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Construction of a ^{19}F-lectin biosensor for glycoprotein imaging by using affinity-guided DMAP chemistry

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ABSTRACT

In this study, assisted by affinity-guided DMAP strategy, we developed a novel ^{19}F-modified lectin as a biosensor for specific detection and imaging of glycoproteins. Exploited the large chemical shift anisotropy property of ^{19}F nuclei, glycoproteins detected by our ^{19}F-biosensor are signatured by broadened peaks in ^{19}F NMR, hence enabled the distinction between glycoproteins and small molecule saccharides. Such signal on/off switching was also applied to glycoprotein imaging by ^{19}F MRI.

^{19}F NMR/MRI is anticipated to serve as an intriguing detection alternative to the ^{1}H NMR/MRI owing to its high sensitivity (83% relative to ^{1}H) and the absent background signals in animal bodies.[1–3] In addition, high environmental sensitivity is another attractive property of ^{19}F nuclei. Taking advantages of these strengths, research on protein-based ^{19}F biosensors started sprouting in recent years, where genetic or non-genetic methods were elaborately utilized to site-specifically incorporate ^{19}F-probes into proteins.[4,5] In most cases, such biosensors sensitively monitored protein conformational change and/or ligand binding events through the chemical shift change in ^{19}F NMR spectroscopy.

We herein propose a novel ^{19}F-based lectin biosensor for selective detection of glycoproteins. Protein glycosylation, one of the most important and complicated post-translational modifications, is known to be involved in controlling a wide range of protein stabilities and functions.[6] Therefore, biosensors those are able to specifically detect glycoprotein are undoubtedly important from both fundamental science and clinical diagnosis perspective. In our strategy, lectins, a family of sugar-binding proteins, were employed as the desired sensor scaffold owing to their remarkable specificity for saccharides. We intended to exploit the large chemical shift anisotropy property of ^{19}F nuclei which intrinsically yields in the intimate relationship between the transverse relaxation time of ^{19}F NMR signals and the apparent molecular mass (M_r) to ^{19}F-probe.[7] It is thus reasonably expected that glycoproteins detection could be carried out through the transverse relaxation time change of ^{19}F-labeled lectins, and we also expect to distinguish glycoproteins from other small molecule saccharides on the basis of their M_r difference (Fig. 1a).

Assisted by affinity-guided DMAP (AGD) strategy previously reported by us (Fig. 1b),[8,9] construction of this unprecedented ^{19}F biosensor was achieved by incorporating a ^{19}F-probe with strict site-specificity in order to generate the sharp NMR signal of ^{19}F-modified lectin. Congerin II (CongII), a galactin isolated from skin mucus of conger eel,[10] was selected as the model lectin for the purpose of principle validation. Based on the knowledge that CongII selectively binds to Lactose/LacNAc with moderate affinity, the AGD reagent consisting of lactose for specific CongII recognition and DMAP moiety for acyl-transfer catalysis was designed (**1**, Fig. 1b).[8] To fulfill the intention of introducing ^{19}F probes to lectin, two thioester-type acyl donors containing 3,5-bis(trifluoromethyl)benzene moiety that carries six magnetically equivalent ^{19}F nuclei were synthesized and tested (**2** and **3**, Fig. 1b). Labeling reaction was carried out in buffer solution at 37 °C. After 4 h incubation, the labeling yield monitored by MALDI-TOF MS achieved 60% using acyl donors **2** whereas the labeling yield associated with shorter linker type acyl donors **3** was only about 30% (Fig. S1). Subsequent NMR measurements revealed **2**-labeled CongII as a sharp single peak at −62.7 ppm (Fig. 2a) whereas **3**-labeled CongII failed to produce any NMR peaks corresponding to the ^{19}F probe (Fig. 2b). In the contrary to **2**-labeled CongII, randomly modified CongII pre-

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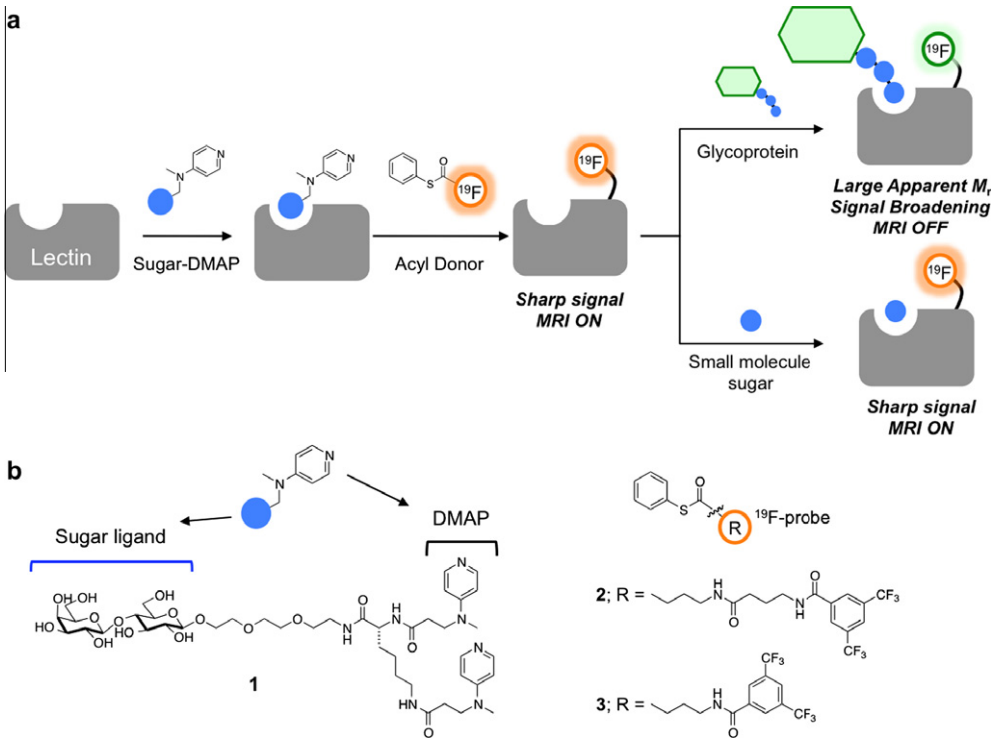

Figure 1. AGD catalyst-mediated selective and site-specific chemical protein labeling. (**a**) Schematic illustration of ^{19}F based lectin biosensor construction strategy. Signal of ^{19}F probe is expected to change upon the specific glycoprotein binding due to the apparent M_r change whereas no such NMR signal change is assumed upon the small sugar molecule binding. (**b**) Chemical structures of AGD catalyst **1**, and acyl donors **2** and **3** used in this study.

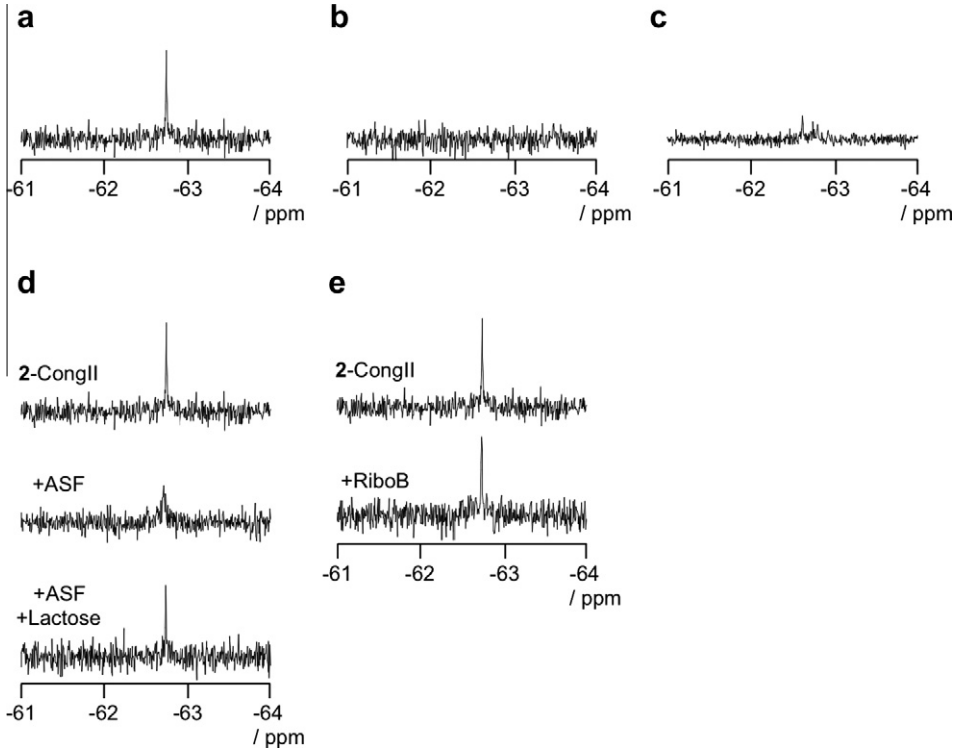

Figure 2. (**a–c**) ^{19}F NMR spectra of purified ^{19}F-labeled CongII (50 μM); (**a**) **2**-CongII prepared with **1** and **2**, (**b**) **3**-CongII prepared with **1** and **3**, (**c**) randomly-labeled CongII prepared with an excess amount of **2** alone for long time incubation. (**d**,**e**) ^{19}F NMR spectra of **2**-CongII upon glycoprotein binding experiments. (**d**) ^{19}F NMR spectra of **2**-CongII (50 μM) alone, in the presence of ASF (50 μM) and in the presence of both ASF and Lactose (2 mM) (top, middle and bottom, respectively). (**e**) ^{19}F NMR spectra of **2**-CongII (50 μM) alone and in the presence of RiboB (50 μM) (top and bottom, respectively). All experiments were performed in 50 mM HEPES buffer (pH 8.0, 0.2 mM TFA, 10% D_2O (v/v)) at 25 °C.

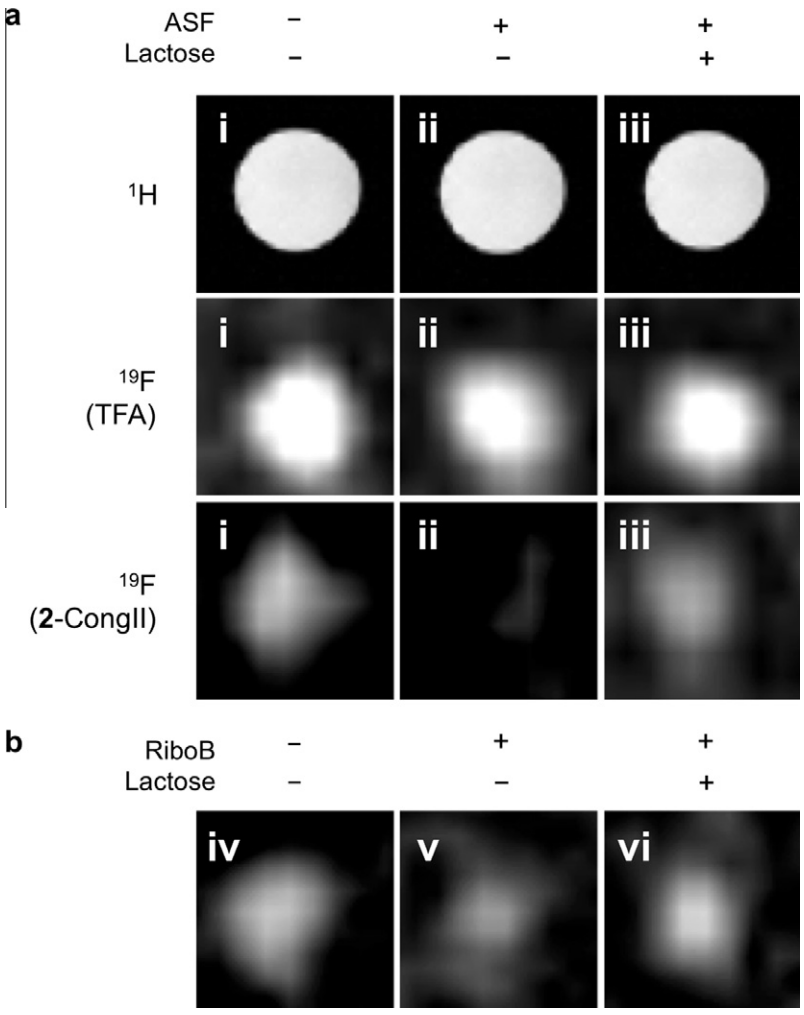

Figure 3. Magnetic resonance images of the samples containing **2**-CongII. (**a**) ^{1}H MR images (top), ^{19}F MR images corresponding to TFA (around at −75.6 ppm, middle) and ^{19}F MR images corresponding to **2**-CongII (around at −62.7 ppm, bottom) of the samples containing **2**-CongII alone (100 μM) (i), in the presence of ASF (100 μM) (ii) and in the presence of both ASF and lactose (4 mM) (iii) (**b**) ^{19}F MR images corresponding to **2**-CongII (around at −62.7 ppm) of the samples containing **2**-CongII alone (100 μM) (iv), in the presence of RiboB (100 μM) (v) and in the presence of both RiboB and lactose (4 mM) (vi). All the experiments were performed in 50 mM HEPES buffer (pH 8.0, 0.2 mM TFA) at 25 °C.

pared by incubating CongII with excessive amount of acyl donor **2** gave multiple weakly-intense NMR peaks (Fig. 2c), strongly indicating the importance of site-specific probe incorporation. Assisted by tyrpsin-digestion in combination with MS/MS analysis, labeling site of **2**-CongII was determined to be dominantly Tyr51 which locates at the sugar-binding pocket (Fig. S2).[8] These results altogether implied that DMAP-catalyzed protein engineering method works successfully for ^{19}F-labeled lectin construction.

Taking labeling efficiency as well as NMR peak quality into consideration, **2**-labeled CongII was employed for following glycoprotein detection studies. Glycoproteins that possess galactoside-rich saccharides at glycan terminals are anticipated to interact with CongII.[10a] As expected, gentle mixing of **2**-CongII with asialofetuin (ASF), a N-type glycoprotein with binding-affinity of 1.3×10^{7} M^{-1} towards CongII,[11] resulted in remarkable peak broadening depicted in ^{19}F NMR spectrum (Fig. 2d). Similar result was obtained when **2**-CongII biosensor was used for another known CongII-binding glycoprotein ovalbumin (OVA)[12] detection (Fig. S3a). In contrast, ^{19}F NMR peak intensity of **2**-CongII remained unaffected in the presence of ribonuclease B (RiboB) (Fig. 2e), a mannose rich glycoprotein,[13] or β-galactosidase (β-Gal) (Fig. S3b), a non-glycoprotein with large molecular weight. This unmistakable ^{19}F NMR difference hence strongly supported that the distinctive peak broadening resulted from ASF or OVA binding is ascribed to specific interaction between **2**-CongII and its binding glycoprotein partners, demonstrating that **2**-CongII works successfully for specific glycoprotein detection. These signal changes were observed in a saturation manner, suggesting that the present method would be theoretically applicable for quantitative determination of the glycoprotein concentration. Interestingly, the broadened ^{19}F NMR peak of **2**-CongII with ASF was almost completely recovered to its natural shrarpness again by the subsequent addition of an excess amount of lactose (Fig. 2d, bottom). These results therefore not only provided the evidence of specific interaction between **2**-CongII and galactoside sugars on ASF, but also brought up the importance of apparent molecular mass (M_r) on the peak broadening phenomenon. Indeed, M_r of **2**-CongII-ASF complex is estimated to be 100 kDa, which is more than threefold larger than **2**-CongII alone or **2**-CongII-lactose complex (about 30 kDa, CongII exist in a homodimer[10a] in the solution). Such significant M_r increment therefore substantially reduced the transverse relaxation time (T_2) of ^{19}F nucleus according to chemical shift anisotropy principle,[7] which in turn was reflected in NMR as broadened peak. Since small CongII-binding molecules such as lactose cause negligible M_r increment, NMR peak sharpness was preserved. In fact, T_2 measurements further supported the proposed glycoprotein detection

mechanism, that is, the T_2 value of **2**-CongII reduced from 0.34 sec to 0.14 sec upon the binding of ASF and recovered to 0.62 sec right after the subsequent addition of lactose.

Moving on from ^{19}F NMR experiments, more visually apprehensible and clinically applicable MRI studies were carried out on glycoprotein detections with the employment of our ^{19}F-based lectin biosensor. For each set of experiment, MR images of proton (^{1}H, Fig. 3a, top), ^{19}F attributed to lectin biosensor (Fig. 3b, bottom) and ^{19}F contributed by internal standard TFA (Fig. 3a, middle) were collected from the samples containing **2**-CongII. Prior to addition of glycoproteins, all ^{1}H and ^{19}F images of samples represented themselves as well-recognizable images (samples (i) and (iv)). After adding ASF, the image attributed to ^{19}F biosensor diminished, leaving only background signal level to be revealed (Fig. 3a, bottom, sample (ii)). Such phenomenon is due to significantly shortened T_2 value as proposed previously and it well agreed with the corresponding NMR results. The intensity was resumed later in the presence of excess amount of lactose (Fig. 3a, bottom, sample (iii)). On the other hand, mixing of ^{19}F biosensor and RiboB caused no much impact on the image intensity (Fig. 3b, samples (v) and (vi)), a predictable outcome judging from NMR results. Therefore, these results clearly indicated that our turn-off type ^{19}F-biosensor is a powerful tool for visualizing glycoprotein specifically.

In conclusion, we succeeded to develop the ^{19}F-based lectin biosensor for specific detection of glycoproteins by using the saccharide-tethered DMAP catalyst and ^{19}F-acyl donors. Constructed biosensor could distinguish glycoproteins from small molecule saccharides for the peak broadening in ^{19}F NMR directly relates to the M_r of each glycoconjugate. Furthermore, this distinct signal on/off switching was applied to visualize the lectin-glycoprotein interaction in a ^{19}F MRI phantom. We envision further applications of such chemically modified ^{19}F-biosensor for detection of various protein–protein interaction pairs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.038.

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