



Figure 2 – figure supplement 3: **Exploration of the effect of species total amounts in allowing for biphasic responses in different modification systems.** There are two kinds of parameters in substrate modification systems: intrinsic rate constants and species total amounts. Analytical work has characterized the possibility or impossibility of obtaining biphasic responses and the dependence on intrinsic rate constants. Semi-analytical work (presented here and in Figure 2 – figure supplement 4) provides further analysis into how species amounts can allow for biphasic responses. For each of the systems considered, we first fix intrinsic kinetic rate constants at or close to those seen experimentally in a reference system (see [6]). We then semi-analytically explore the steady state equations by systematically reducing them and requiring the presence of a biphasic response. In all cases, the presence of biphasic responses (or specifically the onset of biphasic responses, i.e. biphasic peak) is shown by a curve involving two concentration variables. Each point along this curve denotes a concentration variable pair, that will guarantee the onset of biphasic response at the given value. Using these concentrations, the total amounts of all relevant species can be back calculated (for every point on this curve) easily to ascertain what total amounts are to be needed for the behavior (also see Figure 2 – figure supplement 4). Sample calculations for a representative point on each curve is presented in the Supplementary Maple document 2. It is shown by sampling a given point from each of these graphs, that the resulting total amounts of substrates and enzymes are also within the ranges of those accessible by experiments (see Maple document for more detail). All concentrations shown are in  $\mu M$  (A) Coupled covalent modification cycles (B) Two tier enzymatic cascade (C) Double site modification system with common kinase and separate phosphatase (D) Double site modification system with separate kinase and common phosphatase (E) Double site modification with common kinase and common phosphatase (note that  $\epsilon = K/P$ ). The values of total amounts of one of the enzymes (and in where multiple enzymes (kinases/phosphatases) are involved, two of the enzymes), are fixed at levels in physiological ranges and the total amounts of other species are explored. This is to allow for the creation and easy visualization of contour plots.