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Computational Studies on Response and Binding Selectivity of Fluorescence Sensors

George A. Hudson, Lei Cheng, Jiamei Yu, Yong Yan, Daniel J. Dyer, Matthew E. McCarroll, and Lichang Wang*

Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL 62901

Abstract

Using a computational strategy based on density functional theory calculations, we successfully designed a fluorescent sensor for detecting Zn^{2+} [*J. Phys. Chem. B* **2006**, *110*, 22991–22994]. In this work, we report our further studies on the computational design protocol for developing Photoinduced Electron Transfer (PET) fluorescence sensors. This protocol was applied to design a PET fluorescence sensor for Zn^{2+} ions, which consists of anthracene as the fluorophore connected to pyridine as the receptor through dimethylethanamine as the linker. B3LYP and time-dependent B3LYP calculations were performed with the basis set 6-31G(d,p), 6-31+G(d,p), 6-311G(d,p), and 6-311+G(d,p). The calculated HOMO and LUMO energies of the fluorophore and receptor using all four basis sets show that the relative energy levels remain unchanged. This indicates that any of these basis sets can be used in calculating the relative molecular orbital (MO) energy levels. Furthermore, the relative MO energies of the independent fluorophore and receptor are not altered when they are linked together, which suggests that one can calculate the MO energies of these components separately and use them as the MO energies of the free sensor. These are promising outcomes for the computational design of sensors, though more case studies are needed to further confirm these conclusions. The binding selectivity studies indicate that the predicted sensor can be used for Zn^{2+} even in the presence of the divalent cation, Ca^{2+} .

1. Introduction

Recently we proposed a new approach to design photoinduced electron transfer (PET) fluorescence sensors that can be used for a wide variety of applications.¹ The first step of our new approach is to perform computational studies to predict the potential sensor candidates for a given task. Once the sensor candidates are selected computationally, they will then be synthesized, characterized, and subsequently refined. As a proof of concept, we have successfully developed a PET fluorescent sensor for detecting Zn^{2+} using the new approach.¹ The computational selection of the sensor candidates in that work is based on the comparison of the energies between the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) of fluorophore, free receptor, and bound receptor using B3LYP/6-31G(d,p). The success of our strategy, however, leaves many questions unanswered on this simplest computational framework. For instance, does a bigger basis set affect the outcome? Do energies of the HOMO or LUMO of the fluorophore change when it is linked to the receptor, and if it does, how much will it change? Does the bound target molecule change the energy of the HOMO or LUMO of the fluorophore? Will the synthesized sensor work if other metal cations are also present? Furthermore, the future success of our design approach depends very much on the success of computational prediction. Therefore, it is critical to examine the issues discussed above and to develop a

*To whom the correspondence should be addressed. lwang@chem.siu.edu. Phone: (618)4536476.

general computational protocol for fluorescence sensor design with strategies that can balance the computing accuracy and resources. In this work, we used the fluorescence sensor for Zn^{2+} as a prototype to address the above issues.

The design of metal sensors has been traditionally undertaken by many different techniques. These techniques include the application of combinatorial methods² as well as the intuition driven method of rational design followed by organic synthesis.³⁻⁸ The fluorescence mechanism is usually not investigated by the traditional techniques. It is generally believed that an off/on fluorescence sensor works if the HOMO and/or LUMO energy levels of the receptor lie out of the HOMO and LUMO energy range of fluorophore upon binding to the target molecules. Thus, the molecular orbitals, especially the HOMOs and LUMOs of fluorophore and receptor need to be studied if such a mechanism works. One of the advantages of our new approach is that the computational synthesis also provides understanding of the fluorescence mechanism and in turn helps to search for more efficient sensors, such as enhancement of the fluorescence intensity or an increase in detection limits.

Sensors for Zn^{2+} have been under investigation mainly in consideration of zinc's biological function in neurons in which free Zn^{2+} has been found. The metal generally acts as a center for protein chelation, however, in certain organs a significant amount of free Zn^{2+} is present such as the brain.⁹ In the brain Zn^{2+} is believed to be responsible for neuronal regulation and death.¹⁰ Burdette et al. developed a sensor to detect neuronal Zn^{2+} using a fluorescein moiety. The sensor is reported to work rather nicely for detecting zinc but is produced in rather low yields (4%) and has background fluorescence at physiological pH ranges. The sensor developed by Burdette et al. also exhibits fluorescence enhancement for Zn^{2+} in the presence of other metal ions such as Ca^{2+} .⁴ The Komatsu et al. group also reported on Zn^{2+} sensor for neuronal research that was based on the fluorescein moiety. The sensors proposed by Komatsu et al. had varying degrees of affinity for the ion which were engineered to determine the varying concentrations of the Zn^{2+} ion in certain parts of the brain. Though these sensors are reported to work well and even to be selective for Zn^{2+} in the presence of Ca^{2+} the production of them still seems to be plagued by difficult synthetic procedures and low yields.⁶ Other examples of fluorescent sensor for detecting Zn^{2+} are in the literature^{3-8,11-14} but it seems that little attention has been paid to the selective detection of Zn^{2+} in the presence of other divalent metals found in biological systems such as Ca^{2+} and Mg^{2+} .

The sensor (**1**) in Fig. 1 is relatively simple to synthesize. Using this proposed sensor as an example, we performed the Becke's three-parameter exchange functional with the Lee-Yang-Parr correlation functional (B3LYP)¹⁵⁻¹⁸ and time-dependent B3LYP (TD-B3LYP) calculations with various basis sets to study the response and binding selectivity of the sensor for divalent Zn^{2+} ions, in addition to develop a general computational sensor synthesis protocol. B3LYP has mainly been used to calculate structural parameters, energetics, and molecular orbital energies including the HOMO and LUMO energies.^{1,19-22} In the sensor design we are concerned with excited states of the fluorophore and the transitions to these excited states upon adsorption of photons, as these excited states and the transitions are important to understanding the fluorescent behavior of the sensor, both in the presence and absence of the target molecules. As such, TD-B3LYP²³⁻²⁷ is used to calculate the excitation transitions of the proposed sensor and comparison is made between the results obtained using B3LYP and TD-B3LYP.

2. Computational Details

A fluorescence sensor usually consists of a fluorophore, a receptor, and a linker that connects the fluorophore and receptor. In the proposed sensor (Fig. 1), the fluorophore,

receptor, and the linker are anthracene, pyridine, and dimethylethanamine, respectively. The sensor in Fig. 1 differs slightly from the one discussed in the original work¹ in that the linker of the current sensor has one more CH₂. We expect, and as our study has also shown, that this minor difference would not affect any of the computational predictions. In order to investigate whether the HOMO and LUMO energy levels will be changed when the fluorophore and receptor are linked and the role of the linker, we studied the extended fluorophore (**2**), the extended receptor (**3**), shown in Fig. 2, and the sensor (**1**) (shown in Fig. 1). Five systems, i.e. the extended fluorophore, the extended receptor, free sensor, and the sensors bound to Zn²⁺ and Ca²⁺, were studied using B3LYP and TD-B3LYP. As the B3LYP has been rather successful in studying organic molecules, we choose this functional.

All calculations of the above systems were performed using Gaussian03.²⁸ Four basis sets, i.e. 6-31G(d,p), 6-31+G(d,p), 6-311G(d,p), and 6-311+G(d,p), were used in the calculations of the extended fluorophore and receptor, in order to examine whether a small basis set is feasible to use. The calculations for the rest of the systems were carried out using the 6-311+G(d,p) basis set. All calculations were performed in solution with acetone as the solvent, which was treated using the Tomasi's polarized continuum model, except when otherwise mentioned. Full geometry relaxations were performed without any constraints. The SCF convergence was 10⁻⁸ a.u.; the gradient and energy convergence was 10⁻⁴ a.u. and 10⁻⁵ a.u., respectively.

In the TD-B3LYP calculations, we used 6 states to calculate excitation energies. Our test of using 5 and 6 states to calculate the excitation energies of fluorophore and receptor showed that using 5 states can give accurate results and may be used in future work of similar systems. A detailed comparison will be discussed when the excitation data is presented in section 3.1. The first two excitation energies will be discussed, as these will be sufficient for the sensor discussed here. Certainly, if multi-excitation sensors are to be designed, more excitation states need to be included in the calculations.

3. Results

3.1 Computational method investigation: Basis set and TDDFT

The first area of research we considered was a comparison of total energy, HOMO, and LUMO of the (extended) fluorophore and the (extended) receptor, as shown in Fig. 2, obtained using four basis sets. The comparison was made to examine the application of smaller, and thus less time consuming, basis sets for future work on these types of computational studies. The data obtained for fluorophore (anthracen-9-yl)-N,N-dimethylmethanamine can be seen in Table 1.

The calculated total energy, as expected, decreases considerably with the increase of the basis set size. More critical to the sensor design is the HOMO and LUMO energy levels and the HOMO-LUMO energy gap. Data in Table 1 show that the HOMO and LUMO levels become nearly unchanged using the 6-31+G(d,p), 6-311G(d,p), and 6-311+G(d,p) basis set.

The same observations can also be made based on the data obtained for the receptor, which are shown in Table 2. Furthermore, the receptor's HOMO and LUMO levels obtained using all four basis sets are out of the range of the fluorophore's. This indicates that any of the basis sets can be used when the relative HOMO and LUMO levels are concerned, though using of small basis set can save a great deal of computing time. For the future calculations, the above results illustrated that 6-31+G(d,p) is the optimal choice of basis set to model similar systems. The rest of the data reported were obtained with the 6-311+G(d,p) basis set although the use of a smaller basis set would give accurate results.

The excitation energies of the fluorophore or the receptor can be directly obtained from the HOMO-LUMO energy gap, which was provided in Tables 1 and 2, respectively. The comparison of the energy gaps between the fluorophore and receptor in these Tables illustrated that different excitation energies will be required. We also performed TD-B3LYP calculations to obtain the excitation energy of fluorophore and receptor, as the TD-B3LYP calculations will also provide more than one excitation energies, which is useful in the sensor design to examine for potential overlaps. The TD-B3LYP results for the receptor are provided in Table 3.

The data in Table 3 show that the energy required to excite the electron in the HOMO, Molecular Orbital (MO) 25 to the LUMO, MO26, is 5.30 eV. On the other hand, the B3LYP calculation (Table 2) gave 6.06 eV. This is a significant difference in the values of the excitation energies between the B3LYP and TD-B3LYP cases. The TD-B3LYP gives a value that is smaller than B3LYP. This reflects the excitation being a dynamical process thus the use of TD-DFT gives a more accurate value. It is interesting to point out that the excitation from MO24 requires 4.92 eV while the excitation from MO25 to MO26 requires 5.30 eV. This is totally different from the prediction that B3LYP will provide, in which the excitation energy is obtained by subtracting two orbital energies.

We also provided the excitation energies for the fluorophore in Table 4. As previously shown, the excitation energy from the HOMO to LUMO calculated using TD-B3LYP (3.09 eV) is smaller than the value calculated using B3LYP (3.48 eV as shown in Table 1).

The excitation energy values obtained for the fluorophore and receptor either from B3LYP or TD-B3LYP show that they have distinct excitation energies. Therefore, we expect a specific excitation based on the results of isolated studies. We will examine next whether the excitation energies will be changed when the fluorophore and receptor are linked to form a sensor.

Finally, we point out that 5-state and 6-state calculations provided essentially the same results, as shown in Tables 3 and 4. This indicates that one can choose the 5-state in the future calculations.

3.2 Computational sensor design protocol: Isolated components of the sensor vs free sensor

Computational synthesis of fluorescence sensors consists of two parts: thermodynamics and kinetics. In this work, we focus on the thermodynamics of sensor design. In the computational synthesis strategy, we take three steps toward completing the thermodynamics studies. The first step is to select the candidates as fluorophores and receptors by computing and comparing the HOMO and LUMO energy levels and excitation energies of potential fluorophores and receptors. After comparing the relative levels, we choose one or a few promising pairs to construct the sensor. The second step is to compute the above mentioned properties for the sensor and examine whether the conclusions drawn for the individual parts are valid when the fluorophore and receptor are connected as a sensor. The third step is to study the HOMO and LUMO energies of the sensor that binds to the analytes of interest and the selectivity, both response and binding, of the sensor. Calculations and analysis of molecular orbitals and the excitation energies are an important part of the computational sensor design. We emphasize that it is useful and important to perform the work described in step 1, i.e. calculations of isolated fluorophores and receptors. This will allow us to set up a data base that can be used for different projects if the results show that the HOMO and LUMO levels are not perturbed after forming a sensor. In this section, we investigate how much the perturbation to the HOMO and LUMO will be when the fluorophore and receptor are linked for the particular sensor of Zn^{2+} .

The MOs for the 2-methylpyridine (**3**) can be seen in Fig. 3 with the corresponding orbital energies. Among the three occupied orbitals, i.e. MO23-25, the involvement of the linker unit, CH₃, increases from MO23 (no involvement at all) to MO25. Between the two unoccupied orbitals, MO27 involves linker CH₃ group. But the majority of the electrons are confined within the pyridine molecule.

The MOs of (anthracen-9-yl)-N,N-dimethylmethanamine (**2**) are illustrated in Fig. 4 together with their corresponding energies. Among the six MOs in Fig. 4, the electron contour plots show that MO62 mostly belongs to the linker. The first excitation (at 401 nm) of the fluorophore, as shown in Table 4, is composed of the transitions 61→65, 62→64, and 63→64 with coefficients of 0.10401, 0.13144, and 0.63474, respectively. The dominant excitation occurs between MO63 (HOMO) and MO64 (LUMO). Two minor excitations also take place. The excitation from MO62 → MO64 involves charge transfer from the linker to the fluorophore molecule, which may reduce the fluorescence efficiency of the sensor. The other minor excitation takes place between MO61 and MO65. When this occurs, the excited electron will not come back to MO61 with a fluorescence signal because MO26 of the receptor energetically lies between MO61 and MO65 of the fluorophore, which opens a non-radioactive deactivation pathway. These MO energy levels were obtained separately and they may be altered when the fluorophore and receptor are linked together. In what follows we will discuss the MOs in the linked case (free sensor).

Eight MOs of the free sensor together with the orbital energies are shown in Fig. 5. Among these orbitals, three MOs, i.e. MO84, MO89, and MO91, belong to the receptor, while MO86 belongs to the spacer, and the rest belong to the fluorophore. These orbitals are quite similar to the orbitals obtained using isolated components. In fact, we can find eight matching pair orbitals from the comparison of these orbitals and the isolated cases (Fig. 3 and Fig. 4): MO84 and MO25, MO85 and MO61, MO86 and MO62, MO87 and MO63, MO88 and MO64, MO89 and MO26, MO90 and MO65, and MO91 and MO27. Not only the shapes of these orbitals resemble each other closely, the orbital energies also remain largely unchanged, which can be seen clearly in Fig.6.

Figure 6 depicts the MOs energy levels obtained from both isolated components **2** and **3** and the free sensor (**1**). Indeed, the MO energies obtained from isolated components (the left columns in Fig. 6) and the free sensor (the right columns in Fig.6) show that their differences are negligible. This indicates that these molecular orbitals are localized and the linker does not significantly change the orbital energy or shape. These results are promising and indicate that we can make direct comparisons among the molecular orbitals of the isolated fluorophore and receptor. This energy diagram also shows clearly that the MO26 lies in between MO61 and MO65, or equivalently, MO89 in between MO85 and MO90. The excitation from MO61 to MO65 (or from MO85 to MO90) in the presence of acceptor will result in an off fluorescence signal. Next, we investigate the oscillator strength of such an excitation from TD-B3LYP calculations.

The first two excitation energies of the free sensor (**1**) were obtained using TD-B3LYP and the results are summarized in Table 5. The first excitation (at 401 nm) is composed of transitions from MO85→90 and 87→88, which have determinants of 0.1064 and 0.6399, respectively. The first of these transitions will not produce the fluorescence due to MO89 corresponding to an empty MO of the receptor, which will accept the excited electron and hinder fluorescence of the anthracene ring. However, the latter transition will result in background fluorescence of the sensor because this excitation is from the HOMO to the LUMO and there is no 'receptor' MO that lies between them. The second excitation (at 378 nm) of the free sensor is a charge transfer process and is irrelevant to the subject of current interest.

3.3 Sensor Selectivity

In this section, we present results on whether the free sensor (**1**) shown in Fig.1 can be used for detecting Zn^{2+} , which depends on the sensor's response and binding selectivity upon the presence of Zn^{2+} . The structure of Zn^{2+} bounded species is illustrated in Fig. 7.

The calculated MOs of the Zn^{2+} chelated fluorescent sensor can be seen in Fig. 8. Most of the MOs still resemble those of the free sensor, such as MO100 (Fig. 8) to MO85 (Fig.5), but significant differences can be observed due to the presence of Zn^{2+} . For instance, MO102 is very different from any of the MOs of the free sensor. The first and second excitations of the Zn^{2+} chelated sensor are provided in Table 6.

The first excitation (at 458 nm) occurs with an oscillation strength of 0.0809 and is composed of the transition from MO101→102. This transition is a charge transfer process. The second excitation (at 381 nm), more relevant to the fluorescence signal, is composed of transitions from 101→103. As none of the receptor's MOs lies in between MO101 and MO 103, we can predict that this excitation will result in fluorescence. This is demonstrated clearly in Fig. 9.

The above data suggests that the proposed sensor will work in an off/on manner. This same type of comparison was applied to a sensor previously reported¹ and matches the experimentally collected data quite well in that the off/on behavior is predicted by this model and observed by experiment.

Due to its presence in biological systems we must also consider the sensor interacting with the Ca^{2+} ion. The structure of the Ca^{2+} chelated sensor is shown in Fig. 10 and the MOs that are relevant to the fluorescence sensor are depicted in Fig. 11. We can see that Ca^{2+} alters the electron distribution considerably. Table 7 provides two excitation energies and the corresponding MOs involved in these excitations.

The first excitation (at 416 nm) of the sensor when chelated to the Ca^{2+} ion occurs with a strength of 0.0919 and is composed of the transitions from MO96→97 and 96→99 with determinants of 0.64216 and 0.10824, respectively. The first transition, MO96 to MO97, will produce a fluorescent effect but the other transition will not due to MO98 corresponding to a 'receptor' MO. Also, it is worth noting that the wavelength is different from those of Zn^{2+} . The second excitation (at 336 nm) of the sensor occurs with the oscillation strength of 0.0040 and is composed of transitions from MO96→98, which is a charge transfer process and thus produces no fluorescence. As the excitation wavelength for these two cations are different, it indicates that the proposed sensor has response selectivity towards Zn^{2+} over Ca^{2+} . There is also another effect which should be taken into account and that is the binding selectivity of the sensor.

The calculated values in the gas and solution phase using B3LYP with the 6-311+G(d,p) basis set can be seen in Table 8. From the data shown Table 8, upon chelation of Zn^{2+} and Ca^{2+} , 327 kcal/mol and 182 kcal/mol of energy will be released, respectively. This implies that the formation of the host-guest complex is favored thermodynamically over the free Zn ion. The rather large energy difference makes intuitive sense because the Zn atom is more electronegative than the Ca atom, the trend should hold for the 2+ species of both atoms, thus it will form a more stable complex when binding to the electron rich nitrogen centers of the sensor. Evidence for improved binding of Zn^{2+} due to being more electronegative than Ca^{2+} can be found in examining the ionization energies of the Zn and Ca atoms. The 1st ionization energy for Zn is 216.6 kcal/mol and that for Ca is 141.0 kcal/mol. The 2nd ionization energy for Zn is 414.3 kcal/mol and that for Ca is 273.8 kcal/mol.²⁹ This shows that the electrons are more tightly bound to zinc than calcium, which shows Zn^{2+} to be more

electronegative than Ca^{2+} . The above analysis is based on the gas phase studies. The data for the complexes in acetone also show that the host-guest complex formation is favorable process with less energy being released with respect to that in gas phase. Our calculations show that the sensor will release 27 kcal/mol of energy when binding to the Zn^{2+} ion and 18 kcal/mol of energy when binding to the Ca^{2+} ion, thus the sensor will favor binding to the Zn^{2+} ion over the Ca^{2+} ion. Experimental confirmation of this prediction is in progress.

4. Conclusions

Density functional theory and TDDFT calculations using B3LYP were performed on fluorescence sensor (**1**) bound and unbound to the Zn^{2+} ion with the basis set 6-31G(d,p), 6-31+G(d,p), 6-311G(d,p), and 6-311+G(d,p). The calculated HOMO and LUMO energies of fluorophore (anthracene) and receptor (pyridine) using the above four basis sets show that the relative energy levels using the last three basis sets remain unchanged. This indicates that any of the basis sets, 6-31+G(d,p), 6-311G(d,p), or 6-311+G(d,p), can be used in calculating the relative molecular orbital (MO) levels. The fact that the difference among basis sets was comparatively small supports the idea that the smaller basis sets can be applied with reasonable accuracy. As we expected, the comparison between DFT and TDDFT showed significant differences with the use of TDDFT giving a smaller value for the excitation energies. The comparison of both HOMO-LUMO energy levels and the excitation energies between the free and bound sensors illustrated the off/on mechanism. In the binding selectivity studies, our calculations show that the sensor will release 27 kcal/mol of energy when binding to the Zn^{2+} ion and only 18 kcal/mol of energy when binding to the Ca^{2+} ion, thus the sensor will favor binding to the Zn^{2+} ion over the Ca^{2+} ion. Therefore, the sensor can be used for Zn^{2+} even in the presence of the divalent cation, Ca^{2+} .

We have provided a computational approach to the design of fluorescence sensors based on PET. Furthermore, the relative MO energies of the independent fluorophore and sensor are not altered in the case of Zn^{2+} sensor when they are linked together. This is very encouraging, as it suggests that one can calculate the MO energies of these components separately and use them as the MO of the free sensor. More prototype studies are needed to further confirm these conclusions. In comparison with the actual synthesis and characterization to be performed, we can improve upon various aspects of the computational protocol (virtual design). We believe that, with the establishment of a rigorous virtual synthesis method, our approach on the design of fluorescence sensors, which consists of virtual design, directed synthesis, and characterization, will not only become a successful model, but also provide better understanding of the fluorescence mechanisms.

Acknowledgments

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Figure 1.
The Zn^{2+} fluorescence sensor (**1**). The blue, grey, and white balls represent nitrogen, carbon, and hydrogen atoms, respectively.

Figure 2. The extended sensor fluorophore, (anthracen-9-yl)-N,N-dimethylmethanamine (**2**), and extended sensor receptor, namely 2-methylpyridine (**3**). The blue, grey, and white balls represent nitrogen, carbon, and hydrogen atoms, respectively.

Figure 3.
Molecular orbitals (MOs) of the extended receptor (**3**).

Figure 4.
Molecular orbitals (MOs) of the extended fluorophore (**2**).

Figure 5.
Molecular orbitals (MOs) of the free sensor (**1**).

Figure 6. The energy diagram of molecular orbitals obtained through calculations of (A) the isolated fluorophore (2) and receptor (3) and (B) free sensor (1).

Figure 7.
The Zn²⁺ chelated fluorescent sensor. The blue, grey, white, and grey-blue balls represent nitrogen, carbon, hydrogen, and zinc atoms,

Figure 8.
Molecular orbitals (MOs) of the Zn^{2+} chelated sensor.

Figure 9.

An MO energy diagram of B) the free sensor and C) the Zn²⁺ chelated sensor. The energies of all MOs of the free sensor are relative to MO87 and those of chelated sensor are relative to MO101.

Figure 10.
The Ca^{2+} chelated fluorescent sensor. The blue, grey, white, and yellow balls represent nitrogen, carbon, hydrogen, and calcium atoms, respectively.

Figure 11.
Molecular orbitals (MOs) of the Ca^{2+} chelated sensor.

Table 1

A comparison of the calculated values for the total energy, HOMO (molecular orbital 63), LUMO (molecular orbital 64), and HOMO-LUMO energy gap (ΔE), of the extended sensor fluorophore (anthracen-9-yl)-N,N-dimethylmethanamine (**2**) using four basis sets

Basis Set	Energy (a.u.)	HOMO (eV)	LUMO (eV)	ΔE (eV)
6-31G(d,p)	-712.832087	-5.21	-1.69	3.52
6-31+G(d,p)	-712.855402	-5.46	-2.00	3.45
6-311G(d,p)	-712.978111	-5.46	-1.95	3.51
6-311+G(d,p)	-712.985286	-5.53	-2.05	3.48

Table 2

A comparison of the calculated values for the total energy, HOMO (molecular orbital 25), LUMO (molecular orbital 26), and HOMO-LUMO energy gap (ΔE), of the extended sensor receptor 2-methylpyridine (**3**) using four basis sets

Basis Set	Energy (a.u.)	HOMO (eV)	LUMO (eV)	ΔE (eV)
6-31G(d,p)	-287.622961	-6.83	-0.60	6.23
6-31+G(d,p)	-287.635736	-7.09	-1.02	6.07
6-311G(d,p)	-287.685351	-7.06	-0.88	6.18
6-311+G(d,p)	-287.690415	-7.15	-1.09	6.06

Table 3

Calculated excitation energies, oscillator strengths, and molecular orbitals (MOs) involved in the excitation for 2-methylpyridine (**3**) using 6-311+G(d,p)

Energy (eV)	Wavelength (nm)	Oscillator Strength	MOs	Coefficient
5.30	234	0.0874	23 → 27	0.31389
			25 → 26	0.62205
4.92	252	0.0054	24 → 26	0.68616

Note: Whenever the data obtained from the 5-state calculations differ from those from the 6-state calculations, the 5-state data are presented in parentheses.

Table 4

Calculated excitation energies, oscillator strengths, and molecular orbitals (MOs) in the excitation for fluorophore, (anthracen-9-yl)-N,N dimethylmethanamine (**2**), using 6-311+G(d,p)

Energy (eV)	Wavelength (nm)	Oscillator Strength	MOs	Coefficient
3.09	401	0.1043 (0.1042)	61 → 65	0.10418 (0.10401)
			62 → 64	−0.12910 (0.13144)
			63 → 64	0.63506 (0.63474)
3.26	380	0.0027 (0.0028)	62 → 64	0.69056 (0.69009)
			63 → 64	0.10643 (−0.10849)

Note: Whenever the data obtained from the 5-state calculations differ from those from the 6-state calculations, the 5-state data are presented in parentheses.

Table 5

Calculated excitation energies, oscillator strengths, and molecular orbitals (MOs) involved in the excitation for free sensor (**1**) using 6-311+G(d,p)

Energy (eV)	Wavelength (nm)	Oscillator Strength	MOs	Coefficient
3.09	401	0.1034	85 → 90	0.10640
			87 → 88	0.63990
3.28	378	0.0012	86 → 88	0.69750

Table 6

Calculated excitation energies, oscillator strengths, and molecular orbitals (MOs) involved in the excitation for Zn^{2+} chelated sensor using 6-311+G(d,p)

Energy (eV)	Wavelength (nm)	Oscillator Strength	MOs	Coefficient
2.71	458	0.0809	101 $\rightarrow$ 102	0.65946
3.25	381	0.0121	101 $\rightarrow$ 103	0.68587

Table 7

Calculated excitation energies, oscillator strengths, and molecular orbitals (MOs) involved in the excitation for Ca^{2+} chelated sensor using 6-311+G(d,p)

Energy (eV)	Wavelength (nm)	Oscillator Strength	MOs	Coefficient
2.98	416	0.0919	96 $\rightarrow$ 99	0.10824
			96 $\rightarrow$ 97	0.64621
3.28	336	0.0040	96 $\rightarrow$ 98	0.69750

Table 8

Calculated energies for the free ions, free sensor and ion-bound sensors in acetone solution and gas phase

	Energy (a.u.) (sol.)	Energy (a.u.) (gas)
Zn ²⁺	-1779.067031	-1778.331426
Ca ²⁺	-677.4992992	-676.9057847
Free Sensor	-999.4662866	-999.4417791
Sensor-Zn ²⁺	-2778.54091	-2778.301297
Sensor-Ca ²⁺	-1676.927055	-1676.645544