IB-083

Antibody-oligonucleotide conjugation based on cyanine dyes as cross-linking reagents

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Antibody-oligonucleotide conjugates represent a new class of synthetic chimeric biomolecules with unique properties. They are widely used in various fields of medicine and biotechnology, such as therapeutic cell targeting, multiplexed protein diagnostic assays (immuno-PCR, PLA), targeted delivery, etc. One approach to conjugation is the use of strain-promoted alkyne-azide cycloaddition (SPAAC), in which a bicyclo[6.1.0]non-4-yne (BCN) moiety activated antibody is covalently linked to an azide-modified DNA-matrix.

Our method is based on the application of asymmetric water-soluble cyanine dyes Cy3 and Cy5 as cross-linking reagents. This is feasible due to the combination of functional groups, such as an activated ester for reaction with the amino groups of proteins and oligonucleotides, and an azide group or a bicyclononyne moiety for subsequent conjugation.

The introduction of fluorescent labels into the studied biomolecules make it possible to determine the number of introduced functional groups, the ratio of biological objects in the resulting conjugate, and also use resonance energy transfer to monitor the kinetics of product formation. Reaction conditions and purification processes was optimized to achieve maximum yield.

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Synthetic Raman spectra to improve quantitative real-time monitoring models for alcoholic yeast fermentation

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In bioprocessing, traditional off-line measurements of nutrient concentrations and cell density provide a limited and delayed overview of the process. This underlines the need for process analytical technologies (PAT) that measure real-time information and improve process monitoring, characterization, and control. Raman spectroscopy is a valuable PAT tool for automated monitoring during upstream process development, as it provides detailed chemical information. However, to effectively implement Raman spectroscopy, it is necessary to improve chemometric model calibration. Current data-driven statistical models require extensive data collection and face challenges such as inherent cross-correlations between biological molecules that are integrated into models, resulting in non-specific models.

This study demonstrates the calibration of partial least squares (PLS) models using synthetic Raman spectra for glucose and ethanol quantification in alcoholic yeast fermentation. By preprocessing empirical pure component spectra of glucose and ethanol to isolate the characteristic peaks and combine them in silico in varying ratios to simulate different concentrations, we generate synthetic spectra. These spectra are validated through comparison with experimentally determined spectra of glucose and ethanol mixtures, and are subsequently used to calibrate PLS models. The models are compared with those calibrated using experimental batch cultivation data, by calculating the Root Mean Square Error of Prediction (RMSEP) and analysing regression coefficients to assess model specificity.

This project aims to streamline the calibration process by reducing the need for extensive batch runs that are traditionally required for model calibration. Consequently, it enables a faster and more cost-effective data collection process, accelerating the implementation of chemometric models.

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Development of a bioprocess for the production of a herbicidal sugar as sustainable alternative to glyphosate

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The negative impact on the environment, insects and humans by massive glyphosate application as a herbicide worldwide is heavily discussed. Therefore, an effective as well as ecologically acceptable alternative is urgently wanted. Such a novel herbicidal candidate is 7-deoxy-sedoheptulose (7dSh) which was first described as such by Brilisauer et al. (2019). The chemoenzymatic synthesis of this sugar has a low yield of 20% and is uneconomical due to high substrate costs. A natural producer strain is Streptomyces setonensis with a titer of 23 mg/L (Brilisauer et al., 2019).

Therefore, we aim to develop a microbial process with S. setonensis for the large-scale production of 7dSh. To evolve a scalable, well-characterized bioprocess for an unknown microbial system, cultivation conditions were examined using a high-throughput microbioreactor system (BioLector) as well as a design of experiments approach in shake flasks to investigate the reciprocal influence of cultivation factors. To gain a deeper understanding of the metabolism the oxygen transfer rate is monitored with RAMOS® (Respiration Activity MOnitoring System), showing 7dSh production after the growth phase when glucose concentration is low. Results suggest that elevated osmolarity enables higher product titers. Surprisingly so does phosphate abundance, although in other Streptomyces strains synthesis of secondary metabolites is coupled to phosphate limitation (J.F. Martin, 2004). Moreover, a fed-batch operation regime was investigated while scaling the process up to stirred-tank reactors to prolong synthesis with a glucose feeding rate of 0.6 g/h. In summary, the product titer was increased by factor 100 to <3 g/L.

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Improved syngas fermentation to ethanol by recycling of acetate and biomass

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