Available online at www.sciencedirect.com
ScienceDirect
Coordination Chemistry Reviews 250 (2006) 3118–3127
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

Indicator–displacement assays

Binh T. Nguyen, Eric V. Anslyn *

Chemistry and Biochemistry Department, The University of Texas, 1 University Station, A5300 Austin, TX 78712-0165, USA

Received 3 May 2005; accepted 14 April 2006
Available online 25 April 2006

Contents

1. Introduction 3118
   1.1. Indicator–displacement assay 3118
2. Colorimetric indicator–displacement assays 3119
   2.1. Applications in anion sensing 3119
   2.2. Biological examples 3021
3. Fluorescent indicator–displacement assays 3122
   3.1. Applications in anion sensing 3122
   3.2. Biological examples 3122
   3.3. Imprinted polymers 3123
4. Metal complexing indicator–displacement assays 3123
   4.1. Applications in anion sensing 3123
   4.2. Biological examples 3125
5. Conclusion 3125
   References 3125

Abstract

Indicator displacement assays (IDAs) are now a popular method for converting most any synthetic receptor into an optical sensor. In this review many such assays are highlighted, along with biological counterparts. The focus is upon colorimetric, fluorescent, and metal containing IDAs. The power of the method can be readily appreciated by the large diversity of analytes that have been targeted with this technique. It is clear that the method is now well accepted and will continue to be one of many methods used to create optical detection methods from synthetic receptors.

Keywords: Receptors; Sensors; Indicators; Supramolecular chemistry; Molecular recognition

1. Introduction

Traditionally, the most widely used approach for chemosensors is the indicator–spacer–receptor approach (ISR). In this approach, an indicator (chromophore or fluorophore) is covalently attached to a receptor through a spacer (Fig. 1). Commonly with organic structures, introduction of an analyte that binds to the receptor would induce measurable changes in fluorescence or absorbance. These measurements can be used to obtain binding constants and stoichiometries of binding [1].

Although it is the most popular, the ISR approach has limitations. The major limitation is that attachment of the indicator to the receptor may require difficult syntheses. An alternate approach that circumvents this problem is the indicator–displacement assay (IDA). Herein, we present a summary of examples, advantages, and applications of IDAs.

1.1. Indicator–displacement assay

In an IDA, an indicator is first allowed to bind reversibly to a receptor. Then, a competitive analyte is introduced into the system causing the displacement of the indicator from the host, which in turn modulates an optical signal [2] (Fig. 2). Based on this principle, the major requirement for an IDA is that the

* Corresponding author. Tel.: +1 5124710068; fax: +1 5124717791.
E-mail address: anslyn@ccwf.cc.utexas.edu (E.V. Anslyn).

0010-8545/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2006.04.009

constants and stoichiometries of binding.

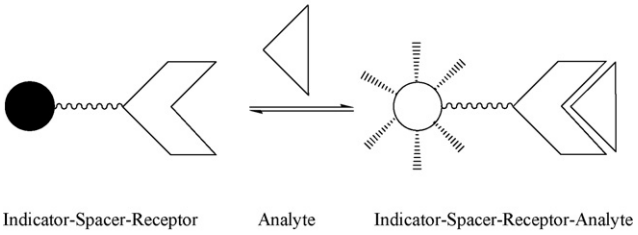


Fig. 1. Schematic of the indicator–spacer–receptor approach.

affinity between the indicator and the receptor be comparable to that between the analyte and the receptor.

Signal modulation in an IDA is possible based on several mechanisms: photoinduced electron transfer (PET) [3,4], fluorescence resonance energy transfer (FRET) [5], electronic energy transfer (EET) [6,7], or simple changes in local ionic strength or pH [8]. The common interactions between the indicator or analyte and the host are H-bonding [9–15], electrostatic interactions [16–27], and complexing with metal centers [28,29]. These interactions are dependent on the geometry of the guest, its charge, its hydrophobicity, and the solvent system [30].

The IDA offers many advantages over traditional sensing assays. First, the method does not require the indicator to be covalently attached to the receptor. Second, because there are no covalent bonds between the receptor and the indicator, one can employ several different indicators with the same receptor. Third, the assay works well in both organic and aqueous media, and lastly, the assay is easily adapted to different receptors and platforms for quick analysis [2]. In this review we classify three types of IDAs. The colorimetric IDA (C-IDA) which employs colorimetric indicators. The second class is the fluorescent IDA (F-IDA) which uses fluorescent indicators, and the third class is the metal complexing IDA (M-IDA) that utilizes a metal center with either a colorimetric or fluorescent indicator. Hence, an M-IDA is a subset of both a C-IDA and a F-IDA.

IDAs have been used to sense both cations and anions. However, the majority of IDAs have been for anions. Anions play fundamental roles in many phenomena, including biological processes such as the transport of hormones, proteins biosynthesis, DNA regulation, and the activity of enzymes [31]. Recognition or sensing of anions is a current goal of molecular recognition [1]. The important roles of anions have inspired chemists to devote significant efforts toward the designs of practical chemosensors for the detection of various anions, both qualitatively and quantitatively.

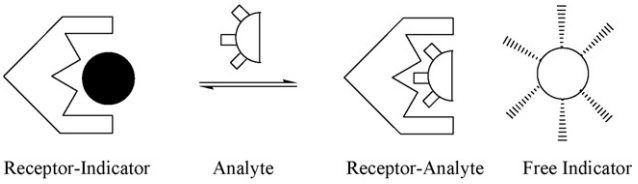


Fig. 2. Schematic of the indicator–displacement assay.

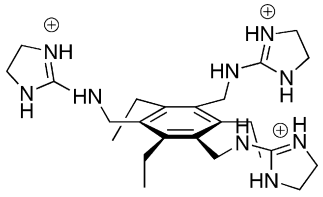
2. Colorimetric indicator–displacement assays

Naked-eye detection of various chemicals has been the inspiration for the development of C-IDAs. In a C-IDA, the indicator's color varies depending on whether it is free or bound to the receptor. Change in the color of the indicator modulates the optical signal, and thus, makes the detection of binding events possible. Prior to the development of this method by our group, there were few examples of C-IDAs in the literature. Two examples we took our lead from were the detection of acetylcholine by Inouye and Shinkai [32,33].

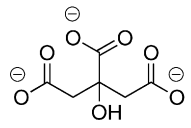
2.1. Applications in anion sensing

Previous studies showed that boronic acids form reversible bonds with 1,2-diols and α-hydroxy carboxylates [34], and guanidinium groups bind carboxylates through H-bonds or charge-pairing interactions [35,36]. Optimizing these advantages, we designed a series of receptors that incorporated both boronic acid and guanidinium moieties to detect different carboxylate-containing compounds.

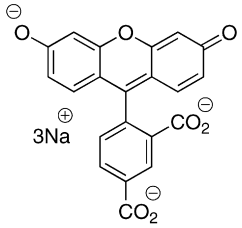
The design of receptor **1** was based on the "pinwheel" scaffold 1,3,5-trisubstituted-2,4,6-triethylbenzene. It contains three guanidinium groups, allowing it to bind to citrate **2** (a tricarboxylate at neutral pH) [3], a major component of the Krebs Cycle that produces intermediates from a variety of biosynthesis processes [37]. Citrate is also found in citrus-containing drinks [38]. The "sensing ensemble" for the detection of citrate includes the receptor **1** and the indicator 5-carboxyfluorescein **3**. When the solvent was changed from water to methanol–water (3:1), the binding constant between **1** and **2** increased an order of magnitude. This result agreed with other studies that showed decreases in polarity and H-bonding ability of the solvent lead to increases in binding [35]. The binding constant for **2** was found to be $2.9 \times 10^5\ M^{-1}$ by UV–vis spectroscopy [39].



1

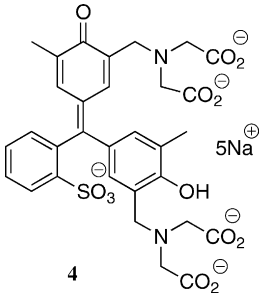


2



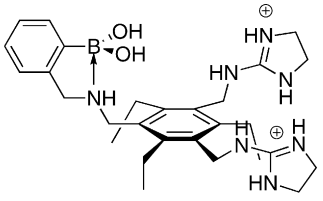
3

By combining C-IDAs with an artificial neural network analysis (ANN), the receptor **1** with xylenol orange **4** was used to detect citrate and calcium [40]. In this study, an ANN was trained to recognize the patterns of absorbances at various combinations of concentrations of calcium and citrate. The pattern was used to determine the concentration of these two analytes in flavored vodkas.

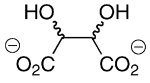


4

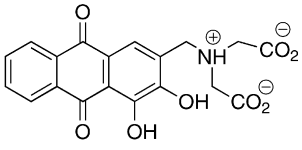
Receptor **5**, with one pendent boronic acid, was used to analyze for tartrate **6**, a natural product in grape juices and wines [41]. Tartrate contains two carboxylates and one diol. The guanidinium moieties and the boronic acid were incorporated to bind the carboxylates and the diol, respectively. A binding constant of $5.5 \times 10^4\ M^{-1}$ was obtained using the ensemble of alizarin complexone **7** and **5**. Receptor **5** was also used in the threshold detection of malate in Pinot Noir grapes [42]. In this method, the ratio of the receptor–indicator was adjusted so that it creates color changes that occurred closer to the point at which one equivalent of malate had been added.



5



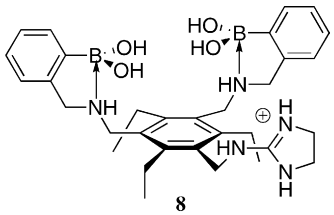
6



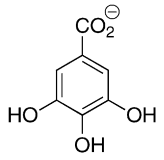
7

Receptor **8**, with two pendent boronic acids, was designed to bind the carboxylate and the hydroxyphenyl groups of gallate **9** [43], a tris-hydroxybenzoic acid derivative found in Scotch whiskies. Using pyrocatechol violet **10**, it was determined that the binding constant between **8** and **9** was $1.0 \times 10^4\ M^{-1}$. It was later found that receptor **8** had higher affinity to tartrate than gallate using the same ensemble [44]. The binding constant for tartrate was $1.4 \times 10^5\ M^{-1}$.

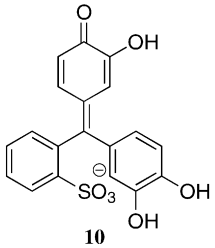
Knowing that the receptor **8** has high affinity for tartrate, we adapted the C-IDA into paper test strips that could produce color upon the introduction of tartrate-containing droplets [2]. This "portable" C-IDA is the first example that a C-IDA can be easily transitioned into different media for naked-eye detection. The ensemble of **8** and pyrocatechol violet **10** was also used to track the kinetics of reactions that produce tartrate [2]. Among reactions studied were the dihydroxylation of maleic acid and fumaric acid catalyzed by osmium tetroxide in water, as well as the acid-catalyzed hydrolysis of dimethyl tartrate [45].



8

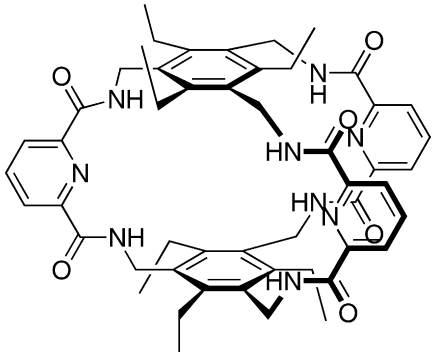


9

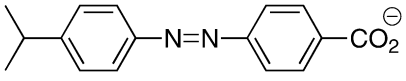


10

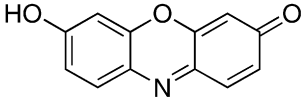
On the basis of H-bonding, receptor **11** was designed to bind nitrate, a C_3-symmetric anion [46]. The ensembles **11**:**12** or **11**:**13** in organic solvent were studied, and a moderate association constant for nitrate was determined ($500\ M^{-1}$).



11

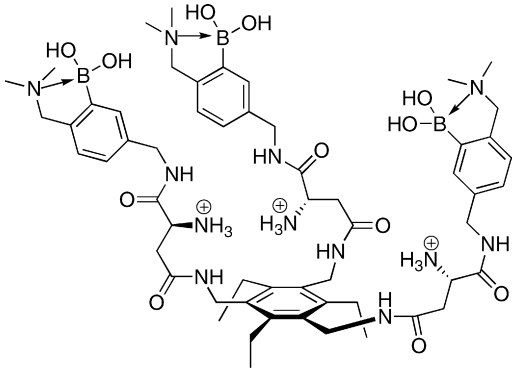


12

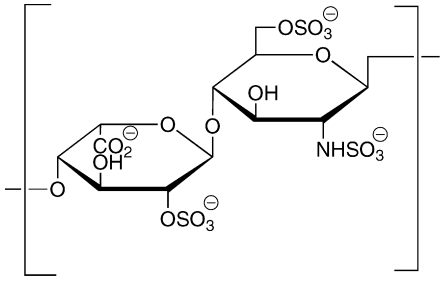


13

Using the same hexasubstituted benzene, receptor **14** was created. This receptor was effective at binding heparin **15**, an anticoagulant [47]. Utilizing the charge-pairing interactions between heparin and ammoniums [48–50], and the widely used boronic acids in sensing of saccharides [51–58], the ensemble of **14**:**10** was used in this study. The binding constant for heparin was $3.8 \times 10^4\ M^{-1}$. The results also revealed that the boronic acids were important in this assay because a receptor lacking these groups yet retaining the ammoniums had very low affinity for heparin.

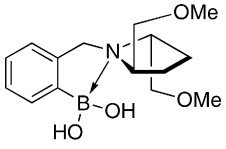


14

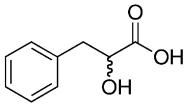


15 (major unit)

A recent extension of the C-IDA in our group allowed us to quantify enantiomeric excess and concentration of α-hydroxy acids [59]. Using the chiral receptor **16** with either alizarine complexone or pyrocatecholviolet, the concentration of **17** and its enantiomeric excess were obtained. This facile approach offers quick analysis of chiral analytes without lengthy syntheses.



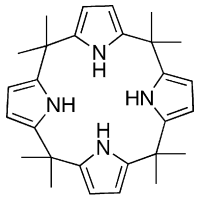
16



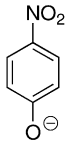
Phenyllactic acid

17

One of the prime targets in sensing is halogen anions. Sessler and coworkers developed pyrrolic receptor **18** for this purpose [60]. This *meso*-octamethylcalix[4]pyrrole has higher affinity for fluoride anion than for other halides. It binds to the anion on the basis of H-bonds through the pyrrolic hydrogens. Using 4-nitrophenolate anion **19**, Sessler was able to detect the association of fluoride with the receptor based on the color change that results from the displacement of indicator **19**.



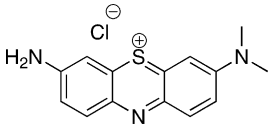
18



19

2.2. Biological examples

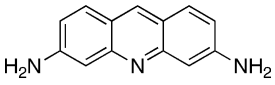
The C-IDA has also found biological applications. In the quantitation of plasma protamine, a coagulant that is administered during cardiopulmonary bypass and cardiac catheterization to reverse the anticoagulant activity of heparin [61,62], Yang and coworkers showed that protamine effectively displaced indicator **20** from its complexation with heparin [63].



Azure A

20

Another biological application is the study of the binding of proflavin **21** to human α-thrombin [64]. Brick and coworkers studied the binding of proflavin to the specificity pocket of the complex α-thrombin-hirugen by displacing the indicator with PPACK, an inhibitor of α-thrombin [65].



21

Efforts in using C-IDA to develop aptamer-based colorimetric probes for cocaine are also underway. On the basis of cation–nucleotide interactions, the Stojanovic group used aptamer **22** and cyanine dye **23** to detect cocaine **24** (Fig. 3) [66]. The cyanine dye first binds to the specific loop of the aptamer. Then, competitive binding of cocaine to the loop causes the displacement of the dye. The binding of **23** to **22** and its displacement from **22** modulates the signal change in this assay.

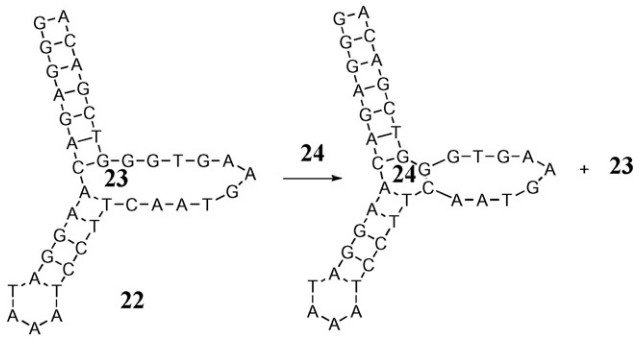
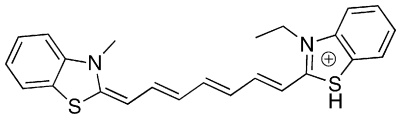


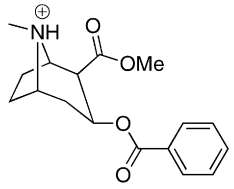
Fig. 3. Displacement of indicator by cocaine.

Beside C-IDA, F-IDA (fluorescent indicator–displacement assay) is also widely used in analytical sciences, particularly due to its high sensitivity.



Diethylthiotricarbocyanine iodide

23



24

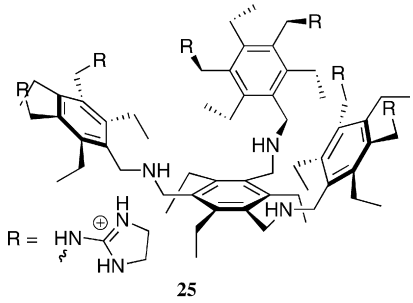
3. Fluorescent indicator–displacement assays

Similar to a C-IDA, in a F-IDA a fluorescent indicator is displaced from a receptor upon the introduction of an analyte. However, unlike a C-IDA, changes in emission of the indicator are measured instead of the absorbance. In general, F-IDA is more sensitive than C-IDA. It can potentially measure concentrations that are one million times smaller than can be determined by an absorbance method [4].

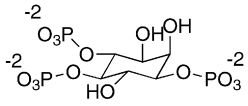
3.1. Applications in anion sensing

We developed receptor **25** that utilized a F-IDA to sense inositol-1,4,5-triphosphate **26**, a polyanionic secondary messenger in cellular processes [67,68]. The receptor **25** contains four units of 1,3,5-trisubstituted-2,4,6-triethylbenzene in addition to guanidinium groups attached to each unit. Previous studies showed that guanidiniums not only bind to carboxylates but also to phosphates [69]. This cleftlike receptor had high affinity toward **26** with a binding constant of $1.0 \times 10^8\ M^{-1}$. The indicator 5-carboxyfluorescein was used in this study [3].

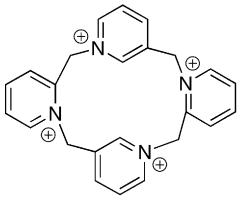
The importance of ATP in biological systems has inspired many chemists to find suitable sensors. Akkaya and coworkers found that calixpyridinium tetracationic receptor **27** had high affinity for ATP. The tetraanionic indicator **28** was used for fluorescent detection [70]. Due to the cationic nature of **27**, the indicator **28** was a suitable choice for ion-pairing interactions. Upon the introduction of ATP, **28** was displaced from **27** and higher fluorescence emission was observed. The binding constant was found to be $2.87 \times 10^4\ M^{-1}$.



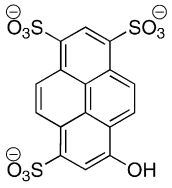
25



26



27

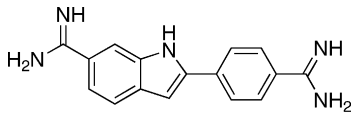


8-Hydroxy-1,3,6-pyrenetrisulphonate

28

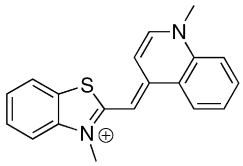
3.2. Biological examples

F-IDAs have also been applied to biological studies. Using indicator **29** and designed dsDNA, Kwalczykowski and coworkers monitored *Escherichia coli* Rec A protein activity on dsDNA [71]. Rec A Protein plays a critical role in the recombination and repair pathways of *E. coli* [72]. Kwalczykowski measured helicase activity with thiazol orange **30** [73]. Helicase has many functions in biological processes [74,75]. One of its functions is unwinding DNA during replication to produce ssDNA. Thus, its activity can be monitored based on the amount of dsDNA present. The indicator **30** binds to dsDNA better than ssDNA and the fluorescence intensity of **30** is higher when it is bound to dsDNA than that when it is unbound.



4',6-diamidino-2-phenylindole

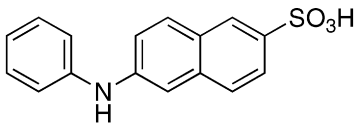
29



Thiazole orange

30

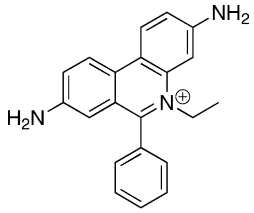
The same method was used by Mock and coworkers to study biotin–avidin interactions based on the displacement of indicator **31** [76]. Biotin and avidin are common agents that are used in histochemistry and in purification of variety of biological substances [77].



2-Anilinonaphthalene-6-sulphonic acid

31

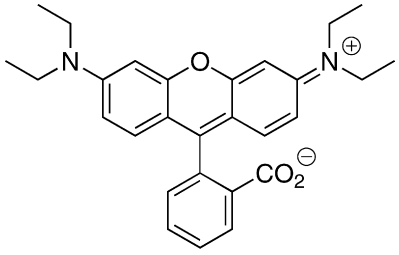
Recent development in high-thoughput selection of small molecules that bind DNA using a F-IDA was pioneered by Boger [78,79]. This method is also called fluorescent intercalator displacement (FID) because the fluorescent indicator is also an intercalator. In this method, using either indicator **30** or ethidium bromide **32** and hairpin DNA, one can establish DNA binding affinity, sequence selectivity, and binding stoichiometry upon introduction of DNA binding compounds.



32

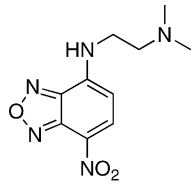
3.3. Imprinted polymers

F-IDAs are also used as a form of displacement chromatography which relies on the displacement of the indicator from molecular imprinted polymer (MIP) chromatographic stationary phases. Wolfbeis has applied this method to quantify and monitor enantioselective binding of amino acids, particularly chiral phenylalanine and phenylalaninamide, to a MIP [80]. Rhodamine B was the indicator used in this study.



Rhodamine B

In another study, Shimizu used a MIP array to differentiate different aromatic amines based on colorimetric response patterns [81]. These patterns were generated upon the displacement of a benzofurane-based amino dye **33**.



33

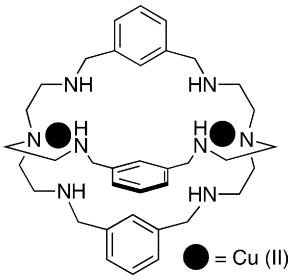
We have summarized work in C-IDA and F-IDA. In the remainder of this review, studies of metal complexing indicator–displacement assay (M-IDA) are discussed.

4. Metal complexing indicator–displacement assays

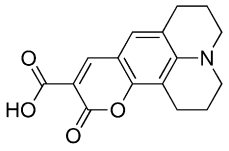
In a M-IDA, a metal is complexed with a receptor. Then, an indicator (chromophore or fluorophore) is allowed to coordinate with both the metal center and the receptor. Addition of an analyte to the system causes the displacement of the indicator from the metal and the receptor. This results in optical changes that can be measured to derive binding affinity. Zinc and copper have been most effectively used.

4.1. Applications in anion sensing

The advantages of a M-IDA are that it can operate in highly polar and solvating solvents (aqueous ethanol or pure water), and displays strong affinity toward anionic substrates [82]. One of the pioneers of this method is the Fabbrizzi group. Fabbrizzi and coworkers have shown that by using the dicopper(II) complex **34** and indicator coumarin **35**, selective carbonate detection in water an be achieved [83]. The carboxylate group of **35** is capable of bridging the two copper centers of **34**. This coordination quenches the fluorescence of **35**. Addition of carbonate anion to the solution regenerated the fluorescence due to the displacement of **35** from **34** by carbonate anion.

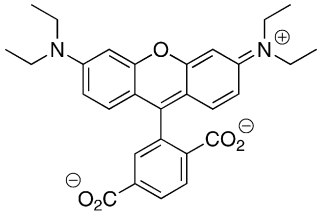


34



35

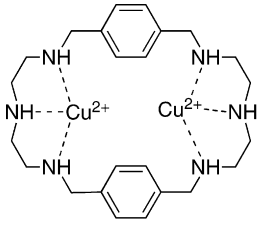
The Fabbrizzi group has also shown that polyamine cage **34** can be successfully used to detect glutamate (a neurotransmitter) in water at pH 7 [84]. The copper ion acts as a fluorophore quencher due to its partially filled 3d orbitals [85]. When compared to other amino acids (aspartate, glycine, alanine, γ-aminobutyric acid), glutamate was bound best to **34** because it has the right length between carboxylates. These characteristics make the inclusion of glutamate in **34** more favorable than other amino acids. The indicator **36** was used in this competition assay.



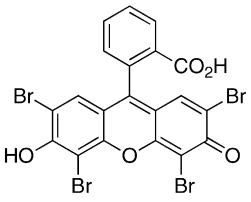
6-Carboxytetramethyl rhodamine

36

The same strategy was used by Fabbrizzi in the detection of pyrophosphate and histidine in water [86,87]. Pyrophosphate anions play important roles in bioenergetic and metabolic processes [88]. Receptor **37** was designed to incorporate two copper ions to gain affinity for the analytes. Eosine **38** was one of the indicators used in this study. Results from these studies showed that **37** binds to pyrophosphate better than monophosphate due to the ability of pyrophosphate to coordinate the two copper centers. The same receptor was also able to discriminate histidine from glycine, phenyl alanine, valine, leucine, and proline. This is because histidine possesses an imidazole residue which coordinates to dicopper centers resulting in higher selectivity.

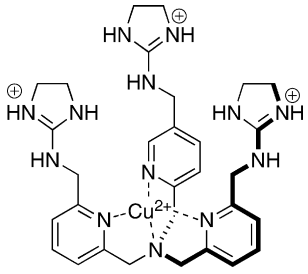


37



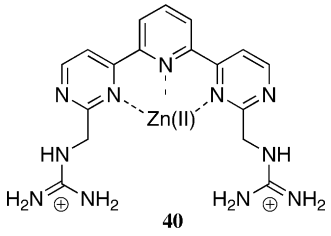
38

Recent studies by our group showed that the designed C_{3v}-symmetric receptor **39** can bind to inorganic phosphate and phosphoesters [89,90]. The copper center and peripheral guanidiniums were designed to complement tetrahedral oxyanions of phosphate. The binding constants for inorganic phosphate and phosphotyrosine were 1.5×10^4 and 11×10^3 M^{-1}, respectively. The indicator in this study was 5-carboxyfluorescein.



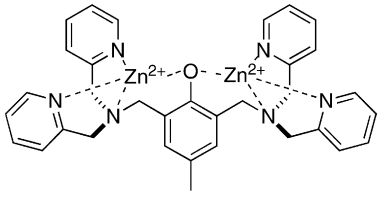
39

Using zinc metallo-receptor **40** together with pyrocatechol violet, we were able to achieve large color changes in response to the presence of aspartate [91]. The appended guanidinium on the receptor is believed to impart selectivity for aspartate.



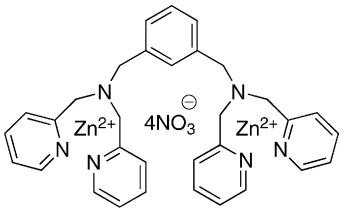
40

The detection of phosphate was also found in work by Kim and coworkers. Kim has shown that receptor **41** has high affinity for phosphate anion (1.12×10^4 M^{-1}) when using a M-IDA that employs pyrocatechol violet [92]. In this assay, binding of the catechol of the indicator to the zinc centers is reversed by the addition of phosphate. Due to this displacement, Kim was able to achieve a color change in phosphate detection.

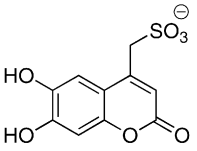


41

Another example is the fluorogenic chemosensing system **42** designed by Smith and coworkers. This dinuclear-zinc receptor has high affinity and selectivity toward pyrophosphate under physiological conditions [93]. The reported binding constant was 1.5×10^7 M^{-1}. The sulphonate **43** was the indicator. When changing the positions of the two arms in **42** from 1.3 to 1.4 or when there is only one arm, receptor **42** showed no binding to pyrophosphate. Also, when reducing the positive charge density of **42** by replacing the core benzene with phenolic anion, the binding affinity of pyrophosphate is lower.

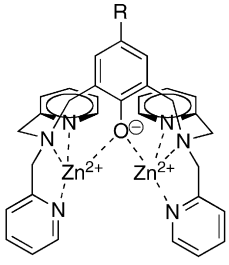


42



43

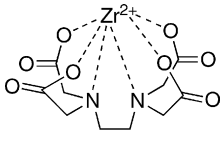
Pyrophosphate was also a target in Hong's studies [94,95]. Using receptor **44** that possessed two zinc centers, high affinity for pyrophosphate was achieved ($2–6 \times 10^8\ M^{-1}$). When *p*-nitrophenylazo group was incorporated as a fluorescent reporter, a significant color change was observed for pyrophosphate. There was no color change for phosphate, acetate, or fluoride. In addition, when the fluorescent reporter was naphthalene, high discrimination for pyrophosphate over ATP was found.



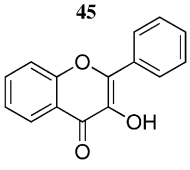
44

R = p-nitrophenylazo or naphthalene

Sensing halides is also a goal in applications of M-IDAs. Suzuki and coworkers discovered that simple fluorometric system **45** that contains zirconium(IV)-EDTA and the indicator flavonol **46** can be used to detect fluoride anion [96]. The complex of **46**:**45** exists through the coordination between the hydroxy group and the ketone of **46** and Zr(IV) of **45**. Addition of two fluoride anions displaces **46** from the complex. This in turn, produces fluorescent changes. The detection limit was approximately 3×10^{-3} M.

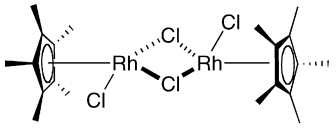


45

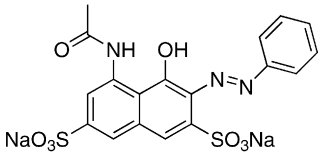


46

Attempts to use other metals beside copper and zinc in an M-IDA were also explored. Severin and coworkers took advantage of the commercially available rhodium-based receptor **47** complexing with azophloxine **48** for sequence-selective detection of histidine- and methionine-containing peptides in water at neutral pH [97].



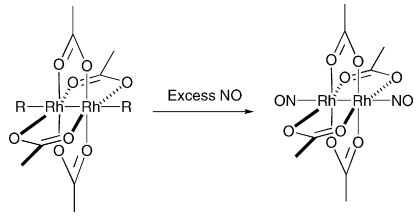
47



48

4.2. Biological examples

Lippard has used a dirhodium receptor **49** that incorporates dansylimidazole or dansylpiperrazine. The complex was used as a nitric oxide sensor [98]. Nitric oxide is involved in several physiological processes, such as vasodilation [99], carcinogenesis [100,101], and neurodegenerative disorders [102]. In this system, addition of NO displaces the coordinated fluorophore, which gives rise to fluorescence from unbound fluorophore. The detection limit for NO was approximately 4–8 μM.



49

R = Dansylimidazole or Dansylpiperazine

5. Conclusion

In summary, the breadth of the examples given above makes it clear that an IDA is a useful and facile technique for the creation of optical sensors. Receptors designed to exploit hydrogen-bonding, metal coordination, ion-pairing, and hydrophobic interactions have been ameanable to the use of an IDA. This tool provides scientists with an optical interrogation method for the study of many kinds of binding phenomena, followed by extension to a quantitative method. With the demand for accurate and quickly implemented assays in chemical detection, the simple IDA approach is becoming the method of choice for many applications. We believe this technique will continue to grow in its use and applicability for years to come.

References

[1] S.M. Roberts, Molecular Recognition: Chemical and Biochemical Problems, Royal Society of Chemistry, Cambridge, 1989.
[2] B.T. Nguyen, S.L. Wiskur, E.V. Anslyn, Org. Lett. 6 (2004) 2499.

[3] S.L. Wiskur, H. Ait-Haddou, J.J. Lavigne, E.V. Anslyn, Acc. Chem. Res. 34 (2001) 963.
[4] F. Sancenon, R. Martinez-Manez, Chem. Rev. 103 (2003) 4419.
[5] P.D. Beer, D.K. Smith, Prog. Inorg. Chem. 46 (1997) 1.
[6] A. Taglietti, L. Rarodi, P. Pallavicini, M. Licchelli, L. Fabbrizzi, Transition Metals in Supramolecular Chemistry, John Wiley & Son Ltd., New York, 1999.
[7] P. De Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley, C.P. McCoy, J.T. Rademacher, T.E. Rice, Chem. Rev. 97 (1997) 1515.
[8] T. Aoyagi, A. Nakamura, H. Ikeda, H. Mihara, A. Ueno, Anal. Chem. 69 (1997) 659.
[9] A. Echevarren, A. Galan, J.-M. Lehn, J. De Mendoza, J. Am. Chem. Soc. 111 (1989) 4994.
[10] G. Deslongchamps, A. Galan, J. De mendoza, J. Rebek Jr., Angew. Chem. Int. Ed. 31 (1992) 61.
[11] A. Metzger, V.M. Lynch, E.V. Anslyn, Angew. Chem. Int. Ed. 36 (1997) 862.
[12] M. Berger, F.P. Schmidtchen, J. Am. Chem. Soc. 121 (1999) 9986.
[13] S. Camiolo, P.A. Gale, M.I. Ogden, B.W. Skelton, A.H.L. White, J. Chem. Soc., Perkin Trans. 2 (2001) 1294.
[14] S. Shinoda, M. Tadokoro, H. Tsukube, R. Arakawa, Chem. Commun. (1998) 181.
[15] L.O. Abouderbala, W.J. Belcher, M.G. Boutelle, P.J. Cragg, J. Dhaliwal, M. Fabre, J.W. Steed, D.R. Turner, K.J. Wallace, Chem. Commun. (2002) 358.
[16] H. Boerrigter, L. Grave, J.W.M. Nissink, L.A.J. Chrisstoffells, J.H. Van Der Mas, W. Verboom, F. De Jong, D.N. Reinhoudt, J. Org. Chem. 63 (1998) 4174.
[17] F. Werner, H.J. Schneider, Helv. Chim. Acta 83 (2000) 465.
[18] P. Buhlmann, S. Nishizawa, K.P. Xiao, Y. Umezawa, Tetrahedron 53 (1997) 1647.
[19] S. Sasaki, M. Mizuno, K. Naemura, Y. Tobe, J. Org. Chem. 65 (2000) 275.
[20] P.A. Gale, J.L. Sessler, V. Kral, Chem. Commun. 1 (1998) 1.
[21] C. Bucher, R.S. Zimmerman, V. Lynch, J.L. Sessler, J. Am. Chem. Soc. 123 (2001) 9716.
[22] C.J. Woods, S. Camiolo, M.E. Light, S.J. Coles, M.B. Hursthouse, M.A. King, P.A. Gale, J.L. Sessler, J. Am. Chem. Soc. 124 (2002) 8644.
[23] J.L. Sessler, J.M. Davis, Acc. Chem. Res. 34 (2001) 989.
[24] J.L. Sessler, T.D. Mody, D.A. Ford, A. Lynch, Angew. Chem. Int. Ed. 31 (1992) 452.
[25] J.L. Sessler, M. Cyr, H. Furuta, V. Kral, T.D. Mody, T. Morishima, M. Shionoya, S. Weghorn, Pure Appl. Chem. 65 (1993) 393.
[26] M. Takeuchi, T. Shioya, T.M. Swager, Angew. Chem. Int. Ed. 40 (2001) 3372.
[27] P.A. Gale, S. Camiolo, C.P. Chapman, M.E. Light, M.B. Hursthouse, Tetrahedron Lett. 42 (2001) 5095.
[28] A. Metzger, V.M. Lynch, E.V. Anslyn, Angew. Chem. Int. Ed. 36 (1997) 862.
[29] M. Berger, F.P. Schmidtchen, J. Am. Chem. Soc. 121 (1999) 9986.
[30] P.D. Beer, P.A. Gale, Angew. Chem. Int. Ed. 40 (2001) 486.
[31] A. Bianchi, K. Bowman-James, E. Garcia-Espana, Supramolecular Chemistry of Anions, Wiley–VCH, New York, 1997.
[32] M. Inouye, K. Hashimoto, K. Isagawa, J. Am. Chem. Soc. 116 (1994) 5517.
[33] K.N. Koh, K. Araki, A. Ikeda, H. Otsuka, S.J. Shinkai, J. Am. Chem. Soc. 118 (1996) 755.
[34] G. Wulff, Pure Appl. Chem. 54 (1982) 2093.
[35] B.R. Linton, M.S. Goodman, E. Fan, S.A. Van Arman, A.D. Hamilton, J. Org. Chem. 66 (2001) 7313.
[36] M. Berger, F.P. Schmiduchen, J. Am. Chem. Soc. 121 (1999) 9986.
[37] R.H. Garrett, C.M. Grisham, Biochemistry, Saunder College Publishing, New York, 1995.
[38] S.L. Wiskur, J.J. Lavigne, A. Metzger, S.L. Tobey, V. Lynch, E.V. Anslyn, Chem. Eur. J. 10 (2004) 3792.
[39] K.A. Connors, Binding Constants, The Measurement of Molecular Complex Stability, John Wiley & Sons, New York, 1987.
[40] S.C. McCleskey, P.N. Floriano, S.L. Wiskur, E.V. Anslyn, J.T. McDevitt, Tetrahedron 59 (2003) 10089.
[41] J.J. Lavigne, E.V. Anslyn, Angew. Chem. Int. Ed. 38 (1990) 3666.
[42] A.M. Piatek, Y.J. Bomble, S.L. Wiskur, E.V. Anslyn, J. Am. Chem. Soc. 126 (2004) 6072.
[43] S.L. Wiskur, E.V. Anslyn, J. Am. Chem. Soc. 123 (2001) 10109.
[44] S.L. Wiskur, PhD Thesis. The University of Texas, Austin, Texas, 2003.
[45] The reaction rate constant for the dihydroxylation of fumaric acid catalyzed by osmium tetroxide was mistakenly reported as $6 \times 10^{-4}\ \mathrm{min}^{-1}$ which should be $6 \times 10^{-4}\ \mathrm{h}^{-1}$.
[46] K. Niikura, A.P. Bisson, E.V. Anslyn, J. Chem. Soc., Perkin Trans 2 (1999) 1111.
[47] Z. Zhong, E.V. Anslyn, J. Am. Chem. Soc. 124 (2002) 9014.
[48] B. Mulloy, R.J. Linhard, Curr. Opin. Struc. Biol. 11 (2001) 623.
[49] R.E. Hileman, J.R. Fromm, J.M. Weiler, R.J. Lindhard, Bioessays 20 (1998) 156.
[50] R. Sasisekharan, G. Venkataraman, Curr. Opin. Chem. Biol. 4 (2000) 626.
[51] T.D. James, K.R.A.S. Sandanayake, S. Shinkai, Angew. Chem. Int. Ed. 35 (1910).
[52] A. Sugasaki, K. Sugiyasu, M. Ikeda, M. Takeuchi, S. Shinkai, J. Am. Chem. Soc. 123 (2001) 10239.
[53] L.A. Cabell, M.K. Monahan, E.V. Anslyn, Tetrahedron Lett. 40 (1999) 7753.
[54] M. Takeuchi, M. Ikeda, A. Sugasaki, S. Shinkai, Acc. Chem. Res. 34 (2001) 865.
[55] T. Mizuno, T. Fukumatsu, M. Takeuchi, S. Shinkai, J. Chem. Soc., Perkin. Trans. 1 (2000) 407.
[56] H. Kobayashi, K. Nakashima, E. Ohshima, Y. Hisaeda, I. Hamachi, S.J. Shinkai, Chem. Soc., Perkin. Trans. 2 (2000) 997.
[57] M. Ikeda, S. Shinkai, A. Osuka, Chem. Commun. (2000) 1047.
[58] S. Penade, Topics in Current Chemistry, Springer-Verlag, New York, 2002.
[59] L. Zhu, E.V. Anslyn, J. Am. Chem. Soc. 126 (2004) 3676.
[60] P.A. Gale, L.J. Twyman, C.I. Handlin, J.L. Sessler, Chem. Commum. 1999 (1851).
[61] A.G. Goodman, L.S. Gilman, A. Gilman, The Pharmacological Basis of Therapeutics, McMillan, New York, 1980.
[62] L.B. Jaques, J. Can. Med. Assoc. 108 (1973) 1291.
[63] V.C. Yang, Y.Y. Fu, C.L.C. Teng, S.C. Ma, J.N. Shanberge, Thromb. Res. 74 (1994) 427.
[64] E. Conti, C. Rivetti, A. Wonacott, P. Brick, FEBS Lett. 425 (1998) 229.
[65] W. Bode, I. Mayor, U. Bauman, R. Huber, S.R. Stone, J. Hofsteenge, EMBO J. 8 (1989) 3467.
[66] D.W. Landry, M.N. Stojanovic, J. Am. Chem. Soc. 124 (2002) 9678.
[67] B.V.L. Potter, D. Lampe, Angew. Chem. Int. Ed. 34 (1933).
[68] M.J. Berridge, Nature 361 (1993) 315.
[69] B. Dietrich, D.L. Fyles, T.M. Fyles, J.-M. Lehn, Helv. Chim. Acta 62 (1979) 2763.
[70] S. Atilgan, E.U. Akkaya, Tetrahedron Lett. 45 (2004) 9269.
[71] E.N. Zaitsev, S.C. Kowalczykowski, Nucl. Acids Res. 26 (1998) 650.
[72] A. Revzin, The Biology of Nonspecific DNA–Protein Interactions, CRC Press, Boca Raton, 1990.
[73] A.K. Eggleston, N.A. Rahim, S.C. Kowalczykowski, Nucl. Acids Res. 24 (1996) 1179.
[74] S.W. Matson, K.A. Kaiser-Roger, Annu. Rev. Biochem. 59 (1990) 289.
[75] S.W. Matson, Progress in Nucleic Research and Molecular Biology, Academic Press, New York, 1991.
[76] D.M. Mock, G. Langford, D. Dubois, N. Criscimagna, P. Horowitz, Anal. Biochem. 151 (1985) 178.
[77] N.M. Green, Avd. Protein Chem. 29 (1975) 85.
[78] Y.W. Ham, W.C. Tse, D.L. Boger, Bioorg. Med. Chem. Lett. 13 (2003) 3805.
[79] W.C. Tse, D.L. Boger, Acc. Chem. Res. 37 (2004) 61.
[80] S.A. Piletsky, E. Terpetschnig, H.A. Andersson, I.A. Nicholls, O.S. Wolfbeis, Fresenius J. Anal. Chem. 364 (1999) 512.
[81] N.T. Greene, K.D.J. Shimizu, J. Am. Chem. Soc. 127 (2005) 5695.
[82] A. Taglietti, M. Licchelli, L. Fabbrizzi, Dalton Trans. (2003) 3471.

[83] A. Taglietti, A. Leone, L. Fabbrizzi, Angew. Chem. Int. Ed. 40 (2001) 3066.
[84] M. Bonizzoni, G. Piovani, A. Taglietti, L. Fabbrizzi, Tetrahedron 60 (2004) 11159.
[85] L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, A. Taglietti, D. Sacchi, Chem. Eur. J. 2 (1996) 75.
[86] A. Taglietti, F. Stomeo, N. Marcotte, L. Fabbrizzi, Angew. Chem. Int. Ed. 41 (2002) 3811.
[87] M.A. Hortala, N. Marcotte, F. Stomeo, A. Taglietti, L. Fabbrizzi, J. Am. Chem. Soc. 125 (2003) 20.
[88] W.N. Lipscombe, N. Strater, Chem. Rev. 96 (1996) 2375.
[89] S.L. Tobey, E.V. Anslyn, Org. Lett. 5 (2003) 2029.
[90] T. Zhang, E.V. Anslyn, Tetrahedron 60 (2004) 11117.
[91] H. Ait-Haddou, S.L. Wiskur, V.M. Lynch, E.V.J. Anslyn, Am. Chem. Soc. 123 (2001) 11296.
[92] M.S. Han, D.H. Kim, Angew. Chem. Int. Ed. 41 (2002) 3809.
[93] R.G. Hanshaw, S.M. Hilkert, H. Jiang, B.D. Smith, Tetrahedron Lett. 45 (2004) 8721.
[94] D.H. Lee, J.H. Im, S.U. Son, Y.K. Chung, J.I. Hong, J. Am. Chem.Soc. 125 (2003) 7752.
[95] D.H. Lee, S.Y. Kim, J.I. Hong, Angew. Chem. Int. Ed. 43 (2004) 4777.
[96] Y. Takahashi, D.A.P. Tanaka, H. Matsunaga, T.M. Suzuki, J. Chem. Soc., Perkin Trans. 2 (2002) 759.
[97] A. Buryak, K. Severin, Angew. Chem. Int. Ed. 43 (2004) 4771.
[98] S.A. Hilderbrand, M.H. Lim, S.J. Lippard, J. Am. Chem. Soc. 126 (2004) 4972.
[99] L.J. Ignaro, Angew. Chem. Int. Ed. 38 (1870).
[100] P.K. Lala, Cancer Metatasis Rev. 17 (1998) 1.
[101] D.A. Wink, Y. Vodovotz, F. Laval, M.W. Dewhirst, J.B. Mitchell, Carcinogenesis 19 (1998) 711.
[102] V. Calabrese, T.E. Bates, A.M.G. Stella, Neurochem. Res. 25 (2000) 1315.