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Biochip for astrobiological applications: Investigation of low energy protons effects on antibody performances

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ABSTRACT

Antibody-based micro-arrays instruments are very promising tools for the search for biomarkers in planetary exploration missions. Since such instruments have never been used in this context, it is important to test their resistance to space constraints. In particular, cosmic particles might be deleterious. In the present study, we have investigated the effect of low energy protons (2 MeV) on antibody performances with fluences levels much greater than expected for a typical mission to Mars. We show that these particles do not alter significantly the antibody recognition capability for both free (in solution) and grafted (covalently bound to the support) freeze–dried antibodies. Details of the freeze–dried drying process used to optimize antibody performances during our experiments are also presented.

1. Introduction

Searching for signs of past or present life in our Solar System (in particular on Mars, Europa, Enceladus and Titan) is a major challenge in planetary exploration. One approach to conduct the search consists of finding an organic material. The problem is then to determine whether it is the product of an organism or abiotic chemical processes.

McKay (2011) argued that biology is distinguishable from chemistry in that it selects some organic molecules and uses them in preference to other molecules. Indeed, distributions of organic molecules have different patterns when produced by abiotic or biological processes. Moreover, different life forms are likely to have different patterns. So, to detect unambiguously signs of life in extraterrestrial samples, it would be necessary to search for a wide diversity of organic molecules with concentrations widely different from those found in samples known to be free of biological organism. This pattern characterization should be also very important to discriminate a real alien life form (dead or alive) from a merely contamination (by the probe or instruments).

As a consequence, a good strategy to design an ideal instrument for the search for life in the Solar System should be to favor techniques that are able to detect and determine concentrations of a wide number of organic molecules. This technique should also be non-destructive regarding these target molecules.

One of the most promising technologies is based on affinity sensors. A biochip is a miniaturized device composed of molecular recognition tools (or affinity receptors) like antibodies or aptamers, which allows the detection of hundreds of different compounds in a single assay. Antibody and aptamers have been selected toward a wide variety of targets from single molecules (including nucleotides, nucleosides, amino acids, carbohydrates, etc.) to complex target mixtures or whole organisms (Tang, 2007; Nimjee et al., 2005). Several instruments based on this technology are under development. For example, the Life Marker Chip (LMC) (Sims et al., 2005; Martins, 2011), planned for the ESA ExoMars rover mission, and the Signs Of LIfe Detector (SOLID) (Parro et al., 2011) use both antibodies whereas the Biochip for Organic Matter Analysis in Space (BiOMAS) proposes to combine antibodies and aptamers (Le Postollec et al., 2007; Baqué et al., 2011).

One major concern in the methodology adopted in the BiOMAS project is to determine the biochip resistance to space constraints and to define well-adapted protections (Le Postollec et al., 2007). We focus here on one of the space constraints (cosmic radiation) and one affinity sensor (antibody). Indeed, the target detection sensitivity is in

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particular related to the quantity of functional antibody. Thus, to develop a "space biochip", it appears necessary to ensure antibodies survival against space hazards and, in particular, to determine their behavior under cosmic particles irradiation. Le Postollec et al. (2009a) recently performed simulations with the Geant4 toolkit to determine the radiation environment that a biochip would face if it is placed into a rover dedicated to explore Mars surface. Ionizing doses accumulated in the biochip and fluxes of particles entering have been established for both the Earth–Mars transit and the journey at Mars surface. Neutrons and gammas appear as dominant radiation species on Mars soil whereas protons dominate during the interplanetary travel.

Following these original simulation results and considering the lack of experimental data on radiation effect on antibodies, our team decided to investigate the effects of different types of particles at several energies. In a first study, Le Postollec et al. (2009b) performed neutrons irradiation of both antibodies and fluorescein dyes (used for detection of recognition events) at two energies (0.6 and 6 MeV) and with different fluences. Sample analyses demonstrated that, in tested conditions, neutrons do not affect antibody recognition capability and fluorescence dye intensity. In the present paper, we broaden this study from neutron to proton to test the effect of protons on antibody ability to link their target (recognition capability).

Section 2 details proton irradiation parameters used to perform the experiment. Analytical requirements that led to choose the biochip model are presented in Section 3. Section 4 describes samples preparation and analysis protocols. Section 5 gives results. The last section draws conclusions and brings out the perspectives to this study.

2. Proton irradiation

2.1. The external beam line of the CENBG/AIFIRA irradiation facility

The Centre d'Etudes Nucléaires de Bordeaux-Gradignan (CENBG) hosts the "AIFIRA" ion beam platform. This facility is equipped with a 3.5 MV Singletron electrostatic ion accelerator, designed by the HVEE^{TM} company, able to deliver high intensity beams of light ions (H^+, D^+, He^+) up to 50 μA, with a strong beam brightness (>15 A/rad^2 m^2 eV) and a high energy stability ($\Delta E/E \approx 10^{-5}$). Five beam lines are available, one of them being dedicated to non-destructive ion beam analysis techniques: the external beam line. In this line, the ion beam is guided under vacuum using slits, collimators and beam steerers. For this work, we used standard irradiation conditions as followed. Protons were delivered at 3 MeV by the electrostatic accelerator. The beam diameter was set to 2 mm before exiting the vacuum tube of the external beam line. The beam extraction back into air was made through a 5.4 μm Kapton® foil coated with a gold layer of 80 nm.

As described hereafter, the beam line was simulated with Geant4 in order to estimate beam energy and shape all along the irradiation system. Diameter, homogeneity and energy of the beam have been simulated at different distances from the extraction window in order to optimize the positioning of the samples.

2.2. Geant4 modeling of the beam extraction

The external beam line was simulated using the most recent version (9.3) of the Geant4 toolkit (Agostinelli et al., 2003; Allison et al., 2006). Physics processes taken into account are multiple scattering and ionization using the standard electromagnetic package of Geant4. The incident beam diameter was set to 2 mm, as described above, and the beam energy was shot following a Gaussian distribution with a 3 MeV mean value and a standard deviation of 5×10^{-5} MeV.

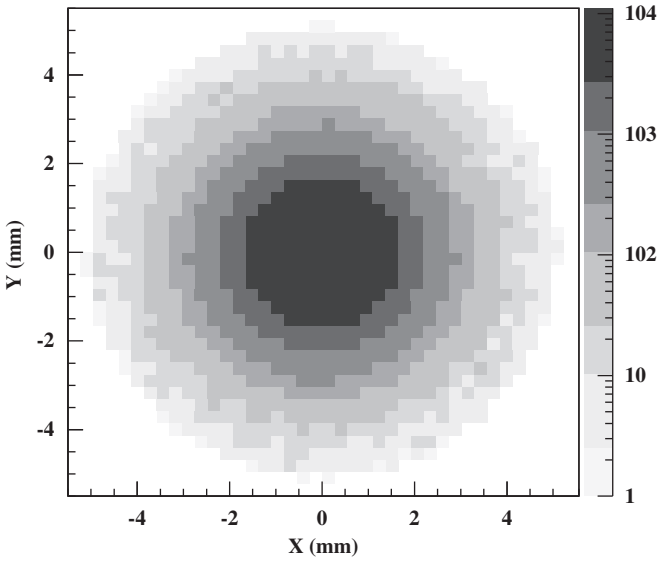

Fig. 1. Two-dimensional beam distribution, 6 cm away from the extraction window.

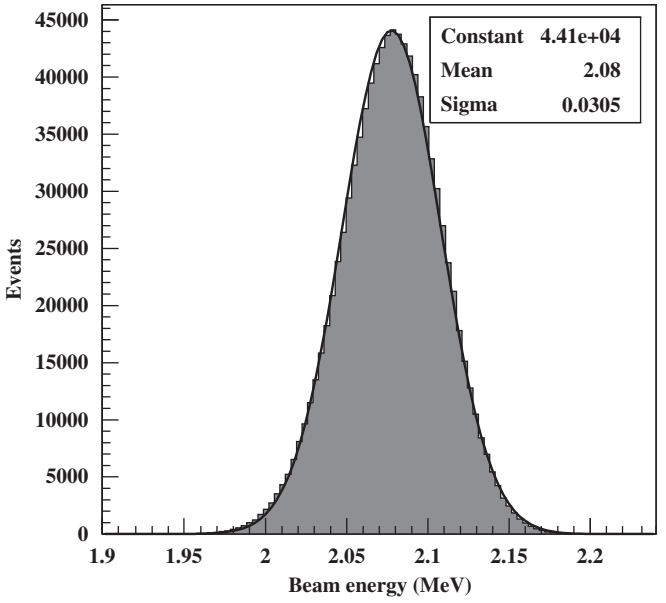

Fig. 2. Beam energy distribution at sample position. A Gaussian fit to the distribution is shown giving a mean value of 2.06 MeV. The fit parameters are given in the top right inset.

One million incident protons were simulated and results are shown in Figs. 1 and 2 for samples positioned in ambient air 6 cm away from the beam extraction window. Fig. 1 shows the two-dimensional beam distribution at this location. The corresponding beam spread at this position is estimated using a Gaussian fit with a standard deviation of 1 mm. Such a beam allows for a targeted irradiation of individual wells (diameter 6.6 mm) containing the samples minimizing irradiation of adjacent wells. On this irradiated surface the beam energy follows a Gaussian distribution with a mean value of 2.06 MeV, as reported in Fig. 2.

2.3. Mars mission simulation

Prior to this experiment, several simulations were performed using two Geant4-based applications: GRAS (Santin et al., 2005) and PLANETOCOSMICS (Desorgher et al., 2005). The objective was