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Review

# Nucleic acid aptamers as aptasensors for plant biology

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**Our knowledge of cell- and tissue-specific quantification of phytohormones is heavily reliant on laborious mass spectrometry techniques. Genetically encoded biosensors have allowed spatial and some temporal quantification of phytohormones intracellularly, but there is still limited information on their intercellular distributions. Here, we review nucleic acid aptamers as an emerging biosensing platform for the detection and quantification of analytes with high affinity and specificity. Options for DNA aptamer technology are explained through selection, sequencing analysis and techniques for evaluating affinity and specificity, and we focus on previously developed DNA aptamers against various plant analytes. We suggest how these tools might be applied in planta for quantification of molecules of interest both intracellularly and intercellularly.**

## Highlights

DNA aptamers provide a versatile option for recognising and measuring a wide range of specific analytes in biological systems, and they have begun to be exploited in biomedicine.

Aptamers have been selected against many phytohormones as well as against plant pathogens.

Diverse aptamer selection techniques have been developed, and some promote selections of conformationally active candidates.

Affinity maturation techniques allow for guided improvements of specificity and affinity.

DNA aptamers as aptasensors offer advantages over other quantitative and analytical techniques, including the potential for measurements *in vivo*.

## Quantitative biosensors in plants

A biosensor is a synthetic device that produces a measurable signal upon interaction with its target ligand, usually a biological analyte [1,2]. Biosensors consist of two main components, a biological recognition moiety that interacts with the analyte with high sensitivity and specificity, and a signalling module designed according to the sensor's application. Examples of biosensors are numerous and diverse. For example, the medical sciences have pioneered the use of many biosensors, such as the enzyme-based blood glucose biosensor being used by patients with diabetes worldwide [3]. Another example is a peptide used to recognise thrombin in human blood [4] giving a fluorescence output correlating with thrombin concentration in blood plasma samples, and there are many examples of antibodies used as the biological recognition moiety linked to diverse output modules such as gold nanoparticles, holograms, and electrical devices [5].

In plant biology, genetically encoded biosensors have become the established tools for spatial and temporal monitoring of many analytes, including sugars and phytohormones [6–10]. For example, monosaccharide reporters were developed from bacterial periplasmic-binding proteins. These proteins change conformation when binding their substrates and when fused to a pair of fluorophores with overlapping emission spectra give Förster resonance energy transfer (FRET)-based nanosensors [8]. A sensor based on a mutated bacterial tryptophan repressor protein coupled with fluorophores also allows monitoring of cytoplasmic auxin concentrations [10]. Similarly, a set of FRET-based sensors for abscisic acid (ABA) has been engineered from the ABA receptor complex [5,11–13]. A semiquantitative ratiometric fluorescent sensor for the phytohormone auxin was constructed by coupling the auxin-sensitive Domain II of an Aux/IAA protein with the fluorescent protein Venus alongside the fluor Tomato which was coupled to a mutated, auxin-insensitive Domain II to give the sensor named R2D2 [7]. These and other genetically encoded biosensors work well in certain intracellular compartments, generally giving semiquantitative data at high spatial resolution [9,11,12,14,15].

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For quantitative measurements of small molecules in plants, at both tissue level and from isolated and sorted cells, the method of choice remains mass spectrometry [16]. Electrochemical microbiosensors have been developed for measurements of phytohormones and compounds in plants [1,16], although most have been aimed at the food processing industries rather than studies of plant biology. An exception is the use of cytokinin oxidase (cytokinin dehydrogenase) coupled with a platinum microelectrode to successfully measure cytokinin concentrations in tomato (*Solanum lycopersicum*) root exudate [17].

It is clear that protein-based sensors have proved remarkably helpful in many plant systems, but they also have their limitations. For example, phytohormone measurements within the apoplast remain essentially unexplored [18]. Genetically encoded ratiometric pH sensors for the apoplast are advancing [19] and apo-pHusion makes use of the pH sensitivity in some of the GFP variants [20]. Hence, there is the prospect of new, durable biosensor proteins active in this space. At the same time, nucleic acid aptamers are also being developed as the recognition moiety for biosensors of many types and they offer an alternative resource for analyte measurements in the apoplast.

An aptamer (from the Latin *aptus*, meaning 'to fit') is a short, synthetic oligonucleotide selected to have a specific binding interaction with target molecules [21]. Aptamers offer comparable sensitivity and specificity to antibodies while having significant advantages, including ease of synthesis, well-known chemistry, and cost-effectiveness (Table 1). Since the 1990s, numerous aptamers have been identified for a wide spectrum of target ligands ranging from small molecules to whole cells (Table 2). The versatility of aptamers allows them to be coupled with diverse sensing platforms for different applications, such as enzymatically linked oligonucleotide assays (ELONAs), electrochemical devices, nanoparticles for microscopy and diagnostics, and surface plasmon resonance (SPR) [22–25]. In this review, we will collate the information on aptamers directed at plant targets and look forward to how the versatility of these reagents might be harnessed, with a particular emphasis on the phytohormones.

## Aptamer selection methods

Aptamers are selected by systematic evolution of ligands by exponential enrichment (SELEX; Box 1). The choice of selection and partition techniques during SELEX depends on various factors, including the nature of target analyte and the intended application of the aptamer (Table 2). Frequently, ligands are immobilised onto agarose or magnetic beads to facilitate the separation of analyte-binding sequences from the nonbinders [26]. FluMag-SELEX introduces a fluorescent tag using a labelled primer, allowing sequence partitioning and recovery

Table 1. Features of nucleic acid aptamers versus monoclonal antibodies

| Features | Aptamers | Antibodies |
|---|---|---|
| Synthesis | Chemically synthesised in a short period | Complex extensive processing, often requiring immunisation |
| Cost | Inexpensive | High production cost |
| Stability | Nuclease degradation<br>Degradation via UV exposure | Short biological half-life<br>Specific storage conditions |
| Target | Wide range from whole cell to small molecules | Wide range from whole cell to small molecules |
| Specificity | High | High |
| Affinity | High | High |

Table 2. Comparison of different types of SELEX selection processes for aptamers and their targets

| SELEX types | Examples of target ligands | Methods | Refs |
|---|---|---|---|
| Direct SELEX | Proteins Macromolecules | Immobilisation of target ligand onto agarose or magnetic beads to facilitate separation of specific binders from the unbound pool. | [16,87,88] |
| FluMag-SELEX | Proteins Macromolecules | Fluorescent tag introduced in PCR amplification allows monitoring of DNA recovery through SELEX rounds. | [27] |
| Solution SELEX | Small molecules Toxins | A complementary oligonucleotide is used to hybridise to and immobilise the aptamer library. After washing steps, incubation with the target ligand allows ligand binders to refold around the target for collection in solution. | [28,39] |
| Cell SELEX | Whole cells | The DNA library is incubated with whole cells for the selection process. Separation of bound sequences can be performed by centrifugation. | [68] |
| Capillary Electrophoresis SELEX | Proteins Higher-molecular weight ligands | Ligand-specific sequences are separated due to the difference in size and charge of the complex. | [89] |
| GO-SELEX | Proteins | GO sheet absorbs and removes unbound sequences through π–π stacking. | [29] |

efficiency to be monitored at each cycle (Figure 1A) [27]. However, immobilisation of small-molecule analytes can pose a serious drawback as modifications to these compounds can significantly change their surface chemistry and this is the case for all the phytohormones. Alteration of available functional groups changes the biophysical properties of small molecules, thus reducing the chance of selecting appropriate aptamers.

An attractive alternative is solution SELEX, where the DNA library is hybridised onto a short complementary oligonucleotide that is displayed on magnetic beads. This allows incubation with free, unaltered target ligand and promotes selection of aptamers that change conformation upon analyte binding, leading to loss of hybridisation with the captured oligonucleotide [28]. These analyte-released aptamer candidates are then readily recovered for sublibrary amplification.

Graphene oxide-SELEX (GO-SELEX) offers further refinement to the selection and partition of aptamer candidates. This method selects aptamers without any immobilisation of either library or target analyte [29]. After incubation of the DNA library with the target, the solution is introduced to GO whereupon unbound sequences are removed by absorption onto GO, leaving aptamer candidates that are folded around their target free for amplification.

The number of SELEX rounds necessary for selecting specific aptamers varies greatly but is generally above five (Figure 1). It is also common in later rounds for selection stringency to be raised or for counterselection steps to be included. These will tend to improve the affinity and the specificity of successive sublibraries, respectively.

Aptamer sequences can be highly enriched at the end of the SELEX process, but more frequently they are present at less than 1% of the remaining diversity (Figure 1B). While this can represent enrichments of over $10^{12}$-fold, secondary selection processes are necessary to identify the best target binders. Therefore, sequence analyses also play an important role in aptamer selections. Conventional cloning and sequencing methods have been used extensively to sequence aptamer pools after the last round of SELEX, with the limiting factor being the finite number of sequences that can be handled with these methods. In recent years, high capacity next-generation

Box 1. Systematic evolution of ligands by exponential enrichment (SELEX)

Aptamers, which can be RNA or DNA [79,85], are selected through SELEX, where target-specific aptamer sequences emerge through sequential affinity-based enrichments from a starting highly diverse (~$10^{15}$), naïve oligonucleotide library (Figure I). The variable sequence is typically 40 bases flanked by primers for amplification. Each round of SELEX consists of three phases: selection, partition, and amplification. Selection allows ligand-specific aptamers to bind. In some cases, counterselection steps can be introduced by incubating the library with control molecules to eliminate nonspecific binders from successive sublibraries. A range of partitioning techniques can be used to separate ligand-specific aptamers from the pool (see Table 2 in main text). The separated aptamers are then amplified and subjected to further rounds of SELEX.

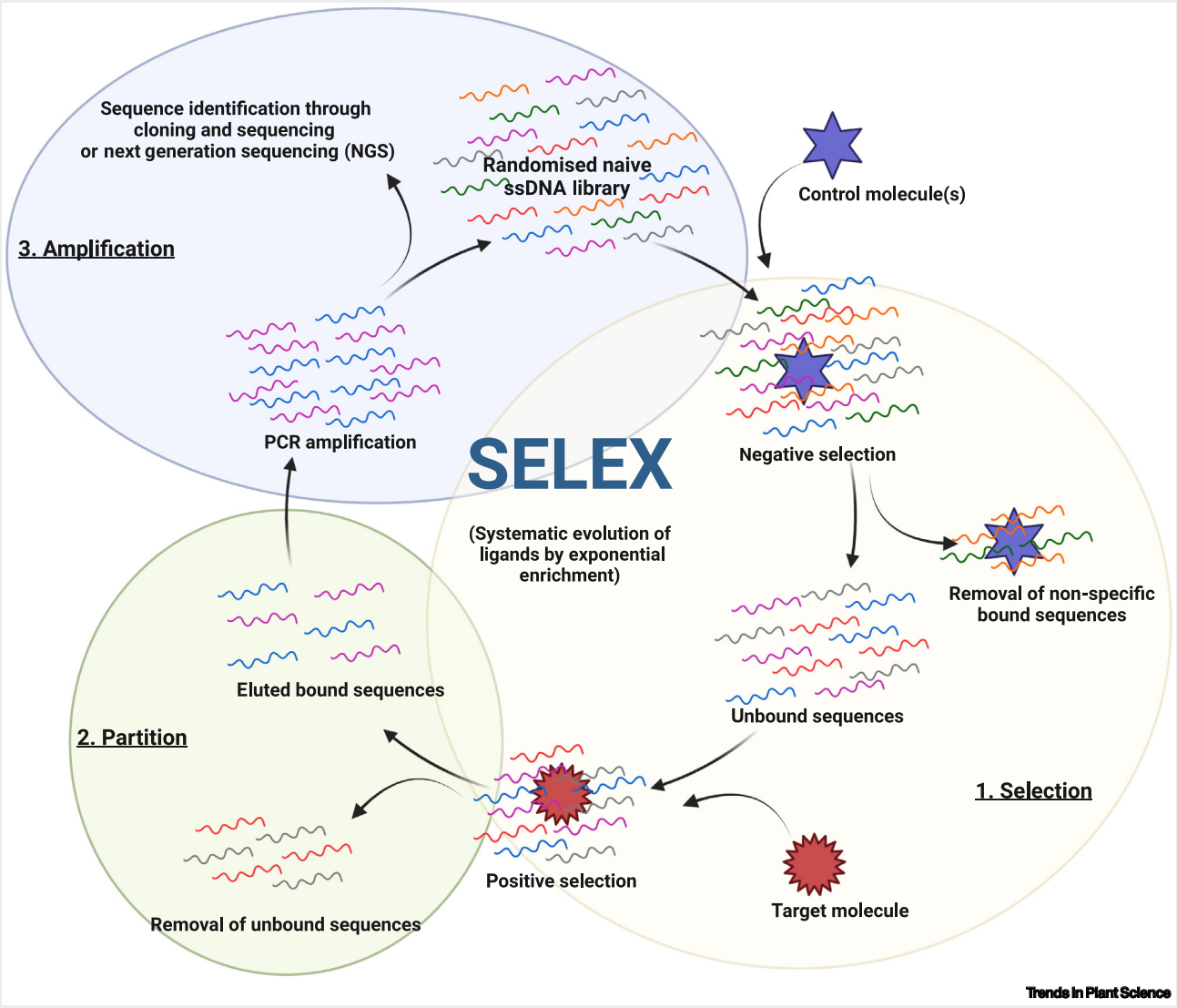


Figure I. General schematic illustration of the SELEX process for DNA aptamers. Figure created with BioRender.com.

sequencing (NGS) has allowed the sublibraries of many rounds of SELEX to be sequenced in one reaction. Unique oligonucleotide 'barcodes' can be added to each sublibrary as part of sample preparation, allowing them to be deep sequenced on the same NGS run [21]. This not only identifies the sequences enriched most, but can also illustrate the enrichments of certain sequences through SELEX rounds (Figure 1C) [21]. Helpfully, NGS can also reveal particular motifs segregating with target binding [30,31].

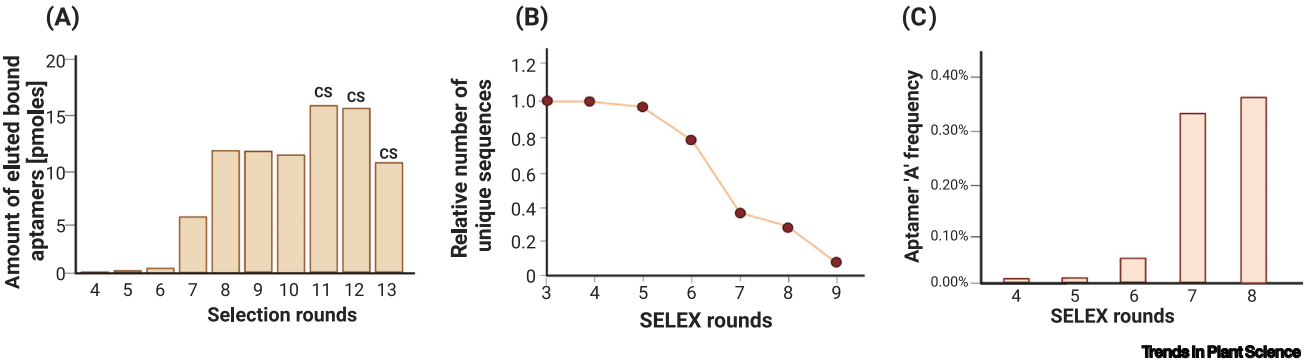


Figure 1. Enrichment of target-binding aptamer sequences through SELEX. (A) Fluorescence measurements from each round of FluMag-SELEX was used to monitor recovery efficiency of single-stranded DNA eluted by the target molecule. CS indicates SELEX rounds with a counterselection step [27]. (B) NGS data reveal the drop in the relative number of unique sequences after round 5 of SELEX, indicating enrichment of sequences as the SELEX rounds proceed. (C) The frequency or relative abundance of aptamer 'A' through rounds of SELEX indicating the specificity of aptamer 'A' after round 7 [86]. Figure created with BioRender.com. Abbreviations: NGS, next-generation sequencing; SELEX, systematic evolution of ligands by exponential enrichment.

## Aptamer targets

In the biomedical arena, aptamers directed against macromolecules have been developed for diagnosis or therapy for many years [28,32–35], and there is a growing literature on aptamers directed against small-molecule targets [26,36–41]. In the field of plant developmental biology, the analytes of widest interest are the phytohormones, which are all small molecules. All phytohormones are believed to transit around plants in either the transpiration stream or the phloem [42], and whichever route they take they must pass through the apoplast to reach their target cells. As noted earlier, there are genetic biosensors that will report on changes in concentration of specific targets on arrival, intracellularly, but there remains a need for biosensors that can report on the vital, less tractable, local extracellular concentrations. The potential of nucleic acid aptamers as the sensing module of biosensors (hence aptasensors) for studying small-molecule analytes in different subcellular and intracellular compartments in plants needs to be evaluated. The following sections summarise the availability of suitable aptamers with their binding affinities summarised in Table 3.

Table 3. Aptamers against small phytoactive molecules: binding affinities and techniques used for their characterisation

| Ligands | Estimated $K_D$ | Techniques used | Refs |
|---|---|---|---|
| ATP | 6 μM | Ultrafiltration | [37] |
| | 16.4 μM | ITC | [90] |
| | 34.4 μM | Microscale thermophoresis | [91] |
| | 30.98 μM | SPR | [92] |
| *Trans*-zeatin | 3.85 μM | ITC | [38] |
| | 0.23–13.68 μM range | Fluorescence assay | [53] |
| | 0.1–100 nM range | Photoelectrochemical | [54] |
| ABA | About 0.9 μM | Displacement assay | [59] |
| SA | 4.7 μM | Nanopore film sensor | [36] |
| ASPV MT32 | 55 nM | SPR | [70] |
| ASPV PSA-H | 83 nM | SPR | [70] |

## ATP aptamers

ATP is increasingly being recognised as a signalling molecule in plants [43–46] as it is in animals [47,48] and a fluorescent, genetically encoded ATP biosensor has been used to map tissue gradients and stress responses in arabidopsis (*Arabidopsis thaliana*) [49]. Importantly, an aptamer to ATP has been the most widely studied small-molecule DNA aptamer, making its story a useful reference for this review (Box 2) even though it has yet to be used for plant biology.

### ATP aptasensors for plants?

Intracellular ATP is well known to be the main source of energy for both plants and animals. However, as extracellular ATP (eATP), it also serves as a signalling compound involved in many aspects of plant growth, development and the regulation of responses to pathogens [43–45], which can be monitored through cytosolic $Ca^{2+}$ signalling [49]. During cell damage caused by biotic or abiotic stress, high levels (millimolar range) of eATP released to the apoplast act as a wound signal [43] and putative receptors for eATP have been described [50,51]. Pollen germination can be regulated by eATP in a concentration-dependent manner, with pollen growth enhanced at low ATP

### Box 2. Selecting aptamers to ATP

ATP was immobilised onto agarose beads for conventional direct SELEX [37]. From a starting library with a diversity of $2 \times 10^{14}$ ssDNA sequences, 21% of the ssDNA input was eluted by ATP. All 17 clones sequenced from this output were different. The accumulating aptamer literature shows that the population of aptamer hits after eight to 12 rounds of SELEX is seldom more than a few percent of the total sequence diversity, and secondary selection is necessary.

The ssDNA starting library consisted of 72 random bases flanked by 20-mer extensions on each end for primer-binding sites [37]. Two of the aptamer clones were synthesised and shown to bind to immobilised ATP. One was selected for affinity maturation studies, which included sequence truncations and mutagenesis to determine binding motifs and to enhance the binding affinity, experiments later extended by others [90–92]. The ATP-binding site was first determined to be within the 72 random bases, but mutagenesis allowed the aptamer length to be shortened to 45 and then to 25 bases, which formed two stable, stacked G-quadruplexes. Further mutagenesis showed that these G-quadruplexes dominate the structure [90] and later work showed that two ATP molecules intercalate into this framework as the basis for recognition and affinity [90,93].

The observed dissociation constant ($K_D$) of the ATP aptamer varies with the detection method used. Initial analysis using ultrafiltration yielded an estimated $K_D$ of 6 μM with variants giving values between 1 and 10 μM [37]. Later ITC gave comparable low micromolar values [90]. Poorer affinities were observed using microscale thermophoresis and SPR techniques with $K_D$ values of 34.4 M and 30.98 μM, respectively [91,92].

The specificity of the ATP aptamer is dependent on the purine and ribose moieties of ATP, not on the phosphate groups. There is minimal binding with other nucleic acid bases, nor with many other diverse purines. However, the ATP aptamer does significantly bind to ADP, AMP, and adenosine [37,91] and so its application as an aptasensor for measuring ATP *in vivo* needs careful calibration (Figure I).

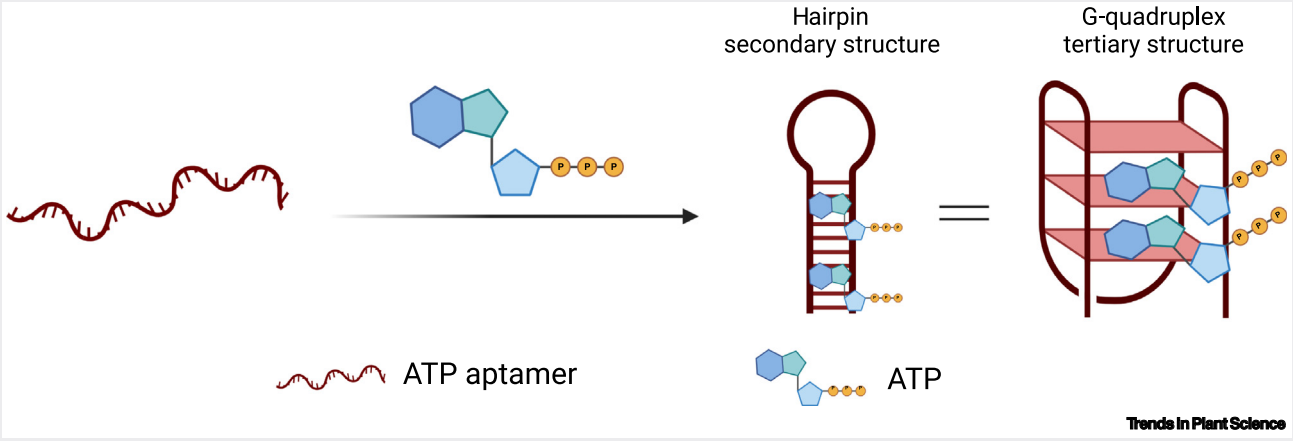


Figure I. A scheme of structure switching for the aptamer against ATP. In the presence of ATP, the target molecule-induced conformational change promotes a more stable G-quadruplex structure in the aptamer. Figure created with BioRender.com.

concentrations and limited at much higher, millimolar concentrations [44,52]. It will be highly beneficial to monitor eATP concentrations in the apoplast and an eATP aptasensor could be robust and sufficiently sensitive for the task.

## Cytokinin aptamers

As with ATP, cytokinins are purines and various aptamers have been selected against cytokinins. A modified direct SELEX method with *trans*-zeatin immobilised onto Sepharose [38] selected highly specific binders after 24 rounds of SELEX. Selection stringency was increased at each round, using counterselection steps with L-histidine, increasing numbers of washes, increasing volumes of counterselection beads, a decreasing single-stranded DNA (ssDNA) pool for each SELEX round, and shorter elution times with decreasing volumes of the target molecule. After sequencing and secondary structure analysis, sequence truncations and mutagenesis were performed to further enhance the aptamer's binding affinity, resulting in a 35-base aptamer with a dissociation constant ($K_D$) for *trans*-zeatin of 3.85 μM measured by isothermal titration calorimetry (ITC). This aptamer is highly selective for *trans*-zeatin with very low binding with *cis*-zeatin and dihydrozeatin, but some binding with *trans*-zeatin riboside.

As noted earlier, a biosensor needs the recognition event converted into a quantifiable signal and several read-out technologies have been reported for cytokinin aptamers. A GO sensor platform was developed initially, providing a 'turn-on' fluorescence measurement upon cytokinin-aptamer binding [38]. The same aptamer was further developed in a structure-switching assay to successfully quantify *trans*-zeatin, whereby a fluorescein-labelled aptamer was paired with a hybridising oligonucleotide linked to a quencher [53]. When hybridised, the aptamer was 'open' and quenched. Binding of *trans*-zeatin leads to a conformational change in the aptamer as it forms a stable hairpin incorporating the target, releasing the oligo-quencher and activating fluorescence. This format of assay had a detection limit of 135 nM for *trans*-zeatin, which might not be sensitive enough to record endogenous tissue cytokinin concentrations, but an electrochemical sensor using this cytokinin aptamer has also been reported with an impressive linear response range from 0.1 to 100 nM and limit of detection of 0.03 nM [54].

### Cytokinin aptasensors for plants?

Cytokinin is undoubtedly one of the vital phytohormones, promoting growth, cell division, germination, shoot and root development, and leaf senescence among its repertoire [55]. Both *trans*-zeatin and *cis*-zeatin are biologically active, although the former is reported to be the most abundant and is significantly more active than its *cis*-isomer [56]. It is crucial that quantitative techniques are sufficiently specific to distinguish the isomers. As for other phytohormones, chromatography coupled with mass spectrometry is used to quantify cytokinins [16,57]. Nonetheless, this method is labour intensive and does not reveal cell- or tissue-specific details. Application of cytokinin aptasensors could provide an alternative temporally resolved and cell-/tissue-specific quantification of cytokinin concentrations. Unfortunately, to date none of these cytokinin aptasensors has been tested *in planta*, but the sensitivity and selectivity of this aptamer make it a very attractive candidate for such applications if a suitable vehicle can be found. A riboswitch RNA aptamer has also been reported to be sensitive to endogenous cytokinin [58] but has not been developed further.

## Abscisic acid aptamers

Highly sensitive and specific ssDNA aptamers have been selected against ABA for quantitative assay development [59]. Despite being a small molecule, direct SELEX was implemented by attaching a biotinylated linker at the C4 position of ABA. A counterselection step with the linker was implemented after the fourth round to avoid enriching for nonspecific binders and two

aptamers were selected with $K_D$ values estimated at 0.98 μM and 0.80 μM, respectively, using a radiolabelled ABA displacement binding assay. The assays revealed a high level of aptamer specificity for the (+)2-*cis*, 4-*trans* ABA enantiomer, with minimal binding to the (–)2-*cis*, 4-*trans* ABA enantiomer or other isomers.

### ABA aptasensors for plants?

The aforementioned ABA aptamers were selected primarily for medical use as ABA has been found to be an endogenous cytokine in human granulocytes [60]. However, given that ABA is one of the important regulatory phytohormones, especially important for abiotic stress responses [61], these reagents could be adopted by plant science. Low nanomolar concentrations of ABA are observed in plants during 'nonstressful' conditions but its concentration can spike to hundreds of micromolar levels during seed maturation and extrinsic stresses [62], concentrations that are consistent with the working affinity of ABA aptamers. Importantly, ABA is a long-distance mobile signal carried in the xylem and this can be a challenging compartment from which to measure local ABA concentrations as genetic reporters [6] are not available within these cells.

The concentration of ABA and its metabolites in plant tissues can be accurately measured using chromatography techniques coupled with mass spectrometry or by immunological assay techniques [16,57]. However, these methods require compound isolation from tissue samples and can be time-consuming. In general, they do not reflect the temporal nor spatial distribution of the hormone. DNA-based aptasensors for ABA have been developed to work in microfluidic flow cells [63] which could be good alternatives for quantitative measurements of ABA *ex planta* without sample workup. The system uses minimal sample volumes, and ABA concentrations were measured between 4 and 40 μM in plant extracts. Another assay developed for ABA measurements was designed based on aptamer-coated gold nanoparticles (Box 3).

## Salicylic acid aptamers

A DNA aptamer has been developed against salicylic acid (SA; 2-hydroxybenzoic acid) with high binding affinity and specificity [26]. Solution SELEX was chosen for aptamer selection due to the limitations of the small size and few functional groups presented by SA. Counterselection steps with 4-hydroxybenzoic acid after the fourth round increased selection stringency and eliminated nonspecific binding sequences. Stringency was also increased by extending the length of the capture oligo from seven to nine bases in the later SELEX rounds. Sequences from NGS gave details of enrichment from rounds 6, 13, and 15 [36].

The aptamer was coupled to a gold-coated nanopore film to provide a measurement of SA concentration based on an optical shift. The kinetic binding data were fitted to a two sites model with reported $K_D$ values of 34.57 nM and 4.703 μM for the two sites. However, without additional data for two sites, the higher of these two values is more consistent with the experimental data provided, suggesting micromolar sensitivity. There were minimal optical shifts observed with SA metabolites, suggesting that the SA aptamer has good selectivity.

### Salicylic acid aptasensors for plants?

SA is one of the phytohormones responsible for the immune response in plants as well as contributing to thermogenesis, flowering and circadian clock function [64]. Analytical techniques including chromatography coupled with mass spectrometry and a genetically encoded biosensor have been developed in efforts to quantify SA concentrations both in cells and in tissues [65]. However, the SA aptamer has a $K_D$ comparable to endogenous SA receptors [66] and offers alternative sensor options [63].

Box 3. A gold nanoparticle aptamer assay for ABA

In the presence of ABA, the aptamers leave the surface of gold nanoparticles that then aggregate, a process recorded by SPR (Figure I).

The method gave a linear dose response to ABA concentrations between 0.5 and 50 μM. The assay results show that the aptamers are sensitive and selective, but this has not yet been adapted into an aptasensor for use *in planta*. However, the gold nanoparticles suggest a delivery vehicle if a suitable readout can be devised.

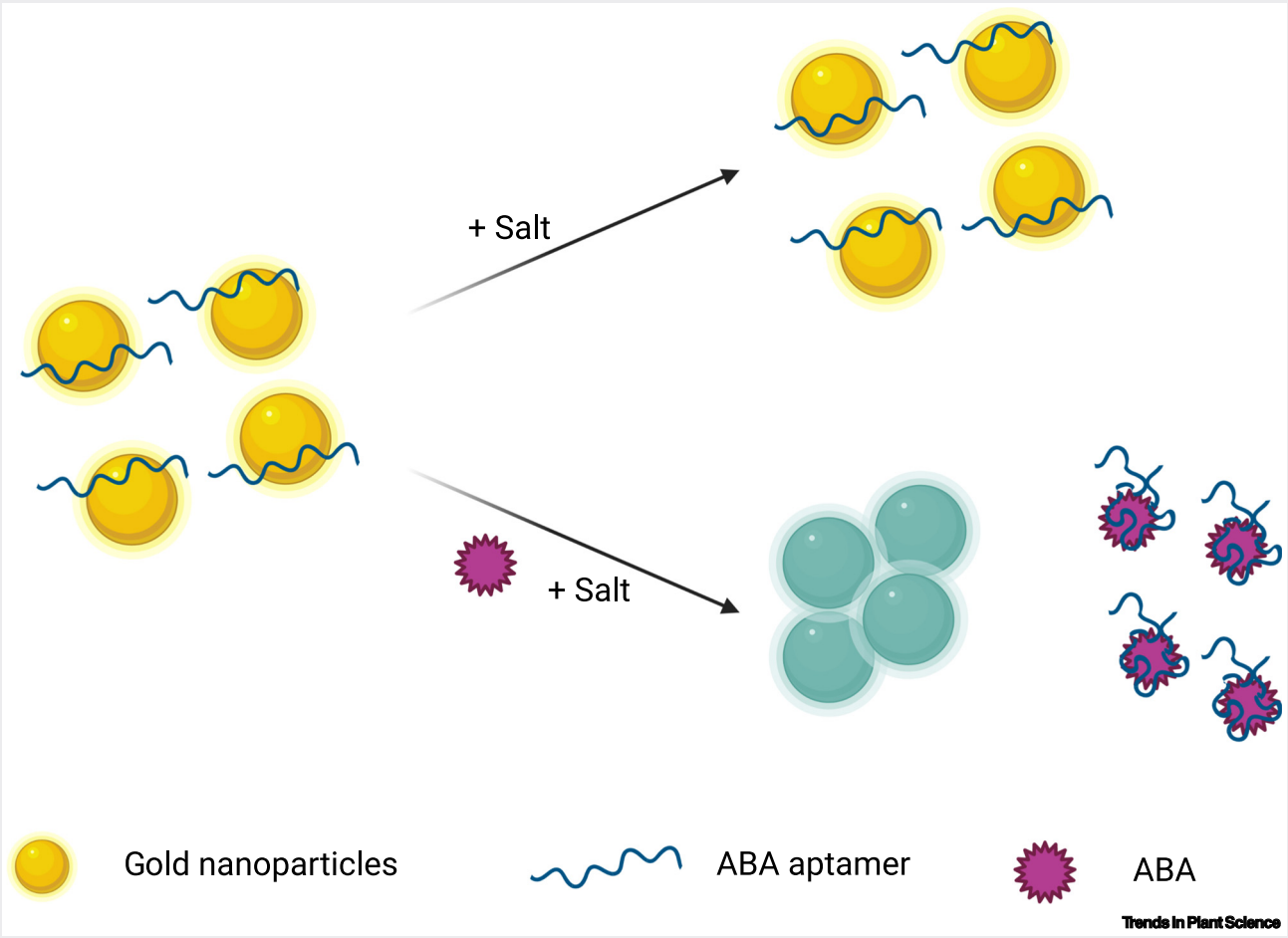


Figure I. Schematic illustration of the localised SPR assay as used with ABA aptamers. Figure modified from [94] and created with BioRender.com.

## Apple stem pitting virus aptamers

In medicine and forensics, the majority of aptamers are developed against macromolecular targets, such as proteins or cell-surface determinants [33,35,67,68]. Plant science has also started selecting for aptamers as diagnostic agents. For example, a modified version of direct SELEX was used for aptamers against two different coat proteins of the apple stem pitting virus (ASPV) known as MT32 and PSA-H [69]. MT32 and PSA-H proteins were cloned with polyhistidine affinity tags allowing immobilisation on nickel beads for the selection cycles. Removal of nonspecific binders was done by addition of poly(deoxyinosinic-deoxycytidylic) acid and BSA during library incubations. Selection stringency was increased throughout the selection process by reducing the amount of capture protein. Characterisation of the selected aptamers with SPR revealed

## Box 4. Aptasensor concepts for applications *in planta*

Well-established chemistries for linking DNA aptamers at their 3′ and 5′ ends to diverse partner molecules create many options for aptasensor development and three options are illustrated inFigure I. The smallest will be conformationally active aptamers to be conjugated at opposite ends to complementary fluors which will exhibit FRET as either empty (shown in Figure IA) or full conformers. Fluorescence-based aptasensors are among the most popular applications because they give positional data as well as analyte quantitation using turn-on or turn-off mechanisms. Fluorescent nanoparticles can be used both as FRET donor and as delivery vehicle, perhaps holding the sensor in the apoplast, for example (Figure IB). Electrochemical biosensors developed as microelectrodes (shown in Figure IC), but also as part of scanning tunnelling microscope probes offer many sensor scales and geometries [95]. In the microelectrode system shown, the aptamer will be conjugated to a gold electrode at one end, and the proximity of an electroactive compound at the other (e.g., methylene blue) is detected as a change in electrical signal by the electrode [96]. Electrodes allow precise positioning of the sensor within tissues, such as for measuring hormone transients in xylem sap or the apoplast around mesophyll cells in the leaf.

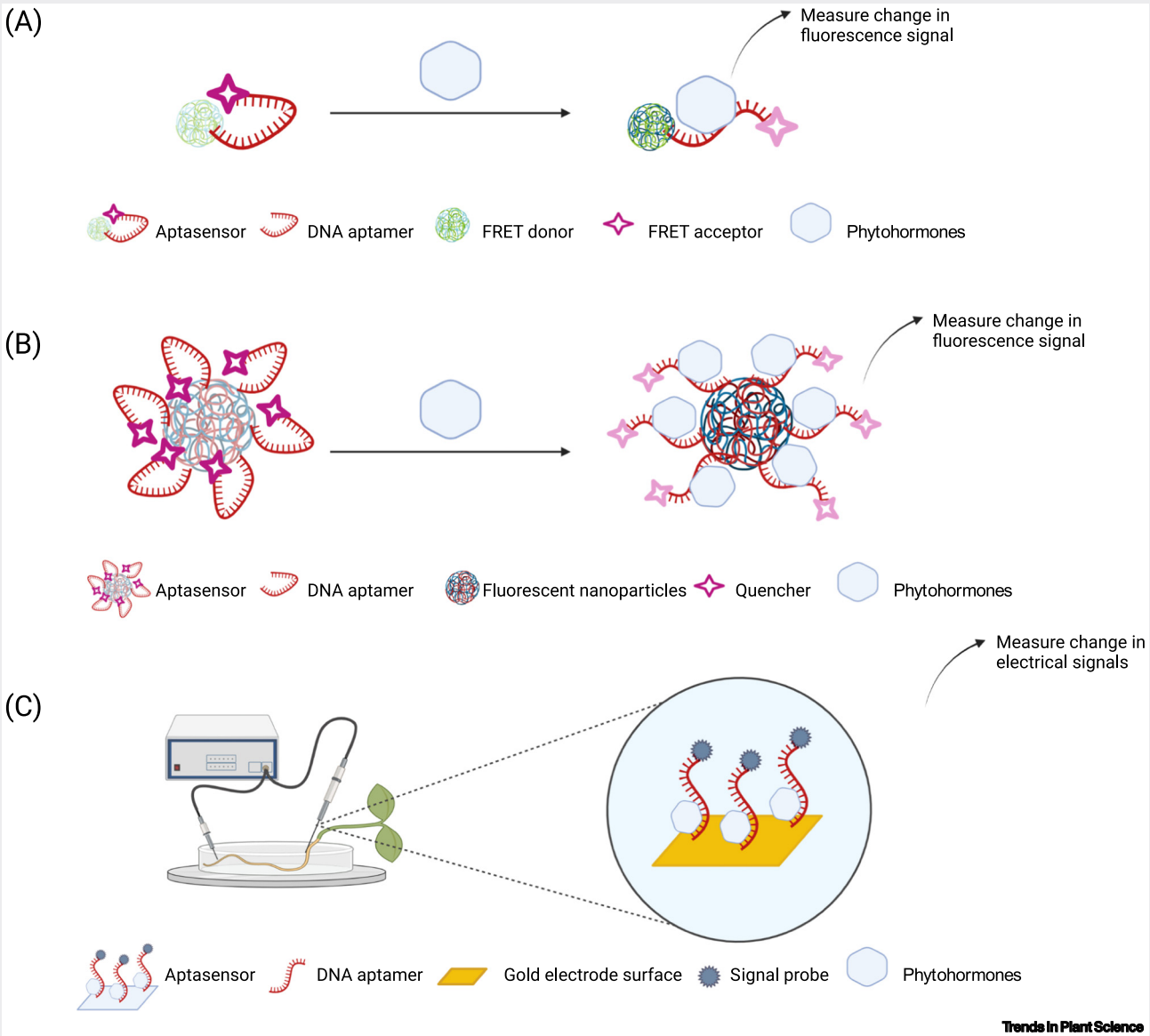


Figure I. Various aptasensor designs for *in planta* detection of phytoactive molecules. Figure created with BioRender.com.

that the MT32 aptamer binds to both MT32 and PSA-H with affinities ($K_D$) estimated at 55 and 83 nM, respectively. The aptamers also possess high specificity and differentiate other viruses including apple mosaic virus and apple chlorotic leaf spot virus. The aptamers have been used for SPR imaging and an ELONA sensing platform has been developed [70,71].

### ASPV aptasensors for plants?

ASPV is a pathogenic virus infecting many pome and stone fruit species [72]. Symptoms vary depending on host species, from symptomless to severe, such as xylem pitting, red mottling, and downward growth [73]. It is advantageous to be able to detect and identify such a pathogen before it severely impairs the performance of the crop. Previous ASPV detection techniques have been limited to grafting onto indicator plants, which is very time-consuming and slow [74]. In-field RT-PCR methods can be highly sensitive but lack high throughput [75]. ASPV aptasensors could detect latent pathogen with high sensitivity, high specificity, and high throughput.

## Concluding remarks and future perspectives

### Aptasensor development for use *in planta*

The applications summarised earlier have all been for *ex vivo* sample measurements, but biosensors can be used to monitor physiological phenomena in virtually any biological system [22,76], including *in vivo,* with little to no trauma to the organism [77,78]. To convert established aptamers into sensing tools that will work *in planta*, many parameters need to be considered. For example, the stability of DNA in the testing environment is vital. As free oligonucleotides, ssDNA aptamers are at risk of degradation by nucleases, but conjugation on both 5′ and 3′ ends, such as with fluors, allows shielding from exonucleases, while base modifications will minimise endonuclease degradation [79]. Aptamers incorporated as part of DNA tetrahedron structures are also reported to resist nuclease attacks and this stability has made them useful as aptamer beacon sensors in mammalian cells [80]. DNA tetrahedrons have yet to be evaluated in plants. Furthermore, DNA tetrahedron structures can be designed with self-assembling properties, encouraging complex 'aptachain' formations that can resist enzymatic degradation [81]. These DNA engineering possibilities are attractive propositions as aptasensor scaffolds, but need testing.

As with all sensors, the application should dictate how aptasensors are developed. Many platforms have been developed for measurements on extracted samples, making aptamers amenable for rapid, facile diagnostic kits [24,26,36,63,70,71,78,82]. However, for measurements *in planta,* a fluorescent optical biosensor suited to confocal microscopy could give high spatial resolution and sensitivity (Box 4). There is little need to compete with genetically encoded fluorescent sensors such as those developed for intracellular monitoring of phytohormones [12], but the plant apoplast remains a poorly explored compartment and it is not readily amenable to most intracellular probes. Aptasensors might provide the ideal tool for these spaces and nanoparticles are an attractive option as delivery vehicles. Polymer nanoparticles (<40 nm) are known to penetrate into and through plant cell walls [83,84] and to pass into the xylem. Therefore, aptamer-coated nanoparticles as aptasensors could open up opportunities for studying the behaviours of phytoactive compounds in ways that have been prohibited previously [67] (see Outstanding questions).

### Outstanding questions

How long before an aptasensor is developed for reporting analyte concentrations *in planta*?

Will aptamers prove to be sufficiently robust in the mildly acidic conditions of apoplast?

Which aptasensor design (Box 4) will prove most versatile for plants? For example, will aptasensors based on nanoparticles reside in the cell wall matrix to sample the apoplast? Will different nanoparticle designs pass through the plasma membrane to the cytoplasm?

Will aptasensors passage to all parts of a plant in the vasculature to provide sensing options throughout the plant?

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**Declaration of interests**

No interests are declared.

## References


1. Sadanandom, A. and Napier, R.M. (2010) Biosensors in plants. *Curr. Opin. Plant Biol.* 13, 736–743
2. Bhalla, N. *et al.* (2016) Introduction to biosensors. *Essays Biochem.* 60, 1–8
3. Teymourian, H. *et al.* (2020) Electrochemical glucose sensors in diabetes management: an updated review (2010–2020). *Chem. Soc. Rev.* 49, 7671–7709
4. Ramjee, M.K. (2000) The use of fluorogenic substrates to monitor thrombin generation for the analysis of plasma and whole blood coagulation. *Anal. Biochem.* 277, 11–18
5. Sharma, S. *et al.* (2016) Antibodies and antibody-derived analytical biosensors. *Essays Biochem.* 60, 9–18
6. Jones, A.M. *et al.* (2014) Abscisic acid dynamics in roots detected with genetically encoded FRET sensors. *eLife* 3, e01741
7. Liao, C.Y. *et al.* (2014) Reporters for sensitive and quantitative measurement of auxin response. *Nat. Methods* 12, 207–210
8. Chaudhuri, B. *et al.* (2011) Dynamic imaging of glucose flux impedance using FRET sensors in wild-type *Arabidopsis* plants. *J. Exp. Bot.* 62, 2411–2417
9. Isoda, R. *et al.* (2020) Sensors for the quantification, localization and analysis of the dynamics of plant hormones. *Plant J.* 105, 542–557
10. Herud-Sikimić, O. *et al.* (2021) A biosensor for the direct visualization of auxin. *Nature* 592, 768–772
11. Rizza, A. *et al.* (2017) *In vivo* gibberellin gradients visualized in rapidly elongating tissues. *Nat. Plants* 3, 803–813
12. Balcerowicz, M. *et al.* (2021) Fluorescent biosensors illuminating plant hormone research. *Plant Physiol.* 187, 590–602
13. Waadt, R. *et al.* (2014) FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in *Arabidopsis*. *eLife* 3, e01739
14. Samodelov, S.L. *et al.* (2016) StrigoQuant: a genetically encoded biosensor for quantifying strigolactone activity and specificity. *Sci. Adv.* 2, e1601266
15. Larrieu, A. *et al.* (2015) A fluorescent hormone biosensor reveals the dynamics of jasmonate signalling in plants. *Nat. Commun.* 6, 6043
16. Novak, O. *et al.* (2017) Zooming in on plant hormone analysis: tissue- and cell-specific approaches. *Annu. Rev. Plant Biol.* 68, 323–348
17. Tian, F. *et al.* (2014) A highly selective biosensor with nanomolar sensitivity based on cytokinin dehydrogenase. *PLoS One* 9, e90877
18. Shinoda, H. *et al.* (2018) Fluorescent proteins for investigating biological events in acidic environments. *Int. J. Mol. Sci.* 19, 1548
19. Moreau, H. *et al.* (2022) Genetically encoded fluorescent sensors adapted to acidic pH highlight subdomains within the plant cell apoplast. *J. Exp. Bot.* Published online May 23, 2022. http://doi.org/10.1093/jxb/erac210
20. Gjetting, K.S. *et al.* (2012) Live imaging of intra- and extracellular pH in plants using pHusion, a novel genetically encoded biosensor. *J. Exp. Bot.* 63, 3207–3218
21. Mayer, G. (2016) *Nucleic Acid Aptamers: Selection, Characterization, and Application*, (vol. 1380). Humana Press
22. Gooch, J. *et al.* (2017) Developing aptasensors for forensic analysis. *Trends Anal. Chem.* 94, 150–160
23. Le, T.T. *et al.* (2014) Determination of minimal sequence for binding of an aptamer. A comparison of truncation and hybridization inhibition methods. *RSC Adv.* 4, 47227–47233
24. Kim, S. *et al.* (2018) An aptamer-aptamer sandwich assay with nanorod-enhanced surface plasmon resonance for attomolar concentration of norovirus capsid protein. *Sensors Actuators B Chem.* 273, 1029–1036
25. Nguyen, B. *et al.* (2007) Biosensor-surface plasmon resonance: quantitative analysis of small molecule-nucleic acid interactions. *Methods* 42, 150–161
26. Mckeague, M. *et al.* (2014) Selection and characterization of a novel DNA aptamer for label-free fluorescence biosensing of ochratoxin A. *Toxins (Basel)* 6, 2435–2452
27. Stoltenburg, R. *et al.* (2005) FluMag-SELEX as an advantageous method for DNA aptamer selection. *Anal. Bioanal. Chem.* 383, 83–91
28. Stoltenburg, R. *et al.* (2012) Capture-SELEX: selection of DNA aptamers for aminoglycoside antibiotics. *J. Anal. Methods Chem.* 2012, 415697
29. Park, J.W. *et al.* (2012) Immobilization-free screening of aptamers assisted by graphene oxide. *Chem. Commun.* 48, 2071–2073
30. Hoinka, J. *et al.* (2018) AptaSUITE: a full-featured bioinformatics framework for the comprehensive analysis of aptamers from HT-SELEX experiments. *Mol. Ther. Nucleic Acids* 11, 515–517
31. Afgan, E. *et al.* (2018) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses. *Nucleic Acids Res.* 46, W537–W544
32. Khati, M. *et al.* (2003) Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'F-RNA aptamers. *J. Virol.* 77, 12692–12698
33. Kaur, H. and Yung, L.Y. (2012) Probing high affinity sequences of DNA aptamer against VEGF165. *PLoS One* 7, e31196
34. Misono, T.S. and Kumar, P.K. (2005) Selection of RNA aptamers against human influenza virus hemagglutinin using surface plasmon resonance. *Anal. Biochem.* 342, 312–317
35. Sypabekova, M. *et al.* (2017) Selection, characterization, and application of DNA aptamers for detection of *Mycobacterium tuberculosis* secreted protein MPT64. *Tuberculosis* 104, 70–78
36. Chen, C. *et al.* (2019) Development of a structure-switching aptamer-based nanosensor for salicylic acid detection. *Biosens. Bioelectron.* 140, 111342
37. Huizenga, D.E. and Szostak, J.W. (1995) A DNA aptamer that binds adenosine and ATP. *Biochemistry* 34, 656–665
38. Qi, C. *et al.* (2013) G-quadruplex DNA aptamers for zeatin recognizing. *Biosens. Bioelectron.* 41, 157–162
39. Reinemann, C. *et al.* (2016) Generation and characterization of quinolone-specific DNA aptamers suitable for water monitoring. *Biosens. Bioelectron.* 77, 1039–1047
40. Zheng, X. *et al.* (2015) A saxitoxin-binding aptamer with higher affinity and inhibitory activity optimized by rational site-directed mutagenesis and truncation. *Toxicon* 101, 41–47
41. Malhotra, S. *et al.* (2014) Selection of aptamers for aflatoxin M1 and their characterization. *J. Mol. Recognit.* 27, 493–500
42. Park, J. *et al.* (2017) Plant hormone transporters: what we know and what we would like to know. *BMC Biol.* 15, 93
43. Tanaka, K. *et al.* (2014) Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. *Front. Plant Sci.* 5, 446
44. Chivasa, S. *et al.* (2005) Extracellular ATP functions as an endogenous external metabolite regulating plant cell viability. *Plant Cell* 17 3019–2034
45. Tanaka, K. *et al.* (2010) Extracellular ATP signaling in plants. *Trends Cell Biol.* 20, 601–608
46. Pietrowska-Borek, M. *et al.* (2020) New insight into plant signaling: extracellular ATP and uncommon nucleotides. *Cells* 9, 345
47. Schwiebert, E.M. and Zsembery, A. (2003) Extracellular ATP as a signaling molecule for epithelial cells. *Biochim. Biophys. Acta (BBA) Biomembr.* 1615, 7–32
48. Dale, N. (2021) Real-time measurement of adenosine and ATP release in the central nervous system. *Purinergic Signal.* 17, 109–115
49. De Col, V. *et al.* (2017) ATP sensing in living plant cells reveals tissue gradients and stress dynamics of energy physiology. *eLife* 6, e26770
50. Choi, J. *et al.* (2014) Identification of a plant receptor for extracellular ATP. *Science* 343, 290–294
51. Pham, A.Q. *et al.* (2020) *Arabidopsis* lectin receptor kinase P2K2 is a second plant receptor for extracellular ATP and contributes to innate immunity. *Plant Physiol.* 183, 1364–1375
52. Zhou, J. *et al.* (2014) Extracellular ATP is involved in the initiation of pollen germination and tube growth in *Picea meyeri*. *Trees* 29, 563–574
53. Liu, C. *et al.* (2016) Target-induced structure switching of a hairpin aptamer for the fluorescence detection of zeatin. *Anal. Methods* 8, 5957–5961
54. Wang, Y. *et al.* (2018) Photoelectrochemical apta-biosensor for zeatin detection based on graphene quantum dots improved photoactivity of graphite-like carbon nitride and streptavidin induced signal inhibition. *Sensors Actuators B Chem.* 257, 237–244
55. Akhtar, S.S. *et al.* (2019) Role of cytokinins for interactions of plants with microbial pathogens and pest insects. *Front. Plant Sci.* 10, 1777

56. Prerostova, S. *et al.* (2018) Cytokinins: their impact on molecular and growth responses to drought stress and recovery in *Arabidopsis*. *Front. Plant Sci.* 9, 655
57. Muller, A. *et al.* (2002) A multiplex GC-MS/MS technique for the sensitive and quantitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. *Planta* 216, 44–56
58. Grojean, J. and Downes, B. (2010) Riboswitches as hormone receptors: hypothetical cytokinin-binding riboswitches in *Arabidopsis thaliana*. *Biol. Direct* 5, 60
59. Grozio, A. *et al.* (2013) Selection and characterization of single stranded DNA aptamers for the hormone abscisic acid. *Nucleic Acid Ther.* 23, 322–331
60. Bruzzone, S. *et al.* (2007) Abscisic acid is an endogenous cytokine in human granulocytes with cyclic ADP-ribose as second messenger. *Proc. Natl. Acad. Sci.* 104, 5759–5764
61. Chen, K. *et al.* (2020) Abscisic acid dynamics, signaling, and functions in plants. *J. Integr. Plant Biol.* 62, 25–54
62. Shu, K. *et al.* (2018) Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. *Front. Plant Sci.* 9, 416
63. Feng, S. *et al.* (2021) Optical aptamer-based sensors for detecting plant hormones. *IEEE Sensors J.* 21, 5743–5750
64. Rivas-San Vicente, M. and Plasencia, J. (2011) Salicylic acid beyond defence: its role in plant growth and development. *J. Exp. Bot.* 62, 3321–3338
65. Huang, W. *et al.* (2016) Quantitative *in situ* assay of salicylic acid in tobacco leaves using a genetically modified biosensor strain of *Acinetobacter* sp. ADP1. *Plant J. Cell Mol. Biol.* 46, 1073–1083
66. Klessig, D.F. *et al.* (2016) Multiple targets of salicylic acid and its derivatives in plants and animals. *Front. Immunol.* 7, 206
67. Thiviyanathan, V. and Gorenstein, D.G. (2012) Aptamers and the next generation of diagnostic reagents. *Proteomics Clin. Appl.* 6, 563–573
68. Sefah, K. *et al.* (2010) Development of DNA aptamers using Cell-SELEX. *Nat. Protoc.* 5, 1169–1185
69. Balogh, Z. *et al.* (2010) Selection and versatile application of virus-specific aptamers. *Fed. Am. Soc. Exp. Biol. J.* 24, 4187–4195
70. Lautner, G. *et al.* (2010) Aptamer-based biochips for label-free detection of plant virus coat proteins by SPR imaging. *Analyst* 135, 918–926
71. Komorowska, B. *et al.* (2017) Application of nucleic acid aptamers for detection of apple stem pitting virus isolates. *Mol. Cell. Probes* 36, 62–65
72. Martelli, G.P. and Jelkmann, W. (1998) *Foveavirus*, a new plant virus genus. *Arch. Virol.* 143, 1245–1249
73. Ma, X. *et al.* (2019) Functional analysis of apple stem pitting virus coat protein variants. *Virol. J.* 16, 20
74. Gugerli, P. and Ramel, M.E. (2004) Production of monoclonal antibodies for the serological identification and reliable detection of apple stem pitting and pear yellow vein viruses in apple and pear. *Acta Hortic.* 657, 59–69
75. Jelkmann, W. and Keim-Konrad, R. (1997) Immuno-capture polymerase chain reaction and plate-trapped ELISA for the detection of apple stem pitting virus. *J. Phytopathol.* 145, 499–503
76. Gooch, J. *et al.* (2021) Generating aptamers towards human sperm cells using massively parallel sequencing. *Anal. Bioanal. Chem.* 413, 5821–5834
77. Vieira, P.A. *et al.* (2019) Ultra-high-precision, *in-vivo* pharmacokinetic measurements highlight the need for and a route toward more highly personalized medicine. *Front. Mol. Biosci.* 16, 69
78. Ferguson, B.S. *et al.* (2013) Real-time, aptamer-based tracking of circulating therapeutic agents in living animals. *Sci. Transl. Med.* 5, 213ra165
79. Odeh, F. *et al.* (2019) Aptamers chemistry: chemical modifications and conjugation strategies. *Molecules* 25, 3
80. Xie, N. *et al.* (2016) Competition-mediated FRET-switching DNA tetrahedron molecular beacon for intracellular molecular detection. *ACS Sensors* 1, 1445–1452
81. Neves, M.A.D. *et al.* (2019) A proof of concept application of aptachain: ligand-induced self-assembly of a DNA aptamer. *RSC Adv.* 9, 1690–1695
82. Qi, Y. *et al.* (2020) An aptamer-based colorimetric sensing of acetamiprid in environmental samples: convenience, sensitivity and practicability. *Sensors Actuators B Chem.* 304, 127359
83. Sun, X.D. *et al.* (2020) Differentially charged nanoplastics demonstrate distinct accumulation in *Arabidopsis thaliana*. *Nat. Nanotechnol.* 15, 755–760
84. Parkinson, S.J. et al. Polymer nanoparticles pass the plant interface. *bioRxiv*. Published online March 27, 2022. http://doi.org/10.1101/2022.03.24.485656.
85. Ellington, A.D. and Szostak, J.W. (1990) *In vitro* selection of RNA molecules that bind specific ligands. *Nature* 346, 818–822
86. Blank, M. (2016) Next-generation analysis of deep sequencing data: bringing light into the black box of SELEX experiments. In *Nucleic Acid Aptamers* (Vol. 1380) (Mayer, G., ed.), pp. 85–95, Humana Press
87. Yoshitomi, T. *et al.* (2020) Binding and structural properties of DNA aptamers with VEGF-A-mimic activity. *Mol. Ther. Nucleic Acids* 19, 1145–1152
88. Mayer, G. and Hover, T. (2009) *In vitro* selection of ssDNA aptamers using biotinylated target proteins. In *Nucleic Acid and Peptide Aptamers* (Mayer, G., ed.), pp. 19–32, Humana Press
89. Mendonsa, S.D. and Bowser, M.T. (2004) *In Vitro* evolution of functional DNA using capillary electrophoresis. *J. Am. Chem. Soc.* 126, 20–21
90. Zhang, Z. *et al.* (2017) New insights into a classic aptamer: binding sites, cooperativity and more sensitive adenosine detection. *Nucleic Acids Res.* 45, 7593–7601
91. Entzian, C. and Schubert, T. (2016) Studying small molecule-aptamer interactions using microscale thermophoresis (MST). *Methods* 97, 27–34
92. Chang, A.L. *et al.* (2014) Facile characterization of aptamer kinetic and equilibrium binding properties using surface plasmon resonance. *Methods Enzymol.* 549, 451–466
93. Zhang, Z. and Liu, J. (2018) An engineered one-site aptamer with higher sensitivity for label-free detection of adenosine on graphene oxide. *Can. J. Chem.* 96, 957–963
94. Wang, S. *et al.* (2017) Localized surface plasmon resonance-based abscisic acid biosensor using aptamer functionalized gold nanoparticles. *PLoS ONE* 12, e0185530
95. Rodríguez-Galván, A. and Contreras-Torres, F.F. (2022) Scanning tunneling microscopy of biological structures: an elusive goal for many years. *Nanomaterials* 12, 3013
96. Wer, X. *et al.* (2022) Sensitive identification of microcystin-LR via a reagent-free and reusable electrochemical biosensor using a methylene blue-labeled aptamer. *Biosensors* 12, 556