



Figure 2 – figure supplement 4: **Semi-analytical approach for determining total amounts of species for realizing biphasic responses.** This figure provides complementary information to Figure 2 – figure supplement 3 into how species total amounts can allow for biphasic responses. For each of the systems considered, as discussed in Figure 2 – figure supplement 3 we first fix intrinsic kinetic rate constants at or close to those seen experimentally in a reference system. 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 in different ways. On one hand, we show the curve relating two concentration variables (as shown in Figure 2 – figure supplement 3). On the other hand the curve has also been translated into curves in total amounts and that is shown in a separate panel. This demonstrates that biphasic responses can be readily obtained for biologically reasonable total amounts (all total amounts and concentrations are in μM). (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 ($\epsilon = K/P$). 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 countour plots.