Lab-on-chip systems for integrated bioanalyses

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Biomolecular detection systems based on microfluidics are often called lab-on-chip systems. To fully benefit from the miniaturization resulting from microfluidics, one aims to develop 'from sample-to-answer' analytical systems, in which the input is a raw or minimally processed biological, food/feed or environmental sample and the output is a quantitative or qualitative assessment of one or more analytes of interest. In general, such systems will require the integration of several steps or operations to perform their function. This review will discuss these stages of operation, including fluidic handling, which assures that the desired fluid arrives at a specific location at the right time and under the appropriate flow conditions; molecular recognition, which allows the capture of specific analytes at precise locations on the chip; transduction of the molecular recognition event into a measurable signal; sample preparation upstream from analyte capture; and signal amplification procedures to increase sensitivity. Seamless integration of the different stages is required to achieve a point-of-care/point-of-use lab-on-chip device that allows analyte detection at the relevant sensitivity ranges, with a competitive analysis time and cost.

Introduction

Top-down cleanroom microfabrication techniques were developed to process integrated electronic circuits. Specialized processes allowed the extension of these techniques to process microelectromechanical systems (MEMSs), in which three-dimensionality and mechanical functionality are added at the scale of microfabrication processes (from micrometres to nanometres) [1,2]. The development of technology to fabricate microchannel networks of high complexity and with high degrees of fluidic control [3,4] led to the rapidly growing field of lab-on-chip (LoC)/microfluidics research and applications. In particular, microfluidics development has been potentiated by the use of soft-lithography techniques, which offer ease of prototyping and low costs [3,4].

Miniaturization of fluidic operations is particularly compelling for analytical applications in the biomedical, environmental, and food and feed safety fields [5–7] where the assays are typically performed using benchtop equipment. The following characteristics, either taken in isolation or combined, can be decisive in establishing an advantage for a miniaturized LoC device with respect to standard benchtop equipment: portability for point-of-use or point-of-care applications, capability of multiplexing several analytes (e.g. biomarkers), easy integration with data acquisition and processing, ability to integrate all steps 'from sample to answer', the potential for low cost due to low reagent and sample volumes and finally, the possibility of simple operation, i.e. does not require a trained operator.

The simple implementation of a biosensor in microfluidics would require fluidic handling to interface the solution under analysis with the microfluidic network and carry the solution into a reaction zone so that this solution is made to contact a molecular recognition layer, which selectively captures the analyte; in addition, a transduction mechanism is required to allow the conversion of the event of molecular capture into a signal (usually electronic) that can then be processed and displayed. In general, this simple implementation alone is not sufficient for a successful analysis: it may be necessary to add stages of

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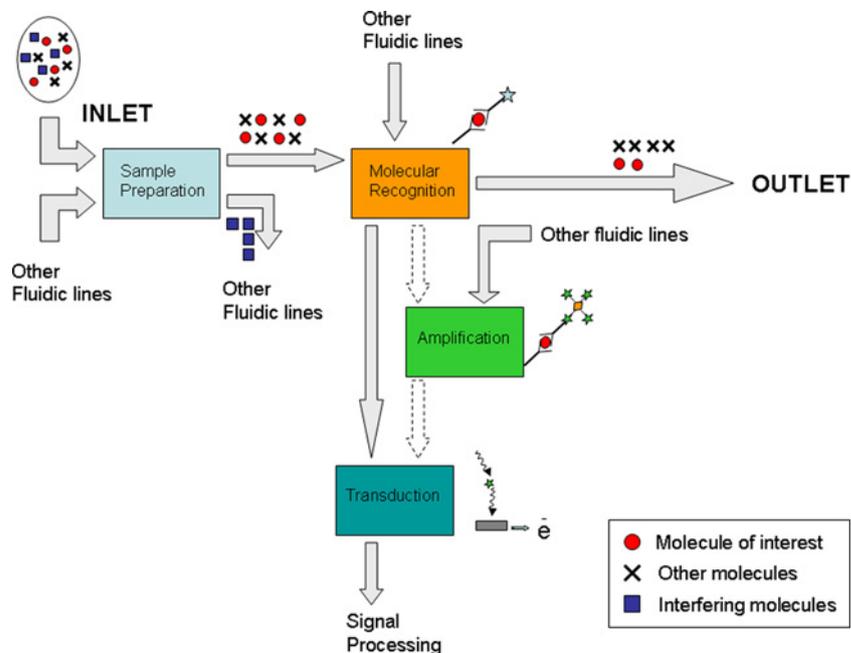
Figure 1. Schematic diagram of an integrated LoC biosensor

A major challenge is to develop a microfluidic-based platform that can carry out these different steps while keeping the requirements of performance, portability and cost.

sample preparation that can (i) remove contaminant or interfering molecules, (ii) extract or expose the analyte, e.g. from within a cell, and/or (iii) contribute to the pre-concentration of the analyte of interest. Often, only trace levels of target analytes are present in the samples, which may also require a strategy of signal amplification to increase the sensitivity of the assay. Figure 1 presents a schematic diagram of an integrated LoC system that includes all of these different components, which will be discussed in the following sections.

Fluidic handling

In an LoC microfluidic network, the flow rates must be controlled in every location of the network and at every given time of the assay. How this spatial and temporal control is implemented has a profound impact on the design of the overall integrated system. The first key choice is whether the fluidic pumping will be achieved using an external pump or pumped internally by capillarity. The large surface-to-volume ratio of miniaturized fluidic systems, compared with their macroscopic counterparts, allows the efficient design of integrated capillary pumps [8,9]. External pumps (such as syringe or peristaltic pumps) allow a wider range of flow rates, higher operation times, and no dependence on the detailed chemical and physical properties of the inner microchannel surfaces. Capillary pumps are autonomous and require no connections to exterior mechanical systems and hence are particularly suitable for simple inexpensive disposable miniaturized analytical systems. The second key choice is whether or not integrated valves should be part of the system. Integrated valves allow active on-chip flow control, which may be required for complex fluidic handling, but significantly increases the complexity of the microfluidic device and its control system [10,11]. For ultimate fluidic control, and only desirable in complex fluidic chips for high-value applications, the pumping itself could be integrated on-chip [10,12]. In addition to the pumping and valving, along with their integration into the fluidic system, the next aspect of fluidic handling, which requires attention for the precise detection of cellular and molecular events, relies on molecule and cell manipulation and detection with these systems, including single-molecule and single-cell addressing [13]. Such high-precision bioanalytical applications using microfluidic systems range from high-throughput
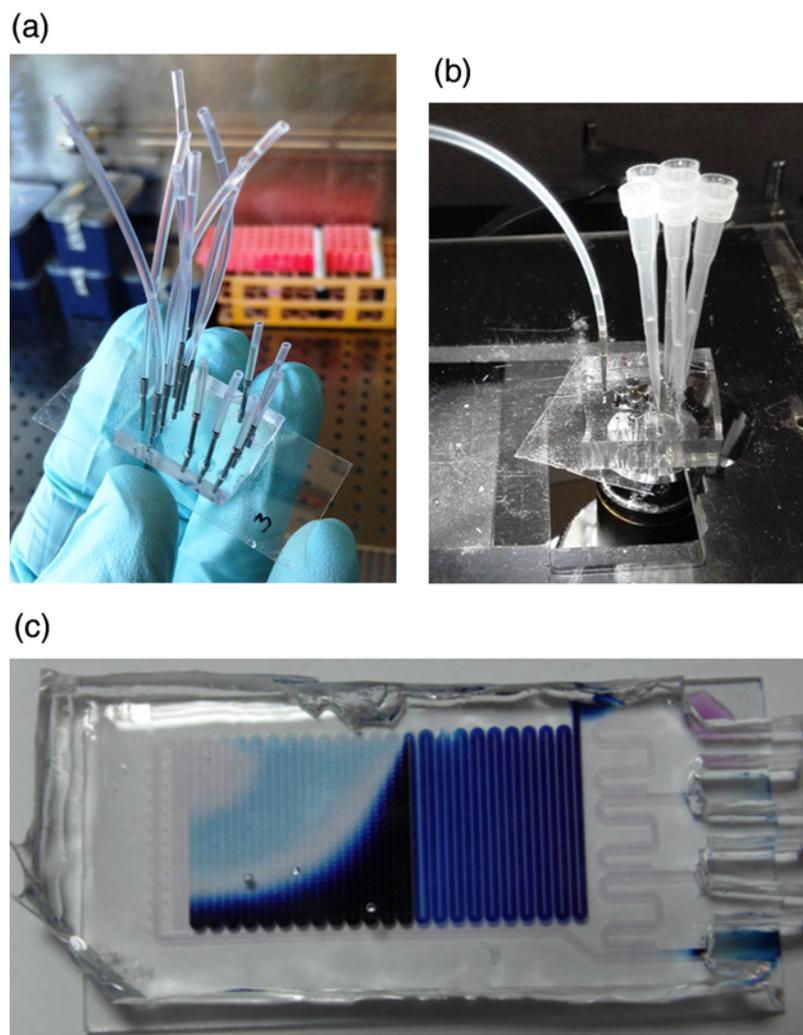
Figure 2. Typical research devices which use a set of tubes to allow the insertion of fluids and valve control

(**a**) A microfluidic device for cell manipulation with integrated pneumatic valves and external pumping. The connections on the left are for valve control. The others are fluidic connections for cell and medium insertion and removal. (**b**) A microfluidic device for multiplex toxin detection with external pumping. The inlet connections are the pipette tips on the right, and there is a single negative pressure (suction) pumping on the left. Magnetic valves can be seen in the centre of the chip. (**c**) A capillary device with a sequence of integrated pumps that allow for different flow rates. The fluids are inserted on the right, and a coloured liquid has been pumped by a two-level capillary pumping scheme.

single-molecule analysis [14] and single-molecule polymerase chain reaction (PCR) [15] to cellular micropatterning [16] and the generation of protein interaction networks [17].

The discussion in the previous paragraph has already hinted that, for biosensing, another crucial design consideration is its interface with the outside world, and how this type of interface matches the particular requirements of the application in terms of allowable complexity and cost. It can be argued that one of the major difficulties faced in the market implementation of LoC biosensors is the complexity and lack of standardization of the fluidic connections between the chip and the outside world. A typical research device such as those shown in Figures 2(a) and 2(b) uses a set of tubes that allow the insertion of fluids and the control of valves. The effort, care and technique required to attach and couple to these tubes without compromising both the device and the analysis is not compatible with a practical commercial operator-independent microfluidic analysis system. Even a capillary chip requires an operator to place the different fluids needed for the analysis at the inlet to the chip, either with a manual pipette (Figure 2c) or a robotic

apparatus, and, although automated microfluidic systems exist, they require both complex fluidic connections and custom-made controllers [18].

Target capture

In the previous section, the need to achieve spatial and temporal control of the flow rates in all locations of the microfluidic network was highlighted. This need for fluidic control is crucial to what is perhaps the key event of the biosensing process: the capture of the target analyte by an immobilized probe. This capture is based on the principle of molecular recognition – the probe and target have a set of specific spatial, chemical and physical interactions that should, under optimized conditions, simultaneously give a high affinity for the specific probe–target binding, and a high selectivity with respect to other (possibly very similar) molecules that may be present in the solution.

The probe – which is usually a molecule but can also be an entire cell [19–21] – is immobilized at the capture area of the microfluidic network, most commonly on the channel surface. There are two main considerations that are necessary to understand the target capture process [22–24]. The first is that the target must reach the probe molecule, which occurs most commonly by diffusion to the channel surface. In microfluidic systems, this diffusion usually occurs as the target-bearing solution flows past the immobilized probe at a constant flow rate. For typically used flow rates of the order of μl/min, channel cross sections with widths of the order of a few hundred micrometres and heights of the order of a few tens of micrometres, the transport is dominated by the convection process along the channel, and only a small fraction of the target molecules reach the surface by diffusion (Péclet numbers in the hundreds to thousands). The second important consideration is that, once the target molecule has reached the surface, it has to interact with the probe. This interaction with the probe is modelled as an equilibrium process, and here the dissociation constant K_D of the probe–target complex is the main parameter. K_D has the dimensions of a concentration, and this number indicates a target concentration above which essentially all the probes will be saturated with target molecules and below which the fraction of occupied probes in equilibrium will be correspondingly reduced [22–24].

The discussion in the previous paragraph results in two important observations for the design of an LoC assay: the first is that the flow rates should be such that the target capture is reaction-limited (and not diffusion-limited); and the second is that a lower value of K_D (and, correspondingly, a higher value for the probe–target affinity constant $K_A = 1/K_D$) is very advantageous if high sensitivity is desired. The first condition, expressed through the dimensionless Damköhler numbers (Da) that relate the reaction rate with the transport rate, is usually observed in microfluidics. The second condition depends on the characteristics of the probe selected for molecular recognition and target capture. Note that the probe affinity to the target may also be modulated by the immobilization procedure, for example if changes of affinity occur due to conformal changes or if there are physical limitations in the access to the binding sites. For a particular target, there are a number of probes that can be considered: antibodies, antibody fragments, peptamers, aptamers, etc. The necessary condition for the sensitivity of these probes is an appropriate value of K_D. Although the dissociation constant affects the relative amount of recognition sites occupied at equilibrium, the absolute signal measured is also a function of the total amount of probes. Another key parameter in probe evaluation is its tendency to bind to non-target molecules that may be present in the solution. These non-specific interactions will severely decrease the signal-to-noise ratio of the assay and limit its sensitivity. The same effect may also be derived from the non-specific binding of signalling molecules to non-passivated regions of the substrate. Thus, it is also important to select an appropriate strategy to ensure a complete passivation of the free/unoccupied surface using, for example, a blocking agent such as bovine serum albumin (BSA), casein or salmon sperm DNA. Figure 3 shows the dose–response curve for the capture of prostate-specific antigen (PSA) for a fixed amount of immobilized probes using a microfluidic platform [25].

In this section, the capture of the target by an immobilized probe was discussed. Note that there was no mention of the origin of the target-containing solution. This solution could be the untreated sample containing the molecule of interest, or the integrated LoC system may include sample preparation modules. Sample preparation steps, which are often required to remove molecules that interfere with or even impair the molecular recognition process, or to achieve a pre-concentration of the target molecule to enhance sensitivity, will be discussed in a later section.

Transduction

In the previous section, the capture of the target by an immobilized probe through a molecular recognition process was discussed. Although central to the biosensing process, this stage does not complete the assay – one needs now
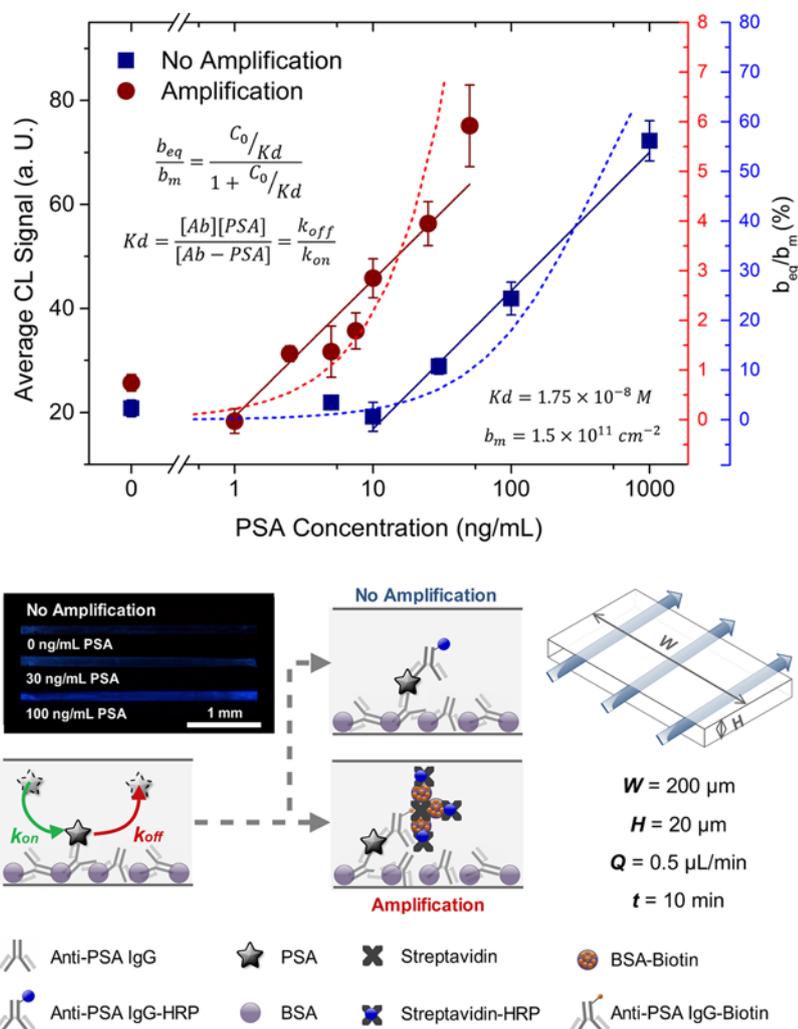
Figure 3. Microfludic detection of prostate-specific antigen (PSA)

A capture antibody (anti-PSA IgG) is adsorbed on the surface of the microchannel. In the 'No Amplification' curve, the signal is obtained by the chemiluminescence generation from an HRP-labelled anti-PSA IgG that binds to a different epitope of the PSA molecule than that used for capture molecular recognition. A strategy for signal amplification is schematically highlighted at the bottom, and the corresponding signal indicated in the 'Amplification' curve. The dashed curves correspond to the theoretical predictions in terms of ratio of probes occupied in equilibrium (b_{eq}) per total number of probes on the surface (b_m) using the equation shown in the plot [17]. From the theoretical analysis it can be observed that for constant K_d and b_m values, the amplification strategy provides an absolute chemiluminescence (CL) signal 10-fold higher than in non-amplified conditions for the PSA concentrations providing the same b_{eq}.

to transduce this molecular event into a physical signal that can be measured. The selected mode of transduction is central to the overall design of the integrated LoC system. The impact of the mode of transduction goes beyond the simple integration of the transducer into the system. It requires careful co-design with the other stages of the assay such as the fluidic handling, sample preparation, and, naturally, the target capture and signal amplification stages.

A vast number of transduction methods can be found in the literature [4,26–29], many of which are discussed in this volume. Often, these methods are first classified as either 'label-free' methods, or methods involving a label. A label-free method involves only the target molecule of interest and does not require further action beyond the process of target capture by the immobilized probe. Examples of label-free methods include the use of surface plasmon resonance (SPR), sensitive to changes in the complex dielectric constant in the channel in the vicinity of typically a gold (Au) layer [30,31]; field-effect transistors (FETs) in which the charges of captured molecules
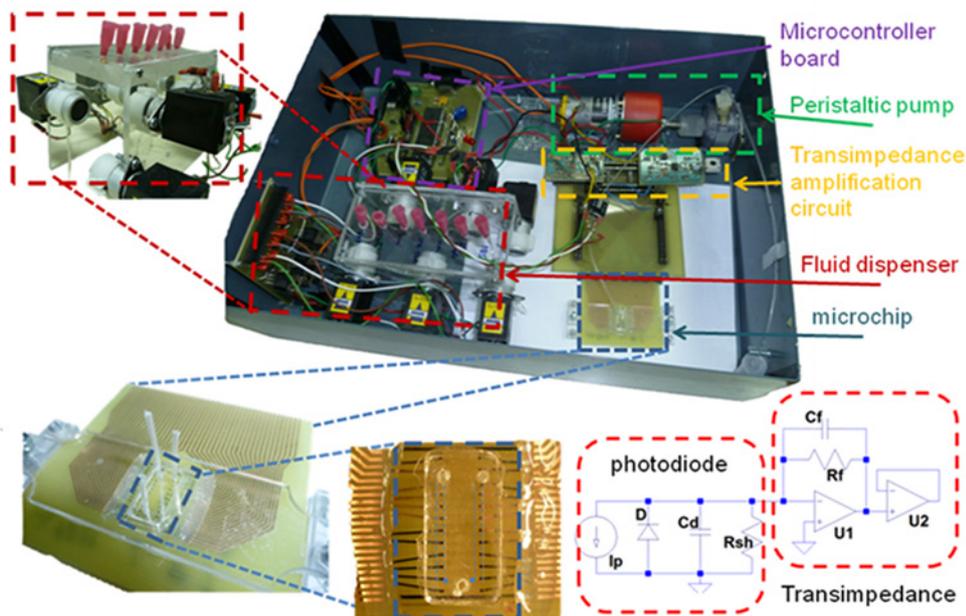
Figure 4. Picture of a prototype integrated LoC system showing its main components

A close-up of the microchip (bottom) shows the U-shaped microfluidic chamber aligned with two rows of photodiodes located underneath. Schematic diagrams of the transimpedance circuit with feedback resistor R_f and capacitor C_f together with the simulated photodiode with current source I_p, diode D, diode capacitor C_d and shunt resistor R_{sh}.

modulate the electrical conductivity of a film or nanowire [32,33]; and MEMs, in which the change of mass or mechanical stress due to the capture of molecules is monitored [34–36]. The gold standard for labelled transduction methods is a miniaturized version of micro-well plate immunoassays: after the capture of the target, a secondary probe – usually an antibody – that recognizes a free region of the target is circulated in the microchannel and captured by the bound target. This secondary probe is linked to a fluorophore, or to an enzyme (enzyme-linked immunosorbent assay – ELISA). What is detected is either the fluorescence from the fluorophore, or the chemiluminescence or optical absorption produced by reactions of the enzymatic label. Many other labels have been proposed, such as magnetic nanoparticles [37–39] or redox-active molecules [40–44]. A main issue with label-free methods is that, in general, any non-specifically adsorbed species contributes to the signal, whereas labelled methods offer an additional step of specificity (at the cost of a more complex sequence of analysis steps and the use of more chemicals – with the corresponding increased cost and analysis time). As will be discussed in a later section, labels often contribute to a more effective transduction – for example, when an enzymatic label such as horseradish peroxidase (HRP) is used, and a tetramethylbenzidine (TMB) substrate is flowed, a single enzyme produces approximately 7000 highly optically absorbing product molecules that precipitate in the channel and can be detected by transmission measurements [45].

A second consideration in the design of the integrated LoC system is whether or not the sensor must be in physical contact with the solution containing the target. Transduction methods such as SPR, MEMs or electrochemical redox measurements all require physical contact. Other methods, which, although, in principle, could be contact-free, require such short distances between the molecular recognition event and the transducer as to make them essentially contact methods (among these are magnetic detection of superparamagnetic nanoparticle labels, and FET and impedance measurements). In general, optical measurements do not require contact between the channel and the photodetector. This issue is of great relevance in the microfluidic chip design and in the interface between the chip and the addressing electronics: an optical sensor can be a fixed part of a detection system involving a disposable microfluidic chip, while an SPR disposable microfluidic chip must have a patterned Au surface and a redox transducer microfluidic chip that must be electrically interfaced with the detection system. Figure 4 shows a laboratory prototype microfluidic system. A macro-scale fluid dispenser and a peristaltic pump are used for fluid handling, and an array of thin-film silicon photodiodes are used to transduce the molecular reaction event that occurs on the

straight microfluidic channels via the acquisition of the resulting chemiluminescence photons. Note the additional contribution of the signal handling components (transimpedance amplification circuit) and the overall control and communication centre of the system (microcontroller board), which are not discussed in this review.

To close this section, a final remark needs to be made regarding the importance of considering and controlling non-specific binding in the molecular recognition/transduction section of the microfluidic chip. Often, the ultimate sensitivity of the system is considered as the ability to detect a single molecular recognition event, and the transducer system is designed to try to approach this limit. Nevertheless, one should keep in mind that there is considerable interference by either other molecules in solution or by the secondary detection probes themselves that result in an important non-specific signal. This non-specific signal, always present in realistic biosensing situations, is usually a key factor in determining the sensitivity of the integrated LoC system.

Sample preparation

The majority of the literature on LoC systems for biosensing in medical, environmental, food/feed safety and other applications focus on strategies for target capture and transduction. The analytical target is usually in a well-defined buffer solution. On the other hand, standard well-established macro-analytical processes for these applications usually involve a complex series of steps to prepare the samples for analysis. This is because in real samples the target molecules are never in isolation. There is a complex molecular and cellular matrix that will severely interfere with the target capture and transduction. An integrated 'from sample-to-answer' LoC system will have to include a stage of sample preparation upstream from target capture (as indicated in Figure 1). Such a stage will increase the complexity of the microfluidic network, fluidic handling, and interface between the chip and the control box.

As with typical benchtop analytical procedures, different samples and or analytes of interest require different preparation methods. Hence it is clear that one cannot develop a 'universal sample preparation stage' that can be integrated to subsequent stages of an integrated LoC system. Since there is an enormous variability of matrix/target analyte pairs that can be considered, it seems that, in the near future, the research on microfluidic integrated sample preparation processes will focus on a case-by-case basis. For example, in blood analysis, separation of the cells from the plasma or serum is often required because of interference with optical transduction; in the analysis of mycotoxins in wine, the polyphenols present can complex with the antibodies and must be removed; and in the analysis of milk, it is the relatively high content of lipid molecules that interferes with the target capture and transduction [46–48]. A few strategies have been implemented and are worth mentioning, such as the use of integrated filters on the chip [49,50], the liquid-solid capture of either the interferents or the target in large 3D areas in the chip, achieved via nanostructuring or the use of packed micro- or nano-beads [51]; the use of partitioning in integrated liquid-liquid extraction techniques, in particular, using immiscible fluids or aqueous two-phase systems (ATPSs) [52,53]; separation using either magnetic or electrical fields, in particular, dielectrophoresis (DEP) [54,55]; or even the extraction and purification of DNA from cells [56]. For example, Figure 5 shows an integrated ATPS sample preparation module that is used for the upstream cleaning of a raw wine sample prior to an indirect competitive immunoassay performed within the same microchannel network, for detecting ochratoxin A (OTA) (Figure 5a) [53]. This immunoassay can be transduced using either a fluorophore or an enzyme, via luminol oxidation-induced chemiluminescence, which is then acquired by an integrated photodetector (Figures 5b and 5c).

However, in the many cases that the required analysis starts from a solid source – for example, the detection of toxins in cereals – it is hard to avoid the necessity of an off-chip extraction process, which should be simple, fast and require as little laboratory equipment as possible. Another aspect is that a sample preparation stage may also aim at target pre-concentration, which may be required in samples where the target is present in highly diluted concentrations. Examples of pre-concentration approaches include work on in-line microfluidic pre-concentration using electrophoretic principles or miniaturized solid-phase extraction (SPE) by packing micron-sized beads in microchannels or fabricating high-density monolithic features [57].

Amplification

The analysis design in an integrated LoC system always involves a set of compromises. These compromises may be 'external', such as considerations related to cost or complexity or 'internal', involving intrinsic limitations in the
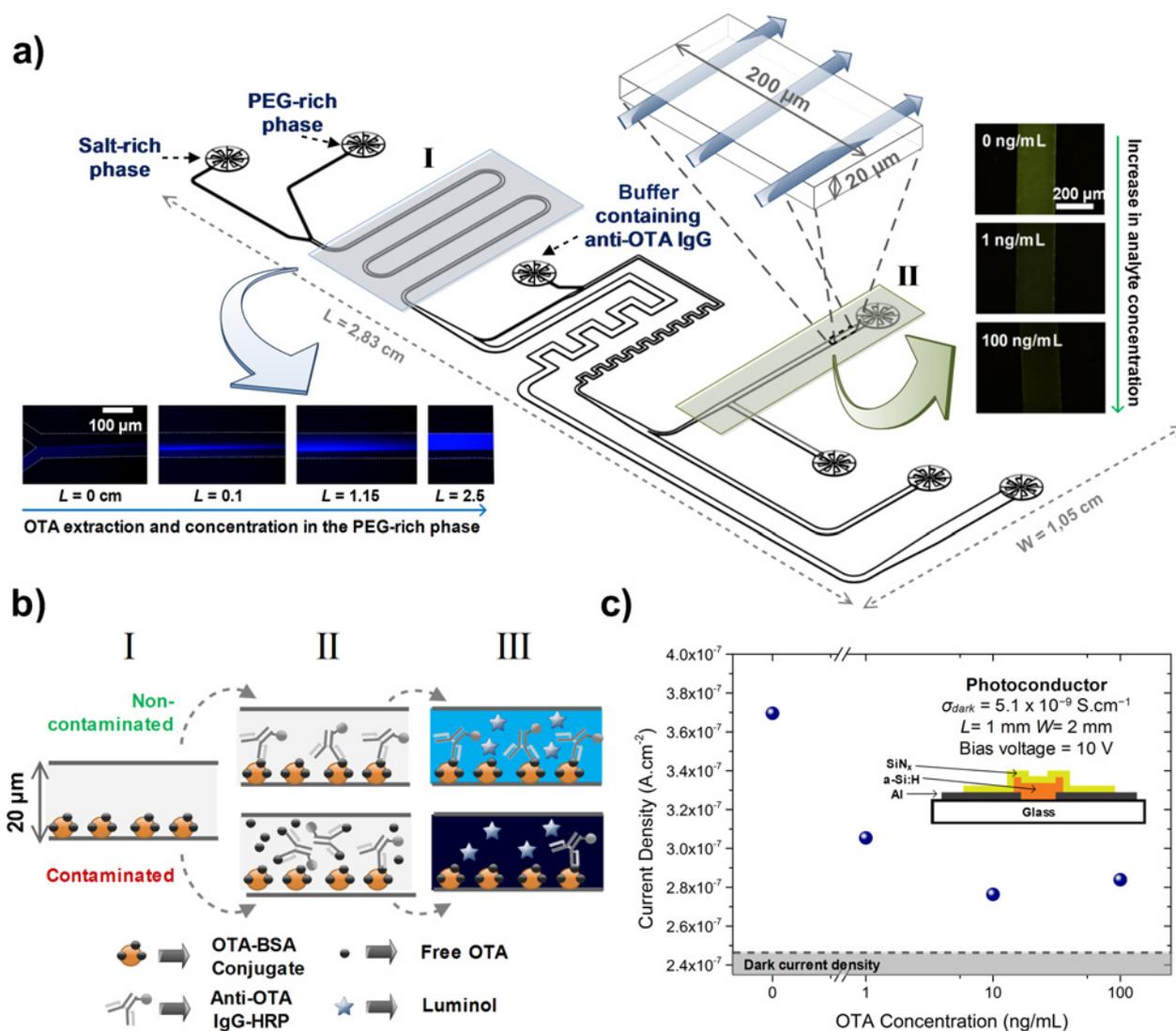
Figure 5. An integrated ATPS sample preparation module used for the upstream cleaning of a raw wine sample

(**a**) Schematic diagram of a microfluidic network with an OTA extraction and concentration ATPS, followed by a stage where an indirect competitive ELISA (as schematically detailed in (**b**) is used to quantify the OTA. A miniaturized photodetector can be integrated in the molecular recognition area to transduce the labelled fluorescence or chemiluminescence (**c**).

performance of one or more stages (such as the limited removal of interfering species in the sample preparation stage, or limited affinity of the probe to the target), and may result in a limited range of analytical sensitivity.

Recently, considerable attention has been devoted to designing processes that can amplify the signal from each individual molecular recognition event. This amplification will result in an increase in assay sensitivity.

There are many amplification strategies being developed [58–60]: from secondary probes connected to a polymer chain carrying several fluorophore molecules, to self-assembled structures that grow from the secondary probe and include several fluorophores, enzymes or nanoparticles (as shown in Figure 3), to metal nanoparticles connected to a secondary probe to enhance SPR or mass-detection transduction, to metal–fluorophore plasmonic interactions [61–63]. Such intrinsic amplification strategies targeting assay redesign and engineering might go a long way in reaching clinically and biologically desired sensitivities. The gain in the signal resulting from such approaches could be intelligently tagged with novel high-sensitive and design-specific tools such as real-time single molecule detectors [64–67], thus complementing the methods mentioned in the Transduction section, aimed at further enhancement in resolution and detection.

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As in the engineering design of an integrated LoC system, there are also trade-offs in these amplification strategies. The gain in sensitivity comes at an increase in the cost of analysis by requiring extra chemicals, and an increase in time and complexity. Each added step will also increase the chance of errors and decrease reliability. The inclusion of a signal amplification stage needs to be carefully pondered within the context of the requirements of the specific application.

Conclusions

The design of an integrated LoC analysis system takes as a starting point a set of specifications, such as the starting sample, the relevant target(s) detection range, portability requirements, desired level of operator competence, required time of analysis and the target cost per analysis. Based on these specifications, the design will consider the different stages described in this review: fluidic handling, target capture, transduction, sample preparation and amplification. The analysis systems described can also be viewed as modules that can be coupled to other microfluidic platforms. To achieve a truly integrated lab-on-a-chip, it is possible to have a biosensor area in a device designed for cell or tissue culture and analysis. In this way, cell culture, assays and read-outs can be performed in a single system, as opposed to the several bulky and expensive instruments that are nowadays common in the biology laboratory. For a newcomer to the field, there are a bewildering variety of technical choices, but in the horizon of a decade, the perspective is the accelerated development of a well-established set of procedures or standards for the analysis and synthesis of integrated microfluidic analytical systems (as is the case, for example, in electronics). These procedures will bridge microfluidic mechanics, bioanalytical chemistry, sensors and transducers, molecular biology, signal processing and electronic engineering.

Summary

- Integrated lab-on-chip (LoC) systems are required for fast, portable, simple-to-operate and cost-effective 'sample-to-answer' analysis in the medical, veterinarian, food and feed and environmental sectors.

- The design of integrated LoC systems requires a combination of fluidic handling, sample preparation, target capture by molecular recognition, transduction of target capture to a measureable signal and, possibly, amplification of the target signal.

- The particular design implementation will depend on the analysis requirements (such as starting sample, analytical range sought, portability requirements, required speed of analysis, number of targets to analyse, level of operator qualification and cost limits).

Abbreviations

ATPS, aqueous two-phase system; FET, field-effect transistor; HRP, horseradish peroxidase; LoC, lab-on-chip; MEMS, microelectromechanical system; OTA, ochratoxin A; PSA, prostate-specific antigen; SPR, surface plasmon resonance.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Madou, M.J. (2002) Fundamentals of microfabrication: the science of miniaturization, 2nd edn, CRC Press, Boca Raton
2 Judy, J.W. (2001) Microelectromechanical systems (MEMS): fabrication, design and applications. Smart Mater. Struct. **10**, 1115–1134 CrossRef

3   Whitesides, G. (2014) The lab finally comes to the chip. Lab Chip **14**, 3125–3126 CrossRef PubMed

4   Kelley, S.O., Mirkin, C.A., Walt, D.R., Ismagilov, R.F., Toner, M. and Sargent, E.H. (2014) Advancing the speed, sensitivity and accuracy of biomolecular detection using multi-length-scale engineering. Nat. Nanotechnol. **9**, 969–980 CrossRef PubMed

5   Sackmann, E.K., Fulton, A.L. and Beebe, D.J. (2014) The present and future role of microfluidics in biomedical research. Nature **507**, 181–189 CrossRef PubMed

6   Atalay, Y.T., Vermeir, S., Witters, D., Verbruggen, B., Verboven, P., Nicolai, B.M. et al. (2011) Microfluidic analytical systems for food analysis. Trends Food Sci. Technol. **22**, 386–404 CrossRef

7   Foudeh, A.M., Fatanat Didar, T., Veres, T. and Tabrizian, M. (2012) Microfluidic designs and techniques using lab-on-chip devices for pathogen detection for point-of-care diagnostics. Lab Chip **12**, 3249–3266 CrossRef PubMed

8   Zimmermann, M., Schmid, H., Hunziker, P. and Delamarche, E. (2007) Capillary pumps for autonomous capillary systems. Lab Chip **7**, 119–125 CrossRef PubMed

9   Li, X., Ballerini, D.R. and Shen, W. (2012) A perspective on paper-based microfluidics: current status and future trends. Biomicrofluidics **6**, 011301 CrossRef

10 Unger, M.A., Chou, H.P., Thorsen, T., Scherer, A. and Quake, S.R. (2000) Monolithic valves and pumps by multilayer soft lithography. Science **288**, 113–116 CrossRef PubMed

11 Kwang, W.O. and Chong, H.A. (2006) A review of microvalves. J. Micromech. Microeng. **16**, R13–R39 CrossRef

12 Iverson, B.D. and Garimella, S.V. (2008) Recent advances in microscale pumping technologies: a review and evaluation. Microfluid. Nanofluid. **5**, 145–174 CrossRef

13 Zhao, Y., Chen, D., Yue, H., French, J.B., Rufo, J., Benkovic, S.J. et al. (2013) Lab-on-a-chip technologies for single-molecule studies. Lab Chip **13**, 2183–2198 CrossRef PubMed

14 Kim, S., Streets, A.M., Lin, R.R., Quake, S.R., Weiss, S. and Majumdar, D.S. (2011) High-throughput single-molecule optofluidic analysis. Nat. Methods **8**, 242–245 CrossRef PubMed

15 Leng, X., Zhang, W., Wang, C., Cui, L. and Yang, C.J. (2010) Agarose droplet microfluidics for highly parallel and efficient single molecule emulsion PCR. Lab Chip **10**, 2841–2843 CrossRef PubMed

16 Madaboosi, N., Uhlig, K., Schmidt, S., Jaeger, M.S., Moehwald, H., Duschl, C. et al. (2012) Microfluidics meets soft layer-by-layer films: selective cell growth in 3D polymer architectures. Lab Chip **12**, 1434–1436 CrossRef PubMed

17 Gerber, D., Maerkl, S.J. and Quake, S.R. (2009) An *in vitro* microfluidic approach to generating protein-interaction networks. Nat. Methods **6**, 71–74 CrossRef PubMed

18 Gómez-Sjöberg, R., Leyrat, A.A., Pirone, D.M., Chen, C.S. and Quake, S.R. (2007) Versatile, fully automated, microfluidic cell culture system. Anal. Chem. **79**, 8857–8563

19 Bousse, L. (1996) Whole cell biosensors. Sens. Actuators B Chem. **34**, 270–275 CrossRef

20 Sorenen, S.J., Burmolle, M. and Hansen, L.H. (2006) Making bio-sense of toxicity: new developments in whole-cell biosensors. Curr. Opin. Biotechnol. **17**, 11–16 CrossRef

21 Pasco, N.F., Weld, R.J., Hay, J.M. and Gooneratne, R. (2011) Development and applications of whole cell biosensors for ecotoxicity testing. Anal. Bioanal. Chem. **400**, 931–945 CrossRef PubMed

22 Squires, T.M., Messinger, R.J. and Manalis, S.R. (2008) Making it stick: convection, reaction and diffusion in surface-based biosensors. Nat. Biotechnol. **26**, 417–426 CrossRef PubMed

23 Gervais, T. and Jensen, K.F. (2006) Mass transport and surface reactions in microfluidic systems. Chem. Eng. Sci. **61**, 1102–1121 CrossRef

24 Zimmerman, M., Delamarche, E., Wolf, M. and Hunziker, P. (2005) Modeling and optimization of high-sensitivity, low-volume microfluidic-based surface immunoassays. Biomed. Microdevices **7**, 99–110 CrossRef PubMed

25 Madaboosi, N., Soares, R.R.G., Chu, V. and Conde, J.P. (2015) A microfluidic immunoassay platform for the detection of free prostate specific antigen: a systematic and quantitative approach. Analyst **140**, 4423–4433 CrossRef PubMed

26 Gopinath, S.C.B., Tang, T.H., Cirtartan, M., Chen, Y. and Lakshmipriya, T. (2014) Current aspects in immunosensors. Biosens. Bioelectron. **57**, 292–302 CrossRef PubMed

27 Myers, F.B. and Lee, L.P. (2008) Innovations in optical microfluidic technologies for point-of-care diagnostics. Lab Chip **8**, 2015–2031 CrossRef

28 Cumhur Tekin, H. and Gijs, M.A.M. (2013) Ultrasensitive protein detection: a case for microfluidic magnetic bead-based assays. Lab Chip **13**, 4711–4739 CrossRef PubMed

29 Choi, S., Goryll, M., Sin, L.Y.M., Wong, P.K. and Chae, J. (2011) Microfluidic-based biosensors toward point-of-care detection of nucleic acids and proteins. Microfluid. Nanofluid. **10**, 231–247 CrossRef

30 Guo, X. (2012) Surface plasmon resonance based biosensor technique: a review. J. Biophotonics **5**, 483–501 CrossRef PubMed

31 Lee, H.J., Nedelkov, D. and Corn, R.M. (2006) Surface plasmon resonance imaging measurements of antibody arrays for the multiplexed detection of low molecular weight protein biomarkers. Anal. Chem. **78**, 6504–6510 CrossRef PubMed

32 Patolsky, F. and Lieber, C.M. (2005) Nanowire nanosensors. Mater. Today **8**, 20–28 CrossRef

33 Choi, K., Kim, J-Y., Ahn, J-H., Choi, J-M., Im, M. and Choi, Y-K. (2012) Integration of field effect transistor-based biosensors with a digital microfluidic device for a lab-on-a-chip application. Lab Chip **12**, 1533–1539 CrossRef PubMed

34 Kim, Y.I., Park, T.S., Kang, J.H., Lee, M.C., Kim, J.T., Park, J.H. et al. (2006) Biosensors for label free detection based on RF and MEMS technology. Sens. Actuators B Chem. **119**, 592–599 CrossRef

35 Arlett, J.L., Myers, E.B. and Roukes, M.L. (2011) Comparative advantages of mechanical biosensors. Nat. Nanotechnol. **6**, 203–215 CrossRef PubMed

36 Haun, J.B., Yoon, T-J., Lee, H. and Weissleder, R. (2010) Magnetic nanoparticle biosensors. WIREs Nanomed. Nanobiotechnol. **2**, 291–304 CrossRef

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37 Patil, S.B., Vögtli, M., Webb, B., Mazza, G., Pinzani, M., Soh, Y. et al. (2015) Decoupling competing surface binding kinetics and reconfiguration of receptor footprint for ultrasensitive stress assays. Nat. Nanotechnol. **10**, 899–907
      CrossRef PubMed

38 Fernandes, A.C., Duarte, C.M., Cardoso, F.A., Bexiga, R., Cardoso, S. and Freitas, P.P. (2014) Lab-on-chip cytometry based on magnetoresistive sensors for bacteria detection in milk. Sensors **14**, 15496–15524 CrossRef PubMed

39 Niemirowicz, K., Markiewicz, K.H., Wilkzewska, A.Z. and Car, H. (2012) Magnetic nanoparticles as new diagnostic tools in medicine. Adv. Med. Sci. **57**, 196–207 CrossRef PubMed

40 Willner, I.I. and Katz, E. (2000) Integration of layered redox proteins and conductive supports for bioelectronic applications. Angew. Chem. Int. Ed. Engl. **39**, 1180–1218 CrossRef PubMed

41 Chen, I-J. and White, I.M. (2011) High-sensitivity electrochemical enzyme-linked assay on a microfluidic interdigitated microelectrode. Biosens. Bioelectron. **26**, 4375–4381 CrossRef

42 Dechtrirat, D., Gajovic-Eichelmann, N., Wojcik, F., Bier, F.F. and Scheller, F.W. (2014) Electrochemical displacement sensor based on ferrocene boronic acid tracer and immobilized glycan for saccharide binding proteins and *E. coli*. Biosens. Bioelectron. **58**, 1–8 CrossRef PubMed

43 Peng, L., Utesch, T., Yarman, A., Jeoung, J.H., Steinborn, S., Dobbek, H., Mroginski, M.A., Tanne, J., Wollenberger, U. and Scheller, F.W. (2015) Surface-tuned electron transfer and electrocatalysis of hexameric tyrosine-coordinated heme protein. Chemistry **21**, 7596–7602 CrossRef PubMed

44 Chunglok, W., Khownrumit, P., Rijiravanich, P., Somasundrum, M. and Surareungchai, W. (2011) Electrochemical immunoassay platform for high sensitivity protein detection based on redox-modified carbon nanotube labels. Analyst **136**, 2969–2974
      CrossRef PubMed

45 Gouvêa, A., Pereira, A.T., Pimentel, A.C., Prazeres, D.M.F., Chu, V. and Conde, J.P. (2008) Colorimetric detection of molecular recognition reactions with an enzyme biolabel using a thin-film amorphous silicon photodiode on a glass substrate. Sens. Actuators B Chem. **135**, 102–107 CrossRef

46 Song, Y., Sauret, A. and Shum, H.Ch. (2013) All-aqueous multiphase microfluidics. Biomicrofluidics **7**, 061301 CrossRef

47 Kersaudy-Kerhoas, M. and Sollier, E. (2013) Micro-scale blood plasma separation: from acoustophoresis to egg-beaters. Lab Chip **13**, 3323–3346 CrossRef PubMed

48 Labuz, J.M. and Takayama, S. (2014) Elevating sampling. Lab Chip **14**, 3165–3171 CrossRef PubMed

49 Broyles, B.S., Jacobson, S.C. and Ramsey, J.M. (2003) Sample filtration, concentration and separation integrated on microfluidic devices. Anal. Chem. **75**, 2761–2767 CrossRef PubMed

50 Andersson, H., Ahmadian, A., van der Wijngaart, W., Nilsson, P., Enoksson, P., Uhlen, M. et al. (2000) Micromachined flow-through filter-chamber for solid phase DNA analysis. In Micro Total Analysis Systems 2000 (van der Berg, A., Olthuis, W. and Bergveld, P., eds), pp. 473–476, Springer, Dordrecht CrossRef

51 Oleschuk, R.D., Shultz-Lockyear, L.L., Ning, Y. and Harrison, D.J. (2000) Trapping of bead-based reagents within microfluidic systems: On-chip solid-phase extraction and electrochromatography. Anal. Chem. **72**, 585–590 CrossRef PubMed

52 Kralj, J.G., Sahoo, H.R. and Jensen, K.F. (2007) Integrated continuous microfluidic liquid-liquid extraction. Lab Chip **7**, 256–263 CrossRef PubMed

53 Soares, R.R.G., Novo, P., Azevedo, A.M., Fernandes, P., Aires-Barros, M.R., Chu, V. et al. (2014) On-chip sample preparation and analyte quantification using a microfluidic aqueous two-phase extraction coupled with an immunoassay. Lab Chip **14**, 4284–4294 CrossRef PubMed

54 Pommer, M.S., Zhang, Y., Keerthi, N., Chen, D., Thomson, J.A., Meinhart, C.D. et al. (2008) Dielectrophoretic separation of platelets from diluted whole blood in microfluidic channels. Electrophoresis **29**, 1213–1218 CrossRef PubMed

55 Cetin, B. and Li, D. (2011) Dielectrophoresis in microfluidics technology. Electrophoresis **32**, 2410–2427 CrossRef PubMed

56 Hong, J.W., Studer, V., Hang, G., Anderson, W.F. and Quake, S.R. (2004) A nanoliter-scale nucleic acid processor with parallel architecture. Nat. Biotechnol. **22**, 435–439 CrossRef PubMed

57 Giordano, B.C., Burgi, D.S., Hart, S.J. and Terray, A. (2012) On-line sample pre-concentration in microfluidic devices: a review. Anal. Chim. Acta **718**, 11–24 CrossRef PubMed

58 Escosura-Muniz, A., Parolo, C. and Merkoci, A. (2010) Immunosensing using nanoparticles. Mater. Today **13**, 24–34 CrossRef

59 Tansil, N.C. and Gao, Z. (2006) Nanoparticles in biomolecular detection. Nano Today **1**, 28–37 CrossRef

60 Anker, J.N., Hall, W.P., Lyandres, O., Shah, N.C., Zhao, J. and van Duyne, R.P. (2008) Biosensing with plasmonic nanosensors. Nat. Mater. **7**, 442–453 CrossRef PubMed

61 Kedia, N., Roy, S.G., De, P. and Bagchi, S. (2014) Synthesis of a polymer bearing several coumarin dyes along the side chain and study of its fluorescence in pure and binary solvent mixtures as well as aqueous surfactant solutions. J. Phys. Chem. B **118**, 4683–4692 CrossRef PubMed

62 Robin, M.P. and O'Reilly, R.K. (2015) Strategies for preparing fluorescently labelled polymer nanoparticles. Polym. Int. **64**, 174–182 CrossRef

63 Guo, L., Jackman, J.A., Yang, H., Chen, P., Cho, N. and Kim, D. (2015) Strategies for enhancing the sensitivity of plasmonic nanosensors. Nano Today **10**, 213–239 CrossRef

64 Uemura, S., Aitken, C.E., Korlach, J., Flusberg, B.A., Turner, S.W. and Puglisi, J.D. (2010) Real-time tRNA transit on single translating ribosomes at codon resolution. Nature **464**, 1012–1017 CrossRef PubMed

65 Clarke, J., Wu, H.-C., Jayasinghe, L., Patel, A., Reid, S. and Bayley, H. (2009) Continuous base identification for single molecule nanopore DNA sequencing. Nat. Nanotechnol. **4**, 265–270 CrossRef PubMed

66 Wanunu, M., Dadosh, T., Ray, V., Jin, J., McReynolds, L. and Drndic, M. (2010) Rapid electronic detection of probe-specific microRNAs using thin nanopore sensors. Nat. Nanotechnol. **5**, 807–814 CrossRef PubMed

67 Patolsky, F., Zheng, G., Hayden, O., Lakadamyali, M., Zhuang, X. and Lieber, C.M. (2004) Electrical detection of single viruses. Proc. Natl. Acad. Sci. U.S.A. **101**, 14017–14022 CrossRef PubMed