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Recent Progress in Zn(II)-Coordinated Molecular Probes for Pyrophosphate Detection

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Abstract: Pyrophosphate (PPi) is an important biomarker involved in numerous intracellular processes and is associated with several human diseases. It is critically involved in a variety of biological reactions, particularly in the formation of nucleic acids (DNA and RNA). Thus, the advancement of selective receptors for PPi ions that allow real-time monitoring of intracellular PPi levels in living systems is of great importance. PPi is found in synovial fluid, blood plasma, and urine at concentrations that effectively inhibit abnormal calcification by acting as a natural suppressor of hydroxyapatite formation. It is also involved in essential biological functions such as cell growth, chromosomal elongation, energy storage and transduction, and iron delivery. However, elevated levels of PPi can lead to complexation with calcium ions, resulting in the formation of microscopic calcium pyrophosphate crystals that deposit in joints and cause calcium pyrophosphate deposition (CPPD) disease. Due to its vital biological functions and pathological relevance, the development of novel chemosensors capable of selectively detecting PPi in aqueous environments under physiological conditions continues to be a compelling and dynamic research focus. Over time, numerous chemosensors have been reported for PPi detection, with fluorescent systems receiving special interest due to their exceptional sensitivity, accurate detection, real-time measuring potential, and relevance in bioimaging applications. Among luminescent probes, Zn(II)-based metal complexes stand out for their excellent biocompatibility and favorable water solubility. This review systematically compiles and discusses Zn(II) metal complex–based chemosensors designed for the selective recognition of pyrophosphate ions.

Keywords: Pyrophosphate, molecular recognition, sensing, PPi, bio-imaging, Zn(II) complex

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1. Introduction:

Phosphate and phosphate-derived anions occupy a central position in chemistry, biology, and materials science. In living systems, inorganic phosphate (Pi) and its condensed forms regulate a wide spectrum of essential processes, including redox balance, intracellular signaling, energy metabolism, enzymatic regulation, skeletal development, and biomineralization.[1,2,3,4] Additionally, they were crucial in the synthesis of other biomolecules, including DNA and RNA.[5] A significant component of membrane lipids, or phospholipids, is phosphates. DNA polymerization, cellular breakdown of adenosine triphosphate (ATP), and other metabolic processes produce pyrophosphate (PPi).[6,7] It is a crucial marker for the diagnosis of a number of clinical disorders, such as the aberrant increase in PPi levels seen in individuals with calcium pyrophosphate crystal deposition illness. Pi is generated during the polymerization reaction of the nucleotides and also developed during the biosynthesis of various biomolecules viz., cAMP, cGMP, and steroids.[8] Enzymes like pyrophosphatase regulate the PPi concentration in body fluid and dysfunction of these enzymes elevated the PPi level that induces several diseases such as osteoarthritis and pseudogout.[9] Additionally, PPi participates in the biosynthesis of guanosine 3'-diphosphate-5'-diphosphate (ppGpp), a crucial regulatory mechanism controlling gene expression, in a number of bacteria and plants.[10] The release of PPi during nucleotide incorporation has also been exploited in real-time DNA sequencing technologies and cancer diagnostics, further underscoring its biomedical relevance.[11] PPi also possesses notable industrial importance. It has been investigated as a functional material in lithium battery electrodes and as a catalyst for the generation of maleic anhydride from *n*-butane.[12] The broad biological, clinical, and technological significance of PPi has therefore stimulated sustained efforts toward the development of efficient recognition and sensing strategies.

Despite this interest, selective recognition of PPi remains fundamentally challenging. The anion exhibits exceptionally high hydration energy (−2765 kJ mol^{-1}) and strong solvation in aqueous media, which significantly weakens receptor–anion interactions.[13] Furthermore, its high charge density and structural similarity to other biologically relevant phosphates such as ATP, ADP, and inorganic phosphate complicate selective discrimination. Traditional hydrogen-bond-based receptors often suffer from limited performance in competitive aqueous environments, highlighting the need for alternative recognition strategies. Metal-coordination approaches have proven particularly powerful in addressing these challenges.

Nature itself provides an instructive model: the bacitracin–Zn(II)–lipid complex demonstrates how Zn^{2+} ions coordinate with peptide backbones while simultaneously binding to pyrophosphate oxygen atoms, forming stable metal–oxygen frameworks. This coordination-driven recognition illustrates the strong affinity of Zn^{2+} toward oxygen-rich phosphate species and has inspired the rational design of synthetic zinc-based receptors.[14,15]

Zinc(II) is especially attractive for PPi sensing due to its hard Lewis acid character, flexible coordination geometry, moderate binding strength, and biocompatibility. Zn^{2+} preferentially interacts with hard Lewis bases such as oxygen donors, enabling robust Zn–O coordination with pyrophosphate. Importantly, zinc complexes often exhibit good aqueous solubility and minimal redox activity under physiological conditions, making them suitable for biological applications. Over the past two decades, these characteristics have been strategically exploited in the development of mono-, di-, and multinuclear Zn(II) complexes capable of selectively binding PPi even in highly competitive media. A diverse range of ligand frameworks—including dipicolylamine (DPA), terpyridine, quinoline derivatives, phenolate-based systems, and macrocyclic architectures—has been incorporated into Zn(II)-based chemosensors. These systems employ various photophysical transduction mechanisms such as photoinduced electron transfer (PET), chelation-enhanced fluorescence (CHEF), intramolecular charge transfer (ICT), restriction of intramolecular rotation (RIR), and excited-state intramolecular proton transfer (ESIPT). Through these mechanisms, PPi binding can generate fluorescence “turn-on,” “turn-off,” ratiometric, or cxolorimetric responses. Many reported probes demonstrate nanomolar detection limits and remarkable selectivity over structurally analogous anions, representing substantial progress in the field.

Nevertheless, challenges remain in achieving high selectivity in fully aqueous environments, minimizing interference from ATP and other polyphosphates, improving real-time imaging capability, and enhancing structural tunability continue to drive research efforts. Consequently, systematic evaluation of structure–function relationships in Zn(II)-based systems is essential for advancing next-generation PPi sensors. This review provides a comprehensive overview of Zn(II)-based molecular receptors for PPi detection, encompassing mononuclear, dinuclear, and trinuclear architectures. Particular emphasis is placed on coordination design principles, photophysical signaling mechanisms, binding stoichiometry, analytical performance, and biological applications. By critically examining recent advances, this review aims to highlight key design strategies, identify remaining

limitations, and outline future directions for the development of selective and sensitive sensing platforms for PPi in water and biological mediums.

2. Design and development of chemosensors

Over the decades researchers observed nature as a source of inspiration for the design of efficient binding sites for both cations and anions. Bacitracin provides a representative example, featuring a well-organized binding pocket composed of Zn(II) and Na(I) centers in conjunction with amino acid residues from the peptide framework, enabling effective recognition of lipid PPi anions. The crystallographic study shows that the peptide backbone of the Bacitracin–Zn(II)–lipid complex folds around and encapsulates the PPi group of the lipid substrate. Zn(II) centre of the Bacitracin–Zn(II)–lipid complex coordinates with two 'O' atoms of the PPi moiety and other oxygens coordinates with the amino fragment of the peptide backbone and water molecule (Figure 1).[16]

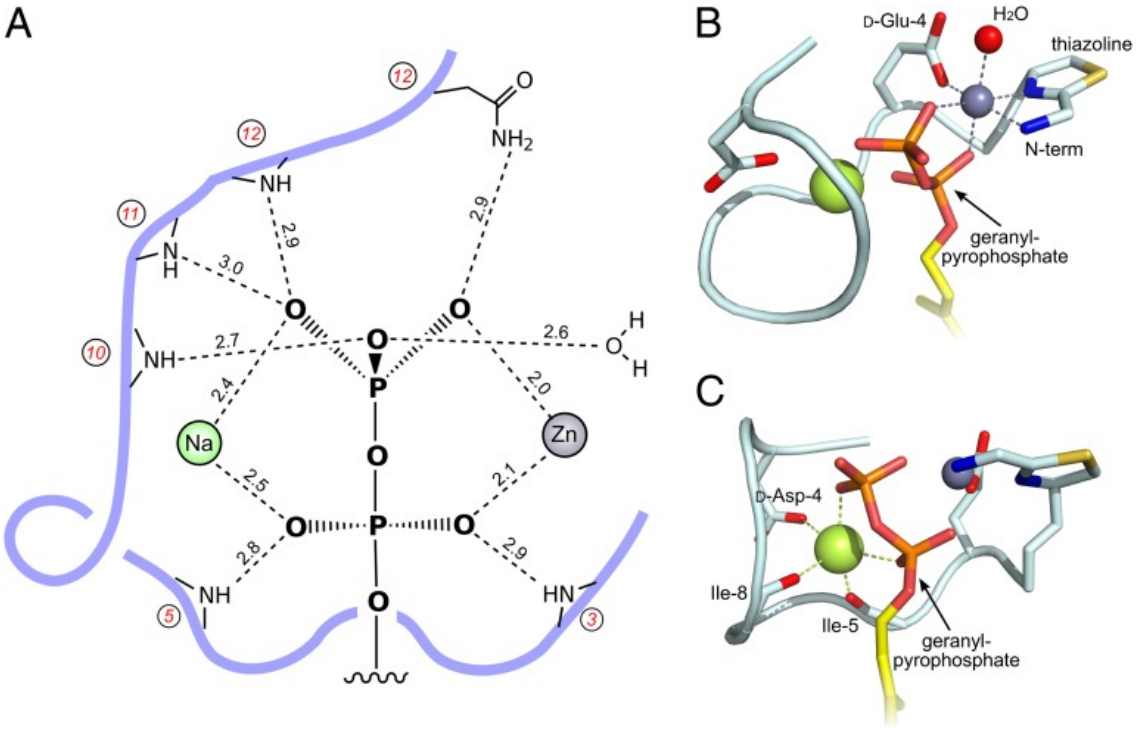


Figure 1: (A) Interaction of bacitracin with the lipid pyrophosphate moiety, highlighting interatomic distances (Å) between ligand oxygen atoms, hydrogen-bond–donating groups of the antibiotic, and associated metal ions. Residue numbers corresponding to specific backbone or side-chain groups of bacitracin are indicated by red circled labels. **(B)** Zinc ion coordination environment. **(C)** Sodium ion coordination environment. The color scheme

matches that used in Fig. 1A.[16] Reproduced with permission from ref. 16. Copyright 2013, Springer nature.

The coordination behavior of natural complexes, including Bacitracin–Zn(II)–lipid complex, has inspired biomimetic approaches toward the synthesis of zinc-based probes for the selective recognition of PPi. Further, Zn^{2+} complexes are widely used for the detection of PPi because Zn^{2+} has a unique combination of coordination chemistry, binding strength, selectivity, and compatibility with sensor designs**.** PPi is highly negatively charged ($P_4O_7^{4-}$) and contains multiple oxygen atoms.

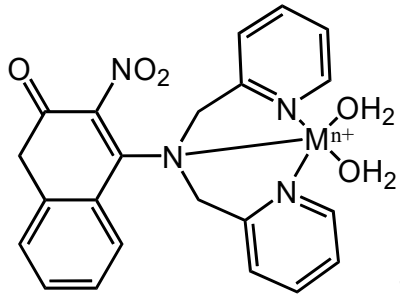
Zn^{2+} is a hard Lewis acid**,** so it preferentially binds to hard Lewis bases**,** especially oxygen donors. This makes Zn^{2+}–O coordination particularly strong and stabilizes Zn^{2+}–PPi complexes.

2.1. Zn (II) based chemosensors for the recognition of PPi

2.1.2. Mono Zn(II)-complexes

Chemosensors having one Zn(II) centre are widely used for the selective detection of PPi anion. The Zn(II) centre has vacant coordination sites which could coordinate with the PPi anion. Researchers commonly used dipicolyl-amine, terpyridine, hydroxyl amine, diamino pyridine based ligand an then complexed with Zn(II) ion for the detection of PPi anion.

Das and colleagues attached coumarin derivative with the dipicolylamine (DPA) moiety and used for cation recognition.[17] They observed turn-on emission response for Zn(II) and Cd(II). In order to explore recognition of anions and corresponding potential bioanalytical application the group further developed Zn(II) and Cd(II) containing metal complexes of the ligand (**1a** and **1b**; Figure 2) . Both the complexes selectively recognize pyrophosphate (PPi) in water medium. Analysis of the fluorescence titration data revealed relative binding affinities for **1a** and **1b**, towards PPi and the respective association constant values are 3.18 x 10^5 M^{-1} and 2.68 x 10^4 M^{-1} respectively. Benesi–Hildebrand plot and mass spectra analysis confirmed 1 : 1 adduct formation between the host and the guest. Authors presume that, the higher electron density around the guest makes it superior for binding towards M^{2+} centre over ATP and Pi. Finally these chemosensors were also used for the evaluation of the enzyme activity of alkaline phosphatase.

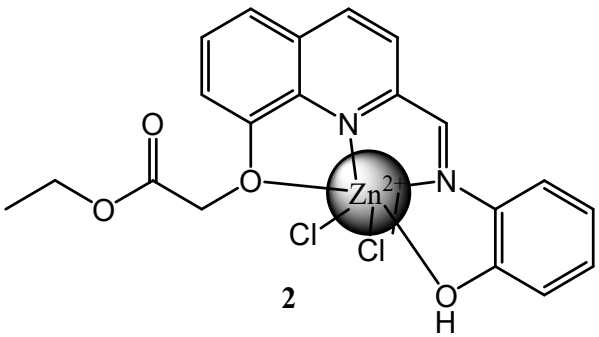


1a; M= Zn

1b; M= Cd

Figure 2: Schematic representation of chemosensor **1a** and **1b**. Reproduced with permission from ref. 17. Copyright 2011, Royal society of Chemistry.

Li and coworkers developed a mononuclear Zn(II) complex of 8-hydroxyl quinoline derivative (**2**; Figure 3) and used for the colorimetric recognition of pyrophosphate in aqueous medium.[18] They revealed the crystal structure of the Zn(II) complex obtained from single crystal X-ray diffraction. In presence of PPi in ethanol/water medium the intensity of the UV-vis absorption of **2** at 504 nm diminished and the red colour of the solution becomes colourless. This colour change occours because PPi oxygen strongly coordinated with Zn(II) than the phenolic hydroxyl group of the ligand, thus Zn(II) come out the complex and the ligand becomes free. Among the various ions tested, the metal complex selectively detects PPi. Furthermore, nanofiber of the complex was successfully prepared via electrospinning, and its reddish colour also becomes colourless after immersion into the pyrophosphate solution. The nanofibers also selectively detect PPi.



2

Figure 3: Schematic representation of chemosensor **2**. Reproduced with permission from ref. 18. Copyright 2015, Royal society of Chemistry.

Zhang and co-workers developed a single conical nanochannel immobilized with a terpyridine derivative (TPYD) via covalent attachment (**3**; Figure 4).[19] When terpyridine functionalized nanochannel exposed to Zn^{2+}, the transmembrane ionic current was decreased. This decreased ionic current is due the formation of the Zn^{2+} complex which increased the positive charge on the nanochannel surface. Zn^{2+}- TPYD complex immobilized nano channel was used for the selective detection of pyrophosphate (PPi). Upon treatment with PPi, the ionic current increased as the nanochannel surface became more negative upon PPi binding. The nanochannel exhibited excellent selectivity for Zn^{2+} and PPi.

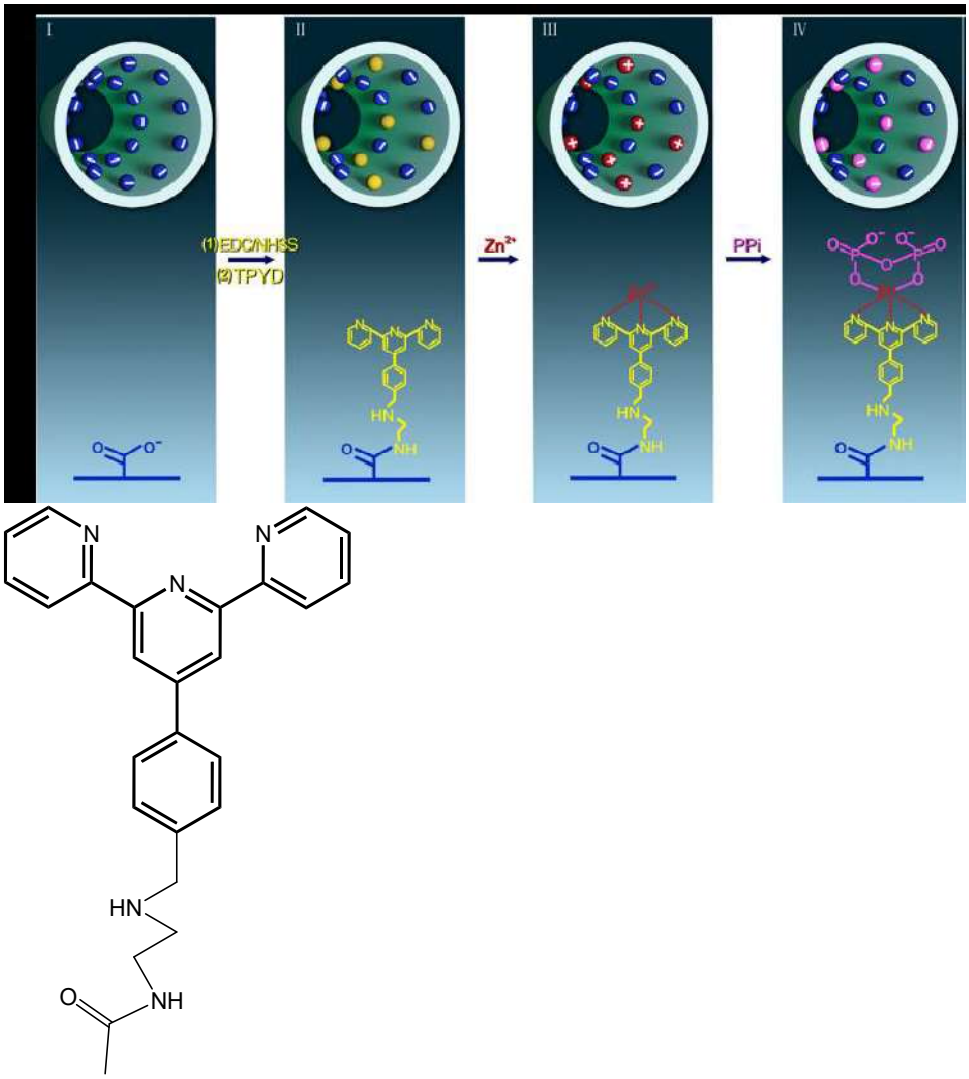


Figure 4: Schematic representation of chemosensor **3.** Reproduced with permission from ref. 19. Copyright 2017, Wiley.

In order to develop selective chemosensor pyrophosphate in aqueous medium Rissanen and coworkers developed a Zn(II)-terpyridine complex (**4**; Figure 5).[20] The chemosensor exhibited a remarkable luminescence response (~500 times enhancement) at 591 nm in presence of 50 μM of PPi and could be able to detect even 0.8 nM of PPi. This Zn(II) complex could also be able to detect PPi in live HeLa cells using confocal fluorescence microscopy. Furthermore, the complex also self-assembled into a hydrogel which further utilized to make paper strip based chemosensor device, for easy and low-cost detection of pyrophosphate.

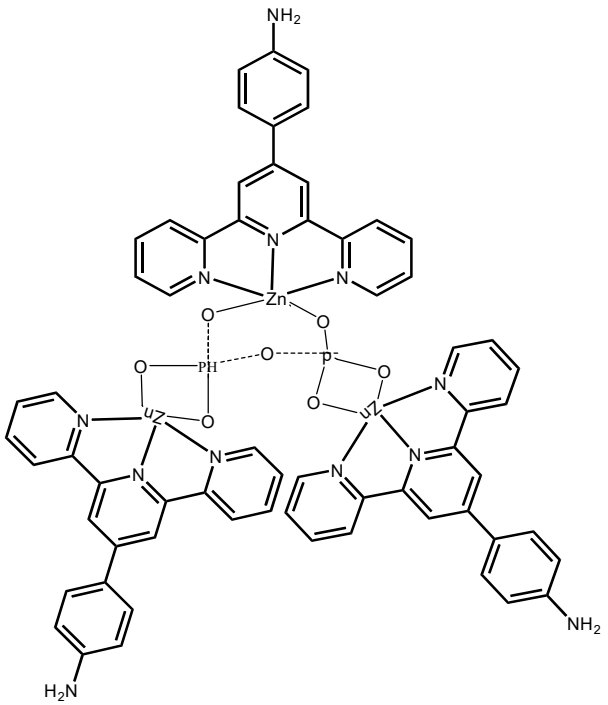
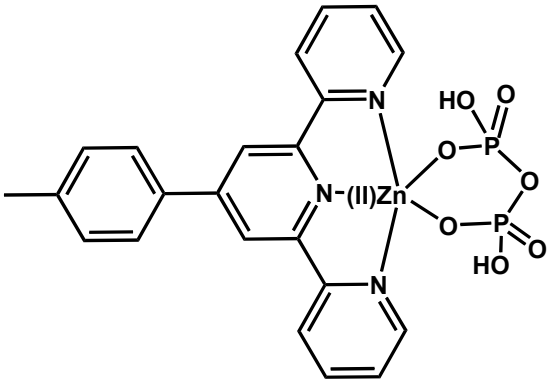


Figure 5: Schematic representation of the Zn(II)-terpyridine based chemosensor **4**. Reproduced with permission from ref. 20. Copyright 2014, American chemical society.

Huang et al. developed Zn(II) complex of 4-(methylphenyl)-2,2':6',2''-terpyridine (mptpy) (**5**, Figure 6) for the selective detection of PPi.[21] Among the many anions tested in aquoues media, spectrophotometric analyses showed that the Zn(II) complex specifically identified PPi. Upon gradual increase of PPi the luminescence of the complex at 406 nm was 18 nm hypsochromic shifted, with enormous enhancement in the emission The complex's luminescence at 406 nm was blue shifted to 388 nm with a significant increase in emission intensity upon a progressive increase in PPi. The metal complex could amazingly

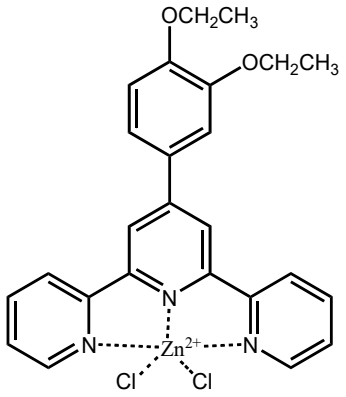
differentiate PPi among other competitive biological phosphates viz, ATP, UTP, CTP, GTP, Pi and some other inorganic anions. This extreme selectivity of the metal complex towards PPi in aqueous medium is quite extraordinary in the field of molecular recognition. The group claimed that the synthetic variation of terpyrine based ligand could produce more efficient chemosensors for the important biological target anion PPi.



5

Figure 6: Schematic representation of chemosensor **5.** Reproduced with permission from ref. 21. Copyright 2012, The Royal society of Chemistry.

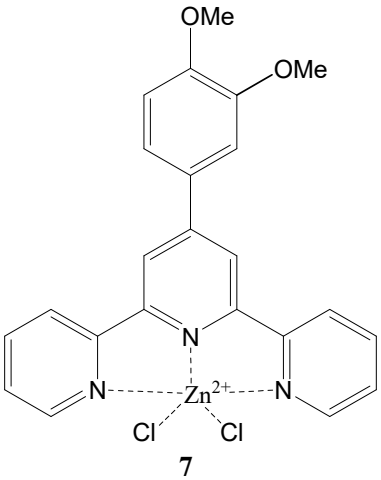
In order to create a selective chemosensor for PPi, the Ghosh group synthesized a novel luminous terpyridine derivative **6** (Figure 7), which subsequently complexed with Zn(II).[22] Numerous analytical methods, including NMR, mass spectroscopy, CHN analysis, and DFT investigation, were used to characterize the chemosensor. When ten equivalents of other competing anions were present, the chemosensor was able to identify PPi in an aqueous media. The chemosensor made a 2:1 adduct formation with PPi. The receptor was able to recognize even as low as ~ 27.89 nM concentration of PPi. Additionally, the receptor **7** was used for antioxidant qualities, antiproliferation research on human breast cancer MDAMB-231 cells, and real-time PPi measurement. With an IC50 value of 53.85 μM, the chemosensor demonstrated outstanding cytotoxic action against MDAMB-231 cells.



6

Figure 7: Schematic representation of chemosensor **6.** Reproduced with permission from ref. 22. Copyright 2023, Elsevier.

The Kar group synthesized a novel Zn(II) complex of a terpyridine derivative (**7**; Figure 8) and employed it as a chemosensor for anion detection in aqueous media.[23] The sensor selectively recognized PPi in aqueous medium under physiological conditions over other common anions. Upon PPi binding, the sensor exhibited a 50-fold enhancement in emission at 502 nm, accompanied by visible cyan fluorescence. Job plot analysis verified a 1:3 binding stoichiometry between the PPi guest and the Zn(II)-based host. Competitive experiments showed that the presence of other anions did not interfere with PPi detection. The group proposed that this chemosensor could be a useful probe for identifying PPi in ecological and biological materials. Additionally, the sensing mechanism of the Zn(II)-terpyridine complex was elucidated through DFT calculations.



7

Figure 8: Schematic representation of chemosensor **7.** Reproduced with permission from ref. 23. Copyright 2021, Elsevier.

Chen group also used a Zn^{2+}-terpyridine (Zn(II)-tpy) based luminescent chemosensor for the selective recognition of PPi.[24] They have reported a series of six Zn(II)-tpy complexes for this purpose. The group varied the no of electron donating groups in the benzene ring of the tpy moiety, also they varied hydrophobicity of the sensors. Among the Six different Zn^{2+}-complexes the sensor having -O-CH_3 groups at the meta positions of the benzene ring (**8;** Figue 9) can recognize PPi in water medium. This probe showed good selectivity for pyrophosphate over competing nucleoside triphosphates . Further the presence of an extra –$OCH_2CH_2CH_3$ at the para to the phenyls group (**9;** Figue 9) increases the emission signal speed of the sensor towards the analyte.

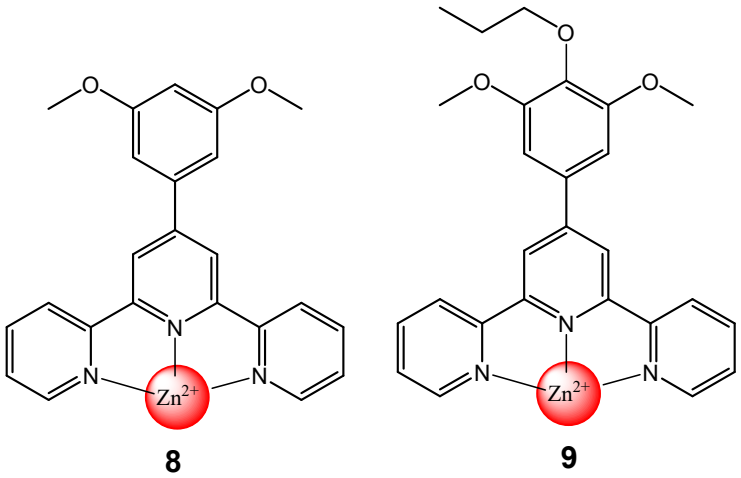


Figure 9: Schematic representation of chemosensors **8** and **9.** Reproduced with permission from ref. 24. Copyright 2025, Elsevier.

Very recently Liu *et al.* developed a multifunctional near-infrared fluorescent chemosensor (DHP) (9a; Figure 9a) for the sequential detection of Zn^{2+} and pyrophosphate (PPi).[25] The probe was constructed by integrating a dicyanoisophorone fluorophore with a di(thiophen-2-yl)ethyl amide recognition unit and exhibited prominent aggregation-induced emission (AIE) characteristics. In a THF/HEPES buffer system, DHP selectively recognized Zn^{2+}, producing a significant fluorescence enhancement at 660 nm through a fluorescence “turn-on” response. The sensor demonstrated a rapid response time of approximately 4.7 s and a low detection limit of 0.13 μM, together with a large Stokes shift of 160 nm, which minimized excitation-light interference. The resulting DHP–Zn^{2+} complex subsequently acted as a fluorescent probe for PPi, affording a detection limit of 0.39 μM. The Zn^{2+}- and PPi-sensing mechanisms were elucidated using ^{1}H NMR titration, FT-IR, HRMS and DFT calculations. Importantly,

the probe showed practical applicability in smartphone-assisted RGB detection of Zn^{2+} in real water samples, portable swab-based analysis, Zn^{2+} determination in plant tissues and fluorescence imaging of Zn^{2+} and PPi in living cells. Overall, this work highlights the potential of an AIE-active dicyanoisophorone platform for rapid, sensitive and multifunctional detection of biologically and environmentally relevant species.

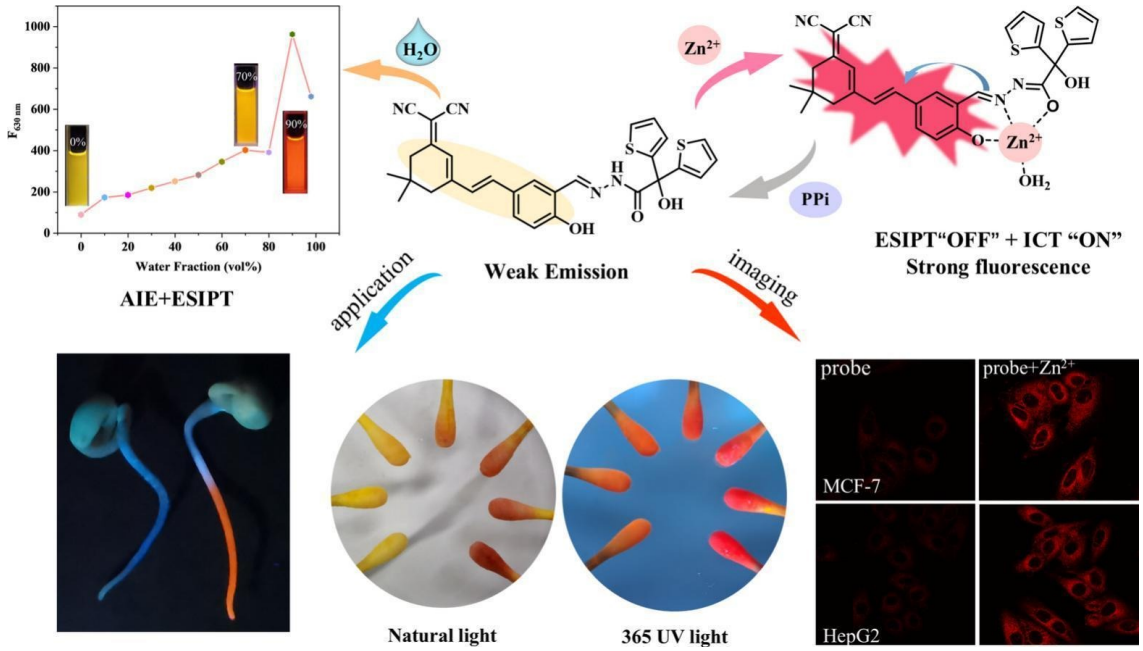


Figure 9a: Schematic representation of sequential recognition of Zn2+ and PPi by the sensor **9a** and corresponding experiments. Reproduced with permission from ref. 25. Copyright 2026, Elsevier.

2.1.2. Dinuclear Zn(II) complexes

Various researchers has used bis-Zn(II) complexes for the detection of pyrophosphate. The presence of two Zn^{2+} ions made the chemosensors more soluble in an aqueous media. In fluorometric or colorimetric sensors, binding to bis-Zn systems often leads to much larger signal changes because PPi bridges the two zinc centers and induces a distinct structural/electronic change. Bis-Zn receptors often maintain strong PPi binding even in physiological buffers or saline solutions, while mono-Zn ones lose affinity due to competitive solvation and ionic strength. This is especially important for real biological assays (cell culture, diagnostics, etc.) where conditions are far from ideal for simple receptors.

Two terpyridine–Zn(II) complex–based luminescent probes, designated **10** and **11**, were created by Thenmozhi et al. for the recognition of PPi ions in aqueous medium and in live cells.[26] Selectivity studies conducted in HEPES buffer (pH 7.4) showed that both probes preferentially recognize PPi even in the presence of competing anions and nucleotides. When PPi was gradually added, probes **10** and **11** (Figure 10) displayed a linear increase in luminescence intensity, which was explained by suppression of the PET process. Density functional theory (DFT) calculations and fluorescence emission and NMR titration measurements verified the host-guest interaction between the sensor and PPi. The Zn(II) complexes and PPi have a 1:2 binding stoichiometry, according on job plot analysis. Association constants of 7.72×10^5 M^{-2} for probe **8** and 6.74×10^5 M^{-2} for probe **9** were calculated using emission titration data. Importantly, both probes exhibited ultralow detection limits for PPi, reaching 0.47 nM and 0.17 nM, respectively. The biocompatibility of the sensor was confirmed by Cytotoxicity studies in MCF-7 breast cancer cells.

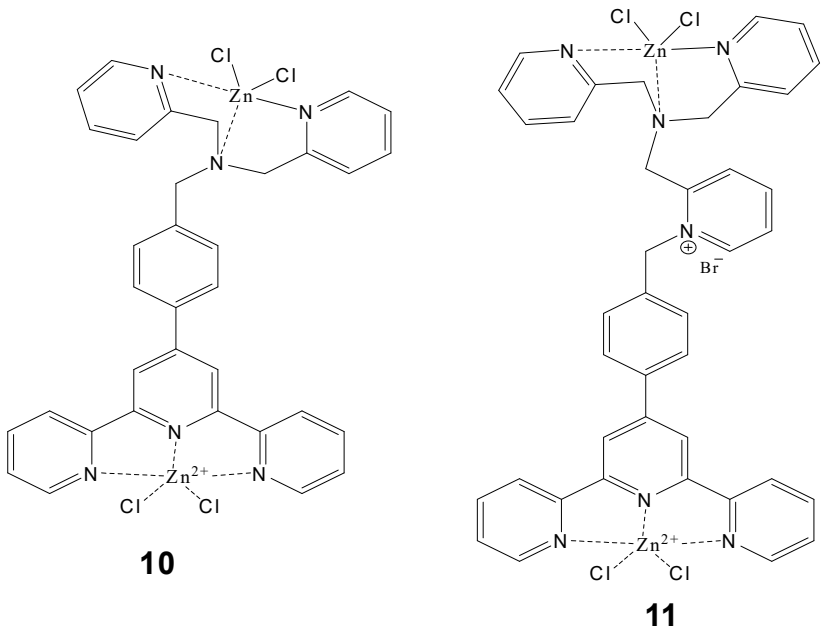
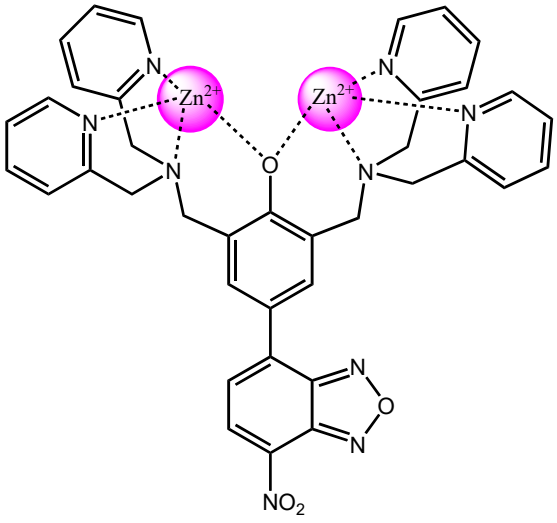


Figure 10: Schematic representation of chemosensors **9** and **10.** Reproduced with permission from ref. 26. Copyright 2023, Elsevier.

Yang and colleagues synthesized a unique NBD chromophore incorporating DPA–Zn^{2+} (**12**; Figure 11) probe for the selective colorimetric sensing of PPi pure water medium.[27] The

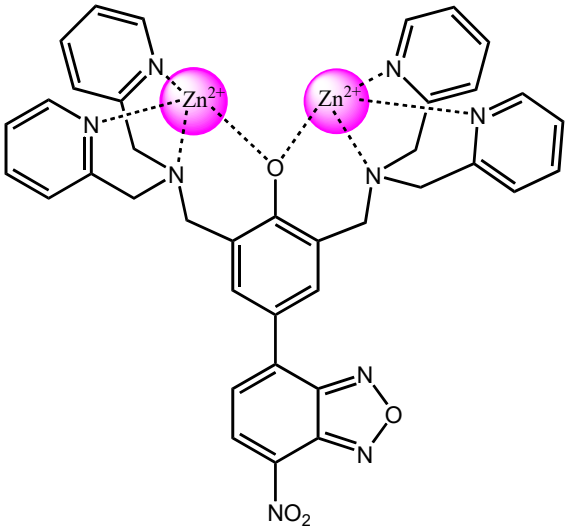
sensor **12**, which appears red, immediately turned purple when one equivalent of PPi was added, which confirmed the 1:1 complex formation between receptor and analyte. UV-vis spectrophotometric titration provided more evidence for this complexation, where substantial PPi-induced changes were observed in the absorption profile of **12**, matching the visible color shift. The absorbance at 501 nm gradually diminished and exhibited a pronounced 25 nm bathochromic shift, while a new peak at 600 nm grew steadily until reaching saturation when excess of one equivalent of the anion was added, which confirm a 1:1 binding stoichiometry (2.9×10^8 M^{-1}). ^{31}P NMR titration additionally showed that both sets of oxygen atoms attached to each phosphorus in PPi interact equally with **12**. The group also showed that the probe could also be able to monitor PPase activity.



12

Figure 11: Schematic representation of chemosensor **12.** Reproduced with permission from ref. 27. Copyright 2026, The Royal Society of Chemistry.

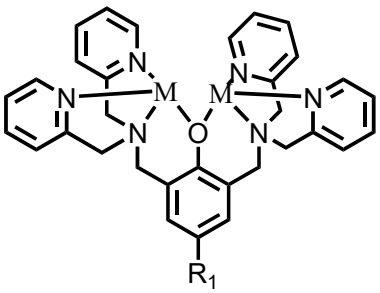
Delgado and co-workers employed a macrocyclic dizinc(II) receptor **13** contain two quinoline moiety as signaling unit for PPi recognition (Fig. 12).[28] Analysis of the absorption and emission spectral data analysis confirmed 1:1 and 1:2 complex formation with PPi (log K_{11} = 6.23; log K_{12} = 4.85) in neutral buffer solution. Introduction of one equiv. of $HPPi^{3-}$ resulted in a pronounced 21-fold rise in emission at 380 nm with a 10 nm blue shift. The chemosensor showed lower detection limit of 300 nM. Upon further exposure of PPi, emission intensity diminished as Zn^{2+} ions were stripped from **13** by $HPPi^{3-}$.



13

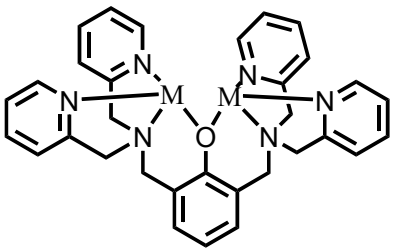
Figure 12: Schematic representation of chemosensor **13.** Reproduced with permission from ref. 28. Copyright 2026, American Chemical Society.

McKenzie group has designed and synthesized three bismetallic compounds containing Ga(III) (**14**), In(III) (**15**) and Zn(II) (**16)** containing bis(2-picolyl)amino)phenolate ligand **(**Figure 13) and PPi was selectively detected in an aqueous media at neutral pH using the IDA method and pyrocatechol violet dye.[29] The dye formed adducts with all three complexes, and upon adduct formation, the UV-Vis spectra underwent a 24 nm red shift from 441 to 603 nm. When a variety of common anions were used to assess the selectivity of the three complexes $\{M_2(bpbp)(H_xPV)\}^{n+}$ M = Ga^{3+}, In^{3+}, Zn^{2+}, it was discovered that the receptor utilizing Ga^{3+} displayed a selective color change following addition of PPi (1 eq.). The In^{3+} or Zn^{2+} complexes were less selective and provide responses to several of the anions. Further the group also reported the crystal structure of the Ga based probe **14** complexed with PPi anion.



$R_1 = CH_3$ **14**

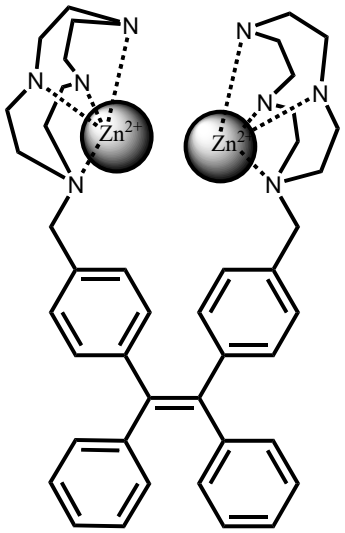
$R_1 = C(CH_3)_3$ **15**



16

Figure 13: Schematic representation of the chemosensor **14**, **15** and **16**. Reproduced with permission from ref. 29. Copyright 2015, The Royal Society of Chemistry.

Yu and colleagues later reported a tetraphenylethylene (TPE)-based dizinc(II) chemosensor incorporating a "cyclen" (1,4,7,10-tetraazacyclododecane) unit (**17**) for PPi detection (Fig. 14).[30] Fluorescence titrations showed that **17** binds PPi in a 2:1 stoichiometry along with association constant K_a = 170.94 M^{-2} and lower detection limit of 22.8 nM. It also showed selectivity over other competitive anions. Addition of 0.5 equivalents of the analyte produced roughly a 15-times luminescence enhancement, due to the restriction rotation of the TPE unit.



17

Figure 14: Schematic representation of the chemosensor **17**. . Reproduced with permission from ref. 30. Copyright 2014, The Royal Society of Chemistry.

In order to recognize PPi evolved in a Polymerase chain reaction (PCR) Anbu and colleagues developped a phenathroimidazole phenolate-based dinuclear Zn(II) complex (**17**) (Fig. 15). [31]The chemosensor can selectively recognizes PPi over other anions tested. Upon binding with the anionic target anion it exhibited ~3.2 times rise in emission at 495 nm because of the CHEF. The chemosensor showed very high association constant with PPi (3.47 x 10^5 M^{-1}), while LOD of 0.91 nm. The group also demonstated the recognition of pyrophosphate from excreated from the PCR reaction.

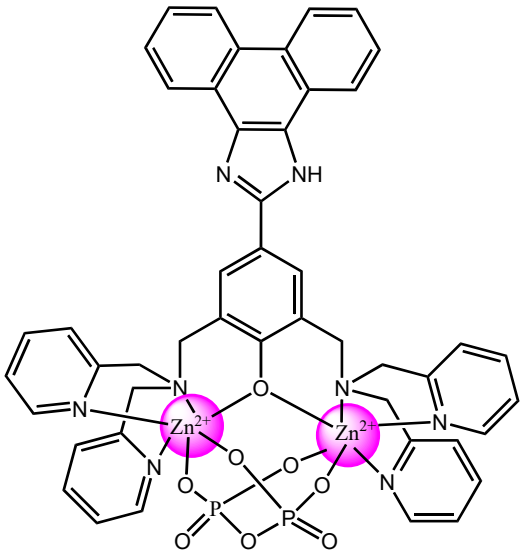


Figure 15: Schematic representation of the mechanism of PPi recognition by the chemosensor **18**. . Reproduced with permission from ref. 31. Copyright 2015, The Royal Society of Chemistry.

In order to get ratiometric luminescence response from PPi Chen group has developed Zn^{2+} complexes of 2-(2-hydroxyphenyl)-1,3-benzoxazole (HBO) containing DPA moiety (**19** and **20**) (Fig. 17).[32] **19** shows bright lumiscence at ~420 nm due to the presence of the HBO unit. **19** exhibited no significant emission response in presence of various anions tested such as $HPO_4^{2-,}$ CH_3COO^-, citrate, etc. except PPi. When PPi is added, the 19 emission at 420 nm experiences a Stokes shift of about 100 nm and appears at 518 nm. The observed phenomenon was attributed to the fact that upon analyte binding the two Zn^{2+}-DPA units were separated away from the phenol unit resulting ESIPT among phenolic 'H' and benzoxazole 'N'. The group further confirmed this ESIPT mechanism upon PPi banding by ^{1}HNMR and mass spectroscopic studies. The mononuclear model compound **20** also exhibited rise in emission at 427 nm upon PPi binding but no Stokes shift in the emission spectrum. This experiment with model sensor **20** confirmed that it is essential to present dinuclear Zn(II) moiety in order to get ESIPT result upon PPi sensing. **19** showed very high association constant of Ka = 9.2 x 10^7 M^{-1} with PPi.

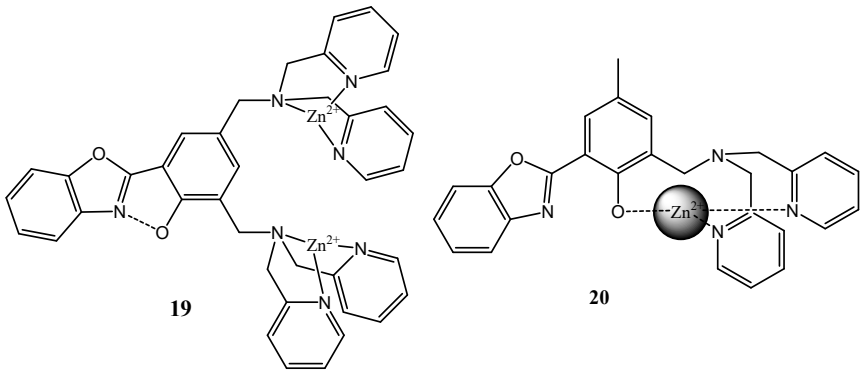


Figure 17: Schematic representation of the chemosensors **19** and **20**. Reproduced with permission from ref. 32. Copyright 2011, American Chemical Society.

Pathberiya et al. designed two dipicolylamine–dizinc(II) complexes, functionalized with 1-naphthyl and 9-anthracenyl groups (**21** and **22**), for the specific detection of PPi under pseudobiological conditions (Fig. 18).[33] Complex **21** showed a concentration-dependent emission at 450 nm when one equivalent of PPi was added, and this response remained selective although there are three equivalents of various competing anions. Upon PPi binding, the fluorescence of **21** increased seven-fold and exhibited a 14 nm Stokes shift from 450 nm. Coordination of PPi to the Zn(II) center increases the charge concentration on the 'O' atom of the phenolate part by weakening the metal–phenolate interaction, thereby facilitating charge migration between the phenolate fragment and the naphthalene unit, which results in a pronounced change in emission. The binding constant for the **21**–PPi complex was calculated as $K_a = 7.2 \times 10^5\ M^{-1}$. The authors also verified that C39 can track the catalytic activity of inorganic pyrophosphatase (PPAse), which cleaves the phosphoester bond of PPi. In contrast, the second chemosensor, **22**, displayed luminescence at 484 nm that was diminished gradually upon PPi addition, accompanied by a 38 nm Stokes shift. The fluorescence quenching was ascribed to PET from the DPA nitrogen atoms to the anthracene unit after PPi binding.

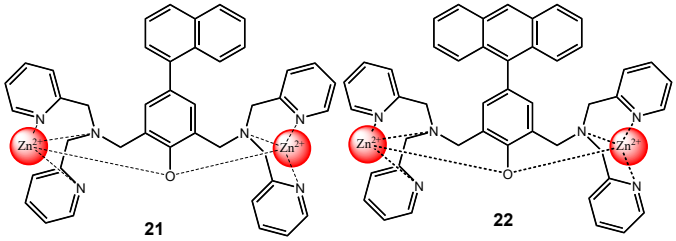


Figure 18: Diagrammatic illustration of chemosensors **21** and **22**. . Reproduced with permission from ref. 33. Copyright 2012, Elsevier.

To detect PPi selectively in a 100% water media, Yang and colleagues created a dicyanomethylene-benzopyran near-infrared (NIR) emissive luminous chemosensor (**23**; Figure 19).[34] Upon excitation at 504 nm **23** showed very low fluorescence intensity at 639 nm. The emission of **23** at 639 nm was suppressed up to 0.5 equivalent when PPi was added, but the luminescence was unexpectedly increased at 654 nm when an additional 4 equivalent excess of PPi was added. The quenching of emission is due to the PET from dipicolyl amine 'N's and benzene ring, whereas the rise of new emission peak is because of ICT process between phenolate oxygen and fluorophore. **23** showed excellent selectivity and sensitivity towards PPi among various competitive anions tested. The sensor also showed excellent limit of detection (LOD) of 42 nM. The sensor could also be able to recognize PPi in 'in vitro' as well as 'in vivo' in A549 cells and rats, respectively.

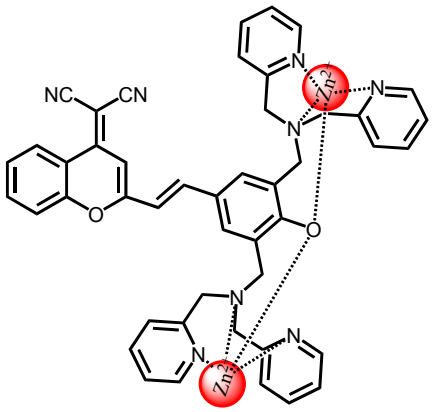
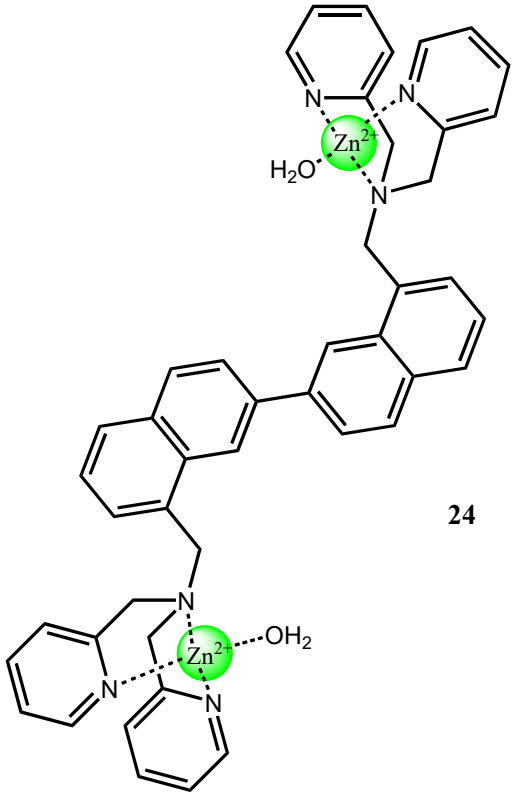


Figure 19: Schematic representation of the chemosensor **23.** . Reproduced with permission from ref. 34. Copyright 2018, Elsevier.

Kondo and colleagues developed a 2,2′-binaphthalene-based chemosensor (**24;** Figure 20) functionalized with DPA groups for stepwise recognition of Zn^{2+} and PPi in water medium.[35] **24** exhibited weak luminescence at 380 nm because of a PET process from the DPA 'N' donors to the binaphthalene core. Fluorescence was significantly enhanced, with emissions at 367 and 380 nm, upon formation of **24**–Zn^{2+} (K_{11} = 5.39 ± 1.70 × 10^6 M^{-1}), and **24**–$2Zn^{2+}$ (K_{12} = 2.43 ± 0.54 × 10^6 M^{-1}) complexes. The study further revealed that **24**–$2Zn^{2+}$ could adopt different conformations depending on pH: a bis-hydrated transoid form [**24**–$2Zn^{2+}(H_2O)_2]^{4+}$ under slightly basic conditions (pH 7.2) and a hydroxy-bridged cisoid form [**24**–$2Zn^{2+}(H_2O{\cdot}OH^-)_3^+$ or **24**–$2Zn^{2+}(OH^-)_3^+$] under mild acidic environment (pH 5.6). The Zn^{2+} complex of **24** interacted readily with anions via the electron deficient zinc centers, forming a **24**–$2Zn^{2+}$–anion complex, as confirmed by both absorption and luminescence titration experiments. Due to the employment of one eq. of H_2PPi, the fluorescence of **24**–

$2Zn^{2+}$ at 367 and 380 nm increased further and plateaued, demonstrating efficient binding. Competitive titrations with other anions (Cl^-, AcO^-, ClO_4^-, NO_3^-, $H_2PO_4^-$) confirmed the high selectivity of **24**–$2Zn^{2+}$ for H_2PPi, evidenced by fluorescence enhancement at 380 nm.



24

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Figure 20: Schematic representation of the chemosensor **24** . Reproduced with permission from ref. 35. Copyright 2014, The Royal Society of Chemistry.

2.1.3. Tri Zn(II) complex based receptor

A quinoline-based luminescent chemosensor (**25**; Figure 21) was synthesized by Ghosh and colleagues for the recognition of Zn^{2+} in an acetonitrile–water medium.[36] Notably, the sensor operates effectively over a pH range of 4–10. The sensing behavior was thoroughly investigated using different analytical techniques, which indicate a 1:3 stoichiometric binding interaction between **25** and Zn^{2+}. This complexation mode was further supported by ^1H NMR experiment. DFT studies provided insights into the 3D structure of the Zn^{2+} complex of **25**, which is presumed to form in situ in an acetonitrile medium. Additionally, X-ray crystallographic analysis reveals the three-dimensional structure of the tris-Zn^{2+} complex of

25. Finally, the resulting tris-Zn^{2+} complex was used as an emission "turn-off" probe for the recognition of PPi in a mixed water–acetonitrile solvent system, exhibiting an exceptionally LOD of 45.37×10^{-9} M.

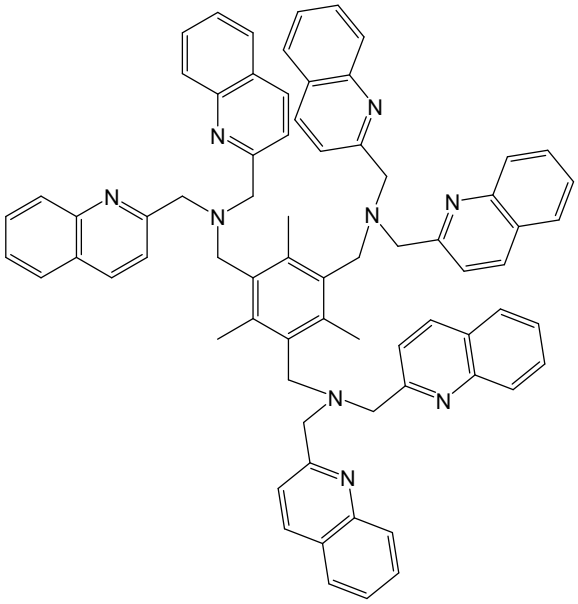

Figure 21: Schematic representation of the chemosensor **25**. Reproduced with permission from ref. 36. Copyright 2018, The Royal Society of Chemistry.

3. Conclusion:

In conclusion, substantial advancements have been achieved in the creation of Zn(II)-based molecular probes for the selective detection of pyrophosphate (PPi). Drawing inspiration from biological systems, researchers have extensively engineered zinc(II)-centered chemosensors to tackle the inherent challenges of PPi recognition. Due to its hard Lewis acid character and strong preference for oxygen-donor ligands, Zn^{2+} readily forms stable coordination complexes with the highly charged $P_2O_7^{4-}$ anion. This coordination-mediated approach has proven particularly effective in aqueous and physiological environments. Both mononuclear and multinuclear Zn(II) complexes exhibit impressive sensitivity and selectivity toward PPi. Mononuclear systems—commonly based on ligands such as dipicolylamine (DPA), terpyridine, quinoline, and related frameworks—enable diverse optical responses including fluorescence "turn-on," "turn-off," ratiometric, and colorimetric signaling. Many of these probes achieve nanomolar detection limits, strong binding affinities, and excellent selectivity over competing anions like ATP, ADP, and inorganic phosphate. Notably, several

have been successfully implemented in live-cell imaging, paper-based sensors, hydrogel matrices, nanochannel platforms, and real-time enzymatic monitoring. Dinuclear Zn(II) complexes further strengthen binding and selectivity through cooperative interactions that bridge the PPi anion. These systems often generate enhanced fluorescence signals, demonstrate robust performance under physiological conditions, and are well suited for tracking biological events such as PCR amplification and pyrophosphatase activity. Sophisticated photophysical mechanisms—including PET inhibition, CHEF, ICT, RIR, and ESIPT—have been strategically employed to maximize signal output. Trinuclear Zn(II) assemblies extend this concept by offering wider operational pH ranges and exceptionally low detection thresholds. Collectively, Zn(II)-based molecular receptors constitute a highly adaptable and effective platform for PPi sensing. Ongoing optimization of ligand architectures, incorporation of multifunctional fluorophores, and integration into practical analytical devices are anticipated to drive further progress. Future efforts are likely to emphasize improved biocompatibility, real-time in vivo applications, and potential clinical translation. Overall, the developments outlined here highlight how rational coordination chemistry coupled with deliberate photophysical engineering can successfully address the challenges of PPi detection in aqueous and biological systems.

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