

New Insights into Carbon Nanodots: Analysis of Ensemble and Single Molecule Fluorescence

A THESIS SUBMITTED

FOR THE DEGREE OF

Doctor of Philosophy

By

Syamantak Khan



School of Basic Sciences

Indian Institute of Technology, Mandi

Himachal Pradesh 175001, INDIA

OCTOBER 2017

Dedicated to
My Beloved Parents



Declaration by the Research Scholar

This is to certify that the thesis entitled “New Insights into Carbon Nanodots: Analysis of Ensemble and Single Molecule Fluorescence”, submitted by me to the Indian Institute of Technology Mandi for the award of the degree of **Doctor of Philosophy (Ph.D)** is a bona fide record of research work carried out by me under the supervision of **Dr. Chayan K. Nandi**. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

Syamantak Khan

Date:

Place: Mandi

Research Scholar
School of Basic Sciences
Indian Institute of Technology, Mandi
Himachal Pradesh -175001, India



Thesis Certificate

This is to certify that the thesis entitled “New Insights into Carbon Nanodots: Analysis of Ensemble and Single Molecule Fluorescence” submitted by **Mr. Syamantak Khan** to the Indian Institute of Technology, Mandi for the award of the degree of **Doctor of Philosophy (Ph.D.)** is a bonafide record of research work carried out by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

Date:

Place: Mandi

Dr. Chayan Kanti Nandi

Associate Professor
School of Basic Science
Indian Institute of Technology, Mandi
Himachal Pradesh -175001, India

Acknowledgements

I take this opportunity to thank all those who have helped me in the successful completion of work leading to this thesis and in making my days memorable during my Ph.D. First and foremost, I express my gratitude to my supervisor, Dr. Chayan Kanti Nandi for his constant and never tiring guidance which brought the best out of me in these years. Especially, I thank him for giving me the opportunity to work with exciting single molecule techniques. Learning and working with him has been one of the most enriching and fruitful experiences of my life which made me a more industrious individual than ever before. I also wish to thank him and his wife Ishita Nandi for making IIT Mandi my second home.

I express my sincere thanks to my institute IIT Mandi and its administration for all the facilities and necessary support for my research. I convey my gratitude to my doctoral committee members, Dr. Ajay Soni, Dr. Aditi Haldar, Dr. Amit Jaswal and Dr. Varun Dutt for their invaluable advice and encouragement. I thank the faculty members in the Department of Chemistry for teaching me various interesting courses and keeping me motivated in my research. I am also grateful to the staff members and operators of various instruments for their help, support and encouraging involvement in my research quests.

It is a great pleasure to acknowledge Dr. Alexey Chizhik and Prof. Joerg Enderline at the University of Gottingen, Germany, for being a wonderful host and making my stay memorable at Gottingen. I am grateful to Dr. Alexey Chizhik, Dr. Nareian Karedla, Mr. Sebastian Isbaner, Mr.

Simon Stein, Mr. Jan Thiart and Dr. Anna Chizhik for helping me accelerate my learning curves and hone my skills during my stay in Gottingen. I would also take this opportunity to thank Dr. Montu Hazra and Mr. Sourav Ghosal at SINP, Kolkata for their enthusiastic involvement in my research and consolidating my experimental data with their excellent computational studies.

I wish to thank my previous and present research group members Dr. Charu Dwivedi, Dr. Abhishek Chaudhary, Dr. Abhishek Gupta, Mr. Navneet Chandra Verma, Ms. Chethana Rao, Mr. Arpit Bharadwaj, Ms. Sanjhal Jain, Ms. Akshita Sharma for their support and encouragement during my research work. Research would have been less exciting without the enthusiastic involvement of those curious minds. Especially, I wish to convey my heartfelt gratitude to Navneet for being my true research partner and a friend in need as well. My sincere thanks to my friends who always enthusiastically shared their knowledge and helped me learn more. I express my gratitude to Mr. Gourab Dey and Mr. Pankaj Gour for taking the challenge to teach me organic chemistry, Mr. Rambabu for helping me with X-ray crystallography and Mr. Manoj Dhiman for trying hard to teach me Matlab.

It is said that good friends are not easy to find, but I have been lucky to have many. Thanks to Manoj Dhiman, Anmol Kothari, Dr. Lakshman Mahato, Gourab Dey, Himadri Chakraborty for making my early days at IIT Mandi truly memorable. I am grateful to have friends like Jalim Singh, Navneet Verma, Dr. Abhishek Gupta, Gourab Dey, Pankaj Gour, Dr. Sougata Sinha, Somnath Acharya, Arpit Baradwaj and Rituporno Gogoi, for their constant help and encouragement. I would like to especially thank Jalim Singh for being my perfect roommate and a true friend with his never-ending support. I also wish to thank my beloved friend Dr. Debarati Bandyopadhyay for her graceful presence in my life with her unique ability to find faults in me and my writings.

I am extremely grateful to the peer-reviewers of my accepted and rejected research papers. It would have been impossible for me to carry out my research successfully and write this thesis today without their critical assessment of my work and valuable suggestions to improve them. I am also grateful to the editors for accepting my research papers in their esteemed journals, giving me an opportunity to communicate my research findings. I want to convey my gratitude to the organizers of various international conferences, which I attended in the last few years, to provide me the opportunity to present my research with talks and posters. I also thank the organizers of the ‘RAMS 2016, Hyderabad’ for recognizing my research with a special award.

I would like to take this opportunity to express my gratitude to the great teachers and monks from my college - IIT Kharagpur and school - Ramakrishna Mission Vidyalaya Narendrapur, who made me what I am today. I wish to remember and thank my supervisor at IIT Kharagpur Prof. Sudip Ghosh, and his student Dr. Sintu Samanta for sowing the seeds of research in me with their encouragement and invaluable guidance. I want to thank some of my closest friends including Dr. Sankha Subhra Mukherjee, Soumya Poddar, Sauporno Ghosh, Arjo Ray and Dr. Apurba Pailan for their unfailing support till date in spite of staying in different parts of the world. My sincere thanks to Soumya Poddar for being a wonderful host during my stay in California to attend the International Conference: Advanced Functional Materials 2017, UCLA.

Last but not the least, I am, needless to mention, indebted to my parents, my beloved sister and my family without whose constant encouragement and boundless support this day of mine would have remained a dream.

Abbreviations

Å	Angstrom
AA	Ascorbic Acid
AFM	Atomic Force Microscopy
ATR	Attenuated Total Reflection
CCD	Charge Coupled Device
CNDs	Carbon Nanodots / Carbon dots
CNTs	Carbon Nano Tubes
DAPI	4',6-diamidino-2-phenylindole
DLS	Dynamic Light Scattering
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Di Amine Tetra Acetic Acid
EMCCD	Electron Coupled Charged Coupled Device
FBS	Fetal bovine Serum
FFT	Fast Fourier Transform
FTIR	Fourier Transform Infrared Spectroscopy
GNPs	Gold Nano Particles
GO	Graphene Oxide
GQDs	Graphene Quantum Dots
HRMS	High Resolution Mass Spectroscopy
kD	Kilo Dalton
kV	Kilo Volt
Lys	Lysine
mL	Millilitre
NIR	Near-Infrared
nM	Nano-Molar
nm	Nanometer
PBS	Phosphate Buffer Saline

PDA	Potato Dextrose Agar
PMT	Photomultiplier Tubes
PEG	Poly Ethylene Glycol
pM	Pico-Molar
PVA	Poly Vinyl Alcohol
QDs	Quantum Dots
QY	Quantum Yield
RESOLFT	Reversible Saturable Optical Linear Fluorescence Transitions
RhB	Rhodamine B
RNA	Ribose Nucleic Acid
rpm	Rotation Per Minute
SEM	Scanning Electron Microscopy
SOFI	Super-Resolution Optical Fluctuation Imaging
STED	STimulated Emission Depletion
STORM	Stochastic Optical Reconstruction Microscopy
TCSPC	Time Resolved Single Photon Counting
TEM	Transmission Electron Microscopy
TIRF	Total Internal Reflection
TPDCA	5-oxo-3,5-dihydro-2H-thiazolo [3,2-a] pyridine-3,7-dicarboxylic acid
TRANES	Time Resolved Area Normalized Emission Spectra
TRES	Time Resolved Emission Spectra
TRF	Time Resolved Fluorescence
μ M	Micro Molar
μ L	Microlitre
UV-Vis	Ultraviolet-Visible spectroscopy
XPS	X-Ray Photo Electron Spectroscopy
XRD	X-ray diffraction

Table of Contents

1. Introduction	1
1.1. Objectives and Overview	3
1.2. Quantum Dot and Quantum Confinement	4
1.3. Carbon Nanodots	5
1.4. Origin of Photoluminescence	7
1.4.1. Excitation Dependent Fluorescence of CNDs	7
1.4.2. Emissive Surface States of CNDs	9
1.4.3. Crystalline and Amorphous Core of CNDs	10
1.4.4. Molecular Fluorescence in CNDs	12
1.5. Single molecule Studies	13
1.6. Overview of the Chapters	15
1.7. References	18

2. Materials and Methods	21
2.1. Materials	23
2.2. Methods	24
2.2.1. Synthesis Procedures	24
2.2.1.1. Synthesis of CNDs from Chitosan	24
2.2.1.2. Synthesis of CNDs from Sodium Citrate and Sodium thiosulphate.	24
2.2.1.3. Synthesis of CNDs from PDA	25
2.2.1.4. Synthesis of CNDs from urea and p- phenylenediamine	25
2.2.1.5. Synthesis of Orange emissive CNDs from citric acid and urea	26
2.2.1.6. Synthesis of Methylenesuccinic acid and TPDCA	26
2.2.2. Characterization Techniques	27
2.2.2.1. Transmission Electron Microscopy (TEM)	27
2.2.2.2. Scanning Electron Microscopy (SEM)	28
2.2.2.3. X-ray Diffraction (XRD)	29
2.2.2.4. Atomic Force Microscopy (AFM)	30
2.2.2.5. X-ray Photoelectron Spectroscopy	31
2.2.2.6. High Resolution Mass Spectroscopy (HRMS)	32
2.2.2.7. Nuclear Magnetic Resonance Spectroscopy (NMR).	32
2.2.2.8. Raman Spectroscopy	33
2.2.2.9. Dynamic Light Scattering (DLS)	33
2.2.2.10. Zeta Potential	34
2.2.2.11. UV-VIS Spectroscopy	35
2.2.2.12. Fourier Transform Infrared Spectroscopy (FTIR)	34
2.2.2.13. Steady State Fluorescence Spectroscopy	35
2.2.2.14. Time Resolved Fluorescence Spectroscopy (TRFS).	36
2.2.2.15. Quantum Yield (QY) Calculation	37
2.2.2.16. Centrifugation	38
2.2.2.17. Dialysis	39
2.2.3. Methods and Measurements	40
2.2.3.1. Separation of Fluorescence Components from CNDs	40
2.2.3.2. Confocal Microscopy for Lifetime measurements.	40
2.2.3.3. Single Molecule Transients, Blinking, Photoswitching.	41
2.2.3.4. Single Molecule Imaging	42
2.2.3.5. Fluorescence Correlation Spectroscopy (FCS)	42
2.2.3.6. Cell Culture	43
2.2.3.7. Confocal Imaging of Mammalian Cells	43
2.3. References	44

3. Multi-emissive States in Carbon Nanodots	45
3.1. Abstract	47
3.2. Introduction	47
3.3. Excited State Dynamics	51
3.4. Role of Chemical Environment	57
3.5. Origin of Fluorescence	68
3.6. Conclusion	71
3.7. References	71
4. Reversible Photoswitching of Carbon Nanodots	73
4.1. Abstract	75
4.2. Introduction	75
4.3. Characterization of CNDs	76
4.4. Photoswitching Study	78
4.5. Mechanism of Photoswitching	80
4.6. Conclusion	87
4.7. References	87
5. Origin of Fluorescence Blinking in Single Carbon Nanodot	89
5.1. Abstract	91
5.2. Introduction	91
5.3. Characterization of CNDs	93
5.4. Single Particle Fluorescence in Room Temperature	96
5.5. Single Particle Fluorescence at Cryogenic Temperature	97
5.6. Distribution of OFF- and ON-state	100
5.7. Dual-focus FCS Measurements	101
5.8. Time Resolved Fluorescence of Single CND	103
5.9. Mechanism of Fluorescence Blinking	106
5.10. Conclusion	109
5.11. References	110

6. Single Molecule Imaging of Nucleolus with Carbon Nanodots	113
6.1. Abstract	115
6.2. Introduction	115
6.3. Characterization of CNDs	117
6.4. Confocal Microscopic Imaging	119
6.5. Single Molecule Imaging	122
6.6. Conclusion	126
6.7. References	126
7. Organic Nanocrystals Resemble the Properties of Carbon Nanodots	129
7.1. Abstract	131
7.2. Introduction	131
7.3. Hydrothermal Conversion of Citric Acid	132
7.4. Molecular Fluorophores and Organic Nanocrystals	136
7.5. CND-like Optical Properties	140
7.6. Conclusion	142
7.7. References	142
8. Future Directions	145
8.1. Unification of Multiple Hypotheses	147
8.2. Future Experimental Design	150
8.3. References	151
Appendix	153
Complete List of Publication	155
Permission for the reproduction of published articles	157