

# Comparison of Physical Adsorption and Covalent Coupling Methods for Surface Density-Dependent Orientation of Antibody on Silicon

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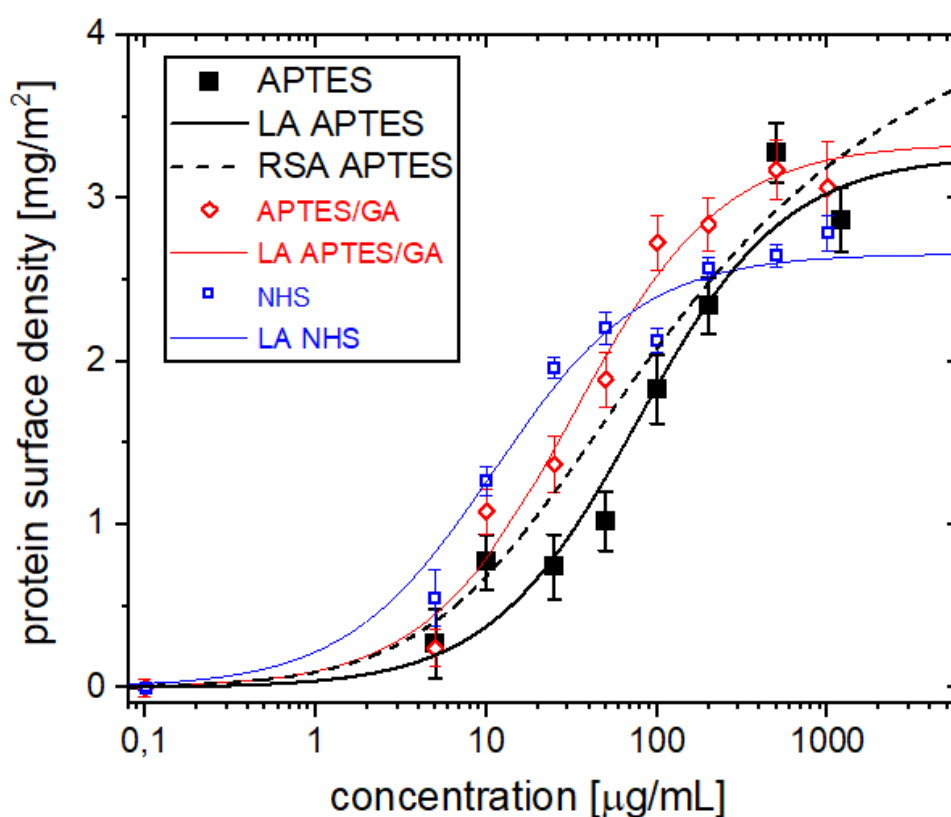
## S1. Adsorption isotherm of IgG on APTES-modified substrate

Surface density values  $\Gamma$  of IgG antibody, immobilized with various concentrations  $c$  of IgG solutions, and determined for SiO<sub>2</sub> substrates modified with APTES (this study, solid squares) are compared with similar results obtained earlier [8] for APTES/GA (open diamonds) and NHS-silanes [8] (open squares) in Fig. S1. The data are described using Langmuir adsorption model with solid lines that are a guide to eye. In addition, description provided by Random Sequential Adsorption (RSA) model for APTES-modified substrate is shown as a dashed line. The data can be described with two adsorption parameters: binding capacity (BC) and affinity constant (AC), provided by both Langmuir (eq. S1) and RSA model (eq. S2) [8]:

$$\Gamma = BC \frac{c}{c + 1/AC} \quad (S1)$$

$$c = \frac{\Gamma}{BC} (1/AC) / \left[ \left( 1 + 0.812 \frac{\Gamma}{BC} + 0.4258 \left( \frac{\Gamma}{BC} \right)^2 + 0.0716 \left( \frac{\Gamma}{BC} \right)^3 \right) \left( 1 - \frac{\Gamma}{BC} \right)^3 \right] \quad (S2)$$

The values of adsorption parameters, determined within both models for IgG adsorbed on APTES-modified substrate, are compared in Table S1 with the values obtained previously [8] for APTES/GA and NHS-silanes.

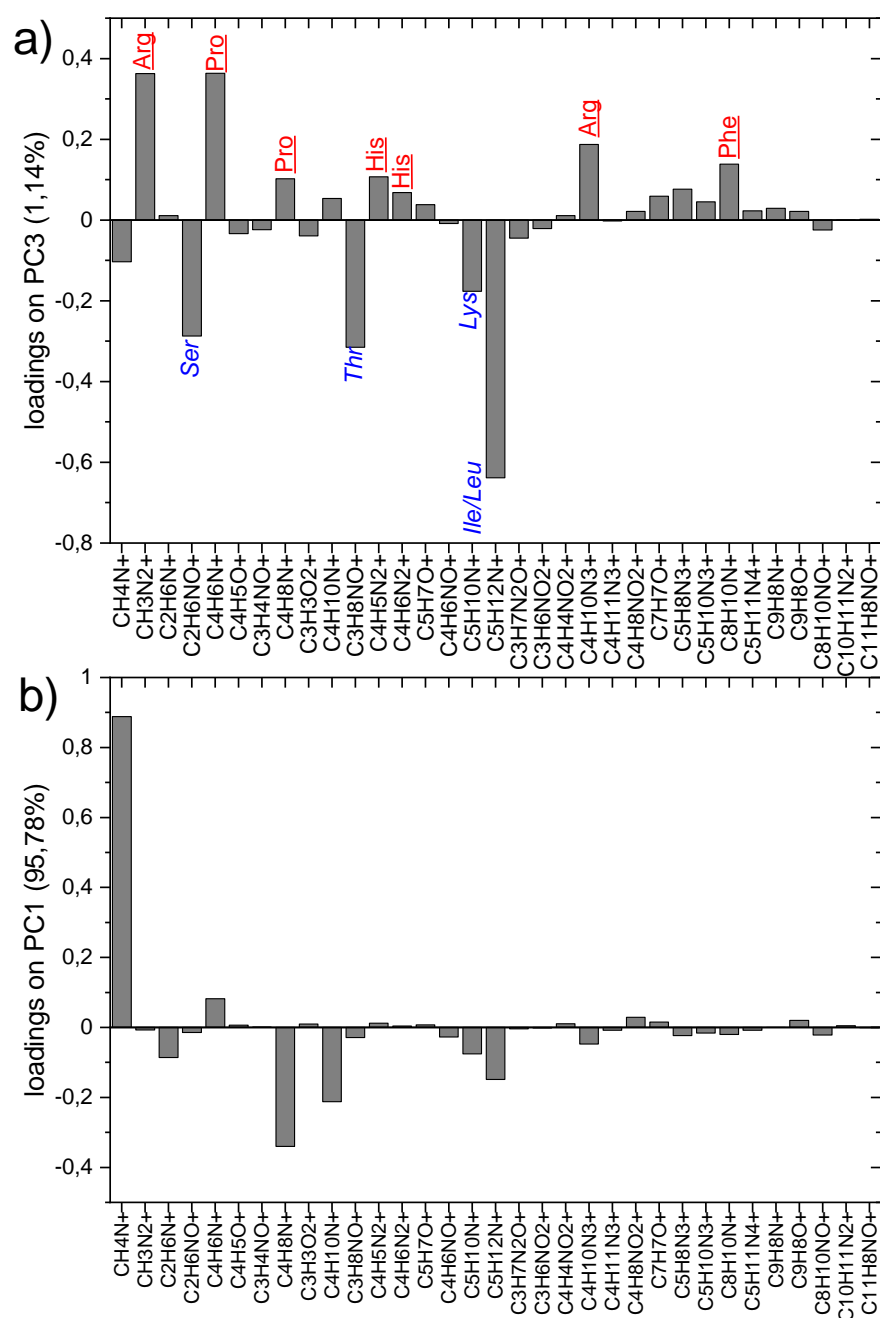


**Figure S1.** Adsorption isotherm of goat anti-rabbit IgG on SiO<sub>2</sub> surface modified with APTES (this study, solid squares), APTES/GA [8] (open diamonds) and NHS-silane [8] (open squares). Solid lines are a guide to eye and describe the experimental data on basis of Langmuir model (LA). In addition, the description provided by Random Sequential Adsorption (RSA) model for APTES-modified surface is marked as a dashed line.

**Table S1.** Binding capacity (BC) and affinity constant (AC), determined within Langmuir and Random Sequential Adsorption model for adsorption data of goat anti-rabbit IgG on SiO<sub>2</sub> surface modified with APTES (this study), APTES/GA [8] and NHS-silane [8].

	Langmuir model		RSA model	
	BC [mg/m <sup>2</sup> ]	AC [ $\times 10^7$ 1/M]	BC [mg/m <sup>2</sup> ]	AC [ $\times 10^7$ 1/M]
APTES	~3.3	~0.2	~4.3	~0.4
APTES/GA	~3.3	~0.5	~3.8	~1.6
NHS	~2.7	~1.4	~3.2	~2.8

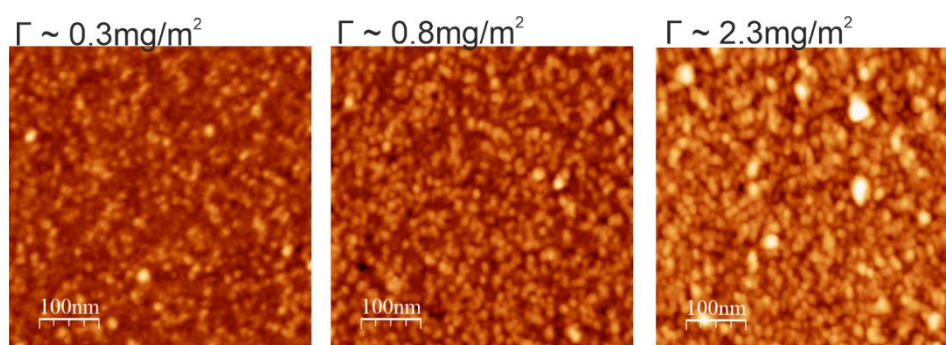
**S2.** Loading plots of the PCA model developed to examine surface density dependent orientation of IgG molecules adsorbed on APTES-modified substrate



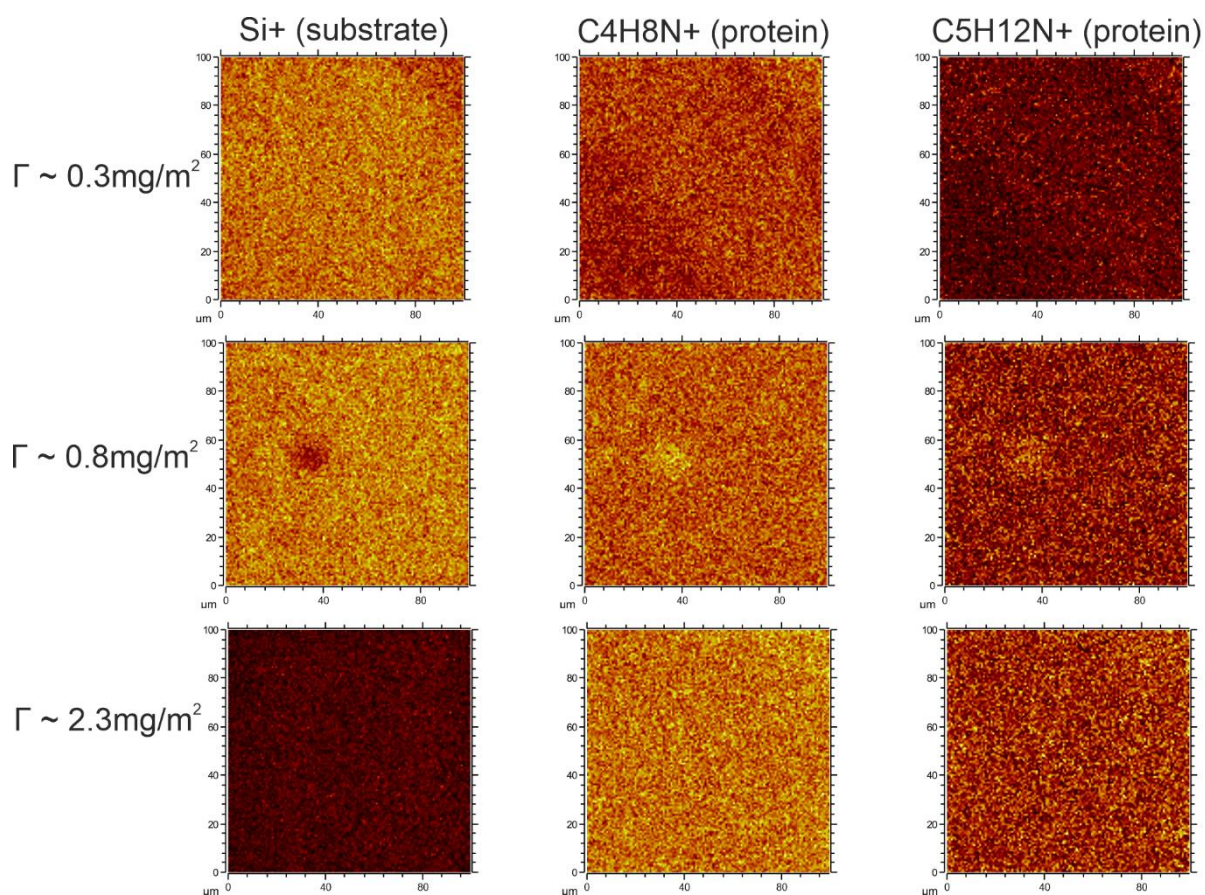
**Figure S2.** TOF-SIMS and PCA analysis of the IgG molecules surface density dependent orientation on APTES modified silicon substrates. PCA loadings plot for the PC3 (a) and for the PC1 (b).

### S3. Evaluation of IgG layers uniformity

To evaluate uniformity of IgG layers topographic AFM images were recorded with an Agilent 5500 microscope working in non-contact mode. AFM probes with spring constant about 2 N/m, resonant frequencies about 70 kHz and tip radius below 7nm were used. Several AFM images were taken at different areas for each sample.



**Figure S3.** Representative AFM topographic images of IgG molecules layers immobilized on SiO<sub>2</sub> substrates functionalized with APTES with different surface density. AFM analysis confirms uniformity of IgG layers.



**Figure S4.** Representative TOF-SIMS maps (Si<sup>+</sup> ion from substrate as well as two protein derived ions C<sub>4</sub>H<sub>8</sub>N<sup>+</sup> and C<sub>5</sub>H<sub>12</sub>N<sup>+</sup>) recorded on IgG molecules layers immobilized on SiO<sub>2</sub> substrates functionalized with APTES with different surface density. TOF-SIMS imaging confirms good uniformity of IgG layers.