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Properties of Hydrogen-Bonded Liquids at Interfaces

<https://doi.org/10.1515/zpch-2018-1110>

Received January 19, 2018; accepted February 16, 2018

Abstract: Effects of interfaces on hydrogen-bonded liquids play major roles in nature and technology. Despite their importance, a fundamental understanding of these effects is still lacking. In large parts, this shortcoming is due to the high complexity of these systems, leading to an interference of various interactions and effects. Therefore, it is advisable to take gradual approaches, which start from well designed and defined model systems and systematically increase the level of intricacy towards more complex mimetics. Moreover, it is necessary to combine insights from a multitude of methods, in particular, to link novel preparation strategies and comprehensive experimental characterization with inventive computational and theoretical modeling. Such concerted approach was taken by a group of preparative, experimentally, and theoretically working scientists in the framework of Research Unit FOR 1583 funded by the Deutsche Forschungsgemeinschaft (German Research Foundation). This special issue summarizes the outcome of this collaborative research. In this introductory article, we give an overview of the covered topics and the main results of the whole consortium. The following contributions are review articles or original works of individual research projects.

Keywords: aqueous solutions; confinement; hydrogen-bonded liquids; macromolecular crowding; mesoporous silica; proteins; reverse micelles; water.

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1 Introduction

The properties of hydrogen-bonded (HB) liquids in nanoscopic confinements are of utmost importance in nature and technology. In living cells, biological functions derive from an interplay of various types of densely packed macromolecules with water and cosolvents in between [1–3]. In nanotribology, technological applications depend on interactions between adjacent surfaces and aqueous solutions [4, 5]. These examples illustrate that understanding the behavior of HB liquids in various types of nano-sized confinements is a crucial issue.

In many cases, the structure and dynamics of liquids in confinement deviate from that in the bulk [6–10]. For instance, molecules can show preferred orientations and slowed motions near interfaces. Likewise, the thermodynamic properties of confined and bulk liquids are often different [11, 12]. Examples are a depression of the freezing point and, for mixtures, enhanced or reduced miscibility. However, a comprehensive understanding of such confinement effects is still lacking. In general, various mechanisms cause altered behaviors of liquids in nanoscopic confinements. Firstly, confined liquids are subjected to geometrical restrictions so that finite-size effects result when intrinsic structural or dynamical length scales are comparable to the spatial extension of the confinement. Secondly, confined liquids are exposed to interactions with the confining matrix, leading to distorted behaviors in the interfacial region, which weaken with increasing distance to the liquid-matrix interface. As a consequence of the latter effect, liquid properties usually strongly vary across a given confinement.

Considering the high complexity of natural and technological HB materials, gradual approaches are advisable. Specifically, in order to obtain fundamental insights, it is useful to systematically increase the level of intricacy from relatively simple models, e.g. pure water in defined confinements, such as mesoporous silica, to relatively complex mimetics, e.g. proteins dissolved in water and cosolvents and confined by macromolecular crowders. This approach involves suitable variation of both liquid and matrix. Here, we move from pure to mixed HB liquids and from small to large molecules. Moreover, we vary the size, hydroaffinity, and rigidity of the matrices and we consider regular as well as irregular confinements. In view of the very rich phenomenology, it is also necessary to collect information from a number of different methods. In this issue, we compile results from various experimental techniques so as to characterize structure and dynamics in wide ranges of time and length scales. Furthermore, we compare data from experiments with results from computational and theoretical studies. Using such a comprehensive approach, detailed knowledge about the effects of various types of nano-sized confinements on the properties of HB liquids, in particular, about the roles of finite-size effects and liquid-matrix interactions can be obtained.

The described concerted approach was taken in the framework of Research Unit FOR 1583 funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation). The consortium comprises preparative, experimentally, and theoretically working scientists organized in ten projects. This issue summarizes the outcome of Research Unit FOR 1583. While this article gives an overview over the covered topics and the main results of the whole consortium, the following contributions are review articles or original works of the individual projects.

This overview article is organized as follows: Section 2 deals with the preparation of suitable confinements. It outlines that confinements of well-defined sizes and shapes can be obtained from mesoporous silica materials and ion-track membranes. Moreover, we show that chemical surface functionalization and atomic layer deposition allow us to tune the properties of the inner surfaces of these matrices. Finally, we discuss that water-in-oil microemulsions provide access to confinements with tunable rigidity, while macromolecular crowders mimic the situation in biological materials. In Section 3, results for pure HB liquids in confinement are presented. Due to its omnipresence, the primary focus is on water. In particular, we exploit that, in severe confinement, the freezing point is significantly lowered and, hence, it is possible to ascertain the properties of liquid water at low temperatures. This is of fundamental importance for an understanding of the anomalies of water, but obscured by rapid crystallization for the bulk liquid. Section 4 deals with results for confined aqueous mixtures. A main aspect is microphase separation near interfaces, in particular, its dependence on the composition of the mixture and its role for the dynamical behavior. In Section 5, biological materials and mimics thereof are addressed. The main emphasis is on confinement effects on protein properties, including protein stability, structure, dynamics, and phase behavior. Moreover, we study protein-solvent couplings. Finally, our conclusions are presented in Section 6.

2 Preparation of tailor-made confinements

2.1 Preparation and chemical functionalization of mesoporous silica; NMR characterization

Mesoscopically organized porous silica materials [13–25] with their typical dimensions between the molecular and the macroscopic regime, i.e. between 1 and 100 nm, and their favorable physico-chemical properties, such as large specific volume and surface, low specific weight, high thermal stability, and tunable pore sizes, have large application potentials in physics, chemistry, biology, and

materials science. Already now, their applications include diverse topics such as catalysis, separation media, gas storage, transporters for drugs, additives for polymers and more [26–30].

Because of their large inner free volumes, relatively narrow pore diameters, and high inner surface areas, mesoporous silica materials are ideal model systems for detailed investigations of confinement effects on pure liquids, multiphase liquids, or solutions, in particular, HB systems. They help in understanding the influence of surface forces, the competition between surface and bulk forces, and finite-size effects on the behavior of the liquids inside the pores [31]. Such studies include the effects of a confinement on the structural, dynamical, and thermo-physical properties such as the freezing and melting points or glass transitions [31–35] of confined molecules such as water, alcohols, carbonic acids, protein solutions [36, 37] and many others. Their three dimensional pore networks can be either sponge-like, leading to disordered glasses such as CPG-10-75 [12, 38] or Vycor, or they can be ordered in the form of a two-dimensional hexagonal array of cylindrical pores, leading to well-known periodic mesoporous silica materials, such as MCM-41 [13] and SBA-15 [39, 40]. Their versatility is mainly due to the fact that their production allows for a precise tuning of the porosity parameters, and the presence of surface-silanol groups of the Q_2 and Q_3 type allows for easy chemical modification of their inner surfaces. Thus, their surfaces can be made to be, e.g. polar or non-polar, hydrophilic or hydrophobic, covered with hydrogen-bond donors or acceptors or with other chemical functions [29]. These surface functionalizations are achieved by either covalently binding functional groups such as amino, amide, carboxyl, phosphate, chloride or peptide functions by post-synthetic grafting, or by co-condensation with appropriate additives during the synthesis [28, 41, 42].

No single physico-chemical technique is able to fully characterize confined liquids inside these pores. Rather, in general, several complementary techniques have to be combined to reveal the complete picture. In recent years the combination of multi-nuclear variable temperature solid-state NMR [43], thermodynamic techniques like differential scanning calorimetry (DSC) or thermogravimetric analysis (TGA) [44, 45], and computational techniques [46] has evolved into the most powerful approach for studies of these systems (see e.g. [36, 43, 47–57]). In such combined approaches, NMR experiments provide insights at the molecular level, in particular when employing the cross polarization (CP) and magic angle spinning (MAS) techniques, thermodynamic techniques into the macroscopic properties, and computation links these two regimes and assists an interpretation of the respective results.

If the effects of severe confinements are to be studied, it is important to investigate materials with narrow pore diameters and very high surface homogeneity.

In this case, MCM-41 materials are to be preferred to SBA-15 materials since the former have smaller pore diameters [13] and smoother inner surfaces [47] than the latter. In the framework of Research Unit FOR 1583, a series of very well-defined MCM-41 materials with tunable narrow pore diameters, starting at 2.2 nm and, if necessary, functionalized by suitable groups were synthesized. For the MCM-41 synthesis, a reaction protocol was employed, which is based on that of Grünberg et al. [58] and Grün et al. [59]. It is described in some detail in the contribution of Brodrecht et al. [60] to this special issue.

While pore volumes, pore sizes, and specific surface areas of mesoporous silica materials can be characterized by nitrogen adsorption, ^{29}Si solid-state NMR spectroscopy yields information about the surface sites. In this special issue, such approaches are described by Brodrecht et al. [61] and Weigler et al. [62]. Figure 1 shows ^{29}Si solid-state NMR spectra of native and functionalized MCM-41 materials [61, 62]. We see that transformations of the silanol groups during functionalization can be detected based on changes from Q-groups to T-groups.

After synthesis, special care has to be taken to ensure complete drying of the samples, as these materials are very hygroscopic and adsorbed water molecules can strongly influence the result of confinement studies [48], in particular when lowly hydrated samples with similar amounts of near-surface and bulk-like water are studied [8, 51, 63, 64]. Therefore, it is important to

Fig. 1: Upper panel: definition of silicon atoms Q_n ($n=2-4$) and T_n ($n=1-3$) in native and functionalized silica materials, respectively. Lower panel: ^{29}Si CP MAS spectra at 10 kHz of (left) native and (right) functionalized silica, showing the appearance of T_n -groups during the functionalization (adapted from Weigler et al. [62]).

check the hydration state before filling of porous materials. For this purpose, ^1H MAS NMR measurements proved particularly useful. In this special issue, Brodrecht et al. [60] show that this method allows one to clearly distinguish water species in different chemical environments and that conventionally stored MCM-41 samples contain a substantial amount of water, confirming the need for an appropriate drying procedures before pore loading. Moreover, these authors describe a suitable protocol for the preparation of “water-free” mesoporous silica samples.

Of large interest are modifications of mesoporous silica with organic molecules, in particular, with biomolecules like peptides [65–68], leading to inorganic/organic hybrid materials which model typical porous systems in biomineralization [69, 70]. Natural examples of such materials are bones consisting of hydroxyapatite and collagen or diatoms consisting of silica and sillafins (polyamines) [71–74].

In this special issue, Brodrecht et al. [61] describe grafting of collagen-like nonapeptide $\text{H}-(\text{Gly-Pro-Hyp})_3-\text{OH}$ to mesoporous silica materials. As described in more detail in previous work [57], its binding to the surface can be proven by a combination of ^{13}C CP MAS and dynamic nuclear polarization (DNP) enhanced ^{15}N CP MAS NMR experiments in natural abundance. ^{13}C NMR spectra are reproduced in Figure 2. The intensity reduction of the peaks of the succinimidyl moiety (ca. 15 ppm, Figure 2a and d) and the appearance of the characteristic peaks for the nonapeptide (Figure 2b) in the carbonyl as well as

Fig. 2: ^{13}C CP MAS NMR (14 Tesla, 5 kHz) spectra of (a) immobilized and (b) free nonapeptide $\text{H}-(\text{Gly-Pro-Hyp})_3-\text{OH}$; (c) pre-activated silica TSTU-silica (at 10 kHz), and (d) COOH-silica (at 10 kHz). Note: Signals marked with * refer to spinning sidebands (adapted from Werner et al. [57]).

the aliphatic regions indicate a successful immobilization of the peptide. The final prove of the formation of a covalent bond between peptide and silica was derived from the DNP enhanced ^{15}N NMR spectra [57].

2.2 Preparation and ALD modification of ion-track channels in polymer membranes

In addition to mesoporous silica materials, Research Unit FOR 1583 studied ion-track polymer membranes as porous systems with very long, parallel oriented, cylindrical nanochannels. While mesoporous silica materials have the advantage of a very high specific surface and a well-ordered pore structure with small diameters below ca. 8 nm and narrow pore diameter distributions, ion-track channels have the advantage that the pore diameter can be adjusted over a very broad range from tens of nm up to μm . Figure 3a gives a sketch of the production of ion-track membranes. The fabrication consists of several steps including the irradiation of a polymer membrane (typically polycarbonate or polyethyleneterephthalate) with high-energy heavy ions followed by chemical etching, which allows one to selectively remove the damaged material created along each ion trajectory. The diameter of the channels is adjusted by selecting suitable etching conditions (e.g. time, concentration, etc.) and the number of the channels is determined by the irradiation conditions, exploiting that the track of each ion is converted into a nanochannel [75–78]. In the next step, the ion track membrane can be coated with silica or other oxides by atomic layer deposition (ALD) [78, 79]. Furthermore, functionalization with linkers such as APTES is possible.

Figure 3b displays SEM pictures of track-etched polycarbonate membrane ion-irradiated under various angles of beam incidence. Owing to the larger pore diameters, the porosity of these materials is much smaller than the porosity of mesoporous silica materials. Due to the smaller inner surface, chemical characterization and monitoring of the functionalization was performed by means of DNP providing higher sensitivity than regular solid state NMR. As experimental examples, the upper data in Figure 3c and d display the DNP enhanced ^{29}Si and ^{13}C solid-state NMR spectra of the material. The ^{29}Si NMR spectrum clearly reveals the presence of T_n -groups, indicating the successful functionalization of the silica-coated surface with APTES, see Section 2.1. Note that without DNP, the intensity of the T_n -groups is too low for their detection in studies on ion-track membranes. The comparison of the ^{13}C solid-state NMR spectra of the sample recorded with (ii) and without (i) microwave irradiation clearly illustrates the suitability of this technique for future studies.

To characterize the pore geometry of track-etched channels, e.g. the pore diameter, and to follow the size reduction upon ALD coating, small angle X-ray scattering (SAXS) proved very useful [78–81]. Combining SAXS and scanning transmission electron microscopy (STEM) provided valuable information about

Fig. 3: (a) Illustration of the steps implemented to prepare amine functionalized silica coated porous polycarbonate-membranes. (b) SEM image (side and top view) of track-etched polycarbonate membrane ion-irradiated under various angles of beam incidence. Cylindrical open channels go through the entire membrane with a thickness of 30 μm. (c) DNP enhanced ^{29}Si CP MAS spectrum (i) reveals the occurrence of the T_n -groups, as clearly visible in the zoom in the spectrum (ii). (d) DNP enhanced ^{13}C CP MAS spectra of the SiO_2 ALD coated polycarbonate membrane, measured at 8 kHz spinning, nominally 110 K, for (i) MW on and (ii) MW off, revealing signal enhancements (adapted from Kumari et al. [61]).

Fig. 4: (a) STEM images of single nanotubes synthesized by ALD coating of polycarbonate nanochannels with a nominal wall thickness of 5 nm of TiO_2 . The outer diameters of the nanotubes are about 18, 31, 42, and 55 nm. (b) Corresponding channel diameter as a function of etching time before and after ALD coating, determined from SAXS measurements. The uncertainties of the SAXS analysis are about 0.2 nm. Solid line is a linear fit to the SAXS diameters for uncoated channels; dashed line is a guide to the eye for the reduced diameters as expected from the nominal thickness of the ALD coating. The figure was adapted from Spende et al. [78].

Fig. 5: Contact angle measurements as a function of the number of ALD cycles. With increasing thickness of the SiO_2 layer the samples become more hydrophilic. Reproduced from Sobel et al. [79].

the homogeneity and deposition rate of ALD in the nanochannels. Figure 4 shows, for example, results obtained for deposition of TiO ALD layers on membranes with different channel diameters [78, 79]. The exact composition of the deposited layer is decisive for the functionality of the coated membrane. It can readily be characterized by X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR), providing evidence for nearly stoichiometric compositions for various coatings [78, 79].

Fig. 6: Molecular-templated ALD applicable to the creation of hydrophobic nanopatterns or nanocavities. Large molecules such as p-tert-butyl-calix[4]arene (calixarene) are covalently bound to the substrate surface by condensation of hydroxyl groups (top) and act as template for the subsequent ALD coating process (middle). Removal of the molecules results in nanocavities (bottom). Image is modified from Sobel and Hess [82].

To demonstrate the potential of the ALD approach for tailoring the hydrophilic/hydrophobic properties of polymer surfaces, we performed contact angle measurements (see Figure 5). Upon ALD coating of PC membranes, the initially hydrophobic surface becomes more and more hydrophilic with increasing thicknesses of the deposited SiO₂ layer [79].

2.3 Nanoscale structuring of surfaces

Based on these coating studies, we further developed strategies for controlled surface patterning [82, 83]. Specifically, ALD-based techniques allowed us the

fabrication of nanocavities on the inner surfaces of mesoporous materials. The process of nanocavity formation is sketched in Figure 6 [83]. It involves the attachment of large molecules such as *p-tert*-butyl-calix[4]arene (CAL) as templates to a TiO_2 monolayer on SBA-15 material followed by Al_2O_3 deposition using ALD treatment and, finally, removal of the CAL molecules by reaction with ozone [83]. In this way, uniform nanocavities of conical shape and 1–2 nm diameter are produced.

2.4 Soft confinements: macromolecular crowders, micelles etc.

Due to macromolecular crowding, biochemical and biophysical measurements performed in dilute solution may fail to reflect the actual scenario taking place *in cellulo* [84–86]. One approach to carry out more relevant measurements would be to use cellular extracts. Such complex extracts contain, however, many kinds of biomolecules so that a coexistence of various types of interactions may hamper gaining detailed mechanistic information about the process under study. Consequently, crowding effects are often mimicked *in vitro* by adding high concentrations of relatively inert molecules such as polyethylene glycol, polysaccharides such as Ficoll and dextran, or proteins such as lysozyme to the biomolecular solutions. Such approach was also taken in this work [84]. The underlying physical mechanism by which macromolecular crowding acts is generally explained in terms of excluded volume, i.e. the volume inaccessible to the biomolecules due to their repulsive interaction with the macromolecular crowding agents. This notion goes back to Asakura and Oosawa [87], who have described depletion forces induced by steric, hard-core interactions. This mechanism was also proposed to explain why small cosolutes, namely protective (compatible) osmolytes, which are preferentially excluded from proteins, shift the protein folding equilibrium towards the natively folded state. However, one must be aware that such artificial crowding agents can sometimes interact weakly with the systems being examined, hence leading to enthalpic effects next to the purely entropic excluded volume effect.

Next to macromolecular crowders, soft matter mesophases such as micelles, hexagonal and cubic lipid phases, microemulsions, and hydrogels provide well-defined containers of tunable size and rigidity for investigations of conformational, dynamical, and hydration properties in confinement [88]. Furthermore, liquid-crystal matrices may serve as anisotropic soft matter confinements. Besides the size and shape of the confinement, the properties of the confining walls may have a strong influence on the system as well and can

be varied. Specific interactions with the wall depend on the chemical nature of the confined solution and the wall, and the interaction between a confined liquid and the confining walls will be increasingly relevant with the decrease of confinement size. Further, while a hard confinement is static in the range of experimentally accessible times, a soft confinement may constantly vary in shape and size. The mutual dynamics of the wall and confined liquid is dependent on the relaxational properties of the wall, which may be hard or soft, referring to much longer or comparable relaxation times of the wall as compared to the confined liquid [88].

3 Pure hydrogen-bonded liquids in confinement

3.1 Water

Confined water is ubiquitous in biology, geology, and technology. Despite its importance, many properties of confined water are still elusive. There are two main causes for this limited knowledge. First, water, in spite of its simple chemical structure, shows a large number of complex properties already as a bulk liquid, e.g. it exhibits various anomalies, including the well-known density anomaly [89, 90]. Second, liquid water forms an extended hydrogen-bond network so that geometrical restriction and interfacial interactions may have particularly strong consequences [91]. It is well established that confined water, depending on the size and hydroaffinity of the confinement, shows a significantly lower freezing point than bulk water [92–94]. In very severe confinement, e.g. in silica pores with diameters below ca. 2 nm, crystallization to hexagonal ice is even completely suppressed. Furthermore, it is known that confined waters usually exhibit substantially altered structural and dynamical properties [9, 95, 96]. However, a fundamental understanding of these differences between confined and bulk waters is still lacking.

To unravel the origin of the anomalies of water, the supercooled regime of the liquid received considerable attention [97]. A main reason for this interest is the theoretical conjecture that the anomalies of water originate from a second critical point in the supercooled regime, which is related to a liquid–liquid (LL) phase transition between high-density and low-density forms [98, 99]. Another main cause is the experimental finding that water evolves from a fragile to a strong glass former upon cooling. Specifically, the temperature dependence of its structural relaxation deviates from an Arrhenius law in the weakly supercooled regime, corresponding to fragile behavior, while it obeys

an Arrhenius law in the deeply supercooled regime, indicative of strong behavior, suggesting a fragile-to-strong (FS) transition of water near 225 K [100, 101]. However, direct experimental observation of the LL and FS transitions is hampered due to immediate crystallization of bulk water in the ‘no man’s land’, at 150–235 K. Therefore, a large number of studies exploited the possibility to suppress crystallization in severe confinement in order to explore the properties of liquid water throughout the supercooled regime [96, 102–104]. The outcome of such studies on confined water remains, however, subject of controversial scientific debate [102]. In particular, it is still vigorously discussed whether changes in the temperature-dependent dynamics of confined water may be taken as evidence for an existence of the proposed LL and FS transitions of bulk water.

In this special issue, theoretical, computational, and experimental approaches are taken to advance our understanding of liquid water. Review articles by Geske et al. [105], Sebastiani [106], and Demuth et al. [107] provide overviews of these activities. Moreover, original works by Brodrecht et al. [60] and Weigler et al. [62] deal with the properties of water in lowly hydrated and peptide functionalized mesoporous silica, respectively.

Simple schematic models proved useful to rationalize the unusual behavior of water and other network-forming liquids [108, 109]. Heckmann and Drossel [110] studied a one-dimensional model, which combines a global density-dependent attractive interaction with a local intermolecular interaction potential featuring a narrow minimum at a distance larger than the distance of next neighbors resulting from the global term. Based on straightforward analytical calculations, they found that this model, depending on the values of its parameters, shows a LL phase transition between high-density and low-density liquids, which results from the competition between increasing the entropy at distances outside the potential minimum and decreasing the potential energy at distances inside. Moreover, they argued that such LL transition can cause a FS transition. The predictions of this model were further explored in molecular dynamics (MD) simulations on water-like [111] and silica-like [112] bulk liquids, where the partial charges of the atoms were purposely scaled to systematically vary the Coulomb interactions.

MD simulations enable detailed investigations on the effects of various types of confinements on the properties of water [95, 96, 113–116]. In particular, spatially resolved analyses of simulation data yield valuable information about degree and range of structural and dynamical distortions. In this special issue, two review articles give an overview of the field [105, 106]. Two types of approaches can be distinguished. One part of the simulation studies strived for perfect reproduction of real systems, e.g. chemically realistic silica pores were generated using

ab-initio methods [117, 118] or sophisticated algorithms [119]. In most cases, it was found that structure and dynamics of water are strongly distorted near realistic solid surfaces [95, 96, 105, 106, 113–118]. Another part of the computational approaches aimed for a fundamental understanding of the phenomena based on purposefully designed artificial systems. For example, Drossel and coworkers showed that water behaviors are substantially different at hydrophobic and hydrophilic surfaces as well as at smooth and rough walls [120, 121]. Moreover, Vogel and coworkers demonstrated that it is a suitable concept to study liquids in ‘neutral’ confinements [122–125]. In such systems, the confined liquid and the confining matrix consist of the same type of particles and, hence, the interactions are not different at the interface. For water and silica [122–125], it was observed that, despite a largely undistorted structure, the dynamics strongly slows down at neutral walls.

In Figure 7, we compare dynamical profiles of water across various types of solid confinements with cylindrical shape. Specifically, we show correlation times τ of the structural relaxation as a function of the distance to the liquid-matrix interface. When studying water in an amorphous silica pore to mimic the experimental situation in mesoporous silica materials [105, 121], it was found that the structural relaxation is about an order of magnitude slower at the pore wall than in the pore center, where bulk behavior is recovered as long as the pore diameter does not drop below ca. 2 nm. For neutral pores [122–124], an even stronger slowdown was reported, indicating that this change of water dynamics cannot be

Fig. 7: Correlation times τ of the structural relaxation of water as a function of the distance to a ‘neutral’ wall (see text for details) [122], a silica wall [105], and a smooth wall [121], as obtained from MD simulations of the SPC/E model of water at 230 K.

attributed to particularly strong water-matrix interactions. In pores with smooth walls [120, 121], a slowdown of water motion in the interfacial region was not observed, even when the potential of the smooth wall matched the average potential of the atomistic wall at a given distance. Based on these and other [105, 125, 126] results for confined liquids, it was concluded that liquids in the vicinity of atomistic surfaces show slowed structural relaxation because they have to adapt to a static energy landscape imposed by immobile surface atoms and, hence, their capability for structural relaxation is impaired. For water in neutral confinement [124], the effects were successfully rationalized in terms of a theory for glass formation.

Calorimetric, dielectric, and scattering studies [63, 92–94, 102–104, 127] prevail when studying the properties of confined water at low temperatures, e.g. to explore conjectures that the anomalies of water arise from peculiarities in the supercooled regime. In this issue [60, 62, 107], it is shown that NMR studies, in particular ^2H NMR approaches [56, 60, 62, 128] can shed new light on this question. Figure 8 compares correlation times τ of water dynamics in narrow silica pores obtained from different experimental methods. All studies report cross-overs in the temperature dependence, but their interpretation is controversial

Fig. 8: Correlation times τ of water reorientation in mesoporous silica from neutron scattering (NS) [127], dielectric spectroscopy (DS) [63], and ^2H NMR spin-lattice relaxation (SLR) and stimulated-echo (STE) studies [128]. The top arrow marks the glass transition temperature of interfacial water observed in positron annihilation lifetime spectroscopy [129]. For comparison, correlation times from thermally stimulated currents studies on hydrated collagen are shown as triangles [130].

[102]. A neutron scattering (NS) study [127] reported a prominent kink at 225 K and took it as evidence for a FS dynamical transition related to a LL phase transition of bulk water, while a dielectric spectroscopy (DS) approach [63] found a mild crossover at 180 K and related it to changes of glassy dynamics. While the ^2H NMR correlation times [128] agree with the NS data near room temperature, they show no sharp kink, but only a mild bending at 225 K. This effect was found to be accompanied by an emergence of an immobile water fraction. Therefore, it was argued that this change in the temperature dependence is not due to a LL transition, but occurs in response to a reduction of pore volume available to the mobile water fraction when the immobile water fraction forms [56, 128]. The ^2H NMR correlation times near and below 225 K agree well with the DS data. Careful analysis of various ^2H NMR observations implied that when decreasing the temperature in the range $T < 225$ K, the data reflect the structural (α) relaxation less and less, which undergoes a glass transition at about 180 K [129], and become dominated by a secondary (β) relaxation, for which the temperature dependence changes as a consequence of the glassy structural arrest. Comparing this β relaxation in various types of aqueous systems, several universal properties were reported [102, 103], in particular, a common low-temperature activation energy of ~ 0.5 eV. These findings demonstrate that it is a difficult task to extract unambiguous information about properties of supercooled bulk water from confinement studies.

3.2 Other liquids

When intending to ascertain the effects of interfaces on HB liquids, glycerol is an ideal test case because it can easily be supercooled as a bulk and a confined liquid and, hence, it is possible to compare the respective properties in broad temperature ranges down to a glass transition. Inconclusive results were obtained in previous studies of confinement effects on glycerol dynamics, explicitly, a speedup or a slowdown of dynamics as well as unchanged correlation times were reported for different types of confinements [131–137]. In this special issue, the roles of the size and the rigidity of the confinement are addressed for glycerol in micellar confinement [107, 138]. Detailed studies revealed that use of an appropriate microemulsion allows one to alter the size of the glycerol droplets by changing the composition and to vary the rigidity of the micellar confinement by modifying the viscosity of the surrounding matrix [139]. In particular, scattering work showed that the droplet phase is stable upon cooling. Scanning calorimetry yielded a glass transition temperature T_g , which hardly depends on the size and rigidity of the confinement. This result is consistent with the outcome

of ^2H NMR measurements in the weakly supercooled regime, which indicate that glycerol reorientation is hardly affected by the used micellar confinements. By contrast, such studies in the deeply supercooled regime reveal substantially accelerated glycerol rotation for some of the studied microemulsions [107, 139]. However, the observed speedup does not result from faster structural relaxation of glycerol molecules within the droplets, but rather from rotational motion of the droplets as whole, which is faster than the molecular reorientation in the ultraviscous liquid for small sizes of the glycerol droplets and low viscosities of the surrounding matrix. The latter effect became particularly clear in work using triplet solvation dynamics (TSD) [139]. In this special issue, Weigl et al. [138] describe the TSD method in some detail. Briefly, a laser pulse is employed to excite dye molecules added at a low concentration into a long lived triplet state. Afterwards, the triggered reorientation of liquid molecules in the solvation shell of the dye molecule are monitored by recording time-resolved phosphorescence spectra.

Confinement to small pores depresses, in general, the melting point of a substance. As a consequence, small aromatic molecules like biphenyl and naphthalene, which are solids at room temperature in their bulk phases, become liquids when confined inside pores. Previous studies of benzene confined in mesoporous silica [32, 49] had revealed a very interesting dynamic behavior. In the framework of Research Unit FOR 1583, the dynamics of biphenyl and naphthalene inside silylated and non-silylated MCM-41 with pore diameters of about 3 nm were investigated [54, 58]. In the case of biphenyl, a strong reduction of the melting point by more than 100 K was observed. In addition, the ^2H NMR spectra slightly below the transition temperature yielded evidence for a pre-melting process in the form of isotropic motions of the biphenyl molecules. In the case of naphthalene, a strong reduction (152 K) of the melting point was also found, but the spectra did not reveal any indications of a pre-melting process. Line-shape analysis implied that the confined naphthalene most probably forms a plastic crystalline phase, which was initially proposed for naphthalene in ball-milled silica [140–143] and later confirmed for naphthalene in mesoporous silica using several experimental methods [144].

4 Mixtures of hydrogen-bonded liquids in confinement

Confinement has considerable influence on the properties of pure liquids, but its effects on binary mixtures are even more intriguing. For confined mixtures, the interactions between the molecular species and the confining wall may be

substantially different and larger or smaller than the interactions between the components in the bulk. In combination with geometrical restriction, these interaction effects can cause disturbed phase behavior [11, 12, 145]. Moreover, they can lead to strong gradients of the molecular concentrations, which in turn, can affect the dynamical properties throughout a confinement. In this issue, such phenomena are addressed for various types of HB liquid mixtures using experimental and computational approaches [105–107].

4.1 Water-alcohol mixtures

Aqueous solutions of low molecular weight alcohols are of large importance in nature and technology. In such mixtures, polar and nonpolar groups coexist, leading to intricate structural and dynamical features as well as manifold phase behaviors, including microphase separation, in the bulk [146, 147], which become even more complex in confinements [148, 149].

MD simulations proved very useful to ascertain concentration gradients of HB liquid mixtures in confinement [126, 150–152]. In Figure 9, we show results from simulations of a water-ethanol mixture confined between slabs comprised of silanol groups [152]. The density profiles of the molecular species across the slit reveal layer formation near the interfaces. Moreover, it is striking that the density of water is reduced in the first layer at the wall, while that of ethanol is increased in this region. This very different behavior of the components is indicative of a spatially selective microphase separation, i.e. a partial demixing of water and ethanol induced by the confining walls. Commonly, surfaces with high silanol

Fig. 9: (Left) Snapshot of a mixture of water and ethanol molecules as between slabs made of silanol groups. (Right) Partial densities of water and ethanol molecules as a function of the distance to the center plane of the slab confinement at the indicated temperatures. Both figures are extracted from Guo et al. [152].

density would be considered hydrophilic, but it turns out that the reduced availability of hydrogen bonds, as compared to the bulk liquid, is sufficient to partially repel water from the wall and to enrich ethanol in the interfacial layer. Further analysis showed a formation of a saturated HB network between the silanol and ethanol O–H groups, with one donor and one acceptor functionality per O–H group, leading to noticeable stabilization of the ethanol-rich layer at the interface [106, 152].

Other MD simulation work [105, 126] investigated microphase separation for mixtures of water and ethylene glycol in amorphous silica pores. Comprehensive insights into the dependence of liquid behavior on the confinement properties were available from consideration of amorphous and crystalline matrices and from modification of the force field parameters, which served to alter the HB network of the liquid and to tune the polarity of the walls. It turned out that the confinement induces microphase separation of the mixtures, which qualitatively and sensitively depends on the properties of both liquid and matrix so that water or alcohol molecules may be preferentially adsorbed at the silica surface. For all studied confinements, pronounced mobility gradients were observed for both components across the pore, with the structural relaxation being several orders of magnitude slower at the pore walls than in the pore center [105, 126].

Experimental studies indicated that various properties of confined water-alcohol mixtures are affected by such microphase separation. For example, an altered concentration dependence of the structural relaxation was taken as indirect evidence for the existence of this phenomenon [148, 149], which is difficult to detect in experiments otherwise. Likewise, a contribution to this special issue [107] rationalizes the puzzling observation that the tendency for crystallization of aqueous alcohol solutions is enhanced rather than reduced in confinement based on partial demixing. It is argued that, due to wall-induced concentration gradients, the water (alcohol) concentration can be sufficiently high (low) in some regions of the confinement to allow for ice formation in these areas even when crystallization does not occur in the corresponding bulk mixture. The fractional crystallization leads to a freeze-concentrated solution, which may show significantly altered dynamics [107, 153]. Hence, different liquid-matrix interactions for the components of a mixture can have subtle effects on the dynamical behavior. Explicitly, they can lead to induced partial demixing and, thus, altered compositions throughout the confinement, in particular, when fractional freezing occurs, so that the dynamical properties can differ from that of the bulk mixture with the same overall composition not only at the interface, but rather throughout the confinement.

As a first direct experimental study of the microphase separation of confined water-alcohol mixtures, room-temperature and low-temperature (110 K) ^1H - ^{29}Si FSLG HETCOR experiments, combined with MD simulations, were performed

on a mixture of water and octanol confined in mesoporous silica SBA-15 (unpublished), similar to the study on another mixture reported in Section 4.2. These experiments employ the heteronuclear dipolar interactions between different types of protons and the silica-surface to distinguish their distribution in the system.

4.2 Water-isobutyric acid (iBA) mixtures

In addition to binary water-alcohol mixtures, Research Unit FOR 1583 investigated also a binary mixture of water and a carboxylic acid confined in mesoporous silica material. Owing to the coexistence of the hydrophobic aliphatic chain and the hydrophilic carboxylic group, these acids are ideal model compounds for studies on the microphase separation of binary liquids inside confinement. Specifically, a mixture of water and 2-methylpropanoic acid (isobutyric acid, iBA) was studied by a combination of NMR spectroscopy and MD simulation. The bulk mixture has a phase diagram with a large miscibility gap, leading to phase separation over a wide concentration range, dependent on temperature. Buntkowsky and coworkers [50, 52, 53] investigated the microphase separation of this binary mixture in mesoporous silica in previous works. Based on NMR data, they developed a structural model, which proposes that, below a critical temperature of 42 °C, two phases coexist inside the pores and form concentric cylinders. Moreover, these authors conjectured that the water-rich phase is close to the pore walls and the iBA-rich phase forms the inner cylinder.

To check this tentative assignment and to understand the mechanism for the microphase separation, Harrach et al. [154] performed refined solid-state NMR studies in combination with MD simulations. The 2D $^{29}\text{Si}/^1\text{H}$ FSLG HETCOR spectrum (Figure 10, left panel) monitors the resolved magnetic dipolar interactions between protons and the silica nuclei on the surface at 100 K, where a frozen momentary picture of the liquid structure inside the pores is observed due to an absence of molecular dynamics. Interestingly, there are cross peaks between the surface ^{29}Si signals and both the aliphatic protons and the hydroxyl and carboxyl protons. The latter two are not resolved in the ^1H dimension, but yield a strong signal at ca. 6.5 ppm, showing that hydroxyl and carboxyl protons are in fast chemical exchange. The low-field shift of this line (relative to pure water) shows that it stems from the proposed iBA-rich phase. This interpretation is corroborated by the strong cross-peak (1.2 ppm) between ^{29}Si signals and aliphatic protons, which proves that a substantial amount of the aliphatic chains of iBA is close to the silica surface. Finally, there is an additional weak signal at 11.5 ppm, which is assigned to non-exchanging protons in the carboxyl group of iBA. Further studies

Fig. 10: (Left) The 2D $^1\text{H}/^{29}\text{Si}$ FSLG HETCOR spectrum of an iBA/ H_2O mixture confined in SBA-15 clearly reveals that both hydroxy and aliphatic protons are in contact with the surface silicon nuclei (56 wt% iBA, 100 K, 8 kHz MAS, 89 kHz FSLG homonuclear decoupling [155]). (Right) Density profiles of iBA and water molecules across the confinement at various temperatures (60 wt% iBA). The center of the pore is at 0 Å and the pore boundary is at 25 Å. Both figures were extracted from Harrach et al. [154].

exploited that the experiment is more sensitive to shorter distances when shorter ‘contact times’ are used. It was found that the hydroxyl protons are mainly correlated with the Q_2 and Q_3 groups and the aliphatic protons mainly with the near surface Q_4 groups [154].

The right panel of Figure 10 shows results from concomitant MD simulations [154]. The density profiles of water and iBA as a function of the distance from the pore center indicate that the iBA density is clearly higher at the pore wall than in the pore center, in particular at low temperatures, confirming the existence of microphase separation. Further analyses revealed that iBA molecules orient preferentially in an inverted brush-like structure in the interfacial region, i.e. radially with the carboxylic group pointing towards the pore wall and the aliphatic chains pointing towards the pore-center [154].

To determine the origin of the microphase separation, enthalpic and entropic details of confined water/iBA mixtures were studied. Analyzing the hydrogen bonds between the molecular species and the pore wall, it was found that the energy decrease per formed hydrogen bond is larger for iBA than for water (see Figure 11, left panel). To understand the energetics in more detail, Harrach et al. [154] started from a non-equilibrium configuration, where iBA is located in the center of the pore, and followed the time evolution of various energy contributions during the equilibration. Exemplary results are shown in the right panel of Figure 11. In the top frame, we see that the fraction of

Fig. 11: Left panel: average change in energy between liquid components and pore per hydrogen bond (with pore). Left hand side: hydrogen bonds between water and pore; right hand side: hydrogen bonds between iBA and pore; middle: hydrogen bonds between iBA and pore, normed by ratio of volume. Right panel: simulated time evolution of a water/iBA mixture (70 wt%) with iBA initially located in the pore center. Upper panel: fraction of water molecules beyond the distance r from the pore center (full lines) and equilibrium values (dashed lines). Middle panel: average number of hydrogen bonds between water molecules per water molecule. Lower panel: change in potential energy of the total system and with regard to electrostatic water–water interaction. Both figures were extracted from Harrach et al. [154].

water molecules in outer pore regions decreases upon equilibration. The bottom frame displays two concurrent enthalpic processes: On the one hand, the electrostatic binding energy between wall and liquid becomes less negative as iBA replaces part of the water (blue line). On the other hand, there is a lowering of the van der Waals (vdW) energy between pore and mixture (green line). Quantitative analysis shows that the latter process dominates the former so that the interaction energy between the pore and the liquid becomes more negative when iBA accumulates at the wall. In addition, there is a concomitant strong reduction of the electrostatic water–water interaction energy (cyan line) since the number of water–water hydrogen bonds increases during equilibration (middle panel). The net effect of these three contribution is a decrease of the total energy of nonbonded interactions (black line). Hence, energetic reasons play the key role for the tendency of the iBA to preferentially wet the pore walls.

Altogether, by combination of solid-state NMR spectroscopy and MD simulation it was possible to unambiguously show that the microphase separation of water/iBA mixtures in mesoporous silica involves an iBA-rich phase close to the pore wall and a water-rich phase in the pore center, in contrast to previous assumptions. The MD simulations reveal that this surprising phase behavior is mainly the result of the minimization of the hydrogen-bond enthalpy.

4.3 Polymer-loaded microemulsions and liquid-crystal systems

Another contribution to this special issue focuses on soft matter confinements that are well defined with respect to their size and shape. Specifically, Kuttich et al. [88] study the structure, dynamics, and phase behavior for binary water mixtures, in particular, aqueous polymer solutions, in soft confinements formed in the droplet phase of inverse microemulsions. In this way, they address the question of the mutual interaction between the confined liquid and a soft confinement. The confinement size could be conveniently tuned by the composition of the microemulsion and resulted in water droplets of well-defined size with a thermally fluctuating surfactant layer. For example, in AOT-based microemulsions the addition of polyethylene glycol (PEG) to the water droplets does not significantly change the average size of the droplets, but increases their polydispersity. Further, the addition of PEG or other polymers has a considerable influence on the stiffness of the surfactant layer of different types of microemulsions [156–158], as indicated by an analysis of the thermal fluctuations, e.g. using neutron spin echo experiments. Furthermore, Kuttich et al. [88] showed for mixtures of water and PEG that the interaction between the polymer and the surfactant layer depends strongly on the type of surfactant used. The findings for PEG-loaded systems indicated that in AOT-based microemulsions PEG is located at the water/surfactant interface, while in the case of $C_{12}E_4$ -based systems the polymer remains in the droplet center. This supports reports suggesting an attractive interaction between the AOT layer and the embedded PEG chain [88].

Moreover, Berghaus et al. [159] studied the effect of hydrostatic pressure on the structure of a bicontinuous microemulsion in the presence of a confining solid interface by X-ray reflectometry, and compared the results to the bulk behavior determined by SAXS. High pressure has been applied to be able to fine-tune molecular shapes (i.e. the molecular packing parameter) and intermolecular interactions. Surface-induced lamellar ordering is observed close to a hydrophilic interface, which persists upon compression. The lamellar domains are compressed, but the correlation length of lamellar order does not change with pressure. Although pressure can cause the formation of highly ordered lamellar phases from ordered bicontinuous cubic phases in the bulk, such a scenario is not observed for the disordered analog studied here. High pressure increases the stiffness of the interfacial surfactant layer, but this is not sufficient to overcome the loss in conformational entropy that would result from a transition to an ordered lamellar phase [159].

Other studies in the framework of Research Unit FOR 1583 exploited interactions between solutes and differently substituted liquid crystals to induce

anisotropic NMR interactions and to determine the influence of hydrogen bonds on these interactions [160–163].

5 Biological materials

Owing to the high concentration of macromolecules of up to about 300 mg mL^{-1} [85, 86], biological cells are highly volume-occupied complex solvents. Such crowded systems have the potential to significantly modulate the stability, conformational dynamics, reaction rates, and transport processes of biomolecules such as proteins. Macromolecular crowding generally refers to effects attributed to excluded volume. Many of these excluded volume effects may be predicted using analytical statistical-thermodynamic models or computer simulations and can be observed experimentally by measurement of the dependence of conformational or reaction equilibria and kinetics on the concentration of inert crowding agents.

Many crowding studies based on the use of artificial macromolecular agents such as Ficoll, dextran, and PEG reported on the ability of the excluded volume effect to stabilize folded proteins, enhance protein-protein associations as well as promote protein polymerization and aggregation [84, 85, 88, 164–167]. For example, Erlkamp et al. [164] applied FTIR spectroscopic, SAXS, and calorimetric measurements to explore the effect of 30 wt% Ficoll on the temperature- and pressure-dependent stability diagram of SNase, which is a monomeric protein, i.e. it consists of a single polypeptide chain. As shown in Figure 12, the temperature- and

Fig. 12: Pressure-temperature stability diagram of the monomeric protein SNase in dilute buffer solution and in the presence of 30 wt% of the macromolecular crowding agent Ficoll 70; 1 MPa = 10 bar (modified from [84, 164]).

pressure-induced equilibrium unfolding of SNase is markedly shifted to higher temperatures and pressures in 30 wt% Ficoll solution. In a similar fashion, it was found that not only temperature-induced equilibrium unfolding of RNase A, but also unfolding induced by pressure is markedly prohibited in crowded dextran solutions, suggesting that crowded environments such as those found intracellularly, will also oppress high-pressure protein unfolding [165]. Moreover, the unfolded protein retains more ordered secondary structure elements in the presence of dextran, which seems to be a manifestation of the entropic destabilization of the unfolded state by crowding as well.

More recently, the discussion revolving around the macromolecular crowding effect includes also the consideration of nonspecific chemical (“soft” or “quinary”) interactions between the solute and crowding agent, which are of enthalpic nature and can also influence the folding stability and dynamics of biomolecules [85, 168–170]. This enthalpic contribution depends on temperature, pressure as well as on the chemical make-ups of the biomolecule, the crowder, and of the cosolvents if present. Soft interactions can increase or decrease the folding stability of a biomolecule [85, 168–170]. For example, electrostatic repulsions can reinforce the stabilizing effect of excluded volume, whereas attractive interactions of electrostatic (including hydrogen bonding) or hydrophobic nature counteract the influence of steric repulsion, because unfolding is accompanied by an increase of solvent-accessible surface area. Using protein crowders and cell lysates, Pielak and coworkers pointed out that the enthalpic contribution of crowding may even dominate and destabilize globular proteins and impede their diffusion [171–173].

For reactions to occur in solution, the reactants must first diffuse together, then they can interact, leading, after overcoming some activation energy in the transition state, to formation of the product. In a highly crowded environment, the generally rapid rate of molecular diffusion may be significantly reduced. Consequently, a reaction that is reaction-controlled in a dilute solution can become diffusion-controlled in the crowded biological cell. Slowing down of diffusion in crowded environments may be, e.g. due to collisions with the crowding agent or due to nonspecific binding to the crowder. At the same time, crowding leads to an increase in the effective concentration (activity) of the reactants, which may, for example, lead to an enhancement of association or polymerization reactions [84]. Limitations of such a scenario are again due to additional enthalpic soft interactions between protein and crowder which may affect the kinetics as well. In fact, Erlkamp et al. [164] showed that the folding rate of the monomeric protein SNase decreases markedly in the presence of Ficoll, which indicates that besides the commonly encountered excluded volume effect, other factors need to be considered when assessing confinement effects on protein folding kinetics. Among those, crowder-induced viscosity changes seem to be prominent.

In the framework of Research Unit FOR 1583 [174], FTIR and NMR experiments were combined to investigate the influence of a cell-mimicking crowding environment on the structure and dynamics of an elastin-like peptide (ELP) with the sequence GVG(VPGVG)₃, which, due to a high number of hydrophobic amino acid side chains, exhibits an inverse temperature transition (ITT), i.e. a decrease of the population of random coil structures and the concomitant increase of type II β -turns with increasing temperature. In this case, the addition of Ficoll leads to a minor destabilizing effect of type II β -turn structures (Figure 13a). This is in contrast to the expected excluded volume effect of macromolecular crowders, but could again be explained by weak interactions of the peptide with the polysaccharide chains of the crowding agent. Further, the macromolecular crowding agent leads to the onset of a reversal of the folding transition at high temperatures, at $\sim 80^\circ\text{C}$. Analyzing site-resolved ^{13}C spin-lattice relaxation times, Weißheit et al. [174] show that core and terminal parts of the peptide backbone have diverse mobility. On the other hand, in agreement with the FTIR analysis, macromolecular crowding does not lead to marked changes in the relaxation behavior [174]. Therefore, it is not straightforward to separate effects due to ELP folding from effects due to concomitant modification of the solvent viscosity. Conversely, in the presence of the compatible osmolyte trimethylamine-*N*-oxide (TMAO), stabilization of the folded structure can be concluded from the decrease of disordered structures and concomitant increase of regular secondary structures, see Figure 13b. Hence, the presence of the osmolyte and nanocrowder TMAO leads to a decrease of the temperature of the ITT.

At high protein concentrations ($>100\text{ mg mL}^{-1}$), self-crowding conditions are encountered. Understanding the intermolecular interaction potential, $V(r)$, of proteins under such conditions is essential for understanding protein

Fig. 13: Temperature effect on the secondary structure content of 10 wt% of the elastin-like peptide GVG(VPGVG)₃; disordered structures (left axis, black symbols) and type II β -turns (right axis, red symbols) in the absence and in the presence of (a) 30 wt% Ficoll and (b) 1 M TMAO [174, 175].

aggregation, crystallization, and protein phase behavior in general. Performing SAXS studies on dense lysozyme solutions as a function of pressure, Winter and coworkers [176–180] showed that the interaction potential changes in a nonlinear fashion over a wide range of temperatures, salt, and protein concentrations. The structural properties of water lead to a modification of the protein–protein interactions at high pressures, which might have significant consequences for the stability of proteins in extreme natural environments [176]. Neither temperature nor protein and salt concentrations lead to marked changes in the pressure dependence of $V(r)$, indicating that changes of the water structure dominate the pressure dependence of the intermolecular forces. Furthermore, by analysis of the temperature, pressure, and ionic strength dependence of the normalized second virial coefficient, B_2 , it was shown that the interaction can be fine-tuned by pressure, which can be used to optimize B_2 values for controlled protein crystallization [177].

The cellular milieu, encompassing macromolecular crowding and natural osmolytes, significantly influences protein aggregation and association processes. As several cell degenerative diseases are related to the self-association and fibrillation of amyloidogenic peptides, an understanding of the impact of macromolecular crowding on these processes is of high biomedical importance. Seeliger et al. [181] studied the self-association of the type-2 diabetes mellitus related human islet amyloid polypeptide (hIAPP) in various crowded environments including network-forming macromolecular crowding reagents and hard-sphere like protein crowders. Two competing processes could be identified: a crowder-concentration and crowder-type dependent stabilization of globular off-pathway hIAPP species and a retarded or even inhibited hIAPP fibrillation reaction. The cause of these crowding effects was revealed to be mainly excluded volume in the polymeric macromolecular crowders, whereas non-specific interactions seemed to be prevailing in protein crowded environments. Complementary cytotoxicity assays revealed non-toxicity for the stabilized globular species, in contrast to the high cytotoxicity imposed by the normal fibrillation pathway. From these findings it can be concluded that cellular crowding is able to effectively stabilize the monomeric native conformation of hIAPP, hence enabling the conduction of its normal physiological function and preventing it from cytotoxic aggregation [181].

As an example of a biologically relevant polymerization process, tubulin, one of the main components of the cellular cytoskeleton, was studied in the absence and presence of the effects of kosmotropic and chaotropic osmolytes, such as TMAO and the metabolic waste product urea, as well as the macro-crowder Ficoll and nanocrowder sucrose [167], see Figure 14. The growth rate of the formation of microtubules was found to be dramatically decreased in the

Fig. 14: Polymerization reaction of 30 μM tubulin at $T = 37^\circ\text{C}$ monitored by turbidimetry in the presence of Ficoll and its monomeric equivalent sucrose at different concentrations. The data are normalized on a scale from 0 to 100% (after Schummel et al. [167]).

presence of urea but significantly increased in the presence of TMAO, and the presence of 10 wt % Ficoll increased the apparent growth rate by one order of magnitude. These data clearly show that the polymerization of tubulin is very sensitive to both the surrounding solvent and crowding. General physico-chemical features of the cytosolic environment can significantly modulate the polymerization behavior of biomolecules such as actin and tubulin, key molecular reactions controlling cell dynamics and motility. Hence, such effects cannot be neglected in any discussion of biomolecular association reactions occurring *in cellulo*.

In a contribution to this special issue, Knop and Winter [182] use FTIR spectroscopy to reveal the effects of different types of cosolvents (TMAO, urea) as well as macromolecular crowding (using the crowding agent Ficoll) on the temperature- and pressure-dependent structures of poly-L-lysine, poly-D-lysine and their racemic mixture. Compared to the effects of cosolvents on the unfolding transition of proteins, their effects on the α -helix to aggregated β -sheet transition of polylysine are quite small. High hydrostatic pressure has been found to favor the α -helical state over the aggregated β -sheet structure which is reflected in a volume decrease of $\Delta V = -32 \text{ mL mol}^{-1}$, indicating that the packing mode is more efficient in the α -helical structure. Addition of both urea and TMAO lead to a decrease in pressure stability of the aggregated β -sheet structure, which is accompanied by a three-fold decrease in ΔV , whereas the macromolecular crowder Ficoll has little effect on the β -to- α transition. The more than 3 kbar higher β -to- α transition pressure of the racemic mixture compared with PLL

Fig. 15: Spatially resolved analyses of reorientation dynamics in elastin-water mixtures with hydration levels of 1.0 and 9.2 g/g at 300 K. (a) Rotational correlation functions of the peptide bonds at the studied hydration levels and for various distances to the interface. Specifically, inner (in) and outer (out) peptide bonds are located near the protein core and the hydrogen shell, respectively. (b) Rotational correlation times τ of water as a function of the distance d to the interface. The horizontal line marks the value for bulk water. The figure is extracted from Geske et al. [105].

confirms the drastic stabilization of β -sheet aggregates if the stereoisomers PLL and PDL are combined. Changes in hydration and packing of the polypeptides occur upon interaction and fine packing of the polypeptide's chains of opposed chirality, which are slightly modulated by the properties of cosolute and crowding, only [182].

MD simulations proved useful to ascertain dynamical couplings between proteins and solvents, which underlie biological functions [183–185]. Even though these computational approaches allowed for valuable tests of theoretical concepts, such as the slaving model [186], the problem remains unsolved to this day. In this special issue [105], MD simulations are employed to investigate dynamical couplings for hydrated elastin in a spatially resolved manner. The studied model comprises the elastin-mimetic polypeptide (VPGVG)₅₀ [187, 188], which has essentially a globular shape without defined secondary structure, solvated by water molecules at various hydration levels. In Figure 15a, we see that a higher water concentration leads to a higher peptide mobility, consistent with the common argument that the hydration shell facilitates protein dynamics. However, there is a strong mobility gradient across the peptide, explicitly, backbone dynamics is more pronounced near the interface with water than in the interior of the globule. Thus, the influence of the hydration shell differs for different amino acids, depending on their position with respect to the interface. In Figure 15b, it is evident that a mobility gradient also exists for water. Specifically, the structural relaxation of water is significantly slower at the

peptide surface than at larger distances, in particular, for lower water content and, thus, lower peptide mobility. These results imply that dynamical couplings of proteins and solvents are governed by strong mobility gradients and, thus, dynamical heterogeneities, which should be considered in improved theoretical approaches.

Compared to such studies on peptides and proteins, knowledge of the effects of hydration and confinement on the structure and dynamics of nucleic acids, such as RNAs, hairpins, G-quadruplexes and ribozymes, is still scarce [189–191]. Revealing the free energy and conformational landscape of such structures, and exploring the forces controlling their conformational dynamics and interactions at crowded solution conditions is prerequisite for understanding their function on a molecular level.

6 Conclusion

In conclusion, interfaces influence HB liquids in manifold ways. Therefore, it is highly advisable to closely link novel preparation strategies and comprehensive experimental characterization with inventive computational and theoretical modeling in order to gain a fundamental understanding. Such concerted approach was successfully taken in Research Unit FOR 1583. Novel methods were developed to prepare tailor-made confinements based on solid and soft materials. It turned out that, in general, the structural, dynamical, and thermodynamic features of HB liquids at interfaces and in confinements are substantially different from that in the bulk. The degree and range of the effects is, however, very sensitive to the properties of the matrix and the type of the liquid. In the framework of Research Unit FOR 1583, the roles of structure, hydroaffinity, and rigidity of the inner surfaces were determined for important pure and mixed HB liquids, including several biological materials. Moreover, theoretical concepts were proposed to improve our understanding of interface and confinement effects, at least for model systems. However, the field is very broad and, hence, it was necessary to restrict the studies to a small part of purposefully chosen classes of systems. Therefore, it is highly desirable to continue such investigations with the aim to broaden further the covered areas towards complex biological and technological materials in the future.

Acknowledgement: Financial support by the Deutsche Forschungsgemeinschaft in the framework of Forschergruppe FOR 1583 through grants Bu-911/18-1/2, Vo-905/8-1/2, Vo-905/9-1/2, Vo-905/10-1/2, and Wi-742/17-1/2 is gratefully acknowledged.

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