**Supplementary Information Text S1**. To a first approximation, the theoretical maximum binding capacity (*Rmax*) of the immobilized ligand on the Biacore chip surface can be estimated using the following equation:

Equation S1: $R\_{max}= \left(\frac{MW\_{A}}{MW\_{L}}\right)×R\_{L}×s$

where *MWA* is the molecular weight of the analyte, *MWL* is the molecular weight of the ligand, *RL* is the response level (RU) of immobilized (captured) ligand, and *s* is the number of binding sites per ligand. Incorporating the actual values for the relevant parameters from the ERK Biacore analysis results in the following:

 Equation S2: $R\_{max}= \left(\frac{0.587 (kDa)}{70.5\pm 0.5 (kDa)\_{ }}\right)×(1100-1400 RU)×1$ = **9–11.5 RU**

The maximal responses for each experiment, corresponding to the observed Biacore traces at the highest analyte concentrations (**8-10.5 RU**), can then be compared with this theoretical value to determine the percent of the maximum capacity attained.

In cases where complete saturation of an immobilized surface is attained (*and* each protein ligand is capable of binding analyte) the percent bound should approach 100% of the theoretical maximum level. This was observed in both single cycle kinetic experiments with inactive WT ERK1 and inactive WT ERK2. Multi-cycle Biacore analysis of both active WT ERK1 (Fig. 4B) and active G169D ERK2 (Supplementary Information Fig. S5) indicated that complete saturation for these surfaces was not reached (~75% of total capacity bound at highest analyte concentration), although based on the fitting results they are both predicted to achieve a level of > 90% (within error) at saturation. Data for the remaining Biacore experiments yielded bound percentages of 87-92% (> 90%, within error) of the maximum level.

It may be worth noting that the total percentage of the maximum analyte bound (vs *Rmax*) does not have any direct impact on the fitted kinetic parameters. However observation of percentages approaching 100% can indicate that the protein is fully active (ie. binding competent) when immobilized on the Biacore chip surface and that complete saturation of the surface has been attained at the highest analyte concentration used. As such, this approximation provides an empirical measure of whether an immobilized ligand is well behaved and additionally serves as a useful guide for evaluating data quality.