Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Tracking complexation state of PBAE polyplexes in cells with super resolution microscopy

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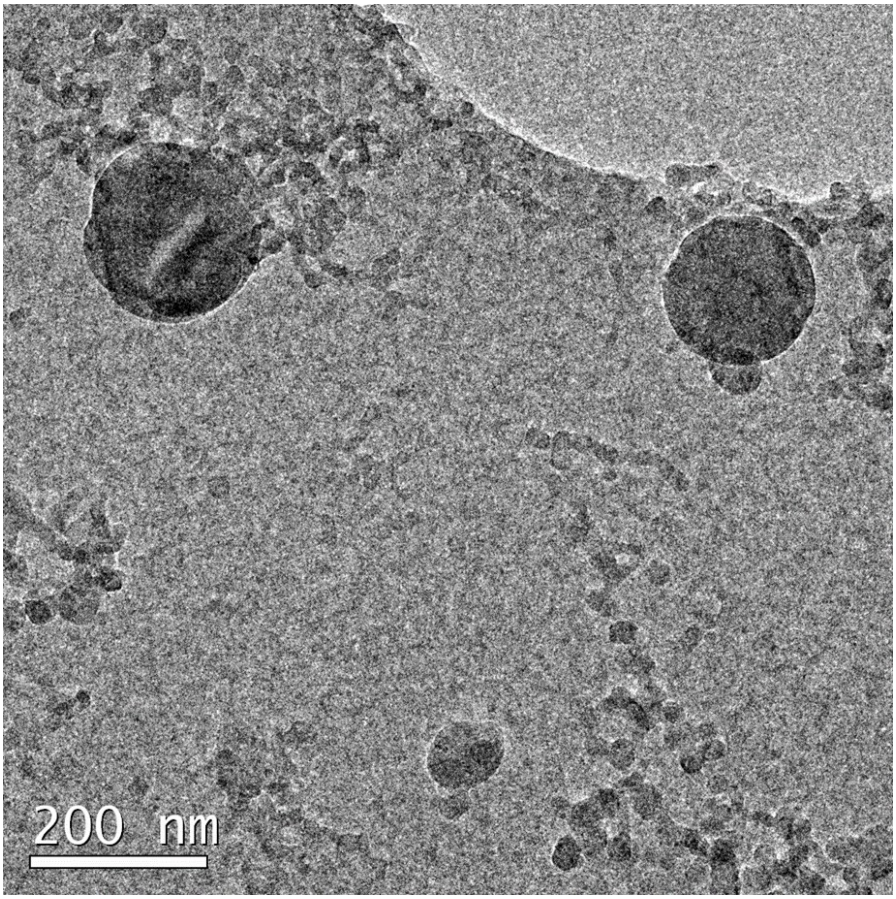

Fig. S1 CryoTEM image of pBAE polyplexes. Two polyplexes are shown, accompanied by other smaller structures that are attributed to be aggregates of polymer.

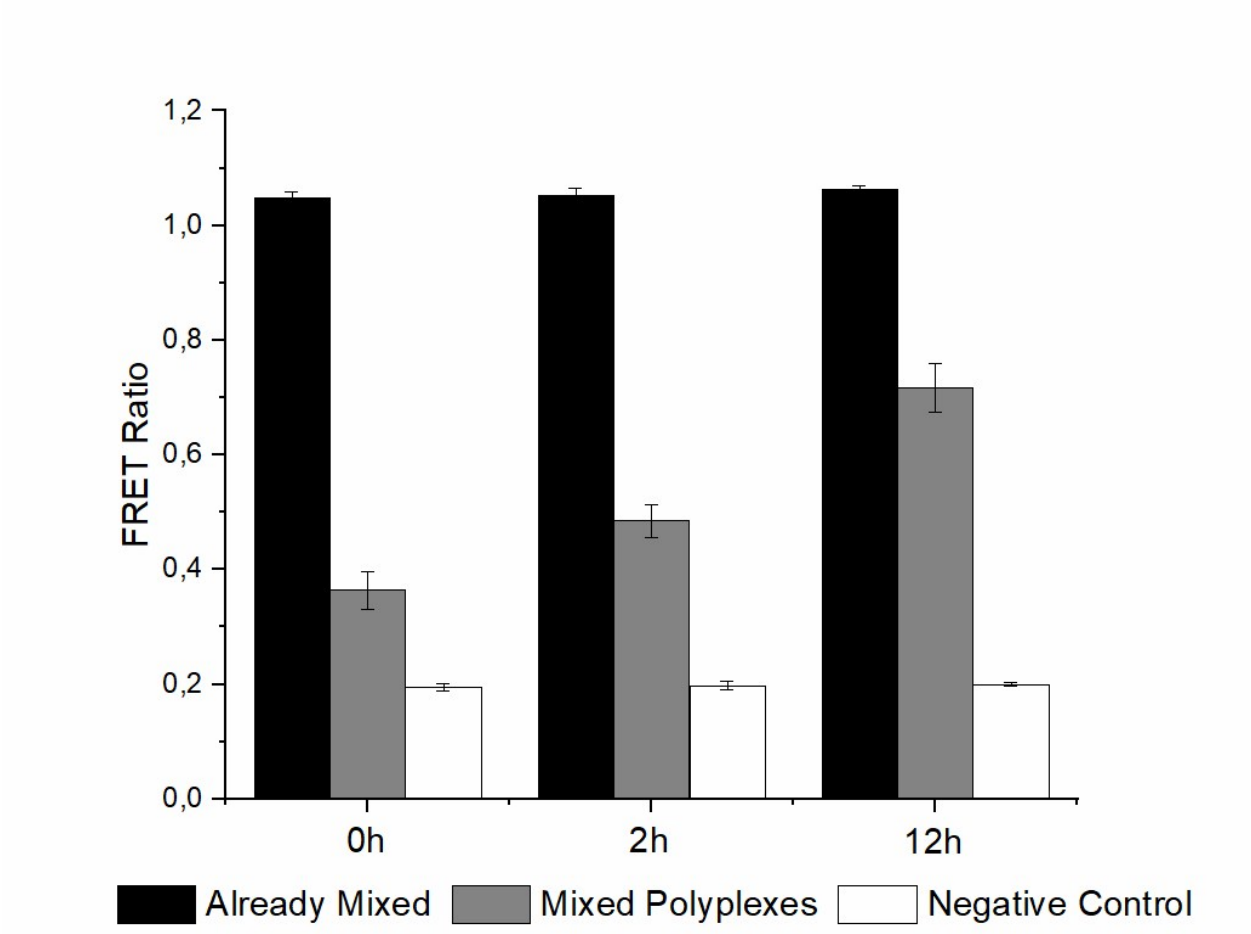

Fig. S2 Dynamic equilibrium stablished between polyplexes and free polymer molecules. FRET signal increases when a Cy3-labelled and a Cy5-labelled batch of polyplexes are mixed. Signal remains stable when polyplexes are already prepared with Cy3-labelled pDNA and Cy5-labelled PBAE.

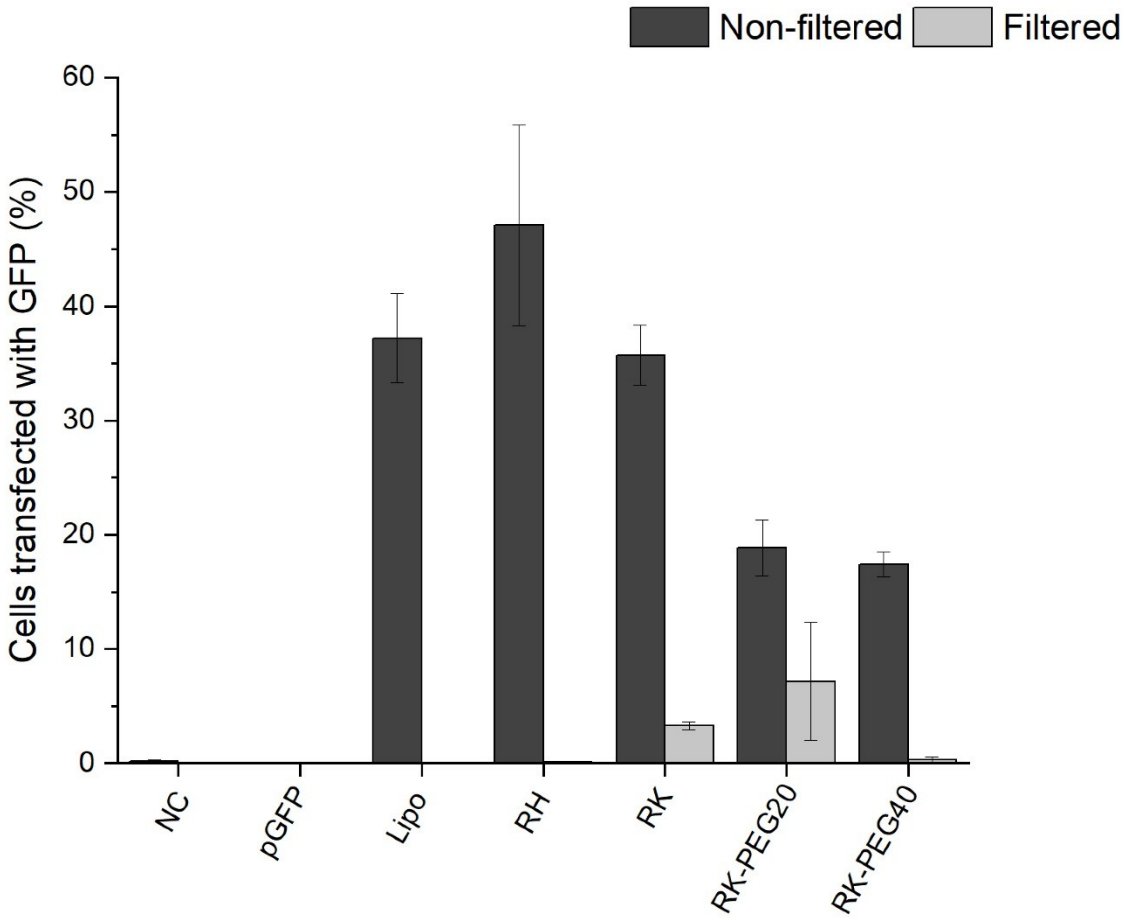

Fig. S3 Transfection efficiencies of different kinds of lyophilized pBAE polyplexes in ARPE cultured cells before and after filtration (R: arginine, H: histidine, K: lysine). Mean and standard deviation are plotted. Negative control with only C_6 polymer (NC) or only pGFP are plotted in the first two columns. The same plasmid is transfected with Lipofectamine as a positive control of transfection. Those controls are only tested non-filtered.

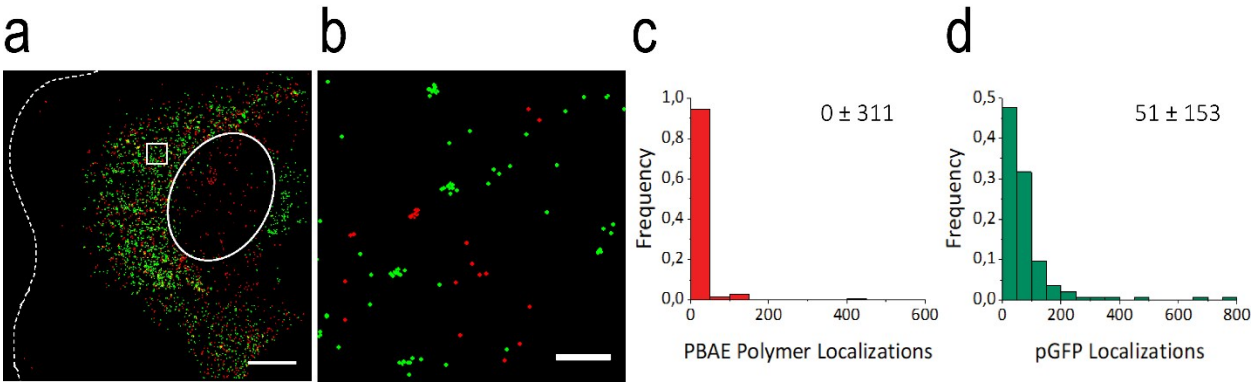

Fig. S4 *d*STORM images quantification at 48h. (a) Representative *d*STORM image of pBAE polyplexes (pBAE in red and pDNA in green) in COS7 cells after 48h incubation. Dashed line delimits the cell membrane and circle the nucleus. (b) Close-up view on white square in a. (c) Quantification of pBAE polymer localizations in each polyplex (median ± SD). (d) Quantification of pDNA localizations in each polyplex (median ± SD). Scale bars, 10 µm (a), 500 nm (b).

Table S1 | PBAE polyplex DLS measurements. Data is shown in mean ± SD.

Sample	Size (nm)	PDI
Fresh	146.1 ± 7.6	0.147 ± 0.025
Lyophilized	169.2 ± 17.3	0.166 ± 0.004
Before Filtering	168.8 ± 21.4	0.338 ± 0.056
Non-filtered fraction	194.6 ± 32.5	0.368 ± 0.145