药明康德 WuXi AppTec

a WuXi AppTec company

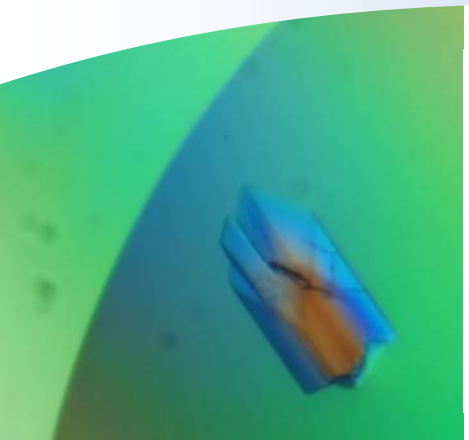
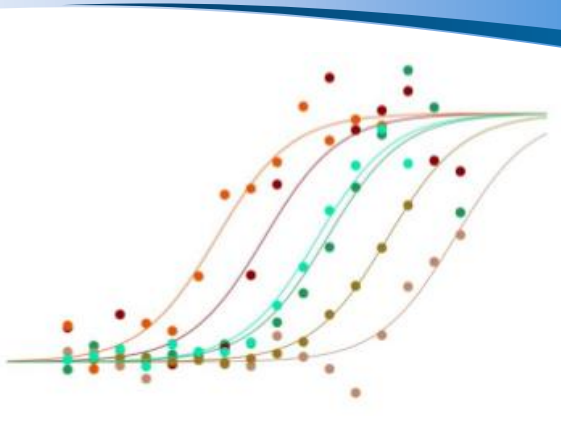
JEDI Program/JDI01

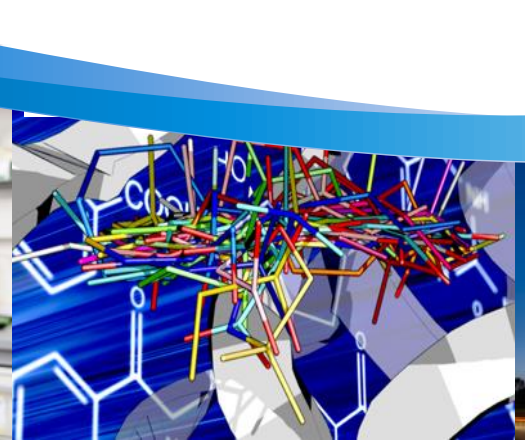
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

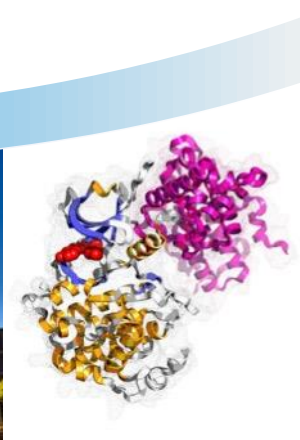
nsp12

nanoDSF / MST/ TRIC measurements

May 27, 2021





Status

- nanoDSF:
  - nsp12 shows good thermal stability (Tm of ~47°C).
  - Reducing agents and detergents have no significant impact on protein thermal stability, and the protein tolerates up to 3% DMSO.
  - However, none of the tool cpds (GS-443902 trisodium = active Remdesivir metabolite), Ribavirin (ICN-1229) and Favipiravir (MCE)) nor ATP show significant effects on protein thermal stability.

- TRIC (Dianthus):
  - Nsp12 was successfully labelled with RED-Tris-NTA dye.
  - However, none of the tool cpds (GS-443902 trisodium = active Remdesivir metabolite), Ribavirin (ICN-1229) and Favipiravir (MCE)) show binding to RED-Tris-NTA labelled nsp12.

- Labelled MST:
  - For comparison, experiments were also run on the NT.115 instrument and are in line with TRIC and nanoDSF results.

➢ Nsp12 alone is insufficient for binding of ATP or tool compounds. Thus, a TRIC/MST assay cannot be established with nsp12.

nanoDSF

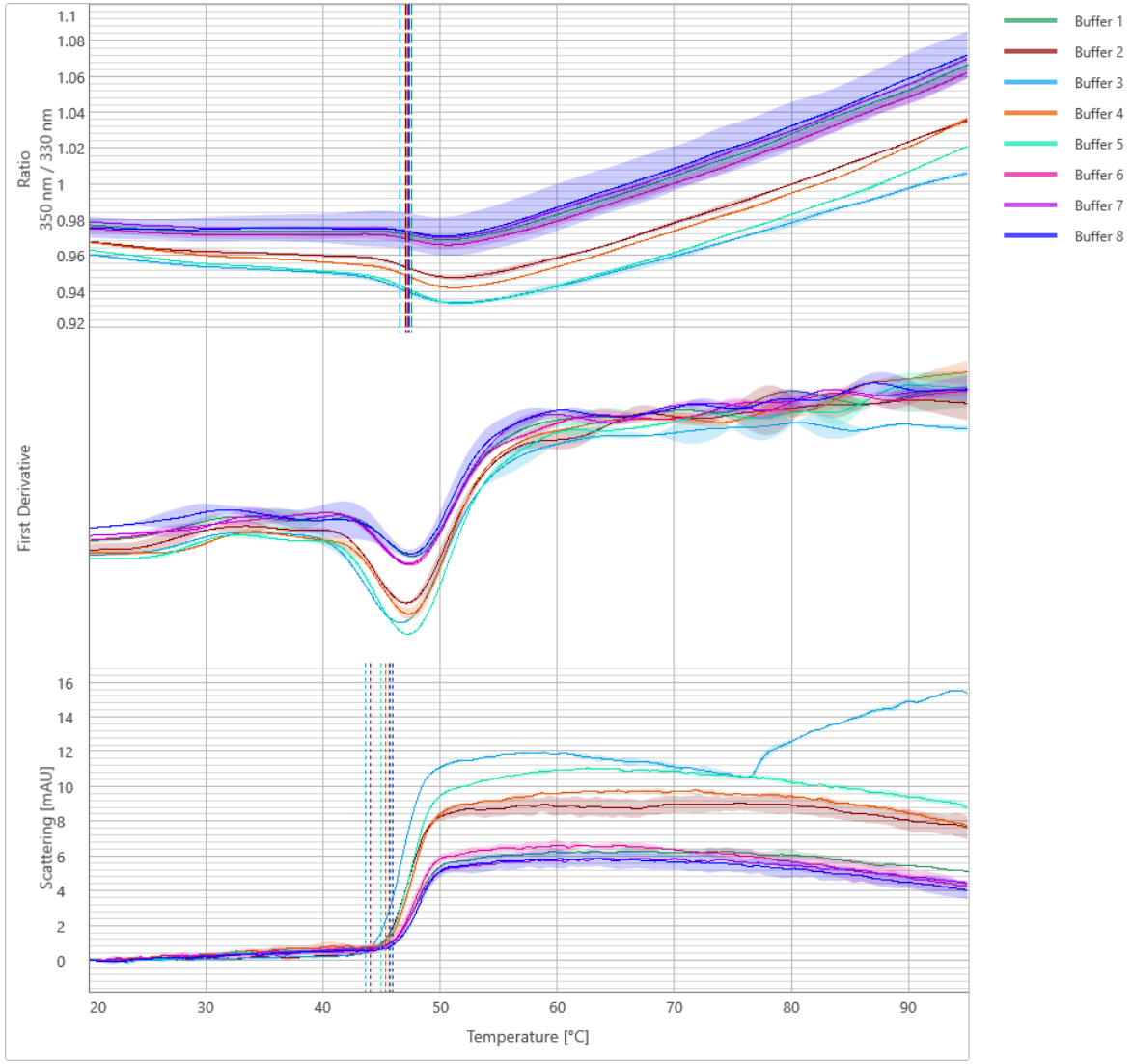
nsp12 (DVT1, PC13929-1)

nanoDSF Assay Conditions

Protein	0.1 mg/ml nsp12 (DVT1, PC13929-1)
Assay Buffer	8 Buffers (see table)
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 50 % Temperature ramp: 2°C/min

	Conditions
Buffer 1	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$
Buffer 2	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **0.005 % Tween20**
Buffer 3	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **0.05 % Tween20**
Buffer 4	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **0.05 % Pluronic**
Buffer 5	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **0.1 % Pluronic**
Buffer 6	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **1 mM TCEP**
Buffer 7	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **2.5 mM DTT**
Buffer 8	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, **2.5 mM GSH**

Effect of reducing agents and detergents on nsp12 (DVT1, PC13929-1) thermal stability



Condition	Ø T_m [°C] [1]	s.d. [°C]	Ø T_{agg} [°C] [1]	s.d. [°C]	Analysis mode
Buffer 1	47.6	0.2	45.8	0.1	ratio
Buffer 2	47.1	0.3	44.1	0.1	ratio
Buffer 3	46.6	0.0	43.7	0.0	ratio
Buffer 4	47.2	0.1	45.4	0.1	ratio
Buffer 5	47.2	0.1	45.0	0.1	ratio
Buffer 6	47.3	0.2	45.6	0.1	ratio
Buffer 7	47.2	0.1	45.3	0.6	ratio
Buffer 8	47.3	0.1	45.9	0.2	ratio

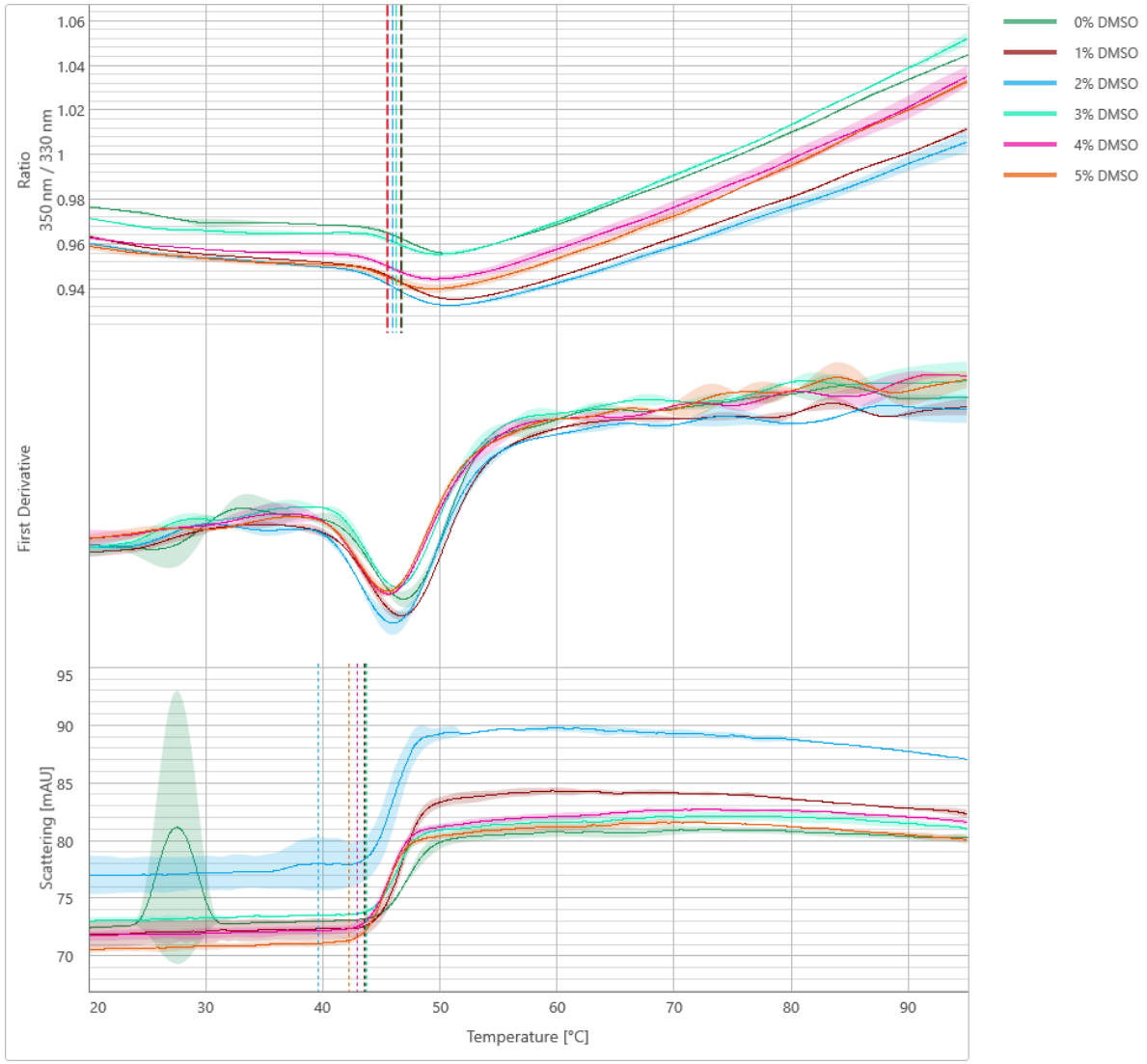
[1] determined in duplicate

- No significant impact of detergents or reducing agents on protein thermal stability was observed

nanoDSF Assay Conditions

Protein	0.1 mg/ml nsp12 (DVT1, PC13929-1)
Assay Buffer	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM MgCl2,2.5 mM DTT, 0.005 % Tween20 DMSO: 0 - 5 %
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 50 % Temperature ramp: 2°C/min

Impact of DMSO on nsp12 (DVT1, PC13929-1) thermal stability



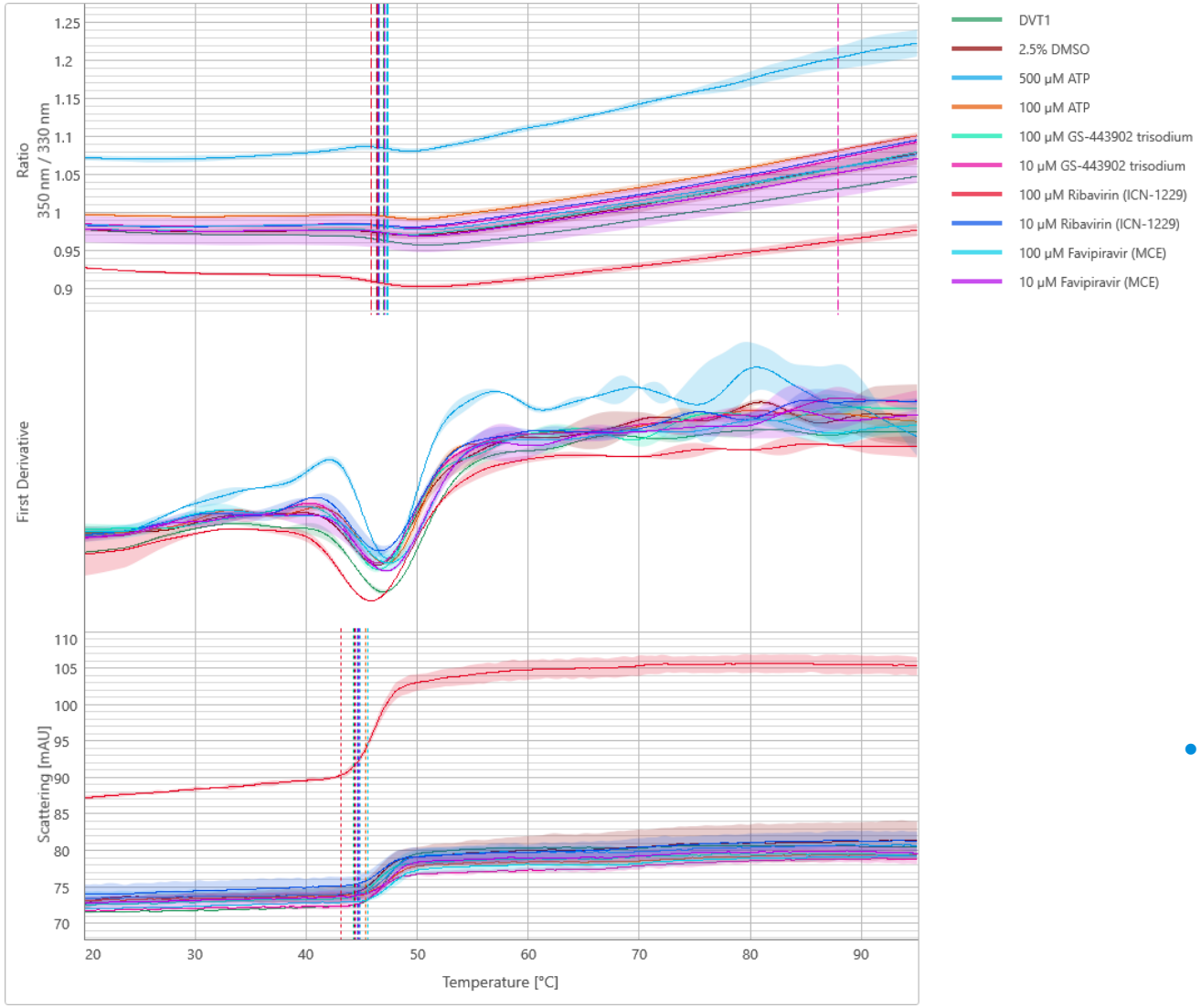
DMSO	T_m [°C]	s.d. [°C]	ΔT_m [°C] [2]	Ø T_{agg} [°C] [1]	s.d. [°C]	Analysis mode
-	46.8	0.1	-	43.6	1.6	ratio
1%	46.6	0.2	- 0.2	43.6	0.3	ratio
2%	46.0	0.1	- 0.8	39.6	4.6	ratio
3%	46.3	0.1	- 0.5	43.7	0.2	ratio
4%	45.6	0.2	- 1.2	42.9	0.1	ratio
5%	45.5	0.2	- 1.3	42.2	0.4	ratio

- Increasing DMSO concentrations destabilize the protein.
- A concentration of 2.5 % was selected for further measurements.

nanoDSF Assay Conditions

Protein	0.1 mg/ml nsp12 (DVT1, PC13929-1)
Assay Buffer	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM MgCl2, 2.5 mM DTT, 0.005 % Tween20 DMSO: 2.5 %
compounds	ATP at 100 µM and 500 µM GS-443902 trisodium (JDI-880) at 10 µM and 100 µM Ribavirin (ICN-1229) (JDI-879) at 10 µM and 100 µM Favipiravir (MCE) (JDI-881) at 10 µM and 100 µM
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 50 % Temperature ramp: 2°C/min

Effect of compounds on nsp12 (DVT1, PC13929-1) thermal stability



Titrant	Ø T_m [°C] [1]	s.d. [°C]	ΔT_m [°C] [2]	Ø T_{agg} [°C]	s.d. [°C]	Analysis mode
Protein alone	46.9	0.1	-	44.7	0.2	ratio
2.5 % DMSO	46.4	0.1	- 0.5	44.3	0.5	ratio
500 µM ATP	47.3	0.1	0.4	44.8	0.5	ratio
100 µM ATP	47.1	0.2	0.2	45.3	0.3	ratio
100 µM GS-443902 trisodium	46.5	0.2	- 0.4	44.3	0.1	ratio
10 µM GS-443902 trisodium	46.5	0.2	- 0.4	44.5	0.0	ratio
100 µM Ribavirin (ICN-1229)	45.8	0.1	- 1.1	43.1	0.0	ratio
10 µM Ribavirin (ICN-1229)	46.6	0.4	- 0.3	44.7	0.1	ratio
100 µM Favipiravir (MCE)	47.4	0.0	0.5	45.6	0.2	ratio
10 µM Favipiravir (MCE)	47.1	0.1	0.2	44.7	0.5	ratio

[1] determined in duplicate
[2] referenced to

- Neither ATP nor any of the tool compounds has a significant impact on protein thermal stability.

nanoDSF Summary: cpds effects on nsp12 (DVT1, PC13929-1)

a WuXi AppTec company

Effect of 3 compounds and ATP on the thermal stability of nsp12 (DVT1, PC13929-1) analyzed by nanoDSF

Target name	Titrant / Condition	Ø T_m [°C]	s.d. [°C]	ΔT_m [°C]	Ø T_{agg} [°C]	s.d. [°C]	Analysis mode
nsp12	Protein alone	46.9	0.1	-	44.7	0.2	ratio
	2.5 % DMSO	46.4	0.1	- 0.5	44.3	0.5	ratio
	500 µM ATP	47.3	0.1	0.4	44.8	0.5	ratio
	100 µM ATP	47.1	0.2	0.2	45.3	0.3	ratio
	100 µM GS-443902 trisodium	46.5	0.2	- 0.4	44.3	0.1	ratio
	10 µM GS-443902 trisodium	46.5	0.2	- 0.4	44.5	0.0	ratio
	100 µM Ribavirin (ICN-1229)	45.8	0.1	- 1.1	43.1	0.0	ratio
	10 µM Ribavirin (ICN-1229)	46.6	0.4	- 0.3	44.7	0.1	ratio
	100 µM Favipiravir (MCE)	47.4	0.0	0.5	45.6	0.2	ratio
	10 µM Favipiravir (MCE)	47.1	0.1	0.2	44.7	0.5	ratio

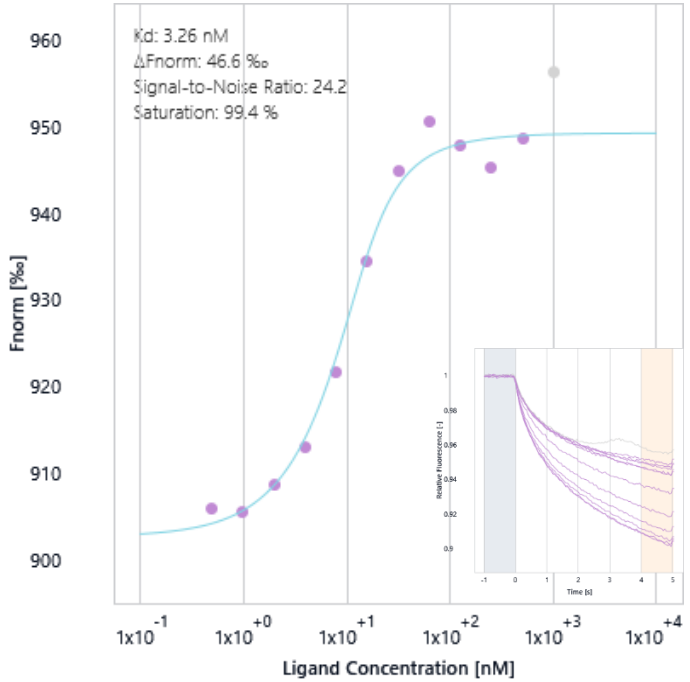
TRIC (Dianthus)

nsp12 (DVT1, PC13929-1)

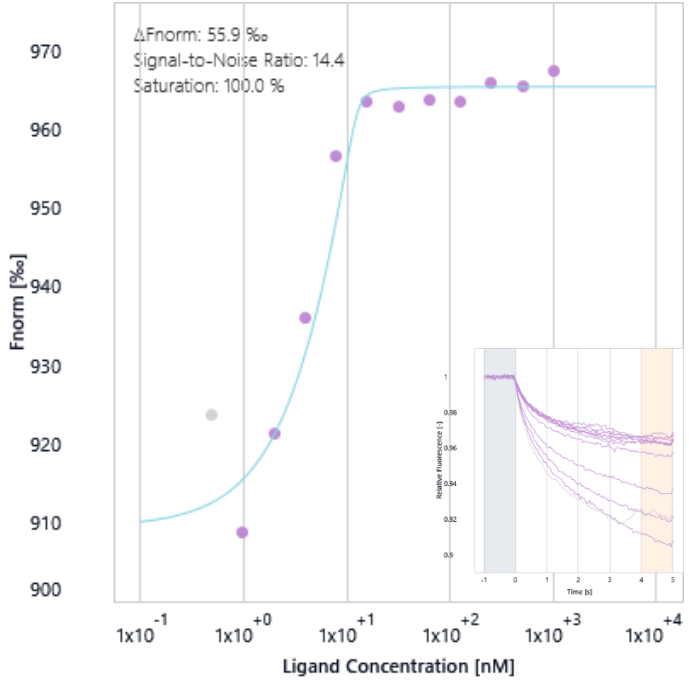
TRIC Assay Conditions

Fluor. Molecule	25 nM RED-tris-NTA 1st gen. 25 nM RED-tris-NTA 2nd gen.	
Fluorophore	RED-tris-NTA 1st and 2nd gen.	
Instrument	Dianthus NT.23PicoDuo	
Measurement parameter	LED Power: 11 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Singlicates	
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.05% Tween20 Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.5 mM ATP, 0.05% Tween20	
Titrant	nsp12 (DVT1, PC13929-1)	1000 – 0.49 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. vs. nsp12 (DVT1, PC13929-1) at Buffer 1



RED-tris-NTA 1st gen.

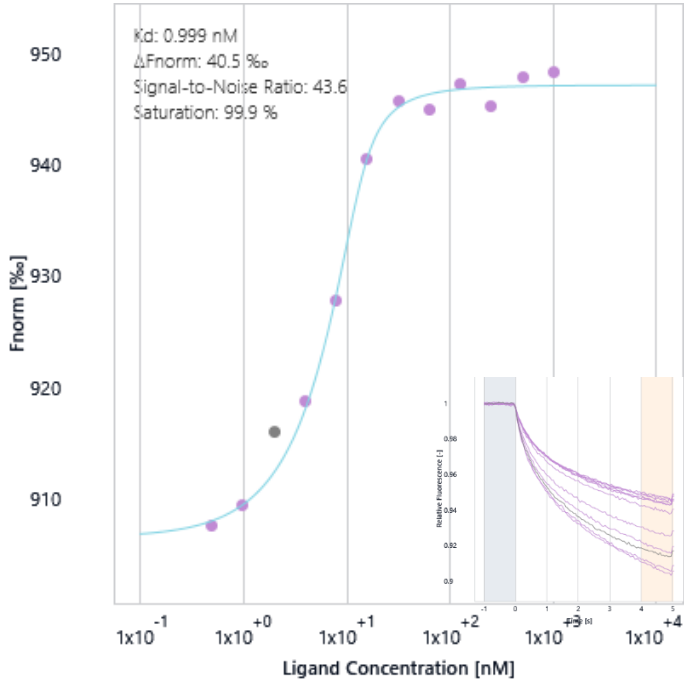


RED-tris-NTA 2nd gen.

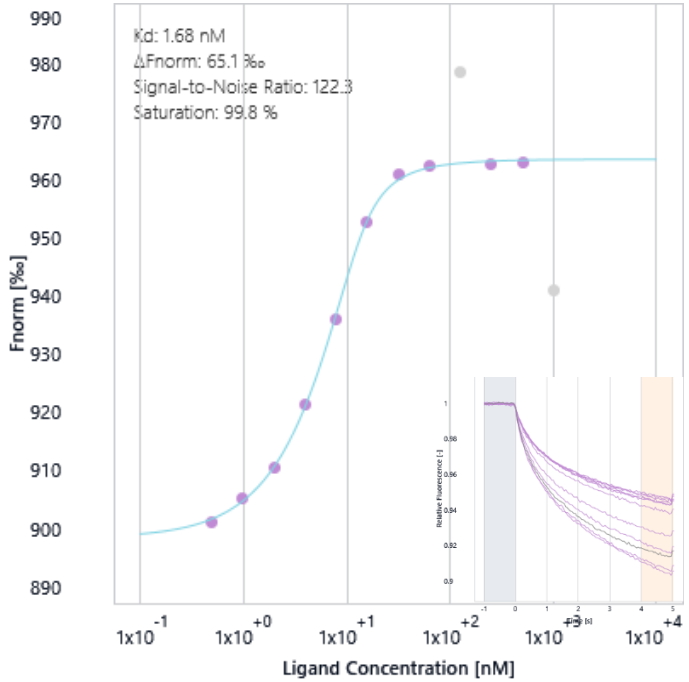
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	RED-tris-NTA 1st gen.	nsp12	3.3E-09	1.8E-09	5.9E-09	46.6	24.2	5	Buffer 1
RED-tris-NTA 2nd gen.	RED-tris-NTA 2nd gen.	nsp12	1.0E-10	1.1E-14	8.8E-07	55.9	14.4	5	Buffer 1

- RED-tris-NTA 1st gen. binds nsp12 with a determined K_D of 3.3 nM.
- RED-tris-NTA 2nd gen. binds nsp12 with a determined K_D of 0.1 nM.
- Both dyes can be used to non-covalently label nsp12.

RED-tris-NTA 1st and 2nd gen. vs. nsp12 (DVT1, PC13929-1) at Buffer 2



RED-tris-NTA 1st gen.



RED-tris-NTA 2nd gen.

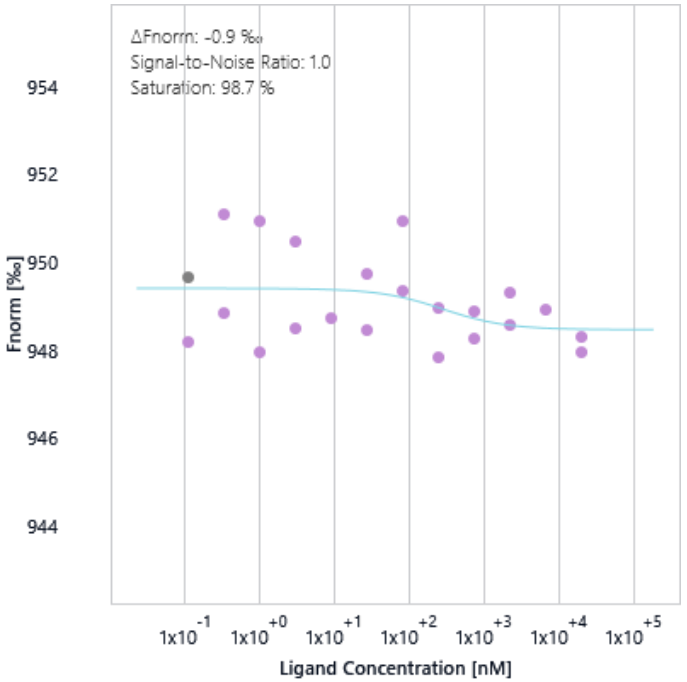
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	RED-tris-NTA 1st gen.	nsp12	1.0E-10	-	-	40.5	43.6	5	Buffer 2
RED-tris-NTA 2nd gen.	RED-tris-NTA 2nd gen.	nsp12	1.7E-09	1.4E-09	2.0E-09	65.1	122.3	5	Buffer 2

- RED-tris-NTA 1st gen. binds nsp12 with a determined K_D of 1.0 nM.
- RED-tris-NTA 2nd gen. binds nsp12 with a determined K_D of 1.7 nM.
- Both dyes can be used to non-covalently label nsp12.

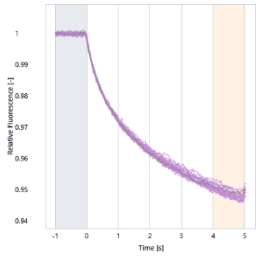
TRIC Assay Conditions

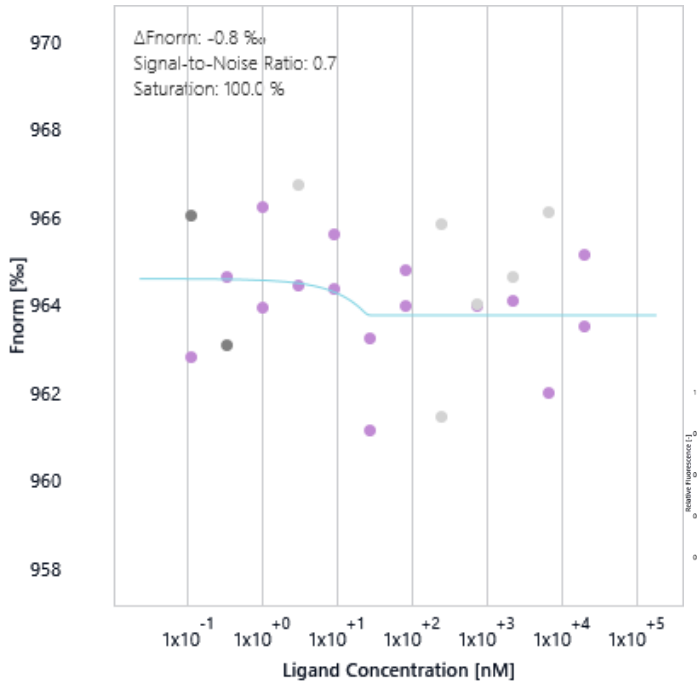
Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	25 nM protein / 12.5 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 23 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.05% Tween20 Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.5 mM ATP, 0.05% Tween20		
Titrant	GS-443902 trisodium (active Remdesivir metabolite)	JDI-880	20 µM – 0.11 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. vs. nsp12 (DVT1, PC13929-1) in Buffer 1

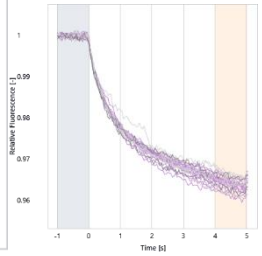


RED-tris-NTA 1st gen.





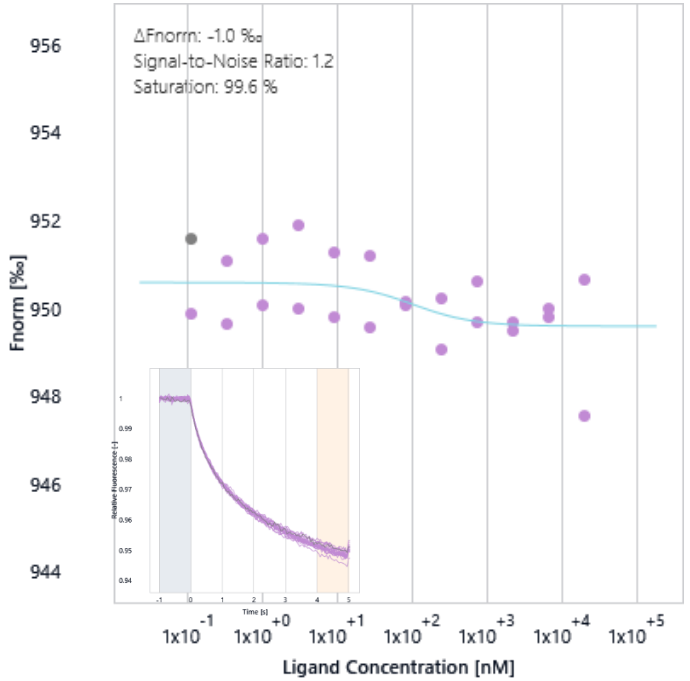
RED-tris-NTA 2nd gen.



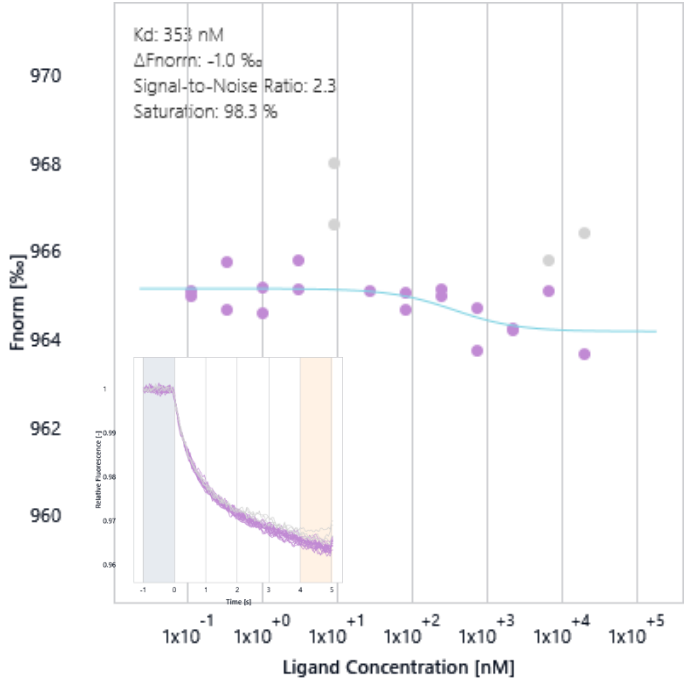
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	nsp12	GS-443902 trisodium	-	-	-	-	-	5	Buffer 1
RED-tris-NTA 2nd gen.	nsp12	GS-443902 trisodium	-	-	-	-	-	5	Buffer 1

- RED-tris-NTA 1st and 2nd gen. labelled nsp12 does not bind GS-443902 trisodium in buffer 1.

RED-tris-NTA 1st and 2nd gen. vs. nsp12 (DVT1, PC13929-1) in Buffer 2



RED-tris-NTA 1st gen.



RED-tris-NTA 2nd gen.

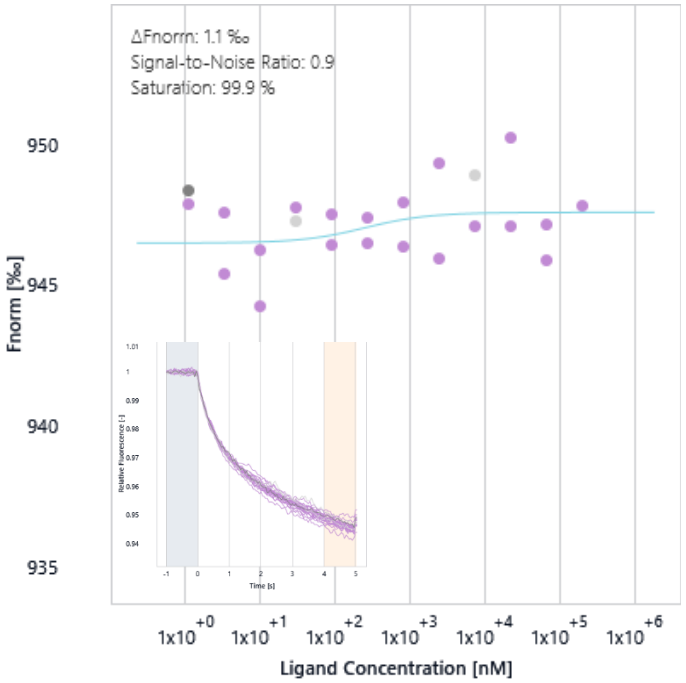
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	nsp12	GS-443902 trisodium	-	-	-	-	-	5	Buffer 2
RED-tris-NTA 2nd gen.	nsp12	GS-443902 trisodium	-	-	-	-	-	5	Buffer 2

- RED-tris-NTA 1st and 2nd gen. labelled nsp12 does not bind GS-443902 trisodium in buffer 2.

TRIC Assay Conditions

Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	RED-tris-NTA 1st		
Labelling conditions	25 nM protein / 12.5 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 20 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.05% Tween20 DMSO: 2.5%		
Titrant	GS-443902 trisodium Ribavirin (ICN-1229) Favipiravir (MCE)	JDI-880 JDI-879 JDI-881	200 µM – 1.13 nM (12 conc.)

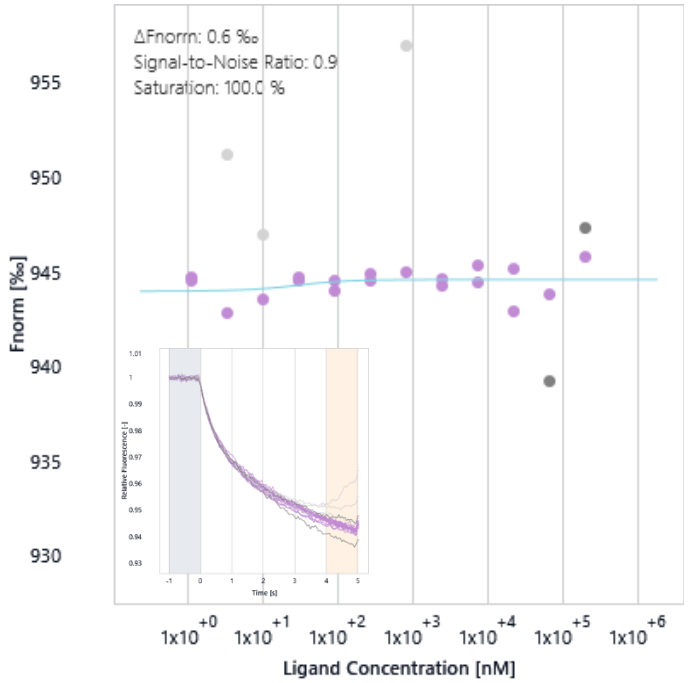
RED-tris-NTA 1st labelled nsp12 (DVT1, PC13929-1) vs. GS-443902 trisodium



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	nsp12	GS-443902 trisodium	-	-	-	-	-	5	

- RED-tris-NTA labelled nsp12 does not bind GS-443902 trisodium.

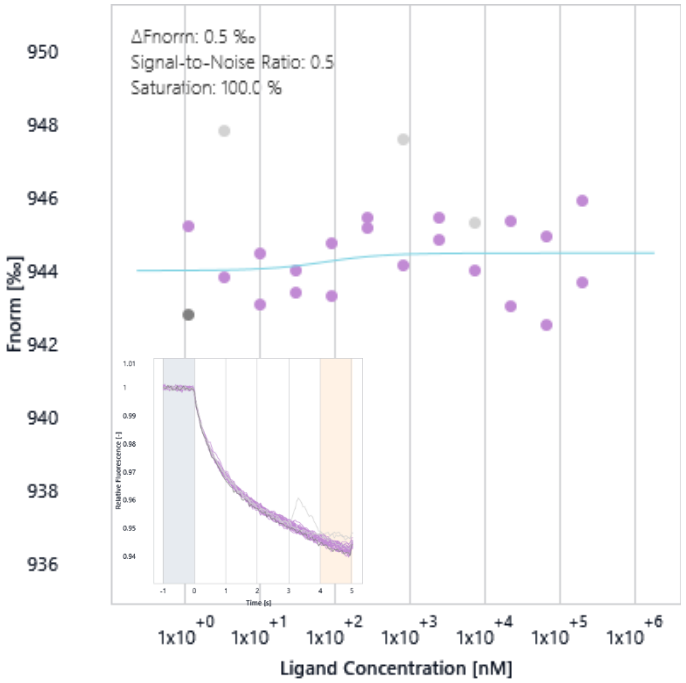
RED-tris-NTA 1st labelled nsp12 (DVT1, PC13929-1) vs. Ribavirin (ICN-1229)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	nsp12	Ribavirin (ICN-1229)	-	-	-	-	-	5	

- RED-tris-NTA labelled nsp12 does not bind Ribavirin (ICN-1229).

RED-tris-NTA 1st labelled nsp12 (DVT1, PC13929-1) vs. Favipiravir (MCE)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	nsp12	Favipiravir (MCE)	-	-	-	-	-	5	

- RED-tris-NTA labelled nsp12 does not bind Favipiravir (MCE).

Labelled MST

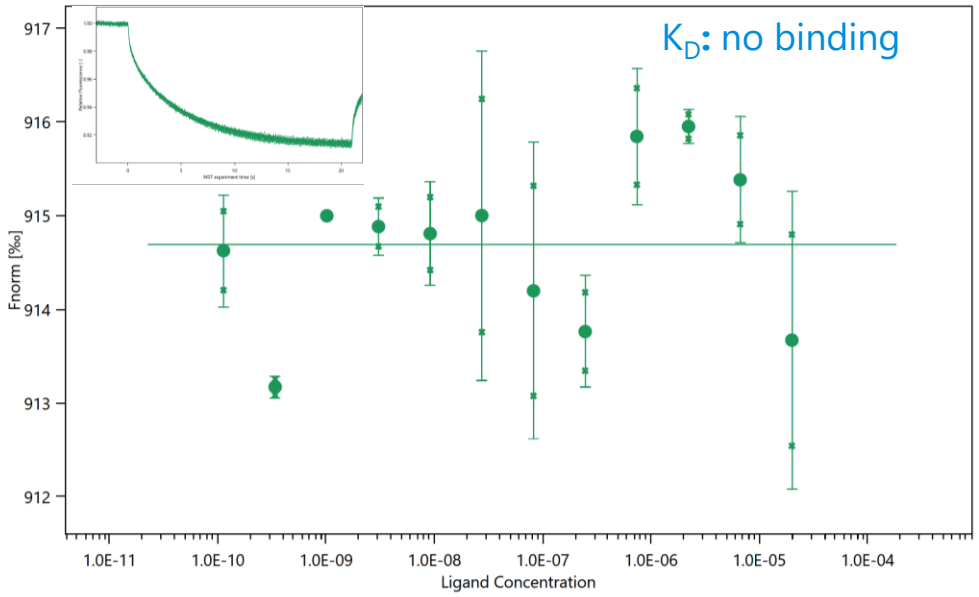
nsp12 (DVT1, PC13929-1)

MST labelled assay conditions

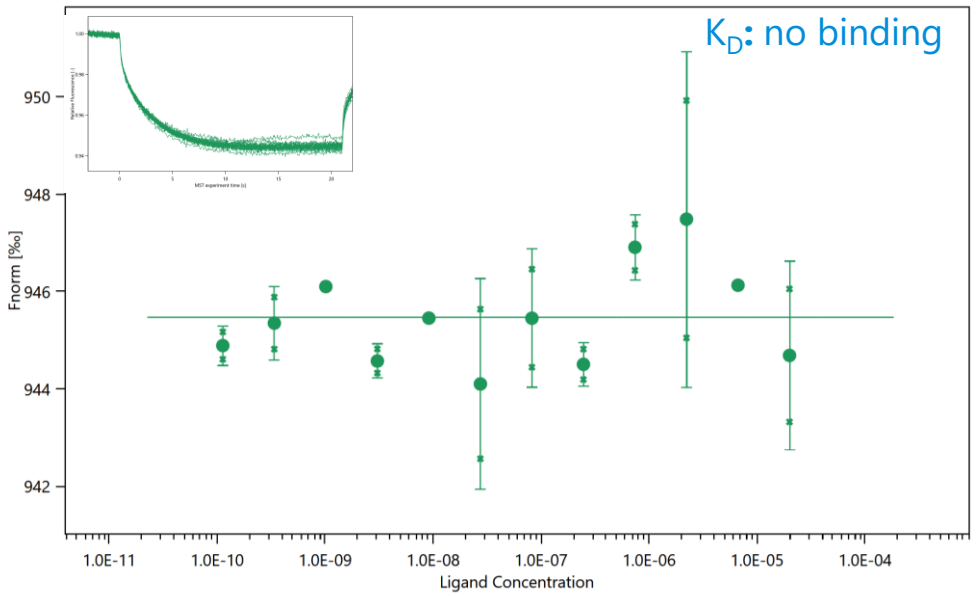
Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	25 nM protein / 12.5 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (03)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 80 % MST Power: 40 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.05% Tween20 Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.5 mM ATP, 0.05% Tween20		
Titrant	GS-443902 trisodium	JDI-880	20 µM – 0.11 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. labelled nsp12 (DVT1, PC13929-1) vs. GS-443902 trisodium in buffer 1

RED-tris-NTA 1st gen.



RED-tris-NTA 2nd gen.

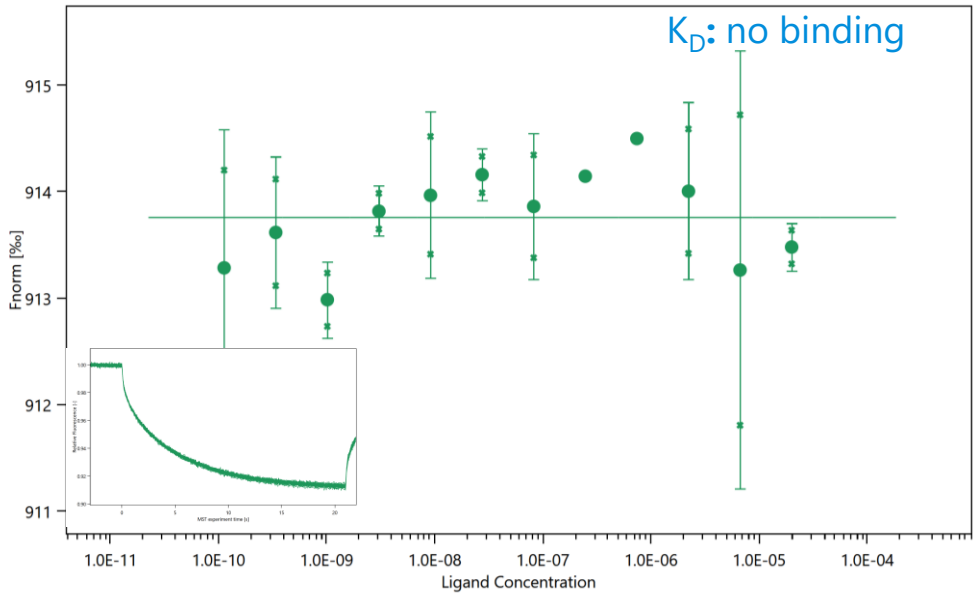


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 1st gen.	nsp12	GS-443902 trisodium	-	-	-	-	20	Buffer 1
RED-tris-NTA 2nd gen.	nsp12	GS-443902 trisodium	-	-	-	-	20	Buffer 1

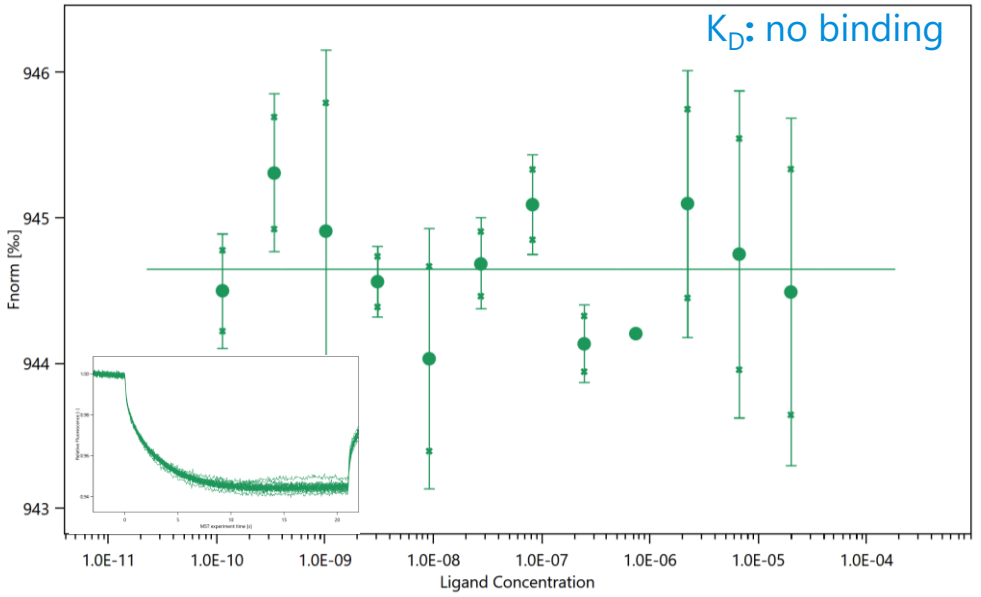
- RED-tris-NTA 1st gen. labeled nsp12 does not bind GS-443902 trisodium.
- RED-tris-NTA 2nd gen. labeled nsp12 does not bind GS-443902 trisodium.

RED-tris-NTA 1st and 2nd gen. labelled nsp12 (DVT1, PC13929-1) vs. GS-443902 trisodium in buffer 2

RED-tris-NTA 1st gen.



RED-tris-NTA 2nd gen.



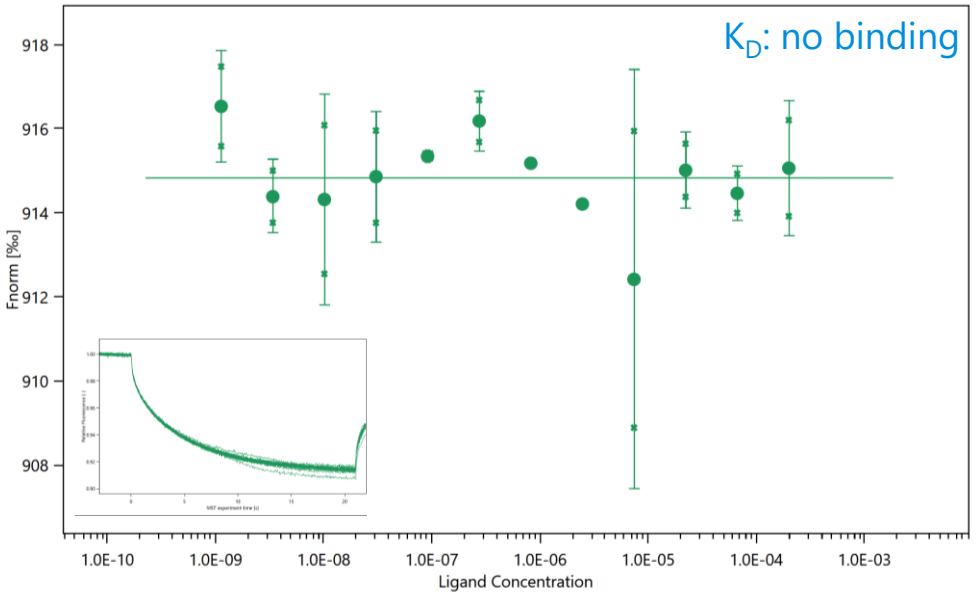
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 1st gen.	nsp12	GS-443902 trisodium	-	-	-	-	20	Buffer 2
RED-tris-NTA 2nd gen.	nsp12	GS-443902 trisodium	-	-	-	-	20	Buffer 2

- RED-tris-NTA 1st gen. labelled nsp12 does not bind GS-443902 trisodium.
- RED-tris-NTA 2nd gen. labelled nsp12 does not bind GS-443902 trisodium.

MST labelled assay conditions

Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	25 nM protein / 12.5 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (03)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 100 % MST Power: 40 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.05% Tween20 DMSO: 2.5%		
Titrant	GS-443902 trisodium Ribavirin (ICN-1229) Favipiravir (MCE)	JDI-880 JDI-879 JDI-881	200 µM – 1.13 nM (12 conc.)

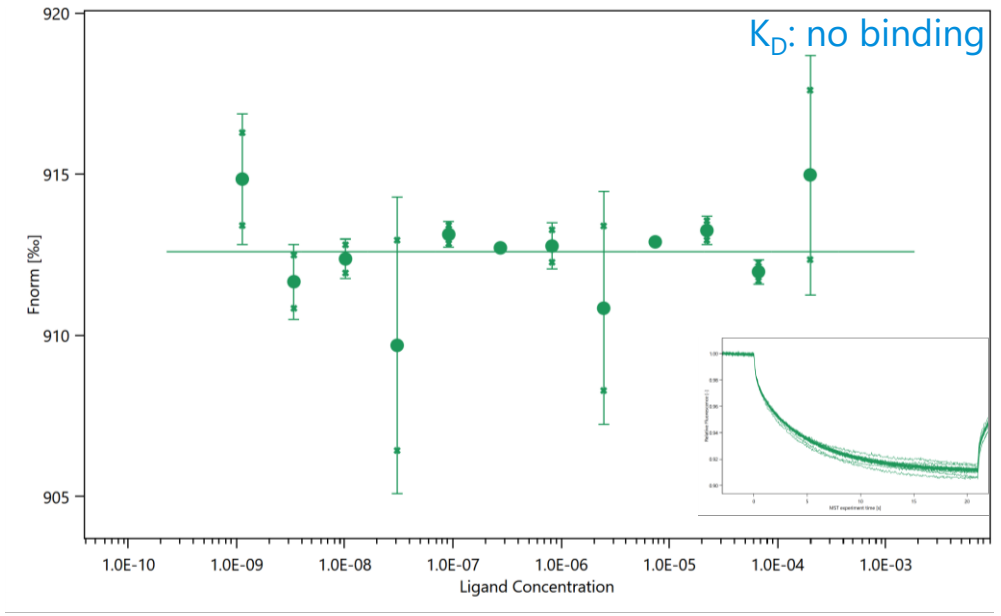
[RED-tris-NTA 1st labelled nsp12 (DVT1, PC13929-1) vs. GS-443902 trisodium



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 1st gen.	nsp12	GS-443902 trisodium	-	-	-	-	20	

- RED-tris-NTA labelled nsp12 does not bind GS-443902 trisodium.

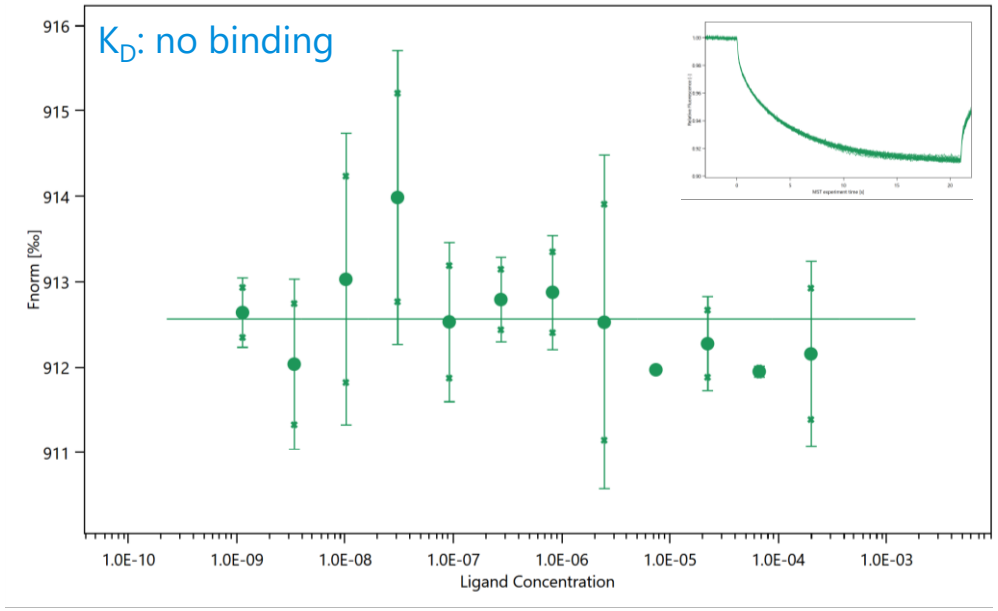
[RED-tris-NTA 1st labelled nsp12 (DVT1, PC13929-1) vs. Ribavirin (ICN-1229)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 1st gen.	nsp12	Ribavirin (ICN-1229)	-	-	-	-	20	

- RED-tris-NTA labelled nsp12 does not bind Ribavirin (ICN-1229).

RED-tris-NTA 1st labelled nsp12 (DVT1, PC13929-1) vs. Favipiravir (MCE)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 1st gen.	nsp12	Favipiravir (MCE)	-	-	-	-	20	

- RED-tris-NTA labelled nsp12 does not bind Favipiravir (MCE).

Next steps

- As neither nsp12 nor the RdRp complex allow biophysical characterization of compound interactions using TRIC (Dianthus), we recommend to stop the project

Crelux GmbH a WuXi AppTec company
Dr. Saskia Villinger
Senior Scientist and Deputy Head of Biophysics & Screening
Am Klopferspitz 19a
82152 Martinsried
Germany

Saskia.Villinger@wuxiapptec.com
www.crelux.com
www.wuxiapptec.com