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Electronic Supplementary Material

Effective SARS-CoV-2 antiviral activity of hyperbranched polylysine nanoparticles

Luigi Stagi[1], Davide De Forni[2], Luca Malfatti[1], Francesca Caboi[3], Andrea Salis[4], Barbara Poddesu[2], Giulia Cugia[2], Franco Lori[2], Grazia Galleri[5], Plinio Innocenzi[1,*]

[1]*Laboratorio di Scienza dei Materiali e Nanotecnologie (LMNT), Dipartimento di Chimica e Farmacia, CR-INSTM, Università di Sassari. Via Vienna 2, 07041 Sassari, Italy.*
[2]*ViroStatics srl, Viale Umberto I, 46, 07100 Sassari, Italy*
[3]*Laboratorio NMR e Tecnologie Bioanalitiche, Sardegna Ricerche, Parco Scientifico e Tecnologico della Sardegna, 09010 Pula (CA), Italy*
[4]*Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, Cittadella Universitaria, SS 554 bivio Sestu. 09042 Monserrato (CA), Italy*
[5]*Dipartimento di Science Mediche, Chirurgiche e Sperimentali, Viale S. Pietro 8, 07100 Sassari, Italy*

**Corresponding author: plinio@uniss.it*

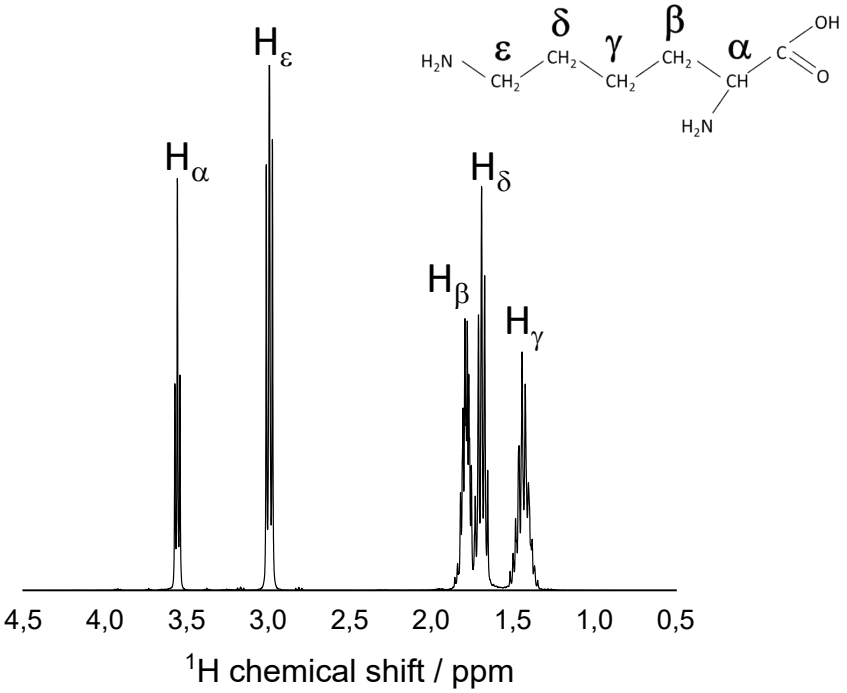


Figure S1. ^{1}H NMR of L-Lysine

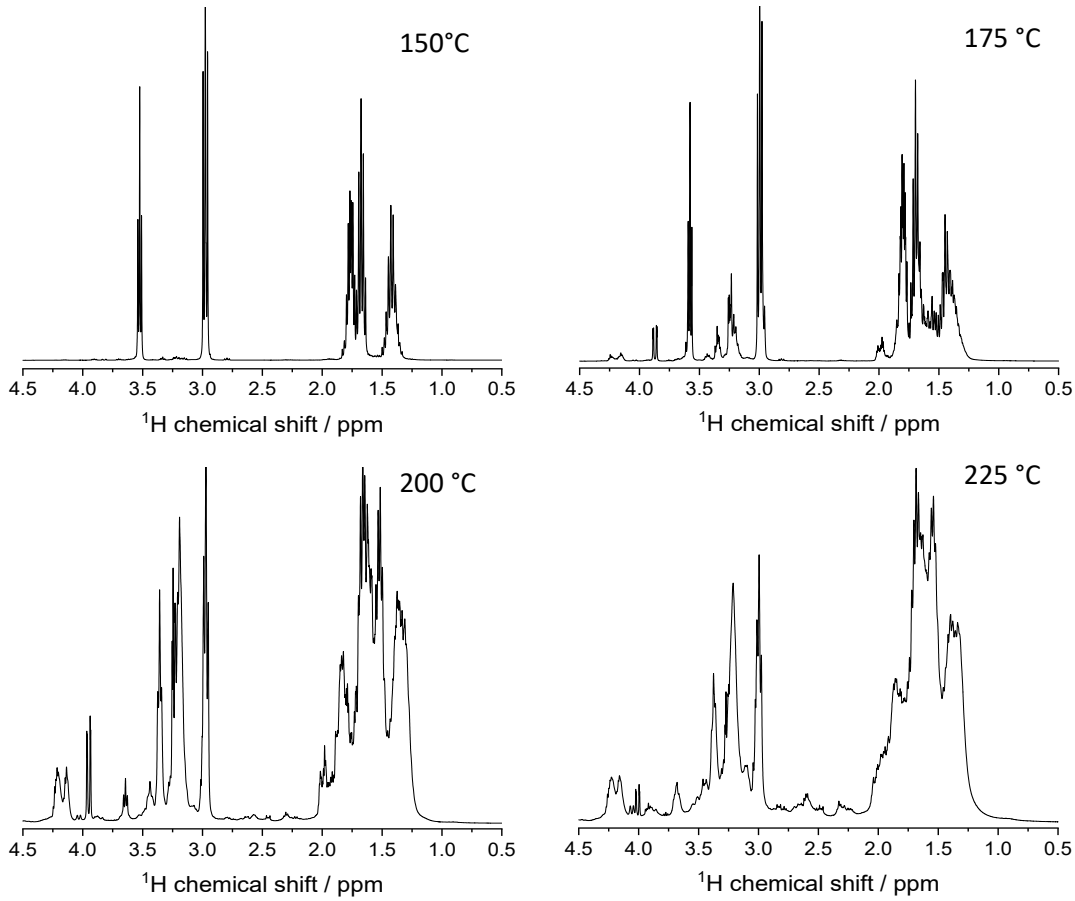
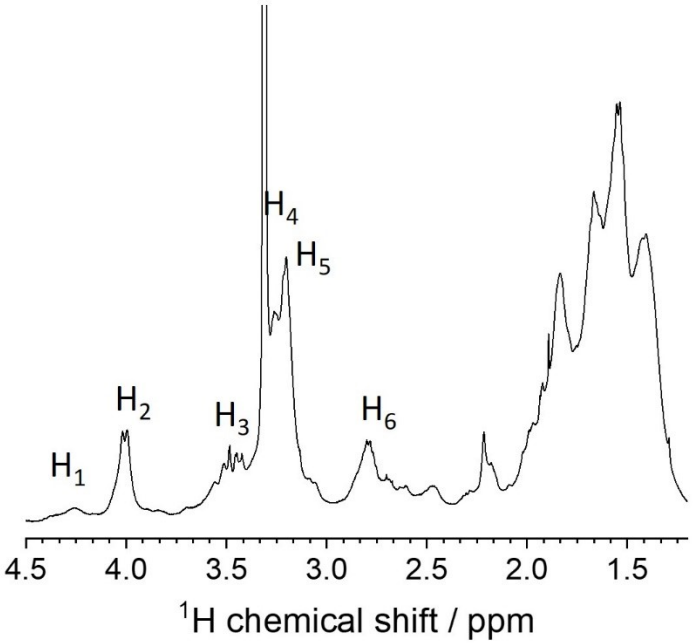


Figure S2. ^{1}H NMR of L-Lysine – H_3BO_3 as a function of the thermal polymerization temperature


H1
H2
H3
H4
H5
H6
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1H chemical shift / ppm

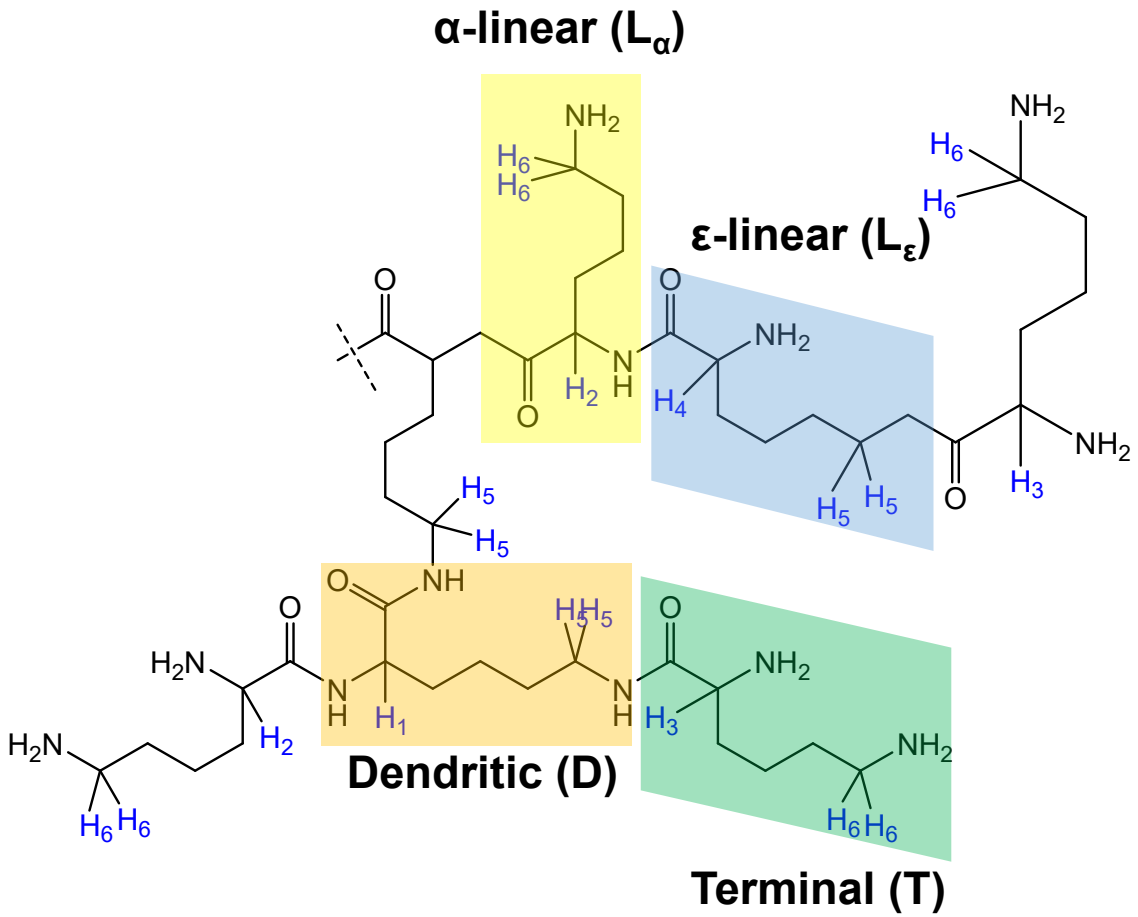


Figure S3. ^{1}H NMR spectra of lysine (top) and hyperbranched polylysine nanopolymers (middle). Attribution of the chemical shifts (bottom) has been done following the data published in: Scholl, M., Nguyen, T. Q., Bruchmann, B., Klok, H.-A. The Thermal Polymerization of Amino Acids Revisited; Synthesis and Structural Characterization of Hyperbranched Polymers from L-Lysine. J. Polymer Sci.: Part A: Polymer Chem., 45, 5494–5508 (2007). The ^{1}H NMR data are consistent with the formation of a hyperbranched polylysine structure via thermal induced amidation reactions, in accordance with FTIR and DSC data.

H_6 2.67 ppm ε–CH_2 group in a α-linear and terminal structural unit

H_5 3.19 ppm ε-CH_2 group next to an ***amide bond*** (dendritic and ε-linear structural unit)

H_4 3.25 ppm α–CH protons of the ε-linear structural units

H_3 3.33 ppm α–CH protons of the terminal structural units

H_2 4.02 ppm α–CH protons of the α-linear structural units

H_1 4.24 ppm α–CH protons of the dendritic protons

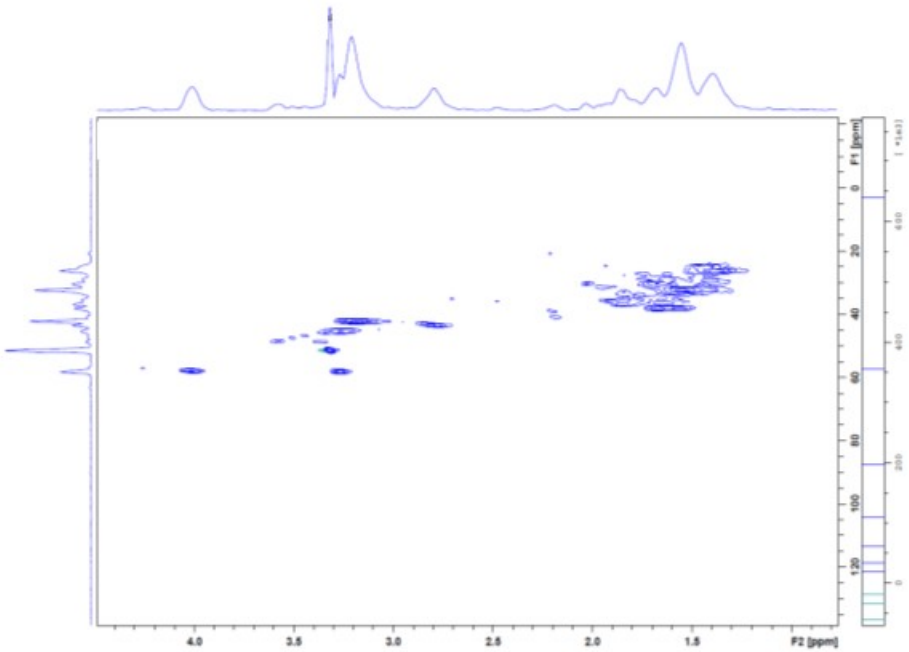


Figure S4. 2D NMR spectra ^{13}C (y axis) ^{1}H (x axis) of HPN in D_2O.

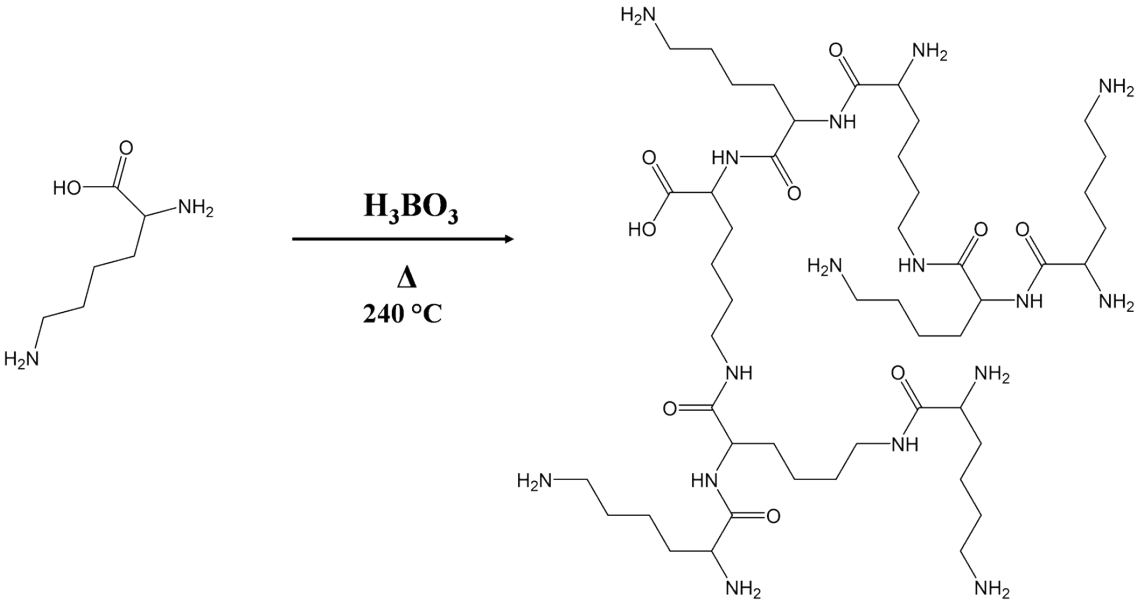


Figure S5. Formation of a hyperbranched polylysine via thermal polymerization.

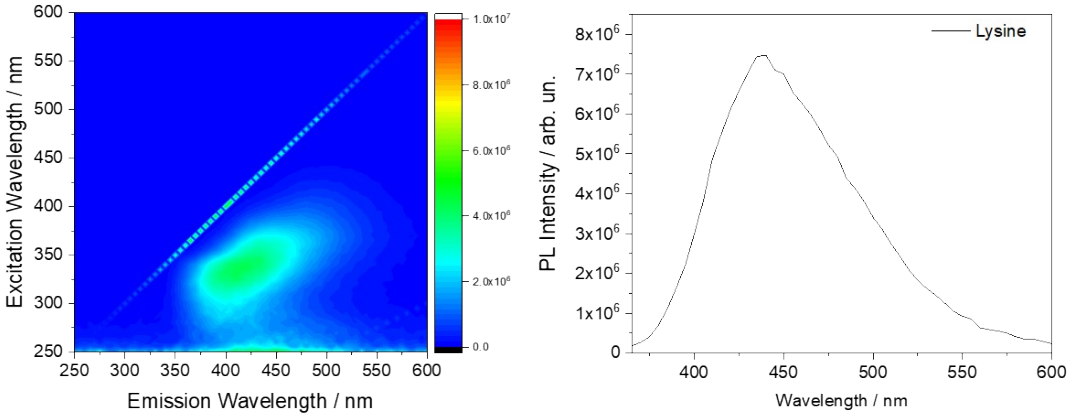


Figure S6. Three-dimensional fluorescence graph [excitation (y)−emission (x)−intensity (z)] of lysine in water (left). Emission spectrum of lysine upon excitation at 350 nm (right).

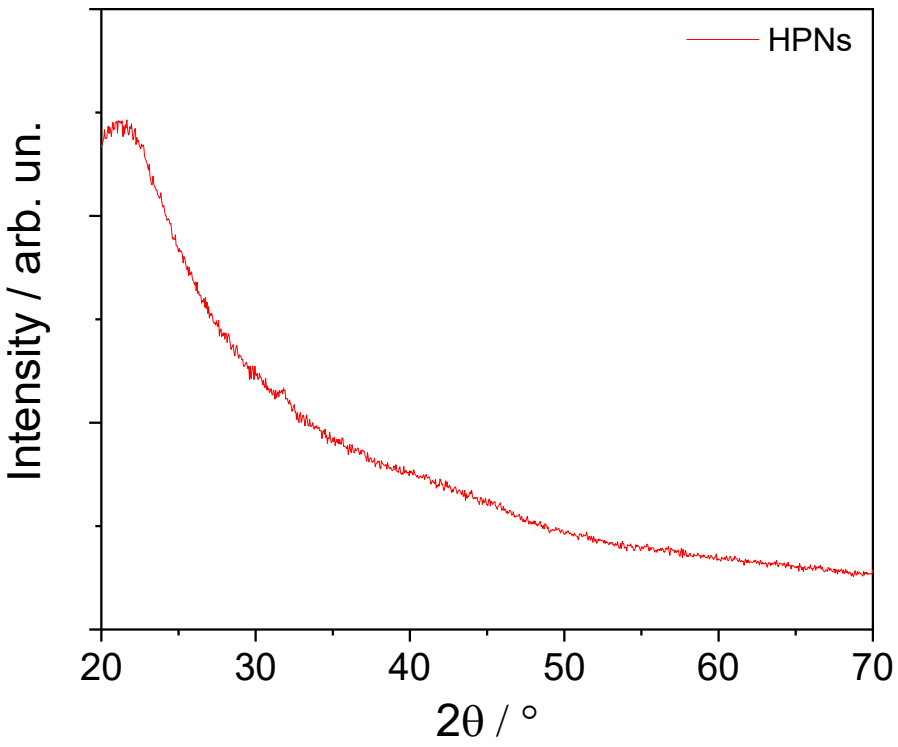


Figure S7. X-ray diffraction analysis of the HPN sample. The diffraction pattern supports the amorphous nature of the HPNs.

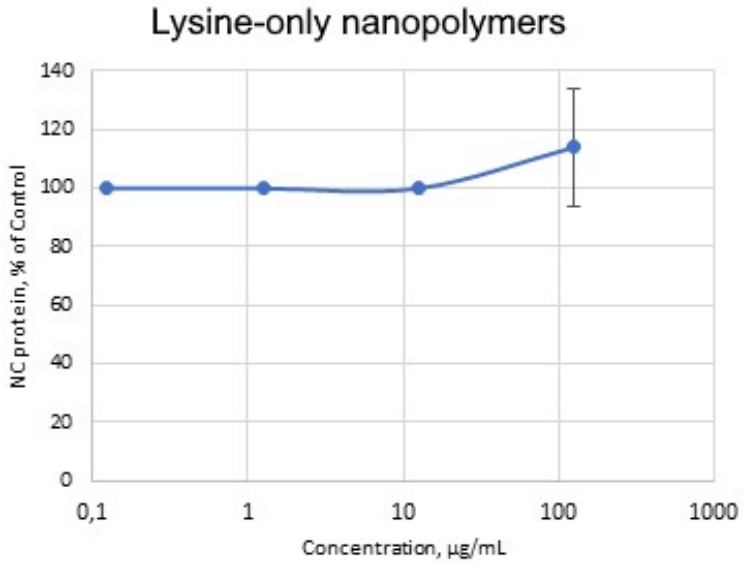


Figure S8. Effect of L-Lysine only polymeric nanoparticles on SARS-CoV-2 replication. The nanomaterial does not show any antiviral activity.

Table S1. Antiviral activity data of test compounds at the different concentrations, i.e., % viral nucleocapsid protein, NC, compared to untreated infected control (= 100%).

Remdesivir		**HPNs**		**Lysine-only nanopolymers**	
Concentration ($\mu g\ mL^{-1}$)	**Antiviral activity (% viral NC protein, compared to untreated control)**	**Concentration ($\mu g\ mL^{-1}$)**	**Antiviral activity (% viral NC protein, compared to untreated control)**	**Concentration ($\mu g\ mL^{-1}$)**	**Antiviral activity (% viral NC protein, compared to untreated control)**
6	14 ± 7	500	14 ± 7	125	114 ± 20
1.2	73 ± 9	50	79 ± 41	12.5	100 ± 0
0.24	96 ± 10	5	95 ± 19	1.25	100 ± 0
0.05	107 ± 23	0.5	94 ± 9	0.125	100 ± 0

Lysine-only nanomaterial was not effective in reducing SARS-CoV-2 viral replication, with a 50% inhibitory concentration (IC_{50}) value higher than 125 $\mu g\ mL^{-1}$.

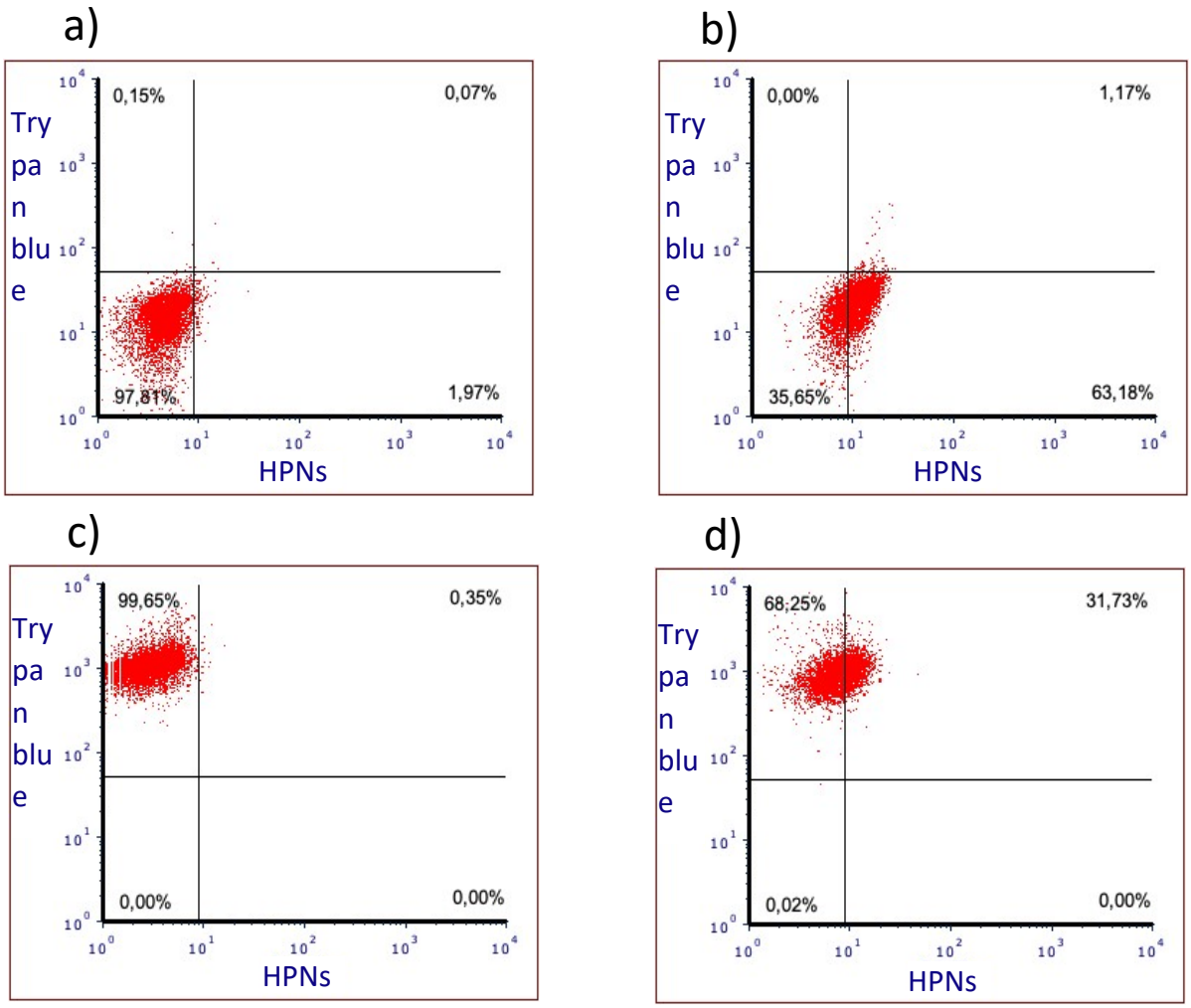


Figure S9. Dot plots for control cells (a) and cells pulsed with 250 µg mL^{-1} of HPNs for 24 hours (b); dot plots for control cells (c) and cells pulsed with 250 µg mL^{-1} of HPNs for 24 hours (d) after treatment with trypan blue 0.025%, showing only partial quenching of extracellular fluorescence and confirming internalization of HPNs.