Supplementary Information

Synergistic Effect of Orientation and Lateral Spacing of Protein G on the On-chip Immunoassay

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Supplementary Tables

Table S1. Calculation of average number of Cy5 dyes per Cy5-labled antibody.

A_{280}	A_{650}	DF		Molar Extinction coefficient ($cm^{-1}M^{-1}$)	Concentration [a] (μM)	Number of dyes per IgG antibody [b]
0.21	0.84	6.0	IgG antibody	$1.70x10^5$ at 280 nm	5.93	3.40
			Cy5 fluorophore	$2.50x10^5$ at 650 nm	20.2	

The absorbance values at 260 and 650 nm were read in the UV/Vis spectrum as shown in Figure S1. Each molar concentration of IgG antibody and Cy5 dye was calculated according to the Beer's law, c = A/εl, where c is the molar concentration, e for the extinction coefficient, and l for the path length.

[a] molar concentration of IgG antibody, $c(\text{IgG antibody}) = (A_{280}\text{-}0.05 \times A_{650}) \times DF/(1.70x10^5\ cm^{-1}\ M^{-1} \times 1.0\ cm)$ and molar concentration of Cy5, $c(\text{Cy5 dye}) = A_{650} \times DF/(2.50 \times 10^5\ cm^{-1}\ M^{-1} \times 1.0\ cm)$, where DF is the dilution factor of the Cy5-labeled antibody during the acquisition of its UV

absorbance.

[b] ratio of the molar concentration of antibody to that of Cy5 dye.

Table S2. Quantification of the fluorescence intensity of the on-chip immunoassay against rIL-2.

Concen. of rIL-2 (pg/ml)	DSC-conjugated, high-density amine surface		DSC-conjugated, 9-acid dendron surface		GMBS-conjugated, 9-acid dendron surface		Ratio of average intensity		
	Protein G(1)	Cys-protein G(2)	Protein G(3)	Cys-protein G(4)	Protein G(5)	Cys-protein G(6)	A(3,4)[a]/ A(1,2)	A(5)/ A(1,2)	A(6)/ A(1)
100,000	21751±379	26054±608	27679±534	29082±320	4692±365	58055±2763	1.1	2.2	2.7
50,000	18075±535	23054±237	25444±243	27974±941	7834±3227	58948±4359	1.2	2.6	3.3
10,000	13959±338	17518±1788	20433±308	21133±299	5062±387	32590±1512	1.2	1.9	2.3
5,000	8604±460	11614±2113	12630±245	15503±331	3446±1327	24602±1551	1.3	2.1	2.9
1,000	2163±109	4328±187	8810±194	5673±97	1657±280	7062±462	1.3	1.6	3.3
200	256±38	481±72	1353±100	947±112	110±28	1819±201	2.0	3.8	7.1
50	20±41	51±10	342±56	243±24	23±7	514±31	4.8	10.1	25.7

[a] A(m, n) denotes the average value of groups m and n at each concentration of rIL-2. The ratio of A(5) to A(1) for the rIL-2 concentration from 1 ng/ml to 100 ng/ml is 2.87±0.39.

Supplementary Figures

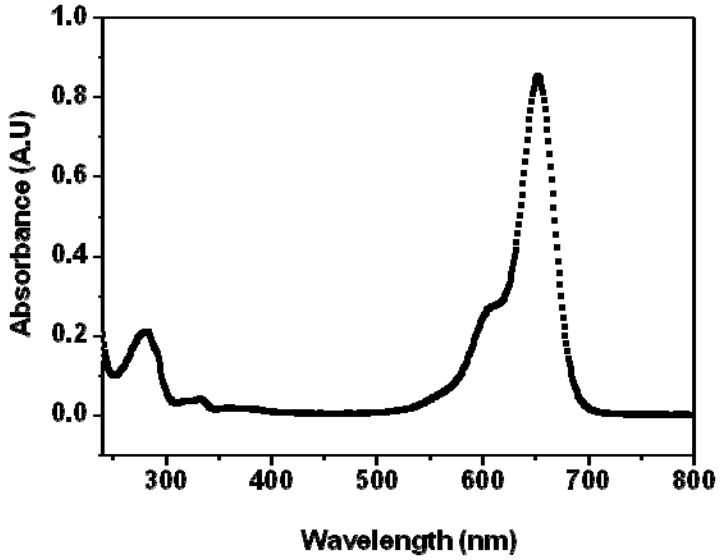
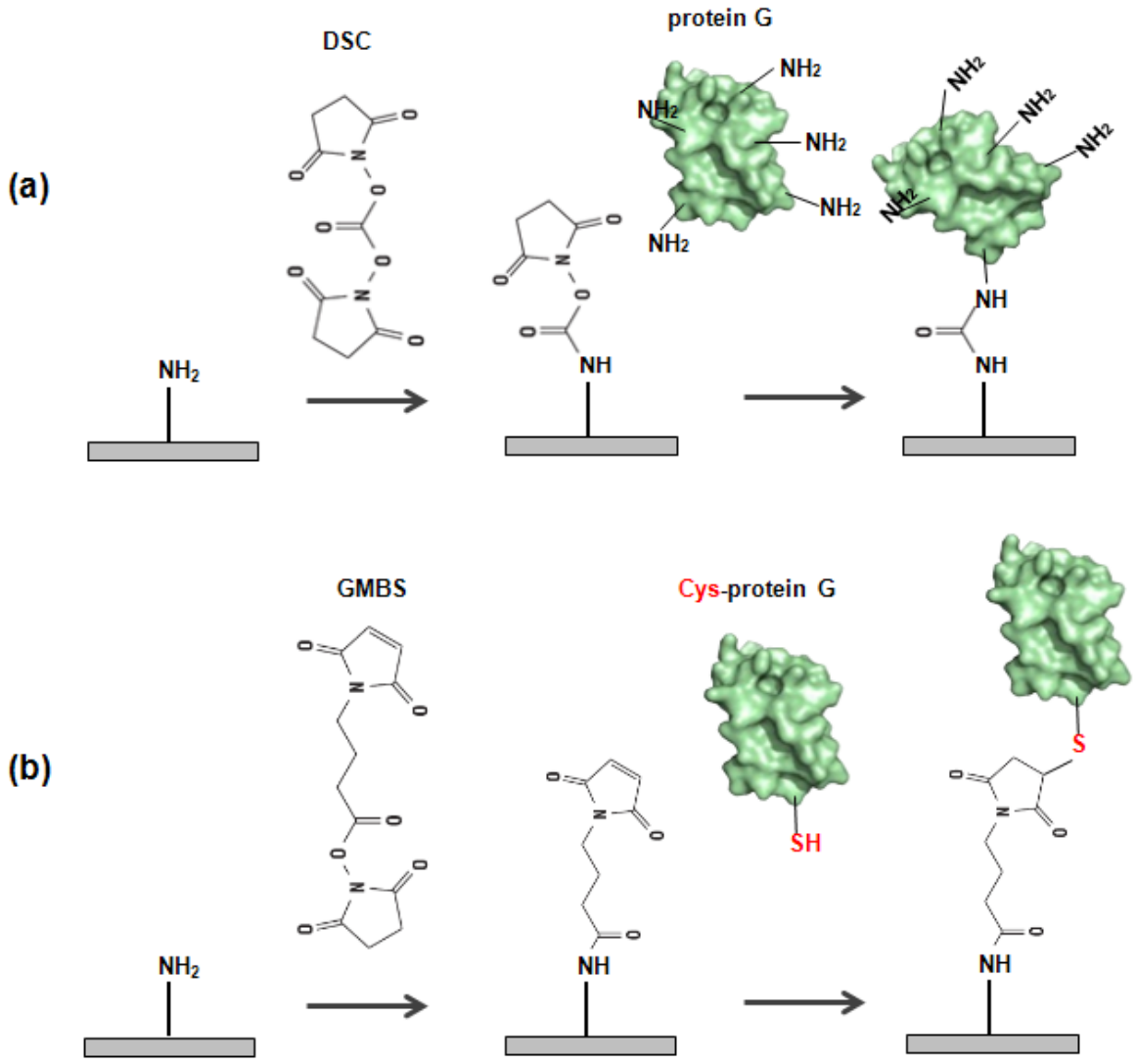


Figure S1. Absorbance curve of Cy5-labled anti-IL-2 IgG_{2b} antibody. The optical absorbance of the Cy5-labled antibody for the light whose wavelength ranged from 240 nm to 800 nm was obtained in a quartz cuvette with a 1.0-cm-long path length.



Scheme S1. Covalent immobilization of protein G and Cys-protein G. (a) The primary amine group of the solid surface could react with DSC to produce the NHS ester functionalized surface. The amine group of one of lysines in the protein G was supposed to be covalently immobilized to form an amide bond. (b) The NHS group of GMBS is to react with the amine group on the surface followed by the conjugation of a thiol group (in red) of the N-terminal cysteine of Cys-protein G to the thiol-reactive maleimide group.

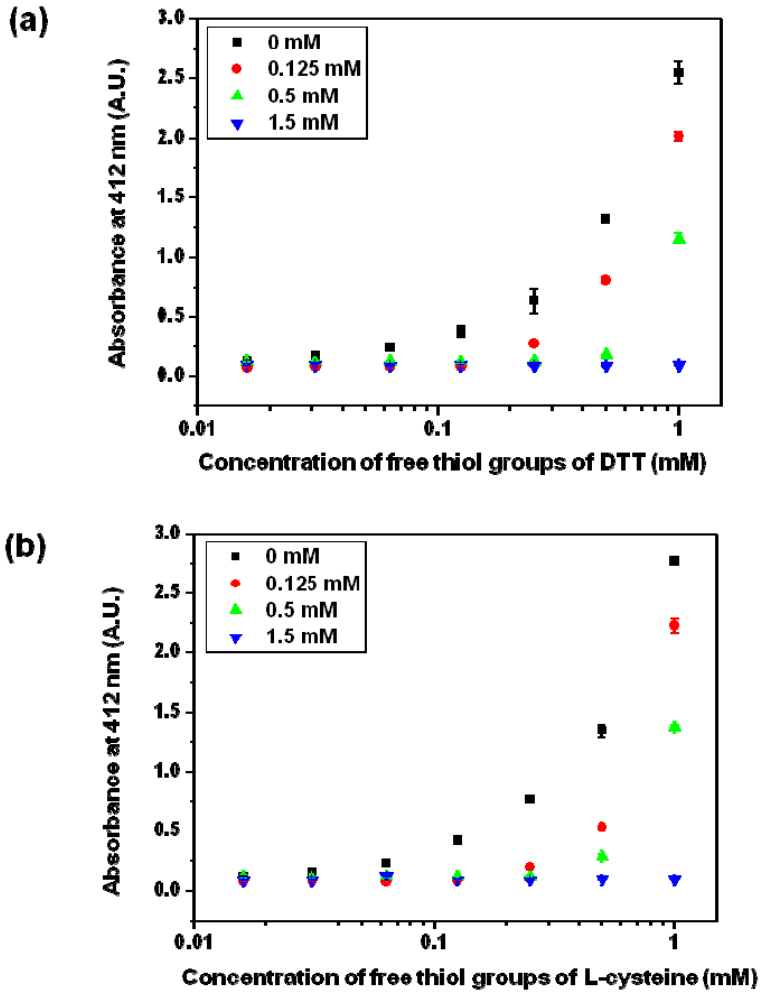


Figure S2. Conjugation of GMBA to free thiol groups in DTT and L-cysteine. The free thiol groups of (a) DTT or (b) L-cysteine in PBS were coupled to thiol-reactive GMBA (0.125, 0.5, or 1.5 mM) in DMF and incubated for 30 min at room temperature. The 20 μl of Ellman's reagent (4 mg/ml) dissolved in 0.1 M sodium phosphate buffer (pH 8.0) was added to the labeled DTT and L-cysteine solution of 100 μl as well as the unlabeled ones (0 mM) in a 96-well plate. After the incubation for 30 min at RT, the plate was placed in a plate reader (SpectraFluor Plus, TECAN) to measure the optical absorbance at 412 nm. The absorbance from three independent measurements was represented by mean±SD and plotted along the concentration of the free thiol group prior to the GMBA conjugation.

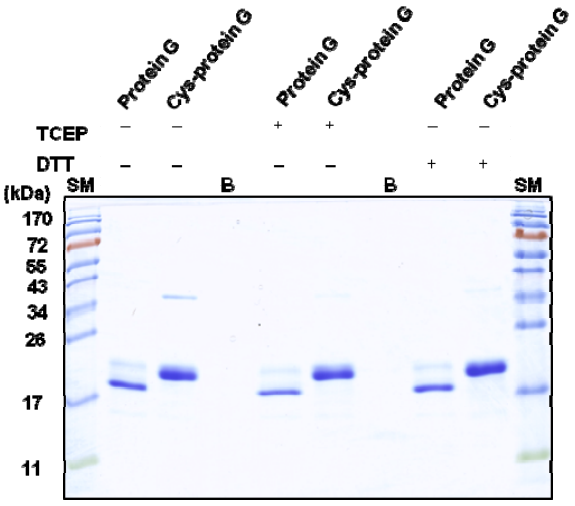


Figure S3. Gel electrophoresis of protein G and Cys-protein G. Protein Gs treated with or without TCEP or DTT were separated on a 15% SDS-polyacrylamide gel. The blank lanes (denoted by B) were intentionally placed between the neighboring groups of the protein Gs to avoid the cross-contamination of the reducing agents during gel electrophoresis. The size makers (SM) from 11 to 170 kDa were also run at both ends of the gel.

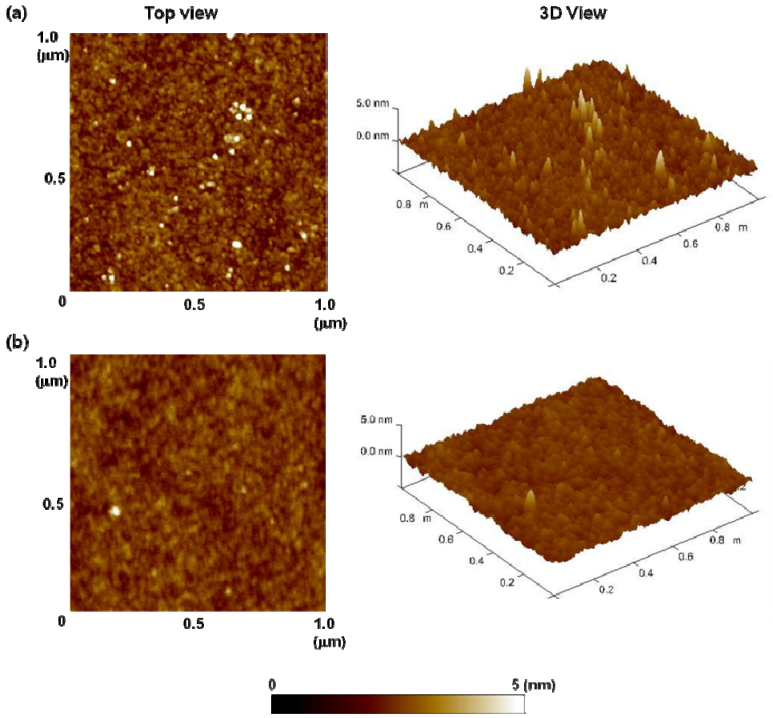


Figure S4. Topographic images of the high-density amine (a) and 9-acid dendron (b) surfaces by AFM. The height was scaled by the graded bar as shown at the bottom.

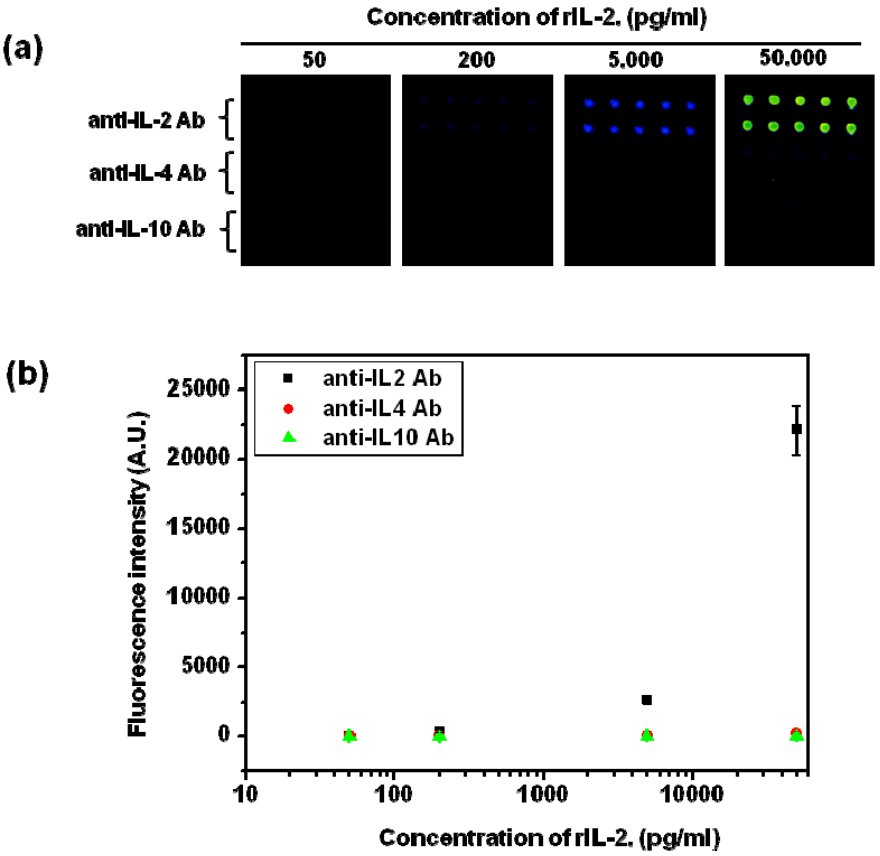


Figure S5. Detection of the target antigen using the capture antibody that was directly immobilized to the surface. (a) Three kinds of capture probes including anti-IL-2, anti-IL-4, and anti-IL-10 antibodies were spotted directly on the DSC-activated 9-acid dendron surface followed by the incubation of rIL-2 at the concentration ranging from 50 pg/ml to 50 ng/ml. The Cy5-labled anti-IL-2 detection antibody was used to detect the rIL-2 bound to the capture antibody. (b) Quantification of rIL-2 detection in each antibody spot.

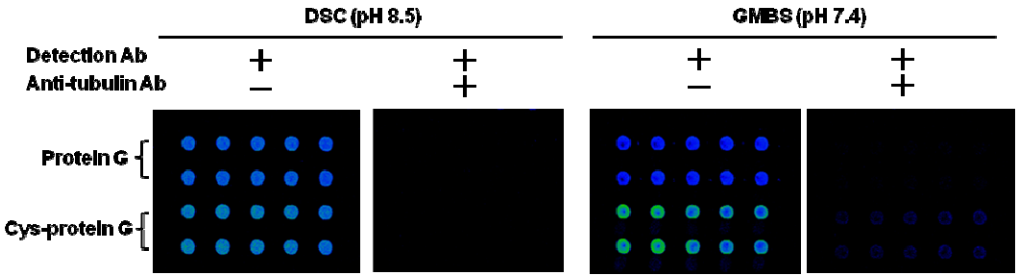


Figure S6. Employment of anti-tubulin IgG_{2a} antibody as a blocking molecule. Even in the absence of rIL-2, the protein Gs immobilized on either DSC-activated or GMBS-activated 9-acid dendron surface showed the detectable Cy5 level when the Cy5-labled detection antibody was incubated. The antibody mixture with the 1:1 molar ratio of the unlabeled anti-tubulin IgG_{2a} antibody to Cy5-labeled detection antibody could decrease the Cy5 signal to the undetectable level in the absence of rIL-2.

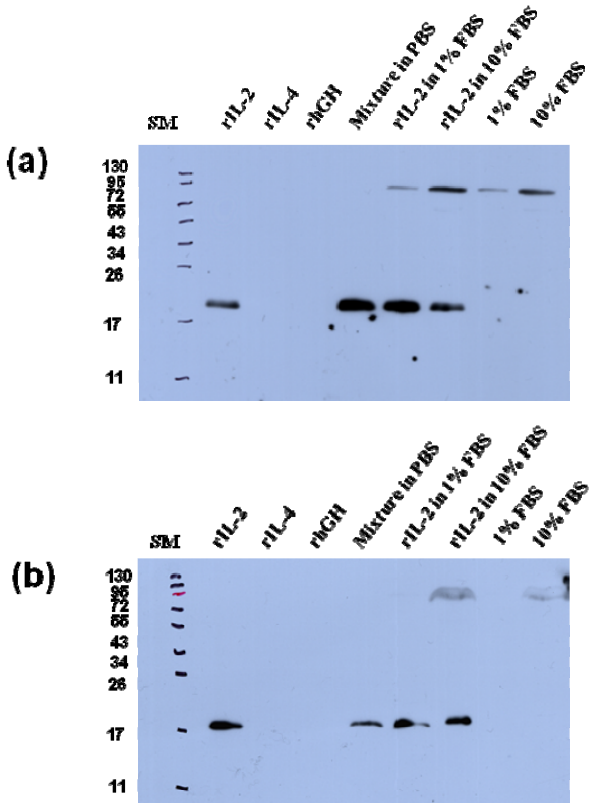


Figure S7. Immunoblotting against rIL-2 in the protein mixture solution or diluted serum solution. Scanned film images are shown when the membrane was probed with either the anti-IL-2 capture antibody (a) or anti-IL-2 detection antibody (b).