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in kemijsko tehnologijo

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VABILO NA PREDAVANJE
V OKVIRU DOKTORSKEGA ŠTUDIJA
KEMIJSKE ZNANOSTI / INVITATION TO THE
LECTURE WITHIN DOCTORAL PROGRAMME IN
CHEMICAL SCIENCES

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z naslovom / title:

High-Throughput methods for production, purification and interaction of proteins

v sredo, 24. 5. 2023 ob 15. uri /
on Wednesday, 24. 5. 2023 at 15.00
v predavalnici 1 v 1. nadstropju Fakultete za kemijo in kemijsko tehnologijo, Večna pot 113 **/ in lecture room 1,** 1st floor at the Faculty of Chemistry and Chemical Technology, Večna pot 113

Vljudno vabljeni! / Kindly invited!

Abstract:

The HTP protein production and interaction facility of the AFMB offers custom and automated protocols (in 96/384 format) covering all the stages of cloning, protein production (in *E. coli*), purification (µg to tens of milligrams) as well as *in vitro* study of protein-protein, protein-peptide or protein-DNA interactions. The facility purified more than 10.000 proteins for research teams, French (>10 ANR) or international networks (>10 EU grants).
We developed and validated protein purification protocols at a pace of >1.000 cultures and purifications per week (1, 2). This is used to either improve the soluble level of difficult proteins (3) or to purify protein librairies such as transcription factors (4), the 5.000 disulfide rich animal toxins of the EU VENOMICS project (5), hundreds of CAZYmes (6) or the full repertoire of the 266 human PDZ domains (7).
To characterize proteins at a pace and scale that is compatible with hundreds of proteins in micrograms, several custom-made protocols have been developed such as a new *in vitro* protein-DNA interaction assay (HTP SELEX (4)) and HTP quantitative *in vitro* protein-protein or protein-peptide interaction assays (HTP Hold-up (7)) able to determine thousands of affinities per day. Using holdup, we published the biggest quantitative dataset for PDZ-pbm interactions (8) and were the only ones to identify the human PDZ binders of SARS-CoV-2 (9).
The most recent developments of the holdup will open the way to the systematic deciphering of the Full human- PDZ-pbm quantitative interactome and could be adapted to many more interactomes.

1) High-throughput protein expression screening and purification in *Escherichia coli*. Vincentelli R, Cimino A, Geerlof A, Kubo A, Satou Y, Cambillau C. **Methods**. 2011 Sep;55 (1):65-72

2) High throughput quantitative expression screening and purification applied to recombinant disulfide-rich venom proteins produced in *E. coli*. Saez, NJ, Nozach H, Blemont, M, Vincentelli, R. **JOVE**, 2014 Jul 30;(89):e51464.

3) Vincentelli R, Romier C. Expression in Escherichia coli: becoming faster and more complex. **Curr Opin Struct Biol.** 2013 Jun;23(3):326-34.

4) DNA-binding specificities of human transcription factors. Jolma A, Yan J, Whitington T, Toivonen J, Nitta KR, Rastas P, Morgunova E, Enge M, Taipale M, Wei G, Palin K, Vaquerizas JM, Vincentelli R, Luscombe NM, Hughes TR, Lemaire P, Ukkonen E, Kivioja T, Taipale J., **Cell**. 2013 Jan 17;152 (1-2):327-39.

5) High-throughput expression of animal venom toxins in Escherichia coli to generate a large library of oxidized disulphide-reticulated peptides for drug discovery. J. Turchetto*; A. F. Sequeira*; L. Ramond*; F. Peysson*; J. L.A Bras; N. J Saez; Y.Duhoo; M. Blémond; C. I.P.D Guerreiro; L.Quinton; E. De Pauw; N. Gilles; H. Darbon; C. M.G.A Fontes; R. Vincentelli, **Microbial Cell Factories**, . 2017 Jan 17;16(1):6.

6) Helbert W, Poulet L, Drouillard S, Mathieu S, Loiodice M, Couturier M, Lombard V, Terrapon N, Turchetto J, Vincentelli R, Henrissat B. Discovery of novel carbohydrate-active enzymes through the rational exploration of the protein sequences space. **Proc Natl Acad Sci** U S A. 2019 Mar 26;116(13):6063-6068

7) Quantifying domain-ligand affinities and specificities by high-throughput holdup assay. Vincentelli R*, Luck K*, Poirson J, Polanowska J, Abdat J, Blémont M, Turchetto J, Iv F, Ricquier K, Straub ML, Forster A, Cassonnet P, Borg JP, Jacob Y, Masson M, Nominé Y, Reboul J, Wolff N, Charbonnier S, Travé G. **Nat Methods**. 2015 Aug;12(8):787-93

8) G. Gogl, B. Zambo, C. Kostmann, A. Cousido-Siah, B. Morlet, F. Durbesson, L. Negroni, P. Eberling, P. Jane, Y. Nomine, A. Zeke, S. Østergaard, E. Monsellier, R.Vincentelli, G. Trave. Quantitative fragmentomics allow affinity mapping of interactomes, **Nat. Commun**. 2022 Dec 7;13(1):7555

9) Caillet-Saguy C, Durbesson F, Rezelj VV, Gogl G, Tran QD, Twizere JC,Vignuzzi M, Wolff N*, Vincentelli R *. Host PDZcontaining proteins targeted by SARS-CoV-2 . **FEBS J** . 2021 Apr 17.

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