pharmaceutics

Supplementary Materials: Amphiphilic Anionic Oligomer-Stabilized Calcium Phosphate Nanoparticles with Prospects in siRNA Delivery via Convection-Enhanced Delivery

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Structural analysis of pristine oligomer

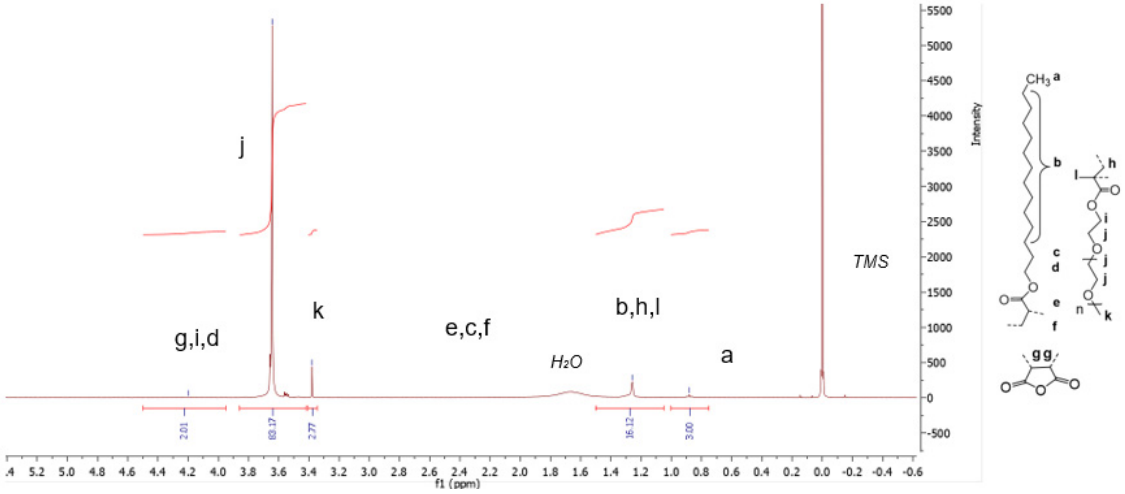


Figure S1. ^{1}H-NMR of o14PEGMA (04/04/10) in $CDCl_3$ with 0,03%TMS, c = 0.5 mg/ml

δ [ppm] 0.75-1.00 ;1.05-1.50; 3.35-3.40; 3.42-3.86; 3.95-4.50

Size exclusion chromatography of pristine oligomer stabilizer

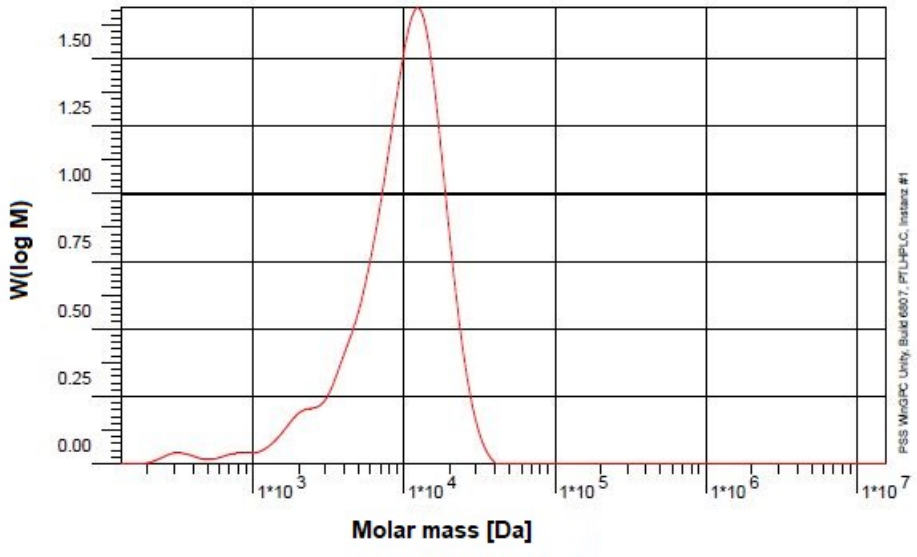
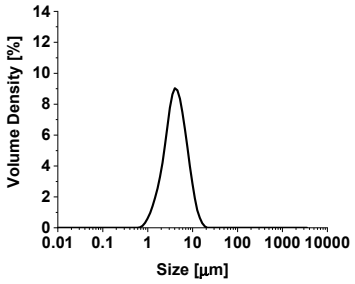
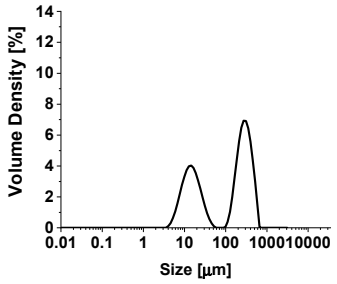
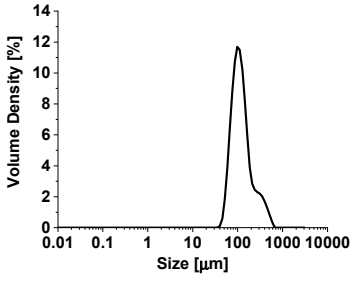
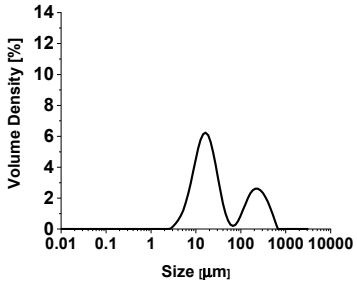
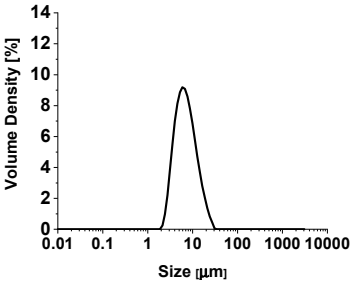
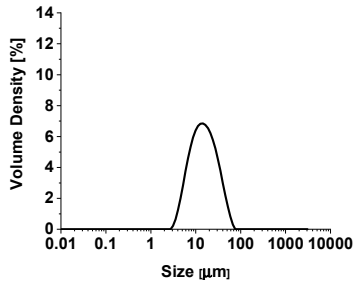
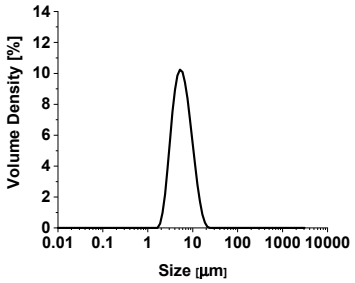
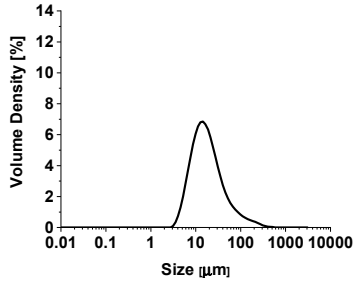
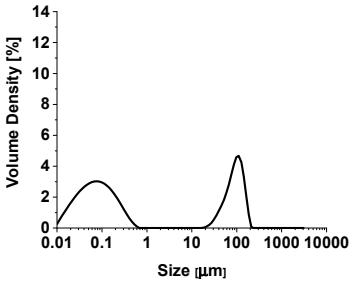
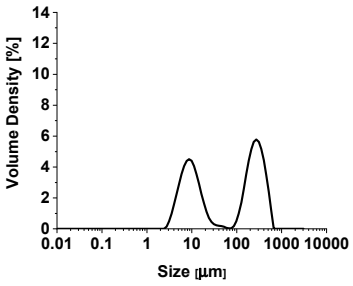
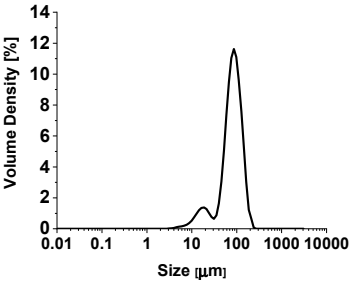
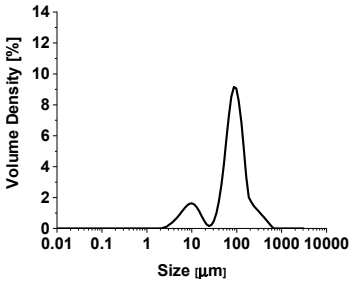
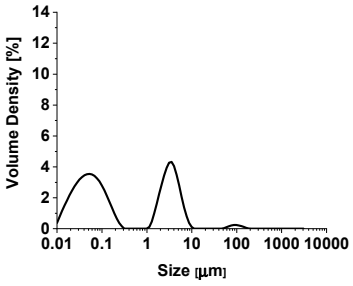
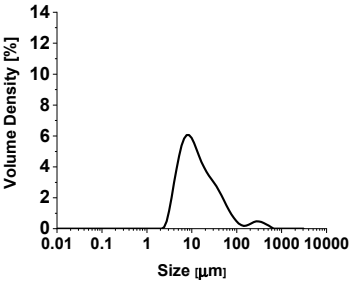
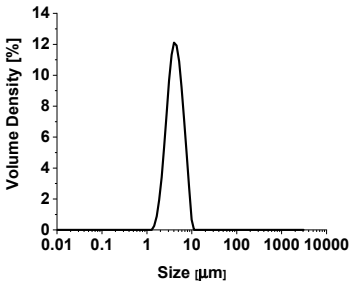
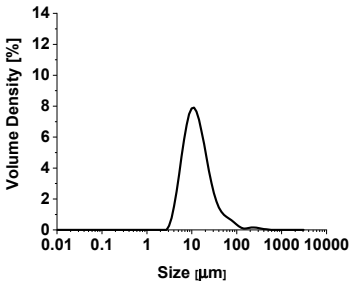


Figure S2. Gel permeation chromatography chromatograms of o14PEGMA(1:1:2.5) to determine size distribution c = 10mg/ml in THF ; V = 40 µl; flow rate=1ml/min; n=3

Impact of oligomer concentration on CaP-NP stabilization.

			Size distribution	
			t = 5 min	**t = 30 min**
2 µmol/L o14PEGMA(1:1:2.5)_NH₃	**1.5 mmol/L PO_4^{3-}**	**Method 1**	 D[4;3] = 5.05 µm	 D[4;3] = 194 µm
		Method 2	 D[4;3] = 151 µm	 D[4;3] = 88.4 µm
	6.0 mmol/L PO_4^{3-}	**Method 1**	 D[4;3] = 8.45 µm	 D[4;3] = 19.1 µm
		Method 2	 D[4;3] = 6.72 µm	 D[4;3] = 29.8 µm

			Size distribution	
			t = 5 min	t = 30 min
5 µmol/L o14PEGMA(1:1:2.5)_NH_3	1.5 mmol/L PO_4^{3-}	Method 1	 D[4;3] = 39.6 µm	 D[4;3] = 166 µm
		Method 2	 D[4;3] = 89.2 µm	 D[4;3] = 104 µm
	6.0 mmol/L PO_4^{3-}	Method 1	 D[4;3] = 3.21 µm	 D[4;3] = 31.4 µm
		Method 2	 D[4;3] = 4.77 µm	 D[4;3] = 20.6 µm

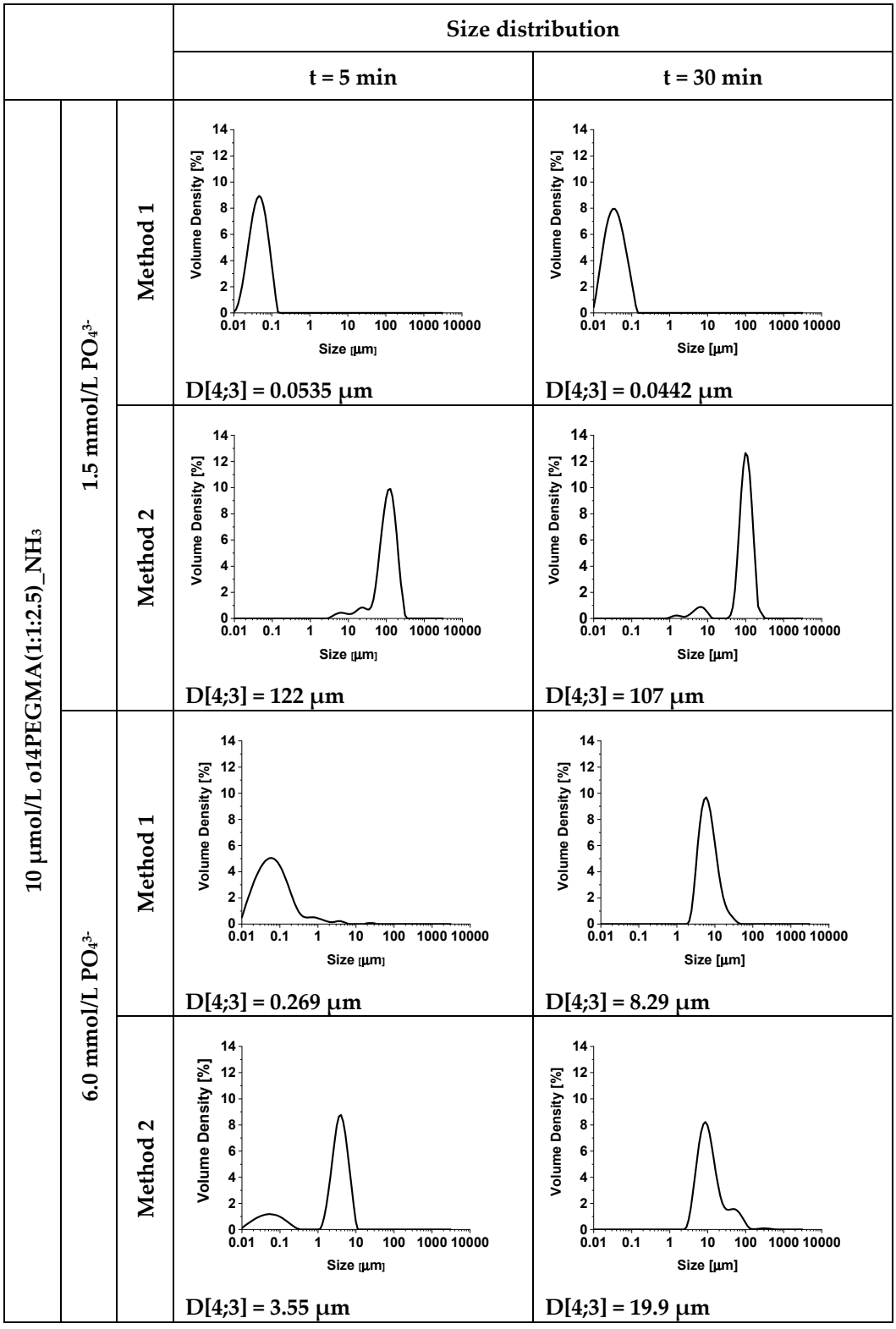
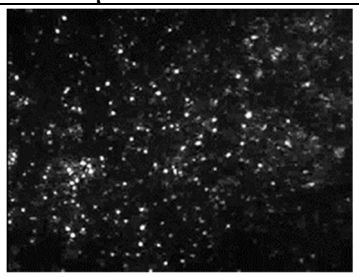
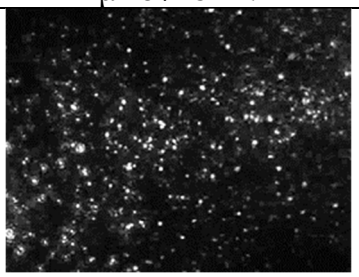
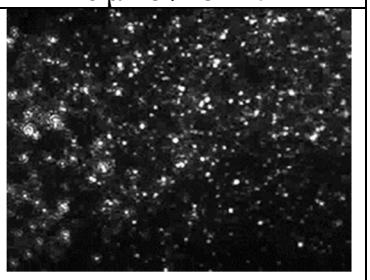
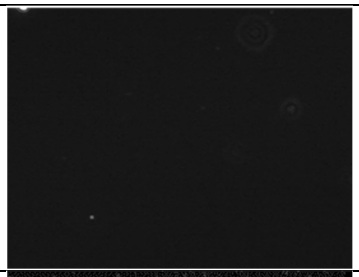
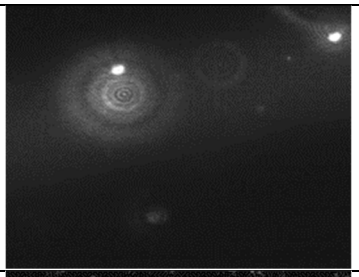
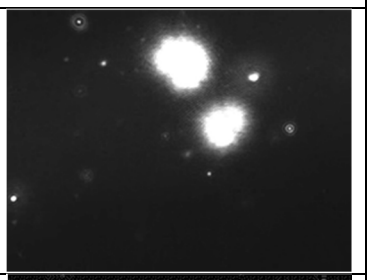
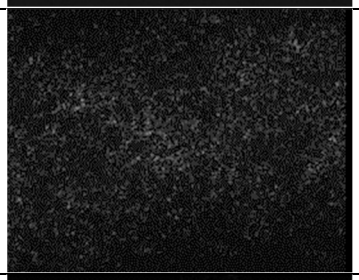
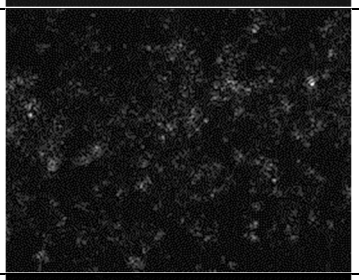
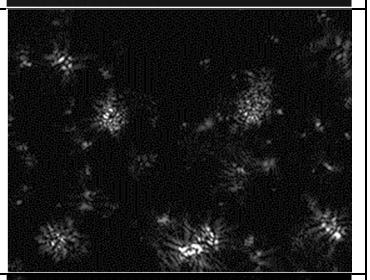
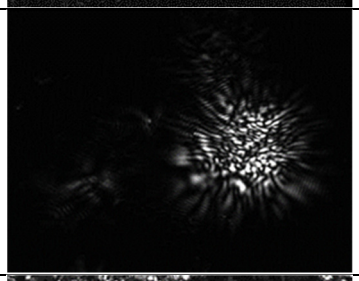
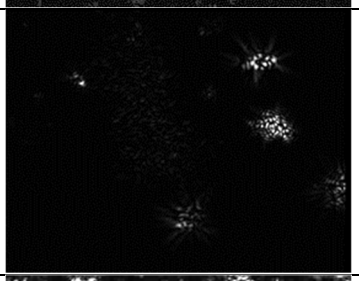
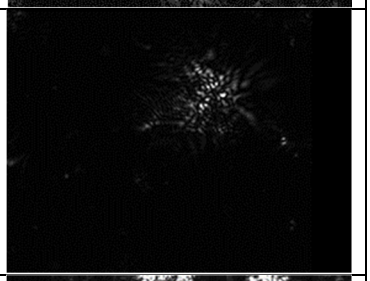
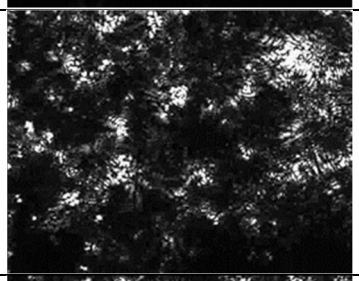
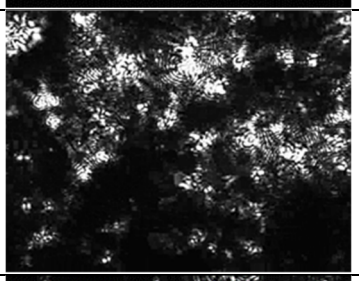
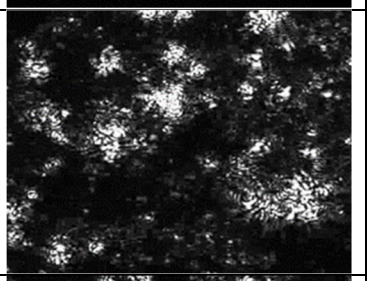
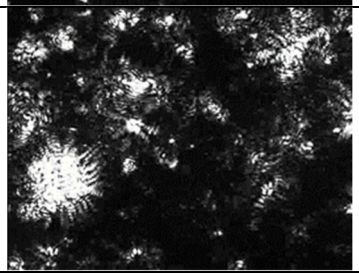
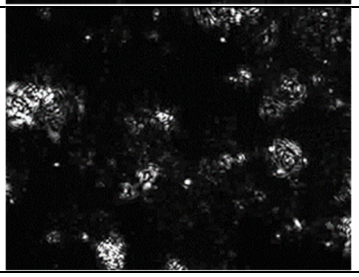
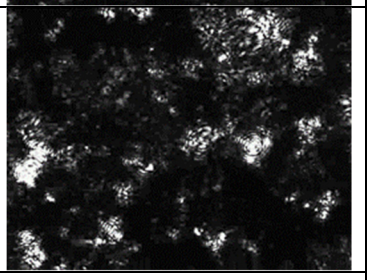


Figure S3. Influence of oligomer concentration on size distribution and aggregation of CaP particles determined by Laser diffraction analysis.

NTA screenshots for analysis of aggregation status

			0 µmol/L siRNA	1 µmol/L siRNA	10 µmol/L siRNA
10 µmol/L o14PEGMA(1:1:2.5)_NH_3	1.5 mmol/L PO_4^{3-}	Method 1			
		Method 2			
	3.75 mmol/L PO_4^{3-}	Method 1			
		Method 2			
	6.0 mmol/L PO_4^{3-}	Method 1			
		Method 2			
			0 µmol/L siRNA	1 µmol/L siRNA	10 µmol/L iRNA

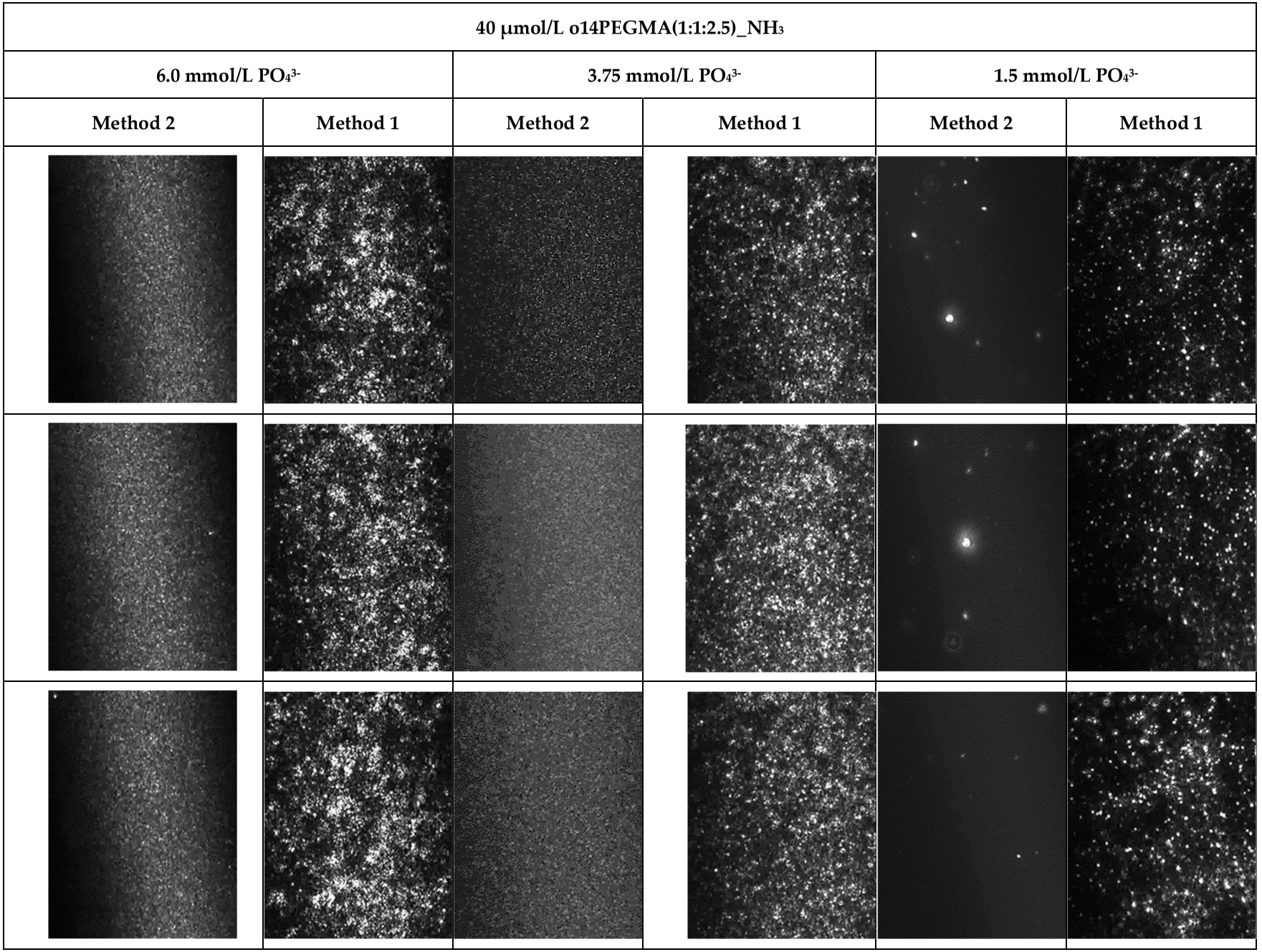


Figure S4. NTA screenshots of CaP-NP formulation 30 min after fabrication.

Impact of cell culture medium & serum proteins on size distribution of CaP-NP

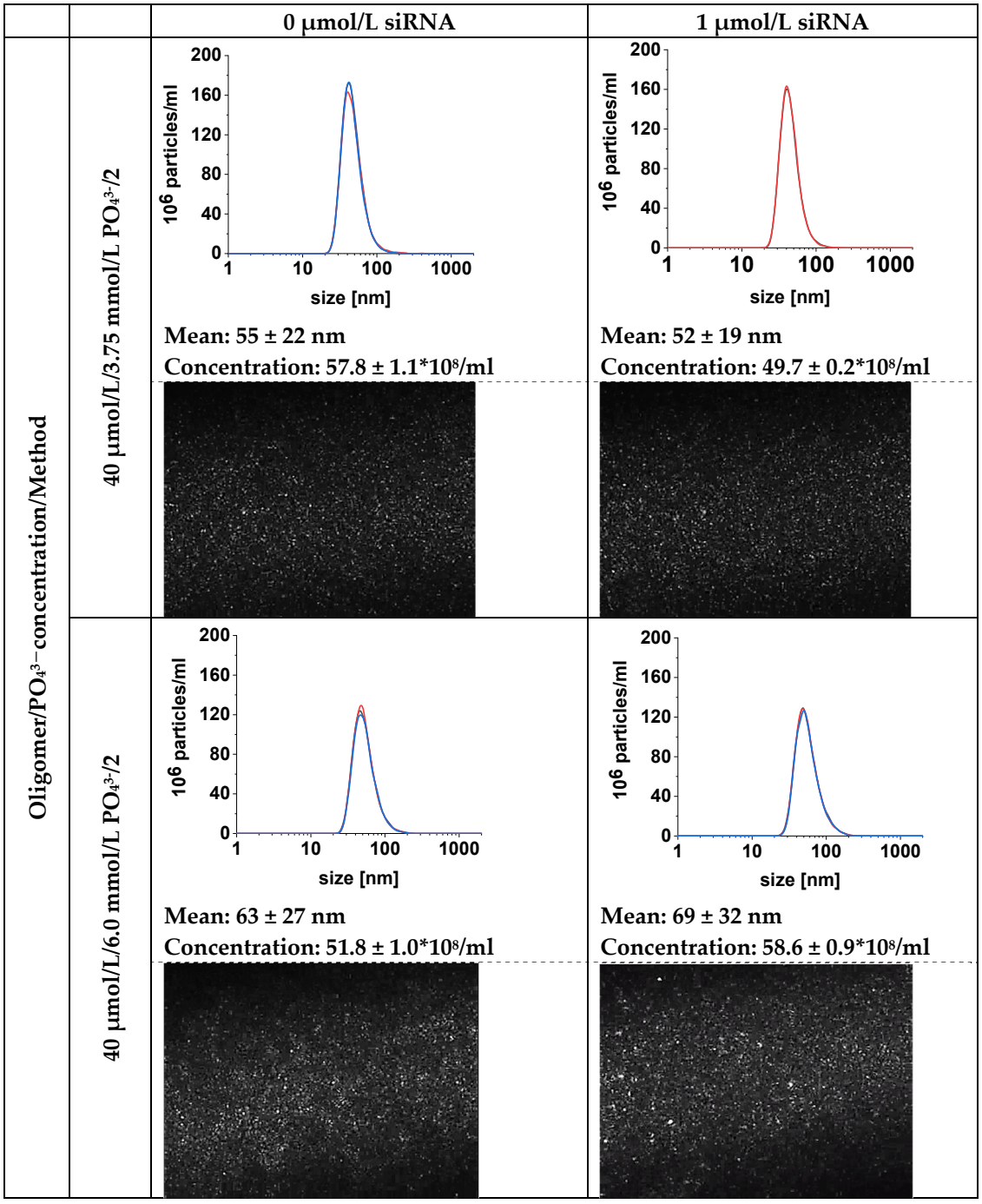


Figure S5. Effect of serum proteins and cell culture medium on stability of o14PEGMA(1:1:2.5)_NH_3-stabilized CaP-NP after 5 h.

Cellular Uptake of CaP-NP in F98 cells

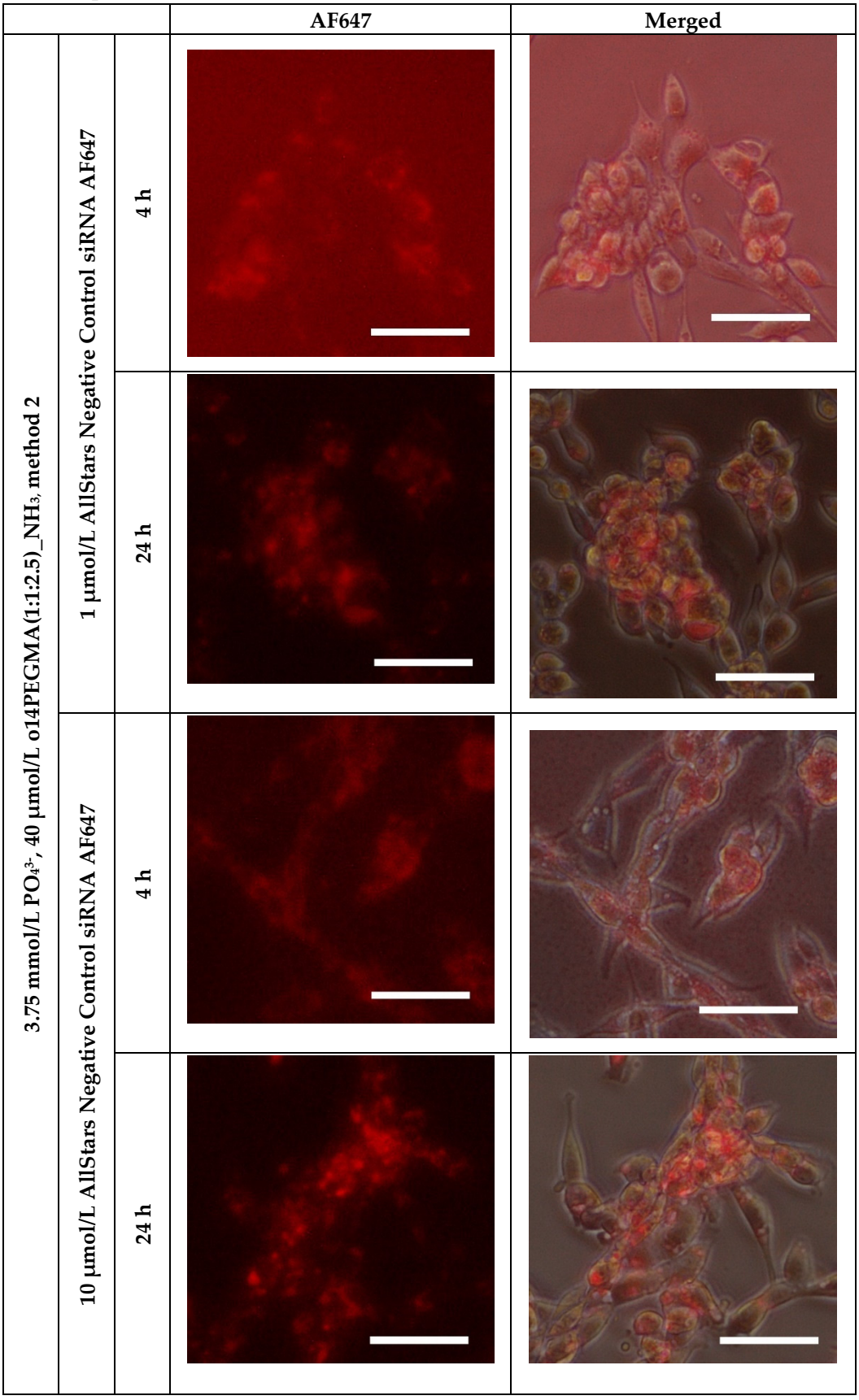
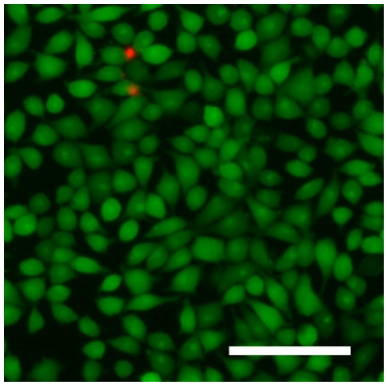
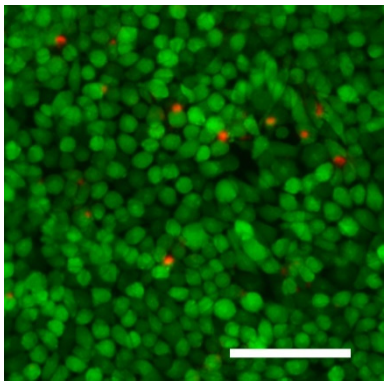
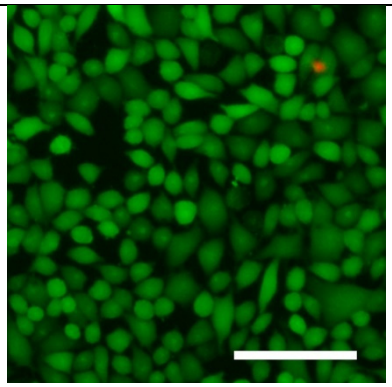
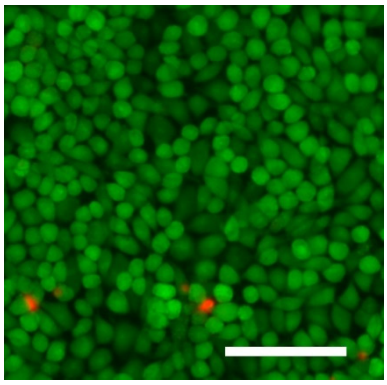
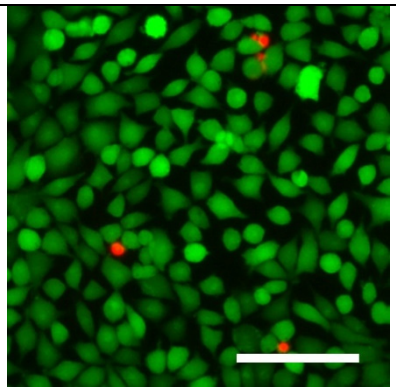
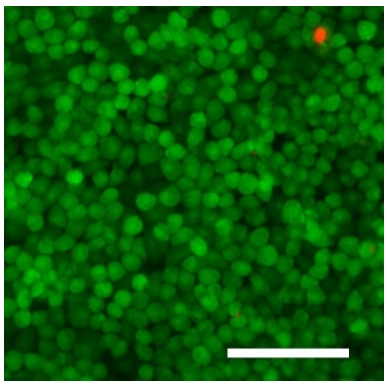


Figure S6. Uptake of siRNA (AlexaFluor™ 647 labeled)-loaded o14PEGMA(1:1:2.5)_NH_3-stabilized CaP-NP in F98 cells. Scale bars represent 100 µm.

Live/Dead staining

	Group		24 h	72 h
Non-concentrated	Control	Untreated		
	3.75 mmol/L PO_4^{3-}, 40 μM o14PEGMA(1:1:2.5)_NH_3, method 2	0 μmol/L siRNA		
		1 μmol/L siRNA		

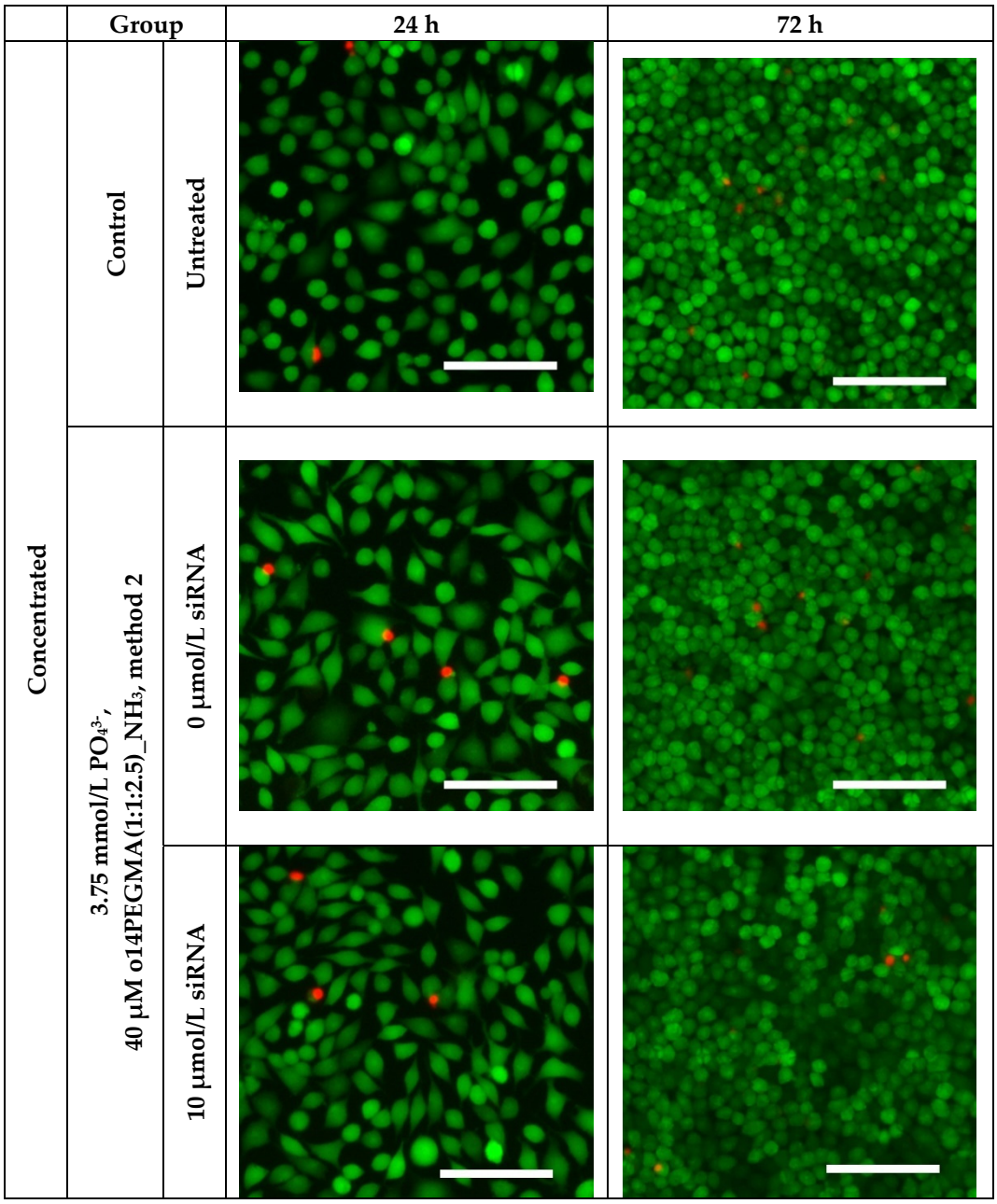


Figure S7. *In vitro* biocompatibility testing of CaP-NP in L929 cells: Live/Dead staining. Scale bars represent 100 µm.

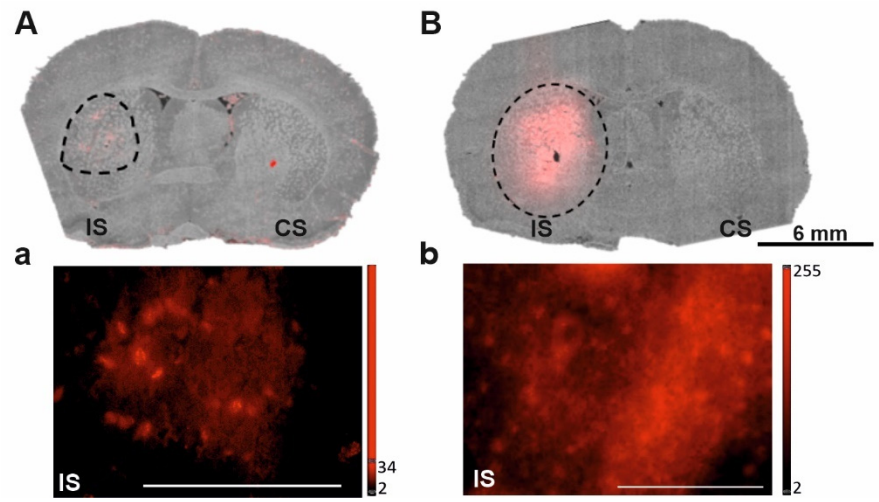


Figure S8. *In vivo* biodistribution of CaP-NP loaded with 10 μmol/L AllStars Negative Control siRNA AF647 (A, a) or AllStars Negative Control siRNA AF647 alone (B, b) 1 hour post-CED in healthy brain (n=2). Representative brain sections of fluorescence distribution in the whole slide (A, B) and fluorescence of the AllStars Negative Control siRNA AF647 at the injection site within the CaP-NP (a) and alone (b). IS: ipsilateral side (CED injection), CS: contralateral side (control) (scale bars represent 150 μm, x40 magnification).