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SpADS and SNAP-NAPPA Microarrays towards Biomarkers Identification in Humans: Background Subtraction in Mass Spectrometry with *E.coli* Cell Free Expression System

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Abstract

We present a useful approach towards for biomarkers identification in an innovative self-assembling protein microarray based on "Nucleic Acid Programmable Protein Array" (NAPPA) and SNAP tag coupled to *E.coli* cell free expression system. This approach prove capable to resolve the "background" problem associated to the above label free detection system for the identification of proteins and of protein-protein interaction in humans that could become used in clinical practice.

Keywords: Mass Spectrometry; Electrophoresis; Protein

Introduction

In the last decade, Mass Spectrometry has played a key role in the advance of proteomics [1,2]. As research moves toward more sophisticated systems, it is urgent to develop protein analysis and identification techniques to meet the high-throughput demand [3-8]. The integration of microarrays with MS has generated a powerful new tool to deal with the problems in this area [9]. The flight time between the laser striking the array surface and the molecules reaching the detector at the end of the tube depend on the m/z of the proteins, thus enabling the system to accurately determine the mass of the protein species present in the sample [1,10]. One reported successful example is the ProteinChip® System of Ciphergen Biosystems Inc consisting in a SELDI-TOF-MS instrument equipped with a pulsed UV nitrogen laser source. Upon laser activation, the proteins at the array surface are desorbed and ionized, and subsequently accelerated by an electric field down the flight-tube, before reaching the detector. The patent (i.e. EU Patent No. 1 354 203) describes using mass spectrometry to detect certain protein biomarkers that are present in patients with bladder cancer versus patients who do not have bladder cancer. The high specificity of MS means that the signals of minute proteins or peptides that are undetectable using traditional techniques can be measured. As a result, SELDITOF MS has been applied to the screening of tumor biomarkers such as ovarian cancer, urinary bladder cancer, lung cancer, prostate cancer, colon cancer, breast cancer and liver cancer. Another example of the detection of a biomarker was the identification of CD8 cell anti-HIV factor (CAF). It has been known for more than a decade that certain HIV-1-infected individuals who are immunologically stable secrete a soluble factor, CAF, which suppresses HIV-1 replication. Although considerable work has been done, their identity was still obscure. Zhang et al. used the SELDI technique to discover a cluster of small proteins that were secreted when CD8 T cells from longterm non-progressors with HIV-1 infection were stimulated [11]. Although the SELDI protein chip has many advantages, including simple operational procedures, speed, high sensitivity and abundant information, it faces several challenges, including the normalization of sample collection and experimental procedures, identification and verification of biomarkers efficiently, and proper interpretation of sophisticated SELDI-MS data. In addition, most proteins in serum have a very low concentration and are difficult to detected by the SELDI technique. This may require pre-enrichment or separation using beads, LC and electrophoresis [12].

In previous researches [1,13,14] we carried out feasibility studies of MALDI-TOF MS analysis of different kind of Nucleic Acid Programmable Protein Array (NAPPA). The NAPPA method allows for functional proteins to be synthesized in situ directly from printed cDNAs just in time for assay. The use of purified proteins was substituted with the use of cDNAs encoding the target proteins for the microarray. In our research we employed two different mass spectrometry (MS) techniques, the Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) MS and Liquid Chromatography-Electrospray Ionization MS (LC-ESI-MS). The last goal of our research is to develop a standardized analysis procedure, able to analyze the protein-protein interactions occurred on NAPPA array in a label free manner.

In the present manuscript we present the data obtained by a bioinformatics analysis of MALDI-TOF MS data carried out utilizing a specific "PURE system" database. Taking advantage of the full characterization of PURE Express system, starting from the list of its components we constructed a database of the entire triptych fragment belonging to PURE system molecules. Using SpADS algorithms we subtracted from our experimental mass lists the background peaks belonging to PURE system molecules. Once this procedure will prove successful serum proteomic profiles and emerging protein-protein interactions computed from MALDI-TOF of NAPPA SNAP arrays in association with QCM_D nanoconductimetry [7,8] could be measured by MALDI TOF MS along with classification tree established via our software in order to help us to provide a more accurate approach for diagnosis and clinical staging of cancers.

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Materials and Methods

For all concerns production and expression of NAPPA and MS analysis refer to [6].

NAPPA SNAP

We analyzed different kind of NAPPA, in the last improved version the proteins were synthesized with the addition of a SNAP tag – therefore we named SNAP_NAPPA this kind of array - and translated using a reconstituted Escherichia coli coupled cell-free expression system. The addition of a SNAP tag to each protein enabled its capture to the array through an anti-SNAP antibody printed simultaneously with the expression plasmid. SNAP tag is a 20 kDa mutant of the DNA repair protein O6-alkylguanine-DNA alkyltransferase that reacts specifically and rapidly with benzylguanine (BG) derivatives, leading to irreversible covalent labeling of the SNAP tag. SNAP tag has a number of features that make it ideal for a variety of applications in protein labeling, in particular its substrates are chemically inert towards other proteins, avoiding nonspecific labeling in cellular applications. Moreover also the chemistry and the printing of the NAPPA have been improved [15]. 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).

"PURE system" database construction

To reduce the sample complexity (i.e. the amount of biological material due to NAPPA chemistry and to the expression system) the *in vitro* translation-transcription (IVTT) system we used was from *E.coli*. The PURE system represents an important 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. The PURE system has the capacity for a yield of more than 100 μg/ml is today exclusively licensed to New England Biolabs (Ipswich, MA, USA) under the trade-name "PURExpress" [16]. Moreover the *E.coli* IVTT lysate is totally characterized, which could be a fundamental advantage for the subsequent analysis of the results.

The base to realize the "PURE system" database was the full knowledge of PURE EXPRESS composition (reported in Table 1). Through Expasy databank (www.expasy.org) search we identified the peptide sequences for each component. These sequences were *in silico* trypsin digested by means of the software Sequence Editor included into the Biotools package. Hereafter the concentrations of the components used in the PURE system [17].

I_recombinant proteins			
IF1	RF3	GlnRS	AspS
IF2	RRF	TrpRS	AlaRS
IF3	ArgRS	TyrRS	GlyRS
Methionyl-tRNA formyltransferase	CysRS	HisRS	PheRS α2β2
EF-Tu	IleRS	ProRS	Creatine kinase
EF-Ts	LeuRS	ThrRS	Nucleotide diphosphate. Kinase
EF-G	MetRS	SerRS	Myokinase
RF1	ValRS	LysRS	Inorganic pyrophosphatase
RF2	GluRS	AsnRS	T7 RNA polymerase
II_ribosomal proteins			
30S ribosomal subunit protein S1	50S ribosomal subunit protein L17	50S ribosomal subunit protein L5	30S ribosomal subunit protein S17
30S ribosomal subunit protein S2	50S ribosomal subunit protein L18	50S ribosomal subunit protein L6	30S ribosomal subunit protein S18
30S ribosomal subunit protein S3	50S ribosomal subunit protein L19	50S ribosomal subunit protein L7/L12	30S ribosomal subunit protein S19
30S ribosomal subunit protein S4	50S ribosomal subunit protein L20	50S ribosomal subunit protein L9	30S ribosomal subunit protein S20
30S ribosomal subunit protein S5	50S ribosomal subunit protein L21	50S ribosomal subunit protein L10	30S ribosomal subunit protein S21
30S ribosomal subunit protein S6	50S ribosomal subunit protein L22	50S ribosomal subunit protein L11	30S ribosomal subunit protein S22
30S ribosomal subunit protein S7	50S ribosomal subunit protein L23	50S ribosomal subunit protein L13	50S ribosomal subunit protein L1
30S ribosomal subunit protein S8	50S ribosomal subunit protein L24	50S ribosomal subunit protein L14	50S ribosomal subunit protein L2
30S ribosomal subunit protein S9	50S ribosomal subunit protein L25	50S ribosomal subunit protein L15	50S ribosomal subunit protein L3
30S ribosomal subunit protein S10	50S ribosomal subunit protein L27	50S ribosomal subunit protein L16	50S ribosomal subunit protein L4
30S ribosomal subunit protein S11	50S ribosomal subunit protein L28	50S ribosomal subunit protein L32	30S ribosomal subunit protein S13
30S ribosomal subunit protein S12	50S ribosomal subunit protein L29	50S ribosomal subunit protein L33	30S ribosomal subunit protein S14
50S ribosomal subunit protein L35	50S ribosomal subunit protein L30	50S ribosomal subunit protein L34	30S ribosomal subunit protein S15
50S ribosomal subunit protein L36	50S ribosomal subunit protein L31	30S ribosomal subunit protein S16	
III_ribosomal RNAs			
23S Rrna	5S rRNA	16S rRNA	

IV_bulk tRNAs			
TRNAalaT	tRNAmetT	tRNAglyX	tRNAglnX
TRNAalaU	tRNAmetU	tRNAglyY	tRNAgltT
TRNAalaV	tRNAmetV	tRNAhisR	tRNAgltU
TRNAalaW	tRNAmetW	tRNAileT	tRNAgltV
TRNAalaX	tRNAmetY	tRNAileU	tRNAgltW
TRNAargQ	tRNAmetZ	tRNAileV	tRNAglyT
TRNAargU	tRNApheU	tRNAileX	tRNAtyrU
TRNAargV	tRNApheV	tRNAileY	tRNAtyrV
TRNAargW	tRNAproK	tRNAleuP	tRNAvalT
TRNAargX	tRNAproL	tRNAleuQ	tRNAvalU
TRNAargY	tRNAproM	tRNAleuT	tRNAvalV
TRNAargZ	tRNAsec	tRNAleuU	tRNAvalW
TRNAasnT	tRNAserT	tRNAleuV	tRNAvalY
TRNAasnU	tRNAserU	tRNAleuW	tRNAvalZ
TRNAasnV	tRNAserV	tRNAleuX	tRNAglyU
TRNAasnW	tRNAserW	tRNAleuZ	tRNAglyV
TRNAaspT	tRNAserX	tRNAlysQ	tRNAglyW
TRNAaspU	tRNAthrT	tRNAlysT	tRNAglnV
TRNAaspV	tRNAthrU	tRNAlysV	tRNAglnW
TRNAcysT	tRNAthrV	tRNAlysW	tRNAtrpT
TRNAglnU	tRNAthrW	tRNAlysY	tRNAtyrT
TRNAlysZ	tRNAvalX		

Table 1: Pure Express composition

Mass spectrometry

To this aim we employed a MALDI-TOF mass spectrometer for NAPPA analysis (Figure 1). The PURE system. Protein biosynthesis proceeds in three steps: initiation, elongation, and termination. In *E.coli*, the translation factors responsible for completing these steps are three initiation factors (IF1, IF2, and IF3), three elongation factors (EF-G, EF-Tu, and EFTs), and three release factors (RF1, RF2, and RF3), as well as RRF for termination. However, RF2 is not required for the translation of genes terminating with the codons UAG or UAA. The PURE system includes 32 components that we purified individually: IF1, IF2, IF3, EF-G, EF-Tu, EF-Ts, RF1, RF3, RRF, 20 aminoacyl-tRNA synthetases (ARSs), methionyl-tRNA transformylase (MTF), T7 RNA polymerase, and ribosomes. In addition, the system contains 46 tRNAs, NTPs, creatine phosphate, 10-formyl-5,6,7,8-tetrahydrofolic acid, 20 amino acids, creatine kinase, myokinase, nucleoside-diphosphate kinase, and pyrophosphatase [17]. The presence of "background" molecules, in fact, represents the main obstacle to the data interpretation and bioinformatic tools are necessary to improve them. For this reason new matching software have been implemented.

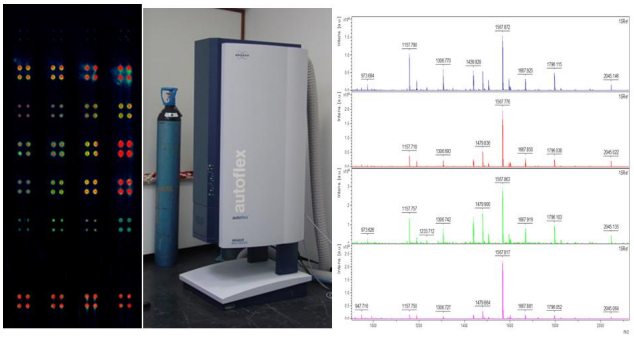

Figure 1: Experimental set-up. (left) Samples were printed on a gold coated glass slides; the array printing was realized in a special geometry for MS analysis. The spots of 300 microns were printed in 12 boxes of 10x10 printed with SNAP genes (p53, CDK2, Src-SH2 and PTPN11-SH2), lower boxes were printed with master mix as negative control. (cente) SNAP-NAPPAs were analyzed by MALDI-TOF MS. For Bruker MS analysis the matrix was mixed with the trypsin digested fragment solutions directly on the slides and let to dry before the analysis. (right) Mass spectra summation with the arrows pointing at the theoretical peak position.

SpADS was used for the subtraction of the Master Mix spectrum from p53 and ptp spectra respectively [13]. The options used for the preprocessing of these latter two spectra were a binning window of 100 and peak extraction. No Region of Interest were selected, i.e. the whole range of the spectra were used. Finally, before the background subtraction, a peak alignment was performed.

SpADS an R implementation of preprocessing algorithms for data reduction and noise suppression was used in order to filter results from background noise i.e. master mix MS spectrum. Moreover, this latter was used coupled to and R implementation of the K Means clustering (Figure 2).

Results

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 Autoflex. 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 with espect to 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. For this latter reason SpADS: An R Script for Mass Spectrometry Data Preprocessing before Data Mining an ad hoc script was implemented. SpADS provides useful preprocessing functions such binning, peak extractions, spectra background subtraction and dataset managing. Moreover, in its final version, it is able to perform peak recognition

and amplitude independent subtraction functions were implemented [13].

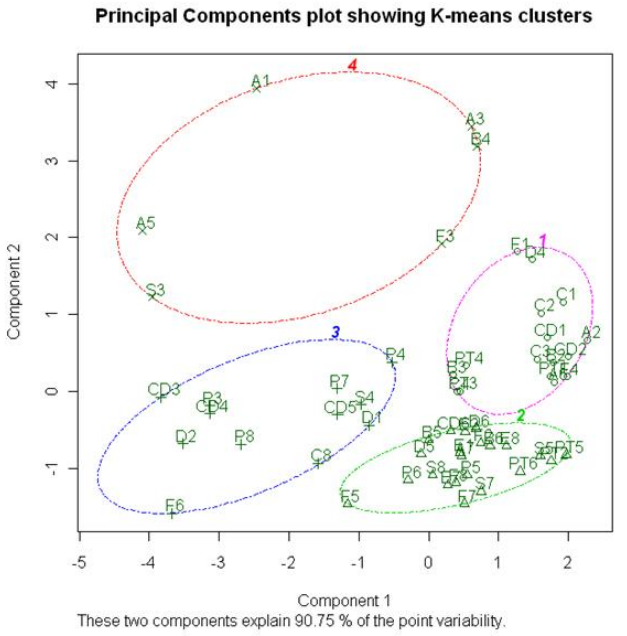

Figure 2: SpADS and Clustering solution for a specimen of 56 protein samples of raw data. Only binning preprocessing function was performed before cluster analysis run on the ROI 1000/1200.

Results are showed in Figures 3-6. To reduce the sample complexity (i.e. the amount of biological material due to NAPPA chemistry and to the expression system) the *in vitro* translation-transcription (IVTT) system we used was from *E.coli*. The PURE system represents an important 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. The PURE system has the capacity for a yield of more than 100 μg/ml is today exclusively licensed to New England Biolabs (Ipswich, MA, USA) under the trade-name "PURExpress" [17] Moreover the *E.coli* IVTT lysate is totally characterized, which could be a fundamental advantage for the subsequent analysis of the results.

The base to realize the "PURE system" database was the full knowledge of PURE EXPRESS composition. Through Expasy databank (www.expasy.org) search we identified the peptide sequences for each component. These sequences were *in silico* trypsin digested by means of the software Sequence Editor included into the Biotools package. Hereafter the concentrations of the components used in the PURE system [17].

The proteins immobilized on the SNAP are synthesized with a SNAP tag and a FLAG tag that could also contribute to the difficulty in matching spectra with databases that are based on tryptic digests of natural proteins. It was then useful to consider strategies that compensate for this.

We have, then, modified the sequence of our proteins, adding the tag sequences (the full protein sequences were obtained from NEB). We used this modified sequence to perform a new fingerprint: the theoretical mass lists of the chimeras after trypsin digestion by means of the software SequenceEditor included into the Biotools package. We matched the experimental mass lists with these theoretical mass lists.

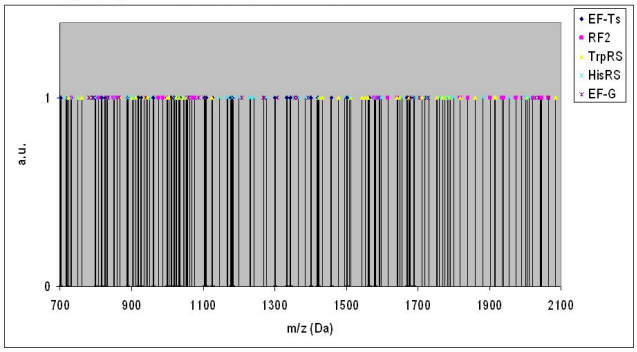

Figure 3: Reconstructed MS spectrum obtained adding five different theoretical mass lists of PURE express components (reported in the legend)

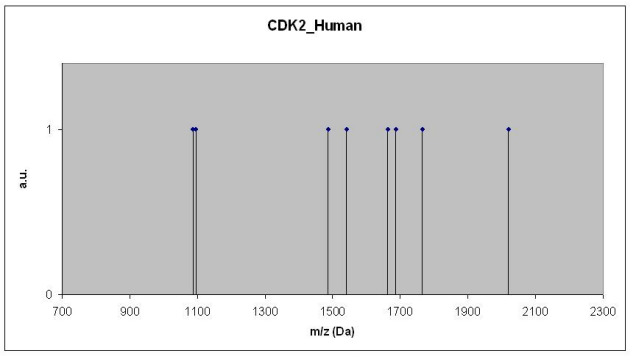

Figure 4: Reconstructed MS spectrum obtained subtracting from CDK2 experimental mas list the PURE express compnents theoretical mass lists

In Figure 1 it is reported a theoretical mass spectrum, reconstructed starting from the theoretical mass list of different PURE system components (as reported in the figure legend), after trypsin digestion, by means of Microsoft Excel software. It is evident the high complexity of such kind of analysis without the aid of a specific software.

In Figures 2 and 3 are reported the experimental mass spectra of Cdk2 and p53 tryptic digested samples obtained by Microsoft Excel software after the subtraction of the theoretical mass lists of tryptic fragments of all the PURE systems components. For PTPN11 SH2 and SRC SH2 no peak remained after the background subtraction that is probably due to a lower level of expression of these proteins. In summary out of the total 140 lists summarized in Tables 1 and 2 only 5 different theoretical mass lists (reported in the side legend) of the PURE express components bacterial lysate are shown in Figure 3.

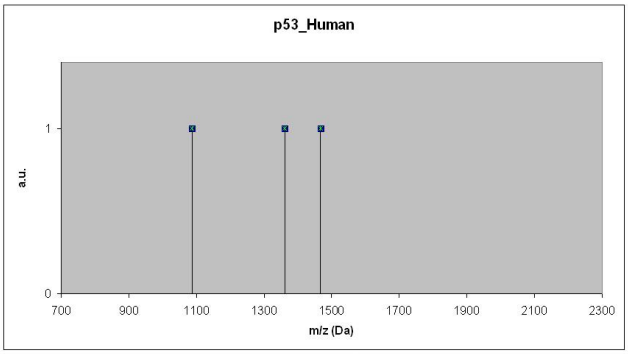

Figure 5: Reconstructed MS spectrum obtained subtracting from p53 human experimental mas list the PURE express components theoretical mass lists

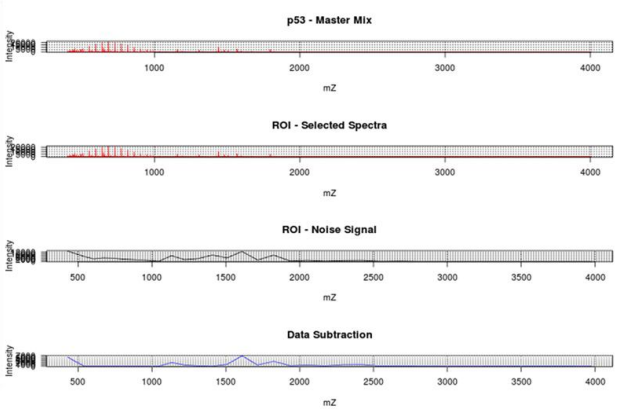

Figure 6: The bottom image is produced subtracting from p53 spectra the Master mix spectra properly alligned. The software cannot instead produce significant results automatically subtracting the bacterial lysate from the NAPPA spectra

Translation components	Concentration (µg/µl)
AlaRS	13
ArgRS	10
AsnRS	30
AspRS	22
CysRS	25
GlnRS	36
GluRS	26
GlyRS	30
HisRS	30
IleRS	20
LeuRS	22
LysRS	35
MetRS	27
PheRS	23
ProRS	16
SerRS	17
ThrRS	19
TrpRS	11
TyrRS	22
ValRS	20
MTF	12
IF1 3	37
IF2	35
IF3	1.5
EF-G	20
EF-Tu	7
EF-Ts	9
RF1	2.9
RF3	41
RRF	15

Table 2: Peptide Sequence of the component I of PURE Express

And even these few are difficult to distinguish. Figures 4 and 5 represent the reconstructed MS spectra obtained subtracting respectively from CDK2 and p53 experimental mas lists all the peaks of the PURE express components theoretical mass lists, after a very long work utilizing excel. For the experimental mass lists of SRC e PTP genes samples nothing remains visible (not shown). A satisfactory result is that some peaks are still present in half of our sample genes, suggesting that with the aid of ad hoc software this kind of analysis will improve significantly the end results. Encouragingly Figure 6 show in the bottom image a similarly good result is obtained by subtracting from p53 spectra the experimental Master mix spectra when properly alligned. The software cannot instead produce significant results automatically subtracting the bacterial lysate Master Mix from the NAPPA spectra.

Conclusions

In the present manuscript we have successfully carried out a proof of principles which however need further optimization of the experimental layout in progress. Recent development the monitoring of gene-gene [6,7,14,18,19] and protein-protein [20] interactions in SNAP NAPPA microarray by QMC_D nanoconductimetry [8], Mass Spectrometry [10], Anodic Porous Allumina, [21] and Bioinformatics [14] open new avenues in functional proteomics overcoming the critical limits of fluorescence clinical studies using Nucleic Acid Programmable Protein Arrays or similar [22]. It appears thereby of fundamental importance to combined Nanogenomics and Nanoproteomics to warrant significant advancements in clinical

research in general and in cancer treatment in particular. Our main pertinent findings characterizing several model system and several nanotechnologies support these conclusions and progress achieved in the improvement of automated label free biomarkers detection in NAPPA SNAP microarrays by Mass Spectrometry and subsequent sophisticated data acquisition and processing.

Acknowledgments

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References

1. Spera R, Josh L, Nicolini C (2011) MALDI-TOF Characterization of NAPPA Generated Proteins. Journal Mass Spectrometry 46: 960–965.
2. Spera R, Nicolini C (2011) CHO Proteome Alterations Induced By Reverse Transformation. Cell Biochem Biophys 61: 731-737.
3. Weston AD, Hood L (2004) Systems biology, proteomics, and the future of health care: toward predictive, preventative, and personalized medicine. J Proteome Res 3: 179-196.
4. Bragazzi N, Nicolini C (2014) Nanogenomics Enabling Personalized Medicine: An Application To Kidney Transplantation. Cell and Molecular Biology.
5. Bragazzi N, Spera R, Pechkova E, Nicolini C (2014) NAPPA-Based Nanobiosensors for the Detection of Proteins and of Protein-Protein Interactions Relevant to Cancer. Journal of Carcinogenesis and Mutagenesis.
6. Bragazzi NL, Pechkova E, Nicolini C (2014) Proteomics and proteogenomics approaches for oral diseases. Adv Protein Chem Struct Biol 95: 125-162.
7. Spera R, Terencio T, Bezerra Correiab, Nicolini C (2013) Nappa Based Nanogravimetric Biosensor: Preliminary Characterization. Sensors Actuators B 182: 682– 688.
8. Spera R, Festa F, Bragazzi NL, Pechkova E, LaBaer J, et al. (2013) Conductometric monitoring of protein-protein interactions. J Proteome Res 12: 5535-5547.
9. Nicolini C, Spera R, Festa F, Belmonte L, Chong S, et al. (2013) Mass Spectrometry And Florescence Analysis Of Snap Arrays. J Nanomed Nanotechnol 4: 181.
10. Nicolini C, Correia TB, Stura E, Larosa C, Spera R (2013) Atomic Force Microscopy And Anodic Porous Allumina Of Nucleic Acid Programmable Protein Arrays. Recent Pat Biotechnol 7: 111-121.
11. Zhang X, Zhang J, Li Q, Wu XY, Wang T, et al. (2013) SELDI-TOF-MS in chronic obstructive pulmonary disease. Chinese Science Bulletin 58: 634-640.
12. Yu X, Xu D, Cheng Q (2006) Label-free detection methods for protein microarrays. Proteomics 6: 5493-5503.
13. Belmonte L, Spera R, Nicolini C (2013) SpADS: An R Script for Mass Spectrometry Data Preprocessing before Data Mining. J Comput Sci Syst Biol 6: 298-304.
14. Bragazzi NL, Nicolini C (2013) A Leader Genes Approach-based Tool for Molecular Genomics: From Gene-ranking to Gene-network Systems Biology and Biotargets Predictions. J Comput Sci Syst Biol 6:165-176.
15. Ramachandran N, Raphael JV, Hainsworth E, Demirkan G, Fuentes MG, et al. (2008) Next-generation high-density self-assembling functional protein arrays. Nat Methods 5: 535-538.
16. Pechkova E, Chong S, Tripathi S, Nicolini C (2010) Cell free expression and APA for NAPPA and protein crystallography, in Functional Proteomics and Nanotechnology-based Microarrays, Pan Stanford Series on Nanobiotechnology.
17. Shimizu Y, Kanamori T, Ueda T (2005) Protein synthesis by pure translation systems. Methods 36: 299-304.
18. Nicolini C, Pechkova E (2010) Nanoproteomics for nanomedicine. Nanomedicine (Lond) 5: 677-682.
19. Jovanovic V, Giacomelli L, Sivozhelezov V, Degauque N, Lair D, et al. (2010) AKT1 leader gene and downstream targets are involved in a rat model of kidney allograft tolerance. J Cell Biochem 111: 709-719.
20. Spera R, Badino F, Hainsworth E, Fuentes M, Srivastava S, et al. (2010) Label-free detection of NAPPA via mass spectrometry, in Functional Proteomics and Nanotechnology-based Microarrays, Pan Stanford Series on Nanobiotechnology.
21. Nicolini C, LaBaer J, Nanotechnology applications of Nucleic Acid Programmable Protein Arrays, in Functional Proteomics and Nanotechnology-based Microarrays, Pan Stanford Series on Nanobiotechnology.
22. Nicolini C, Bragazzi N, Pechkova E (2012) Nanoproteomics enabling personalized nanomedicine. Adv Drug Deliv Rev 64: 1522-1531.