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COMMUNICATION

Light controlled cell to cell adhesion and chemical communication in minimal synthetic cells

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T. Chakraborty,[a‡] S. M. Bartelt,[a‡] J. Steinkühler,[b] R. Dimova[b] and S. V. Wegner[a*]

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Decorating GUVs, used as minimal synthetic cell models, with photoswitchable proteins allows controlling the adhesion between them and their assembly into multicellular structures with light. Thereby, the chemical communication between a sender and a receiver GUVs, which strongly depends on their spatial proximity, can also be photoregulated.

Cell to cell communication is a central function in life and is an important aspect to consider in the context of bottom-up synthetic biology, which aims to understand basic features of life through the construction of minimal synthetic cells from molecules *in vitro*.[1, 2, 3] In local intercellular communication (known as paracrine signalling in biology) a sender cell releases a chemical signal that is perceived by a receiver cells. A prime example of such communication is observed in neurons, where neurotransmitters are released from presynaptic neuron into synaptic cleft and bind to receptors on the postsynaptic neuron resulting in transduction of the signal into the cell. As also evident from this example, only cells that are near one another can sense the signal, as such chemical signals can only travel relatively short distances before they become too diluted. Therefore, cell to cell communication in minimal synthetic cells involves i) controlling signal transduction from the sender to the receiver cell and ii) building multicellular networks with defined spatial structures bringing sender and receiver cells in proximity.

Minimal synthetic cells with different communication modes have provided insight into how to use cell–to-cell communication to program collective and multicellular behaviour in communities of minimal synthetic cells. For example, DNA-based communication has been used for information processing in consortia of synthetic cells,[4, 5] quorum sensing behaviour has been achieved with minimal synthetic cells through diffusive transcription factors[6] and predatory behaviour through direct contact and signal transduction has been implemented in synthetic cell communities.[7] Likewise, chemical signals have been transduced from sender to receiver compartments per enzyme cascades and diffusible payload using membrane permeable signals or pore forming proteins such as α-hemolysin [5, 8-10] These examples showcase the general concepts of cell to cell communication and the potential of these in producing emerging properties.

The distance between the sender and the receiver cell in a multicellular network is the second central aspect to consider in local cell to cell communication. The importance of spatial organization of different minimal synthetic cells has been highlighted in recent studies where the sender and receiver cells were placed in defined geometries using microfluidics or optical tweezers.[11] The alternative bottom-up approach to self-assemble multicellular structures relies on specific adhesions between different cell mimics and mirror principles of tissue organisation in multicellular organisms[12]. The possibility to trigger and dynamically alter adhesions in response to external stimuli such as metal ions,[13] temperature or light makes it possible to spatiotemporally change these multicellular structures. Moreover, optically modulating the interaction of two membranes can also result in membrane area increase[14] and fusion.[15, 16] For these reasons controlling the spatial arrangement of sender and receiver cells by controlling the adhesions between them represents a powerful way to regulate local cell-to-cell communication.

In this study, we show how controlling the adhesion between a sender and receiver cell using light can be used to also control local cell-to-cell communication (Fig. 1). In implementing photoswitchable adhesions between the cells, we can form multicellular assemblies on demand and disassemble them in the dark. The photoregulation with visible light is particularly attractive as it provides high spatiotemporal control, is noninvasive, and allows tuning interactions.[17, 18] The light responsive assembly of these consortia thereby also allows controlling the local cell-to-cell communication since receiver cells in close proximity to sender cells will perceive the released chemical signal.

In our design, we used giant unilamellar vesicles (GUVs) as cell mimetic compartments due to their similarity to eukaryotic cells in size and membrane structure. As adhesion molecules, we employed the proteins iLID and Nano, which bind to each other under blue light (480 nm) and dissociate from each other in the dark.[18] Such

[a] *Max Planck Institute for Polymer Research, 55128, Mainz, Germany*
Email: wegners@mpip-mainz.mpg.de
[b] *Department of Theory and Biosystems, Max Planck Institute of Colloids and Interfaces, 14476, Potsdam,Germany*
‡ *These authors contributed equally to the work*
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photoswitchable protein-protein interactions have widely been used in optogenetics to control diverse cell functions with light and are becoming valuable molecular building blocks in the context of bottom-up synthetic biology.[17-19] Moreover, these photoswitchable proteins are biocompatible, have high specificity for their binding partner and operate in complex aqueous environments.

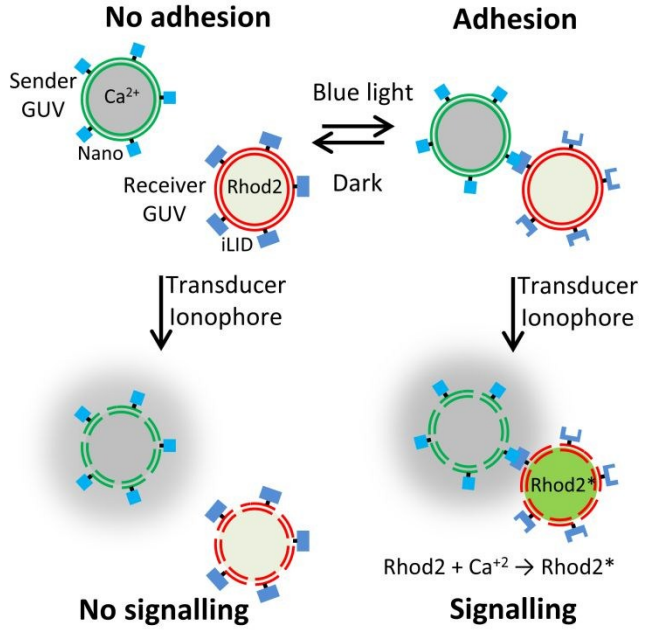

Fig. 1 The transmission of a signal from a sender GUV to a receiver GUV depends on their proximity, which can be controlled by GUV-GUV adhesion. The adhesion between sender and reviver GUV can be reversibly controlled with blue light by decorating the GUVs with the proteins Nano and iLID, respectively, which bind to each other under blue light. When the sender and the receiver GUVs adhere to each other and an ionophore is added as a transducer past the membranes, the chemical signal, Ca^{2+}, can be communicated from the sender GUV to the receiver GUV, which becomes fluorescent upon Rhod2 binding to Ca^{2+}.

In the first step to build photoswitchable cell-to-cell adhesion, the proteins iLID and Nano were immobilized on the outer membrane of two separate GUV populations containing 0.1% of Ni^{2+}-NTA-DGS using the binding of the His-tags on the proteins to the Ni^{2+}-nitrilotriacetic acid (Ni^{2+}-NTA) head group of the lipid (Fig. 2a), as previously reported.[17, 19] Each population of GUVs was fluorescently labelled with a membrane dye to differentiate them (iLID-GUV: DiD, Ex/Em: 644/665 nm, shown in red, and Nano-GUV: DiI, Ex/Em: 549/565 nm, shown in green). These fluorophores were chosen such that their excitation did not result in photoactivation of the blue light switchable iLID/Nano interaction. The two GUV populations were mixed in 1:1 ratio in the dark and were allowed to settle on a glass surface. Statistically GUVs of opposite types came into close proximity but did not interact strongly in the dark (Fig. 2a, Movie 1). Upon turning on blue light, which activated the iLID-Nano protein interaction, the GUVs adhered to each other within a few minutes, as evidenced by an increased overlap of the two vesicle membranes and the deformation of one of the vesicles. To observe the light dependent adhesion, it was important that the two GUVs were in close proximity so that the proteins are close enough to interact. Additionally, one of the GUV populations was osmotically deflated so that upon adhesion the GUVs could deform and yield a large adhesion site. The adhesions between the GUVs were stable once formed over the duration of the blue light illumination, without showing apparent fusion. Moreover, also in bulk mixed population of the iLID and Nano functionalized GUVs formed large multi-GUV clusters under blue light but not in the dark (Fig. S4a, Fig. S4c). Overall, these finding demonstrate that the specific adhesion between iLID and Nano functionalized GUVs can be triggered with blue light.

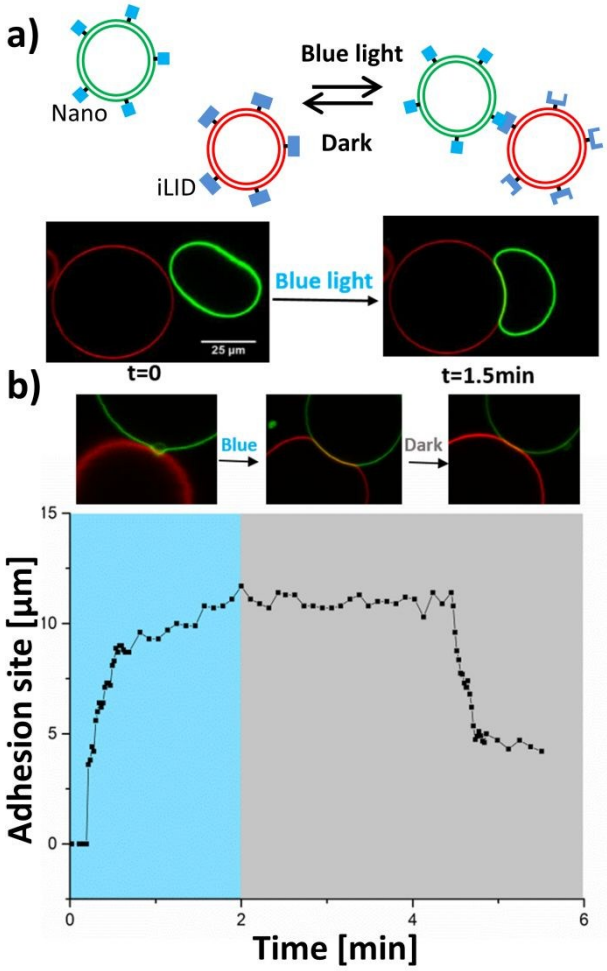

Fig. 2 Photoswitchable GUV-GUV adhesions. a) Microscopy images of Nano (membrane in green) and iLID (membrane in red) functionalized GUVs, which interact with each other upon blue-light illumination, visible through the increase in adhesion zone and the deformation of the deflated GUV (right). b) Adhesion between Nano and iLID decorated GUVs is reversible in the dark. The GUV-GUV adhesion zone (arc length) over time under blue light (shaded in blue) and in the dark (shaded in grey) for the GUVs shown above.

Reversibility of adhesions is an important feature as it allows disassembly of multicellular structures in response to changing stimulation and the separation of the sender and receiver cells at a desired time point. The GUV-GUV adhesions based the iLID-Nano protein interactions are expected to be reversible, since the two proteins dissociate from each other in the dark.[20] Indeed, when GUVs that adhered to each other under blue light were placed in

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the dark, the adhesion became less prominent up to complete detachment within a few minutes (Fig. 2b, Movie 2). This was evident from a decrease in overlay of the membranes of the two interacting GUV and the complete (Fig. S1a) or partial (Fig. S1b) reversion of the bowl shaped deformation at the adhesion site. Whether reversion occurred, strongly depended on the extent the GUVs were deflated and deformed upon adhesion. In cases where the deflated GUV deformed extensively into a bowl shape, the energy barrier became too high for the reversal of the deformation and these GUVs did not separate in the dark within a period of 20 min (Fig S1c). Such a barrier might be provided by local enrichment of the adhesion molecules in the adhering membrane segment. In this way the adhered state becomes kinetically trapped. Such a barrier might be provided by local enrichment of the adhesion molecules in the adhering membrane segment and the adhered state may become kinetically trapped.[12]

The kinetics of the blue light triggered adhesion and its reversion in the dark were analysed by measuring the length of the arc of its maximal cross section of adhesion site between the two GUVs over time under changing illumination (Fig. 2b). GUV-GUV adhesions formed within the first minute of blue light activation, where two GUVs of opposite type coming into proximity expelling the water gap in between appears to be the rate limiting step and not the activation of the iLID protein with blue light, which happens within seconds of blue light illumination.[18] Once the interaction partners iLID and Nano were in proximity so that the first contact occurred, the adhesion formed abruptly within a few seconds (Fig. 2b, Fig. S2a). The final length of adhesion site depended on the size of the two GUVs and the amount of excess membrane of the deflated GUV. In cases where the GUV-GUV adhesions reversed in the dark, it was observed after a few minutes and with a similar sudden profile as the adhesion formation (Fig. 2b, Fig. S2b).

After having established the specific and photoswitchable GUV-GUV adhesions, we wanted to control cell to cell communication through the spatial organization of sender and receiver cells. We proposed that the exchange of a chemical signal would be more efficient when the sender and receiver GUV adhere to each other under blue light (Fig. 1). Calcium ions play an important role in cellular signalling and have been implicated in the birth of cell sized lipid vesicles.[21] In our design, the sender cells were GUVs containing Ca^{2+} (2 µM) as a chemical signal and were functionalized with Nano at their outer surface. The receiver cells were deflated GUVs loaded with the Ca^{2+} sensitive dye Rhod2 (500 nM), which becomes fluorescent upon Ca^{2+} binding and were functionalized with iLID at their outer surface. These GUVs were prepared using spontaneous swelling and loaded with the respective cargo by adding Ca^{2+} or Rhod2 into the rehydration buffer. Later, excess cargo on the outside of the GUVs was removed in repeated washing steps. For the Ca^{2+} to be transferred from the sender and receiver GUV across the lipid membranes, we used the calcium selective ionophore, ionomycin (830 nM), as a transducer.[22] Indeed, when the ionophore was added to receiver GUVs in Ca^{2+} containing buffer, the Rhod2 fluorescence increased within a minute (Fig. S3), as detected with a plate reader and under the microscope, showing that effective transfer of Ca^{2+} across the membrane.

To demonstrate that the signal transfer from the sender to the receiver GUVs depends on the adhesions between them, we mixed sender and receiver GUVs in equal amounts and incubated them for 1 h either in the dark or under blue light. Subsequently, we added the ionophore to these samples and measured the increase in the fluorescence of the Ca^{2+} sensitive dye Rhod2 (Ex/Em : 552/581 nm) using a plate reader. We observed that the fluorescence increased more for the sender/receiver GUV mixture illuminated with blue light, where the GUVs form clusters, than the one kept in the dark, where the GUVs do not interact (Fig. 3). This observation shows that the adhesion of a population of sender and receiver GUV lead to a more effective transfer of the Ca^{2+} signal observed as an increase in Rhod2 fluorescence. The increase in fluorescence was very rapid and complete within a minute. Moreover, the fluorescence was stable over longer periods indicating a stable transfer of the signal.

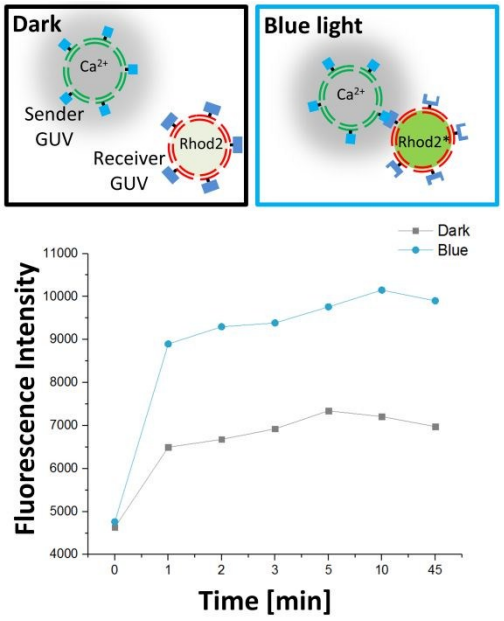

Fig. 3 Adhesion dependent GUV-GUV communication. Sender GUVs (2 µM Ca^{2+}) and receiver GUVs (500 nM Rhod2) were incubated for 1 h either under blue light or in dark before adding ionomycin and measuring the fluorescence of the Ca^{2+} sensitive dye Rhod2.

To gain further insight how the blue light dependent adhesions between sender and receiver GUVs alter Ca^{2+} signalling, we investigated this process for individual GUVs. For this purpose, the sender GUVs (2 µM Ca^{2+}, functionalized with Nano) with the membrane dye DiI (shown in green) and receiver GUVs (500 nM Rhod2, shown in green, functionalized with iLID) with the membrane dye DiD (shown in red) were prepared. Subsequently, equal numbers of sender and receiver GUVs were mixed and either illuminated for 15 min under blue light (488 laser) or kept in the dark (Fig. 4a). It should be noted that the fluorescent dyes DiI in the membrane of the sender GUV and Rhod2 inside the receiver GUV emit at similar wavelengths and were detected at once, yet, their distinct spatial localization made it possible to differentiate them. We observed that sender and receiver GUVs adhered to each other under blue light (Fig. S4a) but not in the dark (Fig. S4c) after 1 h incubation. Further, the addition of ionomycin resulted in an increased Rhod2 fluorescence inside the receiver GUVs, which interacted with sender GUVs under blue light (Fig. 4a, Fig. S4b). On

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the other hand, the increase in Rhod2 fluorescence was less prominent in non-adhering GUVs in the dark (Fig. S4d). These observations were quantified by measuring the mean fluorescence intensity inside randomly picked receiver GUVs (n=25) in the samples described above before and after adding the ionophore. To assure that the Ca^{2+} signal originates in the sender GUVs and not from the surrounding sender GUV buffer, receiver GUVs in the surrounding buffer of the sender GUVs was used as a control. This quantification showed that the mean Rhod2 intensity in the receiver GUVs increased significantly for samples kept under blue light but not for samples kept in the dark or the control sample after initiating the Ca^{2+} transfer by adding the ionophore (Fig. 4b). These results confirmed that successful chemical communication between sender and receiver GUVs strongly depends on their adhesion to each other.

As the distance between the sender and receiver GUV plays a pivotal role in the transfer of the chemical signal, we investigated differences in response of receiver GUVs that were in direct contact (proximal) and were not interacting (distal > 10 µm away) with a sender GUV under blue light illumination (Fig. 4c). We noted that the increase in Rhod2 signal was larger for proximal receiver GUVs than for distal GUVs after initiating the transfer of Ca^{2+} by adding the ionophore (Fig. 4d). Actually, the response of the distal receiver GUVs was comparable the non-interacting GUVs in the dark. Similarly, when the response of individual GUVs was tracked over time, GUVs that formed direct contact with a sender GUV had faster and stronger response than a GUV which was further away from the sender GUV once the ionophore was added (Fig S5).

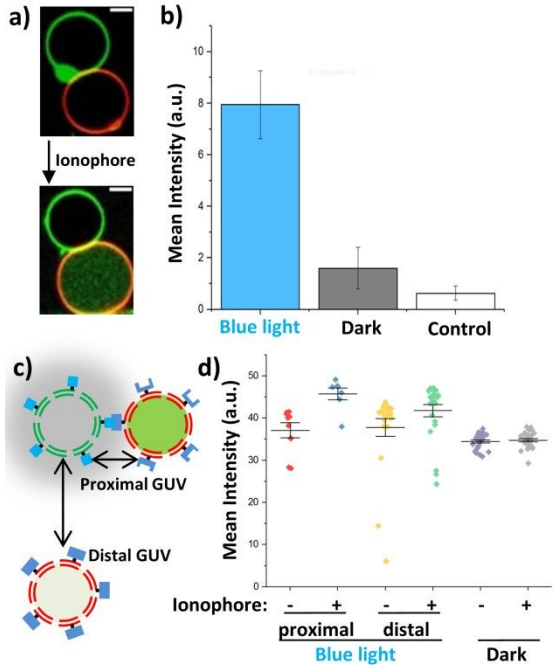

Fig. 4 Proximity controlled GUV-GUV communication. a) Microscopy images of an interacting sender GUV (Ca^{2+}, membrane in green) and a receiver GUV (Rhod2 in green, membrane in red) before and after adding ionomycin. Rhod2 fluorescence increases inside the receiver. b) Change in Rhod2 fluorescence inside the receiver GUVs in the presence of sender GUVs upon addition of ionomycin measured for individual GUVs in confocal microscopy images (n= 25). Buffer surrounding sender GUVs and no sender GUVs was used as a control. c) Receiver GUVs in direct contact with sender GUVs (proximal GUVs) perceive the chemical signal more effectively than receiver GUVs at a further distance (distal GUV). d) Rhod2 intensity in confocal microscopy images for proximal and distal GUVs under blue light and in the dark before (-) and after (+) adding ionomycin.

In summary, we demonstrate how proximity between sender and receiver GUVs can be reversibly controlled by light using photoswitchable proteins iLID and Nano as adhesion molecules. Furthermore, the photoswitchable adhesions between sender and receiver GUVs provide a general approach to control their proximity and hence local cell-to-cell communication in minimal synthetic cells. The reversibility and the high spatiotemporal control provided by the photoswitchable adhesions between two different types of GUVs are important elements in assembling minimal synthetic cells housing different life-like processes into prototissues with high precision and to alter them dynamically. The molecular players in the study presented here are highly modular and can be implemented into other minimal synthetic cells. In particular, ionophores such as ionomycin are an attractive alternative to pore forming protein α-hemolysin to achieve selective permeability for particular ions across lipid bilayers. As demonstrated in this study cell-to-cell adhesion is a key actor to regulate chemical cell-to-cell communication also in the context of bottom-up synthetic biology.

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References

1. S. Hennig, G. Rodel and K. Ostermann, *J. Biol. Eng.*, 2015, **9**, 13.
2. R. Lentini, N. Yeh Martin and S. S. Mansy, *Curr. Opin. Chem. Biol.*, 2016, **34**, 53.
3. Y. Elani, A. Gee, R. V. Law and O. Ces, *Chem. Sci.*, 2013, **4,** 3332.
4. A. Joesaar, S. Yang, B. Bogels, A. van der Linden, P. Pieters, B. Kumar, N. Dalchau, A. Phillips, S. Mann and T. F. A. de Greef, *Nat. Nanotechnol.*, 2019, **14**, 369.
5. K. P. Adamala, D. A. Martin-Alarcon, K. R. Guthrie-Honea and E. S. Boyden, *Nat. Chem.*, 2017, **9**, 431.
6. H. Niederholtmeyer, C. Chaggan and N. K. Devaraj, *Nat. Commun.*, 2018, **9**, 5027.
7. Y. Qiao, M. Li, R. Booth and S. Mann, *Nat. Chem.*, 2017, **9**, 110.
8. C. E. Hilburger, M. L. Jacobs, K. R. Lewis, J. A. Peruzzi and N. P. Kamat, *ACS Synth. Biol.*, 2019, **8**, 1223.
9. T. D. Tang, D. Cecchi, G. Fracasso, D. Accardi, A. Coutable-Pennarun, S. S. Mansy, A. W. Perriman, J. L. R. Anderson and S. Mann, *ACS Synth. Biol.*, 2018, **7**, 339.
10. V. Noireaux and A. Libchaber, *Proc. Natl. Acad. Sci. U.S.A.*, 2004, **101**, 17669.
11. G. Bolognesi, M. S. Friddin, A. Salehi-Reyhani, N. E. Barlow, N. J. Brooks, O. Ces and Y. Elani, *Nat. Commun.*, 2018, **9**, 1882.
12. S. Villringer, J. Madl, T. Sych, C. Manner, A. Imberty and W. Romer, *Sci. Rep.*, 2018, **8**, 1932.
13. S. Sun, M. Li, F. Dong, S. Wang, L. Tian and S. Mann, *Small*, 2016, **12**, 1920.
14. V. N. Georgiev, A. Grafmuller, D. Bleger, S. Hecht, S. Kunstmann, S. Barbirz, R. Lipowsky and R. Dimova, *Adv. Sci.*, 2018, **5**, 1800432.
15. L. Kong, S. H. Askes, S. Bonnet, A. Kros and F. Campbell, *Angew. Chem.*, 2016, **55**, 1396.
16. Y. Suzuki, K. H. Nagai, A. Zinchenko and T. Hamada, *Langmuir.*, 2017, **33**, 2671.
17. S. M. Bartelt, J. Steinkuhler, R. Dimova and S. V. Wegner, *Nano Lett.*, 2018, **18**, 7268.
18. E. Chervyachkova and S. V. Wegner, *ACS Synth. Biol.*, 2018, **7**, 1817.
19. S. M. Bartelt, E. Chervyachkova, J. Steinkuhler, J. Ricken, R. Wieneke, R. Tampe, R. Dimova and S. V. Wegner, *Chem. Commun.*, 2018, **54**, 948.
20. G. Guntas, R. A. Hallett, S. P. Zimmerman, T. Williams, H. Yumerefendi, J. E. Bear and B. Kuhlman, *Proc. Natl. Acad. Sci. U.S.A.*, 2015, **112**, 112.
21. F. Tao and P. Yang, *Sci. Rep.*, 2015, **5**, 9839.
22. M. A. Kolber and D. H. Haynes, *Biophys. J.*, 1981, **36**, 369.

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