Supporting Information

Manipulation and Quantification of Graphene Oxide Flake Size: Photoluminescence and Cytotoxicity

*Brian R. Coleman†, Timothy Knight†‡, Valerie Gies†, Zygmunt J. Jakubek†, Shan Zou†#**

†Measurement Science and Standards, National Research Council Canada, 100 Sussex Drive, Ottawa, Ontario K1A 0R6, Canada; ‡Department of Physics, McMaster University, 1280 Main Street W, Hamilton, Ontario L8S 4L8, Canada; #Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada,*corresponding author, Email: shan.zou@nrc-cnrc.gc.ca.

Additional data on sonication energy and size dependence, AFM images, and GO dispersion stability testes were included.

Flake Size Measurements by AFM. Processing and analysis of AFM images was performed with Gwyddion, an open source software with well-documented operation and functions.[1] Details of the processing and determination of flake sizes can be found in the supporting info. Briefly, to facilitate automated analysis, images were flattened in Gwyddion to correct for drifts during scanning and the z scale zero was set at the lowest z-level in the image. The flattening was executed using the polynomial background removal function that was only applied to pixels corresponding to the substrate, but not those corresponding to the GOs or contaminants, which were masked out. This allowed application of high order flattening (up to the degree of 20 split between x and y directions) without introducing unwanted processing artifacts or flake size and shape alteration. Next, each image was opened in ImageJ and converted to 8-bit greyscale. A threshold was set at a half of an average flake gray value, which corresponds to height, and flakes were selected by masking all pixels above the threshold. Finally, the maximum Feret's diameter (the maximum distance between two parallel lines tangent to the flake circumference)[2] or the flake area were measured for each masked flake. Flake areas were next converted to equivalent circle (circle with the same area as that of a flake) diameters using the $d = 2\sqrt{area/\pi}$ formula. The Feret's diameter was further used as a flake size descriptor for comparison with DLS results. Only isolated flakes were measured; those in contact with the edge of the image or perceived to extend outside the image were excluded from the size analysis, as their entire area could not be measured. Since the procedure passively favored smaller flakes as they were less likely to touch or extend past an edge of the image, images were typically

recorded with a large scan area (20 µm by 20 µm) to minimize the bias. Features with the area smaller than 4 pixels were excluded as these could not be distinguished from measurement artifacts at large scan sizes such as 20 µm by 20 µm. The AFM size analyses were automated to speed up processing of large data sets and reduce analyst bias. Workflow of the AFM analysis is illustrated in Figure S1. A representative AFM image of Graphenea GO flakes (0.002 mg/mL dispersion, no sonication) on freshly cleaved mica is shown in Figure S1A. Figure S1B shows the AFM image converted to 8-bit gray scale with the flakes selected by masking all pixels with gray values above the threshold set at half of the average flake height. Figure S1C shows a representative flake size (Feret's diameter) histogram of 520 flakes, where a peak value of ~1 µm was observed.

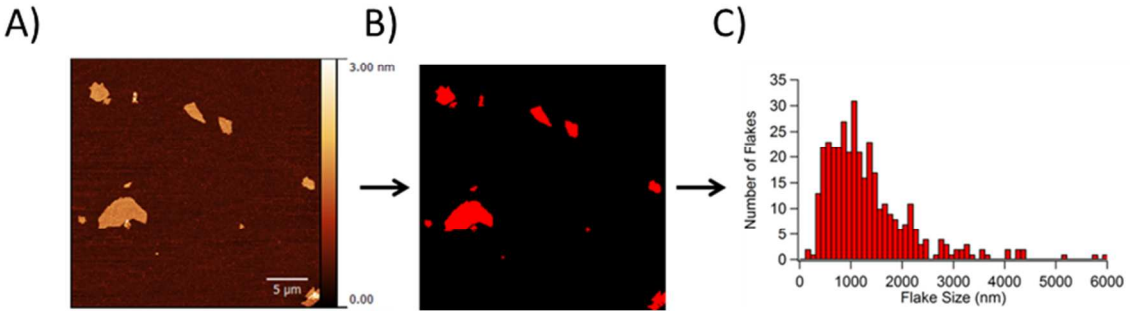

Figure S1. (A) Representative AFM image of unsonicated Graphenea GO on mica. Scale bar represents 5 µm. (B) Thresholded image displaying masked GO flakes. (C) Flake size (Feret's diameter) histogram from measurement of 520 flakes.

Height Measurements. Forty-five isolated flakes were chosen for height measurements with Gwyddion. To minimize analyst bias in the measurement and to average noise effects and variability of GO flake flatness, flake height was measured by taking three profiles along the slow AFM scan direction for each flake, one at the top, one in the middle, and one at the bottom of a flake (Figure S2A). Variation of the vertical displacement value in the flake and background

areas resulted from variation of flake thickness and residual background curvature and roughness, respectively, as well as from noise and other instrumental and ambient effects. The three profiles measured along these lines are shown in Figures S2B–D. The minimum and maximum height values were recorded for each trace and the average value was calculated to represent the flake thickness (the background is assumed to be 0).

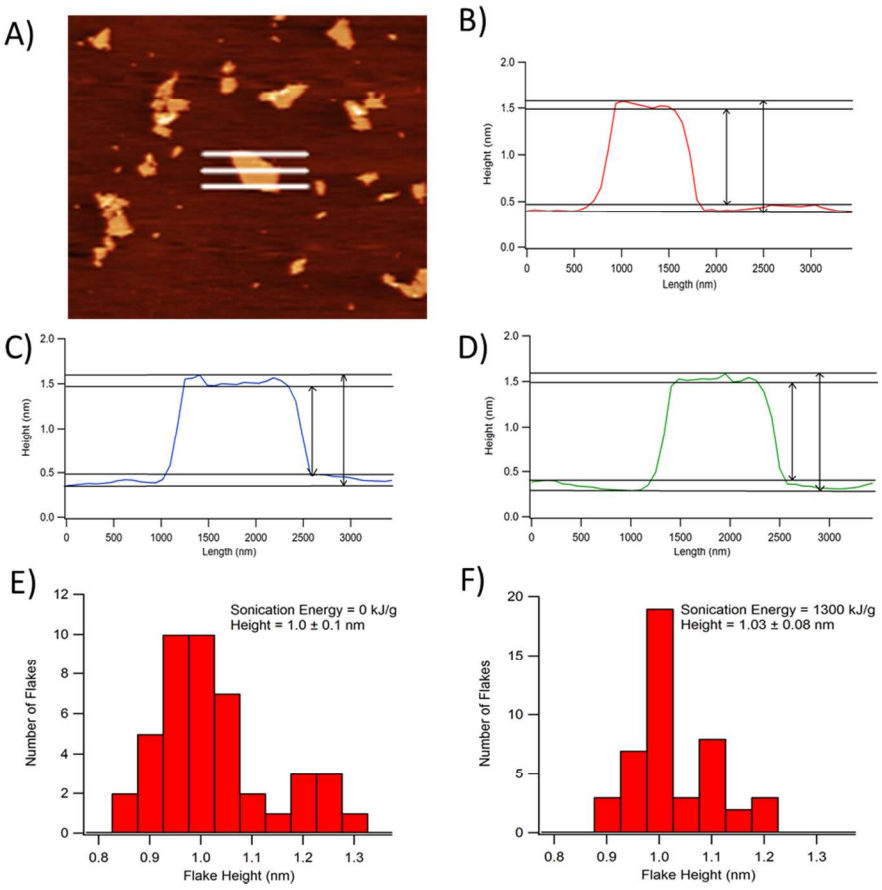

Figure S2. (A) Representative image of GO on mica displaying 3 height profiles. (B) Profile from top of flake. (C) Profile from middle of flake. (D) Profile from bottom of flake. Final flake height was assigned as the average of the minimum and maximum heights for the 3 profiles. (E) and (F) histograms displaying results for 0 and 1.3 MJ/g sonicated GO, respectively.

Table S1. The measured Feret's diameter values determined from AFM analysis of Graphenea and Angstrom GO samples: presented as the highest frequency population of the histogram, h; and the number weighted average values, N_A. Error stated refers to the standard deviation of 3 measurements.

GO Supplier	Sonication Energy (kJ/g)	h (nm)	N_A (nm)
Graphenea	0	773	1358 ± 111
	400	465	712 ± 37
	1240	450	579 ± 22
	2120	275	472 ± 21
	7250	53	159 ± 138
	9940	143	254 ± 17
	12000	60	129 ± 25
	16000	67	125 ± 11
	18000	47	92 ± 7
Angstrom	100	98	329 ± 41
	200	65	295 ± 62
	300	60	268 ± 27
	500	73	249 ± 13
	600	60	295 ± 17
	3000	60	290 ± 17
	4000	53	188 ± 25
	5000	60	202 ± 19
	7000	60	126 ± 15
	8000	40	106 ± 5
	9000	40	156 ± 3
	10000	40	150 ± 21
	12000	40	152 ± 7
	14000	67	183 ± 36
	20000	63	205 ± 18

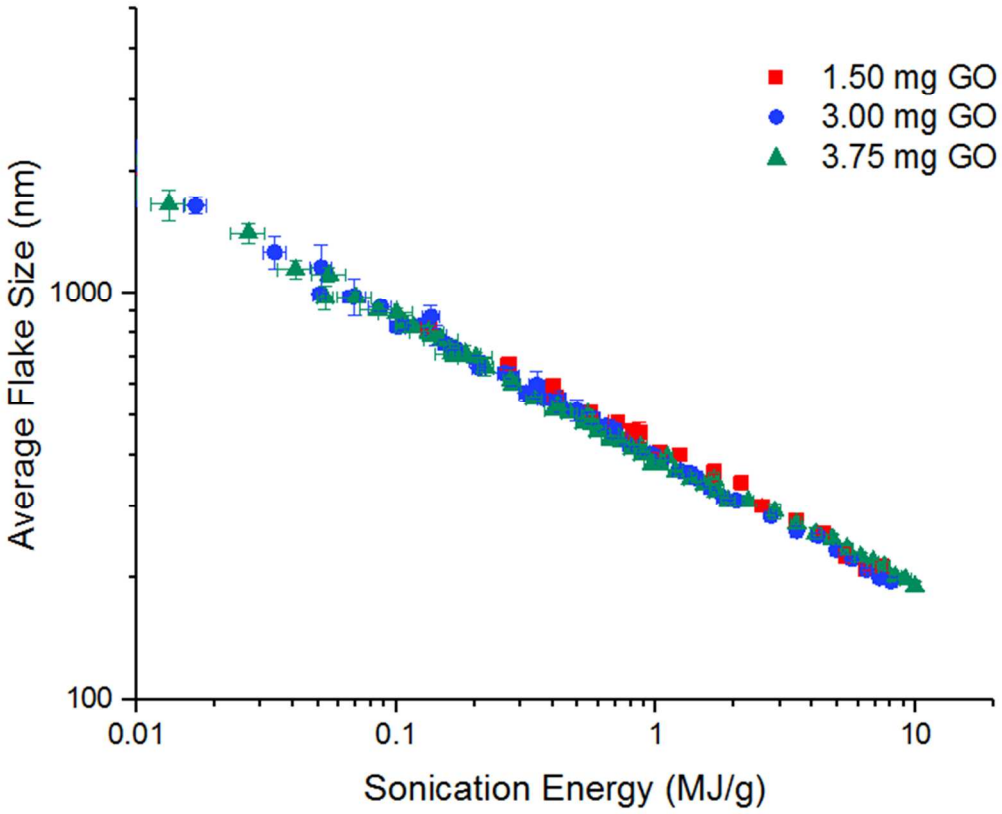

Figure S3. Log-log plot of average DLS size (hydrodynamic diameter) as a function of sonication energy for samples with three different masses of Graphenea GO.

Table S2. Average flake size (hydrodynamic diameter) measured by DLS for Graphenea GO aqueous dispersions at various sonication energies. Error stated refers to the standard deviation of 3 measurements. Multiple measurements were performed at 0 kJ/g in order to establish the initial flake size.

Sonication Energy (kJ/g)	Average Flake Size (nm)	Sonication Energy (kJ/g)	Average Flake Size (nm)	Sonication Energy (kJ/g)	Average Flake Size (nm)	Sonication Energy (kJ/g)	Average Flake Size (nm)
0	2128 ± 252	164	716 ± 24	676	462 ± 7	2010	310 ± 255
0	1864 ± 11	167	708 ± 20	692	462 ± 3	2059	309 ± 17
0	2161 ± 24	169	731 ± 30	708	485 ± 3	2124	345 ± 10
0	1961 ± 10	185	711 ± 44	717	440 ± 2	2264	310 ± 3
0	2034 ± 16	203	697 ± 268	725	440 ± 3	2547	302 ± 12
0	2058 ± 25	206	681 ± 34	738	435 ± 4	2766	284 ± 5
0	1974 ± 3	208	657 ± 57	804	423 ± 4	2866	291 ± 5
0	1955 ± 190	221	659 ± 24	812	415 ± 79	3450	277 ± 3
0	2009 ± 5	261	638 ± 32	824	452 ± 57	3484	260 ± 2
13	1661 ± 20	271	671 ± 46	864	456 ± 45	3485	272 ± 1
17	1648 ± 5	276	613 ± 5	871	411 ± 30	4119	247 ± 6
27	1410 ± 6	277	628 ± 30	871	420 ± 26	4213	253 ± 5
34	1264 ± 14	280	598 ± 16	885	410 ± 20	4384	258 ± 3
41	1146 ± 225	316	570 ± 11	887	403 ± 20	4772	249 ± 170
51	994 ± 8	340	550 ± 2	966	382 ± 35	4954	234 ± 10
51	1160 ± 9	341	572 ± 4	966	402 ± 25	5349	227 ± 7
53	978 ± 10	348	597 ± 5	1025	409 ± 22	5444	236 ± 13
55	1113 ± 8	372	550 ± 13	1030	381 ± 30	5707	222 ± 4
67	982 ± 4	400	596 ± 334	1053	390 ± 16	6135	225 ± 12
69	982 ± 10	402	515 ± 3	1105	396 ± 68	6349	211 ± 3
70	978 ± 2	412	559 ± 6	1193	367 ± 42	6472	209 ± 9
85	912 ± 6	419	530 ± 4	1235	403 ± 35	6847	219 ± 5
87	927 ± 50	421	555 ± 8	1239	367 ± 32	7250	200 ± 8
100	894 ± 27	428	524 ± 6	1361	350 ± 9	7386	214 ± 6
102	826 ± 28	495	517 ± 7	1363	363 ± 7	7582	213 ± 2
105	833 ± 19	515	509 ± 12	1427	353 ± 1	8000	226 ± 7
108	842 ± 10	531	481 ± 2	1534	339 ± 8	8341	196 ± 5
116	826 ± 20	546	503 ± 3	1618	334 ± 9	9125	199 ± 2
124	831 ± 25	558	511 ± 7	1672	356 ± 12	9937	191 ± 4
136	874 ± 239	566	478 ± 219	1673	367 ± 7	12900	217 ± 18
136	788 ± 81	571	494 ± 11	1677	350 ± 4	14000	217 ± 3
143	788 ± 118	598	458 ± 5	1712	327 ± 10	16000	156 ±3
149	770 ± 158	647	473 ± 3	1812	316 ± 5	18000	201 ± 7
154	752 ± 104	667	438 ± 5	1896	310 ± 7	20000	174 ± 4

Table S3. Average flake size (hydrodynamic diameter) measured by DLS for Angstrom GO aqueous dispersions at various sonication energies. Error stated refers to the standard deviation of 3 measurements.

Sonication Energy (kJ/g)	Average Flake Size (nm)
0	331 ± 18
100	194 ± 7
200	189 ± 14
300	188 ± 11
500	186 ± 12
600	181 ± 15
1000	159 ± 11
2000	152 ± 18
3000	134 ± 7
4000	140 ± 10
5000	138 ± 21
7000	174 ± 5
8000	157 ± 5
9000	141 ± 3
10000	171 ± 8
14000	140 ± 6
15000	150 ± 5
16000	180 ± 4
18000	137 ± 13
20000	141 ± 1

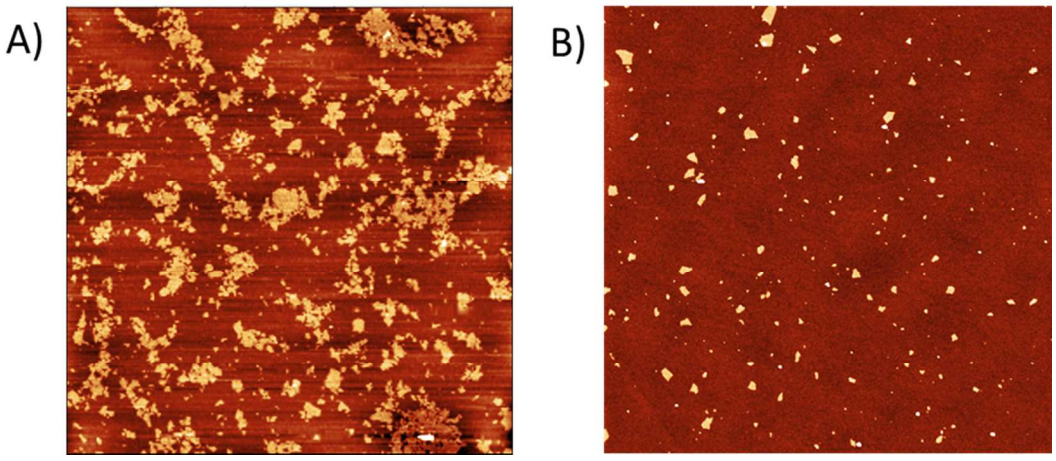

Figure S4. 10 µm x 10 µm AFM images of (A) Angstrom and (B) Graphenea GO sonicated with 16 MJ/g energy.

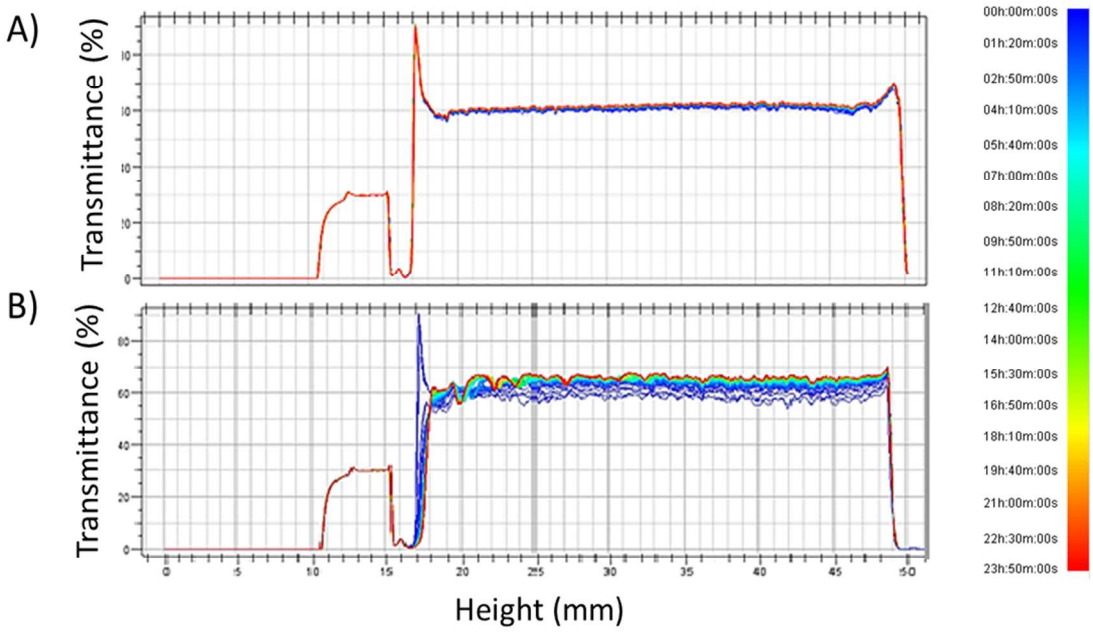

Figure S5. Stability measurements showing transmittance of light over a 24 h period for aqueous GO dispersions at (A) pH = 12.3 and (B) pH =12.8. Increased transmittance over time suggests sedimentation of large particles.

Table S4. Sonication energies for 10 mL of 2 mg/mL Graphenea GO solutions for use in cell culture study.

Sonication Energy (kJ/g)	DLS Flake Size (nm)	AFM Flake Size (nm)
60	896±9	867±29
450	220.1±0.6	239±12
2550	147±4	158.7±7.0

References

(1) Necas, D.; Klapetek, P. An Open-Source Software of SPM Data Analysis, *Open Phys.* **2012**, *10*, 181-188.
(2) Merkus, H. G. Particle Size Measurements: Fundamentals, Practice, Quality, Springer, New York, 2009.