**Systematic investigation of the effects of macromolecular crowding on glycolytic enzymes from *Saccharomyces cerevisiae***

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The goal of bottom-up systems biology is to generate high-quality predictive models that enhance our understanding of cellular behaviour. For mathematical models of metabolism to accurately simulate experimental data, the conditions under which the enzyme parameter values are obtained should be close to the actual *in vivo* environment. However, this is traditionally not the case, many enzyme kinetic studies are carried out under conditions which are optimal for the enzyme being studied and can be far removed from the actual native conditions the enzyme would be found in. An aspect of the intracellular environment which is quite often overlooked is the effect of the large quantity of different macromolecules which occupy it, known as macromolecular crowding. In order to better understand how the complex heterogeneous environment of the cell influences enzyme kinetics, we exposed reactions catalysed by hexokinase (HXK), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase (ENO) and pyruvate kinase (PYK) to inert synthetic polymers of different shapes and size at varying concentrations. Raw kinetics data were acquired either from spectrophotometric assays with microtitre plates or from Nuclear Magnetic Resonance (NMR) spectroscopy time courses. Enzyme kinetic parameter estimates were obtained by fitting initial rate kinetics and NMR time-course data to a kinetic model based on a rate equations for each respective enzyme. The presence of the synthetic polymers (Dextran70, Ficoll70 and PEG35) had different effects on the $V_{max}$ and $K_m$-values for the different enzymes. In some cases, significant changes in kinetic parameters were observed in the crowded solutions relative to the baseline uncrowded solutions, e.g., high concentrations of crowding agents decreased the $V_{max}$ -values. Changes in kinetic parameters depended on the size and shape of the crowding agent used. Future work will focus on the characterisation of outstanding glycolytic enzymes and incorporating these data into an overall kinetic model of glycolysis. This will enable us to assess on an encompassing scale the importance of macromolecular crowding in determining the output of such a model.

**Keywords**: macromolecular crowding, systems biology, metabolism, enzyme kinetics