Multifunctional Programmable Self-Assembled Nanoparticles in Nanomedicine

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Multifunctional Programmable Self-Assembled Nanoparticles in Nanomedicine

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

by

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Abstract

Developing methodologies to control the architecture of nanoparticles (NPs) at the atomic level prevents their inhomogeneity and leads to a variety of expected functions. Rationally designed nanoparticles can either be programmed or crystallized structures into pre-determined structures achieving tunable particle pore size and physiochemistry. In this dissertation, two broad classes of multifunctional nanoparticles are developed, metal-organic frameworks and DNA-NP aggregates.

Metal-organic frameworks are a novel class of highly porous crystalline materials built from organic linkers and metal cluster-based secondary building units. However, applications in bioremediation have not been developed very well especially in applications regarding drug delivery systems (DDS). The first studies in this dissertation aimed to develop a new MOF DDS that can be applied to photodynamic therapy (PDT) and achieved the difficult targeted treatment of triple-negative breast cancer (TNBC) cells, tumor-associated macrophages (TAM), and pancreatic cancer cells. The successful development of DDS for MOFs with maltotriionate potassium was the first step in the studies. Then the development of a new stable and effective PDT ligand in the oxidative environment and a bioconjugation method was further developed. Finally, the MOF platform was completed for the dual therapy MOF, which can be used for PDT and chemotherapy treatments, showed a more significant effect to kill cancer cells than with only PDT.

Another study was the development and analysis of programable architecture with DNA and multiple kinds of metal NPs. The well-defined base-pairing interactions of DNA allow it to behave as a programmable bond on a nanoparticle scaffold. Binary metal NPs have been increasingly receiving significant attention due to the surface plasmonic response (SPR) and
multifunctional abilities. However, the technique of dual-metal NP synthesis and studies in the quantum field have not been established. As the second study, binary and multiple-metal NP structure in programmed spatial organization using DNA-based technology was developed.
Acknowledgments

First of all, I’ve encountered various adversity and challenges preventing me from getting Ph.D.; still, I would like to thank the University of Arkansas for giving me the opportunity and platforms and for supporting me to the end to get my Ph.D. in a foreign country. In particular, I am indescribably grateful to my mentor, Professor Sakon, who has been understanding me and guiding me to this accomplishment. Not only research but also a better way of life with sustainable physical and mental health did he teach me. Without him, it was impossible to pursue my Ph.D. continuously. I thank Professor. Jin-Woo Kim for his kindness and time to accept and guide me as my co-advisor. While at this university, I belonged to a wide range of laboratories in organic chemistry, protein chemistry, cell experiments, and DNA-nanotechnology, and I was able to learn the differences in analysis methods and research management methods. It was an immeasurable experience. I thank my committee members for their guidance in both my research and professional matters. I also acknowledge my undergraduate students who spent their precious time on some part of my research: special thanks to Zachary Heidrick, Olivia Watson, Christopher Salmon, Miu Tsuji, and Konomi Sasaki. I am also grateful for the Xiaogang Peng Foreign Graduate Student Fellowship, which was awarded to Yoshie Samaki during 2021 Fall semester.

I thank my husband John Ozdemir to support me and overcome our Ph.D. journey together.

Last but not least, I am grateful to my family for their unwavering love and trust during this challenging doctoral journey.
Dedication

For your infinite compassion and love, strength and support, strong faith and encouragement,

I would like to dedicate my Ph.D.

To my Parents, Yasuo and Mina Kubo

To my late Grandpa, Kazuo Sakamaki (passed Sep 15th, 2021)

To Dr. Daisaku Ikeda
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List of Published Papers

   - Some part of the article was used as chapter II of this dissertation
   - *First co-authors (memo of my contribution attached)

   - Entire article was used as chapter III of this dissertation

   - Entire article was used as chapter IV of this dissertation
   - *First co-authors (memo of my contribution attached)
Chapter I - Introduction

In 2018, cancer was responsible for 9.6 million deaths, and 18.1 million were newly diagnosed worldwide.\textsuperscript{1} Breast cancer is the most common cancer in American women, with the exception of skin cancers. Currently, the average risk of a woman in the United States developing breast cancer at sometimes in her life is about 12%. For 2019, the American Cancer Society estimated that 268,600 new cases of invasive breast cancer will be diagnosed in women, about 62,930 new cases of carcinoma in situ (CIS) will be diagnosed (CIS is non-invasive and is the earliest form of breast cancer), and about 41,760 women will die from breast cancer. In recent years, incidence rates have increased slightly (by 0.4% per year). Currently there are more than 3.1 million breast cancer survivors in the United States. This includes women still being treated and those who have completed treatment. Death rates from female breast cancer dropped 40% from 1989 to 2016. Since 2007, breast cancer death rates have been steady in women younger than 50 but have continued to decrease in older women.\textsuperscript{2-5} These decreases are believed to be the result of finding the breast cancer earlier in the screening process, as well as better treatments. However, in different breast cell lines, there are less and more aggressive cell lines. Tumor subtypes and cell types are similar. MCF7 and T-47D were classified Luminal A subtype which classifies it as less aggressive, while BT549, HS578T, MDA-MB468, and MDA-MB231 cell lines are classified as triple negative breast cancer (TNBC), which is more aggressive. Depending on the tumors subtype, the applied treatments are different due to the different expressions of receptors.
Figure 1. Tumor subtypes.  

Luminal A subtype expresses estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2(HER2). Luminal A cancers are low-grade, tending to grow slowly and have the best prognosis. Luminal A breast cancers are likely to benefit from hormone therapy and may also benefit from chemotherapy. However, TNBC lacks ER, PR, and HER2 receptors and does not benefit from targeted therapy. TNBC comprises 15-20% of all breast cancers. Compared with patients with hormone receptor positive or HER2 positive breast cancer, patients with TNBC are typically younger in age (age <50 years), African American and have a higher incidence of BRCA1/2 mutations. The clinical course is frequently characterized by early relapse and poor overall survival. The TNBC phenotype is impervious to therapies commonly used in other breast cancer subtypes, including hormonal therapy and HER2 receptor antagonism.

Table 1. Molecular classification of breast carcinoma.
Cytotoxic chemotherapy remains the only approved treatment. More than 80% of women with TNBC are treated with chemotherapy regimens that include anthracyclines such as doxorubicin. However, doxorubicin has been known to be ineffective in the treatment of TNBC, and can cause cardiotoxicity as a serious side effect. Furthermore, chemotherapy treatment of breast cancer cell lines using either 5-FU, cisplatin, paclitaxel, doxorubicin, or etoposide showed multi-drug resistance. Compared to other breast cancer cell lines such as MDA-MB468, MDA-MB231 was reported to have significant multi-drug resistance and develop drug resistance due to high P-glycoprotein (P-gp) expression, which reduces the effects of anti-cancer drugs. The clinical treatment of MDA-MB231 in patients is frequently characterized by early relapse and poor overall survival. TNBC including MDA-MB231 represents an unmet clinical need with its aggressive clinical course and paucity of effective treatment options. There are no specific treatment guidelines for TNBC patients and there are no effective targeting chemo drugs.

Also, pancreatic cancer is one of the leading causes of cancer mortality, with an overall Mortality/Incidence ratio of 98% and an overall five-year survival rate of about 6%. This high mortality is due to several factors, including the late stage at which most patients are diagnosed and the difficulty and dangers associated with surgical resection, which is currently the most effective treatment. Based on the United States National Cancer Institute’s data for pancreatic cancer, more than half (52%) of cases were diagnosed at the distant stage, at which point the cancer has already spread to distant parts of the body. Pancreatic cancer cell lines were also chosen for targeted cancer.

Nanotechnology could approach for this unmet medical need cancer treatment. Recently, the engineering of metal-organic frameworks (MOFs) for photodynamic therapy (PDT) was reported for the use for PDT. Photodynamic treatment kills cancer cells by activating a drug termed
a photosensitizer or photosensitizing agent with light. The light source is a laser or another kind of light source, such as LEDs. Because photosensitizers accumulate in aberrant cells and the light is focused specifically on them, photodynamic treatment causes little harm to healthy cells. It is beneficial for patients with skin malignancies and precancers because photodynamic treatment does not leave scars. However, PDT has the potential to damage normal cells in the treatment region, resulting in adverse consequences. The light utilized in photodynamic treatment has a maximum penetration depth of approximately 1/3-inch, or 1 centimeter. Thus, PDT is limited to the treatment of malignancies located on or just under the skin, or on the lining of internal organs or cavities. Due to the limited penetration of light into big tumors, photodynamic therapy is less effective in treating them.

However, well-designed MOF crystals would be able to solve problems with current photosensitizer (PS) and offer certain multi properties such as size controllability, biodegradability, drug delivery system (DDS) with base frameworks and huge inner surface area, enhanced generation of reactive oxygen species (ROS) with heavy atom effect and without aggregation between PS, active targeting with MOF’s surface area, and theragnostic. Establishment of new MOF’s with these multi-functions would be a new novel approach for PDT and chemotherapy. MOFs for the use for targeting treatment was not developed yet. The utilization of MOF as a drug carrier which could capsulate chemo drugs also can treat not only solid cancer but also metastatic cancer.

The three objectives of this dissertation work are the following:

1. Developing a DDS method of MOF to target TNBC.

2. Synthesizing a new linker to absorb longer wavelength for deeper tissue treatment and developing a new bioconjugation method.
3. Designing and synthesizing a new MOF to deliver chemo drugs and PS to target pancreatic cancer.

Another nanotechnology was also studied in this dissertation. Localized surface plasmon resonance (LSPR) caused by the interaction between metallic NPs induces red-shifted wavelength and absorbs near-infrared wavelength. The optical properties of plasmonic metal nanoparticles are determined by the oscillation of free electrons in resonance with the incident electromagnetic field, a phenomenon known as localized surface plasmon resonance (LSPR), the frequency of which is proportional to the size and geometry of the nanoparticles.\(^\text{14}\) Additionally, the LSPR frequency is influenced by both the dielectric constant of the surrounding medium and the dielectric constant of the nanoparticle. With increasing refractive index (RI) of the medium, a redshift in LSPR is observed, which has proven extremely useful as an optical sensing tool. Because the pair of plasmonic nanoparticles is an anisotropic nanostructure, they exhibit plasmonic coupling,\(^\text{15}\) which can be effectively controlled by varying their interparticle distance. Apart from the dielectric medium's influence, the LSPR frequency is also influenced by the interaction of plasmonic nanoparticles. However, synthesizing such anisotropic nanostructures and studying the properties are tremendous endeavors, so simulation studies have been performed more than practical research.\(^\text{15-18}\) Thus, the interaction between plasmonic nanoparticles and their environment garners critical attention in biomedical applications such as DNA origami and colorimetric biomolecule sensing. Additionally, these dimers are utilized to fabricate gratings for high-resolution space observatories and computer circuits. However, even the preliminary study of plasmonic alloy nanoparticles as dimers, which forms the basic foundation for this current work, is extremely rare.
Several developed techniques for synthesizing nano dimers include the seed growth approach, the Vander Waals method, and the high-pressure method. However, those methods require purification, organic solvent, or high-level facilities for the synthesis. In addition, those methods are neither possible nor reasonable to control the distance between nanoparticles nor to construct further complicated 2D and 3D structures. As the other option, origami technology has been reported to be a good candidate for this solution. The origami platform can make 3D structures as they are designed. However, the origami technology has a disadvantage in that it cannot arrange NPs close enough to cause SPR effects on the origami platform.

A synthesis of anisotropic ssDNA-linked NP was reported by Kim et al. in 2011. None of other reported methods could come to close the way so far after that report. Its approach, “one step at a time” conjugation of DNA, allows different ssDNA to bind to AuNP (3 nm) at +x, -x, +y, -y, and +z by step by step. Although it causes a chirality problem at sixth ssDNA, five different kinds of ssDNA could make 3D structural anisotropic DNA-linked NP.

This research aimed to expand this method to other sizes and types of metallic NPs and synthesize bimetal dimers to obtain practical results instead of a simulation study. Since metallic NPs of gold (Au), silver (Ag), and copper (Cu) are free-electron-like metals and have more pronounced SPR properties, Au, Ag, Cu, and iron-oxide (Fe₃O₄) were selected for studying SPR interaction and surface-enhanced Raman spectroscopy (SERS) between NPs. Furthermore, those metallic NPs have some theragnostic effects such as anti-bacterial effect, anti-cancer, fluorescence, and MRI. Thus, this study will develop this field study of the anisotropic structure and synthesize the multifunctional biosensor.

The additional object of this dissertation work is the following:
4. Developing the method of anisotropic DNA-linked NPs which can create further 3D structures and investigating their LSPR.

Overall, by highly controlling NPs’ structures, that research will help show new possible applications and approaches such as cancer treatment and biosensors. Furthermore, it will lay the foundation for future research, where the impact of factors such as unmet medical needs treatment, invasive cancer treatment, and the study in quantum of 3D structure and physiological properties.
1.1 Reference


Chapter II - Literature Review

2.1 Metal-Organic Framework for Photodynamic Therapy (PDT)

This article from section 2.1 to 2.11 has been accepted for publication in [Metal–Organic Frameworks and Covalent Organic Frameworks as Platforms for Photodynamic Therapy], published by Taylor & Francis. Right managed by Taylor & Francis

As described above, cancer caused 9.6 million deaths in 2018, with 18.1 million new diagnoses that same year.1 Preventative lifestyle choices and early detection of cancer can greatly reduce an individual’s risk of mortality but the staggering amount of cancer related deaths motivates intense scientific investigation into cancer treatments. The conventional methods of cancer treatment include a conjunction of chemotherapy and radiation treatment that cause indiscriminate cell death leading to debilitating side effects. Surgical options to treat cancer tumors are also intensive and can cause a major off-target damage. Alternatively, therapy using nontoxic light (visible -and near-IR (NIR) light) rather than harmful irradiation has been developed along with the use of light dependent drugs. Together, these new tools have undergone clinical trials and their potential is quickly being realized. Phototherapy in general utilizes light-catalyzed chemical reactions to treat diseases. The use of phototherapy for treatment of diseases stretches back more than 100 years with a Nobel Prize in 1903 being awarded to Niels R. Finsen for his work on phototherapy to treat symptoms of tuberculosis.2 More specifically, PDT as a treatment for cancer relies on the chemical reactions caused by three factors: light, oxygen, and a PS. The PS is a material which can produce reactive oxygen species (ROS) upon exposure of light of a specific wavelength. The treatment of cancer by PDT begins with PS injection. The PS then accumulates at the targeted region either passively or via directed targeting of the cancerous tissue.3-9 Upon
illumination of the targeted area, ROS are then generated and initiate the apoptotic process, and sometimes, depending on light and dye dose, necrosis can also occur.\textsuperscript{10-15}

The therapeutic effect of PDT depends on the photochemical and physiological attributes of the PS.\textsuperscript{16-18} Methods for improving the photodynamic effectiveness and biocompatibility of the PS are widely investigated to improve PDT treatment. The development of nanotechnology to achieve an effective PDT treatment is the key for overcoming these challenges.\textsuperscript{16, 19-21} Studies in nanomedicine have moved towards solving problems such as hydrophobicity, PS retention, and aggregation.\textsuperscript{20, 22-24} Delivery by nanocarriers not only improves pharmacokinetics but also increases the PS concentration in tumor cells.\textsuperscript{16, 25} Nano-sized metal–organic frameworks, or “nMOFs,” are inorganic and organic hybrid materials showing promise for PDT nanomedicines \textit{i.a.} because of the EPR effect (see below). PDT-capable MOFs have recently been developed and are being studied.\textsuperscript{21, 26-55} In this \textit{Critical Review}, we explain the basic mechanism of PDT, the biological effects of ROS, the history of PDT, clinical uses, MOFs, and the benefits of using MOFs. We will focus on MOF's effective use for PDT and its development. We will emphasize the potential use of nMOF scaffolds as agents for PDT delivering nanomedicine and discuss the use of another family of porous crystals, COFs, for use in PDT as described in the recent literature.\textsuperscript{46} (Figure 1)
2.2 Photodynamic Reaction

PDT uses light to initiate chemical reactions in the body. Light in the 600–1200 nm region is referred to as the “optical window of tissue” (OWT) because of its ability to penetrate the body. The wavelength range of light effective for PDT is 600–800 nm\textsuperscript{56-58}, light above 800 nm does not have enough energy to initiate PDT.\textsuperscript{59} PDT utilizes photosensitive compounds called photosensitizers (PS), which upon irradiation to an excited singlet state can then reach an excited triplet state. This triplet state is reached by undergoing intersystem crossing. (Figure 2) It is this triplet excited state of the PS that can generate toxic reactive oxygen species (ROS). Two reaction pathways of therapeutic interest produce ROS capable of initiating cells death. Type I consists of electron transfer and radical induced hydrogen removal, while Type II is the direct formation of singlet oxygen.\textsuperscript{10}
Figure 1. 2 PDT proposed mechanism

In Type I reactions, the triplet excited state of the PS can reduce $O_2$ to the superoxide anion radical ($O_2^-$). Superoxide anion radicals in the body lead to the generation of various ROS. $O_2^-$ is spontaneously or enzymatically converted to cytotoxic $H_2O_2$. $O_2^-$ can reduce $Fe^{3+}$ to make $Fe^{2+}$ and $H_2O_2$ by the Harber-Wiess reaction, followed by $Fe^{2+}$ mediated breaking of the $O-O$ bonds in $H_2O_2$ forming $OH^-$ and $OH^-$ in what is known as the Fenton reaction. The net reaction is $O_2^- + H_2O_2 \rightarrow OH^- + OH^- + O_2$. Using appropriate metals can help to produce more ROS and $O_2$. An example of such a MOF is Fe(III)MIL-100. Iron(III) has the capability to catalyze the decomposition of hydrogen peroxide, which tends to be over-secreted in tumor sites. As the Fenton reaction proceeds, free iron(III) ions are released and $H_2O_2$ is rapidly decomposed to generate an ample amount of $O_2$. This $O_2$ generation is beneficial because the efficiency of PDT depends on
the availability of $O_2$, which tends to be scarce in the environments surrounding cancerous tissues due to tumor hypoxia.

Evidence suggests that $Cu^{2+}$ and $Cu^+$ may also play this role in the body.$^{61}$ $OH^-$ is the most reactive of all the ROS as there is no enzymatic system to defend living organisms against it. In a cellular system, $OH^-$ will bind a proton by most likely abstracting one from a substrate.$^{62}$ Abstraction of a proton from this substrate is a cytotoxic oxidation. All three of these species ($O_2^-$, $H_2O_2$ and $OH^-$) are considered ROS capable of being generated by PDT.

The *Type II* reaction forms singlet oxygen ($^1O_2$) by direct transfer of energy to a nearby oxygen molecule. An electron in the degenerate $\pi^*$ antibonding orbital of triplet dioxygen will have its spin inverted forming $^1O_2$. Since the reactivity of $^1O_2$ is higher than $O_2^-$ and PDT induced generation of $^1O_2$ occurs at a higher rate ($k \approx 1-3 \times 10^9 M^{-1} s^{-1}$) than generation of $O_2^-$ ($k \approx 1 \times 10^7 M^{-1} s^{-1}$) it is $^1O_2$ that is often considered the most important ROS in PDT.$^{63}$ It has been shown that production of $^1O_2$ and other ROS species exist in equilibrium and practical implementations of PDT focused on the production of *type I* reaction sequence.$^{11}$ After light excitation, the PS may also return to the ground state by emission of a photon, which is optimal for bio-imaging applications, or by vibrational relaxation and the corresponding dissipation of heat.

### 2.3 Tumor Destruction and Biological Response

The end result of ROS creation by PS is localized cell death. PS buildup in various organelles initiates intracellular production of ROS which can cause cell death by either apoptosis or necrosis. The mechanism of cell death is dependent on the location of the PS within the cells.$^{64}$ The ROS generated are capable of causing irreversible damage if generated inside cells, particularly inside subcellular organelles such as the mitochondria and the Golgi apparatus.
¹O₂ has a half-life of 0.03–0.18 ms and only has a radius of diffusion of <0.02 µm. This means PS must be concentrated around the target area for highest efficacy.⁶⁵,⁶⁶ Indeed, singlet oxygen has a radius of destruction measured in nanometers, and photodynamic damage will then occur only very close to the intracellular location of the PS.⁶⁷

Cancerous tumors have the ability to grow blood vessels by a process called angiogenesis. Without targeting of these blood vessels along with the cancer cells, the PS cannot be effective.⁶⁸ The effect of PDT can vary on the vasculature of cancer cells depending on the PS used.⁶⁹ Because of this effect, PDT should be used in combination with angiogenic inhibitors as validated by Ferrario et al.⁷⁰

The tumor-specific immune response triggered by damage from ROS has also been investigated.¹³-¹⁵ The localized damage caused by PDT will release mediators that initiate an inflammatory response by the immune system to eliminate the source of inflammation.⁷¹ For example, one pathway includes PDT-induced damage of phospholipids, which starts a series of processes, that releases various inflammatory mediators stimulating a response macrophages, neutrophils, and mast cells of the immune system.⁷²

2.3 ROS Neutralization Mechanism (Glutathione Neutralization)

Reactive oxygen species are produced constantly in healthy cells by the electron transport chain of mitochondria. To prevent damage, these ROS are neutralized by enzymes in the body. PDT must compete with these anti-oxidative processes. A primary cellular defense mechanism against ROS is intracellular glutathione (GSH). A redox reaction between H₂O₂ and GSH forms H₂O, O₂, and glutathione disulfide (GSSG). The enzymes superoxide dismutase (SOD) and superoxide catalase also neutralize ROS in the body.
2.4 Obstacles for the Development of an Ideal Photosensitizer

2.4.1 Photosensitivity after Treatment and Effective Targeting Leads to High Targeting Expectations

The activation of a PS by otherwise harmless light to initiate cell death is the crux of PDT. However, the activation of this process can occur during a patient’s normal daily routine when exposed to any source of emission that produces the corresponding wavelength of light. With this in mind, one must take into consideration the length of time the PS will remain in the body and how long the patient will remain photosensitive. For instance, the skin and eyes of patients injected with the second-generation PS temoporfin (under the brand name Foscan) will remain photosensitive for up to 15 days while direct exposure of Foscan on the skin can cause photosensitivity for 3 months.\(^7^3\) Patient detriment due to long term photosensitivity emphasizes the need to isolate the PS in the tumor by effective targeting. Most administered PS are porphyrins or porphyrin derivatives. The basic underlying structure is porphin, a highly conjugated macrocycle with 18-\(\pi\) electrons. It can be custom-designed through addition of various functional groups to an innumerable amount of different porphyrin molecules.\(^7^4\) The nature of porphyrin as planar organic molecule results in PS that are hydrophobic and susceptible to aggregation due to \(\pi-\pi\) stacking.\(^2^1\) Since the exact reasons for the selectivity of PSs to tumor cells are not well understood, targeting tumors is a serious limitation to the development of new PS compounds.

2.4.2 Treatment Efficiency Depends on Accurate Light Delivery to the Tumor

The need for strong illumination of the PS by near infrared light leads to a challenge for prospective PDT as tumors deep inside of the body could only be illuminated strongly by endoscopy.\(^7^5\) PDT also requires the presence of oxygen for the PS to cause cell death. Therefore,
tumors surrounded by dead tissue or especially dense tumors have yet to be effectively treated with PDT.\textsuperscript{76}

\textbf{2.4.3 Tumor dispersion in Metastatic Cancers}

Not all cases of cancer involve a single tumor. Cancer cells can spread from one location to many others through a process called “metastasis”. In metastatic forms of cancer, cancer cells separate from a primary tumor and can travel through the circulatory or lymphatic system to another organ and resume growth.\textsuperscript{77} The result of metastasis is a patient with tumors of various sizes distributed over the entire body. For a PS to be successful in this scenario, it must overcome three hurdles. Firstly, solubility and aggregation issues mentioned previously. Secondly, production of ROS by a PS dispersed across the entire body could be very damaging to the patient. Lastly, exposure to deeply penetrating light of a specific wavelength to the entire body is another unique challenge.

\textbf{2.4.4 Tissue Oxygenation is Crucial to the Photodynamic Effect}

Solid tumor masses within an organ consistently show a decrease in oxygen levels from the healthy tissue surrounding them. This phenomenon is called “tumor hypoxia” and reduces the effectiveness of a wide range of cancer therapies including PDT.\textsuperscript{78}

There are two situations of oxygen depletion relevant to PDT. The first is when the tumor is hypoxic prior to the administration of the PS. Alternatively, the tumor may experience localized hypoxia as a result of oxygen consumption by the irradiated PS.\textsuperscript{79} To gauge the effectiveness of PDT treatment, oxygen levels before and during activation of PS must be measured. In practice,
this means monitoring tissue necrosis along with real time observation of oxygen levels of effected tissue and surrounding blood.\textsuperscript{80,81}

Methods to quantify the amount of ROS produced in the tumor by the PS in real time is also a fundamental need to gauge the effectiveness of PDT treatment. \(^1\)O\(_2\) creation by the PS in the complex environment of the body is difficult to model because the optical properties of tissue are not adequately developed for predictions to be made regarding ROS generation during PDT. Attempts to calculate the amount of \(^1\)O\(_2\) produced have been made by accounting for the concentrations of PS and oxygen in the region the light is applied, but remains inaccurate compared to direct dosimetry calculations of radiotherapy.\textsuperscript{82-84} Singlet oxygen luminescence detection (SOLD) is an analytical method developed to detect the luminescence of \(^1\)O\(_2\) at 1270 nm \textsuperscript{85,86} and was preformed using a single optical fiber in 2013.\textsuperscript{87} Direct observation methods are technological advances that are beneficial for the observation of PDT. However, methods to detect the amount of \(^1\)O\(_2\) produced \textit{in vivo} might not require direct observation. For instance, monitoring the photobleaching of porphyrin photosensitizers can be used to indirectly determine the amount of \(^1\)O\(_2\) produced.\textsuperscript{88}
2.5 History of the Photosensitizer

2.5.1 First Generation

The first generation of PS was developed in the 1970’s and 80’s. (Figure 3) They consist of interlinked porphyrin molecules. Porphyrins have two regions of strong photon absorption which include bands in the 380-500 nm range called the “Soret bands,” and bands in 500–750 nm region called the “Q-bands”.  

The absorption values of porphyrin can be easily manipulated. The chemical structure of the porphyrin macrocycle allows for numerous modifications, including metalation of the nitrogen
containing center. Due to the huge diversity of porphyrin macrocycles, it is now possible to synthesize porphyrin structures that can absorb in any region of the visible spectrum.\textsuperscript{90} The first PDT drug used clinically was the porphyrin-based porfimer sodium, sold as \textit{Photofrin}. This oligomeric mixture of ether and ester linked hematoporphyrins with absorbance in the 630 nm range of light was approved for clinical use for treatment of recurrent papillary tumors in 1993.\textsuperscript{91}

2.5.2 Second Generation

Unlike the first generation of PS, second generation PS are chemically pure and absorb light at higher wavelengths. The introduction of chemical purity allowed for effective purification methods and a standardized synthetic product.\textsuperscript{93} Second generation PS also included chlorin and bacteriochlorin molecules, which are reduced porphyrins that absorb light of higher wavelengths than porphyrin.\textsuperscript{94} By absorbing at higher wavelengths, deeper penetration of light was achieved making the PS more effective. Other second-generation molecules include phthalocyanines, naphthalocyanine and – as a PS precursor - amino-levulinic acid (ALA). In the body ALA is transformed to Protoporphyrin IX. Temoporfin a chlorin molecule which strongly absorbs red light at 652 nm, showed increased uptake into tumor cells over 72 hours after injection into a patient and was approved for clinical use for certain cases of head and neck cancer by the European Union in 2001. The improvements in the timeframe of tumor uptake, stronger absorption, and the chemical purity of Foscan places it in the second generation of PDT drugs along with other pure porphyrin and chlorin molecules. \textsuperscript{82,94,95}
Figure 3. Absorption of main PS for PDT $^{18, 57, 96, 97}$
2.5.3 Third Generation

The third generation of PS molecules is distinct from the previous two because of their use of conjugation of PS compounds to various biomolecules such as somatostatin analogs, antibodies and single chain Fv fragments. This results in higher selectivity in targeting cancer cells. Sugars have also been used to increase the selectivity of PS molecules. Binding of photosensitizers to sugars such as maltotriose, mannose, and glucose led to increased accumulation of the photosensitizer in tumor cells. Glucose bound chlorin has higher antitumor effectiveness than the second generation talaporfin chlorin molecule. The third generation has come to include PS capable of binding to biological carriers in vivo, thus, increasing delivery by the bloodstream. For instance, boronated metallochlorins can interact with serum albumin making prior conjugations to biological targeting moieties unnecessary. (Figure 3)

2.5.4 Nanoparticles as Fourth Generation Photosensitizers

Nanoparticles (NP) are materials whose dimensions are between 1-100 nm in size. Nanoparticles created for the delivery of PS materials are included in the fourth generation of PS. Examples of fourth generation PS include; liposome mimicking porphyrin-phospholipid conjugate nanoparticles developed in 2011 by Lovell et al. In 2016 Liu, Zhang and Yang synthesized peptide synthesized self-assembled PS nanosphere held together by electrostatic interactions, π-π stacking, hydrophobic interactions and covalent linkage, the weak PS-peptide interactions could be reactive to changes in pH, redox potential and enzyme concentration leading to targeted PS release.

Encapsulation or incorporation of the PS by a cancer targeting NP can reduce the distribution of the PS in the rest of the body causing mitigation of side-effects. In addition, it also
increases the efficiency of the PS meaning smaller quantities are needed for treatment.\textsuperscript{108} When used as a PS, nanoparticles add benefits, such as large surface to volume ratios and the ability to include both diagnostic and therapeutic functionality in one particle. These benefits are also found in MOFs. In the past decade organic and inorganic nanoparticles have been employed as third and fourth generation photosensitizers for PDT.\textsuperscript{82, 109-111} The ability to bind polar moieties onto the surface of nanoparticle polymers makes it possible to create water soluble nanoparticles that can be transported easily through the bloodstream.\textsuperscript{112} Organic NPs engineered towards drug delivery and non-covalent encapsulation of the PS include liposomes, micelles, and polymeric nanoparticles that are usually biocompatible and able to release PS in body.\textsuperscript{16, 110, 113-115} Inorganic materials include: gold NPs, quantum dots, zinc oxide NPs, titanium dioxide NPs, X-ray activatable NPs, and up-conversion NPs. These materials can be used as multifunctional nanocarriers, which can be capable of contributing to the activation mechanism of PS excitation.\textsuperscript{12, 16, 114, 116-120} Very Recently, Lin’s group, followed by Zhou and Liu’s groups, have demonstrated that nanometric inorganic-organic hybrid networks, known as “metal-organic framework nanoparticles” (nMOFs, NMOFs, MOF NPs or nanoMOFs) are effective platforms for PDT.\textsuperscript{21, 39, 44, 48, 49}

2.6 Targeting

2.6.1 Passive Targeting: EPR Effect

Passive targeting of cancer refers to the permeability of NPs through cell membranes due to their small size. Maeda et al. first identified this passive targeting as the “enhanced permeation and retention (EPR) effect” and it has been dubbed as the “gold standard” for tumor-selective drug delivery in anti-cancer agent design.\textsuperscript{4, 121} The passive accumulation of NPs is the result of this EPR
effect. The accumulation of NPs in tumor happens because of the leaky vasculature (enhanced permeation of NPs) and impaired lymphatic drainage (increased retention of NPs) of the tumor.\(^5\) To make the passive targeting most effective, the size of NPs should be between 10–200 nm. NPs with the size smaller than 10 nm can be excreted via kidney. While, NPs with size bigger than 200 nm can be easily trapped by reticuloendothelial systems (RES) such as macrophages.\(^6, 12^2\) Furthermore, NPs need to retain stability in blood until penetration into the tumor by the EPR effect. By controlling the size of NPs to smaller than 30 nm accumulation and penetration into pancreatic tumors, which are less susceptible to the EPR effect, can be improved.\(^7\) Furthermore, optimal moieties such as poly(ethylene glycol) (PEG) conjugated to NPs can enhance delivery of NPs to a tumor by avoiding interactions with blood components like serum albumin.\(^8\)

### 2.6.2 Active Targeting

Accumulation of NP by passive targeting does not isolate NPs in tumors only. NP can distribute to the healthy organ tissue as well.\(^9\) However, in active targeting, NPs can target cancer cells by use of peripherally conjugated targeting moieties such as sugars, antibodies, folate, and cRGD, which are recognized by receptors overexpressed in cancer cells and other components of the tumor microenvironment.\(^8\) To maximize NP accumulation in tumor cells, active and passive targeting should be used in conjugation. Moreover, porphyrins can be combined with biological vectors such as proteins, steroids, toxins, carbohydrates, peptides, and functionalized nanoparticles to overcome the problem of systemic prolonged photosensitization syndrome shown in healthy tissues. (Figure 5)\(^12^3\)
2.7 Clinical PDT for Cancer

Applications of PS compounds in the body can also be diagnostic as shown in experiments with 5-amino-levulinic acid (ALA) as a precursor of protoporphyrin IX. This compound fluoresces red when exposed to violet light between 375–440 nm, accumulates in tumor cells in a 20:1 ratio compared to normal cells, and is a good example of the theranostic employment of PS compounds.\textsuperscript{124,125} Certain parts of human anatomy lend themselves towards the facile application of light, therefore, tumors in these regions can be more effectively treated by PDT. The use of ALA-PDT for treatment of actinic keratosis skin showed a response rate of 89\% compared to 13\% for placebo. Testing of ALA-PDT for basal cell cancer (BCC) showed a complete positive response in 92\% of superficial BCC and a 71\% complete positive response in nodular BCC.\textsuperscript{59}

The use of Foscan for treatment of cancers in the head and neck was evaluated by doctors at Tata Memorial Hospital, Mumbai and shown to completely treat 30\% of patients with tumors
that were less than 10 mm deep and that could be fully illuminated with no ill side effects. 61% of patients experienced significant increases in quality-of-life.\textsuperscript{126}

Tumors in the esophagus have also been treated clinically by PDT. From an experimental group of 102 patients afflicted with Barrett’s dysplasia or mucosal adenocarcinoma, 56% underwent complete ablation following a single PDT treatment with the Photofrin PS.\textsuperscript{127} In a separate study of PDT for treatment esophageal cancer, the use of hematoporphyrin followed by illumination of the affected area with 630 nm light resulted in 37% of patients entering complete remission through use of the hematoporphyrin PDT alone and 82% of patients administered both hematoporphyrin PDT and radiotherapy entered complete remission.\textsuperscript{128}

In a study of patients with prostate cancer, administration of a porphyrin photosensitizer followed by application of at least 23 J cm\textsuperscript{-2} across 90% of the entire prostate resulted in 8 of 13 patients being cancer negative 6 months after treatment as determined by biopsy.\textsuperscript{129} Inoperable lung cancer is one arena where PDT by porphyrin photosensitizers performs exceptionally well. A PDT study consisting of 59 patients used Photofrin as the PS in order to treat early stage lung cancer with tumors located in the center of the lung. The results showed that treatment brought 50 patients (84.8%) to complete remission, and out of those, only 5 had cancer recur.\textsuperscript{130}

\subsection*{2.8 Metal Organic Frameworks (MOFs)}

The development of MOF synthesis in 1995 introduced the first hybrid organic-inorganic coordination polymers consisting of periodic formations of organic carboxylate linkers coordinated to metal-oxide clusters.\textsuperscript{131,132} This unifying motif of positively charged metal oxide secondary building units (SBUs) with multiple coordination sites connected by coordination of an anionic organic building unit (OBU) is what defines MOFs. The SBU and OBU can be considered
the building blocks of a MOF. The traditional synthesis involves solvothermal reaction of the OBU and SBU in a sealed chamber. MOF porosity exceeds that of any other material as determined by surface area through BET analysis. The MOFs NU-109 and NU-110 broke the world record for highest surface area of any material in 2012. As the number of SBUs and OBUs incorporated into MOFs increased, the number of identified MOF structures grows, with an estimated 70,000 MOF structures entered into the Cambridge Structure Database (CSD) by 2016. Structural diversity is amplified by MOFs’ isoreticular nature where the general topology of the crystal stays the same while functionalities and pore dimensions differ. In practice, this is when a linker of certain length is replaced by a linker with the same number of coordination sites and similar geometry but with a different length. Replacing linkers in this fashion results in channels of similar shape but larger in size. The pore size of UiO-66 is 8–11.5 Å meaning that molecules larger than this cannot be effectively absorbed into UiO-66, but the isoreticular nature of MOFs allows for the synthesis of UiO-67 with OBU’s roughly twice as long. The larger pore sizes of 11-23Å then allowed larger molecules to fit in the cavities of the alternative MOF. The larger pore size made possible the entrapment of tris-(2,2′-bipyridine)ruthenium(II) hexafluorophosphate (Ru(bpy)₃(PF₆)) into UiO-67. Utilizing the 4, 4′-biphenylidicarboxylate OBU gives UiO-67 and using 4, 4′, 4″-triphenylidicarboxylate OBU results in UiO-68, an example of the isoreticular pathway to developing new MOFs with the same SBU.

The number of SBUs and OBUs capable of incorporation into a MOF allows for the strategic design of application specific materials. When designing MOFs, one must consider both the topology of the MOF and the supramolecular assembly of the crystal structure. Applications where performance of MOFs stand out include gas absorption, tandem catalysis, and thin films. The structure of MOFs can be further engineered towards
optimal performance by *de novo* selection of relevant SBUs and OBUs, post-synthetic modification and crystal size control.

### 2.8.1 De Novo SBU Selection

MOFs can be engineered for specific functions by careful selection of the building blocks; that is, selecting specific metals for the SBU and relevant linkers for the OBU that fit desired needs. The two most utilized SBU families include the Me-O paddlewheel cluster (Me = Cu, Zn, Ni, Co)\textsuperscript{150-152} and metal oxides of the group 4 metals (Me\textsuperscript{IV}-MOFs/ Me = Ti, Zr, Hf)\textsuperscript{152-154}. SBUs are usually formed *in situ* during the MOF synthesis. Stability is an important characteristic manipulated by SBU selection.

It is known that metals with higher oxidation states make for more stable SBUs.\textsuperscript{155, 156} The most well-known of the highly stable Me\textsuperscript{IV}-MOFs is UiO-66 with zirconium oxide “brick” SBU of formula Zr\textsubscript{6}O\textsubscript{4}(OH)\textsubscript{4}(CO\textsubscript{2})\textsubscript{12}.\textsuperscript{157} UiO-66 is formed by the connection of 12 1,4-benzene dicarboxylates to each Zr-oxide SBU.

### 2.8.2 De Novo OBU Selection

OBUs are the organic component of MOFs. OBUs coordinate to the SBUs with varying degrees of topicity. Topicity in this case refers to the number of sites available for coordination to the SBUs. OBUs utilized range from ditopic to octatopic. Unlike the SBUs, OBUs are synthesized prior to MOF synthesis. The first MOFs synthesized utilized the simple and inert 1,4-dicarboxylate as the OBU. Advantageous use of OBUs for their catalytic, mechanical, or physical properties inspired the use of OBUs with rising complexity.\textsuperscript{158, 159} A complex cyclic carbohydrate has even been incorporated into MOF as an OBU.\textsuperscript{160} Porphyrins have become important OBUs for the planned design of function specific MOFs. Porphyrins are cable of holding
a metal atom in the center of their heterocycle, thus, bringing another transition metal center into a MOF. The use of custom designed porphyrins in MOFs began with the development of PIZA-1 which comprised a Co(III) metalloporphyrin bridging linker between Co(II) carboxylate SBUs for zeolite-analogous selective absorption. Metalloporphyrin ligands utilized as OBUs has expanded towards applications including heterogeneous catalysis and light harvesting.

2.8.3 Post synthetic modification

The addition of new functional groups to the OBU of MOF materials is possible through post synthetic modification. The ability for post synthetic modification is another factor towards the diversity of MOFs. Post synthetic modification of OBUs began in 2009 by Wang and Cohen. The practice now includes modification of amine and aldehyde groups, post synthetic metal exchange, solvent assisted ligand exchange, and metal addition to SBUs.

2.8.4 Nanoscale MOF (nMOF)

Size control methods for the synthesis of nanoscale materials is a critical aspect of crystal engineering. Materials that are typically totally insoluble become easily dispersed in solution when brought to the nanoscale. For biological applications, nanoscale materials are especially relevant because of their passive uptake into cells. Nanoscale MOFs (nMOFs) have been used for biomedical imaging and drug delivery. Several techniques exist for the synthesis of nMOFs, including limiting solvation of the starting materials, manipulation of thermal conditions, and use of nanoemulsions. One method for size controlled synthesis of nMOFs has been shown to be expandable to 8 MOF frameworks and involves the dropwise addition of linker and metal solution into the synthetic mixture.
2.9 Heavy Atom Effect

For PDT, the heavy atom effect has widely been utilized. Among ROS, the main cytotoxic agent, $^{1}\text{O}_2$, is produced via the reaction between the long-lived triplet state of the PS and molecular oxygen. The quantity of generated $^{1}\text{O}_2$ is dependent on the efficiency of intersystem crossing (ISC) since population of the triplet state of PS is the result of ISC from the photoexcited singlet PS. It is known that the presence of heavy atoms can enhance spin-orbit coupling subsequently increasing the rate of ISC. There are means by which to incorporate heavy atoms into PS: addition into the chromophore (internal heavy atom effect) and peripheral to the chromophore (external heavy atom effect). The internal heavy atom effect has been mainly used for PDT by covalently incorporating heavy atoms into the PS chromatophore. Generally, in internal heavy atom effect, because of the increased probability of intersystem crossing, the presence of heavy atoms (Br, I) as substituents of aromatic molecules results in fluorescence quenching. The intersystem crossing is favored by spin-orbit coupling whose efficiency has a $Z^4$ dependence ($Z$ is the atomic number). Heavy metal incorporation into a PS can boost $^{1}\text{O}_2$ production by increasing the level of intersystem crossing. A closed-shelled diamagnetic metal is better suited than an open-shelled paramagnetic metal because the latter shortens the triplet lifetime and makes certain chromogens photo-inactive. Some nMOFs mentioned preform conjugation of the PS to I and Zn for this reason. The intermolecular external heavy atom effect can also be considered as a promising strategy. Kim S et al. reported silica nanoparticles with intraparticle heavy-atom effect on the encapsulated PS for PDT. As it was observed for Hf-MOF, coordination of the ligands to Hf on the SBUs leads the decrease in fluorescence intensity resulted from the enhanced intersystem crossing.
2.10 Metal Toxicity

When using MOFs for PDT, the chosen MOF should be biocompatible. Knowledge of toxicity and pharmacokinetics of the MOF used in PDT is essential. When selecting metals for SBU for enhancement of ISC the toxicity of metals should be of concern. Table 1 is a concise chart of select metal toxicities.\textsuperscript{194}

Table 2. Oral LD50 (Rats) and Daily Requirements (Human) of Selected Metals.

<table>
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<th>Metal</th>
<th>LD50 (g/kg) in Rat</th>
<th>Daily Dose (mg) in Human</th>
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<td>Zr</td>
<td>4.1</td>
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<tr>
<td>Ti</td>
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<td></td>
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<tr>
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<tr>
<td>Mg</td>
<td>8.1</td>
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As for hafnium (Hf), Liu’s group investigated the rate of clearance of their nMOF, Hf-TCPP-NMOF-PEG by detecting the amount of Hf in feces and urine of mice. Hf was completely excreted from their systems in seven days. This suggested that Hf-TCPP-NMOF-PEG was decomposed \textit{in vivo} and excreted via organs.\textsuperscript{9} Study of Zr-nMOF loaded with Doxorubicin included an examination of adsorption distribution metabolism and excretion of the MOF. The
content of Zr increased in the liver, spleen, kidney, and intestine but reduced in cancer and other tissues at 20 h, indicating nMOFs were metabolized by the liver and kidney. The Zr content recovered to normal levels at 36 h, indicating that nMOFs were cleared rapidly. The biocompatibility with normal tissues and cytotoxicity to tumor tissue indicate that nMOFs represent an effective probing and therapy system for potential translation to clinical applications.35
2.11 Gold Nanoparticles

Gold nanoparticles (GNP) are diverse from gold particles. The bigger estimate may be a yellow inactive strong, but GNP could be a wine ruddy compound with antioxidant properties. GNP appears different sizes within the extend of 1 nm to 8 μm, whereas at the same time sub-octahedral, round, octahedral, icosahedron multi-twin, deca-hedral, multi-twin, tetrahedral, unpredictable shape, nanotriangles, hexagons. Appears diverse shapes such as platelets, nanorods, nano crystals, etc. Gold nanoparticles have been the subject of considerable research around the world due to their potential applications in various zones counting gadgets, nanotechnology and biomedicine. They are described in detail as having weak antibacterial activity compared to other metal nanoparticles that have been talked about. In any case, the measured gold NP is very low (about 2 nm) and has a significant antibacterial effect. The antibacterial movement of gold nanoparticles is thought to be due to the era of reactive oxygen species (ROS), which escalate the oxidative stretch of microbial cells. Photo thermal therapy (PTT) can be connected to enhance the antibacterial activity of AuNP. When gold nanoparticles are irradiated with a laser, they become warm due to the excitation of electrons. It allows them to be used as anti-cancer or antibacterial agents. For example, Au NP has been reported to improve its bactericidal effect on S. aureus when exposed to laser vitality.

2.12 DNA-based nano system

Synthetic nanoparticles (NPs) have isotropic and average properties. On the other hand, NPs in living systems are unique; for example, spherical peptides have both lipophilic and lipophobic regions. Because of this anisotropic property, spherical peptides have functionality such as recognition, transmission, and activation via interaction, binding, and catalyzing. By drawing inspiration from the complicated anisotropic structures and functions of nanoparticles in living
systems, the field of DNA computation and DNA-guided self-assembly originated.\textsuperscript{197} DNA-based computing is greatly aided in performing computations by the natural characteristics of DNA molecules; for example, the template-matching hybridization reaction between DNA oligonucleotides can perform Turing-universal computation.\textsuperscript{198} The DNA base pair (Adenine (A), Guanine (G), Cytosine (C), and Thymine (T)) are used when performing DNA-based computations. The base-pair sequences play the role of source code which is then compiled into a set of interparticle interactions (machine code), resulting in programmable construction of complex structures and functions.\textsuperscript{199} Utilizing biological materials for engineered systems opens the possibility of computing and constructing anisotropic structures closer in complexity to what is found in nature. Further inspired by nature is the self-assembly of information-coded structures like DNA. In biology, self-assembly is a ubiquitous process that plays a variety of crucial roles and underpins the production of a wide range of complex biological structures. For instance, peptides play a role as the building blocks to self-assemble complex structures. Also, for the controlled synthesis of NPs ensembles, self-assembly has emerged as a powerful and feasible method. For the past two decades, materials scientists have sought to use nature's assembly principles to build artificial materials with hierarchical structures and customized properties that can be used to fabricate functioning devices.\textsuperscript{200} Significantly, DNA-applied NPs hold a high promise to self-organize into a computation-capable design owing to the specificity of DNA’s unique molecular recognition properties. That specificity has been demonstrated in experiments and simulations of DNA bricks and showed that even thousands of distinct DNA bricks could reliably self-assemble into specified shapes with a high yield.\textsuperscript{201,202} Programmable self-assembled NPs with DNA have been reported, including DNA tiles and DNA origami \textsuperscript{203-215,216-226}, or DNA-grafted NPs as building blocks.\textsuperscript{227-250} Despite the promise and recent progress in DNA-directed NP
self-assembly, the controlled assembly of designed nanostructures with specific shapes and functions remains challenging to attain because of inherent randomness in the experimental environment and the complexity of design and analysis.

Nevertheless, the goal is clear: to develop a DNA-based nanosystem that can be programmed to achieve complicated shape and function. There should be sufficient DNA sequences in this system to build large structures. One challenge in generating these assemblies is that the DNA oligonucleotides must not interfere with each other, as this would lead to errors and defects. The DNA oligonucleotides should be incorporated into the basic building blocks so that programmability is enabled. This means that the locations and orientations of the individual DNA sequences in the building block should be controlled and repeatable for anisotropic structures. Once this is obtained, then applications must be explored. New models and design methodologies that can overcome the inherent randomness and potential for disorder in complex systems should be developed along the way. This chapter focuses on the fundamental challenges for programmable and scalable nanostructure self-assembly with DNA and NPs and the new reported anisotropic structures. We do not overview other anisotropic nano-building blocks such as patchy or Janus particles. After a brief overview of fundamental challenges in the status of nanostructure self-assembly, we describe the two kinds of strategies that can attain predicted DNA-NP self-assembly structures. Here, as the smallest DNA-templated units, DNA-grafted NP and origami-DNA-NP are remarked.

2.13 Fundamental Challenges in Nanostructure Self-Assembly: Anisotropic

Two publications in *Nature* from 1996 initiated the study of DNA-based nanosystems.\(^\text{227}\).\(^\text{228}\) The utilization of DNA for linking to NPs generates crystal structures.\(^\text{227-250}\) For the introduction of advanced nanobiotechnology, precise control over the structure of metal nanomaterials is
essential. Various approaches to the assembly of nanoparticles into structured blocks have been widely explored. Nanocrystals with regulated three-dimensional structures, on the other hand, have been challenging to make. The purpose of DNA in nature is to store information in a uniform template, and the structural complexity of synthetic DNA nanostructures now far exceeds what nature has built with DNA.

These synthetic DNA sequences, unlike genetic DNA, do not usually code for proteins. Instead, they transport data, which includes the intensities and specificity of the interactions that govern self-assembly. The first challenge to design a DNA-based nanosystem is synthesizing a unit of DNA-NP (NP building blocks) or DNA-origami template. This smallest unit of NP building blocks should self-assembly and be compatible with each other to increase complexity. Controlling the number and position of binding sites along with the relative orientation of DNA on the surface of NP building blocks would provide for more control over the shape and function of final self-assembled structures. Given the significant efforts in self-assembly research and recent advances in NP synthesis, methods in this area remain restricted. This would also improve the reproducibility and scalability of self-assembly processes, paving the way for the creation of “enabling” multifunctional nanostructures at all sizes, from nano to micro to meso to macro, and in all dimensions, from 1D to 3D. The other method to achieve a desired structure is the DNA-origami template. Seeman introduced the logical design of an immobile Holliday junction in the 1980s,251 which transformed DNA into a nanoscale polymer stretching in two dimensions rather than simple 1D double helices. This study marked the beginning of DNA nanotechnology, enabling the massively parallel synthesis of well-defined nanostructures, with picomole-scale synthesis yielding $10^{12}$ copies of a product. Since then, numerous DNA nanostructures have been created using the junction of multiple short single-stranded DNAs, including double-crossover,
triple-crossover,\textsuperscript{252} $4 \times 4$\textsuperscript{253}, and three-point star structures (ssDNA).\textsuperscript{254} These DNA tiles can then be constructed into periodic superstructures such as nanotubes, 2D lattices,\textsuperscript{255} and 3D polyhedra, hydrogels, and crystals.\textsuperscript{226, 256, 257} In this method, NPs could be arranged in prescribed locations by the specific DNA sequences.

The present difficulty in DNA-NP self-assembly is linking structure to function. The potential for DNA-directed integration of diverse NP into nanostructures of variable sizes and shapes to achieve desired functions based on the features of the NPs, such as the plasmonic capabilities of linked metallic NPs, is exciting. Engineering numerous discrete nanoscale particles (NPs) into single multifunctional nanoscale structures with specified physical, chemical, or biological properties has the potential to revolutionize a wide range of research disciplines, from optoelectronics and nanophononics to nanomedicine.\textsuperscript{258} Multiple activities and functionalities might be performed in succession or simultaneously because of the content of potential NP compositions in the nanocomposite structure. This would allow us to make use of the complementary qualities of various imaging and sensing modalities, allowing us to obtain more complete, accurate, and trustworthy data by combining them. Electronic devices,\textsuperscript{259} photonic circuits,\textsuperscript{258, 260-264, 265} and medicinal theranostics, for example, all benefit from these characteristics.\textsuperscript{258, 260-264, 265} As a result, there has been consistent interest in developing multifunctional nanocomposites with specified shapes and sizes that include a variety of nanocomponents for specific purposes.\textsuperscript{258, 260-264} Nevertheless, it is difficult to achieve optimal structural designs, let alone those that incorporate function. Furthermore, although DNA-NP assembly has been developing, computing systems to predict the change of optimal physicochemical properties also need to improve. For example, when DNA-NP structures become more complex, understanding the local morphology of the quantum plasmon effect is far from perfect and thus requires further
studies.\textsuperscript{266-270} Linking a structure to a predicted function is still challenging and based on trial and error.

2.14 Anisotropic DNA-NPs (One-after-Another Conjugation of DNA)

As a consequence of the significant development in the syntheses of inorganic NPs with controlled morphologies and adjustable sizes over the last two decades, a variety of inorganic NP building blocks of various forms and compositions\textsuperscript{225,271,272,273-277} are now accessible. Despite these advancements in NP syntheses, the precise, scalable, and high-rate assembly of heterogeneous nanocomponents into multifunctional nanoarchitecture with specified shapes and sizes remains a problem in nanotechnology. The site-specific functionalization of multiple DNA to the NP's surface in a well-defined and stoichiometric manner without affecting the intended function of the DNA linkers is the critical technology for programming the spatial arrangement of NP building blocks into the final self-assembled nanostructures. On an NP, DNA linkers should be positioned at particular angles to one another, resulting in well-defined spatial configurations of DNA on the NP building block. Different DNA linkers should be put at other places on the NP, enabling fine control of the spatial positioning of individual nano-building blocks and enhancing the complexity of the structure to be produced. The DNA-conjugation chemistry should also be tenable and straightforward and generalizable to a variety of NPs. The ability to manipulate matter at the molecular level in such a controlled manner holds enormous promise for synthesizing NP building blocks with the necessary properties for programmable matter.\textsuperscript{278,279}

Different functionalization techniques have been attempted to regulate the number of DNA on an NP since Alivisatos and co-workers first demonstrated mono functionalization of DNA to gold NPs based on maleimide conjugation chemistry.\textsuperscript{227-250} A defined number of short DNA oligonucleotides with a thiol group at either the 3' or 5' end were conjugated to gold-NPs, which
were then capped with N-propylmaleimide, either by restricting the surface area of an NP using very small NPs or by using significant separation and purification steps, such as gel electrophoresis and extraction.\textsuperscript{227-250} As a result, the DNA-linked NPs self-assembled into simple homo-, hetero-, and trimeric NPs, as well as three-dimensional structures including four different-sized NP complexes. Other studies \textsuperscript{227-250} demonstrated the self-assembly of heterostructures such as cat-paw, satellite, and dendrimer-like structures by attaching DNA linkers to NPs in an anisotropic manner based on the geometric constraint. These methods, while impressive, were unable to regulate the amount and shape of DNA on an NP at the same time, which is required to create programmable matter. An anisotropic functionalization method for attaching two distinct DNAs to an NP in a particular configuration directed by a DNA geometric template has been described.\textsuperscript{227-250} Another way exhibited different self-assembled complexes, including cross-shaped complexes, using a one-pot technique to regulate DNA number and position to synthesize NPs with one to five DNA binding sites.\textsuperscript{227-250}
Figure 5. Representative examples of rational self-assembly of nanostructures by DNA-linked NP building blocks in 1D (panels a-e), 2D (panels f-o), and 3D (panels p-w). Note that some of original images were amended by cropping, re-coloring, and rotating them. See the original articles for scale bars and experimental details. Figures were adapted with permission from: Copyright 2009 Royal Society of Chemistry. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
Figure 6. Anisotropic functionalization of DNA on an NP

However, it was recently discovered that two robust strategies govern the capacity to anisotropically organize DNA linkers on an NP at precise angles and synthesize NP building
blocks with well-defined DNA configurations in all three dimensions (Figure 4a).© 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

The first strategy is to create the final programmable three-dimensional structure by synthesizing the smallest unit that is anisotropically mono-functionalized. The number of DNA linkers on an NP may be adjusted by attaching DNA one by one. With careful study of the interplay of physicochemical interactions among the components, capping ligands, DNA, and NP, the angles between DNA and NP may be regulated in the functionalization procedure. These include steric hindrance and electrostatic interactions between DNA and an NP surface, which are typically capped with charged chemical ligands to be mono-disperse in the solution phase. A careful design of the twist of the DNA helix might also regulate the angles between NPs in self-assembled structures. Different DNA linkers might be placed at various places on an NP to achieve anisotropy. The length of the DNA linkers may also influence the distance between NPs. Modulating DNA-NP interactions in such a precise manner should enable us to harness NP building blocks with maximum programmability, allowing tailored nanostructures with desired characteristics of all sizes, ranging from basic 1D dimers to 3D crystals.

On the other hand, anisotropic functionalization is not simple. Not only must many physicochemical and structural parameters be taken into account, but a careful, rational design is required to develop DNA-linked nano-building blocks for the self-assembly of multifunctional nanostructures with arbitrary shapes and functions. This technique is referred to as "one-after-another DNA conjugation" because it changes the number of DNA molecules on NP one by one. The studies by Kim et al. showed a system of colloidal gold NP building blocks conjugated with DNA oligonucleotide linkers with symmetry up to sixfold. Control was achieved through an aqueous-phase ligand replacement strategy that combined optimized water-soluble gold NP
precursors with a mixed layer of dimethyl aminopyridine and mercaptoethanol sulfonic acid capping ligands using a previously described silica-gel-based sequential solid-phase functionalization approach. DNA oligonucleotides were linked to the NP strand-by-strand, or one at a time, during the building block synthesis. By repeating the procedure, it was possible to control DNA strands' quantity and spatial orientation on an NP. for detailed physicochemical analyses of the DNA-linked NP assembly).

To summarize, ethidium bromide-stained agarose gel imaging showed DNA association with NPs and increased their molecular weight. The degree of functionalization increased, suggesting that the number of DNA strands on an NP may be successfully controlled. The optical spectrum analysis findings were consistent with those obtained by gel electrophoresis. To determine the molecular geometry of the functionalized DNA on NPs, each DNA-linked NP was hybridized with an NP containing one complementary DNA strand. Transmission electron microscopy (TEM) image analysis revealed that the resulting self-assembled composites have a defined number of DNA at defined angles from one another up to sixfold symmetry, implying that the sequential solid phase monofunctionalization strategy exerts excellent control over the orientation and spatial arrangement of DNA. Due to the sequential nature of the ligand replacement approach, DNA linkers segregated maximum via mutual exclusion and repulsion, resulting in an NP with linkers at 90° or 180° angles.

After this report, a similar way of this "one-after-another DNA conjugation" was also demonstrated by Ma et al. in Nature publication. In this report, the double-stranded DNA modified with the desthiobiotin group was conjugated with the monoavidin group on the magnetic nanoparticle (MNP) surface. In contrast, the other end with thiol group bound onto AuNS. Attained AuNS-1DNA was then used for the next monofunctionalization. Each step attaches one DNA.
AuNS-nDNA becomes AuNP-(n+1) DNA as same as the former "one-after-another DNA conjugation". This process was repeated until they gained AuNS-5DNA. Applying MNP for this system instead of silica-gel helps the separation of unattached AuNS easier from the MNP-DNA-AuNS in solution. The constant crystallization on both the branch and the branch was created after the branch was established (Figure 4b). The continuous crystallization on both the branch and the nanoseed produced the branching AuNC shape after the branch was grown. Simply by changing HAuCl4 concentrations, the dimensions of the branch in the AuNC guided by the dsDNA were tunable in the ranges of 1.3 ±0.7–25.1 ±3.0 nm in length and 2.4 ±0.3–10.9 ±1.1 nm in cross-sectional diameter. This study's control level is similar to the most recent advancement in the bottom-up synthesis of colloidal nanostructures. This research revealed that DNA-directed nanocrystal synthesis could control the metallic crystallization process in the desired direction.
Figure 7. “One step at a time” DNA conjugation
a) Molecular geometry of nBLOCK visualized by TEM and its schematic representation. Each nBLOCK was hybridized with a monofunctionalized nBLOCK with a single complementary DNA oligonucleotide. Left panels: TEM images of the self-assembled structures. Bottom right panels: magnified TEM images. Top right panels: schematic illustration of the assembled structures, showing nBLOCK up to sixfold symmetry (all x, y, and z directions). In the schematic depictions, the solid lines represent double-stranded DNA and the gray dotted circles in the 2D plane. Scale bars represent 20 nm. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim b) Scheme showing DNA-directed crystallization of a AuNC (i–v) Illustrations and TEM images of star-shaped AuNCs with controlled branches from 1 to 5, respectively. Scale bars, 10 nm. Scale bars, 100 nm. The pictures were adapted. © 2016, The Author(s) Ma et.al.

2.15 Geometrically Diverse Plasmonic Building Blocks

The second strategy for governing the capacity to anisotropically organize DNA linkers on an NP at precise angles and synthesize NP building blocks is coordinating isotropic spherical nanoparticles through shape-induced directed contacts aided by DNA recognition. In this case, the
NPs are assembled into well-defined predefined patterns using a different method. According to the reports,\textsuperscript{248, 250} along with increasing the range of NP superstructures available, such assemblies can introduce a new degree of parallelism between ordinary chemical molecules and NP superstructures. Furthermore, they enable the selective attachment of spherical NPs to nonspherical components in a regioselective manner. Similar to chiral assemblies, the regioisomers are anticipated to vary in terms of the geometric arrangement of the various segments and their optical, chemical, and perhaps other characteristics. Xu et al. reported regiospecific assemblies made from NPs and gold nanorods (NRs). As a result, NPs are arranged in a more sophisticated manner than before, which is conceptually comparable to the strong regioselectivity seen in organic chemistry. As far as such assemblies are concerned, three basic configurations should be addressed. When it comes to NRs, NPs may be linked to the ends alone or attached to the sides only, or they can be connected to both the backs and sides. As used in this text, superstructures categorized as End, Side, and Satellite assemblies will be referred to as such from here on out (Scheme 5a). The fact that minor differences between geometrical locations and the number of NPs linked may be considered is noteworthy. Among them are the situations in which a single NP is connected to one end of the NR and a variety of asymmetric placements of NPs along the sides of the NR in various configurations. These different systems were synthesized in three different ways with modification with ssDNA. Since the CTAB coating is thinner towards the ends,\textsuperscript{282, 283} the oligonucleotides only covalently bind at the “end” locations when thiolated DNA is present in low quantities. Increasing ssDNA concentration causes NR surface alteration on both ends and sides, resulting in the Satellite route in these assemblies. Furthermore, with low and high concentrations of ssDNA, various oligonucleotides may be used for the modification processes. As a result, they utilized ASY1 (red) and a “helper” DNA (black). The complementary ASY2
strand is used to bind NPs to ASY1. Helper DNA is employed to regulate the assembly process' regioselectivity, since otherwise, thiolated ssDNA linked to the ends could spontaneously diffuse all over the NR surface, causing the NP placement selectivity to be thrown off. A layer of covalently bonded helper DNA provides a considerably more significant barrier to DNA transport than an ionically adsorbed CTAB layer. As a result, when NRs are first exposed to a low concentration of helper DNA, the ends get occluded. Following the insertion of ASY1, the connection along the sides occurs. The NPs will be connected to the sidewalls as well. In this way, they demonstrated 2D regiospecific self-assembly.

According to the findings of Lu et al., when the proper regimes of nanoparticle sizes and DNA shells are used, a well-defined three-dimensional (3D) lattice of blocks and spheres could be created using cubic and octahedral nanoblocks (Figure 5b-e). Furthermore, the ordering of spherical nanoparticles could be controlled by directed linkages supplied by DNA-encoded faceted nanoscale ‘blocks,’ which can guide the movement of the particles. Bonding directionality is naturally ruled by the block's anisotropic form and appealing facets, as shown. Consequently, clusters of spheres may be formed in line with the geometry of the shaped item. At some point, large-scale binary lattices may be produced predictably. We demonstrate this idea in this research by assembling spherical nanoparticles using directed bindings supplied by polyhedral nanoblocks, either cubes (CBs) or octahedrons (OCs). The reciprocal attraction of particles with disparate shapes is needed to prevent phase separation. In this respect, both spherical and polyhedral particles were functionalized with DNA strands, thus ‘encoding' particle interactions through DNA sequence. Favorable interactions between different-shape particles through DNA complementary interactions was imposed, while same-shape particles are repulsive due to entropic chain effects.
This suggested technique does not need sophisticated manufacturing of spherical particles and may use a broad range of existing nanoscale-shaped objects.²⁸⁶
Figure 8. Geometrically Diverse Plasmonic Building Blocks
a) Scheme of schematics of synthetic method for regiospecific NPs assemblies and representative TEM images of End, Side, and Satellite assemblies. Reprinted (adapted) with permission from Liguang Xu, Hua Kuang, Chuanlai Xu, Wei Ma, Libing Wang, and Nicholas A. Kotov *Journal of the American Chemical Society* **2012** 134 (3), 1699-1709 DOI: 10.1021/ja2088713 Copyright 2016 American Chemical Society.

(b) SNPs (yellow unit) are isotropic objects with high symmetry. ANPs (grey unit) have low-order symmetry; for example, CB or OC as shown in electron micrographs (inner frame length of images is 100 nm). Regulated interactions between particles are induced by functionalizing SNPs and ANPs with complementary DNA strands. The directional bonding of ANPs coordinates SNPs in a local structure (clusters). (c top) Schematic and (bottom) SEM images illustrate the shape-induced directional bonding of cubic blocks in a cluster-dimension-extending history (from left to right). The scale bars, 50 nm.

d) High-magnification SEM images of binary superlattices formed by 38-nm SNPs/46-nm CBs with 30S DNA. Scale bar, 200 nm.

e) Schematic and (bottom) SEM images illustrating the directing role of octahedral blocks in the formation of clusters, and their merging into larger-scale structures (from left to right). Scale bars, 50 nm.

f) SEM images of SNP/OC-assembled superlattice fragments. Scale bar, 100 nm. The pictures were adapted. © 2015, Nature Publishing Group.
2.16 Current Status and Future Challenge

DNA-grafted NP building blocks showed promise in having the prerequisite aforementioned properties for programmable matter, allowing for the self-assembly of arbitrary, anisotropic shapes, including in 3 dimensions. NP spacing can be tuned by controlling the lengths of DNA strands and angles of twisting in the DNA helix. The overall geometry of DNA can be controlled by controlling the extent of electrostatic repulsion between DNA through adjusting net charges of the DNA strands and anisotropy achieved by attaching different DNA linkers at different locations on NPs. Some remaining challenges include repeated binding of DNA and chirality problems when binding six alien DNA strands. We can bind different DNA strands on an NP for the +x, -x, +y, -y, and +z, axis but not -z according to the developed method, this is because upon attaching the sixth DNA at -z axis, chiral products are generated, and the separation of these chiral products is difficult.

Shape-induced directed contacts aided by DNA recognition result in the formation of a 3D crystal lattice. However, this shape-induced three-dimensional crystal still has a flaw in that the lattice’s size and shape cannot be regulated. This issue can be resolved by combining several NP building blocks with alien DNA along each axis. As an example, we will demonstrate how to construct the 2x2x2 box form. Furthermore, with the advantage of “one step at a time” conjugation of DNA,” the length of DNA and kinds of metals NP can be easily replaced. This technique allows for the creation of three-dimensional mesoscale building blocks from which various nanoparticles structure with different functions may be formed. Not only is the advantage of nBLOCK that DNA and NPs could be flexibly modified in terms of size, but it should also be highlighted that the interaction between these NPs and DNA is covalent, resulting a strong structure.
2.17 Application

Even though the subject of DNA nanotechnology evolved from the idea of crystallizing proteins into ordered 3D structures, great effort has been made to incorporate many functional nanomaterials into a range of real-world applications. Controlled integration of various functions into a single structure through multiple NPs can revolutionize many areas of study, including biology, chemistry, physics, medicine, and materials science and engineering. This may open up new opportunities for nanostructure self-assembly in biosensing, photovoltaics, and other areas, resulting in novel materials with better characteristics for medical theranostics, medication delivery, and energy conversion, to mention a few. Among these, diagnostic imaging and therapeutic contrast agents designed to include several NPs to combat illness are among the most alluring applications for self-assembly of the multifunctional nanocomposite. The ability to engineer nanocomposites' sizes and structural configurations enable the production of “customizable” plasmonic nanomaterials. The optical characteristics of nanomaterials, namely their extinction and scattering spectra, are highly affected by geometric variables like form and size, material compositions, and the surrounding dielectric environment. Thus, improved optical sensitivity across several wavelength bands may be achieved by designing and organizing metallic nanoparticles of different sizes and shapes into desirable patterns and geometries. The extinction of light in nanostructures with numerous NPs is influenced by their geometries (size and shape) and electromagnetic interaction (modulated by the inter-particle spacing and the surrounding dielectric environment). The DNA-programmable NP building blocks would provide a strong tool for fabricating metallic NP composites with adjustable plasmon resonances for particular purposes. Modulation of the spatial locations and distances between individual NPs in constructed structures is enabled by the DNA-linked NP
building blocks, resulting in self-organized NPs in specified configurations with unique plasmonic signatures. By manipulating the plasmonic interactions between neighboring NPs, plasmonic engineering of NP composites may also result in significant improvements in insensitivity. By perfectly matching the plasmonic spectra of nanocomposites and resonant laser wavelengths, this would enable extremely selective and sensitive diagnosis and treatment. Additionally, multispectral, multi-color imaging is feasible by combining several nanocomposites with precisely controlled plasmonic spectra. The optical tunability and responsiveness in the near-infrared (NIR) region (i.e., 700–1400 nm) promise minimally invasive imaging and treatment of illnesses like malignancies and bacterial infections. In fact, DNA-directed assemblies have been shown to have potential applications in bioimaging, including RNA imaging, bioimaging with live metal ions, and phototherapeutics. DNA molecules govern the arrangement of NPs and switch assembly topologies. The DNA-engineered NP assemblies could have desirable NP configurations and better EM fields for hot electron excitation, improving photothermal conversion efficiency. Different metal NP assemblies performed superior photothermally. For example, the heat transfer efficiency of Au NRs was 20.7 percent, whereas that of Au NR dimers was up to 42.3 percent. Also, Au-Cu NPs incorporating pyramids by using DNA as templates showed better photothermal conversion ability. Not only did DNA-directed assemblies exhibit unique optical properties for analyte interaction, but they could also evade biological sequestration for accumulation in cells or tissues. The ability to manipulate geometric configurations and surface properties offers creative methods to circumvent the obstacle and significantly improve blood circulation times. Shielding particles with biocompatible and opsonin-resistant moieties (by site-specifically linking different ligands on their surface, both biological and chemical, such as polymers such as dextran sulfate and polyethylene glycol) may ensure their prolonged
circulation times and high therapeutic efficacy. Additionally, control over the size and form of the NP complexes may aid in their passage through the bloodstream to reach the target organ. It is worth mentioning that many biological particles, including blood cells and numerous dangerous ones such as bacteria, have evolved to avoid macrophages by becoming transparent to blood opsonins. One may design and build nanocomposites with unique forms and surface properties that allow for prolonged circulation by studying nature. DNA-directed NP assemblies have enabled the design of very particular assembled structures and, ultimately, producing an assembly with acceptable performance. However, difficulties persist in the actual implementation of DNA-driven NP assemblies, necessitating further study. DNA-directed NP assemblies are pH and ionic strength sensitive, making them unsuitable for use in severe environmental conditions. Additionally, DNA molecules were readily changed on the surface of metal NPs due to the presence of stable metal-SH interactions. In contrast, effective modified methods for DNA molecule attachment on the surface of non-metallic NPs should be investigated. Some of the difficulties will be overcome via future research and refinement, and NP assemblies with good biocompatibility, high stability, and increased sensitivity will be utilized in macroscopical materials and photonic devices.
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Chapter III - Maltotriose Conjugated Metal–Organic Frameworks for Selective Targeting and Photodynamic Therapy of Triple Negative Breast Cancer Cells and Tumor Associated Macrophages

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Contribution for this paper:

- Yoshie Sakamaki, first author, designed, decided, and performed all experiments and write,
- John Ozdemir, second author, contributed to writing,
- Alda Diaz Perez, third author, contributed to ELIZA experiment,
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- Miu Tsuji contributed to in vitro experiment,
- Christopher Salmon contributed to in vitro experiment,
- Joseph Batta-Mpouma contributed to get zeta-potential data,
- Anthony Azzun contributed to some purification for few days,
- Valerie Lomonte contributed to in vitro for few days,
- Yuchun Du provided me some cell lines,
- Julie Stenken, provided proof reading,
- Jin-Woo Kim provided some instruments and proof reading,
- M. Hudson Beyzavi submitted this paper.

I provided these data to two of above undergraduate students’ thesis.
3.1 Introduction

Metal–organic frameworks (MOF) are a well-suited platform for drug delivery systems that can affect photodynamic therapy (PDT). A well-designed PDT delivery system to treat cancer can overcome some problems of current photodynamic therapy such as prolonged photosensitivity and tumor specificity. Triple negative breast cancer (TNBC) is difficult to treat with existing chemotherapy and often requires surgery because it quickly metastasizes throughout the body. Tumor associated macrophages (TAMs) are known to promote angiogenesis, matrix remodeling, and metastases. Considerable evidence suggests that most TAMs are more similar to M2-polarized macrophages and although some TAMs are known to be M1 polarized there is evidence that most are M2-like the selective targeting of M2 polarized macrophages would be a necessary
One possible route to target the M2 over the M1 macrophages is to take advantage of the overexpression of CD206 (mannose receptor) on the surface of the M2 macrophages. MOF nanoparticles of dimensions around 50 nm were synthesized by a solvothermal reaction of Mn(III)-tetrakis(4-carboxyphenyl) porphyrin, tetrakis(4-carboxyphenyl) porphyrin and ZrOCl₂. Through post-synthetic modification, Zn(II) was incorporated to the tetrakis(4-carboxyphenyl) porphyrin sites and potassium maltotrionate was conjugated to the empty coordination sites on the Zr(OH)₄O₄. The resultant maltotriose-PCN-224-0.1Mn/0.9Zn was able to specifically target tumor cells and M2-polarized macrophages. Upon irradiation by an LED light MA-PCN-224-0.1Mn/0.9Zn treated triple negative breast cancer (TNBC) and the M2-polarized macrophages selectively by actively targeting via the GLUT and CD206 receptors. The MA-PCN-224-0.1Mn/0.9Zn showed no toxicity towards normal cell lines and no dark toxicity.

Since triple negative breast cancer (TNBC) cells lack the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2); it is difficult to treat with receptor targeting drugs. TNBC comprises 15–20% of all breast cancers, and its clinical course is frequently characterized by early relapse and poor overall survival. Cytotoxic chemotherapy remains the only approved treatment. More than 80% of women with triple negative breast cancer are treated with chemotherapy regimens that include anthracyclines such as doxorubicin (DOX). However, doxorubicin is ineffective in the treatment of TNBC, and can cause cardiotoxicity as a serious side effect. Furthermore, chemotherapy treatment of breast cancer cell lines using either 5-FU, cisplatin, paclitaxel, doxorubicin, or etoposide showed multi-drug resistance. Tumor subtypes and cell types are similar. MDA-MB231 cell lines are classified as triple negative breast cancer (TNBC). The MDA-MB-231 cancer cell line is a highly aggressive
TNBC which express high P-glycoprotein (P-gp) to reduce the effects of anti-cancer drugs.\textsuperscript{11} MDA-MB-231 is also known for its high invasive potential, which is related to its high expression of the GLUT receptor.\textsuperscript{6}

PDT is cancer treatment which relies on a photosensitizer (PS) that upon irradiation by light will generate toxic reactive oxygen species (ROS), such as singlet oxygen ($^1\text{O}_2$) that cause apoptosis in cancer cells.\textsuperscript{7} PDT is garnering attention due to its ability to treat tumors without surgery, radiotherapy or chemotherapy. The porphyrin macrocycle is a known PS that has seen clinical use in the European Union under the name Foscan.\textsuperscript{118} However, these PS do not target specific cancers, which results in less effective treatment and a prolonged photosensitivity. In 2014, porphyrin containing MOFs were reported to treat head and neck cancers for PDT.\textsuperscript{72} MOFs can deliver a PS via two strategies: either loading of the PS within the porous MOF framework or by incorporating the PS into the MOF as the organic linker between the metal rich secondary building units (SBUs). The latter strategy of rigid fixation of a PS into a MOF as the organic linker within the crystal structure prevents aggregation of the PS which increases ROS generation as aggregation results in quenching.\textsuperscript{1} MOF platforms for PDT also take advantage of the heavy atom effect wherein a heavy atom incorporated into the PS change the intersystem crossing during ROS production.\textsuperscript{1} The heavy atom in a MOF may be either the metal in porphyrin center or the metals in the SBU.

Through a phenomenon known as the Warburg effect, cancer cells are known to have an increased the demand of glucose.\textsuperscript{330} Maltotriose is a chain of three glucose molecules which can be used as a cancer targeting moiety in PDT.\textsuperscript{331, 332} The modification with maltotriose aimed to target cancer cells and tumor-associated macrophages (TAM) via GLUT and CD206 receptors, respectively. Tumor-associated macrophages (TAM) are known to be associated with M2-like
phenotype roles, such as angiogenesis, matrix remodeling, and metastases. M2 macrophages are known to be characterized by a specific marker such as macrophage scavenger receptor (CD204 and CD163) and also mannose receptor-1 (CD206)\cite{334,336} The ability to target TAMs is an ability that often used to determine the effectiveness of therapeutic agents in targeted cancer therapy.\cite{126,337,339} However, many of these trials have revealed that the therapies indiscriminately target tumor specific M2-like and M1-like phenotypes. The activated macrophages (M1 phenotype) are important because they serve important roles in the defense mechanisms against bacteria, virus, parasites and so on and so it could be beneficial to inhibit the unintentional destruction of M1-polarized macrophages.\cite{126,340,341} Thus, nonspecific targeting of all macrophages can make the body immunocompromised. MA-PCN-224-0.1Mn/0.9Zn can target the M2-polarized macrophages by the use of maltotriose conjugation to the MOF surface. By targeting M2 macrophages with maltotriose, we can more effectively treat different cancers. Using size-control methodologies the PCN-224-0.1Mn/0.9Zn particles had dimensions within the 50–200 nm regime. Nanoparticles within this size range accumulate in the poor vasculature of tumors by a phenomena known as the enhanced permeability and retention (EPR) effect.\cite{28} This maltotriose-conjugated MOF was designed to be a new novel drug delivery system (DDS) able to target cancer and the CD206 receptor while reducing ROS damage to normal cells and macrophages.

3.2 Result and Discussion

3.3.1 Synthesis

5,10,15,20-Tetrakis (4-carboxyphenyl)porphyrin (H₂TPP) was synthesized and hydrolyzed to 5,10,15,20-Tetrakis (4-carboxyphenyl)porphyrin (H₂TCPP) with known procedures.\cite{342,343} Metal insertion with Mn(III) of the H₂TCPP gave MnTCPP. The MOFs were
synthesized by mixing the ratio (1:9) of MTCPP (M = Mn) and TCPP with ZrOCl₂ in a size-control methodology⁵³ to produce nano-scale MOFs PCN-224-0.1Mn. The MOFs were then treated with ZnCl₂ for insertion of Zn into the H₂TCPP ligands for enhanced ¹O₂ generation by the heavy atomic effect. The heavy atom effect is known to enhance intersystem crossing (ISC) of PS and generate more singlet oxygen.¹ The resultant PCN-224-0.1Mn/0.9Zn was conjugated to maltotronic acid (MA) in potassium maltotriionate acid solution. MA was synthesized from maltotriose as reported with some modifications.⁴⁴ The PCN-224 structure consists of six connected Zr₆-oxo nodes that has another 6 coordination sites still available for conjugation to -COOH moieties.⁴³ Maltotronic acid in an aqueous solution chelates to the Zr₆ node of the PCN-224-0.1Mn/0.9Zn to give the MA-PCN-224-0.1Mn/0.9Zn. Conformation of the maltotronic acid chelation is performed by measurement of zeta-potential and TEM analysis. The initial concentrations of MnTCPP to H₂TCPP in the reaction mixture along with final Mn:Zn ratios along with particle size of the resultant MA-PCN-224-0.1Mn/0.9Zn MOFs are depicted in Figure 2.2.

Scheme 1. The different kinds of PCN-224-0.1Mn/0.9Zn with size and metal.

3.3.2 Characterization

The MOF samples were characterized using powder X-ray diffraction (pXRD), transmission electron microscopy (TEM), inductively couple plasm mass spectrometry (ICP-MS),
and UV-vis, and MA-PCN-224-0.1Mn/0.9Zn was characterized by TEM, zeta-potential, UV-vis, and fluorescence. Structural elucidation of the PCN-224-0.1Mn was performed by matching the pXRD pattern of the synthesized samples with the calculated pXRD patterns of the PCN-224-0.1Mn as shown in Figure 2.3d. The Mn:Zn metal ratios in the MA-PCN-224-0.1Mn/0.9Zn samples were determined to be 1:10.1 (Supporting Information section S4). Using inductively coupled plasma mass spectroscopy (ICP-MS) this result indicates an increased incorporation of H$_2$TCPP over Mn-H$_2$TCPP into the PCN-224-0.1Mn framework. Comparison of particle size and morphology of the MOF particles before and after post-synthetic modification with Zn and MA using TEM can be seen in Figure 2.3 a-b; this analysis reveals that the median particle size of the nMOFs increases from the 40–50 nm range before modification to 50–60 nm after with MA. This ~10nm increase is attributed to a thick layer of MA around the nMOF particles after conjugation with MA. Modification with MA was also analyzed by zeta potential (Supporting Information section S4). Before modification with MA, the surface charge of PCN-224-0.1Mn/0.9Zn was measured to be is -4.22 mV (stv is 0.1173), after modification with MA the surface of PCN-224-0.1Mn/0.9Zn is -32.4 mV (stv is 0.4706). The large negative shift in zeta-potential is also indicative of a large layer of MA in the MA-PCN-224-0.1Mn/0.9Zn; moreover, this increase in negative surface charge will increase electrostatic dispersion in water. Saccharide-conjugated porphyrin displays a widening in the UV-vis absorption spectra which was observed in the collected UV-vis absorption of MA-PCN-224-0.1Mn/0.9Zn. (Figure 2.3e) The change in surface electronics also leads to a widening of the UV-Vis absorption of MA-PCN-224-0.1Mn/0.9Zn.
3.3.3 In vitro

The selective antitumor effect of MA-PCN-224-0.1Mn/0.9Zn was tested in-vitro by comparing the cell toxicity of MA-PCN-224-0.1Mn/0.9Zn towards two cancer cell lines, MDA-MB231 (TNBC) and HeLa (epithelial from cervix) cells, as well as one normal cell line, MCF10a (epithelial from breast). Incorporation of MA onto the PCN-224-0.1Mn/0.9Zn particles target the
GLUT receptor of cancer cells. MA-PCN-224-0.1Mn/0.9Zn solutions were prepared in phosphate-buffered saline (PBS). MA-PCN-224-0.1Mn/0.9Zn was well dispersed in phosphate buffered saline (PBS) stably. (Supplementary information Figure S2.2)

For bio-imaging, the fluorescence spectra of MA-PCN-224-0.1Mn/0.9Zn in PBS was measured at 420 nm excitation with the resultant spectrum shown in Figure 2.4. MA-PCN-224-0.1Mn/0.9Zn has large excitation wavelength of 400 to 440 nm, and 560 nm (Supplementary information Figure S2.1).

Figure 11. The fluorescence emission of MA-PCN-224-0.1Mn/0.9Zn in PBS $\lambda_{ex}=420$ nm.

The PDT effect of MA-PCN-224-0.1Mn/0.9Zn and H2TPP against MDA-MB231, Hela, and MCF-10a cells were measured by WST-8 assay. The number of live cells is directly proportional to the amount of formazan produced. The WST-8 assay was carried out as follows: the three cell lines underwent 24 hours treatment with H2TPP and MA-PCN-224-0.1Mn/0.9Zn respectively then the cells were washed by PBS three times and irradiated by while LED light (100
mW/cm²) for 15 minutes. The comparison of PDT effect for MA-PCN-224-0.1Mn/0.9Zn between cancer cell lines and normal cell lines is shown in Figure 2.5a. MDA-MB231 and Hela cancer cell lines exposed to MA-PCN-224-0.1Mn/0.9Zn and irradiated with LED light were effectively killed 73% and 87% respectively but under the same conditions MA-PCN-224-0.1Mn/0.9Zn showed limited toxicity towards the MCF10a cell lines.

To demonstrate that toxicity is dependent on irradiation with LED light the dark toxicity of MA-PCN-224-0.1Mn/0.9Zn was also tested against all three cell lines used in the study and the results are shown in Figure 2.5b. The dark toxicity of MA-PCN-224-0.1Mn/0.9Zn against all three cell lines was minimal suggesting that the toxicity of MA-PCN-224-0.1Mn/0.9Zn is arising from the irradiation of the PS MOF with light and subsequent ROS generation. Also, the lack of dark toxicity indicates that cellular toxicity of the MA-PCN-224-0.1Mn/0.9Zn could be localized to effected areas by the selective irradiation of those areas with LED light. To determine the effectiveness of the targeting by MA conjugation the all three cell lines were also exposed to the H₂TCPP linker and irradiated, as suspected; without a targeting moiety all cell lines were indiscriminately killed (Figure 2.5c). The apoptosis effect by MA-PCN-224-0.1Mn/0.9/Zn on MDA-MB231 cells was also analyzed by caspase-3 assay as seen in Figure 2.5d. Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease which catalyzes the cleavage of many key cellular proteins.347 In addition to cell viability tests caspase-3 activity was detected in to determine the amount of apoptosis among MDA-MB231 cells exposed to MA-PCN-224-0.1Mn/0.9Zn. The caspase-3 assay data shown in Figure 2.5d correlates with the cell viability tests and shows that as the amount of MA-PCN-224-0.1Mn/0.9Zn is increased more apoptosis is observed.
Figure 12. Bio-activity data of maltotriose-conjugated PCN224
a) PDT effect (LED irradiation for 15 mins 100mW/cm²) of MA-PCN-224-0.1Mn/0.9Zn on MDA-MB231, Hela, and MCF10a b) dark toxicity of MA-PCN-224-0.1Mn/0.9Zn on MDA-MB231, Hela, and MCF10a. c) PDT effect (LED irradiation for 15 mins 100mW/cm²) of H₂TCPP on MDA-MB231, Hela, and MCF10a. d) Caspase-3 activity of MDA-MB231 treated by MA-PCN-224-0.1Mn/0.9Zn (LED irradiation for 15 mins 100mW/cm²). One-way ANOVA was performed with a p<0.05 and Tukey as a post-test in N=3. Significant differences were determined by comparing against control sample. The data represent mean ± SD, n=3.

Further study of MA-PCN-224-0.1Mn/0.9Zn selectivity was performed by detecting cellular uptake of the PS MOF into cancer cells with bio-imaging. By detecting the emission of MA-PCN-224-Mn, the cellular uptake of MA-PCN-224-0.1Mn/0.9Zn on HeLa, MDA-MB231, and MCF10a were observed. MA-PCN-224-0.1Mn/0.9Zn was not detected in MCF10a but was observed in the Hela and MDA-MB231 as seen in Figure 2.6. These images indicate that a MOF
functionalized with MA can be a suitable agent for actively targeting cancer cells. The ability to forgo uptake into normal cells is essential for the ensuring that PS MOFs can not show a prolonged photosensitivity. It also increases the efficiency of the MOF PS such that less material can be administered in treatment when compared to a PS that will not actively target cancer cells.

Figure 13. Bio-imaging
Bio-imaging data of MDA-MB231, Hela, and MCF10a cells. The emission of MA-PCN-224-0.1Mn/0.9Zn was shown as a red color.

In addition to tumor cells the specific targeting of the M2-like macrophage was attempted. The choice to target M2 macrophages which overexpress the CD206 macrophage was done in part
to make the MOF particles more selective to TAMs. Most cancer chemotherapy is not selective and thus also affects M1 phenotype macrophage which are crucial components in the immune defense mechanism (Figure 6a). It has previously been reported that TAM expresses more of the mannose receptor (CD206) than the M1 macrophage does. From THP-1 cell line, M1-polarized and M2-polarized macrophages were transformed by following the reported way. THP-1 cells are immortalized monocyte-like cells derived from the peripheral blood of a 1-year old male child with acute monocytic leukemia. They have been used in the investigation function during a healthy and/or diseased stage. THP-1 cells are known to differentiate into macrophage-like cells using phorbol 12-myristate 13-acetate (PMA). Similar to macrophage cells, THP-1 cells stimulated with PMA have been shown to be used to induce NF-κB in response to lipopolysaccharide (LPS) with an increase of secretion of tumor necrosis factor alpha (TNF-α). For this experiment, CCL2 and IL-10 were measured to confirm the presence of M1/M2 phenotype. Human CCL2 (C-C motif chemokine ligand 2) is known to be involved during a pro-inflammatory response, phagocytosis and monocytic infiltration. Macrophage induced with LPS have shown an increase of the presence of M1 phenotype and also the secretion of CCL2. On the other hand, IL-10 is known to reduce pro-inflammatory response and express M2-like phenotype. For this experiment, IL-4/IL-13 are known modulator to release IL-10 which is known to be secreted by M2 phenotype. Moreover, IL-10, IL-4 and IL-13 is known to down regulated the CCL2 production in activated macrophages. As shown in Figure 2.7b, LPS/IFNγ shown a significant increase of CCL2 comparing with control. Also, it is possible to observe a significant decrease of CCL2 in macrophage-like cells induced with IL-4/IL-13. Moreover, IL-10 was ran in this experiment; however, the sample were below of the detection limit. Even though, the concentration of IL-10 were below of the detection
limit, it is possible to observe that CCL2 concentration were lower for IL-4/IL-13 than the control. Previous research have shown that the activation of M2 can be shown due to the decrease of pro-inflammatory cytokines such as CCL2, and IL-4/IL-13 have been used in the expression of M2-like phenotype.355, 363, 364 After these confirmation, by WST-8 assay, MA-PCN-224-0.1Mn/0.9Zn showed that it has an ability to kill only the M2 polarized macrophages without killing M1 macrophage (Figure 2.7c).
Figure 14. M1 and M2 macrophages-like bio-data
THP-1 cells were activated to macrophage-like by using phorbol 12-myristate 13-acetate. After activation, LPS/IFNγ was added to induce the M1 phenotype or IL-4/IL-13 was added to induce the M2 phenotype. a) The roles of M1 and M2 macrophages. b) Macrophage-like cells induced with LPS/IFNγ and IL-14/IL-13. The culture was incubated for 24 hours at 37 °C in 5% CO2. One-way ANOVA was performed with a p<0.05 and Tukey as a post-test in n=3. Significant differences were determined by comparing against control sample. c) PDT effect (LED irradiation for 15 mins, 100 mW cm⁻²) of MA-PCN-224-0.1Mn/0.9Zn on M1 and M2 phenotype. The data represent mean ± SD, n = 3.
3.4 Conclusion

We have reported that a nano-scaled MOF MA-PCN-224-Mn can selectively target cancer cell lines and tumor associated macrophages due to the action of maltotronic acid conjugated. MA-PCN-224-0.1Mn/0.9Zn is a well-designed drug delivery system that has been proven to actively two kinds of cancer cell lines while showing minimal toxicity toward the normal MCF10a cell lines. MA-PCN-224-0.1Mn/0.9Zn has also shown its ability to target M2 polarized macrophages without killing M1-polarized macrophages. This work lays an essential foundation for utilizing cancer targeting moieties on the surface of MOF for the targeted delivery of a PS to effected cells. In future studies it would be beneficial to perform targeting tests using specifically TAMs that have been polarized as M2 macrophages following the procedure recently developed by Benner et. al. The characterization of MA-PCN-224-0.1Mn/0.9Zn surface charge with zeta potential confirms that there is the dispersive effect of MOF particles can be enhanced by surface functionalization. MA-PCN-224-0.1Mn/0.9Zn should be tested in a living mouse model in future studies to determine if the MA conjugation to the surface of the MOF can sustain cancer targeting effect within the circulation of a living sample. In vivo studies are also necessary in the future so that the metal toxicity of the MOFs can be determined. The degree of ROS generation by monitoring \(^1\text{O}_2\) phosphorescence at 1270 nm also warrants further study so that the heavy atom effect on ROS production compared to the free H\(_2\)TPP is better understood. However, it is clear that the MA-PCN-224-0.1Mn/0.9Zn represents a major proof of concept that molecular targeting of receptors on the surface of cancer cells will mitigate the indiscriminate cellular death associated with PDT. To date most efforts in the synthesis of MOFs for PDT attempt to increase the effectiveness by utilizing different organic PS molecules within the MOF framework. This work is the first to actively target cancer cells and M2-polarized macrophages through a specific receptor...
(CD206). This is a much more efficient approach that must be used in future explorations of MOFs as PDT agents as it brings the PS directly to the cancer cells. Active targeting will also increase the potency of a PS so that less of the MOF needs to be administered reducing any prolonged photosensitivity. Since MA-PCN-224-0.1Mn/0.9Zn also contains the paramagnetic Mn(III) metal is will be an excellent MRI agent that has a cancer targeting effect. The MOF synthesis developed in this work also showcase an excellent procedure of controlling the size of porphyrinic PCN-224 MOF particles.
3.5 References


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3.6 Appendix

Supporting information for

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Maltotriose Conjugated Metal–Organic Frameworks for Selective Targeting and Photodynamic Therapy of Triple Negative Breast Cancer Cells and Tumor Associated Macrophages

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Keywords: photodynamic therapy (PDT), metal-organic framework (MOF), passive and active targeting, triple-negative breast cancer (TNBC), tumor-associated macrophage (TAM)
Materials:

Dulbecco’s modified eagle’s medium (DMEM) was purchased from Life Technologies Corporation. Fetal bovine serums (FBS) USDA APPROVED ORIGIN, sodium chloride and penicillin strep were purchased from VWR. Caspase-3 Assay Kit was purchased from Bioassay systems. Normocin was purchased from InvivoGen. TetraZ Cell Counting Kit and Recombinant Human IL-4 were purchased by BioLegend. Mammary epithelial cell growth medium and detach kit 30 were purchased from PromoCell. Recombinant Human IL-13 was purchased from PEPRO. Cholera toxin *vibrio cholerae* was purchased from Enzo Life Sciences. Disodium hydrogen phosphate dodecahydrate and potassium dihydrogen phosphate were purchased from ACROS Organics. Potassium chloride Propanoic acid were purchased from AVANTOR PERFORMANCE MATERIALS. Dimethyl sulfoxide was purchased from Corning. RPMI-1640 Medium was purchased by ATCC. Phorbol 12-myristate 13-acetate (PMA) was purchased by AdipoGen. INFγ was purchased by BON OPUS BIOSCIENCES MS. BD OptEIA™ was purchased from BD Biosicences. MnCl₂ and ZrOCl₂ • 8H₂O and ZnCl₂ were purchased from Alfa Aesar. Pyrrole was purchased from Alfa Aesar and purified by distillation when used. Methyl 4-formylbenzoate was purchased from TCI America.

Instruments:

Transmission electronmicroscopy (TEM) was carried out on JEOL 1011. SEM and EDX was carried out on FEI Nova Nanolab 200 Dual-Beam Focused Ion Beam. X-ray powder diffraction (PXRD) data was obtained at room temperature (298 K) on RIGAKU II miniflex. Absorbance was measured on BioTeK Synergy H1 Hybrid microplate reader in the WST-8 and caspase 3 assay. ICP was measured on ICAP Q ICP-MSI Thermo Fisher ICP- MS. UV data of
porphyrins were obtained on DU800 (Beckman Coulter). UV data of MOF were obtained on Thermo Scientific NanoDrop 2000c spectrophotometer. Zeta-potential was carried out on Nano-ZS Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). Fluorescence emission was carried out on Jasco FP8500 Spectrofluorometer.

Synthesis of maltotronic acid and Nano-sized MOF:

**Synthesis of 5,10,15,20-Tetrakis (4-carboxymethoxyphenyl)porphyrin (H$_2$tmp):**

5,10,15,20-Tetrakis (4-carboxymethoxyphenyl)porphyrin (H$_2$tmp) was synthesized according to the literature. A round-bottom flask was charged with propionic acid (50 mL), distilled pyrrole (0.65 mL, 9.3 mol, 1.0 equiv), and methyl 4-formylbenzoate (1.5 g, 9.1 mol, 1.0 equiv). The reaction solution was then refluxed overnight and allowed to cool to room temperature. The product, a purple solid, was then collected by vacuum filtration, washed with water, and dried with a vacuum. (20% yield).

**Synthesis of [5,10,15,20-Tetrakis (4-carboxymethoxyphenyl)porphyrinato]manganese (III) chloride (Mn-tmp):**

[5,10,15,20-Tetrakis (4-carboxymethoxyphenyl)porphyrinato]manganese (III) chloride (Mn-tmp) was synthesized by following the reported way. MnCl$_2$ anhydrous (50 mg, 0.397 mmol) and H$_2$tmp (42 mg, 0.0497 mmol) were dissolved in 3 ml of DMF in round bottom flask. The reaction was stirred and refluxed open to air at 150°C for overnight. The reaction was monitored by TLC. Upon completion, cold water was added to the vessel and the precipitate was collected by centrifugation. The collected solid was washed with water 3 times with centrifugation and dried in a vacuum oven. The yield was quantitative.
**Synthesis of 5,10,15,20-Tetrakis (4-carboxyphenyl) porphyrin (H$_2$TCPP):**

The synthesis of 5,10,15,20-Tetrakis (4-carboxyphenyl) porphyrin (H$_2$TCPP) was done according to the reported method with some modification.\(^2\) The obtained H$_2$tmp (80 mg) was stirred in a mixture of THF (5 mL) and MeOH (5 mL). A solution of KOH (100 mg) in H$_2$O (5 mL) was then introduced, and the resulting mixture was refluxed for 12 hours. After cooling down to room temperature, the THF and MeOH were evaporated. The solution was acidified with 1.0 M HCl until no further precipitate was detected. The precipitate was collected by centrifugation and washed with water and dried in vacuum oven. The yield was quantitative.

**Synthesis of [5,10,15,20-Tetrakis(4-carboxyphenyl)porphyrinato]-Mn(III) Chloride (MnTCPP):**

The synthesis of MnTCPP was done according to the reported method with some modification.\(^2\) The obtained Mn-tmp (50 mg) was stirred in a mixture of THF (5 mL) and MeOH (5 mL). A solution of KOH (100 mg) in H$_2$O (5 mL) was then introduced, and the resulting mixture was refluxed for 12 hours. After cooling down to room temperature, the THF and MeOH were evaporated. The solution was acidified with 1.0 M HCl until no further precipitate was detected. The precipitate was collected by centrifugation and washed with water. The solid was dried in vacuum. ESI MS found m/z=[M-Cl] + calc = 843.1282; UV-vis (DCM): \(\lambda_{abs} = 370, 394, 471, 528, 580, 616\) nm, \(\lambda_{max} = 471\) nm\(^2\)
Synthesis of nano-sized PCN-224-0.1Mn/0.9Zn:

Nano-sized PCN-224 was synthesized by a method developed by Park et al.\(^4\) Chemical quantities for the PCN-224-Mn synthesis are shown in chart S1. The starting materials were dissolved in DMF in a 100mL round bottom flask and the mixture was stirred (300 rpm) at 90 °C for 5 h. After the reaction is done, PCN-224-0.1Mn nanoparticles were collected by centrifugation (15000 rpm, 30 min) followed by washing with fresh DMF until the wash fractions were totally clear. The yield was 80%.

<table>
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<th>Porphyrin</th>
<th>ZrOCl₂·8H₂O</th>
<th>Benzoic</th>
<th>DMF</th>
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<td>0.0117 mmol H₂TCPP</td>
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<td></td>
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</tbody>
</table>

Chart S1, Synthesis of nano-sized PCN-224-0.1Mn/0.9Zn

Post-metalation with Zn:

3.5n was added to the H₂TCPP porphyrins by post synthetic modification similar to the reported procedure by Kung et al.\(^5\) PCN-224-0.1Mn was shaken in a 2 ml solution of 2 mM ZnCl₂/DMF at 60 °C and 500 rpm for 24 hours. After being shaken, the resultant PCN-224-0.1Mn/0.9Zn was washed with DMF and ethanol. The metal content of the MOF was then determined by ICP and is depicted in chart S2.
Synthesis of maltotronic acid:

The maltotronic acid was synthesized using a reported procedure with some modifications. Maltotriose (200 mg) was dissolved in hot water (1.5 ml) and diluted with ethanol (400 µl). To this mixture a solution of 200 mg iodine in 2 mL of methanol was added and then sonicated until the solution was homogenous.

0.6 M KOH in methanol was added to the homogenous solution dropwise until the potassium maltotroionate precipitated out of solution. The precipitate was washed by methanol and water 3 times respectively. After washing, the potassium maltotroionates were dissolved in water and freeze-dried to provide a white powder. ESI MS found [M+K]calc = 559.1271.

**Synthesis of MA-PCN-2240.1Mn/0.9Zn**

To conjugate maltatronic acid to the surface of the PCN-224-0.1Mn/0.9Zn a 100 µl of 10 mg/ml aqueous potassium maltotronic acid solution was prepared and 1 mg of PCN-224-0.1Mn/0.9Zn was added. The mixture was sonicated for 5 mins. MA-PCN224-0.1Mn/0.9Zn was then centrifuged at 20000 rpm and the maltotrionate solution was decanted. The MA-PCN-224-0.1Mn/0.9Zn was dried overnight in the air.

---

<table>
<thead>
<tr>
<th>ICP (100 ppb)</th>
</tr>
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<tbody>
<tr>
<td>Mn 2.339 ppb</td>
</tr>
<tr>
<td>PCN224-0.1Mn/0.9Zn</td>
</tr>
<tr>
<td>Zn 23.76 ppb</td>
</tr>
<tr>
<td>Zr 17.65 ppb</td>
</tr>
</tbody>
</table>

Chart S2. ICP
.5 Characterization of PCN224-0.1Mn/0.9Zn and MA-PCN224-0.1Mn/0.9Zn:

**Zeta-potential:**

To analyze the surface charge of PCN-224-0.1Mn/0.9Zn and MA-PCN-224-0.1Mn/0.9Zn, each sample was measured three times by a zeta-sizer. Each measurement consisted of 12 runs over 36 minutes. Mean and standard division were determined by these three measurements. (Chart S3 & S4)

<table>
<thead>
<tr>
<th>PCN-224-Mn/Zn</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement 1</td>
<td>-4.91</td>
<td>0.112</td>
</tr>
<tr>
<td>Measurement 2</td>
<td>-2.74</td>
<td>0.115</td>
</tr>
<tr>
<td>Measurement 3</td>
<td>-5.00</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Mean -4.22  Stv 0.1173

Chart S3. Three times measurements of zeta-potential of PCN-224-0.1Mn/0.9Zn

<table>
<thead>
<tr>
<th>MA-PCN224</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement 1</td>
<td>-30.4</td>
<td>0.459</td>
</tr>
<tr>
<td>Measurement 2</td>
<td>-33.4</td>
<td>0.472</td>
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<tr>
<td>Measurement 3</td>
<td>-33.5</td>
<td>0.481</td>
</tr>
</tbody>
</table>

Mean -32.4  Stv 0.4706

Chart S4. Three times measurements of zeta-potential of MA-PCN-224-0.1Mn/0.9Zn
**Fluorescence emission:**

For bio-imaging study, fluorescence emission of MA-PCN-224-0.1Mn/0.9Zn was measured. 1 mg of MA-PCN-224-0.1Mn/0.9Zn was suspended in 1 ml of PBS. After checking its emission wavelength over excitation wavelength over 200-700 nm at room temperature, $\lambda_{\text{max}}$ of emission was determined at 606 nm. Then, at $\lambda_{\text{em}}$=606, excitation wavelength was also measured. This spectrum showed that MA-PCN-224-0.1Mn/0.9Zn can be excited by wide visible light wavelength. (Figure S1)

![Excitation spectrum of MA-PCN224-0.1Mn/0.9Zn at $\lambda_{\text{em}}$=606](image)

**Cell Culture:**

- MDA-MB231 cells and Hela cells were cultured in high glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100$\mu$g/ml normocin, 100 IU penicillin, and 100 $\mu$g/ml streptomycin. The cultures were maintained in a humidified incubator in a 5% CO$_2$/95% air at 37 °C.
• MCF10a was cultured in mammary epithelial cell growth medium with bovine pituitary extract 0.004 ml/ ml, epidermal growth factor 10 ng/ml, insulin 5 µg/ml, hydrocortisone 0.5 µg/ml, 100µg/ml normocin, and cholera toxin 100 ng/ml. The cultures were maintained in a humidified incubator in a 5 % CO₂/95% air at 37 °C.

• THP-1 cells were grown using RPMI-1640 medium, 10% FBS and 100µg/ml normocin. Monocyte counts were performed using a hemocytometer containing a 1:1 ratio of trypan blue and cell culture medium, and 1.0 x10⁶ cells/mL were added to each well. Monocytes were converted to macrophage-like cells using 320 nM of phorbol 12-myristate 13-acetate (PMA) and incubated for 24 hours at 37 °C in 5% CO₂.

• Macrophage activation were induced to a M1 macrophage using lipopolysaccharide/Interferon-γ (LPS/IFN-γ) or M2 macrophage using interleukin-4/interleukin-13 (IL-4/IL-13) by following the reported way.7 To induce M1 phenotype, macrophage-like cells were induced with 100 ng/mL of LPS with a 20 ng/mL of IFNγ. To induce M2 phenotype, macrophage-like cells were induced with 20 ng/mL of IL-4 with a 20 ng/mL of IL-13. After the macrophage-like cells were induced with their respective inducer, macrophages were incubated for 24 hours at 37 °C in 5% of CO₂.

• Characterization of macrophages occurred by observing attachment of cells to the well surface, then washing with Hank’s solution to rid the culture of any planktonic monocyte remainders. The culture before washing was used for confirmation of M1 and M2 macrophages for ELISA. Subsequent to washing, M1 and M2 macrophages are used for WST-8 assay.
Dark toxicity Test:

Dark toxicity test was carried out by WST-8 assay. MDA-MB231, Hela, and MCF10a cells were seeded in 96-well microtiter plates at a density of 2×10^4 cells/well and incubated for 24 h in an atmosphere of 5% CO2 at 37 ºC. The different concentration of MA-PCN-224-0.1Mn/0.9Zn solutions were prepared in PBS. The different concentrations of MA-PCN-224-0.1Mn/0.9Zn (0.1, 5, 10, 20, 50 µg/ml) were well dispersed in PBS. (Figure S2) The 3 kinds of cells were sequentially incubated with different concentrations of MA-PCN-224-0.1Mn/0.9Zn (0.1, 5, 10, 20, 50 µg/ml) for another 24 h in cell cultural media without FBS. To control wells, the same amount of PBS as MA-PCN-224-0.1Mn/0.9Zn solution was added. Next, the cells were washed by PBS three times to get rid of any MOFs outside of cells. Subsequent to washing, 90 µl of fresh cultural media and 10 µl of cell counting kit solution were added to each well. After 4 hours, the absorbance was measured at 450 nm with microplate reader.

Cell viability was calculated by the following equation.

\[
Cell\ viability\ % = \frac{A_0 - A_b}{A_c - A_b} \times 100
\]  

(1)

As is the OD450 value of treatment group, Ac is the OD450 value of control group and the Ab is the OD450 value of blank well. Each independent experiment was performed in triplicate.

Figure S2. Suspension of MA-PCN-224-0.1Mn/0.9Zn (MA-PCN-224-Mn) in PBS.
Photodynamic therapy test

Photodynamic therapy test was carried out by WST-8 assay. First, on MDA-MB231, a toxicity of LED light was analyzed. MDA-MB231 cells were seeded in 96-well microtiter plates at a density of $2 \times 10^4$ cells/wells and incubated in 180µl of DMEM for 24 h in an atmosphere of 5% CO2 at 37 ºC. Then, MDA-MB231 cells were irradiated by LED light (100 mW/cm²) for 15 mins. After irradiation, 20 µL of cell counting kit solution was added to each well. After 4 hours, the absorbance was measured at 450 nm with microplate reader. OD450 (absorbance value) was compared when irradiation is 100% (Figure S3) Each independent experiment was performed four times.

![Figure S3. Cell toxicity of LED light](image)

MDA-MB231, Hela, and MCF10a cells were seeded in 96-well microtiter plates at a density of $2 \times 10^4$ cells/well and incubated for 24 h in an atmosphere of 5% CO2 at 37 ºC. The different concentration of MA-PCN-224-0.1Mn/0.9Zn solutions were prepared in PBS. The 3 kinds of cells were sequentially incubated with different concentrations of MA-PCN-224-0.1Mn/0.9Zn (0.1, 5, 10, 20, 50 µg/ml) for another 24 h in cell cultural media without FBS. The different concentration
of H₂TCPP solutions were prepared in PBS. The 3 kinds of cells were sequentially incubated with different concentrations of H₂TCPP (20, 40, 60, 80, 100 µg/ml) for another 24 h in cell cultural media without FBS. For control wells, the same amount of PBS as H₂TCPP solution was added. Next, the cells were washed by PBS three times to get rid of MA-PCN-224-0.1Mn/0.9Zn and H₂TCPP outside of cells. Subsequent to washing, 90 µl of fresh cultural media was added to each well, and then the cells were irradiated by LED light (100 mW/cm²) for 15 mins. After irradiation, 10 µl of cell counting kit solution was added to each well. After 4 hours, the absorbance was measured at 450 nm with microplate reader. Cell viability was calculated. Each independent experiment was performed in triplicate.
Confocal imaging

Confocal Imaging: MDA-MB231, Hela, and MCF10a were detached and were incubated in glass bottom 96-well microtiter plates and incubated for 24 hours. After the cells were incubated with MA-PCN-224-0.1Mn/0.9Zn solution (20 µg/ml and 50 µg/ml) for 5 hours and were washed once with PBS and twice with 4% paraformaldehyde PBS solution. For control, the same amount of PBS solution was added and incubated. The confocal images were captured using a confocal laser scanning microscope with an excitation wavelength of 405 nm. (Figure S4 to S6)

Figure S4. Bio-imaging of MA-PCN-224-0.1Mn/0.9Zn in MCF10a
Figure S5. Bio-imaging of MA-PCN-224-0.1Mn/0.9Zn in Hela
Cell apoptosis:

For quantitative analysis of cell apoptosis, QuantiFluo™ Caspase-3 Assay Kit was used. BioAssay Systems’ Caspase-3 Assay Kit measures caspase-3 activity in biological samples. In the assay, caspase 3 cleaves off substrate (N-Ac-DEVD-AFC) forming a highly fluorescent product. The fluorescence intensity ($\lambda_{ex/em} = 400/490$ nm) is proportional to the caspase-3 activity. Briefly, MDA-MB231 cells were seeded in 96-well plates with a density of $2 \times 10^4$ cells/well for 24 h in an atmosphere of 5 % CO2, 95 % air at 37 °C. The different concentration of MA-PCN-224-0.1Mn/0.9Zn solutions were prepared in PBS. The cells were sequentially incubated with different concentrations of MA-PCN-224-Mn/Zn-0.1Mn/0.9Zn (0.1, 5, 10, 20, 50 µg/ml) for
another 24 hours. For control, the same amount of PBS solution was added and incubated. Next, the cells were washed by PBS three times to get rid of MA-PCN-224-0.1Mn/0.9Zn outside of cells. Subsequent to washing, 100 µl of fresh cultural media was added to each well, and then the cells were irradiated by LED light (100 mW/cm²) for 15 mins. After irradiation, media was removed and added 100 µl of working reagent to each well. The 96-well plates were mixed by shaking for a minute at 200 rpm on a plate shaker. For the background well, only working reagent was added to empty well. The cells were incubated for 4 hours in the dark. The fluorescence intensity at λex/em = 400/490nm was measured with microplate reader. For the calculation, the fluorescence intensity value of each sample was subtracted from that of the background well. The ΔF values represent the relative caspase-3 activity. All assays were repeated in triplicate and the data is represented as the mean ± SD. Statistical analysis was performed using one-way ANOVA and a Turkey as a post-test using the Sigma Plot software package. Statistically significant values were defined as p<0.05.

ELISA for CCL2 and IL-10:

After the macrophage-like cells were induced with LPS/IFNγ or IL-4/IL-13, ELISA was run to confirm macrophage activation using the culture supernatants. CCL2 and IL-10 were measured with enzyme-linked immunosorbent assays (ELISA) for human kits according to the manufacturing procedure. Each sample was run in triplicate at 450 nm with a reference of 570 nm. All assays were repeated in triplicate and the data is represented as the mean ± SD. Statistical analysis was performed using one-way ANOVA and a Turkey as a post-test using the Sigma Plot software package. Statistically significant values were defined as p<0.0
Chapter IV - A Bio-Conjugated Chlorin-Based Metal–Organic Framework for Targeted Photodynamic Therapy of Triple Negative Breast and Pancreatic Cancers

John Ozdemir, co-first author

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Contribution for this paper:

- Yoshie Sakamaki, first author, designed, decided, and performed all experiments and write this paper,
- John Ozdemir, co-first author, contributed to writing, helping some porphyrin synthesis, and communicating with Beyzavi after I left the group.
- Zachary Heidrick and Anthony Azzun contributed to organic synthesis,
- Olivia Watson contributed to help synthesis of MOFs and in vitro,
- Miu Tsuji contributed to in vitro experiment,
- Christopher Salmon contributed to in vitro experiment,
- Arvind Sinha and Joseph Batta-Mpouma contributed to get zeta-potential data and UV-vis data of MOFs,
- Zachary McConnell and David Fugitt contributed to some organic synthesis for few days.
- Yuchun Du provided me some cell lines,
- Jin-Woo Kim provided some instruments and proof reading,
- M. Hudson Beyzavi submitted this paper.

I provided this data to one of the above undergrad students’ thesis.
4.1 Introduction

MOFs have been proposed as biodegradable platforms for photodynamic therapy (PDT); in particular, research on the use of metal–organic frameworks (MOFs) in photodynamic therapy has been rapidly developing in the past five years.\textsuperscript{1-8} PDT uses a non-toxic dye called a photosensitizer (PS) in conjunction with harmless visible or near-infrared (NIR) light to produce reactive oxygen species (ROS) in order to kill cancer cells or bacteria. PDT has the advantage of dual selectivity in that the PS can be directed to targeting cells or tissues and the light can be physically directed to the affected area.\textsuperscript{9-11} To be viable as a PS, a compound should achieve a high quantum yield of singlet oxygen generation upon absorption in the long wavelength region (\(~ 800
Additionally, for a PS to be a viable therapeutic, it must have preferential tumor localization, minimal dark toxicity, and simple formulation.\textsuperscript{12} The synthesis of new PSs is of fundamental importance for developing PDT as a usable tumor treatment. Chlorin is a PS that has been clinically tested as an antitumor agent for PDT. The chlorin macrocycle is a reduced porphyrin that has a center of UV absorption blue shifted from the porphyrin absorption region. Both of these macrocycles are studied as therapeutic agents for biomedical uses.\textsuperscript{13} Natural and synthetic chlorins are important because they have unique photophysical properties compared to the parent porphyrin, such as enhanced red light absorption and often improved fluorescence quantum yield.\textsuperscript{13} The chlorin ring, however, can be easily converted back into porphyrin by oxidation, especially during irradiation with light as the singlet oxygen generated may oxidize the unsaturated chlorin ring.\textsuperscript{14} Chlorin macrocycles are formed by the oxidative or reductive removal of a double bond in one of the a pyrrole rings comprising a porphyrin macrocycle.\textsuperscript{15,16} Stabilizing the single bond at the beta position of the pyrroles in a chlorin ring can be achieved by installing a 5-membered pyrrolidine at this position instead of simply adding two hydrogens.\textsuperscript{13}

Incorporating PSs into a MOF also prohibits the PS from forming molecular aggregates; this makes the crystalline MOF framework beneficial because the photophysical study of porphyrin-based macrocycles in aqueous solutions have shown that dimerization and aggregation are prevalent.\textsuperscript{17,18} When the PS is fixed into a supramolecular framework, as is the case in a MOF, then dimerization between the PSs cannot take place. Another issue with current PSs is the inability to discriminate between unhealthy and normal tissues, resulting in non-selective spreading of the PS throughout the body. For instance, some clinical PDT patients must avoid sunlight for at least 4 weeks after treatment.\textsuperscript{19} Glycosylation of porphyrin and chlorin macrocycles serves to make the
molecules amphiphilic, and conjugation to sugars can also target cancer cells by the Warburg effect.

As a continuation of our works on PDT utilizing MOFs, in the present work, the synthesis of a 5-ring C2 chlorin that can be incorporated into an Hf-MOF with topology similar to UiO is described. Surface glycosylation of the nano-scaled MOF crystals with maltotriose sugar gives the nanoparticles active targeting ability. The strategy of conjugation biomolecules to a MOF surface as a method to target cancer cells was explored previously by Zhou and coworkers wherein folic acid was conjugated to the surface of a porphyrinic MOF. The targeting mechanism is based on binding with the glucose transporter (GLUT) receptors, which are highly expressed on cancer cell surfaces. Maltotriose was bioconjugated to the MOF surface using a $S_N Ar$ reaction, which is the first example of such a reaction modifying a MOF surface to the best of our knowledge.

The hybrid composition of MOF frameworks allows for a multitude of functionalities to be installed onto one material. In this work, two MOF platforms have been engineered to target and kill cancer cells by the action of PDT. The two PS MOFs developed in this work, so named MA-HfMOF-PFP-Ni/Zn (MA-HfMOF-PFP) and MA-HfMOF-PFC-Ni/Zn (MA-HfMOF-PFC), were shown to be effective against two aggressive cell lines, MDA-MB-231 (triple negative breast cancer) and MIA-PaCa2 (pancreatic cancer). MDA-MB-231 represents a cancer cell line that is especially difficult to target and the MIA-PaCa2 is a highly aggressive and invasive cancer cell line. The cell death rates of these two cancer lines when treated with the MOF nanoparticles compared to cell death rates during analogous treatment of healthy cells demonstrates how the deliberate design of MOF nanoparticles at the compositional level can successfully enable anticancer activity.
4.2 Results and Discussion

4.2.1 Synthesis of EDA-MA and nano-sized MOFs

The synthesis of the chlorin linker began with preparation of 1-5-(pentafluorophenyl)dipyrromethane (PFP-DPM)\textsuperscript{33} which underwent condensation with methyl 4-formylbenzoate to synthesize 5,15-bis(4-carboxyphenyl)10,20-bis(pentafluorophenyl)porphyrin (Me\textsubscript{2}PFP; \textit{Scheme 1}; Supplementary Information page S4). After generating PFP-DPM, we modified established methods\textsuperscript{33, 34} to produce Me\textsubscript{2}PFP (yield: 300 mg of Me\textsubscript{2}PFP, 18.25\% yield) (Scheme 2; Supporting Information page S2.4). Pure Me\textsubscript{2}PFP was used for hydrolysis, metalation or chlorin synthesis. Metal insertion with Ni(II) of the Me\textsubscript{2}PFP gave 5,15-bis(4-carboxylxyphenyl)-10,20-bis(pentafluorophenyl)porphyrinato-Ni(II) (Ni-Me\textsubscript{2}PFP), and then Ni-Me\textsubscript{2}PFP underwent hydrolysis of esters to give 5,15-bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrinato-Ni(II) (Ni-H\textsubscript{2}PFP)\textsuperscript{33,35} (Scheme 2; Supporting Information page S6). 5,15-Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)chlorin (H\textsubscript{2}PFC) was synthesized through a 1,3-dipolar cycloaddition\textsuperscript{13} reported previously by Cavaliero and coworkers\textsuperscript{36} (\textit{Scheme S1}).
Scheme 2. Synthesis of 5,15-bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrinato-Ni(II) (Ni-H$_2$PFP) and 5,15-bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)chlorin (H$_2$PFC).

Figure 16. UV-VIS spectra of Me$_2$PFC, Zn-Me$_2$PFP, Me$_2$PFP, and Ni-Me$_2$PFP
UV-vis absorption spectroscopy was used to record the absorbance of all the synthesized macrocycles (Figure 1). The H$_2$PFC was stored in amber vials until needed for the MOF synthesis. To compare the effectiveness of chlorin-based MOFs to that of porphyrin-based MOFs for PDT, two kinds of MOFs were prepared. For the porphyrin-based MOFs, HfMOF-PFP-Ni was synthesized by mixing a 1:1 ratio of Ni-H$_2$PFP and H$_2$PFP with HfCl$_4$ and a size-controlled synthetic methodology developed by He et al.\textsuperscript{37} resulted in nano-scale MOFs HfMOF-PFP-Ni. The chlorin-based MOFs (HfMOF-PFC-Ni) were synthesized by mixing a 1:1 ratio of Ni-H$_2$PFP and H$_2$PFC with HfCl$_4$. In order to employ the heavy atom effect that will increase the generation of reactive oxygen species,\textsuperscript{38-40} HfMOF-PFP-Ni and HfMOF-PFC-Ni were treated with ZnCl$_2$, inserting Zn into the H$_2$PFP and H$_2$PFC ligands, respectively (Figure 3.3). Because Zn is a heavy atom, its presence near the PS enhances their intersystem crossing (ISC), causing an increase in $^1$O$_2$ production and improving their efficiency.\textsuperscript{1, 41-44} Since it is known that the metalation of paramagnetic metal to porphyrin causes the decrease of generation of singlet oxygen,\textsuperscript{37} we attempted to coordinate Ni$^{2+}$ only into porphyrin but not the chlorin linkers. EDA-maltotriose (EDA-MA) was covalently bound to the MOF surface by a S$_N$Ar reaction between the sugar and the pentafluorobenzene substituents on the MOF linkers (Figure 3.3) to give MA-HfMOF-PFP and MA-HfMOF-PFC, respectively.
EDA-MA was synthesized through reaction of MA with EDA (Figure 3.4a). A reductive amination at the anomeric position of maltotriose was carried out according to a reported procedure. The reaction progress was monitored by TLC, IR, and $^1$H NMR. During the reaction IR spectroscopy reveals a signal at 1800 cm$^{-1}$ which arises from the C=N bond in the imine intermediate; after the reductive amination the N–H bending vibration at 1600 cm$^{-1}$ is found from the isolated product (Figure 3.4b). The signals associated with the $\alpha$ and $\beta$ anomeric protons of the disappeared on $^1$H NMR as the reaction proceeded (Supporting Information Figure S2.3).
Figure 17. Reductive amination
a). Reductive amination of MA with EDA to yield MA-EDA. The reaction proceeds through an imine intermediate. b) Infrared spectroscopy (IR) of reductive amination. Top left: IR spectra of EDA and top right: maltotriose. Bottom left: IR spectra of the reaction mixture showing the imine intermediate and bottom right: EDA-MA.
4.2.2  MOF Synthesis

The synthetic route developed by He et al\textsuperscript{37} for the synthesis of nanoscale Zr and Hf MOFs was used in this work to synthesize two new MOFs: HfMOF-PFP and HfMOF-PFC. The Hf-MOFs used HfCl\textsubscript{4} as the metal source and were synthesized through a solvothermal reaction in DMF with acetic acid and water modulators (Supporting Information, Table S3.1). For HfMOF-PFP, a 1:1 ratio of H\textsubscript{2}PFP and Ni-H\textsubscript{2}PFP (0.165 mmol) was added to a solution of 0.33 mmol HfCl\textsubscript{4} in a solution of DMF (700 µL), acetic acid (42 µL) and water (17 µL). The same molar ratios used in the HfMOF-PFP synthesis were used in the HfMOF-PFP synthesis but instead of porphyrin, chlorin linkers (H\textsubscript{2}PFC and Ni-H\textsubscript{2}PFC) were used. All MOFs were synthesized in a 15 min reaction at 120 °C; stirring was maintained at 300 rpm. After synthesis, post synthetic modification was carried out to install Zn\textsuperscript{2+} into coordination sites at the center of the H\textsubscript{2}PFP linkers which was followed by the bio-conjugation step.

4.2.3  Bio-conjugation S\textsubscript{N}Ar reaction

Synthetic investigation began by developing model reactions to simulate the S\textsubscript{N}Ar reaction between EDA-maltotriose and a pentafluorophenyl group. First decafluorobiphenyl and EDA were selected as model molecules. Four bases were tested (diethylamine, diisopropylethyleamine, Potassium tert-butoxide and CsF) as well as a control reaction with no base. Ultimately the reaction with no base worked the best according to $^{19}$F NMR (Supporting Information Figure S3.5), and it can be deduced that in this example the EDA was basic enough to undergo the reaction unaided. This result did not hold when Me\textsubscript{2}PFP and EDA-MA were used to attempt the S\textsubscript{N}Ar reaction in DMF or DMSO. UV-vis spectroscopy was used to monitor the reaction, and it is expected that the saccharide-conjugated porphyrin displays a widening in the UV-vis absorption spectra as was observed previously.\textsuperscript{46} Mixtures of Me\textsubscript{2}PFP and EDA-MA were stirred in DMF for 3 hours wherein
UV-Vis at the beginning and end of the reaction time showed no difference (Figure S3.5). When CsF was added to reaction mixture the expected widening of the absorption band was observed after 3 hours (Figure 3.5), which indicated a successful S_NAR reaction.

![UV-Vis spectra](image.png)

Figure 18. UV-vis spectra of Me_{2}PFP dissolved in DMF and the Me_{2}PFP after three hours of mixing with EDA and CsF as base.

Based on the results of small-molecule reactions, surface modification of the MOF crystals was carried out with CsF as a base. In order to bioconjugate the EDA-MA, two separate solutions were prepared. One solution contained 1.5 mg CsF dissolved in 500µL of DMF while the other had 4 mg of the appropriate MOF complex mixed with 1 mg of EDA-MA in 500 µL of DMF. 100 µL of the CsF/DMF solution was pipetted into the MOF suspension. The resultant mixture was
then shaken at 500 rpm for 3 hours. After 3 hours, the reaction was complete, and the resultant bio-conjugated MOF complex was rinsed with DMF and water.

4.2.4 Characterization of MOFs

The MOF samples were characterized using powder X-ray diffraction (pXRD), transmission electron microscopy (TEM), inductively couple plasm mass spectrometry (ICP-MS), and UV-vis. MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-Ni/0Zn were characterized by TEM, zeta-potential and UV-vis. X-ray diffraction patterns of HfMOF-PFC-Ni and HfMOF-PFC-Ni/Zn were compared to calculated diffraction patterns from the single crystal structure of the MOF DBP-UiO synthesized by Lu et al. Unlike DBP-UiO the HfMOF-PFC MOFs synthesized in this work do not completely match the UiO topology but the HfMOF-PFC MOFs do retain structural motifs similar to UiO as evidenced by similarities in the computed and observed patterns (Figure 3.6a). The Ni:Zn metal ratios in the HfMOF-PFP-Ni/Zn and HfMOF-PFC-Ni/Zn samples were determined to be 1:3.1 and 1:2.7 (Table S3.2) using ICP-MS. This result indicates an increased incorporation of \(\text{H}_2\text{PFP}\) over \(\text{Ni-H}_2\text{PFP}\) or \(\text{H}_2\text{PFC}\) into the HfMOF-PFP-Ni and HfMOF-PFC-Ni frameworks. Comparison of particle size and morphology of the MOF particles before and after post-synthetic modification with Zn and EDA-MA using TEM can be seen in Figure 5b. This analysis reveals that the median particle size of the nano MOFs (nMOFs) increases from the 60–70 nm range before modification to 70–80 nm after with EDA-MA conjugation (Figure S3.7).
Figure 19. Characterization of HfMOFs

a) PXRD of HfMOF-PFC-Ni, HfMOF-PFC-Ni/Zn, and the simulated DBP-UiO diffraction pattern. b) The TEM images of HfMOF-PFC-Ni/Zn and MA-HfMOF-PFC-Ni/Zn. This ~10 nm increase is attributed to a coating of EDA-MA around the MOF particles.

Modification with EDA-MA was also analyzed by zeta potential (Table S4). Before modification with EDA-MA, the surface charge of HfMOF-PFP-Ni/Zn was measured to be is
0.651 mV (stv = 0.00146). After modification with EDA-MA, the surface of MA-HfMOF-PFP-Ni/Zn is -5.007 mV (stv = 0.000832). This slight negative shift in zeta-potential is also indicative of EDA-MA conjugating to MOFs. It is assumed that the nitrogen atoms of EDA may capture hydrogen atoms from two alcohols of maltotriose (Figure S3.10) resulting in a mild negative shift; moreover, this increase in negative surface charge will increase electrostatic dispersion in water.

As shown in Figure 3.5, saccharide-conjugated porphyrin displays a widening in the UV-Vis absorption spectra. The metalation of Zn into Me₂PFC shifts the soret band from 650 nm to 630 nm (Figure 3.7a) as is observed in the UV-vis of the isolated Zn-Me₂-PFC (Figure 3.7b). The shift in the soret band when Zn is incorporated into the chlorin is also observed in MA-HfMOF-PFC-Ni/Zn when compared to HfMOF-PFC-Ni (Figure 3.7c).
Figure 20. UV-VIS study for Zn metalization
a) UV-vis spectrum of Me$_2$PFC before and during metalation with Zn. b) UV-viz of Zn-Me$_2$PFC. c) UV-vis of HfMOF-PFC-Ni and MA-HfMOF-PFC-Ni/Zn.
4.2.5 Bioactivity Data

The selective antitumor effect of MA-HfMOF-PFC-Ni/Zn was tested in-vitro by comparing the cell toxicity of MA-HfMOF-PFP-Ni/Zn towards three cancer cell lines: MDA-MB-231 (triple negative breast cancer) cells, MIA-PaCa2 (pancreatic cancer) cells, and HeLa (cervical cancer). MCF-10a (an immortalized human breast epithelial cell line derived from non-tumorigenic breast epithelium) was also tested. Incorporation of EDA-MA onto the MA-HfMOF-PFC-Ni/Zn particles target the GLUT receptor of cancer cells. The PDT effect of MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-Ni/Zn against MDA-MB-231, MIA-PaCa2, Hela, and MCF-10a cells were measured by WST-8 assay where the number of live cells is directly proportional to the amount of formazan produced. The WST-8 assay was carried out as follows: After the four cell lines were treated with MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-Ni/Zn respectively, for 24 hours, the cells were washed with PBS three times and irradiated with a white LED light (100 mW/cm²) for 30 minutes. The comparison of PDT effect for MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-Ni/Zn between cancer cell lines and normal cell lines is shown in Figure 7. MDA-MB-231, MIA-PaCa2, and Hela cancer cell lines were exposed to MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFPC-Ni/Zn and irradiated with an LED light. These cancer cell lines were killed effectively after 80 µg/ml of MA-HfMOF-PFP-Ni/Zn while they were killed dramatically after 20 µg/ml of MA-HfMOF-PFC-Ni/Zn. Both MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-Ni/Zn showed smaller toxicity towards the MCF-10a cell lines than cancer cell lines. To demonstrate that toxicity is dependent on irradiation with LED light, the dark toxicity of MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-Ni/Zn were also tested against two cell lines used in the study, and the results are shown in Figure 3.8. The dark toxicity of MOFs against all three cell lines was minimal, suggesting that the toxicity of MA-HfMOF-PFP-Ni/Zn and MA-HfMOF-PFC-
Ni/Zn arise only during irradiation with light. To gauge the targeting ability of the surface modified MOFs, ICP-MS was used to determine the Hf levels in the treated cells. The amount of elemental Hf in the MDA-MB-231 and MCF-10a cell lines was compared to Hf levels in the HeLa cells to determine MOFs’ ability to target cancer cells via GLUT receptor. MDA-MB-231 is also known for its highly aggressive growth, which correlates to a high expression of the GLUT receptor. The result shows that cellular uptake of the maltotriose-conjugated MOFs is 20 times higher for MDA-MB-231 cells than for MCF-10a cells. This result is a strong indication that the maltotriose functionalized surface causes selective uptake of the MOFs into cancer cells. Cellular uptake of MA-HfMOF-PFC was lower than that of MA-HfMOF-PFP which could be due to the increased polarity of the chlorin linkers of MA-HfMOF-PFC.
Figure 21. PDT effect of MA-HfMOF-PFP-Ni/Zn
4.3 Conclusion

We have reported that the nano-scaled MOF MA-HfMOF-PFC-Ni/Zn can selectively target cancer cells due to maltotrionic acid conjugation on the surface of the MOF. MA-HfMOF-PFC-Ni/Zn is a well-designed drug delivery system that has been proven to actively target three cancer cell lines while showing minimal toxicity toward the normal MCF-10a cell line. The characterization of MA-HfMOF-PFC-Ni/Zn surface charge with zeta potential confirms that the dispersive effect of MOF particles can be enhanced by surface functionalization. Our previous work on this topic involved the modification of MOF nanoparticle surfaces with maltotrionic acid for similar targeted anticancer effect. To determine if the MA conjugation to the surface of the MOF can achieve a cancer targeting effect within the circulation of a living sample, MA-HfMOF-PFC-Ni/Zn should be tested in a mouse model for future studies. In vivo studies by Liu’s group reported that the Hf of their nMOF, Hf-TCPP-NMOF-PEG was excreted in the feces and urine of mice completely in seven days. Understanding the excretion mechanism of the relevant elements will reveal to what extent toxic metal accumulation will determine therapeutic MOF dosages during in vivo experiments. Our chlorin-based nano-MOFs (MA-HfMOF-PFC-Ni/Zn) showed stronger PDT effects than porphyrin-based nano-MOFs (MA-HfMOF-PFP-Ni/Zn). Investigating and incorporating new PSs into multifunctional MOFs is a major proof of concept to make useful nanoparticles for PDT.

To date most efforts towards designing MOFs for PDT are focused on incorporating the most effective organic PS molecules within the MOF frameworks as linkers; and towards this end, more complex and efficient symmetric macrocycles should be synthesized. Inhibiting dimerization and aggregation of PSs fixed into the supramolecular crystalline frameworks means that MOFs serve as the most optimal platform for PDT. Meanwhile, functionalizing nanoparticle surfaces to
increase cancer targeting\textsuperscript{52-54} specificity will do much to broaden the viability of clinical nanomedicine. It is a much more efficient approach that must be used in future explorations of MOFs as PDT agents as it brings the PS directly to the cancer cells. Active targeting will also increase the potency of a PS so that less of the MOF needs to be administered, reducing any prolonged photosensitivity or metal toxicity. Since MA-HfMOF-PFC-Ni/Zn also contains the paramagnetic Ni\textsuperscript{2+} metal, it will be an excellent MRI agent.\textsuperscript{55}
4.4 References


42. Simone, B. C. D.; Mazzone, G.; Russo, N.; Sicilia, E.; Toscano, M., Metal Atom Effect on the Photophysical Properties of Mg(II), Zn(II), Cd(II), and Pd(II) Tetraphenylporphyrin Complexes Proposed as Possible Drugs in Photodynamic Therapy. *Molecules* 2017, 22, 1093.


4.5 Appendix

Supporting information for

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A Bioconjugated Chlorin-Based Metal–Organic Framework for Targeted Photodynamic Therapy of Triple Negative Breast and Pancreatic Cancers

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Keywords: photodynamic therapy (PDT), metal-organic framework (MOF), passive and active targeting, triple-negative breast cancer (TNBC), tumor-associated macrophage (TAM)
Materials and Methods

Instruments

Transmission electronmicroscopy (TEM) was carried out on JEOL 1011. SEM and EDX was carried out on FEI Nova Nanolab 200 Dual-Beam Focused Ion Beam. X-ray powder diffraction (PXRD) data was obtained at room temperature (298 K) on RIGAKU II miniflex. Absorbance was measured on BioTeK Synergy H1 Hybrid microplate reader in the WST-8. ICP was measured on ICAP Q ICP-MSI Thermo Fisher ICP-MS. UV data of porphyrins were obtained on DU800 (Beckman Coulter). UV data of MOF were obtained on Thermo Scientific NanoDrop 2000c spectrophotometer. Zeta-potential was carried out on Nano-ZS Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). Cell homogenization was carried out on Sonifer Cell Disruptor Model SLPe (BRANSON).

Materials

Dulbecco’s modified eagle’s medium (DMEM) was purchased from Life Technologies Corporation. Fetal bovine serums (FBS) USDA APPROVED ORIGIN, sodium chloride and penicillin strep were purchased from VWR. Normocin was purchased from InvivoGen. TetraZ Cell Counting Kit was purchased by BioLegend. Mammary epithelial cell growth medium and detach kit 30 was purchased from PromoCell. Cholera toxin vibrio cholerae was purchased from Enzo Life Sciences. Disodium hydrogen phosphate dodecahydrate and potassium dihydrogen phosphate were purchased from ACROS Organics. Potassium chloride was purchased from AVANTOR PERFORMANCE MATERIALS. Dimethyl sulfoxide was purchased from Corning. HfCl₄ was purchased from BeanTown Chemical and ZnCl₂ was purchased from Alfa Aesar. NiCl₂ was purchased from STREM Chemicals. Pyrrole was purchased from Alfa Aesar and purified by
distillation when used. Methyl 4-formylbenzoate was purchased from TCI America. Pentafluoro benzaldehyde was purchased from Oakwood Chemical. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and Trifluoroacetic acid (TFA) were purchased from Millipore sigma.

Synthesis of EDA-maltotriose and Nano-sized MOF

Scheme S 1. Synthetic scheme for Ni-H$_2$PFP and H$_2$PFC

Synthesis of 1-5- (pentafluorophenyl)dipyromethane (PFP-DPM)

1-5- (Pentafluorophenyl)dipyromethane was synthesized by following a reported method with few modifications. A solution of pentafluorobenzaldehyde (2.0 mL, 16.2 mmol) in freshly distilled pyrrole (50 mL, 720 mmol) was degassed with a stream of N$_2$ for 20 min before adding trifluoroacetic acid (120 µL, 1.62 mmol). The mixture was stirred for 30 min at room temperature, diluted with CH$_2$Cl$_2$ (400 mL), and then washed with 0.1M NaOH (400 mL). The organic phase was washed with water (400 mL) and dried over Na$_2$SO$_4$. Evaporation of the solvent at reduced
pressure gave brown oil. Unreacted pyrrole was removed under high vacuum, yielding a tacky solid that was flushed on a column of silica using a mixture of hexanes:ethyl acetate:triethylamine (80:20:1) as the eluent. Fine white powder was obtained by evaporating the solution. 1HNMR and 19FNMR were matched to the reported one. 1H NMR (400 MHz,CDCl3): δ 5.92 (1H, s, CH), 6.00 – 6.05 (2H, m, ArH), 6.14 – 6.19 (2H, m, ArH), 6.71 – 6.76 (2H, m, ArH), 8.17 (2H, brs, NH); 19F NMR (400 MHz, CDCl3): δ -161.47 (2F, m, ArF), -155.95 – -156.06 (1F, t, $J = 21.0$ Hz, ArF), -141.70 (2F, brd, $J = 20.7$ Hz, ArF).

Synthesis of 5,15- Bis(4-carboxyphenyl)-10,20-bis(pentafluorophenyl)porphyrin (Me$_2$PFP)

5,15- Bis(4-carboxyphenyl)-10,20-bis(pentafluorophenyl)porphyrin was synthesized by following two reported methods with some modifications. Our established method produces solely Me$_2$PFP, completely bypassing all other byproducts. First, 1.141g (3.65 mmol) of PFP-DPM was combined with 799mL of dried DCM under nitrogen. After ten minutes, we added 599 mg (3.65 mmol) of methyl 4-formylbenzoate and increased the nitrogen flow. Twenty minutes later, 479µL (6.25 µmol) of trifluoroacetic acid (TFA) was added. The solution was left to react for 24 hours. The following day, 871 µL (6.25 µmol) of triethylamine was added, followed by 821.5 mg 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) forty minutes later. The products were collected via flash column after three hours (the eluent was a 1:99 ratio of ethyl acetate:dichloromethane). Me$_2$PFP was evaporated from the eluent after being collected from the column. Once evaporated, it was rinsed using MeOH to yield 300mg of Me$_2$PFP, 18.25% yield. The resultant pure Me$_2$PFP was saved for either metalation or chlorin generation. 1HNMR and 19FNMR were matched to the reported one.
\[ H \text{ NMR (376 MHz, CDCl}_3\): } \delta -2.84 \text{ (2H, s, NH), 4.15 (6H, s, CO}_2\text{CH}_3\), 8.33 (4H, d, } J = 8.1 \text{ Hz, ArH), 8.49 (4H, d, } J = 8.1 \text{ Hz, ArH), 8.85 (4H, d, } J = 4.8 \text{ Hz, } \beta \text{H), 8.92 (4H, d, } J = 4.8 \text{ Hz, } \beta \text{H); 19F NMR (400 MHz, in CDCl}_3\): } \delta -162.0 \text{ (4F, m, ArF), -152.3 \text{ (2F, t, } J = 21.2 \text{ Hz, ArF); -137.1 \text{ (4F, dd, } J = 8.1 \text{ Hz, 23.7 Hz, ArF); UV-vis (DCM): } \lambda_{\text{abs}} = 368, 412, 509, 537, 597, 650 \text{ nm, } \lambda_{\text{max}} = 412 \text{ nm.}\\

Synthesis of 5,15- Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrin (H\textsubscript{2}PFP)

5,15- Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrin was hydrolyzed in 2:1 ratio of HCl:TFA for several days at 70 °C by following the reported method.\textsuperscript{8}

\[ H \text{ NMR (400 MHz, in DMSO): } \delta -3.02 \text{ (2H, s, NH), 8.15 (4H, d, } J = 8.0 \text{ Hz, ArH), 8.28 (4H, d, } J = 7.9 \text{ Hz, ArH), 8.97 (4H, d, } J = 4.5 \text{ Hz, } \beta \text{H), 9.29 (4H, d, } J = 4.8 \text{ Hz, } \beta \text{H),13.42 (2H broad); 19F NMR (400 MHz, in DMSO): -162.83 - 162.89 \text{ (4F, m, ArF), -152.16 \text{ (2F, t, } J = 22 \text{ Hz, ArF), -139.49 - 139.54 \text{ (4F, dd, } J = 6.0 \text{ Hz, 25.3 Hz, ArF); UV-vis (DCM): } \lambda_{\text{abs}} = 370, 412, 512, 546, 586, 633 \text{ nm, } \lambda_{\text{max}} = 412 \text{ nm.}\\

Synthesis of 5,15- Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)chlorin (Me\textsubscript{2}PFC)

5,15- Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)chlorin was synthesized by referencing the patent.\textsuperscript{10} To begin, 119mg of Me\textsubscript{2}PFP was combined with 26mg of sarcosine in 50mL of dried toluene. For sixteen hours, the solution was refluxed under nitrogen and in the dark with 26 mg of sarcosine and 26 mg of paraformaldehyde being added every two hours. The final solution was added to chloroform and rinsed with water to remove excess sarcosine and
paraformaldehyde. The resultant Me$_3$PFC was purified by silica gel chromatography, with the eluent being a mixture of hexane and ethyl acetate. The final yield of the racemic mixture was 20.6% with an additional 26mg of Me$_2$PFP that was recycled. UV-vis (DCM): $\lambda_{abs} = 368, 412, 509, 537, 597, 650$ nm, $\lambda_{max} = 412$ nm

Figure 3.S 1 Absorption spectrum of Me$_3$PFC.

Figure S 2. High resolution mass spectrum of Me$_3$PFC with the [M+H]$^+$ and [M+2H]$^{2+}$ peaks labeled.
Synthesis of 5,15- Bis(4-carboxyphenyl)-10,20-bis(pentafluorophenyl)chlorin (H₂PFC)

The obtained 5,15- Bis(4-carbomethoxyphenyl)-10,20-bis(pentafluorophenyl)chlorin was hydrolyzed in 2:1 ratio of hydrochloric acid: TFA for several days at 70 °C by checking TLC. ¹H NMR of NH and five ring hydrogens were referred to the reported peak. The yield of the racemic mixture of products is quantitative (22 mg) ESI MS found m/z=[M+H⁺] + calc = 940.2

Figure S 3. The structures of the two stereoisomers of H₂PFC and ¹H NMR.
Synthesis of 5,15-Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrinato-Ni(II) (Ni-Me2PFP)

The metalation of Me2PFP with Ni was based on previous findings.2 Under constant Nitrogen, 100mg of Me2PFP was combined with 208.9mg of NiCl2·6H2O in 5mL of DMF. The solution was refluxed overnight. Once the reaction was complete, ice was added to the solution. The solution was filtrated. Then, the Ni-Me2PFP was redissolved in dichloromethane (DCM). 1H NMR (500 MHz, Chloroform-d) δ 8.81 (d, J = 4.5 Hz, 4H), 8.72 (d, J= 4.4 Hz, 4H), 8.41 (d, J= 7.8 Hz 2H), 8.13 (d, J = 7.4 Hz, 2H), 4.11 (s, 6H). 19F NMR (376 MHz, Chloroform-d) : δ -161.87 (4F,m,ArF), -152.33 (2F, t, J = 21.2 Hz, ArF); -137.23(4F, dd, J = 8.1 Hz, 23.7 Hz, ArF); UV-vis (DCM): λabs = 409, 521, 561 nm, λmax = 409 nm

Synthesis of 5,15-bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrinato-Ni(II) (Ni-H2PFP):
5,15-Bis(4-carbomethoxyphenyl)-10,20-bis(pentafluorophenyl)porphyrinato]-Ni(II) was hydrolyzed in 2:1 ration of hydrochloric acid:TFA for several days at 70 °C by following the reported method.

Synthesis of 5,15- Bis(4-carboxylphenyl)-10,20-bis(pentafluorophenyl)porphyrinato]-Zn(II) (Zn-Me$_2$PFP):

The metalation of Me$_2$PFP with Zn was performed in the following way: Zn(OAc)$_2$ was added to Me$_2$PFP in DCM and MeOH (1:1) solution. The solution was stirred overnight. The solution was evaporated, and zinc-metallized Me$_2$PFP was washed by water and redissolved in DCM. UV-vis (DCM): $\lambda_{abs} = 395, 414, 546$ nm, $\lambda_{max} = 414$ nm
Synthesis of maltotriose-EDA (Bioligand), reductive amination

In order to synthesize maltotriose-EDA, a previously reported method was followed in which 0.2 mmol (100 mg) of maltotriose was combined with 0.2 mmol (13 µL) of ethylenediamine (EDA) in 1 mL of water, followed by the addition of 0.1 M borate buffer. Then, 0.4 mmol (25 mg) of NaCNBH₃ was added and the resultant solution was stirred constantly for three days. The product was checked by Infrared Spectroscopy (IR) and TLC in vanillin and ninhydrin. The solution was then flooded with 10x its volume of ethanol and the resultant precipitate was washed again with ethanol and collected by centrifugation at -5 ºC. Pure maltotriose-EDA was obtained via lyophilization with 90% yield. ¹H-NMR showed anomer hydrogen disappeared as it was reported. ESI MS found m/z=[M+H⁺] + calc = 549.2499 No dimer (MA-EDA-MA) was detected.
Figure S 5. $^1$H NMR (DMSO-d$_6$) of standard maltotriose (purple) and maltotriose-EDA (brown). After the completion of the reaction, anomeric hydrogens (α and β hydrogen) disappear.

Synthesis of nano-sized HfUiO-PFP and HfUiO-PFC

Nano-sized MOFs were synthesized according to the reported method. All materials in the Table 3.S1 were dissolved in DMF in a 50 mL round bottom flask, and the mixture was stirred (300 rpm) at 120 °C for 15 mins. After the reaction is done, HfUiO-PFP and HfUiO-PFC nanoparticles were collected by centrifugation (15000 rpm, 30 min) followed by washing with
ethanol 3 times. MOFs were dried in a vacuum oven at 60 °C. After being completely dried, about 4.5 mg of HfUiO-PFP and HfUiO-PFC were weighed and analyzed via PXRD, TEM and UV-vis.

<table>
<thead>
<tr>
<th>MOF Post-metalation with Zn</th>
</tr>
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<tr>
<td>After the HfMOFPFP and HfMOF-PFC were isolated, the free porphyrin coordination sites at the H2TCPP centers were metallated with ZnII. With some modification, the method reported by Farha and coworkers for the post-synthetic modification of porphyritic MOFs was followed.6 In order to achieve post-synthetic metalation, HfMOF-PFP-Ni and HfMOF-PFC-Ni were shaken in a 2 mL solution of 2 mM ZnCl2/DMF at 60 °C and 500 rpm for 24 h. After being shaken, the resultant HfMOFPFP-Ni-Zn (HfMOF-PFP) and resultant HfMOF-PFC-Ni-Zn (HfMOF-PFC) were washed with DMF. Analysis of the metalation was done with ICP, and EDX. Zinc and nickel were not detected well by EDX, but they were detected by ICP. Since metals incorporated in porphyrin were not detected well by EDX for HfMOF-PFC, ICP-MS was carried out.</td>
</tr>
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</table>
Table 3.S 2 EDX and ICP-MS

<table>
<thead>
<tr>
<th>MOF Bio-conjugation S_NAr</th>
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</table>

Study of bio-conjugation with model substrates: First, to find the best condition for the $S_NAr$ reaction, the reaction was performed with model substrates. Decafluorobiphenyl and diethylamine were selected for the bio-conjugation reaction. For five conditions, diethylamine, diisopropylethylamine, KOBu, CsF, and no base were chosen. According to the result of $^{19}$F NMR, no base condition was best to generate ideal product since EDA is base.

Figure 3.S 2 Model reaction for bio-conjugation $S_NAr$ reaction
Table S 1. Condition of the model reaction for the reaction shown in Figure S7

<p>| | |</p>
<table>
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<tbody>
<tr>
<td><strong>Temperature</strong></td>
<td><strong>Room temperature</strong></td>
</tr>
<tr>
<td>Decafluorobiphenyl</td>
<td>0.015 mmol</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>0.03 mmol</td>
</tr>
<tr>
<td>Base (Diethylamine, CsF, KOBt, diisopropylethylamine)</td>
<td>0.015 mmol</td>
</tr>
<tr>
<td>DMSO</td>
<td>500 μL</td>
</tr>
<tr>
<td>Time</td>
<td>3 hours</td>
</tr>
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</table>

Figure S 6. $^{19}$F NMR results of the model reactions in Figure S7.
Figure S 7. UV-VIS spectra of S_{N}Ar reaction between Me_{2}PFP and maltotriose-EDA (MA-EDA) without base.

Characterization of bio-conjugated MOFs

Distribution: Analysis of the size distribution of HfUiO-PFP-0.5Ni/0.5Zn and MA-HFUUiO-PFP-0.5Ni/0.5Zn. Each sample was measured by TEM.

Figure S 8. The size distribution of HfMOF-PFP-Ni-Zn (before modification in blue) and MA-HFMOFPFP-Ni-Zn (after modification in green).
The mean size was 62.9 nm before modification and 77.5 nm after modification.

Figure S 9. The size distribution of HfMOF-PFC-Ni-Zn before modification in blue and MA-HfMOFPFC-Ni-Zn after modification in green.
The mean was 68.99 nm before modification and 75.1 nm after modification.

Figure S 10. Analysis of the PXRD patterns of HfMOF-PFP-Ni(purple) and HfMOF-PFP-Ni-Zn(red).
TEM images: Analysis of the crystal shape of HfMOF-PFC-Ni-Zn and MA-HfMOF-PFC-Ni-Zn.

TEM observations can be seen in Figure S13

Figure S 11. The TEM images of a) HfMOF-PFP-Ni-Zn (Before modification) and b) MA-HfMOF-PFPNi-Zn (After modification).

Figure S 12. UV-vis spectra of HfMOF-PFP-Ni-Zn without MA in red and MA-HfMOF-PFC-Ni-Zn with MA in purple.

Zeta-potential: Analysis of the surface charge of HfMOF-PFP-Ni-Zn and MA-HfMOF-PFP-Ni-Zn was carried out by zeta potential. Each sample was measured three times by zeta-sizer.
One measurement consisted of 12 scans and each sample was scanned three times for a total of 36 scans. Mean and standard division were determined by these three scans and can be seen in Table S4.

Table S2. Zeta-potential of HfMOF-PFP-Ni-Zn (HfMOF-PFP) and MA-HfMOF-PFP-Ni-Zn (MAHfMOF-PFP)

<table>
<thead>
<tr>
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<th>Mean</th>
<th>Standard Deviation</th>
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<tr>
<td>Measurement 1</td>
<td>0.653</td>
<td>0.0228</td>
</tr>
<tr>
<td>Measurement 2</td>
<td>0.525</td>
<td>0.0205</td>
</tr>
<tr>
<td>Measurement 3</td>
<td>0.775</td>
<td>0.0201</td>
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Mean 0.651. Stv 0.00146

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
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</thead>
<tbody>
<tr>
<td>Measurement 1</td>
<td>-5.37</td>
<td>0.346</td>
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<tr>
<td>Measurement 2</td>
<td>-4.78</td>
<td>0.358</td>
</tr>
<tr>
<td>Measurement 3</td>
<td>-4.87</td>
<td>0.362</td>
</tr>
</tbody>
</table>

Mean 5.007. Stv 0.00832

In Vitro Cell Culture

MDA-MB231 cells, MIA-PaCa2 cells and Hela cells were cultured in high glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 100µg/ml normocin. MCF10a was cultured in mammary epithelial cell growth medium with bovine pituitary extract 0.004 ml/ml, epidermal growth factor 10 ng/ml, insulin 5 µg/ml, hydrocortisone 0.5 µg/ml, 100µg/ml normocin, and
cholera toxin 100 ng/ml. The cultures were maintained in a humidified incubator in a 5% CO₂/95% air at 37 ℃.

Dark toxicity Test

The dark toxicity test was carried out by WST-8 assay. MDA-MB231, Hela, MIA-PaCa2 and MCF10a cells were seeded in 96-well microtiter plates at a density of 2×10⁴ cells/well and incubated for 24 h in an atmosphere of 5% CO₂, 95% air at 37 ℃. The different concentration of MA-Uio-PFP and MA-Uio-PFC solutions were prepared in PBS. The 3 kinds of cells were sequentially incubated with different concentrations of MA-Uio-PFP and MA-Uio-PFC (10, 20, 40, 80, 100µg/ml) for another 24 h in cell cultural media without FBS. To control wells, the same amount of PBS as MA-PCN224 solution was added. Next, the cells were washed with PBS three times to get rid of MOFs outside of cells. Subsequent to washing, 90 µl of fresh cultural media and 10 µl of cell counting kit solution were added to each well. After 4 hours, the absorbance was measured at 450 nm with microplate reader.

Cell viability was calculated by the following equation.

\[ \text{Cell viability} \% = \left( \frac{A_s - A_b}{A_c - A_b} \right) \times 100\% \]

As is the OD450 value of treatment group, Ac is the OD450 value of control group, and the Ab is the OD450 value of a blank well. Each independent experiment was performed three times.
Photodynamic therapy test

The photodynamic therapy test was carried out by WST-8 assay. MDA-MB231, Hela, MIA-PaCa2 and MCF10a cells were seeded in 96-well microtiter plates at a density of $2 \times 10^4$ cells/well and incubated for 24 h in an atmosphere of 5 % CO2, 95 % air at 37 ºC. The different concentration of MA-UiO-PFP, MA-UiO-PFC solutions were prepared in PBS. The 4 kinds of cells were sequentially incubated with different concentrations of MA-UiO-PFP, MA-UiO-PFC (10, 20, 40, 80, 100µg/ml) for another 24 h in cell cultural media without FBS. Next, the cells were washed by PBS three times to get rid of MA-UiO-PFP, MA-UiO-PFC outside of cells. Subsequent to washing, 90 µl of fresh cultural media was added to each well, and then the cells were irradiated by LED light (100 mW/cm²) for 15 mins. After irradiation, 10 µl of cell counting kit solution was added to each well. After 4 hours, the absorbance was measured at 450 nm with a microplate reader. Cell viability was calculated. Each independent experiment was performed three times.

Cellular Uptake

MDA-MB231 and MCF10a cells were seeded in 6-well microtiter plates at a density of $5 \times 10^5$ cells/well and incubated for 24 h in an atmosphere of 5 % CO2, 95 % air at 37 ºC. After washing with PBS 2 times, 1960µl of media without FBS was added to each well. 40µl of 1mg/ml of MA-UiO-PFP, MA-UiO-PFC solutions in PBS were added to each well. The cells were incubated for further 24 hours and washed with PBS three times, and then collected by using rubber policemen. After centrifugation at 1500 rpm for 10 mins, the cells were washed with miliQ water twice. The cells were then homogenized by using Sonifer Cell Disruptor Model SLPe. Cell
solutions were diluted in 2% HNO₃ for ICP measurement. Hf element was measured by ICP for each sample.

Table S 3. ICP measurements

<table>
<thead>
<tr>
<th></th>
<th>MCF10a</th>
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</thead>
<tbody>
<tr>
<td>MA-Uio-PFP</td>
<td>3.36 ppb</td>
<td>96.08 ppb</td>
</tr>
<tr>
<td>MA-Uio-PFC</td>
<td>19.97 ppb</td>
<td>342.08 ppb</td>
</tr>
</tbody>
</table>
Chapter - V – Iron-Based Metal-Organic Framework for Targeted Photodynamic Therapy and Chemotherapy of Pancreatic Cancer

I provided this data to one of my undergraduate students’ thesis.

5.1 Introduction

Pancreatic cancer is one of the leading causes of cancer mortality, with an overall Mortality/Incidence ratio of 98% and an overall five-year survival rate of about 6%\(^1\). This high mortality is due to several factors, including the late stage at which most patients are diagnosed and the difficulty and dangers associated with surgical resection, which is currently the most effective treatment. Based on the United States National Cancer Institute’s data for pancreatic cancer, more than half (52%) of cases were diagnosed at the distant stage\(^2\), at which point the cancer has already spread to distant parts of the body. Photodynamic therapy (PDT) provides a minimally invasive approach to cancer therapy by employing certain wavelengths of light to initiate chemical reactions in the body to produce localized tissue necrosis. It utilizes three essential components: photosensitive compounds called photosensitizers (PS), light, and oxygen. On their own, these individual components are harmless, but combined they can be used to initiate cell apoptosis. Upon irradiation with light, PSs can reach an excited singlet state that is followed by an excited triplet state via intersystem crossing. In the presence of oxygen, this excited triplet state can in turn generate a toxic reactive oxygen species (ROS) using one of two reaction pathways\(^3\). The end result of ROS creation by the PS is localized cell apoptosis or necrosis, depending on the location of the PS within the cell\(^4\). The activation of PSs is dependent on their activation with certain wavelengths of light. Therefore, upon light irradiation, only tissues with an accumulation of PSs should be damaged. Nanoparticles (NPs) can be created for the delivery of PS materials.
Due to their small size and shape, NPs are able to passively accumulate in tumor cells, a phenomenon known as the “enhanced permeation and retention (EPR) effect.” Through the EPR effect, NPs are able to better accumulate within tumors due to their leaky vasculature and are more likely to be retained due to the impaired lymphatic drainage of tumors.\textsuperscript{5} However, relying on passive accumulation alone allows for the NPs to potentially distribute to healthy tissues as well.\textsuperscript{6} Using active targeting, NPs can specifically target tumors through peripheral conjugation of specific ligands that recognize receptors overexpressed in cancer cells, resulting in the enhanced delivery of the NP.\textsuperscript{6} To maximize the accumulation of the NP in cancerous tissues, active and passive targeting should be used in tandem. Our biofunctionalized MOF complex is designed to treat pancreatic cancer efficiently and safely via photodynamic therapy, combining active targeting, drug-loading, and photosensitivity into a single platform. MIL-101(Fe) (Matérial Institut Lavoisier) was used as the MOF base of the compound. MIL-101(Fe) is preferred over MIL-101(Cr) due to the toxicity of chromium and the biocompatibility of iron.\textsuperscript{7} Solid tumors have an acidic cellular environment and an altered pH gradient across their cell compartments. MIL-101(Fe) is an ideal framework because it lacks stability in acidic environments and thus is biodegradable, giving it the ability to release the photosensitizer within the cancer cells.\textsuperscript{8} An additional benefit of using MIL-101(Fe) is the presence of iron within its framework, as the paramagnetic metal will improve the nanoparticle’s bio-imaging capability in techniques such as magnetic resonance imaging (MRI).\textsuperscript{9} A C2 pentafluoro benzene porphyrin, 5,15-Bis(carboxylphenyl)-10,20-bis(pentafluorophenyl) porphyrin (henceforth referred to as H\textsubscript{2}PFP), served as the organic linker and photosensitizer for the MIL-101(Fe). To actively target pancreatic cancer cells, the compound was peripherally conjugated with maltotrionic acid. The purpose of this modification was to take advantage of a phenomenon known as the Warburg effect, in which
solid tumors, including those found in pancreatic cancer, are known to have increased glucose uptake and glycolysis rates. Theoretically, this bioconjugation should allow the MOF complex to specifically target the glucose transporter-1 (GLUT-1) that is overexpressed in pancreatic cancer cells.\textsuperscript{10, 11} For some trials, MIL-101(Fe) was loaded with doxorubicin, a chemotherapy drug, to determine its effectiveness in a chemo-photodynamic dual therapy approach. Metal-organic frameworks (MOFs) are promising nanoparticle drug carriers for PDT. They are formed by repetitive sequences of strong coordination bonds between metal ions or clusters and organic linker ligands. The resulting large surface-to-volume ratios and highly crystalline structures allow for loading large amounts of PSs and/or drug compounds.\textsuperscript{12, 13, 14} The porous structure of MOFs also facilitates the diffusion of ROS, although the efficacy of PDT is still limited due to the obstacle of tumor hypoxia.\textsuperscript{15} Porphyrins can be used as the organic linkers in MOF structures. In addition to their MOF-linking capabilities, porphyrins are the basis of many leading photosensitizers as they are able to efficiently produce singlet oxygen. However, being planar molecules, porphyrins are hindered by their tendency to self-aggregate due to their strong $\pi$-$\pi$ interactions. By incorporating photosensitizing porphyrins into their structure, MOFs are able to limit this aggregation, resulting in a compound that combines drug delivery and photosensitivity into a single platform for PDT.\textsuperscript{14}

5.2 Result and Discussion

5.2.1 Synthesis of C2 porphyrin with high yield

We began by synthesizing the organic linker and photosensitizer, H$_2$PFP, following a procedure established by Moore, et al. with some adjustments.\textsuperscript{16} In order to synthesize H$_2$PFP, 1-5-(pentafluorophenyl)dipyromethane (PFP-DPM) had to be synthesized. Once PFP-DPM was generated, it was used to synthesize Bis(4-carbomethoxyphenyl)-10,20-
bis(pentafluorophenyl)porphyrin (Me$_2$PFP) following a procedure reported by Kooriyaden, et al. with some modifications.\textsuperscript{17} Me$_2$PFP was purified with MeOH for a yield of 18.25%. The resultant Me$_2$PFP was then hydrolyzed in a 2:1 hydrochloric acid (trifluoroacetic acid) TFA mixture for two days at 70°C. This hydrolysis resulted in the conversion of the bilateral ester ends of the Me$_2$PFP into carboxylic acids, forming H$_2$PFP.

5.2.2. Size-controlling study

The functionality of MOFs for PDT is largely dependent on their size in order to take advantage of the EPR effect in tumors. To be most effective for passive targeting, the nanoparticle should be between 10-200 nm.\textsuperscript{3} Therefore, we tried several different conditions to control the size of MIL-101(Fe). MIL-101(Fe) was synthesized according to earlier studies by Xie et al. and Cui et al with some modifications in an effort to produce homogenous crystals.\textsuperscript{18}\textsuperscript{19} However, their reported crystals were too big to achieve sufficient EPR effects. Also, Wyszogrodzka et al. reported a way to make the crystal smaller by milling the crystals. These three MIL-101(Fe) were also not porphyrin-based. In our first four trials, we tried to control the size of the crystals by adding various volumes of water (0, 27, 54, and 81 μL for Conditions A, B, C, and D, respectively). These volumes of water were each added to 40.5 mg of FeCl$_3$ and 20.6 mg of terephthalic acid in 1.5 mL of DMF then stirred for 20 hours at 110°C (In these size-control trials, terephthalic acid was used as a linker in place of H$_2$PFP in order to be more cost-effective). However, as shown in Figure 1a, increasing the volume of water produced larger crystals that were outside of the acceptable size range. Figures 1b below shows the PXRD for Conditions A-D, respectively. The mean diameters for Conditions A-D were 315 nm, 1032 nm, 1371 nm, and 2798 nm, respectively, indicating that the addition of water increased the size of the crystals past the acceptable range for PDT. Under Condition E, 20.6 mg of terephthalic acid was mixed with 40.5 mg of FeCl$_3$ in 3 mL
of DMF for 10 hours at 110°C while Condition F was the same except that it used 1.5 mL of DMF instead of 3 mL. Condition E produced smaller crystals than Condition F, indicating that limiting the solvation of the starting materials could make size-controlling efforts more successful. **Figures 1c and 1d** show the TEM imaging and PXRD, respectively, for Conditions E and F. Therefore, we decreased the concentration of the starting materials instead of varying the volume of water for our additional size-control trials.

**Figure 22.** Size study of MIL101

a) TEM imaging for MIL-101(Fe) following: A) Condition A, mean 315 nm; B) Condition B, mean 1032 nm; C) Condition C, mean 1371 nm; and D) Condition D, mean 2798 nm. b) The PXRD for MIL-101(Fe) under Conditions A-D. C) TEM images for MIL-101(Fe) under Condition
E (mean 191 nm) and Condition F (mean 401 nm). d) The PXRD for MIL-101(Fe) under Conditions E and F.

Using these findings, three more trials were conducted using different concentrations and thermal conditions. In Condition G, 5 mg of H$_2$PFP was combined with 2 mg of FeCl$_3$ in 600 μL of DMF. The solution was then heated in a vessel at 110°C for 10 hours in an oven. In Condition H, 11.1 mg of H$_2$PFP was combined with 5 mg of FeCl$_3$ in 150 μL of DMF. The solution was then heated in a vessel at 60°C for 10 hours in an oven. In Condition J, 5 mg of H$_2$PFP was combined with 2 mg of FeCl$_3$ in 600 μL of DMF. The solution was then stirred in a round bottom flask at 300 rpm and 100°C for 14 hours in an oil bath. Following each of these reactions, the MIL-101(Fe) was collected via centrifugation then washed with ethanol for 3 hours. Condition J was ultimately found to produce homogenous crystals within the acceptable size range, and its crystals were used for the rest of the project. Figure 2 below shows the TEM imaging of our synthesized MIL-101(Fe) for Conditions G, H, and J. The mean diameters of crystals produced by Conditions G and J were found to be 218 nm and 91 nm, respectively. Condition H did not produce homogenous crystals. The PXRD of Condition G (Figure 3) confirmed the synthesis of MIL-101(Fe). The peak was shifted to the left compared to original terephthalic acid-based MIL-101(Fe). Under Condition J, we succeeded in controlling the size of the MIL-101(Fe) to be less than 200 nm, and the size distribution of the synthesized crystals in this condition is found in Figure 4. However, due to the significantly smaller size of the crystal, we could not obtain clear PXRD for Condition J with our machine. Since we aimed to make the size of the crystal smaller for PDT, we used the crystals produced under Condition J for our biofunctionalized compound. In Figure 5, the UV-VIS spectra of porphyrin-based MIL-101(Fe) are shown in Figure S1.
Figure 23. The TEM images for MIL-101(Fe) under Condition G (mean 218 nm), Condition H (no homogenous size), and Condition J (mean 91 nm).
Figure 24. The PXRD for Condition G, confirming the synthesis of MIL-101(Fe).

Figure 25. The size distribution of the MIL-101(Fe) produced under Condition J. The vast majority of the synthesized crystals were fewer than 200 nm in diameter, leaving them in the acceptable size range for non-specific endocytosis by cells.
5.2.3  Doxorubicin (DOX) encapsulation

Doxorubicin (DOX) was encapsulated following a previously reported method with some modifications.\textsuperscript{20} First, doxorubicin was neutralized with TEA in DMSO to a pH of 7 to prevent the doxorubicin from damaging the MIL-101(Fe). 3 mg of blank MIL-101(Fe) was dispersed in DMSO, and 6 mL of 2 mg/mL DOX-DMSO solution was added dropwise. This mixture was stirred at room temperature and 100 rpm for 48 hours and DOX@MIL-101(Fe) was recovered via centrifugation and dried under a vacuum for 48 additional hours. DOX@MIL-101(Fe) was collected by centrifugation, and the supernatant solution was used for UV-VIS test. The encapsulation and resulting DOX@MIL-101(Fe) was confirmed using UV-VIS spectroscopy. We confirmed the encapsulation of doxorubicin within MIL-101(Fe) using UV-VIS spectroscopy at 500 nm, as shown in Figure S2. By calculating the decrease of the intensity of the DOX peak in the supernatant, the amount of DOX absorbed by MIL-101 was determined. 2.06 mg of DOX was absorbed by 3 mg of MIL-101 theoretically based on the UV-VIS result. The peak around 400 nm is loose porphyrin that leaked from broken MIL-101(Fe) due to DMSO.

5.2.5  MA-MIL101 (Fe) Conjugation

Maltotriionic acid was synthesized following a procedure reported by our previous work.\textsuperscript{21} To conjugate maltotriionic acid to the surface of DOX@MIL-101(Fe), 1 mg of DOX@MIL-101(Fe) was added to 100 µL of 10 mg/mL aqueous potassium maltotriionate solution (pH=7). This mixture was sonicated for 5 minutes then centrifugated at 20,000 rpm, after which the excess maltotriionate solution was decanted. The resulting maltotriionic acid-conjugated DOX@MIL-101(Fe) (henceforth referred to as DOX@MA-MIL-101(Fe)) was used for in vitro procedures. DOX@MIL-101(Fe) and DOX@MA-MIL-101(Fe) were analyzed by zeta-potential. DOX@MIL-
101(Fe) shows -4.60 (Stv -0.3605) while DOX@MA-MIL-101(Fe) shows -49.3 (Stv -3.863) (Table S1)

5.2.6 In vitro results

We determined the effectiveness of MIL-101(Fe) and MA-MIL-101(Fe) for the treatment of pancreatic cancer in vitro using the MIA PaCa-2 cell line. We determined the effectiveness of maltotriose-conjugation for active targeting of pancreatic cancer using MCF-10a, a human mammary epithelial tissue, as a control to represent healthy tissue.

5.2.7 Cellular absorption confirmation

Prior to conjugation with maltotrionic acid, the size-controlled MIL-101(Fe) (condition J) was able to enter the cells via endocytosis based on its small size alone, as evidenced by microscopic imaging in Figure 6a, reflecting the success of our size-control experimentation.

5.2.8 Dark toxicity and photodynamic therapy

The effectiveness of a photosensitizer can be evaluated by comparing cell viability with and without irradiation. The cell viability in the presence of the photosensitizer without irradiation is known as “dark toxicity.” The dark toxicity of H₂PFP alone, MIL-101(Fe), and MIL-101(Fe) loaded with the DOX chemotherapy agent (henceforth referred to as DOX@MIL-101(Fe)) was tested using a WST-8 assay. The dark toxicity tests showed a significant increase in the toxicity of MIL-101(Fe) in concentrations larger than 10 µg/mL, indicating that this dosage is the maximum acceptable therapeutic amount of the compound (Figure 6b).
5.2.9 Photodynamic therapy

To test the photodynamic capabilities of MIL-101(Fe), its “light toxicity” for MIA PaCa-2 cells was measured using a WST-8 assay. For the control wells, the same volume of PBS was added rather than H$_2$PFP, MIL-101(Fe), or DOX@MIL-101(Fe) solution. Irradiation therapy showed a significant decrease in cell viability at the 10 µg/mL doses and upward, although the light toxicity of lower doses (0.1 and 1 µg/mL) were not significantly changed (Figure 6c).

5.2.10 Targeting Specificity

To test the effectiveness of maltotriose conjugation for specific targeting of the overexpressed GLUT-1 receptors in pancreatic cancer cells, the cell viability of MIA PaCa-2 and MCF-10a treated with maltotriose-conjugated DOX@MA-MIL-101(Fe) (henceforth referred to as DOX@MA-MIL-101(Fe)) and light irradiation was tested in a WST-8 assay. MIA PaCa-2 and MCF-10a were each treated with DOX@MA-MIL-101(Fe) using the same light toxicity procedure outlined directly above. However, the MA-DOX@MIL-101(Fe) was only prepared in lower concentrations (0.1, 1, and 10 µg/mL) because these concentrations did not exhibit dark toxicity. When conjugated with maltotrionic acid, DOX@MA-MIL-101(Fe) decreased the cell viability of MIA PaCa-2 at not only the 10 µg/mL dosage, but also at the lower dosages as well (Figure 6d). These results indicate that the conjugation with maltotrionic acid does increase the ability of the compound to actively target the pancreatic cancer cells, increasing the effectiveness of the treatment. By comparing these results with the cell viability of MCF-10a under the same treatment, we can see that the compound is capable of targeting the pancreatic cancer cells while causing only minimal damage to healthy tissues.
Figure 26. Bioactivity data of MIL101
a) Microscopic images of MIA PaCa-2 after treatment with MIL-101(Fe). This demonstrates the ability of the MOF (purple) to be taken up by the cells via endocytosis based on its small size. b) Cell viability of MIA PaCa-2 under dark treatment with H$_2$PFP, MIL-101, and DOX@MIL-101. c) Cell viability of MIA PaCa-2 under light treatment with H$_2$PFP, MIL-101, and DOX@MIL-101. d) Treatment of MIA PaCa-2 and MCF-10a with DOX@MA-MIL-101(Fe) and LED irradiation.

5.3 Conclusion

We successfully made a new way to make a size-controlled, porphyrin-based MIL-101(Fe). The success of our compound in treating MIA PaCa-2 is promising for the future of specific targeting in photodynamic therapy, particularly as a supplemental approach for the treatment of pancreatic cancers. The in vitro experimentation confirmed that drug delivery with our MOF compound increased the effectiveness of the dual photodynamic and chemotherapy approach, and that this effectiveness was further improved by conjugating the MOF’s surface with maltotronic
acid. Although treatment with DOX@MA-MIL-101(Fe) alone may not be enough to completely eradicate pancreatic tumors, our study has shown that it could potentially be used in a neoadjuvant approach combined with chemotherapeutic agents to minimize the size of the tumor, allowing for safer and more feasible surgical resection. Additionally, the incorporation of iron into the framework of the compound gives it potential bioimaging capabilities. Although further testing is required, the success of this multifunctional compound could have wide implications for the application of MOFs within the field of medicine. The next step for this project would be treatment of actual pancreatic tumors in vivo using rats. This would allow us to determine its effectiveness in physiological environments. In vivo experimentation would also allow us to assess the compound’s bioimaging capabilities, as the incorporation of paramagnetic iron into its framework gives it potential diagnostic capabilities using techniques such as magnetic resonance imaging (MRI).
5.4 Reference


5.5 Appendix

Supporting information for Iron-Based Metal-Organic Framework for Targeted Photodynamic Therapy and Chemotherapy of Pancreatic Cancer

Instrumentation

Transmission electronmicroscopy (TEM) was carried out on JEOL 1011. X-ray powder diffraction (PXRD) data was obtained at room temperature (298 K) on RIGAKU II miniflex. Absorbance was measured on BioTeK Synergy H1 Hybrid microplate reader in the WST-8 assay. UV data was obtained on DU800 (Beckman Coulter). Zeta-potential was carried out on Nano-ZS Zetasizer (Malvern Instruments Ltd., Worcestershire, UK).

Materials

Dulbecco’s modified eagle’s medium (DMEM) was purchased from Life Technologies Corporation. Fetal bovine serums (FBS) USDA APPROVED ORIGIN, sodium chloride and penicillin strep were purchased from VWR. Normocin was purchased from InvivoGen. TetraZ Cell Counting Kit WAS purchased by BioLegend. Mammary epithelial cell growth medium and detach kit 30 were purchased from PromoCell. Disodium hydrogen phosphate dodecahydrate and potassium dihydrogen phosphate were purchased from ACROS Organics. Potassium chloride Propanoic acid were purchased from AVANTOR PERFORMANCE MATERIALS. Dimethyl sulfoxide was purchased from Corning. FeCl₃ was purchased from Alfa Aesar. Pyrrole was purchased from Alfa Aesar and purified by distillation when used. Methyl 4-formylbenzoate was purchased from TCI America.
Figure S 13. UV-vis of MIL-101

![UV-VIS of MIL-101](image)

Figure S 14. UV-vis of DOX and DOX@MIL-101, confirming the encapsulation of 2.06 mg of doxorubicin within 3 mg of MIL-101(Fe).
Chart S1. Three times measurements of zeta-potential of DOX@MIL-101 and DOX@MA-MIL101

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<tr>
<th>DOX@MIL-101</th>
<th>Mean</th>
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<tbody>
<tr>
<td>Measurement 1</td>
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<td>Measurement 2</td>
<td>-3.36</td>
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<tr>
<td>Measurement 3</td>
<td>-4.88</td>
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Mean -4.60  Stv -0.3605

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<th>DOX@MA-MIL101</th>
<th>Mean</th>
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<tbody>
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<td>Measurement 1</td>
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<tr>
<td>Measurement 2</td>
<td>-47.4</td>
</tr>
<tr>
<td>Measurement 3</td>
<td>-54.1</td>
</tr>
</tbody>
</table>

Mean -49.3  Stv -3.863

Cell Culture

MIA-PaCa2 cells were cultured in high glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100µg/ml Normocin, 100 IU penicillin, and 100 µg/ml streptomycin. The cultures were maintained in a humidified incubator in a 5% CO$_2$/95% air at 37°C.

MCF-10a was cultured in mammary epithelial cell growth medium with bovine pituitary extract 0.004 ml/ml, epidermal growth factor 10 ng/ml, insulin 5 µg/ml, hydrocortisone 0.5 µg/ml, 100µg/ml Normocin, and cholera toxin 100 ng/ml. The cultures were maintained in a humidified incubator in a 5% CO$_2$/95% air at 37°C.

Dark Toxicity

MIA PaCa-2 was seeded in 96-well microtiter plates at a density of 2x10$^4$ cells/well and incubated for 24 hours in an atmosphere of 5% CO$_2$ at 37°C. Sequential concentrations of H$_2$PFP, MIL-101(Fe), or DOX@MIL-101(Fe) (0.1, 1, 10, 25, and 50 µg/mL) were prepared in PBS and
the MIA PaCa-2 were incubated with these different concentrations for an additional 24 hours. For the control wells, the same volume of PBS was added rather than H₂PFP, MIL-101(Fe), or DOX@MIL-101(Fe) solution. Following incubation, the cells were washed three times with PBS to remove any H₂PFP, MIL-101(Fe), or DOX@MIL-101(Fe) outside of the cells to minimize any absorption due to free floating compounds. 90 µL of fresh media and 10 µL of cell counting kit solution were added to each well, and after 4 hours the absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated according to the following equation:

\[
\text{Cell Viability} \% = \frac{[A_s - A_b]}{[A_c - A_b]} \times 100 \tag{1}
\]

In which \(A_s\) is the OD540 value of the treatment group, \(A_c\) is the OD450 value of the control group, and \(A_b\) is the OD450 value of a blank well. Each independent experiment was performed three times.

Photodynamic Therapy

MIA PaCa-2 were seeded in 96-well microtiter plates at a density of 2x10⁴ cells/well and incubated for 24 hours in an atmosphere of 5% CO₂ at 37°C. Sequential concentrations of H₂PFP, MIL-101(Fe), or DOX@MIL-101(Fe) (0.1, 1, 10, 25, and 50 µg/mL) were prepared in PBS and the MIA PaCa-2 were incubated with these different concentrations for an additional 24 hours. Following incubation, the cells were washed three times with PBS to remove any H₂PFP, MIL-101(Fe), or DOX@MIL-101(Fe) outside of the cells. However, after 90 µL of fresh media was added to each well, the cells were irradiated by a LED lamp (100 mW/cm²) for 15 minutes. 10 µL of cell counting kit solution was then added to each well, and after 4 hours the absorbance was
measured at 450 nm using a microplate reader. Equation 1 was again used to calculate cell viability. Each independent experiment was performed three times.

Targeting Specificity

MIA PaCa-2 and MCF-10a were seeded in 96-well microtiter plates at a density of 2x10^4 cells/well and incubated for 24 hours in an atmosphere of 5% CO₂ at 37°C. Sequential concentrations of the MA-DOX@MIL-101(Fe) (0.1, 1, and 10 µg/mL) were prepared in PBS and the cells were incubated with these different concentrations for an additional 24 hours. Following incubation, the cells were washed three times with PBS to remove any DOX@MIL-101(Fe) outside of the cells. However, after 90 µL of fresh media was added to each well, the cells were irradiated by a LED lamp (100 mW/cm²) for 15 minutes. 10 µL of cell counting kit solution was then added to each well, and after 4 hours the absorbance was measured at 450 nm using a microplate reader. Equation 1 was again used to calculate cell viability. Each independent experiment was performed four times.
Chapter - VI - DNA-linked Multi-Functional Bio-imaging Agents / Tunable Physiochemical Properties

6.1 Introduction

The goal of this project was to make progress in the development of DNA-linked nanoparticles to form multifunctional 1D and 2D structures. The overall strategy was to use the DNA-linked structures as building blocks for these 1D and 2D structures. The foundation of generating these DNA-programmable nanoparticle building blocks includes the fields of DNA computation, DNA-guided self-assembly and nanoparticles in general. The programmable nature of DNA can be utilized in conjunction with the anisotropic nature of NP synthesis (Table 3) to enable the integration of NPs into nanostructures of various sizes and shapes which might serve various functions such as plasmonic capabilities of the linked metallic NPs. Engineering many discrete NPs into a single multifunctional nanoscale structure with specified physical, chemical, or biological properties has the potential to revolutionize a wide variety of research fields, ranging from optoelectronics and nanophononics to nanomedicine. The potential for various NP compositions within a nanocomposite structure makes feasible the ability to combine complementing characteristics of several imaging and sensing modalities to provide more accurate data during diagnostics. This project begins with the development of 1D and 2D structures using the smallest anisotropically monofunctionalized units. Ultimately, this research aims to develop programmable three-dimensional DNA-NP blocks.
Table 3. Overview of the variety of metallic nanoparticles and examples of their cancer immunotherapy applications.¹

<table>
<thead>
<tr>
<th>MNP</th>
<th>Approach</th>
<th>Mechanism</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuprous oxide</td>
<td>Alter tumor microenvironment</td>
<td>Alter expression of drosophila transcription factor</td>
<td>Induced myeloid infiltration and systemic immunity</td>
</tr>
<tr>
<td>Gold</td>
<td>Antigen/adjuvant delivery; Photothermal therapy</td>
<td>Increased CTL responses; tumor ablation released tumor antigens</td>
<td>Reduced tumor growth in vivo; prevented tumor growth in vivo</td>
</tr>
<tr>
<td>Iron oxide</td>
<td>M1 macrophage polarization; Protein delivery; Photothermal therapy MRI</td>
<td>Increased pro-inflammatory macrophage proliferation; IONP-HSP cheperoned antigens to APCs; thermal tumor ablation</td>
<td>Inhibited tumor growth; IONP-HSP led to tumor-specific CTL responses; ablation led to protective immunity</td>
</tr>
<tr>
<td>Silver</td>
<td>Reduce tumor-promoting cytokines</td>
<td>Decreased IL-1β signaling in tumor microenvironment</td>
<td>Inhibited fibrosarcoma tumor growth in vivo</td>
</tr>
</tbody>
</table>


### 6.2 Results and Discussion

Briefly, from the results, this research showed that it improved the reproducibility, versatility, and stability of the originally reported study, and was implemented not only in gold but also in silver, copper, and iron oxide. However, the DMAP-MESA-AuNPs (dmAuNPs) used in the reported method were deficient in several ways and required some modifications for size study and bimetal dimers. NPs used for the purpose of generating further dimensional structure must be stable in the presence of pH changes and buffers. Additionally, all metallic nanoparticles (MNPs) (Au, Ag, Cu, and Fe₃O₄) should have the same stable physiochemical property in their surface
ligand, as this has an effect on the SPR effect. It also simplifies the processing for all metallic NPs. As a result, polyvinylpyrrolidone (PVP), which is widely used to synthesize colloidal NPs, were chosen as their capping ligand. PVP is an excellent stabilizer due to its unique chemical and physical properties, which include high chemical stability, nontoxicity, and solubility in a broad range of polar solvents. The experimental section below describes the newly developed or modified synthetic methods for PVP-capped AuNPs, AgNPs, CuNPs, and Fe₃O₄. All MNPs generated homogeneous NPs with a diameter of 5 to 10 nm. Apart from developing the protocol with various types of metallic NPs, a new COOH-silica-gel was synthesized to obtain high yield DNA-linked NPs (experimental section). On the negatively charged silica-gel surface, the electrostatic reaction between NH3+-DNA and silica-gel-COO must occur efficiently as the first step in the reported “one step at a time” DNA conjugation. This interaction was investigated in a variety of scenarios, but ddH₂O and other buffers (boric buffer, phosphate buffer, and acetic buffer) had no discernible effect on the yield of this interaction. Thus, depending on the type of MNP, the first step was performed in ddH₂O or boric buffer (pH =7). Briefly, after attaching DNA to silica-gel and activating it with SH, the exact molar amount (or excess in case of CuNPs and Fe₃O₄) of NPs was added to bind to SH. The molarity of AuNPs and AgNPs was determined using the UV-VIS intensity.

To begin, a size study was conducted to determine the smallest unit (DNA-linked NP) in various AuNP sizes (3, 5, 10 nm). 3 nm of DNA-linked NP was synthesized as dmAuNPs in the reported method. The 3 nm of dmAuNPs were successfully reproduced dmAuNPs dimers in Figure 27.
Two methods were tried for AuNPs with diameters of 5 and 10 nm. First, 5 and 10 nm dmAuNPs were synthesized using the same method as 3 nm dmAuNPs, with some ligand concentration changes. They were linked to DNA after a simple size separation with centrifugation, but they easily aggregated during the "one step at a time" process shown in Figure 28. Despite the fact that the surface charge of these particles was strongly negative (ranging from -25 to -40 widely), the larger size of dmAuNPs was easily aggregated in the boric buffer. The increased amount of DMAP on the surface of AuNPs could be the cause of this aggregation. Positively charged DMAP and negatively charged MESA are the ligands of dmAuNPs, and DMAP’s charge is affected by pH and buffer. It was also suggested that the presence of two ligands on the surface of AuNPs should result in a low precision in obtaining the same zeta-potential (no data was shown). dmAuNPs were difficult to treat and showed low reproducibility.
Because the aforementioned methods were not able to stabilize DNA-linked AuNPs it was decided to implement the PVP ligand exchange method. As such, commercially available 5 and 10 nm of citrate-capped AuNPs were ligand exchanged to form PVP-AuNPs. While the yield of AuNPs to DNA attachment decreased, the release of DNA from silica-gel was maximized (experimental section). Due to the increased size of NPs, it was assumed and corrected that more than one single-stranded DNA (ssDNA) could attach to one side of PVP-AuNPs. As illustrated in Figures 29 and 30, 5 and 10 nm PVP-AuNPs generated dimers as intended. These results demonstrated that DNA conjugation at the +x axis to 10 nm NPs was feasible.

Furthermore, DNA hybridization between complementary DNAs attached to NPs (complementary DNA building blocks) resulted in dimers, despite the possibility of two ssDNAs being attached to a single NP at the +x axis. However, no SPR effects associated with the formation of AuNP dimers of these different sizes (3, 5, 10 nm) were observed in the UV-vis spectra (Figure 31). Regardless, the length of ssDNA used in this study was 20 bases and the length between NPs which would theoretically provide a 8 nm distance between particles. Surprisingly, TEM images of dimers revealed an average distance of approximately 2 nm between NPs, and no SPR was observed. One explanation for the unexpected distance of 2nm distance between NPs may be the
variation in aqueous (UV-VIS) and dried conditions (TEM). PVP-AuNP (5) dimers, on the other hand, did not have a strong peak at 520 nm, although it still showed the red color of the colloidal gold solution. This phenomenon requires further analysis with the computational study.

Figure 29. TEM images of 5 nm dimers (PVP-AuNPs)

Figure 30. TEM images of 10 nm dimers (PVP-AuNPs)
Additionally, to determine whether DNA can attach to the surface of larger NPs along multiple axes and whether DNA-linked NPs can form multiple NPs architectures, multiple ssDNA conjugation at +x, -x, and +y was investigated using 5 nm PVP-AuNPs. Given the possibility of multiple ssDNAs binding to each axis site, steric hindrance may occur, interfering with the second and third ssDNA conjugation. Though ssDNA could not be visualized by TEM due to its low electric density, the successful construction of a T-shaped two-dimensional structure was confirmed by hybridization with three complementary DNA-AuNPs in Figure 32. The UV-VIS spectrum of this substance is depicted in Figure 32. As can be seen from the spectrum, there is a distinct DNA peak at 260 nm. Interestingly, PVP-AuNPs (5 nm) conjugated with three DNA molecules exhibited a redshifted peak at 580 nm. However, the T shape product hybridized with
the remaining three PVP-AuNPs (10 nm) lacked the peak at 580 nm and the peak at 525 nm of the 1-DNA conjugated PVP-AuNPs (10 nm) lacked the peak at 530 nm.

These fundamental size study with gold nanoparticles resulted in a subsequent study with various types of MNPs. Each PVP capped metallic NP (PVP-AgNP, PVP-CuNP, and PVP-Fe₃O₄) that has optimal size (5 to 10 nm) were synthesized for further study of “one step at a time” method and SPR (experimental section).

Figure 32. TEM images of T shape (+x, -x, and +y)
Following the size study and synthesis of each metallic nanoparticle, each DNA-linked MNP was investigated using the same length ssDNA (20 bases). PVP-CuNPs-DNA and PCP-Fe$_3$O$_4$-DNA, with the exception of PVP-AgNPs-DNA, may be adapted similarly to PVP-AuNPs-
DNA (experimental section). PVP-AgNPs-DNA, on the other hand, required an optimization study in order to be obtained. PVP-AuNP-DNA-PVP-CuNP, PVP-AuNP-DNA-PVP-AgNP, and AuNP-DNA-PVP-Fe₃O₄ were synthesized via hybridization for 1 hour at room temperature (pH=7.4) in 10 mM Tris-HCl buffer after each building block was prepared. As a Figures 34, 35, and 36 show TEM images of these successfully synthesized dimers, while Figures 37 and 38 show their relative UV spectra. Dimers of Au-Ag and Au-Cu produced a new redshifted peak, which agreed with the computational study and the other published study. Furthermore, due to the low solubility of PVP-Fe₃O₄, a single UV-VIS spectrum was obtained in hexane rather than water. After generating Au-Fe₃O₄ dimers, the solubility improved, and the spectra in water shown in Figure 39 were obtained. As demonstrated, Au-Fe₃O₄ dimers generated their SPR effect unambiguously (redshifted peak above 600 nm). These data led to the conclusion that silver, copper, and iron oxide nanoparticles may have larger hot spots than gold nanoparticles and interact with gold nanoparticles, potentially accounting for the observed LSRP effects.
Figure 34. TEM image of Au-Ag dimers
Figure 35. TEM image of Au-Fe₃O₄ dimers

Figure 36. TEM images of Au-Cu dimers (scale bar is 20 nm)
Figure 37. UV-VIS spectra of Au (5)-Cu dimers and Au (10)-Ag dimers

Figure 38. UV-VIS spectra of Fe₃O₄ and Fe₃O₄ - Au (5) dimers
6.3 Experimental Section

6.3.1 Preparation of PVP-AuNP

First, PVP capped AuNP (PVP-AuNP) was prepared by ligand-exchanging from citrate capped AuNPs. Citrate-capped AuNP (5 and 10 nm) were purchased by BBI™ Solutions. 5 nm and 10 nm of citrate-capped AuNPs were ligand-exchanged in the concentration of 40 µL and 2 mM of 40K PVP respectively. The ligand-exchange happened immediately and the PVP-AuNP solutions were stored at 4 °C for further use. The ζ-potentials of 5 nm and 10 nm AuNPs were changed from strong minus to slightly minus in Table 4. The DLS data also showed difference before after ligand-exchange, and PVP coated AuNP showed smaller size (no data shown). In addition, the UV-VIS spectrum indicates this ligand exchange did not cause any aggregation in Figure 39.

Table 4. ζ-potentials of citrate capped and PVP capped AuNPs

<table>
<thead>
<tr>
<th></th>
<th>5 nm</th>
<th>10 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Citrate-capped AuNPs</strong></td>
<td>Mean -24.6</td>
<td>Mean -9.3</td>
</tr>
<tr>
<td></td>
<td>Std Dev 3.11</td>
<td>Std Dev 0.894</td>
</tr>
<tr>
<td><strong>PVP capped AuNPs</strong></td>
<td>Mean -7.23</td>
<td>Mean -0.473</td>
</tr>
<tr>
<td></td>
<td>Std Dev 0.725</td>
<td>Std Dev 0.036</td>
</tr>
</tbody>
</table>
6.3.2 Synthesis of PVP-AgNPs

Next, PVP capped AgNPs (PVP-AgNPs) were synthesized. Silver nanoparticles are not stable and undergo surface oxidation and Ag\(^+\) ion dissolution. Shielding the nanoparticle surface from oxidation and Ag\(^+\) ion release is difficult because most coatings do not completely protect the AgNPs surface, providing no long-term stability in the environmental and biological environments that cause toxicity. At the moment, the most robust method for increasing the long-term stability of AgNPs is to coat them with covalent ligands such as polyvinylpyrrolidone (PVP). There has been no report to make smaller PVP-AgNPs (3 to 10 nm). Thus, this report developed a new synthesis method of smaller stable PVP-AgNPs. The reducing agent solution (3 mM NaBH\(_4\)) was
prepared freshly on ice and used within 1 min. Next, 5 mL of 0.5mM AgNO₃ solution including 1 mM PVP and freshly prepared 5mL reducing agent solution were mixed using two 1 mL pipettes. An unused small glass container or well-rinsed glass beaker was used as a reaction vessel for each synthesis. The stirring speed was kept constant at 300 rpm. The solution is stored at 4 °C under dark for further use. If DLS shows some aggregation above 100 nm, the solution must be centrifuged or filtrated before storage, or they will likely aggregate in one week. Additionally, adding Cl⁻ ion to the PVP-AgNPs solution to be 2 mM Cl⁻ ion contributed to the stability of the PVP-AgNPs.

The PVP-AgNPs intensity of UV kept no difference for two months at least (more comprehensive analysis was not performed). Also, pH must be between 5 to 10.5 during the nBLOCK process for their stability. The AgNPs were analyzed by UV-VIS, z-potential, DLS, and TEM. The PVP-AgNPs have a slightly minus charge compared to naked AgNPs (in the synthesis, no presence of PVP). The size of PVP-AgNPs showed a mean of 14.44 (PDI=0.339) and was confirmed by the TEM image in Figures 40 and 41. Figure 42 showed the UV-VIS of PVP-AgNPs (λ_max=415) and did not show any decrease of intensity and aggregation for two months by adding Cl⁻ ion (2 mM NaCl). The PVP-AgNPs could also be freeze-dried for long-term storage.

Table 5. z-potentials of PVP capped AgNPs and naked AgNPs

<table>
<thead>
<tr>
<th>PVP capped AgNPs</th>
<th>Mean</th>
<th>-2.44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std Dev</td>
<td>0.165</td>
<td></td>
</tr>
<tr>
<td>Naked AgNPs</td>
<td>Mean</td>
<td>-19.6</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>
Figure 40. TEM images of PVP-AgNPs (scale bar is 100 nm)

Figure 41. Size distribution of PVP-AgNPs from TEM images (n=50)
6.3.3 Synthesis of PVP-CuNPs

PVP-CuNPs was synthesized with modification based on the reported method. In a round bottom flask, 200 mL of ddH$_2$O, 0.5 mM of PVP (Mw 40 K), and 0.2 mM of CuSO$_4$ were dissolved. After they were melted, ascorbic acid (4 mM) was added to the flask. The reaction flask was heated at 80 °C under mechanical stirring at 300 rpm. The reaction time did not affect the size of PVP-Cu from 8 hour to 1 day (no data shown here). After the reaction, you can concentrate the solution by freeze-dry or rotary evaporator under vacuum. UV-VIS spectrum ($\lambda_{max}$=298) showed CuNPs formation in Figure 43. About DLS, the size can be not accurate with diluted CuNPs solution. It showed bigger around 10 nm by DLS while concentrated solution by DLS showed 5 nm in Figure 44. The $\zeta$ potential of PVP-CuNPs is 1.43 (Std=0.226).
Figure 43. UV-VIS of PVP-CuNPs.

Figure 44. The size of PVP-CuNPs in different concentration by DLS measurement.
6.3.4 Synthesis of PVP-Fe₃O₄

PVP-Fe₃O₄ NPs were synthesized with modification based on the reported method. The rxn was performed strictly under N₂. 1 mmol of acetylacetonate, 50 mg of PVP (40K), and 5 mmol 1,2-hexanedecandiol were added to degassed 2mL of octyl ether in three-neck round bottom flask. The rxn solution was physically stirred. After confirming the PVP was dissolved in the solution, the mixtures were slowly heated to 120 °C in 1 hour and kept for 10 min. Afterward, the temperature was rapidly raised to 230 °C and refluxed for 1 hour. The black nanoparticles were precipitated and centrifuged to remove the solvent and dispersed in hexane (red to black). It is slightly soluble in ethanol but not in water very well because of 1,2-hexanedecandiol. It made a milky solution in ethanol and did not give clear UV-VIS spectra at that point. Successfully synthesized PVP-Fe₃O₄ should move toward a strong magnet (Figure 45). TEM was analyzed in Figure 46, and the mean of the size was 8.7 nm (Std 2.9 nm).

Figure 45. The magnetic analysis of PVP-Fe₃O₄ solution on a strong magnet.
6.3.5 Synthesis of Silica-gel-NTA

Synthesizing a strongly minus charged silica-gel for the electrostatic interaction with the DNA’s amine group was a key to synthesize DNA building block. By modifying NH₂-silica-gel with nitrilotriacetic acid (NTA) in Scheme 4 or citric acid, synthesized COOH-silica-gel had more carboxylate group than commercially available COOH-silica-gel.

NH₂-silica-gel (2 g), and NTA (1.17 g) were dispersed in 200 mL pyridine in a 500 mL round-bottom flask. The mixture was rotated at 400 rpm in an oil bath at 90 °C with a reflux system for four nights. After it cooled to room temperature, dH₂O, ethanol, and dichloromethane (DCM) were used to rinse the particles twice, respectively. The products were dried at room temperature for 24 h and then stored in a covered bottle before used. A surface charge of synthesized COOH-silica-gel was measured by DLS in ddH₂O and boric buffer (pH 7.4). The reaction time was optimized for four days. The majority of amine on the NH₂-silica-gel needs to react with NTA since NH₂-silical-gel charges positive in a buffer (pH=7) and is reactive with minus charged NPs and DNA’s...
phosphonate group. To bind one DNA to one NP accurately, the synthesized COOH-silica-gel should not have any positive charge on the surface.

Scheme 4. The reaction of NH$_2$-silica-gel and NTA (it can also be citric acid)

**Figure 47** (bottom-line) shows ζ-potential of NH$_2$-silica-gel (starting material), commercially available COOH-silica-gel, Citrate-silica-gel (synthesized between NH$_2$-silica-gel and citric acid), and NTA-silica-gel (synthesized between NH$_2$-silica-gel and NTA) in boric buffer, respectively.

![Diagram](image)

Figure 47. FTIR of silica-gel and ζ-potential of silica-gel in boric acid

In addition, FTIR was analyzed to check the amount of binding carboxylic acid to silica-gel in **Figure 47** (upper-line). The broad peak at 3300 cm$^{-1}$ indicated COOH, and commercially
available COOH-silica-gel showed that it did not have enough COOH on the surface of silica-gel. The commercially available COOH-silica-gel did not work well for the electrostatic reaction. Therefore, a new COOH-silica-gel was synthesized. The synthesized COOH-silica-gel from the reaction between citric acid and NH₂-silica-gel also worked. However, it could cause the decarboxylation step while heating up, and there were no convenient methods to check the reaction process such as TLC, so I chose NTA instead of citric acid at the end. Finally, NTA-silica-gel was mixed with minus-charged AuNPs (PVP-AuNPs or dmAuNP) to confirm if NTA-silica-gel did not react with NPs without DNA, Figure 48 showed amine-silica-gel reacted with AuNPs and became red while successfully synthesized NTA-silica-gel did not interact with dmAuNP or PVP-AuNPs.

![Figure 48. Pictures of NTA-silica-gel and Amine-silica-gel mixed with AuNPs](image)

**6.3.6 “One step at a time” DNA conjugation**

A DNA oligonucleotide (P1) and its complementary sequence (P1c) with chemically modified 3’ and 5’ ends was designed in previous study and purchased from Integrated DNA Technologies. The reported DNA functionalization technique was used with some modification and optimization.
The positively charged amine end of the DNA can electrostatically bind to the negatively charged surfaces of silica gel. Briefly, the reaction mixture consisted of DNA (0.01 µM) and NTA-silica-gel (5 mg) and was incubated overnight with gentle mixing. The DNA-modified NTA-silica-gel (DNA-silica-gel) was washed three times by centrifugation and resuspended in an optimized buffer or solvent. Either TCEP or DTT was used to activate disulfides to SH before adding MNPs. After MNP conjugated to SH of DNA, MNP-DNA-silica-gel was washed by centrifugation with a specific buffer or solvent. Finally, the electrostatically bound DNA was released from silica-gel by adding NaOH with gentle mixing for approximately 30 mins. This step effectively cleaved DNA from silica gel. The resultant monofunctionalized NP block with one DNA strand was neutralized and resuspended in 10 mM tris-HCl buffer (pH=7) for hybridization. The kinds of buffer and pH of NaOH were optimized for each MNP, respectively (Table 6).
Table 6. Optimized process of “one step at a time” DNA conjugation for each MNP

| STEPS | dmAuNPs | PVP-AuNPs | PVP-AgNPs | PVP-CuNPs | PVP-Fe$_3$O$_4$
|-------|---------|-----------|-----------|-----------|-----------
| DNA-Si | DNA in ddH$_2$O to 5 mg NTA-silica gel overnight rxn |
| WASH (T/M) | boric buffer (pH 7.4) second sequence is tris buffer | acetate buffer (pH 5) | 2 mM NaCl | acetate buffer (pH 5) | acetate buffer (pH 5) |
| DNA ACTIVATION | add TCEP to 100 µl acetate buffer | add TCEP to 100 µl 2 mM NaCl buffer | add TCEP to 100 µl acetate buffer | add TCEP to 100 µl acetate buffer |
| INCUBATE | 10 mins | 10 to 60 mins | 10 mins | 10 to 60 mins | 10 to 60 mins |
| WASH 3 TIMES | wash with boric buffer (2nd: tris) | no wash | wash with 2 mM NaCl | no wash | no wash |
| RESUSPEND IN BUFFER | 100 µL | - | 1 mL | - | - |
| ADD NPS | Add dmAuNPs | Add PVP-AuNPs | Add PVP-AgNPs + Aluminum foil | Add PVP-CuNPs (excess) | Add PVP-Fe$_3$O$_4$NPs (excess) |
| INCUBATE | 1 hour to over night | over night | 1 hour | over night | over night |
| STABILIZATION WITH MUA | - | - | add MUA 2.6 mM to make 1.3 mM MUA in a vial | - | - |
| WASH* | boric buffer (pH 7.4) (2nd: tris) | ddH$_2$O | 2 mM NaCl | ddH$_2$O | ddH$_2$O |
| ADD EACH WASH* SOLUTION ADD NAOH | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| NUTRALIZATION AND UV | 40 mM 100 µl | 100 mM 100 µl | 2 mM NaCl pH 10.5 NaOH 100 µl | 100 mM 100 µl | 100 mM 100 µl |
| HYBRIDIZATION | 10 mM Tris HCl (pH 7.4) buffer for 1 hour at rt |
| COLLECT DIMERS | Centrifuge and collect only dimers by 13 K for 15 mins |

MUA*: Mercaptoundecanoic acid, used for stabilizing AgNPs. MUA-AgNPs-DNA was generated. The molarity of AgNPs was referred by an intensity of UV-VIS (c=A$_{max}$/ε). The molarity of AuNPs was determined using the UV-VIS intensity (c=A$_{450}$/ε$_{450}$).
6.4 Material and Instruments

The following chemicals were purchased and utilized without any further purification. 40K polyvinylpyrrolidone (PVP) was purchased by CALBIOCHEM. AgNO₃ was purchased by AMRESCO. CuSO₄ (98%) was purchased by STREM CHEMICALS. Si-Carboxylic Acid (COOH-silica-gel) and Si-Amine (Amine-silica-gel) were purchased by SILICYCLE. NaBH₄ (98%), 11-Mercapto-undecanoci acid (95%) were purchased by Aldrich. Dried pyridine was purchased by EMD. Nitrilotriacetic acid (NTA) was purchased by BTC. Citric acid anhydrous was purchased by SIGMA. 0.5 M Tris-HCl Buffer pH 6.7 was purchased by BIORAD 1,2-hexandecandiol, tris(2,4-pentandionato) iron (III) and n-octyl ether were purchased by TCI.

Transmission electron microscopy (TEM) was carried out on JEOL 1011. UV-VIS spectra were obtained on Thermo Scientific Nanodrop 2000c spectrophotometer. Zeta-potential and DLS were carried out on Nano-ZS Zetasizer (Malvern Instruments Ltd., Worcestershire, UK).

DNA Sequences

<table>
<thead>
<tr>
<th>P1: DNA Template (5'&gt;3')</th>
<th>/5ThioMC6-D/ aacgaccttcagtagtag /3AmMO/</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1c: DNA Complement (5'&gt;3')</td>
<td>/5ThioMC6-D/catactctagaagttcgtt/3AmMO/</td>
</tr>
</tbody>
</table>

6.5 Conclusion and Future Challenges

This work highlights efforts made towards the construction of 1D and 2D multifunctional structures incorporating DNA-linked nanoparticle building blocks. The study expanded efforts in the DNA computation method and DNA-guided self-assembly to include other MNPs, CuNPs, AgNPs, and Fe₃O₄NPs. The major advancement of this work over the previously described efforts is the improvement of stability and reproducibility. The ability to coat all of the MNPs described with PVP capping ligands while maintaining the DNA-SH binding activity enables the MNPs to
be stored in powder form without freeze-drying. Various combinations of MNPs organized into 2D structures reveal unusual physiochemical properties due to LSPR. The practical study of these LSPRs is the first time data has been collected on such materials and prior investigations had been computational. These investigations reveal that the two-dimensional constructions of AuNP-CuNP, AuNP-AgNP, and AuNP-Fe₃O₄NP dimers resulted in AuNPs having a red-shifted absorbance. One interesting trend that was noted is that the rate of NP binding to DNA on the silica-gel decreases as the size of the NPs increased however, the NPs sizes of (3, 5 and 10 nm) were all able to form DNA-AuNP dimers. By optimizing the amount of PVP ligand on the AuNPs of various sizes, high yields of DNA-linked AuNP building blocks of approximately 80 to 100% were obtained when the amount of attached AuNP was set to 100%. These successfully monofunctionalized anisotropic DNA-NP building blocks and LSPR physical data will serve as the foundation for programmable three-dimensional DNA-NP structures. This study expanded the possibility of multifunctional architecture and improved our understanding of its physicochemical properties for use in a variety of imaging and sensing modalities.
6.6 Reference


Chapter -VII- Over All Conclusion

In this dissertation, self-assembly nanoparticles were designed for photodynamic therapy and bioimaging agents. Three kinds of MOFs were developed for multifunctional nanomedicines. By utilizing maltotriose, two types of bioconjugation methods were developed to target cancer cell lines. Maltotriionate modified MOFs showed a better targeting ability than EDA-maltotriose since maltotrionate could bind to the other molecules by hydrogen bonding. Furthermore, the physiological data of MOFs were correlative to the targeting ability and PDT effect. These three different metals consisting MOFs showed low toxicity; however, of Hf, Zr, and Fe, Fe showed the most toxicity. Thus, it concluded its difficulty of developing an MRI agent. These porphyrin-based MOFs were, however, active for fluorescence imaging. For further study of self-assembly nanoparticles for bioimaging, the quantum study of MNPs was required. DNA technique for nBLOCK was optimized for Ag, Au, Cu, and Iron oxides in this study. Those metals were selected as good bioimaging candidates. By making the bimetal dimers, the LSPR between different MNP were observed. This physical data would help understand and develop the potentials of DNA-based multifunctional architecture and its physicochemical properties for use in various imaging and sensing modalities.