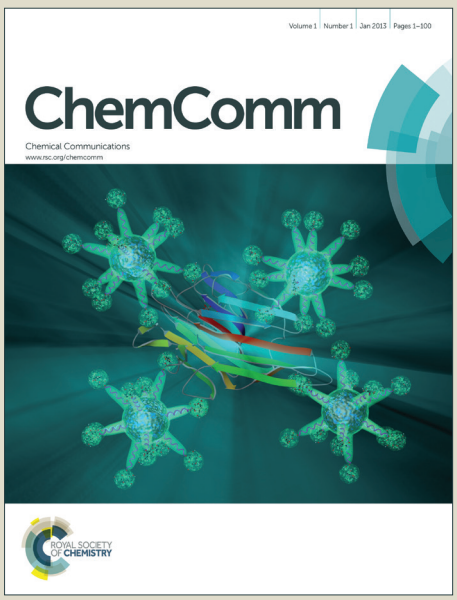
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Chem Commun

RSCPublishing

COMMUNICATION

Optical control over bioactive ligands at supramolecular surfaces

Cite this: DOI: 10.1039/x0xx00000x

J. Voskuhl‡,[a] S. Sankaran‡[a] and P. Jonkheijm*[a]

Received 00th January 2012,
Accepted 00th January 2012

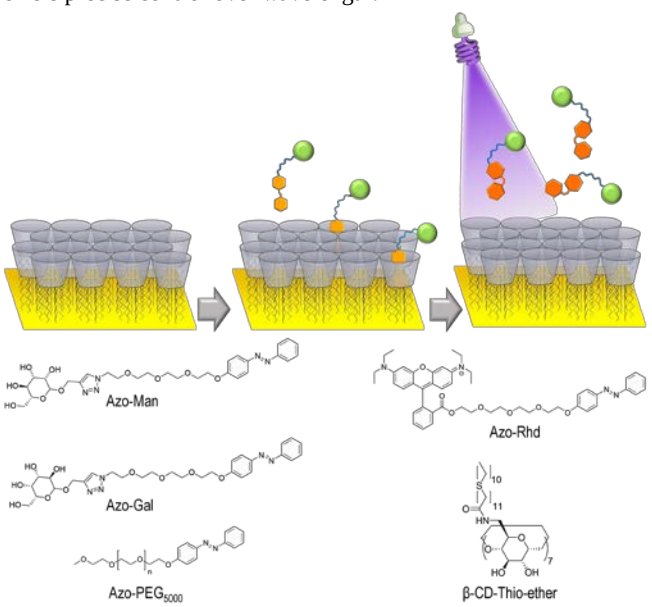
DOI: 10.1039/x0xx00000x

www.rsc.org/

In this communication we report for the first time the use of azobenzene modified glycoconjugates to establish optical control over bioactive ligands at a supramolecular β-cyclodextrin (β-CD) surface. Several studies were conducted to investigate the photoresponsive immobilization of proteins and bacteria on these supramolecular surfaces.

Dynamic arrays of bioactive ligands have recently attracted much interest since they enable real-time control over surface properties by employing external and remote triggers.[1] While arrays of biomolecules have been applied in various areas of biomedical and bioanalytical research,[2] stimuli responsive behaviour of these surfaces can easily be offered by supramolecular chemistry. Employing supramolecular chemistry not only delivers improved specificity, directionality and tunability of the interaction motifs and strengths but responsiveness of the presentation of bioactive ligands at surfaces can be achieved as well. [3]

Supramolecular bioactive surfaces have been recently developed that employ host molecules such as cucurbit[n]urils (CB[n])[4-6] and cyclodextrins.[7, 8] These barrel-like cyclic structures have the ability to encapsulate hydrophobic bulky guest molecules such as adamantane and ferrocene. Approaches to fabricate such supramolecular bioactive platforms involve attaching the host molecules directly to the surface by using covalent silane chemistry on glass[9, 10], thiol chemistry on gold[11, 12] or in the case of CBs, even coordinative chemistry between gold surfaces and the glycouril oxygens has been reported.[13] For example, we supramolecularly printed proteins bearing a ferrocene unit on β-CD monolayers to achieve electrochemical responsive protein arrays.[7, 14] Ferrocene, which binds as neutral species to CD, but does not in the oxidized state, was erased from printed arrays following an external electrochemical stimulus.[7] Alternatively, non-covalent strategies can be employed by fixing the guest molecules to the surface and allowing the host to bind from solution.[15] This strategy has been successfully utilized in the case of electro-responsive peptide surfaces based on CB[8][5, 6, 16] and some CD based systems.[17] Onto this type of supramolecular surfaces, we supramolecularly printed naphthalene and tryptophan modified biomolecules on methylviologen monolayers in the presence of CB[8].[5, 18] These surfaces were used for the supramolecular detachment of cells when RGD peptides were used following an external reductive potential.[5] Although redox-responsive supramolecular bioactive surfaces have been demonstrated to have important biomimetic potentialities, no photoresponsive CD based surface systems have been reported. Photosensitive host-guest complexes are of significant interest as light can be applied in a remote manner as an external stimulus and offers precise control over wavelength.



Scheme 1: Assembly of bioactive ligands with photoswitchable properties on a cyclodextrin monolayer. Given molecular structures are used in this study.

Here we report a novel approach on the use of highly ordered monolayers of β-CD on gold and glass surfaces to immobilize glycoconjugates via a photoresponsive azobenzene moiety. Azobenzenes are well known molecules in the field of supramolecular chemistry since they bear the opportunity to bind to CDs and CBs in the stable *trans* form and can be released from the cavity after UV-irradiation by conversion into the bulkier, more hydrophilic *cis*-azobenzene.[1a, 19-21] Although this behaviour has been successfully used in previous studies to fabricate photoresponsive supramolecular nanoparticles and polymers based on CB[8][22-24] as well as light responsive systems consisting of amphiphilic CDs to induce aggregation,[25] to transport DNA and proteins[26, 27] and to tune the wettability[28, 29] and to modulate the assembly of cells[30] and gels,[31] no previous report has demonstrated optical control over protein and bacterial immobilization on cyclodextrin surfaces. We address whole protein and bacterial immobilization and their optical control on the supramolecular platform, which is significantly more complex than previous photoresponsive supramolecular studies in solutions.

Herein, two glycoconjugates were synthesized bearing an azobenzene unit linked via a tetraethyleneglycol unit to either mannose or galactose (Scheme 1, Azo-Man and Azo-Gal). Mannose is known to bind carbohydrate specific lectins such as concanavalin A (ConA) and the well-known FimH receptor on pathogenic bacteria like *E.-Coli*. Furthermore, a fluorescently labelled azobenzene derivative bearing a rhodamine unit was synthesized (Scheme 1, Azo-Rhd) to visualize azobenzenes assembled onto β-CD surfaces. To measure the binding affinity of azobenzenes to the β-CD monolayer on gold, a titration series of Azo-Man over a range of 25 μM - 1 mM was performed using quartz crystal microbalance (QCM) at a flow rate of 100 μL/min followed by extensive washing with buffer (Fig. 1A). Binding events caused a drop in the resonance frequency of the crystal and the 5th harmonic was followed as it presented the best signal to noise ratio. In all QCM studies performed dissipation was also monitored, but the change appeared negligible. In order to determine the association constant, the values of the changes in frequency at saturation were plotted against concentrations of Azo-Man. Fitting using the Langmuir equation yielded a K_a value of 5.8×10^3 M^{-1} for binding of Azo-Man onto β-CD monolayers (Fig. 1B). This value favourably compares to the binding affinity of azobenzene to β-CD in solution, which was previously determined by isothermal titration calorimetry (ITC) in aqueous media (K_a = 2.4 $\times10^3$ M^{-1}).[26, 27] With this knowledge, the difference in binding between *cis* and *trans*- forms of Azo-Man (Fig. 1E) on the β-CD monolayer was investigated. To this end, a 250 μM solution of Azo-Man was irradiated with UV light (365 nm) for 10 min prior to injection in the flow chamber. As expected, a significant difference in frequency change was observed between the *cis*-and *trans*-forms of Azo-Man, signifying a lower affinity of the *cis*-Azo to the β-CD SAM. Since the photoisomerization efficiency is about 80% as determined from ^{1}H-NMR studies,[26] (see Electronic Supplementary Fig. S7) about 50 μM still remains in the *trans*-form which nicely accounts for the observed residual change in frequency signal of -6 (Fig. 1C). In a final QCM experiment, the binding of ConA onto β-CD SAMs bearing carbohydrate Azo-Man was investigated and using Azo-Gal as negative control. Flowing 1 mM solutions of glycoconjugates over a β-CD SAM on gold, led to asignificant change in frequency similar to what was seen in the previous measurements. Subsequently, a 50μg/mL solution of ConA was led over the carbohydrate bearing surfaces. In the case of the Azo-Man surface, this caused the frequency to dramatically change, corresponding to the adsorption of ConA onto this surface (Fig. 1D). The Azo-Gal control surface exhibited a much smaller QCM response showing that ConA binding occurs specifically to mannose. Washing with buffer resulted in dissociation of ConA, which occurred at a reduced rate compared to the association to Azo-Man. This observation is probably related to the tetrameric nature of ConA that could bind two mannose units on a flat surface. Consequently, this causes it to be able to bind to Azo-Man at the β-CD monolayer in a bivalent manner. Washing with an excess of (D)-mannose leads to a higher dissociation rate of the proteins from the Azo-Man surface, supporting the fact that binding occurs specifically.

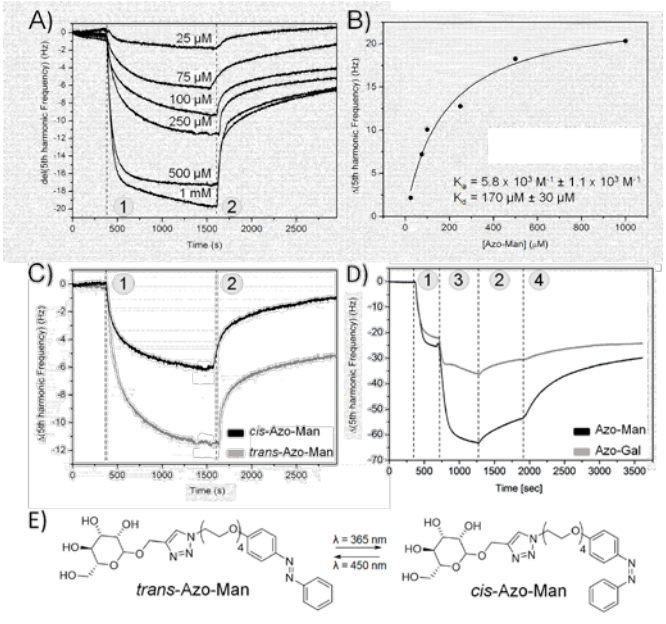


Fig. 1: A) QCM measurements of Azo-Man at varying concentrations on a β-CD-SAM. B) Binding curve of Azo-Man to β-CD-SAM. C) Differences in QCM response between *cis*-Azo-Man and *trans*-Azo-Man on a β-CD-SAM. D) Immobilization of ConA onto Azo-Man and Azo-Gal at β-CD-SAM. Legends: 1. Incubation with Azo-Man or Azo-Gal, 2. Washing with PBS, 3. Incubation with ConA, 4. Washing with (D)-Mannose. E) *trans-cis* isomerisation of Azo-Man.

To visualize the optical control over the assembly of bioactive molecules, microcontact printing was performed on β-CD modified glass substrates and imaged using epifluorescence microscopy. In a first experiment, a 500 μM Azo-Man solution mixed with 2.5 μM Azo-Rhd was printed using a PDMS-stamp. Following brief washing with water patterns of dots that are 100 μm in diameter with a spacing of 100 μm were clearly observed, as seen in Fig. 2A, indicating the successful immobilization of the inked molecule onto the β-CD surfaces. Next, Azo-Man was printed in lines on the β-CD surface and backfilled with protein repellent Azo-PEG$_{5000}$. The patterned substrate was then incubated with a fluorescein-conjugated ConA and washed briefly. This experiment produced clear patterns of immobilized ConA indicating the successful interplay of two orthogonal supramolecular interactions on the β-CD surface (Fig. 2B). As a next step, the suitability of these supramolecular Azo-Man surfaces for binding of bacterial cells was investigated. Two different strains of *E-coli* were selected that differ only in their mannose binding properties to assess whether the mannose-functionalized supramolecular surfaces can interact with *E-coli*. In addition to a strain that binds to mannose (ORN-178), a second strain (ORN-208) was used where its FimH receptor was mutated to eliminate mannose binding. In a first experiment, two β-CD surfaces with immobilized Azo-Man were separately incubated with the two bacterial strains. Both substrates were then incubated with Hoechst H33342 to stain the DNA in the bacteria. Representative images are shown in Fig. 2C and D. From these images it can be seen that only the ORN-178 strain was able to successfully interact with the

surface. The binding of the bacteria to Azo-Man was confirmed using QCM (see Electronic Supplementary Fig. S10). After washing the bacterial cells remained stably bound at the surface. In the case of ORN-208 a negligible number of surface-immobilized bacterial cells were observed, confirming the selectivity of the supramolecular system. Finally, we printed the Azo-Man in line patterns and backfilled the interspace with Azo-PEG_{5000} to avoid non-specific interactions. After DNA staining of ORN-178, clear line patterns of blue bacteria were observable (Fig. 2E) showing the possibility to assemble bacteria in patterns on Azo-Man surfaces.

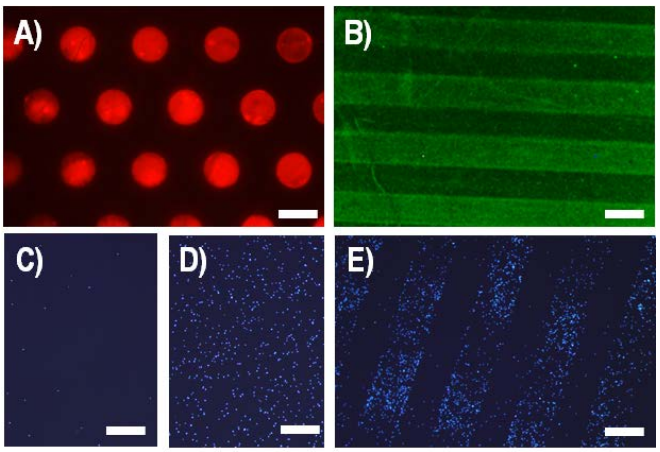


Fig. 2: Fluorescence microscopy images of surfaces where A) Azo-Man/Azo-Rhd (200/1) was printed on a β-CD SAM, Azo-Man was assembled on β-CD SAMs followed by incubation with B) fluorescein-conjugated ConA, C) *E-Coli* strain ORN-208, D) and E) *E-Coli* strain ORN-178. The scale bars represent 100 μm.

To assess the photo-responsiveness of the supramolecular system, patterns of Azo-Man mixed with 1% Azo-Rhd were printed on two β-CD modified glass substrates and imaged with a fluorescence microscope. Both surfaces were then placed in water for 5 mins with one substrate exposed to 365 nm UV light while the other substrate was kept in the dark. Fluorescence images were again recorded (Fig. 3A) and intensity profiles were made and averaged over a 7 mm^2

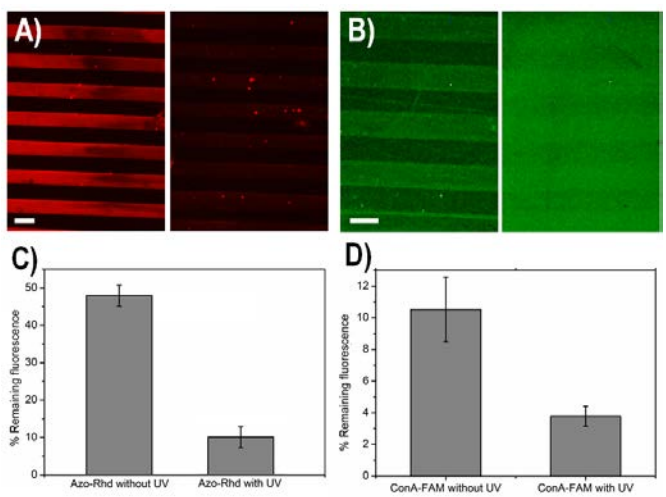


Fig. 3: A) Fluorescence microscopy image of Azo-Rhd immobilized on CD-SAMs before and after irradiation with UV-light. B) Fluorescein labeled ConA immobilized on Azo-Man before and after irradiation with UV light. C) Intensity profile of Azo-Rhd before and after irradiation with UV light. D) Intensity profile of fluorescein labeled ConA immobilized on Azo-Man before and before and after irradiation with UV light. Scale bars represent 100 μm.

area and plotted as percentage of the intensity prior to washing (Fig 3C). The substrate that was not irradiated with the 365 nm light retained nearly 50% of its original fluorescence intensity, which corresponds to the dissociation as observed in our QCM experiments. In strong contrast, the substrate exposed to the UV irradiation, lost almost all its original fluorescence intensity, which can be attributed to the photoisomerization of the azobenzene units. To extend the photo control of the supramolecular system towards ConA protein assembly, a similar experiment was conducted where printed Azo-Man was released from a β-CD surface using UV irradiation and backfilled with Azo-PEG_{5000}. A non-irradiated substrate was used as comparison. Fluorescein-conjugated ConA was then allowed to interact with these surfaces. Fluorescence microscopy images revealed clear line patterns on the non-irradiated surface and very faint patterns under high exposure conditions on the irradiated surface (Fig. 3B and D).

Conclusions

We have described a supramolecular approach for achieving for the first time optical control of biomolecules by an external light stimulus. We were able to successfully determine the binding affinity of glycoconjugates on β-cyclodextrin SAMs by QCM measurements. Furthermore, several fluorescence microscopy images showed the effective immobilization of azobenzene functionalized dyes and carbohydrates that can specifically interact with proteins and bacteria. Furthermore it was shown that azobenzene molecules can be released by an external light stimulus and modulate the assembly of ConA proteins. Taken into account that larger biological entities, such as bacteria, have about 200 FimH receptors on its membrane surface and that ca. 20% of the azobenzene moieties that has been used in this study remain in its *trans*-conformation after UV-light irradiation, a significant number of receptors would hold the binding of bacteria to the substrate. However, further optimization of the azobenzene modification, linker lengths and surface assembly strategy will broaden possible applications of our work. The current work bears the potential for further purposes such as analysis of surface bound proteins by ligands which can be removed under mild conditions.

Acknowledgement

PJ, SS and JV thank the St-ERC 259183 and ERC PoC 31015 for funding. Carmen Stoffelen is acknowledged for supply of the azobenzene-polyethylene conjugate.

Notes and references

‡ Both authors contributed equally to this publication

[a] *Laboratory Group Bioinspired Molecular Engineering, MESA+ Institute for Nanotechnology, Department of Science and Technology, University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands. *Corresponding author: E-mail: p.jonkheijm@utwente.nl; www.jonkheijm.org*

† Electronic Supplementary Information (ESI) available: Details of synthetic procedures, monolayer studies and QCM experiments. See DOI: 10.1039/c000000x/

[1] a) J. Brinkmann, E. Cavatorta, S. Sankaran, B. Schmidt, J. van Weerd, P. Jonkheijm, *Chem. Soc. Rev.*, 2014, DOI: 10.1039/C4CS00034J. b) J. Robertus, W.R. Browne, B.L. Feringa, *Chem. Soc. Rev.*, 2010, **39**, 354-378. c) P. Mendes, *Chem. Soc. Rev.*, 2008, **37**, 2512-2519.

[2] J. Voskuhl, J. Brinkmann, P. Jonkheijm, *Curr. Op. Chem. Biol.*, 2014, **18**, 1–7.

[3] K. Petkau-Milroy, M.H. Sonntag, L. Brunsveld, *Chem. Eur. J.*, 2013, **19**, 10786-10793.
[4] P. Neirynck, J. Brinkmann, Q. An, D.W.J. van der Schaft, L.-G. Milroy, P. Jonkheijm, L. Brunsveld, *Chem. Commun.*, 2013, **49**, 3679-3681.
[5] Q. An, J. Brinkmann, J. Huskens, S. Krabbenborg, J. de Boer, P. Jonkheijm, *Angew. Chem. Int Ed*, 2012, **51**, 12233-12337.
[6] F. Tian, M. Cziferszky, D. Jiao, K. Wahlström, Jin Geng, O.A. Scherman, *Langmuir*, **2011**, ***27***, 1387-1390.
[7] L. Yang, A. Gomez-Casado, J.F. Young, H.D. Nguyen, J. Cabanas-Danés, J. Huskens, L. Brunsveld, P. Jonkheijm *J. Am. Chem. Soc.*, **2012**, ***134***, 19199-19206.
[8] D. Thakar, L. Coche-Guérente, M. Claron, C.H.F. Wenk, J. Dejeu, P. Dumy, P. Labbé, D. Boturyn, *ChemBioChem* 2014, **15**, 377-381.
[9] S. Onclin, J. Huskens, B.J. Ravoo, D.N. Reinhoudt, *Small*, 2005, **1**, 852-857.
[10] A. Perl, A. Gomez-Casado, D. Thompson, H. H. Dam, P. Jonkheijm, D. N. Reinhoudt, J. Huskens, *Nat. Chem.*, 2011, **3**, 317-322.
[11] J.M. Campiña, A. Martins, F. Silva, *Electrochim. Acta*, 2009. **55**, 90-103.
[12] C.A. Nijhuis, F. Yu, W. Knoll, J. Huskens, D.N. Reinhoudt, *Langmuir*, 2005, **21**, 7866-7876.
[13] Q. An, G. Li, C. Tao, Y. Li, Y. Wu, W. Zhang, *Chem. Commun.* 2008, 1989-1991.
[14] D. Wasserberg, D.A. Uhlenheuer, P. Neirynck, J. Cabanas-Danés, J. H. Schenkel, B.J. Ravoo, Q. An, J. Huskens, L.-G. Milroy, L. Brunsveld, P. Jonkheijm, *Int. J. Mol. Sci*. 2013, **14**, 4066-4080.
[15] X.-J. Shi, G.-J. Chen, Y.-W. Wang, L. Yuan, Q. Zhang , David M. Haddleton, H. Chen, *Langmuir*, **2013**, ***29*** , 14188-14195.
[16] F. Tian , N. Cheng , N. Nouvel , J. Geng, O.A. Scherman, *Langmuir*, 2010, ***26***, 5323-5328.
[17] O. Roling, A. Mardyukov, J.A. Krings, A. Studer, B.J. Ravoo, *Macromolecules*, **2014**, DOI: 10.1021/ma500043b.
[18] A. González-Campo, M. Brasch, D.A. Uhlenheuer, A. Gómez-Casado, L. Yang, L. Brunsveld, J. Huskens, P. Jonkheijm, *Langmuir*, 2012, ***28***, 16364-16371.
[19] F. Tian, D. Jiao, F. Biedermann, O.A. Scherman, *Nat. Commun.*, 2012, **3**, 1207.
[20] P. Bortolus, S. Monti, *J. Phys. Chem.* 1987, **91**, 5046-5050
[21] T. Fenske, H.-G. Korth, A. Mohr, C. Schmuck, *Chem. Eur. J.*, 2011, **18**, 738-755.
[22] C. Stoffelen, J. Voskuhl, P. Jonkheijm, J. Huskens, *Angew. Chem. Int Ed*., 2014, **13**, 3400-3404.
[23] Y. Lan, Y. Wu, A. Karas, O.A. Scherman, *Angew. Chem. Int. Ed.*, 2014, **53**, 2166-2169.
[24] J. del Barrio, P.N. Horton, D. Lairez, G.O. Lloyd, C. Toprakcioglu, O.A. Scherman, *J. Am. Chem. Soc.*, 2013, **32**, 11760-11763.
[25] S.K.M. Nalluri, B.J. Ravoo, *Angew. Chem. Int. Ed.*, 2010, **49,** 5371-5374.
[26] S.K.M. Nalluri, J. Voskuhl. J.B. Bultema, E.J. Boekema, B.J. Ravoo, *Angew. Chem. Int. Ed*., 2011, **50**, 9747-9751.
[27] A. Samanta, M.C.A Stuart, B.J. Ravoo, *J. Am. Chem. Soc*., 2012, **134**, 19909−19914
[28] P.B. Wan, Y.G. Jiang, Y.P. Wang, Z.Q. Wang, X. Zhang, *Chem. Commun*., 2008, **44**, 5710-5712.
[29] Y.G. Jiang, P.B. Wan, M. Smet, Z.Q. Wang, X. Zhang, *Adv. Mater.*, 2008, **20**, 1972-1977.
[30 Y.-H. Gong, C. Li, J. Yang, H.-Y. Wang, R.-X. Zhuo, X.-Z. Zhang, *Macromolecules*, 2011, **44**, 7499–7502
[31] H. Yamaguchi, Y. Kobayashi, R. Kobayashi, Y. Takashima, A. Hashidzume, A. Harada, *Nat. Commun.*, 2012, **3**, 603.

