Quantification of circulating cancer biomarkers via sensitive topographic measurements on single binder nanoarrays

Elena Ambrosetti^{1,2,3*}, Pamela Paoletti^{1,4}, Alessandro Bosco⁵, Pietro Parisse¹, Denis Scaini^{1,2}, Elda Tagliabue⁶, Ario de Marco⁷, Loredana Casalis^{1*}

¹ Elettra-Sincrotone S.C.p.A., Nanoinnovation lab, ss 14 km 163.5 in Area Science Park, 34149 Basovizza-Trieste, Italy
² University of Trieste, PhD School in Nanotechnology, Piazzale Europa 1, 34127 Trieste, Italy
³ INSTM-ST Unit, ss 14 km 163.5 in Area Science Park, 34149 Basovizza-Trieste, Italy
⁴ International School for Advanced Studies (SISSA), Via Bonomea 265, 34136 Trieste, Italy
⁵ Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Scheeles väg, 17177 Stockholm, Sweden
⁶ Fondazione IRCCS-Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Via Amadeo 42, 20133 Milano, Italy
⁷ Center for biomedical sciences and engineering, University of Nova Gorica, Dvorec Lanthieri, Glavni Trg 8, 5271 Vipava, Slovenia

* Correspondence: loredana.casalis@elettra.eu; Tel.: +39-040-375-8291; Fax: +39-040-938-0902

^{*} Present address: Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Scheeles väg, 17177 Stockholm, Sweden

Supporting Information



Figure S1 Schematic representation of Antibody-DNA conjugation reaction: first the Ab is functionalized with S-HyNic group and DNA is linked to S-4FB group. Then these two groups react to provide a covalent Ab-DNA conjugate.



Figure S2 Schematic representation of nanobody-DNA conjugation reaction: the Cys thiol group at the C-term of VHH reacts with the maleimide group attached to the 5' end of the DNA sequence.



Figure S3 Schematic representation of the SPR experimental approach: the ssDNA is attached on streptavidin (SA) surface through streptavidin-biotin bond; then the ligand Ab-DNA conjugate is immobilized by hybridization and the ECD-Her2 is run as analyte over the surface.



Figure S4 Histogram created collecting different experiments (number of experiments: 30); data are subdivided into classes of density defined through the ssDNA patches relative height. Error bars represent the standard deviation; red: grafting of ssDNA; green: hybridization of Ab-cDNA conjugate.



Figure S5 Histogram created collecting different experiments (number of experiments: 30); data are subdivided into classes of density defined through the ssDNA patches relative height. Error bars represent the standard deviation; red: grafting of ssDNA; green: hybridization of Nb-cDNA conjugate.



Figure S6 Indirect ELISA. (A) Schematic representation of the assay. (B) Determination of binding affinity between ECD-Her2 and antibodies (MGR2 and MGR3). Absorbance is measured at 450 nm and plotted as a function of Ab concentration (values in duplicate). Data are fitted with Hill equation.