Pharmaceutical BIOPROCESSING

The future for biosensors in biopharmaceutical production

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A defining feature of bioprocesses is the need for measurement, monitoring and control; in the context of biopharmaceuticals this need is further heightened by the absolute requirement to ensure the quality of the product [1]. This is evidenced by the size of bioanalytical endeavor found within the R&D programs of the major biopharmaceutical companies and the supplier industry that caters for this instrumentation need. It is a need that grows at a pace reflected in the initiatives involving the regulatory authorities such as PAT central to the larger vision of QbD. At the core of these attempts to improve biopharmaceutical production is the need for rapid, ideally online, measurement [2]. This would open up a whole range of opportunities for improved control of such processes [3]. In this article we will highlight the relatively small but significant roles biosensors currently play in biopharmaceutical process development and operation and debate the reasons for this in the face of the great potential offered by biosensors. The article will then progress to examine new biosensor concepts deriving from synthetic biology – that of *in vivo* biosensors which may deliver the online information we desire and see biosensors play a much more significant role in the future of bioprocessing.

A biosensor is often defined as 'a device for the detection of an analyte that combines a biological component with a physicochemical detector component'. In many senses, the concept of the biosensor is the magic bullet for the bioanalytical sector; it is perhaps not surprising therefore that to achieve this is not straightforward. This is not to say there have not been significant successes:

- The world's diabetic population depends on blood glucose measurements to administer insulin based on an amperometric based biosensor technology (enzyme electrodes). This represents the largest single biosensor application in terms of numbers of devices and market size;
- Optical biosensors, largely surface plasmon resonance (BIAcore) has become the default method to directly measure protein–protein interactions in the laboratory.

Both of these technologies have been adapted for the bioprocess sector, enzyme electrodes are used to measure metabolites such as lactate and glutamine in mammalian cell culture (e.g., Nova Biomedical). Equally optical transduction technology is now available in a parallel probe type configuration (Fortebio) for use in a microtiter plate format to enable higher throughput protein quantitation (e.g., for product molecules such as antibodies, this technology is directed at process development activities).

The interesting question is why then in the face of such successes is the approach not much more widespread. The fact is that biosensors must be designed for their application to have: the correct selectivity and dynamic range, and the capacity to cope with impurities/interfering compounds likely to be present; this represents a significant challenge. It is in contrast to traditional bioanalytical

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methods we might employ such as HPLC, MS or immunoassays where as much as possible the analyte fits within known methodological approaches. Biosensors conceptually are most closely related to immunoassays, where the creation of an antibody with the right selectivity is the critical step in assay development, from which point somewhat generic procedures can be adopted. To progress to what could be described as a biosensor requires the direct transduction of the analyte–antibody interaction to create a signal. This investment is often difficult to justify unless the sensor can used multiple times requiring a very stable system. This has meant researchers in the area have looked for ways to tackle this problem from applying methods to stabilize antibodies or using antibodies from species which are intrinsically stronger molecules (e.g., camelid), to small so called biomimetic molecules (e.g., triazine dyes), which may offer the stability and lifetime required, through to evolving methods to create a process called molecular imprinting [4].

Despite such challenges, the match between the immediacy of measurement offered by biosensors and the growing need for in-process measurement of multiple biochemical and biological species indicates the need [5]. The potential offered can be seen when using biosensors to monitor fermentation [6] and chromatography operations [7]. The challenge that remains is making the technology robust and accessible. There are some clear issues if such a sensor is to be placed in a process for online sensing. How will the risk of contamination or leaching of sensor components be avoided? And how will calibration be achieved? Such issues are a strong argument for spectroscopic methods that allow for online and non-invasive measurement but the issues here surround the data analysis required to deconvolute specific biological data from the signals, an area of continuing research for in-process modeling for the biopharmaceutical sector [8]. The use of such methods to fingerprint raw materials has already become common. This means the relatively basic needs of product, key metabolites and critical impurities measurement remain off-line, usually laboratory-based at present.

If biosensors are to address this unmet need there is a need to see a step change in the biosensor concept which as noted can frequently be complicated by the need to construct complex surfaces and interfaces to mediate the sensing, the longevity of such sensors is as a result often limiting for this application. The potential to remove the need for this surface is therefore an exciting possibility. The advent of synthetic biology may present such an opportunity; it is an emerging discipline that seeks to apply engineering principles to the design and construction of biological organisms for user-defined purposes [9]. It could mean the transduction element traditionally referred to when describing a biosensor is transposed to features designed within the cellular components which could then be measured remotely by fluorescence for example. This would enable non-invasive measurement of the process alleviating concerns associated with the often conflicting needs of GMP, for example.

This concept of organism design in the field of analytical technology is now starting to produce findings of direct relevance to bioprocessing. It has been shown possible to design dedicated organisms for the purposes of biosensing and to include genetic circuits designed to report on the internal state of the cell within the design of strains for manufacturing [10]. The latter enable the rapid, non-invasive analysis of cell metabolism, including information on nutrient utilization, product formation, and the detection of stress responses. These new types of *in vivo* biosensors could be applied to bioprocess design, used in manufacturing for online processing monitoring and control or both. Since they are derived from or contained within living organisms, they are self-renewing and also avoid the challenges associated with engineering bio-compatible surfaces and interfaces to mediate detection.

In direct analogy to other types of biosensors, *in vivo* biosensors can be thought of as consisting of three components: a sensor, a transducer and an output [11]. The sensor will be responsible for signal recognition and the choice of this element confers the specificity of the biosensor. The transducer (also sometimes called an actuator or a signal processor) converts the signal into a measurable output such as fluorescence, luminescence, a colour change or an electrical current [10]. Most of the examples of *in vivo* biosensors to date rely on an output that can be measured spectroscopically, although steps towards the biological production of an electrical current are underway [12].

One of the engineering principles that synthetic biology has adopted is modularity [9]. When applied to the design of *in vivo* biosensors, this means that the individual components can be designed and characterized separately and then linked together in new combinations to create biosensors for different purposes. Hence, for example, determining the link between arbitrary fluorescence units and the number of molecules of green fluorescent protein in a particular experimental set up allows direct quantification of output from a circuit regardless of the method of sensing and transduction or characterization of the specificity and binding affinity of a particular protein domain allows it reuse in many biosensor designs in different contexts (e.g., [13–15] all use variants of the same binding protein to sense, respectively, glutamine in mammalian cells, arginine in plants, and glutamine in plants).

In principle, any biological macromolecule can be utilized as the sensor component. Proteins are by far the most common example to date, however, nucleic acid aptamers can also be designed to specifically bind to target molecules [10]. Membrane-bound proteins can be used to sense the external environment [16], or proteins can be expressed in the cytoplasm [13] or trafficked to various organelles [17] to sense conditions specifically within the cell or particular microcompartments. Transduction usually begins with a conformational change in the macromolecular structure of the sensor upon interaction with the target analyte (e.g., rearrangement of the 3D structure, dimerization or cleavage). This can, in turn, result directly in a detectable signal on its own (e.g., a change in fluorescence emission,) or enable activation of a downstream process such as transcription, translation, or RNA processing to result in the output [10].

A ratiometric output, where the signal from more than one measurement is compared, can be used to increase the accuracy of measurement. Ratiometric measurements can help reduce variation from other variables in the experimental set up [18] and enhance the accuracy of quantitative measurements. Examples include normalization by the number of cells or optical density of the culture, a signal from a protein which is constitutively produced, or generating multiple output signals from the design of the biosensor itself. An example of the latter strategy is biosensors that rely on Förster resonance energy transfer (FRET). FRET utilizes two separate fluorophores and is therefore an inherently ratiometric technique. *In vivo* biosensors based on FRET have found widespread application in the quantification of intracellular metabolite concentrations [19].

An example of metabolite monitoring using an *in vivo* biosensor design is that of glutamine, an important nutrient source for cells, which is of particular interest to mammalian bioprocessing. Several different biological recognition elements are available as sensing components, the choice of which will impact the actuator. In the majority of designs, the output can also be independently chosen (apart from FRET which inherently relies on fluorescence). For example, if the goal is to measure the extracellular concentration of glutamine, then an *in vivo* biosensor based on a bacterial two-component system could be designed [16]. Two-component systems consist of a transmembrane protein, which senses the target analyte and a response regulator which is phosphorylated upon sensing in order to affect transcription of the genes involved in metabolising the analyte. In this case, the sensor domain would be the membrane protein (specifically the extracellular domain). Actuation would occur by the conformational change upon analyte binding, which leads to dimerization of the membrane protein, phosphorylation of the response regulator, and transcription. The output could be independently chosen to be any reporter gene desired, which could be controlled by any one of the natural promoters that the response regulator controls. Hence, many minor alterations in the design can be explored to alter the sensitivity of the response.

Alternatively, to measure intracellular concentrations of glutamine, a design based on riboswitch-mediated control of translation is possible. Riboswitches are naturally occurring elements of secondary structure in mRNA that are used in nature to tune the expression of genes involved in the metabolism of the target analyte. For this design, the sensing component would be the RNA aptamer that binds to glutamine [20] and actuation occurs via a conformational change upon binding that controls translation of the associated mRNA molecule. The secondary structure formed is reversible, allowing a switch between free and blocked translation of the reporter, which is modulated by the presence of glutamine. Thus, the concentration of the reporter protein is controlled by the concentration of glutamine. In this instance, as with the two-component system, any reporter gene could be used as an output. Sensitivity of the system can be tuned by altering the amount of mRNA produced in the cell, the affinity of the aptamer for glutamine, and the strength of the translation initiation sequence in the mRNA.

Finally, for a ratiometric protein-based *in vivo* biosensor, a FRET design could be employed [13,15]. For FRET, the sensing component would be a protein domain that binds to the target analyte, for instance, a bacterial periplasmic binding protein that binds to glutamine. Upon glutamine binding, actuation occurs via conformational change in the protein which alters the distance between the associated fluorophores and, as a consequence, the fluorescence emission profile. As mentioned previously, the output in this case must be fluorescence; however, there are several fluorophore pairs to choose from in the design phase. The biosensor can also be tuned by altering the linker regions that connect the fluorophores to the binding domain [19].

The three example designs of an *in vivo* biosensor each operate on a different biological principle, and thus have differing response times. The FRET biosensor will have the fastest change in response to analyte concentration because the signal depends only on the conformational change of a pre-existing fusion protein. In contrast, the design based on the two-component systems will require phosphorylation, transcription, translation, and folding of the reporter in order to manifest the signal. Thus, it will have the slowest

response time. The riboswitch-mediated design relies on translation and folding, thus it will be intermediate in response time. Therefore, different applications might require a different choice of design – if rapid changes in metabolite concentration must be monitored, then a FRET design will be required. However, if slower changes are expected, then the other designs offer greater flexibility in the choice of output signal.

It can be seen that *in vivo* biosensors offer a highly versatile analytical platform derived from their modular design based on synthetic biology principles. This offers novel opportunities for monitoring and control in biopharmaceutical production. However the technology is still very much nascent for such applications and for the near future manufacturers are likely to still look to more conventional biosensor formats. Nevertheless the opportunities to develop *in vivo* biosensors towards real-world solutions and further their design toward a whole range of process relevant analytes is one that is available now.

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