TITLE: CC-TRIGGER-FEET: Designing dynamic and controllable feet for a de novo protein robot

Summary: Proteins are nature's nano-robots, catalyzing reactions, recognizing molecules, and transporting cargo. Biomimetic nanomachines have the potential to solve several current real-world problems, however their use is limited by existing folds and inadequate stability of natural proteins. De novo designed proteins are hyper-stable and can achieve properties and shapes not found in nature.

CC-TRIGGER-FEET will combine protein engineering with state-of-the-art deep learning techniques and wet-lab experiments. PhD student will construct dynamic heterodimeric protein elements whose binding state will be controlled either through protein phosphorylation or coiled-coil strand displacement. The heterodimeric "feet" will be rigidly attached to de novo designed fibers. All components are genetically encodable, potentially enabling the designed feet to function in living cells.

The project will enable design of a protein walker robot that can be tightly controlled and will represent a new class of dynamic protein assemblies that do not exist in nature.

Research techniques used: For protein design we will use the latest AI methods: RFDiffusion and AFDesign will create rigid scaffold connections. ProteinMPNN will be used to design side chains. Proteins will be expressed in *E. coli*. Purification will be done using Ni affinity and size-exclusion chromatography. The binding of proteins will be measured using Surface Plasmon Resonance and FRET. Stopped flow for kinetics. The movement of the walker along the track will be measured using single molecule TIRF microscopy. Structures of fibers with rigid feet will be screened using negative-stain TEM microscopy and solved using Cryo-EM.

The reason why the topic is innovative: One of the key innovations is the application of cutting-edge AI protein design methods to design multistate assemblies and molecular robots. Molecular robots have the potential to solve many pressing problems from drug delivery in precision medicine to self-healing and self-cleaning materials. The other unique aspect is using coiled-coil strand displacement to achieve control over walking and open the door to powered molecular machines. Strand displacement has shown promise in DNA nanotechnology and the same principles could be applied to genetically encodable protein machines.

SUPERVISOR: dr. Ajasja Ljubetič, research associate
DEPARTMENT: D12 Department of Synthetic Biology and Immunology

REPUBLIKA SLOVENIJA
MINISTRSTVO ZA VISOKO ŠOLSTVO, ZNANOST IN INOVACIJE
Masarykova cesta 16, SI - 1000 Ljubljana

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