Electronic Supplementary Information

A Fluorescent Nanoparticle Probe Based on Sugar-Substituted Tetraphenylethene for Label-Free Detection of Galectin-3

Zhiling Zhang, Yong Yu, Yueyue Zhao, Ka Ming Ng*

Department of Chemical and Biological Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

*Corresponding author: kekmng@ust.hk

Experimental section

Materials and characterization

All chemical reagents were purchased from commercial sources and used without further purification unless otherwise stated. The β -galactoside sugar derivative LacNAc-sp-NH₂ was purchased from Glyconz Limited. The TPE derivative **1** was synthesized according to our previous work. Galectin-3 was purchased from Abcam (ab50236). ¹H and ¹³C NMR spectra were taken on a Bruker ARX 400 NMR spectrometer in D₆-DMSO/D₂O (4:1). HRMS spectra were recorded on a Bruker Daltonics MicrOTOF ESI mass spectrometer in CH₃CN/H₂O (1:1) with calibrant added. Other reagents were all purchased from Sigma-Aldrich. Size distribution and average particle size were determined using a Brookhaven ZetaPlus potential analyzer. The morphology of the particles was studied by JEOL-6390 SEM. Pure water was produced by Millipore MilliQ Plus Water Purification System. Fluorescence intensity and spectrum were recorded by VarioskanTM LUX multimode microplate reader.

Synthesis of 2 (TPE-(LN)₂)

To a water solution of LacNAc-sp-NH₂ (10 mM, 12 mL) was added TPE derivative **1** (5 mM in DMSO, 10 mL). The mixture was incubated at room temperature for 6 hrs with agitation. The mixture was purified with HPLC to give product **2** as white powder. ¹H NMR (700 MHz, D₆-DMSO/D₂O (4:1)), δ (ppm): 7.17-6.87 (m, 18H), 4.64-4.50 (m, 4H), 4.301 (d, 1H, *J* = 8.3), 4.297 (d, 1H, *J* = 8.3), 4.229 (d, 2H, *J* = 7.1), 3.969 (s, HOD), 3.81-3.26 (m, 32H), 2.533 (m, DMSO), 1.792 (s, NAc), 1.783 (s, NAc), 1.664 (m, 4H). ¹³C NMR (151 MHz, D6-DMSO/D₂O (4:1)), δ (ppm): 171.11, 143.73, 143.65, 142.25, 140.65, 137.66, 130.90, 128.37, 128.26, 127.09, 126.97, 104.03, 101.16, 81.10, 75.79, 75.17, 73.29, 72.37, 70.94, 68.49, 67.42, 60.87, 60.63, 55.05, 39.26 (DMSO), 29.01, 23.12. HRMS (*m*/*z*): 1355.531 ([M+H]⁺ calcd. 1355.524); 678.269 ([M+2H]²⁺/2 calcd. 678.262).

Fluorescence detection

The solution for galectin-3 detection was prepared by mixing 90 μ L of PBS buffer (10 mM, pH 7.4) with 10 μ L TPE-(LN)₂ stock solution (1 mM, in DMSO) thoroughly, followed by addition of galectin-3 stock solution (1 mg/mL). Fluorescence measurement under UV irradiation (365 nm)

was carried out with the prepared solution incubated at 37 °C for 15 min. The fluorescence intensity at 470 nm was read as the fluorescence response for detection. For control experiment, the 1 μ L galectin-3 stock solution was pre-incubated with 1 μ L LacNAc stock solution (1 mM). For interference study, galectin-3 was replaced by BSA, lectin, glucose, maltose, glutamic acid, lysine, Ca²⁺ and Na⁺. For establishment of standard curve, the volume of galectin-3 stock solution added varied between 0-1 μ L to make the final concentration of galectin-3 between 0-10 μ g/mL. For the tests in FBS, the solution for galectin-3 detection was prepared by mixing 85 μ L of PBS buffer, 5 μ L FBS, and 10 μ L TPE-(LN)₂ stock solution thoroughly, followed by addition of 0.2 μ L, 0.6 μ L or 1 μ L galectin-3 stock solution.



Fig. S1 ¹H NMR spectrum of TPE-(LN)₂



Fig. S2 ¹³C NMR spectrum of TPE-(LN)₂



Fig. S3 HRMS spectrum of TPE-(LN)₂. $[M+H]^+ = 1355.531$



Fig. S4 HRMS spectrum of TPE-(LN)₂. $[M+2H]^{2+}/2 = 678.269$



Fig. S5 Fluorescence spectra of TPE-(LN)₂ in DMSO/PBS buffer mixtures with different water fractions (f_w). [TPE-(LN)₂], 100 μ M; Excitation wavelength, 365 nm.