ISSN 2959-409X

Developing a Photoacoustic Probe for in vivo Tumor pH Detection by Gaussian calculation

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Abstract:

pH dysregulation is a major feature of cancer, and the development of advanced photoacoustic probes capable of monitoring pH is important for both diagnosis and treatment of cancer. Previous work (done by Tijana Jokic's group) found that aza-BODIPY molecule has better photoacoustic properties and this was investigated in depth, leading to the development of new pH probes, but the functionality of the molecule can be upgraded again if the photoacoustic properties of the molecule can be further expanded, while the maximum wavelength of the molecule can be further extended to fit the commercial range. This study optimized the previous molecule based on the Gaussian program, and the new molecule with NO_2 attached to R4 showed the best properties in which the maximum excitation wavelength reached 770.85 nm.

Keywords: photoacoustic probe; aza-BODIPY; pH Detection; Gaussian calculation

1. Introduction

Cancer outbreaks in China have become increasingly severe in recent decades. According to the National Bureau of Statistics, there will be 19.29 million new cancer cases worldwide in 2020, of which 4.57 million will occur in China, accounting for 23.7% of new cancer cases worldwide. Therefore, it is urgent to control cancer.

In medicine, cancer is a malignant tumor that originates from epithelial tissue and is the most common type of malignancy. Although there are many different forms of cancer, these cancer cells share some adaptive characteristics. One hallmark of cancer is pH dysregulation, as the pH gradient in cancer shows an "inverse" pattern, meaning that the pH inside the cell is higher than the pH outside the cell. Under healthy physiological conditions in the human body, the pH of blood and tissues is approximately 7.4. In the case of infected or diseased tissues such as tumors, the environment is usually acidic, with a local pH range of 6.5-6.8. This gradient allows cancer to develop by promoting proliferation, evading apoptosis, and adapting to metabolism, migration, and invasion.

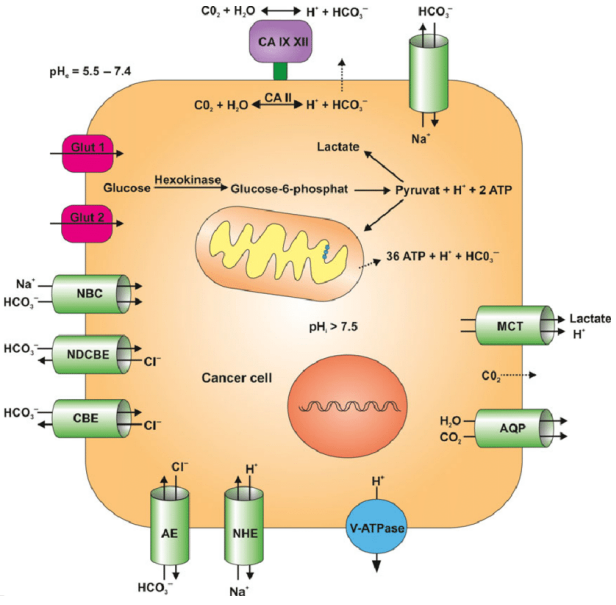

Figure1: Diagram of the pH regulating mechanisms in the tumor microenvironment

Cancer can be diagnosed and treated more effectively by monitoring the pH of the blood. Traditional methods include glass pH electrodes, fiber optic pH microsensors, and magnetic resonance imaging (MRI), but each method has certain limitations at the same time. Therefore, it is essential to optimize pH probes. A photosensitive imaging probe with non-invasive, radiation-free imaging is a good solution.

Previous studies of aza-BODIPYs molecules by Tijana Jo-

kic's group have shown good photostability and great versatility in terms of PET receptor location, as well as better fluorescence and photoacoustic properties. Ultimately, if some of the photoacoustic properties of the molecule can be enhanced, such as expanding the excitation wavelength of the molecule and broadening the shift between wavelengths, the increase in wavelength will bring the spectrum closer to the infrared region, thus increasing the penetration of the molecule and allowing for enhanced performance in the application of the molecule as a pH probe; the reduction in wavelength will at the same time avoid the interference of the organic conjugated small molecules to the organism itself and minimize the energy required for excitation to avoid damaging biological cells, new molecules with better fluorescence properties can be developed. Also, the expanded ones could be better adapted to the commercially often equipped monitorable range (680-900 nm). Therefore, in this study, it is hoped that the maximum excitation wavelength can be expanded to obtain better properties of such molecules.

For this reason, the excitation wavelengths of the newly optimized molecules will be focused on in this study. Through ground state calculations, optimization and excited state calculations by Gaussian program, the goal will be reached theoretically.

2. Methods

Gaussian program (version 6.0) was used to calculate and optimize the ground state and the excited state.

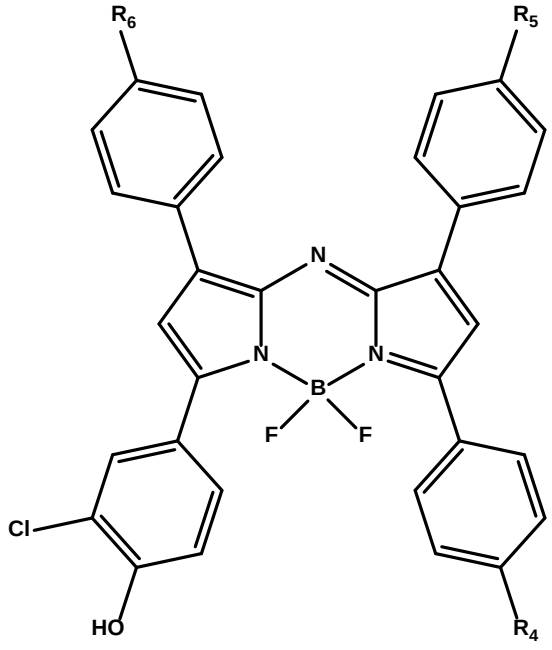

Figure 2: Diagram of the original molecule and the three selected ports of the linking group

The original molecule was aza-BODIPY and three ports of the linking group, R4, R5, R6, was selected (Figure 2).

In Gaussian calculation, the m062x method and def2svp in the DFT algorithm were chosen; in the calculation of the excited state, the same method and basis set chosen at the beginning with the ground state calculated the wrong answer, and then the cam-b3lyp method and 6-311g(d,p) basis set were chosen to calculate the original molecule and the modified new molecule.

3. Results

The molecule pH needs to be calculated in both acid and base environments, and the hydroxyl group on phenol would ionize under alkaline conditions, so the ionization was drawn on the original molecule to indicate the condition in the alkaline environment.

In the calculation of the ground state, the m062x method was finally chosen to try the calculation. Also, Def2-SVP was chosen to try because it has a polarization function on all atoms.

According to the original literature, EtOH/ H_2O was mixed 1:1 in the calculation, so both ethanol and water were used when choosing the solvent.

The excitation wavelengths listed in the original literature for the acid and alkaline cases are 668 nm in acid and 734 nm in alkaline, respectively.

Under the cam-b3lyp method 6-311g(d,p) basis set, the molecule in an alkaline environment, compared to 734 given in the original literature, was calculated for the alkaline case in pure ethanol solution, giving a result of 722.50; however, the results in an acidic environment were not as good as the alkaline, compared to 668 given in the original literature, which In the case of pure water, the excitation wavelengths of both increased to 615.88 nm for the acidic and 726.04 nm for the basic.

To achieve the goal of increasing the excitation wavelength, the book was chosen to consult. In *Modern Physical Chemistry*, we can find that conjugation can be modified by modifying the electron-donating group and electron-absorbing group to the molecule. By looking at the calculated HOMO-LUMO image, I found that it was concentrated on the R4 and R5 parts. Therefore, I considered modifying different groups to the R4, R5 part.

A total of five attempted modification groups were passed, Cl, CN, and NO_2 attached to R4, and NO_2 and Cl-OH attached to R5.

Table 1: Excitation wavelength calculated by cam-b3lyp method 6-311g(d,p) basis set

Modification position and manner	Excitation wavelength*
R4-Cl	618.22 nm
R4-CN	767.36 nm
R4-NO_2	770.85 nm
R5-Cl-OH	750.64 nm
R5-NO_2	536.98 nm

*the solution was chosen as water

Among these five modifications, the new molecule with NO_2 attached on R4 showed the best properties, reaching 770.85 nm, which met the goal of my study.

By the relevant literature, the optimized molecule by Gaussian specific method and modified the molecule to obtain a molecule with an excitation wavelength of 770.85 nm, which achieved my goal: to extend the excitation wavelength as much as possible. Finally, the development of the photoacoustic probe for in vivo tumor pH detection was temporarily completed.

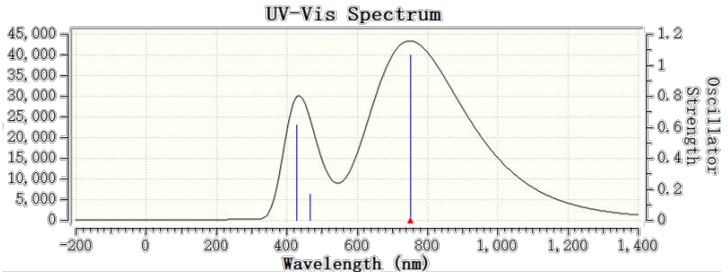

Figure 3: Spectral images of the best molecules

4. Discussion

From the previous studies, I learned that aza-BODIPY molecules are optimized from BODIPY molecules and have more advantages in cell imaging and in vivo use; aza-BODIPY molecules also have many benefits for pH monitoring: for example, they have near-infrared absorption/emission, high reactive oxygen species generation, and photothermal conversion efficiency.

The first step in calculating the molecular properties is to calculate the nature of the ground state of the molecule. Since the DFT (density functional theory) method is the most widely used calculation method and is very accurate, we chose to calculate the ground state of the original aza-BODIPY and the final molecule in the literature using the DFT method.

Since the process of molecular excitation requires a certain amount of time, the TDDFT method was chosen.

At the beginning of the calculation, I applied the method of the base state calculation to this part of the calculation. First, I tried to use m062x and def2svp to calculate the energy and found that both were incorrect. With the help of my mentor, I changed the method and the basis set.

For the second time, the method with cam-b3lyp 6-311g(d,p) was chosen for the calculation with the basis group.

Regarding the different calculations obtained in the acid-base case and in the two solvents, the excitation wavelength calculations obtained in the acidic condition are not as accurate as in the alkaline one. One of the presumed reasons is that the molecules carry protons, and in the acidic case, the molecules with protons are more affected, while in the alkaline case, the effect is not as pronounced. Another reason speculated is that the original solution from which the results were calculated was a one-to-one mixture of ethanol and water, and after calculating the molecules in both pure ethanol and pure water solutions, the results in the literature were not reduced.

However, since no other more suitable method was found and the deviation of the calculation results did not entirely affect the optimization process, i.e., the results calculated in water or ethanol alone were found to be smaller than those in the literature, I will continue to use the basis set of this method, making the optimized theoretical excitation wavelength of 700 nm or more.

In addition, some feasible ideas for future research are presented.

First, the excitation wavelength of this molecule for the alkaline case can be calculated to test whether the maximum value of 900 nm in the commercially available monitoring range mentioned earlier is exceeded, and if so, try whether the two remaining molecules with calculated excitation

wavelengths above 700 nm will form a better-quality option.

Secondly, it is possible to test whether the same group is attached to R4, R5 and R6 as presumed by observing the molecular orbitals: the most effective group is attached to R4.

In addition, there are other requirements for a good photoacoustic probe, such as low quantum yield. In subsequent calculations, it can be calculated to know whether the molecule meets the requirements for a good photoacoustic probe with a low quantum yield. If the quantum yield is too high, it does not provide enough energy, so quantum yield is also an important part of the subsequent calculations.

In future studies, it might be possible to combine probes that monitor different phenomena among them: in the tumor microenvironment, the effects are not only acidosis (low pH), but also hypoxia (low oxygen), nutritional deprivation, vascular abnormalities, etc. A combination of both or more might allow a more accurate test of the tumor.

Last but not least, it is also necessary to test whether the molecule will have the high excitation wavelengths as calculated in theory when actually synthesized, and whether the theory can be verified in real-life synthesized molecules will be a major direction for subsequent in-depth research.

ACKNOWLEDGMENT

Thanks to the organizers of the Yuanpei Junior Scholars event for providing such a valuable research opportunity!

Thanks to Ms. Yang Juan, the subject master of Computational Chemistry and Materials Science, for taking us into the world of computational chemistry and materials science.

Thanks to Mr. Fang Runting, the group mentor of the first group, for patiently teaching us the basics and calculations and lead us through the study.

Thanks to the Jokic T research group, developing this excellent molecule for the basis of my research.

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