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DISCUSSIONS

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Preparation of multivalent glycan micro- and nano-arrays: general discussion

Adam Braunschweig, Joseph P. Byrne, Ryan Chiechi, Yuri Diaz Fernandez, Jeff Gildersleeve, Kamil Godula, Laura Hartmann, Clare Mahon, Yoshiko Miura, Alshakim Nelson, Stephan Schmidt, W. Bruce Turnbull, Daniel Valles, Jin Yu and Dejian Zhou

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Adam Braunschweig opened the discussion of the paper by Jeffrey Gildersleeve: In your contribution to this Discussion, you have shown that the avidity between protein and glycan is sensitively dependent upon glycan–glycan spacing, linker composition, linker length, *etc*. As a result, different microarrays produce different results for specificity and avidity towards a particular lectin. Given this inherent complexity, is there a "true value" for avidity that reflects most accurately how this recognition may be occurring in biology?

Jeff Gildersleeve answered: This is an interesting consideration. A glycan determinant can be present in varying contexts in biology. The avidity will depend on the structure of the determinant as well as the presentation. For example, a lectin might have four binding sites. In some settings, the lectin might only be able to engage two binding sites. In other settings, the lectin might bind glycans using all four of its binding sties. These could both be biologically relevant, but the avidities could be quite different. So, there are likely many "true values" for avidity for each glycan determinant. When connecting apparent K_d values measured on a glycan microarray to biological systems, I think of it as potential – "in the right context, this lectin could bind with an apparent K_d value of X to this glycan". It has the potential to bind, but it will depend on other factors, such as the nature of the carrier chain and the spacing and orientation of the glycans.

Daniel Valles asked: Since there has been much discussion about the varying binding constants throughout different glycan arrays, do you think binding should be defined as a range rather than a single number?

Kamil Godula responded: Of course a single binding constant can be measured for the binding to a single glycan or a multivalent glycopolymer. However, it is not that clear how much practical information these measurements provide if one is considering the presence of a large number of similar

glycoconjugates at the cell surface. Perhaps an apparent binding constant to the glycocalyx surface may be a more meaningful measure.

Jeff Gildersleeve then added: This is a really interesting perspective. There are so many factors that influence binding – monovalent *vs.* multivalent ligand, type and extent of multivalency, nature of the linker or carrier glycan chain, assay conditions, *etc*. As a result, the measured binding interaction can vary quite a lot. How one defines binding or discusses binding depends on the situation. For an individual protein binding to a specific ligand, I would probably refer to a single number. If one is talking about how well a lectin binds its ligands, a range would make more sense.

Clare Mahon asked: To what extent does the observed dissociation constant depend on the technique you use to measure it? Would you expect to determine similar dissociation constants for wholly solution-phase measurements compared to those determined on surfaces?

Jeff Gildersleeve answered: The method can have a significant effect. We refer to the values we measure on the array as "apparent K_d values". One might also refer to them as "surface K_d values". In most cases, they represent the apparent binding avidity of a multivalent binding interaction. Binding in solution to a multivalent conjugate can be quite different. We have discuss this at length in a paper.[1] Briefly, on a surface, a lectin can form a multivalent complex involving 1 lectin and 1 neoglycoprotein, or it can form a multivalent complex bridging 2 or more neoglycoprotein molecules on the surface. In solution, only the 1:1 complex forms. In many cases, lectins can form multivalent complexes with a surface but not with a neoglycoprotein in solution (*ie*. the spacing and orientation of the glycans on the protein may not be suitable to form a 1:1 complex). Binding in solution to a monovalent glycan will often give much weaker binding than interactions with multivalent glycans on a surface.

1 Y. Zhang, Q. Li, L. Rodriguez and J. C. Gildersleeve, An array-based method to identify multivalent inhibitors, *J. Am. Chem. Soc.*, 2010, **132**, 9653–9662.

Dejian Zhou opened the discussion of the paper by Daniel Valles: The use of the right linker to ensure that all sugars are accessible to lectin binding is important to make glycan microarrays more robust. Have you studied how linker length and flexibility affect the lectin binding? Also the sugar density reported in your paper appears quite low, *e.g.* at 0.1–0.6 molecule per nm^2, suggesting that the sugars are rather loosely packed on the surface. How did you determine the sugar density of such surfaces?

Daniel Valles answered: At the moment, we have not done a thorough investigation of different linker lengths. However, we understand that different linkers can lead to more robust systems, which is why we plan to examine this in the future. Unfortunately, the packing is just pure estimation based on the size of the monosaccharide. We decided to show a range from 0.1–0.6 nm^2 to help draw conclusions with our experimental data. The theoretical calculations show that if the molecules were packed at 0.1 nm^2, multivalency could not occur if the glycan

density falls below 20%, which agrees with the experimental data we have shown in the paper.

Ryan Chiechi then enquired: Rather than having to discuss effective binding constants, would it make more sense to measure the binding of an internal standard and report a value relative to that? That way the internal standard experiences the same local environment as the surface-binding event that is under investigation, potentially facilitating comparisons across a variety of experimental platforms. This is a concept that is common in other areas such as colloidal self-assembly, where what matters is the relative strengths of interactions in competition with each other rather than scalar values on an absolute unit scale.

Jeff Gildersleeve replied: This is a really interesting idea and something that could be very helpful. We print a variety of control spots on our array, including IgG, IgM, and IgA. I think at least some other groups also print these controls. These controls could potentially serve as standards for assessing relative affinity between array platforms. I would like to note that the apparent K_d values measured on our array and the CFG array have similar values. Thus, in the majority of cases, the apparent affinities are quite similar; there are just certain specific instances where there are large differences in binding.

Stephan Schmidt asked: The presented results indicate a critical spacing of mannose units above which ConA is bound to the chip surface. Since this mannose spacing correlates with the minimum binding site distance of ConA ($\sim$7 nm), do you think that ConA binds in a chelate-like fashion on the chip? Furthermore, when further increasing the mannose density, there is a proportionality between mannose density and bound ConA. Does this suggest that this linear regime is governed by statistical multivalency, *i.e.* the mannose units contribute additively?

Daniel Valles answered: To the first part of your question, ConA is a tetramer constructed of four monomers, each of which has its own binding site. Due to the location of each binding pocket, we believe that only two sites are available to bind to a monolayer of mannose. That being said, our paper suggests that if both of those pockets cannot reach a mannose attached to the surface, then the overall binding will not be strong enough to remain attached to the surface. In this particular example, it may seem to be linear, but what we expected to see was step-like drops-off of fluorescence when spacing between the immobilized mannose starts to make them further away from one another. The best example of this is the difference between $\chi = 0.3$ and $\chi = 0.2$. There is a sudden drop off to the point where the fluorescence of the ConA binding becomes scarce to the point where it is immeasurable.

Joseph P. Byrne commented: This was a really interesting piece of research. I was fascinated at how your estimated density of sugars on the surface, and hence the average distance between mannose units, correlated well with the experimentally-known distance between binding sites for ConA to interact in a multivalent way. This seems like an encouraging result.

Do you think that this system that you and your colleagues have described could be used as a tool for estimating, or perhaps even quantifying, the distance between binding sites or carbohydrate-recognition domains, in new, less-studied, or even unknown lectins?

Adam Braunschweig replied: Thanks for this question. What we reported in our *Faraday Discussion* manuscript was that no binding between printed mannose and ConA in solution was observed when our estimate of the average spacing between mannosides reached ~7 nm, which is the same as the distance between the binding sites of ConA. So, one could assume that for ConA to persist on the surface after washing, then the protein would need to be able to reach at least two mannosides, so if we consider this assumption valid, then, potentially this method could be used to estimate the distance between binding sites. Although the data are promising, there is, however, considerable uncertainty in estimating the distance between mannosides on the surface. You will see in the paper that we had two estimates for the distance between mannosides on the surface, with the high-density as well as a low-density estimate based upon previous results from our group. A consistent theme in this general discussion was that characterizing the nature of the surface with nanoscale precision remains a major challenge. Without more accurate and easy-to-use methods to characterize directly the average spacing of the mannosides, I would be reticent to draw any firm conclusions, but I do consider this work to be a step forward in the exact direction that your question implies.

Yuri Diaz Fernandez opened the discussion of the paper by Clare Mahon: Within your paper, the thermodynamic parameters for the monomer and the polymer-anchored receptors are very similar at the same temperature. Particularly, we would expect the entropy to be different considering the change in degrees of freedom, going from a monomer to the polymeric receptor. Could you explain why this intuitively expected difference is not observed in your system?

W. Bruce Turnbull then added: Regarding the entropy of binding of the polymers to the CTB lectin, it is important to note that we do not see any apparent enhancement in affinity by calorimetry for the glycopolymers relative to the monovalent GM1 oligosaccharide, even though we do see an enhancement in inhibitory potency. While others studying different systems have often observed affinity enhancements using ITC, we have never seen this for multivalent GM1 ligands binding to CTB regardless of whether the multivalent scaffold is a polymer, dendrimer or tailored glycoprotein. Yet in most of these cases we do see substantial increases in inhibitor potency up to a few thousand times relative to the monovalent ligand. I suspect that the differences we observe between the binding and inhibition experiments are a result of observing distinct processes happening on different timescales: the ITC reports rapid binding to the GM1 while a slower rearrangement to the most stable multivalent configuration during the inhibition assay would be effectively invisible calorimetrically because it would likely have slow kinetics and relatively small net enthalpy changes. The result is that it is challenging to interpret the measured entropies in terms of multivalent binding.

Clare Mahon replied to both: In both cases, the binding of GM1os to CTB is entropically unfavourable – the geometry of the binding site requires rigidification of the oligosaccharide conformation to enable complexation. You are correct to note that we observed a similar value for the entropy of complexation in both cases. This observation may suggest that major reorganisation of the polymer conformation is not required to enable complexation. Below their LCST we expect the polymers to exist in solution as hydrated random coils. In this conformation a significant proportion of GM1os residues are expected to be displayed on the surface of the coil, available for complexation without significant rearrangement of the polymer backbone.

Yuri Diaz Fernandez then asked: Have you observed any effect of the pH on the affinity of the receptor?

Clare Mahon replied: We have not performed any experiments to investigate the complexation of our polymer with CTB under conditions other than physiological pH. We don't expect, however, that changing the pH would alter the affinity of the interaction to a significant extent – provided, of course, that the protein itself was stable at the pH under study.

Dejian Zhou said: The GM1 conjugated thermal response polymer can catch and release CTB in a temperature-dependent manner. Since CTB–glycan binding became weaker at higher temperature, have you tested whether GM1-conjugated to a non-thermal responsive polymer can also do the same job?

Clare Mahon responded: Yes, we had looked at similar receptors constructed on a non-thermoresponsive dimethylacrylamide scaffold and we didn't see a change in the binding stoichiometry at elevated temperature – so the 'catch and release' behaviour is dependent on the thermoresponsive nature of the poly(-NIPAm) scaffold.

Yoshiko Miura asked: The catch and release of CTB was attained by PolyNIPAAm-GM1 conjugates. The system is based on the LCST of polyNIPAAm. Considering the LCST of the polymer, the entropy effect is important. Is it possible to measure thermodynamic parameters like entropy?

Clare Mahon responded: Yes, some techniques allow for the determination of entropic contributions too binding. In our case, we used isothermal titration calorimetry to determine all thermodynamic parameters of interaction. The recognition of GM1os by CTB is entropically unfavourable, as it requires rigidification of the oligosaccharide conformation. We found that the binding of GM1os-decorated polymers to CTB was also unfavourable both above and below the LCST of the polymer.

Yoshiko Miura opened a general discussion by addressing Jeffrey Gildersleeve: Glycan arrays have been examined by many molecules and methods.

When considering the interaction between glycan and protein, it is thought that not only the enthalpy benefits between glycan and protein, but also the mobility of molecules, the shape of clusters, the flexibility of molecules, and so

on, are involved. Do you have any idea about the structure of the linker that links the glycans?

Jeff Gildersleeve replied: The linker certainly affects recognition in many cases. The portion of a glycan that interacts with a lectin or antibody is often referred to as the glycan determinant. In nature, glycan determinants are often attached to a carrier glycan chain or lipid. For synthetic systems, glycan determinants are often attached *via* a non-natural linker to a surface or multivalent scaffold. The carrier chain, lipid, or synthetic linker can all significantly influence recognition. Sometimes, they can provide some additional contacts or interactions that enhance binding. At other times, they can decrease or even prevent recognition – for example, they might block access of the glycan determinant to the binding pocket through steric interference. I am not able to predict these types of interactions, and they likely vary quite a bit from one protein to another. Therefore, I believe variations in linkers and carrier glycan chains are valuable elements of diversity to include on a glycan microarray to allow one to evaluate their effects empirically.

Alshakim Nelson then addressed Jeff Gildersleeve and Kamil Godula: The field has been trying to probe the nature of carbohydrate–ligand interactions (spacing, ligands, linkers, *etc.*), so has anyone looked at using artificial intelligence or machine learning to identify systems to probe these interactions?

Jeff Gildersleeve and **Kamil Godula** have not yet replied.

Jin Yu then enquired: How did you compare between two different array, since the signal-to-noise ratio is different on each slide. In common cases we used the value by subtracting the total signal to local background, but even the same epitope can have different background from different slides, therefore resulting in different observed signal intensities. How did CFG address to this issue?

A follow-up question is: despite your great work with the glycoprotein conjugate microarray, if you could choose again, which method of microarray setup would you prefer; between an NHS coated covalent array, an NGL noncovalent array, a glycoprotein array, or another platform like the Luminex beads system?

Jeff Gildersleeve replied: For the comparison described in our paper, we did not adjust for differences in signal-to-noise ratios. There were several reasons. First, the CFG and our own data both used background-subtracted RFU signals, so this methodology was consistent on both arrays. Second, the noise levels were similar for the vast majority of the data (One way to evaluate noise is to look at the variations in signals for the lowest 50% of RFU values. For example, the lowest 50% of RFU values across the array might vary between +50 and -50 RFU.) There was one set of data where our array had a lot of noise – SNA at 50 μg mL^{-1}. Since there was a lot of noise and our noise was considerably higher than the CFG, we did not include this dataset in our comparison. Third, our analyses were focused on signals that were well above background levels for both arrays. Thus, we concluded that variations due to noise would have only very minor influence on the analysis.

As for your second question, I think each of the platforms has their advantages and disadvantages. The optimal system really depends on the application. One of the really neat features of the NGL platform is the ability of the neoglycolipids to move and adjust to match the spacing of different glycan binding proteins. Because of this feature, one does not have to have the right spacing on the surface – the glycans can adjust to accommodate different proteins. However, there are situations where one would like to distinguish between different proteins that have different spacing preferences. For example, some subpopulations of antibodies in human serum will only bind glycans at high density while others will bind at either high or low density. These different subpopulations can have different clinical significance. To detect or monitor these different subpopulations of antibodies, one needs defined/stable densities of glycans on the surface.

There are also advantages and disadvantages for a bead-based array versus a slide. In terms of sample throughput, the Luminex bead system is really powerful. For example, one can easily evaluate hundreds to thousands of human serum samples. The slide based system has advantages in terms of total number of glycans that can be evaluated and using much less material. For example, one could print 20,000 different glycans on a slide and test a lectin for binding to all of them in a single experiment. The Luminex system can accommodate up to about 500 different glycans in one experiment, but the people I know that use Luminex prefer to just use more like 100–200 different types of beads per experiment. There are several features I really like about the neoglycoprotein system. First, it is easy to translate results from the array to other assays and experiments. When using array binding information to design multivalent inhibitors or reagents, one needs a multivalent presentation that matches the presentation on the array surface. For many array platforms, finding this match can be challenging. For example, what multivalent scaffold should one use – a nanoparticle, liposome, glycopolymer, *etc.*? In addition, what type of linker should one use to attach the glycan to the scaffold? What density of glycan will best mimic the density on the array surface. These are not trivial questions. With the neoglycoprotein platform, we can use the same neoglycoprotein from our array in other experiments. For example, going from the array to an ELISA or Western blot is really simple. Also, one can attach a neoglycoprotein to a Luminex bead and the signals are nearly identical because many features of presentation, such as the linker and glycan density, are preserved. Second, we have flexibility for how we attached glycans to the neoglycoprotein. We don't need a specific group, like a free amine, to get a glycan onto the surface. This allows us to use glycans from many different sources, including glycans with an azide linker, a free lactol, or a carboxylic acid linker. Third, we can print a lot of different entities using the same conditions – we print neoglycoproteins, natural glycoproteins, and DNA on our surfaces using the same conditions, settings, and slide surface. That being said, if I was to start over, I'm not sure I would use bovine serum albumin as the carrier protein for our neoglycoproteins. This protein is a lot more complex than I realized when I first got started. I would also love a system where we have much better control over spacing and orientation of glycans on the surface. We can modulate average differences on the surface, but it would be really neat to control them more precisely on a molecular level.

W. Bruce Turnbull asked: Is there any correlation between lectin architecture and the differences in binding that you observe between the different formats of the glycan microarray? For example cases where binding sites are pointing in the same direction *vs.* in opposing directions?

Jeff Gildersleeve replied: While lectin architecture is likely to be an important contributor, we are not yet able to correlate lectin architecture with differences in binding. The ability to form a multivalent complex depends on many factors, including the spacing and orientation of the lectin binding sites, the density of the glycans on the surface, the linker length, and the linker flexibility. At this point, we don't have a good system to isolate the effects of architecture. In addition, we have very little information about how these lectins are interacting with glycans on the surface for any glycan microarray.

W. Bruce Turnbull then asked: Could the differences in binding you see between glycan microarrays with different architectures provide insights into the native ligands for a lectin? For example, glycolipid *vs.* glycoprotein?

Jeff Gildersleeve responded: Using the differences in binding to gain insight about the native ligands would be very useful, but it is challenging. A key barrier is that we know very little about how the lectins interact with glycans on a microarray surface at a molecular level.

Dejian Zhou commented: The glycan microarrays based on bovine serum albumin (BSA) neoglycoprotein scaffolds appear to be effective in promoting lectin binding. However, given the sugars are conjugated to BSA *via* surface lysine residues which are not evenly distributed throughout the protein surface, it may be difficult to produce a uniform sugar coating with the same inter-sugar distance. In addition, have you checked whether such BSA layers are homogenous on the surface? Do they form a uniform, complete monolayer? Have you considered using other nanoparticle scaffolds (*e.g.* quantum dots or gold nanoparticles) which have uniform surface reactivity toward glycan ligands and therefore may be able to offer potentially better control over the sugar densities and inter-glycan spacing. Moreover, the unique optical properties of these nanoparticles, *e.g.* strong fluorescence for quantum dots[1] and efficient fluorescence quenching for gold nanoparticles,[2] can be harnessed for binding confirmation and quantification. For example, we have recently demonstrated that quantum dots displaying polyvalent specific glycan ligands (glycan = mannose or Man–α-1,2-Man) can act as multifunctional nanoprobes to dissect the exact binding modes of a pair of closely related, almost identical tetrameric lectins, DC-SIGN and DC-SIGNR, *via* a multimodal readout strategy combining fluorescence resonance energy transfer (FRET), transmission electron microscopy and hydrodynamic size analysis. Moreover, they can also be used to quantify the binding affinity with DC-SIGN/R *via* a sensitive, ratiometric FRET readout. We have further revealed that the apparent K_d measured by our FRET method matches well to their inhibition potency (IC_{50}) against DC-SIGN mediated pseudo-Ebola virus infection of target cells.[3,4] Given such potential advantages, are you interested in making glycan microarrays using such functional nanoparticle scaffolds? If so, I will be very happy to collaborate with you on this development.

1 I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher and J. M. Mauro, *Nat. Mater.*, 2003, **2**, 630–638.
2 B. Dubertret, M. Calame and A. J. Libchaber, *Nat. Biotech.*, 2001, **19**, 365–370.
3 Y. Guo, C. Sakonsinsiri, I. Nehlmeier, M. A. Fascione, H. Zhang, W. Wang, S. Pöhlmann, W. B. Turnbull and D. Zhou, *Angew. Chem., Int. Ed.*, 2016, **55**, 4738–4742.
4 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.

Jeff Gildersleeve replied: These are excellent points. The neoglycoprotein format has a number of limitations. We have very minimal molecular level information about our surfaces, but our expectation is that there is heterogeneity to the spacing and orientation of the glycans. In addition, we do not know if we have a monolayer on the microarray surface. We use a print concentration that saturates the surface in its ability to capture neoglycoproteins. This provides consistency from one array to another and from spot to spot, but we have not been able to characterize the surface in great detail. From some prior work, we think the glass surface is pretty rough at the molecular level and that there are a variety of crevices of varying size.[1] We also believe there is a consistent coating of neoglycoprotein over the surface area of the spot.[2]

We have considered many different multivalent formats for constructing arrays. So far, the neoglycoprotein format has been the most convenient. We would be very interested in methods to control spacing and orientation of glycans in a more precise way. The use of your nanoparticles could offer some unique properties and advantages for constructing glycan microarrays. We can talk more later *via* email about a potential collaboration, but it could be pretty interesting.

1 Y. Wang, J. C. Gildersleeve, A. Basu and M. B. Zimmt, Photo- and bio-physical studies of lectin-conjugated fluorescent nanoparticles: Reduced sensitivity in high density assays, *J. Phys. Chem. C.*, 2010, **114**, 14487–14494.
2 Y. Zhang, Q. Li, L. Rodriguez and J. C. Gildersleeve, An array-based method to identify multivalent inhibitors, *J. Am. Chem. Soc.*, 2010, **132**, 9653–9662.

Stephan Schmidt addressed Clare Mahon: Some work suggest that above the LCST carbohydrate functionalized PNIPAM shows higher affinity due to presentation of the carbohydrates on the outside of the hydrophobic globule. This is different for the presented GM1 functionalized PNIPAM, where the higher affinity was observed below LCST. Can you explain the different behaviors?

Clare Mahon replied: There are some studies in the literature where poly(-NIPAm)s have been used as steric 'shields' to prevent or frustrate complexation of a receptor below the LCST of the poly(NIPAm). At temperatures above the LCST these chains collapse, exposing the carbohydrate recognition motifs and consequently increasing the affinity of the receptor to the target lectin.

In our case, we have made linear statistical copolymers of NIPAm and a GM1os-modified monomer unit. When these polymers undergo coil-to-globule collapse we expect they will rearrange to some extent to display their hydrophilic GM1os residues on the outside of these globules. The surface of these globules must display net hydrophobic character, however, as we observe the formation of large aggregates in solution by DLS. The size of these aggregates dictates that they must incorporate many collapsed chains, so overall we have whole polymeric receptors buried within the aggregates. The GM1os residues on the surface of the

aggregate can interact with CTB, but those in the centre cannot, so overall we see a decrease in the avidity of the interaction.

Laura Hartmann addressed Jeff Gildersleeve & Alshakim Nelson: On the one hand the discussion has shown the importance of model systems to address fundamental questions on carbohydrate–lectin interactions such as 2D arrays. On the other hand, it has been discussed that we should think more about the application and what requirements will have to be met outside of an array setting going towards biological systems. In order to potentially bridge these two areas of research, do you think it would be possible to print onto cells and create an array on cells?

Jeff Gildersleeve replied: This is an intriguing idea. We have never tried to print onto cells. Peng Wu is developing a cell-based glycan array platform, although the approach is to construct glycans on the surface of cells *via* enzymatic modification rather than printing. Glycolipids and neoglycolipids can insert into cell membranes under the right conditions, so it might be interesting to print them onto cells.

Alshakim Nelson responded: The advantage of a 2D array is that we can control patterns at the nanoscale with sub-10 nm resolution. We still do not have the tools to produce 3D arrays with the same resolution with spatial control. There is some inspiring work by Kristi Anseth and Cole DeForest (among others), who are developing methodologies that allow one to produce 3D patterns within hydrogels with spatiotemporal control. These developments may be the key to developing model systems that allow us to investigate multivalency at the cell surface.

Ryan Chiechi concluded by remarking: In defense of molecular nanofabrication: Commercial photolithographic technology is not only constrained in the types of structures it can fabricate, but also the materials. Furthermore, the reported feature-size of photolithographic processes is the smallest measurable feature. A 7-nm process does not imply that one can write arbitrary nanostructures in arbitrary materials with 7-nm resolution. Molecular self-assembly can provide Angstrom-level resolution and capture molecule-scale interactions between nano- and micro-scale objects, which is particularly relevant in *vivo*.

Conflicts of interest

Peter Seeberger holds significant shares in GlycoUniverse, the company that produces the automated glycan synthesizer.