TU Delft

Delft University of Technology

Compartmentalizing Supramolecular Hydrogels Using Aqueous Multi-phase Systems

Mytnyk, Serhii; Olive, Alexandre G.L.; Versluis, Frank; Poolman, Jos M.; Mendes, Eduardo; Eelkema, Rienk; van Esch, Jan H.

DOI
10.1002/anie.201706272

Publication date
2017

Document Version
Final published version

Published in
Angewandte Chemie (International Edition)

Citation (APA)
Mytnyk, S., Olive, A. G. L., Versluis, F., Poolman, J. M., Mendes, E., Eelkema, R., & van Esch, J. H. (2017). Compartmentalizing Supramolecular Hydrogels Using Aqueous Multi-phase Systems. *Angewandte Chemie (International Edition)*, *56*(47), 14923-14927. https://doi.org/10.1002/anie.201706272

Important note
To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright
Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy
Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

This work is downloaded from Delft University of Technology.
For technical reasons the number of authors shown on this cover page is limited to a maximum of 10.

Multi-Phase Systems

International Edition: DOI: 10.1002/anie.201706272
German Edition: DOI: 10.1002/ange.201706272

Compartmentalizing Supramolecular Hydrogels Using Aqueous Multi-phase Systems

*Serhii Mytnyk, Alexandre G. L. Olive, Frank Versluis, Jos M. Poolman, Eduardo Mendes, Rienk Eelkema, and Jan H. van Esch**

Abstract: *A generic method is used for compartmentalization of supramolecular hydrogels by using water-in-water emulsions based on aqueous multi-phase systems (AMPS). By forming the low-molecular-weight hydrogel throughout all phases of all-aqueous emulsions, distinct, micro-compartmentalized materials were created. This structuring approach offers control over the composition of each type of the compartments by directing the partitioning of objects to be encapsulated. Moreover, this method allows for barrier-less, dynamic exchange of even large hydrophilic solutes (MW ≈ 60 kDa) between separate aqueous compartments. These features are expected to find use in the fields of, for instance, micro-structured catalysts, templating, and tissue engineering.*

Compartmentalization plays a central role in a variety of biological and industrial processes. For instance, it allows incompatible metabolic reactions to run simultaneously inside eukaryotic cells by prohibiting mutual interference.[1] Additionally, compartmentalization is crucial for encapsulation, delivery and release of active ingredients (drugs, flavors), as well as for structuring various materials through templating and scaffolding.[2,3] In synthetic systems, compartmentalization is generally achieved either by emulsification of immiscible phases, or through encapsulation of solutes inside various vesicles.[4–6]

However, most common approaches have limitations. For example, emulsification techniques are widely used for creating hydrophobic domains in aqueous phases, but are inherently limited in terms of the possible number of accessible types of compartments. On the other hand, vesicle-based approaches can effectively encapsulate various aqueous phases within their membranes and can be used to create numerous different coexisting micro-compartments with excellent control over their composition. However, while hydrophobic lipid- or polymer-based membranes grant vesicles their outstanding properties, they also seriously limit the transport of many polar solutes.[7] Furthermore, in contrast to emulsions, which can be generated and stabilized relatively easily even on a large scale, preparation of loaded vesicles can be a long, not easily scalable process. Therefore, there is a need for a simple and elegant approach that allows the creation of micro-compartments containing polar molecules without introducing any barriers.

One of the most promising directions for barrier-less structuring of aqueous media is to employ aqueous multi-phase systems (AMPS). AMPS are multi-component water-based mixtures generally containing several incompatible polymers and/or salts that can form distinct macroscopic aqueous phases.[8] Aqueous two- and three-phase systems are widely used in biotechnology for extraction and separation of biomolecules, organelles, and even living cells, since they offer a large number of mild, fully aqueous environments.[9] In recent years, the group of Keating has exploited aqueous phase separation as a tool for compartmentalizing biomolecules by encapsulating aqueous two- and three-phase systems within lipid vesicles to create prototype artificial cells.[10–13] Several other examples of stabilization of water-in-water emulsions using various nanoparticles have also been reported;[14–16] however these methods are system-specific and cannot be universally applied to form stable arbitrary AMPS emulsions. An alternative surfactant-free route to stabilize emulsions has been recently demonstrated by the group of Ulijn, who have generated stable oil-in-water emulsions by creating an interfacial supramolecular gel network around the dispersed droplets.[17,18] Therefore, formation of a supramolecular hydrogel network within AMPS emulsions should allow capturing their fine structure, resulting in micro-compartmentalized, fully aqueous hydrogel materials.

In this article, we present a generic method for compartmentalizing aqueous media using aqueous phase separation of incompatible polymers and formation of supramolecular hydrogel. Additionally, by localizing streptavidin in a hydrogel, we suggest a route to biomimetic micro-structuring of soft materials. Since the method presented here has the potential for creating multiple levels of specific compartments without any diffusion-limiting hydrophobic barriers, we expect it to have an impact in the fields of, for instance, micro-structured catalysts, templating, and tissue engineering.

AMPS-based all-aqueous emulsions with micron-sized droplets can be easily prepared even by manual shaking, due to the extremely low interfacial tension between coexisting aqueous phases. Properties of the individual phases, such as interfacial tension, density, and viscosity, determine the time necessary to achieve macroscopic phase separation of any given water-in-water (W/W) emulsion. Therefore, to control the size of the compartments by capturing different stages of phase separation via gelation, the timescales of two key processes have to be considered: first, the timescale of

[*] S. Mytnyk, Dr. A. G. L. Olive, Dr. F. Versluis, Dr. J. M. Poolman, Dr. E. Mendes, Dr. R. Eelkema, Prof.Dr. J. H. van Esch
Department of Chemical Engineering, Delft University of Technology
van der Maasweg 9, Delft, 2629 HZ (The Netherlands)
E-mail: j.h.vanesch@tudelft.nl

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.201706272.

 © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

macroscopic phase separation of a given all-aqueous emulsion (t_{sep}), and second, the timescale of formation of a hydrogel that is meant to trap the structure of such an emulsion (t_{gel}). These two timescales define the distribution and the size of the compartments in the final material. When $t_{gel} \ll t_{sep}$, the gel network forms sufficiently fast to capture the fine structure of corresponding water-in-water emulsion by mechanically fixing the microdroplets in place and preventing them from coalescing. In the opposite case ($t_{gel} \gg t_{sep}$), macroscopic phase separation would be complete before the gel network has formed, thus resulting in a layered material, likely with nearly no micro-compartments.

Herein we focus on the first scenario ($t_{gel} \ll t_{sep}$) to achieve homogeneous hydrogel micro-structuring. To achieve this, we have chosen ATPS with $t_{sep} > 1$ hour and adjusted the t_{gel} to be about 15 minutes (Supporting Information, Figure S1). To be able to control the gelation rate, we used a supramolecular hydrogel that has been previously developed in our group.[19] In short, gelator precursors **1** (aldehyde) and **2** (hydrazide) react in aqueous solution at 3:1 ratio to form hydrogelator **3** (hydrazone), it self-assembles into fibers and forms a supramolecular gel network (Figure 1 a). In this work, we chose the concentration of gelator precursors **1** and **2,** and the pH of the medium such as to ensure rapid gelation after emulsification.

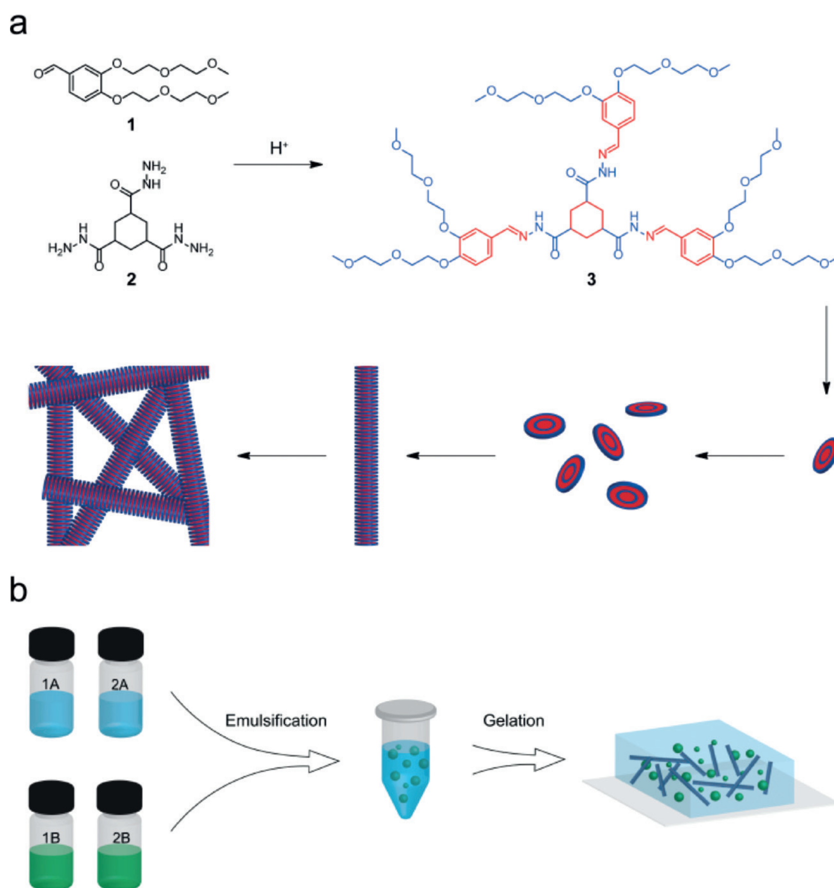

Figure 1. a) Reaction of the gelator precursors **1** and **2** to form tris-hydrazone hydrogelator **3**, followed by its self-assembly into fiber network forming a gel; b) preparation of compartmentalized hydrogels: solutions of compounds **1** and **2** in immiscible polymeric phases A and B are combined to form an emulsion with its consequent gelation.

In a typical experiment, compounds **1** and **2** were introduced into both phases of an aqueous two-phase system formed by poly(2-ethyl-2-oxazoline) (PEtOx) and dextran at pH 5. After combining the polymer solutions in appropriate ratios, they were vigorously stirred to obtain a W/W emulsion and left to form a compartmentalized gel overnight (Figure 1 b). Detailed description of the preparation of polymer solutions, corresponding aqueous two-phase systems (ATPS), compartmentalized hydrogels and their characterization can be found in the Supporting Information.

The obtained PEtOx–dextran emulsions appeared as soft, opaque hydrogels, without any signs of macroscopic phase separation. This observation was confirmed by detailed investigations into the microscopic structure of the materials using confocal laser-scanning microscopy (CLSM). We found that microscopic spherical domains of one of the phases were homogeneously dispersed and immobilized in a second, continuous phase (Figure 2 a–c). Upon increasing the volume fraction of dextran-rich phase (red), we observed the reversal of the phases from dextran-in-PEtOx (Figure 2 a) to PEtOx-in-dextran emulsions (Figure 2 b,c). Interestingly, the structure of prepared compartmentalized hydrogels remained unchanged within 1 week of observation, which indicates that also at longer timescales coalescence of the micro-droplets is effectively hindered by entrapping them in the static fiber network of the supramolecular gel (Supporting Information, Figures S2,S3). Importantly, the size of the micro-compartments depended on the volume fraction of corresponding polymer phases: while small well-dispersed droplets were formed at the volume fraction of the dispersed phase of 25 % v/v, much larger domains were present at 50 % v/v. This phenomenon can be explained by an increase in droplet coalescence rate at high volume fraction of the dispersed phase, which is generally observed for emulsions.[20] This means that t_{sep} decreases, while t_{gel} remains constant, thus resulting in materials with larger compartments.

Having established the dependence of material structure on the ratio of the phases, we investigated the scope of the method by extending it to other ATPS, namely poly(*N*-vinyl pyrrolidone) (PVP)–dextran and Ficoll–dextran. We found that hydrogels, compartmentalized using W/W emulsions formed by these ATPS, behaved similarly to those prepared with the PEtOx–dextran system, although we observed a clear difference in their micro-structure. Compartments in PVP–dextran gels appeared to be larger than in case of PEtOx–dextran, and often possessed inclusions of the continuous phase (Figure 2 d–f). In the Ficoll–dextran system, however, large interconnected pores filled with a dense gel network

 © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

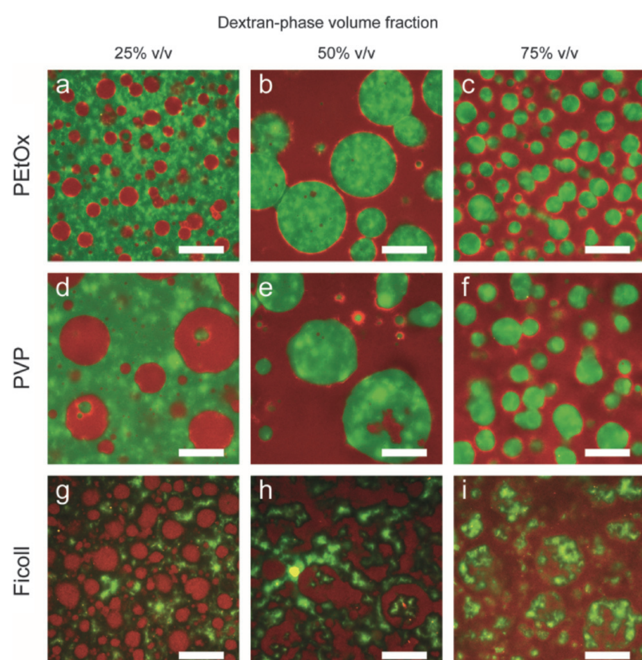

Figure 2. CLSM micrographs of hydrogels structured with a)–c) PEtOx–dextran, d)–f) PVP–dextran, and g)–i) Ficoll–dextran ATPS, using different volume ratios of the polymer phases (left to right: −75:25, 50:50, and 25:75). Dextran phases were labeled with TRITC-dextran (red) and gel network was labeled with fluorescein-modified aldehyde 1 (green).[21] Scale bars: 50 μm.

were observed (Figure 2g–i). Interestingly, both the size and the distribution of micro-compartments varied depending on the combination of the polymers used for compartmentalization. We presume that these differences are caused by the influence of individual polymers on the rate of phase separation of the corresponding emulsions. Thus, a careful choice of ATPS used for hydrogel compartmentalization may allow tuning the nature of micro-compartments and overall structure of these materials.

Importantly, the method presented herein not only allows creating aqueous compartments in a hydrogel, but also offers control over the composition of individual types of compartments. Such control can be achieved either by exploiting the selective partitioning of (bio)molecules in aqueous multiphase systems, or by making use of specific interactions to drive the partitioning in the desired direction. Herein we illustrate this feature by localizing fluorescently labeled streptavidin (SA) in a PEtOx–dextran hydrogel. While normally SA displays a homogeneous distribution between PEtOx and dextran phases, addition of a dextran–biotin conjugate during preparation of the gels drove SA partitioning almost entirely to the dextran-rich phase (Supporting Information, Figure S4). This experiment highlights the potential of our approach to be used for creating biomimetic materials, which enable the simultaneous performance of several catalytic/enzymatic reactions localized to specific droplet microreactors. Since such an application would require a dynamic exchange of the contents of the compartments among the continuous phase and the other compartment types, we further investigated the permeability of the prepared materials.

The permeability of the micro-compartments was estimated by studying the diffusion of several polar molecules through PEtOx–dextran hydrogels. Fast diffusion of charged dyes (Pyranine and Rhodamine B) through the hydrogel compartments confirmed the absence of hydrophobic barriers at the interface of the micro-domains (Supporting Information, Figures S5,S6). Furthermore, compartmentalized hydrogels were even permeable to large polar biomolecules. To illustrate this property, we brought a streptavidin-containing PEtOx–dextran hydrogel in contact with the solution of dextranase, a bacterial enzyme that selectively hydrolyses 1,6-glycosidic linkages of dextran, thus degrading the polymer to glucose monomers. We followed this process by monitoring the dextran-phase fluorescence, which over the course of experiment has changed from highly localized to uniformly distributed (Figure 3). This observation suggests that after the diffusion of dextranase through the material no dextran chains remained, and so the fluorescent label was able to

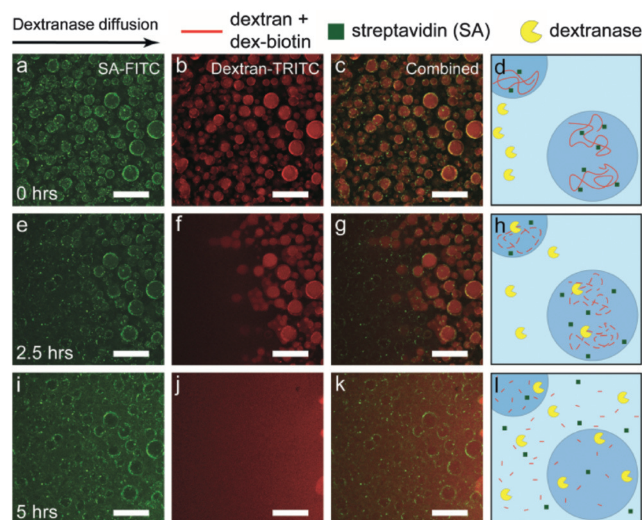

Figure 3. CLSM micrographs of the dextranase diffusion through hydrogel structured with PEtOx–dextran ATPS and containing SA-FITC with dextran–biotin: a)–c) at the start of the experiment, dextranase enters from the left, e)–g) after 2.5 h, i)–k) after 5 h. SA-FITC channel is displayed in green, dextran-TRITC channel in red. Images (d), (h), and (l) are representations of corresponding stages of dextran degradation using dextranase. The volume ratio of PEtOx phase to dextran phase was 75:25. Scale bars: 200 μm.

distribute homogeneously throughout the resulting dextran-free hydrogel. Therefore, dextranase, with a molecular weight of approximately 60 kDa, diffused through ca. 1 mm of the gel within 5 h, while simultaneously hydrolyzing the dextran present in the material. Similarly, streptavidin fluorescence became more evenly distributed, owing to its unhindered diffusion after degradation of dextran–biotin. It should be noted that some residual brighter green regions at the interface of the compartments remained visible, which may be due to the entrapment of some SA-FITC within the denser areas of gel present there. Importantly, the initial structure of

 © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

the compartmentalized hydrogel remained intact and the material retained its solidity. These results confirm that our method allows barrier-less, dynamic exchange of even large hydrophilic solutes between the separate aqueous compartments.

In sharp contrast to conventional emulsification approaches used for the structuring of soft materials, the AMPS-based method is not limited to only two immiscible phases; as many as 16 immiscible aqueous phases in equilibrium have been reported, and systems with 3 to 6 immiscible phases are being continuously discovered.[9,22] To demonstrate the versatility and potential of this technique, we prepared a set of compartmentalized hydrogels using a polyethylene glycol (PEG)–Ficoll–dextran aqueous three-phase system (A3PS). By simply using one of the polymer solutions as a continuous phase and dispersing the other two in it, we were able to access an extremely wide range of structures (Figure 4). Distribution of the gel network and polymer-rich phases appears to be far more complex than in examples discussed above. We observed structures ranging from

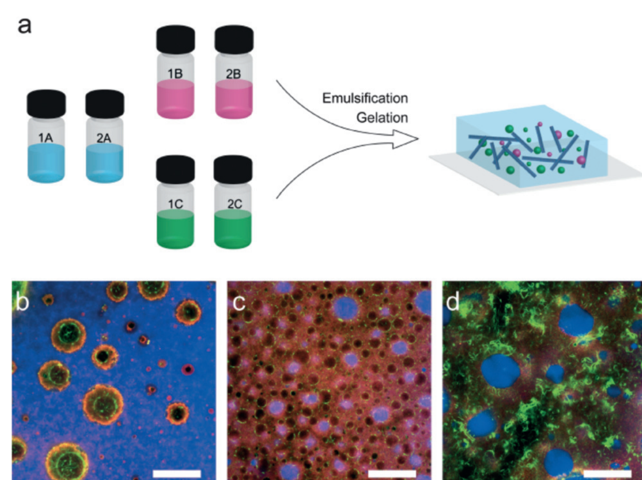

Figure 4. a) Compartmentalizing hydrogels with aqueous three-phase system (A3PS) formed by polymers A, B, and C; b–d) CLSM micrographs of hydrogels structured with PEG–Ficoll–Dextran A3PS with phase-volume ratios of PEG/Ficoll/dextran equal to: b) 4:1:1, c) 1:4:1, and d) 1:1:4. Dextran phase: green, Ficoll: red, gel network: blue. Scale bars: 50 μm.

homogeneously distributed two types of spherical compartments (Figure 4a) to core–shell-like domains displayed in Figure 4b. These results demonstrate the potential of using higher-order AMPS to create complex, artificially compartmentalized hydrogel materials reminiscent of biological tissues. Furthermore, while the actual laws governing the formation of these complex composites are yet to be fully investigated and understood, we expect that the main design considerations ($t_{gel} \ll t_{sep}$) will also apply to other aqueous multi-phase systems.

In conclusion, we have reported a versatile, generic approach for creating compartmentalized water-based materials by forming a supramolecular hydrogel within mixtures of aqueous phase-separating polymers. We have successfully applied our method to several all-aqueous emulsions and we expect it to be easily extended to the majority of aqueous multi-phase systems, thus opening access to a large number of tunable aqueous micro-environments. Along with the potential of such hydrogel materials to mimic biological compartmentalization of biomolecules, we have demonstrated their permeability to even large polar solutes. We envision that these features may find use in, for instance, the design of novel biomimetic hydrogel catalysts, for templating of porous soft materials or in fabrication of supramolecular hydrogel scaffolds for tissue engineering.

Acknowledgements

The authors gratefully acknowledge the financial support of the EC 7th Framework Programme Marie Curie Actions via the European ITN SMARTNET No. 316656 (S.M.), and the Netherlands Organization for Scientific Research (R.E. and F.V. through NWO-Vidi grant; A.G.L.O. and J.M.P. through NWO-ECHO grants).

Conflict of interest

The authors declare no conflict of interest.

Keywords: multi-phase systems · aqueous systems · colloids · compartmentalization · gels

How to cite: *Angew. Chem. Int. Ed.* **2017**, *56*, 14923–14927
Angew. Chem. **2017**, *129*, 15119–15123

[1] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, Garland Science, New York, **2002**.
[2] L. Schoonen, J. C. M. van Hest, *Adv. Mater.* **2016**, *28*, 1109–1128.
[3] A. H. Chen, P. A. Silver, *Trends Cell Biol.* **2012**, *22*, 662–670.
[4] B. T. Kelly, J.-C. Baret, V. Taly, A. D. Griffiths, *Chem. Commun.* **2007**, 1773.
[5] M. Li, X. Huang, T.-Y. D. Tang, S. Mann, *Curr. Opin. Chem. Biol.* **2014**, *22C*, 1–11.
[6] M. Massignani, H. Lomas, G. Battaglia, *Modern Techniques for Nano- and Microreactors/-reactions*; Springer, Berlin, **2010**, pp. 115–154.
[7] S. S. Mansy, J. P. Schrum, M. Krishnamurthy, S. Tobé, D. A. Treco, J. W. Szostak, *Nature* **2008**, *454*, 122–125.
[8] A. L. Grilo, M. Raquel Aires-Barros, A. M. Azevedo, Y. Zhu, D. Chen, X. Wang, L. Huang, D. Peng, A. Sattar, M. A. B. Shabbir, et al., *Sep. Purif. Rev.* **2016**, *45*, 68–80.
[9] P.-A. Albertsson, *Partition of Cell Particles and Macromolecules*, 3rd ed., Wiley, New York, **1986**.
[10] C. D. Keating, *Acc. Chem. Res.* **2012**, *45*, 2114–2124.
[11] P. Torre, C. D. Keating, S. S. Mansy, *Langmuir* **2014**, *30*, 5695–5699.
[12] W. M. Aumiller, B. W. Davis, N. Hashemian, C. Maranas, A. Armaou, C. D. Keating, *J. Phys. Chem. B* **2014**, *118*, 2506–2517.
[13] W. M. Aumiller, C. D. Keating, *Adv. Colloid Interface Sci.* **2017**, *239*, 75–87.
[14] B. T. Nguyen, T. Nicolai, L. Benyahia, *Langmuir* **2013**, *29*, 10658–10664.
[15] A. Gonzalez-Jordan, T. Nicolai, L. Benyahia, *Langmuir* **2016**, *32*, 7189–7197.

© 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[16] M. Vis, J. Opdam, I. S. J. van't Oor, G. Soligno, R. van Roij, R. H. Tromp, B. H. Erné, *ACS Macro Lett.* **2015**, *4*, 965 – 968.
[17] S. Bai, C. Pappas, S. Debnath, P. W. J. M. Frederix, J. Leckie, S. Fleming, R. V. Ulijn, *ACS Nano* **2014**, *8*, 7005 – 7013.
[18] I. P. Moreira, I. R. Sasselli, D. A. Cannon, M. Hughes, D. A. Lamprou, T. Tuttle, R. V. Ulijn, *Soft Matter* **2016**, *12*, 2623 – 2631.
[19] J. Boekhoven, J. M. Poolman, C. Maity, F. Li, L. van der Mee, C. B. Minkenberg, E. Mendes, J. H. van Esch, R. Eelkema, *Nat. Chem.* **2013**, *5*, 433 – 437.
[20] D. J. McClements, *Food Emuls*, CRC, Boca Raton, FL, **2004**.
[21] J. M. Poolman, C. Maity, J. Boekhoven, L. van der Mee, V. A. A. le Sage, G. J. M. Groenewold, S. I. van Kasteren, F. Versluis, J. H. van Esch, R. Eelkema, *J. Mater. Chem. B* **2016**, *4*, 852 – 858.
[22] C. R. Mace, O. Akbulut, A. A. Kumar, N. D. Shapiro, R. Derda, M. R. Patton, G. M. Whitesides, *J. Am. Chem. Soc.* **2012**, *134*, 9094 – 9097.

Manuscript received: June 20, 2017
Revised manuscript received: August 14, 2017
Accepted manuscript online: August 16, 2017
Version of record online: September 12, 2017

 © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim