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COMMUNICATION

Selection of a synthetic glycan oligomer from a library of DNA-templated fragments against DC-SIGN and inhibition of HIV gp120 binding to dendritic cells†‡

Mihai Ciobanu,[a] Kuo-Ting Huang,[a] Jean-Pierre Daguer,[a] Sofia Barluenga,[a] Olivier Chaloin,[b] Evelyne Schaeffer,[b] Christopher G. Mueller,[b] Daniel A. Mitchell[c] and Nicolas Winssinger*[a]

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We report the synthesis of a nucleic acid-encoded carbohydrate library, its combinatorial self-assembly into 37 485 pairs and a screen against DC-SIGN leading to the identification of consensus ligand motifs. A prototypical example from the selected pairs was shown to have enhanced binding. A dendrimer incorporating the selected motifs inhibited gp120's binding to dendritic cells with higher efficiency than mannan.

Despite early success with the synthesis and screening of carbohydrate libraries,[1] most attention has been devoted to glycan arrays containing collections of natural glycan motifs.[2] In this study, we aimed at preparing a library of modified synthetic glycans in a combinatorial fashion using peptide nucleic acid[3] (PNA) encoding,[4] assembling the library in different dimeric combinations and screening for improved binding to DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin). DC-SIGN is a tetrameric C-type lectin mainly present at the surface of immature dendritic cells, which modulates the immune response against different pathogens.[5,6] Its affinity for high mannose glycans has been exploited by several opportunistic pathogens,[7] including HIV[8] which displays a high density of the Man-α1,2-Man motif on its gp120 envelope protein.[9] This has engendered a significant interest in developing strategies to antagonize this interaction in order to inhibit the pathogen entry.[10] Good success has been achieved with scaffolds which display multiple copies of mannose-based glycans,[11] notably with dendrimers which are particularly effective to recapitulate the avidity of multimeric interactions.[12]

The library design was based on the previous observation that mannose disaccharides appended at the α- and ε-nitrogen of lysine could be dimerized onto a DNA-template to recapitulate the binding of gp120's high mannose glycan.[13] To further probe binding to DC-SIGN, we designed a library of PNA-encoded glycans as shown in Fig. 1. A resin containing three orthogonally protected branch points was prepared (**1**): a Fmoc-protected amine for PNA oligomerization, and the α- and ε-nitrogen of lysine protected as an azide and with Mtt, respectively, for sequential coupling of different glycan fragments. The library synthesis commenced with the introduction of the first PNA codon followed by reduction of the azide and coupling of the first glycan fragment. Five glycan fragments (Fig. 1, panel II) derivatized with 2-hydroxy acetic acid at the reducing end were coupled by standard amide-bond formation. Four of the glycan fragments contain one or two azides for further diversification. After the second cycle of split and mix, the azides were modified by either, a simple reduction and temporary protection (Boc), copper catalyzed cycloaddition[14] with different alkynes or reduction and acetylation or benzoylation (Fig. 1, panel III) followed by PNA-encoding. Deprotection of the Mtt and two more cycles of split and mix reiterating the glycan coupling and azide diversifications afforded the final library. Cleavage from the resin (TFA) followed by global deprotection of the glycans (NH_3 in MeOH) afforded the final library of 441 different PNA-encoded glycan dimers [while the library is made up of $5 \times 5 \times 5 \times 5$ building blocks affording 625 permutations, the fact that fragment E (Man-α1,2-Man) does not contain an azide reduces the library size while providing an oversampling of this fragment].

We have recently shown that PNA-encoded small molecule fragments could be combinatorially assembled onto a library of DNA templates and screened against an immobilized target to identify the fittest fragment pairs.[15] We reasoned that the same approach could be used to screen a large collection of assemblies of the PNA-encoded glycan (Fig. 2). Thus, a library of single strand DNA was used to combinatorially pair a subset of the glycan library (105 members containing arbitrarily J2 as the last element of diversity) with a different subset (357 members omitting J2 as the last element of diversity) to afford a solution theoretically containing 37 485 glycan pairs. While this library does not cover the entire solution set of permutations, all library members are minimally paired with Man-α1,2-Man disaccharide, which represents the starting point of the library. Next, DC-SIGN was immobilized on magnetic beads and presented with the DNA-templated library in an HBS–$CaCl_2$ buffer for 30 min. Following the removal of the supernatant and accompanying washes, the resin was heat-denatured to recover the DNA

[a] *Institut de Science et Ingenierie Supramoleculaires (ISIS), Université de Strasbourg—CNRS, 8 allee Gaspard Monge, 67000 Strasbourg, France. E-mail: winssinger@isis.u-strasbg.fr*
[b] *Institut de Biologie Moleculaire et Cellulaire Université de Strasbourg—CNRS (UPR9021), Strasbourg, France*
[c] *Warwick Medical School, University of Warwick, UK*

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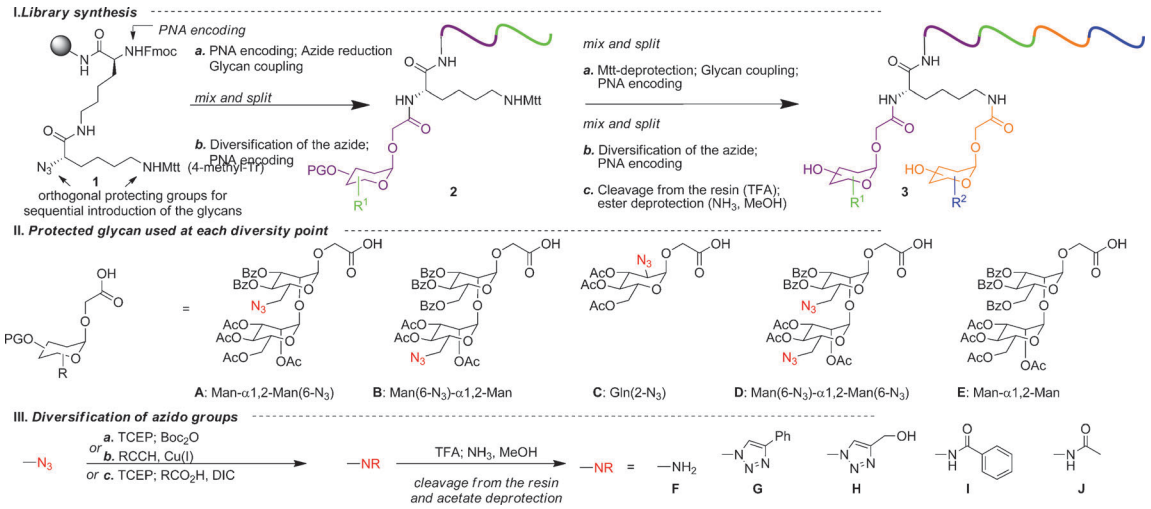

Fig. 1 Split and mix synthesis of a PNA-encoded library of glycans.

templates of the best binders. The same experiment was performed with underivatized beads as a negative control. The sequence of the DNA, hence the structure of the selected fragment, was first determined by microarray hybridization. The microarray contains 625 sequences complementary to each PNA but does not provide information as to the pairing of the PNAs. As can be seen in Fig. 2, while there was no obvious selection against the beads alone (red, false coloring), a clear pattern emerged for the selection against DC-SIGN (green, false coloring). Notably Man(6N_3)-α1,2-Man was highly selected as the first (B1) and second (B2) glycan while azides derivatized as a phenyl triazole (G1, G2) or benzoyl amide (I1, I2) were preponderant. To obtain further information about the pairing of selected assemblies, the selected DNA was cloned and 10 random clones were sequenced. Analysis of the sequences showed a consensus motif in good agreement with the microarray data with a very strong selection for B1 as the first glycan in both PNAs but a weaker selection for the second glycan in either position. Nevertheless, Man(6N_3)-α1,2-Man is still the most or second most selected fragment. Notably, underivatized Man-α1,2-Man is clearly underrepresented in the results of the selection despite its oversampling in the library.

In order to validate the results of the screen, three assemblies were prepared as single entities to measure their binding kinetics by surface plasmon resonance and calculate a dissociation constant (K_D). The first assembly (**4**, see Fig. 3 for the structure) was present in the clone sequencing and is representative of the selected motifs identified on the microarray; the second (**5**) is made up of two units of a Man-α1,2-Man dimer and as such constitutes a positive control and a starting point of the library; the third (**6**) is a homodimer of the first PNA from assembly **4**. As the DC-SIGN interaction with a carbohydrate is known to be calcium-dependent, the affinities were evaluated in a buffer with and without calcium in order to validate that the measured affinity is indeed a product of the foreseen interaction. All three assemblies showed a measurable binding affinity only in the presence of calcium

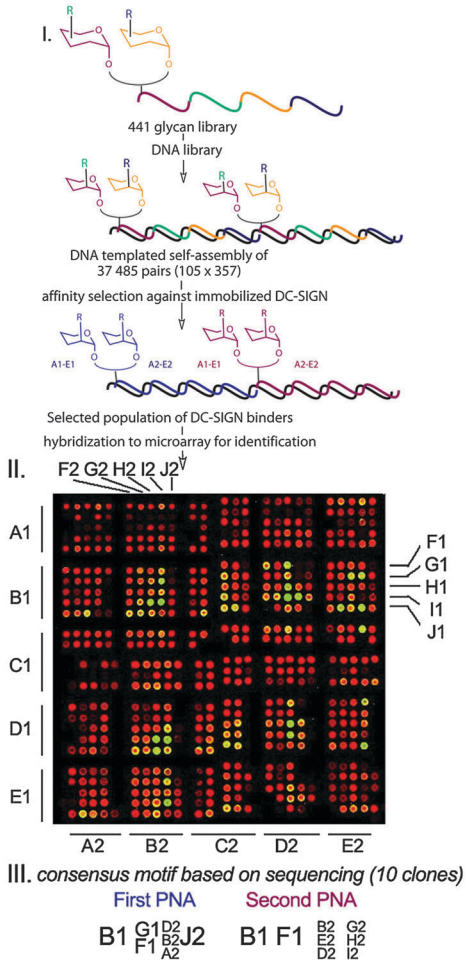

Fig. 2 DNA-templated combinatorial assembly of the glycan library and screening against DC-SIGN. (I) The library of 441 PNA-encoded glycans is assembled onto a library of DNA templates and the best binders are obtained by affinity purification against immobilized DC-SIGN. (II) Microarray analysis of the selected population: the selection against DC-SIGN is shown in green whereas a control selection against BSA is shown in red (false color scheme). The microarray contains all the complementary sequences to the library of glycans. Each PNA encodes for a dimer of glycans denoted as A1-E1 for the first one and A2-E2 for the second one. Each glycan derivatization is denoted as F1-J1 for the functional group corresponding to the first glycan and F2-G2 for the functional group corresponding to the second glycan (see Fig. 1 for structures). (III) Sequencing of 10 clones from the selection against DC-SIGN was analyzed providing the shown consensus motif.

Entry	Assembly	K_D (μM)	buffer
1	4: B1F1B2J2-B1F1B2G2	0.127	HBS-P with 5 mM $CaCl_2$
2		no binding	HBS-P
3	5: E1E2-E1E2	4.09	HBS-P with 5 mM $CaCl_2$
4		no binding	HBS-P
5	6: B1F1B2J2-B1F1B2J2	2.09	HBS-P with 5 mM $CaCl_2$
6		no binding	HBS-P

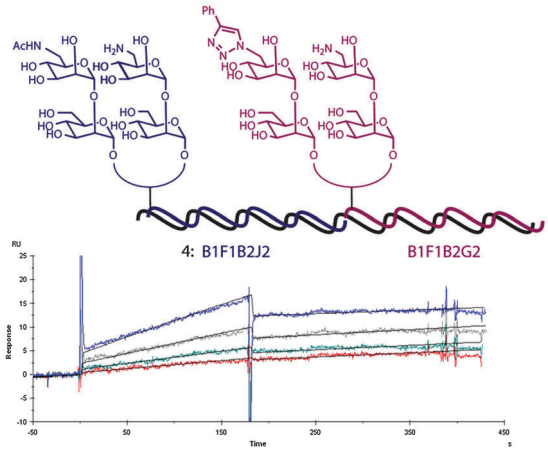

Fig. 3 Affinity of three assemblies for DC-SIGN measured by SPR. Top: calculated K_D of three assemblies in two buffers (the letters denote the fragments, see Fig. 1 for the structure); Middle: structure of assembly **4** identified in the screen, sensogram of assembly **4** at 25, 12.5, 6.25 and 3.12 μM (entry 1).

(Fig. 3, entries 1, 3, 5 *vs* 2, 4, 6). Gratifyingly, assembly **4** had over 30 fold improved affinity relatively to the positive control (assembly **5**) concurring the microarray and sequencing data. To validate whether the selected modified glycan would antagonize the interaction of gp120 with dendritic cells, the selected fragments were resynthesized without the PNA and coupled to a 5th generation PAMAM dendrimer (**8**, Fig. 4, see ESI‡ for experimental details). Mass spectroscopic analysis of the product suggested that each dendron statistically contained 6 units of each glycan. The dendron **8** was found to inhibit the interaction of gp120 with dendritic cells in a dose dependent manner. As shown in Fig. 5, dendron **8** completely inhibited the interaction of gp120 with dendritic cells at 10 μM whereas a natural polymer of mannose (mannan) only partially inhibited this interaction at 100 μg mL^{-1} (500 μM in mannose).

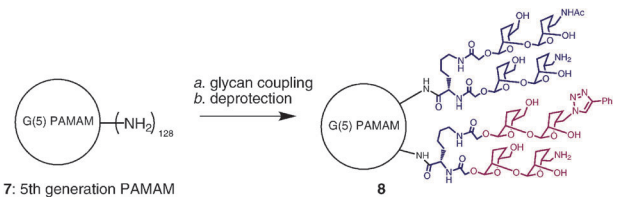

Fig. 4 Synthesis of a glycodendrimer **8** derivatized with glycan fragments of assembly **4**.

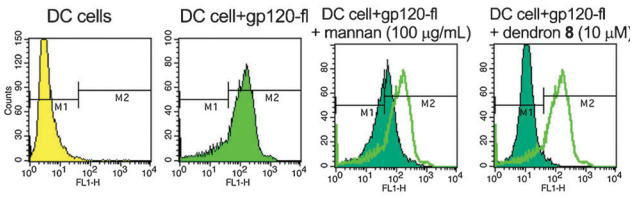

Fig. 5 Inhibition of gp120 binding to dendritic cells (DC cells).

To the best of our knowledge, this is the first example of a split and mix combinatorial synthesis of a nucleic acid encoded glycan library. The ability to pair such encoded glycan library in a combinatorial fashion onto DNA provides a rapid means to generate a large diversity of assemblies to probe and decode binding leveraged on multimeric interactions. While the present pilot library focused specifically on mannose, and only explored limited modifications, the results do suggest that a substitution with an aryl group at the 6-position of the terminal mannose is beneficial for DC-SIGN binding. In the larger context, the results also suggest that this approach should be broadly applicable to different glycoconjugates including mammalian oligosaccharides and microbial peptidoglycans.

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Notes and references

1 P. H. Seeberger and D. B. Werz, *Nat. Rev. Drug Discovery*, 2005, **4**, 751; R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, A. Smith, K. Biswas, W. C. Still and D. Kahne, *Science*, 1996, **274**, 1520.

2 P. H. Seeberger and D. B. Werz, *Nature*, 2007, **446**, 1046; J. C. Paulson, O. Blixt and B. E. Collins, *Nat. Chem. Biol.*, 2006, **2**, 238; O. Blixt, S. Head, T. Mondala, C. Scanlan, M. E. Huflejt, R. Alvarez, M. C. Bryan, F. Fazio, D. Calarese, J. Stevens, N. Razi, D. J. Stevens, J. J. Skehel, I. van Die, D. R. Burton, I. A. Wilson, R. Cummings, N. Bovin, C. H. Wong and J. C. Paulson, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 17033.

3 M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden and P. E. Nielsen, *Nature*, 1993, **365**, 566.

4 J. L. Harris and N. Winssinger, *Chem.–Eur. J.*, 2005, **11**, 6792.

5 L. Wu and V. N. Kewal Ramani, *Nat. Rev. Immunol.*, 2006, **6**, 859.

6 A. Imberty, Y. M. Chabre and R. Roy, *Chem.–Eur. J.*, 2008, **14**, 7490.

7 S. Pohlmann, J. Zhang, F. Baribaud, Z. W. Chen, G. Leslie, G. Lin, A. Granelli-Piperno, R. W. Dom, C. M. Rice and J. A. McSKeating, *J. Virol.*, 2003, **77**, 4070; L. Tailleux, O. Schwartz, J. L. Herrmann, E. Pivert, M. Jackson, A. Amara, L. Legres, D. Dreher, L. P. Nicod, J. C. Gluckman, P. H. Lagrange, B. Gicquel and O. Neyrolles, *J. Exp. Med.*, 2003, **197**, 121.

8 T. B. H. Geijtenbeek, D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. F. van Duijnhoven, J. Middel, I. Cornelissen, H. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor and Y. van Kooyk, *Cell (Cambridge, Mass.)*, 2000, **100**, 587; H. Feinberg, D. A. Mitchell, K. Drickamer and W. I. Weis, *Science*, 2001, **294**, 2163; D. A. Mitchell, A. J. Fadden and K. Drickamer, *J. Biol. Chem.*, 2001, **276**, 28939.

9 K. J. Doores, C. Bonomelli, D. J. Harvey, S. Vasiljevic, R. A. Dwek, D. R. Burton, M. Crispin and C. N. Scanlan, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 13800.

10 C. N. Scanlan, J. Offer, N. Zitzmann and R. A. Dwek, *Nature*, 2007, **446**, 1038.

11 G. Tabarani, J. J. Reina, C. Ebel, C. Vives, H. Lortat-Jacob, J. Rojo and F. Fieschi, *FEBS Lett.*, 2006, **580**, 2402; M. Sanchez-Navarro and J. Rojo, *Drug News Perspect.*, 2010, **23**, 557; S. Sattin, A. Daghetti, M. Thepaut, A. Berzi, M. Sanchez-Navarro, G. Tabarani, J. Rojo, F. Fieschi, M. Clerici and A. Bernardi, *ACS Chem. Biol.*, 2010, **5**, 301; S. K. Wang, P. H. Liang, R. D. Astronomo, T. L. Hsu, S. L. Hsieh, D. R. Burton and C. H. Wong, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3690; C. R. Becer, M. I. Gibson, J. Geng, R. Ilyas, R. Wallis, D. A. Mitchell and D. M. Haddleton, *J. Am. Chem. Soc.*, 2010, **132**, 15130.

12 R. Roy, *Curr. Top. Struct. Biol.*, 1996, **6**, 692; Y. M. Chabre and R. Roy, *Curr. Top. Med. Chem.*, 2008, **8**, 1237.

13 K. Gorska, K. T. Huang, O. Chaloin and N. Winssinger, *Angew. Chem., Int. Ed.*, 2009, **48**, 7695.

14 M. Meldal and C. W. Tornoe, *Chem. Rev.*, 2008, **108**, 2952; H. C. Kolb and K. B. Sharpless, *Drug Discovery Today*, 2003, **8**, 1128.

15 J. P. Daguer, M. Ciobanu, S. Alvarez, S. Barluenga and N. Winssinger, *Chem. Sci.*, 2011, **2**, 625.