Nanoparticle Drug Delivery Methods via DNA Nanotechnology

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Introduction

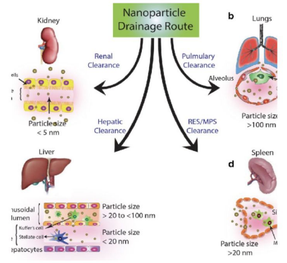
The use of gold coated magnetic nanoparticles (gold coated MNPs) for targeted cancer treatment has shown to be promising, however, it poses one detrimental problem; nanoparticles can cause extensive damage to the human body as they become toxic when left inside for an extended period of time. In an effort to counteract this toxicity, we provide creative alternatives to ensure a less toxic route of clearance. Our team aims to make the use of gold nanoparticles for drug delivery a safe reality by designing a system that delivers the nanoparticles into the body and carries them out before they can cause trouble. Our proposal consists of three main mechanisms: release strand, magnetic field, and Callback BUS. The release strand is responsible for carrying the nanoparticles into the body, to their target location, and then releasing them to perform their intended task (the release of a drug). The magnetic field extracts the nanoparticles from their aggregation in the cell once treatment has been delivered. The BUS is a second DNA structure that will capture the particles and carry out a safe removal utilizing the body's natural extraction processes. Our BUS structures utilize the concepts of DNA nanotechnology and DNA origami to form a safe and structurally sound vessel for nanoparticle transportation.

Background Information on DNA Nanotechnology

"DNA nanotechnology" is a broad term that refers to the use of DNA as a literal building material. DNA nanostructures are utilized for their unique structural qualities, not as carriers of genetic information. "DNA origami" refers to the folding of DNA to create nanostructures. This technique has been used to create both two-dimensional and three-dimensional structures (our team utilizes a three-dimensional structure for the BUS). DNA origami takes advantage of the base pairing nature of DNA and ability to bind numerous helices together with staple strands to form structures capable of containing cargo. The general process involves the folding of a long strand held together at specific points by staple strands to fold itself into a two-dimensional or three-dimensional structure, much like the concept of folding a flat piece of paper into a three-dimensional piece of art (Seeman). Their ability to hold cargo makes DNA nanostructures potential tools for carrying out safe drug delivery, and our research proposes a specific method for accomplishing this task.

Toxicity and Proposed Solution

The main focus of the various nanostructure drug delivery methods that have been considered throughout our research is to ensure that nanoparticles can be removed from cells after they have successfully delivered their payload (medication, DNA, RNA, etc.). The most prominent issue that our method aims to resolve is regarding the nanoparticles getting stuck in the cell. The aggregation of nanoparticles within the cell leads to toxicity-related consequences due to the interaction of this foreign material with various biological systems. The release and callback method involves sending treatment gold coated, magnetic nanoparticles to their target location via DNA nanotubes called Release Strands and through a series of chemical signals upon contact with the tumor site, the nanoparticles are released and can deliver their treatment to target cells (i.e. cancer cells). Once the treatment nanoparticles are released and are in the process of the treatment delivery, the call back method is initiated by researcher administering a solution containing Callback DNA Structures via injection that will bind to the tumor wall and researchers will induce an external magnetic field on the body to pull the nanoparticles of the cells which will the be collected by the Callback Buses. Once the nanoparticles are secured in the Callback Buses, the BUS can then detach and will be carried out of the body via the bloodstream.



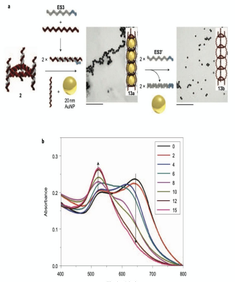
(Bose)

Release Method

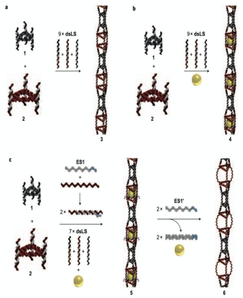
The Release Method involves encapsulating gold coated magnetic nanoparticles in a DNA nanotube known as the Release Strand that is designed to receive an RNA strand specific to the target for which when the strand is connected to the release strand, the DNA unravels and the nanoparticle is thus released. (Sleiman) The Sleiman's Lab developed this release strand (DNA nanotube) that can be loaded with nanoparticles and once in contact with a specific sequence of RNA the nanoparticle will be released (Sleiman).

The nanoparticles are conjugated with a ssDNA aptamer as a targeting method to help with the cellular uptake of nanoparticles. The nanoparticles are conjugated with a signal based linker (ph, UV light linkers, etc.) and once the nanoparticle encounters the corresponding chemical signal (certain ph, certain concentration of UV light, etc.) inside the target cell which causes the linker to be cleaved off the nanoparticles which causes the treatment attached to said linker to be released in the cell (Kong).

The proposed release and callback methods require the DNA nanostructures and nanoparticles to have certain shapes, sizes, and composition in order to effectively execute their designed task while ensuring the safety of the biological systems that the nanostructures encounter. The optimal size of the nanoparticles needs to be approximately 15-20 nm to ensure that the release strand can securely contain the nanoparticle. (Luo) The main issue that arises is whether the nanoparticles are too big to be excreted from the body (along with the Callback BUS). The process of renal excretion begins with filtration through the glomerulus which is designed to filter blood to form urine and acts much like a strainer (Zhang).As the nanoparticles travel through the afferent arteriole, they run into a barrier of the pores of the glomerulus, which only allows nanoparticles around <6nm to be filtered through. The larger nanoparticles will enter the liver through the portal vein, but run into issues upon entrance. A solution to this issue is to alter the surface charge and conjugating peptides on the nanoparticle surfaces to allow for larger particles to be filtered . A positive surface charge ensures a faster clearance, since Kuppfer cells engulf more negatively charged nanoparticles. The nanoparticles will also be conjugated with PEG protein peptides that can act as camouflage and prevent the Kupffer Cells from recognizing them as foreign bodies. Unfortunately, filtration of nanoparticles through hepatocytes can be toxic to the body. Depending on the nanoparticle's physicochemical properties, it may take between a few hours and many months to excrete. In order to accelerate the duration of hepatic clearance, conjugating the nanoparticles with a slightly positive charge, galactose receptors (which attract hepatocytes), and RGD peptides will enhance cellular uptake and increase the nanoparticle's probability of filtration. If the nanoparticles do not pass through the liver, they will be filtered by the spleen (Yang).



Construction of DNA nanotubes T2a and T2b, and ultraviolet-visible spectra of T2a. a, Two linking strands that connect together triangles **2** into large nanotubes were modified to contain an eight-base overhang. Assembly of triangles **2** and the appropriate linking strand plus the new linking strands **S31** in the presence of 20 nm AuNP resulted in DNA nanotubes **T2a** with uniformly encapsulated and closely spaced AuNPs. Selective opening of the DNA nanotubes **T2a** with specific added DNA strands **S32*** led to nanotubes **T2b** in their single-stranded form that spontaneously released their particle cargo. TEM images showed the line pattern of AuNPs in close proximity in nanotubes **T2a** and the loss of ordering of AuNPs 15 minutes after the addition of strand **S32*** (scale bars = 200 nm). **b**, Ultraviolet-visible spectra of nanotubes **T2a** recorded at various times after addition of weaker strand **S32*** at room temperature from zero to 15 minutes (arrows show increase and decrease in intensity with time). (Sleiman)



Construction of triangular DNA nanotubes 3, 4, 5 and 6. a, Nine **dsL5** of appropriate sequence were designed to assemble pairs of small triangles **1** and large triangles **2** to give triangular DNA nanotubes **3** with longitudinal variation and alternating larger (14 nm) and smaller (7 nm) capsules along the tube length. **b**, Size-selective encapsulation of AuNPs (gold spheres) gave nanotubes **4** that encapsulated lines of nanoparticles in the large capsules, with precise positioning of the particles 65 nm apart. **c**, Two linking strands that connected together triangles **2** into the large capsule were modified to contain an eight-base overhang (**S31**). Assembly of triangles **1** and **2**, the appropriate linking strands and the new linking strands (**S31**) in the presence of 15 nm AuNPs resulted in DNA nanotubes **5**. Selective opening of DNA nanotubes **5** with specific added DNA eraser strands **S31***, which were fully complementary to **S31**, led to nanotubes **6** in their single-stranded form that spontaneously released their particle cargo. (Sleiman)

Callback

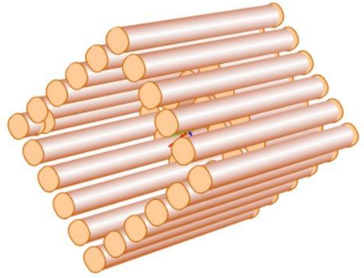
The process of removing the nanoparticles from the cells is vital in reducing the toxicity derived from nanoparticle aggregation. In order to initiate this removal process, a sufficient external force is necessary to pull the nanoparticles out of the cell, hence the need for magnetic nanoparticles. Pure magnetic nanoparticles have a few shortcomings, including a somewhat underwhelming conjugation ability. A solution to this problem arises when Fe, Fe_3O_4, and Fe_2O_3 (three forms of magnetic nanoparticle) are coated with gold, these magnetic nanoparticles gain not only increased biocompatibility, but also the conjugation ability that makes pure gold nanoparticles incredibly useful (Lunnoo). Alongside the conjugation of treatment, gold coated MNPS can be conjugated with single stranded DNA (ssDNA) (Maeda). This ssDNA, small strands of which are often called 'sticky ends' for their tendency to attach strongly to their counterparts, are composed of sequences of nitrogenous base pairs which, when exposed to a complementary strand, will quickly and firmly attach, forming a double helix. These magnetic, sticky ended callback nanoparticles can then be attached to a DNA structure through the use of tight DNA 'nanocages', which can capture the nanoparticles upon contact which will encapsulate the treatment nanoparticles (Luo). This structure is vital to the collection of the treatment nanoparticles and is called the Callback BUS. A solution with these structures will be injected into the bloodstream within a brief window after the initial treatment has been administered.

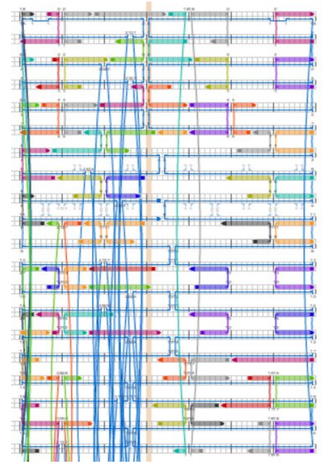
The Callback nanoparticles will be outfitted with the same aptamers (ssDNA) used by the Release Strand (Belyanina), which will attach the BUS to the tumor site when encountered in the bloodstream. This will allow a large quantity of Callback BUSes to congregate around the tumor site, which will increase the odds of treatment nanoparticle recollection once they are drawn out of the cell via an external magnetic field. The process of removing treatment nanoparticles from the cell itself is where the magnetic nature of the treatment particles becomes important. After enough time has passed for the Callback BUSes to congregate around the tumor (this will likely be in the range of five minutes, as blood circulates the entire body roughly once every 13 second) (Tarr), an external magnetic field will be generated outside of the body focused on the site of the tumor. This field will attract the magnetic nanoparticles, drawing them out of the cell. The tumor site at this point should be surrounded with Callback BUSes and upon contact these BUSes will bind with the tumor site. The magnetic field will draw the treatment nanoparticles out of the cell after delivery, so one can predict that the nanoparticles will come into contact with at least one BUS. The odds of this event occurring are increased by the opposing charges of the treatment and the Callback, gold coated MNPs which will be embedded in the Callback Buses. The ssDNA conjugation forces the treatment nanoparticles and callback nanoparticles to bind. Eventually, the aptamers on the Callback BUSes will be dislodged from the tumor site and released into the bloodstream, where the body's natural systems will take over.

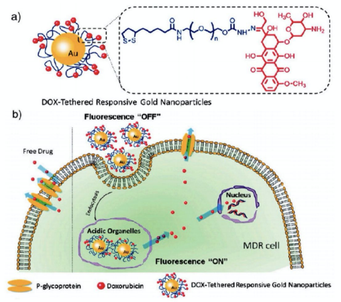
Mechanics

Our initial design for the Callback BUS was a box structure made from DNA of approximately 40 by 60 nanometers. This design poses certain limitations, the first of which being the size. Any DNA box structure will begin to lose its structural integrity if the dimensions exceed 100 by 100 nanometers.Another option that was considered was the icosahedron model which has numerous advantages compared to the box. The first of which being that the size is more flexible. The inherently different structure, the icosahedron model has not been shown to become floppy at larger scales, which removes size as a limiting factor. The next most prominent advantage to using this model would be the openness of its structure. Unlike the box, the icosahedron does not contain walls, it is more of a skeleton. The lack of walls makes for plenty of space for nanoparticles to flow in or out of the structure. This can be accomplished with the box as well, but for the box, this involves careful consideration of the size of the staple stands connecting each DNA scaffold to ensure that the nanoparticles fit in the gaps and make their way in and out of the structure.

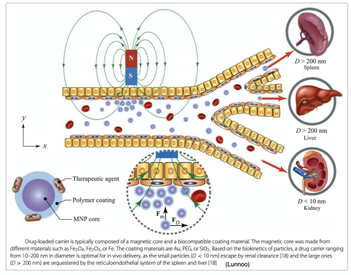
The final design of the BUS consisted of a box structure made up of a total of 36 individual DNA strands, woven together with a series of staple strands. This structure was designed in CaDNAno, a modeling program chosen for its popularity among industry and research in the development of three-dimensional DNA structures. Overall, the process of designing and validating the BUS served as a proof of concept for the ability of the CaDNAno program to produce consistent, regular geometry through the manipulation of DNA strands. The final product suggests that creating a DNA nanostructure capable of fulfilling the responsibilities of our proposals Callback BUS is realistic.







(a) Schematic illustration of DOX-tethered responsive gold nanoparticles (Au NPs);



Drug-loaded carrier is typically composed of a magnetic core and a biocompatible coating material. The magnetic core was made from different materials such as Fe_3O_4, Fe_2O_3, or Fe. The coating materials are Au, PEG, or SiO_2. Based on the biokinetics of particles, a drug carrier ranging from 10–200 nm in diameter is optimal for in vivo delivery, as the small particles (D < 10 nm) escape by renal clearance [18] and the large ones (D > 200 nm) are sequestered by the reticuloendothelial system of the spleen and liver [18]. (Lunnoo)

Future work

When considering where to take this project in the future, experimental testing of the concepts described will be instrumental in determining how to proceed. Following those tests, there are a number of directions future research may take. One issue of note worth investigating is the necessity of magnetizing the callback nanoparticles. Since the entire region surrounding the tumor will be exposed to magnetic fields strong enough to pull nanoparticles out from the cells themselves, it is not unreasonable to believe that the BUS itself may be dislodged. The question that emerges is whether the magnetic field will dislodge the BUSes, and if so what a secure alternative would be to ensure the treatment nanoparticles will be attracted to, and thus attach to, the BUS. Another direction to pursue will be artificially deactivatable aptamers, which would allow the release of the Callback BUSes from the tumor site immediately after ending the magnetic field, which could prevent degradation of the Callback BUSes and nanoparticle conjugations.

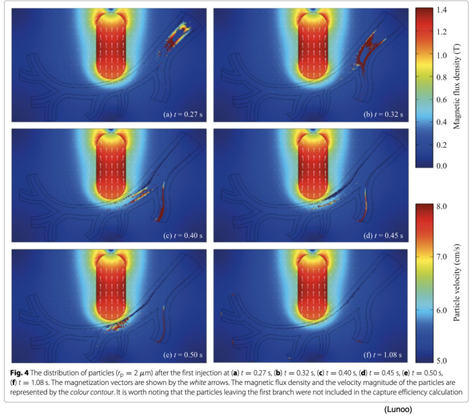
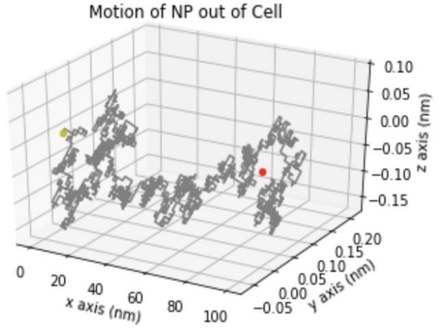


Fig. 4 The distribution of particles ($D_p = 2\ \mu m$) after the first injection at (**a**) $t = 0.27$ s, (**b**) $t = 0.32$ s, (**c**) $t = 0.40$ s, (**d**) $t = 0.45$ s, (**e**) $t = 0.50$ s, (**f**) $t = 1.08$ s. The magnetization vectors are shown by the white arrows. The magnetic flux density and the velocity magnitude of the particles are represented by the *colour contour*. It is worth noting that the particles leaving the first branch were not included in the capture efficiency calculation

(Lunoo)

Python Simulation of Nanoparticle Motion

In order to demonstrate that nanoparticle callback using magnetic attraction is feasible, we developed a Python simulation that approximates the forces acting on a magnetic nanoparticle free in a cell under the effect of an external rotating magnetic field. The field simulated had a field strength of 8000 A/m and a rotational frequency of 50 Hz. This rotating field applies a torque to the deformed nanoparticle with a strength given by $N = (1.4\pi 0ab2H0)^2\sin(2\theta) = 3\pi ab(Belyanina et. al.)$ where a is the long axis of the deformed particle, b is the short axis,H0 is the magnetic field strength, and θ is the angle of the long axis of the nanoparticle to the field lines. This torque can be combined with the equation for the force caused by the pulling due to the spinning F = 1.5πb (Belyanina et. al.) to find the translational force experienced and therefore the distance travelled. The other forces experienced by the particle are the random force due to Brownian motion impacts and drag force, for which we used the Einstein relation for the drag coefficient $D = k_bT/6\pi\eta r$ where Tis the temperature, η is the dynamic viscosity, and r is the radius of the particle (Kardar). The distance travelled for this drag coefficient is $x = b2Dt$ (Kardar) where b is randomly either -1 or 1 and t is the time step used between particle collisions. By superimposing the two distances, we can obtain the actual distance travelled in each direction for a timestep.



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References

Aktas, Y., et al. "Overview of Nano-Drugs Characteristics for Clinical Application: the Journey from the Entry to the Exit Point." Journal of Nanoparticle Research, Springer Netherlands, 1 Jan. 1970, link.springer.com/article/10.1007/s11051-014-2527-7.

Bujold, Katherine E., et al. "Sequence-Responsive Unzipping DNA Cubes with Tunable Cellular Uptake Profiles." Chem. Sci., vol. 5, no. 6, 2014, pp. 2449–2455., doi:10.1039/c4sc00646a.

Kang, Huizhi, et al. "Single-DNA Molecule Nanomotor Regulated by Photons." Nano Letters, vol. 9, no. 7, 2009, pp. 2690–2696., doi:10.1021/nl9011694.

Lo, Pik Kwan, et al. "Loading and Selective Release of Cargo in DNA Nanotubes with Longitudinal Variation." Nature News, Nature Publishing Group, 14 Mar. 2010, www.nature.com/articles/nchem.575.

Luo, Xin, et al. "Encapsulation of Gold Nanoparticles into DNA Minimal Cages for 3D-Anisotropic Functionalization and Assembly." Small, vol. 14, no. 5, 2017, p. 1702660., doi:10.1002/smll.201702660.

Seeman, Nadrian C. Structural DNA Nanotechnology. Cambridge University Press, 2016.

Sreejivungsa, Kanikunn, et al. "Light-Regulated Release of Entrapped Drugs from Photoresponsive Gold Nanoparticles." Journal of Nanomaterials, vol. 2016, 2016, pp. 1–7., doi:10.1155/2016/4964693.

Sreejivungsa, Kanikunn, et al. "Light-Regulated Release of Entrapped Drugs from Photoresponsive Gold Nanoparticles." Journal of Nanomaterials, vol. 2016, 2016, pp. 1–7., doi:10.1155/2016/4964693.

Strogatz, Steven. Nonlinear Dynamics and Chaos: with Applications to Physics, Biology, Chemistry, and Engineering. CRC Press, Taylor Et Francis Group, 2019.

"Unique Roles of Gold Nanoparticles in Drug Delivery, Targeting and Imaging Applications." Molecules, vol. 22, no. 9, 2017, p. 1445., doi:10.3390/molecules22091445.

Yang, C., et al. "Peptide Modified Gold Nanoparticles for Improved Cellular Uptake, Nuclear Transport, and Intracellular Retention." Nanoscale, vol. 6, no. 20, 2014, pp. 12026–12033., doi:10.1039/c4nr02535k.

Zhang, Ys-Nan, et al. "Nanoparticle–Liver Interactions: Cellular Uptake and Hepatobiliary Elimination." Journal of Controlled Release, Elsevier, 13 Jan. 2016, www.sciencedirect.com/science/article/abs/pii/S0168365916300190.