Hybrid Organic-Inorganic Bridged Silsesquioxane Nanoparticles for Cancer Nanomedicine

Dissertation by

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In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

King Abdullah University of Science and Technology, Thuwal,
Kingdom of Saudi Arabia

October, 2017
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ABSTRACT

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It is well established that cancer is one of the leading causes of death globally. Its complete eradication requires early detection and intensive drug treatment. In many cases it might also require surgery. Unfortunately, current medicine is still more focused on cancer treatment rather than elimination of its reason. The mechanism of tumor emergence and development is quite complicated, although, we are constantly advancing in this field.

Nanomedicine is envisioned as the silver bullet against cancer. Thus, nanoscale systems with therapeutic and diagnostic modalities can simultaneously perform several functions: accurate detection of tumor site, precise targeting, and controlled drug release inside abnormal cells and tissues while being nontoxic to healthy ones. Moreover, surface modification of such nanoparticles allows them to be invisible to the immune system and have longer blood circulating time.

The performed research in this dissertation is completely based on hybrid organic-inorganic bridged silsesquioxane (also known as organosilica) nanomaterials, therefore comprising “soft” organic/bioorganic part which can imitate certain biorelevant structures and facilitates successful escape from the immune system for more efficient accumulation in cancer cells, while “hard” inorganic part serves as a rigid and stable basis for the creation of cargo nanocarriers and imaging agents. This dissertation discusses the
critical points of safe biodegradable nanoplanformas, delivery of large biomolecules, and
cytotoxicity regarding the shape of nanoparticles. As a result novel fluorescent
biodegradable oxamide-based organosilica nanoparticles were developed, light-triggered
surface charge reversal for large biomolecule delivery was applied with hollow bridged
silsesquioxane nanomaterials, and biocompatibility of periodic mesoporous organosilicas
with different morphologies was studied. Furthermore, the current achievements and
future perspectives of mesoporous silica organosilica, and silsesquioxane nanoparticles
were considered in regards to their biomedical applications and summarized in two
reviews.
ACKNOWLEDGEMENTS

The very first people I would like to thank are the closest ones, my parents. Doing science does not promise you high salary and great perspectives in my country and generally around the world. Despite these things my parents have never regretted about my choice (and me too), they believe that knowledge is one of the most valuable and powerful things in the world. I sincerely appreciate their continuous support throughout these years.

I would like to thank my Ph.D. advisor, Prof. Niveen M. Khashab, for her genuine patience towards me. We frequently had different opinions on the same topics. However, I could always freely voice my thoughts without fear of punishment or prejudice. In science, it is absolutely normal to be wright or wrong. Her policy of laissez-faire allowed me to be an independent researcher with a great variety of things to work at. And, of course, I should mention the participation in Lindau Nobel Laureate Meeting 2017, which would be impossible without the nomination by Prof. Niveen M. Khashab.

Indeed, teaching and proper conveyance of knowledge to other people are subjects of significant importance. Therefore, I want to express gratitude to those colleagues and friends who successfully performed these tasks for the last four years of my Ph.D. pursuit: Dr. Khachatur Julfakyan introduced me to the working process in the lab, our collaboration resulted in my first publication during KAUST period, numerous scientific discussions, that we had, improved my deductive logic; Dr. Jonas Croissant gave me intensive training in sol-gel synthesis and developing of organosilica drug delivery systems, he is the one who taught me how to do both clear presentation and well-
structured publication; Dr. Valentina Carboni acquainted me with multiple synthetic techniques in organic chemistry and showed herself as dedicated teacher in many other subjects. Moreover, I should thank more than 60 members of Smart Hybrid Materials group whom I met during this time. I am sure I studied something from every one of them. KAUST faculty Prof. Valentin Rodionov, Prof. Luigi Cavallo, Prof. Kazuhiro Takanabe, and Prof. Omar F. Mohammed also made an important input to my background during chemistry courses.

My research publications would be impossible without the help of next people: Dr. Basem Moosa, Shahad Alsaiari, Kholod Alamoudi, Dr. Lin Deng, Dr. Dalaver H. Anjum, Dr. Andrei Gurinov, Dr. Abdul-Hamid Emwas, Haneen Omar, and Jie Lu. They demonstrated real devotion to solving scientific problems.

I appreciate committee members Prof. Niveen M. Khashab, Prof. Markus Antonietti, Prof. Mohamed Eddaoudi, and Prof. Jorge Gascon for their time investment and helpful suggestions during defense process.

I thank our group assistants Katerina Bartonova and Alena Synkova who supported me many times in dealing with bureaucratic issues.

Finally, last but not least, I am thankful to my friend on the other side of the world, Evgenii Grebennikov, who introduced me to KAUST and helped a lot with an application process.

KAUST is an excellent place to be focused solely on research. It removes the burden of constant domestic problems like in outside world. I truly enjoyed the opportunity to stay here and do what I like, do science.
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LIST OF ABBREVIATIONS

Chapter 1

BSSO bridged silsesquioxane
BTSE 1,2-bis(triethoxysilyl)ethane
CAPIR circulation – accumulation – penetration – internalization – release
COSSO cube-octameric silsesquioxane
CTAB cetyltrimethylammonium bromide
DMF dimethylformamide
EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EPR enhanced permeability and retention
FDA US Food and Drug Administration
GNR gold nanorod
ISO/TC International Organization for Standardization/ Technical Committee
LPMO large-pore mesoporous organosilica
MOF metal-organic framework
nab nanoparticle albumin-bound
NHS N-hydroxysuccinimide
NP nanoparticle
PLGA-PEG poly(lactic-co-glycolic acid)-b-poly(ethylene glycol)
POM polyoxometalate
PCOSSO polymeric cube-octameric silsesquioxanes
PRINT particle replication in the non-wetting template
siRNA small interfering RNA
TEOS tetraethoxysilane

Chapter 2

CMC critical micellar concentration
Cpt camptothecin
DOX doxorubicin
GM Goeppert-Mayer units of the molecular two-photon cross-section
GOx glucose oxidase
GSH glutathione
HIFU high intensity focused ultrasound
H-MONs core-shell hierarchical MSN@MON particles
HMONs hollow mesoporous organosilica nanoparticles
HPMO hollow periodic mesoporous organosilica
L-Arg L-Arginine
<table>
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<tr>
<td>MDR</td>
<td>multidrug resistance</td>
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<td>MONs</td>
<td>mesoporous organosilica nanoparticles</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MSNs</td>
<td>mesoporous silica nanoparticles</td>
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<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
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<tr>
<td>NIR</td>
<td>near-infrared</td>
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<tr>
<td>ONs</td>
<td>organosilica nanoparticles</td>
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<tr>
<td>ORMOSIL</td>
<td>organically modified silica</td>
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<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
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<td>PEI</td>
<td>polyethylenimine</td>
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<td>P-gp</td>
<td>P-glycoproteins</td>
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<td>PMO</td>
<td>periodic mesoporous organosilica</td>
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<td>SBF</td>
<td>simulated body fluid</td>
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<td>SDAs</td>
<td>structure directing agents</td>
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<td>SERS</td>
<td>surface enhanced Raman scattering</td>
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<td>SSO</td>
<td>silsesquioxane</td>
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<td>UV-Vis</td>
<td>ultraviolet–visible</td>
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**Chapter 3**

<table>
<thead>
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<th>Acronym</th>
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<td>APTES</td>
<td>3-aminopropyltriethoxysilane</td>
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<tr>
<td>BET theory</td>
<td>Brunauer–Emmett–Teller theory</td>
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<td>CCK-8</td>
<td>cell counting kit-8</td>
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<tr>
<td>CLSM</td>
<td>confocal laser scanning microscopy</td>
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<tr>
<td>CP/MAS</td>
<td>cross-polarization/magic angle spinning</td>
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<tr>
<td>DAPI</td>
<td>4,6-diamino-2-phenylindole</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<td>DLS</td>
<td>dynamic light scattering</td>
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<tr>
<td>DPBS</td>
<td>Dulbecco’s phosphate buffered saline</td>
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<td>EELS</td>
<td>electron energy-loss spectroscopy</td>
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<tr>
<td>EMEM</td>
<td>Eagle’s minimum essential medium</td>
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<td>ESI</td>
<td>electrospray ionization</td>
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<td>EtOH</td>
<td>ethanol</td>
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<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
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<td>FITC</td>
<td>fluorescein 5(6)-isothiocyanate</td>
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<tr>
<td>FTIR</td>
<td>Fourier-transform infrared</td>
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<td>HAADF</td>
<td>high-angle annular dark-field</td>
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<td>HPDEC</td>
<td>high powered decoupling</td>
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<tr>
<td>HRTEM</td>
<td>high-resolution TEM</td>
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<tr>
<td>LTQ</td>
<td>linear trap quadrupole</td>
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MS  mass spectrometry
NMR  nuclear magnetic resonance
OBA  oxamide-bridged alkoxysilane
PBAE  poly(β-amino ester)
PLA  polylactic acid
SEM  scanning electron microscopy
ssNMR  solid state NMR
STEM  scanning transmission electron microscopy
TEA  trimethylamine
TEM  transition electron microscopy
TGA  thermal gravimetric analysis
TMS  tetramethylsilane

Chapter 4
DIPEA  N,N-diisopropylethylamine
DTG  derivative of the thermogravimetric curve
GFP  green fluorescent protein
PBA  photoresponsive bridged alkoxysilane
PDI  polydispersity in DLS analyses
PDT  photodynamic therapy
pDNA  plasmid DNA
Rho B  rhodamine B
SI  spectrum imaging
ssDNA  single stranded DNA

Chapter 5
BJH method  Barrett-Joyner-Halenda method
BR-PMO  PMO bended nanorods
DMSO  dimethylsulfoxide
FPMO  fluorescent PMO
IC$_{50}$  half maximal inhibitory concentration
IUPAC  International Union of Pure and Applied Chemistry
R-PMO  PMO nanorods
S-PMO  PMO nanospheres
THF  tetrahydrofuran
W-PMO  PMO nanowires
XRD  X-ray diffraction
LIST OF ILLUSTRATIONS

[*]References are omitted

Chapter 1

Figure 1.1. All hybrid nanomaterials can be divided into three groups: hybrids with separated localized organic and inorganic components like dumbbell and Janus NPs (A1), core-shell nanostructures (A2), yolk and core-satellite NPs (A3 and A4 respectively), as well multiple-core nanoarchitectures (A5); hybrids with separated continuing phases, e.g. derivatives of layered double hydroxides (B); and nanomaterials with hybridization at the molecular level, for example, bridged silsesquioxane (BSSO) NPs (C1), polymer NPs based on polyoctameric silsesquioxane (POSSO) (C2), cluster-based hybrids (C3), and metal-organic frameworks (MOFs) (C4). Note: the figure does not fully represent all studied hybrid nanomaterials.

Figure 1.2. Hybrid nanomaterials attract the interests of both academic and industrial sectors.

Figure 1.3. Schematic representation of the chemical routes for the synthesis of hybrid organic-inorganic nanomaterials. Adapted with permission.[*] Copyright 2011, the Royal Society of Chemistry.

Figure 1.4. CAPIR cascade of nanomedicine includes five steps: circulation in the blood compartments, tumor accumulation through the enhanced permeability and retention (EPR) effect, penetration, and subsequent cellular internalization and intracellular drug release. Reproduced with permission.[*] Copyright 2014, Wiley-VCH.

Figure 1.5. The timeline of key achievements in the cancer nanomedicine.[*] FDA: US Food and Drug Administration; nab: nanoparticle albumin-bound; PLGA-PEG: poly(lactic-co-glycolic acid)-b-poly(ethylene glycol); PRINT: particle replication in non-wetting template; siRNA: small interfering RNA. Adapted with permission.[*] Copyright 2017, Nature Publishing Group.

Figure 1.6. Current synthetic strategies toward novel hybrid organic-inorganic nanomaterials for cancer treatment: nanoscale MOF for combined photodynamic and radiation therapy (DMF stands for dimethylformamide) (A), gold nanorod (GNR) embedded into large-pore mesoporous organosilica (LPMO) nanospheres for gene and photothermal cooperative therapy (CTAB stands for cetyltrimethylammonium bromide, BTSE – 1,2-bis(triethoxysilyl)-
ethane, TEOS – tetraethoxysilane, PEI – polyethylenimine) (B), and crosslinked POSSO-nanohybrids for imaging-guided photodynamic therapy (NHS stands for N-hydroxysuccinimide, EDC – 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) (C). Adapted with permission.[*] Copyright 2016, Elsevier (A). Adapted with permission.[*] Copyright 2017, the Royal Society of Chemistry (B). Adapted with permission.[*] Copyright 2017, the Royal Society of Chemistry (C).

Chapter 2

Figure 2.1. Detailed statistics on the number of publications in the area of nonporous (A) and porous (B) hybrid ONs versus MSNs (C). The performed data evaluate numbers of publications in all fields, biomedical fields, and animal studies from 2007 to 2017. Only research articles are considered, no reviews. Data received from the Scopus database (the last update 01/10/17). The simplified illustration unites various organosilica NPs under the one synthetic scheme (D).

Figure 2.2. The extensive classification of hybrid organic-inorganic siliceous nanomaterials with the focus on chemical composition, morphology, and synthetic strategy. A: T<sub>8</sub>-SSO, B: T<sub>12</sub>-SSO, C: crystal-like SSO NPs, D: polymeric SSO NPs or ONs (?), E+F: MONs or PMO NPs (periodic mesoporous BSSO NPs), G+H: nonporous BSSO NPs, I+K+M+O: nonporous ONs, J+L+N+P: MONs, Q: nonporous ONs, also ORMOSIL, R: vesicular ONs, also cerasomes.

Figure 2.3. The hydrolysis and condensation of TEOS (A) and bridged organoalkoxysilane (B) in the sol-gel process lead to the formation of silica and organosilica materials, respectively.

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Figure 2.5. Representation of the morphologies (left side) and chemical compositions (right side) of various nonporous ONs and BSSO NPs (with molar percentages in NPs) for biomedical applications. The particle biomedical applications are indicated in the upper-right corners (e.g. “CD” for cargo delivery, see legend at the bottom). Adapted with permissions from American
Figure 2.6A. Various chemical compositions of MONs and PMO NPs for biomedical applications. The percentage of components is represented in regard to their molar ratios inside nanoparticles. In the upper-right corner of every cell the biomedical application is shown (e.g. “CD” for cargo delivery, see legend at the bottom). PB: Prussian blue, MNP: magnetic NP, UCNP: upconversion NP, ND: nanodiamond. Composition of inorganic core: PB: K₄[Fe(CN)₆], MNP1: Fe₃O₄, MNP2: BaFe₁₂O₁₉, UCNP1: NaGdF₄:Yb, Tm@NaGdF₄, UCNP2: NaGdF₄:Yb,Er.

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Figure 2.8. Redox-mediated degradation of disulfide- (A–F), and tetrasulfide-based ONs (G, H); hypoxia-triggered disintegration of ONs (I–J); trypsin-cleavable nonporous oxamide- (K–L) and tri-L-lysine-BSSO NPs (M–N) for controlled drug deliveries; pH-mediated degradation of prodrug NPs with covalently encapsulated doxorubicin via hydrazone bonds (O–P). The arrows indicate the addition of different triggers: glutathione (A–B, E–H), mercaptoethanol (C–D), cytochrome P450 reductase (I–J), trypsin (K–N), and acidic medium with pH5 (O–P). Adapted with permissions from American Chemical Society, Royal Society of Chemistry, and Wiley.

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**Figure 2.11.** In vivo applications of therapeutic nanovectors based on ONs: combinational anticancer therapy (A),[*] delivery of gaseous transmitters (B),[*] fluorescent imaging (C),[*] thermal imaging (D),[*] MR imaging (E),[*] and ultrasound imaging (F).[*] Adapted with permissions from American Chemical Society,[*] Royal Society of Chemistry,[*] Elsevier,[*] and Wiley.[*]

**Chapter 3**

**Figure 3.1.** Schematic representation of the synthesis and enzymatic degradation of BSSO NPs via the cleavage of amide bonds.

**Figure 3.2.** Synthetic pathway toward the OBA precursor.

**Figure 3.3.** $^1$H (A) and $^{13}$C (B) NMR spectra of the OBA precursor. $^1$H-NMR: (CDCl$_3$, 500 MHz) $\delta$ (ppm) 7.57 (s, J = Hz, 2H), 3.80 (q, J = 7 Hz, 12H), 3.29 (t, J = 6.7 Hz, 4H), 1.66 (m, J = 7.7 Hz, 4H), 1.20 (t, J = 7.1 Hz, 18H), 0.63 (t, J = 8.3 Hz, 4H). $^{13}$C-NMR: (CDCl$_3$, 125 MHz) $\delta$ (ppm) 159.9, 58.6, 42.1, 22.9, 18.3, 7.8.

**Figure 3.4.** FTIR spectrum of the OBA precursor. FTIR (cm$^{-1}$): 3317, 3017, 2973, 2936, 2895, 1668, 1522, 1449, 1371, 1276, 1203, 1110, 950, 783, 703, 572, 481.

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**Figure 3.7.** Typical background subtracted electron energy-loss spectrum of BSSO NPs (A). STEM-EELS elemental mapping (silicon, oxygen, nitrogen, carbon, sulfur) of a representative BSSO and BSSO-FITC NPs (B and C, respectively). Scale bar of 50 nm. Comparison of the sulfur signal in the BSSO-FITC and BSSO NPs demonstrating the homogeneous FITC dye incorporation in BSSO-FITC NP (D). *Asterisks encompass the diameter of NP.

**Figure 3.8.** FTIR spectra for the OBA precursor, BSSO, and BSSO-FITC NPs.

**Figure 3.9.** ssNMR of $^{29}$Si and $^{13}$C nuclei via CP/MAS and HPDEC sequence in BSSO and BSSO-FITC NPs (A and B, respectively). In the carbon spectrum of BSSO NPs, number indicate the followings: 1: Si-OCH$_2$CH$_3$, 2: HOCH$_2$CH$_3$, 3: CTAB residues. In the carbon spectrum of BSSO-FITC NPs, asterisks indicate Si-
OCH$_2$CH$_3$ and CTAB residues. FITC peaks are most likely in the noise of the spectrum because of the low content of the incorporated dye, of which the presence is unambiguously demonstrated by UV-Visible spectroscopy (Figure 3.15) and in vitro imaging (Figure 3.17).

**Figure 3.10.** TGA data for BSSO (A) and BSSO-FITC NPs (B).

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**Figure 3.18.** CLSM image (3D) of HeLa cells after incubation with BSSO-FITC NPs displaying the partial endocytosis and cell adhesion of BSSO-FITC NPs after 6 h of incubation.

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**Chapter 4**

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Chapter 1: Introduction

1.1 Significance of the Research

The crucial importance of a medicine as a life-saving science and practice was realized by humans many thousands of years ago. Back in that times, first doctors used plants, soils, and clays as primitive forms of healing agents. This medicinal knowledge was passed and accumulated from generation to generation resulting in the foundation of modern medicine. Although the first findings were done by trial and error, we are still getting benefits from it. The twentieth century revealed new paradigms and techniques for drug discovery. Thus, groundbreaking discoveries in human biology and chemistry, determination of structure-function properties of chemical compounds and automated drug screening was all made possible.

Despite the fact that we have gotten smarter in combating diseases, nature showed us “ace up its sleeve” especially when considering multidrug resistance. Therefore, nowadays it is not enough to provide a drug just with a single therapeutic property and sufficient concentration.

After ischemic heart disease and stroke, cancer is the second leading cause of death globally (according to the World Health Organization, 2015).1 It requires early detection, targeted delivery, controlled drug release, and minimum side effects for effective cancer eradication.2-5 Moreover, recent results prompt to speak about personalized treatment rather than a general one with an attachment to the individual genome.6-7

To tackle aforementioned problems, nanomedicine was born. Its diverse portfolio of nanoscale systems (1 – 200 nm) with therapeutic and diagnostic modalities can
simultaneously perform several functions: accurate detection of tumor site, precise targeting, and controlled drug release inside abnormal cells and tissues while being nontoxic to healthy ones. Moreover, surface modification of such nanoparticles allows them to be invisible to the immune system and have longer blood circulating time. Importantly to note, that nanomedicine is a relatively young field with a lot of questions asked, and a lot more to answer, but it is still one of the fastest growing research areas with a number of promising applications.

The research work in this dissertation is aimed to address: 1) the critical issue of nanoparticle biodegradation and solve it with a nature-inspired approach, 2) the light-triggered surface charge reversal for large biomolecule delivery, 3) the development of a new synthetic procedure for the nanoparticles with different morphologies to tune toxicity and, finally, 4) the construction of supramolecular nanosystems for efficient cell penetration and drug delivery. The performed research is based on hybrid organic-inorganic bridged silsesquioxane nanomaterials, therefore comprising the best of both worlds: the rigid inorganic silica framework and multifunctional organic moieties.\textsuperscript{8-12} It is an excellent contribution to the global nanomedicine rush with two main focuses on safety and designed synthesis of nanoparticles. Indeed, all designed systems encompass only one type of hybrid materials, nevertheless, same strategies can be realized in a large number of other nanostructures.
1.2 What are Hybrid Nanomaterials?

1.2.1 The Edge of “Nano”. According to a famous Chinese proverb, “A journey of a thousand miles begins with a single step”, for us this single step will be the definition of two widely used scientific terms: nanoscale and nanomaterial. Technical Committee (TC) 229 of the International Organization for Standardization (ISO) is giving next definitions:

- **nanoscale**: size range from approximately 1 nm to 100 nm (1 nm = 10^{-9} m);
- **nanomaterial**: material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale;

Although ISO/TC determines nanoparticle (NP) as nano-object with all three external dimensions in the nanoscale, it is not typical for majority scientific publications where the same term usually covers nanosphere, nanorod, nanofiber and other morphologies.

The uniqueness of nanomaterials is coming from the fact that their physical and chemical properties can’t be evaluated by simple extrapolations from a larger size. Usually, but not exclusively, such phenomena are observing in the size range from approximately 1 nm to 100 nm. However, in the case of nanomedicine, this range should be extended up to 200 nm following the recent analysis of nanoparticle delivery to tumors. Thus, it was shown that more than 80% of publications in the field during the period 2005 – 2015 were conducted with particles within the size limits 1 – 200 nm. Therefore, further on any material with at least one external dimension 1 – 200 nm will be considered as a nanomaterial.
1.2.2 Classification of Hybrid Nanomaterials. There is a sort of conceptual similarity between “nanocomposite” and “hybrid nanomaterial”. Therefore, it is important to make a separate line between the two of them. Both types include different constituents in their structures. However, the nanocomposite is a physical mixture of nanoscale dispersion and micron level matrix, with minimal interactions between individual parts. On the other hand, hybrid nanomaterial, which demonstrates chemical bonding between its nanoscale and/or molecular level constituents, exhibits new properties not necessarily found in the individual components and usually prepared by chemical reactions. Nanko developed the classification of hybrid materials\textsuperscript{15} as listed below, although it may not generally reflect all fields of chemistry:

A) structurally-hybridized materials;

B) materials hybridized in chemical bond;

C) functionally-hybridized materials;

Gomez-Romero, Sanchez, and Kickelbick divided hybrids based on the type of chemical bonding between their parts. Class I consists of hybrids with weak electrostatic interactions, van der Waals or hydrogen bonding, while class II comprises of hybrids with strong chemical bonding between its components.\textsuperscript{16-18}

Probably, there is no perfect way to classify hybrid NPs, nevertheless previously described categorizations can be combined and complemented considering phase separations (Figure 1.1): A) hybrids with separated localized organic and inorganic phases (Janus, yolk, core-shell NPs, etc.); B) hybrids with separated continuing phases; C) and nanomaterials without phase separation, hybridization is observed at the molecular level.
Figure 1.1. All hybrid nanomaterials can be divided into three groups: hybrids with separated localized organic and inorganic components like dumbbell and Janus NPs (A1), core-shell nanostructures (A2), yolk and core-satellite NPs (A3 and A4 respectively), as well multiple-core nanoarchitectures (A5); hybrids with separated continuing phases, e.g. derivatives of layered double hydroxides (B); and nanomaterials with hybridization at the molecular level, for example, bridged silsesquioxane (BSSO) NPs (C1), polymer NPs based on polymeric cube-octameric silsesquioxanes (PCOSSO) (C2), cluster-based hybrids (C3), and metal-organic frameworks (MOFs) (C4). Note: the figure does not fully represent all studied hybrid nanomaterials.
1.2.3 The Current State of Things. The idea to integrate both organic and inorganic parts in one material has created an immense number of opportunities for research and application. Although we are not the first one in this area, natural selection spent billions of years to endow certain bio-inorganic hybrid materials such as teeth, bones, ivory, and shells with optimal durability, mechanical properties, density, etc.\textsuperscript{19} Nowadays man-made hybrid materials offer a wide spectrum of functional properties and gradually consolidate their positions in diverse academic and industrial domains such as energy,\textsuperscript{20} electronics,\textsuperscript{21} photonics,\textsuperscript{22} catalysis,\textsuperscript{23} health,\textsuperscript{24} and many more (Figure 1.2).\textsuperscript{25-32}

Speaking of general chemistry of hybrids, usually, the inorganic part is represented with metal oxide NPs, metal-oxo polymers, or metal-/nonmetal-oxo clusters like cube-octameric silsesquioxanes (COSSOs) and polyoxometalates (POMs). Both organic monomers and macromolecules can be part of the hybrid network, incorporated via sol-gel polymerization, solvo- or hydrothermal growth, self-assembly and other synthetic techniques. Over the last decades, various chemical approaches were developed to tailor complex hybrid nanosystems and set in advance their size, composition, functionality, and morphology (Figure 1.3). Thus, three main chemical paths are used to generate hybrids:\textsuperscript{33} A) sol-gel chemistry and hydrothermal synthesis; B) the hybridization of well-defined nanobuilding blocks; C) self-assembly of amphiphilic molecules or polymers with further polycondensation. Moreover, advanced methods as electrospinning, dip-pen lithography, ink-jet, nano-imprinting, and micro-molding are currently exploited to elevate the complexity of novel hybrid materials and introduce them to various fields of life associated with sustainable development, medicine, housing, automatics, etc.
Figure 1.2. Hybrid nanomaterials attract the interests of both academic and industrial sectors.

Figure 1.3. Schematic representation of the chemical routes for the synthesis of hybrid organic-inorganic nanomaterials. Adapted with permission.© Copyright 2011, the Royal Society of Chemistry.
1.3 The Ascent of Nanomedicine

Nanoscale materials have evoked “scientific tsunami” in the academic and industrial communities, and the field of biomedical applications is no exception. The unique ability to control physicochemical properties and outcome of the NPs in predetermined biorelevant conditions upraised nanomedicine to the tops of cancer research. Furthermore, bottom-up chemistry makes possible to realize both imaging and drug delivery modalities within single nanosystem, so called theranostic nanosystem.

The effectiveness of cancer nanomedicine is determined by successful accomplishment of CAPIR cascade: circulation in the blood compartments (C), accumulation in the tumor (A), subsequent penetration deep into the tumor tissue (P), internalization inside tumor cells (I), and finally intracellular drug release (R) (Figure 1.4). To maximize the overall drug delivery efficiency of NPs their chemical composition, inner volume, surface chemistry, size, and geometrical shape should be considered as a playground for improvement. Thus, stimuli-degradable NPs will fully release their drug in targeted site, porous and hollow NPs can transport large amounts of anticancer or therapeutic bioactive molecules, the pegylation of the surface will help NPs to avoid macrophage capture, sub-200 nm NPs can accumulate mainly in cancer cells, and the shape of NPs may facilitate the penetration into the tumor.

The necessity to be flexible regarding circumstances and 50 years old history of cancer nanomedicine (Figure 1.5) are expressed in an impressive arsenal of nanomaterials like liposomes, gold, magnetic, polymer, carbon, and mesoporous silica/organosilica NPs.
Figure 1.4. CAPIR cascade of nanomedicine includes five steps: circulation in the blood compartments, tumor accumulation through the enhanced permeability and retention (EPR) effect, penetration, and subsequent cellular internalization and intracellular drug release. Reproduced with permission. Copyright 2014, Wiley-VCH.

Figure 1.5. The timeline of key achievements in the cancer nanomedicine. FDA: US Food and Drug Administration; nab: nanoparticle albumin-bound; PLGA-PEG: poly(lactic-co-glycolic acid)-b-poly(ethylene glycol); PRINT: particle replication in the non-wetting template; siRNA: small interfering RNA. Adapted with permission. Copyright 2017, Nature Publishing Group.
1.4 New Powerful Weapon in Arms Race against Cancer

The story of the war between living organisms and cancer is an ancient battle. Rothschild’s team using X-ray analysis detected bone cancer in 70 million years old dinosaur vertebrae. He showed that those tumors were similar to human ones, demonstrating that cancer emerged in living beings ages ago.\textsuperscript{96}

Multiple mutations, multidrug resistance, and metastasis can fire back all our attempts to fully eradicate cancer, especially during late stages. Thus, novel sophisticated strategies are required, like those in nanomedicine, which combine multifunctional components to cheat cancer defense system, destroy genetic information and cell compartments, and perform continuous imaging/sensing.\textsuperscript{3}

Hybrid nanomaterials completely satisfy the aforementioned requirements. Their “soft” organic/bioorganic part imitates certain biorelevant structures and facilitates successful escape from the immune system to penetrate and accumulate in cancer cells, while the “hard” inorganic part serves as a cargo carrier, imaging agent or heat inducer. However, the number of functions performed by organic and inorganic components are much broader than discussed before and will be further considered in the next chapters.

In recent years, hybrid nanomaterials based on MOF,\textsuperscript{97} organosilica,\textsuperscript{98} PCOSSO,\textsuperscript{99} gold,\textsuperscript{100} and iron oxide\textsuperscript{101} showed excellent results during in vivo experiments. Liu et al. demonstrated that nanoscale MOF based on photosensitizer can be used for photodynamic therapy,\textsuperscript{97} gold nanorods embedded into organosilica shell were exploited for photothermal effect and gene delivery,\textsuperscript{98} and cubic silsesquioxane cages can simply serve as a cement to build nanoarchitectures (Figure 1.6).
Figure 1.6. Current synthetic strategies toward novel hybrid organic-inorganic nanomaterials for cancer treatment: nanoscale MOF for combined photodynamic and radiation therapy (DMF stands for dimethylformamide) (A), gold nanorod (GNR) embedded into large-pore mesoporous organosilica (LPMO) nanospheres for gene and photothermal cooperative therapy (CTAB stands for cetyltrimethylammonium bromide, BTSE – 1,2-bis(triethoxysilyl)ethane, TEOS – tetraethoxysilane, PEI – polyethylenimine) (B), and crosslinked PCOSSO-nanohybrids for imaging-guided photodynamic therapy (NHS stands for N-hydroxysuccinimide, EDC – 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) (C). Adapted with permission. Copyright 2016, Elsevier (A). Adapted with permission. Copyright 2017, the Royal Society of Chemistry (B). Adapted with permission. Copyright 2017, the Royal Society of Chemistry (C).
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Chapter 2. Hybrid Siliceous Nanomaterials as Innovative Platforms for Cancer Nanomedicine


2.1 The Best of Both Worlds

As it was mentioned earlier in Chapter 1.1, hybrid organic-inorganic silsesquioxanes comprise the best of both worlds: the rigid inorganic silica-like framework and multifunctional covalently incorporated organic moieties. It is frequently acceptable to put silsesquioxane materials under the umbrella of a much larger organosilica class. Nonetheless, such approach is only partially correct and the nuances of this question will be later discussed. However, to give you a broad vision of the hybridization influence on physicochemical properties of materials, the topic of this Chapter 2 will be extended to organosilica nanoparticles (ONs). Thus, nowadays they are considered as the next generation of functional materials, the successors of silica nanomaterials in all areas starting with catalysis and ending biomedicine. Moreover, the aim of this dissertation is to show that in terms of many biomedical applications including bioimaging and therapeutic delivery, organosilicas are more promising when compared to other nanomaterials. There are several reasons for such statement. First, ONs have a controllable chemical composition, size, morphology, porosity, and surface chemistry. Second, being partially inorganic, these NPs can be employed as robust theranostic (therapeutic+diagnostic) nanovectors for bioimaging and delivery of small size cargos and larger guests, as proteins and DNAs, without the leakage of contents, which is a complicated task for fully organic nanovectors with their soft and fragile nature. On the other hand, potential risks related to longer bioaccumulation should not be underestimated. Therefore, the third reason is controlled degradation of ONs via proper selection of organic moieties covalently incorporated into the hybrid organic-inorganic
framework. The kinetics of degradation and clearance of ONs can be tuned from a few days to weeks. The last but not the least, the large variety of ONs can be prepared out of commercially available and cost-effective precursors in a scalable manner. All these factors have expressed in the rapid growth of organosilica research in the last decade.

Every two weeks three new research articles appear in the area of ONs. Although these numbers are not as impressive as those for mesoporous silica NPs (MSNs) alone (3 articles per day), the annual geometrical growth of the number of ONs publications are observed for the last decade (2007 – 2017) comparing to linear one in the MSNs area (Figure 2.1A-C). These facts allow to assume that in the next decade the number of publications in two competitive areas will be equalized. Speaking about ONs, the special accent should be done on the particular interest toward biomedical fields. Thus, 42% and 57 % of research articles about nonporous and porous ONs, respectively, are devoted to biomedical studies (the average values for the last decade are considered). Obviously, porous materials have a great advantage over nonporous ones in regard to their larger surface area. Inner porosity is a critical issue for multiple cargo loading and release strategies when it comes to nanomedicine. Interestingly, in the case of porous organosilica nanohybrids, the percentage of biomedical publications already exceeds the value of 43% for MSNs. The great variety of organic functional moieties that can be embedded into the organosilica framework will make this gap even larger in the near future.

Organosilicas are the vast class of hybrid materials and yet their chemical compositions and synthetic routes can be displayed in a simple scheme, which obligatory
Figure 2.1. Detailed statistics on the number of publications in the area of nonporous (A) and porous (B) hybrid ONs versus MSNs (C). The performed data evaluate numbers of publications in all fields, biomedical fields, and animal studies from 2007 to 2017. Only research articles are considered, no reviews. Data received from the Scopus database (the last update 08/10/17). The simplified illustration unites various organosilica NPs under the one synthetic scheme (D).

includes one or several organoalkoxysilanes/ bridged-organoalkoxysilanes and optionally TEOS (Figure 2.1D). The porosity of NPs can be regulated via the addition of structure directing agents (SDAs) as surfactants. Such flexibility in regard to physicochemical properties provides numerous opportunities for production of nanomaterials with predesigned biomedical properties. Classification and synthesis of ONs will be thoroughly considered in Chapters 2.2 and 2.3.
2.2 Classification of Hybrid Siliceous Nanomaterials

The trial to make clear classification of hybrid organic-inorganic siliceous materials may appear at some point perplexed. The reason is an absence of strictly followed universal nomenclature. In the following paragraphs, this attempt will be done through comprehensive analysis of published literature, chemical compositions, morphologies, and synthetic strategies related to silsesquioxane and organosilica nanohybrids.

Let’s start with important definitions: 1) a silsesquioxane is a class of hybrid materials comprises of cage-type, polymeric, and crystal-like structures with the chemical formula \([R-(SiO_{1.5})_m]_n\), where \(R\) stands for an organic group, both “\(n\)” and “\(m\)” are natural numbers, when \(m=1\) and \(n=[8, 10, 12, ...]\) the silsesquioxane has cage-type structure, for \(m\geq 2\) and \(n=[\text{large number}]\) the polymeric structure has place and the material is called bridged silsesquioxane, in this situation “\(m\)” can be considered as a connectivity of an organic bridge; 2) an organosilica is any hybrid polymer structure which involves covalent incorporation of \(R-(SiO_{1.5})_m\) units.\(^3\)\(^4\)

The cage-type silsesquioxanes commonly synthesized by hydrolysis and subsequent condensation of organotrichlorosilanes.\(^3\) However, an alternative way via the condensation of organoalkoxysilanes is also applicable.\(^4\) The cage-type silsesquioxanes \([R-SiO_{1.5}]_n\) are usually classified as \(T_8\), \(T_{10}\) or \(T_{12}\) (\(n=8, 10\) and \(12\), respectively) where “\(T\)” points on tetrahedral vertexes in the geometry of cage. The number “\(n\)” will determine the shape of silsesquioxane cage (or vice versa the shape of the cage will determine the number “\(n\)” , rather philosophical question). Thus, \(T_8\)-cage approximately will be a cube (Figure 2.2A) and \(T_{12}\)-cage – rectangular parallelepiped (Figure 2.2B) where every silicon
atom is located in the tetrahedral vertex of a polyhedron and connected to three oxygen atoms which in turn bonded to other silicon atoms. The “R” groups are directed out of the cage. Importantly, one silsesquioxane cage can have several different organic groups. The example of T₈-cage may be found in Chapter 1.5 (Figure 1.6C), usually, such compounds are called cube-octameric silsesquioxanes (COSS)⁶-⁷ or simply polyhedral oligomeric silsesquioxanes (POSS),⁸-⁹ although, the first name is more precise. To make the name even more informative it should be transformed into COSSO: “C” for cubic (the shape of cage in regard to the position of silicon atoms), “O” for octameric (number of elemental units RSiO₁.₅ in the cage structure), “S” for sil- (from silicon), “S” for –sesqui- (from Latin 1.5), and “O” for –ox- (oxygen), the abbreviation of suffix –ane, which points to the presence of C-C bond, is not practiced in chemical literature, therefore it will be omitted. Importantly to note, that COSSO cages per se can be considered as molecular nanoparticles standing on the edge of nano- and molecular worlds with the size ~1 nm (for T₈-cage).¹⁰ Further on such COSSO cages can assemble into larger NPs through non-covalent (crystal-like NPs, Figure 2.2C)¹¹-¹² and covalent interactions (polymeric COSSO (PCOSSO), Figure 2.2D).¹³-¹⁴

The large variety of hybrid siliceous materials are usually united under the name organosilica (Figure 2.2E-R). However, differentiation should be done. Thus, organosilica nanomaterials types E-H and K-R are obtained via initial co-condensation of single or multiple organoalkoxysilanes and optional addition of TEOS, whereas organosilica types I-J are prepared via the grafting of organic groups onto previously synthesized silica NPs (porous and nonporous ones).¹⁵-¹⁶ The types I-J will not be further considered in the
dissertation for the negligible amount of organic fraction in comparison to the total silica content.

For the same chemical composition, ONs with two morphologies can be produced: mesoporous (MONs, Figure 2.2E, F, L, N, P) and nonporous materials (nonporous ONs, Figure 2.2 G, H, K, M, O). The ONs synthesized from pure organoalkoxysilane (Figure 2.2Q, R) are not included in this list, because none of the mesoporous analogues are reported. The formation of hollow structures (Figure 2.2R) is possible due to the self-assembly and condensation of organic components into lipid bilayer membranes, such structures coined the name cerasomes because of their similarity with ceramics and liposomes.\textsuperscript{17-21}

Coming back to the porosity of ONs, which is tuned via the addition of SDAs such as surfactant micelles (e.g. CTAB,\textsuperscript{22} Pluronic F127,\textsuperscript{23} Pluronic P123,\textsuperscript{24} fluorocarbon surfactant FC4\textsuperscript{25}) and the ratio between organoalkoxysilanes. Usually, the introduction of TEOS component along with surfactant facilitates the formation of mesoporous structures. However, the disadvantage of the TEOS addition lies in the fact that it will decrease the relative content of the organic component in the organosilica framework and therefore minimize the beneficial effect of hybrid structure. Organoalkoxysilanes with bulky groups are hardly arranged in the space to form ordered mesoporous structures unless sort of non-covalent interactions (e.g. hydrogen bonding, \(\pi-\pi\) stacking) will be introduced between them.\textsuperscript{26-28}

Organosilica NPs types E and F are frequently called MONs\textsuperscript{29-30} or periodic mesoporous organosilica (PMO), in the case of well-organized mesopores.\textsuperscript{24, 31} The types G and H can occur in the literature under different names and abbreviations like bridged
Figure 2.2. The extensive classification of hybrid organic-inorganic siliceous nanomaterials with the focus on chemical composition, morphology, and synthetic strategy. A: T₈-SSO (COSSO or POSSO), B: T₁₂-SSO (POSSO), C: crystal-like SSO NPs, D: PCOSSO NPs or ONs (?), E+F: MONs or PMO NPs (periodic mesoporous BSSO NPs), G+H: nonporous BSSO NPs, I+K+M+O: nonporous ONs, J+L+N+P: MONs, Q: nonporous ONs, also ORMOSIL, R: vesicular ONs, also cerasomes.
silsesquioxane (BS) NPs,\textsuperscript{32} polysilsesquioxane (PSQ) nanoparticles,\textsuperscript{33} or bridged polysilsesquioxanes (BPS).\textsuperscript{34} Further, in the text, these types of organosilicas NPs will be distinguished as nonporous bridged SSO (BSSO) NPs. Thus, types E and F also can be considered as mesoporous BSSO NPs. Organosilica types K, M, O should be called nonporous ONs, and L, N, P – MONs, however, the confusion may happen in regard to types E and F. Moreover, types K-P occasionally appears in the literature under the common silica name.\textsuperscript{35-37} Type Q is also known as ORMOSIL (organically modified silica).

The open question is how proper to assign the type D of nanohybrids (see Figure 2.2) to organosilica NPs. From the chemical side, types D, E, and G are indistinguishable. However, the difference appears at the structural level where PCOSSO NPs consists of covalently interlinked T\textsubscript{8} COSSO cages, however, the same is not necessary for types E and G.\textsuperscript{38} Another question should be raised about how similar are types C and Q. Again their chemical composition can be the same, but structurally they can be different. These questions are not currently well explained in scientific literature and may bring new aspects to the research in the field of nanohybrids.

For the sake of simplicity, the proposed classification scheme does not include nanostructures with a higher hierarchy (e.g. hollow, core-shell, yolk-shell, Janus, satellite structures) or multiconnected organic bridges (m≥3). Although, the abundant number of complex structures will be presented throughout the dissertation.

The majority of organosilica nanomaterials discussed in Chapter 2 and generally in the dissertation will consider types E – H and K – R with occasional examples of the rest types. Although, the research part of the dissertation based only on BSSO NPs, it will be
useful to have a comprehensive overview of other hybrid organosilica materials though they have relatively smaller organic content when it comes to BSSO. This will highlight the fact that even small organic doping may significantly enhance the performance of nanomaterials for biomedical purposes.

2.3 Synthesis of Organosilica Nanomaterials

The typical synthesis of organosilica materials involves so called sol-gel method in aqueous solution. This process consists of two steps: the hydrolysis and condensation of alkoxy silanes/organooalkoxy silanes in aqueous solution under basic or acidic conditions. As it was previously described, the synthetic methods for organosilicas can optionally include the alkoxy silanes Si(OX)$_4$ (where X is typically OEt or OMe) and obligatorily use the organooalkoxy silanes R(Si(OX)$_3$)$_m$ (where R is an organic group, m $\geq$ 1). As examples, the syntheses of silica (SiO$_2$) and bridged silsesquioxane (organosilica types E or G, Figure 2.2) materials will be provided. Thus, the hydrolyses of Si(OEt)$_4$ (Figure 2.3A) and RSi$_2$(OEt)$_6$ (Figure 2.3B) take place in basic media and lead to reactive silanolates species (≡Si-O-) which gradually condense forming covalent siloxane bonds (Si-O-Si) and progressively larger oligomers. Over the time, the sol-gel process produces silica (SiO$_2$) or BSSO materials (O$_{1.5}$Si-R-SiO$_{1.5}$). However, in order to obtain mesoporous nanomaterials, scientists use surfactants such as cetyltrimethylammonium bromide (also known as CTAB). The application of surfactants as SDAs was briefly mentioned during classification process (Chapter 2.2). Being amphiphilic, surfactant molecules form spherical micelles at a concentration above the critical micellar concentration (CMC). Their hydrophilic heads
Figure 2.3. The hydrolysis and condensation of TEOS (A) and bridged organoalkoxysilane (B) in the sol-gel process lead to the formation of silica and organosilica materials, respectively.

“look” in aqueous solution, while hydrophobic tails “hide” inside the micelle. At concentrations higher than the CMC, spherical micelles become cylindrical and eventually can form hexagonal packings with increasing concentrations of surfactant. These supramolecular structures may have the scale of several hundred nanometers, therefore they found application as a template for growing mesoporous nanoparticles. During the sol-gel process, the condensation of silanolate species is observed around micellar packings since electrostatic interactions are involved. The addition of surfactant to the process displayed in Figure 2.3A will produce MSNs. However, a mixture of TEOS and organoalkoxysilane(s) will form MONs, while the templated approach to the process in Figure 2.3B may lead to the growth of PMO NPs or MONs, in an absence of ordered mesoporosity. Importantly, the micellar template has to be removed from mesoporous
ONS for the purposes of cargo loading and possible toxicity of surfactant. The calcination is recommended to avoid since the organically-bridged structures are not stable above 450 °C.\(^{44}\) As a facile method, the ion exchange under sonication with ethanolic solution of ammonium nitrate can be used for extracting processes.\(^{45-47}\)

The templating methods can not only determine the porosity of nanoparticles but also determine their geometrical shapes (Figure 2.4). Thus, Kumar et al. applied microemulsion method (Figure 2.4A) to spatially confine the condensation process of vinyltriethoxysilane, aminopropyltriethoxysilane, and near-infrared (NIR) fluorophore to produce 25 nm ORMOSIL NPs with a narrow size distribution.\(^{48}\) SDAs-assisted synthesis of porous ONs (Figure 2.4B) was already discussed, therefore it can be skipped. However, it worthy to note, that the mixture of surfactants can generate hollow MONs (HMONs) via the combination of vesicle-templating and liquid-templating processes by FC4 and CTAB surfactants, respectively (Figure 2.4C). Hollow magnetic ethylene-bridged MONs were obtained in such method within the size range 100-200 nm.\(^{25}\) Nonetheless, the soft-templating strategy is limited for the large scale production of hollow NPs, while hard-templating method (Figure 2.4D) with solid silica spheres can afford synthesis of highly monodisperse core-shell NPs in large quantities. The solid template can be later removed by etching in basic conditions (Na\(_2\)CO\(_3\) solution) or with HF solution, which will lead to the formation of HMONs with incorporated organic groups R inside the shell (R\(^1\)=phenylene, R\(^2\)=ethynylene, R\(^3\)=ethylene, R\(^4\)=tetrasulfide).\(^{49-52}\)

To visualize the inner structure of MONs, the process of sol-gel synthesis and templating method should be recalled. The organization of silanolates around surfactant
Figure 2.4. Schematic representation of typical strategies for the syntheses of non-hollow (A, B) and hollow ONs (C, D). The inclusion of micellar templating leads to the growth of mesoporous organosilica structures which will allow leveraging its full biomedical potential. The illustration of inner ONs structures promotes the understanding of their physicochemical properties and novel approaches toward more efficient drug nanocarriers (E).
micelles, their further condensation, and template extracting will leave MONs with organic groups homogeneously distributed along pore walls.\textsuperscript{54} Similar picture will be observed for the non-templated synthesis of ONs, the only exception will be the distribution of organic moieties throughout the whole volume of NP (Figure 2.4E).

### 2.4 Physicochemical Properties of Organosilica Nanomaterials

The synthetic methods developed for porous and nonporous silica NPs have been also applied to produce various nonporous ONs, MONs, BSSO and PMO nanomaterials. The large choice of commercially available organoalkoxysilanes and ongoing research in the synthesis of novel ones allow to design and generate ONs with controlled sizes, morphologies, surface chemistry as well as a limitless number of functional properties via the covalent incorporation of various organic groups (Figures 2.5 and 2.6A-B).

As a result of the sol-gel process, two types of organosilica NPs can be produced: nonporous and porous nanomaterials. The higher surface area of mesoporous materials is a playground for the development of cargo nanocarriers. However, that does not imply the useless of nonporous ONs. Thus, the internal and surface cargo loadings were investigated. Such materials utilized various strategies for controlled drug delivery and release, which will the topic of further discussion. Hu et al. designed nonporous 260 nm BSSO NPs (Figure 2.5H) with the light-mediated release of adsorbed plastic antibodies via the surface charge reversal due to the cleavage of nitrophenylene groups under UV-light.\textsuperscript{34} Although, the most common approach for drug delivery with nonporous ONs includes internal cargo encapsulation through one-pot synthesis-loading or exploiting
organoalkoxysilanes with covalently attached drug (prodrug strategy). In this scenario, sustainable drug release will be observed only upon the stimuli-mediated degradation of ONs in the presence of redox (Figure 2.5B, 55 E 56) or enzyme triggers (Figure 2.5F, 57 G 57).

This effect can be achieved, for example, via the incorporation of disulfide- (Figure 2.5B, C, 36 D 37) and ester bonds (Figure 2.5F, G) inside the hybrid organosilica framework.

**Figure 2.5.** Representation of chemical compositions (left side, only structural units) and morphologies (right side) of various nonporous ONs and BSSO NPs (with molar percentages in NPs) for biomedical applications. The particle biomedical applications are indicated in the upper-right corners (e.g. “CD” for cargo delivery, see legend at the bottom). Adapted with permissions from American Chemical Society, 34, 48, 57 Royal Society of Chemistry, 36-37 Elsevier, 56, 58-59 Springer, and Wiley. 55, 60-61
However, it should be mentioned, that the most frequent application of nonporous ONs lies in the area of biomedical imaging (Figure 2.5I, J, K), photodynamic and photothermal therapy (Figure 2.5A, L). Obviously, these applications do not require high surface areas to perform sufficiently enough their functions. The covalent encapsulation of NIR-fluorophore inside 25 nm ONs (Figure 2.5I) by Kumar et al. make possible to conduct biodistribution and clearance studies on nontumored nude mice and to show hepatobiliary excretion of the NPs over the period of 15 days. Enhanced tumor therapy efficiency was achieved with propyl iodine/porphyrin nanohybrids (Figure 2.5A) which yielded $^1$O$_2$ and heat generation upon 650 nm irradiation, as a result – necrosis of tumor tissue and inhibition of tumor growth. But not exclusively organosilica component can serve for imaging purposes, in the core-shell structures, magnetic (Figure 2.5J) and plasmonic component (Figure 2.5K) can be used for magnetic resonance imaging (MRI) and surface enhanced Raman scattering (SERS), respectively.

It can be noted from Figure 2.5 that a large fraction of ONs contains additionally TEOS, as a source of silica. In some cases, high content of organic groups along with their bulky size may hinder the formation of NPs. Nevertheless, it is justified sacrifice to reach nanoscale region with the impactful enough number of organic functional groups. The second reason of adding TEOS is the purpose to synthesize mesoporous hybrid materials, which further will be the topic of our discussion. Although, it should be said, that using of TEOS is not always necessary to produce porous ONs (e.g. PMO). The incorporation of organic groups will determine both the shape and physicochemical behavior of NPs. Thus, Yang et al. prepared 30 nm MONs with large pores (Figure 2.6A-B, C) via a mixture of
TEOS and bis(3-triethoxysilylpropyl)tetrasulfide where the former one also played the role of co-surfactant. PMO NPs prepared by Guan et al. with only bridged organosilanes allow to observe the change of porous structure of PMO: methylene, ethylene, ethynelene, and phenylene bridging units led to three-dimensional hexagonal ($P6_3/mmc$), cubic ($Pm\bar{3}n$) ($A4^{31}$), two-dimensional hexagonal ($P6mm$) ($E4^{31}$), and wormlike structures, respectively.$^{31}$ Ethylene-bridged PMO nanocubes with highly-ordered cubic structure have been prepared with astonishing surface areas up to 2370 m$^2$·g$^{-1}$. Importantly, the chemical nature of organoalkoxysilane can have a crucial impact on the final shape and morphology of organosilica NPs. Thus, Croissant et al. reported that the sol-gel process with 1,2-bis(triethoxysilyl)ethane resulted in rod-shaped PMO NPs, whereas bis(3-triethoxysilyl-propyl)disulfide generated non-porous BSSO NPs, their mixtures in various ratios generated either shorter rod-shaped or spherical PMO NPs.$^{64}$ On the other hand, phenylene-ethynelene-bridged PMO NPs ($D6^{65}$), which formed multipodal NPs in same synthetic conditions. Moreover, the experimental conditions can have a radical influence on the synthesis and growth of PMO nanomaterials for a given organoalkoxysilane. Fatieiev et al. described the preparation phenylene-bridged PMO NPs with spherical, rod-shaped, bended rod-shaped, and wire-like morphologies by changing organic co-solvent additives presented in the sol-gel mixture (Figure 2.6A-B, $D5^{44}$, $E5^{44}$, $F5^{44}$). The stirring speed could also change the geometry of NPs from spherical to monopodal as it was shown for phenylene-bridged PMO NPs ($C5^{53}$) and Janus ($C6^{53}$) phenylene-ethynelene-bridged PMO NPs at low steering speed (300 rpm).
Figure 2.6A. Various chemical compositions (only structural units) of MONs and PMO NPs for biomedical applications. The percentage of components is represented in regard to their molar ratios inside nanoparticles. In the upper-right corner of every cell the biomedical application is shown (e.g. “CD” for cargo delivery, see legend at the bottom). PB: Prussian blue, MNP: magnetic NP, UCNP: upconversion NP, ND: nanodiamond. Composition of inorganic core: PB: K₄[Fe(CN)₆], MNP1: Fe₃O₄, MNP2: BaFe₁₂O₁₉, UCNP1: NaGdF₄:Yb, Tm@NaGdF₄, UCNP2: NaGdF₄:Yb,Er.
Figure 2.6B. TEM and SEM images display the variety of morphologies and structures of MONs and PMO NPs for biomedical applications. The shape of organosilica NPs can vary from nanospheres to nanowires, including hollow, yolk-shell, core-shell, Janus, satellite and multipod structures in regard to the biomedical application.
The incorporation of functional groups into the pore walls impacts the chemical properties of the nanohybrids. The incorporation of ethane (Figure 2.6A-B, A1, B1, A4, B4, C4, D4), ethylene (E4, F4), benzene groups (C1, A5, B5, C5, D5, E5, F5) and their various combinations (C6, D6) leads to tunable hydrophobicity/hydrophilicity in the pore walls allowing the loading of hydrophobic or hydrophilic drugs in huge contents (0.6-1.2 g of drug per g of NPs). Unlike silica which has an isoelectric point around 2 and 3, it was demonstrated that PMO NPs become positively charged near pH 5.5, which was harnessed for the autonomous pH-controlled release and delivery of drugs with various PMO NPs (Figure 2.6A-B, D1, E1, F1). Biodegradable organosilica nanovehicles can be prepared by adding disulfide (D2, E2, F2, B6), tetrasulfide (A3, B3, C3, D3, E6), and oxamide bridges (F6) into the pore walls of MONs or PMO NPs. Organosilica NPs were also endowed with optical properties. Multifluorescent MONs were prepared by co-condensing TEOS with biphenylene-, anthracene-, and/or naphthalene-based bridged organoalkoxysilanes (A2). Two-photon NIR absorbing MONs and PMO nanotheranostics (B2, C2, A6) with two-photon-sensitive conjugated bridging units were designed for the three-dimensional spatiotemporal imaging.

Similarly to other types of NPs, core-shell nanostructures were also designed with mesoporous organosilica shells for multiple theranostic applications. For example, magnetic core-shell NPs can be applied for hyperthermia and temperature-triggered cargo release (Figure 2.6A-B, B7, C7). Plasmonic core-shell NPs with gold core are usually applied for photodynamic therapy and imaging due to their plasmonic properties.
Prussian blue core-shell NPs were studied for magnetic resonance and photoacoustic imaging, as well as photothermal therapy (E322). Molybdenum sulfide (MoS$_2$) nanosheet core-shell vectors were reported to display remarkable photothermal effects (F891). Upconversion core-shell NPs possess an excellent light conversion from the NIR to the ultraviolet–visible (UV-Vis) region and can be utilized in tandem with UV-Vis-sensitive molecules (e.g. azobenzenes, spiropyrans) for light-triggered controllable drug release (D792, E793). Another example is nanodiamond core-PMO shell vectors which were recently found to generate reactive oxygen species upon two-photon excitation (E894).

2.5 Biological Behaviors

Indeed, the current amount of data regarding the biological behavior of organosilica NPs is limited. And yet, we are able to make preliminary conclusions and feel the gaps by drawing parallels with MSNs. Thus, the toxicity of ONs may be attributed to two possible factors: 1) the presence of silanolates on the surface of NPs, which facilitate the membranolysis and 2) the generation of reactive oxygen species leading to the cell death. These two main factors are regulated by the surface charge, porosity, NPs size, surface chemistry, and level of dispersibility. Thus, variety of MONs and PMO NPs demonstrated extremely low hemolytic behavior and high biocompatibility even at high concentrations (>1000 µg·mL$^{-1}$).95-96 These results may look surprisingly if you think about common MSNs, however, there is not trick in terms of novel surface chemistry with organic groups embedded throughout the whole volume. Recent studies showed in vitro
biocompatibility of PMO NPs with phenylene, ethylene, phenylene-porphyrin,\textsuperscript{30} and ethylene-butadienediphenyl bridges.\textsuperscript{88} Moreover, it is known fact that pegylation could improve the biocompatibility of ONs via lowering the interactions between negatively charged silanolates and positively charged trimethylammonium groups of the lipid bilayer of red blood cells.\textsuperscript{97-98} Thus, Hu et al. demonstrated that Janus Au@PMO-PEG NPs composed of ethylene bridges were well tolerated by 4T1 murine breast cancer cells.\textsuperscript{89} Pegylated HMONs with bis(propyl)disulfide groups had a minimal cytotoxicity in 4T1 cancer cells up to at least 200 \(\mu\text{g} \cdot \text{mL}^{-1}\).\textsuperscript{29} The latter in vivo experiments with healthy Kunming mice showed no pathological changes after intravenous administration of HMONs-PEG at high doses (5, 10 and 20 \(\text{mg} \cdot \text{kg}^{-1}\)). The same excellent in vivo biocompatibility was found for disulfide-doped MONs (dose: 30 \(\text{mg} \cdot \text{kg}^{-1}\)),\textsuperscript{79} disulfide-doped pegylated MONs-PEG with covalently incorporated metalloporphyrins (dose: 20 \(\text{mg} \cdot \text{kg}^{-1}\)), and for phenylene/tetrasulfide-bridged hollow PMO NPs (dose: 50 \(\text{mg} \cdot \text{kg}^{-1}\)).\textsuperscript{50} The question of ONs in vivo biocompatibility is a question of many factors, and yet, it should be noted the formation of protein corona as a one, which can shed the light on this problem, although, currently it is terra incognito within organosilica research.\textsuperscript{1}

However, it is important to mention, that low cytotoxicity is, of course, necessary factor but alone is not sufficient to reach all purposes of nanomedicine. As soon as the intravenous injection is done, the NPs are on their own to find the way to the tumor cite (with the exception of magnetically actuated systems). This leads us to the next question: how efficient we can be in the precise targeting. Well, Wilhelm et al analyzed research literature for the past decade in the field of solid tumor treatment with NPs and found
Figure 2.7. Biocompatibility and biodistribution studies of vinyl-ONs (A-B),\textsuperscript{48} core-shell mercaptopropyl-ONs (C-D),\textsuperscript{59} and phenylene/tetrasulfide-HPMO NPs (E-F).\textsuperscript{50} All organosilica NPs were excellently tolerated by cancer cells (A, C, E) and in vivo studies showed preferable accumulation in liver and spleen 24 h after the intravenous administration into mice (B, D, F). Adapted with permissions from American Chemical Society\textsuperscript{48, 50} and Elsevier.\textsuperscript{59}
that only 0.7% (median) of administered NPs successfully deposit in pathological areas. Although this report includes only those studies with pharmacokinetics data, it is critical to grasp the idea of only partial NPs delivery. The large fraction of NPs will be eventually accumulated in various organs of the body like liver, kidney, spleen, and lungs. Therefore, the biodistribution data is so meaningful on the way to clinical translation of nanomedicine. In the recent years, the results of animal studies on organosilica NPs encouraged researchers to continue the work on the development of advanced theranostic nanohybrids. For example, multimodal 25 nm vinyl-ONs (Figure 2.5l) were well tolerated by pancreatic cancer cells (Figure 2.7A) and showed preferable liver and skin accumulation at 24 h post injection (Figure 2.7B), which can be further used for passive drug delivery, while core-shell Au@ORMOSIL NPs (Figure 2.5K) with mercaptopropyl groups had low cytotoxicity with MDA-MB-231 cells (Figure 2.7C) and due to the EPR effect were passively targeted to the tumor in 0.5 h after injection (Figure 2.7D). The comprehensive work by Chen et al. discovered that hollow PMO (HPMO) NPs (Figure 2.6A-B, E6) with phenylene- and tetrasulfide-incorporated groups possessed excellent biocompatibility with MCF-7/ADR cancer cells even at the high concentrations (96% cell viability at 600 µg·mL⁻¹, 48h) (Figure 2.7E), while in vivo studies demonstrated relatively high accumulation in tumor cite (Figure 2.7F).

No doubts, that description of ONs biological behavior will be incomplete without raising the topics of biodegradation and clearance. The in-depth discussion of organosilica biorelated degradation may be found elsewhere. Briefly, there are two categories of parameters which determine the kinetics of ONs degradation: 1) the inner characteristics,
**Figure 2.8.** Redox-mediated degradation of disulfide- (A–F)\(^{57, 64, 100}\) and tetrasulfide-based ONs (G, H);\(^{50}\) hypoxia-triggered disintegration of ONs (I–J);\(^{101}\) trypsin-cleavable nonporous oxamide- (K–L)\(^{47}\) and tri-L-lysine-BSSO NPs (M–N) for controlled drug deliveries;\(^{102}\) pH-mediated degradation of prodrug NPs with covalently encapsulated doxorubicin via hydrazine bonds (O–P).\(^{103}\) The arrows indicate the addition of different triggers: glutathione (A–B, E–H), mercaptoethanol (C–D), cytochrome P450 reductase (I–J), trypsin (K–N), and acidic medium with pH5 (O–P). Adapted with permissions from American Chemical Society,\(^{50}\) Royal Society of Chemistry,\(^{37, 100, 103}\) and Wiley.\(^{47, 64, 101-102}\)
like surface area, pore size, wall thickness, dispersibility, incorporated organic groups, surface chemistry, etc. and 2) the outer characteristics with a medium, pH, temperature, static or continuous flow, etc.\textsuperscript{2} In particular, the role of the covalent incorporation of organic moieties will be under the spot. The selection of organic group can be done in regard to the biomedical purposes. Thus, if the one demands the nanocarrier with long circulating time, ethylene/curcumin bridged PMO NPs can be exploited (stability at least 28 days).\textsuperscript{73} Negligible dissolution (2 wt\%) were also shown for large-pore phenylene-bridged PMO nanospheres after 6 days in a physiological medium.\textsuperscript{104} On the other hand, the fast decomposition of ONs can be achieved with next stimuli-responsive organic moieties: disulfide-\textsuperscript{,37, 64, 100} tetrasulfide-\textsuperscript{,50} azo-\textsuperscript{,101} oxamide-\textsuperscript{,47} tri-L-lysine-\textsuperscript{,102} and hydrazone bonds (Figure 2.7).\textsuperscript{103} The stimuli-mediated degradation of ONs was also proved to be the strategy for sustainable and controlled drug release (e.g. doxorubicin).\textsuperscript{50, 103} Unfortunately, nowadays there are no studies of ONs clearance. Thus, I expect that readers of the dissertation will find this gap attractive enough to be filled and advance the field of hybrid nanomaterials.
2.6 Biomedical Applications

2.6.1 Cargo Loading and Delivery Strategies

Hybrid organosilica nanovehicles with their small size, high surface area, facile chemical functionalization, biocompatibility, and tunable degradability are considered as perspective nanoplatforms for controlled cargo delivery and release inside cancer cells and tissues.\textsuperscript{2, 105-106} There are two main loading strategies applied to realize this approach: surface loading and pore loading, regarding the intrinsic structure of nanomaterial. Moreover, such materials as PMO NPs maintain not only aforementioned strategies but also allow pore-wall engineering strategy to reach exceptionally high cargo content (up to 1.2 g per g of NPs) without further leakage even in the absence of pore capping agents.\textsuperscript{47, 53} Taking into the account that majority cancer drugs are hydrophobic (e.g. camptothecin, doxorubicin, gemcitabine, paclitaxel) this was not possible with common MSNs, while tunable hydrophobic/hydrophilic nature of PMO pore walls make possible strong non-covalent pore-drug interactions. However, another pathway can be utilized for nonporous materials, like an internal encapsulation of cargos via one-pot syntheses\textsuperscript{107} or prodrug design with degradable linkers.\textsuperscript{103, 108} Apart from that, nanomaterials with hollow, core-shell, and yolk-shell structures can possess both high surfaces and additional theranostic features, therefore frequently exploited to maximize therapeutic efficiency.\textsuperscript{49, 52, 78, 88, 94, 109-110} Various release strategies include internal (pH, redox reactions, enzymes) and external actuations (light, magnetic, ultrasound triggers) depending on tumor location and its type (Figure 2.9).\textsuperscript{72, 106, 111-112} The brief overview of these strategies for porous and nonporous organosilica NPs will be given further in the chapter.
Figure 2.9. The overview of the cargo loading and release strategies with porous and nonporous organosilica NPs for biomedical applications.

**pH-Triggered Release.** The systematic comparison between healthy and cancer cells reveals significant difference regarding their intrinsic pH. Thus, tumor tissue maintains acidic extracellular pH due to lactate secretion from anaerobic glycolysis. The pH-controlled release of drugs inside cancer cells can be achieved due to electrostatic repulsions between drug and nanocarrier or pH-cleavable linkers either between cargo and nanoplatform or within its pore-wall structure. The research performed by Croissant et al. with PMO NPs (Figure 2.6A-B, B6) showed the instantaneous release of doxorubicin (DOX) in solution experiments at pH 5.5. This effect was observed due to electrostatic repulsion between DOX and positively charged PMO NPs (zeta potential +27 mV). Same trend was observed with another HMONs delivering DOX (Figure 2.10A). HMONs showed 4.4 wt% release of DOX at pH 7.4 and almost 15 times higher release at pH 5.0.
after 48 h. The introduction of pH-responsive groups like imine, hydrazone, hydrazide can regulate the graduate release of the drug into the acidic intracellular environment.\textsuperscript{115} This concept was realized by Xu et al. with DOX-prodrug NPs.\textsuperscript{103} Drug was covalently incorporated within nonporous organosilica NPs (Figure 2.8M) via hydrazone bonds. It was shown the negligible release of DOX at pH7.4, while acidic pH5.0 resulted in 70% cumulative release after 120 h.

**Enzyme-Triggered Release.** Porous and nonporous organosilica nanovectors could also be actuated by enzymes via an ester,\textsuperscript{108, 116} peptide,\textsuperscript{102} and oxamide bond\textsuperscript{47, 117} cleavages. For example, camptothecin–organosilica nanoconjugates with 50 and 200 nm diameter (Figure 2.10B, Cpt50 and Cpt200, respectively) demonstrated twice higher release of drug in the presence of esterase comparing the same kinetics with phosphate buffered saline (PBS) buffer alone due to ester bonds.\textsuperscript{108} The research group of De Cola imitated the natural process of protein digestion and incorporated tri-L-lysine peptide\textsuperscript{102} inside organosilica nanomaterial. The enzyme-mediated degradation was shown with trypsin in solution (Figure 2.8O-P). However, the low porosity of these BSSO NPs due to the presence of bulk organic bridges did not allow high loading capacity. This limitation was overcome by Croissant et al. with trypsin-responsive oxamide-based PMO nanovectors (Figure 2.6A-B, F6). These highly porous (850 m$^{2}$·g$^{-1}$) NPs with unprecedented loading capacities up to 0.84 g of drug per 1 g of NPs demonstrated controlled release of both hydrophilic and hydrophobic anticancer drugs.\textsuperscript{47}

**Redox-Triggered Release.** The design of nanosystems with triggered release may consider the fact that the concentration of glutathione (GSH) bioreducer is usually higher
in cancer cells than that in normal cells and difference between the extracellular (2-20 μM) and intracellular (2-10 mM) media. Therefore, redox-responsive organosilica platforms can be developed with disulfide and tetrasulfide linkers.\textsuperscript{33, 35-37, 100, 103} Hollow sub-50 nm PMO NPs with disulfide bonds (Figure 2.10C) possessed tumor-specific degradability at 10 mM GSH concentration and as a result graduate release of a cancer drug.\textsuperscript{29} Another approach included surface functionalization with redox-cleavable disulfide anchor for siRNA delivery (Figure 2.10D). Gel retardation electrophoresis with 10 mM GSH demonstrated clear breakage of disulfide groups and release of siRNA from the HMONs.\textsuperscript{122} The redox-triggered release was also realized with oxaliplatin-based BSSO NPs (Figure 2.5E) in reducing tumor environments (the presence of glutathione and cysteine). The process involved the reduction of Pt\textsuperscript{IV}-based prodrug BSSO NPs to Pt\textsuperscript{II}-complex and its further escaping from the silsesquioxane framework.\textsuperscript{56, 123}

**Light-Triggered Release.** Light-responsive organosilica NPs exploit several main mechanisms to release a cargo: photoisomerization, photolysis, and photothermal conversion. The common example of photoisomerizable systems is azobenzene-modified nanovectors. However, this process involves UV-light irradiation (351 nm) which is not biorelevant, therefore, the transition to NIR-responsive systems are necessary. To solve this problem, the light-responsive system was additionally doped with two-photon fluorophores which allowed NPs to perform effective two-photon cross section (300-100 GM at 700-750 nm). The MONs with such design (Figure 2.6A-B, \textbf{B2, C2}) demonstrated controlled opening of azovalves after NIR-irradiation and consecutive drug release.\textsuperscript{82, 124} The elegant work was conducted by Hu et al. with light-mediated cargo release from the
**Figure 2.10.** Controlled release strategies applied to porous and nonporous organosilica nanovectors: pH- (A),114 enzyme- (B),108 redox- (C, D),29, 122 light- (E),77 and ultrasound-triggered releases (F).51 Cpt: camptothecin, PBS: phosphate buffered saline, SBF: simulated body fluid. Adapted with permissions from American Chemical Society,108 Royal Society of Chemistry,51, 114 Elsevier,29, 77 and Wiley.122
surface of nonporous BSSO NPs (Figure 2.5H) via surface charge reversal from \(-45 \text{ mV}\) to \(+33 \text{ mV}\) (\(1.9 \text{ mW-cm}^{-2}, \text{254nm}, 20 \text{ min}\)). This phenomenon was observed due to photolysis of the o-nitrobenzyl group and unprotecting amine-bridged organosilica NPs under UV-light.\(^{34}\) Among photothermal nanoplatforms, those core-shell and Janus structures with organosilica shells and plasmonic or upconversion cores are reported.\(^{85-87, 89, 92-93, 125}\) These inorganic constituents allow to trigger drug release with NIR-light and realize deeper laser tissue penetration. The main factor of NIR-triggered release in these cases is the heat generated by the inorganic core. In fact, a dramatic increase of Er@MSNs temperature from 20 to 45 °C was experimentally measured after 15 min of infrared irradiation.\(^{126}\) Lu et al. explored the potential of copper sulfide (CuS) doped tetrasulfide-based HMONs for NIR-triggered mild hyperthermia and drug delivery (Figure 2.10E). The temperature of 41 – 42 °C was maintained after 30 min of low power NIR-irradiation (808 nm) and targeted delivery of DOX was successfully performed in tumor cells.\(^{77}\)

**Magnetic-Triggered Release.** The process of magnetic-controlled release involves the interaction of magnetic nanoparticles with external alternating magnetic field and the conversion of its energy into the dissipating heat. Unfortunately, there are no reports yet in this field. The potential system for magnetic induced cargo release can be considered the one with ethylene-bridged HPMO and embedded Fe\(_3\)O\(_4\) NPs (Figure 2.6A-B, B7). Thus, drug loading and release were shown for this nanomaterial with aspirin and ibuprofen, although in the absence of magnetic field.\(^{25}\) Another potential magnetic nanovector was studied by Yang et al. and represented Fe\(_3\)O\(_4\) magnetic core with poly(vinylsilane) as the
outer shell. These nanorattles showed strongly enhanced T₂-weighted MRI in the liver tissue, but their therapeutic potential was not exploited.¹²⁷

**Ultrasound-Triggered Release.** It’s relatively new approach regarding hybrid organosilica nanovectors, which implies the exploiting of high intensity focused ultrasound (HIFU). This technique attracts special attention of researchers, which is caused by its non-invasive nature and ability to perform simultaneous imaging and site-specific therapy with much lower sides effects than current cancer therapies, moreover, it is already FDA-approved technique.¹²⁸ However, the insufficient transfer of energy from HIFU source to tumor cite requires additional enhancement agents (EAs), which increase the therapeutic efficacy.¹²⁹ Thus, hollow MONs and PMO NPs proved to be not only efficient EAs due to cavity effect but were also used as drug delivery vectors. Interestingly, TEM studies did not reveal any damages in the hollow organosilica structure after HIFU irradiation, which allows drug release in graduate manner and not in abrupt one in case of structural collapsing.⁵¹ The additional experiment studied DOX releasing profiles at different temperatures. The results showed similar patterns regardless the temperature, assuming either mechanical or/and cavitation effects involved in the release process.⁵¹ Furthermore, several in vivo studies confirmed a practical aspect of the HIFU-triggered release of hydrophobic DOX (Figure 2.10F)⁵¹ or paclitaxel⁵² drugs from phenylene-based HPMO NPs. For example, HPMO NPs demonstrated 70% DOX release after 10 s of 200 W HIFU irradiation (Figure 2.10F). Although such nanosystems can effectively accumulate large amounts of hydrophobic drugs, on the other hand, their significant passive leakage was observed even without ultrasound.⁵⁰⁻⁵²
2.6.2 Small-Sized Cargo Delivery

The comprehensive overview of small cargo delivery was done along the discussions in Chapters 2.4, 2.5 and 2.6.1. The multiple examples of hybrid nanosystems with small anticancer drugs (e.g. DOX, Cpt) were represented in Figures 2.8 and 2.10. To summarize aforementioned information, next advantages of organosilica nanocarriers over other nanosystems should be mentioned: 1) the sophisticated combination of rigid inorganic component and multifunctional organic moieties; 2) higher loading capacity of hydrophobic drugs (up to 1.2 g per g of NPs\textsuperscript{47, 53} which is not possible with fully inorganic NPs (e.g. MSNs) and 3) the tunable degradation behavior of ONs via incorporation of various stimuli-responsive organic groups.\textsuperscript{102, 130}

2.6.3 Gene or Protein Delivery

The delivery of small molecules such as anticancer drugs via porous and nonporous ONs has been extensively discussed throughout this dissertation. The delivery of larger bioactive molecules such as proteins and genes has been comparatively much less investigated with ONs. The main reason for such delay is the delivery nanoplatform, which could both safely deliver and release the cargo in a controlled manner. To solve this problem several strategies were applied: 1) cargo delivery via adsorption on the external surface of ONs;\textsuperscript{34, 131} 2) loading of large-pore MONs\textsuperscript{121-122} and 3) the in situ or post encapsulation of genes and proteins into the both hollow cavity or organosilica shell of stimuli-degradable nanovectors.\textsuperscript{55, 68}
2.6.4 Combination Anticancer Therapy

Among the defensive mechanisms of cancer cells, the one, in particular, multidrug resistance (MDR) is occurred to be the way to diminish all benefits of anticancer drugs and endanger the life of patients. Therefore, combination therapy with the administration of not single but several active agents as well as the application of drug/siRNA complex was developed to tackle the MDR problem. The simultaneous co-delivery of drug/siRNA complex can eradicate cancer cells and silence the overexpression of drug efflux transporters which are responsible for MDR mechanism in cancer cells.\(^ {132-134}\) As a part of redox-triggered release discussion, the work of Wu et al. was considered.\(^ {122}\) It describes the knocking down P-glycoproteins (P-gp) with siRNA modulator and co-delivery of DOX with large-pore HMONs (Figure 2.10D). The initial pore size (4 nm) of phenylene-bridged HPMO NPs was enlarged to 24 nm by hydrothermal treatment via the partial breakage of Si-C bonds. This was done in order to load the siRNA inside the large pores and protect it from the premature enzyme degradation in the surrounding microenvironment.\(^ {122}\) The release mechanism included the redox cleavage of disulfide bond as a connector between HMONs and the positively charged capping poly (β-amino esters), which in turn, accompanied by the release of attached siRNA. Only upon the high level of intracellular GSH, the controlled siRNA release was observed. At the end of the experiment, the in vivo studies demonstrated 14 times decrease of tumor volume in comparison with a control group without DOX/siRNA combinational therapy (Figure 2.10D).\(^ {122}\) Another design of nanocarriers was developed by Huang et al. and involved elaborate 70 nm core-shell hierarchical MSN@MON particles (H-MONs) with biodegradable tetrasulfide-
incorporated groups (Figure 2.11A). Thus, DOX was loaded inside the MSN-core and siRNA for down-regulation of P-gp expression was encapsulated in large mesopores (diameter 4-9 nm) of organosilica shell. Animal studies on mice bearing MCF-7/ADR xenograft showed significant decrease in tumor growth (87% inhibition rate) after the administration of DOX/siRNA@H-MONs vectors in comparison to the mice in the group of free DOX (51% inhibition rate), while combinational therapy as well demonstrated much lower side effects compared to the free DOX injection.

2.6.5 Cancer Therapy via Gaseous Molecule Delivery

This section will be devoted to the recent achievements in the delivery of gaseous transmitter, namely nitric oxide (NO) with MONs. Nitric oxide molecules can perform beneficial and toxic effects in regard to their concentration and location. However, it is challenging quest, firstly, to enrich the nanocarrier with unstable NO bearing molecules, secondly, to deliver certain amounts of gaseous molecules to the targeted place circumventing possible side effects. In the context of these problems, MONs can afford their hybrid nature to find the proper solution.

In the recent study biodegradable hollow MONs were loaded with L-arginine amino acid and then coated with glucose oxidase enzymes to perform a synergistic cancer therapy via the intracellular production of H\textsubscript{2}O\textsubscript{2} and NO species (Figure 2.11B). Glucose molecules which serve as a fuel for tumor metabolism, were used to trigger the delivery process. Thus, intratumoral glucose was converted into gluconic acid and toxic H\textsubscript{2}O\textsubscript{2} by the glucose oxidase, whereas the production of H\textsubscript{2}O\textsubscript{2} caused the death of U87MG
glioblastoma cancerous cells. The continuous consumption of glucose cut the energy supply chain of tumor and made it to “starve”. Moreover, the generation of H$_2$O$_2$ resulted in later oxidation of L-arginine and generation of NO gas for combined H$_2$O$_2$/NO therapy.$^{137}$

In the relative biomedical field of bacterial inhibition, the strategy of postfunctionalization was developed to enrich MONs with NO molecules via covalent modification. The group of Polarz used PMO NPs where phenylene-bridges additionally contained thiol groups for covalent attachment of NO via the formation of S-nitrosothiol functionalities.$^{138}$ This material in combination with $^{1}$O$_2$ producer performed high antibacterial efficiency under the sunlight against pathogenic bacterium 	extit{Pseudomonas aeruginosa}. The observed results can be explained by the production of highly reactive peroxynitrite molecules (ONOO$^-$) via the reaction between superoxide radicals (O$_2^•$) and NO.$^{138}$ Shin et al. used TEOS and N-(6-aminohexyl)aminopropyltrimethoxysilane to obtain ONs and then converted amino groups into N-diazeniumdiolate NO donors under the pressure of NO (5 atm).$^{139}$ This method generated ONs with NO loading up to 1.8 μmol·mg$^{-1}$. Although, the alternative approach allowed to reach much higher NO loading up to 11.3 μmol·mg$^{-1}$ via the prior synthesis of N-diazeniumdiolate NO donor out of aminoalkoxysilane and its further condensation with TEOS.$^{140}$

It is noteworthy to say about another gasotransmitter, carbon monoxide (CO), which so far has not been delivered with MONs, however, multiple examples of MSNs-based nanosystems with carbonyl complexes are reported.$^{141-142}$
Figure 2.11. In vivo applications of therapeutic nanovectors based on ONs: combinational anticancer therapy (A),\textsuperscript{135} delivery of gaseous transmitters (B),\textsuperscript{137} fluorescent imaging (C),\textsuperscript{143} thermal imaging (D),\textsuperscript{60} MR imaging (E),\textsuperscript{144} and ultrasound imaging (F).\textsuperscript{145} Adapted with permissions from American Chemical Society,\textsuperscript{144} Royal Society of Chemistry,\textsuperscript{145} Elsevier,\textsuperscript{135} and Wiley.\textsuperscript{60,137,143}
2.6.6 Bioimaging and Theranostic Nanosystems

The virtually unlimited number of organic moieties which can be embedded inside the organosilica framework and their optical properties resulted in a large variety of nanohybrids suitable for in vivo imaging: 1) fluorescent imaging (Figure 2.11C); 2) NIR-imaging; 3) two-photon imaging; 4) thermal imaging (Figure 2.11D). The facile synthesis of core-shell structures paved the way to MRI (Figure 2.11E) and SERS imaging with magnetic iron oxide and plasmonic gold NPs, respectively. The hollow structure of MONs and PMO NPs was explored as EAs for ultrasound imaging (Figure 2.11F). Moreover, the chemistry of organosilica materials is not limited to a single organic group, it can be further extended by incorporation of stimuli-degradable groups or covalently attached drugs. All these possible theranostic features make organosilica nanomaterials a promising candidate for clinical trials.
Post Scriptum

Chapter 2 is up-to-date reporting of scientific achievements in the field of organosilica NPs and their biomedical applications, while Chapters 3-5 are based on several years old publications, hence you may find their introductions as retrospective ones.
2.7 References


3.1 Introduction

Nanomedicine has emerged as an extensive subject of investigation and applications around the world due to the discovery of the unique diagnostic and therapeutic advantages of nanomaterials.\textsuperscript{1-14} The nanoscale dimension of such materials endows them with long blood circulation times as well as preferential accumulation in cancerous tissues through EPR effect.\textsuperscript{15-16} However, one of the crucial challenges to be addressed for their future applications is the ability to degrade in biological environment. Biodegradability of nanomaterials is indeed expected to be the safest route to avoid largely-unknown and potentially harmful side-effects of accumulated NPs in the human body. Thus, the cytotoxicity of both nanomaterials and their degradation products should be very low, and NPs should be degraded shortly after the fulfillment of their biomedical purpose.\textsuperscript{17}

Biodegradable NPs could be divided into three groups: polymeric, inorganic and organic-inorganic hybrid nanomaterials. Among polymeric particles, polylactic acid (PLA),\textsuperscript{18} polylactide-co-glycolide (PLGA)\textsuperscript{19} and poly(\(\beta\)-amino ester) (PBAE)\textsuperscript{17} are the most utilized.\textsuperscript{20} Calcium phosphate,\textsuperscript{21} manganous phosphate,\textsuperscript{22} and porous silicon\textsuperscript{23} are examples of biodegradable inorganic NPs. Additionally, biodegradable organic-inorganic hybrid NPs such as periodic mesoporous organosilica have been reported.\textsuperscript{24} The hybrid nature of organosilica was designed to increase the biodegradability of silica\textsuperscript{13,25-26} with a high content of organic functionalities in the matrix for biomedical applications. Effective biodegradation could be achieved by the homogeneous distribution of cleavable functional groups\textsuperscript{27} via different stimuli such as redox reactions,\textsuperscript{24} pH change,\textsuperscript{28} and light actuation.\textsuperscript{29}
Among organic-inorganic hybrid nanomaterials bridged silsesquioxane (BSSO) nanoplatforms have been recently described for few biomedical applications. Indeed, such materials of general formula $O_{1.5}Si-R-SiO_{1.5}$ are constituted of high contents of organic R bridging groups (~40-60 wt%) in a robust and easily functionalized silica matrix. Thus, the properties of BSSO NPs could be widely tuned according to the organic groups presenting in the bridged organoalkoxysilane sol-gel precursors. It is noteworthy that the synthesis of BSSO NPs for biomedical application is challenging since the absence of silica source (e.g. tetraethoxysilane) during the condensation of bridged-organoalkoxysilanes generally leads to bulk, micro-sized particles, or aggregated NPs. In this context, Shea et al. reported BSSO NPs with light-triggered charge reversal features for anti-body release. Lin et al. reported BSSO NPs composed of oxaliplatin bridges for cancer therapy. Croissant and coworkers prepared two-photon sensitive BSSO NPs for efficient photodynamic therapy and bioimaging applications. Wong Chi Man et al. reported pH-sensitive BSSO NPs for the release of cyanuric acid attached via hydrogen bonds with the BSSO matrix as a model of drug delivery system in lysosomal acidity. Ester-bridged silsesquioxane-coated liposomes were applied for drug delivery. Slow degradation in the presence of glutathione was shown for nonporous redox-cleavable BSSO NPs. Only one study has been reported on biodegradable BSSO NPs which consisted of gadolinium-complexed bridges with disulfide linkages which can be cleaved by glutathione for MRI imaging. However, the variety of stimuli-degradable groups, in particular enzymatically degradable ones, can be additionally extended with carbamate, ester, and amide linkers, which only recently started to appear in BSSO NPs.
3.2 Design

Herein we report the controlled synthesis of biodegradable sub-200 nm non-aggregated dense BSSO NPs by sol-gel reaction of an oxamide-bridged alkoxy silane (OBA) precursor inspired from Nature with the common enzymatically-catalyzed metabolism processes (Figure 3.1). Moreover, fluorescent BSSO-FITC NPs were obtained through an incorporation of fluorescein isothiocyanate moieties inside the siloxane framework. Both materials were fully characterized by various techniques displaying the high organic content and functionalities of the NPs. Additionally, the enzymatically mediated degradation of NPs in simulated biological media was demonstrated, and fluorescent BSSO nanoprobe were applied for in vitro imaging in cancer cells.

Figure 3.1. Schematic representation of the synthesis and enzymatic degradation of BSSO NPs via the cleavage of amide bonds.
3.3 Experimental Section

3.3.1 Materials. Absolute ethanol, 3-aminopropyltriethoxysilane (APTES), sodium hydroxide, fluorescein 5(6)-isothiocyanate (FITC), trimethylamine (TEA), sodium sulfate, ammonium nitrate, cetyltrimethylammonium bromide (CTAB), phosphate buffered saline (PBS, pH 7.4), and Cell Counting Kit-8 (CCK-8) were purchased from Sigma-Aldrich. Oxalyl chloride was obtained from Alfa Aesar. Anhydrous dichloromethane (DCM) was purchased from Acros. All chemicals were used without further purification. Deionized water was used in all procedures. The human cervical tumor cell line (HeLa) was purchased from ATCC. Eagle’s minimum essential medium (EMEM), Dulbecco’s phosphate buffered saline (DPBS, pH 7.4), fetal bovine serum (FBS), penicillin-streptomycin, paraformaldehyde, trypsin, 4,6-diamino-2-phenylindole (DAPI), and CellMask™ deep red plasma membrane dye were purchased from Invitrogen.

3.3.2 Methods: Synthesis and Characterization. The OBA precursor was synthesized by coupling 1 equivalent of oxalyl chloride with 2 equivalents of APTES in DCM using TEA as a base catalyst at 0 °C (Figure 3.2). BSSO nanomaterials were then prepared by the addition of an ethanolic solution of the OBA to an aqueous basic solution (pH 12) containing the CTAB surfactant (Figure 3.1). Besides, positively-charged fluorescent BSSO-FITC NPs were obtained in similar conditions through the co-condensation of the OBA, FITC-alkoxysilane and APTES. A number of physicochemical and biological methods was applied to characterize comprehensively the OBA precursor and derivative nanomaterials. The obtained data will be discussed further in the chapter.
Synthesis of OBA. First, a 50 mL round-bottom flask was dried under vacuum using a heat gun. Dry DCM (10 mL) was added to the flask via canula followed by the addition of TEA (0.42 mL, 3 mmol). Then, APTES (0.834 mL, 2.1 mmol) was added to this solution and flask cooled to 0 °C in an ice bath. The DCM solution of oxalyl chloride was added dropwise (0.5 mL, 1 mmol, 2 M). After the complete addition, the reaction mixture was removed from the ice bath and stirred at room temperature for 1 h; the colorless solution gradually became yellowish. All synthetic steps were done under N₂ atmosphere. Then, the mixture was diluted with DCM and washed with water and brine. The two phases were separated and the organic phase with the product was dried over Na₂SO₄, filtered, and concentrated under reduced pressure by a rotary evaporator. The obtained product was crystalline (475 mg, 95%) which is not characteristic for silanes and could be due to intermolecular hydrogen bonding reported previously for oxalic acid diamides.⁴²-⁴⁴

![Synthetic pathway toward the OBA precursor.](image)

Synthesis of FITC-APTES.⁴⁵ FITC (2.7 mg, 6.9 μmol) was dissolved in EtOH (1.5 mL) and APTES (6 μL, 25.6 μmol) was added. The reaction was conducted with magnetic stirring at room temperature for 2 h. The obtained product, FITC-APTES, was used directly without purification.
**Synthesis of BSSO NPs.** The synthesis was performed according to a modified procedure of Croissant et al.\cite{24} A mixture of CTAB (6.4 mg, 17.5 µmol), distilled H\textsubscript{2}O (1.5 mL), EtOH (0.1 mL), and NaOH (6.68 µL, 1.28 M) was stirred at 75 °C for 50 min at 1000 rpm in a 10 mL glass bottle. Then, the stirring speed was increased to 1400 rpm and an ethanolic solution of the OBA precursor (0.3 mL, 48 µmol, 0.16 M) was added. After adding the bridged alkoxy silane the temperature was raised to 80 °C. The condensation process was conducted for 2 h. Then, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and the NPs were collected by centrifugation for 10 min at 14000 rpm. The sample was washed twice with an ethanolic solution of NH\textsubscript{4}NO\textsubscript{3} (6 g·L\textsuperscript{-1}),\cite{46} and three times with ethanol, water, and ethanol. Each washing step involved a sonication of 10 min at 40 °C; the collection was carried out in the same manner. The sample was finally dried under vacuum for few hours.

**Synthesis of BSSO-FITC NPs.** A mixture of CTAB (6.4 mg, 17.6 µmol), distilled H\textsubscript{2}O (1.5 mL), EtOH (0.1 mL) and NaOH (6.68 µL, 1.28 M) was stirred at 75 °C for 50 min at 1000 rpm in a 10 mL glass bottle. Then, the stirring speed was enhanced to 1400 rpm and a mixture of alkoxy silanes was added (24 µmol of OBA in 150 µL of EtOH and 0.7 µmol of FITC-APTES in 150 µL of EtOH). After the addition, the temperature was increased to 80 °C, and the condensation process was conducted for 2 h. Then, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and the NPs were collected by centrifugation for 10 min at 14000 rpm. The washing and collection procedures were carried out in the same manner as for BSSO NPs. The sample was finally dried under vacuum for few hours.
**Nuclear Magnetic Resonance (NMR) Spectroscopy.** $^1$H NMR and $^{13}$C NMR spectra were performed at 500 MHz and 127 MHz with CDCl$_3$ solutions at 5 mg·mL$^{-1}$ with an Avance™ III Bruker™ Corporation instrument. Solid state NMR (ssNMR) spectra were recorded using a Bruker WB Avance™ III HD spectrometer operating at magnetic field strength of 9.39 T, corresponding to Larmor frequency of 400 MHz for $^1$H nuclei, and equipped with a Bruker 3.2 mm cross-polarization/magic angle spinning (CP/MAS) probehead rotating the sample at 15 kHz rate. $^{13}$C spectra were measured directly, while for $^{29}$Si cross-polarization technique was used with contact pulse durations of 2 ms. Chemical shifts were referenced to external tetramethylsilane (TMS).

**UV-Vis-IR Spectroscopy.** Fourier-transform infrared (FTIR) spectra were recorded on a Thermo Scientific™ Nicolet™ iS™10 FT-IR Spectrometer. UV-Vis absorption spectra were recorded on a Varian Cary 5000 spectrophotometer. All UV-Vis spectra were acquired in a quartz cell (sealed by Teflon cap), background correction was done with deionized water. The concentrated solution of the OBA precursor was prepared in absolute ethanol, placed on the face of KBr pellet, dried, and used to obtain FTIR spectrum. The powder samples of CTAB, BSSO and BSSO-FITC NPs were grounded with KBr to prepare pellets for FTIR analyses.

**Dynamic Light Scattering (DLS) and Zeta Potential Analyses.** DLS and zeta potential measurements were performed using a Malvern Nano ZS instrument at 25 °C. Each measurement was conducted three times to validate the numbers.
**Mass Spectrometry (MS).** The sample was dissolved in methanol and 100 µL of the solution was injected into Thermo Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Mass Spectrometer.

**Electron Microscopy.** Transition electron microscopy (TEM) images were recorded with a Technai™ T12 (FEI Company) microscope operated at 120 kV. A drop of an aqueous dispersion of NPs was placed directly on a carbon-coated copper grid and dried at room temperature. Scanning electron microscopy (SEM) images were recorded with a field emission Nova™ NanoSEM 630 microscope (FEI Company). A drop of an aqueous dispersion of NPs was placed directly on a carbon film attached to aluminum pin stub and dried at room temperature. The elemental mapping and high-resolution TEM of particles were carried out with another TEM of model Titan™ G² 80-300CT from (FEI Company) which was equipped with a post-column energy filter of model GIF Tridiem® 863 (Gatan Inc.). The GIF was used in electron energy-loss spectroscopy (EELS) mode combined with high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM). Moreover, Si-L$_{23}$ (99 eV), S-L$_{23}$ (165 eV), C-K (283 eV), N-K (401 eV) and O-K (532 eV) energy-loss edges were utilized in making Si, S, C, N and O elemental maps, respectively. A typical EELS spectrum showing the energy loss edges of these elements extracted from a spectrum image dataset is shown in Figure 3.7A. While the elemental maps of above listed elements from a typical size NP are shown in Figure 3.7B.

**Confocal Laser Scanning Microscopy (CLSM).** CLSM images were acquired with Zeiss LSM 710 (Carl Zeiss) upright confocal microscope.
Thermal Gravimetric Analysis (TGA). TGA was done on TG 209 F1 Iris® (Netzsch) under nitrogen atmosphere from 30 to 800 °C, with a heating rate of 10 °C·min⁻¹.

Nitrogen Adsorption-Desorption Analysis. N₂ sorption isotherms and corresponding pore size distributions were acquired to characterize the mesoporous structures of BSSO and BSSO-FITC NPs on a Micromeritics ASAP® 2420 instrument. The total pore volumes and surface areas were determined by Brunauer–Emmett–Teller (BET) theory.

Cells Viability of HeLa Cells Treated with BSSO NPs. The cytotoxicity of BSSO NPs incubated with HeLa cells was evaluated using the CCK-8 assay. Cells were seeded at a density of 1×10⁴ cells per well in 96-well flat bottom plates and incubated with EMEM containing 10% FBS and 0.1% penicillin-streptomycin at 37 °C in a humidified 5% CO₂ atmosphere for 12 h. After cell attachment, they were washed with DPBS and incubated with the suspension of BSSO NPs in EMEM at different concentrations (100, 10, 1, 0.1, 0.01, and 1×10⁻³ µg·mL⁻¹) for 24 h. Cell viability was evaluated with CCK-8 procedure.

Intracellular Localization and Internalization of BSSO-FITC NPs. HeLa cells were seeded on glass cover slides, and cultured in EMEM containing 10% FBS and 0.1% penicillin-streptomycin at 37 °C in a humidified 5% CO₂ atmosphere. After cell attachment, the medium was replaced with fresh medium containing 10 µg·mL⁻¹ of BSSO-FITC, followed by incubation for 6 h. Cells on cover slides were washed twice with DPBS, then fixed with 4% paraformaldehyde for 1 h and washed 3 times with DPBS. Then, nuclei were stained with DAPI for 3 min and then washed 3 times with DPBS. The cell membrane was stained with CellMask™ deep red plasma membrane dye for 3 min and then washed 3 times with DPBS. Finally, cells were observed with CLSM.
3.4 Results and Discussion

The successful synthesis of the OBA precursor was confirmed by NMR and FTIR spectroscopies in conjunction with mass spectrometry (Figures 3.3-3.5). Therefore, the OBA precursor was further used to generate both BSSO and BSSO-FITC NPs. The physicochemical characteristics of nanomaterials were then characterized by various techniques. SEM and TEM images displayed nearly monodisperse spherical BSSO and BSSO-FITC NPs with average sizes of 125 and 92 nm respectively (Figure 3.6). In regard to composition, the successful incorporation of organic moieties within the siloxane framework was investigated by STEM-EELS method (see a typical spectrum in Figure 3.7A). The uniform distribution of oxamide groups within BSSO and BSSO-FITC NPs was demonstrated by STEM-EELS elemental mappings of silicon, oxygen, nitrogen and carbon atoms (Figure 3.7B-C). Moreover, such analysis also proved the homogeneous distribution of the sulfur-containing FITC dyes (Figure 3.7C), with a clear signal of sulfur in BSSO-FITC NPs when compared to BSSO NPs (Figure 3.7D). Such an observation is often a requirement in dye doped nanomaterials in order to maintain intense fluorescence properties. The preservation of oxamide groups was confirmed by the presence of the $\nu_{C=O}$ stretching mode of the amide-I at 1668 cm$^{-1}$ in the OBA precursor and 1669 cm$^{-1}$ in BSSO NPs and BSSO-FITC NPs (Figure 3.8). Besides, with red-shift of the $\nu_{Si-O}$ mode from 1080 cm$^{-1}$ in the OBA precursor to 1090-1140 cm$^{-1}$ in the BSSO and BSSO-FITC NPs indicates the high condensation of the siloxanes. Such conclusions are further confirmed by ssNMR of $^{13}$C and $^{29}$Si nuclei, displaying the environment of the oxamide as well as the major proportion of $T^2$ and $T^3$ silicon environments for the nanomaterials (Figure 3.9A-B).
Figure 3.3. $^1$H (A) and $^{13}$C (B) NMR spectra of the OBA precursor. $^1$H-NMR: (CDCl$_3$, 500 MHz) δ (ppm) 7.57 (s, J = Hz, 2H), 3.80 (q, J = 7 Hz, 12H), 3.29 (t, J = 6.7 Hz, 4H), 1.66 (m, J = 7.7 Hz, 4H), 1.20 (t, J = 7.1 Hz, 18H), 0.63 (t, J = 8.3 Hz, 4H). $^{13}$C-NMR: (CDCl$_3$, 125 MHz) δ (ppm) 159.9, 58.6, 42.1, 22.9, 18.3, 7.8.
Figure 3.4. FTIR spectrum of the OBA precursor. FTIR (cm$^{-1}$): 3317, 3017, 2973, 2936, 2895, 1668, 1522, 1449, 1371, 1276, 1203, 1110, 950, 783, 703, 572, 481.

Figure 3.5. LTQ-Orbitrap MS full scan: +ve ESI of the OBA precursor. MS (ESI+) m/z (%): 497.3 (100) [MH$^+$], 451.2 (55) [M$^-$- one ethoxy fragment].
Figure 3.6. SEM (A-B) and TEM (C-D) images of BSSO and BSSO-FITC NPs. TEM statistical size distributions of BSSO (E) and BSSO-FITC NPs (F).
Figure 3.7. Typical background subtracted electron energy-loss spectrum of BSSO NPs (A). STEM-EELS elemental mapping (silicon, oxygen, nitrogen, carbon, sulfur) of a representative BSSO and BSSO-FITC NPs (B and C, respectively). Scale bar of 50 nm. Comparison of the sulfur signal in the BSSO-FITC and BSSO NPs demonstrating the homogeneous FITC dye incorporation in BSSO-FITC NP (D). *Asterisks encompass the diameter of NP.

Figure 3.8. FTIR spectra for the OBA precursor, BSSO, and BSSO-FITC NPs.
Figure 3.9. ssNMR of $^{29}$Si and $^{13}$C nuclei via CP/MAS and HPDEC sequence in BSSO and BSSO-FITC NPs (A and B, respectively). In the carbon spectrum of BSSO NPs, number indicate the followings: 1: Si-OCH$_2$CH$_3$, 2: HOCH$_2$CH$_3$, 3: CTAB residues. In the carbon spectrum of BSSO-FITC NPs, asterisks indicate Si-OCH$_2$CH$_3$ and CTAB residues. FITC peaks are most likely in the noise of the spectrum because of the low content of the incorporated dye, of which the presence is unambiguously demonstrated by UV-Visible spectroscopy (Figure 3.15) and in vitro imaging (Figure 3.17).
Thermo-gravimetric analyses depicted weight loss of ca. 50% between 400 and 700 °C associated with the decomposition of the high content of organic bridges (Figure 3.10A-B).\textsuperscript{47-48} The initial stage of calcination includes for both nanomaterials loss of ethanol and water as solvents, which were used for syntheses and washings.

The texture and structure of BSSO and BSSO-FITC NPs were further assessed by nitrogen adsorption-desorption analysis. The CTAB surfactant, which was found necessary to obtain non-aggregated nanospheres, was partially extracted in the resulting NPs as shown by \textsuperscript{13}C NMR and FTIR analyses (Figures 3.9 and 3.11), residues remaining entrapped in the materials.\textsuperscript{46, 49} Dried BSSO and BSSO-FITC NPs were non-porous via porosimetry measurements with surface areas under 25 m\textsuperscript{2}g\textsuperscript{-1} (data not shown) which are typical of self-assembled BSSO.\textsuperscript{50-52} Accordingly, the incorporation of organic bridges into the silica framework leads to the formation of parallel nanosized channels as shown by high-resolution TEM (HRTEM, see Figure 3.12). This is most-likely resulting from intermolecular hydrogen bonds between oxamide groups, as reported with macroscale BSSO materials.\textsuperscript{53-55} Besides, the oxamide group is known to be a crucial component in designing organic gelators.\textsuperscript{42-44}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{tga_data}
\caption{TGA data for BSSO (A) and BSSO-FITC NPs (B).}
\end{figure}
Figure 3.11. FTIR spectra for BSSO, BSSO-FITC NPs, and CTAB.

Figure 3.12. HRTEM image of a representative BSSO NP at high magnification displaying the nanoscale periodicity arising from the self-assembly of amide groups within the nanomaterial.
The biodegradability of BSSO NPs was then investigated in simulated biological media. The suspensions of NPs in Tris buffer (pH 4), PBS buffer (pH 7.4), and trypsin enzyme in PBS (pH 7.4) buffer were stirred at 37 °C during 24 and 48 h. Experimental results showed that BSSO NPs were only degraded in the presence of trypsin enzymes. This conclusion was supported by TEM images before and after enzymatic degradation (Figure 3.13A-B), as well as DLS measurements after 24 and 48 h which depicted the expected decrease of the NPs size (Figure 3.13C). The initial average hydrodynamic diameter of 295 nm turned into 122 nm after 48 h stirring with trypsin-PBS. The same effect was observed in BSSO-FITC nanomaterials (Figure 3.14). The trypsin enzyme is known to cleave amino-acids into carboxylate and ammonium groups,\(^{56}\) which is the most likely mechanism in the degradation as suggested by the decrease of oxamide vibration modes in the FTIR spectrum of degraded NPs (see Figure 3.15).

Fluorescent BSSO-FITC NPs were then applied as nanoprobes in cancer cells. Indeed, BSSO-FITC NPs have the yellow fluorescence of fluorescein which absorbs light at 505 nm (Figure 3.16A) and could thus be envisioned for biomedical diagnosis. BSSO-FITC NPs were also designed to be positively-charged with aminopropyl groups on their surface in order to increase the cellular uptake as reported in the literature.\(^{57-58}\) While BSSO NPs were negatively-charged (-38 mV) due to silanolate groups, the zeta potential of BSSO-FITC NPs was thus increased to +45 mV (Figure 3.16B). The HeLa cell line was selected, and the biocompatibility of BSSO NPs was first demonstrated with a complete cell survival up to 100 \(\mu g\cdot mL^{-1}\) (Figure 3.17A). Secondly, fluorescent NPs were incubated and tracked in cancer cells. Nuclei were stained in blue with DAPI dyes (Figure 3.17B), and cell membrane
in red with CellMask™ (Figure 3.17C). After only 6 h of incubation, a significant population of the NPs was endocytosed (see Figure 3.17D-E) as witnessed by the intense green fluorescence of the designed nanoprobe within cells (Figures 3.18 and 3.19).

Figure 3.13. TEM images of BSSO NPs in PBS buffer (A), and in trypsin-PBS buffer (B) after 24 h. DLS analyses of the as prepared BSSO NPs before and after 24 and 48 h of enzymatic degradation (C).
Figure 3.14. TEM images of BSSO-FITC NPs in the trypsin-PBS buffer after 24 h (A) and 48 h (B). DLS analyses of BSSO-FITC NPs before and after 24 and 48 h of enzymatic degradation (C).

Figure 3.15. FTIR spectra for BSSO and enzymatically-degraded BSSO NPs.
Figure 3.16. UV-Visible spectra comparison of BSSO and BSSO-FITC NPs displaying the absorption of the dye at $\lambda_{ex}=505$ nm in BSSO-FITC nanomaterial (A). Zeta potential charge distributions of BSSO and BSSO-FITC NPs (B).

Figure 3.17. In vitro studies of BSSO-FITC NPs. Cell cytotoxicity of HeLa cells incubated with BSSO-FITC NPs for 24 h (A). CLSM images of BSSO-FITC NPs. Nuclei are stained in blue with DAPI dyes (B), cells membranes in red with CellMask™ (C), and BSSO-FITC NPs appear with the green fluorescence (D, merged in E). Scale bars of 20 μm.
Figure 3.18. CLSM image (3D) of HeLa cells after incubation with BSSO-FITC NPs displaying the partial endocytosis and cell adhesion of BSSO-FITC NPs after 6 h of incubation.

Figure 3.19. CLSM images (Z-stack gallery) of HeLa cells after incubation with BSSO-FITC NPs.
3.5 Conclusions

In summary, we describe for the first time enzymatically-degradable bridged silsesquioxane hybrid nanomaterials based on nature-inspired oxamide bridges with the organosilica framework. The designed nanomaterials were non-aggregated with biologically relevant sizes for preferential accumulation in tumors. The unique constitution of the materials with a very high organic content (~50%) was found to be homogenously distributed within individual particle which is probably key for the degradation behavior of the system. The biodegradation of NPs was demonstrated in the presence of the trypsin enzymes in simulated biological media. Furthermore, such nanoplatform could be rendered fluorescent by fluorescein dyes in order to image cancer cells. This work was extended by Croissant et al. through the introduction of a porosity and studying its impact on the extent and kinetics of the biodegradability of oxamide-based BSSO NPs to achieve safer biomedical nanotools.59
3.6 References


Chapter 4. Photoresponsive Bridged Silsesquioxane Nanoparticles with Tunable Morphology for Light-Triggered Plasmid DNA Delivery

4.1 Introduction

BSSO nanomaterials with chemical structures $O_{1.5}$Si-R-SiO$_{1.5}$, where R stands for organic group, are emerging as the next generation of organosilica hybrid materials.\textsuperscript{1-9} Nonetheless, it remains a challenge to control the kinetics in sol-gel processes, which generally leads to macroscale nonporous BSSO functional materials.\textsuperscript{10-12} Their design involves kinetically controlled sol-gel processes of bis- or multi-organoalkoxysilanes which yield to hybrid materials with very high organic contents depending on the type of organic groups.\textsuperscript{1} BSSO materials substantially differ with organically-modified silica, also called ORMOSIL, which are organically-doped silica materials and thus possess lower organic contents. Consequently, the BSSO matrix photophysical, chemical, thermal and mechanical properties are governed by the homogenously distributed organic fragments within the siloxane network.\textsuperscript{13} Therefore, BSSO materials with features such as luminescence,\textsuperscript{14} magnetism,\textsuperscript{15} and self-assembly were designed\textsuperscript{16} for various applications including catalysis,\textsuperscript{17} solid-state lightning,\textsuperscript{18} energy, and electronics.\textsuperscript{13}

Nanoscale BSSO materials are thus highly desirable in these days of miniaturized devices and functional nanomaterials. Shea et al. pioneered BSSO NPs with photodeformable particles based on coumarin dimer bridges.\textsuperscript{3} The material was composed of 100 nm aggregated spherical particles. The same group later reported bipyridinium- and ethylenediamine-based BSSO spherical monodisperse particles,\textsuperscript{4} as well as phenylene, alkylene, and aminoalkyl bridges.\textsuperscript{19} Recently, several studies described BSSO NPs for biomedical applications, namely drug delivery with cis-platin bridges as pro-drug,\textsuperscript{20} photothermal drug release with sandwich-like molybdenum sulfide/mesoporous
ethane-based BSSO nanosheets, in vitro fluorescent imaging with biodegradable BSSO nanoprobes comprised of oxamide bridges, MRI imaging with BSSO NPs composed of gadolinium-complexed bridges, Hayashi et al. performed in vivo photodynamic therapy (PDT) with spherical BSSO particles incorporating tetra-alkoxysilylated porphyrin and iodopropyl groups. Croissant et al. reported BSSO and Au@BSSO core-shell NPs designed from a tetra-alkoxysilylated two-photon photosensitizer and efficiently applied them for two-photon fluorescence imaging and photodynamic therapy in cancer cells. They recently designed disulfide-based biodegradable BSSO NPs chemically-doped with diphenylbutadiene and porphyrin photosensitizers for two-photon photodynamic therapy and imaging in vitro. BSSO NPs with charge reversal from negative to positive values were reported with aminopropyl-bridges with o-nitrobenzyl pending groups and applied for light-triggered hydrogel assembly and plastic antibody release. Reported BSSO nanomaterials were almost exclusively dense nanospheres, with the exception of perylenediimide-bridged nanoribbons endowed with electronic properties obtained by Hammer and co-workers, and several microfibers with diameters of several hundred nanometres. The aggregation of BSSO NPs is often observed and remains a major drawback for future biomedical applications. Ideally, BSSO NPs should be non-aggregated sub-200 nm nanomaterials to benefit the EPR effect and thus accumulate in cancerous tissues and organs. The delivery of plasmid DNA was achieved with ORMOSIL and mesoporous silica NPs, but not with BSSO NPs, and necessitated complex multistep postfunctionalizations of polymer and coupling agent.
4.2 Design

Herein we report the controlled syntheses of sub-200 nm non-aggregated BSSO nanospheres with tunable morphology with dense or hollow nanostructure designed from sol-gel processes of a photoresponsive bridged alkoxy silane (PBA) precursor (see Figure 4.1). The unique constitution of these BSSO NPs enables the on-demand surface charge reversal via light-trigger from positive to negative charges. Furthermore, this feature was harnessed to apply BSSO nanocarriers without further functionalization for the first time for light-triggered plasmid DNA delivery in cancer cells. The delivery of pDNA was studied through the production of the green fluorescent protein as a reporter gene. As well, single stranded DNA labeled with Cy3 was used to assess the intracellular penetration of BSSO NPs.

**Figure 4.1.** The design of BSSO and hollow BSSO NPs via the sol-gel reactions of the PBA precursor, as shown by TEM micrographs.
4.3 Experimental Section

4.3.1 Materials. Absolute ethanol, 3-aminopropyltriethoxysilane (APTES), 4-(bromomethyl) 3-nitrobenzoic acid, dimethylformamide (DMF), sodium hydroxide, sodium sulfate, ammonium nitrate, cetyltrimethylammonium bromide (CTAB), phosphate buffered saline (PBS, pH 7.4), ethyl acetate, hexane, rhodamine B, and Cell Counting Kit-8 (CCK-8) were purchased from Sigma-Aldrich. Oxalyl chloride and N,N-diisopropylethylamine (DIPEA) were obtained from Alfa Aesar. Anhydrous dichloromethane (DCM) was purchased from Acros. All chemicals were used without further purification. Deionized water was used in all procedures. The human cervical tumor cell line (HeLa) was purchased from ATCC. Eagle’s minimum essential medium (EMEM), Dulbecco’s phosphate buffered saline (DPBS, pH 7.4), fetal bovine serum (FBS), penicillin-streptomycin, paraformaldehyde, trypsin, 4,6-diamino-2-phenylindole (DAPI), agarose, gel loading buffer, SYBR® Gold nucleic acid gel stain, and TBE buffer were purchased from Invitrogen. Plasmid DNA (pDNA, 4.5 kbp) was purchased from GeneCopoeia, while Cy3-labeled single stranded DNA (ssDNA) was purchased from IDT.

4.3.2 Methods: Synthesis and Characterization. The PBA precursor was first synthesized and characterized. The synthesis involved a soft chlorination of 4-(bromomethyl)-3-nitrobenzoic acid followed by a coupling with 2 equivalents of APTES in DCM under base-catalysis at 0 °C (Figure 4.2).²⁹ BSSO nanomaterials were then synthesized via sol-gel method. Controlled design of BSSO NPs with different sizes was achieved via a modified Stöber method.³⁰ An aqueous mixture containing CTAB, NaOH, and EtOH as a co-solvent was prepared at 75 °C. Then, the hydrolysis-condensation of the
PBA precursor was conducted for 2 h. Such conditions lead to the formation of BSSO NPs with size under 200 nm, as shown by TEM (see top image in Figure 4.1). Interestingly, we unexpectedly found that without the use of CTAB in a certain range of experimental conditions (pH, PBA concentration, etc.) the formation of the hollow BSSO nanostructures was observed (see the bottom image in Figure 4.1). A number of physicochemical and biological studies was performed to describe fully the PBA precursor and derivative nanomaterials. The obtained results will be discussed further in the chapter.

**Synthesis of PBA.** To a DCM solution (5 mL) of 4-(bromomethyl) 3-nitrobenzoic acid (200 mg, 0.77 mmol) was added an oxalyl chloride solution in DCM (0.42 mL, 0.84 mmol, 2 M). Catalytic drops of dried DMF were added to initiate the reaction. The reaction mixture was stirred for 2 h at 0°C. Then, DCM with the excess amount of oxalyl chloride was removed under vacuum. The dried product was dissolved in DCM (2 mL) and added to a DCM solution (5 mL) of APTES (0.38 mL, 1.62 mmol) with DIPEA (0.30 mL, 1.70 mmol) at 0°C. After complete addition, the reaction mixture was removed from the ice and stirred at room temperature for 14 h. The reaction mixture was washed with cold water, extracted thrice with DCM (5 mL), and dried with Na$_2$SO$_4$. The crude liquid was purified via column chromatography using ethyl acetate/ hexane (1:1) as an eluent to yield a yellow oil of the PBA (311 mg, 67%).

![Figure 4.2. Synthetic pathway to design the photosensitive PBA precursor.](image)
Synthesis of dense BSSO NPs. The synthesis was performed according to a modified procedure of Croissant et al. A mixture of CTAB (6.4 mg, 17.5 µmol), distilled H₂O (1.5 mL), EtOH (0.1 mL) and NaOH (13 µL, 1 M) was stirred at 75 °C for 50 min at 1000 rpm in the dark. Then, the stirring speed was increased to 1400 rpm and an ethanolic solution of the PBA (3 mL, 0.05 mmol, 0.167 M) was added, and the sol-gel process was conducted for 2 h at 80 °C. The solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and NPs were collected by centrifugation for 15 min at 14000 rpm. The sample was then washed three times with ethanol, water, and ethanol. The sample was then dispersed in water in a propylene tube, sonicated 20 min, and allowed to sediment for 20 min. The supernatant containing the stable colloidal NPs was taken out, centrifuged 15 min at 14000 rpm and dried under vacuum few hours. It was found out by DLS analyses that with increasing of CTAB concentration BSSO NPs lose their monodispersity with an increase of the average size. The same effect was observed when the pH was increased (see Table 1).

Synthesis of hollow BSSO NPs. The key difference of this procedure was the absence of CTAB in the mixture which allowed the hollow structure formation in certain conditions. A mixture of distilled H₂O (1.5 mL) and NaOH (13 µL, 1 M) was stirred at 75 °C for 50 min at 1000 rpm in the dark. Then, the stirring speed was increased to 1400 rpm and an ethanolic solution of the PBA (0.18 mL, 24 µmol, 0.133 M) was added, and the condensation process was conducted for 2 h at 80 °C. The solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and the NPs were collected by centrifugation for 15 min at 14000 rpm. The sample was washed three times
with ethanol, water, and ethanol. The sample was then dispersed in water in a propylene tube, sonicated 5 min, and allowed to sediment for 20 min. In comparison with dense BSSO NPs, hollow BSSO NPs were sonicated for a shorter period (5 vs 20 min) in order to avoid the possible NPs degradation through a cavitation effect. The supernatant containing the stable colloidal NPs was taken out, centrifuged 15 min at 14000 rpm and dried under vacuum few hours.

**NMR Spectroscopy.** $^1$H NMR and $^{13}$C NMR spectra were performed at 500 MHz and 127 MHz with CDCl$_3$ solutions at 5 mg·mL$^{-1}$ with an Avance™ III Bruker™ Corporation instrument. Chemical shifts were referenced to external TMS.

**UV-Vis-IR Spectroscopy.** FTIR spectra were recorded on a Thermo Scientific™ Nicolet™ iS™10 FT-IR Spectrometer. UV-Vis absorption spectra were recorded on a Varian Cary 5000 spectrophotometer. All UV-Vis spectra were acquired in a quartz cell (sealed by Teflon cap), background correction was done with deionized water. The concentrated solution of the PBA precursor was prepared in absolute ethanol, placed on the face of KBr pellet, dried, and used to obtain FTIR spectrum. The powder samples of CTAB, BSSO and BSSO-FITC NPs were grounded with KBr to prepare pellets for FTIR analyses.

**DLS and Zeta Potential Analyses.** DLS and zeta potential measurements were performed using a Malvern Nano ZS instrument at 25 °C. Each measurement was conducted three times to validate the numbers.

**Electron Microscopy.** TEM images were recorded with a Technai™ T12 (FEI Company) microscope operated at 120 kV. A drop of an aqueous dispersion of NPs was placed directly on a carbon-coated copper grid and dried at room temperature. SEM images were
recorded with a field emission Nova™ NanoSEM 630 microscope (FEI Company). A drop of an aqueous dispersion of NPs was placed directly on a carbon film attached to aluminum pin stub and dried at room temperature. The elemental mapping and high-resolution TEM of particles were carried out with another TEM of model Titan™ G² 80-300CT from (FEI Company) which was equipped with a post-column energy filter of model GIF Tridiem® 863 (Gatan Inc.). The GIF was used in EELS mode combined with HAADF-STEM. Moreover, Si-L₂3 (99 eV), S-L₂3 (165 eV), C-K (283 eV), N-K (401 eV), O-K (532 eV), and Cl-L₂3 (200 eV) energy-loss edges were utilized in making Si, S, C, N, O and Cl elemental maps, respectively.

**CLSM.** CLSM images were acquired with Olympus™ FluoView FV1000 confocal microscope.

**TGA.** TGA was done on TG 209 F1 Iris® (Netzsch) under nitrogen atmosphere from 30 to 800 °C, with a heating rate of 10 °C·min⁻¹.

**Spectroscopic Monitoring of the Photoreaction.** Photoresponsive experiments were performed via the following laser: OmniCure® UV curing system, series 2000 (Lumen Dynamics):

- **Monitoring via UV-Vis Spectroscopy.** A solution of the PBA was diluted with an ethanol to 0.12 mg·mL⁻¹ prior to light irradiation. Then, this diluted solution (2.5 mL) was placed in a quartz cell at 10 cm from the 365 nm laser. The fluence was of 24.6 mW·cm⁻². At various time intervals, the cell was moved to measure the UV-Vis spectrum. The distance between the sample and the laser and the irradiation parameters were identical for all irradiation experiment in this work.
• Monitoring via $^1$H NMR Spectroscopy. The photoreaction was also confirmed by comparing the $^1$H NMR spectrum of the PBA before and after irradiation. The PBA was dissolved in CDCl$_3$ at 5 mg·mL$^{-1}$ and the NMR tube was placed 10 cm below the laser. After the irradiation, the sample was analyzed by $^1$H NMR.

• Monitoring via FTIR Spectroscopy. A KBr plate was coated with a drop of an ethanolic solution of the PBA (0.04 mg·mL$^{-1}$) and dried on the air for 2 h. The process was repeated several times to afford a measurable amount of the PBA via FTIR. Then, the KBr-PBA plate was placed under the 365 nm laser with a fluence of 24.6 mW·cm$^{-2}$. At various time intervals, the plate was moved to measure the FTIR spectrum. The distance between the sample and the laser and the irradiation parameters were identical for all irradiation experiment in this work.

BSSO-Plasmid DNA NPs Conjugation. Plasmid DNA (pDNA, 4.5 kbp) was introduced to an aqueous suspension of hollow BSSO NPs (300 µL, 1.1 mg·mL$^{-1}$). The suspension was agitated at room temperature for 3 h. Then, the solution was washed three times with PBS buffer (10x).

BSSO-ssDNA NPs Conjugation. Cy3 labeled ssDNA (cy3-aaaaaaaaaaaaaaaaaaaaaaaaatgcggccagatgtgacgcagatggccagagtcaggc, 1 µL, 280 ng·mL$^{-1}$) was introduced to an aqueous suspension of hollow BSSO NPs (300 µL, 1.1 mg·mL$^{-1}$). The suspension was agitated at room temperature for 3 h. Then, the suspension was washed three times with PBS buffer (10x).

Gel Electrophoresis. Gel electrophoresis was carried out to confirm the conjugation of pDNA and to identify the optimal pDNA coating on BSSO NPs. The mixture was
electrophorized on an agarose gel (2%). The free pDNA migrates in the gel whereas bound pDNA remains in the wells. The optimal ratio of BSSO NPs over pDNA was found to be 1 to 1 (µg·µg⁻¹).

**Cell Culture and Cell Viability Study.** HeLa cells were cultured in EMEM containing 10% of FBS and penicillin-streptomycin at 37 °C in a humidified 5% CO₂ atmosphere. The cytotoxicity of BSSO NPs was evaluated using the CCK-8 assay. HeLa cells were seeded at a density of 5×10³ cells per well in 96-well flat bottom plates and incubated for 12 h. Cells were washed with DPBS buffer and incubated in the culture media with BSSO NPs for 12 h at 37 °C. Cell viability was evaluated by the CCK-8 colorimetric procedure.

**CLSM Study for pDNA Delivery.** HeLa cells were seeded in CLSM dish and cultured in EMEM containing 10% of FBS and 0.1% of penicillin–streptomycin at 37 °C in a humidified 5% CO₂ atmosphere. After cell attachment, BSSO-pDNA NPs 25 µg·mL⁻¹ were added and incubated for 6 or 12 h. Then, cells were fixed in 4% of paraformaldehyde. Finally, nuclei were stained with DAPI for 10 min and then washed three times with DPBS. The delivery of pDNA was assessed by the GFP fluorescence, and the transportation of single ssDNA was tracked via Cy3 labelling.
4.4 Results and Discussion

The chemical structure of the precursor was confirmed via $^1$H, $^{13}$C NMR, and FTIR spectroscopies (Figures 4.3-4.4). The successful synthesis of the PBA precursor allowed us further to apply it in the generation of BSSO nanomaterials with dense and hollow morphologies. The physicochemical properties of the BSSO NPs were then characterized by various techniques.

As it was mentioned earlier (Chapter 4.3.2) we surprisingly discovered that without the use of CTAB in a certain range of temperature, pH, and PBA concentration the formation of the hollow BSSO nanostructures was observed, contrary to the modified Stöber method. In fact, the diameter of NPs as well the formation of the hollow or non-hollow structure could be tuned by varying four parameters: pH, temperature, PBA concentration, and type of an alcohol co-solvent (see Figure 4.5, Tables 1 and 2). The formation of hollow particles could be achieved from 50 to 90 °C and pH 11-12 (Figure 4.5B-C), while no correlation was observed between the size and temperature. The possibility to form hollow BSSO NPs with methanol, ethanol, and i-propanol as co-solvents (and not from butanol) suggests the formation of nanoemulsions in specific conditions which promote the growth of hollow particles (Figure 4.5D).

The size, morphology, and composition of BSSO nanomaterials are the subjects of further discussion. Thus, SEM micrographs displayed relatively monodisperse dense and hollow BSSO spherical NPs (Figure 4.6A-B), with an average hydrodynamic diameters of 190 and 106 nm respectively (Figure 4.6C-D), which is in accordance with microscopy images and the hydration layer on NPs.
Figure 4.3. $^1$H (A) and $^{13}$C (B) NMR spectra of the PBA precursor. $^1$HNMR (500 MHz, CDCl$_3$) δ (ppm) 8.32 (d, J = 1.4 Hz, 1H), 8.02 (d, J = 2.1 Hz, 1H), 7.73 (d, J = 10.1 Hz, 1H), 6.88 (m, 1H), 4.06 (s, 2H), 3.80 (m, 12 H), 3.34 (m, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.76 (m, 2H), 1.73 (m, 2H), 1.20 (m, 18H), 0.72 (t, J = 9.6 Hz, 3H), 0.64 (m, 3H). $^{13}$C NMR (125.7 MHz, CDCl$_3$) δ (ppm) 165.0, 148.9, 139.2, 134.8, 131.6, 131.4, 123.2, 58.7 (3C), 58.5 (3C), 52.4, 50.6, 42.4, 23.4, 22.8, 18.4 (3C), 18.3 (3C), 7.9 (2C).
Figure 4.4. FTIR spectrum of the PBA precursor. FTIR (cm\(^{-1}\)): 3722, 3456, 3100, 2973, 2929, 2884, 1643, 1538, 1450, 1350, 1310, 1097, 1097, 950, 785, 674.

Table 1. Synthesis of the BSSO NPs from the monomer PBA using different conditions. The amount of the PBA is constant (0.05 mmol). *PDI=polydispersity in DLS analyses. *Average NPs size determined by DLS analyses. "Synthesis of the BSSO NPs was described in Chapter 4.3.2."
<table>
<thead>
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<th>Sample</th>
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<th>PBA (µmol)</th>
<th>NaOH (µL)</th>
<th>Alcohol (µL)</th>
<th>Temp. (°C)</th>
<th>D avg (TEM)* (nm)</th>
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<td>13 (1 M)</td>
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<td>35</td>
</tr>
<tr>
<td>3</td>
<td>hollow</td>
<td>12.5</td>
<td>13 (1 M)</td>
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*Table 2. The tunability of the size and morphology of the BSSO nanomaterials according to the experimental conditions. *Average size of NPs was determined by statistical TEM analyses (400 NPs).
Figure 4.5. The influence of the PBA concentration (A), temperature (B), pH (C), and alcohol co-solvent type (D) on the diameter of NPs. Black and white circles respectively indicate the formation of dense and hollow BSSO NPs. No CTAB was used for all syntheses.

Figure 4.6. SEM micrographs and DLS size distributions of dense (A, C) and hollow BSSO NPs (B, D).
The incorporation of organic bridges into the silica framework of dense and hollow BSSO NPs was proved by FTIR spectroscopy with the $\nu_{\text{C=O}}$ at 1645 cm$^{-1}$, $\nu_{\text{N-H}}$ at 3289 cm$^{-1}$, $\nu_{\text{N-O}}$ at 1535 cm$^{-1}$, as well as aliphatic and aromatic C-H stretching modes (Figure 4.7). The high degree of condensation of siloxanes was confirmed by the shift of the $\nu_{\text{Si-O}}$ value from 1090 to 1130 cm$^{-1}$. TGA data for BSSO NPs confirmed the high organic content of the nanohybrids with two significant weight losses at 220 and 480 °C according to the first derivative of the thermogravimetric (DTG) curve, leading to a total weight loss of 46-48% (Figures 4.8A-B). The initial stage of calcination includes, for both nanomaterials, loss of ethanol and water as solvents, which were used for syntheses and washings. Moreover, we utilized spectrum imaging (SI) in STEM combined with EELS to assess the homogeneity in the composition of hollow BSSO NPs. Spectra were acquired at each pixel image (~1 nm) on a representative particle, and the elemental mappings of silicon, oxygen, nitrogen, and carbon were extracted (Figure 4.9A). The results clearly exhibit the homogenous dispersion of the organic moieties within the NPs framework. This conclusion was further supported by the homogeneous composition of these elements along the diameter of hollow BSSO NPs (Figure 4.9B). Additionally, we also successfully prepared rhodamine B-doped hollow BSSO NPs of 50 nm with the homogenous composition of dye in the nanostructure (Figure 4.10).

The light-responsiveness of the BSSO nanomaterials was then assessed and monitored spectroscopically. In a typical experiment, an aqueous suspension of NPs was irradiated with UV-light at 365 nm (24.6 mW·cm$^{-2}$). We first analysed the irradiation of the positively-charged o-nitrophenylene-ammonium PBA precursor which caused a
classic internal photocleavage leading to the formation of the neutral the nitrosophenylene-imine derivative (Figure 4.11A). The comparison of the 4.1-3.75 ppm areas of the $^1$H NMR spectra of the PBA precursor before and after irradiation demonstrated the disappearance of the peak at 4.05 ppm (Ph-CH$_2$-NH-, see Figure 4.12). Also, the generation of the nitrosophenylaldehyde was confirmed by monitoring the increase of the broad peak around 300 to 400 nm with its further decreasing when the imine was formed (see Figure 4.11B). The photochemical changes were additionally supported by the shift of the symmetric stretching $\nu$$_{N-O}$ vibration mode from 1540 to 1550 cm$^{-1}$ (Figure 4.13). Besides, the same trends were observed in BSSO nanomaterials (Figure 3B-C, see FTIR $\nu$$_{N-O}$ shift in Figure 4.14). The photo-reaction monitored via UV-Visible spectroscopy was found to necessitate 10 minutes of irradiation for a full conversion (see Figure 4.11C), which was accompanied by a progressive modification of the surface charge of BSSO NPs from +46 to -5, -30 and -39 mV after 4, 8, and 10 minutes of irradiation respectively (Figure 4.15A). Upon illumination, o-nitrophenylene-ammonium moieties on the surface of BSSO NPs turned into neutral nitrosophenylene-imine moieties, which resulted in a negative surface charge afforded by silanolates groups (Figure 4.16).

The unique charge reversal feature was thus applied for the electrostatic binding of negatively charged pDNA and its subsequent delivery to cancer cells (Figure 4.15B-D). BSSO NPs demonstrated biocompatibility with HeLa cells, as shown by less than 10% of cytotoxicity up to 20 $\mu$g·mL$^{-1}$ (Figure 4.17A). The electrostatic binding of pDNA (4.5 kbp, ~20 nm) on positively charged BSSO NPs was confirmed via gel electrophoresis and zeta potential measurements, with 7.5 ng of bound pDNA per 1 $\mu$g of NPs (Figure 4.17B-C).
Figure 4.7. FTIR spectra for dense and hollow BSSO NPs.

Figure 4.8. TGA data for dense (A) and hollow BSSO NPs (B).
Figure 4.9. STEM-EELS elemental mapping (silicon, oxygen, nitrogen, carbon) of a representative hollow BSSO NP. The merged image consists of silicon, nitrogen, and oxygen (A). Scale bar of 50 nm. Spectrum image of a representative hollow BSSO NP (A) for the elemental analyses of carbon, nitrogen, oxygen, and silicon (B) along the diameter of the particle (ca. 120 nm).
Figure 4.10. Schematic representation of rhodamine B-doped hollow BSSO NPs (A). The dye was clearly observed via UV-Visible spectroscopy with a typical absorption maximum at 560 nm (B). TEM micrograph (C) and statistical size analysis of BSSO NPs (D). STEM-EELS elemental mapping (nitrogen, carbon, oxygen, silicon, chlorine) revealing the homogeneity of the hybrid composition of the dye-doped BSSO NPs from a representative spectrum image (SI, top left) (E).
Figure 4.11. Photoreaction of the PBA precursor (A). UV-Visible spectroscopy monitoring of the photoreaction in the PBA in absolute ethanol (B) and in an aqueous suspension of the BSSO NPs (C) before and after irradiation for different time intervals.

Figure 4.12. $^1$H NMR 4.1-3.75 ppm area of the PBA precursor before and after irradiation with UV-laser (A and B respectively). The photoreaction involves most likely the intermediate presence of the primary amine and the nitroso-phenylene aldehyde.$^{34-36}$
Figure 4.13. Monitoring of the photoreaction of the PBA via FTIR spectroscopy in KBr pellet before and after irradiation with UV-laser for different time intervals.

Figure 4.14. Monitoring of the photoreaction within BSSO NPs via FTIR spectroscopy in KBr pellet with various durations of UV-irradiation.
Figure 4.15. Zeta potential measurements on hollow BSSO NPs before and after irradiation, depicting the NPs surface charge reversal (A). Schematic representation of positively-charged BSSO NPs (B) which electrostatically bind pDNA (C) for light-triggered delivery (D). The negative charge of NPs results from the neutralisation of the charge of the organic bridges.

Figure 4.16. The proposed mechanism of the reversal charge of BSSO NPs upon UV-irradiation.
Figure 4.17. HeLa cells viability study with the incubation of BSSO NPs at various concentrations (A). Gel electrophoresis of BSSO-pDNA NPs with different NPs over pDNA ratios (µg·µg⁻¹) demonstrating the binding of the pDNA on BSSO NPs (B). Zeta potentials of BSSO and BSSO-pDNA NPs before (red and black curves respectively) and after 40 s of UV-irradiation (green and blue curves respectively). The negative surface charge of BSSO-pDNA NPs after UV-irradiation (blue curve) confirmed the partial release of pDNA from its surface, otherwise, it should be positive (green curve) (C).

Hollow BSSO NPs were found to be tolerated by in HeLa cells, as shown by less than 10% of cytotoxicity up to 20 µg·mL⁻¹ (Figure 4.17A). The electrostatic binding of pDNA (4.5 kbp, ~20 nm) on positively charged BSSO NPs was confirmed via gel electrophoresis and zeta potential measurements, with 7.5 ng of bound pDNA per 1 µg of NPs (Figure 4.17B-C). HeLa cancer cells were then incubated with BSSO-pDNA NPs at 11 µg·mL⁻¹ for 12 h. Nuclei were stained with DAPI and the delivery of pDNA was assessed via the production of the green fluorescent protein (GFP) as a reporter gene. Indeed, the fluorescence of GFP necessitates the transcription of pDNA in the nucleus with subsequent mRNA translation, which implies the detachment of pDNA from BSSO NPs. CLSM images were thus acquired to determine the pDNA delivery capability of the designed hollow BSSO NPs (see Figure 4.18). Before irradiation, a small amount of GFP could be seen, which indicated that a
small fraction of DNA were autonomously delivered to cells via NPs (Figure 4.18A). However, when the UV-irradiation was turned on (365 nm, 24.6 mW·cm$^{-2}$, 40 s), a sufficient amount of pDNA was delivered in the cytosol (see Figure 4.18B) through photoinduced electrostatic repulsions (Figure 4.15C-D). This effect was also clearly seen after only 6 h of incubation (Figure 4.19). Notably, zeta potential measurements for BSSO NPs didn’t show full charge reversal from positive to negative after 40 s of UV-irradiation. Although, it was enough to decrease electrostatic binding and consequently increase repulsions between negatively charge silanolate groups and pDNA. As a result, partial release of pDNA from the surface of BSSO NPs was observed (Figure 4.17C). Note that, ssDNA was also attached to BSSO NPs (53 ng·mg$^{-1}$), transported, and tracked via Cy3 cyanine labelling, while bright field images showed the intracellular co-localizations of aggregated BSSO NPs and ssDNA-Cy3 fluorescence (Figure 4.20).

**Figure 4.18.** CLSM images of HeLa cells after 12 h of incubation with non-irradiated (A) and irradiated BSSO-pDNA NPs (B). Nuclei are stained in blue with DAPI. Plasmid DNA is tracked via GFP fluorescing in green, thus proving the pDNA delivery from BSSO NPs. Scale bars of 40 µm.
Figure 4.19. CLSM images of HeLa cells incubated with BSSO-pDNA NPs after 6 h of incubation. Plasmid DNA is tracked via GFP fluorescing in green after translation in the nuclei, thus proving the pDNA delivery from BSSO NPs. Scale bars of 40 µm.

Figure 4.20. CLSM images of HeLa cells incubated with BSSO-ssDNA NPs after 12 h of incubation. Nuclei are stained with DAPI and ssDNA is tracked via green fluorescence of Cy3. Arrows indicate internalized BSSO-ssDNA NPs.
4.5 Conclusions

In summary, we report the syntheses of bridged silsesquioxane nanomaterials with tunable size and morphology, affording non-aggregated dense or hollow nanospheres. The organic-inorganic nanomaterials possessed a very high organic content (~50%) of photoresponsive fragments which enabled the on-demand charge reversal from positive to negative values. The hybrid compositions of the designed materials were investigated via various techniques and found to be homogenous in the NPs, while its photoresponsiveness was monitored spectroscopically. As a proof of concept, these BSSO NPs were exploited for the biomedical application. The light-triggered delivery of plasmid DNA with BSSO-pDNA NPs to cancer cells in culture was demonstrated for the first time. The light-actuation was found to be effectively delivering plasmid DNA while the non-irradiated nanomaterials did not induce significant gene expressions. Dye-doped hollow BSSO NPs are envisioned for biomedical imaging while the use of a near-infrared fluorophore could extend its potential for in vivo biomedical applications.
4.6 References


Chapter 5. Cellular Internalization and Biocompatibility of Periodic Mesoporous Organosilica Nanoparticles with Tunable Morphologies: from Nanospheres to Nanowires

5.1 Introduction

Among a large variety of porous functional nanomaterials, MSNs have attracted a lot of interests for possessing unique chemical and physical properties, thermal and mechanical stabilities, and a high biocompatibility.\(^1\)\(^-\)\(^4\) MSNs are characterized by large specific surface areas, tunable and uniform pore sizes, controllable morphologies, and the well-established chemistry of silica which accounts for the extensive research reported in the past two decades on the subject.\(^2\)\(^,\)\(^5\) The chemical modifications of MSNs with organic moieties is usually carried out to impart new features to the nanomaterials for specific applications. The resulting inorganic-organic silica hybrid nanomaterials combine the advantages of a robust porous organic/inorganic framework, along with the intrinsic properties of the organic fragments designed for various applications.\(^6\)\(^-\)\(^8\)

Organically-bridged PMO were first introduced independently by three research groups in 1999 as an alternative to organically-doped mesoporous silica.\(^9\)\(^-\)\(^11\) Unlike conventional silica (SiO\(_2\)) or organically-doped silica, the bridged silsesquioxane structure of PMO maximizes the organic content in the materials which dramatically affects their physicochemical properties and performances.\(^12\)\(^-\)\(^13\) PMOs are prepared solely by hydrolysis and condensation of bridged-organoalkoxysilane precursors without using a classical silica precursor (e.g. TEOS or TMOS).\(^12\) However, the condensation of bridged-organoalkoxysilanes is quite challenging and often leads to nonporous bulk materials or particles with low porosities\(^13\)\(^-\)\(^14\) which are often aggregated.\(^15\)\(^-\)\(^16\) These obstacles to control the morphology and porosity of PMO NPs account for the much fewer studies reported on the subject when compared with MSNs.\(^12\)\(^,\)\(^17\)
Despite these challenges, nanoscale PMOs have been increasingly reported in the past few years.\textsuperscript{6, 18-25} Compared with MSNs, enhanced properties were obtained with PMO NPs in drug,\textsuperscript{23, 26-28} gene,\textsuperscript{29} and protein\textsuperscript{30} deliveries, catalysis,\textsuperscript{31-33} energy\textsuperscript{34} and separation.\textsuperscript{35} This was fueled by the unique characteristics and properties arising in the porous silsesquioxane frameworks of PMO NPs which were limited or foreign to MSNs.\textsuperscript{6, 12, 24} For example, the tunable hydrophilicity/hydrophobicity of the silsesquioxane pores enables unprecedentedly high cargo loading capacities of 80-100 wt\%,\textsuperscript{36-37} enhanced catalytic activities,\textsuperscript{38-41} outstanding carbon dioxide capture\textsuperscript{20} via PMO materials. The type of organic bridge can lower\textsuperscript{42-43} or trigger\textsuperscript{23, 26, 36} the degradability of PMO NPs in physiological conditions. The variety of properties and applications of organic molecules are theoretically available for PMO NPs, as long as their synthesis would permit it.\textsuperscript{12}

The versatility of syntheses and applications of PMO nanomaterials has been recently reviewed.\textsuperscript{12} Dense and hollow spherical PMO particles, as well as rod- and fiber-like morphologies, were described depending on the type of organic bridge. Helicoidal nanobundles of micrometer lengths were reported with ethylene-(\text{-CH}_2\text{-CH}_2\text{-}), ethynylene-(\text{-CH}=\text{CH-}), meta- and para-phenylene-bridged PMO via chiral organogelator templates.\textsuperscript{44} Helicoidal ethynylene-bridged PMO NPs were prepared with CTAB and (S)-\text{-\beta-}citronellol template.\textsuperscript{45} Ethenylene-disulfide-bridged PMO nanorods were also synthesized via CTAB templates.\textsuperscript{26} The controlled synthesis of ethane-based PMO nanorods with adjustable pore sizes from 2.6 to 7.3 nm was also reported.\textsuperscript{46} To the best of our knowledge, there are no reports on phenylene-bridged NPs with rod-like and wire-like nanostructures.
5.2 Design

Herein we report for the first time the controlled syntheses of para-phenylene-bridged PMO with various morphologies from nanospheres to bended rods, nanorods, and nanowires (Figure 5.1). The sol-gel reaction was carried out at low stirring speed with the 1,4-bis(triethoxysilyl)benzene precursor. All nanomaterials were fully characterized via various techniques and displayed mesostructures with 2.4-2.6 nm wide pores. The addition of different organic co-solvents with the PMO precursor was essential to modify the final shape of PMO NPs. The secondary roles of the stirring speed, temperature, and organosilica concentrations were also discussed. The influence of the morphology of the PMO NPs on their cellular internalization and biocompatibility was then studied. The control of the morphology of PMO nanomaterials is envisioned to foster biomedical investigations in regard to the role of the shape of PMO particles on their in vivo biodistribution and theranostic applications.

**Figure 5.1.** Schematic representation of the syntheses of PMO NPs with controlled morphologies via the addition of organic co-solvents (A), and their biocompatibility and cellular internalization studies (B).
5.3 Experimental Section

5.3.1 Materials. Cyclohexane, toluene, dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetone, tetrahydrofuran (THF), absolute ethanol, ammonium nitrate, sodium hydroxide, 1,4-bis-(triethoxysilyl)benzene, 3-aminopropyltriethoxysilane (APTES), fluorescein isothiocyanate (FITC), 3-trihydroxysilylpropyl methylphosphonate (THMP) cetyltrimethylammonium bromide (CTAB), doxorubicin (DOX), and Cell Counting Kit-8 (CCK-8) were purchased from Sigma-Aldrich. All chemicals were used without further purification. Deionized water was used in all procedures. The human cervical tumor cell line (HeLa) was purchased from ATCC. Eagle’s minimum essential medium (EMEM), Dulbecco’s phosphate buffered saline (DPBS, pH 7.4), fetal bovine serum (FBS), penicillin-streptomycin, paraformaldehyde, trypsin, and Hoechst 33342 were purchased from Invitrogen.

5.3.2 Methods: Synthesis and Characterization. PMO nanomaterials were first synthesized in a basic aqueous solution (pH 12) with CTAB as a structure directing agent at 75 °C, with stirring speed 450 rpm. The formation of PMO nanomaterials with various morphologies was controlled by the dissolution of the 1,4-bis(triethoxysilyl)benzene sol-gel precursor in various organic co-solvents: toluene, cyclohexane, and DMF. All organic co-solvents were aprotic to avoid the premature hydrolysis and condensation of the alkoxysilanes and had various polarities. PMO nanowires (W-PMO) were obtained with pure toluene (100 %) co-solvent, nanorods (R-PMO) with pure cyclohexane (100 %), bended nanorods (BR-PMO) with a mixture of toluene/DMF (40/60 %, v:v), and nanospheres (S-PMO) with pure DMF (100 %).
Preparation of W-PMO Nanomaterial. A mixture of CTAB (125 mg, 3.43·10^{-1} mmol), distilled water (60 mL), and sodium hydroxide (438 µL, 2 M) was stirred at 75 °C for 30 min at 450 rpm in a 100 mL round bottom flask. Then, 1,4-bis(triethoxysilyl)benzene (150 µL, 3.75·10^{-1} mmol, 1 M) in 375 µL of toluene was added to the previous solution, and the condensation process was conducted for 2 h. Then, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation during 15 min at 14 krpm. The as-prepared sample was sonicated twice with an ethanolic solution of ammonium nitrate (6 g·L^{-1}) and washed three times with ethanol, water, and ethanol. Each washing step involved a sonication of 20 min at 40 °C; the collection was carried out in the same manner. The as-prepared CTAB-free PMO material was finally dried under vacuum for few hours.

Preparation of R-PMO, BR-PMO, and S-PMO nanomaterials. A similar procedure was used for the syntheses of R-PMO, BR-PMO, and S-PMO nanomaterials. A mixture of CTAB (125 mg, 3.43·10^{-1} mmol), distilled water (60 mL), and sodium hydroxide (438 µL, 2 M) was stirred at 75 °C for 30 min at 450 rpm in a 100 mL round bottom flask. Then, 1,4-bis(triethoxysilyl)benzene (150 µL, 3.75·10^{-1} mmol, 1 M) in 375 µL of toluene was added to the previous solution, and the condensation process was conducted for 2 h. Then, the material was collected, extracted, washed, and dried as described for W-PMO material.

Preparation of Fluorescent PMO (FPMO) nanomaterial. Following the synthesis and purification of every PMO nanomaterial, a certain amount of solid product (10
mg for each type of PMO NPs) was redispersed in distilled water (6 mL). Then, FITC-APTES (180 µL in EtOH, 5.00·10⁻³ mmol) and THMP (20 µL, 5.26·10⁻² mmol) were added to each aqueous dispersion of PMO NPs and stirred for 45 min at 60 °C. After this time, dispersions were cooled to room temperature while stirring, and FPMO samples were collected and extracted as described for W-PMO material.

**UV-Vis-IR Spectroscopy.** FTIR spectra were recorded on a Thermo Scientific™ Nicolet™ iS™10 FT-IR Spectrometer. UV-Vis absorption spectra were recorded on a Varian Cary 5000 spectrophotometer. All UV-Vis spectra were acquired in a quartz cell (sealed by Teflon cap), background correction was done with deionized water. The powder PMO samples were grounded with KBr to prepare pellets for FTIR analyses.

**DLS and Zeta Potential Analyses.** DLS and zeta potential measurements were performed using a Malvern Nano ZS instrument at 25 °C. Each measurement was conducted three times to validate the numbers.

**Electron Microscopy.** TEM images were recorded with a Technai™ T12 (FEI Company) microscope operated at 120 kV. A drop of an aqueous dispersion of NPs was placed directly on a carbon-coated copper grid and dried at room temperature. SEM images were recorded with a field emission Nova™ NanoSEM 630 microscope (FEI Company). A drop of an aqueous dispersion of NPs was placed directly on a carbon film attached to aluminum pin stub and dried at room temperature.

**TGA.** TGA was done on TG 209 F1 Iris® (Netzsch) under a nitrogen atmosphere from 30 to 800 °C, with a heating rate of 10 °C·min⁻¹.
**Nitrogen Adsorption-Desorption Analysis.** N\textsubscript{2} sorption isotherms and corresponding pore size distributions were acquired to characterize the mesoporous structures of PMO NPs on a Micromeritics ASAP\textsuperscript{®} 2420 instrument.

**Low and Wide Angle X-ray diffraction (XRD).** Powder XRD measurements were performed using a PANalytical X’Pert Pro X-ray powder diffractometer using the Cu K\textalpha radiation (40 V, 40 mA, \(\lambda = 1.54056 \, \text{Å}\)) in a \(\theta - \theta\) modes from 0° to 5° and 10° to 60° (2\(\theta\)).

**CLSM.** CLSM images were acquired with FluoView FV1000 confocal microscope.

**Cell Culture and Cell Viability Study.** HeLa cells were cultured in EMEM containing 10% of FBS and penicillin-streptomycin at 37 °C in a humidified 5% CO\textsubscript{2} atmosphere. The cells were detached and collected for further counting and plating. Cells were seeded in 96 well plates at a density of 5\cdot10^{3} per well. After 24 h, cells were treated with different concentrations of FPMO NPs and incubated for 24 h at 37 °C. The culture medium was then discarded and 100 \(\mu\text{L}\) of CCK8 solution in DMEM was added to each well and incubated for 4 h in darkness. The absorbance values were measured at 590 nm using the xMark™ microplate absorbance spectrophotometer.

**Cell Transfection Study by CLSM.** HeLa cells were seeded on coverslips placed in 6 well plates and cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in a 5% CO\textsubscript{2} humidified atmosphere. After cell attachment, W-FPMO, R-FPMO, BR-FPMO, and S-FPMO NPs were incubated with the cells for 6 h at a final concentration of 10 \(\mu\text{g}\cdot\text{mL}^{-1}\). Cells were washed 3 times with DPBS buffer and nuclei were stained with Hoechst 33342 according to the manufacturer’s instructions. Cells were then washed again and fixed with 4% paraformaldehyde before imaged by upright CLSM.
5.4 Results and Discussion

SEM and TEM images were then used to characterize the size and the morphologies of the particles. Electron microscopy techniques displayed relatively uniform PMO NPs with the controlled wire, rod, and spherical particle morphologies (see Figures 5.2-5.4). The toluene/DMF mixture leads to the mixture of various morphologies from bended rods to gyroid particles (Figure 5.5).\textsuperscript{47-49} The average particles dimensions were determined by statistical counts with TEM images (Figure 5.2) for PMO nanospheres (154 nm), bended nanorods (110×184 nm), nanorods (117×537 nm), and nanowires (104×916 nm, though this morphology contained a minor fraction of rod-like NPs, Figure 5.2A). In some cases, the length of nanowires could reach up to 16 µm with a mesoscopic periodicity throughout all its length (Figure 5.6). Similar trends were obtained with DLS measurements (Figure 5.7).

The composition of the nanomaterial was assessed by FTIR spectroscopy and TGA. The comparison of the FTIR spectra of PMO nanomaterials consistently showed the same vibrations modes for all morphologies. The preservation of para-phenylene groups in all PMO NPs was supported by the presence of the aromatic $\nu_{\text{C=}}$ and $\nu_{\text{Si-C}}$ stretching vibration modes at 1638 cm$^{-1}$ and 1156 cm$^{-1}$, respectively, and the out-of-plane aromatic $\nu_{\text{Csp2-H}}$ bending modes at 918, 763, and 549 cm$^{-1}$ (Figure 5.8). Besides, with red-shift of the $\nu_{\text{Si-O}}$ mode from 1060 cm$^{-1}$ in 1,4-bis(triethoxysilyl)benzene precursor to 1070-1090 cm$^{-1}$ in the PMO NPs indicated the high condensation degree of the siloxanes. The silanolates groups on the surface of the NPs also account for the negative surface charge of all PMO NPs (Figure 5.9).
Figure 5.2. Evolution of morphology of phenylene-bridged PMO NPs with the variation of the organic co-solvent(s) in the reaction media (A). Representative TEM images depict the uniformity of PMO NPs (B), and statistical averages of the length (L) and diameter (D) of each nano-objects is plotted for each nanomaterial (C). For BR-PMO NPs the value L is taken as the longest distance between two outer points.

Figure 5.3. SEM images of PMO NPs with different morphologies: W-PMO (A), R-PMO (B), BR-PMO (C), and S-PMO NPs (D).
Figure 5.4. TEM images of W-PMO (A), R-PMO (B), BR-PMO (C), and S-PMO NPs (D).

Figure 5.5. Representation of gradual growth of BR-PMO NPs into gyroids based on TEM images.
Figure 5.6. TEM images of W-PMO at various magnifications confirming the mesoporous periodicity of the nanomaterial.
Figure 5.7. DLS measurements of W-PMO, R-PMO, BR-PMO, and S-PMO NPs.

Figure 5.8. FTIR spectra of PMO nanomaterials revealing vibration modes consistent with the phenylene-bridged silsesquioxane PMO structure.
Figure 5.9. Zeta potential measurements of PMO NPs showing their negative surface charge.

Figure 5.10. TGA of PMO nanomaterials revealing mass losses consistent with the phenylene-bridged silsesquioxane PMO structure.

Such conclusions were further confirmed by TGA which depicted two weight loss stages for all samples. The first stage between room temperature and 90 °C with a weight loss of 20 % can be assigned to the dehydration of the samples, the second one between 400 and 800 °C with a weight loss of about 30 % is associated with the decomposition of the organic bridges which theoretically account for 39 wt% in fully condensed PMO nanomaterials (Figure 5.10).18, 37, 50
The porosity of PMO nanomaterials was then assessed by nitrogen sorption analysis. The CTAB surfactant, which was found necessary to obtain porous nanomaterials, was fully extracted in the resulting NPs as shown by FTIR analyses (Figure 5.11). The total pore volumes and surface areas (determined with BET theory) of PMO respectively ranged from 0.36 cm$^3$·g$^{-1}$ and 520 m$^2$·g$^{-1}$ for W-PMO NPs to 0.86 cm$^3$·g$^{-1}$ and 1100 m$^2$·g$^{-1}$ for S-PMO NPs (Figure 5.12). All hysteresis loops belong to type H4 according to the IUPAC convention, with the characteristic step-down at P/P$_0$ near 0.4. Importantly, all the phenylene-based PMO NPs have medium-sized mesopores of diameter between 2.4 and 2.6 nm, which is slightly higher than those of previously reported phenylene-bridged PMO nanospheres. Furthermore, the low and wide angle XRD patterns confirmed the presence of molecular and mesoscopic periodicity in all PMO NPs, except the spherical particles which tend to have a disordered/radial mesoporosity. Low angle XRD patterns showed the typical peaks at low angles corresponding to pore to pore distances of 5 to 5.6 nm (Figure 5.13A, C, E, G), which is equal to the sum of pore diameter and the wall thickness. This was additionally supported by high magnification TEM images of PMO NPs (Figures 5.14-5.17). The periodic arrangement of the phenylene bridges and the formation of crystalline domains (7.5, 5.8, 9.6, and 4.7 nm for W-, R-, BR-, and S-PMO respectively) was demonstrated for all nanomaterials by wide-angle XRD (7.4-7.6 Å peaks) and the Scherrer’s equation (Figure 5.13B, D, F, H). The minor increase of pore sizes obtained in our process is the result of the co-solvent addition which then acts as a swelling agent inside surfactant-silicate micelles, similarly to mesoporous silica and organosilica materials.
Figure 5.11. FTIR spectra of PMO nanomaterials (W: PMO wires; R: PMO rods; BR: PMO bended rods; S: PMO spheres) before and after the CTAB extraction confirming the role of the template of CTAB molecules.

Figure 5.12. $N_2$-adsorption(▲)-desorption(●) isotherms and pore size distribution of W-PMO (A,E), R-PMO (B,F), BR-PMO (C,G) and S-PMO NPs (D,H). The BET calculations and Barrett-Joyner-Halenda (BJH) pore size distributions obtained from adsorption branches demonstrate the mesoporous nature of all the nanomaterials.
Figure 5.13. XRD diffractograms of PMO nanomaterials (W: PMO wires; R: PMO rods; BR: PMO bended rods; S: PMO spheres) at low (A, C, E, G) and wide angles (B, D, F, H).
Figure 5.14. TEM image of W-PMO confirming the mesoporous periodicity of the nanomaterial.

Figure 5.15. TEM image of R-PMO confirming the mesoporous periodicity of the nanomaterial.
Figure 5.16. TEM image of BR-PMO confirming the mesoporous periodicity of the nanomaterial.

Figure 5.17. TEM image of S-PMO confirming the mesoporous periodicity of the nanomaterial.
This feature is especially important for several applications such as separation, protein or gene delivery and biocatalysis. To demonstrate the availability of the porosity of the PMO nanomaterials, 30 wt% of doxorubicin (DOX) was loaded into the NPs and the cargos were retained inside the framework in aqueous neutral conditions (Figure 5.18). Dried PMO NPs were also readily and stably redispersed in water (Figure 5.19). These features support the potential for these nanomaterials for various applications such as separation, catalysis, and cargo deliveries.

The mechanism of the formation of PMO NPs with various morphologies was then investigated, and few trends were proposed. First, the PMO mesostructures observed are in line with the well-established hexagonally-arranged surfactant-silicate micellar template mesoporous silica. The role of CTAB molecules as molecular templates was confirmed by FTIR spectroscopy, as a high content of CTAB could be removed from the particles after the extraction of the surfactant (Figure 5.11). It was then observed that the addition of a polar organic co-solvent, such as DMF which miscible with water, leads to the formation of spherical NPs. Note that, spherical NPs were also observed with the addition of other polar solvents such as DMSO and acetone (data not shown). On the contrary, it was observed that non-polar co-solvents (toluene, cyclohexane) induced the formation of anisotropic phenylene-bridged PMO NPs. To confirm the influence of the co-solvent polarity on the final shape of PMO NPs, we conducted several control experiments. As already mentioned, the growth of bended nanorods was observed in a mixture of toluene and DMF (40/60 %) which could be explained by the competition between the
nonpolar toluene and polar DMF solvents. The toluene is proposed to assist the formation of long-range ordered surfactant-silicate micelles while DMF would promote their curvature\textsuperscript{55} (Figure 5.5). We demonstrated this hypothesis with the variation of the toluene/DMF ratios (100/0; 80/20; 60/40; 40/60; 20/80; 0/100) which confirmed the progressive change of morphology from nanowires to nanospheres (Figure 5.20). The use of THF, which is significantly more polar than toluene but not as much as DMF, also induced the formation of bended nanorods (Figure 5.21). Note that, the syntheses of anisotropic NPs were carried out at low stirring speed (450 rpm), but only spherical NPs were formed at higher ones (e.g. 1200 rpm) consistently with a previous report (Figure 5.22).\textsuperscript{18} It was also previously described that slow stirring speed promotes the formation of nanofibers via microemulsions.\textsuperscript{59} These facts thus suggest that the preparation of PMO NPs involves a microemulsion process highly dependent on the oil phase, similarly to silica colloids.\textsuperscript{59-61} Non-polar co-solvents are stabilized within rod-like CTAB micelles and favor the accumulation of the hydrophobic 1,4-bis(triethoxysilyl)benzene precursor. We suggest the following mechanism: as opposed to polar and water soluble co-solvent(s) which lead to isotropic PMO formation, non-polar ones may lead to hydrolysis and condensation preferentially along the micellar axis, as the silanes may have been mainly supplied from the interior of the micelles due to their hydrophobic affinity. This suggested mechanism also implies that the diameter of the hexagonally-arranged rod-like micelles would govern the final diameter of the PMO NPs, which accounts for the similar diameter of rod- and wire-like PMO nanomaterials (~110 nm).
Figure 5.18. DOX loading capacities in PMO NPs before and after aqueous washings revealing the high adsorption capability of the hydrophobic PMO pores and the absence of DOX leakage in neutral aqueous conditions.

Figure 5.19. Photograph of PMO NPs dispersions in water, demonstrating the excellent dispersibility of the NPs after drying.
Figure 5.20. TEM images of PMO NPS are displaying the impact of addition of organic co-solvents on the morphology of the particles. Toluene (T) and DMF (D) were used as organic co-solvents in different volume ratios.

Figure 5.21. TEM images of PMO bended nanorods obtained in the presence of THF (A-B).

Figure 5.22. TEM images of spherical PMO NPs obtained in the presence of cyclohexane at 1200 rpm stirring speed (A-B).
All types of PMO NPs were endowed with green fluorescence to facilitate theirs in vitro studies. Thus, PMO surface was grafted with fluorescein-alkoxysilane and additionally trihydroxysilylpropyl methylphosphonate was introduced to reduce aggregation and increase the stability of FPMO NPs in aqueous suspension. The incubation of HeLa cells with different concentrations of FPMO NPs showed the influence of the morphology on the in vitro toxicities of these NPs (Figure 5.23A). Interestingly, toxicities of FPMO NPs are following the same trend as it was previously described for polymer micelle assemblies, HeLa cells demonstrated higher viability with wire-like FPMO (W-FPMO, IC₅₀=65 µg·mL⁻¹) comparing one with spherical FPMO NPs (S-FPMO, IC₅₀=32 µg·mL⁻¹). This observation is the result of hindered cell penetration of W-FPMO NPs due to the presence of longitudinal interactions with cell membranes. As well, the internalization of FPMO NPs was studied with CLSM. Fluorescein moieties were successfully coupled to the PMO surface, so the FPMO NPs could be tracked by their green fluorescence (Figure 5.23B-E). CLSM images showed efficient uptake for all types of FPMO NPs in 6 h.
Figure 5.23. HeLa cell viability studies with the incubation of FPMO NPs at various concentrations (A). CLSM images of HeLa cells incubated with W-FPMO (B), R-FPMO (C), BR-FPMO (D), and S-PMO NPs (E) after 6 h of incubation. Nuclei are stained in blue with Hoechst 33342 dye and FPMO NPs appear with the green fluorescence. Scale bars of 20 μm.
5.5 Conclusions

In summary, we have developed for the first time a method to control the morphology of phenylene-bridged PMO NPs with the type of organic co-solvent used to solubilize the sol-gel precursor. Uniform spherical, rod- and wire-like mesoporous organosilica nanomaterials were obtained and fully characterized with various techniques. The anisotropic growth mechanism of PMO NPs was explained by the formation of microemulsion due to the significant difference in solvent polarities and solubilisation in micelles. The simple selection of the organic co-solvent thus allows the researcher to tune the morphology of phenylene-bridged PMO nanomaterials. The high surface areas from 500 to 1000 m$^2$·g$^{-1}$, the medium sized pores, the high loading of cargos, and excellent water dispersibility of the particles support their potential application in various fields. Phenylene-bridged PMO NPs were endowed with fluorescent properties and the role of their shapes was investigated in regard to the in vitro toxicities. Spherical particles demonstrated higher toxicity than wire-like particles, which is in good agreement with previously published results for polymer materials. These results raise the interest to design the long-circulating drug delivery systems based on anisotropic PMO NPs. The question of PMO biodistribution in regard to particle shape-dependence is now open for in vitro and in vivo studies.
5.6 References

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Chapter 6: Summary and Perspectives

This dissertation consists of two parts: the first one (Chapters 1 and 2) gives a brief, although informative, introduction to hybrid nanomaterials with a focus on biomedical applications of organosilica nanosystems, the second part (Chapters 3-5) includes a thorough description of three novel hybrid organic-inorganic bridged silsesquioxane nanoplatforms. Each of nanoplatform possessed the combination of unique features which were appropriately exploited for in vitro imaging, controlled release and delivery of active biomolecules, and biocompatibility studies. The enzymatically-degradable oxamide-bridged silsesquioxane nanomaterials (Chapter 3) demonstrated preferential accumulation in cancer cells and significant degradation in biorelevant conditions after 48 h. Moreover, such nanoplatform could be rendered fluorescent by fluorescein dyes in order to perform in vitro imaging. The hollow non-aggregated nanospheres of nitrobenzene-based hybrid organosilica (Chapter 4) showed on demand light-triggered delivery of plasmid DNA via surface charge reversal. The limitation of previously described systems is their absence of porosity, therefore the research was further conducted with phenylene-bridged PMO NPs (Chapter 5). The simple selection of the organic co-solvent allowed us to tune the synthesis and morphology of PMO nanomaterials. These nanoparticles were later endowed with fluorescent properties for cellular internalization and biocompatibility studies. Thus, spherical NPs demonstrated higher toxicity than wire-like NPs, which was shown for polymer materials in previously published works.

The performed research does not intend to claim the real application of obtained systems in the human body, rather demonstrate the proof of concept with further
application in more sophisticated materials. Besides, the materials presented in Chapters 3 and 4 had low surface area and could not be employed for sufficient cargo delivery. In addition, enzyme-triggered degradation was observed only in a test tube, hence the possible outcome for in vitro and in vivo studies is unknown. While we can arrange a limited set of experimental parameters in a test tube (e.g. concentration of NPs, enzyme), it is the task of significant complexity to reproduce the same conditions as in living organisms with their variety of microenvironments, proteins, and pHs. Speaking in general, there are very few articles about in vivo applications of organosilica, and in particular, bridged silsesquioxane NPs, for the last 10 years. The statistics for the period 2007 – 2017 shows 328 publications in the field of organosilica nanomaterials, however, only 30 of them were devoted to animal studies (Chapter 2, Figure 2.1A-B). Comparing these numbers with those for common mesoporous silica NPs – 561 publications among 8808 were dedicated to in vivo work (Chapter 2, Figure 2.1C). Another issue is the complexity of PMO NPs synthesis with bulk organic bridges. Usually, the generation of porous hybrid nanostructures requires the addition of organoalkoxysilane component (more than 90 mol%) with short phenylene, ethylene, or ethenylene-bridges. This fact substantially reduces the possible impact of complex organic moieties on the final physicochemical properties of hybrid materials. Obviously, we have way more data on the biological behavior of mesoporous silica NPs than we have for siliceous hybrid organic-inorganic NPs. Nevertheless, the ability to solve aforementioned problems, the relatively young age of this area and its steep growth in the number of publications anticipate a
broad range of smart organosilica nanosystems with a high potential to reach clinical trials.

The merge of organic and inorganic components in organosilica nanohybrids may imply sort of additive properties, however, the emergence of unique features can also be observed. It is crucial to foresee the outcome of multiple interactions between constituents of organosilica and surrounding biological media en route their biomedical implementation. Indeed, the gathered in vitro and in vivo data on the behavior of such NPs gives us only the overall impression – the intermediate processes are not known so far. And yet, there are many questions that need answers as there is a great potential to design interesting systems with an enormous range of active organic moieties incorporated within inorganic silica framework. These hybrid moieties could hold the key to the next generation of smart nanomaterials that could have a considerable impact on many aspects of our life especially extremely sensitive biomedical applications.
APPENDICES

A) Published Articles


This work is discussed in Chapter 5

**Abstract:** This work describes the sol–gel syntheses of para-substituted phenylene-bridged PMO nanoparticles with tunable morphologies ranging from nanowires to nanospheres. The findings show the key role of the addition of organic co-solvents in the aqueous templates on the final morphologies of PMO NPs. Other factors such as the temperature, the stirring speed, and the amount of organic solvents also influence the shape of PMO NPs. The tuning of the shape of the PMO nanomaterials made it possible to study the influence of the particle morphology on the cellular internalization and biocompatibility.
Abstract: We describe biodegradable mesoporous hybrid nanoparticles in the presence of proteins and their applications for drug delivery. We synthesized oxamide phenylene-based mesoporous organosilica nanoparticles in the absence of a silica source which had remarkably high organic content and high surface areas. Oxamide functions provided biodegradability in the presence of trypsin model proteins. MON displayed exceptionally high payloads of hydrophilic and hydrophobic drugs (up to 84 wt%), and a unique zero premature leakage without the pore capping, unlike mesoporous silica. MON were biocompatible and internalized into cancer cells for drug delivery.

**Abstract:** Despite the worldwide interest generated by bulk materials, the design of PMO nanomaterials with controlled morphology remains largely unexplored and their properties unknown. In this work, we describe the first study of PMO NPs based on *meta*-phenylene bridges, and we conducted a comparative structure–property relationship investigation with *para*-phenylene-bridged PMO NPs. Our findings indicate that the change of the isomer drastically affects the structure, morphology, size, porosity and thermal stability of PMO materials. We observed a much higher porosity and thermal stability of the *para*-based PMO which was likely due to a higher molecular periodicity. Additionally, the *para* isomer could generate multipodal NPs at very low stirring speed and upon this discovery we designed a phenylene–ethylene bridged PMO with a controlled Janus morphology. Unprecedentedly high payloads could be obtained from 40 to 110 wt% regardless of the organic bridge of PMOs. Finally, we demonstrate for the first time the co-delivery of two cargos by PMO NPs. Importantly, the cargo stability in PMOs did not require the capping of the pores, unlike pure silica, and the delivery could be autonomously triggered in cancer cells by acidic pH with nearly 70% cell killing.

This work is discussed in Chapter 4

Abstract: Bridged silsesquioxane nanocomposites with tunable morphologies incorporating o-nitrophenylene–ammonium bridges are described. The systematic screening of the sol–gel parameters allowed the material to reach the nanoscale with controlled dense and hollow structures of 100–200 nm. The hybrid composition of silsesquioxanes with 50% organic content homogeneously distributed in the nanomaterials endowed them with photoresponsive properties. Light irradiation was performed to reverse the surface charge of nanoparticles from +46 to −39 mV via a photoreaction of the organic fragments within the particles, as confirmed by spectroscopic monitoring. Furthermore, such nanoparticles were applied for the first time for the on-demand delivery of plasmid DNA in HeLa cancer cells via light actuation.

**Abstract:** We describe BSSO hybrid nanomaterials with an unusually high organic content (ca. 50%) based on oxamide components mimicking amino acid bio cleavable groups. Unlike most bulk BSSO materials, the design of sub-200 nm nearly monodisperse nanoparticles was achieved. These enzymatically degradable BS NPs were further tested as promising imaging nanoprobes.

**Abstract:** Polydopamine-coated FeCo nanocubes (PDFCs) were successfully synthesized and tested under microwave irradiation of 2.45 GHz frequency and 0.86 W·cm² power. These particles were found to be non-toxic in the absence of irradiation, but gained significant toxicity upon irradiation. Interestingly, no increase in relative heating rate was observed when the PDFCs were irradiated in solution, eliminating nanoparticle (NP)-induced thermal ablation as the source of toxicity. Based on these studies, we propose that microwave-induced redox processes generate the observed toxicity.
B) Published Reviews


**Abstract:** Predetermining the physico-chemical properties, biosafety, and stimuli-responsiveness of nanomaterials in biological environments is essential for safe and effective biomedical applications. At the forefront of biomedical research, mesoporous silica nanoparticles and mesoporous organosilica nanoparticles are increasingly investigated to predict their biological outcome by materials design. In this review, we first chronicle how the nanomaterial design of pure silica, partially hybridized organosilica, and fully hybridized organosilica governs not only the physico-chemical properties but also the biosafety of the nanoparticles. The impact of the hybridization on the biocompatibility, protein corona, biodistribution, biodegradability, and clearance of the silica-based particles is described. Then, the influence of the surface engineering, the framework hybridization, as well as the morphology of the particles, on the ability to load and controllably deliver drugs under internal biological stimuli (e.g. pH, redox, enzymes) and external non-invasive stimuli (e.g. light, magnetic, ultrasound) are presented. To conclude, trends in the biomedical applications of silica and organosilica nanovectors are delineated, such as unconventional bioimaging techniques, large cargo delivery, combination therapy, gaseous molecule delivery, antimicrobial protection, and Alzheimer’s disease therapy.
Abstract: The biorelated degradability and clearance of siliceous nanomaterials have been questioned worldwide, since they are crucial prerequisites for the successful translation in clinics. Typically, the degradability and biocompatibility of mesoporous silica nanoparticles have been an ongoing discussion in research circles. The reason for such a concern is that approved pharmaceutical products must not accumulate in the human body, to prevent severe and unpredictable side-effects. Here, the biologically degradability and clearance of silicon and silica NPs are comprehensively summarized. The influence of the size, morphology, surface area, pore size, and surface functional groups, to name a few, on the degradability of silicon and silica NPs is described. The noncovalent organic doping of silica and the covalent incorporation of either hydrolytically stable or redox- and enzymatically cleavable silsesquioxanes is then described for organosilica, bridged silsesquioxane, and periodic mesoporous organosilica NPs. Inorganically doped silica particles such as calcium-, iron-, manganese-, and zirconium-doped NPs, also have radically different hydrolytic stabilities. To conclude, the degradability and clearance timelines of various siliceous nanomaterials are compared and it is highlighted that researchers can select a specific nanomaterial in this large family according to the targeted applications and the required clearance kinetics.
C) Research Projects in Progress

*The list of participants in alphabetical order

- **Title**: Time-resolved SERS studies of supra-molecular systems on gold surface
  
  *Participants*: Carboni, V.; Fatieiev, Y.; Khashab, N. M.
  
  *Status*: Research in progress

- **Title**: Periodic mesoporous organosilica nanowires for gas adsorption and separation
  
  *Participants*: El Tall, O.; Fatieiev, Y.; Khashab, N. M.; Omar, H.
  
  *Status*: Research in progress

- **Title**: Assembly of gold nanoparticles into spherical isokinetic structures
  
  *Participants*: Carboni, V.; Fatieiev, Y.; Khashab, N. M.
  
  *Status*: Research in progress

- **Title**: Supracolloidal self-assembly of biodegradable organic–inorganic nanohybrids
  
  *Participants*: Fatieiev, Y.; Carboni, V.; Muhammed, H.; Khashab, N. M.
  
  *Status*: Research in progress

- **Title**: Biodegradable PMO nanoparticles exhibit excellent tumor accumuration and enable efficient delivery of anticancer drugs in the chicken egg tumor model
  
  *Participants*: Croissant, J.; Fatieiev, Y.; Khashab, N. M.; Tamanoi, F.; Vu, B.
  
  *Status*: Draft

- **Title**: Singlet oxygen-responsive anthracene-doped periodic mesoporous organosilica nanoparticles
  
  
  *Status*: Draft

- **Title**: Gold nanorod core periodic mesoporous organosilica shell nanoparticles and their Pickering emulsion
  
  
  *Status*: Draft
D) Conferences and Meetings

