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Meeting Abstracts

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Proceedings ELBA NW Nanoforum XLI. Part-III: Concluding Remarks

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Part-3 Concluding Remarks

LB Based Nanocrystallography at the Frontiers of Proteomics

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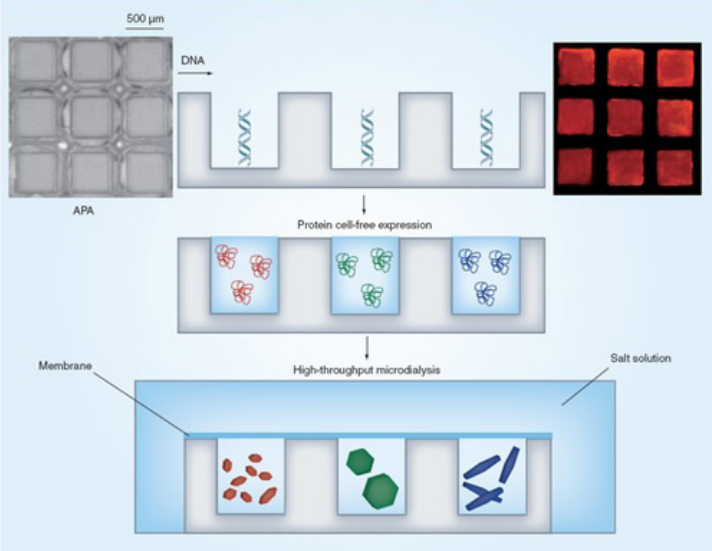
Label-free Nucleic Acid Programmable Protein Array (NAPPA) technology, in combination with protein nanobiocrystallography and its possible future development using anodic porous alumina (APA) along with a cell-free expression system (as summarized here in Figure), appear to form a single approach capable of effectively solving the numerous problems still present in medical diagnosis and therapy. The Anodic Porous Alumina (APA) surface is prepared by a suitable electrolytic process designed to obtain a regular distribution of deep micrometric holes. The high aspect ratio (depth/width ratio) of the pores makes this material also a natural wave guide for any fluorescent molecule present on the bottom of the pores, avoiding crosstalk of many point-light sources too close as frequently in fluorescent NAPPA. The dielectric properties of Al_2O_3 makes this structure optimal for the realization of an electrically anisotropic system; The application of APA-NAPPA approach as an advanced "on chip laboratory" could result in challenging application - the cell free expressed protein molecules, trapped in the pores, after adding the precipitate can become protein nanocrystals useful for upcoming frontier XFELs technology for protein structure determination. The LB nanotemplate deposited onto the APA surface could be used to triggered protein nanocrystals formation. In principle, the crystallization in the pore could be achieved by means of microdialysis or batch crystallization methods. In the LB approach, nucleation is initiated at the interface between the high surface density LB film and protein/precipitant solution. *In situ* micro- and nano- GISAXS (Grazing Incidence Small Angle X-ray Scattering) studies confirms the film re-organization when in contact with protein/precipitant solution, resulting in nanocrystal formation, even those unobtainable by classical methods. Previously, LB grown crystals (up to submicron dimensions) were proved to be radiation stable in comparison those grown by classical method. This phenomenon could be fully exploited in serial femtosecond crystallography without cryocooling, reducing the radiation damage effects to the protein structures.



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QMCD Nanoconductimetry and Mass Spectrometry Using SNAP Arrays of Genes for Cancer Control

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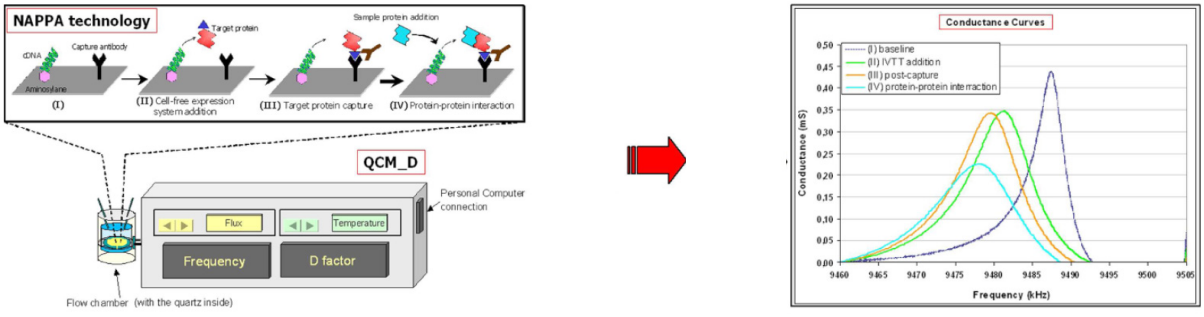
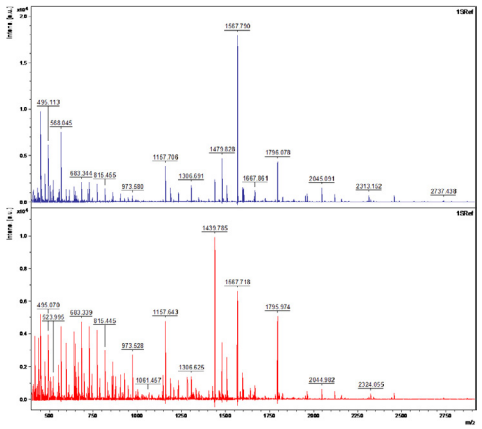
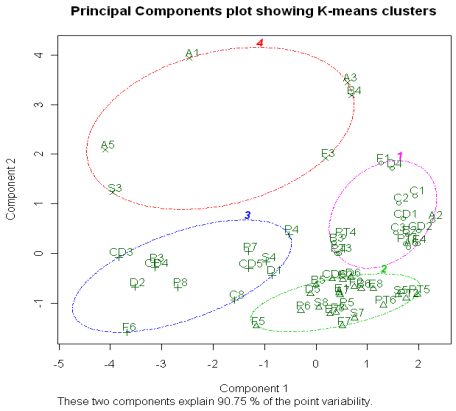
Nanobiotechnology Moscow State University and Foreign Member Russian Academy of Sciences Using the New England BioLabs (NEBL) SNAP-based Genes Expression in conjunction with our "sub-micron arrays" (Anodic Porous Allumina and/or Kapton based Nanopores), we exploit our proprietary microarrays scanner (DNASER, DNA analyzer) and Label Free Nanotechnologies to carry out the construction of SNAP-based Genes Nanoarrays, using gold surface coated for 10 minutes with 2% solution of 32) Aminopropyltriethoxysilane (APTES) in acetone, rinsed in acetone and dried with filtered air. Full length complementary DNAs (cDNAs) for oncosuppressor 53 (p53), Cyclin-dependent kinase 2 (CDK2), SH2 (Src Homology 2) domain of the proto-oncogene trosine-protein kinase (Src) and tyrosine-protein phosphatase non-receptor type 11 (PTPN11) were amplified and cloned. Printing mix was prepared with 0.66 μg/μl DNA capture reagent BG-PEG-NH2 for the one-step synthesis of SNAP-tag substrates from esters on labels or surfaces. Determination of Protein-Protein Interaction for the chosen cancer following the identification of leader genes (or hub genes, investigated with theoretical ab initio bioinformatics analysis using in-house software and algorithms, and then experimentally confirmed via DNASER). These genes are expressed by PURE (Protein synthesis Using Recombinant Elements) Express in spots less than 1 micron size piezo-microdispensed and then characterized via (a) Label Free proprietary Autoflex Mass Spectrometry (MS) integrated with ad hoc software, namely the Spectrum Analyzer and Data Set manager (SpADS) and (b) a proprietary Quartz Crystal Micro-balance with Dissipation factor monitoring (QCM_D) Nanoconductimetry, enabling to describe properties such as changes in frequency and conductance, viscoelasticity and dissipation factor. Solutions without DNA were prepared (called Master Mix, MM), as negative controls, in printing mix. Negative controls were prepared with a varying concentration range of SNAP capture reagent. As a positive control (for fluorescence analysis) mouse IgG or rabbit IgG (Pierce, IL, USA) were added in a printing mix instead of DNA. The above two nanotechnologies gave birth over the years to Label Free Functional Nanoproteomics enabling personalized nanomedicine.



a_ Label Free QCM_D Nanoconductimetry of SNAP Genes Arrays





b_ Label Free Autoflex Mass Spectrometry of SNAP Genes Arrays

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Conclusions

With the above two conclusive papers this 12th edition since 1991 of the Joint Meeting of Russian and European scientists symbolizes a constant search for a world peace and scientific cooperation extended now to North American science leading to this first Joint Nanoforum among the Nanoworld Journal and Conference in USA and the LXI edition of the Nanoworld Institute Labs of Fondazione ELBA Nicolini in Europe. At the end of the Conference as one of its concrete outcomes the possible integration is outlined for its participants into future ERC-EU, R01-USA, Small Business USA and Small Business Russian Federation Grants Applications in progress respectively by the Fondazione EL.B.A. Nicolini and its President USA citizen since 1976 and Russian Academy Sciences member since 2008.

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