

Structure and Function: Ubiquitination of NEMO and Optineurin

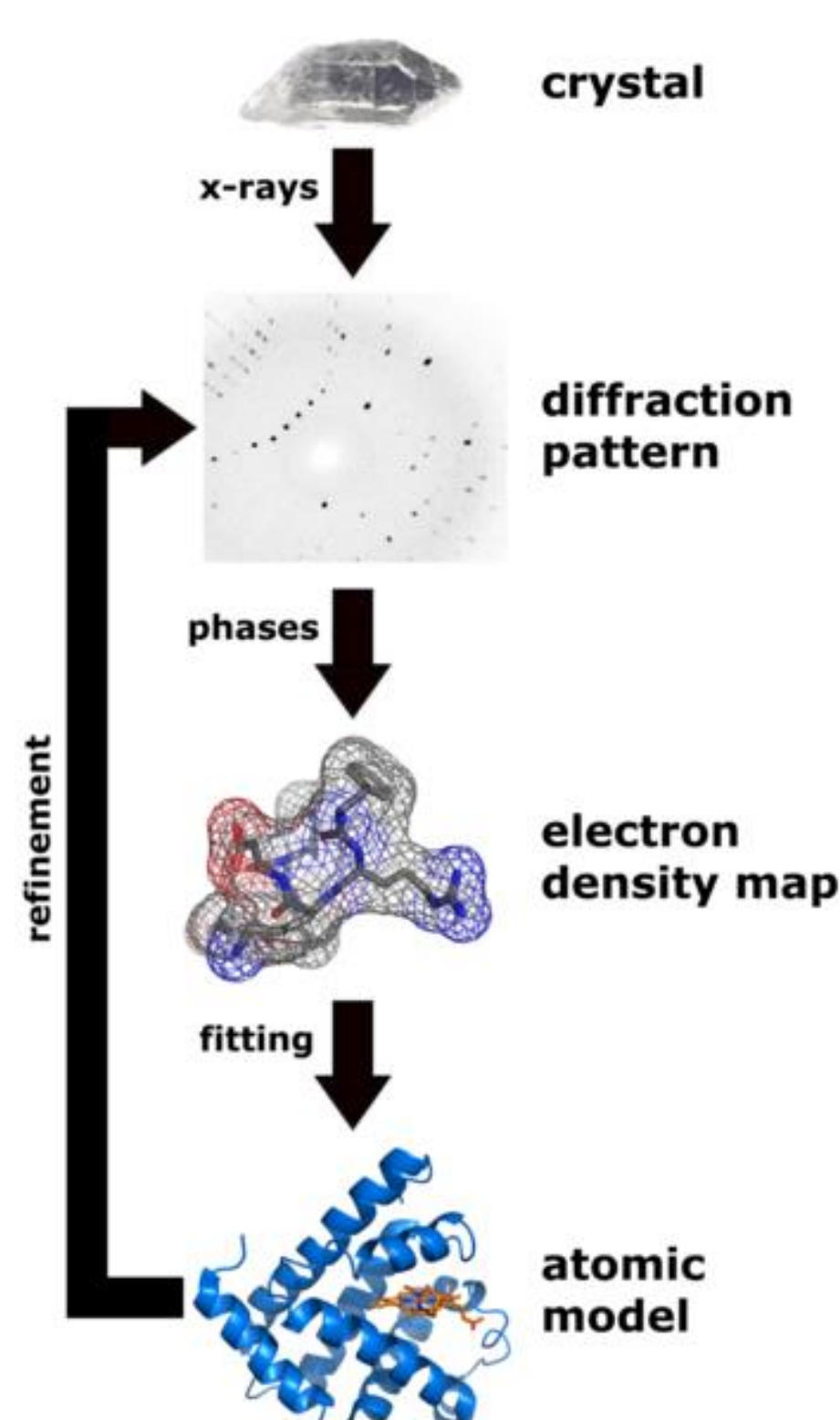
Introduction

- NF- κ B-essential-modulator (NEMO) and Optineurin (OPTN) are two proteins which rely on Ubiquitin to carry out the degradation of other macromolecules
- NEMO, a regulatory subunit, is ubiquitinated, beginning a signal pathway leading to transcription of immune-response genes
- OPTN binds to ubiquitinated substrates, leading to autophagy of degraded proteins or structures (prominently in nervous tissue)
- Mutations (OPTN and Ubiquitin), phosphorylation, and oligomerization state (Ubiquitin) have been shown to impact protein ubiquitination affinity
- The purpose of this project was to determine the effects of such variations on ubiquitination affinities, and explain effects structurally (via X-ray crystallography)

Keywords: NEMO, OPTN, Ubiquitin.

Methods

- Transform *E. coli* mutants with plasmid encoding for protein of interest, GST or His tag, antibiotic resistance (*lac* operon)
- Grow *E. coli* in LB + Antibiotic media, scale up to several liters (37 ° C)
- Express protein overnight with IPTG (25 ° C)
- Spin down cells, lyse
- Attach tag-binding GS4B or TALON substrates to protein tags
- Purify and concentrate (Nickel columns, FPLC, Nanodrop, SDS-PAGE)
- Crystallize and collect structural data (X-ray diffraction affinity data Resonance)



Results

OPTN

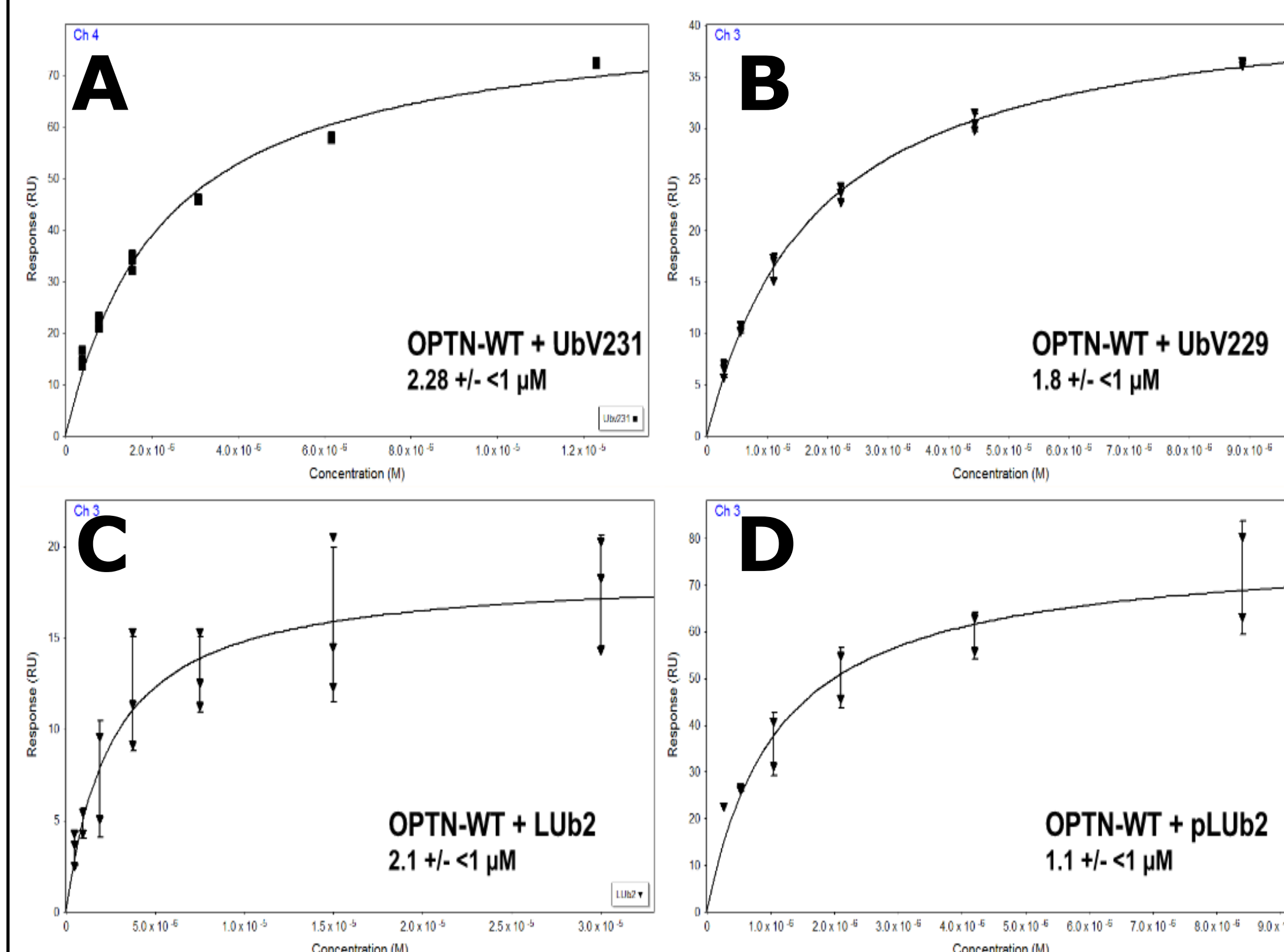


Figure 1. Wild-type Optineurin (OPTN) binding affinity with mono-ubiquitin mutants (UbV) and linear di-ubiquitin dimer (LUb2). Bottom-right numbers correspond to dissociation constants. A-B, C-D differences are significant. Data acquired using Biopix Surface Plasmon Resonance machine. Error bars represent standard deviation. For each concentration, N = 3.

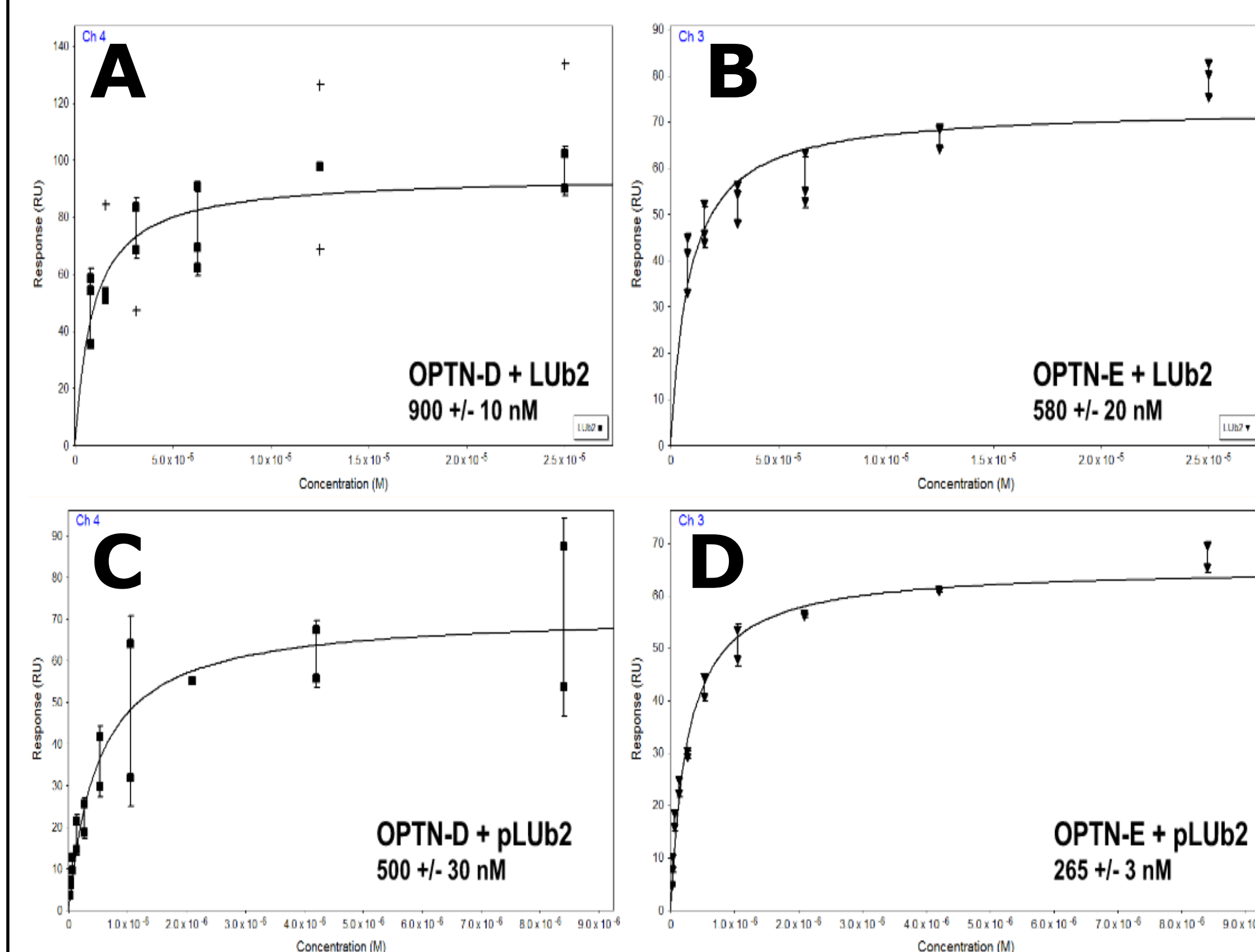


Figure 2. OPTN Mutants (S473D, S473E) binding affinities with phosphorylated and un-phosphorylated LUb2 dimer. Bottom-right numbers correspond to dissociation constants. All differences are significant, except B-C. Data acquired using Biopix Surface Plasmon Resonance machine. Error bars represent standard deviation. For each concentration, N = 3.

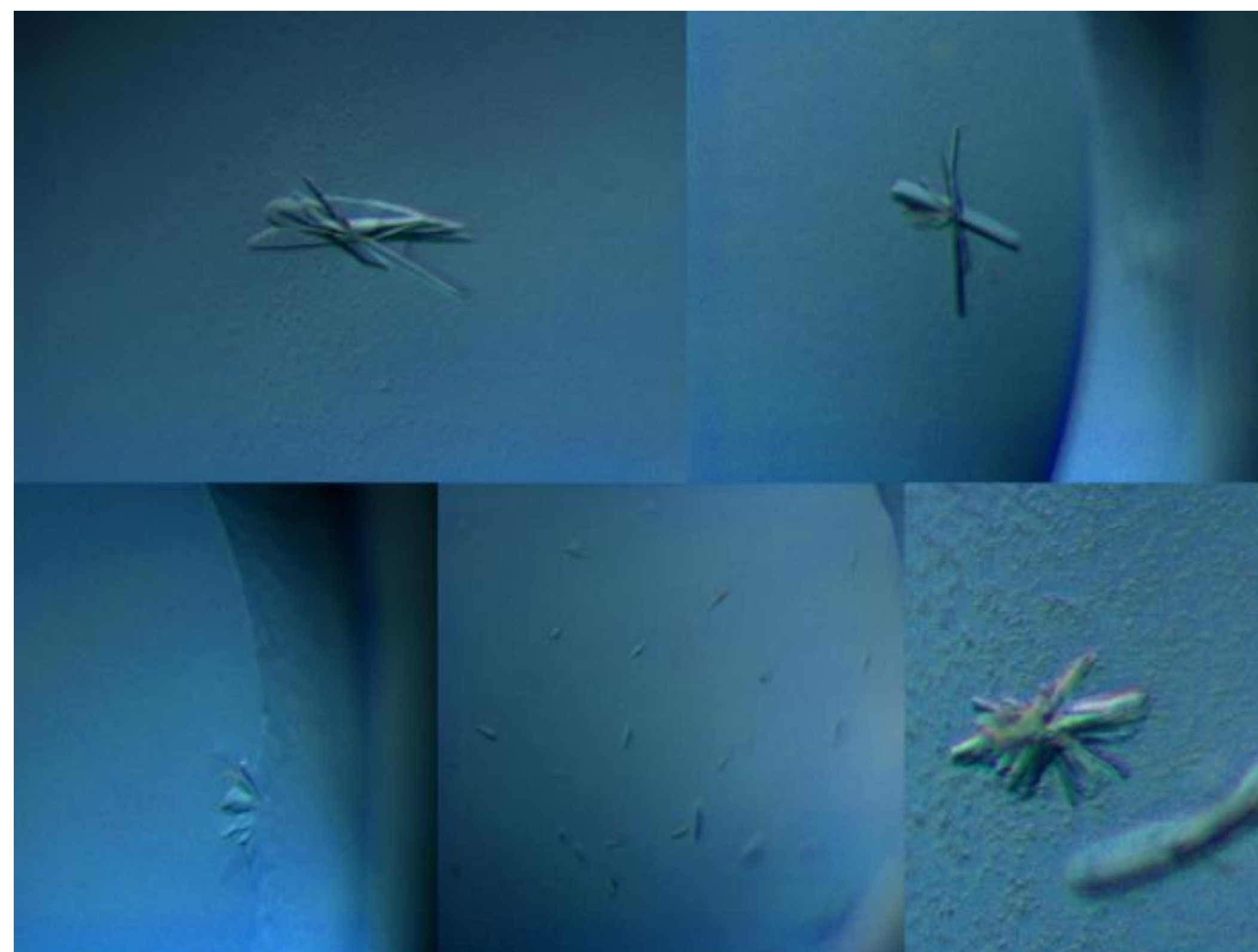


Figure 3. OPTN-S473D + LUb2 crystals. Crystals were acquired via 1:1 molar co-crystallization in 96-well plates, using the Art Robbins Crystal Gryphon machine, and 1-week incubation in 20 ° C conditions.

- Collected 1 X-ray diffraction dataset on OPTN-S473D + LUb2 at $< 3 \text{ \AA}</math>$

NEMO

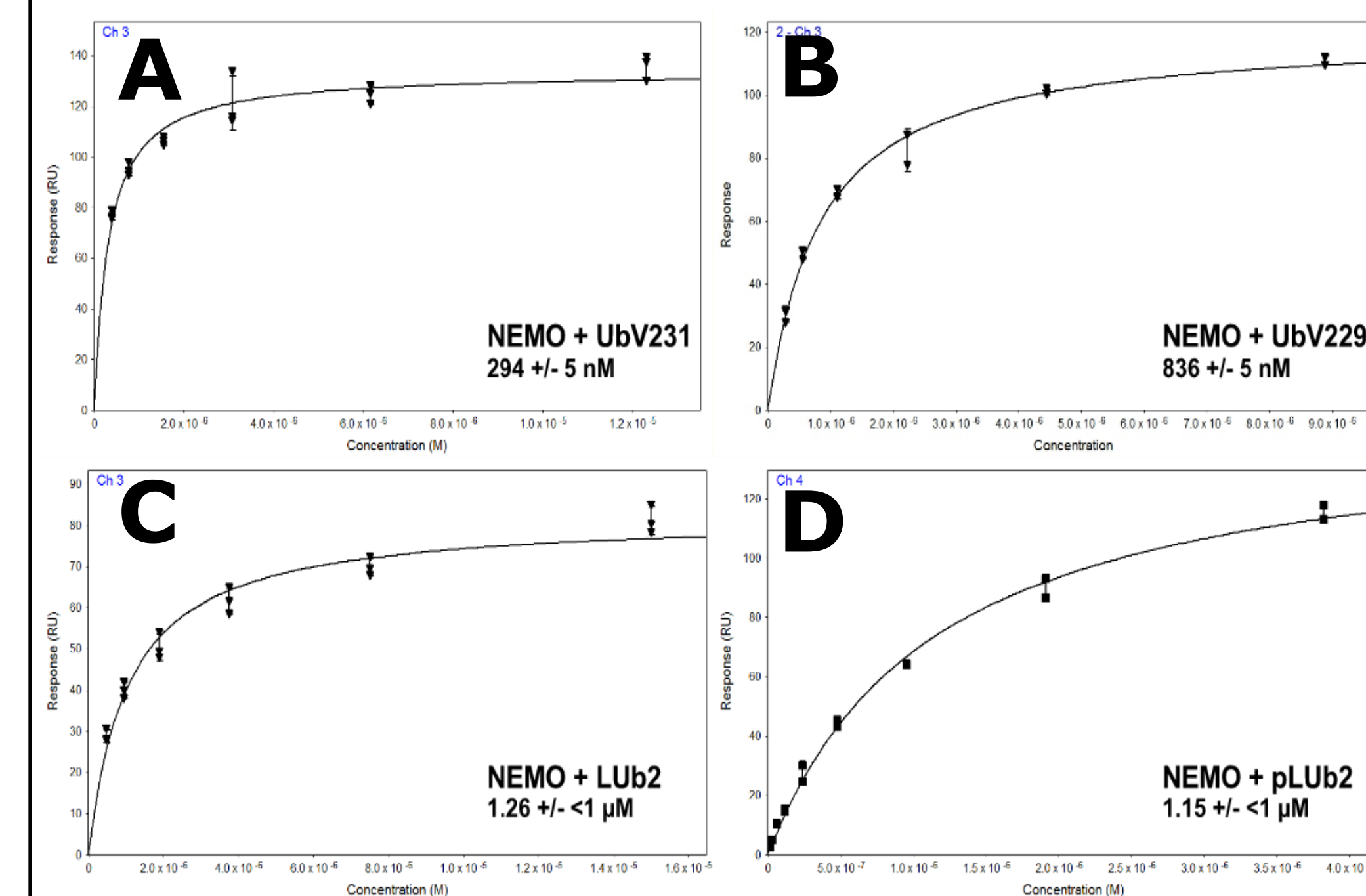


Figure 4. NEMO binding affinity with UbVs and LUb2. Bottom-right numbers correspond to dissociation constants. All differences are significant, except C-D. Data acquired using Biopix Surface Plasmon Resonance machine. Error bars represent standard deviation. For each concentration: N = 3.



Figure 5. NEMO + UbV 229 crystals. Crystals were acquired via 1:1 molar co-crystallization in 96-well plates, using the Art Robbins Crystal Gryphon machine, and 1-week incubation in 20 ° C conditions.

Conclusions

- OPTN mutants bind to LUb2 with higher affinity than WT
 - Negative charges on Asp and Glu are "phospho-mimetic"
- Phosphorylation of LUb2 enhances binding affinity in OPTN-WT and mutants, but not in NEMO
- Mono-ubiquitin mutants show higher affinity to NEMO than LUb2, also bind to OPTN
- OPTN-S473D + LUb2 structure will be first published structure involving OPTN mutant
 - OPTN-WT structure not yet published
- Crystals not obtained for OPTN-S473E + LUb2, nor NEMO + UbV 231

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