Specific labeling of Sialic Acids on Living Cells by Quantum Dot Tagged with Phenylboronic Acid

Introduction:

Sialic acids (SA) are commonly found at the terminal position of the glycan structures on the cell membrane. Changes in SA expression are closely associated with various disease states such as cancer, cardiovascular, and neurological diseases. Phenylboronic acid (PBA) was found to form favorable binding with SA among common carbohydrates (mannose, glucose, galactose, and SA) on cell surfaces at the physiological pH of 7.4. To specifically and efficiently label SA on living cells, PBA was linked to the surface of Quantum Dots (QDs) and the resulting conjugates was used for cancer cell staining. QD staining produces better imaging and are resistance to photobleaching, allowing microscopic observation of cells for extended periods of time (up to more than five months).

Materials:

Cells grown on a surface (for example, culture plates, chamber slides, cover slips, etc) with a confluency >50%; QD-PBA (Ocean's Catalog # QBO); SA (Sigma); sialidase (Neuraminidase from *Clostridium perfringens*, Sigma); 10 mM PBS buffer pH 7.2; Ocean's blocking buffer that has been optimized with Ocean's nanoparticles, (Catalog # BBB), to minimize non-specific binding of the QDs.

Procedure:

1. **Sialidase treatment for negative control samples:** Incubate cells on glass cover slips in RPMI without FBS and containing sialidase at a final concentration of 40 mU/mL for 30 min at 37 ºC.
2. **Free SA competition for negative control samples:** Incubate cells on glass cover slips in PBS containing 10 mM free SA for 10 min at RT.
3. **QD incubation:** Add QBO (QD600-PBA) at a final concentration of 20 nM to the cover slips in 1 and 2. Incubate at RT for 30 min. When positive, the cell membrane will appear reddish orange which is the color of the QD.
4. **Washing:** wash cells with BBB twice and PBS once for 5 min each time
5. **DAPI staining to see the cell nucleus (appears green):** Put one drop of 10 ug/ml DAPI solution on the cover slips and wait for 5 min. Wash off DAPI solution with DI water.
6. **Mounting:** place 90% glycerol on a fresh clean microscope glass slide and face down (the cells must get buried on the glycerol). Seal the cover slip with nail polish and allow to dry at RT.
7. **Observation:** Mount the slide on a microscope and observe for fluorescence under a UV light source.

Results

The results in Figures 1 and 2 are typical for cells treated with Ocean's QBO. In Figure 1 A and Figure 2A, the cell membrane appear bright red when the cells were incubated with the QBO. The negative control in Figure 1B, Figure 1C, Figure 2B, and Figure 2C, which were with free SA and sialidase respectively, did not get stained with the QBO. These results indicate that we can see the cells expressing SA very clearly with the QBO.

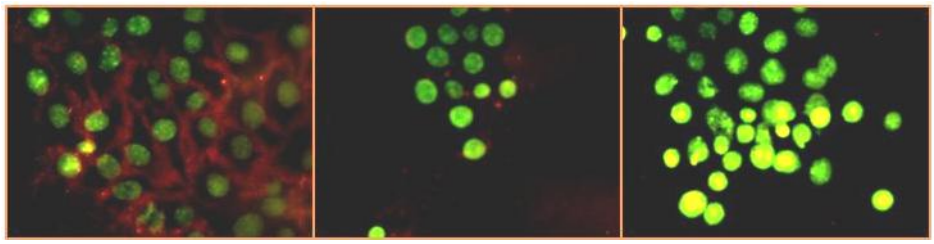

Figure 1. Mouse cancer 4T1 cells stained with QBO600. 4T1 cells were treated with PBS (A), free SA (B) or sialidase (C) before staining with 20 nM of QBO600 (magnification 40×).

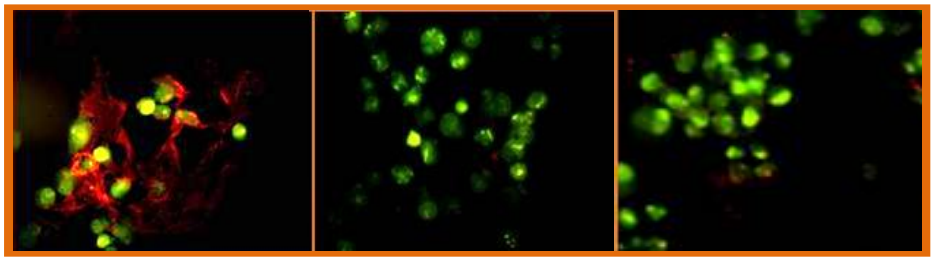

Figure 2. Human breast cancer MDA-MB-231 cells stained with QBO600. MDA-MB-231 cells were treated with PBS (A), free SA (B) or siliadase (C) before staining with 20 nM of QBO600 (magnification 40×).