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Nabila Laroui, Maëva Coste, Dandan Su, Lamiaa Ali, Yannick Bessin, et al.. Cell-selective siRNA delivery using glycosylated dynamic covalent polymers self-assembled in situ by RNA-templating. *Angewandte Chemie International Edition*, 2021, 60 (11), pp.5783-5787. 10.1002/anie.202014066 . hal-03113520

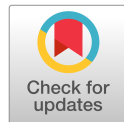
HAL Id: hal-03113520

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A Journal of the Gesellschaft Deutscher Chemiker

Angewandte Chemie

GDCh

International Edition

www.angewandte.org

Accepted Article

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This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.202014066

Link to VoR: <https://doi.org/10.1002/anie.202014066>

Cell-selective siRNA delivery using glycosylated dynamic covalent polymers self-assembled *in situ* by RNA-templating

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Dedicated to Prof. Eric T. Kool on the occasion of his 60th birthday.

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Abstract: Dynamic covalent libraries enable exploring complex chemical systems from which bioactive assemblies can adaptively emerge through template effects. In this work, we studied dynamic covalent libraries made of complementary bifunctional cationic peptides, yielding a diversity of species from macrocycles to polymers. Although polymers are typically expressed only at high concentration, we found that siRNA act as a template in the formation of dynamic covalent polymers at low concentration in a process guided by electrostatic binding. Using a glycosylated building block, we were able to show that this templated polymerization further translates into the multivalent presentation of carbohydrate ligands, which subsequently promotes cell uptake, and even cell-selective siRNA delivery.

Dynamic Covalent Libraries (DCLs) result from the reversible covalent association of complementary building blocks.^[1] DCLs are constitutionally complex chemical systems, colloquially called “mixtures”, from which networks, bioactive assemblies, and even life-like behaviors can all emerge spontaneously.^[2] The past few years have witnessed a growing interest in using DCLs in delivery applications, in particular for finding smart delivery vectors that self-assemble *in situ* and are capable of adaptation throughout the delivery process.^[3]

DCLs made of bifunctional building blocks can generate macrocycles (“rings”) or polymers (“chains”), and quite often those two species co-exist. However, such ring-chain equilibrium is strongly affected by concentration.^[4] It is well-known that high concentrations (≥ 50 mM) favor chains (Dynamic Covalent Polymers,^[5] DCPs),^[6] while low concentrations favor rings.^[7] Rare examples of *in situ* polymerization in aqueous media take place at low concentration. Rao and co-workers used rigid monomers to achieve *in situ* polymerization with 80% conversion into polymers at concentration as low as 0.1 mM.^[8] Template effects, a hallmark of supramolecular chemistry, can affect ring-chain equilibria by increasing the local concentration of pre-associated monomers. Previous reports have shown that nucleic acids (NA) can act as templates and trigger a templated polymerization.^[9]

RNA interference therapy uses small interfering RNA (siRNA) which can be delivered into cells by cationic DCPs.^[10] However, despite two recent approvals (Onpatro[®], Givlaari[®]) and continuous progresses,^[11] the targeted delivery of siRNA remains a critical issue. In this context, we have evidenced constitutional adaptation of vectors in the presence of NA,^[12] but few examples discussed how NA templates can affect ring-chain equilibria. Bouteiller, Sollogoub and co-workers described a supramolecular system based on capped β -cyclodextrins that undergoes DNA-templated cooperative supramolecular polymerization by preventing self-inclusion of the heterobifunctional monomer.^[13] siRNA complexation and delivery was shown at a monomer concentration as low as 0.4 mM. Using amphiphilic dendrimers, Peng and co-workers have also reported that siRNA templates supramolecular assemblies.^[14] However, Aida and co-workers have provided the only example, followed by others,^[15] of a dynamic covalent system that undergoes siRNA-templated polymerization.^[16] The system is based on homobifunctional dithiol monomers bearing guanidinium groups that lead to cationic polydisulfides through a siRNA-templated oxidative polymerization, promoted by KI/I₂, occurring at a monomer concentration of about 0.9 mM. While siRNA activity using those DCPs rivalled the transfection reagent DharmaFECT, reaching about 30-35% luciferase activity, the amount of siRNA taken up was five-fold smaller. Conjugation to oligoarginines (Arg₆) provided a significant improvement, suggesting that a limitation of such electrostatically-driven siRNA-templated polymerization arises from the resulting polyplexes lacking an excess of cationic groups which is often beneficial for triggering cell uptake but unfortunately can elicit cytotoxicity as well. We hypothesize that a multivalent display of carbohydrate ligands such as *D*-mannoses onto DCPs could improve the cell uptake, possibly in a selective manner, by a receptor-mediated internalization pathway.

The mannose receptors (MR) are transmembrane proteins belonging to the C-type lectin superfamily of carbohydrate-binding receptors.^[17] While they bind single sugars with low affinity, they are capable of clustering and thus susceptible to multivalent binding effects.^[18] MR are interesting targets because they participate in endocytosis,^[17] being able to activate

the cell uptake of nanoparticles, especially in cancer cells where they can be over-expressed.^[19] Glycosylation has been proposed to regulate the internalization of vectors,^[20] but, to date, there are only two reports on glycosylated DCPs.^[21]

Herein, we report on DCLs made of two complementary bifunctional cationic peptides (Figure 1). We show that siRNA-

templated polymerization take place at low concentration, yielding DCPs through a poly-condensation process. Using a clickable building block appended with *D*-mannose, we show that the siRNA-templated polymerization translates into a multivalent presentation of *D*-mannoses that promote cell uptake and cell-selective siRNA delivery.

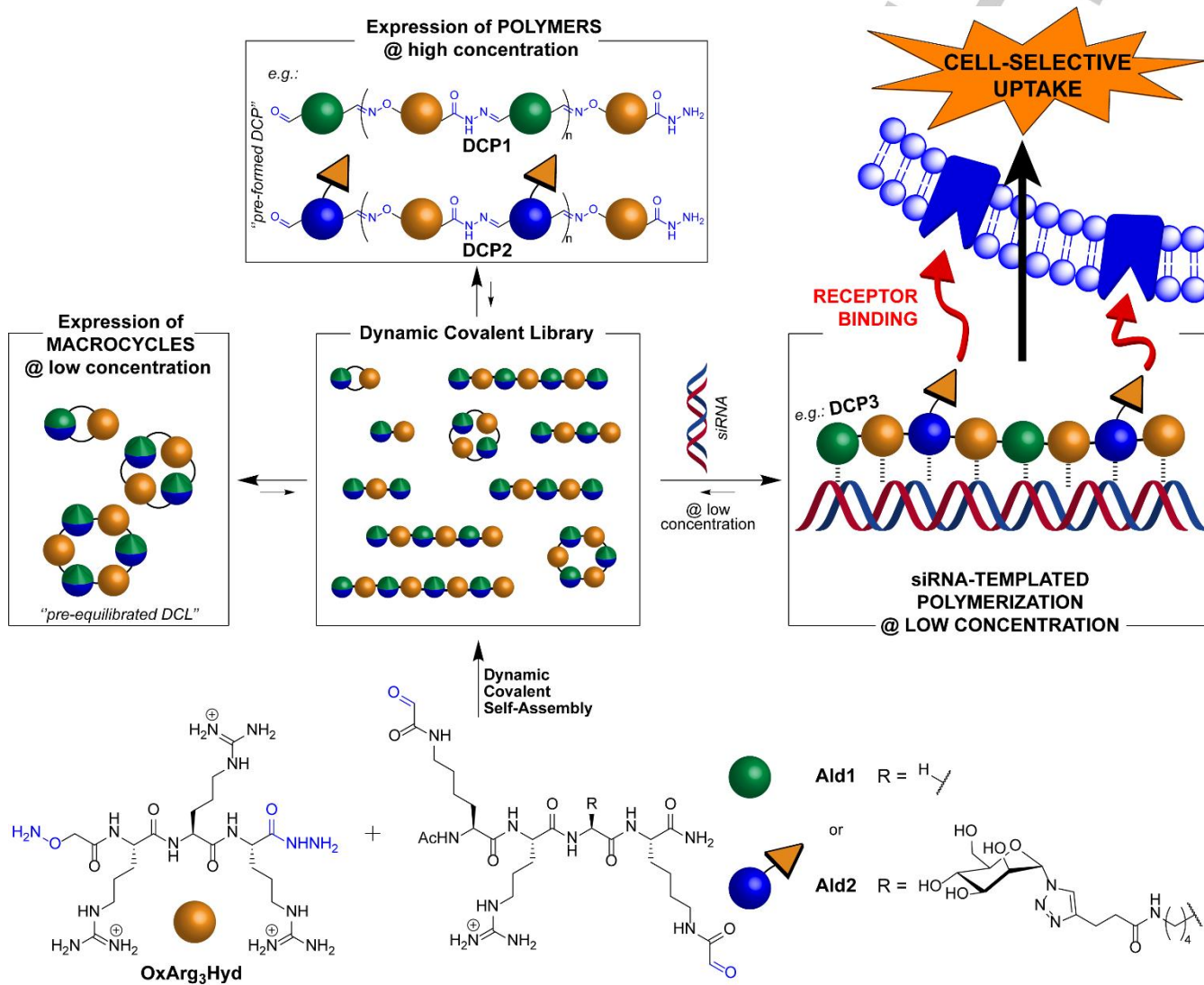


Figure 1. Representation of the dynamic covalent self-assembly of **Ald1** and **Ald2** with **OxArg₃Hyd** that produces dynamic covalent libraries (DCLs; **DCL1**: **Ald1** + **OxArg₃Hyd**; **DCL2**: **Ald2** + **OxArg₃Hyd**; **DCL3**: **Ald1** (0.8 equiv.) + **Ald2** (0.2 equiv.) + **OxArg₃Hyd**) from which macrocycles or dynamic covalent polymers (DCPs) can be expressed depending on the concentration or the presence of a nucleic acid template. Herein we demonstrate that the siRNA-templated polymerization results in a multivalent presentation of *D*-mannoses that trigger cell-selective uptake.

The building blocks **Ald1**, **Ald2**, and **Ox-Arg₃Hyd** consist in modified cationic peptides and were obtained through solid-phase peptide synthesis (see Supporting Information). While **Ald1** is a non-functionalized bisaldehyde, **Ald2** bears a *D*-mannose. **Ox-Arg₃Hyd** is a complementary building block, made of three *L*-arginines, with hydrazide and aminoxy groups at, respectively, the C and N termini. Our previous work showed that such cationic peptide-based building blocks lead, through acylhydrazone and oxime ligations, to macrocycles or DCPs at, respectively, low and high concentrations – the latter being

oligomers (DP \approx 7-10) that are able to complex DNA through multivalent binding and deliver siRNA.^[7, 22]

We tested *in situ* siRNA complexation by mixing, at room temperature, **Ald1** and **Ox-Arg₃Hyd** at low concentration (0.4 mM of each), in the presence of siRNA. The formation of this **DCL1** was carried out in aqueous buffer at pH = 5.5 (100 mM AcONa), which is optimal for both oxime and acylhydrazone ligations with glyoxylic aldehydes, and different N/P ratios^[23] were screened. After an initial overnight incubation, we observed, by gel electrophoresis, a complete complexation of siRNA at N/P = 20 (Figure 2A, left). The **DCL2** formed at the same low

concentration (0.4 mM) by combining **Ald2** and **Ox-Arg₃-Hyd** displayed a similar behavior, with a slightly worse degree of complexation at N/P = 10 (Figure 2A, right). Kinetic monitoring showed that complete complexation is reached 30 min after mixing (Figure 2B). The presence of an excess of methoxyamine (MeONH₂) in the **DCL1** completely suppresses siRNA complexation, pointing out the role of condensation reactions (Figure 2C). To elucidate whether macrocycles or polymers are responsible for siRNA complexation, we pre-formed **DCP1** at high concentration (50 mM) and tested siRNA complexation which was complete at N/P = 20 (Figure 2D). When testing **DCL1** at low concentration (0.4 mM) in the absence of siRNA ("pre-equilibrated **DCL1**"), only a partial complexation was initially observed (Figure 2D), showing that the constitution expressed at low concentration is less active towards siRNA complexation than the one formed at high concentration – in other words macrocycles are less effective than DCPs. However, a complete complexation was observed at longer incubation times (≥ 16 h) (Figure 2D), suggesting that constitutional adaptation take place in the presence of siRNA, by rearranging macrocycles into DCPs. These results show that **DCP1** and **DCP2** are the active components of the DCLs for siRNA complexation and that their expression results from a templated polymerization (Figure 1).

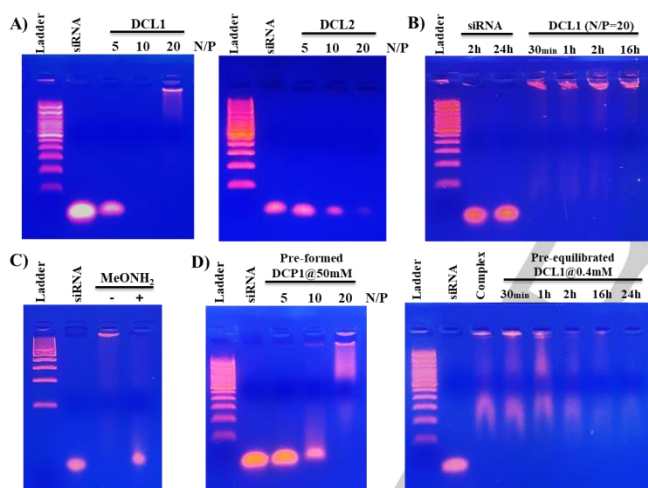


Figure 2. Gel electrophoresis analysis of siRNA complexation: A) by **DCL1** (left) and **DCL2** (right) formed *in situ* at low concentration in the presence of siRNA – N/P = 5, 10, and 20 tested; B) kinetic monitoring of siRNA complexation at N/P = 20 by **DCL1** formed *in situ* at low concentration; C) by **DCL1** (N/P = 20, 30 min incubation) in the absence (-) and in the presence (+) of methoxyamine (67 mM, 170 equivalents, with respect to **Ald1**); D) by pre-formed **DCP1** at N/P = 5, 10, and 20 (left), and kinetic monitoring of siRNA complexation (N/P = 20) by pre-equilibrated **DCL1** (low concentration (0.4 mM) in the absence of siRNA) (right).

Nanoparticles were observed by dynamic light scattering (DLS) when mixing pre-formed **DCP1** with siRNA (190.9 nm), the aforementioned siRNA-templated **DCL1** and the self-adapted **DCL1** (pre-equilibrated **DCL1** incubated with siRNA for 16 hours) both giving nanoparticles of similar and larger size (respectively, 387.3 nm and 319.0 nm), which further prove the ability of **DCL1**, and especially of **DCP1**, to complex siRNA (Table 1 and Figure S10). The polyplex made from pre-formed **DCP1** gave a positive ζ -potential (20.9 mV) while the siRNA-

templated and self-adapted **DCL1** tend to neutral values (1.25 and 2.49 mV, respectively), indicating that electrostatic interactions are likely the main driving force for polyplex formation and that template effects operate until charge neutralization (Table 1). Considering the number of guanidinium groups in each building block and the fact that each siRNA strand is 21-mer, we expect that oligomers of DP ≈ 10 are formed, which is in line with the DP previously observed for this class of DCPs,^[7, 22] and also consistent with the observation of Aida and co-workers that hexamers of their tetra-guanidylated monomers were formed by siRNA-templated polymerization.^[16]

Table 1. Characterization of polyplexes (N/P = 20) by dynamic light scattering and ζ -potential measurements.

Compounds	Size \pm SD (nm)	PDI \pm SD	ζ -potential \pm SD (mV)
siRNA	n.d.	n.d.	-26.9 \pm 1.3
DCL1 ^[a]	n.d.	n.d.	11.9 \pm 1.6
Pre-formed DCP1 ^[b]	190.9 \pm 2.6	0.27 \pm 0.01	20.9 \pm 5.7
siRNA-templated DCL1 ^[c]	387.3 \pm 92.8	0.39 \pm 0.07	1.25 \pm 0.59
Pre-equilibrated DCL1 ^[c]	319.0 \pm 64.0	0.41 \pm 0.1	2.49 \pm 0.89

[a] without siRNA; [b] from **DCL1** prepared at 50 mM, 30 min incubation with siRNA; [c] incubation 16 hours in the presence of siRNA. SD: standard deviation; PDI: polydispersity index.

The cellular activity of DCPs, made from siRNA-templated DCLs as described above, was assessed through knock-down experiments of luciferase activity on HCT-116-Luc colorectal cancer cells which overexpress the mannose receptors.^[19a] Unfortunately, neither **DCP1** nor **DCP2** showed activity. We hypothesized that the density of *D*-mannose could be too high in **DCP2** and could hinder siRNA complexation and/or receptor-binding – a mannose density of 21% being best for promoting cell uptake using polymeric nanoparticles.^[24] The copolymer **DCP3** was prepared from a siRNA-templated DCL made of 1 equiv. **Ox-Arg₃-Hyd**, 0.8 equiv. **Ald1** and 0.2 equiv. **Ald2**, and a dose-dependent silencing of luciferase activity was observed, down to 47% luciferase activity at a siRNA concentration much lower (0.2 vs. 5 μ M) than in a previous study reporting similar activity (Figure 3).^[13] Moreover, no significant cytotoxicity was observed in these conditions, which contrast with the more effective but cytotoxic Lipofectamine RNAiMax (Figure 3). In contrast, no siRNA activity was achieved using **DCP2** and **DCP3** on MCF-7 human breast adenocarcinoma cells which do not overexpress the MR (Figure S11).^[19a]

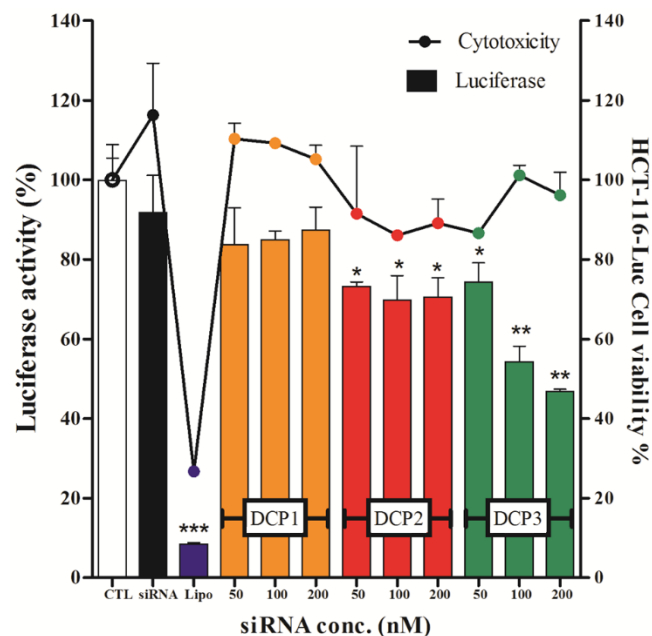


Figure 3. Knock-down of luciferase activity by DCPs/siLuc on HCT-116-Luc colorectal cancer cells (bars), and cell viability (dots). DCPs are made *in situ* from siRNA-templated DCLs. White bar: HCT-116-Luc cells alone; black bar: cells incubated with siLuc alone at 100 nM; colored bars: cells transfected with control lipoplex (Lipofectamine RNAiMax/siLuc at 50nM concentration of siRNA), DCPs/siLuc at N/P = 20 at various siLuc concentrations (50, 100 and 200 nM) and DCPs concentrations (10, 21 and 42 μ M, respectively). The luciferase activity was adjusted by cell viability. Experiments were done in triplicates (means \pm SEM), * p < 0.05, ** p < 0.005, *** p < 0.0005.

Confocal fluorescence microscopy showed that **DCP3** promotes cell uptake of a cy5-labelled siRNA (siCY5) in HCT-116 cancer cells (Figure 4A). A competition experiment using an excess of *D*-mannose completely suppressed cell uptake, showing the key role of the interaction with MR (Figure 4B). Interestingly, cell uptake was much weaker on MCF-7 cells (Figure S12). An overlap with LysoTracker[®] Green was seen, indicating the presence of the polyplex in the endolysosomal compartments and suggesting an endocytotic internalization pathway (Figure 4A). While this endolysosomal trapping can limit siRNA activity, it has been shown that endosomal escape at low efficiency (1-2%) can be sufficient to trigger a significant siRNA effect.^[25] Overall, these results confirm that **DCP3** is an effective delivery vehicle of siRNA displaying improved uptake and cell-selective delivery thanks to a receptor-mediated internalization.

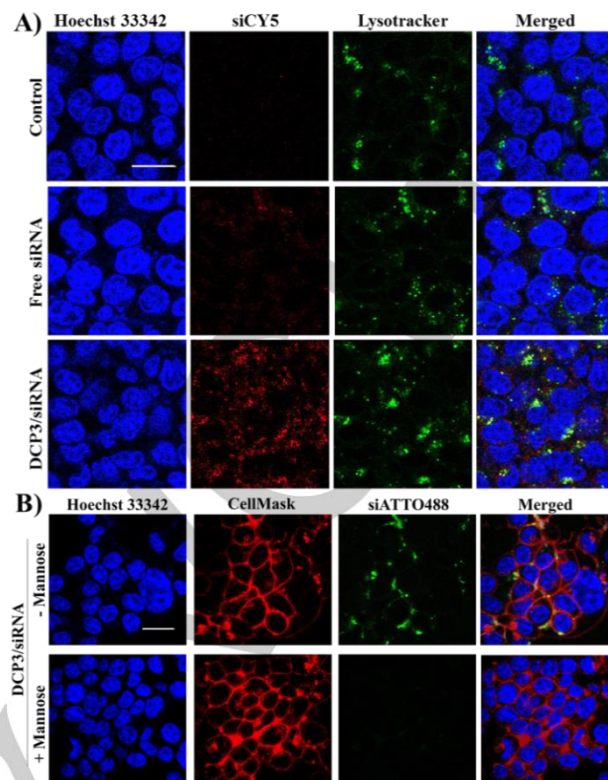


Figure 4. Cell uptake of siRNA in HCT-116 cancer cells visualized by confocal fluorescence microscopy: A) uptake of red stained siCY5 alone and complexed by **DCP3**, and co-localization with LysoTracker[®]; B) competition experiments of siATTO488 (100 nM) delivery by **DCP3** in absence (-) or in presence (+) of excess *D*-mannose (40 mM). Scale bars: 20 μ m.

In conclusion, we described a new generation of DCPs made of clickable cationic peptides that can be expressed *in situ* by siRNA templating. By clicking a *D*-mannose group onto one peptide building block, we showed that the corresponding siRNA-templated DCPs promoted cell-uptake through binding to mannose receptors. Finally, cell-selective siRNA delivery in cell lines that over-express the MR was achieved using **DCP3**. We believe that the versatility of this templated self-assembly approach coupled to the ability to click functional groups will unleash wider perspectives toward self-fitted DCP-based vectors of nucleic acids.

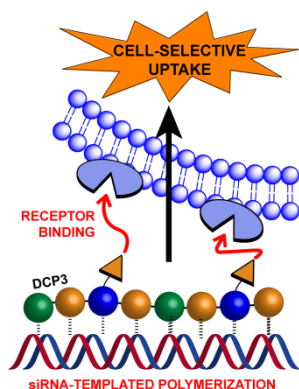
Acknowledgements

We thank the ANR (ANR-17-CE07-0042-01), the LabEx CheMISyst (ANR-10-LABX-05-01) and the Ligue Nationale Contre le Cancer, Comité de l'Hérault, for funding. NL thanks the Ministère Algérien de l'Enseignement Supérieur et de la Recherche Scientifique for a PhD fellowship. We thank Montpellier RIO Imaging platform (ANR-10-INBS-04), member of FranceBioImaging, for imaging facilities, and IRCM Cell culture unit (INCa-DGOS Inserm 6045) for providing HCT-116-Luc cells.

Keywords: Dynamic covalent polymer • siRNA delivery • mannose targeting • template effects • self-assembly

- [1] a) W. Zhang, Y. Jin, *Dynamic Covalent Chemistry: Principles, Reactions, and Applications*, Wiley-VCH, Weinheim, **2017**; b) Y. H. Jin, C. Yu, R. J. Denman, W. Zhang, *Chem. Soc. Rev.* **2013**, *42*, 6634-6654; c) F. B. L. Cougnon, J. K. M. Sanders, *Acc. Chem. Res.* **2012**, *45*, 2211-2221; d) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J. L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652-3711; e) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, J. F. Stoddart, *Angew. Chem. Int. Ed.* **2002**, *41*, 898-952.
- [2] a) J. Solà, C. Jimeno, I. Alfonso, *Chem. Commun.* **2020**, DOI: 10.1039/D1030CC04170J; b) J. M. Lehn, *Angew. Chem. Int. Ed.* **2015**, *54*, 3276-3289; c) J. M. Lehn, *Angew. Chem. Int. Ed.* **2013**, *52*, 2836-2850; d) J. W. Li, P. Nowak, S. Otto, *J. Am. Chem. Soc.* **2013**, *135*, 9222-9239; e) S. Otto, *Acc. Chem. Res.* **2012**, *45*, 2200-2210; f) N. Giuseppone, *Acc. Chem. Res.* **2012**, *45*, 2178-2188; g) R. A. R. Hunt, S. Otto, *Chem. Commun.* **2011**, *47*, 847-858.
- [3] a) J. Lopez-Andarias, J. Saabach, D. Moreau, Y. Y. Cheng, E. Derivery, Q. Laurent, M. Gonzalez-Gaitan, N. Winssinger, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2020**, *142*, 4784-4792; b) Y. Y. Cheng, L. L. Zong, J. Lopez-Andarias, E. Bartolami, Y. Okamoto, T. R. Ward, N. Sakai, S. Matile, *Angew. Chem. Int. Ed.* **2019**, *58*, 9522-9526; c) S. Ulrich, *Acc. Chem. Res.* **2019**, *52*, 510-519.
- [4] H. Jacobson, W. H. Stockmayer, *J. Chem. Phys.* **1950**, *18*, 1600-1606.
- [5] a) Y. Liu, J. M. Lehn, A. K. H. Hirsch, *Acc. Chem. Res.* **2017**, *50*, 376-386; b) F. Garcia, M. M. J. Smulders, *J. Polym. Sci. Pol. Chem.* **2016**, *54*, 3551-3577; c) N. Roy, B. Bruchmann, J. M. Lehn, *Chem. Soc. Rev.* **2015**, *44*, 3786-3807.
- [6] a) G. S. Pulcu, N. S. Galenkamp, Y. J. Qing, G. Gasparini, E. Mikhailova, S. Matile, H. Bayley, *J. Am. Chem. Soc.* **2019**, *141*, 12444-12447; b) G. Gasparini, E. K. Bang, G. Molinard, D. V. Tulumello, S. Ward, S. O. Kelley, A. Roux, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2014**, *136*, 6069-6074; c) E. K. Bang, G. Gasparini, G. Molinard, A. Roux, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2013**, *135*, 2088-2091.
- [7] C. Bouillon, D. Paolantoni, J. C. Rote, Y. Bessin, L. W. Peterson, P. Dumy, S. Ulrich, *Chem. Eur. J.* **2014**, *20*, 14705-14714.
- [8] L. Cui, S. Vivona, B. R. Smith, S.-R. Kothapalli, J. Liu, X. Ma, Z. Chen, M. Taylor, P. H. Kierstead, J. M. J. Fréchet, S. S. Gambhir, J. Rao, *J. Am. Chem. Soc.* **2020**, *142*, 15575-15584.
- [9] M. Surin, S. Ulrich, *ChemistryOpen* **2020**, *9*, 480-498.
- [10] a) Y. Zhang, Y. Qi, S. Ulrich, M. Barboiu, O. Ramström, *Mater. Chem. Front.* **2020**, *4*, 489-506; b) D. Su, M. Coste, A. Diaconu, M. Barboiu, S. Ulrich, *J. Mater. Chem. B* **2020**, DOI: 10.1039/d10300tb01836h.
- [11] Y. W. Dong, T. Z. Yu, L. Ding, E. Laurini, Y. Y. Huang, M. J. Zhang, Y. H. Weng, S. T. Lin, P. Chen, D. Marson, Y. F. Jiang, S. Giorgio, S. Pricl, X. X. Liu, P. Rocchi, L. Peng, *J. Am. Chem. Soc.* **2018**, *140*, 16264-16274.
- [12] a) E. Bartolami, Y. Bessin, V. Gervais, P. Dumy, S. Ulrich, *Angew. Chem. Int. Ed.* **2015**, *54*, 10183-10187; b) E. Bartolami, Y. Bessin, N. Bettache, M. Gary-Bobo, M. Garcia, P. Dumy, S. Ulrich, *Org. Biomol. Chem.* **2015**, *13*, 9427-9438.
- [13] P. Evenou, J. Rossignol, G. Pemboung, A. Gothland, D. Colesnic, R. Barbeyron, S. Rudiuk, A. G. Marcelin, M. Menand, D. Baigl, V. Calvez, L. Bouteiller, M. Sollogoub, *Angew. Chem. Int. Ed.* **2018**, *57*, 7753-7758.
- [14] X. X. Liu, J. H. Zhou, T. Z. Yu, C. Chen, Q. Cheng, K. Sengupta, Y. Y. Huang, H. T. Li, C. Liu, Y. Wang, P. Posocco, M. H. Wang, Q. Cui, S. Giorgio, M. Fermeglia, F. Q. Qu, S. Pricl, Y. H. Shi, Z. C. Liang, P. Rocchi, J. J. Rossi, L. Peng, *Angew. Chem. Int. Ed.* **2014**, *53*, 11822-11827.
- [15] J. Zhou, L. Sun, L. P. Wang, Y. C. Liu, J. Y. Li, J. Y. Li, J. Li, H. H. Yang, *Angew. Chem. Int. Ed.* **2019**, *58*, 5236-5240.
- [16] a) A. Kohata, P. K. Hashim, K. Okuro, T. Aida, *J. Am. Chem. Soc.* **2019**, *141*, 2862-2866; b) P. K. Hashim, K. Okuro, S. Sasaki, Y. Hoashi, T. Aida, *J. Am. Chem. Soc.* **2015**, *137*, 15608-15611.
- [17] L. East, C. M. Isacke, *Bba-Gen Subjects* **2002**, *1572*, 364-386.
- [18] L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Angew. Chem. Int. Ed.* **2006**, *45*, 2348-2368.
- [19] a) M. Gary-Bobo, Y. Mir, C. Rouxel, D. Brevet, I. Basile, M. Maynadier, O. Vaillant, O. Mongin, M. Blanchard-Desce, A. Morere, M. Garcia, J. O. Durand, L. Raehm, *Angew. Chem. Int. Ed.* **2011**, *50*, 11425-11429; b) D. Brevet, M. Gary-Bobo, L. Raehm, S. Richeter, O. Hocine, K. Amro, B. Loock, P. Couleaud, C. Frochot, A. Morere, P. Maillard, M. Garcia, J. O. Durand, *Chem. Commun.* **2009**, 1475-1477.
- [20] L. Dutot, P. Lécorché, F. Burlina, R. Marquant, V. Point, S. Sagan, G. Chassaing, J.-M. Mallet, S. Lavielle, *J. Chem. Biol.* **2010**, *3*, 51-65.
- [21] a) I. Gallego, A. Rioboo, J. J. Reina, B. Diaz, A. Canales, F. J. Canada, J. Guerra-Varela, L. Sanchez, J. Montenegro, *Chembiochem* **2019**, *20*, 1400-1409; b) P. Morelli, E. Bartolami, N. Sakai, S. Matile, *Helv. Chim. Acta* **2018**, *101*, e1700266.
- [22] C. Bouillon, Y. Bessin, F. Poncet, M. Gary-Bobo, P. Dumy, M. Barboiu, N. Bettache, S. Ulrich, *J. Mater. Chem. B* **2018**, *6*, 7239-7246.
- [23] The N/P ratio corresponds to the total number of cationic guanidinium groups in solution divided by the number of phosphodiester groups brought by the siRNA.
- [24] S. Chu, C. Tang, C. H. Yin, *Biomaterials* **2015**, *52*, 229-239.
- [25] J. Gilleron, W. Querbes, A. Zeigerer, A. Borodovsky, G. Marsico, U. Schubert, K. Manygoats, S. Seifert, C. Andree, M. Stoter, H. Epstein-Barash, L. G. Zhang, V. Koteliensky, K. Fitzgerald, E. Fava, M. Bickle,

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Dynamic covalent polymers are expressed at low concentration by siRNA templating from peptide-based dynamic covalent libraries. Using a glycosylated building block, we evidenced that this templated polymerization further translates into the multivalent presentation of carbohydrate ligands, which subsequently promotes cell uptake, and even cell-selective siRNA delivery.