Investigating the Binding Mechanism of DRAQ5

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Background

Fluorescence is a fascinating phenomenon where light is absorbed and then emitted at a less energetic wavelength. Many animals exhibit naturally occurring fluorescence, with that number being greater than one might expect. These include, scorpions, platypuses, various species of birds like puffins, Gouldian finches, and even parrots, the hawkbill turtle, and the Virginia opossum. Within all these species the reason for fluorescence also varies depending on the species. Some possible reasons include hunting, protection, mating, or something to do with nocturnal behavior [15].

DRAQ5 is a synthetic fluorescent dye that binds to DNA in live or fixed cells and can often be used alongside other dyes. It is often used in flow cytometry, high content screening, and laser scanning microscopy in the fields of immunocytochemistry, immunofluorescence, and immunohistochemistry. Some of the purposes include measuring cell cycle distribution, measuring nuclear integrity, and assessing intracellular DNA content. It has also been used in both prokaryotic and eukaryotic cells. Both information about cell cycle and DNA sequencing can be obtained from the use of DRAQ5 in flow cytometry. More information about the uses is shown in **Figure 4.** The connection between the many purposes for natural fluorescence and the many applications of synthetic fluorophores like DRAQ5 cannot be ignored. DRAQ5 binds to double stranded DNA between the A-T minor grove. Because it binds to DNA intercalatedly, once the dye is attached, it eventually leads to the death of the cell. DRAQ5 has a $Ex_{\lambda max}$ at 647nm and an $Em_{\lambda max}$ at 681/697 nm as shown in **Figure 5**. It can also be excited at lower wavelengths including 488 nm. Two values are listed for the $Em_{\lambda max}$ to show that a red shift occurs when it binds to DNA. The fact that it fluoresces in deep red zone is useful because unlike other dyes which require near UV light to fluoresce. UV excitation of the fluorescent dye leads to an increase in cell death.

Research Question

Upon a brief inspection of the literature surrounding DRAQ5, there seemed to be some inconsistencies when talking about the binding mechanism in particular. DRAQ5 can be seen to be described as having both an intercalant binding mechanism, as well as binding between the A-T minor groove. These two different binding mechanisms are sometimes used interchangeably, while other times they are used to mean different things. This research would like to explore the binding mechanism of DRAQ5 in order to reconcile these inconsistencies within the general understanding of how DRAQ5 works.

Methods

In order to discover the impacts that the binding mechanism has, previous literature will be consulted and analyzed to determine any possible oversights areas in which additional conclusions are able to be drawn. Research about this topic involved specifically looking at the analysis of the binding mechanism as it is described in the existing literature as well as the circumstances that warranted those conclusions. The results of various pieces of work were fit together in a way that was able to form a conclusion based on what was consistently observed. While CAS SciFinderⁿ and ScienceDirect were used for an initial examination of the existing literature, most of the articles were found simply using Google or by looking at the references that were used in an article that was determined to be useful.

Results and Conclusion

Upon an initial search for DRAQ5, there were 200 results on SciFinder. Most of these proved to not be of particular use since they were more about the cell samples that DRAQ5 was used to analyze, and not about the properties of DRAQ5 itself. The type of cells used in the different articles were not considered in this research. Only a limited number of the found sources were consulted and the overall results should be considered from the perspective of this limited sample size. Certain ambiguities were expressed in articles about DRAQ5 about the binding mechanism. These offered no clear answer about what is actually happening when compared to each other.

Figure 1. Image from manufacturer showing product packaging [9].

Figure 2. Image of DRAQ5 product before being used. Product appears blue despite fluorescing red. [10]

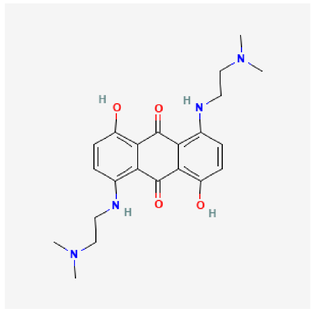

Figure 3. Chemical structure of DRAQ5 [7].

APPLICATION	PRODUCT	DRAQ5™
Cytometry	Flow Cytometry	✔
	Slide-/plate-based cytometry	✔
	Imaging Flow Cytometry	✔
	Cell Cycle Analysis	✔
Screening	Image-Based Screening	✔
	High-Throughput Flow Cytometry	✔
	In-Cell Westerns	✔
Imaging	Immuno-fluorescence microscopy	✔
	Live-Endpoint Cell/Tissue Imaging	✔
	Live Organism Imaging	
In Vitro Toxicology	Image-based	✔
	Flow Cytometry-Based	✔
In-Cell Westerns		✔
Cell Sorting		✔
Apoptosis	Flow Cytometry-based	✔
	Image-based	✔
Cell Health	Image-based	✔
	Flow Cytometry-based	
Hypoxia	Image-based	
	Flow Cytometry-Based	

Figure 4. Chart showing the uses for DRAQ5 [13].

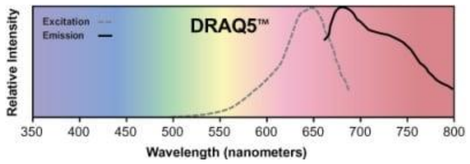

Figure 5. Excitation and emission spectra of DRAQ5 with visible light spectra in that background for reference. $Ex_{\lambda max}$ is at 646 nm and $Em_{\lambda max}$ is at 681 nm [8].

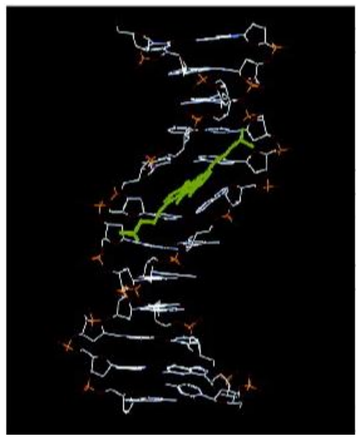

Figure 6. Simulated model of DRAQ5 binding to DNA [5]. Binding mechanism not specified.

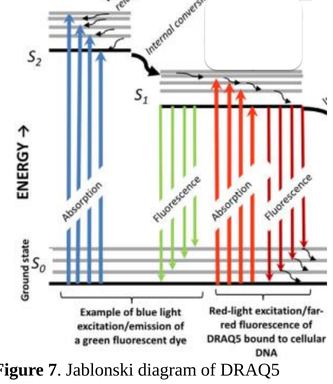

Figure 7. Jablonski diagram of DRAQ5 (modified) [12].

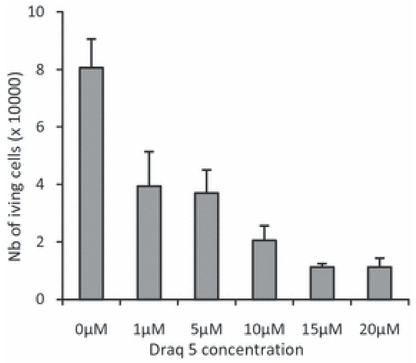

Figure 8. Shows the cytotoxic effect of different concentrations of DRAQ5 over 24 hours [4].

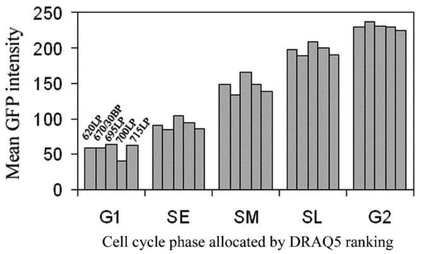

Figure 9. Using green fluorescent protein to show the reporting of each phase in the cell cycle at different emission filters. DRAQ5 was used at a concentration of 20 µM. [5]

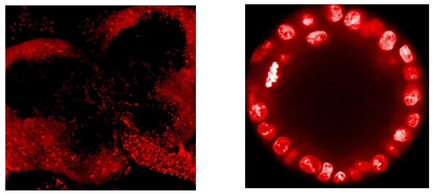

Figure 10 a,b. Examples of DRAQ5 stain [8] [11].

	Concentration of DRAQ5 used			
Binding Mechanism	<5 µM	>5 µM	Both	Doesn't say
A-T Minor Groove	••	••		
Intercalated	•	••••	•	
Both		•	•	•

Figure 12. Sample of distribution of stated binding mechanism from the various consulted literature [1],[2],[3],[4],[5],[6],[8],[16],[17],[18].

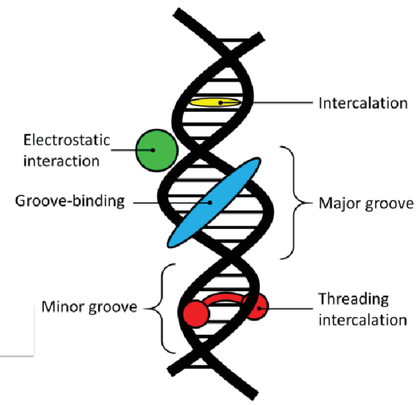

Figure 11. Examples of what different modes of binding could look like [14].

Results and Conclusions Cont.

In one source DRAQ5 was stated as intercalating when it was used at concentrations of 1 µM [3]. Another source described it as binding stoichiometrically which is indicative of AT binding at concentrations of 10 µM [6]. Both these sources only report one binding mechanism. There were, however, some that listed two binding mechanisms. Most briefly, it was told to have a high affinity for intercalation at 5 µM while also reported to bind into the minor groove [4]. More in depth, one more article described DRAQ5 as having the potential for multiple binding modes. That article said it had a preference for binding at the AATT sites as well as intercalating with a threading method which means there is a side chain in each groove [5]. This article used DRAQ5 at 20 µM. Results summarized in **Figure 12.**

One article had a more comprehensive potential explanation than the others. DRAQ5 was found to have a concentration dependent binding mechanism. At concentrations less than 5 µM it binds to the A-T minor groove. At concentrations greater than that it binds intercalated or in between the base plates [1]. The wording suggests the possibility of it not being a distinct switch in mechanism but rather in the presentation in which mode dominates. Despite there still being some inconsistencies in the previous results, this explanation was regarded as the strongest one currently found. The effects of this discovery have not been explored or elaborated in the years following. How does this affect the cell? What does this mean for the permanency of DRAQ5's binding?

DRAQ5 concentration has been reported to show greater cell death at higher concentrations [4], however it also shows the highest number of cells in the G2 phase. [5] That was taken however, at a concentration of 20 µM. At 1 µM DRAQ5 showed no drastic cytotoxic effects just slowed down proliferation [3].

Future Work

One proposed future research method for determining the effects would be to perform an experiment that partially replicates the experiment described by Martin, Leonhardt, and Cardoso [3]. The main difference between the proposed experiment and the already existing research is that this new experiment would only analyze DRAQ5 and would include different concentrations. The proposed concentrations for this experiment are 1, 4,5,6, 10, and 20 µM. The main experiment would be the one about the toxicity of DRAQ5 to the cell [3]. The source of the toxicity seems to be due to the unwinding of the DNA in order to bind between the base plates which interrupts the DNA replication process, What effect does concentration have on the toxicity? Is there still toxicity at lower concentrations that do not bind between the base plates?

One of the experiments to be replicated is the one from Njoh et al. that uses green fluorescent protein in combination with DRAQ5 to visualize the effects of DRAQ5 on the cell cycle [5], specifically the experiment that produced **Figure 9.** This also connects to the goal of the previous experiment to be reproduced in the future. It would be interesting to compare the results from this experiment with the results from Richard et al that are shown in **Figure 8.** [4]. when both experiments are done on the same concentrations. The goal would be to determine what would happen if **Figure 8** and **Figure 9** were combined. It appears like there could be a conclusion to be drawn from repeating the experiment to show the number of cells in each phase in the cell cycle at the different concentrations previously listed.

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