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JEDI Program/JDI01

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

8-point MST screen Screen

SARS-CoV-2-Nucleocapsid protein

October 22, 2021

Status

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▪ MST Labelled

- 8-pt-screening on RED-TRIS-NTA 2nd gen. labelled SARS-CoV-2-Nucleocapsid protein was successfully established using VHH E4-3 (NTD nanobody) as a positive control.
- 119 compounds were screened for binding to RED-TRIS-NTA 2nd gen. labelled SARS-CoV-2-Nucleocapsid protein using 8-pt dilution series (singlicate) from 100 µM – 45.7 nM, showing the following results:

Category	20 sec MST On Time*
Binder	33 (28%)
Weak Binder	23 (19%)
Aggregation	4 (3%)
Autofluorescence	1 (1%)
Non-Binder	37 (31%)
Potential Binder	21 (18%)

* Some compounds analyzed at 2.5s or 5s

- K_D values were estimated from the 8-pt-data, but need to be confirmed using 12-pt-titrations in duplicates

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Labelled MST

SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)

Labelled assay conditions:

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Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	RED-tris-NTA 2nd gen.		
Protein Labelling	100 nM protein / 25 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: 90 % MST Power: 60% 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, 0.1% PEG 8000, 0.05% Tween20, 2 mM DTT DMSO: 2.5%		
Tool compound	VHH E4-3 (NTD nanobody) 119 cpds	EEF1 (PD14989-1) (stored at 4°C) See next page	500 nM – 0.23 nM, 500 nM – 3.91 nM (8 conc.) 100 µM – 45.7 nM (8 conc.)

Compound list

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Compound	Crelux code	Dilution series
N_virS283046	JDI-482	100 µM – 45.7 nM (8 conc.)
N_vir81522176	JDI-483	100 µM – 45.7 nM (8 conc.)
N_mli03333297	JDI-484	100 µM – 45.7 nM (8 conc.)
N_vir22895394	JDI-485	100 µM – 45.7 nM (8 conc.)
N_virS5561807	JDI-486	100 µM – 45.7 nM (8 conc.)
N_vir90530145	JDI-487	100 µM – 45.7 nM (8 conc.)
N_virS7649529	JDI-488	100 µM – 45.7 nM (8 conc.)
N_mli63842155	JDI-489	100 µM – 45.7 nM (8 conc.)
N_virS567325	JDI-490	100 µM – 45.7 nM (8 conc.)
N_mli02198304	JDI-491	100 µM – 45.7 nM (8 conc.)
N_vir10573874	JDI-492	100 µM – 45.7 nM (8 conc.)
N_mli70078856	JDI-493	100 µM – 45.7 nM (8 conc.)
N_virS1728196	JDI-494	100 µM – 45.7 nM (8 conc.)
N_vir88769404	JDI-495	100 µM – 45.7 nM (8 conc.)
N_virS3121055	JDI-496	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
N_mli90888336	JDI-497	100 µM – 45.7 nM (8 conc.)
nps5-05397113	JDI-498	100 µM – 45.7 nM (8 conc.)
N_mli85056505	JDI-499	100 µM – 45.7 nM (8 conc.)
N_mli23144428	JDI-500	100 µM – 45.7 nM (8 conc.)
N_mli53397017	JDI-501	100 µM – 45.7 nM (8 conc.)
N_mli04851030	JDI-502	100 µM – 45.7 nM (8 conc.)
N_vir57660628	JDI-503	100 µM – 45.7 nM (8 conc.)
N_vir26573770	JDI-504	100 µM – 45.7 nM (8 conc.)
N_mli35944272	JDI-505	100 µM – 45.7 nM (8 conc.)
N_mli67968109	JDI-506	100 µM – 45.7 nM (8 conc.)
N_mli55472868	JDI-507	100 µM – 45.7 nM (8 conc.)
N_vir28697689	JDI-508	100 µM – 45.7 nM (8 conc.)
N_mli96196880	JDI-509	100 µM – 45.7 nM (8 conc.)
N_vir28515645	JDI-510	100 µM – 45.7 nM (8 conc.)
N_vir88287350	JDI-511	100 µM – 45.7 nM (8 conc.)

Compound list

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Compound	Crelux code	Dilution series
N_vir28578150	JDI-512	100 µM – 45.7 nM (8 conc.)
N_mli10136998	JDI-513	100 µM – 45.7 nM (8 conc.)
N_virS3222347	JDI-514	100 µM – 45.7 nM (8 conc.)
N_mli1777542	JDI-515	100 µM – 45.7 nM (8 conc.)
N_mli98112840	JDI-516	100 µM – 45.7 nM (8 conc.)
N_vir6018734	JDI-517	100 µM – 45.7 nM (8 conc.)
N_mli82816373	JDI-518	100 µM – 45.7 nM (8 conc.)
N_mli4976341	JDI-519	100 µM – 45.7 nM (8 conc.)
N_mli8141378	JDI-520	100 µM – 45.7 nM (8 conc.)
N_mli77484258	JDI-521	100 µM – 45.7 nM (8 conc.)
N_mli59963320	JDI-522	100 µM – 45.7 nM (8 conc.)
N_vir81857663	JDI-523	100 µM – 45.7 nM (8 conc.)
nps5-1883445	JDI-524	100 µM – 45.7 nM (8 conc.)
N_vir12432176	JDI-525	100 µM – 45.7 nM (8 conc.)
N_mli14744111	JDI-526	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
N_mli85170109	JDI-527	100 µM – 45.7 nM (8 conc.)
N_mli63697292	JDI-528	100 µM – 45.7 nM (8 conc.)
N_vir76654816	JDI-529	100 µM – 45.7 nM (8 conc.)
N_mli8051337	JDI-530	100 µM – 45.7 nM (8 conc.)
N_mli95654891	JDI-531	100 µM – 45.7 nM (8 conc.)
N_mli52788352	JDI-532	100 µM – 45.7 nM (8 conc.)
N_vir20581701	JDI-533	100 µM – 45.7 nM (8 conc.)
N_mli0326111	JDI-534	100 µM – 45.7 nM (8 conc.)
N_mli28683431	JDI-535	100 µM – 45.7 nM (8 conc.)
N_mli69018947	JDI-536	100 µM – 45.7 nM (8 conc.)
N_mli96977340	JDI-537	100 µM – 45.7 nM (8 conc.)
N_mli3468538	JDI-538	100 µM – 45.7 nM (8 conc.)
N_virS3392340	JDI-539	100 µM – 45.7 nM (8 conc.)
N_vir29273343	JDI-540	100 µM – 45.7 nM (8 conc.)
N_mli14851671	JDI-541	100 µM – 45.7 nM (8 conc.)

Compound list

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Compound	Crelux code	Dilution series
N_mli23269068	JDI-542	100 µM – 45.7 nM (8 conc.)
nps5-36126069	JDI-543	100 µM – 45.7 nM (8 conc.)
N_mli62721963	JDI-544	100 µM – 45.7 nM (8 conc.)
N_mli06391133	JDI-545	100 µM – 45.7 nM (8 conc.)
nps5-23032612	JDI-546	100 µM – 45.7 nM (8 conc.)
N_vir71031069	JDI-547	100 µM – 45.7 nM (8 conc.)
N_mli78615777	JDI-548	100 µM – 45.7 nM (8 conc.)
N_vir17530618	JDI-549	100 µM – 45.7 nM (8 conc.)
nps5-44255312	JDI-550	100 µM – 45.7 nM (8 conc.)
N_vir12978576	JDI-551	100 µM – 45.7 nM (8 conc.)
N_mli40170382	JDI-552	100 µM – 45.7 nM (8 conc.)
N_mli17233350	JDI-553	100 µM – 45.7 nM (8 conc.)
N_mli10177485	JDI-554	100 µM – 45.7 nM (8 conc.)
N_vir93990858	JDI-555	100 µM – 45.7 nM (8 conc.)
N_mli74895785	JDI-556	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
N_vir19657070	JDI-557	100 µM – 45.7 nM (8 conc.)
N_mli24324649	JDI-558	100 µM – 45.7 nM (8 conc.)
N_mli46366081	JDI-559	100 µM – 45.7 nM (8 conc.)
N_mli17081372	JDI-560	100 µM – 45.7 nM (8 conc.)
N_mli91949254	JDI-561	100 µM – 45.7 nM (8 conc.)
N_vir64136516	JDI-562	100 µM – 45.7 nM (8 conc.)
N_mli95750138	JDI-563	100 µM – 45.7 nM (8 conc.)
N_vir50513282	JDI-564	100 µM – 45.7 nM (8 conc.)
N_mli53143159	JDI-565	100 µM – 45.7 nM (8 conc.)
N_mli29841695	JDI-566	100 µM – 45.7 nM (8 conc.)
nps5-15399474	JDI-567	100 µM – 45.7 nM (8 conc.)
N_mli50324464	JDI-568	100 µM – 45.7 nM (8 conc.)
N_mli06842807	JDI-569	100 µM – 45.7 nM (8 conc.)
N_mli57710940	JDI-570	100 µM – 45.7 nM (8 conc.)
N_mli76891947	JDI-571	100 µM – 45.7 nM (8 conc.)

Compound list

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Compound	Crelux code	Dilution series
N_vir2052994	JDI-572	100 µM – 45.7 nM (8 conc.)
N_mli0529479	JDI-573	100 µM – 45.7 nM (8 conc.)
N_vir31956114	JDI-574	100 µM – 45.7 nM (8 conc.)
nps5-1514688	JDI-575	100 µM – 45.7 nM (8 conc.)
N_vir35792872	JDI-576	100 µM – 45.7 nM (8 conc.)
N_mli90699919	JDI-577	100 µM – 45.7 nM (8 conc.)
N_vir24029780	JDI-578	100 µM – 45.7 nM (8 conc.)
N_mli29092492	JDI-579	100 µM – 45.7 nM (8 conc.)
N_mli19997575	JDI-580	100 µM – 45.7 nM (8 conc.)
N_mli66038635	JDI-581	100 µM – 45.7 nM (8 conc.)
N_mli73875714	JDI-582	100 µM – 45.7 nM (8 conc.)
N_mli81170343	JDI-583	100 µM – 45.7 nM (8 conc.)
N_vir42139901	JDI-584	100 µM – 45.7 nM (8 conc.)
N_mli84819808	JDI-585	100 µM – 45.7 nM (8 conc.)
N_vir59350848	JDI-586	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
N_mli59499651	JDI-587	100 µM – 45.7 nM (8 conc.)
N_vir21851273	JDI-588	100 µM – 45.7 nM (8 conc.)
N_mli1962727	JDI-589	100 µM – 45.7 nM (8 conc.)
N_mli75388875	JDI-590	100 µM – 45.7 nM (8 conc.)
N_mli83969946	JDI-591	100 µM – 45.7 nM (8 conc.)
nps5-21028425	JDI-592	100 µM – 45.7 nM (8 conc.)
N_vir37574304	JDI-593	100 µM – 45.7 nM (8 conc.)
N_vir41794128	JDI-594	100 µM – 45.7 nM (8 conc.)
N_vir35275625	JDI-595	100 µM – 45.7 nM (8 conc.)
N_vir29881608	JDI-596	100 µM – 45.7 nM (8 conc.)
N_mli00238613	JDI-597	100 µM – 45.7 nM (8 conc.)
N_vir27248244	JDI-598	100 µM – 45.7 nM (8 conc.)
N_vir18499351	JDI-599	100 µM – 45.7 nM (8 conc.)
N_virS7029430	JDI-600	100 µM – 45.7 nM (8 conc.)

RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. VHH E4-3 - Tool compound day 1

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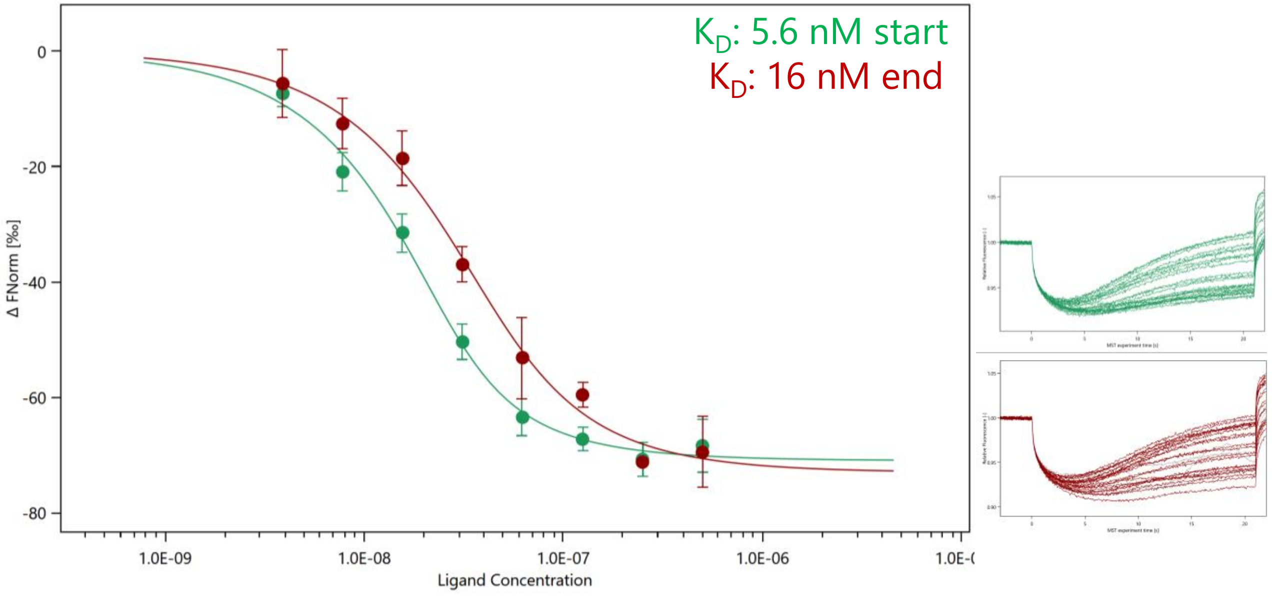
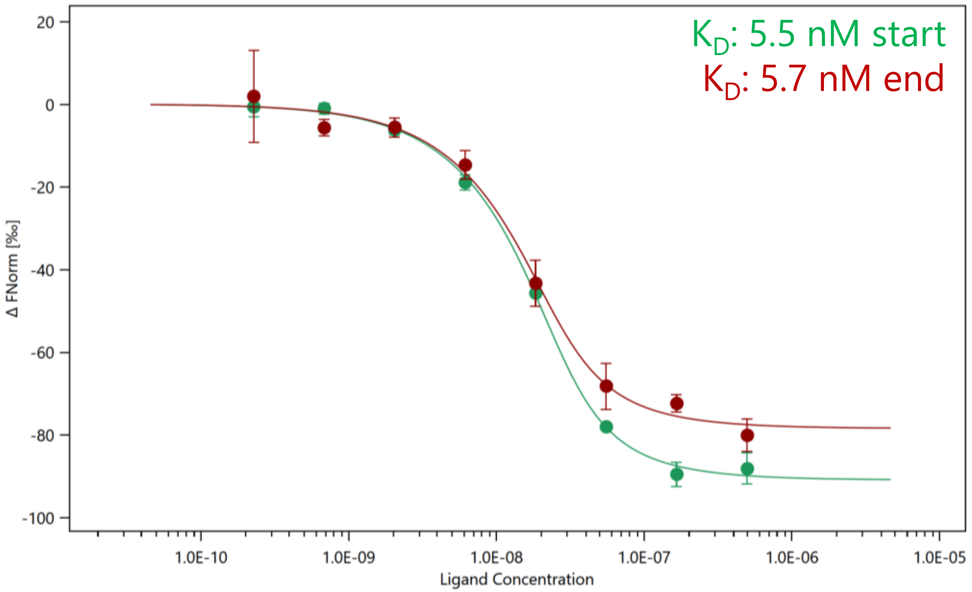
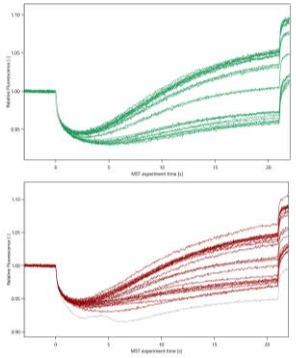


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	VHH E4-3	5.6E-09	2.2E-09 – 1.4E-08	71	41.1	20	Start (green)
RED-TRIS-NTA 2nd gen.	Nucleocapsid	VHH E4-3	1.6E-08	5.6E-09 – 4.6E-08	73	34.8	20	End (red)

VHH E4-3 binds to Nucleocapsid with estimated K_D value of 5.6 and 16 nM and similar ΔFnorm and signal-to-noise over the entire screen.

RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. VHH E4-3 - Tool compound day 2





Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	VHH E4-3	5.5E-09	3.1E-09 – 9.8E-09	91	75.5	20	Start (green)
RED-TRIS-NTA 2nd gen.	Nucleocapsid	VHH E4-3	5.7E-09	1.5E-09 – 2.2E-08	79	32.2	20	End (red)

VHH E4-3 binds to Nucleocapsid with estimated K_D value of 5.5 and 5.7 nM and similar ΔFnorm and signal-to-noise over the entire screen.

RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. VHH E4-3 - Tool compound day 3

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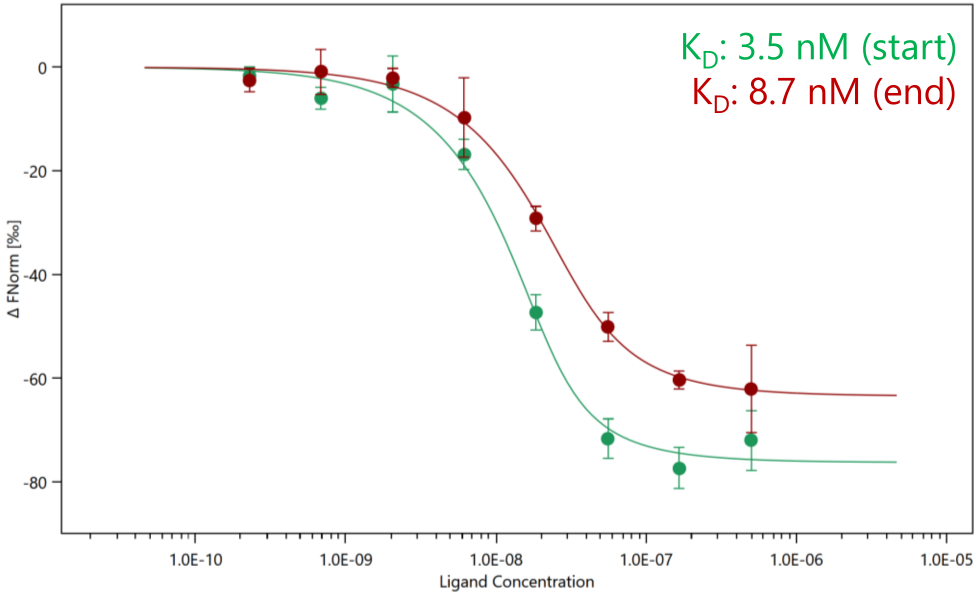

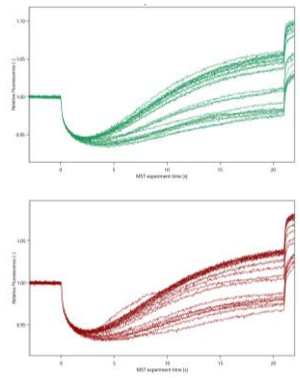

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	VHH E4-3	3.5E-09	1.6E-09 – 7.8E-09	76	26.4	20	Start (green)
RED-TRIS-NTA 2nd gen.	Nucleocapsid	VHH E4-3	8.7E-09	4.2E-09 – 1.8E-08	63	53.2	20	End (red)

VHH E4-3 binds to Nucleocapsid with estimated K_D value of 3.5 and 8.7 nM and similar ΔFnorm and signal-to-noise over the entire screen.

RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. compounds – examples Binders

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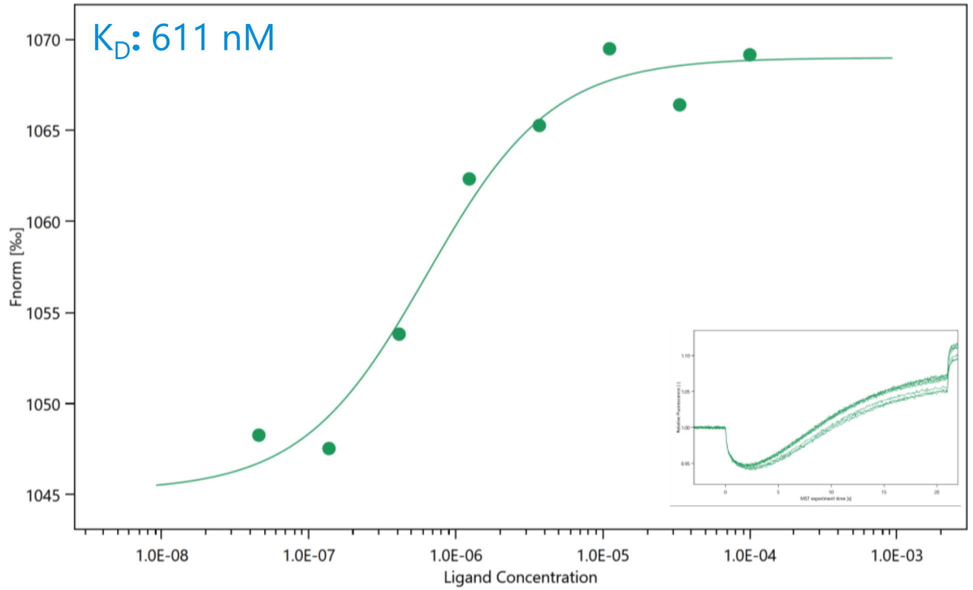
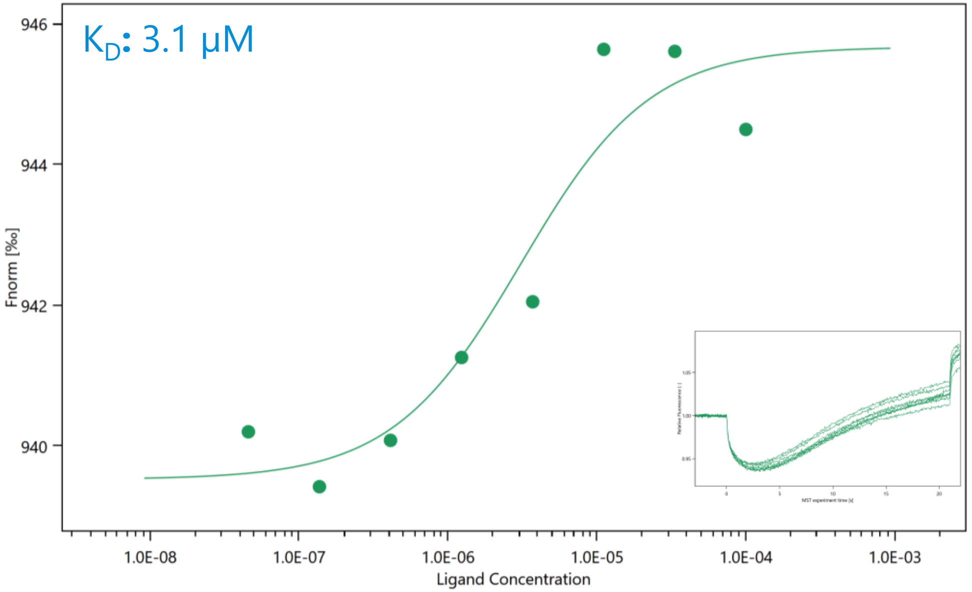
N_mli8141378 (JDI-520)



N_vir24029780 (JDI-578)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_mli8141378	6.1E-07	23.8	15.9	20	Binder
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_vir24029780	3.1E-06	6.1	8.1	2.5	Binder

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds N_mli8141378 with a determined K_D of 611 nM.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds N_vir24029780 with a determined K_D of 3.1 µM.

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RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. compounds – examples Weak Binders

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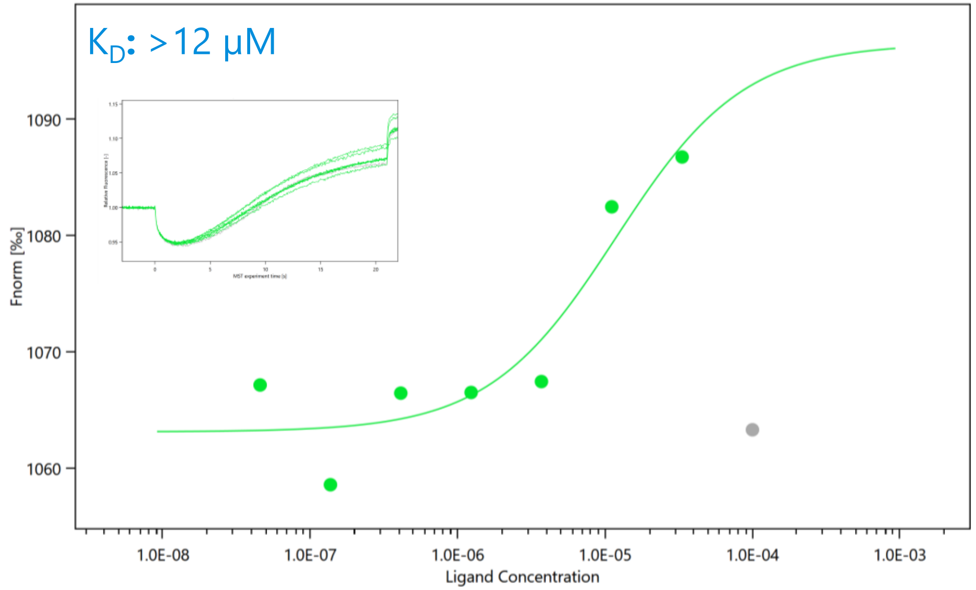
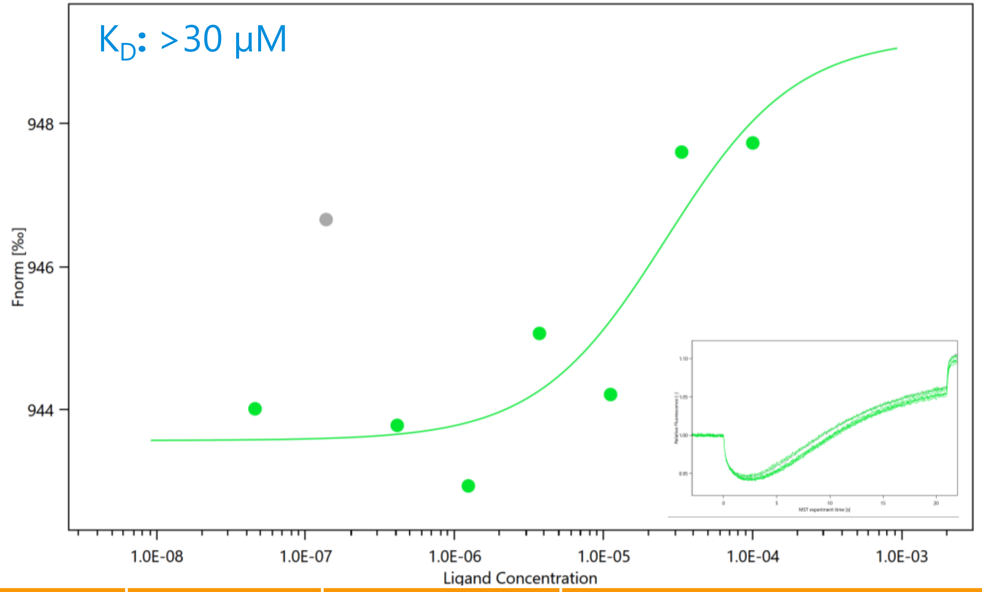
N_virS3222347 (JDI-514)



N_mli67968109 (JDI-506)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_virS3222347	>1.2E-05	33.4	9.9	20	Weak binder, no saturation reached
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_mli67968109	>2.6E-05	5.6	7.3	2.5	Weak binder, no saturation reached

- RED-tris-NTA 2nd gen. labelled Nucleocapsid weakly binds N_mli23144428 with a determined K_D >12 µM without reaching saturation.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid weakly binds N_mli23144428 with a determined K_D >30 µM without reaching saturation.

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RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. compounds – examples Potential Binders

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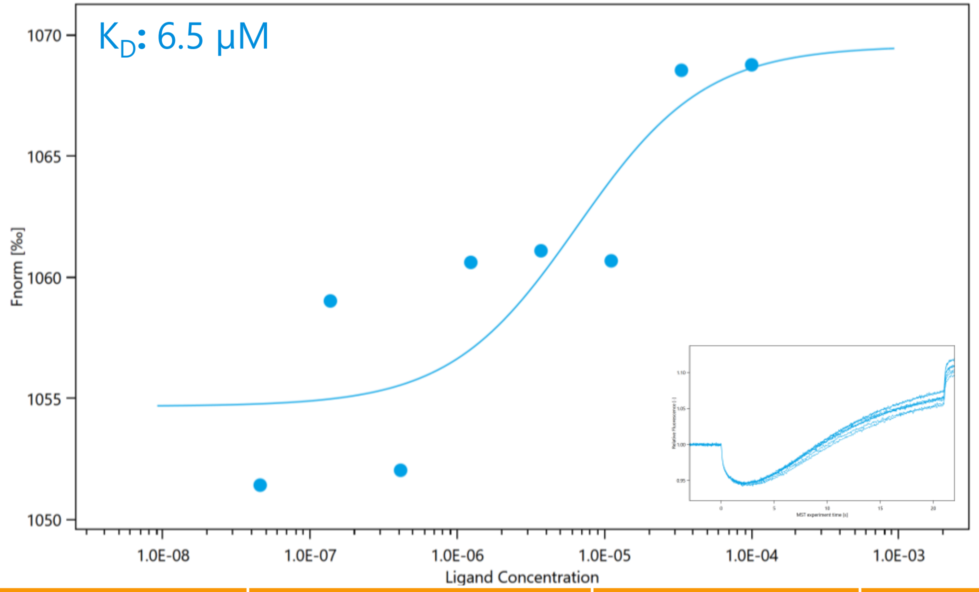
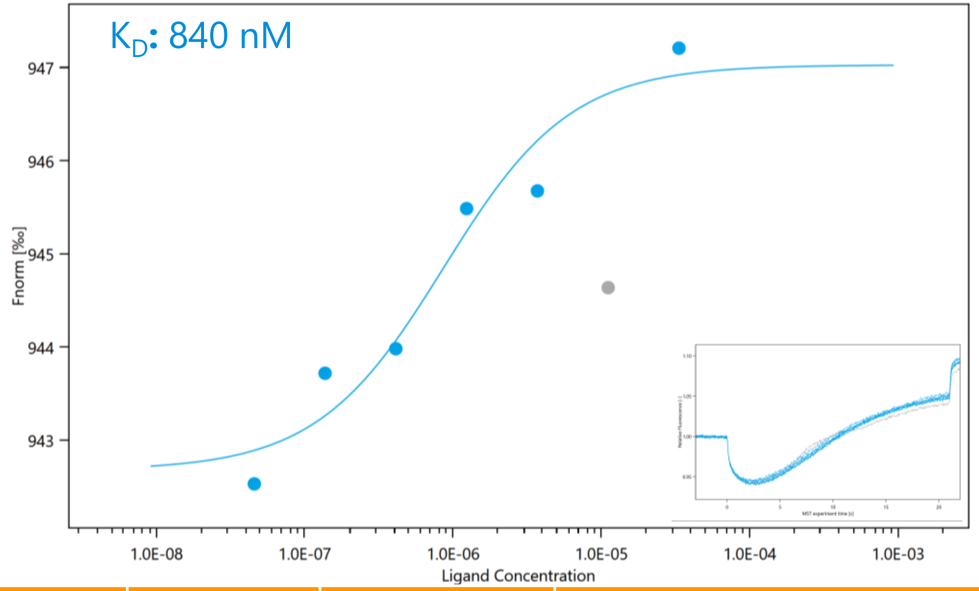
N_mli1777542 (JDI-515)



N_mli76891947 (JDI-571)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_mli1777542	6.5E-06	14.9	4.8	20	Potential binder, low signal-to-noise
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_mli76891947	8.4E-07	4.4	11.0	2.5	Potential binder, low delta Fnorm

- RED-tris-NTA 2nd gen. labelled Nucleocapsid potentially binds N_mli1777542 with a determined K_D > 7 µM, low signal to noise.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid potentially binds N_mli76891947 with a determined K_D of 840 nM, low delta Fnorm.

RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. compounds – examples Potential Binder: N_vir17530618 (JDI-549)

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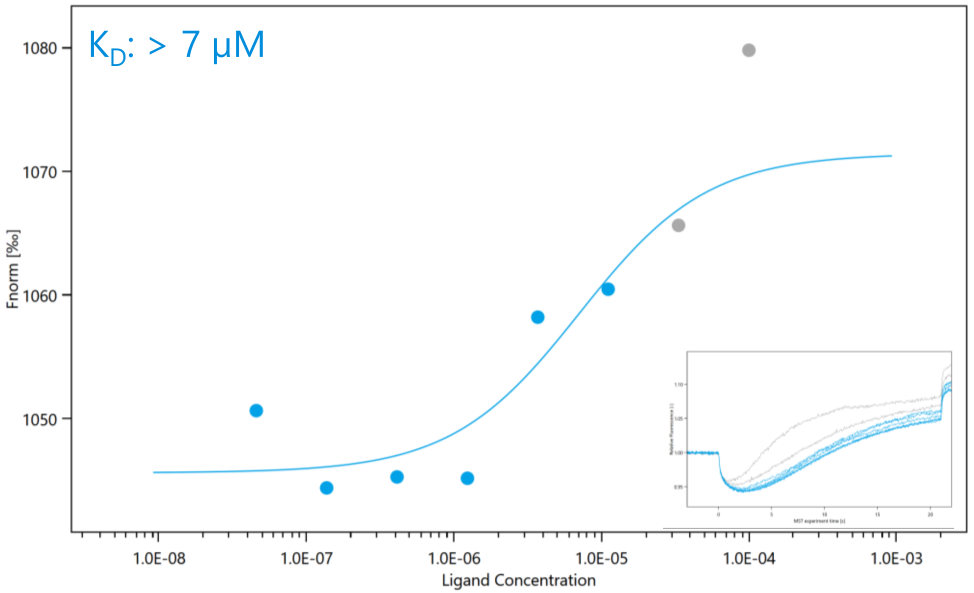
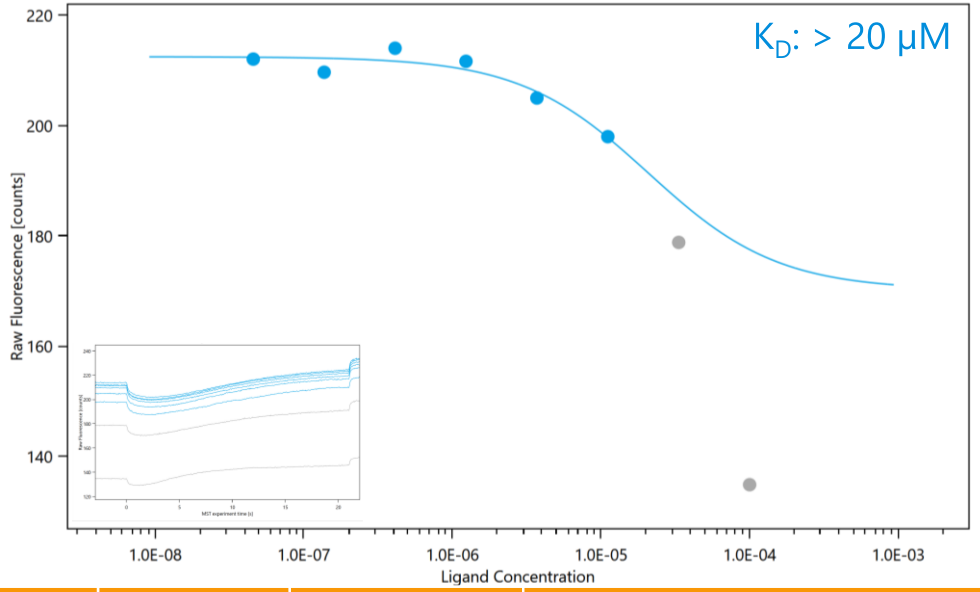
MST



initial fluorescence



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_vir17530618	>7.1E-06	25.8	7.3	20	Potential binder, Quenching
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_vir17530618	>2.1E-05	42.3	23.8	initial fluorescence	Potential binder, Quenching

- RED-tris-NTA 2nd gen. labelled Nucleocapsid potentially binds N_vir17530618 with an estimated K_D > 7 µM. The compound induces fluorescence quenching that may be binding-related or non-specific, which can be tested by titration against a non-related protein.

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RED-TRIS-NTA 2nd gen. labelled Nucleocapsid vs. compounds – examples Potential Binders

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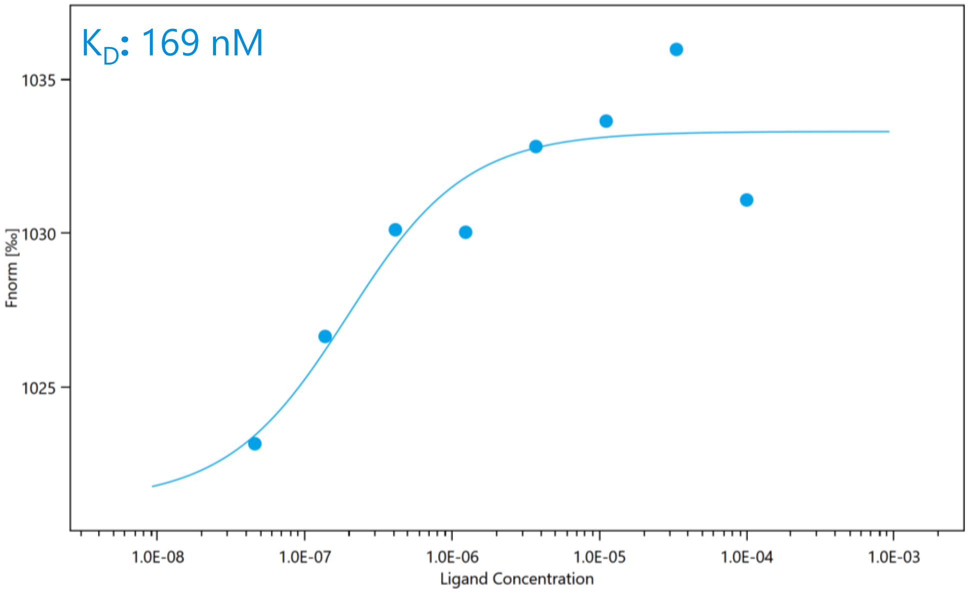
N_mli17081372 (JDI-560)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Nucleocapsid	N_mli17081372	1.7E-07	12.0	7.9	20	Potential binder, missing unbound state

- RED-tris-NTA 2nd gen. labelled Nucleocapsid potentially binds N_mli17081372 with a determined K_D of 170 nM. The unbound state is missing, suggesting that this compound may bind with high affinity. Repetition with lower compound concentrations is needed.

Next steps

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- We suggest to measure all binders (33 cpds) and potential binders with missing unbound state (12 cpds, potential high affinity) in 12-pt K_D validation in duplicates a WuXi AppTec company

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