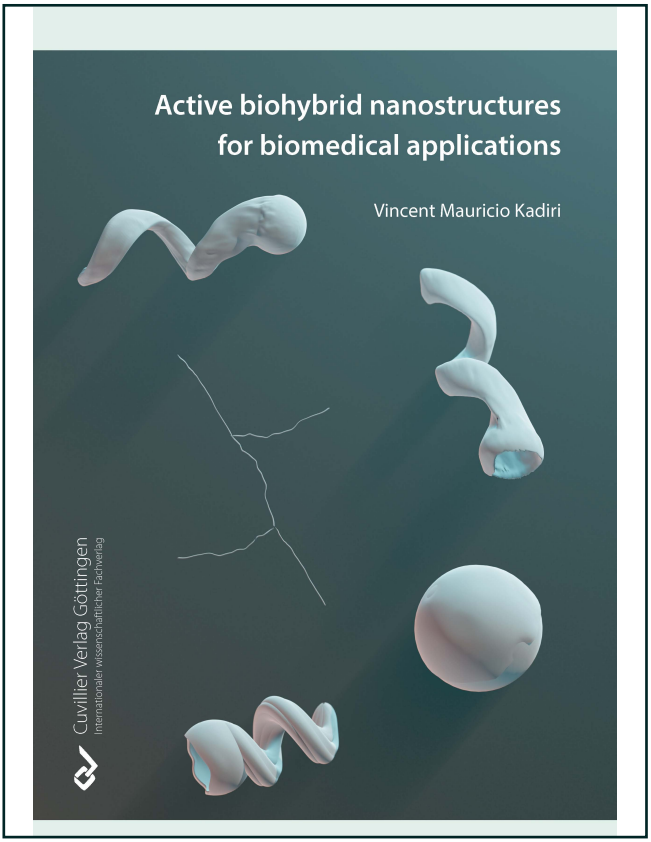
Vincent Mauricio Kadiri (Autor)

Active Biohybrid Nanostructures For Biomedical Applications



https://cuvillier.de/de/shop/publications/8579

1

INTRODUCTION

The first documented surgeries were performed 5000 years ago in ancient Egypt. [16] Many practices of modern medicine can similarly be traced back to ancient African or Asian origin (*e.g.* proto-vaccines [17] or specialized doctors for eyes, teeth or surgery [16]), yet medicine has clearly undergone many transformations since then. Over the centuries, progress through serendipitous discoveries and inherited knowledge was steady yet mostly slow. Certain innovations such as germ theory and quarantining the sick, however, were nothing short of revolutionary when they were proposed by Ibn Sina (alias Avicenna) and contemporaries in the 11th century. [18] In time, more and more debilitating diseases became treatable with novel pharmacoactive compounds and treatments.

Further rapid progress ensued due to the rigorous application of the scientific method in the last 250 years to finally yield modern medicine as we know it, [19] and directly aided in making modern living standards possible and extending life expectancy. [20] Ultimately, the path led to the recent rapid development, testing and approval of mRNA-based vaccines against a novel coronavirus (Sars-Cov-2) in the span of just under a year. [21]

Multiple new disruptive biomedical technologies are still being developed. Immunotherapy, for instance, targets diseases through a modification of the adaptive immune system. Thymus-derived lymphocytes (T-cells) express receptors that bind antigen fragments of either pathogens (pathogen-associated molecular pattern (PAMP)) or diseased cells (damage-associated molecular pattern (DAMP)). Certain diseases such as cancers, however, find ways to hijack or circumnavigate these immune defenses, thereby suppressing an immune response. These surreptitious diseases currently require lengthy and unpleasant treatments that often put the patient at risk. [22] The idea behind immunotherapy is, therefore, to identify and promote receptors capable of binding these antigens, allowing the patient's body

to recognize the disease. To this end, T-cells extracted from the patient's blood are genetically modified to express these receptors. After culturing (*i.e.* multiplying) the T-cells, they are re-injected and target otherwise undetectable tumor cells or pathogens. One hopes that immunotherapy could eventually avoid or complement more dangerous treatments, like systemically administered chemotherapy, this way. [23] Biomedical information technology is also expected to have an impact on the medical field. Artificial intelligence (AI), *e.g.*., is proposed as a complement to the repertoires of doctors and is intended to aid with diagnoses. [24] Bacteriophage therapy has long been discussed as a possible new pharmaceutical alternative to using antibiotics, especially in the face of multi-resistant bacterial strains spreading globally. Bacteriophages, or phages for short, are viruses that unlike *e.g.* influenza viruses or severe acute respiratory syndrom coronavirus 2 (Sars-Cov-2) are bactericidal (*i.e.* target and kill bacteria) while being harmless to animals. While promising, safety concerns against using viruses as a treatment have mostly prevented their large scale application to date. [25, 26] Bacteriophages exhibit many desirable traits (such as chemical and genetic modifiability combined with a short generation time) that could make them relevant to nanomedicine beyond their direct application as a pharmaceutical compound.[4] Therefore, M13 bacteriophages are considered in this thesis as a possible material for the construction of biomedical nanodevices. A more thorough introduction to M13 bacteriophages will be given in section 4.1.

The list of advanced biomedical technologies being developed today is extensive (see Fig. 1.1). Stem cell therapies, [28] high throughput and integrated -omics (genomics, transcriptomics, metabolomics *etc.*), [29] lab-on-a-chip microfluidics, [30, 31] robotics, [24] and gene-editing are all expected to work in tandem to bring about a more personalized, technologized medicine of the future. They could enable newer, more precise treatments, *in vivo* models, surgical robots or point-of-care devices. [32, 33, 34] Changes are thus on the horizon for medicine of the 21st century and beyond. Likewise, the biomedical application of nanotechnology, nanomachines or nanodevices (hereafter referred to as nanomedicine) aims to transform how we diagnose diseases or administer therapeutics. [35] Current challenges in nanomedicine, as well as possible ways to address them from a physical chemist's perspective are the focus of this thesis.

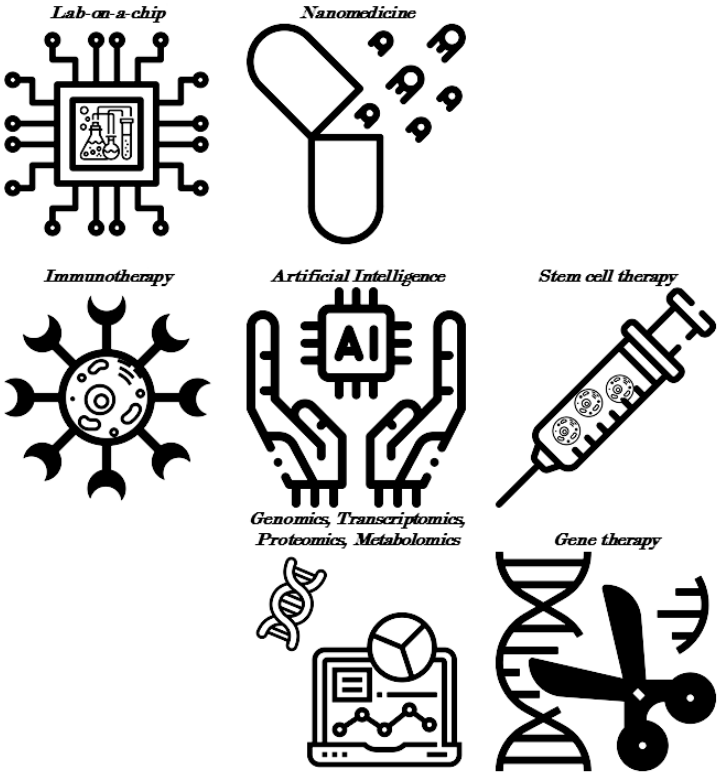


Figure 1.1: Overview of new medical developments and nanotechnology in the early 21st century. Icons adapted from [27]

The idea behind nanomedicine sounds strikingly simple: micrometer or nanometer-sized devices or machines are loaded with therapeutic cargo (DNA, RNA, drugs *etc.*) and injected into the human body to deliver their payload with high, possibly cellular precision; ideally while also giving diagnostic feedback. A similar concept of nanomedicine, commonly attributed to Albert Hibbs and

Richard Feynman, has been around for decades. In his talk "There's plenty of room at the bottom" Feynman called it "swallowing the surgeon". [36] Presumably starting with the 1966 science fiction movie "Fantastic Voyage" (later adopted into a book by the late Isaac Asimov [37]), this idea entered into modern popular culture. Today's public discourse - both positive and negative - and media with science fiction elements subscribe to this vision and often already take intelligent surgical "nanobots" for granted. [38, 39]

Despite this popularity, the reality of biomedical research in nanotechnology does not align with the vision of intelligent nanomachines stated above. Selected nanoparticle-based compounds and preparations from hand-sanitizers to drug delivery systems and vaccines did reach clinical trials or application stages over the last years. [40, 21] However, while promising, these examples of nanoparticulate carriers (1-100 nm) are markedly different from the envisioned (but not yet existing) intelligent autonomous nanomachines. More critical studies even describe them as imprecise and even the enhanced permeability and retention (EPR) effect, which claims tumors preferentially internalize nanoparticles, may have little factual basis. [41] Currently available nanoparticles don't yet exhibit cellular precision, autonomy or diagnosis aspects. [40] One state-of-the-art nanomedicine - liposomes - can shed light on why nanomedicines lack these features and what it would take to realize them.

Drug loaded liposomes obtained approval by the United States Food and Drug Administration (FDA) as far back as 1995. [42] They are vesicles that self-assemble with one or more lipid bi-layers. Since they are typically utilized in aqueous media, the hydrophobic chains sequester on the inside of a lipid-bilayer while the hydrophilic ends are oriented away from it, facing the medium or cargo respectively. A schematic of a liposome is pictured in Fig. 1.2a. As depicted, liposomes can incorporate a payload on the inside of either the vesicle (hydrophilic payload) or by solubilizing it between the hydrophobic chains. This makes them easy to manufacture and highly scalable drug or gene carriers. Additionally, being fabricated from lipids, liposomes can easily be metabolized and degraded after *e.g.* gene delivery. [21, 42, 43]

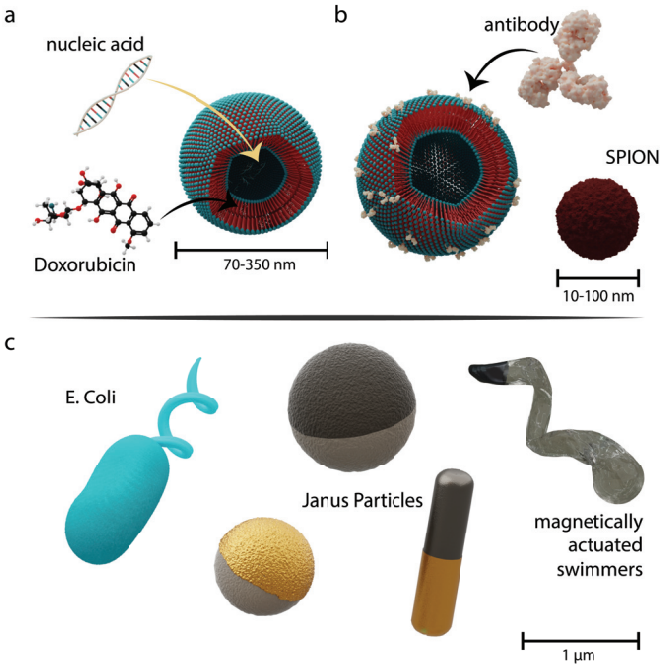


Figure 1.2: a) Liposomes are 70-350 nm biomedical nanoparticles that consist of a lipid bilayer, can load genes or drugs as cargo, and rely on Brownian diffusion to reach target sites. b) Targeted drug carriers can be achieved by equipping liposomes with antibodies or by magnetically pulling superparamagnetic iron oxide nanoparticles (SPION)s. c) Natural systems like Escherichia coli (E. Coli) exhibit a precise and sophisticated targeting system: chemotaxis. Inspired by this natural precision, active nano- and microswimmers such as chemically-powered Janus particles and magnetically actuated propellers are being investigated as next-generation biomedical cargo carriers. Scale bar in c) is 1 µm.

While certainly efficacious, the potential of liposomes to target a specific tissue or cell — apart from controlling the injection site — is limited since liposome diffusion in the body is completely passive. Diffusion is governed by either Brownian motion

or external flows in the body (*e.g.* blood). Generally, the payload only reaches an intended therapeutic target by chance.

Nevertheless, progress is being made as researchers are investigating how to enable targeting nanoparticles as shown in Fig. 1.2b. [44] Future liposome applications might very well copy a mechanism found in nature, namely the antigen-antibody interaction to improve targeting precision. Studies show that integrating antibodies onto nanoparticle carriers can increase accumulation in specific tissues or cell types. [45] Liposomes, while being useful drug carriers, thus currently fall short of nanomedicine's goals stated above in two regards:

1. targeting and cellular precision
2. diagnostics or signalling

Fortunately, natural pathogens such as bacteria can serve as model systems on how to achieve these criteria. Certain microorganisms evolved sophisticated mechanisms of targeting and signalling in the active motion and taxis of bacteria.[46] E. Coli (depicted in Fig. 1.2c) utilize chemical energy to rotate their flagella in a corkscrew-like fashion, which actively propels them forward trough the medium. [47] Swimming at the micron-scale will be discussed in detail in section 2.1. As Howard Berg observed in 1972, a tumbling event (caused by one flagellum changing direction) will change the bacterium's orientation once every second. As soon as the flagellar motor starts back up, the bacterium will resume swimming in a straight line (run) until the next tumbling event. Berg noted that E. Coli augmented their run-and-tumble foraging if a food source was present. The run intervals became longer when moving towards the food source and shorter while moving away. [47] This meant that over time the bacteria exhibited directed active motion up a chemical gradient, a process known as chemotaxis. The bacterium is still subject to Brownian motion, however, through active motion it can explore space more efficiently and direct its path away from a negative stimulus or toward a food source. Taxis is something many natural systems exploit. Beside chemical gradients other taxes include aerotaxis, gravitaxis, magnetotaxis or phototaxis, *i.e.* sensing oxygen, gravity, magnetic fields or light respectively. [46] Taxis is typically observed in more

complex biological systems or organisms. E. Coli *e.g.* have an entire taxis apparatus. Since both taxis and active motion are important aspects of nature's specificity and precision, attempts are being made at realizing nanomedicines that copy these processes. [48]

Active motion can thus not only be achieved by biological systems but also synthetically. Chemical swimmers are a form of active matter that operate away from thermodynamic equilibrium (see also sections 2.1.5 and 4.3) and continually transform energy, typically from a chemical fuel (H_2O_2, glucose, *etc.*) into motion. One geometry that allows active artificial micro- and nanoswimmers to actively propel at the micron-scale are Janus particles, named after the two-faced Roman God (of *inter alia* doors and time) Janus who has one face that looks into the past while the other looks into the future. Janus particles (shown in 1.2c) also exhibit two faces, one catalytic and one chemically inert face. The asymmetric catalysis of a surrounding fuel enables propulsion *via* self-phoresis (discussed in section 2.1.5). [49] The active motion of Janus particles can result in collective phenomena such as ordered movement, spontaneous assembly of active colloids, and directed motion. [50]

One advantage of using inorganic structures is that synthetic human-made technologies are less limited in the choice of material. One key requirement is that they should be biocompatible, *i.e.* not cause toxic side effects, however. One inorganic system explored in this thesis are actively propelling chemical motors (see chapter 6). Another type of inorganic nanoparticle that has been extensively investigated in the literature in recent years are superparamagnetic iron oxide nanoparticles (SPION)s (Fig. 1.2b). [51, 44] While these are not actively swimming, they can be 'pulled' to a target site by the magnetic gradient of an external magnet. SPIONs are relatively easy to fabricate and functionalize, and are promising for diagnosis and therapy (nuclear magnetic resonance imaging (MRI) contrast, magnetothermal destruction of tumor sites, delivery by magnetic control, biodegradability, and biocompatibility). [51] However, a disadvantage is that SPIONs require a strong magnetic field gradient, which could make them difficult to utilize *in vivo*. For this reason it could be advantageous to instead use weak rotating homogenous magnetic fields that can be realized experimentally over larger distances. This thesis therefore also investigates nanopropellers that copy E. Coli's helical shape to propel (shown

in Fig. 1.2c)). For external manipulation, a magnetic section is incorporated into these structures. A weak (mT) rotating magnetic field is generally sufficient to actuate the nanopropellers through rotation-translation coupling, allowing physical targeting by varying the magnetic field direction (see also section 2.1). [52]

After discussing some state of the art nanomedicines, it becomes clearer which attributes nanoscopic or microscopic devices for *in vivo* diagnoses, therapy and targeting would need to fulfil:

1. Materials and nanostructures should be fully biocompatible and non-toxic.
2. Structures should permit a rational *de novo* design and ideally self-assemble.
3. Nanodevices should move actively and precisely at cellular size scales.
4. Nanodevices should chemically and specifically target tissues.
5. Nanodevices should be biodegradable or have pathways out of the organism.

Liposomes and SPIONs fulfil several of these criteria. If the vision of nanomedicine is to be realized, one of the remaining challenges is the functional combination of all of these traits into one singular, or a collection of nanoparticulate systems working in tandem.

This thesis will present two systems of active biohybrid nanostructures which could serve as a basis for the construction of such functional nanomedicines. Part I prefaces this work with both an overview of the state of the art (this chapter) and a treatment of the basic theory behind the discussed applications (chapters 2, 3, and 4).

Part II (see page 57) will discuss magnetic nanomotors which hold great potential for facilitating nanomedicine applications due to their precision targeting capabilities. Previous iterations, however, paid little attention to material selection and biocompatibility. Thus, commonly utilized materials are incompatible with biomedical applications due to toxicity (Ni) or they are impractical due to low magnetic moments (FeO_x).

Chapter 5 will therefore focus on the fabrication and characerization of wholly biocompatible, hard magnetic biomedically functional $L1_0$ FePt-based magnetically

actuated nanopropellers and their application for *in vitro* gene delivery. Chapter 6 then aims to expand the functionality of FePt-based nanodevices by presenting light- and magnetically actuated structures that exhibit catalytic properties.

Part III (see page 97) will focus on schemes to make nanodevices biodegradable and to realize a gene therapy platform for primary eye cells and non-carcinoma cell lines.

Part IV (see page 117) will focus on self-assembly of entirely biologically-derived nanodevices based on M13 bacteriophages. M13 bacteriophages were selected for their biocompatibility and relative ease of genetic modification. They thus yield self-assembled, highly modular toolboxes of biological or organic building blocks. Enzymatic nanonets for repeated enzyme recovery and biocatalysis will be discussed in chapter 8.

The concluding chapter 9 (see page 141) will critically discuss the findings presented in the publications. It will aim to give a brief outlook on remaining challenges and future research (such as a possible extension to DNA origami, phage origami) as well as emphasize the progress made with the biomedical nanodevices discussed in this thesis.