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Supporting Information for

High-throughput quantitative microscopy-based half-life measurements of intravenously injected agents

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This PDF file includes:

Figures S1 to S4
Tables S1 and S2

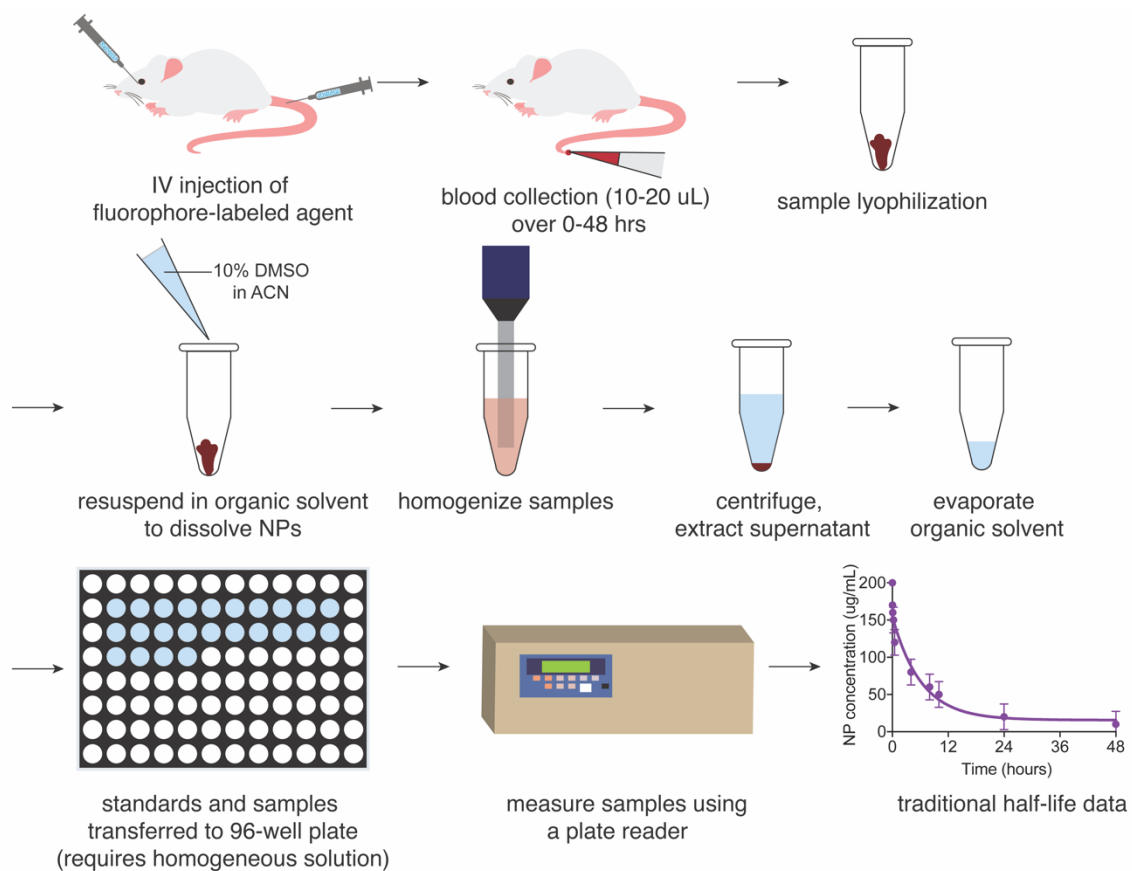


Fig. S1. Schematic illustrating the workflow for half-life measurements and end-point analyses using a traditional microplate method.

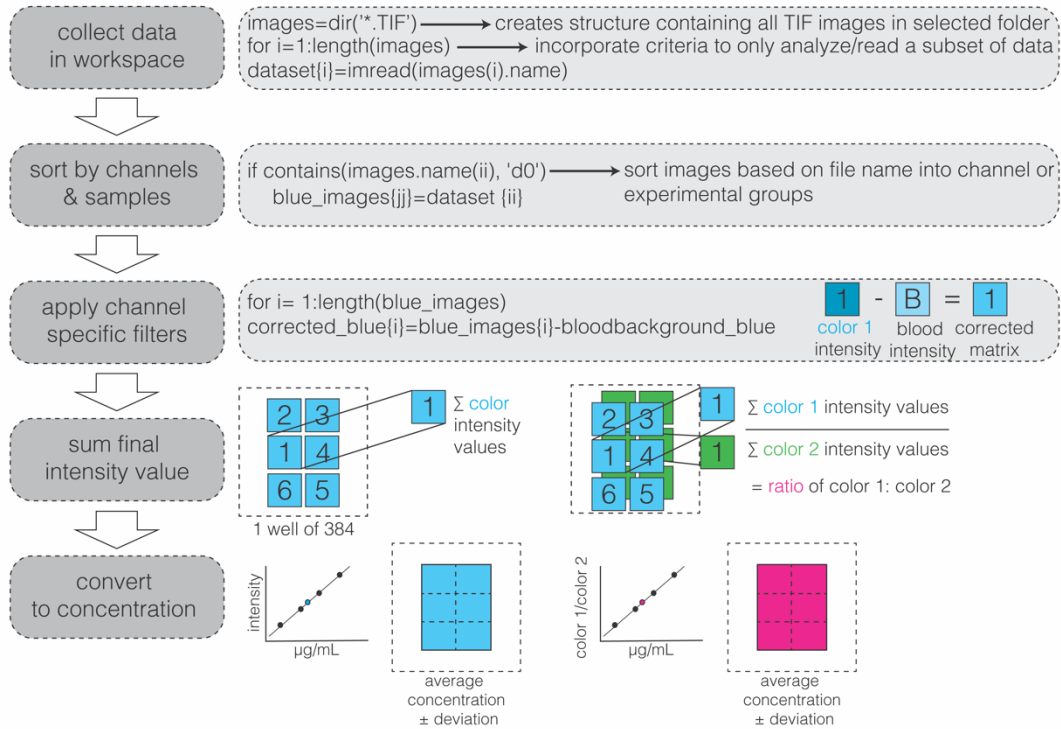


Fig. S2. MATLAB program workflow for quantitative image analysis.

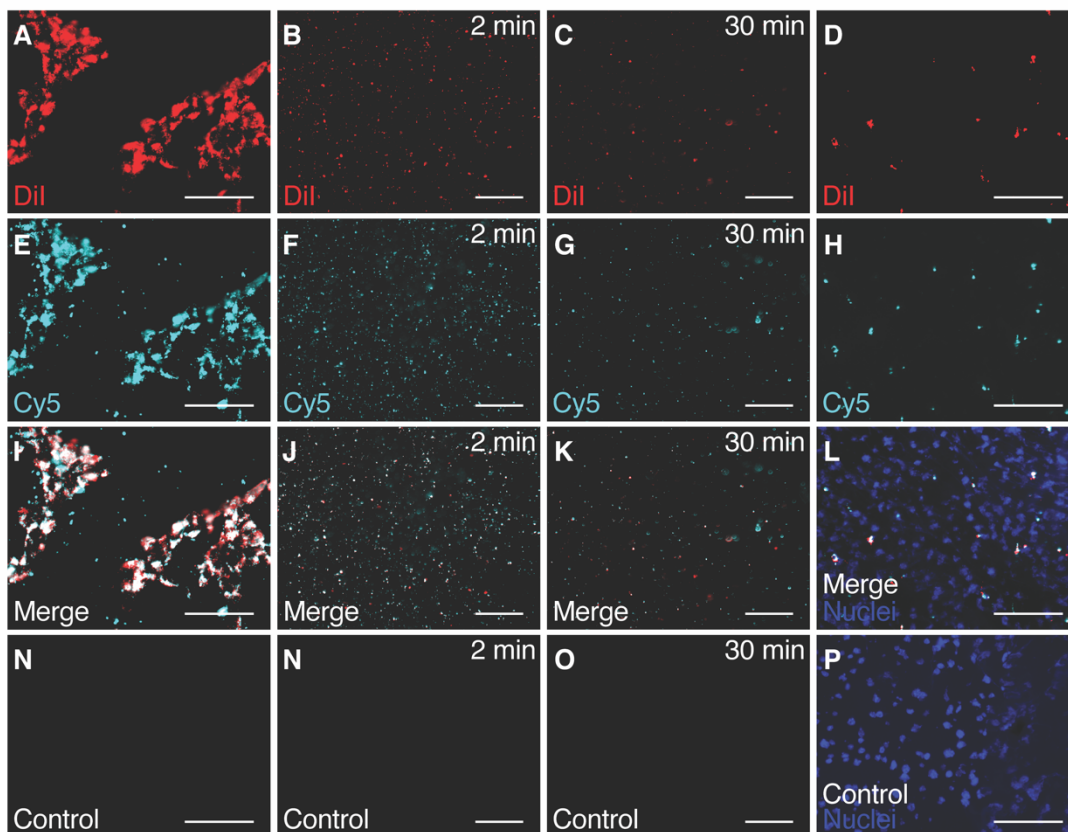


Fig. S3. Dual-color NPs demonstrate colocalization of polymeric vehicle and dye cargo. Representative fluorescence images of dual color NPs PLGA-Cy5 loaded with Dil administered *in vitro* in HEK293 cells and IV *in vivo*. (A-D) Dil signal in (A) HEK293 cells 24 hours after treatment, (B) blood collected 2 minutes after IV NP administration, (C) blood collected 30 minutes after IV NP administration, (D) liver sections 48 hours after IV NP administration. (E-H) Cy5 signal in the samples described in (A-D). (I-L) Merged Dil, Cy5, and Nuclei (where applicable) signal in the samples described in (A-D). (M-P) Merged Dil and Cy5 signal in control samples. Scale bars, 100 μm .

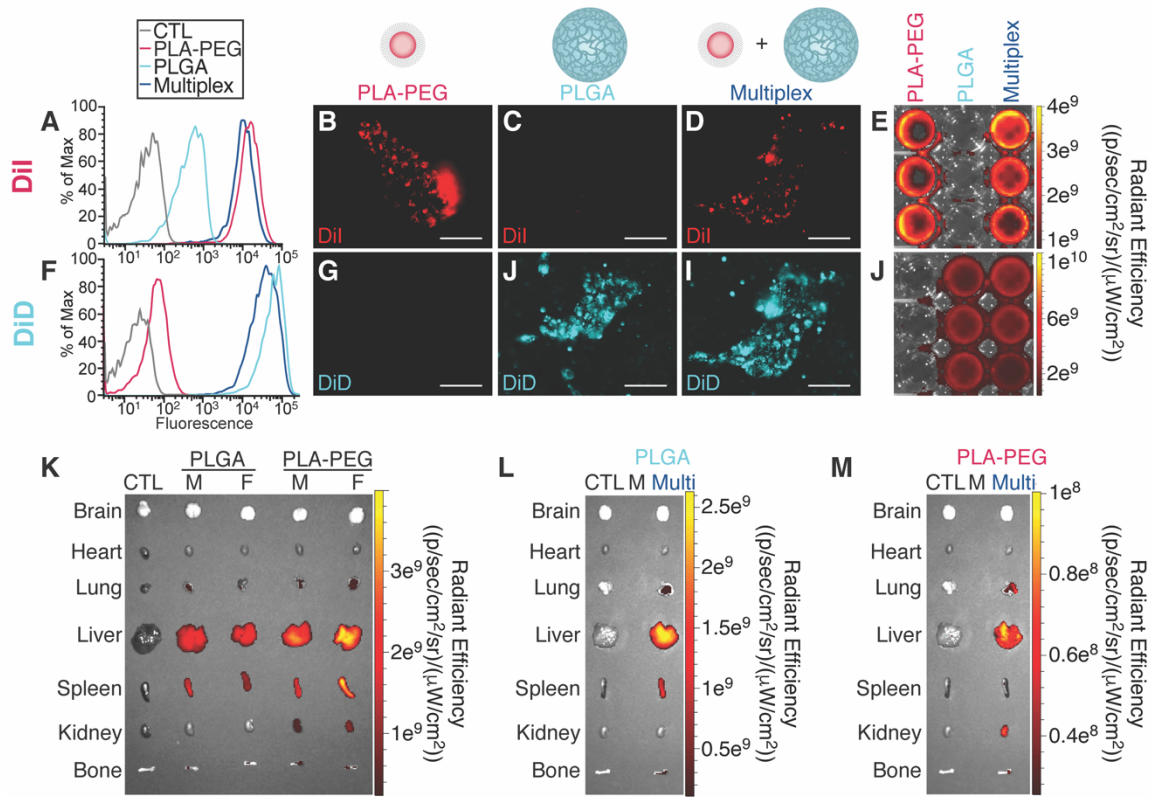


Fig. S4. Quantitative fluorescent measurements demonstrate feasibility of multiplexing. (A-J) Representative flow cytometry, microscopy, and IVIS data following treatment of HEK293 cells with DiI-loaded PLA-PEG, DiD-loaded PLGA, or combined DiI PLA-PEG and DiD PLGA NPs at 0.2 mg/mL. Panels (A-E) indicate signal obtained in the red channel (DiI signal). Panels (F-J) indicate signal obtained in the far red channel (DiD signal). Together, these data demonstrate that NP formulations encapsulating different fluorescent dyes can be distinguished from one another even when combined in the same experiment. (K-M) IVIS images of DiD or DiI NP-treated animals 48 hours after *in vivo* IV administration with untreated control animals. (K) DiD signal in the organs of male and female animals administered with DiD-loaded PLGA or PLA-PEG NPs. (L) DiD signal in the organs of a male animal administered with DiI-loaded PLA-PEG NPs and DiD-loaded PLGA NPs. (M) DiI signal in the organs of the animal in panel (L). Scale bars, 100 μm .

Table S1. Characterization data for dye-loaded PLA-PEG and PLGA NPs and the Anti-CD45 IgG1 antibody.

Agent	Diameter (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
PLA-PEG DiD	173 ± 1	0.140	-24.0 ± 0.9
PLA-PEG DiO	184 ± 5	0.140	-19.2 ± 0.6
PLA-PEG DiI	183 ± 1	0.150	-23.8 ± 0.5
PLGA DiD	273 ± 2	0.190	-17.2 ± 0.7
PLGA DiO	262 ± 2	0.140	-20.2 ± 0.6
PLGA-Cy5 DiI	339 ± 2	0.180	-19.2 ± 0.5
mouse-anti-human IgG1 antibody (anti-CD45)	~10 (150-170 kDa)	-	-

Table S2. NP concentration values calculated for each image (n=6) of each blood sample (n=3) per time point (n=10) following retro-orbital or tail-vein IV PLA-PEG NP administration.

Time	NP concentration (µg/mL)							
	RO M1	RO M2	RO M3	Average RO	TV M1	TV M2	TV M3	Average TV
2 min	119.5	117.7	118.2		235.1	236.1	223.9	
	129.6	130.4	131.9		270.7	255.4	246.4	
	132.3	130.5	122.0		254.0	251.7	231.4	
	114.7	115.7	108.3		223.5	226.1	204.6	
	152.0	136.4	128.5		226.8	241.0	220.7	
	151.0	134.5	132.1		238.6	244.4	234.0	
average	133.2	127.5	123.5	128.1	241.4	242.4	226.8	236.9
standard deviation	15.6	8.7	9.3	4.9	17.9	10.6	14.1	8.7
15 min	208.5	213.7	203.6		186.8	245.0	216.9	
	224.0	225.7	216.9		228.0	260.2	223.4	
	219.3	227.8	218.9		207.8	252.7	213.5	
	205.8	204.0	196.9		186.6	237.3	201.0	
	240.6	238.0	224.2		197.6	251.9	222.6	
	220.5	238.5	227.6		204.5	250.6	237.3	
average	219.8	224.6	214.7	219.7	201.9	249.6	219.1	223.5
standard deviation	12.4	13.6	12.0	5.0	15.5	7.7	12.0	24.2
30 min	257.4	236.9	260.2		230.5	192.9	232.9	
	270.3	273.0	288.4		259.4	210.3	274.3	
	273.9	255.1	287.3		256.8	204.7	250.2	
	243.1	223.7	260.3		222.2	185.0	208.4	
	266.6	242.0	282.3		243.3	200.6	222.9	
	273.3	245.1	280.0		244.4	198.9	248.9	
average	264.1	246.0	276.4	262.2	242.8	198.7	239.6	227.0
standard deviation	11.9	16.8	12.9	15.3	14.5	8.9	23.2	24.6
1 hr	205.8	176.1	155.4		191.1	132.5	178.1	
	219.2	198.3	167.1		203.3	147.8	204.9	
	211.5	180.3	165.6		198.5	146.6	195.0	
	197.8	160.7	153.6		184.3	130.9	171.5	
	224.6	169.6	161.6		199.8	138.6	184.0	
	228.9	182.5	163.8		203.4	140.5	188.7	
average	214.6	177.9	161.2	184.6	196.7	139.5	187.1	174.4
standard deviation	11.8	12.7	5.5	27.3	7.6	7.0	12.0	30.6
2 hr	135.1	149.8	149.3		152.4	118.1	124.4	
	147.5	152.3	151.7		152.4	128.2	139.4	
	146.6	152.1	148.6		152.4	130.4	128.9	
	131.7	148.0	135.2		152.4	121.7	120.0	
	122.5	140.1	125.5		152.4	113.5	113.1	

	128.5	144.2	136.2		152.4	111.4	110.5	
average	135.3	147.8	141.1	141.4	152.4	120.5	122.7	131.9
standard deviation	10.0	4.8	10.4	6.2	0.0	7.7	10.7	17.8
4 hr	99.9	126.1	96.5		109.7	95.8	125.3	
	115.5	142.4	116.2		122.2	122.0	141.3	
	99.9	129.1	115.9		119.2	113.8	137.1	
	93.8	118.0	99.4		107.8	66.1	120.3	
	94.7	112.3	88.9		101.2	62.0	112.4	
	109.5	115.7	89.4		103.3	85.1	113.0	
average	102.2	123.9	101.1	109.1	110.6	90.8	124.9	108.8
standard deviation	8.6	11.0	12.3	12.9	8.5	24.5	12.2	17.1
8 hr	118.7	91.6	87.6		105.6	82.5	97.3	
	135.0	102.6	97.9		114.2	91.9	119.4	
	123.7	100.2	95.8		110.8	93.0	117.0	
	113.6	91.5	88.7		104.2	85.2	91.3	
	104.3	84.3	80.6		102.1	80.4	79.5	
	105.6	90.2	84.3		107.5	81.1	89.8	
average	116.8	93.4	89.1	99.8	107.4	85.7	99.1	97.4
standard deviation	11.6	6.8	6.6	14.9	4.4	5.5	15.9	11.0
10 hr	54.2	54.9	45.2		69.0	77.5	91.9	
	65.8	63.7	55.0		80.4	87.0	45.7	
	65.9	62.9	53.5		72.4	85.9	112.2	
	56.3	56.0	44.7		66.0	78.8	99.3	
	50.4	52.3	40.3		61.4	73.0	85.9	
	52.3	53.2	43.3		64.7	75.6	92.6	
average	57.5	57.2	47.0	53.9	69.0	79.6	87.9	78.8
standard deviation	6.8	4.9	5.9	6.0	6.8	5.6	22.5	9.5
24 hr	41.0	41.7	45.8		33.0	46.1	43.7	
	49.8	49.4	54.9		36.4	51.5	51.0	
	45.0	48.2	49.6		34.2	51.2	47.3	
	39.8	41.9	45.0		32.4	46.4	43.2	
	37.1	37.3	42.0		31.7	43.1	39.4	
	38.3	39.1	42.6		33.4	45.2	40.1	
average	41.8	43.0	46.7	43.8	33.5	47.3	44.1	41.6
standard deviation	4.8	4.9	4.9	2.5	1.7	3.4	4.4	7.2
48 hr	17.9	16.0	15.5		12.4	19.1	15.8	
	21.4	19.0	18.2		13.9	22.2	19.0	
	19.8	18.4	17.8		13.2	22.2	18.7	
	17.7	15.8	15.6		12.1	19.2	15.9	
	16.4	14.6	14.3		11.5	17.4	14.6	
	16.4	15.0	14.6		11.6	18.4	14.5	

average	18.2	16.5	16.0	16.9	12.5	19.7	16.4	16.2
standard deviation	2.0	1.8	1.6	1.2	0.9	2.0	2.0	3.6