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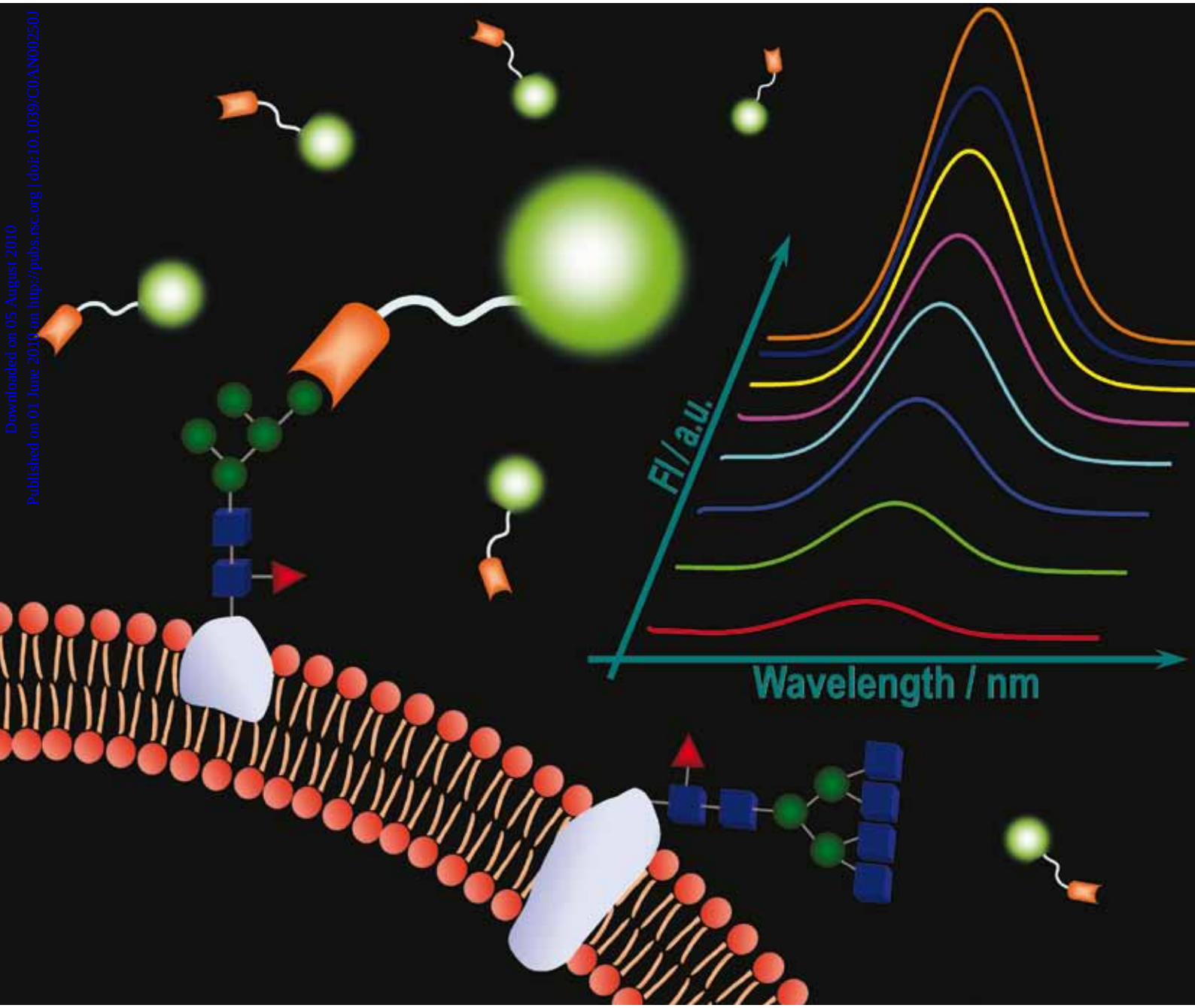

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A simple fluorescent strategy for *in situ* evaluation of cell surface carbohydrate with a quantum dot–lectin nanoprobe†

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A simple and rapid fluorescent method was developed for the *in situ* evaluation of cell surface carbohydrate by homogeneous specific recognition of a quantum dot–lectin nanoprobe to mannosyl groups on the cell surface. The strategy was further used for dynamically monitoring the alteration of cell surface carbohydrate expression in response to drugs.

Carbohydrates, a major class of biomacromolecules, play important roles in cell growth and development, immune recognition and response, tumor growth and metastasis, anti-coagulation, cell–cell communication and microbial pathogenesis.[1] Aberrant carbohydrate expression is closely related to diseases such as cancer. However, the study for glycobiology has been impeded, mainly because of the inherent difficulties in glycan analysis.[2] As a result, developing simple and sensitive strategies for the evaluation of carbohydrate expression on living cells is of great importance for deciphering glycodes.

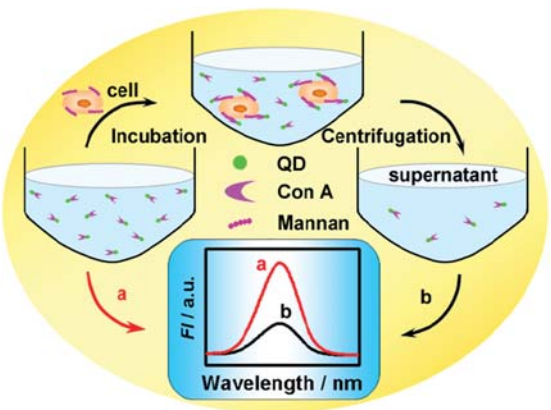
Current efforts for carbohydrate analysis have been focused on the usage of mass spectrometry and chromatography.[3] Although mass spectrometry can supply structural information, it is unsuitable for the analysis of carbohydrates on living cells due to its destructiveness. A lectin array-based platform has been used to identify and differentiate the structure of carbohydrates on living cells by means of the specific recognition of lectins to carbohydrates.[4] However, these analytical systems generally involve the fluorescent labeling of cells, which disturbs the cellular behaviors. In addition, the autofluorescence of cells also affects the sensitivity of these methods. Moreover, because of the inaccessibility of the active sites, the sensitivity and stability of these methods were impaired. In this work, a simple, rapid and low-cost strategy was developed for *in situ* cell surface carbohydrate assay by combining a quantum dot (QD)–lectin fluorescent nanoprobe with a specific and homogeneous recognition process.

QDs have been adopted for analytical and biological applications as excellent *in vivo* and *in vitro* fluorescent probes,[5] owing to their unique optical properties, small size, long-term stability and good water solubility. The combination of QDs with lectin introduced a specific carbohydrate recognition ability to QDs, thus providing stable and efficient fluorescent nanoprobes for the *in situ* interrogation of carbohydrate on cell surfaces without suffering from steric effects.

Herein, the mercaptopropionic acid (MPA)-capped CdTe QD was linked to concanavalin A (Con A, a lectin with specificity for mannosyl groups) for the fabrication of a mannose-specific nanoprobe (see ESI†). The prepared QD–lectin nanoprobe could bind to cells by specific recognition of Con A for cell surface mannosyl groups using leukemic K562 cells as a model. After homogeneous incubation of cells with the nanoprobe solution and subsequent removal of the nanoprobe-bound cells, the decrease in fluorescence intensity (*FI*) of the nanoprobe solution was related to the cell amount and the expression extent of the mannosyl groups on the K562 cells (Scheme 1). At saturated binding, the change in *FI* could be used to quantify the average number of mannosyl groups on the cell surface. To further demonstrate the feasibility of the proposed strategy, it was applied for the dynamic monitoring of the change of carbohydrate expression on cancer cells in the presence of a drug. The proposed method possesses promising applications in clinical diagnosis and the therapy of human cancers.

The synthesis of water-soluble MPA-capped CdTe QDs was carried out using the method reported previously.[6] For preparation of the nanoprobe, 700 μL MPA-capped CdTe QD solution (7.6 μM) was mixed with EDC (1 mg in 50 μL phosphate buffered saline, PBS) and Con A (250 μL, 1 mg mL^{-1} in PBS). After incubation for 3 h at 25 °C under shaking in the dark, the resulting mixture was ultrafiltrated at 3000 *g* for 12 min at 4 °C to remove the non-conjugated QDs and by-product. After the obtained conjugates were washed with 50 mM pH 7.4 Tris-HCl buffer and then 10 mM pH 7.4 PBS for three times by ultrafiltration, the resulting nanoprobe was diluted to 700 μL and kept at 4 °C.

The prepared QD–Con A nanoprobe was characterized by UV-vis spectrometry and Fourier transform infrared spectrometry (FT-IR) (Fig. S1 in ESI†). Successful conjugation was verified by the existence



Scheme 1 Schematic representation of the fluorescent strategy for monitoring cell surface carbohydrate expression.

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† Electronic supplementary information (ESI) available: experimental details, characterization of quantum dot–lectin nanoprobe and experimental optimization. See DOI: 10.1039/c0an00250j

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of the characteristic absorption peak of the QDs at 520 nm in the spectrum of the nanoprobe due to the much smaller size of the free QDs, which could be removed during the ultrafiltration process. The IR spectrum of the nanoprobe showed two peaks located at 1643 and 1535 cm^{-1}, which could be ascribed to the carbonyl stretching vibration (amide band I) and the N–H bending vibration (amide band II) of protein, respectively. Thus the MPA-capped QDs were bound to Con A.

Considering that the 1 : 1 QD to Con A ratio could be expected,[7] the concentration of QD–Con A nanoprobes was obtained by measuring the Con A concentration using the Bradford method,[8] which is a spectroscopic analytical procedure widely used to measure the concentration of protein in a solution. Using Con A as the model protein, the binding of Coomassie brilliant blue G-250 to the lectin led to an absorption peak at 595 nm. Its intensity is proportional to the concentration of the protein, resulting in a calibration curve for the determination of the QD–Con A nanoprobe concentration (Fig. S2 in ESI†). As a result, the concentration of the QD–Con A nanoprobe solution was 7.2 μM.

The fluorescence spectra of the prepared nanoprobe in PBS containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} showed an obvious fluorescent emission peak at 552 nm, owing to the luminescent behavior of the QDs (Fig. 1A). Upon increasing the nanoprobe concentration from 0.54 to 1.80 μM, the *FI* increased linearly, with a linear relation coefficient of 0.998 ($n = 8$) (Fig. 1B).

A blocking experiment was performed on a 24-well plate to demonstrate the specific binding of the nanoprobes to the cell surface mannosyl groups (Fig. 2). In order to simplify the manipulation, a type of adherent tumor cell, BGC-823, was used, with which the redundant reagents could easily be removed without a centrifuging step after reaction with nanoprobe or Con A followed with nanoprobe. After the BGC-823 tumor cells were incubated with the QD–Con A nanoprobe for 1 h and subsequent washed with PBS, the cells displayed distinct fluorescence emission from the QDs. Nevertheless, initial reaction of the BGC-823 tumor cells with Con A led to the occupation of the mannose sites on the cell surface, which prevented subsequent binding of the QD–Con A nanoprobe, and thus the fluorescence emission could hardly be detected.

With the nanoprobe in hand, the mannose expression on the K562 cell surface was evaluated through comparing the *FI* of the nanoprobe solution before and after cell incubation. QD–Con A nanoprobe solutions (0.5 mL) at different concentrations were firstly mixed with K562 cell suspensions of 10^4 cells mL^{-1} containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} in a 24-well cell culture cluster (Costar, USA), respectively, for optimizing the nanoprobe concentration to obtain saturated binding of the nanoprobe to the cell surface mannose moieties. The homogeneous recognition was performed at 25 °C under gentle shaking in the dark. The optimal incubation time was 1 h (Fig. S3 in ESI†), during which the fluorescence of the nanoprobe solution in the presence of 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} was stable. After the nanoprobe was bound to the cell surface owing to the specific recognition of the lectin-contained nanoprobe to the mannose moieties, the cells was separated from the mixed solution by centrifugation at 1000 rpm for 10 min. The *FI* of the supernatant was measured, and a decrease of *FI* compared with the initial nanoprobe solution could be observed. The difference in fluorescence intensity (Δ*FI*) was directly related to the amount of QD–Con A nanoprobe that was captured by cells. As shown in curve **a** (Fig. 3A), with the increase of the nanoprobe concentration, the

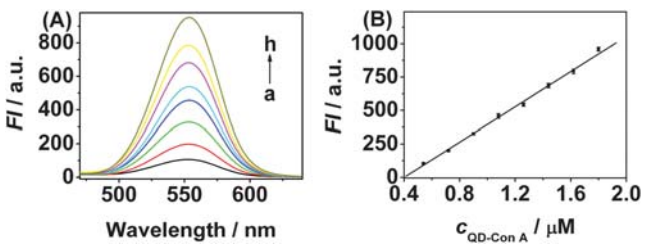

Fig. 1 (A) Fluorescence spectra of 0.54, 0.72, 0.90, 1.08, 1.26, 1.44, 1.62, 1.80 μM QD–Con A nanoprobes in PBS containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} (from a to h); (B) linear calibration plot of *FI vs.* the concentration of QD–Con A nanoprobe.

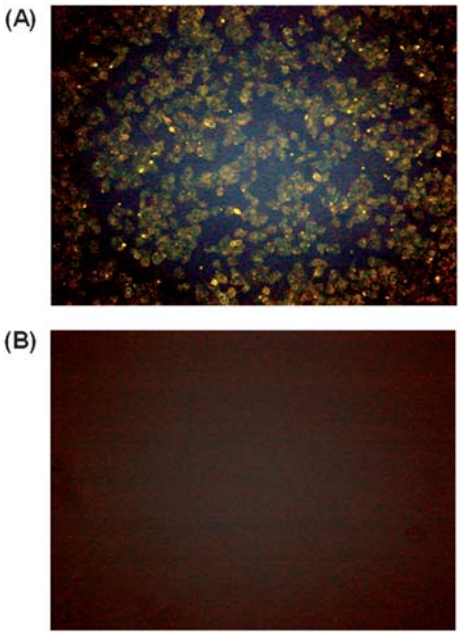

Fig. 2 Fluorescence images of BGC-823 cells after reaction with (A) 0.5 μM QD–Con A nanoprobe for 1 h and (B) 0.5 μM Con A for 30 min and then 0.5 μM QD–Con A nanoprobe for 1 h. The incubation was performed in the presence of 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}.

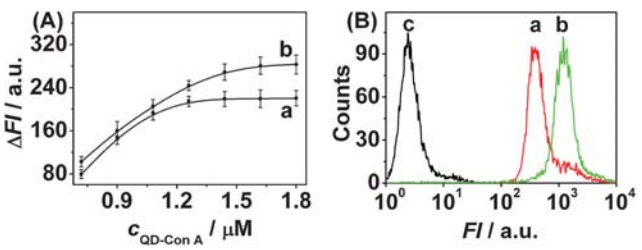

Fig. 3 (A) Plots of Δ*FI* of QD–Con A nanoprobe *vs.* the initial concentration of nanoprobe upon incubation with (**a**) normal K562 cells and (**b**) Swainsonine (SW)-treated K562 cells; (B) flow cytometric analysis of Con A-binding sites expressed on the K562 cell surface (**a**) before and (**b**) after treatment with SW for 2 days, and (**c**) autofluorescence of unlabeled K562 cells. The recognition was performed in the presence of 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}.

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ΔFI value increased and reached a plateau at *ca.* 1.2 μM, indicating the saturated binding of the nanoprobe by the cells.

Using the fluorescent calibration curve (Fig. 1B), the maximum amount of nanoprobe bound to the cell surface, corresponding to the plateau value of ΔFI, could be obtained to be 1.01×10^{14}. Since the amount of cells for incubation was fixed at 5000 cells, the saturated amount of the cell-bound nanoprobe was associated with the expression extent of mannosyl groups on the K562 cells. Thus the average number of mannosyl groups expressed on the cell surface for four determinations could be calculated to be 2.01×10^{10} with a relative standard deviation of 7.9%, showing a good reproducibility. This result was in good agreement with that of 2.3×10^{10} reported in previous work.[9] The presented strategy detected the fluorescence of the nanoprobe in cell-free solution, and thus it was not influenced by cell autofluorescence. The homogeneous recognition system also avoided the steric effect during the binding process.

The proposed method could be used for not only evaluating the carbohydrate expression, but also for dynamically monitoring its change on living cells. Swainsonine (SW), a well-established mannosidase inhibitor, was chosen as the model drug to treat the cells for verifying the ability of dynamic monitoring. SW could increase the expression of terminal high-mannose-type glycan on the cell surface, and is a potential anti-metastasis drug.[10] SW-treated K562 cells were obtained by culturing the cells in a medium containing SW (2 μg mL^{-1}) for 2 days. After homogeneously incubating the nanoprobe with the SW-treated cells at the same concentration as that of the normal cells for 1 h, the *FI* of the supernatant was measured to obtain the ΔFI value. The variation tendency of ΔFI *vs.* the nanoprobe concentration was similar to the normal cells (curve **b**, Fig. 3A). The saturated binding occurred at the nanoprobe concentration of 1.5 μM, indicating a greater expression of mannosyl groups on SW-treated K562 cells. The average amount of mannose moieties on the SW-treated K562 cell surface could be evaluated in the same way. Compared with normal cells, the mannose moieties increased by 28.4%, giving a statistically significant change ($p < 0.05$, calculated by one-way ANOVA[4a]). The increase of the carbohydrate expression monitored by the designed fluorescent method was consistent with that obtained by flow cytometric analysis using fluorescein isothiocyanate (FITC)-labeled lectin for recognition (Fig. 3B).

The time-dependent variation of carbohydrate expression upon SW treatment was also monitored using the proposed strategy. During treatment with SW over 56 h, the SW-treated cells revealed a progressive increase of ΔFI at the nanoprobe concentration of 1.6 μM, and tended to a plateau at 48 h (Fig. 4). This result indicated that Con A-recognizing carbohydrates on cell surfaces were sensitive to SW treatment.

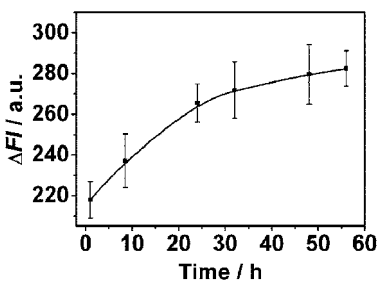

Fig. 4 Plot of ΔFI of QD–Con A nanoprobe upon incubation with SW-treated K562 cells *vs.* SW treatment time, using the nanoprobe concentration of 1.6 μM.

In conclusion, a simple and rapid fluorescent method was proposed for the *in situ* evaluation of carbohydrate expression on living cells by use of a QD–Con A nanoprobe in a homogeneous recognition system. The nanoprobe could efficiently bind to the K562 cells by the specific recognition of cell surface mannosyl groups to Con A. The average amount of mannosyl moieties on cell surface could be obtained by fluorescently evaluating the nanoprobe-binding capacity of the K562 cell. The method was used for monitoring the alteration of cell surface glycans in response to a drug, showing high sensitivity, good reproducibility and acceptable accuracy. The fluorescent detection was not influenced by cell autofluorescence. In addition, the recognition system was homogeneous, and therefore all the mannose groups on the cell surface are accessible during the binding process. The method was low cost and could be conveniently extended for the analysis of other carbohydrates on the cell surface, thus providing a basis for monitoring the cancer disease progression at the molecular level.

Acknowledgements

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