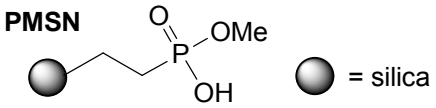
Supplementary information for Manuscript ID TB-ART-03-2015-000555

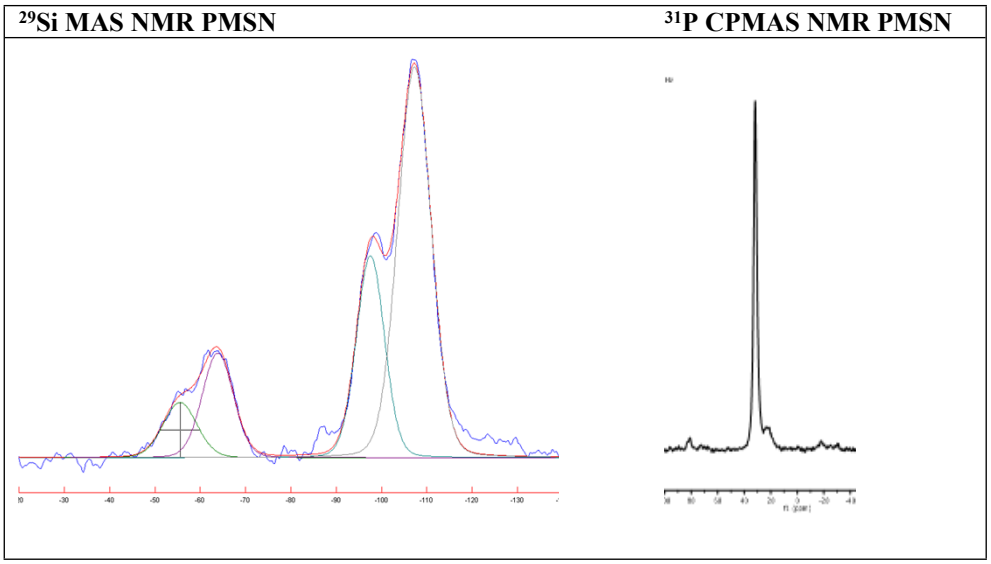
Paper: Intracellular Delivery of BSA by Phosphonate@Silica Nanoparticles

Sai Prakash Maddala, Diana Velluto, Giulia Mastroianni and Alice C. Sullivan*

School of Biological and Chemical Science Queen Mary University of London Mile End Road London E14NS

S1: Solid state NMR of PMSN



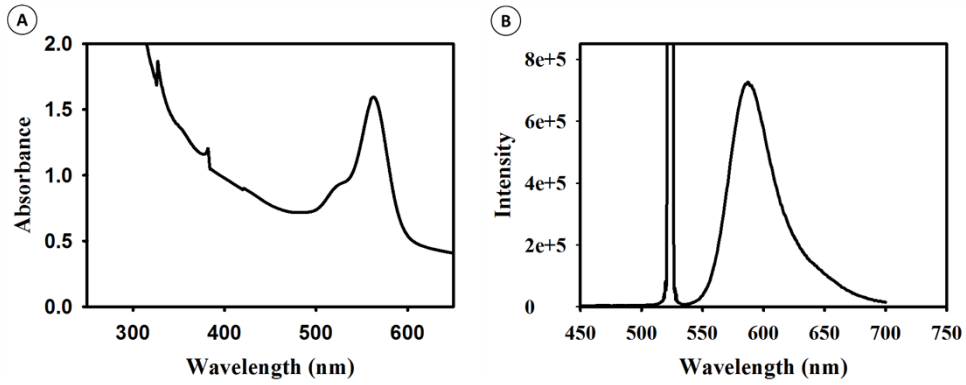


Solid state spectra were obtained using a Bruker AMX 600MHz instrument: ^{29}Si HPDec MAS, frequency 119.2 MHz, spinning speed 12 kHz; 1 minute recycle delay, with 2 μs ~ 45° pulse; ^{31}P CPMAS; frequency 242.9 MHz, spinning speed 12 kHz, 1 ms contact time, 1.5 s delay, 90° pulse for 2.0 μs.

Solid state ^{31}P and ^{29}Si NMR data with deconvolution analysis for PMSN				
phosphonate mmol /g = 2.5 molar ratio P:Si = 0.22)	δ_{29Si} T^2-55.3 , T^3,-63.9 ,Q^3,-98.6 ,Q^4 -106.9 ((%)T^2 8, T^3 14,Q^3 24,Q^4 54)	% Condensation in T,Q sites 88,92	(%)T of surface coverage[a] T^2+ T^3/(Q^3+ T^2+ T^3) =48%	δ_{31P} 35.6

[a] S. Huh, J. W. Wiench, J. C. Yoo, M. Pruski, and V. S. Y. Lin, *Chem. Mater.*, 2003, **15**, 4247-4256.

S2:UV-vis absorption spectra and fluorescence spectra of PMSN*

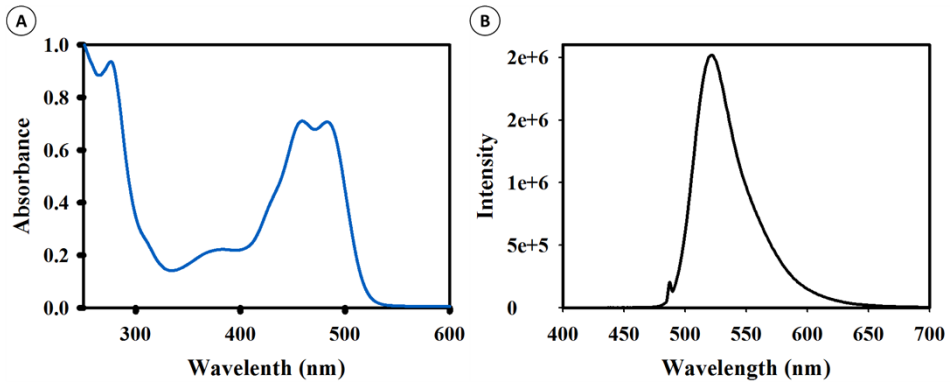


A) UV-Vis spectrum PMSN* (in PBS (pH 7.40) at 1 mg/mL)

B) Fluorescence spectrum PMSN* in PBS (pH 7.40) at 1 mg/mL (λ_{ex} 524nm)[a]

[a]K. Hashimoto, M. Hiramoto and T. Sakata, *J. Phys. Chem.*, 1988, **92**, 4272-4274.

S3: UV-vis absorption spectra and fluorescence spectra of BSA#



A) UV-Vis spectrum of BSA# (in PBS (pH 7.40) at 1 mg/mL)

B) Fluorescence spectrum of BSA# in PBS (pH 7.40) at 0.3 mg/mL (λ_{ex} 488 nm)[a]

[a]M. Bruchez Jr., M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science*, 1998, **281**, 2013-2016.

S4: BSA and BSA$^{\#}$ adsorption study (Langmuir fit)

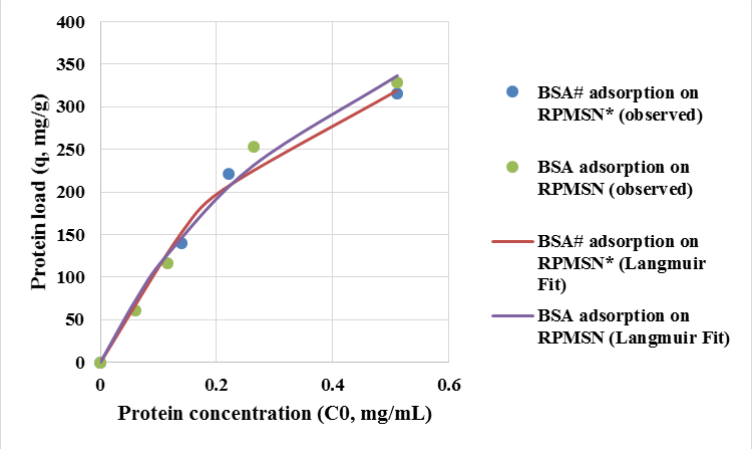
The effect of fluorescent dye labelling on the adsorption properties on the nanoaparticles and proteins was studied and the data was fitted using Langmuir equation (equation 1). While both samples showed similar adsorption profiles and exhibited Langmuir type adsorption, the maximum protein load on the nanoparticles (calculated at C_0 0.5 mg/mL protein concentration) was lower for BSA$^{\#}$ adsorption on PMSN*.

$$q = \frac{q_m K_L C_0}{1 + K_L C_0} \quad \text{------ Equation 1[a]}$$

C_0 is the equilibrium concentration, q_m is maximum load, K_L is the Langmuir constant

Protein	**Nanoparticles**	**Maximum load* (q_m, mg/g)**	**Langmuir constant (K_L, mL/mg)**	**R^2 (Langmuir fit)**
BSA	PMSN	653.0	2.08	0.996
BSA$^{\#}$	PMSN*	544.4	2.79	0.986

* At protein concentration of 0.5 mg/mL



[a]S. H. Brewar, W. R. Glomm, M. C. Johnson, M. K. Knag and S. Franzen, *Langmuir*, 2005, **21**, 9303-9307.

S5: Time dependent uptake of the PMSN* nanoparticles

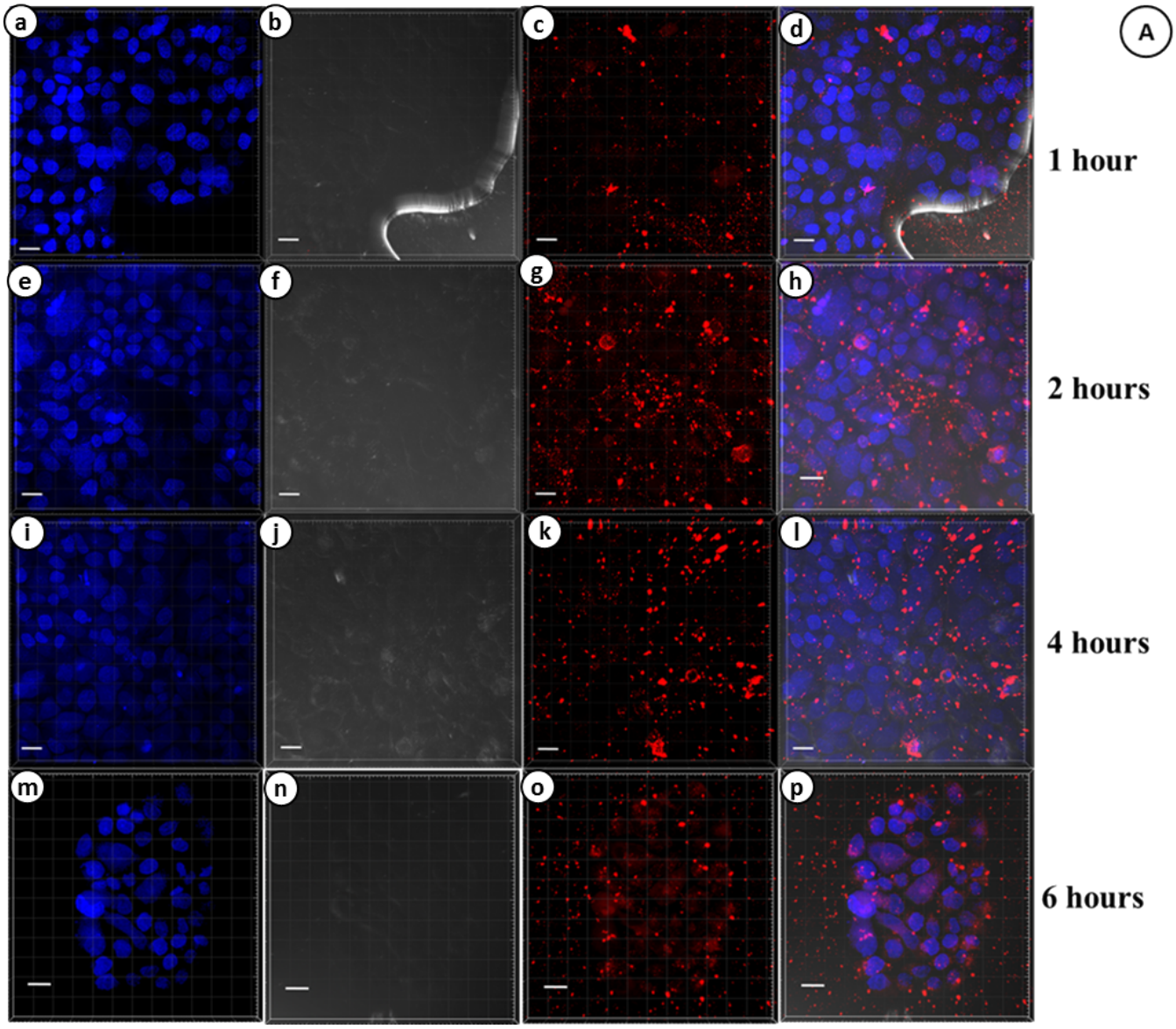


Figure S5: **A**) Time dependent uptake of PMSN* nanoparticles by HeLa cells (left to right for each time point) (Scale 20 µm) Blue features are DAPI stained cell nuclei and red spots are due to Rhodamine labelled nanoparticles PMSN*, merged images on right.

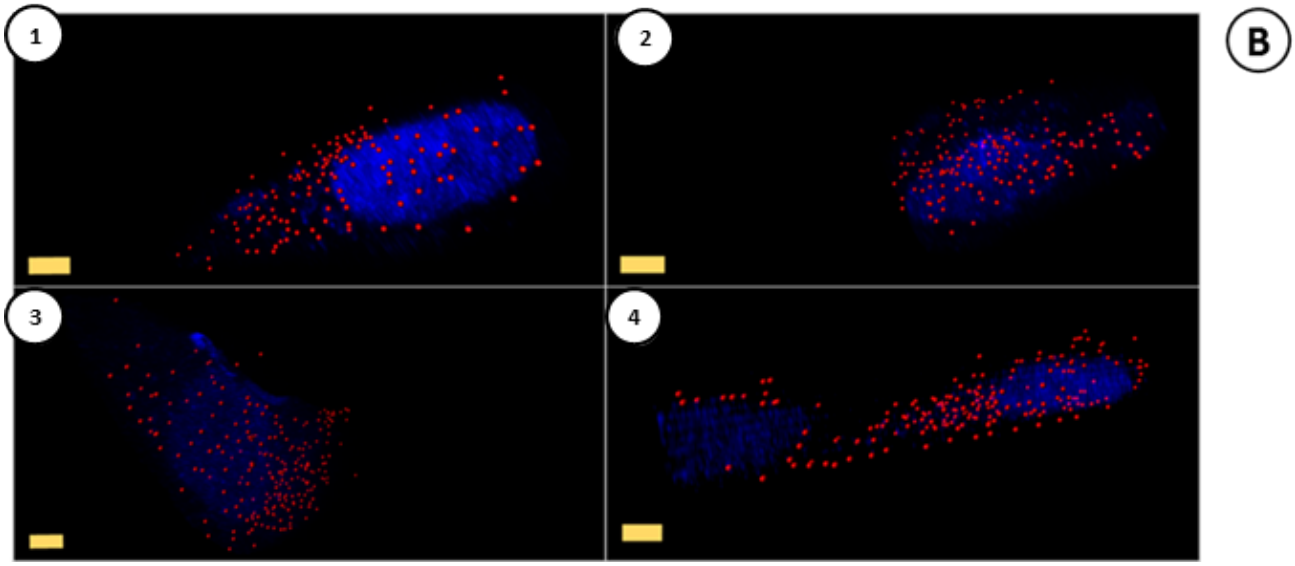


Figure S5: B) 3D distribution of PMSN* nanoparticles in HeLa cells at different exposure times (Scale bar 10 μm in all images); **1**) 1 hour; **2**) 2 hours; **3**) 4 hours and **4**) 6 hours. Blue features are DAPI stained cell nuclei and red spots are due to Rhodamine labelled nanoparticles PMSN*.

S6: Time dependent uptake of the BSA$^{\#}$@PMSN* nanoparticles

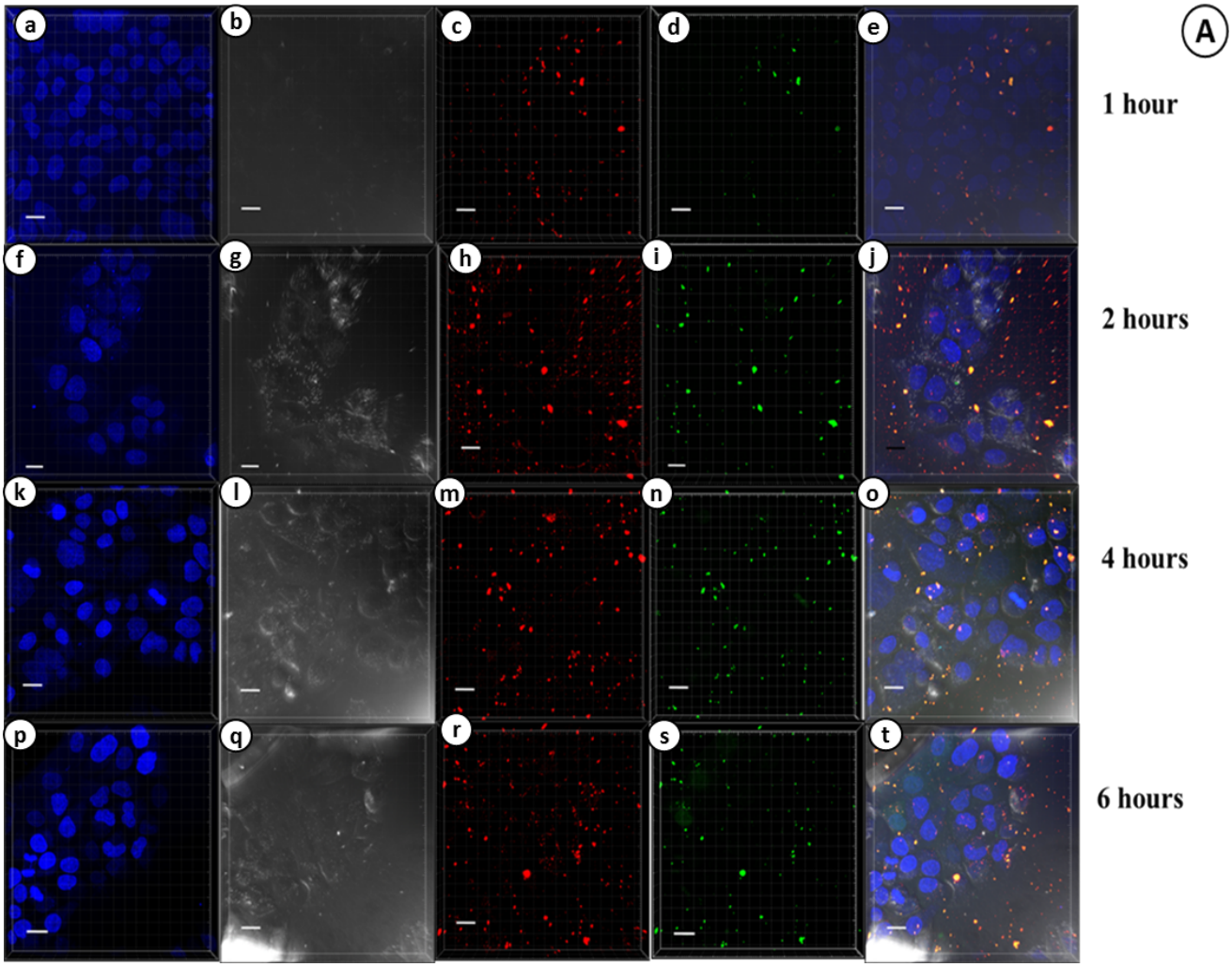


Figure S6: A) Time dependent uptake of BSA$^{\#}$@PMSN* by HeLa cells, (left to right for each time point) (Scale 20 µm). Blue features are DAPI stained cell nuclei, red spots are due to Rhodamine labelled nanoparticles PMSN* and the green spots are due to Fluorescein labelled protein BSA$^{\#}$, merged images on the right hand side.

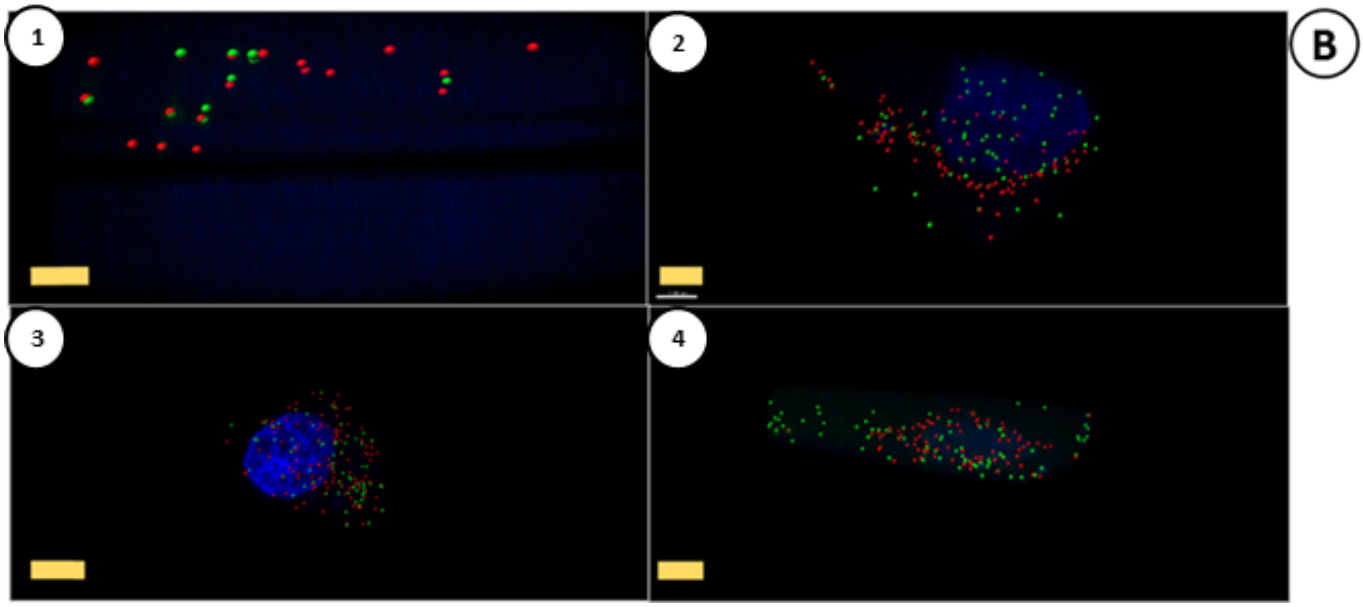


Figure S6: B) 3D distribution of BSA$^{\#}$@PMSN* in HeLa cells at different exposure times; **1**) 1 hour (Scale bar 5 µm); **2**) 2 hours (Scale bar 7 µm); **3**) 4 hours (Scale bar 10 µm) and **4**) 6 hours (Scale bar 5 µm). Blue features are DAPI stained cell nuclei, red spots are due to Rhodamine labelled nanoparticles PMSN* and the green spots are due to Fluorescein labelled protein BSA$^{\#}$.

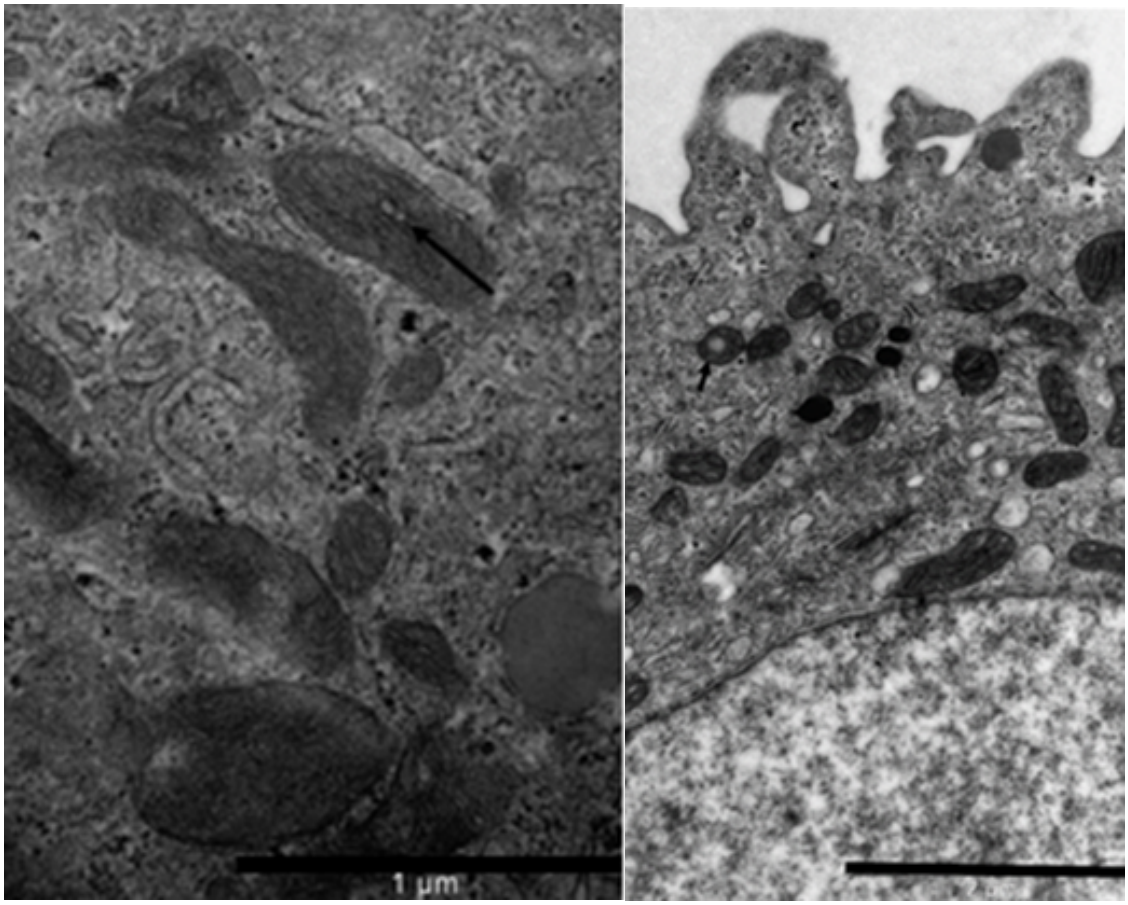


Figure S6: C) **Additional TEMs showing possible localisation (black arrows) of BSA$^{\#}$@PMSN* nanoparticles in mitochondria (scale bar = 1µm).**

S7: BSA$^{\#}$ load dependent uptake of the BSA$^{\#}$@PMSN* nanoparticles

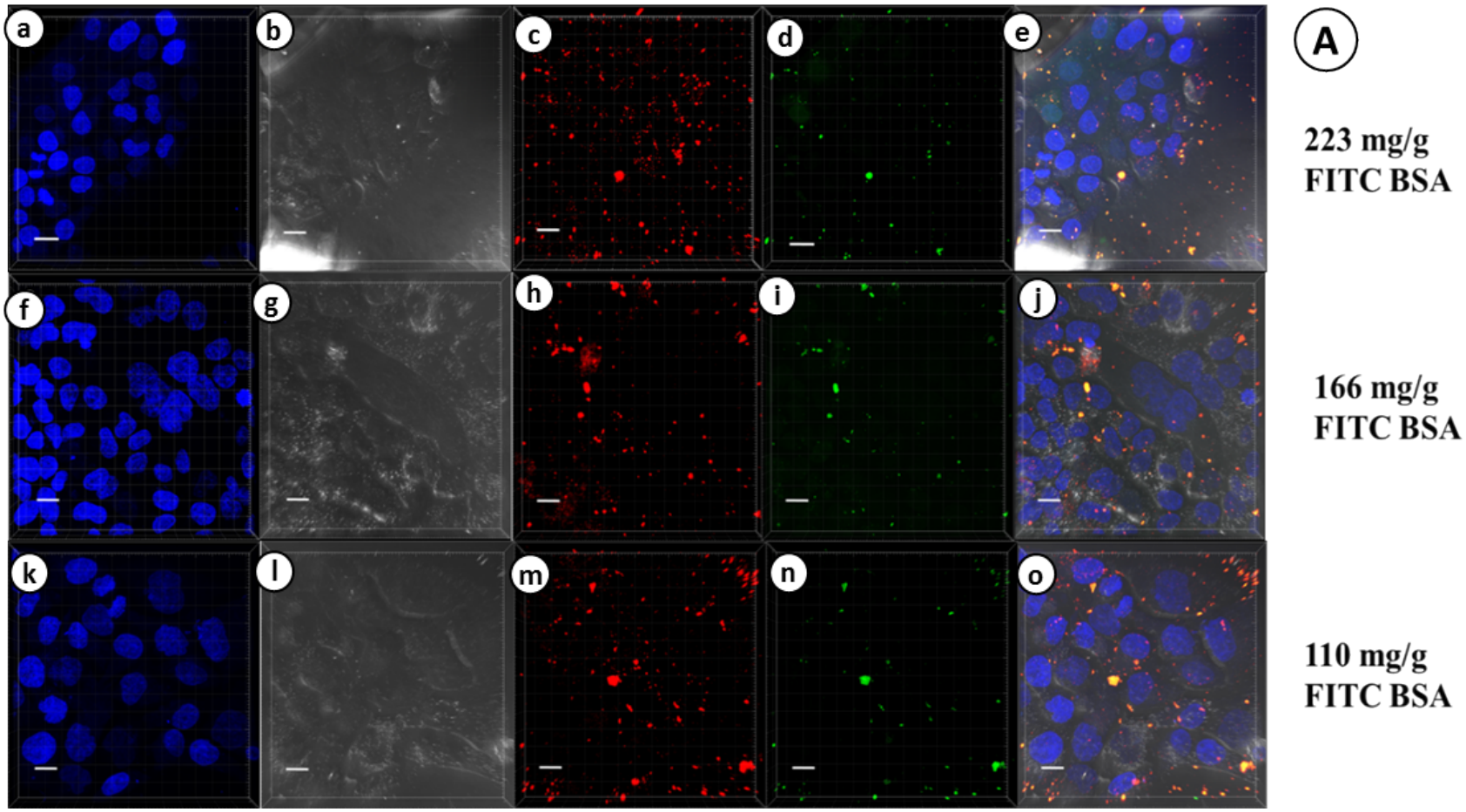


Figure S7: A) Confocal microscope images showing the effect of protein load in BSA$^{\#}$@PMSN* particles on particle uptake, (left to right for each BSA$^{\#}$ load) (Scale bar 20 μm). Blue features are DAPI stained cell nuclei, red spots are due to Rhodamine labelled nanoparticles PMSN* and the green spots are due to Fluorescein labelled protein BSA$^{\#}$, merged images on the right hand side.

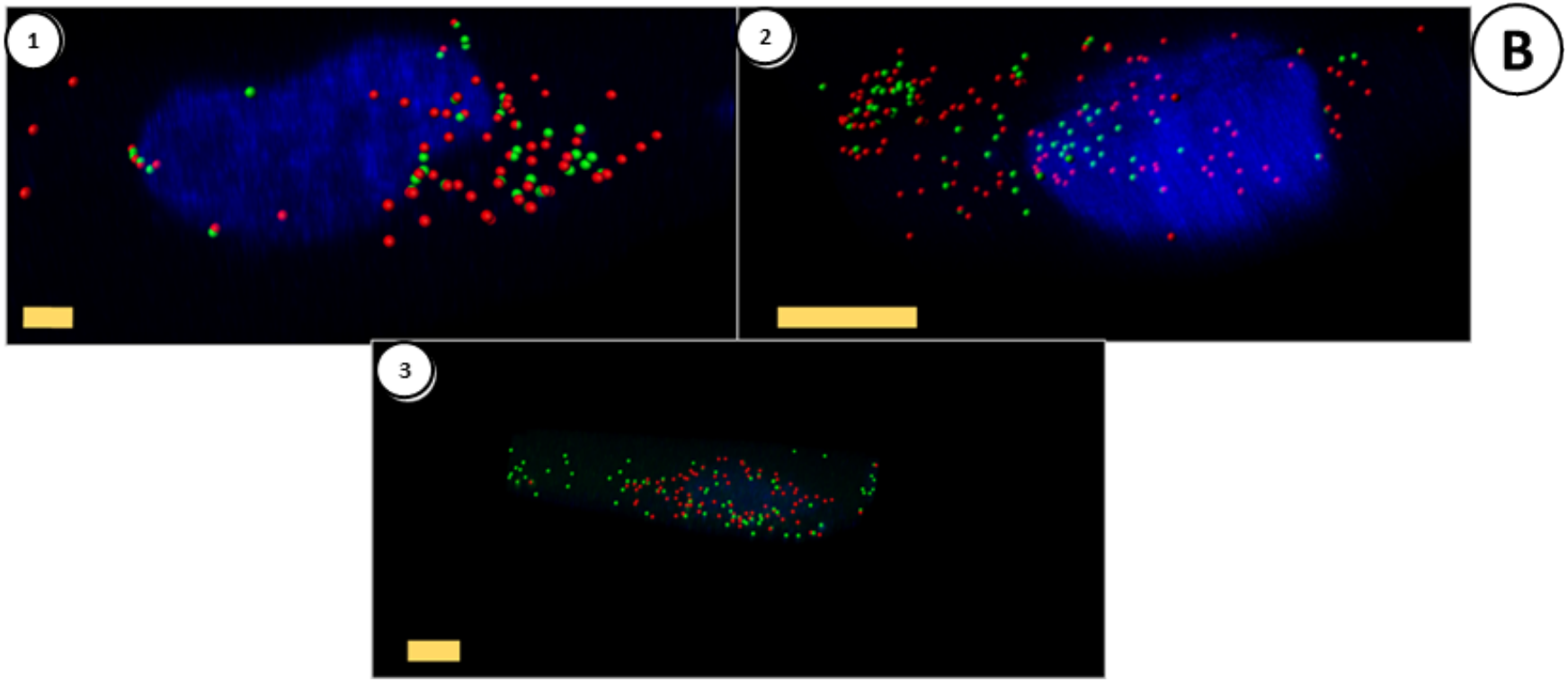


Figure S7 B) 3D distribution of BSA[#]@PMSN* nanoparticles in HeLa cells at different protein load; **1**) 111.1 mg/g (Scale bar 5 µm); **2**) 166.7 mg/g (Scale bar 5 µm); **3**) 223.0 mg/g (Scale bar 10 µm); Blue features are DAPI stained cell nuclei, red spots are due to Rhodamine labelled nanoparticles PMSN* and the green spots are due to Fluorescein labelled protein BSA[#].

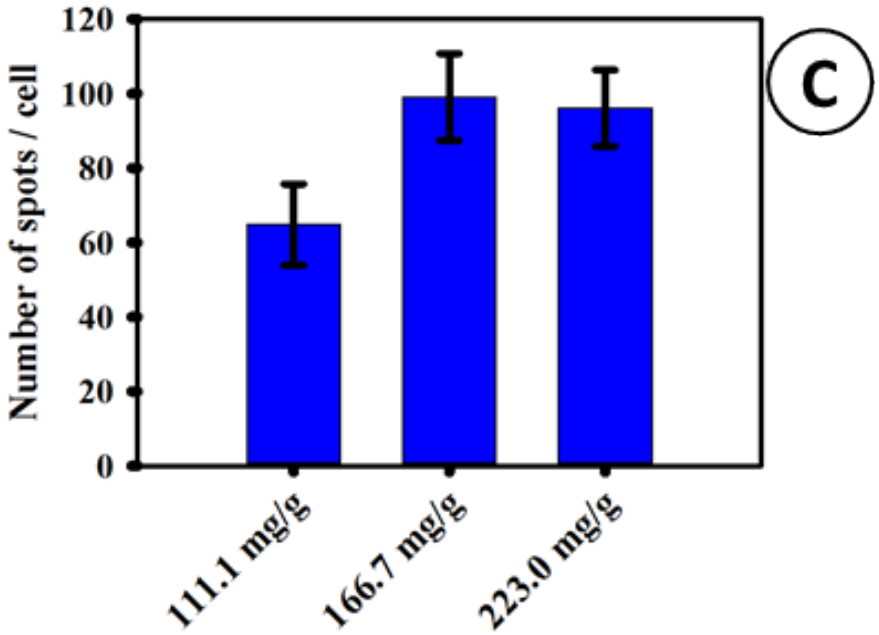


Figure S7 C) BSA[#]@PMSN* Particle uptake versus protein load. (n = 20 ± SEM.)

S8: Effect of incubation in DMEM on PMSN* loaded HeLa cells

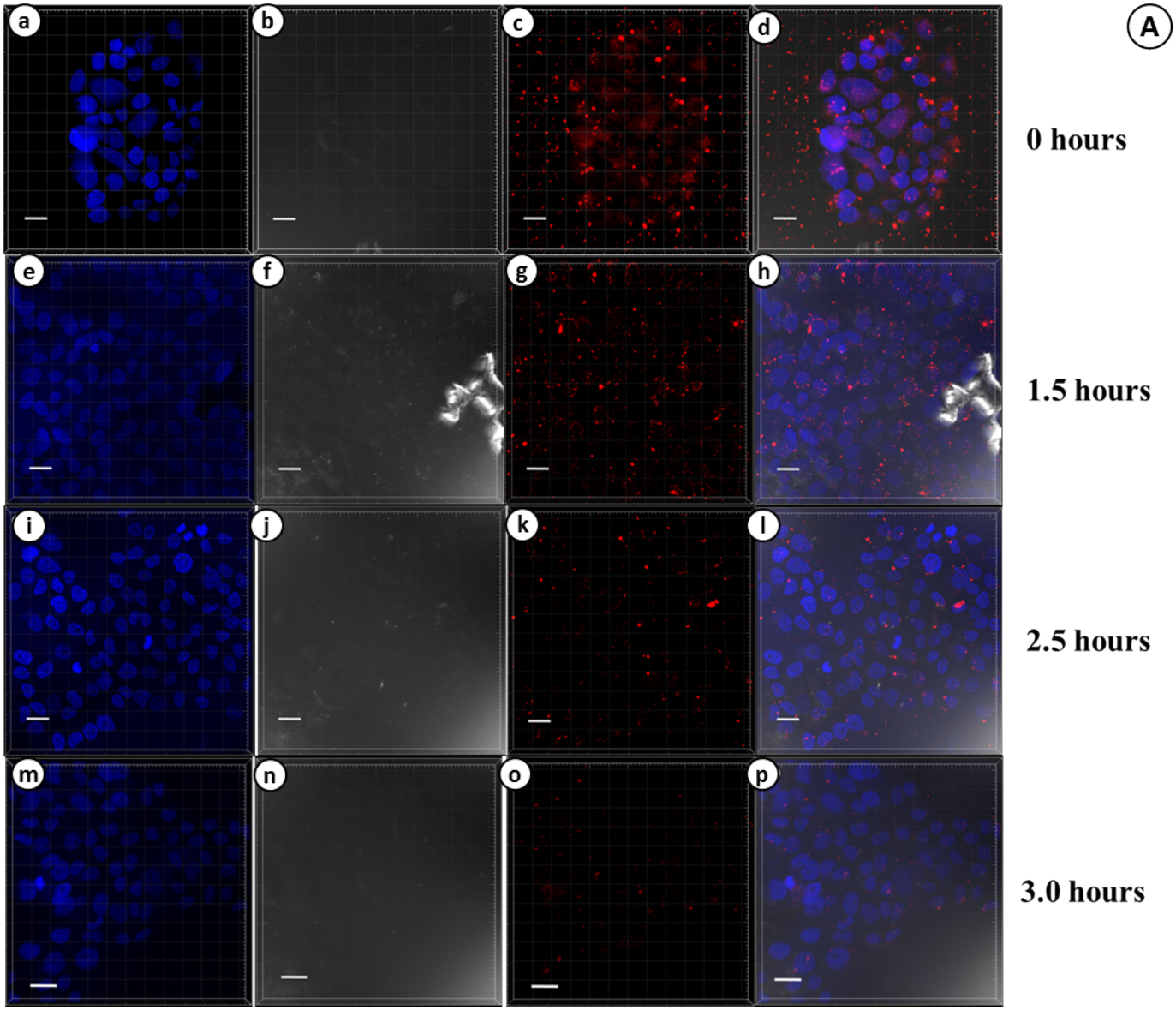


Figure S8: **A)** Effect of incubation in DMEM on PMSN* in HeLa cells, (left to right for each time point, control at 0 hours) (Scale 20 µm). Blue features are DAPI stained cell nuclei and red spots are due to Rhodamine labelled nanoparticles PMSN*, merged images on right.

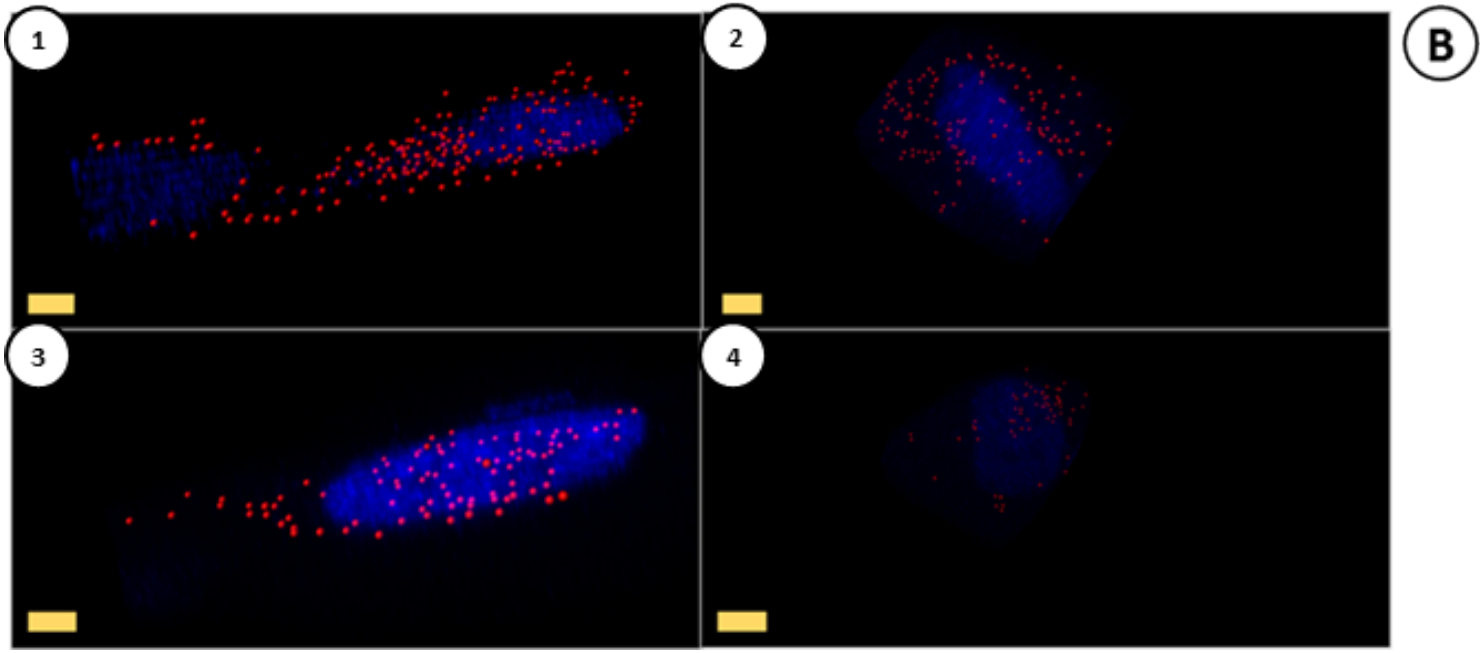


Figure S8: B) 3D Distribution of PMSN* nanoparticles in HeLa cells at different incubation times in particle free media; **1**) 0 hours (Scale bar 10 µm); **2**) 1.5 hours (Scale bar 10 µm); **3**) 2.5 hours (Scale bar 10 µm) and **4**) 3 hours (Scale bar 10 µm).). Blue features are DAPI stained cell nuclei and red spots are due to Rhodamine labelled nanoparticles PMSN*.

S9: Effect of incubation in DMEM on BSA#@PMSN* loaded HeLa cells.

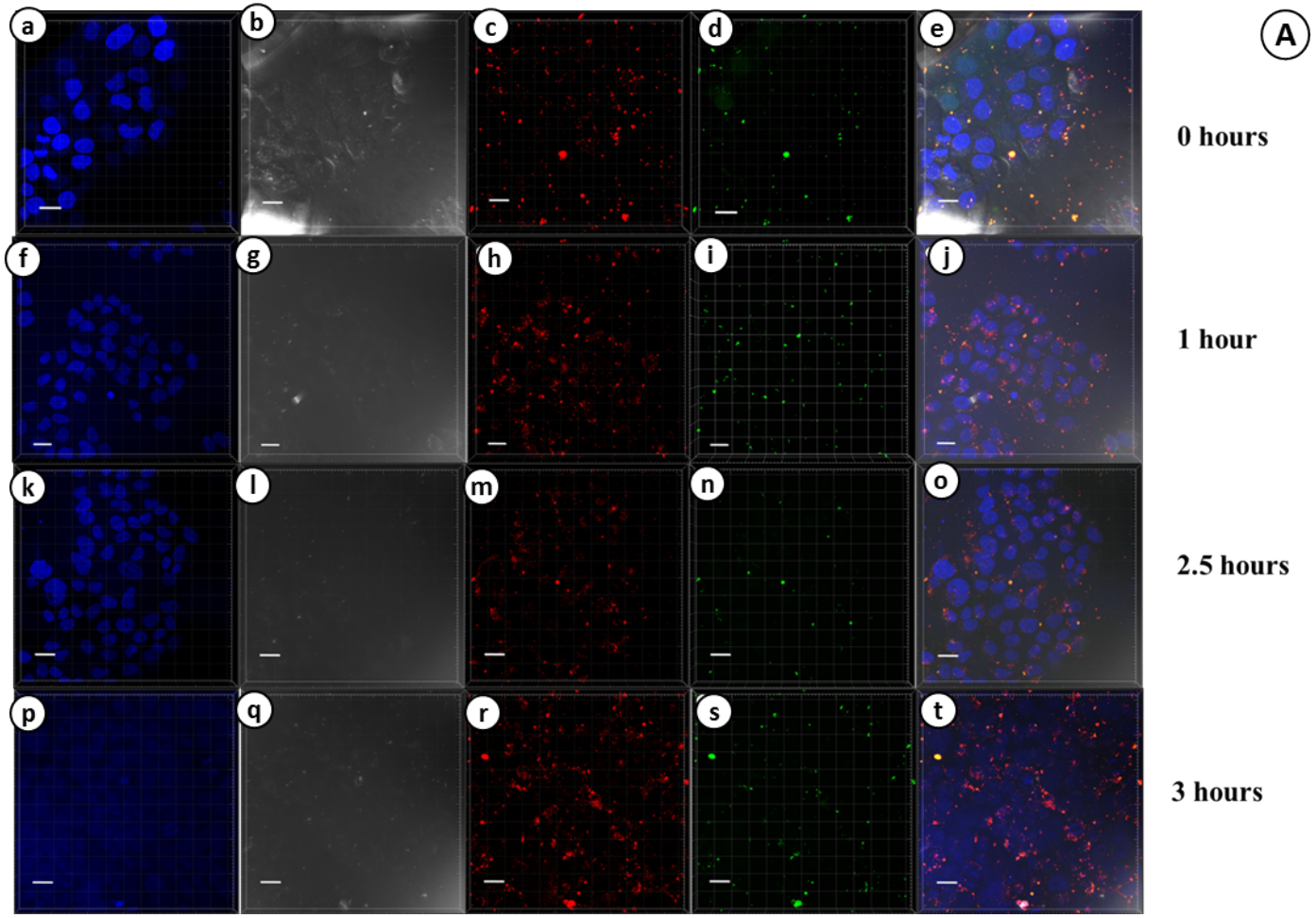


Figure S9: A) Effect of incubation in DMEM on BSA#@PMSN* in HeLa cells, (left to right for each time point, control at 0 hours) (Scale 20 µm). Blue features are DAPI stained cell nuclei, red spots are due to Rhodamine labelled nanoparticles PMSN* and the green spots are due to Fluorescein labelled protein BSA#, merged images on the right hand side.

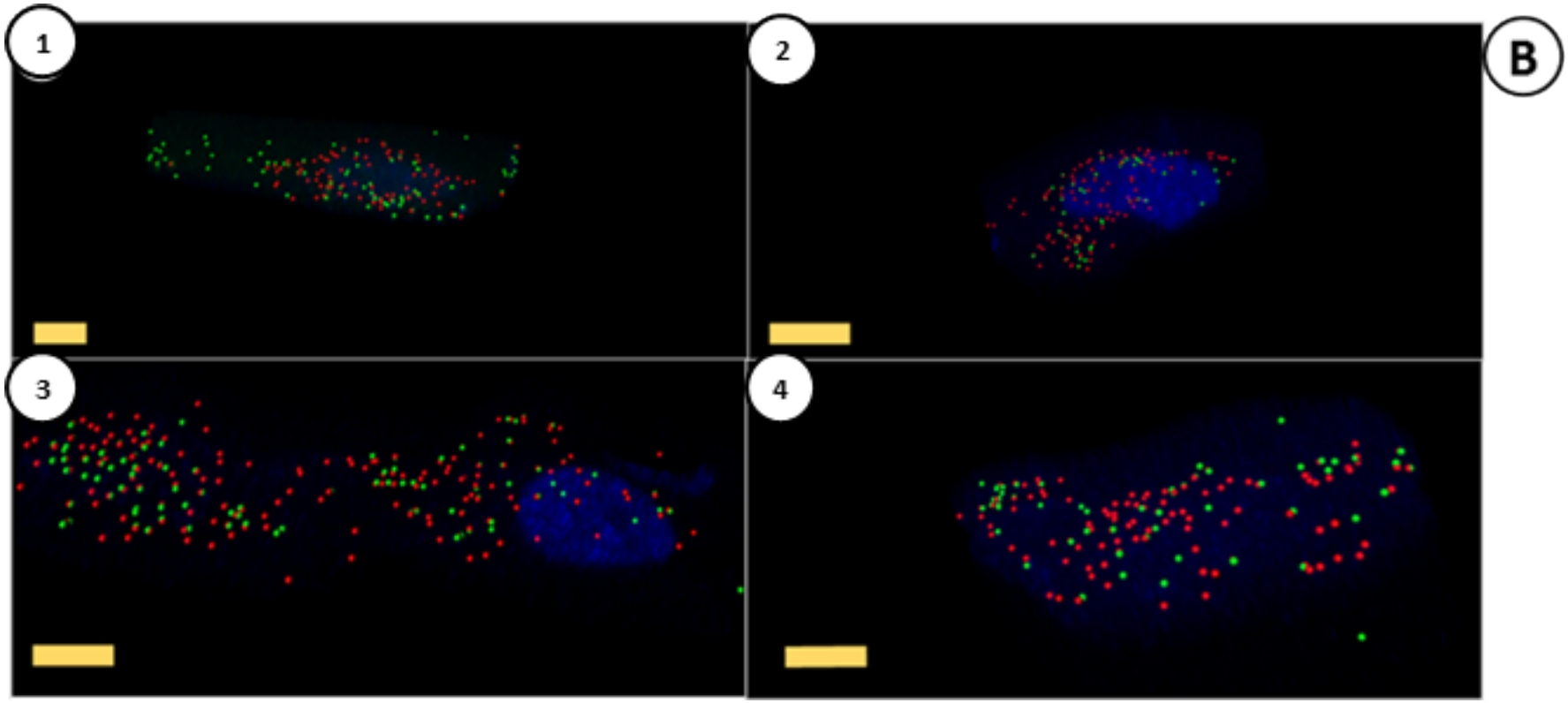


Figure S9: B) 3D Distribution of BSA$^{\#}$PMSN* nanoparticles in HeLa cells at different incubation times in particle free media; **1**) 0 hours (Scale bar 10 µm); **2**) 1.5 hours (Scale bar 10 µm); **3**) 2.5 hours (Scale bar 10 µm) and **4**) 3 hours (Scale bar 10 µm). Blue features are DAPI stained cell nuclei, red spots are due to Rhodamine labelled nanoparticles PMSN* and the green spots are due to Fluorescein labelled protein BSA$^{\#}$.