National
Institute of Chemistry
Slovenia

Načrtovanje bioloških sistemov – možnosti in uporaba

Sintezna biologija

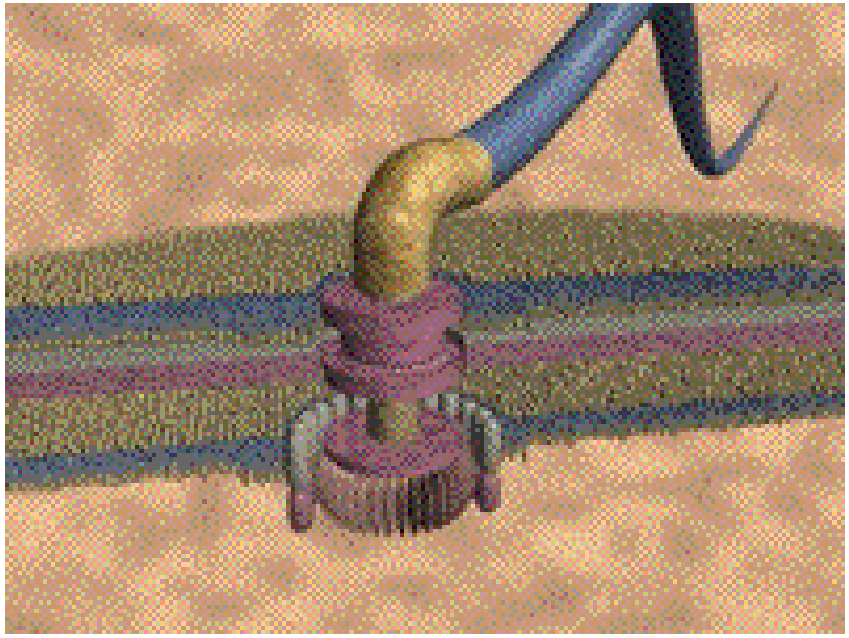
za razumevanje narave in novo tehnološko revolucijo

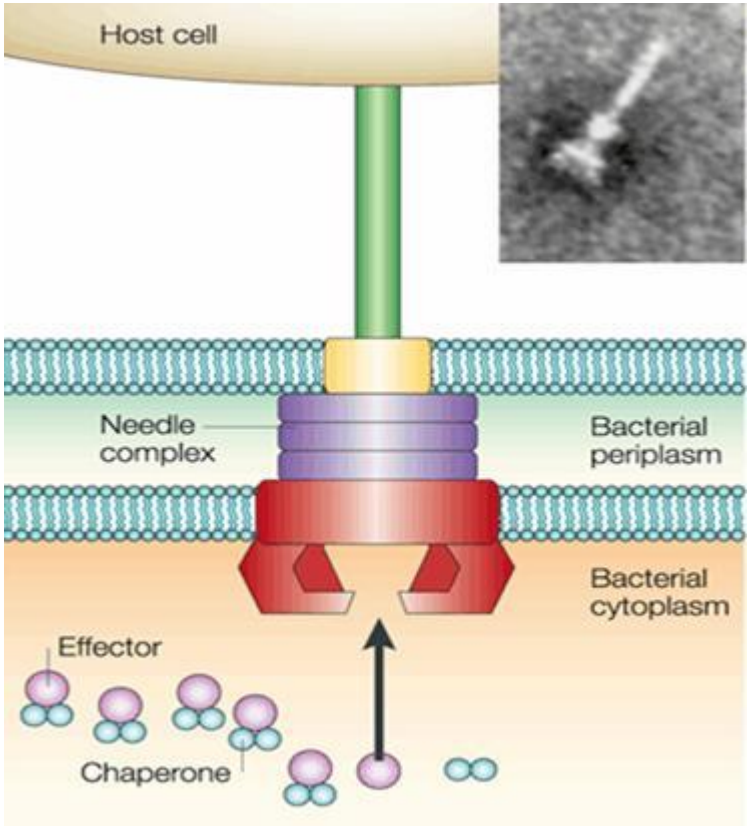
Roman Jerala

Kemijski inštitut


Izjemen potencial semena

Naravni stroji, ki so organizirani na atomskem nivoju

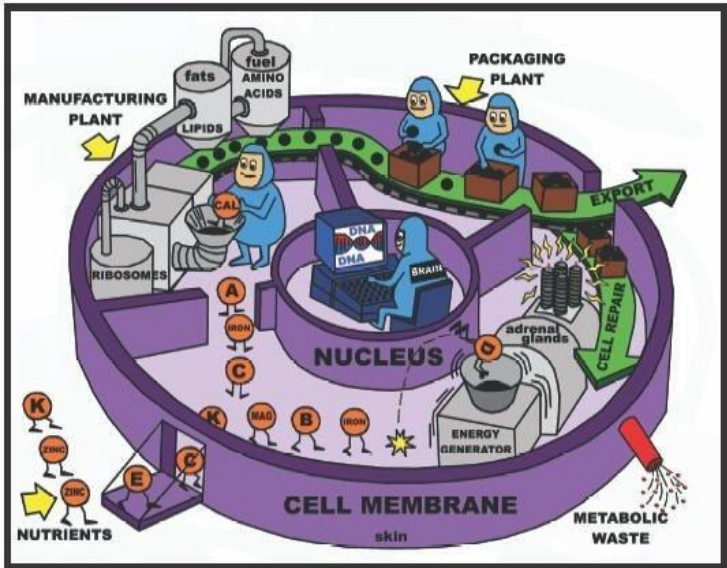




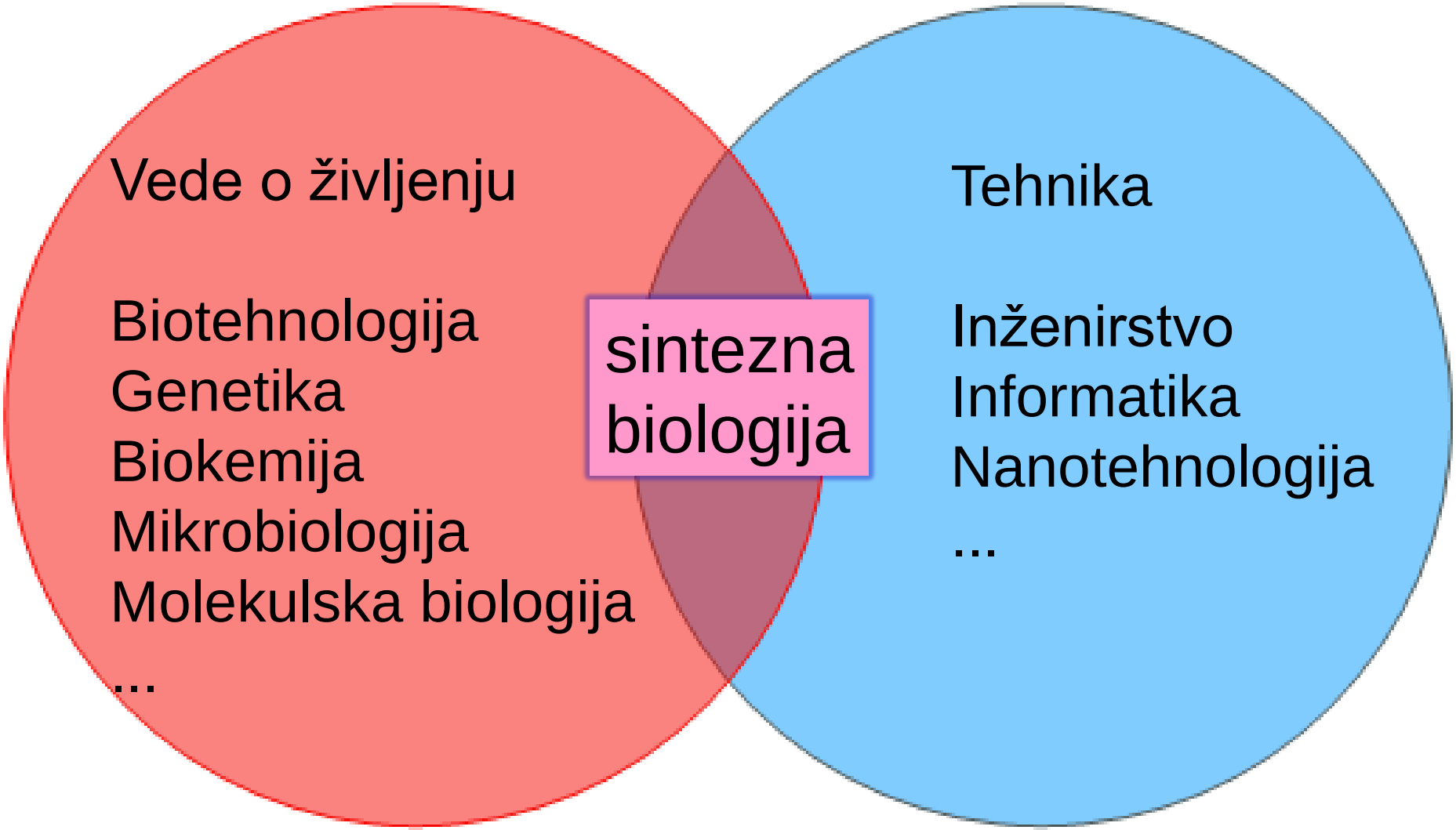
http://www.arn.org/mm/mm.htm

Ugodne tehnološke lastnosti bioloških sistemov

- Struktura določena na nanometrskem merilu
- Samopodvojevanje
- Recikliranje sestavin in gradnikov
- Energetska učinkovitost
- Sposobnost odzivanja in prilagajanja na okolje
- Robustnost
- Evolucija



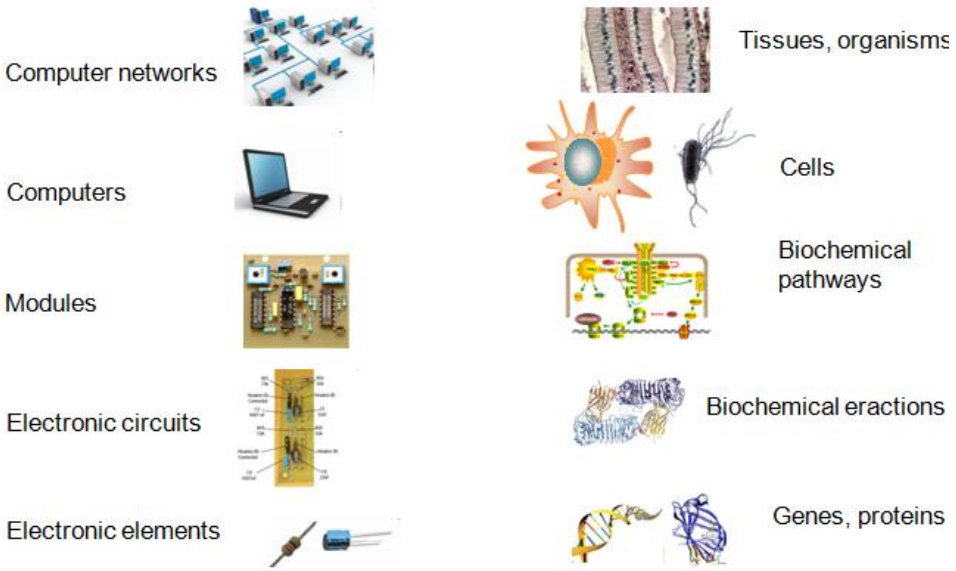
Sintezna biologija kot biološko inženirstvo



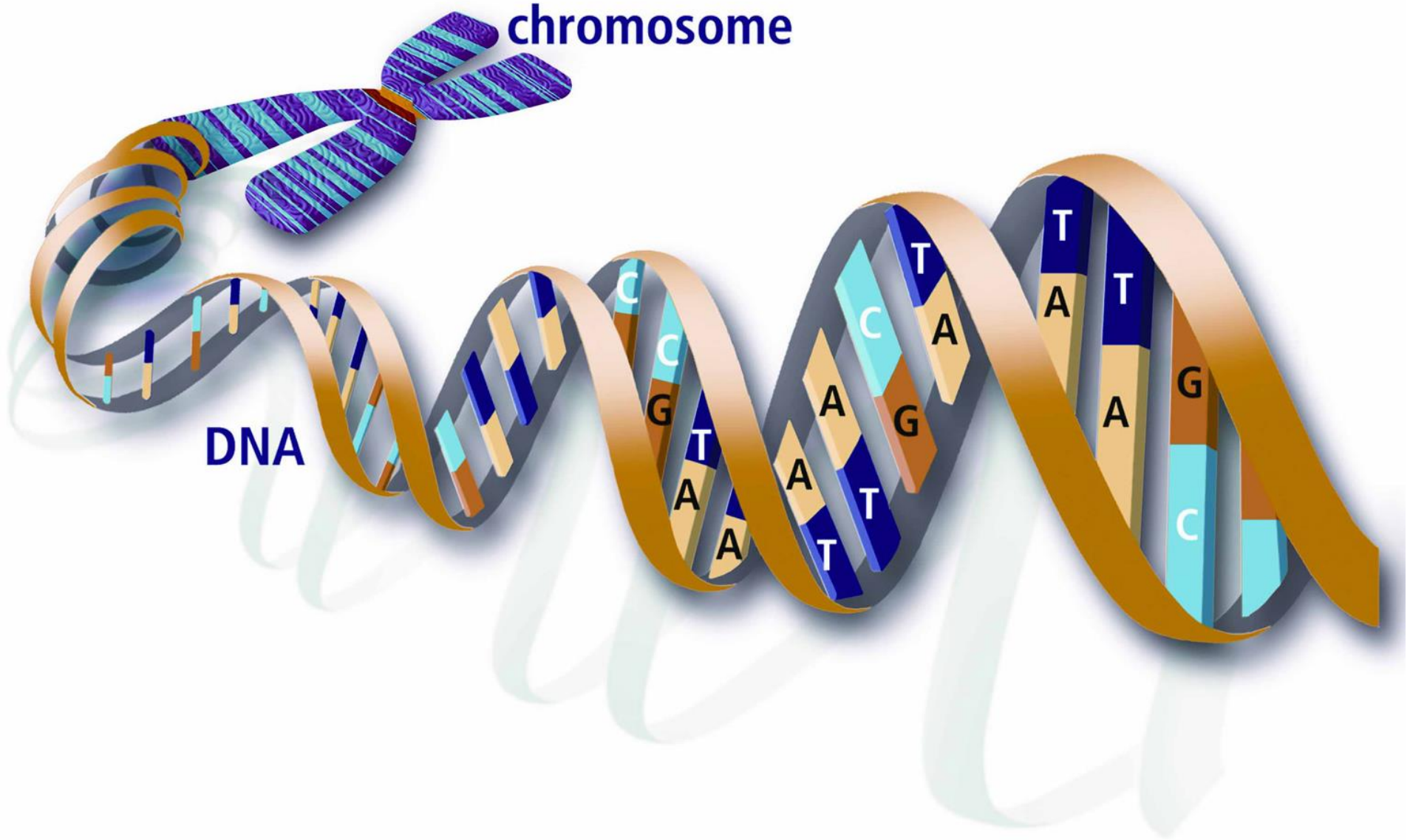
Inženirski pristop

- Uporaba inženirskih pristopov v bioloških sistemih

- Pomembni inženirski principi:
  - Modularnost
  - Abstrakcija
  - Zanesljivost
  - Napovedljivost
  - Standardizacija

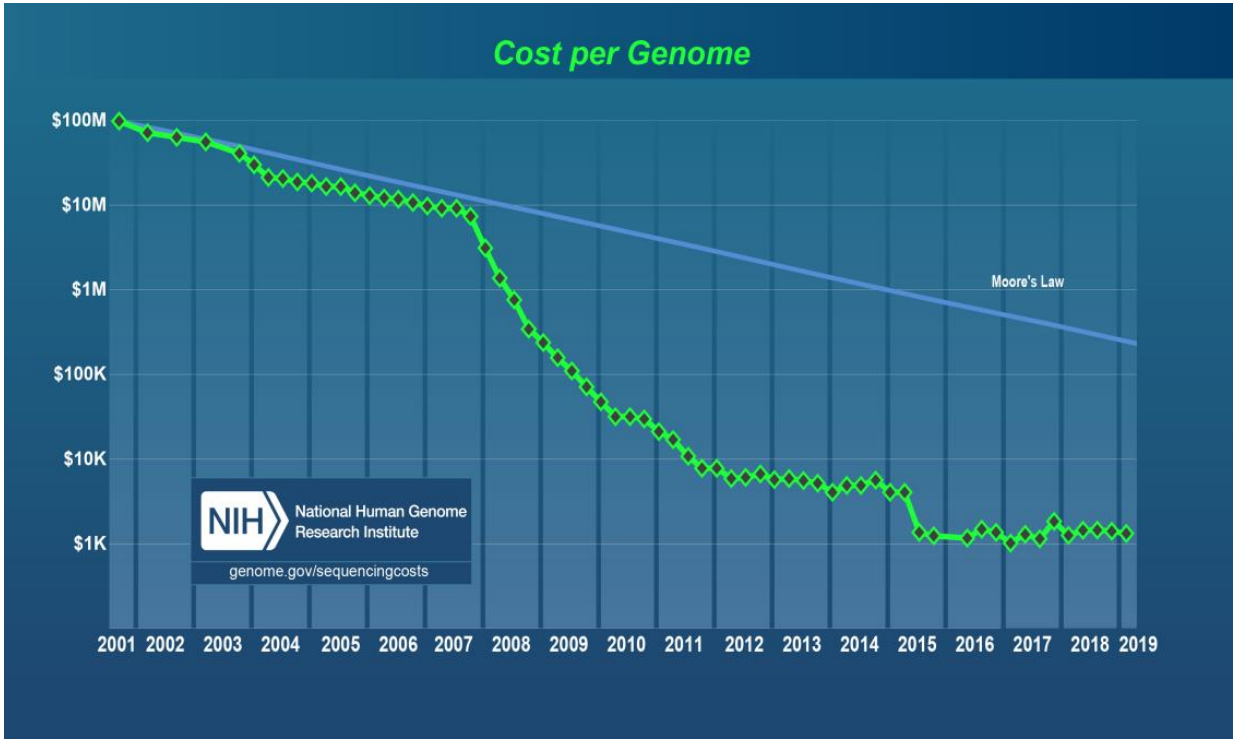


DNK kot program življenja

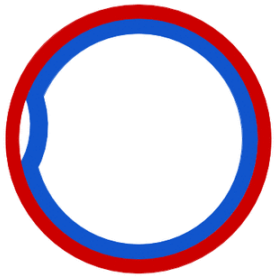


Določanje DNK zaporedja genomov

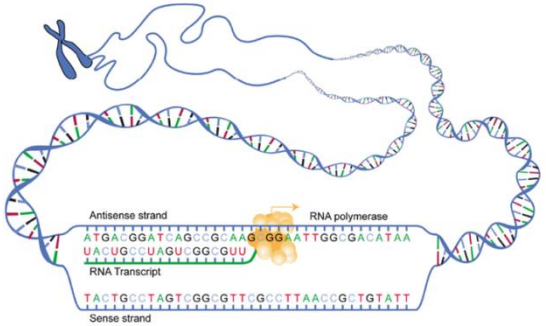
2001 Človeški genom



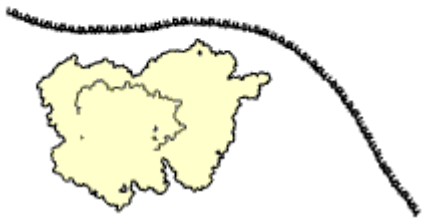
Kako pridemo od DNK do proteinov



Podvojevanje DNK

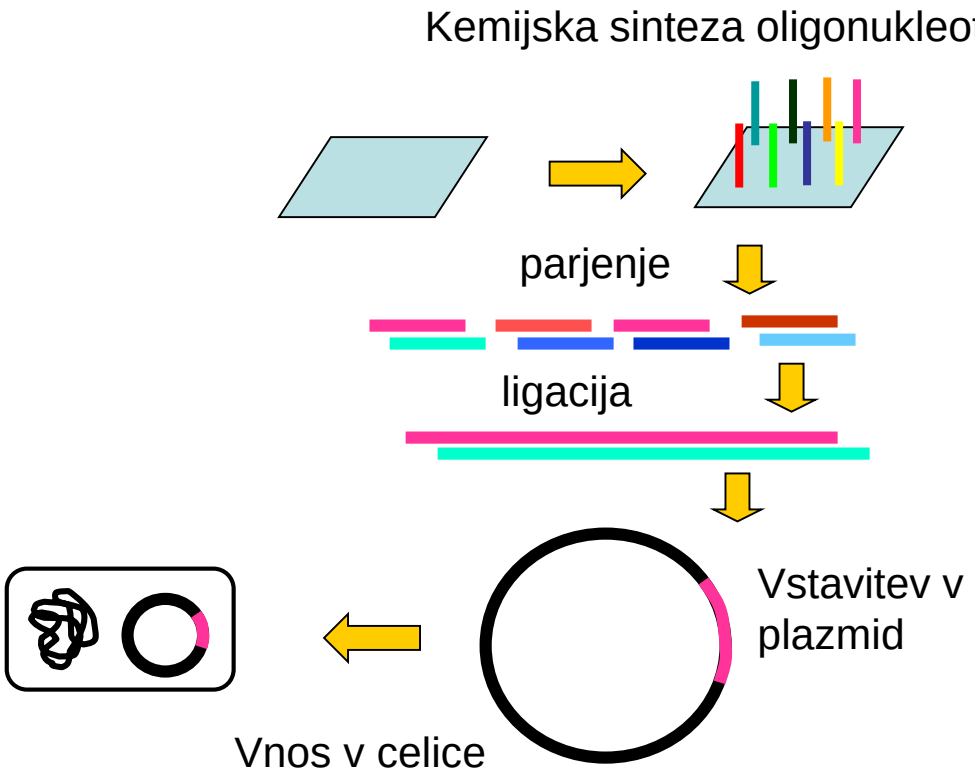


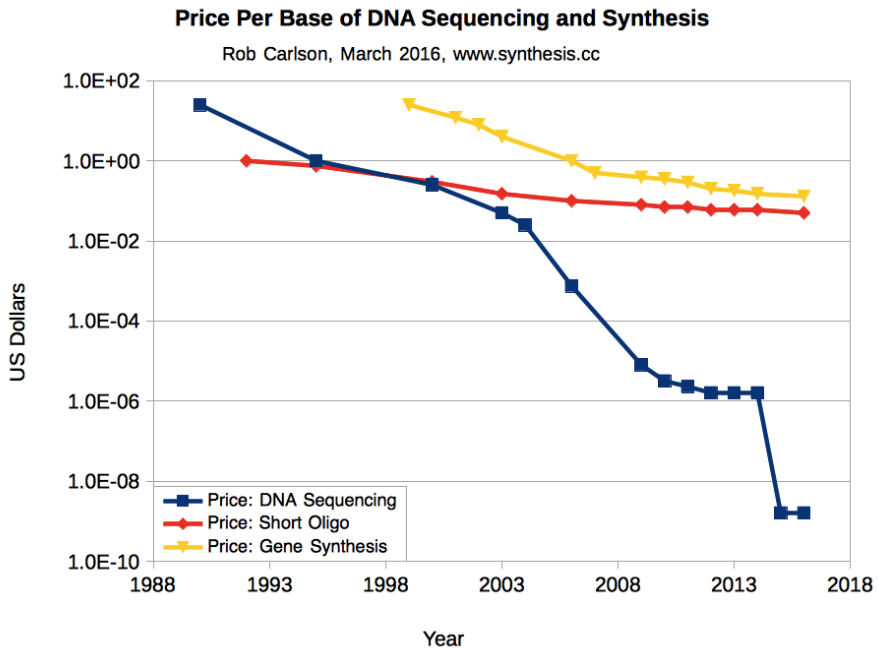
Prepisovanje DNK v RNK



Prevajanje RNK v proteine

Sintetična DNK

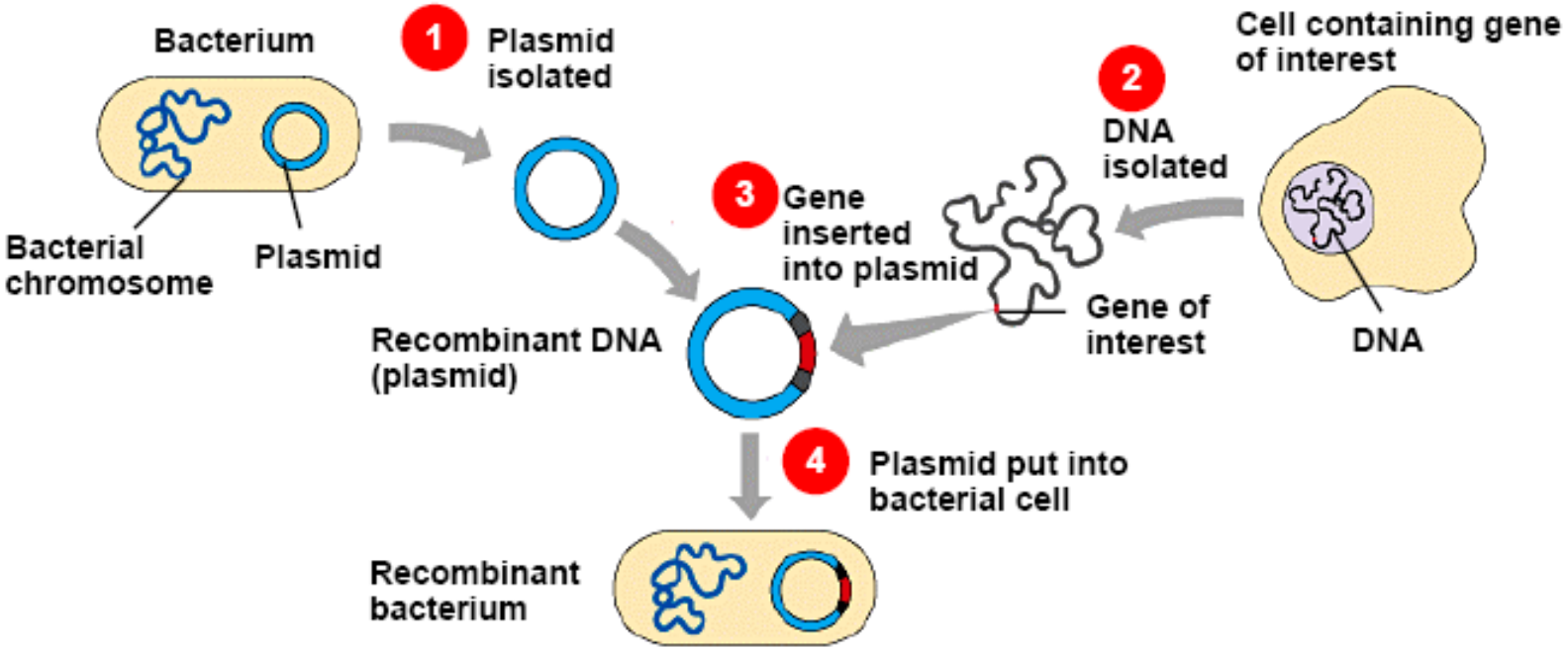




1985 prvi sintetični gen v tedanji Jugoslaviji pr. 400 nukleotidov – danes je komercialno dostopen v nekaj dneh

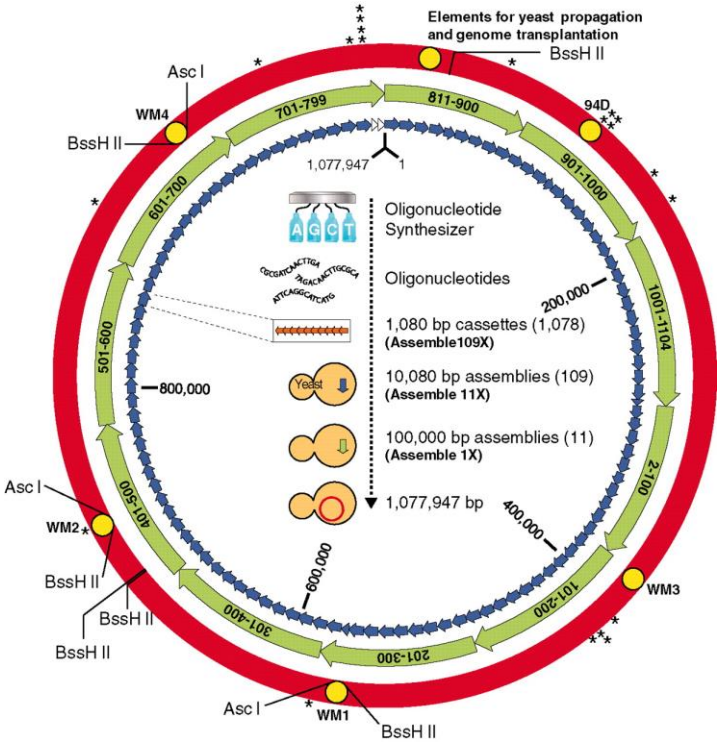
Kako reprogramiramo organizem z DNK ?

DNK lahko poljubno kombiniramo – režemo, sestavljamo, spreminjamo posamezne nukleotide – uporabljamo **metode rekombinantne DNK**

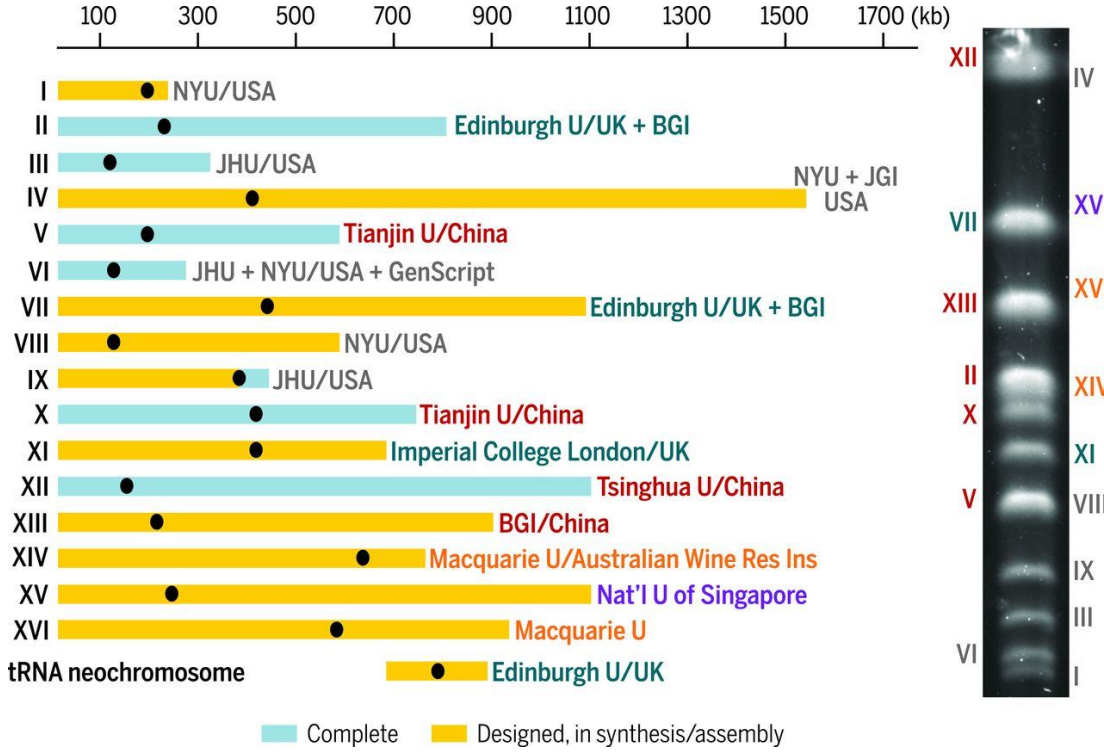


Koščke DNK lahko vstavljamo v celice (bakterijske, človeške...) kjer ta DNK deluje enako kot lastna

Sestavljanje sintetičnega genoma mikobakterije in kvasovke



1 Mbp



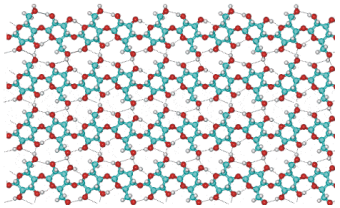
12 Mbp

Kaj lahko sintezna biologija stori za nas

- obnovljivi viri energije
- novi materiali & bionanomateriali
- diagnostika in zdravljenje bolezni
- procesiranje informacij
- biosenzorji in bioremediacija okolja...

Obnovljivi viri energije iz biomase

CO_2

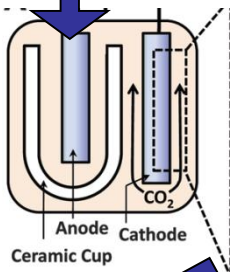


celuloza

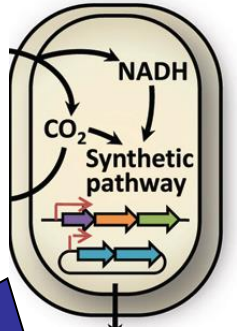
encimi

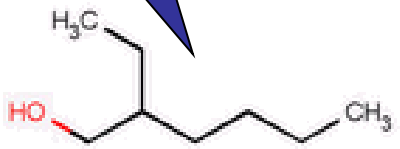
sladkorji

CO_2



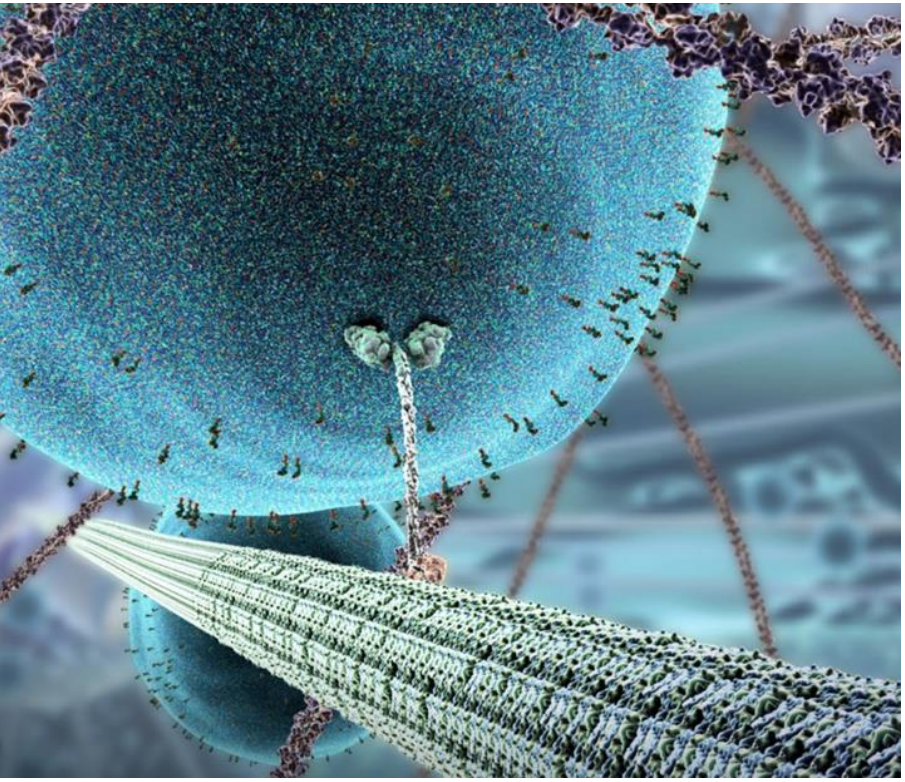
Mikrobna pretvorba

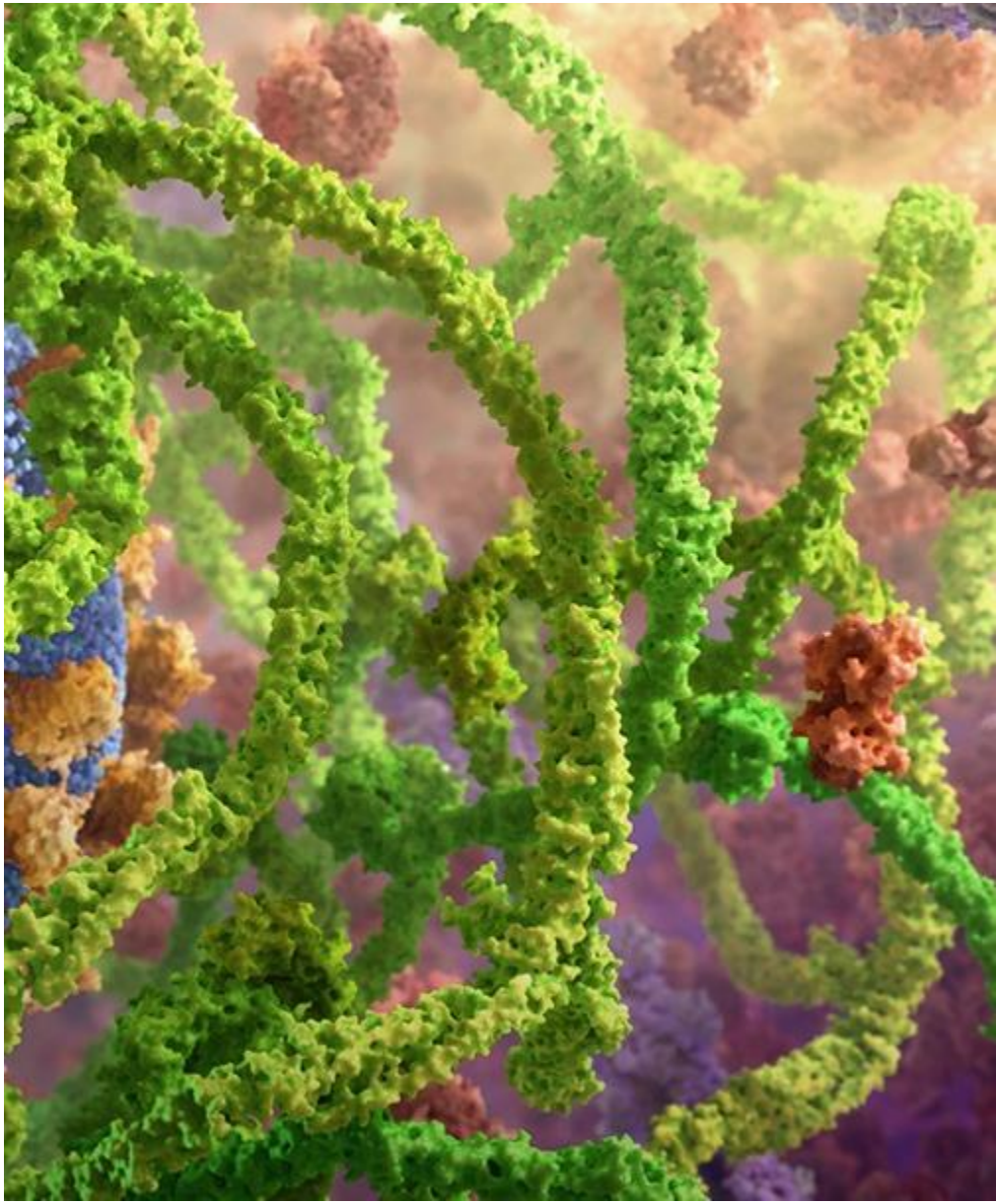




gorivo

Kompleksne nanostrukture in celični stroji

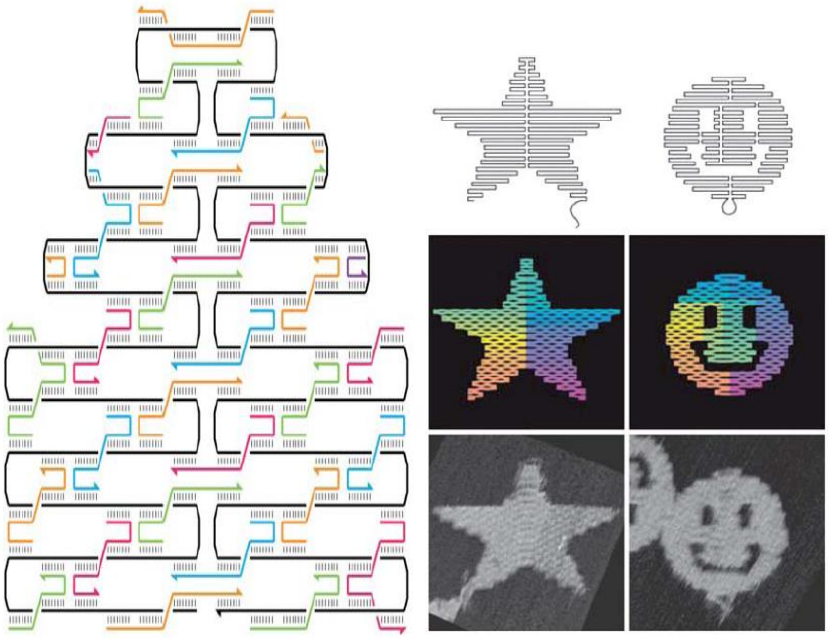




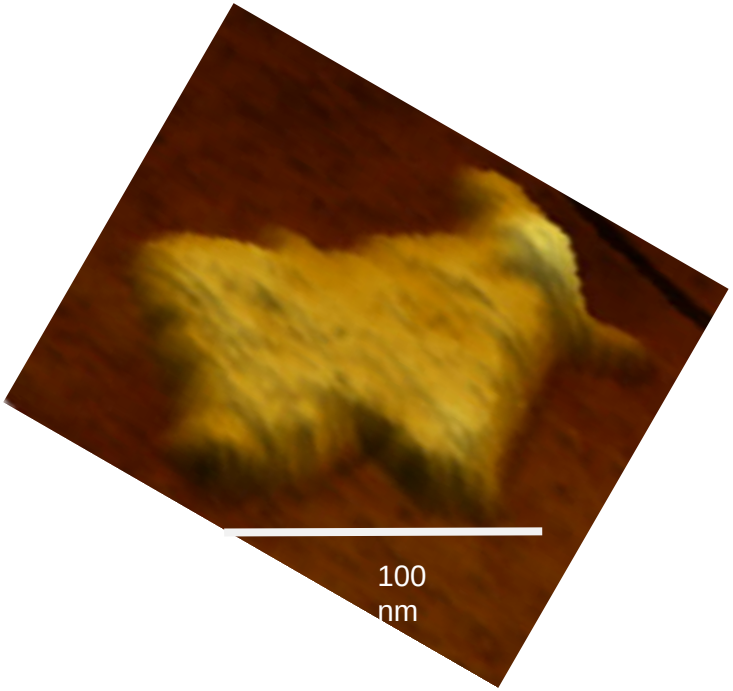
Nanostrukture na osnovi DNK

2D DNA origami

- Long DNA + oligonucleotides



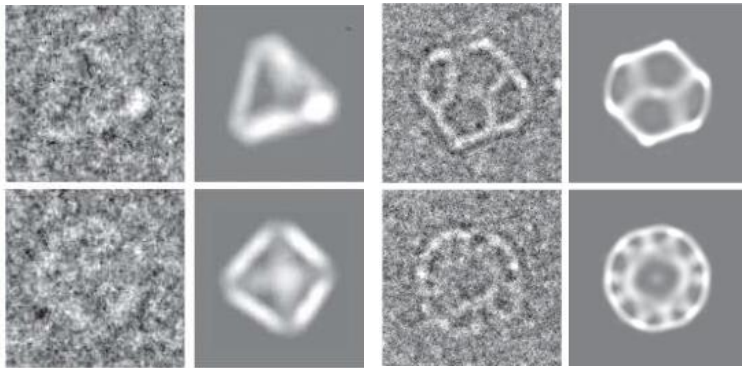
Kuzuya et al., Nanoscale, 2010

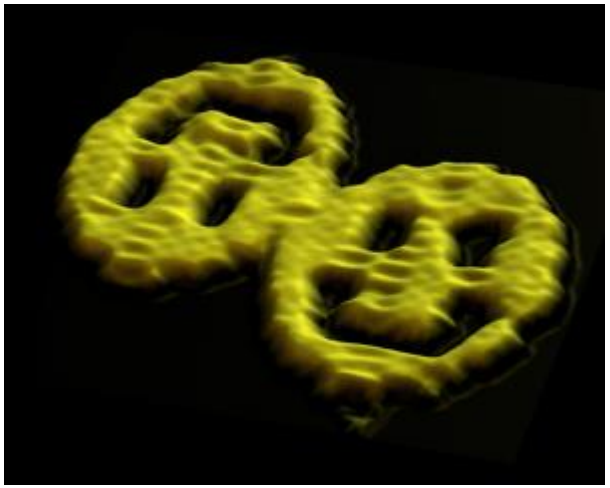


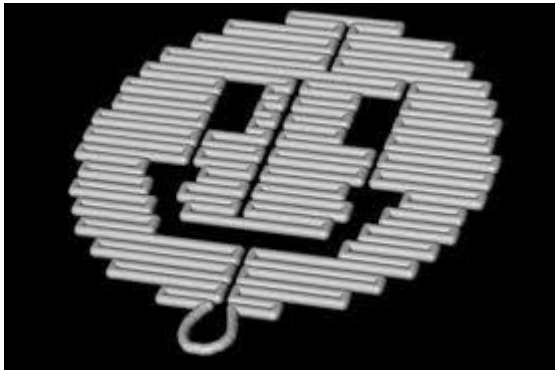
Jerala et al., ActaChimSlov 2010

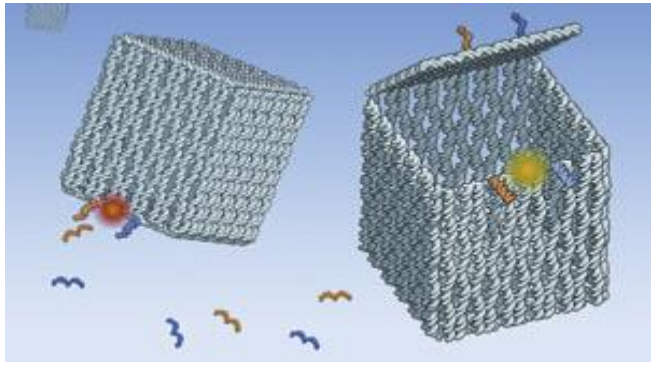
Nanostrukture na osnovi DNK

- DNA origami



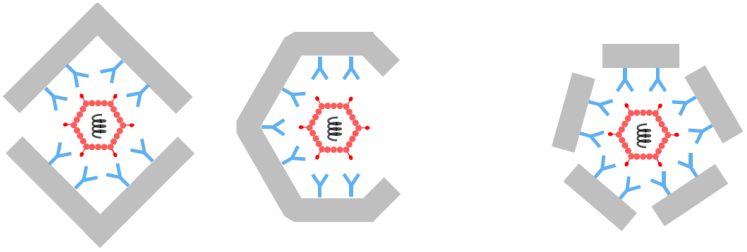


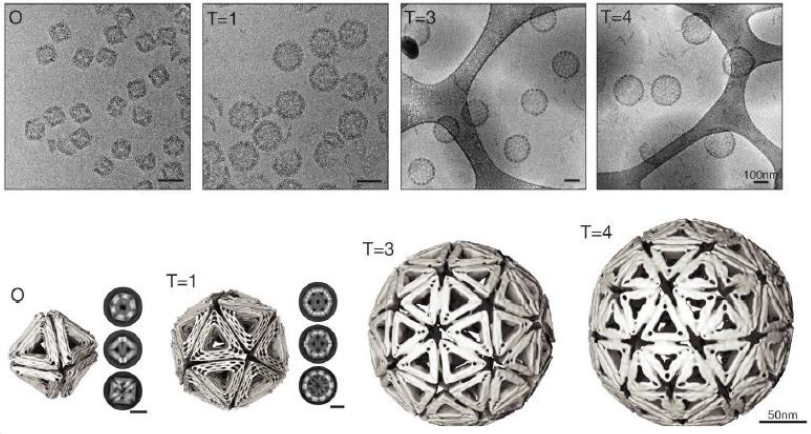




He et al., Nature 2008, ChemComun 2006

Virofight – boj proti virusnim okužbam s kletkami za viruse



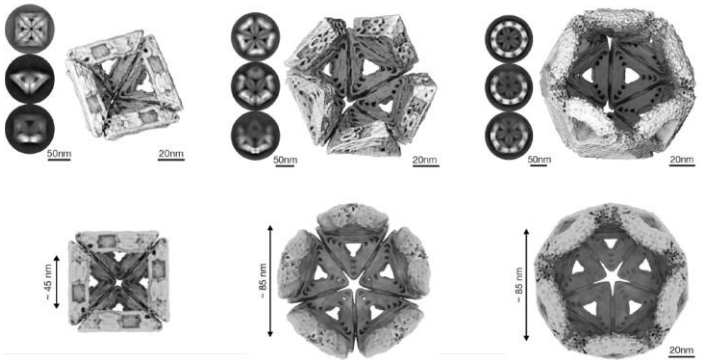


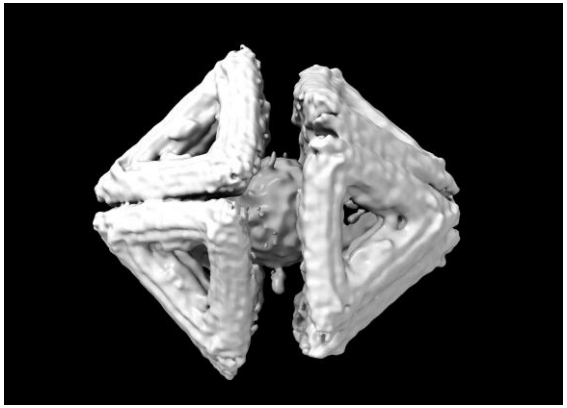
Material za kletke:
- DNA
- polipeptidi

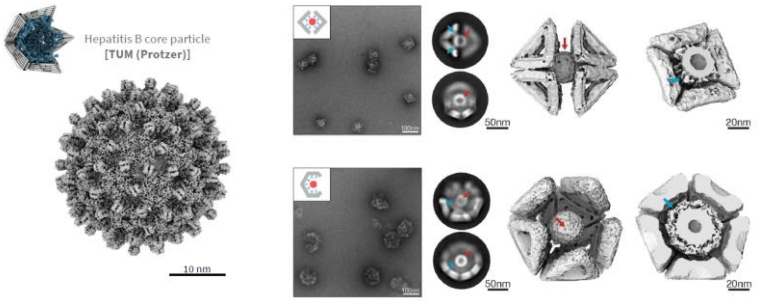
Virofight

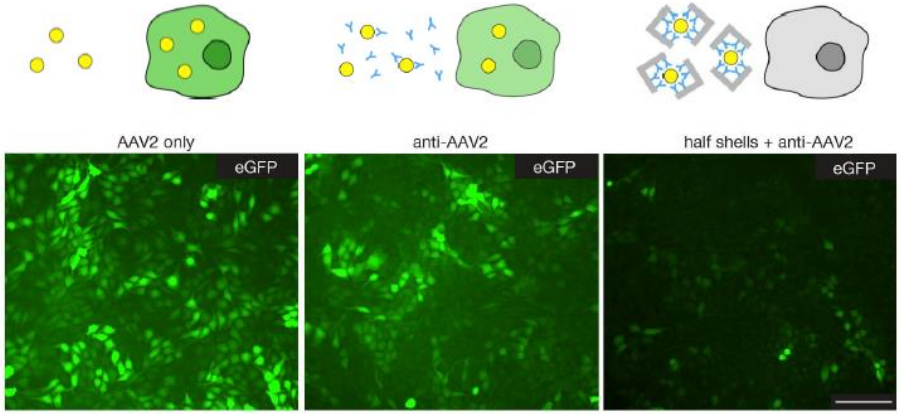
https://www.virofight.eu/

Virofight – DNA kletke



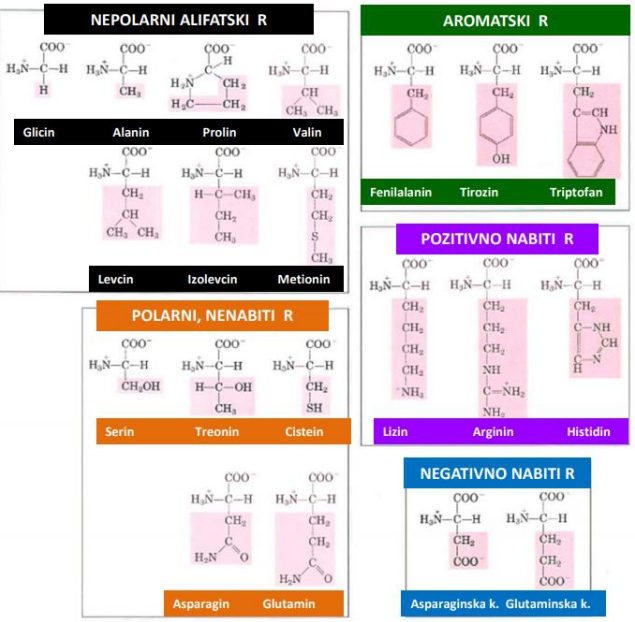


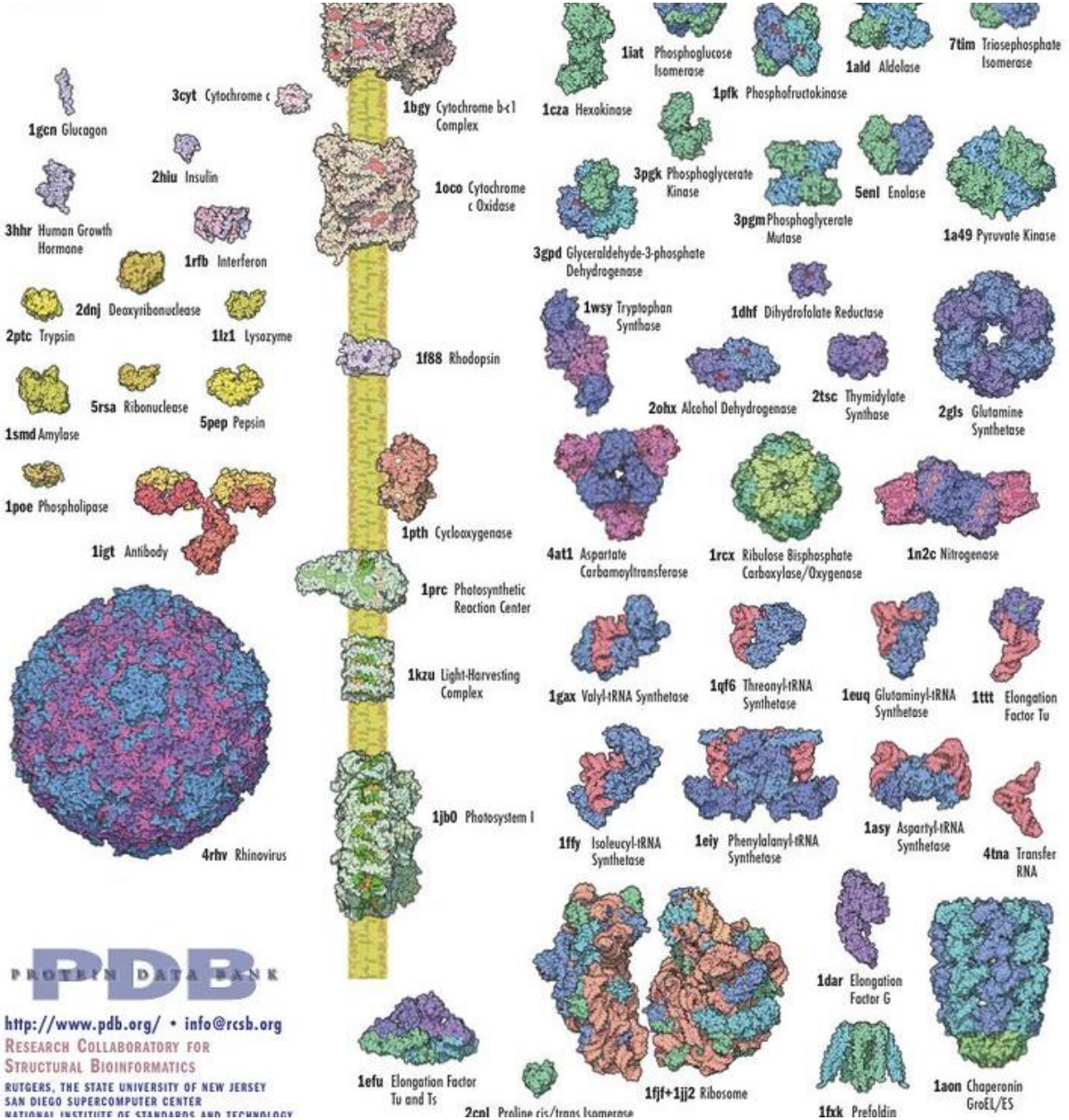




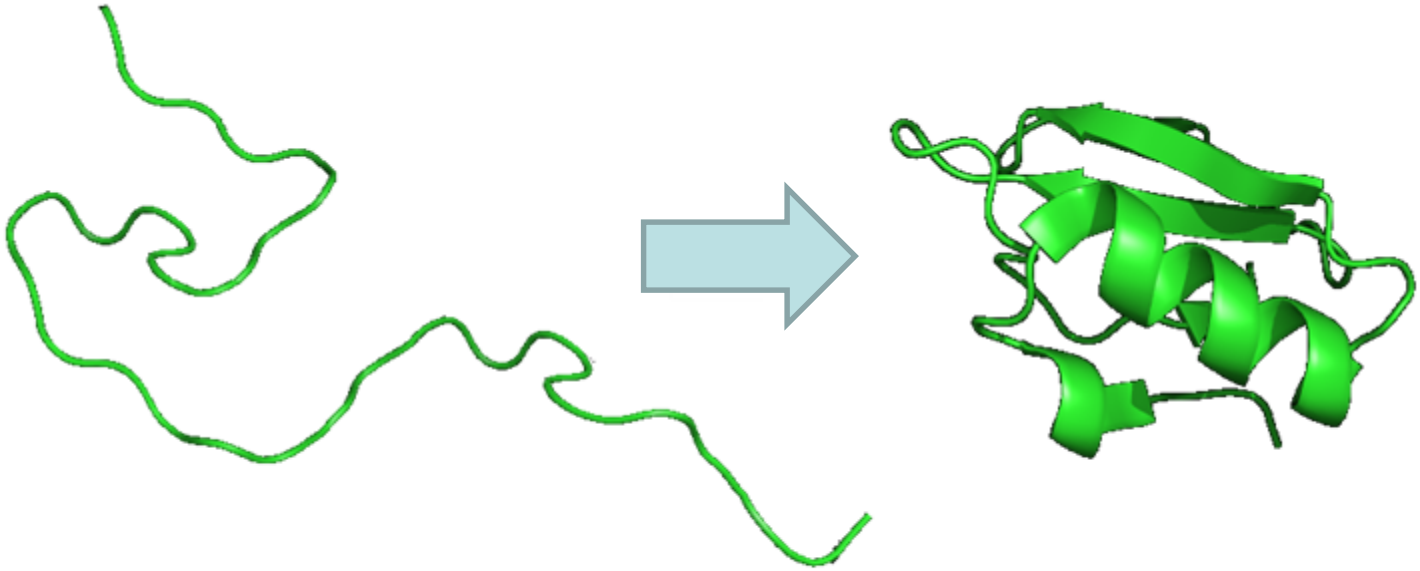
Dietz laboratorij, TUM

Raznolikost naravnih proteinov





Naravni proteini



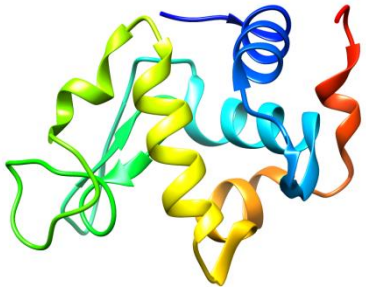
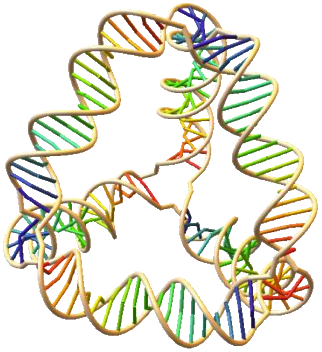
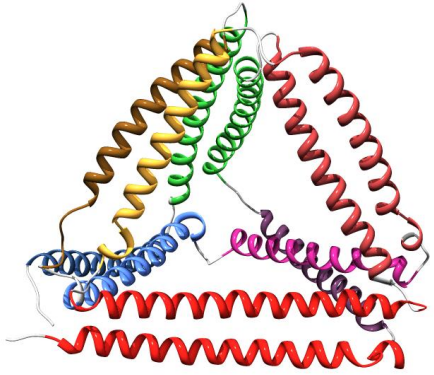
Raziskovanje temne snovi proteinskega vesolja

(le majhen delež od vseh možnih zaporedij polipeptidov se je lahko kadarkoli pojavil v naravi)

Številno možnih proteinov dolgih 100 aminokislin : 10^{130}

Število atomov v vesolju: 10^{50}

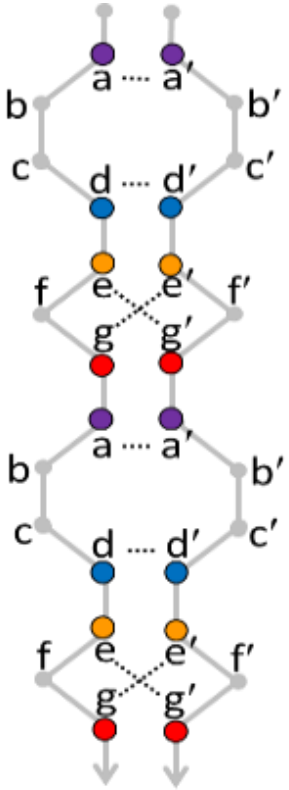
Naravne in dizajnirane nanostrukture

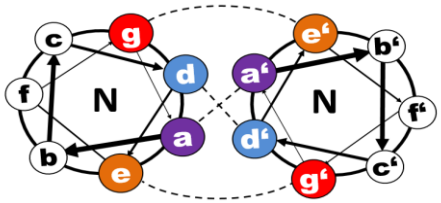
	DNA	Protein
Evolved compact fold		
Modular fold		

Modularni dimerni gradniki

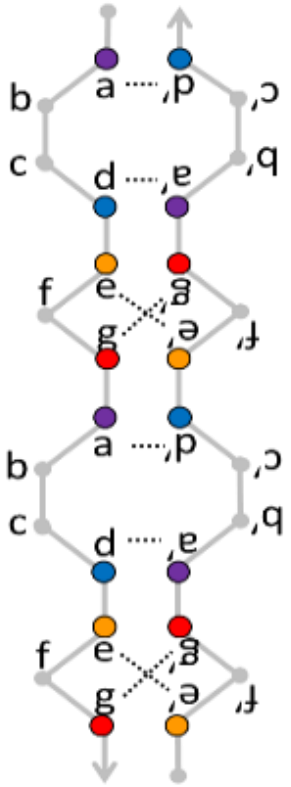
Dimeri obvitih vijačnic

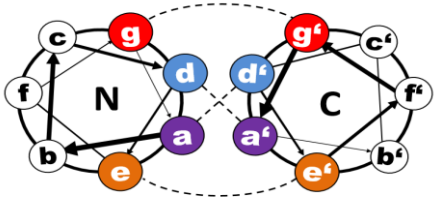
paralelni dimer





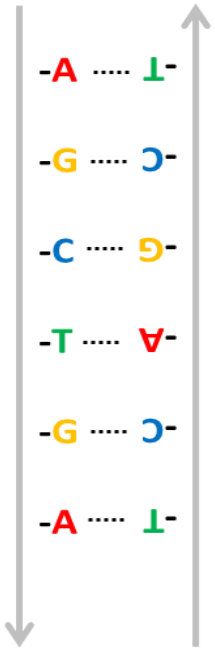
antiparalelni dimer

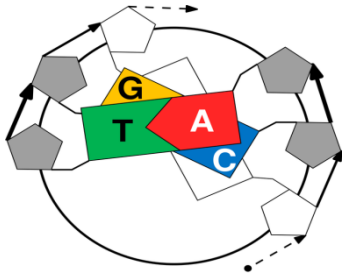




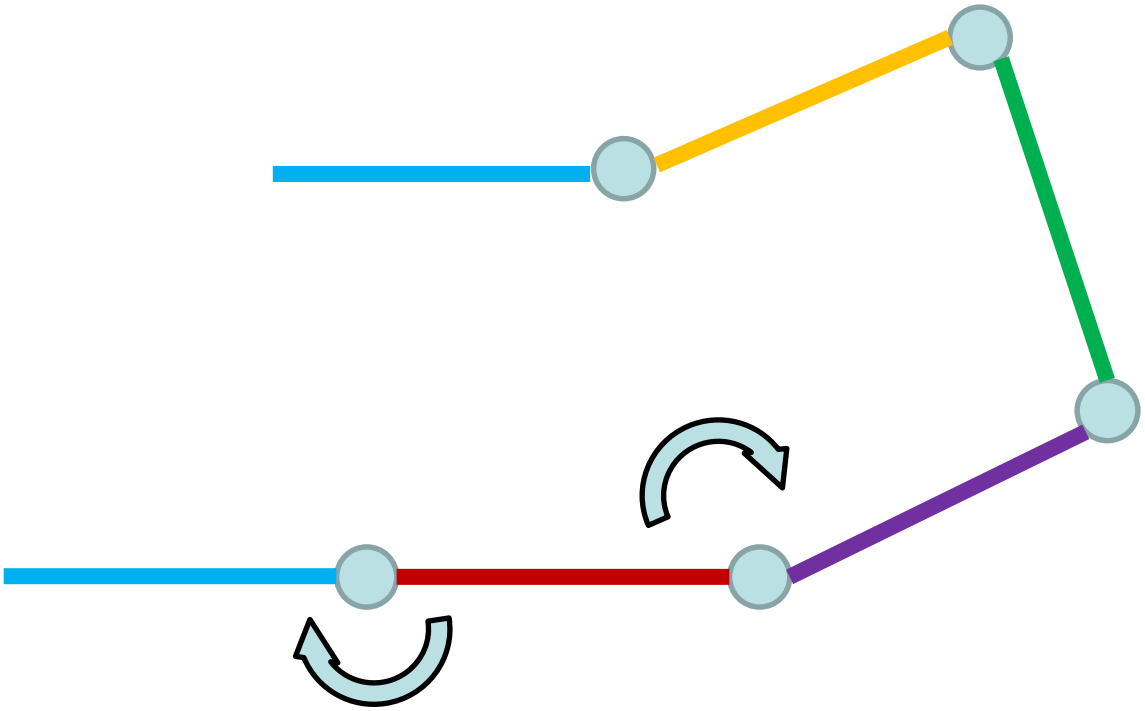
DNK

antiparalelni dimer

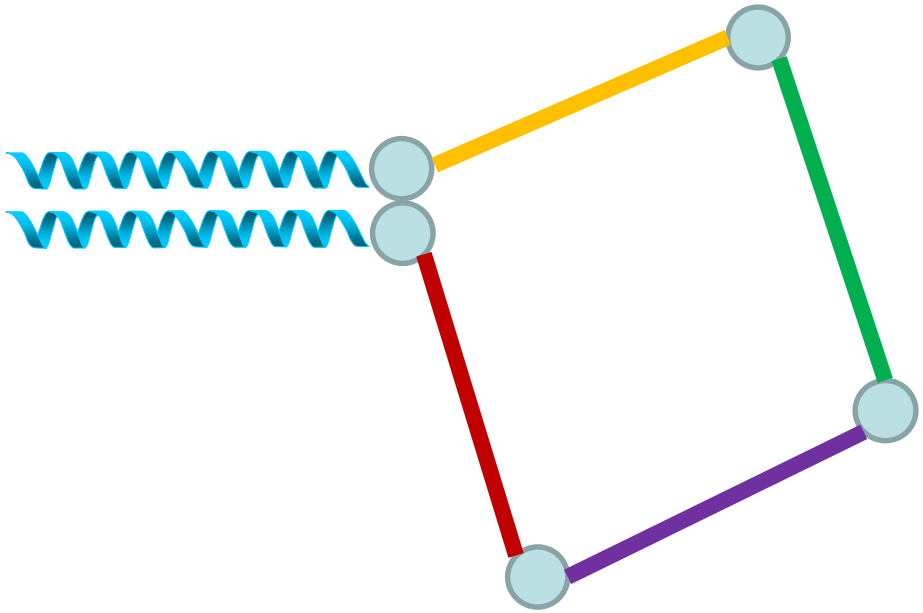




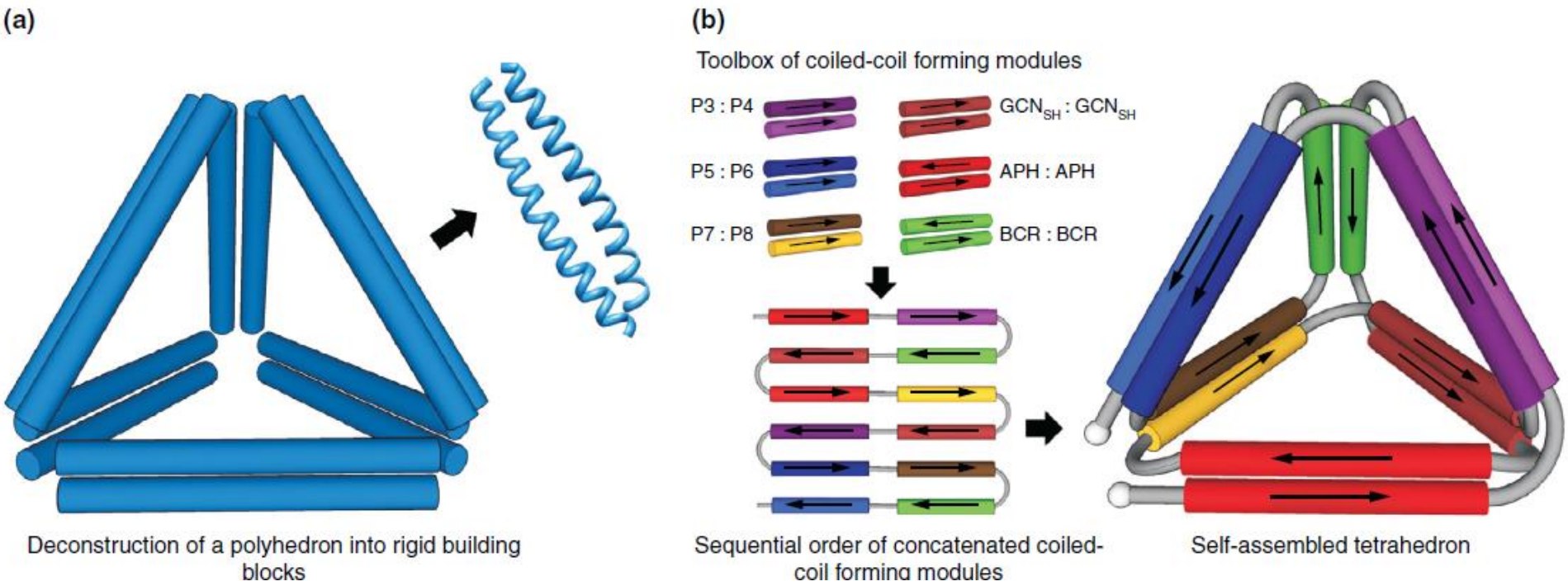
Peptidni gradniki povezani v verigo



Peptidni gradniki povezani v verigo



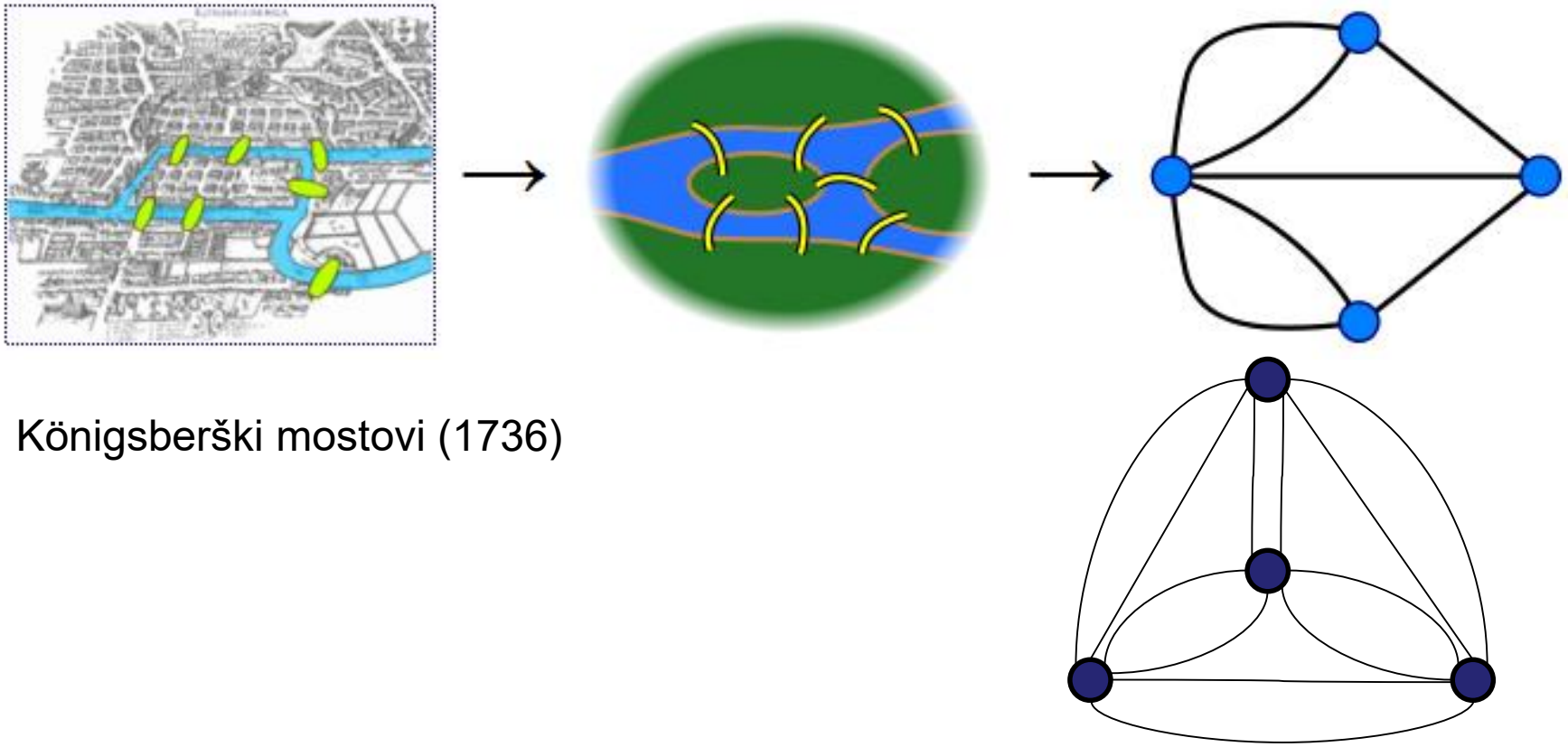
Gradnja strukture iz modulov



Božič-Abram et al., Cur.Op.Chem.Biol. 2013

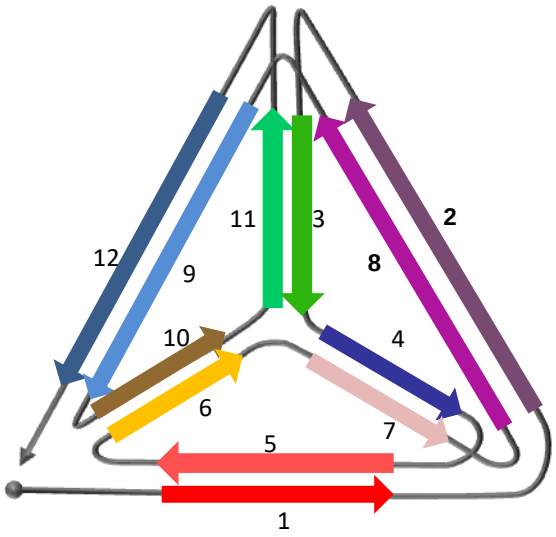
Matematična topološka analiza

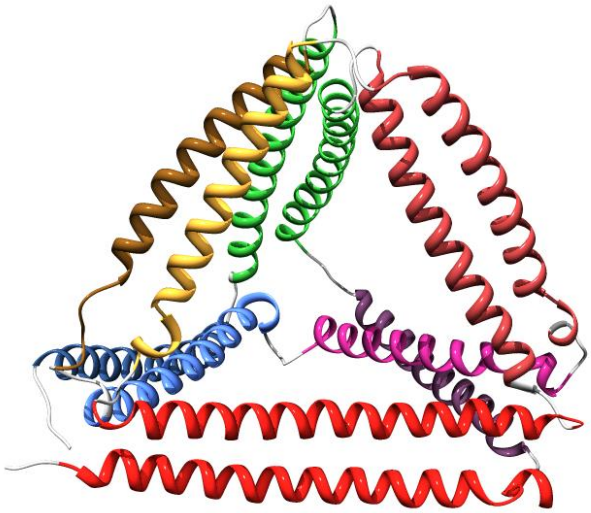
Eulerjev obhod

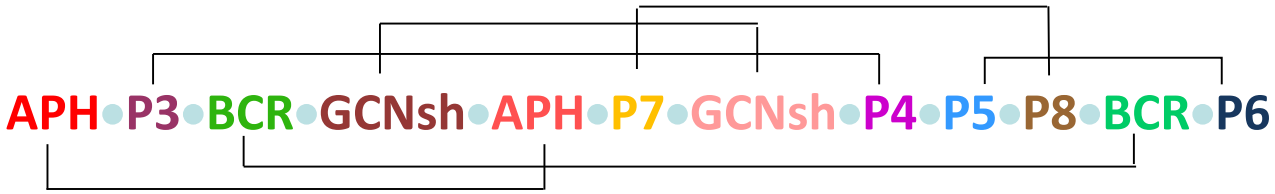


Königsberški mostovi (1736)

Dizajn polipeptidnega tetraedra



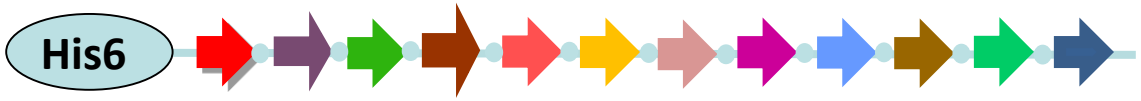




4 paralelni dimeri

2 antiparalelna dimera

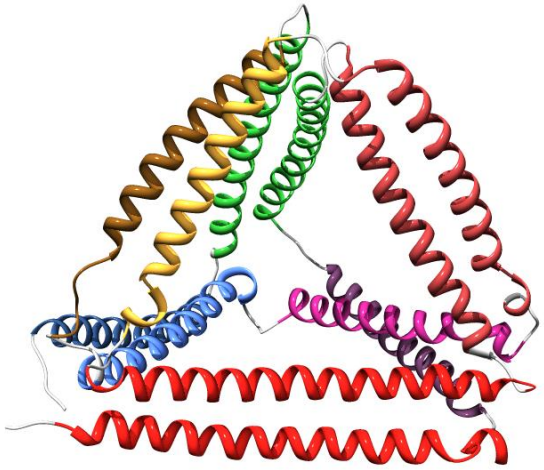
TET12

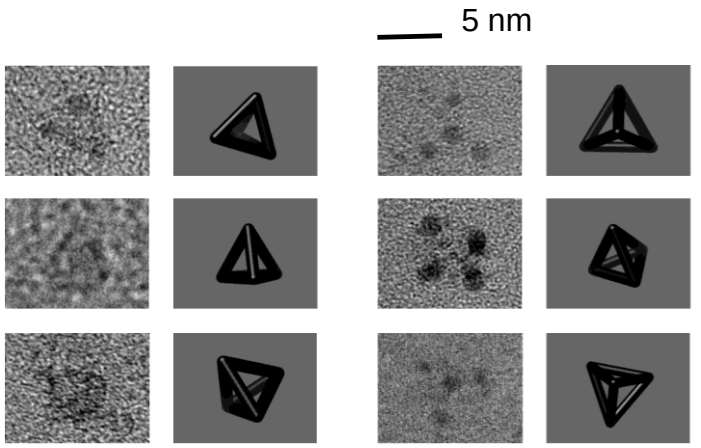


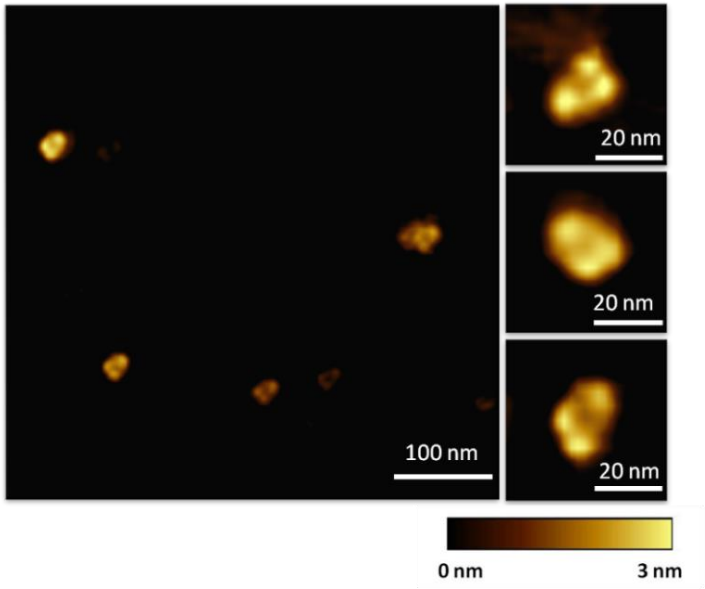
- Gibljiv povezovalni peptid

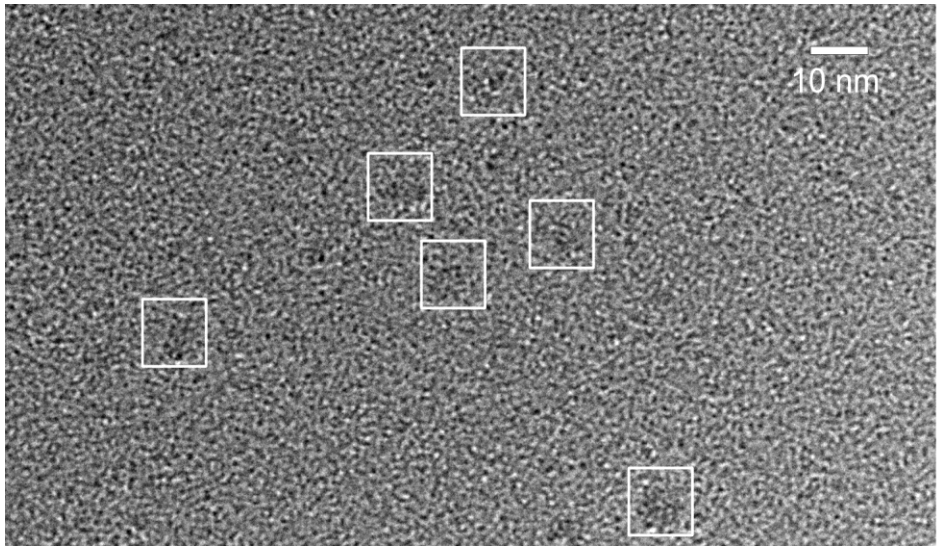
SGPG

TEM in AFM analiza



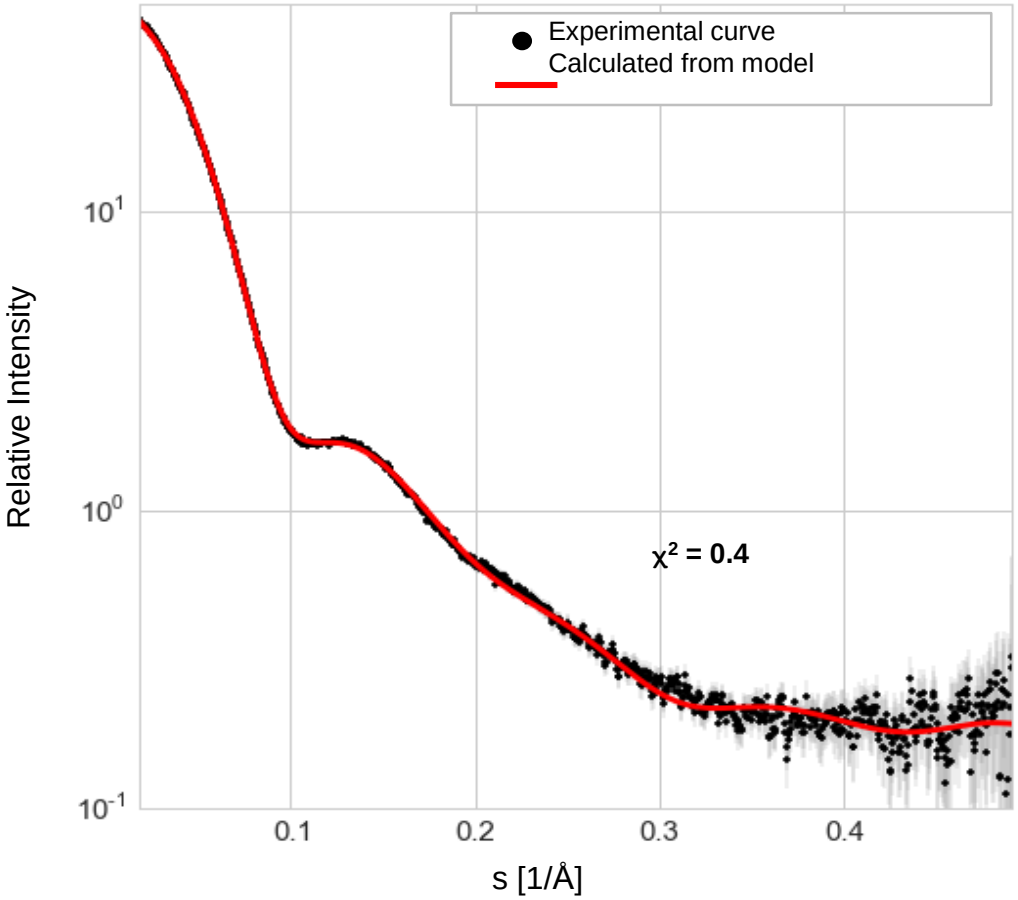


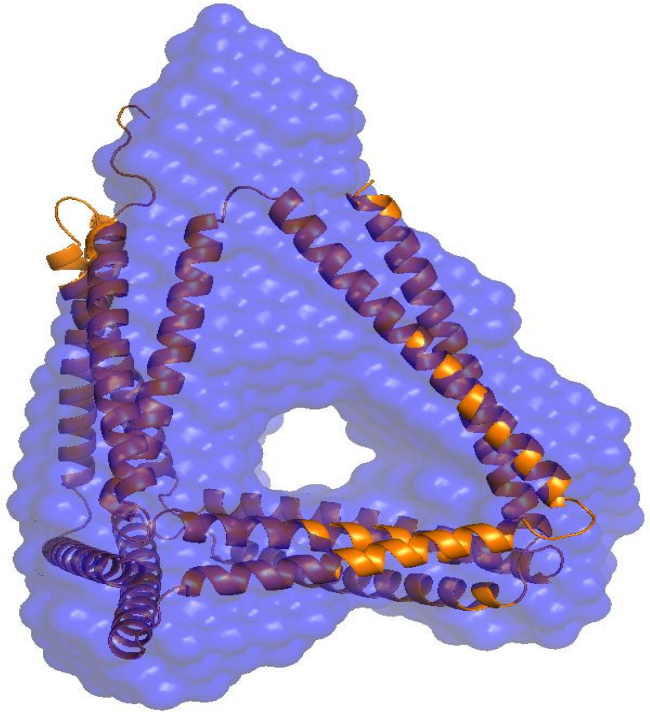




Gradišar et al., Nature Chem. Biol. 2013

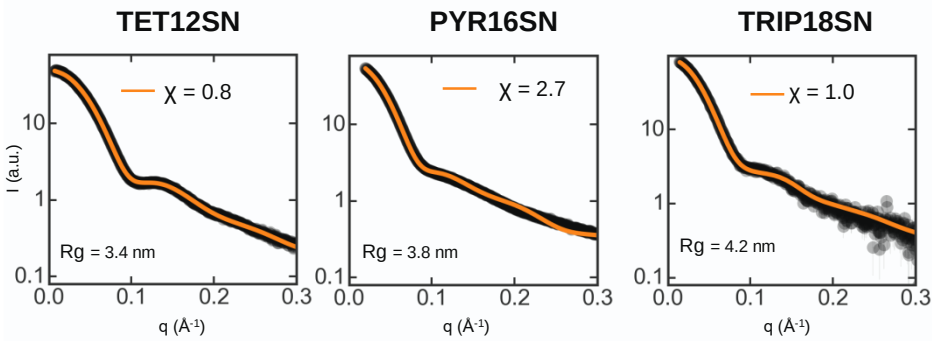
SAXS analiza TET12SN

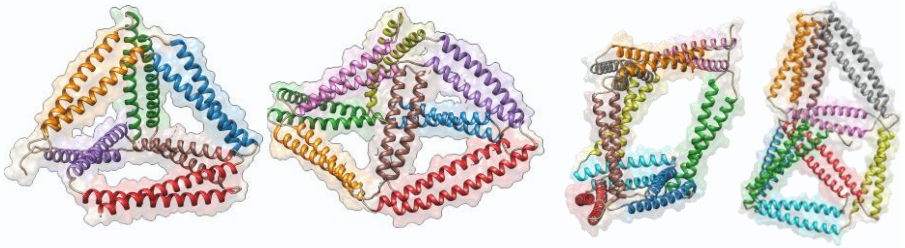


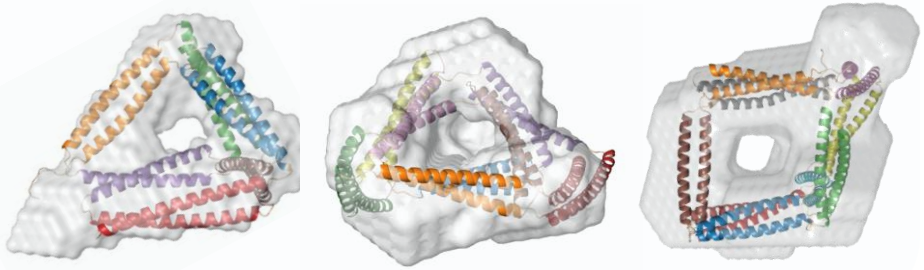


Struktura proteinskih kletk

SAXS

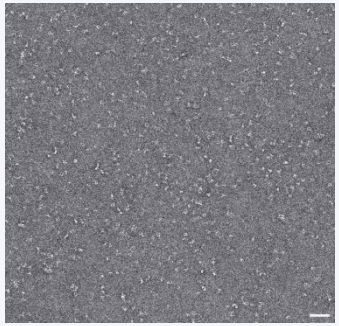


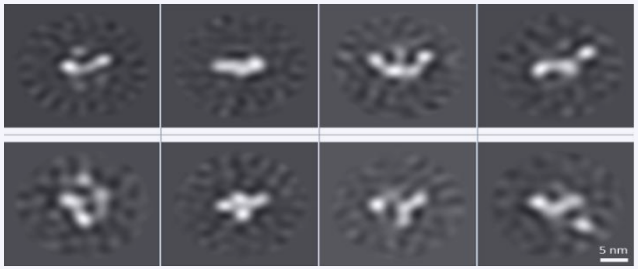




TEM

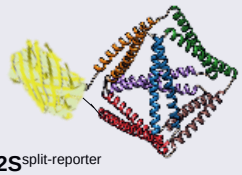
Potrjena struktura kletk s sipanjem svetlobe in elektronsko mikroskopijo





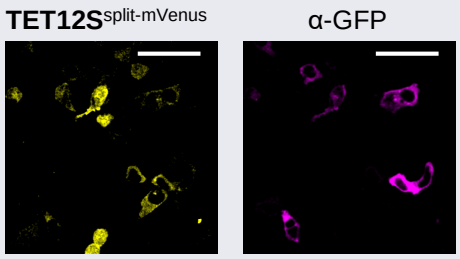
Načrtovani proteini v sesalskih celicah

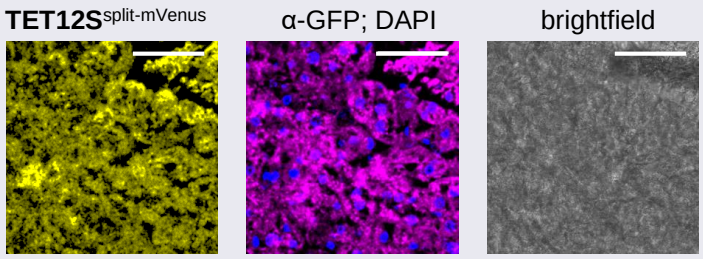
Fluorescent reporter reconstitution

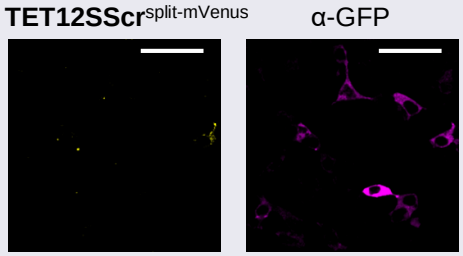


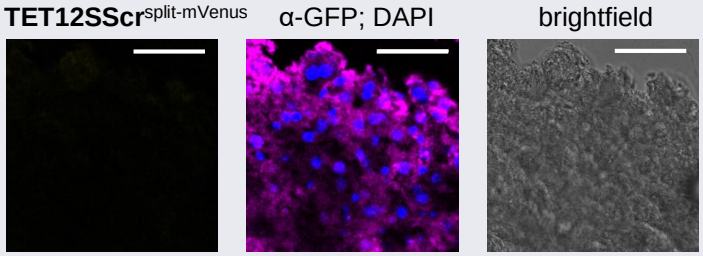
TET12S$^{split\text{-}reporter}$

TET12SScr$^{split\text{-}reporter}$

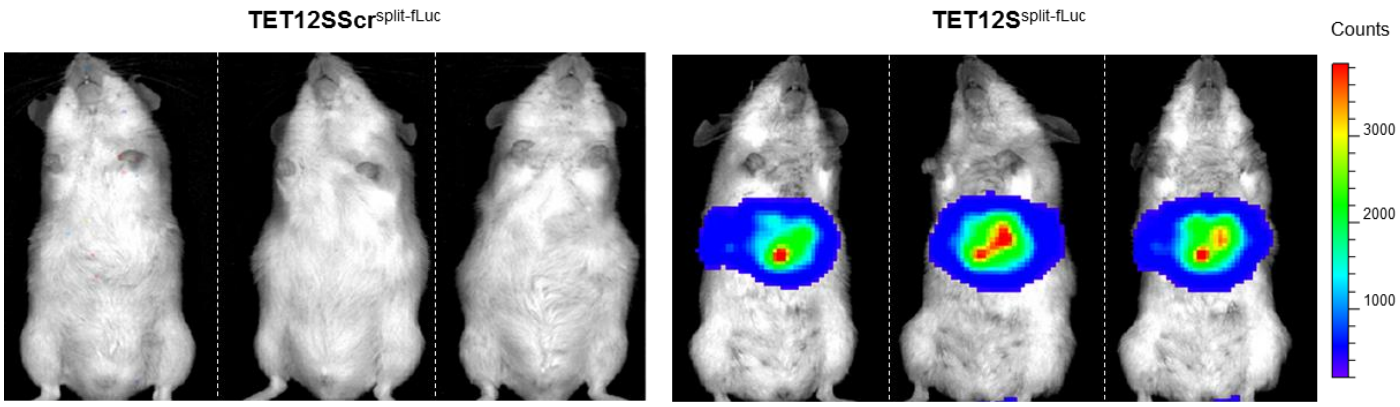








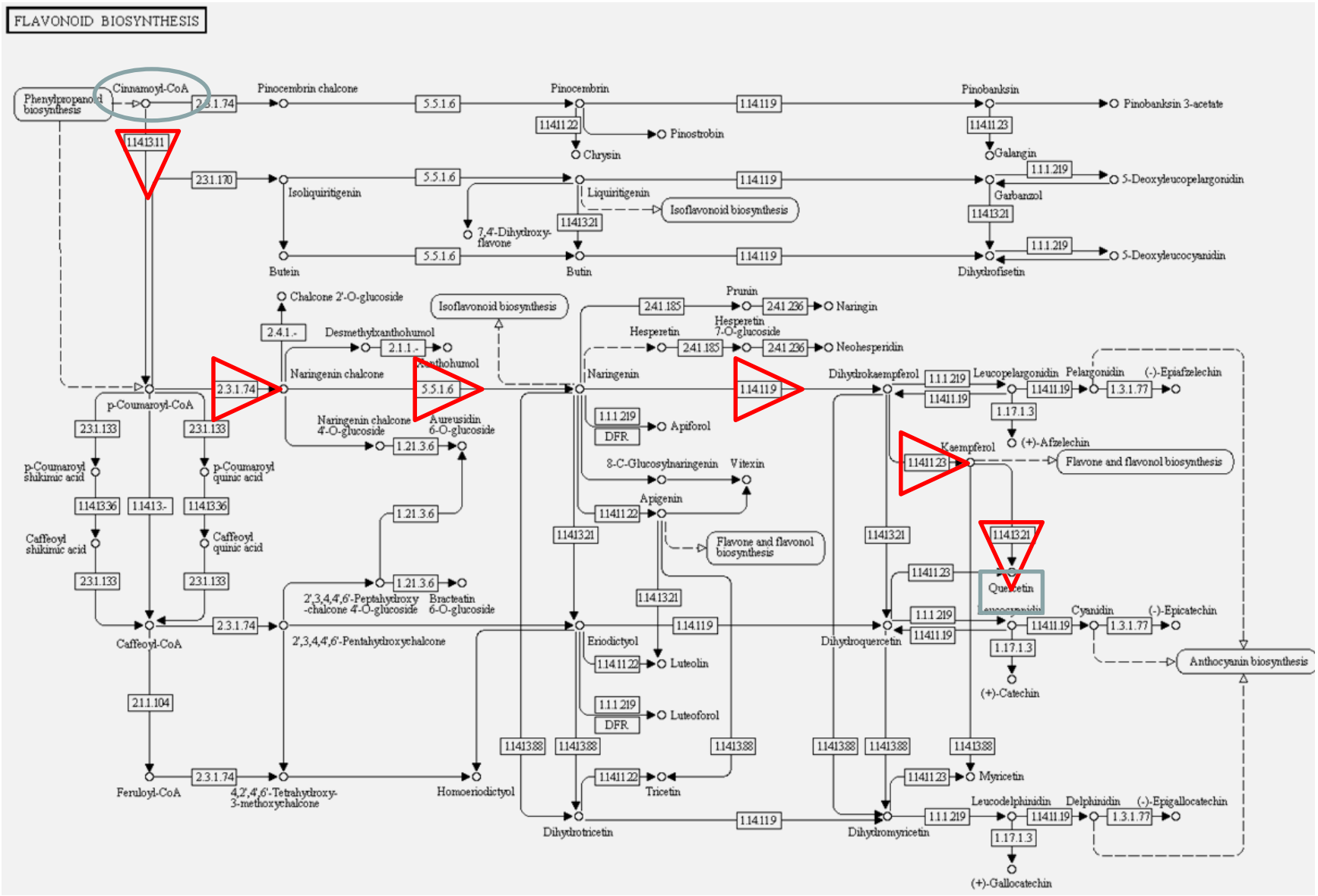
Protein cages correctly folded in mice without inflammation



Medicinska uporaba sintezne biologije

- Boljši način priprave zdravilnih učinkovin
- Priprava novih zdravilnih učinkovin
- Terapevtske celice
- Diagnostika
- Raziskava bioloških procesov s sintezno biologijo
- ...

Večstopenjske biosintezne poti



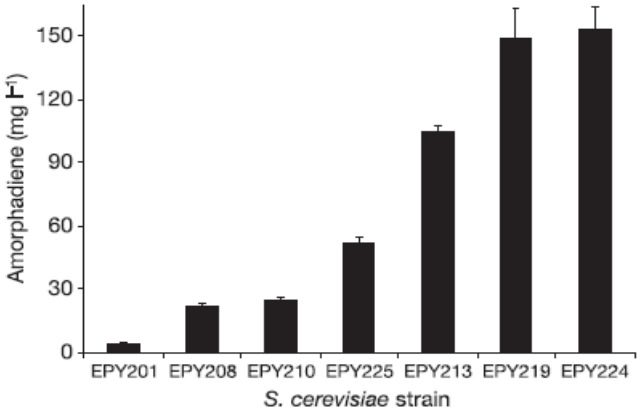
Prenos biosintezne poti v bakterije s pomočjo sintezne biologije

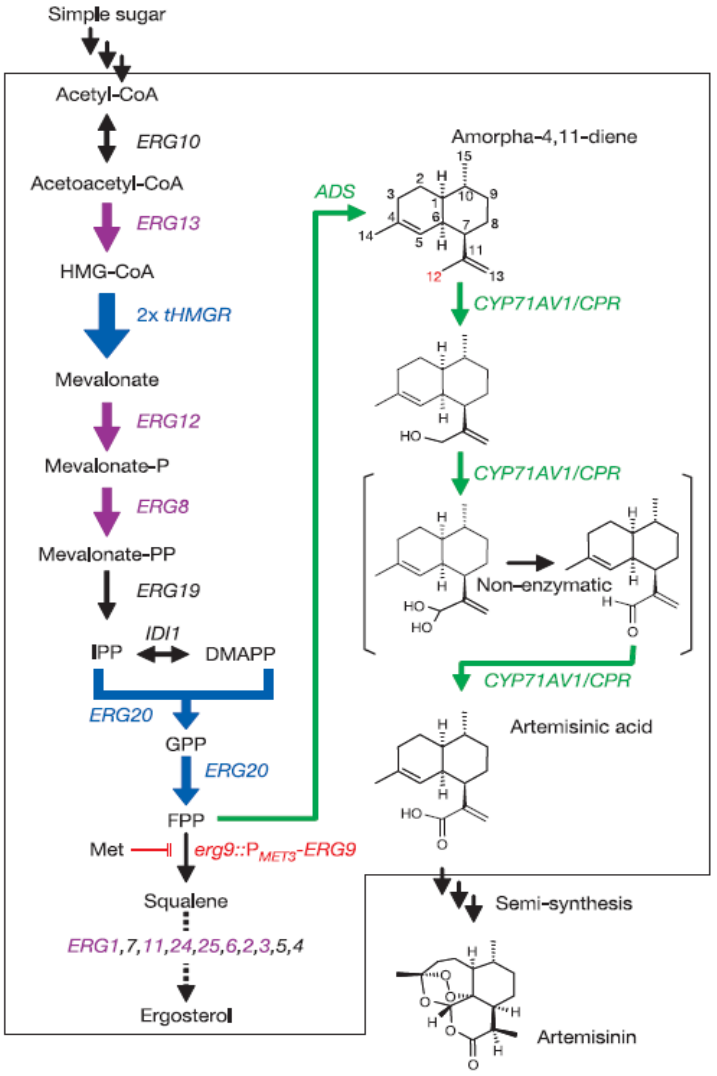
nature Vol 440|13 April 2006|doi:10.1038/nature04640

LETTERS

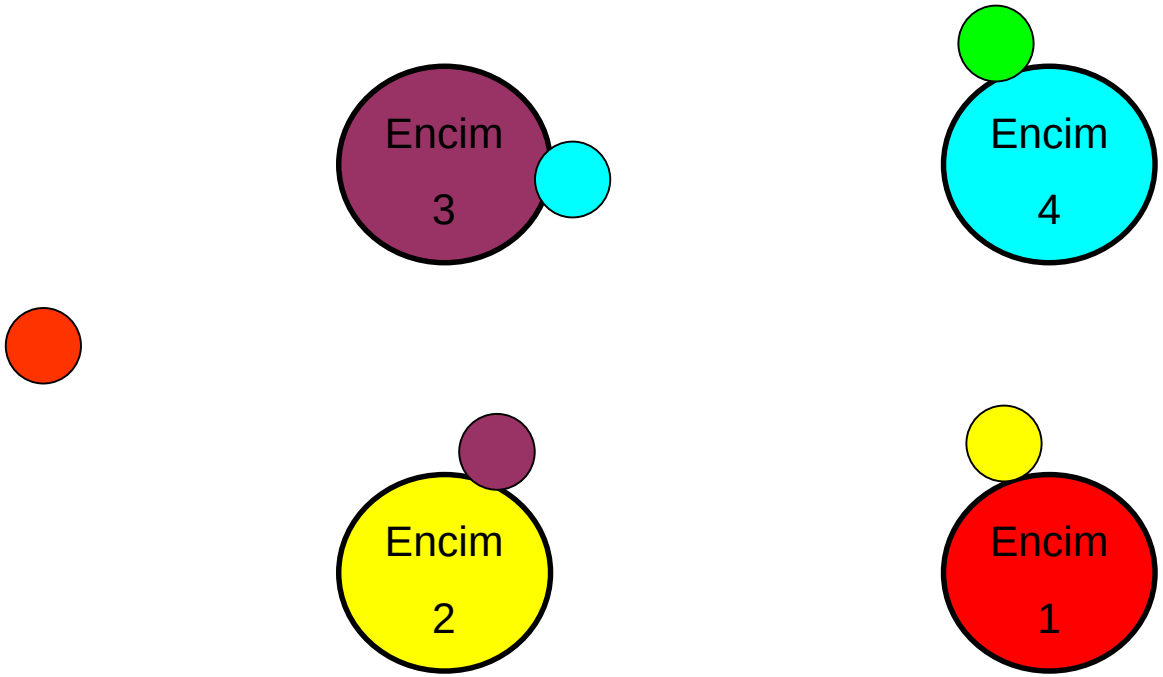
Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]

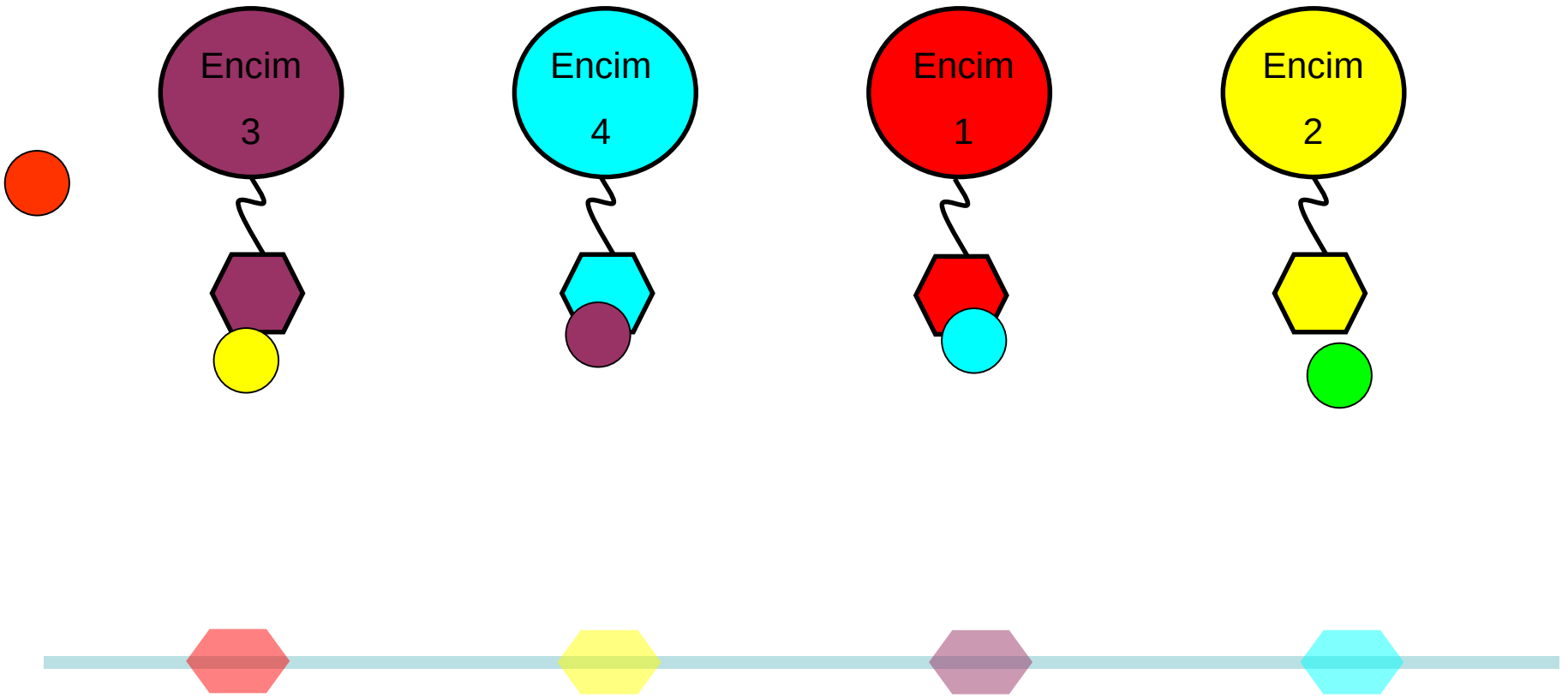




Omejitve več-encimskih katalitskih reakcij

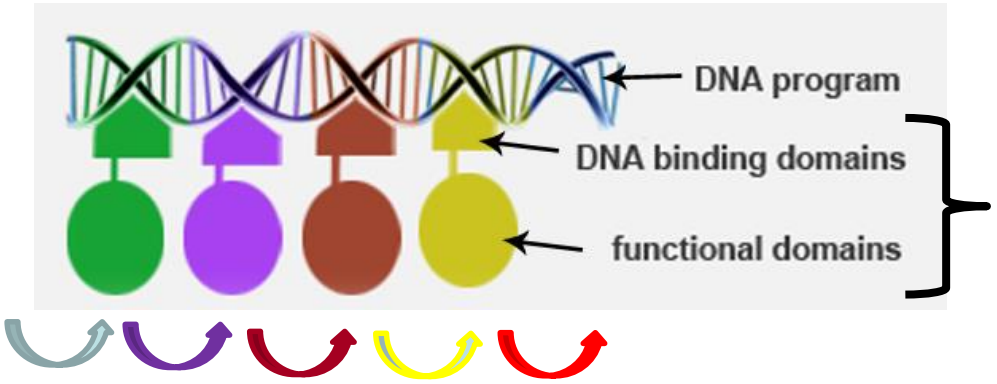


Ogrodje in urejenost biosintetskih encimov za izboljšanje večstopenjske biosinteze



Izboljšana biosinteza s pomočjo DNK ogrodja

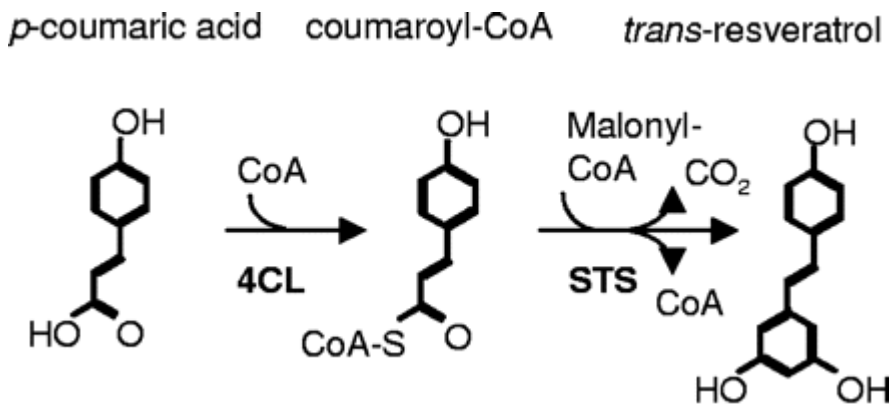
Vrstni red DNK določa zaporedje vezave funkconalnih proteinov

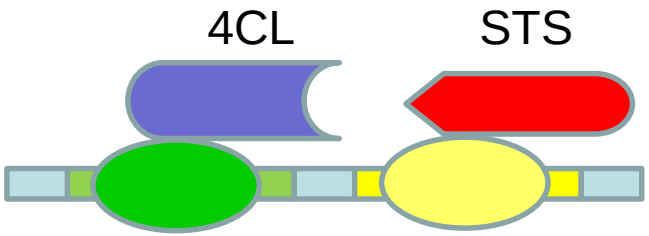


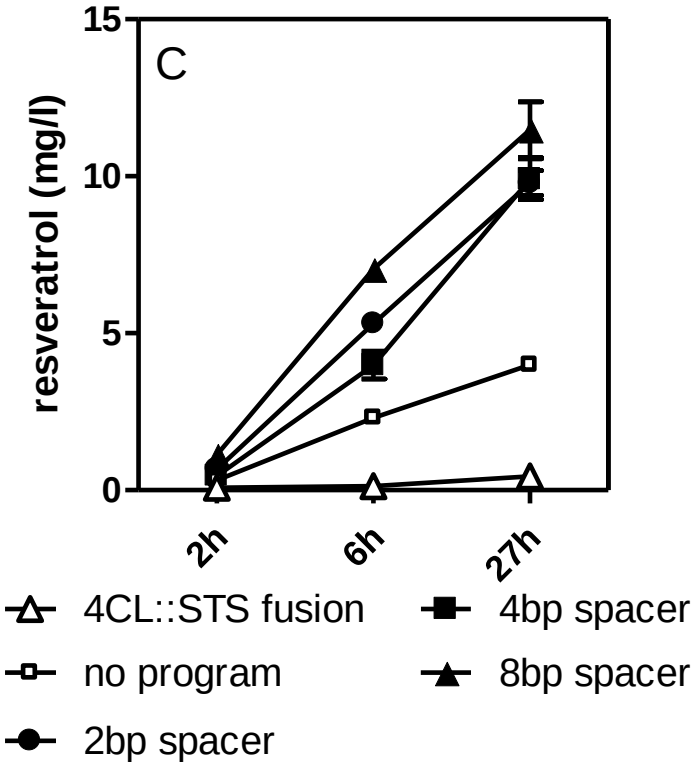
fuzijski proteini med DNK vezalno domeno in encimi

Vezani encimi izvedejo zaporedno modifikacijo substrata

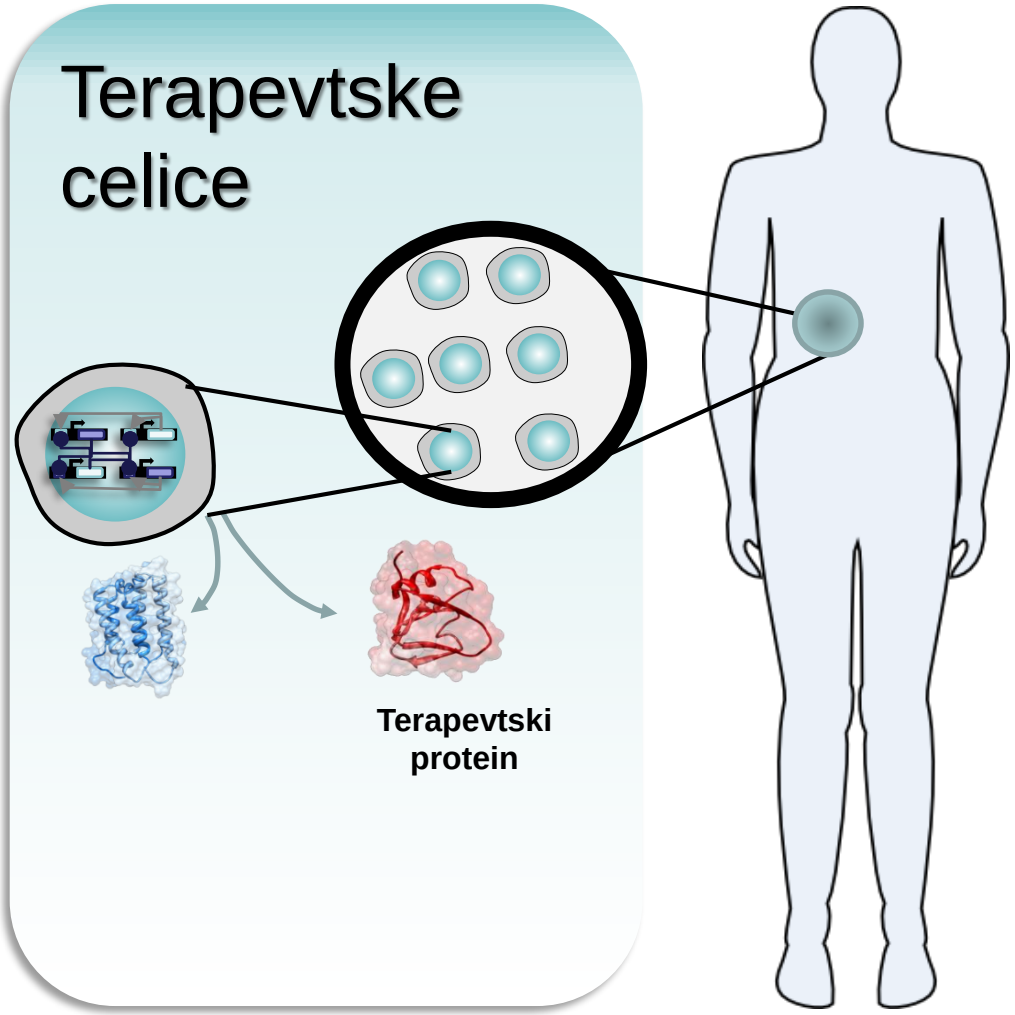
Izboljšana biosinteza s pomočjo DNK ogrodja





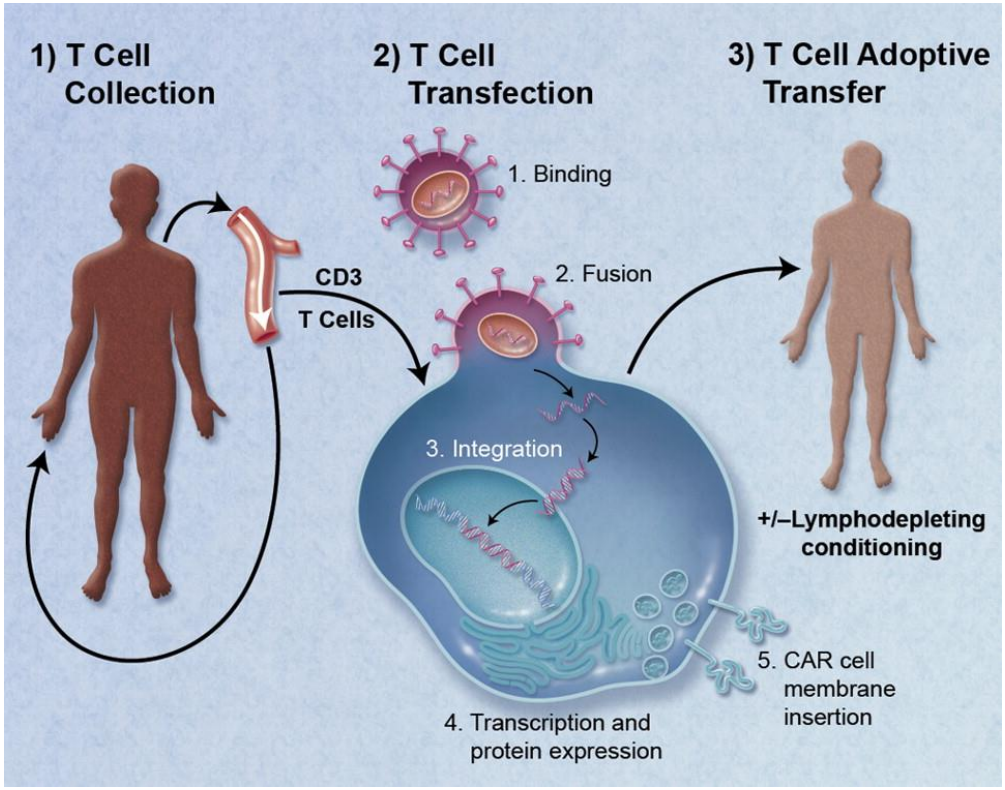


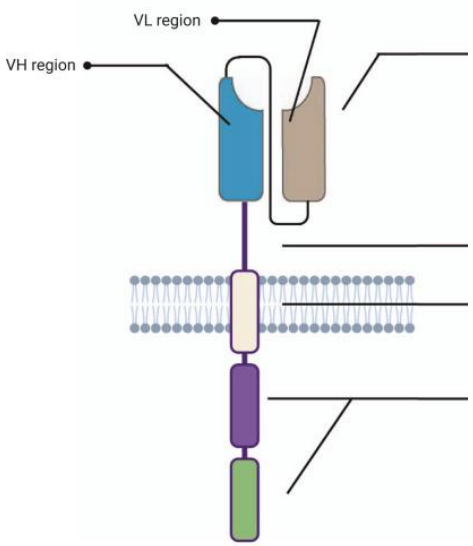
Genska in celična terapija



- Zaznavanje stanja v tkivu
- Uravnavanje z zunanjimi signali
- Zaznavanje kombinacije signalov za večjo specifičnost (npr. rakave celice)
- Celice proizvajajo zdravilne učinkovine v tkivu

Imunoterapija raka s celicami CAR T





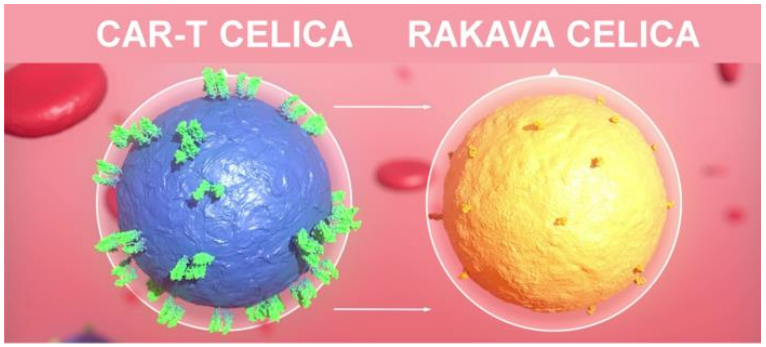
Prepoznavanje tačnega proteina na rakavih celicah (scFv domena protitelesa)

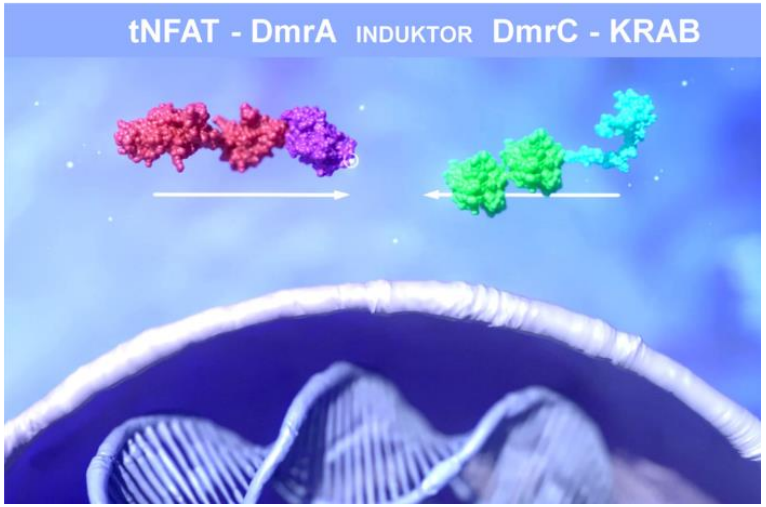
Transmembranska domena

Domene za aktivacijo signalnih poti celic T

Himerni antigenski receptor za prepoznavanje tarčnih celic

Uravnavanje aktivacije celic CAR T za zdravljenje raka



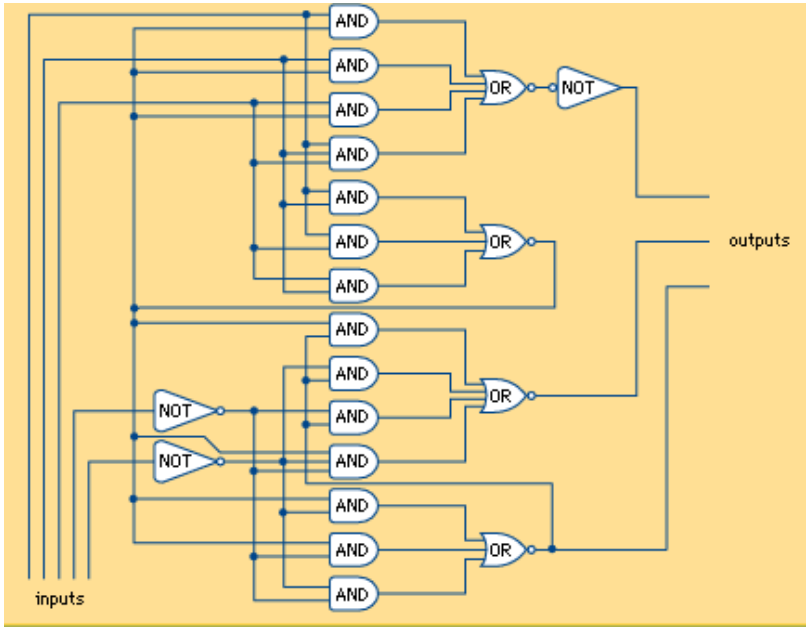


Lainšček in sod., patentna prijava

Procesiranje informacij v electronskih in celičnih vezjih

Electronska vezja

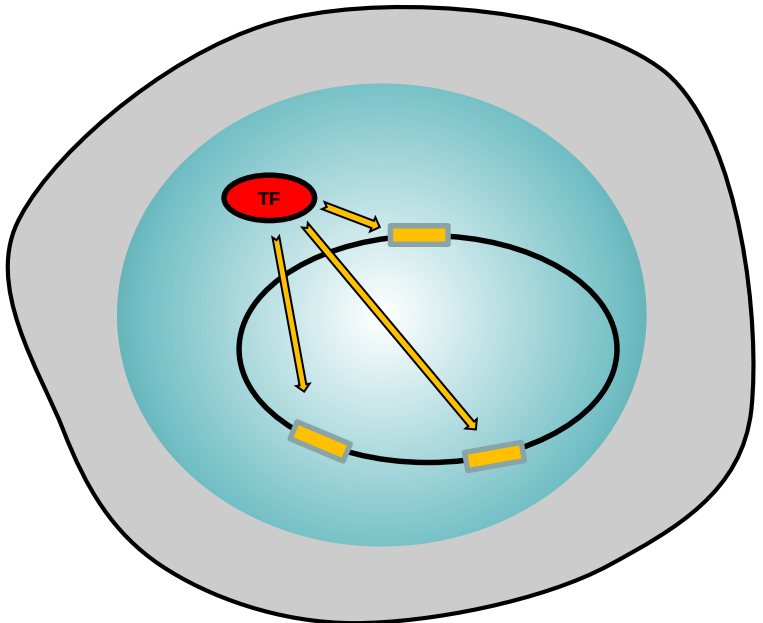
Prevodne žice prenašajo informacijo



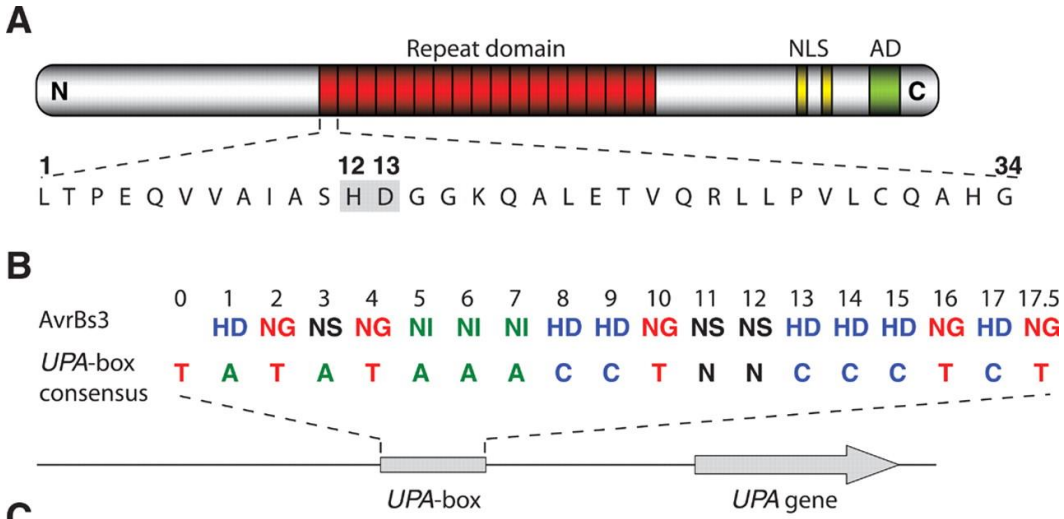
Celice

Difuzija molekul: transkripcijski dejavniki se vežejo na DNK vezalna mesta

ORTOGONALNOST

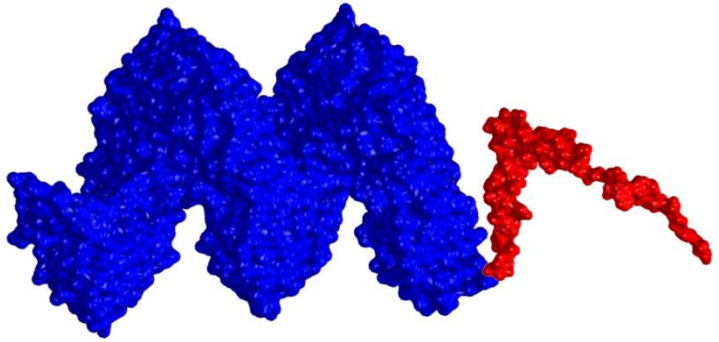


TALE proteini prepoznajo zaporedje DNK



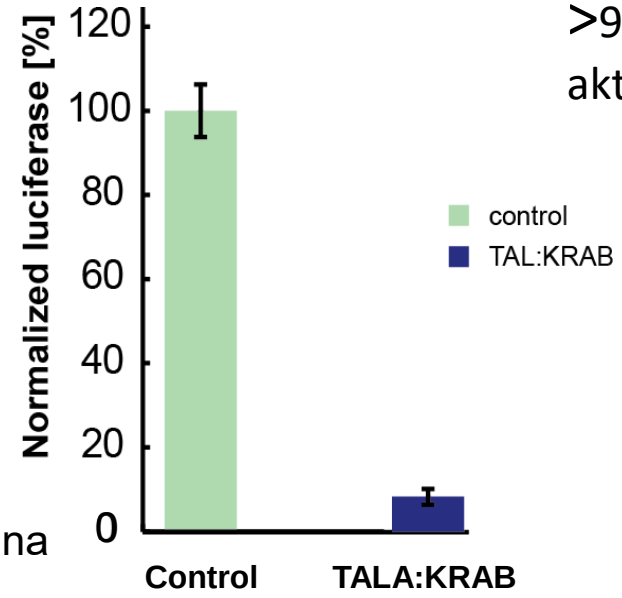
Bloch et al., Science 2009

Umetni TALE aktivatorji in represorji

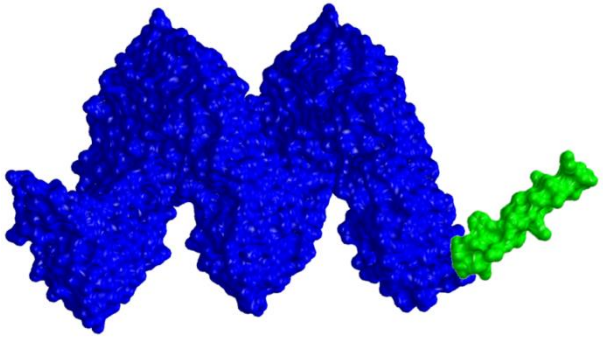


TAL **KRAB**

KRAB: Krueppel-associated box – utišanje kromatina

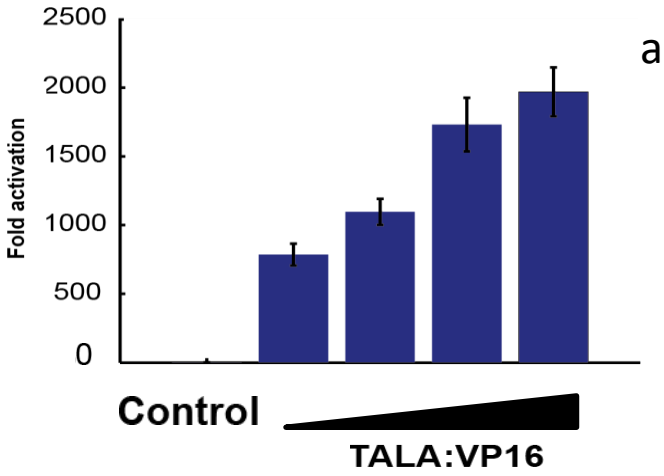


>90% znižanje aktivnosti



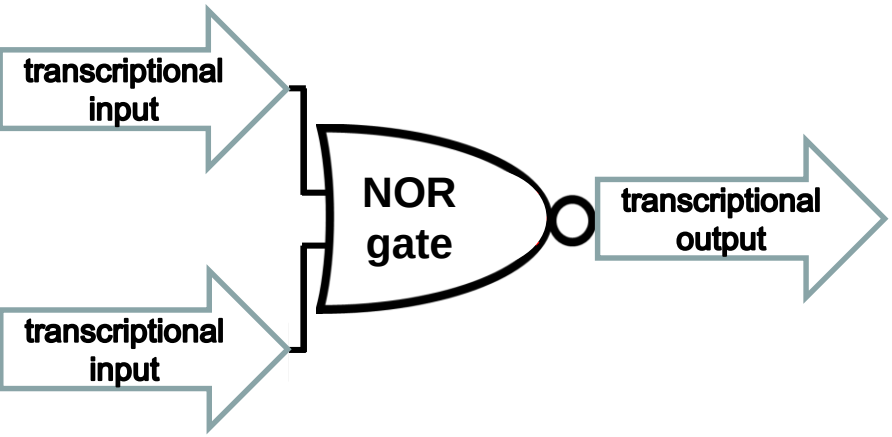
TAL **VP16**

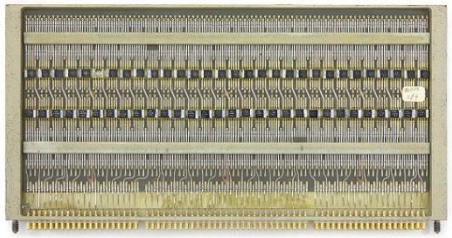
VP16: pritegnitev transkripcijskega kompleksa



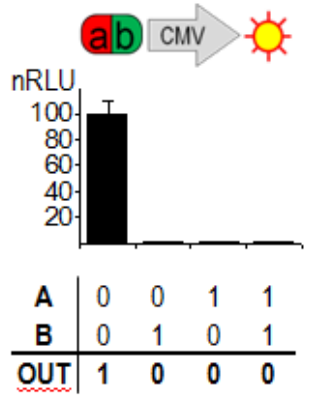
>1500-kratna aktivacija

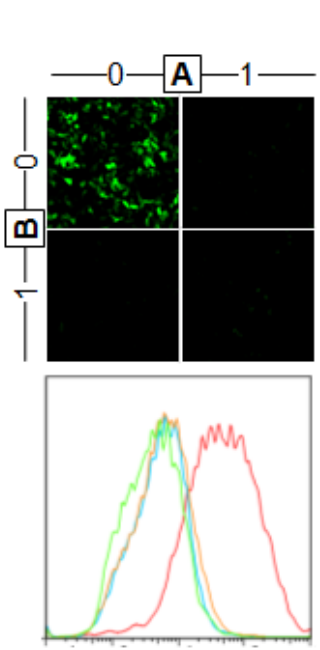
Dizajnirana NOR logična vrata

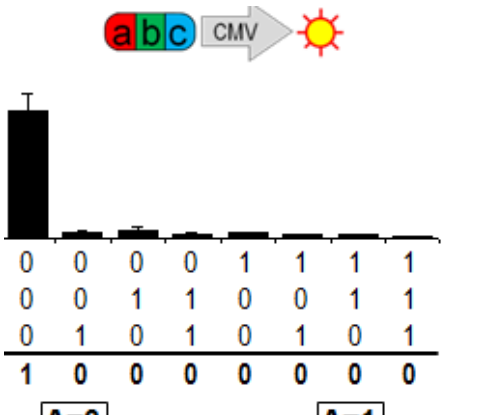


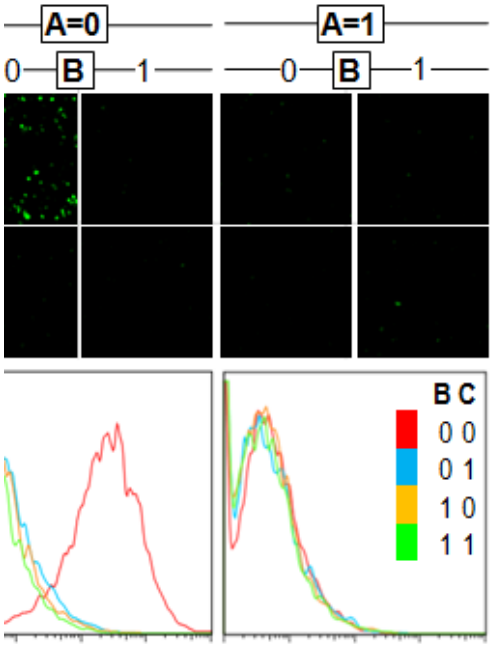


Apollo računalnik
Micrologic chip –
5600 trojnih NOR vrat

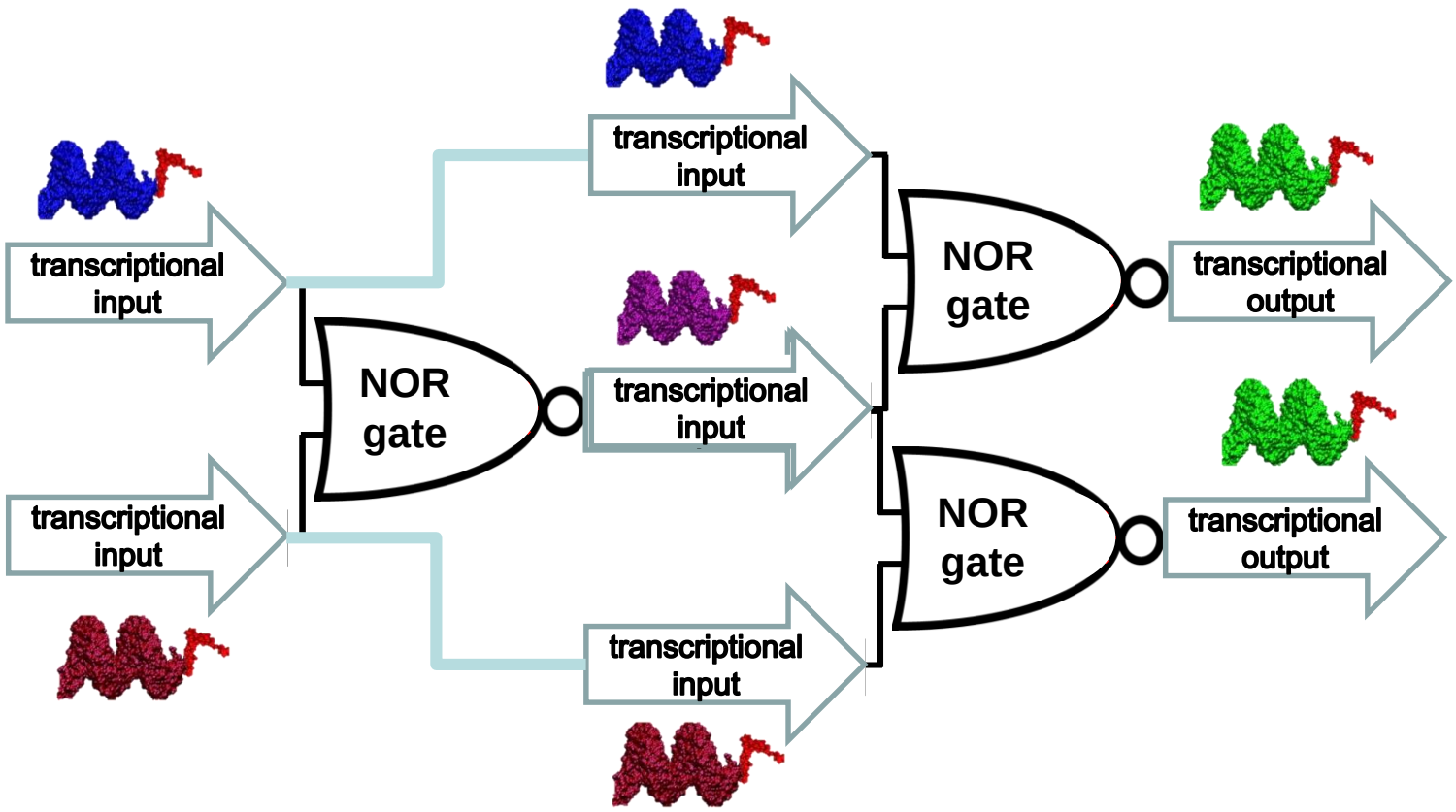




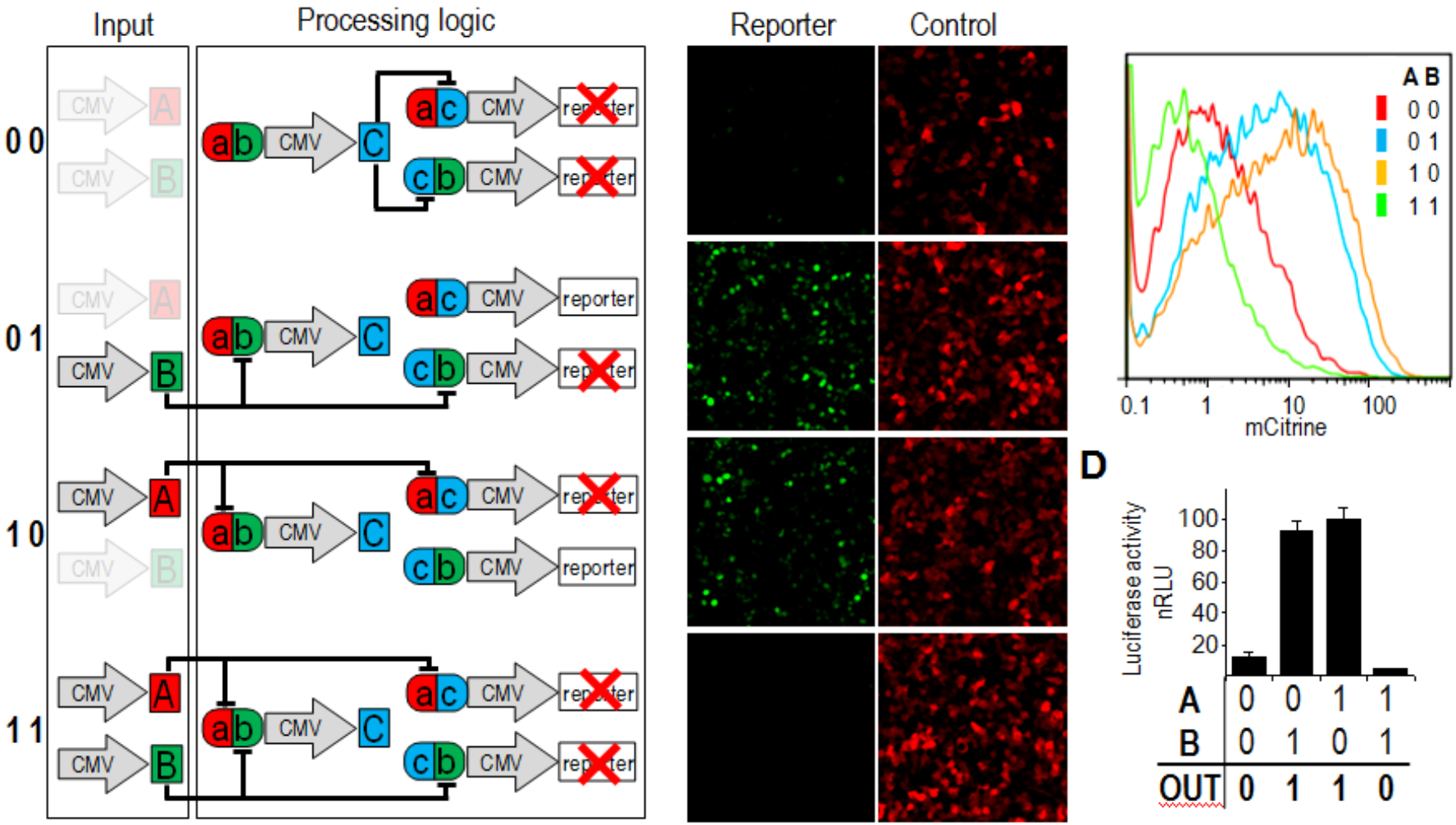




Povezovanje NOR vrat za druge funkcije

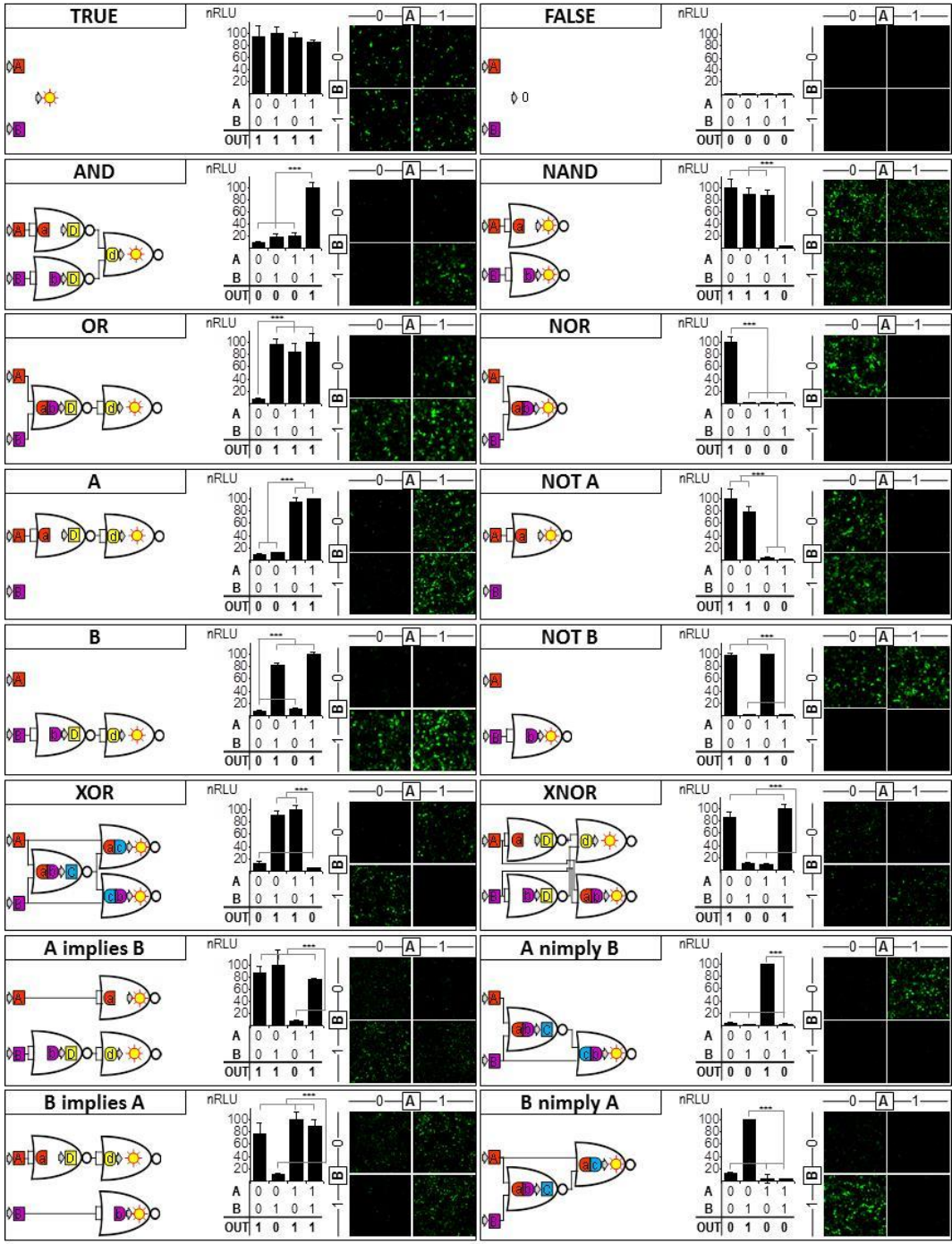


XOR funkcija

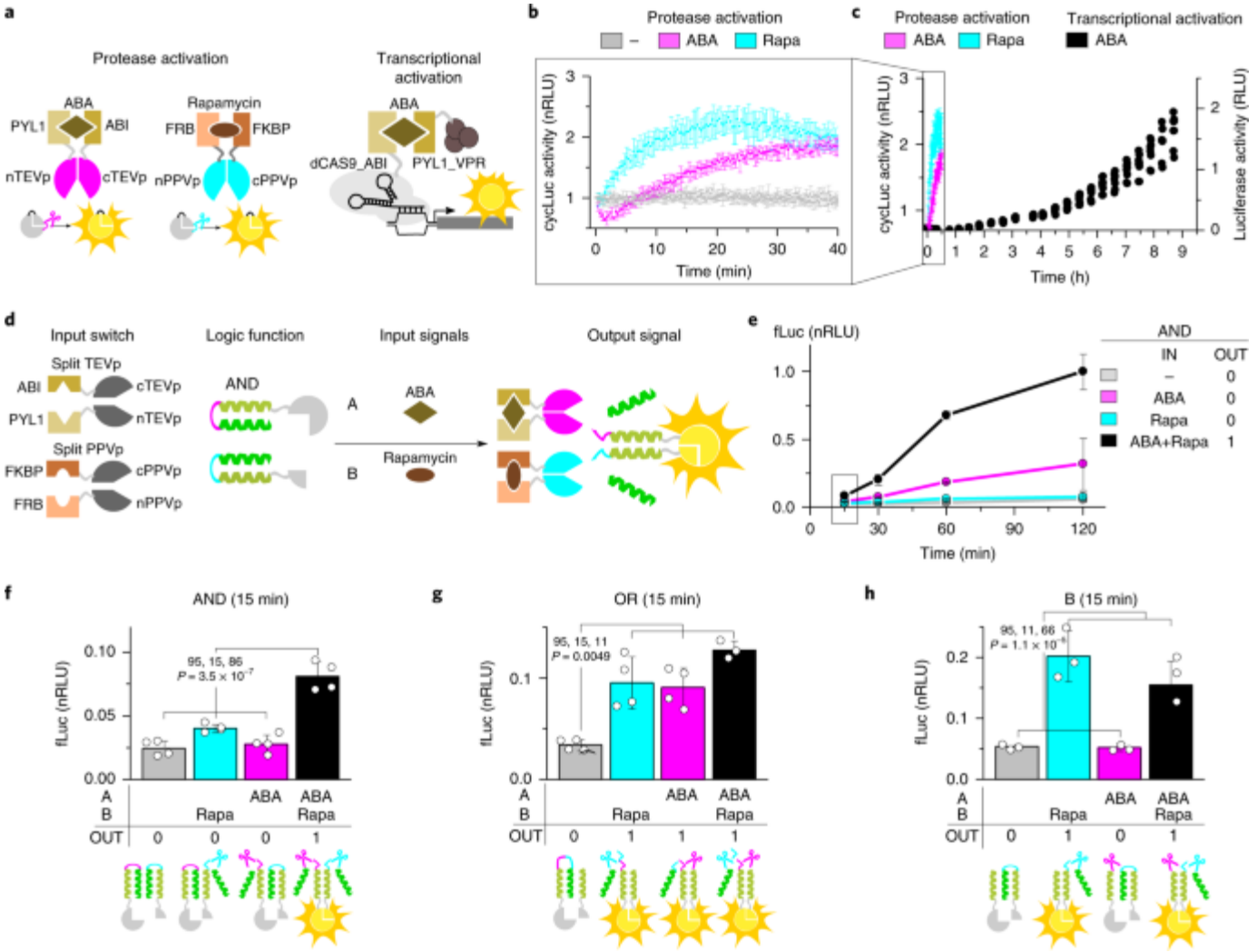


Logične funkcije

Implementacija vseh 16 dvovhodnih logičnih funkcij na osnovi NOR vrat



Hitro odzivna celična vezja



Fink in sod. **Nature Chemical Biology** 2019

Vizija

- Sintezna biologija ter vede o življenju bodo pomembno oblikovale prihodnost (industrija, medicina)
- Na področju medicine bosta vedno večjo vlogo igrali genska in celična terapija

Vizija sintezne biologije kot posnemanja bioloških sistemov

“See things not as they are, but as they might be” Robert Oppenheimer

Hvala vam za pozornost

ter sodelavkam in sodelavcem za sodelovanje in skupne uspehe