A new fluorescent sensor for the visual detection of anions

Detecting the presence of anions such as phosphate and nitrate is very important, with a range of clinical, environmental and biological applications. Dr Nicola Edwards and her team at Misericordia University of Dallas, Pennsylvania, US, developed a fluorescent sensor for the detection of anions based on terbium (Tb) and europium (Eu). Anion detection was achieved visually and monitored using spectroscopic techniques.

Dr Nicola Edwards and her team at Misericordia University of Dallas, Pennsylvania, US, developed a fluorescent sensor for the detection of phosphates, nitrates, and other anions. The sensor fluoresces green under UV light; its fluorescence is quenched in the presence of anions. The extent of this quenching is dependent on the type of anion that is being detected and its concentration. Anion detection was achieved with just the naked eye and monitored using well-established spectroscopic techniques.

Chemosensors (or sensors for short) are molecules that interact uniquely with a substance of interest by means of a chemical interaction. This interaction can be used to detect or 'sense' the presence of the substance of interest, called the analyte. Optical sensors detect changes in the amount of light absorbed or emitted by the molecule in the presence of the analyte. The capabilities of these sensors are tremendous: they make the previously invisible analyte suddenly visible, shedding light on whether the analyte is present in the medium being tested. With just the naked eye, one can detect the presence of the analyte without the use of chemical instrumentation, or the personnel needed to operate them.

Sensors perform two main functions: they determine if an analyte is present in the medium being tested and, if the analyte is present, they determine its concentration. This ability to monitor chemical species of interest has become a significant tool in our arsenal and has been applied in medicinal and environmental arenas as well as in forensics. Sensors are used every day, by scientists and non-scientists alike, for example in checking water quality, the contents of pools or for disease detection from blood or urine samples. This article will provide an overview of the steps that researchers use to design and construct sensors, showcasing a particular system that was designed and used for the detection of anions.

WHY THE DETECTION OF ANIONS IS IMPORTANT

Anions are chemical entities bearing an overall negative charge. The names of anions can be recognised as they have the endings 'ide', 'ite', and 'ate'. Figure 1 shows the structures of anions that were used in this study. The detection and quantification of anions has attracted widespread interest among researchers due to their wide-ranging applications. In environmental settings, monitoring levels of nitrate and phosphate – common components of fertilisers – is crucial in determining levels of pollution in waterways. Some diseases and conditions lead to imbalances in the concentration of anions in biological fluids and hence monitoring these anion concentrations can serve as a means of diagnosing these conditions and monitoring treatment plans. For example, high concentrations of phosphate in blood are correlated with kidney disease and high concentrations of pyrophosphate in synovial fluid are correlated with a rheumatologic disorder called calcium pyrophosphate dehydrate disease (CPDD). Pyrophosphate concentrations in blood are also inversely correlated with the amount of vascular calcification in patients with chronic kidney disease.

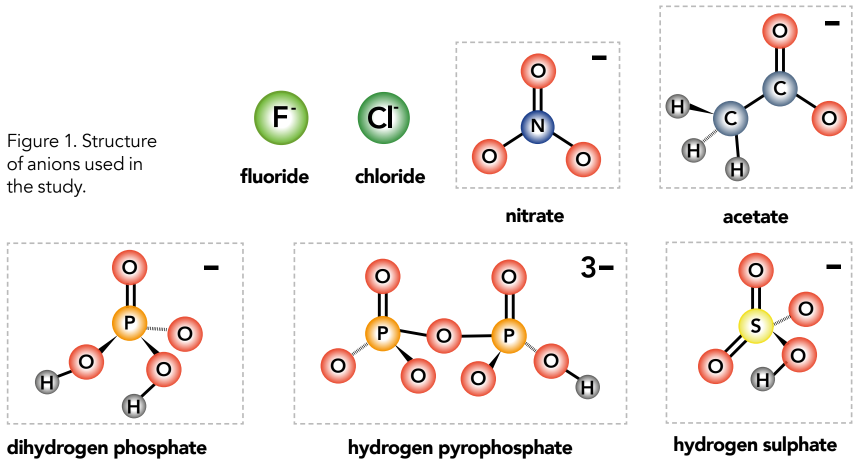

Figure 1. Structure of anions used in the study.

DESIGNING AND CONSTRUCTING ANION SENSORS

The design of a sensor for an anion or any analyte of interest is based on the construct shown in Figure 2 and is composed of two main parts: a receptor that contains binding sites tailored to the analyte of interest. The job of the receptor is to maximise the likelihood of binding or attachment of the analyte to the sensor. The sensor also contains a signalling unit. The signalling unit is usually a coloured compound or one which is fluorescent, i.e. it emits a glow upon excitation with light. There also must be a mechanism in place to allow for communication between the signalling unit and the receptor, such that a change is registered in the signalling unit (in its colour or fluorescence) when the anion/analyte is bound to the receptor. The types of changes (seen in Figure 2) are: i) increase in intensity of an existing colour or fluorescence, labelled a 'turn on' response; ii) decrease in intensity of an existing colour or fluorescence, labelled a 'turn off' response; and iii) a change in colour.

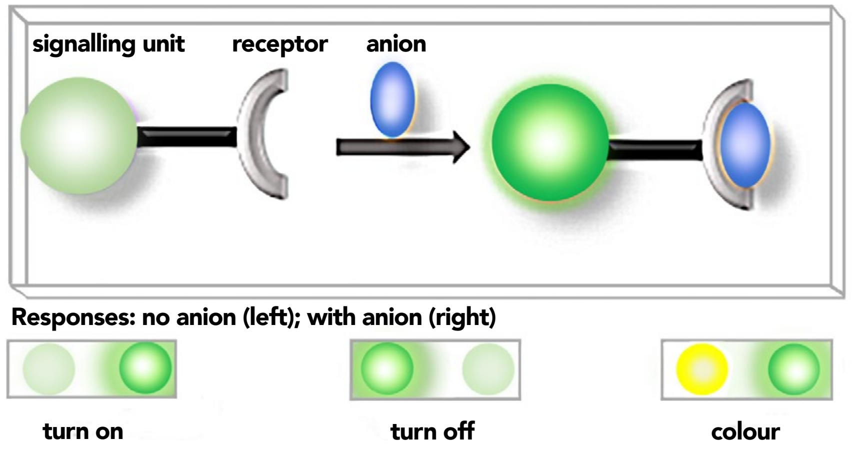

Figure 2. General construct of a sensor and possible responses.

Binding of anions to the receptor is based on the concept of 'opposites attract'. The negatively-charged anion is attracted by moieties (groups of atoms) possessing full or partial positive charges. In one approach, non-covalent interactions are used to ensure that the anion binds to the receptor. Amine, amide, and urea are common moieties used for anion binding; these moieties use hydrogen bonding interactions. In a second approach, a metal ion (as part of a metal ligand complex) that possesses a full-blown positive charge is used to bind the anion. It can either form a new metal ion-anion complex or the anion can bind to the existing metal-ligand complex, such that it allows for the registered output to be changed.

Lanthanides, a group of metals in the middle of the periodic table (elements with atomic numbers 58–71), are frequently employed in the construction of anion sensors. To harness their remarkable properties, they must be attached to an antenna –a ligand capable of exciting the lanthanide so as to turn on its fluorescence (Figure 3). The solvent used for the study is also an important design consideration as the receptor must outcompete the solvent to bind to the anion.

DEVELOPING ANION RECEPTORS AS THE FIRST STEP

Calixarenes have proven to be versatile scaffolds for the development of anionic receptors and sensors, as it can be functionalised at the lower and upper rims with anion recognition moieties. The scaffold allows these moieties to work in tandem for enhanced binding of anion to receptor. Edwards and her team perfected a four-step method for the modification of calixarenes with linkers containing amides and pyridine moieties at the lower rim of the calixarene (Figure 4, left panel). Computational studies were used to design the structure of the receptors and nuclear magnetic resonance (NMR) spectroscopy was used to test the binding of the anions to the modified calixarenes. Pyridine-functionalised calixarenes served as an important model system as it was simpler to study computationally and experimentally. The receptor was found to recognise dihydrogen phosphate and carboxylates in a highly competitive solvent, dimethyl sulfoxide (DMSO).

DEVELOPING ANION SENSORS

The team then turned its attention to adding the signalling unit. This was tantamount to 'swapping' the pyridine for terpyridine moieties; this process used a slightly different synthetic scheme than was used in their 2013 study. Additionally, the calixarenes were chelated (forming a compound comprised of a ligand with a central metal atom) with Tb and Eu salts, two lanthanides which emit colours in the visible range of the spectrum, when attached to a suitable antenna. (Figure 4:

The team reported that the sensor was responsive towards a variety of anions.

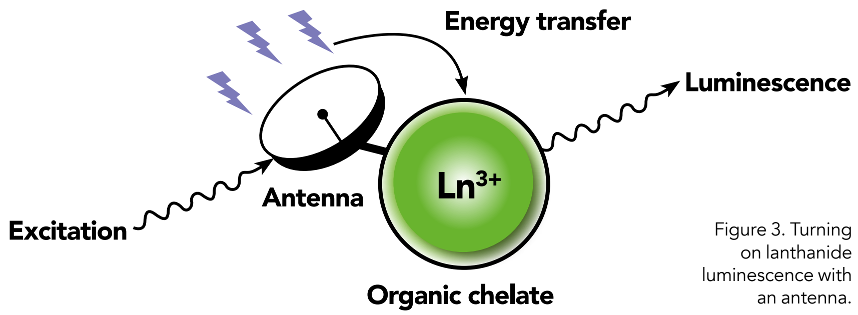

Figure 3. Turning on lanthanide luminescence with an antenna.

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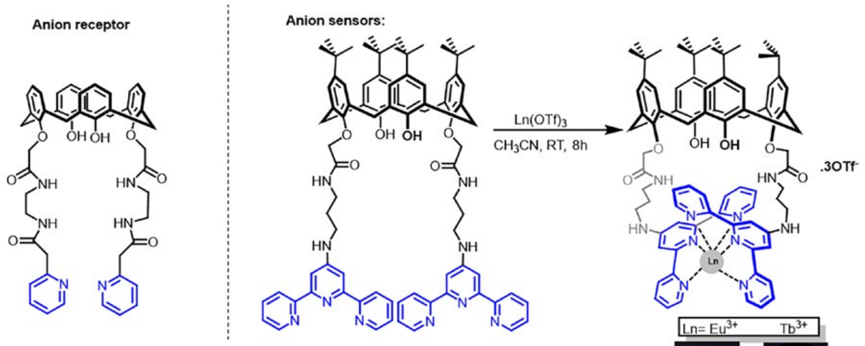

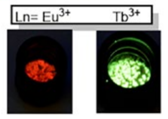

Figure 4. Pyridine-functionalised calixarenes developed for use as anion receptors and terpyridine-functionalised calixarenes developed for use as anion sensors.

right panel). Edwards and her colleagues also studied the photophysical properties and anion-sensing capabilities of the lanthanide complexes and reported their results in a 2020 article.

HOW THE SENSOR WORKS AND ITS CAPABILITIES

The sensing capabilities of the Tb complex were tested due to its higher luminescence output relative to its Eu counterpart. This was done by means of visual detection, UV-Vis and fluorescence studies. The Tb complex formed a green, fluorescent solution in acetonitrile (visible under UV light); its fluorescence was quenched in the presence of anions, indicating that this sensor exhibits a turn-off response towards this group of analytes.

Shown in Figure 5 is a summary of the changes that occur when the anion is bound to the sensor along with the visual appearance of the sensor in the absence (left) and the presence (right) of one of the anions in the study, dihydrogen phosphate.

The sensor responds to a variety of anions: hydrogen sulphate, nitrate, the phosphate derivatives (dihydrogen phosphate and hydrogen pyrophosphate), the halides (chloride and fluoride), and the acetate ion. The sensor exhibited the best responses as measured by the degree of quenching towards pyrophosphate, followed next by fluoride and then hydrogen sulphate. Generally, there was good correlation between the sensor response and the basicities of the anions except for hydrogen sulphate which presented with a greater sensor response than would be expected if basicity was the only factor at play. This indicated that the presence of OH bonds (with the ability to quench the fluorescence of the Tb) was also an important factor in the sensor response to anions.

With just the naked eye, one can detect the presence of the analyte without the use of chemical instrumentation.

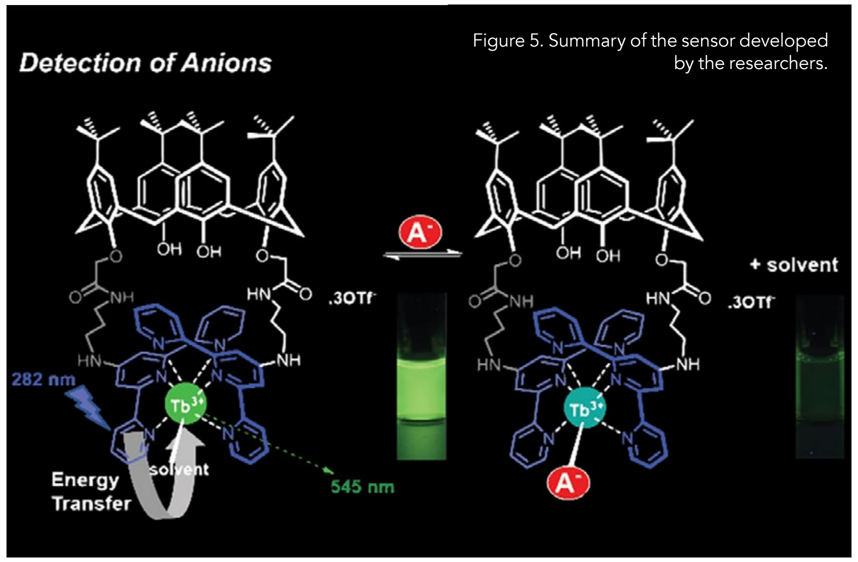

Figure 5. Summary of the sensor developed by the researchers.

Fluorescence studies were used to obtain a quantitative measure of the binding of the sensor to anions. The hypersensitive peak of the Tb luminescence was used as a probe for these studies. For each anion, a binding isotherm (fluorescence intensity at 545nm vs concentration of anion) was obtained. From this data, binding constants were extracted. These studies indicated that the binding of anion to sensor was strong; binding constants for anions were above the limit of what could be measured for some anions and even the notoriously difficulty nitrate had a high binding constant (log K= 5.60(0.16)).

WHAT IS NEXT FOR THE RESEARCH GROUP?

The group is currently working on developing a pattern-based sensing array for anions based on their sensor. Pattern-based sensor arrays use a series of sensors that work in tandem to produce composite signals for the analytes of interest, anions in this case. Essentially an array creates 'fingerprints' for each anion, allowing for their detection and (in some cases) quantification in unknown samples. Following this, the team will explore extensions of this system to other media and analytes of interest.

Edwards and her team have successfully developed a terpyridine-functionalised calixarene that acts as an anion sensor when it is complexed with Tb(III). Anion sensing results in a change in the luminescent properties of the complex, that can be detected with the naked eye. The anion-sensing ability of the Tb(III) complex was evaluated via visual detection, UV-vis and fluorescence studies. The team reported that the sensor was found to be responsive towards a variety of anions. It will ultimately work towards a system that can potentially be used for disease diagnosis in clinical settings.

Behind the Research

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W: Nicola Edwards - Faculty Opinions

Research Objectives

Dr Nicola Edwards and her team developed a fluorescent sensor for the detection of phosphates, nitrates and other anions.

Detail

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Bio
Dr Nicola Edwards is an associate professor of chemistry at Misericordia University. She obtained her B.A. in chemistry from Rice University and a Ph.D. in chemistry from the University of California, Los Angeles. She was a postdoctoral fellow at the University of Texas at Austin. Her research interests are centred around the development of detection methods for anions, cations and small molecules of interest.

Funding
Misericordia University Faculty Research Grant Program

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Personal Response

How close is your team to developing the sensor array? Have you got specific clinical applications in mind for the array under development?

" Sensor arrays utilising optical sensors are extremely powerful tools in our arsenal, allowing for the detection and quantification of substances in complex media. This can be done with just the naked eye and obviates the need for chemical instrumentation or the personnel needed to operate them. With our anion sensor in hand and all the lessons learned from our studies, we are excited to be at the stage where we are diversifying the system by creating the library of sensors for use in the sensor array. We hope to then fine-tune our system, ie, maximise the discrimination for anions and apply it to other media including water and biological fluids. "

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