

Synthetic reference materials for extracellular vesicles

Zoltán Varga^{1,*}, Edwin van der Pol^{2,3,4}, Rienk Nieuwland^{2,3}

¹Biological Nanochemistry Research Group, Research Centre for Natural Sciences - Budapest, Hungary,

²Laboratory of Experimental Clinical Chemistry, ³Department of Biomedical Engineering and Physics and

⁴Vesicle Observation Center, Amsterdam University Medical Centers, Location AMC, Amsterdam, the Netherlands

* Corresponding author: varga.zoltan@ttk.hu



Introduction

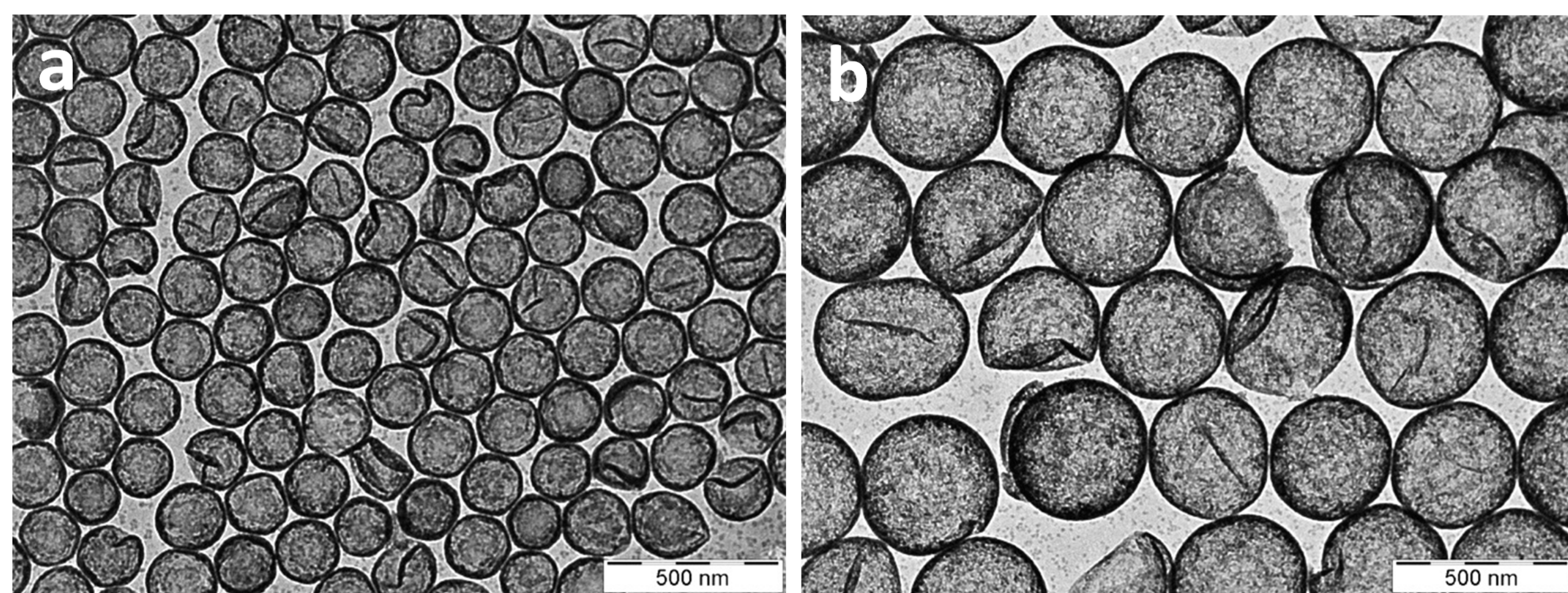
There is an unmet need for standardization of concentration measurements of extracellular vesicles (EVs). **Flow cytometry** remains the clinically most applicable method for measuring it. To compare concentration measurements of EVs between flow cytometers, solid **polystyrene reference beads** are used. However, these polystyrene beads **lead to false size determination of EVs** due to the **mismatch in refractive index** between the beads and EVs.

Essentials

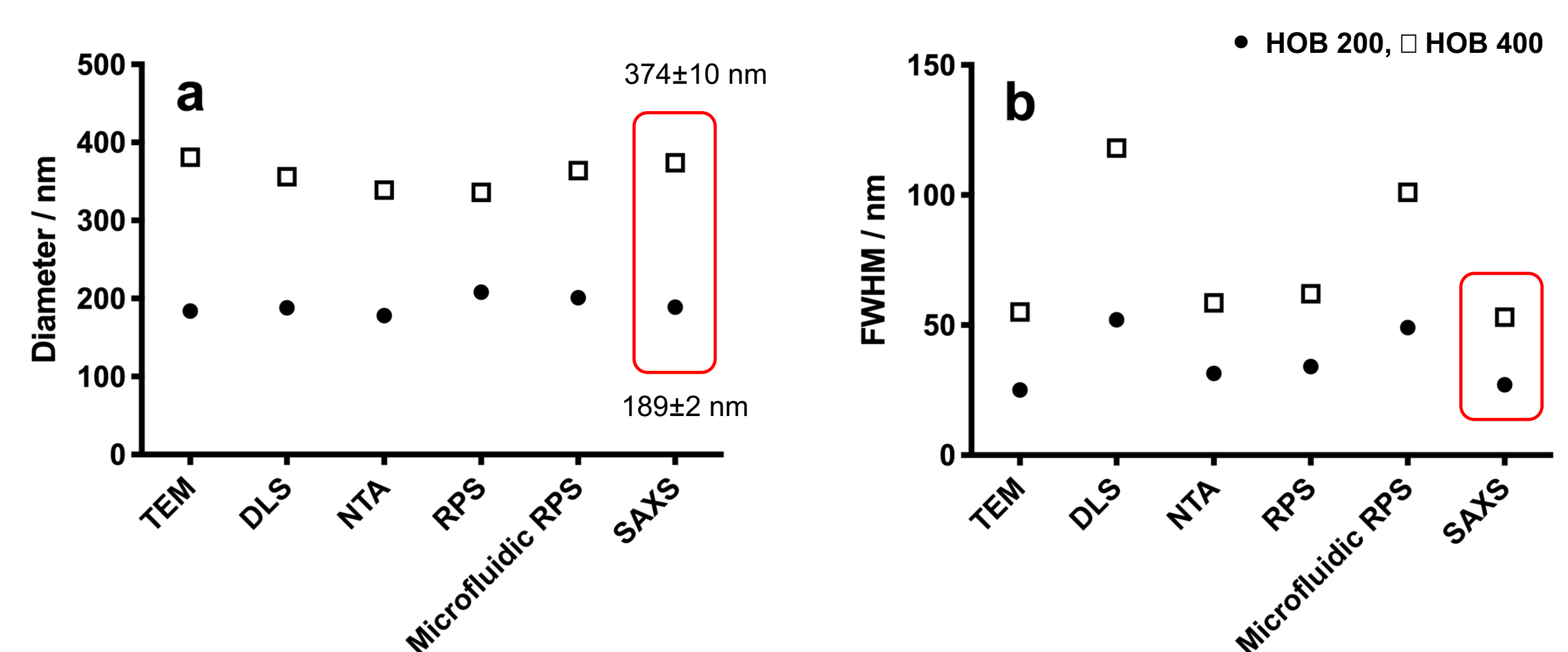
- Standardization of EV measurements by flow cytometry needs improvement
- Hollow organosilica beads (HOBs) were prepared, characterized, and tested as reference particles for EVs
- HOBs resemble the structure and the light scattering properties of EVs
- HOBs can be used to set size gates in nanometers independent from the optical configuration of a flow cytometer.

Hollow organosilica beads (HOBs)

HOBs were prepared by a hard template sol-gel method and extensively characterized for morphology, size distribution and colloidal stability.



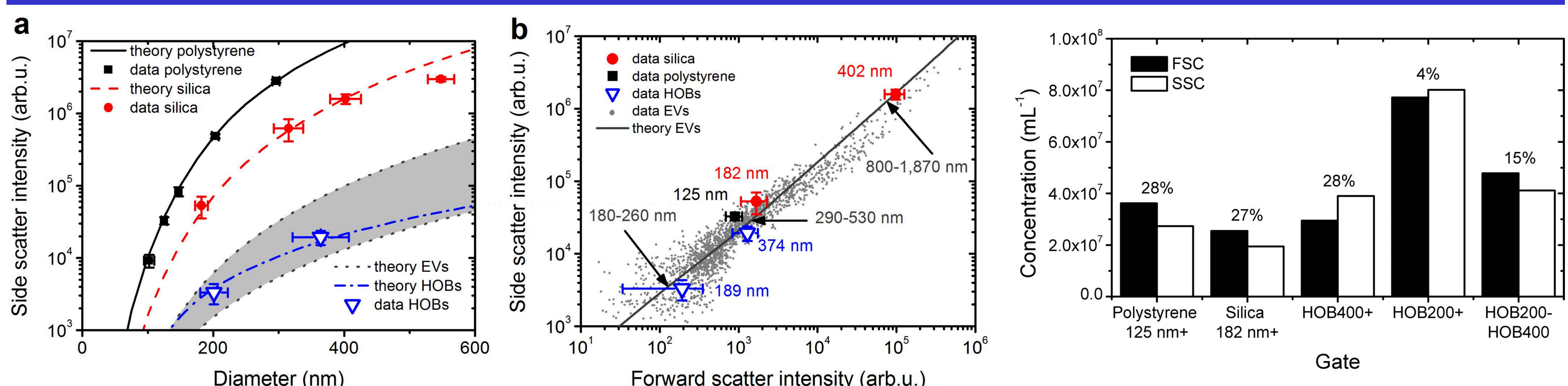
Transmission electron microscopy (TEM) analysis of HOBs prepared by using nominal 200 nm (HOB200, a) and 400 nm (HOB400, b) sized silica templates.



Size distribution of HOBs as determined by TEM, dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), resistive pulse sensing (RPS), microfluidic resistive pulse sensing (MRPS), and small-angle X-ray scattering (SAXS). (a) Mean diameters. (b) Full width at half maximum values of the size distributions.

HOBs proved to be monodisperse with homogeneous shell thickness with mean diameters of (189±2) nm and (374±10) nm for HOB200 and HOB400, respectively, with a polydispersity below 15%.

Flow cytometry of EVs and HOBs



Light scattering properties of polystyrene beads (squares), silica beads (circles), **HOBs (triangles)**, and **platelet-derived (CD61+) EVs (dots)** from human plasma measured (symbols) by flow cytometry and calculated (lines) by Mie theory. (a) Side scatter versus diameter. (b) Side scatter versus forward scatter.

Concentration of platelet-derived extracellular vesicles (EVs) within gates set by polystyrene beads, silica beads, and hollow organosilica beads (HOBs) for the forward scattered light (FSC) and side scattered light (SSC) detector.

Outlook

Among liposomes and low refractive index solid nanoparticles, the applicability of HOBs as synthetic reference materials for EVs will be further investigated in the METVES II project.

Acknowledgement

This work was supported under grant numbers PD 121326 and NVKP_16-1-2016-0007 by NKFIH (Hungary) and 18HLT01 METVES II by the European Metrology Programme for Innovation and Research (EMPIR). The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States. ZV was supported by the János Bolyai Research Fellowship.