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Rational design of a highly selective fluorescent sensor for L-histidine detection in aqueous solution†

Yan Qiao,[a,b] Bin Chen,[a] Yangyang Yang,[a] Xin Wang,[a] Yufang Xu*[a] and Honglin Li*[a]

Computational studies in combination with experimental research were used to design a new rapid, selective and sensitive "turn-on" fluorescent sensor (H3) for L-histidine, which can be first quenched by Ni^{2+} and then recovered upon addition of His.

Among the twenty proteinogenic amino acids, histidine plays a pivotal role in biochemistry due to its aromatic imidazole moiety, which is a common coordinating ligand in metalloproteins and a part of the catalytic site in certain enzymes.[1] Histidine is active in maintaining healthy tissues and protecting nerve cells that transport messages from the brain to various other parts of the body.[2] An abnormal histidine level is an indicator of many diseases[3] such as chronic kidney disease,[3a] acute liver failure,[3b] and rheumatoid arthritis.[3c] Therefore, the development of high-quality methods for histidine detection is extremely necessary and important. Numerous studies have dealt with the detection of histidine using techniques such as voltammetry,[4] UV/vis spectroscopy,[5] luminescence spectroscopy methods,[6] and fluorescence spectroscopy.[7] However, most of the available probes exhibit poor selectivity or require sophisticated detection systems such as the use of organic solvents. The development of reliable, rapid and accurate methods for the determination of histidine is still a highly challenging area of research.

The addition of transition metal ions to the chemosensor ensemble can usually change fluorescence signals due to the chelation of metal ions by the chemosensors. The subsequent snatching of the chelated metal ions from the ensemble by some analytes can again change the fluorescence signal. The two-step signal change phenomenon has been used for the development of some novel fluorescent probes for biologically or environmentally relevant analytes.[8] From a theoretical point of view, if the analyte can snatch the chelated metal ion, the binding energy for the ion must be lower than that of the chemosensor. This inspires us to theoretically predict and then experimentally confirm rationally designed highly selective chemosensors for amino acids such as His. We propose that a chemosensor that can bind a metal ion more weakly than His but more strongly than all other amino acids can be used for selective His detection. If true, this hypothesis would certainly provide valuable insights for the rational design of highly selective chemosensors for other analytes.

Previous studies have revealed that His is always a good ligand in Ni^{2+} containing metal enzymes; furthermore, the octahedral complex of Ni^{2+} with free His molecules has been crystallized previously.[9] Examination of the crystal structure shown in Fig. 1 shows that all three His functional groups, *i.e.*, NH_2, COOH, and the imidazole group, can coordinate with Ni^{2+}, indicating that the binding interaction of His with Ni^{2+} should be sufficiently strong. Moreover, as a paramagnetic metal ion, Ni^{2+} has a pronounced quenching effect on fluorophores, with this characteristic leading to its wide use as a chelated metal ion in probe design.[10] Therefore, Ni^{2+} is an ideal choice for use as the chelated metal ion in the present work. Due to the excellent intrinsic fluorescence characteristics and the fluorescence quenching effect triggered by coordination with paramagnetic metal ions, 7-hydroxycoumarin and

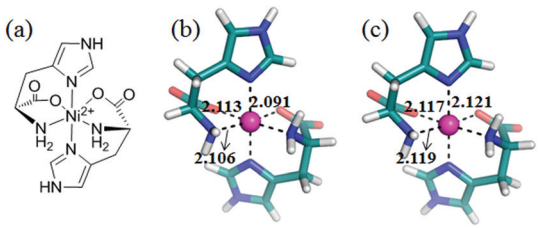
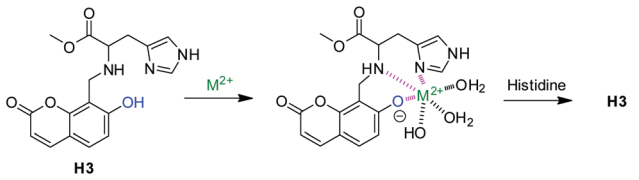


Fig. 1 Complex of Ni^{2+} with 2His. (a) Schematic illustration of the Ni-2His complex. (b) Crystal structure of the Ni-2His complex. (c) Optimized structure for the Ni-2His complex. All distances are in Å.

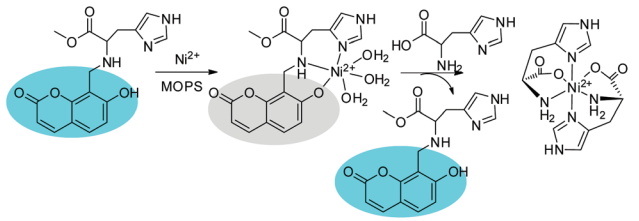
[a]*Shanghai Key Laboratory of New Drug Design, State Key Laboratory of Bioreactor Engineering, School of Pharmacy, East China University of Science & Technology, Shanghai 200237, China*

[b]*Pathophysiology Department, Basic Medical College of Zhengzhou University, Zhengzhou 450001, China*

†Electronic supplementary information (ESI) available: Computational details, General experimental procedure, experimental procedures, spectroscopic properties, ^1H NMR, ^{13}C NMR and HRMS of compounds. See DOI: 10.1039/c5dt04214c



Scheme 1 Illustration of the designed chemosensor **H3**.



Scheme 2 Schematic illustration of a speculated fluorescent signal change.

its fluorescent derivatives have been widely used as indicators of various metal ions.[11] Thus, 7-hydroxycoumarin is considered a good candidate fluorophore. Herein, considering these two factors, we devised a chemosensor (**H3**) (Scheme 1) incorporating His into 7-hydroxycoumarin *via* a methylene group. First, to preserve the inherent sensitivity of the fluorophore, we reserve the whole 7-hydroxycoumarin group in the devised chemosensor and assume that the 7-hydroxyl group can coordinate with Ni^{2+}. Second, to improve the selectivity, the ability of the devised chemosensor to bind Ni^{2+} should be between that of His and all other amino acids. Because the 7-hydroxyl group has been assumed as a coordinating group, we preserve the imidazole and NH groups of His as Ni^{2+} ligands but protect the third ligand, *i.e.*, the carboxyl group, from coordinating with Ni^{2+}. Therefore, in the devised chemosensor **H3**, the carboxyl group of His was protected by methylation, and the methylene group is used as a linker to connect His with 7-hydroxycoumarin in order to set the 7-hydroxyl group at an appropriate position for coordination with Ni^{2+}. We assume that the intrinsic fluorescence emitted by chemosensor **H3** can be first quenched by Ni^{2+}, and then recovered by addition of histidine (as shown in Scheme 2). To the best of our knowledge, this is the first report of a computationally designed chemosensor for selective detection of His. The successful strategy outlined in the present work can be used in rational design of highly selective chemosensors for other analytes.

Prior to the synthesis of **H3**, we performed quantum mechanical calculations to compare the Ni^{2+} binding abilities of **H3**, His and other amino acids. Here, we do not need to consider all the amino acids, but only His, Asp and Glu, which are most commonly involved in coordinating with Ni^{2+}. To compare the binding abilities more directly, we can keep one molecule of His coordinating with Ni^{2+} in all the systems considered and replace the other His molecule with **H3** or other amino acids such as Asp and Glu. Therefore, four models were considered: Ni-2His, Ni-His-**H3**, Ni-His-Asp, and Ni-His-Glu. The optimized

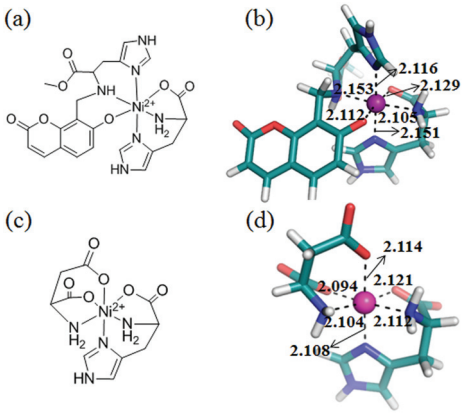


Fig. 2 (a and b) Binding mode and optimized structures of the Ni-His-**H3** complex. (c and d) Binding mode and optimized structures of the Ni-His-Asp complex. All the distances are in Å.

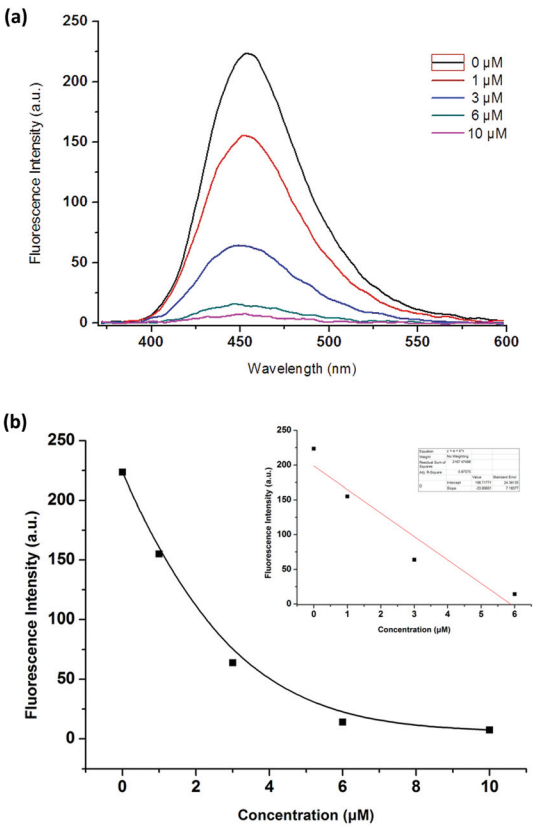


Fig. 3 Fluorescence emission spectra of **H3** upon addition of Ni^{2+}. (a) **H3** concentration in MOPS buffer (50 mM, pH 7.2) was 5 μM. Ni^{2+} concentration was 1–10 μM. Excitation and emission wavelengths were 336 and 455 nm, respectively. Slit: 2.5 nm, 5 nm. (b) Graph of the fluorescence responses to the Ni^{2+} ion (1 μM–10 μM). Inset shows fluorescence intensity of **H3** (5 μM) at 455 nm as a function of Ni^{2+} concentration (0–6 μM).

structures of Ni-His-**H3** and Ni-His-Asp complexes are shown in Fig. 2. In all calculations, we assumed that the coordination mode of Ni^{2+} with the ligands is similar to that found in the Ni-2His crystal structure, *i.e.*, hexacoordinated. All calculations were performed in the framework of density functional theory (DFT) using Gaussian 09 software.[12] The M06-L functional[13] with a def2-TZVP basis set[14] for the metal ion and a 6-31+G* basis set for light atoms was employed in structural optimizations, while a combination of 6-311++G** for light atoms and def2-TZVP for the metal ion was used in energy refinement. Solvation effects were taken into consideration by using the SMD solvation model[15] with water as the solvent. The details of the interaction energy calculation method are summarized in the ESI (Tables S1, S2, and Fig. S1†). According to the calculation results, the interaction energies for the Ni-2His, Ni-His-**H3**, Ni-His-Asp, and Ni-His-Glu models are −71.4, −70.4, −67.8, and −65.1 kcal mol^{-1}, respectively, indicating that the binding of Ni^{2+} with **H3** should be weaker than that of His but stronger than that of other amino acids such as Asp and Glu.

The fluorescence changes in the **H3**-Ni^{2+} complex upon the addition of amino acids were then investigated (Fig. 3). We first prepared a solution of the **H3**-Ni^{2+} complex by mixing the **H3** solution and a $Ni(ClO_4)_2$ solution (**H3**/Ni^{2+}, 1/1 equiv.). The **H3**-Ni^{2+} complex was relatively insensitive to pH in the 6.5–8.0 range (Fig. S4†). Fluorescence emission spectra of histidine titration were measured under physiological conditions (50 mM MOPS) with λ_{ex}: 350 nm. It was found that the fluorescence intensity increased with the addition of histidine (Fig. 4). The **H3**-Ni^{2+} complex exhibited a maximum absorption at 360 nm, which gradually shifted to a shorter wavelength

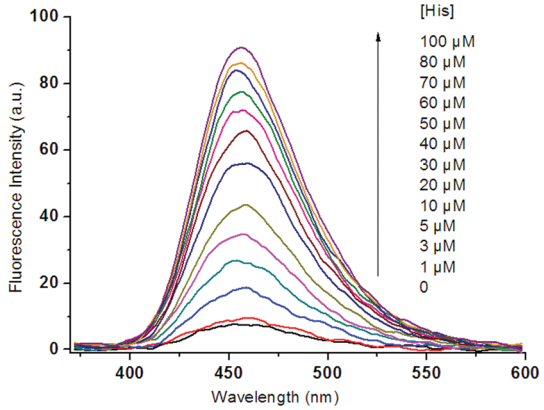


Fig. 4 Emission spectra of the **H3**-Ni^{2+} complex in the presence of histidine (0–100 μM) in MOPS (50 mM, pH 7.2) solution. Excitation and emission wavelengths were 350 and 454 nm, respectively. Slit: 2.5 nm, 5 nm. The **H3**-Ni^{2+} complex was prepared *in situ* by mixing **H3** and $Ni(ClO_4)_2$ (**H3**: 5 μM, $Ni(ClO_4)_2$: 5 μM).

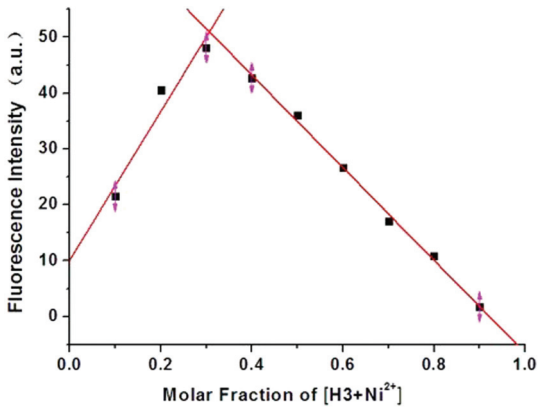


Fig. 6 Job's plot of the [**H3** + Ni^{2+}] complex in MOPS (50 mM, pH 7.2) buffer solution. The total concentration of [**H3** + Ni^{2+}] and histidine was 10 μM. Excitation and emission wavelengths were 350 and 454 nm, respectively, slit: 2.5 nm–5 nm.

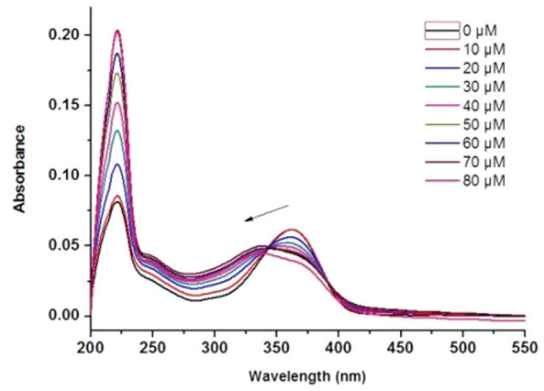


Fig. 5 Absorption spectra of [**H3** + Ni^{2+}] (5 μM) for different concentrations of histidine (0–80 μM) in MOPS (50 mM, pH 7.2) buffer solution. Excitation and emission wavelengths were 350 and 454 nm, respectively. Slit: 2.5 nm, 5 nm.

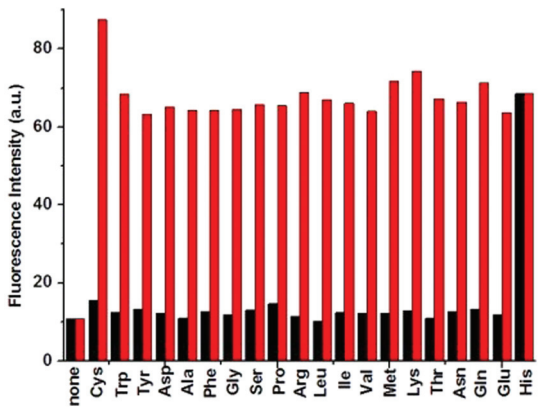


Fig. 7 Fluorescence emission spectra of the **H3**-Ni^{2+} complex (5 μM) upon addition of different amino acids (100 μM) in MOPS (50 mM, pH 7.2) buffer solution. Concentration of histidine was 50 μM. Excitation wavelength was 350 nm. Black bars represent addition of different amino acids to **H3**-Ni^{2+} buffer solution. Red bars represent subsequent addition of 50 μM histidine to the above solution.

at 330 nm close to the **H3** absorption with the sequential addition of histidine. Fig. 5 illustrates that histidine displaced **H3** to bind with Ni^{2+}, liberating **H3** from the quenching environment and turning the fluorescence "on". Two isosbestic points at 344 nm and 384 nm were also observed (Fig. 5). A Job's plot analysis was also performed indicating that the ratio of **H3**-Ni^{2+} and L-histidine was 1 : 2 (Fig. 6).

To confirm the specificity of the fluorescence response of the **H3**-Ni^{2+} complex to histidine, we next measured the fluorescence spectra. As shown in Fig. 7 (black column), while no fluorescence intensity changes were observed in the emission spectra of the other 19 amino acids, a significant fluorescence enhancement was observed in the presence of histidine under identical conditions (red column); the observed fluorescence increased immediately upon the addition of histidine into solutions that already contained the different amino acids and the **H3**-Ni^{2+} complex.

Further evidence for **H3** chelating the Ni^{2+} ion with 1 : 1 stoichiometry is provided directly by ESI Mass (Fig. S6†), and it can also be seen that the 7-hydroxyl of coumarin in the resulting **H3**-Ni^{2+} complex is deprotonated. To prove that the 7-hydroxyl is indeed involved in coordination, we synthesized the reference compound **H3-1** (Scheme 3), where 7-hydroxyl is replaced by 7-methoxy, and tested its ability to bind different metal ions by UV-absorbance and fluorescence spectroscopy (Fig. S7†). We found that the Ni^{2+} ion had nearly no or low response to **H3-1** (Fig. 8). The unchanged absorbance and fluorescence indicated that **H3-1** cannot be effectively combined with the above metal ions. These results proved that the 7-hydroxyl is one of the chelating sites in **H3** in the process of coordination with these metal ions.

In summary, based on the computational predictions, a new highly selective "turn-on" fluorescent sensor (**H3**-Ni^{2+}) for L-histidine was designed and synthesized. Experimental data proved that the **H3**-Ni^{2+} complex exhibits excellent selectivity for L-histidine over the other 19 amino acids in a neutral buffer solution. The recognition mechanism was also deduced; this consists of two L-histidine molecules binding with Ni^{2+} and releasing **H3** to enable fluorescence recovery. This methodology shows great potential for practical application, such as the detection of other disease-associated biomarkers and pharmaceuticals and in clinical analysis.

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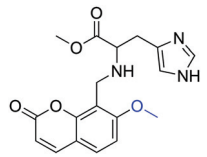

H3-1

Scheme 3 The structure of **H3-1**.

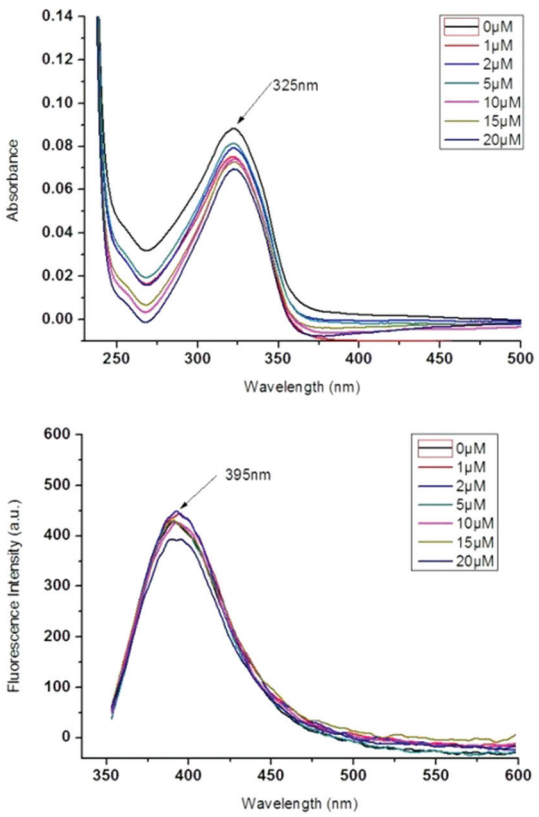

Fig. 8 UV-absorbance and fluorescence emission spectra of compound **H3-1** in response to Ni^{2+} ions in MOPS (50 mM, pH 7.2) buffer solution. Excitation and emission wavelengths were 325 and 395 nm, respectively, slit: 10 nm–10 nm.

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