



Konferenca NAK – za učitelje naravoslovnih predmetov

## Vede o življenju za tehnologije prihodnosti

dr. Helena Gradišar, Kemijski inštitut  
CO EN-FIST



CENTER ODLIČNOSTI

4. konferenca učiteljev naravoslovnih predmetov – NAK 2017



REPUBLIKA SLOVENIJA  
MINISTRSTVO ZA IZOBRAŽEVANJE,  
ZNANOST IN ŠPORT



EVROPSKA UNIJA  
EVROPSKI  
SOCIALNI SKLAD  
NALOŽBA V VAŠO PRIHODNOST

Naložbo sofinancirata Republika Slovenija in Evropska unija iz Evropskega socialnega sklada

# Odsek za sintezno biologijo in imunologijo



# Pregled predstavitve

- Kratak uvod v sintezno biologijo
- Biosintezni tekoči trak
- Celični računalnik - logične operacije in stikalo
- **Proteinske nanostrukture**





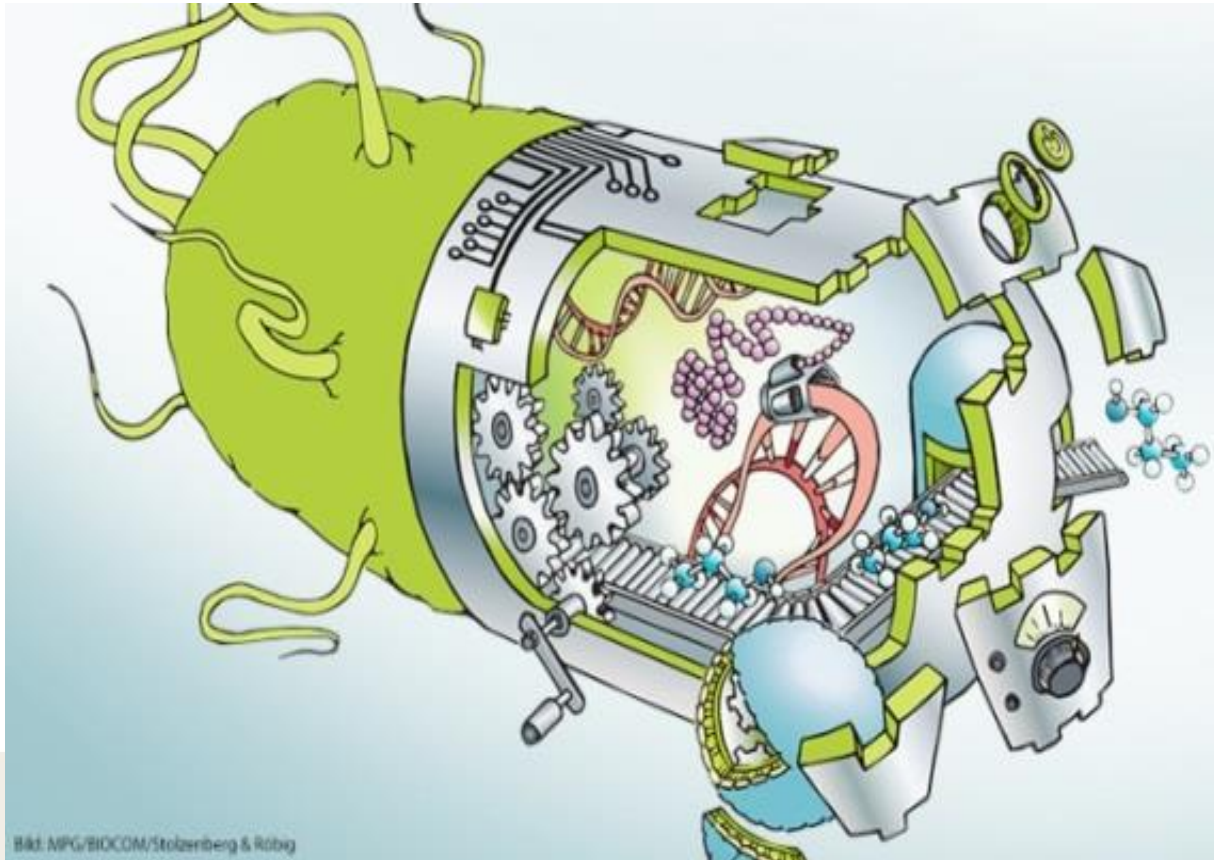
“What I cannot create, I do not  
*understand!*”

Fizik Richard Feynman



# KAJ JE SINTEZNA BIOLOGIJA ?

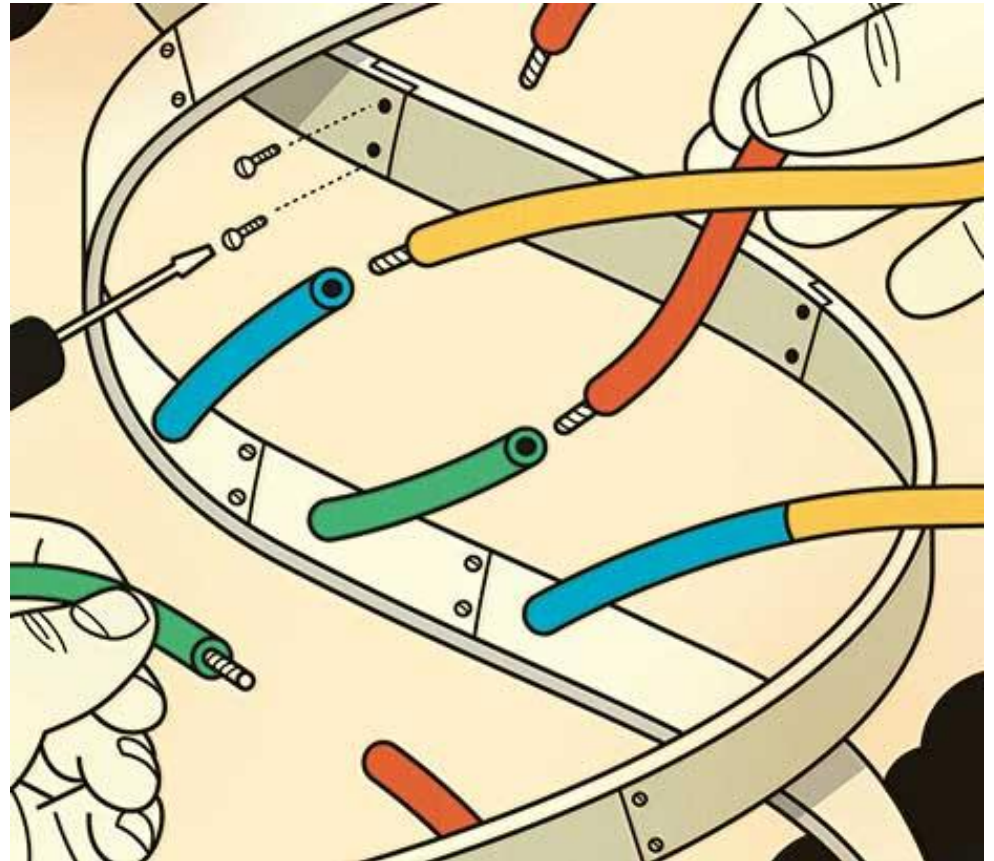
- Interdisciplinarna veda, združuje znanja ved o življenju
- Nove biološke funkcije in sistemi, ki v naravi ne obstajajo



# Sintezna biologija kot raziskovalno orodje

## Inženirski principi

- modularnost
- standardizacija
- abstrakcija
- zanesljivost
- predvidljivost



# Uvaja inženirske pristope v biologijo

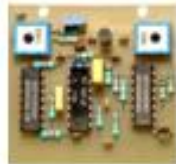
Računalniška omrežja



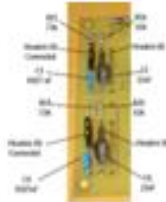
Računalniki



Moduli



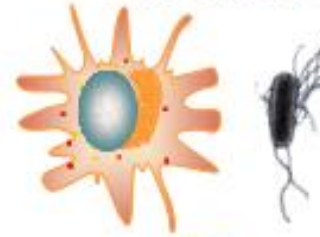
Elektronska vezja



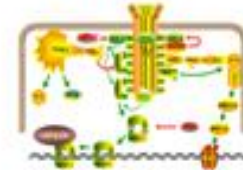
Elektronski elementi



Tkivo, organizem



Celice



Biokemijske poti



Biokemijske reakcije

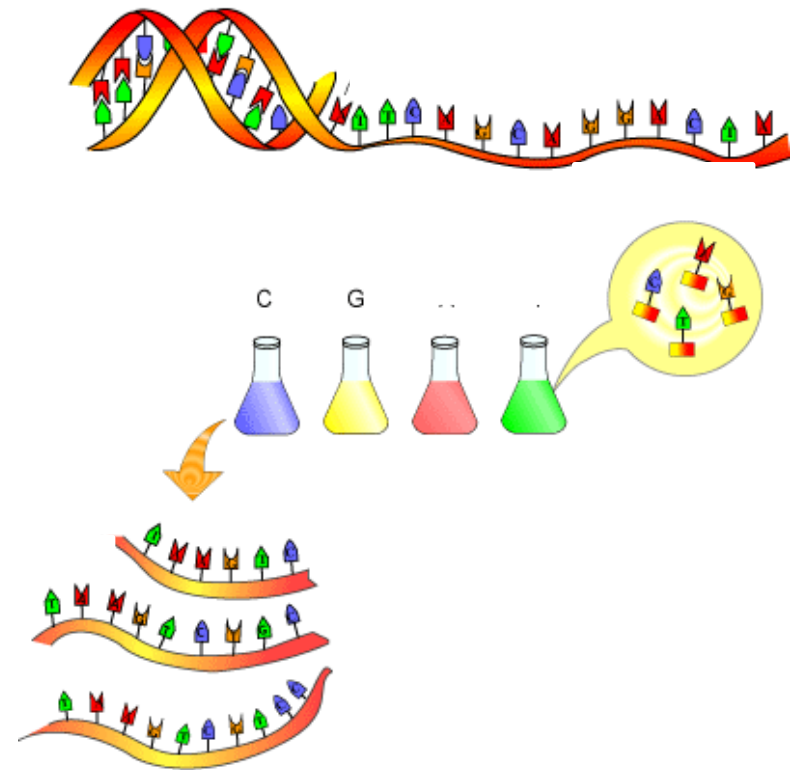


**MODULI** –  
geni, proteini



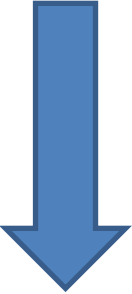
# Metode v sintezni biologiji

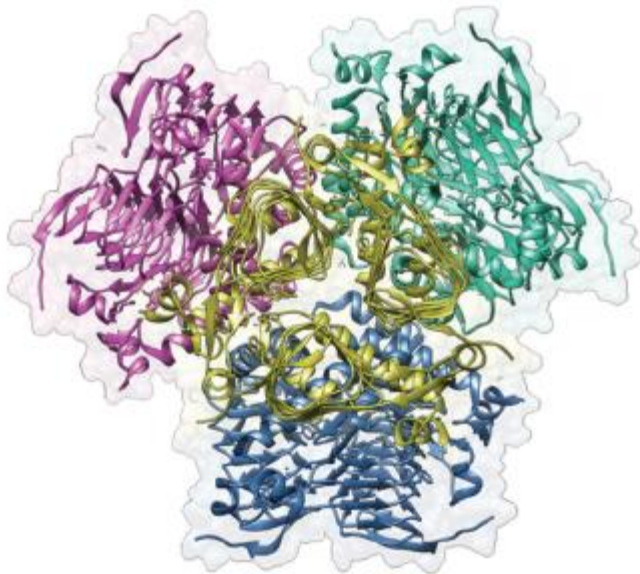
- Sekveniranje DNA zaporedij
- Sinteza umetnih DNA zaporedij
- Metode molekularne biologije
- Določanje struktur proteinov
- Računalniško modeliranje



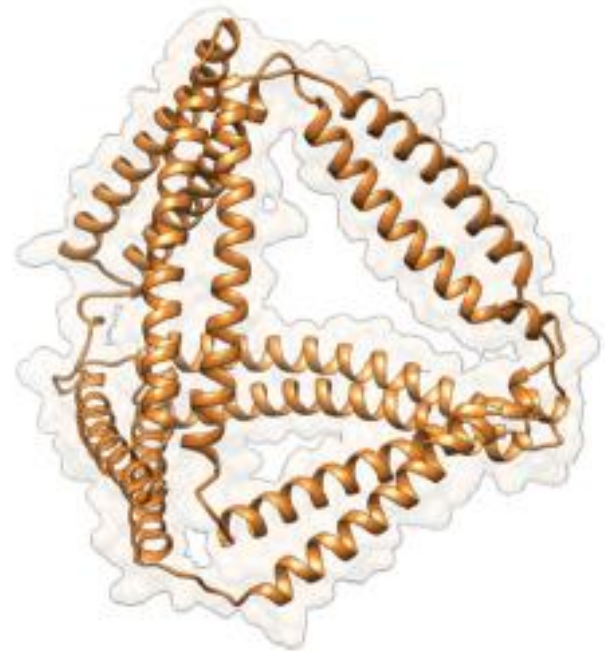


# Dva različna pristopa v sintezni biologiji

- 
- nadgradnja obstoječega ogrodja



- 
- sestavljanje iz modulov



# Sintezna biologija – prihajajoča tehnološka revolucija na različnih področjih

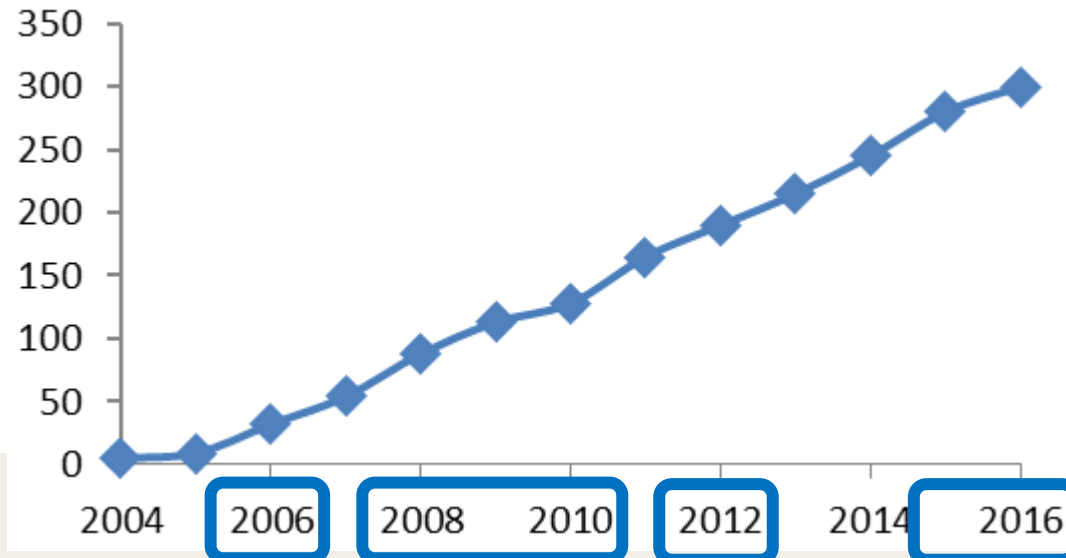
- Medicina (zdravljenje in diagnostika)
- Biotehnološki procesi
- Novi materiali in bionanomateriali
- Procesiranje informacij
- Biosenzorji
- Obnovljivi viri energije
- Varstvo okolja
- Novo razumevanje življenja



# international Genetically Engineered Machines

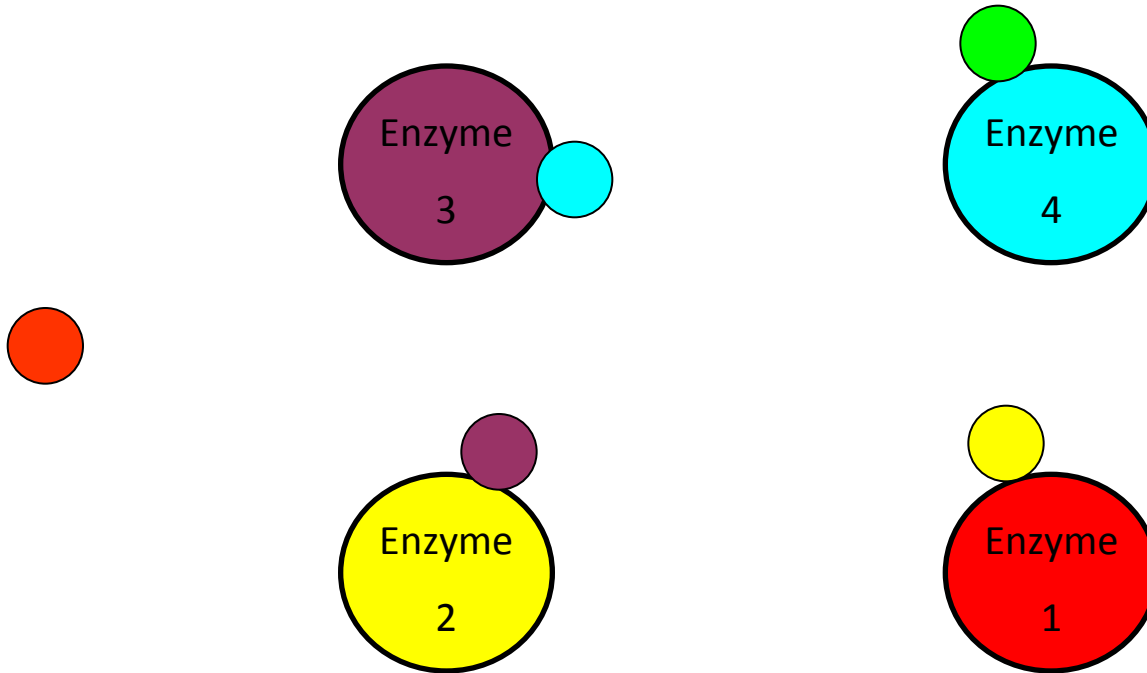


- študentsko tekmovanje v sintezni biologiji, MIT, Boston
- Slovenija 3x Grand prize

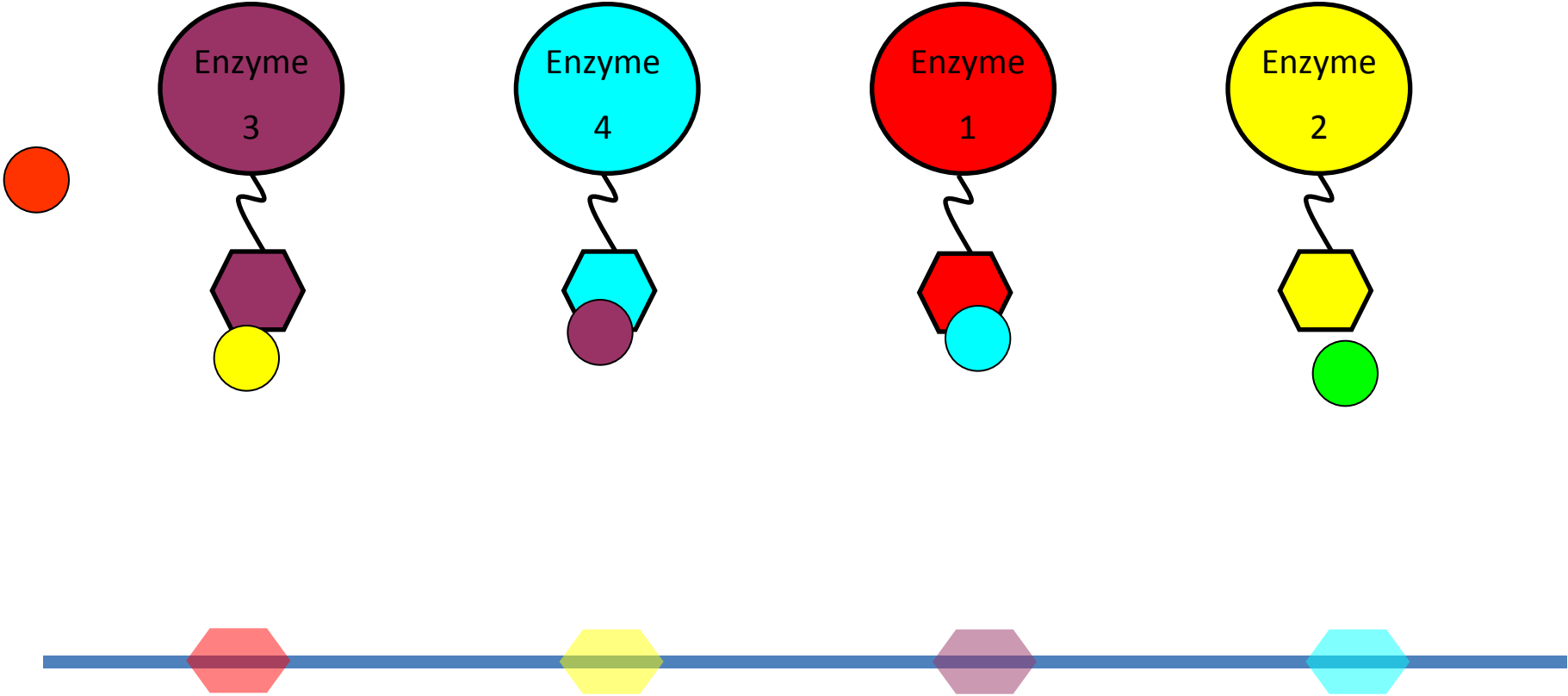


# 1. BIOSINTEZNI TEKOČI TRAK

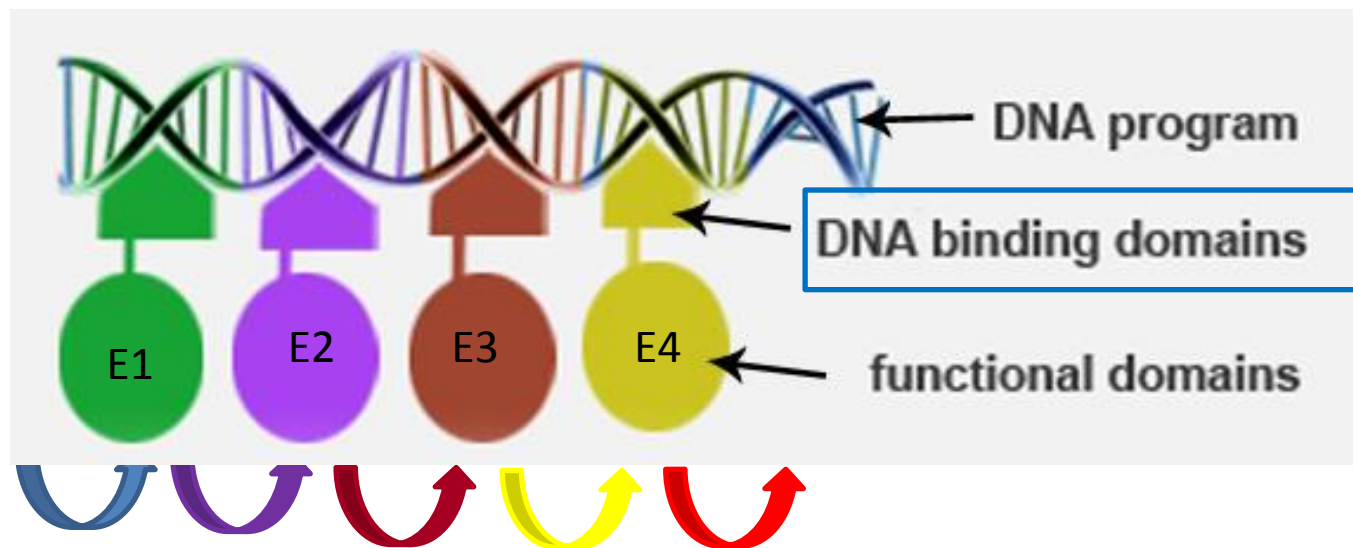
- Večstopenjske katalizirane reakcije



# Ureditvev encimov na ogrodje



# Fuzija funkcionalnih encimov in DNA-vezavnih domen



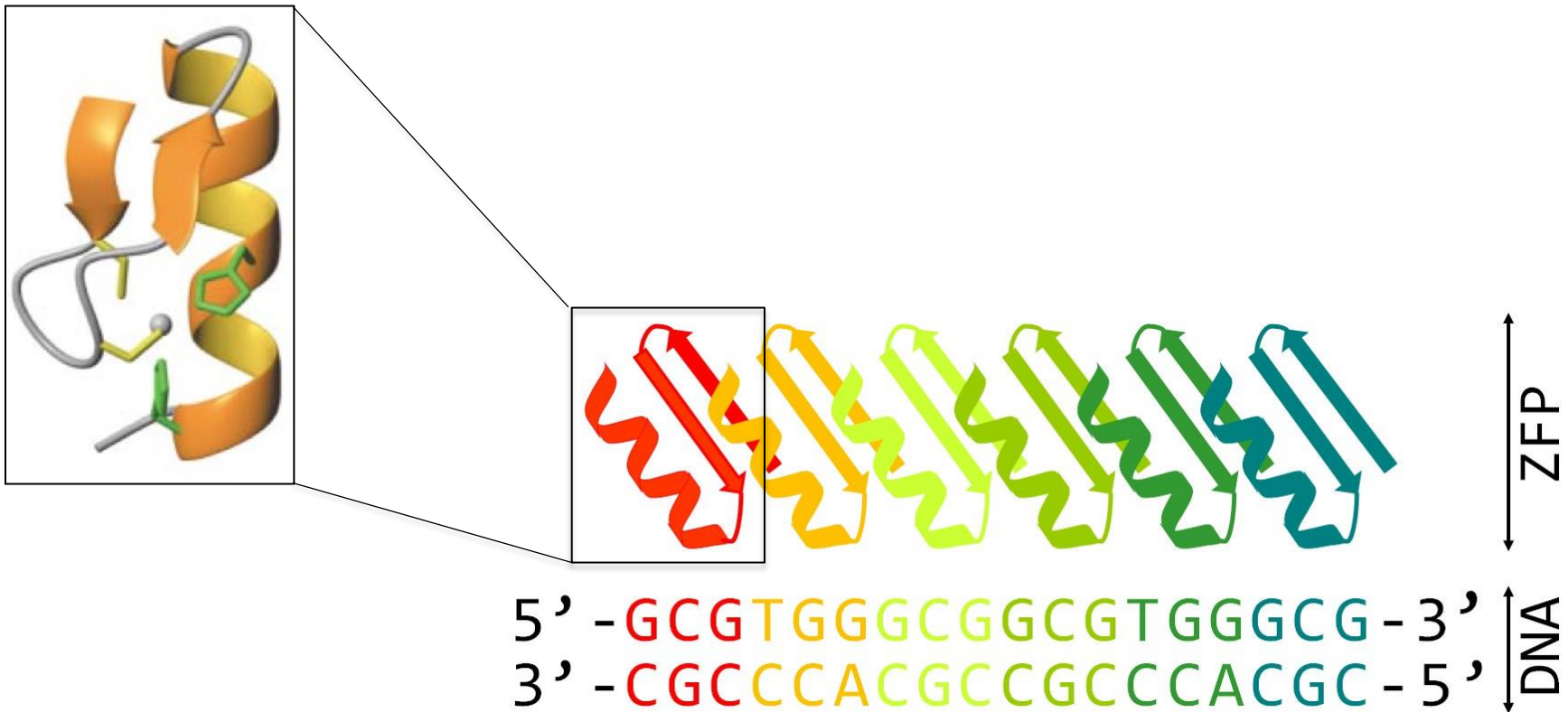
Izhodna spojina

Produkt



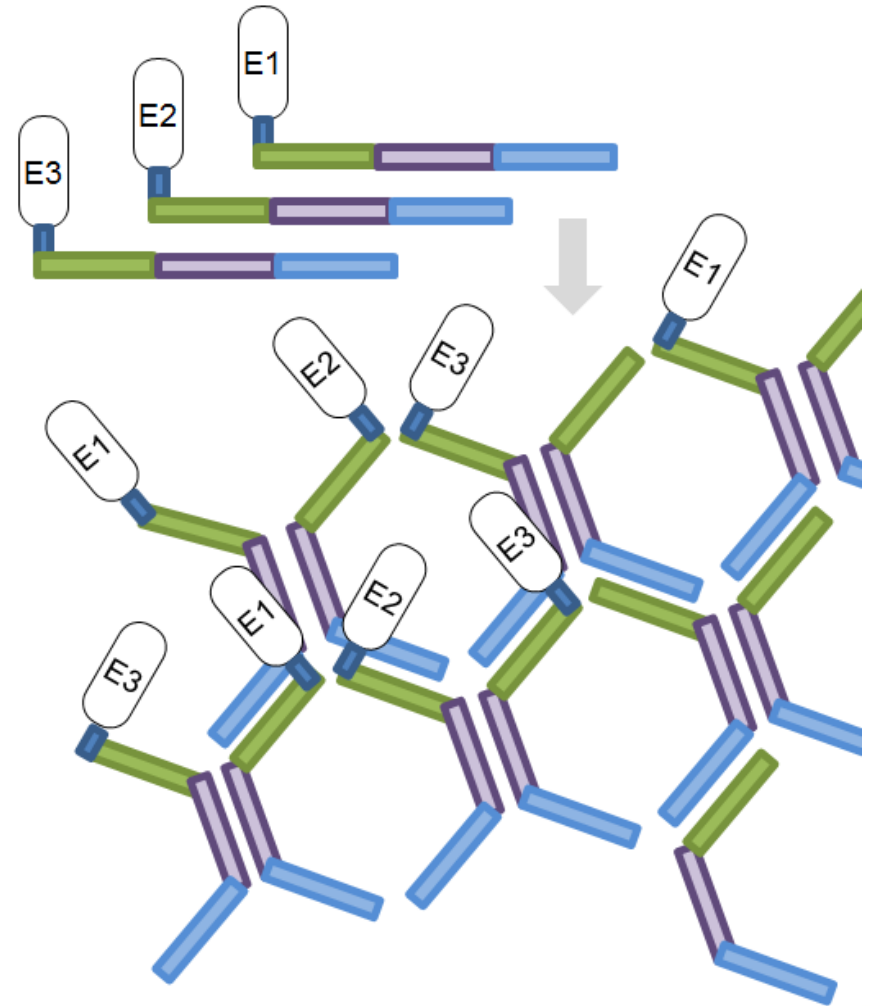
# Proteini z motivi zinkovih prstov (ZFP)

- Modularnost
- Prepoznavajo specifično zaporedje DNA



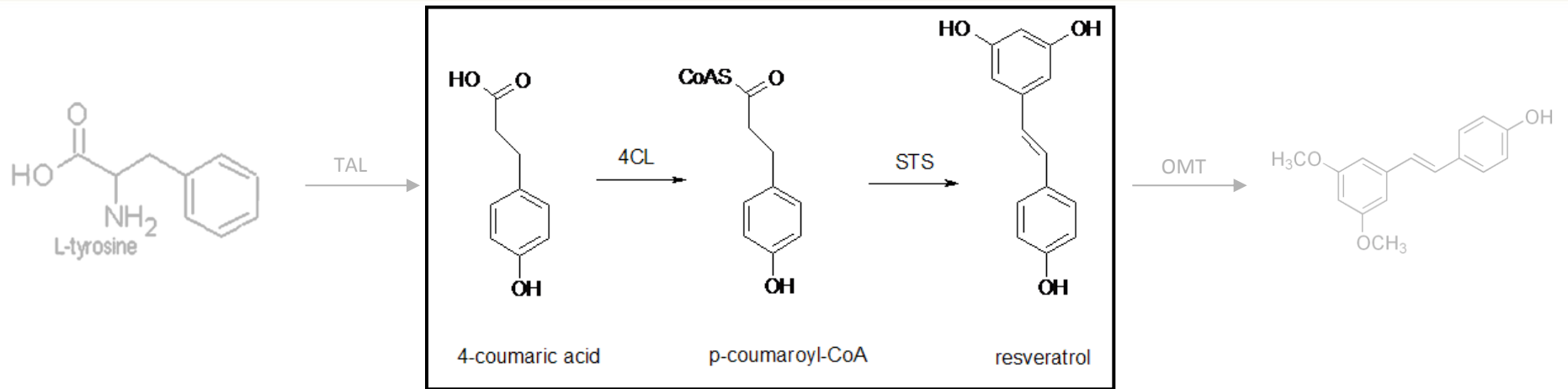
# Vezava funkcionalnih encimov na proteinsko ogrodje

- Večji izkoristki reakcije
- Hitreje do produkta
- Manj stranskih produktov

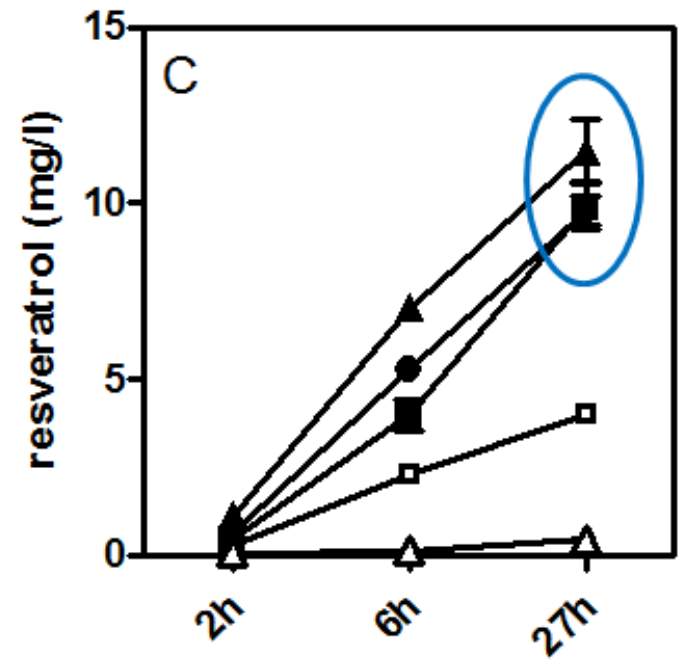




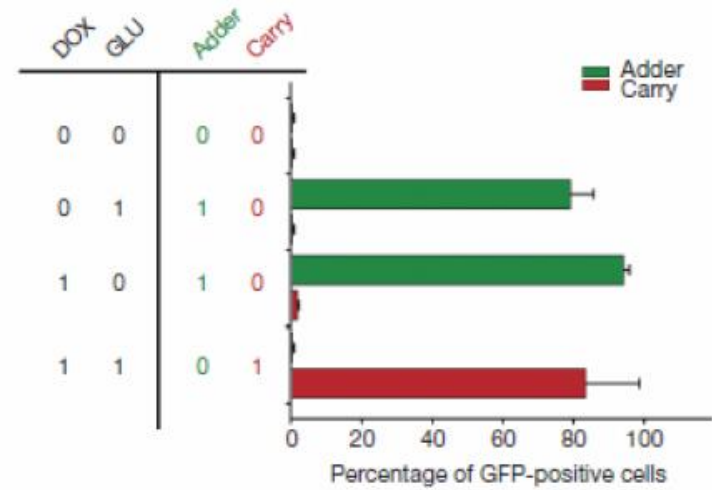
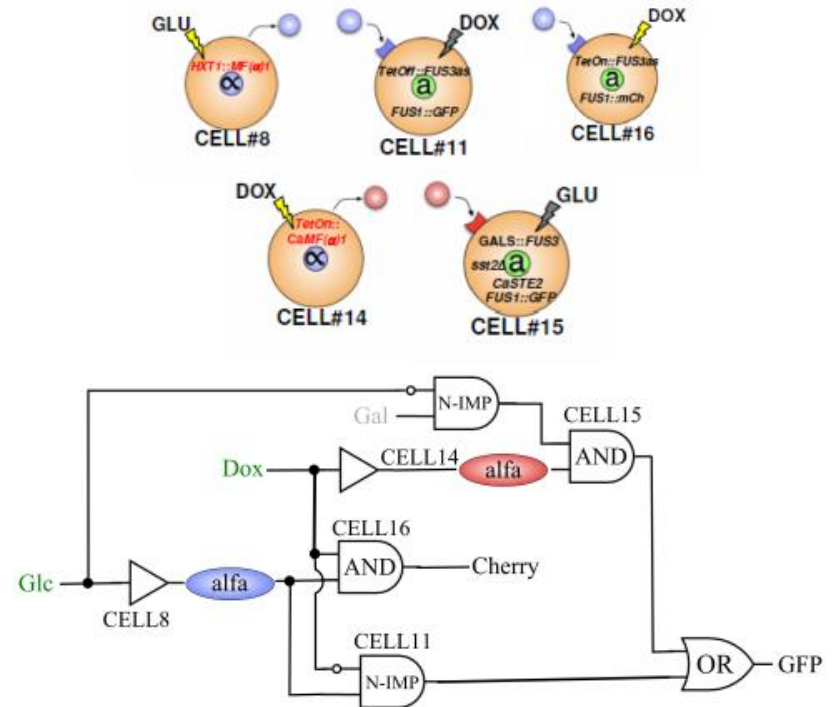
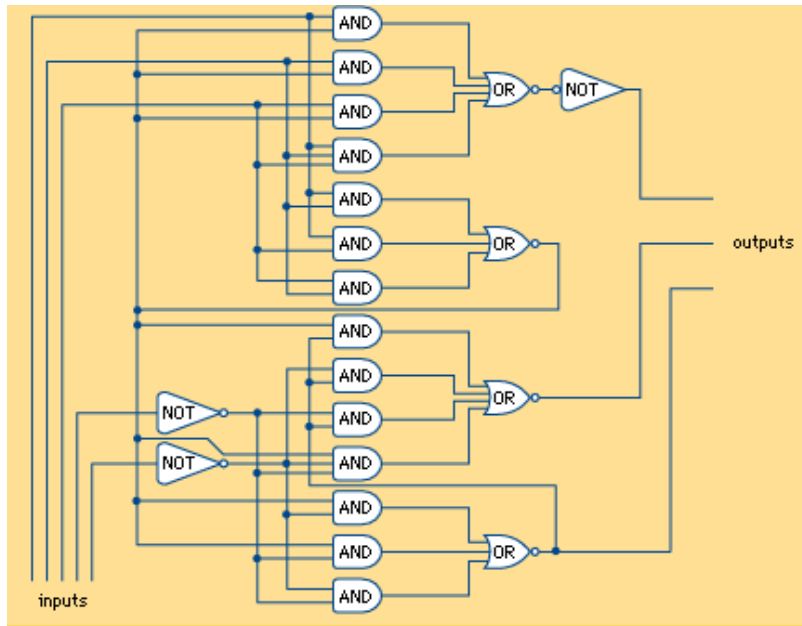
# Izboljšanje biosinteze resveratrola



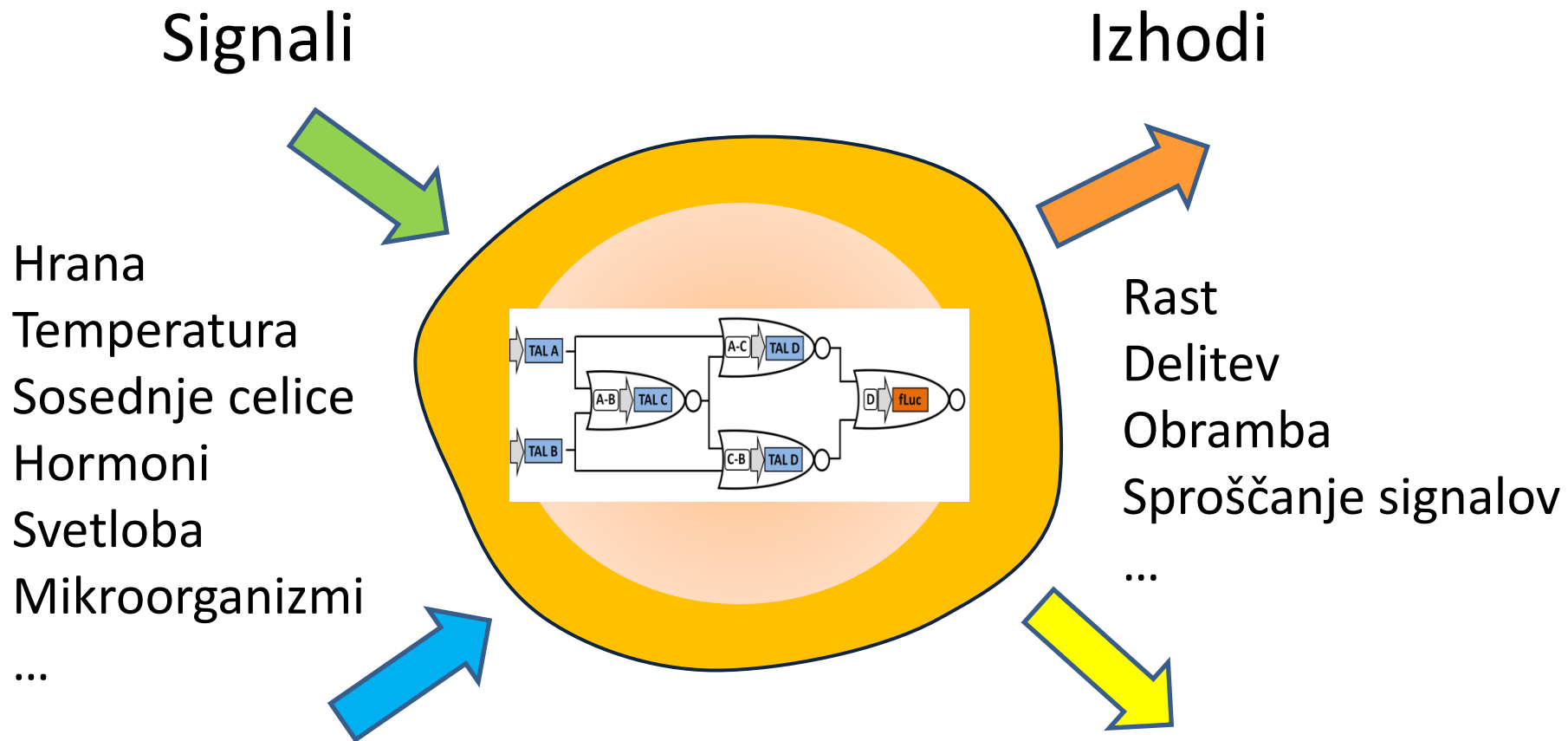
Team Slovenia - iGEM 2010 project  
**DNA coding beyond triplets**



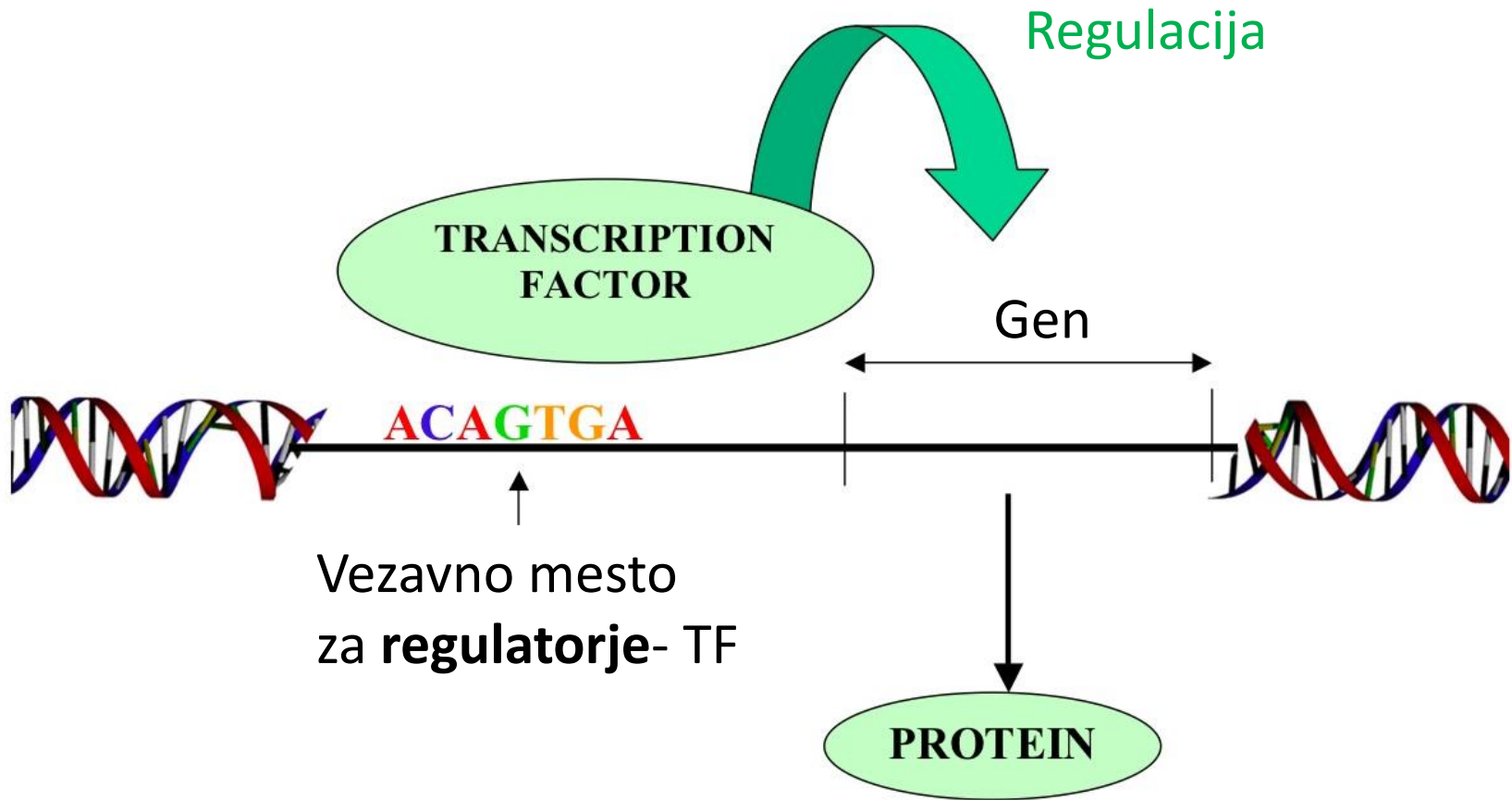
# 2. CELIČNI RAČUNALNIK



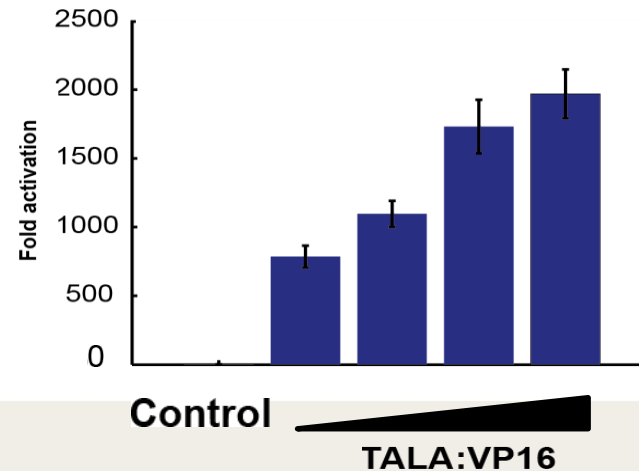
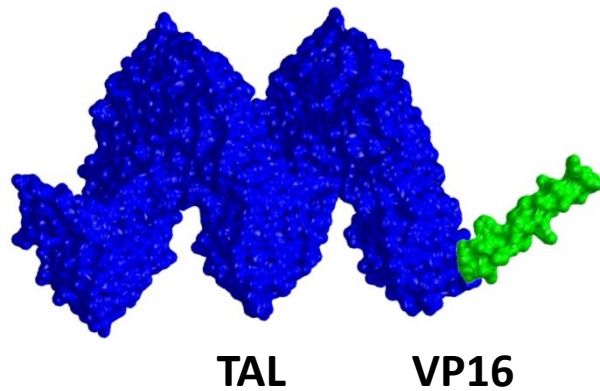
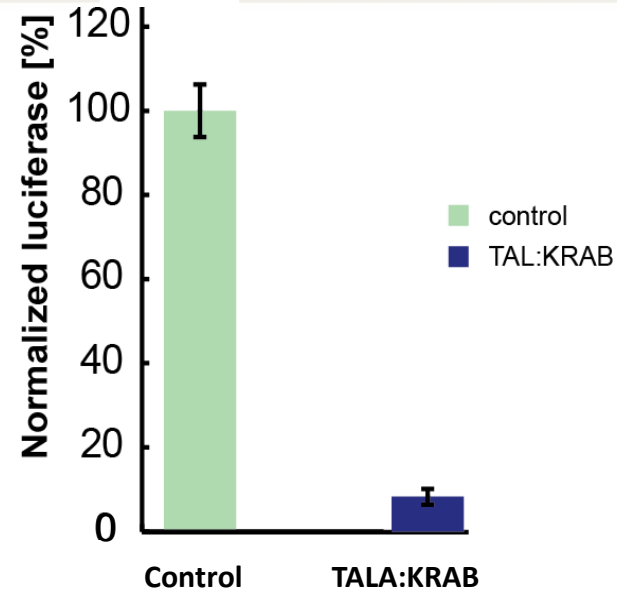
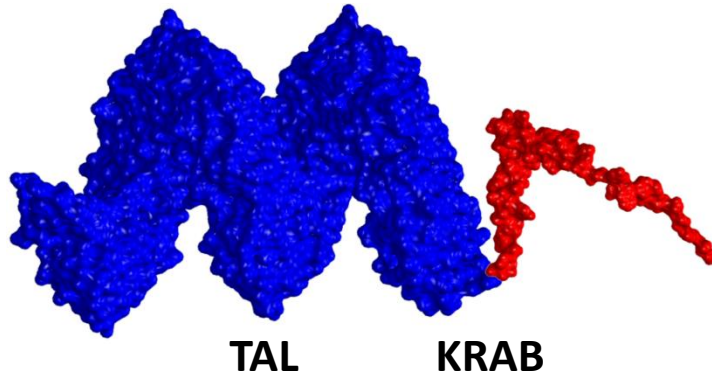
# Celice kot procesorji informacij



# Celični posredniki informacij

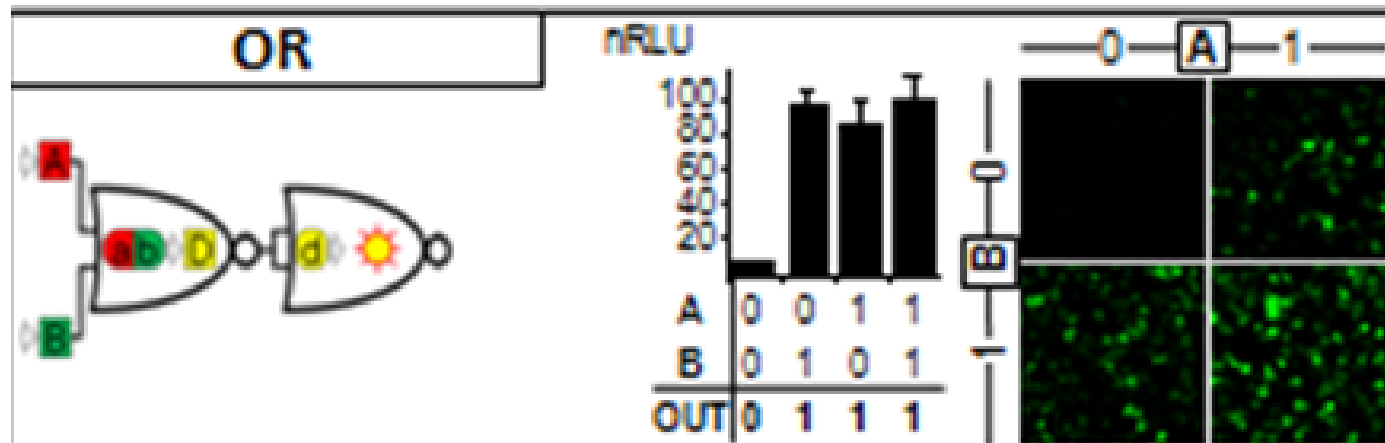
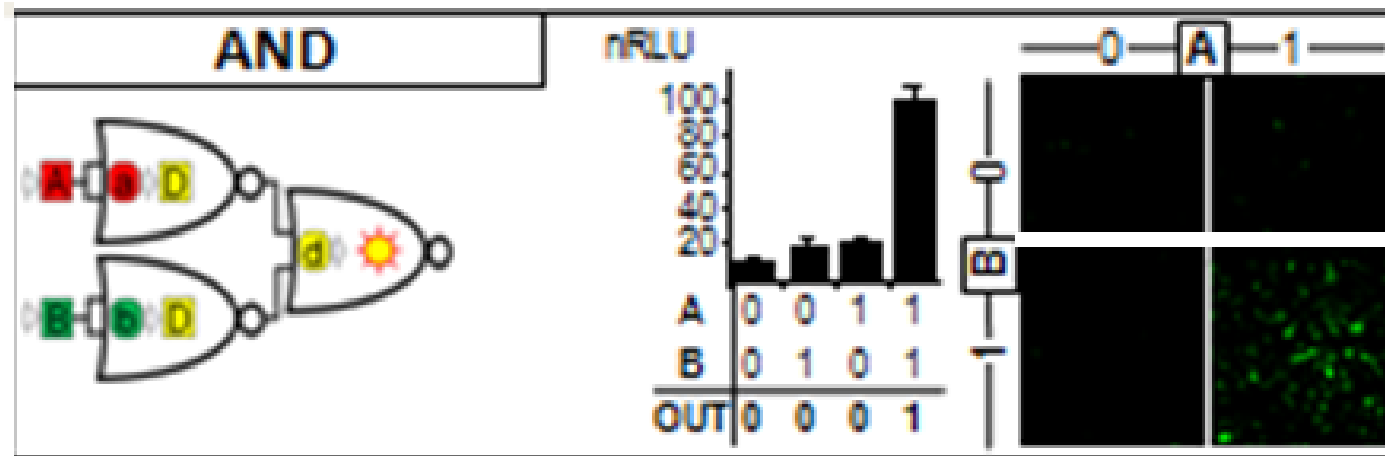


# Regulatorji TAL proteini: represorji in aktivatorji

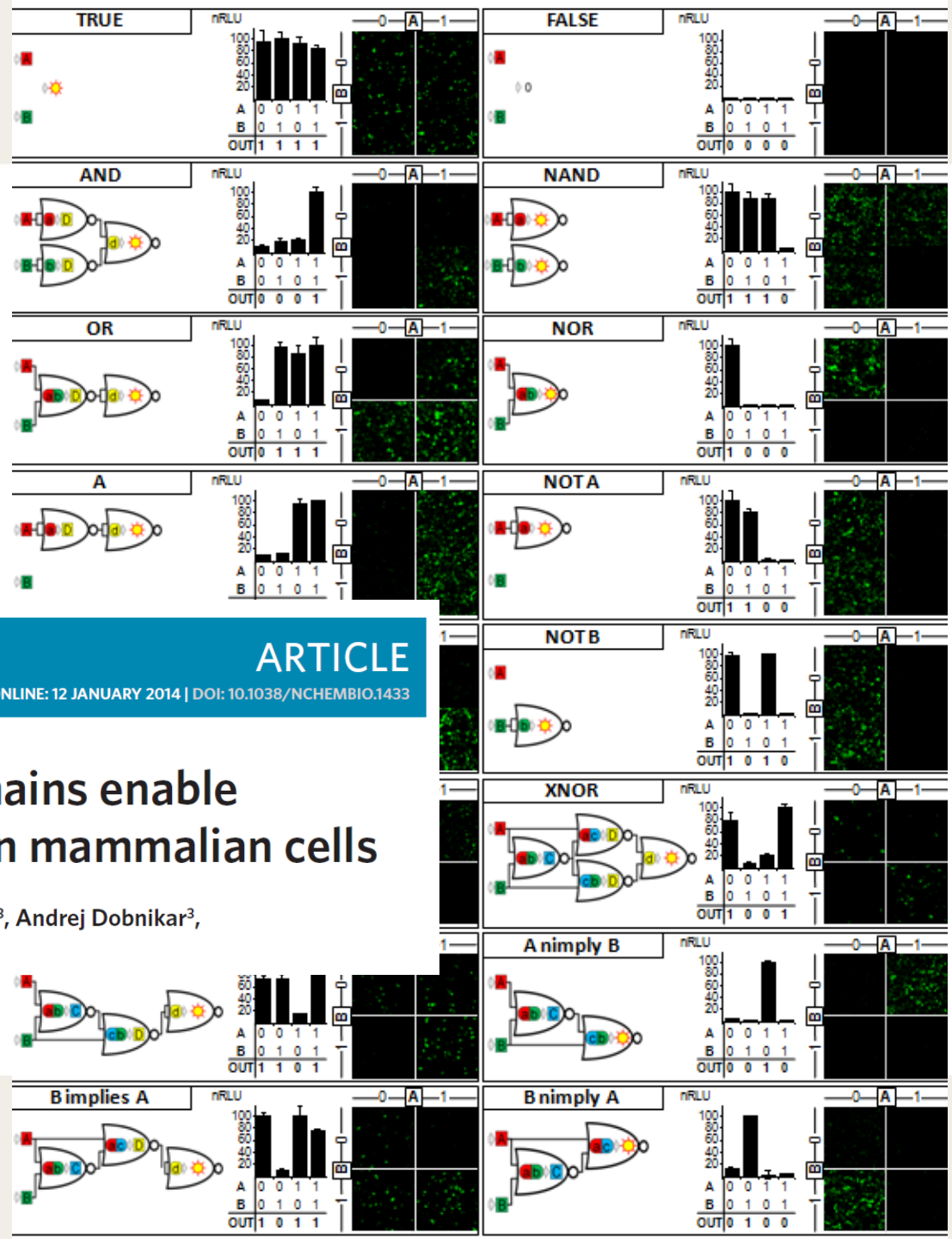


TAL - Transcription activator-like effector

# Vrata AND in OR



- 16 logičnih operacij z dvema vhodoma

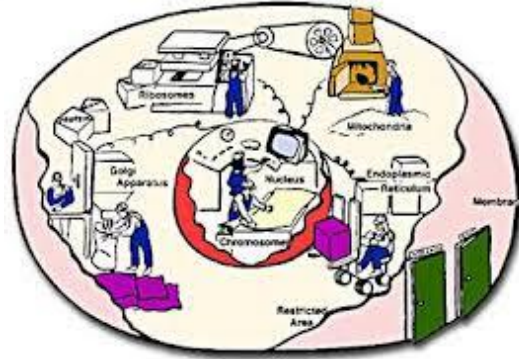


## Designable DNA-binding domains enable construction of logic circuits in mammalian cells

Rok Gaber<sup>1,2,4</sup>, Tina Lebar<sup>1,2,4</sup>, Andreja Majerle<sup>1,2</sup>, Branko Šter<sup>3</sup>, Andrej Dobnikar<sup>3</sup>,  
Mojca Benčina<sup>1,2</sup> & Roman Jerala<sup>1,2\*</sup>

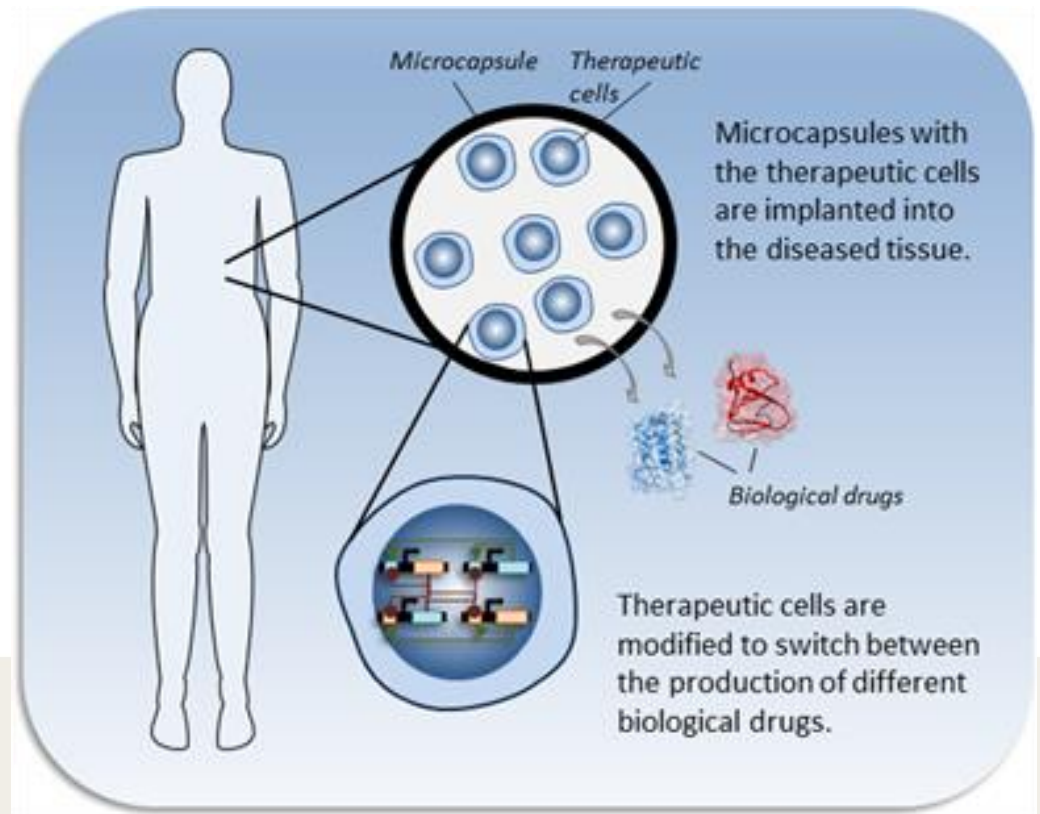


# Zakaj bi potrebovali digitalne celične računalnike?



Upravljanje celic:

- „tovarne“
- senzorji
- zdravila



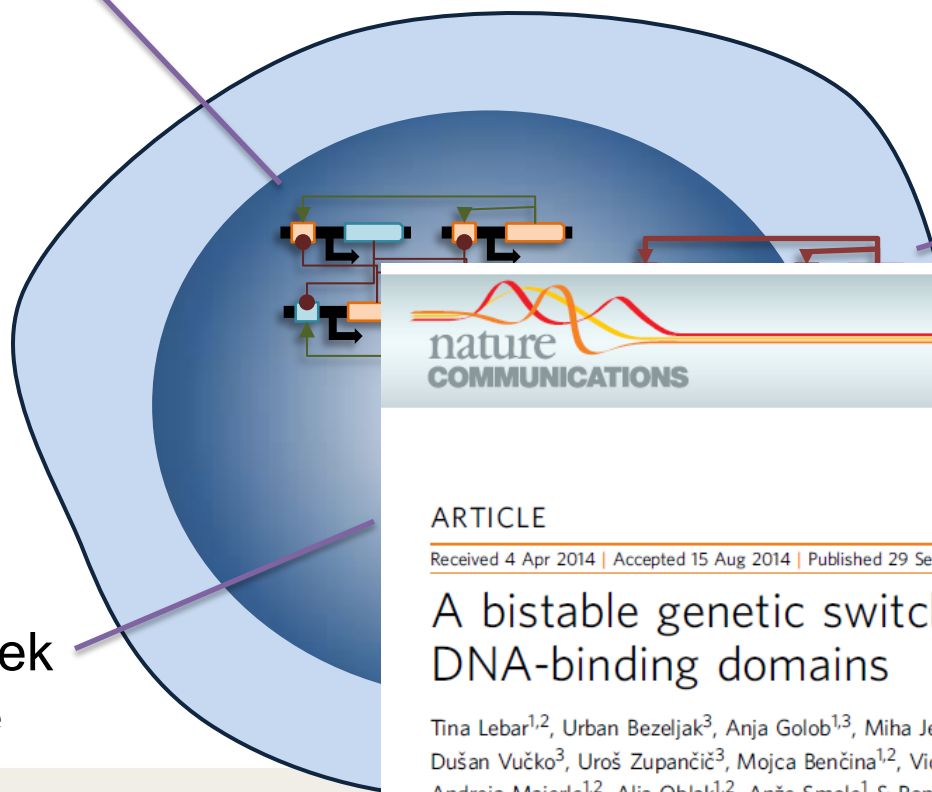


# Potreba po več stikalih



Produkcija prve učinkovine

Produkcija druge učinkovine



Zaključek terapije

nature COMMUNICATIONS

ARTICLE

Received 4 Apr 2014 | Accepted 15 Aug 2014 | Published 29 Sep 2014

DOI: 10.1038/ncomms6007

## A bistable genetic switch based on designable DNA-binding domains

Tina Lebar<sup>1,2</sup>, Urban Bezeljak<sup>3</sup>, Anja Golob<sup>1,3</sup>, Miha Jerala<sup>3</sup>, Lucija Kadunc<sup>1,3</sup>, Boštjan Pirš<sup>3</sup>, Martin Stražar<sup>3,4</sup>, Dušan Vučko<sup>3</sup>, Uroš Zupančič<sup>3</sup>, Mojca Benčina<sup>1,2</sup>, Vida Forstnerič<sup>1</sup>, Rok Gaber<sup>1,2</sup>, Jan Lonzarič<sup>1,2</sup>, Andreja Majerle<sup>1,2</sup>, Alja Oblak<sup>1,2</sup>, Anže Smole<sup>1</sup> & Roman Jerala<sup>1,2</sup>

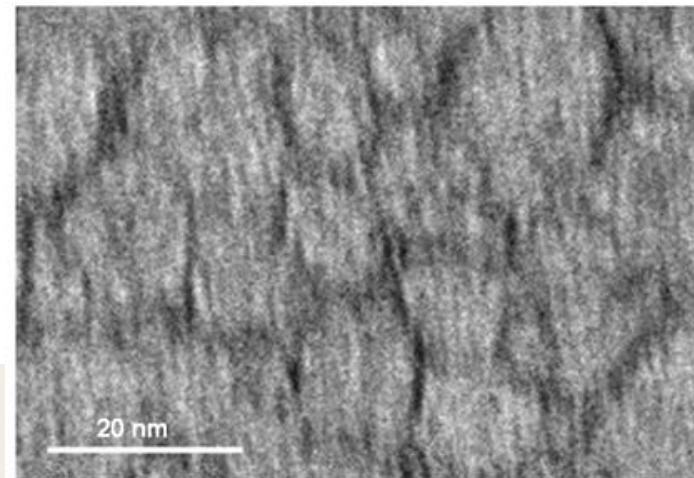
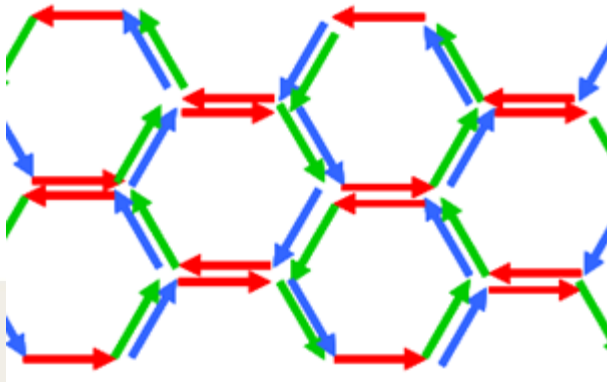
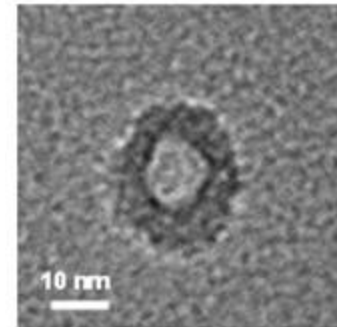
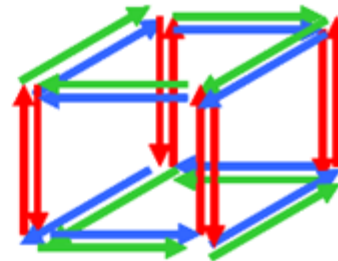
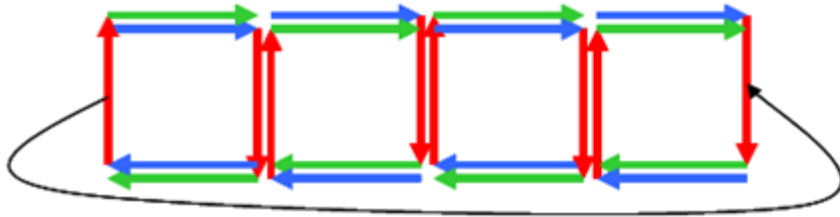


# 3. PROTEINSKE NANOSTRUKTURE, MREŽE



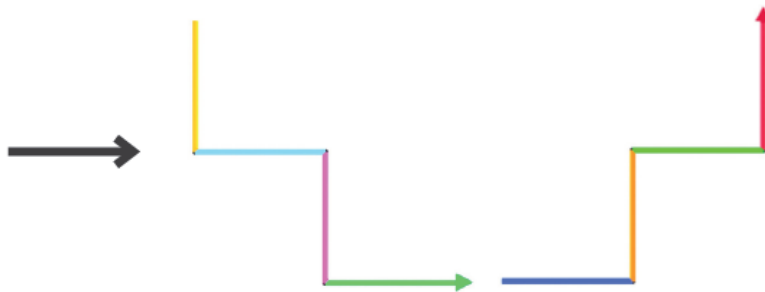
nano  
**BRICKS [pro]**  
iGEM 2009  
team **Slovenia**

[think outside the box]



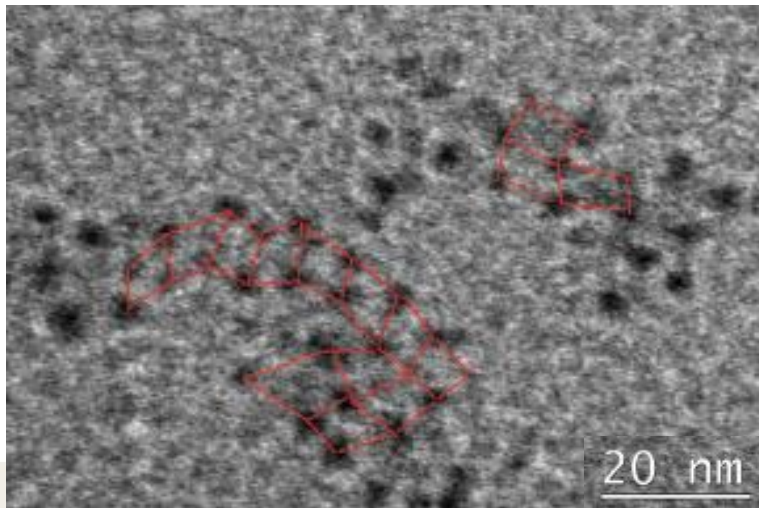
# 2D proteinske nanomreže

-  P3
-  P4
-  P5
-  P6
-  P7
-  P8
-  P9
-  P10

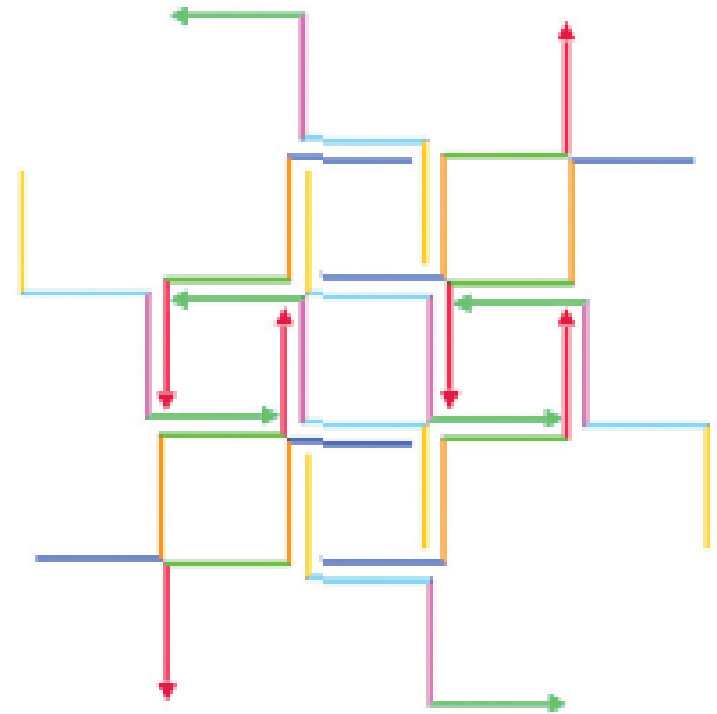


Moduli (peptidi)

Gradniki (proteini)



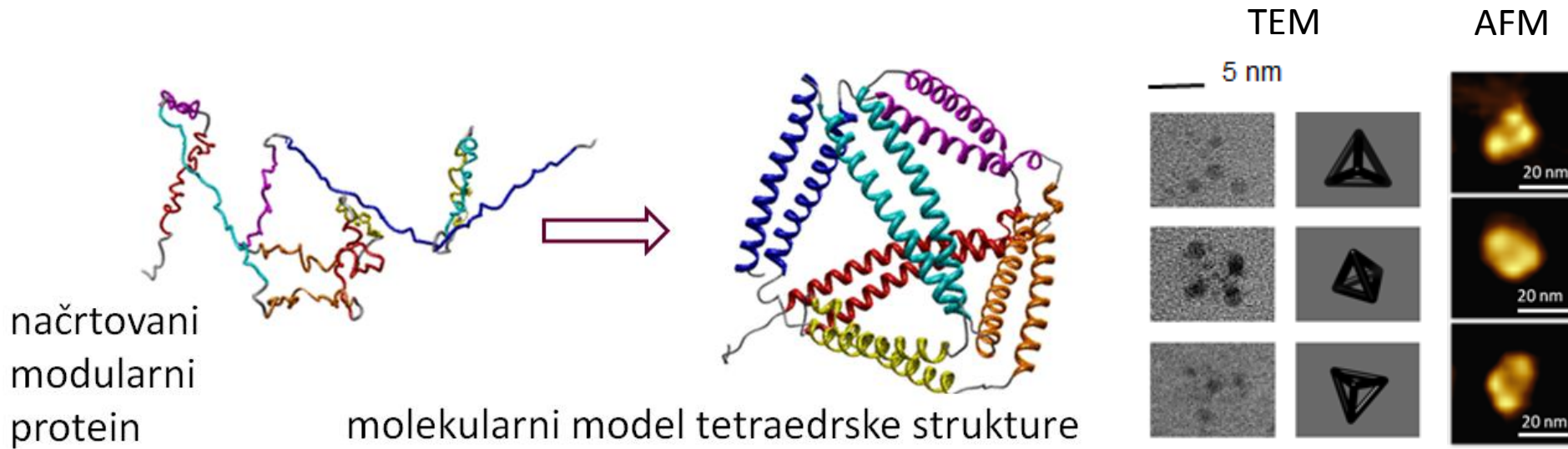
TEM



Samosestavljiva kvadratna mreža



# Nova strategija za samosestavljive nanokletke



(2013)

ARTICLE

PUBLISHED ONLINE: 28 APRIL 2013 | DOI: 10.1038/NCHEMBIO.1248

nature  
chemical biology

## Design of a single-chain polypeptide tetrahedron assembled from coiled-coil segments

Helena Gradišar<sup>1,2</sup>, Sabina Božič<sup>1</sup>, Tibor Doles<sup>1,2</sup>, Damjan Vengust<sup>3</sup>, Iva Hafner-Bratkovič<sup>1</sup>, Alenka Mertelj<sup>3,4</sup>, Ben Webb<sup>5-7</sup>, Andrej Šali<sup>5-7</sup>, Sandi Klavžar<sup>4,8</sup> & Roman Jerala<sup>1,2,9\*</sup>



# Proteinski origami

nova generacija umetnih bionanostruktur



Design of coiled-coil protein-origami cages that self-assemble *in vitro* and *in vivo*

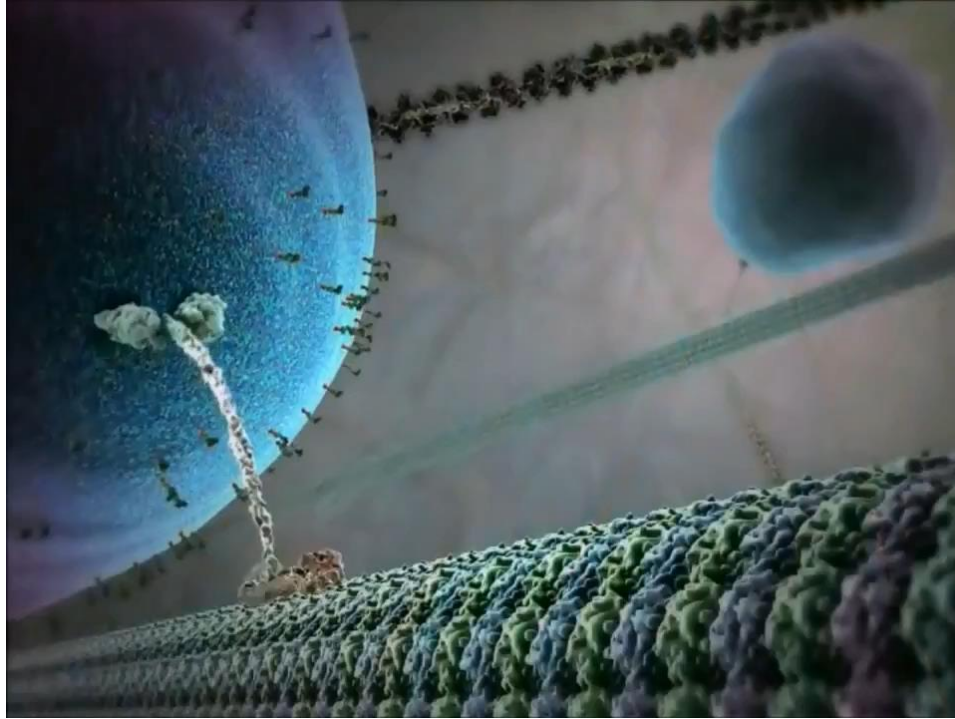
Ajasja Ljubetič<sup>1,10</sup>, Fabio Lapenta<sup>1,2,10</sup>, Helena Gradišar<sup>1,3</sup>, Igor Drobnak<sup>1</sup>, Jana Aupič<sup>1,4</sup>, Žiga Strmšek<sup>1</sup>, Duško Lainšček<sup>1</sup>, Iva Hafner-Bratkovič<sup>1,3</sup>, Andreja Majerle<sup>1</sup>, Nuša Krivec<sup>1</sup>, Mojca Benčina<sup>1</sup>, Tomaž Pisanski<sup>5</sup>, Tanja Čirković Veličković<sup>6</sup>, Adam Round<sup>7,8</sup>, José María Carazo<sup>9</sup>, Roberto Melero<sup>9</sup> & Roman Jerala<sup>1,3</sup> 



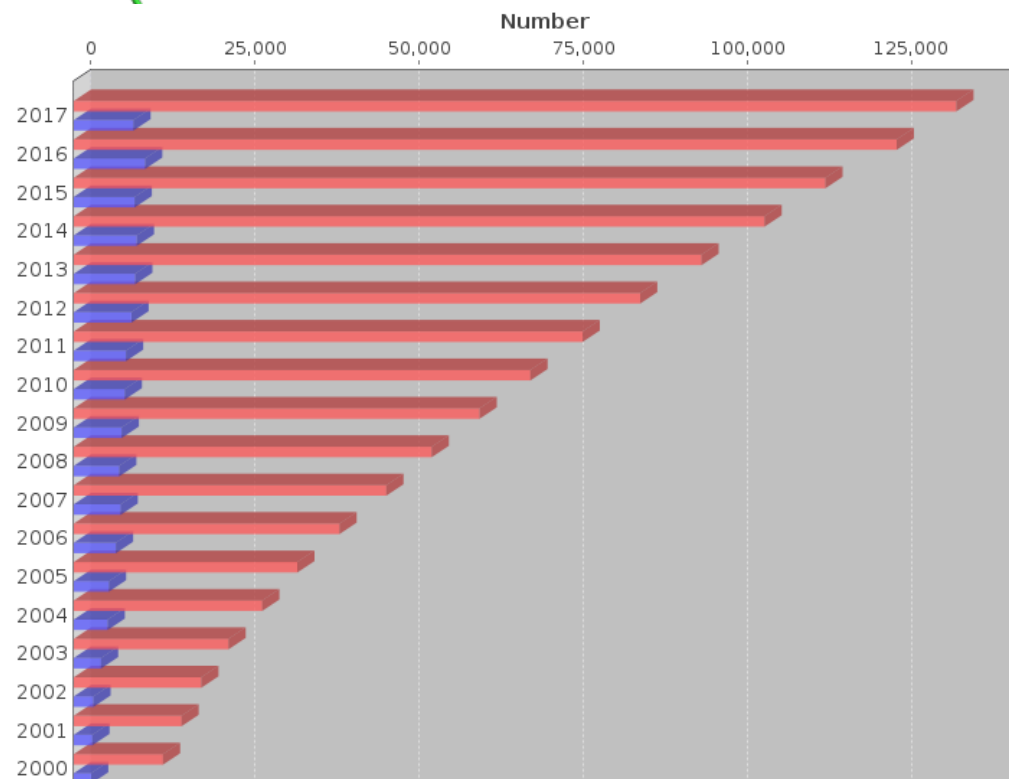
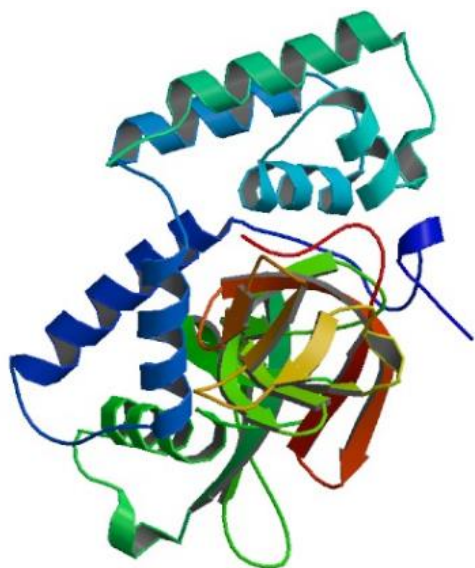
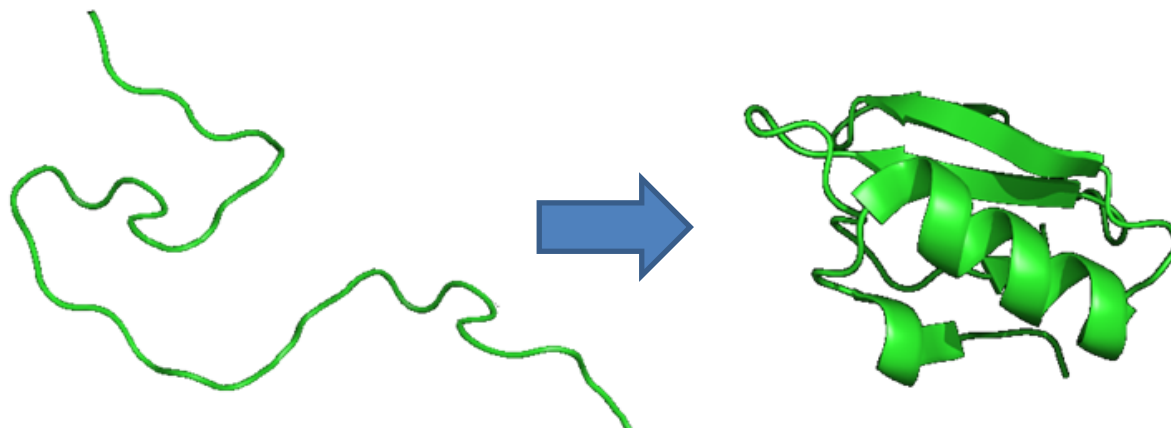
(2017)

**nature**  
**biotechnology**

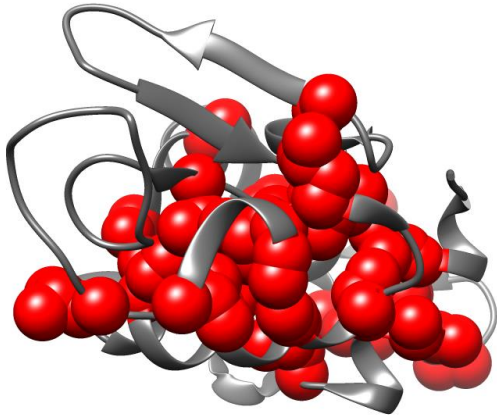
# Celice – zakladnica proteinskih nanostrojev



# Naravne proteinske nanostrukture

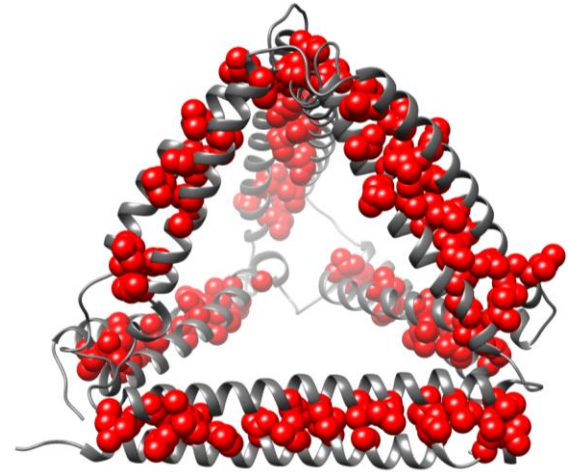


## Naravni proteini



Kompaktno jedro

## Načrtovani proteini

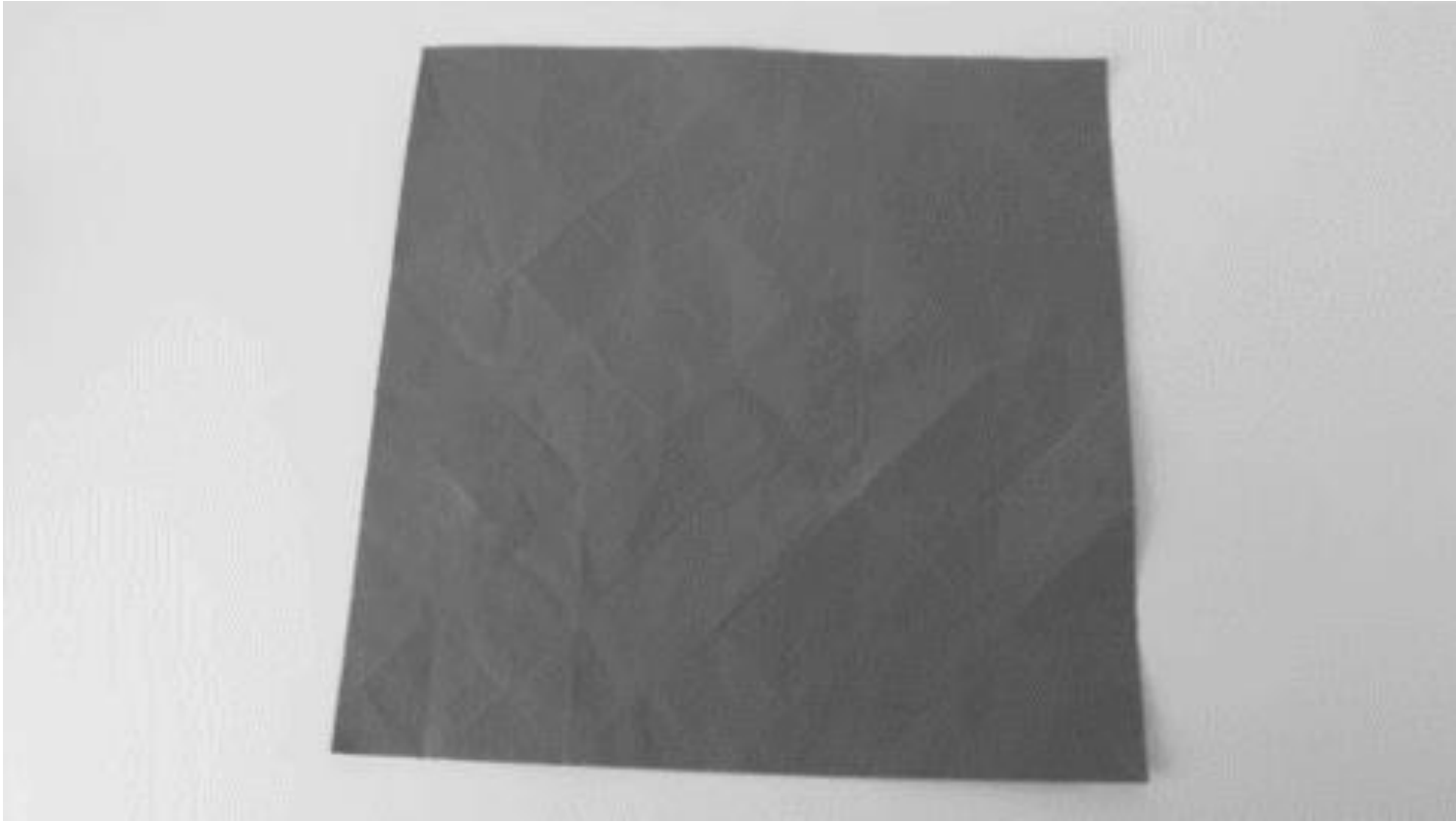


**Nanokletka**  
**Proteinski origami**

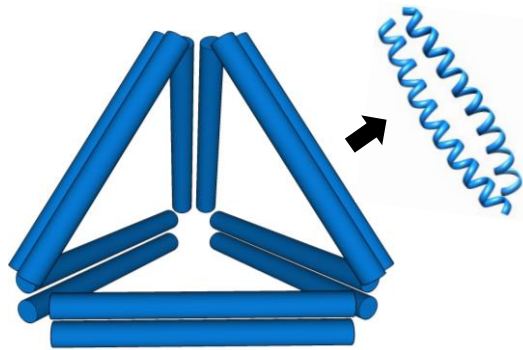




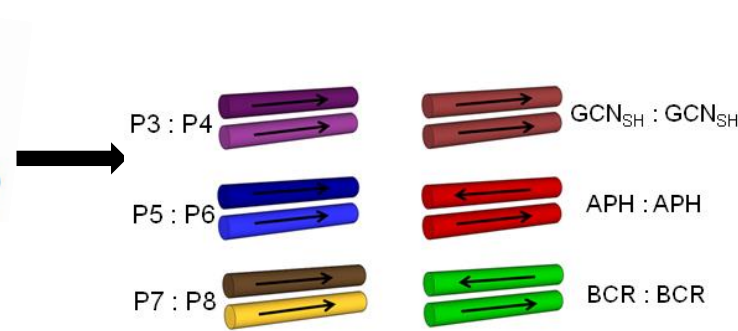
# Origami



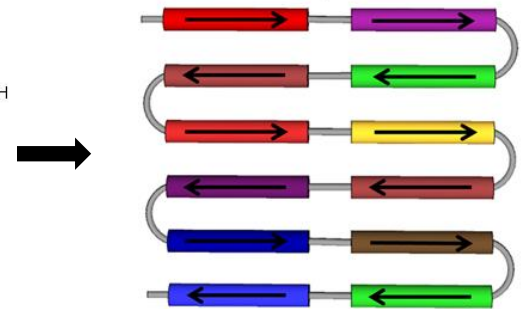
# Načrtovanje tetraedrske strukture iz ene verige



Modularnost

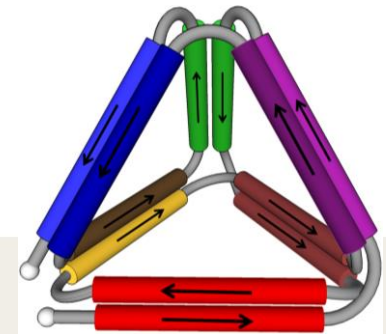


Set ortogonalnih parov



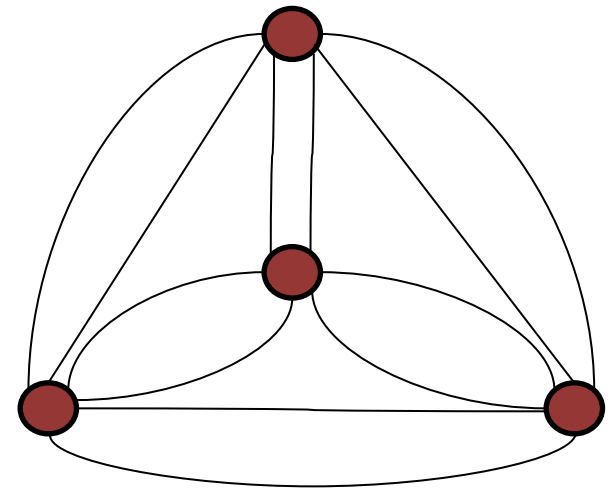
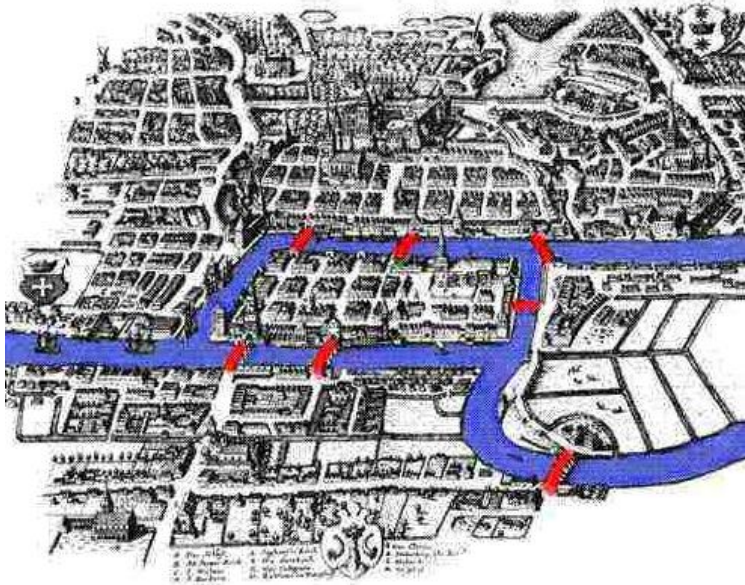
Določen vrstni red

Samosestavljivi tetraeder

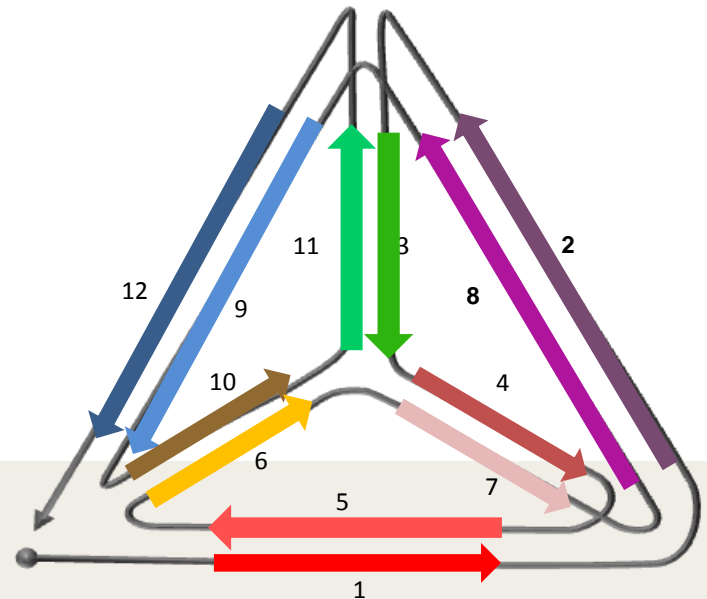


# Matematični problem – topološka analiza

## Eulerjevi obhodi



Teorija grafov  
Matematično modeliranje



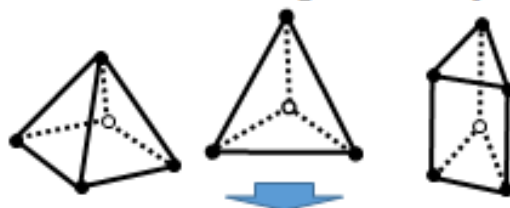
# CoCoPOD

Coiled-Coil  
Protein Origami  
Design platform

SPLET: [github.com/NIC-SBI/CC\\_protein\\_origami](https://github.com/NIC-SBI/CC_protein_origami)

## CoCoPOD platforma

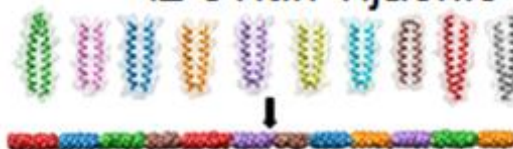
Določitev geometrije



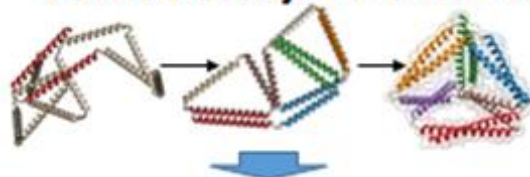
Selekcija optimalne topologije



Selekcija gradbenih modulov  
iz ovitih vijačnic



Konstrukcija 3-D modela



*in silico* validacija dizajna

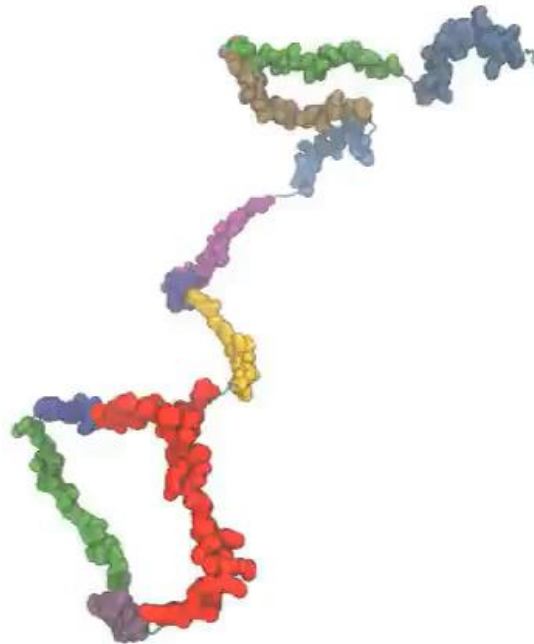


## Št. topologij

Tetraeder	3
K. piramida	52
T. prizma	25
T. bipiramida	470
Kocka	40
Oktaeder	21479



# Simulacija zvitja v tetraedrsko strukturo



# Ovite vijačnice – rigidni moduli



- Načrtovanje aminokislinske sekvence

SPEDENAALEEKIAQLKQKNAALKEEIQALEYG  
SPEDKNAALKEEIQALEEENQALEEKIAQLKYG

SPEDEIQALEEKNAQLKQEIAALEEKNQALKYG  
SPEDKIAQLKEENQQLEQKIQALKEENAALYEG

Itd.



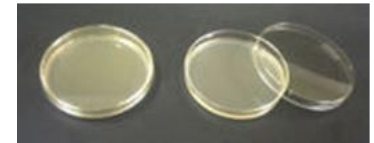
# Delo v laboratoriju

## Aminokislinska sekvenca

MKQLEKELKQLEKELQAI EKQLAQLQWKAQARKKKL  
AQLKKKLQASGPGSPEDEIQQLEEEIAQLEQKNAALK  
EKNQALKYSGPGDIEQELERAKASIRRLQE VNQER  
SRMAYLQTL LAKSGPGQLEDKVEELLSKNYHLENEVA  
RLKKLVGSGPGMKQLEKELKQLEKELQAI EKQLAQLQ  
WKAQARKKKLAQLKKKLQASGPGSPEDEIQALEEKN  
AQLKQEI AALEEKNQALKYSGPGQLEDKVEELLSKN  
YHLENEVARLKKLVGSGPGSPEDKIAQLKQKI QALKQE  
NQQLEENAALEYGSGPGSPEDENAALEEKIAQLKQK  
NAALKEEIQALEYSGPGSPEDKIAQLKEENQQLEQKI  
QALKEENAALEYGSGPGDIEQELERAKASIRRLQE V  
NQERSRMAYLQTL LAKSGPGSPEDKNAALKEEIQALE  
EENQALEEKIAQLKYGH HHHHHH

## Sintetični gen

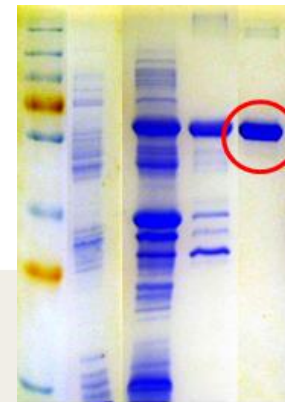
DNA



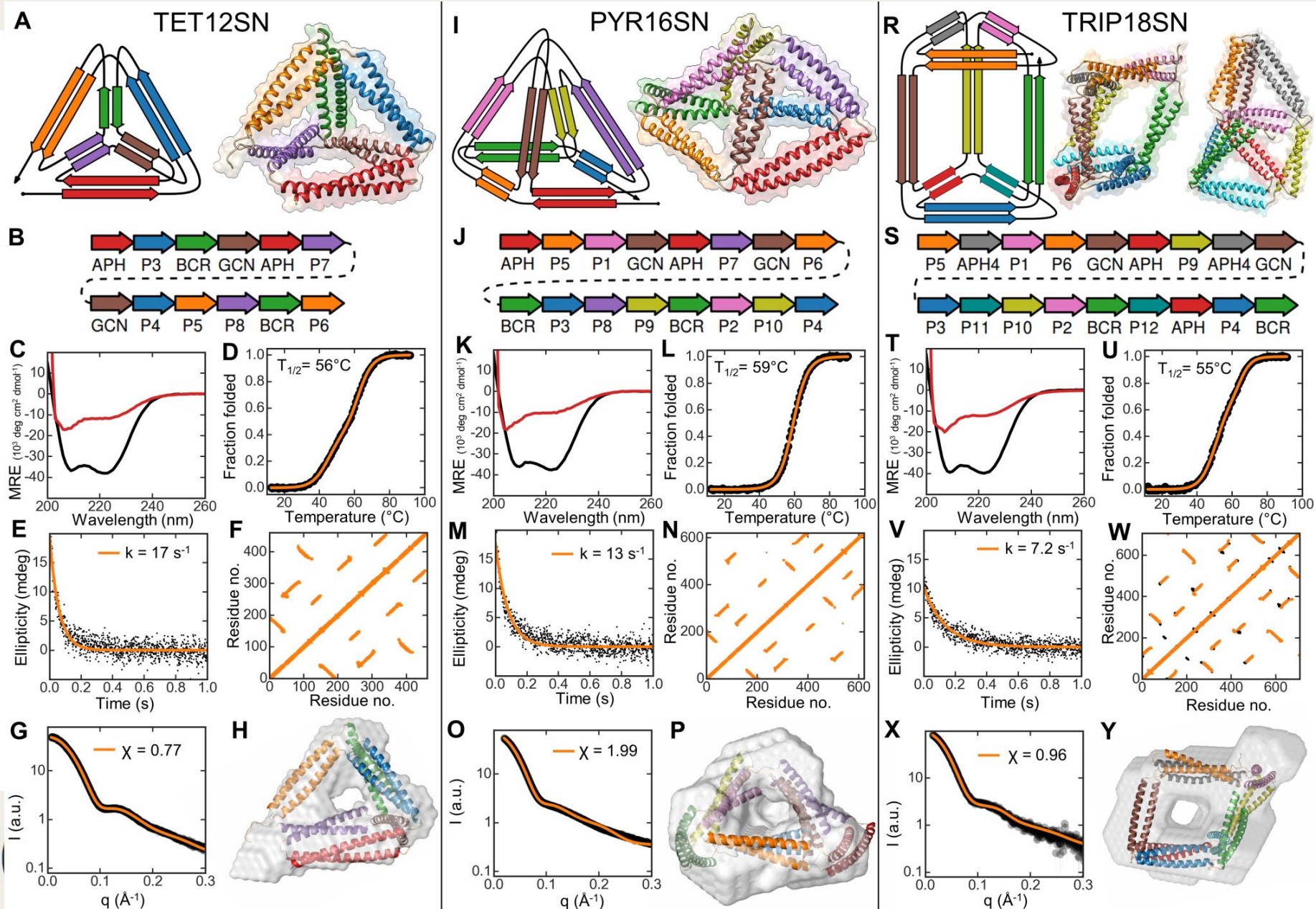
## Produkcija v bakterijah



## Izolacija proteina

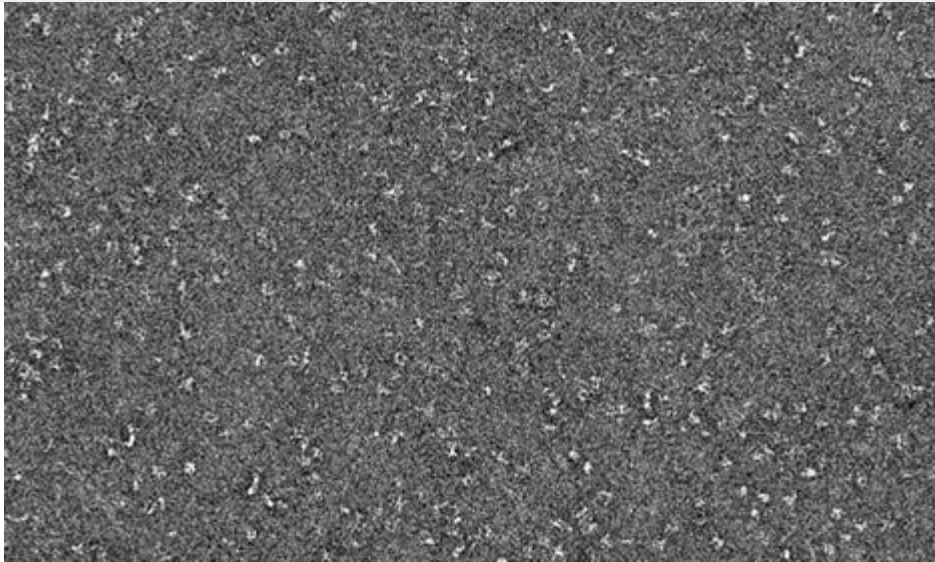


# Bio-fizikalna karakterizacija

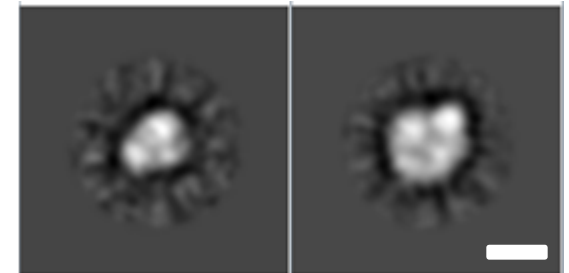




# Elektronska mikroskopija

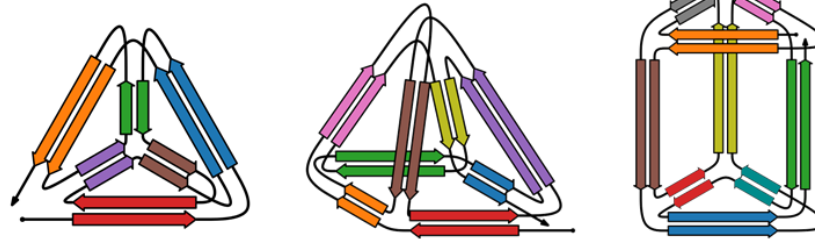


Single particle analysis

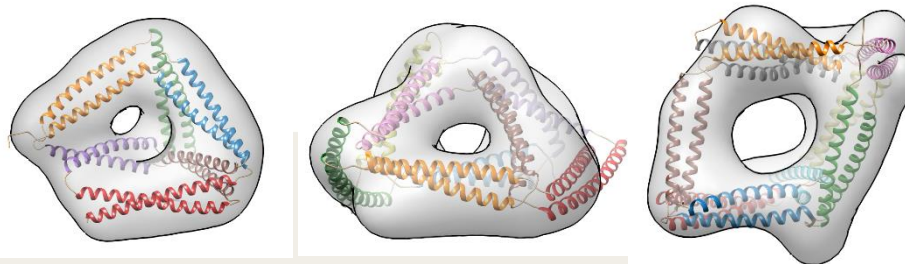


5 nm

Design



TEM

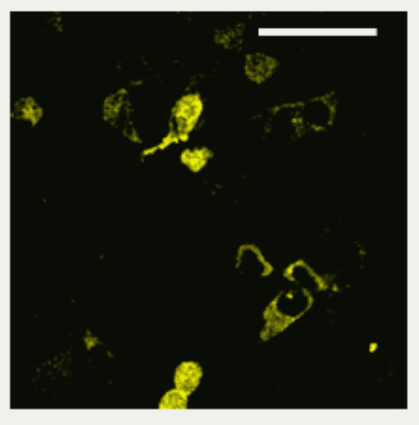
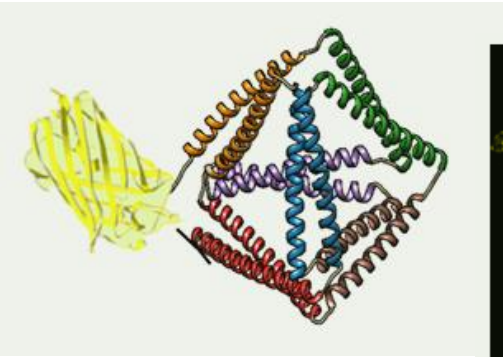


**Potrdili votlo  
notranjost !**

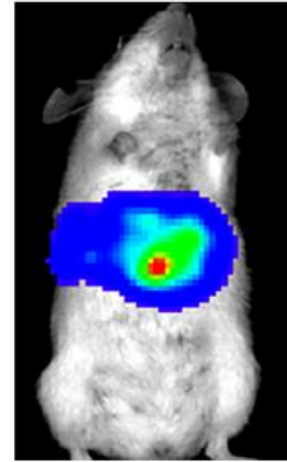


# In vivo sestavljanje

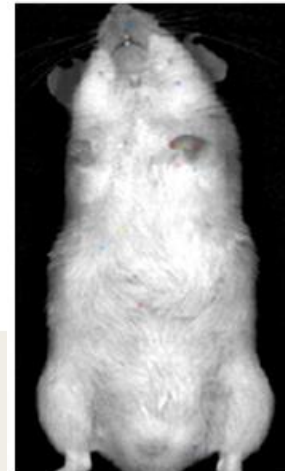
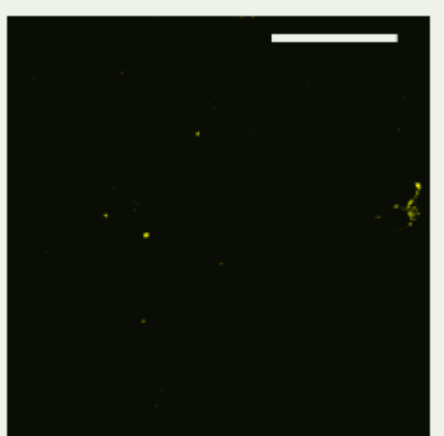
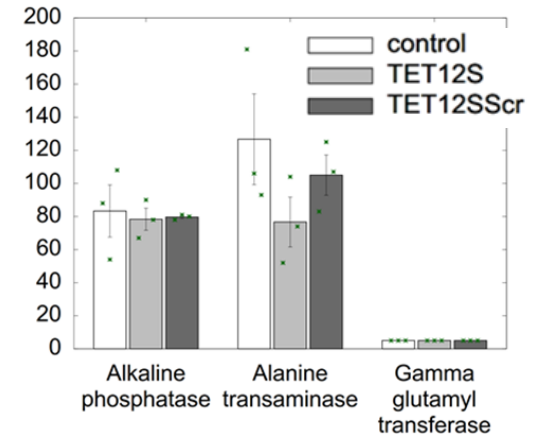
## Sesalske celice



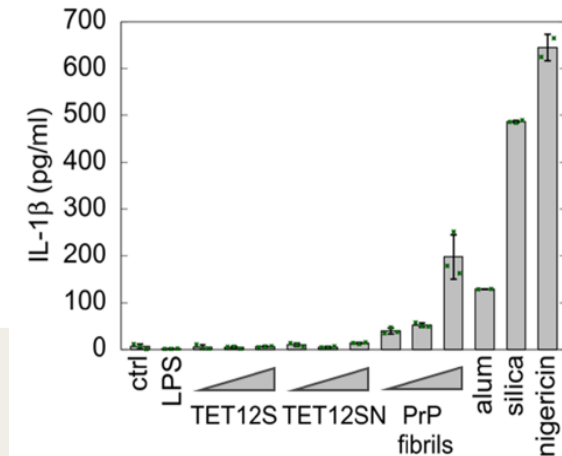
## Miši



## Jetrni encimi



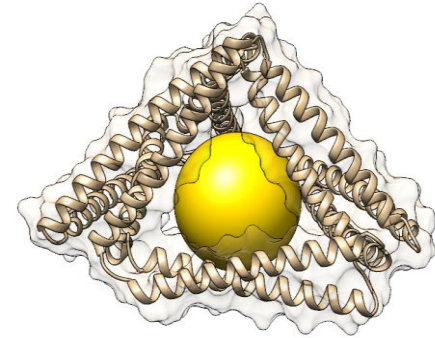
## Imunski odziv



# Potencialne aplikacije

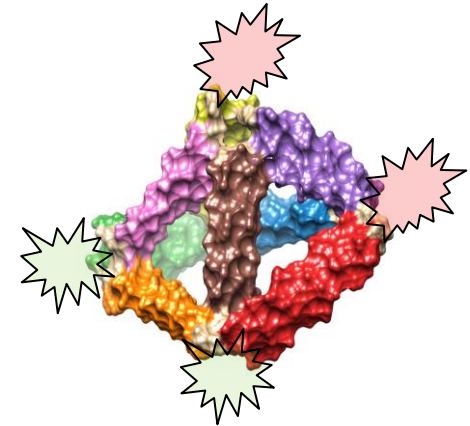
## KLETKE

- Enkapsulacija encimov, enkapsulirana kataliza
- Dostava zdravil



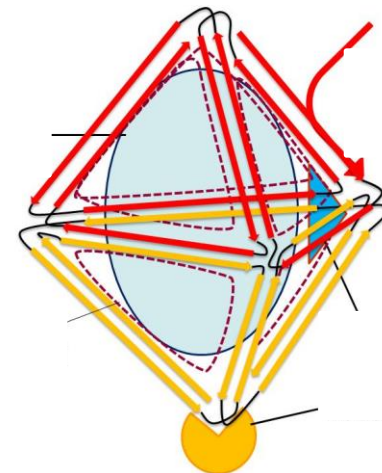
## OGRODJE

- Biokompatibilno strukturno ogrodje
- Razvoj vakcin



## NANOMATERIALI

- Načrtovani molekularni stroji
- Biomaterial, ki se lahko programira
- Biosenzorji



# ZAHVALA

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REPUBLIKA SLOVENIJA  
MINISTRSTVO ZA IZOBRAŽEVANJE,  
ZNANOST IN ŠPORT

