Supplementary Material

Materials and Methods

Cloning

PrA nanobody sequences were codon-optimized for expression in *E. coli* and cloned into pCR2.1 after gene synthesis (Eurofins MWG Operon), incorporating BamHI and XhoI restriction sites at 5' and 3' ends, respectively. Nanobody sequences were then subcloned into a pET21-based vector containing a pelB leader sequence, using BamHI and XhoI restriction sites [1]. For constructs incorporating a C-terminal cysteine, the nanobody sequences were PCR amplified with primers encoding a cysteine residue immediately following the nanobody sequence.

PrA sequences were cloned by PCR from wild-type *S. aureus* protein A. Constructs comprised the PrA E domain (1xPrA), E and D domains (2xPrA), and E, D, A, and C domains (4xPrA).

Structural modeling

The structure of the LaP-1 nanobody was modeled using the I-TASSER web server [2; 3; 4], and was aligned to the Fab chain of the 1DEE structure [5]. The structure was visualized using PyMOL software (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC).

Protein purification

pelB-fused nanobodies were purified as previously described [1]. Briefly, proteins were expressed in Arctic Express (DE3) cells (Agilent), induced with IPTG at a final concentration of 0.1 mM. Cells were induced at an OD_{600} = 0.5-0.6 for 18-20 hours at 12°C, then pelleted by a 10 min spin at 5,000 x g. The periplasmic fraction was then isolated by osmotic shock [6]. This fraction was bound to His-Select nickel affinity resin (Sigma),washed with His wash buffer (20 mM sodium phosphate pH 8.0, 1 M NaCl, 20 mM imidazole), and eluted with His elution buffer

(20 mM sodium phosphate pH 8.0, 0.5 M NaCl, 0.3 M imidazole). The elution was then dialyzed into PBS.

Recombinant PrA was expressed in BL21 DE3 Gold cells, which were induced at OD_{600} = 0.5-0.6 with 1 mM IPTG, for 4 hrs at 30°C. Cells were centrifuged for 10 min at 5,000 x g, then resuspended in 20 mM HEPES pH 7.4 / 300 mM NaCl / 10 mM imidazole / 0.1 M PMSF. The cells were lysed in a microfluidizer, then centrifuged at 40,000 rpm for 45 minutes in a 50.2 Ti rotor. The supernantant was then bound to His-Select nickel affinity resin (Sigma),washed with PrA wash buffer (20 mM HEPES pH 7.4 / 0.3 M NaCl, 10 mM imidazole), and eluted with PrA elution buffer (20 mM HEPES pH 7.4 / 0.3 M NaCl / 0.3 M imidazole). The elution was then dialyzed into PBS.

K_D determinations

SPR measurements were obtained on a Proteon XPR36 Protein Interaction Array System (Bio-Rad). Recombinant 1xPrA, 2xPrA, and 4xPrA was immobilized on a ProteOn GLC sensor chip: the chip surface was first activated with 50 mM sulfo-NHS and 50 mM EDC, run at a flow-rate of 30 µl/min for 300 sec. The ligand was then diluted to 2-6 µg/ml in 10 mM sodium acetate, pH 5.0, and injected at 25 µl/min for 75 sec. Finally, the surface was deactivated by running 1 M ethanolamine-HCl (pH 8.5) at 30 µl/min for 300 sec. This led to immobilization of approximately 200-600 response units (RU) of ligand.

K_Ds of recombinant nanobodies were determined by injecting 4 concentrations of each protein, in triplicate, with a running buffer of 20 mM HEPES, pH 8.0 / 150 mM NaCl / 0.01% Tween. Proteins were injected at 25 µl/min for 640 sec, followed by a dissociation time of 900 sec. Between injections, residual bound protein was eliminated by regeneration with 4.5 M $MgCl_2$ in 10 mM Tris, pH 7.5, run at 100 µl/min for 90 sec. Binding sensorgrams from these injections

were processed and analyzed using the ProteOn Manager software. Binding curves were fit to the data with a Langmuir or two-state binding model, using grouped k_a, k_d, and R_{max} values.

Dynabead conjugations

IgG was conjugated to epoxy-activated magnetic Dynabeads (Life Technologies) as previously described [7]. Recombinant nanobodies were conjugated to epoxy-activated magnetic Dynabeads with minor modifications to published coupling conditions [1]; 10 µg of recombinant nanobody protein was used per 1 mg of Dynabeads, with conjugations carried out in 0.1 M sodium phosphate, pH 8.0 and 1 M ammonium sulfate, with an 18-20 hour incubation at 30°C.

Recombinant PrA nanobodies were conjugated to amine magnetic Dynabeads (Life Technologies) with N-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) as follows. Amine Dynabeads were washed two times and then resuspended in PBS / 1mM EDTA. SPDP (Thermo Fisher) was reconstituted in DMSO to 20mM and added to the resuspended beads. The beads were incubated for 30 min at room temperature, then washed once with PBS/1mM EDTA.

Recombinant PrA nanobodies with C-terminal cysteines were prepared at 1 mg/ml in PBS with 1 mM EDTA. Immobilized TCEP resin (Thermo Fisher) was equilibrated in PBS / 1mM EDTA, combined with the nanobody, and incubated at room temperature for 1 hr. The nanobody solution was then taken off the beads, added to the SPDP-activated Dynabeads, and incubated overnight at room temperature. After this incubation, beads were washed once with PBS and blocked with PBS / 5mM cysteine for 15 minutes at room temperature. This was followed by two 5 minute washes in PBS, a 5 minute wash in PBS/0.5% Triton, and finally a 15 minute wash in PBS/0.5% Triton.

PrA Binding Assay

PrA nanobodies were tested for affinity to PrA. Nanobodies were expressed in Arctic Express Cells (Agilent); cells were grown at 37°C until they reached OD_{600} 0.6-0.7, at which point they were induced with 1 mM IPTG at 18°C overnight. Cultures were then pelleted, and periplasmic fractions were obtained [6]. NaCl was added to 300 mM, and these fractions were then applied to PrA-coupled Sepharose resin (Life Technologies) equilibrated in PBS, and incubated at 4°C for 30 min. After incubation the resins were washed twice with 20 mM sodium phosphate, pH 7.4 / 500 mM NaCl. Bound protein was then eluted by boiling beads in LDS for 10 min. Samples from the periplasmic fraction, PrA-Sepharose flow-through, and elution were run on a gel, which was stained in Coomassie Blue.

Affinity isolation of PrA tagged protein complexes

Affinity isolations were performed according to previously published protocols with some modifications [7; 8]. Either 250 mg (Nup84-PrA, Cdc33-PrA) or 50 mg (RNA pol II) of cell powder was resuspended in the following binding buffers: Cdc33-PrA, 1 mL of 20mM HEPES pH 7.4 / 500mM NaCl / 0.1% Tween-20 / 0.5% Triton X-100 / 0.05% RNasin / 0.1 M PMSF / 3 µg/mL pepstatin A; Nup84-PrA, 2.25 mL of 20mM HEPES pH 7.4 / 500mM NaCl / 2 mM $MgCl_2$ / 0.1% CHAPS / 0.1 M PMSF/ 3 µg/ml pepstatin A / 1:500 PIC; RNA pol II, 500 µL of 20mM HEPES pH 7.4 / 150 mM NaCl / 0.1% Tween-20 / cOmplete EDTA-free protease inhibitor cocktail (Roche). For RNA pol II pullouts, the resuspended powder was sonicated for 10 sec. All lysates were centrifuged for 10 min at 14,000 rpm, and the supernatants were added to 10 µL of Dynabead slurries, previously washed 3 times in the corresponding binding buffer. The beads were incubated rotating at 4°C for 10 min. (Cdc33-PrA and RNA pol II) or 30 min. (Nup84-PrA). After incubation beads were washed three times with binding buffer.

For epoxy-conjugated Dynabeads, protein was eluted by incubating in LDS at room temperature (IgG beads) or 75°C (nanobody beads) for 10 minutes. For SPDP-activated LaP-1 Dynabeads, protein was eluted twice with 50 uL volumes of binding buffer containing 25 mM DTT, each time

shaking at room temperature for 10 min. LaP bead elutions were then pooled and TCA precipitated. After native elution, PrA beads were heated in LDS at 75°C for 10 min to elute remaining protein.

To determine affinity isolation yields, samples of resuspended lysate supernatants were taken before and after Dynabead binding. These were run on a 4-12% Novex Bis-Tris gel in MES running buffer (Life Technologies), and probed by Western blotting using mouse anti-GFP antibody (Roche). Band intensities were quantified using ImageJ software.

Supplementary Figure Legends:

Supplementary Figure 1. LaP affinity for PrA or TAP. (A) Four candidate LaP nanobodies were expressed in bacteria, and periplasmic extracts were incubated with PrA-Sepharose. For each nanobody, input (I), flow-through (F), and elution (E) samples from the Sepharose binding were run by SDS-PAGE and Coomassie-stained. (B) The 2xLaP-1 dimer or rabbit IgG were conjugated to epoxy-activated magnetic beads and used to isolate *S. cerevisiae* Nup53-PrA or Nup53-TAP. Contaminating IgG heavy chain (HC) and light chain (LC) or 2xLaP-1 are labeled.

Supplementary Figure 2. SPR sensorgrams are shown for injections of multiple concentrations of LaP-1 or 2xLaP-1 nanobody over immobilized 1xPrA, 2xPrA, or 4xPrA. Curves fit from a Langmuir model (2xLaP-1) or two-state model (LaP-1) are shown in black.

References:

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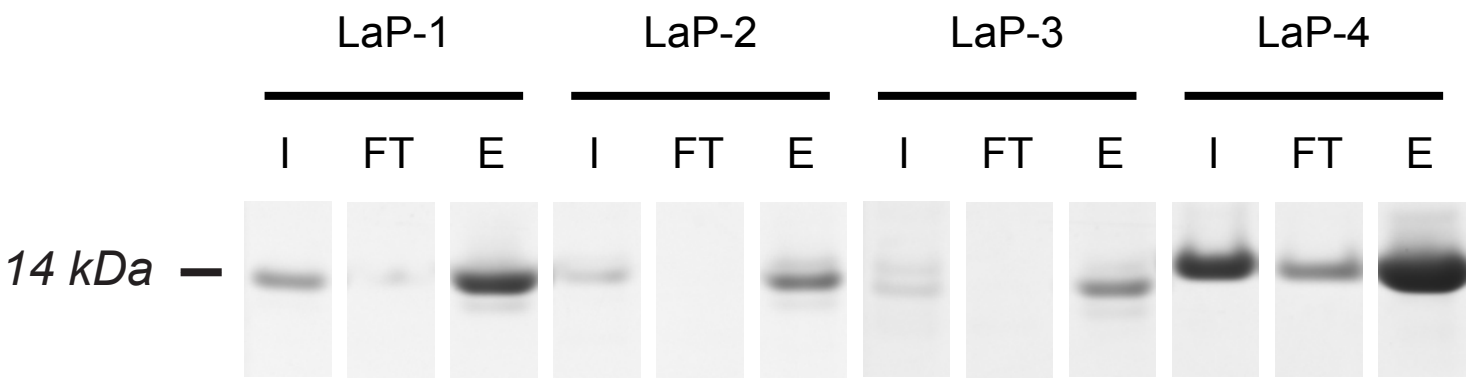
A



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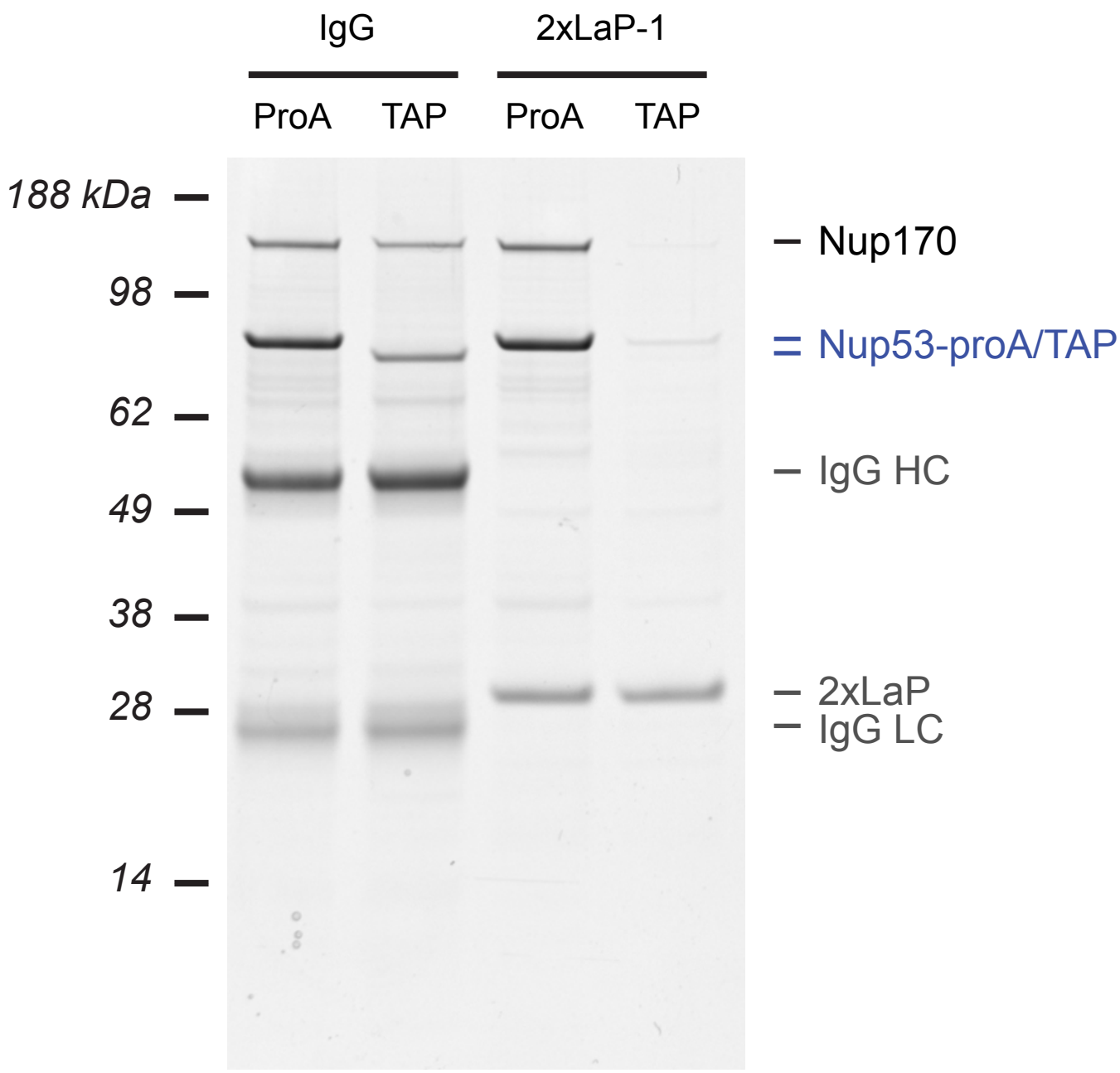
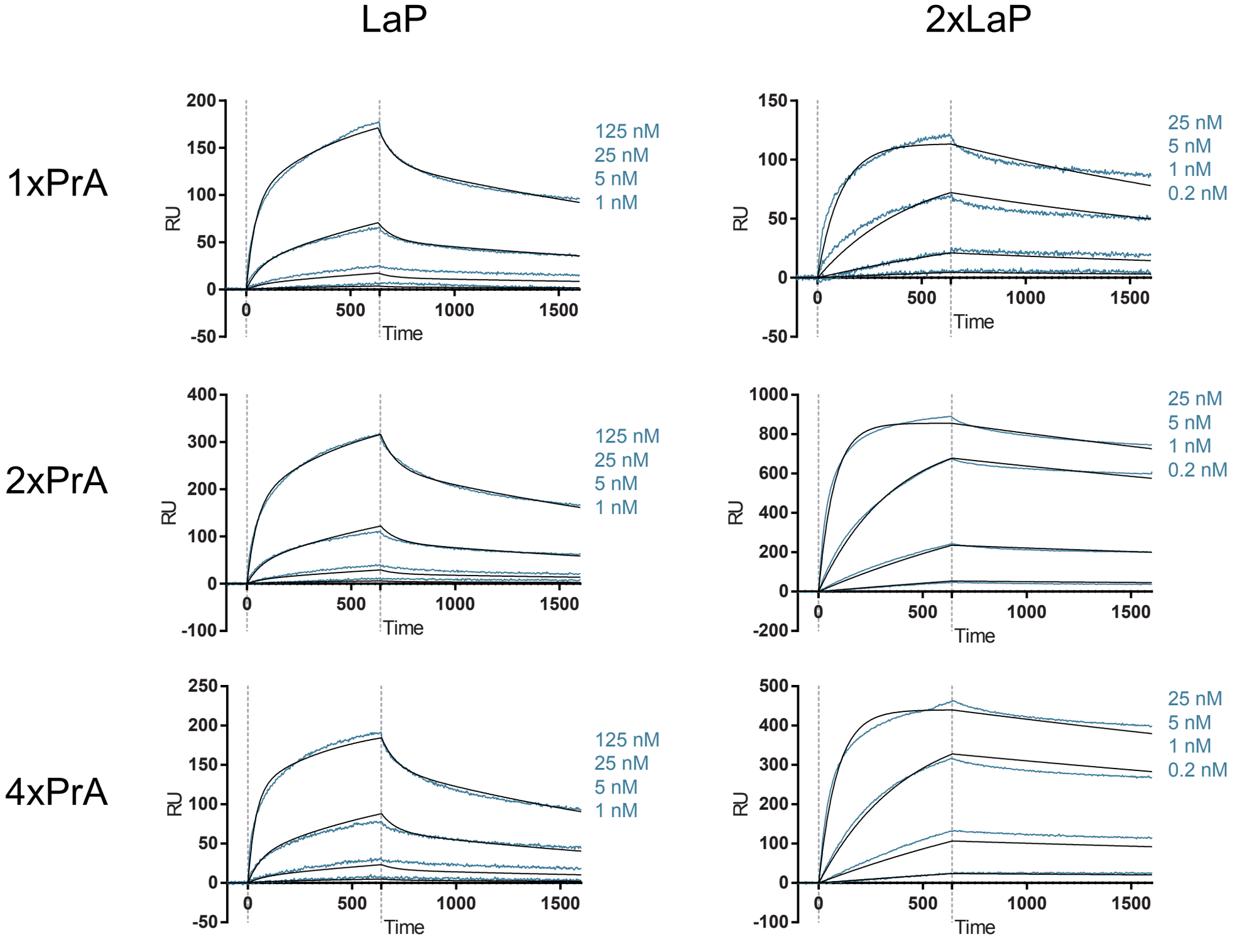


Supplementary Fig. 1



Supplementary Fig. 2