

Sizing experiments and bio-nano interactions: method matters



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INTRODUCTION

Nanomedicine characterization can pursue different levels of complexity. Consequently, leading scientific and regulatory bodies in the pharmaceutical field have acknowledged that the lack of established methods to provide reliable preclinical data represents the bottleneck in the process of bringing promising nanomedicines to the market despite intensive research work in this field. Therefore, the aim of the presented research was to perform a thorough analysis of the selected nanosystem (nanoemulsion) focusing on size estimation and particle-protein interaction applying several state-of-the-art techniques, highlighting important factors for a reliable analysis.

Table 1. Qualitative and quantitative composition of the selected nanoemulsion

Ingredient	Concentration (%)
Medium-chain triglycerides	10
Polysorbate 80	9
Soybean lecithin	1
Ultrapurified water	10

RESULTS

Table 2. Nanoemulsion: Comparative overview of the values of the selected parameters of the DLS measurement

Sample type	Dilution	Z-ave (nm)	PDI	Attenuation	Count rate (kcps)	Intercept of the correlation function	T (°C)
Nanoemulsion diluted in water	1:10	86.11±0.87	0.289±0.007	7	148.3	0.912	25
	1:20	85.93±0.81	0.276±0.011	8	365.7	0.9	25
	1:50	86.02±0.83	0.268±0.006	8	204.9	0.915	25
	1:100	85.78±0.71	0.249±0.011	9	298.1	0.907	25
	1:1000	88.93±1.17	0.231±0.010	11	440.2	0.892	25
Nanoemulsion diluted in PBS	1:10	98.39±0.50	0.131±0.015	8	424.7	0.896	25
	1:20	93.88±0.63	0.131±0.012	8	323.4	0.909	25
	1:50	95.22±0.35	0.135±0.009	9	440.4	0.901	25
	1:100	93.25±0.53	0.125±0.015	9	255.8	0.915	25
	1:1000	95.89±0.78	0.122±0.016	11	327.9	0.897	25

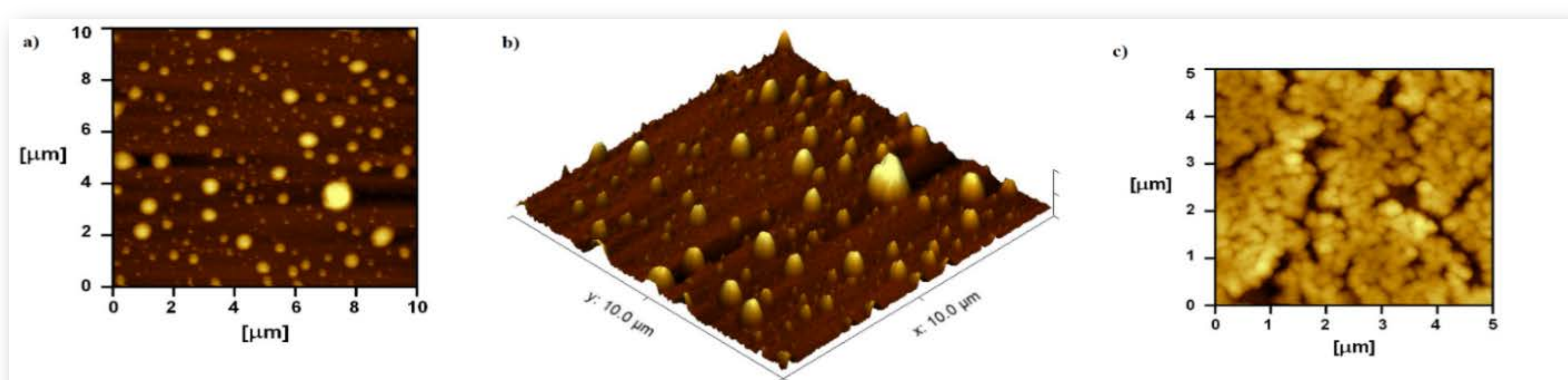


Figure 2. AFM micrographs of the nanoemulsion without HSA (a: 2D image; b: 3D image) and after incubation with HSA (c)

Acknowledgement

This research was supported by the Science Fund of the Republic of Serbia, Grant No. 7749108, Neuroimmune aspects of mood, anxiety and cognitive effects of leads/drug candidates acting at GABAA and/or sigma-2 receptors: In vitro/in vivo delineation by nano- and hiPSC-based platform — *NanoCellEmoCog* and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200026)

METHODOLOGY

As a model nanosystem, previously developed nanoemulsion was used (Table 1), prepared via spontaneous emulsification procedure. To assess nanoparticle-protein interaction, nanoemulsion was incubated with human serum albumin (HSA, Sigma Aldrich), in the final protein concentration of 2.5 mg/ml, during 1h, at 37 °C, under constant mixing. Same analysis were performed with nanoemulsion *per se*, and after incubation with proteins. Size and size distribution were evaluated applying batch mode dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments, UK), while morphological evaluation of the samples and additional sizing experiments were performed applying AFM as a high-resolution technique (NTEGRA Prima atomic force microscope, NT-MDT, Moscow, Russia). In order to assess the structure of the nanoparticles and, especially, interactions in a biorelevant surrounding, a laboratory X-ray setup was applied (Bruker Nanostar, Bruker AXS GmbH, Karlsruhe, Germany).

MAIN CONCLUSIONS

- In this research, it has been demonstrated how important is to carefully select **measurement conditions** even for DLS – a commonly used and the only standardized method, in order to keep the measurements meaningful.
- **Not every method is capable of detecting some specific bio-nano interactions.**
- Aiming to generate reliable datasets, *condition sine qua non* is to perform **complementary and orthogonal techniques with increasing complexity.**

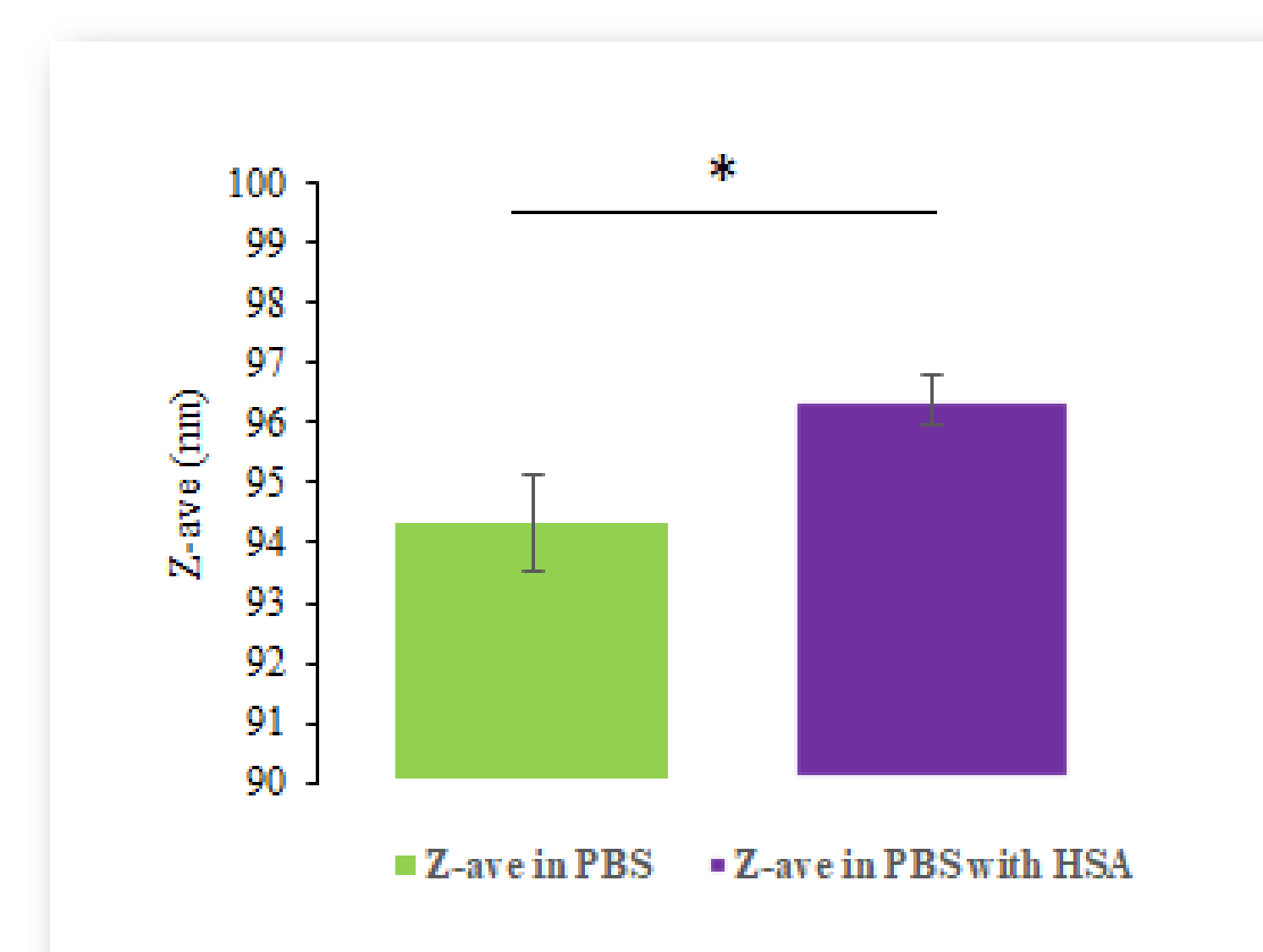


Figure 1. Z-ave of nanoemulsion determined by dynamic light scattering in PBS: changes in size caused by the HAS adsorption; *statistically significant difference (Student t-test; p<0.05)

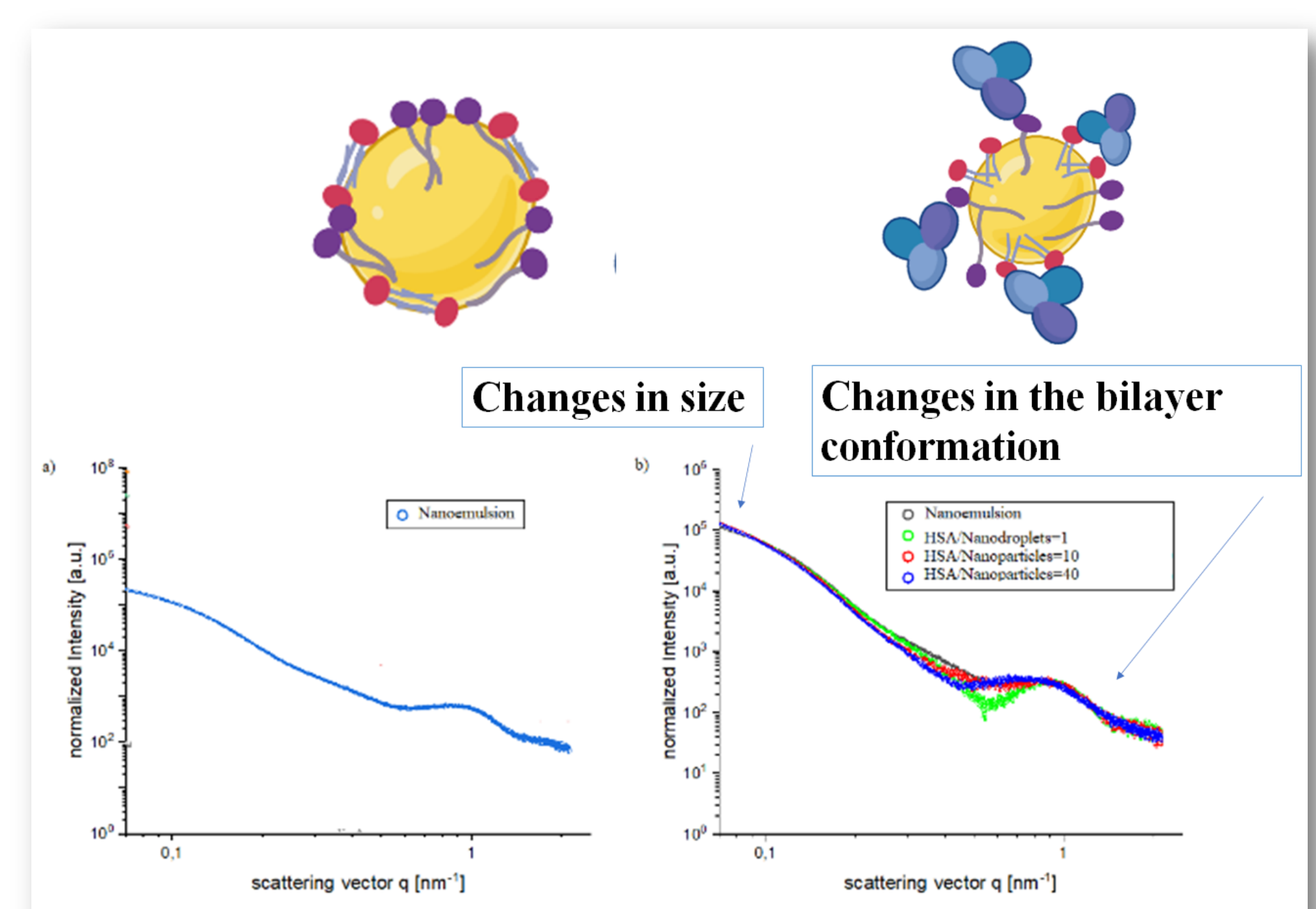


Figure 3. SAXS intensities of the nanoemulsion *per se* (a) and in different HSA/nanoparticle ratios (b); q values in the higher region depict bilayer structure