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Light-Controlled Gene Expression in Yeast

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Abstract : Light as a stimulus provides the capability to develop regulation techniques for customizable gene expression. A great advantage is the extremely flexible and accurate dosing that can be performed in a non invasive and sterile manner even for high throughput technologies. Therefore, light regulation in a multiwell microbioreactor system was realized providing the opportunity to control gene expression with outstanding complexity. A light-regulated gene expression system in Saccharomyces cerevisiae was designed applying the strategy of caged compounds. These compounds are photo-labile protected and therefore biologically inactive regulator molecules which can be reactivated by irradiation with certain light conditions. The “caging” of a repressor molecule which is consumed after deprotection was essential to create a flexible expression system. Thereby, gene expression could be temporally repressed by irradiation and subsequent release of the active repressor molecule. Afterwards, the repressor molecule is consumed by the yeast cells leading to reactivation of gene expression. A yeast strain harboring a construct with the corresponding repressible promoter in combination with a fluorescent marker protein was applied in a Photo-BioLector platform which allows individual irradiation as well as online fluorescence and growth detection. This device was used to precisely control the repression duration by adjusting the amount of released repressor via different irradiation times. With the presented screening platform the regulation of complex expression procedures was achieved by combination of several repression/derepression intervals. In particular, a stepwise increase of temporally-constant expression levels was demonstrated which could be used to study concentration dependent effects on cell functions. Also linear expression rates with variable slopes could be shown representing a possible solution for challenging protein productions, whereby excessive production rates lead to misfolding or intoxication. Finally, the very flexible regulation enabled accurate control over the expression induction, although we used a repressible promoter. Summing up, the continuous online regulation of gene expression has the potential to synchronize gene expression levels to optimize metabolic flux, artificial enzyme cascades, growth rates for co cultivations and many other applications addicted to complex expression regulation. The developed light-regulated expression platform represents an innovative screening approach to find optimization potential for production processes.

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