N1-0125 - Chemically enhanced coiled-coil protein origami: towards designed molecular machines | Kemično izboljšan zviti proteinski origami: v smeri oblikovanih molekularnih strojev

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1. DESCRIPTION OF THE PROJECT | OPIS PROJEKTA:

Proteins are highly advanced molecular machines evolved by the selection process through random mutations. Driving all the functions in the cell, proteins have been extensively studied, yet recent scientific advances open numerous possibility for *de novo* design of new protein folds. One approach is modular protein design by which protein secondary structure motifs can be used as building blocks to construct novel proteins. Dimeric coiled-coils were utilized to design single-chain polyhedral cages: coiled-coil protein origami (CCPO) which showed to self-assemble in bacteria and mammalian cell without interfering with cellular functions.

This project aims to further expand the chemical and structural space of novel CCPOs. Novel topologies of protein origamis will be designed by selecting series of naturally occurring or designed higher-order coiled-coils. The selected coiled-coils have the purpose to increase the overall stability of the CCPOs and enabling the design of novel 2-D and 3-D topologies. The protein models will be obtained by molecular modelling while their structures will be characterized by state-of-the-art techniques. In addition to single-chain protein folds, the aim is to design and develop stable dimeric proteins for externally assisted self-assembly, for example via nanobody binding.

The continuation of the project includes selecting CCPO topologies and certain residue positions for chemical modification with different functional groups. A variety of the selected functionalizations incorporated onto CCPOs will enable the screening of modified CCPOs for numerous different applications such as the delivery of hydrophobic drugs or respiratory gases, cell targeting, etc.

Proteini so zelo napredni molekulski stroji, ki so se razvili s postopkom selekcije z naključnimi mutacijami. Zaradi vseh funkcij v celici so proteini temeljito preučeni, vendar nedavni znanstveni napredek odpira številne možnosti *de novo* načrtovanja novih proteinskih zvitij. Eden od pristopov je modularna zasnova proteinov, s katero se motivi sekundarne strukture proteinov lahko uporabijo kot gradniki za izdelavo novih proteinov. Dimerne obvite vijačnice so bile uporabljene za načrtovanje enoverižnih poliedrov: proteinski origami z obvitimi vijačnicami (CCPO), ki se sami sestavljajo v bakterijah in sesalskih celicah, ne da bi pri tem posegali v celične funkcije.

Cilj tega projekta je nadalje razširiti kemijski in strukturni prostor novih CCPO. Nove topologije proteinskega origami bodo oblikovane tako, da izberemo vrsto naravnih ali načrtovanih obvitih vijačnic. Namen izbranih obvitih vijačnic je povečati splošno stabilnost CCPO in omogočiti zasnovo novih 2-D in 3-D topologij. Proteinske modele bomo dobili z molekularnim modeliranjem, njihove strukture pa bodo določene z najsodobnejšimi tehnikami. Poleg enojnih verižnih zvitij želimo oblikovati in razviti stabilne dimerne s sposobnostjo samo-zvijanja z zunanjim nadzorom, na primer z vezavo nanotelesc.

Nadaljevanje projekta vključuje izbiro topologij CCPO in nekaterih položajev ostankov za kemijsko modifikacijo z različnimi funkcionalnimi skupinami. Različne izbrane funkcionalizacije, vključene v CCPO, bodo omogočile pregled modificiranih CCPO za različne aplikacije, kot so dostava hidrofobnih zdravil ali dihalnih plinov, prepoznavanje celic itd.

a. Basic information about funding | Osnovna informacija o viru financiranja:

The project is funded by ARRS within category D for the period of two years (1.1.2020 – 31.12.2021) with 1658 hours per year. | Projekt financira ARRS v kategoriji D za obdobje dveh let (1.1.2020 - 31.12.2021) s 1658 urami na leto.

b. Project information with the link to SICRIS | Povezava v bazo SICRIS

Postdoctoral research project N1-0125; principal investigator at the National Institute of Chemistry: 53047, Tamara Šmidlehner, http://www.sicris.si/search/rsr.aspx?lang=slv&id=49436

2. PROJECT STAGES AND AIMS | FAZE IN CILJI PROJEKTA

The project lasts for two years and includes several aims:

- **Year 1**

  Aim 1: Selection of natural and designed coiled-coils and de *novo* design of different CCPO topologies with enhanced properties

  Aim 2: Method development for introducing non-natural chemical moieties into selected positions within CCPO

- **Year 2**

  Aim 3: Production and full characterization of novel CCPOs of different topologies

  Aim 4: Determination of functionalized CCPOs delivery applicability

Projekt traja dve leti in ima več ciljev:

- **1. leto**

  Cilj 1: Izbira naravnih in načrtovanih obvitih vijačnic in *de novo* načrtovanje različnih topologij CCPO z izboljšanimi lastnostmi

  Cilj 2: Razvoj metode za uvajanje nenaravnih kemijskih skupin na izbrana mesta v CCPO

- **2. leto**

  Cilj 3: Pridobivanje in karakterizacija novih CCPO različnih topologij

  Cilj 4: Določitev uporabnosti funkcionaliziranih CCPO

3. REFERENCES RELATED TO THE PROJECT | REFERENCE, BIBLIOGRAFIJA

Weijun Zhou, **Tamara Šmidlehner**, Roman Jerala, Synthetic biology principles for the design of protein with novel structures and functions, FEBS Letters, 2020, 2199-2212 https://febs.onlinelibrary.wiley.com/doi/full/10.1002/1873-3468.13796

4. LOGO OF THE ARRS | LOGO FINANCERJA

arrs

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REPUBLIKE SLOVENIJE