

Diabetes Treatment: Selective Synthetic Receptor for Glucose

Bahareh Shirinfar* and Nisar Ahmed*[a]

A new synthetic receptor has selective and strong interactions with glucose, directing towards future diabetes management. These studies pave the way to design future selective receptors that can potentially be modified with combinations of urea

walls having multiple H-binding sites to generate hydrophilic affinities, and the incorporation of promising aromatic systems for hydrophobic π -interactions with glucose CH.

Diabetes is a major medical concern, affecting approximately 5% of the global population.^[1] Although it can be managed, for example by insulin injections, there is a continual risk that glucose concentrations will stray outside safe boundaries. The monitoring of blood glucose levels is thus critical for effective treatment. Analysis using test strips on withdrawn blood samples is well-established, but this can only be performed a few times each day. As a result, it is difficult to avoid high average blood glucose concentrations, and these cause serious long-term complications (heart disease, blindness, kidney damage, stroke, nerve damage etc.). There is also a danger of excessively low glucose levels, which can be fatal. Diabetes management would be greatly improved by a long-term implantable glucose sensor, which could give continuous read-out of glucose levels.

The main element of a glucose monitor is a molecule which binds glucose selectively and sends out a signal as it does so. In mainstream technology this role is played by an enzyme, glucose oxidase, but this natural molecule is not readily adaptable to continuous operation. Like most enzymes it is not indefinitely stable. Moreover it does not just bind glucose but also catalyzes its oxidation, and this leads to technical issues for sensor design. For continuous operation it is preferable to use a molecule which binds glucose in a non-destructive, equilibrium process, and which sends a signal which does not depend on a chemical reaction.

The selective binding of glucose in water is challenging due to its hydrophilic and hydromimetic nature, as well as its complex three-dimensional structure. Both natural lectins,^[2] and "synthetic lectins"^[3,4,5] have low affinities to glucose ($K_a \sim 500 \text{ M}^{-1}$ and $K_b \sim 250 \text{ M}^{-1}$ respectively). Also, the structural resemblance of glucose to other saccharides leads to low selectivities. The synthetic receptors incorporating boronic acids

bind more strongly with glucose. However, they have a tendency to complex other polyols and are pH-sensitive.^[1,6]

The design of synthetic glucose receptors is intrinsically challenging, because carbohydrate moieties are highly hydrophilic and thus problematic to identify from water. However, over the past few years the team of A. P. Davis in Bristol has reported several synthetic lectins which bind glucose and related carbohydrates with good affinities and excellent selectivities.^[4,5] The early versions were complex polycyclic structures that were difficult to synthesize, and did not provide an easily-read signal for glucose monitoring.^[4] In 2012, a new system was discovered which largely solved the aforementioned problems.^[5] The molecule concerned, termed AnR 1, is a simple macrocycle in which two anthracene units are separated by rigid isophthalamide spacers (see Figure 1). AnR 1 is accessible in just 6 steps from simple starting materials. The anthracenes can take up a parallel orientation, and in this conformation a glucose molecule can slide between them, making favorable polar and apolar contacts with different parts of the receptor. The binding constant K_a is modest, at $\sim 60 \text{ M}^{-1}$, but this value is suitable for discriminating between glucose concentrations in the range of interest ($\sim 0\text{--}20 \text{ mM}$). Other carbohydrates such as galactose and mannose are bound very weakly. A particular advantage of AnR 1 is the presence of the anthracene units, which are highly fluorescent. When the glucose enters the cavity the conformation changes, and this affects the fluorescence output (which increases nearly three-fold). The receptor thus possesses a built-in reporting system which can be used to follow glucose concentrations.

The low binding constant of AnR 1 places limits on potential applications. There has been a long effort in the Davis research group to find a glucose receptor that binds selectively and strongly, allowing greater scope for the design of devices for glucose-monitoring or insulin-dispensing. In a recent development, they at the University of Bristol and the company Ziylo (a new company name, Carbometrics) have achieved a remarkable improvement on past performance. They disclose a synthetic receptor that selectively recognizes glucose in aqueous biological media with affinities for far stronger than their previously reported systems for carbohydrate recognition (see Figure 2).^[7] The new receptor is also highly selective, showing

[a] Dr. B. Shirinfar, Dr. N. Ahmed
School of Chemistry, Cardiff University
Park Place, Main Building, Cardiff CF10 3AT (UK)
E-mail: shirinpostech@gmail.com
AhmedN14@cardiff.ac.uk

© 2018 The Authors. Published by Wiley-VCH AG. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

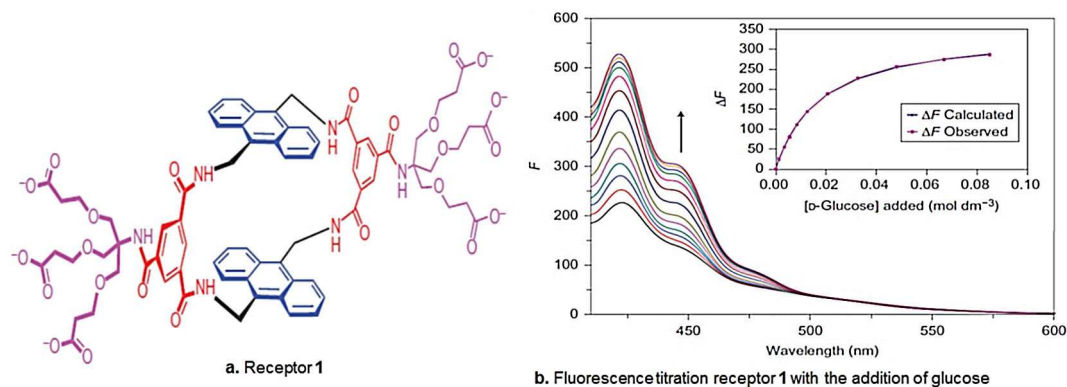


Figure 1. a) The receptor (AnR) 1 contains the central polar (red) and hydrophobic (blue) regions. The binding region is surrounded by dendrimers (pink) that make the receptor soluble in water. b) Fluorescence titration of 1 with the addition of glucose in phosphate buffer solution (pH 7.1, 0.1 M) at 298 K. Emission intensity increases as additions proceed. Reproduced with permission from Ref [5]. Copyright (2012) Nature Publishing Group.

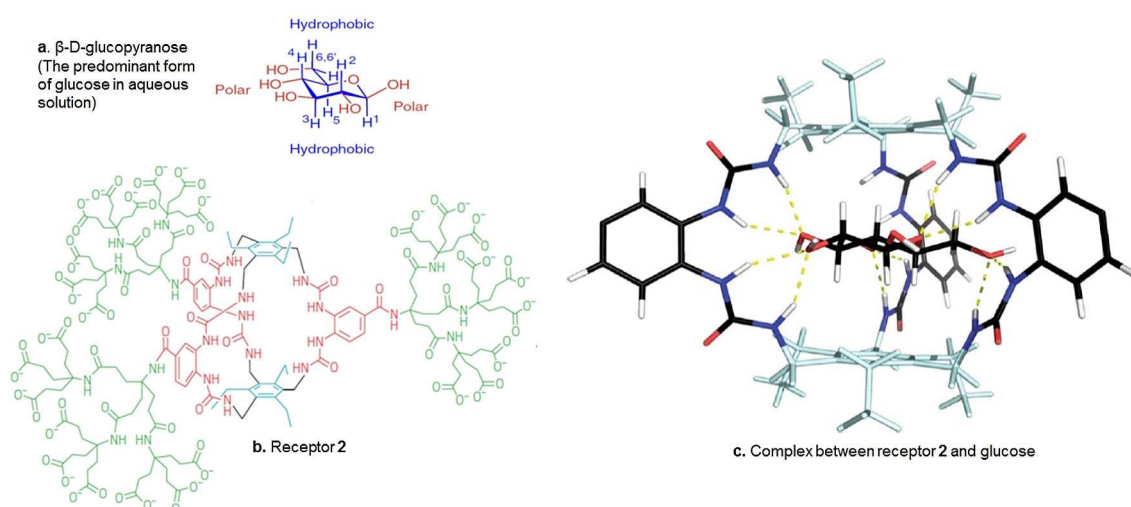
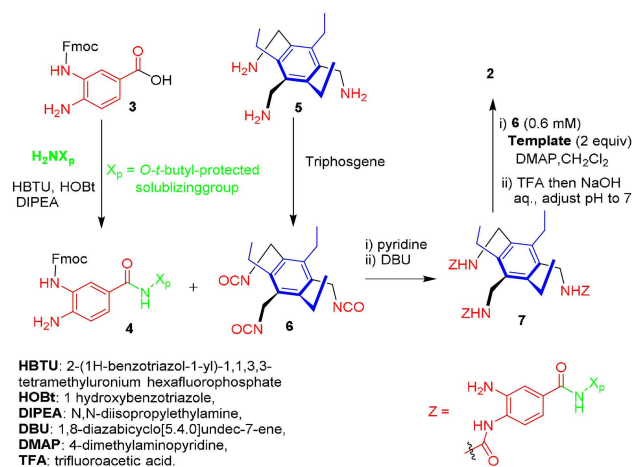


Figure 2. a) β -D-glucopyranose. b) The receptor 2 contains the central polar (red) and hydrophobic (blue) regions. The binding region is surrounded by three dendrimers (green) that make the receptor soluble in water. c, the complex features ten intermolecular hydrogen bonds, shown as yellow broken lines. The triethylmesitylene units are colored pale blue, and the dendritic side-chains are omitted for clarity. Reproduced with permission from Ref [7]. Copyright (2018) Nature Publishing Group.

hundred times greater affinities for glucose as compared to closely related molecules such as mannose or galactose.

The new synthetic receptor 2 has been designed as a compact template shaped structure that has a hollow space inside and has been decorated with three dendrimers for aqueous solubility. In contrast to previous receptors, the new receptor has six urea spacer groups capable of forming multiple H-bonds. These are used to connect substituted aromatic triethylmesitylene moieties that serve as floor and roof of the binding site. Significantly, these aromatic parts generate hydrophobic π -interactions with glucose axial hydrogens during complexation. The numerous urea moieties of the receptor are arranged in such a way as to develop strong H-bonding with glucose through oxygen atoms. The new receptor 2 was synthesized in a few steps from known compounds 3 and 5, as shown in scheme 1. The yield of receptor 2 was significantly increased to ~50% through a templating process using an organic-soluble glucoside.



Scheme 1. Synthetic route to receptor 2.^[7]

NMR and Isothermal Titration Calorimetry (ITC) titrations were performed to measure affinities of receptor **2** with a variety of substrates. From NMR titrations, the binding constant for glucose was quantified as $K_a = 18,600 \text{ M}^{-1}$ and ITC showed similar affinity for glucose ($K_a = 18,200 \text{ M}^{-1}$). To obtain the selectivity of new receptor **2**, a number of substrates with close similarity to glucose were tested (see Figure 3). Methyl β -D-

Substrates	$K_a (\text{M}^{-1})$	
	ITC	NMR
D-Glucose	18,200	18,600
Methyl β -D-glucoside	7,900	7,500
D-Glucuronic acid	5,300	ND
D-Xylose	5,800	ND
2-Deoxy-D-glucose	725	ND
D-Galactose	180	130
D-Mannose	140	140
D-Ribose	220	270
D-Fructose	60	51
D-Cellobiose	30	31

Figure 3. Substrates and binding affinities (K_a) for receptor **2** measured in D_2O containing phosphate buffer (10 mM, pH = 7.4) at $T = 298 \text{ K}$. ND: not determined.

glucoside, glucuronic acid and xylose possess pyranose moieties with all-equatorial geometries and showed moderate affinities $> 5000 \text{ M}^{-1}$ as compared to glucose. Other substrates bound far more weakly. Furthermore, the receptor **2** was not only stable in biological media but also showed reasonable binding to glucose. For example, affinities in cell culture media were measured at $K_a \sim 5300 \text{ M}^{-1}$. The reduction in affinities compared to water might be due to interference from Ca^{2+} and Mg^{2+} ions. The receptor also has no toxicity towards HeLa cells up to 1 mM concentration.

In summary, we have highlighted the new synthetic receptor that is stable, easy to synthesize and shows selective binding to glucose that could help in diabetes treatment through glucose monitoring^[6] and glucose-responsive insulin.^[8] The core structure of receptor perfectly complements the all-equatorial β -pyranoside substrate (glucose). It binds glucose with an affinity of $K_a \sim 18,000 \text{ M}^{-1}$, comparing well with natural receptor systems and shows selectivities at biological levels. These studies pave the way to design future selective receptors that can potentially be modified with combinations of urea walls having multiple H-binding sites to generate hydrophilic

affinities, and the incorporation of promising aromatic systems for hydrophobic π -interactions with glucose CH. Furthermore, the incorporation of fluorescent systems with extended conjugation in the receptor's core could be useful for *in vivo* monitoring of glucose.

Acknowledgements

Marie Skłodowska-Curie Actions COFUND (Grant No 663830) to Dr. Nisar Ahmed is gratefully acknowledged. We also thank Royal Society funding for Newton Fellowship to Dr. Bahareh Shirinfar (School of Chemistry, University of Bristol).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: glucose · synthetic lectins · urea spacers · diabetes · receptors

- [1] T. D. James, M. D. Phillips, S. Shinkai, *Boronic Acids in Saccharide Recognition*, RSC, Cambridge, **2006**.
- [2] E. J. Toone, *Curr. Opin. Struct. Biol.* **1994**, *4*, 719.
- [3] The lectins, from which this term derives, are the major family of carbohydrate-binding proteins.
- [4] a) E. Klein, M. P. Crump, A. P. Davis, *Angew. Chem. Int. Ed.* **2005**, *44*, 298; b) Y. Ferrand, M. P. Crump, A. P. Davis, *Science* **2007**, *318*, 619; c) Y. Ferrand, E. Klein, N. P. Barwell, M. P. Crump, J. Jimenez-Barbero, C. Vicent, G. J. Boons, S. Ingale, A. P. Davis, *Angew. Chem. Int. Ed.* **2009**, *48*, 1775; d) B. Sookcharoenpinyo, E. Klein, Y. Ferrand, D. B. Walker, P. R. Brotherhood, C. F. Ke, M. P. Crump, A. P. Davis, *Angew. Chem. Int. Ed.* **2012**, *51*, 4586; *Angew. Chem.* **2012**, *124*, 4664e) T. J. Mooibroek, *Nat. Chem.* **2016**, *8*, 69; f) P. Rios, et al. *Angew. Chem. Int. Ed.* **2016**, *55*, 3387; g) P. Rios, *Chem. Sci.* **2017**, *8*, 4056.
- [5] C. Ke, H. Destecroix, M. P. Crump, A. P. Davis, *Nat. Chem.* **2012**, *4*, 718.
- [6] a) X. L. Sun, T. D. James, *Chem. Rev.* **2015**, *115*, 8001.
- [7] R. A. Tromans, T. S. Carter, L. Chabanne, M. P. Crump, H. Li, J. V. Matlock, M. G. Orchard, A. P. Davis, *Nat. Chem.* **2018**, *10*, DOI: 10.1038/s41557-018-0155-z.
- [8] Q. Wu, L. Wang, H. J. Yu, J. J. Wang, Z. F. Chen, *Chem. Rev.* **2011**, *111*, 7855.

Manuscript received: December 4, 2018

Revised manuscript received: December 27, 2018

Version of record online: ■■■, ■■■■