

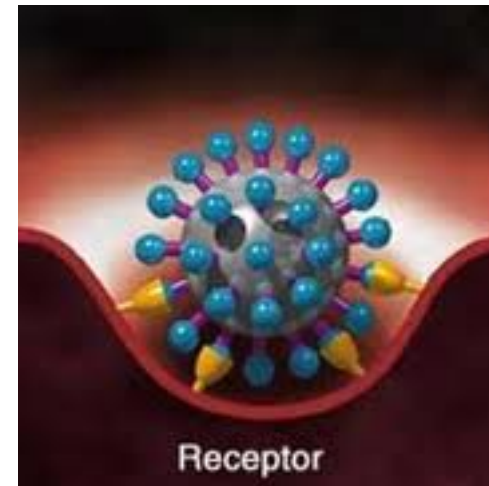
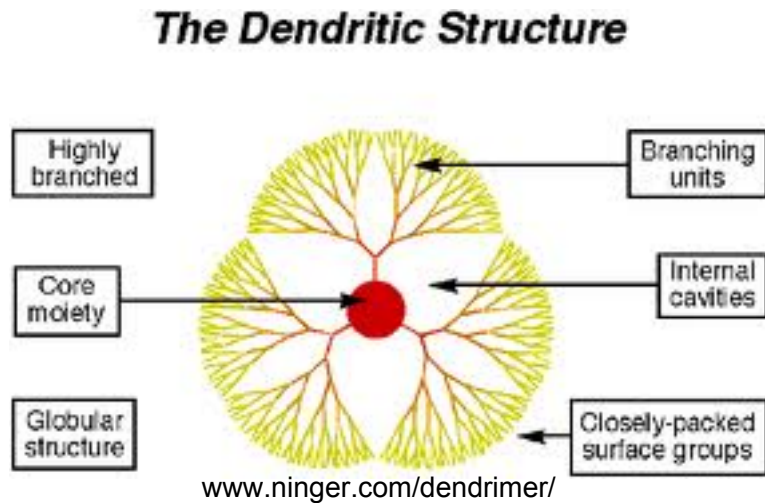
Riboflavin-Based Cell Targeting: Riboflavin Binding Protein Binding Studies

Andrew Harmon, Seok Ki Choi, Brad Orr
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Overview

- Background
 - Developing cell targeting nanoparticle
 - Multivalent Dendrimer - small molecule approach



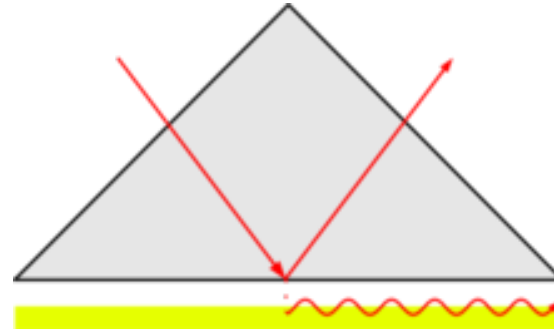
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- G5 (dendrimer) - RF drug complex

Overview - SPR

Surface Plasmon Resonance

- Tool: SPR
 - Mechanism



en.wikipedia.org/wiki/Surface_Plasmon_Resonance

Series S Sensor Chip CM5

The diagram shows a cross-section of the sensor chip. A yellow layer is on top of a grey substrate. Above the yellow layer is a matrix of red spheres connected by grey lines, representing the carboxymethylated dextran matrix. Below the yellow layer is a blue liquid layer.

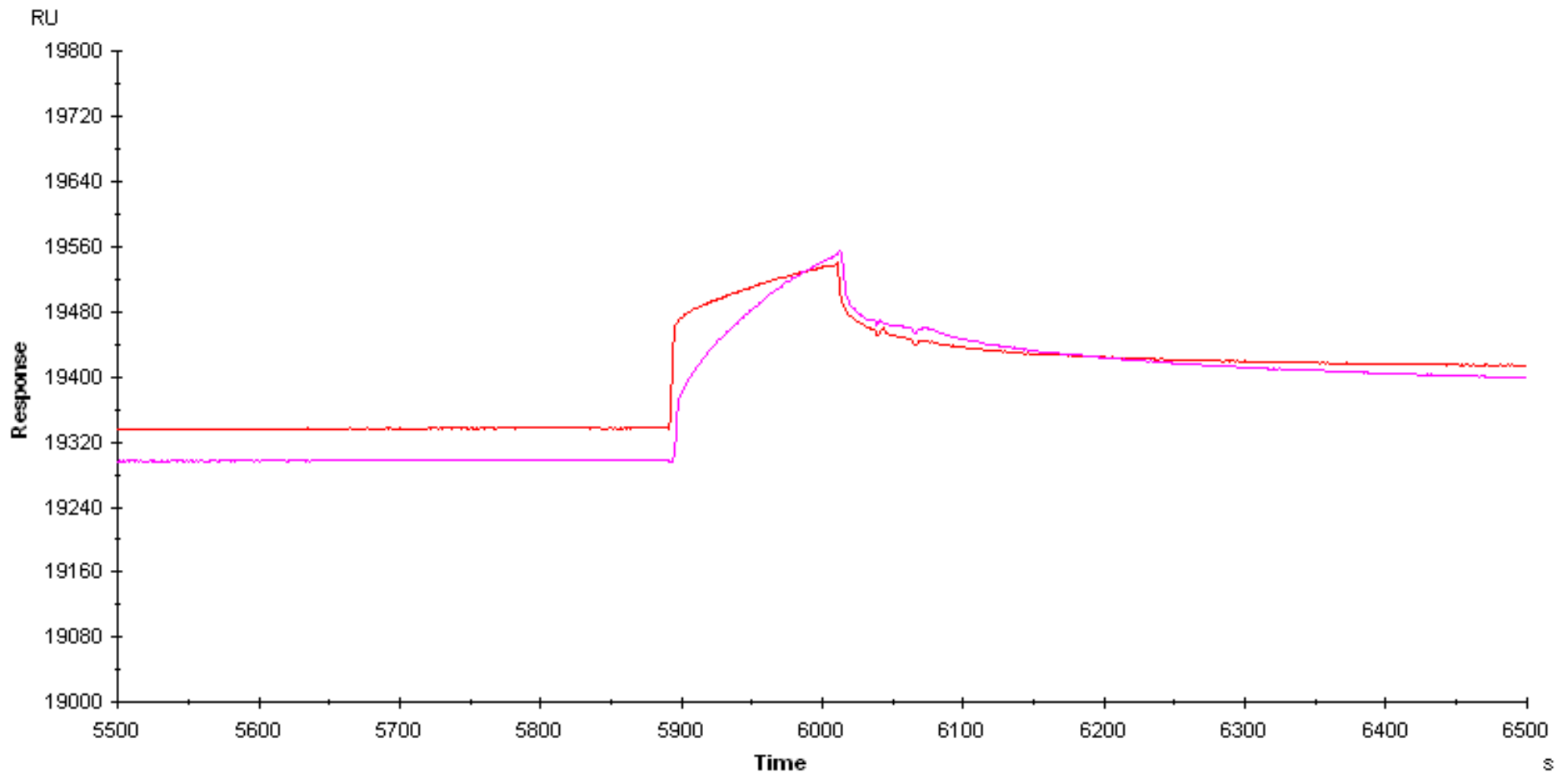
- Carboxymethylated dextran matrix
- The most versatile chip available
- Excellent chemical stability

BIACORE

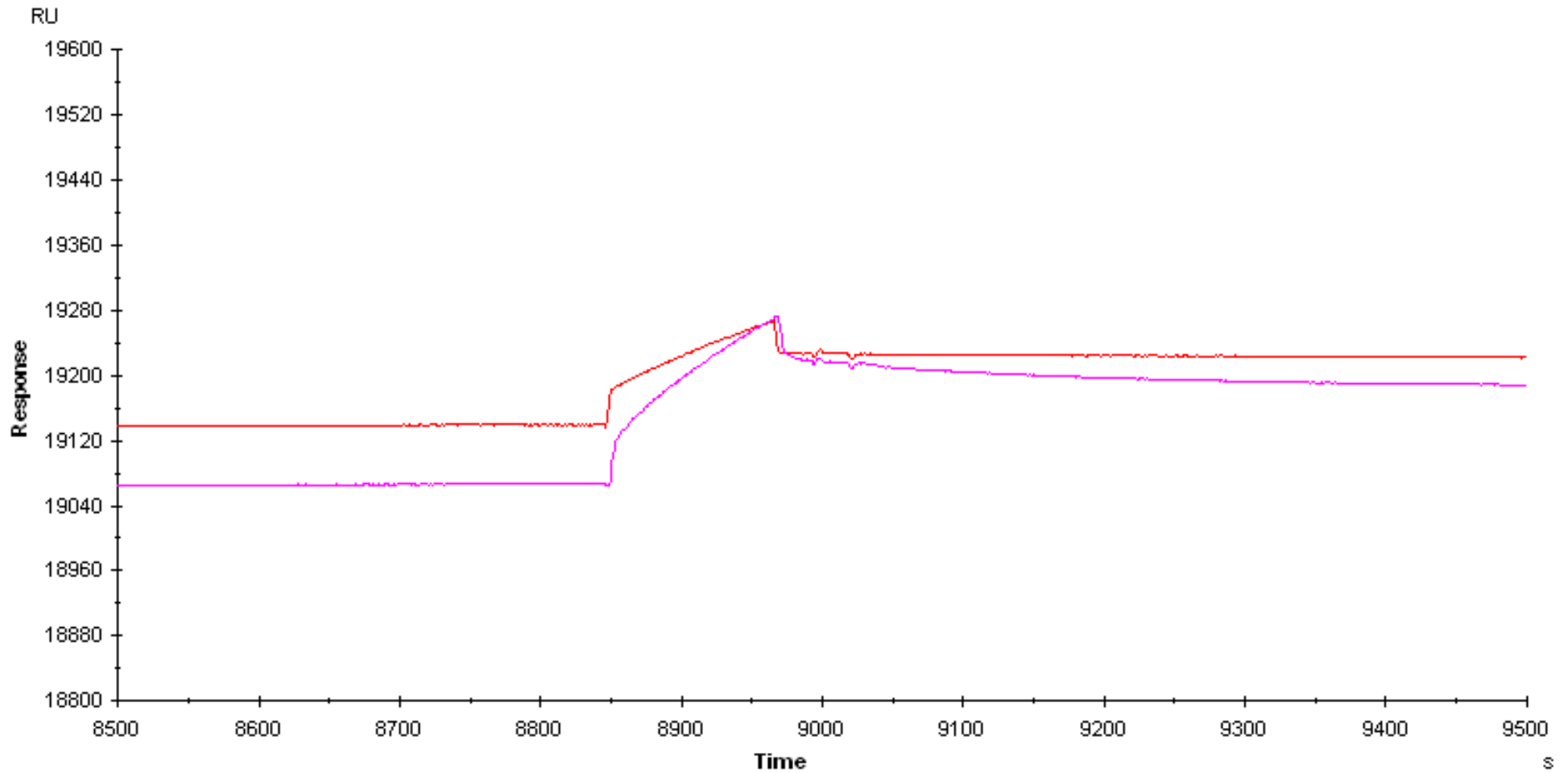
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RFBP and Competitive Binding SPR

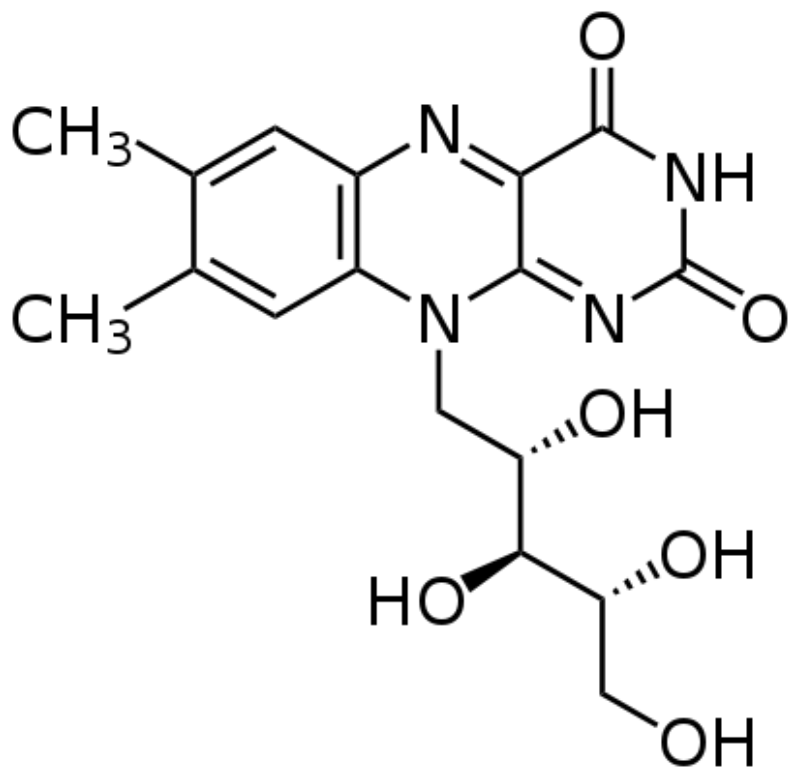
- RFBP competition with free RF □
 - RF in solution competing with RF linked to chip
- RFBP competition with RF mimics
 - RF mimics compete with RF linked to chip.



RFBP
RFBP at 11 μM

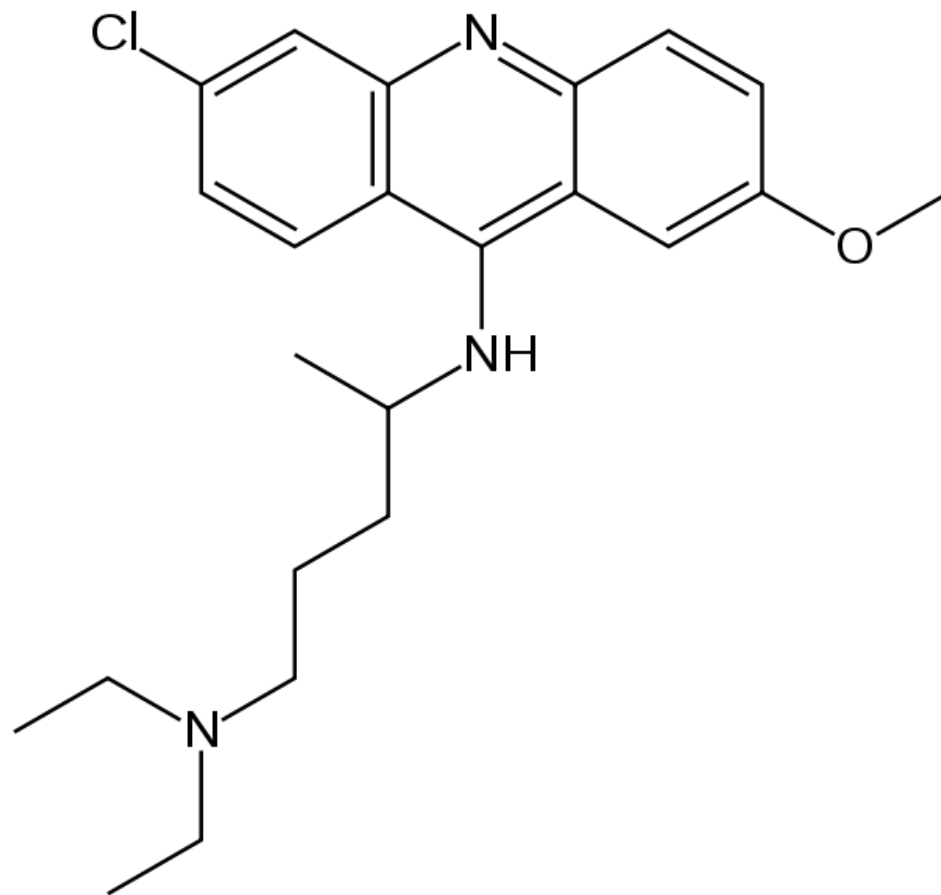


RFBP with free RF
RFBP at 5.5 μ M; RF at 6.7 μ M



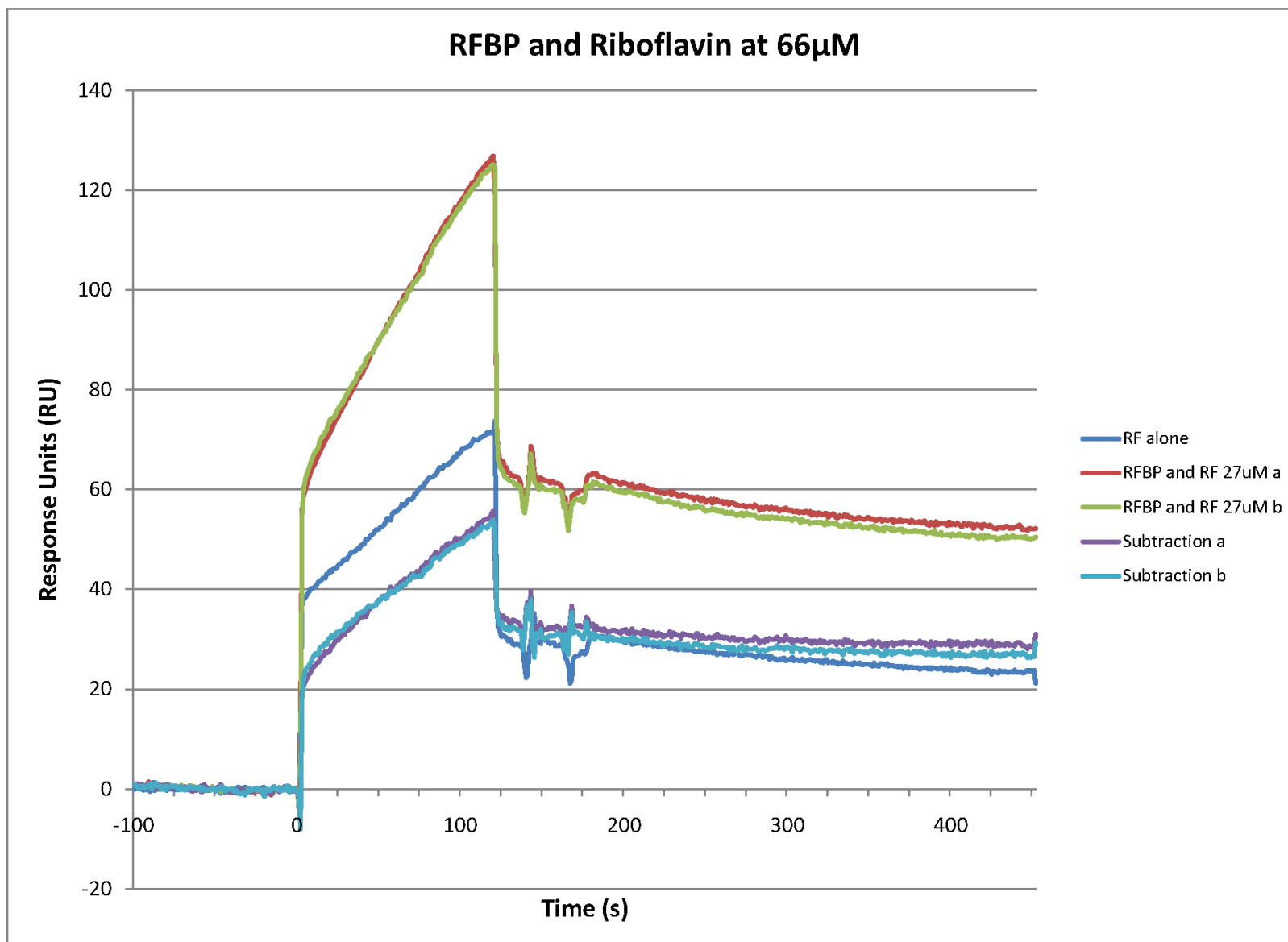
Riboflavin

Mimic chosen for aromatic similarity; also a known drug that signals apoptosis

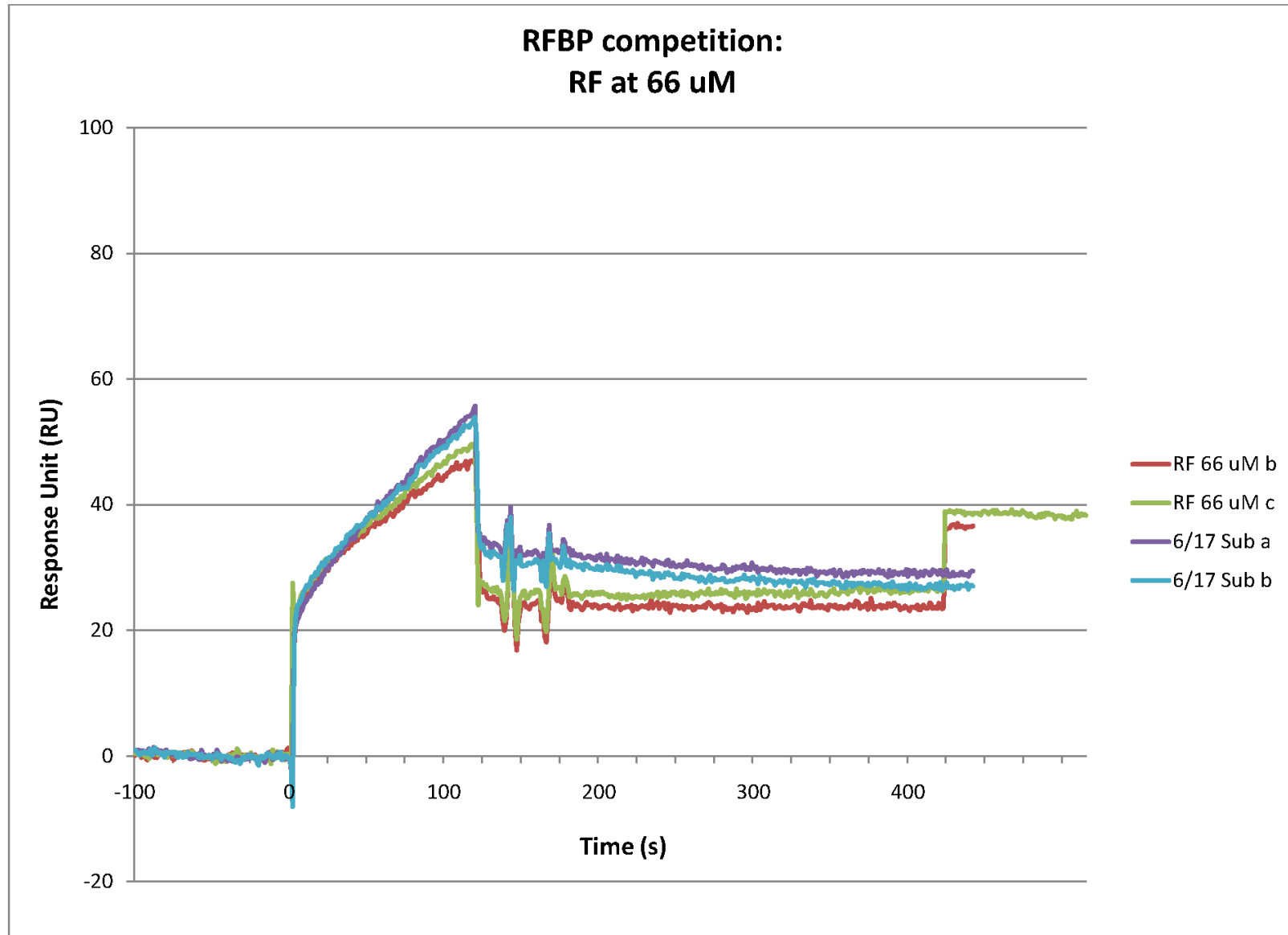


Quinacrine

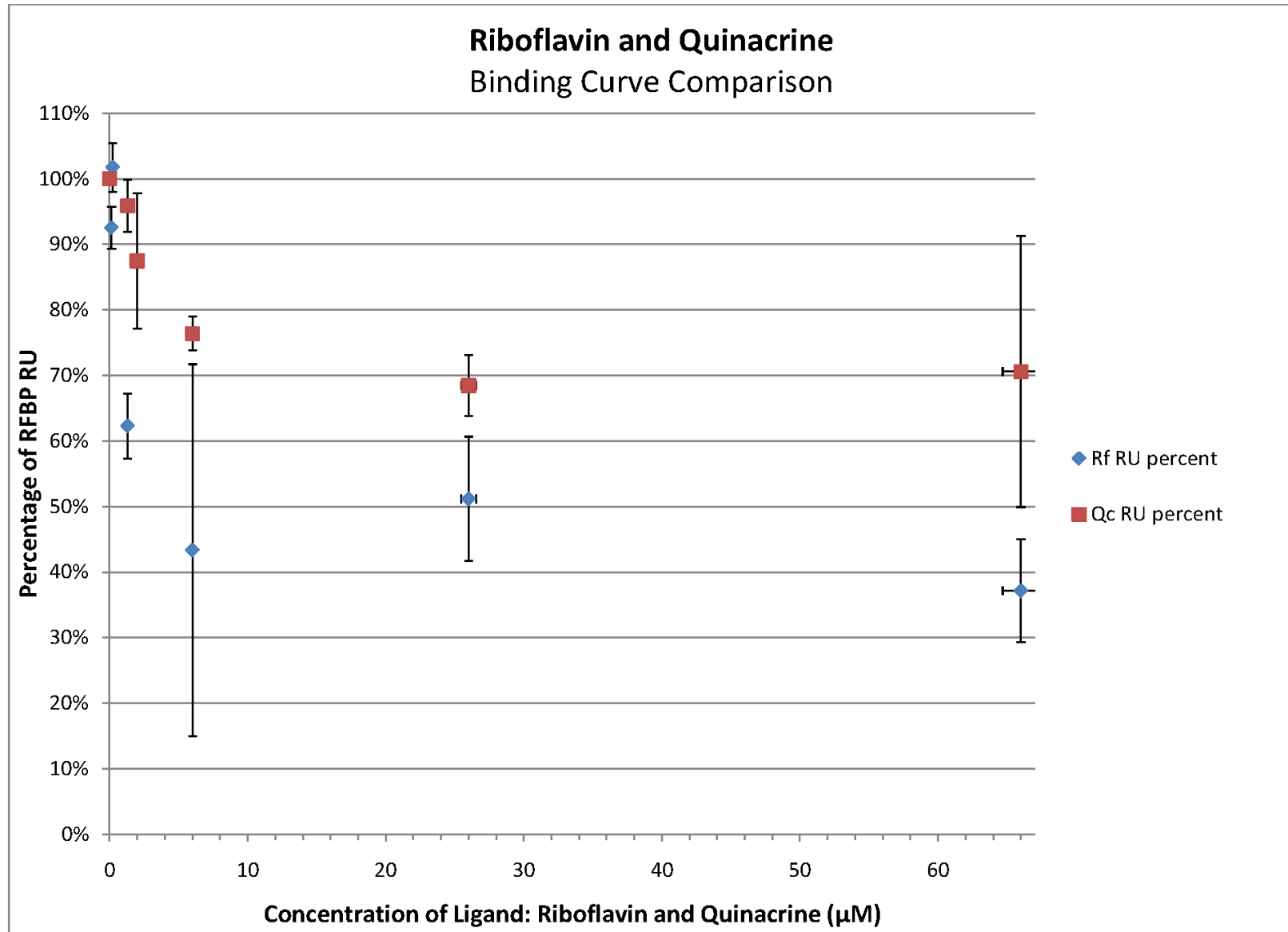
One concentration - working towards a binding curve



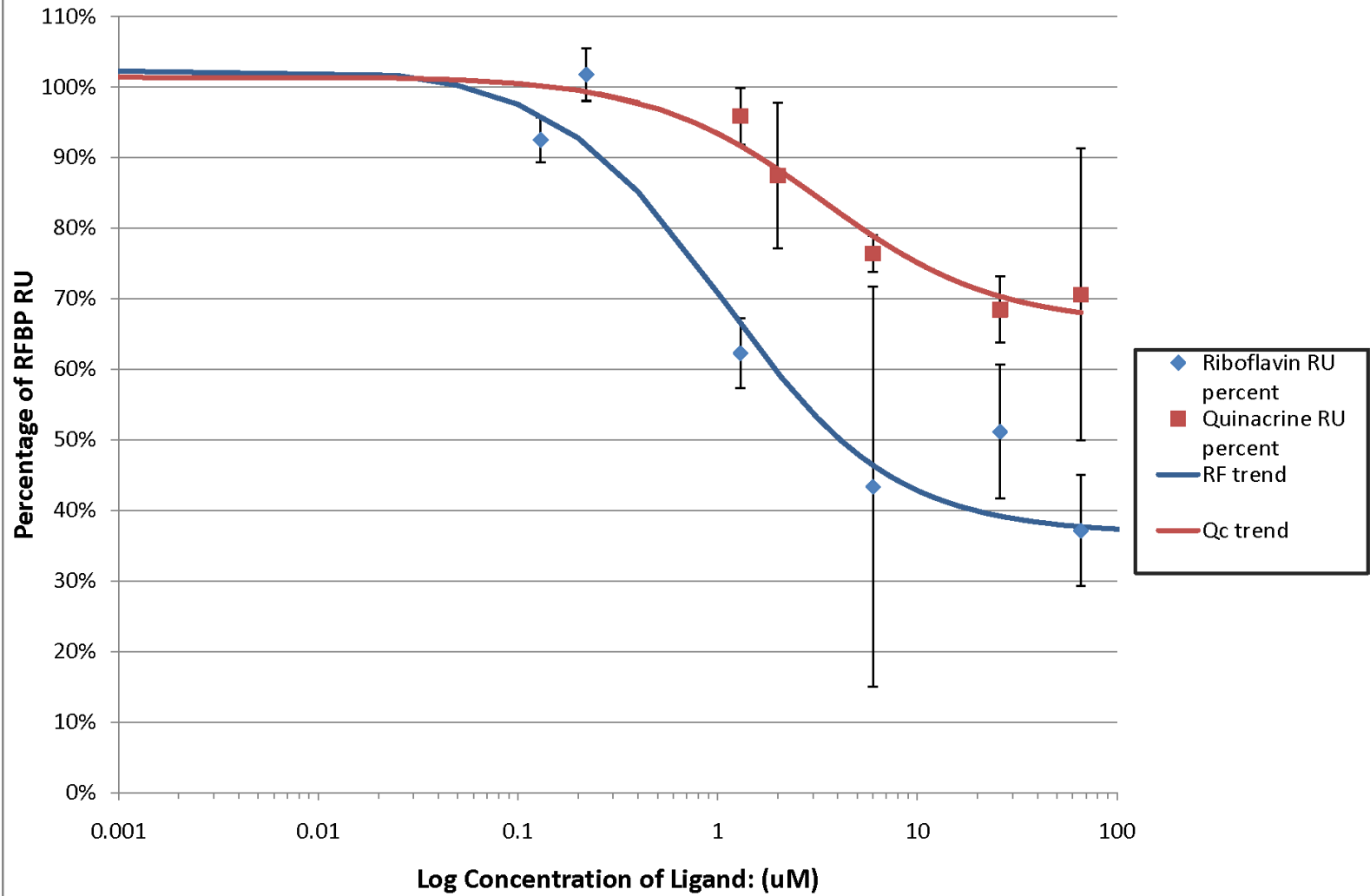
One concentration - working towards a binding curve after subtraction



Riboflavin Binding Studies: Riboflavin - RFBP competition



Riboflavin and Quinacrine Binding Curve Comparison - Logarithmic



Riboflavin Binding Studies

- IC_{50} □ Competition with RF linker for RFBP active site
 - RFBP - Riboflavin $IC_{50} \sim 1.05 \mu M$
 - RFBP - Quinacrine $IC_{50} \sim 28.0 \mu M$
- Quinacrine seems about one order of magnitude weaker binding (for this ligand-protein system)

Moving on

- Work on SPR system:
 - Immobilize RFBP on chip
- Multivalent system
 - dendrimer with RF (and drug?)
 - dendrimer with Qc
 - Quinacrine's 1 order of magnitude deficiency may be vastly outweighed by Multivalent binding cooperativity
 - Quinacrine has been shown as apoptotic inducer

Thank you all

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