Electronic supplementary information for

Non-invasive study of the three-dimensional structure of nanoporous triblock terpolymer membranes

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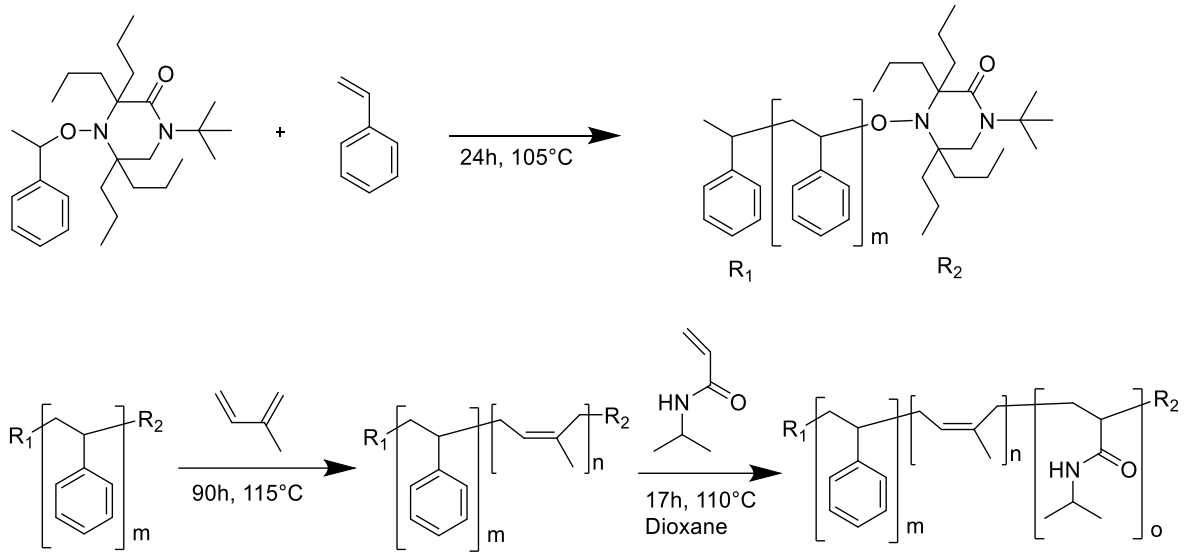
Outline:

1. Triblock terpolymer synthesis and characterization
2. Membrane preparation
3. Environmental scanning electron microscopy
4. Chord length analysis
5. Sample preparation for SPOT
6. SPOT setup
7. Stage drift correction

1. Triblock terpolymer synthesis and characterization



Scheme S1 Synthesis of the herein used triblock terpolymer using sequential nitroxide-mediated polymerization

The triblock terpolymer PS_{43}-*b*-PI_{40}-*b*-$PNIPAAm_{17}$ was synthesized by nitroxide-mediated polymerization (NMP) as described in this section.

In a 10 mL overpressure vial 3 mL unstabilized styrene (800 eq) and 16.6 mg *1-tert*-Butyl-3,3-dipropyl-5,5-diethyl-4-(1-phenylethoxy)-piperazin-2-one (NMP-Initiator)[1] was degassed in three freeze pump thaw cycles and heated to 105°C for 24h. After cooling to room temperature, the reaction mixture was precipitated twice in methanol. After filtration, 1.25 g of a white powder (yield 45%) was obtained. The number weight molar mass was determined *via* size-exclusion chromatography (SEC) using polystyrene standards resulting in 29 300 g/mol and dispersity Đ = 1.15.

The polystyrene macroinitiator was dissolved in 7.05 mL Isoprene (2000 eq) and degassed using three freeze pump thaw cycles and heated for 90 h to 115 °C. After cooling to room temperature, the reaction mixture was precipitated twice in methanol. After filtration, 1.77 g of a white powder (yield 30%) was obtained. The number weight molar

mass was determined *via* SEC using a polystyrene standard resulting in 41 300 g/mol and Đ = 1.32 (Fig. S1). The composition was determined *via* proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) resulting in PS_{48}-*b*-PI_{52}.

For the formation of the triblock terpolymer 450 mg of the polystyrene-*block*-polyisoprene (PS-*b*-PI) macroinitiator and 308 mg N-*iso*propylacrylamide (250 eq) were dissolved in dioxane, degassed with argon for 30 min and heated to 110 °C for 17 h. After cooling to room temperature, the reaction mixture was precipitated twice in hot water. After filtration, 500 mg of white flakes (yield 66%) was obtained. The molar mass was determined *via* SEC using a polystyrene standard resulting in 49600 g/mol and Đ = 1.27 (Fig. S1). The composition was determined *via* ^{1}H-NMR resulting in the triblock terpolymer polystyrene-*block*-polyisoprene-*block*-poly(*N*-*iso*propylacrylamide) (PS_{43}-*b*-PI_{40}-*b*-$PNIPAAm_{17}$, subscripts denote the weight fraction in % of the corresponding block). The absolute molar mass is then calculated from a combination of SEC (PS) and ^{1}H-NMR measurements (PI, PNIPAAm). For the determination of the triblock terpolymer composition, the signals at 7.2 – 6.2 ppm (styrene, 5H), 5.3 – 4.6 ppm (isoprene, 1,5H) and 4.2 – 3.8 ppm (NIPAAm, 1H) were compared. From the integrals the composition was calculated and further the amount of repetition units for each block, which resulted in a molar mass of 61100 g/mol (see Table S1).

The SEC measurements were carried out on a Shimadzu-SEC using chloroform, isopropanol and triethylamine (94/2/4) using polystyrene calibration and a refractive index detector. As can be seen in Fig. S1, the triblock terpolymer still contains residual PS-*b*-PI diblock copolymer macroinitiator. However, we expect this contamination to be trapped within the membrane matrix. The ^{1}H-NMR measurements were carried out in deuterated chloroform on a 300 MHz Bruker AVANCE NMR. A summary of the characterization results of the triblock terpolymer are given in Table S1.

Table S1 Summary of the analysis of the triblock terpolymer. a) Shimadzu-SEC using chloroform, isopropanol and triethylamine (94/2/4) with a polystyrene standard and a refractive index detector, b) 300 MHz Bruker NMR in $CDCl_3$, c) numbers refer to the respective degrees of polymerization

	M_n [g/mol][a)]	$Đ$[a)]	M_n [g/mol][b)]	Composition [c)]
PS	29 300	1,15	-	PS_{288}
PS-*b*-PI	41 300	1,32	48 000	PS_{288}-*b*-PI_{258}
PS-*b*-PI-*b*-PNIPAAm	49 600	1,27	61 100	PS_{288}-*b*-PI_{258}-*b*-$PNIPAAm_{115}$

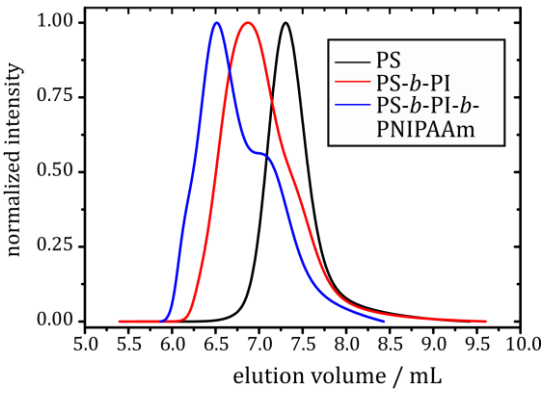


Figure S1 SEC elution traces of the herein used triblock terpolymer

2. Membrane preparation

The triblock terpolymer PS_{43}-*b*-PI_{40}-*b*-$PNIPAAm_{17}$ was synthesized by NMP as described above and then further processed to form membranes *via* self-assembly and non-solvent induced phase separation (SNIPS). A 15 wt % terpolymer solution in a 1:1 mixture of THF and DMF was cast onto a pre-cleaned glass substrate using a doctor blade with a step-

height of 200 µm. This was carried out in a humidity-controlled chamber at 22 °C temperature and a relative humidity of 50 %. After casting, the solvent was allowed to evaporate for 60 s before immersing the proto-membrane into a non-solvent bath of deionized water (pH ≈ 6.0). After several hours, the resulting membrane was removed and stored in high-purity water (Milli-Q).

3. Environmental scanning electron microscopy

Water inflated and cooled pieces of the membrane were placed in the sample chamber of an electron microscope (FEI Quanta FEG 250, Thermo Fisher Scientific) that was operated in the environmental scanning electron microscopy (eSEM) mode. The chamber was chilled to 2°C for 20 minutes and flushed twice with water vapour. After that, it was stabilized at a pressure of 715 Pa corresponding to a humidity of about 100%. Under these conditions, the membrane pores are completely filled with water and therefore, cannot be imaged by the electron microscope. In order to reveal the pores and to ensure that the drying process itself does not alter the structure, the moisture was gradually reduced by lowering the pressure within the sample chamber. An example of an eSEM micrograph of the membrane surface as a function of the humidity is shown in Fig. S2. The contrast and richness of the surface details were sufficiently enhanced at a humidity of 62%, while the structure remains unaltered.

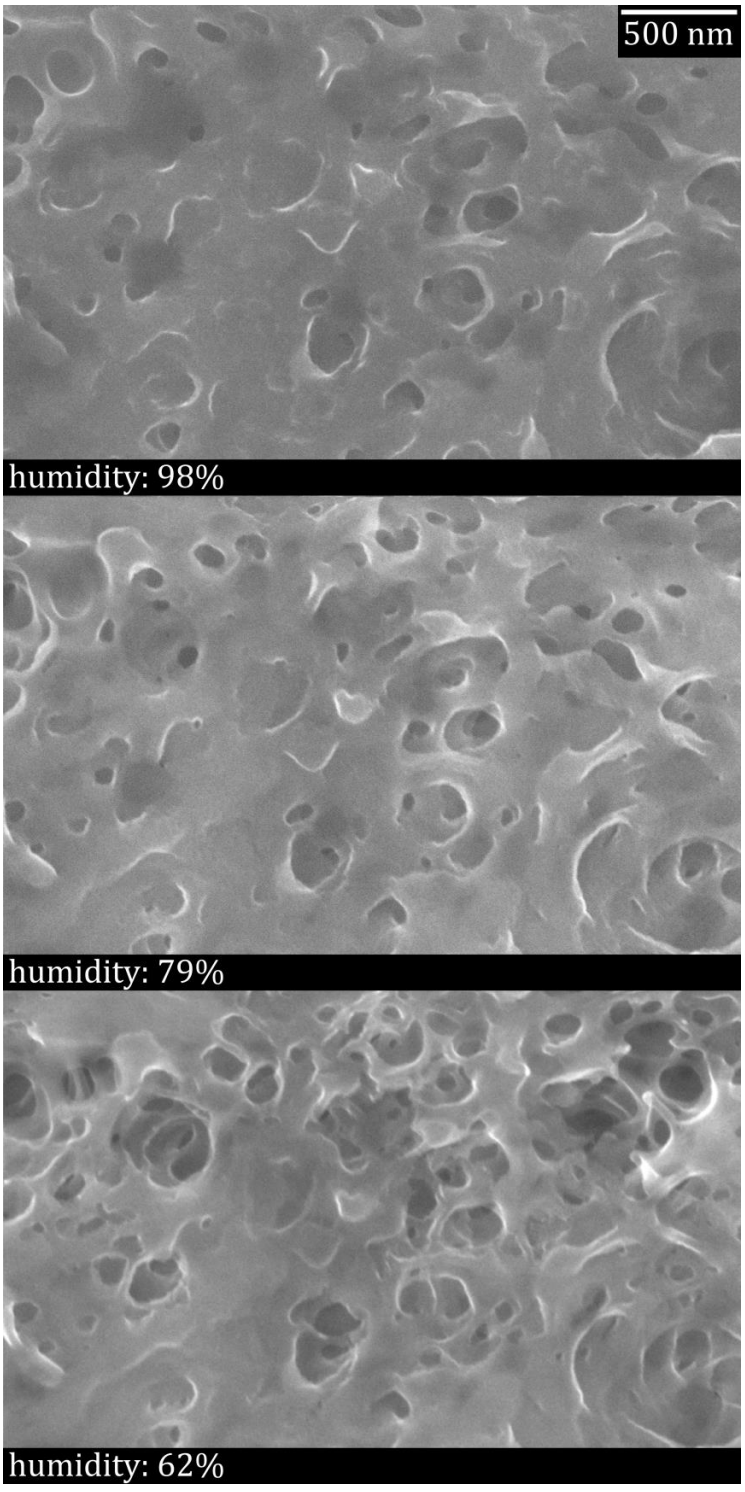


Figure S2 eSEM micrograph of the membrane surface for decreasing the humidity in the environment from 98% to 62% from top to bottom. The humidity is controlled by reducing the pressure from 697 Pa *via* 560 Pa to 437 Pa at a constant temperature of 2 °C. (Voltage: 10.00 kV, Magnification: 40 000x)

4. Chord length analysis

The chord length analysis (CLA) is a digital image processing technique for characterizing the length scales occurring within an inhomogeneous structure. It is based on a binarized image, where black areas represent the membrane wall and white areas indicate void spaces. Within the void space a given number (actually some hundreds or thousands) of points are randomly selected. From these starting points, straight lines are expanded pixel by pixel in different directions until they hit the membrane wall. Lines leading in opposite directions are merged in one chord and a histogram over the length L of this chords is interpreted as the morphological pore size distribution *P(L)*.

The benefit of this objective and fully automatized evaluation is often hampered by the difficulty of correctly binarizing the image. After enhancing the contrast of the images, binarization is usually done by thresholding. Several algorithms are available for determining a suitable greyscale level. We chose a threshold that is between the maximum of the greyscale histogram and its steepest descent and fed it into the adaptive binarization method of MATLAB (The Mathworks, Inc., Version R2016a). Then all voids that touch the limits of the imaged region are removed. But still the method might lead to misinterpretations concerning what is recognized as a wall or a void. Therefore, for each region assigned as a void the area A and the perimeter u were determined from the pixelated image from which the parameter $m = 4\pi A / u^2$ was calculated. Note that for a perfect circular void $m = 1$. Given the membrane formation process it is expectable to deal with round pores, and structures with irregular contours featuring $m \leq 0.5$ are rejected from the analysis. This criterion is independent of the size of the pores which are discriminated exclusively according to their shapes.

5. Sample preparation for SPOT

For the SPOT experiment, we mixed 2,2'-thiodiethanol (TDE) with buffered water (sodium phosphate buffer at pH = 8.0) in the ratio 1:1 to obtain a viscous, high-refractive-index medium with a distinct pH and a known ionic strength of 13 mM. Furthermore, we added a small amount of the non-ionic detergent Triton X-100 yielding a total concentration of Triton X-100 in the buffer of 0.1% (vol/vol). The dynamic viscosity of this mixture was determined to 4.9 mPa s with a rolling-ball viscosimeter (AMVn, Anton Paar GmbH) and density and sound velocimeter (DSA 500 M, Anton Paar). The refractive index of the mixture was determined to $n_{\mathrm{Buffer}} = 1.43$ *via* a refractometer (Abbemat, Anton Paar GmbH). Using this buffer solution, nanoparticle suspensions with number concentrations of 7pM were prepared. We used fluorescent polystyrene nanoparticles with a nominal diameter of 25, 50 and 100 nm stained with Dark Red or Nile Red and with a carboxylate-modified surface as tracers (FluoSpheres® Carboxylate-Modified Microspheres, Thermo Fisher Scientific, $Ø_{\mathrm{nom}} = 25 \pm 4$ nm, Dark Red, F8783; Red Fluorescent Carboxylated PS Latex, $Ø_{\mathrm{nom}} = 50 \pm 18$ nm, CAFR050NM, Magsphere.inc; Red Fluorescent Carboxylated PS Latex, $Ø_{\mathrm{nom}} = 100 \pm 20$ nm, CAFR100NM, Magsphere.inc). The actual size distributions of the tracer particles were determined by dynamic light scattering (DLS) and the CONTIN analysis.[2] The obtained particle distributions are given in Fig. S3.

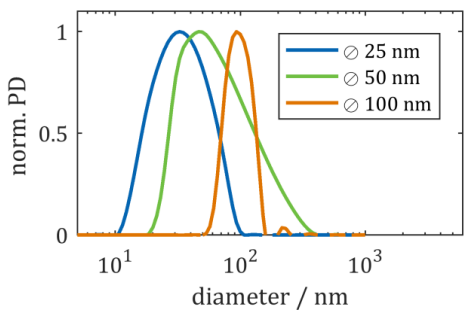


Figure S3 Particle diameter distribution determined *via* the CONTIN analysis of DLS experiments

For the SPOT experiments, a small piece of the membrane (≈ 0.5 cm × 0.5 cm) was dried under moderate nitrogen flow. Subsequently, a droplet (≈10 µL) of sodium phosphate buffer at pH = 8.0 was placed onto the membrane and allowed to soak in for 5 minutes. The membrane was dried again under nitrogen, wetted with 5 µL of the nanoparticle suspension and subsequently sandwiched between two microscope cover slips. Both slips, separated by a thin PDMS (polydimethylsiloxane) ring, constitute the sample cell that was mounted on the piezo-stage.

6. SPOT setup

The experimental setup single-particle orbit tracking consists of a home-built fluorescence microscope, that is extended by a deflection unit for the excitation light for creating two parallel light orbits that are shifted with respect to each other along the optical axis in the detection volume. The deflection unit consists of three acousto-optical deflectors (AOD, DTSX-400-532, driver DRFA10Y-B-0, amplifier AMPA-B-30, Pegasus Optik GmbH). First a linear polarized laser beam with a wavelength of λ = 635 nm (Radius 635-25, Coherent Inc.) or λ = 532 nm (Monopower™-532-100-SM. Alphalas) is directed into the first AOD and deflected vertically with a frequency of 1 kHz.

The deflected beam is guided along the optical axis *via* a relay unit, before it is then split by a 50:50 beam splitter. For each arm, the beam is then projected into a secondary AOD. The secondary AODs are oriented perpendicular with respect to the primary one, and each one superimposes a horizontal deflection with 1 kHz and a $\pi/2$ phase shift on the initial deflection of the light beam. This results in two laser beams that each rotate on a cone with its apex inside the second deflector crystals. In each light pathway, a telecentric lens system with the ratio of the focal lengths of 1:5 expands the beam diameter and projects the apex of the deflection cone into a plane that corresponds to the back focal

plane of an infinity-corrected microscope objective (UPLSAPO 60×, NA = 1.2, water immersion, Olympus Corp.). The desired axial displacement of the two light orbits along the optical axis is achieved by a slight deadjustment of each of the second telecentric lenses in opposite directions. Within the focal lengths of the last telecentric lens the two beams are combined with a polarizing beam splitter cube which requires a $\lambda/2$-waveplate in one of the paths. Subsequently, the combined beams are directed through a $\lambda/4$-waveplate for generating circularly polarized light. Then the excitation passes a dichroic mirror and enters the aforementioned microscope objective. The fluorescence from the sample is collected by the same objective and reflected by the dichroic mirror and focused by a lens (f = 250 mm) into a pinhole (Ø50µm), which is imaged further by another lens (f = 50 mm) onto an avalanche photodiode (APD, SPCM-AQR-14, PerkinElmer Inc.). As an option to take an image from the sample, the excitation light can be defocused with a lens in the excitation path, and the signal can be reflected via a flip mirror in the detection path through a tube lens (f = 200 mm) towards a CCD camera (pixelfly, PCO AG). The sample itself is mounted on a three-axis piezo stage (Tritor 102, Piezosystem Jena GmbH). During a SPOT experiment, the excitation intensity of each of the focal spots is set to approximately 7kW cm^{-2}. The whole setup is controlled by home-written software running on a programmable real-time I/O system (ADwin Gold II, Jäger Computergesteuerte Messtechnik GmbH). The real-time processor recorded the particle positions at a constant sampling rate of 250 Hz and stored them on hard disk. Typically, trajectories could be recorded for several minutes. All trajectories used for this report consist at least of $5 \cdot 10^4$ data points and were acquired at room temperature.

7. Stage drift correction

Our SPOT setup suffers from the fact that we use an open-loop piezo stage without positioning sensors and the piezo stage undergoes unavoidably characteristic creeping. Because the extend of this creeping depends on the voltage differences that are applied for driving the piezo this movement features a memory. As a consequence of this, the experimental trajectories appear as a superposition of the diffusive movements of the tracer particles and a movement of the particles that appears like a drift but which is in fact a movement of the stage. In order to correct the trajectories for the underlying creeping of the piezo stage we calculated a coarsened copy of the trajectory by smoothing adjacent positions over an interval of 60 seconds with a Savitzky-Golay filter of second degree. This smoothed trajectory is then subtracted from the measured trajectory revealing the stage drift-corrected trajectory. This is justified, because in an equilibrated sample without further forces a particle will not change its mean position monotonically. The result of this correction is demonstrated in Fig. S4 for an immobilized particle. As a matter of fact, it turned out that this correction has only a minor effect on the calculated MSDs on the relevant timescales of our experiments.

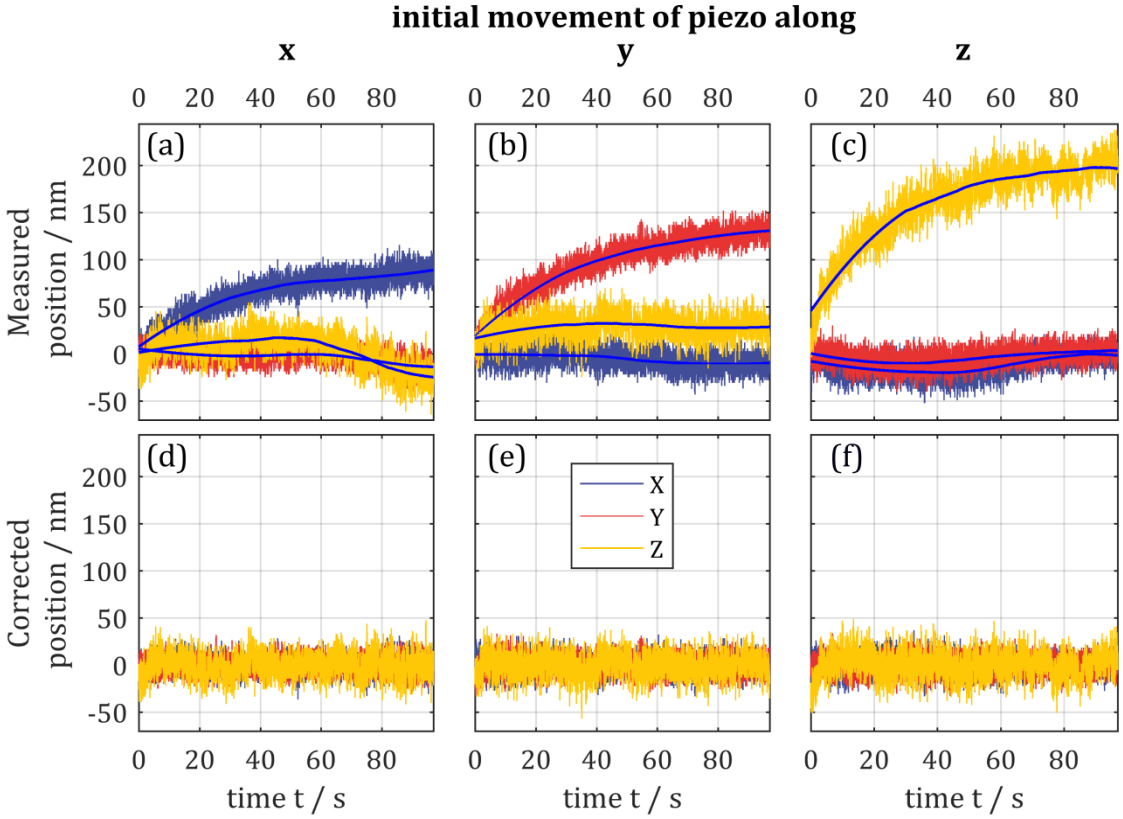


Figure S4 Particle coordinates of an immobilised particle. Top row: Raw data of the trajectory coordinates for x (blue), y (red) and z (yellow) for a 10 µm excursion of the piezo stage along the (a) x, (b) y and (c) z direction, prior to the tracking experiment. The blue solid lines correspond to the smoothed trajectory created with the Savitzky-Golay filter applied on time intervals of 60 s duration The preferential creeping of the piezo along the direction of the preceding excursion is obvious. Bottom row (d-f): Trajectory coordinates after subtracting the fits.

References

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2 A. Scotti, W. Liu, J. S. Hyatt, E. S. Herman, H. S. Choi, J. W. Kim, L. A. Lyon, U. Gasser and A. Fernandez-Nieves, *J. Chem. Phys.*, 2015, **142**, 234905.