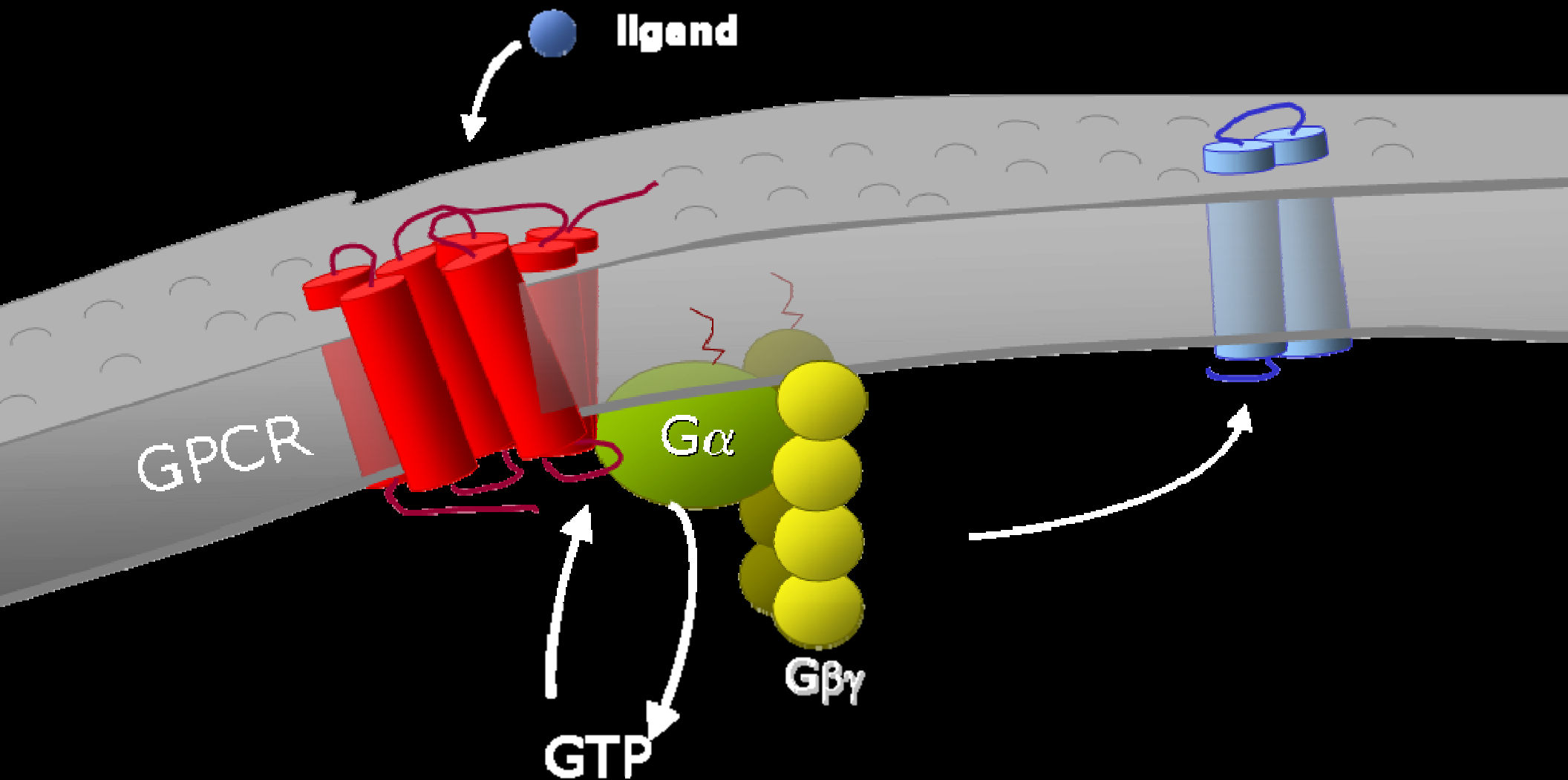


the combination
of SPR and
SPR $K_D = 37 \text{ s}^{-1} / 10 \text{ pM}$

Fragment Screening against GPCRs with SPR

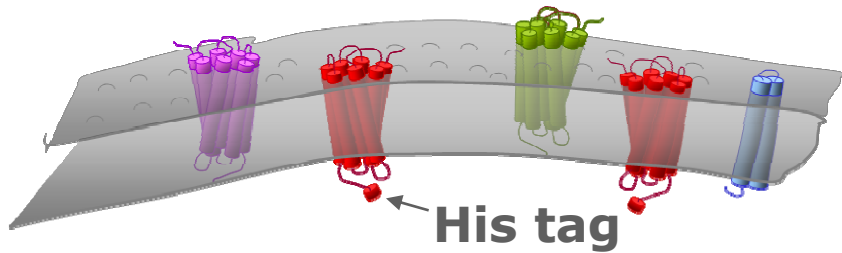


Challenging targets

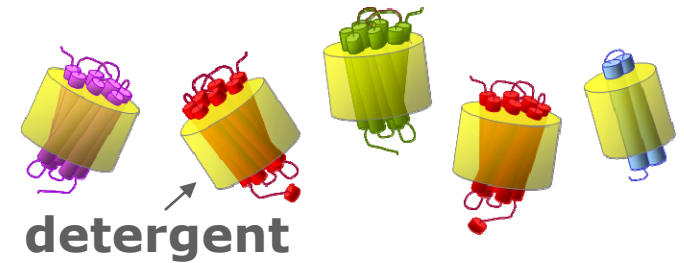


Assay Design

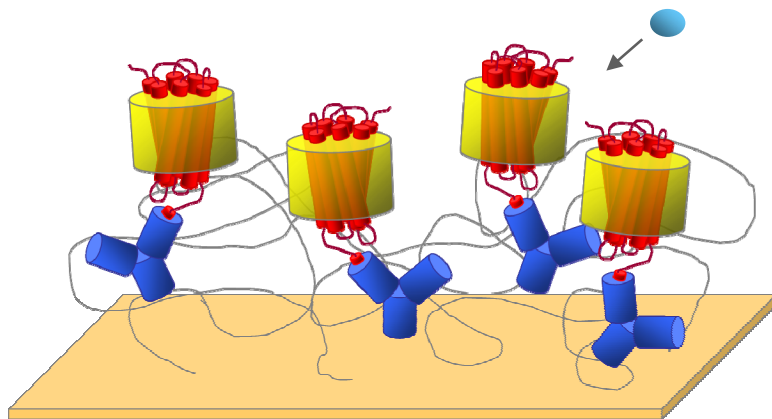
1. Clone, tag, express



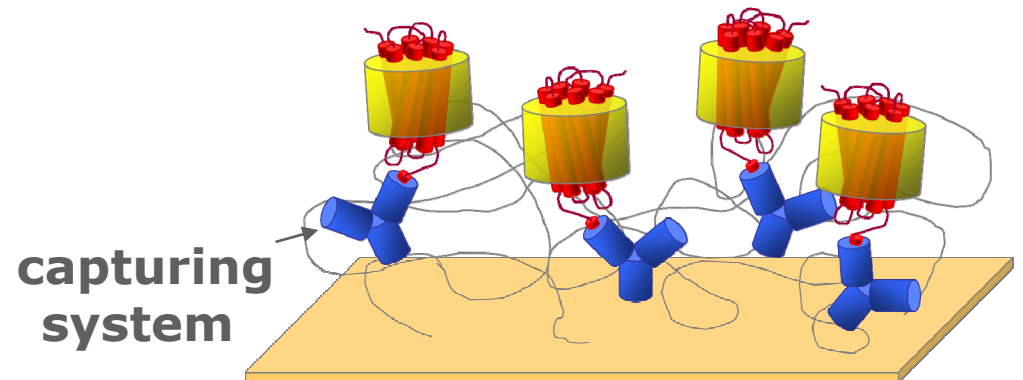
2. Solubilize



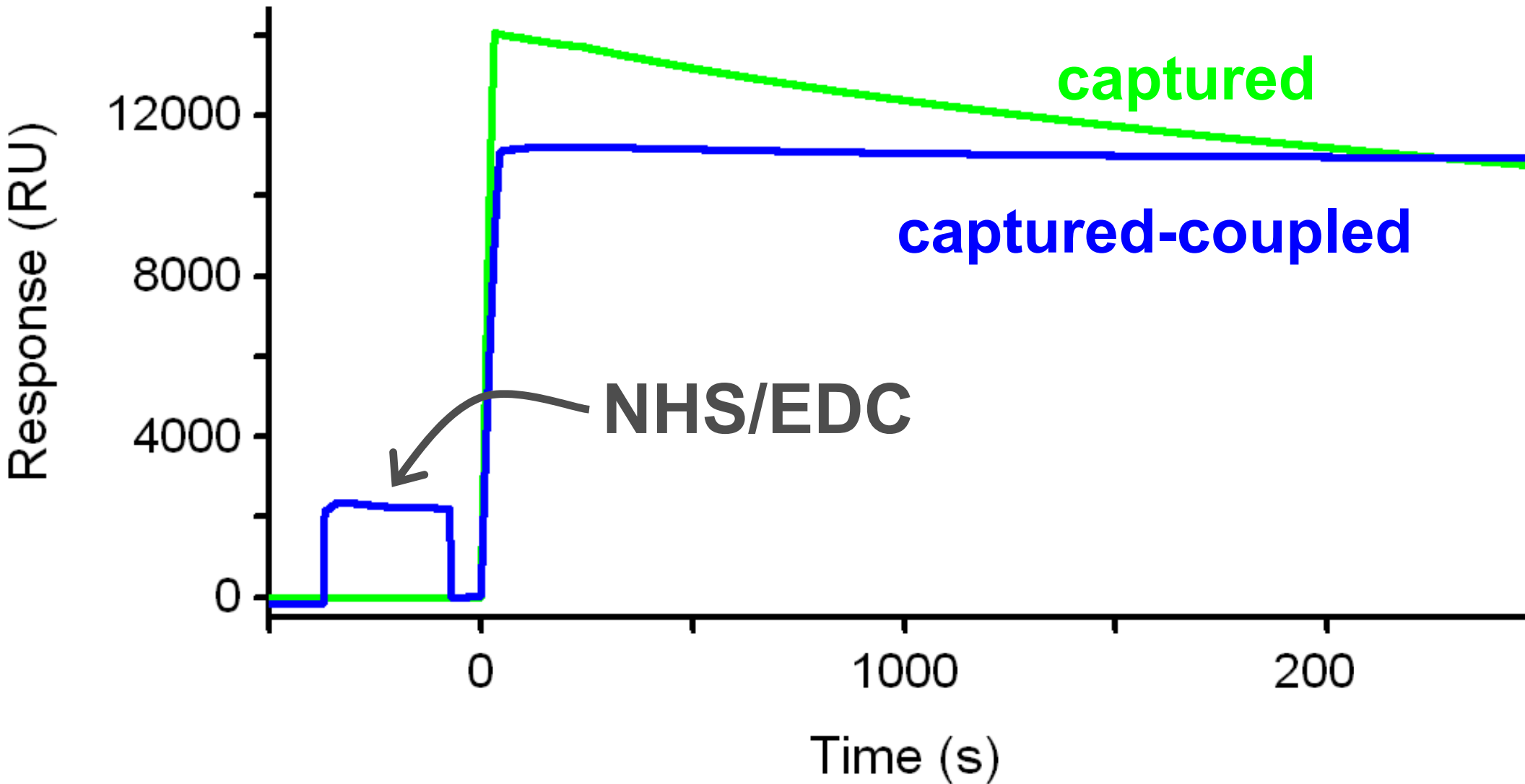
4. Test binding



3. Capture



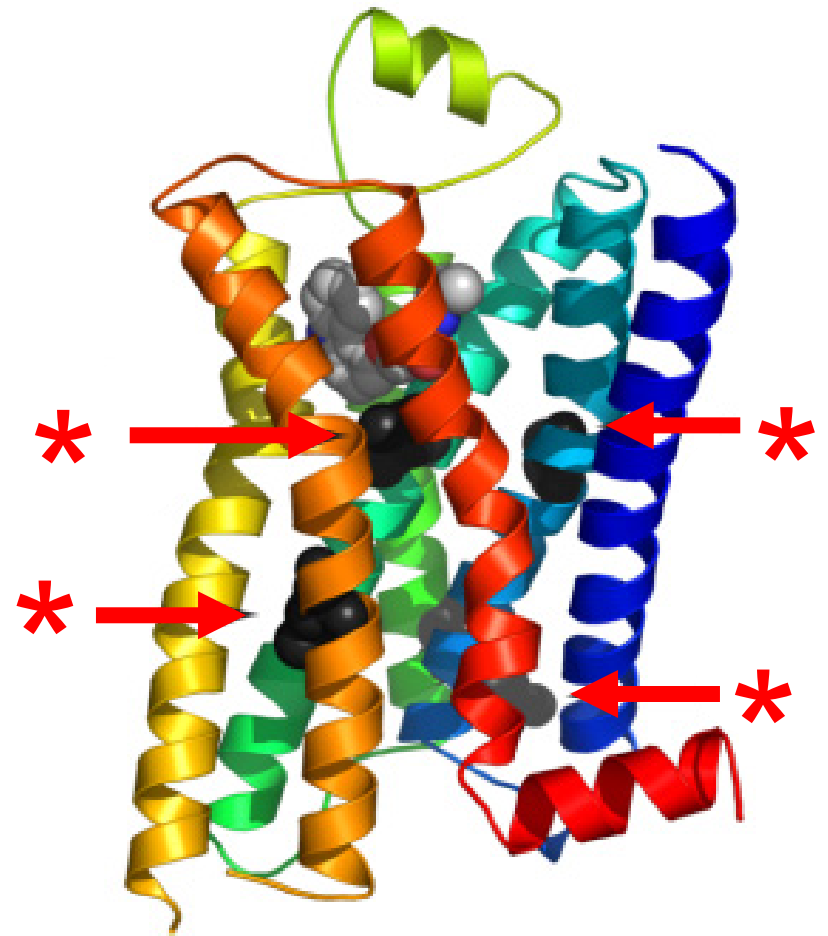
Optimize Captures



Stabilized GPCRs

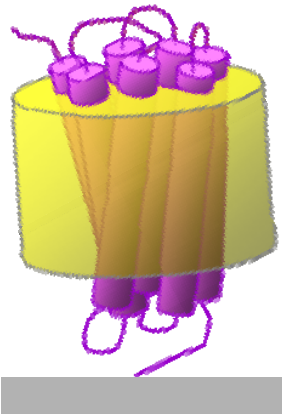
HEPTARES therapeutics

 StaRs

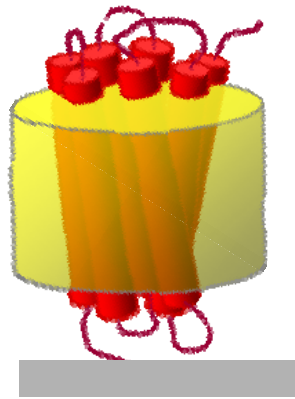


Stabilized GPCRs

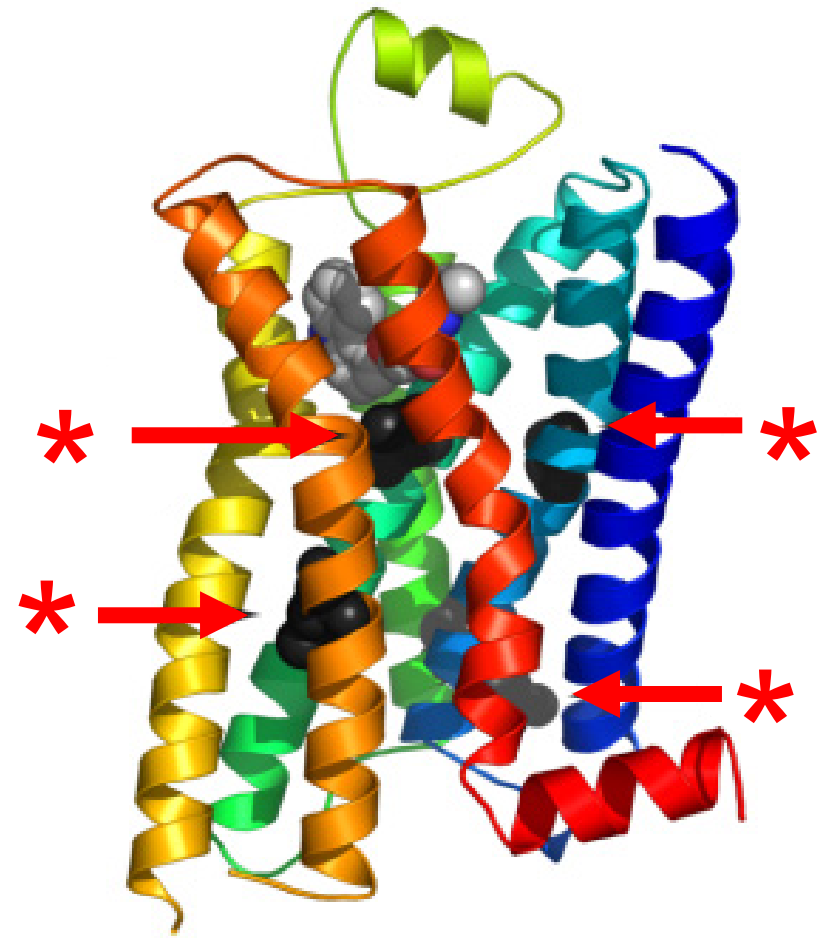
$A_{2A}R$



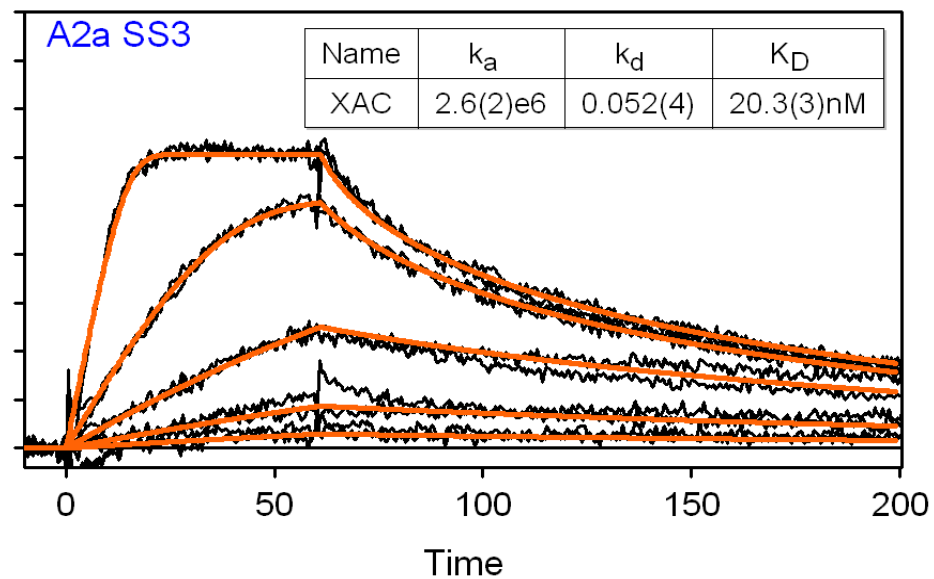
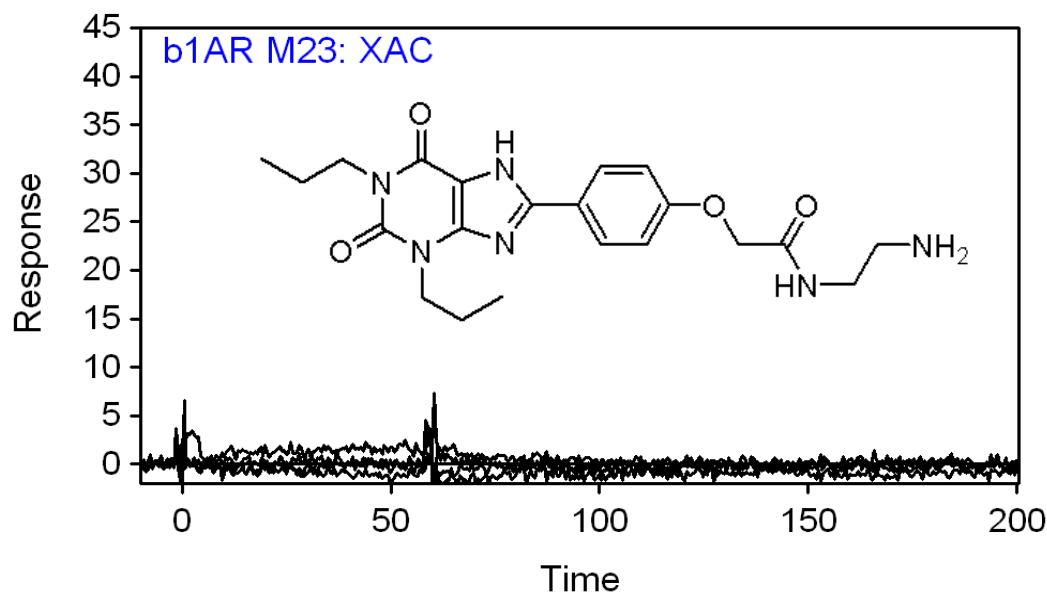
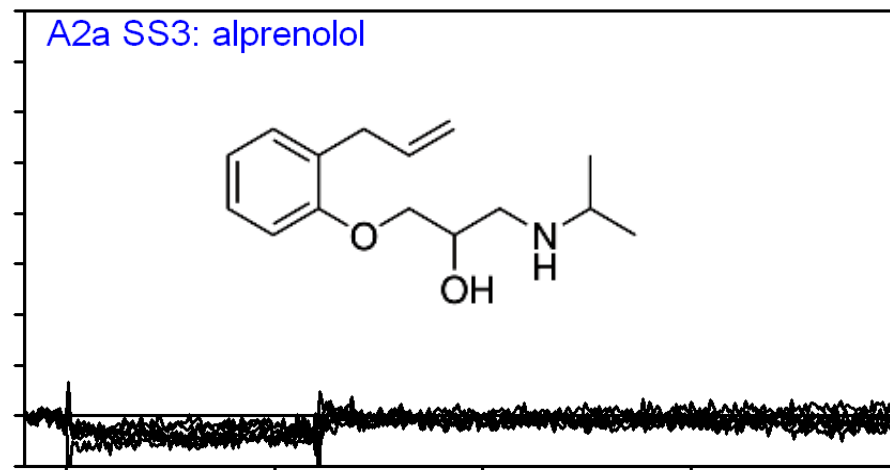
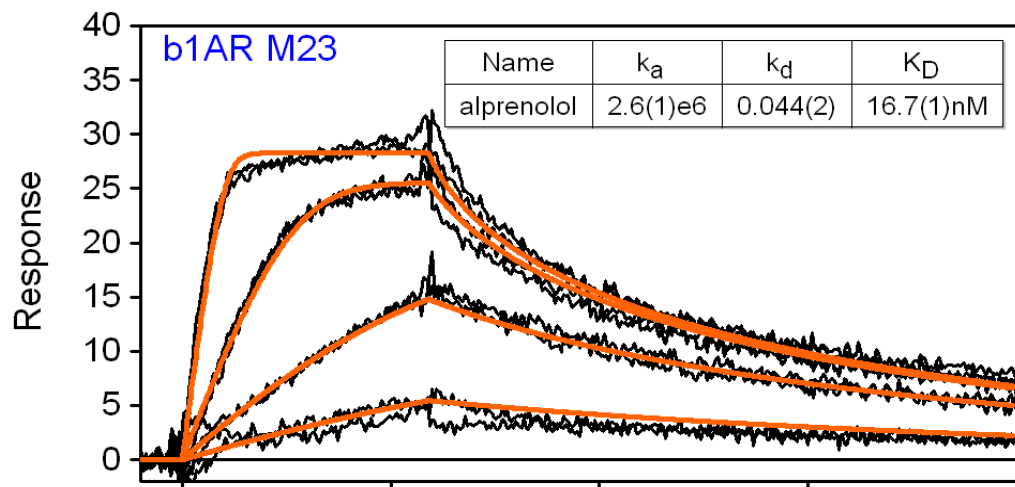
β_1AR



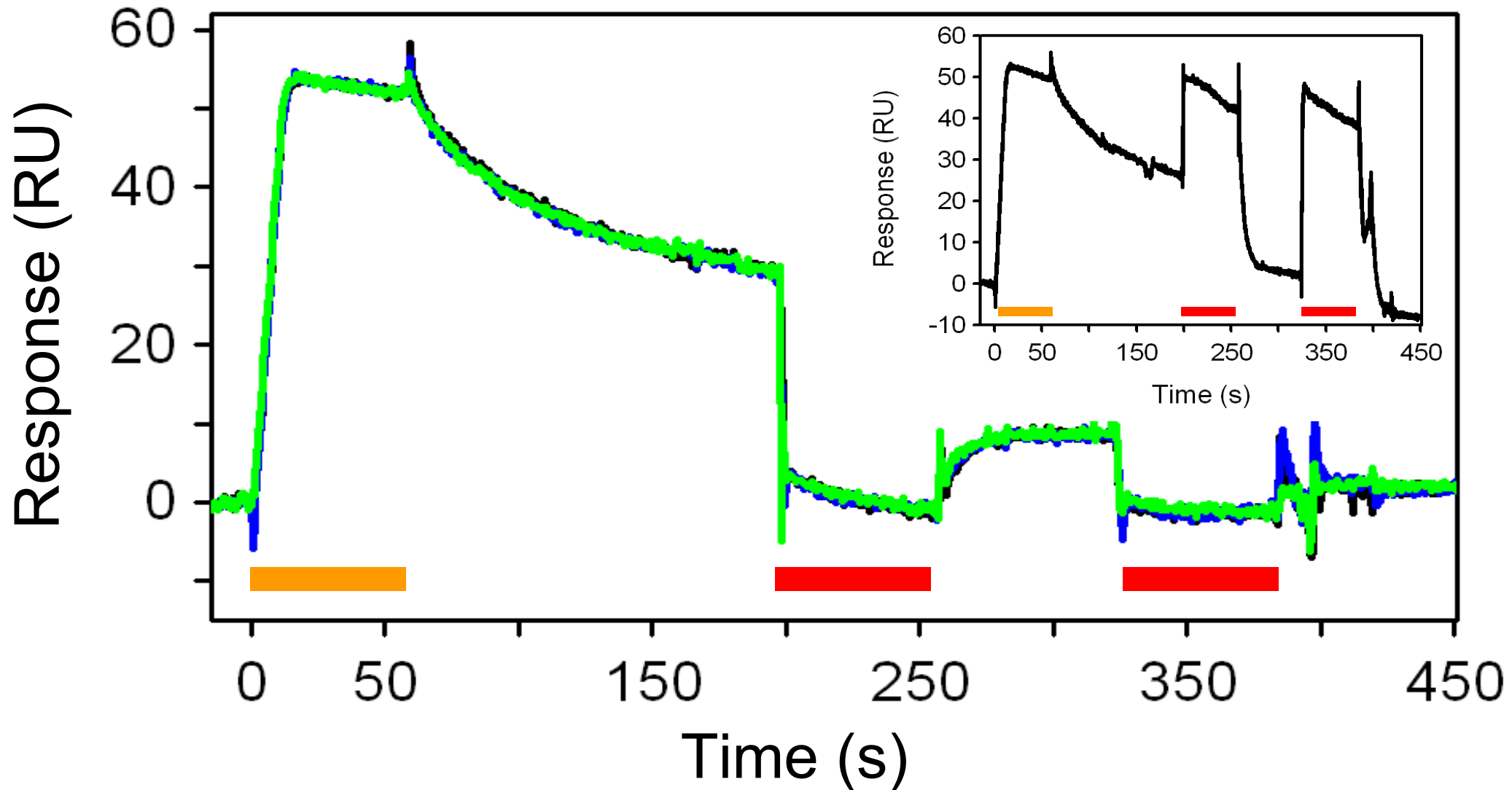
ref



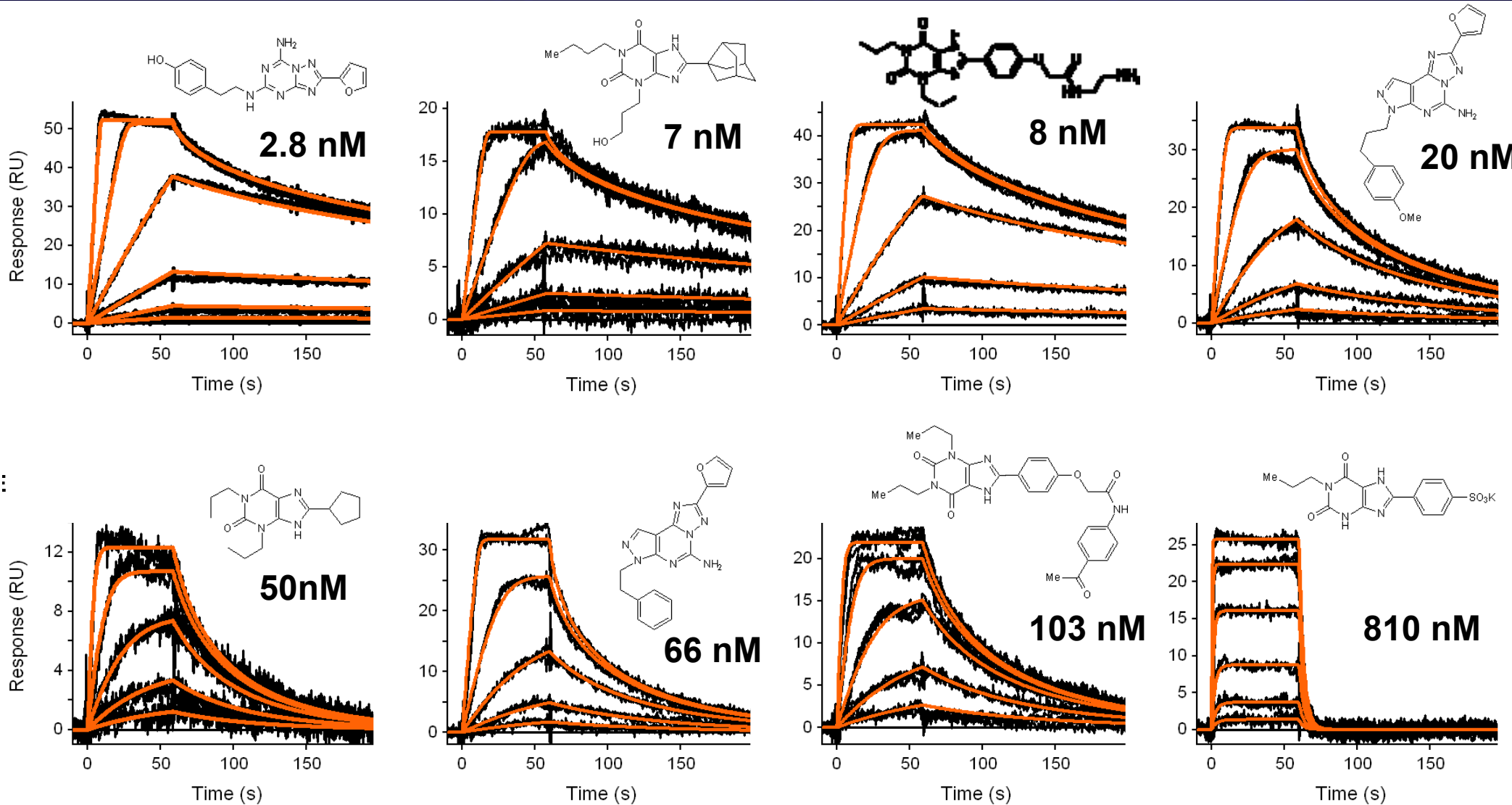
Activity & Specificity



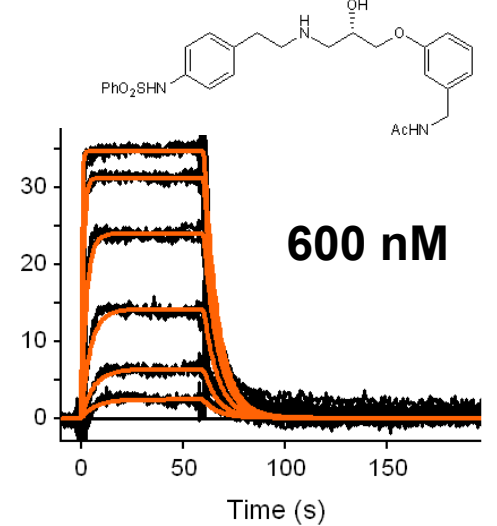
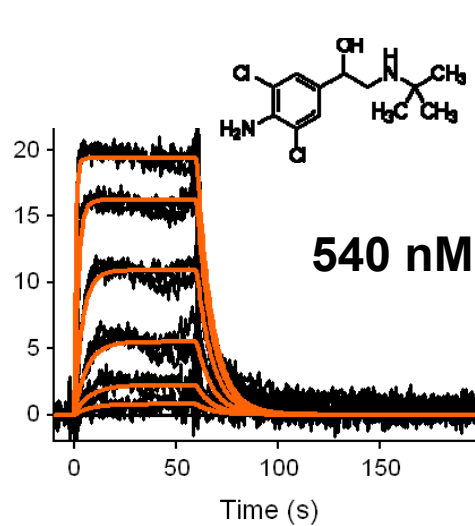
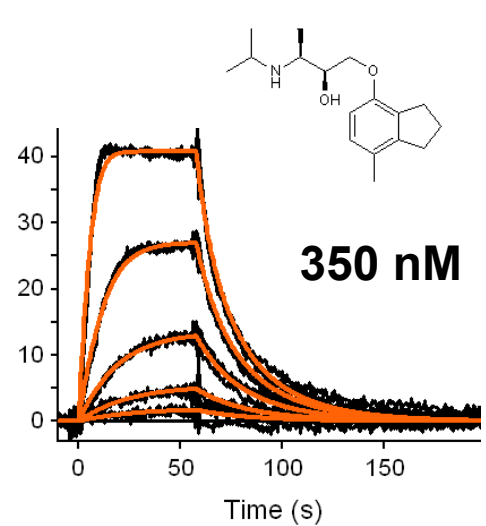
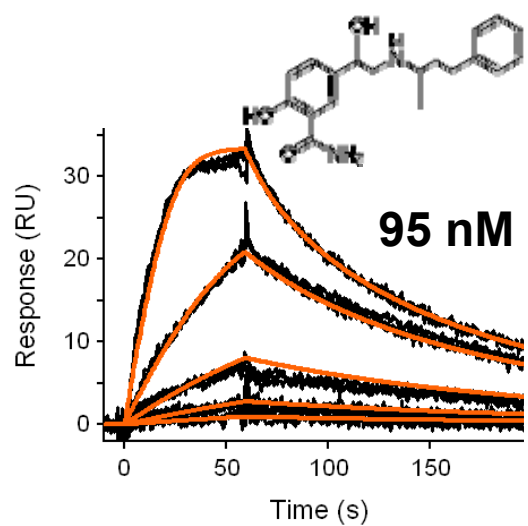
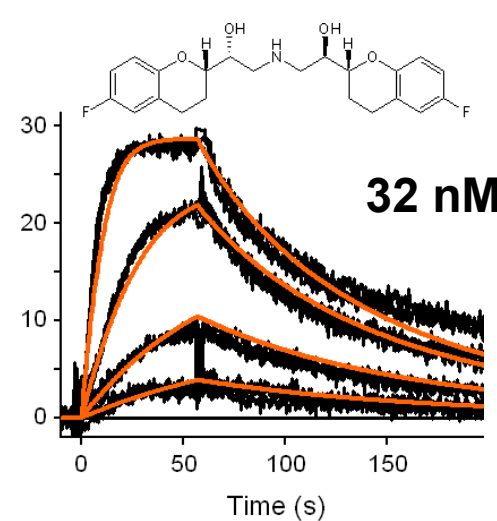
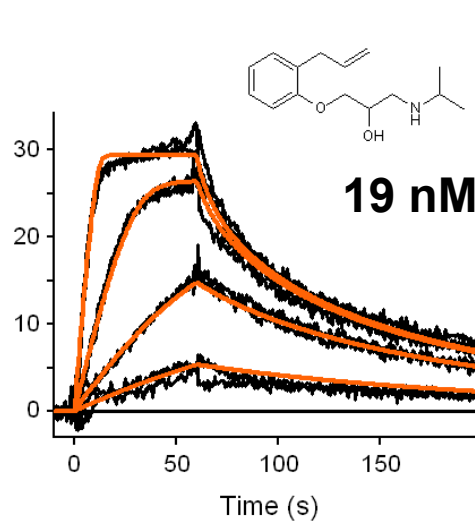
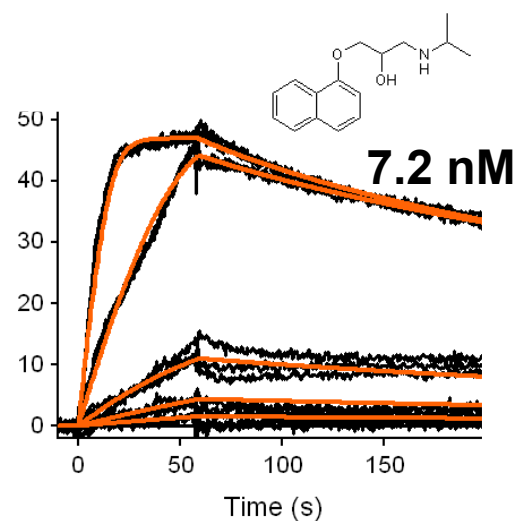
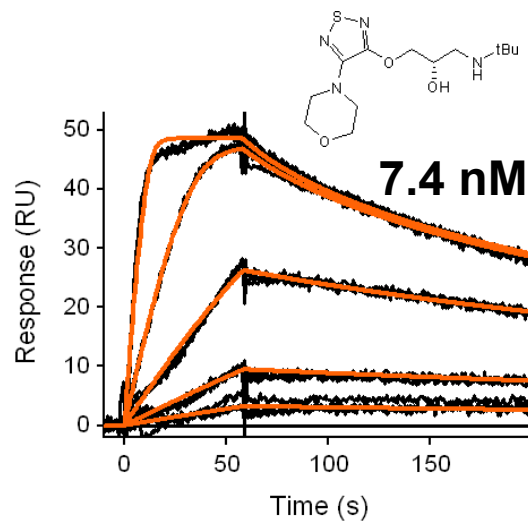
Regeneration



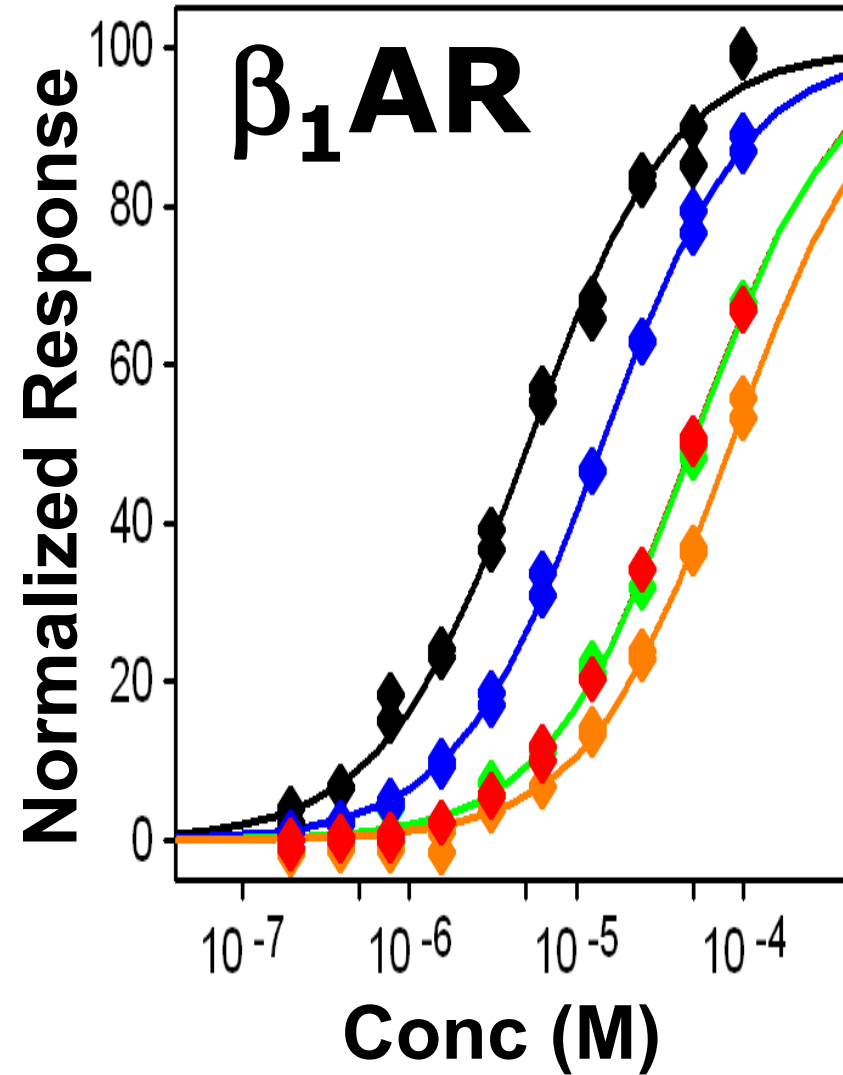
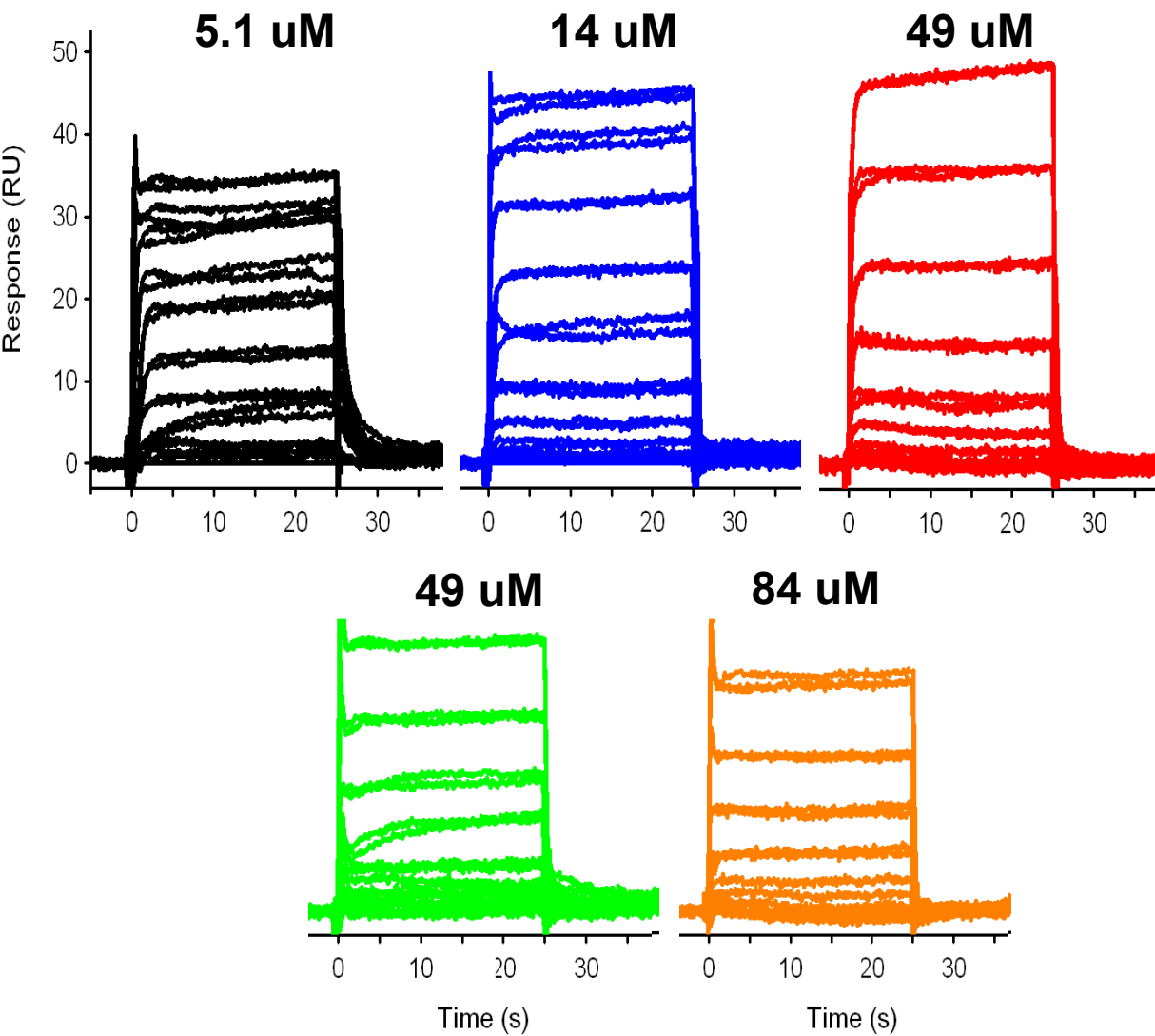
A_{2A} Receptor



β_1 AR Receptor



Weaker interactions



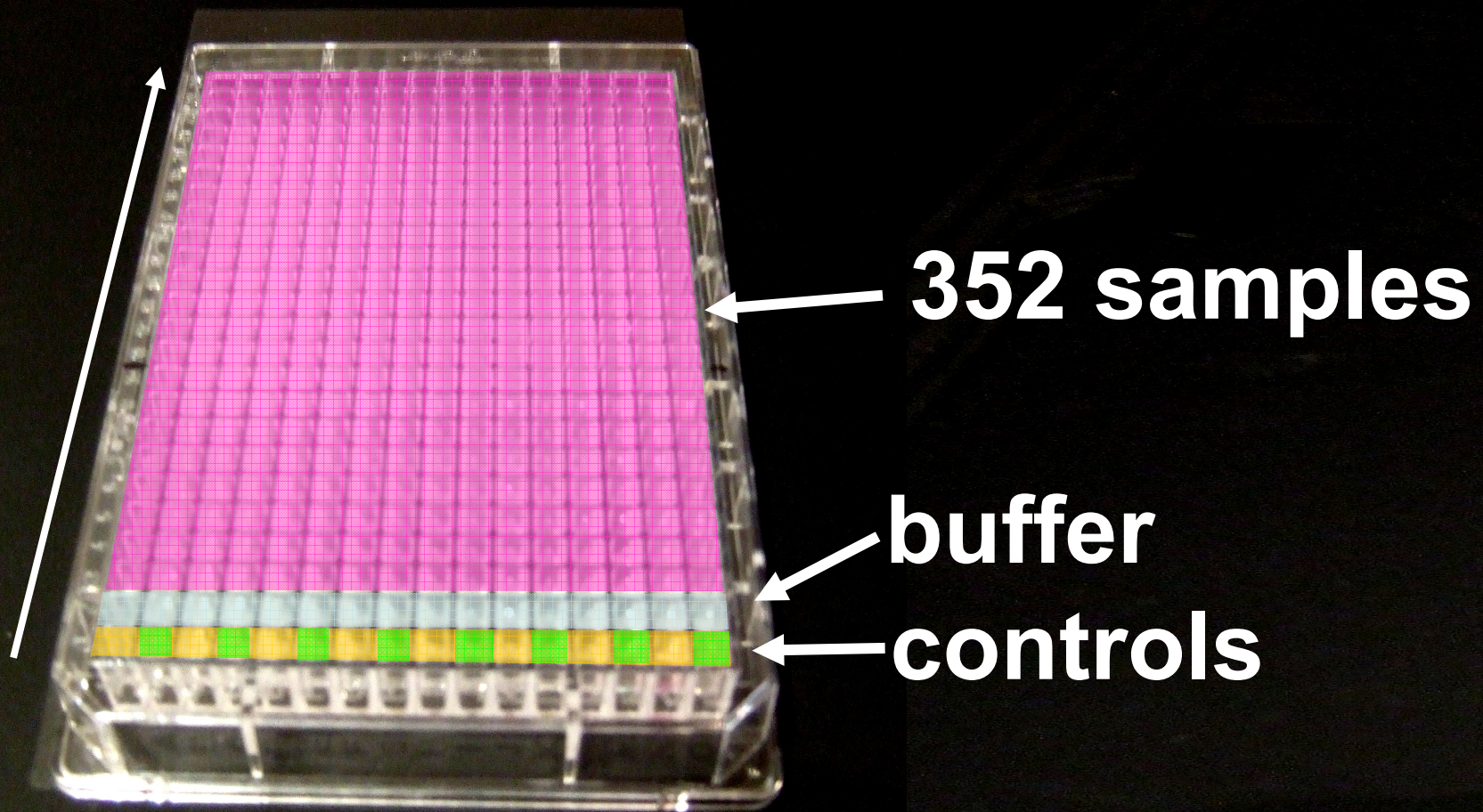
Maybridge Ro3

1500 Diversity Fragment Library

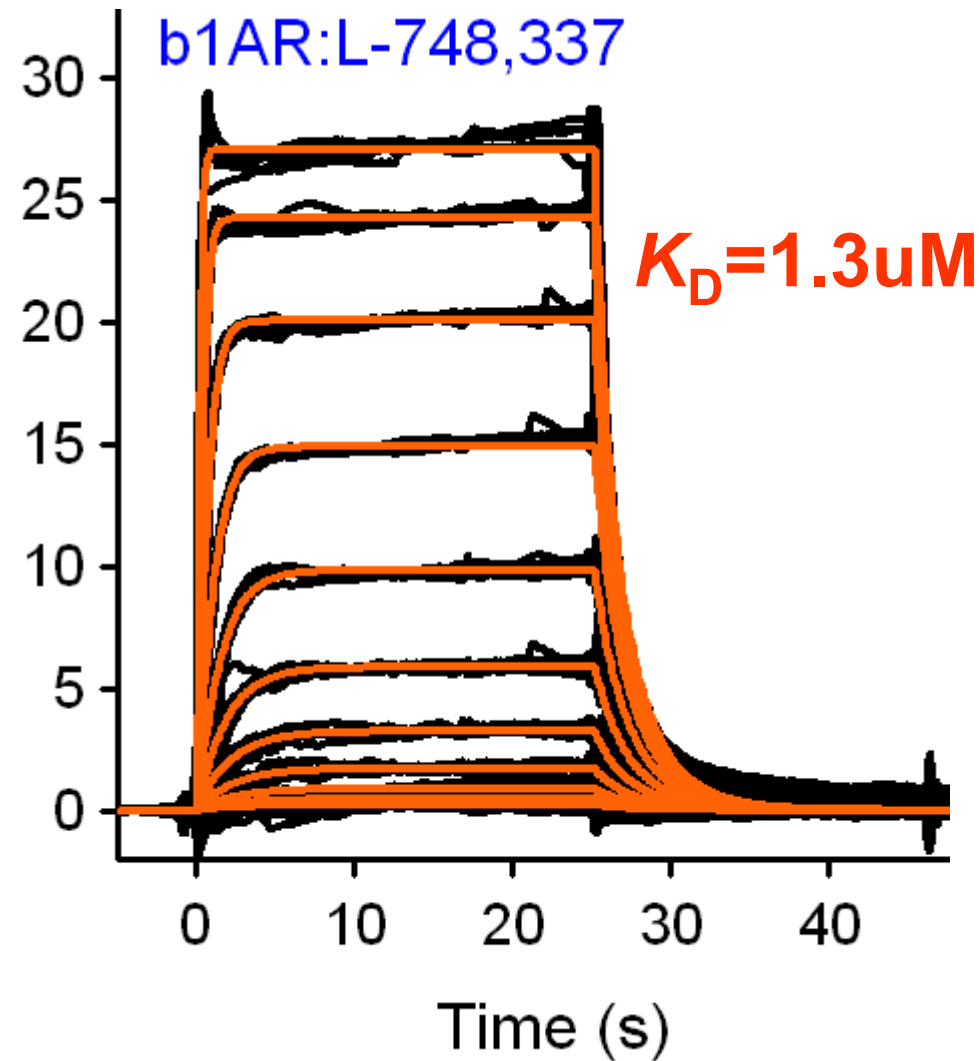
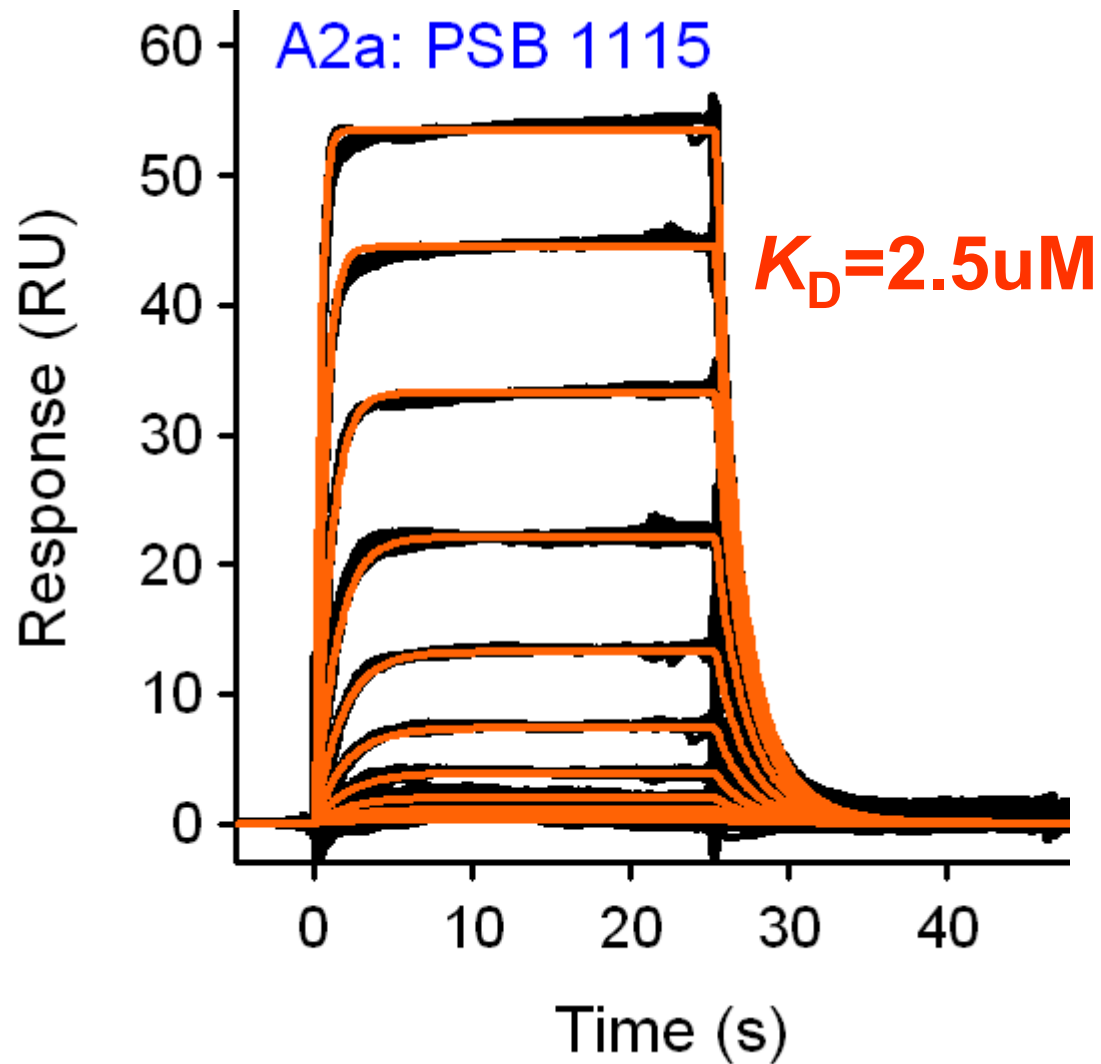
50 μ M in
5% DMSO



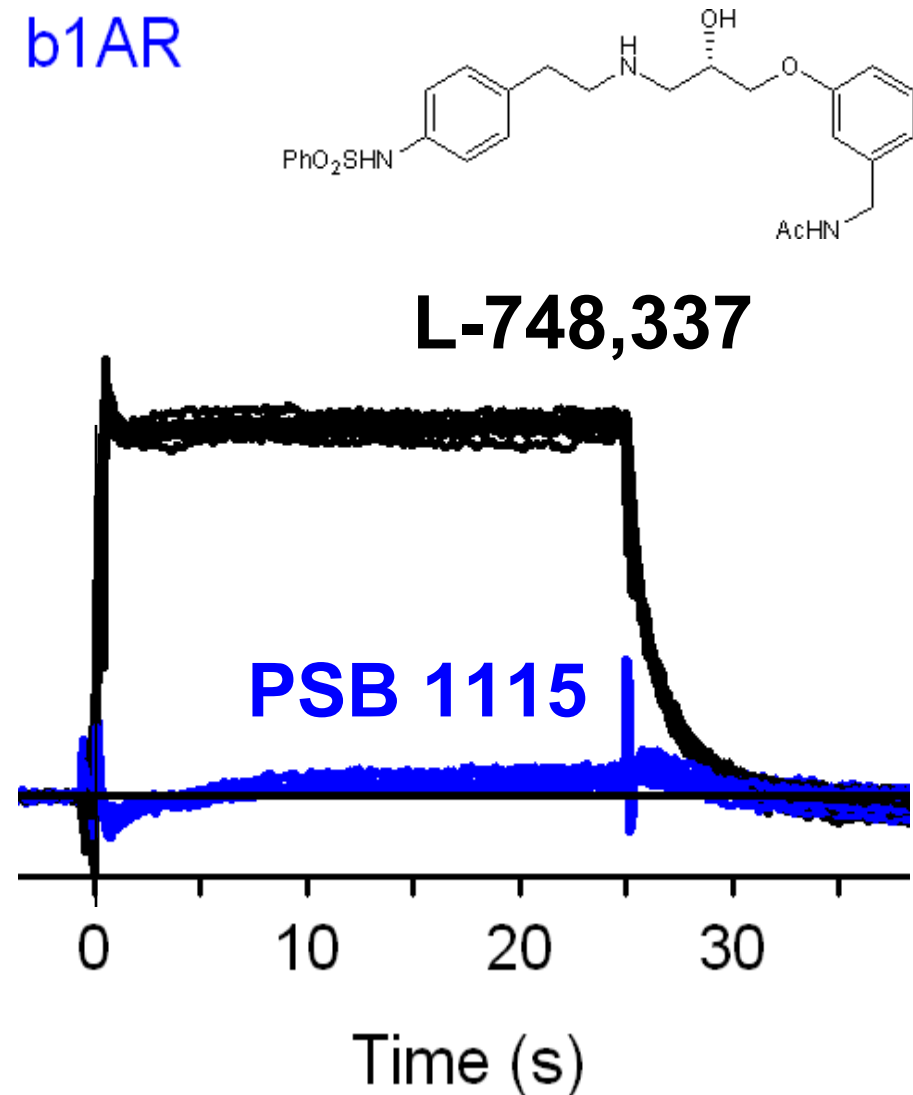
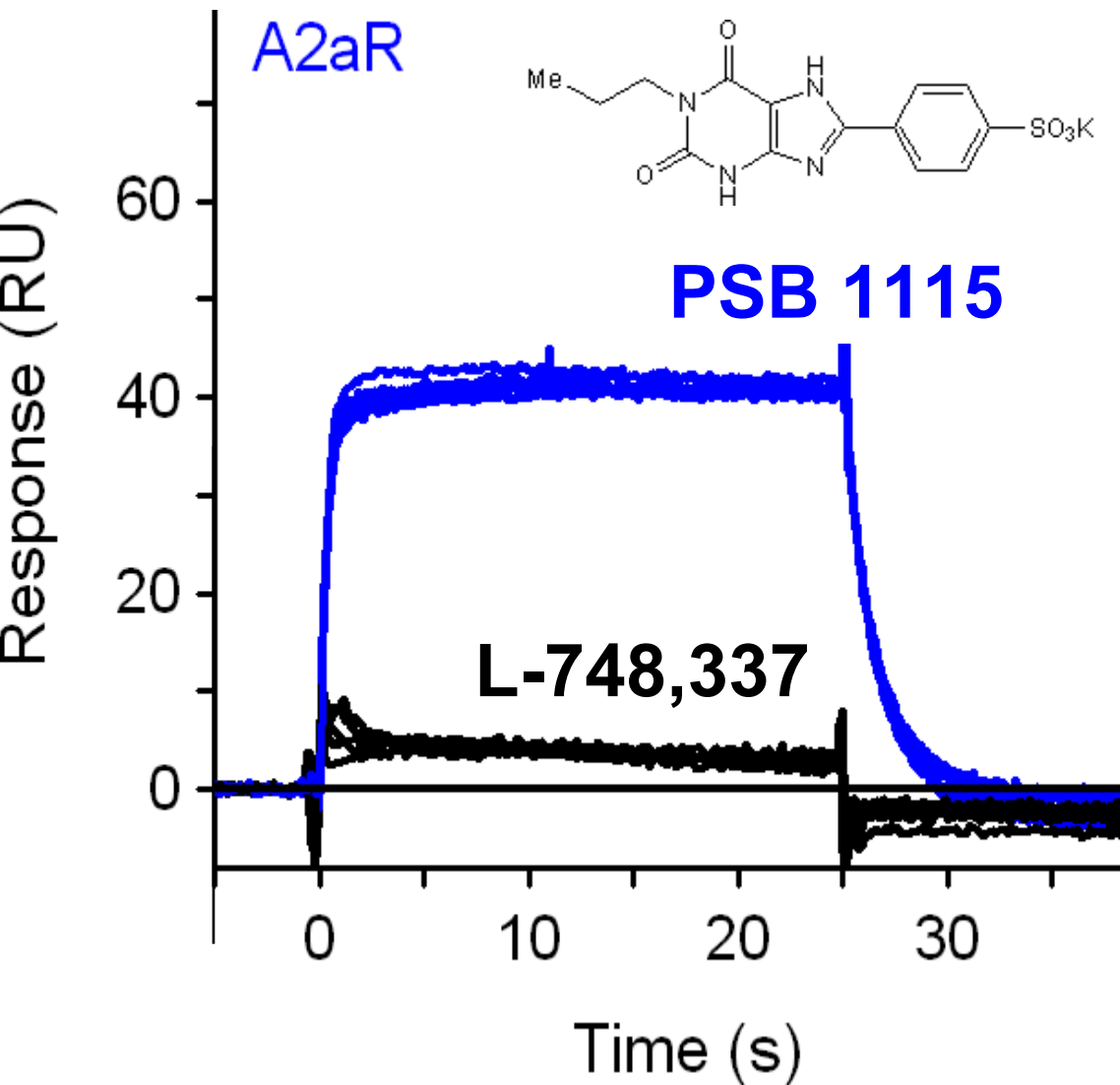
Plate set up



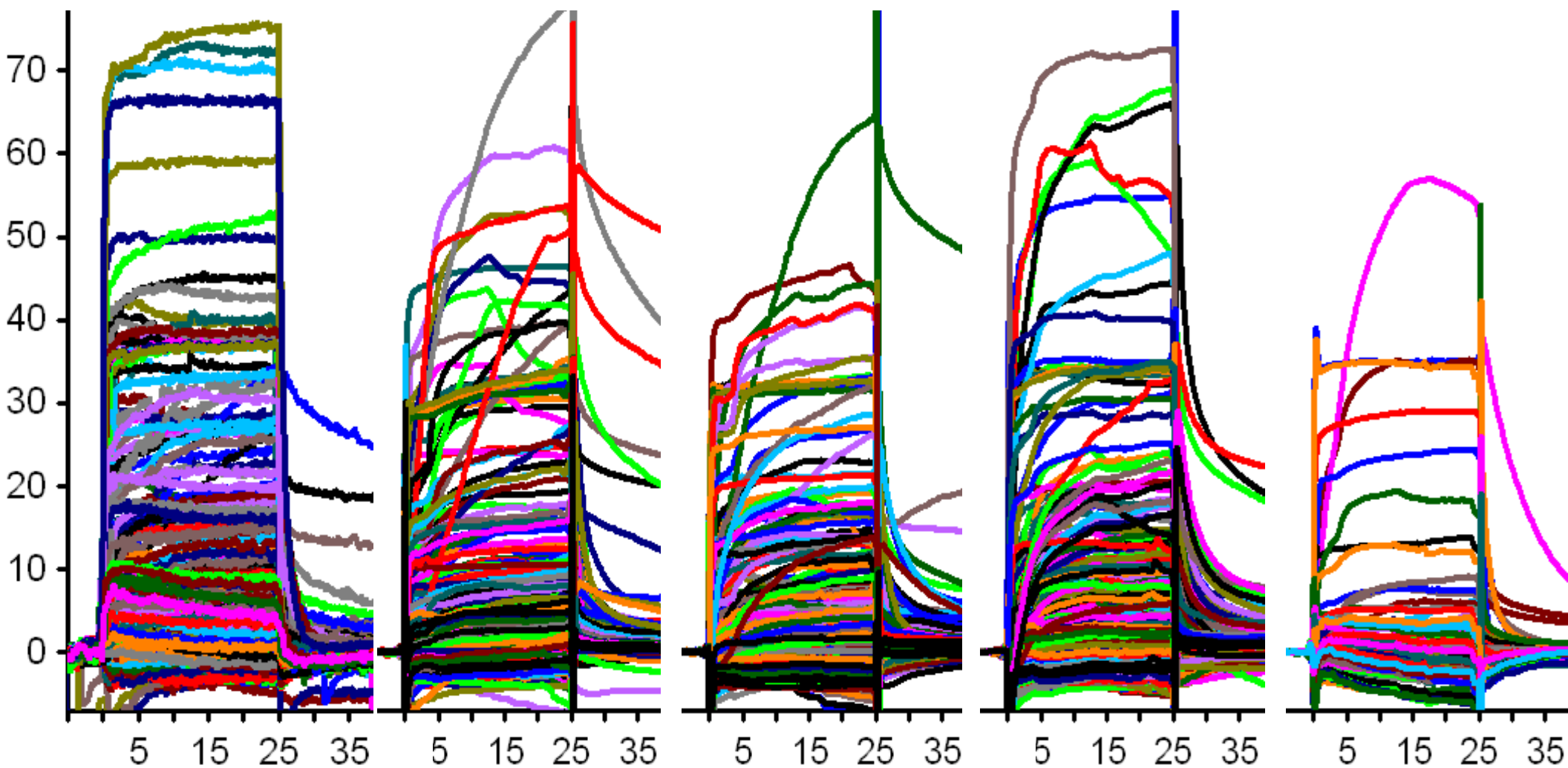
Controls



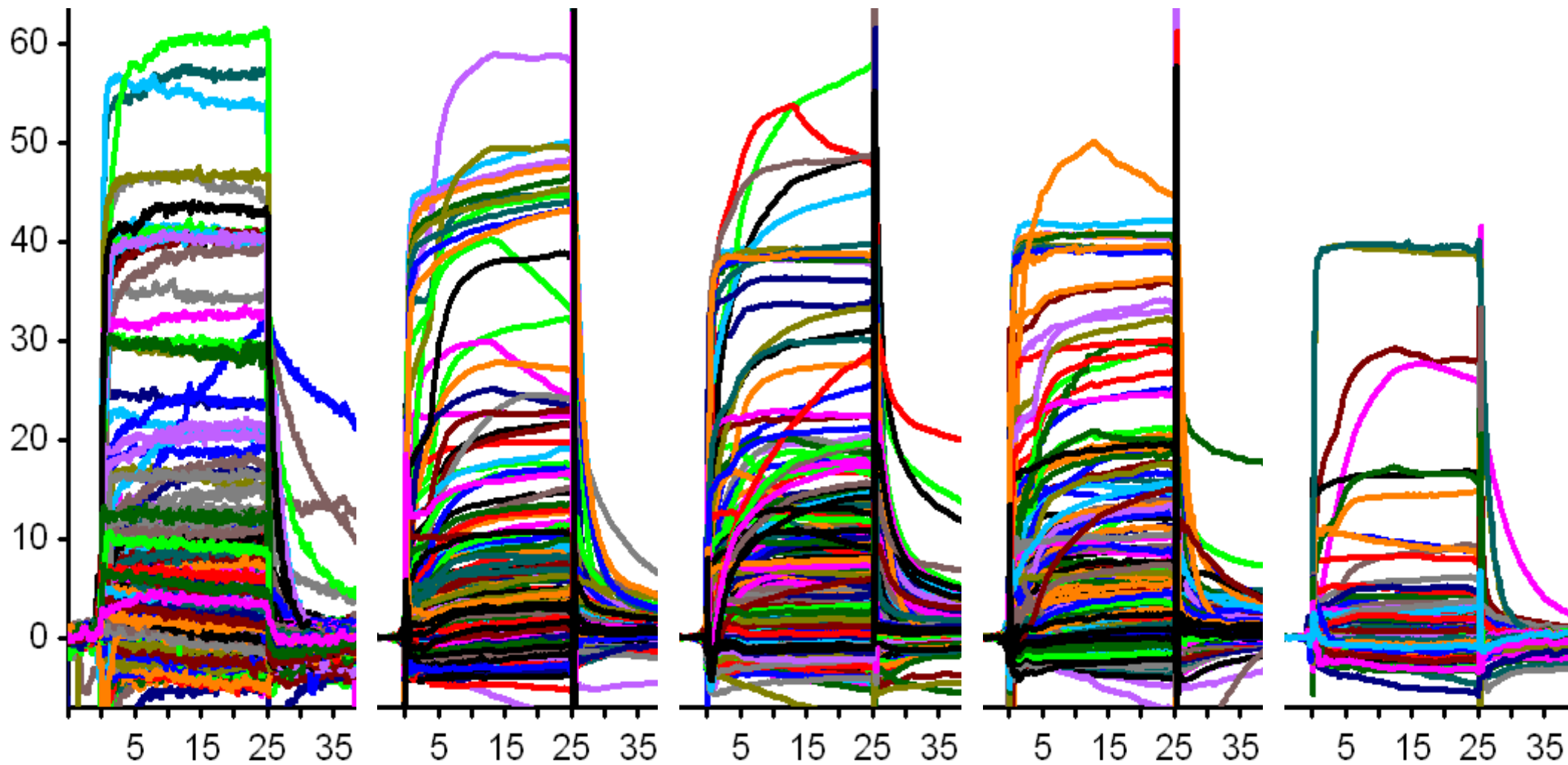
Controls



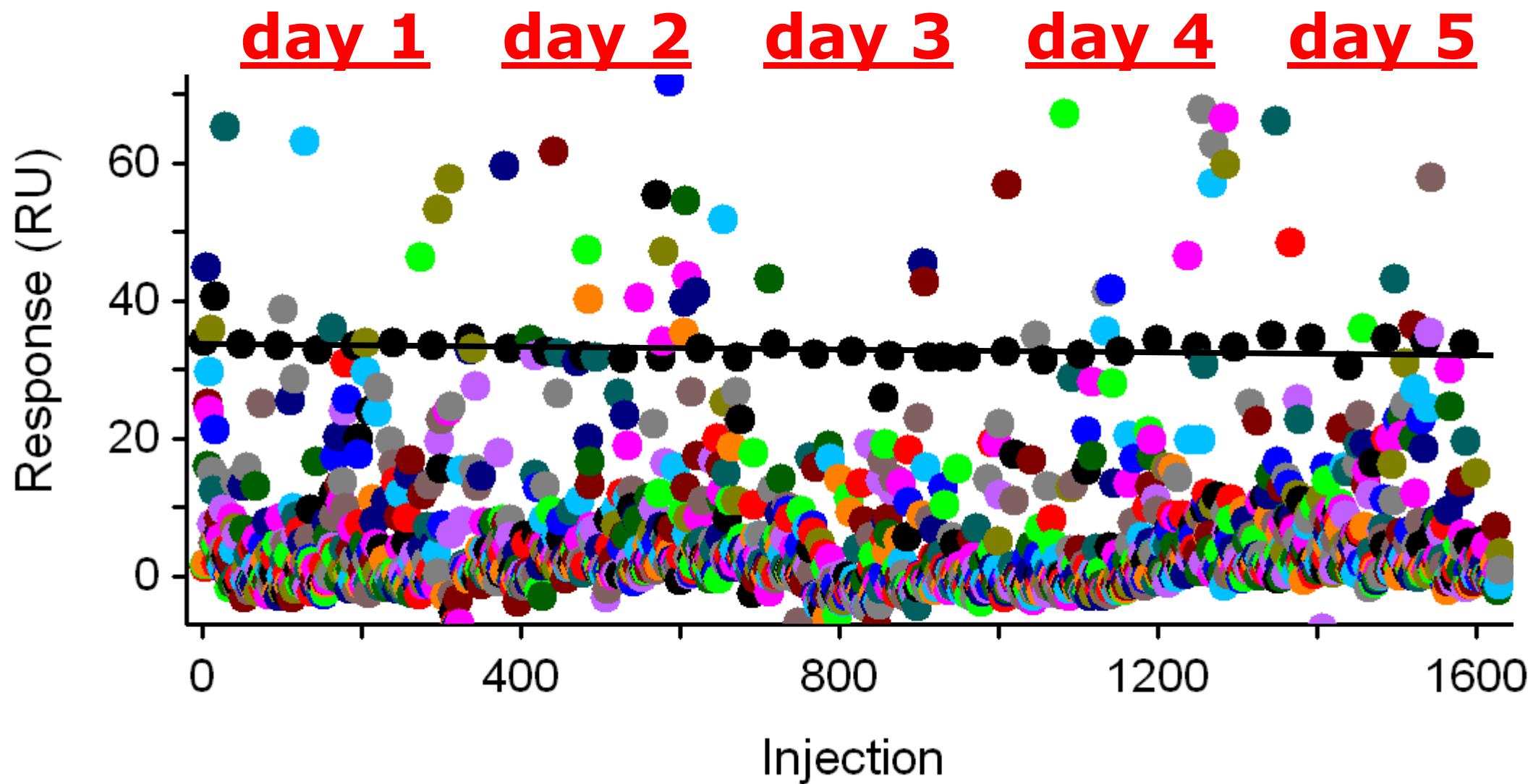
β_1 AR Sensorgrams



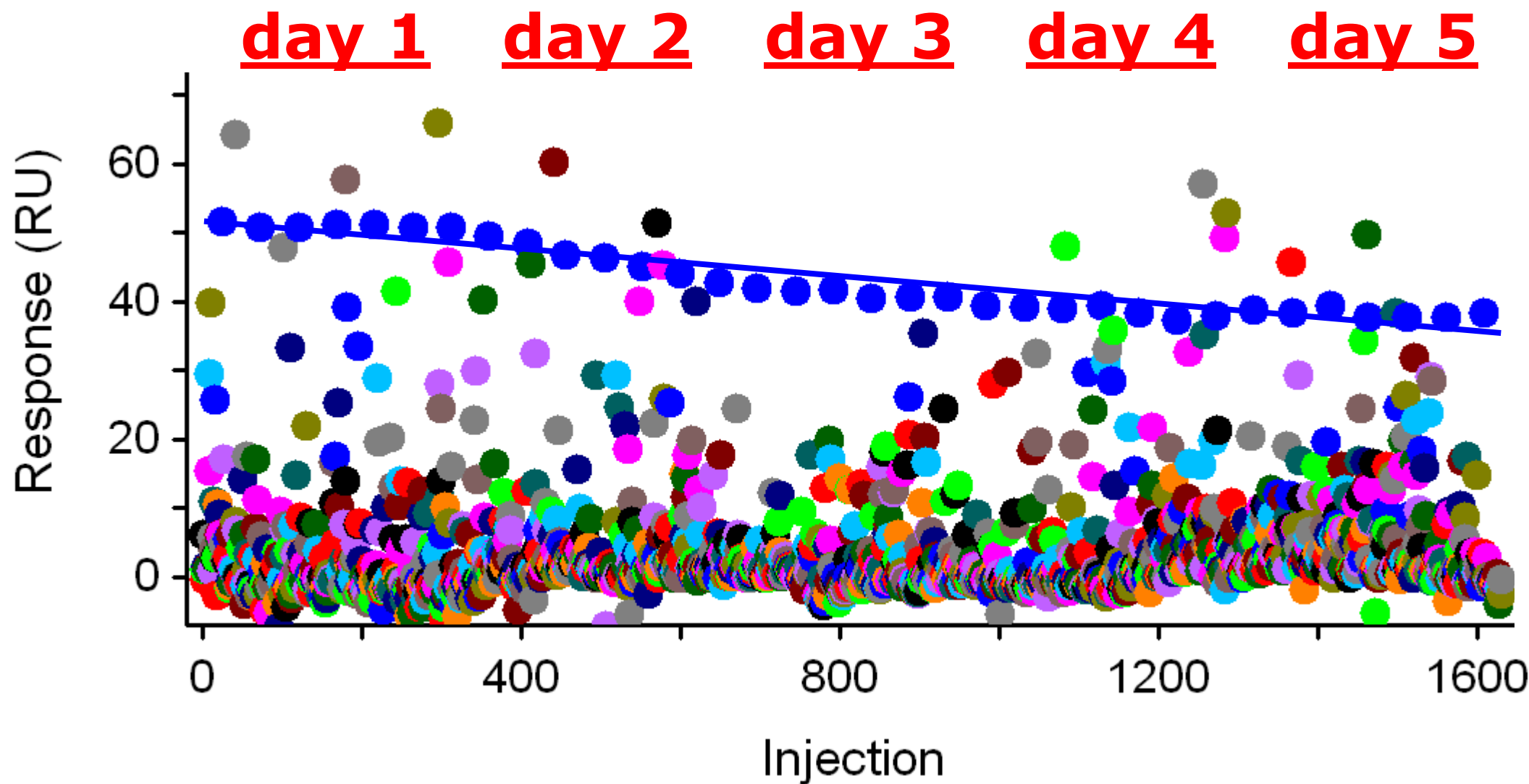
A_{2A}R Sensorgrams



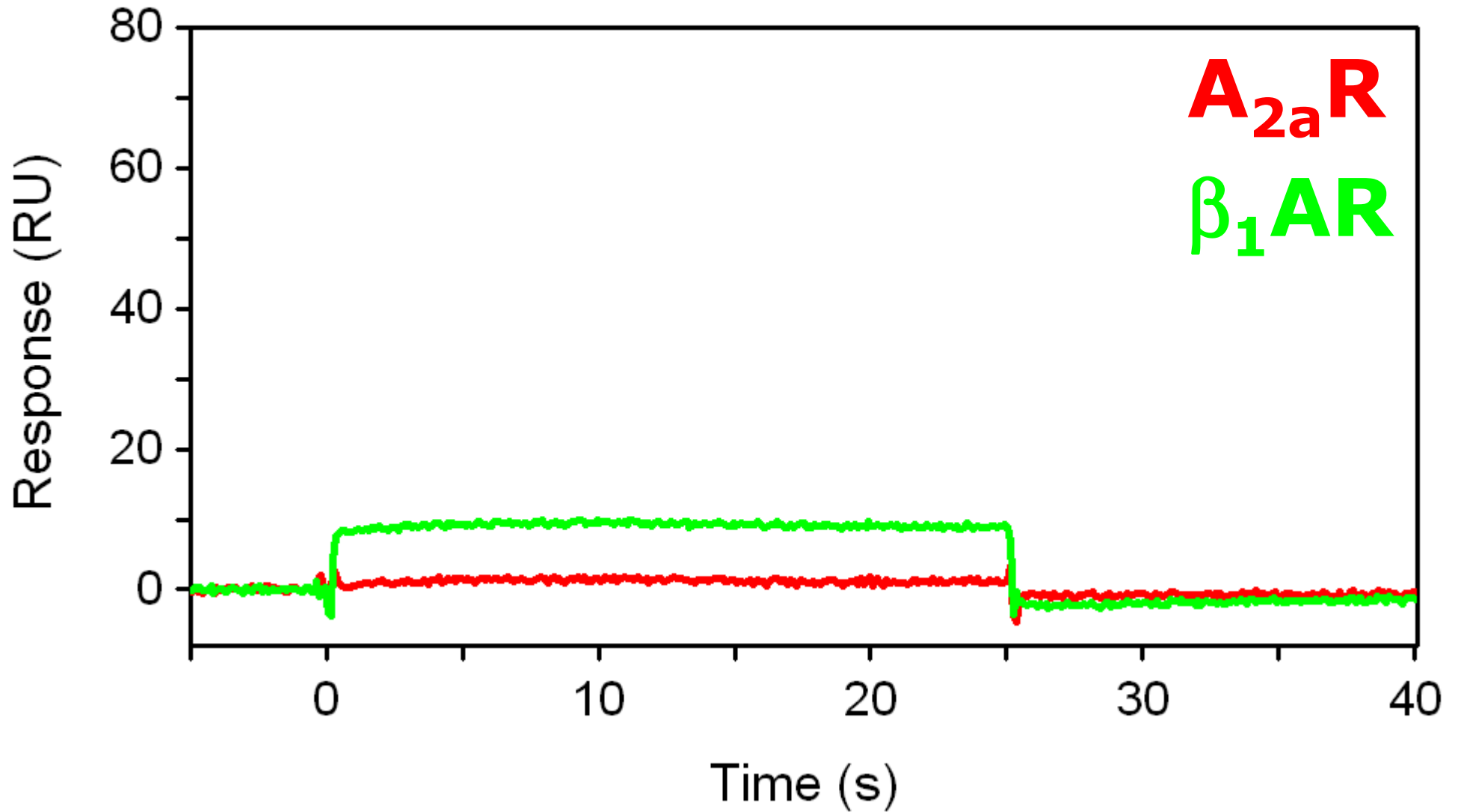
β_1 AR trend plot



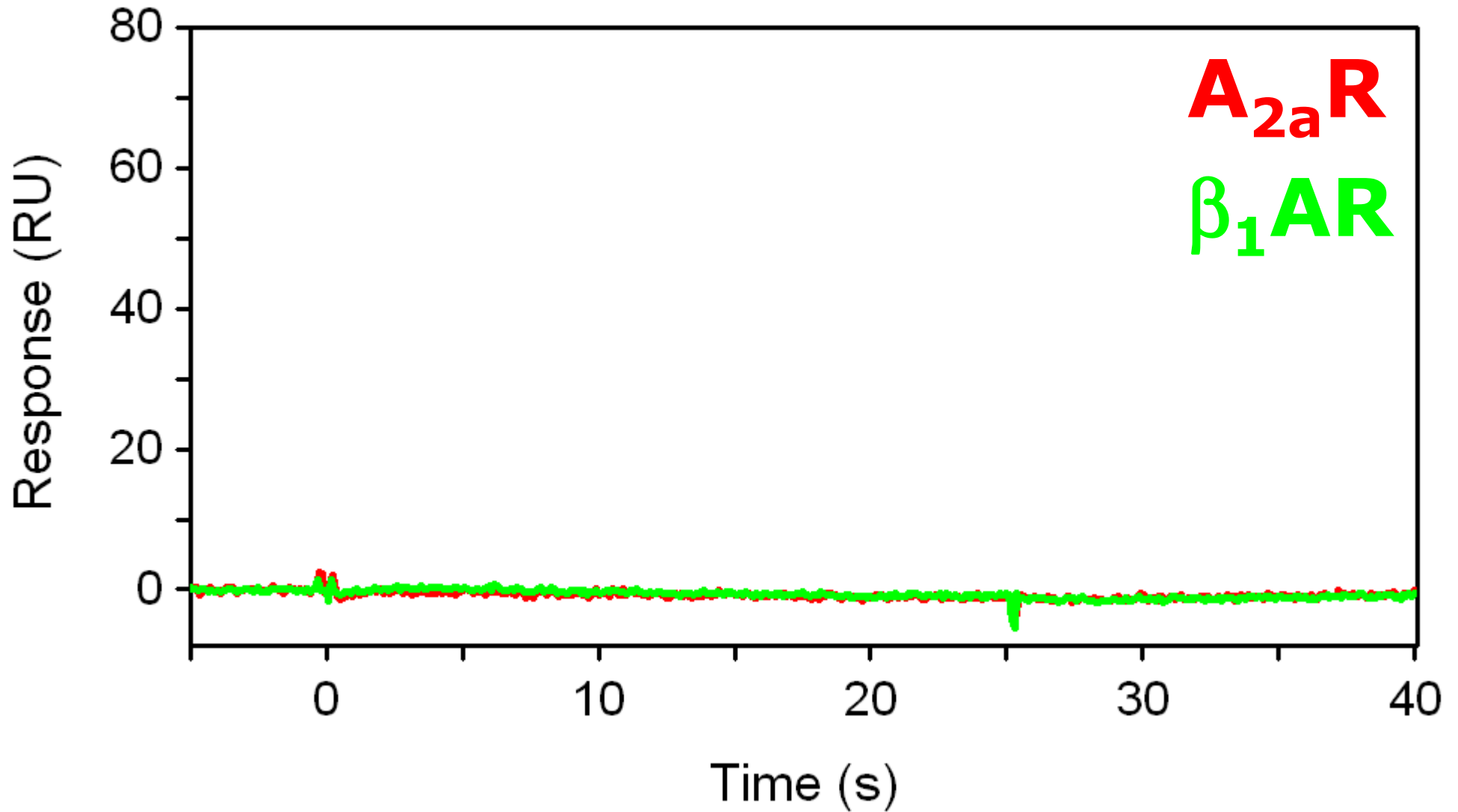
A_{2a}R trend plot



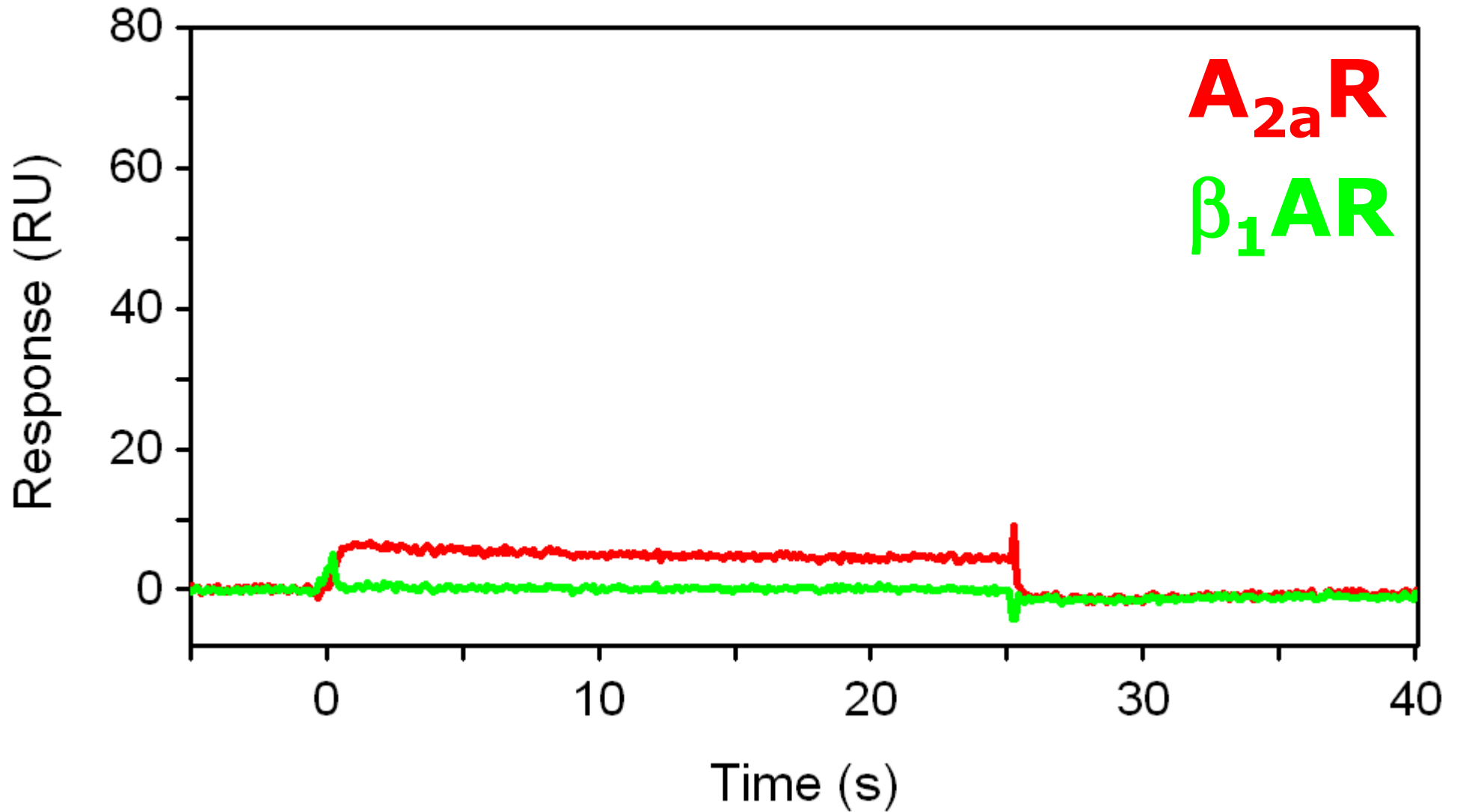
Fragments



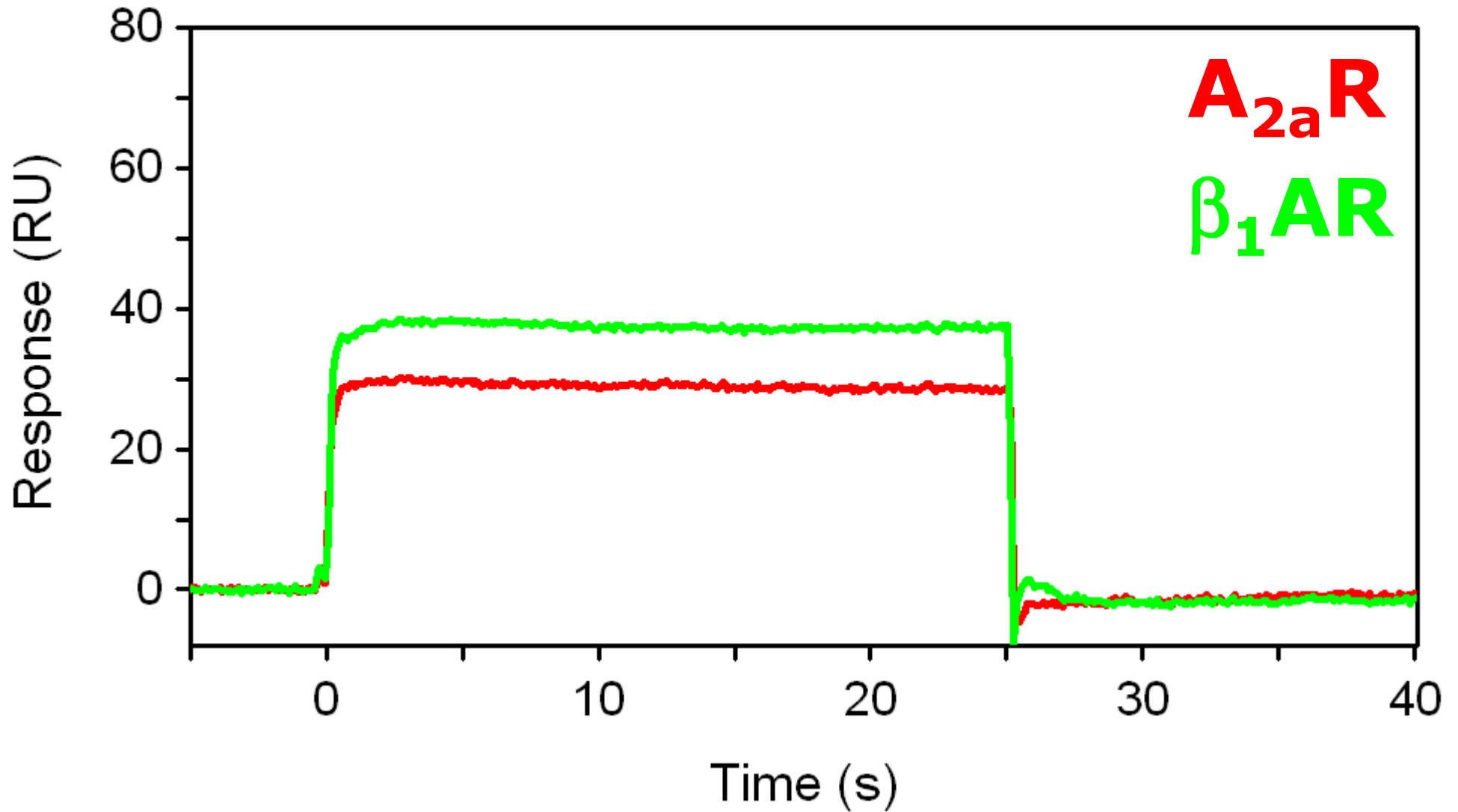
Fragments



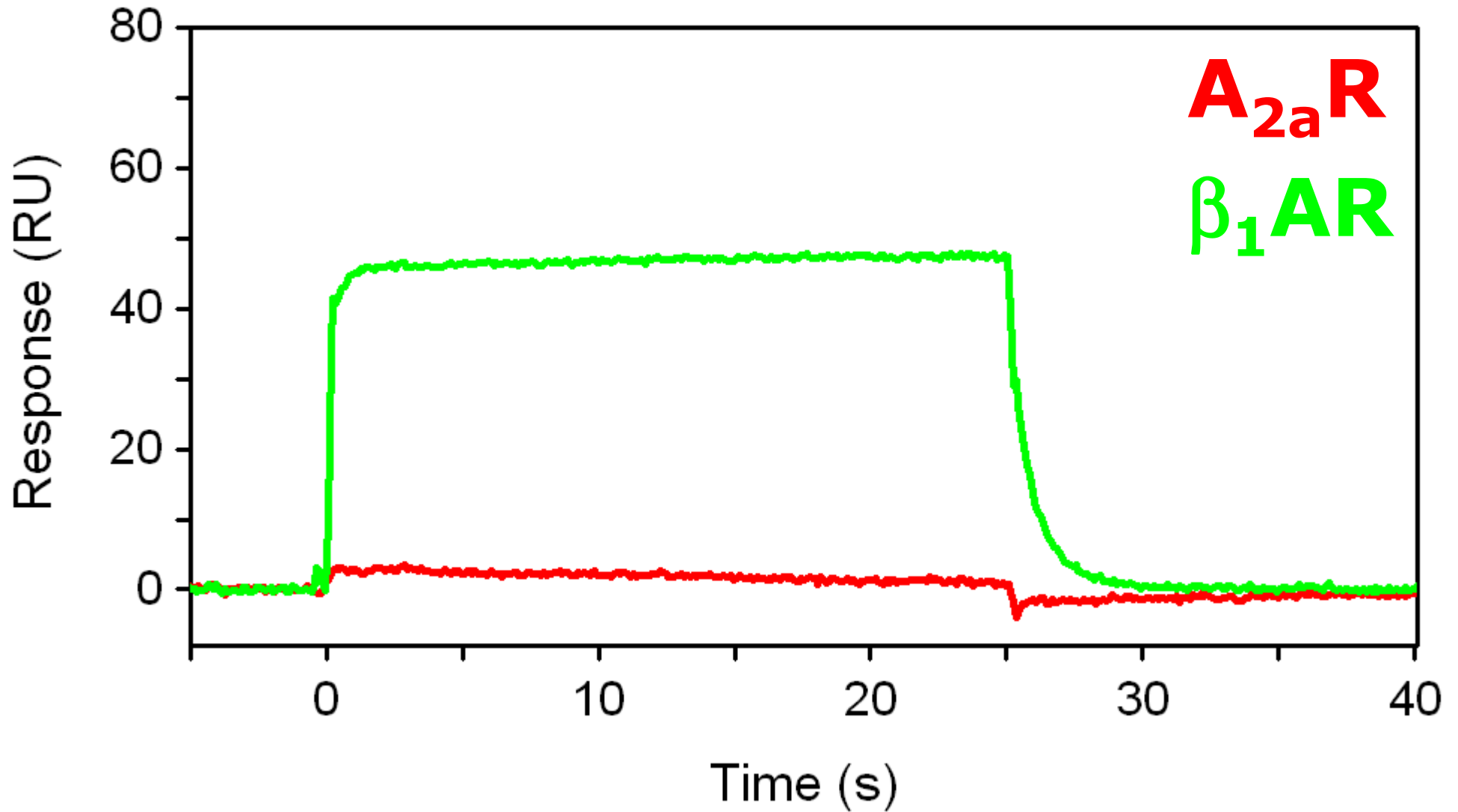
Fragments



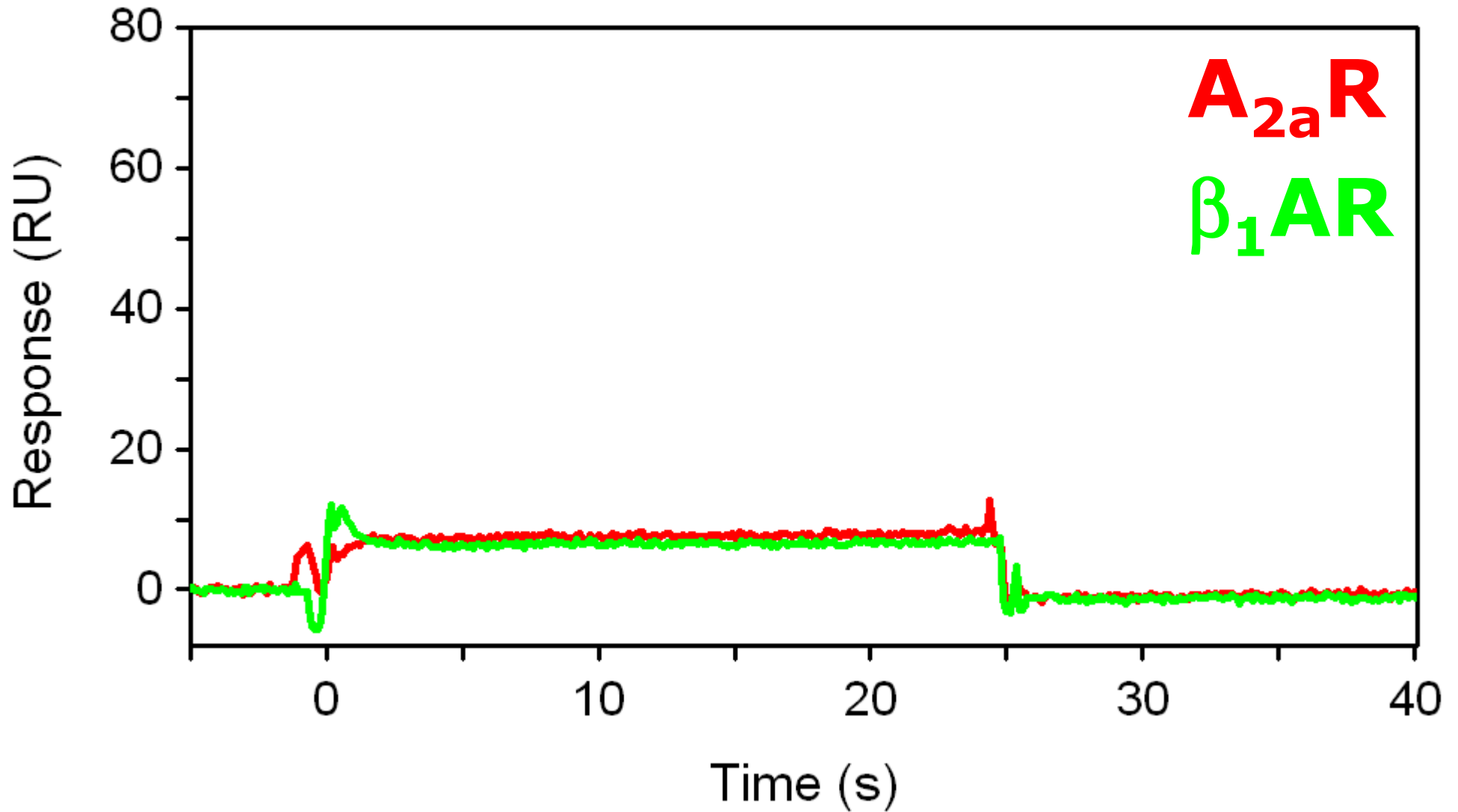
Fragments



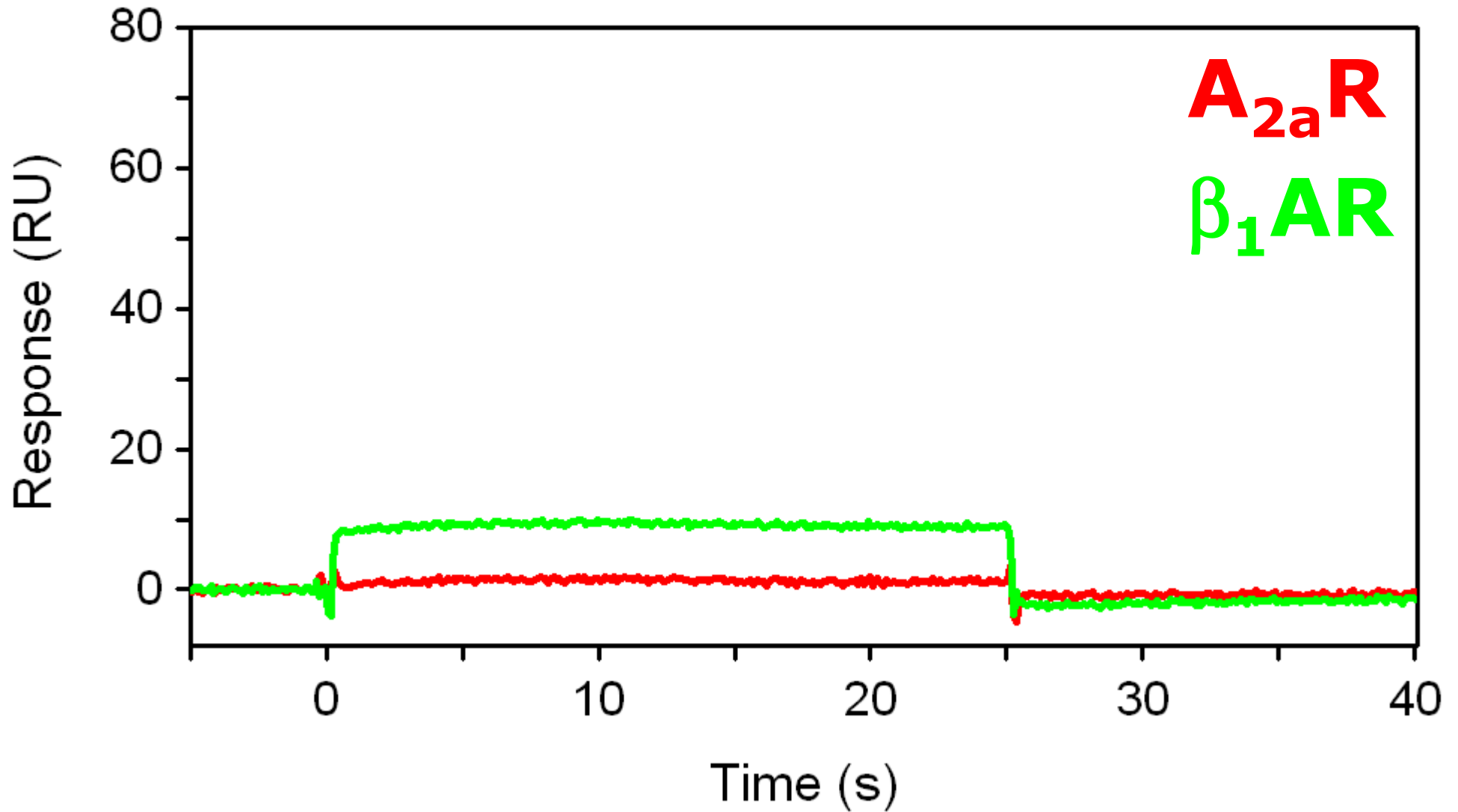
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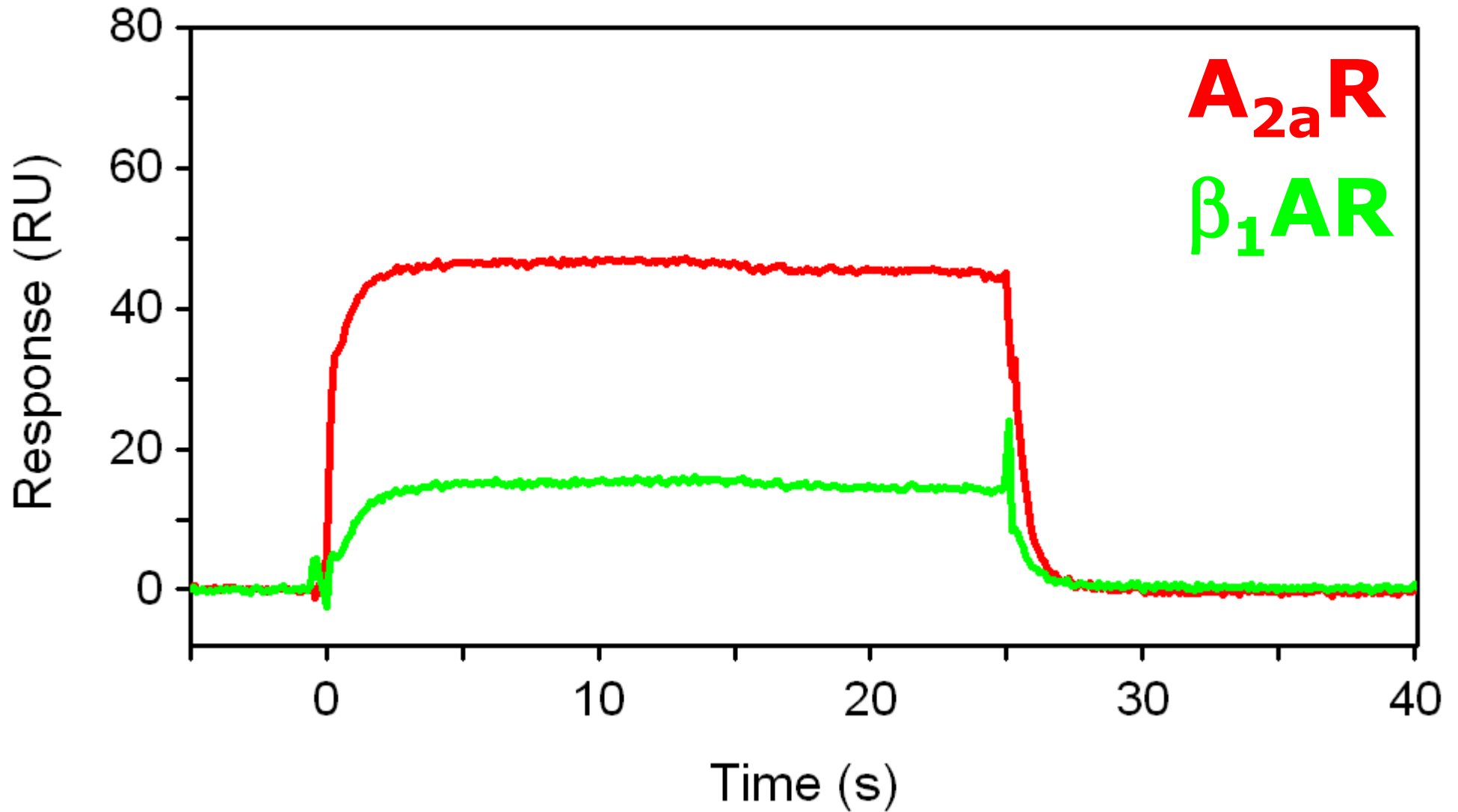
Fragments



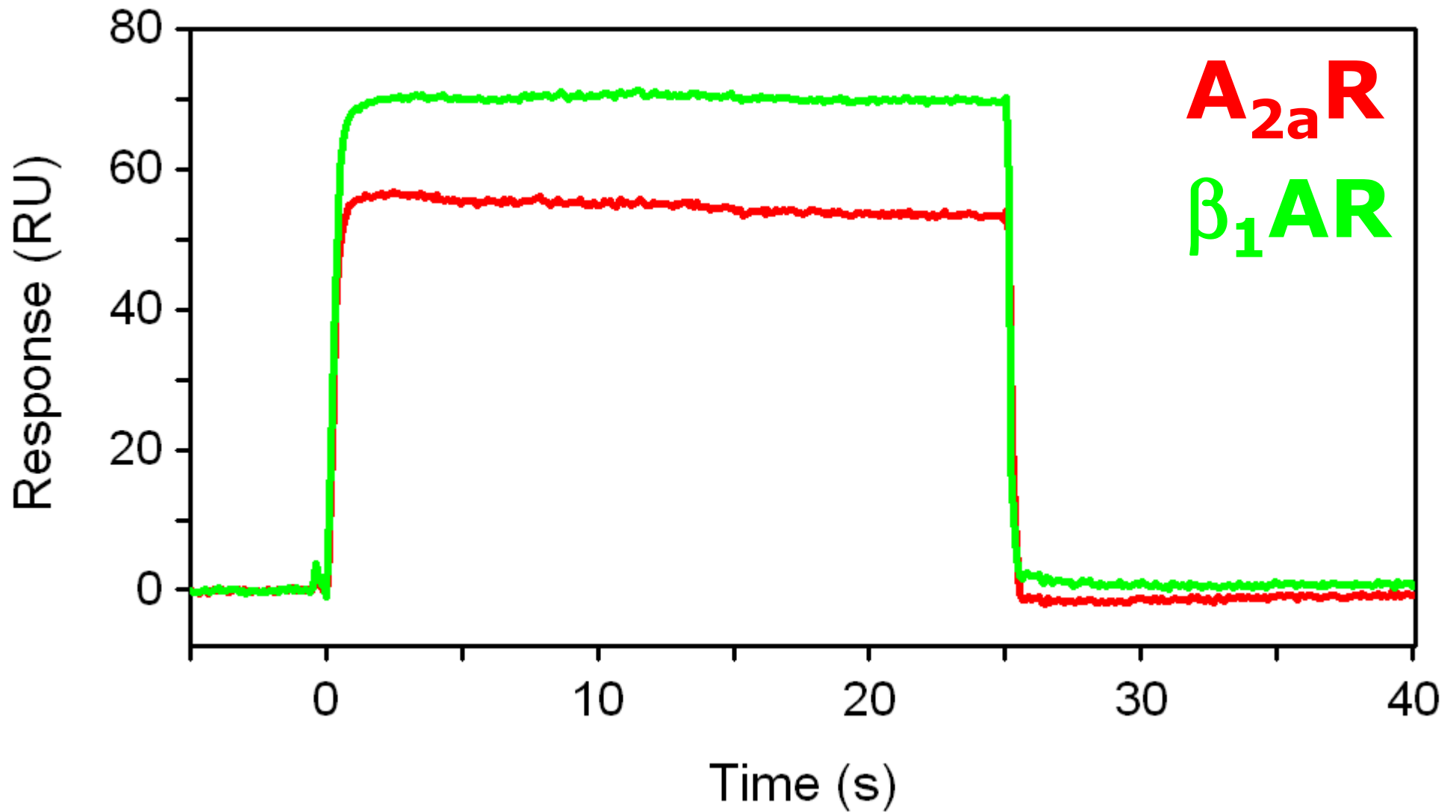
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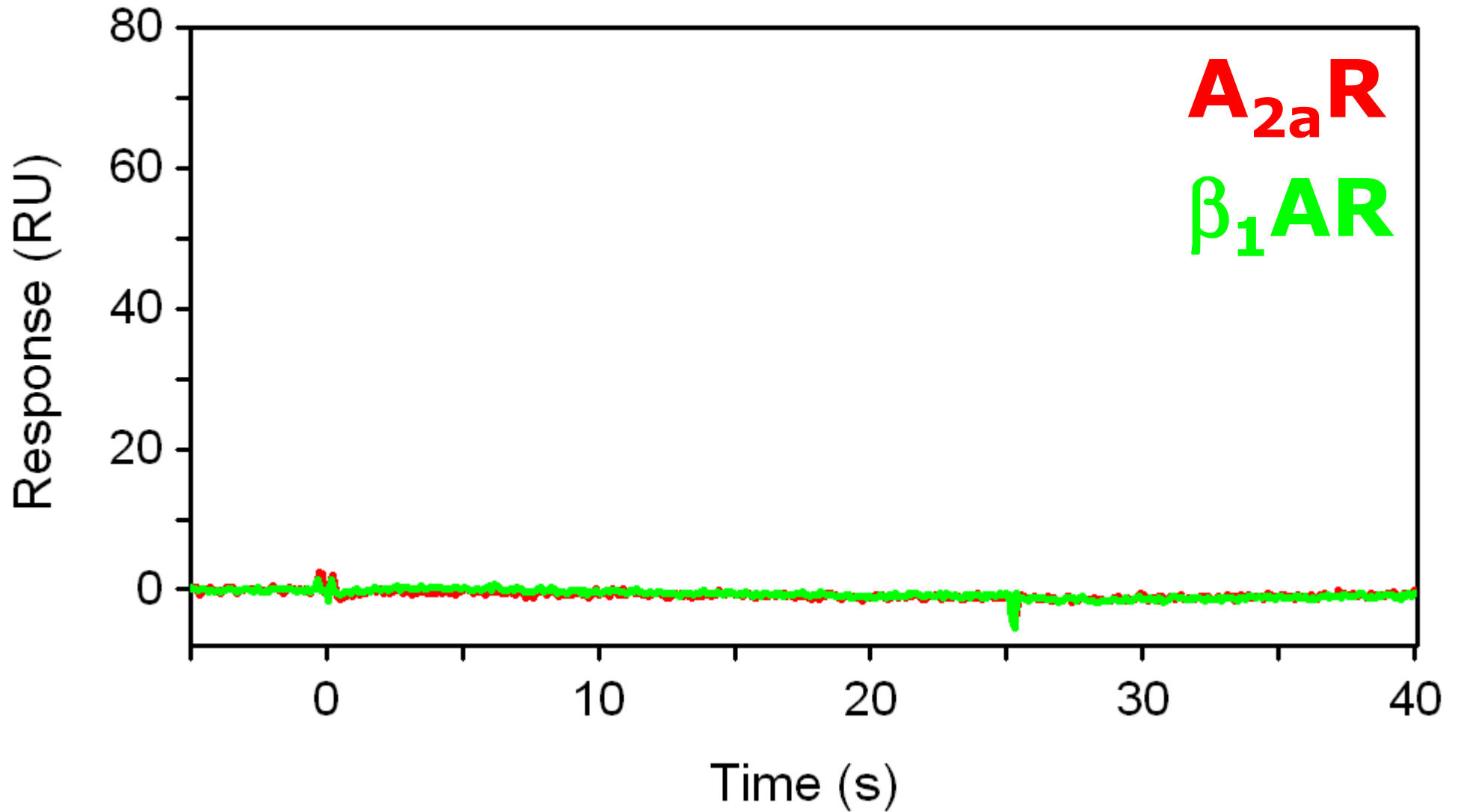
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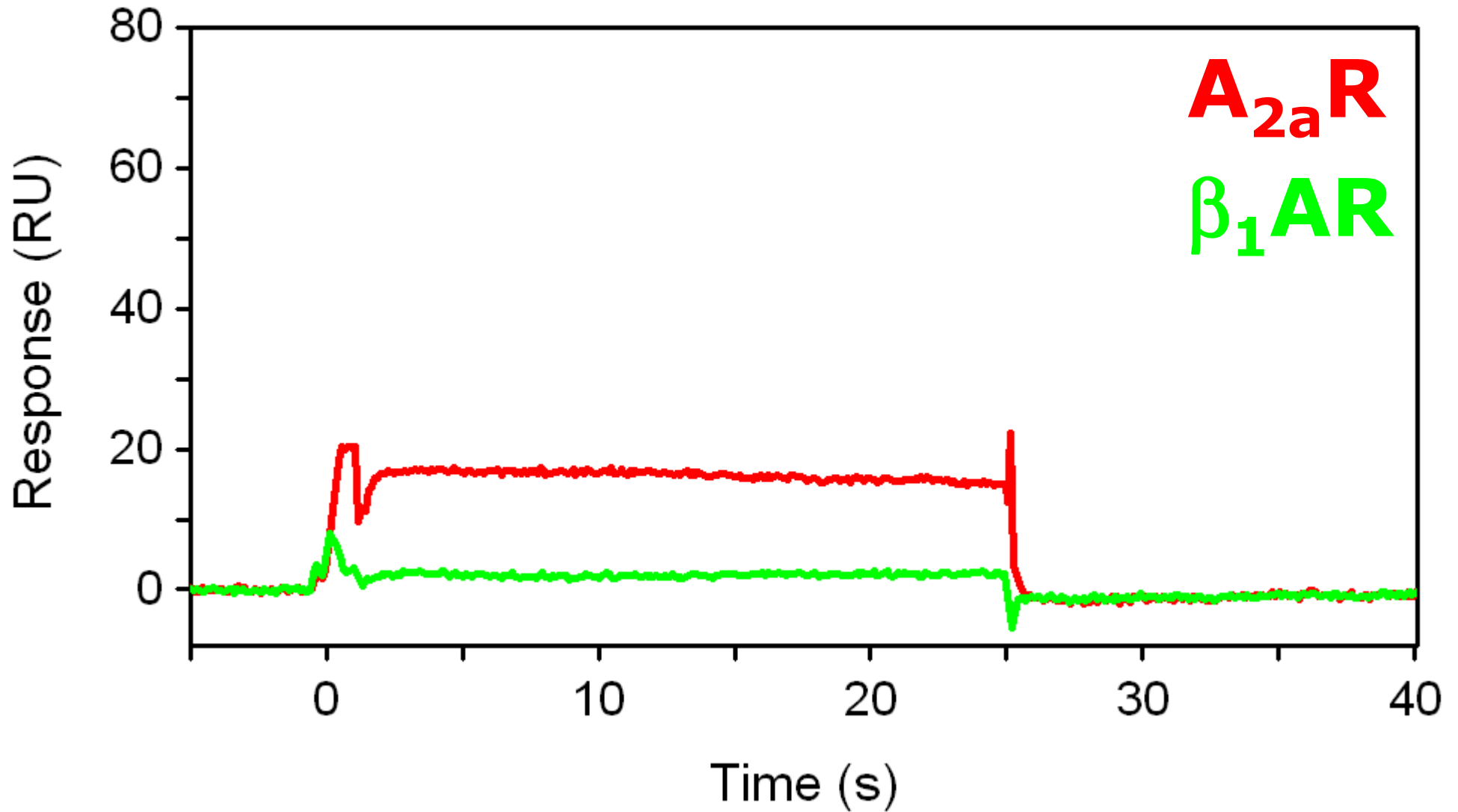
Fragments



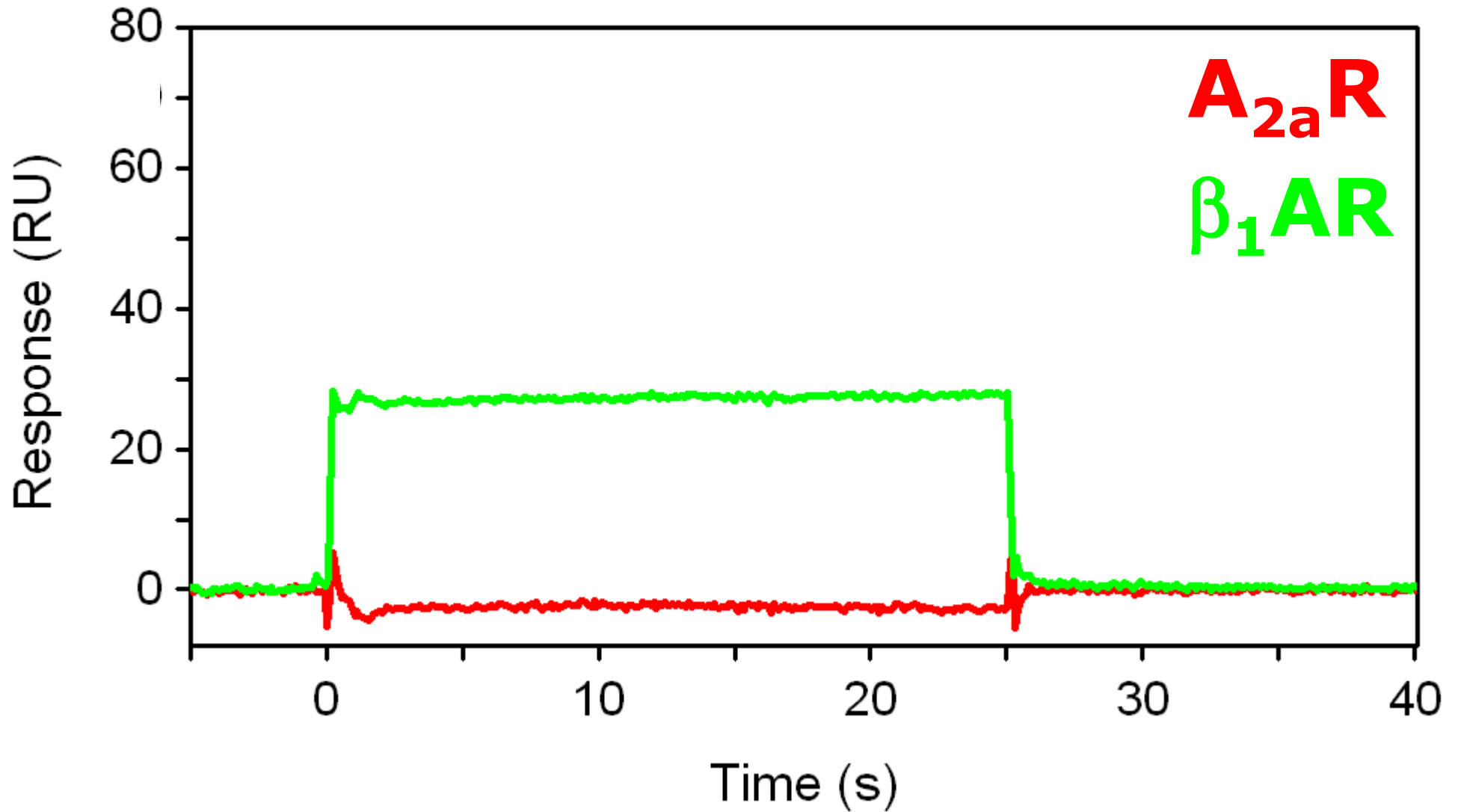
Fragments



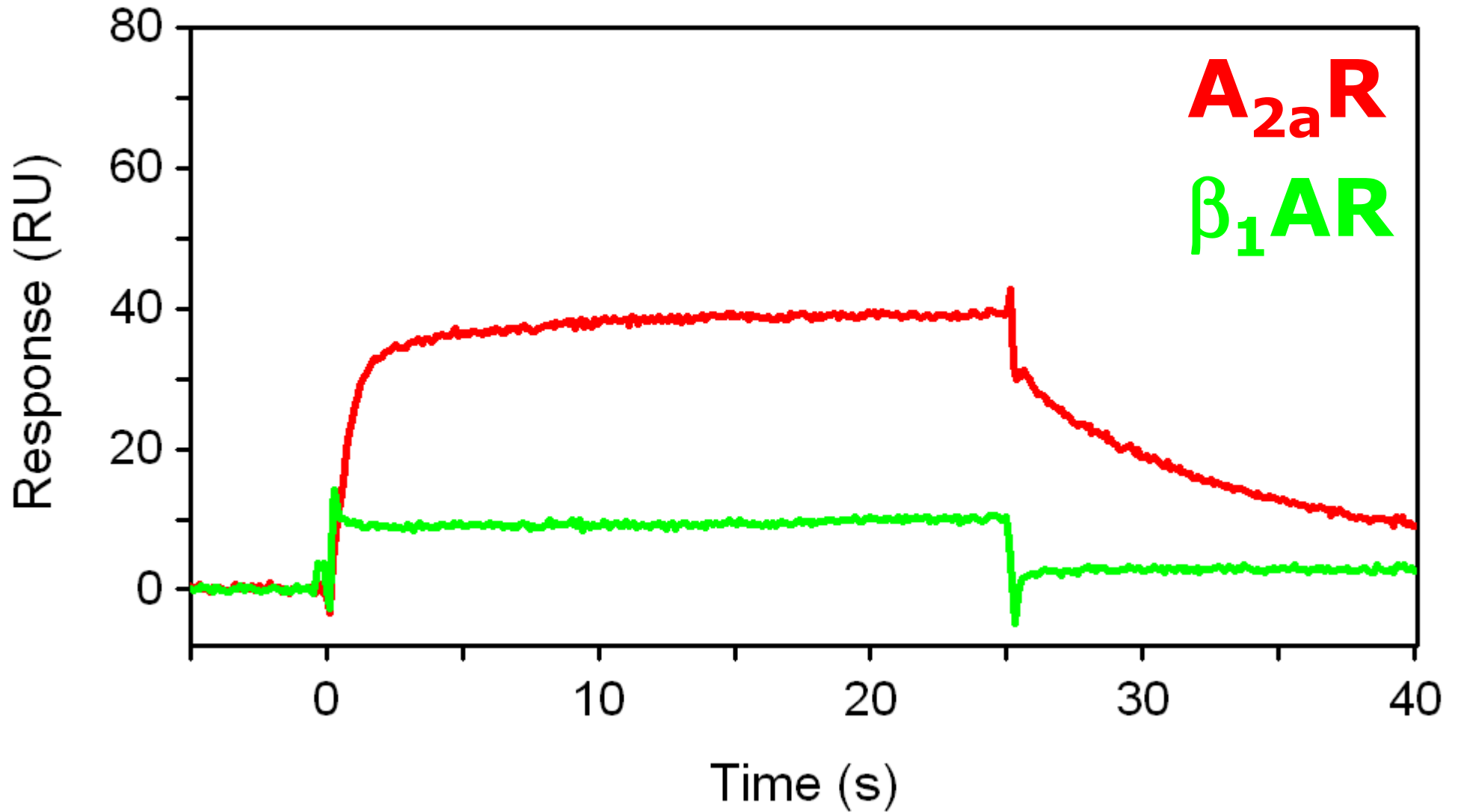
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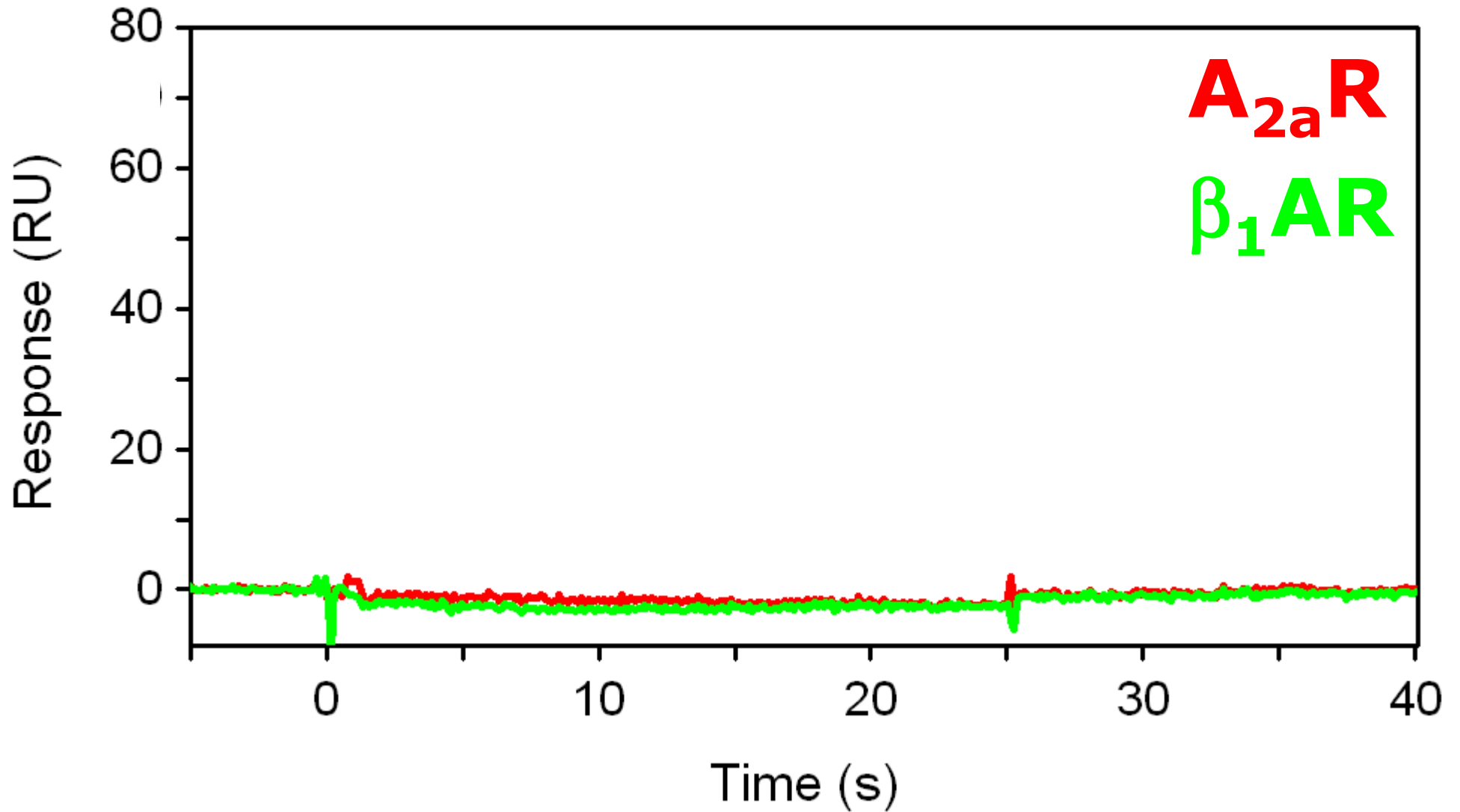
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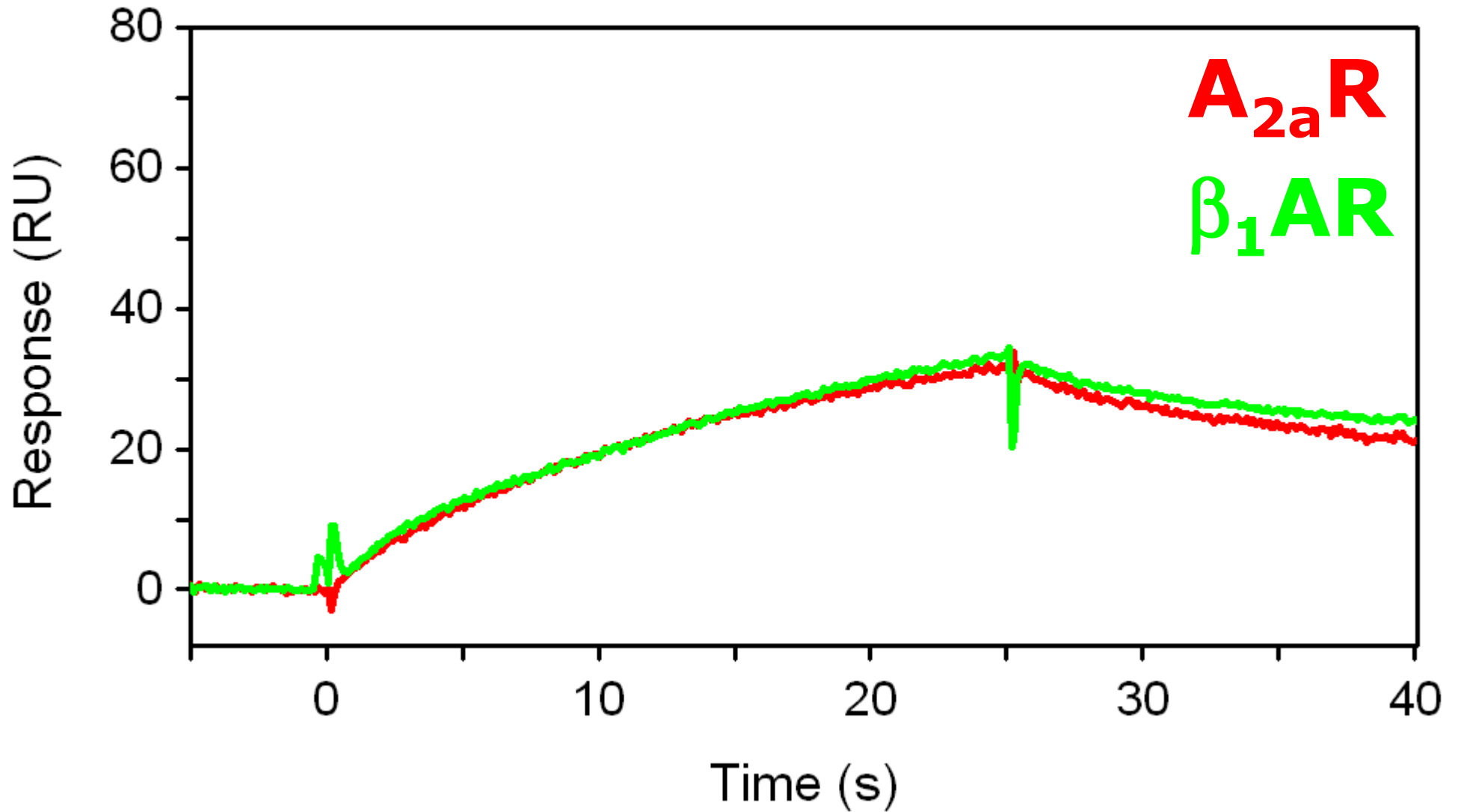
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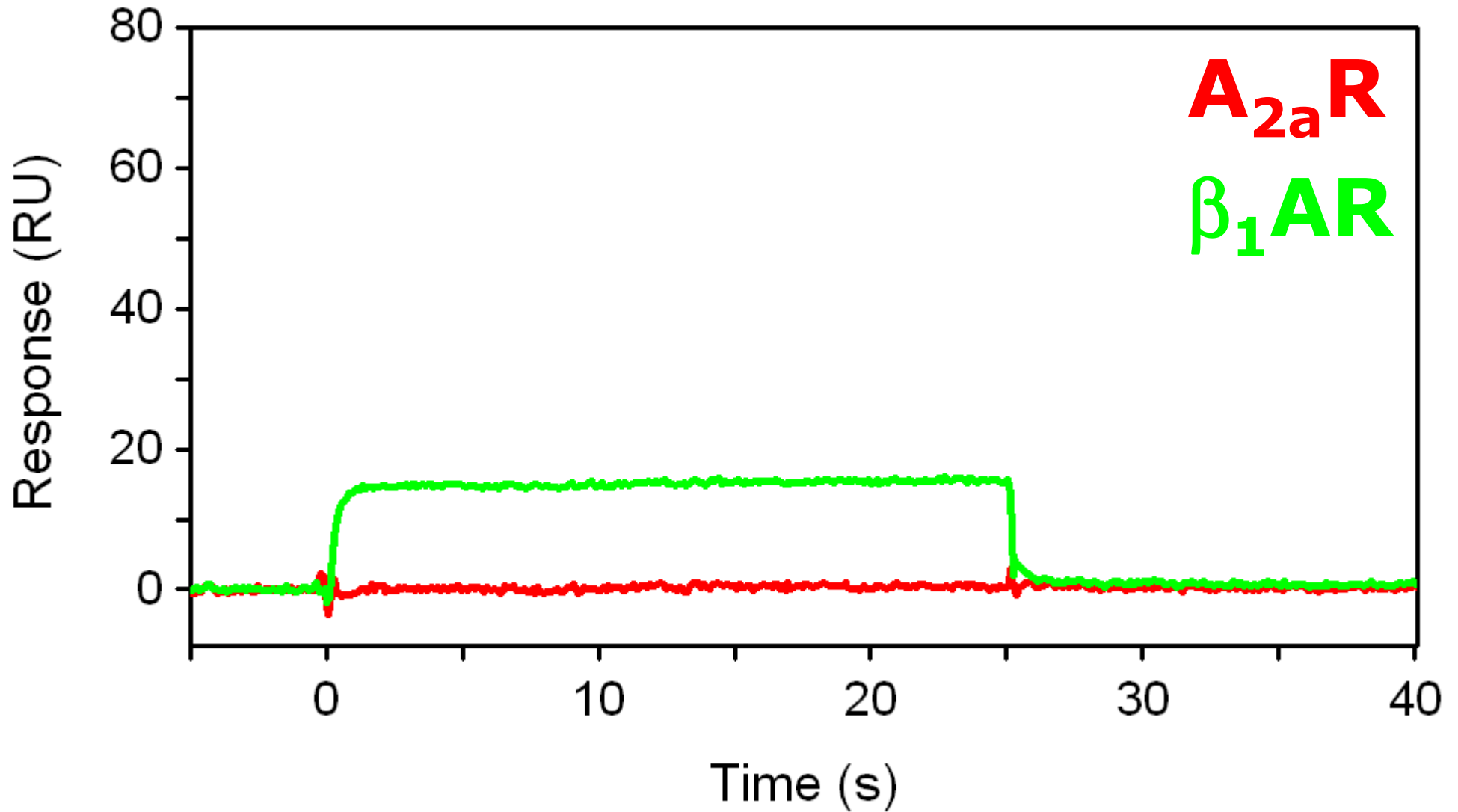
Fragments



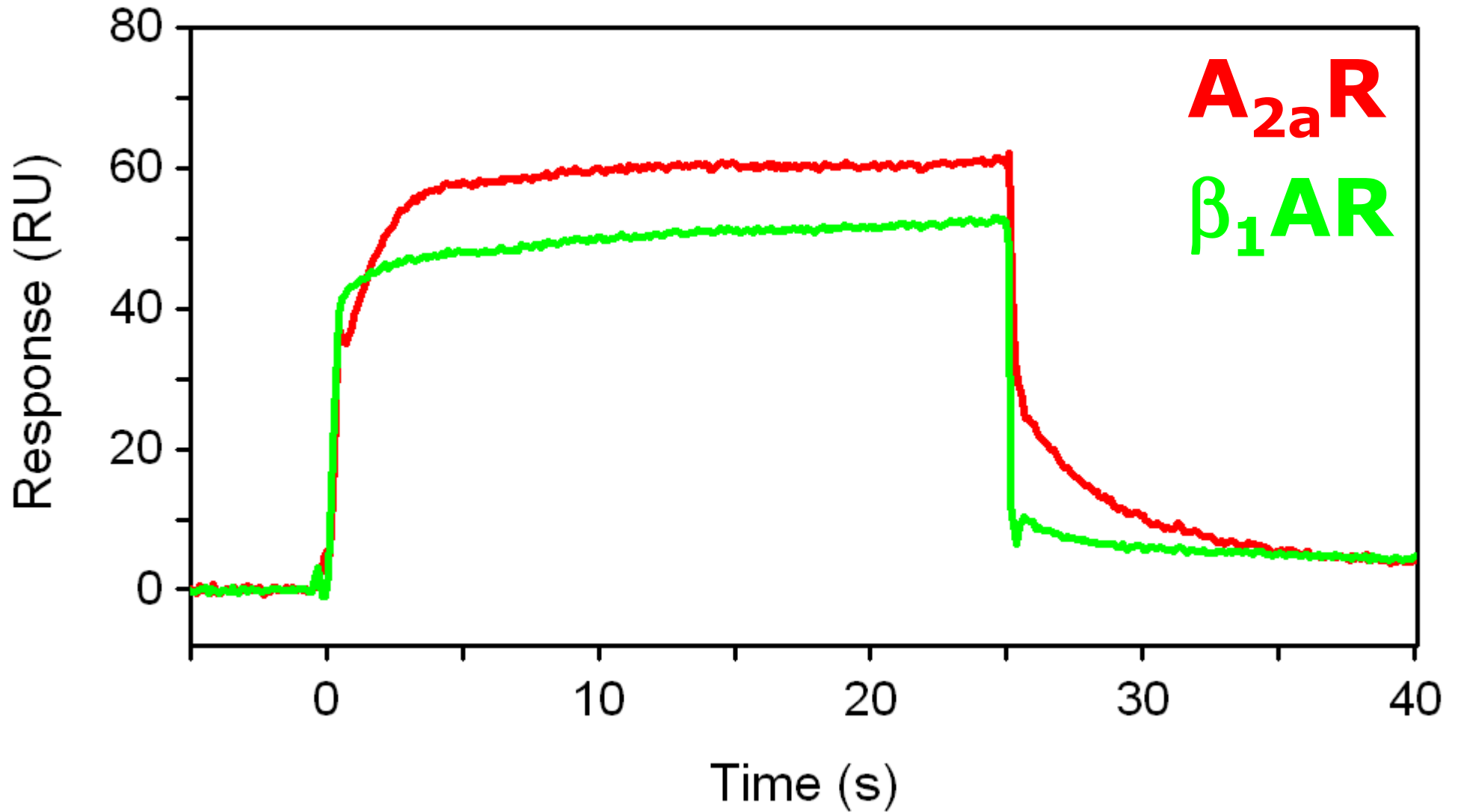
Fragments



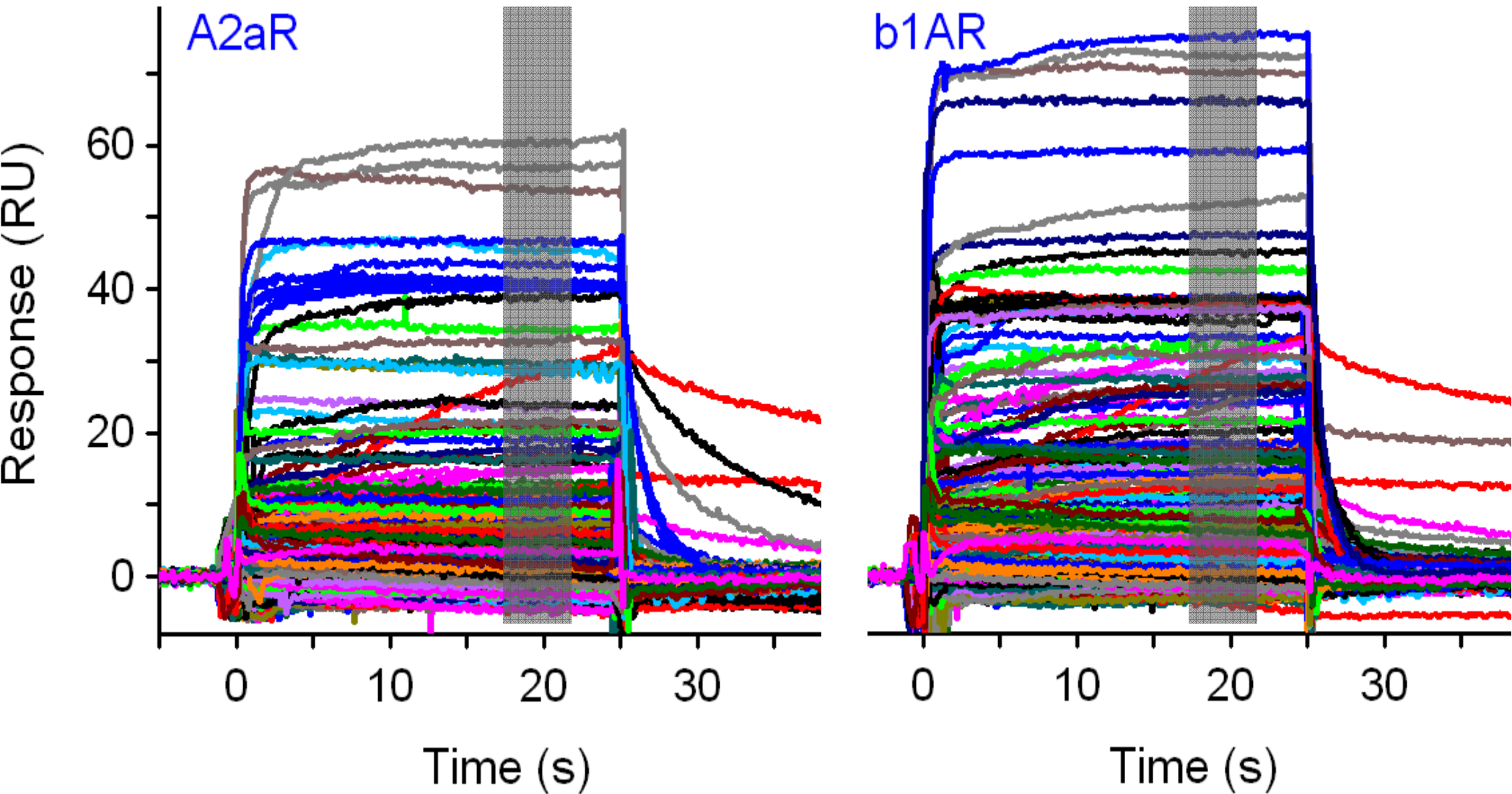
Fragments



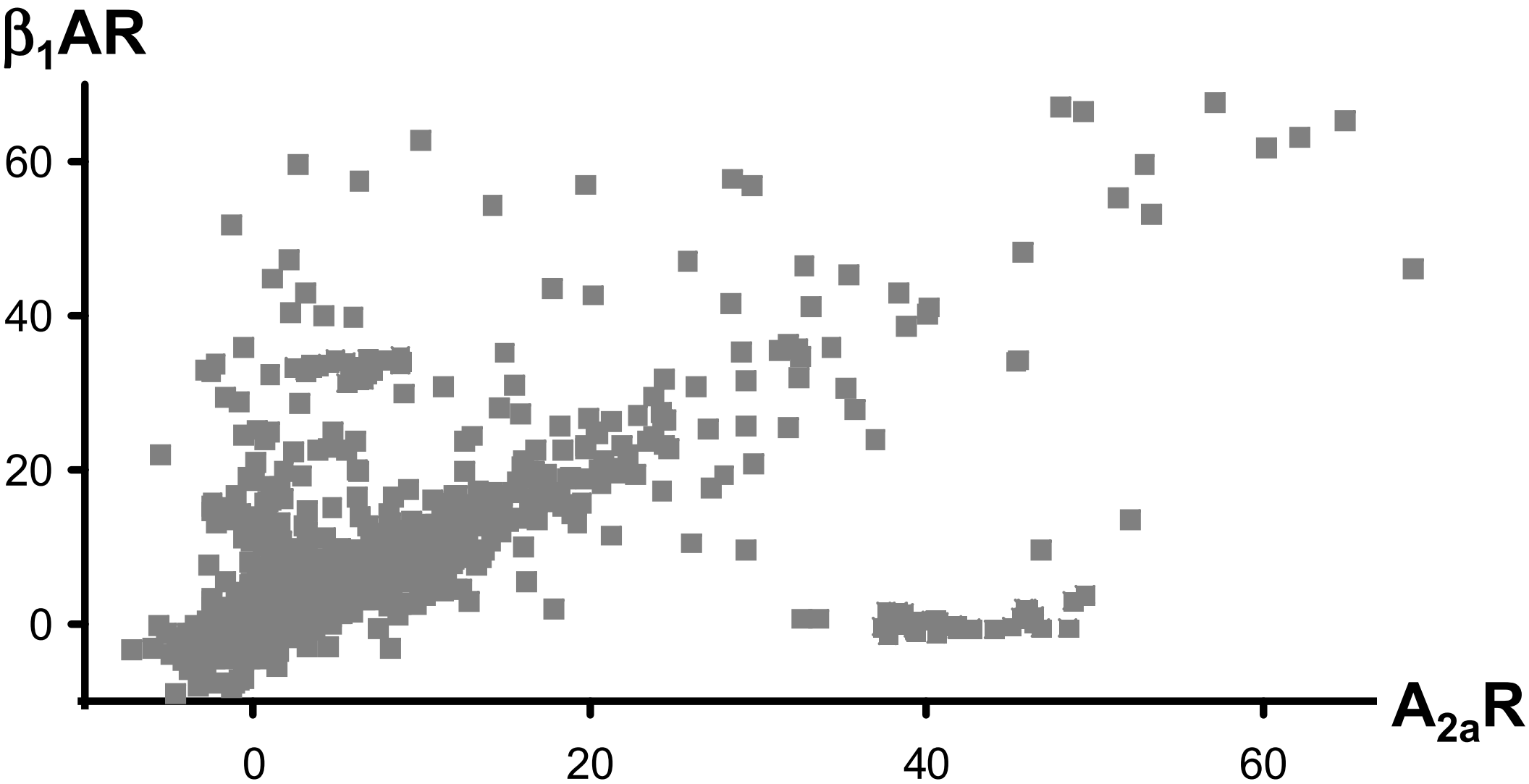
Fragments



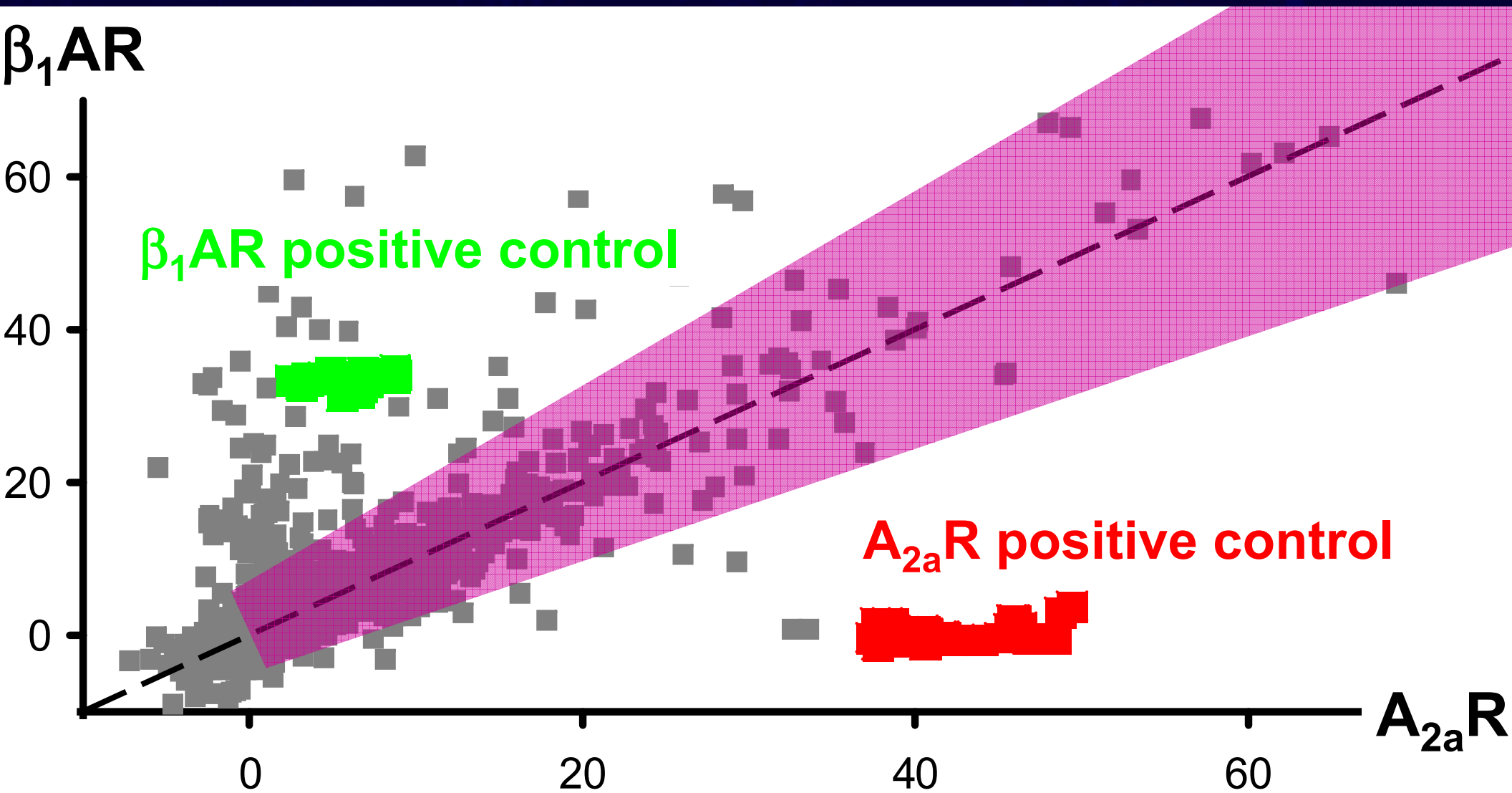
Selectivity



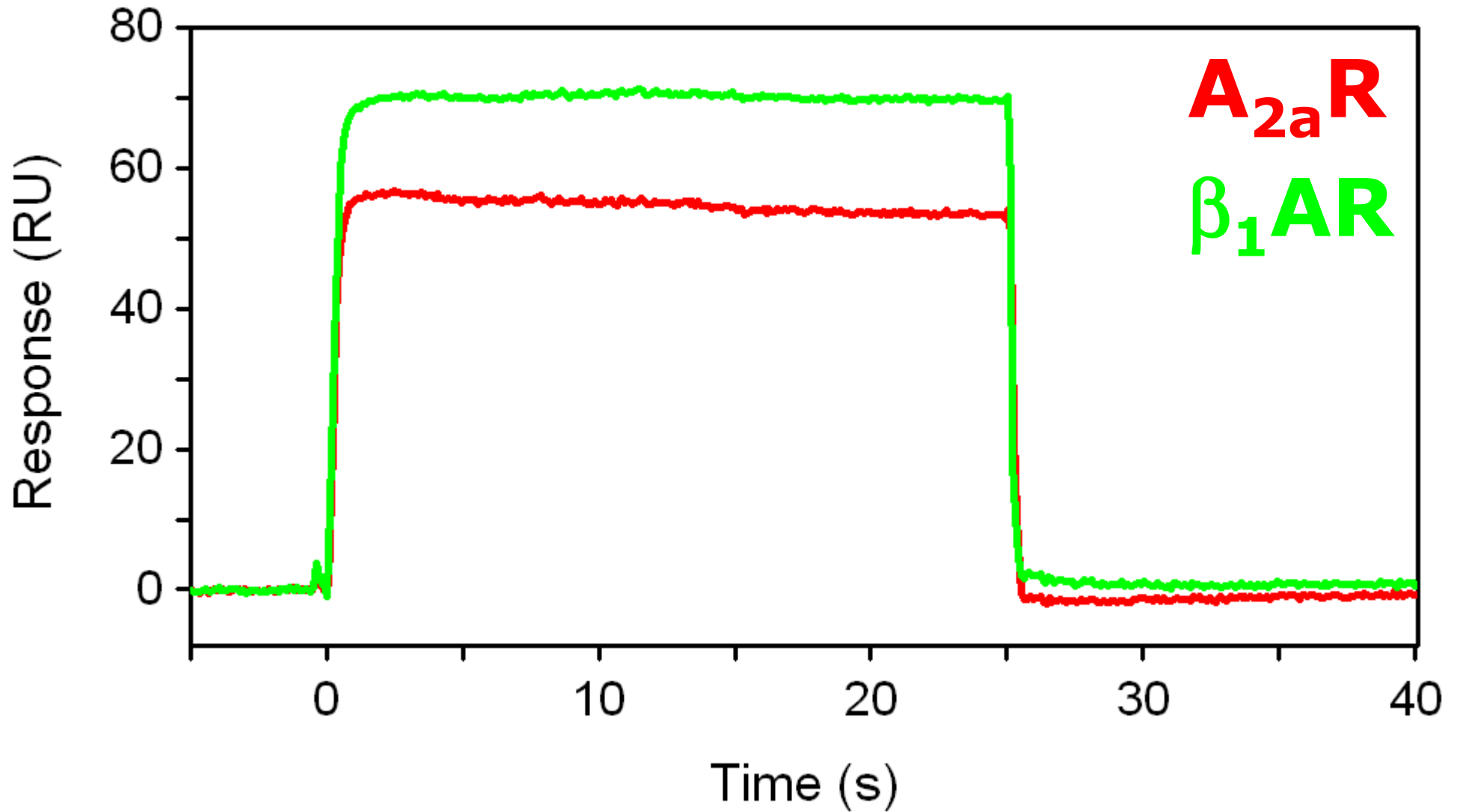
Selectivity



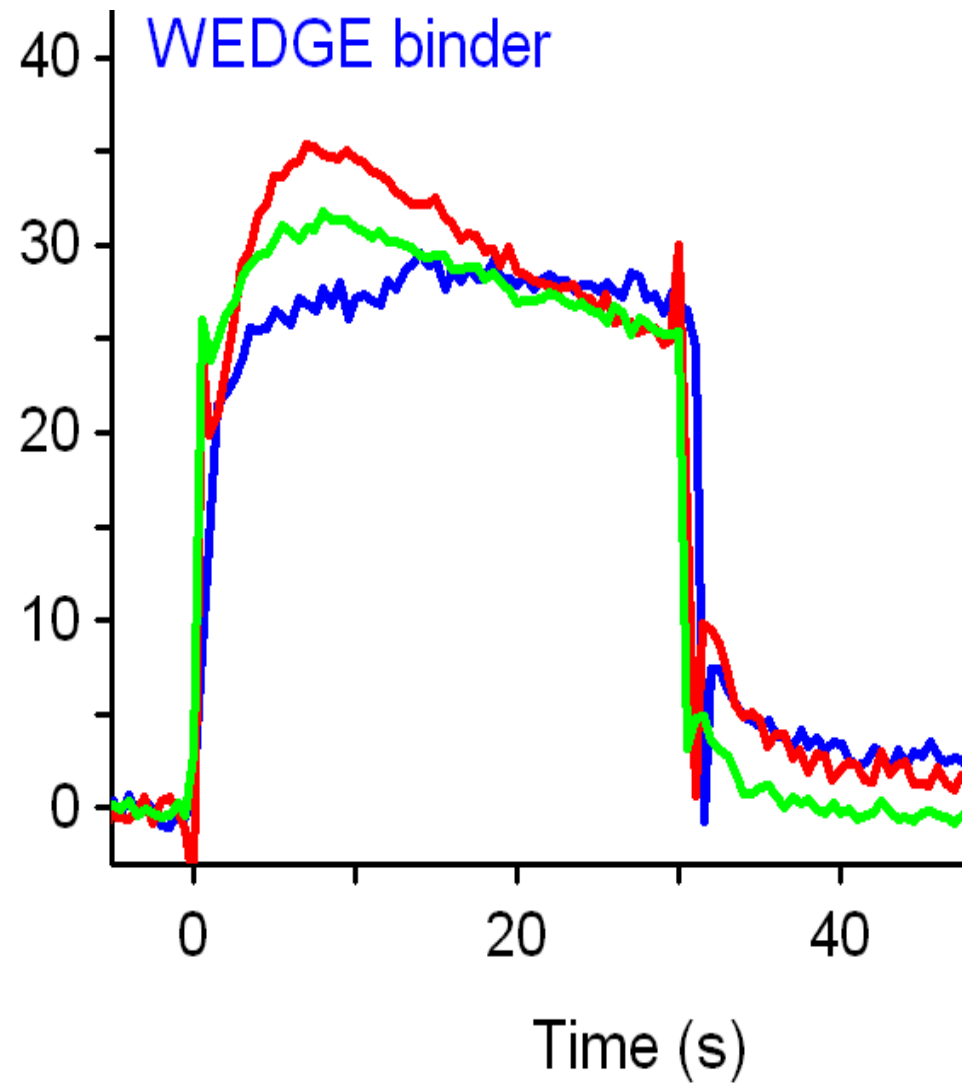
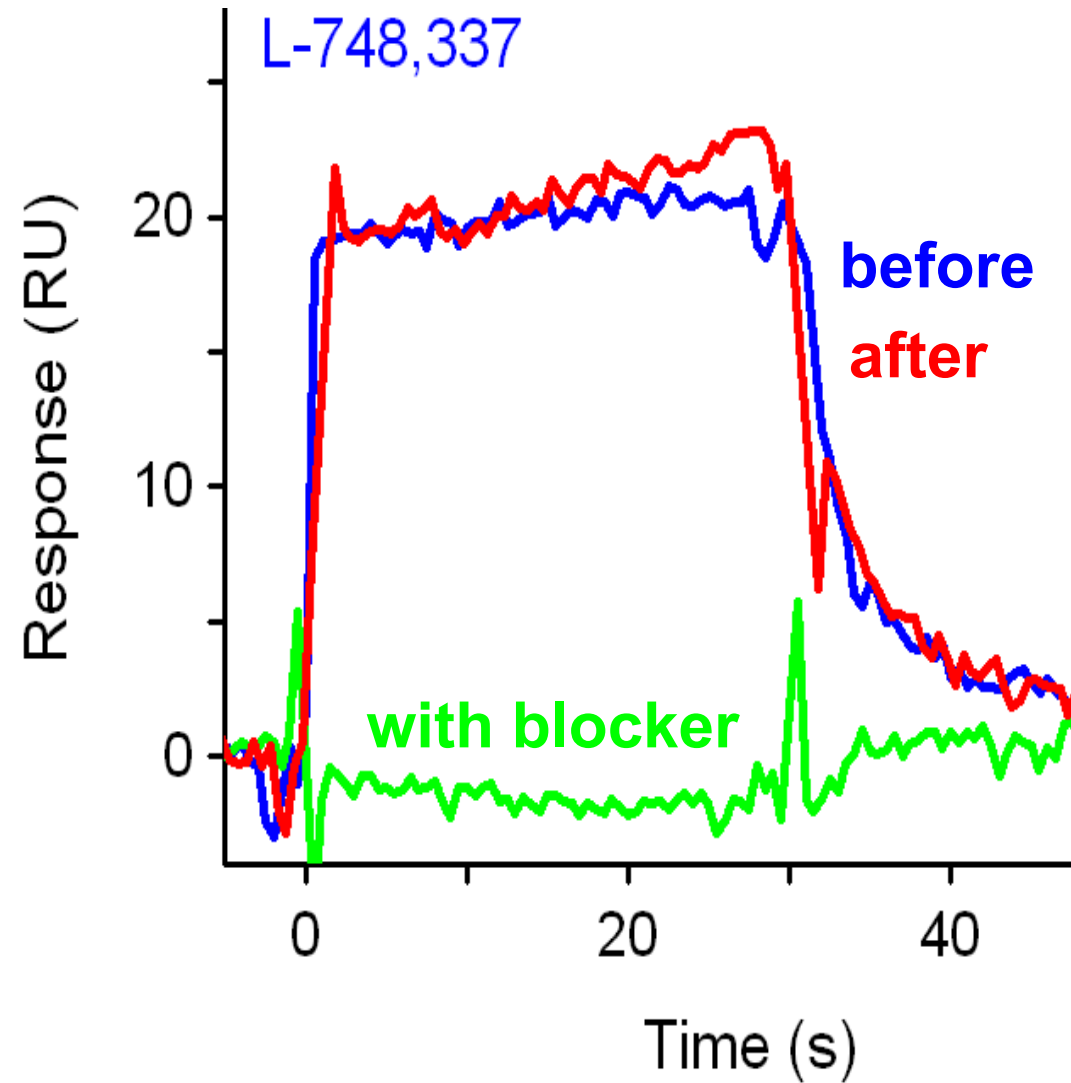
Selectivity



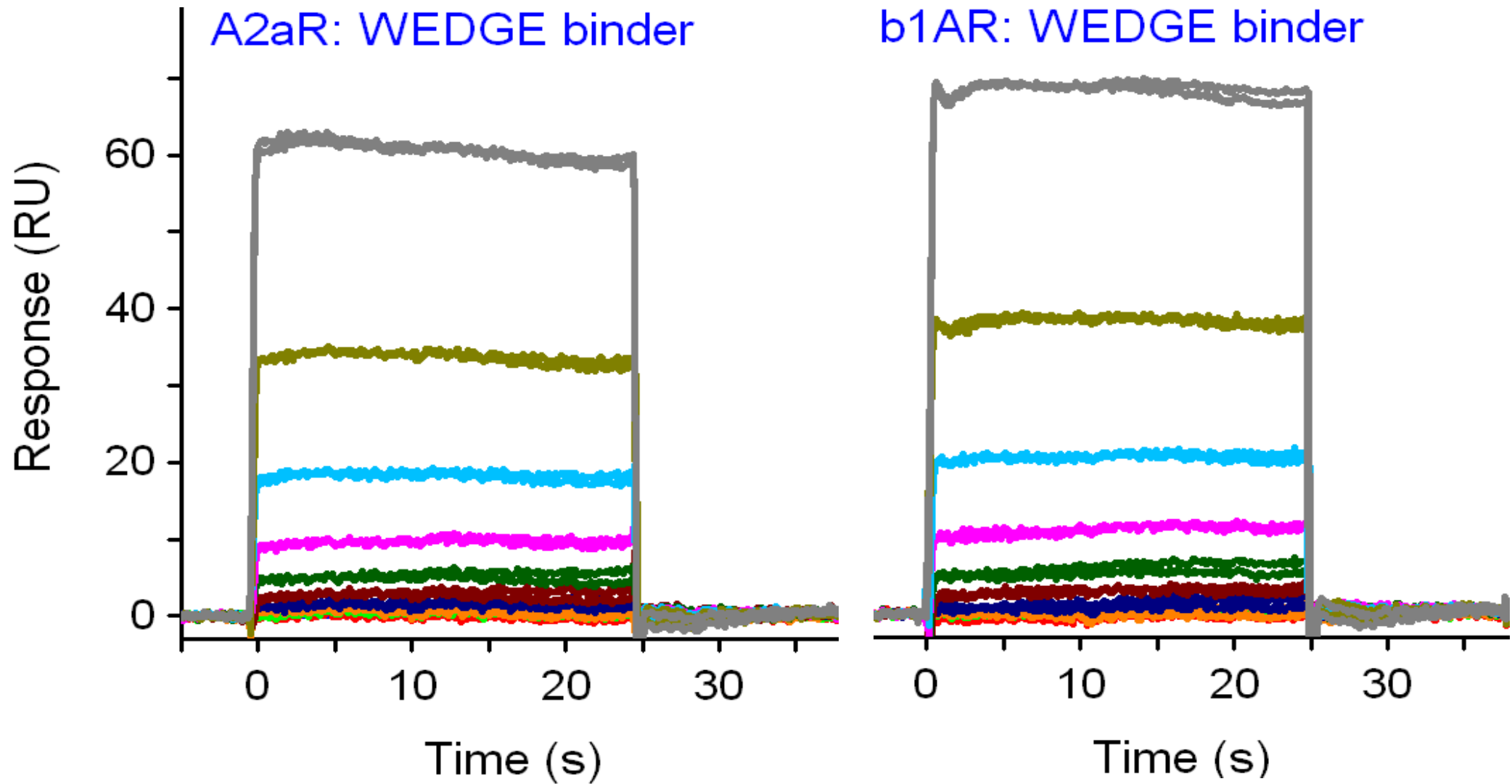
Wedge binders



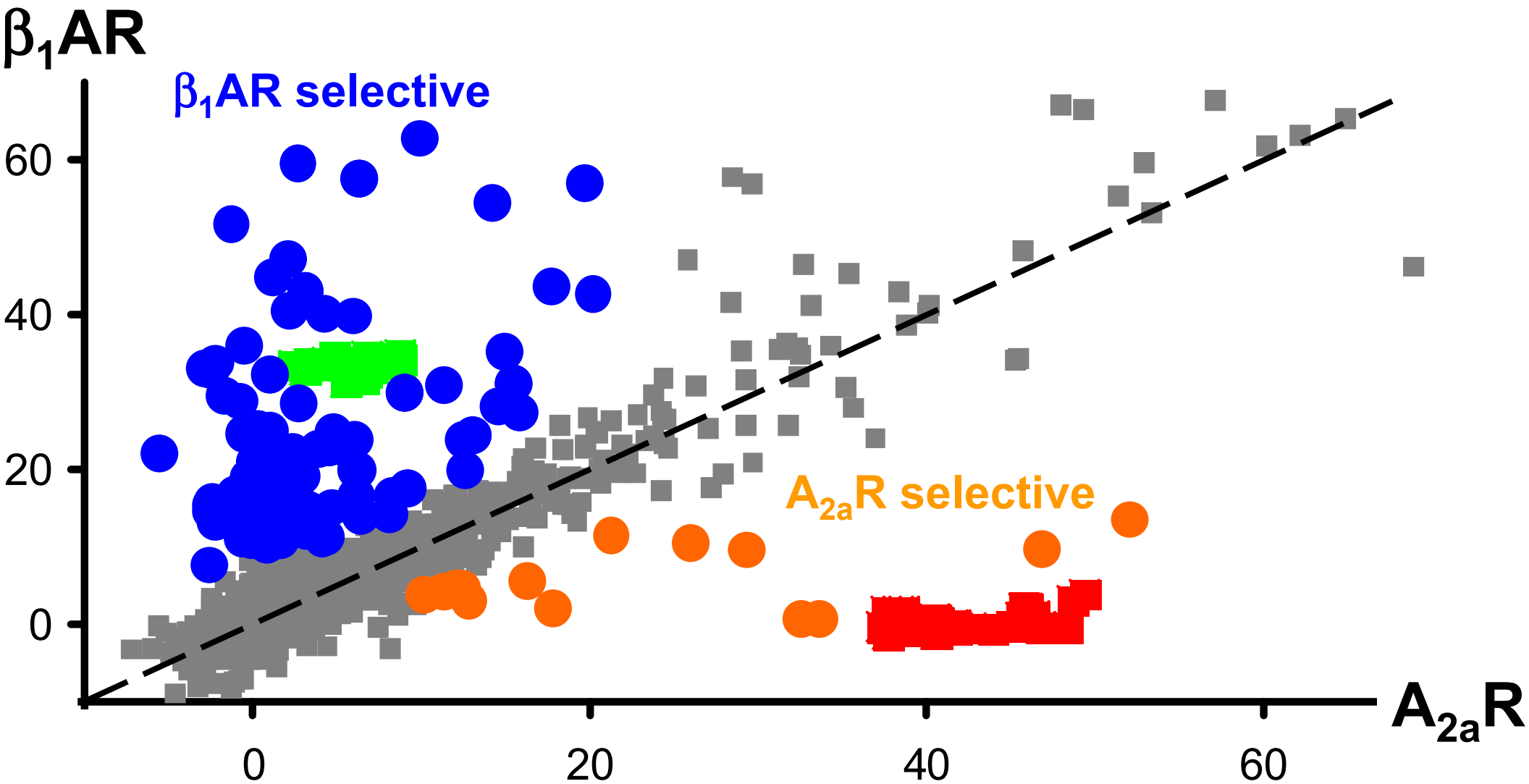
Site competition



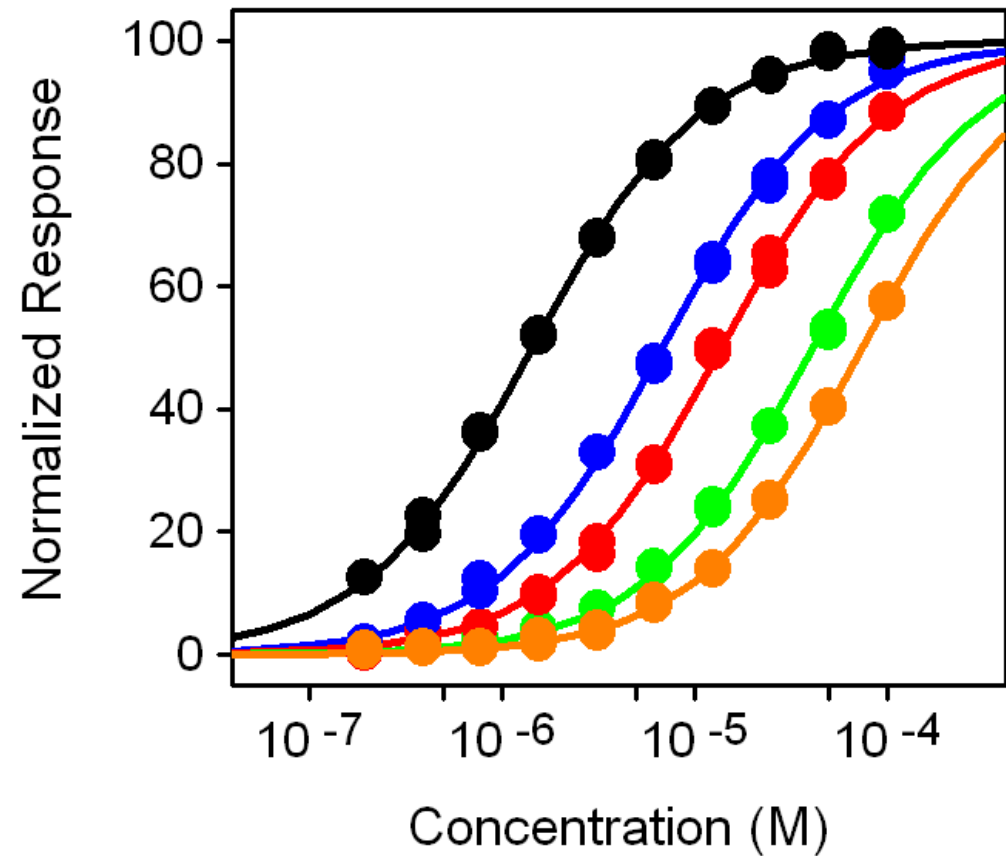
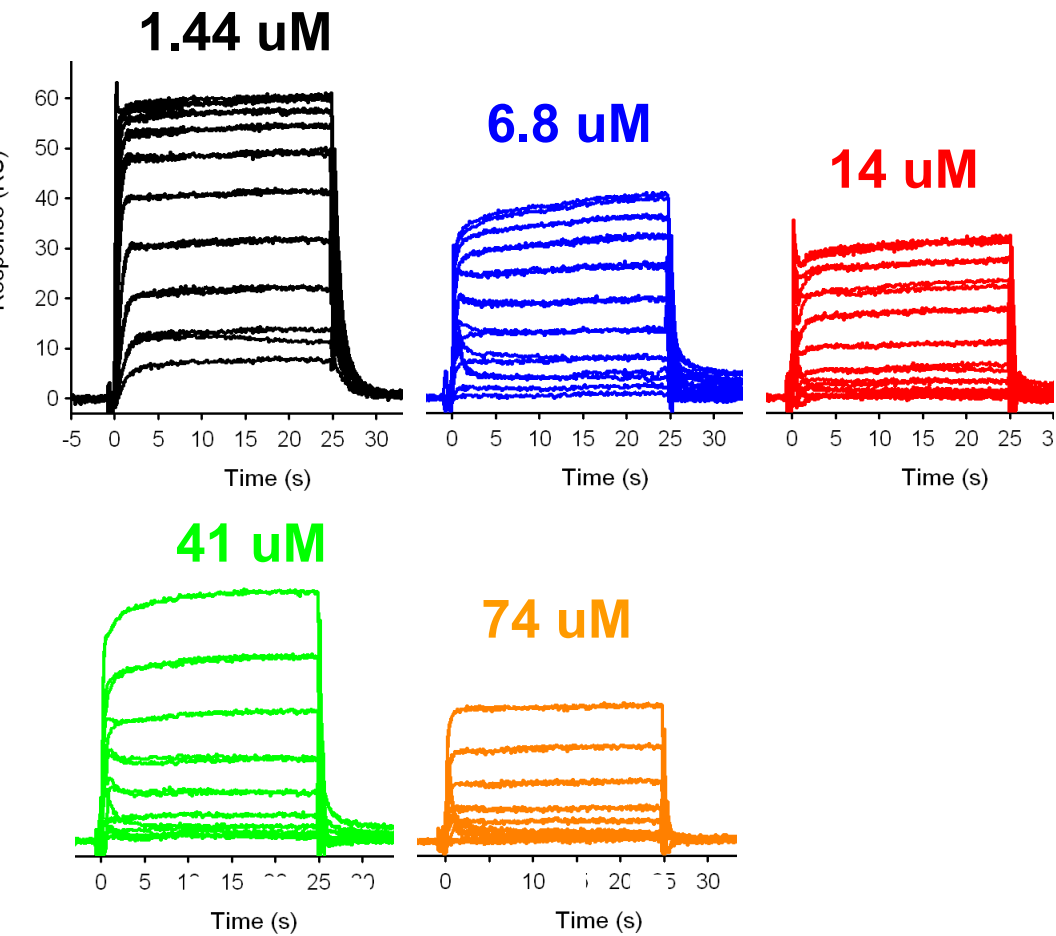
Not Saturable



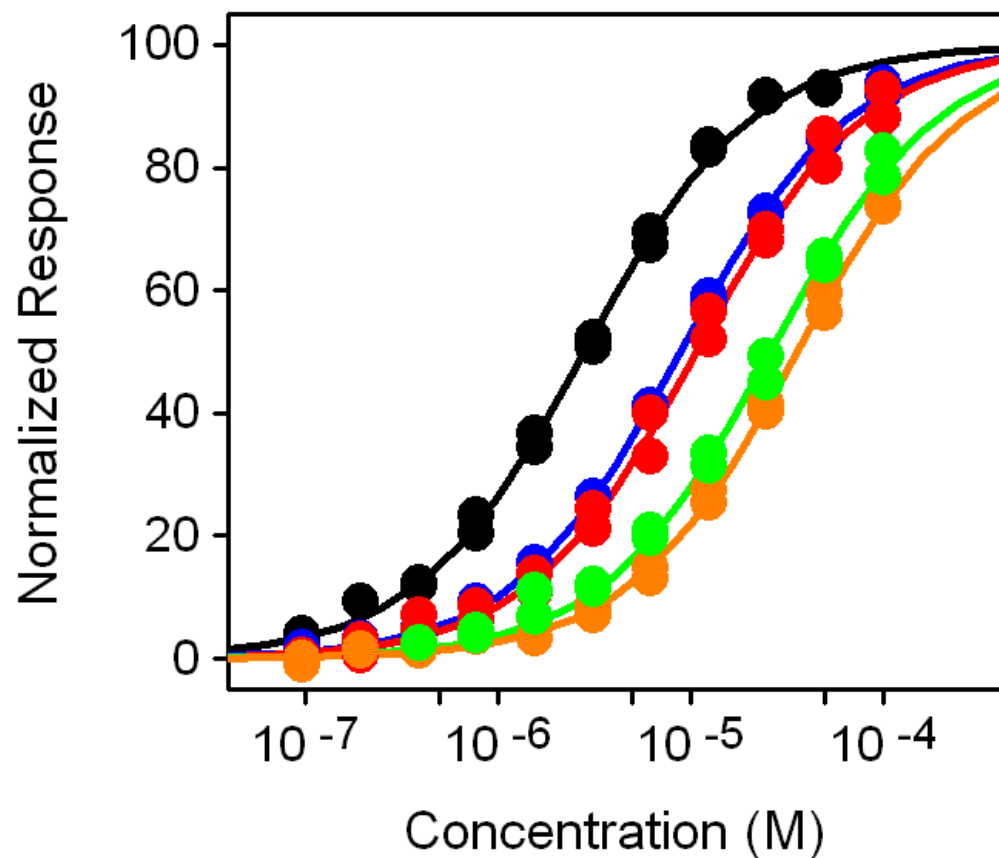
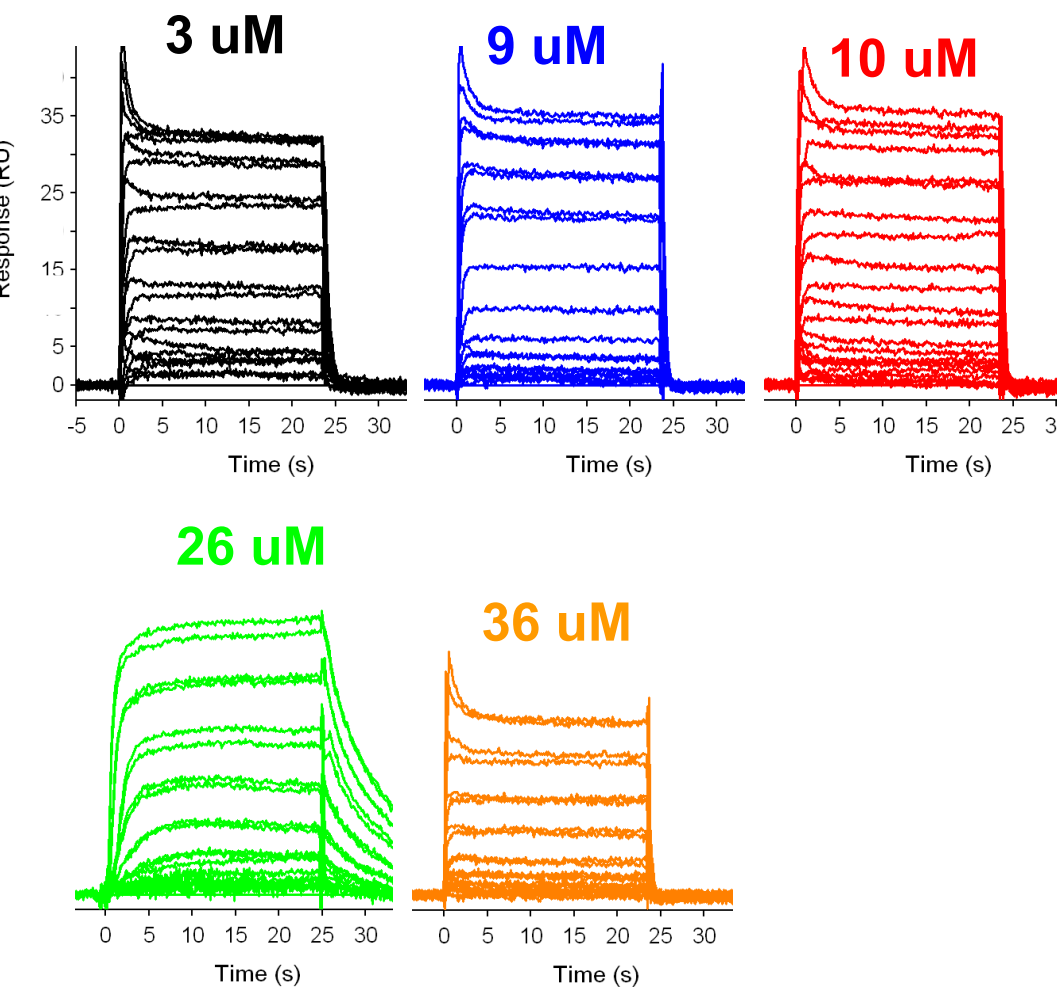
Selective Hits



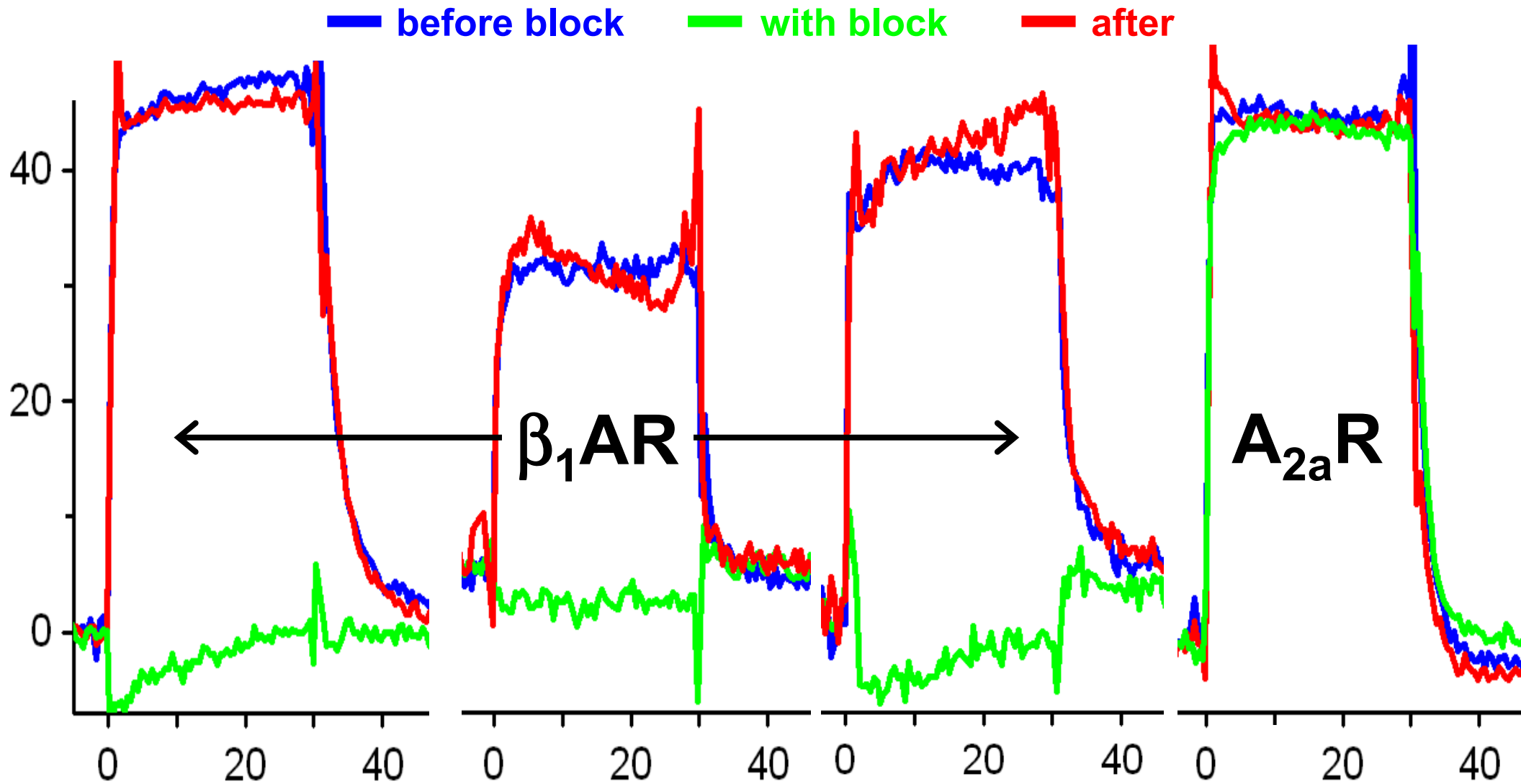
Novel β_1 AR binders



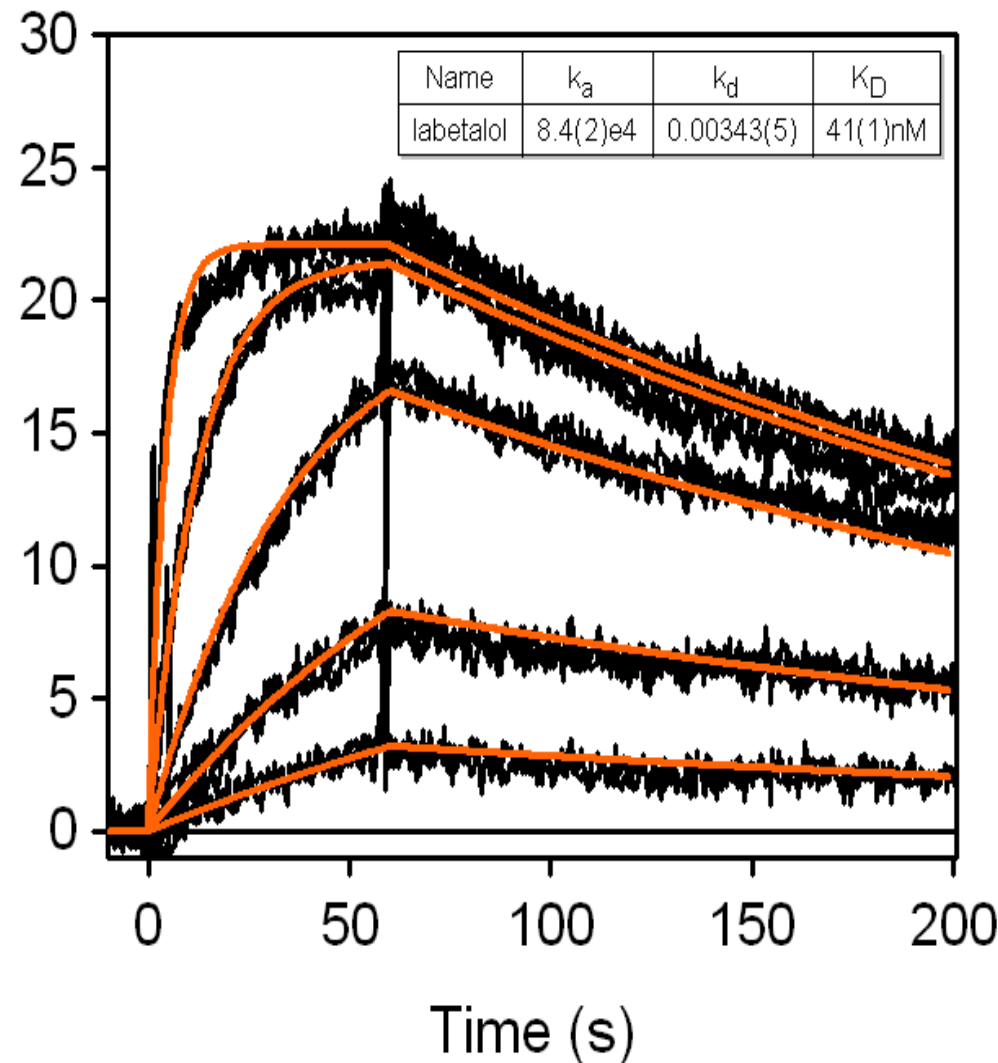
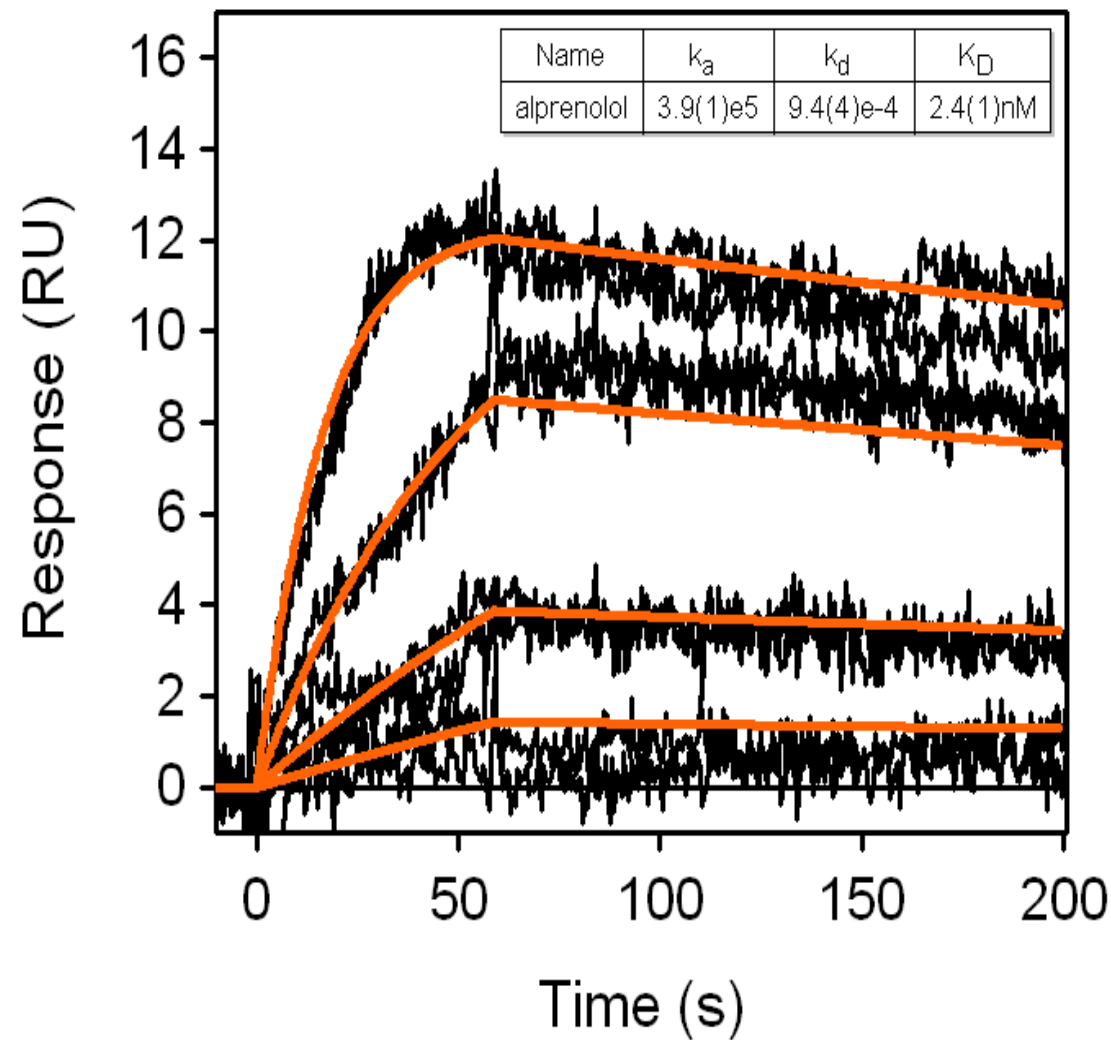
Novel $A_{2A}R$ binders



Competition



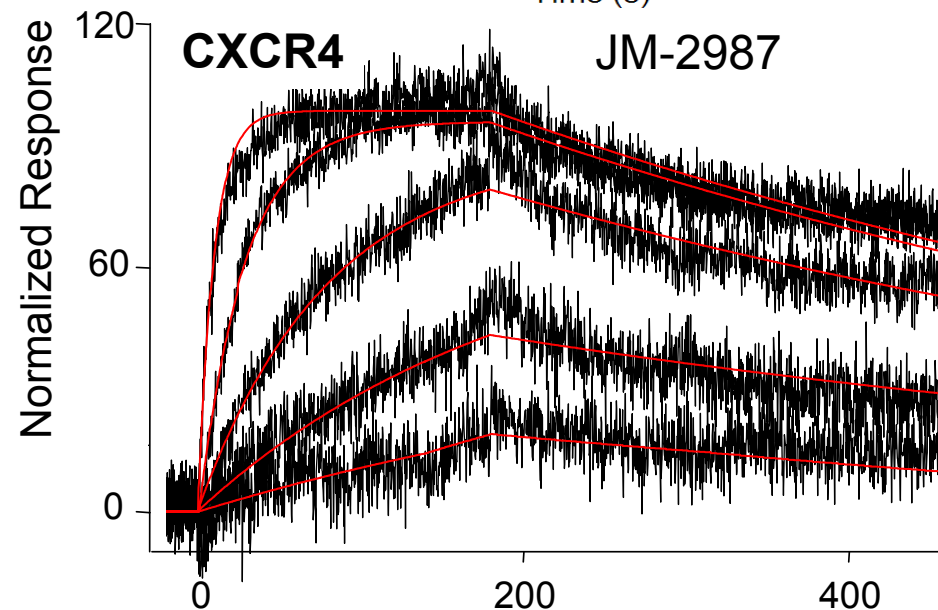
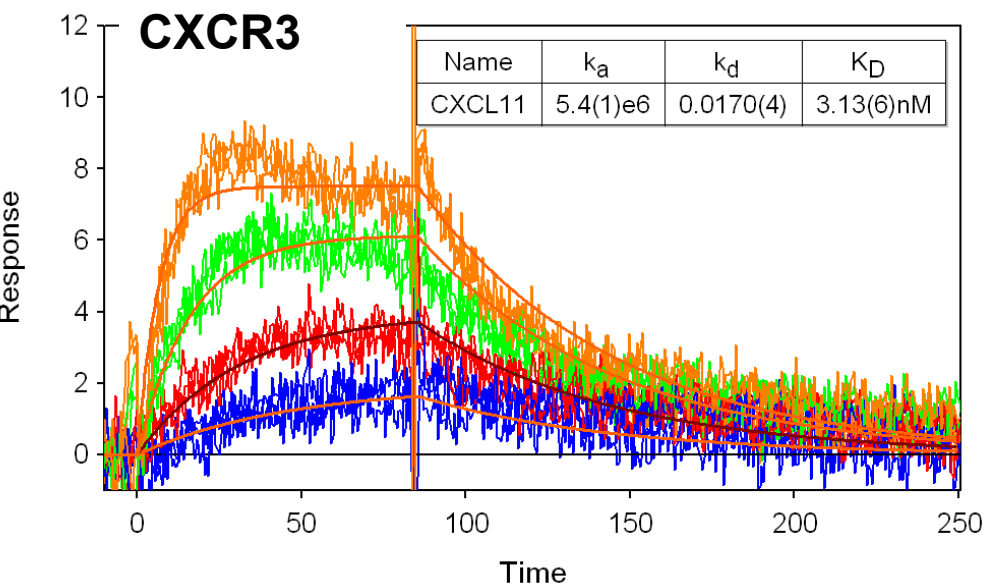
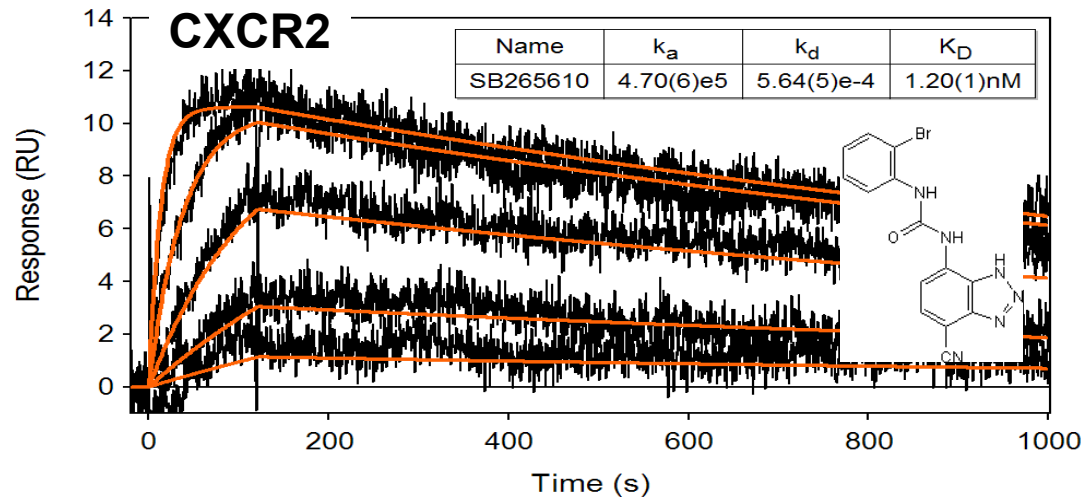
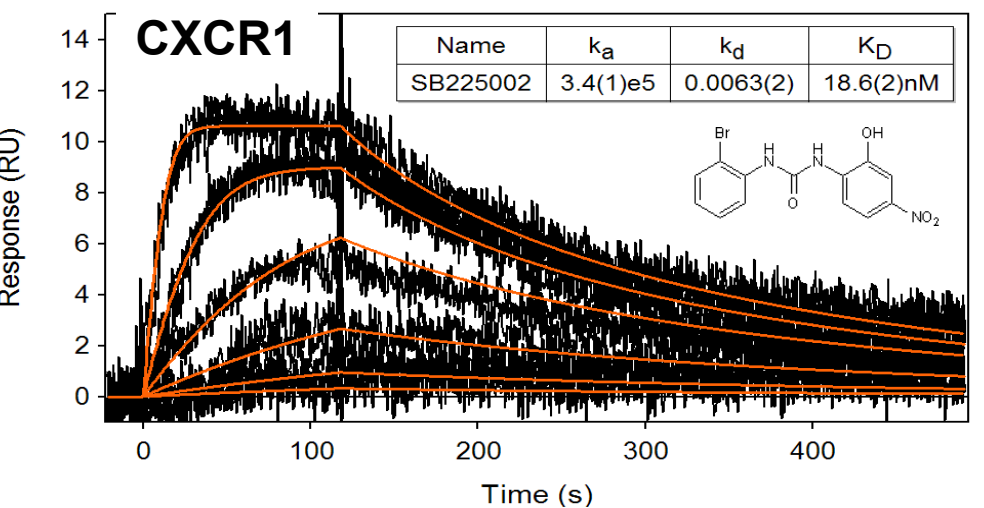
Wild type β_1 AR



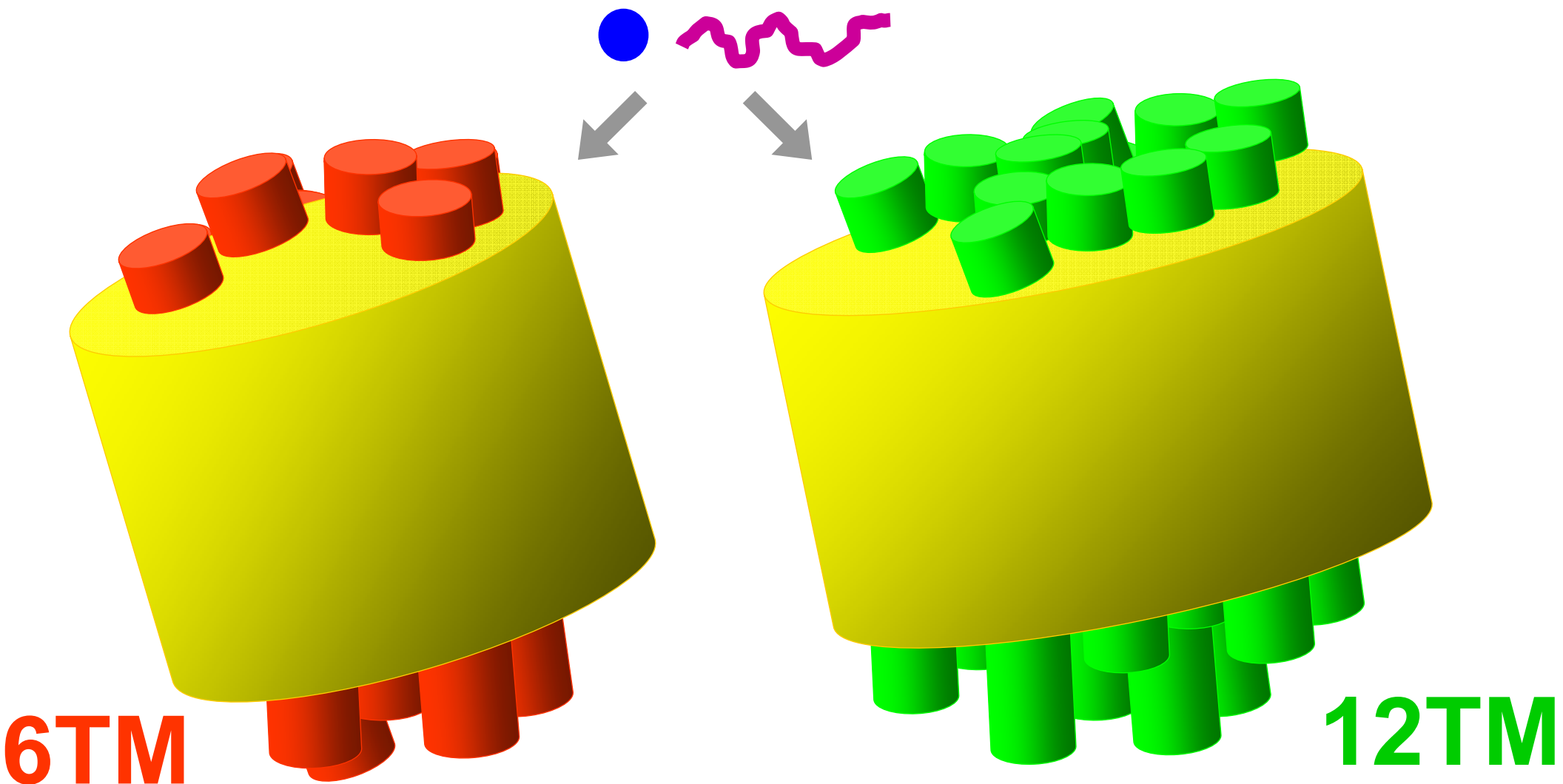
In progress

| | |
|--------------|--------------------------------|
| CXCR1 | β_2AR |
| CXCR2 | α_1AR |
| CXCR3 | α_2AR |
| CXCR4 | glucagon |
| CCR5 | glucagon-like |

Chemokine receptors

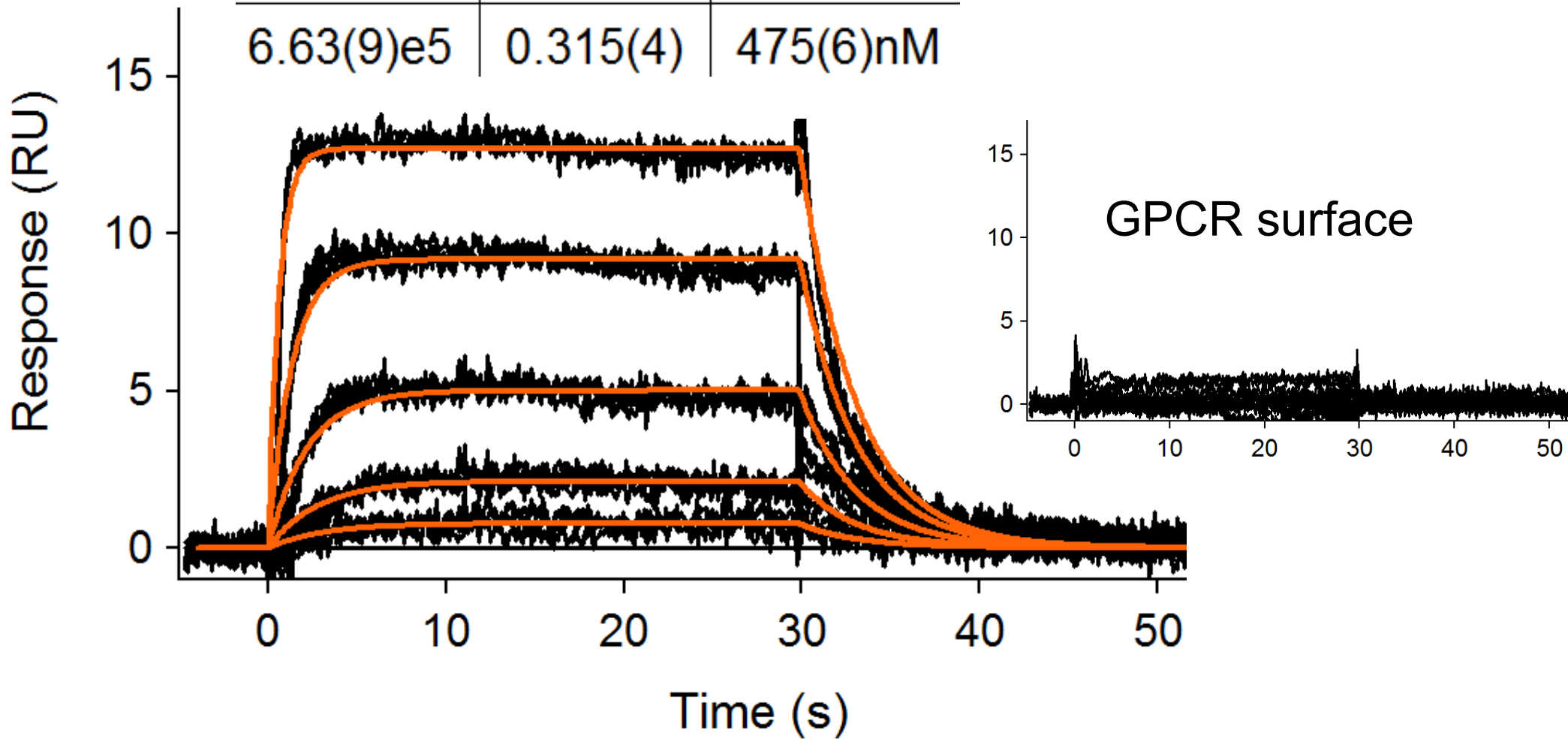


More Membrane Systems

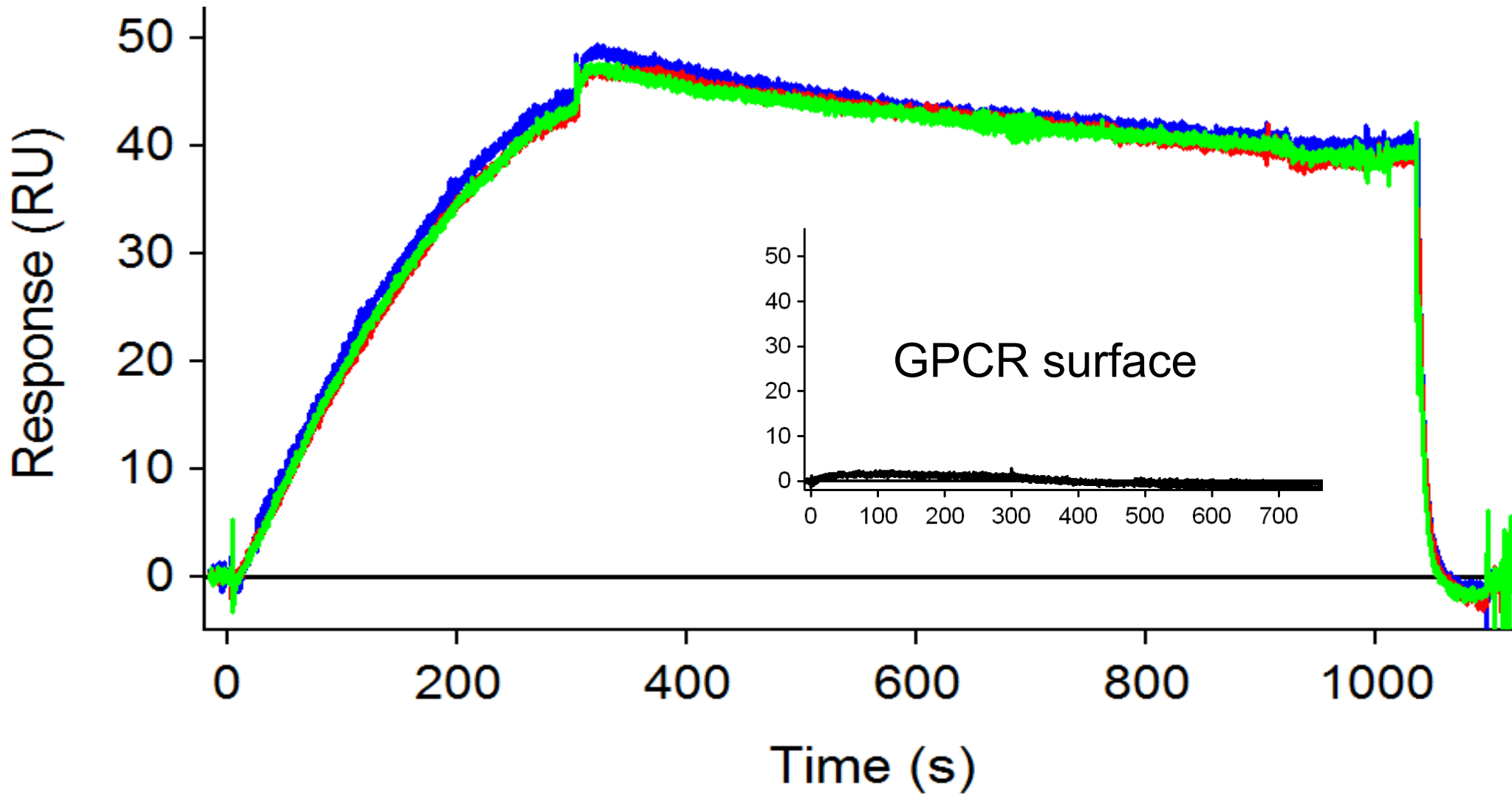


6TM / small molecule

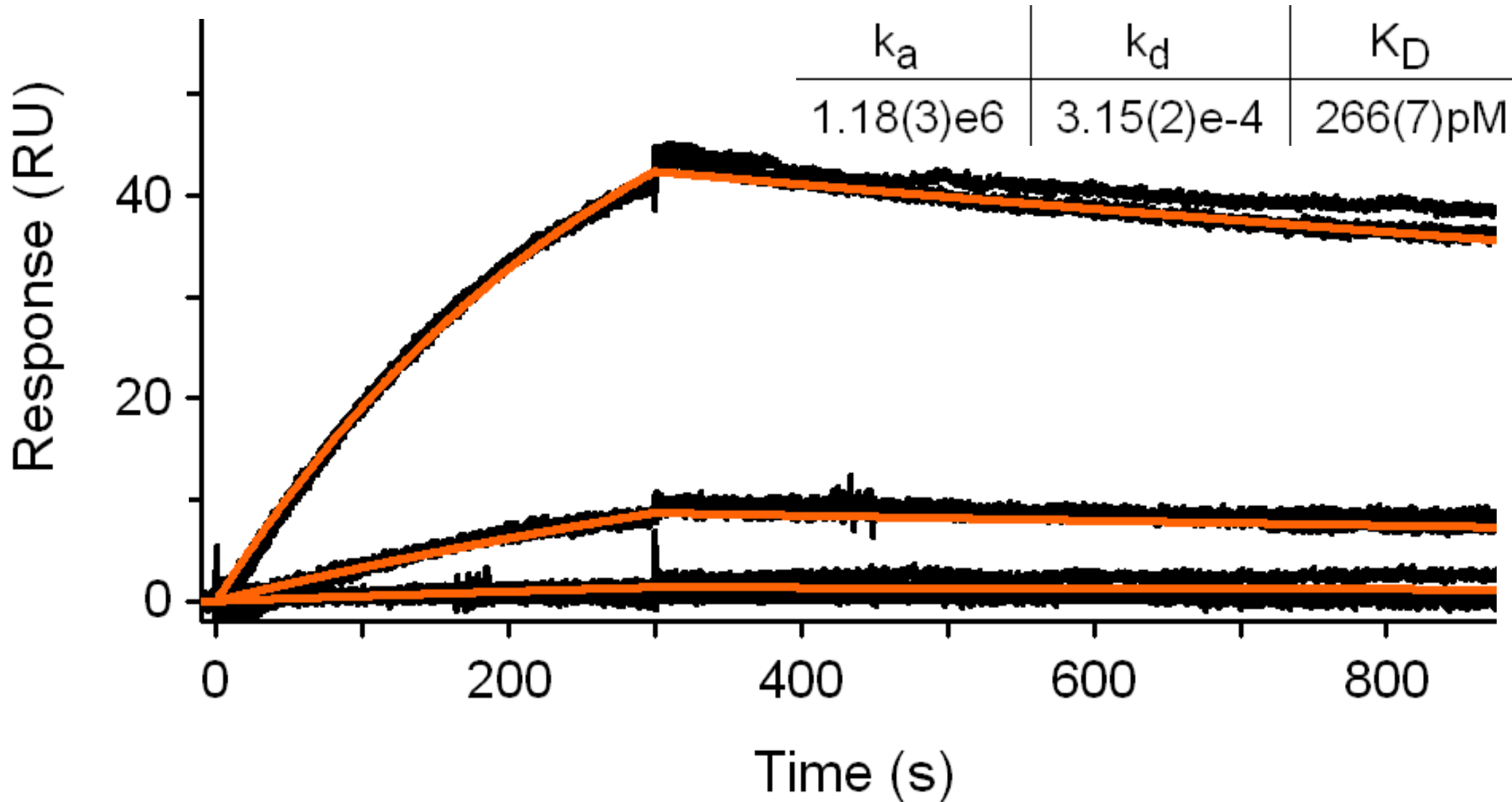
| k_a | k_d | K_D |
|-------------|------------|------------|
| $6.63(9)e5$ | $0.315(4)$ | $475(6)nM$ |



6TM/peptide



6TM/peptide



Summary

Test multiple targets at once

Identify selective hits

**Determine K_D s of hits
($<1 \mu\text{M}$ to $>>100\mu\text{M}$)**

~ 350 fragments/day

rrich@cores.utah.edu

