The AptaReport™
Newsletter
SUMMER 2018

## Phototoxic Aptamers that Target and Neutralize Epithelial Cancer Cells

Normally, oligonucleotides on their own cannot cross a cell membrane. However, once developed into aptamers that bind to specific surface markers/portals, entry into the cell can be achieved. Epithelial cancer cells produce a unique O-glycan-peptide called MUC1 that is expressed on the cell surface. MUC1 is specific to only epithelial cancer cells and is not found on the surface of healthy epithelial cells. MUC1 is regularly recycled through the cell via the trans-Golgi network to undergo further modifications to its glycan structure.

By using aptamers to target MUC1, Ferreira et al. were able to achieve cancer cell specific intracellularizaiton of aptamers 5′ modified with the phototoxic agent chlorin e6 (Ce6). The addition of Ce6 to the 5′ end of the aptamer transforms the aptamer into a targeted drug delivery system that can selectively neutralize cancer cells. Ce6 is a small organic molecule that releases toxic reactive oxygen species (1O2) upon 664 nm light activation. A scheme depicting the mechanism of action for Ce6-aptamer targeting and neutralizing epithelial cancer cells containing the MUC1 glycan-peptide is shown below.

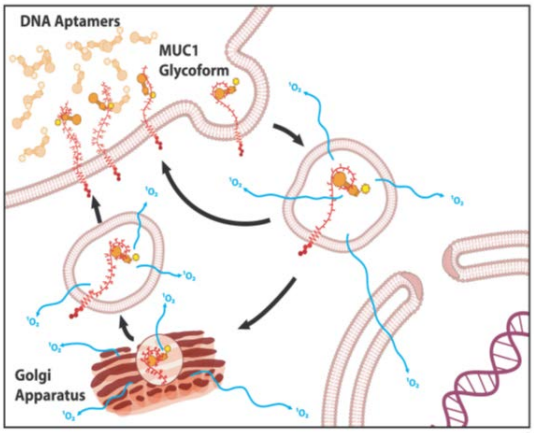


*Figure (adapted)* Aptamers (bound-dark orange, unbound- light orange) bound to MUC1 (red branched structure) are internalized eventually undergo endocytosis. Upon activation of 664nm light, reactive oxygen species (1O2 blue) are released from attached Ce6 (yellow). These reactive oxygen species are cytotoxic thus inducing cancer cell specific cell death.*

-B.R.

*Reference: Apta-Index™ ID #635

## GCGR Protein regulation via aptamer to potentially treat Type II Diabetes

Glucagon receptor (GCGR) is a trans-membrane protein that plays a critical role in the regulation of blood glucose levels. Inhibiting GCGR activity could potentially reduce excess glucose production, suggesting that an aptamer that binds and inhibits GCGR could provide possible therapeutic properties to help treat diabetes mellitus.

No glucagon receptor antagonists or antibodies have made it through final market approval to date. Drug candidates often fail due to problems related to high toxicity or lack of specificity. Wang et al recognized this clinical need, and that aptamers have been shown to produce no immunogenic response—and thus have no toxicity profile—and are generated based on their selective properties. As a result, Wang et al selected for aptamers targeting GCGR protein via cell-SELEX. Of their selected aptamer candidates, GR-3 showed the most promise, and was used in subsequent studies to verify target and determine effect of temperature on aptamer binding. A knockdown assay was run and verified that GCGR was GR-3's target, and the temperature study showed no significant change in aptamer activity between 4-37 degrees Celsius.

Aptamers have advantages in biological systems over other therapeutic agents because they are small, target-specific, non-immunogenic, and easily modified for increased stability. Thus, GR-3 has great therapeutic potential as a molecular probe and a possible tool for treatment and/or management of diabetes mellitus.

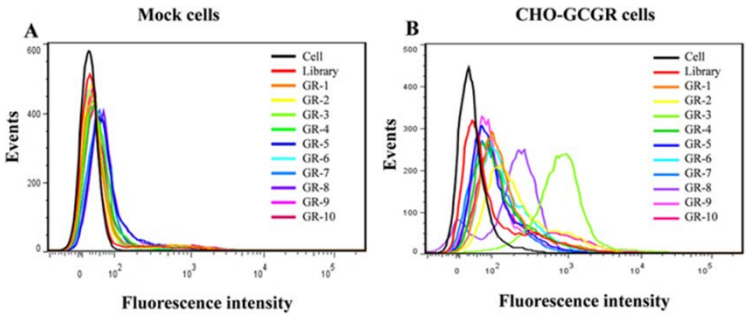


*Figure (adapted)**
*A) Binding of aptamer candidates to Mock cells (control cells) was analyzed by flow cytometry. The unselected initial ssDNA library was used as negative control.*
*B) Binding of CHO-GCGR cells (target cells) was analyzed. GR-3 proving to be the strongest candidate.*

-A.E.

*Reference: Apta-Index™ ID #632

## *Bifidobacterium* bifidum detection via high throughput colorimetric Enzyme Linked Aptamer Assay (ELAA).

B. bifidum has been reported to potentially prevent inflammatory bowel disease, reduce cholesterol, prevent diarrheal disease in infants, and even impact the evolution and maturation of their host's immune response. As a result, B. bifidum and other lactic acid bacteria are often sought for use in probiotic products. However, currently available approaches identifying bacteria for industrial use are both laborious and time-consuming. An aptamer-based detection method could potentially alleviate these issues.

Hu et al. successfully identified aptamer CCFM641-5 against B. bifidum for use in the development of an enzyme linked aptamer assay (ELAA), an aptamer-based ELISA. A proteinase study allowed Hu et al. to determine that the target of the aptamer was likely a membrane protein since binding ability was reduced after cell treatment.

For ELAA development, CCFM641-5 was used to both capture and detect B. bifidum. Since multiple copies of any given membrane protein will likely exist on a single cell's surface, its capture should not inhibit/block detection. A microplate reader was employed to measure the resulting optical density (OD) of the aptamer along a titration of B. bifidum concentrations. This ELAA format is easy to use and allows high-throughput analysis of samples, making bacterial identification cheaper and more efficient.

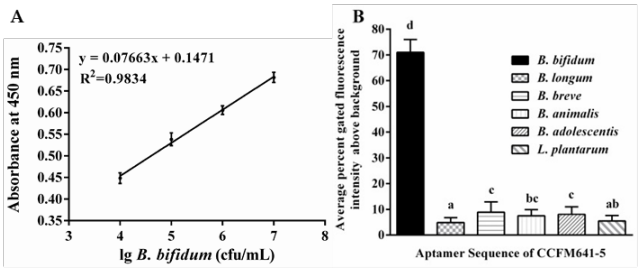


*Figure (adapted)**
*A) Calibration curve of varying concentrations of B. bifidum. OD values determined by microplate reader at 450 nm wavelength. The absorbance at 450 nm represents the mean ± SD of three independent experiments.*
*B) Histogram of the percent gated fluorescence intensity above background for aptamer CCFM641-5. The values of aptamer binding represent the mean ± SD of three independent experiments. Bars with different letters are significantly different ($p < 0.05$).*

-A.E.

*Reference: Apta-Index™ ID #631

## Using Nanobots to Target and Destroy Tumors with Aptamers

One pervasive problem when treating cancer is the inability to distinguish tumor cells from healthy cells. A new strategy has been developed to address this issue by cutting off the tumor's blood vessel rather than target the individual cells. In this strategy, nanobots made from a DNA origami sheet would carry thrombin to the site of the tumor, causing a blood clot that will lead to tumor tissue death. To prevent random blood clotting in healthy parts of the body, aptamers are used to ensure high specificity to the tumor site. The aptamers would target a protein called nucleolin. While nucleolin is produced normally in a healthy body, tumor endothelial cells produce the protein at a much higher rate. At the same time, it is not found on the surface of healthy cells, exponentially increasing the likelihood of the nanobot finding the tumor site. In vivo tests showed dramatic reduction in tumor size and did not show any side effects. Within hours post-injection, the nanobots were able to surround the tumor. Once the thrombin is released and clotting begins, the nanobots can be cleared from the site while the thrombin in the tumors continues to initiate clotting over the course of three days. This is one of the first fully autonomous, DNA robotic systems used for very precise drug delivery and targeted cancer therapy.

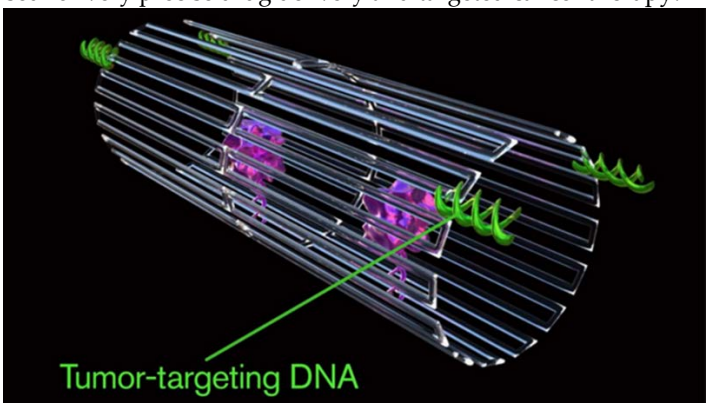


*Figure (adapted)**
*The nanobot is created from a DNA origami sheet (grey), containing thrombin (purple) to the tumor site. The DNA sheet wraps around the thrombin to prevent activation. Aptamers (green) allows the nanobot to target the tumor site to allow thrombin to be deployed in the correct location.*

-J.P.

*Reference: Apta-Index™ ID #290

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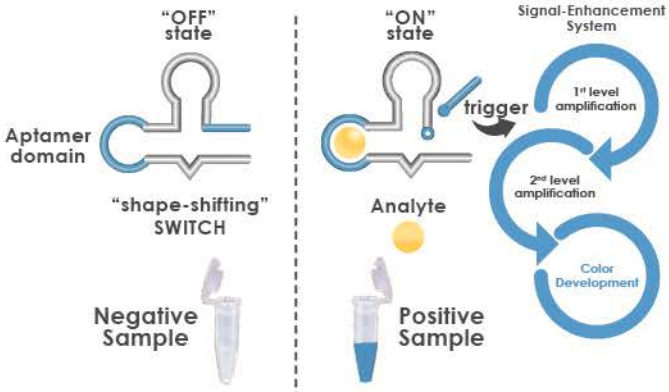


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## Technology Comparison Chart

| | Antibodies | Aptamers | Apta-Beacons™ |
|---|---|---|---|
| Stability/ Refolding | | ++++ | ++++ |
| HIGH Affinity | ++++ | ++++ | ++++ |
| HIGH Selectivity | + | ++++ | ++++ |
| Unknown Biomarkers | | ++++ | ++++ |
| Small Target Selectivity | + | ++++ | ++++ |
| Difficult Target Selectivity | | ++++ | ++++ |
| One-Step Detection | | | ++++ |
| In-Solution Detection | | | ++++ |

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