Multi-walled carbon nanotubes as a platform for Immunoglobulin G attachment

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Table S1. BET surface area (S_{BET}) of the MWCNT with different diameters.

Diameter range [nm]	S_{BET} [$m^2 g^{-1}$]	Reference
< 10	185	(1)
10–20	73	(2)
20–40	67	(2)
60–100	35	(2)

(1) Silva CG, Faria JL. Photocatalytic oxidation of benzene derivatives in aqueous suspensions: Synergic effect induced by the introduction of carbon nanotubes in a TiO2 matrix. Applied Catalysis B: Environmental. 2010; 101, (1–2):81-89. https://doi.org/10.1016/j.apcatb.2010.09.010

(2) Silva CG, Tavares APM, Dražić G, Silva AMT, Loureiro JM, Faria JL. Controlling the Surface Chemistry of Multiwalled Carbon Nanotubes for the Production of Highly Efficient and Stable Laccase-Based Biocatalysts. Chempluschem. 2014; 79(8):1116–22. https://doi.org/10.1002/cplu.201402054

Table S2. Estimated number of IgG in the initial samples of rabbit serum.

Dilution	Estimated number of IgG molecules per 100 µL of diluted rabbit serum
1:5	6.14×10^{14}
1:15	2.05×10^{14}
1:20	1.54×10^{14}
1:30	1.02×10^{14}
1:50	6.14×10^{13}

Table S3. Attachment of RSA present in rabbit serum using 2 mg of MWCNTs (external diameter of 10-20 nm) at different serum dilutions, during 60 min of contact time and pH 5.0.

Dilution	RSA attachment on MWCNTs (%)	Amount of RSA* attached (µg)
1:5	45.9	321.3
1:15	40.6	94.8
1:20	35.8	62.6
1:30	41.2	48.1
1:50	67.8	47.5

*Considerations: albumin concentration in rabbit serum of 35 mg/mL (3)

(3) Barbosa LR, Ortore MG, Spinozzi F, Mariani P, Bernstorff S, Itri R. The importance of protein-protein interactions on the pH-induced conformational changes of bovine serum albumin: a small-angle X-ray scattering study. Biophys J. 2010 Jan 6;98(1):147-57. https://doi.org/10.1016/j.bpj.2009.09.056

Table S4. Estimated number of IgG with different orientations (Fig. S1) that can absorb on the surface of the different diameter MWCNTs

MWCNT diameter (nm)	S_{BET} (m^2 g^{-1})	Estimated number of IgG molecules that can adsorb on the surface of MWCNT (2 mg)		
		"end-on/head-on"	"side-on"	"lying-on"
< 10	185	3.3×10^{15}	3.3×10^{15}	1.3×10^{15}
10-20	73	2.6×10^{15}	2.6×10^{15}	1.0×10^{15}
20-40	67	2.4×10^{15}	2.4×10^{15}	9.6×10^{14}
60-100	35	1.2×10^{15}	1.2×10^{15}	5.0×10^{14}

Considerations: "head on/end on" orientation the single IgG molecules could cover ~ 56 nm area on MWCNT surface"; "side-on" orientation could also cover ~ 56 nm area; and "lying-on" could cover ~ 140 nm area (4).

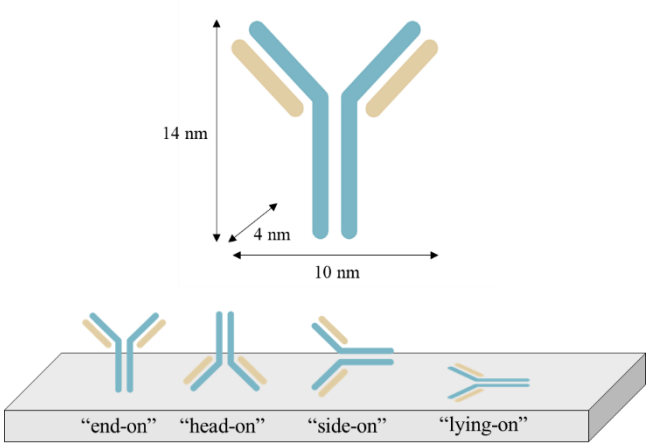

Figure S1 – IgG dimensions and orientations. Adapted from (4).

(4) Nicholas G. Welch, Judith A. Scoble, Benjamin W. Muir, Paul J. Pigram. Orientation and characterization of immobilized antibodies for improved immunoassays (Review), Biointerphases 12, 02D301 (2017). https://doi.org/10.1116/1.4978435

Table S5. Amino acid reactivity (*LIGRe)* for IgG: from pH 5.0 to 8.0.

			LIGRe			
Amino acid residue	**Chain**	**p*K*$_a$**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 8.0**
LYS_{13}	H	10.37	0.00	0.00	0.00	0.00
LYS_{19}	H	10.62	0.00	0.00	0.00	0.00
LYS_{105}	H	10.38	0.00	0.00	0.00	0.00
LYS_{213}	H	10.36	0.00	0.00	0.00	0.00
LYS_{218}	H	10.46	0.00	0.00	0.00	0.00
LYS_{222}	H	10.55	0.00	0.00	0.00	0.00
LYS_{235}	H	10.38	0.00	0.00	0.00	0.00
LYS_{287}	H	10.44	0.00	0.00	0.00	0.00
LYS_{305}	H	11.18	0.00	0.00	0.00	0.00
LYS_{307}	H	9.05	0.00	0.00	0.01	0.09
LYS_{336}	H	10.73	0.00	0.00	0.00	0.00
LYS_{339}	H	10.71	0.00	0.00	0.00	0.00
LYS_{341}	H	10.81	0.00	0.00	0.00	0.00
LYS_{345}	H	10.63	0.00	0.00	0.00	0.00
LYS_{445}	H	10.02	0.00	0.00	0.00	0.01
LYS_{478}	H	10.44	0.00	0.00	0.00	0.00
LYS_{13}	K	10.27	0.00	0.00	0.00	0.01
LYS_{19}	K	10.51	0.00	0.00	0.00	0.00
LYS_{213}	K	10.39	0.00	0.00	0.00	0.00
LYS_{218}	K	10.52	0.00	0.00	0.00	0.00
LYS_{221}	K	10.50	0.00	0.00	0.00	0.00
LYS_{222}	K	10.56	0.00	0.00	0.00	0.00
LYS_{235}	K	10.44	0.00	0.00	0.00	0.00
LYS_{287}	K	10.26	0.00	0.00	0.00	0.01
LYS_{305}	K	10.38	0.00	0.00	0.00	0.00
LYS_{307}	K	10.41	0.00	0.00	0.00	0.00
LYS_{336}	K	11.40	0.00	0.00	0.00	0.00
LYS_{339}	K	10.83	0.00	0.00	0.00	0.00
LYS_{341}	K	10.78	0.00	0.00	0.00	0.00
LYS_{360}	K	10.60	0.00	0.00	0.00	0.00
LYS_{445}	K	10.15	0.00	0.00	0.00	0.01
LYS_{107}	L	10.44	0.00	0.00	0.00	0.00
LYS_{126}	L	10.61	0.00	0.00	0.00	0.00
LYS_{145}	L	10.40	0.00	0.00	0.00	0.00

LYS_{149}	L	11.34	0.00	0.00	0.00	0.00
LYS_{169}	L	10.52	0.00	0.00	0.00	0.00
LYS_{183}	L	10.55	0.00	0.00	0.00	0.00
LYS_{188}	L	10.51	0.00	0.00	0.00	0.00
LYS_{190}	L	10.43	0.00	0.00	0.00	0.00
LYS_{126}	M	10.58	0.00	0.00	0.00	0.00
LYS_{145}	M	10.36	0.00	0.00	0.00	0.00
LYS_{149}	M	11.18	0.00	0.00	0.00	0.00
LYS_{169}	M	10.59	0.00	0.00	0.00	0.00
LYS_{183}	M	10.50	0.00	0.00	0.00	0.00
LYS_{188}	M	10.55	0.00	0.00	0.00	0.00
LYS_{190}	M	10.54	0.00	0.00	0.00	0.00

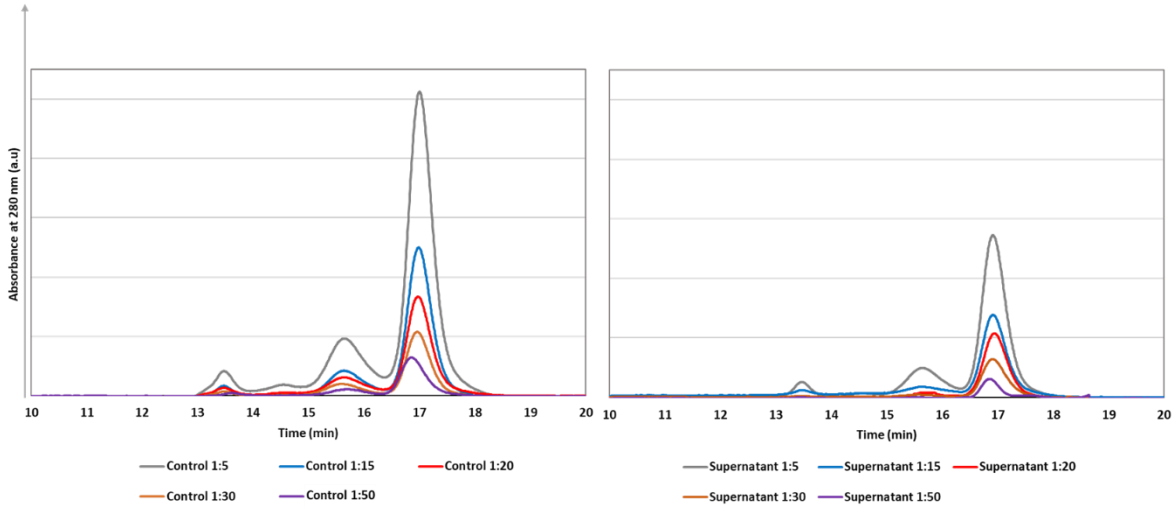

Figure S2. Size-exclusion HPLC chromatograms of rabbit serum diluted 1:5, 1:15, 1:20, 1:30, and 1:50 before (control) and after contact with MWCNTs (supernatant). The IgG and albumin peaks are characterized by a retention time around 15.7 and 17.0 min, respectively.