

# LOCALIZED OPTICAL ACTIVATION OF THERAPEUTIC GENE RELEASE IN BREAST CANCER CELLS USING PHOTOTHERMAL NANOCRESCENTS AS OLIGONUCLEOTIDE-CARRIERS

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## Abstract

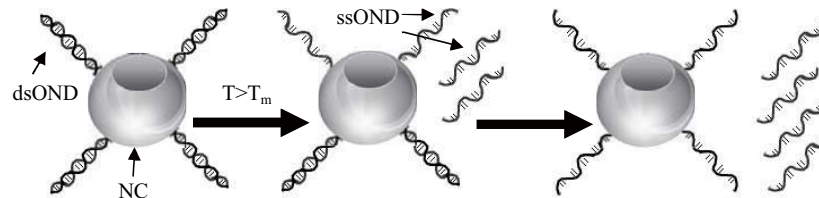
Optical activation of photothermal nanoparticles permits remotely-triggered surface biomolecular reactions with high spatiotemporal resolution. We present a new method to selectively and locally control protein expression by internalizing photothermal nanocrescent particles carrying therapeutic genes within living cells.

*Keywords: gene delivery, molecular nanomedicine, photothermal nanoparticles*

## 1. Introduction

Remotely controllable nanoscale gene delivery vehicles can greatly benefit the study of molecular medicine. In particular, therapeutic gene delivery at specific locations is highly desired for precise gene therapy. We describe a new photoactive, nanoscale gene delivery vehicle based on the oligonucleotide-conjugated Au nanocrescents as photothermal nanoparticles. DNA hybridization and dehybridization has previously been triggered through the use of ultraviolet, photo-active chemical modifications [1] and inductive coupling of a radio frequency (RF) magnetic field to metal nanocrystals [2]. Our approach differs from other approaches in that our gene delivery method offers the combined advantages of high spatial resolution, remote gene release, longer penetration depth, and enhanced photothermal effect. Firstly, in order to provide quantitative control of gene delivery, improvements are needed in the area of spatial resolution (1 microns or less). This method could potentially be used to deliver genes from a single nanoparticle. In this way, we can selectively release genes with optical actuation at desired locations of cultured cells without disturbing the surrounding areas. Such selective gene therapy would be useful to treat cancerous cells in the presence of normal cells in tissue. Secondly, Au-nanocrescent (NC) particles are ideal for near infrared (NIR) activation. Because of the ideal geometry of the nanoparticles, large local field enhancement is seen at the crescent tips of the particle [3]. Finally, NIR activation is also ideal for remote gene delivery because NIR light has a longer penetration depth in human tissue.

Our method uses specialized nanocrescent (NC) particles, which specifically absorb NIR light, in order to optically activate oligonucleotides (OND), such as antisense OND and small interfering RNA (siRNA). Once the temperature on the NC particle reaches the critical melting temperature of ONDs due to photothermal heating, the double-stranded ONDs denature, thereby releasing the ONDs to interfere with mRNA translation within a cell (Fig. 1). We demonstrate the first demonstration of NIR activation of cancer therapeutic gene release using NC particles.



**Fig.1 Concept of gene release using photothermal NC particles** – Double-stranded ONDs (dsOND) are covalently attached to gold nanoparticles. Illumination is used photothermally heat the nanoparticles, causing the double-stranded OND to denature as the temperature reaches a threshold  $T_M$  on the nanoparticles. Because of the unique geometry of the NC particle, local field enhancement occurs at the tips of the crescents and ssONDs are first released from this area. Further photothermal heating will eventually cause all single-stranded ONDs (ssOND) to be released.

## 2. Experimental

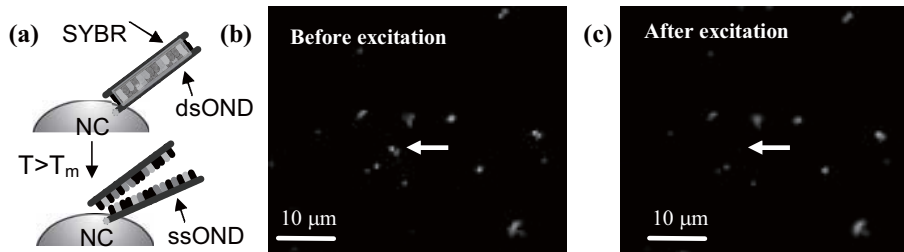
**OND-NC particle conjugation** – Au NC particles are prepared as described elsewhere [3]. The oligonucleotides sequences for EGFR-2 (HER-2) were purchased from Integrated DNA Technologies (Coralville, IA). The sequences are 3'-GTGAGCACCATGGAG-5'-SH and 3'-CTCCATGGTGCTCAC-5'. In order to reduce disulfide bonds, thiolated oligonucleotides are incubated at room temperature with dithiothreitol (DTT) in a 1:100 (OND: DTT) ratio for two hours. To separate the DTT from the ONDs, the mixture is then run through NAP-5 gel column (GE Healthcare) and the eluted ONDs are collected. 70  $\mu$ l of 100  $\mu$ M reduced, thiolated ONDs is then incubated with 500  $\mu$ l of nanoparticles ( $3 \times 10^8$  particles/ml) on a rocker for 40 hours. 100  $\mu$ l of 0.5M PBS is added every 12 hours to decrease negative charge on the ONDs and increase the OND packing.

**OND hybridization** – Excess ONDs in solution are removed by centrifugation and the pellet is resuspended in PBS. 70  $\mu$ l of 100  $\mu$ M complementary ONDs are then added. For hybridization, this mixture is first heated in an 80°C water bath for 2 minutes and then heated in a 65°C water bath for 15 minutes. To allow enough time for hybridization of all ONDs on the particles, the mixture is incubated at room temperature for 24 hours.

## 3. Results and discussion

**In vitro photothermal release** – To demonstrate the *in vitro* release of therapeutic genes from NC particles, double-stranded ONDs are covalently attached to the NC particles through a thiol (-SH) group on the end of one strand. The conjugates are then immobilized on a glass microscope slide. When immersed in solution, the particles remain attached to the glass surface. Fluorescence dye, SYBR Green I, is used to stain double-stranded OND. Laser illumination (10 mW, 785 nm) is then used to locally excite and release the ONDs from the NC particles.

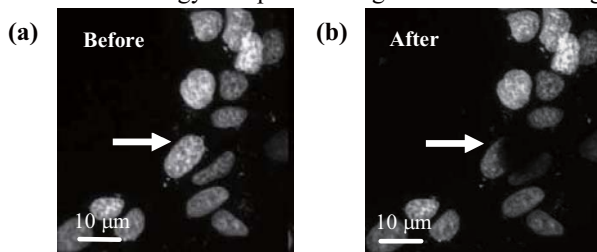
Figure 2 (b) and (c) show NC particles before and after excitation using laser NIR light. The arrow indicates the point of laser excitation. Decrease in the fluorescence is clearly limited to area of laser exposure, which is due to release of single-stranded antisense OND from the NC due to photothermal heating. This shows that ONDs can be successfully delivered with high spatial resolution.



**Fig 2. In vitro release of OND from NC particles.** (a) Concept of double-stranded OND visualization using SYBR dye, and fluorescent images of OND attached to NC particles (b) before NIR excitation and (c) after NIR excitation.

**Intracellular photothermal release** – The OND-NC particles are internalized inside cultured MCF-7 breast cancer cells which overexpress membrane receptor protein EGFR-2 (HER-2). SYBR Green I staining is used to visualize double-stranded ONDs attached to the particles. A 10 mW laser beam (785 nm) is then used to induce photothermal heating at specific locations. When the temperature on the particle reaches the melting temperature of the OND, the double-stranded OND denatures.

We show that after 1 minute of illumination, the fluorescence intensity decreases dramatically, indicating that the double-stranded OND has denatured and the antisense ONDs has been released to subsequently interfere with HER-2 mRNA translation. Control experiments show that the decrease in fluorescence is not due to the photobleaching or thermal quenching. The cells were also viable after illumination since most of the thermal energy dissipates during the OND denaturing.



**Fig. 3. Intracellular release of OND from NC particles.** Fluorescence in cells is from double-stranded ONDs attached to NCs. Fluorescent images (a) before NIR excitation and (b) after NIR excitation.

#### 4. Conclusions

The novel hybrid integration of NC particles and biomolecules has been developed for the optical activation of cancer therapeutic gene release. This work may also permit the remote triggering of surface-enhanced biomolecular reactions with high spatiotemporal resolution and minimal perturbation to surrounding cells, which can be applied in future quantitative systems biology and medicine.

#### References

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- [3] G. L. Liu et al. *Nature Materials*, 5 (2006) 27-32.