Chemistry
A European
Journal

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

Title: A New Phenolate-Ion-Typed Two-Photon Near Infrared Fluorophore Based Biosensor for High-Performance Detection of HNO

Authors: Zhiyun Lu, Moyun He, Chenghui Li, Zhenguo Pang, Kuan Chen, Yanfei Tan, and Yan Huang

To be cited as: *Chem. Eur. J.* 10.1002/chem.202002783

Link to VoR: https://doi.org/10.1002/chem.202002783

WILEY-VCH

10.1002/chem.202002783

WILEY-VCH

A New Phenolate-Ion-Typed Two-Photon Near Infrared Fluorophore Based Biosensor for High-Performance Detection of HNO

Moyun He,[#[a]] Chenghui Li,[# [b]] Zhenguo Pang,[[a]] Kuan Chen,[[a]] Yanfei Tan,*[[c]] Yan Huang,[[a]] and Zhiyun Lu*[[a]]

[a] M. He, Z. Pang, K. Chen, Prof. Y. Huang, Prof. Z. Lu
Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University, Chengdu 610064, P. R. China.
E-mail: *luzhiyun@scu.edu.cn.*
[b] Dr C. Li.
Analytical & Testing Center, Sichuan University, Chengdu, 610064, PR China.
[c] Asst. Prof. Y. Tan
National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, PR China.
E-mail: *tanyf@scu.edu.cn.*
# These authors contributed equally.

Supporting information for this article is given via a link at the end of the document.

Abstract: While (*E*)-4-(2-(4-(dicyanomethylene)-4*H*-chromen-2-yl)vinyl)phenolate anion (**DCPO**$^-$) has emerged to be a potential near infrared (NIR) biosensor signaling unit recently, the pK_a value of its conjugative acid of **DCPOH** (~9) was relatively high, which will lead to relatively low concentrations of **DCPO**$^-$ in physiological conditions and hence unsatisfactory sensitivity of **DCPO**$^-$-based bio-probes. Herein, by difluoro-substitution on **DCPO**$^-$, we exploit a novel fluorophore of ***o*-FDCPO**$^-$ whose conjugate acid has a much lowered pK_a value of 7.42. Meanwhile, ***o*-FDCPO**$^-$ is NIR-emissive with λ_{em} of 693 nm, together with a 0.76 time higher fluorescence efficiency than that of **DCPO**$^-$. The significant superiority of ***o*-FDCPO**$^-$ over **DCPO**$^-$ in sensitivity for NIR bio-sensor applications has been confirmed by comparative studies on two HNO probes, namely ***o*-FDCPO-P** and **DCPO-P** bearing signaling units of ***o*-FDCPO**$^-$ and **DCPO**$^-$, respectively. Moreover, ***o*-FDCPO-P** has been demonstrated to be a high-performance HNO probe with high selectivity, high sensitivity (detection limit: 50 nM) and rapid response, together with a two-photon NIR-excitation imaging capability.

Owing to their low light scattering, deep tissue penetration and minimized interference from background endogenous fluorescence, near infrared (NIR) fluorescence probes have been considered to be more promising than their visible counterparts for in vivo visualization applications.[1] To develop high-performance NIR fluorescence bio-probes, the prerequisite is the exploitation of luminogens capable of fluorescing efficiently in the NIR region (λ_{em} > 650 nm) in physiological conditions (pH, polarity, etc.).[2]

Among the NIR fluorophores developed so far, (*E*)-4-(2-(4-(dicyanomethylene)-4*H*-chromen-2-yl)vinyl)phenolate anion (**DCPO**$^-$, structure shown in Figure 1) has emerged recently to be a potential signaling unit in the construction of NIR biosensors,[3] since in aqueous system, it was reported to show both good photostability and efficient NIR fluorescence (λ_{em}: ~ 700 nm). Nevertheless, according to a literature report[3d], the pK_a value of **DCPOH**, the phenolic conjugate acid of **DCPO**$^-$, is as high as 8.70. Thus in normal physiological conditions (pH = 6.8-7.4),[2a, 4] **DCPO**$^-$ should suffer from relatively low concentrations due to its efficient back-conversion into **DCPOH**, an orange rather than NIR fluorophore.[3a] This will be adverse to the acquirement of highly sensitive NIR bio-probes.

To obtain a **DCPO**$^-$ derivative capable of showing higher concentrations in physiological pH environments, a simple yet effective way is increasing the acidity of the phenolic hydroxyl group of its conjugated acid.[2a] Inspired by the literature reports that for chromophores containing phenolic hydroxyl groups, the acidity of their phenol functional groups can be significantly enhanced by means of difluoro-substitution on the phenolic ring,[5] herein, we consider grafting two F atoms on the phenolic ring of **DCPOH**, so that a compound bearing a more acidic phenol site hence having a lowered pK_a value may be obtained. Nonetheless, the challenge is how to maintain the NIR photoluminescence (PL) characters of the resultant phenolate-anion species while decrease the pK_a value of its phenolic conjugated acid.

Consequently, to determine the grafting positions of the two fluorine atoms, theoretical calculations are carried on the deprotonated species of two **DCPOH** derivatives bearing either an *ortho*- or a *meta*-difluorinated phenol moiety. The computation results reveal that the *ortho*-difluorinated compound owns a nearly identical optical bandgap (E_g) to that of **DCPO**$^-$, but the E_g of the *meta*-modified one is calculated to be slightly widened for ca. 0.1 eV (Figure S1). Therefore, to acquire a NIR fluorophore, the two fluorine substituents should be grafted at the two *ortho*-sites with respect to the phenolic hydroxyl group of **DCPOH**. Accordingly, the *ortho*-difluorinated ***o*-FDCPOH** is synthesized.

To determine whether the difluoro-substitution on **DCPOH** will result in a phenol derivative with enhanced acidity, the pH-dependent UV-vis absorption spectra of ***o*-FDCPOH** in PBS are measured. As shown in Figures 1b, S2 and S3, in relatively acidic conditions (pH ≤ 5.87), the ***o*-FDCPOH**-based sample has its absorption bands predominantly located in 300-530 nm and hence shows a color of pale yellow. Upon gradually neutralization to pH = 7.38, a new lower-energy absorption band with λ_{abs} of 507 nm that corresponds to the ***o*-FDCPO**$^-$ species is observed to be intensified, leading to a color change from pale yellow to light red. Therefore, in normal physiological conditions (pH = 6.8-7.4), ***o*-FDCPO**$^-$ can exist in the ***o*-FDCPOH**-based samples at perceptible concentrations.

On the contrary, for the **DCPOH**-based samples, in a typical physiological condition with pH of 7.38, no obvious spectral signals corresponding to the **DCPO**$^-$ species could be discerned, and the solution color remains to be pale yellow. Only in alkaline conditions (pH = 9.29) does the color of the **DCPOH**-based sample change into light red (Figure S5), indicative of the presence of **DCPO**$^-$ with a perceptible concentration. Based on

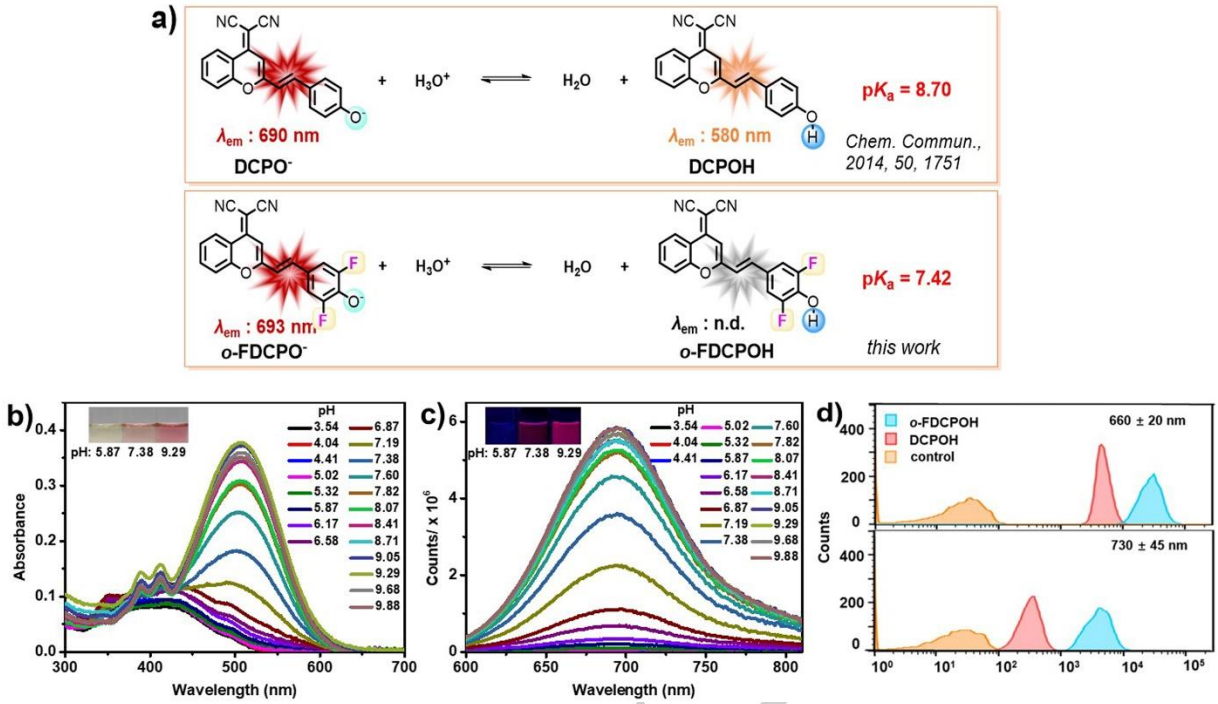


Figure 1. a) Molecular structures of **DCPOH**, **DCPO**$^-$, ***o*-FDCPOH** and ***o*-FDCPO**$^-$. b) pH-dependent UV-vis absorption spectra of ***o*-FDCPOH**. c) pH-dependent fluorescence spectra of ***o*-FDCPOH** (λ_{ex} = 510 nm). Inset: photographs of **DCPOH** and ***o*-FDCPOH** in PBS with different pH at 25 °C (λ_{ex} = 365 nm). d) Cell flow cytometry characterization results of **DCPOH**-incubated and ***o*-FDCPOH**-incubated Raw264.7 cells in different optical channels.

these pH-dependent absorption profiles, the pK_a of **DCPOH** is calculated to be 9.33, which is close to its reported data of 8.70;[3d] but under the very similar experimental conditions, the pK_a of ***o*-FDCPOH** is determined to be 7.42, indicative of the much stronger acidity of ***o*-FDCPOH** than **DCPOH** in their ground states.

In respect of the PL properties, in relatively acidic conditions (pH ≤ 5.87), the ***o*-FDCPOH**-based samples are nearly non-fluorescent (Table S1), but in physiological pH conditions, an intense NIR PL band corresponding to the ***o*-FDCPO**$^-$ species is discernable under excitation of 510 nm (Figure 1c). Yet for the **DCPOH**-based sample, only in alkaline conditions could the NIR PL band be perceptible (Figure S4d). It is noteworthy that under higher-energy excitation (490 nm or 365 nm, vide Figures 1c, S4d and S6a), the ***o*-FDCPOH**-based sample only exhibits one NIR PL band in a stimulated physiological condition (pH = 7.40), but for the **DCPOH**-based sample, it shows an orange/NIR dual PL band that corresponds to the **DCPOH** and **DCPO**$^-$ species, respectively. Note that the predominated orange PL band of the **DCPOH**-based sample implies the inefficient conversion from **DCPOH** to **DCPO**$^-$ in physiological conditions.

On the basis of these pH-dependent PL spectral profiles, the pK_a value of ***o*-FDCPOH** is determined to be 7.25, while that of **DCPOH** is calculated to be 8.90 under very similar experimental conditions. Therefore, in their excited states, the acidity of ***o*-FDCPOH** is also much stronger than **DCPOH**. Meanwhile, in good agreement with the calculation results, the λ_{em} of ***o*-FDCPO**$^-$ is quite similar to that of **DCPO**$^-$ (693 nm vs. 690 nm). Consequently, in physiological pH environments, ***o*-FDCPO**$^-$ should be a more promising NIR signaling fluorophore than **DCPO**$^-$, because its back-conversion to its conjugated acid will be significantly supressed, leading to a higher concentration. This deduction is further validated by comparative PL studies on ***o*-FDCPOH** and **DCPOH**. As illustrated in Figures S6b and S7, under excitation on their corresponding phenolate anion species, in comparison with the **DCPOH**-based sample, the ***o*-FDCPOH**-based one shows a more than 10 folds more intense NIR PL band in simulated physiological environments (pH = 7.40), but only one time higher PL intensity in an alkaline environments with pH of 9.88.

In addition to the much higher concentrations of ***o*-FDCPO**$^-$ than **DCPO**$^-$, in physiological conditions, the relative PL efficiency (φ_{PL}) of ***o*-FDCPO**$^-$ is 0.76 times higher than that of **DCPO**$^-$, and the absolute φ_{PL} of ***o*-FDCPO**$^-$ is 0.90 times higher than that of **DCPO**$^-$ (Table S1). Besides, ***o*-FDCPO**$^-$ shows good photostability (Figure S8). Consequently, owing to its good photostability, potentially higher concentration in physiological environments as well as higher φ_{PL}, ***o*-FDCPO**$^-$ should be a more promising NIR fluorophore than **DCPO**$^-$ for biosensor applications.

To evaluate quantitatively the differences between ***o*-FDCPO**$^-$ and **DCPO**$^-$ in cellular imaging, flow cytometric analyses are carried out. As illustrated in Figure 1d, under excitation at 488 nm, the PL intensity of the ***o*-FDCPOH**-incubated Raw264.7 cells is an order of magnitude higher than that of the **DCPOH**-incubated ones in both the 660 ± 20 nm and the 730 ± 45 nm NIR channels. Consequently, when being used as the luminogen to construct NIR fluorescent biosensors, ***o*-FDCPO**$^-$ should be much superior to **DCPO**$^-$ in cell-labelling sensitivity.

Recently, nitroxyl (HNO), a cellular reactive nitrogen species produced from NO· synthases through oxidation of N-hydroxy-L-arginine or via reduction of NO· by mitochondrial xanthine oxidase,[6] has been demonstrated to be associated with particular physiological or pathological states.[7] However, although significant variations in the level of HNO were considered to be detrimental,[8] the chemistry and biological activities of HNO are still not comprehensively understood.[9] Therefore, it is crucial to develop HNO biosensors capable of measuring HNO levels in live cells. Inspired by a recent report that

DCPO$^-$ could act as the signaling unit to construct a HNO bio-probe, namely **DCPO-P**,[3h] herein, ***o*-FDCPO-P** bearing a similar HNO recognition unit of (diphenylphosphino)benzoate[6, 10] is synthesized (structure shown in Figure 2a), and comparative studies on the optical responses of ***o*-FDCPO-P** and **DCPO-P** to HNO in PBS are carried out to verify whether ***o*-FDCPO**$^-$ is superior to **DCPO**$^-$ as the NIR signaling unit of bio-sensors. Under excitation at either 445 nm or 520 nm, **DCPO-P** and ***o*-FDCPO-P** are both nearly non-emissive due to either the PL quenching effect from the diphenylphosphine moiety, or the relatively low concentrations of their NIR phenolate-ion fluorophores (Figure S10, Table S1). But upon gradual addition of Angeli's salt (HNO donor, $Na_2N_2O_3$, AS), ***o*-FDCPO-P** shows an obvious PL turn-on response toward AS in the NIR region (λ_{em} = 695 nm, Figure 2b). The PL intensity of the ***o*-FDCPO-P**-based sample at 695 nm ($I_{695\ nm}$) shows a good linear correlation with the concentration of AS in the range of 0.2-40 μM, and the detection limit (LOD) of ***o*-FDCPO-P** toward HNO is as low as 50 nM (Figure S11). According to kinetic studies, the reaction equilibrium of ***o*-FDCPO-P** (10 μM) with AS (200 μM) could be attained within 10 min in physiological conditions (Figure S12), confirming the relatively rapid HNO-response capability of ***o*-FDCPO-P**. Moreover, as illustrated in Figure 2c, only HNO could trigger distinct NIR PL turn-on in the ***o*-FDCPO-P**-based sample, whereas other biological relevant species, such as ions (Na^+, K^+, Mg^{2+}, F^-, NO_3^-, NO_2^-, S^{2-}), reactive nitrogen species ($ONOO^-$, NO), reactive oxygen species (H_2O_2, ClO^-, HA, $HO\cdot$), and some common amino acids (Cys, Hcy, GSH), show negligible interference effect, implying the high selectivity of ***o*-FDCPO-P** toward HNO. In line with the literature reports,[10c] with the aid of HR-MS (Figure S13) and ^{1}H NMR (Figure S14) spectral characterizations, the signaling mechanism of ***o*-FDCPO-P** to HNO is safely attributed to an intramolecular Staudinger reaction beween ***o*-FDCPO-P** and HNO (Figure S15).

Under the very same experimental conditions, the **DCPO-P**-based sample also shows a PL turn-on response (Figure S16a). In line with the literature report,[3h] according to our calculations, the LOD of **DCPO-P** toward HNO is as high as 1.25 μM. Based on the fact that the LOD of ***o*-FDCPO-P** is 25 times lower than that of **DCPO-P**, in physiological environments, **DCPO-P** should show a much worse HNO-responding sensitivity than ***o*-FDCPO-P**. This should be ascribed to the severe back-conversion of the yielded NIR fluorophore of **DCPO**$^-$ into its conjugated acid form of **DCPOH**. To verify whether ***o*-FDCPO-P** is superior to **DCPO-P** in detecting HNO in live cells, comparative flow cytometric analyses are carried out. As depicted in Figure 2d, in the presence of AS, the ***o*-FDCPO-P**-incubated live Raw264.7 cells show much stronger fluorescence than the **DCPO-P**-incubated ones in both the 660 ± 20 nm and the 730 ± 45 nm NIR channels, confirming the much better HNO detection capability of ***o*-FDCPO-P** than **DCPO-P** in bio-systems. This deduction is further confirmed by confocal fluorescence observations in live Raw 264.7 cells. In the absence of AS, Raw264.7 cells incubated with both ***o*-FDCPO-P** and **DCPO-P** are non-fluorescent (Figure S19). Upon addition of AS, the **DCPO-P**-incubated cells show relatively weak fluorescence (Figure 2eiii), while those incubated with ***o*-FDCPO-P** can emit much stronger PL in the NIR single-photon channel (Figure 2eiv). More importantly, similar findings can be obtained in two-photon (TP) fluorescence imaging experiments (TP channel, vide Figures 2ev and 2evi). Besides, similar confocal fluorescence imaging results are obtained in live HeLa cells (Figures S20 and S21). All these results demonstrate that ***o*-FDCPO-P** is much superior to **DCPO-P** in HNO detection in bio-systems.

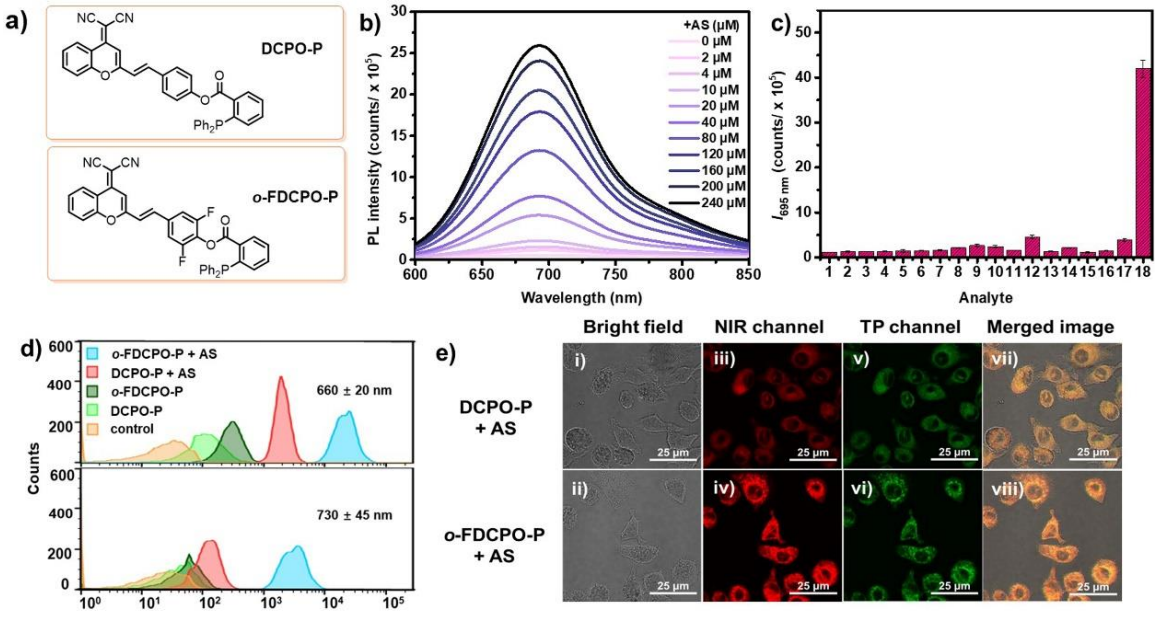


Figure 2. a) Molecular structures of **DCPO-P** and ***o*-FDCPO-P**. b) Fluorescence spectra of 10 μM ***o*-FDCPO-P** (λ_{ex} = 510 nm) in PBS (V_{DMSO}/V_{H2O} = 3/7, pH = 7.38, 37 ± 0.1ºC) in the absence or presence of AS. c) The $I_{695\ nm}$ of 10 μM ***o*-FDCPO-P** PBS solution (V_{DMSO}/V_{H2O} = 3/7, pH = 7.46, 37 ± 0.1 ºC) in the presence of various analytes for 30 min. 1: free; 2-10 (1 mM): Na^+, K^+, Mg^{2+}, F^-, NO_2^-, NO_3^-, S^{2-}, Cys, Hcy; 11-14 (5 mM): GSH, $ONOO^-$, NO, ·OH; 15-17 (1 mM): H_2O_2, ClO^-, HA; 18 (0.15 mM): HNO. d) Flow cytometry characterization results of live Raw264.7 cells incubated with 20 μM ***o*-FDCPO-P** or 20 μM **DCPO-P** in the absence or presence of 200 μM AS in different optical channels. e) One-photon (pictures iii and iv, λ_{ex} = 488 nm, pseudocolored in red) and two-photon (pictures v and vi, λ_{ex} = 980 nm, pseudocolored in green to distinguish from the corresponding single-photon channels) confocal fluorescence images of live Raw264.7 cells incubated with 20 μM **DCPO-P** or 20 μM ***o*-FDCPO-P** in the presence of 200 μM AS for 30 min; vii) and viii): overlay of i, iii, v and vii ; ii, iv, vi, respectively. Scale bar = 25 μm

In summary, by difluoro-substitution on **DCPOH**, ***o*-FDCPOH** bearing a more acidic phenolic site than that of **DCPOH** is acquired. By taking advantage of the more preferable pK_a of ***o*-FDCPOH** than **DCPOH** (ground state: 7.42 vs. 9.33; excited state: 7.25 vs. 8.90), in physiological conditions, ***o*-FDCPOH** can undergo a more thorough deprotonation reaction than **DCPOH**, leading to much higher concentrations of its phenolate-anion-typed fluorophore. Besides, ***o*-FDCPO$^-$** is also NIR-emissive, and the PL efficiency of ***o*-FDCPO$^-$** is 0.76-0.90 time higher than that of **DCPO$^-$**. The significant superiority of ***o*-FDCPO$^-$** over **DCPO$^-$** in constructing NIR bio-sensors is validated by comparative studies on two HNO probes, namely ***o*-FDCPO-P** and **DCPO-P**, because ***o*-FDCPO-P** shows 24 times enhanced fluorescence response sensitivity than **DCPO-P** in both vitro environments and live cells. Actually, ***o*-FDCPO-P** is a high-performance HNO probe with combined high selectivity, high sensitivity (detection limit: 50 nM) and rapid response (10 min), together with the capability of two-photon NIR-excitation imaging. Our results will shed light on the molecular design of high-performance one-photon and two-photon NIR fluorescence biosensors for a great deal of other analytes.

Acknowledgements

We acknowledge the financial support from the National Natural Science Foundation of China (Nos. 21672156, 21875148, 21705114). We thank Dr. P. Wu and Ms. H. Sun of Analytical & Testing Center, Sichuan University for photophysical characterization.

Keywords: near infrared fluorophore • difluoro-substitution • pK_a value • HNO probe • two-photon imaging

[1] a) K. G. Chernov, T. A. Redchuk, E. S. Omelina and V. V. Verkhusha, *Chem. Rev.,* **2017**, *117*, 6423-6446; b) Z. Li, Y. F. Wang, C. Zeng, L. Hu and X. J. Liang, *Anal. Chem.,* **2018**, *90*, 3666-3669; c) Y. Qi, Y. Huang, B. Li, F. Zeng and S. Wu, *Anal. Chem.,* **2018**, *90*, 1014-1020; d) P. Reineck and B. C. Gibson, *Adv. Opt. Mater.,* **2017**, *5*, 1600446; e) X. Wang, Z. Guo, S. Zhu, Y. Liu, P. Shi, H. Tian and W. H. Zhu, *J. Mater. Chem. B,* **2016**, *4*, 4683-4689; f) L. Xiong, J. Ma, Y. Huang, Z. Wang and Z. Lu, *ACS Sens.,* **2017**, *2*, 599-605; g) J. W. Yan, J. Y. Zhu, K. X. Zhou, J. S. Wang, H. Y. Tan, Z. Y. Xu, S. B. Chen, Y. T. Lu, M. C. Cui and L. Zhang, *Chem. Commun.,* **2017**, *53*, 9910-9913; h) J. Zheng, Y. Shen, Z. Xu, Z. Yuan, Y. He, C. Wei, M. Er, J. Yin and H. Chen, *Biosens. Bioelectron.,* **2018**, *119*, 141-148.

[2] a) B. Bao, M. Liu, Y. Liu, X. Zhang, Y. Zang, J. Li and W. Lu, *Tetrahedron,* **2015**, *71*, 7865-7868; b) Y. Dong, H. Xiao, L. Xing, C. Wu, J. Zhou, Z. Zhou, Y. Liu, S. Zhuo and P. Li, *Talanta,* **2020**, *209*, 120549; c) M. Fang, S. Xia, J. Bi, T. P. Wigstrom, L. Valenzano, J. Wang, W. Mazi, M. Tanasova, F. T. Luo and H. Liu, *Chem. Commun.,* **2018**, *54*, 1133-1136; d) J. Lee and M. H. Lee, *Tetrahedron Lett.,* **2017**, *58*, 3178-3182; e) G. Parisio, A. Marini, A. Biancardi, A. Ferrarini and B. Mennucci, *J. Phys. Chem. B,* **2011**, *115*, 9980-9989; f) J. Yin, M. Peng, Y. Ma, R. Guo and W. Lin, *Chem. Commun.,* **2018**, *54*, 12093-12096; g) F. Yu, X. Jing and W. Lin, *Anal. Chem.,* **2019**, *91*, 15213-15219; h) X. Zheng, W. Zhu, F. Ni, H. Ai, S. Gong, X. Zhou, J. L. Sessler and C. Yang, *Chem. Sci.,* **2019**, *10*, 2342-2348.

[3] a) K. Gu, Y. Xu, H. Li, Z. Guo, S. Zhu, S. Zhu, P. Shi, T. D. James, H. Tian and W. H. Zhu, *J. Am. Chem. Soc.,* **2016**, *138*, 5334-5340; b) G. Jiang, M. Li, Y. Wen, W. Zeng, Q. Zhao, C. Chen, H. Yuan, C. Liu and C. Liu, *ACS Sens.,* **2019**, *4*, 434-440; c) H. Li, Q. Yao, J. Fan, J. Du, J. Wang and X. Peng, *Biosens. Bioelectron.,* **2017**, *94*, 536-543; d) M. Li, X. Wu, Y. Wang, Y. Li, W. Zhu and T. D. James, *Chem. Commun.,* **2014**, *50*, 1751-1753; e) J. Ma, J. Fan, H. Li, Q. Yao, J. Xia, J. Wang and X. Peng, *Dyes Pigm.,* **2017**, *138*, 39-46; f) J. Wang, B. Li, W. Zhao, X. Zhang, X. Luo, M. E. Corkins, S. L. Cole, C. Wang, Y. Xiao, X. Bi, Y. Pang, C. A. McElroy, A. J. Bird and Y. Dong, *ACS Sens.,* **2016**, *1*, 882-887; g) P. Wang, Y. Wang, N. Li, J. Huang, Q. Wang and Y. Gu, *Sens. Actuators B-Chem.,* **2017**, *245*, 297-304; h) H. Zhang, Z. Qiao, N. Wei, Y. Zhang and K. Wang, *Talanta,* **2020**, *206*, 120196; i) W. Zhang, F. Huo and C. Yin, *J. Mater. Chem. B,* **2018**, *6*, 6919-6929.

[4] C. Jiao, J. Pang, L. Shen, W. Lu, P. Zhang, Y. Liu, J. Li, X. Jia and Y. Wang, *RSC Adv.,* **2019**, *9*, 20982-20988.

[5] a) Y. Luo, C. Li, W. Zhu, X. Zheng, Y. Huang and Z. Lu, *Angew. Chem. Int. Ed.,* **2019**, *58*, 6280-6284; b) W. Sun, K. Gee, D. Klaubert and R. Haugland, *J. Org. Chem. 62*, 6469-6475; c) W. Sun, K. Gee and R. Haugland, *Bioorg. Med. Chem. Lett.* **1998**, 3107-3110.

[6] F. Ali, S. Sreedharan, A. H. Ashoka, H. K. Saeed, C. G. W. Smythe, J. A. Thomas and A. Das, *Anal. Chem.,* **2017**, *89*, 12087-12093.

[7] A. T. Wrobel, T. C. Johnstone, A. Deliz Liang, S. J. Lippard and P. Rivera-Fuentes, *J Am Chem Soc* **2014**, *136*, 4697-4705.

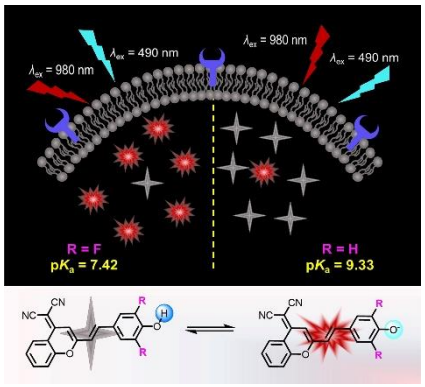
[8] M. Ren, B. Deng, K. Zhou, J. Y. Wang, X. Kong and W. Lin, *J Mater Chem B* **2017**, *5*, 1954-1961.

[9] R. Smulik-Izydorczyk, K. Debowska, J. Pieta, R. Michalski, A. Marcinek and A. Sikora, *Free Radic Biol Med* **2018**, *128*, 69-83.

[10] a) X. Jin, X. Sun, X. Di, X. Zhang, H. Huang, J. Liu, P. Ji and H. Zhu, *Sens. Actuators B-Chem.,* **2016**, *224*, 209-216; b) K. Kawai, N. Ieda, K. Aizawa, T. Suzuki, N. Miyata and H. Nakagawa, *J. Am. Chem. Soc.,* **2013**, *135*, 12690-12696; c) H. Li, Q. Yao, F. Xu, N. Xu, X. Ma, J. Fan, S. Long, J. Du, J. Wang and X. Peng, *Anal. Chem.,* **2018**, *90*, 4641-4648; d) T. Wang, Y. Chai, S. Chen, G. Yang, C. Lu, J. Nie, C. Ma, Z. Chen, Q. Sun, Y. Zhang, J. Ren, F. Wang and W.-H. Zhu, *Dyes Pigm.,* **2019**, *166*, 260-265; e) H. Zhang, R. Liu, Y. Tan, W. H. Xie, H. Lei, H. Y. Cheung and H. Sun, *ACS Appl. Mater. Interfaces,* **2015**, *7*, 5438-5443; f) K. Zheng, H. Chen, S. Fang and Y. Wang, *Sens. Actuators B-Chem.,* **2016**, *233*, 193-198.

10.1002/chem.202002783

Entry for the Table of Contents



A new phenolate-anion-typed high-performance NIR fluorophore is developed for biosensor applications due to the enhanced acidity of its conjugation acid hence increased concentrations in physiological conditions.