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Supporting Information

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Probing Endosomal Escape Using pHlexi Nanoparticles.

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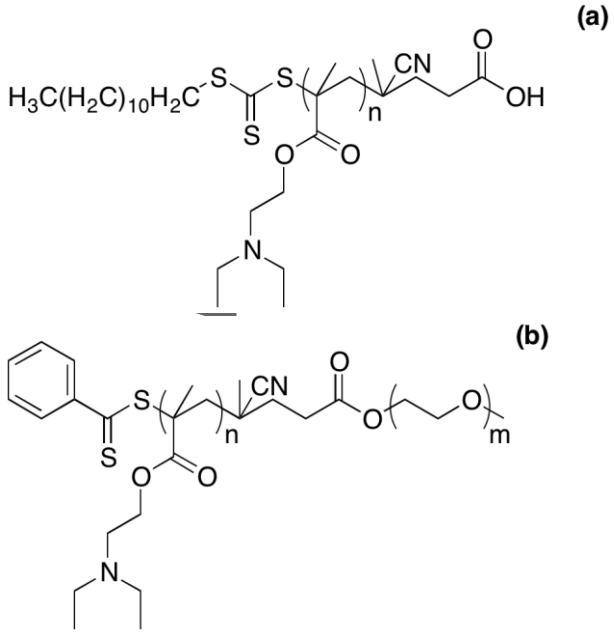

Figure S1. Chemical structure of a) poly [2-(diethylamino)ethyl methacrylate)] (PDEAEMA) homopolymer and b) poly[2-(diethylamino)ethyl methacrylate)]-b-poly(ethylene glycol) (PDEAEMA-b-PEG) copolymer.

Table S1. PDEAEMA characterization via NMR spectroscopy.

PDEAEMA molecular weight (kDa)	Ratio of RAFT agent and monomer	Polymerization time (h)	Percent conversion (%)
7	1:200	4	15%
27	1:1000	4	15%
56	1:500	48	74%
106	1:10000	48	65%

Table S2. PDEAEMA Characterization via Gel Permeation Chromatography using methyl methacrylate standards.

PDEAEMA molecular weight measured via NMR (kDa)	Number Average Molecular Weight (kDa)	Weight Average Molecular Weigh (kDa)	Polydispersity index (PDI)
8	4	5	1.24
34	19	26	1.35
56	22	31	1.43
106	33	42	1.26

Table S3. Particle Diameter of pHlexi nanoparticles without and with OVA-647 at various PDEAEMA molecular weights

PDEAEMA molecular weight (kDa)[a]	pHlexi particle without OVA-647 Diameter (nm)[b]	pHlexi particle with OVA-647 Diameter (nm)[c]
7	137 ± 10	132 ± 8
27	121 ± 3	164 ± 2
56	144 ± 6	194 ± 1
106	128 ± 3	172 ± 3

[a] The number average molecular weight of PDEAEMA as determined by nuclear magnetic resonance spectroscopy [b] Mean particle diameter of pHlexi particle without OVA-647 measured by DLS [c] Mean particle diameter of pHlexi particle with OVA-647 measured by DLS

Table S4. Zeta Potential of OVA pHlexi particles

PDEAEMA molecular weight (kDa)[a]	Zeta Potential (mV)[b]
8	3 ± 7
34	4 ± 5
56	3 ± 6
106	5 ± 6

[a] The number average molecular weight of PDEAEMA as determined by nuclear magnetic resonance spectroscopy [b] Zeta potential of OVA pHlexi particle measured by DLS

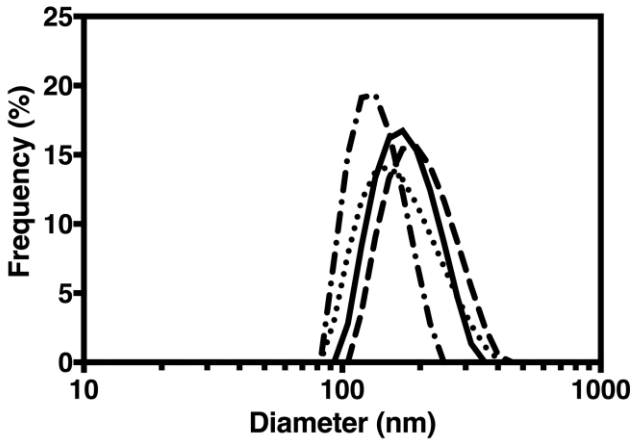

Figure S2. Mean particle diameter of OVA pHlexi nanoparticles as measured by dynamic light scattering. Particles were prepared with PDEAEMA M_n of 7 kDa Da (· − ·), 27 kDa (·····), 56 kDa (- - -), 106 kDa (——).

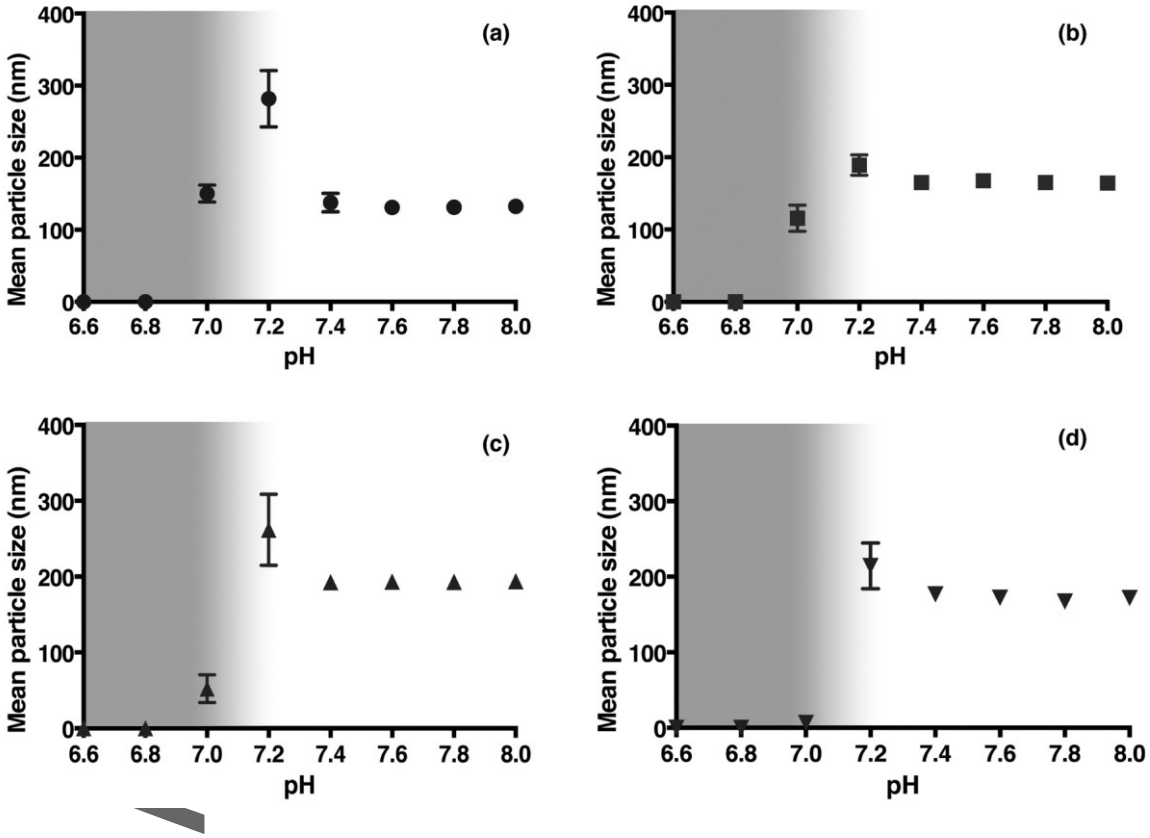

Figure S3. Degradation curves of OVA pHlexi particles, prepared using PDEAEMA of M_n: (a) 7 kDa; (b) 27 kDa; (c) 56 kDa; and (d) 106 kDa. Mean particle size as a function of PBS pH was measured

by DLS. Measurements +/- errors were averaged from 3 readings.

Equation S1. The calculation of percentage ovalbumin loading from UV-Vis spectra at 658nm relative to fluorescence intensity of 106kDa OVA-pHlexi particles.

$$Percentage\ Loading = \frac{absorbance\ at\ 658\ of\ Ova\ pHlexi\ particles}{abosorbance\ at\ 658\ of\ 106000\ Ova\ pHlexi\ particles} \times 100$$

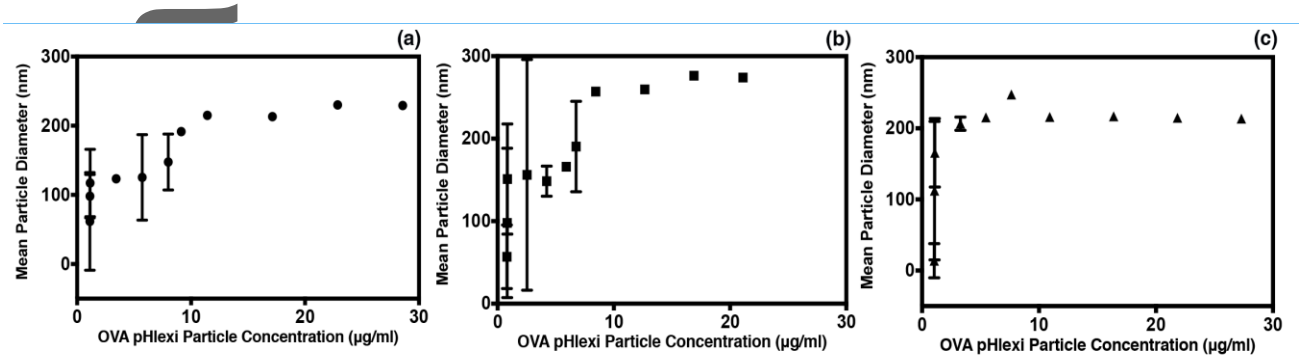

Figure S4. The CMC curves of OVA pHlexi particles, prepared using PDEAEMA of M_n: (a) 6kDa; (b) 30kDa and (c) 106kDa. Mean particle size as a function of PBS pH was measured by DLS. Measurements +/- errors were averaged from 3 readings.

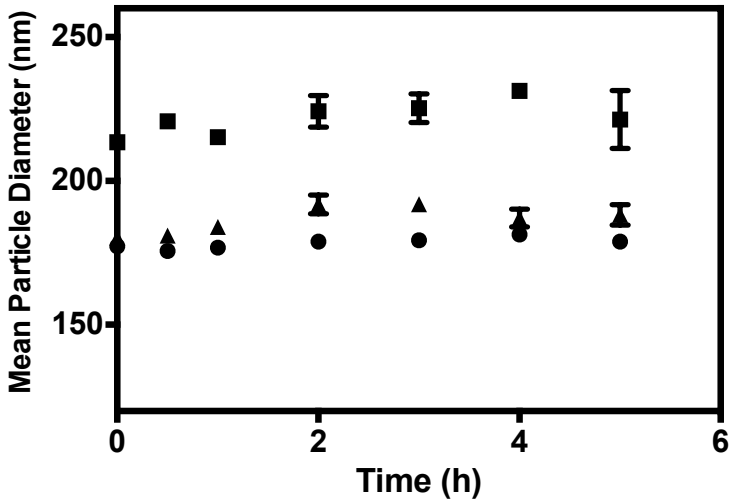

Figure S5. The stability of OVA pHlexi particles in Dulbecco's Modified Eagle Medium (DMEM) with high glucose, heat inactivated fetal bovine serum, penicillin/ streptomycin (10000 U/mL). pHlexi particles were prepared using PDEAEMA of M_n 6kDa (●), 27kDa (■) and 106kDa (▲). Mean particle size was measured by DLS. Measurements +/- errors were averaged from 3 readings.

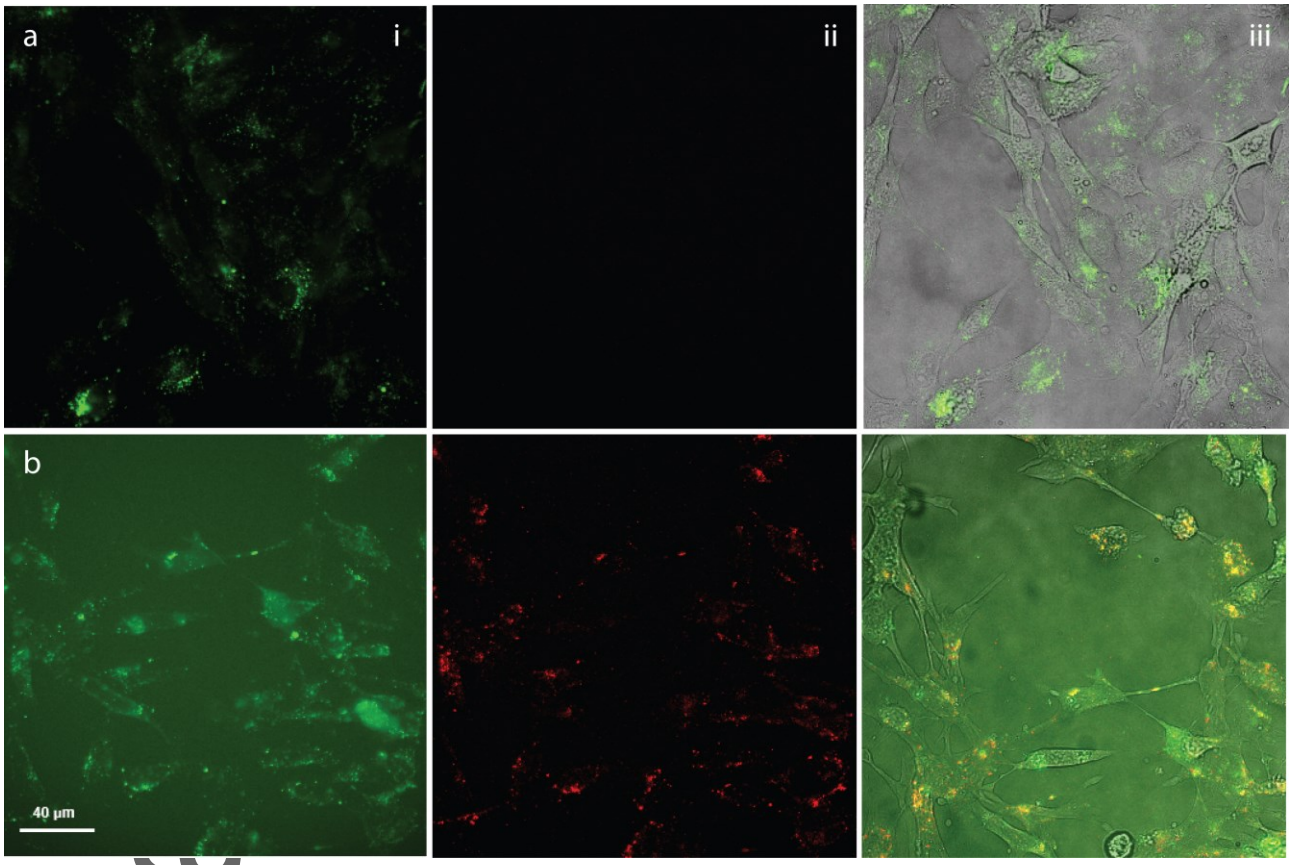

Figure S6. Fluorescence microscopy images of 3T3 cells incubated with calcein alone (a) or calcein and OVA-encapsulated pHlexi particles using a second PDEAEMA 7 kDa (b). The images show calcein fluorescence (i), OVA-647 fluorescence (ii), and merged calcein, OVA-647 and bright field channels (iii).

Table S5. Quantification of induced endosomal escape of calcein from 3T3 fibroblast cells using a second 7 kDa PDEAEMA.

Sample	Number of cells	Cells with released calcein	% Endosomal escape
Calcein control	1550	0	0%
7 kDa Ova pHlexi	1429	50	3%

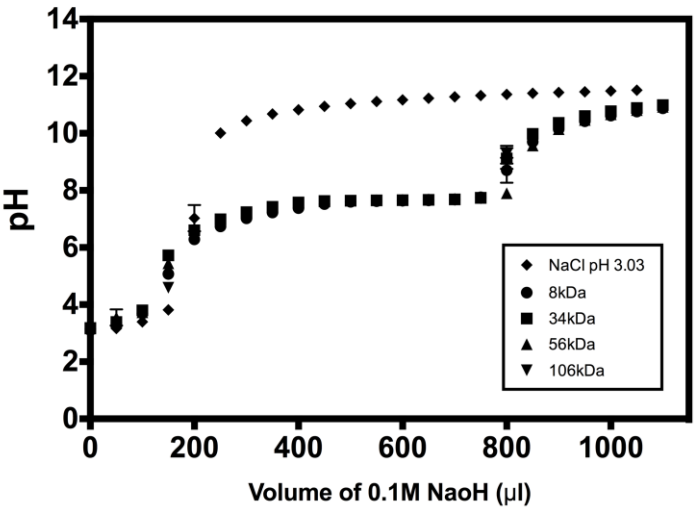

Figure S7. The PDEAEMA buffering curve, prepared using PDEAEMA of M_n 8kDa (●), 34kDa (■), 56kDa (▲), 106kDa (▼) and 150mM NaCl pH 3 (◆).

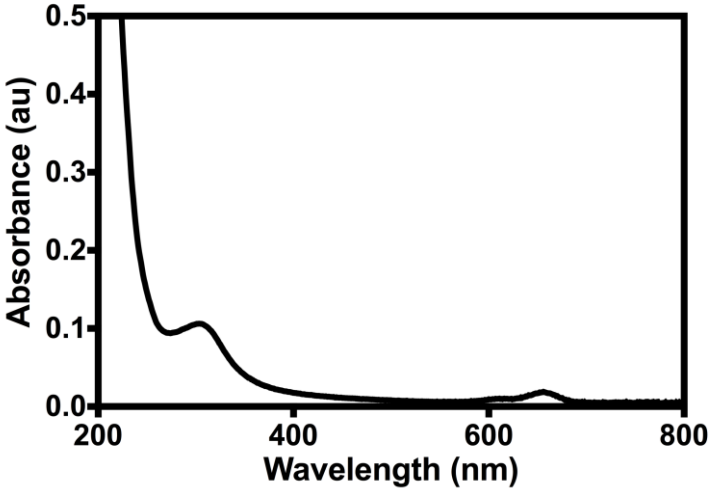

Figure S8. The absorbance spectra of OVA-647 encapsulation in pHlexi particle prepared using PDEAEMA 106kDa.

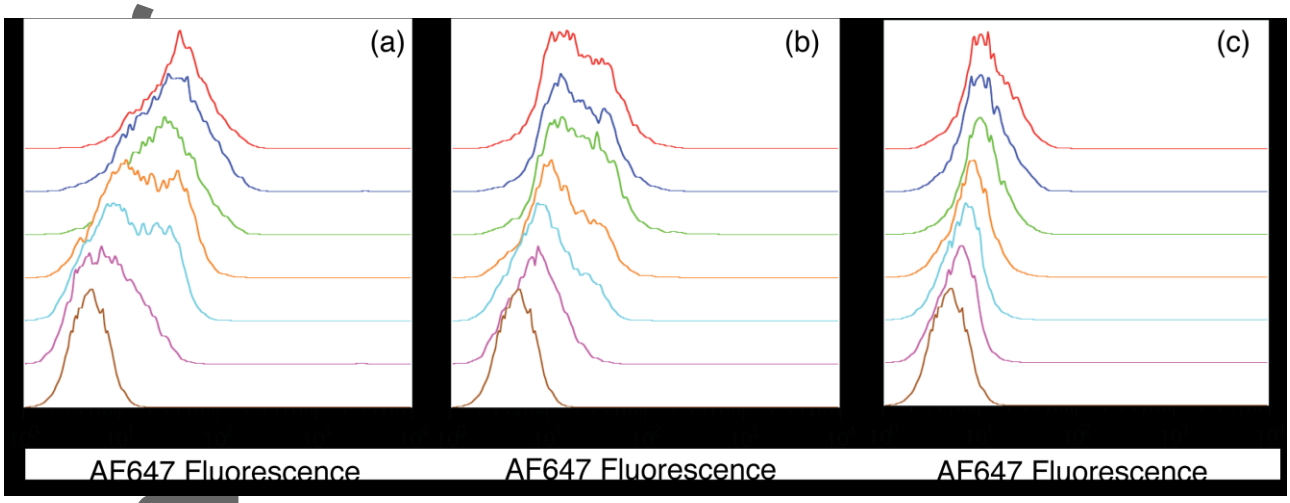

Figure S9. The association of the pHlexi nanoparticles with 3T3 cells increased with decreasing PDEAEMA molecular weight. The histograms of 3T3 cell fluorescence intensity as a result of OVA-647 pHlexi particle association. Particles were prepared with PDEAEMA M_n of 7kDa (a), 27kDa (b) and 106kDa (c).

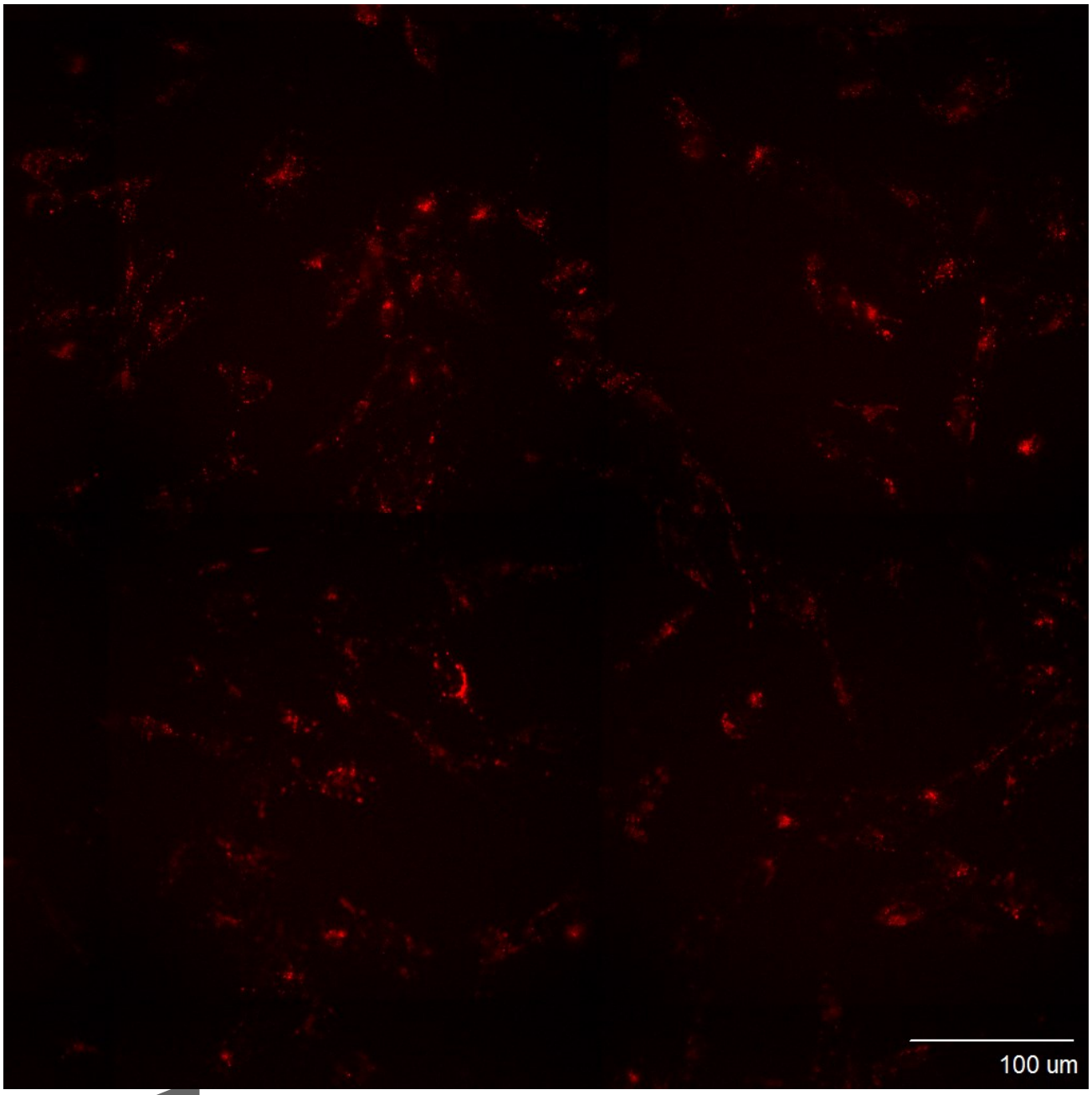

Figure S10. Calcein release assays showed intensity of OVA-647 in 7 kDa particles. Fluorescence microscopy images of 3T3 cells incubated with OVA-encapsulated pHlexi particles (concentration of 20 μg/mL) using PDEAEMA 7 kDa.

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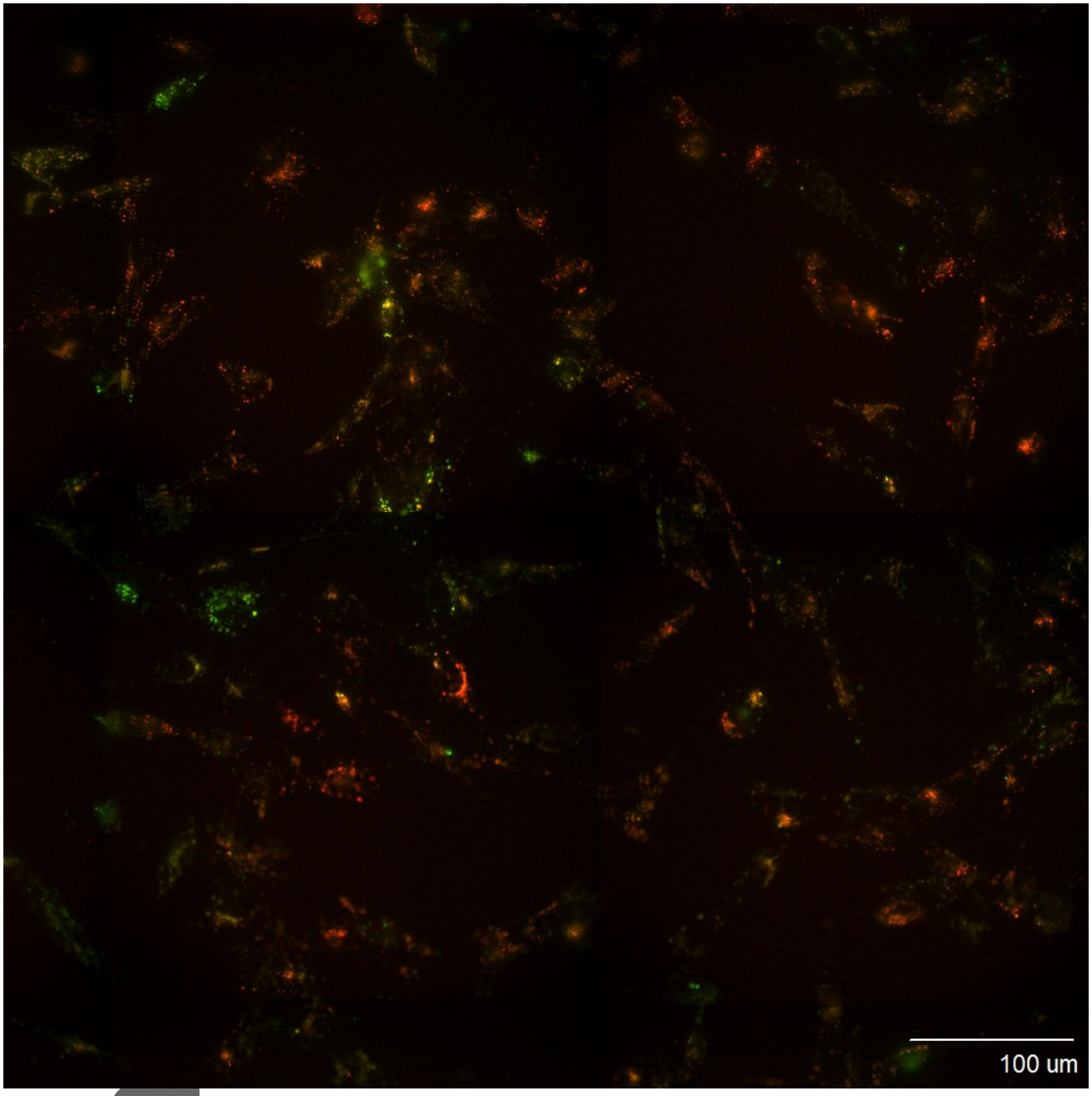

Figure S11. Calcein release assays showed merged calcein and OVA-647 in 7 kDa particles. Fluorescence microscopy images of 3T3 cells incubated with OVA-encapsulated pHlexi particles (concentration of 20 μg/mL) using PDEAEMA 7 kDa.

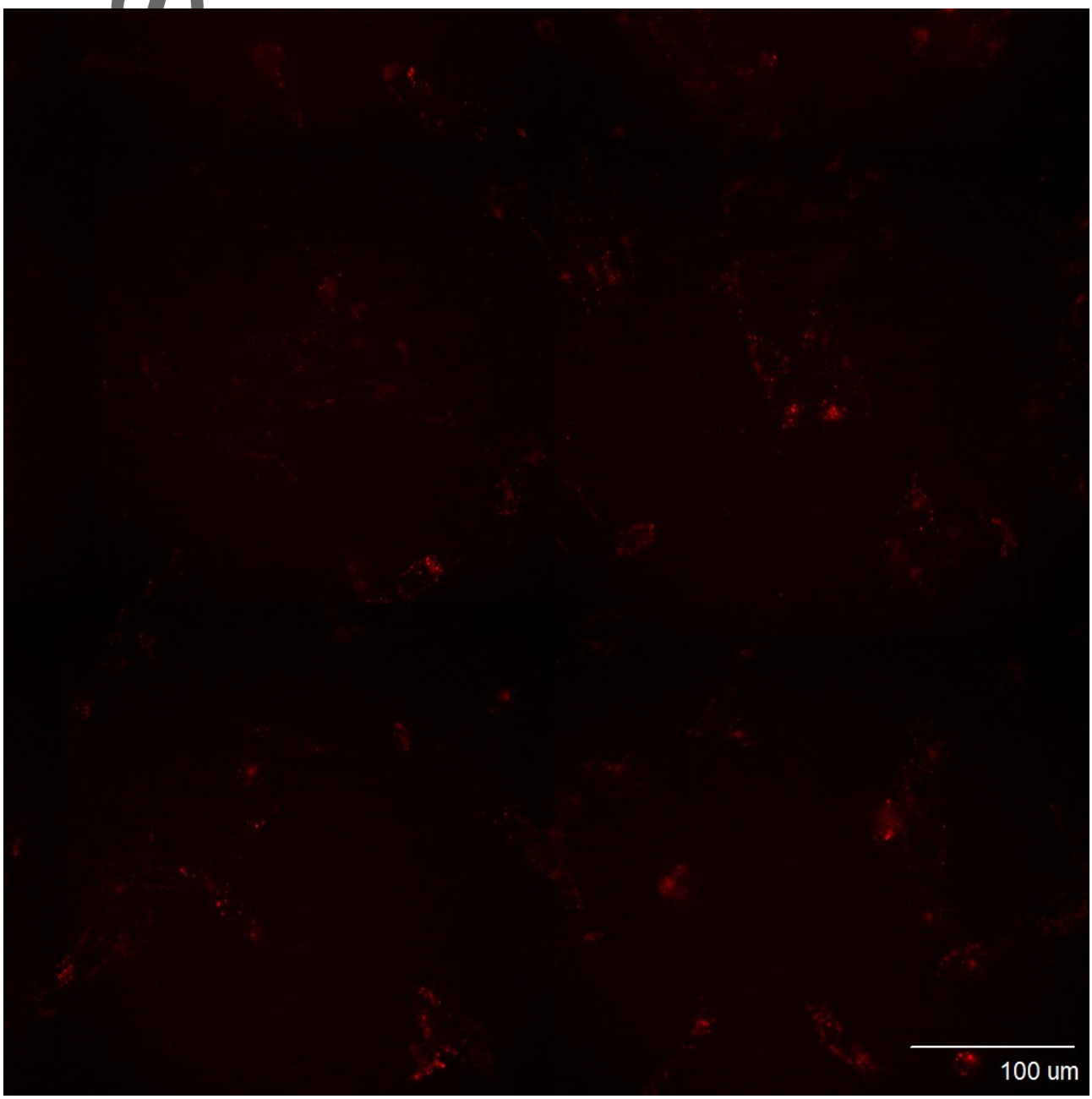

Figure S12. Calcein release assays showed intensity of OVA-647 in 27 kDa particles. Fluorescence microscopy images of 3T3 cells incubated with OVA-encapsulated pHlexi particles (concentration of 20 μg/mL) using PDEAEMA 27 kDa.

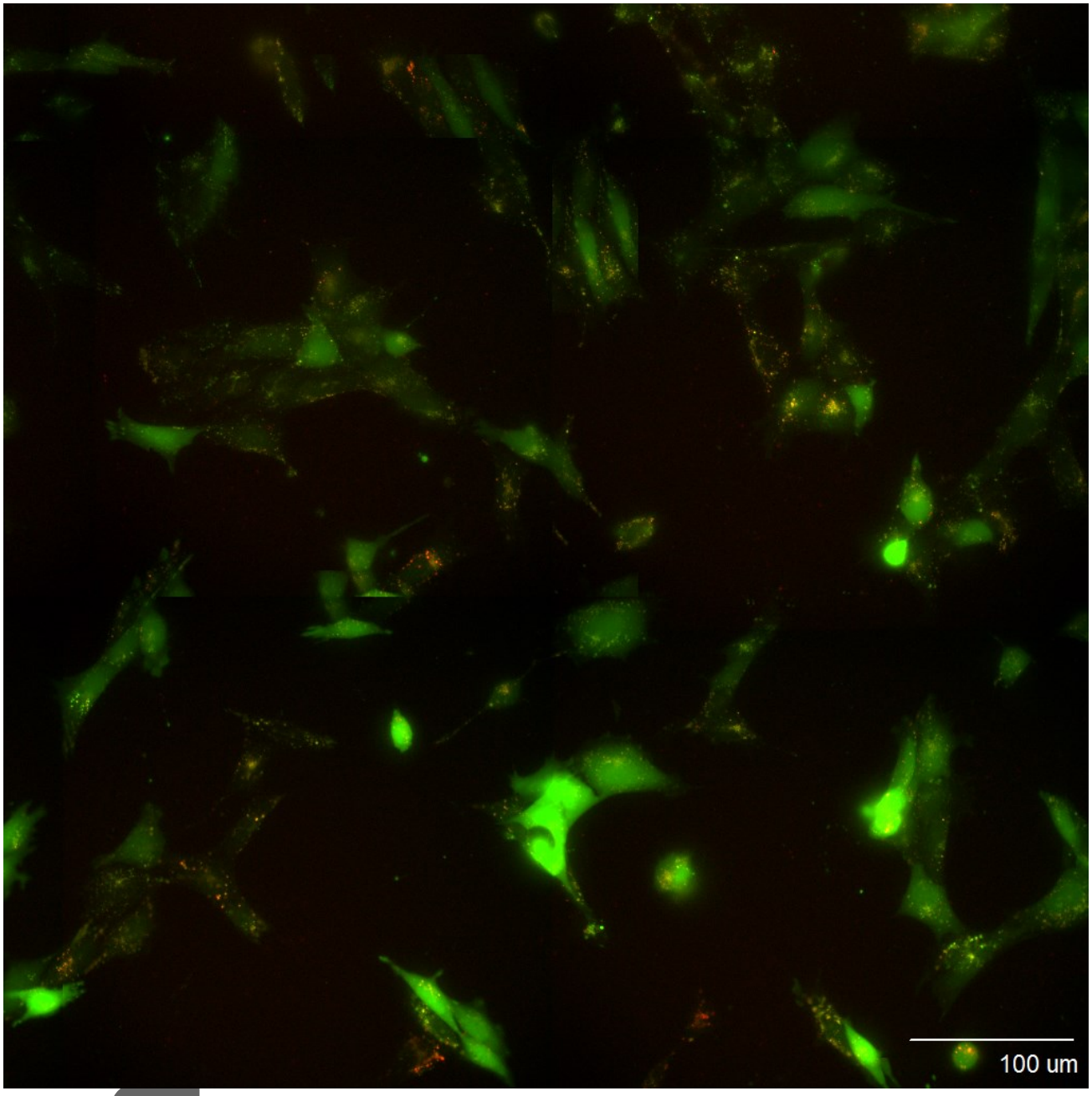

Figure S13. Calcein release assays showed merged calcein and OVA-647 in 27 kDa particles. Fluorescence microscopy images of 3T3 cells incubated with OVA-encapsulated pHlexi particles (concentration of 20 μg/mL) using PDEAEMA 27 kDa.

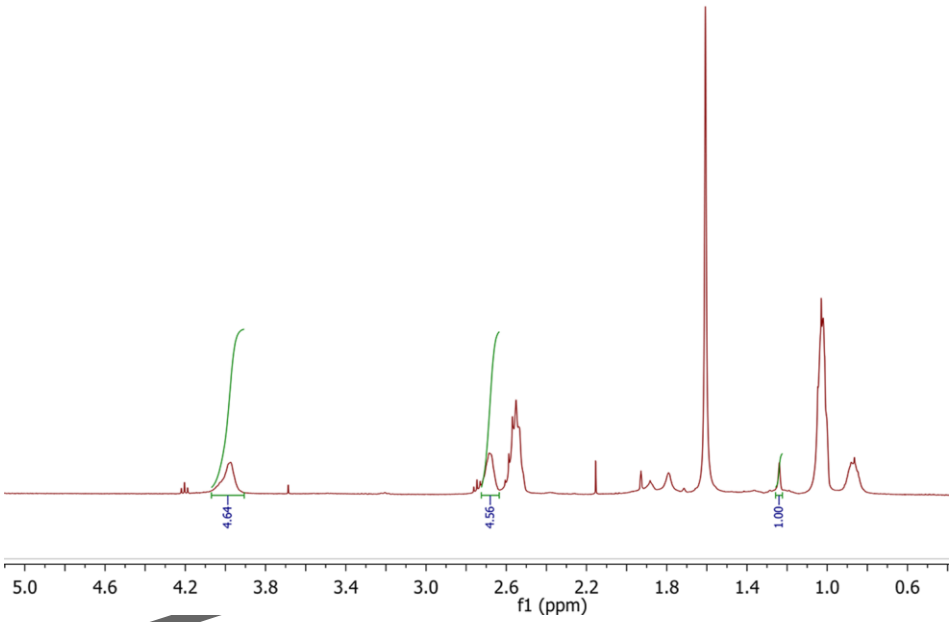

Figure S14 : The NMR spectrum of precipitated PDEAEMA 7kDa in deuterated chloroform, additional peaks at 2.17 and 1.56 ppm are acetone and H_2O respectively.

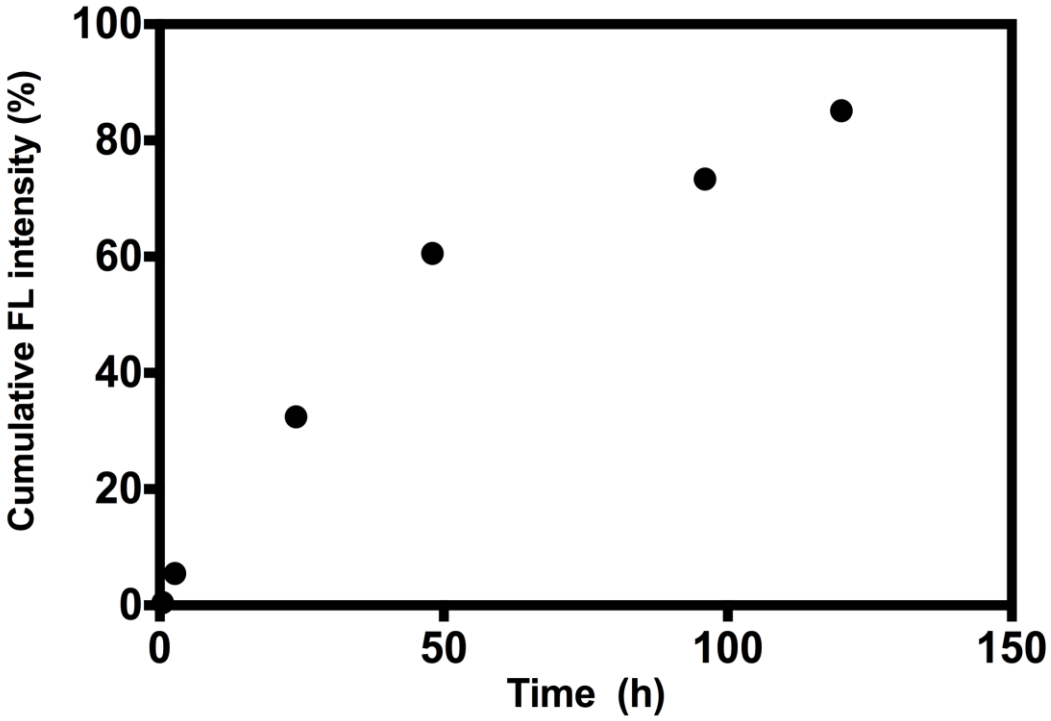

Figure S15: The percentage release of ovalbumin (OVA) (98 mg) at pH 7.