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Supporting information for article:

The evolving story of AtzT, a periplasmic binding protein

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MGSSHHHHHSSGLVPRGSHMQEPLKVAFVYAGPVSDAGYTYAHDQGRLAMEKNLGAKV
KSSYVENPEGADAERVIRKLAADGNKLIFTTSFGFMNPTERVAKAFPNVVFEHATGVKLAK
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WVSTWYDPAKERQAAETLIAQGADVLTQNTNSPATLQVAQEKGKYAFGCDADMSKFAPKA
HLTASISNWGDFYTKTAQAVMAGTWKSEEVHWGMAEGMVKMAPLNAAVPPDAAKLFEEK
KAAMVSGKIKPFQGPLKDQSGAVKVAAGSDLPLASLKGMNWYVQGVEGTIPK

Figure S1 Protein sequence of the AtzT from *Pseudomonas* sp. strain ADP, lacking the tat signal in N-terminal region. In red is shown the 6xHis-tag and the thrombin cleavage sites added through the cloning.

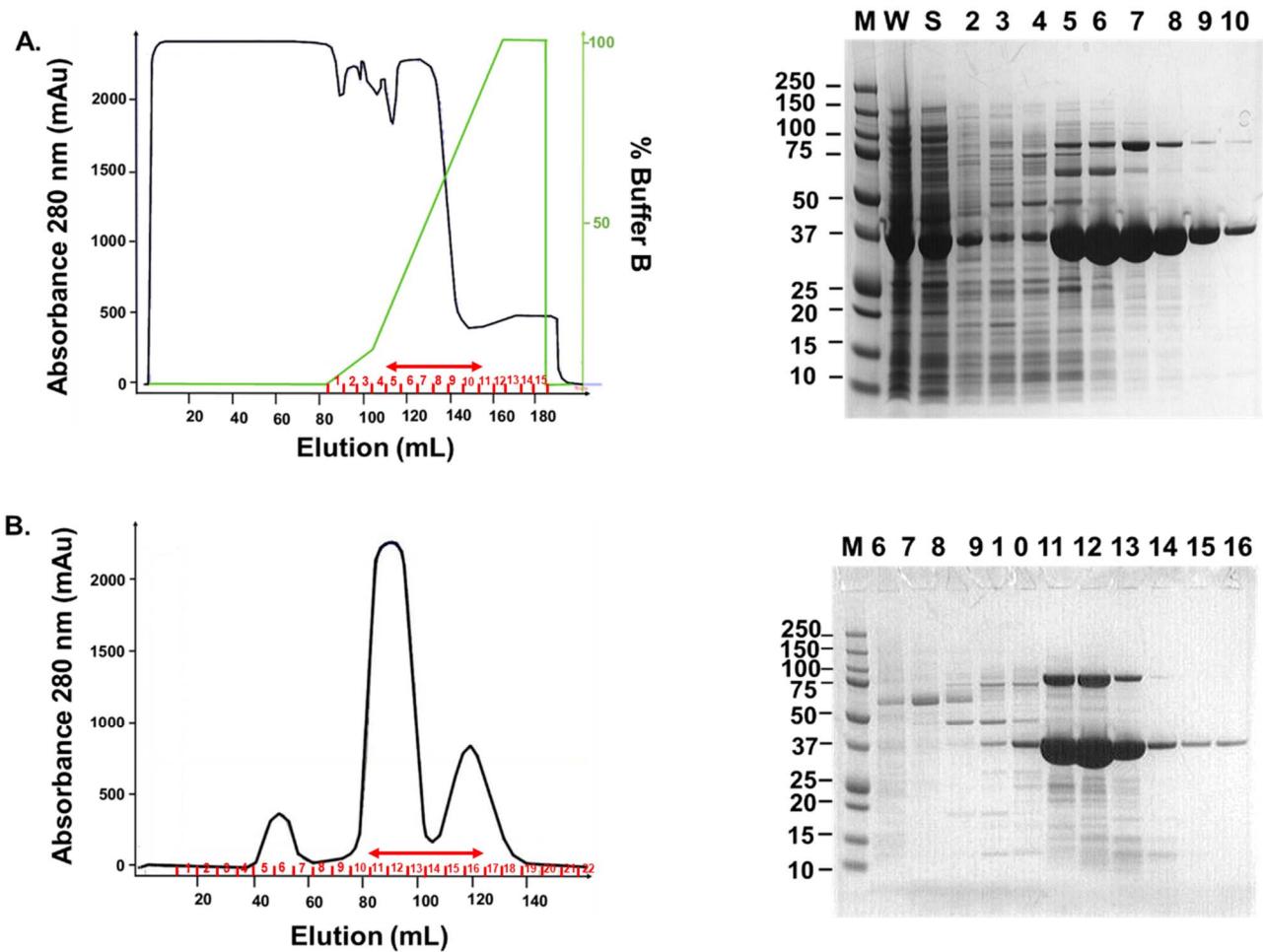


Figure S2 Purification of AtzT. A) NiNTA trace and SDS-PAGE analysis of the fractions 2–10, fractions 5–10 were pooled and are shown by the red arrow at the bottom of the trace, they were concentrated before being run on the S200 size exclusion column; B) Size exclusion trace and SDS-PAGE analysis of the fractions 6–16, fractions 11–16, shown by the red arrow at the bottom of the trace, were concentrated and used for further work; M: mw markers, W: whole cell extract; S: soluble extract, the expected size of AtzT is 38.7 kDa.

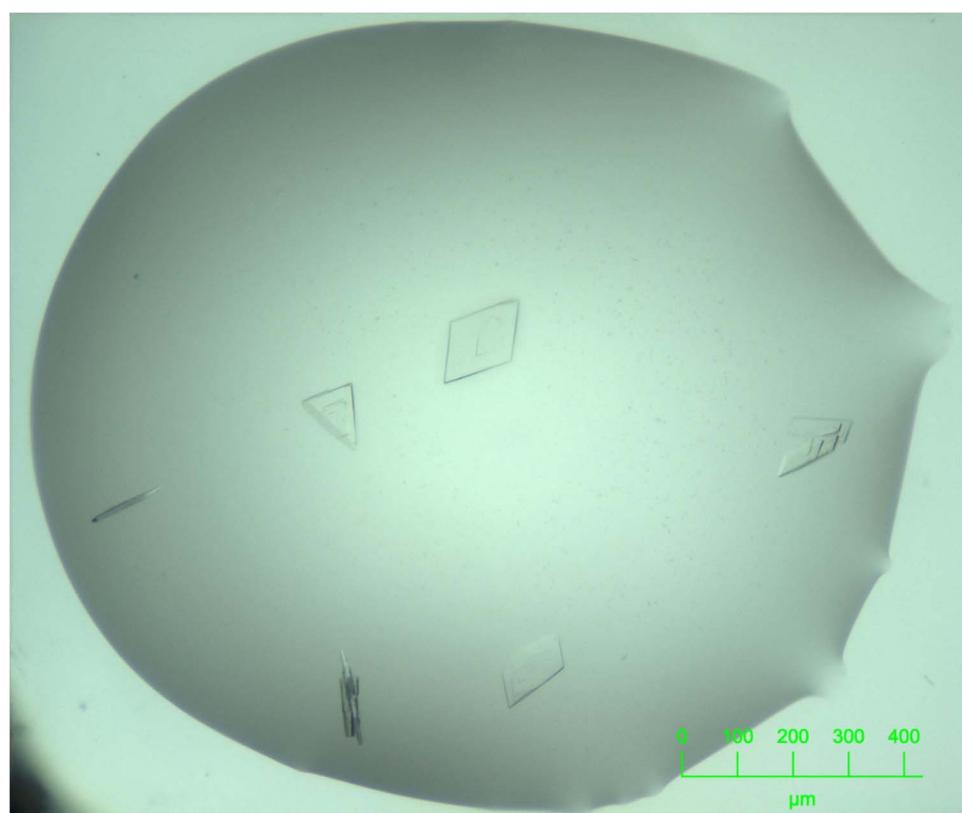
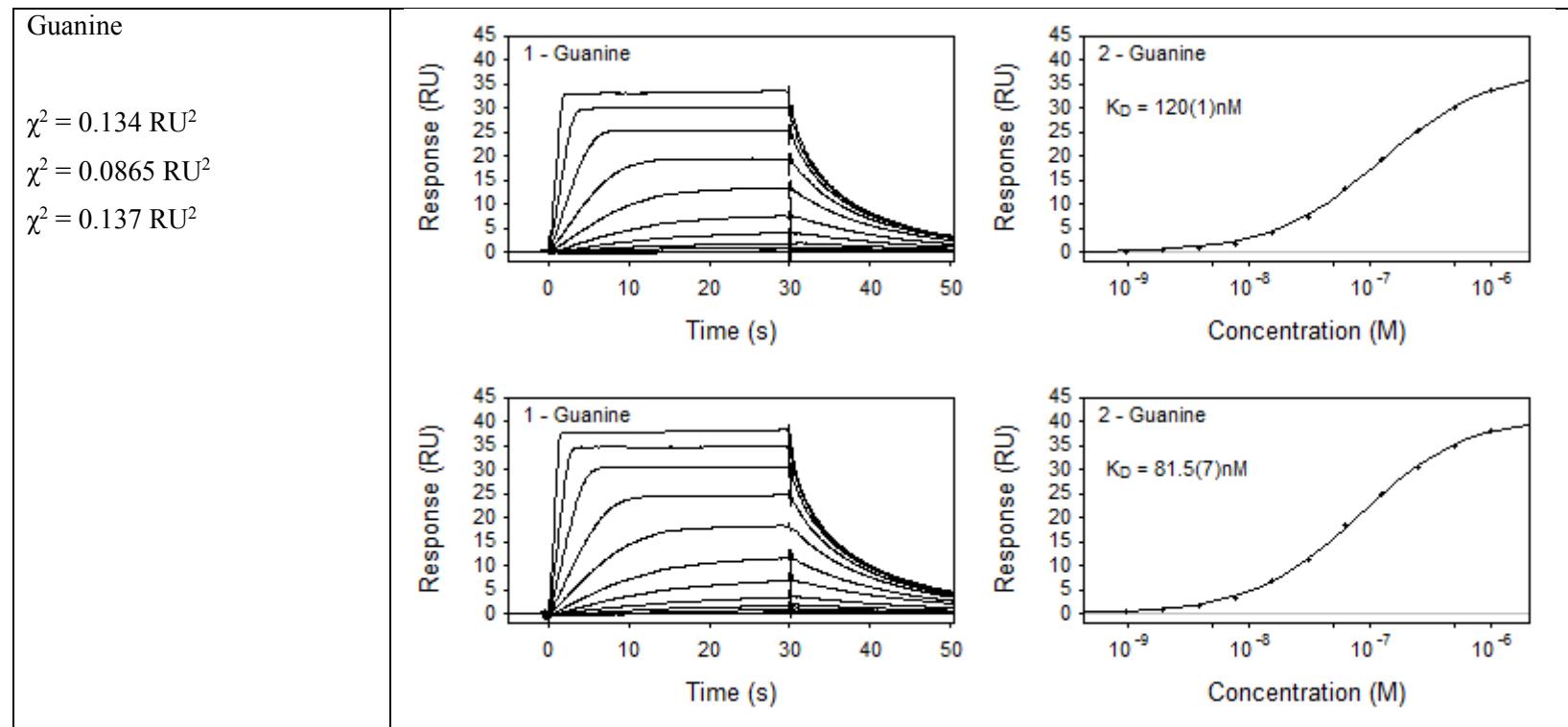
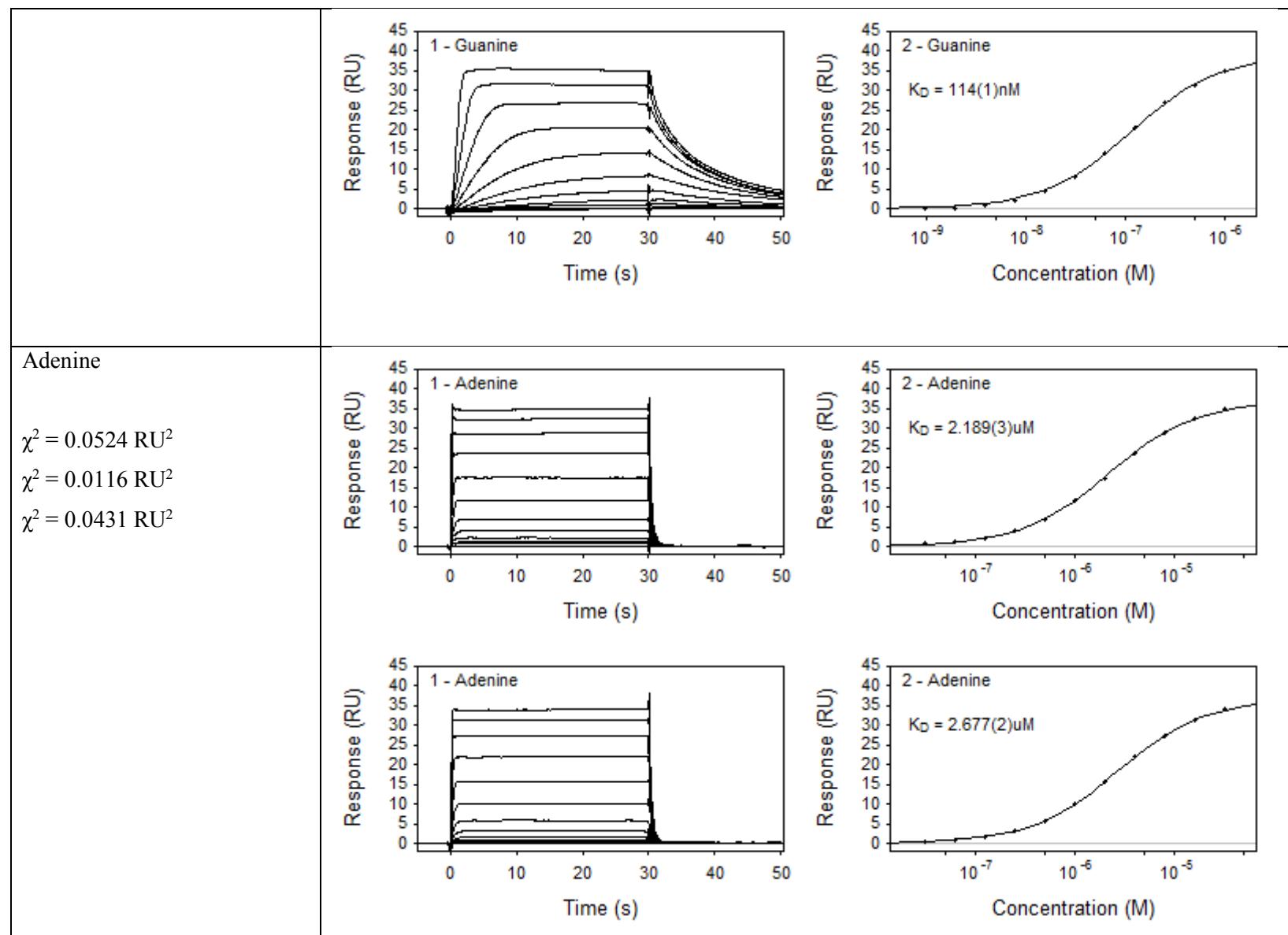
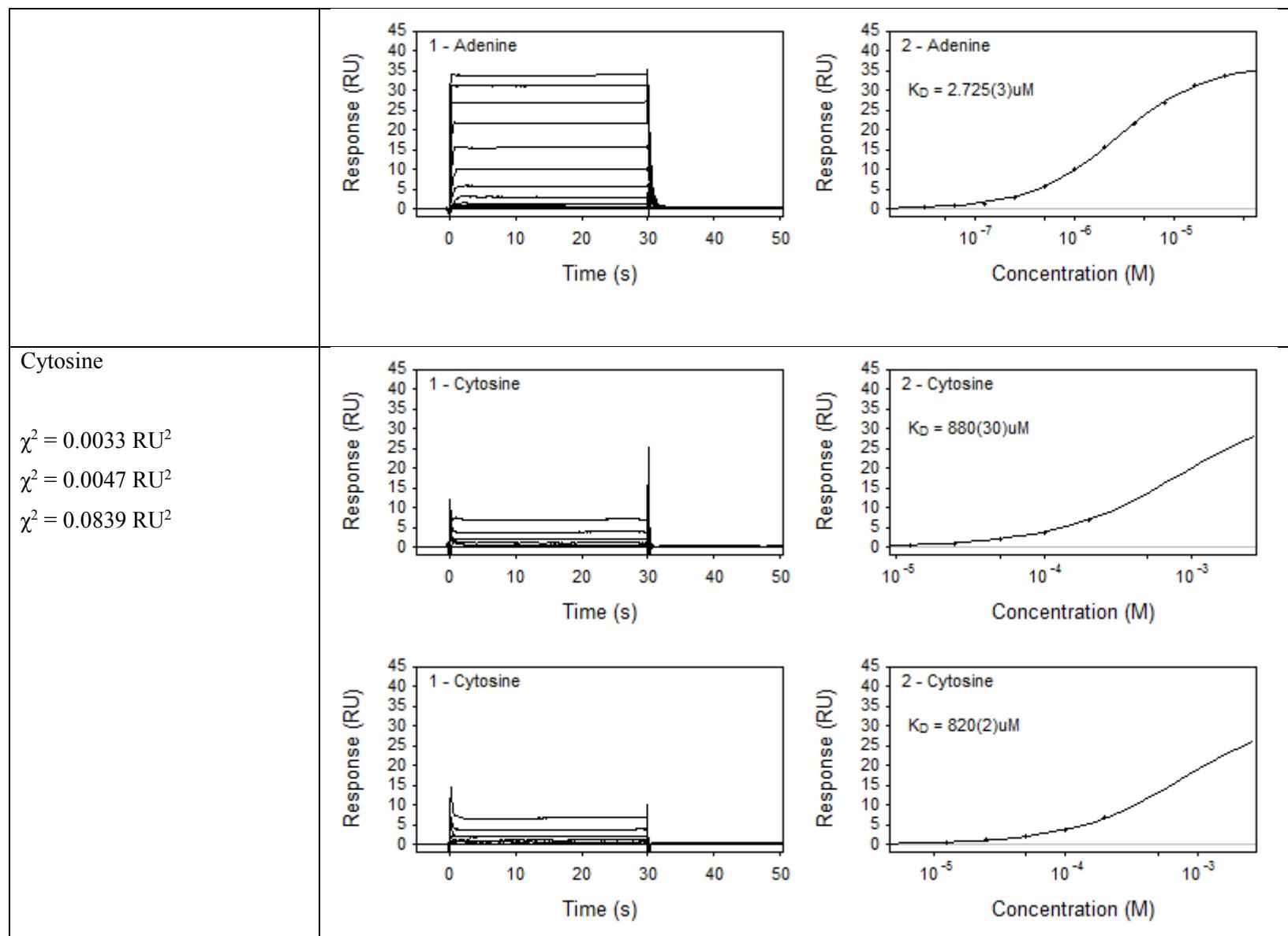


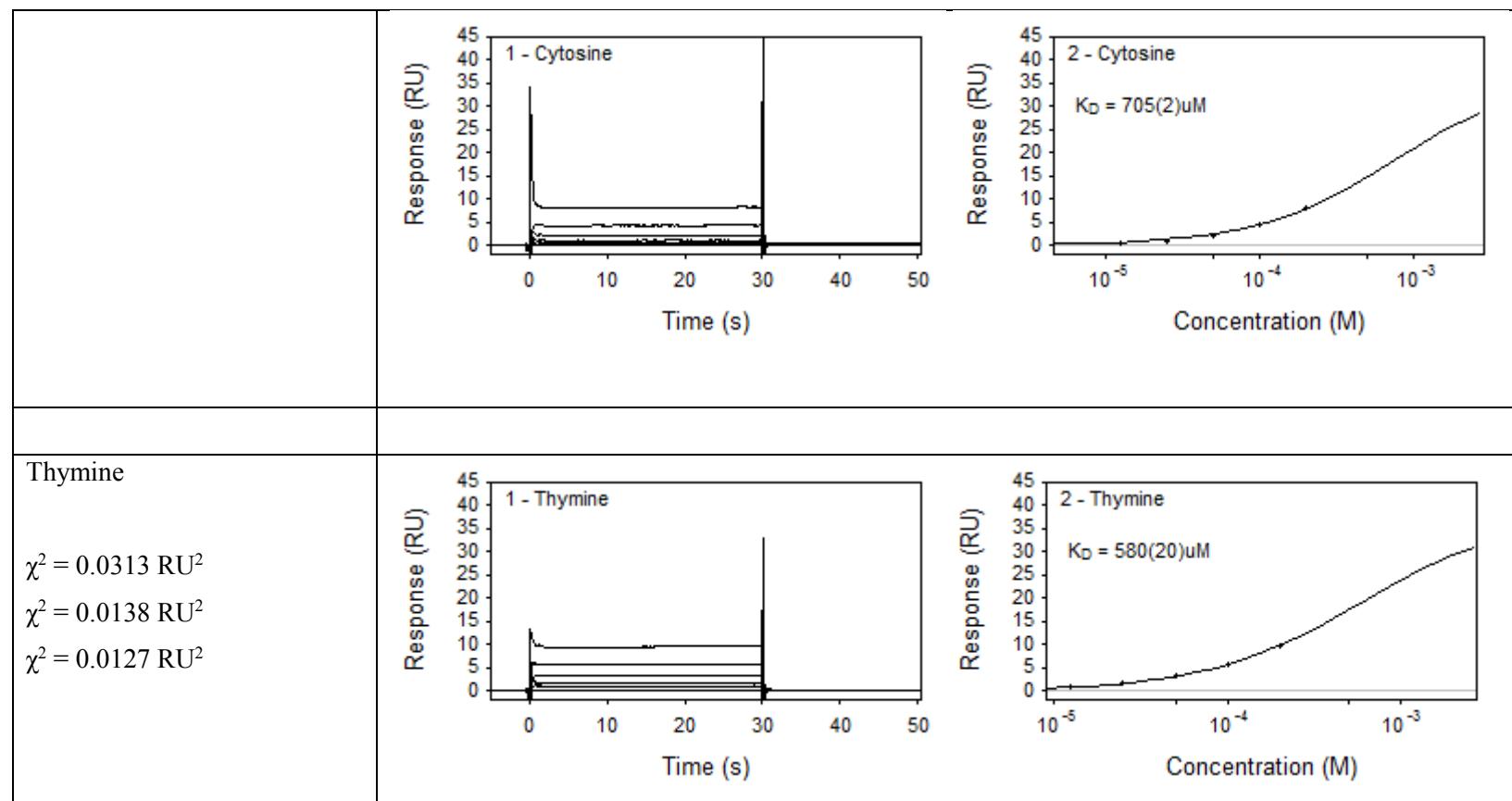
Figure S3 Crystals of AtzT grown in 7.39% (v/v) 2-methyl 2,4-pentanediol, 4.16% (v/v) polyethylene glycol 400, 1.15 M trisodium citrate. Drop consisted of 200 nL protein at 20 mg/mL, 180 nL reservoir solution and 20 nL of a seedstock. Crystal droplet was set up in SD-2 plates from Molecular Dimensions, UK, and was grown at 293 K.

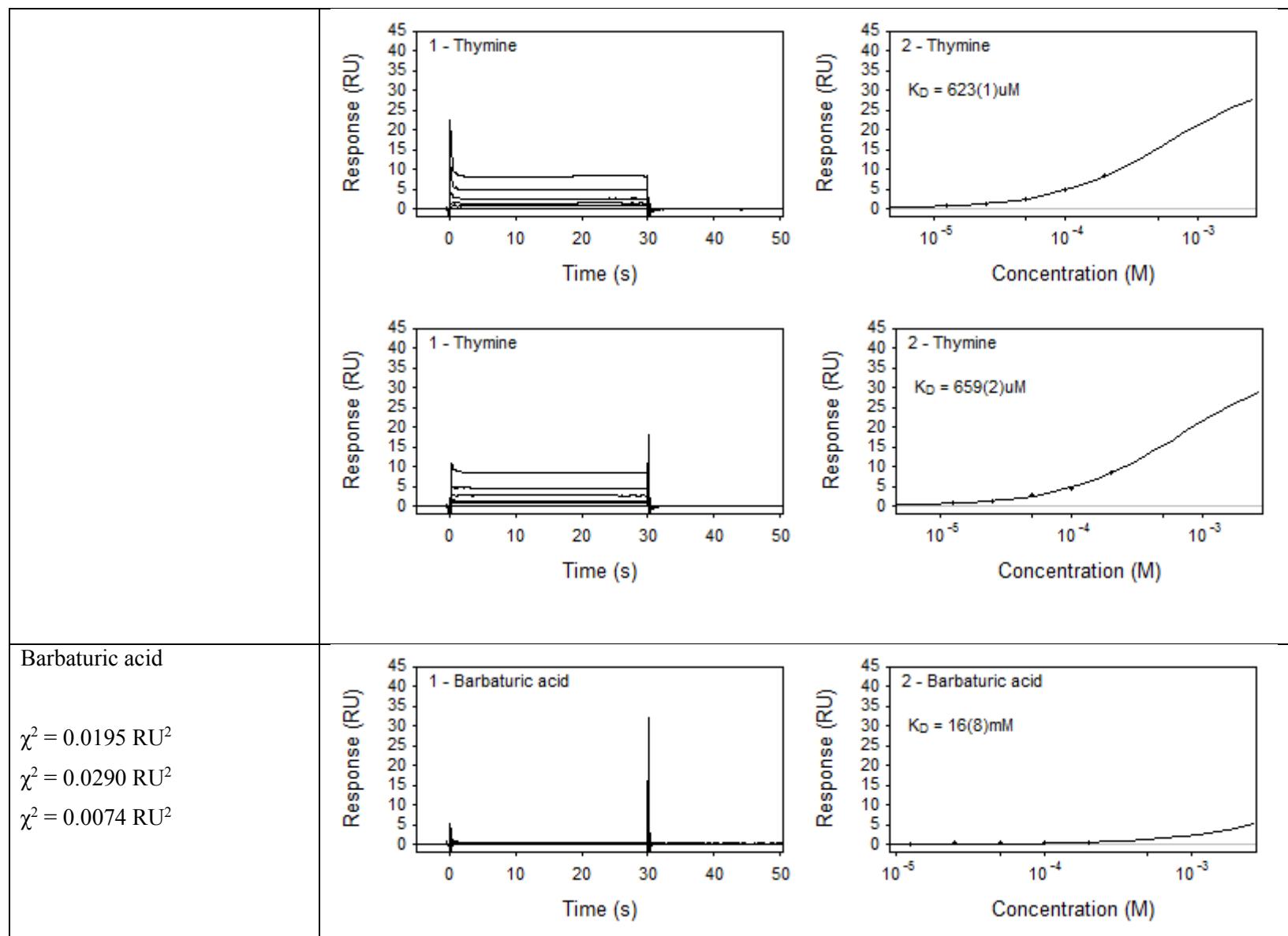
Figure S4 SPR sensorgrams (left) and fit to a 1:1 steady-state model (right). χ^2 values are shown with respect to each replicate.

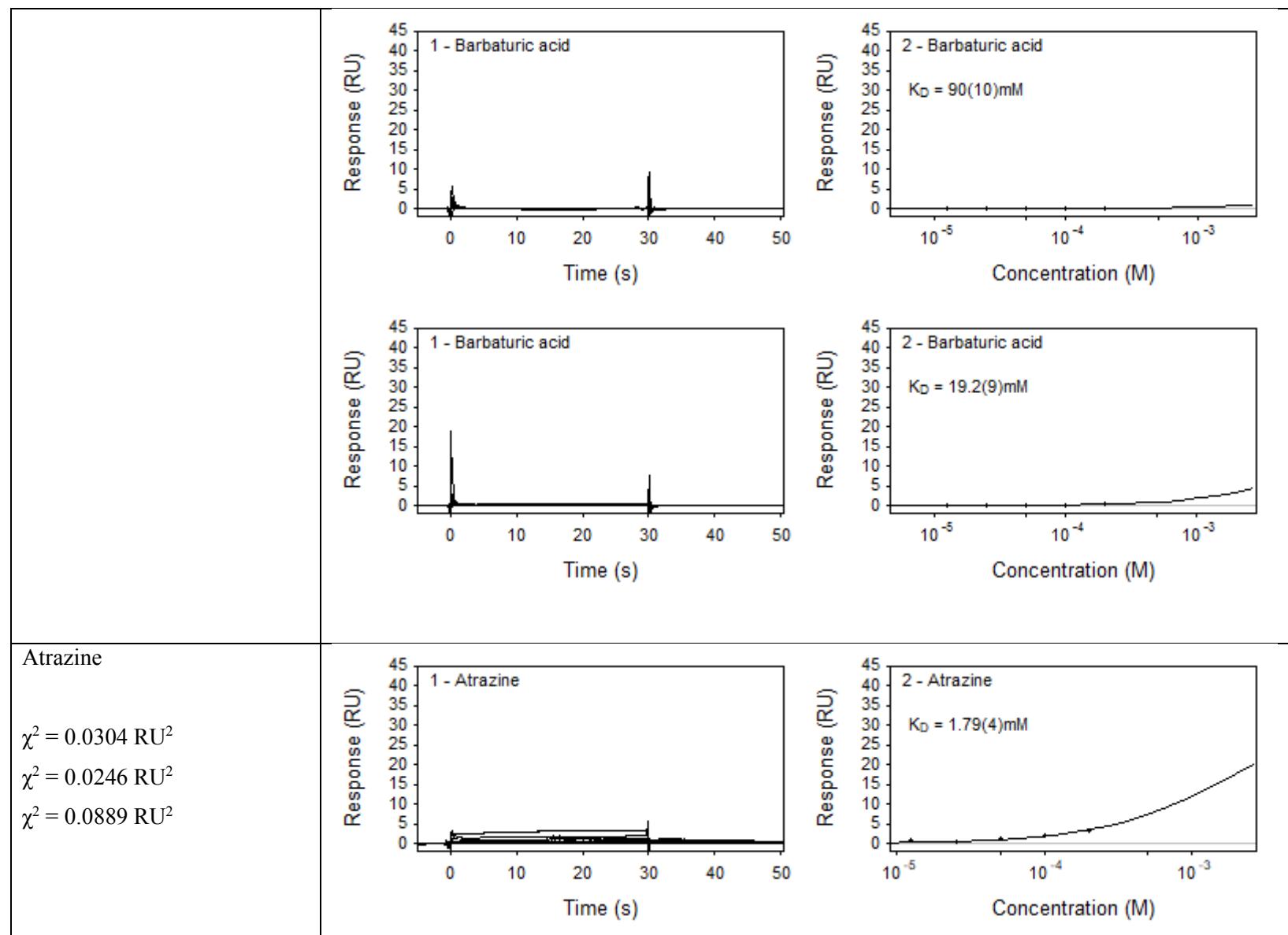


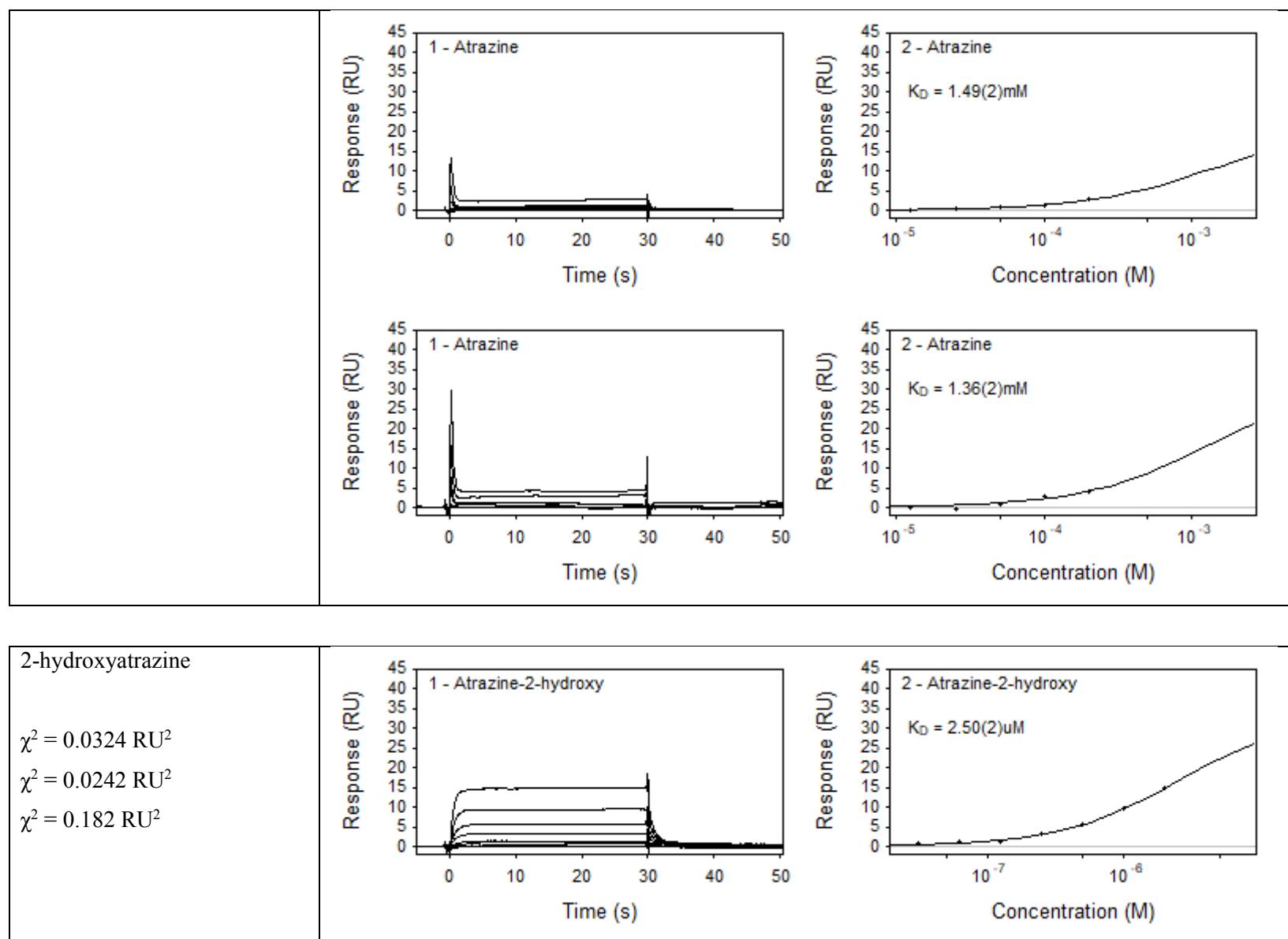


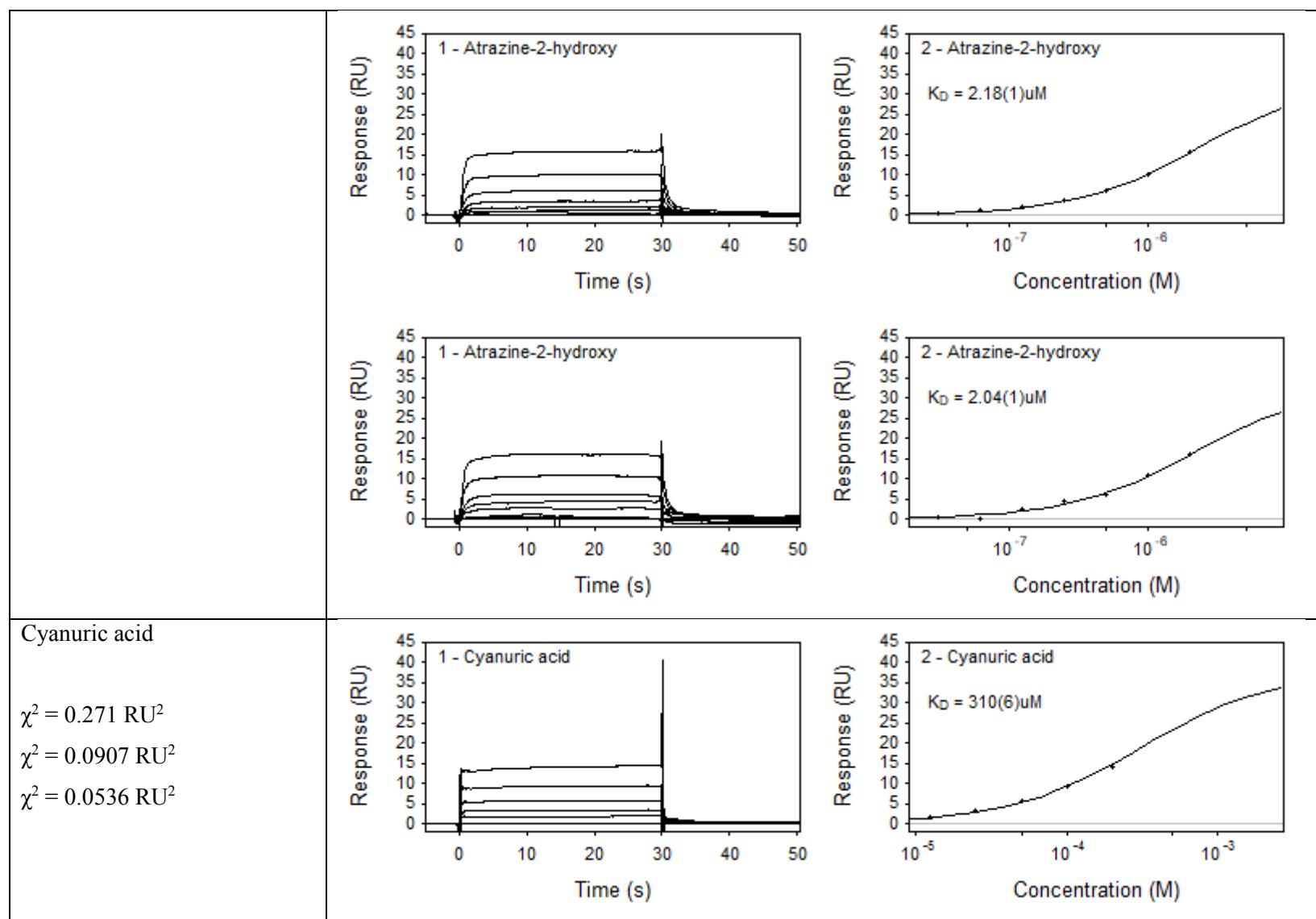


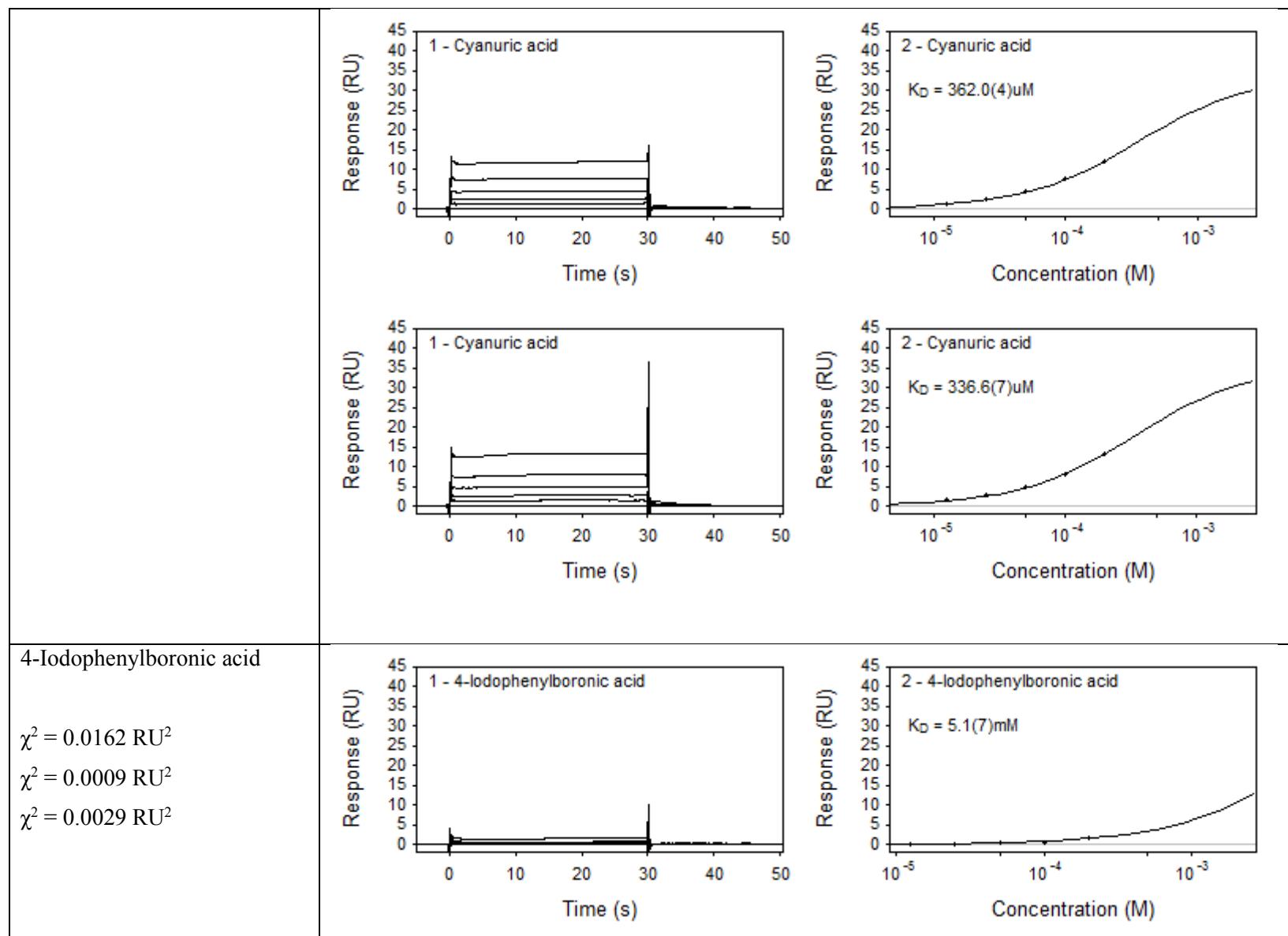


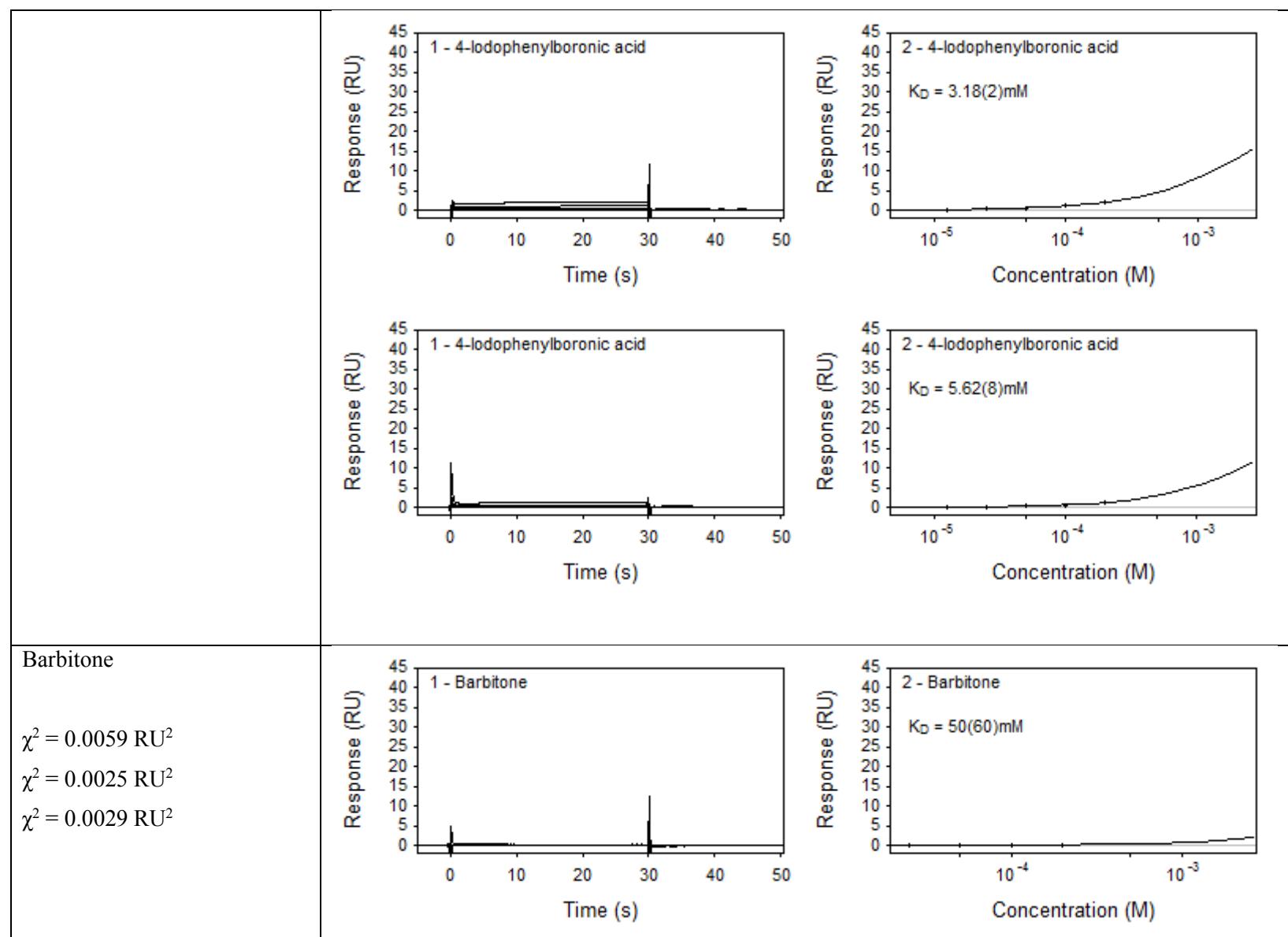


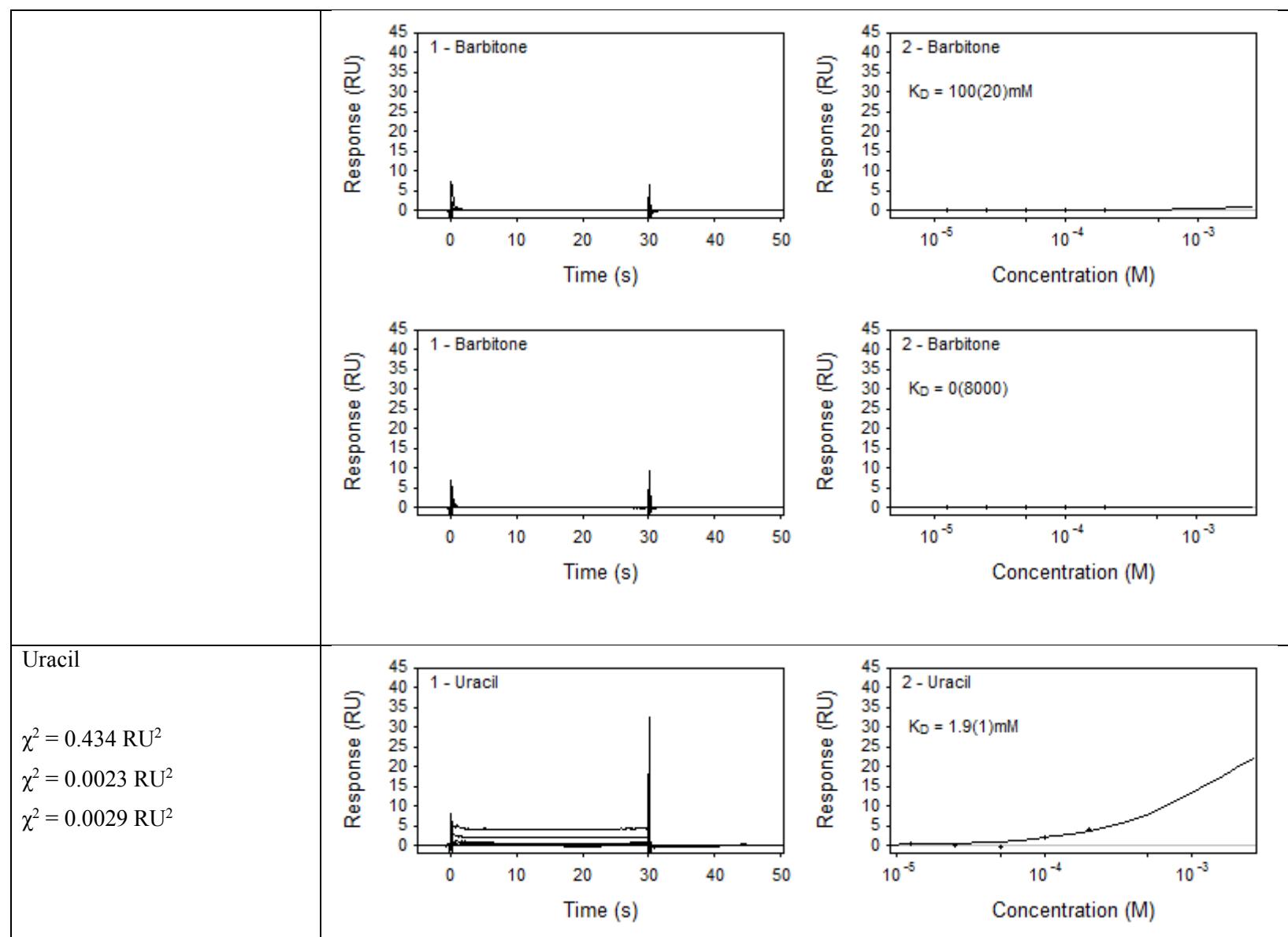


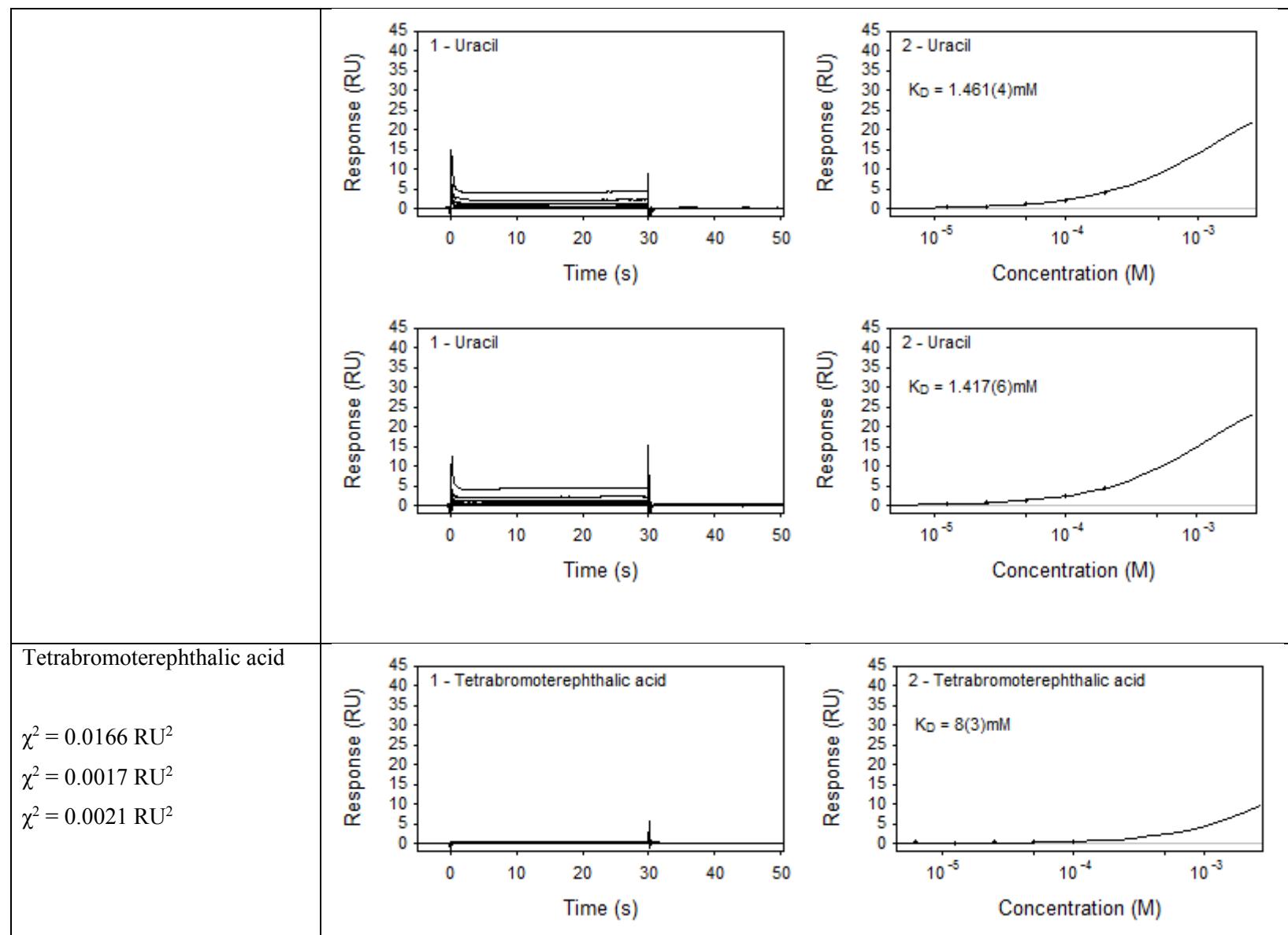


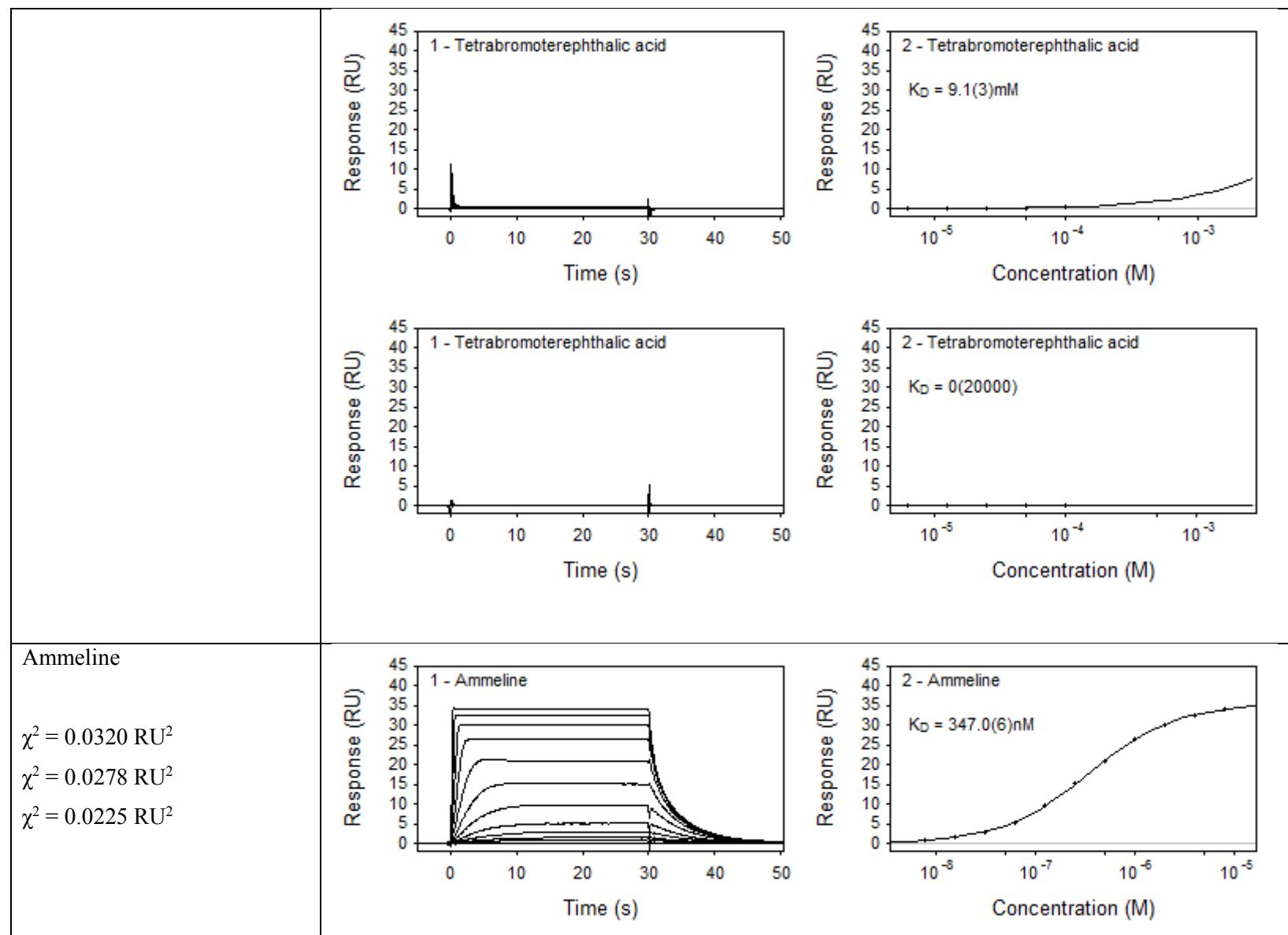


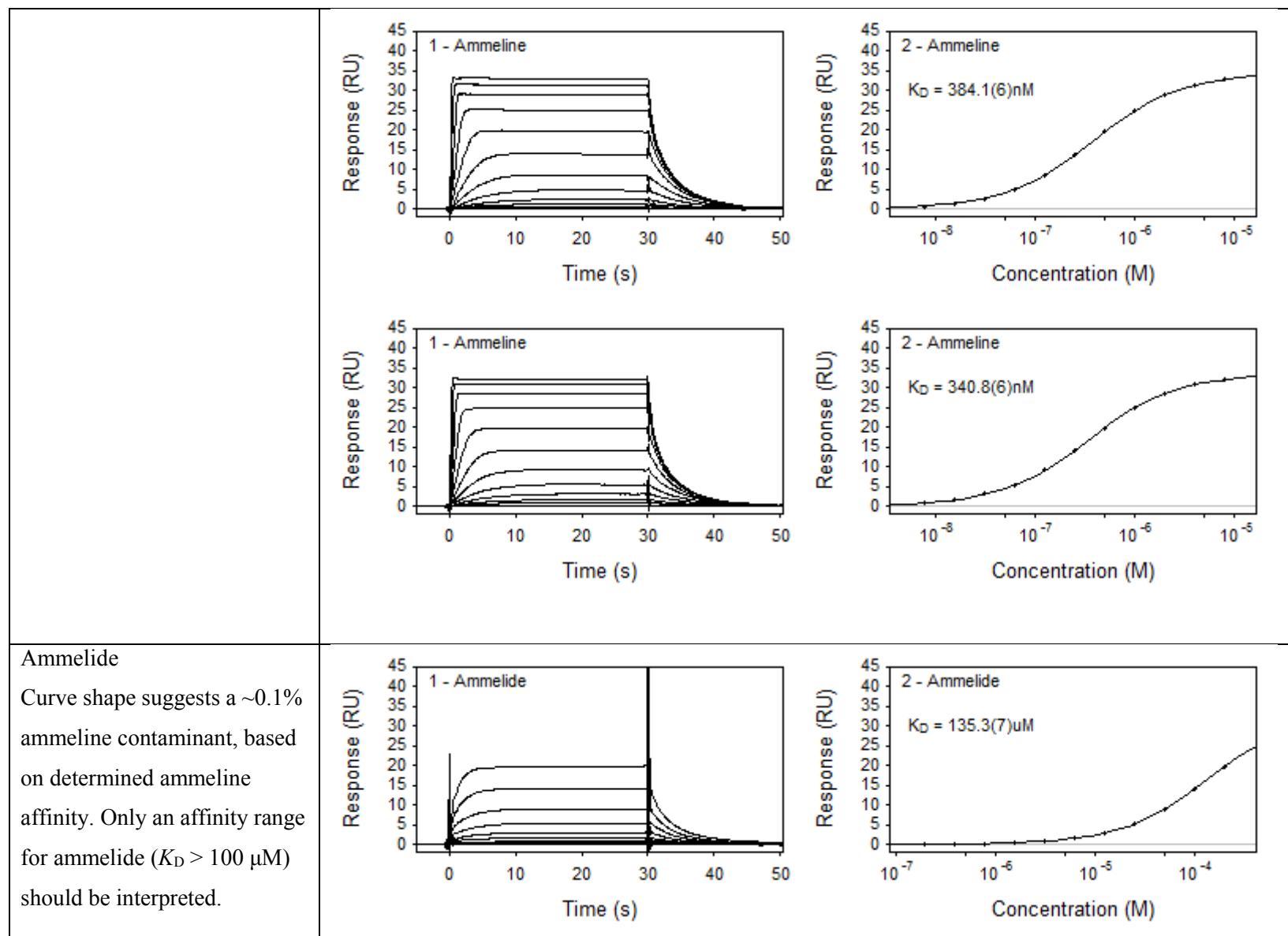


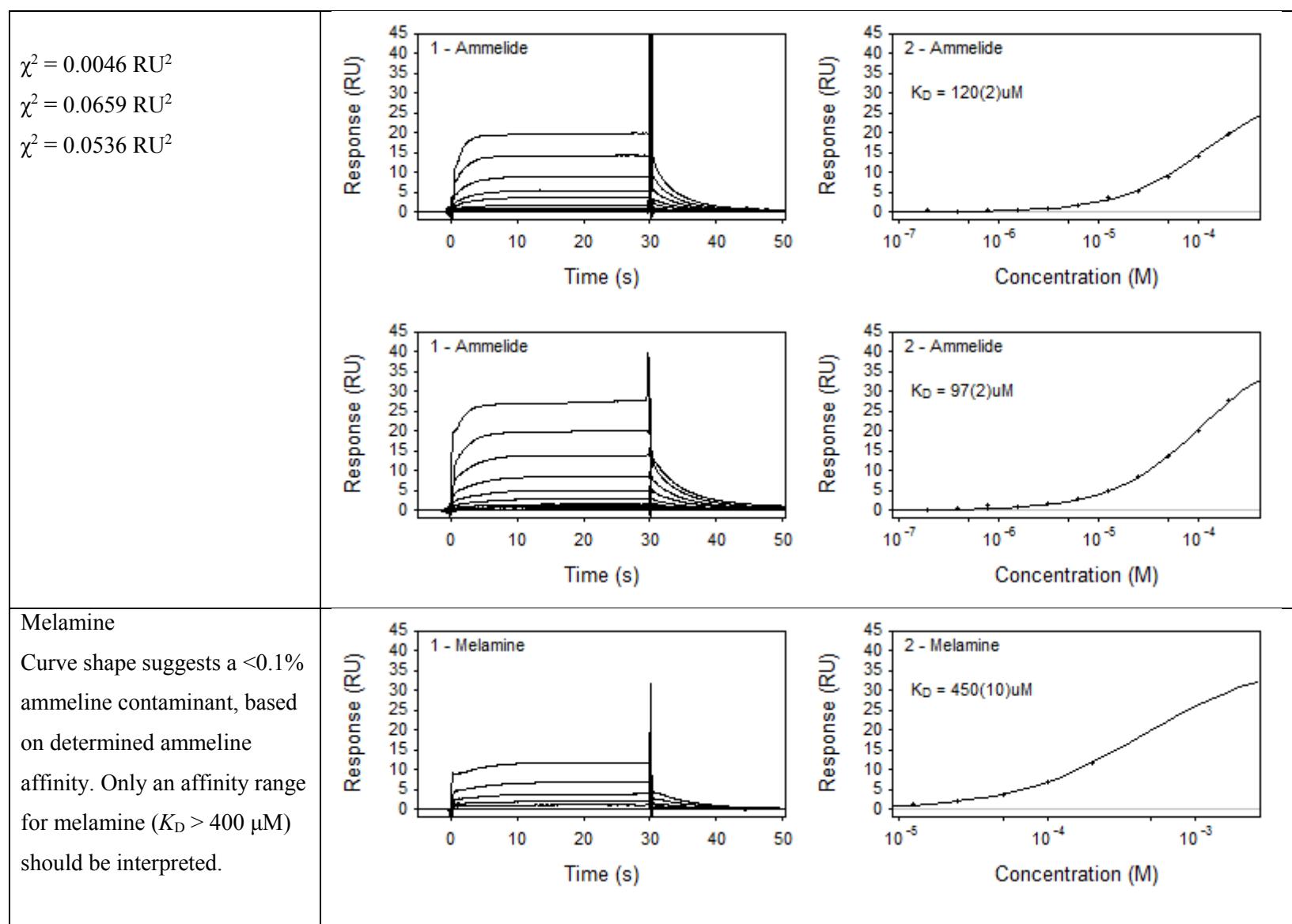


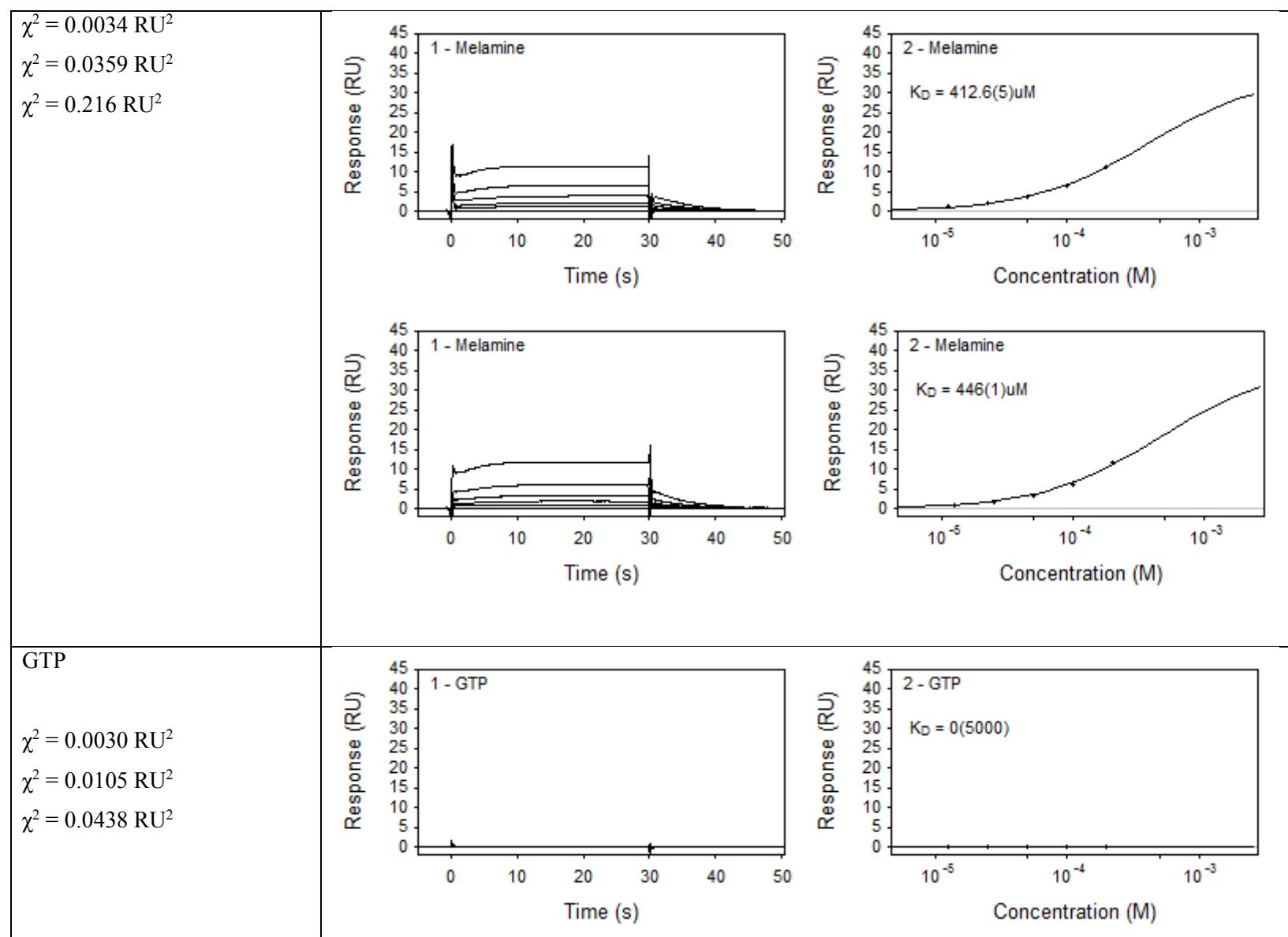












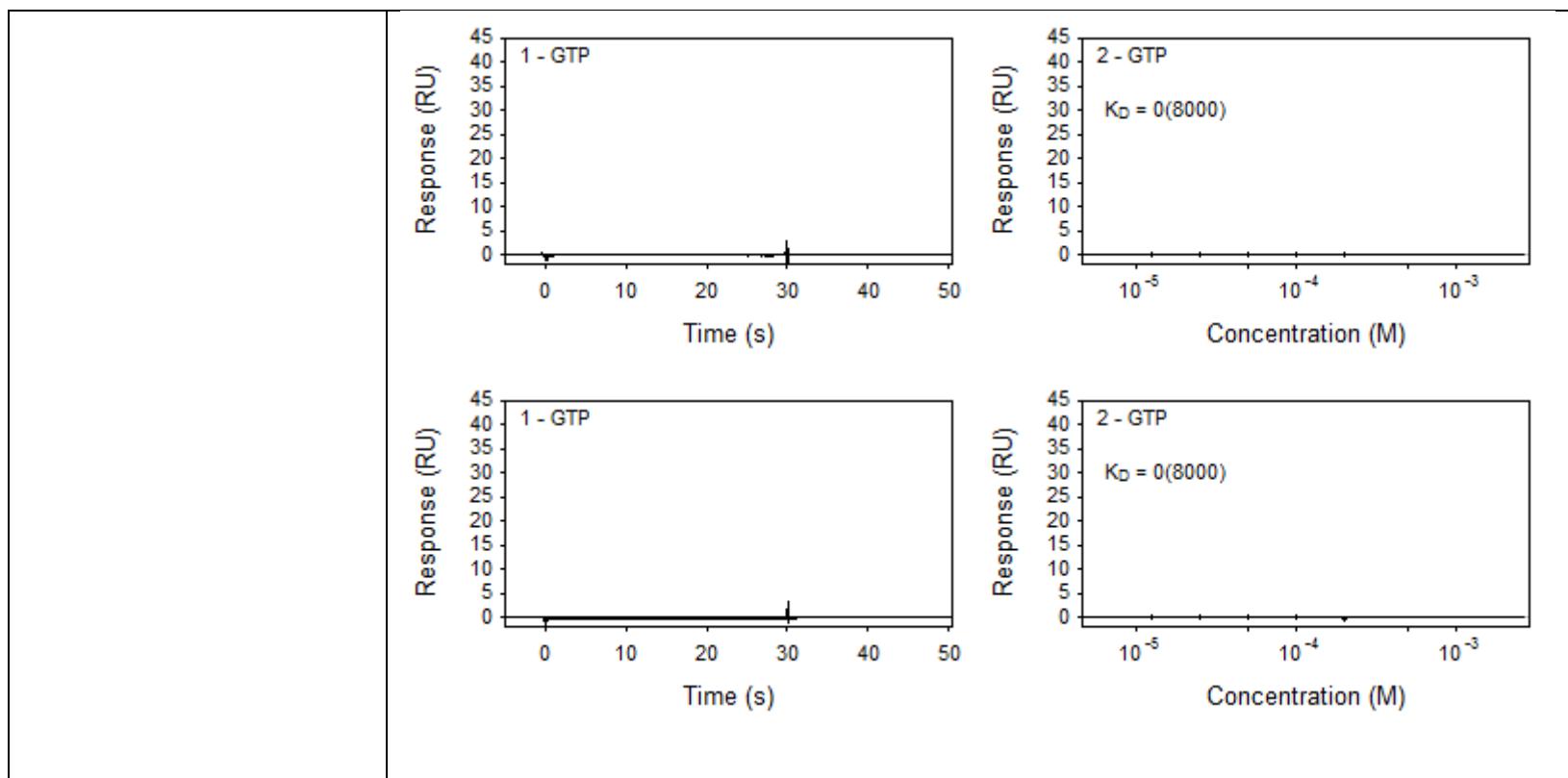


Table S1 Type and number of substrate binding proteins found in the *Pseudomonas* sp. strain ADP genome.

Type of substrate binding protein found in <i>Pseudomonas</i> sp. ADP	Number
amino acid	46
spermidine/putrescine	19
sulfur	6
polyamine/nitrate	4
metal (iron, molybdate)	7
sugar/carbohydrate	3
phosphore	3
multidrug resistance	1
unassigned SBP	23