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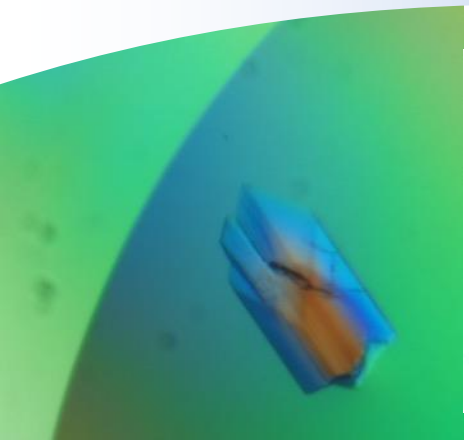
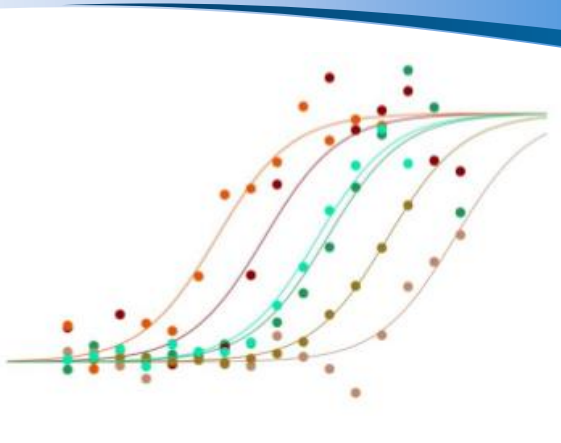
a WuXi AppTec company

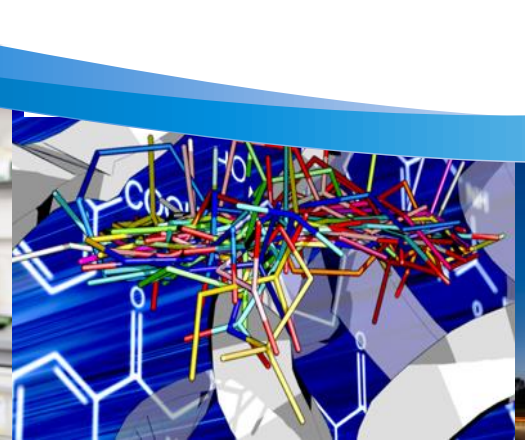
JEDI Program/JDI01

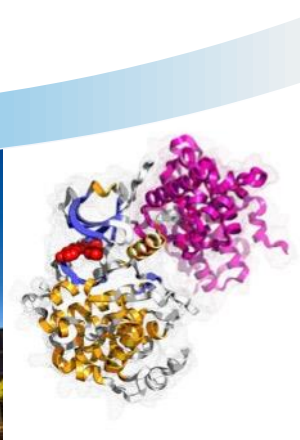
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

Nsp12 - TRIC Based 12-Point K_D Validation

September 16, 2021





Status

- TRIC (Dianthus)

  12-pt K_D validation on RED-TRIS NTA 2nd gen. labelled nsp12 was performed with the 8 most promising hits from 8-pt screen. Compounds were measured from 250 µM, showing the following results:

  - Two weak binders were identified: nsp12S5746081 and nsp1295277234
  - Three compounds are non-binders
  - Three compounds do not show binding, but lead to aggregation

TRIC (Dianthus)

Nsp12 (DVT1, PC13929-1)

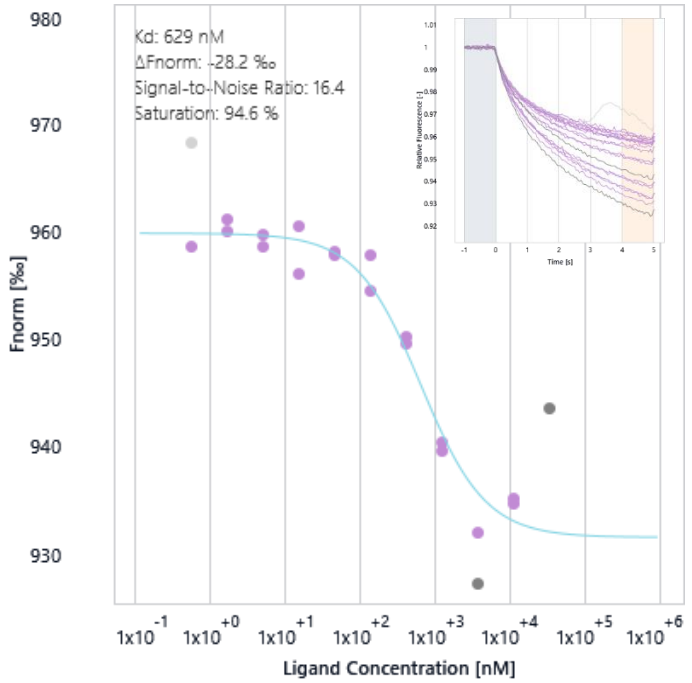
vs. 8 Hits

TRIC Screening Conditions:

Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	12.5 nM RED-TRIS NTA 2nd gen.		
Labelling conditions	Labelling buffer: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM MgCl2, 2.5 mM DTT, 0.005% Tween 25 nM protein / 12.5 nM dye Incubation time: 60 min Centrifugation: 10 min at 15000g		
Preparation steps	Dilute Fragments with the Integra Viaflow Head (12-Point dilution series) Spotting of 500 nl compound with Mosquito into Dianthus Assay plate Buffer/labelled protein were added using Integra Pipettes and mixed with BioShake 3000 elm		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 24 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM MgCl2, 2.5 mM DTT, 0.005% Tween DMSO: 2.5 %		
Titrant	8 Hits Suramin	See list	250 µM – 1.41 nM (12 concentrations) 100 µM – 0.56 nM (12 concentrations)

Compounds
JDI-38
JDI-44
JDI-49
JDI-80
JDI-88
JDI-92
JDI-137
JDI-147

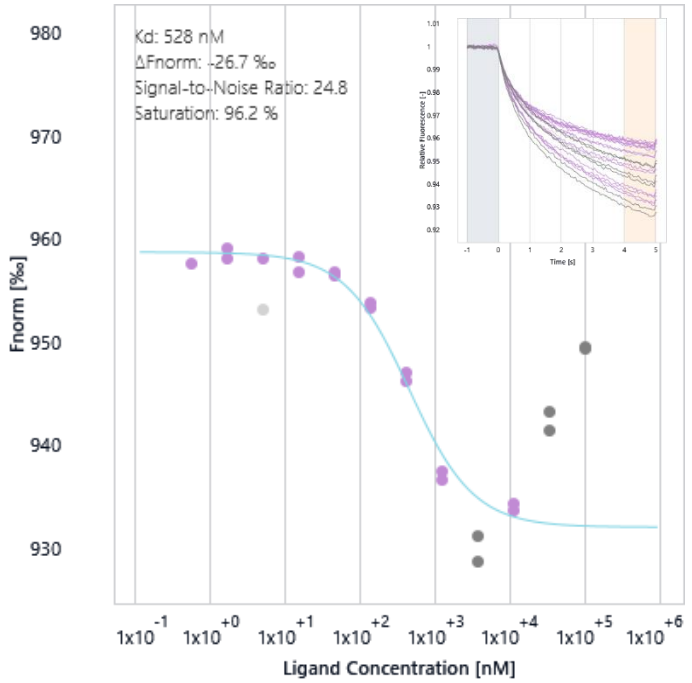
RED-TRIS NTA 2nd gen. labelled nsp12 vs. Suramin (Detector 1)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	Suramin	6.3E-07	4.2E-07	9.4E-07	-28.2	16.4	5	

Suramin binds to RED-TRIS NTA 2nd gen. labelled nsp12 with estimated K_D value of 629 nM and stable ΔFnorm and signal-to-noise over the entire assay plate.

RED-TRIS NTA 2nd gen. labelled nsp12 vs. Suramin (Detector 2)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	Suramin	5.3E-07	4.1E-07	6.9E-07	-26.7	24.8	5	

Suramin binds to RED-TRIS NTA 2nd gen. labelled nsp12 with estimated K_D value of 528 nM and stable ΔFnorm and signal-to-noise over the entire assay plate.

RED-TRIS NTA 2nd gen. labelled nsp12 vs. weak binder

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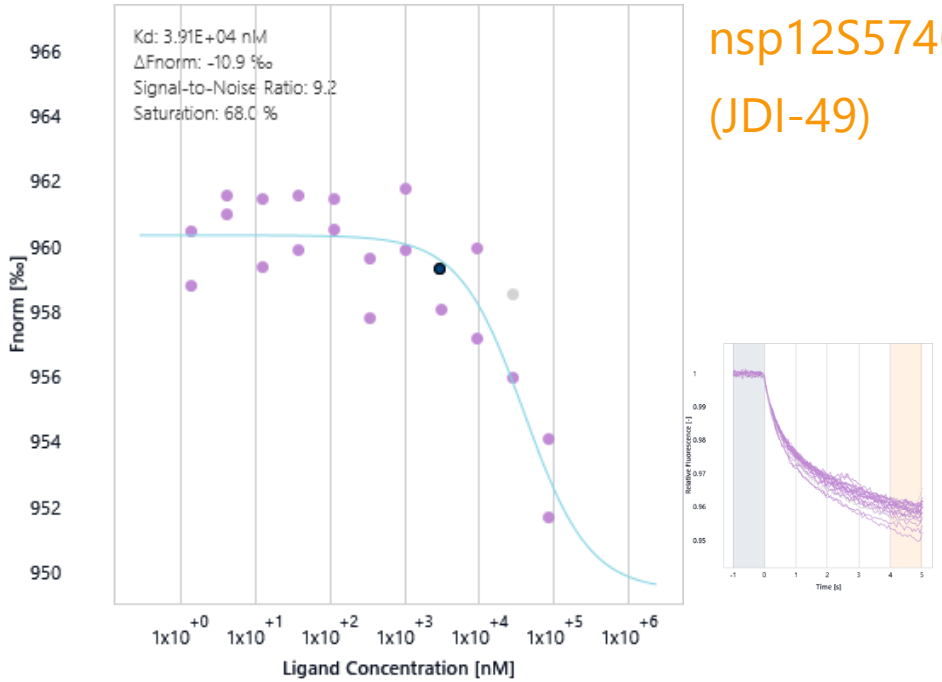
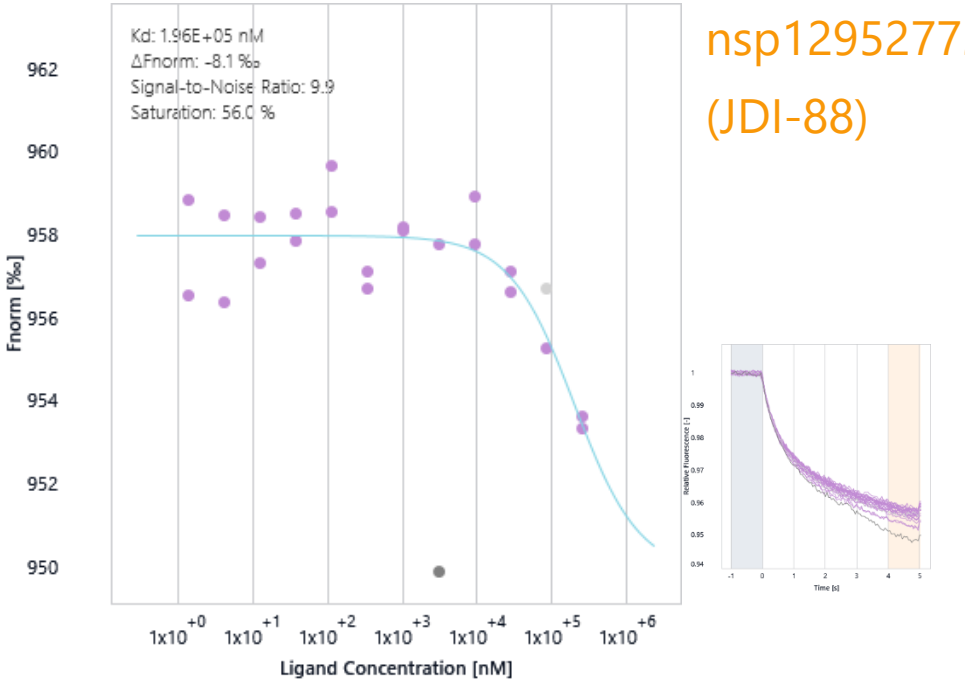


nsp12S5746081

(JDI-49)



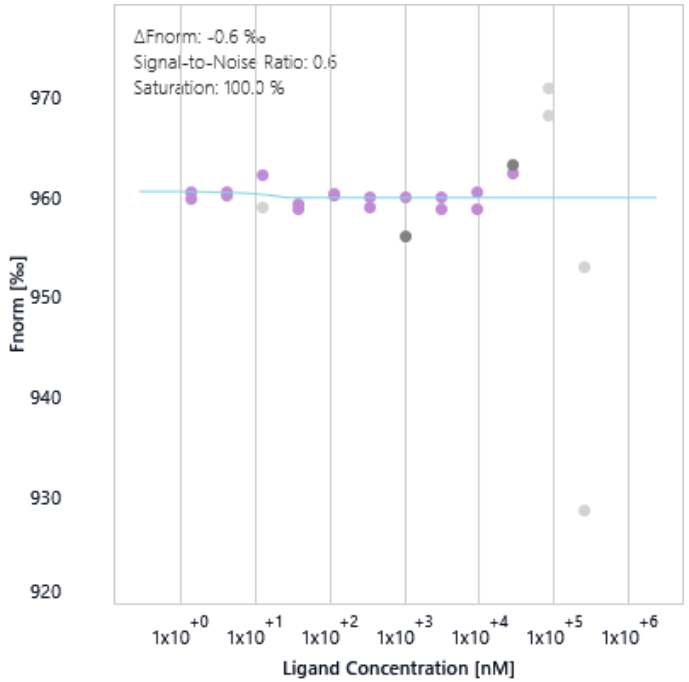
nsp1295277234

(JDI-88)

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5746081 (JDI-49)	>3.9E-05	-10.9	9.2	5	Weak Binder, no saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1295277234 (JDI-88)	>2.0E-04	-8.1	9.9	5	Weak Binder, no saturation

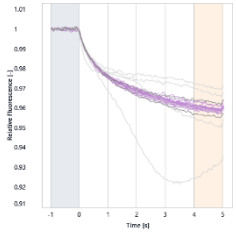
- RED-TRIS NTA 2nd gen. labelled nsp12 weakly binds nsp1242190400 with an estimated K_D > 40 µM without reaching saturation.
- RED-TRIS NTA 2nd gen. labelled nsp12 weakly binds nps5-45651217 with an estimated K_D > 200 µM without reaching saturation.

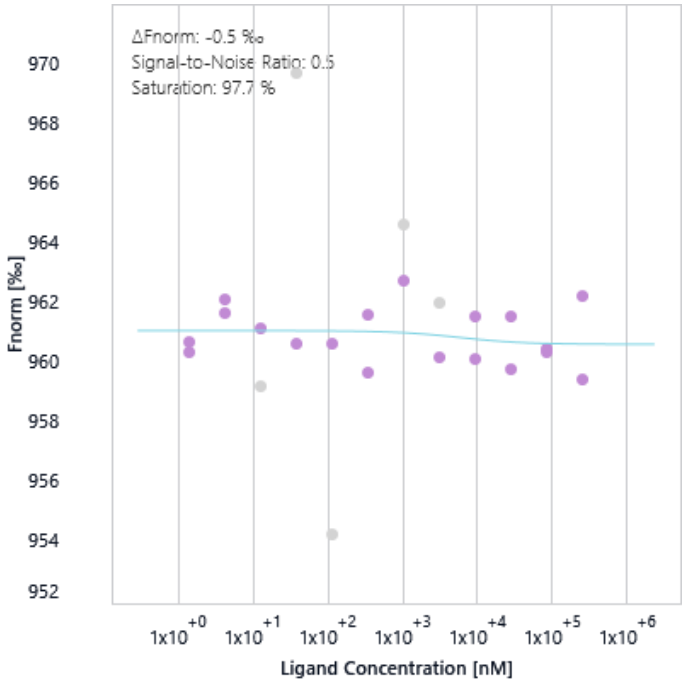
RED-TRIS NTA 2nd gen. labelled nsp12 vs. Aggregation



nsp1242190400

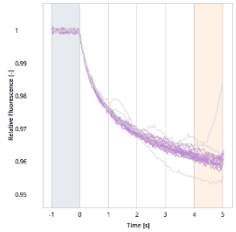
(JDI-38)





S_mli69415177

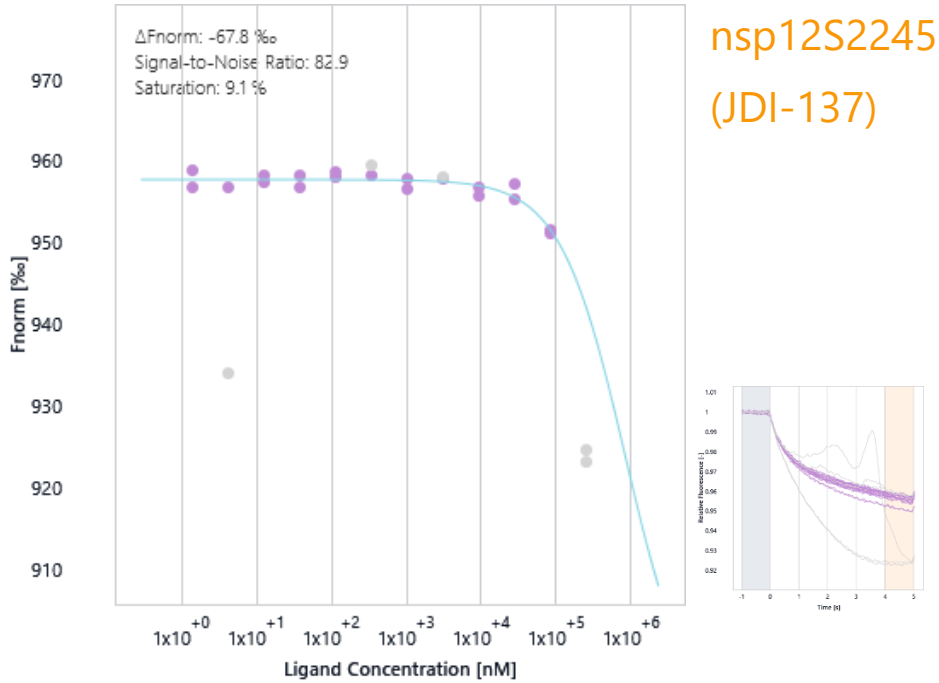
(JDI-80)



Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	N_mli9974624 (JDI-120)	Aggregation	-	-	5	Aggregation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S6174387 (JDI-132)	Aggregation	-	-	5	Aggregation

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp1242190400, aggregation detected
- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind S_mli69415177, aggregation detected

RED-TRIS NTA 2nd gen. labelled nsp12 vs. Aggregation



nsp12S2245233

(JDI-137)

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S2245233 (JDI-137)	Aggregation	-	-	5	Aggregation

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp12S2245233, aggregation detected

RED-TRIS NTA 2nd gen. labelled nsp12 vs. Non-Binder

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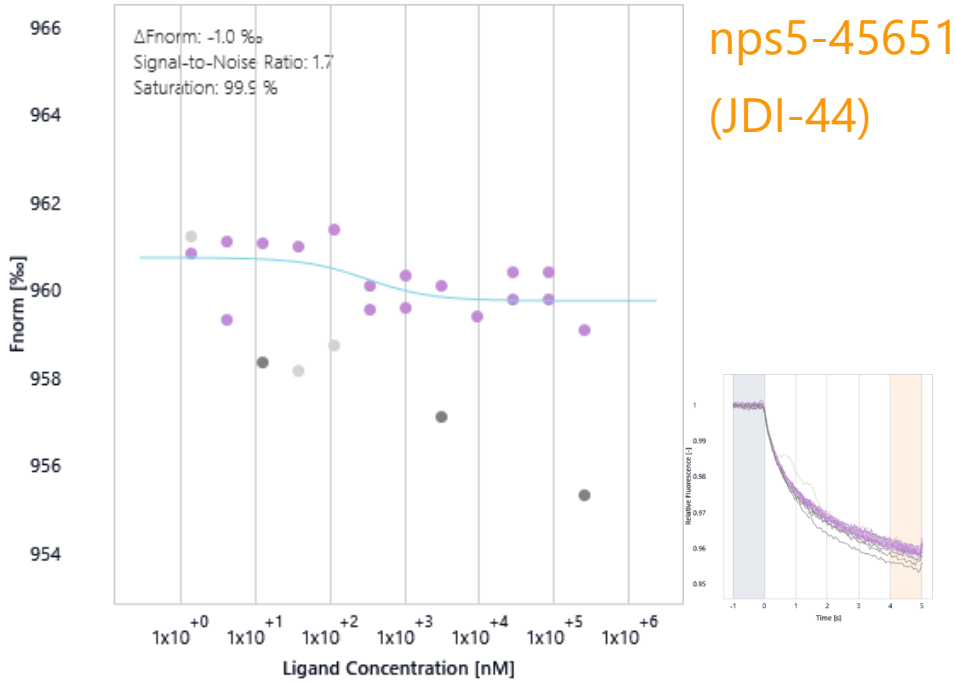
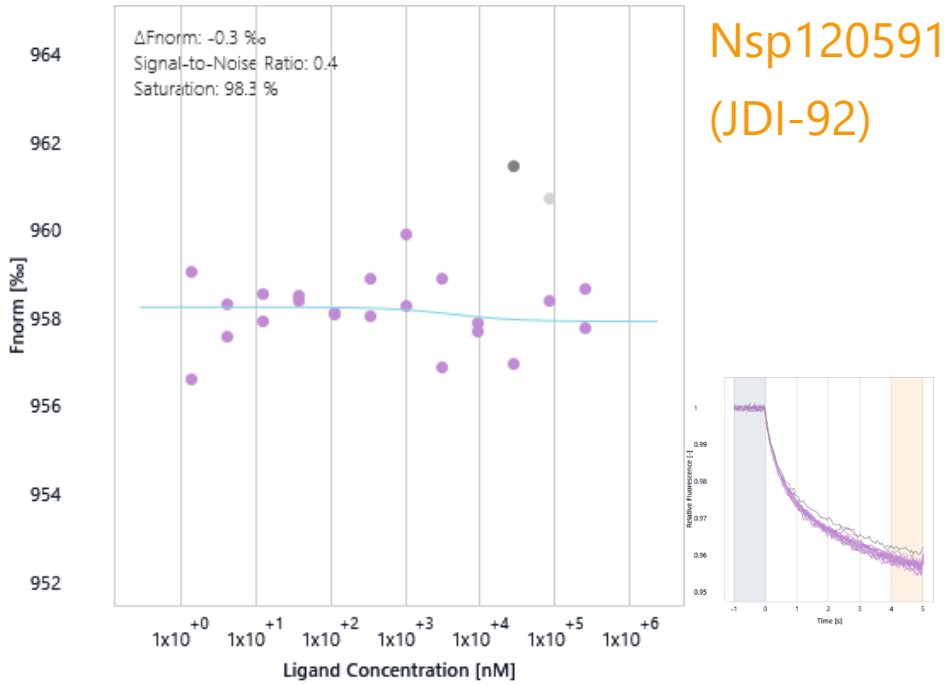


nps5-45651217

(JDI-44)



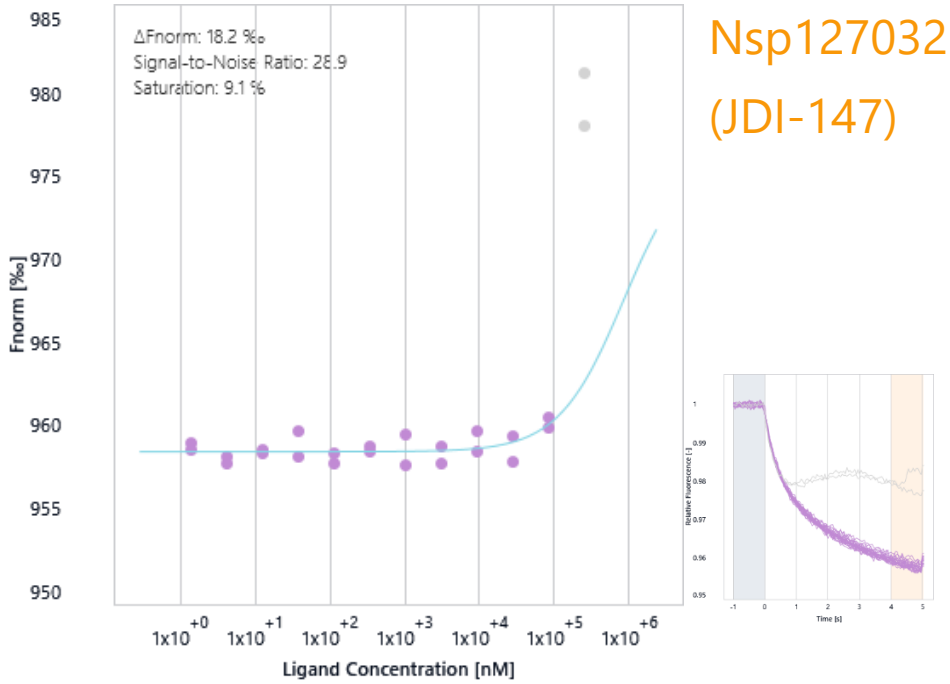
Nsp1205917961

(JDI-92)

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nps5-45651217 (JDI-44)	-	-	-	5	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	Nsp1205917961 (JDI-92)	-	-	-	5	ΔFnorm is insignificant

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nps5-45651217, ΔFnorm is insignificant
- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind Nsp1205917961, ΔFnorm is insignificant

RED-TRIS NTA 2nd gen. labelled nsp12 vs. Non-Binder



Nsp1270326070 (JDI-147)

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1270326070 (JDI-147)	-	-	-	5	Insufficient saturation

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp1270326070, insufficient saturation

TRIC summary

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5746081 (JDI-49)	>3.9E-05	-10.9	9.2	5	Weak Binder, no saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1295277234 (JDI-88)	>2.0E-04	-8.1	9.9	5	Weak Binder, no saturation
RED-TRIS NTA 2nd gen.	Nsp12	N_mli9974624 (JDI-120)	Aggregation	-	-	5	Aggregation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S6174387 (JDI-132)	Aggregation	-	-	5	Aggregation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S2245233 (JDI-137)	Aggregation	-	-	5	Aggregation
RED-TRIS NTA 2nd gen.	Nsp12	nps5-45651217 (JDI-44)	-	-	-	5	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	Nsp1205917961 (JDI-92)	-	-	-	5	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1270326070 (JDI-147)	-	-	-	5	Insufficient saturation

Next steps

- Discuss the data and next steps

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