Supplementary material

Integrated chemiluminescence-based lab-on-chip for detection of life markers in extraterrestrial environments

Augusto Nascetti^{1*}, Mara Mirasoli^{2*}, Elisa Marchegiani², Martina Zangheri², Francesca Costantini¹, Alessandro Porchetta³, Lorenzo Iannascoli¹, Nicola Lovecchio⁴, Domenico Caputo⁴, Giampiero de Cesare⁴, Simone Pirrotta⁵, Aldo Roda²

¹ School of Aerospace Engineering, Sapienza University of Rome, Via Salaria 851, 00138 Rome, Italy

² Department of Chemistry "Giacomo Ciamician", Alma Mater Studiorum - University of Bologna,

via Selmi 2, Bologna, Italy

³ Chemistry Department, University of Rome, Tor Vergata, Via della Ricerca Scientifica, 00133, Rome, Italy

⁴ Department of Information Engineering, Electronics And Telecommunications, Sapienza University of Rome, via Eudossiana 18, 00185 Rome, Italy

⁵ Italian Space Agency, Via del Politecnico, 00133 Roma, Italy

Corresponding Authors

Augusto Nascetti

School of Aerospace Engineering, Sapienza University of Rome

Via Salaria 851, 00138 Rome, Italy

augusto.nascetti@uniroma1.it

Mara Mirasoli

Department of Chemistry "Giacomo Ciamician"

Alma Mater Studiorum - University of Bologna, via Selmi 2, Bologna, Italy

mara.mirasoli@unibo.it

Results and discussion

Competitive immunoassay for ATP detection

An indirect competitive ELISA method for the detection of ATP exploiting the PLEIADES chip was developed. The assay was based on the competition of ATP present in the sample and ATP conjugated to ovalbumin immobilized into the microchannel for binding anti-ATP primary antibody. The detection was performed using a secondary HRP-labelled antibody and adding the proper CL substrate. Fig.S1 reported the signals obtained for different concentrations of ATP in a range comprised between 0.3 nM and 20 μ M. For concentrations lower than 10 nM, no significant changes in CL signal were observed with respect to the maximum signal obtained in the absence of the target analyte. The calibration curve was than generated by normalizing the CL signal obtained for each concentration (B) respect to the signal measured in the absence of the target analyte (B0) which represent the maximum value. The B/B0 ratio values were plotted against the log of analyte concentration and the experimental data were fitted with a four-parameter logistic function.



Fig. S1: CL signals related to different amounts of ATP

Bioluminescence luciferase assay

The calibration curve for the bioluminescence luciferase assay (Fig. S2) was generated by integrating the measured bioluminescence signal obtained at different ATP concentrations. The limit of detection was calculated as the concentration corresponding to the signal of the blank sample (measured when the BL cocktail was poured in a well without the analyte) plus three times its standard deviation.



Fig. S2: Calibration curve obtained related to BL luciferase assay using disposable multiwell

cartridges with integrated a-Si:H photosensors