



Spectroscopic and microscopic characterization of porphyrin-carbon nanostructures interactions in the presence of metal nanoparticles

Leticia Alexandra Verbustel

Thesis to obtain the Master of Science Degree in

Chemical Engineering

Supervisors: Doctor Suzana Maria de Andrade Sousa Paiva

Examination Committee

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ACKNOWLEDGEMENTS

I would like to express my biggest gratitude towards my research supervisor and external promoter Dr. Suzana Maria de Andrade Sousa Paiva, for her time, patience, kindness and encouragement, for generously sharing her knowledge, for her help and advice, and for reading and correcting my thesis countless times.

I am thankful to the Grupo de Fotoquímica for access to their facilities and instrumentation.

I'm also grateful to Ms. Jolien D'aes, my internal promoter, who helped me with reading and correcting my dissertation and who helped me with the countless interschool issues I encountered during the course of this master thesis.

Further, I want to thank the wonderful people at IST, who took me under their wings and became my friends. I acknowledge Carlos, David, Emilio, Pedro and Vanda, for bringing some joy to my days at IST and making this the most pleasant working experience.

Finally, I would like to thank my mother, Linda, for making my stay in Lisbon possible. To Nick I wish to express my love and thank him for his endless patience and support.

My endless gratitude to all.

ABSTRACT

The focus of this dissertation is to achieve better sensing performances by using carbon based nanostructures (CBN). These structures are known for their excellent properties (e.g. thermal and electronic), combined with great mechanical strength and impressive surface area. These materials also have the ability to bind noncovalently to aromatic organic molecules through π - π interaction. One type of aromatic molecules is used here, i.e. porphyrins. They preform several vital functions in nature, e.g. in the light-harvesting complex of photosynthetic organisms and in oxygen transport and storage. The combination of both CBN and porphyrins is thus expected to lead to new devices with potential applications in sensing and photocatalysis.

This thesis gives insight into the interactions between four different porphyrins (TMpyP, ZnTMpyP, TSPP and TPP) and three carbon based nanostructures (functionalized single wall carbon nanotubes, multi wall carbon nanotubes and functionalized graphene sheets). The interactions between hybrid systems of the same four porphyrins and gold nanoparticles (AuNP) adsorbed to a chosen carbon based nanostructure, were also investigated. Gold nanoparticles help prevent aggregation of the carbon based structures and confer additional stability and functionality to the systems.

Distinct spectroscopies were employed to characterize porphyrins and carbon based nanostructures with and without AuNP in solution: UV/vis absorption and fluorescence (steady-state and time-resolved). Additionally, Fluorescence Lifetime Imaging Microscopy (FLIM), was used to characterize the films of such systems based on differences of fluorescence lifetimes. Last but not least, Transmission Electron Microscopy (TEM) was employed to check whether adsorption between the different components took place and to gain additional information about the size and morphology of some of the samples.

Keywords:

Porphyrins, Carbon based nanostructures, Gold nanoparticles, Fluorescence, Sensors

RESUMO

O *focus* desta dissertação é o de conseguir obter sistemas com capacidade melhorada como sensores usando nanoestruturas de carbono (CBN). Estas estruturas são conhecidas pelas suas excelentes propriedades (por exemplo: térmicas e electrónicas) combinadas com uma elevada força mecânica e uma area superficial extensa. Estes materiais têm ainda a capacidade de se associarem de modo não covalente com moléculas orgânicas aromáticas através de interacções do tipo π - π . Como exemplo dessas moléculas temos as porfirinas. Estas desempenham diversas funções vitais na Natureza, como por exemplo, nos sistemas colectores de luz dos organismos fotosinteticos ou no transporte e armazenamento de oxigénio. Espera-se assim que a combinação das CBN com porfirinas resulte em novos dispositivos com potenciais aplicações em sensores e em fotocatálise.

O trabalho apresentado nesta Tese engloba o estudo das interacções estabelecidas entre quatro porfirinas distintas (carregadas de base livre ou metaladas; neutra de base livre) e três nanoestruturas de carbono (nanotubos de carbono de parede única e folhas de grafeno ambos funcionalizados com grupos carboxílicos; e nanotubos de carbono de parede múltipla). As interacções entre os sistemas híbridos contendo as mesmas porfirinas e nanopartículas de ouro (AuNP) adsorvidas a uma dada nanoestrutura de carbono foram igualmente estudadas. As nanopartículas de ouro ajudam a prevenir a agregação das estruturas de carbono e conferem aos sistemas estabilidade adicional e funcionalidade.

A caracterização dos sistemas contendo porfirina, nanoestruturas de carbono com ou sem AuNP em solução foi feita recorrendo a distintas espectroscopias: absorção UV-Vis e fluorescência (em estado estacionário e transiente). Adicionalmente, a caracterização dos sistemas depositados foi realizada por microscopia de fluorescência confocal (FLIM) tendo por base diferenças entre os tempos de vidas de fluorescência. Por fim, recorreu-se à microscopia de transmissão electrónica (TEM) para avaliar a adsorção entre os diferentes componentes e para obter informação quanto ao tamanho e morfologia das nanopartículas nos sistemas em estudo.

Palavras-chave:

Porfirinas, Nanoestruturas de carbono, Nanopartículas de ouro, Espectroscopia de fluorescência, Sensores

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ABSTRACT (NL)

Deze masterthesis focust op het verkrijgen van betere detectie prestaties door middel van uit koolstof bestaande nanostructuren. Deze structuren staan bekend om hun uitstekende eigenschappen (zoals thermische en elektronische), gecombineerd met imposante mechanische sterkte en oppervlakte. Deze materialen hebben eveneens de eigenschap om niet-covalent te kunnen binden aan organische aromatische moleculen door middel van π - π -interacties. Eén soort aromatische moleculen werd in dit onderzoek gebruikt, namelijk porfyrines. Deze moleculen hebben enkele belangrijke functies in de natuur, zoals bij het oogsten van licht in fotosynthetische organismen en bij het transport en de opslag van zuurstof. Het combineren van uit koolstof bestaande nanostructuren en porfyrines zou dus kunnen leiden tot nieuwe apparaten met mogelijke toepassing in gevoeligheid en fotokatalyse.

Dit rapport geeft een inzicht in de interacties tussen vier verschillende porfyrines (TMpyP, ZnTMpyP, TSPP en TPP) en drie uit koolstof bestaande nanostructuren (gefunctionaliseerde enkelwandige nanobuizen, meerwandige nanobuizen en gefunctionaliseerde grafeen lagen). Er werd ook onderzoek verricht naar interacties tussen hybride systemen bestaande uit dezelfde vier porfyrines en structuren met gouden nanopartikels die geadsorbeerd zijn aan een gekozen nanostructuur. De gouden nanopartikels worden toegevoegd om aggregatie van de uit koolstof bestaande nanostructuren te helpen vermijden en leveren bijkomend stabiliteit en functionaliteit aan de systemen.

Enkele specifieke types van spectroscopie werden gebruikt om de oplossingen van porfyrines en uit koolstof bestaande nanostructuren, met en zonder gouden nanopartikels, te karakteriseren, namelijk UV/vis absorptie en fluorescentie (statische en tijdsgeresolveerde), Bijkomend werd Fluorescence Lifetime Imaging Microscopy (FLIM) gebruikt om de filmen van deze systemen te karakteriseren op basis van verschillen in verblijftijd. Als laatste werd Transmission Electron Microscopy (TEM) ingezet om te controleren of adsorptie tussen de verschillende componenten heeft stand gehouden en om extra informatie te vergaren betreffende de grootte en de morfologie van enkele stalen.

Sleutelwoorden:

porfyrines, uit koolstof bestaande nanostructuren, gouden nanopartikels, fluorescentie, sensoren

CONTENTS

ACKNC	DWLEDGEMENTS	I
ABSTR	ACT	II
RESUN	ИО	
ABSTR	ACT (NL)	IV
LIST O	F COMMON ABBREVIATIONS AND SYMBOLS	VII
LIST O	F FIGURES	VIII
LIST O	F TABLES	XII
CHAPT	ER 1 INTRODUCTION	1
1.1	Porphyrins	2
1.2	Carbon based nanostructures (CBN)	4
1.2.1	Carbon nanotubes (CNT)	4
1.2.2	Graphene sheets	5
1.3	Metal nanoparticles: Gold nanoparticles (AuNP)	6
СНАРТ	ER 2 TECHNIQUES AND PRINCIPLES	8
2.1	UV/vis absorption spectroscopy	8
2.1.1	Lambert-Beer's law	9
2.1.2	Effects on absorption spectra	10
2.1.3	Components of a spectrum	11
2.1.4	Surface plasmon resonance in metal nanoparticles	12
2.2	Steady-state fluorescence spectroscopy	13
2.2.1	Effects on fluorescence spectrum	14
2.2.2	Quenching of fluorescence	15
2.3	Time-resolved fluorescence: Time-correlated single photon counting (TCS	PC)17
2.4	Fluorescence Lifetime Imaging Microscopy (FLIM)	18
2.5	Transmission Electron Microscopy (TEM)	19
CHAPT	ER 3 EXPERIMENTAL PART	20
3.1	Materials	20
3.2	Sample preparation and synthesis	20
3.2.1	Preparation of porphyrin stock solutions and dilutions	20
3.2.2	Preparation of solid stock solutions	20
3.2.3	Synthesis of gold nanoparticles and its adsorption on a solid	21
3.3	Instrumentation	24

3.3.1	Spectrophotometer	24
3.3.2	Spectrofluorometer (Fluorolog®)	24
3.3.3	MicroTime 200	25
3.3.4	Electron microscope	26
3.4	Experimental methods	27
3.4.1	Spectroscopy	27
3.4.2	Spectroscopy and fluorescence emission: Interaction between porphyrin and solid	27
3.4.3	Lifetimes measurements using Fluorolog®	29
3.4.4	FLIM measurements	31
3.4.5	TEM images	32
CHAPT	ER 4 RESULTS AND DISCUSSION	33
4.1	Porphyrin interaction with carbon based nanostructures	34
4.1.1	Porphyrin - fCNT interaction in aqueous solution, in DMF and in buffer	34
4.1.2	Porphyrin - fGRAPH interaction in water/DMF mixtures	47
4.1.3	Porphyrin - MWCNT/PEG interaction in water/DMF mixtures	53
4.2	Porphyrin interaction with CNT decorated with gold nanoparticles	57
4.2.1	AuNP and AuNP/PEG with fCNT in water	57
4.2.2	AuNP/PEG with MWCNT in water/DMF mixtures	58
4.3	FLIM-images	66
4.3.1	ТМруР	66
4.3.2	ZnTMpyP	67
CHAPT	ER 5 CONCLUSIONS AND PERSPECTIVES	69
5.1	Conclusions	69
5.1.1	Porphyrin interaction with carbon based nanostructures	69
5.1.2	Porphyrin interaction with CNT decorated with gold nanoparticles	70
5.1.3	General conclusion with and without decoration of AuNP	71
5.2	Perspectives	71
REFER	ENCES	73
APPEN	DIX A CONCENTRATIONS OF STOCK SOLUTIONS	78

LIST OF COMMON ABBREVIATIONS AND SYMBOLS

a.u.	Arbitrary units
AuNP	Gold nanoparticles
CBN	Carbon based nanostructures
CNT	Carbon nanotubes
DMF	N,N-dimethylformamide
fCNT	Functionalized carbon nanotubes
fGRAPH	Functionalized graphene (sheets)
FLIM	Fluorescence Lifetime Imaging Microscopy
In situ	Simultaneously preparation of a mixture
MWCNT	Multi wall carbon nanotubes
PEG	Poly(ethylene glycol)
Preformed	Separate preparation of several components and mixed later
SWCNT	Single wall carbon nanotubes
TEM	Transmission Electron Microscopy
TCSPC	Time-correlated Single Photon Counting
ТМруР	Meso-tetra(4-N-methyl-pyridyl)porphine tetratosylate salt
TPP	Tetraphenylporphine
TSPP	Meso-tetrakis(p-sulfonatophenyl)porphyrin
UV/vis	Ultra Violet/visible region
ZnTMpyP	Zinc tetramethylpyridylporphyrin
λ_{emi}	Emission wavelength
λ _{exc}	Excitation wavelength
λ _{max}	Maximum wavelength
τ	Fluorescence lifetime
Ka	Association or binding constant
Ksv	Stern-Volmer quenching constant
k _a	Bimolecular quenching rate constant
S ₀	Ground state
S ₁ . S ₂	First and second excited state, respectively
V	Static quenching constant

LIST OF FIGURES

Figure 1.1 Structure of the simplest porphyrin, porphin [9]	2
Figure 1.2 Structure of TMpyP [11]	3
Figure 1.3 Acid-base equilibrium of TSPP in aqueous solution [10]	3
Figure 1.4 Structure of ZnTMpyP [12]	3
Figure 1.5 Structure of TPP [11]	4
Figure 1.6 Structures of fCNT (left) and MWCNT (right) [11, 18]	5
Figure 1.7 Structure of fGRAPH	6
Figure 1.8 Different shapes of metal nanoparticles	6
Figure 2.1 Diagram showing different energy states of a molecule. Between each electronic state s	S
(with S_0 the ground state and S_1 or higher the excited states), there lie vibrational levels (V), which are	е
sub-divided into several rotational levels (R) [26]	8
Figure 2.2 Schematic representation of Lambert-Beer's law [28]	9
Figure 2.3 Representation of four types of transition. Contains: σ bonding molecular orbital (MO), τ	Π
bonding MO, non-bonding n MO, σ^* anti-bonding MO and π^* anti-bonding MO [26]	0
Figure 2.4 Typical UV/vis absorption spectrum of a porphyrin [31]1	1
Figure 2.5 Example of the resonance band for gold nanospheres with a diameter of 20 nm [33] 12	2
Figure 2.6 Perrin-Jablonski diagram that gives a general overview of possible molecular processe	s
[38]1	3
Figure 2.7 Representation of Rayleigh and Raman scattering [26]	5
Figure 2.8 Example of a TCSPC histogram during an experiment	8
Figure 2.9 Example of lifetime and intensity images acquired during FLIM [9]	9
Figure 3.1 Set up with N ₂ -gas	2
Figure 3.2 Fluorolog® tau-3 spectrofluorometer2	4
Figure 3.3 Intermediate display when measuring fluorescence emission spectra	5
Figure 3.4 MicroTIme 200 from PicoQuant [56]2	6
Figure 3.5 Electron microscope used for obtaining TEM images2	7
Figure 3.6 General example of a corrected absorption spectrum with (1) the uncorrected spectrum, (2	2)
the empirical scattering function and (3) the corrected spectrum [54]	8
Figure 3.7 (A) average lifetime scale, (B) pattern for FLIM	2
Figure 4.1 UV/vis absorption spectrum (A) and normalized spectrum (B) of TMpyP/water (2 μ M) upon	n
addition of fCNT/water (0 - 0,1 mg/ml), represented by the arrow	4
Figure 4.2 UV/vis absorption spectrum (A) and normalized spectrum (B) of TMpyP/DMF (2 μ M) upon	n
addition of fCNT/DMF (0 - 0,05 mg/mL), represented by the arrow	5
Figure 4.3 Dependence of TMpyP in water at absorbance at 465 nm (left) and in DMF at absorbance	е
at 458 nm (right) on [fCNT]. The lines represents the fits of Eq. 4.1 (see text for details)	6
Figure 4.4 Fluorescence emission spectra of (A) TMpyP/water (2 μ M) with increasing concentration c	of
fCNT/water (in mg/ml) and (B) TMpyP/DMF (2 μ M) with increasing concentration of fCNT/DMF (0)-
0,075 mg/ml), represented by the arrow; λ_{exc} = 435 nm and 415 nm, respectively	6

Figure 4.5 Fitting data of fluorescence quenching for TMpyP in water (left) and in DMF (right) with
fCNT in the corresponding solvent using Eq. 2.6 (see text for details)
Figure 4.6 Fluorescence decays of TMpyP (2 μ M) without and with fCNT (0.006 mg/mL) in A) water
and B) DMF; $\lambda_{exc} = 445 \text{ nm}, \lambda_{emi} = 710 \text{ nm}.$ 39
Figure 4.7 UV/vis absorption spectrum (A) and normalized spectrum (B) of ZnTMpyP/water (2 µM)
upon addition of fCNT/water (0 - 0,1 mg/ml), represented by the arrow
Figure 4.8 Dependence of ZnTMpyP absorbance at 481 nm on [fCNT]. The line represents the fit of
Eq. 4.1 (see text for details)
Figure 4.9 Fluorescence emission spectrum of ZnTMpyP/water (2 $\mu\text{M})$ with increasing concentration of
fCNT/water (0-0,1 mg/ml) represented by the arrow; λ_{exc} = 445 nm
Figure 4.10 Fluorescence decays of ZnTMpyP (2 $\mu\text{M})$ without and with fCNT (0.05 mg/mL) in water;
$\lambda_{\text{exc}} = 445 \text{ nm}, \lambda_{\text{emi}} = 630 \text{ nm}42$
Figure 4.11 UV/vis absorption spectrum of TSPP/water (2 μM) upon addition of fCNT/buffer (0-0,07
mg/ml), represented by the arrow. Inset: dependence of TSPP absorbance at 440 nm on [fCNT]. The
line represents the fit of Eq. 4.1 (see text for details), pH=7.4
Figure 4.12 Fluorescence emission spectrum of TSPP/water (2 $\mu\text{M})$ with increasing concentration of
fCNT/buffer (0-0,1 mg/ml) represented by the arrow; λ_{exc} = 415 nm. Inset: Fitting data of fluorescence
quenching for TSPP with fCNT using Eq. 2.6 (see text for details)
Figure 4.13 Fluorescence decays of TSPP (2 $\mu\text{M})$ without and with fCNT (0.025 mg/mL) in buffered
solution (pH=7.4); λ_{exc} = 445 nm, λ_{emi} = 650 nm
Figure 4.14 The plot of intensity quenching upon the quencher concentration C for fCNT
Figure 4.15 UV/vis absorption spectra of (A) TMpyP/water (2 μ M), (B) ZnTMpyP/water (2 μ M), (C)
TSPP/water (2 $\mu M)$ and (D) TPP/DMF (2 $\mu M)$ upon addition of fGRAPH/water-DMF (in mg/ml), the
legend is applicable for all four spectra
Figure 4.16 Normalized fluorescence emission spectrum of TMpyP/water (2 $\mu\text{M})$ with increasing
concentration of fGRAPH/water-DMF (0-0,025 mg/ml) represented by the arrow; λ_{exc} = 438 nm 48
Figure 4.17 Normalized fluorescence emission spectrum of ZnTMpyP/water (2 $\mu\text{M})$ with increasing
concentration of fGRAPH/water-DMF (0-0,02 mg/ml) represented by the arrow; λ_{exc} = 454 nm
Figure 4.18 Fluorescence emission spectra of (A) TSPP/water (2 $\mu\text{M})$ and (B) TPP/DMF (2 $\mu\text{M})$ with
increasing concentration of fGRAPH/water-DMF, the legend is applicable for both A and B; λ_{exc} = 425
nm and 427 nm, respectively
Figure 4.19 Fluorescence decays of (A) TMpyP, (B) ZnTMpyP, (C) TSPP and (D) TPP without and
with fGRAPH/water-DMF (0,02 mg/mL), [Porphyrin]= 2 μ M; λ_{exc} =445 nm, λ_{emi} = 710, 630, 650 and 650
nm, respectively
Figure 4.20 The plot of intensity quenching upon the quencher concentration C for fGRAPH52
Figure 4.21 TEM images of MWCNT/PEG containing long thin stretched darker grey strings which are
the nanotubes (1) and PEG surrounding the nanotubes, which are lighter grey areas (2), A) is the
general image with a scale of 100 nm and B) is a close up with a scale of 50 nm
Figure 4.22 UV/vis absorption spectra of (A) TMpyP/water (2 $\mu M)$ and (B) ZnTMpyP/water upon
addition of MWCNT/water-DMF (in mg/ml), the legend is applicable for both spectra

Figure 4.23 Fluorescence emission spectra of (A) TMpyP/water (2 µM) and (B) ZnTMpyP/water (2 µM) with increasing concentration of MWCNT/water-DMF (0-0,025 mg/ml) represented by the arrow; λ_{exc} = 441 nm and 456 nm, respectively54 Figure 4.24 Fitting data of fluorescence quenching for TMpyP (left) and ZnTMpyP (right) with MWCNT Figure 4.25 Fluorescence decays of (A) TMpyP and (B) ZnTMpyP, without and with MWCNT (0.025 mg/mL) in water-DMF mixtures. [Porphyrin]= 2 μ M; λ_{exc} = 445 nm, λ_{emi} = 710 and 630 nm, respectively Figure 4.27 TEM images of AuNP with fCNT (preformed) with A) a general image with a scale of 500 nm containing a dark background which is the grid (1a), an almost white background which are the holes in the grid (1b) and dark spheres which are the nanoparticles (2); and B) a close up with a scale Figure 4.28 TEM image of AuNP/PEG with fCNT (preformed) with a scale of 200 nm containing the Figure 4.29 TEM images of AuNP/PEG with MWCNT (preformed) with A) a general image with a scale of 500 nm containing the grid (1a), the holes in the grid (1b) and an example of aggregation (4); and Figure 4.30 TEM images of AuNP/PEG with MWCNT (in situ) with A) a general image with a scale of 500 nm containing the grid (1a), the holes in the grid (1b); and B) a close up with a scale of 100 nm Figure 4.31 UV/vis absorption spectra of TMpyP/water (2 µM) upon addition of AuNP/PEG/MWCNT in water-DMF (in mg/ml) using the preformed method (A) and the in situ method (B), the legend of A is Figure 4.32 Fluorescence emission spectra of TMpyP/water (2 µM) with increasing concentration of AuNP/PEG/MWCNT in water-DMF (0-0,025 mg/ml) represented by the arrow using the preformed Figure 4.33 Fitting data of fluorescence quenching for TMpyP and AUNP/PEG/MWCNT using the Figure 4.34 Fluorescence decays of TMpyP (2 μ M) without and with AuNP/PEG/MWCNT (0.025 mg/mL) in water/DMF mixtures prepared using preformed and *in situ* methods; $\lambda_{exc} = 445$ nm, $\lambda_{emi} =$ Figure 4.35 UV/vis absorption spectra of ZnTMpyP/water (2 µM) upon addition of AuNP/PEG/MWCNT in water-DMF (in mg/ml) using the preformed method (A) and the in situ method (B), the legend of A is Figure 4.36 Fluorescence emission spectra of ZnTMpyP/water (2 µM) with increasing concentration of AuNP/PEG/MWCNT in water-DMF (0-0,025 mg/ml) represented by the arrow using the preformed Figure 4.37 Fitting data of fluorescence quenching for ZnTMpyP and AUNP/PEG/MWCNT using the

Figure 4.38 The plot of intensity quenching upon the quencher concentration C for MWCNT decorated
with AuNP65
Figure 4.39 FLIM images and respective lifetime histograms of TMpyP deposited from (A) an aqueous
solution, (B) MWCNT (0.02 mg/mL), and (C) AuNP/PEG/MWCNT (0.025 mg/mL) prepared in situ. The
lifetime scale is shown on the left. Image bar scale 10 μm (2.5 μm for image B)
Figure 4.40 FLIM images and respective lifetime histograms of ZnTMpyP deposited from (A) an
aqueous solution, (B) fCNT (0.025 mg/mL), and (C) AuNP/PEG/MWCNT (0.025 mg/mL) prepared in
situ. The lifetime scale is shown on the left. Image bar scale 10 µm (0.75 µm for image B)

LIST OF TABLES

Table 3.1 Comparison of the experimental ϵ and the ϵ found in the literature [57, 58]
Table 4.1 Comparison of pH between pure solvent and solutions with fCNT
Table 4.2 Fluorescence lifetimes of TMpyP/water (2 μ M) with fCNT in water; λ_{exc} = 445 nm, λ_{emi} = 710
nm
Table 4.3 Fluorescence lifetimes of TMpyP/DMF (2 μ M) with fCNT in DMF; λ_{exc} = 445 nm, λ_{emi} = 710
nm
Table 4.4 Fluorescence lifetimes of ZnTMpyP/water (2 μ M) with fCNT in water; λ_{exc} = 445 nm, λ_{emi} =
630 nm
Table 4.5 Fluorescence lifetimes of TSPP/water (2 μ M) with fCNT in buffer; λ_{exc} = 445 nm, λ_{emi} = 650
nm
Table 4.6 Association constant K_a (obtained using absorption and emission data), Stern-Volmer
quenching constant K_{SV} , and bimolecular quenching constant k_q for different porphyrins with fCNT 46
Table 4.7 Fluorescence lifetimes of TMpyP/water (2 μ M) with fGRAPH in water/DMF mixtures; λ_{exc} =
445 nm, λ _{emi} = 710 nm
Table 4.8 Fluorescence lifetimes of ZnTMpyP/water (2 μ M) with fGRAPH in water/DMF mixtures; λ_{exc} =
445 nm, λ _{emi} = 630 nm
Table 4.9 Fluorescence lifetimes of TSPP (grey) and TPP (white) (both 2 μ M) with fGRAPH in
water/DMF mixtures; λ_{exc} = 445 nm, λ_{emi} = 650 nm (a scattering component was taken into
consideration)
Table 4.10 Fluorescence lifetimes of TMpyP (grey) and ZnTMpyP (white) (both 2 μ M) with fGRAPH in
water/DMF mixtures; λ_{exc} = 445 nm, λ_{emi} = 710 and 650 nm, respectively
Table 4.11 Stern-Volmer quenching constant K_{SV} , bimolecular quenching constant k_q , volume of
sphere V and association constant K_a for TMpyP and ZnTMpyP with MWCNT
Table 4.12 Fluorescence lifetimes of TMpyP/water (2µM) with AuNP/PEG/MWCNT in water/DMF
mixtures, prepared using the preformed method (grey) and the <i>in situ</i> method (white); λ_{exc} = 445 nm,
λ_{emi} = 710 nm (a scattering component was taken into account)
Table 4.13 Fluorescence lifetimes of ZnTMpyP/water (2µM) with AuNP/PEG/MWCNT in water/DMF
mixtures, prepared using the preformed method (grey) and the <i>in situ</i> method (white); $\lambda_{exc} = 445$ nm,
λ_{emi} = 630 nm (a scattering component was taken into account)
Table 4.14 Stern-Volmer quenching constant K_{SV} , bimolecular quenching constant k_q and volume of
sphere V for different porphyrins and different preparing methods of AuNP/PEG/MWCNT
Table XV Concentrations of the stock solutions of the porphyrins
Table XVI Concentrations of the stock solutions of the solids 78

CHAPTER 1 INTRODUCTION

This dissertation focuses to achieve better sensing performances by using carbon based nanostructures (CBN), more exactly carbon nanotubes (functionalized single wall carbon nanotubes and multi wall carbon nanotubes) and functionalized graphene sheets. Carbon based nanostructures have excellent optical, thermal and electronic properties, combined with terrific mechanical strength and a remarkable surface area.

An important aspect is the ability of these materials to noncovalent bind to aromatic organic molecules through π - π interaction. This is the reason why it is used in many applications, such as for tuning electronic and transport properties, for adsorbing and removing dye pollutants in the treatment of wastewater, etc. Phthalocyanine, fluorescein, pyrene and porphyrin are examples of aromatic molecules that are used in today's research. This dissertation focuses on one kind of aromatic molecules, porphyrins. These two-dimensional aromatic systems also possess very interesting optical, photophysical, photochemical, and electrochemical properties.

However, because of strong van der Waals forces, carbon based nanostructures can aggregate, which turns their processing and purification into a very difficult task. Aggregation can be prevented with the decoration of CBN with metal nanoparticles. In this thesis gold nanoparticles (AuNP) are used, and hybrid systems of CBN/AuNP with porphyrins are built. These hybrid systems with multifunctionality have potential applications as solar cells, (bio)sensors and optoelectronics.

The first step of this research was to solubilize the carbon based structures in the proper solution, to which then several porphyrins were added. Interactions were monitored using several spectroscopic and microscopic techniques such as UV/vis absorption, steady-state and time-resolved fluorescence and transmission electron microscopy (TEM). The next step was to synthesise CBN decorated with gold nanoparticles using two methods, preformed and *in situ*, to which also porphyrins were added. The same techniques as mentioned above are used to monitor interactions between the components. [1, 2]

1

1.1 Porphyrins

Porphyrins, metalloporphyrins and related compounds are omnipresent in nature. They are involved in many different biological activities in which photophysical and redox processes take place.

Porphyrins have very interesting properties, mainly optical, photophysical and -chemical, and electrochemical. Because of this, porphyrins have a great potential as photoactive substances in the development of new molecular materials. Porphyrins and metalloporphyrins have one major medical application as singlet oxygen photosensitizers in photodynamic therapy (PDT). [3-6] PDT is a promising therapeutic procedure for the treatment of solid tumours. The photosensitizing agent, the porphyrin, localizes the tumour and is activated by light of a specific wavelength. This results in a sequence of photochemical and biological processes that cause irreparable damage to the tumour tissues. [7, 8]

Porphyrins are also one of the most promising candidates to be used as fluorescent near infrared probes for non-invasive diagnosis. This could open the possibility to simultaneously detecting and treating of a tumour.

The porphin ring is a two-dimensional aromatic system. The structure of a porphyrin is quite simple: it consists of four pyrrole units connected to their α -carbon through methine bridges. The structure of porphin, the simplest porphyrin, is given in figure 1.1.

Because of the chemical structure of the circular macromolecule, porphyrins are very adaptable, they can be easily modified. Functionalization can take place at their meso- and β -positions (see fig. 1.1). The porphyrin can also be metallated, i.e. hold a metal atom in its center. More components and features can be introduced with the new obtained coordination chemistry properties.

In addition to their great synthetic flexibility, porphyrins also gained a high interest in supramolecular chemistry due to their suitability to integrate supra-organized structures. [3-6]



Figure 1.1 Structure of the simplest porphyrin, porphin [9]

In this dissertation, four different porphyrins were used, with the first being meso-tetra(4-N-methyl-pyridyl)porphine tetratosylate salt or TMpyP. It is a water-soluble, positively charged porphyrin. It is free-base, i.e. no metal is in the center. [10]



Figure 1.2 Structure of TMpyP [11]

The second porphyrin is meso-tetrakis(p-sulfonatophenyl)porphyrin or TSPP. Like TMpyP, TSPP is water-soluble and a free-base porphyrin, but TSPP is negatively charged. Figure 1.3 shows the acid-base equilibrium of TSPP. In acidic solutions TSPP has a zwitterionic nature, which has the tendency to aggregate more than TSPP under neutral pH conditions. As a consequence, a difference can be distinguished between Soret and Q-bands. [10]



Figure 1.3 Acid-base equilibrium of TSPP in aqueous solution [10]

The third porphyrin used is zinc tetramethylpyridylporphyrin, abbreviated by ZnTMpyP or ZnTMpyP⁴⁺, which is a metallated porphyrin or metalloporphyrin. [12]



Figure 1.4 Structure of ZnTMpyP [12]

The fourth and last porphyrin in this dissertation is tetraphenylporphine or TPP, which is a free-base porphyrin that has no charge and therefore is not water soluble.



Figure 1.5 Structure of TPP [11]

1.2 Carbon based nanostructures (CBN)

Carbon based nanostructures have very attracting electronic properties, outstanding mechanical strength and an impressive surface area. These characteristics make carbon based nanostructures interesting materials in the context of energy conversion, offering new opportunities for the design of composite materials with tailored properties. [13]

Two examples of carbon based nanostructures are carbon nanotubes (CNT) and graphene sheets, which will be discussed in the following sections.

1.2.1 Carbon nanotubes (CNT)

Carbon nanotubes were unintentionally discovered by lijima in 1991 while doing a study about the surface of graphite electrodes. Since their discovery, they have had a fundamental role in nanotechnology due to their great thermal, electrical, mechanical and electrocatalytic properties. These properties are associated with the one-dimensional nanometer-size scale that carbon nanotubes obtain. As a consequence, CNT have a lot of potential applications in science and technology. Some examples of already existing applications of CNT are micro-and nano-electronics, batteries with improved lifetimes, extra strong and conductive fibers, structural composite materials and targeting drug delivery. [14, 15]

Two kinds of carbon nanotubes were studied in this dissertation: single wall carbon nanotubes (SWCNT) and multi wall carbon nanotubes (MWCNT).

An important difference between SWCNT and MWCNT is the size: SWCNT typically have a diameter of about 1 nm, while the diameters of MWCNT are typically in the range of 5 to 50 nm. The tube lengths can be altered to be many thousands of times longer. While SWCNT are more flexible than MWCNT, they are harder to make, and therefore more expensive. However, the structure of MWCNT is more complex and has more variety, making it less well understood than SWCNT. SWCNT are considered to perform up to ten times better in

CHAPTER 1 Introduction

specific applications. MWCNT on the other hand are regarded as better electron donors, but SWCNT are more favourable and they have been studied more extensively already by spectroscopic techniques. SWCNT also have a specific imprint in absorption spectra which MWCNT do not have. [15, 16]

The single wall carbon nanotubes in this dissertation were functionalized with carboxylic acid groups (-COOH), making them fCNT, functionalized carbon nanotubes. This carboxylic functionalization is known to improve dispersion properties of the carbon nanotubes. Functionalization has the advantage of preventing carbon nanotubes from aggregating due to strong van der Waals interactions which would lower the active surface area significantly. The electronic structure of carbon nanotube, and therefore its transport and optical properties, is preserved when using non-covalent functionalization. [15, 17]



Figure 1.6 Structures of fCNT (left) and MWCNT (right) [11, 18]

1.2.2 Graphene sheets

Graphene sheets are single-layered and two-dimensional carbon based nanostructures. Graphene has been studied since its establishment in 1940, but single layers of graphene were only successfully identified in 2004 by Novoselov, Geim and co-workers at Manchester University.[19] This material possesses excellent electrical and thermal conductivity, mechanical flexibility and optical transparency. Many of its applications can be found in the field of nanoelectronics, fuel cells, biosensors, drug delivery and H₂ storage. [13, 20]

Graphene can also be functionalized, which prevents agglomeration and facilitates the formation of stable dispersions. Functionalization of graphene can be performed by using covalent and noncovalent modification of graphene oxide, followed by reduction. Despite the advantages of functionalization, it has been observed that the electrical conductivity and the surface area decrease significantly compared to pure graphene. In an attempt to prevent this, studies have been made to try to prepare graphene in a one-step process directly from graphite.[21]

Functionalized graphene can be applied as biosensors, supercapacitors, transistors, drug delivery and polymer nanocomposites. [22]

In this dissertation functionalized graphene sheets (fGRAPH) were indeed used. Its functional groups, which are carboxylic acid groups, are on the edge of the graphene rather than in the basal plane (see fig. 1.7). [13]



Figure 1.7 Structure of fGRAPH

1.3 Metal nanoparticles: Gold nanoparticles (AuNP)

Metallic nanoparticles are a focus of interest because of their potential in nanotechnology. They can be synthesized and modified with various chemical functional groups which allow them to be conjugated with a variety of things. Because of this, nanoparticles have a wide range of potential applications; e.g. in biotechnology, magnetic separation, targeted drug delivery and even diagnostic imaging.

Optical properties of metal nanoparticles are dependent on the metallic element, size and shape of the nanoparticle. Both alkali and noble metals are used, but noble metal nanoparticles are more easily to handle and thus are the most used. A variety of shapes have been produced for gold and silver nanoparticles, e.g. rods, triangles, cubes and stars.



Figure 1.8 Different shapes of metal nanoparticles

The focus in this dissertation is on spherical gold nanoparticles (AuNP). Gold nanoparticles are of special interest due to the following things:

- High biocompatibility
- High chemical stability
- Easy surface modification or conjugation to biomolecules [22-25]

CHAPTER 2 TECHNIQUES AND PRINCIPLES

2.1 UV/vis absorption spectroscopy [26-31]

Electronic UV/vis absorption is regularly used by scientists for several things, for example:

- Determination of the concentration of a substance when its molar extinction coefficient (ε) is known
- Examination of molecular aggregation
- Determination of association constants between different species

The UV/vis region of the spectrum can be divided into three sub-domains: near UV (185-400 nm), visible (400-700 nm) and near infrared (700-1100 nm).

The origin of absorption in this domain is the interaction from electromagnetic radiation with molecules or ions of the sample. When a molecule or ion absorbs a photon from the UV/vis region, the corresponding energy is captured by one or more of the molecules exterior electrons. This results in a modification of its electronic energy (E_{elec}). This modification will also change the energy or rotation (E_{rot}) and the energy of vibration (E_{vib}), all three are components which make up the total mechanical energy of the molecule (E_{tot}).

The alterations result in a large collection of possible transitions.



Figure 2.1 Diagram showing different energy states of a molecule. Between each electronic state S (with S_0 the ground state and S_1 or higher the excited states), there lie vibrational levels (V), which are sub-divided into several rotational levels (R) [26]

The captured energy during photon absorption can be transmitted again through different processes, together with emission of a photon. Transformations of the latter kind are fluorescence and phosphorescence, from which the first one will be exploited further in the dissertation.

2.1.1 Lambert-Beer's law

The operating principle of this technique is based on Lambert-Beer's law, which gives the relation between the absorption of light and the properties of the material through which the light is travelling. The well-known expressions are the following:

$$A(\lambda) = -\log T$$
 and $T = \left(\frac{I}{I_o}\right)$ (2.1)

Thus

$$A(\lambda) = \log\left(\frac{I_0}{I}\right) \tag{2.2}$$

And

$$A(\lambda) = \varepsilon_{\lambda}. l. C \tag{2.3}$$

With

- $A(\lambda)$ is the absorbance or optical density (O.D.), which has no units, and is wavelength dependent
- *I*₀ and *I* are the intensity of the incident light and that of the transmitted light, respectively, also without units
- *T* is called the transmittance, without units
- ε_λ is the molar absorptivity or extinction coefficient, given in M⁻¹.cm⁻¹, and is wavelength dependent
- / is optical path length through the sample, given in cm
- C is the molar concentration of the sample, given in mol/L



Figure 2.2 Schematic representation of Lambert-Beer's law [28]

Absorption spectra are plots of the intensity of light absorbed as a function of the incident wavelength. These spectra contain information about some fundamental electronic properties of the sample, for instance energy levels and probabilities for forbidden and allowed transitions. $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ are the most common transitions observed in the UV/vis range.



Figure 2.3 Representation of four types of transition. Contains: σ bonding molecular orbital (MO), π bonding MO, non-bonding n MO, σ^* anti-bonding MO and π^* anti-bonding MO [26]

2.1.2 Effects on absorption spectra

Chromophores are the functional groups of organic compounds which are responsible for absorption in UV/vis region.

Chromophores can be divided in two groups:

- Isolated chromophores: a molecule possesses one or more non-interacting chromophores. The absorption bands are constant or the overlapping between each chromophore is observed.
- Conjugated chromophore systems: there is interaction between chromophores. This
 can result in two displacements of the absorption spectrum: it is displaced towards
 longer wavelengths, this is called the bathochromic effect or the 'red' shift, and it has
 an increase in absorption intensity, which is called the hyperchromic effect.

The position and the intensity of absorption bands are dependent on the used solvent, because each solvent has its own characteristic polarity. This can also result in a bathochromic effect, for less polar compounds, or in a hypsochromic effect when the chromophore is more polar, this is when the absorption maxima is displaced to shorter wavelengths and is also called the 'blue' shift.

The pH of the used solvent can also have an important effect on the spectrum.

Three types of errors can be recognized due to the instrument.

- Background noise of the light source
- Background noise of the photodiode
- Stray light, which is light that reaches the detector but does not pass through the sample. It decreases the measured concentration of the sample.

2.1.3 Components of a spectrum

As mentioned above, absorption spectra are plots of the intensity of light absorbed as a function of the incident wavelength.

The absorption spectrum of a typical porphyrin (see fig. 2.4) consists of:

- a strong transition from the ground state to the second excited state (S₀ to S₂) at around 400 nm. This is called the Soret band or B band. It is an intense peak in the blue wavelength region and corresponds to the wavelength where maximum absorption occurs.
- a weak transition from the ground state to the first excited state (S₀ to S₁) at around a wavelength of 550 nm, these are called the Q bands and there are usually four of them.

The S and the Q bands both are the result of $\pi \rightarrow \pi^*$ transitions.



Figure 2.4 Typical UV/vis absorption spectrum of a porphyrin [31]

Upon metallation of a porphyrin, e.g. in the case of ZnTMpyP originating from TMpyP, several differences appear in the absorption spectrum: a shift to longer wavelengths of the maximum absorbance peak occurs and the number of Q-bands is reduced from four to two. This is due to the fact that ZnTMpyP holds more symmetry than TMpyP.

Also, pH conditions can influence the placement and the shape of the spectrum, this is especially the case with TSPP (see chapter one).

2.1.4 Surface plasmon resonance in metal nanoparticles

Gold nanoparticles, metal nanoparticles in general, have a typical surface plasmon resonance band in the visible region of the spectrum. This localized surface plasmon resonance (LSPR) is the collective coherent oscillation of the free electrons of metal nanoparticles against a restoring force, which resulted from Coulombic attraction between electrons and nuclei, upon excitation by an oscillating electromagnetic field of light. [32]

The intensity and wavelength of the resonance is very dependent on the size and shape of the nanoparticles, for example larger nanoparticles come with an increase in scattering efficiency and a red-shifted emission. When the nanoparticles have an anisotropic shape, the plasmon resonance extinction is stronger than for nanoparticles with an isotropic shape.

Because of this dependency of LSPR on the dimensions of the nanoparticles, UV/vis absorption spectroscopy gives a good idea about these parameters.



Figure 2.5 Example of the resonance band for gold nanospheres with a diameter of 20 nm [33]

2.2 Steady-state fluorescence spectroscopy [9, 26, 27, 38-44]

As said earlier, when a photon gets absorbed by an atom or molecule, an electron is promoted to a higher excited state. After absorption, the excess energy is re-emitted again, together with emission of a photon, nearly immediately in the form of radiation.

A Perrin-Jablonski diagram (fig. 2.6) gives a general overview of the possible molecular processes that can occur after light absorption.



Figure 2.6 Perrin-Jablonski diagram that gives a general overview of possible molecular processes [38]

Radiative transitions are represented as straight arrows, going either up or down, nonradiative transitions are represented by curly arrows. The energy levels are the following: S_0 (electronic ground-state), S_1 and S_2 (first and second singlet excited states, respectively), and T_1 and T_2 (first and second triplet states, respectively). Triplet states are more stable than the corresponding singlets. Triplet states have a multiplicity of three, while singlet states have a multiplicity of one. Two types of radiationless transitions can be distinguished: internal conversion (IC), which takes place between states of the same multiplicity, and intersystem crossing (ISC), which occurs between states of different multiplicity.

After excitation, the light intensity lowers extremely fast: emission of photons from S_1 to S_0 is very rapid, and it is classified as fluorescence; emission of photons from T_1 to T_0 can also occur with a much slower decay, and it is classified as phosphorescence. The state T_1 produces the delay of the electron returning to its fundamental state. The transition between S_1 and T_1 happens through intersystem crossing. Because transitions between states of different multiplicity are not allowed, the decays of phosphorescence are longer

CHAPTER 2 Techniques and principles

(microseconds to even seconds instead of picoseconds and nanoseconds as in the case of fluorescence).

An emission spectrum is a plot of the fluorescence intensity, which is proportional to the concentration of the analyte, as a function of wavelengths, for a fixed excitation wavelength. These spectra give information about how molecules relax from the higher excited state to the ground state. Using this technique, the excited state of a fluorophore can be used to investigate inter- or intramolecular processes and interactions with its surroundings. These interactions can lead to the increase or decrease (quenching) of emission intensity.

2.2.1 Effects on fluorescence spectrum

2.2.1.1 Inner filter effects

Inner filter effects occur with measurements of samples containing a fluorophore concentration that is too high, i.e. above the typical limit of 1-10 μ M. This can result in non-linear increases in the emission intensity.

Two categories of inner filter effects can be recognized:

- Primary inner filter effects: the incident light is absorbed before reaching the point in the sample at which fluorescence is observed, usually in the centre of the sample
- Secondary inner filter effects: when the photons emitted by one excited fluorophore are reabsorbed by other molecules of the fluorophore in the ground-state

To prevent inner filter effects, it is recommended to keep the absorbance at the excitation wavelength below 0,1 - 0,2 (see chapter 3).

2.2.1.2 Rayleigh scattering and Raman diffusion

When the wavelengths of emission and of excitation are not far apart, confusion can arise between the actual fluorescence and two interferences originating from the solvent in which the fluorophore is dissolved: Rayleigh scattering and Raman diffusion.



Figure 2.7 Representation of Rayleigh and Raman scattering [26]

Rayleigh scattering is the re-emission of a small fraction of the absorbed excitation light in all directions at the same wavelength by the solvent. Its intensity is dependent on the polarizability of the molecules of the solvent.

Raman scattering occurs when a part of the excitation radiation energy is transferred to the solvent molecules. These molecules re-emit photons with a lower energy than the ones used to excite them. The energy difference between the absorbed photons and the re-emitted photons is constant for each solvent. Also dependent on the solvent is the shift in energy between the excitation wavelength, to which Raman scattering is directly proportional, and the Raman peak. For example, the Raman peak of water is always located approximately 3400-3600 cm⁻¹ lower in energy than the excitation wavelength.

The Raman emission band is shifted towards longer wavelengths, while the Rayleigh scattering is placed towards shorter wavelengths. Raman scattering is much weaker than Rayleigh scattering, in the order of 10^2 to 10^3 times.

2.2.2 Quenching of fluorescence

Fluorescence quenching is any process that has the consequence of decreasing the fluorescence intensity of a sample or the fluorescence lifetime. Analysis of the phenomena of quenching can provide information on the surroundings of a fluorophore, both quantitatively and qualitatively.

Some examples of quenching processes:

- Ground state complex formation
- Electron, proton and energy transfer
- Excited state reactions

The focus here lies on quenching by intermolecular processes that are in competition with fluorescence, leading to its quenching.

Quenching can be divided into two subdivisions: static and dynamic quenching.

Dynamic quenching occurs when a fluorophore and the quencher come in contact during the lifetime of the transient excited state. Static quenching generally refers to a complex formation of the fluorophore and the quencher in the ground state. In the static quenching, the fluorescence lifetime remains constant with only a decrease in fluorescence intensity whereas in the case of dynamic quenching fluorescence lifetime also decreases.

One type of dynamic quenching, collisional quenching, can be fitted using the Stern-Volmer equation:

$$\frac{\tau_0}{\tau} = 1 + K_{SV}[Q] = 1 + k_q \tau_0[Q]$$
(2.4)

With

- K_{sv} is the Stern-Volmer quenching constant. It is an indicator of the sensitivity of the fluorophore to the quencher. For example, K_{sv} is larger for flurophores free in solution than for fluorophores buried in a macromolecule, where it is inaccessible to water-soluble quenchers.
- k_q is the bimolecular quenching rate constant
- τ_0 is the unquenched lifetime of the excited fluorophore, τ is the quenched lifetime
- [Q] is the concentration of the quencher

The equation for static quenching also obeys to the Stern-Volmer equation, but with K_S , the static quenching constant, replacing K_{SV} :

$$\frac{I_0}{I} = 1 + K_S[Q]$$
 (2.5)

• *I*₀ and *I* are the fluorescence intensities of the fluorophore in the absence and the presence of the quencher, respectively.

When both static and dynamic quenching co-exist, equations 2.4 and 2.5 can be brought together into one modified form of the Stern-Volmer equation:

$$\frac{I_0}{I} = (1 + K_S[Q])(1 + k_q \tau_0[Q])$$
(2.6)

in which the first term, $(1 + K_s[Q])$, represents the contribution from static quenching and the second term, $(1 + k_q \tau_0[Q])$, represents that of dynamic quenching.

2.3 Time-resolved fluorescence: Time-correlated single photon counting (TCSPC) [38, 45-47]

Time-correlated single photon counting (TCSPC) is a widely used method to measure emission decays in nano- and picosecond time ranges. TCSPC was regularly used in this dissertation for lifetime measurements in solution.

The sequence of a TCSPC experiment is as follows:

- First the sample is excited by an excitation source.
- The sample emits photons and these are collected one by one
- The time between the excitation and emission is measured

This is repeated over many excitation/emission cycles. The excitation light is generated in that kind of way so that less than one photon (in average) reaches the detector. This single photon probability condition is the basic principle of this technique. A very important feature of the TCSPC-technique is that the arrival time of a photon pulse can be determined with great precision since the probability of emission of more than one photon is very low.

After the collection of many photons over sufficient counts, a histogram of the arrival times of the photons is formed. This histogram corresponds to the waveform of the optical pulse response, i.e. the distribution of the probability for a photon emission, along with time. From this histogram one can retrieve the fluorescence decay parameters. It is important to have an instrumental response function to mathematically withdraw this response from the decay and so obtain the lifetimes using, for example, a sum of exponentials or another model.

Fluorescence lifetime can be defined as the inverse of the sum of the rate constant of a radiative process (k_R) and the overall rate constant for all non-radiative processes (k_{NR})

$$\tau = \frac{1}{k_R + k_{NR}} \tag{2.7}$$

 k_R can be replaced by k_F , the fluorescence rate constant, whereas k_{NR} is the sum of the rate constant for internal conversion (k_{IC}) and the rate constant for intersystem crossing (k_{ISC})

$$k_{NR} = k_{IC} + k_{ISC} \tag{2.8}$$

CHAPTER 2 Techniques and principles



Figure 2.8 Example of a TCSPC histogram during an experiment

2.4 Fluorescence Lifetime Imaging Microscopy (FLIM) [9]

Fluorescence Lifetime Imaging Microscopy (FLIM) is a technique that operates in the time domain, more exactly TCSPC (see 2.3), and it uses confocal detection.

Fluorescence lifetime contrast-based imaging has a wide range of applications because the lifetimes are highly dependent on the physical conditions in the fluorophore's local environment, these conditions are for instance:

- temperature
- oxygen levels
- pH
- polarity
- binding to macromolecules
- ion concentration

On the other hand, the lifetimes are in general not dependent on factors that affect steadystate measurements such as probe concentration, light scattering and the amount of excitation intensity. This makes the use of FLIM interesting for studying dynamic processes, for instance in living cells.



Figure 2.9 Example of lifetime and intensity images acquired during FLIM [9]

2.5 Transmission Electron Microscopy (TEM) [48, 49]

Transmission electron microscopy allows for a detailed investigation of the morphology of many things, such as minerals, metals, polymers, etc. A transmission electron microscope can image at a much higher resolution than a common light microscope, thanks to the small de Broglie wavelength of electrons.

In TEM a beam of electrons with high energies is transmitted through a sample. The electron beam is affected by the interactions inside the radiated sample. These interactions are detected and transformed into an image. This image is then enhanced and focused onto an imaging device. The whole process from source to screen is under vacuum.

The condenser lens system of the microscope controls the specimen illumination, going between two extremes:

- a uniform illumination of a large area at low magnification
- a strong focusing power for high magnification

CHAPTER 3 EXPERIMENTAL PART

3.1 Materials

All the glass work (beakers, vials, cuvettes, ...) was carefully washed before and after experiments with tap water and distilled water. Sometimes ethanol was used. The glass work was left to dry or dried with the aid of N_2 -gas.

The used solvents and chemicals were from spectrophotometric grade. The water used for making the stock solutions and the dilutions was distilled or ultrapure water. The synthesis of functionalized graphene was done elsewhere and was as follows:

- 1,25 gr of graphene is suspended in 125 ml of nitric acid
- The suspension is brought to 135-137 °C and was continually stirred for 4h
- It is cooled down to room temperature for 30-40 min and then filtered and washed with about 5 L of water
- Finally it is dried in an oven for at least 24h [50]

ZnTMpyP was also prepared elsewhere by refluxing TMpyP with zinc oxide (ZnO) in water. [51]

Waste, excess solvents or finished samples were disposed of in the correct manner.

3.2 Sample preparation and synthesis

3.2.1 Preparation of porphyrin stock solutions and dilutions

A stock solution of 5.10^{-4} M of the porphyrin was made (2-5 mL) by weighing the calculated amount and adding the suitable solvent. When it is not being used, it has to be kept in the dark so no interaction with light can occur.

From this stock solution, a range of 5 ml-dilutions were prepared with concentrations between 10^{-7} and 10^{-5} M.

3.2.2 Preparation of solid stock solutions

A stock solution of the solid was made in the right solvent (distilled water, DMF, buffer and/or a mixture could be used). This stock solution can be used directly or left aside for several days. Before using, a submerging of half to one hour in the ultrasonic bath is required.

3.2.3 Synthesis of gold nanoparticles and its adsorption on a solid

Experiments with gold nanoparticles (AuNP) are one of the main objectives in this dissertation. They can be synthesised in two different ways: preformed and *in situ*.

When using the preformed method, the gold nanoparticles are made separately and later added to the solid. When using the *in situ* method, gold nanoparticles are formed already with the solid present.

3.2.3.1 Preformed method

A. Synthesis of gold nanoparticles

Citrate-capped gold nanoparticles can be obtained by a chemical reduction of a metal precursor salt which is induced with sodium citrate. When an oxidation number of 0 is reached, the gold nanoparticles start to grow. This is noticeable by the colour change: the solution turns from yellowish to a whine red. The reduction determines the stability, the rate and the path of how the nanoparticles grow.

The steps are the following for a total volume of 30 ml (ultrapure or mili-q water is used here instead of distilled water):

- 20 ml of a 1 mM sodium tetrachloroaurate(III) solution is directly added in an volumetric flask with a round bottom
- 10 ml for a 1 mM sodium citrate solution is prepared in a separate volumetric flask and is heated a little. Sodium citrate is used because it is stable.
- The 2 solutions are mixed and the mixture is stirred and heated for at least half an hour at 100 °C, the reaction is spontaneous.
 - Only a temperature of about 55 °C could be reached. As a consequence, bigger particles could be produced. This has to be confirmed with absorption spectroscopy
 - The colour change from yellowish to a whine red is noticeable

Directly after this, an absorption spectra is taken (with the Lambda 35 spectrophotometer) and the solution is placed in the dark for at least one night (light can influence any still ongoing reaction). Before using, another absorption spectra is measured to determine if any changes occurred. Also, the location of the maximal absorbance gives an indication of the size of the gold nanoparticles.

This procedure is repeated for a second solution of gold nanoparticles, but with PEG, poly(ethylene glycol). PEG is added to ease solubility and dispersion in procedures that
follow. The preparation is the same except for one additional step of adding 1 g of PEG (MW~4600) in the volumetric flask right before adding the citrate.

B. Adsorption of gold nanoparticles on a solid

The next step is to try to let the gold nanoparticles adsorb with one of the solids. The procedure is the following for a volumetric flask of 50 mL:

- 1 g PEG is weighted and dissolved in a small amount of ultrapure water
- 10 mg of solid is weighted and ultrapure water is added
 - \circ The flask cannot be filled completely, space is needed for the next steps
- The solution is set in the ultrasonic bath for 20-30 minutes and N₂-gas is bubbled through to replace the air inside the flask
 - This has two purposes: N₂-gas is inert and will not influence the reaction while air might do that; it makes the solution more homogeneous
 - How: a septum is put over the opening of the flask, one needle is put in to let the air out, one needle is put in the middle to provide the flow of N_2 -gas
- 3 ml of the AuNP solution is added
- The solution is again set in the ultrasonic bath, this time for one and a half hours



Figure 3.1 Set up with N₂-gas

After this, a dispersion is obtained, containing the AuNP adsorbed to the used solid, free solid and solvent. The complexes of AuNP with solid need to be separated from the other two components. This was done by centrifugation or, if the first resulted in a substandard separation, by using a rotavapor.

Evaporation or centrifugation is done four times for at least half an hour for each run:

• Once for the original solvent (distilled water). The supernatant is drained with a Pasteur pipette and kept in case further analysis is necessary.

• Three times with ±20 ml washing solvents (one time with distilled water, two times with ethanol): the material is washed by adding the solvent so it re-dissolves, to help this matter it's submerged in the ultrasonic bath for 2 minutes or put in a vortex.

After the separation, the combined material of gold nanoparticles and solid is recovered. It is dried in an oven at 50 °C for at least one night. Afterwards, the material is scraped from the inside of the flask, crushed using a mortar and pestle to downsize the particles and used to make the stock solutions.

Extra, some of the material is used for obtaining TEM images (see further) to confirm if the gold nanoparticles adsorbed to the solid. This should be done before using it in other experiments.

3.2.3.2 In situ method

The *in situ* method is the following:

- 1 g PEG is weighted and dissolved in a small amount of ultrapure water in flask 1
- 10 mg of solid is weighted and ultrapure water is added, also in flask 1
- 3 mg sodium tetrachloroaurate(III) and 10 mg sodium citrate are weighted in another flask, flask 2, and ultrapure water is added
- Both flasks are submerged separately in the ultrasonic bath for 5 minutes
- The contents of the flasks are put together in one flask and put in the ultrasonic bath for four hours, together with N₂-gas bubbling through the solution.

The obtained dispersion contains the gold nanoparticles adsorbed to the used solid, free solid and solvent. The components are separated the same way as with the preformed method, either with centrifugation or by using a rotavapor. After separation, drying and crushing, the material is again used for making stock solutions and for obtaining TEM images.

3.3 Instrumentation

3.3.1 Spectrophotometer

The absorption spectra were carried out on a double-beam Perkin Elmer Lambda 35 UVvisible absorption spectrophotometer.

During the experiments, a wavelength range from 800 to 300 nm was applied and an optical path length of 1 cm was used. The bandwidth was set to 1 nm for every experiment. The used cuvettes were made of quartz and measurements were performed at room temperature. Both the UV lamp (pre-aligned deuterium) and the visible lamp (tungstenhalogen) were turned on and used. [52]

3.3.2 Spectrofluorometer (Fluorolog®)

3.3.2.1 Fluorescence emission data

Fluorescence emission spectra were obtained with a SPEX® Fluorolog® Tau-3 spectrofluorometer (HORIBA Jobin Yvon) in a FL3-11 configuration. It contains a 450 W Xenon lamp, which acts as a high intensity light source. This lamp has to be stabilized for at least 30 minutes before using it for any measurements. Quartz cuvettes of 1 cm were used.

Fluorescence emission spectra were measured between 580 and 850 nm. The wavelength of excitation, λ_{exc} , depends on the absorption spectra of the samples and is the wavelength where the absorbance of the Soret band isn't higher than 0,2 due to inner filter effects (see chapter 2).

The instrumental response for emission spectra was corrected automatically with the aid of a correction function, which can be found in the program (FluorEssence) and which was provided by the manufacturer.



Figure 3.2 Fluorolog® tau-3 spectrofluorometer

When the instruments runs, an intermediate display shows the progress. It's important to check this a few times in the beginning of the experiment so errors can be corrected as early as possible. It shows three signals (S1, R1, S1c/R1c) as a function of the wavelengths. The latter signal is the one needed for further calculations.

S1c/R1c is the ratio of the corrected signal of detector S1 (S for signal) to the corrected signal of detector R (R for reference), given in CPS/MicroAmps. This ratio is chosen rather than S1 on its own because it corrects the lamp profile or temporary fluctuations caused by the light source. [53]



Figure 3.3 Intermediate display when measuring fluorescence emission spectra

3.3.2.2 Lifetimes

Lifetime measurements were also obtained with the same Fluorolog® Tau-3 spectrofluorometer. Instead of the Xenon lamp, a NanoLED pulse diode is used as a light source and a photomultiplier tube is used for photon detection. The data rate was set proportional to the source repetition rate up to $\sim 2\%$ to avoid photon pileup artifacts. Typical measurements involved accumulation of a maximum count per channel of at least 10 000. The used program is called DataStation.

3.3.3 MicroTime 200

FLIM measurements were obtained with a confocal microscope from PicoQuant GmbH called MicroTime 200 (MT200). This instrument uses the time-correlated single-photon counting (TCSPC) technique.

The instrument contains an inverted microscope which provides image resolution up to 50 nm per pixel. The scanning of the sample can be performed either by sample scanning (moving the sample) or by objective scanning (moving the objective). In this specific case, the objective is fixed and the sample holder placed above the objective can be moved in the X-Y plane.

The excitation light of the measurements is 635 nm, provided by a pulsed picosecond laser diode. Detection is performed by single-photon avalanche diodes and the acquisition of the data is obtained with a computer equipped with a special TCSPC board. [54, 55]



Figure 3.4 MicroTlme 200 from PicoQuant [56]

3.3.4 Electron microscope

A Hitachi H-8100 electron microscope was used to obtain TEM images. The instrument contains a thermionic electron gun which emits electrons into the vacuum and accelerates them between the positive and negative electrode through a selected potential difference. An electromagnetic condenser-lens system controls magnification and electrons focusing. [55]



Figure 3.5 Electron microscope used for obtaining TEM images

3.4 Experimental methods

3.4.1 Spectroscopy

In the first experiments, absorption spectroscopy was used to determine the molar extinction coefficient, ε , of the used porphyrin, which was then compared with the ε found in the literature. Table 3.1 gives a comparison of the experimental ε and the ε found in the literature. The law of Lambert-Beer was used (see chapter 2).

Porphyrin	ε _{lit} (.10 ⁵ Μ ⁻¹ .cm ⁻¹)	ε _{exp} (.10 ⁵ M ⁻¹ .cm ⁻¹)	λ (nm)
ТМруР	2,26	1,97 for in water	422
		2,16 for in DMF	
ZnTMpyP	2,21	1,88	437
TSPP	5,1	1	413
TPP	4,2	1	418

Table 3.1 Comparison of the experimental ε and the ε found in the literature [57, 58]

3.4.2 Spectroscopy and fluorescence emission: Interaction between porphyrin and solid

A number of samples are made with concentrations ranging from 0 to 0,5 mg/ml of solid, but with the same concentration of porphyrin, 2μ M, for each sample. Also, a blank of the sample with the highest concentration is made, this is the sample but without any porphyrin. The

blank is used to check what of the spectra is specific to the porphyrin and what to the solid.

After the samples have settled for half an hour, the absorption spectra are measured and right after the emission data is acquired. This is done consecutive rather than in two separate times so the circumstances are the same.

3.4.2.1 Data correction of absorption spectra

More than once the spectra presented background turbidity or scattering. This background light scattering was corrected by subtracting the following empirical scattering function from each spectrum:

$$corr(\lambda) = \frac{a}{\lambda^b} + c$$
 (3.1)

The correction is a function of the wavelength λ of the incident radiation, whereas *a*, *b*, and *c* are empirical parameters. Figure 3.4 shows a general example of an absorption spectrum with background scattering before and after correction.



Figure 3.6 General example of a corrected absorption spectrum with (1) the uncorrected spectrum, (2) the empirical scattering function and (3) the corrected spectrum [54]

The methodology consists in fitting an exponential to the free baseline of the spectra. The empirical parameters *a*, *b*, and *c* stand for the following:

- *a* is a proportionality factor
- *b* depends on the size of the scattering particles. The value of *b* should vary between
 1 and 2 for very large particles and 4 for small particles. During this research *b* always had a value of 2
- *c* is the offset which simply corrects for the background, for slight differences in the cell position in the spectrophotometer for example. This parameter should be very close to zero.

These parameters were adjusted by an iterative fitting procedure. [54, 59]

Another method was possible to correct the background scattering. Normally, it is necessary to do a blank, i.e. the sample but without porphyrin, for each sample, but a correlation was found between the blank and the concentration of solid. Because of this, the data of the one blank that was done of the sample with the highest concentration, could be transferred to all samples. The correction was made by subtracting the absorbances of the blank, which were multiplied with the following factor, taking into account the suitable concentrations:

 $\frac{C_{sample}}{C_{blank}}$ (3.2)

3.4.3 Lifetimes measurements using Fluorolog®

First a prompt is done at the wavelength of the NanoLED pulse diode (445 nm). This is the same for all the experiments. A prompt represents the profile of the light source and the material used to get this prompt is LUDOX®, which is a light-scattering standard and has a lifetime of 0 ns.[53]

The emission wavelength for the samples depends on the used porphyrin and was as follows for every experiment with that specific porphyrin:

- TMpyP: 710 nm
- ZnTMpyP: 630 nm
- TSPP: 650 nm
- TPP: 650 nm

The bandpass or slit width, is adjusted in function of α , which should be around 1 (0.88-1.32 usually) and represents the count rate. The bandpass can range from 0 to a maximum of 29,4 nm.

3.4.3.1 Data analysis

Decays are automatically fitted with reconvolution of a profile of the light source. This is the prompt and is done in the beginning of every measurement, as said before.

An extra fitting was done with the aid of the program DAS6. The chosen fit type is called '1-5 exponentials': this fitting adds more exponentials to the fitting, the maximum used in this dissertation is 3. The generic expression is the following, with the decay as a function of time:

$$F(t) = \sum_{i} B_{i} * exp({-t/T_{i}})$$
(3.3)

 B_i are the amplitudes, T_i is the lifetime value, index *i* stands for number of exponentials used. The program automatically recommends these parameters. [60]

Logically, the more exponentials that are added, the better the fitting, but it's important to see how much improvement each added exponential brings to the fitting. This evaluation is based on several parameters which are given by the software:

- 1. XSQ, chisq, chi-squared or χ^2 this has to be close to 1. This numerical value reflects the overall goodness of fit. [53]
 - a. If more exponentials don't improve XSQ by much, they are discarded
- 2. The relative amplitude of the lifetimes
 - a. By adding exponentials, more lifetimes are possible. These lifetimes have a certain percentage of probability, this is the relative amplitude.
 - A fair amount of relative amplitude is needed to accept the number of exponentials. For example in the case of 2 exponentials: 99 against 1 is discarded, but 80 against 20 can be possible.
 - c. A negative relative amplitude means a new species is forming in the excited state instead of decaying.
- 3. The Durbin-Watson parameter, this should be around 1,7.

Also an even distribution of the weighted residuals is favourable, which was monitored by visual inspection.

When decided how many exponentials are used for the fitting, information and parameters given by the software are used for calculating the average lifetime (τ_{av}) of each sample. The next formulas are used:

$$A_i = \frac{B_i}{\sum B_i} \tag{3.4}$$

$$F_i = \frac{A_i \tau_i}{\sum A_i \tau_i} \tag{3.5}$$

$$\tau_{av} = \Sigma F_i \tau_i \tag{3.6}$$

 B_i are the amplitudes given by the program, A_i are the normalized amplitudes, and τ_i are the lifetimes in ns, also given during the fitting.

Lifetimes depend a lot on the environment of the sample, such as the composition of the solvent, viscosity, pH, polarity, etc..

3.4.4 FLIM measurements

3.4.4.1 Slide preparation

A special preparation is required before FLIM measurements.

- First a 'Piranha'-solution is prepared, which consists out of 3 parts sulphuric acid p.a., and one part hydrogen peroxide 30 %. A total volume of 200 mL is prepared.
 - When mixing these two components, an exothermic reaction is induced and the temperature increases very fast. To prevent the glass beaker from shattering, it is recommended to add the hydrogen peroxide in small portions, while keeping it in a bath filled with cold water and stirring the beaker around so it can cool off.
- Special microscope cover glasses (slides) with a thickness of 0,13-0,16 mm that are needed for FLIM are put in a special holder one by one. Attention is paid to not to contaminate them.
- The holder with the slides is submerged in the 'Piranha'-solution for a minimum of five hours.
- After, the holder with slides is rinsed with distilled water and submerged in a beaker with also distilled water.
- One by one the slides are dried using N₂-gas.
- One drop or about twelve µL of the samples is placed on top of the slides
 - The sample should spread well and in all directions. This means the slide is very hydrophilic, what it needs to be, due to the hydroxylation of the surface.
- The slides with samples are placed in a petri-dish of which the bottom is covered with Parafilm. This is to prevent the slides from sticking.
- The petri-dish is covered with some punctured Parafilm and left to dry for at least one night.

After this the microscope cover glasses with the sample can be examined using FLIM.

3.4.4.2 Data analysis

With FLIM, images of the sample are projected on a screen. These images consist of areas with different colours, whereby each colour represents the average lifetime (see figure 3.7 A). The scale can be altered, but by default the colour blue always represents the lowest average lifetime and the colour red represents the highest one.

Using these colours, an analysis can be made. The goal is to find specific structures that represent the sample and by using the given software, calculating lifetimes more precisely.

To get a better picture of the sample as a whole, measurements are done on each sample on 9 different spots like the pattern shown in the next figure (figure 3.7 B).

The used software is called SymPhoTime, provided by the manufacturer.



Figure 3.7 (A) average lifetime scale, (B) pattern for FLIM

3.4.5 TEM images

3.4.5.1 Sample preparation

Preparation of the sample for TEM is quite simple: a little of the solid material (powder) is put in a vial and a quickly evaporating solvent is added, usually ethanol. This solution is submerged in the ultrasonic bath for 5 minutes.

Afterwards, a drop of this solution is deposited and air-dried on a carbon/Formvar-coated copper grid. The sample has to be very thin, otherwise the beam of electrons cannot penetrate it.

3.4.5.2 Data analysis

The equipment operates in a bright field imaging mode. In this mode more dense regions of the sample or regions with a higher atomic number produce a dark contrast, whereas the areas without any specimen are brighter. [55]

CHAPTER 4 RESULTS AND DISCUSSION

This chapter will handle the results and discussion of the experiments as described in chapter three. The concentrations of the stock solutions of the used porphyrins and solids are given in appendix A. The molar extinction coefficients can be found in chapter three (table 3.1 p. 27).

A pH measurement was performed for the stock solutions with fCNT. When comparing the pH of solely the solvent and the solvents plus fCNT (table 4.1), it came to light that fCNT makes the pH drop. This is an important fact to take into account.

Table 4.1 Comparison of pH between pure solvent and solutions with fCNT

Solvent	pH without fCNT	pH with fCNT
Water	6,03	5,5
Buffer	7,4	6,86

If the absorption spectrum of a system shows evidence of a complex, it is possible to calculate the binding or association constant K_a with the aid of the next equation, representing the fit [57]:

$$A_T(\lambda) = [Porf]_0 \frac{\varepsilon_p(\lambda) + \varepsilon_c(\lambda)K_a [CBN]^n}{1 + K_a [CBN]^n}$$
(4.1)

With

- $A_T(\lambda)$ = absorbance at measured wavelength λ
- $[Porf]_0$ = departure concentration of the porphyrin = 2 μ M
- $\varepsilon_{\rho}(\lambda)$, $\varepsilon_{c}(\lambda)$ = extinction coefficients of the porphyrin monomer and complex at measured wavelength λ , respectively
- [CBN] = concentration of CBN
- n = term that gives an idea of the process, whether there is cooperativity or not (n=1 no cooperativity, 1:1 complex)

By optimising the fit using MS Excel, K_a can be calculated.

Likewise, the Stern-Volmer quenching constant K_{SV} and the bimolecular quenching constant k_q can be calculated from fluorescence lifetime and fluorescence intensity measurements using the Stern-Volmer equation (eq. 2.6 p. 16). The latter equation is applicable when we have evidence of complex formation. For high concentrations of quenchers static quenching may occur even though no complex is formed. This is due to close distance between the

fluorophore and the quencher. According to this model the quencher remains nearby the fluorophore in a sphere at the moment of excitation instead of forming a complex. Based on Poisson distribution, for this model, a modified Stern-Volmer equation is used:

$$\frac{I_0}{I} = (1 + K_{SV}[Q]) \cdot e^{V[Q]}$$
(4.2)

With *V* being the static quenching constant, which represents the volume of the sphere. [61, 62, 63]

4.1 Porphyrin interaction with carbon based nanostructures

4.1.1 Porphyrin - fCNT interaction in aqueous solution, in DMF and in buffer

4.1.1.1 TMpyP



A. UV/vis absorption spectrum

Figure 4.1 UV/vis absorption spectrum (A) and normalized spectrum (B) of TMpyP/water (2 μ M) upon addition of fCNT/water (0 - 0,1 mg/ml), represented by the arrow

Fig. 4.1 B shows the normalized UV/vis absorption of TMpyP/water with increasing concentration of fCNT/water, taking into account correction for the scattering. There is a red shift of the Soret band with increasing amount of fCNT/water, from 422 nm to 444 nm, which is accompanied by a broadening of the band.

The red shift of the Soret band is usually connected with

- Protonation of the porphyrin pyrrole nitrogens.
- Formation of porphyrin J-aggregates
- Formation of porphyrin/SWNT self-assemblies.

Protonation of the porphyrin ring can be excluded due to the lack of a change in number of Q-bands, which would have been reduced from four to two. The band shows a widening instead of a narrowing, which one would expect in the case of formation of J-aggregates, and thus this option can also be excluded. Therefore, the changes in the spectrum can be assigned to the formation of porphyrin/SWCNT self-assemblies or complexes. [64] These complexes are noncovalently bonded through π - π interactions and/or by electrostatic interactions, which take place between monomeric TMpyP and fCNT.



Figure 4.2 UV/vis absorption spectrum (A) and normalized spectrum (B) of TMpyP/DMF (2 μ M) upon addition of fCNT/DMF (0 - 0,05 mg/mL), represented by the arrow

Fig. 4.2 shows the normalized UV/vis absorption of TMpyP/DMF with increasing concentration of fCNT/DMF, taking into account correction for the scattering. Also here a red shift of the Soret band is present with increasing amount of fCNT/DMF, going from 425 nm to 431 nm, accompanied by a widening of the band.

When compared to the system with water, it can be seen that the red shift is smaller (a difference of 6 nm instead of 22 nm), plus the broadening of the band is less pronounced in the case of DMF. The conclusion can be made that also here the change in spectrum can be assigned to self-assemblies between TMpyP and fCNT, but the responsible π - π interactions are not as strong as in water and electrostatic interactions are probably absent in the case of DMF.



Figure 4.3 Dependence of TMpyP in water at absorbance at 465 nm (left) and in DMF at absorbance at 458 nm (right) on [fCNT]. The lines represents the fits of Eq. 4.1 (see text for details)

The binding constant K_a has a value of 61,7 mL/mg and 93,1 mL/mg for TMpyP and fCNT in water and in DMF, respectively (fig. 4.3). Even though the interactions between porphyrin and CBN were less strong in the case of DMF according to the absorption spectra, K_a values show that the binding capability between TMpyP and fCNT is higher in DMF, yet they are in the same order of magnitude.



B. Fluorescence emission spectrum

Figure 4.4 Fluorescence emission spectra of (A) TMpyP/water (2 μ M) with increasing concentration of fCNT/water (in mg/ml) and (B) TMpyP/DMF (2 μ M) with increasing concentration of fCNT/DMF (0-0,075 mg/ml), represented by the arrow; λ_{exc} = 435 nm and 415 nm, respectively

The fluorescence spectrum TMpyP/water (fig. 4.4 A) shows one, almost structureless emission band ($\lambda_{max} \sim 713$ nm), while the spectrum of TMpyP/DMF (fig. 4.4 B) contains two vibrational resolved emission bands ($\lambda_{max} \sim 654$ nm and ~718 nm). According to Vergeldt F.J. *et al.* [65] this difference is due to an intramolecular mechanism involving mixture between the first excited state, S₁, and a nearby CT (charge-transfer) state, with the polarity of the

solvent greatly influencing the amount of mixing.

The fluorescence intensity of (A) first increases a little, where after it drops with the further increase of fCNT/water. The intensity of (B) decreases immediately with the increase of fCNT/DMF.

Neither of the spectra show any other changes, i.e. formation of a new emission band, although in both cases there was a red shift in the absorption spectra, suggesting the existence of a complex (see above). This result indicates that both the TMpyP/fCNT-complexes in water and in DMF do not emit and the changes in the emission spectra are due to the TMpyP monomer.



Figure 4.5 Fitting data of fluorescence quenching for TMpyP in water (left) and in DMF (right) with fCNT in the corresponding solvent using Eq. 2.6 (see text for details)

The Stern-Volmer quenching constant K_{SV} has a value of a 0 mL/mg for the quenching process in water and in DMF (fig. 4.5). Therefore it is plausible to exclude the dynamic contribution to the quenching process of TMpyP by fCNT in this solvent. The confirmation has to be made based on fluorescence lifetime measurements. The lifetimes are not influenced much (see below). Moreover, the K_a parameter is relatively higher (dotted black line in Fig. 4.5) than that obtained through absorption measurements (full black line in Fig. 4.5), especially in the case of TMpyP. Taking into consideration that in the case of fluorescence we can only use 4 quencher concentrations for the determination of the quenching parameters, the latter must have high errors associated. In the case of DMF there is a good correspondence between K_a withdrawn from both set of data, where a similar range of quencher concentrations was used.

C. Lifetime measurements

[fCNT/water] mg/mL	F ₁	τ ₁ (ns)	F ₂	τ ₂ (ns)	τ _{av} (ns)	XSQ
0	0.77	5.21	0.23	1.02	4.97	1,19
0.002	0.69	5.29	0.31	1.22	4.91	1,25
0.004	0.65	5.35	0.35	1.29	4.88	1,18
0.006	0.63	5.50	0.37	1.23	5.01	1,24

Table 4.2 Fluorescence lifetimes of TMpyP/water (2 μ M) with fCNT in water; λ_{exc} = 445 nm, λ_{emi} = 710 nm

Fluorescence decays are fitted using two exponentials in both the absence and the presence of fCNT, having a lifetime of ~4,96 ns. Since the lifetime values stay consistent, this confirms that the TMpyP-fCNT complex in water must be not fluorescent or has a fluorescence quantum yield much lower than that of the monomer and therefore it is not detectable for these low quencher concentrations. (Note that measurements with higher quencher concentrations were not possible to be made, due to the low intensity of such samples under the laser power excitation available).

Table 4.3 Fluorescence lifetimes of TMpyP/DMF (2 μ M) with fCNT in DMF; λ_{exc} = 445 nm, λ_{emi} = 710 nm

[fCNT/DMF] mg/mL	F ₁	τ ₁ (ns)	F ₂	τ ₂ (ns)	F_3	τ ₃ (ns)	τ _{av} (ns)	XSQ
0	0.19	10.4	0.23	3.53	0.57	0.55	7.63	1.18
0.002	0.43	11.5	0.21	0.36	0.36	0.78	9.98	1.08
0.004	0.33	12.0	0.18	0.49	0.49	0.72	9.97	1.07
0.006	0.44	11.3	0.17	0.40	0.40	0.53	9.99	1.07

In the absence of fCNT/DMF the lifetime has a value of 7,63 ns, fitted using three exponentials. Upon addition of fCNT/DMF, the lifetimes increases untill ~9,98 ns, also fitted with three exponentials. Since there is no dependence on the amount of fCNT added and the fluorescence spectrum showed no proof of a complex, the complex TMpyP-fCNT in DMF is not fluorescent, likewise to the complex in water.

Noticeable here is the dependence of lifetime measurements towards their environment: the lifetimes obtained in DMF are notably higher than the ones obtained in water.



Figure 4.6 Fluorescence decays of TMpyP (2 μ M) without and with fCNT (0.006 mg/mL) in A) water and B) DMF; $\lambda_{exc} = 445$ nm, $\lambda_{emi} = 710$ nm.

4.1.1.2 ZnTMpyP



A. UV/vis absorption spectrum

Figure 4.7 UV/vis absorption spectrum (A) and normalized spectrum (B) of ZnTMpyP/water (2 μ M) upon addition of fCNT/water (0 - 0,1 mg/ml), represented by the arrow

The normalized UV/vis absorption of ZnTMpyP/water with increasing concentration of fCNT/water is shown in the figure above (fig. 4.7). Also this spectra shows a red shift of the Soret band with increasing amount of fCNT/water, going from 436 nm to 459 nm. This shift is accompanied by a widening of the band.

The change in the spectrum can be assigned to self-assemblies between ZnTMpyP and fCNT, likewise to TMpyP and fCNT.



Figure 4.8 Dependence of ZnTMpyP absorbance at 481 nm on [fCNT]. The line represents the fit of Eq. 4.1 (see text for details)

The K_a value of ZnTMpyP ~870 mL/mg, is much higher than that obtained for the free base porphyrin under the same solvent conditions. This greater ability of fCNT to form assemblies with ZnTMpyP may come from specific interactions of the central metal ion of the porphyrin with the substituents carboxylic groups of fCNT.



B. Fluorescence emission spectrum

Figure 4.9 Fluorescence emission spectrum of ZnTMpyP/water (2 μ M) with increasing concentration of fCNT/water (0-0,1 mg/ml) represented by the arrow; λ_{exc} = 445 nm. Inset: Fitting data of fluorescence quenching for ZnTMpyP with fCNT using Eq. 2.6 (see text for details)

The fluorescence intensity of ZnTMpyP/water upon addition of fCNT/water shows an interesting change (fig. 4.9): the intensity lowers with increasing concentration of fCNT/water followed by changes in the relationship between the two vibronics. All together this points to the formation of an emissive ZnTMpyP/fCNT complex.

Confirmation can be obtained from fluorescence lifetimes, Table 4.4. Addition of fCNT up to 0.0075 mg/mL does not lead to significant changes in the average fluorescence lifetime of ZnTMpyP. However, above this concentration, there is a clear increase of this parameter at

the expenses of the increasing contribution of a longer lifetime component of around 5 ns, which we may tentatively assign to the emission of the complex, which was possible to detect at higher quencher concentrations where you have more complex formed, by contrast to the case of the free-base porphyrin, shown above.

Eq. 2.6 was used to obtain the rate constants for the quenching process. K_{SV} has the value of 0 mL/mg due to no decrease in the fluorescence lifetimes. As for the static quenching, K_a , using the value of K_a obtained from absorption data (K_a being ~870 mL/mg), the equation can describe the quenching effect only up to 0.01 mg/mL of fCNT (full black line, inset Fig. 4.9). Above this quencher concentration the values of Io/I obtained are much higher than those predict by eq. 2.6. At these quencher concentrations we are in the range where quencher and fluorophore have similar concentrations and therefore there is no longer a linear relationship.

C. Lifetime measurements

Table 4.4 Fluorescence lifetimes of ZnTMpyP/water (2 μ M) with fCNT in water; λ_{exc} = 445 nm, λ_{emi} = 630 nm

[fCNT/water] mg/mL	F ₁	τ ₁ (ns)	F ₂	τ ₂ (ns)	F ₃	τ ₃ (ns)	τ _{av} (ns)	XSQ
0	0.74	1.38	0.26	0.533	-	-	1.28	0.889
0.0025	0.67	1.43	0.33	0.858	-	-	1.30	0.958
0.005	0.45	1.51	0.55	0.953	-	-	1.27	0.963
0.0075	0.35	1.59	0.65	0.968	-	-	1.26	1.19
0.01	-	-	0.98	1.17	0.02	4.87	1.45	1.20
0.025	-	-	0.93	1.29	0.07	4.76	2.01	1.21
0.05	-	-	0.86	1.22	0.14	5.00	2.76	1.19



Figure 4.10 Fluorescence decays of ZnTMpyP (2 μ M) without and with fCNT (0.05 mg/mL) in water; λ_{exc} = 445 nm, λ_{emi} = 630 nm.

4.1.1.3 TSPP



A. UV/vis absorption spectrum

Figure 4.11 UV/vis absorption spectrum of TSPP/water (2 μ M) upon addition of fCNT/buffer (0-0,07 mg/ml), represented by the arrow. Inset: dependence of TSPP absorbance at 440 nm on [fCNT]. The line represents the fit of Eq. 4.1 (see text for details), pH=7.4

Fig. 4.11 shows the change in the UV/vis absorption of TSPP/water with fCNT/buffer. Upon addition of fCNT/buffer, a shoulder appears at around 435 nm. This is also the peak for the di-acid species of TSPP, which is formed upon acidification. The increase of this new band is accompanied by the decrease of the Soret band, located at 414 nm. At ca. 422 nm, an isosbestic point is observable.

If these changes were related with formation of the di-acid species, this would mean that due to symmetry changes, the absorption spectrum would change from 4 Q-bands to 2 Q-bands upon addition of fCNT. However, due to the increase of solution scattering in that region, together with the very low extinction coefficient of such bands, we cannot conclude anything. Assuming the possibility of a complex, we used Eq. 4.1 and obtained a value of $K_a \sim 37.9$ mL/mg. Therefore, only about 20 % transformation of 2 µM TSPP is caused by 0,07 mg/mL of fCNT/buffer.



B. Fluorescence emission spectrum

Figure 4.12 Fluorescence emission spectrum of TSPP/water (2 μ M) with increasing concentration of fCNT/buffer (0-0,1 mg/ml) represented by the arrow; λ_{exc} = 415 nm. Inset: Fitting data of fluorescence quenching for TSPP with fCNT using Eq. 2.6 (see text for details)

The higher the concentration of fCNT/buffer which is added to TSPP/buffer, the lower the intensity until the curves' maximum peaks flatten completely (fig. 4.12).

The absence of an additional peak at ~674 nm [66] indicates that the new absorption band at 435 nm (see above) is probably not due to the di-acid porphyrin but due to a complex which is non fluorescent and therefore, the emission obtained is due to TSPP monomer.

A value of 48.5 mL/mg could be obtained for K_a , which is in line with the value obtained using absorption data. On the other hand, the Stern-Volmer constant obtained has a much higher value than that obtained with fluorescence lifetimes (K_{sv} ~1.7 mL/mg, see below).

Attention should be pointed out to the different range of concentrations used in lifetime measurements.

If we keep K_{sv} constant and equal to that obtained through lifetime measurements (~1.7 mL/mg) and use K_a ~38 mL/mg withdrawn from absorption data, then we can only describe

the quenching process until up a concentration of 0.025 mL/mg fCNT (full black line, inset of Fig. 4.12). Again, the experimental values are much higher than those predicted by equation 2.6.

C. Lifetime measurements

Table 4.5	Fluorescence	lifetimes of	TSPP/water	(2 µM) with	n fCNT in	buffer; $\lambda_{exc} =$	445 nm,	λ _{emi} =
650 nm								

[fCNT/buffer] mg/mL	A ₁	τ ₁ (ns)	A ₂	τ ₂ (ns)	τ _{av} (ns)	XSQ
0	0.81	9.82	0.19	1.46	9.53	1.19
0.0025	0.81	9.79	0.19	1.65	9.48	1.20
0.005	0.70	9.70	0.30	0.701	9.42	1.17
0.0075	0.70	9.72	0.30	0.928	9.37	1.19
0.01	0.72	9.75	0.28	1.16	9.37	1.14
0.025	0.59	9.74	0.41	1.07	9.12	1.17

The lifetime of TSPP obtains a value of 9,53 ns, which systematically decreases until 9,12 ns. The number of exponentials needed for the fitting stays the same, two, suggesting no second species emits, meaning the complex is non emissive. The systematic decrease in lifetime suggests dynamic quenching takes place. Furthermore, there is no evidence for the formation of the di-acid species which has a fluorescence lifetime of ~3.8 ns.



Figure 4.13 Fluorescence decays of TSPP (2 μ M) without and with fCNT (0.025 mg/mL) in buffered solution (pH=7.4); λ_{exc} = 445 nm, λ_{emi} = 650 nm.

4.1.1.4 Comparison between porphyrins for fCNT

The three porphyrins show interaction in the ground state with fCNT and suggest selfassemblies between porphyrins and fCNT. Unfortunately, due the lack of isosbestic and isoemissive points in the absorption and emission spectra of TMpyP and ZnTMpyP anything can be said whether they are in equilibrium. The scattering interference in the UV/vis spectra could be the reason for not detecting an isosbestic point because its account is not straightforward.

The broadening of the Soret band on the other hand, can be interpreted as a sign of the existence of more than one component in the sample. [67] TSPP did have an isosbestic point and showed that the transformation to the complex was not complete, there was only about 20 % transformation.

In the excited state, however, no changes were observed that suggest that the complexes are non fluorescent and the emission was solely caused by the porphyrin monomers, except for the case of ZnTMpyP. For the latter, we may consider the possibility of interaction between the central metal ion and the carboxylic groups of fCNT.

Regarding solvent for TMpyP, water is preferred because more changes in the spectra are observed and thus suggesting stronger interactions. Contributing to these interactions is hydrogen bonding and electrostatic interactions between positively-charged TMpyP and the negatively charged carboxylate groups of the functionalized carbon nanotubes. Although these interactions are not as strong as normal covalent bonds, they are strong enough to cause differences in the properties of the solution.

The figure below (fig. 4.14) shows the effect of fCNT concentration on the fluorescence intensity ratio of different porphyrins. The plots of I_0/I to C (the quencher concentration) are neither linear or parabolic, except for TSPP, which seems to have a linear correlation between F_0/F and the concentration of fCNT.

Based on the slope change, the sequence of the ability to bind with fCNT in the ground state is believed to be ZnTMpyP > TMpyP/water > TSPP ~ TMpyP/DMF. This sequence is corrected when looking at the values of K_a (see table 4.6), from which it appears that the sequence is actually ZnTMpyP > TMpyP/DMF ~ TMpyP/water > TSPP. However, in the raking based on K_{SV} , representing the ability to interact with fCNT in the excited state, the sequence is TSPP ~ ZnTMpyP > TMpyP/water >> TMpyP/DMF.

45





Table 4.6 Association constant K_a (obtained using absorption and emission data) and Stern-Volmer quenching constant K_{SV} for different porphyrins with fCNT

	TMpyP/water	TMpyP/DMF	ZnTMpyP	TSPP
<i>K_a</i> (mL/mg)-abs	61.7	93.1	869	37.9
K _{SV} (mL/mg)	0	0	0	1.7
K _a (mL/mg)-Flu	286	83.0	1110	48.5

4.1.2 Porphyrin - fGRAPH interaction in water/DMF mixtures



4.1.2.1 UV/vis absorption spectra

Figure 4.15 UV/vis absorption spectra of (A) TMpyP/water (2 μ M), (B) ZnTMpyP/water (2 μ M), (C) TSPP/water (2 μ M) and (D) TPP/DMF (2 μ M) upon addition of fGRAPH/water-DMF (in mg/ml), the legend is applicable for all four spectra

Fig. 4.15 shows the UV/vis absorption spectra of TMpyP/water, ZnTMpyP/water, TSPP/water (solutions were made using buffer as solvent) and TPP/DMF, all with a concentration of 2 μ M, with increasing concentration of fGRAPH in a water/DMF mixture (2:1). The Soret bands are located at ca. 422 nm, 440 nm, 415 nm and 418 nm, respectively. There are small differences in intensity of the peaks and C and D have a small shoulder in the blue region. Although small differences are present, the shape of the bands and their positions did not show any observable changes. Upon normalization (not shown), a nearly complete overlap

of the peaks could be observed.

These reasons, together with the fact that no new absorption band is formed, do not indicate the formation of a complex porphyrin-fGRAPH in water.

4.1.2.2 Fluorescence emission spectrum

A. TMpyP



Figure 4.16 Normalized fluorescence emission spectrum of TMpyP/water (2 μ M) with increasing concentration of fGRAPH/water-DMF (0-0,025 mg/ml) represented by the arrow; λ_{exc} = 438 nm.

Fig 4.16 shows the normalized fluorescence emission spectrum of TMpyP/water upon addition of fGRAPH/water-DMF. Noticeable is that the spectrum changes from a nonstructured emission band, with its maximum at ca. 712 nm, to two ill-defined vibrational resolved emission bands ($\lambda_{max} \sim 712$ nm and ~658 nm).

These changes can be accounted by the concomitant increase of DMF of the bulk solvent upon addition of the quencher, thus assignable to changes in the physicochemical properties of the solvent mixture.

B. ZnTMpyP



Figure 4.17 Normalized fluorescence emission spectrum of ZnTMpyP/water (2 μ M) with increasing concentration of fGRAPH/water-DMF (0-0,02 mg/ml) represented by the arrow; λ_{exc} = 454 nm.

The normalized fluorescence spectrum of ZnTMpyP/water with increasing amount of fGRAPH/water-DMF is given in fig. 4.17. Two peaks at ~636 nm and ~672 nm are detected. When fGRAPH/water-DMF increases, there is a small decrease of the left hand peak, together with a 2 nm-shift to shorter wavelengths.



C. TSPP and TPP

Figure 4.18 Fluorescence emission spectra of (A) TSPP/water (2 µM) and (B) TPP/DMF (2 µM) with increasing concentration of fGRAPH/water-DMF, the legend is applicable for both A and B; λ_{exc} = 425 nm and 427 nm, respectively

The addition of fGRAPH shows a remarkable effect on the fluorescence intensity of TSPP and TPP (fig. 4.18). With the increase of fGRAPH, the emission intensities increased. Noticeable is that no new emission band appeared, suggesting no complex is formed in the excited state, and hence the emission can be assigned solely to the free porphyrin monomers sensing a slight different environment.

4.1.2.3 Lifetime measurements

Table 4.7 Fluorescence lifetimes of TMpyP/water (2 µM) with fGRAPH in water/DMF mixtures; $\lambda_{exc} = 445 \text{ nm}, \lambda_{emi} = 710 \text{ nm}$

[fGRAPH] (mg/mL)	F ₁	τ ₁ (ns)	F ₂	τ ₂ (ns)	F ₃	τ ₃ (ns)	τ _{av} (ns)	XSQ
0	0.20	1.45	0.80	5.26	-	-	5.01	1.25
0.001	0.14	1.50	0.81	4.99	0.05	7.55	5.02	1.16
0.005	0.20	1.45	0.74	5.11	0.06	7.60	5.08	1.24
0.01	0.16	1.22	0.76	4.99	0.08	7.71	5.15	1.06
0.015	0.23	1.24	0.68	5.01	0.09	8.18	5.20	1.15
0.02	0.28	1.18	0.62	5.25	0.10	8.23	5.50	1.04

Table 4.7 shows solely TMpyP could be fitted with two exponentials. Upon addition of fGRAPH, a third exponential is needed for the fitting and the fluorescence lifetime increases to a lifetime of 5,5 ns for a concentration of 0,02 mg/mL of fGRAPH. This addition of an extra exponential confirms the changes in the porphyrin environment due to the presence of a higher amount of DMF in which the porphyrin lifetime is longer (see ahead).

Table 4.8 Fluorescence lifetimes of ZnTMpyP/water (2 μ M) with fGRAPH in water/DMF mixtures; λ_{exc} = 445 nm, λ_{emi} = 630 nm

[fGRAPH/water-DMF] (mg/mL)	F ₁	т ₁ (ns)	XSQ
0	1	1,34	0,88
0,005	1	1,34	0,85
0,01	1	1,35	0,87
0,015	1	1,35	0,85
0,02	1	1,36	0,83

Fluorescence decays of ZnTMpyP with fGRAPH were fitted using just one exponential (table 4.8). There is small increase in lifetime. However, despite the small increase in lifetime, the decay is still single exponential, suggesting only one emitting species exists.

These results confirm what the fluorescence emission spectra already indicated: ZnTMpyP and fGRAPH did not form a complex in the excited state.

Table 4.9 Fluorescence lifetimes of TSPP (grey) and TPP (white) (both 2 μ M) with fGRAPH in water/DMF mixtures; λ_{exc} = 445 nm, λ_{emi} = 650 nm (a scattering component was taken into consideration)

[fGRAPH] mg/mL	F ₁	τ ₁ (ns)	XSQ	F ₁	τ ₁ (ns)	XSQ
0	1	9.73	1.19	1	10.57	1.38
0.001	1	9.74	1.26	1	10.50	1.21
0.005	1	9.88	1.14	1	10.55	1.29
0.01	1	10.1	1.19	1	10.57	1.38
0.015	1	10.3	1.12	1	10.60	1.24
0.02	1	10.4	1.07	1	10.58	1.19

In the absence of fGRAPH, emission decay of TSPP show a mono-exponential behaviour with a τ of 9,73 ns (table 4.9 - grey). In the presence of fGRAPH, the lifetime increases systematically to 10,4 ns for a concentration of 0,02 mg/mL, but the decay still remains

mono-exponential, just reflecting subtle changes in the environment due to the small increase of DMF introduced upon addition of the quencher.

This result indicates the same as the fluorescence emission spectrum, namely that TSPP did not form any complex in the excited-state with fGRAPH. The same can be said for TPP, which was also fitted using just one exponential (table 4.9 - white) and shows a lifetime of \sim 10,57 ns. There are small fluctuations, yet they are in the margin of error of this technique (10 % difference).



Figure 4.19 Fluorescence decays of (A) TMpyP, (B) ZnTMpyP, (C) TSPP and (D) TPP without and with fGRAPH/water-DMF (0,02 mg/mL), [Porphyrin]= 2 μ M; λ_{exc} =445 nm, λ_{emi} = 710, 630, 650 and 650 nm, respectively.

These results confirm that interactions in the excited state of each of the four porphyrins studied with fGRAPH must be much less effective than those evidenced with fCNT.

4.1.2.4 Comparison between porphyrins for fGRAPH

The figure below (fig. 4.20) shows the effect of fGRAPH concentration on the fluorescence intensity ratio of the different porphyrins. The plots of I_0/I vs. the quencher concentration, are either close to 1 or decrease below 1, except for ZnTMpyP. Taking into consideration that lifetime measurements gave indication of an absence of dynamic quenching, only for ZnTMpyP we see a decrease in intensity. The curves could not be fitted using a Stern-Volmer equation (Eq. 2.6 or Eq. 4.2). As a final remark, it is pertinent to say that although we used a solvent mixture of distilled water and DMF, the availability of the carbon material

(fGRAPH) towards interaction with porphyrins is probably not as good as for the case of fCNT which is much more water soluble.



Figure 4.20 Plot of changes in the intensity of the porphyrins upon addition of fGRAPH.



4.1.3 Porphyrin - MWCNT/PEG interaction in water/DMF mixtures

Figure 4.21 TEM images of MWCNT/PEG containing long thin stretched darker grey strings which are the nanotubes (1) and PEG surrounding the nanotubes, which are lighter grey areas (2), A) is the general image with a scale of 100 nm and B) is a close up with a scale of 50 nm

PEG was added to MWCNT to improve dispersion. Figure 4.21 above shows that PEG, which are the lighter grey areas (2), surrounds the multi wall nanotubes, represented by long thin stretched darker grey strings (1).



4.1.3.1 UV/vis absorption spectra

Figure 4.22 UV/vis absorption spectra of (A) TMpyP/water (2 μ M) and (B) ZnTMpyP/water upon addition of MWCNT/water-DMF (in mg/ml), the legend is applicable for both spectra

Fig. 4.22 shows the UV/vis absorption spectra of TMpyP/water and ZnTMpyP/water, both with a concentration of 2 μ M, with increasing concentration of MWCNT in a water/DMF mixture (2:1). The Soret bands are located at ca. 425 nm and 439 nm, respectively.

There are no changes in the position or the shape of the bands, nor is there a formation of a new absorption band. The conclusion can be made that no interaction occurred in the ground state between either TMpyP or ZnTMpyP and MWCNT.



4.1.3.2 Fluorescence emission spectra

Figure 4.23 Fluorescence emission spectra of (A) TMpyP/water (2 μ M) and (B) ZnTMpyP/water (2 μ M) with increasing concentration of MWCNT/water-DMF (0-0,025 mg/ml) represented by the arrow; λ_{exc} = 441 nm and 456 nm, respectively

The change in fluorescence intensity of TMpyP/water and ZnTMpyP/water, both 2 μ M, upon addition of MWCNT/water DMF (2:1) is shown in fig. 4.23. The intensity lowers significantly with increasing amount of MWCNT/water-DMF. Except for the drop in intensity, nothing changed about the spectra: the maximum of the peaks stayed the same (A: ~655 and ~717 nm, B: ~630 and ~670 nm) and no new emission band was formed, suggesting no complex occurred between porphyrin and MWCNT. The emission can be assigned to porphyrin monomers.



Figure 4.24 Fitting data of fluorescence quenching for TMpyP (left) and ZnTMpyP (right) with MWCNT using Eq. 4.2 and 2.6, respectively (see text for details)

For TMpyP with MWCNT, the value of K_{SV} is ~0 mL/mg (fig. 4.24), consistent with the lifetime data (see below) that show independence on the concentration of MWCNT. The static

contribution was accounted for by an association constant and the value obtained was Ka~47.2 mL/mg.

In the case of ZnTMpyP interaction with MWCNT there seems to be a concomitant decrease in the fluorescence lifetimes according to the data in Table 4.10, but the decrease lies inside the error range of ± 0.15 (10%). Taking this result into account, and using Eq. 2.6 for the lo/I data, a value of 45.1 mL/mg is obtained for the association constant between ZnTMpyP and MWCNT which is quite similar to that obtained with the free-base porphyrin.

4.1.3.3 Lifetime measurements

Table 4.10 Fluorescence lifetimes of TMpyP (grey) and ZnTMpyP (white) (both 2 μ M) with fGRAPH in water/DMF mixtures; λ_{exc} = 445 nm, λ_{emi} = 710 and 650 nm, respectively

[MWCNT] mg/mL	F ₁	τ ₁ (ns)	XSQ	F ₁	τ ₁ (ns)	XSQ
0	1	11.1	1.26	1	1.51	0.863
0.001	1	10.8	1.16	1	1.50	0.802
0.005	1	10.8	1.18	1	1.48	0.820
0.01	1	10.8	1.17	1	1.47	0.862
0.015	1	10.8	1.19	1	1.46	0.854
0.02	1	10.8	1.12	1	1.46	0.901
0.025	1	10.8	1.15	1	1.45	0.965

The fluorescence decay of TMpyP is monoexponential with a lifetime of 11,1 ns (table 4.10 - grey). In the presence of MWCNT, the lifetime is lowered to 10,8 ns for all concentration and from which the decays were also fitted by one exponential.

Quenching does occur due to the presence of MWCNT, but the lifetimes stay constant, thus there is no proof of a complex, which the fluorescence spectrum already suggested.

The fluorescence decay of ZnTMpyP is also monoexponential with a lifetime of 1,51 ns (table 4.10 - white). The presence of MWCNT lowers the lifetime systematically to 1,45 ns. The decays stay monoexponential.

Again here quenching does occur due to the presence of MWCNT, but the lifetimes after addition are consistent inside the margin of error. Therefore, no dynamic quenching was observed.



Figure 4.25 Fluorescence decays of (A) TMpyP and (B) ZnTMpyP, without and with MWCNT (0.025 mg/mL) in water-DMF mixtures. [Porphyrin]= 2 μ M; λ_{exc} = 445 nm, λ_{emi} = 710 and 630 nm, respectively

4.1.3.4 Comparison between porphyrins for MWCNT

Based on the plots of F_0/F vs. [MWCNT] of TMpyP and ZnTMpyP (fig. 4.26), it is hard to say which one has the highest ability of the intensity quenching since the slopes match each other. When comparing the values of K_a , summarised in table 4.11, it is detected that that the values are quite similar and therefore both porphyrins have the same ability to form complexes with MWCNT.



Figure 4.26 Plot of changes in the intensity of the porphyrins upon addition of MWCNT.

Table 4.11 Stern-Volmer quenching constant K_{SV} , association constant K_a for TMpyP and ZnTMpyP with MWCNT

	ТМруР	ZnTMpyP
<i>К_{sv}</i> (mL/mg)	0	0
K _a (mL/mg)	47,2	45,1

4.2 Porphyrin interaction with CNT decorated with gold nanoparticles

A 1b 1a B -2 500 nm D 200 nm

4.2.1 AuNP and AuNP/PEG with fCNT in water

Figure 4.27 TEM images of AuNP with fCNT (preformed) with A) a general image with a scale of 500 nm containing a dark background which is the grid (1a), an almost white background which are the holes in the grid (1b) and dark spheres which are the nanoparticles (2); and B) a close up with a scale of 200 nm containing long thin stretched light grey strings which are the nanotubes (3)

The figure above (figure 4.27) shows the TEM images of AuNP with fCNT, which was prepared using the preformed method. It contains the following things:

- A darker grey background (1a, A), which is the grid whereon the sample is suspended
- A lighter, almost white background (1b, A), which are the holes in the grid
- Really dark, almost black spheres (2, A), these are the gold nanoparticles with a diameter of approximately 10-20 nm [68]
- Long, thin stretched light grey strings (3, B), these are the carbon nanotubes with a width of approximately 4-5 nm [69]

Both nanotubes and nanoparticles are present in the solution, but they did not interact with each other. The same applies for AuNP/PEG with fCNT, also prepared with the preformed method (see fig. 4.28). A possible explanation for this is that these AuNP are too big to interact with these thin fCNT.

An alternative is to use other nanotubes such as multi wall carbon nanotubes (MWCNT). These nanotubes are wider than fCNT, thus increasing the chance of interaction. Another
advantage is that the π - π interactions among these structures are less strong, whereby dispersion will be easier. Note that the MWCNT is not functionalized.

Another important aspect concerns the use or not of the polymer: the system with PEG is more preferable because it helps the dispersion. For this reason, the study involving MWCNT is performed only with AuNP/PEG (see next part).



Figure 4.28 TEM image of AuNP/PEG with fCNT (preformed) with a scale of 200 nm containing the nanoparticles (1) and the nanotubes (2)

4.2.2 AuNP/PEG with MWCNT in water/DMF mixtures

AuNP/PEG was introduced to MWCNT in two different ways: preformed, where both components are prepared separately and mixed afterwards, and *in situ*, where AuNP is simultaneously prepared with MWCNT (see 3.2.3).

Figure 4.29 and 4.30 give TEM images of AuNP/PEG with MWCNT, prepared preformed and *in situ*, respectively. The images show that the nanoparticles (2,B) and the carbon nanotubes (3,B) did adsorb as expected because multiwall nanotubes are wider than single wall nanotubes. MWCNT have a width of approximately 8-21 nm [70].

The images show that the nanotubes have the tendency to aggregate together, whereas the particles are spread homogeneously.

Comparing the two methods of preparing the nanoparticles, there are no real differences between preformed and *in situ*: both methods give a good interaction between nanotubes and nanoparticles. One could say that the preformed method produces more nanoparticles and debundled nanotubes, but one must bear in mind that the images cannot speak for the whole solution.

One difference, except for the method itself, is that in the preformed case there is a better dispersion of the system than in the *in situ* one. Both methods are used for further experiments.



Figure 4.29 TEM images of AuNP/PEG with MWCNT (preformed) with A) a general image with a scale of 500 nm containing the grid (1a), the holes in the grid (1b) and an example of aggregation (4); and B) a close up with a scale of 100 nm containing the nanoparticles (2) and the nanotubes (3)



Figure 4.30 TEM images of AuNP/PEG with MWCNT (*in situ*) with A) a general image with a scale of 500 nm containing the grid (1a), the holes in the grid (1b); and B) a close up with a scale of 100 nm containing the nanoparticles (2) and the nanotubes (3)

4.2.2.1 TMpyP



A. UV/vis absorption spectrum

Figure 4.31 UV/vis absorption spectra of TMpyP/water (2 μ M) upon addition of AuNP/PEG/MWCNT in water-DMF (in mg/ml) using the preformed method (A) and the *in situ* method (B), the legend of A is also applicable to B

The figure above (fig. 4.31) shows the UV/vis absorption of TMpyP/water and AuNP/PEG with MWCNT/water-DMF using 2 different methods: preformed and *in situ*. Both graphs have the Soret band at a wavelength of 435 nm and an almost complete overlap of the curves. A and B are almost identical to each other.

There are no changes in the position or the shape of the bands, nor is there a formation of a new absorption band. This result suggests no interaction occurred in the ground state between TMpyP and MWCNT decorated with gold, using either the preformed or the *in situ* method.



B. Fluorescence emission spectrum

Figure 4.32 Fluorescence emission spectra of TMpyP/water (2 μ M) with increasing concentration of AuNP/PEG/MWCNT in water-DMF (0-0,025 mg/ml) represented by the arrow using the preformed method (A) and the *in situ* method (B); λ_{exc} = 439 nm

The change in fluorescence intensity of TMpyP/water upon addition of AuNP/PEG with MWCNT/water-DMF (2:1), using the preformed and *in situ* method, is shown in fig. 4.32. The intensity lowers significantly with increasing amount of AuNP/PEG with MWCNT/water-DMF. Except for the drop in intensity, nothing changed about the spectra: the maximum of the peaks stayed the same (~650 and ~712 nm for both A and B) and no new emission band was formed. The emission can be assigned to the porphyrin monomers.



Figure 4.33 Fitting data of fluorescence quenching for TMpyP and AUNP/PEG/MWCNT using the preformed (left) and *in situ* method (right) using Eq. 4.2 (see text for details)

The quenching effect was taking into account using Eq. 2.6. K_{SV} has a value of a about 0 mL/mg for the preformed method (fig. 4.33). This is in agreement with the obtained lifetime data (see below) in which it can be seen that there is no dependence on the concentration of AuNP/PEG/MWCNT. Therefore, in this case we have the contribution of static quenching only.

Using the *in situ* method, there is a change in the fluorescence lifetime of TMpyP when in presence of AuNP-MWCNT, but no further difference is noted upon increasing the concentration of the quencher (see below).

The association constants obtained are in the same order of magnitude for both methods of preparation of AuNP. Moreover, in the case of the in-situ preparation this value does not differ from the system without the nanoparticles.

C. Lifetime measurements

Table 4.12 Fluorescence lifetimes of TMpyP/water (2µM) with AuNP/PEG/MWCNT in water/DMF mixtures, prepared using the preformed method (grey) and the *in situ* method (white); λ_{exc} = 445 nm, λ_{emi} = 710 nm (a scattering component was taken into account)

[AuNP/PEG/MWCNT] mg/mL	F ₁	τ ₁ (ns)	XSQ	F ₁	τ ₁ (ns)	XSQ
0	1	11.2	1.19	1	11.2	1.19
0.001	1	11.2	1.29	1	10.5	1.17
0.005	1	11.2	1.17	1	10.5	1.15
0.01	1	11.1	1.20	1	10.5	1.21
0.015	1	11.0	1.21	1	10.6	1.21
0.02	1	11.0	1.18	1	10.6	1.12
0.025	1	11.0	1.13	1	10.6	1.17

Fluorescence decays of TMpyP with AuNP/PEG/MWCNT in water-DMF mixtures using the preformed method were fitted using just one exponential. (table 4.12 - grey).

This result, together with the fluorescence spectrum, indicates the same: TMpyP and MWCNT decorated with AuNP did not form an assembly in the excited state using the preformed method. A similar conclusion can be made about the *in situ* method (table 4.12 - white).



Figure 4.34 Fluorescence decays of TMpyP (2 μ M) without and with AuNP/PEG/MWCNT (0.025 mg/mL) in water/DMF mixtures prepared using preformed and *in situ* methods; λ_{exc} = 445 nm, λ_{emi} = 710 nm

4.2.2.2 ZnTMpyP



A. UV/vis absorption spectrum

Figure 4.35 UV/vis absorption spectra of ZnTMpyP/water (2 μ M) upon addition of AuNP/PEG/MWCNT in water-DMF (in mg/ml) using the preformed method (A) and the *in situ* method (B), the legend of A is also applicable to B

Fig. 4.35 shows the UV/vis absorption of ZnTMpyP in the presence of increasing amounts of AuNP/PEG/MWCNT in water-DMF using the preformed and *in situ* method. Both graphs show the Soret band peak at a wavelength of 439 nm and an almost complete overlap of the curves. A and B are again almost identical to each other. Upon addition of MWCNT decorated with AuNP no changes occurred in position or shape of the bands, there are small changes in intensity.

For these reasons one can conclude neither the preformed nor the *in situ* method resulted in an assembly between ZnTMpyP and MWCNT decorated with gold nanoparticles.



B. Fluorescence emission spectrum

Figure 4.36 Fluorescence emission spectra of ZnTMpyP/water (2 μ M) with increasing concentration of AuNP/PEG/MWCNT in water-DMF (0-0,025 mg/ml) represented by the arrow using the preformed method (A) and the *in situ* method (B); λ_{exc} = 457 nm

The drop in in fluorescence intensity of ZnTMpyP/water upon addition of AuNP/PEG with MWCNT/water-DMF using the preformed and *in situ* method, is shown in fig. 4.36. Whereas in the case of the preformed method there is a concomitant decrease of ZnTMpyP fluorescence intensity with increasing amounts of AuNP/PEG/MWCNT, for the *in situ* method a more heterogeneous dependence is detected.

Neither A or B spectra changed regarding position or shape ($\lambda_{max} \sim 632$ and ~ 672 nm), nor did a new emission band form, suggesting the emission is only due to the free ZnTMpyP monomers, hence suggesting no assembly occurred between ZnTMpyP and MWCNT decorated with AuNP.



Figure 4.37 Fitting data of fluorescence quenching for ZnTMpyP and AUNP/PEG/MWCNT using the preformed (left) and *in situ* method (right) using Eq. 4.2 (see text for details)

Once more, we have used Eq 2.6 to obtain the rate constants for the quenching process.

 K_{SV} has a value of a 0 mL/mg for both the preformed as the *in situ* method (fig. 4.37). This is in agreement with the obtained lifetime data (see below), where there is no dependence on the concentration of AuNP/PEG/MWCNT for either method. As for the static component, again the association constants obtained are within the same order of magnitude, 12.3 and 17.4 mL/mg, respectively for the pre-formed and the in situ methods. Noticeable, in this case they are both lower than those obtained for the system without AuNP.

C. Lifetime measurements

All the samples (table 4.13 - grey) are fitted with one exponential and all have a constant value of ~1,52 ns, whether in the presence or in the absence of AuNP/PEG with MWCNT/water-DMF.

The same conclusion can be made about the *in situ* method (table 4.13 - white), which also has a lifetime value of ~1,52 ns, suggesting that there is no dynamic quenching effect in either cases.

(write), $\Lambda_{exc} = 445 \text{ min}$, $\Lambda_{emi} = 650 \text{ min}$ (a scattering component was taken into account)						
[AuNP/PEG/MWCNT] mg/mL	F ₁	τ ₁ (ns)	XSQ	F ₁	τ ₁ (ns)	XSQ
0	1	1.51	1.03	1	1.51	0.95
0.001	1	1.52	1.02	1	1.50	0.90
0.005	1	1.52	1.03	1	1.52	0.92
0.01	1	1.52	1.03	1	1.50	0.97
0.015	1	1.52	1.01	1	1.50	1.01

Table 4.13 Fluorescence lifetimes of ZnTMpyP/water (2µM) with AuNP/PEG/MWCNT in water/DMF mixtures, prepared using the preformed method (grey) and the in situ method (white),) - 115 nm) - 620 nm (a coattaring component was taken into account)

4.2.2.3 Comparison between porphyrins for AuNP/PEG with MWCNT

Neither of the two porphyrins interacted with MWCNT decorated with AuNP the ground state, nor did they interact in the excited state.

The figure below (fig. 4.38) shows the effect of MWCNT decorated with AuNP concentration on the fluorescence intensity ratio of the different porphyrins. The plots of F₀/F to C, which represents the quencher concentration, are almost linear. Based on the slope change, the sequence of the ability of the intensity quenching is TMpyP - in situ > TMpyP - preformed > ZnTMpyP – in situ > ZnTMpyP – preformed. This result indicates that the quenching effect of the AuNP/MWCNT system is more pronounced for TMpyP than ZnTMpyP and preparing AuNP in situ results in more quenching, probably due to the closer proximity of the two species adsorbed to MWCNT like suggested in the difference in τ_{av} (see above). This conclusion can also be seen in the obtained K_a and from which a review is represented in table 4.14.



Figure 4.38 The plot of intensity quenching upon the quencher concentration C for MWCNT decorated with AuNP

	TMpyP - preformed	TMpyP - <i>in situ</i>	ZnTMpyP - preformed	ZnTMpyP - <i>in situ</i>
K _{SV} (mL/mg)	0	0	0	0
K _a (mL/mg)	36.9	45,8	12.3	17.4

Table 4.14 Stern-Volmer quenching constant K_{SV} and association constant K_a for different porphyrins and different preparing methods of AuNP/PEG/MWCNT

4.3 FLIM-images

The microscopy technique of FLIM was used in order to obtain further information about the structure and components' interactions in films deposited on glass.

4.3.1 TMpyP

In figure 4.39 it is depicted the data obtained with TMpyP. For the porphyrin alone (A) we see an almost homogeneous image with an average lifetime of 3 ns which is shorter than that obtained for TMpyP free in aqueous solution probably due to some aggregation derived from solvent evaporation or even an interaction with the glass surface.

The interaction of TMpyP with MWCNT (B) causes fluorescence quenching with the average lifetime of the porphyrin around 1.3 ns obtained by point measurement in the "tubular" region. The effect of the addition of AuNP on TMpyP fluorescence depends on the method of preparation of NP (C): when preformed AuNP are added to the system there is a decrease of both fluorescence intensity and average lifetime; whereas when AuNP are grown *in situ*, a huge increase of the fluorescence intensity is observed together with a shortening of the average lifetime. This last behaviour is indicative of a possible effect of metal enhanced fluorescence emission (MEF). This phenomenon can occur when the dye is located at suitable distances from the metal NP. It seems that in the case of the pre-formed NP the distance between dye and NP is not suitable for MEF. Probably, an electron transfer process may occur which leads to a quenching of both intensity and lifetime.



Figure 4.39 FLIM images and respective lifetime histograms of TMpyP deposited from (A) an aqueous solution, (B) MWCNT (0.02 mg/mL), and (C) AuNP/PEG/MWCNT (0.025 mg/mL) prepared *in situ*. The lifetime scale is shown on the left. Image bar scale 10 μ m (2.5 μ m for image B).

4.3.2 ZnTMpyP

Data was also obtained using the metallated porphyrin, fig. 4.40. A homogeneous image was retrieved for the porphyrin deposited from an aqueous solution with an average lifetime of 1.1 ns (A), which is only slightly shorter than that obtained for the porphyrin free in solution, thus ruling out aggregation during solvent evaporation. The presence of fCNT (B) leads to a change in the histogram profile which shows a higher contribution from short lifetimes as compared to water.

Upon addition of AuNP/MWCNT/PEG, we see a more heterogeneous image (but similar for the two different method of NP preparation) but with quite a distinct histogram: in both cases there is a shortening of the fluorescence lifetimes and an increase in fluorescence intensity as compared to the dye alone. However, the enhancement is far more intense when the *in situ* method is used to obtain AuNP. The fact that NP spreading seems to be better reached for the latter method as denoted in TEM images, may contribute to ensure dye –AuNP interaction and thus leading to a statistically measurable effect of MEF.



Figure 4.40 FLIM images and respective lifetime histograms of ZnTMpyP deposited from (A) an aqueous solution, (B) fCNT (0.025 mg/mL), and (C) AuNP/PEG/MWCNT (0.025 mg/mL) prepared *in situ*. The lifetime scale is shown on the left. Image bar scale 10 μ m (0.75 μ m for image B).

CHAPTER 5 CONCLUSIONS AND PERSPECTIVES

5.1 Conclusions

5.1.1 Porphyrin interaction with carbon based nanostructures

The three porphyrins used (TMpyP, ZnTMpyP and TSPP) show interaction in the ground state with fCNT and suggest self-assemblies between porphyrins and fCNT. This conclusion is based on the broadening of the Soret band, which can be interpreted as a sign of the existence of more than one component in the sample. Only TSPP had an isosbestic point, from which a quantification can be made: transformation to the complex is noticeable but not complete, there was only about 20 % transformation.

In the excited state, however, no changes were observed that suggest a complex exists, except for the case of ZnTMpyP. Therefore it can be concluded that for the other porphyrins the complexes are non fluorescent and the emission was solely caused by the porphyrin This conclusion is confirmed by lifetime monomers. measurements. Regarding solvent choice for TMpyP, water is preferred because more changes in the are observed and thus suggesting stronger spectra interactions. ZnTMpyP has the highest ability to bind with fCNT in the ground state, to which specific interactions between the Zn in the porphyrin and the carboxylic groups of fCNT may contribute.

Neither of the four porphyrins interacted with fGRAPH in the ground state, nor did they interact in the excited state. For all the porphyrins, the emission is due to free porphyrin monomers.

Both porphyrins have the same ability to form complexes with MWCNT.

General conclusion

Absorption spectra revealed that strong π - π binding with the ground state (S₀) of the porphyrins occurred for fCNT but not for fGRAPH or MWCNT.

Ground-state interactions between porphyrins and CBN can be influenced by the geometry of the latter. In this context, fGRAPH being partially planar should be more suitable for π - π interactions with the also planar porphyrins moiety, as compared to the tubular structures of the CNT. Between these two, fCNT and MWCNT, one may consider that with the latter π - π interactions are more prone due to its wider structure, as compared with the thinner

functionalized single-wall (fCNT). Moreover, the COOH groups present in the functionalized materials may sterically hinder π - π interactions. However, one must consider that for the aqueous systems, opposite charged porphyrins may also establish additional electrostatic interactions with the charged CBN (fCNT and fGRAPH). The fact that major spectral alteration occur in the case of fCNT and then fGRAPH confirms the importance of such interactions.

The excited-state interactions are primarily influenced by the reduction potential of CBN. According to previous reports in literature [15, 70, 71] the dynamic quenching involving these structures is due to a photoinduced electron transfer (PET) process between the electron donor, the porphyrin, and the electron acceptor, the CBN. The free energy change ΔG for PET depends on the oxidation potential of the donor (E_{oxi}), and the reduction potential of the acceptor (E_{red}). The lower the redox potential, the more the PET process is favoured so that dynamic quenching is most efficient.

MWCNT contains mainly unsaturated sp^2 carbons and so the electron deficiency makes them better electron acceptors. In turn, fCNT, is heavily substituted by carboxyl groups, and so contains essentially sp^3 as well as some sp^2 carbon atoms. Thus, making fCNT as the less efficient electron acceptor CBN used in this study. The case of fGRAPH is an intermediate one, since carboxyl functionalization occurs solely on the edges leaving the basal plane with its sp^2 carbon hybridization intact.

Altogether, data seems to indicate that the PET process is not the most efficient for either system studied and complexation is highly favourable for the functionalized CBN.

5.1.2 Porphyrin interaction with CNT decorated with gold nanoparticles

Gold nanoparticles did not adsorb to fCNT because AuNP were too big for the thin fCNT. This is why we switched to MWCNT, which are wider, thus enhancing the chance of interaction. TEM images showed adsorption of AuNP did happen to the surface of MWCNT. Further, PEG addition helps the dispersion.

In solution, there seemed to be no interaction of the porphyrins (TMpyP and ZnTMpyP) with MWCNT/AuNP/PEG in the ground state, nor in the excited state, using the preformed and the *in situ* method. The *in situ* method did result in more quenching, probably due to the closer proximity of the two species adsorbed to MWCNT, especially with TMpyP.

However, in the hybrid films it was possible to detect a clear difference between the two methods used to add the AuNP to the system. In the case of the *in situ* method, a clear decrease of the fluorescence lifetime together with an increase of fluorescence intensity could be observed. By contrast, the films of the hybrid prepared by addition of pre-formed AuNP, there was a clear quenching effect with a decrease of both fluorescence lifetime and intensity. These two situations have already been reported for similar systems [55] and their occurrence is greatly influenced by the distance between NP and the dye besides other parameters which include the NP size.

5.1.3 General conclusion with and without decoration of AuNP

It was possible to design porphyrin/fCNT nanohybrids, from which the properties were studied using UV/vis absorption, steady state and time-resolved spectroscopy, as well as by FLIM. The CBN fGRAPH and MWCNT did not result in the same. Also, no hybrid systems could be made using MWCNT decorated with gold, using either the preformed preparing method or the *in situ* method in solution.

5.2 Perspectives

From the carbon based nanostructures without decoration of AuNP, fCNT is the only CBN that showed interaction with all of the porphyrins. Because AuNP were too big for the thin fCNT, it failed to adsorb and there was a switch to wider MWCNT. A more efficient control of NP size is required to facilitate interaction with such thin carbon nanotubes which would require a more extensive study of temperature dependence.

It is also advised to monitor the pH of the solutions carefully from the beginning, especially when using TSPP. Several experiments were meaningless to put in this report because the solutions were not buffered, creating non-stable solutions with very deviating results. To further facilitate the solubility of the CBN in aqueous solution, the addition of polyelectrolytes can be recommended. They also assist in the posterior functionalization with porphyrins of opposite charge.

Future work

Assemblies of CBN with porphrins can lead to the formation of supramolecular assemblies, with potential applications in sensing, solar energy conversion and opto-electronic devices. These flexible next-generation systems would have several advantages compared to the inorganic solar cells used nowadays, with the most important one being low-cost. The latter can be achieved because the high costs associated with the fabrication procedures of

conventional solar cells (e.g. reaching high elevated temperatures, creating high vacuum, etc.) can be avoided.

Other advantages would be the high-efficiency, thermal stability, being environmentally friendly and the reduced weight. [72, 73]

REFERENCES

- Shao X., Zhang X.-F., π-π binding ability of different carbon nano-materials with aromatic phthalocyanine molecules: comparison between graphene, graphene oxide and carbon nanotubes, *J. Photochem. Photobiol. A: Chem.*, 278, 2014, p. 69-74.
- Andrade S.M., Metal nanoparticles growth on carbon nanostructures with photoactive molecules towards optoelectronic applications, Master thesis proposal, IST, Lisbon, Portugal, 2013-2014.
- Gale P.A., Steed J.W., Supramolecular Chemistry: From Molecules to Nanomaterials, John Wiley & Sons Ltd., UK, 2012.
- 4. Boscencu, R., *et al.*, *Biomedical Engineering From Theory to Applications*, InTech Edm., USA, 2011.
- 5. Griffith M. J., *et al.*, Porphyrins for dye-sensitised solar cells: new insights into efficiency-determining electron transfer steps. *Chem. Comm.*, 48, 2012, p. 4145-4162.
- Eguchi M., *et al.*, Porphyrin photochemistry in inorganic/organic hybrid materials: Clays, layered semiconductors, nanotubes, and mesoporous materials. *J. Photochem. Photobiol. C: Photochem.*, 7, 2006, p. 104-126.
- 7. Nishiyama N., *et al.*, Light-harvesting ionic dendrimer porphyrin as new photosensitizers for photodynamic therapy, *Bioconjugate Chem.*, 14, 2003, p. 58-66.
- Dougherty T.J., *et al.*, Photodynamic therapy, *J. Natl Cancer Inst.*, 12, 1998, p. 889-905.
- 9. Teixeira R.A.M., Optical spectroscopy and imaging of porphyrins and phthalocyanines: self-aggregation, microencapsulation and plasmon enhanced emission, PhD dissertation IST, Lisbon, Portugal, 2012.
- 10. Andrade S.M., et al., Self-aggregation of free base porphyrins in aqueous solution and DMPC vesicles, *Biophys. Chem.* 133, 2008, p. 1-10.
- 11. Catalog,Sigma-AldrichCo.,[Webpage],2014,<http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do>,[Visited2/03/2014].2/03/2014].
- Togashi D.M., Costa S.M.B., Absorption, fluorescence and transient triplet-triplet absorption spectra of zinc tetramethylpyridylporphyrin in reverse micelles and microemulsions of aerosol OT-(AOT), *Phys. Chem. Chem. Phys.*, 2, 2000, p. 5437-5444.
- 13. Andrade S.M. et al., Characterization of metal nanoparticles on graphene oxide functionalized with photoactive molecules (*to be submitted*)

- Properties and applications of single-, double- and multi-walled carbon nanotubes, Sigma-Aldrich Co., [Webpage], 2014, http://www.sigmaaldrich.com/technicaldocuments/articles/materials-science/single-double-multi-walled-carbonnanotubes.html, [Visited 23/07/2014].
- 15. Andrade S.M., *et al.*, Polyelectrolyte-assisted noncovalent functionalization of carbon nanotubes with ordered self-assemblies of a water-soluble porphyrin, *ChemPhysChem*, 13, 2012, p. 3622-3631.
- 16. *Carbon nanotubes*, Nanocyl Inc., [Webpage], 2014, http://www.nanocyl.com/en/CNT-Expertise-Centre/Carbon-Nanotubes, [Visited 24/07/2014].
- Gulotty R., *et al.*, Effects of functionalization on thermal properties of single-wall and multi-wall carbon nanotube-polymer nanocomposites, *Am. Chem. Soc. - Nano*, 7, 2013, p. 5114-5121.
- Carbon Nanotubes, IndiaMART intermesh Ltd, [Webpage], 2014, http://www.indiamart.com/reinste-nano-venture/carbon-nanotubes.html, [Visited 7/07/2014].
- 19. Novoselov K.S., Geim A.K., *et al.*, Electric field effect in atomically thin carbon films. *Science* 306, 2004, p. 666-669.
- 20. Kuila T., *et al.*, Chemical functionalization of graphene and its applications, *Prog. Mater. Sci.*, 57, 2012, p. 1061-1105.
- 21. Malig J., *et al.*, Direct exfoliation of graphite with a porphyrin creating functionalizable nanographene hybrids, *Chem. Comm.* 48, 2012, p. 8745-8747.
- 22. Mody V.V., *et al.*, Introduction to metallic nanoparticles, *J. Pharm. Bioallied Sci.*, 4, 2010, p. 282-289.
- 23. Xiang H.P., et al., Size-dependent plasmonic resonances from large-scale quantum simulations, *J. Phys. Chem. Lett.*, 5, 2014, p. 1163-1169.
- 24. Gaultier J., *et al.*, Design strategies of hybrid metallic nanoparticles for theragnostic applications. *Nanotechnol.*, 24, 2013, p. 432002.
- 25. Teixeira R., *et al.*, Plasmon-enhanced emission of a phthalocyanine in polyelectrolyte films induced by gold nanoparticles, *J. Phys. Chem.*, 115, 2011, p. 24674-54680.
- 26. Rouessac A., Rouessac F., *Chemical Analysis Modern instrumentation methods and techniques*, John Wiley & Sons Ltd, West Sussex, United Kingdom, 2007.
- 27. Lakowicz J.R., Principles of Fluorescence Spectroscopy, Springer, UK, 2006.
- 28. Vanderauwer P., *Spectrofotometrie*, course lab instrumental analysis, faculty of Industrial sciences, Artesis hogeschool, Antwerp, Belgium, 2012.
- 29. Crols M., *Biomoleculen 3 lab*, course lab biomolecules, faculty of Industrial sciences, Artesis hogeschool, Antwerp, Belgium, 2011.

- 30. Correia R.F., *Cell membrane models made up from amphiphiles and proteins*, PhD dissertation IST, Lisbon, Portugal, 2012.
- 31. Thappily P., *Soret band B-band*, [Webpage], 7/02/2012, http://unconventionalelectronics.blogspot.pt/2012/02/soret-band.html, [Visited 30/05/2014].
- 32. Rao C.N.R., Müller A., Cheetham A.K., *Nanomaterials Chemistry: Recent Developments and New Directions*, Wiley-VCH, Weinheim, Germany, 2007.
- Cottancin E., *et al.*, Optical properties of noble metal clusters as a function of the size: Comparison between experiments and a semi-quantal theory. *Theor. Chem. Acc.*, 116, 2006, p. 514-523.
- Jain P.K., *et al.*, Noble metals on the nanoscale: optical and photothermal properties and some applications in Imaging, sensing, biology and medicine. *Acc. Chem. Res.*, 41, 2008, 1578-1586.
- 35. Zhang Y.J., Investigation of gold and silver nanoparticles on absorption heating and scattering imaging. *Plasmonics*, 6, 2011, p. 393-397.
- Cao W., *et al.*, Localized surface plasmon resonance of single silver nanoparticles studied by dark-field optical microscopy and spectroscopy. *J. Appl. Phys.*, 109, 2011, p. 034310.
- 37. Murphy C.J., Jana N.R., Controlling the aspect ratio of inorganic nanorods and nanowires. *Adv. Mater.*, 14, 2002, p.80-82.
- 38. Valeur B., *Molecular Fluorescence: Principles and Applications*, Wiley VCH, Weinheim, Germany, 2001.
- 39. Alaa S., et al., Macromolecules Containing Metal and Metal-like Elements, Photophysics and Photochemistry of Metal-Containing polymers, John Wiley & Sons, Inc., New Jersey, Canada, 2010.
- 40. Klán P., Wirz J., *Photochemistry of Organic Compounds: from Concepts to Practice*, John Wiley & Sons, Ltd., UK, 2009.
- 41. Henry B.R., Siebrand W., *Organic Molecular Photophysics*, Birks J.B. Ed., John Wiley & Sons, London, UK, vol.1, 1973, p. 154.
- 42. Zhang G.W., *et al.*, Interaction of the irisflorentin with bovine serum albumin: a fluorescence quenching study. *J. Mol. Struct.*, 891, 2008, p. 93-97.
- 43. Eftink M.R., *Topics in Fluorescence Spectroscopy* (Lakowicz, J.R., ed.), Kluwer Academic Publishers, UK, 2002.
- Behera G.B., *et al.*, Fluorescent probes for structural and distance effect studies in micelles, reversed micelles and micro-emulsions, *Adv. Colloid and interface Sci.*, 82, 1999, p. 1-42.

- 45. Tkachenko N.V., *Optical Spectroscopy: Methods and Instrumentations*, Elsevier, UK, 2006.
- 46. O'Connor D.V.O., *et al.*, Deconvolution of fluorescence decay curves. A critical comparison of techniques. *J. Phys. Chem.*, 83, 1979, p. 1333-1343.
- 47. O'Connor D.V.O., Philips D., *Time-correlated single photon counting*, Academic Press Inc., London, United Kingdom, 1984.
- 48. Stefanaki E., Voutou B., *Electron Microscopy: The Basics*, course in Physics of advanced materials, IST, Portugal, 2008.
- 49. Kohl H., Reimer L., *Transmission Electron Microscopy: Physics of Image Formation*, Springer, UK, 2008.
- 50. Bueno-Alejo C., research department of photochemistry, IST, e-mail, 21/05/2014.
- 51. O. Herrmann, S. H. Mehdi and A. Corsini, Heterogeneous metal-insertion novel reaction with porphyrins. *Can. J. Chem.*, 1978, 56, 1084.
- 52. *Lambda 25, 35, 45 User's guide*, user's guide PerkinElmer Ltd, Buckinghamshire, United Kingdom, 2000.
- 53. *Fluorolog*® *Tau-3 lifetime system Operation manual*, user's guide Horiba Jobin Yvon Inc., Germany, 2001.
- 54. Correia R.F., *Cell membrane models made up from amphiphiles and proteins*, PhD dissertation IST, Lisbon, Portugal, 2012.
- 55. Teixeira R.A.M., Optical spectroscopy and imaging of porphyrins and phthalocyanines: self-aggregation, microencapsulation and plasmon enhanced emission, PhD dissertation IST, Lisbon, Portugal, 2012.
- Fluorescence Microscopes, MicroTime 200, PicoQuant, [Webpage], 2014, http://www.picoquant.com/products/category/fluorescence-microscopes/microtime-200-time-resolved-confocal-fluorescence-microscope-with-unique-single-molecule-sensitivity, [Visited 23/05/2014].
- 57. Andrade S.M., Costa S.M.B., Spectroscopic studies of water-soluble porphyrins with protein encapsulates in bis(2-ethylhexyl)sulfosuccinate (AOT) reverse micelles: aggregation versus complexation, *Chem. Eur. J.*, 12, 2006, p. 1046-1057.
- 58. *Products*, TCI America, [Webpage], 2014, ">www.tcichemicals.com/eshop/pt/br/commodity/A5012/>, [Visited 27/03/2014].
- 59. Castanho M.A., Loura L.M.S., Santos N.C., Separating the turbidity spectra of vesicles from the absorption spectra of membrane probes and other chromophores, *Eur. Biophys. J.*, 26, 1997, p. 253-259.
- 60. DAS6 Fluorescence decay analysis software user guide, user's guide Horiba Jobin Yvon Inc., Germany, 2012.

- 61. Shao X., Zhang X.-F., π-π binding ability of different carbon nano-materials with aromatic phthalocyanine molecules: comparison between graphene, graphene oxide and carbon nanotubes, *J. Photochem. Photobiol. A: Chem.*, 278, 2014, p. 69-74.
- 62. Eftink M.P., Ghiron C.A., Fluorescence quenching studies with proteins, *Anal. Biochem*, 114, 1981, p. 199-227.
- 63. Andrade S.M., Costa S.M.B. Fluorescence quenching of acridine orange in microemulsions induced by the non-steroidal anti-inlammatory drug Piroxicam. *Photochem. Photobiol. Sci.*, 2, 2003, 605-610.
- 64. Kubat P., et al., Self-assemblies of cationic porphyrins with functionalized watersoluble single-walled carbon nanotubes, *J. Nanosci. Nanotech.*, 9, 2009, p. 5795-5802.
- 65. Vergeldt F.J., *et al.*, Intramolecular interactions in the ground and excited states of tetrakis(N-methylpyridyl)porphyrins, *J. Phys. Chem.*, 99, 1995, p. 4397-4405.
- 66. Andrade S.M., Costa S.M.B., Spectroscopic studies on the interaction of a water soluble porphyrin and two drug carrier proteins, *Biophys. J.*, 82, 2002, p. 1607-1619.
- 67. Togashi D.M., *et al.*, Self-aggregation of lipophilic porphyrins in reverse micelles of aerosol OT *J. Phys. Chem. B*, 108, 2004, p. 11344-11356.
- 68. Bueno-Alejo C. et al., manuscript under submission.
- Carbon nanotube, single-walled, carboxylic acid functionalized, Sigma-Aldrich Co. LLC, [Webpage], 2014, http://www.sigmaaldrich.com/catalog/product/aldrich/652490?lang=en®ion=BE, [Visited 22/03/2014].
- 70. Serp P., Toulouse, France, responsible for the synthesis.
- 71. Malig J., *et al.*, Toward multifunctional wet chemically functionalized grapheneintegration of oligomeric, molecular, and particulate building blocks that reveal photoactivity and redox activity, *Acc. Chem. Res.*, 46, 2013, p. 53-64.
- 72. Zhang X.-F., Xi Q., A graphene sheet as an efficient electron acceptor and conductor for photoinduced charge separation, *Carbon*, 49, 2011, p. 3842-3850.
- 73. Huynh W.U., *et al.*, Hybrid nanorod-polymer solar cells, *Science*, 295, 2002, p. 2425-2427.

APPENDIX A CONCENTRATIONS OF STOCK SOLUTIONS

Table XV Concentrations of the stock solutions of the porphyrins

Stock solution of	$C_{\text{stock}}(.10^{-4} \text{ M})$
TMpyP in water	3,34
TMpyP in DMF	4,23
ZnTMpyP in water	3,317
TSPP in water	3,5
TPP in DMF	0,817

Table XVI Concentrations of the stock solutions of the solids

Stock solution of	C _{stock} (mg/ml)
fCNT in water	0,098
fCNT in DMF	0,1012
fCNT in buffer	0,0996
fGRAPH in water/DMF (2:1)	0,5012
MWCNT/PEG in water/DMF (2:1)	0,0988
AuNP/PEG with MWCNT in water/DMF (2:1) - preformed	0,0505
AuNP/PEG with MWCNT in water/DMF (2:1) - in situ	0,0408