Dynamic glycovesicle systems for amplified QCM detection of carbohydrate-lectin multivalent biorecognition†

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We describe multivalent biorecognition of adsorbed lectin layers by biomimetic sensing nanoplatforms based on dynamic glycovesicles in a continuous flow QCM setup.

Molecular recognition in biological systems occurs mainly in interfacial environments such as at membrane surfaces, enzyme active sites, or in the interior of the DNA double helix. At the cell membrane surface, carbohydrate–protein recognition principles apply to a range of specific non-covalent interactions including immune response,[1] cell proliferation, adhesion and death,[2] cell–cell interaction and communication. Protein–protein recognition meanwhile accounts for signalling processes[3] and ion channel structure.[4–7]

Surface based systems for biosensing in terms of real-time label-free measurement of biointeractions have been extensively developed which exploit electro-optical (Surface Plasmon Resonance – SPR), piezoelectric (Quartz Crystal Microbalance – QCM) and electrochemical transduction properties. The piezoelectric phenomenon by which QCM sensors work is a mechanical–electrical effect first reported in 1880, describing the generation of electrical charges on the surface of solids caused by pulling, pushing or torsion.[8] It was later demonstrated by Sauerbrey that there exists a linear relationship between mass adsorbed on piezoelectric crystal surfaces and the crystal's resonant frequency in air or vacuum.[9] Development of this observation toward the study of biological interactions was realised with the design of solution based systems and combination of these with microfluidics and surface chemistry. Consequently, QCM has become a highly relevant analytical technique due to its sensitive solution–surface interface measurement capability and so is highly applicable in label free analysis of biological binding events.[10] It possesses a wide detection range which at the low end can detect monolayer coverage by small molecules. Although the relationship described by Sauerbrey is only valid for a thin, uniform, rigidly attached mass in vacuum, new models to explain the mass–frequency relationship in liquid environments and for non-rigid materials were developed in later theoretical works.[11–13] Besides direct measurement of adsorbed mass, concentration dependent measurements of the frequency shift together with the assumption of a linear relationship between ΔF and Δm allow thermodynamic and kinetic parameters of binding events as well as active concentrations to be determined. Thus QCM has been used in the areas of DNA hybridisation, immunological systems and also in the areas of protein–protein and protein–carbohydrate interactions.[14] A general limitation, however, on sensor technologies sensitivity is the mass of the analyte which is to be adsorbed from solution. At present commercially available sensing systems do not have intrinsic small molecule sensitivity. Amplification methods may be employed in order to study certain biorecognition processes. The most common amplification method is 3-dimensional surfaces, *e.g.* porous films, molecular imprinted polymers, multilayers *etc.*[10c,17] A second strategy concerns the mass increase by the introduction of nanoplatforms such as nanoparticles,[15] vesicles[16] to carry the recognising small molecule element.

Vesicular aggregates exhibit an important advantage as a biological sensing platform in that they mimic the cell membrane – the site of molecular docking, ligand–receptor binding and other vital natural processes such as exosomes. In the last two decades an interest has developed in these liposomal aggregates as drug delivery systems with their major advantage being the physiological origin of their components leading to high systemic tolerance.[18] A third point is that they readily incorporate small molecular species either in their aqueous cavity or lipophilic wall. Surveying specifically protein–carbohydrate interaction studies the approaches used have been to modify the phospholipid building blocks[16] or to incorporate glycolipids or cholesterol carbohydrates in the bilayer.[19–24] Vesicles have been used to demonstrate molecular recognition on the QCM platform for biotin–streptavidin affinity driven adsorption.[25,26]

Referring specifically to lectin recognition by small unilamellar vesicles there are some noteworthy advantages to using this platform in QCM: (a) the phospholipid surface is anti-adhesive towards lectin proteins and so non-specific binding can be avoided;[27] (b) glycolipids should easily partition into bilayers;[28] (c) a small unilamellar vesicle has an average molar mass of around 1.5×10^6 g mol^{-1}.[29]

Herein we report vesicle biorecognition of lectin layers in a continuous flow QCM setup. Simple alkyl glycosides **1–4** (Scheme 1) were used to confer the highly specific "glycocode" to the recognising vesicle nanoplatforms which will present to the multivalent lectin receptor Concanavalin A – Con A. This strategy allows QCM investigation of processes with biologically relevant approximation of *in vivo* parameters, thereby providing a means to attaining an enriched understanding of biorecognition events.

For these reasons, alkyl glycosides **1–4** were prepared to act as synthetic molecular analytes.† They were prepared using "click chemistry" *i.e.* glycosyl azides–tetradecyne

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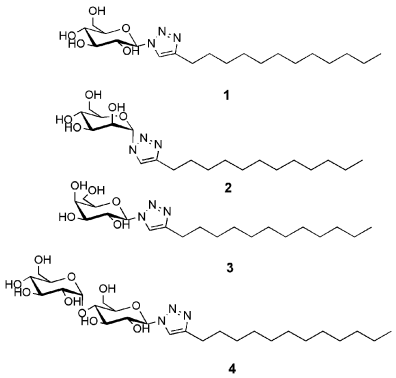

Scheme 1 Alkyl glycosides for vesicle functionalisation: β-glucoside **1**, α-mannoside **2**, β-galactoside **3**, and β-maltoside **4**.

Huisgen-cycloaddition to give a 1,4-di-substituted 1,2,3-triazole unit [30–32] with their anomeric configurations determined by NMR. The vesicle solutions were prepared following a known protocol for SUV's.[33],‡ The functionalised vesicles **V1–V4** were prepared by incubation of the vesicular solutions with different amounts of alkyl glycoside **1–4** for 24 h at 4 °C.§ Small unilamellar vesicles **V1–V4** (25–35 nm in diameter) were tested for interaction with the Con A layers by QCM.

With Con A layers prepared *in situ* on a QCM gold electrode by specific recognition of a polymannoside surface (see ESI†), vesicle solutions displaying an alkyl glycoside type at different concentrations were injected into the QCM setup (Fig. 1). The vesicle solutions had a concentration of 200 μM in POPC and various molar ratios of alkyl glycosides **1–4** to phospholipid were tested.

As shown in Fig. 2 only "maltovesicles" **V4** adsorbed specifically while all the others **V1–V3** showed little adsorption up to the 10 μM concentrations of **1–3**. The first point of interest in these results is that the "mannovesicles" **V2** (mannose: well known binder to Con A)[10,24] derived systems show no apparent affinity whereas the "maltovesicles" **V4** do. A likely cause for this is the accessibility or the orientation of the alkyl glycosides **1–4** at the bilayer surface. The maltoside ligand **4**, bearing a terminal α-Glc moiety could protrude more from the bilayer compared to the monosaccharide ligands **1–3**, which may be buried in the external (or internal) hydrophilic part of the phospholipid layer. The triazole unit just next to the glycoside may have an influence. It could be expected that the triazole ring would confer stabilizing interactions between aromatic aglycon and protein amino acids nearby as has been

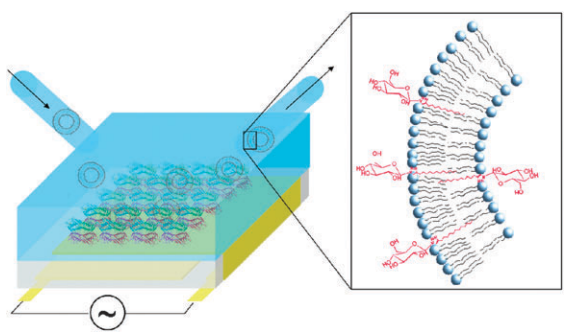

Fig. 1 Con A layers exposed to functionalised vesicle solutions within a QCM system.

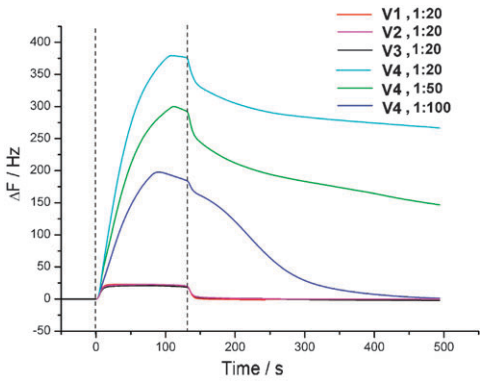

Fig. 2 QCM adsorption profiles for alkyl glycoside functionalised vesicles at different molar ratio of alkyl glycosides **1–4** to phospholipids (1 : 20/mol : mol = 10 μM in glycoside).

described for aryl glycosides.[34] In any case, based on the literature it would not be expected to have a negative effect on binding.

Another interesting characteristic is that the glycosides **1–4** might present an enhanced fluidity in the phospholipids bilayer, with reference to glycolipids or cholesteryl-carbohydrates.[24] The "maltovesicles" **V4** may allow a carbohydrate external microdomain formation with multivalent presentation.

The result of fitting "maltovesicles" **V4** adsorption on Con A to the Langmuir model gives an association constant of $K_a = 8.4 \times 10^4\ M^{-1}$ (see ESI†).[34] Con A is well known to selectively bind α-mannosides and α-glucosides as well as substituted α-mannoside analogues.[35] This demonstrated an affinity enhancement when compared with the Me-α-Glc monosaccharide which has shown in calorimetry experiments a $K_a = 2.4 \times 10^3\ M^{-1}$, even more so when compared with D-maltose $K_a = 1.6 \times 10^3\ M^{-1}$.[35a] Me-α-Man shows a higher affinity at $7.6 \times 10^3\ M^{-1}$ while the corresponding β-glycosides of both Man and Glc showed no measurable affinity.[35b] This 40-fold increase can be attributed to multivalent glycoside presentation, allied to their fluidity within bilayers.[36]

The dynamic hydrophobic interface between carbohydrate molecules and bilayers might mediate the structural self-correlation of supramolecular sugar clusters by virtue of their basic constitutional behaviours. The resultant dynamic system can undergo continual change in its constitution, through dissociation/reconstitution of different mesophases during the vesicle recognition process.[37–39] Their dynamic constitution might render the emergence of recognition events; synergetic and vesicular systems may adapt their external surface distribution allowing ConA to access more than one binding site simultaneously. This concept can be related to a sort of "*Chemical collectivism*"[40a] or to the "Dynamic interactive systems"[40b–d] characterized by their aptitude to macroscopically organize (self-control) their distribution in response to external stimuli in coupled equilibria.

In conclusion, signal amplification through coupling of vesicular nanoplatforms to QCM has been demonstrated to be a versatile method, especially given their facile preparation and propensity to partition amphiphilic molecules. At present commercially available QCM systems do not demonstrate small molecule sensitivity and vesicles offer a route to large

mass amplification allied to their presentation of a dynamic biomimetic interface. This work showed ligand design is, as expected, an important feature and multivalent "biomimetic" longer chain glycolipids could show even higher affinity amplifications.

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Notes and references

‡ 1.5 mg of POPC was dissolved in isopropanol (50 µl). This solution was then injected into 1 ml of buffer (PBS or Tris) (pH 7.4) (NaCl 100 mM) with rapid shaking on a "paramix II" shaker for one minute resulting in a clear solution. This solution was then ultrasonicated for 30 min at 0 °C before being diluted (×10) with buffer giving a final concentration of 200 µM in POPC.

§ 10 µl of alkyl glycoside (200 µM) in EtOH–H_2O (1 : 1) were injected into 990 µl of a 200 µM POPC vesicular solution to give a ratio of (1 : 100) POPC : alkyl glycoside. The solution was then left overnight at 4 °C followed by testing of its lectin specificity by continuous flow QCM analysis.

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