Electronic Supplementary Material (ESI) for Nanoscale.

PExM: Polyplex Expansion Microscopy for cell trafficking studies

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Supplementary information

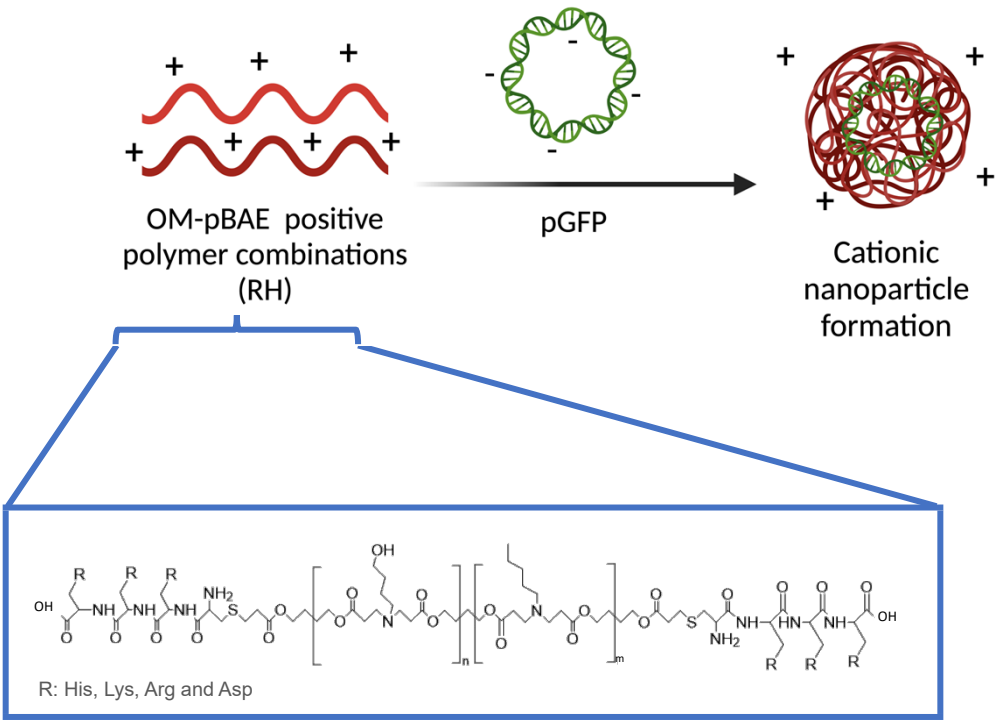

Figure S1. Poly(β-amino esters) nanoparticles components and their chemical structure.

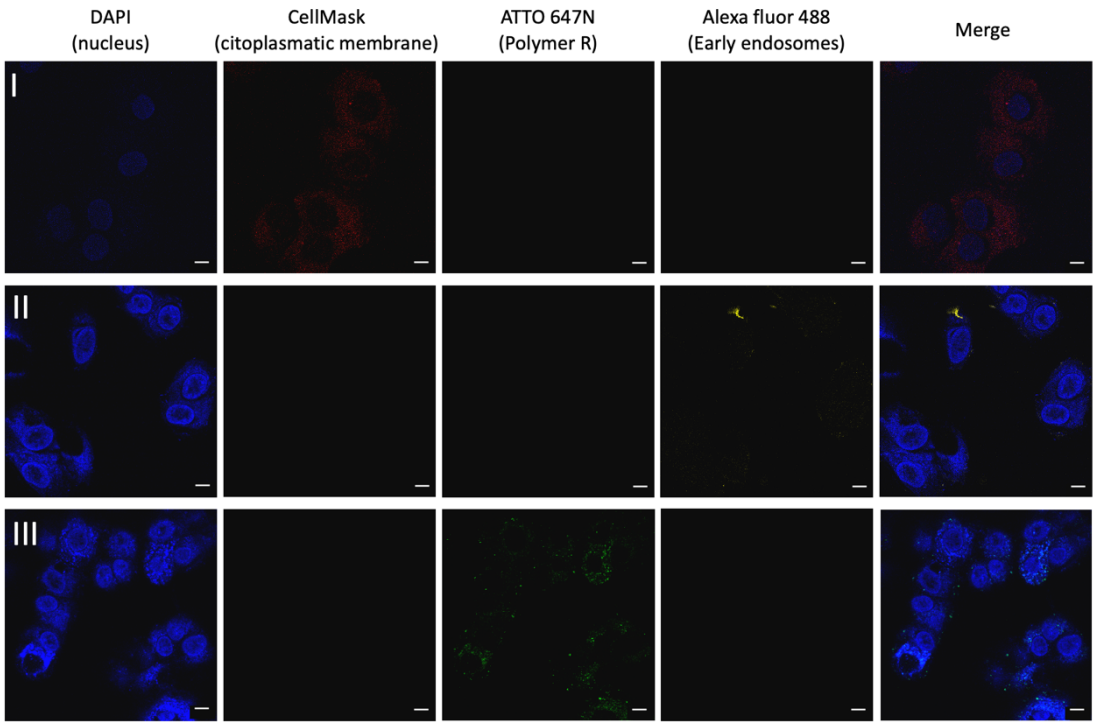

Figure S2. Fluorophore controls for PExM. A549 cells were transfected, using R constructs, after 2 hours of expansion**.** Images taken by a 40X confocal microscope objective. Scale bar: 20 µm. I) Cell mask control. II) Alexa fluor 488 control. III) Atto647N control. DAPI channel is shown in blue, CellMask in red, Atto647N in green, Alexa fluor 488 in yellow and the last channel merge the previous channels. Scale bar 20µm and 40X objective.

Table S1. Labels used for the PNExM protocol, their labelling target, incubations and concentrations.

Label	Attached organelle/molecule	Concentration (ug/mL)	Incubation time (h)	Ex/Em maxima (nm)
DAPI	Nuclei	5	0,3	358/461
Rab5	Early endosomes	5	1	-
AF488	Secondary Ab from Rab 5	5	0,5	488/525
CellMask Orange	Plasmatic membrane	20	0,3	554/567
Atto 647N	Polymeric Nanoparticles	150	12	647/663

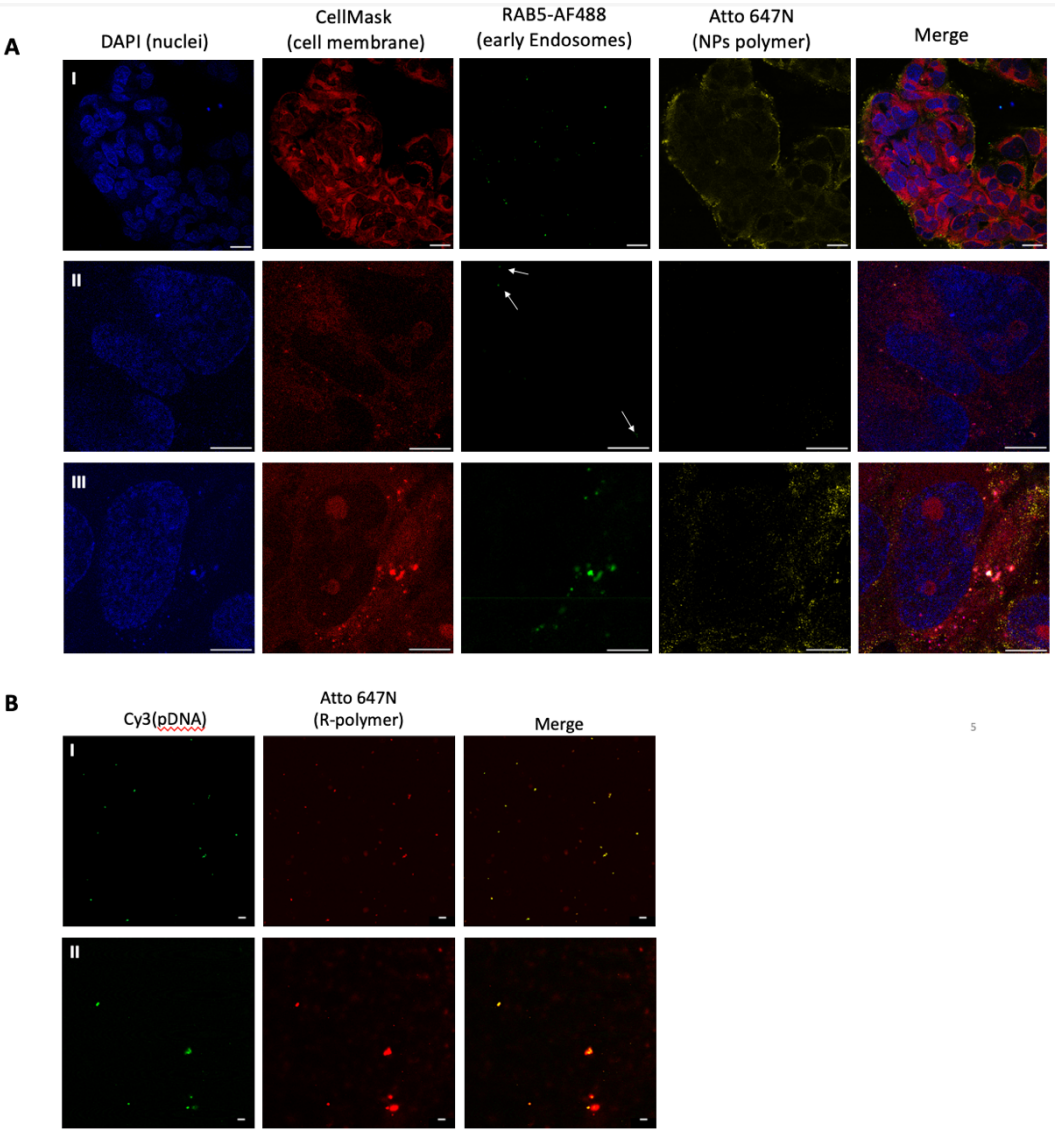

Figure S3. PExM improvements. A) BEAS-2B cells transfected with pBAE NPs I) Illustrates normal confocal microscopy (no expansion) in where there is no single particle visualization, scale bar of 20µm, 63x objective. In A II) Protein ExM conventional protocol in where it is difficult to visualize the nanoparticles (signalized with a white arrow) and there is no expansion of them at all. A III) we can see an image of our protocol in where the particles are expanded among with cells allowing single particle visualization. Both II) and III) post-expanded images with scale bar of 92 µm B) demonstrates the expansion of the nanoparticles without cells. I) pre-expanded nanoparticles and II) post-expanded particles with a 10µm scale bar.

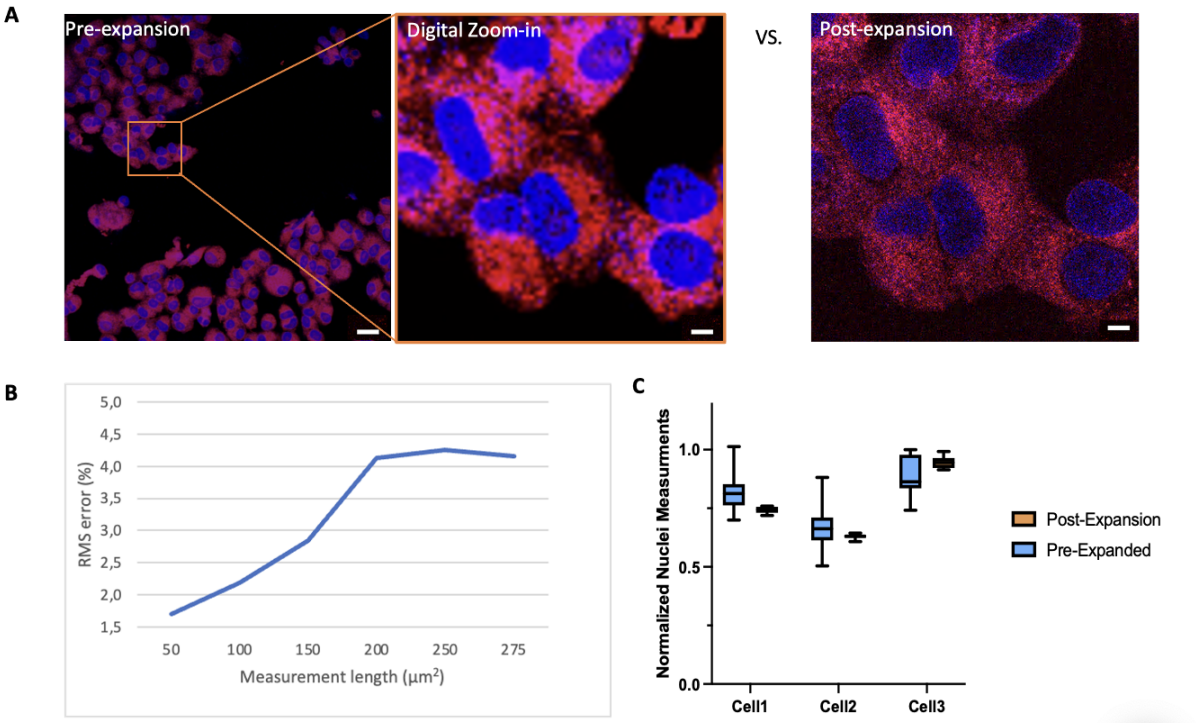

Figure S4. Representative images used for distortion analysis. A) pre-expansion image with a digital zoom-in of A549 cells and post-PExM A549 cells using PExM, Scale bar images are 5µm for the pre-expanded image and 23µm for the zoom-in and post-expanded images. B) Root Mean Square error calculated with the images pre and post expansion from Figures A with Matlab RMS image calculator. C) Distortion measurements of 3 different nucleus of different samples. Each nucleus has been measured 10 times with pre-expanded images and post expanded images.

A)

	Incubation (h)	Overlapping coefficient (%)	P Value
RH	2h	34,3 ± 1,5	0,004 (**)
	4h	28,6 ± 1,1	

B)

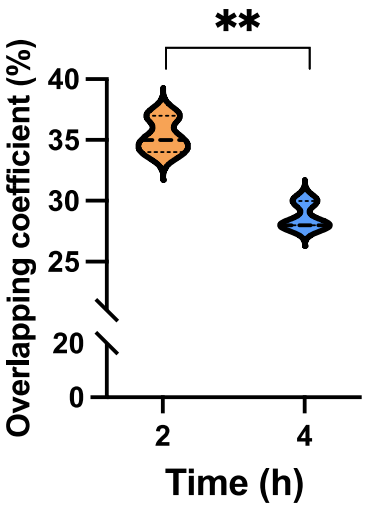

Figure S5. Fiji JACoP Pluggin using overlapping coefficients for the two channels, pBAE nanoparticles and RAB5-AF488 early endosomes for A549 cells transfected with RH NPs. *P* value < 0.0001 (****), *p* value < 0.001 (***), *p* value < 0.01(**) and *p* value < 0.05 (*). A) Table; B) Violins plot