Electronic Supplementary Material (ESI) for Lab on a Chip
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ELECTRONIC SUPPLEMENTARY INFORMATION

On-Chip Bioorthogonal Chemistry Enables Immobilization of In Situ Modified Nanoparticles and Small Molecules for Label-Free Monitoring of Protein Binding and Reaction Kinetics

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Determination of GST conjugate stability and loading:

Fig. S1. GST amino acid sequence and crystal structure showing lysine residues

PDB ID: 1Y6E

```
1   spilgywkik glvqptrlll eyleekyeeh lyerdegdkw rnkkfelgle fpnlpyyidg
61  dvkltqsmai iryiadkhnm lggcpkerae ismlegavld irygvsriay skdfetlkvd
121 flsklpemlk mfedrlchkt ylngdhvthp dfmlydaldv vlymdpmcld afpklvcfkk
181 rieaipqidk ylksskyiaw plqgwqatfg ggdhppksdl vpr
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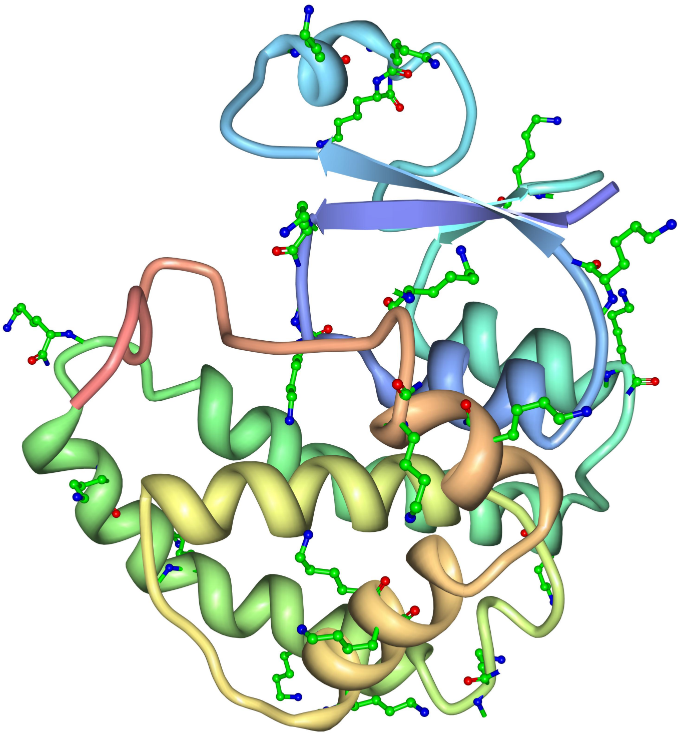

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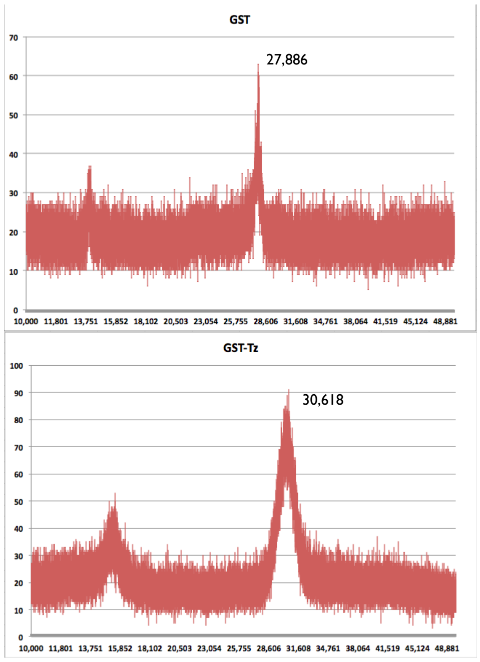


Fig. S2. MALDI-TOF spectra showing the mass for GST and Tz-modified GST. The difference in mass is indicative of Tz loading.

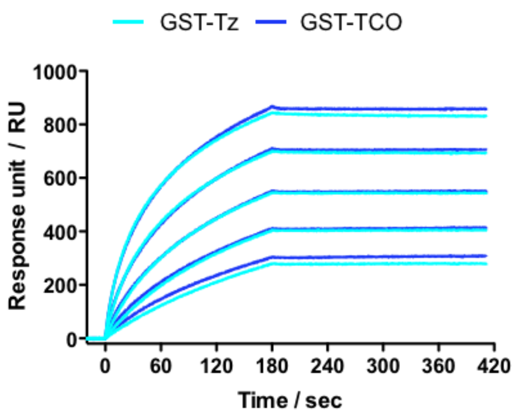
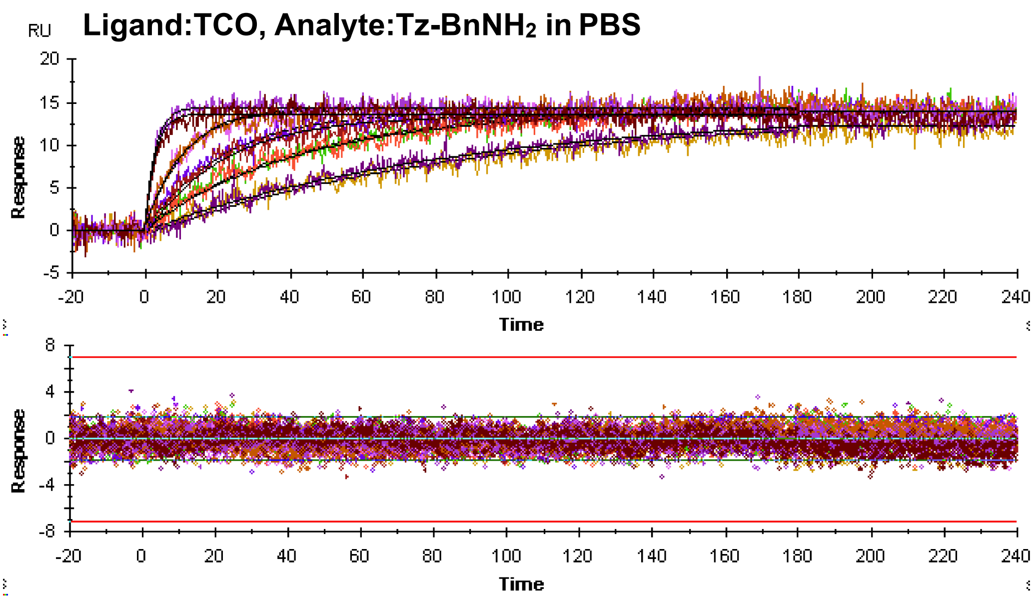


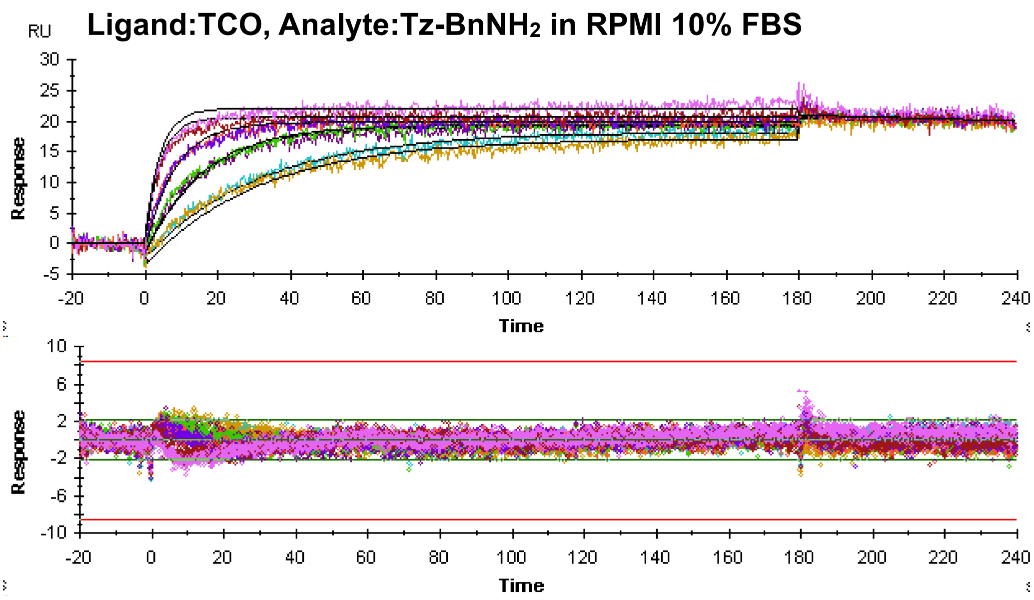
Fig. S3 Overlay of sensograms showing GST-Tz and GST-TCO capture and stability.

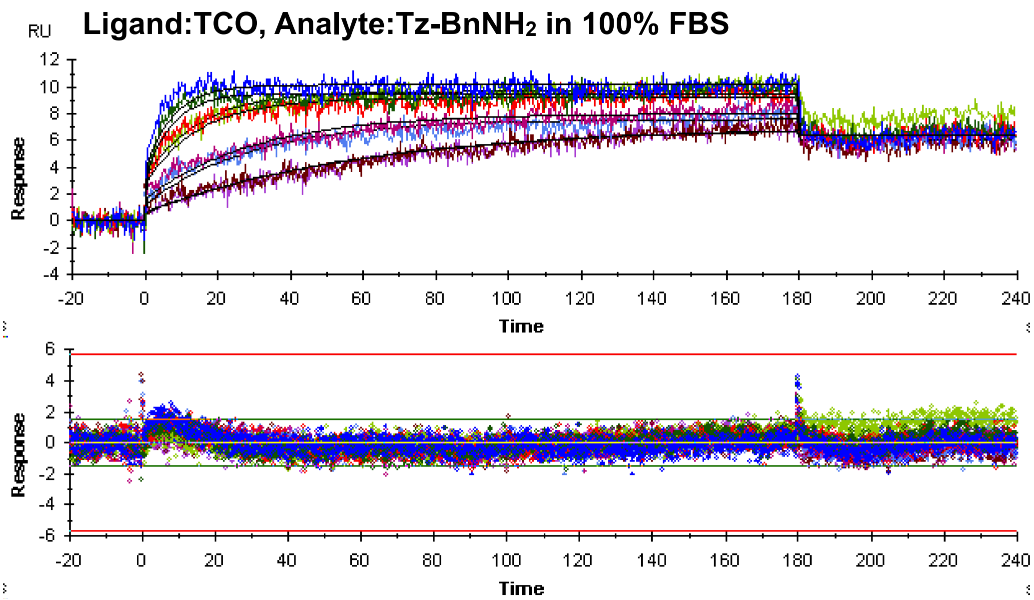
Measurements of small molecule cycloaddition rates:

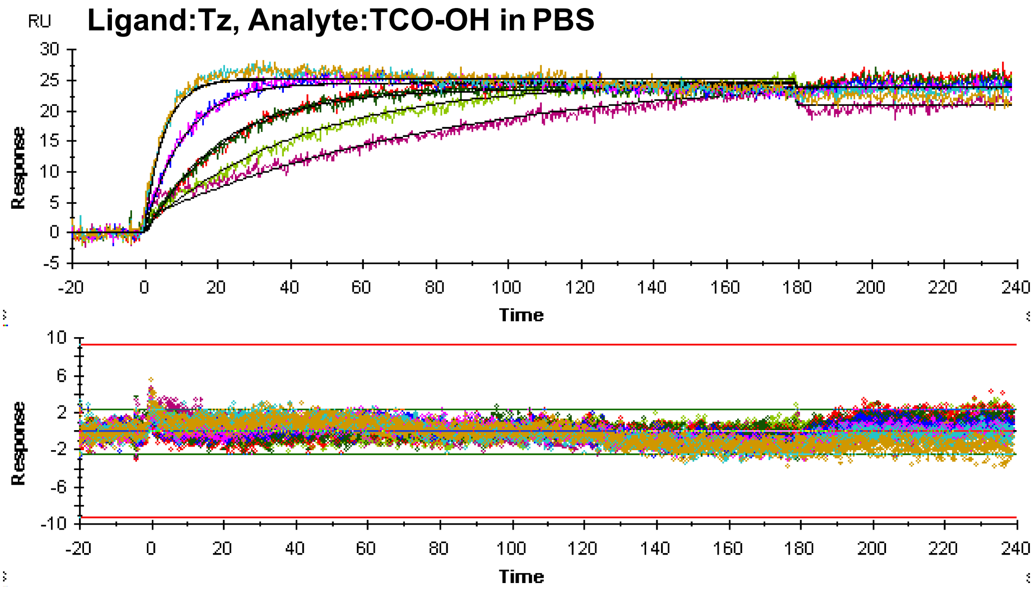
Table S1. Summary of cycloaddition (association) rate constants, k_a for small molecules in various buffer conditions.

Buffer	Analyte	Ligand	k_a $M^{-1}s^{-1}$	SEM
PBS	Tz-$BnNH_2$	TCO	34,062	1,536
RPMI, 10% FBS	Tz-$BnNH_2$	TCO	23,740	180
100% FBS	Tz-$BnNH_2$	TCO	11,943	2,987
PBS	TCO-OH	Tz	18,880	1,570
RPMI, 10% FBS	TCO-OH	Tz	12,775	925
100% FBS	TCO-OH	Tz	2,415	111




Ligand:TCO, Analyte:Tz-BnNH2 in RPMI 10% FBS
RU
Response
Time
Response
Time


Ligand:TCO, Analyte:Tz-BnNH2 in 100% FBS
RU
Response
Time
Response
Time


Ligand:Tz, Analyte:TCO-OH in PBS
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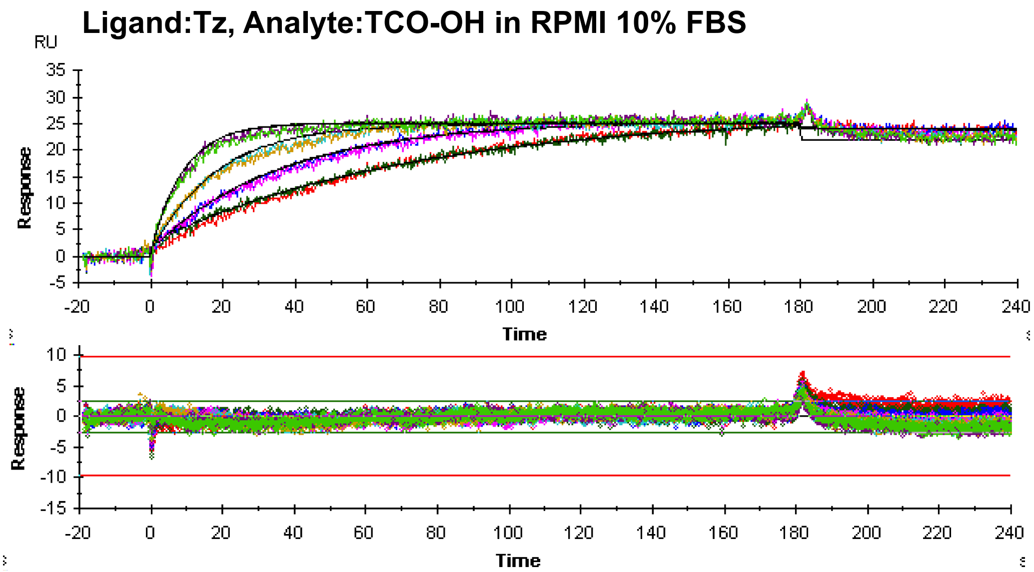
Response
Time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Response
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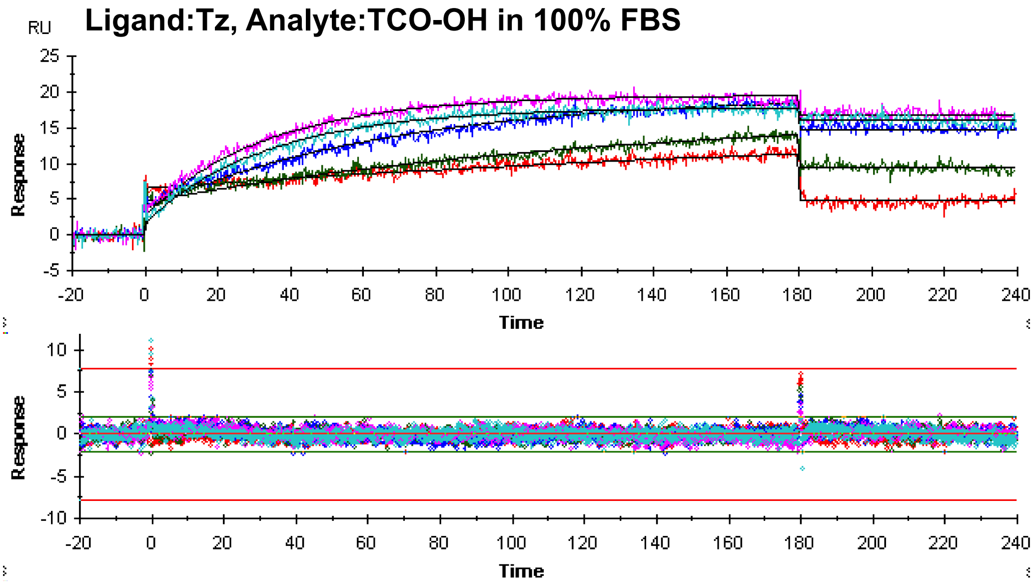
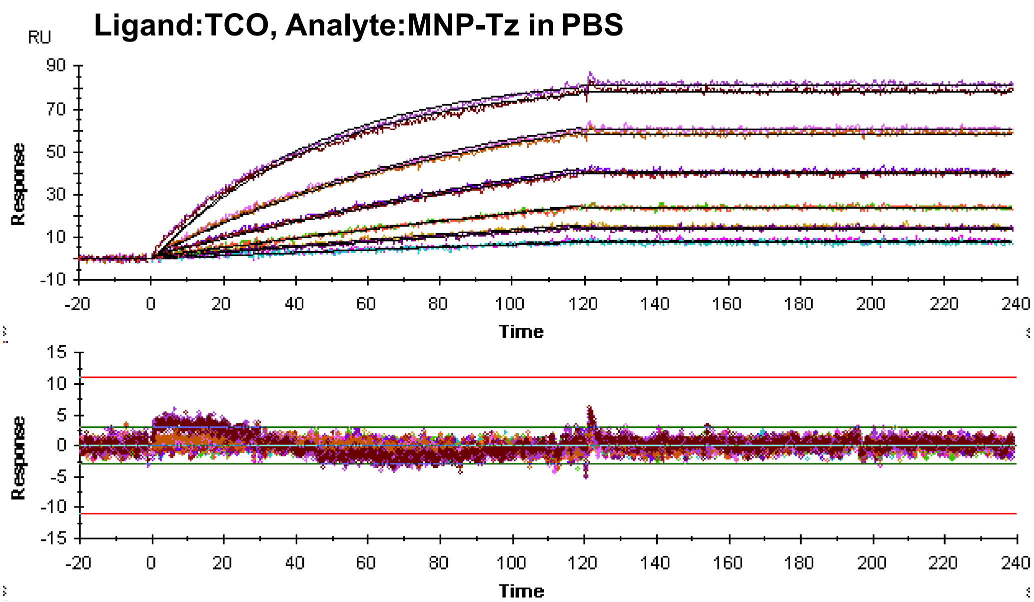
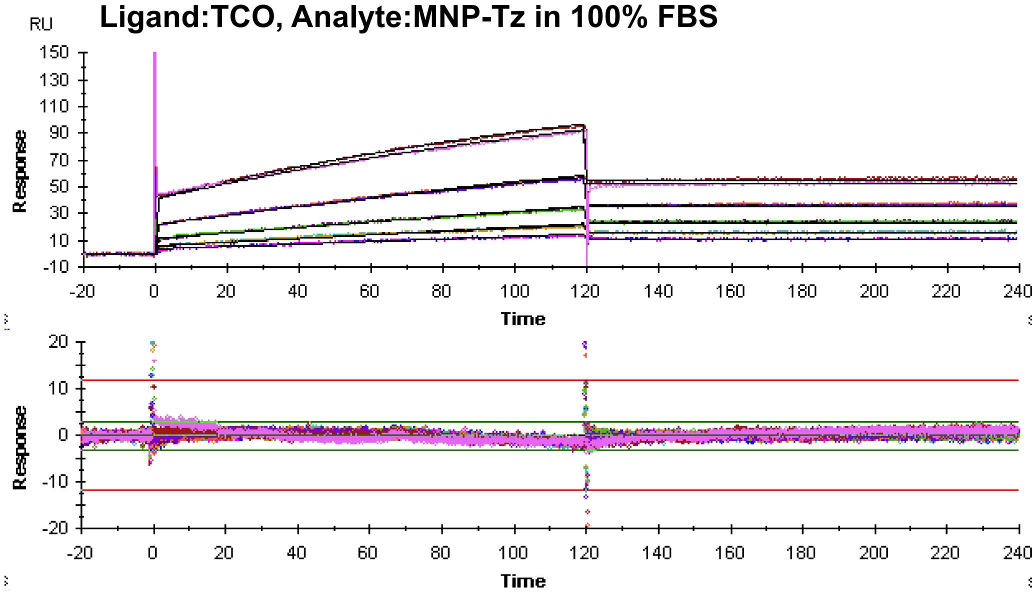

Ligand:Tz, Analyte:TCO-OH in 100% FBS
RU
Response
Time
Response
Time

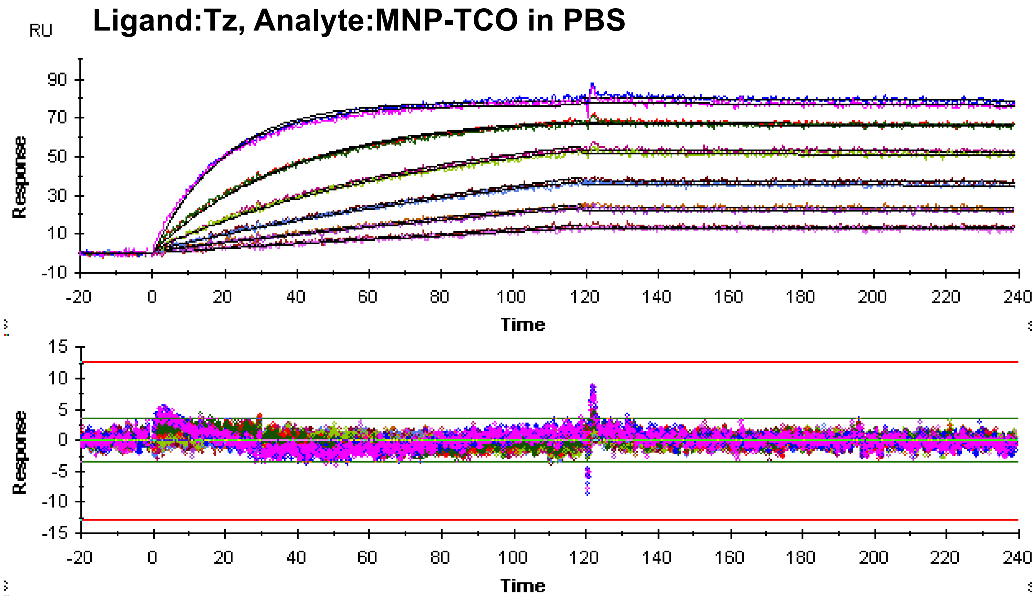
Measurements of MNP-conjugate cycloaddition rates:

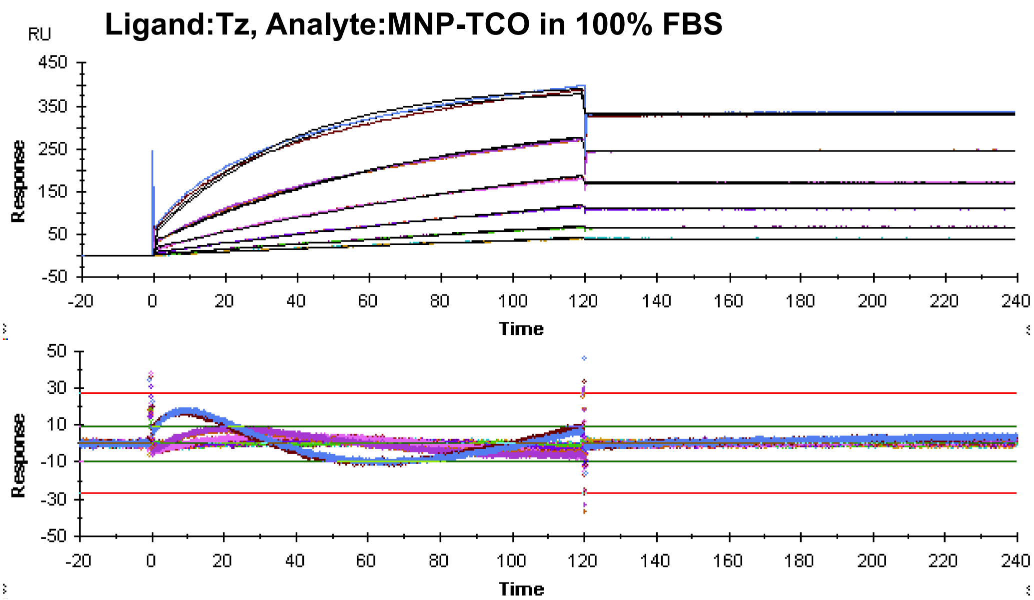
Table S2. Summary of cycloaddition (association) rate constants, k_a for MNPs in various buffer conditions.

Buffer	Analyte	Ligand	k_a $M^{-1}s^{-1}$	SEM
PBS	MNP-Tz	TCO	89,573	4,412
100% FBS	MNP-Tz	TCO	28,705	1,145
PBS	MNP-TCO	Tz	235,440	4,554
100% FBS	MNP-TCO	Tz	118,200	5,500

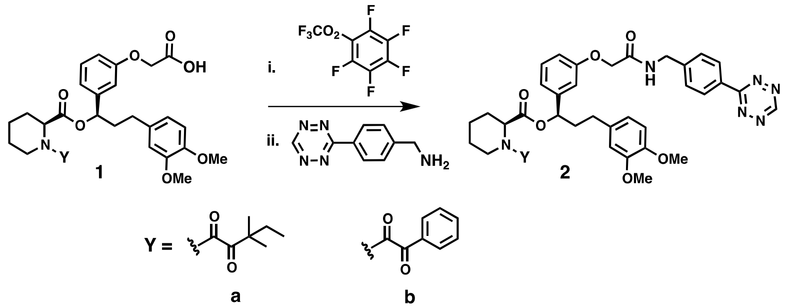



Ligand:TCO, Analyte:MNP-Tz in 100% FBS
RU
Response
Time

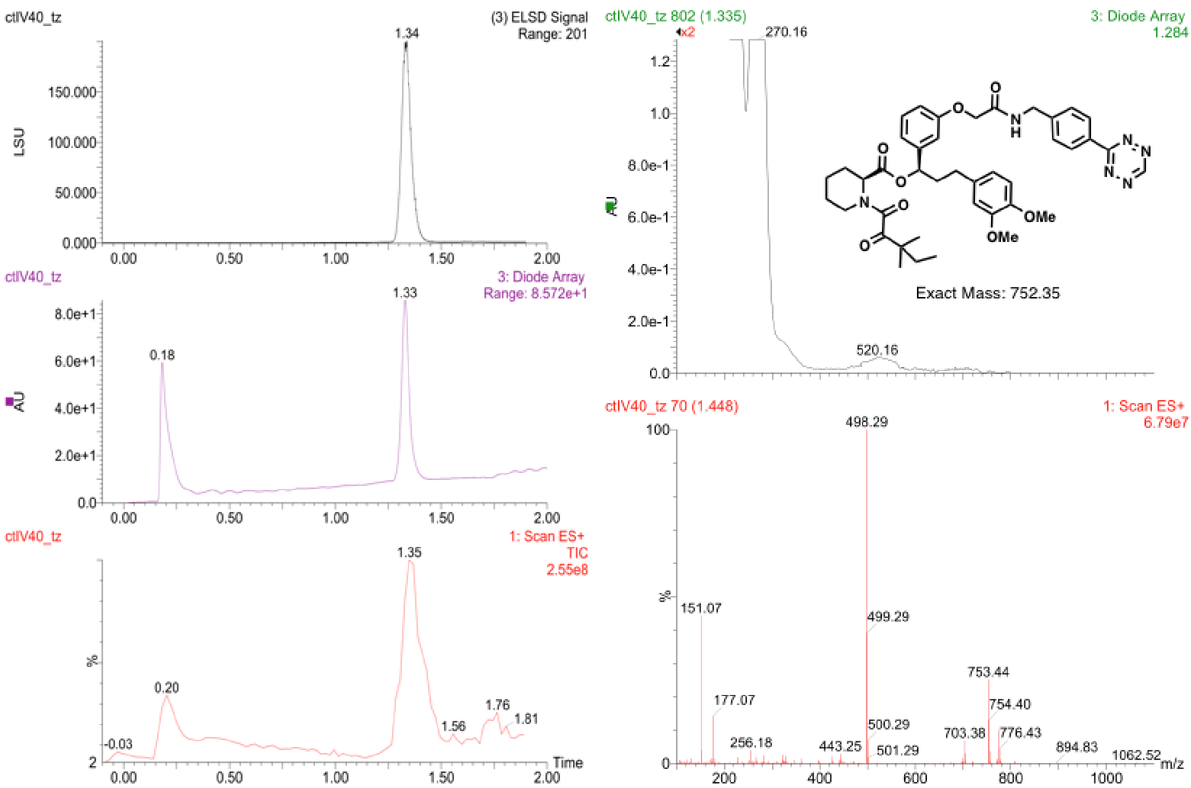

Ligand:Tz, Analyte:MNP-TCO in PBS
RU
Response
Time


Ligand:Tz, Analyte:MNP-TCO in 100% FBS
RU
Response
Time
Response
Time

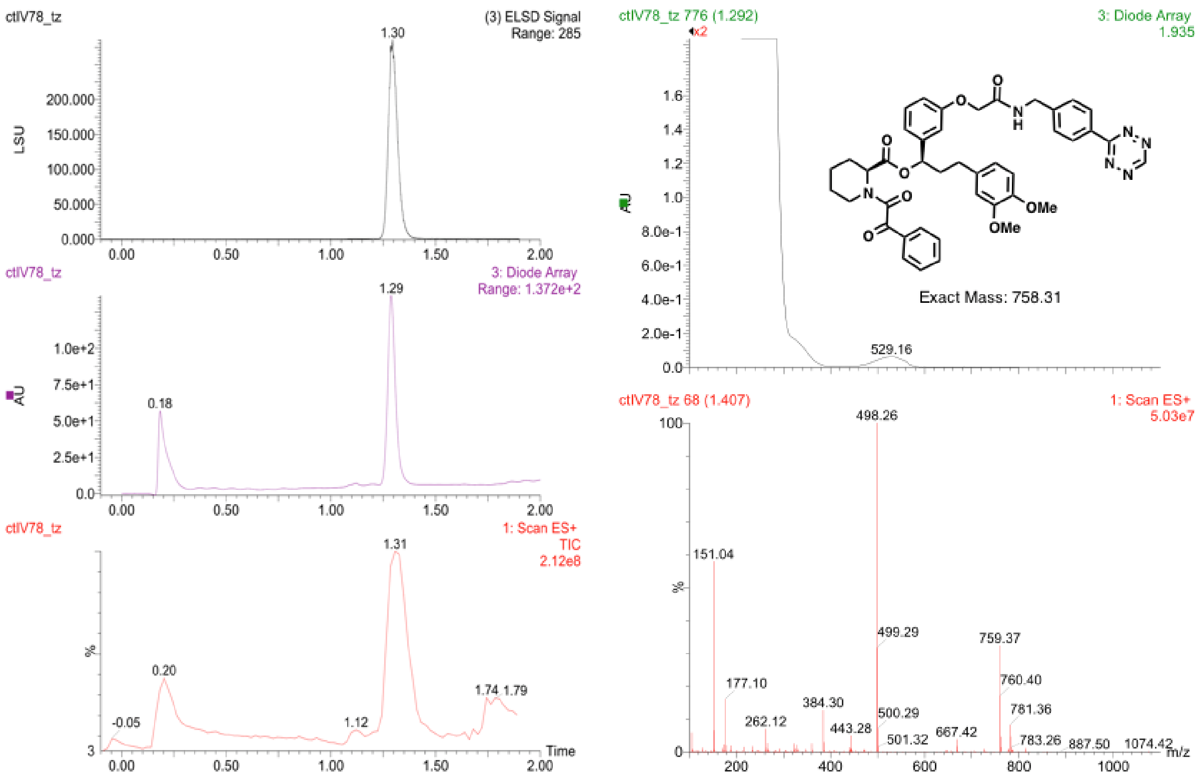
Analytical LC/MS chromatograms and spectra for FKBP12 binding ligands:



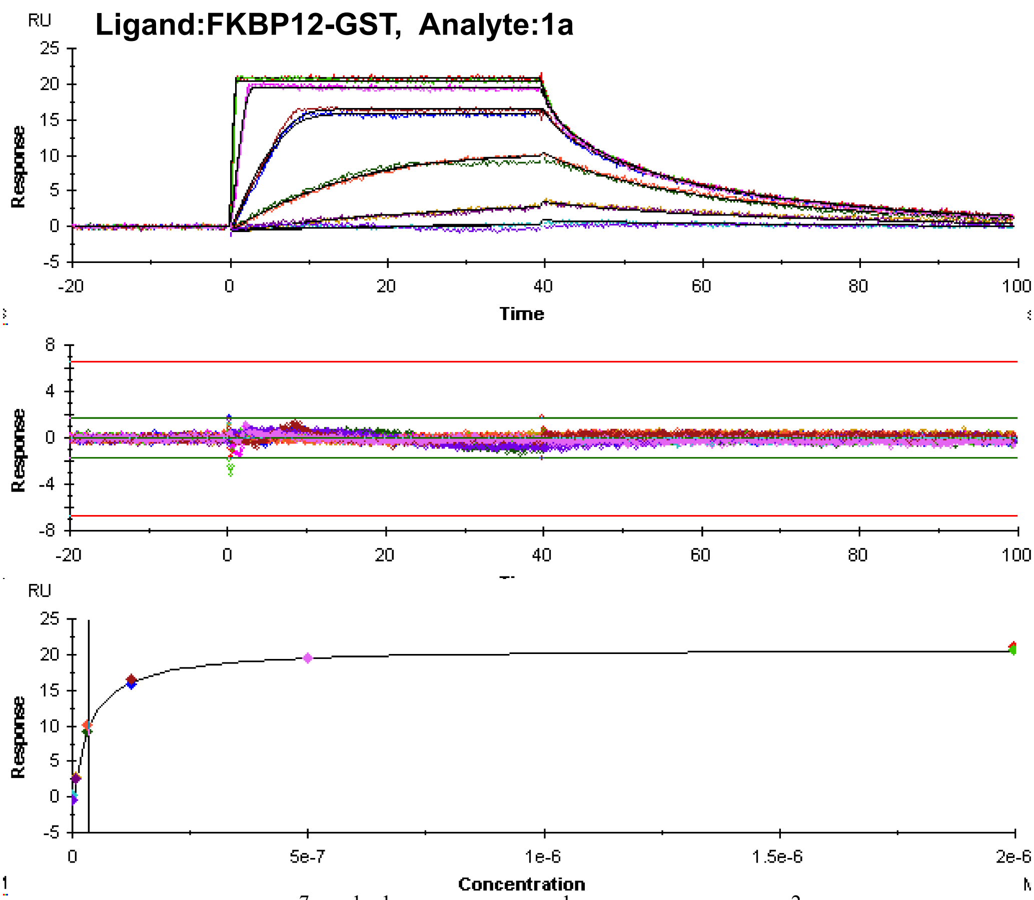
(*S*)-(*R*)-1-(3-(2-((4-(1,2,4,5-tetrazin-3-yl)benzyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl 1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (2a).



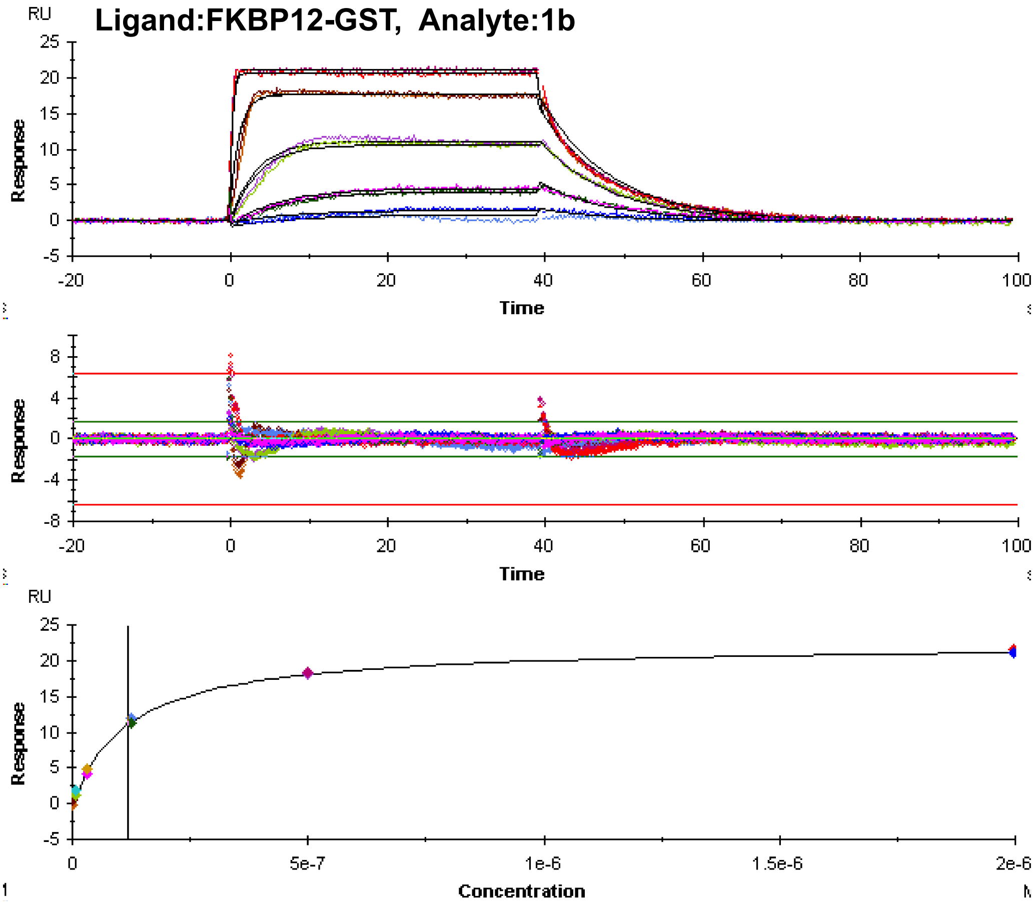
(*S*)-(*R*)-1-(3-(2-((4-(1,2,4,5-tetrazin-3-yl)benzyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl 1-(2-oxo-2-phenylacetyl)piperidine-2-carboxylate (2b).



The binding of small-molecules 1 to immobilized FKBP12:

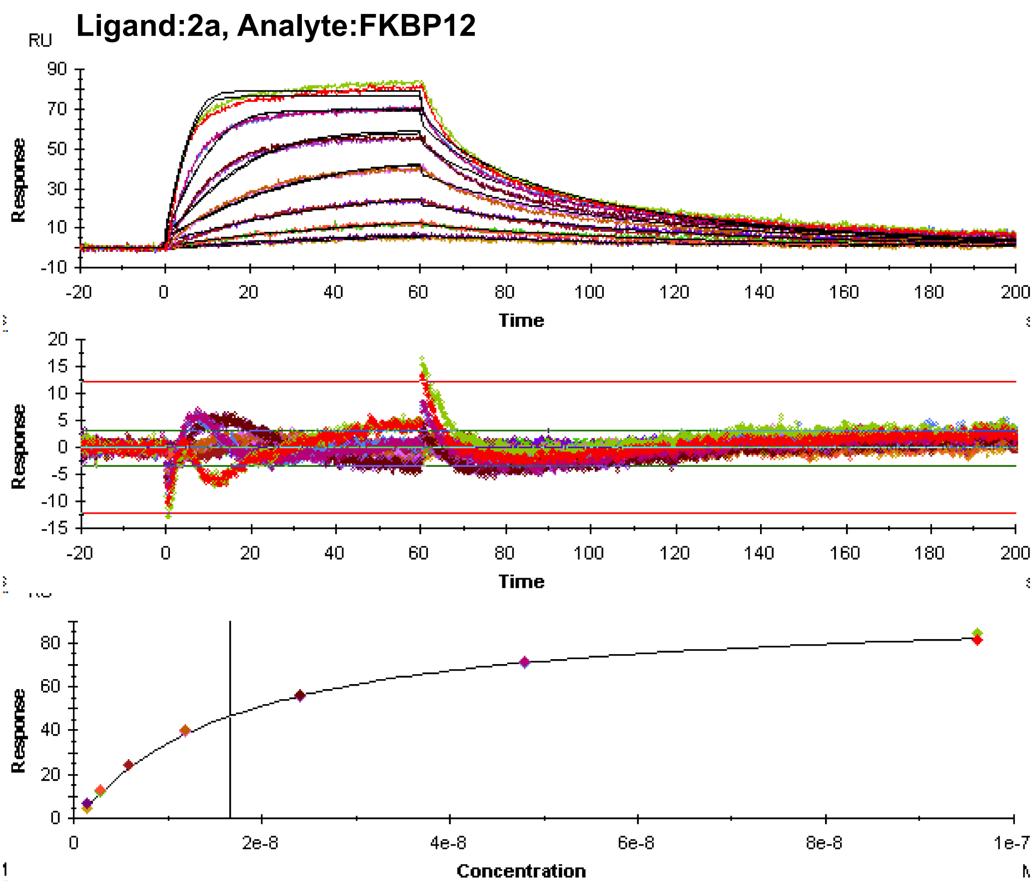


Kinetic analysis: k_a = 1.83 x 10^7 $M^{-1}s^{-1}$, k_d = 0.553 s^{-1}, K_D = 30.3 nM, χ^2 = 0.08
Steady state analysis: K_D = 34.2 nM, χ^2 = 0.14

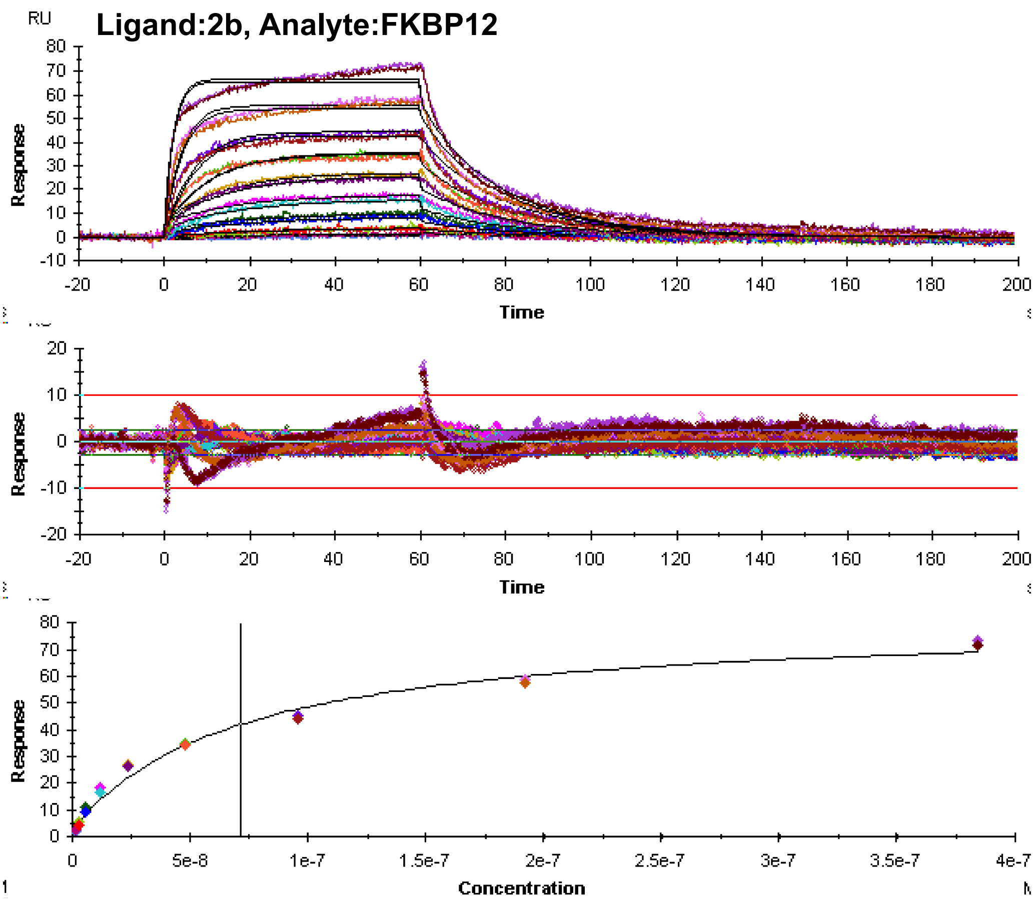


Kinetic analysis: k_a = 1.48 x 10^6 $M^{-1}s^{-1}$, k_d = 0.114 s^{-1}, K_D = 76.8 nM, χ^2 = 0.20
Steady state analysis: K_D = 117 nM, χ^2 = 0.13

The binding of FKBP12 to immobilized small molecules 2:

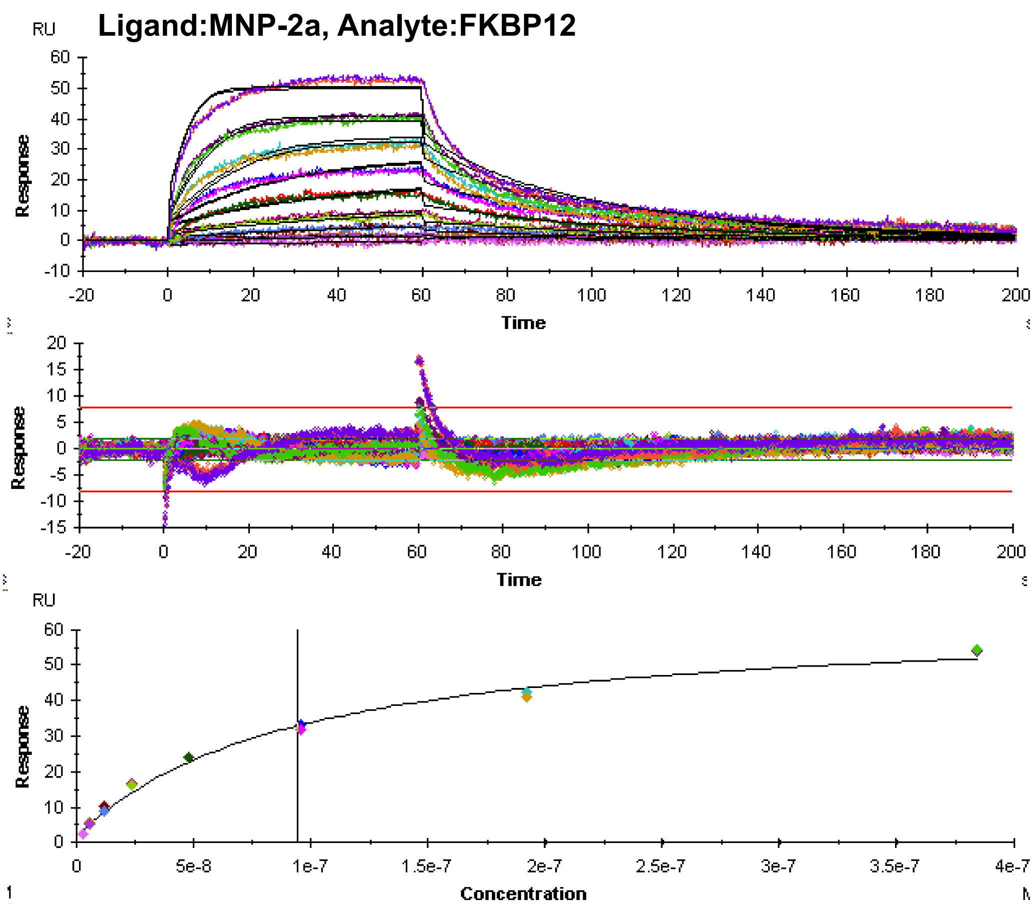


Kinetic analysis: k_a = 5.98 x 10^6 $M^{-1}s^{-1}$, k_d = 5.55 x 10^{-2} s^{-1}, K_D = 11.0 nM, χ^2 = 3.49
Steady state analysis: K_D = 16.7 nM, χ^2 = 0.89

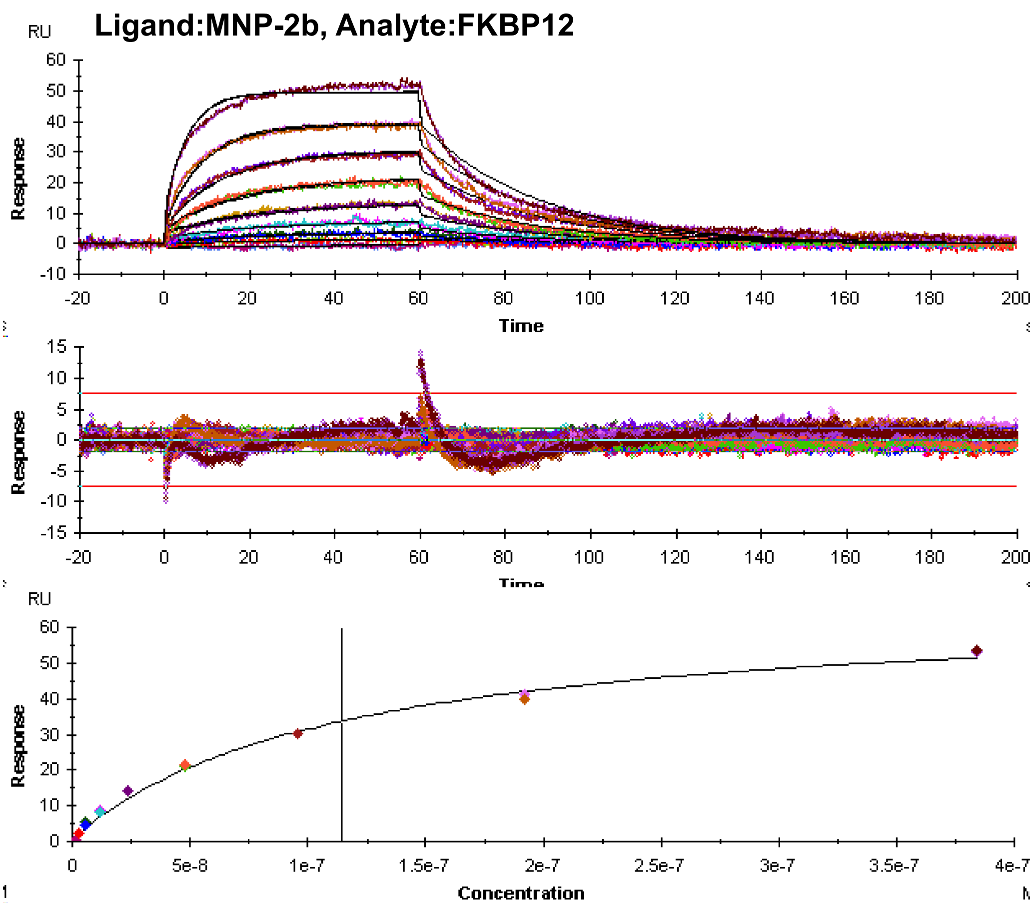


Kinetic analysis: k_a = 1.22 x 10^6 $M^{-1}s^{-1}$, k_d = 6.36 x 10^{-2} s^{-1}, K_D = 52.3 nM, χ^2 = 3.37
Steady state analysis: K_D = 71.6 nM, χ^2 = 8.75

On-Chip nanoparticle derivatization and binding of FKBP12:



Kinetic analysis: $k_a = 8.82 \times 10^5$ $M^{-1}s^{-1}$, $k_d = 3.76 \times 10^{-2}$ s^{-1}, K_D = 42.6 nM, χ^2 = 2.61
Steady state analysis: K_D = 94.2 nM, χ^2 = 2.61



Kinetic analysis: $k_a = 3.91 \times 10^5\ M^{-1}s^{-1}$, $k_d = 3.63 \times 10^{-2}\ s^{-1}$, $K_D = 92.7$ nM, $\chi^2 = 1.56$
Steady state analysis: $K_D = 114$ nM, $\chi^2 = 1.89$