Insights from Swarm-Robotic Experiments on the Penetration of Targeted Nanoparticles into Tumors

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Abstract

Targeted nanoparticles are engineered for the delivery of therapies and diagnostics directly to tumors. By design, they can leak out of vessels into tumors, diffuse through tissue, bind to receptors over-expressed on cancer cells, and undergo endocytosis. Their slow diffusion and strong binding often result in nanoparticles accumulating in the first cells encountered after extravasation, thereby leading to poor treatment of deep-seeded tumor cells. Computer simulations were used to show the impact of binding affinity on the penetration of nanoparticles into tissue. Rather than fine-tune binding kinetics, we propose to delay binding until after the nanoparticles have diffused throughout the tissue. A swarm of over 100 kilobots was used to visualize nanoparticle strategies in robotico. Through embodied experiments, we were able to identify key advantages in delaying nanoparticle binding that could result in lower injected doses and safer therapies. In robotico experiments provide an intuitive and physical instantiation of nanoparticle dynamics that bioengineers can use to build intuition and drive innovation.

Introduction

Cancer causes over 8 million deaths annually in the world (Stewart, 2014). To treat cancer, bioengineers are designing nanoparticles that can deliver drugs and therapeutics directly to tumors. Their size, typically between 10nm and 100nm, allows them to escape the leaky vessels in tumors where they are retained due to the high-pressure environment (Maeda et al., 2012). This drives nanoparticles to passively accumulate in tumors while reducing side effects on healthy tissue (Jain and Stylianopoulos, 2010). Nanoparticles can be loaded with therapeutics that are released in a controlled fashion, or coated with targeting ligands that allow them to uniquely identify and bind to receptors over-expressed on certain cancer cells, thereby driving their internalization (Bao et al., 2013). Note that in reality, many mechanisms exist to drive cells to uptake nanoparticles (Cheng et al., 2012).

The behavior of each nanoparticle depends on its design (size, material, coating and cargo) and the resulting interactions in the body. The collective behavior of trillions of such nanoparticles interacting in a complex tumor environment ultimately defines their success as diagnostic or treatment agents. Predicting and engineering these collective behaviors is often counterintuitive and empirical. Previous work relied on realistic computer simulations based on our expertise in nanomedicine (Kwong et al., 2013; von Maltzahn et al., 2011) and thorough validation from the bioengineering community (Hauert et al., 2013). Our results showed that nanoparticles optimized to strongly bind and accumulate in cancer cells, concentrate in the first cells they encounter after leaking into the tumor environment (Fig. 1). The resulting collective behavior is poor tissue penetration, or so called binding-site barriers, with deep-seeded tumor cells left untreated. Weaker nanoparticle binding could lead to better system outcome. Rather than fine-tune binding kinetics, we propose a generalizable guideline that relies on nanoparticles waiting until they have freely diffused deep into tumor tissue before unveiling their binding moieties (Fig. 2).

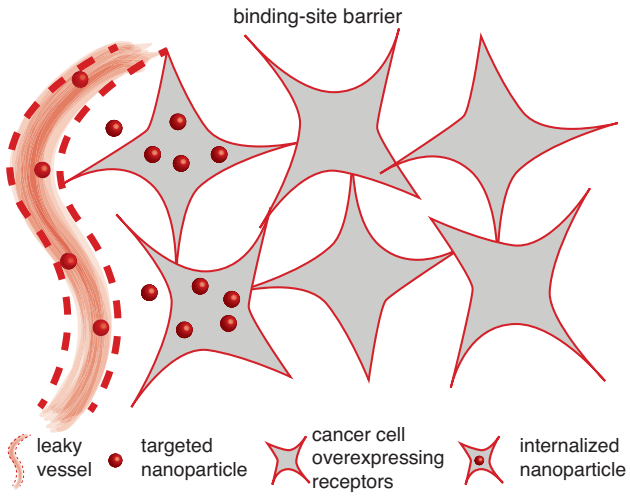

Figure 1: Binding-site barrier. After leaking from vessels in tumors, nanoparticles carrying chemotherapies and decorated with ligands targeted to receptors over-expressed on cancer cells will rapidly bind and internalize. This leads to poor treatment of cells deep in tumor tissue.

One major hurdle to building a working intuition for

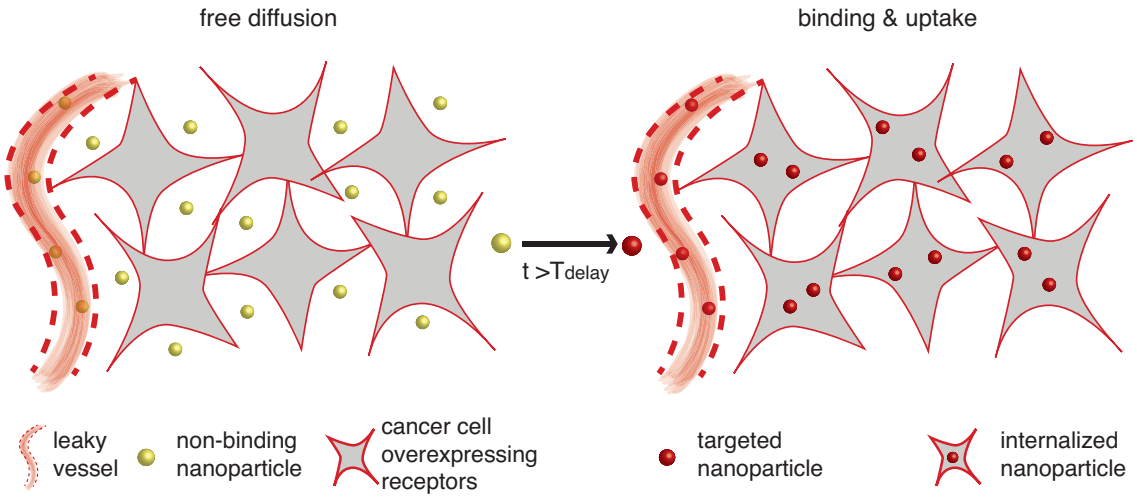

Figure 2: Delayed-binding strategy. Nanoparticles are shielded from binding for a duration T_{delay} after injection, thereby allowing them to diffuse freely throughout the tumor tissue. After this diffusion period, the shielding is degraded based on pH, enzymatic activity, injected chemicals, or external energy sources to unveil intact targeting ligands. The nanoparticles are then able to bind to the tumor cells.

these systems is visualizing nanoparticle dynamics. Validating nanoparticle strategies at the bench is time and cost intensive, and typically relies on static images to infer nanoparticle motion. NanoDoc[1], a game to crowdsource nanomedicine, addresses this issue by providing a visual interface where users can see the simulated behavior of nanoparticles in a virtual tumor and iteratively design or tweak the nanoparticle system dynamics to reach a desired outcome. As an intermediate step between simulation and in vivo work, we implement nanoparticle behaviors on robotic swarms. Robots serve as an easy to see physical instantiation of particles that bioengineers can use to build intuition. They also provide evidence that the treatment strategies designed in simulation can be translated to a physical system that only approximates the idealized virtual behavior.

In particular, we demonstrate two different binding strategies on a swarm of over 100 kilobots (Rubenstein et al., 2013a). Results in robotico show that both the fine-tuning of nanoparticle kinetics, and the delayed-binding strategy, enable nanoparticle-robots to overcome binding-site barriers and penetrate deep into the "tumor tissue". Beyond validating this expected behavior, bioengineers observed that nanoparticle-robots with fine-tuned kinetics were having trouble binding to cell-robots deep in tumor tissue. This observation lead to a new realization that fine-tuning the binding kinetics of nanoparticles, while improving their tissue penetration, made it difficult for them to accumulate in cells far from the vasculature. Indeed, many nanoparticles need to reach the deep-seeded cells for few to accumulate in them. To the contrary, nanoparticle-robots implementing a delayed-binding strategy were able to easily accumulate in all cell-robots. This benefit was further confirmed through stochastic simulations with realistic parameters for nanomedicine. Results show this insight could lead to a reduction of the required injected nanoparticle dose. Further engineering the collective behaviors of nanoparticles could result in emergent cooperative behaviors typically seen in self-organized systems and towards improvements in biomedical applications.

Deep Penetration of Targeted Nanoparticles

Computer simulations can help engineer nanoparticle designs by rapidly predicting experimental outcomes for a large set of design parameters. In previous work, we studied the impact of diffusion and binding kinetics on the ability of targeted nanoparticles to accumulate in cells deep in tumors (Hauert et al., 2013). After injection, nanoparticles are assumed to leak out of vessels and into the tumor tissue where the uniform pressure renders their motion diffusive. Because of their coating, nanoparticles can specifically bind to receptors over-expressed on the surface of tumor cells, leading to their uptake. The probability of binding to a tumor cell depends on the dissociation constant of the nanoparticle. The larger the dissociation constant ($K_D = k_d/k_a$), the weaker the binding. Deterministic and stochastic reaction-diffusion models were implemented to simulate the transport, binding kinetics, and internalization of nanoparticles in a section of tumor tissue. Rather than model the entire tumor, we focus on a challenging scenario whose solution has the potential to generalize to a wide variety of tumor environments. Specifically, we consider a scenario where nanoparticles with a diffusion coefficient of $D = 10^{-8} cm^2/s$ must accumulate

[1]NanoDoc: http://nanodoc.org

at lethal levels (600 nanoparticles) in the 20 cells of a linear tumor section, resulting in a tissue penetration depth of $200\mu m$ from a vessel (Fig. 3a). Each cell is included in a region in which the following reaction network is implemented:

$NP_F + R \overset{k_a,k_d}{\longleftrightarrow} C \xrightarrow{k_i} NP_I + R$

The species in the reaction network are defined as NP_F, free nanoparticles; NP_I, internalized nanoparticles; R, receptors; and C, nanoparticle-receptor complexes. Free nanoparticles diffuse between cell regions with diffusion coefficient D. All parameters for the simulation can be found in Hauert et al. (2013).

Fig. 3b shows the ability of nanoparticles to penetrate in the tumor tissue over time and accumulate at lethal levels in cells. Ranges for dissociation constants are taken from the literature and are realistic for the field of nanomedicine. Interestingly, most nanoparticle formulations would perform poorly in our scenario, resulting in binding-site barriers. Of the seven formulations, only one type of nanoparticle with weak binding ($K_D = 100nM$) is able to accumulate at lethal levels in all cells. Fine-tuning nanoparticle kinetics to reach these requirements can however be detrimental to nanoparticle function. As an alternative, we propose a generalizable guideline that prevents nanoparticles from binding for 24 hours (circulation time of the nanoparticles) and then restores their binding properties (Fig. 2). Fig. 3c shows that a nanoparticle with binding kinetics that would typically result in a binding-site barrier, is now able to diffuse over the tumor tissue and uniformly accumulate in the tumor cells upon activation of its binding functionality. Previous work showed that by using this strategy, all nanoparticle formulations with poor tissue penetration in Fig. 3b are able to accumulate at lethal level in all tumor cells in this scenario (Hauert et al., 2013).

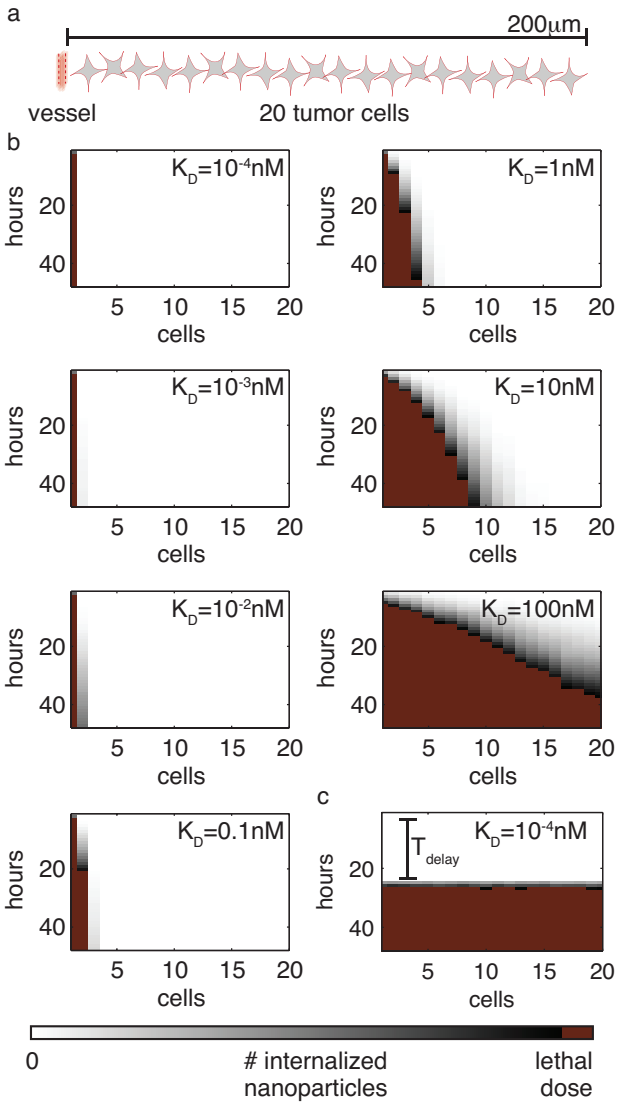

Figure 3: Strategies to improve deep tissue penetration by targeted nanoparticles. a) Simulation scenario in which nanoparticles escaping a vessel must accumulate at lethal levels (600 nanoparticles) in each of the 20 tumor cells in a linear tissue section. b) Stochastic simulations showing the tissue penetration profile of nanoparticles with different dissociation constants. Of the seven nanoparticle formulations, only one is able to kill all twenty cells in the tumor model. c) Stochastic simulation of a nanoparticle implementing the delayed-binding strategy. For the first 24 hours (T_{delay}) of the simulation, the nanoparticle diffuses freely through the tumor tissue. Binding is the reinstated to a strong level, allowing nanoparticles to rapidly accumulate in the tumor cells.

In Robotico Experiments

Robot swarms have been used in the past as a stepping stone towards understanding complex systems. Examples include robot swarms to study social insects (Bonabeau et al., 2000), chemistry (Napp et al., 2011) or cellular systems (Nagpal, 2008). Because of their ability to interact with the physical world, robots instantiate a unique perspective that is more intuitive to understand than computer simulations. They also provide validation that swarm behaviors designed in simplified virtual worlds can translate to physical systems that are subject to noise and unexpected interactions. A swarm of robots could give insights regarding the dynamics of nanoparticles in tumor tissue in a way that is not possible through simulations or lab work. Large numbers of robots are however needed to provide useful insight regarding the workings of nanosystems.

The kilobot swarm developed by Rubenstein et al. provides an ideal testbed with up to 1024 simple robots that can emulate nanoparticle functions in terms of diffusion and binding kinetics. The system was engineered for fully autonomous operation from power-up to power-down (Rubenstein et al., 2012). As a result, it is possible to reprogram

Figure 4: A swarm of over 100 kilobots was used to visualize the binding-site behavior, as well as two strategies to improve deep penetration of targeted nanoparticles. The first strategy relied on fine-tuning the binding kinetics of the nanoparticle-robots (green) until they were able to penetrate through the tumor tissue and bind and internalize (purple) in each tumor cell-robot (red) over a 4min trial. Nanoparticles are initialized in the tumor vessel (wall of the arena). The second strategy prevents nanoparticle-robots from binding to cell-robots until they have had time to diffuse through the tumor tissue. Binding is then restored to a strong level. The full video can be found in the submission material.

the robots and run swarm experiments in a fully hands-off manner. Individual kilobots can set their velocity, illuminate an LED, communicate with and sense distance to neighboring robots and measure surrounding lighting levels. Using this platform, researches have explored multi-robot transport (Rubenstein et al., 2013b; Becker et al., 2013).

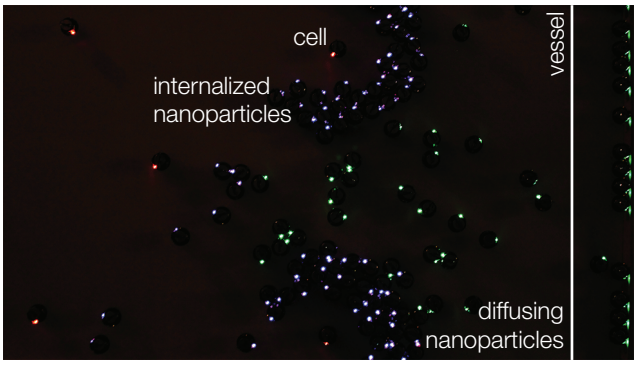

Figure 5: In robotico embodiment of fluorescent markers typically used in biomedical systems. This picture shows a binding-site barrier with freely diffusing nanoparticle-robots (green) binding and internalizing (purple) in tumor cell-robots (red).

Robots are separated into two categories, nanoparticle-robots and cell-robots representing the tumor cells (red). Cell-robots are uniformly positioned in the environment while nanoparticle-robots are positioned "in the vessel" along the wall to one side of the kilobot arena. Upon initializing a swarm experiment, freely-diffusing nanoparticle-robots (green) move randomly across the tumor environment. When receiving messages from cell-robots within communication range, nanoparticle-robots can either virtually bind to the cell-robot (blue) or not. Bound nanoparticle-robots are immobile until they unbind. They can also be internalized within the cell where they will remain indefinitely (purple). Experiments were run for four minutes or until all nanoparticles had been internalized. Running the experiments in the dark, as shown in Fig. 5, results in imagery that is reminiscent of fluorescent nanoparticles moving through tumor tissue from our laboratory and visualized under the microscope (Fig. 6). The main advantage is that now the nanoparticle-robot dynamics can be observed over time. In the first experiment, nanoparticle robots are programmed to

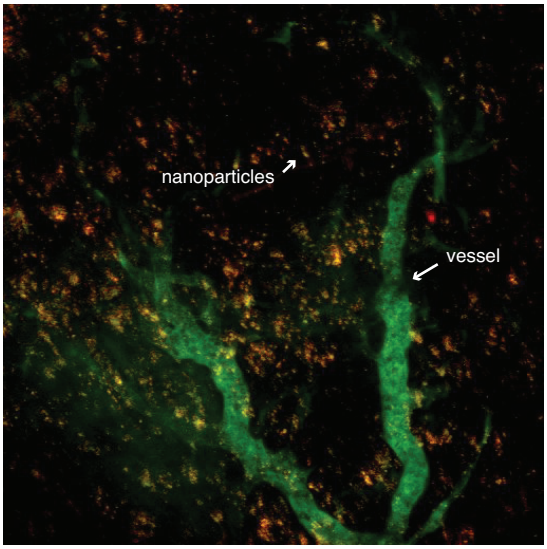

Figure 6: Intravital imaging of ovarian tumor performed on Olympus FV1000 multiphoton laser scanning confocal microscope (25X objective lens). Blood vessels shown in green (70 kDa FITC-Dextran) and gold nanoparticles shown in yellow. Gold nanoparticles were administered intravenously and accumulated within the tumor microenvironment over a period of several days.

strongly bind to cell-robots. The result is a binding-site barrier with nanoparticle-robots internalized in the first cells after extravasation (Fig. 5 and Fig. 4). Fine-tuning the binding kinetics of the robots, in this case by reducing their probability of binding to a cell-robot, was used as a first strategy to enable the robots to penetrate deeper into the tumor tissue and bind to all cells in the environment. Rather than fine-tune nanoparticle dynamics, Fig. 4 shows how a delayed-binding strategy can also enable nanoparticle robots to accumulate in all cells. Specifically, nanoparticle-robots are allowed to freely-diffuse throughout the environment. When the robots are well distributed, their binding capabilities are restored to the levels that originally led to binding-site barriers. A video of the experiments can be found here: https://www.dropbox.com/s/dnsbl6h08wsu5nl/ALIFE.mov

Gained Insight

The usefulness of swarm-robotic testbeds becomes apparent when unique insight is gained from the experience. While watching the nanoparticle-robots with fine-tuned kinetics, we realized it was very difficult for them to efficiently internalize in the deep-seeded tumor cell-robot. At least four nanoparticle-robots entered the communication range of the cell-robot with only one binding over the course of four minutes (Fig. 7). The internalization of the unique nanoparticle-robot was due to a lengthened interaction that emerged from a collision with the cell-robot.

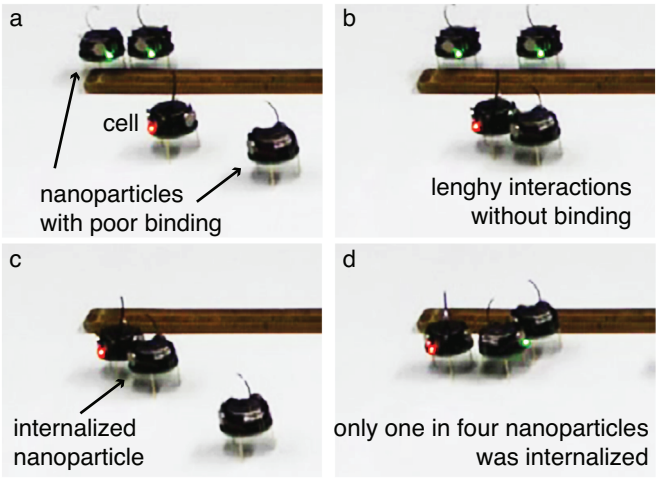

Figure 7: Nanoparticle-robots with fine-tuned kinetics are unable to efficiently internalize in deep-seeded tumor cells. a) Three nanoparticle-robots (green) are within the binding range of a cancer cell (red) without being able to bind to it. b) The nanoparticle-robots continue diffusing, with one robot finally impacting the cell-robot. This extended interaction finally enables the nanoparticle to bind and internalize (c). d) A forth nanoparticle approaches the cell-robot again without successfully binding or internalizing.

The delayed-binding strategy instead allowed nanoparticle-robots to rapidly internalize in deep-seeded cell-robots once binding was activated (Fig. 8). More nanoparticle-robots also seemed to penetrate deeper in the tissue. Delaying binding could therefore have an added benefit that was not initially apparent when doing stochastic simulations. This could in turn lead to lower injected doses than what is needed for nanoparticles with fine-tuned kinetics.

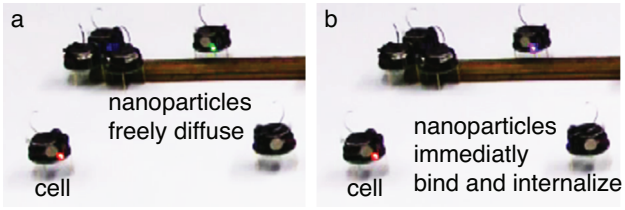

Figure 8: Nanoparticle-robots that follow a delayed-binding strategy are able to rapidly accumulate in deep-seeded tumor cells. a) The nanoparticle-robots freely diffuse (green). b) After binding is initiated ($t > T_{delay}$), all nanoparticles within binding range rapidly internalize (purple) in the cell-robot (red).

Given that robotic experiments are not performed within scales realistic for nanomedicine, additional experiments in simulation are needed to validate the gained insight. Fig. 9 shows the minimum injected dose needed to kill all 20 tumor cells for nanoparticle formulations explored in Fig. 3 using the delayed-binding strategy. In comparison, nanoparticles with fine-tuned kinetics ($K_D = 100nM$) require an injected dose of at lease 19.7mg/kg, which is nearly three times higher than particles that implement the delayed-binding strategy. This is due to the fact that nanoparticles with fine-tuned kinetics have more trouble reaching deep-seeded tumor cells. Once there, they are unable to rapidly accumulate in the cells. Parameters for the simulations are realistic for the field of nanomedicine and are based on work by Hauert et al. (Hauert et al., 2013). Results show the added benefit of the delayed-binding strategy in reducing the required injected dose for the same end-result in terms of cell death. This insight can therefore potentially lead to reductions in toxicity of the overall treatment.

Conclusions

One of the main challenges for bioengineers in nanomedicine is to understand the dynamics of nanoparticle motion and resulting interactions in complex tumor environments. Visualizing nanoparticle dynamics in the lab is mostly done through fluorescent imaging of multiple tumor slices. Simulations have been instrumental in helping engineer and visualize these complex systems, stochastic simulations however lack physical grounding and are difficult to grasp. Swarm robotic testbeds can serve as a

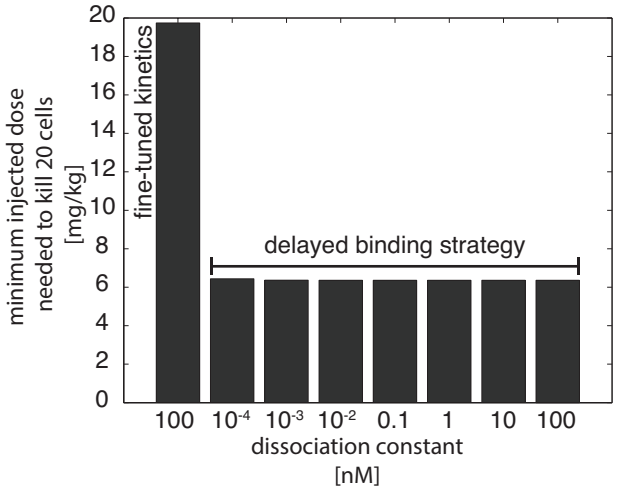

Figure 9: Injected dose needed to kill all 20 cells in the linear tumor tissue for all formulations presented in Fig. 3 when implementing the delayed-binding strategy. Results show that this strategy results in lower required injected doses of nanoparticles than the fine-tuned kinetics strategy.

powerful tool to bridge simplified stochastic simulations and the physical world. In this paper we show how robot swarms provide a visual intuition for nanoparticle dynamics that was used to generate a working hypothesis regarding strategies to increase the penetration of targeted nanoparticles in tumors. Further simulations based on realistic parameters for nanomedicine showed that insight generated through swarm robotics could enable treatments with lower injected doses and resulting toxicity. In the future, we aim to interface swarm robotic testbeds with the NanoDoc game to crowdsource nanomedicine. Remote swarm robotic testbeds could prove useful in helping non-experts learn about nanoparticle dynamics. Additional efforts are needed to perform in robotico experiments that can mathematically scale to experiments in nanomedicine. Nanoparticle designs will ultimately need to be translated to in vivo experiments by experts in the field.

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