

View Article Online View Journal

# ChemComm

# Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: E. Cavatorta, M. Verheijden, W. van Roosmalen, J. Voskuhl, J. Huskens and P. Jonkheijm, *Chem. Commun.*, 2016, DOI: 10.1039/C6CC01693F.



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about *Accepted Manuscripts* in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard <u>Terms & Conditions</u> and the <u>Ethical guidelines</u> still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



www.rsc.org/chemcomm

Published on 06 May 2016. Downloaded by University of Wollongong on 09/05/2016 16:32:33

# CROYAL SOCIETY

## Journal Name

### COMMUNICATION



Received 00th January 20xx, Accepted 00th January 20xx

Emanuela Cavatorta,<sup>†</sup> Mark L. Verheijden,<sup>†</sup> Wies van Roosmalen, Jens Voskuhl, Jurriaan Huskens<sup>\*</sup> and Pascal Jonkheijm<sup>\*</sup>

www.rsc.org/

Multiple naphthol ligands were installed on the glycocalyx of white blood cells via metabolic labeling and subsequent strain promoted azide-alkyne cycloaddition. Only when cucurbit[8]uril was present to drive the formation of ternary complexes, cells specifically assembled on a methylviologen functionalized supported lipid bilayer through multivalent interactions.

The development of strategies to engineer cell-surface interactions is essential to achieve control over cell-cell and cell-material interactions. These interactions are mediated by e.g. cell receptors and their ligands and are dynamic and vital for correct cell behaviour.<sup>1, 2</sup> Supramolecular chemistry provides dynamism, reversibility and responsiveness to the design of cell-surface interactions.<sup>3-6</sup> Installing non-natural supramolecular receptors and ligands on the exterior of cell membranes provides access to programmable cell behaviour.<sup>7-9</sup> In addition, cell adhesion can be controlled in space and time when the supramolecular interaction motifs are sensitive to remote stimuli.<sup>10-15</sup>

Several methods have been described to install non-native motifs on cell membranes without interfering with natural cell receptors and signalling, e.g. by engineering the cell genome,<sup>16</sup> by performing covalent chemistry on membrane proteins,<sup>17</sup> by inserting artificial lipids into the cellular lipid bilayer,<sup>1, 18-24</sup> or by metabolic labeling of the glycocalyx.<sup>25, 26</sup> For example, non-natural sugars containing a bio-orthogonal azido group<sup>27</sup> have been metabolically incorporated into the glycocalyx structure on the cell membrane, as depicted in Fig. 1. These azido-modified cell membranes were reacted with DNA strands via either strain-promoted azide-alkyne cycloaddition (SPAAC), copper(I)-catalyzed Huisgen azide-alkyne cycloaddition

(CuAAC) or Staudinger ligation. This elegant metabolic approach has enabled the site-specific assembly of living cells labeled with single DNA strands on surfaces displaying complementary DNA sequences,<sup>28, 29</sup> but also multiple cell types have been used to form microtissues with controlled stoichiometry.<sup>7</sup>



Fig. 1 At the top, schematic representation of a Jurkat cell modified via metabolic labeling and SPAAC to display naphthol moieties on the cell membrane. In the middle, the naphthol (guest) functionalized Jurkat cell assembles on a methylviologen (guest) bearing supported lipid bilayer in the presence of CB[8] (host) by the formation of multiple ternary host-guest complexes. At the bottom, structures of the molecules used in this study.

Although DNA-immobilization strategies hold great potential, the use of synthetic supramolecular host molecules provides access to cellular assemblies responsive to remote stimuli and avoids susceptibility to nucleases. Among the supramolecular

Molecular Nanofabrication Group, MESA+ Institute for Nanotechnology, Department of Science and Technology, University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands. E-mail: <u>j.huskens@utwente.nl</u> and

<sup>&</sup>lt;u>p.jonkheijm@utwente.nl</u>

<sup>+</sup> These authors contributed equally.

Electronic Supplementary Information (ESI) available: Synthetic procedures, cell preparation, live-death assay, flow cytometry data and calculations for ligands coverage and fabrication of SLBs. See DOI: 10.1039/x0xx00000x

Published on 06 May 2016. Downloaded by University of Wollongong on 09/05/2016 16:32:33

host molecules that are suitable to apply under physiological conditions are the pumpkin-shaped macrocyclic cucurbit[n]uril (CB[n]) host molecules.<sup>6, 30-32</sup> For example, we previously used CB[8] (n=8) as the host to anchor guest Trp-Gly-Gly-Arg-Gly-Asp-Ser (WGGRGDS) peptides via their N-terminal tryptophan surface-bound guest methylviologens as ternary to complexes.<sup>12</sup> Detachment of the cells that adhered to these supramolecular RGD surfaces occurred under electrochemical control. Clearly, this cellular self-assembly strategy relies on the ability to bind RGD ligands to their natural occurring integrin receptors on the cell membrane. This strategy is integrin-dependent and therefore lacks in flexibility when dealing with different cell types with different adhesiveness characteristics. Therefore strategies are required that install non-native supramolecular motifs on cell membranes that enable programmable assembly protocols orthogonal to natural cell receptor-ligand interactions. Very recently we have incorporated CB[8]-binding motifs at the bacterial surface by genetically modifying a transmembrane protein to form multiple intercellular ternary complexes leading to the assembly of bacteria.15

Here we report the installation of supramolecular CB[8]binding ligands on the cellular membrane using metabolic labeling and the subsequent CB[8]-mediated assembly on a supported lipid bilayer (SLB). To achieve specific CB[8]mediated cell adhesion, this receptor-independent strategy was combined with the use of a suspension cell line, such as blood cells. Supramolecular naphthol (Nph) guest moieties were introduced onto the membrane of white blood cells by using metabolic oligosaccharide engineering.<sup>29</sup> Administration of peracetylated N-azidoacetyl-p-mannosamine (MAN) to living Jurkat cells incorporates azido groups into the glycocalyx on the cell surface (Fig. 1). These azido-functionalized cells were then reacted with bicyclononyne-functionalized naphthols (NphBCN, for synthetic details see ESI) in a SPAAC reaction<sup>26</sup> to install the naphthol groups on the cell surface. Finally we show that specific cellular supramolecular interactions established by heteroternary complex formation between CB[8] and two different guests, i.e. SLB-bound methylviologen and cell surface-bound naphthol causing cellular assembly at surfaces. The metabolic introduction of azido groups and the progress of the subsequent reaction with bicyclononyne derivatives was validated using flow cytometry. Jurkat cells were incubated for 3 days with 50  $\mu M$  MAN in cell culture medium and subsequently, after washing, a PBS solution of 48 µM bicyclononyne functionalized fluorescein (fluoBCN) was added for 1 h at 37°C (see ESI for details) for a SPAAC reaction. Subsequently, the cells were washed and analyzed by flow cytometry (Fig. 2a-b). As controls, cells were reacted either with only MAN (condition +MAN-fluoBCN), or with only fluoBCN (condition -MAN+fluoBCN), or with neither of these two (condition -MAN-fluoBCN). All controls consistently showed low fluorescence intensities with respect to the condition when both MAN and fluoBCN (condition +MAN+fluoBCN) were present indicating that the reaction occurred specifically. Moreover, inspection of the cells that were treated with both MAN and fluoBCN using confocal

fluorescence microscopy revealed green emission that was localized on the cellular membrane (Fig. S11). These results confirm earlier findings that metabolic labeling procedures yield azido groups at the cell surface that can react with fluorescent molecules carrying complementary reactive groups for the SPAAC reaction. To verify the availability of functional groups after metabolic labeling and subsequent SPAAC, the change in mean fluorescent intensity in the flow cytometry data was followed as function of reaction time and storage time. After incubation of the cells with azido-functionalized mannose (MAN), cells were washed, kept in culture medium for one more day without MAN prior to SPAAC coupling with fluoBCN. Similarly, the azido-functionalized mannose was administered to cells and directly reacted with fluoBCN, and then kept in culture medium for an additional day without any reactant. In both cases, most of the fluorescence intensity was



lost, yet remained higher than the intensity of their

Fig. 2 Analysis of Jurkat cell surface functionalization after 3 day incubation with 50 µM MAN and subsequent SPAAC with fluoBCN. (a) Distributions of the green fluorescence intensity of cells labeled with 48 µM fluoBCN and controls as assessed by flow cytometry. (b) Average fluorescence intensities over three independent experiments is shown for all conditions: for each individual measurement the mean fluorescence intensity was normalized against a set of calibration beads (error bars = standard deviation) (c) FluoBCN concentration dependence of the fluorescence intensity of labeled Jurkat cells as measured by flow cytometry.

Next, we correlated the concentration of fluoBCN to the number of fluorophores on the cell. Flow cytometry (Fig. 2c) shows a linear trend of increasing fluorescence intensity of cells treated with increasing concentrations of fluoBCN in the range of 10 to 240  $\mu$ M. This result indicates that the number of supramolecular CB[8]-binding ligands on the cell membrane can be modulated. In fact, the number of molecules at the cell surface can be determined quantitatively (Fig. S14-S16). When using for example 50  $\mu$ M of MAN and 48  $\mu$ M of fluoBCN, the number of fluorescein groups per cell was determined to be 1.1\*10<sup>6</sup>, in agreement to values found in literature.<sup>7</sup> As a result this approach seems promising to understand the thermodynamic and kinetic parameters that control cell-cell

DOI: 10.1039/C6CC01693F

Journal Name

Published on 06 May 2016. Downloaded by University of Wollongong on 09/05/2016 16:32:33

#### Journal Name

and cell-surface interactions via defining the density of the supramolecular guest molecules on the cell surface.

We envisioned that such modified cells with artificial supramolecular naphthol guest moieties would interact in a multivalent fashion with a surface displaying methylviologen as complementary guest and CB[8] as the host that binds both Nph and MV groups in a ternary complex. We selected to introduce the complementary methylviologen guest moieties on a supported lipid bilayer to benefit from its excellent nonfouling characteristics as we have demonstrated previously in forming heteroternary complexes between methylviologen and azobenzene-modified sugars at the SLB as an example of cell-material interactions based on a naturally occurring receptor-ligand combination.<sup>33</sup>

SLBs were produced on glass from vesicles that adsorb, fuse and rupture, forming a supported lipid bilayer covering the surface. Vesicles were made of DOPC, a maleimide headgroup modified lipid (MCC, 0.5 mol%) and a DHPE lipid labeled with Texas Red (TR, 0.2 mol%) mixed in chloroform. After drying and rehydration in PBS buffer the vesicles were extruded through a polycarbonate membrane with 100 nm pore size (see SI for details and Fig. S17 for DLS results). To incorporate methylviologen into the vesicles, 100 µM of thiolfunctionalized methylviologen was added to a suspension of the maleimide-functionalized vesicles during the rehydration step. The pH of the rehydration buffer was set to 7.4, which is suitable for the Michael addition reaction to occur.<sup>34</sup> After reacting for 2 h at room temperature, the lipid suspension was extruded and the resulting vesicles were used for the formation of SLBs bearing MV groups. The presence of MV on the surface of these vesicles was verified using zeta potential measurements (Fig. 3). Vesicles incorporating the doubly positively charged MV moieties showed a positive potential. A negative potential originating from the negatively charged MCC and TR lipids was retained in a control experiment where paraguat, which bears no thiol, was used during the reaction. A decreased positive potential was detected after adding CB[8] to the MV-bearing vesicles indicative of complexation of CB[8] and MV, which would result in shielded positive charges. Together these results confirmed that methylviologen was incorporated in the vesicles and that complexation with CB[8] can occur.

To test whether the formation of multiple ternary complexes between MV:CB[8]:Nph would drive the selective adhesion of cells on the SLB, a flow channel setup was used (see ESI for details). Vesicles (0.1 mg/ml) bearing MV functionalities were prepared as described above and flown over the activated glass bottom of the PDMS flow channel. After extensively washing with PBS, a uniform SLB was formed and imaged by fluorescence microscopy (Fig. S19).

Jurkat cells were functionalized with naphthol ligands using the same procedure that was used to equip the Jurkat cells with fluorescein but now using NphBCN instead of fluoBCN. Prior to flowing the naphthol-functionalized cells, a solution of 20  $\mu$ M CB[8] was injected in the flow channel and allowed to interact with the methylviologen on the SLB via host-guest interactions. The formed CB[8]-methylviologen complex

constituted the artificial receptor for the naphtholfunctionalized Jurkat cells. These cells were then loaded in the flow channel in the presence

of 20  $\mu$ M CB[8]. In a period of 10 min without flow the Jurkat



Fig. 3 Zeta potential characterization of vesicles of DOPC:MCC:TR (99.3:0.5:0.2 mol%). On the left, a vesicle bearing MV before and after complexation by CB[8] is depicted



schematically. On the right, zeta potential results are shown for vesicles reacted with MV (vesicles + MV) and added of CB[8] (vesicles + MV + CB[8]). As control, vesicles which were not reacted with MV but which were still containing negatively charged MCC and TR were measured in the presence of paraquat (vesicles + paraquat). Error bars are standard deviations based on 3 measurements.

Fig. 4 Interaction of Nph-modified Jurkat cells on MV-modified SLBs in a flow channel device in the presence of 20  $\mu$ M CB[8] or 20  $\mu$ M CB[7]. Jurkat cells reacted with and without MAN, but both not with NphBCN, were introduced both in presence of 20  $\mu$ M CB[8]. Top panel, representative bright field images at increasing flow rates for the conditions indicated. Scale bars 200  $\mu$ m (inset: 40  $\mu$ m). Bottom panel, dependence of the number of immobilized cells on the applied flow rate. Between 300 and 700 cells were counted per condition using Imagel software in triplicate experiments.

cells settled on the SLB (Fig. 4). Subsequently, a low flow rate was applied and then stepwise increased while monitoring the adhesion of the cells in the chip using bright field microscopy

DOI: 10.1039/C6CC01693F

#### COMMUNICATION

(Error! Reference source not found.). As negative controls, the same procedure was applied but altered either by replacing the CB[8] with CB[7], or to have unmodified cells, or to have cells with MAN but for which the naphthol moieties were missing. Cells in all negative controls already started to disappear from the SLB at flow rates as low as 0.25  $\mu\text{l/min}$  and beyond a flow rate of 1 µl/min hardly any cells remained visible at the SLB (Error! Reference source not found.). On the contrary, in the presence of CB[8], cells displaying Nph ligands selectively adhered on the MV functionalities on the lipid bilayer and cells started to flow away from the SLB when flow rates were applied higher than 10 µl/min. Only beyond 100  $\mu$ l/min all of the initially adhered Jurkat cells could be flushed away. The results demonstrate that the display of CB[8]binding moieties that we introduced via metabolic labeling and strain promoted azide-alkyne cycloaddition seem sufficiently stable to provide supramolecular ligands at the cell surface within the time scale of the surface assembly, and that these ligands interact in a specific manner.

In conclusion, we presented a system that can simplify the investigation of cell/surface interactions through the use of synthetic ligands and receptors. Such a system provides a bioorthogonal modification of the cell surface independently from natural cell ligands and with numbers of supramolecular handles that can in principle be modulated. We have demonstrated the successful use of host/guest chemistry to introduce non-natural ligand/receptor interactions while it also enables the specific control over cell adhesion, possibly even on a spatial and temporal scale. This artificial yet biomimetic approach paves the way for quantifying the relation between the number of receptors and the forces involved in the supramolecular cell adhesion.

#### Notes and references

Published on 06 May 2016. Downloaded by University of Wollongong on 09/05/2016 16:32:33

§ We thank C. Breukers for assistance. PJ thanks support received through St-ERC grant (259183) and NWO-VIDI (723.012.106).

- 1. E. J. Lee, W. Luo, E. W. L. Chan and M. N. Yousaf, *PLoS ONE*, 2015, **10**.
- T. Satav, J. Huskens and P. Jonkheijm, *Small*, 2015, **11**, 5184-5199.
- 3. J. J. Reczek, A. A. Kennedy, B. T. Halbert and A. R. Urbach, J. *Am. Chem. Soc.*, 2009, **131**, 2408-2415.
- D. A. Uhlenheuer, K. Petkau and L. Brunsveld, Chem. Soc. Rev., 2010, 39, 2817-2826.
- K. Petkau-Milroy and L. Brunsveld, *Org. Biomol. Chem.*, 2013, 11, 219-232.
- J. Brinkmann, E. Cavatorta, S. Sankaran, B. Schmidt, J. Van Weerd and P. Jonkheijm, *Chem. Soc. Rev.*, 2014, 43, 4449-4469.
- Z. J. Gartner and C. R. Bertozzi, Proc. Natl. Acad. Sci. U. S. A., 2009, 106, 4606-4610.
- A. B. Rode, T. Endoh, H. Tateishi-Karimata, S. Takahashi and N. Sugimoto, *Chem. Commun.*, 2013, 49, 8444-8446.
- K. Vogel, M. Glettenberg, H. Schroeder and C. M. Niemeyer, Small, 2013, 9, 255-262.

- 10. Y. H. Gong, C. Li, J. Yang, H. Y. Wang, R. X. Zhuo and X. Z. Zhang, *Macromolecules*, 2011, **44**, 7499-7502.
- P. Neirynck, J. Brinkmann, Q. An, D. W. J. Van Der Schaft, L. G. Milroy, P. Jonkheijm and L. Brunsveld, *Chem. Commun.*, 2013, 49, 3679-3681.
- Q. An, J. Brinkmann, J. Huskens, S. Krabbenborg, J. De Boer and P. Jonkheijm, *Angew. Chem. Int. Ed.*, 2012, **51**, 12233-12237.
- a) J. Voskuhl, S. Sankaran and P. Jonkheijm, *Chem. Commun.*, 2014, **50**, 15144-15147. b) L. Yang, A. Gómez-Casado, J. F. Young, H. D. Nguyen, J. Cabanas-Danés, J. Huskens, L. Brunsveld, P. Jonkheijm, *J. Am. Chem. Soc.* 2012, **134**, 19199-19206.
- S. Gürbüz, M. Idris and D. Tuncel, *Org. Biomol. Chem.*, 2015, 13, 330-347.
- S. Sankaran, M. C. Kiren and P. Jonkheijm, ACS Nano, 2015, 9, 3579-3586.
- 16. Y. Peng, D. H. Kim, T. M. Jones, D. I. Ruiz and R. A. Lerner, *Angew. Chem. Int. Ed.*, 2013, **52**, 336-340.
- 17. P. Gong, W. Zheng, Z. Huang, W. Zhang, D. Xiao and X. Jiang, *Adv. Funct. Mater.*, 2013, **23**, 42-46.
- D. Dutta, A. Pulsipher, W. Luo, H. Mak and M. N. Yousaf, Bioconjugate Chem., 2011, 22, 2423-2433.
- N. S. Selden, M. E. Todhunter, N. Y. Jee, J. S. Liu, K. E. Broaders and Z. J. Gartner, J. Am. Chem. Soc., 2012, 134, 765-768.
- H. R. Zope, F. Versluis, A. Ordas, J. Voskuhl, H. P. Spaink and A. Kros, Angew. Chem. Int. Ed., 2013, 52, 14247-14251.
- K. Gabrielse, A. Gangar, N. Kumar, J. C. Lee, A. Fegan, J. J. Shen, Q. Li, D. Vallera and C. R. Wagner, *Angew. Chem. Int. Ed.*, 2014, 53, 5112-5116.
- W. Luo, A. Pulsipher, D. Dutta, B. M. Lamb and M. N. Yousaf, Sci. Rep., 2014, 4.
- 23. A. Pulsipher, D. Dutta, W. Luo and M. N. Yousaf, Angew. Chem. Int. Ed., 2014, **53**, 9487-9492.
- P. J. O'Brien, W. Luo, D. Rogozhnikov, J. Chen and M. N. Yousaf, *Bioconjugate Chem.*, 2015, 26, 1939-1949.
- 25. E. Saxon and C. R. Bertozzi, Science, 2000, 287, 2007-2010.
- J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. Van Hest, D. J. Lefeber, P. Friedl and F. L. Van Delft, *Angew. Chem. Int. Ed.*, 2010, 49, 9422-9425.
- 27. A. Borrmann and J. C. M. Van Hest, *Chemical Science*, 2014, 5, 2123-2134.
- R. A. Chandra, E. S. Douglas, R. A. Mathies, C. R. Bertozzi and M. B. Francis, *Angew. Chem. Int. Ed.*, 2006, **45**, 896-901.
- 29. S. T. Laughlin and C. R. Bertozzi, *Nat Protoc*, 2007, **2**, 2930-2944.
- S. J. Woo, E. Kim, H. K. Young, I. Hwang, W. L. Jae, S. Y. Kim, H. J. Kim and K. Kim, *Angew. Chem. Int. Ed.*, 2005, **44**, 87-91.
- J. Lagona, P. Mukhopadhyay, S. Chakrabarti and L. Isaacs, Angew. Chem. Int. Ed., 2005, 44, 4844-4870.
- 32. F. Tian, D. Jiao, F. Biedermann and O. A. Scherman, *Nat. Commun.*, 2012, **3**.
- a) S. Sankaran, J. Van Weerd, J. Voskuhl, M. Karperien and P. Jonkheijm, *Small*, 2015, **11**, 6187-6196. b) J. van Weerd, S. Sankaran, O. Roling, S. Sukas, S. Krabbenborg, J. Huskens, S. le Gac, B. J. Ravoo, M. Karperien and P. Jonkheijm, *Adv. Mater. Interf.* 2016, DOI: 10.1002/admi.201600055.
- 34. M. Brinkley, Bioconjugate Chem., 1992, 3, 2-13.