In Vitro Activity Studies of Hyperthermal Near-Infrared NanoGUMBOS in MDA-MB-231 Breast Cancer Cells

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Figure S1. ESI MS of the folate product after synthesis reveals that the folic acid precursor is higher in concentration by two fold. (The inset is a scan of the folate.)



Figure S2. ¹⁹F NMR was used to observe the anion exchange from the anion precursor $[BF_4]$ to non-fluorinated product [Folate] for the (A) [1048] and (B) [1061] GUMBOS.



Figure S3. Absorbance measurements were conducted for comparison of a fixed concentration of 32 μ g mL⁻¹ (blue) and of the top aqueous layer of post-centrifugation nanoGUMBOS (red). (A) [1048][Folate] was calculated to be 92.92% insoluble, and (B) [1061][Folate] was calculated to be 88.75% insoluble. (Note: Absorbance shoulder between 1150-1200 nm was determined to be from the folate anion.)



Figure S4. In vitro hyperthermal experiments were conducted in a 96 well plate.



Figure S5. Photothermal measurements were recorded by a thermocouple placed about 2 mm above the laser beam.





Figure S6. The LC_{50} was calculated from the linear slope of the cytotoxicity in different cell lines of NIR absorbing nanoGUMBOS.



Figure S7. The absorbencies of 77.6 μ g mL⁻¹ (A) [1048][Folate] and (B) [1061][Folate] GUMBOS reference and nanoGUMBOS within cells were dissolved in DMSO and measured at 1000 nm. (Note: A 3-median smoothing technique was used for better clarity in the "Digested Cells with NanoGUMBOS" spectrum.)



Figure S8. Concentration absorbance at 1000 nm gradient of percent of 77.6 μ g mL⁻¹ (A) [1048][Folate] and (B) [1061][Folate] nanoGUMBOS in DMSO was used to determine the concentration of nanoGUMBOS that entered the MDA-MB-231 cells.

Table S1. The cytotoxicity of nanoGUMBOS in non-malignant, HS-578-BST, and malignant, MDA-MB-231, human breast cell lines was performed by a fluorescence assay. The concentrations were compared against a Live control (0 μ g mL⁻¹), and the percent viability average and +/- standard deviations are given.

	NanoGUMBOS Percent Viability						
	HS-57	8-BST	MDA-MB-231				
Concentration (µg mL ⁻¹)	[1048][Folate]	[1061][Folate]	[1048][Folate]	[1061][Folate]			
0	100±4.2						
12.3	98.8±1.7	91.8±2.6	102.3±2.5	101.7±5.4			
44.1	96.0±1.2	88.3±7.0	88.6±5.9	88.7±2.1			
77.6	88.2±2.0	88.6±4.2	90.2±3.6	75.9±3.3			
124.9	75.0±4.8	83.0±9.8	59.5±1.2	54.4±2.6			
179.6	59.5±6.1	68.4 ± 2.8	40.4 ± 4.8	9.8±1.6			
Dead	8.6±5.7						

Table S2. The percent viability of MDA-MB-231 human breast cells under hyperthermia was monitored using different nanoGUMBOS, irradiation time lengths, and concentrations. The concentrations were compared against a Live control (no irradiation and 0 μ g mL⁻¹), and the percent viability average and +/- standard deviations are given.

	Hyperthermia Percent Viability - MDA-MB-231						
	[1048][Folate]			[1061][Folate]			
Concentration (µg mL ⁻¹)	Irradiation Time Length			Irradiation Time Length			
	10 min	20 min	30 min	10 min	20 min	30 min	
0	93.3±16.3	72.4±10.8	54.2±12.5	93.3±16.3	72.4±10.8	54.2±12.5	
77.6	70.7±4.6	33.2±6.9	12.0±16	80.0±2.4	22.6±1.2	15.6±14.6	
124.9	54.7±4.3	7.3±6.3	19.2±13.4	61.7±11.4	9.2±3.1	19.2±13.2	
179.6	10.8±6.4	7.3±1.4	9.8±5.3	11.5±8.3	10.0±4.1	10.6±3.0	

Table S3. The photothermal temperature generation of nanoGUMBOS upon continuous irradiation over a set period of time at different concentration was recorded. The concentrations were compared against a Live control (no irradiation and 0 μ g mL⁻¹), and the percent viability average and +/- standard deviations are given.

	Photothermal Temperature (°C)							
	[1048][Folate]			[1061][Folate]				
Time (s)	77.6 μg mL ⁻¹	124.9 μg mL ⁻¹	179.6 μg mL ⁻¹	77.6 μg mL ⁻¹	124.9 μg mL ⁻¹	179.6 μg mL ⁻¹		
0	25.1±0.7	25.1±0.5	23.5±1.0	23.3±1.3	24.4±0.5	25.2±0.4		
10	26.3±0.5	26.6±0.6	25.0±0.6	25.3±1.6	26.5±1.7	27.7±0.6		
30	30.3±3.9	36.2±2.5	30.8±4.5	34.2±3.0	37.7±1.9	38.6±1.5		
60	33.3±6.2	44.4±6.2	34.9±7.6	40.7±3.5	45.6±2.1	45.8±1.8		
120	38.3±8.1	51.5±5.1	42.5±7.4	48.6±3.8	55.4±2.6	55.2±2.3		
300	46.2±6.6	58.6±5.0	56.4±3.5	55.6±3.5	64.5±2.7	65.1±2.0		
600	50.4±5.3	59.9±2.7	58.3±4.2	56.2±3.2	64.6±2.4	66.1±2.4		
1200	49.2±4.9	59.6±1.1	57.3±4.5	55.7±3.7	63.5±2.4	64.6±1.9		
1800	48.1±4.2	59.9±1.9	57.5±6.2	55.4±4.0	63.2±2.4	64.3±2.2		