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SUPPORTING INFORMATION

Bioinspired polydopamine nanoparticles: Synthesis, nanomechanical properties, and efficient PEGylation strategy

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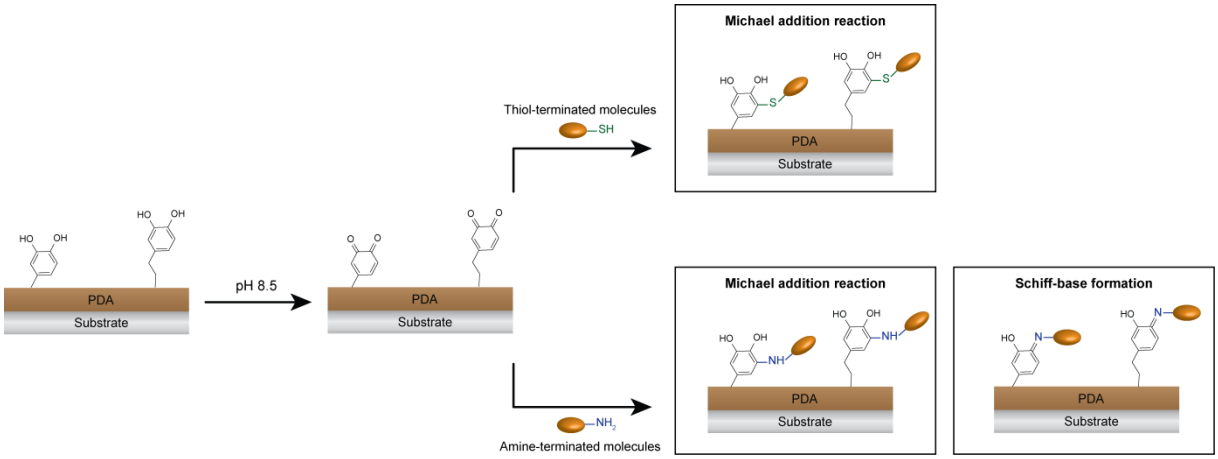


Figure S1: Polydopamine-coated surface functionalization with thiol or amine-terminated molecules, thiol-terminated ligands can be grafted on the PDA catechol groups via Michael addition reaction, while amine-terminated ones are coupled via Michael-addition reaction or Schiff-base condensation reaction.

	Reaction time = 24 H	Reaction time = 30 H
D_h (nm)	164 ± 1	168 ± 4
Yield (%)	31.1	31.8

Table S1: Impact of polymerization reaction time on the size and yield of PDA NPs prepared with [DA] = 3.2 mg/ml and [Ammonia]/[DA] = 25 at 25°C.

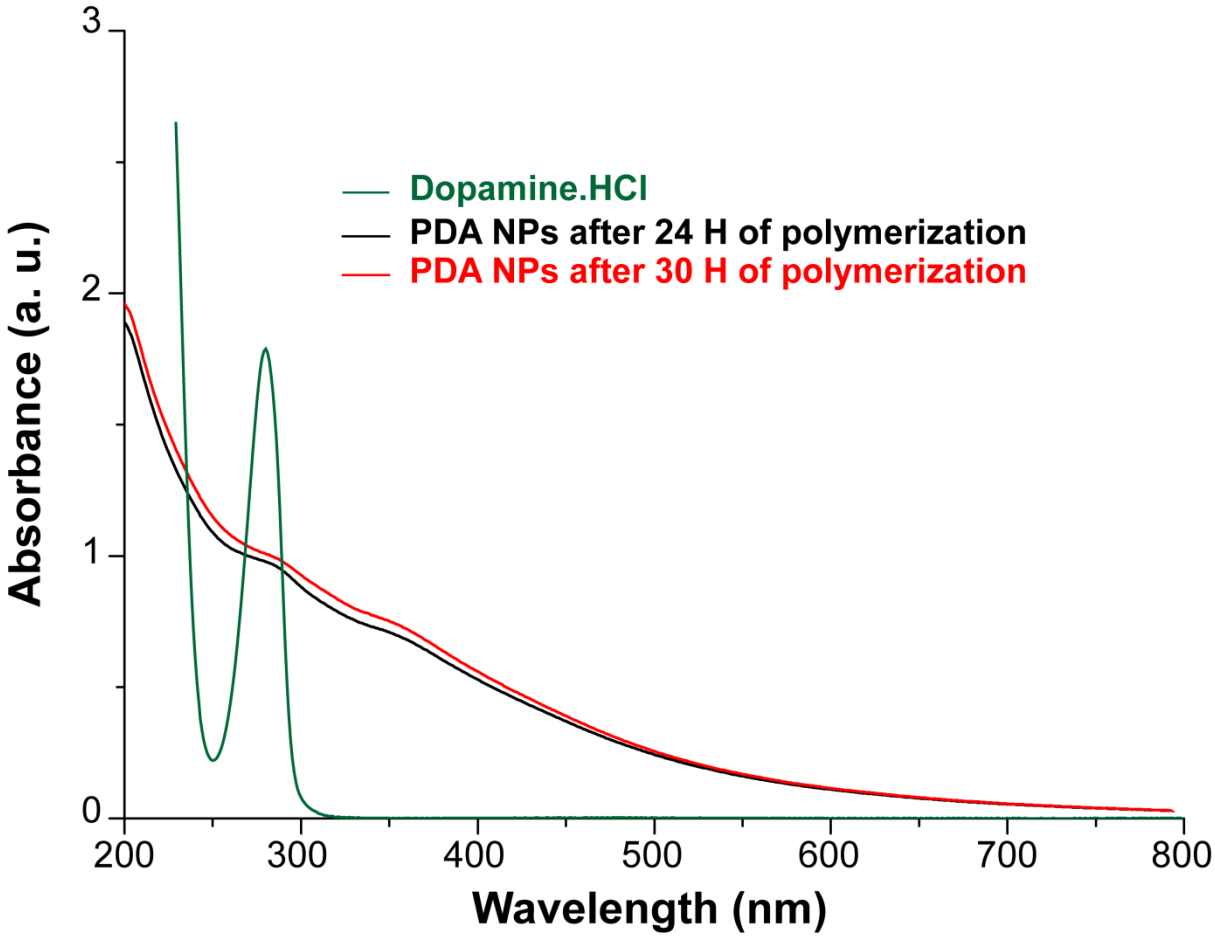


Figure S2: UV-visible absorption spectra of purified PDA NPs obtained after 24 hours (black) or 30 hours (red) of reaction time. Spectra are presented in comparison to that of a dopamine hydrochloride aqueous solution (green).

Supplementary equations

Response surface models generated by central composite analysis for PDA NPs formulation optimization study, T is the experimental temperature (°C) and R the Ammonia/Dopamine molar ratio.

$$\boldsymbol{D_h}\ (\boldsymbol{nm}) = -140.89087 + 58.00798\ \mathrm{T} - 18.45587\ \mathrm{R} - 0.041939\ \mathrm{T\ R} - 1.78885\ T^2 + 0.335141\ R^2 + 0.002083\ T^2\ \mathrm{R} - 0.000845\ \mathrm{T}\ R^2 + 0.016389\ T^3 - 0.001973\ R^3$$

$$\boldsymbol{Yield}\ (\%) = -178.23531 + 18.55453\ \mathrm{T} - 0.828241\ \mathrm{R} + 0.001854\ \mathrm{T\ R} - 0.497238\ T^2 + 0.008004\ R^2 + 0.000245\ T^2\ \mathrm{R} - 0.000155\ R^2 + 0.004404\ T^3 - 7.90\mathrm{E}{-06}\ R^3$$

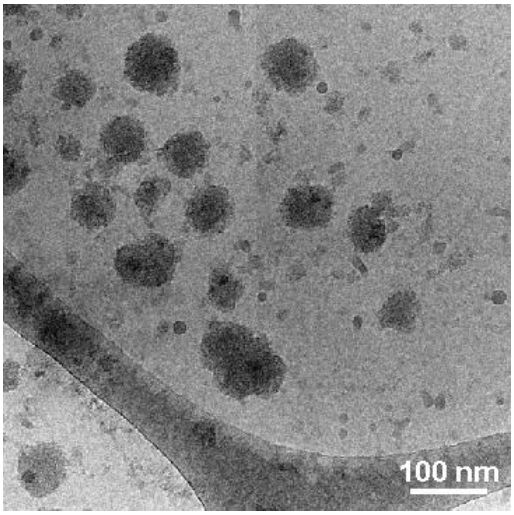
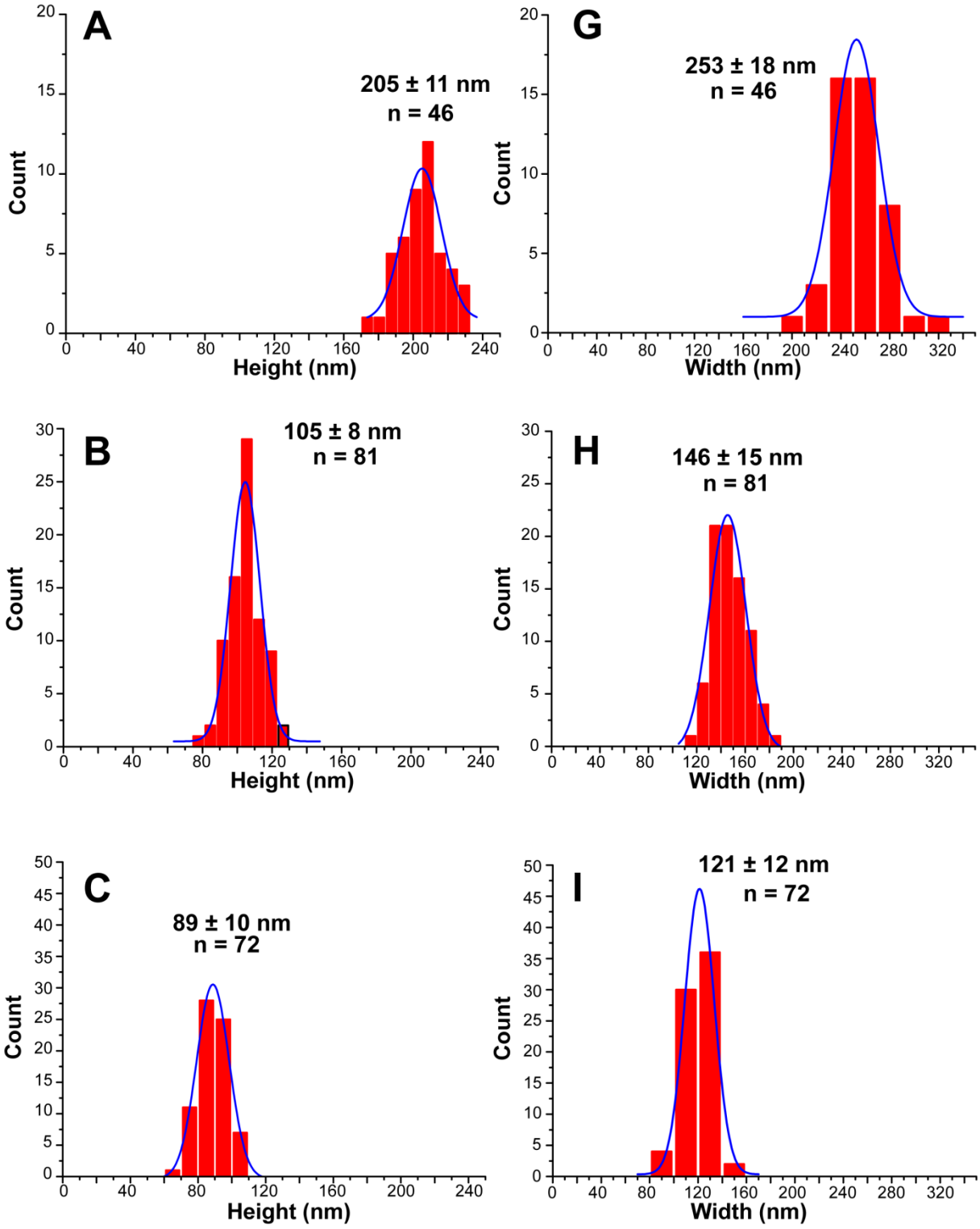


Figure S3: Cryogenic Transmission Electronic Microscopy (Cryo-TEM) image of PDA NPs prepared with [DA] = 3.2 mg/ml and [Ammonia]/[DA] = 25 at 25°C.


A
205 ± 11 nm
n = 46
Count
Height (nm)
G
253 ± 18 nm
n = 46
Count
Width (nm)
B
105 ± 8 nm
n = 81
Count
Height (nm)
H
146 ± 15 nm
n = 81
Count
Width (nm)
C
89 ± 10 nm
n = 72
Count
Height (nm)
I
121 ± 12 nm
n = 72
Count
Width (nm)

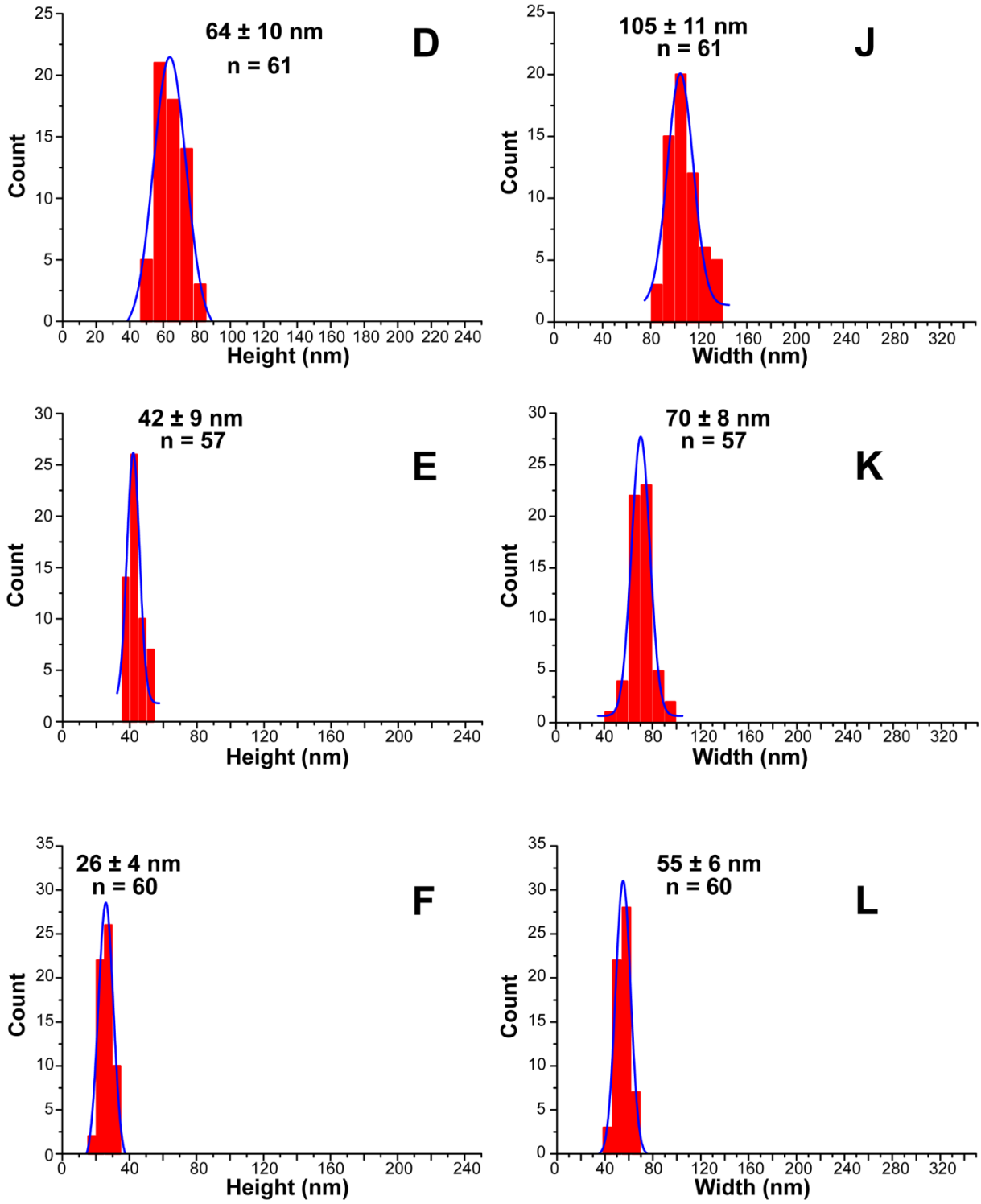


Figure S4: Histograms distributions with the Gaussian fits (blue lines) of the heights (A-F) and widths (G-L) of PDA NPS as measured from the AFM images in air using AM-AFM. These NPs were prepared at [DA] = 3.2 mg/ml and a temperature of 25°C at different [Ammonia]/[Dopamine] ratios (A,G: 10; B,H: 20; C,I: 25; D,J: 30; E,K:38; F,L: 50).

[Ammonia]/ [Dopamine]	T° = 25 °C		T° = 30 °C		T° = 40 °C		T° = 50 °C	
	D_h (nm)	PdI	D_h (nm)	PdI	D_h (nm)	PdI	D_h (nm)	PdI
R10	294±17	0.02±0.10	279±8	0.03±0.01	236±13	0.03±0.02	206±37	0.10±0.03
R20	184±13	0.03±0.01	193±17	0.05±0.01	133±7	0.08±0.01	129±5	0.09±0.03
R25	162±19	0.03±0.02	149±5	0.06±0.01	87±9	0.16±0.03	111±10	0.14+0.03
R30	138±14	0.05±0.01	124±6	0.06±0.01	82±4	0.17±0.04	88 ± 9	0.18±0.02
R38	101±8	0.07±0.02	83±3	0.12±0.04	55 ± 9	0.25±0.10	70 ± 11	0.30±0.02
R50	73±7	0.14±0.05	70±10	0.18±0.06	39 ± 16	0.35±0.13	49 ± 12	0.50±0.17

Table S2: Hydrodynamic diameters and the polydispersity indexes (PdI) of the different PDA nanoparticles prepared in this work. Each reported value is the average ± standard deviation of 3 different batches.

Impact of reaction temperature on the size and yield of PDA nanoparticles

Groups	Size		Yield	
	F-value	***p-value***	***F-value***	***p-value***
R10	31	1.4E-09	12.8	0.2E-02
R20	62.4	9.1E-13	20	0.4E-03
R25	73.7	1.8E-14	23.4	0.3E-03
R30	83.8	3E-15	31.8	8.5E-05
R38	50	3.5E-12	28.8	0.1E-03
R50	17.7	6E-07	29.8	0.1E-03

Table S3: One-way analysis of variance (ANOVA) between the groups of PDA nanoparticles prepared at different temperatures for each [Ammonia]/[DA] ratio. *P*-value less than 0.01 was considered statistically significant.

Impact of [Ammonia]/[Dopamine] molar ratio on the size and yield of PDA nanoparticles

Groups	Size		Yield	
	F-value	***P-value***	***F-value***	***P-value***
25°C	290.5	1.26E-34	23.6	7.9E-06
30°C	629.5	1.64E-42	18.4	2.9E-05
40°C	422.8	1.9E-38	7.2	0.2E-02
50°C	83.4	1.3E-21	**0.1**	**0.99**

Table S4: One-way analysis of variance (ANOVA) between the groups of PDA nanoparticles prepared at different [Ammonia]/[DA] ratios for each temperature. *P*-value less than 0.01 was considered statistically significant.

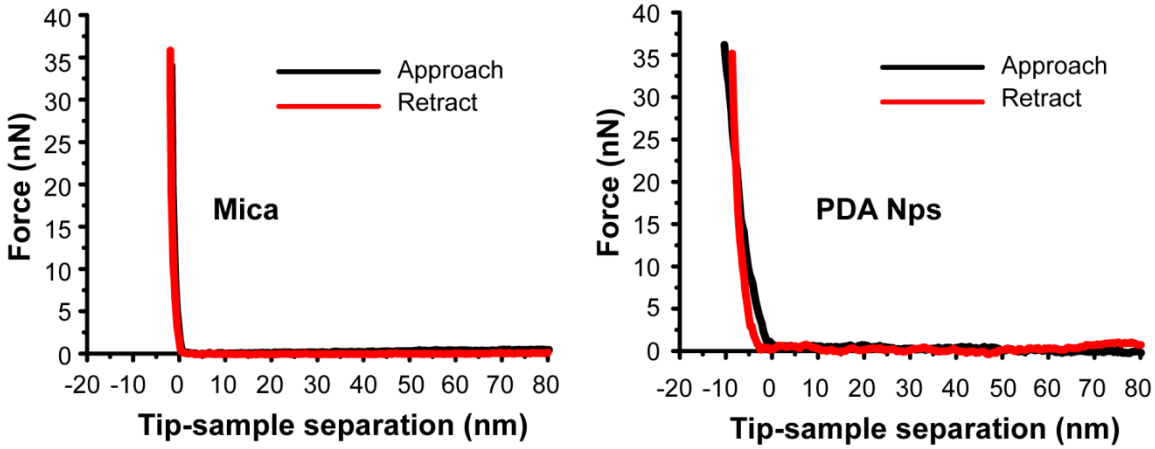


Figure S5: Typical Force/Tip-sample distance curves recorded either on mica (left) or on the top of polydopamine nanoparticle (right) obtained with the Quantitative Imaging (QI) mode at a speed of 50 µm/s and a force setpoint of 35 nN.

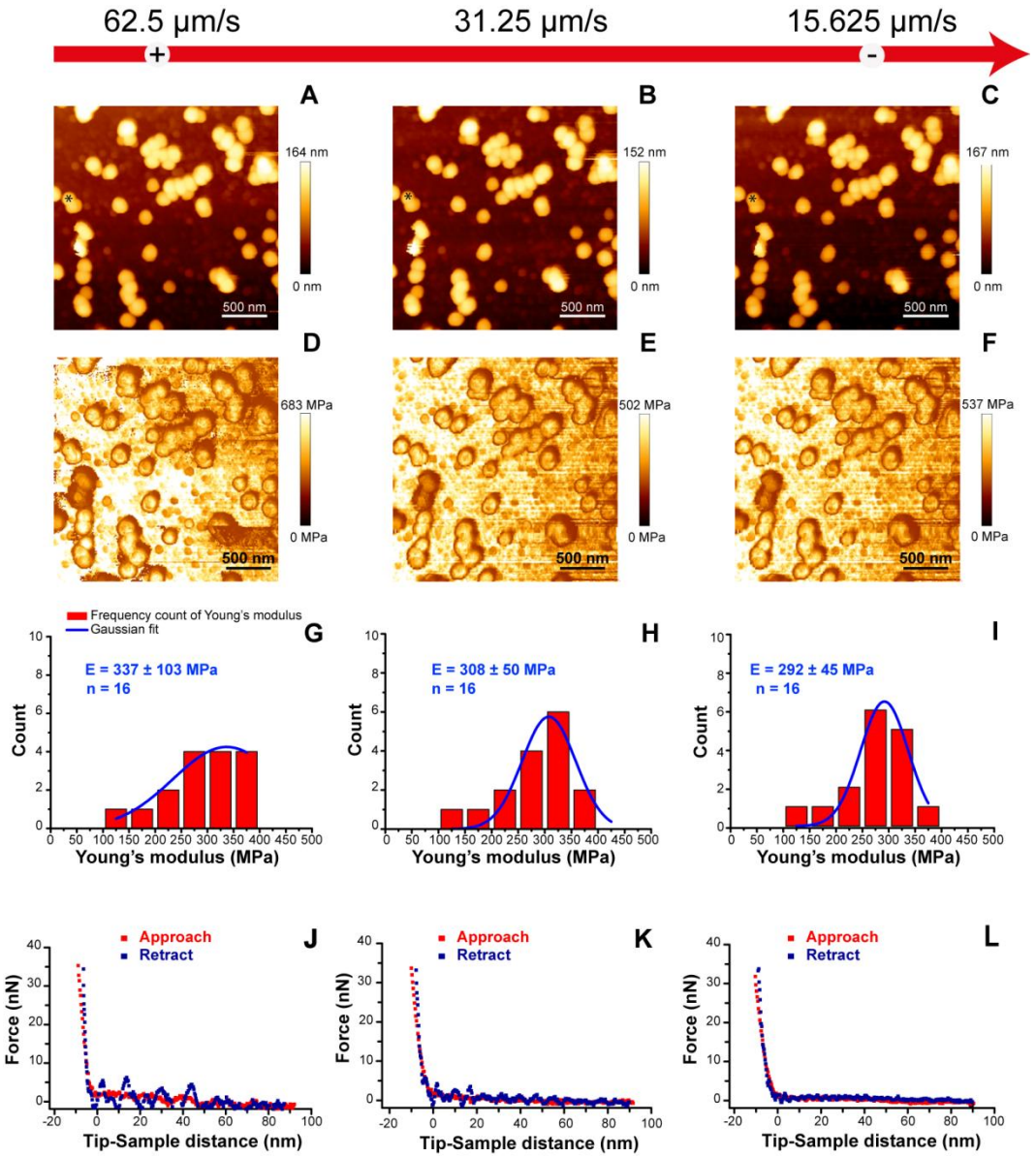


Figure S6: AFM height images (A,B,C) in Tris buffer (10mM, NaCl 150 mM) of covalently attached PDA NPs (D_H = 131±2 nm) and their corresponding Young's modulus maps (D,E,F). These data were obtained with JPK Quantitative imaging™ (QI) mode at a force setpoint of 35 nN and at different indentation speeds of 62.5µm/s (A,D), 31.25 µm/s (B,E) and 15.625µm/s (C,F). G, H, I represent the histograms distribution of the Young's modulus determined at different indentation speeds of NPs with Gaussian fits. J, K, L, are typical force/Tip-sample distance curves recorded on the same PDA nanoparticle marked with a star in figures A, B and C.

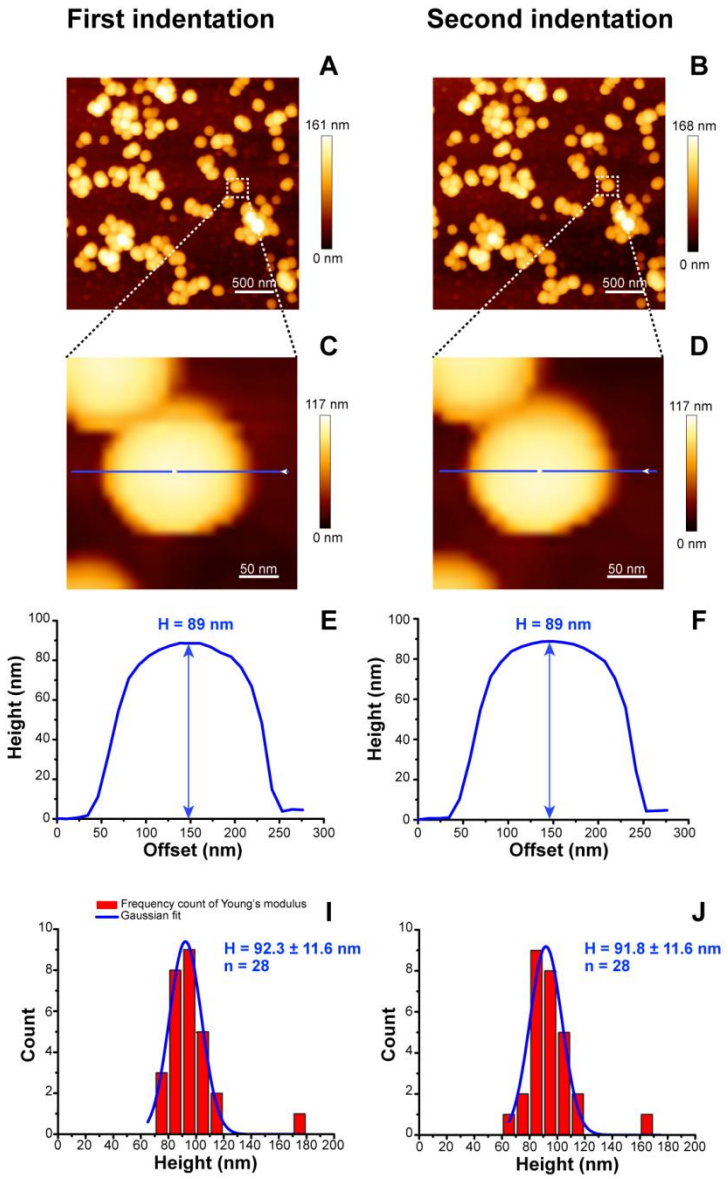


Figure S7: Comparison of the height changes of covalently attached PDA NPs (D_H = 131±2 nm) in Tris buffer (10mM, NaCl 150 mM) following two consecutive AFM images recorded using JPK Quantitative imaging™ (QI) mode at a force setpoint of 35 nN and at an indentation speed of 50µm/s.

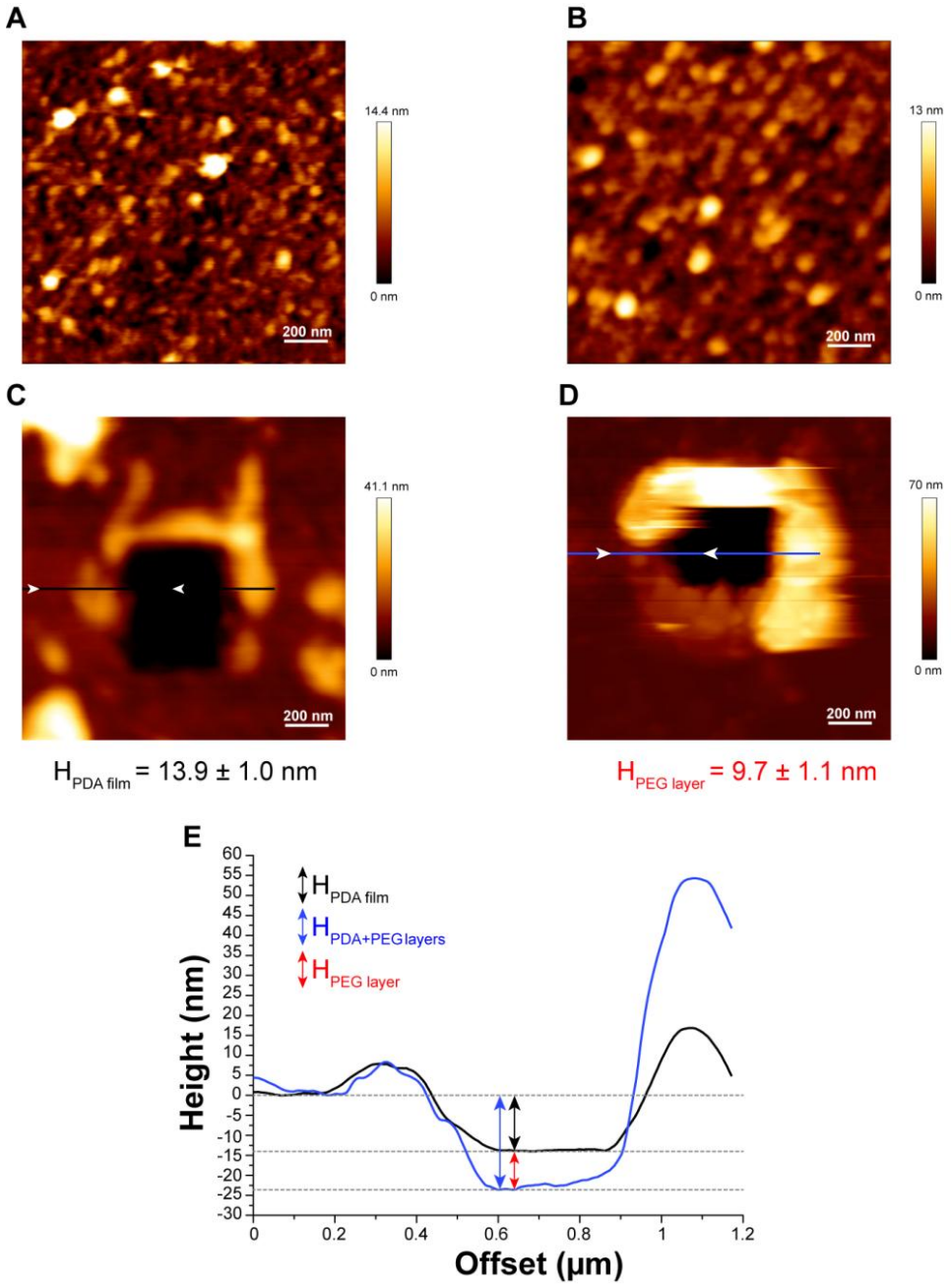


Figure S8: AFM height images (AM-AFM) of a PDA film deposited on a SiO_2 surface. (A) before and (B) after PEGylation with SH-PEG_{2000}-COOH in Tris buffer (10 mM, 150 mM NaCl, pH 8.5). (C) and (D) are the images obtained after nanoscratching experiments of PDA and PEGylated PDA films respectively. The cross sections analyzed along the scratched areas (black line for PDA film and blue one for the PEGylated one) are illustrated in E.

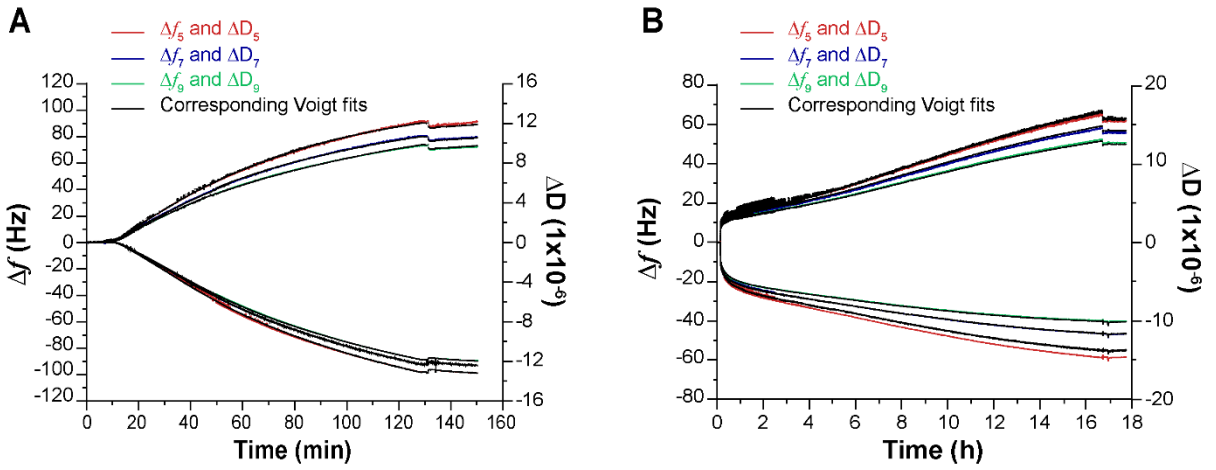


Figure S9: Normalized frequency (Δf_n) and dissipation (ΔD_n) changes as a function of time for the three overtones n= 5 (red), 7 (blue) and 9 (green), for (A) PDA film deposition on SiO_2-coated quartz, with their corresponding Kelvin-Voigt fitting results (black curves) and (B) its subsequent PEGylation using SH-PEG_{2000}-COOH in Tris buffer (10 mM, 150 mM NaCl, pH 8.5).

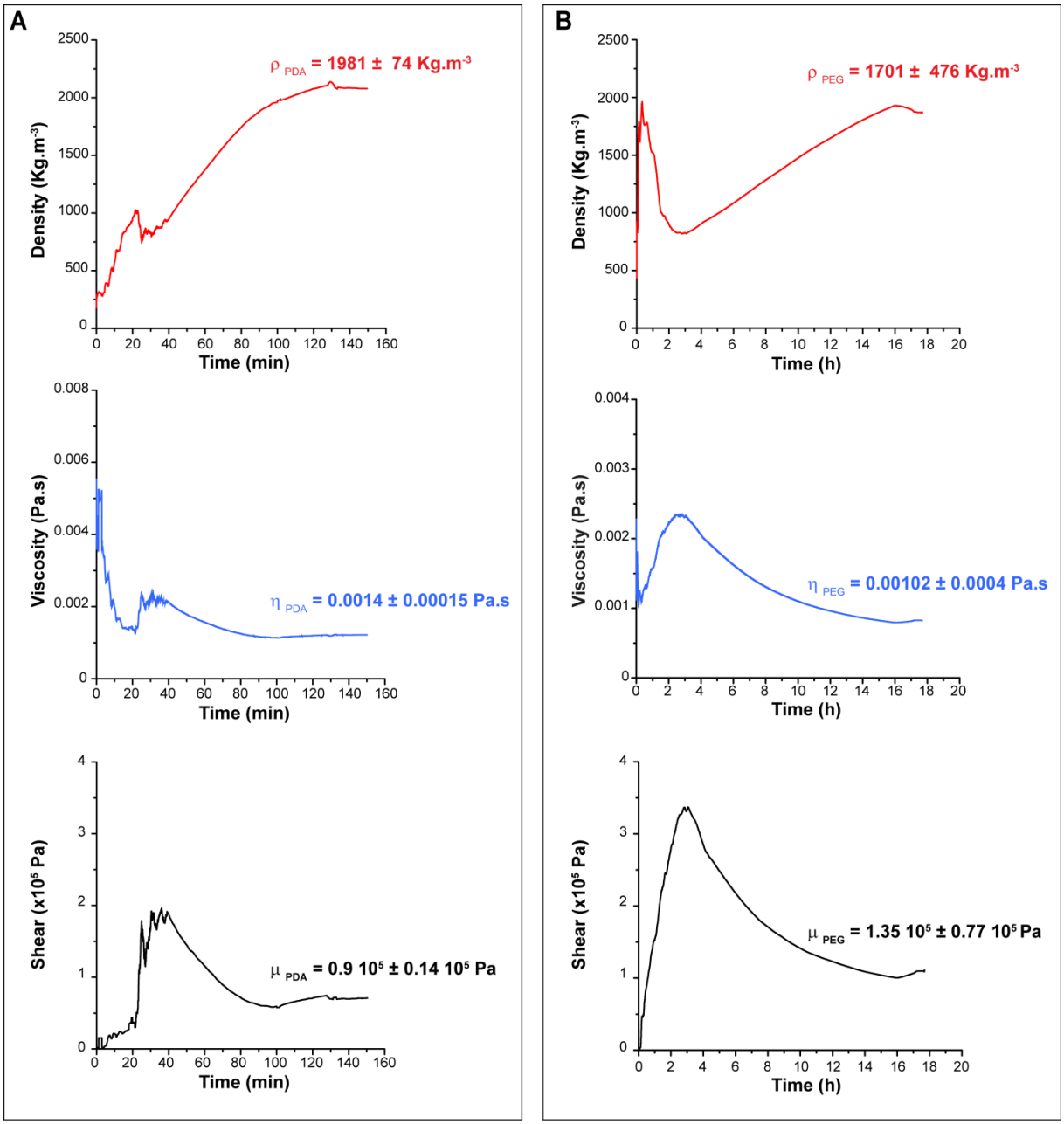


Figure S10: Density ρ, viscosity η and shear modulus μ plots of (A) PDA film and (B) PEG layer grafted in Tris buffer (10 mM, 150 mM NaCl, pH 8.5), generated by Kelvin-Voigt model applied on the frequency and dissipation shifts corresponding to the three overtones n = 5, 7 and 9 during QCM-D experiments.

Determination of PEG quantity needed for PDA NPs PEGylation, calculated based on QCM-D results:

The mass of PEG optimized with QCM-D experiments and allowing the obtention of brush PEG chains on the surface (1 cm^2) was used to calculate the quantity of PEG needed for PDA NPs functionalization under the same conditions. Assuming that PDA NPs are spherical, the surface exposed by PDA NPs in the suspension can be deduced from the following equation (1):

$$S_{NPs} = \frac{V_{NPs}}{V_{NP}} 4 \pi {R_h}^2 \qquad (1)$$

where V_{NPs} / V_{NP} represents NPs number, V_{NPs} the total volume of PDA NPs (m^3) determined based on PDA density given by QCM-D data analysis, V_{NP} the volume of one PDA NP (m^3) and R_h the NPs hydrodynamic radius obtained by DLS (m).

For PDA NPs of an average diameter around 140 nm, the mass needed for PEGylation of 1 mg of PDA NPs is ~ 360 µg. The NPs PEGylation conducted in presence of 10 mg of PEG molecules for 1 mg of PDA NPs guaranties thus a similar grafting density of PEG on the surface.

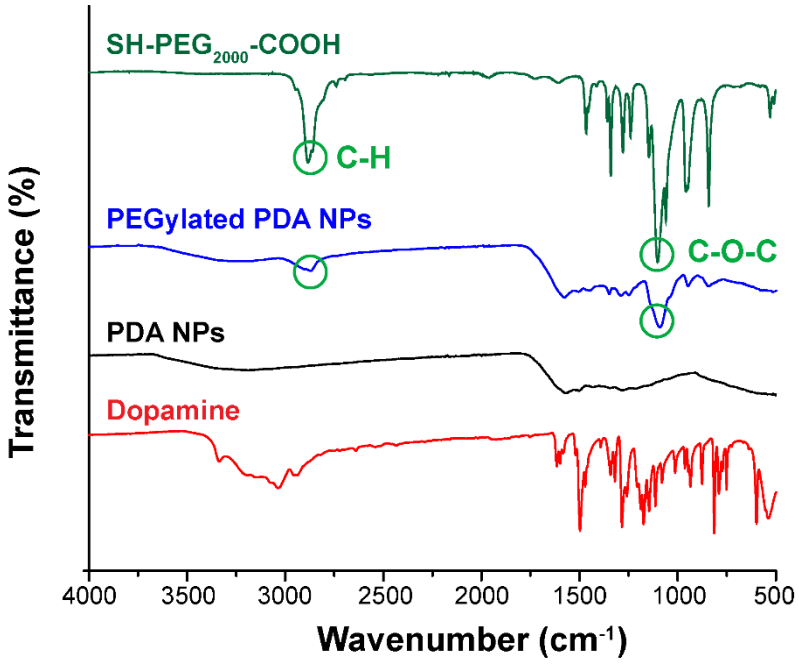


Figure S11: Fourier transform infrared spectroscopy (FTIR) spectra of PDA NPs (black) and PEGylated PDA NPs (blue) lyophilizates, obtained in comparison to dopamine hydrochloride (red) and SH-PEG_{2000}-COOH (green) powders.