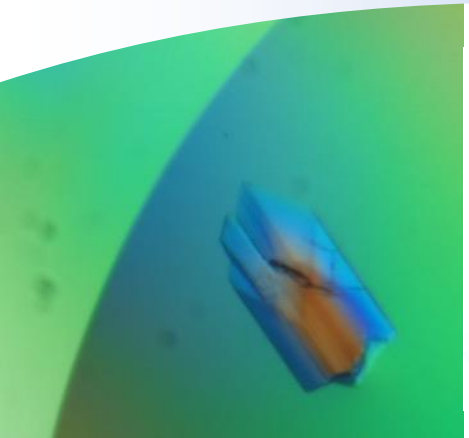
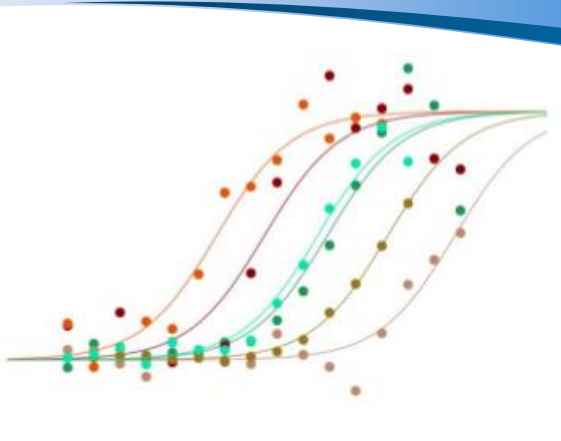
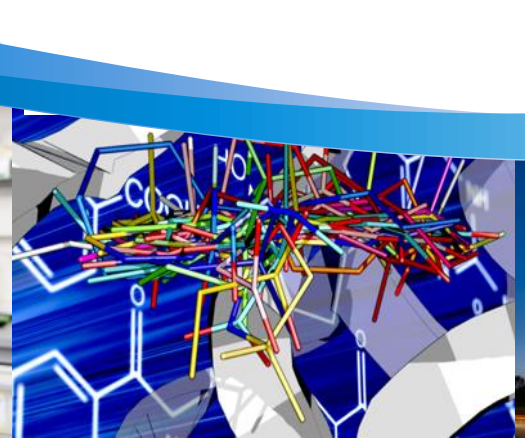
JEDI Program/JDI01

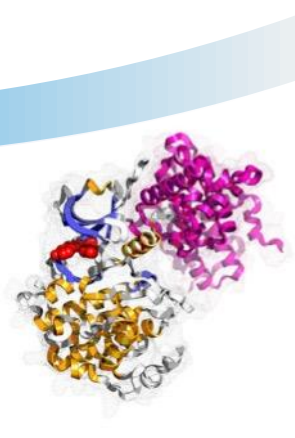
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

Primary Screening of 147 compounds

August 27, 2021





Status

- TRIC (Dianthus)

  8-pt-screening on RED-TRIS NTA 2nd gen. labelled nsp12 was performed in order to identify the most promising hits showing a dose-response.

  - 147 compounds were screened for binding to RED-TRIS NTA 2nd gen. nsp12 using 8-pt dilution series (singlicate) from 100 µM, showing the following results:

Category	5 sec TRIC On Time	
(Potential) Binder	8	5.4%
Weak Binder	2	1.4%
Non-Binder	113	76.9%
Aggregation	24	16.3%

  - **Please note:** The reported K_D values of 8-Point singlicates are only estimates. The goal of the 8-point screening is to identify hits showing a dose-response. A precise K_D determination will be performed as a 12-point duplicates.

  - We recommend to measure all binders and „specific“ potential binders (8) as 12-point duplicates

TRIC (Dianthus)

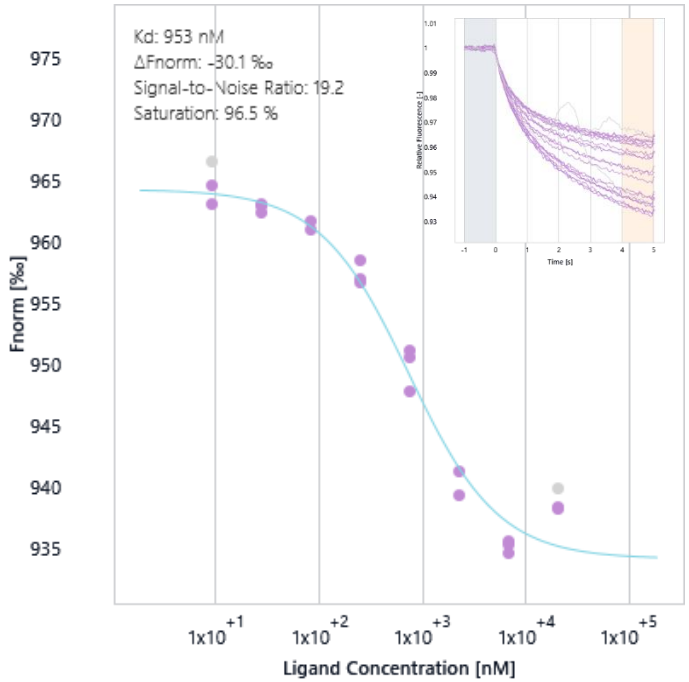
Nsp12 (DVT1, PC13929-1)

vs. 147 Hits

TRIC Screening Conditions:

Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	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 (8-Point dilution series) Spotting of 500 nl compound with Integra Viaflow Head 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: 23 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Singlicate		
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	147 Hits Suramin	JDI-01 – JDI-147	100 µM – 45.72 nM (8 concentrations) 20 µM – 9.14 nM (8 concentrations)

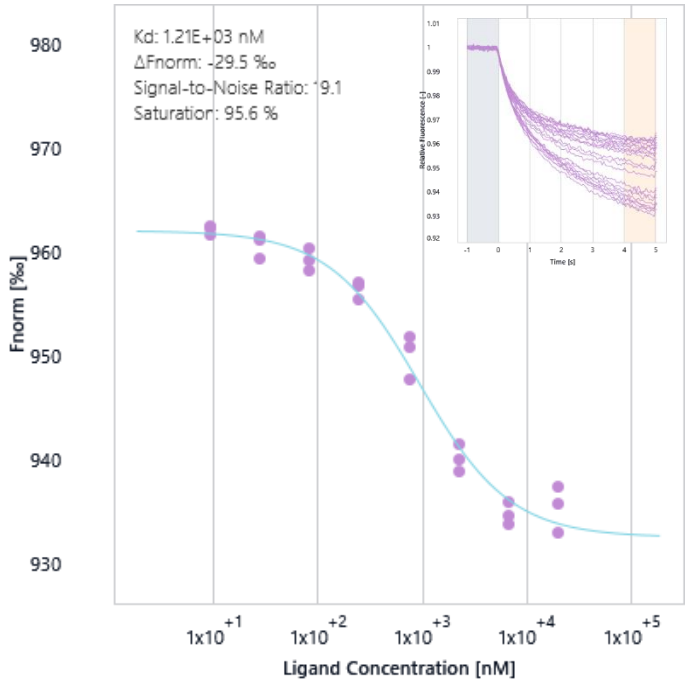
RED-TRIS NTA 2nd gen. labelled nsp12 vs. Suramin (Detector 1)



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	9.5E-07	7.1E-07	1.3E-06	-30.1	19.2	5	

Suramin binds to RED-TRIS NTA 2nd gen. labelled nsp12 with estimated K_D value of 953 nM and stable ΔFnorm and signal-to-noise over the entire assay plate (n=10).

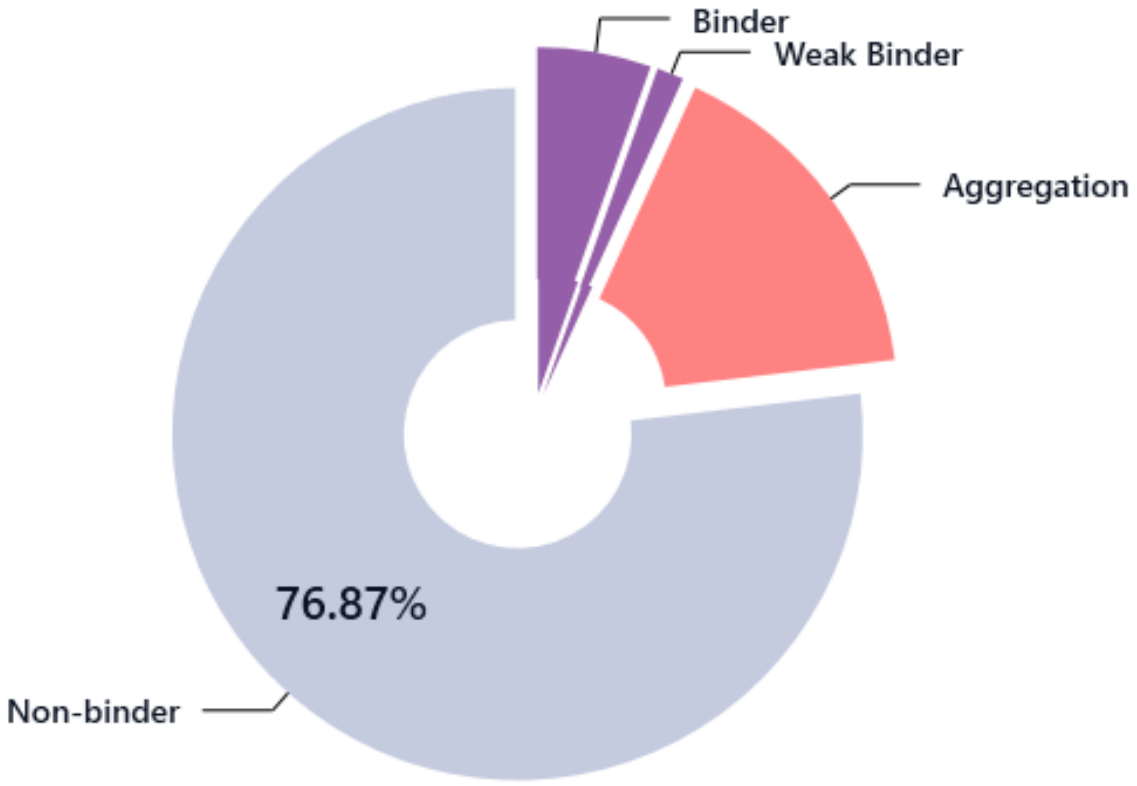
RED-TRIS NTA 2nd gen. labelled nsp12 vs. Suramin (Detector 2)



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	1.2E-06	9.1E-07	1.6E-06	-29.5	19.1	5	

Suramin binds to RED-TRIS NTA 2nd gen. labelled nsp12 with estimated K_D value of 1.2 µM and stable ΔFnorm and signal-to-noise over the entire assay plate (n=10).

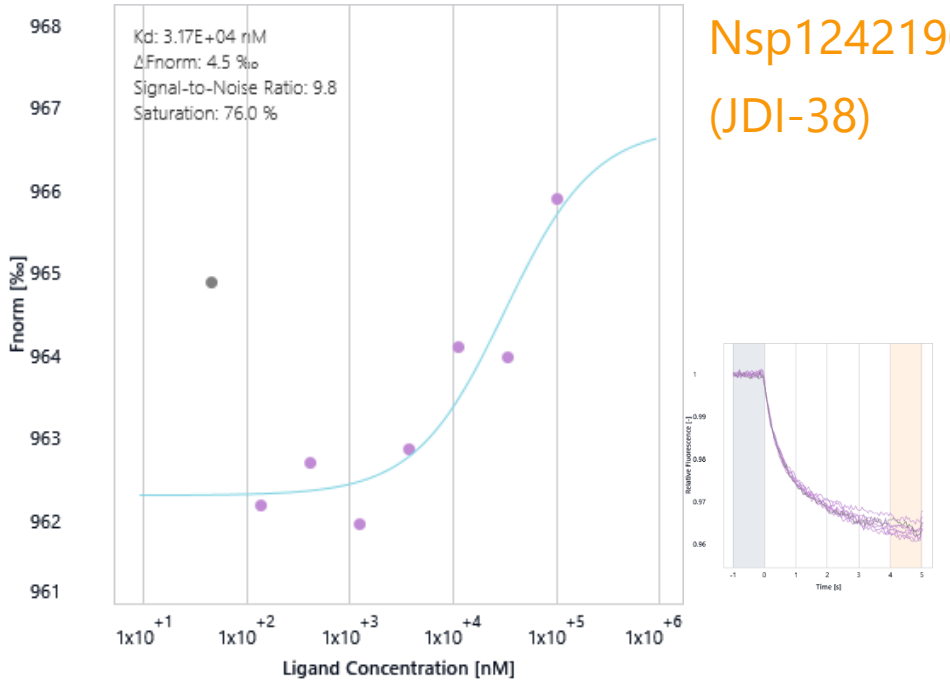
Summary: Single Point Screening of RED-TRIS NTA 2nd gen. labelled nsp12 vs. 147 Hits



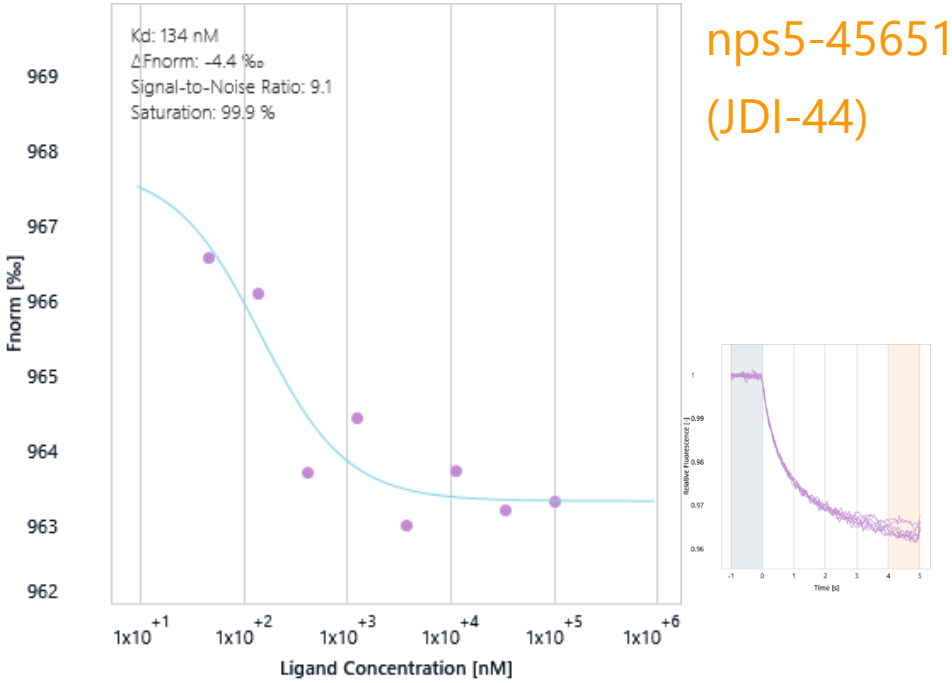
Binder	8
Weak Binder	2
Aggregation	24
Non-binder	113
Total	147

For detailed results see Excel file

RED-TRIS NTA 2nd gen. labelled nsp12 vs. (Potential) Binder



Nsp1242190400 (JDI-38)

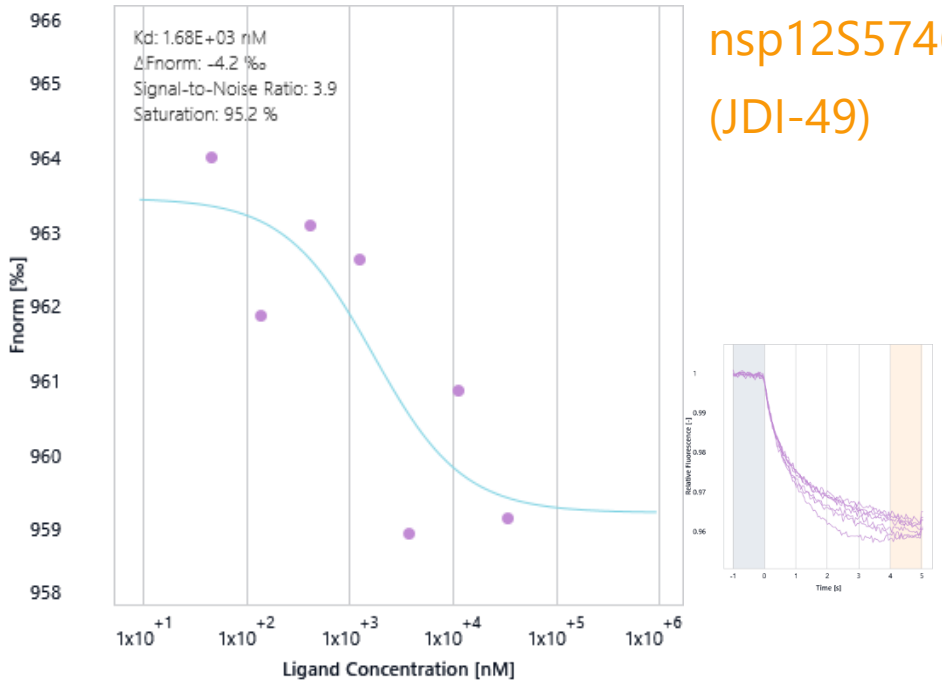


nps5-45651217 (JDI-44)

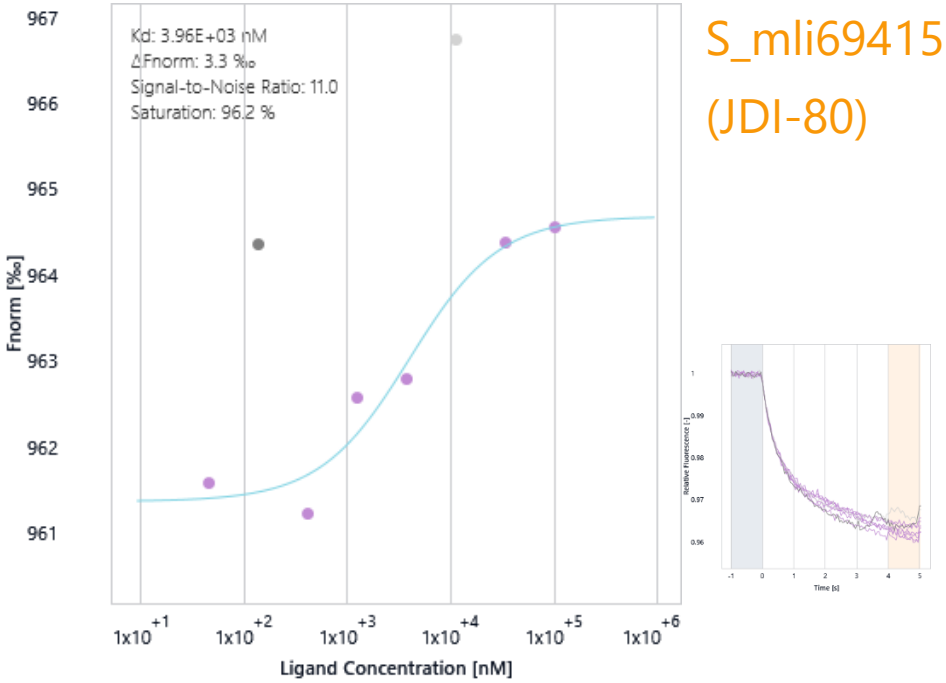
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1242190400 (JDI-38)	3.2E-05	4.5	9.8	5	Potential Binder, low delta Fnorm
RED-TRIS NTA 2nd gen.	Nsp12	nps5-45651217 (JDI-44)	1.3E-07	-4.4	9.1	5	Potential Binder, low delta Fnorm

- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds nsp1242190400 with an estimated K_D of 32 µM, low delta Fnorm.
- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds nps5-45651217 with an estimated K_D of 134 nM, low delta Fnorm.

RED-TRIS NTA 2nd gen. labelled nsp12 vs. (Potential) Binder



nsp12S5746081 (JDI-49)

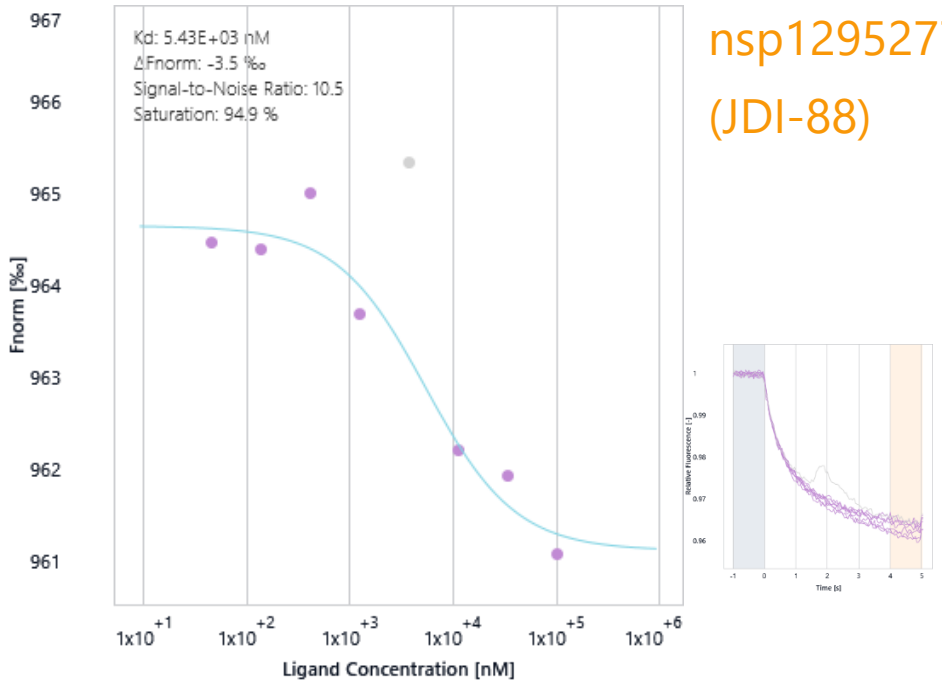


S_mli69415177 (JDI-80)

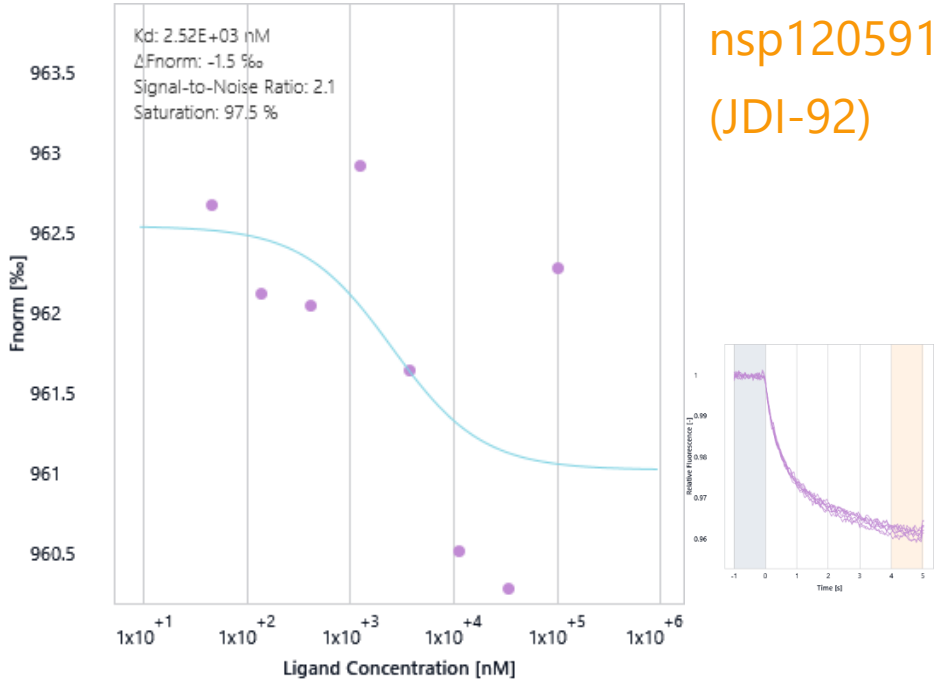
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5746081 (JDI-49)	1.7E-06	-4.2	3.9	5	Potential Binder, low delta Fnorm, low signal to noise
RED-TRIS NTA 2nd gen.	Nsp12	S_mli69415177 (JDI-80)	4.0E-06	3.3	11.0	5	Potential Binder, low delta Fnorm

- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds nsp12S5746081 with an estimated K_D of 1.7 µM, low delta Fnorm and low signal to noise.
- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds S_mli69415177 with an estimated K_D of 4.0 µM, low delta Fnorm.

RED-TRIS NTA 2nd gen. labelled nsp12 vs. (Potential) Binder



nsp1295277234 (JDI-88)

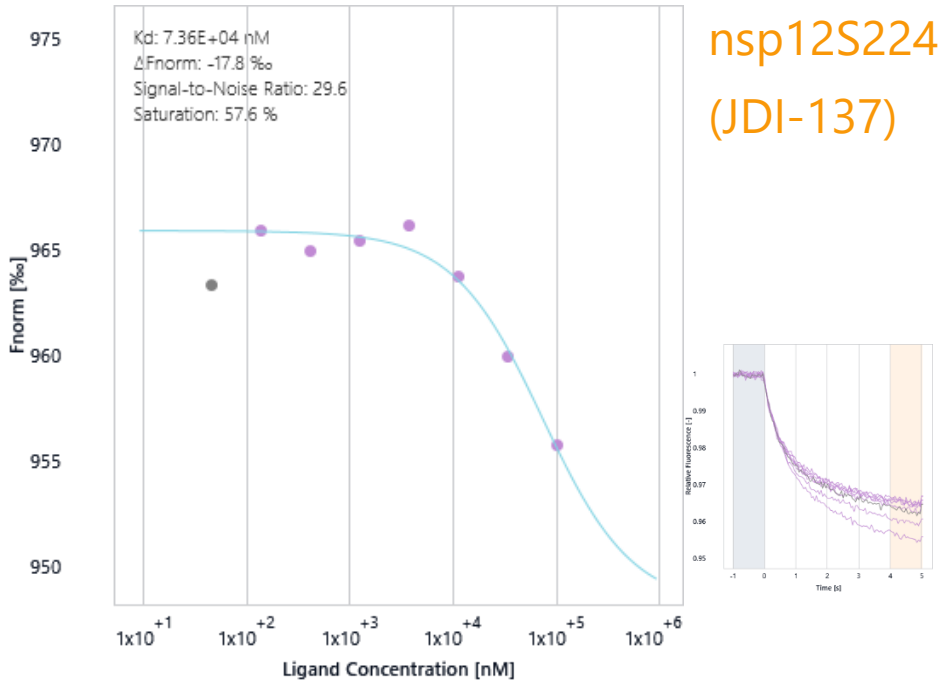


nsp1205917961 (JDI-92)

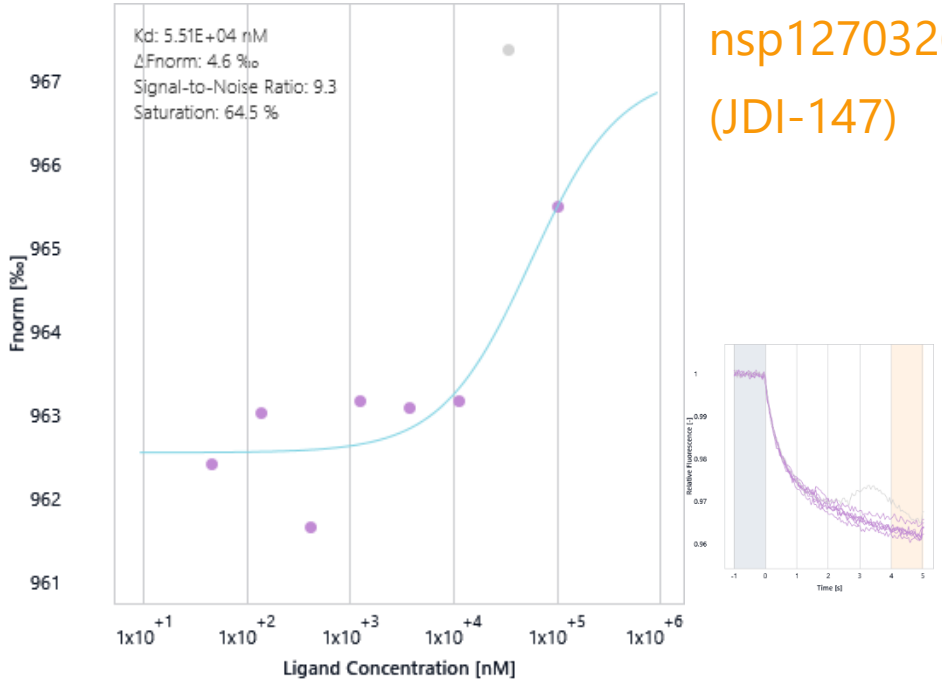
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1295277234 (JDI-88)	5.4E-06	-3.5	10.5	5	Potential Binder, low delta Fnorm
RED-TRIS NTA 2nd gen.	Nsp12	nsp1205917961 (JDI-92)	2.5E-06	-1.5	2.1	5	Potential Binder, low delta Fnorm, low signal to noise

- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds nsp1295277234 with an estimated K_D of 5.4 µM, low delta Fnorm.
- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds nsp1205917961 with an estimated K_D of 2.5 µM, low delta Fnorm and signal to noise.

RED-TRIS NTA 2nd gen. labelled nsp12 vs. (Potential) Binder



nsp12S2245233
(JDI-137)



nsp1270326070
(JDI-147)

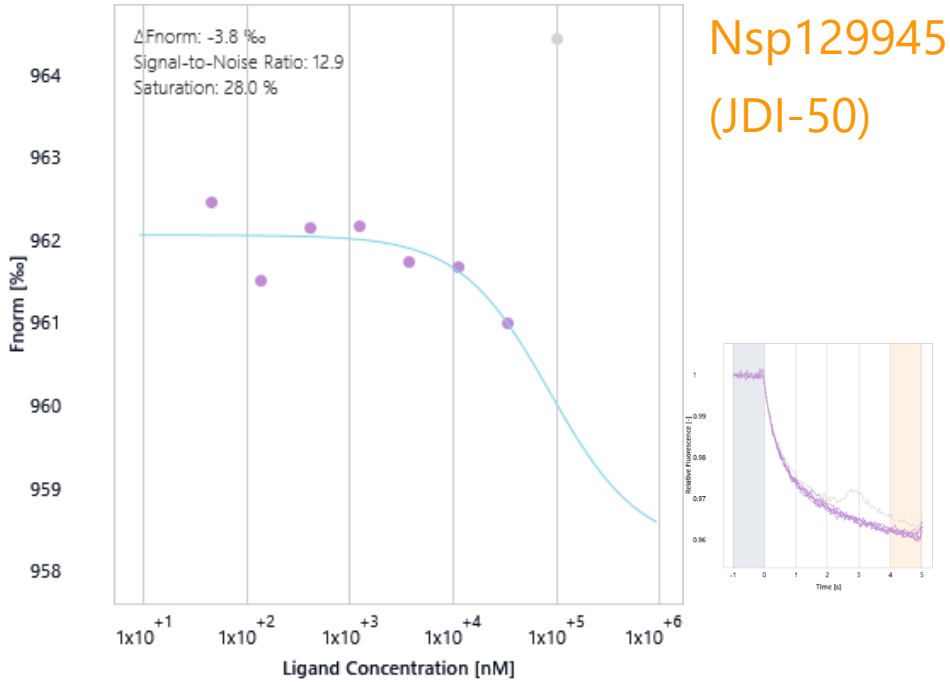
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S2245233 (JDI-137)	>7.4E-05	-17.8	29.6	5	No saturation reached
RED-TRIS NTA 2nd gen.	Nsp12	nsp1270326070 (JDI-147)	5.5E-05	4.6	9.3	5	Potential Binder, low delta Fnorm

- RED-TRIS NTA 2nd gen. labelled nsp12 binds nsp12S2245233 with an estimated K_D > 74 µM without reaching saturation.
- RED-TRIS NTA 2nd gen. labelled nsp12 potentially binds nsp1270326070 with an estimated K_D of 55 µM, low delta Fnorm.

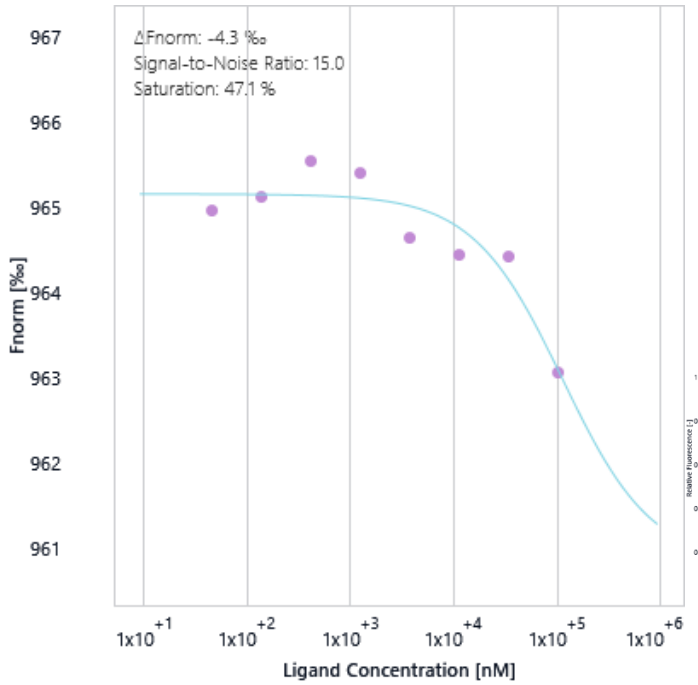
TRIC summary: Binders

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1242190400 (JDI-38)	3.2E-05	4.5	9.8	5	Potential Binder, low delta Fnorm
RED-TRIS NTA 2nd gen.	Nsp12	nps5-45651217 (JDI-44)	1.3E-07	-4.4	9.1	5	Potential Binder, low delta Fnorm
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5746081 (JDI-49)	1.7E-06	-4.2	3.9	5	Potential Binder, low delta Fnorm, low signal to noise
RED-TRIS NTA 2nd gen.	Nsp12	S_mli69415177 (JDI-80)	4.0E-06	3.3	11.0	5	Potential Binder, low delta Fnorm
RED-TRIS NTA 2nd gen.	Nsp12	nsp1295277234 (JDI-88)	5.4E-06	-3.5	10.5	5	Potential Binder, low delta Fnorm
RED-TRIS NTA 2nd gen.	Nsp12	nsp1205917961 (JDI-92)	2.5E-06	-1.5	2.1	5	Potential Binder, low delta Fnorm, low signal to noise
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S2245233 (JDI-137)	7.4E-05	-17.8	29.6	5	No saturation reached
RED-TRIS NTA 2nd gen.	Nsp12	nsp1270326070 (JDI-147)	5.5E-05	4.6	9.3	5	Potential Binder, low delta Fnorm

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



Nsp1299456066 (JDI-50)



nsp1223221670 (JDI-113)

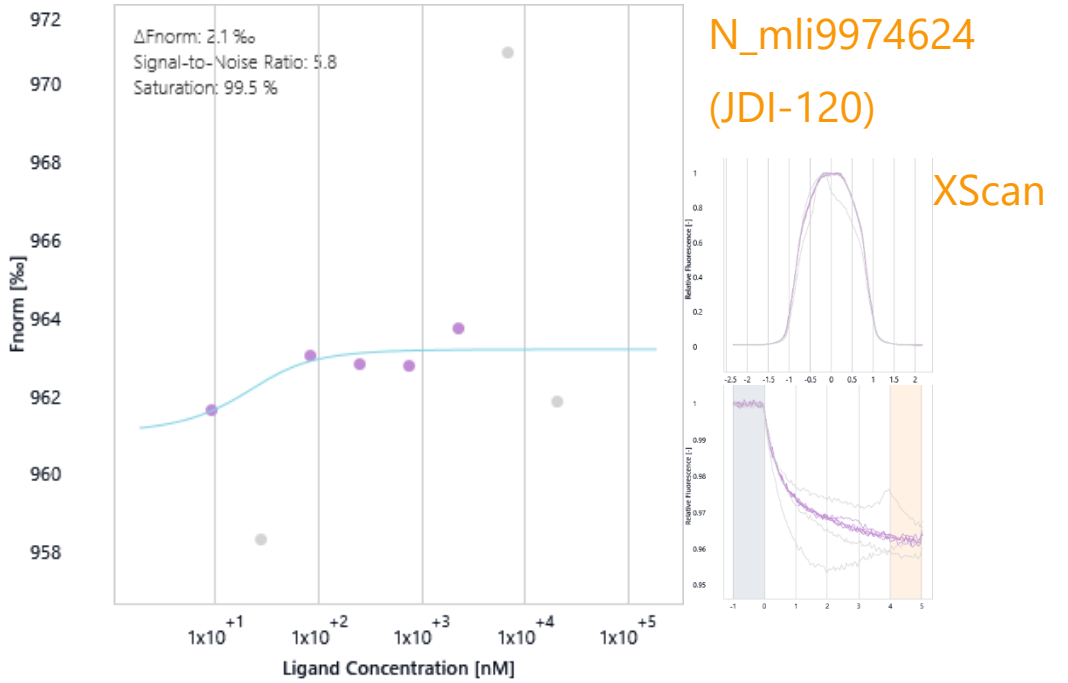
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1299456066 (JDI-50)	> 8.6E-05	-	-	5	without reaching a saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1223221670 (JDI-113)	> 1.1E-04	-	-	5	without reaching a saturation

- RED-TRIS NTA 2nd gen. labelled nsp12 weakly binds nsp1299456066with an estimated K_D > 86 µM without reaching a saturation.
- RED-TRIS NTA 2nd gen. labelled nsp12 weakly binds EN300-211968_FP-04_F18 with a estimated K_D > 112 µM without reaching a saturation.

TRIC summary: Weak binders

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1299456066 (JDI-50)	> 8.6E-05	-	-	5	without reaching a saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1223221670 (JDI-113)	> 1.1E-04	-	-	5	without reaching a saturation

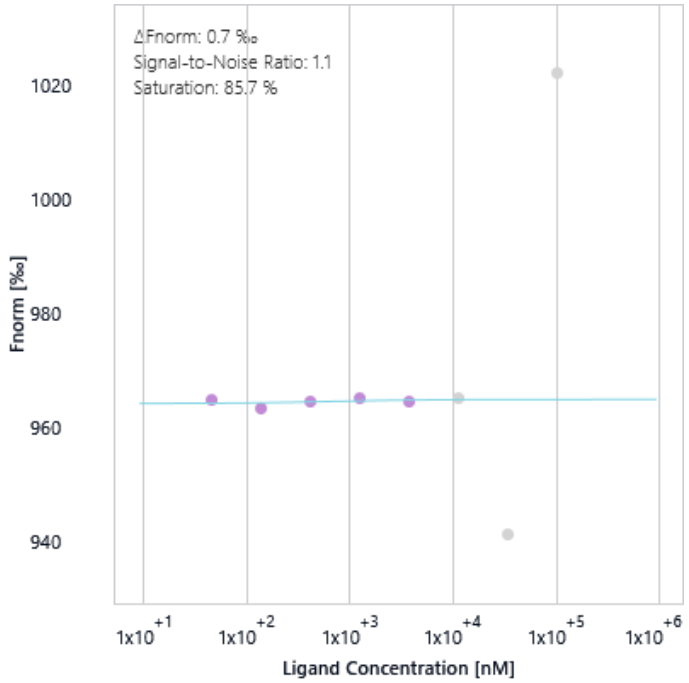
RED-TRIS NTA 2nd gen. labelled nsp12 vs. Aggregation (examples)



N_mli9974624

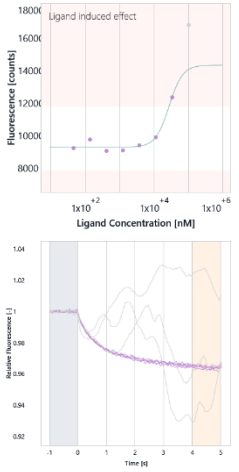
(JDI-120)

XScan



nsp12S6174387

(JDI-132)

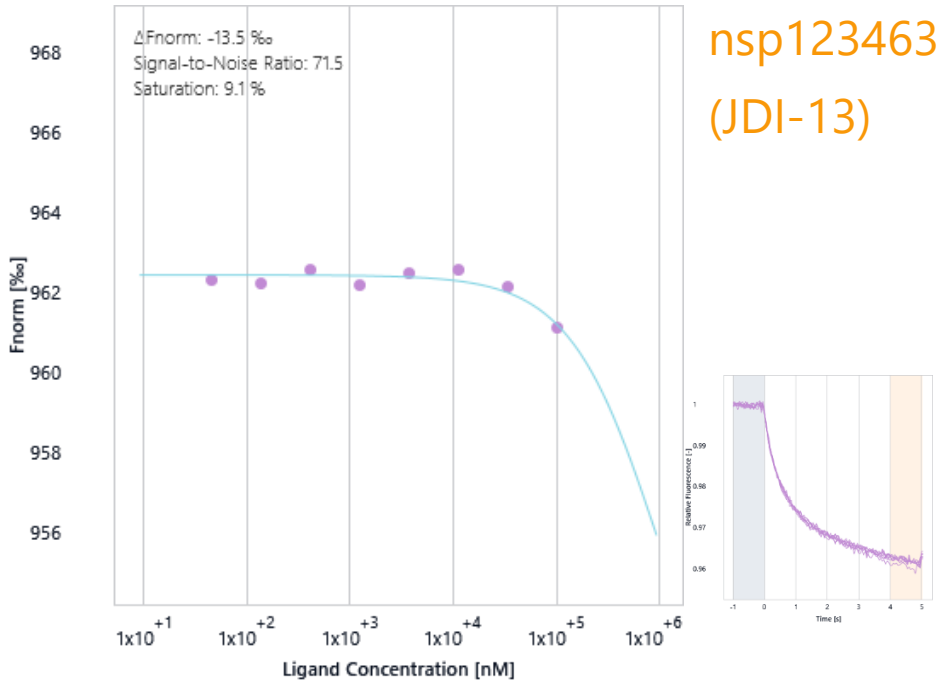


Fluores-cence

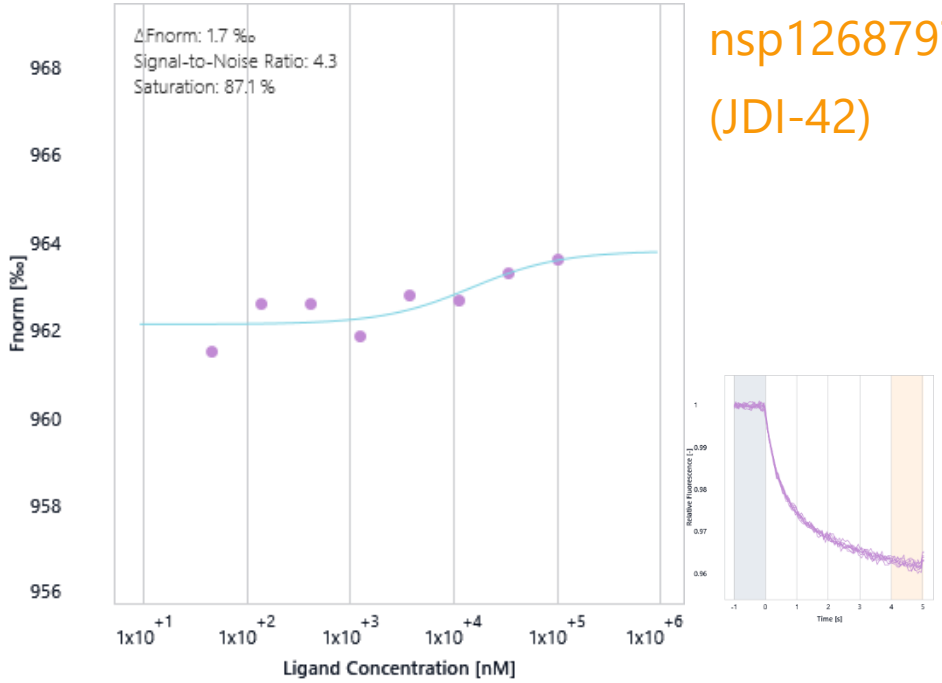
Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	N_mli9974624 (JDI-120)	Aggregation	-	-	5	Aggregation, scan anomaly
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S6174387 (JDI-132)	Aggreation	-	-	5	Aggregation, auto-fluorescence

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind N_mli9974624, aggregation and scan anomaly detected
- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp12S6174387, aggregation and auto-fluorescence detected

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



nsp1234633838 (JDI-13)

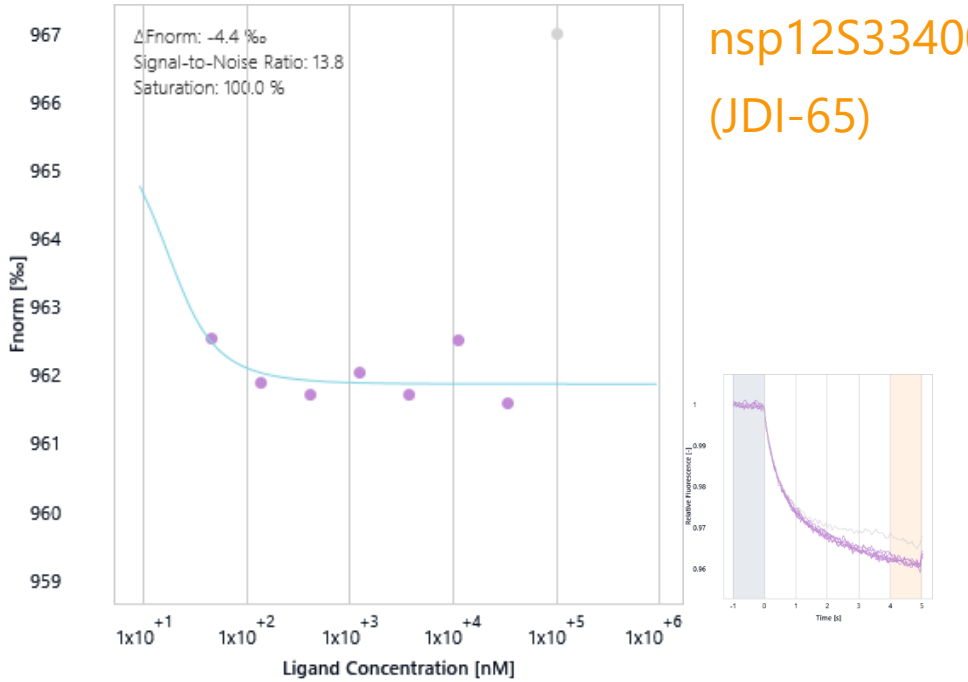


nsp1268797571 (JDI-42)

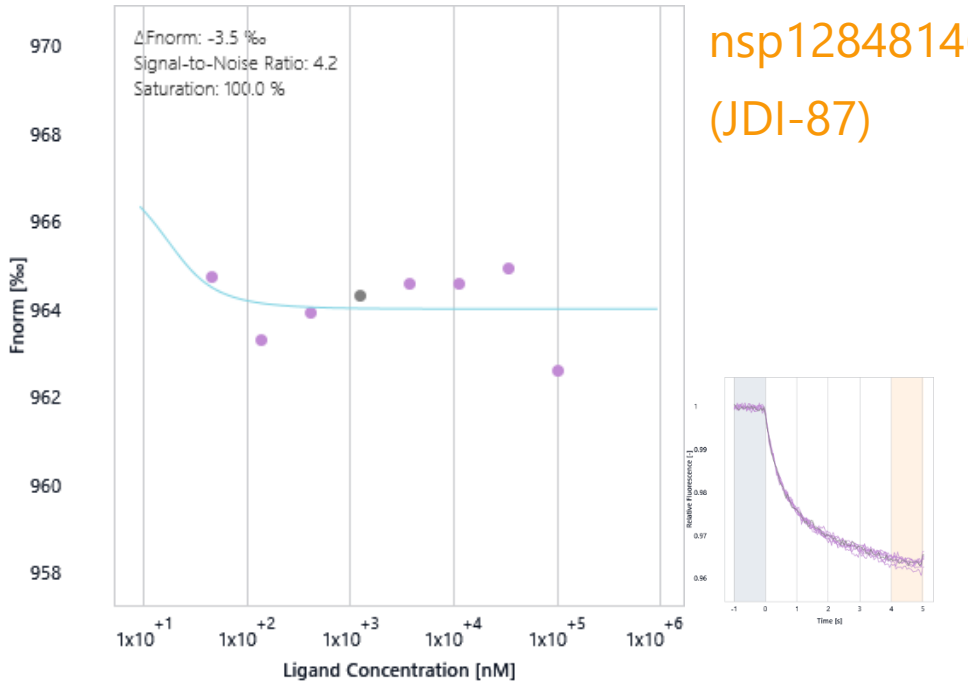
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1234633838 (JDI-13)	-	-	-	5	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1268797571 (JDI-42)	-	-	-	5	ΔFnorm is insignificant

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp1234633838, insufficient saturation
- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp1268797571, ΔFnorm is insignificant

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



nsp12S334004 (JDI-65)



nsp128481463 (JDI-87)

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
Maleimide-NT650	DCAF15	nsp12S334004 (JDI-65)	-	-	-	5	Missing unbound state
Maleimide-NT650	DCAF15	nsp128481463 (JDI-87)	-	-	-	5	Signal-to-noise ratio is too low

- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp12S334004, Missing unbound state
- RED-TRIS NTA 2nd gen. labelled nsp12 does not bind nsp128481463, Signal-to-noise ratio is too low

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1230660873 (JDI-01)	Non-binder	-	3.4	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1224136750 (JDI-02)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1292031246 (JDI-03)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	mixed22121037 (JDI-04)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	S_mli02816443 (JDI-05)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1201658532 (JDI-06)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	AAK1_88452506 (JDI-07)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1286935371 (JDI-08)	Non-binder	-	1.5	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	AAK1_84017292 (JDI-09)	Non-binder	-	0.5	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1204116440 (JDI-10)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1283649163 (JDI-11)	Non-binder	-	2.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1200128500 (JDI-12)	Non-binder	-	0.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1234633838 (JDI-13)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S9563578 (JDI-14)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1217792061 (JDI-15)	Non-binder	-	1.2	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1220196305 (JDI-16)	Non-binder	-	_	5.0	Insufficient saturation

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1296588278 (JDI-17)	Non-binder	-	1.5	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nps5-35493900 (JDI-18)	Non-binder	-	-	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1297152100 (JDI-19)	Non-binder	-	2.4	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1272147962 (JDI-20)	Non-binder	-		5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1227745845 (JDI-21)	Non-binder	-	5.7	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1214469027 (JDI-22)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1271529887 (JDI-23)	Non-binder	-		5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1283265105 (JDI-24)	Non-binder	-	1.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1235930431 (JDI-25)	Non-binder	-	0.9	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nps5-66664305 (JDI-26)	Non-binder	-	0.6	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1257698137 (JDI-27)	Non-binder	-	-	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nps5-08124617 (JDI-28)	Non-binder	-	3.7	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1234837110 (JDI-29)	Non-binder	-	-	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1241216994 (JDI-30)	Non-binder	-	1.5	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S4614385 (JDI-31)	Non-binder	-	0.4	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1240415958 (JDI-32)	Non-binder	-	-	5.0	Insufficient saturation

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1215933248 (JDI-33)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	mixed43465160 (JDI-34)	Non-binder	-	2.7	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1276240 (JDI-35)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	S_mli40772722 (JDI-36)	Non-binder	-	3.8	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1261439245 (JDI-37)	Non-binder	-	0.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1220739343 (JDI-39)	Non-binder	-	2.8	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1285673191 (JDI-40)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nps5-9957601 (JDI-41)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1268797571 (JDI-42)	Non-binder	-	4.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1281665688 (JDI-43)	Non-binder	-	0.4	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1278715557 (JDI-46)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1293052993 (JDI-47)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1210442440 (JDI-48)	Non-binder	-	1.1	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1274041604 (JDI-52)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1253081558 (JDI-53)	Non-binder	-	0.6	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1265686924 (JDI-54)	Non-binder	-	2.2	5.0	ΔFnorm is insignificant

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S3978859 (JDI-55)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	tmprsOS931 (JDI-56)	Non-binder	-	0.8	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1221337338 (JDI-57)	Non-binder	-	2.1	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1256042724 (JDI-58)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1291373487 (JDI-59)	Non-binder	-	0.7	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1299144809 (JDI-60)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nps5-93332042 (JDI-61)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	N_mli70183082 (JDI-62)	Non-binder	-	2.0	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1245037220 (JDI-63)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1207339866 (JDI-64)	Non-binder	-	4.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S334004 (JDI-65)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp129924336 (JDI-66)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5341753 (JDI-67)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S8126062 (JDI-68)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	AAK1_56030161 (JDI-69)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp124827235 (JDI-70)	Non-binder	-	1.7	5.0	ΔFnorm is insignificant

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1250158152 (JDI-71)	Non-binder	-	2.4	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1270460351 (JDI-72)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1284750594 (JDI-73)	Non-binder	-	-	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1209878108 (JDI-74)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	mixed2401365 (JDI-76)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1283597814 (JDI-77)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5920925 (JDI-79)	Non-binder	-	1.7	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1202887949 (JDI-82)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1265686406 (JDI-84)	Non-binder	-	-	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1256841708 (JDI-85)	Non-binder	-	-	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1232883165 (JDI-86)	Non-binder	-	1.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp128481463 (JDI-87)	Non-binder	-	-	5.0	Signal-to-noise ratio is too low
RED-TRIS NTA 2nd gen.	Nsp12	nsp1260760338 (JDI-90)	Non-binder	-	2.2	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	S_mli15215226 (JDI-91)	Non-binder	-	1.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1281969668 (JDI-93)	Non-binder	-	19.8	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1282441565 (JDI-94)	Non-binder	-	-	5.0	Insufficient saturation

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1281162716 (JDI-95)	Non-binder	-	0.2	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1233210093 (JDI-96)	Non-binder	-	1.0	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1265249867 (JDI-97)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1283718396 (JDI-98)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1277324411 (JDI-99)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	nsp1210239754 (JDI-100)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1248258494 (JDI-101)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nps5-05536401 (JDI-102)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1269441180 (JDI-103)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1230285814 (JDI-104)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1277253392 (JDI-106)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1201138389 (JDI-107)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	S_mli569120 (JDI-108)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1209951541 (JDI-109)	Non-binder	-	0.8	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1286174798 (JDI-110)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp125196887 (JDI-111)	Non-binder	-	_	5.0	Insufficient saturation

TRIC summary: Non-Binders

Fluorophore	Fluor. Molecule	Titrant	Category	K_D [M]	Signal / Noise	TRIC On [s]	Comment
RED-TRIS NTA 2nd gen.	Nsp12	nsp1229962097 (JDI-114)	Non-binder	-	0.6	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	S_mli19882654 (JDI-115)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	AAK1_47886013 (JDI-116)	Non-binder	-	2.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1270864599 (JDI-117)	Non-binder	-	0.9	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	mixed91758238 (JDI-121)	Non-binder	-	2.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1278320467 (JDI-122)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp122683351 (JDI-123)	Non-binder	-	_	5.0	Missing unbound state
RED-TRIS NTA 2nd gen.	Nsp12	S_mli71871300 (JDI-126)	Non-binder	-	4.9	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1297654858 (JDI-129)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1264022862 (JDI-130)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1256026779 (JDI-131)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp1296848779 (JDI-133)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	nsp12S5567309 (JDI-135)	Non-binder	-	2.1	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1235865401 (JDI-136)	Non-binder	-	_	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp1226020623 (JDI-140)	Non-binder	-	2.3	5.0	ΔFnorm is insignificant
RED-TRIS NTA 2nd gen.	Nsp12	nsp123795502 (JDI-141)	Non-binder	-	_	5.0	Insufficient saturation
RED-TRIS NTA 2nd gen.	Nsp12	mixed95470405 (JDI-144)	Non-binder	-	_	5.0	Insufficient saturation

Next steps

- Select compounds for the 12-pt screening with nsp12, our suggestions:
  - All (Potential) Binder (8 cpds) and if desired, Weak Binder (2 cpds)

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