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Poster Abstract – D.67

AN OPTIMIZED, CHEMICALLY REGULATED GENE EXPRESSION SYSTEM FOR *CHLAMYDOMONAS*

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Cytochrome c_6, luciferase, nickel, copper, chelating agents

Chlamydomonas reinhardtii is a model system for algal biology and is used for biotechnological applications, such as molecular farming or biological hydrogen production. The *Chlamydomonas* metal-responsive *CYC6* promoter is repressed by copper and induced by nickel ions. However, induction by nickel is weak in some strains, poorly reversible by chelating agents like EDTA, and causes, at high concentrations, toxicity side effects on *Chlamydomonas* growth. Removal of these bottlenecks may encourage the wide use of this promoter as a chemically regulated system for the expression of heterologous genes. Using a codon-optimized *Renilla* luciferase as a reporter gene, we explored several strategies to improve the strength and reversibility of *CYC6* promoter induction. Use of the first intron of the *RBCS2* gene or of a modified TAP medium increases the strength of *CYC6* induction up to 20-fold. In the modified medium, induction is also obtained after addition of specific copper chelators, like TETA. At low concentrations (10 µM) TETA is a more efficient inducer than Ni, which becomes a very efficient inducer at higher concentrations (50 µM). Neither TETA nor Ni show toxicity effects at the concentrations used. Unlike induction by Ni, induction by TETA is completely reversible by micromolar copper concentrations, thus resulting in a transient "wave" in luciferase activity, which can be repeated in subsequent growth cycles.

In the currently used system, downregulation of Photosystem II activity, leading to hydrogen production, is triggered by cycling *Chlamydomonas* cultures between sulphur-replete and sulphur-depleted medium. The method presents evident challenges, such as the difficulty of centrifuging the huge volumes of algal cultures needed for making hydrogen production economically interesting. The use of the TETA/Cu reversible system described here could be used to trigger several subsequent cycles of gene expression/silencing in a cheap, energy-efficient way.