

Concept Paper

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Toward Self-Optimizing Bioprocesses: Real-Time Biosensing by Riboswitches Enables Autonomous Cell Factories

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Abstract

Industrial bioprocesses remain constrained by their inability to directly monitor what happens inside cells in real time. Instead, they rely on external measurements such as nutrient or metabolite levels in the culture medium that only provide delayed and indirect clues about the cell's internal state. Riboswitches, RNA elements that respond to specific small molecules, offer a powerful alternative. Acting as intracellular biosensors, they can detect metabolites at nanomolar to micromolar concentrations within milliseconds. When coupled with gene regulatory systems, riboswitches enable dynamic feedback control, allowing cells to sense imbalances and autonomously adjust their metabolism. This transition from external, delayed monitoring to internal, responsive regulation has the potential to transform microbial production systems into self-optimising factories that are more efficient, adaptive, and resilient.

Keywords: bioprocess; real-time biosensing; riboswitch; autonomous cell factory

1. Introduction

Can microbial production systems truly regulate themselves? While the idea may once have seemed speculative, it is rapidly gaining relevance as biotechnology advances toward autonomous, cell-based manufacturing. Importantly, this vision does rely on the cell's innate ability to monitor and respond to its own biochemical state. A major challenge in industrial bioprocessing has been the absence of direct, real-time access to intracellular metabolite levels, forcing reliance on external measurements that provide only delayed and indirect insights. Riboswitches—naturally occurring RNA elements that detect specific small molecules—offer a compelling solution. Acting as intracellular sensors, they can capture metabolic signals as they arise and immediately adjust gene expression. In doing so, riboswitches pave the way for a new generation of microbial factories that are not only self-regulating but also adaptive and resilient.

2. The Biosensing Challenge in Bioprocessing

Traditional bioprocess monitoring relies on external indicators such as pH, dissolved oxygen, and cell density. While these measurements are useful, they provide limited insight into the cell's internal metabolic state. This becomes a serious limitation when toxic by-products like acetate or butanol accumulate inside the cells, reducing productivity well before any changes are detectable by external sensors [1]. Because intracellular metabolite buildup often precedes detectable changes in the extracellular environment, corrective actions are typically delayed by which time productivity has already declined.

Current biosensing strategies for detecting small molecules in bioprocessing face major limitations. Extracellular sensors are unable to access the intracellular environment where key metabolic events occur. Invasive sampling methods compromise cell integrity and yield only discrete

snapshots, not continuous data. Even high-sensitivity techniques like mass spectrometry require extensive sample preparation, making them unsuitable for real-time monitoring of live cultures. This persistent sensing gap has become a major bottleneck in efforts to develop bioprocesses that are genuinely responsive and adaptive.

3. Riboswitches: Nature's Intracellular Biosensors

Riboswitches represent evolution's solution to continuous intracellular biosensing. These RNA regulatory elements function as highly specific molecular sensors, directly binding small molecule analytes such as amino acids, nucleotides, vitamins, and metabolic intermediates with affinities ranging from nanomolar to micromolar concentrations [2]. Riboswitches offer direct and rapid sensing, with response times in the millisecond range, unlike protein-based sensors that rely on complex, multi-step signal transduction [3]. This makes riboswitches well-suited for use as intracellular biosensors in continuous monitoring applications.

Riboswitches sense target molecules through ligand-induced conformational changes in their RNA secondary structure, directly modulating gene expression. Binding of a specific small molecule to the aptamer domain stabilises distinct RNA conformations that either activate or repress translation, transcription, or mRNA stability. This enables a direct biosensing-to-response mechanism, free from the signal amplification artifacts often associated with multi-component systems. The outcome is a proportional, reversible response that closely mirrors real-time intracellular analyte levels. Recent advances in riboswitch engineering have extended their sensing capabilities beyond natural ligands. Through directed evolution and computational design, researchers have developed riboswitches that respond to non-natural small molecules, including industrial chemicals, pharmaceuticals, and synthetic metabolites [4,5]. High-throughput screening has enabled the development of riboswitches with finely tuned sensitivity and improved dynamic range, making them adaptable tools for custom biosensing in engineered microbes.

4. Real-Time Metabolic Sensing in Action

Riboswitch-based continuous biosensors have already shown substantial benefits in bioprocess control. In *Escherichia coli*, engineered riboswitches sensing acetate accumulation can rapidly trigger metabolic shifts, diverting flux from overflow pathways before toxic levels build up [6]. This represents a shift from reactive to predictive control, allowing cells to adjust their metabolism in real time based on the direct sensing of critical analytes.

pH-responsive riboswitches highlight the potential of continuous intracellular biosensing for optimising bioprocesses. By detecting intracellular buildup of organic acids such as lactate and acetate ahead of measurable pH shifts in the culture medium, they allow early activation of acid tolerance pathways [7]. The ability to sense changes inside the cell—spotting issues at their origin rather than relying on bulk measurements—can be crucial for maintaining productivity and preventing process failure.

Multi-analyte sensing is the next step in riboswitch-based biosensing. Recent studies have shown that integrating multiple riboswitches within a single cell can create biosensor networks capable of monitoring several metabolites at once [8]. This approach allows for more advanced metabolic control, enabling cells to balance competing pathways by sensing multiple intracellular metabolites in real time.

5. From Biosensing to Autonomous Bioprocesses

The shift from passive biosensing to active bioprocess control is where riboswitches show their greatest promise. By linking continuous intracellular sensing with dynamic metabolic responses, these systems form autonomous feedback loops that optimise bioprocess performance without outside input. Cells carrying riboswitch biosensors can detect metabolic imbalances, trigger corrective actions, and maintain ideal production conditions through constant self-monitoring.

In a riboswitch-controlled biofuel production system, sensors continuously track intracellular levels of toxic alcohols and trigger efflux pump expression as thresholds near. At the same time, other riboswitches monitor precursor availability, adjusting pathway flux to sustain optimal production rates. This multi-parameter sensing and control occurs entirely within the cell, delivering response times far faster than any external control system.

Riboswitch-based biosensing offers clear advantages for industrial bioprocessing through its natural scalability. Unlike external sensor arrays, which become more complex and expensive as scale increases, riboswitches scale proportionally with cell numbers. Because each cell houses its own sensing and response system, this approach overcomes the spatial heterogeneity challenges faced by large-scale processes relying on external sensors.

6. Challenges and Future Directions

Despite their potential, riboswitch-based biosensing systems face several challenges before they can be widely adopted. Genetic stability is a major issue, as selective pressure may cause cells to lose metabolically costly sensing circuits during prolonged cultivation [9]. Strategies to preserve riboswitch functionality include integrating them into essential genes and designing circuits resistant to evolutionary loss.

Cross-reactivity with structurally similar molecules can reduce the specificity of riboswitch biosensors in complex metabolic settings. Advances in engineering, such as computational design of more selective aptamer domains, are rapidly improving ligand discrimination [10]. Additionally, creating orthogonal riboswitch families helps minimise interference among multiple biosensors functioning within a single cell.

Regulatory requirements for genetically modified microorganisms with engineered biosensors add complexity to commercial use. However, the contained nature of intracellular biosensing and their resemblance to natural regulatory systems may ease regulatory approval [11]. Demonstrating the safety and stability of riboswitch-based biosensors in pilot-scale studies will be essential for progressing these technologies toward commercial use.

7. Conclusion

The integration of real-time continuous biosensing via riboswitches marks a fundamental shift in bioprocess control—from external monitoring to cellular self-awareness. By allowing cells to continuously detect and autonomously respond to their metabolic state, riboswitch-based biosensors convert bioprocesses into truly intelligent, self-optimising systems.

Moving forward, progress will depend on advancing riboswitch engineering tools, thoroughly characterising biosensor performance in industrial settings, and demonstrating long-term stability in production. Achieving success will require close collaboration among biosensing experts, synthetic biologists, and industrial bioprocess engineers to translate riboswitches' sensing capabilities into reliable, scalable biotechnology platforms.

The future of autonomous bioprocessing depends not on external automation, but on equipping cells with the ability to monitor and optimise their own function. Riboswitches, as nature's most advanced intracellular biosensors, offer the molecular intelligence needed to bring this vision of self-optimising microbial factories to life.

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