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DISCUSSIONS

New directions in surface functionalization and characterization: general discussion

Helena S. Azevedo, [iD] Adam B. Braunschweig, Ryan C. Chiechi, Shelley A. Claridge, [iD] Leroy Cronin, Yuri Diaz Fernandez, Ten Feizi, Laura Hartmann, Mia Huang, Yoshiko Miura, Matteo Palma, Xinkai Qiu, Bart Jan Ravoo, [iD] Stephan Schmidt, W. Bruce Turnbull, [iD] Carsten Werner, Zijian Zheng and Dejian Zhou

Yuri Diaz Fernandez opened discussion of the paper by Zijian Zheng: In your schematic diagram of the initiator transfer process, you located the initiator on top of the first polymer layer (DOI: 10.1039/c9fd00013e). Considering that your initiator molecule contains thiols, have you observed any exchange at the gold surface, between the initiator and the polymer? A molecular exchange mechanism could also explain the time dependence observed in the thickness of the final polymer brush, controlled by the initiator-transfer step.

Zijian Zheng answered: We did not observe an exchange between the thiol molecules and the polymer. The polymer should be more thermally stable than a thiol molecule.

Adam Braunschweig opened discussion of the paper by Matteo Palma: One of the major challenges in using nanopatterns to study biological properties is that often hundreds of different parameters must be explored, and to achieve statistical significance, this will involve repeats. So, making any significant statements should involve thousands of different data points. Does your system have the versatility and the throughput to acquire these thousands of data points?

Matteo Palma responded: One of the major challenges in using nanopatterns to study biological properties is the ability to systematically change different parameters with single-molecule control, nanoscale spatial resolution, and multivalent ability; this is what the DNA nano arrays presented here permit. Additionally, the system has the versatility to acquire multiple and numerous data points when merged with high throughput fabrication methods. Future research will be focused on merging this bottom-up ability with top-down lithography, allowing insight into many different chemical and physical properties, on the same chip.

Laura Hartmann continued the discussion of the paper by Zijian Zheng: Did you test the adsorption of the initiator molecules and the potential to graft on biopolymers or hydrogels?

Zijian Zheng noted: We tried hydrogels but the thiol molecules can easily absorb into the porous network of the hydrogel, meaning that no polymer growth was observed.

Laura Hartmann returned to the discussion of Matteo Palma's paper: You very nicely demonstrated control in spacing in the *x-y*-direction; is it also possible to control positioning in the *z*-direction?

Matteo Palma commented: As of today, we cannot fabricate 3D nano arrays with the same control demonstrated here in *x* and *y*. Moving forward, we are thinking about potential strategies to achieve this

Dejian Zhou asked: The use of DNA origami displaying a well-controlled number of specific ligands to study interactions with cells appears to be very attractive. However, given that the origami are DNA-based, they can be degraded by various nuclease enzymes, especially under intra-cellular or *in vivo* conditions; have you tested their stability under such conditions? Are these DNA origami readily taken up by cells and endocytosed into cells during incubation?

Matteo Palma responded: The DNA origami we emptied were immobilised (covalently) on surfaces. The origami were exposing peptides that were interacting with trans-membrane cell proteins. We do not have evidence of degradation of the origami. When trying to work with the same DNA origami in solution, we did not see evidence of cell uptake – for that, you would rather employ 3D DNA origami, such as tetrahedrons.

Dejian Zhou followed up by asking: You have produced three labels on each DNA origami, with relatively large inter-label spacing. Can you also make denser tether sites, so as to create higher label valencies on each origami scaffold? I think they could also be used as potential scaffolds to controllably display varying numbers of our recently developed polyvalent glycan nanoparticles[1] and study how these stimulate dendritic cell immune responses. We will be happy to collaborate on this if you are interested.

1 Y. Guo, I. Nehlmeier, E. Poole, C. Sakonsinsiri, N. Hondow, A. Brown, Q. Li, S. Li, J. Whitworth, Z. Li, A. Yu, R. Brydson, W. B. Turnbull, S. Pöhlmann and D. Zhou, *J. Am. Chem. Soc.*, 2017, **139**, 11833–11844.

Matteo Palma answered: We labelled multiple sites on individual origami with single-molecule control – here, we did so with up to 18 molecules per origami. In particular, we picked 60, 30, and 20 nm spatial separation for investigating integrin activation (avβ3 integrins have, in the past, been shown to exhibit a preferential spacing of 60 nm; this was done by employing nano-patterned metal dots functionalised with RGD peptides). I think there is potential to explore what you suggest: combing your glycan nanoparticles and

the DNA origami. I am definitely interested in exploring this possibility together with you.

Adam Braunschweig continued the general discussion: I am very sceptical about the rush to adopt machine learning methods as a substitute for more reliable experimental data. Garbage in, garbage out. What is needed are thousands of data points and more reliable data. One of the first things that you learn in a statistics course is that significance increases with n, the number of data points. So, a priority with all these novel surface modification strategies must be to develop approaches where thousands of different data points can be acquired, ideally, on the same surface in order to minimize batch-to-batch variation.

Zijian Zheng agreed: Without reliable data and accurate data analysis, it would be very difficult to build up a database for machine learning. This requires a tremendouss amount of work.

Matteo Palma added: I also agree. I would add that the level of control is important (single-molecule, nanoscale, and multivalent), and this is what the nanoarray platform I have presented permits .

Ryan Chiechi remarked: The problem with machine learning vis-à-vis classifying images of cell growth and adhesion is indeed a small, heterogeneous set of training data from which probabilities are extracted. The comparison to machine vision systems in, for example, self-driving cars is not really apt because a car only needs to differentiate between a person and a hedgehog with a reasonable degree of certainty over many frames of video. In five seconds of video, it may classify the person as a penguin or an elephant in a few frames, before concluding that, on average, it is most likely a person. That sort of classification does not seems useful for studying the morphology of cells.

Zijian Zheng commented: It is difficult to comment or to give a clear answer to that. But indeed, it is way more complicated to understand biological structure and behaviour.

Adam Braunschweig returned to the discussion of Zijian Zheng's paper: Achieving feature diameters of 25 nm is extremely impressive, especially in additive scanning probe methods like yours, where you put a material onto a surface. The first question is, how did you achieve such small feature diameters? Also, given the importance of multiplexing and testing various different conditions to resolve biological problems, how do you envision creating patterns with multiple different inks while maintaining this ~25 nm resolution? Could you, as well, get different materials with controlled spacing between different materials?

Zijian Zheng responded: The sharp tips of scanning probes provide us with the ability to pattern very small features. In principle (as well as in several nice examples), one can pattern the surface with multiplexed materials at sub-100 nm resolution.[1,2] The difficulty is how to resolve the registration between the different features/materials in a easy way. Additive printing provides a promising solution

to this, and it would be important to control the ink flow/transport onto and out from the tips.

1 L. Chen, Z. Xie, T. Gan, Y. Wang, G. Zhang, C. A. Mirkin and Z. Zheng, *Small*, 2016, **12**, 3400–3406.
2 Z. Zheng, W. L. Daniel, L. R. Giam, F. Huo, A. J. Senesi, G. Zheng and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2009, **48**, 7626–7629.

Mia Huang continued the general discussion: The cell and its components are already nanoscale and multivalent. What are the possibilities for developing tools to study the precise nature of their nanoscale and multivalent organization?

Matteo Palma answered: The platform we published in ACS Nano[1] and in this Faraday Discussions paper (DOI: 10.1039/c9fd00023b) is exactly the sort of tool needed to study biomolecular interactions with nanoscale spatial resolution, single-molecule control, and multivalent capability. In particular, we focused on cell matrix interactions dictated by trans-membrane protein integrins and their synergistic work with other membrane receptors to modulate normal cell and cancer cell behavior, as well as cardiomyocyte adhesion. It has been already demonstrated, employing metal nano dots functionalised with peptides (RGD), that there is nanoscale preferential spacing between integrins that favour cell adhesion (60 nm: see work by Joachim Spatz)[2,3] and that there is a minimal adhesion unit of at least 4 integrins per cluster in fibroblast cell adhesion, *i.e.* clustering is important.[4] What was missing was the ability to perform these kinds of experiments with multivalent capability and real single-molecule control, maintaining nanoscale spatial resolution: our DNA origami nanoarray platform is a tool to do exactly this. (By the way, the cell is microscale; nanoscale would include, for example, the integrin head (10 nm) and the spacing between integrins that favour cell adhesion; and nanoscale and multivalent are the interactions at the cell-ECM interface, as well as the interactions present in many other biologically relevant systems).

1 D. Huang, K. Patel, S. Perez-Garrido, J. F. Marshall and M. Palma, *ACS Nano*, 2019, **13**, 728–736.
2 M. Arnold, E. A. Cavalcanti-Adam, R. Glass, J. Blummel, W. Eck, M. Kantlehner, H. Kessler and J. P. Spatz, Activation of Integrin Function by Nanopatterned Adhesive Interfaces, *ChemPhysChem*, 2004, **5**, 383.
3 E. A. Cavalcanti-Adam, *et al*. Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands, *Biophys. J.*, 2007, **92**, 2964–2974.
4 M. Schvartzman, M. Palma, J. Sable, J. Abramson, X. Hu, M. P. Sheetz and S. J. Wind, *Nano Lett.*, 2011, **11**, 1306–1312.

Zijian Zheng added: For surface analysis, a tool should be flexible enough to create structures with multiple functionalities at high resolution and registration. This is the critical challenge in nanoscale patterning of functional materials.

Adam Braunschweig opened discussion of the paper by Bart Jan Ravoo: I really appreciate the use of supramolecular methods to address biological questions, specifically because they give you the ability to systematically vary certain parameters that you may not be able to do with natural systems. Your cyclodextrin vesicles are a perfect example of this. One phenomenon that occurs is that the lipids move around in response to binding to form rafts. I was wondering if the lipids in your cyclodextrin vesicles are similarly mobile.

Bart Jan Ravoo responded: The cyclodextrin vesicles are highly dynamic structures, much like typical liposomes. There is rapid lateral diffusion in the bilayer as long as the vesicles are kept above their T_g (which is around 0 °C for the vesicles used here). Furthermore, there is flip-flop and even some exchange of molecules between vesicles, although this is slow compared to lipids due to the high molecular weight and low water solubility of these amphiphiles with seven hydrophobic chains. We also know that the cyclodextrins can be mixed with other components in the membrane, such as lipids[1] or other amphiphilic macrocycles,[2] which can give rise to interesting dynamic phenomena, such as ligand-induced receptor clustering to bind multivalent guest molecules. Furthermore, one should keep in mind that the host–guest complexes of cyclodextrins are also highly dynamic, so that guest molecules can rapidly diffuse along the vesicles surface.

1 U. Kauscher, M. C. A. Stuart, P. Drücker, H.-J. Galla and B. J. Ravoo, *Langmuir*, 2013, **29**, 7377–7383.
2 Z. Xu, S. Jia, W. Wang, Z. Yuan, B. J. Ravoo and D.-S. Guo, *Nat. Chem.*, 2019, **11**, 86–93.

Xinkai Qiu remarked: The hydrogel showed quite significant fatigue over the four magneto- and photo-switching cycles, in the sense that the storage modulus of the hydrogel decreases dramatically after each cycle. Does the speaker perhaps have more insight to the origin of the fatigue in his system?

Bart Jan Ravoo replied: The observed fatigue is most likely caused by inhomogeneities in the hydrogels. The magneto-response tends to diminish over time due to the precipitation of a fraction of the magnetic nanoparticles during each magnetization cycle. The photo-response tends to diminish as the cycle time is slightly too short for complete recovery after green light irradiation. Furthermore, it is difficult to optimize the dual magneto- and photo-response, *i.e.* larger changes in moduli and less fatigue are obtained if the hydrogel is optimized towards a single response.

Laura Hartmann asked: In your experiment, testing the effect of the magnetic field on the mechanical properties of the hydrogel, based on the proposed alignment of magnetic nanoparticles along the magnetic field, do you expect there to be a difference if the magnetic field and shear are aligned in parallel or perpendicular?

Bart Jan Ravoo noted: There should be a large difference indeed. In our experimental set-up, the field is perpendicular to the shear direction, so that in principle we would expect a maximum effect because the alignment of nanoparticles would also occur perpendicular to the shear direction. It would be interesting to systematically vary the direction of alignment, but unfortunately our set-up does not allow that.

Yoshiko Miura opened discussion of the paper by Carsten Werner: Glycosaminoglycan-based hybrid gels have lots of potential in nanomedicine and tissue engineering. Considering the role of GAGs in natural systems, it is possible to develop a GAG gel that has specific affinity to cytokine or a growth factor. Have you investigated the binding specificity of cytokines for these GAG gels? Have you investigated growth factor binding to the GAG gel?

Carsten Werner answered: While our reported approach aims at designing selectively cytokine-affine GAG–hydrogel systems the obtained specificity will hardly be as high as, for example, in receptor–ligand or antibody–antigen recognition. For growth factor binding to different biohydride gel types (containing different heparin derivatives) see ref. 1–3.

1 A. Zieris, K. Chwalek, S. Prokoph, K. R. Levental, P. B. Welzel, U. Freudenberg and C. Werner, *Journal of Controlled Release*, 2011, **156**, 28–36.
2 A. Zieris, R. Dockhorn, A. Röhrich, R. Zimmermann, M. Müller, P. B. Welzel, M. V. Tsurkan, J.-U. Sommer, U. Freudenberg and C. Werner, *Biomacromolecules*, 2014, **15**, 4439–4446.
3 U. Freudenberg, Y. Liang, K. L. Klick and C. Werner, *Adv. Mater.*, 2016, **28**, 8861–8891.

Dejian Zhou enquired: Have you investigated how binding affects the cytokine diffusing through the hydrogel? Is there any correlation between the cytokine–hydrogel binding affinity and their diffusion rate in the hydrogel?

Carsten Werner answered: Yes, we have studied cytokine transport. It not only depends on the specific binding to the gel (as described by P1 and P2, DOI: 10.1039/c9fd00016j), but also on the geometric constraints (mesh size) of the polymer network.

Adam Braunschweig opened discussion of the paper by Shelley Claridge: I have several questions related to pattering using your lipids. The first: the polar groups on your lipids are involved in forming the monolayers. In one example, you showed an inositol headgroup, which is a proto-carbohydrate. Presumably, you want to use these in binding. Are your headgroups available for binding and have you tried any such experiments? Second, a really interesting aspect of your monolayers is the ability to precisely control spacing on the nanoscale, while making patterns that extend across the macroscale. Have you developed a method yet to form patterns composed of different materials?

Shelley Claridge replied: I agree, the availability of the headgroup to interact with the environment is critical. The first experiments we did with striped phases related to wetting,[1–3] which is very much about the availability of the headgroup. What we have found is that some surprising aspects of the striped phase structure, like the position of the polymerizable group within the chain, can impact how available the headgroup is to impact wetting. Wetting of carboxylic acid stripes also decreases substantially in buffers that contain small concentrations of divalent cations, which would be consistent with the headgroups complexing the ions, a very simple form of binding. Similarly, the structure of a phospholipid *versus* a simpler single-chain amphiphile is important, since it can help the terminal functional group project from the interface, presumably something it is doing in a related but different way at the membrane periphery. More recently, we are beginning to ask questions about headgroup binding to more complex adsorbates in the environment, which also appears to be possible.

1 J. J. Bang, K. K. Rupp, S. R. Russell, S. W. Choong and S. A. Claridge, Sitting phases of polymerizable amphiphiles for controlled functionalization of layered materials, *J. Am. Chem. Soc.*, 2016, **138**, 4448–4457.

2 T. A. Villarreal, S. R. Russell, J. J. Bang, J. K. Patterson and S. A. Claridge, Modulating wettability of layered materials by controlling ligand polar headgroup dynamics, *J. Am. Chem. Soc.*, 2017, **139**, 11973–11979.
3 S. W. Choong, S. R. Russell, J. J. Bang, J. K. Patterson and S. A. Claridge, Sitting phase monolayers of polymerizable phospholipids create dimensional, molecular scale wetting control for scalable solution based patterning of layered materials, *ACS Appl. Mater. Interfaces*, 2017, **9**, 19326–19334.

In terms of making multi-component patterns, we know it is possible to co-transfer multiple amphiphiles, which is a step in that direction, and certainly it would be possible to lithographically pattern multiple classes of molecules.

Stephan Schmidt reopened the discussion of Carsten Werner's paper: In your experience, what are the advantages and challenges of 3D *vs.* 2D cell assays?

Carsten Werner responded: 3D culture is often a more realistic tissue model, however, the exogenous cues acting on cells are more difficult to control in 3D.

Mia Huang addressed Shelley Claridge, Bart Jan Ravoo, and Carsten Werner: Serendipitously, I think the three of you can make an artificial cell! Bart can provide the encapsulation provided by cell membranes, Shelley can provide long-range order found in actin filaments, and Carsten can impart three-dimensional order. I think there are tremendous applications for your technologies that could be employed to understand outstanding questions in biology. For Carsten, especially, using polysialic acid-based hydrogels would be very enabling in understanding neuronal interactions.

Carsten Werner responded: Exploring biohybrid gels containing polysialic acid is an excellent idea - we will definitely do that. Thanks!

Shelley Claridge replied: Thanks, Mia. We have not explored adhesion to striped phases in the sense of actin, but we have definitely been interested recently in the adhesive properties of the stripes more generally, and we are always open to collaboration.

Xinkai Qiu returned to the discussion of the paper by Shelley Claridge: The speaker introduced a useful method to pattern molecules that show long-range order for potential application. In the field of molecular electronics, self-assembled monolayers of organic molecules on polycrystalline substrates, more often than not, show stable and reproducible charge transport properties in the absence of long-range order of the molecules. In that sense, the long-range order of the molecules does not have any impact on the practicality of SAMs. Could the speaker comment on how we should justify long-range order of molecules when concerning the practicality of SAMs?

Shelley Claridge responded: That is a great point. Absolutely, there are applications like you are describing that do not require long-range ordering. At the same time, for many applications, it would be vital. Take, for instance, a situation where you want to use patterns in the nanometer-scale chemistry of the monolayer in the xy plane to control ordering of other objects at the interface. In some of our work, we are using nanometer-wide stripes of functional groups to orient inorganic nanostructures that are microns in length, as prototype circuit elements.[1] In order to orient each

micrometer of the nanostructure, you need an aligned row about 1000 molecules in length, which is already reasonably long-range ordering. For interaction with biological structures using something like a carbohydrate-functionalized striped phase, you might need ordering over distances representing tens to hundreds of thousands of molecules in the monolayer. It all depends on what you want to do with the surface. The nice surprise has been that it is possible to generate such long-range order, which is really opening up the range of problems we can address.

1 A. G. Porter, T. Ouyang, T. R. Hayes, J. Biechele-Speziale, S. R. Russell and S. A. Claridge, *Chem*, 2019, **5**, 2264–2275.

Laura Hartmann asked: After polymerization of the lipids on the surface, is it possible to take them off the surface and isolate them as ribbons or sheets?

Shelley Claridge replied: We have been curious about that as well. It certainly seems possible that this would be a way of creating amphiphilic structures that would be hard to synthesize in other ways. A significant limitation using our current methods would be throughput – a square meter of monolayer of this type represents just a milligram of material. But perhaps scaling can be achieved using graphite flakes or other high surface area supports.

Adam Braunschweig addressed Carsten Werner and Bart Jan Ravoo: These nanoscale systems will be perennial models until we can make them heterogeneous mixtures, to which we can add different components. Carsten, in the case of your system, you have to worry about how additives affect gelation, and Bart, in yours, you have to be concerned about the ability of heterogeneous mixtures to form vesicles. My question is: to what extent have you attempted to make heterogeneous gels and vesicles by incorporating materials that may add new functionality.

Carsten Werner responded: We are working on the formation of multiphasic microgel-in-gel materials with microgels of different characteristics and have incorporated collagen fibrils in the gels. Moreover, we use the rapidly (Michaeal-type addition) crosslinking variant of the gelation to apply the biohybrids as bioinks in additive manufacturing schemes.

Bart Jan Ravoo replied: I would say that a highly attractive feature of our system is that we use a modular strategy in which components can be easily added, substituted, or modified to add or change functionality without a complete redesign and optimization of the entire system. We know that we can change the composition of the vesicles, *e.g.* by adding lipids or by adsorbing magnetic nanoparticles, as shown in the present paper (DOI: 10.1039/c9fd00012g). This also applies to the peptide gelators: we can vary the percentage of guest-tagged peptide to tailor the cross-linking density and we are also exploring the incorporation of two orthogonal guests, one responsive and one inert, so that we can make gels with shape memory.

Adam Braunschweig continued the discussion of the paper by Bart Jan Ravoo: Many vesicles are used to deliver contents that are encapsulated within the interior. Do your vesicles encapsulate different molecules, and have you thought of using your vesicles for a type of stimuli-responsive triggered release?

Bart Jan Ravoo said: The cyclodextrin vesicles are rather leaky but we have explored various ways to make them less permeable, *e.g.* by mixing the cyclo-dextrins with phospholipids[1] or by wrapping the vesicles in a polymer shell.[2] We have also shown that the vesicles can enter cells rather efficiently through endocytosis and we have made redox-responsive polymer shells containing disulfide crosslinks, so that a triggered intracellular release is obtained.[2] In an alternative approach, we have decorated the vesicles with photoswitchable guest molecules that bind, for example, proteins, so that these can be captured and released in response to irradiation.[3]

1 U. Kauscher, M. C. A. Stuart, P. Drücker, H.-J. Galla and B. J. Ravoo, *Langmuir*, 2013, **29**, 7377–7383.
2 W. C. de Vries, D. Grill, M. Tesch, A. Ricker, H. Nüsse, J. Klingauf, A. Studer, V. Gerke and B. J. Ravoo, *Angew. Chem. Int. Ed.*, 2017, **56**, 9603–9607.
3 A. Samanta, M. C. A. Stuart and B. J. Ravoo, *J. Am. Chem. Soc.*, 2012, **134**, 19909–19914.

Ten Feizi addressed Carsten Werner: How would you approach bringing cells in your system?

Carsten Werner answered: Cells are incorporated in our gel system by mixing the cell suspension with maleimide-prefunctionalized GAG component, gel formation results within seconds after combining this mixture with the solution of peptide-functionalized PEG. This procedure was, for example, in the bio-printing of cell-containing gel materials (see, e.g., ref. 1).

1 R. Zimmermann, C. Hentschel, F. Schrön, D. Moedder, T. Büttner, P. Atallah, T. Wegener, T. Gehring, S. Howitz, U. Freudenberg and C. Werner, *Biofabrication*, 2019, **11**, 045008.

Ten Feizi continued by asking: What is the diffusivity of your materials into the hydrogels?

Carsten Werner answered: Transport phenomena of cytokines - and GAG-binding as well as non-binding proteins within our bihybrid GAG-PEG gels are being studied by experiments and by simulations. A systematic study will be published soon.

Ten Feizi asked: Are you looking at any different glycoso-amino-glycans?

Carsten Werner answered: So far we have been using heparin, and different selectively desulfated heparin derivatives as building blocks of our gels. Moreover, we have been using hyaluronic acid, dermatan sulfate and chondroitine sulfate for gel formation with similar crosslinking chemistries. Beyond that, we are planning to use CRISPR-based GAG variants for the preparation of gel matrix libraries.

Helena Azevedo communicated in response to the discussion of Carsten Werner's paper: It is known that, in general, positively charged materials tend to be cytotoxic and cell viability assays show cell death after 24 h. However, if cells are maintained in culture for longer periods of time (1 week), cells are able to recover as proteins from the serum and produced by cells adsorb onto the materials shielding the charge. We may need to revise how we assess biocompatibility of materials *in vitro*.

Stephan Schmidt continued the discussion of the paper by Shelley Claridge: What is the advantage of self-assembly pattering methods in comparison to mass-scale top-down lithography methods in the semiconductor industry?

Shelley Claridge answered: So, if Intel is getting to nodes at or below 10 nm,[1] why bother, right? There are some pretty important reasons, actually. The fabrication processes required to get down to feature scales that small are incredibly expensive, and often require several sequential lithographic steps, so it is not like you can just draw any arbitrary pattern you want, and generate it. In contrast, biomolecules routinely generate patterns at sub-10 nm scales. However, I think the other, even more significant reason (at least for me), is the chemistry. The surface of the cell membrane or a protein binding pocket creates exquisitely precise chemical environments that are the basis for molecular recognition and catalysis. These chemical environments come from positioning functional groups not just in big blocks, but in well-defined geometries relative to other complementary functional groups. To me, that is one of the end goals of molecular lithography, combining that sort of precision with scalability.

1 https://www.extremetech.com/computing/291029-intel-will-launch-7nm-chips-in-2021-ice-lake-ships-in-june.

Mia Huang enquired: Have you thought about using your microscale system to study piezo proteins?

Shelley Claridge replied: No, but we are definitely interested in mechanical signal transduction, so that would be a great thing to look into. Thanks!

W. Bruce Turnbull commented: I really like the idea of patterning the glycolipids on a nanometer scale that could match the distance between binding sites for various lectins, as it could provide a different strategy for selective binding to different lectins. Please can you comment on whether the patterning methodology could be extended beyond phospholipids to natural glycolipids with ceramide tails? Is it possible to functionalize the hydrophobic stripes on the surface to reduce any potential non-specific binding to proteins?

Shelley Claridge replied: Much of the ordering in striped phases arises from the interaction between the alkyl chain and the substrate, so from that perspective, I would also expect ceramides to be capable of forming stripes. The relative width of the head and tail segments of the molecule can also be important in long-range ordering, so, for instance, a molecule with a very wide headgroup might need to be co-assembled with another molecule to achieve a stable striped phase. Usually, if we were going to try assembly of a new molecule that was precious, we would model the likely stripe structure first to see if there are obvious steric factors that would preclude a stable assembled structure. In regards to functionalizing the hydrophobic stripes, there are indications that it is possible to do reactions with the polymer backbone, which would be one way of changing the chemistry to reduce non-specific binding.

Conflicts of interest

There are no conflicts to declare.