SETTING-UP AN ANTIBODY NUCLEIC-ACID PROGRAMMABLE PROTEIN ARRAY (NAPPA) ANTIBODY MICROARRAY PLATFORM

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Background: Antibody microarrays have emerged as a powerful platform for the quantification of protein abundance and post-translational protein modifications. Nucleic-acid programmable protein arrays (NAPPA) constitute an alternative to usual protein arrays where the proteins are expressed directly in the array using the plasmids codifying for the proteins as source of DNA and rabbit reticulocyte as cell-free expression system. The aim of this work has been to set up an antibody NAPPA protein array using monoclonal recombinant antibodies (scFvs) to avoid the complex step of expressing and purifying the antibodies.

Methods: Seven scFvs against gastrin, CXCL1, CXCL3, MMP7, SPARC, EphB2 and FGFR3 together with their targets were sub-cloned in the vector pANT7_cGST to be expressed in the surface of the array using rabbit reticulocyte. The seven protein targets were purified to homogeneity to test the scFvs binding by ELISA, western blotting and NAPPA scFv microarray experiments.

Results: The seven different scFvs, which possess different frameworks, were "in vitro" expressed and correctly folded, maintaining their binding properties against their respective purified antigens by direct and indirect ELISA. We were able to detect as low as 100 ng/mL of antigen in indirect and direct assays using specific antibodies against the targets and the antigens labelled to biotin or Alexa Fluor 555, respectively. The GST-tagged scFvs in the C-terminal end were correctly displayed in the surface of the array, keeping at the same time both, the folding and the functionality for their respective targets in indirect assays using the purified antigens.

Conclusions: These results demonstrated that the scFvs, GST-tagged in the C-terminal, were able to recognise their respective targets in the NAPPA antibody array. These experiments might suppose the starting point for a NAPPA antibody array that could be used to quantify complex proteomes increasing the amount and diversity of the antibodies printed in the array.