Structurally engineered biocompatible molecular probes for live cell-imaging and localization of native DNA

A Thesis

submitted

by

Pankaj Gaur (Roll No: D13006)

for the award of the degree of

Doctor of Philosophy

School of Basic Sciences
Indian Institute of Technology Mandi, India

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To the memories of my Godly Sister

Anju Bala

&

To my beloved Parents

Indian Institute of Technology Mandi
Mandi, Himachal Pradesh – 175001

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Declaration by the Research Scholar

This is to certify that the thesis entitled "**Structurally engineered biocompatible molecular probes for live cell-imaging and localization of native DNA**", submitted by me to the Indian Institute of Technology Mandi for the award of the degree of Doctor of Philosophy is a bonafide record of research work carried out by me under the supervision of Dr. Subrata Ghosh. 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.

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Thesis Certificate

This is to certify that the thesis entitled "**Structurally engineered biocompatible molecular probes for live cell-imaging and localization of native DNA**", submitted by Mr. Pankaj Gaur to the Indian Institute of Technology, Mandi for the award of the degree of Doctor of Philosophy 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.

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Mandi 175001
Date

Research Guide

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Table of Contents

Abbreviations

EM ……..……..……...electromagnetic

QY……..……..…… ..quantum yield

D……..……...……..donor

A……..……..……acceptor

UV……..……..……ultraviolet

Vis……...visible

IR……..infra-red

EL……..electroluminescence

m.p……melting point

CT……..……charge transfer

°C……..degrees Celsius

DCM……....dichloromethane

DMF…….....dimethylformamide

DMSO……...dimethylsulfoxide

ESI……..electron spray ionization

HOMO……highest occupied molecular orbital

HRMS……high resolution mass spectrometry

LUMO…….lowest unoccupied molecular orbital

μM…….……..micromolar

mM…… milimolar

NMR……..nuclear magnetic resonance

TD-DFT……..time-dependent density functional theory

ICT……intramolecular charge transfer

TICT……twist intramolecular charge transfer

CT……..charge transfer

RIM……...restricted intramolecular rotation

DAPI……4',6-diamidino-2-phenylindole

PI……propidium iodide

PBS……...phosphate buffer saline

FBS……..fetal bovine serum

A-T……adenine-thymine

dsDNA……double stranded deoxy ribonucleic acid

ssDNA……..single stranded deoxy ribonucleic acid

DNase…… ……deoxy ribonuclease

RNase…….. ……..ribonuclease

RNA ……ribonucleic acid

CD……..circular dichroism

MTT……3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

LOD……limit of detection

Ka……...association constant

MFI……mean fluorescence intensity

FACS……...fluorescence activated cell sorting

W……watt

MW……..microwave

LEDs……light emitting diodes

Abstract

Because of their vitality toward genetic inheritance, governing and functioning of cellular activities, nucleic acids have earned the prime focus out of other biomolecules. Therefore, deep insight into their structural dynamics and its influence on the cellular functioning could be of great importance in the physiological and pathological investigations. Hence, it became crucial to visualize their dynamics in the cellular milieu, and the same directed immense attention of the researcher for the development of useful techniques for their real time monitoring at cellular level. In this continuation, fluorescence microscopy using emissive molecular probes has grown as a non-invasive optical tool for *in-vitro*/*in-vivo* cellular imaging to understand the role of various biomolecules toward physiological changes and their influences at the cellular level. The promising molecular markers with enhanced optical properties, cytocompatibility, photobleaching resistance and target specificity constitute the backbone of fluorescence microscopy. Although, the dedicated efforts led to the appreciable development of the molecular probes and their successful employment for visualization of nucleic acids, the common shortcomings such as poor photostability, cell-impermeant nature and photoinduced toxicity restrict their real applicability in the biological fields. Moreover, the incorporation of aforementioned requisite parameters into a single molecular probe has been rare and a challenging task. In this concern, we became interested to crack these challenges and to bring the appreciable contributions toward the advanced biochemical research. The molecular probes with tailored donor-acceptor conjugated (D-π-A and D-π-A-π-D pull-push systems) molecular architectures have been devised and synthesized. Their optical behavior was studied both in solution as well as cellular milieu to explore their candidature as promising DNA markers.

The thesis entitled "**Structurally engineered biocompatible molecular probes for live cell-imaging and localization of native DNA**" begins with the development of indole based "turn-

on" green fluorescent molecular probes **PA1-PA5** with rationally designed D-π-A framework. Thorough study of their optical behavior in solution and cell-based assays successfully demonstrated that how a single atom modification (from carbon to selenium through oxygen and sulfur) in the acceptor unit can govern their biocompatibility and affinity toward DNA.

The thesis also emphasizes on the importance of rationally incorporated chemical-functionality and structurally devised crescent geometry toward the selective DNA binding of a molecular probe as compared to other biomolecules. In this context, probes **P1-P5** were developed and further their structure-interaction relationship investigation established the importance of rational chemical functionality towards DNA binding. Further, the development of **RD2** through the synthesis of a well-planned chemical library **RD1-RD8** and a careful structure-interaction relationship study led us to report that probe **RD2** having more curved geometry than others exhibited stronger binding affinity towards DNA. Additionally, **RD2** established its standing as a highly photostable DNA marker for chromosomal staining and micronuclei detection.

Molecular simulation studies employing density functional theory (DFT) contributed toward the understanding of the electronic delocalization in pull-push system and the worth of crescent geometry of the probe towards DNA specificity. Additionally, Autodoc 4.0 and circular dichroism (CD) helped to establish the binding mode and nature of interactions between DNA-probe ensembles.

At last, the study includes the development of a structurally engineered orange emissive probe **RF1** through the synthesis of a series of probes **RF1-RF10.** Thorough photophysical study clearly established that the improved optical properties (high quantum efficiency, brightness and especially long range excitation/emission) can be tuned by a rational design of highly conjugated pull-push system (D-π-A). Additionally, the steric influence of the substituents towards the DNA

binding affinity of the cationic moiety was also explored. Furthermore, a careful cellular investigation established the promising candidature of **RF1** as the first DNA marker used for the real-time monitoring of the reproductive and proliferative potential of the live cells beyond nine days without involving any tedious transfection.

Hence, the thesis presents an extended combinatorial approach which includes synthesis of rationally designed molecular probes as DNA markers, photophysical investigations in steady as well as excited state, understanding of intramolecular charge transfer (ICT) through DFT studies followed by time-resolved fluorescence measurements, establishment of binding mechanism employing molecular modeling supported by CD experiment and evaluation of the biological utility of the synthesized probes for live cell imaging and various biomedical applications.