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# CRITERION BASED OPTIMAL DESIGN OF ISOTOPE FEEDING EXPERIMENTS

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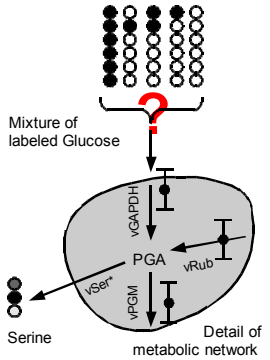
## INTRODUCTION

Steady state metabolic flux analysis based on $^{13}C$-isotope measurements has emerged as a powerful tool for metabolic engineering. The statistical analysis of parameter estimation and progress in analytical and computational methods[1,2] have given flux measurements based on isotope labeling experiments a quantitative foundation. Quantitative steady state flux studies are now routinely applied to plant systems, and metabolic flux maps have been determined for various tissues and plant species[3]. Amongst the most popular model systems are embryo cultures due to the economical importance of seed filling. The information richness of a labeling experiment depends on the network, flux values, metabolite measurements made and choice of label. Although experimental design studies for optimal substrate label have been performed for microbial experiments[4], the results of these studies cannot be assumed to be applicable to plants. The combined differences in network structure, carbon substrate and flux values suggest that a good substrate label design for a bacterial strain may be a poor one for a developing plant embryo. We present optimal experimental isotope label designs for developing *Brassica napus* embryos based on established optimality criteria.




Metabolic flux estimation is an inverse problem, where simulated measurements are least squares fitted to measured values Eqn 1. The calculation of the label measurements from a given set of flux estimates Θ (forward problem) has been solved analytically[2].

$$\min_{\Theta} \left\| F_w(\Theta) - w \right\|^2_{\Sigma_w} + \left\| F_y(\Theta) - y \right\|^2_{\Sigma_y} \qquad (1)$$

In Eqn 1, $F_w$ and $F_y$ are the simulated flux measurements and isotope label expressions respectively, w are measured fluxes and $\Sigma_w$ and $\Sigma_y$ measurement errors.

## METHODS

The scalar-based evaluation of experimental designs uses the parameter covariance matrix defined in Eqn 2[1].

$$Cov = \left[ \left( \frac{\partial F_y}{\partial \Theta} \right)' . \Sigma_y^{-1} . \left( \frac{\partial F_y}{\partial \Theta} \right) \right]^{-1} \qquad (2)$$

Experimental designs were evaluated using the A- and D-criterion, which are defined as the trace of the covariance matrix and the determinant of the covariance matrix respectively. The information content *I* is defined as the criterion value scaled to one standard deviation, relative to a reference experiment[5].

## INFORMATION CONTENTS OF OPTIMAL DESIGNS

Optimal label designs greatly outperformed labeling strategies designed for microbial systems, demonstrating the importance of a tailored optimal label design.

| | Optimal design | | Szyperski | Schmidt |
|---|---|---|---|---|
| | A | D | '95 | '98 |
| $I_A$ | 1.00 | 1.11 | 5.48 | 6.49 |
| $I_D$ | 1.00 | 1.01* | 5.50 | 3.85 |

Table 1. Relative information contents of optimal designs compared with label designs from literature. Information content values are scaled to an A-optimal design.

A second label experiment, using an optimal complementary design led to a further decrease of the parameter uncertainty by an additional ~70%.

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