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反应型铜离子荧光探针的研究进展

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摘　要

铜离子在生命体内发挥重要作用，其含量异常与多种疾病关系密切。对生命体内铜离子进行原位实时检测具有重要意义。荧光成像作为一种非侵入性的新型检测方法，具有高灵敏度、高选择性、响应快速等优点，在生物医学领域有重要应用。本文按照探针响应铜离子的不同反应机理进行分类，对近五年报导的反应型铜离子荧光探针进行了综述，以期铜离子荧光探针能收获更多重要成果。

关键词

荧光探针，铜离子，反应型探针

Review on the Development of Reaction-Based Fluorescent Sensor for Copper Ion

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Abstract

Copper ion plays important roles in living system, whose abnormality relates closely with many diseases. Thus it's meaningful to detect changes of copper ion in living systems *in situ* and in real-time. Fluorescent imaging is gaining great development in life science, which is characterized

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as non-invasive, high sensitivity and specificity, short response-time. We summarized the reaction-based fluorescent sensors for copper ion reported within the past five years, with the hope of more important works about fluorescent sensors for copper ion in the near future.

Keywords

Fluorescent Sensor, Copper Ion, Reaction-Based Sensor

1. 引言

铜离子是生命体内含量第三的过渡金属离子，在许多生理过程中发挥着重要作用[1]。Cu^{2+}含量异常与多种疾病密切相关。过量的Cu^{2+}可能诱发神经退行性疾病，如阿尔茨海默症[2]、威尔逊病[3]、帕金森病[4]、肌萎缩侧索硬化症(ALS) [5]等。Cu^{2+}缺乏则可能致使生长和代谢紊乱，导致冠心病[6]和贫血[7]。因此，检测生命体内铜离子含量具有重要意义。传统的检测方法包括原子吸收光谱法(AAS) [8]、电感耦合等离子体质谱法(ICP-MS) [9]、循环伏安法(CV)等[10]，这些方法在样品前处理、响应时间和价格等方面有很多局限性，需要复杂的设备、繁琐和耗时的预处理程序，无法对生命体内铜离子进行原位实时检测。因此，开发一种能够原位、快速、准确响应生命体内铜离子的方法具有重要意义。

荧光成像作为一种非侵入性的新型检测方法，具有高选择性和高灵敏度，操作简单、响应快速等优点，在生物医学领域得到广泛应用[11]。设计对 Cu^{2+}特异性响应的荧光探针已成为人们关注的重点。到目前为止，人们根据不同的机理设计出多种 Cu^{2+}荧光探针。利用 Cu^{2+}的顺磁性可猝灭荧光[12]，报道了多例开关型荧光探针[13] [14]，利用Cu^{2+}能诱导罗丹明螺内酯环开环反应报导了多例增强型荧光探针[15]。按照识别机理不同，可以把 Cu^{2+}探针分为两大类：基于配位相互作用的配位型荧光探针和基于化学反应的反应型荧光探针。配位型探针与 Cu^{2+}通过配位键结合，配位前后探针的荧光性质发生改变。通过增减配位原子的个数可以调节探针与 Cu^{2+}的配位能力，设计出不同线性响应范围的荧光探针[16]。配位作用一般是可逆的快速过程，因此这类探针有望实现对生命体内 Cu^{2+}的快速原位跟踪。通常选择性不高是配位型荧光探针的缺陷。反应型探针在 Cu^{2+}存在时发生特异性化学反应，生成的产物与探针有显著的荧光性质差异从而实现对 Cu^{2+}的检测[17]。鉴于大部分化学反应不可逆，反应型荧光探针通常较难实现可逆响应，但反应的高特异性使得这类探针通常具有较高选择性，对铜离子的识别不易受其他物种干扰[18]。本文重点对近五年来反应型铜离子荧光探针进行综述。

2. Cu^{2+}诱导螺环开环的反应型探针

罗丹明、荧光素等形成分子内螺环结构时，荧光团的大共轭结构被打破，探针没有荧光。分子内螺环打开后大共轭结构恢复，探针发出荧光信号。外部诱导剂，如 Lewis 酸等可以诱导螺环的开环反应。铜离子属于 Lewis 酸，具有催化活性，可以诱导分子中的取代基亲核进攻螺环并使螺环打开，生成有强烈荧光信号的产物。螺环结构的染料中，罗丹明及其类似物具有荧光量子产率高，光稳定性好，摩尔吸收系数大等显著优点，被广泛应用于荧光探针的开发。酰肼基团能特异性识别铜离子，常被用作 Cu^{2+}识别基团。以罗丹明及其类似物为荧光团，酰肼为识别基团，人们开发了一系列具有高选择性和高灵敏度

的 Cu^{2+}荧光探针。

2019 年，黄等人[19]报导了一种 Cu^{2+}荧光探针 BCX-Cu。探针分子内存在一个螺内酰肼结构，探针几乎没有荧光信号。当 Cu^{2+}存在时，探针发生螺内酰胺开环和酰肼水解反应，生成含有共轭大 π 结构荧光团的产物，在 570 nm 处释放出强烈的荧光信号并实现对 Cu^{2+}的高灵敏度响应(图 1)。该探针在生命体相关 pH 条件下荧光信号稳定，且不受其他生命体常见金属离子的干扰，对 Cu^{2+}的检测限较低，达到 88.7 μM。在 L929 细胞中探针能够响应外源性 Cu^{2+}浓度的变化。动力学实验表明加入铜离子后探针荧光信号 40 min 左右达到稳定，响应时间较长，不利于实时检测 Cu^{2+}变化，后续有望开发响应更迅速、更灵敏的荧光探针。

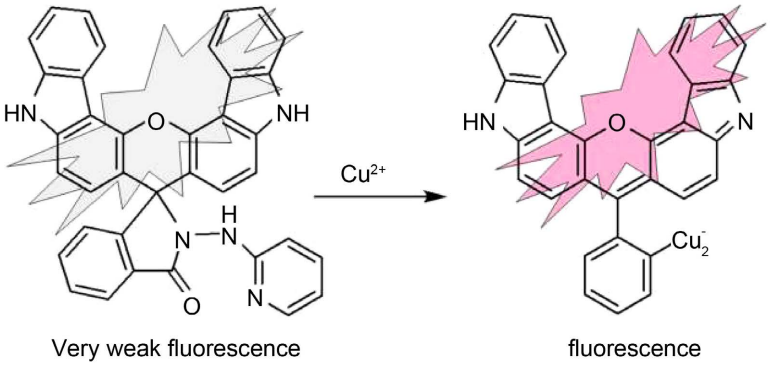

Figure 1. Sensing mechanism of probe BCX-Cu to Cu^{2+} [19]
图 1. 探针 BCX-Cu 对 Cu^{2+}的识别机理[19]

2021 年，Erman Karaku 等人[20]通过罗丹明与肼基膦酸酯基相结合设计了荧光探针 RhP。探针中罗丹明以螺内酰肼结构存在，探针几乎没有荧光信号。Cu^{2+}存在时与探针分子中的 N、O 和 P 原子同时发生配位作用，引发罗丹明的螺环开环反应，同时酰胺键断裂生成罗丹明 B，在 584 nm 处释放出强烈的荧光信号从而实现对铜离子的识别(图 2)。该识别过程需要 20 min 完成。由于罗丹明对环境 pH 变化敏感，该探针在中性及碱性环境下几乎没有荧光信号，而酸性环境下有明显的荧光信号产生，一定程度上减弱了探针对 Cu^{2+}的选择性，降低了信噪比。探针与 Cu^{2+}配合物的荧光信号较为稳定，在 pH 4 到 8 的范围内没有显著变化。同时生命体其他常见金属离子存在时探针也没有明显的响应，包括 Cu^{+}、Zn^{2+}、Cd^{2+}等。探针孵育的 HCT-116 细胞中几乎没有荧光信号，用探针和 Cu^{2+}共孵育后细胞中出现了明显的荧光信号，显示该探针能够响应细胞中外源性 Cu^{2+}浓度的变化。

2023 年杜等人[21]报导了一例含喹啉－荧烷杂合荧光团的探针 QFH。该探针亦是通过 Cu^{2+}诱导螺内酰胺的开环和水解反应释放荧光信号，从而实现对铜离子的选择性识别(图 3)。与罗丹明类探针不同的是，喹啉－荧烷杂合的荧光团本身对环境 pH 变化不敏感，pH 条件的改变不会使探针内的螺环开环而改变荧光信号，提高了探针的选择性。由于共轭结构更大，该荧光团的激发波长和发射波长较罗丹明而言都发生了红移，且斯托克斯位移近 80 nm，成像时受激发光干扰小。反应产物的荧光信号亦不受 pH 变化影响，其他常见金属离子不干扰探针对 Cu^{2+}的识别，因此探针 QFH 对 Cu^{2+}的响应具有高度选择性，且检测限(LOD)低至 1.54 nM。在 HeLa 细胞中探针能够响应外源性 Cu^{2+}浓度的变化。动力学实验表明铜离子存在时该探针的荧光信号需要 60 min 才能达到平衡，响应时间比较长，难以实时检测铜离子的变化。

2023 年孙等人[22]报导的一例探针 PEGR 能快速响应铜离子，30 s 内探针荧光强度增强近 29 倍，大大快于同类型的其他探针[19] [20] [21]。酰肼基取代的罗丹明其螺环闭环时探针没有荧光信号，铜离子与酰肼基团配位并导致酰胺键水解断裂，螺环开环给出罗丹明的荧光信号(图 4)。生命体常见物种中仅 Cu^{2+}

能特异性诱导这一开环反应，且铜离子加入后探针荧光信号 15 min 左右达到平衡，说明此探针能特异性快速响应 Cu^{2+}。在 HepG2 细胞和斑马鱼及食物中探针实现了对 Cu^{2+}浓度变化的响应。探针 PEGR 结构简单，对 Cu^{2+}的响应迅速，灵敏度高，线性响应范围宽(0~200 μM)，应用前景广泛。

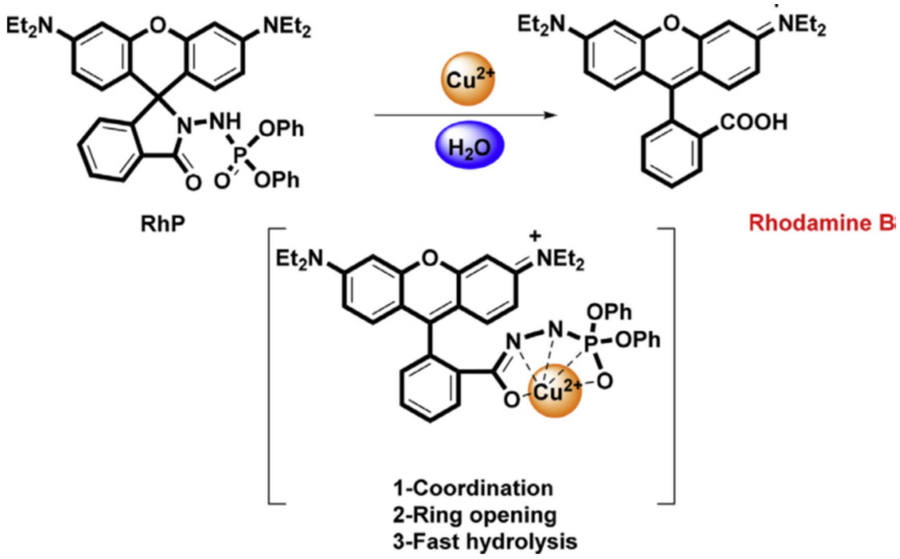

Figure 2. Sensing mechanism of probe RhP to Cu^{2+} [20]
图 2. RhP 探针与 Cu^{2+}离子传感机制[20]

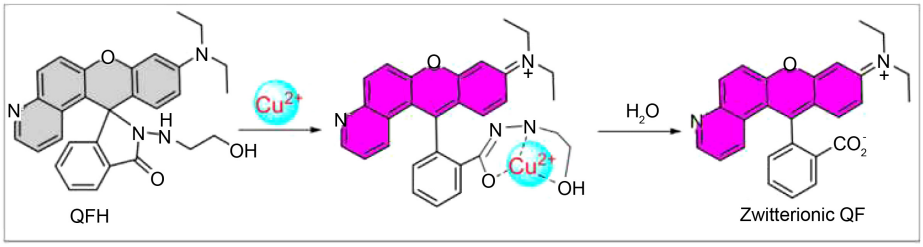

Figure 3. Sensing mechanism of probe QFH to Cu^{2+} [21]
图 3. QFH 探针与 Cu^{2+}的识别机制[21]

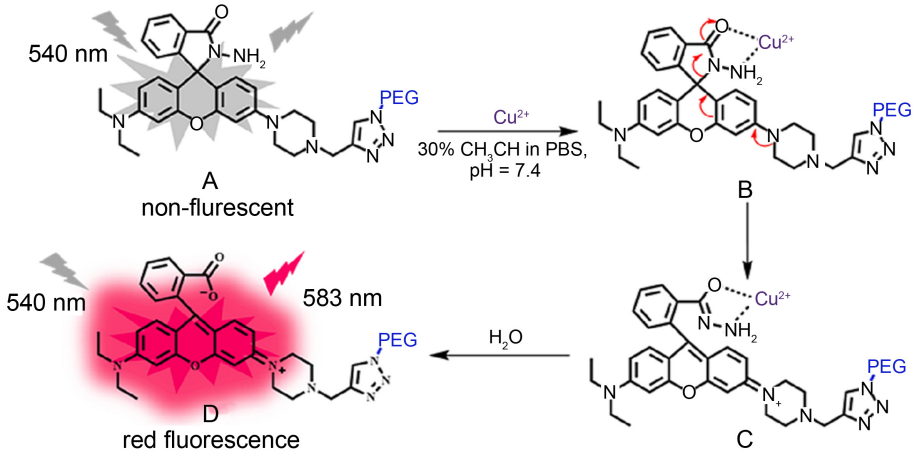

Figure 4. Sensing mechanism of probe PEGR to Cu^{2+} [22]
图 4. 探针 PEGR 对 Cu^{2+}的响应机理[22]

基于这一机理，还有多个课题组设计出选择性响应铜离子的荧光探针[23]-[38]，这一反应机理的探针都表现为荧光增强型，具有较高的响应灵敏度，能检测细胞内或环境样品中铜离子含量的变化。表 1 显示了这些探针的光物理性质、Cu^{2+}检出限(LOD)以及响应时间等。由于这类探针大多以罗丹明及其类似结构的染料为荧光团，激发波长大多在 600 nm 以下，发射波长在可见光区，活体成像应用时受到极大限制。事实上，所报道的此类探针除了少数在斑马鱼中进行了实验，几乎没有其他更深层次组织或活体中成像的报导。

Table 1. Property of ring opening reaction-based Cu^{2+} probes
表 1. 部分 Cu^{2+}诱导螺环开环的反应型探针的性质

Ref	Solvent	$\lambda_{ex}/\lambda_{em}$	Response time	Sensor type	LOD
[23]	$CH_3CN:H_2O$ (2:8, pH 7.2)	390/ [470↓/575↑]	80 min	Ratiometric response	152 nM
[24]	CH_3CN:HEPES (1:9, pH 7.4)	550/572	20 min	Turn-on	30.75 nM
[25]	CH_3CN:HEPES (2:3, pH 7.4)	530/593	30 min	Turn-on 35-fold fluorescence	78 nM
[26]	CH_3CN:HEPES (1:9, pH 7.4)	500/580	5 min	9 folds fluorescence enhancement	71.2 nM
[27]	CH_3CN:HEPES (1:1, pH 7.4)	510/585	30 min	Fluorescence enhancement	35.4 nM
[28]	CH_3CN:PBS (3:7, pH 7.4)	530/580	-	21 folds fluorescence enhancement	0.67 μM
[29]	$CH_3CN:H_2O$ (7:3)	550/618	-	Fluorescence enhancement	-
[30]	DMF:ACN:H_2O (8:1:1)	532/580	-	Turn-on	300 μM
[31]	$CH_3CN:H_2O$ (1:2)	520/575	-	30-fold fluorescence enhancement	89.9 nM
[32]	$MeCN/H_2O$ (1:1)	500/580	5 min	Turn-on	4.7 nM
[33]	EtOH:phosphate (1:1, pH 7.4)	530/579	60 min	384 folds fluorescence enhancement	120 nM
[34]	-	360/575	-	Turn-on	-
[35]	-	360/ [449/488]	30 min	Turn-on	-
[36]	CH_3CN:HEPES (1:1, pH 7.4)	555/585	10 min	Fluorescence enhancement	0.48 μM
[37]	CH_3CN	495/554	-	Fluorescence enhancement	19.1 nM
[38]	PBS (pH 7.4)	480/548	-	7-fold fluorescence enhancement	-

3. Cu^{2+}诱导配位水解的反应型探针

探针中的 N、O 等杂原子可以与金属离子配位，使探针的荧光性质变化以指示金属离子的存在。由于生命体内存在多种可与 N、O 原子配位的过渡金属离子，导致配位型探针的选择性不高，容易受到其他性质相近的金属离子干扰。对于配位水解型铜离子探针而言，Cu^{2+}与探针配位的同时进一步诱导环境中的水分子与探针分子反应，促使探针中的酯键、酰胺键等发生水解反应生成新的荧光分子。与单纯的配位型探针相比，这类探针受到其他金属离子的干扰较小，选择性明显提高。

2018 年付等人[39]报导了一例萘酰亚胺为荧光团的铜离子荧光探针 P，该探针通过 Cu^{2+}独特的催化水解机理发挥作用。萘酰亚胺本身荧光信号微弱，当 6 位被席夫碱取代的肼基占据时，探针 P 在近 550 nm 处发出强烈的荧光信号。Cu^{2+}能特异性引发探针 P 上 6 位的肼基发生水解反应，生成荧光信号微弱的萘酰亚胺，故这一探针为“开－关”形式的铜离子探针(图 5)。同时探针 P 及水解产物的紫外－可见吸收峰有明显位移，故铜离子诱导水解反应发生前后探针溶液的颜色有明显变化，这使得探针可以通过比色法和荧光法双模式检测铜离子(图 6)。由于光漂白等因素也能使荧光信号减弱，双模式检测一定程度上弥补了“开－关”型探针的缺陷。细胞实验显示在 293T 细胞中探针与溶酶体染料有较好的共定位效应，且随着孵育溶液中铜离子浓度的增大，细胞中荧光信号逐渐减弱，表明该探针能够响应外源性铜离子浓度的变化。

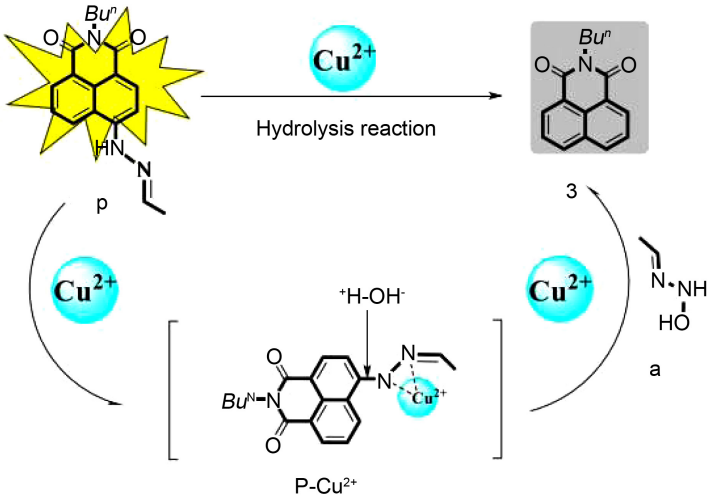

Figure 5. Sensing mechanism of probe P to Cu^{2+} [39]
图 5. 探针 P 与 Cu^{2+}离子的识别机理[39]

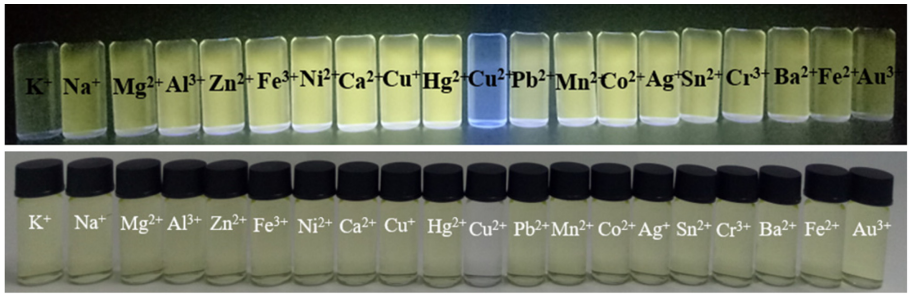

Figure 6. Photos of probe P with various metal ion under UV-light (up) and Visible light (down) [39]
图 6. 不同金属离子存在时探针 P 在紫外灯(上)及可见光(下)模式下的照片[39]

2020 年该课题组报导了另一例铜离子荧光探针 3 [40]。探针 3 与探针 P 结构上相似，都以腙为 Cu^{2+} 识别基团，探针 3 的腙基上增加了可参与 Cu^{2+}配位的噻吩(图 7)。Cu^{2+}存在时探针 3 的水解反应 3 min 即达到平衡，大大快于探针 P 的水解反应速率(近 20 min)，这应该是噻吩参与配位后，增强了探针与 Cu^{2+} 的配位作用，显著加速了水解反应，这一设计策略有助于设计快速响应的配位水解型铜离子探针，有利于对生命体内铜离子含量变化的快速实时检测。

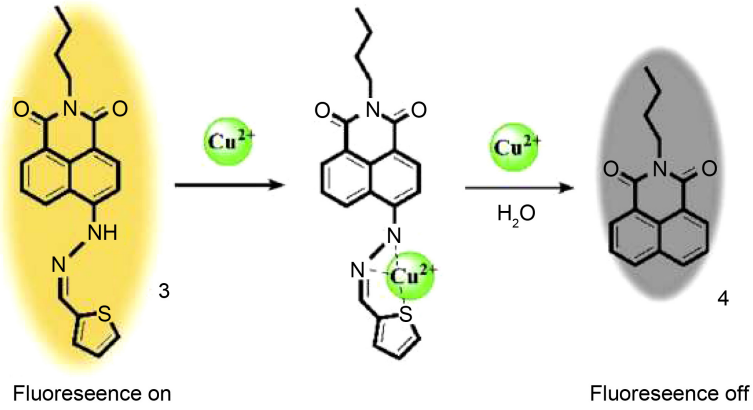

Figure 7. Sensing mechanism of probe 3 to Cu^{2+} [40]
图 7. 探针 3 对 Cu^{2+}的传感机制[40]

2022 年 Nadeem Ahmed 等人[41]报导了一例低检出限的铜离子荧光探针 NC-Cu。探针是由香豆素酮与 6-肼基萘酰亚胺通过缩合反应生成的腙类有机物，本身几乎没有荧光。Cu^{2+}能特异性诱导探针中的亚胺键水解，生成 6-肼基萘酰亚胺和香豆素酮，其中香豆素酮具有强烈的荧光信号，由此指示铜离子的存在(图 8)。生命体常见物种包括金属离子和还原性物种中，只有铜离子能够诱导水解反应发出明显的荧光信号。由于生成了 CuS 沉淀，所以选择性测试中 HS^-与 Cu^{2+}等量共存时没有观察到荧光信号，如果 Cu^{2+} 过量，应该可以观察到配位水解产物的荧光信号，即 HS^-不会干扰探针识别 Cu^{2+}含量异常升高。该探针在 KYSE30 活细胞中实现了对外源性铜离子浓度变化的响应。

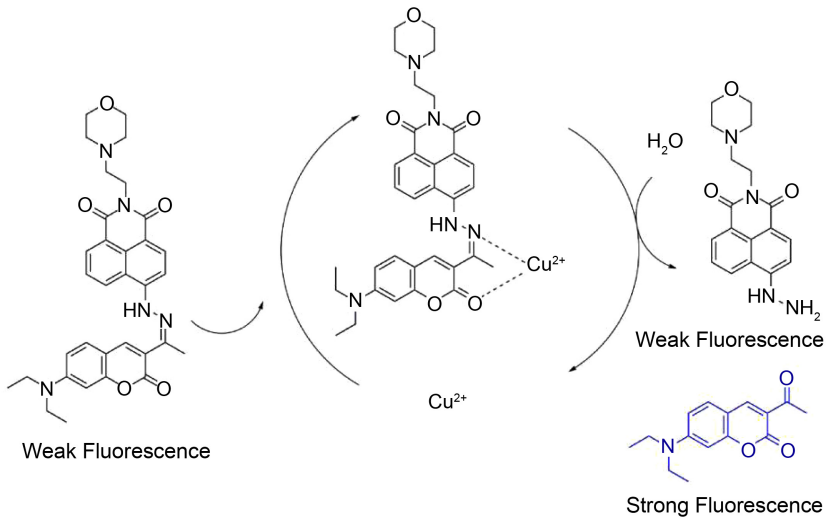

Figure 8. Sensing mechanism of probe NC-Cu to Cu^{2+} [41]
图 8. 探针 NC-Cu 与 Cu^{2+}的反应机理[41]

2023 年何等人[42]报导了以氨基脲为响应基团的铜离子探针 CAA。探针中存在 PET 效应所以荧光信号微弱，与 Cu^{2+}配位后发生水解反应，酰胺键断裂生成具有 ICT 效应的 7-氨基香豆素，438 nm 处出现明显的荧光信号指示 Cu^{2+}的存在(图 9)。这一配位水解反应对铜离子有选择性，当 Cu^{2+}在 0.1~30 μM 范围内，探针 438 nm 处的荧光强度与 Cu^{2+}浓度之间有良好的线性关系。用探针 CAA 孵育的 HeLa 细胞中荧光信号微弱，用 10 μM $CuCl_2$ 预处理后再用探针孵育，细胞中出现明显的荧光信号，提示该探针能够响应 HeLa 细胞中 Cu^{2+}浓度变化。

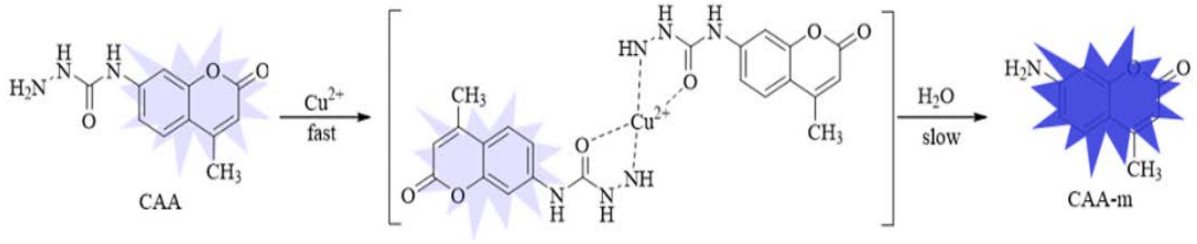

Figure 9. Sensing mechanism of probe CAA to Cu^{2+} [42]
图 9. 探针 CAA 对 Cu^{2+}的传感机制[42]

2-吡啶甲酰胺、腙都能与 Cu^{2+}选择性配位，2023 年 Akarasareenon 等人[43]报导了铜离子荧光探针 JP，以 N-吡啶酰基－腙为 Cu^{2+}识别基团，可同时与多个 Cu^{2+}配位。探针本身荧光信号微弱，Cu^{2+}与探针中 N、O 原子配位后，荧光团上的亚胺键水解并生成 9-醛基久洛尼定，在 420 nm 处给出明显的荧光信号(图 10)。这一配位水解反应同样对铜离子有选择性，探针能选择性识别铜离子。尽管探针与 Cu^{2+}以 1:2 比例配位，但这并没有能够加速探针的配位水解响应速率，对 Cu^{2+}的响应需要 60 min 才达到平衡。

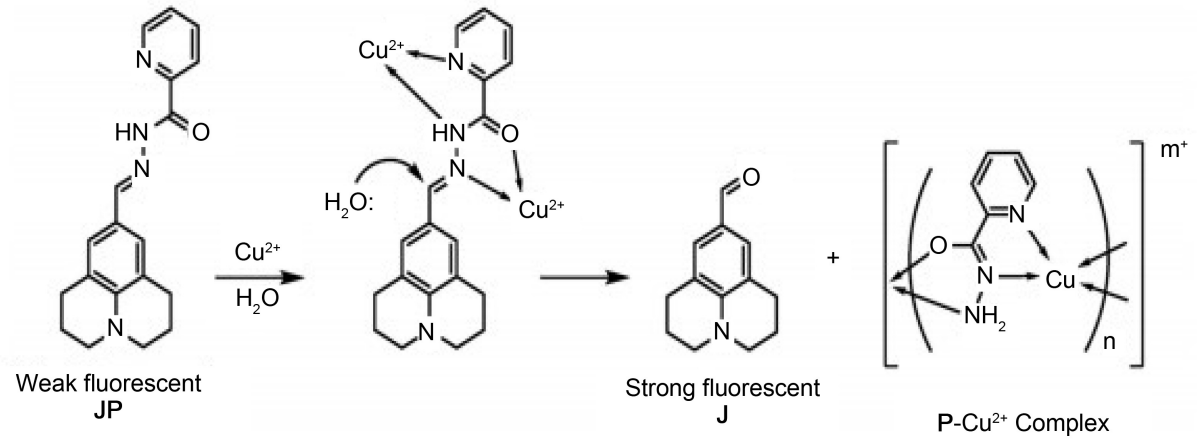

Figure 10. Sensing mechanism of probe JP to Cu^{2+} [43]
图 10. 探针 JP 对 Cu^{2+}离子的感应机制[43]

基于这一机理还报导了多例铜离子荧光探针[44]-[63]，表 2 罗列了这些探针的激发发射波长、检测铜离子的响应时间和检出限(LOD)等。尽管所报导的多例探针激发和发射波长属于可见光区甚至紫外光区，配位水解机理的探针可以选择激发与发射波长位于近红外区域的荧光基团，实现对活体深层次组织中的 Cu^{2+}检测。同时此类探针识别选择性高，对于响应迅速的配位水解型近红外铜离子探针，有望得到更多的研究和应用。

Table 2. Property of hydrolysis reaction-based Cu^{2+} probes
表 2. 基于 Cu^{2+}诱导配位水解反应型探针的性质

probe	Solvent	$\lambda_{ex}/\lambda_{em}$	Response time	Sensor type	LOD
44	Tris-buffer:DMSO (1:9, pH 7.0)	340/378	20 min	130-fold enhancement	59.3 nM
45	HOAc-NaOAc (1% DMSO, pH 5.0)	370/440	40 min	100-fold enhancement	19.4 nM
46	DMSO:Tris-buffer (1:1, pH 7.4)	470/570	15 min	Turn-on	200 nM
47	-	620/686	-	Turn-on	19.1 nM
48	HEPES buffer (pH 7.2)	460/510	300 min	13-fold enhancement	-
49	PBS (0.2% DMSO, pH 7.4)	475/580	12 min	Turn-on	30 nM
50 (NHB)	HEPES (pH 7.4)	410/540	15 min	-	0.06 μM
50 (NHC)	HEPES (pH 7.4)	460/555	15 min	-	0.03 μM
51	PBS (pH 7.4)	620/669	40 s	Turn-on	1.93 nM
52	PBS (pH 7.4)	430/570	-	Turn-off	493 nM
53	PBS (pH 7.4)	344/515	15 min	Turn-on	31 nM
54	HEPES (pH 7.4)	405/[495/560]	2 min	Turn-on	0.62 μM
55	HEPES (pH 7.4)	560/602	20 min	Turn-on	13 nM
56	H_2O/CH_3CN (4:1)	350/443	6 min	65-fold enhancement	8.5 nM
57	TBS solution (40% DMSO, pH 7.4)	500/618	40 min	Turn-on	0.12 μM
58	PBS-DMSO (1:1, pH 7.4)	560/676	40 min	Turn-on	20 μM
59	HEPES (pH 7.4)	350/523	20 min	Turn-on	-
60	Phosphate (pH 7.4)	515/630	15 min	Turn-on	52 nM
61	H_2O/CH_3CN (3:2, pH 7.0)	405/[461/474]	15 min	Ratiometric response	0.52 μM
62	H_2O:DMSO (9:1, pH 7.4)	580/610	15 min	Turn-on	54 nM
63	Ethanol:HEPES (1:1, pH 7.4)	360/453	-	Turn-off	52 nM

4. Cu^{2+}诱导氧化环化的反应型探针

环化反应是指在有机化合物分子中形成新的碳环或杂环的反应，也称闭环或成环缩合。环化反应是制备此类荧光探针的关键，需要巧妙的设计。目前，这类探针报道较少[64]-[69]。

2022 年，纪等人[64]通过香豆醛与 1-羟基-2-乙酰萘的缩合反应，制备了一种光稳定性好、量子产率高的“开启”型探针 1。探针本身表现出微弱的荧光特性，加入 Cu^{2+}后在 540 nm 处可以观察到明显增强的荧光信号。这是铜离子诱导的氧化环化反应生成类黄酮中间体(图 11)，这一机理由 NMR 和 ESI-MS 实验得到了验证。动力学跟踪显示 Cu^{2+}诱导的探针反应需要近 2 小时才能达到平衡，响应速率慢不利于实时跟踪成像。其他生命体常见金属离子和还原性物种单独存在于探针溶液中时不会干扰探针的荧光信号，但 Ag^{+}与 Cu^{2+}共同存在时，会显著抑制探针对 Cu^{2+}的识别响应。在 HeLa 细胞中该探针能够响应外源性 Cu^{2+}浓度的变化。

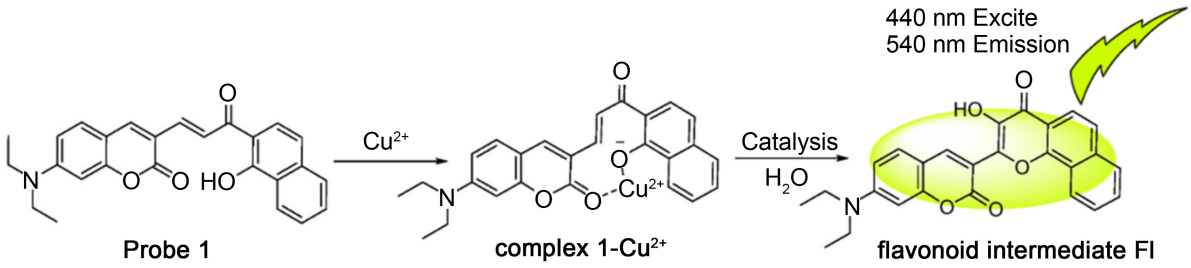

Figure 11. Sensing mechanism of probe 1 to Cu^{2+} [64]
图 11. 探针 1 对 Cu^{2+}的识别机理[64]

2022 年 Okamoto 等人[65]报导了一例高选择性检测 Cu^{2+}的新型荧光探针 OAHP。该探针本身荧光信号微弱，铜离子存在下探针中酰胺键断裂同时分子内发生环化反应，生成荧光信号强烈的恶二唑衍生物(图 12)。该探针对铜离子的识别具有高选择性和高灵敏度，成功应用于自来水样品及 HepG2 细胞内 Cu^{2+}的监测。与纪等人[64]所报导的荧光探针达到平衡需要 2 h 相比，这一探针 40 min 就能达到平衡，大大缩短了响应时间，检出限也更低，探针灵敏度更高。

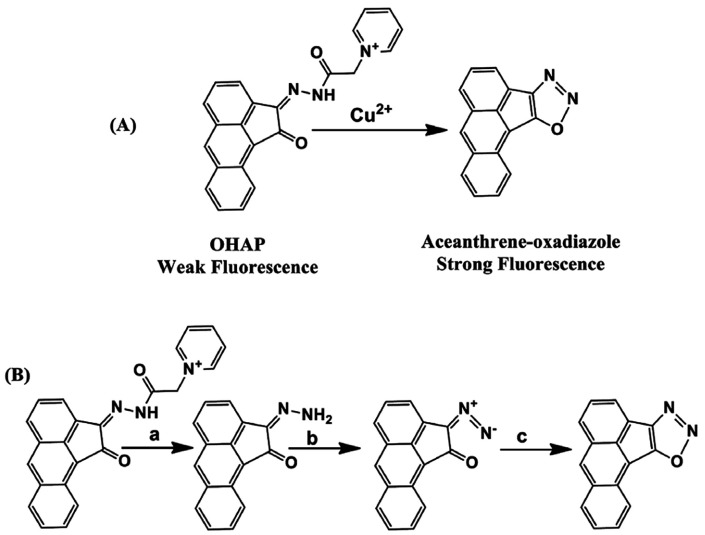

Figure 12. Sensing mechanism of probe OHAP to Cu^{2+} [65]
图 12. 探针 OHAP 对 Cu^{2+}离子的识别机制[65]

5. 总结与展望

本文主要对近五年报导的反应型铜离子荧光探针进行了综述，举例阐述了各类探针的响应机理。尽管反应型铜离子探针已经报导了上百例，但仍有较大的探索空间，特别是氧化环化反应型探针。由于铜离子发挥着重要的生理、病理作用，从活体成像的需求来看，探针除了要满足生物毒性小，光稳定性好，激发及发射光位于近红外光区等条件以外，由于生命体内铜离子始终处于动态变化过程中，原位实时动态跟踪的需求要求探针能够对铜离子可逆成像，即反应型探针与铜离子之间的反应需要可逆，已经报导的探针中尚没有满足这一要求，未来或许有望解决这一需求，取得更多重要成果。

致　谢

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