Molecular materials for photonics

Ville Pale
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Abstract

Billions of years of evolution has produced a variety of functional components that enable life on Earth. The notable examples are chlorophylls that have a vital role in converting the energy of the Sun into chemical energy, and nucleobases that act as the building blocks in DNA/RNA, which store and convey the genetic information in living organisms.

Thematically, this thesis is divided into two parts. The first part studies how the natural components, chlorophyll and uracil molecules, could be utilized in modern photonic applications. Publication I investigates the properties of supramolecular Zn chlorin-poly(4-vinylpyridine) assemblies that mimic the biological antenna structure. Moreover, this approach was used to create assemblies with macroscopically homogeneous pigment distribution. Publication II studies how FRET-mediated energy-transfer could be used to enhance the performance of the materials studied in Pub. I. In Publications III and IV, atomic/molecular layer deposition is established as a method to create fundamentally new three-dimensional sodium networked uracil assemblies with novel optical properties.

The second part investigates how silver nanoparticle assemblies could be utilized in surface enhanced spectroscopic techniques. Generally, Publications V and VI investigate two economical nanofabrication methods to create large-scale plasmonic substrates. In Publication V, azopolymer lithography was utilized to create periodic plasmonic nanoparticle arrays for fluorescence enhancement. In Publication VI, a method utilizing cryogenic deep reactive ion etching with inductively coupled plasma was used to create a plasmonic substrate for surface enhanced Raman scattering applications.

The results obtained in this thesis could pave the way for new biomimetic photonic materials and enable the utilization of economic and large-scale nanofabrication methods in creating new plasmonic materials. Especially, molecular layer deposition was established as a promising and scalable method to create materials with novel structural and optical properties.

Keywords chlorophyll, FRET, uracil, nucleobase, atomic/molecular layer deposition, red-edge excitation shift, plasmonics, metal enhanced fluorescence, surface enhanced Raman scattering
Evolutoon on tuottanut miljöön vuosien aikana valtavasti funktionaalisia yhdisteitä, jotka mahdollistavat elämän maapallolla. Tunnetumia esimerkkejä näistä komponenteista ovat klorofyllit, joilla on olennainen rooli prosesseissa, joissa auringon valo muutetaan kemiallisesti energiaksi. Lisäksi DNAa ja RNAa emäskit toimivat geneettisen information varastoinnissa ja välittämisessä elävissä organismissa.


Työssä esitetty tulokset viitoittavat tietä uusille biomimeettisille fotonisille materiaaleille ja mahdollistavat sellaisten nanovalmistusmenetelmien käyttöä, joilla on mahdollista valmistaa suuria pinta-aloja plasmoniilla rakenteilla. Erityisesti osoitetaan atomi/molekyyliferroskatuksen soveltuvuus menetelmään, jolla pystytään luomaan rakenteellisesti ja optimisesti uudenhallias materiaaleja.

Avainsanat: klorofyll, FRET, urasilli, typpiema, atomi/molekyyliferroskatuus menetelmä, REES, plasmoniikka, metalliavhisteinen fluoresenssi, pintavahvisteinen Raman-siironpintavahviste

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This thesis consists in the work carried out between the years 2011-2016 in the Micro and Quantum Systems (MQS) group. When starting my journey towards the doctorate, the goal was vague and distant. Even which path to follow was shrouded in darkness, but somehow, little by little a path started forming, constantly evolving. Naturally, as in life in general and especially in science, this journey has been accompanied by numerous of missteps and failures. I am, nevertheless, very grateful of all these problems as they have solidified my beliefs in the importance of hard work and perseverance in order to achieve the goals that you have set for yourself.

I am deeply thankful for Professor Ilkka Tittonen for giving me the opportunity to work in the realm of nanoscience and allowing me to pursue my own ideas, no matter how crazy or stupid they may have been. You always had time for a chat and provided support when I required.

I would also like to thank Dr. Sami Kujala for acting as the instructor for this thesis. I am very grateful for your excellent advices and suggestions. I would like to express my gratitude towards the pre-examiners of this thesis, Professor Jouko Korppi-Tommola and Professor James Rice, for their thorough inspection of this thesis.

All of the work in this thesis has been done in collaboration, all whom without this thesis would have never completed. I would like to thank all my co-authors who contributed to the articles. I am very grateful for Dr. Taru Nikkonen and Dr. Juho Helaja (Laboratory of Organic Chemistry, University of Helsinki) for their essential input in organic chemistry and synthesis. I am also very thankful for Dr. Jaana Vapaavuori and Professor Arri Priimagi (Chemistry and Bioengineering, Tampere University of Technology) for all the collaboration and discussions. I am very grateful for Zivile Giedraityte and Professor Maarit Karppinen (Department of Chemistry, Aalto University) for the friendly and productive collaboration we had together. A special thanks go to the Aalto Nanofabrication center at Micronova for all the effort in maintaining the research infrastructure.

For financial support, I would like to acknowledge Academy of Finland (projects 129043 and 285972) Aalto ELEC Doctoral School and Emil Aaltonen Foundation. I also want to thank VTT for giving me the opportunity
to finalize this thesis.

I would like to express my sincere gratitude towards the people of MQS group for creating an enjoyable atmosphere to work. Without your support, these years would have been much bleaker, and I am not even sure if this thesis would have been ever completed. Especially, I would like to thank Mikko Ruoho, my partner in crime, for sharing the office with me and your friendship. I would also like to thank Mikhail Erdmanis for showing me what true dedication is, Osmo Vänskä for our long chats related to work and life, Jorma Selin for your honest criticisms and discussions on the work we did together, Taneli Juntunen for sharing the passion for music and your witty humour, Camilla Tossi for knowing what a Vorlon is. Nerds rule! We have all shared lots of great moments outside the work. And of course I would like to thank all the former MQS members: Nikolai Chekurov, Ossi Kimmelma, Päivi Sievilä, Tuomas Rossi, Zhengjun Liu, Julius Nieminen, Thomas Lindvall and Mika Koskenvuori.

I had the privilege to work with lots of talented people in Micronova during these past years. I would like to thank Professor Harri Lipsanen, Professor Markku Sopanen, Professor Zhipei Sun and Docent Hanne Ludvigsen for all the discussions and advices during the years. Also, I would like thank my former colleagues: Veer Dhaka, Ya Chen, Christoffer Kauppinen, Ali Shah, Susoma Jannatul, Nagarajan Subramaniyam, He Yang, Marco Mattila, Sami Suihkonen, Henri Jussila, Alexander Kravchenko, Igor Shavrin and John Rönn.

I would like to thank all my friends, Sakke, Stigu, Jani, Mikko, Markku, and Epe, for giving me a healthy counterbalance for academic life and work. Or, quoting Berthold Auerbach: “Let the music wash away from the soul the dust of everyday life”.

I want to express my warmest thanks to my parents, Maiju and Harri, and my brother Jyri for all the encouragement and support.

I owe my deepest gratitude for my family, Elina, Sofie, Jasiini and Anton, for giving this work a meaning. And most importantly, reminding me everyday what really is the most precious in life.

Espoo, May 4, 2017,

Ville Pale
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This thesis consists of an overview and of the following publications which are referred to in the text by their Roman numerals.


V Ville Pale, Christroffer Kauppinen, Jorma Selin, Markku Sopanen, and Ilkka Tittonen. Fluorescence-enhancing plasmonic silver nanos-

Author’s Contribution

Publication I: “Biomimetic zinc chlorin–poly(4-vinylpyridine) assemblies: doping level dependent emission–absorption regimes”

The author contributed significantly to the original idea, fabricated samples for optical measurements, carried out ellipsometric, SEM and optical characterization of the samples and analysed the results from these measurements. The author had a significant role in writing the manuscript.

Publication II: “Light-harvesting zinc chlorin-poly(4-vinylpyridine) complexes”

The work was planned and done by the author, except the chemical synthesis of the chlorin molecules, which was done by TN. The author analyzed the results and wrote the manuscript.

Publication III: “Three-dimensional uracil network with sodium as a linker”

The author did the optical measurements (except FTIR), analyzed the results and wrote the corresponding text in the manuscript. The manuscript was drafted together by all authors.

Publication IV: “Excitation-dependent fluorescence from atomic/molecular layer deposited uracil-sodium thin films”

The author did the optical measurements (except FTIR) and analyzed the results. The author wrote the first version of the manuscript and coordi-
Author’s Contribution

nated the writing process.

Publication V: “Fluorescence-enhancing plasmonic silver nanostructures using azopolymer lithography”

The author contributed significantly to the original idea, fabricated the samples (except the lithography), performed the measurements (confocal, steady-state fluorescence and absorption were done together with JS) and analyzed the experimental results. The author was in charge of writing the manuscript.

Publication VI: “Improved SERS Intensity from Silver-Coated Black Silicon by Tuning Surface Plasmons”

The author participated in analyzing the results and drafting the manuscript.
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>Atomic force microscope</td>
</tr>
<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>Aluminium oxide</td>
</tr>
<tr>
<td>ALD</td>
<td>Atomic layer deposition</td>
</tr>
<tr>
<td>APIL</td>
<td>Azopolymer interference lithography</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>BChl</td>
<td>Bacteriochlorophyll</td>
</tr>
<tr>
<td>BSi</td>
<td>Black Silicon</td>
</tr>
<tr>
<td>Chl</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FC</td>
<td>Franck-Condon</td>
</tr>
<tr>
<td>FRET</td>
<td>Förster resonance energy transfer</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>IRF</td>
<td>Instrument response function</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International union of pure and applied chemistry</td>
</tr>
<tr>
<td>LHC</td>
<td>Light-harvesting complex</td>
</tr>
<tr>
<td>LSPR</td>
<td>Localized surface plasmon resonance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest occupied molecular orbital</td>
</tr>
<tr>
<td>MEF</td>
<td>Metal enhanced fluorescence</td>
</tr>
<tr>
<td>MLD</td>
<td>Molecular layer deposition</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>NB</td>
<td>Nucleobase</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NP</td>
<td>Nanoparticle</td>
</tr>
<tr>
<td>OLED</td>
<td>Organic light-emitting diode</td>
</tr>
<tr>
<td>P18</td>
<td>Purpurin 18</td>
</tr>
<tr>
<td>P4VP</td>
<td>Poly(4-vinylpyridine)</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>RC</td>
<td>Reaction center</td>
</tr>
<tr>
<td>REES</td>
<td>Red-edge excitation shift</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface enhanced Raman scattering</td>
</tr>
<tr>
<td>SRG</td>
<td>Surface relief grating</td>
</tr>
<tr>
<td>TCSPC</td>
<td>Time-correlated single photon counting</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Ti:S</td>
<td>Titanium-sapphire</td>
</tr>
<tr>
<td>TRES</td>
<td>Time-resolved emission spectra</td>
</tr>
<tr>
<td>ZnPPME</td>
<td>Zn pyro-pheophorbide a methylester</td>
</tr>
<tr>
<td>Zn-3(^1)-OH-PPME</td>
<td>Zn-3(^1)-pyro-pheophorbide a methylester</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$A$</td>
<td>Dielectric constant factor</td>
</tr>
<tr>
<td>$a$</td>
<td>Onsager cavity radius [m]</td>
</tr>
<tr>
<td>$b$</td>
<td>Peak height</td>
</tr>
<tr>
<td>$E_0$</td>
<td>Incident electric field [V/m]</td>
</tr>
<tr>
<td>$E(r, \lambda)$</td>
<td>Electric field at position $r$ [V/m]</td>
</tr>
<tr>
<td>$E_{\text{FRET}}$</td>
<td>Energy transfer efficiency</td>
</tr>
<tr>
<td>$F(\lambda)$, $F_D(\lambda)$</td>
<td>Fluorescence spectra</td>
</tr>
<tr>
<td>$G$</td>
<td>Raman enhancement factor</td>
</tr>
<tr>
<td>$H(\lambda)$</td>
<td>Normalized fluorescence spectrum</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck constant [$\approx 6.626 \cdot 10^{-34}$ kgm$^2$/s]</td>
</tr>
<tr>
<td>$I'(t)$</td>
<td>Time-dependent fluorescence intensity</td>
</tr>
<tr>
<td>$I'(\lambda, t)$</td>
<td>Time-resolved emission spectra</td>
</tr>
<tr>
<td>$I(t)$</td>
<td>Fluorescence decay</td>
</tr>
<tr>
<td>$I_C(t)$</td>
<td>Calculated fluorescence decay</td>
</tr>
<tr>
<td>$I_m(t)$</td>
<td>Measured fluorescence decay</td>
</tr>
<tr>
<td>$I_{\text{IRF}}(t)$</td>
<td>Instrument response function</td>
</tr>
<tr>
<td>$J_{\text{DA}}$</td>
<td>Overlap integral [nm$^4$/M cm]</td>
</tr>
<tr>
<td>$k$</td>
<td>Boltzmann constant [$\approx 1.38 \cdot 10^{-23}$ kgm$^2$/Ks$^2$]</td>
</tr>
<tr>
<td>$k_m$</td>
<td>Metal induced non-radiative decay rate</td>
</tr>
</tbody>
</table>
List of symbols

\( k_{\text{nr}} \)  Non-radiative decay rate

\( M \)  Spin multiplicity

\( M_{\text{dye}} \)  Molar mass [kg/mol]

\( N \)  Number of datapoints

\( N_A \)  Avogadro constant \( \approx 6.022 \cdot 10^{23} \) \( \text{1/mol} \)

\( p \)  Number of fitting parameters

\( R, r \)  Distance [m]

\( R_0 \)  Förster distance [m]

\( S \)  Total spin angular momentum

\( S_n \)  Singlet electronic state

\( T \)  Temperature [K]

\( t \)  Time [s]

\( T_n \)  Triplet electronic state

\( w_i \)  Mass fraction

\( \langle \tau \rangle \)  Intensity-weighted average lifetime [s]

\( \alpha(\omega) \)  Polarizability [Cm\(^2\)/V]

\( \alpha_i \)  Amplitude component

\( \Gamma \)  Radiative decay rate

\( \gamma \)  Asymmetry parameter

\( \Gamma_m \)  Metal induced radiative decay rate

\( \gamma_{\text{enh}} \)  Fluorescence rate enhancement

\( \gamma_{\text{exc}} \)  Field enhancement

\( \Delta \)  Width parameter

\( \Delta \lambda(t)^2 \)  Spectra full width at half maximum [m]

\( \Delta \mu \)  Dipole moment change [Cm]
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta \nu$</td>
<td>Inhomogeneous broadening function [1/cm]</td>
</tr>
<tr>
<td>$\epsilon(\omega)$</td>
<td>Dielectric function</td>
</tr>
<tr>
<td>$\epsilon_A(\lambda)$</td>
<td>Molar extinction coefficient [m$^2$/mol]</td>
</tr>
<tr>
<td>$\epsilon_m$</td>
<td>Surrounding medium dielectric constant</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Inclination angle</td>
</tr>
<tr>
<td>$\kappa^2$</td>
<td>Orientation factor in FRET</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength [m]</td>
</tr>
<tr>
<td>$\lambda_{cg}$</td>
<td>Spectrum center of gravity</td>
</tr>
<tr>
<td>$\lambda_p$</td>
<td>Peak wavelength [m]</td>
</tr>
<tr>
<td>$\mu_{ge}$</td>
<td>Transition dipole moment [Cm]</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Frequency [Hz] or Vibrational quantum number</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density [kg/m$^3$]</td>
</tr>
<tr>
<td>$\rho_n$</td>
<td>Number density [1/m$^3$]</td>
</tr>
<tr>
<td>$\sigma_k$</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Fluorescence lifetime [s]</td>
</tr>
<tr>
<td>$\bar{\tau}$</td>
<td>Amplitude-weighted average lifetime [s]</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Fluorescence quantum yield</td>
</tr>
<tr>
<td>$\phi_m$</td>
<td>Fluorescence quantum yield in MEF</td>
</tr>
<tr>
<td>$\chi^2_{R}$</td>
<td>Reduced chi-square parameter</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency [rad/s]</td>
</tr>
</tbody>
</table>
1. Introduction

For centuries, scientists have been amazed by Nature’s ability to create complex and functional structures that enable life on Earth. This vast selection of renewable and inexpensive materials with tunable and novel properties has inspired the development of photonic and electronic devices. [1–3] In most cases, biomolecules are highly specialized functional moieties with intrinsic order that can potentially provide unique device properties or even enhanced performance. In this thesis, I will concentrate in more detail on two molecular families that are crucial to life on Earth.

One of the notable examples are chlorophylls that store the energy of the Sun into chemical energy. Chlorophylls are very versatile molecules as they can perform the harvesting of light and transfer the excitation to the catalytic sites, where charge separation takes place. [4] More remarkably, a single chlorophyll molecule can play any of these roles, depending from its molecular environment. Chlorophyll molecules with different functionalizations offer diverse supramolecular self-assembling strategies; i.e., the self-assembly can be coordinated by other pigments or by the surrounding protein environment. [5] For instance, the biological chlorophyll assemblies have specialized and carefully optimized structural geometries in order to fulfil their respective functions. The green sulphur bacteria serves as an excellent example of this optimization as it has a photosynthetic apparatus exhibiting near unity energy transfer efficiencies with carefully optimized intermolecular pigment distances and orientations. [6]

Second important class of biomolecules are the nucleotides; the building blocks of DNA/RNA. [7] Together with the 20 amino acids, they constitute the fundamental elements from which all living organisms are formed. The interplay between the amino and nucleic acids, allow a vast range of different molecular assemblies to be formed that exhibit a diverse spec-
Introduction

Nanotechnology has the potential to integrate different biological constituents into inorganic systems with novel functionalities. For instance, the strongly emerging atomic/molecular layer deposition method has proven to be capable in fabricating of three-dimensional inorganic-organic assemblies in a layer-by-layer fashion. In principle, this deposition technique is also compatible with standard semiconductor fabrication techniques, which further increases its applicability.

Nanoscale structures can be used to dramatically enhance the interaction between light and molecules, such as improving the emission or the excitation rates of the molecular moiety. Especially, metallic nanostructures are good candidates to confine and control light on nanoscale due to the strong interaction between the conduction electrons of the metal and light, giving rise to surface-plasmon polaritons. From application point-of-view, this nanoscale control of light can be used to make low quantum yield processes, such as Raman scattering, more efficient.

This thesis consists of a collection of scientific studies, which investigate the applicability of molecular systems for photonic applications. Publications I and II present two different biomimetic supramolecular zinc chlorin-poly(4-vinylpyridine) assemblies and their applicability for emitting and absorbing light. Publications III and IV demonstrate the intriguing excitation-dependent emission behaviour in atomic/molecular layer deposited crystalline sodium-uracil thin films. Publications V and VI illustrate two large-scale fabrication methods to fabricate plasmonic substrates for fluorescence enhancing and surface enhanced Raman scattering applications, respectively.

This thesis is organized in following way. Chapter 2 presents the key optical properties of organic molecules. It describes the principal molecular phenomena affecting their optical characteristics, which are utilized in the scientific studies. Chapter 3 describes the fundamentals of time-resolved fluorescence and time-resolved emission spectra measurements. Chapter 4 introduces the structural and optical properties of chlorophyll molecules, and describes the properties of biological supramolecular chlorophyll assemblies. It also summarizes the results obtained in Publications I and II. Chapter 5 presents nucleobases and atomic/molecular layer deposition used to grow inorganic-organic thin films. It also summarizes the results shown in Publications III and IV. Chapter 6 describes the fun-
fundamentals of localized surface plasmon enabled enhanced spectroscopic techniques and covers the key properties of the utilized process steps. It also summarizes the results shown in Publications V and VI. Chapter 7 summarizes the results presented in the whole thesis.
Absorption, emission or scattering processes of electromagnetic radiation by atoms or molecules forms the basics in optical spectroscopy. These different optical characterization schemes provide indispensable source of information for scientific research. Moreover, fluorescence as a single spectroscopic technique, has become the dominant technology in the numerous fields of clinical chemistry, biotechnology and medical research. [14–17] The basic processes affecting the emission of a molecule, such as environmental effects, energy transfer and quenching, are well recognized. [18,19]

This chapter presents the basic properties of molecules for understanding the complex cascade of processes that take place after absorption, leading to the emission of light from molecules. Optical processes in liquids and solids are discussed. Rotational effects, pertinent mostly to gases, are left out of scope. Moreover, the physical properties of fluorophores that influence their light emission are covered.

Details of fluorescence quenching is discussed on a general level, while introducing the resonance energy transfer and the aggregation induced changes in the spectral properties in more detail. The chapter also presents rarer optical phenomena, related to the complex interactions between the fluorophore and the molecular environment leading to the excitation-dependent emission shifts.

### 2.1 Energy levels and transitions

When a molecule is excited with light, a variety of processes will take place given that the energy of the exciting photon is suitable to be absorbed by the given molecule. For organic molecules, the molecular orbitals that are closely involved in absorption and fluorescence are the
highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). [20] For some molecules, such as chlorins and bacteriochlorins, higher unoccupied molecular orbitals also play a pivotal role and must be taken into consideration. [21]

Figure 2.1 illustrates a Jablonski diagram that shows the typical processes that occur when a molecule is excited with light. In the figure, the electronic states are termed either with a symbol S or T corresponding to the singlet and triplet states, respectively. The electronic ground state is depicted with the symbol $S_0$, whereas the excited states are expressed as $S_1, S_2, T_1$ and $T_2$. For a singlet state, the electron spins of a molecular system are paired, which means that the total spin angular momentum ($S = \sum s_i$) is zero. This in turn gives the spin multiplicity ($M = 2S + 1$) a value of one, which describes the possible spin orientations in external magnetic field. Respectively, the total spin angular momentum for a triplet state is $S = 1$ as the spins are unpaired yielding a spin multiplicity of $M = 3$, thus the name triplet. [22, 23] The spin pairing and unpairing is also schematically illustrated in Fig. 2.1, where the arrows inside the grey boxes show the orientation of the electron spin. In most cases, the singlet state $S_0$ is the ground state for a molecule, but exceptions exist. For example, the ground state of a diatomic oxygen molecule is a triplet. [20] Moreover, due to the Pauli exclusion principle, the energy

![Figure 2.1. A schematic Jablonski diagram depicting the possible relaxation pathways for a fluorophore after excitation. Electronic states are termed as S for singlet state and T for triplet state. The arrows inside the grey boxes schematically illustrate the spin configuration for each electronic state. Solid and dashed lines correspond to radiative and non-radiative transitions, respectively.](image-url)
Optical properties of molecules of a triplet state in an isolated molecule is generally lower than the singlet state of the same configuration in accordance with Hund’s rule. [24]

Polyatomic molecules also contain vibrational degrees of freedom, and each electronic state consists of harmonic-oscillator-like vibrational states, also shown in Fig. 2.2. [25] Moreover, as the nuclei are more massive than the electrons, the electrons are assumed to follow the nuclei instantaneously, forming the basis for the Born-Oppenheimer approximation. This allows the molecular wavefunction to be separated into the electronic and nuclear wavefunctions. [23] Due to the mass difference between the electrons and nuclei, the time scale for electronic transition is approximately $10^{-15}$ s, whereas the time scale for molecular vibrations is $10^{-10} - 10^{-12}$ s. [18] Hence immediately after the electronic transition, the nuclear configuration of the molecule experiences no significant change. This state is referred to as the Franck-Condon (FC) state. Moreover, the Franck-Condon principle states that transitions between vibrational states with similar nuclear coordinates are more probable. The quantum mechanical interpretation reveals that the transition probability is highest when the overlap between the vibrational wavefunctions is maximized. [26] Figure 2.2 demonstrates this principle for a diatomic molecule.

At room temperature, thermal energy is not large enough to significantly populate the excited vibrational levels. [20, 26] Hence, absorption usually originates from the lowest vibrational state. Depending from the energy of the exciting photon, an electron can be excited to either $S_1$ or $S_2$ electronic states. Immediately after the absorption, the molecule will undergo rapid vibrational relaxations to the ground vibrational state for given multiplicity, dissipating the excess energy as heat. The molecule can also non-radiatively relax between the electronic states known as internal conversion (IC), which can occur if the vibronic wavefunctions overlap sufficiently. The timescale of this process is on the order of picoseconds. With a few exceptions, the molecule will relax to the lowest vibrational level of $S_1$. As the fluorescence lifetimes are usually in the order of nanoseconds, all relaxation processes complete before the photon emission. This is also known as the Kasha’s rule, which states that the majority of photon emission occurs from the lowest excited state of a given multiplicity. [27] All these aspects lead to the fact that emission in most cases occurs at lower energy compared to absorption, which is also known as the Stokes shift.

From the lowest vibrational level of $S_1$, the molecule can relax either via radiative or non-radiative decay. The radiative transitions are termed
as fluorescence and phosphorescence. Fluorescence is a singlet-singlet transition, whereas phosphorescence is a triplet-singlet transition. The transition between the different spin multiplicities are referred to as intersystem crossing, and usually consist in singlet-triplet transitions (or vice versa). These transitions are forbidden in the electric-dipole approximation as they require a spin flip. This means that the processes are very unlikely to happen, with timescales ranging from microseconds to seconds. [28] However, the presence of heavy atoms can increase the probabilities of intersystem crossing via the spin-orbit coupling. [29] For example, the presence of heavy atoms in molecules, such as halogens and many transition metals, can promote intersystem crossing and induce phosphorescence. [18]

The processes behind non-radiative relaxation are various but the most frequent processes are internal conversion, photophysical processes (photobleaching, photoisomerization). Also, intermolecular quenching effects

![Figure 2.2](https://example.com/figure2.png)

**Figure 2.2.** The images illustrates Franck-Condon principle for a diatomic molecule and the electronic transitions between the ground state $S_0$ and excited state $S_1$, stating that the electronic transitions that exhibit a minimal change in the nuclear coordinates are most probable. This means that the absorption is most strongest between the states $S_0, \nu_0 \rightarrow S_1, \nu_4$, whereas the transition $S_0, \nu_0 \rightarrow S_1, \nu_0$ is less probable. The absorption is given in blue color and fluorescence in red color.
Optical properties of molecules can induce additional decay pathways for non-radiative processes. Some of these will be investigated in more detail in the following sections.

2.2 Fluorescence quantum yield and lifetime

The quantum yield ($\Phi$) and fluorescence lifetime ($\tau$) are probably the most important figures of merit for a fluorophore. Quantum yield is defined as the ratio of emitted photons to absorbed photons, making it a measure of the fluorophore brightness. [18] Hence, the substances with quantum yields close to unity, such as rhodamines, display the brightest fluorescence. For a fluorophore, the fluorescence lifetime is also very important characteristic, as it determines the available time to interact and diffuse with its environment before emitting a photon.

Figure 2.3 shows a simplified situation for fluorescence, where the explicit processes leading to the relaxation of $S_1$ state are omitted for clarity and all the possible non-radiative processes are grouped under a single rate constant $k_{nr}$. Using the notations of Fig. 2.3, the quantum yield can be defined as the fraction of molecules that decay by emitting a photon over all decay routes, yielding

$$\Phi = \frac{\Gamma}{\Gamma + k_{nr}},$$  

(2.1)

where $\Gamma$ is the radiative decay rate.

Fluorescence lifetime is defined as the average time that the fluorophore stays in the excited state. In an ensemble of molecules, the emission of a photon via fluorescence is a random event, where each emission event

![Diagram of fluorescence](image)

**Figure 2.3.** A schematic diagram of fluorescence. $k_a$, $\Gamma$ and $k_{nr}$ are the rate constants for absorption, radiative decay and non-radiative decay, respectively. $h$ is the Planck's constant. $\nu_a$ and $\nu_f$ are frequencies of the absorbed and emitted photon, respectively.
Optical properties of molecules

occurs independently from each other. Using the notations in Fig. 2.3, we can write out the rate equation for the excited state as

\[ \frac{dN_{S_1}(t)}{dt} = k_a N_{S_0}(t) - (\Gamma + k_{nr}) N_{S_1}(t). \]  

(2.2)

Now if we assume that the pump is turned off at time \( t = 0 \), we can set \( k_a = 0 \) in Eq. 2.2. The resulting differential equation has the familiar exponential solution that yields an exponential time dependency for the excited state population

\[ N_{S_1}(t) = N_{S_1}(0) \exp(-t/\tau), \]  

(2.3)

where the lifetime is given as

\[ \tau = \frac{1}{\Gamma + k_{nr}}. \]  

(2.4)

Now we should emphasize the probabilistic nature of the emission processes and that the lifetime is a statistical ensemble average. This means that for a large collection of fluorophores, some fluorophores emit quickly after the excitation and some later than \( \tau \).

2.3 Quenching

Section 2.1 described the intrinsic deactivation pathways for a molecule after excitation with light. The intermolecular, or extrinsic, processes that decrease the fluorescence are referred to as quenching. Quenching of fluorescence can take place in a variety of ways and typically this phenomenon is divided into two distinct cases; static or dynamic quenching based. The discrimination between the static or dynamic quenching is based on, whether the process takes place either in the ground- or excited-state, respectively.

In static quenching, a fluorophore forms a stable complex with another molecule referred to as a quencher. For this reason, static quenching is also known as contact quenching. If the resulting ground-state complex is non-fluorescent, the fluorophore has been quenched statically. For example, the aggregation of molecules in micellar systems [30] or the fluorescence behaviour for fluorescent-reporter molecules immobilization on nucleid acids [31] can be explained by static quenching.

On the other hand, dynamic quenching processes take place when the molecule is excited. These processes include energy transfer (Dexter and Förster), collisional quenching and excited-state reactions. [18] In collisional quenching, the excited fluorophore collides with another molecule
resulting in the deactivation of the fluorophore. Typical quencher molecules include molecular oxygen [32], iodide ions [33] and acrylamide [34]. Energy transfer induced quenching will be investigated in more detail in the next section.

To distinguish between the two types of quenching requires investigating either steady-state and time-resolved fluorescence measurements, or absorption measurements. Since the formation of fluorophore-quencher complex in static quenching changes the spectral properties, whereas dynamic quenching does not, absorption spectroscopy is a valuable tool to discriminate between these two types of quenching mechanisms (cf. Section 2.5). The behaviour between static and dynamic quenching in fluorescence measurements is illustrated in Fig. 2.4. Since static quenching forms only non-fluorescent complexes, the non-quenched molecular moieties still emit light with unaltered lifetime. Therefore, the only noticeable change is the diminished fluorescence intensity. However for dynamic quenching, the interaction between the quencher and fluorophore does not quench the fluorescence completely. Therefore, the observed decrease in fluorescence intensity is accompanied by a shortened lifetime as the function of the quencher concentration.

To investigate the kinetics of quenching, one can plot $F_0/F$ ($F_0$ is fluorescence yield without quencher and $F$ is fluorescence yield with quencher present) as the function of the quencher concentration. This is known as

![Figure 2.4](image-url)

*Figure 2.4.* Schematic illustration of the behaviour seen in a steady-state measurement (left), time-resolved measurement (center) and Stern-Volmer plot (right) for (a) static and (b) dynamic quenching. The initial situation in the fluorescence measurements is depicted with red color and after quenching in blue color. In both cases the steady-state intensity decreases, but the lifetime decreases only for dynamic quenching, which is illustrated by the distinguishable behaviour in the Stern-Volmer plots for each quenching type.
the Stern-Volmer relationship. The fluorescence lifetime can be used for the analysis by calculating the $\tau_0/\tau$ ratio instead of $F_0/F$. The different behaviour between static and dynamic quenching is illustrated in Fig. 2.4 (rightmost images). However, these plots describe only the simplest cases and the studied sample can simultaneously exhibit both of these quenching types, in which case the curves need not to be linear.

This analysis has been used in Publication I to verify that the ordering of the molecules is macroscopically homogeneous in the polymer matrix at low dye concentrations as the non-homogeneous ordering would involve the creation of aggregates resulting in static quenching behaviour.

2.4 Förster resonance energy transfer

Fluorescence or Förster resonance energy transfer (FRET) is a non-radiative interaction process, where energy is transferred between particles over distances that are more than molecular (or atomic) radii long. FRET is named after a German scientist Theodor Förster, who continued the work of Jean Perrin [35] and formulated the current form of the theory and also verified it experimentally. [36–38] FRET has found numerous applications in biological, physical and chemical sciences, such as a molecular ruler in biological systems [39], in situ monitoring of proteins in a living cell [40], or optical gain tuning via FRET in a biopolymer laser [41].

Classically, FRET is considered as a long range dipole-dipole interaction between two molecules, a fluorescent donor and an acceptor, which does not need to be fluorescent. The long range refers to a situation, where no orbital overlap between molecules exist, but the interaction still exhibits a near-field character, i.e., within the range from 10 Å to 100 Å.

The efficiency of the energy transfer depends mainly from three factors; from the spectral overlap between the donor fluorescence and acceptor absorption spectrum, the value of the donor quantum yield and the acceptor molar absorption coefficient, and from the orientational factor $\kappa^2$ that describes the relative orientation in space between the donor and acceptor transition dipoles.

Usually, the exact $\kappa^2$ value is unknown and one must settle for approximative values based on different averaging schemes. Depending on the relative orientations between the donor and acceptor, this value can range from 0 to 4. [42] The typical values are $\kappa^2 = 2/3$ (isotropic random orientations) or $\kappa^2 = 0.476$ (static orientations). [43, 44] Moreover, fluorescence
anisotropy measurements can be used to determine suitable limits for $\kappa^2$ and thus minimize the errors when calculating distances. [45]

The energy transfer efficiency is given by [46,47]

$$ E_{\text{FRET}} = \frac{1}{1 + (R/R_0)^6}, \quad (2.5) $$

where $R_0$, the Förster distance, defines the donor-acceptor distance when the efficiency given by Eq. 2.5 is 50%. The Förster distance is also defined as

$$ R_0^6 = \frac{9000(\ln 10) \kappa^2 \phi_I}{128\pi^5 n^4 N_A} J_{DA} \, (\text{Å}^6), \quad (2.6) $$

where $n$ is the refractive index of the host medium, $N_A$ is the Avogadro constant, $\phi_I$ is the intrinsic donor fluorescence quantum yield and $J_{DA}$ is the overlap integral between the donor and acceptor spectra defined as

$$ J_{DA} = \int_0^\infty F_D(\lambda)\epsilon_A(\lambda)\lambda^4 d\lambda. \quad (2.7) $$

Usually, the fluorescence spectrum $F_D$ is normalized to unity and $\epsilon_A$ is the molar extinction coefficient of the acceptor. With the access of time-resolved measurement data, the energy transfer efficiency in Eq. 2.5 can be also defined as

$$ E_{\text{FRET}} = 1 - \frac{\tau_{DA}(q)}{\tau_D}, \quad (2.8) $$

where $\tau_D$ is the lifetime in the absence of acceptor and $\tau_{DA}(q)$ is the lifetime of the donor with a given acceptor concentration $q$. As will be discussed in Section 3.1, the amplitude-weighted lifetimes given by Eq. 3.5 can be used to calculate the FRET efficiency in Eq. 2.8.

### 2.5 Aggregation induced spectral shifts

The close proximity of the molecules can induce extensive changes to the electronic transition energies of the chromophores, which are also distinguishable as altered spectral properties and can be used to determine the degree of aggregation. [48, 49] Qualitatively, the spectral shifts can be explained by the exciton model, originally presented by Kasha et al. [50]. In this model, the closely-packed molecules are approximated as point dipoles, and the intermolecular Coulombic interaction causes the electronic transitions to split into states with altered transition energies and oscillator strengths. For a dimer consisting of identical molecules with parallel transition dipole moments $\mu_{ge}$, the excitonic band splitting term is given as

$$ \Delta E = \frac{2 |\mu_{ge}|^2}{r^3} \left(1 - 3 \cos^2 \theta\right), \quad (2.9) $$
where $\theta$ is the inclination angle between the molecules and $r$ is the center-to-center distance. In equation 2.9, the excitonic splitting term is directly proportional to the transition dipole moment. Therefore, the excitonic splitting is significant only for strong transitions. Also, the inclination angle determines whether the excitonic coupling results in a higher H-type dimer or lower J-type dimer transition energy (cf. Fig. 2.5), compared to monomeric moieties. Note, that the exciton theory is valid only when the molecules can be treated as point dipoles, i.e., no intermolecular orbital overlap is present.

![Figure 2.5. Schematic exciton energy band diagram for a dimer of identical molecules with parallel transition dipoles. The dashed line with the red crosses corresponds to the forbidden out-of-phase dipole arrangement.](image)

The effect of aggregation in the absorption spectrum is illustrated in Fig. 2.6 that shows the absorption spectra (restricted for $Q_y$ band) for the isolated (red) and aggregated (dashed, blue) moieties of Zn-3$^1$-OH-PPME molecule. The formed aggregate has a J-type configuration, since upon the aggregation the absorption peak exhibits ca. 55 nm bathochromic shift in comparison to the isolated molecule. However, the measured aggregate is not a “pure” J-type aggregate, since the spectra do not show the characteristic line sharpening commonly observed for J-aggregates.[51,52] Similar type of head-to-tail assemblies have been observed for chlorophyll derivatives and porphyrins that exhibit J-type self-assembly.[53, 54] However, typically the studied systems may consist of different states of molecular aggregates (dimers, trimers, oligomers) and hence, the spectral shifts do not allow for a quantitative inspection of the molecular structure, but can be very useful to detect the onset of aggregation.
2.6 Red-edge excitation shifts

The molecular environment induce profound effects on the spectral properties of molecules affecting the fluorescence spectra, lifetime or the quantum yield. [55] For example, increasing solvent polarity has a tendency to shift the emission towards lower energies, due to the interaction between the excited state dipole moment and the polar solvent molecules. [56] Also, the immobilization to proteins or membranes, and binding to ligands can alter the spectral properties. [57]

Typical solvent relaxation times are ~10 ps for common polar solvents, whereas the fluorophore emission lifetimes are several nanoseconds. [58] Therefore, these system reaches equilibrium before any emission events take place. If the reorganization of the solvent molecules becomes restricted, it can give rise to red-edge excitation shift (REES), a phenomenon that describes the shifting of the fluorescence spectrum as a function of the excitation wavelength taking place in the red-edge of the absorption spectrum. [59] REES has been observed for a range of fluorophores and host materials, such as highly viscous and glass forming liquids [60, 61], polymer matrices [62, 63], and organized assemblies, such as micelles [64, 65] and proteins [66, 67].

The first prerequisite to observe REES, is the slowing of the molecular environment relaxation time down to the same magnitude with the excited state lifetime. [18] The second prerequisite is the inhomogeneous broadening of the absorption spectrum, which requires a distribution of solute-solvent interaction energies. Especially in condensed or viscous media, the molecules do not necessarily experience an identical environ-
Optical properties of molecules

ment, leading to the inhomogeneous broadening of the absorption and emission spectra. [59] This broadening is especially pronounced for dipolar molecules in polar media, which can be illustrated by the Onsager sphere approximation, where the width of the inhomogeneous broadening function $\Delta \nu$ is given as

$$\Delta \nu = A \Delta \mu a^{-3/2} (kT)^{1/2}. \quad (2.10)$$

$A$ is a constant that depends on the dielectric constant of the solvent, $\Delta \mu$ is the change of molecule dipole moment upon excitation, $a$ is the Onsager cavity radius, $k$ is the Boltzmann constant and $T$ is the temperature. [68] Terms $A$ and $\Delta \mu$ in Equation 2.10 illustrate that the inhomogeneous broadening increases in polar environments and for molecules with intense transitions, respectively. Additional broadening effects can be induced by specific interactions, such as hydrogen bonding and electrostatic interactions. [69,70]

Figure 2.7 schematically illustrates the temporal relaxation encountered in REES. Initially, the molecule is in ground-state and the molecular environment is in equilibrium meaning, that the dipole orientation is random.

![Figure 2.7](image)

**Figure 2.7.** (a) Schematic presentation of the continuous model of solvent relaxation showing the relaxation of the solvent molecules around the excited dipole from $t = 0$ to $t = \infty$. (b) Schematic Jablonski diagram, where the symbol I refers to the intermediate states between the initial FC state and the fully relaxed (R) state. $\nu_0$, $\nu_1$, and $\nu_\infty$ refer to the frequencies belonging to the FC, intermediate and relaxed states, respectively. $\lambda_{FC}$ and $\lambda_{\infty}$ correspond to the wavelength maxima of these states, respectively.

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At $t = 0$, the molecule is excited to the FC state that starts to interact with the environment. It minimizes the interaction energy between the fluorophore and the molecular environments by either reorienting the fluorophore or solvent molecules until the system is at equilibrium ($t = \infty$). Due to the temporal evolution of the relaxation, the REES effects are usually investigated with the help of time-resolved emission spectra, which is presented in Section 3.2.
3. Time-resolved optical measurements

The optical properties and phenomena presented in Chapter 2, can be studied using fluorescence or absorption spectroscopy. While the absorption and steady-state fluorescence measurements are relatively easy to measure using commercial spectrometers, the time-resolved measurements require more attention. Therefore, the next two Sections present the basics of time-resolved fluorescence and time-resolved emission spectra measurements.

3.1 Time-resolved fluorescence

Time-resolved fluorescence measurements form a vital part of any fluorescence study, as they reveal information normally excluded in steady-state fluorescence measurement. Time-resolved fluorescence can be studied either in the time or frequency domain. Here I will concentrate on time domain measurements.

In the time domain measurement, the sample is first excited with a short pulse that has a duration preferably much shorter than the lifetime of the sample. This is followed by recording the time-dependent fluorescence intensity decay profile $I(t)$. From the slope of the $\log I(t)$ plot, the lifetime can be determined as shown in Fig. 3.1a.

While in principle the intensity decay profile could be measured from a single excitation-emission cycle, this is practically impossible due to the limitations of modern electronics and detectors and the required temporal resolution to measure the lifetimes of organic molecules. [18, 71] Therefore, most of time-resolved measurements in time domain are done using a technique referred to as the time-correlated single photon counting (TCSPC).

Instead of direct recording of the fluorescence intensity decay, TCSPC
relies in collecting data over multiple excitation-emission cycles and then reconstructing the intensity decay profile as a histogram, which is illustrated in Fig. 3.1a. One of the prerequisites in a TCSPC measurement is that the excitation has to be attenuated so that a single excitation pulse has high probability to produce only a single fluorescence photon. This is necessary, as after detecting a photon, the detector and the read-out electronics have a dead time of few a nanoseconds. During this time period the system cannot register any photons. If this prerequisite is not met, early photons are over-represented in the histogram also known as the pulse pile-up. To avoid pulse pile-up, the counting rate should be kept low, for example near 1 %. [18]

Figure 3.1b illustrates how the intensity decay profile is formed in a TCSPC measurement. The time between the emission and excitation is measured by the single-photon counting stopwatch electronics. This cycle is then repeated several times, to form a histogram from the emission-excitation time difference readings.

The system used throughout this thesis is illustrated in Fig. 3.1c. The excitation source is a modelocked, frequency-doubled Ti:S femtosecond laser (Coherent, Mira 900-F) with repetition rate of 76 MHz, center wave-

![Figure 3.1](image-url)

**Figure 3.1.** (a) Top image: Schematic presentation of the fluorescence decay profile obtained from a time-resolved measurement shown in red color and the excitation pulse in green color. Center image: The photon counts at different time intervals registered at the detector. Bottom image: The obtained histogram from the TCSPC measurement used to reconstruct the fluorescence decay profile. (b) The measurement of the start-stop times in reverse mode TCSPC. (c) The schematic picture of the used TCSPC setup used in this thesis.
length 400 nm and a pulse width of 200 fs. Microchannel plate photomultiplier tube (Hamamatsu) was used as the detector for the emitted fluorescence. The whole optical system has a time resolution of 200 ps, determined from the measured instrument response function (IRF) from Ludox that consists in colloidal silica in water.

In the most simplest case, the fluorescence decay follows a single exponential decay. An example of such a situation would exist for a dilute (~1 μM) fluorophore solution. However in more complex situations, the observed decay has a multi-exponential character that usually stems from different molecular environment for each fluorophore or different populations of fluorophores within the studied sample. In this situation, the fluorescence intensity profile decay can be constructed from the sum of single exponential decays for $n$ components as

$$I(t) = \sum_{i=1}^{n} \alpha_i \exp\left(-t/\tau_i\right),$$

(3.1)

where $\alpha_i$ and $\tau_i$ are the amplitude and lifetime of the component $i$, respectively. In most cases, double or three exponential model is sufficient to gain a satisfactory fit to the measured data. To evaluate how many components are needed for a good fit is not a straightforward task. The goodness of the fit in Eq. 3.3 can be used as a guideline, but the most important criteria is always the knowledge of the physical model behind the observed phenomenon.

In practice, the observed fluorescence decay $I(t)$ is distorted by the IRF $I_{\text{IRF}}$ that consists of the time profile of the excitation source and the response function of the monochromator and detector. Hence, the observed fluorescence decay is described by the convolution [72]

$$I_M(t) = \int_0^t I_{\text{IRF}}(t-t')I(t')dt' = I_{\text{IRF}}(t) * I(t).$$

(3.2)

IRF can be acquired from a scattering sample, such as Ludox. In this thesis, the TCSPC data are analyzed using Fluofit software (Picoquant) that uses a reconvolution algorithm and least-squares analysis to obtain a fit to measured data. The goodness of the fit can be acquired from the reduced chi-square parameter that is given as [18]

$$\chi^2_R = \frac{1}{N-p} \sum_{k=1}^{n} \frac{1}{\sigma_k} [I_M(t_k) - I_C(t_k)]^2,$$

(3.3)

where $N$ is number of datapoints, $p$ is the number of fitted parameters, $I_M(t_k)$ is the measured data, $I_C(t_k)$ is the calculated decay for a given
number of datapoints used for the analysis and $\sigma_k$ is the standard deviation for each datapoint. Since the emission of a photon is a random process occurring independently from each other, a TCSPC measurement follows Poisson statistics. Thus, the standard deviation is simply the square root of the photon count as $\sigma_k = \sqrt{I_M(t_k)}$.

When working with multi-exponential decays, in most cases it is worthwhile to determine an average lifetime. In the analysis, the sum of amplitude components $\alpha_i$ are usually normalized to unity ($\sum \alpha_i = 1$). Moreover, two widely used expressions for the “average lifetime” are referred to as intensity-weighted and amplitude-weighted lifetimes, which are intermixed quite freely in the literature. However to be specific, when discussing average lifetime, only the intensity-weighted average lifetime should be used. It is defined as

$$\overline{\tau} = \frac{\sum_i \alpha_i \tau_i^2}{\sum_i \alpha_i \tau_i}.$$  

(3.4)

The amplitude-weighted lifetime is defined as

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \propto \int_0^\infty I(t),$$  

(3.5)

which is proportional to the area of the decay curve. This makes the amplitude-weighted lifetime useful when analysing the efficiencies of energy transfer as shown in Section 2.4.

### 3.2 Time-resolved emission spectra

The time-resolved emission spectra (TRES) can be recorded directly using pump-probe or upconversion methods or either indirectly using TCSPC. TRES can be thought to represent the fluorescence spectra taken at discrete times after a pulsed excitation. The procedure starts by measuring the time-resolved decays at different emission wavelengths $\lambda_{em}$ across the fluorescence spectrum $F(\lambda)$. In this thesis, the intensity decays were recorded using 5 nm step size in the monochromator. With 500 $\mu$m slit width, the monochromator spectral resolution was approximately 1.5 nm. These time-resolved decays are used to construct a two-dimensional matrix with wavelength and time as its dimensions, given by

$$I(\lambda, t) = \sum_{i=1}^n \alpha_i(\lambda) \exp \left[ -t/\tau_i(\lambda) \right].$$  

(3.6)

Again, the amplitudes are normalized to unity. Next, a new set of intensity decays need to be calculated so that the time-integrated intensity at
each wavelength is equal to the steady-state intensity at that wavelength

\[ H(\lambda) = \frac{F(\lambda)}{\sum_i \alpha_i(\lambda) \tau_i(\lambda)}. \]  

(3.7)

Equations 3.6 and 3.7 are used to obtain the normalized intensity decay functions at each wavelength, which are used to calculate TRES as in

\[ I'(\lambda, t) = H(\lambda)I(\lambda, t) = F(\lambda) \frac{\sum_i \alpha_i(\lambda) \exp\left[-t/\tau_i(\lambda)\right]}{\sum_i \alpha_i(\lambda) \tau_i(\lambda)}. \]  

(3.8)

The time-resolved emission spectra are now the curves obtained from Eq. 3.8 taken at time \( t_i \). The TRES data \( I'(\lambda, t_i) \) can be improved by fitting a log-normal line shape function to the data, where the log-normal function is given as [73,74]

\[ I_{t_i}(\lambda) = b \exp\left\{-\ln 2 \left( \frac{\ln [1 + 2\gamma(\lambda - \lambda_p)/\Delta]}{\gamma} \right)^2 \right\}, \]

(3.9)

where the variables \( b, \lambda_p, \gamma \) and \( \Delta \) denote the height of the peak, peak wavelength, asymmetry parameter and width parameter, respectively. The fitting is performed using a least-squares fitting function. An example of the fitting result is given in Fig. 3.2 that shows the log-normal line shape function as a red, dashed line fitted to the measured data points.

![Figure 3.2](image-url) A plot showing the results of the log-normal function (Eq. 3.9) fitting procedure.

After the aforementioned procedures, the same data can be used to extract more information about the temporal evolution of the studied system, such as the center of gravity and the full width at half maximum (FWHM) of TRES as done in Publication IV. The center of gravity of time-dependent spectrum is calculated as

\[ \lambda_{cg}(t) = \frac{\sum_i I'(\lambda_i, t)\lambda_i}{\sum_i I'(\lambda_i, t)}, \]

(3.10)
where $\sum_i I'(\lambda_i, t)$ (Eq. 3.8) is the summation over the time-resolved emission spectrum at some instant of time. The time-dependent full width at half maximum (FWHM) is defined as

$$\Delta \lambda(t)^2 = \frac{\sum_i [\lambda_i - \lambda_{cg}(t)]^2 I'(\lambda_i, t)}{\sum_i I'(\lambda_i, t)},$$

where the summations are calculated in a similar fashion as the emission center of gravity. The examination of $\Delta \lambda(t)^2$ can reveal information about the nature of the relaxation process. For example, a constant FWHM during the spectral relaxation may suggest a continuous process, whereas changes during the intermediate times could indicate a two-state process. [75,76]
4. Chlorophylls as nanophotonic components

Through the evolutionary process, the green pigments in plants, algae and bacteria, referred to as chlorophyll molecules, have become the vital components by which the photosynthetic organism convert solar energy into chemical energy. [77, 78] In photosynthesis, chlorophylls participate in the light-harvesting, energy transfer and charge separation processes. Nearly 100 different chlorophyll and bacteriochlorophyll pigments are known today, where the majority of these pigments exist in anoxygenic bacteria. [79]

This Chapter is organized in the following way: Section 4.1 presents the structural and optical properties of chlorophyll molecules. Section 4.2 presents the typical assemblies found in nature. Finally, Sections 4.3 and 4.4 present the results from Publications I and II, respectively, in which the potential of supramolecular zinc chlorin – polymer assemblies for photonic applications has been studied.

4.1 Chlorophylls

4.1.1 Structure

Most of the chlorophyll (Chl) molecules belong to the family of chlorin pigments, which are derived from the porphyrin macrocycle. In the IUPAC nomenclature the name “porphyrin”, describes heterocyclic molecules composed of four pyrrole molecules connected together with methine bridges as shown in Fig. 4.1a. [80, 81] Chlorins have a saturated bond between C-17 and C-18 in ring D and are systematically named as 17,18-dihydroporphyrins. A further reduction in bond between C-7 and C-8 in ring B forms the bacteriochlorin macrocycle, which is systematically referred to as 7,8,17,18-tetrahydroporphyrin.
In nature, chlorophylls and bacteriochlorophylls are rarely met in the forms shown in Fig. 4.1a. Instead, as an additional component, they have an isocyclic five-membered ring E fused to the parent macrocycle as presented in Figs. 4.1b and 4.1c. Moreover, (B)Chls generally occur as Mg\(^{2+}\) coordination complexes and a sesqui- (C-15) or di-terpenoid (C-20) long hydrocarbon chain esterified to the C-17 propionic acid chain (Fig. 4.1d), but exceptions to both of these characteristics exist.

The macrocycle in all tetrapyrroles consists in alternating single and double bonds between the carbon atoms, and has the ability to delocalize the \(\pi\)-orbitals around the macrocycle forming a highly stable cyclic \(\pi\)-orbital structure. The cyclic macrocycle also gives rise to property called aromaticity in all tetrapyrroles (porphyrins, chlorin, bacteriochlorins) that is manifested by exceptional stability, low reactivity, planarity...
of the macrocycle, uniform bond distances, optical properties and diamagnetic ring currents. [84,85]

The central metal in (B)Chls has a decisive influence in the formation of natural and artificial assemblies via the metal-ligand coordinate bond. The typical ligands in biological systems are different amino acid residues (histidine, glutamine, asparagine), water and other Chls. [86, 87] The Mg atom can be found in penta- or hexacoordinated form with one or two axial ligands, respectively, whereas Zn(II) as the central metal is only found as tetracoordinated (without ligands) or pentacoordinated (one axial ligand) forms. [88]

The peripheral substituents can provide additional bonding sites for hydrogen bonding or coordinative bonding with the central metal. For example, the electronegative atoms in the periphery of the macrocycle (e.g. carbonyl group at 13) can act as hydrogen bond acceptor and an electronegative atom with covalently bonded hydrogen (e.g. hydroxy group at 3) can act as the hydrogen bond donor. Moreover, due to the aromaticity of the (B)Chls, they are prone to $\pi-\pi$ interactions that usually take place together with other interactions. Also, the long hydrocarbon tail plays important role in the (B)Chl assembly in biological systems via hydrophobic interactions. [83]

### 4.1.2 Optical properties

The Chl molecules have strong absorption bands in the blue and red wavelength regions, also referred to as the Soret band (~380-430 nm) and the Q bands (~500-800 nm), respectively. These bands arise originate from the $\pi \rightarrow \pi^*$ transitions. [89] This is illustrated in Fig. 4.2 that presents the molar absorption coefficient spectrum ($\epsilon_A(\lambda)$) for a Chl $a$ derivative ZnPPME in tetrahydrofuran (THF). On the other hand, the Chls show little absorption in green spectral region (approx. 500-600 nm), thus giving a green or blue-green colour for the pigment. Because of this distinct feature, the molecules have inherited their name from Greek words (chloros “green”) and (phyllos “leaf”).

The properties of (B)Chls are highly tunable, as the saturation of the macrocycle bonds and the changes in the peripheral substituents can strongly affect the optical properties. For example, the saturation of the 7,8-double bond in BChls can increase the absorption in the visible and NIR range even 2-3 times extending the usable spectral range without additional alterations in the molecule. [21,90,91]
4.2 Biological chlorophyll assemblies

In the photosynthetic systems (PS), Chl molecules participate in the light-harvesting, funneling the excitation energy towards the reaction center (RC) and in the initial charge transfer process in the reaction center to create a proton gradient across the thylakoid membrane. [92, 93] In this sense, the Chl molecules are quite versatile, as they can act in any of the roles depending from the distance between the pigments or in the molecular environment. The photosystems are large protein assemblies consisting of light-harvesting complexes (LHC) surrounding a single RC. The RC's are highly specialized pigment-protein complexes with low pigment density. On the other hand the LHC surrounding the RCs, also known as the antennae, have high pigment density containing hundreds of pigments per RC. [94]

The most abundant chlorophylls in nature are Chl $a$ and Chl $b$ occurring in a ratio of 3:1. [95] Chl $a$ can be found from the peripheral and core antenna complexes, where it performs light-harvesting and also participates in the electron transfer within the reaction center. On the other hand, Chl $b$ is only found in the peripheral antenna complexes. As shown in Fig. 4.1b, the Chl $a$ and Chl $b$ differ only by their peripheral components. In position $R_7$, Chl $a$ has a methyl group and Chl $b$ has a formyl group. However, this small difference is sufficient to induce pronounced alterations in the optical spectrum between these pigments broadening the usable spectral bandwidth for photosynthesis. Therefore with subtle alterations in the precursor molecule, nature has developed many vari-
Chlorophylls as nanophotonic components

In algae and plants, the pigments are precisely organized by the protein matrix to facilitate efficient excitation energy transfer that takes place via the Förster mechanism. In general, this means that the pigments are organized close together and in correct orientations leading to very optimized structure that forms local energy funnels, avoids energy gaps and minimizes concentration quenching. Moreover, the average distance between the Chl molecules is 10 Å in most antenna complexes. [96] Figure 4.3 shows an example of fragment of the LHCII found in spinach, in which the Chls are bound to the proteins by the coordination bond between Mg and various amino acid residues.

However, the green sulphur bacteria have adopted totally different approach in their antennae structure. These bacteria live in extremely low-light conditions and still create their energy through the photosynthetic process. They possess the most efficient antenna structure found from nature, also called as the chlorosome, which is connected to the RC by the Fenna-Matthews-Olson complex. These structures are self-assembled into densely packed aggregates with highest known pigment density in nature. [97] Furthermore, due to the small distances between the pigments, the excitonic couplings between the molecules are strong leading to $J$-type of aggregation. The small distances also enable the excitation energy to delocalize over multiple pigments, leading to efficient coherent energy transport with quantum efficiencies close to unity. [6, 98] Artificial assemblies that mimic the chlorosomal structure have been prepared by utilizing the self-assembly of suitable functionalized metallochlorins.
and porphyrins via the hydrogen bonding and metal-oxygen electron pair coordination as illustrated in Fig. 4.4. [53,54,99]

4.3 Mimicking nature - Emissive and absorption regimes in Zn chlorin-poly(4-vinylpyrine) assemblies

Supramolecular dye-materials are particularly promising for creating functional materials for photonics. [100–102] However, the aggregative tendency of the dyes usually sets limitations for the usable doping levels in non-interacting host materials. Hence, there has been an increasing interest in supramolecular materials that can overcome the aggregative dye-dye interactions. [103,104] Motivated by the pigment-protein analogy from natural plants, in Publication I we studied supramolecular polymer dye materials exhibiting functional similarities as their biological counterparts. These results will be reviewed in this section.

As the host material we chose poly(4-vinylpyridine) polymer (P4VP). The pyridine group in P4VP has a free electron pair that can either act as a ligand for the central metal in coordinative bond or serve as a hydrogen bond acceptor. The binding of Zn chlorin to P4VP is also illustrated in Fig. 4.5. The binding of Chls and metalloporphyrins to P4VP in solution has been evidenced by several studies. [105, 106] Also recently, it has been shown by Arulkashmir et al. that the P4VP host can prevent the aggregation of Zn porphyrin in solid films even with high porphyrin load-
Figure 4.5. (a) Schematic illustration of the non-covalent binding of Zn chlorin in P4VP polymer matrix [Publication I]. (b) Zn Chlorin molecules studied in Publication I: Zn pyro-pheophorbide a methylester (ZnPPME) and Zn 3\textsuperscript{1}-OH-pyro-pheo-phorbide a methylester (Zn-3\textsuperscript{1}-OH-PPME). © The Royal Society of Chemistry, Publication I]
Chlorophylls as nanophotonic components

structure is changed into lamellar morphology that is a clear indication of the selective coordination of the Zn chlorins to the pyridine moieties even with 1 equivalents. Primarily, the change originates from the volume fraction increase of the P4VP domains due to the Zn chlorin binding.

Next, the optical properties of the Zn chlorin-P4VP assemblies were studied thoroughly with absorption and fluorescence spectroscopy. The required Zn chlorin-P4VP ratio was prepared in THF solution and spun on glass substrates, which were heated at 80°C for 2 h to remove residual solvent. Also, samples for ellipsometer and scanning electron microscopy (SEM) measurements were prepared on silicon substrates. The measurements revealed that the spun films were smooth and approximately 200 nm thick.

Figure 4.6 shows the absorption spectra for Zn chlorin-P4VP assemblies and Zn chlorins alone. Clearly, without the polymer the Zn chlorins form aggregates, as evidenced by the line broadening and strong bathochromic shift for the Q_y band. The shifts from the monomer to aggregate correspond to 666 → 677 nm and 655 → 699 nm for ZnPPME and Zn-3′-OH-PPME, respectively. The absorption spectra remains relatively constant for the loading values between 1:100 and 1:2 equiv., indicating that the P4VP host is able to keep the pigments in their monomeric form. However, for both pigments the spectra at 1 equiv. doping level exhibits aggregation characteristics, whereas the Zn-3′-OH-PPME Q_y band exhibits an additional shoulder around 700 nm, indicating stronger aggregation tendency induced by the 3′-hydroxy group.

The emission properties were first investigated using steady-state fluorescence measurements. From the steady-state measurement, a linear decrease in the fluorescence intensity with increasing chlorin concentration. Moreover, a linear quenching behaviour for both chlorins (Fig. 4.7a) was observed in Stern-Volmer type of plots. Moreover, in the SV plot for the Zn-3′-OH-PPME at 6-10 wt% doping, the quenching strength increases. This indicates that the self-aggregation due to the 3′-hydroxy group starts competing with the P4VP leading to enhanced coupling between the dyes, which is in line with the results of the absorption measurements.

Figure 4.7b presents the measured amplitude-weighted average fluorescence lifetimes as a function of pigment loading, which were calculated using Eq. 3.5. The fluorescence lifetimes remain quite constant up to 0.5 wt% (~0.002 equiv.) concentration, but decrease drastically when the concentration increases. Moreover, the decay modes change to biex-
Figure 4.6. The normalized absorption spectra of (a) P4VP(ZnPPME) and (b) P4VP(Zn-3\textsuperscript{1}-OH-PPME) for different pigment concentrations. The black and dashed lines correspond to the pure chlorin aggregates without the polymer as a reference. [© The Royal Society of Chemistry, Publication I]

ponential after the 0.5 wt% loading. Based on this, we can identify that at low loading, the pigments mainly remain in monomeric form, i.e., no substantial coupling between the dyes. For higher concentrations, the intermolecular distance is sufficiently short, enabling coupling via FRET.

To continue the analysis, the loading values were transformed to intermolecular chlorin-chlorin distances with the chromophore number density as [108]

$$r = 3\sqrt[3]{\rho_n^{-1}} = 3\sqrt[3]{\frac{w_i\rho N_A}{M_{dye}}}$$

(4.1)

where $\rho_n$ is the number density, $w_i$ is the mass fraction of the pigments in the polymer, $\rho$ is the density of the host material and $M_{dye}$ is the pigment molar mass. FRET efficiency was calculated using Eq. 2.8, based on the measured lifetime values. By fitting Eq. 2.5 to the calculated data, Förster distance $R_0$ could be determined for the assemblies as demonstrated in Figs. 4.7c and 4.7d. The analysis yielded $R_0$ values of 44.7 Å and 45.2 Å for ZnPPME and Zn-3\textsuperscript{1}-OH-PPME, respectively. These values correspond reasonably well to the reported literature values of 47 Å and 57 Å for Chl
Figure 4.7. (a) Stern-Volmer plots and (b) Fluorescence lifetimes for both pigment assemblies given as a function of dye weight percent. The energy transfer efficiency as the function of the intermolecular distance for (c) Zn-3′-OH-PPME and (d) ZnPPME in P4VP using a macroscale approximation. The Förster distance ($R_0$) was determined for both dyes by fitting the energy transfer function $f(R) = R_0^6/(R_0^6 + R^6)$ to the measured data. [© The Royal Society of Chemistry, Publication I]

$b$-Chl $a$ and Chl $a$-Chl $a$ pairs in a LHCIib complex, respectively. [109]

### 4.4 FRET mediated energy transfer in Purpurin 18-Zn chlorin-poly(4-vinylpyridine) assemblies

In Publication I, the emission and absorption regimes for the Zn chlorin-P4VP assemblies were identified, showing that this type of system could be utilized as an absorbing antenna structure or as an emissive moiety. However, the small Stokes shift and the low optical density of the assemblies limits their usability in emitting applications. For emitting applications, both of these factors could be enhanced with donor-acceptor architecture by integrating another pigment acting as the emitter in the Zn chlorin-P4VP matrix, illustrated in Fig. 4.8a. Naturally, this emitting pigment should fluoresce with lower energy than the absorbing moiety.

In Publication II, we wanted to study systems, where energy transfer in donor-acceptor assemblies mediated by FRET could be used to enhance the emissive properties of the material. These results are reviewed next.
In the study, we selected Purpurin 18 (P18) and ZnPPME as the acceptor and donor moieties, respectively. Figure 4.8b presents the P18 molecule and also the absorption coefficient for both the P18 and ZnPPME demonstrating the spectral differences between these pigments. The additional experimental details can be be found from Publication II.

The study was started by investigating the spectral overlap between ZnPPME and P18. Figure 4.9a shows both the absorption and fluorescence spectra for ZnPPME and P18. The spectral overlap extends from 640 – 740 nm, being highest at ca. 678 nm. The absorption and fluorescence peaks can be found from $\lambda_{Qy} = 666$ nm and $\lambda_{em} = 675$ nm, respectively. Correspondingly for P18, the absorption and fluorescence peaks are $\lambda_{Qy} = 704$ nm and $\lambda_{em} = 712$ nm, respectively. Therefore, the Stokes shift for this hybrid assembly is increased from 9 nm to 48 nm by using P18 as an acceptor moiety.

To investigate whether FRET is taking place within the assemblies, we performed excitation fluorescence scans with a spectral range of 400 – 600 nm, which are shown in Fig. 4.9b. The excitation spectra were collected for emission wavelengths 675 nm and 712 nm corresponding to the fluorescence maxima of ZnPPME and P18, respectively. Distinctively, the excitation spectrum of P18 has a good correspondence to the P18 ab-
Chlorophylls as nanophotonic components

Figure 4.9. (a) Absorption (dashed) and fluorescence (solid) spectra for ZnPPME (blue) and P18 (red) in P4VP. (b) Fluorescence excitation spectra for P18-ZnPPME-P4VP (dashed) and P18-P4VP (solid) assemblies. [© IEEE, Publication II]

Figure 4.10 shows the fluorescence spectra of the P18-ZnPPME-P4VP assembly with different ZnPPME:P18 ratios. Clearly, two peaks at 675 nm and 712 nm are visible when the samples are excited with $\lambda_{\text{exc}} = 430$ nm. As the P18 concentration increases, the ZnPPME emission decreases, while the P18 emission increases proportionally. Due to poorly chosen excitation wavelength, some direct P18 excitation might occur, but nevertheless, the absorption spectrum spectral details. Likewise, the excitation spectrum for the P18-ZnPPME hybrid collected at $\lambda_{\text{em}} = 675$ nm reproduces the spectral properties of ZnPPME absorption spectrum. The situation, however, is drastically changed when the excitation scans are collected at $\lambda_{\text{em}} = 712$ nm. The Soret band is significantly broadened, consisting from both P18 and ZnPPME peaks, indicating that the energy of ZnPPME is effectively transferred to P18.

Figure 4.10. The normalized fluorescence spectra of P18-ZnPPME-P4VP assembly with different ZnPPME:P18 ratios. [© IEEE, Publication II]
observed trend clearly demonstrates the existence of FRET within the assemblies.

Finally, the time-resolved measurements were carried out for the assemblies. The ZnPPME concentration was fixed at 2 wt%, while the ZnPPME:P18 ratio was altered. Table 4.1 presents the lifetime analysis results, together with the calculated effective energy transfer efficiency, calculated using Eq. 2.8. The donor lifetime was 1.31 ns. The obtained results clearly demonstrate that even at low acceptor concentrations, an efficient FRET process is taking place, which enables P18 to efficiently quench the ZnPPME fluorescence. This is also supported by the experimentally determined $R_0$ value of 42.2 Å. The FRET efficiency calculations show that the effective efficiency reaches a value of 82% for the ZnPPME:P18 ratio of 2:1. Moreover, the data also demonstrates that the FRET efficiency can be controlled by changing the ratio between ZnPPME and P18.

Table 4.1. The fluorescence lifetimes of ZnPPME and the effective FRET efficiencies for the P18-ZnPPME-P4VP complex with different ZnPPME:P18 ratios. [c IEEE, Publication II]

<table>
<thead>
<tr>
<th>ZnPPME:P18 ratio</th>
<th>$\tau_D$ (ns)</th>
<th>$E_{\text{FRET}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20:1</td>
<td>0.60</td>
<td>0.54</td>
</tr>
<tr>
<td>6:1</td>
<td>0.69</td>
<td>0.47</td>
</tr>
<tr>
<td>3:1</td>
<td>0.26</td>
<td>0.80</td>
</tr>
<tr>
<td>2:1</td>
<td>0.23</td>
<td>0.82</td>
</tr>
</tbody>
</table>
5. Nucleobases for photonics

Nucleotides are the building blocks in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). These polymers convey and store all genetic information in living organisms. DNA has been utilized quite extensively in electronic and photonic applications, for instance, as the electron transport layer in organic light-emitting diodes (OLEDs) [110], polymer waveguides [111], nonlinear optical devices [112] and as the gate dielectric in organic field-emitting transistors [113], to mention a few. On the other hand, nucleobases (NBs), the constituent moieties of DNA, have gained significantly less attention. This is surprising, as monomeric entities they are compatible with vacuum-based deposition techniques, and their properties are more reproducible in comparison to polymeric DNA. [3]

This Chapter is arranged in the following way. First, Section 5.1 will discuss some general properties of nucleobases. Second, Section 5.2 will present atomic and molecular layer deposition technique, which can be used to fabricate inorganic-organic assemblies. Finally, Section 5.3 will summarize the results of Publications III and IV, in which crystalline Na-uracil assemblies exhibiting excitation-dependent emission were studied.

5.1 Nucleic acids and nucleobases

Figure 5.1 illustrates the double-helix structure of DNA polymer that consists of hydrogen-bonded nucleotide pairs. The nucleotides are composed of unique nucleobases, pentose sugar molecule and a phosphate group. The helical structure of DNA is enabled by the complementary Watson-Crick pairing between the base pairs. The base pairs consist of purine bases (also known as nucleobases); guanine and adenine, and pyrimidine bases; thymine and cytosine. On the other hand in RNA, the thymine is replaced by uracil (pyrimidine). RNA, the single-stranded nucleic acid
polymer, is mainly responsible in translating the genetic information of DNA into proteins.

The nucleobases (NBs) exhibit quite large HOMO-LUMO bandgaps in the energy range from 3.6 to 4.1 eV. [114] According to the reported studies, the relative ionization potentials are ordered in a following manner; guanine < adenine < cytosine < thymine < uracil. From the NBs, guanine has the smallest HOMO and LUMO levels, making it good for hole transport and electron blocking, whereas uracil has the highest HOMO level for hole blocking and LUMO level for good electron transport. [115, 116]

Typically, NBs are non-luminescent due to the extremely low quantum yields, caused by the extremely efficient internal conversion. [117, 118] As an example, the typical fluorescence quantum yields of pyridimines in water are around 0.01. [119] However, earlier research also suggests that small variations to the NB conjugated system could dramatically increase quantum yields, opening new pathways to create more emissive species. [120,121]

![Figure 5.1](image)

**Figure 5.1.** Left-hand side illustrates the double-helix structure of DNA polymer composed of hydrogen-bonded NB pairs stacked between the sugar-phosphate polymer chains. Right-hand side shows the nucleobases of RNA and DNA, where the complementary hydrogen bonding sites in Watson-Crick pairing are highlighted.
5.2 Atomic/Molecular layer deposition

Atomic layer deposition (ALD) is a chemical vapour deposition technique that relies on the two-step, self-terminating surface reactions. [122, 123] Due to the sequential, self-limiting deposition steps, ALD can be used to deposit conformal films onto even most demanding surface topologies, such as nanostructured and high-aspect ratio surfaces and flexible substrates with atomic level thickness control. [124, 125] ALD deposited films have been shown to be uniform and pinhole-free with typical growth rates of 1 Å per reaction cycle. [126, 127]

The introduction of sequential, self-limiting processes utilizing organic precursors has made it possible to grow organic films with similar growth characteristics as the ALD deposited materials. This variant of ALD is referred to as molecular layer deposition (MLD) as actual molecular fragments are deposited. [128] Moreover, the combined action of ALD and MLD techniques enables the deposition of hybrid inorganic-organic assemblies, where the organic molecules are covalently bound to the metal atoms to form periodic, interlinked inorganic-organic chains. [129–131] These hybrid films may not necessarily possess just the combined properties of their host materials, but can also exhibit totally novel material properties.

![Figure 5.2](image.png)

**Figure 5.2.** (a) Schematic presentation of the four ALD/MLD steps; 1. pulsing of the inorganic precursor, 2. purge of the excess precursor and byproducts, 3. pulsing of the organic precursor and 4. purge. (b) Resulting inorganic-organic hybrid assembly with full monolayer coverage, *i.e.* the ideal case.
The typical ALD/MLD cycle is depicted in Fig. 5.2a that consists of four steps; precursor pulsing steps and purging steps. Also, Figure 5.2b shows a schematic illustration of a hybrid material, assuming a full monolayer coverage. The full monolayer coverage is an idealized case that should be achievable with sufficiently long precursor pulses. However, in practice there are many reasons why partial coverage takes place. One example could originate from the tilting or folding of linear, flexible organic molecules, which will reduce the amount of active sites on the surface. [129]

5.3 Excitation dependent emission in three-dimensional sodium networked uracil assemblies

Publications III and IV employed the ALD/MLD technique to fabricate hybrid inorganic and organic assemblies in layer-by-layer fashion. In this case, the utilized inorganic moiety was sodium cation and the organic moiety was uracil.

Uracil is known to assemble in a specific crystal structure (shown in Figure 5.3), which is stabilized via the hydrogen bonds and stacking interactions. [132, 133] Moreover, a tetrametric configuration has been shown to be favourable in the crystallization of NBs. [134] Uracil exhibits a tendency to bind alkali metals, such as sodium via the oxygen atoms. [135] In this sense, an inclusion of metal ion to form a stable crystalline nucleobase structure has a novel character, which has not been demonstrated before.

![Figure 5.3](image-url) Planar uracil structure described in Refs. [132, 133] and the labelling of the constituent atoms: oxygen (red), nitrogen (blue), hydrogen (white) and carbon (black).
The films were grown on the substrates using ALD/MLD layer-by-layer method taking place via ligand-exchange reactions between the uracil and Na(thd) (thd: 2,2,6,6-tetramethyl-3,5-heptadionate) precursors. The step height determined by AFM was 95 nm for a film deposited with 200 cycles, yielding a growth-per-cycle (the average increase of thickness in one ALD/MLD cycle) value of ca. 4.8 Å/cycle.

The structural properties were analyzed using Fourier transform infrared spectroscopy (FTIR). The FTIR spectra of uracil reference and Na-uracil is shown in Figure 5.4. The most pronounced red shifts are detected for modes $\nu(C=O)$ from 1710 to 1699 cm$^{-1}$ and $\nu(N-H)$ from 3129 to 2918 cm$^{-1}$ indicating that $C=O$ and $N-H$ are hydrogen bonded. On the other hand, the $\nu(C=O)$ red shift could also originate from the binding of sodium ions through oxygen. The band at 1630 cm$^{-1}$ belongs to $\nu(C=O)$ with a mixture of ring modes, although a contribution of adsorbed water could be present. The red shift of this band to 1608 cm$^{-1}$ could originate from the carbonyl group interaction with the Na ion. The blue-shifted resonance from 1414 to 1427 cm$^{-1}$ is assigned to the N–H···O hydrogen-bonding, as reported earlier. Moreover, the N–H bond shift 1507–1553 cm$^{-1}$ provides additional evidence in the collaborative hydrogen-bonding between the uracil molecules.

![Figure 5.4. The FTIR spectra from uracil reference powder (green) and Na-uracil thin film (blue). Figure (a) shows the whole measured specturm and (b) shows a selected wavenumber area.](image-url)

The films were found to be crystalline, as indicated by the sharp diffraction peaks in the grazing incidence X-ray diffraction measurements (GIXRD) in Fig. 5.5a. However, the peaks could not be fitted to any specific crystal structure, due to the complex and polycrystalline nature of
Nucleobases for photonics

Figure 5.5. (a) GIXRD spectrum of the sodium-uracil thin film deposited on silicon. (b) The proposed unit cell of the Na-uracil tetrametric structure, where four uracil molecules are bounded to one sodium ion (cyan). The hydrogen bonds are depicted with dashed lines.

these nucleic acid derived hybrid films.

Since GIXRD analysis was not able to shed light on the crystal structure of the Na-uracil films, density functional theory (DFT) calculations were used to investigate the structural parameters. Based on previous literature, the explored geometry was limited to planar tetrametric configuration that is capable of forming stable 2D structure as illustrated in Fig. 5.3. Based on the FTIR and NMR data, the structure in Fig. 5.5b was proposed. In this configuration, three neutral and one protonated uracil bound to Na cation in the central space was found to form a remarkably stable structure.

Following the structural investigations, the samples were studied optically. Figure 5.6a shows the measured absorption spectra of the uracil reference and the Na-uracil thin film both deposited on quartz. The uracil reference sample has two peaks at 200 nm and 260 nm, which are assigned to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions according to previous research, respectively. [118] In the case of Na-uracil, the 260 nm peak shifts to 277 nm and becomes broader. In addition, a long spectral trail is visible that extends to the visible wavelength range.

To gain more information about the optical properties, the absorption spectra of uracil and Na-uracil was calculated using time dependent DFT. As illustrated in Fig. 5.6, the measured spectra agrees well with the calculated ones. The observed bathochromic shift could originate from the planar tetrametric configuration of the uracil molecules. In addition, the
spectral trail could originate from the lower energy peaks similar to the one observed at 350 nm, but broadened by the structural heterogeneity in the Na-uracil film.

The steady-state fluorescence spectra are presented in Fig. 5.7. Surprisingly, the samples exhibited strong and intense fluorescence signals. The strongest signal was obtained using 280 nm pump wavelength. As illustrated in Fig. 5.7a, the Na-uracil films showed an excitation-dependent fluorescence in a broad wavelength range that starts taking place after the 310 nm excitation wavelength. To further study the shifting behaviour, the emission peak wavelengths were plotted as a function of the excitation (Fig. 5.7b). The fitted curve is linear ($R^2 = 0.988$) with a slope of 0.68 between the emission and excitation wavelengths. The slope stays essentially constant up to 540 nm excitation wavelength.

The shifting behaviour of fluorescence taking place at the red edge of the

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Figure 5.6. The absorbance of uracil reference (red) and uracil-sodium thin film (blue). (b) Calculated absorption spectra of uracil (red) and Na-uracil (blue).

Figure 5.7. Images from uracil sodium assemblies exhibiting a broad excitation dependent emission. (a) Normalized emission spectra with various excitation wavelengths. (b) A graph of the emission dependency from the excitation wavelengths including a linear fit with fitting parameters.
absorption spectrum is also known as the REES effect. This phenomenon is described in more detail in Section 2.6. To investigate in more detail this phenomenon, the assemblies were characterized in the time domain. According to the REES model, one should observe a continuous relaxation in TRES (cf. Section 3.2). To construct the TRES matrix, fluorescence intensity decay data was collected across the whole emission spectrum for 400 nm excitation wavelength. The fluorescence decay data was analyzed using a three-exponential model.

Figure 5.8a shows a collection of normalized TRES spectra calculated at different times after the excitation. The observed shifting of the TRES spectra to longer wavelengths is in accordance with the REES model. REES model also predicts that species excited to the FC state should emit at higher energies than the R state. Moreover, the emitting species at R state should exhibit longer lifetimes than the species in the FC state. This is exactly what is observed in Fig. 5.8b.

The figure shows intensity weighted lifetimes (Eq. 3.4) recorded at different wavelength values of the emission spectrum. The lifetime increases linearly from 1.7 to 2.4 ns, illustrating that the FC species emit their light earlier than the relaxed moieties. One can also conclude that the transi-
tion takes place in a continuous manner.

To strengthen the evidence for the REES model, the time-dependent fluorescence center of gravity and the fluorescence spectrum half width were calculated, shown in Figures 5.8c and 5.8d, respectively. The results are consistent with the REES model that requires continuous spectral changes. The center of gravity demonstrates a dynamic Stokes shift, where the majority of changes take place within 1 ns. Moreover, the spectral half width stays quite constant during the spectral relaxation.

Typically, the NB lifetimes are in the picosecond range due to the extremely efficient internal conversion. However, the measured uracil lifetimes for our films are three orders of magnitude higher than the reported values for uracil in aqueous solution. [117,141] Thus, based on the definition of lifetime (Eq. 2.4), one of the rate constants, $k_{nr}$ or $\Gamma$ must decrease. The most probable explanation would be the decrease of the non-radiative component, as the samples also exhibited bright fluorescence. This behaviour could stem from the interactions between uracil and sodium, or the extensive hydrogen bonding network between uracil molecules, which can inhibit the internal conversion process in uracil.
6. Plasmon enhanced spectroscopy

Plasmonics refers to the phenomena that arise from the coupling between electromagnetic fields and the conduction electrons of metallic interfaces or metallic nanoparticles. For various nanophotonic applications, this coupling is highly beneficial as it allows confining light into nanometer scale. This in turn enables new possibilities to use light to manipulate and interact with single nanoscale objects with small absorption cross-sections (e.g. molecules), which would otherwise be impossible. \[24\]

To date, metallic nanostructures have been employed in various applications, such as sensing elements for chemical and biological experiments \[142, 143\], enhanced spectroscopies (e.g. fluorescence and Raman scattering) and to enhance nonlinear processes \[144, 145\]. Moreover, single molecule detection sensitivity has been demonstrated \[146\], resulting in a wealth of applications utilizing metal enhanced fluorescence (MEF) \[147, 148\] and surface enhanced Raman scattering (SERS) \[149, 150\].

This Chapter is organized in following way. The first section discusses the properties of surface plasmon resonance and the influencing factors. Next section presents the foundations for metal enhanced fluorescence and surface enhanced Raman scattering. The last sections will review the results of economical and large-scale plasmonic substrates development for fluorescence enhancement and surface enhanced Raman scattering applications published in Publications \textit{V} and \textit{VI}, respectively.

6.1 Localized surface plasmon resonance

When light interacts with a metallic particle, it can give rise to the collective oscillation of the conduction electrons, also referred to as localized surface plasmon resonance (LSPR), which is illustrated in Fig. 6.1. These coupled modes of electrons and the incident electromagnetic field are lo-
Plasmon enhanced spectroscopy

calized close to the metal-dielectric interface with exponentially decaying electric field in a direction perpendicular to the interface. Resulting from this localization, LSPs can concentrate the incident light to nanometer scale and provide orders-of-magnitude enhancements to the local electromagnetic field strength. [12]

This local field enhancement enables plasmonic nanoparticles (NP) to be utilized as antennas that can efficiently focus the incoming electromagnetic radiation into small volumes. For isolated particles, the focusing effect is considered to consist of the plasmonic enhancement at the resonance frequencies and the lightning rod effect, which is related to the shape of the particle. The plasmonic enhancement effect takes place by accumulating of surface charges upon resonance that produces the strongly localized electric fields. The lightning rod effect is a phenomenon in which sharp features of an electrically charged particle produce the strongest electric fields. [12,24]

![Schematic illustration of the localized surface plasmon oscillation for metal nanoparticle in a sinusoidally varying electric field.](image)

**Figure 6.1.** Schematic illustration of the localized surface plasmon oscillation for metal nanoparticle in a sinusoidally varying electric field.

The resonance wavelength is affected by multitude of different factors, such as shape and size of the particle, composition and local environment. Generally, the increase in the particle size tends to red shift the resonance. Moreover, the extinction spectrum of a NP consists of scattering and absorption that exhibit $R^6$ and $R^3$ dependency on the particle radius, respectively, meaning that for small particles ($R < 25$ nm) the extinction is mostly dominated by the absorption, whereas for large particles the scattering dominates. [151]

In the quasi-static approximation ($d \ll \lambda$, i.e. the particle is much smaller than the wavelength of incident light), the polarizability for a
Plasmon enhanced spectroscopy

A spherical particle is given as [152]

$$\alpha(\omega) = 4\pi R^3 \frac{\epsilon(\omega) - \epsilon_m}{\epsilon(\omega) + 2\epsilon_m},$$  \hspace{1cm} (6.1)$$

where $\epsilon_m$ is the dielectric constant of the surrounding medium and $\epsilon(\omega)$ is the dielectric function of the metal particle. Although Eq. 6.1 is only applicable to small, spherical particles, it demonstrates the strong dependence of the resonance frequency on the dielectric environment, which is the basis for LSPR sensors in biological and chemical experiments. Typically, when the value $\epsilon_m$ increases, the resonances will red shift. [153]

In the visible spectral range, the most popular materials are gold (Au) and silver (Ag) due to their low losses. From these two materials Ag has lower losses, but it’s strong chemical reactivity has favoured Au in practical applications. Also recently, aluminium [154] and copper [155] have been utilized as cheap and abundant plasmonic materials for UV-Vis and NIR spectral range, respectively.

Coupled NP systems can provide improved tunability and control of spectral properties in respect to single NP systems. The coupling effects between plasmonic NPs can lead to spectral changes and additional field enhancements. For example in a particle dimer, by adjusting the distance between the particles and shifting between the two orthogonal polarizations of the incident light, the resonance can be tuned anisotropically. [156] Also in particle dimers (or oligomers) with few nanometer gaps, the near-field coupling can create intense field enhancements, also referred to as “hot spots”, enabling single molecule sensing [157] or supercontinuum generation [158]. Moreover, the ordered arrays of NPs can exhibit extremely narrow and intense extinction lineshapes due to the collective/coupled resonance modes that consist of the single NP LSPRs and the diffraction orders of the array [159,160]. Moreover, these ordered arrays can be tuned for spectrally [161] or spatially [162] selective responses. Finally, ordered clusters of NPs can support complex collective oscillations that can provide very broad plasmonic resonances with tunable Fano resonances for sensing purposes. [163]

6.2 Plasmon enhanced spectroscopy techniques

In this thesis, the investigated surface enhanced spectroscopic techniques are MEF and SERS. Both of these phenomena can be described within the same theoretical quantum optical framework, differentiated only by the
molecule-NP distance. \[164, 165\] In principle, the observed enhancement effects in both techniques consist of two factors that are:

1. Increased excitation rate for the molecule.

2. Improved emission to the far field.

The contribution of these factors takes place in a following way. First, the metallic NP will concentrate the incoming radiation into nanoscale volumes that intensifies the local field and increases the excitation rate. In the presence of metal particle, the local field enhancement for a molecule situated at \(r\) is given as

\[
\gamma_{\text{exc}} \propto \left| \mathbf{E}(r, \lambda) \right|^2 / \left| \mathbf{E}_0 \right|^2, \tag{6.2}
\]

where \(\mathbf{E}_0\) is the electric field without the nanoparticle present.

Second, the excited fluorophore induces a mirror dipole in the NP, which can reradiate the emission more efficiently to the far field resulting in enhanced luminescence. It has been considered that this coupling takes place via the scattering modes of the NP. This theory has been supported by the observation of angular-dependent and \(p\)-polarized emission in MEF measurements indicating that the far-field emission originates from a coupled plasmon-fluorophore system. \[166, 167\] In addition, due to the scattering efficiency dependency in the particle size, particles in which the scattering dominates over the absorption, have been shown to be more ideal for MEF applications. \[168\]

As a summary in both cases, the NPs can be thought to act as an antenna that can function either in the focusing or collecting mode. The following of this section will be devoted in a more detailed description of the principles of MEF and SERS.

### 6.2.1 Metal enhanced fluorescence

In addition to the aforementioned factors, the plasmonic NPs also change the local photonic mode density leading to changes in the radiative and non-radiative decay pathways as illustrated in Figure 6.2. These changes play an important role in the fluorescence enhancement.

The quantum yield and lifetime for an isolated molecule were introduced in Section 2.2. The modified quantum yield for a fluorophore in the vicin-
Figure 6.2. Schematic Jablonski diagram for (a) an isolated fluorophore and (b) a fluorophore adjacent to a metal nanoparticle. \( \Gamma \) and \( k_{nr} \) are the radiative and non-radiative decay rates for an isolated molecule, whereas \( \Gamma_m \) and \( k_m \) are the radiative decay rate and non-radiative decay rate for a molecule next to a plasmonic NP, respectively. \( k_a \) and \( \gamma_{exc} \) are absorption rates for an isolated molecule and a molecule-plasmonic NP moiety, respectively.

The efficiency of a plasmonic NP is expressed as [19]

\[
\Phi_m(d) = \frac{\Gamma_m(d) + \Gamma}{\Gamma_m(d) + k_m(d) + \Gamma + k_{nr}},
\]

(6.3)

where \( \Gamma_m \) and \( k_m \) are the radiative decay rate and non-radiative decay rate induced by the metal NP, respectively. Moreover, both \( \Gamma_m \) and \( k_m \) depend on the distance \( d \) between the NP and the fluorophore. Similarly, the lifetime changes to

\[
\tau_m(d) = \frac{1}{\Gamma_m(d) + k_m(d) + \Gamma + k_{nr}}.
\]

(6.4)

Both \( \Gamma_m \) and \( k_m \) will approach zero with increasing fluorophore-metal distance, and thus the \( \Phi_m \) will approach the value of \( \Phi \) at far enough separation. The contribution of \( k_m \) dominates at distance smaller than 2 nm due to the efficient unidirectional energy transfer to the metal particle, which will quench the fluorophore effectively. However, the effectiveness of \( k_m \) decreases more rapidly than \( \Gamma_m \) at longer distances. Because of this, MEF experiments often employ a dielectric spacer layer to prevent quenching. Moreover, various single molecule experiments in which the emission intensity has been measured as a function of the particle-molecule distance, have given a strong evidence that the fluorescence enhancement has a local maximum. [169–171]
In addition, the relative orientation of the fluorophore to the metal and the degree of spectral overlap between the LSPR and the absorption/emission spectra is an important factor determining the fluorescence enhancement strength. [172] Recent investigations indicate that the maximum enhancement value is obtained when the fluorophore emission peak is slightly red shifted from the plasmon resonance of the particle. [173] Finally summing up all the contributions, the fluorescence rate enhancement using Eqs. 6.2 and 6.3, is given as \( \gamma_{\text{enh}} = \frac{\gamma_{\text{exc}} \Phi_m}{\Phi} \).

The plasmonic NP induced quantum yield increase always leads to shortening of the molecule lifetime. This also means that the fluorophore stays less time in the excited state, which in turn decreases the probability that a fluorophore can decay by another pathway or photobleach, which improves the photostability of the molecule. [10, 19]

It is worth noting that if the intrinsic quantum yield of a fluorophore is already close to unity (e.g. Rhodamine 6G [174] or terrylene in p-terphenyl matrix [175]), the achievable enhancement values are much more modest, 10-20-fold. [170, 171] However, for fluorophores with lower quantum yields, the obtainable enhancement values can be substantially higher. For instance, a 40-fold enhancement has been obtained for IR800 fluorophore with gold nanoshells [148], a 100-fold enhancements have been measured for cyanine dyes situated in periodic silver nanoparticle arrays [176] and even 400-fold enhancements have been obtained for phthalocyanine molecule with a silver island film with 15 nm fatty acid spacer layer [177].

### 6.2.2 Surface enhanced Raman scattering

Raman scattering is an inelastic scattering phenomenon in which a photon is coupled to the vibrational modes of the molecule via the polarizability of a molecule. [178, 179] This process is schematically illustrated in Fig. 6.3. In a classical scattering phenomenon, most light is scattered elastically (Rayleigh scattering) meaning that the incident and scattered photons have same energy. When a part of the incident photon’s energy is transformed into vibrational quanta of the molecule, this process is termed as Stokes scattering. In this case, the scattered photon has lower energy than the incident photon. Also, there might exist a possibility that the molecule already lies at an excited vibrational state, and the scattering takes place to the ground state. In that case the scattered photon has higher energy than the incident photon. This type of scat-
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scattering is referred to as anti-Stokes scattering and it is generally weaker than the Stokes scattering process. The resulting Raman spectrum provides unique “fingerprint” for a given molecule and provides a powerful label-free measurement scheme, which has enabled the widespread use of Raman in physical, chemical and materials sciences. [180, 181]

![Figure 6.3. Schematic Raman scattering diagram.](image)

Since, the Raman scattering cross-section ($\sim 10^{-30} \text{ cm}^2$) is 14-15 orders-of-magnitude smaller than the fluorescence cross-section ($\sim 10^{-15} \text{ cm}^2$), surface enhanced Raman scattering (SERS) has emerged as a powerful method to enhance the Raman signals. [182, 183] When a molecule is brought close to a roughened metal surface or plasmonic nanostructure, the Raman signal can be increased by 4-14 orders of magnitude in comparison to bulk, enabling single molecule sensitivity. [13, 146] The enhancement in a SERS experiment consists of the plasmon-molecule coupling that was discussed in the beginning of this section and from chemical enhancement. The chemical enhancement can provide roughly 10-100 times boost to the Raman signal. [184] Since the frequency shifts of the Raman scattered light are usually much lower than the plasmon resonance linewidth, the total enhancement factor can be approximated to mainly consist of the local field enhancement as $G = \left| \gamma_{\text{exc}}(\omega) \right|^4$. [24] Especially, the “hot spots” between adjacent metal nanostructures provide the highest enhancement. Recent studies have also shown that they also contribute disproportionally large portion of the observed Raman signal. [185, 186] Furthermore, as both of the enhancement mechanisms require close proximity to the plasmonic particle in order to work effectively, the SERS enhancement decreases very rapidly as the function of distance.
6.3 Plasmonic materials for surface enhanced spectroscopy

The majority of periodical nanostructures are currently fabricated using e-beam lithography (EBL) or focused ion beam (FIB) milling. [187–189] In EBL, a photoresist is sequentially exposed using an electron beam, which is followed by development and lift-off steps. In FIB, a metal film or bulk is milled with energetic ions causing site specific sputtering to define the desired structure. Both methods provide high-resolution and structural design freedom making them ideal for prototyping. However, due to their serial nature, both methods have very high fabrication costs, rendering them unfeasible for patterning areas larger than a couple of mm\(^2\). [176, 190] This high-cost can be amended with other techniques, such as nanoimprint lithography (NIL), where a large master stamp is used to pattern structures on a soft resist. [191] However, this approach limits the freedom in design and the high-cost related in the patterning of the stamp is still present. Moreover, imprinting high-aspect ratio nanostructures is not a trivial task, even with dedicated facilities.

Other approaches to produce periodical structures include bottom-up methods and interference lithography. Bottom-up methods utilize self-assembly of colloidal spheres to form a mask or template for metal deposition. A notable example of this is the Fischer pattern, which is created using self-assembled polystyrene sphere acting as the shadow mask and a subsequent metal evaporation. However, the creation of sufficiently large and defect-free structures is challenging, which limits the applicability of this approach. [192]

Optical interference lithography (IL) is an established technique that has several advantages in fabricating periodic structures. [193–195] IL utilizes an interference pattern to expose photoresist created by two or more intersecting, coherent light sources. As a technique, IL is relatively simple and inexpensive and capable of exposing a whole wafer at once without a need for a photomask. The downsides of traditional IL are related to the sensitivity of the post-processing steps, e.g., the used resists are sensitive to stray light, temperature and moisture. Thus, all the post-processing steps must be conducted immediately after the exposure and preferably in facilities (e.g. cleanrooms), where the temperature and moisture are controlled. Moreover, even moderately reflective surfaces can lead to the generation of standing wave patterns in the photoresist, which are unwanted in the exposure process. [194, 196] The amplitude of
this standing wave pattern can be decreased by reducing the reflection of the substrate surfaces with highly absorbing and antireflective coatings. [194] Also, increasing absorption of the resist (e.g. by incorporating dyes) can decrease the standing wave effect.

Figure 6.4 illustrates the Lloyd mirror interferometer setup used in Publication V, where the mirror is perpendicular to the substrate creating the second beam. In this type of interferometer, the directly incident and the reflected beams will create the interference pattern on the surface of the photoresist film with a periodicity of $\Lambda = \lambda/2 \sin \theta$. [197, 198] By rotating the whole mirror stage with respect to the incident beam, the periodicity $\Lambda$ can be adjusted from tens of microns to the half wavelength. However, if the beam divergence angle is not zero, this will introduce error in the interference pattern periodicity away from the mirror. [198] The Lloyd mirror setup is quite robust to mechanical vibrations and air turbulence, since only the optical path variations between the closely spaced mirror and sample give rise to interference pattern disturbances.

Figure 6.4. Schematic illustration of the interferometer setup using the Lloyd mirror.

For SERS applications, non-ordered structures have gathered considerable attention due to the ease of fabrication. The selection of utilized structures is quite broad, such as colloidal metal particles in solution [13, 146], roughened surfaces (metal islands [199], chemically etched surfaces [200]), metallic nanowires [201] and silicon nanowires coated with metal [202] to mention a few. These disordered aggregates can provide very high enhancement factors due to the abundance of “hot-spots”, but a trade-off between the reproducibility and enhancement is often encountered. The highest enhancement factors for SERS have been obtained for colloidal materials, but this approach is hindered by the aggregation of the metal particles before the analyte is introduced.
6.3.1 Azopolymer based fabrication scheme for large-scale plasmonic arrays

Polymers containing azobenzene molecules, also referred to as azopolymers, are particularly promising as an alternative photosensitive material in interference lithography. The advantage of azopolymers is their ability to form a surface relief gratings (SRG) when illuminated with an interference pattern that has either polarization or spatial intensity gradients. [203] The resulting SRG is an exact replica of the incident irradiation pattern and thus azopolymer-based processes do not necessarily require additional post-processing steps. [204, 205]

The SRG formation originates from the reversible cis-trans photoisomerization of azobenzene molecules upon exposure to light also shown in Fig. 6.5. [206] The azopolymers normally exist in the straight trans-state, but upon absorbing a photon of suitable energy they can rapidly switch to the bent cis-state. The separation of the para-carbon atoms can be even from 5.5 to 10 Å between the cis-trans-state as illustrated in Fig. 6.5, respectively. [207] The relaxation back to the trans-state can take place thermally or by absorbing a photon with different wavelength. [208] Thus, the continuous exposure to light usually leads to a reversible switching between the photoisomerization states enabling macroscopic light-induced mass transport in azopolymer films below the glass transition temperature. [209]

The azobenzene molecules in trans-state are more efficiently excited with light that is polarized along the molecules. This polarization sen-

![Figure 6.5. The trans-cis photoisomerization of pseudo-stilbene molecule.](image-url)
Plasmon enhanced spectroscopy

sitivity mitigates their sensitivity to stray light and enables processing in normal lighting conditions. [210] In addition, the polarization sensitivity also eliminates the standing pattern, which is present when performing exposures on reflective surfaces with conventional photoresists. Finally, films prepared using azopolymers are not overly sensitive to over exposure enabling more advanced mask designs with multiple exposures. [204]

To date, azopolymer interference lithography (APIL) has proven to be a versatile technique for nanofabrication, as well as in micro and nanophotonic applications. [211] APIL has been employed to pattern gold [205] and silicon surfaces [204], for sub-wavelength tapered holes and wavelength-scale conical structures from gold [212] and as an template to grow GaAs nanowires [213].

Motivated by these properties of APIL, in Publication V we studied the potential of azopolymers in fabricating large-scale plasmonic nanostructures for enhancing molecular fluorescence. These results will be reviewed in the following section. In Publication V we used poly(disperse red 1 acrylate) azopolymer (pDR1A) that has pseudo-stilbene side-chain attached to the acrylate polymer chain.

**Process flow**
The whole process flow is schematically illustrated in Fig. 6.6. More detailed description of the fabrication process can be found from Publication V. Diced and cleaned borosilicate glass substrates were coated with 40 nm of silver followed by 20 nm of gold using e-beam evaporation. Next, the azopolymer was spun on the substrates and dried for 2 h at 85°C yielding ~100 nm polymer films. The 2D grating was created by two subsequent exposures in a Lloyd mirror interferometer ($\lambda_{\text{exp}} = 488$ nm). The second exposure was performed for a 90° rotated sample. SRGs were partially etched using a reactive ion etcher (RIE) with oxygen to expose the Au surface. The purpose of the Au layer was solely to protect Ag from oxidation in the $O_2$ etch step. To transcribe SRG onto the metal films and form the nanostructures, RIE etching using argon was performed. The etching was done in steps (30 s etch, 2 min cooling) to prevent the polymer mask melting down during the RIE etching. The resulting structures were annealed in nitrogen atmosphere (300°C) and coated with 10 nm of ALD alumina to provide a quenching spacer layer and protect the Ag nanostructures from oxidation. A photograph of the ready substrate is shown in Fig. 6.6.
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Figure 6.6. The schematic process flow using the azopolymer as an etching mask: a) deposition of Ag (40 nm) and Au (20 nm) using e-beam evaporation; b) spin coating of the azopolymer (100 nm); c) inscription of the SRG on the azopolymer; d) etching in O\textsubscript{2} to expose the Au surface; e) dry etching in Ar; and g) Annealing and ALD deposition of 10 nm thick Al\textsubscript{2}O\textsubscript{3}. (g) Photograph of a 2D grating (\(\Lambda = 370\) nm) fabricated on a 2 \(\times\) 2 cm\textsuperscript{2} glass substrate. [© The Royal Society of Chemistry, Publication V]

Characterization and results

Two samples with periodicities of \(\Lambda = 370\) nm and \(\Lambda = 1\) \(\mu\)m were fabricated. The morphology of the fabricated structures was analysed using SEM and atomic force microscope (AFM). Figure 6.7 shows a collection of SEM and AFM images of the fabricated structures for both gratings. These results demonstrate that the structures are mainly homogeneous in shape and size, with a small degree of ellipticity and imperfections. The slight ellipticity might originate from the variations between the two subsequent exposures. The SEM analysis also reveals the highly symmetrical nature and good long-range order of the samples. The statistical SEM image analysis gave an average diameter of \(d = 142 \pm 3\) nm and \(d = 175.6 \pm 3.8\) nm for the samples with \(\Lambda = 370\) nm and \(\Lambda = 1\) \(\mu\)m, respectively. Moreover, the AFM analysis gave average height of \(h = 67 \pm 5.5\) nm and \(h = 144 \pm 11\) nm for \(\Lambda = 370\) nm and \(\Lambda = 1\) \(\mu\)m, respectively.

Based on the measured extinction spectra, Rhodamine 6G (R6G) and Cascade Blue (CB) were selected as fluorescence probes for the \(\Lambda = 370\) nm and \(\Lambda = 1\) \(\mu\)m gratings, respectively. The reference sample in the confocal and fluorescence measurements was ALD alumina coated glass substrate to minimize any errors originating from varying degrees of immobilization on different surfaces. Moreover, the reference and the NP sample were dipped using same procedure parameters. Edge-filters were utilized in the measurements to block unnecessary excitation light.

The stage-scanning confocal images and the fluorescence spectroscopy results are shown in Fig. 6.8. The scan areas in the confocal measurements were 500 \(\times\) 500 \(\mu\)m\textsuperscript{2} and 250 \(\times\) 250 \(\mu\)m\textsuperscript{2} from samples with \(\Lambda = 370\) nm and \(\Lambda = 1\) \(\mu\)m, respectively. The uniformity of fluorescence
in the large-area confocal scans clearly demonstrates the spatial homogeneity in both nanoparticle gratings, and provides additional evidence to the good long-range order of the samples. The enhancement factor was determined by calculating the ratio of the averaged fluorescence between the nanoparticle sample and the reference sample, while keeping the excitation irradiance constant during the measurements. The enhancement factor for R6G on the $\Lambda = 370$ nm grating is 14-fold in the confocal measurement and approximately 11-fold in the fluorescence measurement, which are quite close to each other. Correspondingly, the enhancement factor for CB on $\Lambda = 1 \, \mu m$ grating is only 2-fold in the confocal measurement. However, the enhancement factor for CB is roughly 6.5 in the fluorescence measurement. Most likely, this difference could be explained by the higher collection efficiency and the different collection geometry in the fluorescence measurement.

As discussed earlier, the molecular fluorescence enhancement is usually accompanied by the shortening of lifetime. Based on this information, we measured the lifetimes for R6G and CB on the nanoparticle gratings, on glass reference and in solution. The measured lifetimes are presented in Table 6.1. Emission wavelengths of 560 nm and 420 nm were used for R6G and CB, respectively. The obtained lifetimes for R6G and CB in DIW were 3.98 ns and 3.86 ns, respectively, and could be satisfactorily fitted using a single exponential model. Especially, the lifetime of R6G corresponds exceptionally well to the values reported in the literature. [214] Upon the deposition on glass, the lifetimes of R6G and CB are decreased
Figure 6.8. Stage-scanning confocal microscope and fluorescence scans using R6G (a-c) and CB (d-f) as the probe fluorophores. Figures (a and d) are the confocal microscope scans from glass reference samples with deposited molecules, and figures (b and e) present the confocal microscope scans from samples, in which the molecules are deposited on the fabricated nanostructures. For image pairs (a-b) and (d-e) the upper and lower limits are set to the same values to more clearly demonstrate the measured fluorescence intensity difference. The used excitation wavelengths were 532 nm for (a-b) and 403 nm for samples (d-e), and the average excitation power was 72 μW. Images (c) and (f) show the fluorescence spectra of R6G molecules and CB molecules, respectively, deposited on glass substrate and on 2D nanoparticle array. The excitation wavelength used for R6G was 520 nm and 350 nm for CB. [© The Royal Society of Chemistry, Publication V]

to 1.53 ns and 2.42 ns, respectively. As CB is designed to be more resistant to quenching effects, the relative drop in lifetime is lower than for R6G. Moreover, the fluorescence decay gains a biexponential character upon the deposition on glass. As expected, when the fluorophores are placed on the nanoparticle grating, the lifetimes are decreased. The lifetime of R6G is decreased to 0.51 ns and the lifetime of CB to value of 0.93 ns. For both fluorophores this corresponds to approximately 3-fold decrease in the lifetime in comparison to the glass reference.

The lifetime decrease upon the glass deposition originates from the changed photonic mode density of glass versus to the solution and also the quenching effects of oxygen. Especially on glass samples, the longer lifetime component is proportional to the reduced relative local density of states of the molecules on alumina coated glass surface (factor of ~1.4-1.5). [215] Interestingly, when the molecules are deposited on the nanoparticle grating, the shorter lifetime component accounts approximately 80%
of the total decay indicating a strong nanoparticle-fluorophore coupling. Thus, the lifetime analysis results are in qualitative agreement with the measured fluorescence enhancement values.

**Table 6.1.** The parameters for the fluorescence lifetime analysis showing the lifetime and amplitude components \((\tau_i, ns)\) and \(x_i\) for the fit, respectively, mean lifetime \((\bar{\tau}, ns)\) and goodness of the fit \(\chi^2_R\). [© The Royal Society of Chemistry, Publication V]

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\tau_1 (x_1))</th>
<th>(\tau_2 (x_2))</th>
<th>(\bar{\tau})</th>
<th>(\chi^2_R)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhodamine 6G</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in 1 (\mu)M DIW solution</td>
<td>3.98 (100%)</td>
<td>-</td>
<td>3.98</td>
<td>0.97</td>
</tr>
<tr>
<td>on glass</td>
<td>1.72 (63%)</td>
<td>0.62 (37%)</td>
<td>1.53</td>
<td>1.16</td>
</tr>
<tr>
<td>on nanoparticles</td>
<td>0.79 (20%)</td>
<td>0.15 (80%)</td>
<td>0.51</td>
<td>1.22</td>
</tr>
<tr>
<td><strong>Cascade Blue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in 1 (\mu)M DIW solution</td>
<td>3.86 (100%)</td>
<td>-</td>
<td>3.86</td>
<td>1.06</td>
</tr>
<tr>
<td>on glass</td>
<td>2.44 (94%)</td>
<td>0.41 (6%)</td>
<td>2.42</td>
<td>1.11</td>
</tr>
<tr>
<td>on nanoparticles</td>
<td>1.35 (23%)</td>
<td>0.25 (77%)</td>
<td>0.93</td>
<td>1.91</td>
</tr>
</tbody>
</table>

**6.3.2 Black silicon as a SERS substrate**

Black silicon (BSi) is used to refer to a silicon substrate with nanoscale surface features. These features can be fabricated using a vast selection of dry and wet etching techniques. Mostly, the nanostructured BSi surfaces consist of conical shaped features (or even spikes) that lead to efficient (e.g., 99% [216]) absorption of light in the visible and infrared spectral range. Due to the efficient light absorption, the surface of the material appears black, hence the name black silicon.

Originally, these nanoscale surface features were considered as unwanted side-effects of dry etching, but lately BSi has gathered a lot of attention as a cost-efficient and large-scale way of producing nanostructures. This is because these structures can be fabricated with maskless dry etching, which should be available in any silicon processing facility. BSi has been utilized to increase the absorption in silicon solar cells enabling, record high efficiencies [217], creating superior black surfaces with minimal reflection \((R < 0.5\%)\) in the spectral range of 350 nm−2000 nm [218] and as SERS substrates [219–222]. However, the effect of the spike structure and the metal coating thickness on the plasmonic behaviour on these conical BSi SERS substrates has not been thoroughly investigated. Therefore, in Publication VI we investigated how these parameters influence the SERS
enhancement. These results are reviewed in this section.

The BSi samples were fabricated on a RCA-1 cleaned wafers using a cryogenic deep reactive ion etching with inductively coupled plasma (ICP-RIE) using $\text{SF}_6/\text{O}_2$ plasma at a temperature of $-110^\circ\text{C}$. Two different BSi substrates with different cone geometry and density were fabricated by varying the process parameters in the ICP-RIE etching. The inductive plasma power was a constant 1 kW, whereas the other process parameters are listed in Table 6.2. After sample cleaving, the BSi substrates were coated with silver using e-beam evaporation. Figure 6.9a shows a photo of a 4 inch BSi wafer and a silver coated BSi sample piece, demonstrating the uniformity of the process.

<table>
<thead>
<tr>
<th>Table 6.2. The used ICP-RIE parameters in fabricating the two different BSi substrates.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>BSi01</td>
</tr>
<tr>
<td>BSi02</td>
</tr>
</tbody>
</table>

R6G was used to evaluate the SERS enhancement. The R6G was deposited on the samples by incubating the substrates in 1 $\mu\text{M}$ R6G with 0.75 mM NaCl in DIW for 10 minutes. To remove excess R6G, the samples were rinsed with DIW and dried in ambient conditions. A pre-cleaned Si substrate was used as the reference. R6G is one of the oldest Raman probe materials used in the literature and thus its spectral properties are well known. However, the Raman community is currently discouraging the use of R6G in SERS measurements due to its enormous Raman cross-section, which can lead to inordinately high SERS signal especially in resonant Raman measurements. [223, 224] Nevertheless, this should not pose any problems, as the main contribution of Publication VI lies in identifying the effect of the sample geometry and Ag coating thickness in the SERS enhancement on BSi related materials.

First, the effect of the varying silver layer thickness to the SERS enhancement was studied. Different nominal silver thicknesses were deposited on the BSi01 sample set ranging from 35 nm to 150 nm. The Raman spectra for these samples are presented in Fig. 6.9b. The plain Si reference clearly demonstrates the Raman peak of crystalline silicon at 520 cm$^{-1}$, but no R6G Raman peaks are visible. On the other hand, the Raman spectra collected from the Ag coated BSi01 samples, distinctively shows all the prominent Raman peaks of R6G. [225] This evidently shows that the enhanced Raman signal is due to the Ag coated nanos-
structures on the BSi substrate. Figures 6.9c and 6.9d further illustrate how the varying Ag thickness affects the intensity of the Raman and the background signals. The Raman signal was acquired from 614 cm$^{-1}$ peak and the background intensity taken was at 591.8 cm$^{-1}$, respectively. A local maximum is achieved at a thickness of 80 nm and a similar trend is observed for the background intensity.

To link the observed SERS enhancement with the substrate topology, the samples were studied using SEM. Figure 6.10 presents a collection of SEM figures of BSi structures coated with different nominal silver thickness. As expected, a thinner silver coating enables the formation of nanoparticles, but a thicker film results in a continuous layer of silver. For a coating thicknesses of 35 nm and 55 nm, the appearance of the samples is quite similar explaining the comparable Raman enhancement. For 80 nm thick coating, the nanoparticles are still discernible, but the formation of a continuous film starts taking place. For thicker samples, the deposited silver forms mainly a continuous coating.

The electric field distribution near the structures was calculated using a commercial Optiwave finite-difference time-domain (FDTD) simulation software. Based on the SEM analysis, four different geometries were used. The FDTD simulations agree really well with the observed SERS measurements; i.e., the highest electric fields are produced for a structure consisting of a combination of thin silver film with nanoparticles. This would correspond to the nominal Ag thickness of 80 nm as indicated by...
Finally, to study how the BSi substrate type influences the SERS enhancement, the Raman performance of BSi01 and BSi02 substrates was compared. The Raman spectra for both substrates with nominal silver thickness of 80 nm is shown in Fig. 6.11a. Figures 6.11b and 6.11c also present the SEM images with the same magnification from BSi01 and BSi02 structures, respectively. Notably, the Raman signal from the Ag coated BSi01 is 8 times higher than obtained from the Ag coated BSi02 substrate. This results from the different substrate geometry that leads to the formation of different Ag nanostructures on the BSi. Also, the density of the spikes on the BSi01 substrate is higher, and moreover, the spikes are sharper. This leads to the abundance of “hot spots” on the BSi01 substrate, which enables higher SERS enhancements to be obtained.
The presented results can be divided into two parts. The first part focuses on the utilization of biological molecular components in photonic applications and the second part investigates the development of versatile nanofabrication methods for plasmon enhanced spectroscopy techniques.

First, biomimetic supramolecular zinc chlorin - P4VP assemblies were investigated, in which the P4VP host acts as a scaffold for the Zn chlorins in the visible wavelength range. The employed approach allows to bind the pigments in the polymer host preventing the aggregation of the dyes. Moreover, this approach also allows the pigments to be distributed in the thin film structure homogeneously in the macroscopic scale. The practical upper limit for the absorbing material was identified at 0.5. eq. (300 wt%). At lower doping levels, the pigments are quenched by the Förster mechanism, where the doping level for the emitting material lies at ca. 0.002 equiv. (ca. 1 wt%).

The incorporation of an acceptor molecule into the P4VP-Zn chlorin assembly was found to increase the Stokes shift and the spectral range of the absorption via the Förster mechanism. The optical analysis of these P4VP-Zn chlorin-P18 complexes suggested the existence of an efficient energy transfer within the assemblies. Both of these assemblies are analogous to chlorophyll-protein complexes.

Second, the strongly emerging ALD/MLD technique was used in growing novel organic-inorganic assemblies, in which a uracil (organic moiety) was linked by sodium (inorganic moiety) to form a crystalline 3D network. The assemblies were found to adopt a planar tetrametric configuration as indicated by DFT analysis and corroborated by the FTIR and $^1$H NMR measurements. The measurements also indicated that the adjacent uracil molecules are hydrogen-bonded.

The lifetime of uracil in the complex was found to be 3 orders of mag-
nitude higher, than the values reported for molecular uracil. Moreover, the assemblies exhibited intense blue fluorescence, which is not common as the quantum yields of NBs are commonly very low. The extraordinary behaviour originates from the hydrogen-bonded network and electrostatic interactions that effectively inhibit the internal conversion process and lead to the decrease of the non-radiative decay rate. Moreover, the assemblies were found to exhibit an excitation dependent fluorescence caused by REES. Qualitatively this is caused by the existence of energetically different species, which is evidenced by the inhomogeneous broadening of the absorption spectra.

Third, large-scale and economic plasmonic substrates were fabricated for MEF and SERS applications. The MEF substrate was fabricated using azopolymer lithography to create periodic and symmetrical 2D gratings from silver that had good long-range order. An enhancement factor of 14 was obtained for R6G with a grating periodicity of $\Lambda = 370$ nm. The intensity was roughly doubled for CB in $\Lambda = 1 \mu m$ grating. The actual interparticle distances are too large in both samples to support efficient coupling between the particles and thus the near-field enhancement is mostly responsible of the observed enhancement. In this respect, the 14-fold enhancement value for R6G on the $\Lambda = 370$ nm grating is comparative or even better when comparing with other published work using similar structures and dyes. On the other hand, the extremely low enhancement values for CB cannot be explained by this model. The lower enhancement values could be partly explained by the lower scattering efficiency of the multipolar plasmonic mode of the nanoparticles in the $\Lambda = 1 \mu m$ grating as suggested by earlier research. [226]

The SERS substrate fabrication employed a maskless etching technique, to form nanoscale structures on silicon substrate for subsequent silver evaporation. The nominal silver thickness of 80 nm was found to produce the highest electric field enhancements as evidenced by the FDTD calculations and by the local maximum in the R6G SERS intensity. Also, higher density of the spikes, as well as sharper cone geometry, was found to provide more “hot spots”, leading to higher SERS enhancement.

The results presented in this thesis can be utilized in a broader context. The proposed schemes utilizing chlorins and nucleobases could be utilized for organic solid state lasers, single photon emitters, optical switches or OLEDs. These results also highlight the advantages of supramolecular systems in developing novel and functional assemblies inspired by Na-
ture. The presented nanofabrication methods can be developed further to be used with a larger material selection and more sophisticated fabrication schemes.


References


References


