Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for: Strong Anchoring and Vertical Adsorption Orientations of Antibodies on graphene

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Summary: Schematic representation of the IgG adsorption orientations (Figure S1), hydrophobic backbone used in the steered molecular dynamics (SMD) simulations (Figure S2), comparison of the final adsorption configuration of the the *Flat* and *Flat*-180°x-flipped orientations (Figure S3), root-mean-square-deviation and gyration-radius-tensor components for the *Flat* and *Head* orientations (Figure S4), comparison of the inter-domain distances as measured by MD and AFM (Figure S5), secondary-structure components and Ramachandran plots evolution during the adsorption dynamics for all the orientations (Figures S6-S9), example of a manual count of the IgG orientations on graphene as measured by AFM(Figure S10).

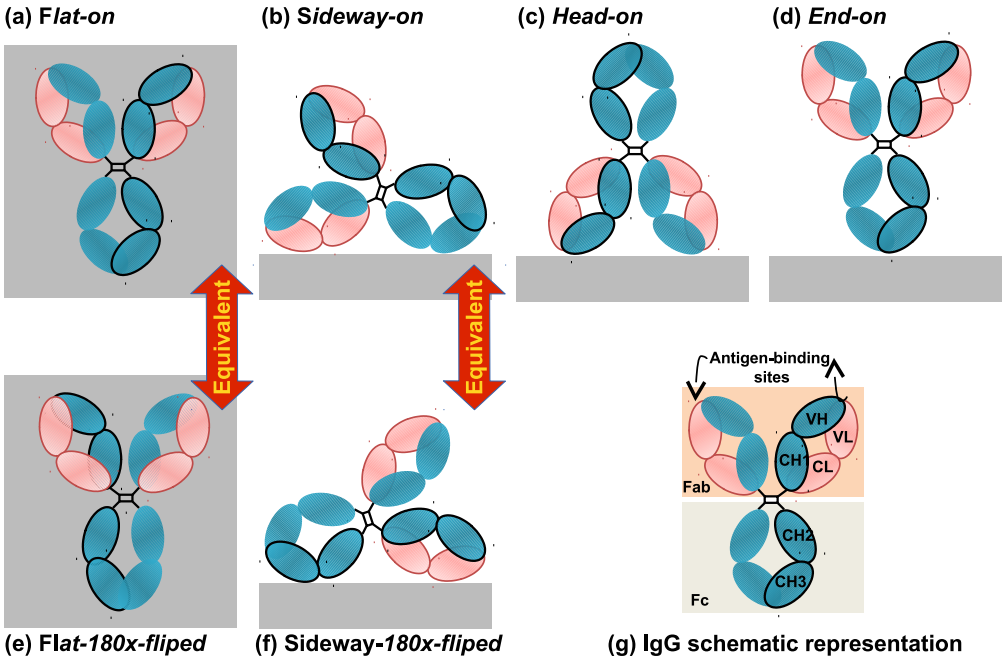

Figure S1: Schematic structure of IgG placed on top of a defect-free and hydrophobic surface. Here we follow the previously established nomenclature[1,2] for naming each of the orientations. Orientations used in our simulations, i.e. the four independent molecular orientations: (a) *Flat*, (b) *Sideway*, (c) *Head* and (d) *End*. Orientations that result in a identical adsorption mechanism and final configurations: (e) *Flat*-180°x-fliped and (f) *Sideway*-180°x-fliped. These orientations can be obtained from orientation (a) and (b), respectively, by a 180^{0} rotation around the horizontal axis lying in the paper plane. In S3, one can observe that there is no fundamental difference upon adsorption for the orientations (a) and (e). The same applies for orientations (b) and (f). At last, in the image (g) we represent the IgG's regions, domains and antigen-binding sites, wich should remain intact in order for the antibody keep its bioactivity.

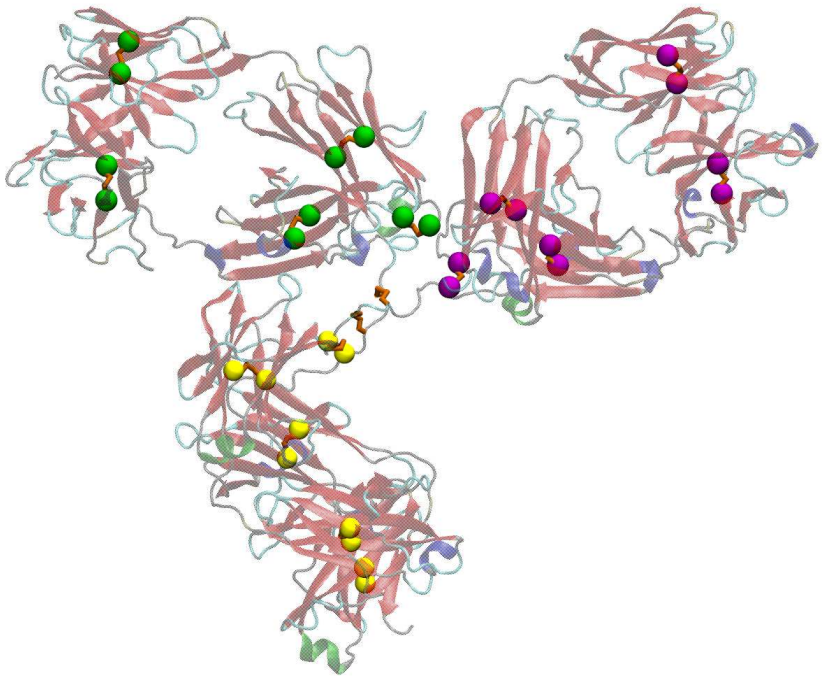

Figure S2: Representation of the hydrofobic backbone used in the steered-molecular-dynamics simulations. The IgG is represented with its secondary structure using a transparent texture. The highlighted atoms are the atoms over which the forces were applied. The atoms in green and purple belong to the Fabs and the atoms in yellow belong to the Fc region. Note that these atoms are the alpha-carbons of cysteines. This choice is motivated by two reasons: (i) these atoms are evenly distributed along the IgG. (ii) Since they belong to cysteines forming very strong S-bonds, they are very resilient to any kind of force applied. In this way we ensure an even force distribution along the protein without affecting its internal structure.

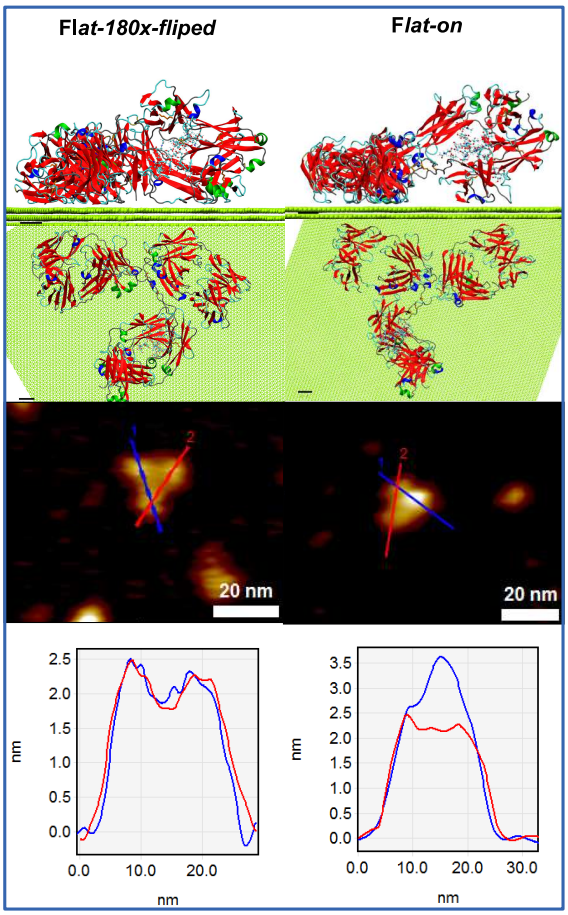

Figure S3: Comparison of the final adsorption configuration of the *Flat* and *Flat*-180°x-flipped orientations (see Figure S1). The images corresponding to the *Flat*-180°x-flipped orientation are on the left column, and the ones corresponding to the *Flat* orientation are on the right column. The first two rows contain: side and top view of the IgG adsorbed after a 152 ns MD simulation. The green atoms bellow belong to a 20×20 nm^2 3-layered graphene surface. The IgG is represented in the first two rows with its secondary structure (same color scheme used in Figure 1 of the article). The two glycan chains present on the Fc domain are represented using ball-stick model, and the sulfur-bridges are represented in dark-orange. The last two rows, from top to the bottom, are: The AM-AFM topographic images, and the height profiles along the red and blue lines represented in the corresponding topography image. These results show that there is no fundamental difference between these adsorption states since both retain their secondary structure and are strongly adsorbed over graphene. Nevertheless, it is interesting to observe that our AFM experiments are capable of distinguishing these two equivalent adsorption orientations. The sole difference between them is that in the *Flat* orientation the Fabs are more strongly adsorbed to graphene.This in turn leads to a torsional upward force on the Fc domain thus leading to a less adsorbed Fc, with respect to the *Flat*-180°x-flipped orientation.

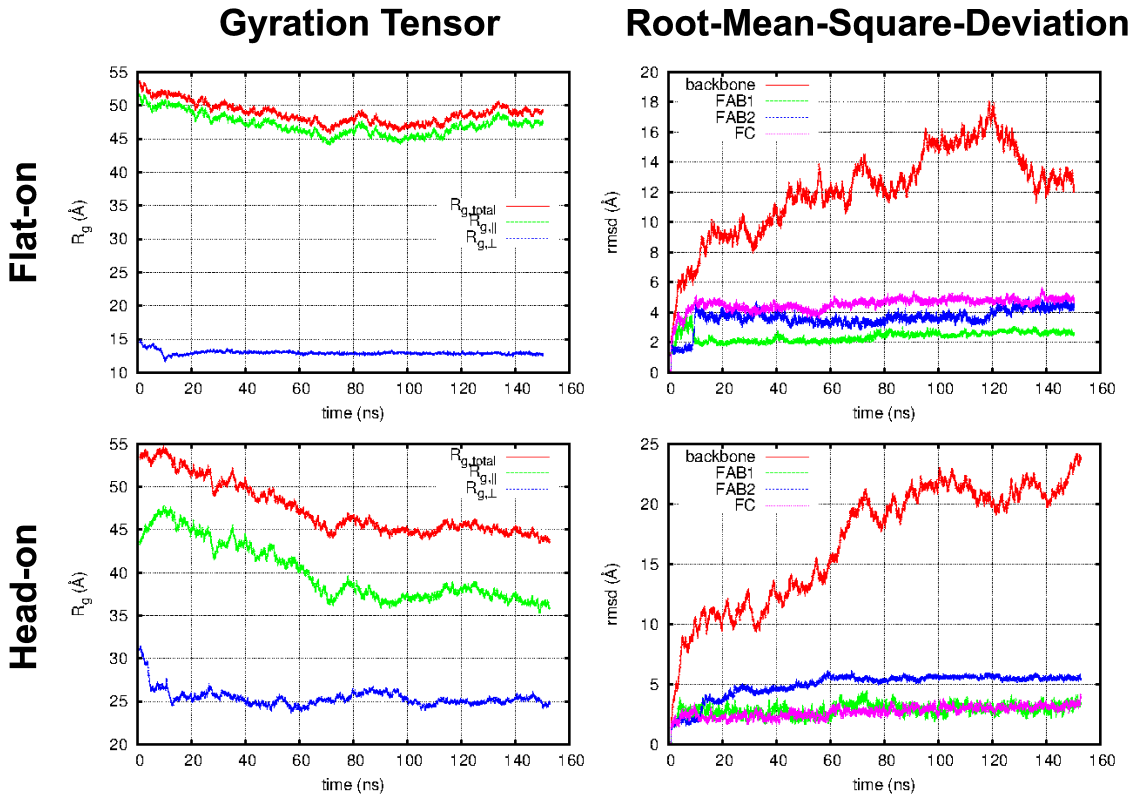

Figure S4: Left column: Evolution during the adsorption dynamics (152 ns) of the total gyration radius ($R^2_{g,total} = (\mathbf{R}^x_g)^2 + (\mathbf{R}^y_g)^2 + (\mathbf{R}^z_g)^2$) (left), the component of the gyration radius parallel to the surface ($R^2_{g,||} = (\mathbf{R}^x_g)^2 + (\mathbf{R}^y_g)^2$) and the component of the gyration radius perpendicular to the surface ($R_{g,\perp} = (\mathbf{R}^z_g)$) for the orientations *Flat* and *Head* (from top to bottom respectively). The term ($\mathbf{R}^{ii}_g$) represents the ii component of the gyration radius tensor. Note that by definition, the $R_{g,\perp}$ tell us how the protein is getting more or less compact along a direction perpendicular to the surface, while the term $R^2_{g,||}$ reveals how the protein is spreading over the surface.[3] Right column: Evolution during the adsorption dynamics of the root-mean-square (rmsd) of all the backbone atoms of the IgG, of the alpha carbons belonging to the Fabs domains (FAB1 and FAB2), and of the alpha carbons belonging to the Fc domain (FC) for the orientations *Flat* and *Head* (from top to bottom respectively).

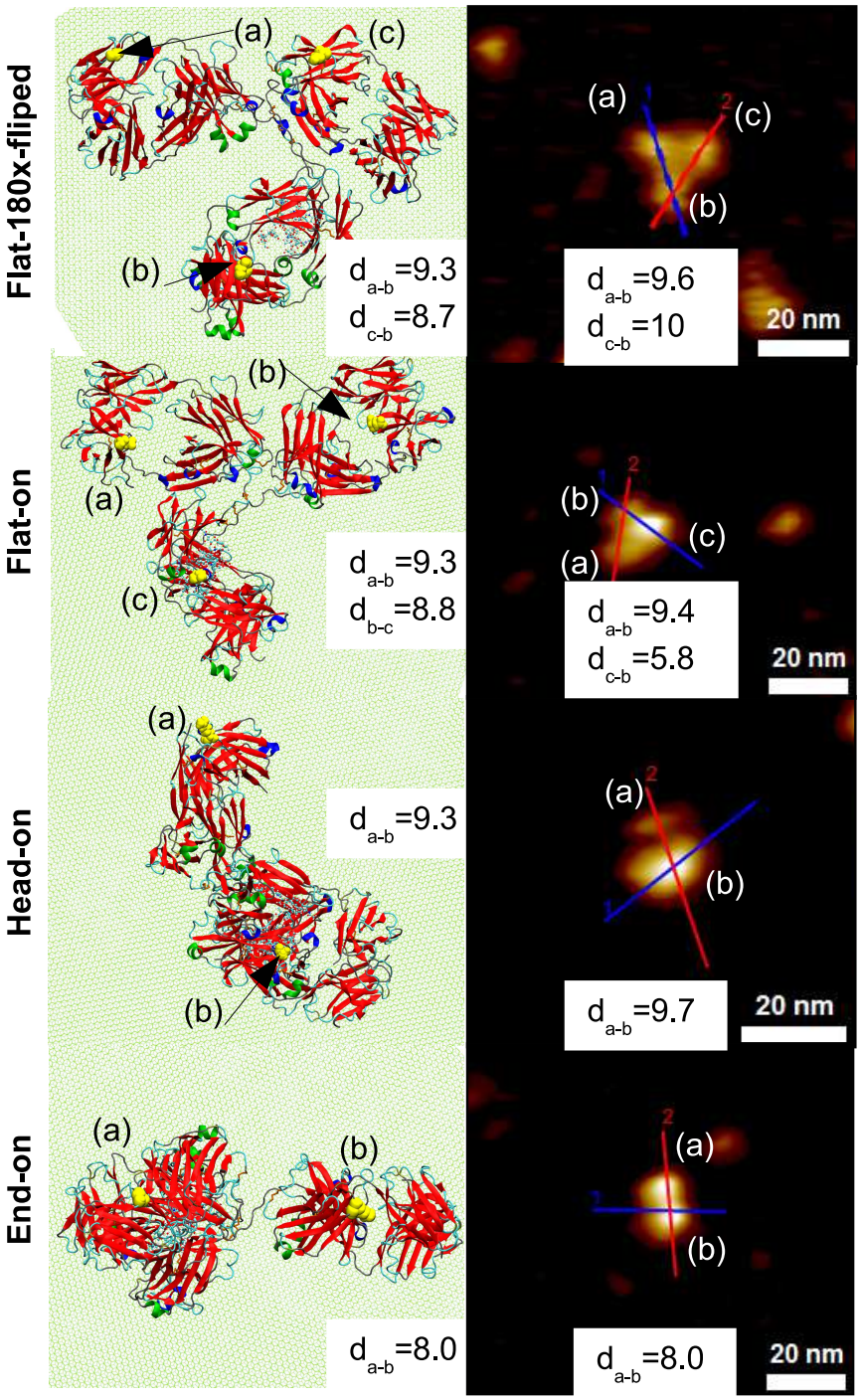

Figure S5: Comparison of the inter-domain distances as measured from the MD simulations (left column) and AFM topography images (right column). In the case of MD simulations, these distances are obtained from the coordinates at the end of the 152 ns equilibration run for each orientation. The points (a,b and c) selected on the MD simulations correspond to maximum height sites, as in the experimental data. Only orientations whose AFM height profile had two peaks have been compared. The assignment of each AFM image to a given orientation is based on both the topography profiles shown in Fig. 1 in the manuscript and the inter-domain distances determined here.

	Thermalization	10ns Eq.	SMD-down	140ns Eq.
Alfa-helix	3.57	2.81	2.58	2.81
Isobeta-bridge	1.44	1.52	2.36	1.44
Beta-strand	46.20	46.05	43.01	44.07
3-pi-helix	2.74	2.51	2.58	3.19
5-pi-helix	0.00	0.00	0.00	0.00
Turn	11.63	12.31	11.40	10.26
Bend	9.04	9.88	10.64	11.70
Random-Coil	25.38	24.92	27.43	26.52

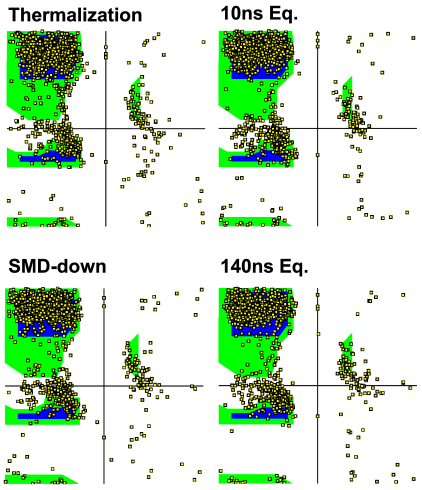

Figure S6: Secondary structure evolution during the adsorption dynamics (150 ns) of the *Flat* orientation. On the left hand side, we present a table containing the percentage of each of the secondary structures evaluated using the DSSP algorithm[4] at major simulation steps, i.e.: after the thermalization, after the 10 ns free adsorption dynamics, after the enhanced adsorption and finally at the last stage of the simulation. On the right hand side, we represent the Ramachandran plots that corresponds to each of the previously mentioned stages.

	Thermalization	10ns Eq.	SMD-down	140ns Eq.
Alfa-helix	3.72	3.72	3.50	3.34
Isobeta-bridge	1.75	1.82	2.13	1.90
Beta-strand	45.59	45.90	45.06	44.98
3-pi-helix	3.19	2.66	2.89	2.66
5-pi-helix	0.00	0.00	0.00	0.00
Turn	10.87	11.93	11.63	12.61
Bend	9.42	9.04	8.97	8.51
Random-Coil	25.46	24.92	25.84	25.99

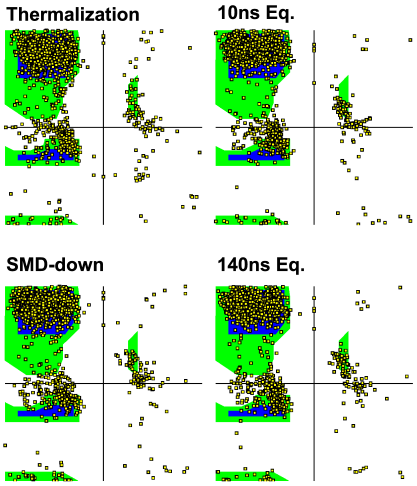

Figure S7: Secondary structure evolution during the adsorption dynamics (152 ns) of the *Head* orientation. Table and Figures have the same meaning as in S6.

	Thermalization	10ns Eq.	SMD-down	140ns Eq.
Alfa-helix	3.57	3.42	2.96	1.98
Isobeta-bridge	1.29	1.82	2.20	1.22
Beta-strand	47.72	46.35	42.48	46.05
3-pi-helix	2.28	2.51	1.60	2.81
5-pi-helix	0.00	0.00	0.00	0.00
Turn	11.93	11.09	13.60	12.84
Bend	9.35	10.18	10.33	10.26
Random-Coil	23.86	24.62	26.82	24.85

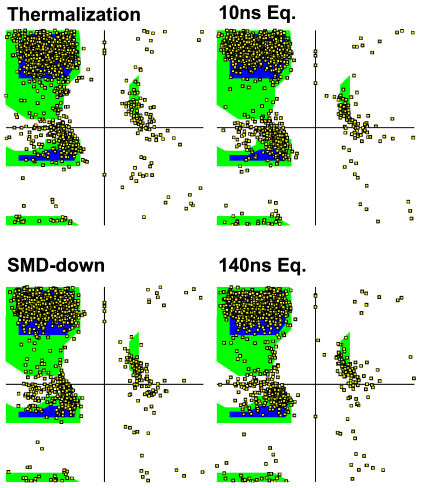

Figure S8: Secondary structure evolution during the adsorption dynamics (150 ns) of the *Sideway* orientation. Table and Figures have the same meaning as in S6.

	Thermalization	10ns Eq.	SMD-down	140ns Eq.
Alfa-helix	3.50	3.65	2.96	2.13
Isobeta-bridge	1.37	1.14	1.37	1.22
Beta-strand	46.81	47.42	46.81	44.68
3-pi-helix	2.96	2.05	3.27	3.19
5-pi-helix	0.00	0.00	0.00	0.00
Turn	11.09	12.16	11.40	12.31
Bend	9.65	9.19	9.95	10.33
Random-Coil	24.62	24.39	24.24	26.14

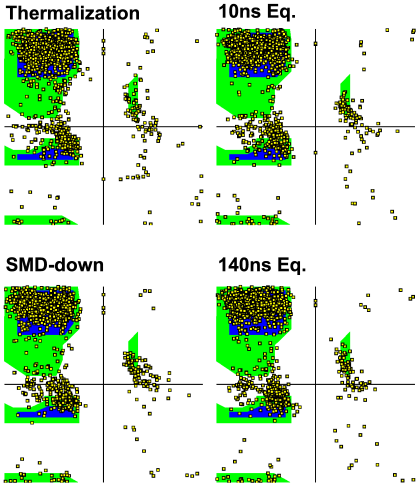

Figure S9: Secondary structure evolution during the adsorption dynamics (150 ns) of the *End* orientation. Table and Figures have the same meaning as in S6.

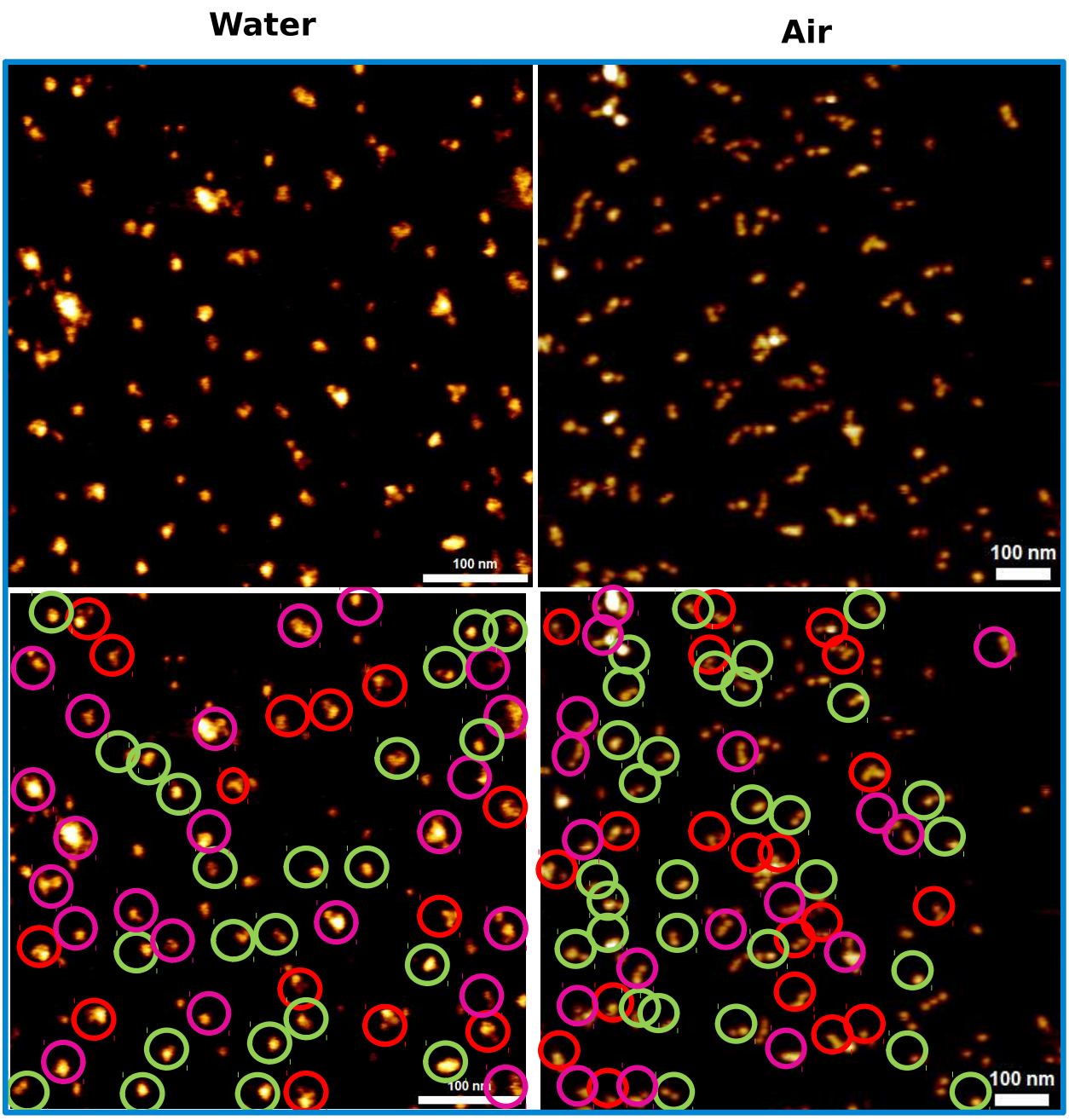

Figure S10: First row from left to right: AM-AFM topographic images of the IgG adsorbed over graphene in water and in air, respectively. On the second row we show a manual count of each of the orientations found in the image on top. The vertical orientations are highlighted with a green circle, the *Flat* orientations with a red circle, and the unresolved ones with a magenta circle.

References

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2. Christine Dupont-Gillain, *Proteins at Interfaces III State of the Art*; ACS Symposium Series; American Chemical Society, 2012; Vol. 1120; pp 453–469.

3. Mücksch, C.; Urbassek, H. M. Enhancing Protein Adsorption Simulations by Using Accelerated Molecular Dynamics. *PLoS ONE* **2013**, *8*, e64883.

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