



## The next generation of smart gold nanobeacons: nanotheranostics is ready for prime time

“These smart two-pair FRET/NSET donor quencher nanoconjugates can be used for universal cancer diagnosis together with gene therapy and drug delivery...”

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### The true meaning of theranostics: making a real impact

The most elementary principle of theranostics (diagnostics and therapy) has been in practice for decades. Since the 1940s, we have been able to image (diagnostics) and treat (therapy) thyroid cancers using radioiodine (also known as the radioisotope Iodine-131) [1]. More recently, advances in molecular biology and medicine together with the cumulative knowledge at the intersection of biomaterials science and innovative imaging has provided a wealth of functional biomarkers for a range of diseases. Theranostics take advantage of these biomarkers to gain specific access to diseased cells/tissues/organs, image and report on the state of the disease, define the presence or absence of a molecular target and deliver therapeutic payloads directly to the disease sites [2]. Today, the field of theranostics is rapidly evolving and simplifying the shift from ‘trial and error’ medicine to personalized and precision medicine, holding great promise for improved patient clinical outcomes [3].

Nanomedical research may now exploit the advancement in nanomaterials design along with the identified molecular disease markers and advanced imaging techniques to translate these concepts into practical solutions for several diseases, especially for cancer diagnosis and treatment. Such material platforms provide for the simultaneous detection of several gene-associated conditions and nanodevices

with the ability to monitor real-time drug action [4]. As cancer is a dynamic and multifactorial disease that is heterogeneous in nature, it can greatly benefit from a treatment that provides diagnosis and is titrated to disease type and state. As such, drug release can be programmed to occur only following target recognition and in a concentration that is commensurate with disease severity. Making use of nanotechnology materials and applications in the theranostics arena, where one material platform reacts in a graded and personalized manner is gaining momentum in the past few years [5].

The majority of theranostic nanosystems [6,7] described so far will most likely revolutionize our understanding of biological mechanisms and push forward the clinical practice through their integration in future clinical platforms. It is now imperative to learn how to translate nanotheranostics data into an actionable clinical strategy; discuss with industry leaders how nanotheranostics is evolving and what the impact is on current research efforts; and last but not least, learn what approaches are proving fruitful in turning promising clinical data into treatment realities [4].

Despite the significant efforts toward the use of nanomaterials for theranostics research, the most primeval meaning of theranostics has to be clarified. The combination of nanocarriers for gene delivery with optical imaging does not represent the true meaning



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of theranostics *per se*. Only a system that, in a single and unique platform (i.e., gold nanobeacons), combines sensing, silencing and treatment at the same time may catapult theranostics to prime time and make a real impact [8].

### Gold nanobeacons: the 'dream' of a smart theranostics platform

It all started in 2011 when we designed a smart and innovative system that could monitor and control RNA synthesis in a quantitative manner and that we called gold nanobeacon [9]. The idea of providing a rapid and sensitive system capable of efficient quantitative monitoring of RNA synthesis and/or inhibition in real time fulfilled our minds with enthusiasm. These gold nanobeacons comprise a stem-loop DNA single-stranded-oligonucleotide that carries a fluorophore and a quencher (gold nanoparticle) at both ends; in absence of target, the stem-loop structure is closed forcing the fluorophore and the quencher to a close proximity, resulting in fluorescence quenching. Upon hybridization to a complementary target at the palindromic region, the stem-loop sequence opens, the fluorophore and quencher are spatially separated and the fluorescence is restored [10].

With this in mind, two sets of gold nanobeacons were designed. One where the loop in the hairpin structure is complementary to a fragment of the *c-Myc* proto-oncogene mRNA produced by *in vitro* transcription (reporter nanobeacons); and a second one (inhibitor nanobeacons) designed to silence gene transcription by inhibiting the T7-RNA polymerase for binding to its specific promoter, thus capable of blocking the transcriptional machinery at the specific promoter site thus obstructing transcription. Combining the use of a reporter and an inhibitor Au-nanobeacon, we were able to create a dual color system to quantify transcription and degree of inhibition in a single reaction vial [9]. Therefore, it was possible to quantify the level of inhibition of the RNA synthesis and associate it to the amount of template being successfully silenced, in other words, assessing actual silencing capability [10]. This gold nanobeacon biosensor, which does not generate a signal from unrelated sequences and discriminates polymerase activities from promoter-specific RNA polymerization, allows for simple, fast, precise and sensitive RNA transcript expression profiling.

Lanza and coworkers also reported on gold nanobeacons used solely for diagnostic procedures, mainly as exogenous contrast agents for detection and signal enhancement in photoacoustic tomography in the near-infrared wavelength window [11,12].

The next logical step was the development of this type of biosensors and explore the advantages provided

by the gold nanobeacons as potential gene silencing vectors. By 2012, Conde *et al.* was able to produce a gold nanobeacon whose hybridization to a target is reported by fluorescence emission enabled by the conformational changes that ensue, therefore blocking the expression of the target gene. As such, besides performing its function as a sensor, the gold nanobeacon also works as an effector therapeutic agent, providing a highly promising theranostics approach [13].

Using these smart gold nanobeacons, Conde *et al.* demonstrate the possibility of efficiently silencing single gene expression, exogenous siRNAs and endogenous miRNAs while yielding a quantifiable fluorescent signal directly proportional to the level of silencing. Depending on the target, gold nanobeacons were used either to knockdown a specific gene or to 'silence the silencers' of a specific pathway, allowing for recovery of previously inhibited gene expression while simultaneously tracking cellular uptake and detecting the cells where silence is happening [13]. The idea of 'silencing the silencers' by inhibiting siRNAs, antisense DNA oligos and endogenous microRNAs turns out to be the 'cream of the crops' in silencing crucial gene pathways: antisense DNA, RNA interference and microRNAs.

Of considerable interest was the observation that these gold nanobeacons can revert the knockdown effect of the specific pathway silencers after a first wave of delivery, indicating that tumor cells were not only receptive to the first therapy but also that their intracellular processing machinery stays proficient.

A significant attribute of these gold nanobeacons is their ability to achieve inhibition of endogenous and exogenous silencers from crucial pathways that regulate gene expression with the delivery of low therapeutic agents concentration noticeably smaller than those necessary for the delivery of free agents, without the use of chemical co-transfectants. This extraordinary efficiency occurs due to the large payload capacity of the nanoparticles and great biocompatible properties in terms of genotoxic, cytotoxic and proteomic effects on exposure to cells [14].

Moreover, the gold nanobeacons can act as a universal theranostic system for the detection and inhibition of a specific gene as may be used to target any specific gene sequence of choice and by playing with the length of both stem and loop, one can achieve a broad range of conditions (e.g., stringency, temperature, and so on) for discrimination and lower signal-to-background ratios [10].

This system evolved from an *in vitro* to an *in situ* approach where a new and versatile concept for gene-specific silencing was devised. Now is the time to validate these results in preclinical applications and upgrade the nanobeacons platform to a trivalent sys-

tem that combines sensing, silencing and drug delivery at the same time, in a single administration using an implantable device [8].

### The next-generation gold nanobeacons: two-pair FRET/NSET nanoswitch to sense, inhibit & deliver drugs

The next generation of gold nanobeacons is here! Now with one single local application using hydrogel scaffolds [15–19] embedded with a two-pair fluorescence resonance energy transfer/NanoSurface energy transfer (FRET/NSET) gold nanobeacons (also known as dark-gold nanobeacons) we are able to overcome drug resistance by detecting and silencing a multidrug resistance protein (MRP1), before chemotherapeutic drug delivery *in vivo*. Our platform contains hydrogel embedded with dark-gold nanoparticles modified with 5-fluorouracil (5-FU)-intercalated nanobeacons that serve as an ON/OFF molecular nanoswitch triggered by the increased MRP1 expression within the tumor tissue microenvironment. This nanoswitch can sense and overcome multidrug resistance prior to local drug release. These nanoprobes comprise a thiol-DNA-hairpin labeled with a NIR dye and a thiol-DNA oligo labeled with a dark quencher (BHQ2), polyethylene glycol, and intercalated drug- all of which are conjugated to a gold nanoparticle core. These dark-gold nanobeacons are then loaded with 5-FU that intercalates in the beacon stem (dsDNA part) of the DNA-hairpin oligo. Under hairpin configuration, the proximity of the NIR dye to the dark quencher leads to fluorescence quenching. Hybridization of the DNA hairpin to a complementary mRNA target restores fluorescence emission due to the gold nanobeacons' conformational reorganization that causes the fluorophore and the quencher to part from each other, yielding a quantitative response. On the other hand, the release of the 5-FU drug can only occur when DNA hairpin hybridizes with the complementary target and can be measured once the distance from the 5-FU and the gold core increases, escalating the drug emission.

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To evaluate the efficiency of the dark-gold nanobeacon probes in sensing and in overcoming multidrug resistance *in vivo*, an orthotopic breast cancer mouse model was developed by injecting 5-FU-resistant MDA-MB-231 cells to the mammary fat pad of female SCID hairless congenic mice. Efficacious and local delivery of the dark-gold nanobeacon probes is achieved by the implantation of a hydrogel disk on top of the triple-negative breast tumors using a polyamidoamine (PAMAM G5) dendrimer crosslinked with dextran aldehyde, which provides enhanced stability of the embedded nanoparticles. Despite the cross-resistance to 5-FU, more than 90% tumor reduction is achieved *in vivo*, following 80% MRP1 silencing compared with the continuous tumor growth following only drug or nonsense nanobeacon administration.

Our approach can be applied to reverse cross-resistance to other chemotherapeutic drugs that are not effective owing to the development of resistance. Developing a new prescription drug and gaining marketing approval often lasts longer than a decade and is estimated to cost more than \$2.5 billion, according to a new study by the Tufts Center for the Study of Drug Development [20]. This value increased by 145% since 2003. From an economic standpoint, restoring the efficacy of existing drugs provides these innovative gold nanobeacons with the potential to be more cost-effective in addition to serving as a smart preclinical theranostics platform. These two-pair FRET/NSET donor quencher nanoconjugates can be used for universal cancer diagnosis together with gene therapy and drug delivery and are now ready for prime time!

### Financial & competing interests disclosure

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