Ionizable Lipids Drive Subcellular Localization and Immune Cell Targeting of Barcoded-Lipid Nanoparticles in Lung Cancer

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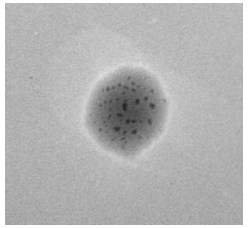
Cancer Therapeutics Gene Delivery

Macrophage-mediated drug delivery system with **nanoparticles**

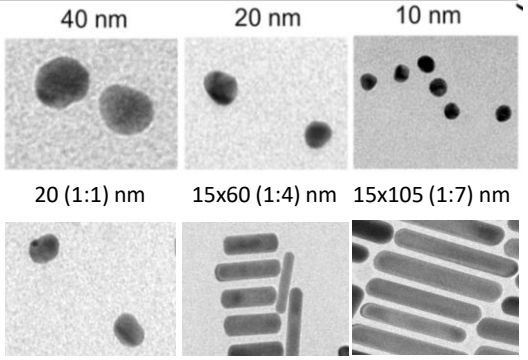
PLGA nanoparticles



- PEG
- Folate
- Dextran

Imaging with signal amplification by DNA barcodes (SAD) and multiplexed imaging technology (MIT) for lung cancer

Gold nanoparticles



Re-distribution of nanoparticles through macrophages

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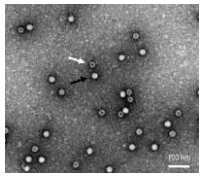
Gene delivery by using photothermal effect of GNPs

Gene delivery by using **recombinant adeno-associated virus (rAAV)**

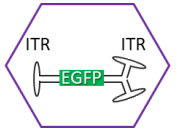
ssAAV and scAAV

TEM of rAAV



ssAAV

scAAV



Only rAAV genome staining with proximity labeling methods

Enhancement of virus production through a key factor related to host cell's gene regulation

Cancer Therapeutics Gene Delivery

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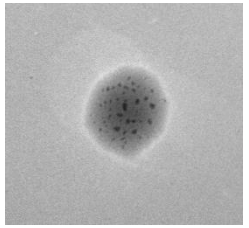

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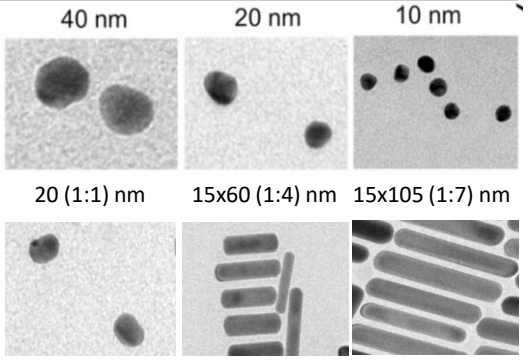

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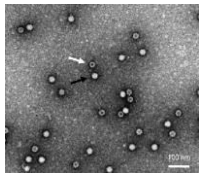
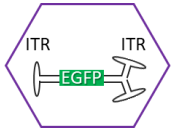


ssAAV

scAAV



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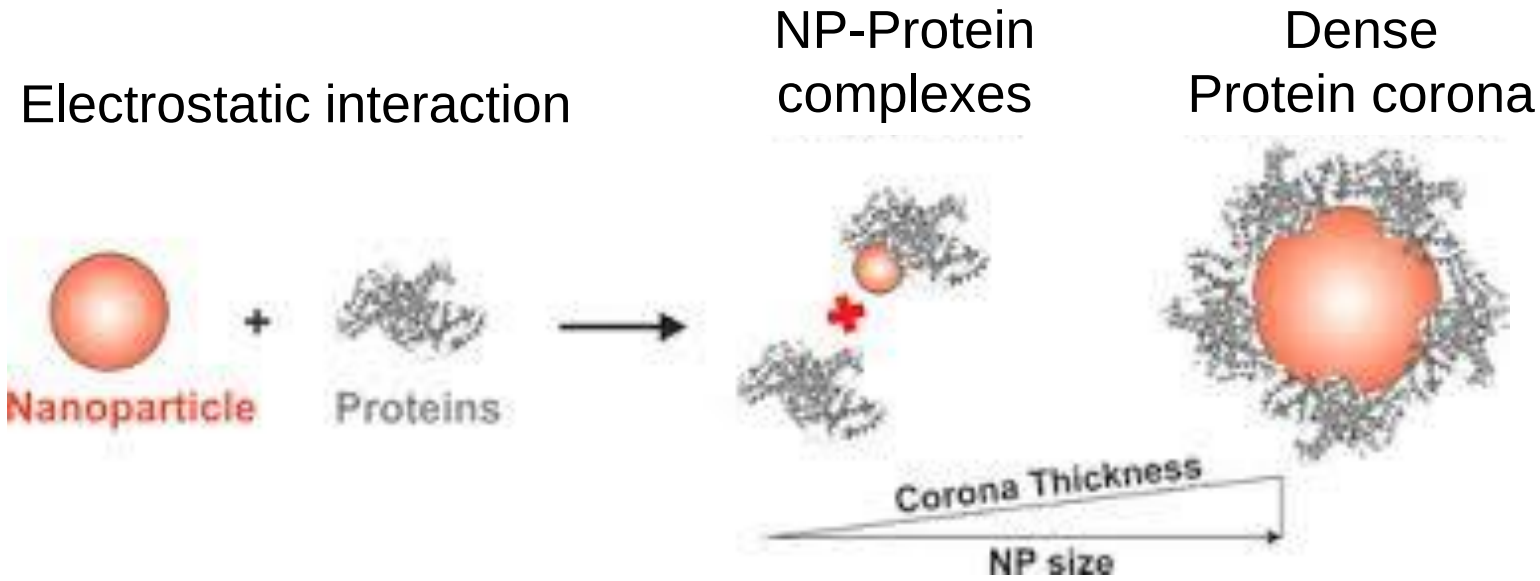
Limitations of Drug Encapsulated Nanoparticle Delivery

- The formation of protein corona



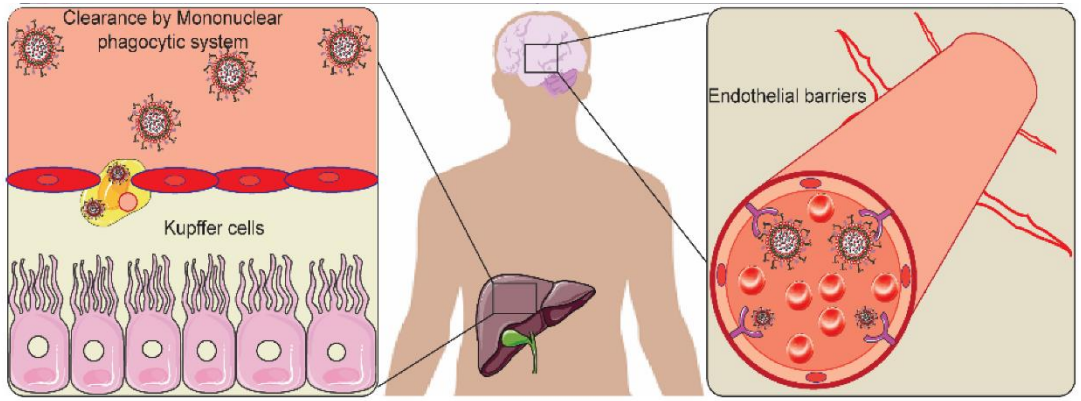
Protein corona composition
- Albumin
- Different fibrinogen types
- Immunoglobulins
- Apolipoproteins
- Apolipoprotein E

Ashkarran, *et al. ACS Pharmacol Transl Sci*. 2024, *7(4)*

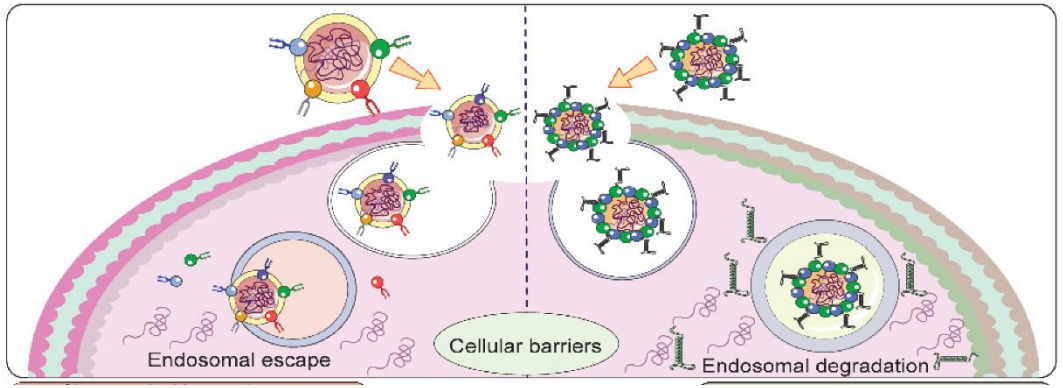
Limitations of Drug Encapsulated Nanoparticle Delivery

The mononuclear phagocytic system (MPS)

1) Clearance by macrophages

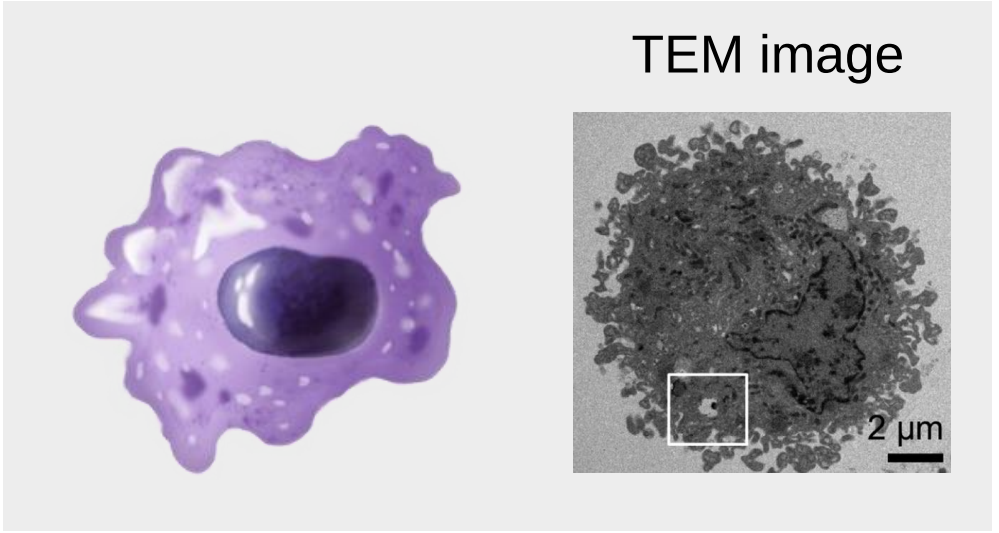


2) Non-specific endocytosis



Irish, et al. Pharmaceutics **2021**, *13*(9), 1433

Macrophages



- Tumor tissue is made of several types of cells such as tumor cell, immune cell, perivascular cell etc.
- Most nanoparticles were taken by tumor associated macrophages

Oh, et al. ACS Nano, 2014, *8*(6), 6232-6241.

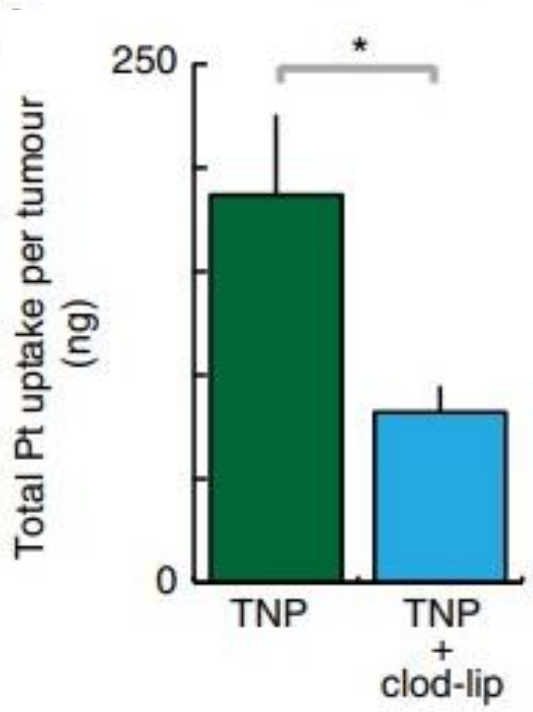
Macrophages is a Key Factor for Nanoparticle Delivery

Control group

No treatment of clodronate liposomes

Experimental group

Treatment of clodronate liposomes



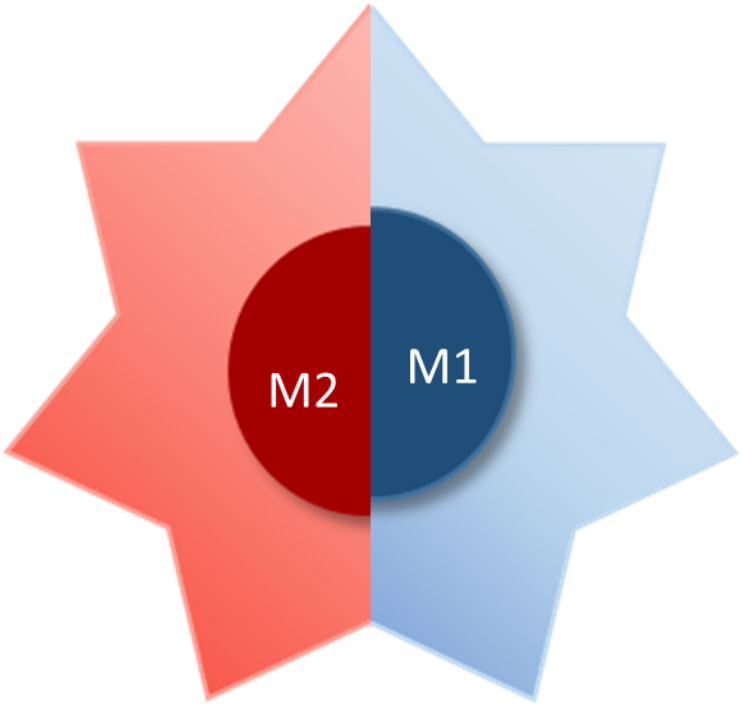
- Clodronate liposomes (clod-lip): drug to deplete macrophage selectively

Miller et al. Nat. comm, 2015, 6, 8692

Macrophage Function

M2
Resolving

- Immunoregulation
- Tissue remodeling
- Tumor promotion



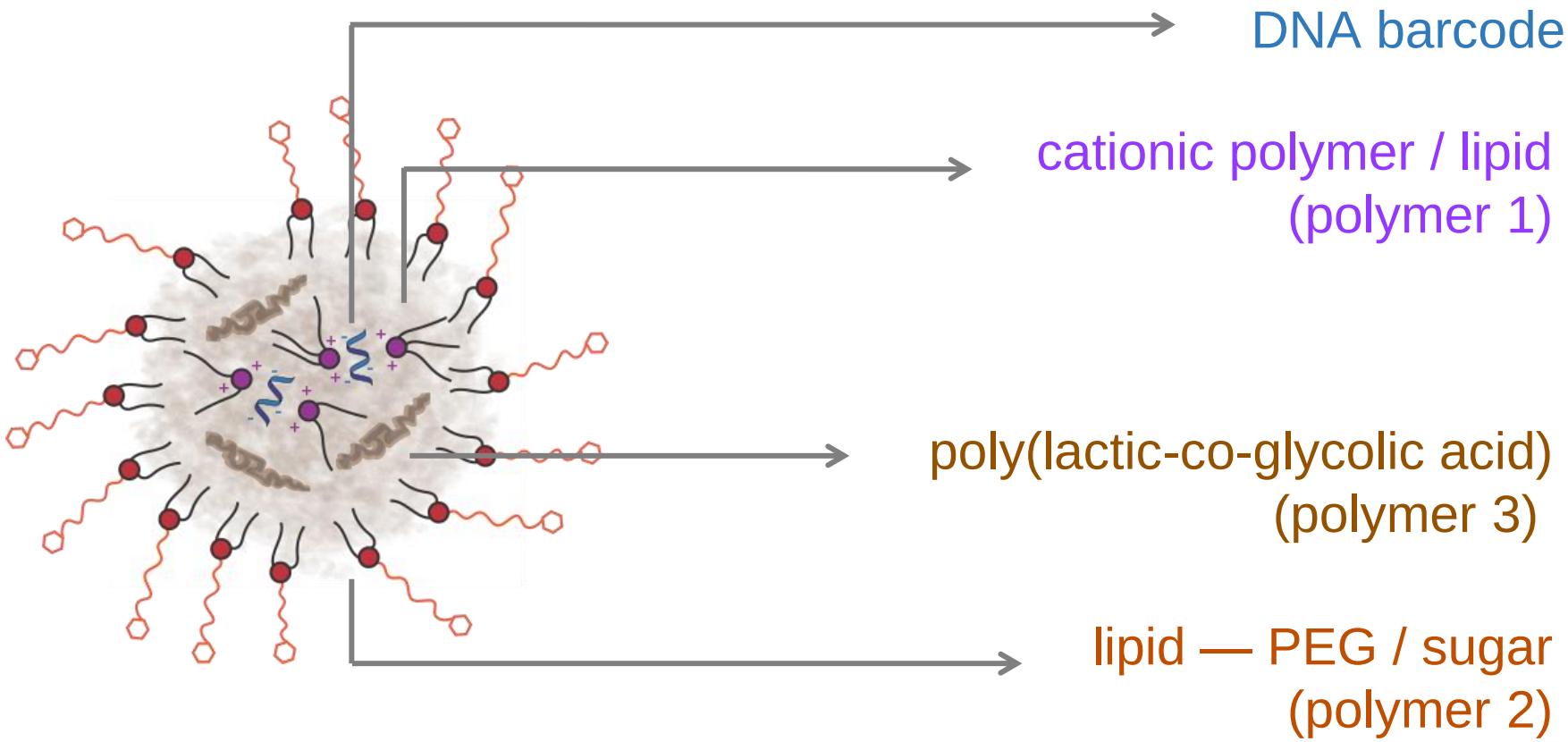
M1
Inflammatory

- Killing of intracellular parasites
- Tissue destruction
- Tumor resistance

Part 1. NANO-SAD for Multiplexed Image Cytometry of Nanoparticle Delivery for Lung Cancer Treatment

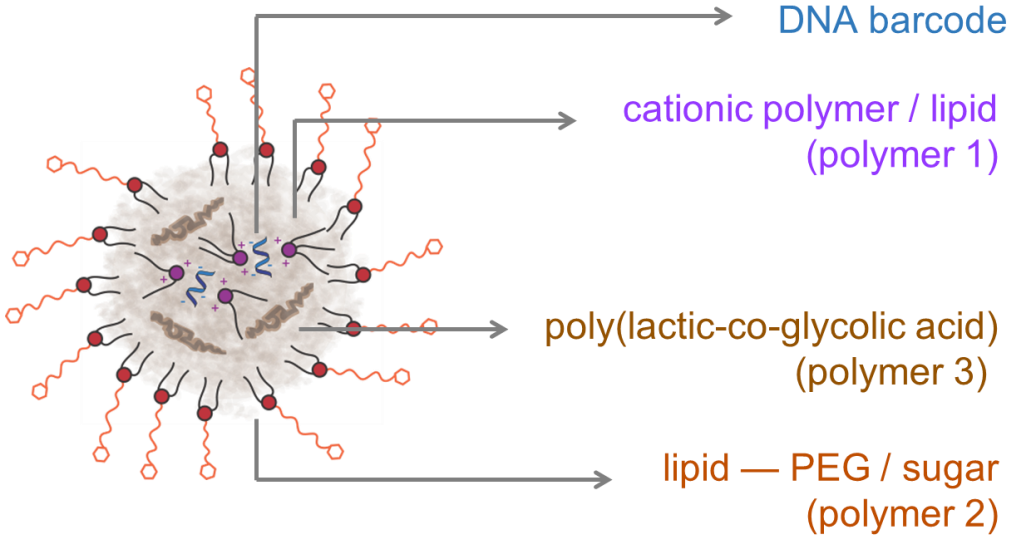
Oh *et al. ACS Nano 2025*

DNA Barcode Encapsulated PLGA Vehicles



- It should be able to represent multiple nanoparticles simultaneously without overlapping with endogenous DNA sequences.

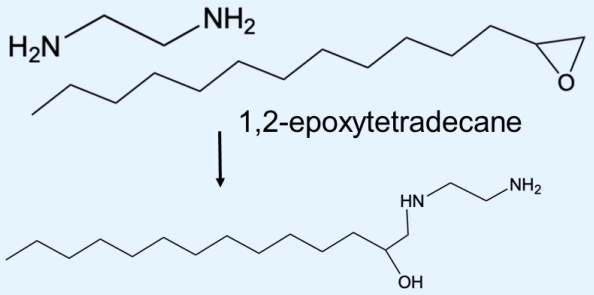
DNA Barcode Encapsulated PLGA Vehicles



Cationic polymer/lipid

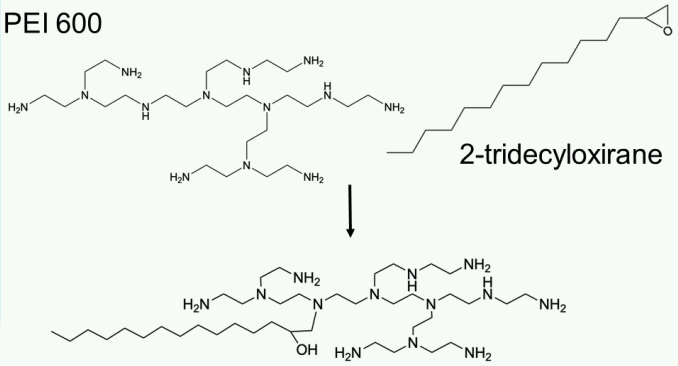
G0-C14

Eethylenediamine



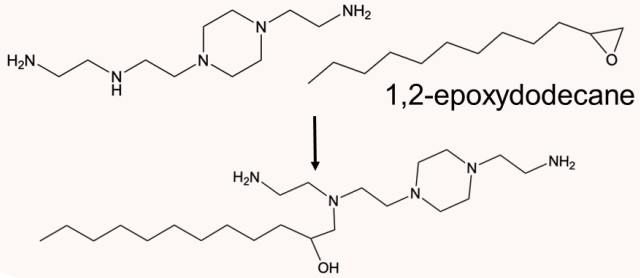
7C1

PEI 600



C12-200

2-(4-{2-[(2-aminoethyl)amino]ethyl}piperazin-1-yl)ethan-1-amine



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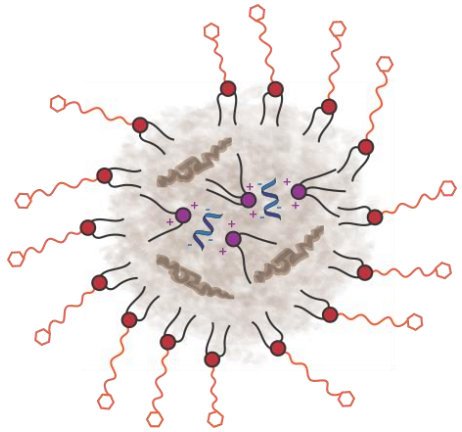
Characterization of DNA Barcode Encapsulated PLGA Vehicles



DNA barcode

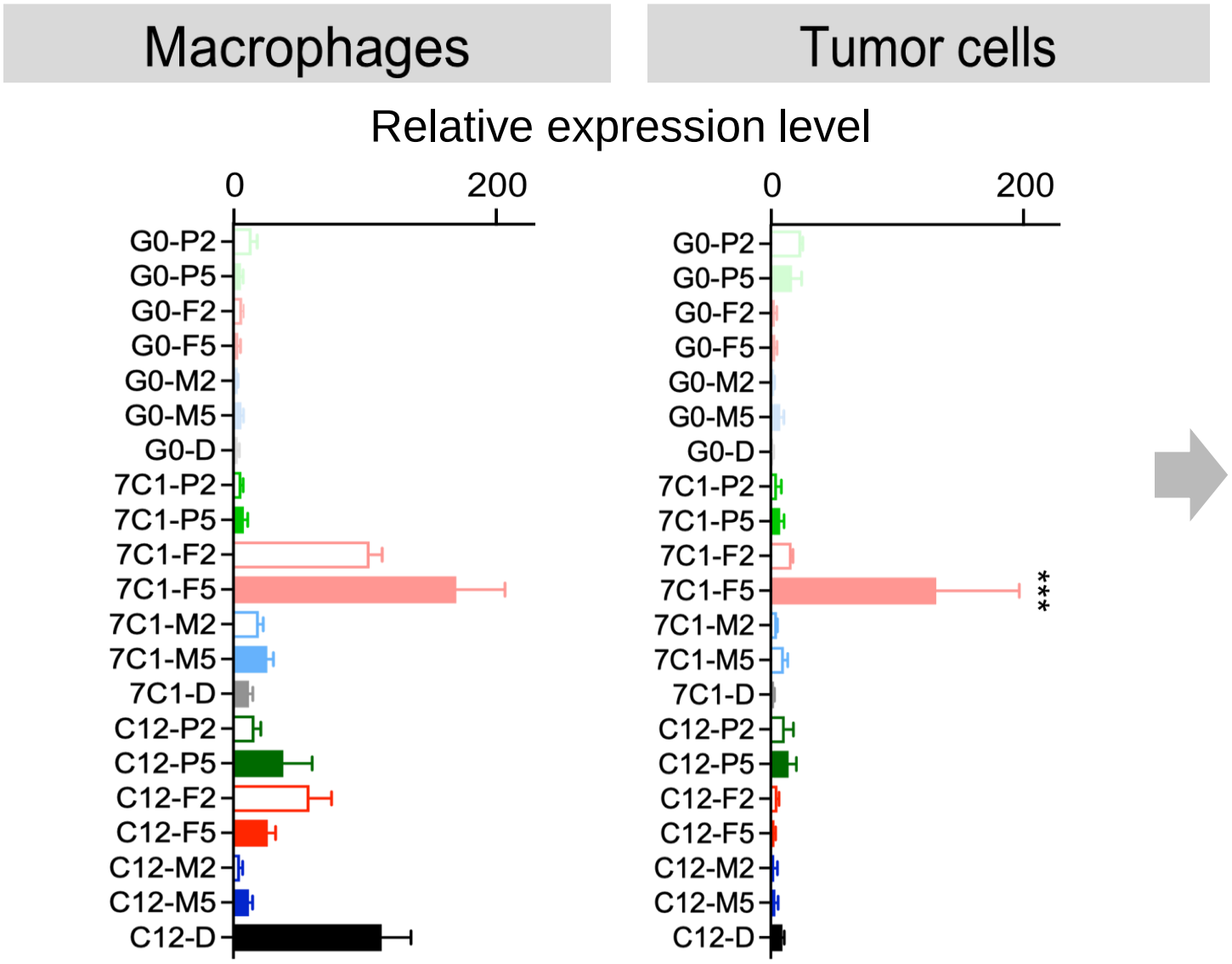
cationic polymer / lipid (polymer 1)

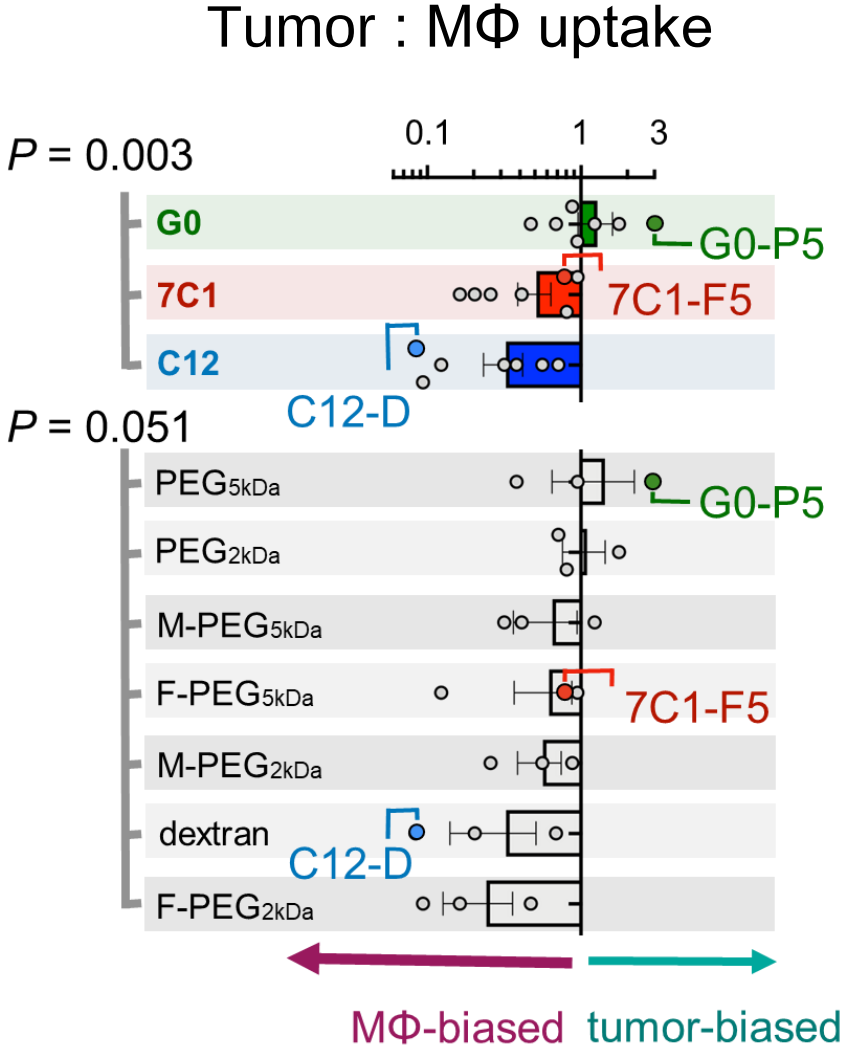
lipid — PEG / sugar (polymer 2)

poly(lactic-co-glycolic acid) (polymer 3)

polymer 1	polymer 3	polymer 2	ZP (mV)	diam. (nm)	PDI	Mϕ uptake	
G0-C14	PLGA	DSPE-PEG$_{2kDa}$	-22	87	0.07	7	12	
		DSPE-PEG$_{5kDa}$	-25	90	0.16	3	9	G0-P5
		DSPE-PEG$_{2k}$-folate	-12	94	0.37	3	2	
		DSPE-PEG$_{5k}$-folate	-12	96	0.26	2	2	
		DSPE-PEG$_{2kDa}$-mannose	-14	91	0.27	2	1	
		DSPE-PEG$_{5kDa}$-mannose	-10	94	0.12	3	4	
		DSPE-dextran	-36	94	0.10	2	1	
7C1	PLGA-COOH	DSPE-PEG$_{2kDa}$	-22	78	0.08	3	3	
		DSPE-PEG$_{5kDa}$	-31	86	0.18	4	4	
		DSPE-PEG$_{2k}$-folate	-19	85	0.02	52	8	
		DSPE-PEG$_{5k}$-folate	-24	80	0.19	84	67	7C1-F5
		DSPE-PEG$_{2kDa}$-mannose	-25	70	0.03	10	3	
		DSPE-PEG$_{5kDa}$-mannose	-20	77	0.08	13	5	
		DSPE-dextran	-27	88	0.19	6	1	
C12-200	PLGA-COOH	DSPE-PEG$_{2kDa}$	-20	85	0.31	8	6	
		DSPE-PEG$_{5kDa}$	-24	88	0.27	19	7	
		DSPE-PEG$_{2k}$-folate	-22	73	0.02	29	3	
		DSPE-PEG$_{5k}$-folate	-19	80	0.10	13	2	
		DSPE-PEG$_{2kDa}$-mannose	-29	79	0.10	3	1	
		DSPE-PEG$_{5kDa}$-mannose	-23	85	0.11	6	2	
		DSPE-dextran	-36	87	0.01	56	5	C12-D

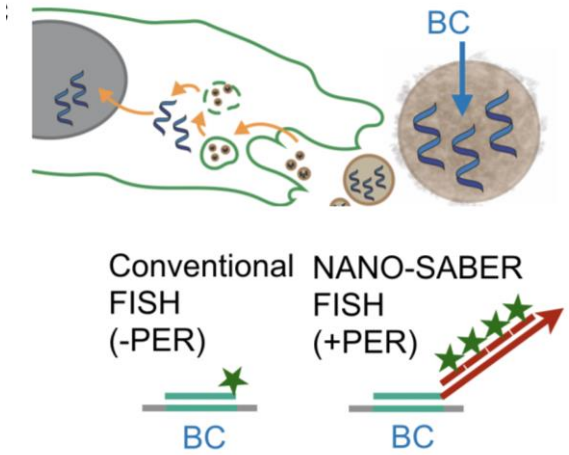
Cell Avidity of DNA Barcode Encapsulated PLGA Vehicles



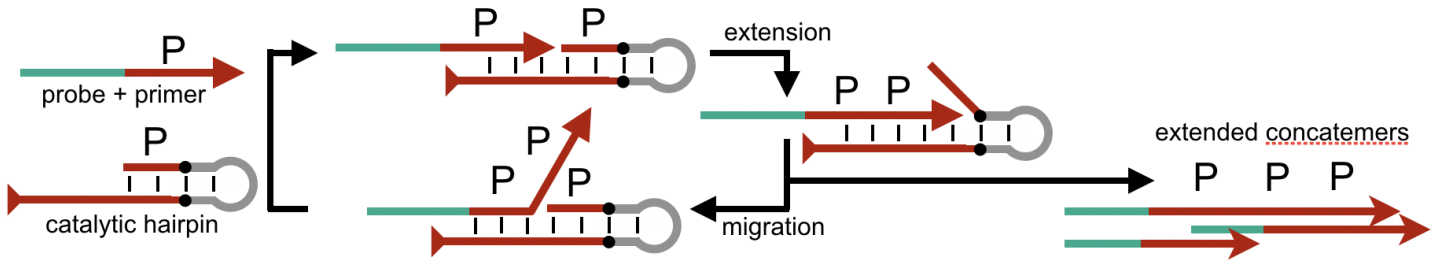


The Strategy of Fluorescence Signal Amplification by DNA Barcodes

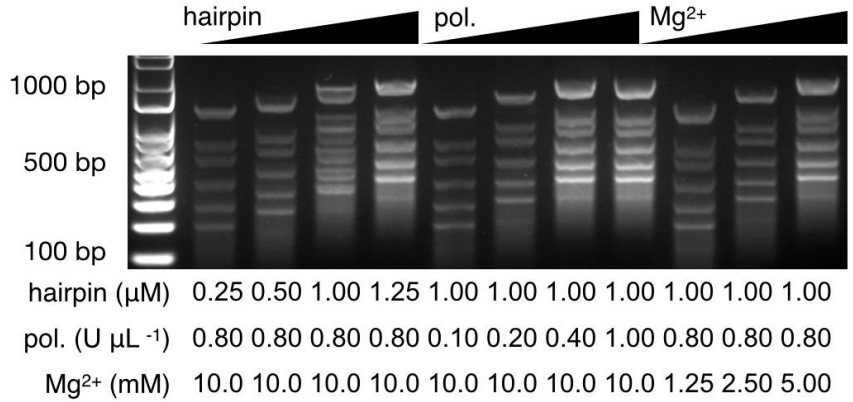
(a)



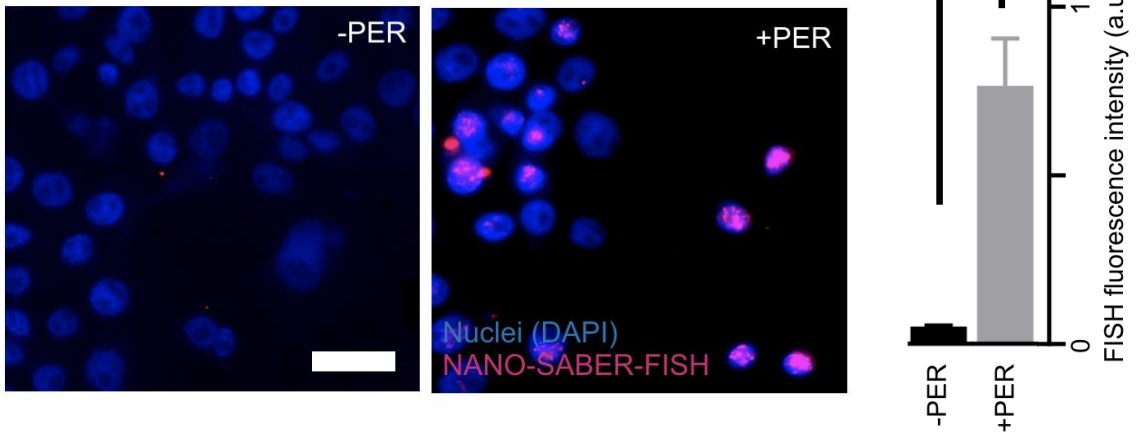
(b) Primer change reaction cycle



(c) Length programmability of PER concatmerers



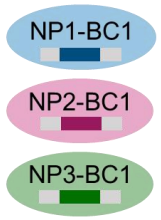
(d) Images to compare the PER function



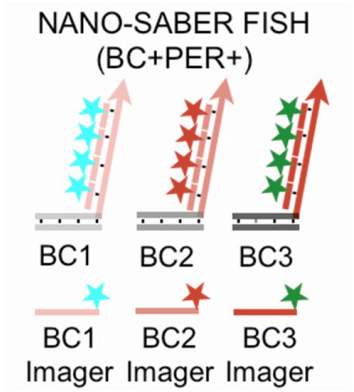
The Strategy for the Spatial Profiling of PLGA nanoparticle vehicles and DNA barcode payloads distribution

Spatial information

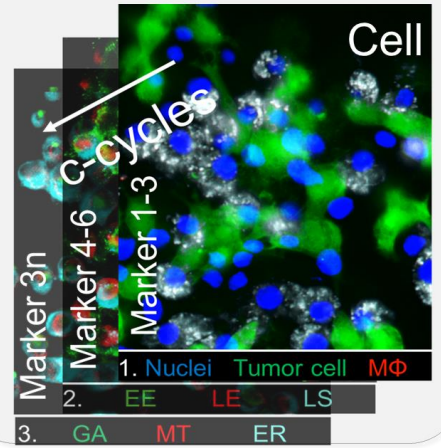
1. PNP vehicles injection

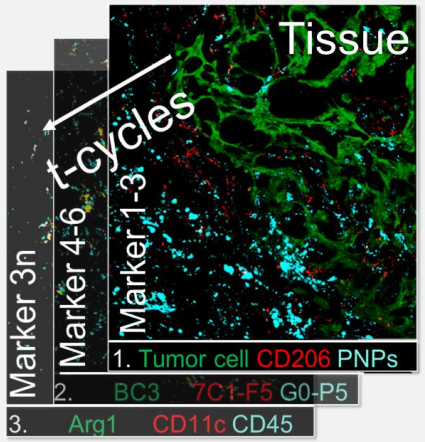


2. Fluorescence signal amplification

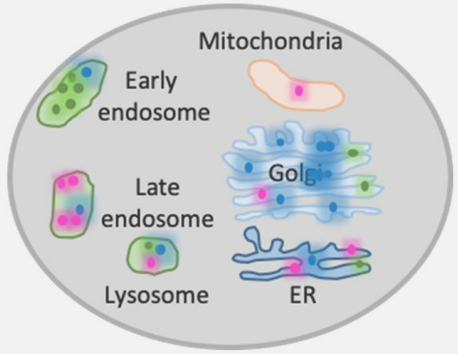


3. Serial IF cycling





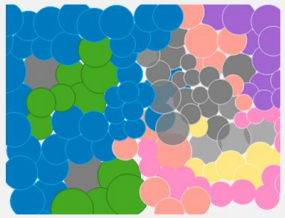
4. Subcellular distribution



Distance

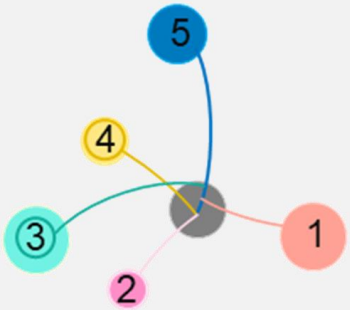
PNPs and BC payloads concentration

5. Phenotype diversity



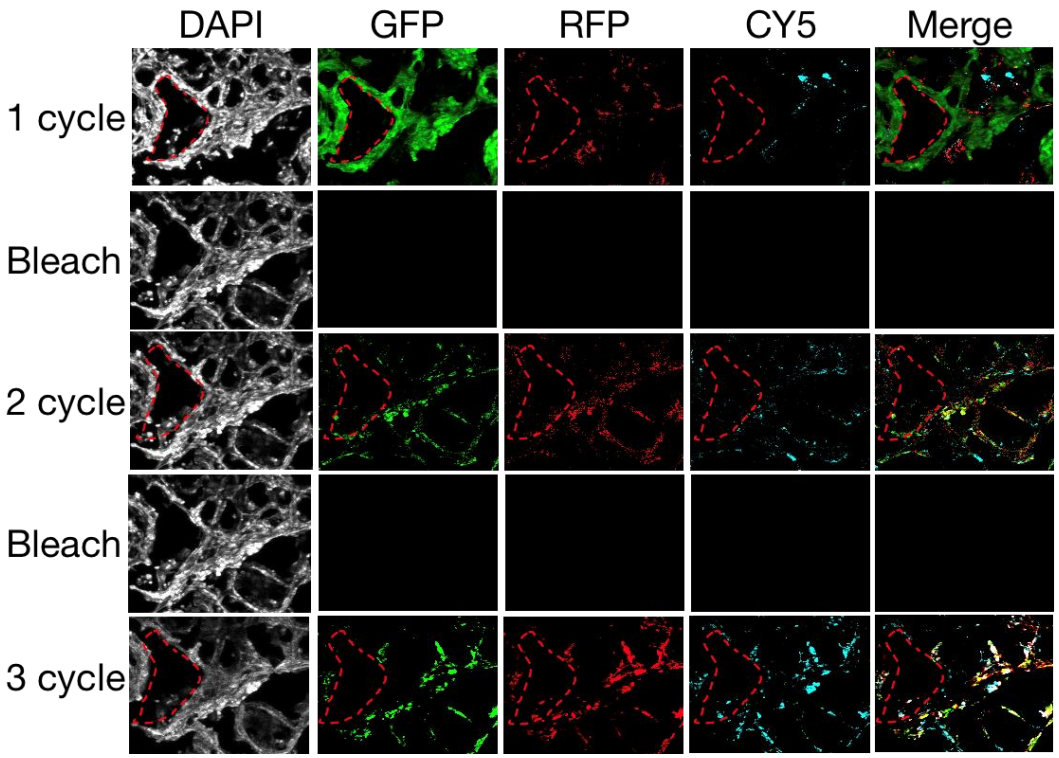
CD206⁺ CD206⁺CD11c⁺
Arg1⁺ Arg1⁺CD11c⁺
CD11c⁺ CD206⁺Arg1⁺CD11c⁺
Tumor CD206⁺Arg1⁺

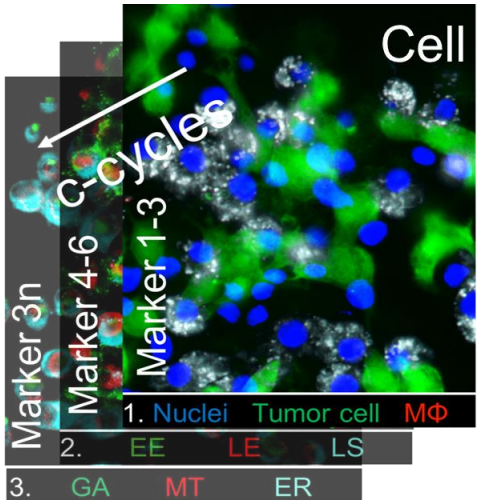
Neighbors



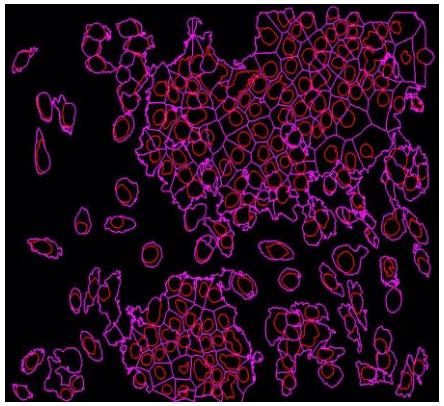
The Process of Multiplexed Imaging Cycling for Tissue

	405	488	546	647
1 cycle	DAPI	GFP	CD206	BODIPY 630
2 cycle		Arg1	PE-CD11c	CD45
3 cycle		G0-P5 (BC1)	7C1-F5 (BC2)	C12-D (BC3)



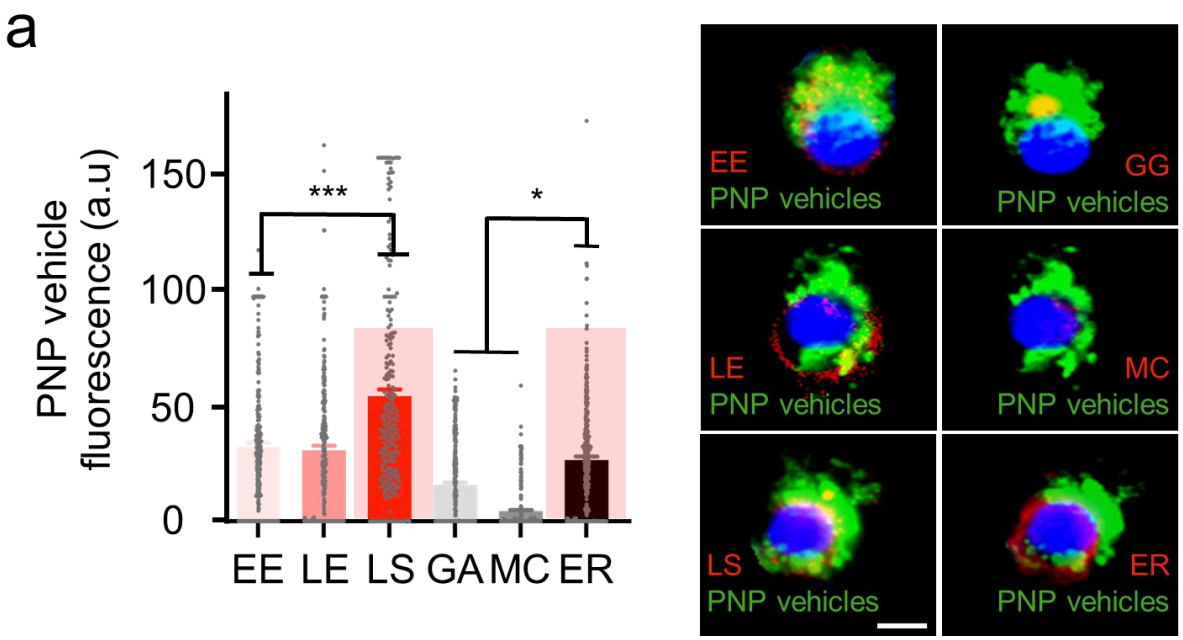


Segmented

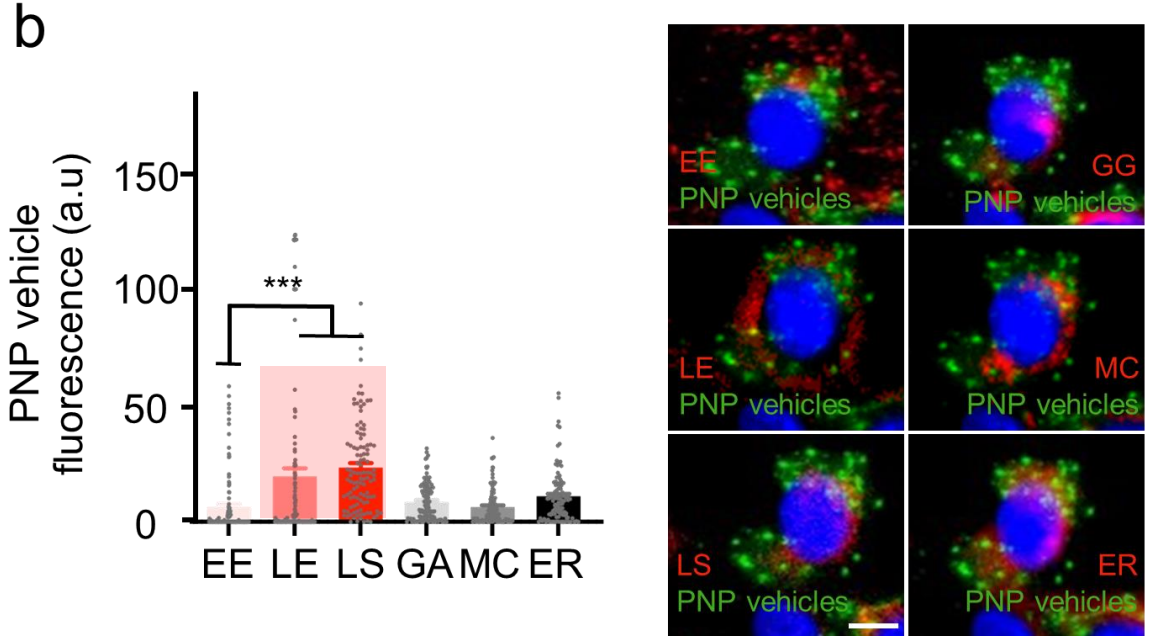


Intracellular Distribution of PNP Vehicles (*In Vitro*)

Macrophage (Raw264.7)



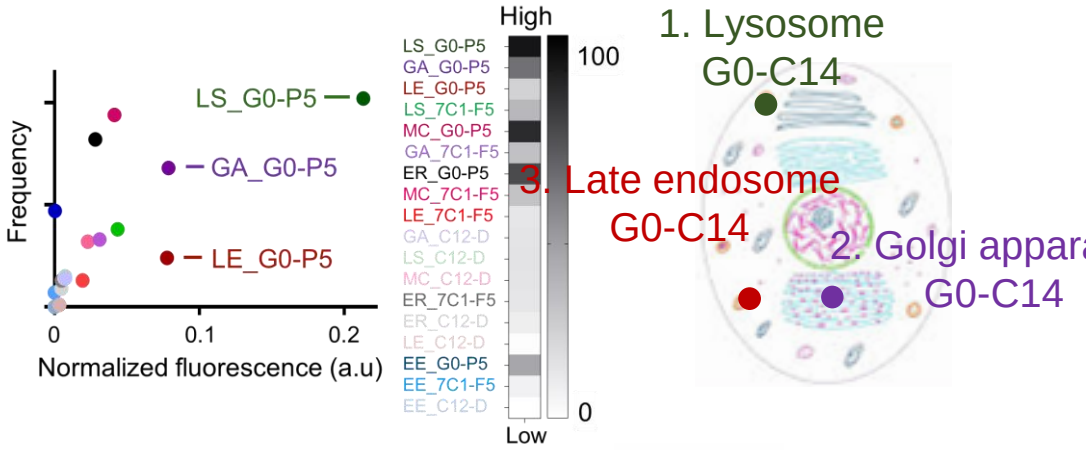
Tumor cell (KP1.9)

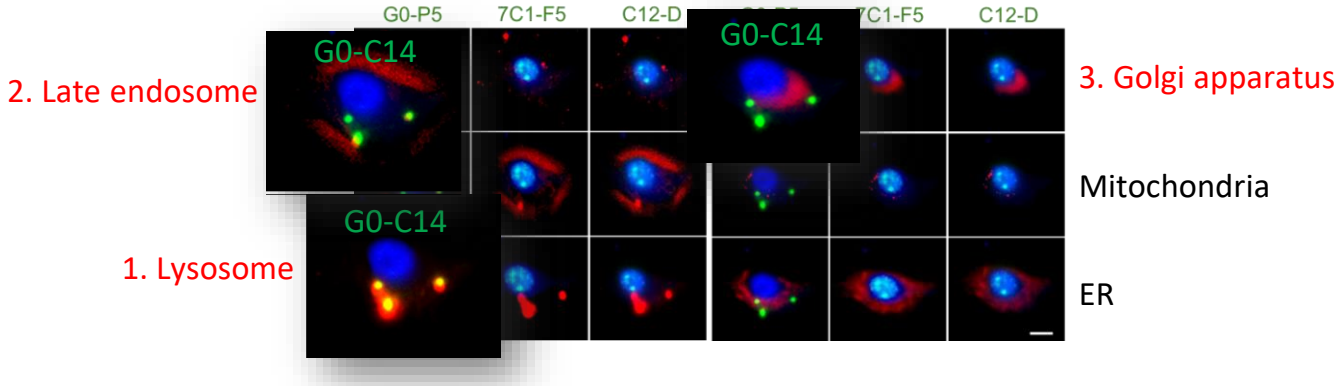


- Macrophages: PLGA nanoparticles (PNPs) were distributed in lysosome (LS) and ER
- Tumor cells: PNPs were positioned in late endosome (LE) and lysosome (LS)

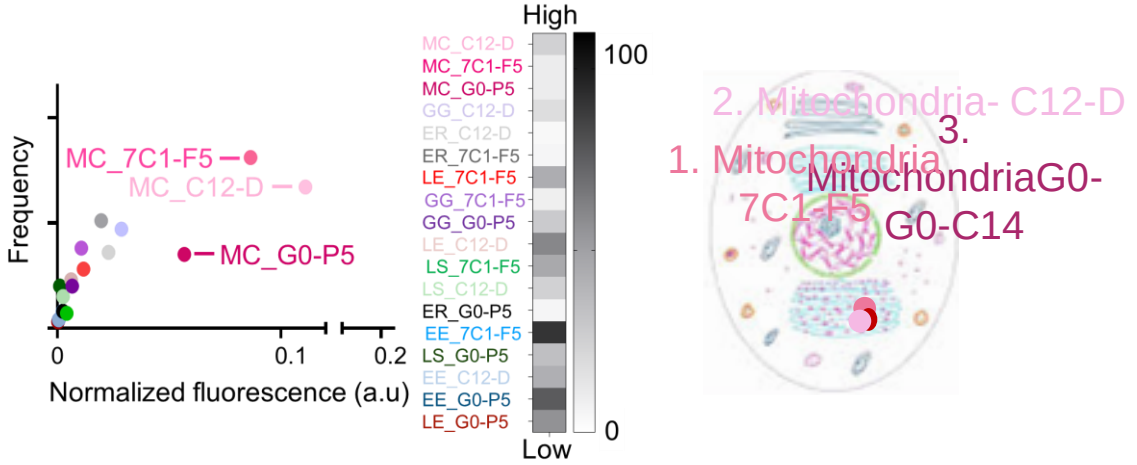
Intracellular Distribution of DNA Barcode Payloads (*In Vitro*)

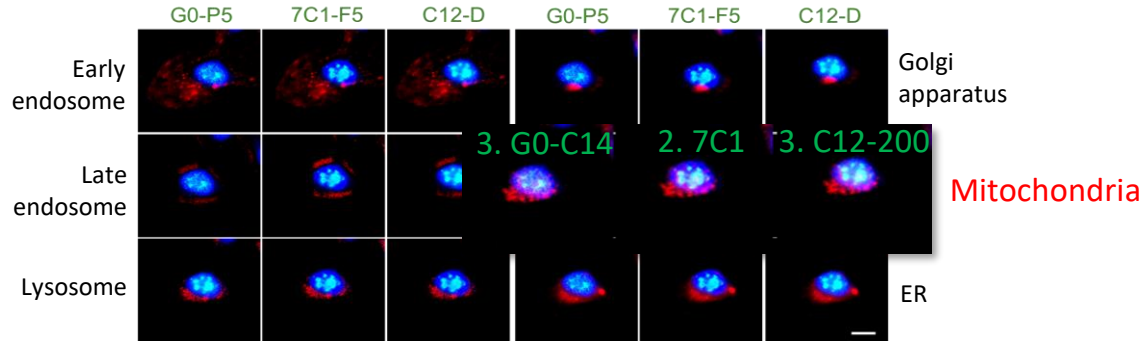
Macrophage (Raw264.7)





Tumor cell (KP1.9)

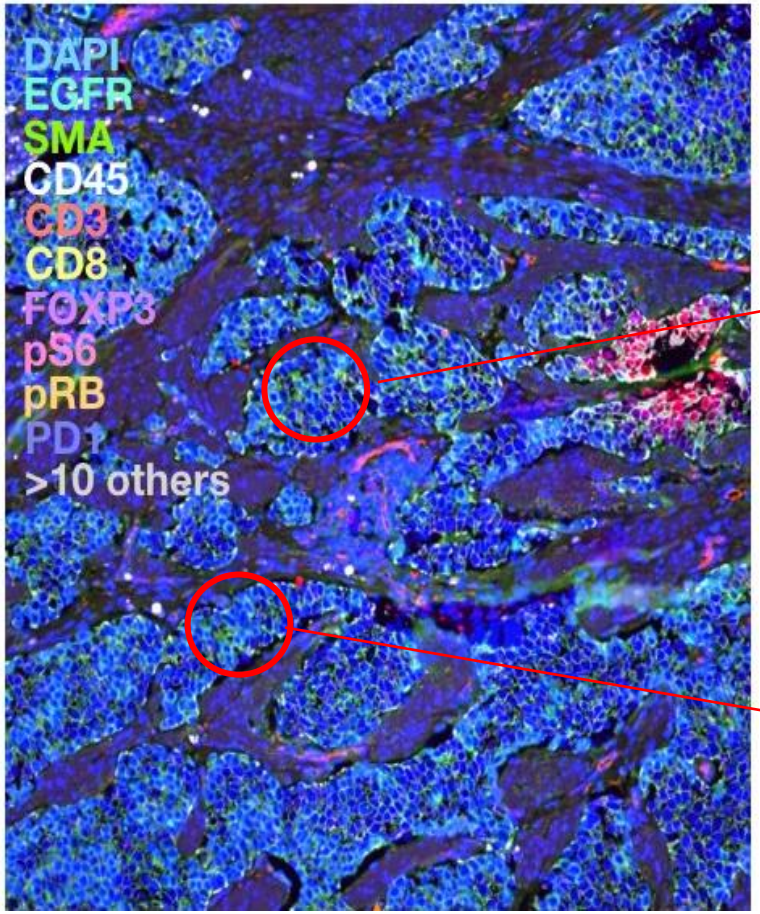




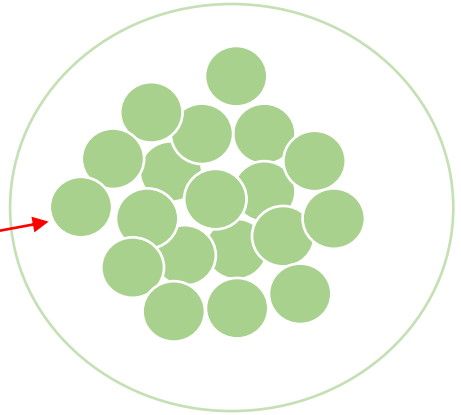
- Macrophages: G0-C14 cationic lipids were distributed in lysosome (LS), Golgi (GA), late endosome (LE)
- Tumor cells: all cationic lipids were located in mitochondria (MC)

The Diversity of Macrophages in Microenvironment

Tissue image



Only tumor cells population

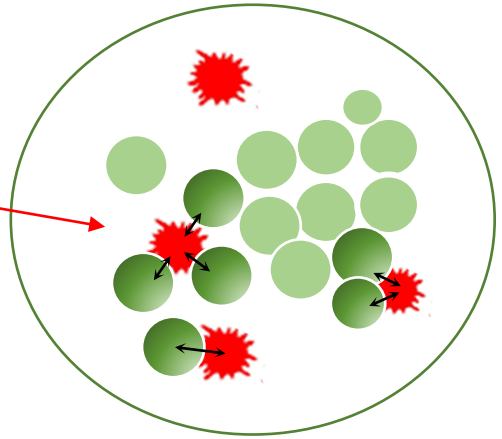


Tumor cell with the same types of antibodies or the same concentration of antibodies

Tumor cell with various types of antibodies or the different concentration of antibodies

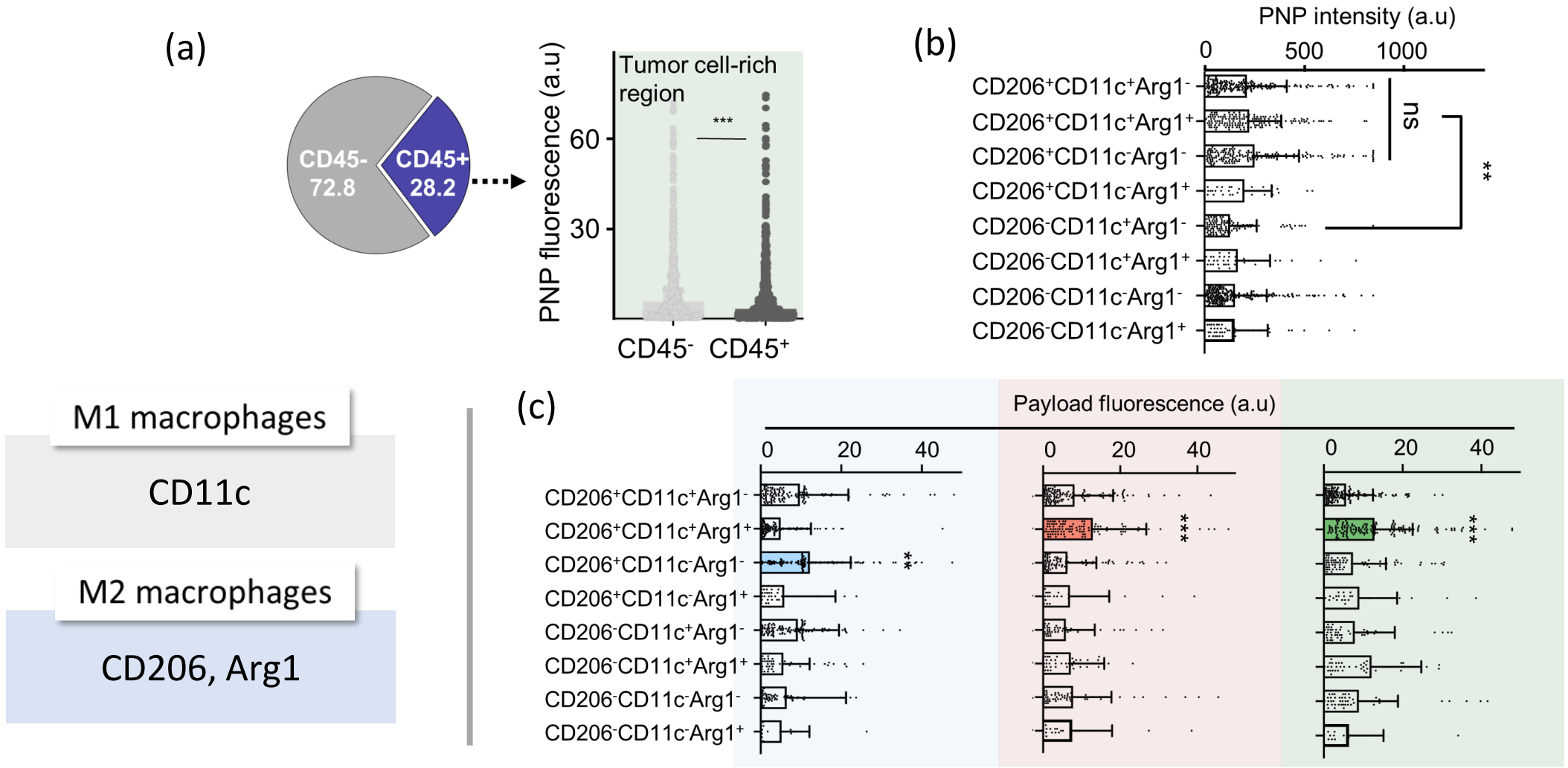
Macrophages

Tumor cells with macrophages



Boibessot *et al, Scientific Reports (2021)*

Intercellular Distribution of PNP Vehicles and BC Payloads

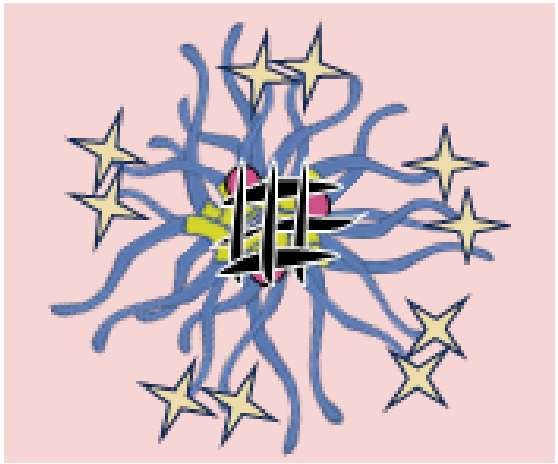


- G0-P5 (BC1) was likely more accumulated with the $CD45^{+}CD206^{+}CD11c^{-}Arg1^{-}$subpopulation
- 7C1-F5 (BC2) and C12-D (BC3) were taken up by $CD45^{+}CD206^{+}CD11c^{+}Arg1^{+}$ subpopulation

Part 2. The Spatial Distribution of Nanoparticles Depends on The Microenvironment between Tumor-Associated Macrophages and Tumor Cells

Investigation of redistribution of sIPNs between macrophages and tumor cells

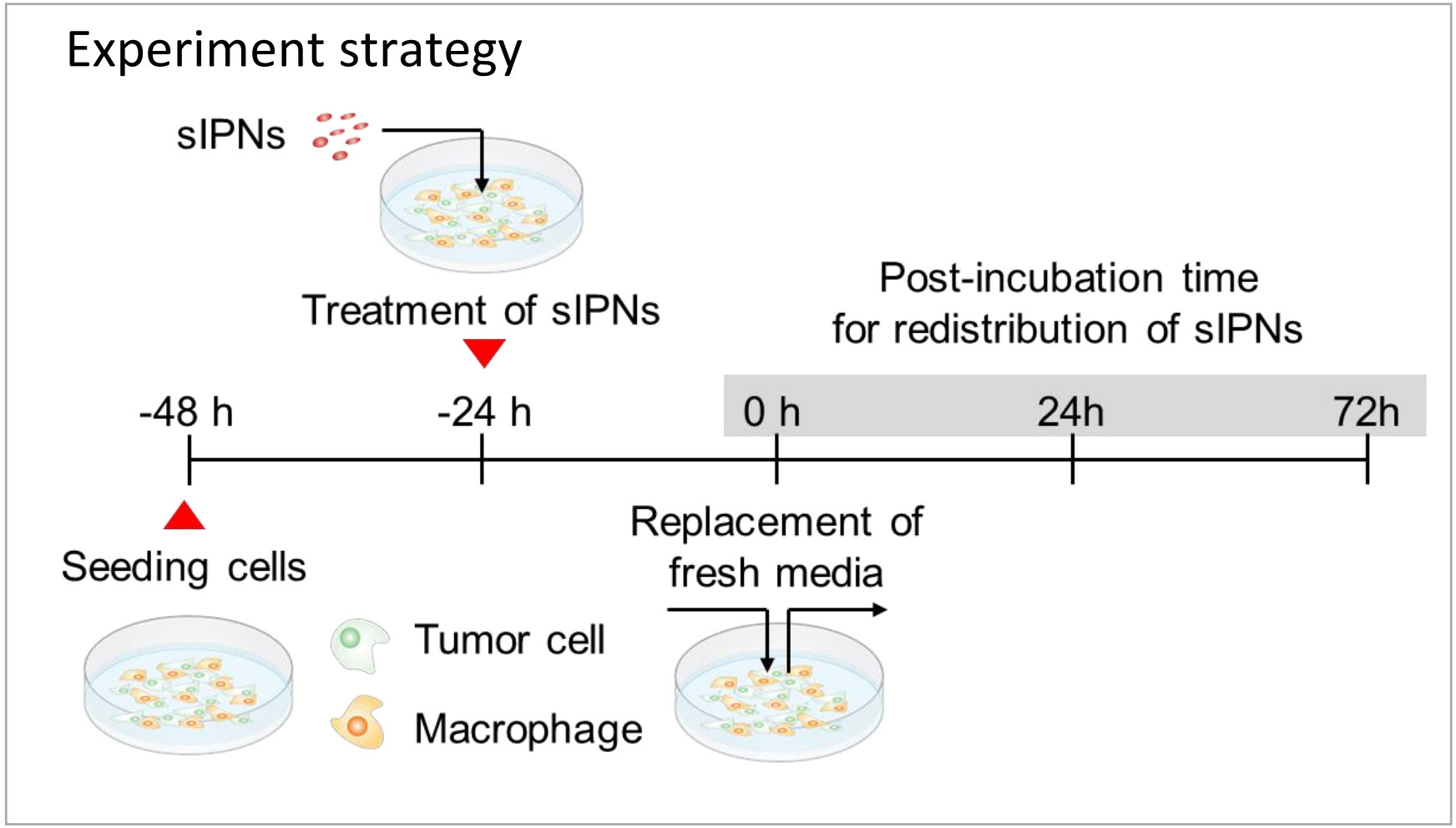
Semi-interpenetrating polymer network (sIPN)



F127

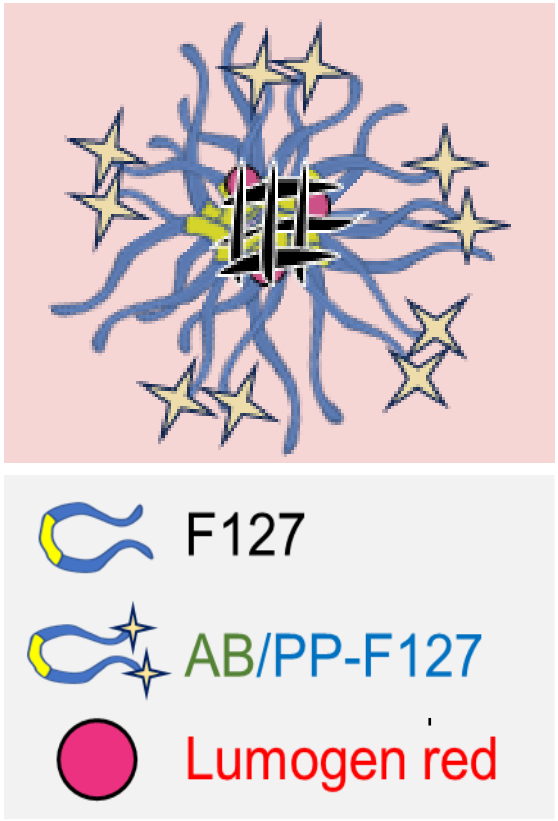
AB/PP-F127

Lumogen red

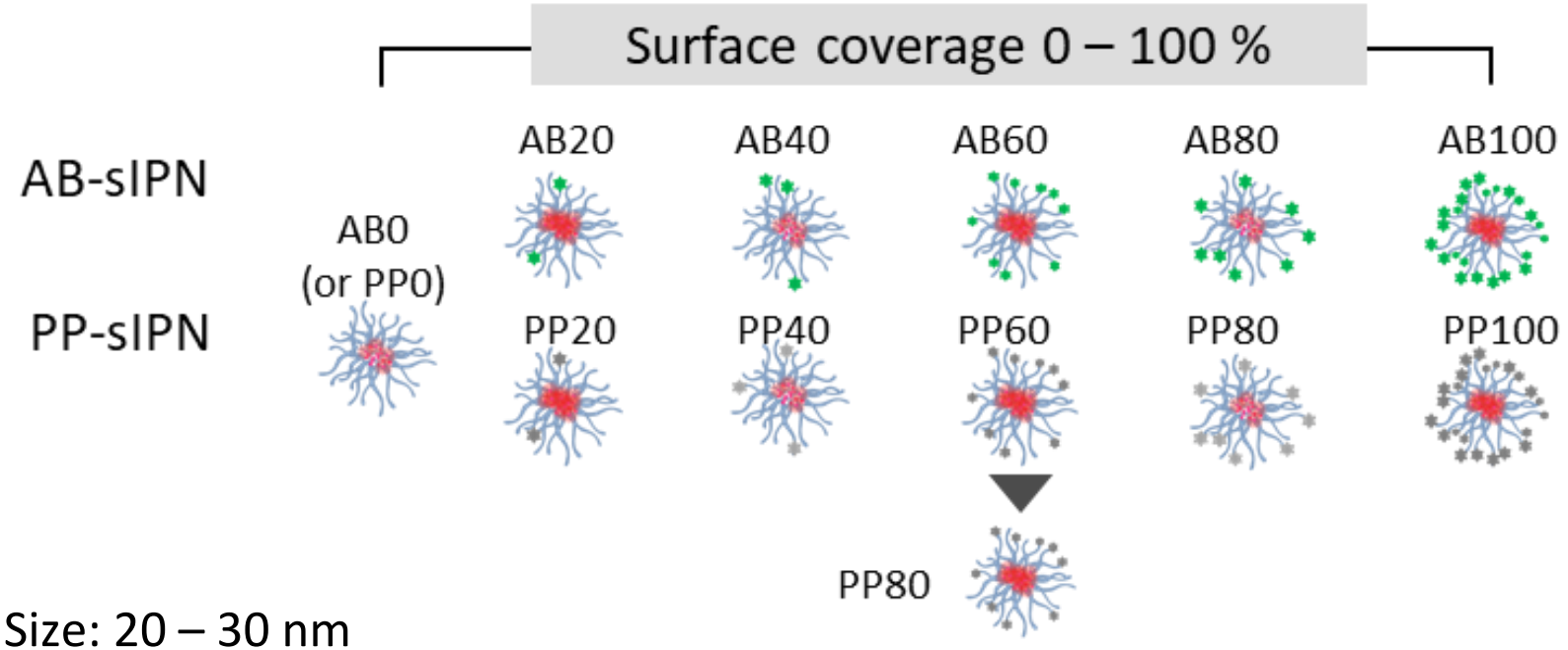


Investigation of redistribution of sIPNs between macrophages and tumor cells

Semi-interpenetrating polymer network (sIPN)



NP library

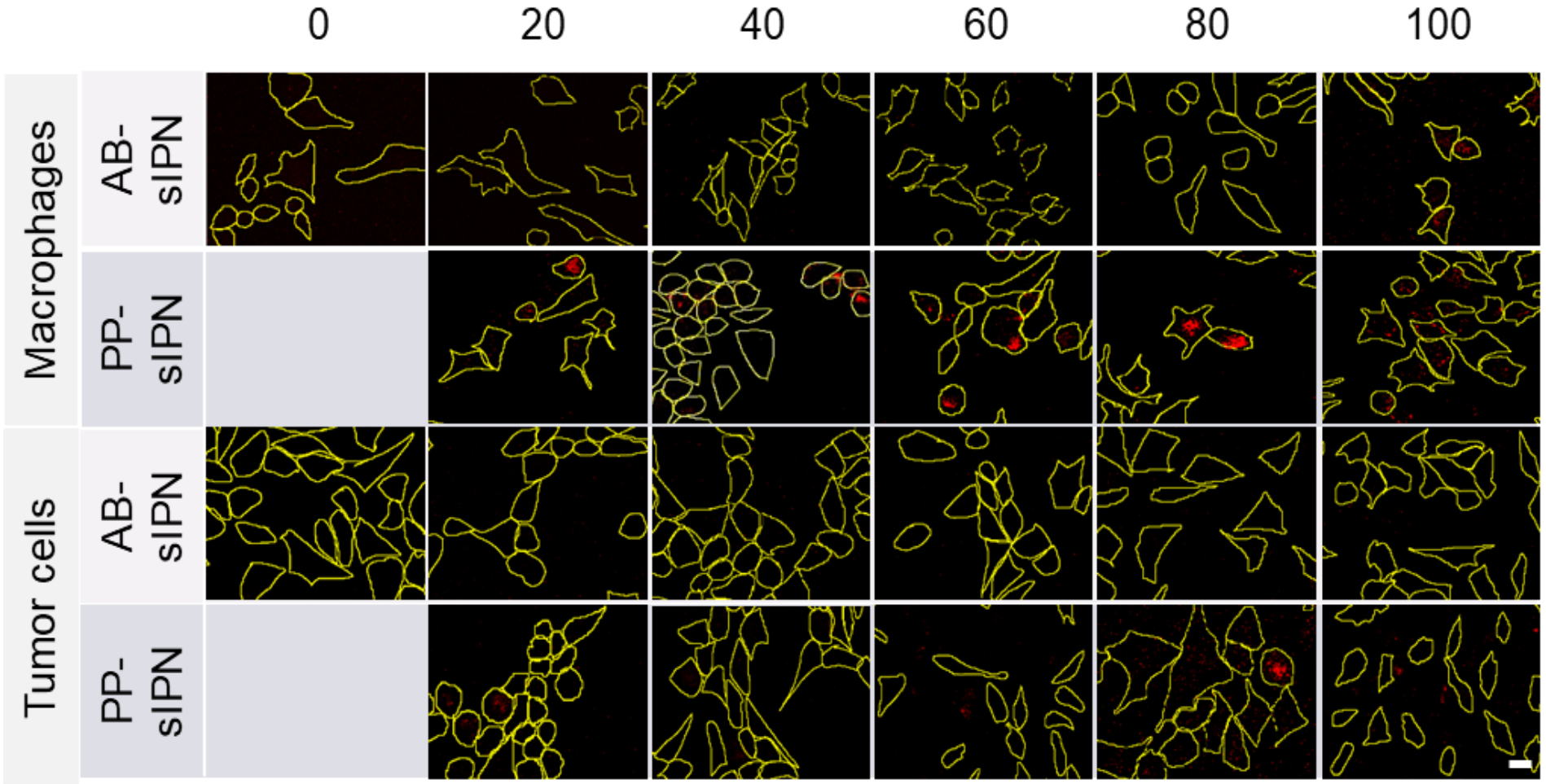


Size: 20 – 30 nm

Surface charge: AB-sIPN (-2.5 – 3.2)

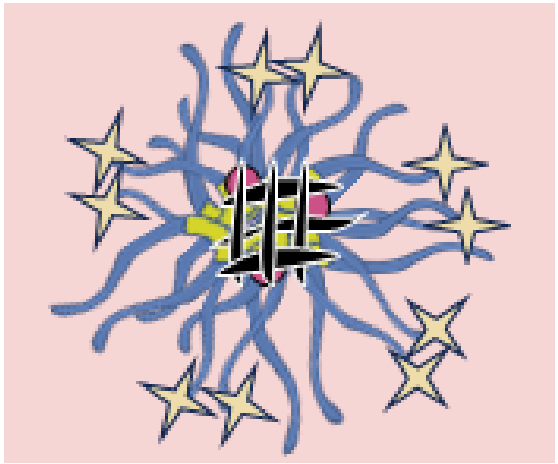
PP-sIPN (-10.0 - -2.5)

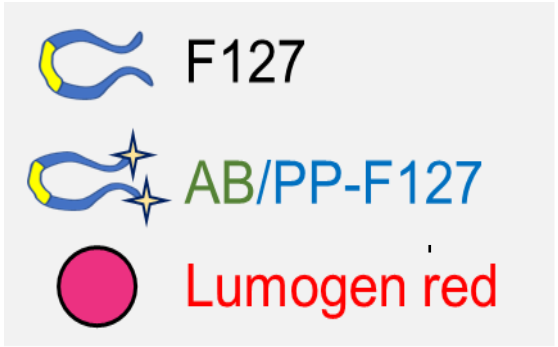
Segmentation of fluorescence images



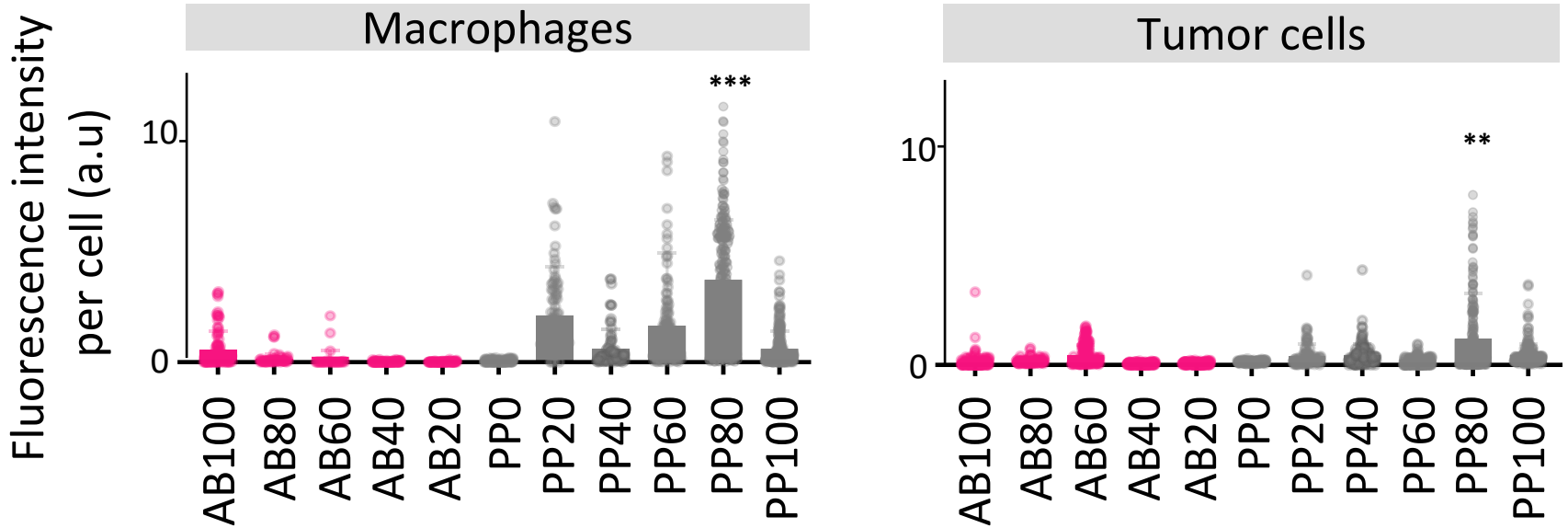
Uptake efficiency of sIPNs in macrophages and tumor cells

Semi-interpenetrating polymer network (sIPN)





Uptake efficiency of sIPN



- PP80-sIPNs were well taken up by tumor cells and macrophages

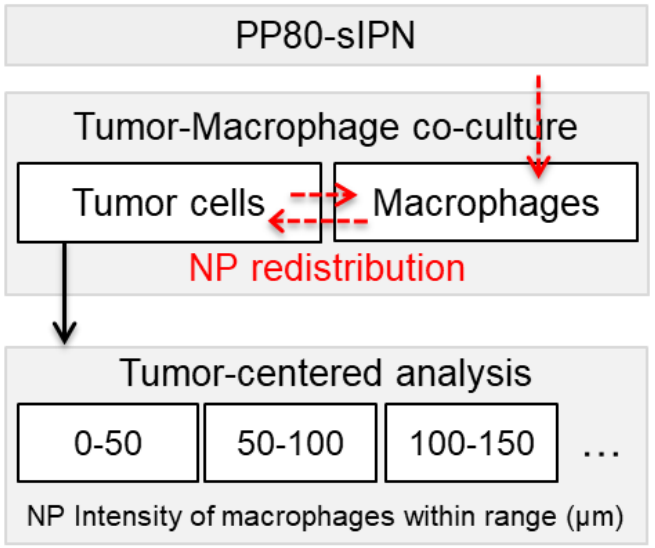
The strategy for the spatial profiling PP80-sIPN

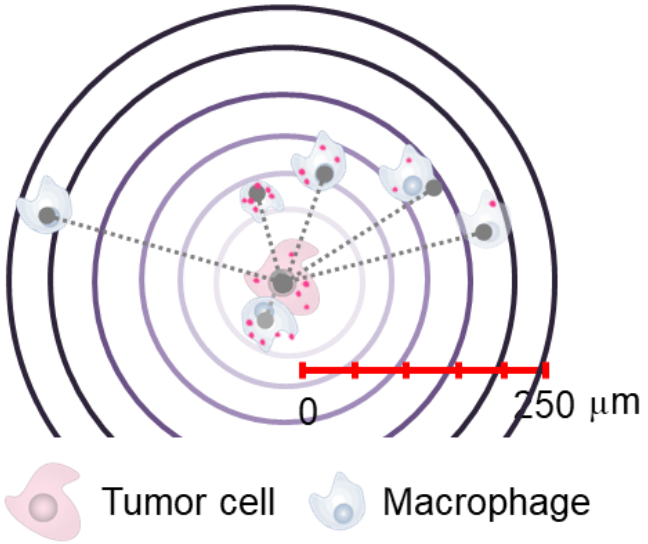
Objective

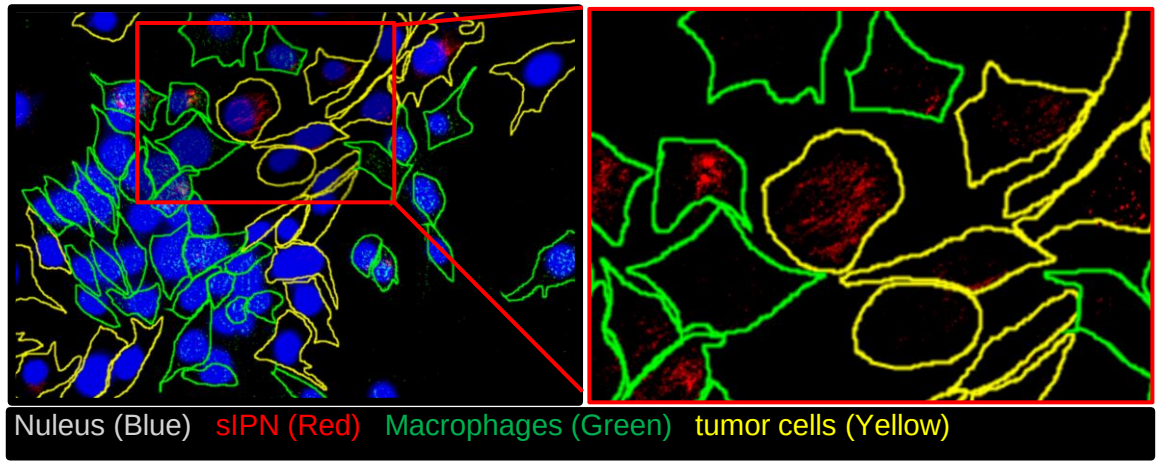
To explore whether sIPN is the key factor driving the most significant migration of tumor cells into macrophages

Factors to be considered

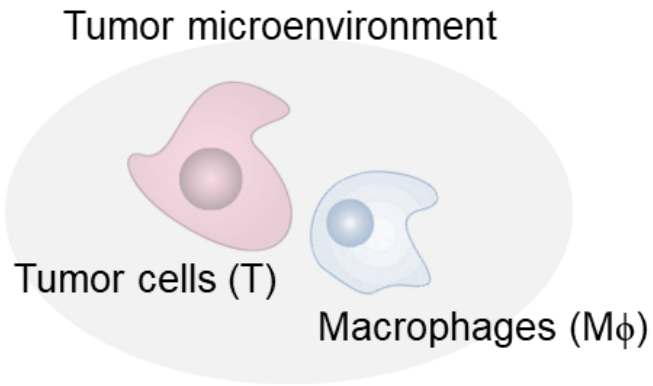
(1) the various compositions of macrophages and tumor cells,
(2) the intercellular distance between tumor cells and tumor neighboring TAMs,
(3) the post-incubation time after replacing the cell culture media without nanoparticles
(4) the concentration of TAM-internalized sIPNs (self-assembled injectable polymeric nanoparticles) in proximity to tumor cells
(5) the number of sIPN-laden TAMs surrounding each tumor cell

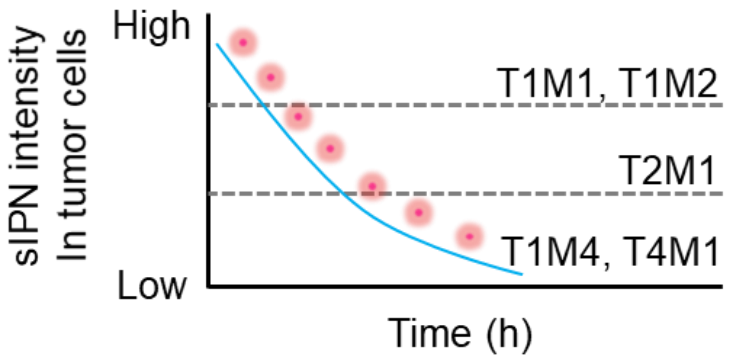


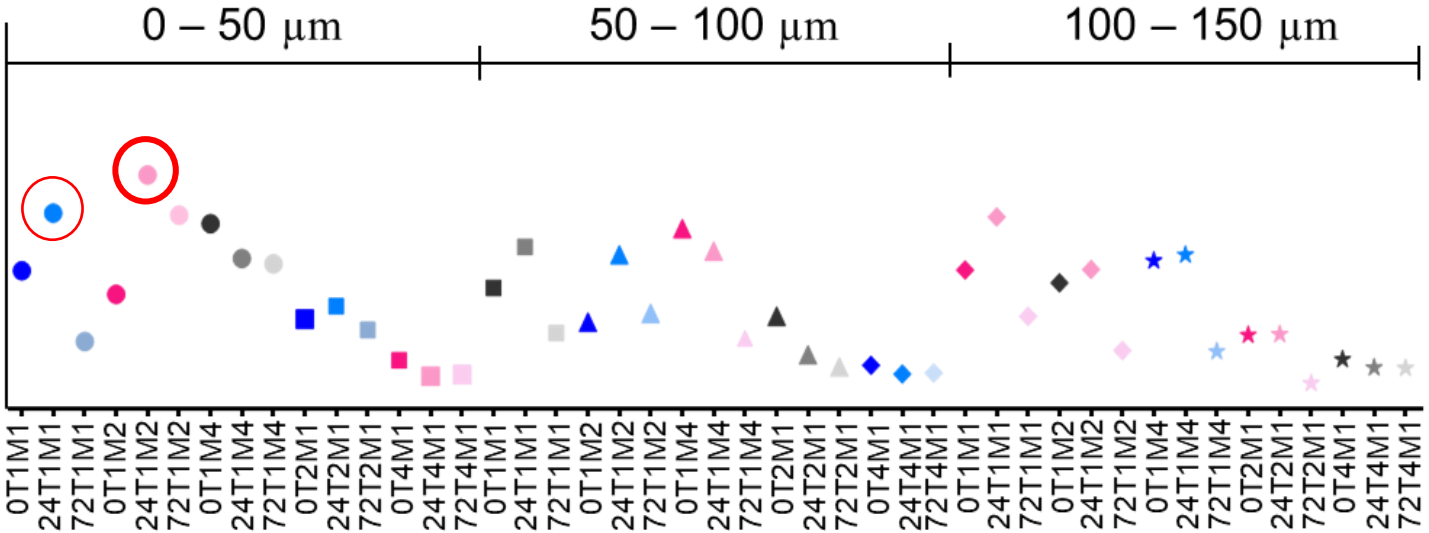




Comparative ranking of tumor cell sIPN intensity across all experimental conditions







- **The composition of tumor cells and macrophages** and **post-incubation time** are one of the critical factors in enhancing NP transfer to target cells.

Conclusions

- **Barcoded LNP Screening Platform Enables High-Throughput In Vivo Profiling**

The use of barcoded LNPs allows simultaneous, multiplexed analysis of multiple formulations in vivo. This platform enables rapid and quantitative evaluation of biodistribution and cell-type specificity, providing a powerful tool for the development of next-generation, precision-targeted LNP therapeutics.

- **LNP Subtypes Influence Subcellular Localization**

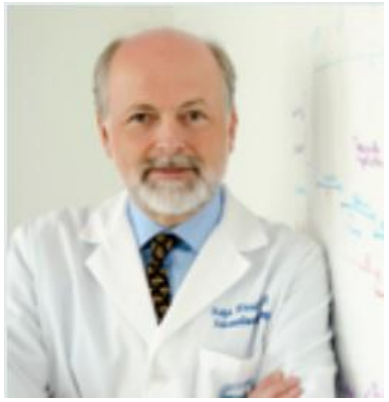
Beyond cellular uptake, different LNP formulations exhibit distinct subcellular localization patterns, including trafficking to the endoplasmic reticulum, lysosomes, or endosomes.

- **LNP Composition Enables Cell-Type Specific Targeting**

The study demonstrates that the type of ionizable lipid used in lipid nanoparticles (LNPs) determines their cellular tropism. Specific formulations preferentially target immune cells such as tumor-associated macrophages (TAMs) and dendritic cells, while others favor uptake by cancer cells.

Support

Harvard Medical School-Massachusetts Medical School
Center for Systems Biology



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Q & A