Supporting Information

Single Quantum Dot-Based Nanosensor for Sensitive Detection of O-GlcNAc Transferase Activity

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Mechanism of the Protein Glycosylation

The O-GlcNAc post-translational modification occurs by the addition of a single N-acetylglucosamine (O-GlcNAc) residue to the serine residues of protein. The OGT uses an ordered bi-bi mechanism with UDP-GlcNAc binding first, followed by the peptide substrate. OGT catalyzes sugar transfer, generating UDP and the O-GlcNAc-modified peptide (Figure S1).[1,2]

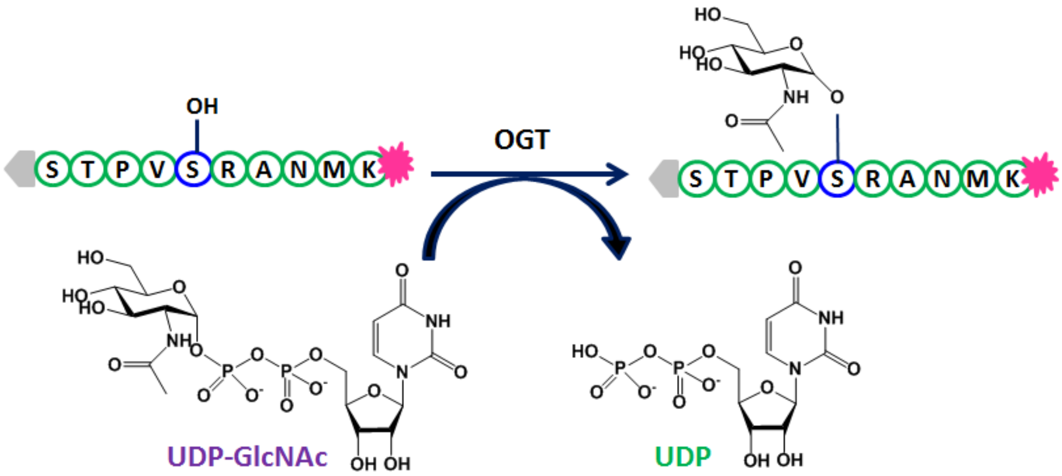

Figure S1. Mechanism of the O-GlcNAc glycosylation of Cy5/biotin-modified peptide. OGT catalyzes the transfer of GlcNAc from UDP-GlcNAc to the serine residues of the substrate peptides, generating the glycosylated peptides.

Analysis of FRET Data

The FRET efficiency (E) can be experimentally obtained from $E = 1 - \frac{F_{DA}}{F_D}$, where F_{DA} is the fluorescence intensity of donor in the presence of the acceptors and F_D is the fluorescence intensity of donor alone in absence of the acceptors. The QD-Cy5 center-to-center separation distance r was estimated by fitting the efficiency E using Förster theory and taking into account that multiple (n) dye acceptors simultaneously interact with the same QD donor. The FRET efficiency for 605QD-peptide-Cy5 nanostructure is given by: $E = \frac{nR_0^6}{nR_0^6 + r^6}$. R_0 is the Förster distance corresponding to E = 50% and is expressed as $R_0 = 0.02108(k^2 \emptyset_D n^{-4} J)^{1/6}\ (nm)$, where κ^2 is the orientation factor (2/3 for randomly oriented dipoles, n is the refractive index of the medium (~1.4 for biomolecules in aqueous solution), Q_D is the quantum yield of the 605QD donor (~0.7), and the spectral overlap integral J is calculated in M^{-1} cm^{-1} nm^4.[3] We calculated the Förster distance for 605QD/Cy5 pair, R_0 = 77 Å.

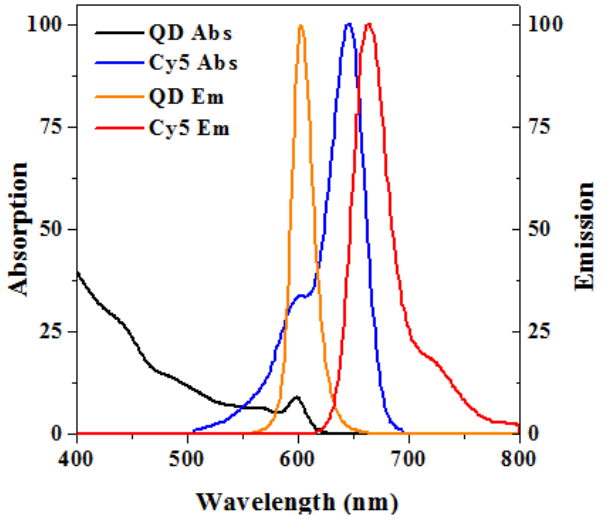

Figure S2. The normalized absorption and emission spectra of the 605QD and Cy5. Black line, absorption spectrum of the 605QD; orange line, emission spectrum of the 605QD; blue line, absorption spectrum of Cy5; red line, emission spectrum of Cy5.

Influence of Substrate-to-QD Ratio on FRET Efficiency in the Ensemble Measurement

We employed the fluorescence measurement to optimize the addition of peptide-to-QD ratio. The biotin/streptavidin-linked QD nanostructure was formed through self-assembly of biotinylated peptides to the streptavidin-coated QDs. The 1 pmol of 605 nm streptavidin-coated QDs was mixed with 0, 6, 12, 24, 36, 42, 48, and 60 pmol of biotinylated peptides and the incubation buffer with a final QD concentration of 10 nM in total volume of 100 μL, followed by incubation at room temperature for 20 min prior to the measurement. The reaction products were subjected to fluorescence measurement. As shown in Figure S3A, with the increase of the biotin/Cy5-modified peptide-to-QD ratio, the fluorescence emission of QD donor at 605 nm decreases, while the Cy5 emission at 670 nm increases, indicating efficient FRET from the QD to Cy5. As shown in Figure S3B, both the FRET efficiency and the Cy5 fluorescence intensity increase with the increasing ratio of the biotin/Cy5-modified peptide to QD from 6 to 48, and reach a plateau beyond the ratio of 48. Additionally, the Cy5 fluorescence intensity shows a linear relationship with the peptide-to-QD ratio in the range from 6 to 48 (red line in Figure S3B). Thus, the addition of peptide-to-QD ratio of 48 is used in the fluorescence measurement.

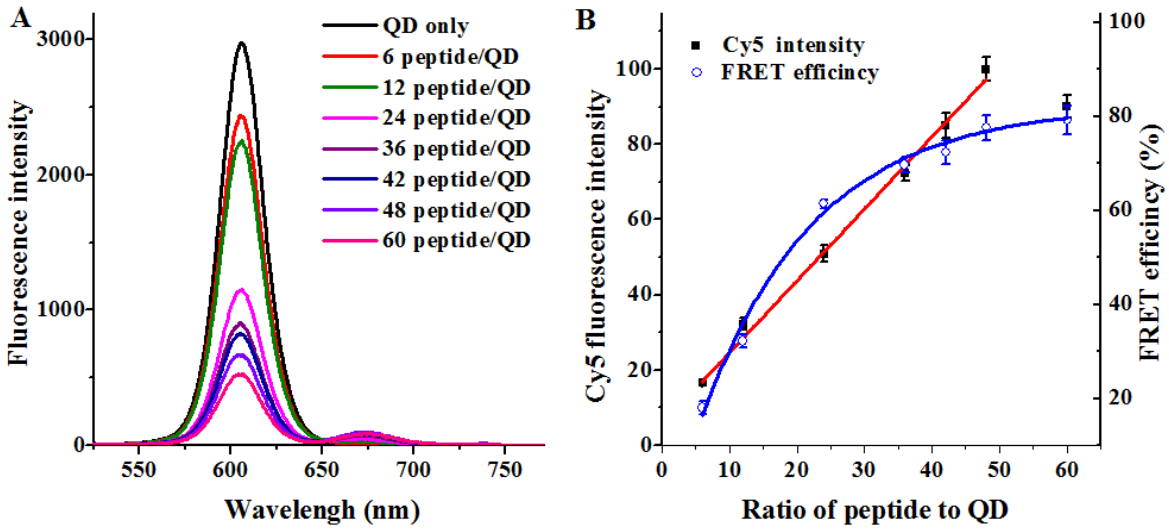

Figure S3. (A) Variance of the QD and Cy5 fluorescence with the increasing peptide-to-QD ratio. (B) Variance of Cy5 fluorescence intensity and FRET efficiency with the increasing peptide-to-QD ratio measured in the ensemble measurement. The QD concentration is 10 nM. Error bars show the standard deviation of three experiments.

Conversion Equation. The Cy5 counts are measured in the experiments. To convert the Cy5 counts to the concentration of UDP-GlcNAc, we performed a series of experiments using various-amount UDP-GlcNAc, high-concentration enzyme and high-concentration peptide to ensure the complete proceeding of reaction.[4] The data were fitted with a linear equation (Figure S4), with the measured Cy5 counts being equivalent to the concentration of UDP-GlcNAc. The consumed UDP-GlcNAc may be calculated from the standard curves.

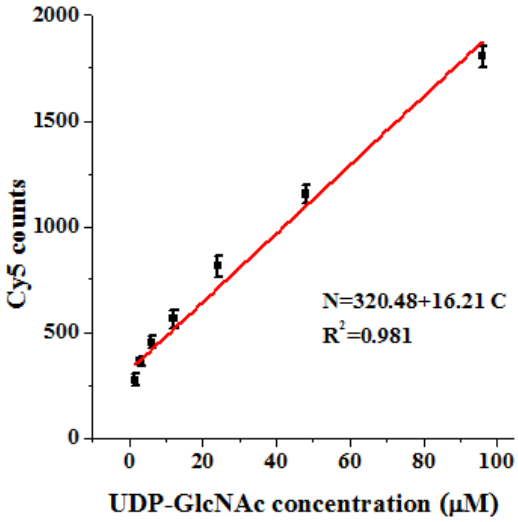

Figure S4. Variance of the Cy5 counts with the concentration of UDP-GlcNAc. Error bars show the standard deviation of three experiments.

Detection of OGT from Cell Extracts.

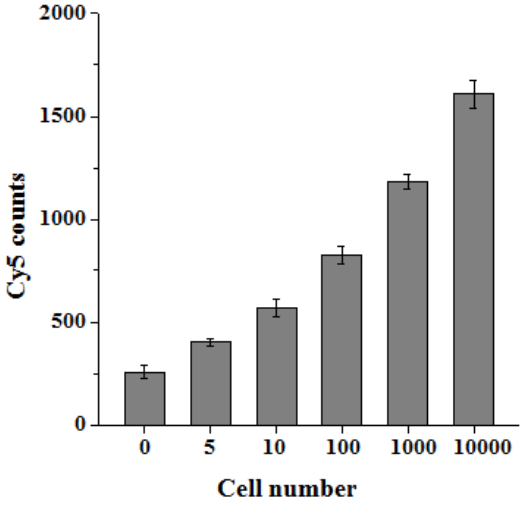

Figure S5. The measured of Cy5 counts in response to the number of HEK-293 cells from 0 to 10000 cells. Error bars show the standard deviation of three experiments.

References

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