Reversible Hill kinetics of bisubstrate reactions

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The behaviour of cooperative enzymes catalysing bisubstrate reactions in cellular metabolism has to date been described in terms of the Monod, Wyman, Changeaux (MWC) and Koshland-Nemethy-Filmer (KNF) models. However, these models are complex and depend on a large number of parameters. Moreover, the MWC model is unable to accurately predict modifier behaviour and does not explain negative cooperativity, which makes its experimental application very cumbersome. In contrast, the published monosubstrate Reversible Hill equation uses very few kinetic parameters, accurately predicts modifier behaviour and allows for negative cooperativity. The current work describes the generalisation of the reversible Hill rate equation to bisubstrate (bi-bi) reactions. We validate the algorithm by comparing computer simulated data to experimental data. The theoretical data are generated with the modelling software package PySCeS, which has been developed in our group, using published substrate/product concentrations of the cooperative enzyme pyruvate kinase from Bacillus stearothermophilus and applying the novel Reversible Hill bi-bi algorithm. These theoretical simulations are then compared to experimental kinetic data for the same enzyme, obtained with phosphorus NMR spectroscopy.