

CrossMark  
click for updatesCite this: *RSC Adv.*, 2015, 5, 80325Received 24th August 2015  
Accepted 15th September 2015

DOI: 10.1039/c5ra17077j

www.rsc.org/advances

# Effect of imidazolium room-temperature ionic liquids on aggregation of amphotericin B: a circular dichroism study†

Laramie P. Jameson and Sergei V. Dzyuba\*

The nature of the anion of imidazolium-based ionic liquids was found to control the aggregate–monomer equilibrium of amphotericin B. This is in contrast to aqueous solutions of inorganic salts and imidazolium-based ionic liquids, which were previously found to favor the disaggregation of amphotericin B. The results further establish the designer solvent ability of ionic liquids, specifically as it relates to their ability to influence intermolecular interactions.

Ionic liquids (ILs) are customizable materials composed entirely of ions, whose chemical and physical properties can be tuned *via* structural modifications within the cationic and anionic counterparts. The applications of ILs are spanned over various disciplines, as these materials have found interesting uses in numerous synthetic, biological, and energy-related processes and systems.<sup>1–8</sup> Recently, the notion of utilizing ILs either as media for solubilizing and stabilizing active pharmaceuticals or producing pharmaceutically active ILs has received considerable attention.<sup>9–21</sup> Thus, understanding the behavior of natural products and synthetic drugs in ILs is critical.

Although a number of studies using ILs as reaction media have illustrated the designer solvent ability of ILs,<sup>22–26</sup> modulation of non-covalent interactions within ILs by simply adjusting the structure constitutes an interesting, yet underdeveloped paradigm. Several studies have demonstrated that ILs can indeed be used to modulate intra- and intermolecular interactions of small molecules.<sup>27–31</sup> The effects of IL–organic solvent or IL–water mixtures on intermolecular assemblies have also been studied.<sup>32–34</sup> Importantly, it was demonstrated that the effects of IL–molecular solvent/water mixtures are distinct from those of inorganic salt–molecular solvent mixtures,<sup>35</sup> as well as neat ILs.<sup>27,34</sup>

Amphotericin B, AmB, (Fig. 1) is a polyene macrolide antibiotic, which has been the subject of interest from pharmacological, synthetic, and structural standpoints.<sup>35,36</sup> AmB is an antifungal drug that is often used against systemic fungal infections, yet despite its high potency, AmB is quite toxic. This has spurred research on various AmB derivatives.<sup>37,38</sup> On the structural level, AmB undergoes self-association/aggregation under a variety of conditions, including temperature, pH, ionic strength, and solvent.<sup>39–43</sup> The aggregation of AmB has been linked to its mode of action and toxicity.<sup>44–46</sup>

The self-assembly of AmB can be monitored spectroscopically using circular dichroism (CD) and absorbance spectroscopies.<sup>39–43,47–49</sup> CD allows for the straightforward detection of AmB aggregates, as the dimer and/or aggregates produce an exciton coupling between the polyene chromophores, which leads to an asymmetric couplet in the 300–400 nm range. At low concentrations, the monomer was reported to be CD silent in this spectral range. However, in some organic solvents, including aliphatic alcohols, the monomer (characterized by positive CD signals) could be observed at 25 mM.<sup>47</sup> Although numerous overlapping absorptions in the absorbance spectra preclude straightforward deconvolution, the 330–350 nm region can be used to confirm the presence of aggregated AmB. Even though the extract structure of the aggregates is widely debated throughout the literature (*i.e.*, head-to-head, head-to-tail, dimer,

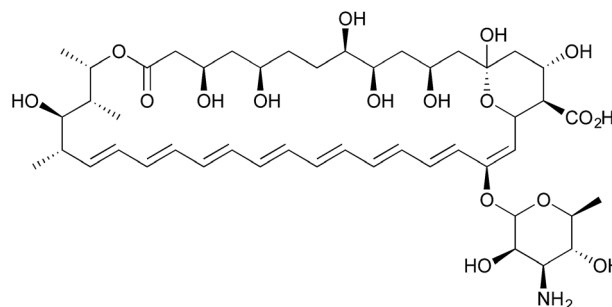


Fig. 1 Structure of AmB.

Department of Chemistry, Texas Christian University, Fort Worth, TX 76129, USA.  
E-mail: s.dzyuba@tcu.edu; Tel: +1 817 257-6218

† Electronic supplementary information (ESI) available: Details on the synthesis of ILs; CD and UV-vis spectra of AmB in ILs and molecular solvents. See DOI: 10.1039/c5ra17077j



oligomer, supramolecular assembly *etc.*), the combination of CD and UV can be used to unambiguously confirm the presence of aggregates.

The nature of the media has been shown to affect the monomer – dimer/aggregate equilibrium of AmB. Specifically, organic solvents were shown to produce the monomeric and oligomeric forms of AmB,<sup>47</sup> while the effects of aqueous solutions of ILs on the self-assembly of AmB depend on the anion of the ILs, as judged by distinct CD spectra.<sup>32</sup> For example, in upto 1 M aqueous solutions of [C<sub>4</sub>-mim]Br, the AmB aggregates were found to be similar to those found in water, whereas in aqueous solutions of [C<sub>4</sub>-mim]BF<sub>4</sub> and [C<sub>4</sub>-mim]NO<sub>3</sub> (1 M solution in water) the AmB aggregates were different from each other and from those found in water. It is noteworthy, however, that increasing the concentration of ILs (from 0.3 to 1 M) favored the formation of the monomeric form of AmB, regardless of the nature of the ILs (this effect was reminiscent to that induced by aqueous solutions of inorganic salts).<sup>50–52</sup> However, even with 1 M ILs, some aggregates were still detected when the concentration of AmB was 10 μM.<sup>32</sup> The effects of neat ILs, or with very low concentrations of water or organic solvents, on the aggregation of AmB have not been investigated, and constitute the focus of this study.

Previously reported results<sup>32</sup> suggested that in order to obtain a measurable CD signal in neat ILs (or with very low water/organic solvent contents), the concentration of AmB would have to be drastically increased. It was shown that at 25 mM AmB existed as a monomer in alcohols (*e.g.*, methanol, ethanol, propanol, *etc.*) and in DMSO, among other solvents. The oligomeric form of AmB was observed in solvents such as water, acetone, and acetonitrile.<sup>47</sup> Thus, we investigated the chiroptical characteristics of an even higher concentration (500 μM) of AmB in DMSO and water, for reference, as we reasoned that the effects of ILs could be elucidated by comparison to those in molecular solvents. Consistent with literature results,<sup>47</sup> in water, AmB exhibited a CD spectrum (bisignate curve with a maximum at 345 nm and several minima above 380 nm) that was typical of the aggregated species (Fig. 2). The corresponding absorption spectrum also confirmed the presence of aggregates, as evidenced from the broad transition and high intensity bands around 340 nm (Fig. 2). On the contrary, in DMSO, monomeric AmB was observed (Fig. 2), which was in accord

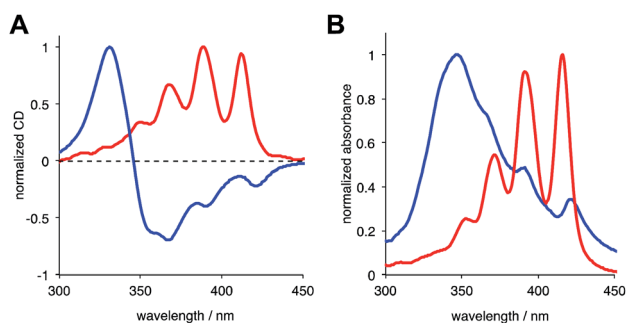


Fig. 2 Circular dichroism (A) and absorption (B) spectra of 500 μM AmB in water (blue) and DMSO (red).

with the disaggregating ability of DMSO and other literature observations.<sup>47</sup> The CD spectrum exhibited sharp, positive transitions in the 370–415 nm range, and no negative signals were observed.

With this point of reference in hand, we investigated the aggregation of AmB in neat ILs, such as [C<sub>4</sub>-mim]BF<sub>4</sub> and [C<sub>4</sub>-mim]NO<sub>3</sub> (Fig. 3), since aqueous solutions of these ILs previously exhibited drastically different effects on the intermolecular interactions of AmB.<sup>32</sup> In neat [C<sub>4</sub>-mim]BF<sub>4</sub>, AmB exhibited spectroscopic features which were consistent with the aggregated form, and which largely resembled the CD spectrum of AmB in water (Fig. 2, blue). The absorbance data also indicated that AmB was highly aggregated in [C<sub>4</sub>-mim]BF<sub>4</sub> (Fig. 3, purple). On the other hand, in [C<sub>4</sub>-mim]NO<sub>3</sub>, monomeric AmB was observed (Fig. 3, orange), and the CD and absorbance spectra resembled those in DMSO (Fig. 1, red). Significantly, the CD signals of AmB in [C<sub>4</sub>-mim]NO<sub>3</sub> and [C<sub>4</sub>-mim]BF<sub>4</sub> were distinct. Thus, it appeared that the equilibrium between the monomeric and aggregated forms of AmB could be controlled by simply adjusting the anion of the ILs. [C<sub>4</sub>-mim]NO<sub>3</sub> appeared to provide a disaggregating environment, which was in contrast to that provided by [C<sub>4</sub>-mim]BF<sub>4</sub>.

In view of the above results, we examined the effects of various anions of [C<sub>4</sub>-mim]-based ILs on the monomer–aggregate equilibrium of AmB (Fig. 4). Specifically, in [C<sub>4</sub>-mim]Br and [C<sub>4</sub>-mim]CF<sub>3</sub>CO<sub>2</sub>, monomeric AmB was observed, as noted by the CD and absorbance spectra (Fig. 4A and Fig. S1 and S2†). Thus, these two ILs exhibited a similar effect as [C<sub>4</sub>-mim]NO<sub>3</sub> (as well as DMSO) on AmB. Notably, small changes in the λ<sub>max/min</sub> are to be expected considering that the polarity of these ILs varies.<sup>53–55</sup>

On the contrary, [C<sub>4</sub>-mim]NTf<sub>2</sub>, and [C<sub>4</sub>-mim]PF<sub>6</sub> favored the formation of AmB aggregates (Fig. 4B and S3, S4†), much like [C<sub>4</sub>-mim]BF<sub>4</sub>. In all cases, the CD spectra appeared to be similar, which suggested that these ILs induced the formation of similar types of AmB aggregates.<sup>34</sup> The absorption data also confirmed the presence of the aggregates, as evident from the strong, dominant transitions around 330–350 nm. Notably, in previously published studies, neat ILs were shown to completely disaggregate small molecules, such as cyanine dyes and porphyrins,<sup>27,34</sup> while a set of ILs seemed to promote the aggregation of AmB in this study.

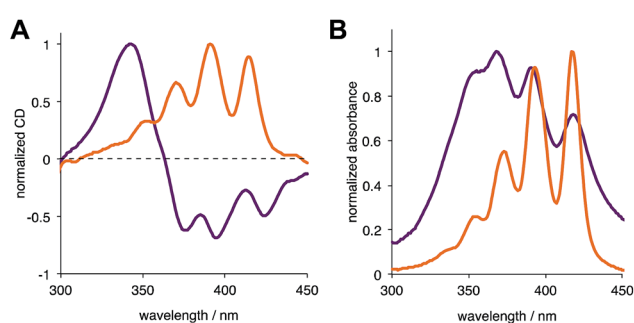


Fig. 3 Circular dichroism (A) and absorption (B) spectra of 500 μM AmB in [C<sub>4</sub>-mim]BF<sub>4</sub> (purple) and [C<sub>4</sub>-mim]NO<sub>3</sub> (orange).



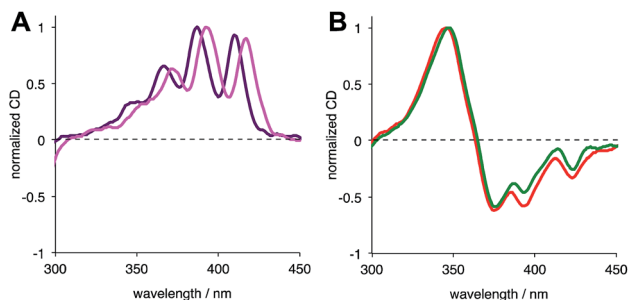


Fig. 4 Circular dichroism spectra of 500  $\mu\text{M}$  AmB in ILs; (A)  $[\text{C}_4\text{-mim}]\text{Br}$  (pink),  $[\text{C}_4\text{-mim}]\text{CF}_3\text{CO}_2$  (purple); (B)  $[\text{C}_4\text{-mim}]\text{NTf}_2$  (red),  $[\text{C}_4\text{-mim}]\text{PF}_6$  (green).

In addition, when we examined the behavior of AmB behavior in  $[\text{C}_4\text{-mim}]\text{OTf}$  (Fig. S5<sup>†</sup>), it appeared that this IL somewhat mimicked the aforementioned two sets of ILs. Based on the CD spectrum, the aggregated form was present as evident by the strong bisignate couplet (with a maximum at 350 nm, and a minimum at 377 nm). Yet, a positive transition at 413 nm indicated the presence of the monomeric form. The absorbance data (Fig. S5<sup>†</sup>) supported that assumption: peaks at 388 and 412 nm (indicative of the monomeric AmB) were more prominent than the 330–350 nm region, which is attributed to aggregated AmB.

The ILs tested in this study appeared to be divided into two distinct groups: those that strongly favored the formation of monomeric AmB ( $[\text{C}_4\text{-mim}]\text{NO}_3$ ,  $[\text{C}_4\text{-mim}]\text{Br}$ , and  $[\text{C}_4\text{-mim}]\text{CF}_3\text{CO}_2$ ) and those that favored the formation of AmB aggregates ( $[\text{C}_4\text{-mim}]\text{NTf}_2$ ,  $[\text{C}_4\text{-mim}]\text{PF}_6$ , and  $[\text{C}_4\text{-mim}]\text{BF}_4$ ). Importantly, a similar grouping was observed in a previous study when the effect of ILs on the intramolecular conformational change of tetracycline was evaluated as  $\text{NTf}_2$ ,  $\text{PF}_6$ , and  $\text{BF}_4$  anions were found to promote the twisted conformation, and  $\text{NO}_3$ ,  $\text{Br}$ , and  $\text{CF}_3\text{CO}_2$  anions were found to promote the extended conformation.<sup>26,27</sup> It is also of interest to note that aqueous solutions of  $[\text{C}_4\text{-mim}]\text{BF}_4$  (upto 1 M) were reported to be potent disaggregating agents of AmB,<sup>32</sup> while in this study, neat  $[\text{C}_4\text{-mim}]\text{BF}_4$  promoted the aggregation of AmB.

Our results suggest that the structure of the ILs is responsible for modulating the monomer–aggregate equilibrium of AmB. However, the explanations for such phenomena are not straightforward, since physical properties of ILs,<sup>26</sup> such as polarity and viscosity, do not correlate with the observed trends. Notably, the effects of ILs are occasionally systematized according to the Hofmeister series,<sup>56–58</sup> even though this series was originally used to describe the ability of inorganic salts to modulate the stability of proteins in aqueous environments. Accordingly, in this study, we found that  $\text{BF}_4^-$ ,  $\text{PF}_6^-$ , and  $\text{NTf}_2^-$ , which could be classified as kosmotropic ions, exerted the opposite effect as  $\text{NO}_3^-$ ,  $\text{CF}_3\text{CO}_2^-$ , and  $\text{Br}^-$ , which could be classified as chaotropic anions. However, as pointed out previously, this description may be too simplistic for complex anions, such as those used here,<sup>57</sup> and might not be applicable for neat ILs.<sup>56</sup> Furthermore, it was noted that the stability/activity of enzymes in neat ILs deviated from the classical

Hofmeister series.<sup>58</sup> Nonetheless, the establishment of some correlations or trends could be useful in making predictions about the effects of ILs on non-covalent interactions of the solutes (especially considering that fact that a similar grouping of ILs was observed in an intramolecular process, *i.e.*, IL-controlled conformational change of tetracycline).<sup>26,27</sup>

On the other hand, several reports have suggested that the aggregation or structural heterogeneity of ILs, which lead to the formation of clusters/aggregates within the ILs,<sup>59–64</sup> can affect various processes within ILs.<sup>21,65</sup> Thus, such nano-domains might also influence non-covalent interactions. Notably, the aggregation of  $[\text{C}_4\text{-mim}]\text{BF}_4$  and  $[\text{C}_4\text{-mim}]\text{NO}_3$  were revealed (based on mass spectrometry and light scattering) to be drastically different,<sup>66</sup> thus tentatively explaining the observed results. It could also be possible that the charged moiety of AmB becomes a part of the IL network, which favors the monomer formation, while AmB aggregates form when such interactions are lacking. Thus, the heterogeneity of ILs could influence the non-covalent interactions of solutes within them. Further studies are required to elucidate the exact nature of the IL–AmB interaction, and will be reported elsewhere.

## Conclusions

Using CD and absorbance spectroscopies, we have shown that by altering the identity of the anion of common, easily accessible ILs, the self-assembly of AmB can be modified. Fundamental investigations towards a better understanding of how neat IL environments affect intermolecular interactions might aid in the development of novel functional materials based on ILs. Further, the establishment of some trends in regard to the effects of ILs on the conformation and self-assembly of small molecules is paramount to the potential applications, and will advance the use of ILs as designer solvents in a variety of fields. Moreover, in this study, the effects of neat ILs were shown to be drastically different from those of IL–water mixtures.<sup>32</sup> Although the exact nature of the AmB aggregates remains to be clarified, our results indicate that the designer solvent ability of ILs might be applicable for controlling intermolecular interactions between small molecules, and shed light on some trends that could be exploited in future studies.

## Acknowledgements

This work was partially supported by NSF (OISE-1316354 to LPJ and CBET-1403226 to SVD). The authors would like to thank Professor O. Annunziata (TCU) for helpful discussions.

## Notes and references

- 1 J. P. Hallet and T. Welton, *Chem. Rev.*, 2011, **111**, 3508.
- 2 M. A. P. Martins, C. P. Clarissa, A. Z. Aniele, D. N. Dayse, N. Zanatta and H. G. Bonacorso, *Chem. Rev.*, 2014, **114**, PR1–PR70, DOI: 10.1021/cr500106x.
- 3 H.-P. Steinrueck and P. Wasserscheid, *Catal. Lett.*, 2015, **145**, 380.
- 4 S. N. Riduan and Y. Zhang, *Chem. Soc. Rev.*, 2013, **42**, 9055.



- 5 J. L. Shamshina, P. S. Barber and R. D. Rogers, *Expert Opin. Drug Delivery*, 2013, **10**, 1367.
- 6 M. Smiglak, J. M. Pringle, X. Lu, L. Han, S. Zhang, H. Gao, D. R. MacFarlane and R. D. Rogers, *Chem. Commun.*, 2014, **50**, 9228.
- 7 S. N. Riduan and Y. Zhang, *Chem. Soc. Rev.*, 2013, **42**, 9055.
- 8 G. Gebresilassie Eshetu, M. Armand, B. Scrosati and S. Passerini, *Angew. Chem., Int. Ed.*, 2014, **53**, 13342.
- 9 S. Domingos, V. Andre, S. Qauresma, I. C. B. Ines, M. F. Minas da Piedade and M. T. Duarte, *J. Pharm. Pharmacol.*, 2015, **67**, 830.
- 10 I. M. Marruchi, L. C. Branco and L. P. N. Rebelo, *Annu. Rev. Chem. Biomol. Eng.*, 2014, **5**, 527.
- 11 P. C. A. G. Pinto, D. M. G. P. Diogo, A. M. O. Azevedo, V. Dela Justina, E. Cunha, K. Bica, M. Vasiliou, S. Reis and M. L. M. F. S. Saraiva, *New J. Chem.*, 2013, **37**, 4095.
- 12 P. D. McCrary, P. A. Beasley, G. Gurau, A. Narita, P. S. Barber, O. A. Cojocar and R. D. Rogers, *New J. Chem.*, 2013, **37**, 2196.
- 13 R. Ferraz, L. C. Branco, I. M. Marrucho, J. M. M. Araújo, L. P. N. Rebelo, M. N. da Ponte, C. Prudêncio, J. P. Noronha and Z. Petrovski, *MedChemComm*, 2012, **3**, 335.
- 14 K. Bica, H. Rodriguez, G. Gurau, O. A. Cojocar, A. Riisager, R. Fehrmann and R. D. Rogers, *Chem. Commun.*, 2012, **48**, 5422.
- 15 J. Stoimenovski, P. M. Dean, E. I. Izgorodina and D. R. MacFarlane, *Faraday Discuss.*, 2012, **154**, 335.
- 16 G. R. Navale, M. S. Dharne and S. S. Shinde, *RSC Adv.*, 2015, **5**, 68136.
- 17 Y. Sahbaz, H. D. Williams, T.-H. Nguyen, J. Saunders, L. Ford, S. A. Charman, P. J. Scammells and C. J. H. Porter, *Mol. Pharmaceutics*, 2015, **12**, 1980.
- 18 D. N. Moreira, N. Fresno, R. Perez-Fernandez, C. P. Frizzo, P. Goya, C. Marco, M. A. P. Martins and J. Elguero, *Tetrahedron*, 2015, **71**, 676.
- 19 J. M. M. Araujo, C. Florindo, A. B. Pereiro, N. S. M. M. Vieira, A. A. Matias, C. M. M. Duarte, L. P. N. Rebelo and I. M. Marrucho, *RSC Adv.*, 2014, **4**, 28126.
- 20 S. V. Dzyuba and R. A. Bartsch, *Tetrahedron Lett.*, 2002, **43**, 4657.
- 21 A. Aggarwal, N. Llewellyn, A. R. Sethi and T. Welton, *Green Chem.*, 2002, **4**, 517.
- 22 M. J. Earle, S. P. Katdare and K. R. Seddon, *Org. Lett.*, 2004, **6**, 707.
- 23 I. Newington, J. M. Perez-Ariandis and T. Welton, *Org. Lett.*, 2007, **9**, 5257.
- 24 C. Hardacre, M. E. Migaud and K. A. Ness, *New J. Chem.*, 2012, **36**, 2316.
- 25 V. Kumar, G. A. Baker and S. Pandey, *Chem. Commun.*, 2011, **47**, 4730.
- 26 L. P. Jameson and S. V. Dzyuba, *J. Nat. Prod.*, 2011, **74**, 310.
- 27 L. P. Jameson and S. V. Dzyuba, *RSC Adv.*, 2013, **3**, 4582.
- 28 L. P. Jameson, J. D. Kimball, Z. Gryczynski, M. Balaz and S. V. Dzyuba, *RSC Adv.*, 2013, **3**, 18300.
- 29 J. D. Kimball, S. Raut, L. P. Jameson, N. W. Smith, Z. Gryczynski and S. V. Dzyuba, *RSC Adv.*, 2015, **5**, 19508.
- 30 M. Ali, V. Kumar, S. N. Baker, G. A. Baker and S. Pandey, *Phys. Chem. Chem. Phys.*, 2010, **12**, 1886.
- 31 P. Dutta, R. Rai and S. Pandey, *J. Phys. Chem. B*, 2011, **115**, 3578.
- 32 L. P. Jameson and S. V. Dzyuba, *Chirality*, 2013, **25**, 427.
- 33 V. Kumar, G. A. Baker, S. Pandey, S. N. Baker and S. Pandey, *Langmuir*, 2011, **27**, 12884.
- 34 Q. X. Wan, Y. Liu and S. S. Wang, *Monatsh. Chem.*, 2009, **140**, 335.
- 35 D. M. Geiser, *Nat. Chem. Biol.*, 2015, **11**, 453.
- 36 R. J. Hamill, *Drugs*, 2013, **73**, 919.
- 37 S. A. Davis, B. M. Vincent, M. M. Endo, L. Whitesell, K. Marchillo, D. R. Andes, S. Lindquist and M. D. Burke, *Nat. Chem. Biol.*, 2015, **11**, 481.
- 38 A. A. Volmer, A. M. Szpilman and E. M. Carreira, *Nat. Prod. Rep.*, 2010, **27**, 1329.
- 39 Y. Kasai, N. Matsumori, Y. Umegawa, S. Matsuoka, H. Ueno, H. Ikeuchi, T. Oishi and M. Murata, *Chem.-Eur. J.*, 2008, **14**, 1178.
- 40 N. W. Smith, O. Annunziata and S. V. Dzyuba, *Bioorg. Med. Chem.*, 2009, **17**, 2366.
- 41 M. Larabi, A. Gulik, J.-P. Dedieu, P. Legrand, G. Barratt and M. Cheron, *Biochim. Biophys. Acta*, 2004, **1664**, 172.
- 42 D. Romanini, G. Avalle, B. Nerli and G. Pico, *Biophys. Chem.*, 1999, **77**, 69.
- 43 Z. Shervani, H. Etori, K. Taga, T. Yoshida and H. Okabayashi, *Colloids Surf., B*, 1996, **7**, 31.
- 44 J. Starzyk, M. Gruszecki, K. Tutaj, R. Luchowski, R. Szlajak, P. Wasko, W. Grudzinski, J. Czub and W. I. Gruszecki, *J. Phys. Chem. B*, 2014, **118**, 13821.
- 45 M. D. Kaminski, *Eur. Biophys. J.*, 2014, **43**, 453.
- 46 P. Wasko, R. Luchowski, K. Tutaj, W. Grudzinski, P. Adamkiewicz and W. I. Gruszecki, *Mol. Pharmaceutics*, 2012, **9**, 1511.
- 47 H. G. Brittain, *Chirality*, 1994, **6**, 665.
- 48 A. Vertut-Croquin, J. Bolard, M. Chabbert and C. Gary-Bobo, *Biochemistry*, 1983, **22**, 2939.
- 49 C. Ernst, J. Grange, H. Rinnert, G. Dupont and J. Lematre, *Biopolymers*, 1981, **20**, 1575.
- 50 M. T. Grijalba, M. Cheron, E. Borowski, J. Bolard and S. Schreier, *Biochim. Biophys. Acta*, 2006, **1760**, 973.
- 51 M. Gagos, S. M. Arczewska and W. I. Gruszecki, *J. Phys. Chem. B*, 2011, **115**, 5032.
- 52 M. Gagos and M. Arczewska, *J. Phys. Chem. B*, 2011, **115**, 3185.
- 53 S. Pandey, S. N. Baker, S. Pandey and G. A. Baker, *J. Fluoresc.*, 2012, **22**, 1313.
- 54 C. Chiappe, M. Malvaldi and C. S. Pmelli, *Pure Appl. Chem.*, 2009, **81**, 767.
- 55 C. Reichardt, *Green Chem.*, 2005, **7**, 339.
- 56 Z. J. Yang, *J. Biotechnol.*, 2009, **144**, 12.
- 57 D. Constantinescu, H. Weingartner and C. Herrmann, *Angew. Chem., Int. Ed.*, 2007, **46**, 8887.
- 58 R. M. Lau, M. J. Sorgedragger, G. Carrea, F. van Rantwijk, F. Secundo and R. A. Sheldon, *Green Chem.*, 2004, **6**, 483.
- 59 J. C. Arague, J. Hettige and C. J. Margulis, *J. Phys. Chem. B*, 2015, DOI: 10.1021/acs/jpcb.5b05506.



- 60 H. K. Stassen, R. Ludwig, A. Wulf and J. Dupont, *Chem.–Eur. J.*, 2015, **21**, 8324.
- 61 M. D. Fayer, *Chem. Phys. Lett.*, 2014, **616–617**, 259.
- 62 Y. Nagasawa and H. Miyasaka, *Phys. Chem. Chem. Phys.*, 2014, **16**, 13008.
- 63 C. C. Weber, A. F. Masters and T. Maschmeyer, *Green Chem.*, 2013, **15**, 2655.
- 64 O. Russina, A. Triolo, L. Gontrani and R. Camintini, *J. Phys. Chem. Lett.*, 2012, **3**, 27.
- 65 C. W. Cameron, A. F. Masters and T. Maschmeyer, *Angew. Chem., Int. Ed.*, 2012, **51**, 11483.
- 66 B. L. Walton, U. Joshi, S. V. Dzyuba, W. J. Youngblood and G. F. Verbeck, *Rapid Commun. Mass Spectrom.*, 2013, **27**, 1954.

