Supplementary Information

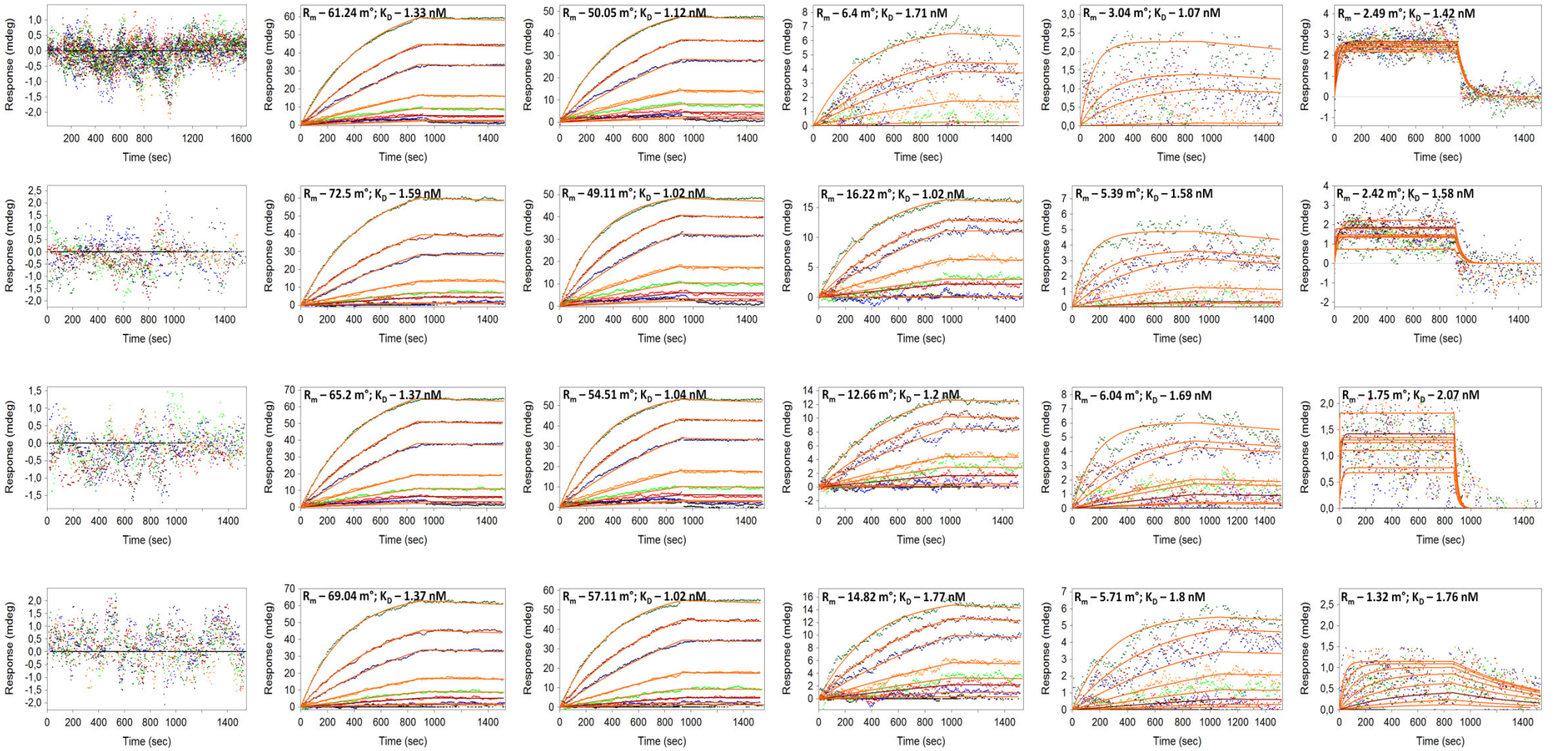
β2M – a-β2M interaction is explained in detail to provide extra information with respect to the article. Since the first column of figure 1S represents buffer spots (spots 1, 7, 13, 19), there is hardly any reaction of analyte which also gives us information about the non-specific binding. The sensorgrams are of various shapes when the spot ligand concentrations are varied and that could be observed here (Fig. 1s). Here the sensorgrams with various analyte concentrations are overlayed and were fitted to the 1:1 interaction model. The respective ligand and analyte concentrations are listed in the main article. At very low spot ligand concentrations (spots 6, 12, 18, 24), the on-rates are much faster as well as faster dissociation. These sensorgrams are typical, which can be observed in the literature for this interactant pair as this might be because of the lower immobilized density (approximately 100 RU). This value corresponds with approximately 10 mdeg which is low and close to the lowest applied spot concentrations. As a rule of thumb 1000 RU SPR units corresponds to 1 ng/mm^2 protein while 1 mdeg <> 10.8 pg/mm^2 protein on the surface (according to calibration supplied by the manufacturer). So if a response is measured of 2.5 mdeg SPR angle shift then in refractive units introduced by Biacore is 25 RU. Since the ligand concentration for creating the spot is not equal to the effective immobilized ligand density because the ligand coupling is not followed in real time , proper quantification of the signal could not be performed in our case. In the article[47], the authors do not use any regeneration step as the signal goes to zero in the dissociation steps. A regeneration step for the low concentration spots is not necessary as we can see that the dissociation curves reach zero (column 6 – Fig 1s). But the higher ligand concentration spots need a regeneration step or a longer time for dissociation as could be

seen in the sensorgrams of the higher spot concentrations (column 2, 3 and 4 of the array – Fig 1s). The estimated affinity for the respective spots is shown in Fig 1s together with its extracted R_m values. The average K_D calculated for the whole array is 1.40 ± 0.26 nM. The obtained affinity is similar to that of the affinity listed in the literature[47]. The basic difference between GE Healthcare's Biacore instruments and IBIS systems are that the Biacore use a flow-through approach for injection of the sample whereas in the latter case in IBIS system a back and forth mixing of a small amount of sample is used. This is advantageous with respect to less sample volume consumption and high and constant mass transport rates but there is also a major disadvantage at the dissociation phase when molecules are dissociating from the surface and intrinsically increasing the concentration from zero to a certain value. Then molecules may rebind to the surface again and the off-rate will be decreased. This might be a reason that we observe some deviation in the fits of dissociation phase at higher ligand concentration spots (ligand spot numbers 2, 3, 8, 9, 14, 15, 20, 21). In the IBIS-iSPR system this problem has been solved by applying both back and forth mixing while at the same time fresh buffer is flown through the flow cell.

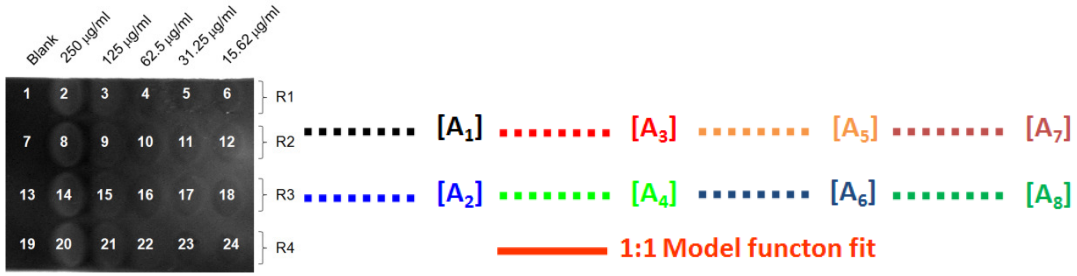
The single injection kinetics results are shown in Fig 2s. When the concentration of analyte is increased for example Fig 2sd for the analyte [A_4], 2 different sensorgram profiles can be observed in which for higher concentration ligand spots, the dissociation seems to be slower. This could be due to the fact that the ligand spot has reached saturation and therefore the applied ligand concentration to create the spots are not linearly correlated always to the effective density. The effective ligand density can be better correlated to the Rm value. The affinity constant estimated using this new approach is shown in Fig 2s. The overall calculated affinity is 1.53 ± 0.63 nM. This is an average

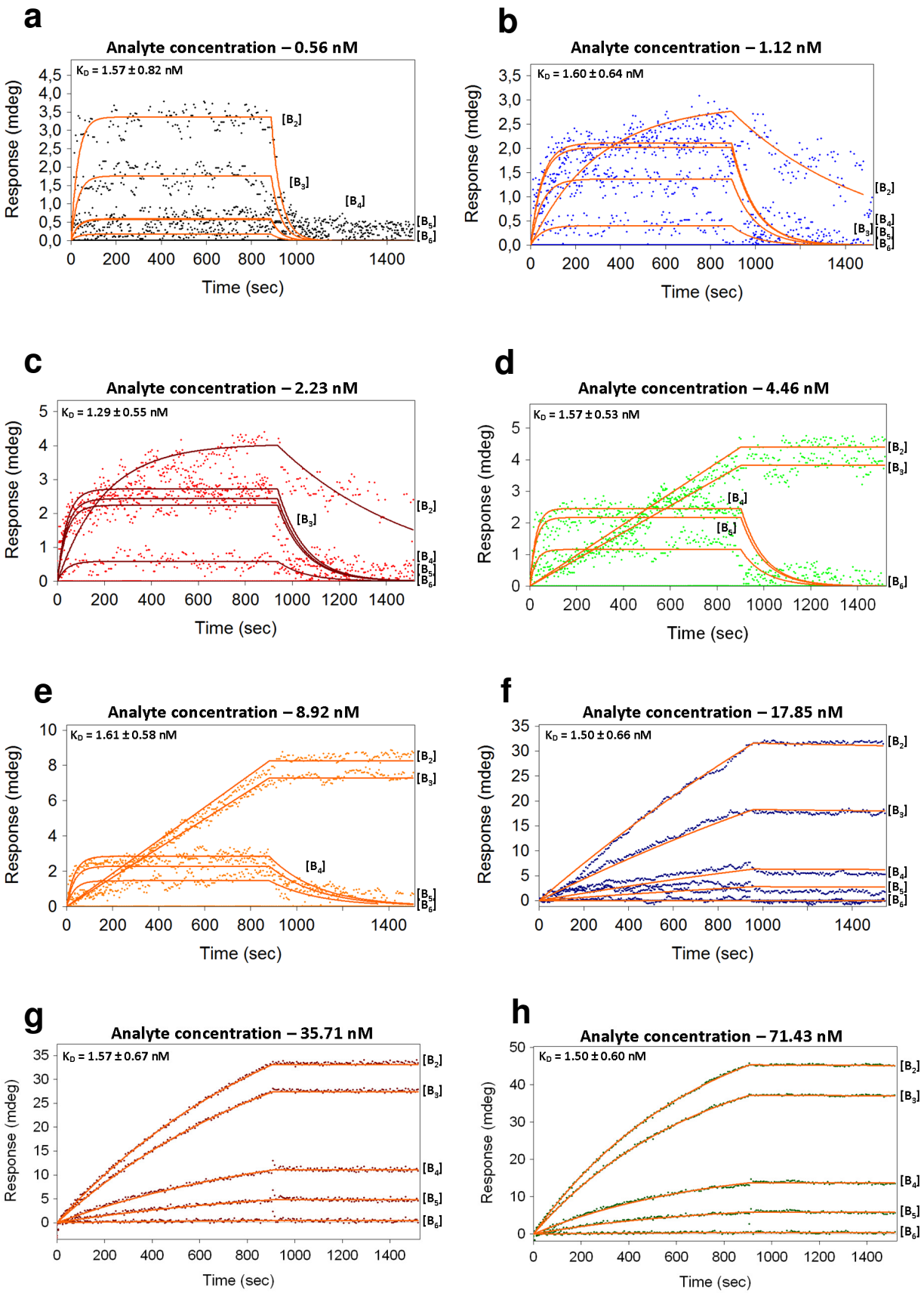
value with standard deviation calculated for 4 series of data with 8 different analytes. This affinity value correlates with the value that was estimated with conventional measurements. The R_m values extrapolated in this method are not a global parameter and therefore linked with each other as the ligand density is different at each spot of the microarray. The extracted R_m values are listed in table 1s with respect to various analyte concentrations for the varying ligand concentrations. Higher noise values affect the kinetic parameter extraction for low ligand density spots. K_D and R_m extracted using conventional analysis are plotted against various ligand concentrations with standard deviation (Fig 3sa). The same profile was already reported for other interactant pairs[50]. K_D extracted using the single injection approach is plotted against various analyte concentrations and standard deviations (Fig 3sb). The best is to restrict injection to a single analyte concentration which is close to the affinity constant value to obtain the kinetics and affinity of the interactant pairs. Normally if the affinity is unknown, then this is the first step to calibrate the interactant pairs with various analyte concentrations and single injection could be useful for a series of measurements. Also this approach is really useful for screening multiple ligands in a microarray and injection of multi-analytes leads to multiple kinetics in the same time which drastically reduces the time and costs of experiments that are in progress in our lab at present.

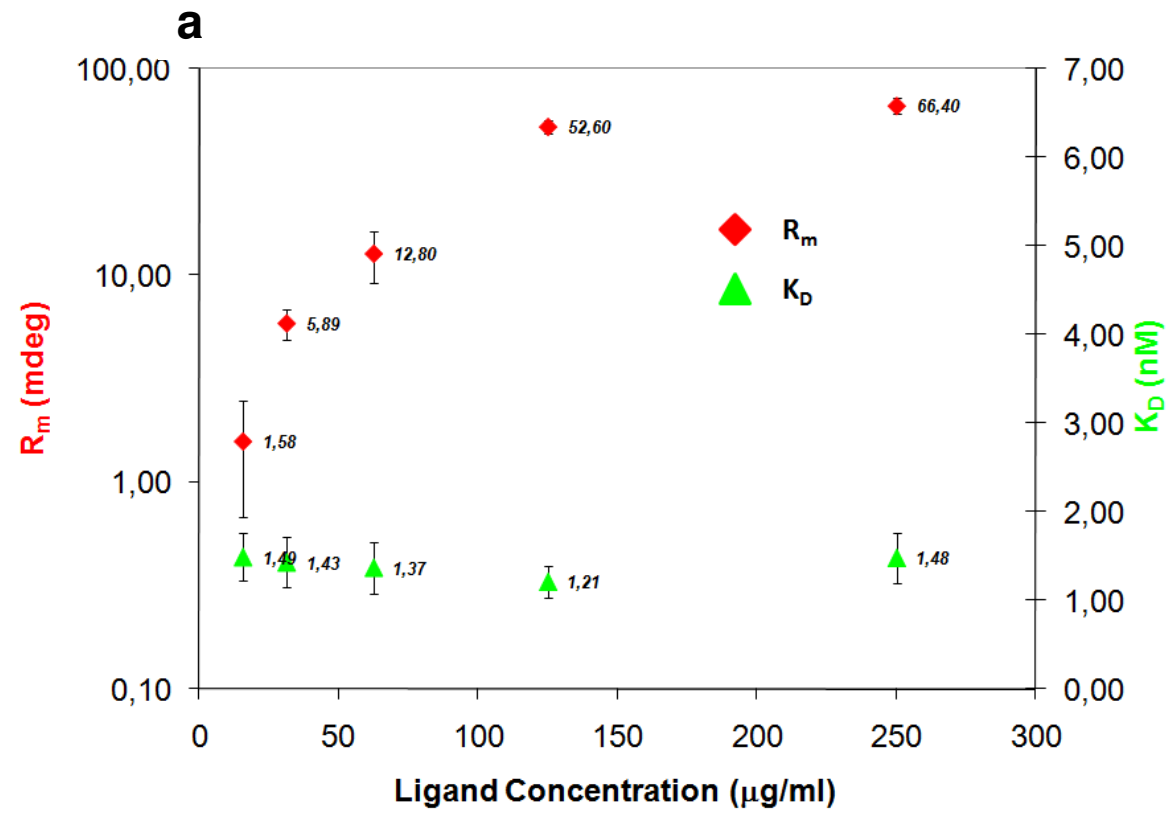
Ligand conc. (µg/ml)	**Analyte conc. (nM)**							
	0.5	1.1	2.2	4.5	9	18	36	72
	Maximum response R_m (mdeg)							
250	1.1	4.4	6.0	29	48	52	61	77
125	1.0	1.1	1.9	9.8	22	42	46	59
62.5	3.6	1.0	3.0	4.7	4.0	6.4	7.6	10.9
31.2	0.6	0.9	3.2	1.3	2.1	3.0	3.3	3.9
15.6	2.0	0.4	0.5	0.9	0.5	0.6	1.3	1.8



[B_1] – 0 µg/ml **[B_2] – 250 µg/ml** **[B_3] – 125 µg/ml** **[B_4] – 62.5 µg/ml** **[B_5] – 31.25 µg/ml** **[B_6] – 15.62 µg/ml**




a
Analyte concentration – 0.56 nM
K_D = 1.57 ± 0.82 nM
b
Analyte concentration – 1.12 nM
K_D = 1.60 ± 0.64 nM
c
Analyte concentration – 2.23 nM
K_D = 1.29 ± 0.55 nM
d
Analyte concentration – 4.46 nM
K_D = 1.57 ± 0.53 nM
e
Analyte concentration – 8.92 nM
K_D = 1.61 ± 0.58 nM
f
Analyte concentration – 17.85 nM
K_D = 1.50 ± 0.66 nM
g
Analyte concentration – 35.71 nM
K_D = 1.57 ± 0.67 nM
h
Analyte concentration – 71.43 nM
K_D = 1.50 ± 0.60 nM
Response (mdeg)
Time (sec)
[B2]
[B3]
[B4]
[B5]
[B6]


a
100,00
10,00
1,00
0,10
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
50
100
150
200
250
300
Rm (mdeg)
KD (nM)
Ligand Concentration (µg/ml)
Rm
KD
66,40
52,60
12,80
5,89
1,58
1,49
1,43
1,37
1,21
1,48

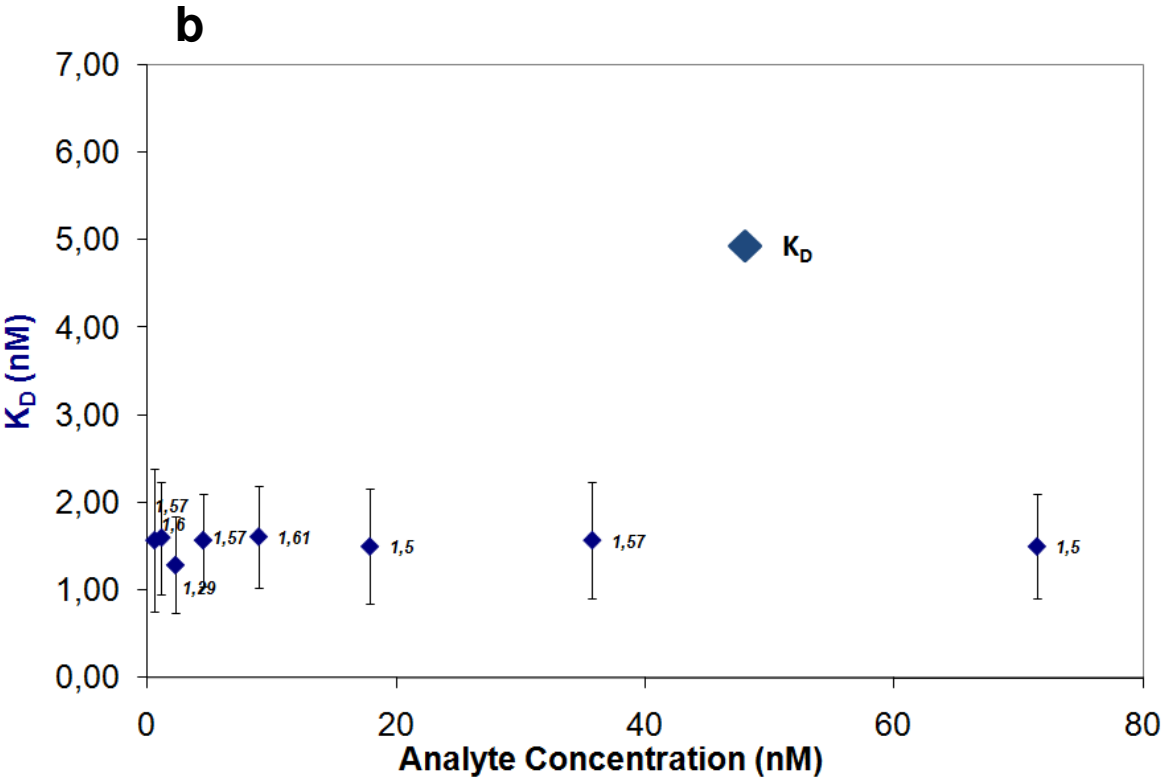

b
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
20
40
60
80
KD (nM)
Analyte Concentration (nM)
KD
1,57
1,6
1,29
1,57
1,61
1,5
1,57
1,5

Table Legend(s)

Table 1s: β2M – a-β2M interactions; R_m extrapolated from global fitting of the data for various ligand concentrations with respect to various analyte concentrations.

Figure Legends

Figure 1s: β2M – a-β2M interaction conventional kinetics measurements using iSPR. The overlayed sensorgrams for the measured responses for the whole array is represented in array format. Various colours in each sensorgram represent various analytes injections. The real time iSPR image of the array is shown.

Figure 2s: β2M – a-β2M interaction single injection kinetics measurements using iSPR. Each plot is the representation of single injection of analyte interacted with the 5 different spots with various ligand concentrations.

Figure 3s: a) Plot of ligand concentration vs. R_m (which represents the ligand density) and K_D for the conventional kinetics measurements. b) Plot of analyte concentration vs. K_D for the single injection kinetics measurements.