Effects of Label-free Fluorescence Aptasensors with Different Aptamer Length on Quenching of Carbon Dots

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Abstract. Label-free fluorescence aptasensors have the advantages of better detection sensitivity, simple manufacturing process and no pollution. In this experiment, the effect of aptamer base number on the performance fluorescence aptasensors has been studied. Thrombin of 15 base number, ATP of 27 base number and DA of 58 base number were selected, and then constructed three kinds of fluorescent probes about Au/Aptamer/CQDs nanostructures with gold nanoparticles and carbon dots. By studying the quenching degree of carbon dots with each probe, the quenching degree of carbon dots fluorescence by ATP probe was the highest, thrombin probe was the worst, and the DA probe was between ATP probe and DA probe. This indicates that with the increase of the aptamer base number in the probe, the more carbon dots are adsorbed on the aptamer and the more fluorescence quenching occurs, but the aptamer will be winding when it has too many bases, leading to the quenching unstable of carbon dots fluorescence. This study provides a useful reference for the construction and detection of fluorescent aptasensors, and has a great potential in the field of small molecule detection.

1. Introduction
As one of the biomarkers for diagnosis of many diseases, small molecular have many biomedical functions, such as storing or transferring information, regulating biological activities, and regulating catalytic reaction[1-3]. Fluorescent aptasensors has a widely used in detecting small molecular because it has better detection sensitivity and accuracy, and the process is relatively simple[4]. Carbon dots have become widely used in fluorescence aptasensors due to their green, no pollution and easy to prepare[5]. The label-free
fluorescence aptasensors have the advantages of fluorescent aptasensors and carbon dots, and it is more simple than the general label fluorescence aptasensors[6].

So the label-free fluorescence aptasensors are gradually catching on. For instance, Saberi et al.[7] structured an label-free fluorescence aptasensors with the cationic carbon dots was prepared by cetrimonium bromide, and then detected the phenylacetamide. Wang et al.[8] constructed AuNPs/Aptamer/N,C-CQDs nanostructure with the positive N,C-dots was prepared by trypsin hydrothermal method, and then for sensitive detection of AFB1. Liang et al.[9]constructed a fluorescence aptasensors with the nitrogen-doped carbon dots was prepared by microwave method, and detected bisphenol A. In fact, there was only one aptamer in the above experiment, some factors were optimized, such as aptamer concentration and salt concentration. However, the sensitivity and specificity of the sensor are also different due to the different aptamer and target, under the same method, so changes in aptamers will cause great changes in sensor performance[10, 11]. At present, no one has made comparison for different aptamers with the same method. Therefore, this experiment studied the influence of different length aptamer on the quenching and recovery of carbon dots fluorescence.

Thrombin of 15 base number[12], ATP of 27 base number [13] and DA of 58 base number [14] were selected as experiment object, through combining with the gold nanoparticles and the carbon dots, the Au/Aptamer/CQDs nanostructure was constructed to explore the effect that the aptamer base number on the quenching of carbon dots.

2. Materials and Methods
2.1. Reagents and materials
Tris (2-carboxyethyl) phosphine (TCEP), thrombin aptamer, adenosine triphosphate aptamer and dopamine aptamer were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), Phosphate buffer solution (PBS, 10mM, pH 7.4). All chemical reagents were of analytical grade and the ultrapure water used throughout the study was prepared by PURELAB Option-R (ELGA Lab Water, UK).

Thrombin aptamer: “5-SH-GGT TGG GGT TGG-3”
ATP aptamer: “5-SH-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3”
DA aptamer: “5-SH -GTC TCT GTG TGC GCC AGA GAA CAC TGG GCC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3”

2.2. The instrument
Fluorescence measurements were performed on a F-7000 fluorescence spectrophotometer (Hitachi, Japan). The ultrasonication during the preparation of CQDs was performed on a SYU-22-500DTD ultrasonic cleaner (Zhengzhou Shengyuan Instrument Co., Ltd., China). All pH values were acquired use FE-20K pH-meter (METTLER TOLEDO, Switzerland). A HZQ -F200 constant temperature shaker (Beijing Donglian haer Instrument Co., Beijing, China) was used for promote the hybridization. Eppendorf centrifuge 5418(Hamburg, Germany) was used to centrifugation.

2.3. Preparation of carbon dots
The carbon dots (CQDs) were synthesized via ultrasonic method according to our previous report[15]. In brief, 10mL glucose aqueous solution (1mol/L) was mixed with 10mL NaOH solution (1.5mol/L) followed by sonicate at 400-watt power for 4 hours to get the raw solution of CQDs. After pH value of the mixture solution was adjusted to 7 with HCl, 100mL
absolute ethyl alcohol was added dropwise with stirring. Then, the solution of CQDs was treated by adding a moderate amount of magnesium sulfate (12 wt%) and stirring for 20 min to remove excess salt.

2.4. Preparation of gold nanoparticles.
Gold nanoparticles (AuNPs) were prepared by reducing chloroauric acid with sodium citrate[16]. Weighing the 250 mL 0.1 mmol·L⁻¹ HAuCl₄ solution in a clean beaker, stirring vigorously until it came to a boil, then added 5 mL 38.8 mmol·L⁻¹ sodium citrate quickly, the color of solution changed from pale yellow to wine red, the reaction continued for 30 min before cooling to room temperature.

2.5. Prepare Au/aptamer/ CQDs probes
ATP aptamer (5 μM) was labelled with S-H bond, and then activated by TCEP (pH=5.4), appropriate amount of Au was added to the activated aptamer, then shook and added trace amounts of citric acid-hydrochloric acid to promote the combination of aptamer and Au. Culturing in the shaking box for 3 hours, centrifuging for 25min(15000rpm), the precipitation was washed with PBS (10 mm pH=7.0) for 3 times. Finally, adding carbon dots, the obtained Au/Aptamer/ c-dot was re-dispersed in PBS (0.1) and uniformly volumetric, stored at 4 °C, reacted 30 min.

3. Results and Discussion
3.1. Method principle
AuNPs is an ideal fluorescence quencher due to it has high molar extinction and wide energy bandwidth[17], AuNPs was synthesized by sodium citrate reduction in this experiment. Carbon dots is an ideal fluorescent group, because it has excellent luminescence and good biocompatibility. The label-free fluorescence aptasensors was composed of AuNPs, CQDs and aptamer, the principle of detection was shown in Figure 1. Firstly, the S-H bond of aptamer was activated in solution, and then bound AuNPs by Au-S bond, getting the Au/Aptamer structure. Secondly, the carbon dots with positive was added to the solution, since the aptamer carried negative charge, the carbon dots can combine with the aptamer through electrostatic adsorption, and assembled into Au/Aptamer/ CQDs-nanostructure. Meanwhile, the fluorescence of the carbon dots was quenched. Finally, the carbon dots fluorescence recovered when the target was added to the solution.

![Figure 1. Schematic of the Carbon dots quenching based on AuNPs](image)

3.2. Method principle
The fluorescence of carbon dots is quenched due to the fluorescence resonance energy transfer (FRET)[18] or internal filtering effect (IFE)[19] occurs between the carbon dots and the gold nano-particles. In order to exploring the relationship between the base number of aptamer and quenching degree of carbon dots, the probe of different length aptamer on fluorescence quenching degree at the same carbon dots concentration were studied.
As shown in Figure 2(a), the fluorescence intensity of pure carbon dots group is the highest at the same carbon dots concentration. In contrast, the probe group of ATP aptamer with 27 base length had the worst fluorescence intensity. The fluorescence intensity of thrombin aptamer probe group of 15 base length was higher than ATP aptamer probe group, and the fluorescence intensity of DA aptamer probe group of 58 base length was between thrombin probe group and ATP probe group.

As shown in Figure 2(b), at the beginning, the fluorescence intensity of aptamer probe decreases as the aptamer base length increases. Referring to Figure 3(a) (b) it is probably that the aptamer of short length was already full of carbon dots, all the carbon dots on the adsorption were quenched, with the increase of aptamer length, the more adsorbed carbon dots, the more quenching degree. However, when the length of the aptamer increased to some degree, the fluorescence intensity of aptamer probe began to increase, referring to Figure 3(c), it may be that the long aptamer bended or folded each other, so the quenching state of DA aptamer probe group was unstable. but the carbon dots on the long aptamer were sparse, and there was not too much quenching phenomenon between the carbon dots[20]. The distant carbon dots adsorbed on the aptamer may not be quenched. In order to explore this reason, the following experiments were conducted with increase the concentration of carbon dots.

**Figure 2.** At the same carbon dots concentration, (a) fluorescence spectra of CQDs, AuNPs/thrombin-aptamer/CQDs, AuNPs/ATP-aptamer/CQDs, and AuNPs/DA-aptamer/CQDs. (b) The peak fluorescence intensity (500nm) of the three probes in Figure 2(a) varied with the length of aptamer.

**Figure 3.** Schematic of (a) thrombin probe. (b) ATP probe. (c) DA probe.
As shown in Figure 4(a), the fluorescence intensity of pure carbon dots increases with the increase of the concentration of carbon dots, and the fluorescence intensity of carbon dots was not fully saturated at this time. As shown in Figure 4(b) and 5(a), as the concentration of carbon dots increases, the fluorescence intensity of both thrombin aptamer probe and ATP aptamer probe was gradually on the rise, it proof that the aptamer of thrombin and ATP are already in the state of saturated quenching, therefore, the fluorescence intensity of thrombin aptamer probe and ATP aptamer probe increased with the increase of carbon dots concentration. As shown in Figure 5(b), the fluorescence intensity of DA probe group was very unstable with the increase of carbon dots concentration. It indicates that the length of DA aptamer is too long, it is easy to bend and fold each other[21], so the quenching state of DA aptamer probe group was unstable.

![Figure 4](image_url)

**Figure 4.** Fluorescence spectra of (a) carbon dots in different concentrations and (b) AuNPs/thrombin-aptamer/CQDs in different carbon dots concentrations

![Figure 5](image_url)

**Figure 5.** Fluorescence spectra of (a) AuNPs/ATP-aptamer/CQDs in different carbon dots concentrations and (b) AuNPs/DA-aptamer/CQDs in different carbon dots concentrations

As shown in Figure 6, the degree of quenching of thrombin, ATP and DA are different for the carbon dots at a certain range of carbon dots concentration. The statement in Figure 2(a) is further verified, the change in aptamer length caused the polytropy of probe, leading to the same effect when the carbon dots concentration changes.
Figure 6. Peak fluorescence intensity (500nm) of CQDs, AuNPs/thrombin-aptamer/CQDs, AuNPs/ATP-aptamer/CQDs, and AuNPs/DA-aptamer/CQDs in different carbon dots concentrations

4. Conclusion
Thrombin fluorescence probes, ATP fluorescence probes and DA fluorescence probes were constructed based on FRET and IFE theory, and then studied the quenching degree of carbon dots. At the same carbon dots concentration, it was found that the fluorescence probe with shorter aptamer length had a more stable quenching ability for carbon dots, the longer of aptamer, the more unstable of carbon dots quenching degree. At different carbon dots concentration, the phenomenon is the same. This indicates that the probe with aptamer of long length has a tendency to winding and have other uncertainty, and the nanostructure is unstable. This conclusion provides an important reference for the construction of fluorescent aptasensors with higher detection accuracy and wider detection range.

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References

[1] A. Drabik, J. Ner-Kluza, K. Piechura, Changes in Protein Glycosylation as a Result of Aptamer Interactions with Cancer Cells, J. SCI. Proteomics. Clinical applications, 14 (2019) e1800186. online

[2] V. Calzada, M. Moreno, J. Newton, Development of new PTK7-targeting aptamer-fluorescent and -radiolabelled probes for evaluation as molecular imaging agents: Lymphoma and melanoma in vivo proof of concept, J. SCI. Bioorg Chem, 25 (2017) 1163-1171.

[3] S. Auslander, D. Fuchs, S. Hurlemann, Engineering a ribozyme cleavage-induced split fluorescent aptamer complementation assay, J. SCI. Nucleic Acids Res, 44 (2016) e94. online

[4] R. Weng, S.T. Lou, L.D. Li, Single-Molecule Kinetic Fingerprinting for the Ultrasensitive Detection of Small Molecules with Aptasensors, J. SCI. Anal Chem, 91 (2019) 1424-1431.

[5] S. Mallakpour, V. Behranvand, F. Mallakpour, Synthesis of alginate/carbon nanotube/carbon dot/fluoroapatite/TiO2 beads for dye photocatalytic degradation under ultraviolet light, J. SCI. Carbohyd Polym, 224 (2019) 115138.

[6] S. Srinivasan, V. Ranganathan, M.C. DeRosa, Label-free aptasensors based on fluorescent screening assays for the detection of Salmonella typhimurium, J. SCI. Anal Biochem, 559 (2018) 17-23.

[7] Z. Saberi, B. Rezaei, A.A. Ensafi, Fluorometric label-free aptasensor for detection of the pesticide acetamiprid by using cationic carbon dots prepared with cetrimonium bromide, J. SCI. Microchim. Acta, 186 (2019) 273.

[8] B. Wang, Y.F. Chen, Y.Y. Wu, Aptamer induced assembly of fluorescent nitrogen-doped carbon dots on gold nanoparticles for sensitive detection of AFB(1), J. SCI. Biosens. Bioelectron, 78 (2016) 23-30.

[9] L. Wang, H.X. Cao, C.G. Pan, A fluorometric aptasensor for bisphenol a based on the inner filter effect of gold nanoparticles on the fluorescence of nitrogen-doped carbon dots, J. SCI. Microchim. Acta, 186 (2019) 28.

[10] M. Kimoto, R. Yamashige, K. Matsunaga, Generation of high-affinity DNA aptamers using an expanded genetic alphabet, J. SCI. Nat Biotechnol, 31 (2013) 453-457.

[11] L.Q. Zhang, Z.Y. Yang, K. Sefah, Evolution of Functional Six-Nucleotide DNA, J. SCI. Journal Of the American Chemical Society, 137 (2015) 6734-6737.

[12] P.S. Prabhakar, R.A. Manderville, S.D. Wetmore, Impact of the Position of the Chemically Modified 5-Furyl-2 'Deoxyuridine Nucleoside on the Thrombin DNA Aptamer-Protein Complex: Structural Insights into Aptamer Response from MD Simulations, J. SCI. Molecules, 24 (2019) 2908.

[13] Y. Ma, F.H. Geng, Y.X. Wang, Novel strategy to improve the sensing performances of split ATP aptamer based fluorescent indicator displacement assay through enhanced molecular recognition, J. SCI. Biosens. Bioelectron, 134 (2019) 36-41.

[14] J. Chen, Y.C. Li, Y.N. Huang, Fluorometric dopamine assay based on an energy transfer system composed of aptamer-functionalized MoS2 quantum dots and MoS2 nanosheets, J. SCI. Microchim. Acta, 186 (2019) 58.

[15] L.J. Ren, P. Zhang, R.B. Qi, Influencing Factors of Luminescence Properties of Carbon Dots Prepared by Ultrasonic, J. SCI. Spectrosc. Spectr. Anal., 37 (2017) 3354-3359.

[16] X.H. Ji, X.N. Song, J. Li, Size control of gold nanocrystals in citrate reduction: The third role of citrate, J. SCI. Journal Of the American Chemical Society, 129 (2007) 13939-13948.

[17] L. Qin, G.M. Zeng, C. Lai, "Gold rush" in modern science: Fabrication strategies and typical advanced applications of gold nanoparticles in sensing, J. SCI. Coordin Chem Rev, 359 (2018) 1-31.

[18] X.L. Wu, Y. Song, X. Yan, Carbon quantum dots as fluorescence resonance energy transfer sensors for organophosphate pesticides determination, J. SCI. Biosens. Bioelectron., 94 (2017) 292-297.
[19] J.L. Wang, Y.G. Wu, P. Zhou, A novel fluorescent aptasensor for ultrasensitive and selective detection of acetamiprid pesticide based on the inner filter effect between gold nanoparticles and carbon dots, *J. SCI. Analyst*, 143 (2018) 5151-5160.

[20] S. Ghayyem, F. Faridbod, A fluorescent aptamer/carbon dots based assay for Cytochrome c protein detection as a biomarker of cell apoptosis, *J. SCI. Methods Appl. Fluoresc.*, 7 (2019) 8.

[21] H.B. Albada, E. Golub, I. Willner, Rational design of supramolecular hemin/G-quadruplex-dopamine aptamer nucleoapzyme systems with superior catalytic performance, *J. SCI. Chem. Sci.*, 7 (2016) 3092-3101.