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荧光传感受体分子的设计、组装与分子识别

孙 向 英

指 导 教 师： 江云宝 教授

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**Design and Assembling of Fluorescent Receptors
for Molecular Recognition and Chemosensing**

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By

Xiang-Ying Sun

Supervisor: Prof. Yun-Bao Jiang

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荧光传感受体分子的设计、组装与分子识别

摘 要

本论文共分五章。

第一章为绪论，介绍了分子识别与荧光传感领域所取得的最新研究成果及发展趋势。包括：(1) 金属离子识别的传感体系；(2) 糖的识别机理及荧光受体分子设计；(3) 激发态非辐射内转换的研究进展；(4) 自组装膜的构建、类型及其应用。

评述了分子识别与荧光传感体系中受体分子的设计思路以及自组装膜的最新研究进展，在此基础上提出论文设想。

第二章，基于激发态非辐射内转换(IC)原理设计荧光分子并建立对溶剂极性等介质环境敏感的识别体系。近期的研究认为 1-N, N-二甲基氨基萘(DMAN) 的荧光量子产率和荧光寿命随溶剂极性减小而减小和缩短的现象是 IC 所致，IC 的发生与相邻的激发态 S_1 , S_2 间能级差有关。改变电荷转移特征的发射态 S_1 的能量，可调控 IC 的发生。本章据此设计合成了在 1-萘胺氮上取代具有不同推电子能力基团的 1-萘胺衍生物，通过改变氮原子上取代基的推电子能力调节胺基的推电子能力，进而调节发射态 S_1 能量和调控 IC 的发生；同时通过改变水-有机溶剂混合比例来调整溶剂极性，进一步调控 IC 过程。

首先合成了 1-萘胺二乙酸钠(NADA)，以两个羧甲基 ($-\text{CH}_2\text{CO}_2\text{Na}$) 替代 DMAN 分子中两个甲基，并通过改变水与有机溶剂(甲醇、乙醇、正丙醇、异丙醇和乙腈)的比例以调整溶剂极性。考察 NADA 在不同极性溶剂中的荧光光谱，发现 NADA 荧光强度及荧光量子产率均随溶剂极性的减小而减小。与

DMAN 相比，两个氨基上推电子能力低于甲基的基团取代使得 NADA 分子中内转换过程加剧。

在此研究基础上，将 DMAN 中 $-CH_3$ 逐个被甲氧酰甲基 ($-CH_2CO_2CH_3$) 取代，合成了 N-甲基-N-甲氧酰甲基-1-萘胺 (MMAN) 和 N, N-二(甲氧酰甲基)-1-萘胺 (DCAN)，详细考察取代基推/拉电子能力和溶剂化效应对内转换的影响。依据 Weller 方程将发射态能量与荧光分子中电子给体的氧化电位相关联，较好地解释了实验结果。同时用 AM1 方法计算分子的结构参数，讨论了结构与性质之间的关系。

实验结果表明，1-萘胺的氨基氮原子上引入推电子能力低于甲基的取代基，提高了发射态能量，使得 IC 过程易于发生。因此可以通过改变电子给体/受体的给予/接受电子能力和溶剂极性来调控 IC 的发生，为建立基于 IC 原理的新的识别传感途径奠定了基础。

已知氨基酸能与铜等金属离子形成稳定的配合物，因此将羧甲基修饰到 1-萘胺分子之氨基氮原子上。合成了 N-(1-萘基)氨基乙酸 (NAA)，1-萘胺二乙酸钠 (NADA)，N-(1-萘基)-N', N'-二羧甲基-乙二胺 (NCEA)，设想通过与金属离子配位的途径改变萘胺分子中胺氮原子的电子云密度方法调控电荷转移 (CT) 态的能量发射和相应的 IC 过程，并据此建立金属离子识别与荧光传感新模式。

第三章首先考察了金属离子 Cu^{2+} 、 Co^{2+} 、 Ni^{2+} 和 Zn^{2+} 对 NAA 荧光光谱的影响，发现金属离子在含 30% 乙醇的水溶液中对 NAA 荧光发生不同程度的猝灭作用，由吸收光谱、猝灭的温度效应和荧光寿命的测定结果推测，金属离子对 NAA 荧光的猝灭作用属静态猝灭，即金属离子与 NAA 形成了稳定的配合物而导致荧光猝灭。 Cu^{2+} 对 NAA 的荧光猝灭远高于其它结构类似的金属离子，且不能与金属离子与 NAA 的配位常数相吻合。进一步实验表明所观察到的静态猝灭实际上可能是经由“作用球”将荧光体和猝灭剂吸引而导致的电子转移猝灭

灭，这种猝灭的发生需要有金属离子与荧光分子间的配位作用和配位组分合适的氧化还原能力匹配，因而赋予了方法对 Cu^{2+} 的高选择性，据此建立了 NAA 对 Cu^{2+} 的荧光传感体系。

类似的实验现象发生于 NADA 分子。由于 NADA 较 NAA 对金属离子具有更强的配位能力，因此金属离子 Cu^{2+} 对 NADA 的荧光猝灭常数更大。实验表明 Cu^{2+} 与 NADA 形成了 1:1 的配合物，配合物结构并由 X-衍射结果确定。

Cu^{2+} 、 Co^{2+} 、 Ni^{2+} 等金属离子与乙二胺的配位常数近似于和氨基乙酸的配位常数。实验发现，这些金属离子对 N-(1-萘基)-乙二胺(NEDA)的荧光猝灭常数基本在同一数量级，且远低于 Cu^{2+} 对 NAA 荧光的猝灭常数。这一现象的产生是由于 NEDA 末端胺具有更强的给电子能力，从而终止了由芳香胺向金属离子发生电子转移而导致的荧光猝灭。基于这一观点，设计了 N-1-萘基-N', N'-二羧甲基-乙二胺(NCEA)，在 NEDA 末端胺上引入羧甲基以降低末端胺氮原子上的电荷密度，使其给电子能力降低，使萘胺向金属离子的电子转移得以发生，同时增强荧光体与金属离子的配位能力。实验表明金属离子对 NCEA 的荧光猝灭程度远远大于对 NADA 的荧光猝灭，但对 Cu^{2+} 识别的选择性较差，这可能由于萘胺与金属离子距离较远而电子转移猝灭作用有所偏离所致。为提高 Cu^{2+} 识别选择性，借助表面活性剂 CTAB 的增敏作用，使得构建的荧光体系作为 Cu^{2+} 传感器可测低至 $1.480 \times 10^{-7} \text{ mol/L}$ 的 Cu^{2+} 。

糖在生物体的新陈代谢中起着重要的作用。因此，准确检测水溶液中一些重要的糖(如果糖、葡萄糖和甘乳糖等)是生物医学研究中的重要内容。基于硼酸对糖的特异性结合作用，以硼酸为糖的受体可建立高灵敏高选择性的糖识别体系。将硼酸基修饰到 1-萘胺荧光体中，合成荧光试剂 N-(邻-硼酸基)-萘基-1-萘胺 (BBAN) 并运用于单糖的识别研究乃第四章重要内容。本章中研究了 BBAN 在不同极性溶剂中的光物理性质。实验发现，在含 30% 甲醇的 $\text{pH}=8.21$

的磷酸盐缓冲溶液中，荧光体 BBNA 与单糖中 D-果糖结合常数最大。随着 D-果糖浓度增加，荧光强度增大，发射光谱略微红移。在 D-果糖存在下，BBNA 的荧光强度与量子产率随溶剂极性的减小而减小，预示着 IC 的存在，并据此建立对 D-果糖的新型识别传感体系。

自组装膜(SAMs)是近年发展迅速、性能优越的超薄膜，已在电分析化学领域显示出极大的优势。但在荧光分析中应用则鲜见报道。论文的第五章介绍了 SAMs 技术在荧光分析中应用的初步的研究工作结果。首先将半胱氨酸组装于金电极表面，控制溶液 pH 调整其存在形式，并将荧光体 NADA 通过与半胱氨酸的静电作用间接组装于金电极表面，成功地观察到了 NADA/Cys/Au 自组装膜/溶液界面荧光，并用电化学、X-光电子能谱和扫描电镜等方法表征自组装膜结构，建立了自组装膜对 Cu^{2+} 的识别与界面荧光传感。结果表明所观察到的自组装膜/溶液界面荧光较 NADA 溶液相荧光蓝移 35nm，可能是由于 NADA 于膜表面所处的环境与在溶液中的不同所致。NADA/Cys/Au 自组装膜测定 Cu^{2+} 的检测限可低至 0.2ppt，比单纯溶液相的(检测限为 599ppm)灵敏度大大提高。该结果为荧光分析提供了一种高灵敏度和高选择性的测定新方法，为发展基于自组装膜的新型界面荧光传感与分子识别方法进行了有益的尝试。

关键词

1-萘胺衍生物，内转换，分子内电荷转移，电子转移，N-取代基效应，溶剂效应，荧光猝灭，自组装膜，界面荧光，荧光传感，分子识别， Cu^{2+} ，糖，硼酸。

Design and Assembling of Fluorescent Receptors for Molecular Recognition and Chemosensing

ABSTRACT

This thesis consists of five chapters.

In Chapter One, the discovery and developments in the field of fluorescent sensing and molecular recognition were briefly reviewed. Examples include (1) important sensing mechanisms for metal ions, (2) design of recognition systems for saccharides, (3) recognition systems based on fast radiationless internal conversion (IC) and (4) fabrication and applications of self-assembled monolayers (SAMs). The research proposals of this dissertation were outlined.

Recent researches have shown that a fast radiationless internal conversion (IC) occurs in 1-dimethylaminonaphthalene (DMAN) *via* vibronic coupling of the proximate S_1 and S_2 states, in which S_1 is the emissive state of intramolecular charge transfer (CT) character. We assumed that varying the energy of the emissive S_1 by modifying DMAN molecular structure or varying solvent polarity could change energy gap ΔE (S_1 , S_2) between S_1 and S_2 states, therefore modulating the IC. In Chapter Two, DMAN derivatives were synthesized to reveal how the electron donating ability of the N-substituent influences the internal conversion.

Sodium 1-naphthylaminodiacetate (NADA) with two carboxymethylene groups substituted at the amino nitrogen atom was synthesized and its fluorescence spectra in aqueous-organic binary solvents with organic components of methanol, ethanol, *n*-propanol, *i*-propanol, and acetonitrile were investigated. It was observed that both the fluorescence intensity and quantum yield of NADA increased with increasing solvent polarity, suggesting that IC indeed occurred with NADA in aqueous-organic binary solvents. A decrease in the energy gap between S_1 and S_2 states was assumed for the enhanced internal conversion with NADA compared to

DMAN.

To further study the influence of N-substitution and solvent on the IC in 1-aminonaphthalene derivatives, we synthesized N-methyl-N-methoxycarbonylmethyl-1-aminonaphthalene (MMAN) and N, N-dimethoxycarbonylmethyl-1-aminonaphthalene (DCAN) replacing N-CH₃-substituents in DMAN by -CH₂CO₂CH₃. Results were analyzed in terms of Weller theory by correlating the emission energy with the oxidation potentials of the electron donor within the investigated fluorophores. Meanwhile, the ground-state structures of DMAN derivatives were optimized with the aid of AM1 calculations. The electron density of the nitrogen atom coincides well with the electrochemistry results which indicate that the amino groups in MMAN and DCAN had obviously lower donating ability that accounted for the enhanced IC. Substitution of -CH₂CO₂CH₃ on the amino group increases the dihedral angle between the amino and naphthyl plane moiety, which was supported by the observation of the blue-shift of the absorption maximum of DCAN and MMAN in organic solvents relative to that of DMAN and the ¹H NMR data of the chemical shifts of proton at C-2 of the MMAN and DCAN that are at lower field than that of DMAN. It was therefore concluded that the IC could be modulated by either modulation of the electron donating/accepting ability by N-substitution or solvatochromic effect.

Because of the impact of the intramolecular charge transfer (ICT) on the IC process, we decided to develop a molecule recognition and fluorescence sensing system of DMAN-like hosts. For this purpose, 2-(1-naphthyl)aminoacetic acid (NAA), sodium 1-naphthylaminodiacetate (NADA) and N-(1-naphthyl)-N', N'-carboxymethylethylenediamine (NCEA) were designed based on the chelating ability to a variety of metal ions of the well-known chelator, ethylenediamine tetraacetic acid (EDTA).

In Chapter three, we investigated the fluorescence spectra of NAA in aqueous solution in the presence of Cu²⁺, Co²⁺, Ni²⁺ and Zn²⁺, and found that the fluorescence of NAA was quenched by these transition metal ions to different

extents, of which the quenching by Cu^{2+} was substantially higher than those of the other metal ions of similar electronic structure, which did not completely followed the order of the stability constants of the metal-NAA complexes. Further investigations showed that there would actually be a kind of “static quenching” occurred within the complex in which the fluorophore and quencher were brought together like in a “sphere of action”. The fluorescence quenching was shown to be due to electron transfer within the metal-NAA complex, which required an optimal combination of high binding affinity and favorable redox properties of the components in the metal-NAA complex and hence afforded selective fluorometric detection of Cu^{2+} . The analytical characteristics of the NAA for Cu^{2+} were also assessed.

Similar results were found with NADA system, but now Cu^{2+} had higher quenching constant towards NADA fluorescence because of the stronger chelating ability of NADA. NADA was found to chelate Cu^{2+} in 1:1 stoichiometry that was supported by the crystal structure determination.

The stability constants of the investigated metal ions with ethylenediamine are similar to those with aminoacetic acid, it is therefore reasonable to assume that the binding properties of metal ions to N-(1-naphthyl) ethylenediamine (NEDA) are similar to those to NAA. We found, however, that the quenching constants to NEDA fluorescence of Cu^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} were all at the same order of magnitude and were much lower than that of Cu^{2+} to NAA fluorescence. These were explained in terms of that oxidation potential of the terminal amine is lower than that of the aromatic amine in NEDA, and hence in metal-NEDA complexes the electron transfer from the terminal amine to metal ions became more favorable, whereas the electron transfer from aromatic amine to metal ion that was assumed to be responsible for the fluorescence quenching would be stopped. To support this assumption, we designed NCEA in which the terminal amine was substituted by $-\text{CH}_2\text{CO}_2\text{H}$ to decrease the electron density at the nitrogen atom so that the electron transfer from aromatic amine to metal ions now became dominant. Experiments

showed that the quenching constants to NCEA fluorescence of the metal ions were much higher than those to NEDA fluorescence, but with poorer selectivity for recognition of Cu^{2+} , which might result from the longer distance between aromatic amine and metal ions in metal-NCEA complexes. The selectivity of NCEA for Cu^{2+} was found to be enhanced in the presence of cationic surfactant, cetyltrimethyl ammonium bromide (CTAB), and a detection limit of 1.480×10^{-7} mol/L for Cu^{2+} was reached.

It is well known that saccharides play a significant role in the metabolic pathways of living organisms, identifying and detecting concentration of biologically important sugars (such as glucose, fructose, galactose, etc) in aqueous solutions is necessary in a variety of medical and industrial contexts. Boronic acid-saccharide covalent complex readily forms in aqueous solutions and represents an important binding force in the recognition of saccharides and related species. Boronic acid based receptors offer the possibility of creating saccharide sensors. In Chapter Four, an internal Conversion (IC) fluorescence probe N-(*o*-boronic acid)benzyl-1-naphthylamine (BBNA) was prepared from 1-naphthylamine and 2-formylbenzeneboronic acid. The fluorescence parameters of BBNA were investigated in a variety of solvents. When BBNA interacted with D-fructose in phosphate buffer solution of pH 8.21/MeOH 2:1 (v/v), its fluorescence intensity of increased and emission maximum red shifted slightly with increasing D-fructose concentration. In the presence of D-fructose, fluorescence quantum yield of BBNA increased with increasing solvent polarity, suggesting that IC occurred with BBNA. The observed order of stability constants (K) of D-fructose > D-galactose > D-glucose, is the same as expected for monoboronic acids. Therefore, a selective recognition system based on IC was constructed for D-fructose.

A great attention has recently been paid to self-assembled monolayers (SAMs) of thiol or disulfide derivatives on gold surface, which are well-ordered, oriented, and easy prepared from a simple one step adsorption from dilute solutions. SAMs have been extensively applied to chemical sensors and biosensors, mostly based on

electrochemical methods. Although the applications of SAMs are effective in diminishing the sample volume with a view of on-chip analysis and have higher analytical sensitivity, little research has been carried out related to the development of fluorescence sensors because the fluorescence at SAMs is readily available quenched by gold substrate. In Chapter Five, an original research was carried out to develop fluorescence sensors based on SAMs. Cysteine was first assembled onto gold electrode. NADA, a fluorescent amino acid designed for selective and sensitive detection of Cu^{2+} in Chapter Three, was then indirectly assembled onto the cysteine modified gold by electrostatic interaction between NADA and cysteine. The self-assembled bilayer membrane NADA/Cys/Au was fabricated and characterized by cyclic voltammetry (CV) in combination with X-ray photoelectron spectra (XPS). The interface fluorescence emission wavelength of NADA (403nm) was found blue shifted from that of NADA solution (438nm), which may be ascribed to the altered environment the fluorophores experienced in the SAMs.

This NADA/Cys/Au electrode was used as fluorescence sensor for Cu^{2+} that showed excellent sensitivity in the determination of trace amount of copper ions, and a detection limit of 0.2ppt, at the order of magnitude lower than in bulk solution. The results indicated that assembling the fluorophores indirectly onto gold surface effectively avoided fluorescence quenching by gold and fluorescence sensors based on SAMs could be successfully fabricated. Results suggested the potentials of SAMs modified electrodes to be used in fluorescence sensors with substantially improved detection sensitivity, and hence the promising prospects in environmental and life science researches.

Keywords

1-Aminonaphthalene derivatives, internal conversion, intramolecular charge transfer, electron transfer, N-substitution effect, solvatochromic effect, fluorescence quenching, self-assembled monolayers, interface fluorescence, fluorescence sensor, molecular recognition, Cu^{2+} , saccharide, boronic acid.

第 一 章 绪 论

1.1 前言

分子识别是有机化学家和生物化学家在分子水平上研究生物体系中的化学问题时提出的。人们从酶与核酸的研究认识到生化系统巧妙的特异性，开始设计和合成一些比较简单的分子来模拟天然化合物的性质。早在 1984 年，Fischer 就曾用“钥匙和锁”的关系形容酶对底物的选择性结合。天然识别过程依据的是自然选择规律，如酶和底物、抗原-抗体、荷尔蒙-受体的反应具有很强的特异性。化学识别是通过分子设计，进行仿生模拟识别。分子识别化学是研究分子或分子集合体对特定分子进行选择继而结合的过程。利用分子彼此间的识别、结合特征，从中发掘高效、高选择性功能。若将具有识别部位的多个分子组合，分子彼此便会寻找最稳定最接近部位的位置，并形成超越单个分子功能的高级结构的分子集合体，称为超分子。在底物(主体)与受体(客体)结合的超分子体系中，维系分子之间的作用力是几种弱相互作用力的协同作用，其强度不次于化学键，而发生在分子之间的选择性结合过程为分子识别，图 1.1.1 简单描述了从分子化学到超分子化学的基本特征[1]。

运用在特定的结构区域内的非共价键，即多种分子间的相互作用(如静电力、氢键、范德华力等)的能量和立体化学方面特性的知识能够设计出人工受体分子，它们可以通过形成超分子结构，即具有明确结构和功能的“超分子”，选择性地与底物结合。它们既是分子组装体信息处理的基础，又是组装高级结构的重要途径之一[2~3]。因此，分子识别是超分子化学的核心研究内容之一。基于分子识别的荧光传感体系是超分子化学与光化学学科相结合的成就，并作为

一种全新的客体识别和分析手段，正迅猛发展。而与生命科学、环境科学、医学等领域密不可分的金属离子及中性分子糖的识别与荧光传感已成为人们所研究的热点之一[4]。本论文首先简单介绍荧光传感与分子识别领域所取得的一些最新研究成果及发展趋势，并在此基础上根据识别对象的不同设计新型荧光传感体系。

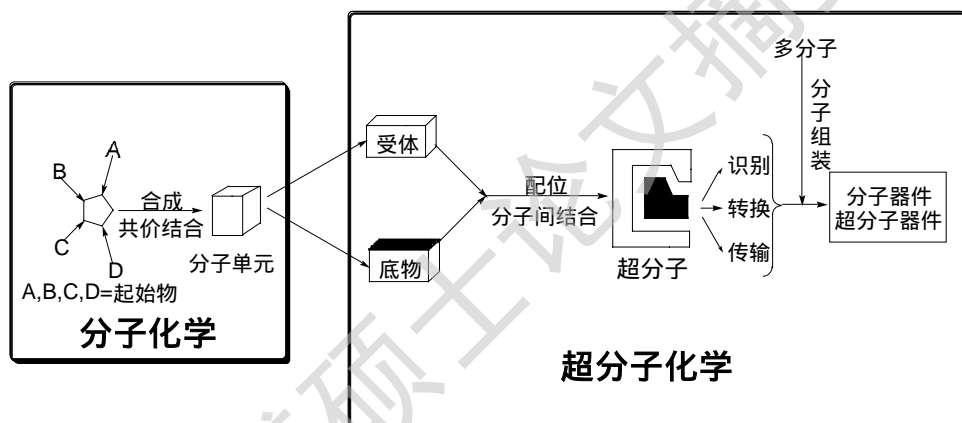


图 1.1.1 从分子化学到超分子化学的基本特征

1.2 金属离子识别与荧光传感

1.2.1 基于 PET 的荧光传感体系

在对金属离子识别体系中，基于光诱导电子转移 PET(Photoinduced Electron Transfer)的模式最为常见[5~8]。

作为荧光开关的 PET 体系由发光基团(fluorophore)、具有客体结合位点的受体 (receptor)和连接臂(spacer)组成，荧光团和受体通过连接臂连结在一起。若受体分子中发光基团的激发态能量足以氧化该分子的受体部分，则当受体分

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