Electronic Supplementary Material (ESI) for RSC Advances.

Selecting analytical tools for characterization of polymersomes in aqueous solution - supplementary information

Joachim Habel, Anayo Ogbonna, Nanna Larsen, Solène Cherré, Søren Kynde, Søren Roi Midtgaard, Koji Kinoshita, Simon Krabbe, Grethe Vestergaard Jensen, Jesper Søndergaard Hansen, Kristoffer Almdal, Claus Hélix-Nielsen

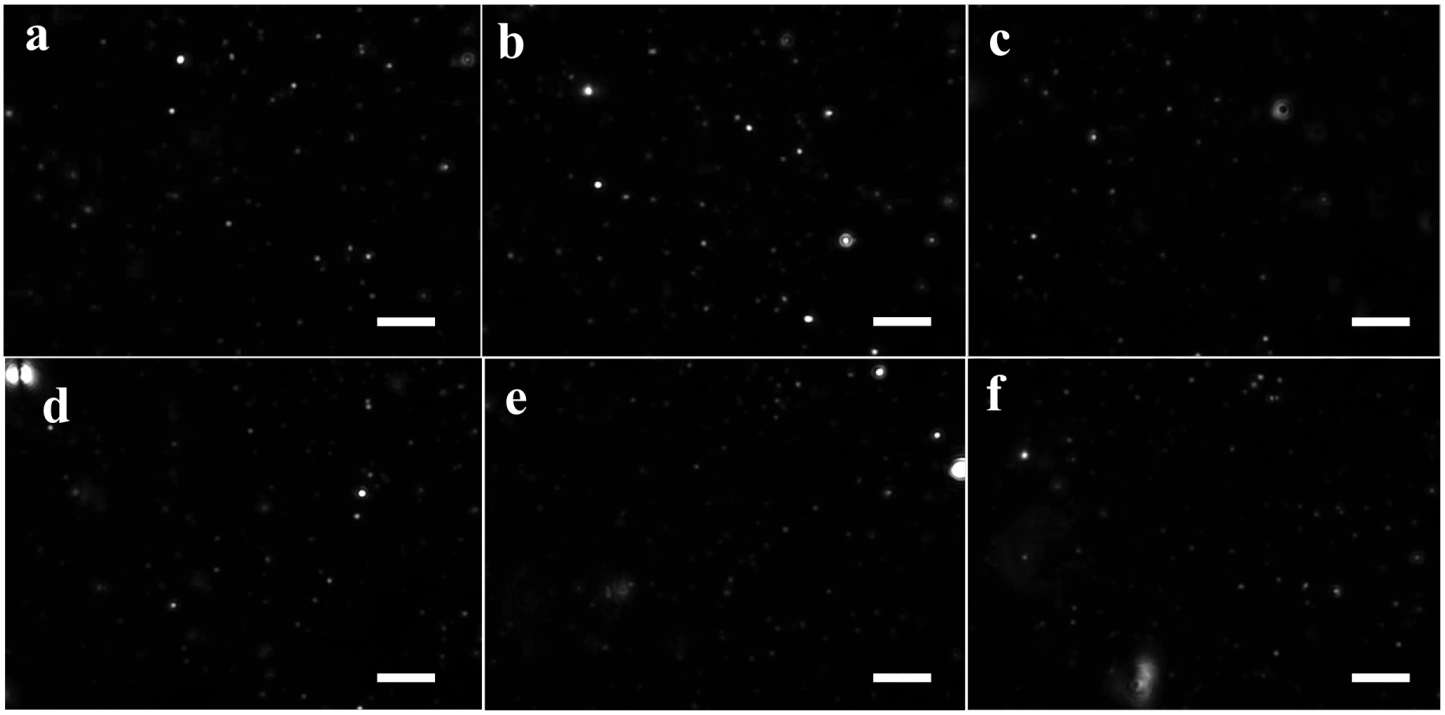

Figure 1: Images of light scattering projections, observed with a camera with 20X magnification sense from nanoparticle tracking analysis (NTA) analysis. a)+b) Mean population of polymersomes, c) smaller polymersome populations, d)+e) highly saturated polymersome projections due to overexposure, visible at the edges of the images, f) diffraction rings due to overexposure at the bottom of the image. Scale bar is 400μm.

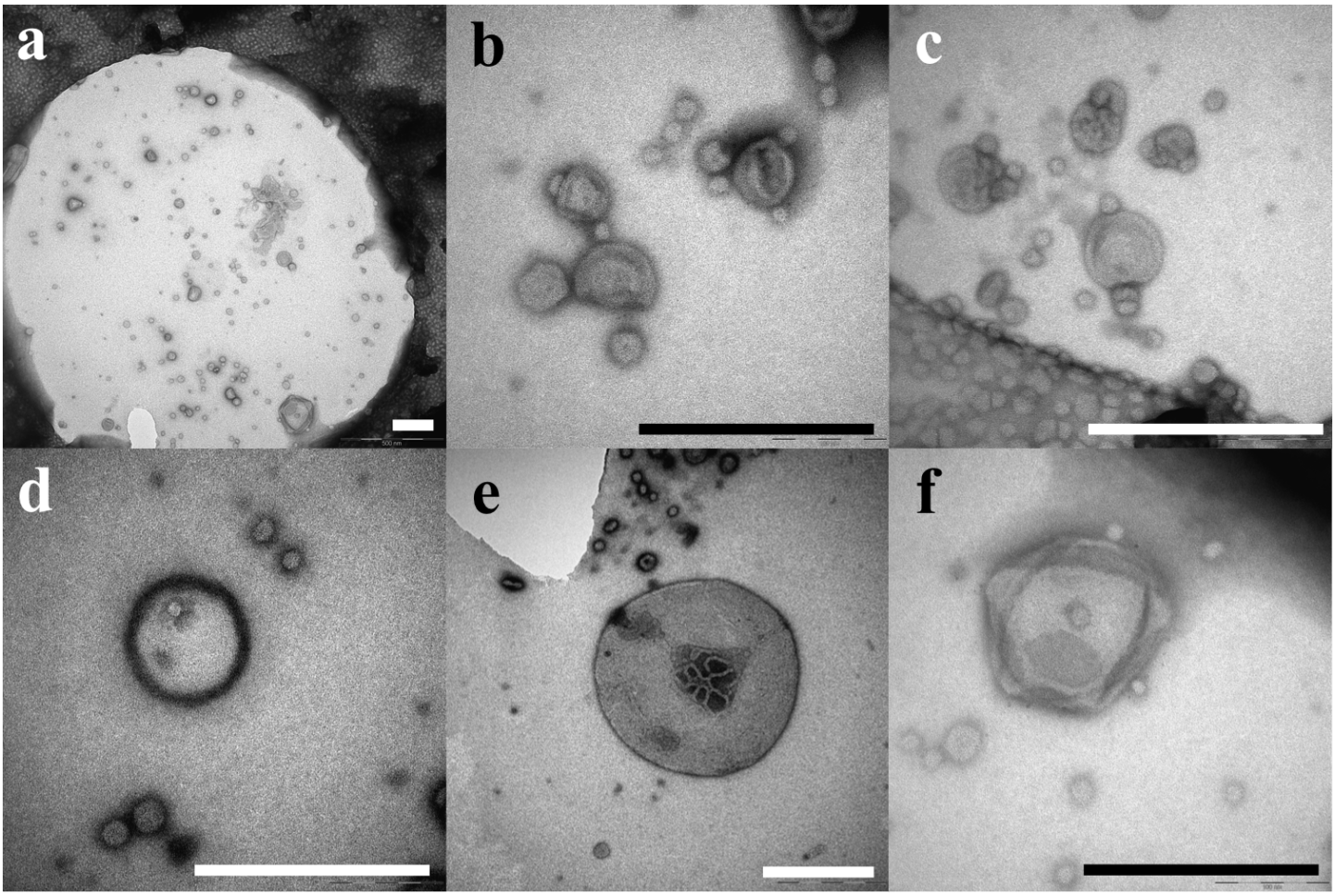

Figure 2: Images of negative-staining transmission electron microscopy (NS-TEM) analysis. a) Overview image containing mainly small polymersomes, a few larger ones and ruptured bilayer sheets, potentially remains of a huge polymersome, b)-d) small and middle-sized polymersomes, e) one of the few intact large polymersomes, f) collapsed large polymersomes. Scale bar is 200nm

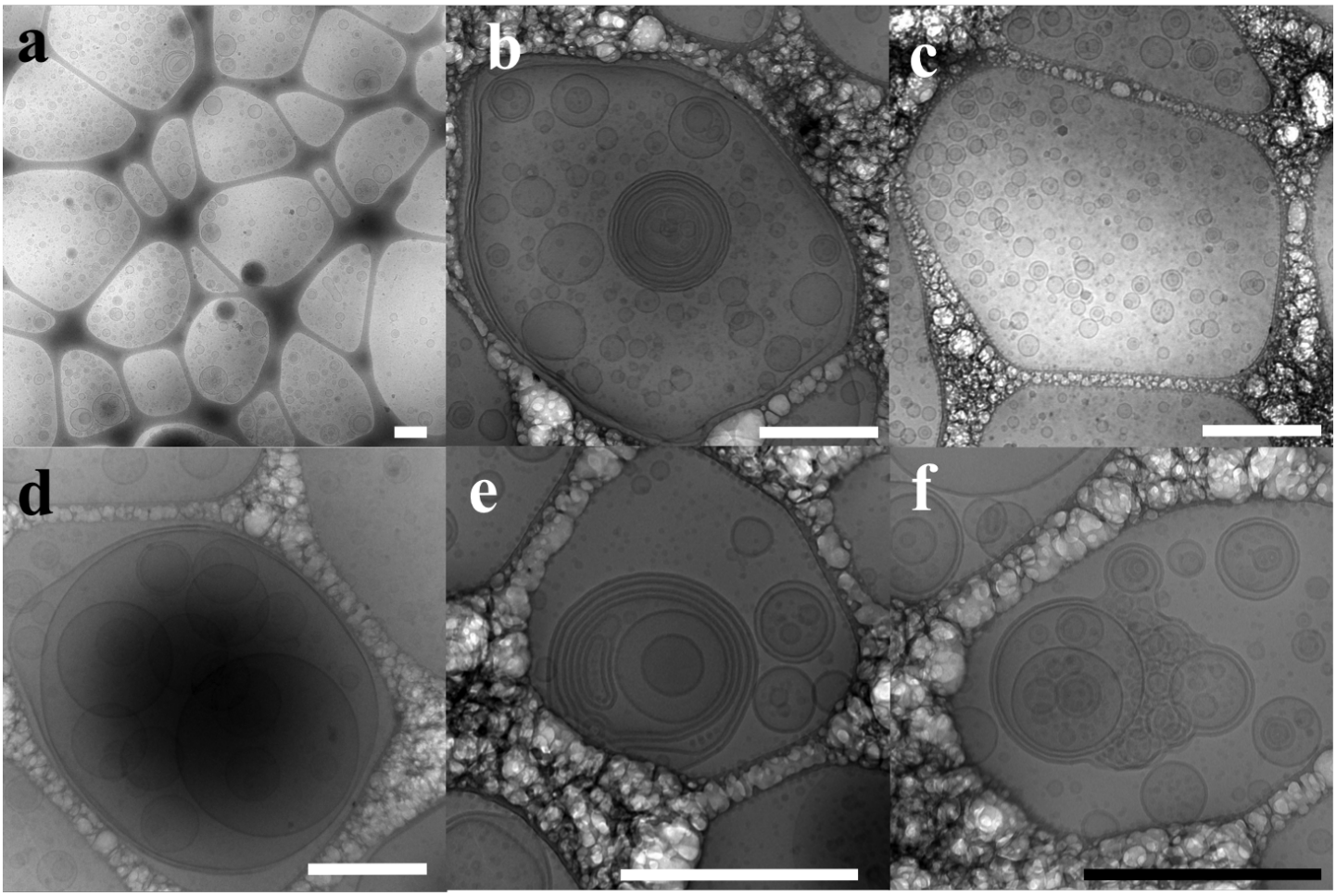

Figure 3: Images of Cryo-TEM analysis. a) Overview image containing all kinds of polymersome sizes and lamellarities, b) carbon hole with highly heterogeneous polymersomes, c) carbon hole with mono disperse polymersomes, d) multi vesiclular polymersomes, e) multilamellar polymersomes, f) polymersome agglomeration of multi vesicular polymersomes. Scale bar is 400nm.

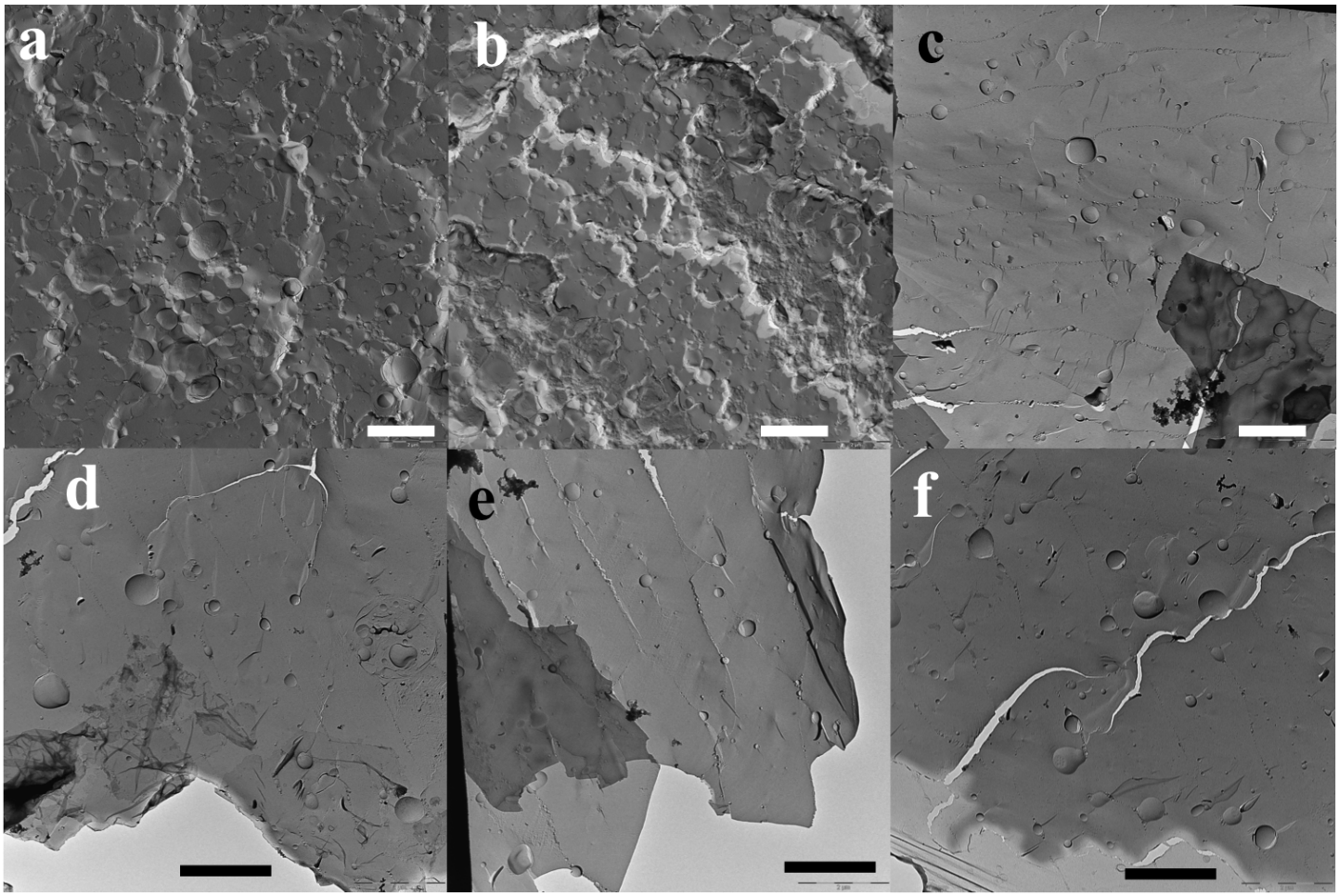

Figure 4: Images of Freeze fracture (FF)-TEM analysis. a)+b) Overview images containing high polymersome populations, where at b) replica cracks could be mainly observed, where there were high polymersome accumulations. c)-f) Small polymersome populations, where d)+e) were on the edges of the replica. Scale bar is 2μm.

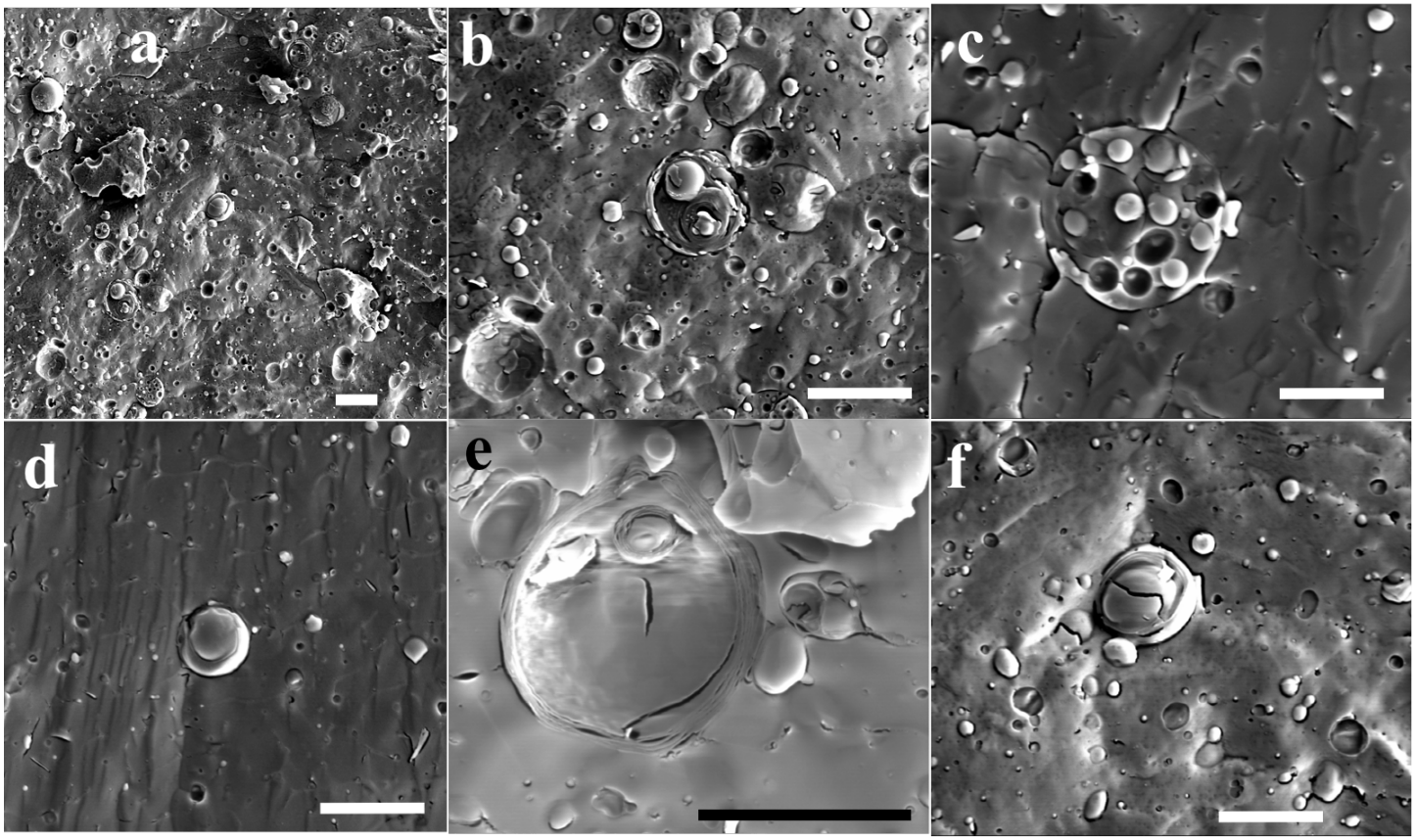

Figure 5: Images of FF-Cryo-scanning electron microscopy (SEM) analysis. a)+b) Overview images containing high polydisperse polymersome populations. b)+c) Multivesicular polymersomes d)+e) multilamellar polymersomes, f) melting of the sample at higher magnification. Scale bar is 3μm.

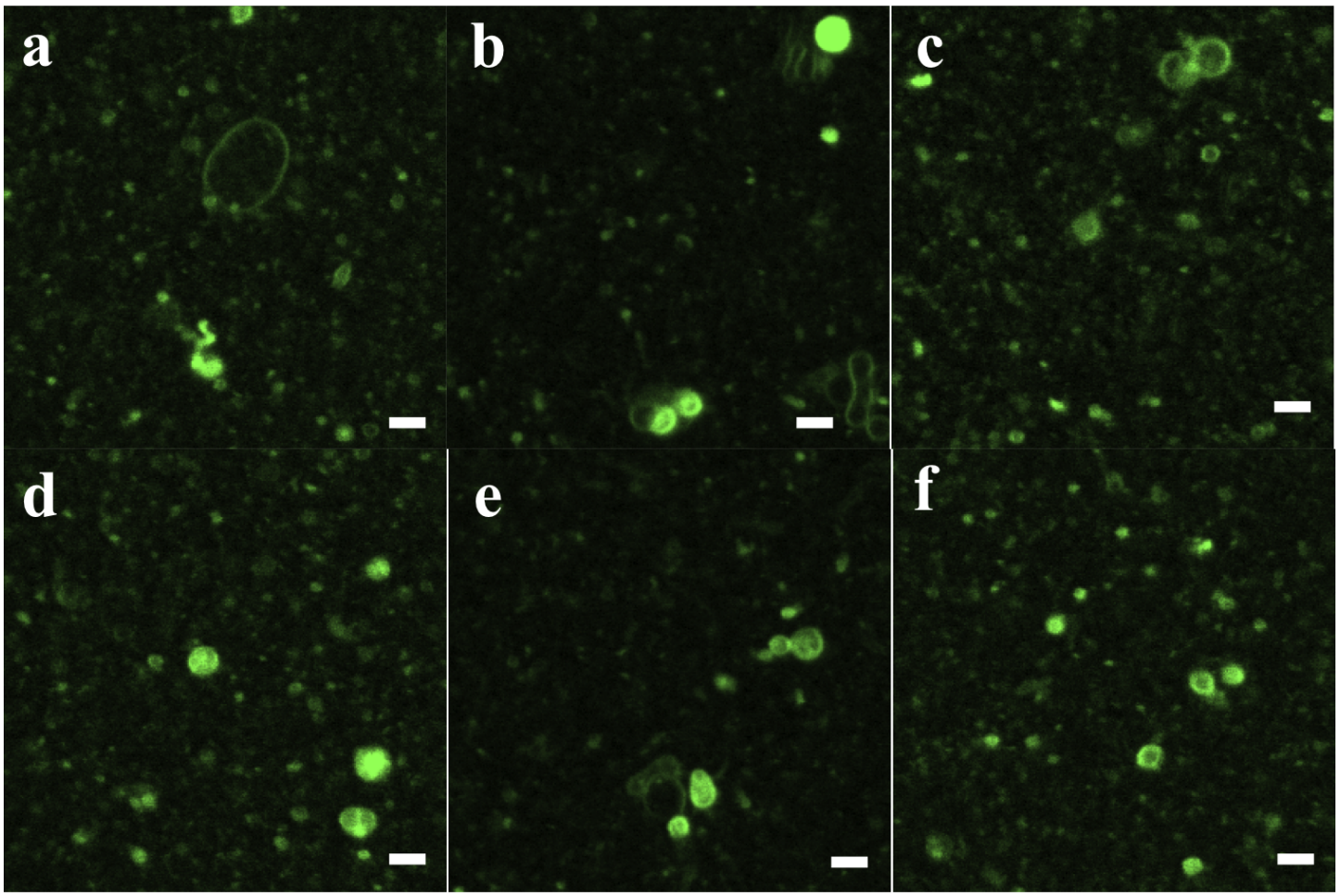

Figure 6: Images of confocal laser scanning microscopy (CLSM) analysis with coumarin 6 labelled polymersomes. a)+b) Mainly small coumarin-filled polymersomes, where there were some large ones, where the bilayer could be observed, b) tubular structures at the right bottom of the image, Overview images containing high polydisperse polymersome populations. c) unfilled and d) filled larger polymersomes, e) a mixture of both, f) high polymersome population. Scale bar is 2μm.

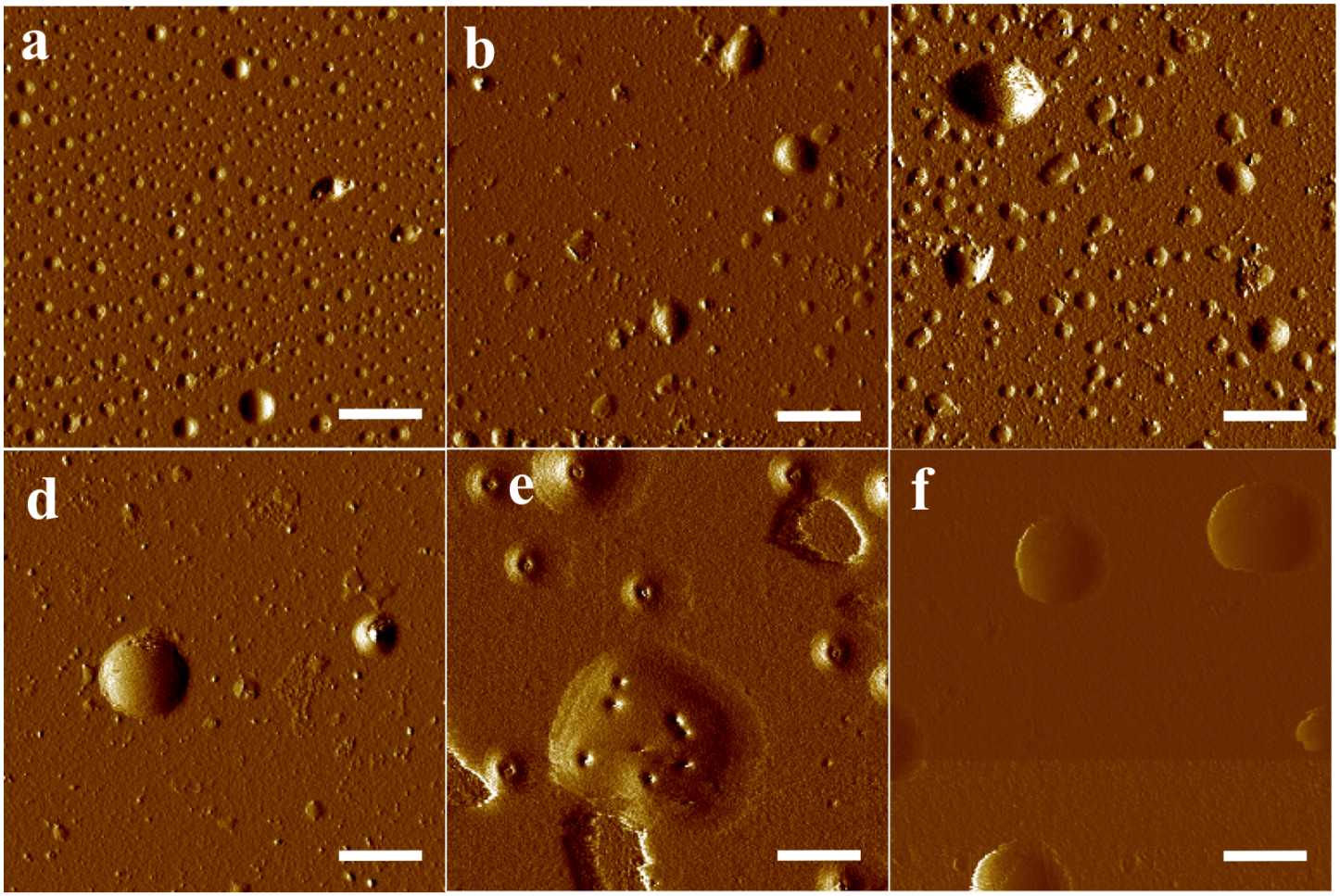

Figure 7: Feedback signal images of atomic force microscopy (AFM) analysis. a) Highly populated and b) rarely populated ares, c) area with polymerosmes of heterogeneous size, d) potential visualization of multilamellar polymersomes at the right side of the image, e) artifacts due to the cantilever on top of the polymersomes, f) large polymersomes. Scale bar is 1μm.

1 Modelling of small-angle scattering

The scattering intensity of the three shell model can be written as a sum of four concentric spheres [1]:

$$I_{3\text{shell}}(q) = \frac{n}{N_\text{P}} \left[\sum_{i=1}^{4} \rho_i V_i \Psi(q, R_i) \right]^2 . \tag{1}$$

where R_i is the radius of the i'th sphere and $V_i = 4\pi/3\, R_i^3$ is its volume, q the x-ray and neutron scattering vector and

$$\Psi(q, R) = 3 \frac{\sin(qR) - qR\cos(qR)}{(qR)^3} \tag{2}$$

is the form factor amplitude of a sphere. The factors ρ_i are given by the scattering length densities of the solvent, ρ_s, the PEO-chains, ρ_peo, and the PB-chains ρ_pb: $\rho_1 = \rho_\text{peo} - \rho_\text{s}$, $\rho_2 = \rho_\text{pb} - \rho_\text{peo}$, $\rho_3 = \rho_\text{peo} - \rho_\text{pb}$ and $\rho_4 = \rho_\text{s} - \rho_\text{peo}$. The intensity is also proportional to the number density of scattering polymersomes which is equal to the number density of the block copolymers, n, divided by the number of polymers per polymersome N_P.

The diffuseness of the polymersome interfaces were accounted for by multiplying the scattering intensity by the function $\exp(-s^2q^2)$, where s describes the width of the interface. With this addition the complete model is described by:

$$I(q) = \exp(-s^2q^2) \int G(R_1, R, \sigma) I_{3\text{shell}}(q, R, C_\text{v}, d_\text{bilayer}, d_\text{P}, n) \text{d}R \tag{3}$$

A number of molecular constraints have been applied to keep the model physically meaningful and reduce the number of free parameters. First, the scattering lengths of PB_{33} and PEO_{18} can readily be calculated from their chemical compositions and good estimates for their partial specific volumes $v_\text{pb} = 3.341$ nm^3 and $v_\text{pb} = 1.098$ nm^3 can be found in the literature [2, 3]. This constrains the scattering length densities. Second, the total volumes of the molecules should sum up to the volume of the shells: $N_\text{P} v_\text{pb} = V_2$ and $N_\text{P}(v_\text{peo} + N_\text{w} v_\text{w}) = V_1 + V_3$. A number N_w of water molecules of volume v_w are allowed in the hydrophilic shells.

2 Abbreviations/Nomenclature

AFM - Atomic force microscopy
CLSM - Confocal laser scanning microscopy
FF - Freeze fracture
I - Scattering intensity
n - Number density of the block copolymers
N_P - Number of polymers per polymersome
NTA - Nanoparticle tracking analysis
NS - Negative staining
q - X-ray / neutron scattering vector
s - Width of polymersome interface
R_i - Radius of the i'th sphere at small-angle scattering modelling
SEM - Scanning electron microscopy
TEM - Transmission electron microscopy
V - Volume of the i'th sphere at small-angle scattering modelling
v - Partial specific volumes of hydrophobic and hydrophilic polymer units or water
$\Psi(q, R)$ - Form facture ampliture of a sphere at small-angle scattering modelling
ρ - Scattering length density

References

[1] Jan Skov Pedersen, *Analysis of small-angle scattering data from colloids and polymer solutions: modeling and least-squares fitting*. Advances in Colloid and Interface Science, 70 (0) **1997**, pp. 171–210.

[2] Craig Bartels, Rafil Basheer, and Malcolm Dole, *Specific volumes of styrene-butadiene block copolymers. 2. Effect of solvent sorption on density measurements*. Macromolecules, 15 (5) **1982**, pp. 1422–1424.

[3] Jan Skov Pedersen and Cornelia Sommer, "Temperature dependence of the virial coefficients and the chi parameter in semi-dilute solutions of PEG". In: *Progress in Colloid and Polymer Science*. Springer Berlin Heidelberg, 2005, pp. 70–78.