Squaraine-Based Optical Sensors: Designer Toolbox for Exploring Ionic and Molecular Recognitions

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Abstract: Small molecule-based chromogenic and fluorogenic probes play an indispensable role in many sensing applications. Ideal optical chemosensors should provide selectivity and sensitivity towards a variety of analytes. Synthetic accessibility and attractive photophysical properties have made squaraine dyes an enticing platform for the development of chemosensors. This review highlights the versatility of modular assemblies of squaraine-based chemosensors and chemodosimeters that take advantage of the availability of various structurally and functionally diverse recognition motifs, as well as utilizing additional recognition capabilities due to the unique structural features of the squaraine ring.

Keywords: squaric acid; squarylium; absorbance; fluorescence; aggregation; chelate; chemodosimeter; host–guest chemistry

1. Introduction: A Case for Squaraine Scaffold

The design and synthesis of small molecule sensors, whose photophysical properties are altered upon interaction with various analytes in differing types of environments, represents one of the most important and active areas of modern chemical research. The tunability of both photophysical properties as well as analyte recognition motifs is crucial for achieving versatile chemosensor designs. Therefore, important consideration lies in the synthetic accessibility of the sensor’s scaffold. These facile, modular routes, based on a minimum number of synthetic steps utilizing readily available materials, are among the scaffold’s most desirable features.

Squaraine dyes (also known as squarylium dyes or 1,3-squaraines) are resonance-stabilized zwitterionic structures that feature donor–acceptor–donor motifs (Figure 1). Squaraines possess several attractive photophysical characteristics, which include strong absorption in the visible–near-infrared (IR) range, emissions in the near-IR region, good quantum yields, as well as good photostabilities. This contributes to their wide use as photonic materials in a variety of fields, including photovoltaics, biological imaging, and labeling, as well as photodynamic therapy [1,2].

From the synthetic standpoint, it could be argued that squaraine dyes are among the easiest ones to access, since the squaraine scaffold could be assembled in a fairly facile and modular manner. Thus, based on the photophysical properties of squaraines, and their synthetic accessibility, squaraines could be placed among the most versatile dyes that are currently available.

A number of reviews have covered the sensing ability of squaraine dyes in a variety of settings and for numerous analytes [3–7]. Yet, often, the recognition of analytes by the sensors is presented from the analyte perspective, while synthetic aspects for the design of chemosensors are given a lesser priority. Arguably, considering how various structural and functional components could be conjugated to a chromophoric/fluorophoric scaffold might provide a more general approach for more efficient sensor design. The dye’s scaffold should provide ample modification points that are amenable to the introduction of multiple
desired functionalities. Despite its fairly simple structure, the squaraine scaffold is fully amenable to diverse functionalization, both pre- and post-squaraine assembly.

![General structures of symmetric and non-symmetric 1,3-squaraines shown in the form of zwitterions. C, C', and N indicate the nature of the bond that connects squaraine ring to the substituents.]

This review will highlight some of the structural and functional motifs that have been used for assembling squaraine-based chemosensors, as an inspiration and a driving force for future designs. It should be mentioned that some of the chemosensors that are presented here could be more accurately described as chemodosimeters [8], based on their covalent interaction with the analyte. However, for the sake of consistency, and uniformity in structure labels, all squaraine-based scaffolds that interact with any analyte, regardless of whether this interaction is covalent or non-covalent in nature, will be referred to as squaraine sensors (SQSs).

2. Synthesis of Squaraine Dyes: General Considerations

Synthetic approaches to 1,3-squaraines are quite robust and modular, although they are not always very efficient, as the yields of the final dyes typically range from fair to moderate. However, this “shortcoming” is compensated by the operational simplicity of the isolation and purification of squaraines, i.e., in many instances, filtration, recrystallization and/or washing are sufficient for obtaining pure products. Importantly, as the examples that will follow demonstrate, synthesis of the squaraine core is typically performed under fairly mild conditions. This assures a high degree of functional group tolerance in the synthesis of squaraines. A summary of the general considerations for the synthesis of symmetric and non-symmetric squaraine dyes is presented below. For more detailed, substrate-specific synthetic approaches, some recent reviews could be consulted [9–11].

2.1. Squaric Acid-Based Syntheses

The synthesis of squaraine dyes typically involves a condensation reaction that proceeds via the nucleophilic addition of electron-rich (hetero)aromatics, compounds with an activated methyl group, or amines, to the electrophilic center on the squaric acid (Scheme 1). Several solvent systems, typically at elevated temperatures, have been utilized. However, the most commonly used solvents are as follows: (a) mixtures of aromatic hydrocarbons (typically toluene or benzene) and alcohols (typically n-butanol),
with the azeotropic removal of water (this is, by far, the most prevalent system) [12–14];
(b) alcohols, in the presence of trialkylorthoformates, which are used as dehydrating
agents [15–17]; (c) typical molecular organic solvents, such as dimethylformamide [18–20].
Non-conventional types of media, such as deep eutectic solvents, have been recently shown
to be suitable for the synthesis of squaraine dyes [21]. Furthermore, solvent-free approaches
to some squaraine dyes have been introduced [22]. These novel approaches might prove to
be a viable complement to traditional synthetic routes to squaraines.

**Scheme 1.** General synthetic approaches to symmetric and non-symmetric squaraines using squaric acid as the starting
material. **Nu**\(^{\text{C}}\)-H and **Nu**\(^{\text{N}}\)-H depict C- and N-nucleophiles, respectively.

Conventionally, the symmetric squaraines **A** and **B** (Scheme 1) are obtained by reacting
the corresponding nucleophiles (ca. 2 equiv.) with squaric acid [12–14]. A few general
comments are noteworthy. When amines are used as N-nucleophilic components (**Nu**\(^{\text{N}}\)-H),
primary amines mostly appear to be viable substrates [12], and only a handful of squaraines
are produced upon the reaction of secondary amines with squaric acid [23]. In the case
of aromatic amines, the electronic nature of substituents on the aromatic ring appears
to have little effect on the efficiency of the reaction. However, only electron-rich aromatic
C-nucleophiles (**Nu**\(^{\text{C}}\)-H) prove to be viable reagents for squaric acid; this is consistent with
the electrophilic aromatic substitution conditions. For the synthesis of the C-arylidene type
of squaraines (Figure 1), condensation reactions typically require the use of organic bases,
such as quinolone and pyridine, to generate suitable nucleophilic species.

Understandably, efficient one-pot syntheses of non-symmetric squaraines are not
feasible [24]. Therefore, in order to obtain non-symmetrical squaraines, such as **C**, **D**, and
**E** (Scheme 1), multistep routes have to be considered. These routes rely on the synthesis
of the corresponding semisquaraines, i.e., **H** and **I** [24–26]. Semisquaraine **H** could be
obtained directly from the reaction between equimolar amounts of primary amines and
squaric acid. It was found that the reaction is quite facile in water, under both conventional and microwave heating [27,28]. In the case of aromatic amines, the presence of electron-donating and electron-withdrawing groups had little effect on the efficiency of the reaction. In the case of 4-aminobenzylamine [28], the reaction proceeded via the aromatic amine, rather than the benzylic one, due to the protonation of the more basic benzylic amine by squaric acid, thus rendering it non-nucleophilic.

On the other hand, one-step synthesis of the corresponding C-semisquaraine I appeared to not be possible, as the reaction between squaric acid and Nu$_1^{C}$-H tends to produce the corresponding alkyl squarate F under various conditions [29,30]. However, the subsequent hydrolysis reaction, either under acidic or basic conditions (typically in the presence of organic solvent, such as acetone, EtOH, or AcOH, to aid the solubility of the starting materials) affords I in 70–90% yields. It should be noted that the direct conversion of F to C was also reported [31].

2.2. Squaric Acid Ester and Squaryl Chloride-Based Approaches

In a number of instances, it was reported that general routes that utilize squaric acid as one of the starting materials fail to produce the desired squaraines (e.g., A and B) and/or semi-squaraines F, G, H, and I (Scheme 1). Therefore, alternative routes to squaraines that involve either dialkyl squarate J or squaryl dichloride K were developed (Scheme 2).

![Scheme 2](image)

**Scheme 2.** General synthetic approaches to semisquaraines via dialkyl squarates and squaryl chloride. Nu$_1^{C}$-H and Nu$_1^{N}$-H depict C- and N-nucleophiles, respectively.

Dialkyl squarate J is produced via refluxing squaric acid in the corresponding alcohol [32–35], although methyl and ethyl squarates, for example, are available from major chemical manufacturers. The C- and N-containing semi-squaric acid esters F and G could be obtained in ca. 60–80% upon a reaction of J with the corresponding Nu$_1^{C}$-H and Nu$_1^{N}$-H (Scheme 2). These semi-squaric acid esters could then undergo hydrolysis, either under acidic or basic conditions, to yield the desired squaraines H and I in moderate to good yields [36–38]. The corresponding squaraines could then be obtained according to general protocols (Scheme 1).

Squaryl chloride K could be efficiently produced upon the treatment of squaric acid with chlorinating agents, such as thionyl chloride [39,40], and, typically, as-prepared.
K could be used for subsequent steps, without any purification. The reaction between K and Nu\textsuperscript{R1-H} or Nu\textsuperscript{R2-H} nucleophiles produces the corresponding semisquaric acid chlorides L \[41\] and M \[42–45\], respectively. The subsequent acidic hydrolysis furnishes semisquaraines H and I \[46–49\], which could then be converted to squaraines following established protocols (Scheme 1).

Interestingly, secondary amines, along with primary amines, were shown to be common, viable nucleophiles towards either \( J \) or \( K \) \[41\]. This is contrary to the reaction between amines and squaric acid (Scheme 1), where primary amines were mostly shown to be viable nucleophiles \[12\].

3. Chelating Group Approach for Sensor Designs

One of the most utilized approaches to chemosensor design relies on the incorporation of an analyte recognition motif into a chromophoric/fluorogenic scaffold (so-called binding unit-signaling unit approach \[50\]). In the case of squaraine dyes, this approach is realized in a fairly straightforward manner, by utilizing analyte recognition motifs that contain either an electron-rich aromatic moiety or amine functionality. Several analyte-specific recognition motifs, which are primarily based on the so-called host–guest interactions, are given below.

3.1. Crown Ether-Containing Squaraine Sensors

Crown ethers, as well as their nitrogen- and sulfur-containing analogues, are well-known receptors for a variety of alkaline and alkaline-earth (crown and aza-crown ethers), and transition and heavy metals (aza-crown and (aza)-thio-crown ethers) \[51\]. Synthetic routes to various functional crown ether motifs have been developed, which makes them suitable building blocks for chemosensor development \[52\]. Crown ether-containing squaraines have been prepared using a general method (Scheme 3) \[53\], following a condensation reaction between squaric acid and N-phenyl crown ethers \[54–56\].

![Scheme 3. Synthesis of aza-crown ether-containing SQS1, SQS2, and SQS3 \[53\].](image)

The interaction of SQS1–SQS3 was investigated with Li\textsuperscript{+}, Na\textsuperscript{+} and K\textsuperscript{+} salts (used as their respective perchlorate salts) in toluene–CH\textsubscript{3}CN mixtures. In general, a decrease in both absorption and emission intensities was noted. In addition, by examining the quenching of quantum yield as a function of the increasing concentration of Li\textsuperscript{+} and Na\textsuperscript{+} salt, it was suggested that SQS2 forms stronger complexes with these metal ions than SQS1. Specifically, SQS1–Li and SQS1–Na appeared to have \( K_\text{d} \) values of ca. 3.7 mM and 10 mM \[53\], respectively, while SQS2–Li and SQS2–Na appeared to have \( K_\text{d} \) values of ca. 0.64 and 0.77 mM \[57\], respectively. Notably, for SQS1, a one-to-one complex was prevalent when \([\text{Li}^+] \leq 10 \text{ mM} \), whereas at \([\text{Li}^+] > 20 \text{ mM} \), a two-to-one complex was suggested \[57\].

Later, the interaction of SQS1, SQS2, and SQS3 with alkaline-earth, heavy, and transition metal ions in CH\textsubscript{3}CN was investigated using absorption spectroscopy \[58\]. Only SQS3 appeared to optically respond to the presence of an equimolar amount of...
Ba$^{2+}$, as was evident by the $\lambda_{ab}^{\text{max}}$ shift from 636 to 586 nm, with the solution changing color from blue to purple. Qualitatively similar changes in the absorption spectrum of SQS3 were noted in the presence of Pb$^{2+}$ (although the addition of larger quantities of Pb$^{2+}$ led to the disappearance of the absorption signal). Interestingly, Cu$^{2+}$, Fe$^{3+}$, and Hg$^{2+}$ (either in equimolar or excess amounts) induced a color change for all these crown ether-containing sensors. Based on stability constants and semiempirical calculations, it was determined that metal ion coordination at the nitrogen led to hypsochromic shifts, whereas bathochromic shifts were induced by metal ion coordination with the oxygen of the squaraine ring. The coordination of two metal ions typically led to the appearance of a broad band at 480 nm, or bleaching of the signal. However, since complex, yet qualitatively similar profiles were noted in all cases, practical sensing considerations that involve these SQSs might be limited.

One-pot, chromatography-free synthesis of an aza-crown ether-containing squaraine, from commercially available compounds, yielded the dye SQS4, with absorption and emission maxima at ca. 635 nm and ca. 665 nm, respectively (Scheme 4) [59]. The 1-Aza-18-crown-6 ionophore on SQS4 provided some preferential interactions with alkaline-earth metal ions over those of the alkaline ones, with Mg$^{2+}$ and Ba$^{2+}$ ions causing the largest decrease in the absorption and emission intensities of the sensor upon binding. It should be noted that the most pronounced changes were noted when a large excess (ca. 1800-fold) of metal ions, with respect to the sensor SQS4, was used. Based on the changes in the absorption spectra of SQS4, aggregation of the dye upon binding to Ba$^{2+}$ was evident from the considerable broadening of the signal. Similar spectral changes were noted when the Mg$^{2+}$–SQS4 complex was allowed to equilibrate overnight. As a follow-up, a more detailed procedure for SQS4, as well as a 1-aza-15-crown-5-containing squaraine, SQS5, which should, in principle, have high affinity towards smaller-sized ions, such as Li$^+$, for example, was also reported (albeit no binding studies were disclosed) [60].

![Scheme 4: One-pot synthesis of aza-crown-containing sensors SQS4 and SQS5](image-url)

Later, it was demonstrated that Li-selective SQS6 could indeed be obtained by incorporating an aza-15-crown-5 moiety into the squaraine scaffold (Scheme 5) [61]. The synthesis of SQS6 was accomplished in a fairly straightforward manner, with step 3 proceeding in a 25% yield, while requiring chromatographic purification of SQS6 (Scheme 5). Specifically, SQS6 was shown to exhibit a ca. 4.5-fold increase in the emission intensity in the presence of a large excess (up to 500-fold) of Li$^+$ ions. It is of interest to point out that even in the presence of a 1000-fold excess of Li$^+$, no significant changes in the absorption spectrum were observed. On the contrary, the absorption spectrum of SQS6 underwent drastic changes in the presence of even a slight excess of Cu$^{2+}$ ions. Specifically, when SQS6 (5 µM in CH$_2$Cl$_2$/CH$_3$CN, 1/4 v/v, mixture) was titrated with Cu$^{2+}$ (from 5.0 µM to 25 µM; the nature of the Cu salt was not specified), a continuous decrease in absorption intensity, up to ca. 13-fold of the original value, was noted.
Structures of symmetric lariat diaza-crown ether-containing SQS6, SQS7, SQS8, and benzo-18-crown-6-containing SQS6 (Figure 2) [62]. Similarly to the cases described above, a decrease in both the absorption and emission intensities of the sensor took place in the presence of increasing amounts of Na\(^{+}\) (0 to 4mM), whereas no spectral changes were noted upon the addition of solutions that contained saturated potassium salts, such as nitrate, perchlorate, and thiocyanate. Based on these spectroscopic changes, a dissociation constant of 0.7 mM (in iPrOH) for the Na–SQS6 complex was determined.

Although, in general, the complexation of softer Lewis acids, such as Hg\(^{2+}\) and Pb\(^{2+}\), would benefit from the presence of softer donor atoms, such as S, dibenzo-18-crown-6-containing SQS8 (Figure 2) exhibited a relatively high sensitivity towards Hg\(^{2+}\), presumably due to the formation of a sandwich-like complex (based on 1/SQS8 to 2/Hg\(^{2+}\) stoichiometry) [63]. This sensor, which was obtained following a general condensation procedure between an electron-rich aromatic component (i.e., dibenzo-18-crown-6) and squaric acid, showed a typical decrease in absorption intensity in the presence of μM amounts of Hg\(^{2+}\) ions. Importantly, no changes in the absorption spectrum of SQS8 were noted in the presence of other typical host ions for crown ethers, such as Na\(^{+}\), K\(^{+}\), and Mg\(^{+}\), which were all used in a 20-fold molar excess [63].

Sulfur-containing crown ethers are known hosts for a variety of heavy metal ions. Specifically, dithia-dioxa-aza-crown ether (Scheme 6, blue structure), which could be prepared in two steps, using commercially available materials [64], has been widely utilized as a Hg\(^{2+}\) and Ag\(^{+}\) recognition moiety on the fluorescent dye scaffolds, including BODIPY, rhodamine, and anthracene [65]. This crown ether was used to prepare SQS9, following a typical condensation procedure (Scheme 6) [66]. In aqueous solution (H\(_2\)O:CH\(_3\)CN 4:1,
metal ions (e.g., Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Ni\(^{2+}\), Fe\(^{3+}\)), i.e., ions with virtually no affinity towards the softer version of crown ethers, did not cause any changes in the absorption spectrum of SQS9, as indicated by the presence of a 647 nm peak, and the formation of a green-colored solution. Not surprisingly, alkali, alkaline-earth, transition, and heavy metal ions (e.g., Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Ni\(^{2+}\), Fe\(^{3+}\)), i.e., ions with virtually no affinity towards the softer version of crown ethers, did not cause any changes in the absorption spectrum of SQS9. The effect of Hg\(^{2+}/Ag^+\) ions on the emission properties of SQS9 (\(\lambda_{\text{em}}^{\text{max}} = 667\) nm, \(\Phi = 0.13\), \(\tau = 0.67\) ns in CH\(_3\)CN) was not investigated [66]. It is worth noting that in neat CH\(_3\)CN, SQS9 did show a bathochromic shift in the presence of Fe\(^{3+}\) (1 equiv.) and a hypsochromic shift in the presence of Cu\(^{2+}\) (1 equiv.) [58].

Polyether bridged squaraine SQS10 was synthesized in a straightforward manner, in five steps, starting with catechol (Scheme 7) [67]. Based on dynamic light-scattering data, as well as the absorption and emission profiles of SQS10 in CH\(_3\)CN/H\(_2\)O mixtures, SQS10 was shown to undergo significant aggregation when the water content was \(\geq 10\%\) (v/v). Thus, all the metal binding studies were conducted in a CH\(_3\)CN/H\(_2\)O mixture with a 9/1 ratio (v/v).

Significant fluorescence enhancement was noted when SQS10 was titrated with Pb\(^{2+}\) salt (\(K_d = 27\) µM), while a number of transition and heavy metal ions, as well as ions that are known typical guests for crown ethers (e.g., Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\)), did not exhibit any prominent increases in emission intensity. It is of interest to mention, however, that the emission intensity of SQS10 notably increased (ca. four-fold) in the presence of Pb\(^{2+}\) (20 equiv.), whereas a ca. seven-fold decrease in emission intensity was noted in the presence of Cu\(^{2+}\) (20 equiv.). Thus, SQS10 could potentially be explored in both "turn-ON" and "turn-OFF" sensing modes [67].
3.2. Podand-Containing Squaraine Sensors

Podands, acyclic analogues of crown ethers (although other structural scaffolds that are capable of adopting specific geometry around the guest molecule have been included in this group), are among the versatile chelating motifs for various ionic and molecular species [68–70].

The podand-based sensor SQS11, with \( \lambda_{\text{ab}}^{\text{max}} = 630 \text{ nm} \) and \( \lambda_{\text{em}}^{\text{max}} = 652 \text{ nm} \), was obtained in a fairly straightforward manner, via condensation between bis-N-phenyl-containing podand and an aniline-containing semisquaraine (Scheme 8) [71]. In the presence of increasing amounts of Ca\(^{2+}\), the sensor SQS11 exhibited the following ratiometric response: a decrease in the 630 nm absorption maximum was accompanied by an increase in the 570 nm transition. Furthermore, a pronounced decrease in emission intensity was even noted in sub-equimolar amounts of Ca\(^{2+}\), with a relatively low estimated dissociation constant (\( K_d = 0.53 \mu\text{M} \)). The observed spectroscopic changes have been attributed to the formation of a foldameric structure, where the two chromophoric squaraine units were brought into close proximity of each other due to Ca-podand complexation, i.e., in the form of a face-to-face π-stacking arrangement. This structure was responsible for the hypsochromic shift, which was consistent with the formation of H-type species.

The sensor SQS11 showed significant selectivity towards Ca\(^{2+}\) in CH\(_3\)CN; for example, only insignificant changes in the absorbance and emission spectra of SQS11 were noted in the presence of Na\(^+\), K\(^+\), Sr\(^{2+}\), Mg\(^{2+}\), or Ba\(^{2+}\). When 0.5 and 1.0 equiv. of Ca\(^{2+}\) were added to SQS11, the quantum yield decreased by ca. 40 and 97%, respectively. On the contrary, when the same amounts of Mg\(^{2+}\) (i.e., 0.5 and 1.0 equiv.) were added, the quantum yield of SQS11 only decreased by ca. 5 and 20%, respectively.

By adjusting the length of the ethylene glycol tether, several other podand-containing squaraine sensors, SQS12–SQS14, were prepared (Scheme 8) [72]. The spectroscopic characteristics of SQS12–SQS14 exhibited some dependence on the length of the tether; for example, the absorption and emission maxima showed negative correlations with increasing numbers of ethylene glycol units in the podand moiety, whereas the quantum yields showed a positive correlation.

Notably, different stoichiometries were realized in regard to the Ca–SQS12 and Mg–SQS12 complexes. The larger cation Ca\(^{2+}\) was not a good fit for the podand’s oxygen and two nitrogen coordination sites, which prompted the formation of a sandwich-like two-to-one complex. On the other hand, the smaller cation Mg\(^{2+}\) appeared to favor the formation of a one-to-one complex. It should also be noted that as the length of the tether increased,
i.e., SQS12 to SQS14, the formation of one-to-one complexes appeared to be preferred, regardless of the identity of the metal ion. Furthermore, the mono-squaraine-containing podands PO1–PO5 (Scheme 8, bottom left) showed no changes in the absorption and emission spectra in the presence of various alkaline or alkaline-earth metal ions. However, $^1$H NMR investigations revealed that these podands do interact with Ca$^{2+}$ and Mg$^{2+}$, while no podand–metal interactions were detected for Li$^+$, Na$^+$, or K$^+$ [72,73].

[Scheme 8. Synthesis of podand-containing optical sensors SQS11–SQS14 (top, [71,72]), and examples of optically silent Ca$^{2+}$/Mg$^{2+}$–hosts PO1–PO4 (bottom left, [72,73]) and thio-containing SQS15 and SQS16, and thio-containing podand PS-1 (bottom right, [74]).]

The incorporation of soft S-donor atoms into the podand structure is known to favor the coordination of various metal ions that are considered to be soft Lewis acids, including mercury (Hg$^{2+}$) and silver (Ag$^+$). Thus, SQS15 and SQS16, along with PS-1 (Scheme 8, bottom right), were synthesized, and their ability to interact with Hg$^{2+}$ was examined [74]. Unlike the oxygen-containing squaraines PO1–PO4, which showed no optical response in the presence Ca$^{2+}$, the thio-containing analogues did show an optical response in the presence of Hg$^{2+}$, and the sensing ability appeared to be dependent on the length of the podand moiety, with SQS16 exhibiting the most pronounced changes in the presence of Hg$^{2+}$ (4 equiv.), while PS-1 was optically silent. Thus, by simply switching the recognition motif,
while retaining the other structural features of the squaraine dye constant, it appeared to be possible to construct sensors that are selective towards distinct analytes.

Additionally, a number of other thio-containing podands, in particular, those containing a thiocarbamate moiety, had been conjugated onto the squaraine’s scaffold and produced Hg$^{2+}$-specific sensors, which appeared to have “turn-ON” responses (Figure 3). Symmetric SQS17 showed strong absorption ($\lambda_{\text{abs max}} = 644$ nm) and emission ($\lambda_{\text{em max}} = 661$ nm) bands in organic solvents (CH$_2$Cl$_2$, CHCl$_3$, AcOH), which were indicative of a monomeric structure [75]. In aqueous solutions, aggregates of SQS17 were noted; for example, the purple-colored solution of SQS17 in the AcOH–H$_2$O mixture (4/6 v/v), with a broad absorption range of 500–700 nm ($\lambda_{\text{abs max}} = 548$ nm), showed no transitions above 650 nm in the emission spectrum. However, upon the addition of a Hg$^{2+}$ salt, a change from a purple-to blue-colored solution was noted, with the appearance of an absorption band at 636 nm, and a strong emission at 660 nm. These spectral changes indicated that the complexation of Hg$^{2+}$ induced the formation of the monomeric form of SQS17. Importantly, other metal ions, which are also considered to be soft Lewis acids (e.g., Ag$^+$, Cd$^{2+}$, Pb$^{2+}$), did not produce any noticeable changes, either in the absorption or in the emission spectra of SQS17. This suggested a lack of interaction with SQS17, as well as an inability of these ions to induce the disaggregation of SQS17. Therefore, SQS17 appeared to be a viable, selective, naked-eye Hg$^{2+}$ sensor, which provided an emission enhancement of ca. 700-fold, affording a detection limit of 7.1 nM.

The related non-symmetric SQS18 maintained high selectivity towards Hg$^{2+}$, but showed only a 10-fold enhancement in emission intensity in the presence of Hg$^{2+}$ (45 nM detection limit) [76]. For some squaraines, i.e., SQS19 and SQS20, the disaggregation effects induced by Hg$^{2+}$ were amplified by the addition of excess amounts of EDTA [77,78]. It is also of interest to note that even one dithiocarbamate moiety on the squaraine scaffold, i.e., SQS20 and SQS21 (Figure 3), still yielded a Hg$^{2+}$-selective response, with detection limits that were somewhat comparable to SQS17 [78,79], albeit with significantly reduced emission enhancement observed upon the addition of Hg$^{2+}$ salt; for example, a ca. four-fold increase in emission intensity in the presence of Hg$^{2+}$ (3 equiv.) was observed in the case of SQS18, while a ca. 700-fold increase was noted for SQS17 in the presence of Hg$^{2+}$ (5 equiv.). However, direct comparisons between SQS17 and SQS18...
should be taken with caution, due to the differences in the nature and composition of the media that were used for spectroscopic and metal ion detection studies, i.e., AcOH–H₂O (4/6 v/v) in the case of SQS17, versus EtOH–H₂O (1/4 v/v) in the case of SQS18. Additional structural manipulation revealed that the position of the dithiocarbamate moiety in the squaraine scaffold, e.g., SQS20 vs. SQS21, had little effect on the sensing ability towards Hg²⁺; both sensors showed good Hg²⁺ selectivity over other metal ions, and the emission intensity enhancements were in the range of 8- to 10-fold.

In contrast, SQS22, SQS23, and SQS24, which feature thio-ether-containing binding motifs, provided “turn-OFF” responses upon interaction with metal ions (Figure 4) [80–82]. Specifically, in CH₃CN, SQS22 exhibited the following spectral characteristics that were consistent with monomeric species: a strong, narrow absorption band (λₐₑₓₘₐₓ = 636 nm) and intense emission (λₑₑₘₐₓ = 670 nm) [80]. However, upon addition of Hg(ClO₄)₂, a gradual decrease in absorption intensity and a hypsochromic shift of λₑₑₘₐₓ to 575 nm, along with a decrease in emission intensity, were noted (in the presence of 20 equiv. of Hg²⁺, SQS22 showed no significant absorption or emission transitions). It is of interest to point out that in the presence of 20 equiv. of Cu²⁺ and Fe³⁺ (but not in the presence of Ag⁺, Pb²⁺, Cd²⁺, Zn²⁺, Ni²⁺, Fe²⁺, Cr³⁺), significant changes in both the absorption and emission spectra of SQS22 were noted. The optical response of SQS22 to Cu²⁺ was identical to that of Hg²⁺, whereas, in the presence of Fe³⁺, a ca. 20 nm hypsochromic shift, with decrease (ca. 1.6-fold) in intensity and moderate broadening, along with a ca. 10-fold decrease in emission intensity, was observed. Yet, in the CH₃CN/H₂O (2/1 v/v) mixture, only Hg²⁺ retained its interaction with SQS22, while all the other ions, including Cu²⁺ and Fe³⁺, did not induce any spectral changes in SQS22’s optical profiles. Thus, an efficient “turn-OFF” Hg sensor was realized [80]. Under these conditions (i.e., 20 equiv. of Hg²⁺, 2/1 (v/v) CH₃CN/H₂O mixture), the sensing ability of SQS22–Hg towards thiophilic species was tested. In the presence of Cys, Hcy, GSH, and His (20 equiv.), significant intensity increases in the absorption (at 642 nm) and emission (at 678 nm) bands were observed, which indicated a “turn-ON” sensing profile.

![Figure 4. Structures of thio-containing sensors SQS22 [80], SQS23 [81], and SQS24 [82].](image-url)

The observed cross-sensitivity of SQS22–Hg towards both SH-containing analytes and His prompted further studies that led to SQS23 [81], which was synthesized following the general routes utilized for non-symmetric squaraines (Scheme 2). Similarly to SQS22, in aqueous media, SQS23 was present in the form of non-emissive aggregates, which exhibited a broad absorption band in the 480–780 nm range. Monomer-to-aggregate equilibrium appeared to be dependent on the amount of water in the H₂O/THF mixtures. Importantly, SQS23–Hg’s Cys-His selectivity could be easily modulated by simply adjusting the water content, with the highest Cys-His selectivity (ca. 6.3-fold) achieved in the 4/1 H₂O/THF mixture. In addition, SQS23 was found to be completely soluble in the aqueous solution that contained β-cyclodextrin (β-CD), and the formation of the inclusion complex was confirmed by the appearance of an induced circular dichroism signal at 650 nm [81]. Encapsulated SQS23 showed the following photophysical characteristics that were consistent with the monomeric form of the dye: a narrow absorption band at 648 nm, and a strong emission (quantum yield of 0.28) band at 675 nm. Similarly to the SQS23–Hg⁴⁺ interaction, the SQS23–β-CD complex was also able to complex with Hg²⁺, which was evident from the decreasing absorption and emission bands of SQS23–β-CD in
the presence of increasing amounts of Hg$^{2+}$. In addition, the SQS23–β-CD–Hg assembly showed ca. three-fold selectivity towards SH-containing analytes (e.g., Cys, Hcys, and GSH) over His [81].

The incorporation of a slightly more elaborate thio-ether-containing binding motif on the squaraine scaffold yielded SQS24 (Figure 4) [82]. The monomeric form of SQS24 was observed in EtOH, yet the formation of J-aggregates (broad peaks with a maximum around 750 nm) was noted in the EtOH/H$_2$O mixtures with a water content above 70% (v/v). This sensor showed moderate selectivity towards Ag$^+$ (ca. 1.4-fold selectivity over other ions) and Hg$^{2+}$ (ca. 3.2-fold selectivity over other ions), as assessed by the changes in emission intensity. It appeared that the “turn-ON” response was induced when EDTA was added to a weakly fluorescent SQS24–Hg complex, but not to a SQS24–Ag complex. Furthermore, various amino acids, including Cys, His, and Trp, induced a significant increase in both the absorption and emission bands when added to SQS24–Hg. This highlighted the inferior selectivity capabilities of SQS24–Hg compared to other thio-containing squaraine sensors.

Thus, depending on the structural features within the podand-like moieties, SQSs that operate in either “turn-ON” or “turn-OFF” modes (Figures 3 and 4) towards ionic and molecular analytes could be realized.

### 3.3. Tetracarboxylate Binding Motif

The introduction of a tetracarboxylate binding motif on two tethered aromatic units produced sensors that allowed for the measurement of cytosolic Ca$^{2+}$, with high selectivity over other divalent ions, especially Mg$^{2+}$ (Figure 5A) [83,84]. Built on the successful application of these sensors for Ca$^{2+}$ sensing, various fluorophores have been decorated with a tetracarboxylate chelating motif that yielded Ca$^{2+}$ sensors [85]. These sensors offer a wide spectroscopic range, which covers green, red and near-IR regions, in both absorbance and emission, while affording emission enhancements of over 100-fold upon Ca binding [85].

![Figure 5. Structures of Ca-specific sensors. (A): original Ca indicators; (B): squaraine-containing SQS25 [86].](image)

The incorporation of the tetracarboxylate motif into the squaraine scaffold produced the sensor SQS25 that exhibited absorption and emission maxima at 698 nm and 733 nm, respectively (Figure 5B) [86]. The construction of SQS25 was accomplished via a chromatography-free two-step procedure, using typical condensation conditions between squaric acid and the more electron-rich aromatic moiety of the t-Bu-protected tetracarboxyl-containing unit, followed by TFA-induced removal of the t-Bu group (Figure 5B; the tetracarboxyl-aromatic moiety was synthesized via a multi-step chromatography-free sequence).
The intensity of the absorption of SQS25 underwent continuous decreases upon the addition of increasing amounts of Ca\textsuperscript{2+} ions, with notable changes (ca. 30% decrease) starting to occur in sub-equivalent amounts of Ca\textsuperscript{2+} \cite{86}. At a 500-fold excess of Ca\textsuperscript{2+}, appreciable broadening of the absorption peak at 698 nm (along with a ca. 80% decrease in intensity, and the appearance of additional peaks below 650 nm), indicative of the formation of various aggregation species, was noted. The dissociation constant was determined to be 3.6 \(\mu\)M. Importantly, however, no significant changes in the absorption spectrum of SQS25 were noted, even in the presence of a large excess (ca. 400-fold) of M\textsubscript{G}\textsuperscript{2+}. Notable changes in the presence of Ca\textsuperscript{2+} ions were also present in the emission spectrum of SQS25 (Figure 5B). About a 30% decrease in emission intensity was observed when a 3.8-fold excess of Ca\textsuperscript{2+} was added, and a large excess of Ca ions led to a further decrease in SQS25’s emission signal, with some flattening of the response above a 75-fold excess of Ca\textsuperscript{2+} \cite{86}.

### 3.4. Nitrogen-Containing Heterocycles as Binding Motifs

N-containing heterocycles, in particular, pyridine, are among the most commonly used moieties that are present in a variety of binding motifs for transition and heavy metal ions \cite{87–90}. The incorporation of such pyridine-containing chelators, such as di-2-pyridylmethylamine (i.e., dipicolylamine), in various dye scaffolds is a proven strategy to obtain viable chemosensors \cite{91–93}.

The introduction of dipicolyl moieties into the squaraine scaffold, to give SQS26, was achieved using a solvent-free, one-step process from commercially available starting materials (Scheme 9) \cite{94}. Notably, although reactions between aliphatic amines and alkyl squarates tend to give squaramides \cite{95}, in this particular case, neat conditions at the elevated temperature appeared to favor an exclusive formation of the symmetric 1,3-disubstituted squaraine. In addition, unlike most squaraine dyes, SQS26 appeared to be completely soluble in water (over a wide pH range), and no aggregation processes were noted, based on \(^1\)H NMR spectroscopy in D\textsubscript{2}O over a period of 8 days. Detailed pH and metal ion binding studies using SQS26 were carried. In addition, a notable (ca. six-fold) increase in the emission intensity of SQS26 (\(\lambda_{em}^{max} = 476\) nm) was observed in the presence of Hg\textsuperscript{2+} (1.05 equiv. added as Hg(NO\textsubscript{3})\textsubscript{2} salt in MES buffer (2.5 mM, pH 5.0)). On the other hand, no prominent changes in the emission spectra of SQS26 were noted in the presence of other metal ions, such as Zn\textsuperscript{2+} and Cu\textsuperscript{2+}, even though these ions are known to complex with the dipicolyl motif.

![Scheme 9. Solvent-free synthesis of SQS26](image_url)

Other pyridine-containing squaraine-based sensors have also been reported. Specifically, SQS27 and SQS28 were prepared in a straightforward manner (Scheme 10) \cite{96}. SQS27 provided a naked-eye determination of Cu\textsuperscript{2+} ions; the absorption maximum of SQS27 (\(\lambda_{ab}^{max} = 514\) nm; pink-colored solution) underwent a drastic shift to the red region (\(\lambda_{ab}^{max} = 680\) nm; blue-colored solution). Upon addition of EDTA, the pink color of SQS27 was restored, thus indicating a reversible nature of the SQS27–Cu interaction. Significantly, no pronounced changes were observed in the presence of 26 metal ions, including alkaline, alkaline-earth, transition, heavy metal, and lanthanides. It was determined that the one-to-one SQS27–Cu complex had \(K_d = 0.5\) \(\mu\)M. Furthermore, Cu selectivity was observed when the emission spectra of SQS27 were recorded in the presence of various metal ions; a six-fold decrease in the emission intensity (\(\lambda_{em}^{max} = 626\) nm) was produced in the
presence of only Cu\(^{2+}\). The possibility of SQS27 having Cu-sensing ability in a biological setting was tested using LL/2 and HepG2 cell lines. A notable decrease in intracellular fluorescence, i.e., “turn-OFF” response, was detected when these cells (incubated with 20 \(\mu\)M SQS27) were treated with CuCl\(_2\) (100 \(\mu\)M). Virtually complete fluorescence recovery was observed upon addition of EDTA (500 \(\mu\)M).

Similarly, in the presence of Cu\(^{2+}\) ions, SQS28 also exhibited red-shifted absorption, albeit less pronounced (from 554 nm to 640 nm, i.e., 86 nm shift) compared to SQS27 (166 nm shift), along with a ca. eight-fold suppression in emission intensity at 651 nm.

The incorporation of a quinoline moiety on squaraine’s scaffold was achieved in three steps, to give SQS29 (Scheme 11) [97]. Unlike many squaraines, SQS29 appeared to be relatively soluble in water, without significant aggregate formation. Specifically, the intensity of a narrow absorption peak (\(\lambda_{\text{ab max}} = 645\) nm) in ethanol (EtOH, 100%) decreased only by ca. 7% when the water content was increased from 0 to 70%, without any appreciable broadening of the peak or shift of the \(\lambda_{\text{ab max}}\). Although, in 100% water, a ca. 1.8-fold decrease in absorption intensity was observed, neither the width nor the \(\lambda_{\text{ab max}}\) of the peak changed. The fluorescent intensity of SQS29 (\(\lambda_{\text{em max}} = 664\) nm) was largely unaffected when the water content varied from 0 to 70% in EtOH.

By screening the interaction of SQS29 with various metal ions, a “turn-OFF” mode towards Hg\(^{2+}\) was observed. The emission intensity gradually decreased in the presence of increasing concentrations of Hg\(^{2+}\); a 10 % decrease was noted in the presence of 4 equiv. of Hg\(^{2+}\), whereas a 90 % decrease was noted in the presence of 24 equiv. of Hg\(^{2+}\). The emission intensity of SQS29 was restored upon addition of EDTA to SQS29–Hg solution (qualitatively similar results were obtained by the addition of a Cys, while GSH and His showed an inferior recovery of SQS29 emission, i.e., ca. 30% of that achieved with Cys).

Bipyridyl is a common binding motif for a variety of transition and heavy metal ions [90]. The incorporation of bipyridyl into the squaraine scaffold was achieved following a multi-step synthetic route, to give SQS30 (Scheme 12) [98]. This sensor was prone to aggregation in the presence of water, as the formation of non-emissive, H-type aggregates, with a low molar extinction coefficient, was observed. Thus, all fluorescent studies involving analytes were conducted in acetone. It was found that in the presence of increasing amounts of Zn\(^{2+}\) ions, a decrease in the emission maximum at 698 nm, with a
concurrent increase in the maximum at 720 nm, was noted. Furthermore, the absorption spectrum of SQS30 revealed a bathochromic shift (from 680 nm to 695 nm) in the presence of Zn$^{2+}$. Based on these spectroscopic data, the detection limit for Zn$^{2+}$ was estimated to be ca. 60 nM. Noteworthy, SQS30 showed a complex fluorescence emission response to other metal ions; for example, SQS30 became completely non-emissive above 680 nm in the presence of Cd$^{2+}$, Cu$^{2+}$, Co$^{2+}$, and Ni$^{2+}$, whereas a ca. 10-fold decrease in emission intensity ($\lambda_{em}^\text{max} = 698$ nm, i.e., no shift in the SQS30 maximum) was observed in the presence of Ag$^+$. 

The incorporation of bipyridyl into the squaraine scaffold was achieved following a straightforward manner, starting with diethyl squarate [99]. In the presence of less than 5 equiv. of tetrabutylammonium fluoride (TBAF, with F$^-$ acting as a base), the yellow-colored solution (due to low/non-existent basicity), NH$_4$OAc was able to induce similar changes in the absorption spectrum of SQS29 (Scheme 13). Unlike many squaraines, SQS30 appeared to show a complex fluorescence emission response to other halides; for example, SQS30 became completely non-emissive above 680 nm in the presence of Ag$^+$, whereas a ca. 10-fold decrease in emission intensity ($\lambda_{em}^\text{max} = 698$ nm, i.e., no shift in the SQS30 maximum) was observed in the presence of Ag$^+$.

**Scheme 11.** Synthesis of symmetric quinoline-containing Hg$^{2+}$-selective SQS29 [97].

Bipyridyl is a common binding motif for a variety of transition and heavy metal ions (due to low/non-existent basicity), NH$_4$OAc was able to induce similar changes in the absorption spectra of SQS29 (both qualitatively and quantitatively).

**Scheme 12.** Synthesis of bipyridyl-containing SQS30 [98] for metal ion recognition.

### 4. Squaraine Scaffolds with Acidic NH

By taking advantage of the fairly acidic nature of NH, a N-nucleophilic-based CO$_2$ sensor SQS31 was introduced (Scheme 13). SQS31 was synthesized in a fairly straightforward manner, starting with diethyl squarate [99]. In the presence of less than 5 equiv. of tetrabutylammonium fluoride (TBAF, with F$^-$ acting as a base), the yellow-colored SQS31 solution in DMSO ($\lambda_{ab}^\text{max} = 445$ nm) changed to a purple-colored solution ($\lambda_{ab}^\text{max} = 525$ nm) SQS31-1. When an excess of F$^-$ was used (ca. 20 equiv. of TBAF), a green solution ($\lambda_{ab}^\text{max} = 655$ nm), attributed to the delocalized di-anion SQS31-2, was obtained. Although other halides did not deprotonate SQS31 (due to low/non-existent basicity), NH$_4$OAc was able to induce similar changes in the absorption spectra of SQS31 (both qualitatively and quantitatively).
Arguably, by adjusting the electronic demands of the substituents on the squaraine core, the acidity of the NH could be modulated to yield sensors towards various bases.

When a solution of SQS31-2 was treated with various amounts of CO2, a blue shift in the absorption maximum, from 655 nm to 525 nm, was observed (Scheme 13, a rapid conversion of SQS31-3 to SQS31 was assumed). The limit of CO2 detection was estimated to be about 0.64 μM. Importantly, the response of SQS31-1 to CO2 appeared to be fairly fast and reversible, which allowed for 30 cycles of switching between 655 nm (SQS31-1) and 525 nm (SQS31-2), via alternating additions of TBAF and CO2. Overall, a naked-eye detection of CO2 and F− by SQS31 was realized.

Subsequently, a more elaborate sensor, SQS32, where a vinlylic amine was conjugated to the squaraine scaffold, was synthesized, following a two-step procedure, using a dimethylaniline-containing semisquaraine as the starting material (Scheme 14) [100]. Upon the treatment of SQS32 with TBAF, intermediate SQS32-1 (λab,max = 405 nm) was produced, whose spectral properties differed drastically from SQS32 (λab,max = 539 nm). This process (Scheme 14) was accompanied by the color change from pink (SQS32-1) to yellow (SQS32-2), which provided the naked-eye detection of F− (similarly to those observed for SQS31; Scheme 13). Changes were also noted in the emission spectra of SQS32 in the presence of F−; the emission intensity of SQS32 (λem,max = 611 nm; red-colored solution under 365 nm UV lamp irradiation) decreased as a function of increasing concentration of F−, with a concomitant increase in SQS32’s emission intensity (λem,max = 485 nm; green-colored solution under 365 nm UV lamp irradiation). Not surprisingly, other halides (i.e., non-basic Cl−, Br−, and I−) did not induce any formation of SQS32-1, as judged by the lack of any changes in the absorption and emission spectra of SQS32 in the presence of these anions [100]. However, it is of interest to note that, similarly to SQS31 (Scheme 14), NH4OAc was also able to produce a deprotonated intermediate SQS32-1, albeit less efficiently than TBAF (a decrease of about 2.5-fold was determined, based on the changes in both the absorption and emission spectra).

When SQS32-1 was exposed to CO2, a conversion to SQS32 was noted in accordance with the changes in the absorption and emission maxima, and the respective intensities [100]. Based on DFT calculations, it was proposed that SQS32-1 was likely to act as a base to generate HO−, which, in turn, would lead to the formation of HCO3− upon the reaction with CO2 (Scheme 14). This was contrary to a more conventional interaction between SQS32-1 and CO2, which would have led to an unstable intermediate SQS32-2, with subsequent generation of HCO3− and SQS32 (Scheme 14).
Overall, the results of these studies indicated that readily accessible squaraine-based sensors could be used for the visual and spectroscopic detection of CO2. In view of the tunable acidity of NH, the design of other multi-analyte squaraine-based platforms should be possible.

5. Electrophilicity of Squaraine Ring’s Carbon as Key Element in “Turn-Off” and “Turn-On” Sensing Paradigms

A number of chemosensors operate in the “turn-OFF” regime when a nucleophilic attack on the electrophilic center of the chemosensor disrupts the conjugation system, and, as such, this leads to a demise of low-energy absorption and emission bands [101,102]. The addition of the nucleophile to the squaraine core produced non-emissive species, while the addition of appropriate analytes led to the elimination of the nucleophile, restored the squaraine conjugation, and regenerated the original photophysical properties (Figure 6A). Squaraine-based chemodosimeters that are responsive to various nucleophilic analytes have been reported, including thiols (Figure 6B) [103–106], nitrogen-containing nucleophiles, such as amines and hydrazine (Figure 6C) [107], as well as cyanide ion (Figure 6D) [108,109]. It should be noted that thiol and hydrazine additions to squaraines could typically be reversed by the addition of thiophilic metal ions or aldehydes, respectively. In some instances, differentiation among similar Nu and X species (Figure 6A) could be achieved by the application of pattern-based recognition methods [110,111]. This approach was exemplified by SQS33, which was able to differentiate the effects of five thiols (e.g., propane thiol, 3-mercaptopropionic acid, naphthalene-2-thio, 2,3-dimercaptopropanol, and 2-acylamino-3-mercaptopropionic acid methyl ester) and five metal ions (e.g., Hg2+, Pd2+, Cu2+, Fe2+, and Ni2+) [103]. In general, the addition of CN− to squaraine’s electrophilic four-membered ring is not reversible.

Structurally, these sensors (Figure 6B–D) range from rather generic (SQS33) and relatively simple (SQS34) to more elaborate sensors, such as SQS35 and SQS36, which possess moieties for metal ion recognition or aggregation-induced emission capabilities, respectively. It should also be kept in mind that, often, selectivity towards a certain analyte is determined by using a set of related species, i.e., the sensor’s selectivity towards a specific ion will be assessed using a series of related or relevant ions. As such, sensors might be simply “branded” for a specific class of analytes. This might potentially limit further studies into the expanded scope of other analytes for this sensor. From the sensor’s utility point of view, however, it might be advantageous to use one sensor for the detection of analytes that belong to different classes of compounds by simply adjusting the media in which the detection process is taking place; for example, simply by manipulating the nature of the media, SQS35 (Figure 6) showed excellent selectivity towards various nucleophilic species, such as thiols (CH3CN/H2O 1/4, v/v; buffered at pH 6 (10 mM MES)) [105]. Under the aforementioned condition, SQS35 did not optically respond to the presence of CN−. However, in neat CH3CN, SQS35 exhibited excellent selectivity towards CN− over other nucleophilic anions, such as F− and AcO− [108]. Yet, it should be noted that SQS35
in CH$_3$CN also optically responded to the presence of metal ions, such as Hg$^{2+}$, Fe$^{3+}$, and Cu$^{2+}$ [58].

A few additional interesting and squaraine-unique cases, in regards to nucleophilic addition to squaraine’s four-membered ring, are detailed below.

**Figure 6.** Some examples of nucleophile-sensitive SQSs. ON–OFF–ON relay of nucleophile-specific chemodosimeter (A). Representative structures of the following SQSs that showed sensitivity towards thiols (B): SQS33 [103], SQS34 [104], SQS35 [105], SQS36 [106]; the following to hydrazine and amines (C): SQS37 [107]; the following to cyanide ion (D): SQS38 [108], SQS39 [109]).

### 5.1. Nucleophilic Addition as a Tool for Controlling Energy Transfer

It was shown (Section 4) that when an acidic NH is present, F$^-$ (in the form of TBAF, for example) could act as a base and elicit the formation of a nucleophilic nitrogen center as a viable precursor for CO$_2$ sensing (Schemes 13 and 14). However, in the absence of acidic NH (i.e., C arene-type squaraines; Figure 1), F$^-$ might be expected to act as a nucleophile towards the electrophilic carbon on the squaraine ring. Indeed, squaraines, which are obtained via Nu$^-$ addition to squaric acid or its congeners (Schemes 1 and 2), could serve as “turn-OFF” sensors for the F$^-$ nucleophile, which could attack the electrophilic center of the squaraine ring [112]. The squaraine scaffold was decorated with an additional fluorophore, thus coupling an energy transfer process (i.e., Förster resonance energy transfer—FRET) with nucleophilic sensing (Scheme 15). SQS39, which was synthesized in just five steps,
was specifically designed as a nucleophile-regulated FRET-ON to FRET-OFF sensor. **SQS39** exhibited the following two absorption maxima: 400 nm, which corresponded to the naphthalimide unit (Scheme 15, moiety shown in blue), and 659 nm, which corresponded to the squaraine unit (Scheme 15, moiety shown in red). Upon excitation of the naphthalimide unit ($\lambda_{\text{ex}} = 400$ nm), FRET to the squaraine unit ($\lambda_{\text{em}} = 667$ nm) was observed with over 90% efficiency (in CH$_3$CN, and in the CH$_3$CN/H$_2$O mixture (9/1, v/v)). As expected, upon titration of **SQS39** (10 μM) with F$^-$ (2 μM–1.2 mM, which acted as a nucleophile), the intensity of the band associated with the squaraine unit decreased, due to disruption of the conjugated system. Not surprisingly, F$^-$ did not have any impact on the naphthalimide, since there were no viable electrophilic centers on this chromophoric unit. The intensity of emission at 667 nm (associated with squaraine) was decreasing, while the emission at 537 nm (associated with naphthalimide) was increasing, as a function of the added F$^-$, which resulted in ratiometric FRET-based sensing. Since a qualitatively similar ratiometric response for **SQS39** was noted upon addition of CN$^-$, it could be argued that **SQS39** (and other related squaraine-based multi-dye systems) could be used for the detection of other nucleophilic species.

![Scheme 15. FRET-based sensor SQS39 for nucleophilic anions [112]. Nu = F$^-$ or CN$^-$, Fluorescence spectra qualitatively demonstrate changes in the emission intensities at 537 nm (naphthalimide moiety, shown in blue) and at 667 nm (squaraine moiety, shown in red) before (left) and after (right) exposure of SQS39 to Nu.](image)

### 5.2. Nucleophile-Induced Skeletal Rearrangement

Several phosphines, e.g., P$^+$Bu$_3$, P(NMe$_2$)$_3$, and P(p-MeO-C$_6$H$_4$)$_3$, were shown to be efficient nucleophiles towards an electrophilic carbon of the squaraine core of **SQS40** (Scheme 16A) [113]. Similarly to the nucleophilic addition of thiols to squaraines, the addition of P(III)-nucleophiles also led to an emission “turn-OFF” response. The P-containing adducts have photophysical characteristics that are drastically different from the initial squaraines, i.e., green–blue color of the squaraine **SQS40** faded away in the presence of the phosphines, and produced a yellow product, **SQS40-1**, as was evident by the disappearance of the absorption band at 656 nm. Notably, when a bulkier P(p-MeOC$_6$H$_4$)$_3$ was used, the nucleophilic addition was found to be reversible, with the P-adduct being favored by lower temperatures. A temperature-dependent NMR study revealed the reversible nature of this P-nucleophilic addition to the squaraine. Interestingly, contrary to the addition of thiols to squaraines, where the association is typically favored by higher temperatures, in the case of phosphine addition, the equilibrium was heavily shifted towards the P-adduct at lower temperatures [113]. Thus, thermochromic applications might be exploited using these zwitterionic P-containing adducts.
Furthermore, in view of the ease of the synthesis and purification/isolation (pure products were obtained by vapor–vapor crystallization) of these P-containing zwitterionic compounds, e.g., **SQS40-1** (Scheme 16A), the use of these compounds as sensors for transition metals was explored. Specifically, a number of transition metal ions, such as Pd$^{2+}$, Rh$^1$, Ag$^+$, Au$^{3+}$, as well as Cu$^+$ and Cu$^{2+}$, were able to induce phosphine elimination and restore the squarine’s structure **SQS40** (confirmed by the appearance of the characteristic color associated with **SQS40, $\lambda_{ab,\text{max}} = 656$ nm**). On the other hand, Ni$^{2+}$, Fe$^{3+}$, Ti$^{3+}$, and Ti$^{4+}$ failed to eliminate the P-containing moiety from **SQS40-1**, as was evident from the persistent yellow color of the solution.

In a subsequent study, it was found that the presence of certain substituents (i.e., OH, NHAc, NHTs, and NHBoc) in the ortho-position of the aromatic rings could induce a cascade of reactions, following P-nucleophilic addition to the squaraine ring, leading to the formation of furanone or oxindole scaffolds (Scheme 16B) [114]. Specifically, P$^{n}$Bu$_3$ addition to NHAc-containing squaraine **SQS41** produced oxindole **SQS41-1** (Scheme 16B).

Similarly to previous PR$_3$ additions to squaraines, P$^{n}$Bu$_3$ addition to **SQS41** was accompanied by a change in color from blue to colorless, thus resulting in a “turn-OFF” sensing mechanism. Importantly, the reversed process, i.e., reformation of the squaraine core, was achieved by the addition of the acid to **SQS41-1** to restore **SQS41**; thus, a “turn-ON” pH-sensing mechanism was realized (Scheme 16B).

### 6. Chemo-Uncaging Strategies for Sensing

Although squarine-based sensors that rely on a nucleophilic attack on the electrophilic carbon of the squaraine ring have been widely utilized, it could be argued that a “turn-OFF” type of response might not be optimal for some applications, especially those related to biological environments. Therefore, developing alternative strategies that yield a “turn-ON” type of squaraine sensor would be advantageous. In order to explore this avenue, the squaraine ring’s susceptibility to nucleophilic attacks must be addressed. To this end, several strategies to protect squaraines from nucleophilic attack have been developed. Specifically, threading squaraine through a macrocyclic scaffold, thus generating a rotaxane-type system (Figure 7A), proved to be a viable approach for not only making squaraines chemically inert to nucleophilic insults, but also preventing the aggregation of squaraine dyes, thus preserving the desired photophysical characteristics of the monomeric state [115]. As such, these squaraine-threaded rotaxane systems have been demonstrated to be useful for various bioimaging applications [116–118]. However, additional synthetic steps are required for the synthesis of the macrocycle, as well as the synthesis of the rotaxane, which might decrease the synthetic accessibility of the desired squaraine-based chemosensor.

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**Scheme 16.** Representative examples of relays that feature phosphine additions to squaraine scaffolds (A): **SQS40** [113] and (B): **SQS41** [114].
Figure 7. Squaraine dyes with reduced reactivity towards thiol nucleophilic attack on the squaraine’s electrophilic center. (A): a representative example of squaraine rotaxane [116]; (B): ortho-substituted squaraines [119]; (C): symmetric arylidene squaraine [120].

Thus, as a complementary approach, chemical modification of the squaraine, to prevent nucleophilic attack on the four-membered ring, was also explored. Arguably, simply incorporating substituents into the ortho-position of the aromatic substituents on the squaraine ring could reduce the electrophilicity of the squaraine’s ring. It appeared that an intricate balance between electronic and steric factors could modulate the rate of thiol addition to the electrophilic center on the squaraine ring (Figure 7B) [119]. Importantly, the presence of a single OH group on each of the aromatic moieties was shown to be sufficient for almost completely inhibiting the nucleophilic attack of N-acetyl cysteine.

Additionally, it was shown that squaraines that feature the arylidene-type moiety (Figure 7C) are not susceptible to nucleophilic attack by dimercaprol, i.e., aliphatic dithiol [120]. This type of squaraine dye, both the symmetric and non-symmetric versions, could be easily obtained from CH-active nucleophiles (i.e., Nu-C-H; Schemes 1 and 2).

It has long been established that the incorporation of an electron-withdrawing moiety on a chromophoric scaffold could quench the fluorophore emission, and, thus, yield the OFF-state of the sensor. Assuming that the removal of that moiety, a process that could also be referred to as either deprotection or uncaging, does not affect the structural integrity of the fluorophore, the ON-state could be realized [121,122]. In particular, the 2,4-dinitrobenzenesulfonyl group, along with the 4-nitrobenzylsulfonyl and pentafluorobenzenesulfonyl groups, when conjugated onto several fluorophoric scaffolds, such as coumarin, rhodamine, and crystal violet, were shown to efficiently quench the emission of the fluorophore, thus giving the OFF-state of the sensor [123,124].

The sensor SQS42, bearing the 2,4-dinitrobenzenesulfonyl moiety as the caging element, was obtained in four linear steps with a 13% overall yield (Scheme 17). Spectroscopically, the caged sensor SQS42 and the related parent SQS42-I exhibited the same absorption and emission maxima (λ_{ab}^{max} = 635 nm, λ_{em}^{max} = 656 nm), yet the quantum yields’ difference was over 30-fold (0.006 and 0.203, respectively) [125].

However, when low-emission SQS42 was treated with increasing amounts of Cys, highly emissive SQS41-I was produced. Notably, higher concentrations of thiols led to a decrease in the absorption of the squaraine chromophore (λ_{ab}^{max} = 635 nm), due to nucleophilic attack on the four-membered ring electrophilic center [125].

In order to inhibit the competing reaction, SQS43 was introduced (Scheme 17) [126]. The arylidene moiety (shown in red) provided enhanced stability towards the nucleophilic attack (in line with model squaraine; see Figure 6C), whereas the glycol tethered carbohydrate moiety (shown in pink) was introduced to enhance the solubility in aqueous media. The thiolate attack was shown to be much more facile at the electrophilic center of the 2,4-dinitrobenzenesulfonyl moiety, rather than at the squaraine’s core. No decrease in either the absorption or fluorescent emission intensities was noted, even when excess amounts of thiol were used [126].
Scheme 17. Synthesis of caged SQS42 [125] and structure of water-soluble, caged SQS43 [126].

It is also important to note that, in aqueous media, around neutral pH, it is possible to differentiate between aromatic and aliphatic thiols, since aromatic thiolates are better nucleophiles than aliphatic thiolates (due to the higher acidity of the S–H bond and, as a result, more facile generation of the thiolate anion). Therefore, chemical uncaging of a probe using aromatic thiols proceeds significantly faster compared to aliphatic thiols [127,128]. Thus, SQS43 could be turned into an aromatic thiol-specific probe by simply adjusting the pH of the media [126].

Recently, a thio-squaraine-specific Hg sensor, SQS44, was introduced, and it featured a Hg$^{2+}$-induced S-to-O- “uncaging” optical sensing mechanism (Scheme 18) [129]. This approach took advantage of the well-established ability of Lawesson’s reagent to convert carbonyl groups into thio-carbonyl-containing ones [130], and the fact that Hg$^{2+}$ could trigger the reverse desulfurization reaction with a high degree of functional group tolerance [8]. Symmetric squaraine SQS44-1 was synthesized following general protocols, and the subsequent incorporation of sulfur atoms on the squaraine’s four-membered ring was achieved using Lawesson’s reagent under standard conditions [130], to give SQS44 (Scheme 18).

Scheme 18. Synthesis of sulfur-containing SQS44 and Hg$^{2+}$-triggered uncaging of SQS44 [129].

Due to the heavy atom effect, i.e., S for O substitution, the emission of sulfur-containing SQS44 appeared to be significantly decreased compared to the oxygen-containing analogue SQS44-1; for example, the quantum yields for SQS44 and SQS44-1 were 0.163 and 0.647, respectively. However, in the presence of increasing amounts of Hg(NO$_3$)$_2$, a substantial increase in emission intensity ($\lambda_{em,max}$ = 657 nm) was noted, thus yielding a “turn-ON” sensor [129]. SQS44 operated in a relatively wide pH range (pH 3.0–7.0, which is suitable
for various biological systems), and provided an acceptable detection limit of Hg$^{2+}$ (estimated at 1.2 nM). Remarkably, other thiophilic metal ions, such as Pb$^{2+}$, Cd$^{2+}$, and Ag$^{+}$, failed to induce any appreciable changes in the emission spectra. Furthermore, due to its relatively low cytotoxicity and acceptable aqueous solubility, SQS44 was subjected to in vitro (HeLa cell) and in vivo (zebra fish) fluorescence imaging studies. In both environments, SQS44 showed good stability and appreciable Hg$^{2+}$-sensing capability [129], which makes it a viable and potentially useful near-IR probe for fluorescence imaging of various biological systems.

7. Squaraine–Metal Scaffold for Sensing

Molecular platforms with multiplexing capabilities are of interest. Arguably, in view of the facile and modular synthetic access to structurally and functionally diverse structures, including post-modification options, squaraine dyes might be among the most versatile scaffolds to design and synthesize sensors for multi-analyte recognition.

A dipicolylamine-containing squaraine, SQS45 (sensing ability beyond interaction with Zn$^{2+}$ is yet to be established), which was prepared following standard protocols, was complexed with Zn$^{2+}$ to give squaraine scaffold SQS46 that showed the ability to sense CO$_2$, picric acid, and phosphates (Scheme 19). It should be noted that SQS46 was not isolated, but rather used as prepared, i.e., CH$_3$CN with excess Zn(ClO$_4$)$_2$ (last step in Scheme 19). In all the cases, coordination of the oxygen atom(s) of the analytes to the Zn center of SQS46 modulated the photophysical properties of the sensing scaffold [131].

When an acetonitrile solution of SQS46 (containing ca. 1% of water, v/v) was purged with CO$_2$, a color change from violet to blue was noted, along with a decrease in the 584 nm absorption band, and an increase in the absorption band at 633 nm. Although the emission of SQS46 in CH$_3$CN is fairly low, in the presence of increasing amounts of CO$_2$, a “turn-ON” response, with $\lambda_{em}^{max} = 659$ nm, was observed [131]. $^{13}$C NMR investigation revealed the presence of a carbonate anion, which indicated that CO$_2$ sensing by SQS46 was conducted in the form of the carbonate. In fact, when SQS46 was treated with carbonate ions, spectroscopic and visual changes in the SQS46 solution, which were similar (yet more rapid) to those observed upon the purging with CO$_2$, were observed. Furthermore, drastically smaller changes, in both the absorption and emission spectra (as well as no color change in the SQS46 solution), were observed in the absence of water in the system, upon prolonged purging with CO$_2$. In addition, it was reported, albeit with little detail, that the bound carbonate could be released in the presence of Ca$^{2+}$. Thus, in principle, an even

Scheme 19. Synthesis of squaraine-based Zn-containing sensing scaffold SQS46 for anion and molecular recognition [131].
more elaborate sensing platform could be realized, which will implement a regeneration of SQS46.

Next, interactions between SQS46 and various phosphate anions were explored [131]. SQS46 allowed for a naked-eye differentiation between cyclic and linear phosphates, including GMP and c-GMP. Specifically, the absorption maximum of SQS46 ($\lambda_{ab}^{\text{max}} = 584$ nm; purple-colored solution) was red-shifted to 615 nm (blue-colored solution) in the presence of cyclic phosphates, and further red-shifted to 633 nm (light-blue-colored solution) in the presence of linear phosphates. The changes in the emission spectra of SQS46 were also pronounced. Similarly to the aforementioned CO$_2$/carbonate case, a “turn-ON” response was noted when SQS46 was titrated with phosphates. Similar levels of emission enhancements were observed in the cases of both cyclic and linear phosphates (i.e., a ca. 8–9-fold increase in emission intensity in the presence of 6–7 equiv. of phosphates), and, in both cases, the ppm levels of the detections were estimated. However, the emission maxima were distinct enough to assure differentiation between cyclic and linear phosphates; for SQS46–c-GMP, the $\lambda_{em}^{\text{max}}$ was 644 nm, and for SQS46–GMP, the $\lambda_{em}^{\text{max}}$ was 659 nm.

In addition to ionic recognition, SQS46 showed appreciable capability in molecular sensing [131]. Specifically, the purple-colored ($\lambda_{ab} = 584$ nm) solution of SQS46 in acetonitrile changed to green ($\lambda_{ab} = 644$ nm) upon addition of an aqueous solution of picric acid. Thus, a naked-eye determination of an explosive was achieved. SQS46 also acted as a fluorescent “turn-ON” sensor for picric acid, as a gradual increase in the emission intensity at 644 nm was noted upon titration of SQS46 solution with picric acid (up to ca. 10-fold emission enhancement was noted in the presence of 15 equiv. of picric acid), and provided a detection limit of 3 ppm. Furthermore, ca. 6.5-fold selectivity over other nitrophenols (e.g., 2-nitro/3-nitro/4-nitro-phenols) was noted.

8. Conclusions

The tunable photophysical properties and synthetic accessibility of squaraine dyes make them one of the most versatile, privileged chromophoric and fluorophoric scaffolds, which are almost ideal for designing and synthesizing various optical chemosensors. This review illustrated a plethora of approaches towards squaraine-based chemosensors that allow for “turn-ON”, “turn-OFF”, and relay-type sensing modes for the detection of various ionic and molecular analytes. In addition, non-symmetric squaraine-based sensors allow for further modulation of photophysical properties and solubilities, as well as providing additional possibilities for the conjugation and immobilization of these sensors on other platforms.

Decorating squaraine dyes with various structurally and functionally diverse moieties has produced a number of chemosensing scaffolds with optical responses that cover a wide spectra window in the presence of various types of ionic and molecular analytes (Table 1). However, as exemplified by a number of accounts, the sensing ability and selectivity of SQSs appear to also be dependent on the media used. Hence, it might be advantageous to evaluate sensors’ analyte recognition capabilities in various types of media, i.e., in regards to the nature of the solvents and/or composition for mixed solvent systems, such as aqueous/organic solvents. Importantly, developing and utilizing standard sets of conditions that would be used for screening specific analytes should allow for meaningful comparisons of the sensing capabilities of various SQSs.

Although the post-functionalization of squaraine scaffolds is possible, most synthetic approaches rely on the condensation of various C/N-nucleophiles, with squaric acid (or its congeners) as the last step in assembling the squaraine. Since the condensation step is performed under relatively mild conditions, many functional groups and structural motifs are tolerated. As such, the diversity of squaraine-based chemosensors, and, hence, the selectivity and sensitivity towards analytes, is only limited by the accessibility of the C/N-nucleophilic components and the researchers’ imagination.
Table 1. A list of SQSs and their recognition motifs for sensing of various analytes.

| Recognition Motif       | SQS# | Analyte                          | Refs.  |
|-------------------------|------|----------------------------------|--------|
| crown ether             |      |                                  |        |
| SQS1                    | Li⁺  | [53]                             |        |
| SQS2                    | Li⁺, Na⁺ | [53]                        |        |
| SQS3                    | Ba²⁺, Pb²⁺, Cu²⁺, Fe³⁺, Hg²⁺ | [58]   |
| SQS4                    | Mg²⁺, Ba²⁺ | [59]                     |        |
| SQS5                    | Hg²⁺  | [63]                             |        |
| SQS6                    | Li⁺   | [61]                             |        |
| SQS7                    | Na⁺   | [62]                             |        |
| SQS8                    | Hg²⁺  | [63]                             |        |
| SQS9                    | Hg²⁺, Ag⁺, Fe³⁺, Cu²⁺ | [58,66] |        |
|SQS10                   | Pb²⁺, Cu²⁺ | [67]                     |        |
| podand                  |      |                                  |        |
| SQS11                   | Ca²⁺  | [71]                             |        |
| SQS12                   | Ca²⁺, Mg²⁺ | [72]                       |        |
| SQS13                   | Ca²⁺, Mg²⁺ | [72]                       |        |
| SQS14                   | Ca²⁺, Mg²⁺ | [72]                       |        |
| SQS15                   | Hg²⁺  | [74]                             |        |
| SQS16                   | Hg²⁺  | [74]                             |        |
| SQS17                   | Hg²⁺  | [75]                             |        |
| SQS18                   | Hg²⁺  | [76]                             |        |
| SQS19                   | Hg²⁺  | [77]                             |        |
| SQS20                   | Hg²⁺  | [78]                             |        |
| SQS21                   | Hg²⁺  | [79]                             |        |
| SQS22                   | Hg²⁺, Cu²⁺, Fe³⁺ | [80]     |        |
| SQS23                   | Hg²⁺, Cys, Hcys, GSH | [81]   |        |
| SQS24                   | Hg²⁺, Ag⁺, Cys, His, Trp | [82]   |        |
| tetracarboxylate        |      |                                  |        |
| SQS25                   | Ca²⁺  | [86]                             |        |
| nitrogen-containing heterocycle |      |                                  |        |
| SQS26                   | Hg²⁺  | [94]                             |        |
| SQS27                   | Cu²⁺  | [96]                             |        |
| SQS28                   | Cu²⁺  | [96]                             |        |
| SQS29                   | Hg²⁺  | [97]                             |        |
| SQS30                   | Zn²⁺, Cd²⁺, Cu²⁺, Co²⁺, Ni²⁺, Ag⁺ | [98] |        |
| acidic NH               |      |                                  |        |
| SQS31                   | F⁻, CO₂ | [99]                        |        |
| SQS32                   | F⁻, CO₂ | [100]                       |        |
| squaraine-ring as electrophile |      |                                  |        |
| SQS33                   | propan-1-thiol, 3-mercaptopropionic acid, 2-acetylamino-3-mercaptopropionic acid methyl ester, 2,3-dimercapto-propane-1-ol, naphthalene-2-thiol; Pd²⁺, Hg²⁺, Ni²⁺, Cu²⁺, Fe³⁺, 4-acetamidothiophenol, N-acetyl-L-Cys, L-Cys methyl ether, L-Cys, 2-(dimethylamino)ethanethiol; Hg²⁺ propanethiol, Cys, Cys-Gly, GSH CN⁻ | [103] |        |
| SQS34                   | propanethiol, Cys, Cys-Gly, GSH | [104] |        |
| SQS35                   | Cys, GSH | [105]                    |        |
| SQS36                   | Cys, GSH | [106]                    |        |
Table 1. Cont.

| Recognition Motif | SQS# | Analyte | Refs. |
|-------------------|------|---------|-------|
|                   | **SQS37** | NH$_2$NH$_2$, HO$^-$, ethanolamine, methylamine, β-phenylethylamine; glyoxal, glutaric dialdehyde, propionaldehyde, pyridine-2-carboxaldehyde, formaldehyde, acetaldehyde | [107] |
|                   | **SQS38** | CN$^-$ | [109] |
|                   | **SQS39** | F$^-$, CN$^-$, P$_{8}$Bu$_{3}$, PN(Me$_2$)$_3$, P(p-MeO-C$_6$H$_4$)$_3$; Pd$^{2+}$, Rh$^+$, Ag$^+$, Au$^{3+}$, Cu$^{3+}$, Cu$^{2+}$ | [112] |
|                   | **SQS40** | P($\text{p-MeO-C}_6\text{H}_4\text{H}_3$); Pd$^{2+}$, Rh$^+$, Ag$^+$, Au$^{3+}$, Cu$^{3+}$, Cu$^{2+}$ | [113] |
|                   | **SQS41** | P$_{8}$Bu$_{3}$; TsOH | [114] |
| caging group      | **SQS42** | Cys, Hcys | [125] |
|                   | **SQS43** | PhSH | [126] |
|                   | **SQS44** | Hg$^{2+}$ | [129] |
| metal-containing scaffold | **SQS45** | Zn$^{2+}$ | [131] |
|                   | **SQS46** | CO$_2$/CO$_3^{2-}$; GMP; c-GMP; picric acid | [131] |

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