

Temperature-responsive properties of pH-sensitive poly(methacrylic acid) grafted brush coatings with controlled wettability for cell culture

Ostap Lishchynskiy^{1,3}, Svitlana Tymetska^{2,4}, Yana Shymborska², Joanna Raczkowska², Kamil Awsiuk², Andre G. Skirtach³, Sergiy Korolko⁵, Anastasiia Chebotar¹, Andrzej Budkowski^{2}, Yuriy Stetsyshyn^{1*}*

¹Lviv Polytechnic National University, St. George's Square 2, 79013 Lviv, Ukraine

²Smoluchowski Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-348, Kraków, Poland

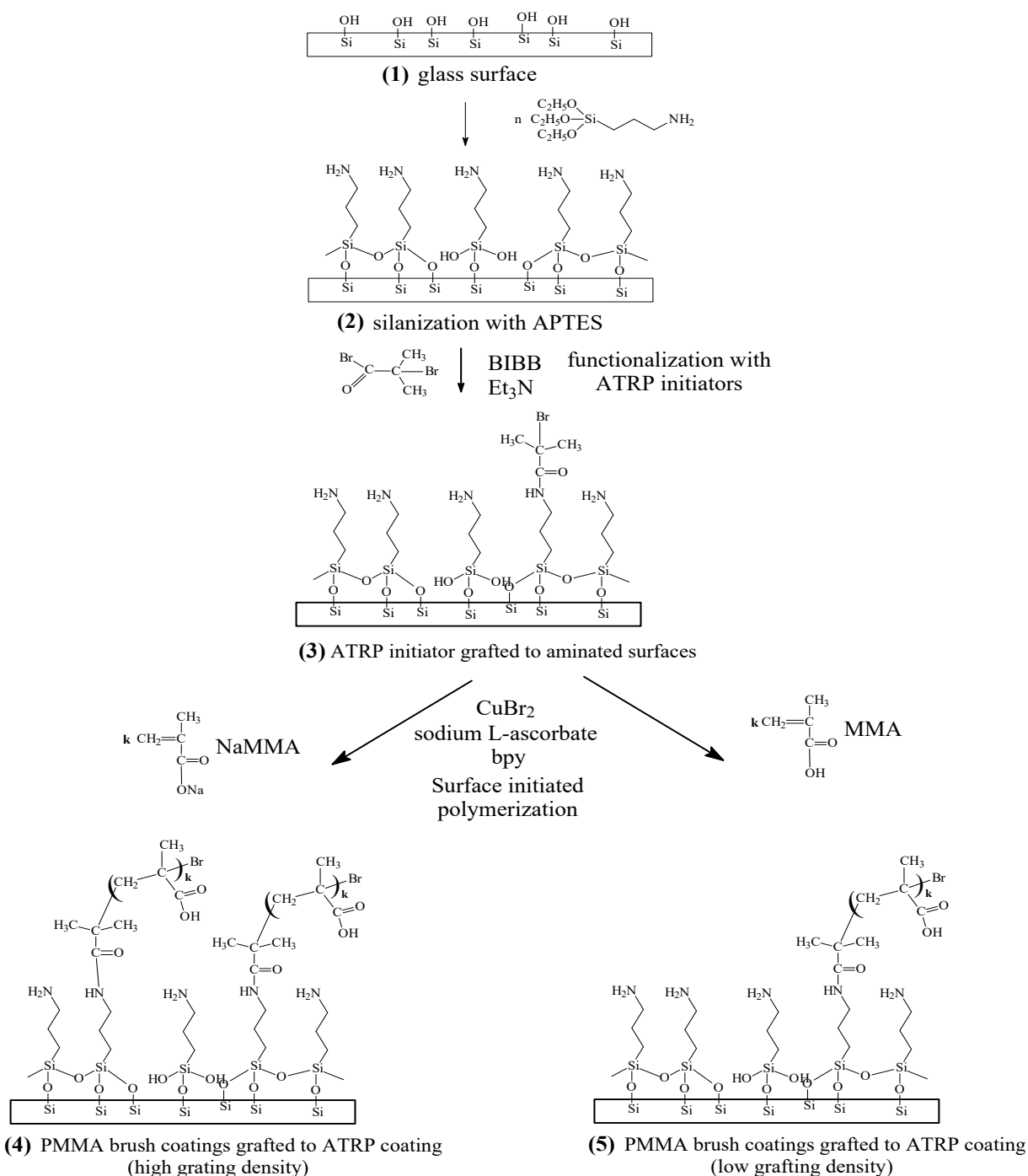
³Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Proeftuinstraat 86, 9000 Ghent, Belgium

⁴Jagiellonian University, Doctoral School of Exact and Natural Sciences, Łojasiewicza 11, 30-348 Kraków, Poland

⁵Hetman Petro Sahaidachnyi National Army Academy, 32, Heroes of Maidan Street, Lviv 79012 Lviv, Ukraine

*Corresponding authors E-mail: Yuriy Stetsyshyn yrstecushun@ukr.net

Andrzej Budkowski andrzej.budkowski@uj.edu.pl



Scheme S1. Functionalization of glass surface **(1)** with amino-terminated APTES film **(2)**, subsequent grafting of ATRP initiator **(3)** and polymerization of NaMAA or MAA, initiated by ATRP initiator, resulting in PMAA brush coatings with high **(4)** and low **(5)** grafting densities.

Table S1. Determination of the coefficients (C_w , C_d) that relate the thickness of the brush [nm] in the wet state ($h_{\text{wet}} = C_w N \sigma^{1/3}$) and the dry state ($h_{\text{dry}} = C_d N \sigma$) with the grafting density σ

[chains/cm²] and the degree of polymerization N (based on [A1]). Determination of the coefficient

C that relates grafting density and the ratio of the wet and dry thickness $h_{\text{wet}}/h_{\text{dry}} = C/\sigma^{2/3}$.

polymer	M_n [g/mol]	ρ [g/mol]	$R_g/N^{3/5}$ (based on Rg and N) [nm]	C_w^a [nm ^{5/3}]	C_d^b [nm]	C (C_w / C_d) [nm ^{2/3}]
PNIPAM	113.2	1.269[A1]	0.17[A1]	0.153[A1]	0.149[A1]	1.03[A1]
PMAA	86.1	1.285[A2]	0.14[A3]	0.110	0.111	0.99

^a using the relation $C_w = 2 \pi^{1/3} (R_g/N^{3/5})^{5/3}$

^b using the relation $C_d = (M_n/\rho)/N_A$, where is the Avogadro's number

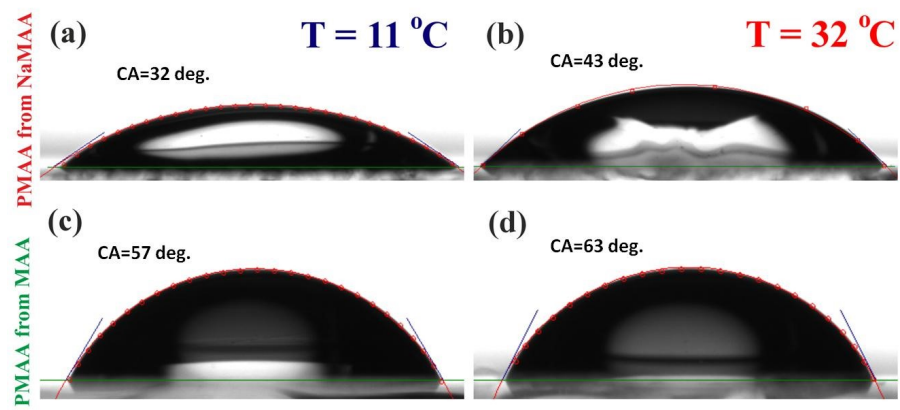
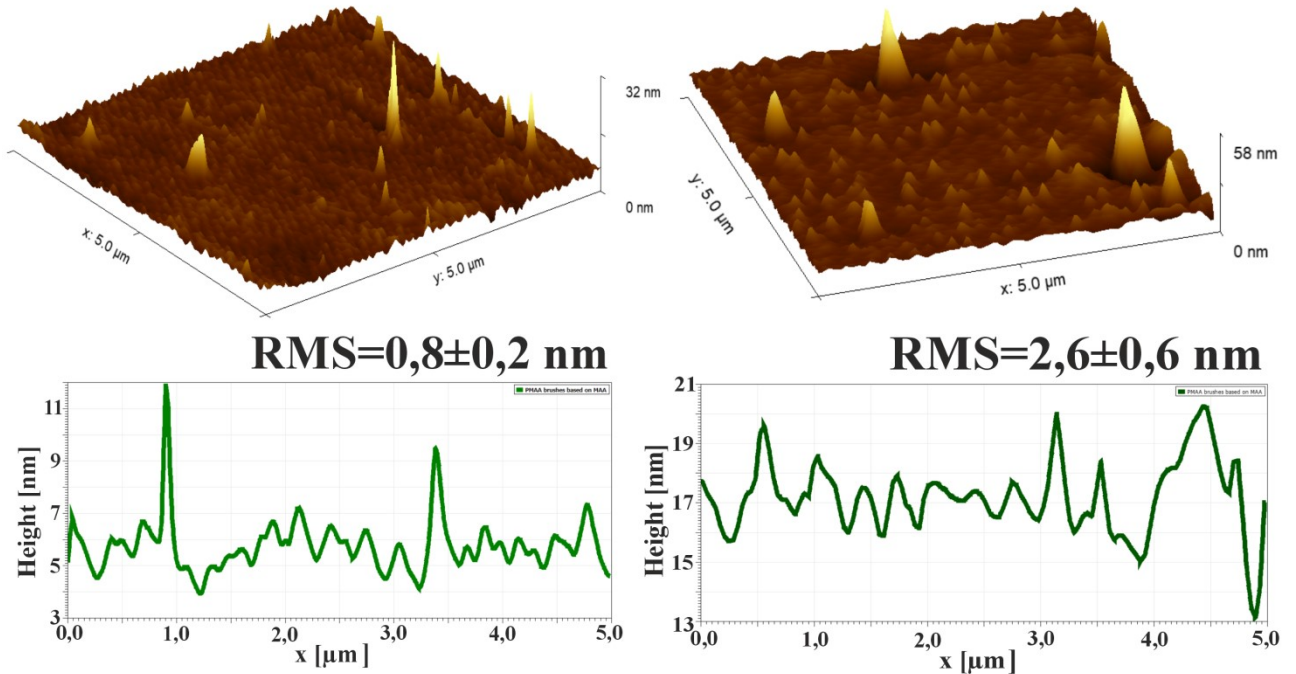


Figure S1. Typical images of wetting contact angles recorded at temperatures 11°C (a and c) and 32°C (b and d) for PMAA grafted brush coating fabricated from NaMAA (a and b) or MAA (c and d) solutions.

Temperature

10 °C 32 °C

PMAA brushes based on MAA



PMAA brushes based on NaMAA

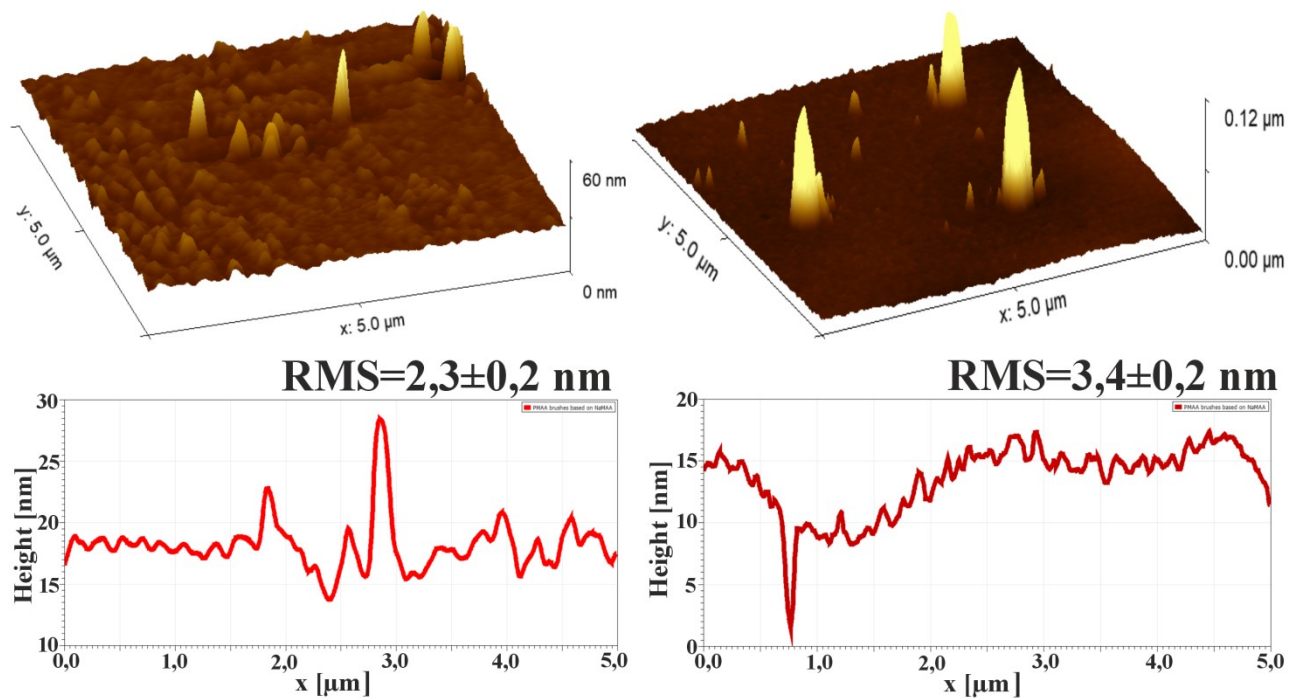


Figure S2. Typical AFM images recorded at temperatures 10°C and 32°C for PMAA grafted brush coating fabricated from MAA or NaMAA solutions.

The integral geometry approach provides full quantitative characterization of 2 dimensional images by means of three morphological (Minkowski) measures, reflecting the coverage, lateral shape (interface length), and connectivity of the white regions. They can also be used to semi-quantitatively analyze protein adsorption examined by fluorescence micrographs, with the fluorescence intensity proportional to the amount of adsorbed proteins.

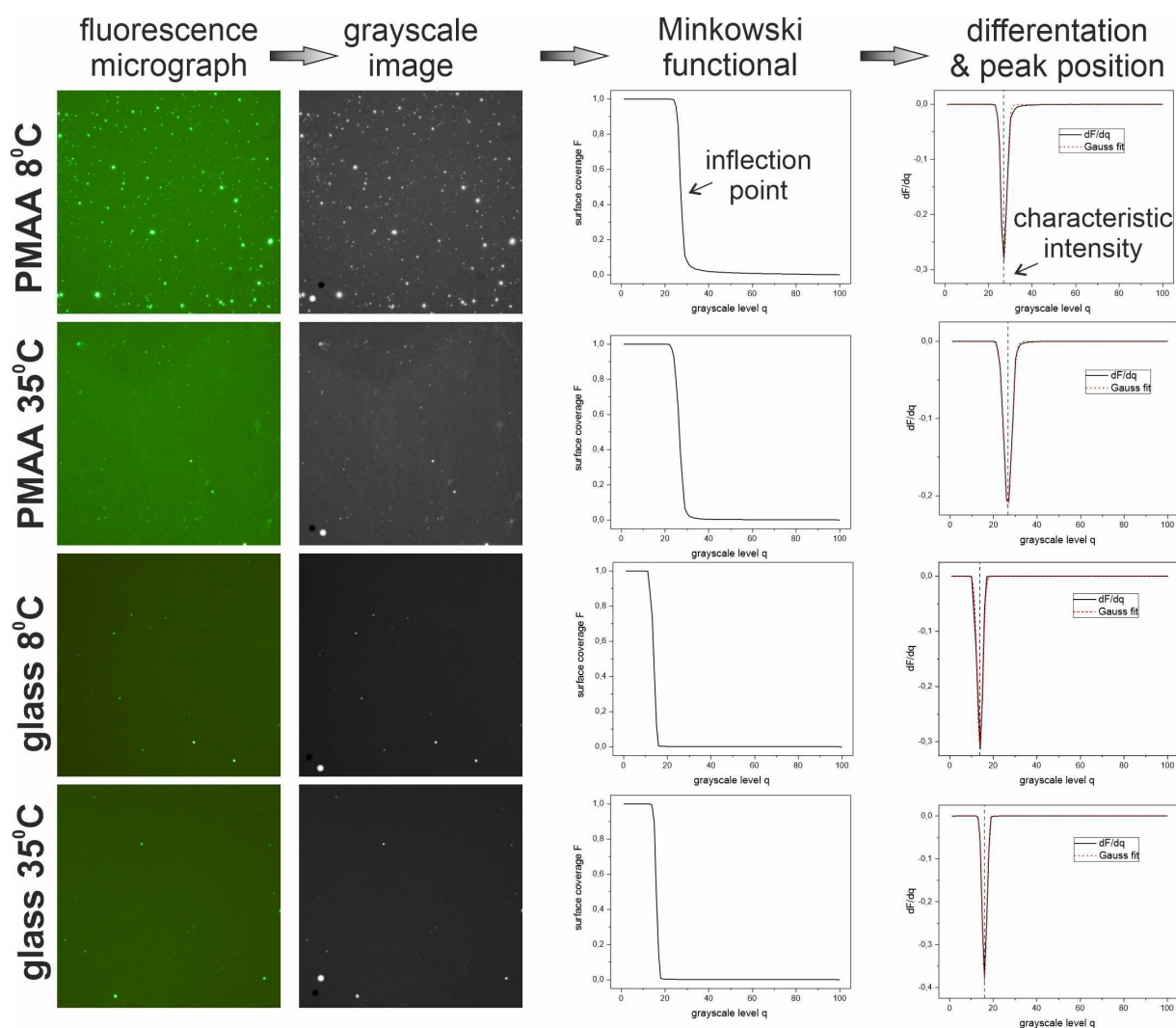


Fig. S3. Fluorescence micrograph transformed into a grayscale image, set of corresponding Minkowski measures and a quantitative analysis providing characteristic fluorescence intensity.

For this purpose, each micrograph (Fig. S3 first column) is transformed into a grayscale image (Fig. S3 second column), and a set of Minkowski measures is calculated as a function of the grayscale level, corresponding to the fluorescence intensity using the software developed in our laboratory and described in details previously [A4, A5]. Additionally, to ensure the same reference scale, for each image two dots, one black and one white are added. Then, the fluorescence intensity characteristic for each image and

proportional to the amount of adsorbed proteins is determined, from the inflection point of surface coverage vs grayscale level curve (Fig. S3 third column), simply by derivative analysis (Fig. S3 last column). The amount of adsorbed proteins is characterized by the value of grayscale level where the deflection point occurs, enabling a quantitative comparison between the set of measurements although the exact amount of proteins is not defined.

References

- [A1] S. Wang and Y. Zhu, Facile Method to Prepare Smooth and Homogeneous Polymer Brush Surfaces of Varied Brush Thickness and Grafting Density, *Langmuir* **2009**, *25*, 13448–13455, <https://doi.org/10.1021/la901785t>
- [A2] Polymer Data Handbook: Second Edition, James E Mark (ed.), Oxford University Press, Oxford 1999
- [A3] Robin, C.; Lorthioir, C.; Fall, A.; Ovarlez, G.; Amiel, C., Unexpected Slow Kinetics of Poly(Methacrylic Acid) Phase Separation in the Semi-Dilute Regime. *Polymers* **2022**, *14*, 4708. <https://doi.org/10.3390/polym14214708>
- [A4] Zemła, J., Lekka, M., Wiltowska-Zuber, J., Budkowski, A., Rysz, J., & Raczkowska, J. (2008). Integral geometry analysis of fluorescence micrographs for quantitative relative comparison of protein adsorption onto polymer surfaces. *Langmuir*, *24*(18), 10253-10258.
- [A5] Raczkowska, J., Rysz, J., Budkowski, A., Lekki, J., Lekka, M., Bernasik, A., ... & Czuba, P. (2003). Surface patterns in solvent-cast polymer blend films analyzed with an integral-geometry approach. *Macromolecules*, *36*(7), 2419-2427.