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Mass spectrometry, SPADs and SNAP-NAPPA micron arrays for biomarkers identification in humans

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We present the analysis of an innovative kind of self-assembling protein microarray, the "*Nucleic Acid Programmable Protein Array*" (NAPPA), express with the SNAP tag *E.coli* coupled self free expression system. The goal is to develop a standardize procedure to identify biomarkers in clininical setting and to analyze the protein-protein interaction occurred on NAPPA array using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Bruker Ultraflex. We employ in the process "*Protein synthesis Using Recombinant Elements*" (PURE) system which due to its high complexity needs ad hoc bioinformatic tools to be analysed. The PURE system represents a step towards a totally defined *in vitro* transcription/translation system, thus avoiding the "black box" nature of the cell extract. The immediate advantage is the significantly reduced level of all contaminating activities and the *E. coli* IVTT respect the RRL or human lysate, which is totally characterized and thereby represents an advantage for the subsequent MS analysis of the results. The presence of "background" molecules, in fact, represents the main obstacle to these MS data interpretation. SpADS: An R Script for Mass Spectrometry Data Preprocessing before Data Mining. SpADS provides useful preprocessing functions such binning and peak extractions, as available tools, and provides functions of spectra background subtraction and dataset managing. The MS samples are obtained from SNAP-NAPPA spots printed on gold coated glass slides in higher density, in order to obtain an amount of protein appropriate for MS analysis. The spots of 300 microns were printed in 12 boxes, each box with 100 identical spots. The sample genes immobilized used as test cases were p53_Human (Cellular tumor antigen p53); CDK2_Human (Cyclin-dependent kinase), 2; Src_Human-SH2 (the SH2 domain of Proto-oncogene tyrosine-protein kinase), PTPN11 (Human-SH2, the SH2 domain of Tyrosine-protein phosphatase non-receptor type 11).

Biography

Evgeniya Peshkova (alias Eugenia Pechkova) is presently an assistant professor of Biochemistry and Biophysics at the University of Genova Medical School. After taking her Doctoral degree in Chemistry at Moscow State Lomonosov University in 1998, and the Ph.D. in Biophysics at University of Genova in 2003, was Scientific Director of Fondazione EL.B.A. (Electronic Biotechnology Advanced) and Principle Investigator of a big FIRB research grant on Organic Nanotechnology. Later she acquired the scientific responsibility of the laboratory of Nanobiocrystallography at the Nanoworld Institute, University of Genova. In 2007 she worked as a Visiting Scientist at the European Synchrotron Radiation Facility (ESRF) in Grenoble in Macromolecular Crystallography and Soft Condensed Matter, remaining up to now one of the PI of Radiation Damage BAG. Author of more than 50 international scientific publications (ISI-SCI), 2 patents, several chapters to books and textbooks. Author of "Proteomics and Nanocrystallography" and editor of "Synchrotron Radiation and Nanobiosciences" (with Claudio Nicolini), "Synchrotron Radiation and Structural Proteomics" (with Christian Riekel) within the Pan Stanford Series on Nanobiotechnology. Her main scientific interest is structural proteomics, functional nanoproteomics and nanocrystallography.

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