

Review

Aptasensors for lysozyme detection: Recent advances

Gheorghe Melinte^{a,b}, Giulia Selvolini^a, Cecilia Cristea^{b,**}, Giovanna Marrazza^{a,c,*}^a “Ugo Schiff” Chemistry Department, University of Florence, Via della Lastruccia 3, Sesto Fiorentino (FI), 50019, Italy^b Analytical Chemistry Department, Faculty of Pharmacy, “Iuliu Hatieganu” University of Medicine and Pharmacy, Strada Louis Pasteur 4, Cluj-Napoca, 400349, Romania^c Istituto Nazionale Biostrutture e Biosistemi (INBB), Unit of Florence, Viale Delle Medaglie D'Oro 305, 00136 Roma, Italy

ARTICLE INFO

Keywords:

Lysozyme
Aptamer
Allergens
Electrochemical sensor
Optical sensor

ABSTRACT

Lysozyme is an enzyme existing in multiple organisms where it plays various vital roles. The most important role is its antibacterial activity in the human body; in fact, it is also called “the body’s own antibiotic”. Despite its proven utility, lysozyme can potentially trigger allergic reactions in sensitive individuals, even in trace amounts. Therefore, lysozyme determination in foods is becoming of paramount importance. Traditional detection methods are expensive, time-consuming and they cannot be applied for fast *in-situ* quantification. Electrochemical and optical sensors have attracted an increasing attention due to their versatility and ability to reduce the disadvantages of traditional methods. Using an aptamer as the bioreceptor, the sensor selectivity is amplified due to the specific recognition of the analyte. This review is presenting the progresses made in lysozyme determination by means of electrochemical and optical aptasensors in the last five years. A critical overview on the methodologies employed for aptamer immobilization and on the strategies for signal amplification of the assays will be described. Different optical and electrochemical aptasensors will be discussed and compared in terms of analytical performances, versatility and real samples applications.

1. Introduction

Lysozyme, also known as muramidase or N-acetylmuramic hydrolase, is a small protein of 129 amino acids with a molecular weight of 14.3 kDa owing a monomeric structure, which is stabilized by four disulfide bonds through eight cysteine residues, lysine and leucine residues as starting and final amino acids, respectively, and an isoelectric point of 11 [1,2]. In the animal kingdom, three major and distinct types of lysozyme can be identified: c-type, g-type and i-type, where the initials stand for “chicken” (or “conventional”), “goose” and “invertebrate”. The c-type and i-type lysozyme have similar molecular weight values (14.3 kDa and 14.7 kDa, respectively), while the g-type has greater molecular weight of 20–22 kDa [3,4]. Nevertheless, the human lysozyme was the first mammal c-type to be sequenced and used as a model protein for a variety of studies. The primary structure of c-type lysozyme is shown in Fig. 1.

The conformational dynamics of the folded and unfolded states and the thermal behavior of enzyme molecular interactions have been investigated using nuclear magnetic resonance [5]; moreover, in order

to determine the structure of lysozyme, infrared spectroscopy technique was also applied. The spectrum of lyophilized lysozyme shows a broad peak centered on 180 cm^{-1} , which may be due to the overlapping of an ensemble of hydrogen bond vibrational modes associated with the peptide backbone; the α -helical backbone vibrational modes have been associated with the peaks observed at 333 cm^{-1} and 375 cm^{-1} , while the peak appearing at 445 cm^{-1} is due to the β -plated sheet [6].

The term “lysozyme” was first coined in 1922 by Alexander Fleming, who demonstrated its antibacterial role while testing the nasal secretion of an acute coryza-suffering patient on blood agar plates and observing that in the first three days no bacterial colony had grown [7]. Lysozyme catalyzes the hydrolysis of the $\beta(1-4)$ -bond between the N-acetylmuramic acid and N-acetylglucosamine residues of bacterial wall, that determines the peptidoglycan integrity; this hydrolysis in turn causes the lysis of the bacteria, making lysozyme to be called the “body’s own antibiotic”. Nevertheless, the antibiotic activity is only valid for Gram-positive bacteria, as Gram-negative ones have an extra lipopolysaccharide outer membrane that makes the peptidoglycan wall inaccessible to the enzyme [3].

* Corresponding author. “Ugo Schiff” Chemistry Department, University of Florence, Via della Lastruccia 3, Sesto Fiorentino (FI), 50019, Italy.

** Corresponding author. Analytical Chemistry Department, Faculty of Pharmacy, “Iuliu Hatieganu” University of Medicine and Pharmacy, Strada Louis Pasteur 4, Cluj-Napoca, 400349, Romania.

E-mail addresses: cristea@umfcluj.ro (C. Cristea), giovanna.marrazza@unifi.it (G. Marrazza).<https://doi.org/10.1016/j.talanta.2021.122169>

Received 6 October 2020; Received in revised form 26 January 2021; Accepted 27 January 2021

Available online 30 January 2021

0039-9140/© 2021 Elsevier B.V. All rights reserved.

Lysozyme is present in almost all human body fluids, with the highest amount found in tears (1267 mg L^{-1}) [8], serum ($0.7\text{--}2.58 \text{ mg L}^{-1}$) [9], gastric juice and mother milk (300 mg L^{-1}) [10], while the lowest quantity is found in urine ($1.7\text{--}123 \text{ } \mu\text{g L}^{-1}$) [9], cerebrospinal fluid ($7.7\text{--}84 \text{ } \mu\text{g L}^{-1}$) [8] and bile (0.4 mg L^{-1}) [11]. As lysozyme concentration can increase over a threshold level in body fluids, tissues, etc. in presence of some pathological conditions, this enzyme is also widely used as a biomarker molecule for a variety of diseases, such as leukemia [12], AIDS [13], pulmonary tuberculosis [14], rheumatoid arthritis and Crohn disease. The monitoring of its concentration in pathological conditions is of paramount importance, as this is also correlated to the disease status and to the outcome of the therapeutic treatment [15,16].

Lysozyme has also a significant importance in food industry, as it is

widely applied as a preservative for its antibacterial activity. In particular, it is useful in wine industry as a stabilizer and fermentation-stopper instead of sulfites, with an allowed limit of 50 g hL^{-1} set by the European Community [17]; it is also added to some cheeses or to beer as prevention for butyric acid bacteria, as well as in meat and shrimps to extend their shelf-life [18]. Unfortunately, lysozyme can potentially trigger adverse reaction of the immune system in sensitive individuals, even if its presence in food is in trace amounts. Therefore, it is considered as one of the five major allergenic proteins (ovomucoid, ovalbumin, ovotransferrin, ovomucin and lysozyme) in the egg of the domestic chicken (*Gallus domesticus*), where it can be found in large amounts in the white part. Its allergic activity has been demonstrated by detecting serum specific immunoglobulin (IgE) produced after its ingestion [19].

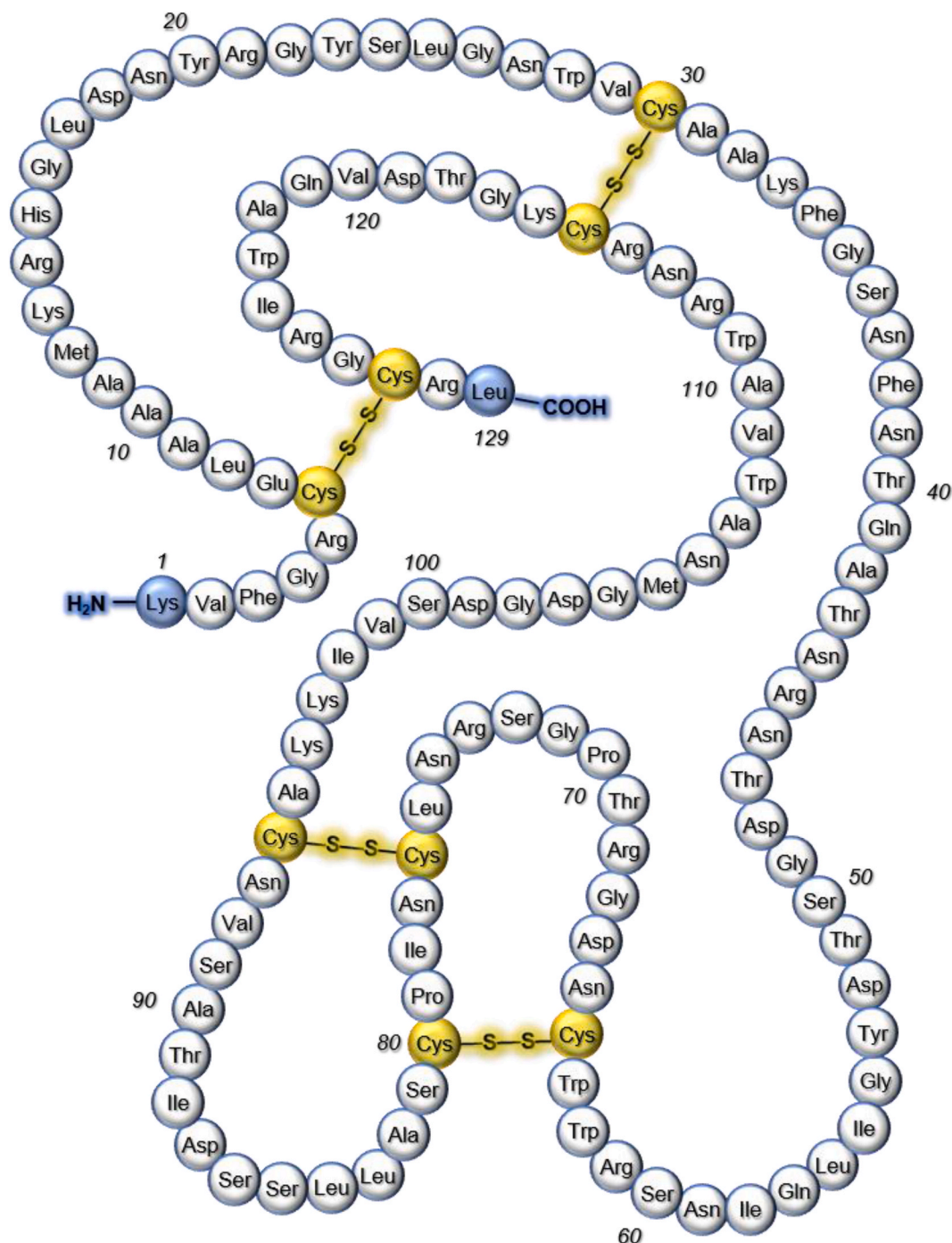


Fig. 1. Scheme of the primary structure of lysozyme.

Food allergy symptoms can range from mild urticaria to anaphylactic shock, even causing the death of the patient [20,21]. In order to prevent this kind of adverse reaction, individuals with a history of allergenic sensibility must avoid becoming in contact with the allergen [22] and the food containing it [20]. Therefore, reliable analytical methods to guarantee consumers protection and to improve the food production in terms of hazard analysis of critical control point, risk assessment and good manufacturing practice are necessary [22].

In this review, without pretending to be exhaustive, the aptamer-based sensors (i.e. aptasensors) designed in the last five years have been reported by a transduction-based classification, which takes into consideration both the electrochemical and the optical approaches, highlighting some of their technical challenges and the new trends by means of a set of selected recent applications.

The existing reviews are mainly focused on the development of electrochemical aptasensors, including and their possible application in serum analysis [18], and on the role of lysozyme in food industry [1].

The present review is specifically designed to consider the latest advances in the development of both electrochemical and optical aptasensors, emphasizing the similarities and differences between them, while also considering the few sensors that are using different or no bioreceptors.

2. Discussion

2.1. Analytical standard methods for lysozyme determination

The first quantification method of lysozyme was the measurement of its enzymatic activity. More precisely, lysozyme was isolated using electrophoresis from urine samples and its capacity of clearing a *Micrococcus lysodeikticus* suspension was then tested. Smolelis et al. used an optical method, based on a comparison of light transmissions of crystalline lysozyme dilutions after the addition of susceptible cells and incubation [23]. A procedure that improved the analytical properties of the previous ones was based on the incubation of lysozyme on Petri dishes filled with *Micrococcus lysodeikticus* colonies. After 12–48 h, the distance cleared by lysozyme was measured and compared with a blank sample [24].

Nowadays, several methods to characterize and directly quantify the enzyme amount have been developed. Differential scanning calorimetry (DSC) was used to study the protein stability, the pH effects on its thermodynamic and energetic stability profiles as well as to understand the presence of its aggregates during the crystallization process. DSC can also be used to predict the interaction between lysozyme and drugs or with other proteins of interest [25,26].

Immunoenzymatic methods, especially the enzyme-linked immunosorbent assay (ELISA), have been mostly used for their specificity, the ability to analyze a large number of samples and high sensitivity, obtaining limit of detection (LOD) values down to $0.2 \mu\text{g L}^{-1}$ [27]. A study conducted by L. Pellegrino and A. Tirelli managed to develop a high-performance liquid chromatography (HPLC) method to quantify lysozyme in milk and cheese samples, obtaining a LOD of $0.8 \mu\text{g L}^{-1}$ [28].

Starting from the aforementioned ELISA and HPLC methods, studies have been conducted by Kerkaert et al. to compare the ELISA assays with HPLC procedure in order to assess the reliability of both for the quantification of lysozyme in complex matrices. Evidences have shown that for the commercial ELISA kit, no reliable results were obtained, neither qualitative nor quantitative. In contrast to the HPLC method, the ELISA was prone to incomplete lysozyme extraction and matrix interferences during the immunological reactions, as the lysozyme interaction with other food proteins would undoubtedly also affect the antibody-antigen interaction and thus the ELISA response. This suggests that, before the quantification, a separation step should be performed, which is an advantage of the HPLC technique [29]. The commercially available lysozyme determination kits are using either antibodies as the

recognition element or *Micrococcus lysodeikticus* cells as the substrate for detecting the active lysozyme only; both assays can be applied to biological samples [30,31].

2.2. (Bio)sensors for lysozyme determination

A biosensor is defined as “a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological or biomimetic recognition element (biochemical receptor or bioreceptor) which is in direct spatial contact with a transducer”. According to the nature of the biorecognition process, biosensors can be classified in two main categories: catalytic biosensors, in which the bioreceptor is constituted by mono- or multi-enzymes, whole cells (i.e. bacteria, fungi or eukaryotic cells) or tissue slices, and affinity biosensors, in which the bioreceptor (e.g. antibodies, nucleic acids, molecularly imprinted polymers – MIPs, peptides and lectins) is capable to bind selectively and reversibly a specific target in order to obtain an useful analytical signal [32].

Different biomolecules (antibodies, oligonucleotide sequences and cells) and biomimetic molecules (e.g., MIPs) have been used as bioreceptors for lysozyme detection.

A photoelectrochemical immunosensor was developed by exploiting a sandwich format in which two antibody-antigen interactions take place; the first between the immobilized primary antibody and lysozyme, the second between lysozyme and the secondary antibody, captured on dopamine-grafted silica nanospheres. Upon acid etching, the grafted dopamine, a good electron donor, was being released and triggered hole-trapping, activating ZnIn_2S_4 -based heterojunctions, which generated the photoelectrochemical signal [33]. A direct determination immunoassay was also developed by immobilizing an anti-lysozyme antibody on gold nanorods and measuring the change in localized surface plasmon resonance (LSPR) signal after having quantified the antibody-antigen interaction [34]. Moreover, MIPs have also been developed and applied as the recognition element in an electrochemical sensor for lysozyme determination [35]. The bacteriolytic effect of lysozyme was also used for its quantification by immobilizing a *Micrococcus lysodeikticus* bacterial colony on a gold SPR chip modified with polycationic poly(diallyldimethyl-ammonium) and negatively charged graphene oxide (GO) [36]. The determination without using any receptor was obtained by SPR and LSPR with analytical performances comparable with the SPR aptasensor presented later in this review [37,38]. Polarized light microscopy was also used for the direct determination of lysozyme, and it can attract more attention for further studies [39]. The aforementioned approaches are summarized in Table 1.

2.3. Aptasensors

In recent years (2016–2020) aptamer-based sensors for allergens and, in particular, for lysozyme determination have attracted more and more attention, with an increasing number of published articles. An all-fields search in Scopus using the words “lysozyme” and “aptamer” generated 3461 result, from which 1799 only in the last 5 years [40] while a search for the terms “allergens” and “aptamer” generated 1266 results, 789 of them falling in the 2016–2020 time period [41].

Aptamers are composed of single-stranded RNA or DNA synthesized *in vitro* from a high number of random base sequences, using a procedure called Systematic Evolution of Ligands by EXponential enrichment (SELEX). Aptamers have been used as substituents for antibodies in biosensing due to their similar recognition properties and thanks to their advantages compared with those of antibodies. Aptamers have better properties like smaller size, 20–25 fold smaller than antibodies, conferring them a better access to biological compartments with great applications in sensors for diagnosis [42]. Other properties, like low immunogenicity, high chemical stability [43] and adaptive modification, make them more suitable as recognition elements in biosensing.

Table 1

Sensors and biosensors for lysozyme detection using different receptors and transducers.

	Platform	Determination Method	Bioreceptor	LOD (nM)	Linearity (nM)	Interferents	Real Samples	Ref.
1.	Ab ₁ /Lyz/Ab ₂ -DA-SiO ₂ NSs	PEC	Antibody	4.2*10 ⁻⁵	1.4*10 ⁻⁴ –35	Ala, BSA, Cys, DA, glucose, UA, urea	serum	[33]
2.	Ab/AuNRs	LSPR	Antibody	–	31.25–16*10 ³	–	–	[34]
3.	CuFe ₂ O ₄ -MIP/GCE	DPV	MIP	0.11	3.5–56	Cyt C, RNase A	urine	[35]
4.	<i>M. lysodeikticus</i> /GO/PDDA/Au chip	SPR	Whole cell	3.5	14–2.8*10 ³	human IgG, penicillin, streptomycin, Try	serum	[36]
5.	GA/AgNPs	LSPR	–	1.5	3–150	BSA, caffeine, urea, glucose, Met, nicotinic acid, Pro	egg white, milk	[37]
6.	(PMAA)NPs/Au chip	SPR	–	0.66	1–50	Hb, Mb	egg white	[38]
7.	Dodecyl β-D-glucopyranoside/LC	PLM	–	7	7–7*10 ³ *	–	–	[39]

Ab – antibody, AgNP – silver nanoparticle, Ala – alanine, AuNR – gold nanorod, BSA – bovine serum albumin, Cys – cysteine, Cyt C – cytochrome C, DA – dopamine, DPV – differential pulse voltammetry, GA – glutamic acid, GCE – glassy carbon electrode, GO – graphene oxide, Hb – hemoglobin, IgG – immunoglobulin G, LC – liquid crystal, LSPR – localized surface plasmon resonance, Lyz – lysozyme, Mb – myoglobin, Met – methionine, MIP – molecularly imprinted polymer, PDDA – poly(diallyldimethylammonium), PEC – photoelectrochemistry, PLM – polarized light microscopy, (PMAA)NP – poly (N-methacryloyl-(L)-phenylalanine) nanoparticle, Pro – proline, RNase A – ribonuclease A, SiO₂NS – silica nanosphere, SPR – surface plasmon resonance, Try – trypsin, UA – uric acid. *Tested concentrations.

Aptamers can be used in almost all immunosensors design with higher stability and better reusability compared with antibodies. Moreover, aptamers show a better flexibility and more types of biosensors can be designed thanks to their intra- and inter-molecular hybridization and exact sequence knowledge [44].

The aptamer sequences used in the discussed electrochemical and optical aptasensors for lysozyme determination are shown in Table 2.

2.3.1. Aptasensors methods

Aptamer immobilization onto a substrate represents the first step in aptasensors design. This step is important in order to assure a high reactivity, appropriate orientation, accessibility and stability of the surface-confined aptamer probe as well as for minimizing non-specific adsorption events. Therefore, the immobilization process plays the major role in determining the overall performance of an aptasensor. It can be performed using a variety of methods such as chemical or physical adsorption, physical entrapment within a membrane or gel, or covalent binding, depending on the assay format and detection principle.

The physical and chemical adsorptions (or chemisorption) on the solid substrate are the simplest and fastest immobilization approaches. However, the aptamer immobilization results in a random orientation, since the orientation of the binding sites is not controlled; moreover, the immobilization process depends on the pH, on the buffer composition or on temperature changes [48–50]. This leads to a decreased selectivity and sensitivity of the aptasensor. Nevertheless, there are examples of good immobilization methods based on chemisorption using noble metal surfaces as the solid substrate [51]. The most successful approaches for assuring high reactivity, proper orientation, and high stability of the surface-confined aptamer sequence are the covalent bond, the affinity reaction and the self-assembled layer using functionalized aptamer sequences with specific molecules or chemical groups (e.g. PEG, –SH, –NH₂, biotin, sugars, etc.) in various positions of the oligonucleotide sequence (2', 3' and 5') [52,53]. Generally, the 3'-end of the oligonucleotide sequences seems to be more appropriate, since it is the primary target for exonucleases, and, consequently, its coupling to the

solid support would simultaneously confer to the sensor some resistance to nucleases themselves [48].

Covalent immobilization is applied using electrode surfaces such as carbon, graphene, silicon or copper by electrografting of aryl diazonium salts [54].

The immobilization by avidin (or streptavidin) and a biotinylated aptamer is also considered a very valuable method. Firstly, avidin is covalently immobilized on the transducer surface (silica, magnetic beads, metal electrodes, quantum dots) and a biotinylated aptamer is then incubated over time [48,55].

Gold or other noble metal surfaces are frequently used as solid substrate because they have the advantage that thiolated aptamers can be immobilized by self-assembling, that guarantees suitable stability, surface coverage and the maintenance of the same binding affinity as that shown in solution. Moreover, the gold surface promotes the single-stranded DNA (ssDNA) binding by non-covalent bonds, such as electrostatic interaction and hydrophobic forces, especially with purine and pyrimidine rings of the DNA bases [53].

Recently, new materials, such as nanoparticles, modified polymers and hybrid nanomaterials have been employed for aptasensor development to perform aptamer immobilization [56].

After aptamer immobilization, a proper blocking step is usually performed in order to prevent the non-specific adsorption of interfering substances [52] before the affinity reaction between the aptamer and the target. The aptamer-analyte complex formation, together with its efficiency, is one of the most important steps when the analyte quantification using aptasensors is desired. It can be described as a process of molecular recognition realized by a specific interaction between the components. The affinity reaction between the aptamer and its target can be seen as a specific folding of the aptamer around a specific part of the target itself. There are many weak non-covalent interactions, like hydrogen bonding or hydrophobic effect, which confer selectivity and affinity for the recognition of the analyte. An important factor is the binding affinity that is constant in the same analytical conditions (pH, buffer, temperature etc.), and can be calculated through a variety of techniques; the aptamers with a higher affinity constant offer a better selectivity [57].

In order to transduce the biorecognition event, different assay approaches can be applied; depending on the properties of the target, they can be divided into labeled and label-free formats [58]. Labeling methods offer high sensitivity in case the analyte itself does not show a specific activity (e.g. electrochemical, optical, etc.) and thus could not be directly quantified. In this case, the aptamer is modified with an electrochemical or optical active compound (e.g. ferrocene, gold nanoparticles, methylene blue, fluorescent probes, etc.) that will be quantified and correlated with the concentration of the desired analyte [59]; labels also include enzymes (e.g. alkaline phosphatase, horseradish

Table 2

DNA aptamer sequences for lysozyme.

	Aptamer Sequence	K _d (nM)	Ref.
1.	5'-GGG AAT GGA TCC ACA TCT ACG AAT TCA TCA GGG CTA AAG AGT GC AGA GTT ACT TAG TTC ACT GCA GAC TTG ACG AAG CTT-3'	29	[45]
2.	5'-GCA GCT AAG CAG GCG GCT CAC AAA ACC ATT CGC ATG CGG C-3'	2.8	[46]
3.	5'-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3'	31	[47]

peroxidase, etc.), with the concentration of the enzymatic product being related to that of the analyte. Labeled assays include different assay formats, such as the sandwich (i.e. the target molecule is captured between two aptamers, of which the capture aptamer is anchored to the sensors surface, while the labeled one is used for detection), the displacement (i.e. the incubation with the target molecule causes the displacement of a complementary sequence, previously hybridized with the immobilized aptamer) and the switch-on/off (i.e. the binding with the target molecule induces a conformational change in the aptamer, decreasing/increasing the distance of the label molecule from the sensor surface). Label-free approaches rely on the modification of the interfacial properties of the aptasensor surface upon the binding between the aptamer and its target molecule. In this case, the analyte could be directly detected and quantified, by recording changes in its own electrochemical or optical activity or by using the signal of a redox probe, for electrochemical aptasensors [60]. The advantage of this approach is the real-time evaluation of the analyte-bioreceptor binding [61]. Both labeled and label-free strategies will be discussed for electrochemical and optical lysozyme aptasensors. A schematic representation of the aptasensors is presented in Fig. 2.

2.3.2. Electrochemical aptasensors

The most used electrochemical techniques as transduction mechanism in aptasensor design are cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and pulsed voltammetric techniques such as differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The advantages of electrochemical aptasensors include their low costs, the short time needed for analysis, the high selectivity and sensitivity, and the possibility of *in-situ* determination, making them a great tool in detecting biological compounds in a fast and sensitive way [62].

EIS represents a non-destructive electrochemical technique used to describe and quantify the properties of materials and substances by characterizing the changes occurred and the electrode/solution interface. Even though it is a simple and sensitive analytical method, its lack of selectivity reduces the number of the possible applications [63,64].

Among all the voltammetric techniques, CV is the most widely used, especially at the beginning of an electrochemical study, as it can rapidly detect the electrochemical redox characteristics of the studied species, providing important information about the thermodynamics of the redox process or the analysis of coupled electrochemical reactions or adsorption processes [65]. Compared to linear voltammetric techniques,

pulsed voltammetric techniques lead to a lowering of the detection limits by increasing the ratio between the faradaic and non-faradaic currents. DPV and SWV are extremely useful techniques for measuring trace levels of organic and inorganic species [66].

Different strategies have been applied in recent years in order to increase the sensitivity of the previous developed aptasensors and to decrease the limit of detection. The lowest limit of detection for lysozyme by means of EIS is of 1.9 fM and was achieved by Rezaei et al.: a glassy carbon electrode (GCE) was modified with multiwalled carbon nanotubes (MWCNTs), reduced graphene oxide (rGO), chitosan, and graphene quantum dots (GQDs) in order to increase its electrochemical active area and its conductivity. Glutaraldehyde was used as a linker, in order to make the carbonyl groups to react with an aptamer modified with an amino group at its 5'-end. Lysozyme was quantified by measuring the variation in the charge transfer resistance (ΔR_{ct}) at the electrode surface in the presence and absence of the analyte. Using the same platform, but DPV as the electrochemical technique, a LOD of 3.7 fM was achieved. Despite the lower LOD obtained and a good selectivity against some usual serum proteins, the sensor developed by Rezaei et al. shows as a disadvantage the impossibility of *in-situ* determination [67], which was overcome by the same group by using screen printed carbon electrodes (SPCE) and the same strategy based on rGO and glutaraldehyde, but replacing MWCNT and GQD with an ionic liquid and amino-mesoporous silica nanoparticles. In this way, a better selectivity in the presence of the same serum proteins was obtained with recovery rates in the range 94.6–106% but slightly higher LOD values (2.1 fM for EIS and 4 fM for DPV, respectively) were retrieved [68]. A schematic comparison of the two aforementioned platforms is reported in Fig. 3.

For the direct voltammetric determination of lysozyme, simple, fast and as-less-as-possible expensive approaches have been developed with almost the same principle of detection. GCE, SPCE or gold electrode surfaces were used as the transducer; different gold nanostructures, like gold nanoparticles (AuNPs) or gold nanocrystals, were employed in order to increase the electroactive surface and to improve the aptamer immobilization. The aptamer was usually immobilized by using chemisorption, S–Au covalent bond or by click chemistry. Different redox probes were used as signal generators: $[\text{Fe}(\text{CN})_6]^{3-/4-}$, methylene blue (MB) or $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$, with the same principle of detection employed in most of the published articles. The redox probe can be either already bound to the aptamer, having a labeled aptamer, or being bound to the aptamer in a separate step and released after the interaction between the analyte and the aptamer. This event resulted in a

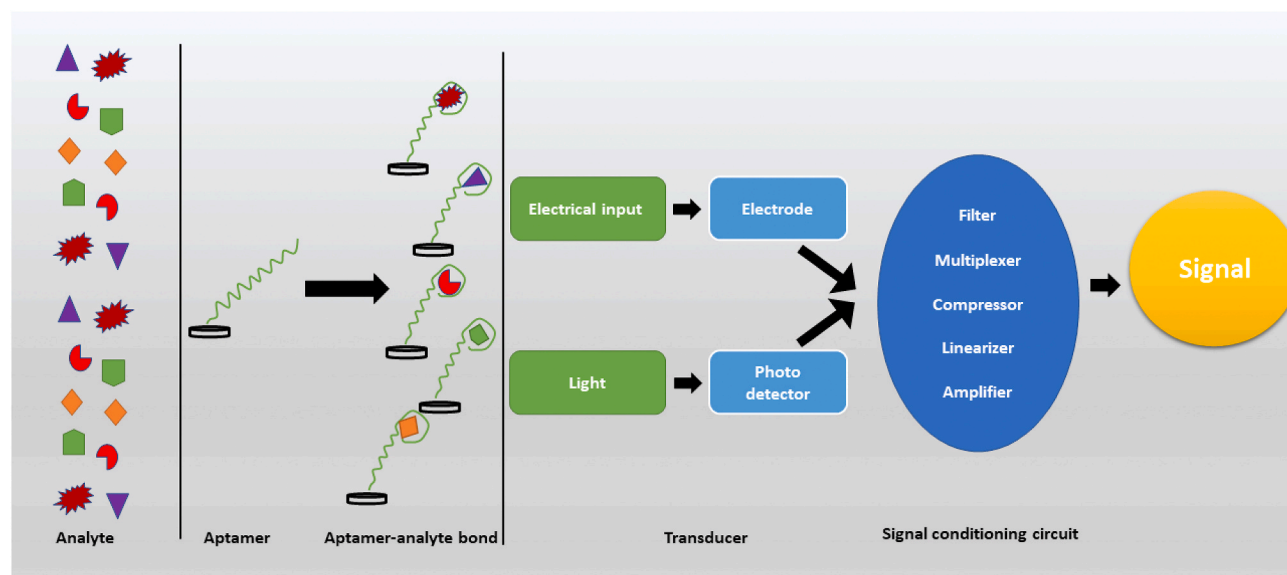


Fig. 2. Schematic representation of an aptasensor, adapted from A.A. Ensafi [54].

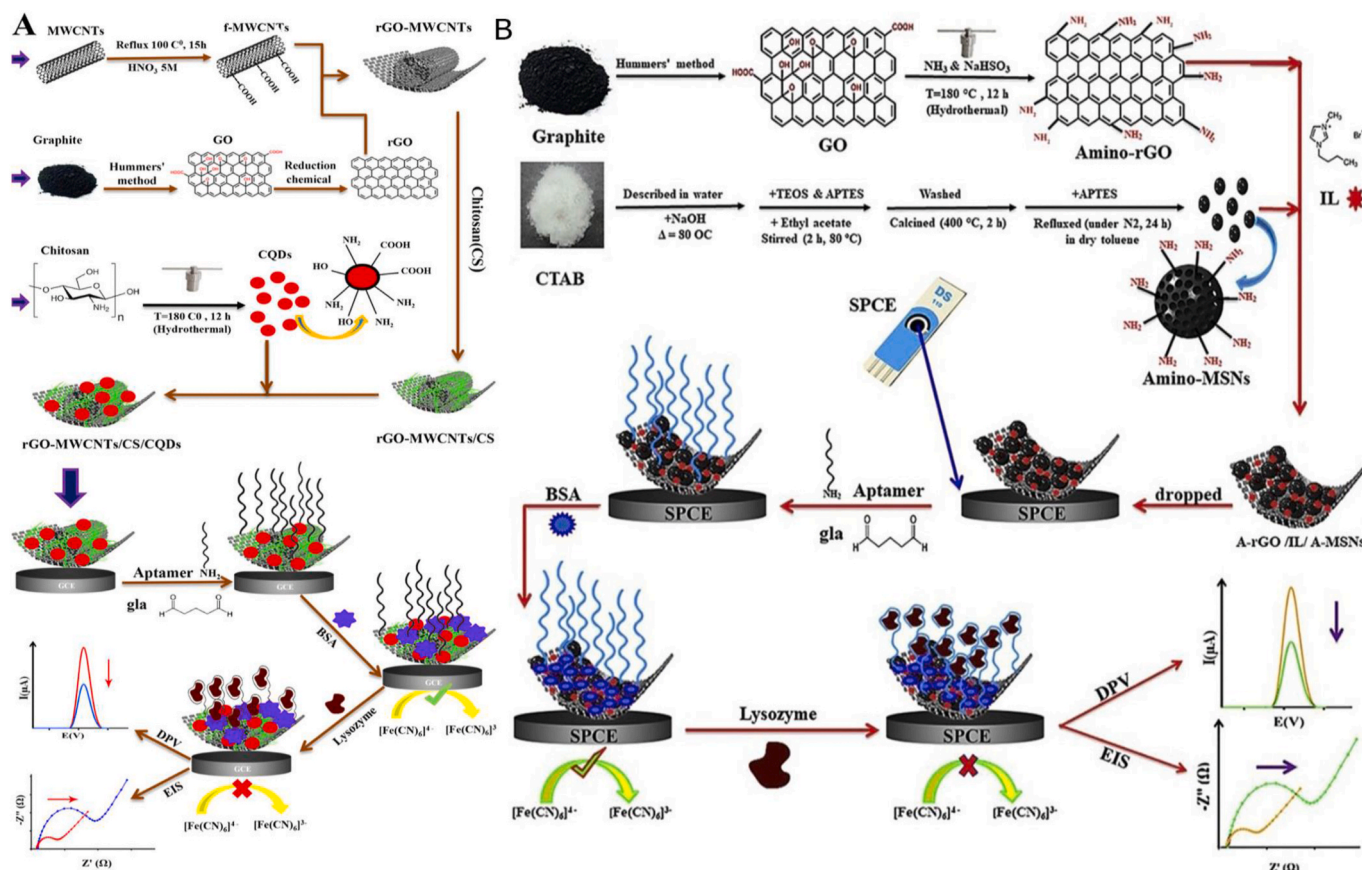


Fig. 3. A comparative representation of the two aptasensing platforms based on reduced graphene oxide developed by Rezaei research group. A) Platform based on multiwalled carbon nanotubes, chitosan and graphene quantum dots on a glassy carbon electrode; B) platform based on ionic liquid and amino-mesosilica nanoparticles on a screen-printed carbon electrode. Adapted from Refs. [67,68] with permission of Elsevier.

decrease of the signal, which was correlated with the increase of lysozyme concentration, thus obtaining a signal-off aptasensor. In all situations, the determination is performed using an electrochemical label, obtaining a labeled aptasensor [69–71]. A schematic representation of

the general approach for direct aptasensor development is reported in Fig. 4.

Regarding the indirect determination, most articles in the recent years present either a sandwich assay or a competitive assay. In the first

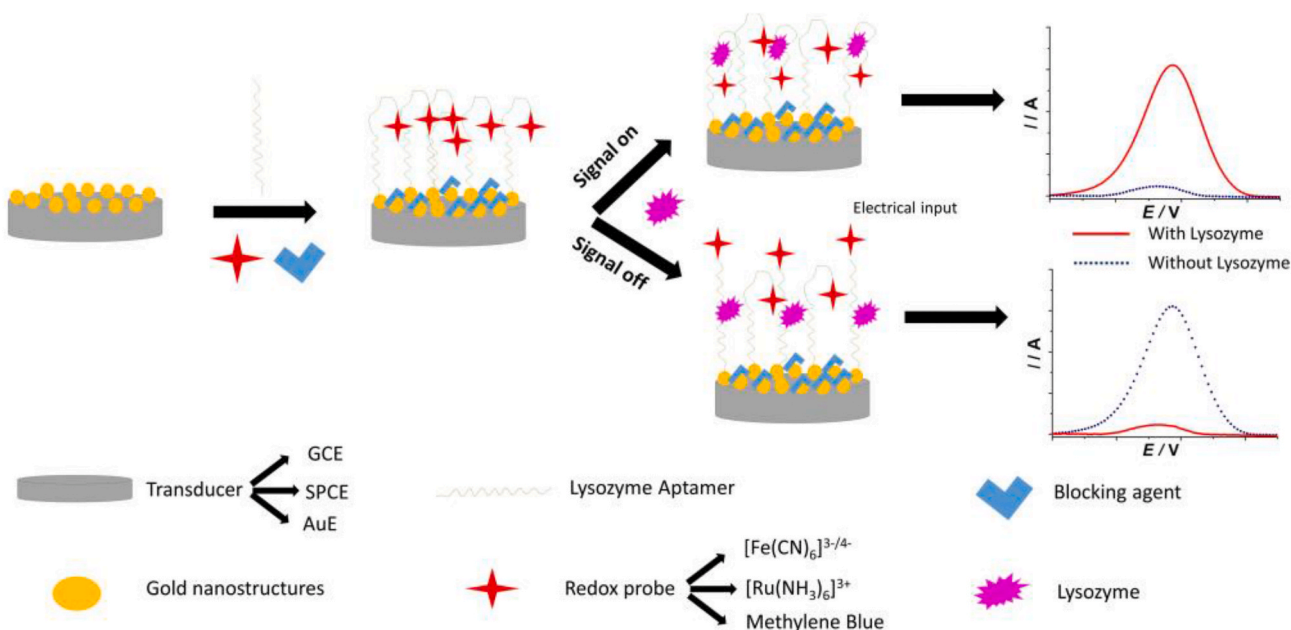


Fig. 4. Schematic representation of the reported electrochemical aptasensors for direct detection of lysozyme.

case, two aptamers or an aptamer-antibody couple can be used [72] for lysozyme binding; the immobilized element is generally used to ensure the binding of the target, while the other to help its quantitative determination [73]. In the second case, an aptamer is used to bind lysozyme and the redox probe, while a secondary DNA sequence is used to create a competition between the first aptamer and lysozyme. A stronger bond with the analyte is then created, thus breaking the first aptamer-lysozyme bond in order to make the redox probe available for producing a signal. Cao et al. developed an aptasensor exploiting three DNA sequences: an aptamer for lysozyme, a first sequence complementary to the aptamer (DNA-A) and a second sequence complementary to DNA-A (DNA-B). Gold nanoparticles were modified with DNA-A, to which the aptamer molecules were bound in absence of lysozyme, while a gold electrode was modified with DNA-B. In presence of lysozyme, the aptamer was released from gold nanoparticles, which were then captured by hybridization between DNA-A and DNA-B and accumulated onto the electrode surface. The AuNPs-DNA sensing units were able to catalyze the reaction of H_2O_2 and HQ, generating the electroactive benzoquinone [74]. Different enzymatic couples are used as the electrochemical signal generator in indirect assays, like alkaline phosphatase/1-naphthyl phosphate couple [72], the oxidation of hydroquinone by hydrogen peroxide [74] or even a double redox probe as $[Ru(NH_3)_6]^{3+}$ /ferrocene, which can be used to increase the limit of detection and the sensitivity of the sensor [75].

An interesting voltammetric approach was presented by Chen and his group that used a double stranded DNA, containing a lysozyme binding aptamer and its complementary DNA, with ethidium bromide (EB) molecules, used for photosensitization, embedded between the dsDNA. After lysozyme recognition by the aptamer, the dsDNA structure was broken, EB was released, and the photosensitization process was turned off proportionally with lysozyme concentration in the 10^{-10} – 10^{-6} pM

range [75].

CV has been used mainly for platform characterization and in a less extensive way for the actual quantification of lysozyme. Nevertheless, in a study conducted by Titou et al. a simple and disposable aptasensor for direct lysozyme detection and quantification using CV was developed. By electrodepositing AuNPs on the surface of a SPCE, the electroactive surface area was increased and the chemisorption of a thiol-modified aptamer was carried out. The formation of the aptamer-lysozyme complex led to steric hindrances and diffusion barriers for the charge transfer of $[Fe(CN)_6]^{3-/4-}$ redox probe at the electrode surface, resulting in a decrease of the anodic peak current, which was taken as the analytical signal and correlated with the amount of lysozyme. A LOD of 23 nM and a linear range between 70 nM and 700 nM were obtained [76]. The developed CV aptasensor has the advantage of a simple and fast approach and the possibility for *in-situ* determination, but the analytical performance was slightly inferior when compared to other electrochemical aptasensors developed in recent years. An example of a photoelectrochemical aptasensor, combining an optical input with an electrochemical output, was also developed by immobilizing the aptamer on a fluorine doped tin oxide substrate; the signal before and after the aptamer-target interaction was measured in order to quantify lysozyme [77]. Electrochemical aptasensors have been applied to detect lysozyme in a variety of real samples, ranging from wine samples to egg. Moreover, some of them were also applied in spiked biological samples analysis, representing promising approaches for further development in order to detect lysozyme directly from patients' serum, urine, or tears. The discussed electrochemical aptasensors are summarized in Table 3.

2.3.3. Optical aptasensors

Determination by means of optical biosensors is a very wide field, including many examples of determination methods based on

Table 3
Electrochemical aptasensors for lysozyme detection.

	Electrode Modification	Determination Method	LOD (pM)	Linearity (pM)	Interferents	Real Samples	Ref.
1.	LBA/CHIT-GO/PGE	EIS	28.5×10^{-3}	–	BSA, Thr	–	[78]
2.	LBA/rGO-AuNP/CHIT-AuNP/MWCNT-AuNP/GCE	EIS	9×10^{-3}	2×10^{-2} –200	–	urine, saliva	[79]
3.	LBA/CNT/SPCE	EIS	6.3	17.5 – 3.5×10^5	BSA, Thr	–	[47]
4.	LBA/diazonium salt/PGE	EIS	1.67×10^6	1.67×10^6 – 5×10^6	BSA, Cyt C	wine	[80]
5.	LBA/ NH_2 -GO/IL/ NH_2 -MSN/GLA/SPCE	EIS	2.1×10^{-3}	10^{-2} – 2×10^5	BSA, IgG, PSA	egg white, serum, tears, wine	[68]
6.	LBA/GO/MWCNT/CHIT-GQD/GLA/GCE	DPV	4×10^{-3}	2×10^{-2} – 5×10^4	BSA, IgG, PSA	egg white, serum, urine, wine	[67]
7.	AuNP/ErGO/GCE	EIS	3.7×10^{-3}	2×10^{-2} – 10^4	–	serum	[81]
8.	LBA1/Lyz/LBA2/diazonium salt/GCE	DPV	0.06	1–104.3	BSA, casein, Cyt C	wine	[82]
9.	OligoB/LBA-Lyz/OligoA-AuNP/AuE	DPV	0.24	9.6–205.5	BSA, BHB, IgG	serum	[74]
10.	HRP-AuNP-LBA/AuE	DPV	4.3×10^{-3}	5×10^{-3} – 5×10^3	BSA, Cyt C, glucose, Thr	serum	[73]
11.	LBA/ Cu_2O /rGO/PpPG/AuE	DPV	60	10^2 – 2×10^5	BSA, casein, Cyt C, Hb, IgG, Thr,	plasma, saliva, urine	[83]
12.	dsDNA/AuNPs-CNPE	DPV	2	10 – 10^6	BSA, Mb, Thr	urine	[75]
13.	LBA/diazonium salt/AuNPs/SPCE	SWV	0.021	0.07–3.5	BSA, cocaine, Thr	egg white	[69]
14.	LBA/AuNC/GCE	SWV	0.1	0.1 – 10^4	BSA, Thr	egg white, serum	[70]
15.	MB/LBA/AuE	SWV	10^{-4}	10^{-4} – 10^2	avidin, BSA, HER2, HSA, IgE, IgG, Thr	serum	[71]
16.	Fc-LBA-RuHEX/AuE	SWV	0.8	10 – 10^5	BSA, Hb, Thr	serum	[84]
17.	LBA/AuNP/SPAuE	CV	2.3×10^4	7×10^4 – 7×10^5	BSA, casein, Cyt C, NA, K^+	wine	[76]
18.	LBA/ VS_2 QDs- Bi_2S_3 /FTO	PEC	1.1×10^{-3}	3.5×10^{-3} –70	ALP, BSA, CIP, GOD, HRP, Try	egg white, serum	[77]
19.	LBA/Au/ Fe_2O_3 /FTO	PEC	3×10^{-3}	10^{-2} – 10^2	BSA, His, Phe, Pro, Try	egg white, serum	[85]

ALP – alkaline phosphatase, AuE – gold electrode, AuNC – gold nanocluster, AuNP – gold nanoparticle, BHB – bovine serum hemoglobin, BSA – bovine serum albumin, CHIT – chitosan, CIP – calf intestinal alkaline phosphatase, CNPE – carbon nanotube paste electrode, CNT – carbon nanotube, Cyt C – cytochrome C, CV – cyclic voltammetry, DPV – differential pulse voltammetry, dsDNA – double stranded DNA, EIS – electrochemical impedance spectroscopy, ErGO – electrochemically reduced graphene oxide, Fc – ferrocene, FTO – fluorine-doped tin oxide, GCE – glassy carbon electrode, GLA – glutaraldehyde, GO – graphene oxide, GOD – glucose oxidase, GQD – graphene quantum dot, Hb – hemoglobin, HER2 – human epidermal growth factor 2, His – histidine, HRP – horseradish peroxidase, HSA – human serum albumin, IgE – immunoglobulin E, IgG – immunoglobulin G, IL – ionic liquid, LBA – lysozyme-binding aptamer, Lyz – lysozyme, MB – methylene blue, Mb – myoglobin, MSN – mesosilica nanoparticles, MWCNT – multiwalled carbon nanotube, NA – neutravidin, Oligo – oligonucleotide, PEC – photoelectrochemistry, PGE – pencil graphite electrode, Phe – phenylalanine, PpPG – plasma-polymerized propargylamine, Pro – proline, PSA – prostatic specific antigen, QD – quantum dot, rGO – reduced graphene oxide, RuHEX – $[Ru(NH_3)_6]^{3+}$, SPAuE – screen printed gold electrode, SPCE – screen-printed carbon electrode, SWV – square wave voltammetry, Thr – thrombin, Try – trypsin.

fluorescence, absorption, refraction or dispersion of light. During spectroscopic experiments, properties like polarization, amplitude, energy and phase can be exploited [86].

A technique that combines both the electrochemical and the optical approaches is electrochemiluminescence (ECL). While chemiluminescence (CL) determination is based on the generation of photons after a chemical reaction, CL aptasensors are using as photons generator the aptamer-analyte interaction and usually a signal enhancer to decrease the limit of detection [87]. The difference in the ECL case is that the photons generation is the result of an electrochemical reaction [88]. Another optical method widely used in biosensing is fluorescence, as it offers good selectivity and sensitivity by monitoring the frequency change of electromagnetic radiation, due to radiation absorption and generation of an excited state. In this way, a bright signal can be easily measured even at single-cell level [89]. A particular type of fluorescent technique is fluorescence resonance energy transfer (FRET). In this case, after a donor fluorophore is excited by incident light, an energy transfer occurs towards an acceptor molecule, which is in close distance with respect to the donor; this transfer leads to a reduction of the donor's fluorescence intensity and of the excited state lifetime [90]. An optical biosensing method offering the advantage of naked-eye detection is the colorimetric assay. This assay does not require expensive materials or instrumentation; moreover, point-of-care devices for *in-situ* determination are easy to be developed, sometimes even by exploiting the technology offered by a smartphone camera [91].

ECL is a very developed field with many different possible strategies to be applied for achieving the same goal. Even in lysozyme determination, all four ECL aptasensors presented in this review use different detection strategies, nevertheless with some similarities, as their development focuses on obtaining a surface with a high active area and good affinity for aptamer immobilization: this includes the use of nanomaterials like AuNPs combined with different graphite-based nanostructures (QDs, GO) and a signal generator or amplifier like Ru, Si or luminol [92–95]. In terms of analytical performances, the reported

ECL aptasensors have a wide linear range, mostly because of the representation of the signal as a function of logarithm of lysozyme concentration. The lowest LOD (0.41 fM) was achieved by Dong et al. using a competitive assay in which a luminol-modified complementary DNA sequence interacted by hybridization with a thiolated aptamer, immobilized on the surface of a gold electrode. When the sensor was incubated with lysozyme, the specific interaction between the enzyme and its aptamer released the luminol-modified DNA from the electrode surface, resulting in a decrease of ECL signal, which was proportional to lysozyme concentration at a logarithmic scale (Fig. 5) [93].

Compared to ECL aptasensors, the CL aptasensors are not well developed and presented in literature. Nevertheless, the principle is almost similar, and the analytical performances are close to those of the ECL aptasensors [96]. A different approach was used by Giuffrida et al., who designed a digital microfluidic-based CL aptasensor that could detect lysozyme into droplets of 20 nL in volume, using a flow system and sample volumes as low as 1 μ L with a detection time of just 10 min [97].

Fluorescence sensors make use of multiple aptamer immobilization techniques and lysozyme detection strategies. Different QDs (Mn–ZnS, CdTe–CdSe, CdTe) have been used for both aptamer binding and signal generation. Usually at the beginning, the aptamer is forming a bound with the QDs; after the interaction with lysozyme, the aptamer is released and the fluorescent signal, generated by the QD, is enhanced [96–98]. AuNPs and AgNPs have also been used for their fluorescent enhancement effect as well as their good reaction with thiol-modified lysozyme binding aptamers [98–100]. Chen et al. presented an original approach, as a double switch aptasensor for the determination of both lysozyme and ATP was developed. By using a lysozyme binding aptamer, an ATP binding aptamer and a DNA containing complementary regions for both these two aptamers, a hybridized dsDNA is obtained. The dsDNA is then modified with methyl violet (MV), captured between its two strands. After the interaction of the analytes with their specific aptamers, by a competition assay, the dsDNA is opening and MV

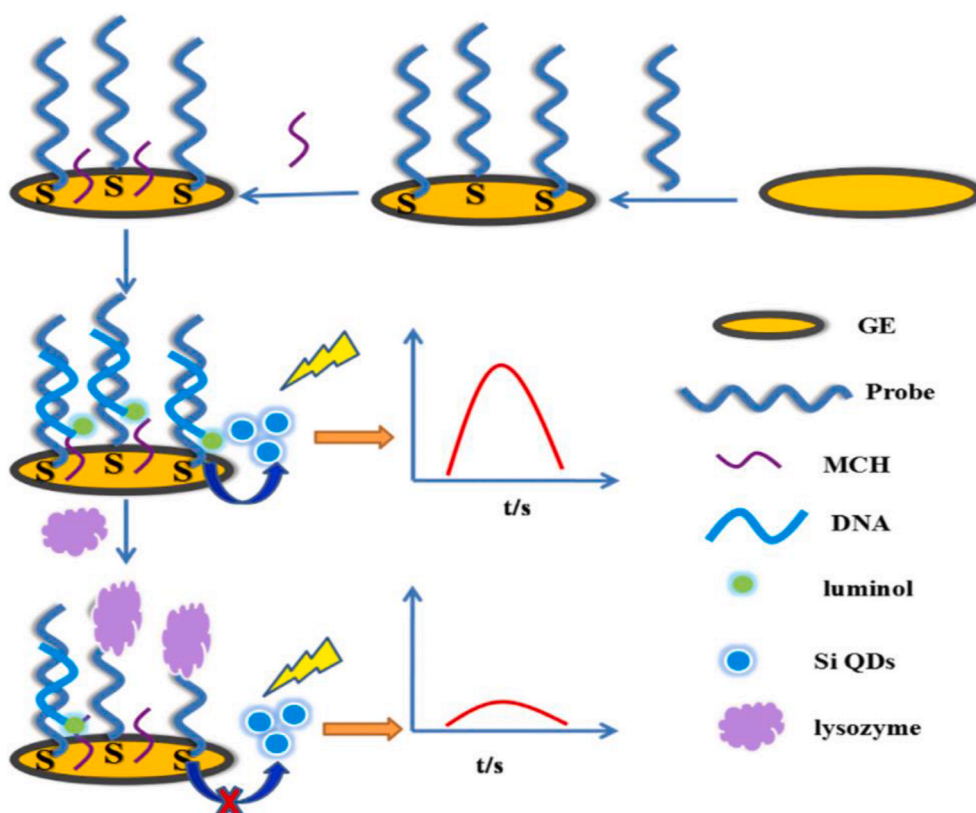


Fig. 5. Schematic representation of a classical ECL aptasensor for lysozyme detection [91] with permission of Elsevier.

is released, amplifying the signal [101].

In FRET aptasensing, different strategies have been applied for lysozyme determination, but the main characteristics are similar to those of the fluorescence sensors. A signal-on, in flow, aptasensor was developed by Sapkota and Dhakal by using just a Cy3 fluorophore-modified aptamer, bound to the flow cell by biotin-streptavidin interaction. When the aptamer-lysozyme coupling takes place, the aptamer suffers a conformational change and the fluorophore goes from a low-FRET to a high-FRET state. Even though the aptasensor had the advantage of flow determination, the LOD of 30 nM is not suitable for real samples analysis [102]. Better analytical performances have been achieved by using nanomaterials such as carbon dots, GO or nanocrystals, as signal generator and enhancer, reaching LODs down to the picomolar range [103,104].

Regarding the colorimetric assays, AuNPs are widely used in this kind of aptasensors development thanks to their optical properties and their simple and efficient mechanism in producing a highly sensitive response [105]. Therefore, in the last five years, most of the colorimetric aptasensors for lysozyme determination made use of AuNPs both for aptamer immobilization and exploiting their optical properties for lysozyme quantification. In the simplest assay format, aptamers with a thiol-ending group are covalently immobilized onto the AuNPs surface and the color shift is measured after the interaction with the analyte. A signal-off aptasensor was developed by Xie et al. with a low limit of detection (54 pM) and a good sensitivity, despite the narrow linear range (0.35–2.4 nM) [106].

The challenge in the AuNPs based colorimetric approach is represented by the aptamer immobilization strategy. Different approaches have been used: Cao et al. previously immobilized a complementary

oligonucleotide strand that worked as a substrate for the binding of the aptamer [74], while Wai's approach used cysteamine to change the AuNPs charge and to increase the aptamer adsorption [107]; in both cases, a wider linear range was obtained.

Enzymatic indirect determination was also studied in recent years. Lou's approach used aptamer-modified bimetallic Cu@AuNPs responsive to iodide, as well as the iodide-catalyzed H_2O_2 /TMB (3,3',5,5'-tetramethylbenzidine) reaction system. In presence of lysozyme, its interaction with the aptamer hindered iodide adsorption on the NPs, causing a higher amount of iodide ions to be available in solution. These ions catalyzed the oxidation of TMB by H_2O_2 , resulting in a change of the solution color [108]. Another enzymatic approach made use of biotin-modified aptamers and the alkaline phosphatase/1-naphthyl phosphate enzymatic couple as the signal generator [109]. The discussed optical aptasensors are listed in Table 4.

2.3.4. Comparison between electrochemical and optical approaches

Different determination methods offer different characteristic to be exploited in sensors development. The most important in the biomedical field is the sensitivity of the sensor; comparing the best LODs obtained by using the different determination methods described above, a good comparison of methods sensitivity can be done.

The electrochemical methods present in general the lowest LOD values, in the femtomolar range, which can be achieved only by using ECL and CL approaches among all the optical techniques, that is, those being actually related with the electrochemical ones. The best limit of detection achieved using SWV is 0.1 fM, obtained through a very simple approach and a direct determination using MB as a redox indicator [71]. Among optical detection methods, ECL and CL offer the possibility of

Table 4
Optical aptasensor for lysozyme detection.

	Platform	Determination Method	LOD (nM)	Linearity (nM)	Interferents	Real Samples	Ref.
1.	RuSiNP/PLL-ssDNA-Au/LBA/AuNPs/3Dgraphene	ECL	7.5×10^{-7}	23×10^{-7} –0.05	BSA, casein, Cyt C, IgG, Thr	wine	[92]
2.	Luminol-ssDNA/LBA-SiQD/AuE	ECL	4.1×10^{-7}	35×10^{-7} –0.35	BSA, HRP, Thr	serum	[93]
3.	LBA/AuNPs/N-GQD/ITO	ECL	8×10^{-7}	10^{-6} –10	Hb, HSA, IgG, Thr	serum	[110]
4.	LBA/Ru/PAA-PAM/HOPG	ECL	7×10^{-3}	0.035–70	BSA, Thr	–	[95]
5.	DNAzyme/LBA/GQDs/GO/CF	CL	8.7×10^{-7}	1.8×10^{-7} –4.6 $\times 10^{-4}$	–	urine	[96]
6.	LBA/AuNPs	CL	4.5×10^{-5}	10^{-5} – 5×10^{-4}	–	serum	[97]
7.	LBA/(Mn–Zn)QD	Fluorescence	0.54	5.5–44.5	BSA, pepsin, Thr, Try	serum, urine	[111]
8.	CdTe–CdSeQD/Hemin–G-quadruplex/DNAenzyme	Fluorescence	2.6	5–500	BSA, Thr,	serum	[112]
9.	LBA-cDNA–AgNC	Fluorescence	5.6	2–500	–	–	[98]
10.	LBA/GO	Fluorescence	6.8×10^{-3}	0.025–0.18	BSA, Cyt C, Hb, IFN- γ , Mb, NF- κ B	saliva, serum	[99]
11.	CdTeQD/Lyz–LBA/AuNPs	Fluorescence	0.55	1–20	BSA, Cys, IgG, Thr	saliva, tears	[100]
12.	(ATPBA + LBA)-dsDNA	Fluorescence	9.3×10^{-3}	5–100	elastase, ovalbumin, papaine, Thr	serum	[101]
13.	ThNI/Lyz/LBA/AuNPs	Fluorescence	10	1–25	BSA, Hb	saliva	[113]
14.	LBA/streptavidin modified	FRET	30	30–2000	BSA, Cyt C, glucose	–	[102]
15.	CD-LBA/GO	FRET	0.07	0.7–140	Ala, BSA, Cys, glucose, UA, urea	serum	[103]
16.	LBA/lanthanide–NC–scintillators	FRET	0.94	3–200	AA, BSA, glucose, HSA, Thr	serum	[104]
17.	LBA/Oligo-A/AuNP	Colorimetric	0.9	5–500	–	–	[74]
18.	LBA/AuNPs	Colorimetric	0.054	0.35–2.4	BSA, GOx, HSA, pepsin	–	[106]
19.	LBA/CysAuNPs	Colorimetric	2.3	37.5–180	BSA, Cyt C, insulin, VEGF	–	[107]
20.	LBA/Lyz/COOH-MBs	Colorimetric	10	5–140	BSA, casein, Cyt C	wine	[114]
21.	IO^{-2} /LBA/Cu@Au core shell	Colorimetric	60	100 – 10^5	BSA, Hb, HRP, Mb, pepsin, Thr	egg white	[108]
22.	LBA/Au chip	SPR	500	10^3 – 10^5	albumin, avidin, BSA, Thr	serum	[115]

AA – ascorbic acid, AgNC – silver nanocluster, Ala – alanine, ATPBA – ATP-binding aptamer, AuE – gold electrode, AuNP – gold nanoparticle, BSA – bovine serum albumin, CD – carbon dot, cDNA – circular DNA, CF – carbon fiber, CL – chemiluminescence, Cys – cysteine, Cyt C – cytochrome C, dsDNA – double-stranded DNA, ECL – electrochemiluminescence, FRET – fluorescence resonance energy transfer, GO – graphene oxide, GOx – glucose oxidase, GQD – graphene quantum dot, Hb – hemoglobin, HOPG – highly oriented pyrolytic graphite electrode, HRP – horseradish peroxidase, HSA – human serum albumin, IFN- γ – interferon- γ , IgG – immunoglobulin G, ITO – indium tin oxide, LBA – lysozyme-binding aptamer, Lyz – lysozyme, MB – magnetic bead, Mb – myoglobin, NC – nanocrystal, N-GQD – nitrogen-doped graphene quantum dot, NF- κ B – recombinant human nuclear factor- κ B, Oligo – oligonucleotide, PAA – polyacrylic acid, PAM – polyamidoic acid, QD – quantum dot, RuSiNP/PLL – Ru(bpy) $_3^{2+}$ -silica@poly-L-lysine, SPR – surface plasmon resonance, ssDNA – single-stranded DNA, ThNI – fluorescent conjugated polymer brush, Thr – thrombin, Try – trypsin, UA – uric acid, VEGF – vascular endothelial growth factor.

much lower LOD than the fluorescence, FRET or UV–Vis methods, that cannot provide LOD in femtomolar order of magnitude, but can go as low as the picomolar one for lysozyme. In conclusion, strictly from the sensitivity point of view, the electrochemical methods represent a better choice for the quantification of trace amounts of lysozyme from biological, food or environmental samples (Table 5).

2.3.5. Real samples applications

In literature, there are several examples of lysozyme aptasensors applied to real-world samples either in biological fluids like serum, saliva, tears or urine [83] or in food samples like egg white [69] or wine. Nevertheless, the aptasensor signal is often negatively affected from matrix effect, non-specific binding and interferences such that qualitative or quantitative measurements are not possible. Therefore, important studies must be devoted in the aptasensor design so that they can be applied in real samples. However, the most used approach is the sample dilution with appropriate buffer at a level where the influence of the interferents is minimum. The main disadvantage of this approach is that the sample dilution, by lowering the final lysozyme concentration, requires a very sensitive aptasensor. In the case of biological samples, serum samples were diluted in the range between 20 and 9000 times [68,81] for the electrochemical aptasensors and in the range 0–100 times for the optical ones [97,101]. Urine samples were analyzed after a dilution of 5–100 times by the electrochemical aptasensors [67,79] and 10^6 times for the optical ones [96]. Regarding the analytical performances in biological fluids, lysozyme was determined in serum down to 4 nM with recoveries in the range 95–100% for the electrochemical aptasensors [68] and 1 pM with 91% recovery using optical transducers [110]. Lysozyme determination from urine yielded a 0.52 nM LOD using electrochemical methods [67] and 90 μ M by optical ones with recoveries in the range 97.5–103.2% [96]. Heydari-Bafrooei et al. compared their electrochemical method with a standard HPLC method. Lysozyme was electrochemically determined down to 0.17 μ M, compared with a 0.15 μ M found in HPLC [79]. Shamsipur et al. compared their method of lysozyme detection in serum with an ELISA kit, retrieving a LOD of 350 μ M lysozyme in diluted serum while the ELISA kit was able to determine 325 μ M lysozyme [81]. A fluorescent aptasensor developed by Qiu et al. was also applied in serum samples and its performance was compared with the results obtained by using an ELISA kit. Standard addition method was used, and concentrations of lysozyme in the range 50–320 nM were obtained in the final solutions. The results showed a recovery in the 85–112% range [112].

Some sensors were also applied in wine industry, mostly in spiked samples, in the majority of cases the amount of lysozyme present could not be determined with the aptasensor [67,68], but acceptable recoveries were obtained (77–102%), even though most of the sensors reported less amount of lysozyme than that added, which might be due to some matrix interferences [67,80]. One interesting application of lysozyme detection was described by Titoiu et al., that monitored the amount of lysozyme in wine during the winemaking for a period of 7 months and compared the results with a HPLC method. The results proved the utility of the aptasensor based on the parallel analysis of a set of 8 samples, as the two methods correlate very well, with a linearity coefficient of 0.992 and a slope very close to 1 [76].

3. Conclusion and perspectives

This review focused on the electrochemical and optical aptasensors developed between 2016 and 2020, and the advances that have been made in these years. For the electrochemical aptasensors, one of the main goals was to improve the analytical performances, such as an increase of the electroactive area and the conductivity, and to facilitate the aptamer immobilization, done by using mainly hybrid nanocomposites obtained by modifying the electrodes with combinations of different nanomaterials like AuNPs or graphene oxide. The other important goal was to create devices for *in-situ* determination in real samples that could

Table 5

Sensitivity of the electrochemical and optical detection methods for lysozyme aptasensors.

Determination Method	LOD (fM)	Reference
EIS	1.9	[67]
DPV	0.2	[73]
SWV	0.1	[71]
ECL	0.41	[93]
CL	0.87	[96]
Fluorescence	6.8×10^3	[99]
FRET	7×10^4	[103]
Colorimetric	5.4×10^4	[106]

CL – chemiluminescence, DPV – differential pulse voltammetry, ECL – electrochemiluminescence, EIS – electrochemical impedance spectroscopy, FRET – fluorescence resonance energy transfer, SWV – square wave voltammetry.

be further used for mass production.

Despite the development of several types of aptasensors for lysozyme, their application in biological samples is yet difficult; in fact, the concentration of the analyte might be low in real samples, and it can become even lower when taking into consideration the dilution steps needed to decrease the effect of other biological compounds. Therefore, even though some aptasensors can reach LOD values as low as the femtomolar range, the selectivity has to be the highest possible in order to decrease the chances of a false positive result [116].

The main goal in sensors development is to achieve the construction of a device that could be bought and sold in the market, and this is still one of the main challenges and perspectives for lysozyme aptasensors. The studies presented in this review report the sensitivity needed for a commercial aptasensor, but the problem of specificity in complex matrices still has to be resolved for industrial development. Therefore, with the miniaturization and development of the optical and electrochemical techniques, commercially aptasensors for *in-situ* determination of lysozyme from food or biological samples can be obtained and this can be an important element in the challenge of offering a safer environment for lysozyme sensitive patients. Nowadays the research on lysozyme is more and more focusing on the discovery of other roles of this enzyme by modifying its monomeric structure. In fact, by obtaining lysozyme oligomers, the preservation role of the enzyme increases; preliminary studies show that it might have also a role in meat preservation. Moreover, recent studies show that lysozyme might be used in the polymers industry for intelligent packaging development, being a key component in the preservation of packed food [117]. Other important uses are still found for lysozyme in industries like food, agriculture, winery or pharmaceutical, and without any doubt its importance will become even higher in the near future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by Italian Government in the framework of PRIN 2017, Prot. 2017YER72 K_005. Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, PN-III-P1-1.2-PCCDI2017-0221 (INTELMAT) within PNCDI III. This paper was published under the frame of European Social Fund, Human Capital Operational Programme 2014–2020, project no. POCU/380/6/13/125171.

References

- [1] T. Wu, Q. Jiang, D. Wu, Y. Hu, S. Chen, T. Ding, X. Ye, D. Liu, J. Chen, What is new in lysozyme research and its application in food industry? A review, *Food Chem.* 274 (2019) 698–709, <https://doi.org/10.1016/j.foodchem.2018.09.017>.
- [2] A.M. Hamdani, I.A. Wani, N.A. Bhat, R.A. Siddiqi, Effect of guar gum conjugation on functional, antioxidant and antimicrobial activity of egg white lysozyme, *Food Chem.* 240 (2018) 1201–1209, <https://doi.org/10.1016/j.foodchem.2017.08.060>.
- [3] L. Callewaert, C.W. Michiels, Lysozymes in the animal kingdom, *J. Biosci.* 35 (2010) 127–160, <https://doi.org/10.1007/s12038-010-0015-5>.
- [4] D. Cao, H. Wu, Q. Li, Y. Sun, T. Liu, J. Fei, Y. Zhao, S. Wu, X. Hu, N. Li, Expression of recombinant human lysozyme in egg whites of transgenic hens, *PLoS One* 10 (2015) 1–15, <https://doi.org/10.1371/journal.pone.0118626>.
- [5] C. Corsaro, D. Mallamace, A Nuclear Magnetic Resonance study of the reversible denaturation of hydrated lysozyme, *Phys. A Stat. Mech. Its Appl.* 390 (2011) 2904–2908, <https://doi.org/10.1016/j.physa.2011.03.038>.
- [6] T. Ding, A.P.J. Middelberg, T. Huber, R.J. Falconer, Far-infrared spectroscopy analysis of linear and cyclic peptides, and lysozyme, *Vib. Spectrosc.* 61 (2012) 144–150, <https://doi.org/10.1016/j.vibspec.2012.02.020>.
- [7] On a remarkable bacteriolytic element found in tissues and secretions, *Proc. R. Soc. Lond. - Ser. B Contain. Pap. a Biol. Character* 93 (1922) 306–317, <https://doi.org/10.1098/rspb.1922.0023>.
- [8] B. Porstmann, K. Jung, H. Schmechta, U. Evers, M. Pergande, T. Porstmann, H. J. Kramm, H. Krause, Measurement of lysozyme in human body fluids: comparison of various enzyme immunoassay techniques and their diagnostic application, *Clin. Biochem.* 22 (1989) 349–355, [https://doi.org/10.1016/S0009-9120\(89\)80031-1](https://doi.org/10.1016/S0009-9120(89)80031-1).
- [9] Immundiagnostik, Lysozyme ELISA for the in Vitro Determination of Lysozyme in Serum, Urine and Lique, 2019. http://www.immundiagnostik.com/fileadmin/pdf/LYSOZYM_Serum_Urin_Liq_1P_K6903.pdf. (Accessed 15 March 2020).
- [10] B. Lönnardal, Biochemistry and physiological function of human milk proteins, *Am. J. Clin. Nutr.* 42 (1985) 1299–1317, <https://doi.org/10.1093/ajcn/42.6.1299>.
- [11] J. Hankiewicz, E. Swierczek, Lysozyme in human body fluids, *Clin. Chim. Acta* 57 (1974) 205–209, [https://doi.org/10.1016/0009-8981\(74\)90398-2](https://doi.org/10.1016/0009-8981(74)90398-2).
- [12] P. Venge, T. Foucard, J. Henriksen, L. Håkansson, A. Kreuger, Serum-levels of lactoferrin, lysozyme and myeloperoxidase in normal, infection-prone and leukemic children, *Clin. Chim. Acta* 136 (1984) 121–130, [https://doi.org/10.1016/0009-8981\(84\)90283-3](https://doi.org/10.1016/0009-8981(84)90283-3).
- [13] M.H. Grieco, M.M. Reddy, H.B. Kothari, M. Lange, E. Buimovici-Klein, D. William, Elevated β 2-microglobulin and lysozyme levels in patients with acquired immune deficiency syndrome, *Clin. Immunol. Immunopathol.* 32 (1984) 174–184, [https://doi.org/10.1016/0090-1229\(84\)90119-3](https://doi.org/10.1016/0090-1229(84)90119-3).
- [14] P.E. Perillie, K. Khan, S.C. Finch, Serum lysozyme in pulmonary tuberculosis, *Am. J. Med. Sci.* 265 (1973) 297–302, <https://doi.org/10.1097/00000441-197304000-00005>.
- [15] K.R. Falchuk, J.L. Perrotto, K.J. Isselbacher, Serum lysozyme in Crohn's disease. A useful index of disease activity, *Gastroenterology* 69 (1975) 893–896, [https://doi.org/10.1016/S0016-5085\(19\)32404-7](https://doi.org/10.1016/S0016-5085(19)32404-7).
- [16] T. Okamoto, K. Ueda, H. Maeda, T. Kambara, Determination of lysozyme activity by fluorescence polarization in rheumatoid synovial fluids and release of lysozyme from polymorphonuclear leukocytes by chemotactic factors, *J. Immunol. Methods* 103 (1987) 221–227, [https://doi.org/10.1016/0022-1759\(87\)90293-6](https://doi.org/10.1016/0022-1759(87)90293-6).
- [17] P. Weber, H. Steinhart, A. Paschke, Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA, *J. Agric. Food Chem.* 55 (2007) 3127–3133, <https://doi.org/10.1021/jf0634366>.
- [18] A. Vasilescu, Q. Wang, M. Li, R. Boukherroub, S. Szunerits, Aptamer-based electrochemical sensing of lysozyme, *Chemosensors* 4 (2016) 1–20, <https://doi.org/10.3390/chemosensors4020010>.
- [19] H.S. Park, D.H. Nahm, New occupational allergen in a pharmaceutical industry: serratal peptidase and lysozyme chloride, *Ann. Allergy Asthma Immunol.* 78 (1997) 225–229, [https://doi.org/10.1016/S1081-1206\(10\)63392-3](https://doi.org/10.1016/S1081-1206(10)63392-3).
- [20] S. Neethirajan, X. Weng, A. Tah, J.O. Cordero, K.V. Ragavan, Nano-biosensor platforms for detecting food allergens – new trends, *Sens. Bio-Sensing Res.* 18 (2018) 13–30, <https://doi.org/10.1016/j.sbsr.2018.02.005>.
- [21] C. ling Yan, B. xiao Ding, X. ming Lan, S. bo Guo, Y. ying Xie, C. bo Wang, The toxicity study on marine low-temperature lysozyme, *Food Chem. Toxicol.* 46 (2008) 604–609, <https://doi.org/10.1016/j.fct.2007.09.001>.
- [22] O. Hosu, G. Selvolini, G. Marrazza, Recent advances of immunosensors for detecting food allergens, *Curr. Opin. Electrochem.* 10 (2018) 149–156, <https://doi.org/10.1016/j.coelec.2018.05.022>.
- [23] A.N. Smolens, S.E. Hartsell, The determination of lysozyme, *J. Bacteriol.* 58 (1949) 731–736. <http://www.ncbi.nlm.nih.gov/pubmed/15395173>. (Accessed 9 March 2020).
- [24] E.F. Osserman, D.P. Lawlor, Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia, *J. Exp. Med.* 124 (1966) 921–952, <https://doi.org/10.1084/jem.124.5.921>.
- [25] A. Blumlein, J.J. McManus, Reversible and non-reversible thermal denaturation of lysozyme with varying pH at low ionic strength, *Biochim. Biophys. Acta Protein Proteomics* 1834 (2013) 2064–2070, <https://doi.org/10.1016/j.bbapap.2013.06.001>.
- [26] M. Nishimoto, U. Komatsu, N. Tamai, M. Yamanaka, S. Kaneshina, K. Ogli, H. Matsuki, Intrinsic interaction mode of an inhalation anesthetic with globular proteins: a comparative study on ligand recognition, *Colloid Polym. Sci.* 289 (2011) 1785–1797, <https://doi.org/10.1007/s00396-011-2491-z>.
- [27] M.L. Vidal, J. Gautron, Y. Nys, Development of an ELISA for quantifying lysozyme in hen egg white, *J. Agric. Food Chem.* 53 (2005) 2379–2385, <https://doi.org/10.1021/jf048692a>.
- [28] L. Pellegrino, A. Tirelli, A sensitive HPLC method to detect hen's egg white lysozyme in milk and dairy products, *Int. Dairy J.* 10 (2000) 435–442, [https://doi.org/10.1016/S0958-6946\(00\)00065-0](https://doi.org/10.1016/S0958-6946(00)00065-0).
- [29] B. Kerkaert, F. Mestdagh, B. De Meulenaer, Detection of hen's egg white lysozyme in food: comparison between a sensitive HPLC and a commercial ELISA method, *Food Chem.* 120 (2010) 580–584, <https://doi.org/10.1016/j.foodchem.2009.10.027>.
- [30] Lysozyme Test Kit | Fast & reliable lysozyme detection, (n.d.). <https://www.romerlabs.com/en/analytes/food-allergens/lysozyme-test-kit/>.
- [31] Lysozyme Activity Kit sufficient for 100 tests | Sigma-Aldrich, (n.d.). <http://www.sigmaaldrich.com/catalog/product/sigma/ly0100?lang=it®ion=IT>.
- [32] D.R. Thévenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: recommended definitions and classification, *Biosens. Bioelectron.* 16 (2001) 121–131, [https://doi.org/10.1016/S0956-5663\(01\)00115-4](https://doi.org/10.1016/S0956-5663(01)00115-4).
- [33] X. Li, X. Wang, L. Zhang, J. Gong, High-throughput signal-on photoelectrochemical immunoassay of lysozyme based on hole-trapping triggered by disintegrating bioconjugates of dopamine-grafted silica nanospheres, *ACS Sens.* 3 (2018) 1480–1488, <https://doi.org/10.1021/acssensors.8b00253>.
- [34] E. Marlow, A. Murphy, R. Pollard, Optimisation of gold nanorod array for sensing applications, *Br. Irish Conf. Opt. Photonics, BICOP 2018 - Proc* 2018 – 1–4, <https://doi.org/10.1109/BICOP.2018.8658344>.
- [35] A. Liang, B. Tang, H.P. Hou, L. Sun, A.Q. Luo, A novel CuFe₂O₄ nanospheres molecularly imprinted polymers modified electrochemical sensor for lysozyme determination, *J. Electroanal. Chem.* 853 (2019) 113465, <https://doi.org/10.1016/j.jelechem.2019.113465>.
- [36] A. Vasilescu, S. Gáspár, M. Gheorghiu, S. David, V. Dinca, S. Petcu, Q. Wang, M. Li, R. Boukherroub, S. Szunerits, Surface Plasmon Resonance based sensing of lysozyme in serum on Micrococcus lysodeikticus-modified graphene oxide surfaces, *Biosens. Bioelectron.* 89 (2017) 525–531, <https://doi.org/10.1016/j.bios.2016.03.040>.
- [37] K. Shrivastava, N. Nirmalkar, M.K. Deb, K. Dewangan, J. Nirmalkar, S. Kumar, Application of functionalized silver nanoparticles as a biochemical sensor for selective detection of lysozyme protein in milk sample, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 213 (2019) 127–133, <https://doi.org/10.1016/j.saa.2019.01.039>.
- [38] Y. Saylan, F. Yilmaz, A. Derazshamshir, E. Yilmaz, A. Denizli, Synthesis of hydrophobic nanoparticles for real-time lysozyme detection using surface plasmon resonance sensor, *J. Mol. Recogn.* 30 (2017) 1–7, <https://doi.org/10.1002/jmr.2631>.
- [39] Y. Wang, Q. Hu, T. Tian, Y. Gao, L. Yu, A nonionic surfactant-decorated liquid crystal sensor for sensitive and selective detection of proteins, *Anal. Chim. Acta* 937 (2016) 119–126, <https://doi.org/10.1016/j.aca.2016.07.010>.
- [40] Scopus - document search results, (n.d.).

- [48] A. De Girolamo, M. McKeague, M. Pascale, M. Cortese, M.C. DeRosa, Immobilization of aptamers on substrates. *Aptamers Anal. Appl.*, 2018, <https://doi.org/10.1002/9783527806799.ch3>.
- [49] E.M. Nelson, L.J. Rothberg, Kinetics and mechanism of single-stranded DNA adsorption onto citrate-stabilized gold nanoparticles in colloidal solution, *Langmuir* 27 (2011) 1770–1777, <https://doi.org/10.1021/la102613f>.
- [50] A. Sassolas, L.J. Blum, B.D. Leca-Bouvier, Optical detection systems using immobilized aptamers, *Biosens. Bioelectron.* 26 (2011) 3725–3736, <https://doi.org/10.1016/j.bios.2011.02.031>.
- [51] Rozenblum, Radrizzani Pollitzer, Challenges in electrochemical aptasensors and current sensing architectures using flat gold surfaces, *Chemosensors* 7 (2019) 57, <https://doi.org/10.3390/chemosensors7040057>.
- [52] A. Ravalli, D. Voccia, I. Palchetti, G. Marrazza, Electrochemical, electrochemiluminescence, and photoelectrochemical aptamer-based nanostructured sensors for biomarker analysis, *Biosensors* 6 (2016), <https://doi.org/10.3390/bios6030039>.
- [53] M. Negahdary, Electrochemical aptasensors based on the gold nanostructures, *Talanta* 216 (2020), 120999, <https://doi.org/10.1016/j.talanta.2020.120999>.
- [54] A.J. Downard, Electrochemically assisted covalent modification of carbon electrodes, *Electroanalysis* 12 (2000) 1085–1096, [https://doi.org/10.1002/1521-4109.\(200010\)12:14<1085::AID-ELAN1085>3.0.CO;2-A](https://doi.org/10.1002/1521-4109.(200010)12:14<1085::AID-ELAN1085>3.0.CO;2-A).
- [55] A. Jain, K. Cheng, The principles and applications of avidin-based nanoparticles in drug delivery and diagnosis, *J. Contr. Release* 245 (2017) 27–40, <https://doi.org/10.1016/j.jconrel.2016.11.016>.
- [56] R. Rapini, G. Marrazza, Electrochemical aptasensors for contaminants detection in food and environment: recent advances, *Bioelectrochemistry* 118 (2017) 47–61, <https://doi.org/10.1016/j.bioelechem.2017.07.004>.
- [57] R. Miranda-Castro, N. De-los-Santos-Álvarez, M.J. Lobo-Castañón, Characterization of aptamer-ligand complexes. *Aptamers Anal. Appl.*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2018, pp. 127–172, <https://doi.org/10.1002/9783527806799.ch4>.
- [58] R. Sharma, K.S.M.S. Raghavarao, Nanoparticle-based aptasensors for food contaminant detection. *Nanomater. Food Appl.*, Elsevier, 2018, pp. 123–145, <https://doi.org/10.1016/B978-0-12-814130-4.00006-3>.
- [59] L. Cheng, C. Xu, H. Cui, F. Liao, N. Hong, G. Ma, J. Xiong, H. Fan, A sensitive homogenous aptasensor based on tetraferrocene labeling for thrombin detection, *Anal. Chim. Acta* 1111 (2020) 1–7, <https://doi.org/10.1016/j.aca.2020.03.017>.
- [60] M. Teriș, G. Melinte, B. Ciui, I. Șimon, R. Știufiuc, R. Săndulescu, C. Cristea, A novel label free electrochemical magnetoinmunosensor for human interleukin-6 quantification in serum, *Electroanalysis* 31 (2019) 282–292, <https://doi.org/10.1002/elan.201800620>.
- [61] S. Sang, Y. Wang, Q. Feng, Y. Wei, J. Ji, W. Zhang, Progress of new label-free techniques for biosensors: a review, *Crit. Rev. Biotechnol.* 36 (2016) 465–481, <https://doi.org/10.3109/07388551.2014.991270>.
- [62] M.A. Tabrizi, The electrochemical aptasensors for the determination of tumor markers. *Graphene Bioelectron*, Elsevier Inc., 2018, pp. 193–218, <https://doi.org/10.1016/B978-0-12-813349-1.00009-3>.
- [63] A.I. Zia, S.C. Mukhopadhyay, Electrochemical Sensing: Carcinogens in Beverages, 2016, <https://doi.org/10.1007/978-3-319-32655-9>.
- [64] R. García-González, M.T. Fernández Abedul, Electrochemical Impedance Spectroscopy for Characterization of Electrode Surfaces, Elsevier Inc., 2020, <https://doi.org/10.1016/B978-0-12-815932-3.00012-7>.
- [65] D.A.C. Brownson, C.E. Banks, *Handbook of Graphene Electrochemistry*, 2016.
- [66] J. Wang, *ANALYTICAL ELECTROCHEMISTRY*, III, John Wiley & Sons, Inc., Hoboken, New Jersey, 2006.
- [67] B. Rezaei, H.R. Jamei, A.A. Ensafi, An ultrasensitive and selective electrochemical aptasensor based on rGO-MWCNTs/Chitosan/carbon quantum dot for the detection of lysozyme, *Biosens. Bioelectron.* 115 (2018) 37–44, <https://doi.org/10.1016/j.bios.2018.05.012>.
- [68] H.R. Jamei, B. Rezaei, A.A. Ensafi, An ultrasensitive electrochemical anti-lysozyme aptasensor with biorecognition surface based on aptamer/amino-rGO/ionic liquid/amino-mesoporous silica nanoparticles, *Colloids Surf. B Biointerfaces* 181 (2019) 16–24, <https://doi.org/10.1016/j.colsurfb.2019.05.030>.
- [69] D. Xie, C. Li, L. Shanguan, H. Qi, D. Xue, Q. Gao, C. Zhang, Click chemistry-assisted self-assembly of DNA aptamer on gold nanoparticles-modified screen-printed carbon electrodes for label-free electrochemical aptasensor, *Sensor. Actuator. B Chem.* 192 (2014) 558–564, <https://doi.org/10.1016/j.snb.2013.11.038>.
- [70] Z. Chen, J. Guo, J. Li, L. Guo, Tetrahedral Au nanocrystals/aptamer based ultrasensitive electrochemical biosensor, *RSC Adv.* 3 (2013) 14385–14389, <https://doi.org/10.1039/c3ra41065j>.
- [71] M. Jarczewska, D. Talarek, E. Malinowska, Electrochemical studies on the binding between surface-tethered DNA aptamers and lysozyme, *J. Electrochem. Soc.* 166 (2019), <https://doi.org/10.1149/2.0481916jes>.
- [72] C. Ocaña, A. Hayat, R. Mishra, A. Vasilescu, M. Del Valle, J.L. Marty, A novel electrochemical aptamer-antibody sandwich assay for lysozyme detection, *Analyst* 140 (2015) 4148–4153, <https://doi.org/10.1039/c5an00243e>.
- [73] Z. Liu, H. Wang, An antifouling interface integrated with HRP-based amplification to achieve a highly sensitive electrochemical aptasensor for lysozyme detection, *Analyst* 144 (2019) 5794–5801, <https://doi.org/10.1039/c9an01430f>.
- [74] X. Cao, J. Xu, J. Xia, F. Zhang, Z. Wang, An electrochemical aptasensor based on the conversion of liquid-phase colorimetric assay into electrochemical analysis for sensitive detection of lysozyme, *Sensor. Actuator. B Chem.* 255 (2018) 2136–2142, <https://doi.org/10.1016/j.snb.2017.09.019>.
- [75] Z. Chen, Q. Xu, G. Tang, S. Liu, S. Xu, X. Zhang, A facile electrochemical aptasensor for lysozyme detection based on target-induced turn-off of photosensitization, *Biosens. Bioelectron.* 126 (2019) 412–417, <https://doi.org/10.1016/j.bios.2018.09.074>.
- [76] A.M. Titoiu, R. Porumb, P. Fanjul-Bolado, P. Epure, M. Zamfir, A. Vasilescu, Detection of allergenic lysozyme during winemaking with an electrochemical aptasensor, *Electroanalysis* 31 (2019) 2262–2273, <https://doi.org/10.1002/elan.201900333>.
- [77] J. Zhang, M. Shang, Y. Gao, J. Yan, W. Song, High-performance VS2 QDs-based type II heterostructured photoanode for ultrasensitive aptasensing of lysozyme, *Sensor. Actuator. B Chem.* 304 (2020) 127411, <https://doi.org/10.1016/j.snb.2019.127411>.
- [78] A. Erdem, E. Eksin, M. Muti, Chitosan-graphene oxide based aptasensor for the impedimetric detection of lysozyme, *Colloids Surf. B Biointerfaces* 115 (2014) 205–211, <https://doi.org/10.1016/j.colsurfb.2013.11.037>.
- [79] E. Heydari-Bafrooei, S. Askari, Ultrasensitive aptasensing of lysozyme by exploiting the synergistic effect of gold nanoparticle-modified reduced graphene oxide and MWCNTs in a chitosan matrix, *Microchim. Acta.* 184 (2017) 3405–3413, <https://doi.org/10.1007/s00604-017-2356-3>.
- [80] D. Ortiz-Aguayo, M. del Valle, Label-free aptasensor for lysozyme detection using electrochemical impedance spectroscopy, *Sensors* 18 (2018) 354, <https://doi.org/10.3390/s18020354>.
- [81] M. Shamsipur, L. Farzin, M.A. Tabrizi, Ultrasensitive aptamer-based on-off assay for lysozyme using a glassy carbon electrode modified with gold nanoparticles and electrochemically reduced graphene oxide, *Microchim. Acta.* 183 (2016) 2733–2743, <https://doi.org/10.1007/s00604-016-1920-6>.
- [82] C. Ocaña, A. Hayat, R. Mishra, A. Vasilescu, M. Del Valle, J.L. Marty, A novel electrochemical aptamer-antibody sandwich assay for lysozyme detection, *Analyst* 140 (2015) 4148–4153, <https://doi.org/10.1039/c5an00243e>.
- [83] S. Fang, X. Dong, H. Ji, S. Liu, F. Yan, D. Peng, L. He, M. Wang, Z. Zhang, Electrochemical aptasensor for lysozyme based on a gold electrode modified with a nanocomposite consisting of reduced graphene oxide, cuprous oxide, and plasma-polymerized propargylamine, *Microchim. Acta.* 183 (2016) 633–642, <https://doi.org/10.1007/s00604-015-1675-5>.
- [84] X. Cao, J. Xia, H. Liu, F. Zhang, Z. Wang, L. Lu, A new dual-signalling electrochemical aptasensor with the integration of “signal on/off” and “labeling/label-free” strategies, *Sensor. Actuator. B Chem.* 239 (2017) 166–171, <https://doi.org/10.1016/j.snb.2016.08.009>.
- [85] Z. Li, C. Su, D. Wu, Z. Zhang, Gold nanoparticles decorated hematite photoelectrode for sensitive and selective photoelectrochemical aptasensing of lysozyme, *Anal. Chem.* 90 (2018) 961–967, <https://doi.org/10.1021/acs.analchem.7b04015>.
- [86] O. Hosu, A. Florea, C. Cristea, R. Săndulescu, Functionalized Advanced Hybrid Materials for Biosensing Applications, Elsevier Inc., 2019, <https://doi.org/10.1016/B978-0-12-815743-5.00006-8>.
- [87] Y. Yao, H. Wang, X. Wang, X. Wang, F. Li, Development of a chemiluminescent aptasensor for ultrasensitive and selective detection of aflatoxin B1 in peanut and milk, *Talanta* 201 (2019) 52–57, <https://doi.org/10.1016/j.talanta.2019.03.109>.
- [88] A.J. Bard, Z. Ding, N. Myung, Electrochemistry and electrogenerated chemiluminescence of semiconductor nanocrystals in solutions and in films, *Struct. Bond* 118 (2005) 1–57, <https://doi.org/10.1007/b137239>.
- [89] M.N. Velasco-Garcia, Optical biosensors for probing at the cellular level: a review of recent progress and future prospects, *Semin. Cell Dev. Biol.* 20 (2009) 27–33, <https://doi.org/10.1016/j.semcdb.2009.01.013>.
- [90] A.R. Clapp, I.L. Medintz, J.M. Mauro, B.R. Fisher, M.G. Bawendi, H. Mattoussi, Fluorescence resonance energy transfer between quantum dot donors and dye-labeled protein acceptors, *J. Am. Chem. Soc.* 126 (2004) 301–310, <https://doi.org/10.1021/ja037088b>.
- [91] Y. Song, W. Wei, X. Qu, Colorimetric biosensing using smart materials, *Adv. Mater.* 23 (2011) 4215–4236, <https://doi.org/10.1002/adma.201101853>.
- [92] F.K. Du, H. Zhang, X.C. Tan, J. Yan, M. Liu, X. Chen, Y.Y. Wu, D.F. Feng, Q. Y. Chen, J.M. Cen, S.G. Liu, Y.Q. Qiu, H.Y. Han, Ru(bpy)3²⁺-Silica/Poly-L-lysine-Au as labels for electrochemiluminescence lysozyme aptasensor based on 3D graphene, *Biosens. Bioelectron.* 106 (2018) 50–56, <https://doi.org/10.1016/j.bios.2018.01.059>.
- [93] Y.P. Dong, J. Wang, Y. Peng, J.J. Zhu, A novel aptasensor for lysozyme based on electrogenerated chemiluminescence resonance energy transfer between luminol and silicon quantum dots, *Biosens. Bioelectron.* 94 (2017) 530–535, <https://doi.org/10.1016/j.bios.2017.03.044>.
- [94] Y.N. Khonsari, S. Sun, A novel label free electrochemiluminescent aptasensor for the detection of lysozyme, *Mater. Sci. Eng. C* 96 (2019) 146–152, <https://doi.org/10.1016/j.msec.2018.11.016>.
- [95] Y. Xu, T. Chen, J. Sun, J. Liu, Multifunctional solid-state electrochemiluminescent chemosensors and aptasensor with free-standing active sites based on task-specific pyrene-terminated polymers via RAFT polymerization, *Anal. Chim. Acta* 1039 (2018) 31–40, <https://doi.org/10.1016/j.aca.2018.07.046>.
- [96] Y. Sun, Y. Lin, R. Han, X. Wang, C. Luo, A chemiluminescence biosensor for lysozyme detection based on aptamers and hemin/G-quadruplex DNAzyme modified sandwich-rod carbon fiber composite, *Talanta* 200 (2019) 57–66, <https://doi.org/10.1016/j.talanta.2019.03.007>.
- [97] M.C. Giuffrida, G. Cigliana, G. Spoto, Ultrasensitive detection of lysozyme in droplet-based microfluidic devices, *Biosens. Bioelectron.* 104 (2018) 8–14, <https://doi.org/10.1016/j.bios.2017.12.042>.
- [98] L.S. Ardekani, T.T. Moghadam, P.W. Thulstrup, B. Ranjbar, Design and fabrication of a silver nanocluster-based aptasensor for lysozyme detection,

- Plasmonics 14 (2019) 1765–1774, <https://doi.org/10.1007/s11468-019-00954-5>.
- [99] X. Liu, Q. Li, P. Gu, S. Su, Y. Huang, X. Feng, Q. Fan, W. Huang, Highly sensitive fluorometric turn-on detection of lysozyme based on a graphene oxide/ssDNA assembly, *IEEE Sensor. J.* 17 (2017) 5431–5436, <https://doi.org/10.1109/JSEN.2017.2730478>.
- [100] L. Chen, N. Xia, T. Li, Y. Bai, X. Chen, Aptasensor for visual and fluorometric determination of lysozyme based on the inner filter effect of gold nanoparticles on CdTe quantum dots, *Microchim. Acta.* 183 (2016) 2917–2923, <https://doi.org/10.1007/s00604-016-1903-7>.
- [101] F. Chen, C. Cai, X. Chen, C. Chen, “Click on the bidirectional switch”: the aptasensor for simultaneous detection of lysozyme and ATP with high sensitivity and high selectivity, *Sci. Rep.* 6 (2016) 1–7, <https://doi.org/10.1038/srep18814>.
- [102] K. Sapkota, S. Dhakal, FRET-based aptasensor for the selective and sensitive detection of lysozyme, *Sensors* 20 (2020) 914, <https://doi.org/10.3390/s20030914>.
- [103] J. Wu, Y. Hou, P. Wang, Z. Wang, Y. Li, S. Wang, M. Yang, Detection of lysozyme with aptasensor based on fluorescence resonance energy transfer from carbon dots to graphene oxide, *Luminescence* 31 (2016) 1207–1212, <https://doi.org/10.1002/bio.3092>.
- [104] X. Ou, Y. Chen, L. Xie, J. Chen, J. Zan, X. Chen, Z. Hong, Y. He, J. Li, H. Yang, X-ray nanocrystal scintillator-based aptasensor for autofluorescence-free detection, *Anal. Chem.* 91 (15) (2019) 10149–10155, <https://doi.org/10.1021/ACS.ANALCHEM.9B02166>.
- [105] O.H. Shayesteh, R. Ghavami, A novel label-free colorimetric aptasensor for sensitive determination of PSA biomarker using gold nanoparticles and a cationic polymer in human serum, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 226 (2020) 117644, <https://doi.org/10.1016/j.saa.2019.117644>.
- [106] Y. Xie, Y. Huang, D. Tang, H. Cui, L. Yang, H. Cao, W. Yun, Sensitive colorimetric detection for lysozyme based on the capture of a fixed thiol-aptamer on gold nanoparticles, *New J. Chem.* 43 (2019) 4531–4538, <https://doi.org/10.1039/c9nj00016j>.
- [107] J.L. Wai, S.Y. New, Cysteamine-coated gold nanoparticles for bimodal colorimetric detection with inverse sensitivity: a proof-of-concept with lysozyme, *RSC Adv.* 10 (2019) 1088–1094, <https://doi.org/10.1039/c9ra07930k>.
- [108] T. Lou, H. Qiang, Z. Chen, Core-shell Cu@Au nanoparticles-based colorimetric aptasensor for the determination of lysozyme, *Talanta* 163 (2017) 132–139, <https://doi.org/10.1016/j.talanta.2016.10.095>.
- [109] R.K. Mishra, A. Hayat, G.K. Mishra, G. Catanante, V. Sharma, J.L. Marty, A novel colorimetric competitive aptamer assay for lysozyme detection based on superparamagnetic nanobeads, *Talanta* 165 (2017) 436–441, <https://doi.org/10.1016/j.talanta.2016.12.083>.
- [110] Y.N. Khonsari, S. Sun, A novel label free electrochemiluminescent aptasensor for the detection of lysozyme, *Mater. Sci. Eng. C* 96 (2019) 146–152, <https://doi.org/10.1016/j.msec.2018.11.016>.
- [111] L. Zuo, G. Qin, Y. Lan, Y. Wei, C. Dong, A turn-on phosphorescence aptasensor for ultrasensitive detection of lysozyme in humoral samples, *Sensor. Actuator. B Chem.* 289 (2019) 100–105, <https://doi.org/10.1016/j.snb.2019.03.088>.
- [112] Z. Qiu, J. Shu, Y. He, Z. Lin, K. Zhang, S. Lv, D. Tang, CdTe/CdSe quantum dot-based fluorescent aptasensor with hemin/G-quadruplex DNzyme for sensitive detection of lysozyme using rolling circle amplification and strand hybridization, *Biosens. Bioelectron.* 87 (2017) 18–24, <https://doi.org/10.1016/j.bios.2016.08.003>.
- [113] P. Gu, X. Liu, Y. Tian, L. Zhang, Y. Huang, S. Su, X. Feng, Q. Fan, W. Huang, A novel visible detection strategy for lysozyme based on gold nanoparticles and conjugated polymer brush, *Sensor. Actuator. B Chem.* 246 (2017) 78–84, <https://doi.org/10.1016/j.snb.2017.01.043>.
- [114] R.K. Mishra, A. Hayat, G.K. Mishra, G. Catanante, V. Sharma, J.L. Marty, A novel colorimetric competitive aptamer assay for lysozyme detection based on superparamagnetic nanobeads, *Talanta* 165 (2017) 436–441, <https://doi.org/10.1016/j.talanta.2016.12.083>.
- [115] Y. Bai, R. Zhao, F. Feng, X. He, Determination of lysozyme by thiol-terminated aptamer-based surface plasmon resonance, *Anal. Lett.* 50 (2017) 682–689, <https://doi.org/10.1080/00032719.2016.1190737>.
- [116] C. Feng, S. Dai, L. Wang, Optical aptasensors for quantitative detection of small biomolecules: a review, *Biosens. Bioelectron.* 59 (2014) 64–74, <https://doi.org/10.1016/j.bios.2014.03.014>.
- [117] G. Leśniewski, T. Yang, Lysozyme and its modified forms: a critical appraisal of selected properties and potential, *Trends Food Sci. Technol.* 107 (2020) 333–342, <https://doi.org/10.1016/j.tifs.2020.11.004>.