ABSTRACT

G-quadruplexes are four-stranded unorthodox nucleic acid structures formed by stacked G-quartets with Hoogsteen base paired guanine bases. The human genome consists of over 370,000 putative G-quadruplex-forming sequences. Most of these are found to localize at genomic regions with important cellular functions, such as the telomere, immunoglobulin switch regions, proto-oncogene promoters, and mRNA untranslated regions. G-quadruplex (G4) formation has been established at telomeres and a significant number of oncogene promoters. The bio-physical and structural characterization of G-quadruplexes has been subject of extensive research thereby providing valuable insights into structural nuances. However, their physiological functions remain open to investigation and interpretation. Small molecules that are capable of selectively binding G4 structures have emerged as important components of quadruplex research. Further, quadruplex-selective fluorescent probes have gained prominence for their ability to visualize quadruplex structures *in cellulo* in real time. The aim of this thesis was to investigate a small molecule fluorescence probe for specific targeting of G4 structures and to examine the scope of use of such a chemical agent. We have developed a novel class of dimeric cyanine dyes in our laboratory. In this thesis, we study the interaction of a dimeric cyanine dye with G4.

The thesis contains four experimental parts. In the first experimental part, interaction of 3,3'-(butane-1,3-diyl)bis(2-methylbenzo[d]thiazol-3-ium) bromide (hereafter dye **1**) with various synthetic G-rich oligonucleotides has been studied. Notably, dye **1** displays nearly 1400-fold fluorescence enhancement upon interaction with specific type of G-rich sequences. This fluorescence enhancement is especially attractive alongside the nearly 30-fold fluorescence enhancement upon interaction with duplex and single stranded DNA molecules. The fluorescence enhancement of dye **1** is attributed to a de-aggregation

induced mechanism wherein fluorescence quenched H-aggregated form of the dye is transformed to a highly fluorescent monomeric form upon binding specific G4 conformations. Interaction of dye is specific to G4 structures and not to the G-rich sequences. Changes in loop lengths and overall length of sequence influence topology of G4 also affecting interaction with **1**. The de-aggregation induced mechanism is sensitive to conformational changes in G4 structures that can be effected by various experimental conditions. Dye **1** is able to convey differences in topology and stability of G4 through the extent of binding and concomitant fluorescence enhancement. Dye binding also stabilizes G4 structures based on increases in melting temperature.

In the second part, *in vitro* interaction of dye **1** is extended to physiologically relevant G-rich sequences that are known to exert G4-mediated activity. The behaviour of **1** against oncogene promoter sequences reinforces our findings from G-rich synthetic oligonucleotides, and also provide support to the *in cellulo* use of **1**. In particular, compared to K-Ras and c-Kit, substantial de-aggregation of dye is observed against c-Myc. The different behaviour of dye **1** in response to c-Myc as opposed to c-Kit or K-Ras is attributable to the G4 topologies formed in each case. Further, the dye is sensitive to the quadruplex-duplex equilibrium that can be effected by altering various conditions including the monovalent ion and choice of sequence. Dye **1** is potent for live cell imaging at low concentrations of usage and the application involves simple incubation. The purported de-aggregation induced fluorescence enhancement mechanism of **1** is used to visualize G4-containing regions of the cell. The staining profile of HeLa cells with the **1** is interesting as bright fluorescence is clearly observed at the nuclear periphery and certain regions of the nucleus. These regions are suggested as corresponding to mitochondrial guanine-rich DNA and nuclear r-DNA, respectively. Our studies support

the ability of dye **1** as being a potent G4-visualizing dye that is comparable to a small number of known G4-selective fluorescent probes.

In the third part, we probe the *in cellulo* concomitant effect of G-quadruplex stabilizing property of dye **1** along with two known anti-cancer drugs on cancer cells. The quadruplex-selective drug piperine and the quadruplex-stabilizing dye **1** apparently compete for the G-rich DNA in cell nucleus. Piperine is found to prevail in this competition as evident from low fluorescence of dye inside the nucleus. Further, binding of the quadruplex-targeting drugs results in changes in cell morphology and/or changes in nucleus thereby permitting staining by the dimeric dye. The effects of piperine and olaparib are different in this regard. The latter produces more dramatic changes in staining profiles of the dimeric dye on drug treated cells. This work presents proof-of-concept for the use of quadruplex-selective staining as indicative of changes in the cellular environment. The sharp contrast between pre- and post-treatment by drugs (that are relatable to quadruplexes directly or indirectly), is not captured as well by a nucleus staining dye DAPI.

In the final part, we deploy dye **1**-DNA complex as a reporter in two different contexts. The dye is used in conjunction with a G4 aptamer as a method of assaying the presence of toxin Bisphenol A. This approach relies on fluorescence quenching of the dye in presence of the aptamer-cognate target complex. Second, we use the dye **1**-DNA complex as a reporter that can be applied for immunosensing applications.

This thesis investigates and establishes scope of use of a G4-selective fluorescence probe thereby adding to a very small set of such known agents. It also poses questions in various contexts that form worthwhile future pursuits in our laboratory.