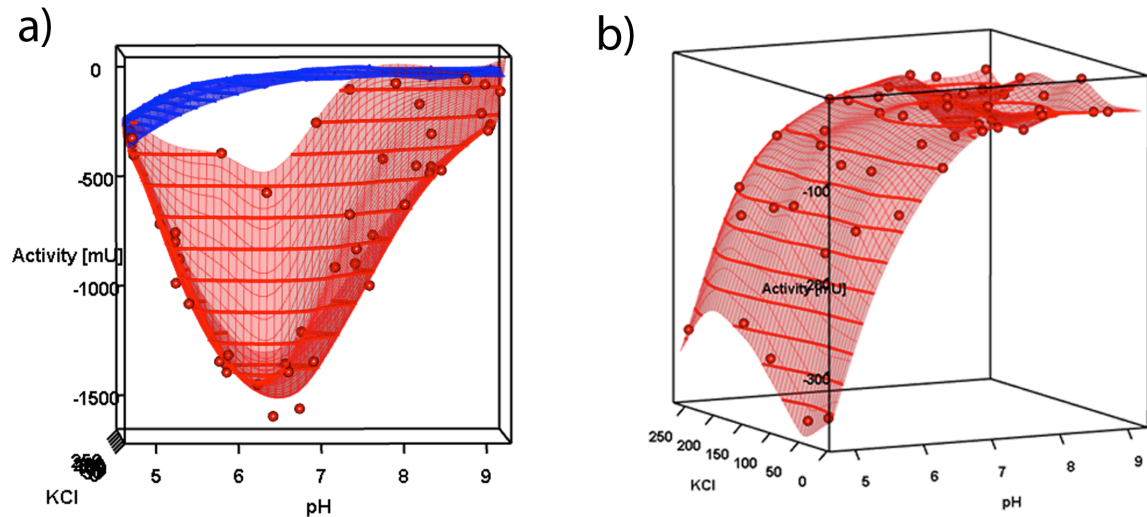




Supplementary Figure 1. Influence of pH and KCl concentration in reaction buffer on spontaneous NADH oxidation. Each dot corresponds to a measurement conducted with one of 48 conditions from the space filling design of the PYK-LDH coupled assay. In all plots, the measured data and the Kriging model fitted surface is shown. a) Blue response surface represents samples without the coupling enzyme LDH; red surface represents samples with all reaction components present (PEP = 1 mM, see Material and Methods). b) Extended view of the response surface model of spontaneous NADH oxidation in samples where LDH is excluded from the reaction (blue response surface in a); but extended scale of z-axes to visualize changes in spontaneous NADH oxidation). Results show a low spontaneous oxidation of NADH at pH<3, which is significantly lower than the changes resulting from enzymatic reactions.