Supplemental Information

Cancer Cell Targeting Via Selective Transferrin Receptor Labeling Using Protein-Derived Carbon Dots

*Sara Strickland, Mychele Jorns, Luke Fourroux, Lindsey Heyd, and Dimitri Pappas**

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409

*Corresponding Author, d.pappas@ttu.edu

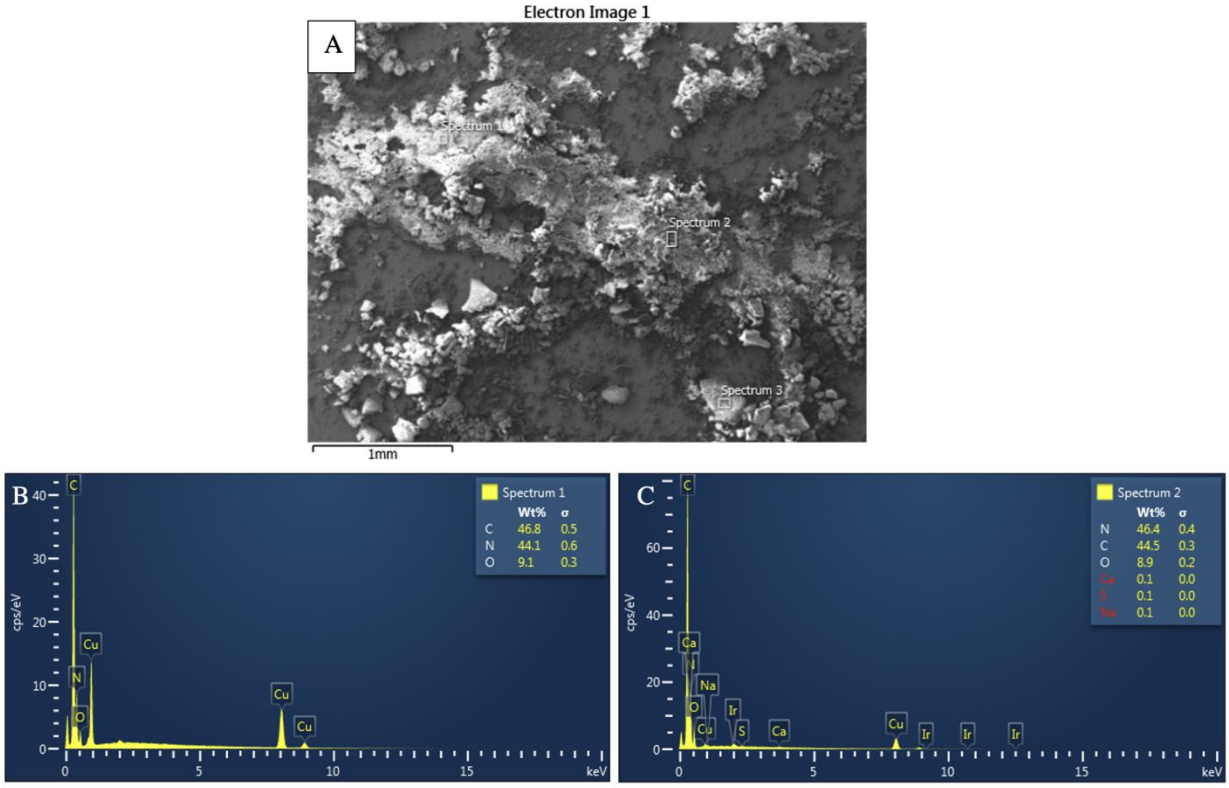


Figure S1. (A) Electron image of first 3 random spot locations. (B) EDS spectrum of electron image 1 location 1. Transferrin derived nanomaterial sample was primary composed of carbon and nitrogen with some oxygen species present. (C) EDS spectrum of electron image 1 location 2. This location showed a similar chemical profile as spectrum 1, however this spot also contained trace amounts of calcium sulfur and sodium. The sulfur is thought to come from the disulfide bonds in the transferrin protein. The calcium and sodium are thought to be contaminants from the water when washing the glassware, the sample was stored in or from the skin of the scientist handling the sample.

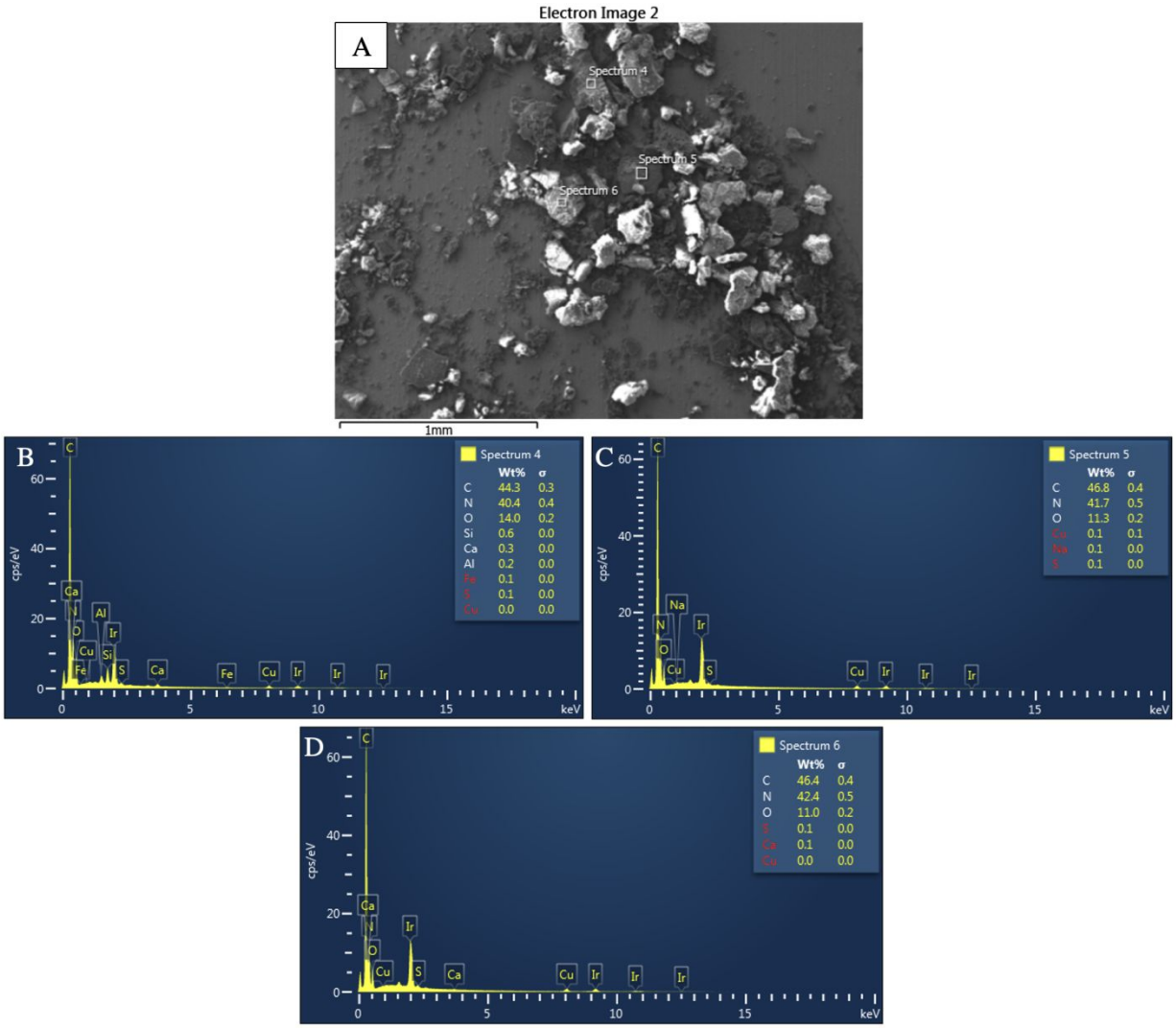

Electron Image 2
A
Spectrum 4
Spectrum 5
Spectrum 6
1mm
B
Spectrum 4
Wt% σ
C 44.3 0.3
N 40.4 0.4
O 14.0 0.2
Si 0.6 0.0
Ca 0.3 0.0
Al 0.2 0.0
Fe 0.1 0.0
S 0.1 0.0
Cu 0.0 0.0
cps/eV
keV
C
Spectrum 5
Wt% σ
C 46.8 0.4
N 41.7 0.5
O 11.3 0.2
Cu 0.1 0.1
Na 0.1 0.0
S 0.1 0.0
D
Spectrum 6
Wt% σ
C 46.4 0.4
N 42.4 0.5
O 11.0 0.2
S 0.1 0.0
Ca 0.1 0.0
Cu 0.0 0.0

Figure S2. (A) Electron image 2 of the transferrin derived nanomaterial with the last 3 random spot locations. (B) EDS spectrum of the 4th location. The ratio of carbon, nitrogen and oxygen species present was similar to the results found in the first electron image in Fig. S1. However, silicon, calcium, aluminum, iron, and sulfur were all found in trace amounts. It is believed that the calcium is a contaminant of the water in the building. The silicon and aluminum contaminants is believed to come from the glass petri dish the sample was stored in and the aluminum spatula the sample was transported with. The sulfur is believed to come from the disulfide bonds in the transferrin protein and the iron could be present due to the use of iron bound holotransferrin as the carbon precursor. (C, D) EDS spectrum of the 5th location and 6th location respectively. Again, the weight % of carbon, nitrogen and oxygen species are homogenous across the sample. Trace amounts of sulfur was detected with minor contamination.

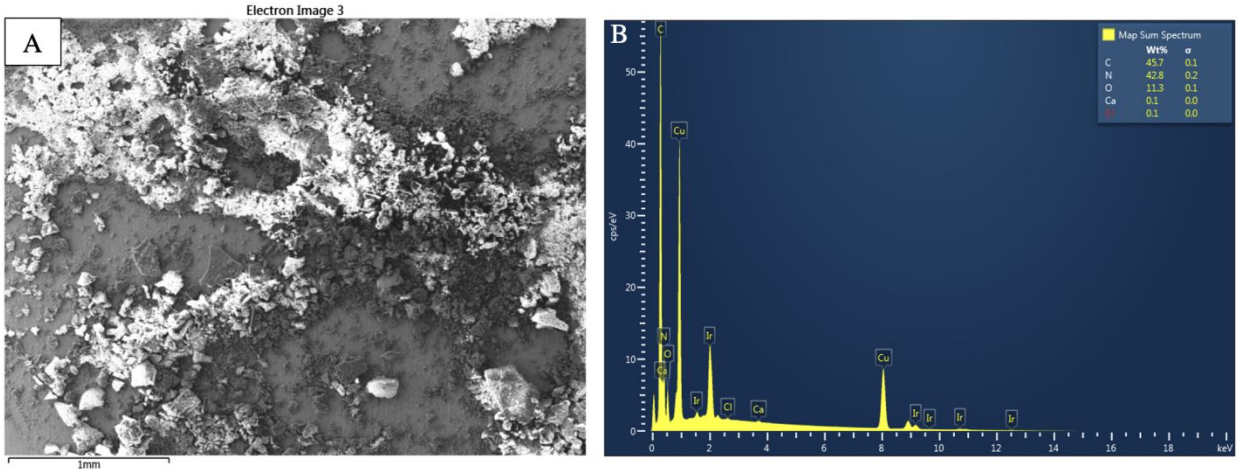


Figure S3. (A) Electron image 3 of transferrin nanomaterial sample. (B) A map sum spectrum was taken of the entire third electron image. Again, the ratio of carbon, nitrogen and oxygen stayed consistent with what was seen at the 6 random spot locations. This indicates synthesis of a homogenous sample.

	dHL60 1% (0.39μM)	HL60 1% (0.39μM)	dHL60 10% (3.90μM)	HL60 10% (3.90μM)	dHL60 25% (9.75μM)	HL60 25% (9.75μM)	dHL60 50% (19.5μM)	HL60 50% (19.5μM)	dHL60 100% (39μM)	HL60 100% (39μM)	dAB CD71-PE	AB CD71-PE
MFI (A.U)	299.76	22.75	349.73	382.03	218.75	392.46	-881.96	366.42	-624.92	130.94	511.35	6514.89
P-Value	1.47614 > 0.05 not statistically significant		0.68344 > 0.05 not statistically significant		0.03147 < 0.05 statistically significant		0.00001 < 0.05 statistically significant		0.00001 < 0.05 statistically significant		0.00001 < 0.05 statistically significant	

Table S1. Background corrected MFI and p-value for every concentration of each of the differentiate and undifferentiated HL60 cell populations.

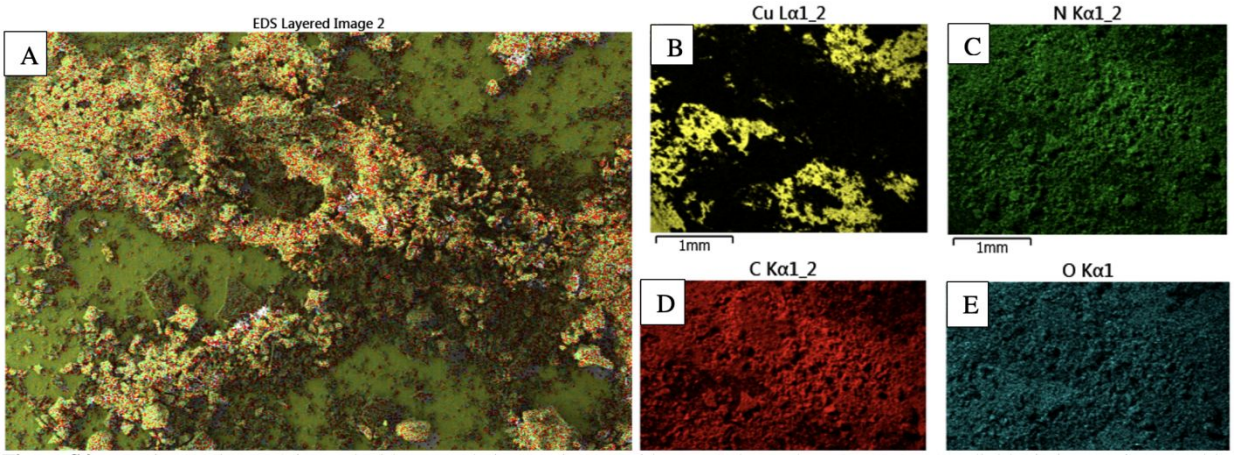
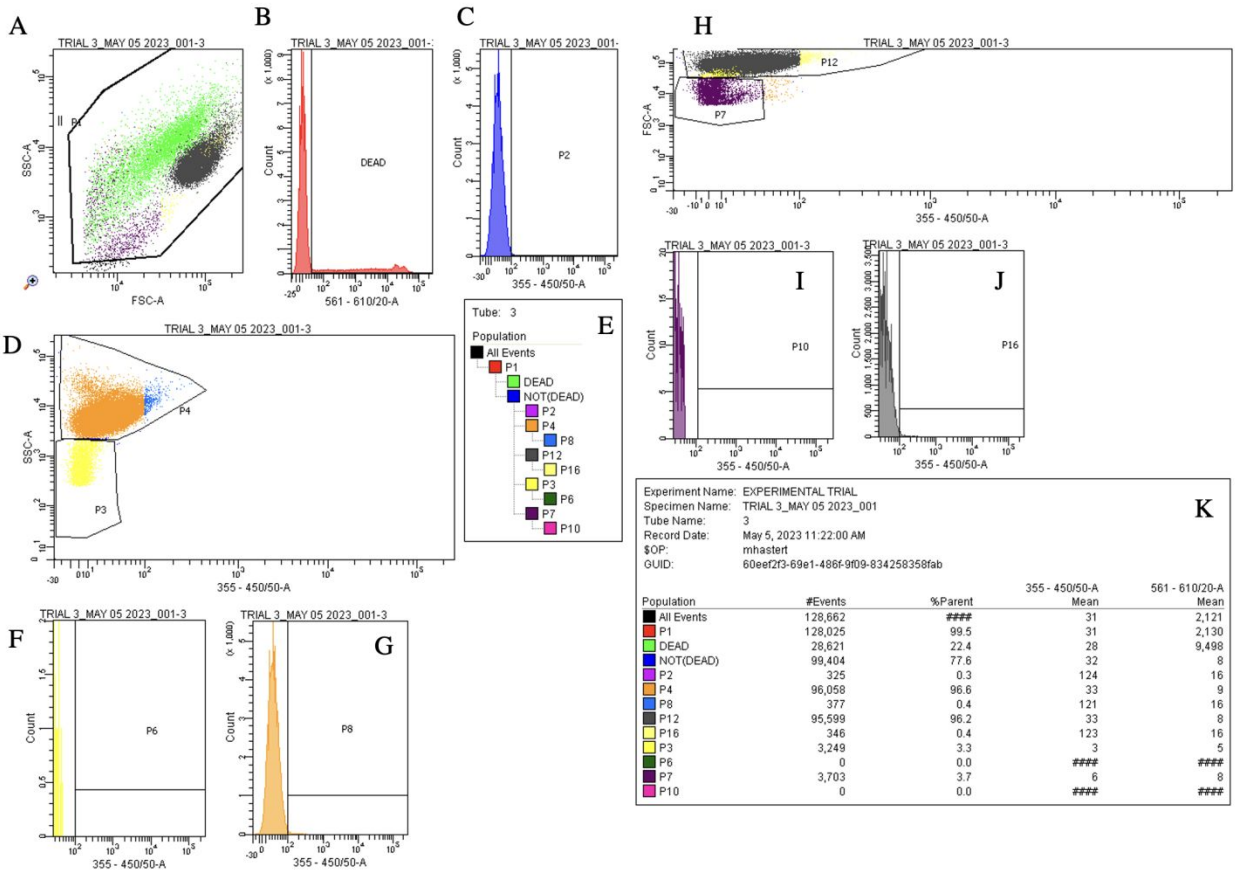


Figure S4. (A) Electron image 3 layered with mapped elemental composition EDS data points. (B, C, D, E) Individual elemental composition maps of electron image 3, showing EDS maps if copper, nitrogen, carbon, and oxygen respectively.



Population	#Events	%Parent	355 - 450/50-A Mean	561 - 610/20-A Mean
All Events	128,662	####	31	2,121
P1	128,025	99.5	31	2,130
DEAD	28,621	22.4	28	9,498
NOT(DEAD)	99,404	77.6	32	8
P2	325	0.3	124	16
P4	96,058	96.6	33	9
P8	377	0.4	121	16
P12	95,599	96.2	33	8
P16	346	0.4	123	16
P3	3,249	3.3	3	5
P6	0	0.0	####	####
P7	3,703	3.7	6	8
P10	0	0.0	####	####

Figure S5. (A) Flow cytometry populations with FSC vs SSC plot of HL60 cells without antibodies or CDs. (B) Histogram of dead cells indicated by PI. (C) Histogram of cells autofluorescence. (D) Dot plot depicting populations of cells indicated by SSC and UV excitation. (E) Chart depicting all the flow cytometry populations and histograms. (F, G) Histograms depicting autofluorescence populations of unstained cells. (H) Dot plot depicting populations of cells indicated by FSC and UV excitation. (I, J) Histograms depicting the autofluorescence populations of unstained cells. (K) Table indicating the total number of events of each population at each excitation wavelength.

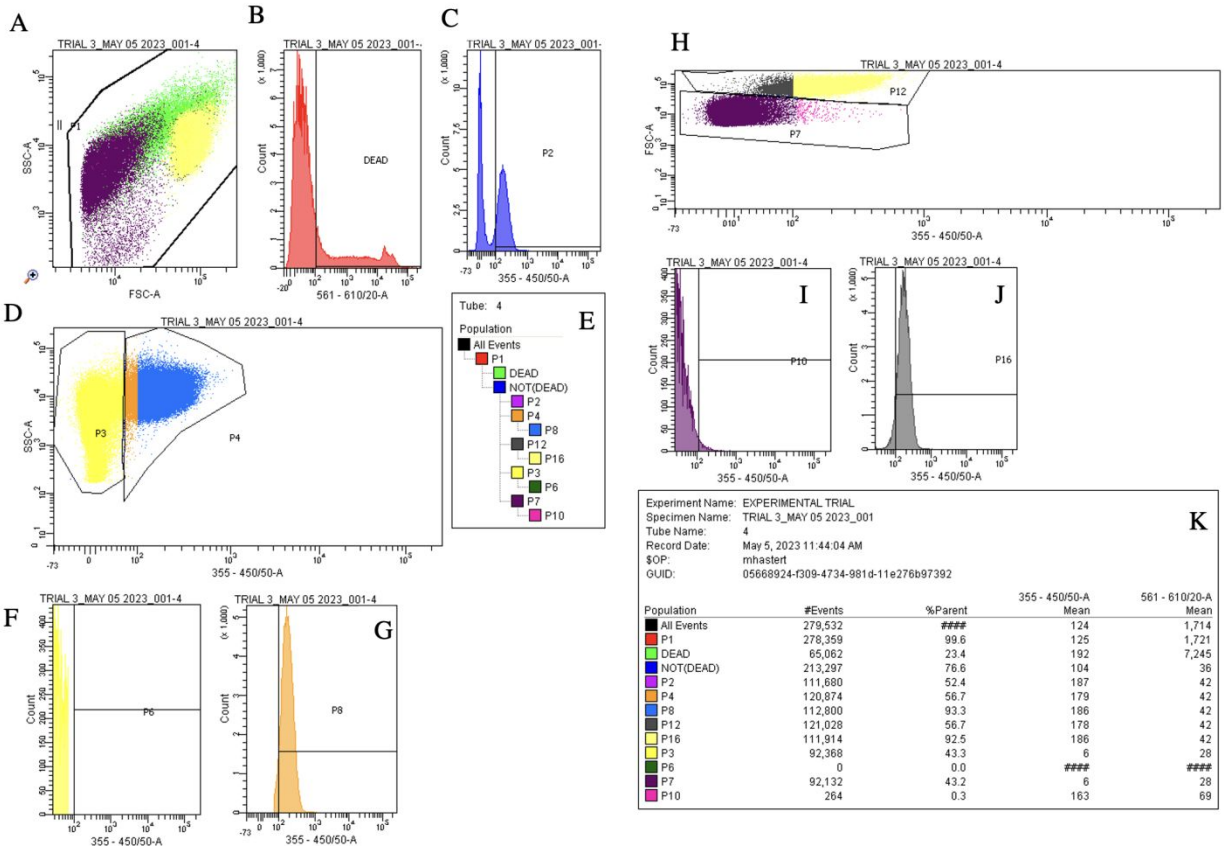
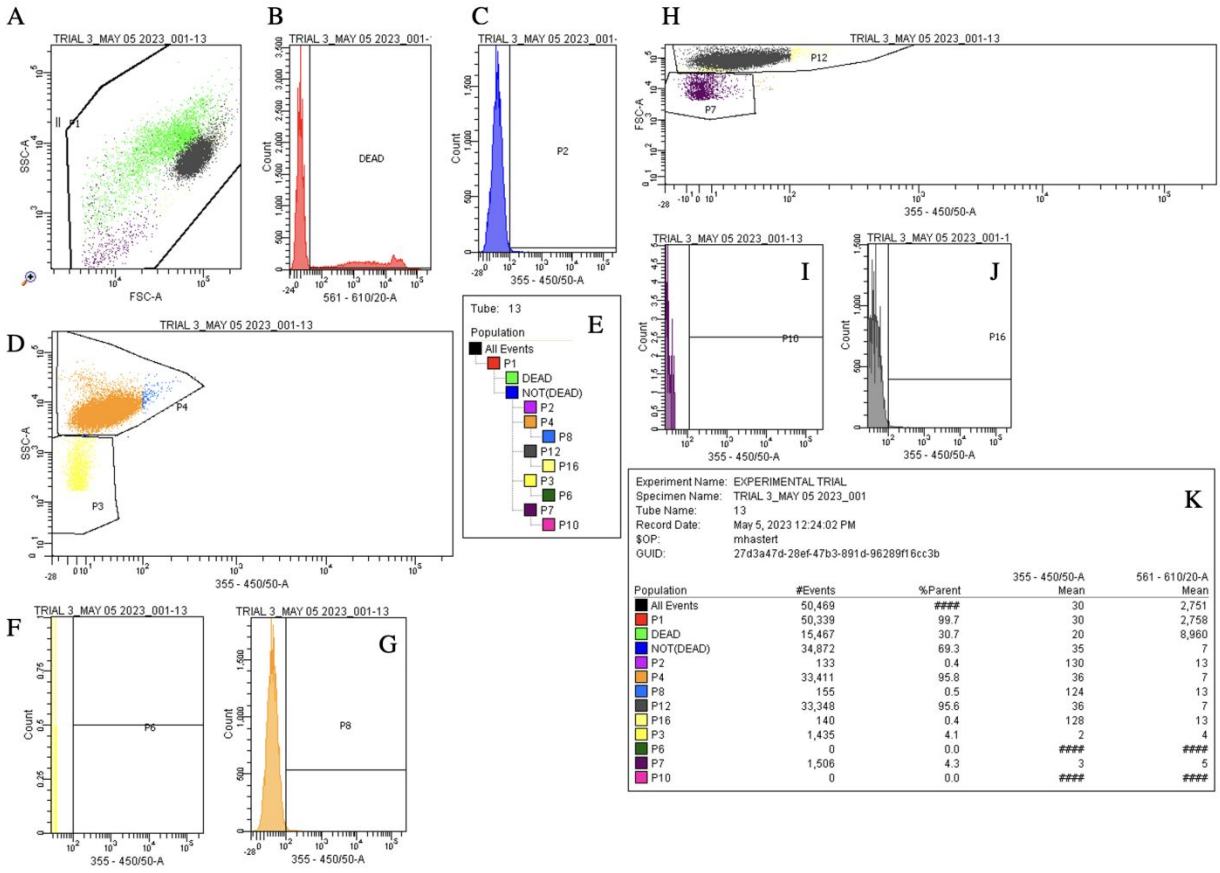


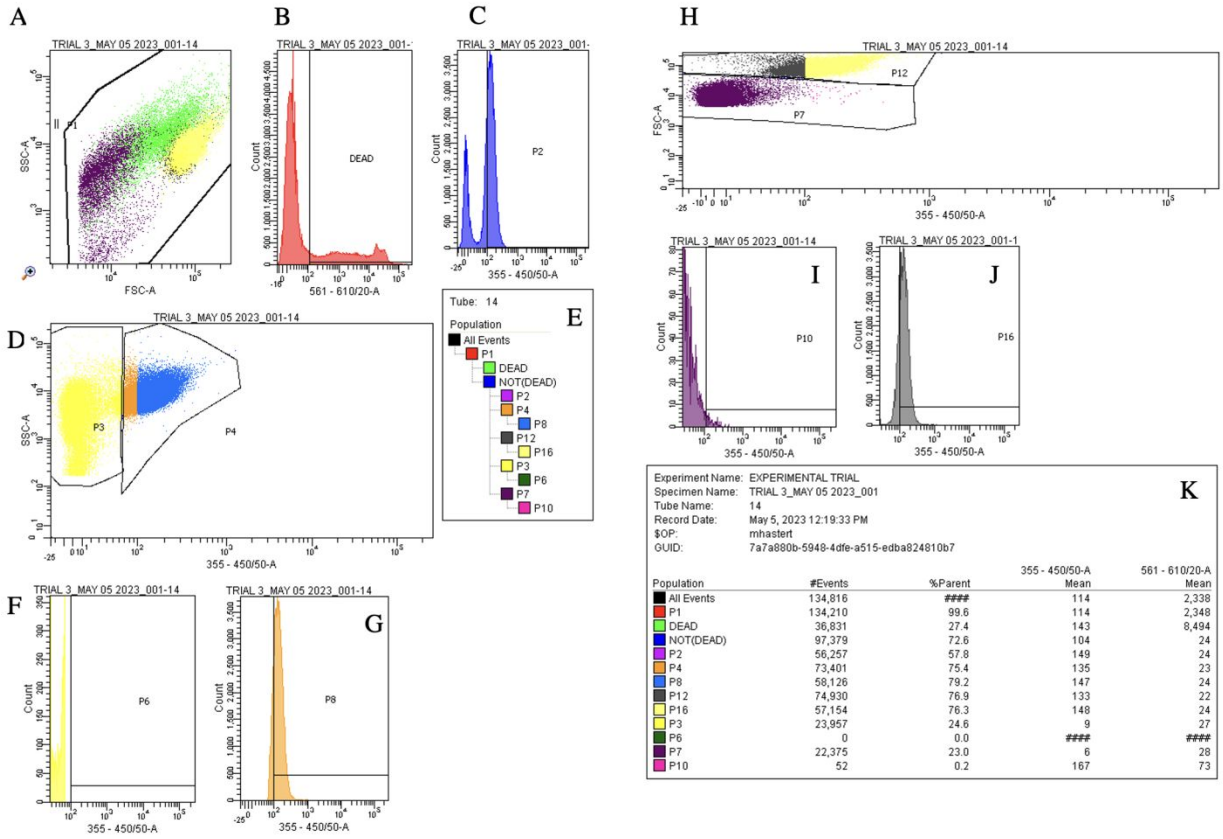
Figure S6. (A) Flow cytometry populations with FSC vs SSC plot of HL60 cells stained with 3.9 μM affinity CDs. (B) Histogram of dead cells indicated by PI. (C) Histogram of cells autofluorescence and the fluorescence of CD71 affinity CDs. (D) Dot plot depicting populations of cells indicated by SSC and UV excitation. (E) Chart depicting all the flow cytometry populations and histograms. (F) Histogram depicting cell autofluorescence and NP fluorescence populations of stained cells. (G) Histogram indicating the fluorescence of transferrin-derived CDs (H) Dot plot depicting populations of cells by FSC and UV excitation. (I, J) Histograms depicting the autofluorescence of cells and NP fluorescence populations of affinity CD stained cells. (K) Table indicating the total number of events of each population at each excitation wavelength.



Population	#Events	%Parent	355 - 450/50-A Mean	561 - 610/20-A Mean
All Events	50,469	####	30	2,751
P1	50,339	99.7	30	2,758
DEAD	15,467	30.7	20	8,960
NOT(DEAD)	34,872	69.3	35	7
P2	133	0.4	130	13
P4	33,411	95.8	36	7
P8	155	0.5	124	13
P12	33,348	95.6	36	7
P16	140	0.4	128	13
P3	1,435	4.1	2	4
P6	0	0.0	####	####
P7	1,506	4.3	3	5
P10	0	0.0	####	####

Figure S7. (A) Flow cytometry populations with FSC vs SSC plot of HL60 cells with 100 µL of CD71 antibody and no CDs. (B) Histogram of dead cells indicated by PI. (C) Histogram of HL60 cells autofluorescence with CD71 antibody. (D) Dot plot depicting populations of cells indicated by SSC and UV excitation. (E) Chart depicting all the flow cytometry populations and histograms. (F, G) Histograms depicting autofluorescence populations of NP unstained cells with CD71 antibody. (H) Dot plot depicting populations of cells indicated by FSC and UV excitation.

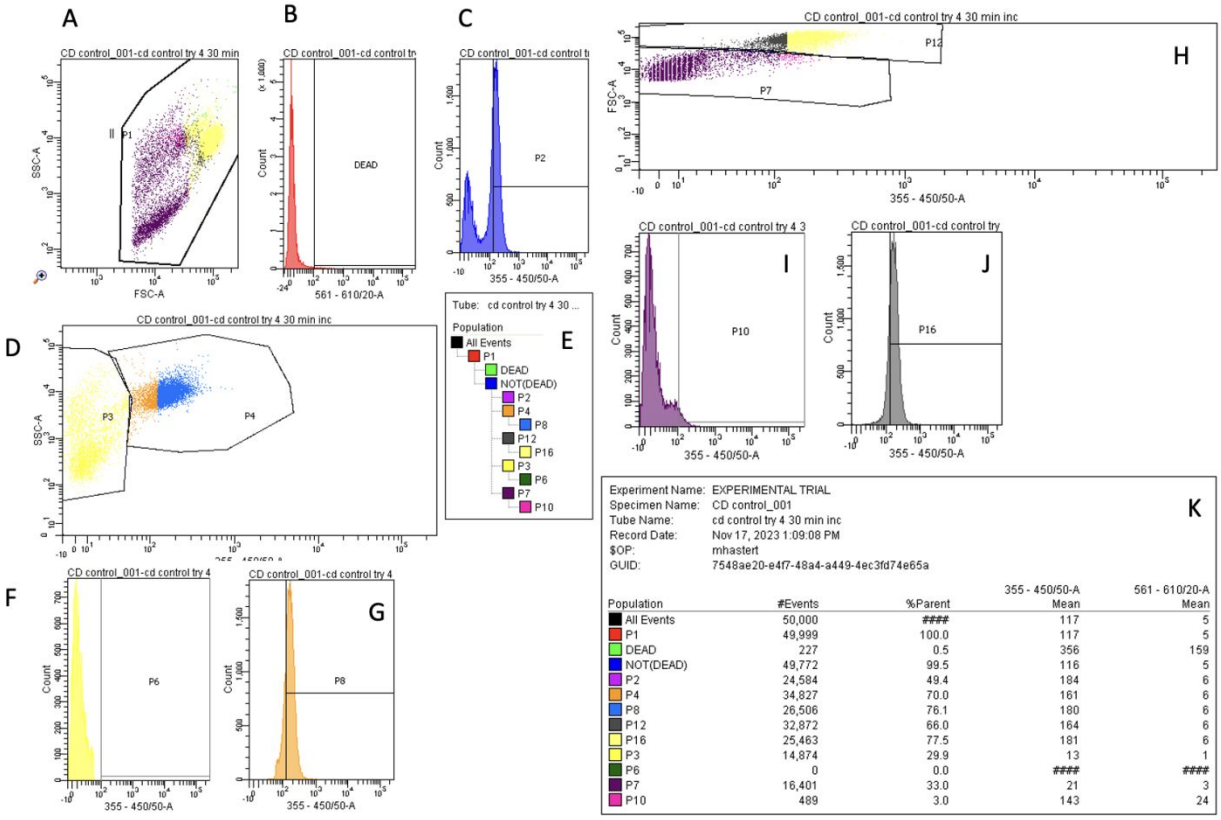
(I, J) Histograms depicting the autofluorescence populations of unstained cells incubated with CD71 antibody. (K) Table indicating the total number of events of each population at each excitation wavelength.



Population	#Events	%Parent	355 - 450/50-A Mean	561 - 610/20-A Mean
All Events	134,816	####	114	2,338
P1	134,210	99.6	114	2,348
DEAD	36,831	27.4	143	8,494
NOT(DEAD)	97,379	72.6	104	24
P2	56,257	57.8	149	24
P4	73,401	75.4	135	23
P8	58,126	79.2	147	24
P12	74,930	76.9	133	22
P16	57,154	76.3	148	24
P3	23,957	24.6	9	27
P6	0	0.0	####	####
P7	22,375	23.0	6	28
P10	52	0.2	167	73

Figure S8. (A) Flow cytometry populations with FSC vs SSC plot of HL60 cells incubated in 100 µL of CD71 antibody and stained with 3.9 µM affinity CDs. (B) Histogram of dead cells indicated by PI. (C) Histogram of cells autofluorescence and the fluorescence of CD71 affinity CDs. (D) Dot plot depicting populations of cells indicated by SSC and UV excitation. (E) Chart depicting all the flow cytometry populations and histograms. (F) Histogram depicting cell autofluorescence and NP fluorescence populations of stained cells. (G) Histogram indicating the fluorescence of transferrin-derived CDs (H) Dot plot depicting populations of cells by FSC and UV excitation. (I, J)

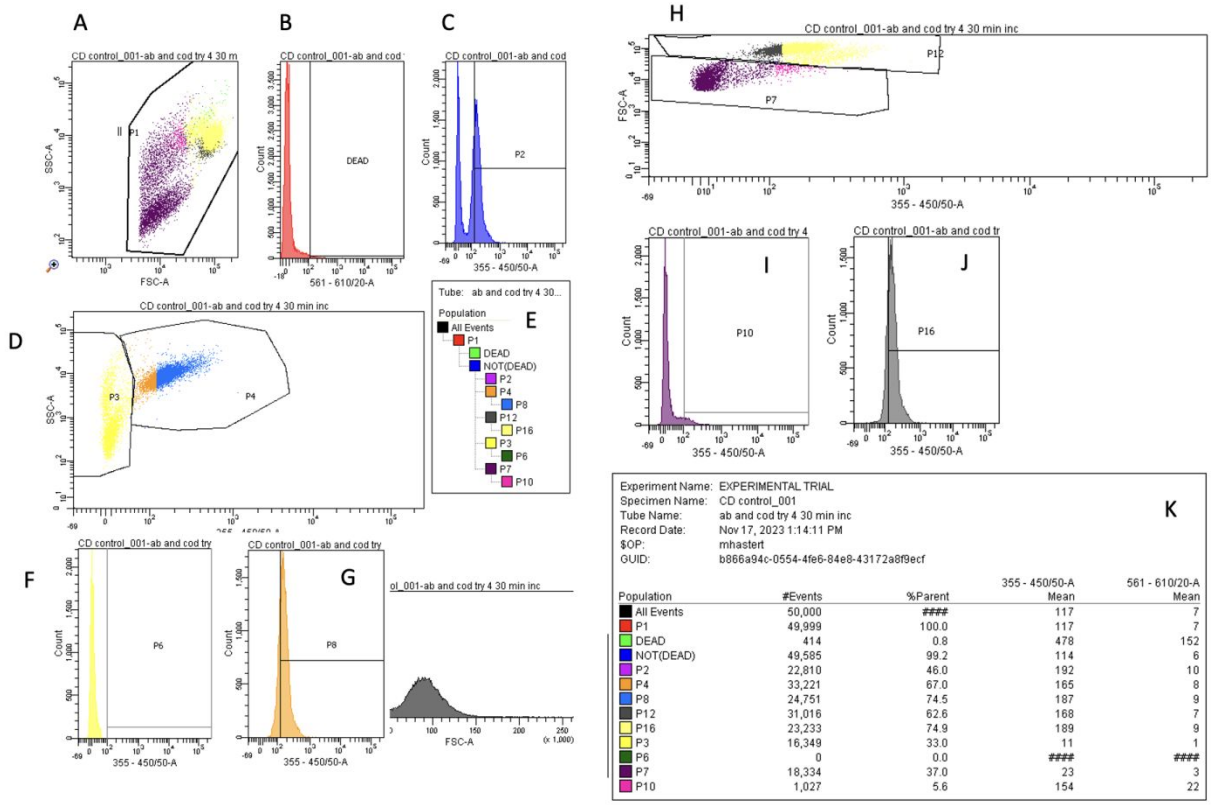
Histograms depicting the autofluorescence of cells and NP fluorescence populations of affinity CD stained cells. (K) Table indicating the total number of events of each population at each excitation wavelength.



Population	#Events	%Parent	355 - 450/50-A Mean	561 - 610/20-A Mean
All Events	50,000	####	117	5
P1	49,999	100.0	117	5
DEAD	227	0.5	356	159
NOT(DEAD)	49,772	99.5	116	5
P2	24,584	49.4	184	6
P4	34,827	70.0	161	6
P8	26,506	76.1	180	6
P12	32,872	66.0	164	6
P16	25,463	77.5	181	6
P3	14,874	29.9	13	1
P6	0	0.0	####	####
P7	16,401	33.0	21	3
P10	489	3.0	143	24

Figure S9. (A) Flow cytometry populations with FSC vs SSC plot of HL60 cells stained with 10% ethylenediamine-formamide derived nanomaterial. (B) Histogram of dead cells indicated by PI. (C) Histogram of cells autofluorescence and the fluorescence of CD71 affinity CDs. (D) Dot plot depicting populations of cells indicated by SSC and UV excitation. (E) Chart depicting all the flow cytometry populations and histograms. (F) Histogram depicting cell autofluorescence and NP fluorescence populations of stained cells. (G) Histogram indicating the fluorescence of transferrin-derived CDs (H) Dot plot depicting populations of cells by FSC and UV excitation. (I, J) Histograms

depicting the autofluorescence of cells and NP fluorescence populations of affinity CD stained cells. (K) Table indicating the total number of events of each population at each excitation wavelength.



Population	#Events	%Parent	355 - 450/50-A Mean	561 - 610/20-A Mean
All Events	50,000	####	117	7
P1	49,999	100.0	117	7
DEAD	414	0.8	478	152
NOT(DEAD)	49,585	99.2	114	6
P2	22,810	46.0	192	10
P4	33,221	67.0	165	8
P8	24,751	74.5	187	9
P12	31,016	62.6	168	7
P16	23,233	74.9	189	9
P3	16,349	33.0	11	1
P6	0	0.0	####	####
P7	18,334	37.0	23	3
P10	1,027	5.6	154	22

Figure S10. (A) Flow cytometry populations with FSC vs SSC plot of HL60 cells incubated in 100 µL of CD71 antibody and stained with 10% ethylenediamine-formamide derived nanomaterial. (B) Histogram of dead cells indicated by PI. (C) Histogram of cells autofluorescence and the fluorescence of CD71 affinity CDs. (D) Dot plot depicting populations of cells indicated by SSC and UV excitation. (E) Chart depicting all the flow cytometry populations and histograms. (F) Histogram depicting cell autofluorescence and NP fluorescence populations of stained cells. (G) Histogram indicating the fluorescence of transferrin-derived CDs (H) Dot plot depicting populations of cells by FSC and UV excitation. (I, J) Histograms depicting the autofluorescence of cells and NP fluorescence populations

of affinity CD stained cells. (K) Table indicating the total number of events of each population at each excitation wavelength.

Corresponding Author

D. Pappas@ttu.edu