Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Linker-free antibody conjugation for sensitive hydrogel microparticle-based multiplex immunoassay

Hyun Jee Lee,[a] Ju Yeon Kim,[a] Yoon Ho Roh,[a] Sun Min Kim[b] and Ki Wan Bong*[a]

[a]Department of Chemical and Biological Engineering, Korea University, Seoul, Republic of Korea.

[b]Department of Obstetrics and Gynecology, Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul, Republic of Korea.

* Author whom correspondence should be addressed.

Email: bong98@korea.ac.kr

I. Mass percentage of the antibody fragments produced in 0.1 mM TCEP

The mass percent of each band in the PAGE gel was approximated by densitometry. The gel analysis function in the ImageJ software was used to generate a histogram of the antibody fragments in 0.1 mM TCEP. The area under each peak corresponds to the intensity of the band times the area of the band. Uneven light exposure is manually excluded out of the data by only measuring the area above the neck of the peak. Densitometry revealed that the composition of the fragments is roughly 37% whole antibodies (2 heavy & 2 light chains), 8% 2 heavy & 1 light chain antibodies, 1% 2 heavy chain antibodies, 30% half antibodies (1 heavy & 1 light chain), 15% 1 heavy chain, and 9% 1 light chain.

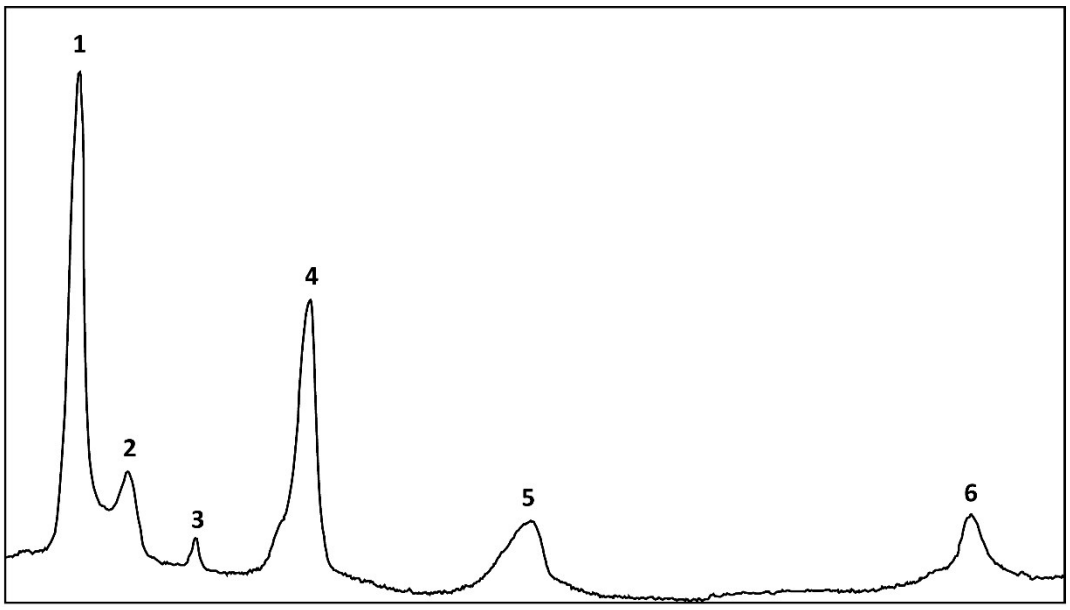

Figure S1 The histogram of the antibody fragments in 0.1 mM TCEP. The mass percentages of each of the six antibody fragments were determined by measuring the area under the corresponding peak.

II. Characterization of the antibody fragments by number

The mass percent of each antibody fragment in 0.1 mM TCEP did not provide a complete picture of the fragmentation events. So, we calculated the relative number of antibody fragments by dividing each mass percent by their corresponding molecular weight. The values were multiplied by 100 to give the least number of relative fragments a value of 1. The result indicated that for every 1 two heavy chain fragment, there are 25 whole antibodies, 6 two heavy and one light chain fragments, 40 half antibodies, 30 heavy chain fragments, and 36 light chain fragments. The summation of the relative number of the heavy chain fragments is 134, which is approximately same value with the total relative number of the light chain fragments, 132. The small discrepancy with the number of light chain fragments is due to the low precision of the densitometry approach. With the relative number of fragments, the relative number of parent antibodies were calculated by tracking pairs of heavy chains. Therefore, there are 67 parent antibodies for every 1 two heavy chain fragment. The percentage of parent antibodies indicated the percentage of the original antibodies that were fragmentized to each form. For example, there are 22% of the original whole antibodies were reduced completely into separate heavy and light chain fragments , which can be obtained by dividing the relative parent antibody number of seperate heavy and light chain into the total number of parent antibody. Lastly, with the assumption that all Fab regions are initially active and interchain disulfide bonds are broken before the intrachain ones, the relative numbers of active Fab regions were calculated by accounting for the number of unbroken heavy-light interchain disulfide bonds.

Table S1 Characterization of the antibody fragments by number

Peak	Ig Chain	Mass Percent	MW	Relative Fragments	Relative Parent Ab		Relative Active Fabs	
		%	kg/mol	n	n	%	n	%
1	2 H + 2 L	37	150	25	25	37	50	52
2	2 H + 1 L	8	125	6	6	9	6	6
3	2 H	1	100	1	1	1	0	0
4	1 H + 1 L	30	75	40	20	30	40	42
5	H	15	50	30	15	22	0	0
6	L	9	25	36				
Total				138	67	100	96	100

III. Improvements in detection sensitivity

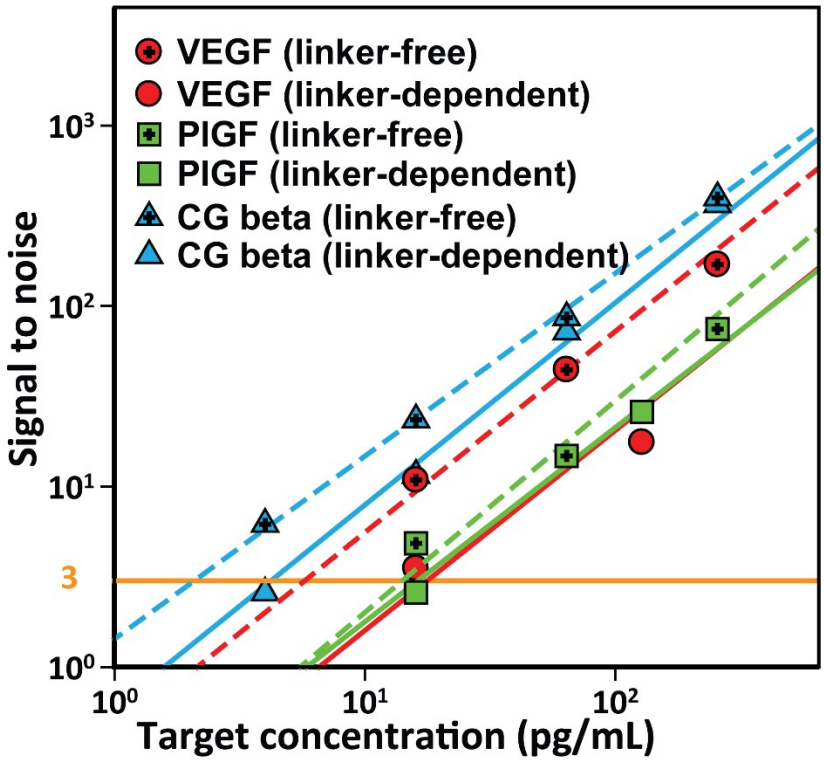

Figure S2 Comparing the signal to noise plots of the linker-free and the linker-dependent antibody conjugation methods. For all three protein targets, the signal to noise ratios of the linker-free particles, which are marked with a cross inside of the symbol, are higher than their linker-dependent counterparts. As a result, the lines of linear regression for the linker-free method (dashed) are above the lines for the linked-dependent method (solid). Likewise, the limit of detection, defined as the target concentration that corresponds to three signal to noise ratio, is lower for the linker-free method.

IV. Immunoassay procedure

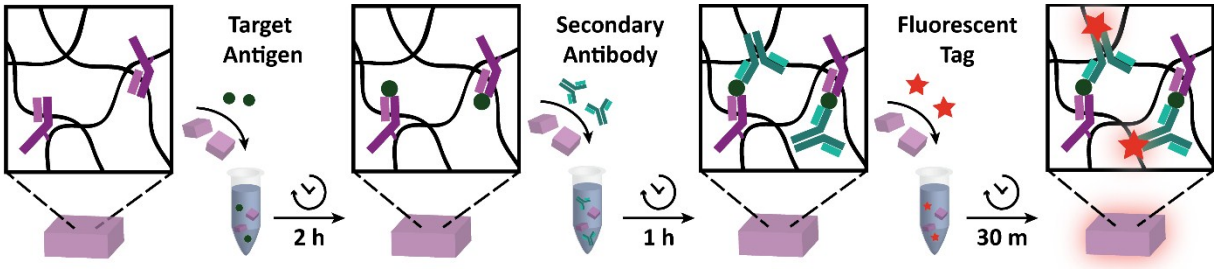

Figure S3 Schematic overview of the immunoassay. The assay sequence correlates with that of the sandwich ELISA. The antibody functionalized particles are mixed into the target sample and antigens are allowed to bind to the capture antibodies. After washing the particles to remove unbounded proteins, secondary antibodies are added. These biotinylated antibodies attach to the target antigen, forming a sandwich unit. Another wash step is performed to remove excess secondary antibodies. Then, streptavidin–phycoerythrin (SA-PE; red) is added to fluorescently label the particle-bound secondary antibodies. Unbound fluorescent tags are washed away in the final wash step.