

Proceeding paper

Amino acid-functionalized polyfluorenes: a class of ultra-sensitive fluorescent sensors favoring $\text{Cr}_2\text{O}_7^{2-}$ †

Wanyi Zhang ¹, Hui Li ¹, Yu Li ², Lei Cao ², Li Sheng ², Ge Zhang ^{2,*} and Shuai Chen ^{1,3,*}

¹ Flexible Electronics Innovation Institute and School of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang, Jiangxi 330013, China;

² School of Chemistry and Chemical Engineering, Jiangxi Science & Technology Normal University, Nanchang 330013, China;

³ Jiangxi Provincial Engineering Research Center for Waterborne Coatings, Nanchang 330013, China;

* Correspondence: author. Tel.: +86-791-83832345 (G. Z.) mail address: zhangge20082006@126.com (G. Z.), shuaichen@jxstnu.edu.cn (S. C.).

Abstract: Conjugated polymers (CPs) are an intriguing material to build fluorescent $\text{Cr}_2\text{O}_7^{2-}$ sensors with excellent sensitivity but often lack specific recognition groups. In this study, several typical amino acids with N and O atom identifying groups were incorporated into fluorene and then six polyfluorene derivatives were synthesized using electrochemical polymerization. Compared to other cations and anions, all of these amino acid-functionalized polyfluorenes have good selectivity towards $\text{Cr}_2\text{O}_7^{2-}$ and enable ultra-trace response with detection thresholds at pM or even fM level.

Keywords: $\text{Cr}_2\text{O}_7^{2-}$; conjugated polymer; polyfluorene; amino acid; fluorescent sensor

1. Introduction

The development of modern industry has caused a large amount of heavy metals to be released into a transitional accumulation of heavy metals in the soil, expanding the area of contamination, thereby affecting the growth of crops and lowering the quality of products. Cadmium ion is more frequently acknowledged as an internationally recognized carcinogen than other heavy metal ions [1]. For example, once its content exceeds the standard in water (existing in the form of $\text{Cr}_2\text{O}_7^{2-}$), it will be enriched in the crops and passed to the human body through the food chain, which will cause various diseases [2,3]. Therefore, it is necessary to detect $\text{Cr}_2\text{O}_7^{2-}$ quickly, easily and accurately.

Among the various methods for the detection of $\text{Cr}_2\text{O}_7^{2-}$, fluorescence analysis has attracted much more attention due to its high sensitivity, simplicity, and rich output signal [4]. Conjugated polymers (CPs) have high molar absorption coefficients and strong light absorption abilities, which are good light energy harvesters [5]. Moreover, these molecules have a unique "molecular line effect" that can amplify the fluorescence response signal hundreds or thousands of times, which makes them stand out in the fields of biomonitoring and environmental analysis [6,7]. Unfortunately, there have been few reports on the specific identification of $\text{Cr}_2\text{O}_7^{2-}$ relying on CPs based fluorescent materials.

Fluorene is a classical blue luminescent polymer material that not only has high fluorescence quantum yield and good optical stability, but also can further improve its photoelectric performance by introducing amino acid groups containing N and O atoms in the side chain [8,9]. Amino acids containing N and O atoms are characterized by high ion binding ability, good selectivity, and a strong pH response, which give them certain advantages in the development of efficient and sensitive ion sensors [10,11]. Therefore, our group proposed a design strategy, selecting the fluorene as the fluorescence group and introducing amino acid moieties at its C9 position as the recognition groups. Then,

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Last-name

Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

six polyfluorene fluorescent derivatives have been successfully fabricated by electrochemical polymerization to construct fluorescence sensors for $\text{Cr}_2\text{O}_7^{2-}$ detection [2,3,10,11].

2. Materials and methods

Six short chains modified with N and O atoms as recognition groups were introduced into fluorene to obtain six monomers (Fmoc-Arg-OH, Fmoc-Glu-OH, Fmoc-GABA-OH, Fmoc-Osu, Fmoc-Ala-OH and Fmoc-Thr-OH) then electrochemical polymerization in the boron trifluoride ethyl ether (BFEE) system (**Figure 1**). Considering that six monomers cannot be electrochemically polymerized in common organic solvents such as tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) containing supporting electrolytes, an attempt was made to add trifluoroacetic acid (TFA) into BFEE as the electrolyte solution. On one hand, BFEE, as a Lewis acid, has a catalytic effect on the electropolymerization of aromatic compounds [2,3,6,11]. On the other hand, the addition of TFA to BFEE leads to the formation of complexes between the monomer and TFA, which reduces the resonance of the aromatic ring and improves the stability of the radical ion while increasing the electrical conductivity [2,3,10,11].

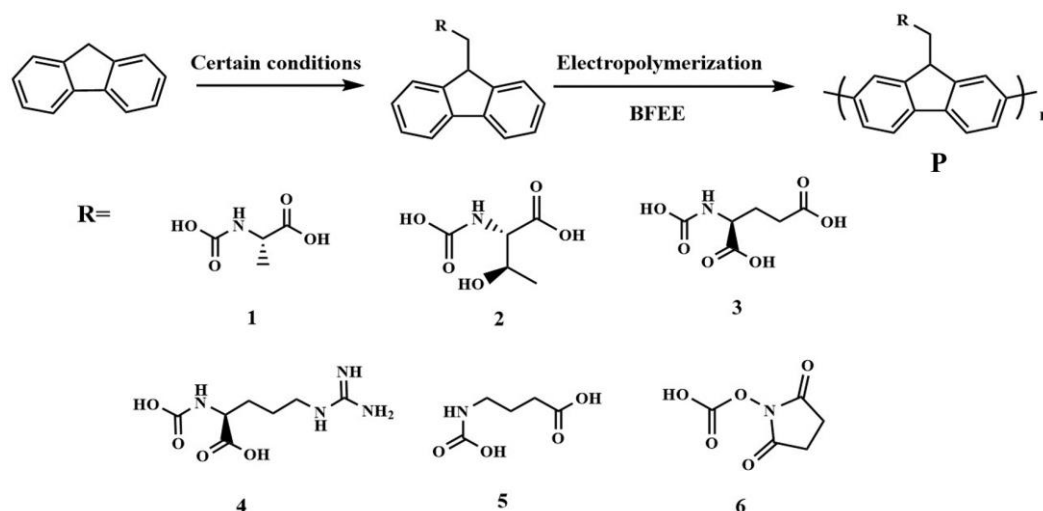


Figure 1. Electrochemical polymerization of six fluorenyl derivatives. (1, Fmoc-Ala-OH; 2, Fmoc-Thr-OH; 3, Fmoc-Arg-OH; 4, Fmoc-Glu-OH; 5, Fmoc-GABA-OH; 6, Fmoc-Osu.).

3. Results and discussion

3.1. Selectivity of polyfluorene derivatives towards $\text{Cr}_2\text{O}_7^{2-}$

To explore the recognition capability of $\text{Cr}_2\text{O}_7^{2-}$, the selective experiment of six polyfluorenes for common anions and cations were studied. It has been experimentally shown that only $\text{Cr}_2\text{O}_7^{2-}$ could quench the fluorescence of six polymers, which indicated these molecules could specific recognition of $\text{Cr}_2\text{O}_7^{2-}$. It is speculated that the N and O atoms may interact with $\text{Cr}_2\text{O}_7^{2-}$, which caused the aggregate of the fluorene skeleton and resulted in its fluorescence quenching [2,3,10,11]. All the polymers also exhibited good selectivity towards $\text{Cr}_2\text{O}_7^{2-}$, which was not interfered by other anions and cations. Besides introducing N and O atoms for chelating $\text{Cr}_2\text{O}_7^{2-}$, another reason for using amino acid groups was to exclude the interference from natural amino acids in agricultural detection. Such good anti-interference ability has been shown in **Figure 2, 3**.

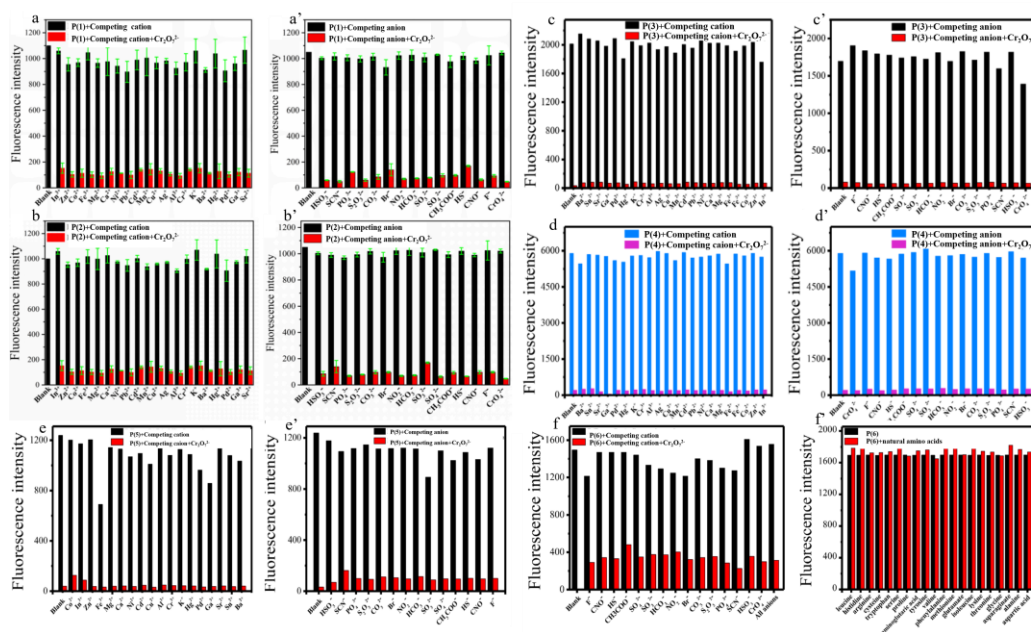


Figure 2. Fluorescence response of P (1) (a, a'), P (2) (b, b'), P (3) (c, c'), P (4) (d, d'), P (5) (e, e') and P (6) (f, f') in the mixed DMSO/PBS (1:1000, v/v) containing various ions. (The black and blue bars represent the addition of the competing ions to a solution of six polymers. The red and pink bars represent the change of the emission that occurs upon the subsequent addition of Cr₂O₇²⁻ to the above solution.) [2,3,10,11].

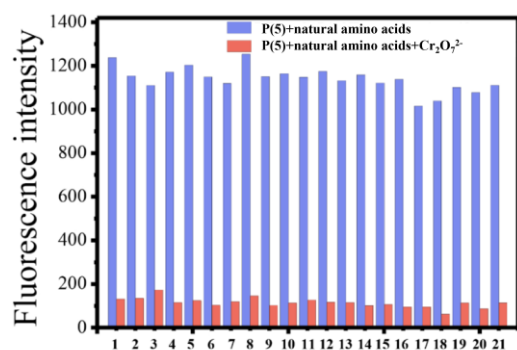


Figure 3. Fluorescence intensity of P(5) upon the addition of 0.1mM Cr₂O₇²⁻ in DMSO-PBS (v/v = 1:500) solution contain 0.1 mM natural amino acids. (The abscissa is from left to right: 1. Blank, 2. Cysteine, 3. Arginine, 4. Histidine, 5. Isoleucine, 6. Asparagine, 7. Glycine, 8. Alanine, 9. Proline, 10. Glutamine, 11. Serine, 12. Aspartic acid, 13. Valine, 14. Lysine, 15. Glutamic acid, 16. Threonine, 17. Tyrosine, 18. Methionine, 19. Leucine, 20. Phenylalanine, 21. Tryptophan.) (Ex = 334 nm).

3.2. Sensitivity test of fluorene and polyfluorene derivatives towards Cr₂O₇²⁻

According to the linear relationship tests between the fluorescence intensity of fluorenes and polyfluorene derivatives in DMSO-EtOH solutions and analyzed Cr₂O₇²⁻ concentrations. We further explored their sensitivity, and the linear relationship between fluorescence intensity and Cr₂O₇²⁻ concentration was studied. As shown in **Table 1**, monomers 1 and 2 have sensitivity to Cr₂O₇²⁻ at the nM level, while the other four monomers only achieve μM. When they were prepared into polymers, their detection sensitivity greatly improved, reaching up to pM and even fM (**Table 1**), which verifies that the molecular wire effect of polymers can greatly improve the sensitivity of the detection of Cr₂O₇²⁻, which further indicates that this type of amino acid-functionalized polyfluorene fluorescence material has the ability of ultra-trace detection of Cr₂O₇²⁻ [2,3,10,11].

Table 1. Limit of detection (LOD) for monomers and polymers.

Monomers	1	2	3	4	5	6
LOD	0.11 nM	0.27 nM	3.3 μ M	16.6 μ M	1.67 μ M	83.3 μ M
Polymers	P1	P2	P3	P4	P5	P6
LOD	1.98 fM	3.72 fM	16.67 pM	3.33 μ M	16.6 fM	8.3 fM

4. Conclusion

In sum, this paper summarizes a design strategy, which constructs a series of amino acid-functionalized polyfluorenes as fluorescent $\text{Cr}_2\text{O}_7^{2-}$ sensors with high selectivity and sensitivity. Thanks to the side-chain groups decorated with N and O atoms, the obtained six monomers and their polymers were not interfered with by common cations, anions, or natural amino acids, which could achieve specific recognition of $\text{Cr}_2\text{O}_7^{2-}$. In comparison, the polymers showed much higher sensitivity with LODs at the pM and fM levels. This work provides an effective way for the design of high-efficiency fluorescent sensors for $\text{Cr}_2\text{O}_7^{2-}$ even in complex environmental systems.

Author Contributions: Conceptualization, G.Z.; methodology, W.Z.; software, W.Z.; validation, W.Z. and G.Z.; formal analysis, S.C.; investigation, H.L., Y.L. and L.C.; resources, G.Z.; data curation, W.Z., H.L., Y.L. and L.C.; writing—original draft preparation, W.Z.; writing—review and editing, S.C. and G.Z.; visualization, W.Z.; supervision, G.Z. and S.C.; project administration, G.Z.; funding acquisition, G.Z.

Funding: We are grateful to the National Natural Science Foundation (5210031010), Jiangxi Provincial Natural Science Foundation of (GJJ2201301, 20212BAB203012), Scientific Fund of Jiangxi Science & Technology Normal University (2022QNBjRC004).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. X. X. Wu, H. R. Fu, M. L. Han, et al., Tetraphenylethylene immobilized metal-organic frameworks: highly sensitive fluorescent sensor for the detection of $\text{Cr}_2\text{O}_7^{2-}$ and nitroaromatic explosives. *Cryst. Growth. Des.*, **2017**, *17*, 6041-6048.
2. F. Li, X. M. Duan, H. Li, et al., Dual effect of aminobutyric acid group and “molecular wire effect” of conjugated polymer enables ultra-trace detection of $\text{Cr}_2\text{O}_7^{2-}$ in fruits. *Microchemical J.*, **2022**, *178*, 107426.
3. F. Li, X. M. Duan, S. X. Hu, et al., Ultra-sensitive detection of $\text{Cr}_2\text{O}_7^{2-}$ in farmland achieved by an electrosynthesized fluorescent poly (Fmoc-succinimide). *Dyes Pigments.*, **2021**, *193*, 109568.
4. Li C., Numata M., Takeuchi M., et al., A sensitive colorimetric and fluorescent probe based on a polythiophene derivative for the detection of ATP. *Angew. Chem. Int. Ed.*, **2005**, *44*, 6371-6374.
5. L. Zou, Y. Wen, H. Zhang, et al., Highly sensitive fluorescent sensor based on electrosynthesized poly (Fmoc-L-serine) enables ultra-trace analysis of $\text{Cr}_2\text{O}_7^{2-}$ in water and agro-product samples. *Sens. Actuators B Chem.*, **2018**, *277*, 394-400.
6. G. Zhang, B. Y. Lu, Y. P. Wen, et al., Facile fabrication of a cost-effective, water-soluble, and electrosynthesized poly(9-aminofluorene) fluorescent sensor for the selective and sensitive detection of Fe(III) and inorganic phosphates. *Sens. Actuators B Chem.*, **2012**, *171*, 786-794.
7. G. Zhang, Y. P. Wen, C. Q. Guo, et al., A cost-effective and practical polybenzanthrone-based fluorescent sensor for efficient de-termination of palladium (II) ion and its application in agricultural crops and environment. *Anal. Chim. Acta.*, **2013**, *805*, 87-94.
8. A. K. Dwivedi, K. M. Prasad, V. Trivedi, et al., Interaction of heme proteins with anionic polyfluorene: insights into physiological effects, folding events, and inhibition activity. *ACS Appl. Mater. Inter.*, **2012**, *4*, 6371-6377.
9. A. K. Dwivedi, G. Saikia, P. K. Iyer, et al., Aqueous polyfluorene probe for the detection and estimation of Fe^{3+} and inorganic phosphate in blood serum. *J. Mater. Chem. A*, **2011**, *21*, 2502-2507.
10. F. Li, G. Zhang, L. Zou, et al., Amino acid groups enable electrosynthesized polyfluorenes to specifically recognize $\text{Cr}_2\text{O}_7^{2-}$. *ACS Appl. Polym. Mater.*, **2022**, *4*, 815-821.
11. H. Li, F. Li, F. Liu, et al., High-quality conjugated polymers achieving ultra-trace detection of $\text{Cr}_2\text{O}_7^{2-}$ in agricultural products. *Molecules*, **2022**, 4294.