Supporting information

Carbohydrate microarray for the detection of glycan-protein interactions using metal-enhanced fluorescence

Jie Yang,[1*] Anne Moraillon,[1] Aloysius Siriwardena,[2] Rabah Boukherroub,[3] François Ozanam,[1] Anne Chantal Gouget-Laemmel[1,*] and Sabine Szunerits[3*]

[1]*Physique de la Matière Condensée, Ecole Polytechnique-CNRS, France*

[2] *Laboratoire de Glycochimie des Antimicrobiens et des Agroressources (LG2A), (FRE 3517-CNRS), Université de Picardie Jules Verne, 33 Rue St Leu, 80039 Amiens, France*

[3] *Institut d'Electronique, de Microélectronique et de Nanotechnologie (IEMN, CNRS-8520), Université Lille 1, Cité Scientifique, Avenue Poincaré – B.P. 60069, 59652 Villeneuve d'Ascq, France.*

[*] To whom correspondence should be addressed: Jie Yang (jie.yang@polytechnique.edu); Anne-Chantal Gouget-Laemmel (anne-chantal.gouget@polytechnique.fr); Sabine Szunerits (sabine.szunerits@iri.univ-lille1.fr)

1. Synthesis of β-propargyl lactoside

In a two-neck round bottomed flask containing a mixture of lactose (5 g, 13.88 mmol) and anhydrous pyridine (85.03 mL) under argon, acetic anhydride (25 mL) was added dropwise at 0°C. After 4 h at room temperature, the mixture was quenched by dropwise addition of aqueous hydrochloric acid (HCl) (12 M). The mixture was extracted with dichloromethane (CH_2Cl_2) and the organic phase was dried over sodium sulphate (Na_2SO_4) and filtered. The solvent was evaporated *in vacuo* to give gel-like white crude product (8.7 g, 80.6%). The practically pure per-O-acetyl-lactose (6.3 g, 9.3 mM) was placed in a two-neck round-bottomed flask under argon and dissolved in dry CH_2Cl_2 (50.65 mL), and then, propargyl alcohol (1.92 mL 30.84 mM) was added followed by dropwise addition of boron trifluoride ethyl etherate (BF_3OEt_2) (5.12 mL, 40.5 mM) at 0°C and then allowed to come to room temperature. After 22 h, the reaction was quenched by slow addition of a saturated sodium bicarbonate ($NaHCO_3$) solution. The mixture was extracted carefully with CH_2Cl_2 and the organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained by flash chromatography using cyclohexane/ethyl acetate (1/1) as eluent (1.01 g, 16.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ=1.90 (s, 3H, -CH_3); 1.978 (s, 3H, -CH_3); 1.980 (s, 3H, -CH_3); 1.987 (s, 3H, -CH_3); 1.994 (s, 3H, -CH_3); 2.06 (s, 3H, -CH_3); 2.08 (s, 3H, -CH_3); 2.39 (t, 1H, ≡CH); 3.72-3.83 (m, 2H, CH-OAc); 3.57-4.041 (m, 4H, CH_2-OAc); 4.27 (d, 2H, -CH_2-C≡); 4.42 (d, 1H, anomeric CH of Galactose Gal); 4.67 (d, 1H, anomeric CH of Glucose Glc); 4.82-5.29 (m, 6H, CH-OAc). ^{13}C NMR δ=20.50-20.84 (7C, CH_3); 55.85 (1C, -CH_2-C≡); 60.81 (1C, -CH_2-OAc); 61.81 (1C, -CH_2-OAc); 66.60 (1C, -CH-OAc); 69.09 (1C, -CH-OAc); 70.71(1C, -CH-OAc); 70.97 (1C, -CH-OAc); 71.30 (1C, -CH-OAc); 72.68 (1C, -CH-OAc); 72.75 (1C, -CH-OAc); 72.83 (1C, -CH-OAc); 75.43 (1C, ≡CH); 76.11 (1C, -C≡); 97.86 (1C, anomeric -CH of Glc); 101.02 (1C, anomeric -CH of Gal); 165.47 (1C, C=O); 166.40 (1C, C=O); 168.78 (1C, C=O); 168.98 (1C, C=O); 169.72 (1C, C=O); 170.03 (1C, C=O); 170.11 (1C, C=O); 170.31 (1C, C=O).

The crude per-*O*-acetyl propargyl-lactoside (1.01 g, 1.5 mmol) was dissolved in dry methanol (MeOH) (60 mL), and sodium methoxide was added (54 mg, 1 mmol). The reaction mixture was stirred vigorously for 2 h, and then treated with Amberlite resin (acid form) until neutral pH is obtained, then filtered and concentrated *in vacuo*. The mixture was purified by flash chromatography using ethyl acetate/MeOH (9/1) (70 mg, 12.3%). ^{1}H NMR (300 MHz, MeOD-d_4) δ=2.87 (t, 1H, ≡CH); 3.35-3.94 (m, 12H, -CH-OH); 4.37 (d, 1H, anomeric –CH of Gal); 4.42 (d, 1H, CH_2-C≡); 4.50 (d, 1H, anomeric –CH of Glc). ^{13}C NMR δ=56.64 (1C, -CH_2-C≡); 61.89 (1C, -CH_2-OH); 62.54 (1C, -CH_2-OH); 70.35 (1C, CH-OH); 72.58 (1C, CH-OH); 74.56 (1C, CH-OH); 74.85 (1C, CH-OH); 76.34 (1C, CH-OH); 76.40 (1C, CH-OH); 76.62 (1C, CH-OH); 77.12 (1C, CH-OH); 79.99 (1C, -C≡); 80.57 (1C, ≡CH); 101.98 (1C, anomeric -CH of Glc); 105.13 (1C, anomeric -CH of Gal).

Figure S1. Linear variation of the fluorescence as a function of ConA and PNA concentrations

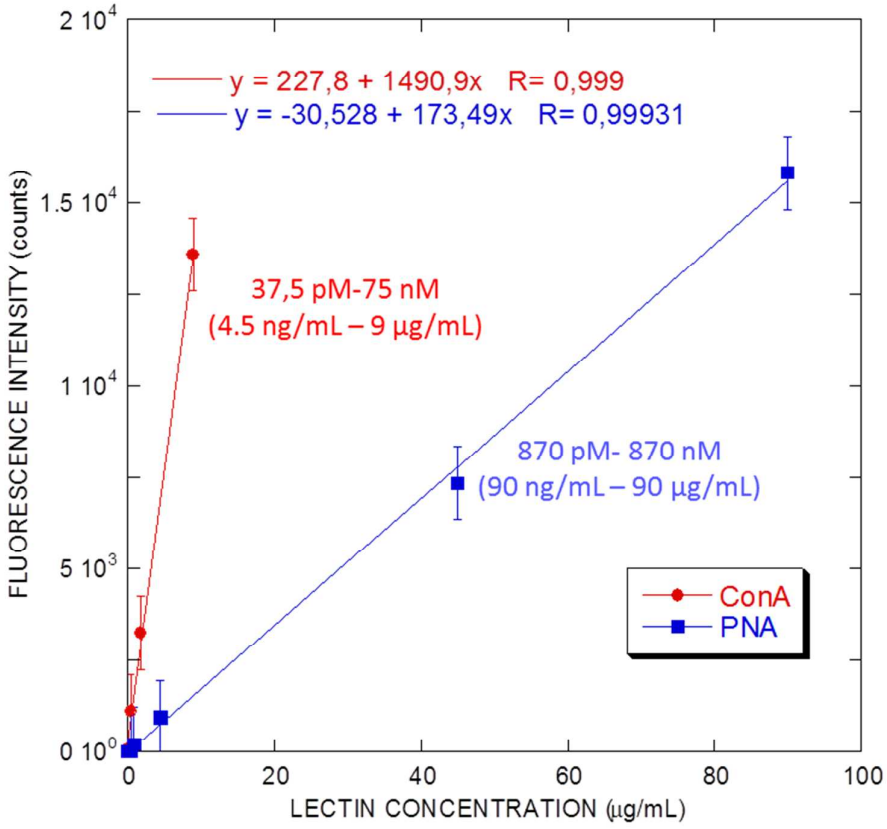

Table S1. Limit of detection (LOD) of some recently reported sensors for glycan-protein binding.

Technique	Binding types	K_D (μM)	LOD	References
E-SPR	PNA-lactose	0.45	41 nM	Gondran et al.[1]
SPR imaging	ConA-mannose	0.18	3.5 nM	Smith et al.[2]
SPR imaging	ConA-maltoheptaose		83 nM	Wang et al.[3]
QCM	ConA-mannose	0.045	130 pM	Lyu et al.[4]
QCM	ConA-mannose	1.15	90 nM	Zhang et al.[5]
Colorimetry	ConA-mannose		190 nM	Hone et al.[6]
Voltammetry	ConA-mannose		7 nM	Sugawara et al.[7]
Impedance spectroscopy	*lens culinaris* lectin -mannose	0.38	5 nM	Szunerits et al.[8]
Cantilever array sensor	ConA-mannose	15.3	9.6 nM	Gruber et al.[9]
Fluorescence microarray	ConA-mannose		9.6 nM	Park et al.[10]
Fluorescence microarray	ConA-mannose	0.08	25 nM	Liang et al.[11]
Fluorescence microarray	ConA-mannose		67 pM	Zhou et al.[12]
Field-effect transistor	*PA-IL* Lectin-galactose	6.8	2 nM	Vedala et al.[13]
Field-effect transistor	*E. Crystagalli* lectin-galactose		1.75 fM	Zhang et al.[14]

1. Gondran, C.; Dubois, M.-P.; Fort, S.; Cosnier, S.; Szunerits, S. *Analyst* **2008,** *133*, 206-212.
2. Smith, E. A.; Thomas, W. D.; Kiessling, L. L.; Corn, R. M. *J. Am. Chem. Soc.* **2003,** *125*, 6140-6148.
3. Wang, H.; Zhang, Y.; Yuan, X.; Chen, Y.; Yan, M. *Bioconjugate Chem.* **2011,** *22*, 26-32.
4. Lyu, Y.-K.; Lim, K.-R.; Lee, B. Y.; Kim, K. S.; Lee, W.-Y. *Chem. Commun.* **2008**, *39*, 4771-4773.
5. Zhang, Y.; Luo, S.; Tang, Y.; Yu, L.; Hou, K.-Y.; Cheng, J.-P.; Zeng, X.; Wang, P. G. *Anal. Chem.* **2006,** *78*, 2001-2008.

6. Hone, D. C.; Haines, A. H.; Russell, D. A. *Langmuir* **2003,** *19*, 7141-7144.
7. Sugawara, K.; Shirotori, T.; Hirabayashi, G.; Kuramitz, H.; Tanaka, S. *J. Electroanal. Chem.* **2004,** *568*, 7-12.
8. Szunerits, S.; Niedziółka-Jönsson, J.; Boukherroub, R.; Woisel, P.; Baumann, J.-S.; Siriwardena, A. *Anal. Chem.* **2010,** *82*, 8203-8210.
9. Gruber, K.; Horlacher, T.; Castelli, R.; Mader, A.; Seeberger, P. H.; Hermann, B. A. *ACS Nano* **2011,** *5*, 3670-3678.
10. Park, S.; Lee, M.-R.; Pyo, S.-J.; Shin, I. *J. Am. Chem. Soc.* **2004,** *126*, 4812-4819.
11. Liang, P.-H.; Wang, S.-K.; Wong, C.-H. *J. Am. Chem. Soc.* **2007,** *129*, 11177-11184.
12. Zhou, X.; Zhou, J. *Biosens. Bioelectron.* **2006,** *21*, 1451-1458.
13. Vedala, H.; Chen, Y.; Cecioni, S.; Imberty, A.; Vidal, S. b.; Star, A. *Nano Lett.* **2011,** *11*, 170-175.
14. Zhang, G.-J.; Huang, M. J.; Ang, J. A. J.; Yao, Q.; Ning, Y. *Anal. Chem.* **2013,** *85*, 4392-4397.
13. Vedala, H.; Chen, Y.; Cecioni, S.; Imberty, A.; Vidal, S. b.; Star, A. *Nano Lett.* **2011,** *11*, 170-175.
14. Zhang, G.-J.; Huang, M. J.; Ang, J. A. J.; Yao, Q.; Ning, Y. *Anal. Chem.* **2013,** *85*, 4392-4397.