Electronic Supplementary Material (ESI) for Analyst.

The Influence of Covalent Immobilization Conditions on Antibody Accessibility on Nanoparticles

Bedabrata Saha[a†], *Pål Songe*[b] *Toon H. Evers*[a] *and Menno W. J. Prins*[a,c‡*]

[a] Philips Research, High Tech Campus, 5656 AE Eindhoven, The Netherlands

[b] Thermo Fisher Scientific, 0379 Oslo, Norway

[c] Department of Biomedical Engineering, Department of Applied Physics, and Institute for Complex Molecular Systems, Eindhoven University of Technology, 5600 MB Eindhoven, The Netherlands

Supporting Information

Characterization of radio-labelling of biomolecules

Fig. S1: Monitoring the ^{125}I radiolabelling reaction as a function of time (a, b), and with different protein molecules (c-e), using thin layer chromatography (TLC) in phosphoimager. The ^{125}I labelling of mAb1 was monitored (a) after 5 min and (b) after 10 min. Similar ^{125}I labelling of (c) mAb2, and (d) protein G was monitored after 10 min reaction time. From the picture it was observed that the reaction was fast, as completed within 5 min. Horizontal dashed line 1 represents the labelled antibody position (> 99.6% of the phosphoimaging intensity), and line 2 represents the free ^{125}I in reaction mixture (< 0.4%).

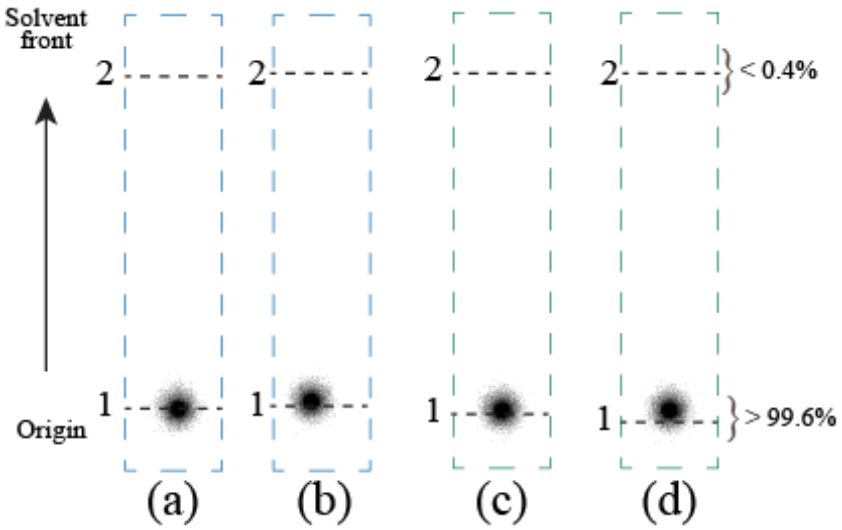


Fig S2: Characterization of anti-troponin antibody and protein G before and after radiolabelling: (A) SDS-PAGE and (B) Radio-imaging in phosphoimager. (A) Lane 1: protein molecular ladder, lane 2: mAb1 non-labelled, lane 3: mAb1 ^{125}I labelled, lane 4: Protein G non-labelled, lane 5: Protein G ^{125}I labelled, lane 6: mAb2 non-labelled, lane 7: mAb2 ^{125}I labelled. (B) Lane 1: ^{125}I labelled mAb1, lane 2: ^{125}I labelled mAb2, lane 3: ^{125}I labelled protein G. The band of protein G appears in SDS PAGE at around 32 kD (in alignment with the report provided by the supplier).

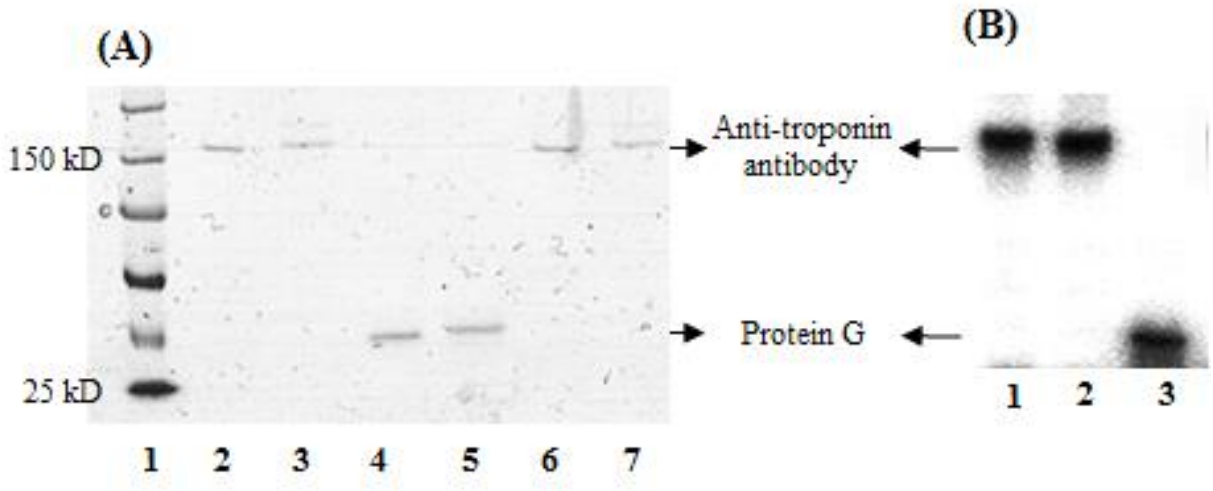
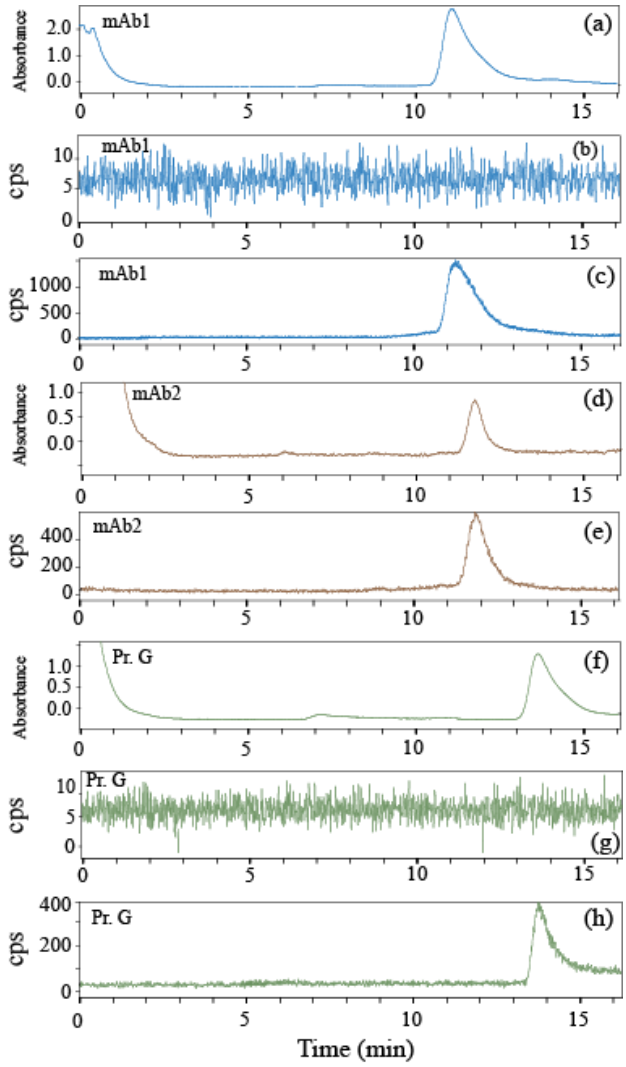


Fig. S3: The biomolecules, labelled with ^{125}I was further analyzed and purified with size exclusion chromatography. Size exclusion chromatography spectra, using Biosep-SEC-S-3000 column with a radiolabel detector, of mAb1 **(a-c)**: (a) UV-Vis detection of eluted ^{125}I labelled mAb1 (similar to unlabelled mAb1, data not shown), radiolabel detection of the elution profile of mAb1 **(b)** before and **(c)** after labelling with ^{125}I; mAb2: (d) UV-Vis detection of eluted mAb2 (similar to unlabelled mAb2, data not shown), **(e)** radiolabel detection of the elution profile of mAb2 after labelling with ^{125}I; protein G (Pr. G): **(f)** UV-Vis detection of eluted protein G, radiolabel detection of the elution profile of protein G **(g)** before and **(h)** after labelling with ^{125}I.



Design of experiment and analysis of antibody immobilization and accessibility

Table S1: Design of experiments of antibody (mAb1) immobilization: different set of experiments for antibody (mAb1) coupling to magnetic nanoparticles performed in this study. In total 19 set of coupling conditions have been used in this set of work, and the amount of immobilized antibodies (mAb1) were quantified in each case. These set of mAb1 coupled nanoparticles were further used for accessibility assays.

	Immobilization parameters			
Experiment Number	**EDC** (equivalent to COOH groups)	**Activation pH** (15 mM MES)	**MAb1 added** (μg per mg of nanoparticles)	**Coupling pH** (15 mM MES)
1	0.5	5.0	20	5.5
2	2.0	5.0	20	5.5
3	0.5	6.0	20	5.5
4	2.0	6.0	20	5.5
5	0.5	5.0	100	5.5
6	2.0	5.0	100	5.5
7	0.5	6.0	100	5.5
8	2.0	6.0	100	5.5
9	0.5	5.0	20	6.5
10	2.0	5.0	20	6.5
11	0.5	6.0	20	6.5
12	2.0	6.0	20	6.5
13	0.5	5.0	100	6.5
14	2.0	5.0	100	6.5

15	0.5	6.0	100	6.5
16	2.0	6.0	100	6.5
17	1.25	5.5	60	6.0
18	1.25	5.5	60	6.0
19	1.25	5.5	60	6.0

Table S2: Second set of design of experiments for antibody (mAb1) immobilization with magnetic nanoparticles performed at higher mAb1 concentration. All the other coupling chemical parameters except mAb1 concentration were kept constant during this set of experiment. In total 5 set of coupling conditions have been used in this work, and the amount of immobilized antibodies (mAb1) were quantified in each case. These set of mAb1 coupled nanoparticles were also further used for accessibility assays.

	Immobilization parameters			
Experiment Number	**EDC** (equivalent to COOH groups)	**Activation pH** (15 mM MES)	**mAb1 added** (μg/mg of nanoparticles)	**Coupling pH** (15 mM MES)
20	1.0	5.5	50	5.5
21	1.0	5.5	100	5.5
22	1.0	5.5	200	5.5
23	1.0	5.5	300	5.5
24	1.0	5.5	500	5.5

Fig. S4. Validity of the contour graphs were analyzed by predicted versus observed plots (with the experimental numbers from Table S1). (a) mAb1 immobilization, (b) Fab domain accessibility, (c) Fc accessibility assay. The correlation coefficients were described in each plot.

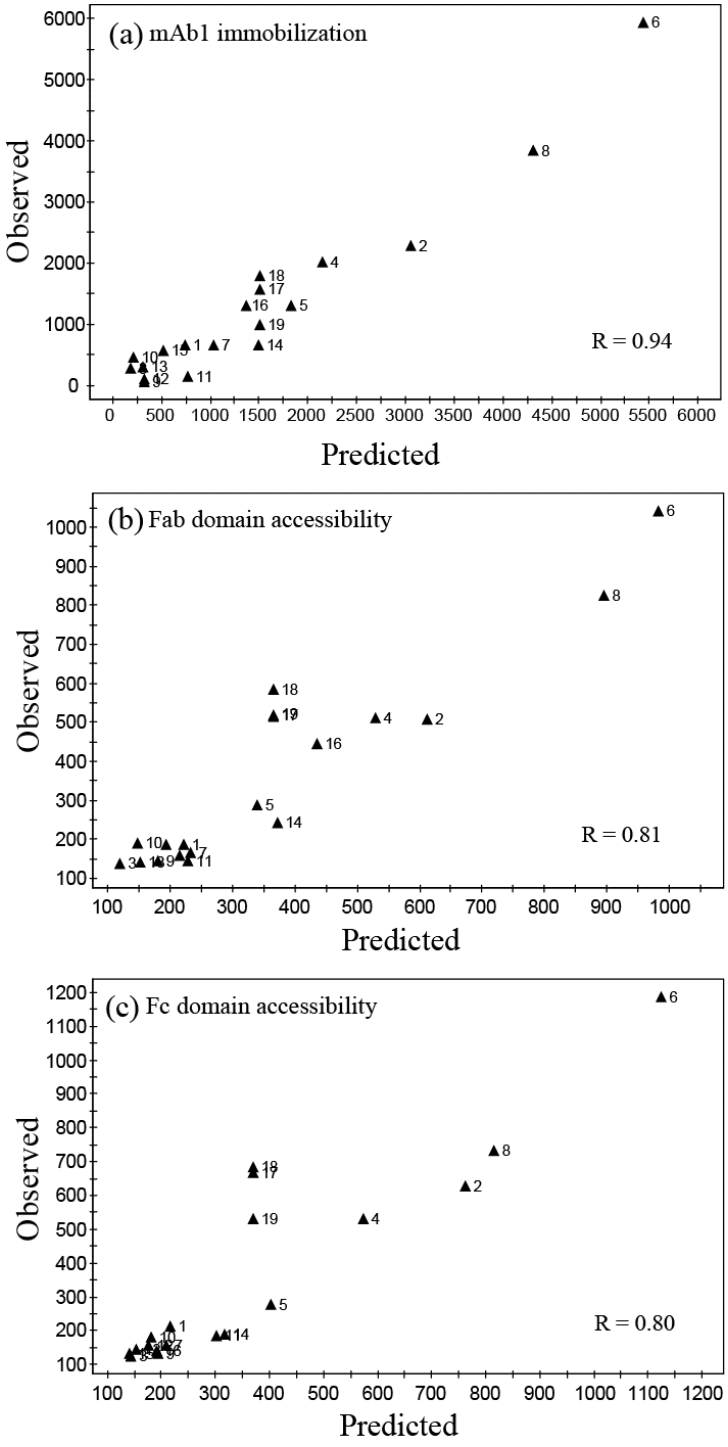


Fig. S5: Coefficient plot (scaled and centered) of the influence of individual coupling parameters (cross-linker concentration, antibody concentration, surface activation pH and coupling pH) on immobilized amount of mAb1 on magnetic nanoparticles, as analyzed by MODDE software (*Umetrics*).

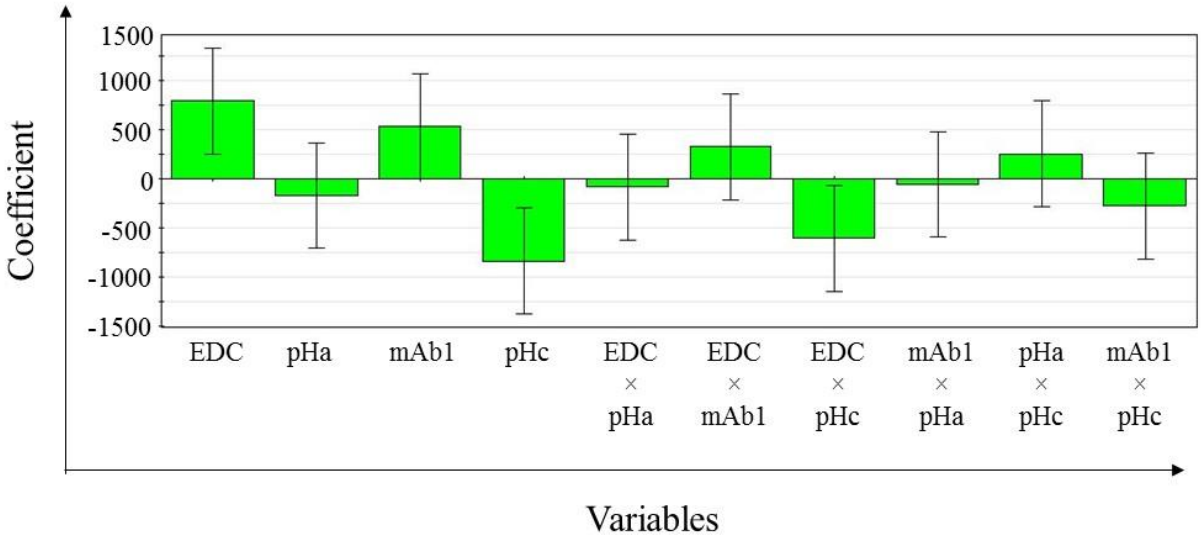


Fig. S6: 4D contour graph of the accessibility assays of mAb1, as a function of mAb1 immobilization condition: (A) Fab domain accessibility assay: molar number ratio (in %) of bound mAb2 to immobilized mAb1, in a cTnI capturing mediated sandwich assay; (B) Fc domain accessibility assay: molar ratio (expressed in %) of bound protein G to immobilized mAb1, in a direct binding assay. Molar ratio was calculated from diving the amount of bound mAb2 and protein G (as quantified from accessibility assays) by immobilized amount of mAb1 per nanoparticle. The data presented in this figure is from the accessibility assays, which were performed with the same set of nanoparticles used for immobilization (from table S1). All the

graphs are plotted as a function of four different mAb1 coupling conditions. Likely, primary X axis: nanoparticle surface activation pH (pHa), primary Y axis: cross-linker (EDC) added in molar equivalence of COOH groups on nanoparticle surface (amount of mmol of COOH group per mg of nanoparticle is provided by the supplier), secondary X axis: antibody coupling pH, secondary Y axis: Amount of mAb 1 (μg) added per 1 mg of nanoparticle for immobilization.

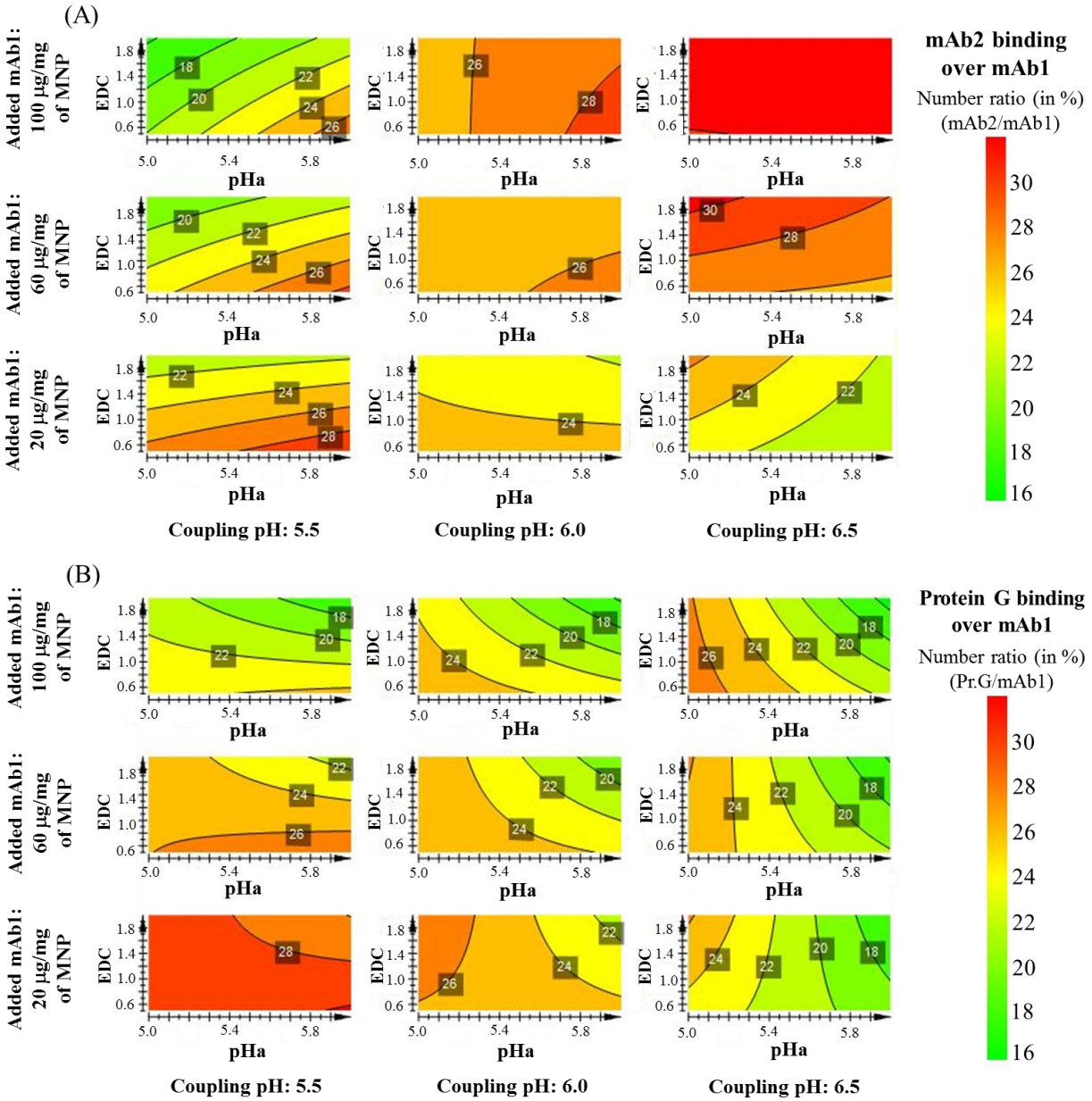


Fig. S7: Coefficient plot (scaled and centered) of accessibility assays: (A) mAb2 binding in a cTnI mediated sandwich assay (Fab domain accessibility assay) and (B) protein G binding in a direct assay (Fc domain accessibility assay), utilizing different set of mAb1 coupled nanoparticles (From table S1 and S2), as analyzed by MODDE software. This plot indicates that, the individual coupling parameters have similar influence on mAb2 and protein G binding in accessibility assays compared to mAb1 immobilization conditions (Comparing with Fig. S4).