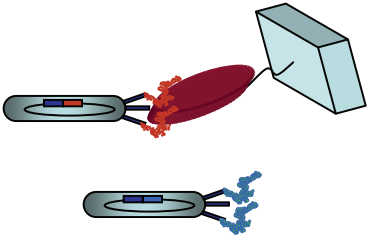
# Selekční proces



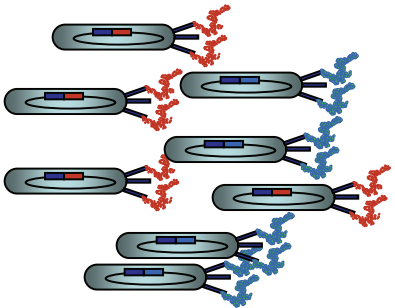
Aplikuje se knihovna na cílový protein



Odmýjí se všechny nenavázané fágy



Provede se eluce navázaných fágů





# Příklad

- Mapování anti-p53 protilátky PAb 240

# Selekce PAb 240 vazebného peptidu z náhodné “phage display” peptidové knihovny

“p53 sequence“

YLDDRNTFRHSVVPYEPP

RHSVIS

FRHSIL

WRHSIV

FKHSIV

LRHSIL

YRHSVI

LRHSVI

YSRHSVYEDGQ

TLRHSIIFGEW

AVRHSVIERTLS

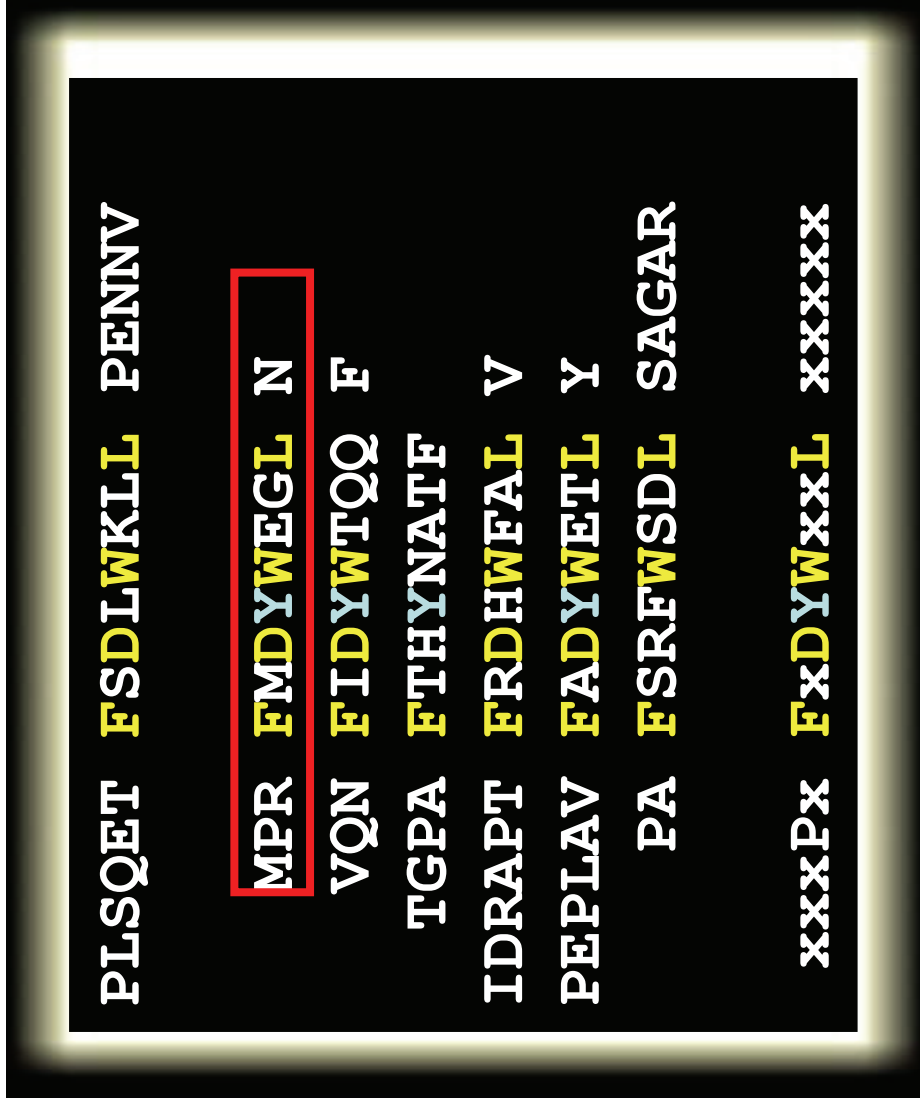
“Phage library  
sequences“

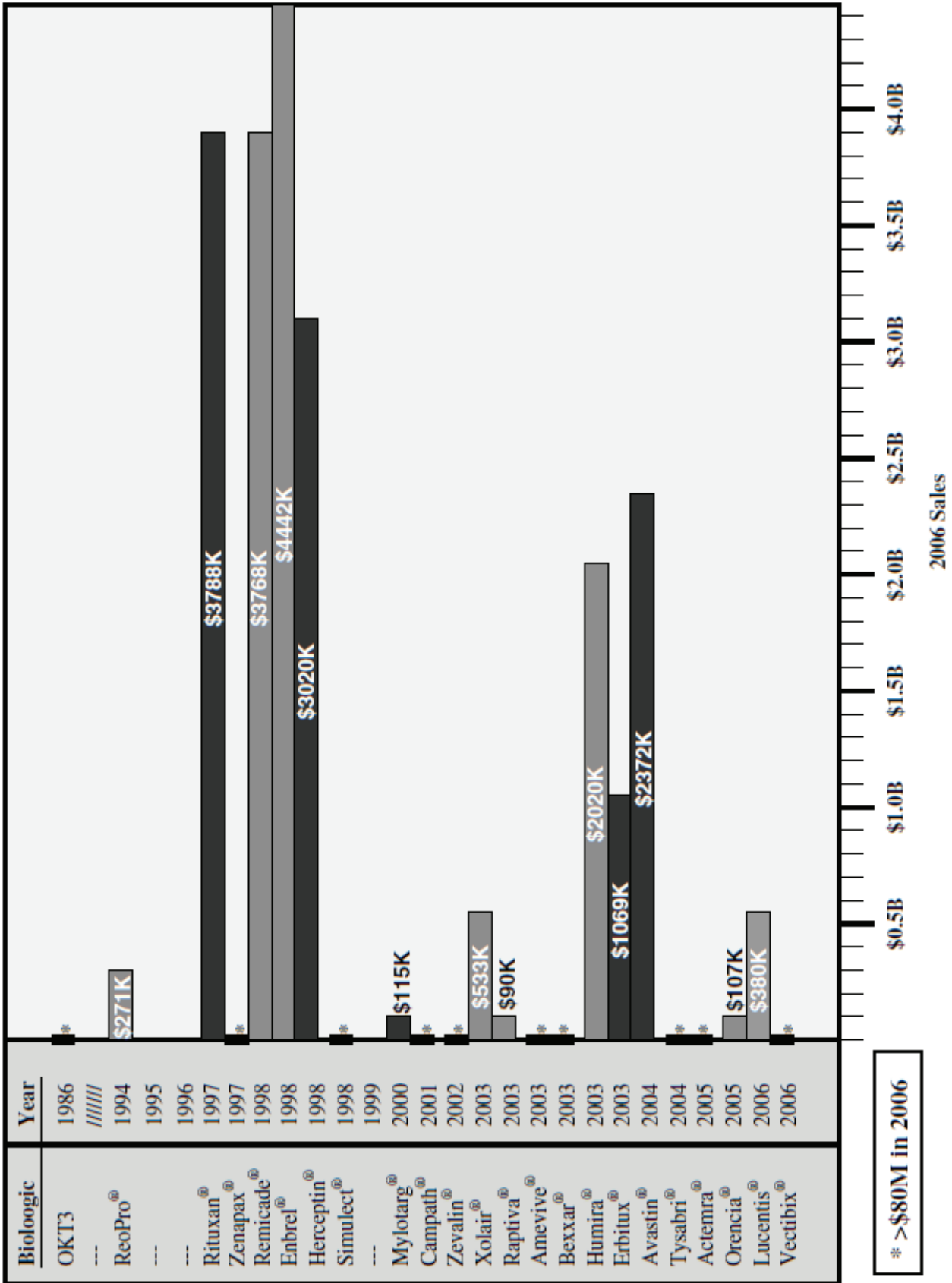
# Selekcce mdm2 vazebných peptidů z náhodné “phage display” peptidové knihovny

“p53 sequence”

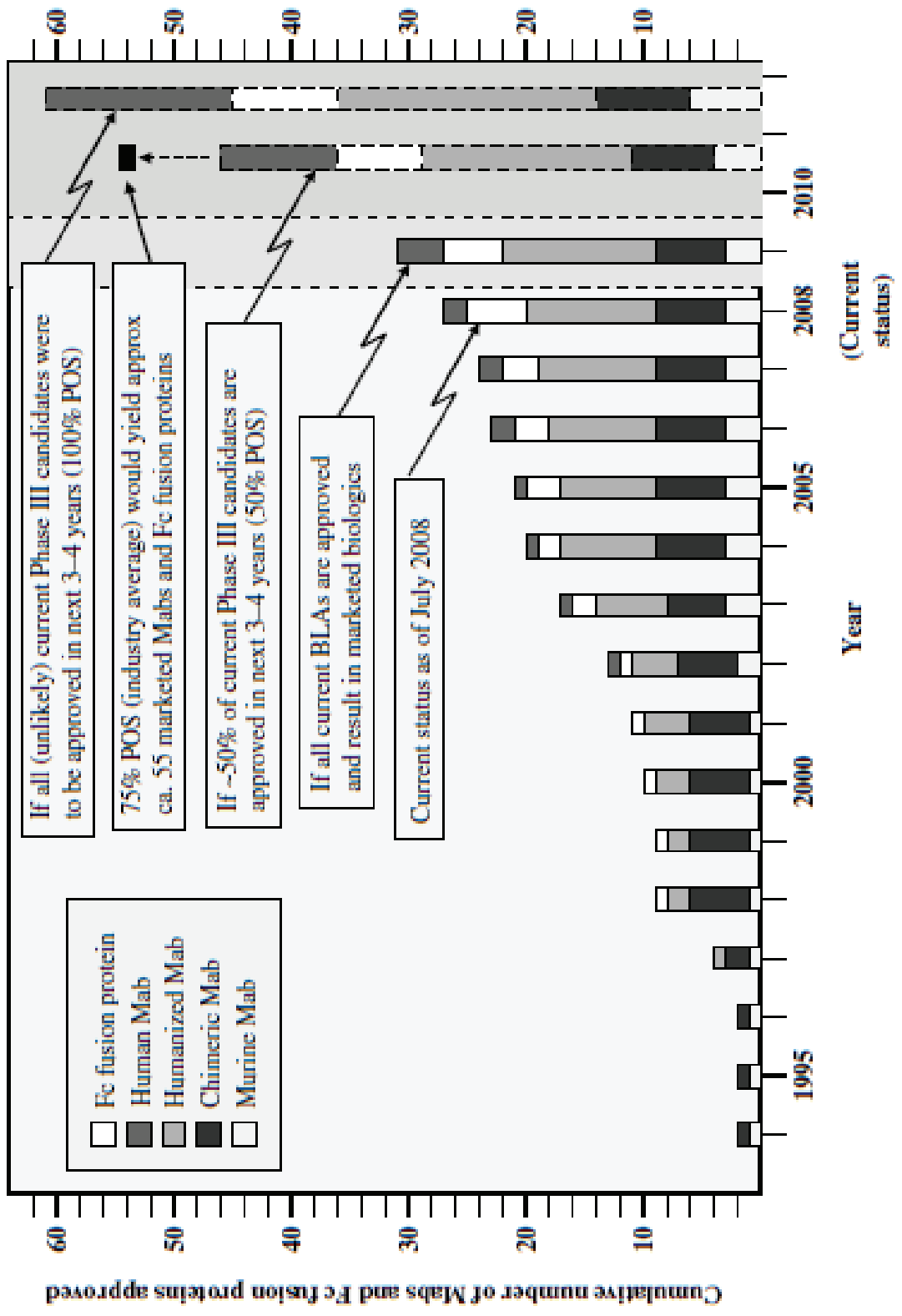
“Phage library  
sequences”

Shoda





\* >\$80M in 2006



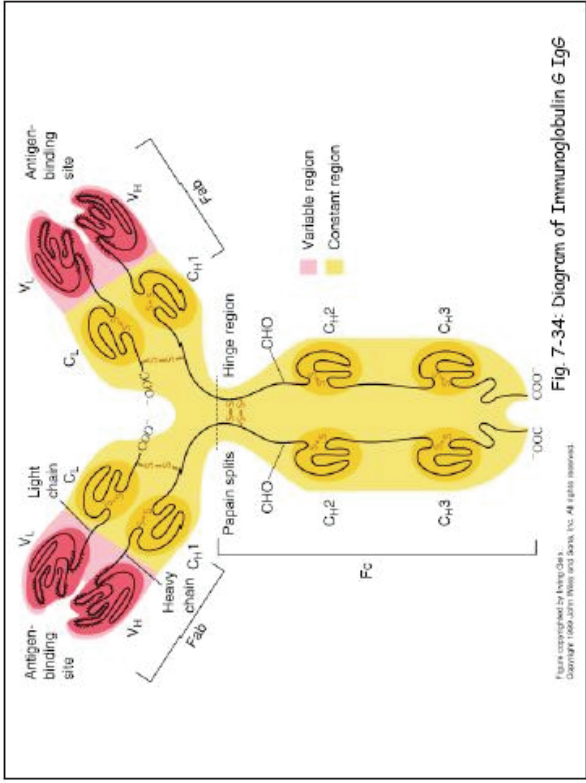
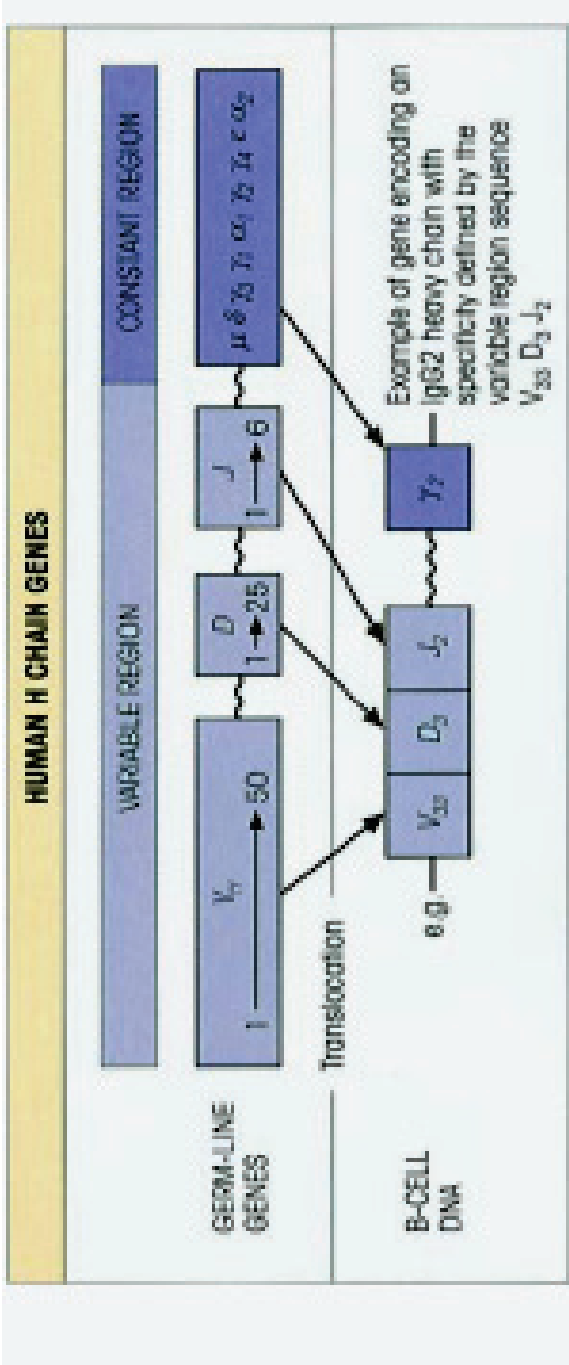
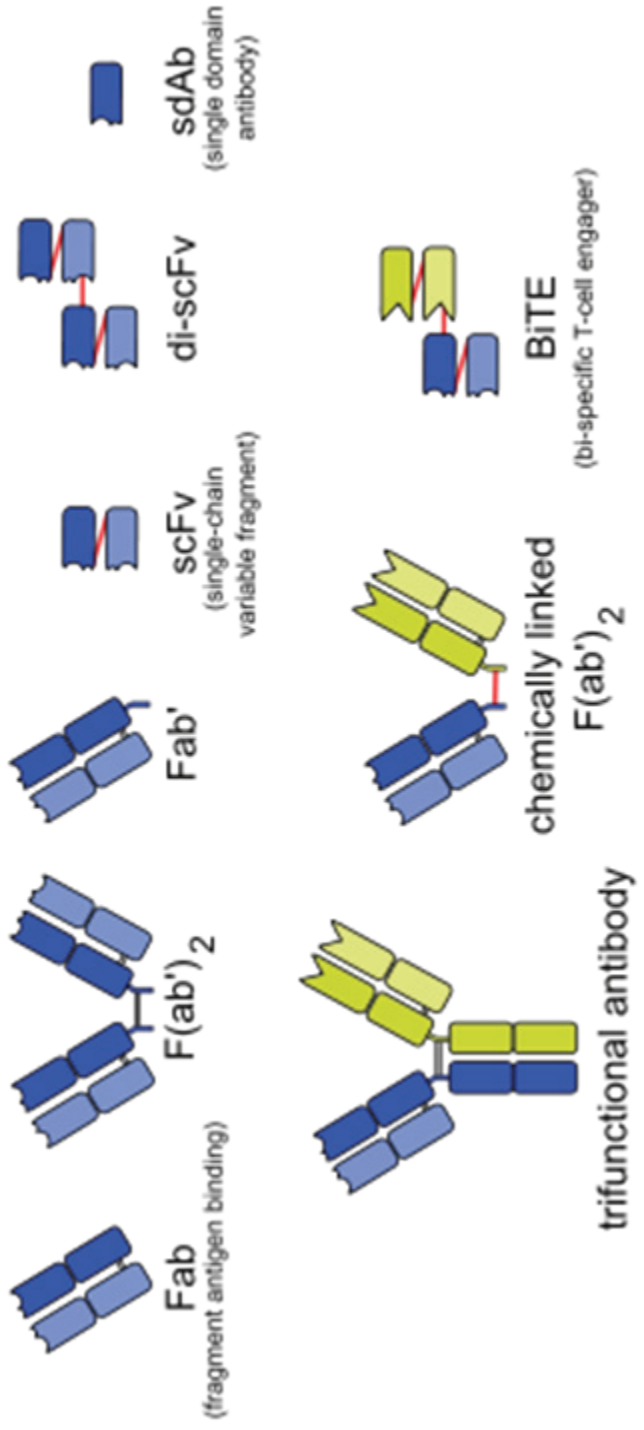
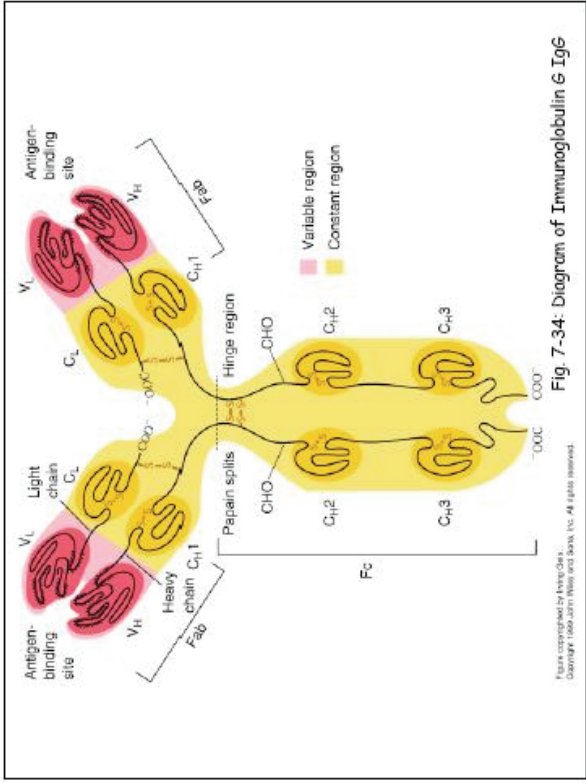


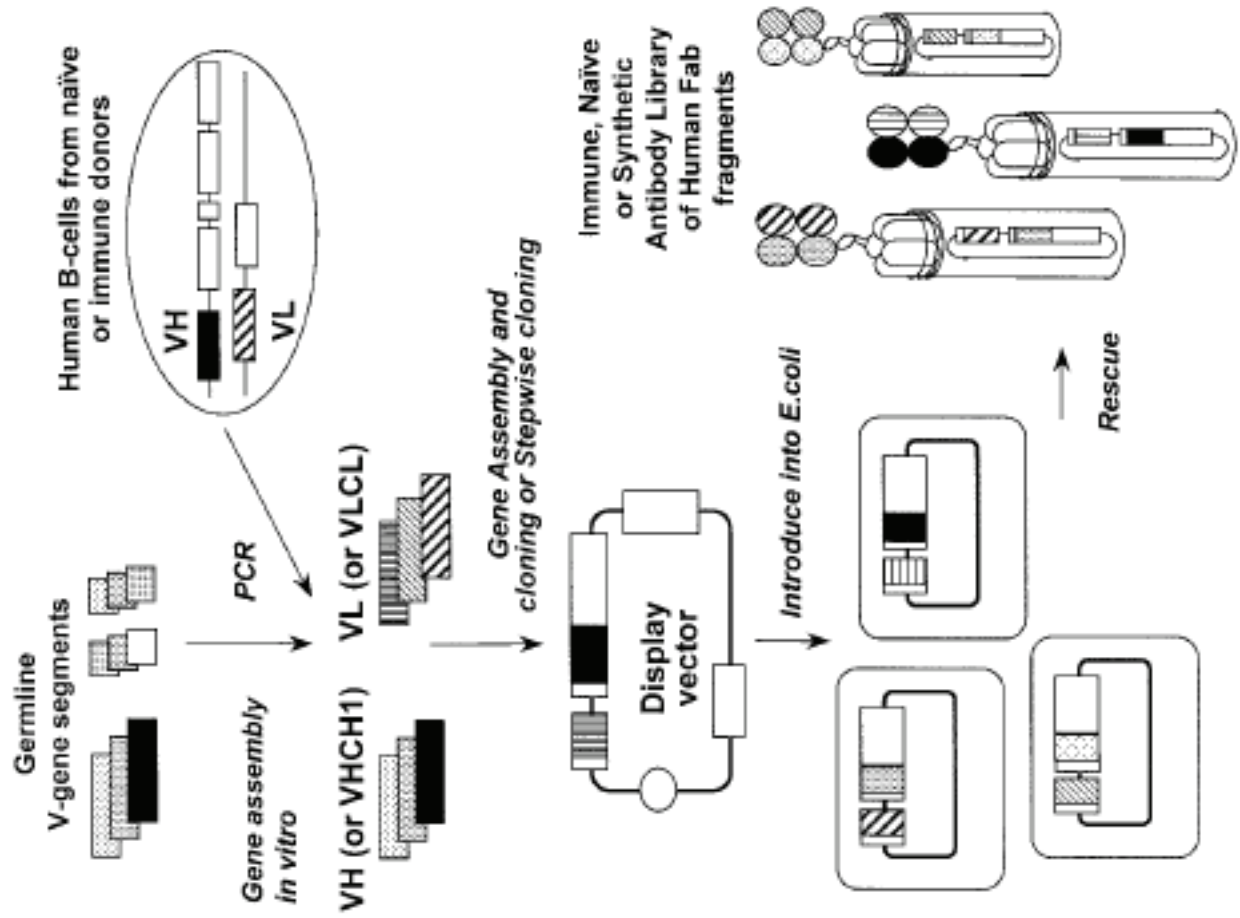
Fig. 7-34: Diagram of Immunoglobulin G (IgG)

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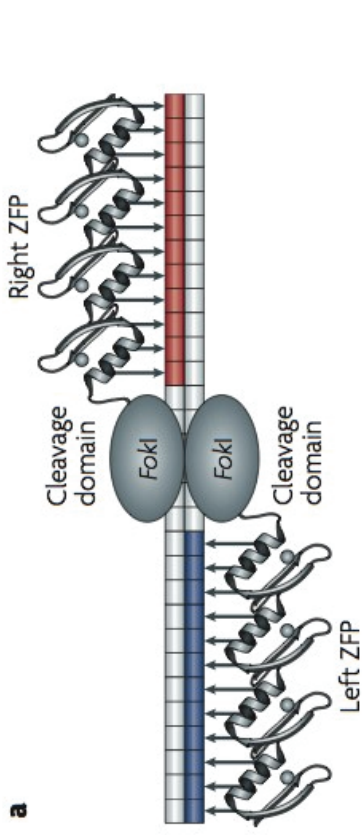
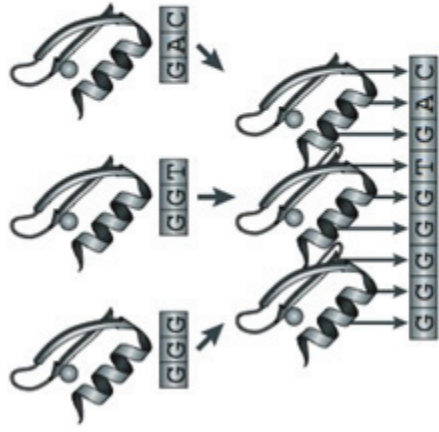




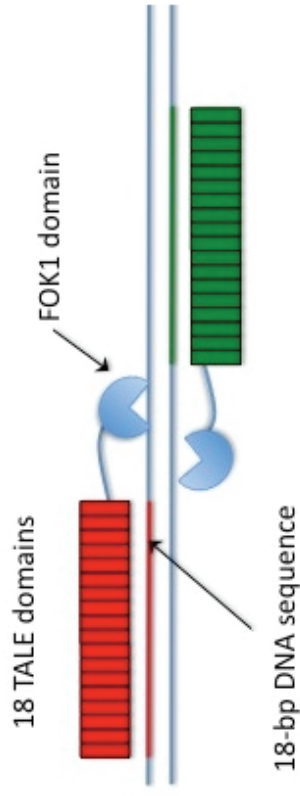
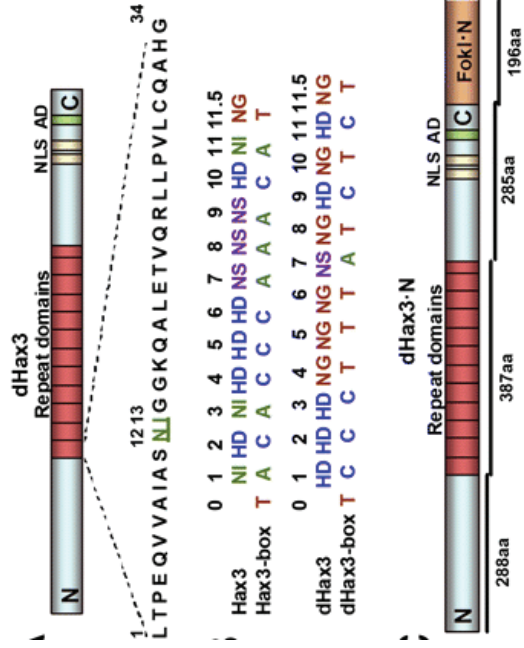




# ZincFinger nucleases



# TALE nucleases



# Transcription Activator-Like Effector Genome Editing

