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In silico-designed nanobodies: from the computer to the bench

VHH antibodies, also known as nanobodies, are the engineered version of antigen-binding domains from heavy-chain-only antibodies found in camelid serum [1]. As the smallest antibody fragments preserving the binding capacity of a whole antibody, they are largely employed for diagnostic and therapeutic applications [2,3]. The small size (~15 kDa) and stability of nanobodies allow their recombinant production in bacterial systems. Lately, a computational-based protocol was developed for the design of VHH sequences against specific epitopes [4].
Here the same algorithm was used to produce nanobodies active against a model protein, the Hen Egg White Lysozyme (HEWL), by starting from a nanobody sequence against an unrelated target. With the aim of improving the in silico design algorithm, complexes between the artificial nanobodies and their target HEWL were studied through structural and biophysical techniques. The VHH protein sequences selected by the algorithm were expressed in Escherichia coli using different protein tags. Their stability was evaluated using a Thermal Shift Assay protocol and results were used to select a suitable buffer for purification and crystallization experiments. An enzymatic inhibition assay was carried out using E. coli cells as substrate of the lysozyme. Despite some predicted overlap between the artificial VHH binding site and the active site of HEWL, no significant inhibition was reported. Nervertheless, for two artificial VHH KD in the nanomolar range were experimentally determined through microscale thermophoresis. The structural characterization of the VHH-HEWL complexes is underway.

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Primary author: NOLASCO, Federico (Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste)

Co-authors: MEDAGLI, Barbara (Centre of Excellence in Biocrystallography Department of Chemical and Pharmaceutical Sciences University of Trieste, Via L. Giorgieri1, 34127 Trieste); LA MANNA, Sara (Dipartimento di Farmacia, Università di Napoli "Federico II"); MARASCO, Daniela (Dipartimento di Farmacia, Università di Napoli "Federico II"); DE ZORZI, Rita (Università degli Studi di Trieste - Dipartimento di Scienze Chimiche e Farmaceutiche); FORTUNA, Sara (CONCEPT Lab, Istituti Italiano di Tecnologia (IIT), Genova); SOLER, Miguel (Dipartimento di Scienze Matematiche Informatiche e Fisiche, Università degli Studi di Udine)

Presenter: NOLASCO, Federico (Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste)

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