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**Nanomachines and Other Caps on Mesoporous Silica  
Nanoparticles for Drug Delivery**

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# Nanomachines and Other Caps on Mesoporous Silica Nanoparticles for Drug Delivery

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## CONSPECTUS

Mesoporous silica nanoparticles (MSNs) are delivery vehicles that can carry cargo molecules and release them on command. The particles used in the applications reported in this Account are around 100 nm in diameter (about the size of a virus) and contain 2.5 nm tubular pores with a total volume of about 1 cm<sup>3</sup>/g. For the biomedical applications discussed here, the cargo is trapped in the pores until the particles are stimulated to release it. The challenges are to get the particles to the site of a disease and then to deliver the cargo on command. We describe methods to do both and we illustrate the applicability of the particles to cure cancer and intracellular infectious disease.

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3 Our first steps were to design multifunctional nanoparticles with properties that allow  
4 them to carry and deliver hydrophobic drugs. Many important pharmaceuticals are hydrophobic  
5 and cannot reach the diseased sites by themselves. We describe how we modified MSNs to make  
6 the dispersable, imagable and targetable and discuss *in vitro* studies. We then present examples  
7 of surface modifications that allow them to deliver large molecules such as siRNA. *In vivo*  
8 studies of siRNA delivery to treat triple negative breast and ovarian cancers are presented.  
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12 The next steps are to attach nanomachines and other types of caps that trap drug  
13 molecules but release them when stimulated. We describe nanomachines that respond  
14 autonomously (with human intervention) to stimuli specific to disease sites. A versatile type of  
15 machine is a nanovalve that is closed at neutral (blood) pH but opens upon acidification that  
16 occurs in endolysosomes of cancer cells. Another type of machine, a snap-top cap, is stimulated  
17 by reducing agents such as glutathione in the cytosol of cells. Both of these platforms were  
18 studied *in vitro* to deliver antibiotics to infected macrophages and *in vivo* and cure and kill the  
19 intracellular bacteria *M. tuberculosis* and *F. tularensis*. The latter is a tier 1 select agent of  
20 bioterrorism.  
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24 Finally, we describe nanomachines for drug delivery that are controlled by externally  
25 administered light and magnetic fields. A futuristic dream for nanotherapy is the ability to  
26 control a nano-object everywhere in the body. Magnetic fields penetrate completely and have  
27 spatial selectivity governed by the size of the field-producing coil. We describe how to control  
28 nanovalves with alternating magnetic fields (AMF) and superparamagnetic cores inside the  
29 MSNs. The AMF heats the cores and temperature sensitive caps release the cargo. *In vitro*  
30 studies demonstrate dose control of the therapeutic to cause apoptosis without overheating the  
31 cells. Nanocarriers have great promise for therapeutic applications, and MSNs that can carry  
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3 drugs to the site of a disease to produce a high local concentration without premature release and  
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5 off-target damage may have the capability of realizing this goal.  
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## 7 8 1. INTRODUCTION AND BACKGROUND 9

10 The development of mesoporous silica nanoparticles in our research group for biomedical  
11 applications began with a completely unrelated line of research: investigation of transparent  
12 matrices for room temperature matrix isolation spectroscopy. The popularity of sol-gel synthesis  
13 of inorganic metal oxides was on the rise, and silica seemed to be a promising matrix candidate.  
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15 The precursors, tetraalkoxy silanes, alcohol and water were readily available, and the formation  
16 of monolithic silica glass by hydrolysis and condensation occurred at room temperature in the  
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18 air.<sup>1</sup>  
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26 Molecules of spectroscopic interest to us dissolved in the initial sol and the glass formed  
27 around them and trapped them. Under acidic conditions and slow drying (to prevent cracking) in  
28 a cuvette, beautiful transparent rectangular prismatic pieces of glass were formed. Because of the  
29 mild synthesis conditions, even delicate biomolecules such as enzymes and other proteins could  
30 be trapped and retain their functions.<sup>2-5</sup>  
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37 The next important step was stimulated by reports that surfactant molecules coupled with  
38 the sol-gel synthesis could template well-ordered pores.<sup>6</sup> Liquid crystalline phases such as rods  
39 or sheets could template tubular and lamellar structures. When the surfactant was removed by  
40 extraction or heating the former phase produced 2-dimensional hexagonal pores. (These  
41 structures were actually reported in the patent literature 20 years earlier but not appreciated at  
42 that time.)<sup>7</sup> The original morphologies were macro-sized translucent pieces, but we wanted  
43 uniform transparent pieces so we explored and develop methods of synthesizing thin films.<sup>8-11</sup>  
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3 We prepared and studied photophysical properties of molecules trapped in films on the order of  
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5 100 nm thick.  
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8 The final step was the discovery that under basic conditions, nanoparticles of the  
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10 templated silica could be prepared.<sup>12</sup> Formation of monodisperse particles was very sensitive to  
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12 not only the concentrations of reactants but also to temperature and stirring speed in a flask. A  
13  
14 cascade of synthetic papers followed and now it is standard to produce particles under 100 nm in  
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16 diameter with highly ordered porosity.<sup>13–18</sup> These particles are generally called “mesoporous  
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18 silica nanoparticles” or MSNs (Figure 1AB). The MSNs that will be described in this account  
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20 typically have a diameter of about 100 nm with 2.5 nm tubular pores, a surface area of about  
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22 1000 m<sup>2</sup>/g and a volume of about 1 cm<sup>3</sup>/g.<sup>19,20</sup>  
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26 As the synthetic challenges were being overcome, the question became what to do with  
27  
28 the particles? In our work, we adapted multiple techniques that we had developed for attaching  
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30 molecules to films to derivatizing the particles’ surfaces (both the outer particle surface and the  
31  
32 huge internal pore surfaces).<sup>19</sup> Attachment of fluorescent dye molecules enabled the particles to  
33  
34 be tracked by optical spectroscopy (even though the particles themselves were only imageable by  
35  
36 transmission electron microscopy). After derivatization and surfactant extraction, the empty  
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38 pores invited being filled with other molecules, and drug molecules brought the MSNs into the  
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40 biomedical world.  
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## 44 2. *IN VITRO* HYDROPHOBIC DRUG DELIVERY

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46 Our first foray into *in vitro* applications of our particles involved hydrophobic anticancer  
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48 drugs as the cargo molecules in the pores.<sup>21</sup> A challenge in cancer therapy is to deliver  
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50 hydrophobic drugs to the sites of the disease. Many important anticancer drugs are poorly  
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52 soluble and some sort of delivery system is needed. (The most important such systems currently  
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3 in use are liposomes and albumins.) We wanted to see if MSNs could be suitable. A problem  
4 immediately arose: MSNs as synthesized aggregated extensively in water and biorelevant fluids.  
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6 To achieve dispersity, we attached and tested many different types of molecules and settled on  
7  
8 phosphonate for our initial studies and fluorescein as our fluorescent label. Anionic phosphonate  
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10 groups were attached by using trihydroxylsilylpropylphosphonate (THMP). This choice was  
11  
12 unconventional because the current lore stated that positively charged particles would be better  
13  
14 taken up by cells. It turned out to be a great choice because the particles had good circulation  
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16 times in mice, were endocytosed readily by cancer cells, and accumulated in xenograft tumors as  
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18 will be discussed later.  
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24 Our first demonstration of hydrophobic drug delivery used camptothecin (CPT), a  
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26 representative anticancer drug that is also fluorescent. Derivatives of CPT are promising and  
27  
28 effective drugs against a wide variety of carcinomas, but have much lower cytotoxicity than  
29  
30 CPT. (FDA approved Irinotecan has a potency of 10% of that of CPT.) CPT was loaded into the  
31  
32 pores by soaking MSNs in a DMSO solution of the drug overnight. The loaded nanoparticles  
33  
34 were sonicated and washed with PBS solution to remove any weakly adsorbed drugs from the  
35  
36 surfaces. A dispersion of the CPT-loaded MSNs was added to PANC-1 cells to determine if the  
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38 nanoparticles transported the CPT into the cells and fluorescence microscopy was used to  
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40 monitor the uptake. The delivery inhibited growth and caused apoptotic cell death.  
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45 We have also utilized mesoporous organosilica nanoparticles (MONs) and a chaperone-  
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47 assisted strategy to facilitate both the loading and the release of hydrophobic drugs from  
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49 nanoparticles. MONs have bridged organoalkoxysilanes in their frameworks that enable a very  
50  
51 high loading of hydrophobic drugs in their pores. We used biodegradable oxamide-phenylene  
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53 based MONs to achieve 84 wt% of hydrophobic CPT and CPT delivery in lung cancer cells.<sup>22</sup>  
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3 For pure MSNs, we recently developed a chaperone-assisted strategy to achieve both substantial  
4 loading and release amounts of the water-insoluble drug clofazimine (CFZ).<sup>23</sup> The interaction  
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6 between the chaperone acetophenone (AP) molecules and CFZ provides the driving force for AP  
7  
8 to carry large concentrations of CFZ into the pores, and thus significantly enhances the release of  
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10 CFZ in buffer solution. *In vitro* studies show that the optimized CFZ-loaded MSNs effectively  
11  
12 kill *Mycobacterium tuberculosis* in macrophages. This chaperone-assisted delivery strategy can  
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14 be applied to the loading and delivery of other hydrophobic drugs with their suitable chaperone  
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16 molecules.  
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### 21 3. MULTIFUNCTIONAL MSNS

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24 The MSNs used in the hydrophobic CPT delivery study had two different molecules attached  
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26 to their surfaces: phosphonate for dispersibility and fluorescein for optical imaging. We wanted  
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28 to push the limits and to demonstrate that more functionality and versatility could be achieved  
29  
30 (Figure 1A). To that end, we synthesized a different type of MSN containing a  
31  
32 superparamagnetic iron oxide core for magnetic manipulation and MRI imaging (Figure 1C).<sup>19</sup>  
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34 The pores were available for filling with cargo. On the outer surface we attached a biomolecule,  
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36 folate, that we wanted to test for active targeting of cancer cells. The resulting paper had a large  
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38 impact because it brought attention to the versatility of MSNs as a platform for multiple actions  
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40 including imaging and drug delivery.  
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45 An important aspect of our explorations of multifunctional MSNs was our investigation  
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47 of active targeting agents. In order to be effective therapeutic agents, MSNs need to be carried  
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49 “passively” by the blood to the site of the tumor. In addition, “active” targeting of specific  
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51 receptors on tumor cells could be achieved by attaching appropriate molecules to the MSNs. Our  
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53 initial studies involved folate and we found that uptake of the particles by cells having folate  
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3 receptors was almost always enhanced *in vitro* but that *in vivo* tumor shrinkage was often but not  
4 always enhanced. Similar results were obtained with MSNs containing attached ferritin and an  
5 RGD peptide.<sup>24</sup> Excellent results with hyaluronic acid are discussed in the following section. It is  
6 clear that active targeting can occur but each type of targeting agent must be evaluated on a case  
7 by case basis.  
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12 We further extended the functionality of MSNs by coating the outside surface with  
13 polymers. The two most important polymers (evidenced by the *in vitro* and *in vivo* studies  
14 described in detail below) were PEG and low molecular weight PEI (Figure 1D). In some cases,  
15 we chemically bonded the PEI to the particles and in others we used electrostatic attraction of the  
16 cationic PEI to the anionic phosphonate-coated surface. We showed that low molecular weight  
17 PEI (1.2 kD) has negligible toxicity compared to that of the high molecular weight polymer.<sup>25</sup> Its  
18 importance lies in its abilities to carry, protect and deliver siRNA (next section) as well as to  
19 facilitate endosomal escape through the proton sponge effect for the nucleotide delivery. The  
20 PEG coatings were used to improve dispersibility and also for their ability to increase circulation  
21 time in mice (stealth particles).<sup>26</sup>  
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#### 33 4. NANOPARTICLE DELIVERY OF SMALL INTERFERING RNAS – FROM BASIC 34 SCIENCE TO CLINICAL APPLICATIONS 35 36 37

38 Recently efforts have begun to use siRNA as a therapeutic agent.<sup>27</sup> Preclinical and  
39 clinical trials have demonstrated that siRNA can be delivered to cells *in vivo* and retain its  
40 function, knocking down target genes in both prostate cancer and melanoma.<sup>27</sup> A drawback to  
41 RNAi-based therapy is that RNA tends to be susceptible to nuclease degradation, and is thus  
42 unstable in the body. Our polymer-coated MSNs proved to be very effective in protecting siRNA  
43 and carrying it to tumors in mice.  
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3 RNA interference (RNAi) using double-stranded RNA was first demonstrated in *C.*  
4 *elegans*.<sup>27,28</sup> In mammals, 21-nucleotide long RNA fragments called small interfering RNAs  
5 (siRNAs) are the effectors of RNAi.<sup>29</sup> These fragments bind to a target mRNA, and then form  
6 the RNA induced silencing complex (RISC).<sup>30</sup> RISC also contains Argonaute proteins,  
7 specifically Ago2, that cleave the mRNA, thus preventing protein translation.<sup>31</sup>

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10 Our earliest successful demonstration of RNA delivery using MSNs involved delivery of  
11 siRNA to drug-resistant KB-V1 cells to silence the expression of efflux transporter P-  
12 glycoprotein (PPgp). We used low molecular weight cationic PEI coating (described in section 3)  
13 in which the anionic siRNA was entrained and protected. Dox was loaded in the pores.<sup>32</sup> The  
14 dual delivery demonstrated that MSN delivery effectively knocked down gene expression of a  
15 drug exporter and increased intracellular DOX levels to improve cytotoxic killing.<sup>32</sup>

16  
17 We have recently shown *in vivo* efficacy of MSN-delivered siRNA in a mouse model of  
18 melanoma. Mice treated with siRNA against *TWIST* had smaller, less vascularized tumors, likely  
19 the result of inhibition of CCL2-driven angiogenesis within the growing tumors.<sup>33</sup> We obtained  
20 similar data in ovarian cancer, where mice treated with siTWIST-MSN plus chemotherapy had  
21 75% less tumor burden than control mice treated with chemotherapy only.<sup>34</sup>

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23 In a second series of studies, we combined active targeting of Cancer Stem Cells (CSC)  
24 with siRNA delivery (Figure 2).<sup>35</sup> Cluster of differentiation 44 (CD44) is a ubiquitously present  
25 glycoprotein on the surface of mammalian cells. Since the discovery that the receptor is over-  
26 expressed in a variety of solid tumors, such as pancreatic, breast and lung cancer, many studies  
27 have focused on methods for targeting CD44 in an attempt to improve drug delivery and  
28 discrimination between healthy and malignant tissue, while reducing residual toxicity and off-  
29 target accumulation. Hyaluronic acid (HA), the primary CD44 binding molecule, has proved a

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3 significant ally in developing nanocarriers that demonstrate preferential tumor accumulation and  
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5 increased cell uptake.  
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8 We studied HA-coated MSNs as a therapeutic approach to deliver oligonucleotides to  
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10 tumors that overexpress CD44+ (Figure 3).<sup>35</sup> We delivered TWIST siRNA to breast and ovarian  
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12 cancer xenografts and reduced tumor burden.<sup>33–35</sup> Our particles inhibited EMT signaling, reduced  
13  
14 tumor burden and exhibited synergistic efficacy in combination with cisplatin.<sup>35</sup> These results  
15  
16 demonstrated a useful therapeutic strategy for chemoresistant ovarian cancer. Successful  
17  
18 application of these types of approaches could pave the way for future RNA-based therapies  
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20 against other targets of interest currently thought to be “undruggable”.  
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## 23 24 5. NANOMACHINES 25

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27 Our next major goal was to design a system that could carry a therapeutic through the blood  
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29 stream to the site of a disease with no leakage, release a high local concentration of the drug,  
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31 release it only on an autonomous or external command, and kill the cancer cells or an infectious  
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33 agents. The release itself required a pore cap that could be closed to trap cargo molecules and  
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35 open in response to a desired stimulus and release the cargo.<sup>13,15,20,36</sup> We named our original  
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37 system a “nanovalve” which consisted of a stalk and a large cyclic molecule that blocked the  
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39 pore in the closed position and slid along the stalk away from the pore opening to release the  
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41 pore’s contents. The large amplitude motion of this nanomachine, like that of any machine,  
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43 needs a power supply. What kinds of motions and what kinds of stimuli could we use that would  
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45 be compatible with a machine deep in the body of an animal? Answering these questions and  
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47 making suitable machines required both imagination and hard work.  
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51 We categorize nanomachines according to the source of the stimulus required to operate  
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53 them (Figure 1E).<sup>14</sup> When the stimulus is a direct result of human intervention, we call it  
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3 “external”. The most important external sources are light, magnetism and ultrasound. Each of  
4 these sources can produce large amplitude motion in molecules by multiple mechanisms. For  
5 example, light energy can cause photochemical reactions, produce heat that causes a chemical  
6 reaction, or be absorbed by “transducer” molecules that change the pH (photoacids) or cause  
7 electron transfer (photooxidants or reductants). Heat production is the primary usable effect of  
8 alternating magnetic fields and ultrasound.  
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11 The second category of operation we call “internal” or “autonomous”. The stimulus for  
12 operation originates from the change in the particles’ surroundings. For example, when the  
13 machine goes from the blood stream to a cell’s endolysosome as a result of endocytosis, the pH  
14 changes from 7.4 to less than 6 and a pH sensitive valve opens. When it enters the cytosol, the  
15 presence of antioxidants such as glutathione cause a redox active valve to open. No external  
16 intervention is required. Autonomous nanomachines such as these are the most widely studied *in*  
17 *vitro* and *in vivo*.  
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20  
21 The most actively studied pH valve is based on the design principle of a cyclic cap that  
22 has a large binding constant to a stalk that is hydrophobic at the pH of blood but becomes  
23 protonated and hydrophilic in the acidic environment of an endolysosome. A widely used  
24 example is shown in Figure 4.<sup>37</sup> The cyclic molecule is  $\beta$ -cyclodextrin ( $\beta$ CD), a cyclic sugar with  
25 a hydrophobic interior. The stalk is a specific benzimidazole with a pKa of about 7. Above pH 7,  
26 the hydrophobic-hydrophobic non-covalent interaction holds the pseudorotaxane together but at  
27 lower pH it dissociates. When the stalk is attached at a pore opening on MSNs, the  $\beta$ CD traps  
28 cargo molecules in the pore but releases them upon acidification. *In vivo* applications of this  
29 autonomous pH nanovalve are discussed in the following section.  
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3 The most-used redox activated system that we named a “snap-top” is based on the design  
4 principle that a redox-sensitive chemical bond can be broken in the presence of reducing agents  
5 in the cytosol of a cell.<sup>38</sup> This simple design utilizes a disulfide stalk containing a bulky cap on  
6 its end (Figure 5). When attached at an MSNs pore surface the cap traps the cargo, but when the  
7 particle escapes the lysosome the reducing agents such as glutathione cause the disulfide to  
8 dissociate into two thiols, releasing the bulky group and the trapped drug molecules. *In vivo*  
9 studies using this redox snap-top are described in the following section.

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19 Many other autonomous nanomachines and capping agents have been studied and several  
20 thorough reviews have been published recently.<sup>13,15,20</sup> One additional example from our group is  
21 the original enzyme-activated snap-top in which a  $\beta$ CD slides on a stalk but is blocked from  
22 leaving by a bulky stopper held in place by a functional group (an ester) that is a substrate for an  
23 enzyme (porcine liver esterase).<sup>39</sup> When the particle encounters the enzyme the bulky group is  
24 “snapped off” and the cargo is released. This type of snap-top is a prototype for a nanocarrier  
25 that could respond only to a specific enzyme overexpressed by a particular cancer cell.

## 36 6. NANOPARTICLES AGAINST INFECTIOUS DISEASES CAUSED BY 37 INTRACELLULAR PATHOGENS

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40 We used both of the nanomachines in Figures 4 and 5 to kill pathogenic bacteria in  
41 phagocytes *in vitro* and *in vivo*. Mononuclear phagocytes, primarily monocytes and tissue  
42 macrophages, are known as professional phagocytes because of their reputation for avidly  
43 ingesting particles of many kinds. Such particles include numerous pathogens that are readily  
44 killed by macrophage antimicrobial armaments. However, one class of pathogens, known as  
45 intracellular parasites, intentionally induce their uptake by macrophages with the aim of  
46 hijacking host cell machinery towards their own end – survival and intracellular replication.

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3 Intracellular pathogens include the agents of tuberculosis and what are referred to by the US  
4 government as Tier 1 Select Agents because of especially high concern that they may be  
5 intentionally employed in a bioterrorist attack. Such pathogens include *Francisella tularensis*,  
6 the agent of tularemia.  
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12 For treatment of many diseases, mononuclear phagocytes – especially those in the liver,  
13 spleen and lung – pose an obstacle to MSN delivery of nanotherapeutics, since they readily  
14 ingest intravenously administered nanoparticles intended for delivery elsewhere, *e.g.* to cancer  
15 cells. MSNs designed for cancer therapy need to be specially designed to evade macrophages,  
16 but for treatment of diseases caused by intracellular pathogens, the avidity with which  
17 macrophages ingest MSNs provides a tremendous advantage – no specific targeting ligands are  
18 necessary to deliver copious amounts of antibiotic-loaded particles to macrophages harboring  
19 intracellular pathogens. Nanotherapeutic delivery of antibiotics maximizes antibiotic delivery to  
20 infected host macrophages while minimizing systemic toxicity.  
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33 Our nanotherapeutics have been designed primarily to treat two diseases caused by  
34 intracellular pathogens – tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb),<sup>23,40,41</sup> and  
35 tularemia, caused by *Francisella tularensis* (Ft).<sup>42,43</sup> Both diseases pose major challenges to  
36 treatment with conventionally delivered antibiotics. TB caused by drug-sensitive organisms  
37 requires 6-9 months treatment and drug-resistant Mtb, which is even more difficult to treat  
38 requires multidrug therapy for up to 2 years. Hence, more efficient delivery of antibiotics to host  
39 macrophages via nanotherapeutics has the potential to shorten the treatment course, improve  
40 adherence, impede the emergence of drug resistance, and reduce systemic drug toxicity.  
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51 Tularemia, a bacterial zoonosis, can manifest as one of several syndromes depending  
52 upon the mode of Ft transmission. Pneumonic tularemia, the most dangerous form and the one of  
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3 greatest concern from a bioterrorism perspective, is contracted via inhalation. With current  
4 therapy, tularemia can still be fatal, resolve slowly, or relapse. Hence functionalized  
5 nanotherapeutics targeting infected macrophages have the potential for more rapid cure, reduced  
6 time in intensive care, and less frequent relapse.  
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12 We designed and evaluated PEI-coated MSNs (~100 nm) loaded with the antibiotic drug  
13 Rifampin for treatment of TB.<sup>41</sup> These MSNs were all avidly ingested by Mtb-infected human  
14 macrophages (Figure 6A). By fluorescence microscopy, the MSNs co-localized with CD63,  
15 indicating uptake into endolysosomes.<sup>41</sup> In *In vitro* studies in which these MSNs were added to  
16 human macrophages infected with the highly virulent Mtb Erdman strain, the MSNs readily  
17 killed Mtb, and they did so significantly more efficiently than an equivalent amount of free drug  
18 (Figure 7A).  
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28 We developed MSNs with pH- and redox potential- sensitive nanovalves for delivery of the  
29 antibiotic moxifloxacin (MXF).<sup>42,43</sup> Both types of MSNs were avidly ingested by Ft-infected  
30 macrophages (Figure 6B) and rapidly killed intramacrophage Ft in a dose-dependent fashion.  
31 These MSNs were evaluated for efficacy in a murine model of lethal pneumonic tularemia in  
32 which mice were infected intranasally with a lethal dose of Ft LVS (6xLD<sub>50</sub>). The mice were  
33 treated with free MXF or MXF-loaded MSNs intravenously every other day for three doses;  
34 euthanized one day later; and Ft CFU assayed in lung, liver, and spleen. In untreated mice,  
35 bacteria multiplied to high levels (~7.5 logs in the lung) and the mice lost 12-24% of their total  
36 body weight. In contrast, mice treated with MXF-loaded MSNs maintained their body weight  
37 and CFU in the lungs decreased by 1-2 logs (Figure 7B).<sup>43</sup> The MXF-loaded MSNs were  
38 significantly more effective than an equivalent amount of free drug in reducing CFU in all  
39 organs; in the lung, they were more effective than ~3 fold greater dose of free MXF.  
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## 7. EXTERNALLY OPERATED NANOMACHINES AND CAPS

A futuristic dream for nanotherapy is the ability to control a nano-object everywhere in the body. Although total control including mobility is far from reality, it is possible to stimulate nanocarriers anywhere and to release therapeutic molecules with spatial and temporal accuracy. The major methods of external control are light, ultrasound and magnetism. For diseases on or near the skin, photothermal therapy is useful, but for tumors deep in the body, magnetism penetrates the best. Externally operated drug release is a step beyond autonomous release because it can be turned on and off at will and does not have to rely on specific chemical changes or the presence of specific molecules in order to operate. It is also more complex than autonomously stimulated release because the external control also requires external instrumentation and an operator. These issues contribute to the sparsity of *in vivo* studies reported to date.

Progress in synthesizing and making functioning nanomachines and caps that can be remotely activated has been rapid.<sup>13,14,20,36</sup> The most prevalent and highly developed systems are light activated. We were interested in using light to power nanomachines, including by direct photochemical bond breaking or photoisomerization and by indirect phototransducers to activate pH and redox valves. Direct photocleavage is a convenient way to remove caps from pores. Molecules in their excited state can transfer protons (photoacids)<sup>44</sup> or electrons (photo-redox)<sup>45</sup> and can be used to activate machines originally designed to be activated by chemical acidification or redox. Excited state energy transfer from molecules with high two photon cross sections can trigger the above reactions using two near infrared photons.

We used our impeller for our first *in vitro* studies.<sup>46</sup> Photo-induced trans-cis isomerization of azobenzene derivatives bonded to the insides of MSN pore walls trap cargo



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3 molecules in the dark but allow them to escape when they wag back and forth.<sup>47,48</sup> We delivered  
4 an anticancer drug into pancreatic cancer cells and observed no killing in the dark but efficient  
5 apoptosis when the impellers were excited.<sup>46</sup>  
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10 Other photo-responsive gatekeepers include azobenzene and cyclodextrin supramolecular  
11 complex,<sup>49,50</sup> photolabile coumarin-based nanovalves,<sup>51</sup> cucurbit[6]uril (CB[6]) and stalk  
12 supramolecular complexes,<sup>52</sup> photoacid molecules and pH-sensitive nanovalves,<sup>44</sup> and photo-  
13 transduced molecules and redox environment-sensitive nanovalves.<sup>53</sup> We later demonstrated two  
14 photon near IR excitation of a transducer molecule that transferred energy to the azobenzene  
15 molecules and released cargo.<sup>54</sup> Interesting progress continues to be made in the two-photon field  
16 for potential biomedical applications.  
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26 In our current research for biological applications, we are focusing on magnetism as the  
27 stimulus, specifically alternating magnetic fields (AMF). Nanoparticles containing  
28 superparamagnetic components are heated by the AMF and thermo-stimulated machines or caps  
29 can be activated by the heat to release cargo. Magnetic heating is currently being used to kill  
30 cells or destroy tumors by hypothermia,<sup>55-57</sup> but it can also create tumor metastasis. We are  
31 focusing on drug delivery by taking advantage of the localized particle heating without bulk  
32 heating.  
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42 To understand what degree of local heating can be attained, we designed nanothermometers  
43 in the MSNs containing the superparamagnetic heaters and measured internal temperature  
44 changes of over 20 °C above that of the surrounding aqueous medium, more than enough to  
45 activate thermosensitive caps without causing hyperthermia.<sup>58-60</sup>  
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51 Our first *in vitro* study used core@shell nanoparticles to carry and deliver drugs actuated by  
52 AMF and used a supramolecular valve comprised of a bulky cucurbit[6]tril (CB[6]) ring and an  
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3 alkylammonium thread attached on the surface of MSNs that acts as a thermo-sensitive  
4 gatekeeper (Figure 8).<sup>61</sup> When the temperature increases, the binding constant between the CB[6]  
5 ring and the alkylammonium thread decreases leading to the detachment of CB[6] from the  
6 thread followed by drug release. The release of drugs was not triggered by bulk heating from the  
7 solutions but by localized heating generated from the interior magnetic cores. We showed that  
8 doxorubicin released intracellularly by AMF stimulation effectively kills MDA-MB-231 breast  
9 cancer cells.  
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19 Very recently, we demonstrated spatial, temporal, and dose control of drug delivery using  
20 superparamagnetic  $\text{MnFe}_2\text{O}_4@\text{CoFe}_2\text{O}_4$  nanoparticles with high specific loss power (1510.8  
21 W/g) and high saturation magnetization (105 emu/g) (Figure 9).<sup>62</sup> Thermo-sensitive gatekeepers  
22 that contain an aliphatic azo moiety release a bulky cap and regulate the release of drugs. The  
23 dose of the drug release was controlled by the AMF exposure time and triggered by the localized  
24 high temperature from the interior magnetic core and not by bulk heating. Multiple sequential  
25 exposure of AMF causes drug release in a step-wise manner. *In vitro* studies show that the drug  
26 delivery platform is biocompatible, that drug-loaded nanocarriers do not kill the cells without the  
27 AMF stimulation, and that cell death was correlated with the AMF exposure time (Figure 10).  
28 Thermo-responsive drug delivery stimulated by an AMF offers the potential of becoming an  
29 innovative chemotherapy that noninvasively, remotely, and precisely controls the dosage of  
30 drugs, avoiding the risk generated when overheating the bulk solution.  
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## 48 8. FUTURE PERSPECTIVES

49 Mesoporous silica is a versatile platform for drug delivery applications. The nanoparticles are  
50 nontoxic, carry large payloads of therapeutic drugs, and can be capped to prevent premature drug  
51 release until the nanoparticles reach their target where they can then be stimulated to release a  
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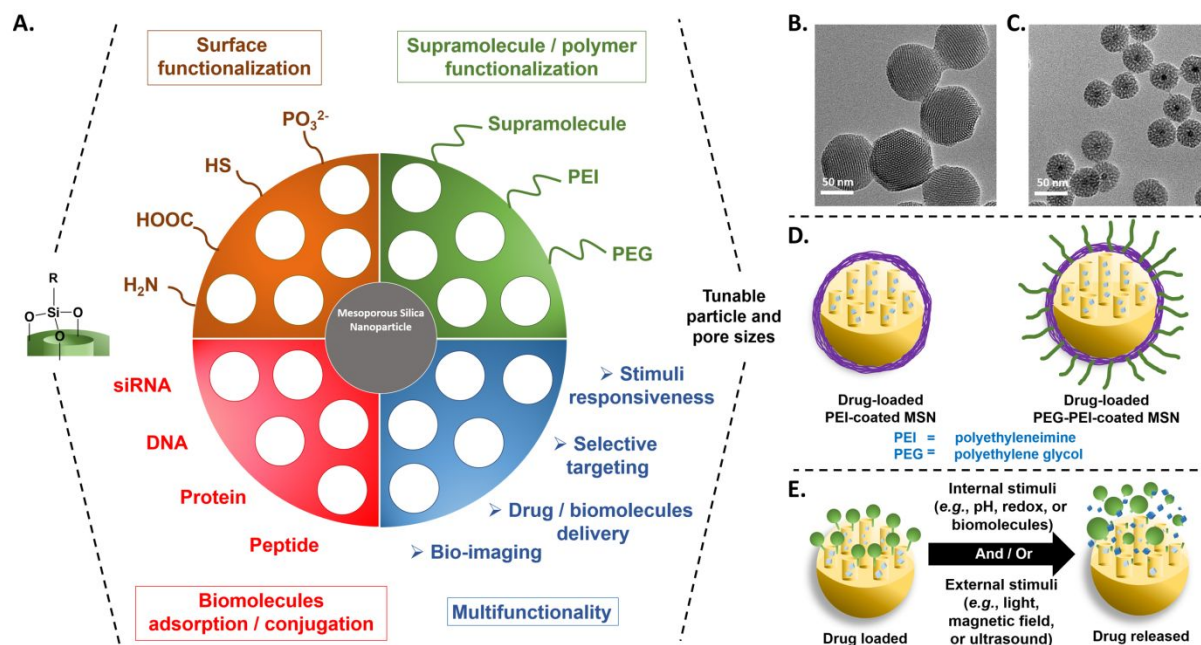
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3 high local concentration of the therapeutic. The particles can carry other important therapeutic  
4 molecules such as siRNA, protect them from enzymes and degradation, and release them  
5 intracellularly. Their potential for curing cancer occupies most current research attention, but  
6 they can be used to treat other diseases including infectious diseases.  
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12 The route of administration is important. We emphasized intravenous administration, but for  
13 treatment of intracellular pathogens, we found that intramuscular injection was extremely  
14 beneficial because of superior pharmacokinetics.<sup>63</sup> Preliminary studies show inhalation  
15 administration to be extremely promising for treatment of lung infections.  
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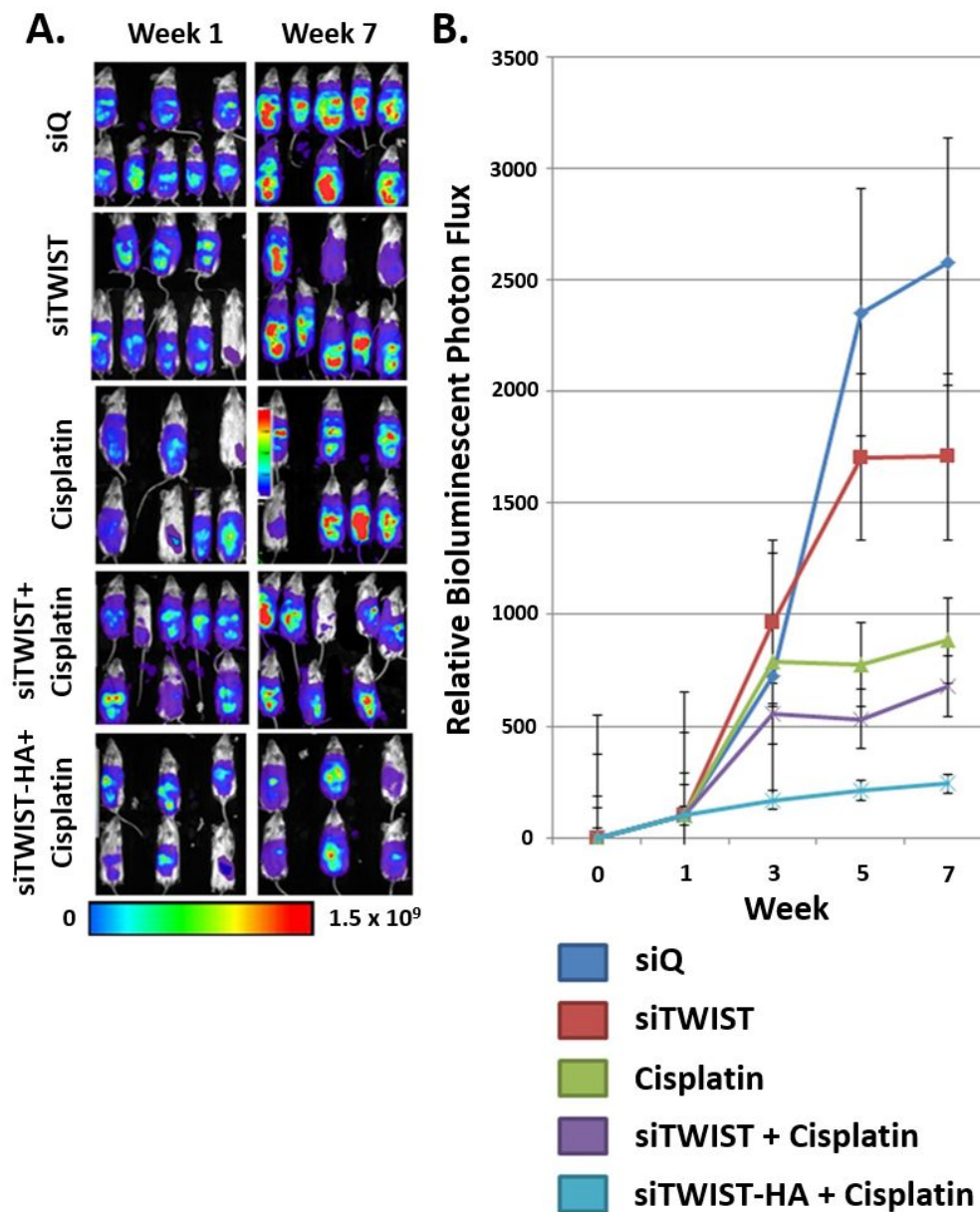
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22 The multifunctionality of nanotherapeutics including MSNs promises to revolutionize drug  
23 delivery. It allows for active targeting of drug-loaded particles (– our best *in vivo* example of a  
24 successful agent is hyaluronic acid – other targeting agents (*e.g.* folic acid, and RGD peptide)  
25 toward their specific cancer cells were also demonstrated). The drug-loaded particles can  
26 additionally contain molecules for image guided therapy, such as paramagnetic metals or  
27 superparamagnetic cores for imaging by MRI, fluorescent dyes (especially in near-IR for optical  
28 imaging), and atoms for PET imaging. The multifunctionality of nanotherapeutics also allows for  
29 theranostics – the combining of therapeutic and diagnostic agents into a single nanoparticle.  
30 Finally, the multifunctionality of nanotherapeutics allows for the delivery of combination drug  
31 therapy at optimal drug dose ratios, thereby enhancing drug synergy and safety.  
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45 It would be extremely beneficial if MSNs could move forward toward clinics. Before then,  
46 many prerequisites need to be fulfilled, such as industrial-scale production of MSNs under Good  
47 Manufacturing Practice (GMP) to guarantee that the properties of the MSNs are reproducible  
48 from batch to batch, detailed investigations of the potential toxicity, bio-distribution, and  
49 clearance from the animal body, and whether drug-loaded MSNs have higher treatment efficacy  
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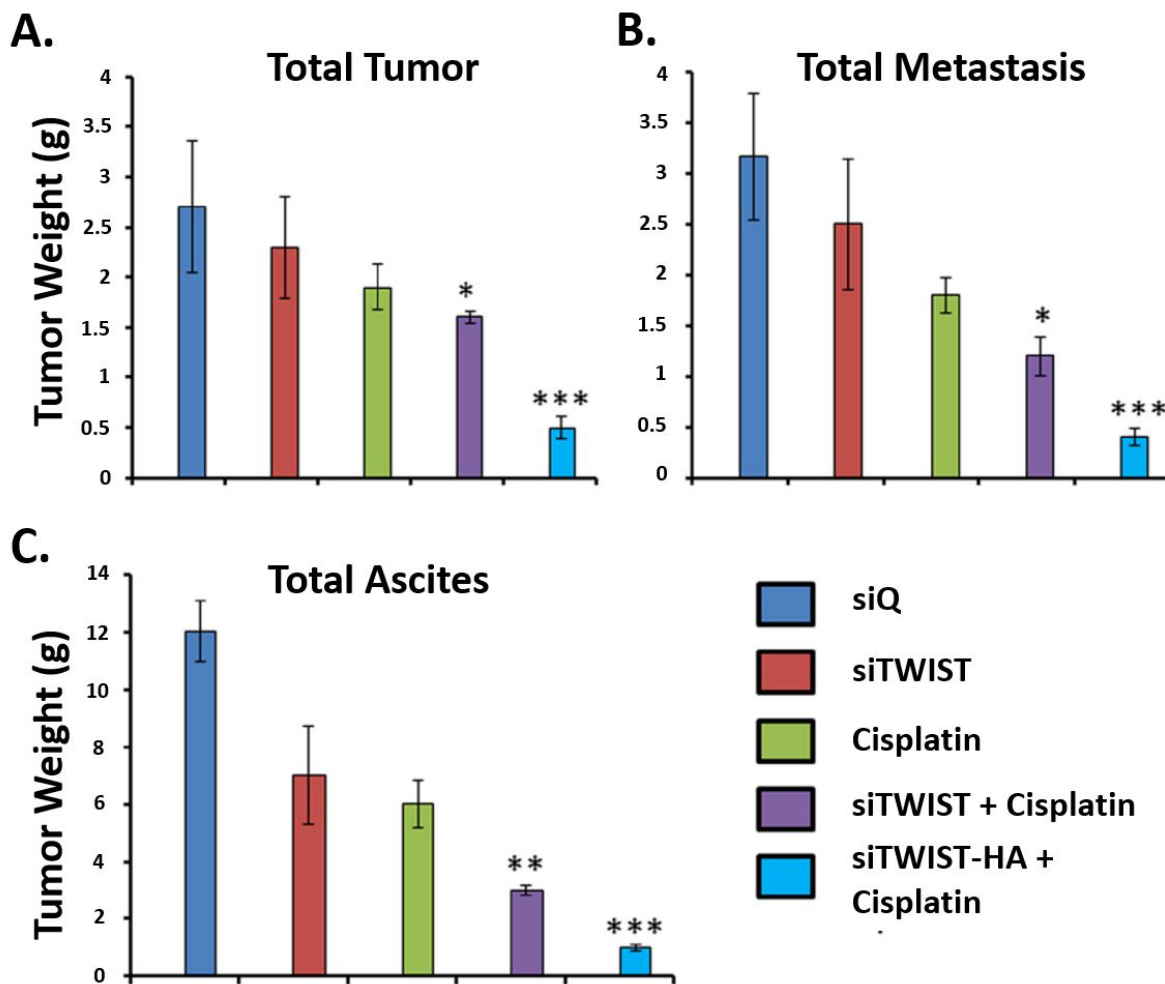
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3 and fewer side effects than those of the free drugs alone. It is a long and complicated road to  
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5 clinical trials and eventual use in the clinic, but the attractive properties make MSNs worthy of  
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7 the attention that they are being given.  
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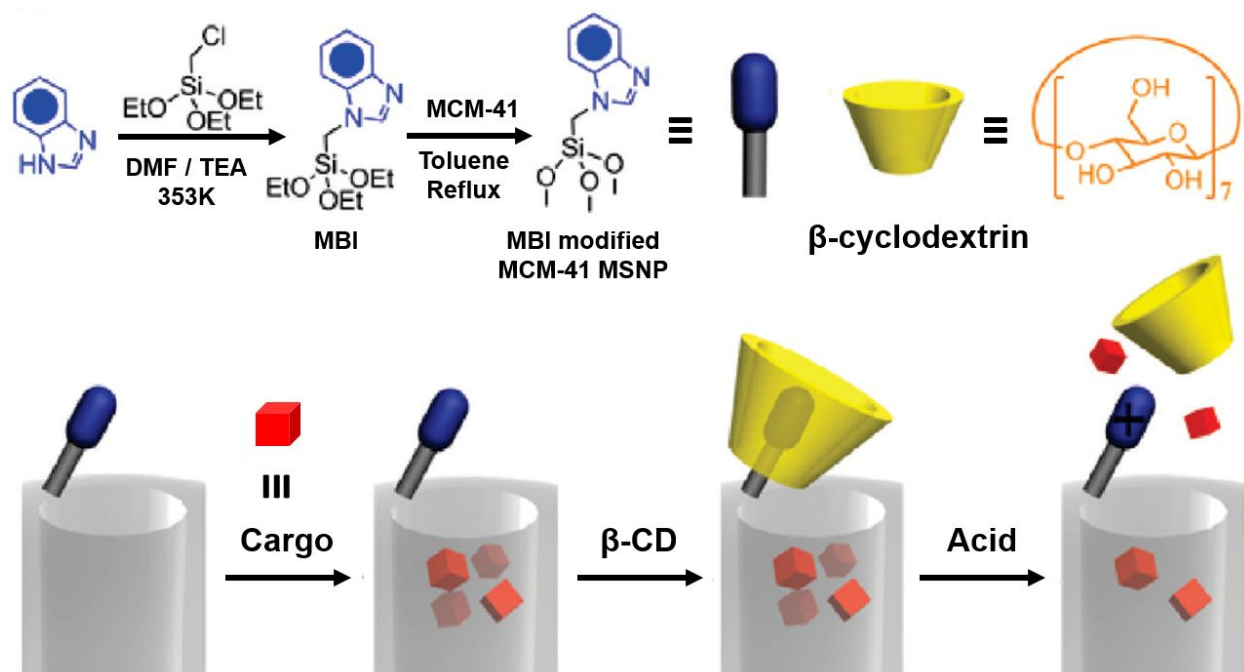
**FIGURE 1.** Collage of MSNs described in this Account. (A) Overview of functionalities of MSNs. Portrait of (B) MSNs and (C) magnetic core@shell nanoparticles. (D) Polymer coating. (E) Stimuli-responsive drug release particles.



**FIGURE 2.** (A) Bioluminescence imaging of Ovar8-IP tumors. Tumors treated with cisplatin emit noticeably weaker signal than siTWIST or siQ only control mice, while those treated with siTWIST-MSNs plus cisplatin exhibit a further loss of signal. siTWIST-MSN-HAs plus cisplatin exhibit a greatest loss of signal. (B) Quantification of bioluminescence depicted in A. Units for luminescence are photons/sec/cm<sup>2</sup>/steradian. Adapted with permission from ref. 35. Copyright 2018 Elsevier.

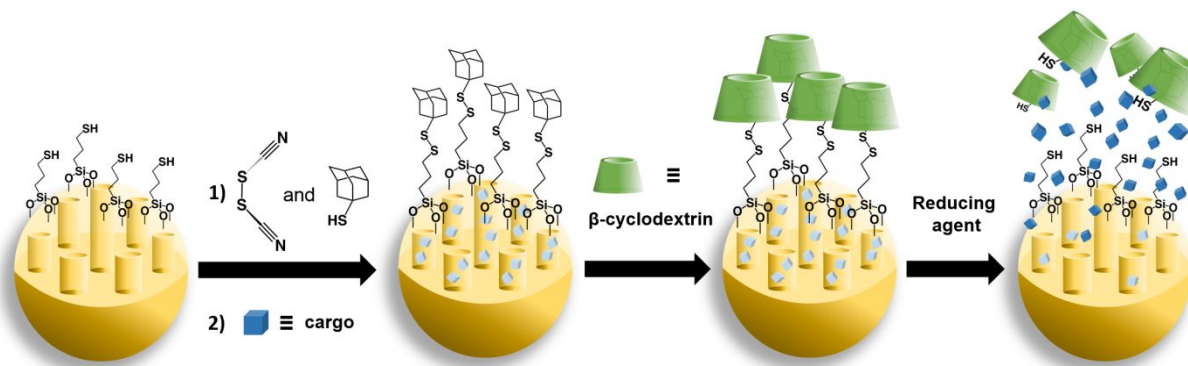


**FIGURE 3.** Quantification of the weight loss of (A) tumor, (B) metastasis, and (C) ascites of mice treated as described in Figure 2. The combination of HA targeted MSNs carrying siRNA and cisplatin was the most efficacious. Adapted with permission from ref. 35. Copyright 2018 Elsevier.



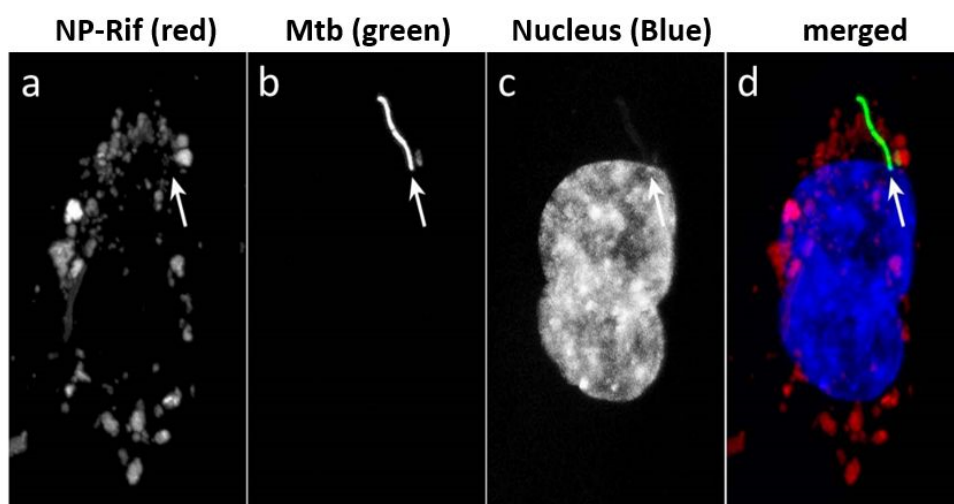
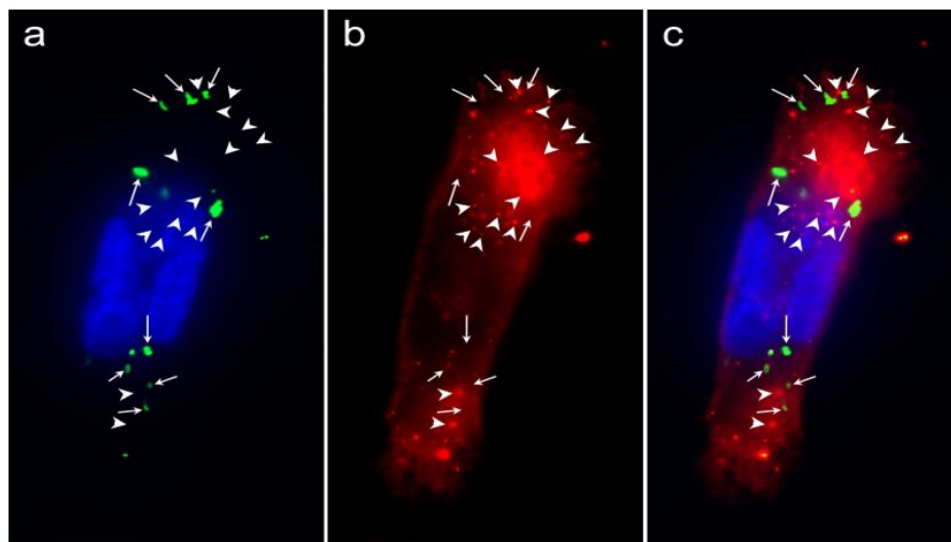
**FIGURE 4.** Schematic illustration of the pH-responsive benzimidazole nanovalve. Adapted with permission from ref. 37. Copyright (2018) American Chemical Society.



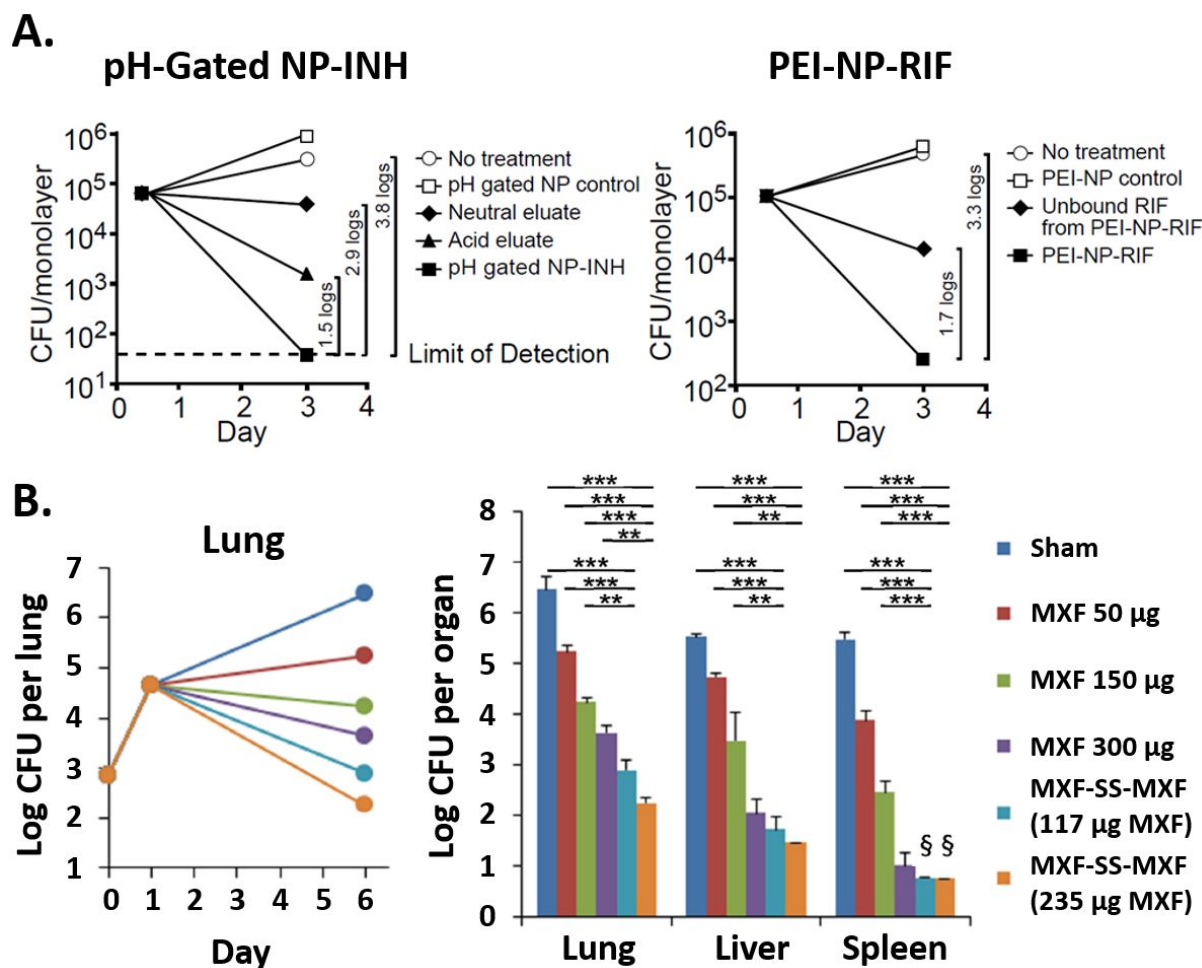


16 **FIGURE 5.** Schematic illustration of the redox-responsive disulfide nanovalve.

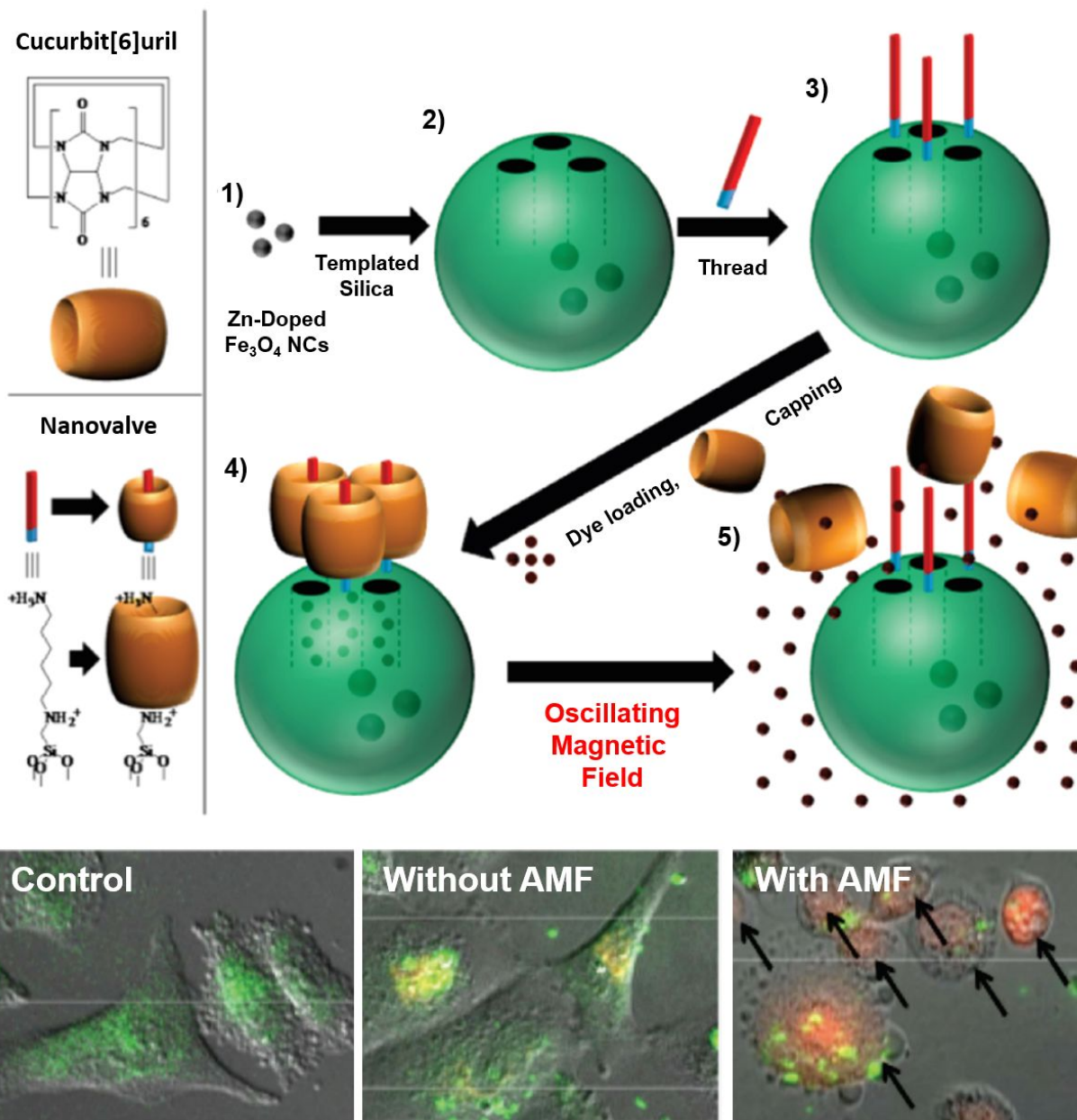
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**A. *M. tuberculosis*****B. *F. tularensis***

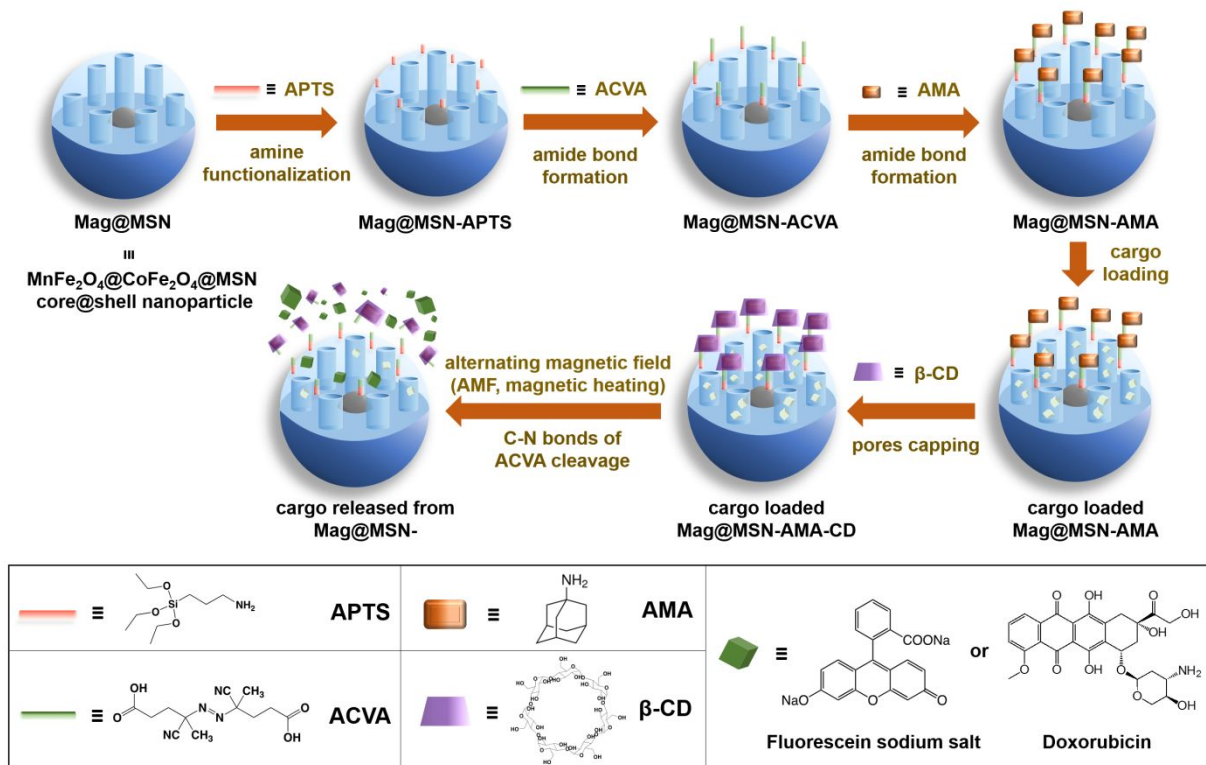
**FIGURE 6.** MSNs are avidly ingested by human macrophages infected with *Mycobacterium tuberculosis* (A) and *Francisella tularensis* (B). (A) Human THP-1 macrophages were infected with GFP-expressing Mtb, incubated with red fluorescent MSNs loaded with rifampin (NP-RIF), fixed, stained with nuclear stain DAPI, and imaged by confocal microscopy. Large numbers of NP-RIF (a) and an Mtb bacillus (b, arrow) are observed in the macrophage, whose nucleus is stained blue (c); the merged image is shown in panel d. Adapted with permission from ref. 41. Copyright 2012 American Society for Microbiology. (B) Human THP-1 macrophages were infected with GFP-expressing Ft, incubated with RITC-labelled MSN-MBI, fixed, stained with DAPI, and imaged by confocal microscopy. Green Ft (a, c, arrows), red MSNs (b, c, arrowheads) and DAPI-stained nucleus (a, c, blue) are seen in individual images (a, b) and merged image (c). Adapted with permission from ref. 42. Copyright 2015 American Chemical Society.



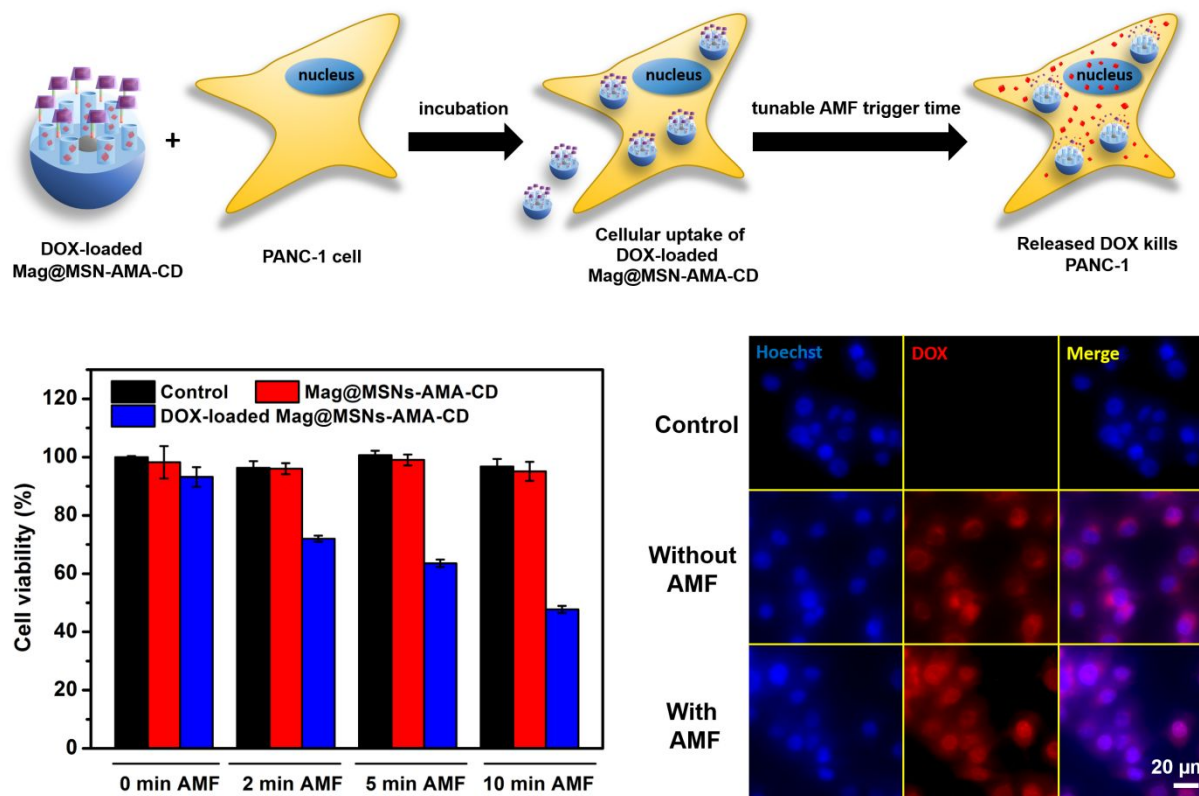
**FIGURE 7.** Antibiotic-loaded MSNs rapidly kill *M. tuberculosis* *in vitro* (A) and *F. tularensis* *in vivo* (B). (A) MSNs loaded with Isoniazid (INH) or Rifampin (RIF) kill Mtb to a greater extent than an equivalent amount of free drug. Compared with no treatment, the INH- and RIF-loaded MSNs reduced the colony-forming units (CFU) by 3.8 and 3.3 log CFU, respectively, and compared with an equivalent amount of free drug, they reduced CFU by an additional 1.5 and 1.7 log CFU, respectively. Adapted with permission from ref. 41. Copyright 2012 American Society for Microbiology. (B) MXF-loaded MSNs rapidly kill Ft *in vivo* in a mouse model of lethal pneumonic tularemia and are much more efficacious in reducing the lung burden of Ft than equivalent amounts of free MXF (Efficacy ratios 3-5:1).<sup>43</sup> The CFUs in lung, liver and spleen were determined on day 6. Adapted with permission from ref. 43. Copyright 2016 Wiley-VCH.



**FIGURE 8.** Schematic illustration of the construction and operation of the thermo-responsive cucurbit[6]uril nanovalve. Fluorescence microscope images show breast cancer cells were killed by the released DOX after the exposure to an AMF. Adapted with permission from ref. 61. Copyright 2010 American Chemical Society.



**FIGURE 9.** Schematic illustration of the synthesis and operation of the thermo-sensitive azo cap. Adapted with permission from ref. 62. Copyright 2019 American Chemical Society.



**FIGURE 10.** Effects of AMF stimulated DOX release on PANC-1 cells. *In vitro* cellular killing and fluorescence microscope images of DOX-loaded nanoparticles after AMF exposure are shown left and right, respectively. Adapted with permission from ref. 62. Copyright 2019 American Chemical Society.

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5 The authors declare no competing financial interest.  
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19 The manuscript was written with contributions by all authors.  
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30

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## Biographical Information

**Wei Chen** was born in Nantou, Taiwan. He received his B.S. and M.S. degrees in chemistry from National Taiwan University, where he did research under Professor Chung-Yuan Mou. He is currently a PhD student at UCLA Chemistry & Biochemistry Department under the supervision of Professor Jeffrey I. Zink. His research focuses on mesoporous silica-based imaging and drug delivery systems.

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3 Illinois. He is the author of more than 500 publications and his current research interests include  
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5 multifunctional nanomaterials and theranostic applications.  
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