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Bio(inspired) strategies for the electro-sensing of β-lactam antibiotics

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Abstract

The dire previsions of the World Health Organization on the so-called "post-antibiotic era" and the continuous and global rise of anti-microbial resistance, spurs our research community to find better ways to fight these threats. In light of this severe threat to human health many attempts have been made to develop efficient methods to detect antibiotic residues in different streams. The use of electrochemistry seems an inviting approach for on-site and fast monitoring. In this critical review, recent developments in the field of (bio) electro-sensing of β-lactam antibiotics will be presented, with a focus on aptamers and molecularly imprinted polymers, the two main promises of a new generation of biosensors, yet to be fulfilled.

Keywords: anti-microbial resistance, β-lactam antibiotics, aptamers, molecularly imprinted polymers, (bio)sensors.

Introduction

The rise of antimicrobial resistance (AMR) in recent years is a major concern for authorities around the world. According to the World Health Organization (WHO), only in Europe about 25.000 patients die annually from infections caused by AMR [1]. In this frame, the European Union and many other regulation agencies around the globe defined maximum residues limits (MRL) for many antibiotics in different food matrices [2–6]. Among all antimicrobial drugs, β-lactam antibiotics are the most effective and used (for an exhaustive review see [7]). The two main classes, penicillins and cephalosporins, account for ca 37% of the European antibiotic consumption. The analytical methods for β-lactam detection can be divided in screening methods and confirmatory methods. The screening tests are sensible enough to detect antibiotics at MRL, however, lack -most commonly- selectivity towards single drugs and are prone to false positive and negative results [8,9]. Electrochemical biosensing seems an inviting approach to recognize β-lactam residues on a single compound level by combining a fast and easy-to-use analytical protocol with enough selectivity and specificity due to the presence of bio-recognition elements. Every antibiotic has its own MRL value and often these values are quite different; the individual quantification of each residues may help authorities to formulate better prevention plans, identifying the possible sources of contamination and avoid wastes. In the present review, the most recent findings in the field of electrochemical biosensing targeting β-lactam antibiotics will be

presented. We will try to look beyond the astonishing figure of merits of the published analytical approaches, underlining what could be the most promising alternatives for robust and reliable (bio)mimetic sensors, guiding the reader towards issues that should be addressed by the scientific community at large. For ease of reference the paper will be divided in sections, each of them devoted to a specific molecular recognition layer.

Antibodies

Despite the huge amount of antibodies available on the market today, only a few have been raised against β-lactam antibiotics [10]. The main challenge, as with other small molecules, lies in the poor immunogenicity of the compounds and the need to couple them with carrier proteins to elicit an immune response in animals. Despite this limitation, some anti-penicillin and anti-cephalosporin antibodies, both monoclonal and polyclonal, are available on the market, and have been used for a variety of biosensing applications [11]. However the reported cross-reactivity with different types of specific antibiotics makes them unsuitable for the selective detection of single β-lactams. A couple of examples of electrochemical immunosensors have been reported in recent years: Merola and coworkers [12] developed two competitive assays based on the immobilization of the molecular recognition layers on an Immobilion membrane, with amperometric readout following the activity of an enzymatic label, horse radish peroxidase (HRP). With both methods a Limit of Detection (LOD) in the low nanomolar range (10^{-10} M) is achieved also in real samples. However even if the class-selectivity is good, the antibodies recognize also other penicillins as well as some cephalosporins. The same research group lately proposed an improved format in which the immobilized penicillin G was conjugated to Bovine Serum Albumin (BSA), exploiting the already investigated competitive assay [13], with a slightly better selectivity. Li et al. [14] instead exploited a mouse anti penicillin G antibody to realize an impedimetric sensor, based on a bilayers lipids membrane (BLM) modified with gold nanoparticles (AuNPs), to promote the immobilization of the antibodies. They report an increase in sensitivity due to the presence of nanoparticles, reaching a LOD of 2.7×10^{-4} ng/L. They tested the interference effect of two other antibiotics, ampicillin and streptomycin, obtaining also good recovery for real milk sample. Karaseva et al. [15] tested both monoclonal and polyclonal antibodies against penicillin G and ampicillin in a piezoelectric based sensor, immobilizing an antibiotic-protein conjugate on the surface of a polypyrrole modified electrode. They tested the sensors in different kind of real samples with good recovery rates (>90%). The intrinsic characteristic of the antibodies (stability, cost, ease of use) however may limit the feasibility of a selective immunosensor for on-site screening even if good results were achieved in term of real sample analysis and LOD [16].

Enzymes

β-Lactamase are bacterial enzymes which represent the most important mechanism of antimicrobial resistance. These enzymes, also called penicillinases, catalyze the hydrolysis of the lactam ring, the core structure of every β-lactam antibiotic, inactivating the drug [17]. The hydrolysis, operated by β-lactamases, involves proton exchange; the idea is to immobilize the enzymes on different transducers to monitor the pH changes associated with the enzymatic reaction. The most common electrochemical readout systems are amperometry and potentiometry; Wu et al. [18] used a pH indicator, hematein, to

monitor the change of H^+ concentration. The amperometric signal was given by the reduction of hematein to hematoxylin and the enzyme in this case was immobilized on graphene nanosheets modified with ionic liquids. Also, Sotomajor and coworkers [19] used a cysteine SAM-modified gold electrode to immobilize a Class B penicillinase enzyme, followed by chronoamperometric detection of the pH changes in solutions upon adding penicillin G. In another paper [20] the same authors exploit cobalt phthalocyanine as an electron mediator, with lower LOD ($7.9*10^{-8}$ M) and wider linear range. As far as potentiometric detection is concerned the group of Ismail used a potentiometric titration approach with different immobilization layers such as poly-vinyl alcohol [21] and polypyrrole [22] or the combination of both [23] together with glutaraldehyde (GLA) and BSA. However, while useful for pharmaceutical formulations, milk sample analysis proved to be problematic and the reported LOD (3 µM) is rather high. All the above mentioned methods, while very sensitive in certain configurations, completely lack selectivity towards different β-lactams antibiotics, that of course have the same core structure.

Proteins

The main antimicrobial activity of β-lactams antibiotics is expressed by the inhibition of the bacterial cell wall synthesis. The core structure of the antibiotics mimic the substrate of penicillin-binding proteins (PBPs), that catalyze the polymerization of the glycan strands to form peptidoglycan[24]. Since this interaction is very specific and efficient (20 M^{-1} s^{-1} for benzylpenicillin) it was used as a molecular recognition layer for antibiotic biosensing. In the last years, the group of Pingarron proposed two different assays exploiting a recombinant PBPs from *Streptococcus pneumoniae* R6. In the first paper the PBP was immobilized on Co^{2+}-tetradentate nitrilotriacetic acid-modified screen printed electrodes [25] while the second employed paramagnetic beads, coated with the complex His-Tag-Isolation [**26] (Figure 1). Quantification of the binding event was performed with a competitive assay between the antibiotic residues and a HRP-labelled tracer. The use of magnetic beads allows better control in the washing and incubation steps and the total analysis time is around 30 minutes. These assays recognize specifically the active form of the antibiotics, an issue rarely considered in antibiotics biosensors literature. The LOD is well below the MRL values (0.7 and 0.9 ng/ml respectively) and the recovery rate from real milk sample is satisfying. The limitation of the shelf-life (up to 10 days) and the rather time consuming protocol to obtain the recombinant PBP, coupled with the lack of specificity, are possible drawbacks for future commercialization of this strategy.

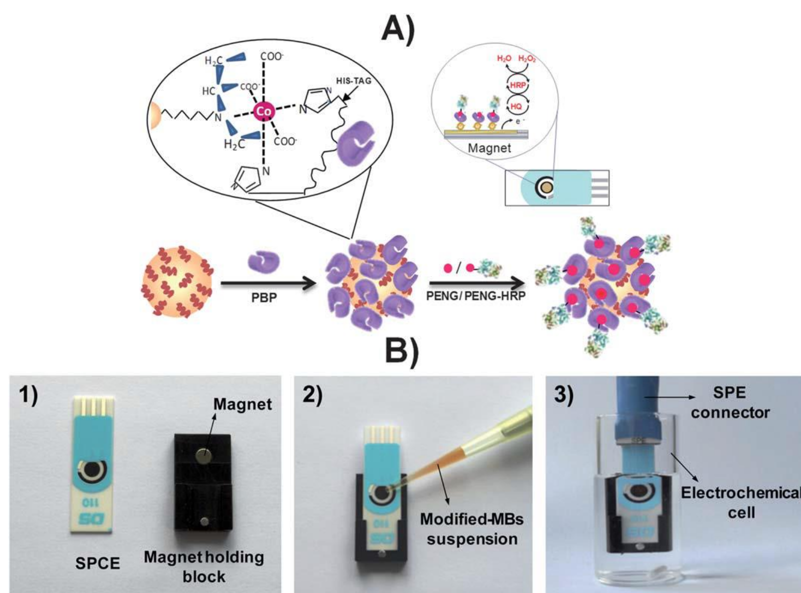

Figure 1. (A) Schematic display of the steps involved in the β-lactam antibiotics affinity magnetosensor developed. (B) Picture showing the SPCE and the homemade magnet holding block (1), the deposition of the modified MBs on the SPCE assembled on the magnet holding block (2) and the assembled SPCE-magnet holding block immersed in the electrochemical cell used for the amperometric measurements (3) (reprinted from [26]).

Aptamers

Aptamers are short strands of ssDNA or RNA that recognized with high specificity the target against which they are selected. Regarded as one of the main innovation in the field of bio(mimetic) materials, aptamers have attracted a lot of attention in recent years, especially for therapeutic applications and drug-delivery [27,28]. They are obtained by a procedure called SELEX (Systematic Evolution of Ligand by Exponential Enrichment) [29] and their applications in electroanalysis have been extensively reviewed [30–32 and reference therein]. Like antibodies, it is challenging to raise aptamers against small molecules[33–35], and there are still very few aptamers available for β-lactams. Song et al. [36] were the first to obtain three different aptamers against ampicillin (AMP4, AMP17 and AMP18). More recently both Noguer et al. [37] and Yoon et al. [38] obtained an aptamer for Penicillin G. Despite this lack of different aptamers, the ampicillin one was used in many analytical configurations in recent years. The aptamer AMP17 was used by Li et al. [39] to develop a dual recycling amplification strategy for ampicillin detection (see Figure 2). The sensor used AMP17 inserted in a tailor-made oligonucleotide probe, labelled with methylene blue (MB) that reacts with Klenow Fragment (KF) polymerase and T7 exonuclease. A similar architecture was used by Huang et al. [40] in a quadratic recycling amplification strategy again with a phi29 polymerase and a nicking endonuclease *Nt.AlwI.* The figures of merit of both sensors are remarkable with a LOD well below the MRL value of ampicillin (4 pM for the dual amplification and 1.09 pM for the quadratic amplification). However the inherent complexity of the analytical approach does not bode well for a possible commercial application. The aptamer AMP17 was also used by Lou et al. [*41] for a signaling-probe displacement sensor. The LOD (10 pM) and the linear dynamic range (100 pM - 1 mM) are indeed very good and also the interference effect of other

antibiotics are minimal. The group of Lai [*42,**43] used another sequence of the aptamer originally selected by Song et al., namely AMP18, to realize two aptasensors for ampicillin, based on a *signal-on* approach. They used a thiolated aptamer, labelled with methylene blue (MB), immobilized on gold electrodes. The conformational change of the aptamer upon binding to ampicillin decreases the distance between the redox probe and the electrode surface, increasing the electron transfer, recorded using alternate current voltammetry (ACV). The first paper [*42] addressed also convincingly a fundamental issue for real world application, the reusability of the sensor. The authors proved that the sensor can be regenerated, up to four times, with a simple washing in DI water. This sensor however does not have the required characteristic in term of LOD (1 µM) and linear dynamic range (5-5000 µM) for antibiotic analysis in real food samples. The same authors, in a second paper [**43], improved the proposed strategy by adding a displacement probe (DP) to increase the distance of the redox mediator and the electrode surface, and thus a gain in signal upon binding with the target. The LOD (30 nM) and the linear dynamic range (0.2-15000 µM) are now in the useful range for antibiotics detection in real samples. However the presence of this sacrificial displacement probe does not allow anymore the regeneration of the modified electrode.

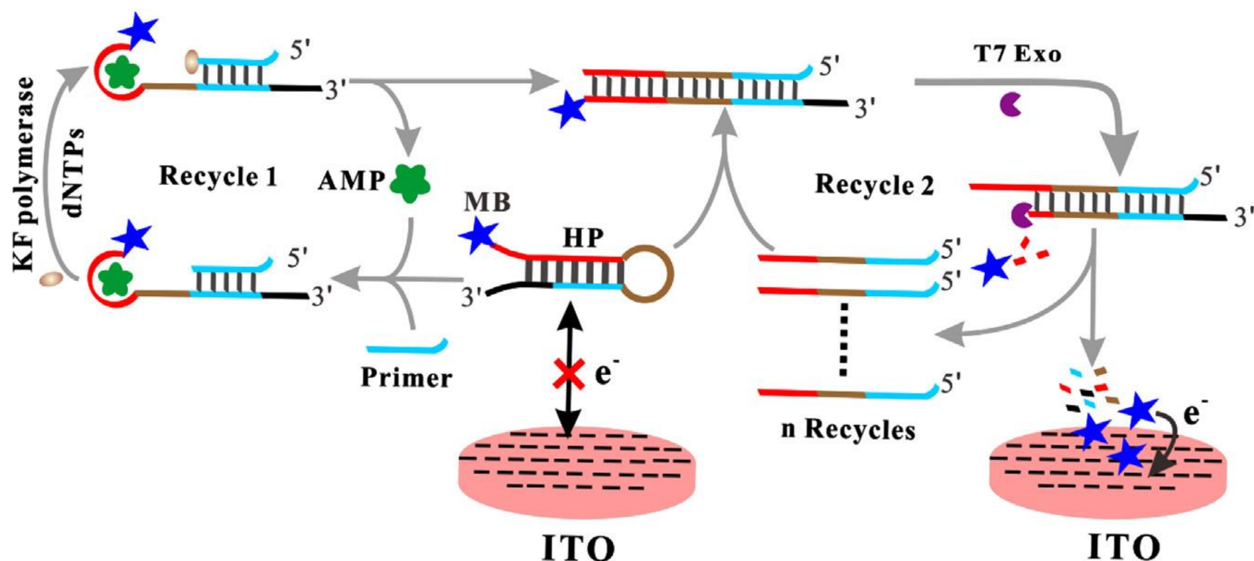

Figure 2. Principle of target-induced and T7 exonuclease-aided recycling amplification homogeneous electrochemical strategy for highly sensitive detection of AMP (reprinted form [39]).

Molecularly imprinted polymers

Along with biomolecules, molecularly imprinted polymers (MIPs) have attracted a lot of attention in recent years for electroanalytical applications. These bio-mimetic materials are mostly used as pre-concentration or extraction step for other analytical techniques, like chromatography [44,45] as well as different kind of electrochemical biosensors [46]. There are two main strategies to synthetize MIPs, electro- and bulk polymerization. In the field of β-lactam sensing, Yang et al. [47] proposed a sensor based on glassy carbon electrode (GC) modified with a gold network doped with a room temperature ionic liquid ([BMIN][BF_4]) coupled with porous platinum nanoparticles and carboxyl graphene. The imprinted polymer is then deposited on this composite by CV using o-phenylediamine as a functional

monomer. The chosen target is cefotaxime, a third-generation cephalosporin used in human medicine as well as, illegally, for food preservation and processing. The sensor shows a good linearity range and detection limit below the MRL value for cefotaxime. Another group developed a MIP-based electrochemical sensor for cefixime, using pyrrole as electroactive monomer. Lutfi Yola et al. [48] modified a glassy carbon electrode with Fe@Au nanoparticles and 2-aminoethanethiol-modified multi walled carbon nanotubes for the electropolymerization of the MIP obtaining a very low limit of detection ($2.2*10^{-11}$ M). Regarding the bulk polymerization approach, the group of Malekzadeh reported two different sensors for the detection of cefixime [49] and ceftazidime [50]. They used a glassy carbon electrode, functionalized with carboxyl-modified multi walled carbon nanotubes and Ag dendrites, to attach the template molecule to the electrode surface. Afterwards they performed a bulk polymerization using acrylamide as a functional monomer, N,N-methylenebisacrylamide as a cross-linker and ammonium persulphate as polymerization initiator. They exploit once again the peculiar electrochemical signal of the target itself for the detection via anodic stripping differential pulse voltammetry (ASDP). The figures of merit of the sensors are remarkable with a reported LOD of 0.5 nM for ceftazidime and 1 nM for cefixime, a very good reproducibility (1.8% and 3.7 respectively) and good recovery rate for real samples (>98%). Another recent application of bulk polymerization is the creation of nanoparticulate molecularly imprinted polymers or nanoMIPs. This new kind of mimetic polymers should overcome many of the limitations of traditional MIPs obtained by bulk polymerization, such as residual template molecules, high binding site heterogeneity and difficult synthetic protocols [51,52]. Karaseva et al. [53] used nanoMIPs to realize a piezoelectric sensor for penicillin G and ampicillin detection. They deposit the synthetized nanoparticles by spin coating on a 10MHz quart resonator, covered with a thin layer of gold, and used the obtained sensor for a direct detection assay, monitoring the change in the resonance frequency before and after the addition of the target molecules. The selectivity study showed that, while the nanoMIPs are very good in discriminating between the target and other structurally different antibiotics (namely cefotaxime and streptomycin), the cross reactivity is quite high for closely related structures (up to 45 % for ampicillin from the penicillin G nanoMIPs). Regardless these limitations, the LOD is below the MRL value, 0.04 µg/mL for penicillin G and 0.09 µg/mL for ampicillin, with a narrow linearity range (0.1 to 1 µg/mL). Given these examples it is clear that the MIP approach could be a feasible alternative for β-lactam detection, especially for third and fourth generation antibiotics (Table S.2). These compounds present many different functional groups and thus more possible anchoring points for the functional monomer to interact with. For less substituted antibiotics, like first and second-generations, particular care should be devoted in the evaluation of interferences, taking into account more structurally related antibiotics before claiming selectivity and specificity of the proposed imprinted polymers. The detection of first and second generation β-lactams (Table S.1 and S.2) is of particular interest since they are still the most used antibiotics both in human and veterinary medicine.

Innovative strategies: photoelectrochemistry

In the last few years photoelectrochemistry has been proven as an innovative strategy to detect many important compounds [54] and recently, De Wael et al. [**55] reported a sensor, based on Zn-phthalocyanine photosensitizers, to detect phenolic antibiotics such as amoxicillin at low concentration

(LOD=20nM). Robust, perfluorinated molecular photosensitizers, resistant chemically yet reactive, have been shown as proof-of-principle efficient enzymes mimics for electrochemical (bio)sensing applications, while favorably enhancing the useful feature of the enzymatic detection mechanism, namely the catalytic formation of an easily detectable product and redox cycling. This approach can be seen as a feasible sensitive alternative for the direct detection of phenol-containing antimicrobial drugs.

Conclusions

The promises of electrochemical biosensors are still far from be fulfilled. Only few of these analytical devices are used by industries and authorities and thus reached the market; while from a basic research point of view, huge improvements have been achieved in the last few years, regarding every aspect of those sensors. However, the efforts to achieve 'better' biosensors should be mitigated by the perspective on their potential for a real world application (responsible research and innovation); in this light MIPs and aptamers seem promising alternatives. Many limitations still remains however; MIPs need a deeper understanding of the characteristics and parameters affecting their performances and their -sometimes- poor selectivity should be improved, for example coupling them with other molecular recognition layers. A systematic and standardized approach to their synthesis is also required. As for the aptamers, the electroanalytical field needs more aptamers, 'better' aptamers. Most of the available aptamers are for bio-medical applications and yet the technology and the know-how is mature enough to tackle challenging targets, like antibiotics or other small molecules. A better understanding of aptamer-target binding mechanisms, along with reliable and accurate characterization techniques, is imperative. Limitations on the availability and the performance of these molecular recognition layers are the possible main cause of the lack of biosensors that can be easily implemented for real world applications.

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References

[1] World Health Organization, Global Action Plan on Antimicrobial Resistance, 2015.
[2] E. Commission, European Commission Regulation 37/2010, (2010).
[3] Codex Alimentarius, Maximum residue limitis (MRLs) and risk management recommendations (RMRs) for residues of veterinary drugs in foods, (2017).
[4] GCC Standardization Organization, Maximum Residues Limits of Veterinary Drugs in Food., (2009).
[5] U.S. Food and Drug Administration, Code of Federal Regulations, TITLE 21, CHAPTER I, SUBCHAPTER E--ANIMAL DRUGS, FEEDS, AND RELATED PRODUCTS, PART 556 TOLERANCES FOR RESIDUES OF NEW ANIMAL DRUGS IN FOOD, 2017.
[6] Food and Agriculture Organization of the United Nations, Basic overview of the

regulatory procedures for authorisation of veterinary medicines with emphasis on residues in food animal species, (2004).
[7] S. Giguere, J.F. Prescott, P.M. Dowling, Antimicrobial Therapy in Veterinary Medicine, 2013. doi:10.1002/9781118675014.
[8] R. Chafer-Pericas, Consuelo, Maquieira, Angel, Puchades, Fast screening methods to detect antibiotic residues in food samples, Trends Trends Anal. Chem. 29 (2010) 1038–1049. doi:10.1016/j.trac.2010.06.004.
[9] M.G. Pikkemaat, Microbial screening methods for detection of antibiotic residues in slaughter animals, Anal. Bioanal. Chem. 395 (2009) 893–905. doi:10.1007/s00216-009-2841-6.
[10] A. Bremus, R. Dietrich, L. Dettmar, E. Usleber, E. Märtlbauer, A broadly applicable approach to prepare monoclonal anti-cephalosporin antibodies for immunochemical residue determination in milk, Anal. Bioanal. Chem. 403 (2012) 503–515. doi:10.1007/s00216-012-5750-z.
[11] V. Gaudin, Advances in biosensor development for the screening of antibiotic residues in food products of animal origin – A comprehensive review, Biosens. Bioelectron. 90 (2017) 363–377. doi:10.1016/j.bios.2016.12.005.
[12] G. Merola, E. Martini, M. Tomassetti, L. Campanella, New immunosensor for Beta-lactam antibiotics determination in river waste waters, Sensors Actuators B. Chem. 199 (2014) 301–313. doi:10.1016/j.snb.2014.03.083.
[13] G. Merola, E. Martini, M. Tomassetti, L. Campanella, Simple and suitable immunosensor for Beta-lactam antibiotics analysis in real matrixes: Milk, serum, urine, J. Pharm. Biomed. Anal. 106 (2015) 186–196. doi:10.1016/j.jpba.2014.08.005.
[14] H. Li, B. Xu, D. Wang, Y. Zhou, H. Zhang, W. Xia, S. Xu, Y. Li, Immunosensor for trace penicillin G detection in milk based on supported bilayer lipid membrane modified with gold nanoparticles, J. Biotechnol. 203 (2015) 97–103. doi:10.1016/j.jbiotec.2015.03.013.
[15] N.A. Karaseva, T.N. Ermolaeva, Piezoelectric immunosensors for the detection of individual antibiotics and the total content of penicillin antibiotics in foodstuffs, Talanta. 120 (2014) 312–317. doi:10.1016/j.talanta.2013.12.018.
[16] A. Chen, S. Yang, Replacing antibodies with aptamers in lateral flow immunoassay, Biosens. Bioelectron. 71 (2015) 230–242. doi:10.1016/j.bios.2015.04.041.
[17] M. Babic, A.M. Hujer, R.A. Bonomo, What ’s new in antibiotic resistance ? Focus on beta-lactamases, Drug Resist. Updat. 9 (2006) 142–156. doi:10.1016/j.drup.2006.05.005.
[18] Y. Wu, L. Tang, L. Huang, Z. Han, J. Wang, H. Pan, A low detection limit penicillin biosensor based on single graphene nanosheets preadsorbed with hematein / ionic liquids / penicillinase, Mater. Sci. Eng. C. 39 (2014) 92–99. doi:10.1016/j.msec.2014.02.012.
[19] L. Moreira, W.F.A. Callera, M.D.P.T. Sotomayor, P.R. Bueno, Penicillinase-based amperometric biosensor for penicillin G, Electrochem. Commun. 38 (2014) 131–133. doi:10.1016/j.elecom.2013.11.022.
[20] M. Thiago, M. V Foguel, L.M. Gonc, P.T. Sotomayor, Beta-Lactamase-based biosensor for the electrochemical determination of benzylpenicillin in milk, Sensors Actuators B. Chem. 210 (2015) 254–258. doi:10.1016/j.snb.2014.12.108.
[21] F. Ismail, S.B. Adeloju, The Use of Poly (Vinyl Alcohol) to Cross-link Penicillinase for the

Fabrication of a Penicillin Potentiometric Biosensor, Electroanalysis. 26 (2014) 2701–2709. doi:10.1002/elan.201400437.

[22] F. Ismail, S.B. Adeloju, A.N. Moline, Fabrication of a Single Layer and Bilayer Potentiometric Biosensors for Penicillin by Galvanostatic Entrapment of Penicillinase into Polypyrrole Films, Electroanalysis. (2014) 2607–2618. doi:10.1002/elan.201400452.

[23] F. Ismail, S.B. Adeloju, Comparison of Single Layer and Bilayer Biosensors Based on Crosslinking of Penicillinase for Potentiometric Detection of Penicillin in Milk and Antibiotics, Electroanalysis. 27 (2015) 1523–1531. doi:10.1002/elan.201500037.

[24] E. Sauvage, M. Terrak, J.A. Ayala, P. Charlier, The penicillin-binding proteins : structure and role in peptidoglycan biosynthesis, Fems Microbiol. Rev. 32 (2008) 234–258. doi:10.1111/j.1574-6976.2008.00105.x.

[25] F. Conzuelo, S. Campuzano, P. Mart, M. Esteban-torres, B. De Rivas, A.J. Reviejo, R. Mun, J.M. Pingarro, Integrated Amperometric A ffi nity Biosensors Using Co 2+ − Tetradentate Nitrilotriacetic Acid Modi fi ed Disposable Carbon Electrodes: Application to the Determination of β ‑ Lactam Antibiotics, Anal. Chem. 85 (2013) 3246–3254.

[**26] M. Gamella, S. Campuzano, F. Conzuelo, M. Esteban-torres, B. De Rivas, An amperometric affinity penicillin-binding protein magnetosensor for the detection of b-lactam antibiotics in milk, Analyst. 138 (2013) 2013–2022. doi:10.1039/c3an36727d.
The authors address an often neglected issue in antibiotics detection in real matrixes: the difference between active and degraded form of the drug and the possible false positive or negative results

[27] K. Chen, B. Liu, B. Yu, W. Zhong, Y. Lu, J. Zhang, J. Liao, Advances in the development of aptamer drug conjugates for targeted drug delivery, Wires Nanomed Nanobiotechnol. 9 (2017) 1–15. doi:10.1002/wnan.1438.

[28] S. Catuogno, C.L. Esposito, V. De Franciscis, Aptamer-Mediated Targeted Delivery of Therapeutics : An Update, Pharmaceuticals. 9 (2016). doi:10.3390/ph9040069.

[29] R. Stoltenburg, C. Reinemann, B. Strehlitz, SELEX — A (r)evolutionary method to generate high-affinity nucleic acid ligands, Biomol. Eng. 24 (2007) 381–403. doi:10.1016/j.bioeng.2007.06.001.

[30] J. Liu, D. Melissa, F.C. Macazo, L.R. Schoukroun-barnes, R.J. White, The Current and Future Role of Aptamers in Electroanalysis, J. Electrochem. Soc. 161 (2014) H301–H313. doi:10.1149/2.026405jes.

[31] H. Malekzad, A. Jouyban, M. Hasanzadeh, Ensuring food safety using aptamer based assays : Electroanalytical approach, Trends Anal. Chem. 94 (2017) 77–94. doi:10.1016/j.trac.2017.07.001.

[32] Y. Xu, G. Cheng, P. He, Y. Fang, A Review : Electrochemical Aptasensors with Various Detection Strategies, Electroanalysis. 21 (2009) 1251–1259. doi:10.1002/elan.200804561.

[33] R. Stoltenburg, N. Nikolaus, B. Strehlitz, Capture-SELEX : Selection of DNA Aptamers for Aminoglycoside Antibiotics, Journa Anal. Methods Cehmistry. 2012 (2012). doi:10.1155/2012/415697.

[34] N. Nikolaus, B. Strehlitz, DNA-Aptamers Binding Aminoglycoside Antibiotics, Sensors. 14 (2014) 3737–3755. doi:10.3390/s140203737.

[35] F. Pfeiffer, G. Mayer, D. Lafontaine, G. Göttingen, K.A. White, G. Mayer, Selection and

Biosensor Application of Aptamers for Small Molecules, Front. Chem. 4 (2016) 1–21. doi:10.3389/fchem.2016.00025.
[36] K. Song, E. Jeong, W. Jeon, M. Cho, C. Ban, Aptasensor for ampicillin using gold nanoparticle based dual fluorescence – colorimetric methods, Anal. Bioanal. Chem. 402 (2012) 2153–2161. doi:10.1007/s00216-011-5662-3.
[37] N. Paniel, G. Istamboulié, A. Triki, C. Lozano, L. Barthelmebs, T. Nouger, Selection of DNA aptamers against penicillin G using Capture-SELEX for the development of an impedimetric sensor, Talanta. 162 (2017) 232–240. doi:10.1016/j.talanta.2016.09.058.
[38] A. Lee, N. Ha, I. Jung, S. Kim, A. Kim, M. Yoon, Development of a ssDNA aptamer for detection of residual benzylpenicillin, Anal. Biochem. 531 (2017) 1–7. doi:10.1016/j.ab.2017.05.013.
[39] X. Wang, S. Dong, P. Gai, R. Duan, F. Li, Highly sensitive homogeneous electrochemical aptasensor for antibiotic residues detection based on dual recycling amplifi cation strategy, Biosens. Bioelectron. 82 (2016) 49–54. doi:10.1016/j.bios.2016.03.055.
[40] H. Wang, Y. Wang, S. Liu, J. Yu, W. Xu, Y. Guo, J. Huang, Target–aptamer binding triggered quadratic recycling amplification for highly specific and ultrasensitive detection of antibiotics at the attomole level, Chem. Commun. 51 (2015) 8377–8380. doi:10.1039/C5CC01473E.
[*41] Z. Yang, X. Ding, Q. Guo, Y. Wang, Z. Lu, H. Ou, Z. Luo, X. Lou, Second generation of signaling-probe displacement electrochemical aptasensor for detection of picomolar ampicillin and sulfadimethoxine, Sensors Actuators B. Chem. 253 (2017) 1129–1136. doi:10.1016/j.snb.2017.07.119.
The influence of passivating agent length for aptamer immobilization was explained in detail and a general strategy to verify its effect on the sensors response was reported
[*42] Z. Yu, R.Y. Lai, A reagentless and reusable electrochemical aptamer-based sensor for rapid detection of ampicillin in complex samples, Talanta. 176 (2018) 619–624. doi:10.1016/j.talanta.2017.08.057.
The sensor proposed in this paper was proven to be reusable up to 4 times without losing signal intensity
[**43]Z. Yu, A.L. Sutlief, R.Y. Lai, Towards the development of a sensitive and selective electrochemical aptamer-based ampicillin sensor, Sensors Actuators B. Chem. 258 (2018) 722–729. doi:10.1016/j.snb.2017.11.193.
The authors reports a general approach to convert singal-off aptasensors in signal-on sensors
[44] A.N. Baeza, J.L. Urraca, R. Chamorro, G. Orellana, M. Castellari, Multiresidue analysis of cephalosporin antibiotics in bovine milk based on molecularly imprinted polymer extraction followed by liquid chromatography-tandem mass spectrometry, J. Chromatogr. A. 1474 (2016) 121–129. doi:10.1016/j.chroma.2016.10.069.
[45] J.L. Urraca, R. Chamorro-mendiluce, G. Orellana, M.C. Moreno-bondi, Molecularly imprinted polymer beads for clean-up and preconcentration of β -lactamase-resistant penicillins in milk, Anal. Bioanal. Chem. 408 (2016) 1843–1854. doi:10.1007/s00216-015-8941-6.
[46] R. Gui, H. Jin, H. Guo, Z. Wang, Biosensors and Bioelectronics Recent advances and future prospects in molecularly imprinted polymers-based electrochemical biosensors, Biosens.

Bioelectron. 100 (2018) 56–70. doi:10.1016/j.bios.2017.08.058.
[47] G. Yang, F. Zhao, B. Zeng, Electrochemical determination of cefotaxime based on a three-dimensional molecularly imprinted film sensor, Biosens. Bioelectron. 53 (2014) 447–452. doi:10.1016/j.bios.2013.10.029.
[48] M. Lüt, T. Eren, N. Atar, Molecularly imprinted electrochemical biosensor based on Fe @ Au nanoparticles involved in 2-aminoethanethiol functionalized multi-walled carbon nanotubes for sensitive determination of cefexime in human plasma, Biosens. Bioelectron. 60 (2014) 277–285. doi:10.1016/j.bios.2014.04.045.
[49] N. Karimian, M.B. Gholivand, G. Malekzadeh, Cefixime detection by a novel electrochemical sensor based on glassy carbon electrode modified with surface imprinted polymer / multiwall carbon nanotubes, J. Electroanal. Chem. 771 (2016) 64–72. doi:10.1016/j.jelechem.2016.03.042.
[50] M. Torkashvand, M.B. Gholivand, G. Malekzadeh, Construction of a new electrochemical sensor based on molecular imprinting recognition sites on multiwall carbon nanotube surface for analysis of ceftazidime in real samples, Sensors Actuators B. Chem. 231 (2016) 759–767. doi:10.1016/j.snb.2016.03.061.
[51] I.E. Tothill, M.J. Abdin, Nano Molecular Imprinted Polymers (NanoMIPs) for Food Diagnostics and Sensor, in: R. Prasad, V. Kumar, M. Kumar (Eds.), Nanotechnol. Food Environ. Paradig., Springer Singapore, Singapore, 2017: pp. 131–151. doi:10.1007/978-981-10-4678-0_8.
[52] F. Canfarotta, A. Poma, A. Guerreiro, S. Piletsky, Solid-phase synthesis of molecularly imprinted nanoparticles, Nat. Protoc. 11 (2016) 443. http://dx.doi.org/10.1038/nprot.2016.030.
[53] N. Karaseva, T. Ermolaeva, B. Mizaikoff, Piezoelectric sensors using molecularly imprinted nanospheres for the detection of antibiotics, Sensors Actuators B. Chem. 225 (2016) 199–208. doi:10.1016/j.snb.2015.11.045.
[54] W. Zhao, J. Xu, H. Chen, Photoelectrochemical bioanalysis : the state of the art, Chem. Soc. Rev. 44 (2015) 729–741. doi:10.1039/C4CS00228H.
[**55] S.M.G.& K.D.W. Stanislav Trashin, Vanoushe Rahemi, Karpagavalli Ramji, Liselotte Neven, Singlet oxygen-based electrosensing by molecular photosensitizers, Nat. Commun. 8 (2017) 1–10. doi:10.1038/ncomms16108.
A novel approach to bio(mimetic) sensing of phenolic compounds and an alternative labelling strategies for biomolecules, with the advantages of an enzymatic reaction without most of their drawbacks.