DETECTION OF BENZO[A] PYRENE IN KEROGEN TYPE IV WITH MULTIPLEX IMMUNOASSAY: RELEVANCE FOR PLANETARY EXPLORATION. M. Moreno-Paz[1], A. Gómez-Cifuentes[1], M. Ruiz-Bermejo[1], O. Hofstetter[2], A. Maquieira[3], S. Morais[3], M. A. Sephton[4], D. Knopp[5], and V. Parro[1], [1]Centro de Astrobiología (CAB, INTA-CSIC), Madrid, Spain, (parrogv@cab.inta-csic.es), [2]Northern Illinois University, DeKalb, IL, USA, [3]Universidad Politécnica de Valencia, Valencia, Spain, [4]Imperial College London, UK, [5]Technische Universität München, Germany.

Introduction: Meteoritic carbonaceous chondrites could have provided organic matter to Mars in the form of amino acids and polyaromatic hydrocarbons (PAHs). Alternatively, any hypothetical martian biota could have provided true molecular biomarkers as aromatic amino acids, steroid and triterpene molecules, or peptides. Most of the methods used to prepare the sample for organic detection in the planetary missions so far have been based on thermal volatilization and pyrolysis. These systems are highly aggressive and very often destroy or modify the target molecules making a cumbersome task their identification. Alternative, milder methods have been proposed over the last decade as those based on liquid extraction and further analysis with biosensor devices[1,2]. Terrestrial kerogen type IV samples exhibit an advanced state of organic matter transformation and it has been proposed as analogue of refractory organic material in carbonaceous chondrites or even Mars organics[3]. Herein we describe the development and validation of a mild, non-destructive, multiplex competitive/inhibitory microarray immunoassay for simultaneous detection of several organic molecules relevant for Mars exploration and/or environmental monitoring. We also have implemented the assay in the SOLID3 (Signs of Life Detector) instrument[4] designed and build for in situ life detection in planetary exploration.

Methods: We have designed a microarray with different small organic aromatic molecules (L and D-Phe[5], modA1_15 aa peptide, benzo[a]pyrene[6], atrazine, sulfametazine, phthalylsulfathiazole, pentahlorophenol, and finasteride[7]) coupled to proteins such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) to form the corresponding hapten-conjugate complexes carrying the small target compound (the hapten). A set of 10 monoclonal and polyclonal antibodies and their corresponding free and hapten-conjugate were used for antibody titration, optimizing the inhibition assay either with single antigen-antibody pair or multiplex microarray immunoassays. The immunoassays were carried out in a nine-array module cassette and in the SOLID instrument[4,8]. The liquid extract from kerogen type IV sample was done by ultrasonication within the extraction cell of the SOLID sample preparation unit.

Results: Most of the antibodies exhibited sensitivities at 1-10 ppb level and some of them even at ppt. A multiplex inhibitory microarray immunoassay (MIMI) capable to distinguish between D and L aromatic amino acids (Phe, Tyr, Trp), benzo[a]pyrene (BAP), pentachlorophenol or sulfone-containing aromatics, was validated with conventional microarray methods as well as with the SOLID instrument.

Detection of benzo[a]pyrene kerogen type IV. The multiplex immunoassay allowed the detection of BAP as well as aromatic sulfones in a water/methanol extract of an early cretaceous sample (c.a. 100 My) enriched in type IV kerogen (Fig. 1). No L/D aromatic amino acids were detected, reflecting the high maturity and the absence of chemical groups. The assay was performed with conventional microarray methods as well as with the SOLID instrument. The MIMI assay permitted to estimate 2-4 ppm concentration of benzo[a]pyrene in the kerogen sample. This was ten times lower than that obtained with organic solvent extraction and analysis with GC/MS. The results demonstrated once more the feasibility of multiplex inhibitory immunoassays and its potential use for in situ analytical instruments in planetary exploration and environmental monitoring.

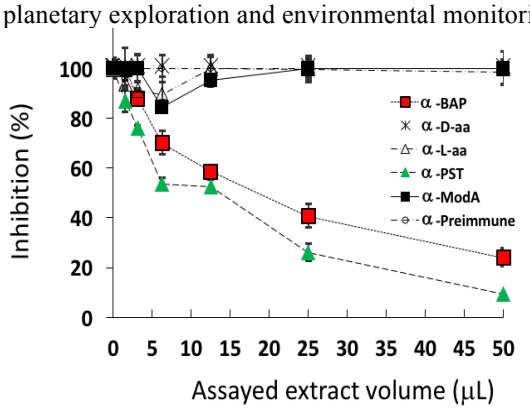

Figure 1. Multiplex Inhibition Microarray Immunoassay detected benzo[a]pyrene and aromatic sulfones from 2.5 mL of an aquoeous extract from 0.5 g of a type IV kerogen sample from early Cretaceous.

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