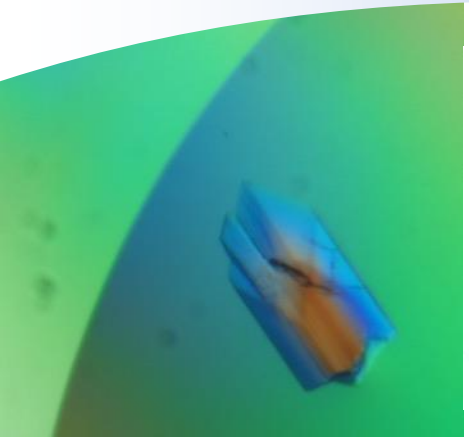
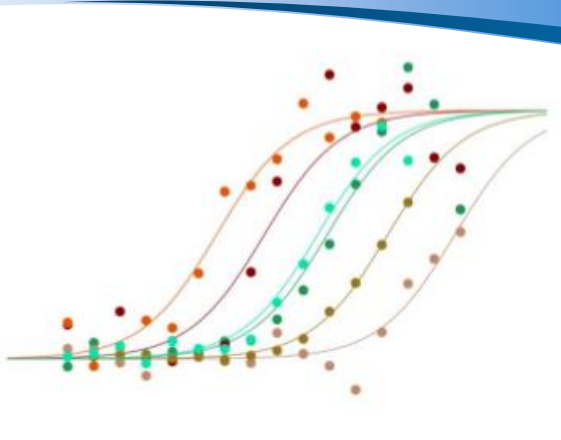
JEDI Program/JDI01

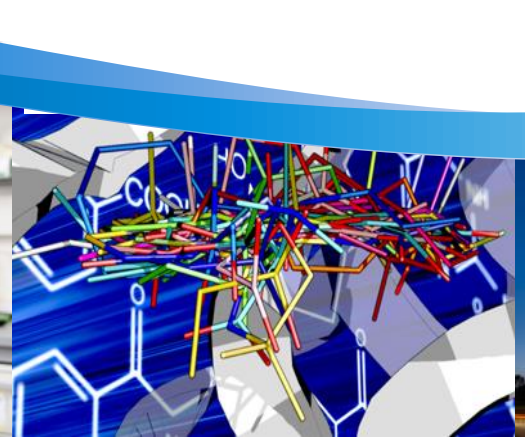
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

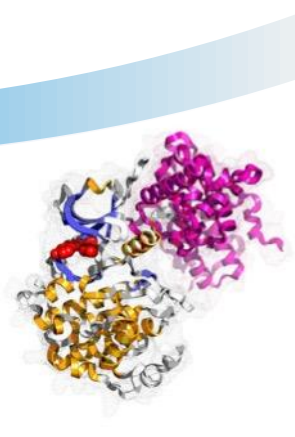
SARS-CoV-2 nsp12

MST/ TRIC measurements

June 24, 2021





Status

- TRIC (Dianthus) / Labelled MST:
  - Binding of Suramin to nsp12 was tested on the Dianthus and NT.115 instrument using the established labelling setup with RED-Tris-NTA dyes.
    - On both instruments Suramin binds to nsp12 with K_D values of 250 nM – 1.3 µM
    - Larger amplitude, signal-to-noise ratio and lower K_D values were observed with the 2nd generation dye, which we recommend to use for compound screening against nsp12.

TRIC (Dianthus)

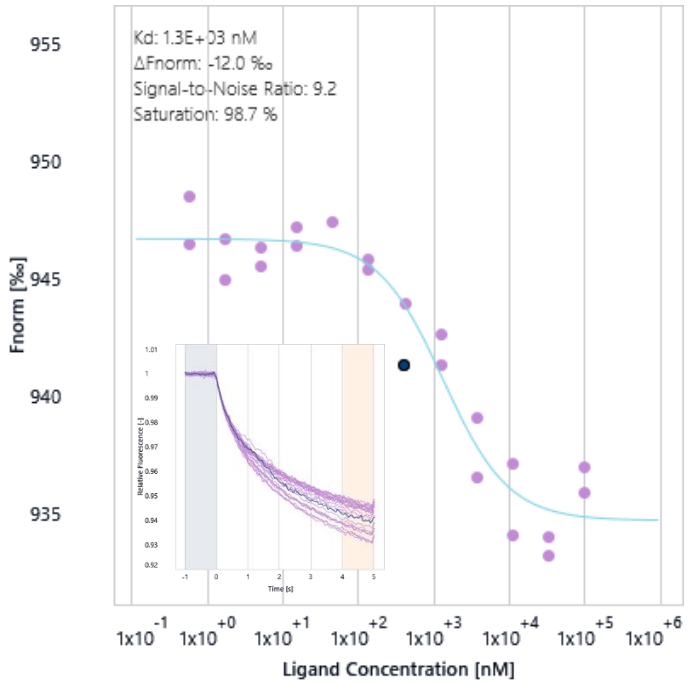
nsp12 (DVT1, PC13929-1)

TRIC Assay Conditions

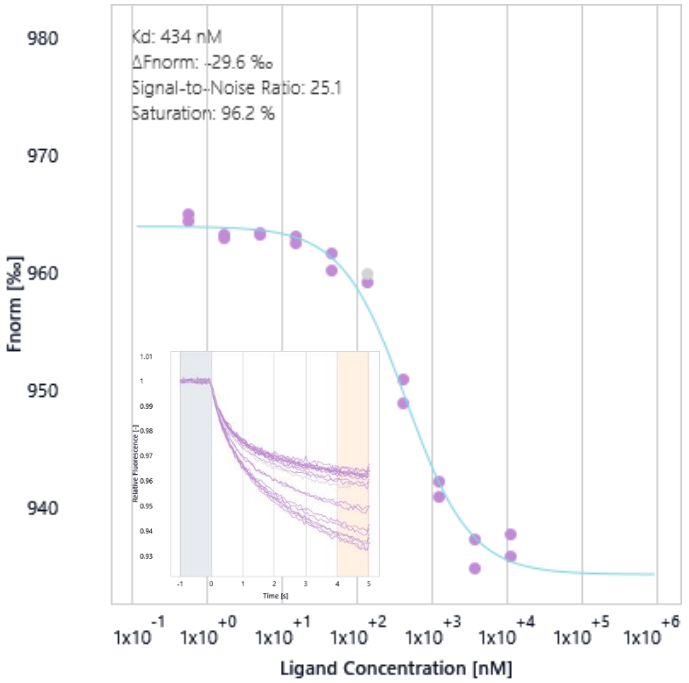
Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	25 nM protein / 12.5 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 18 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.005% Tween20 DMSO: 2.5%		
Titrant	Suramin		100 µM – 0.56 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. labelled nsp12 vs. Suramin

a WuXi AppTec company



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 (DVT1)	Suramin	1.3E-06	7.2E-07	2.3E-6	- 12	9.2	5	
RED-tris-NTA 2nd gen.	nsp12 (DVT1)	Suramin	4.3E-07	3.3E-07	5.6E-7	-29.6	25.1	5	Auto-fluorescence at high cpd concentration

- RED-tris-NTA 1st gen. labeled nsp12 binds to Suramin with a determined K_D of 1.3 µM.
- RED-tris-NTA 2nd gen. labeled nsp12 binds to Suramin with a determined K_D of 434 nM and high ΔFnorm and signal-to-noise ratio. Auto-fluorescence was observed at high cpd concentration (data points excluded).

Labelled MST

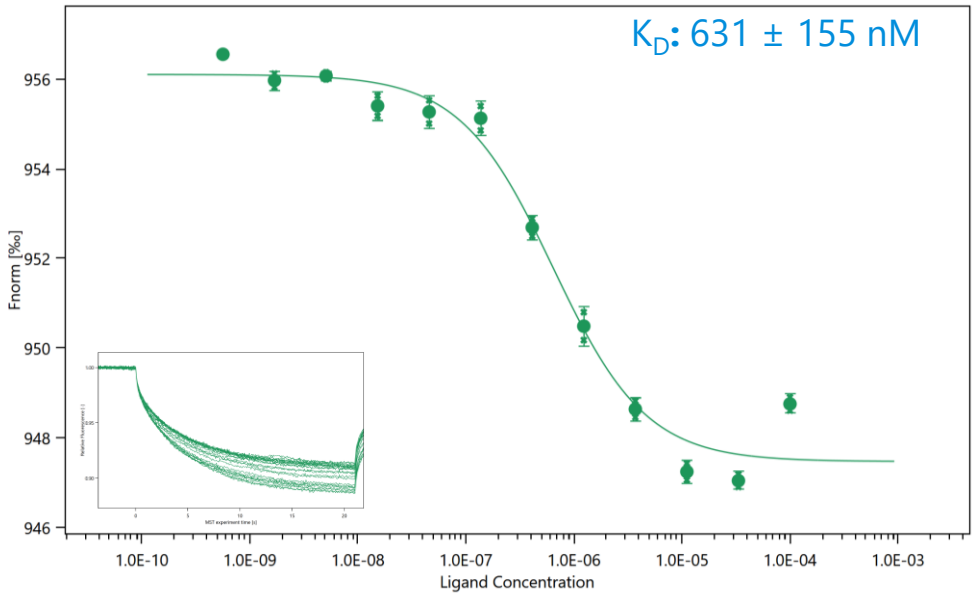
nsp12 (DVT1, PC13929-1)

MST labelled assay conditions

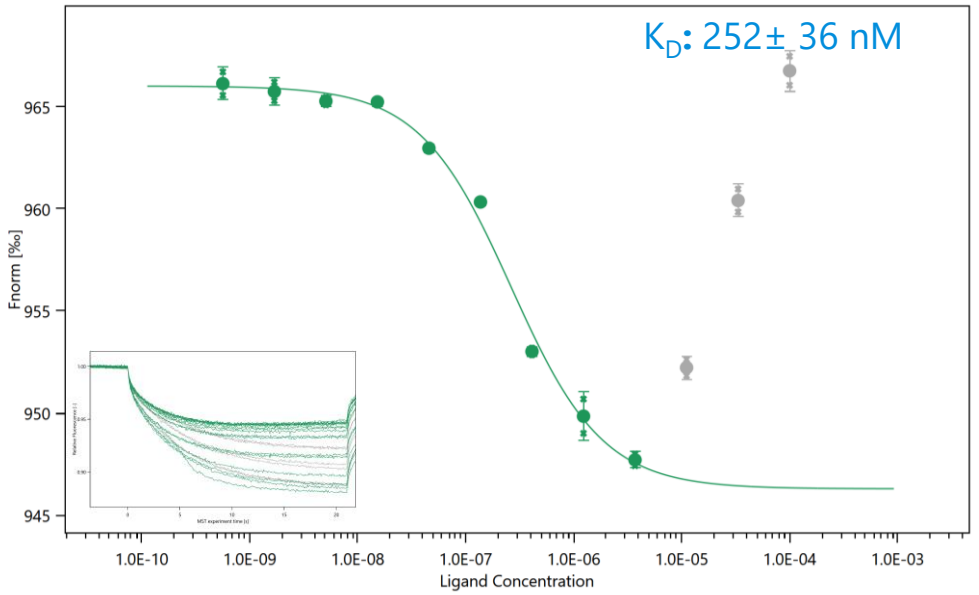
Fluor. Molecule	25 nM nsp12 (DVT1, PC13929-1)		
Fluorophore	RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	25 nM protein / 12.5 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (02)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 60 % MST Power: 40 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 2.5 mM DTT, 0.005% Tween20 DMSO: 2.5%		
Titrant	Suramin		100 µM – 0.56 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. labelled nsp12 vs. Suramin

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 (DVT1)	Suramin	6.3E-07	1.6E-07	8.6	16.1	2.5	
RED-tris-NTA 2nd gen.	nsp12 (DVT1)	Suramin	2.5E-07	3.6E-08	19.7	37.4	2.5	Auto-fluorescence at high cpd concentration

- RED-tris-NTA 1st gen. labeled nsp12 binds to Suramin with a determined K_D of 631 nM.
- RED-tris-NTA 2nd gen. labeled nsp12 binds to Suramin with a determined K_D of 252 nM and high ΔFnorm and signal-to-noise ratio. Auto-fluorescence was observed at high cpd concentration (grey points).

Next steps

- Decide if Suramin can be used as positive control for compound binding to nsp12. For nsp12 we recommend the 2nd generation RED-Tris-NTA dye for labelling.
- Optional: MST/Dianthus assay setup using Pasteur protein (nsp12-nsp7-nsp8 complex) – is at least one of the proteins His-tagged?
- Decide which protein to use for compound screening

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