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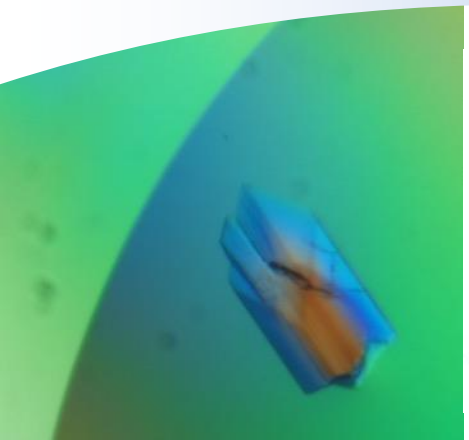
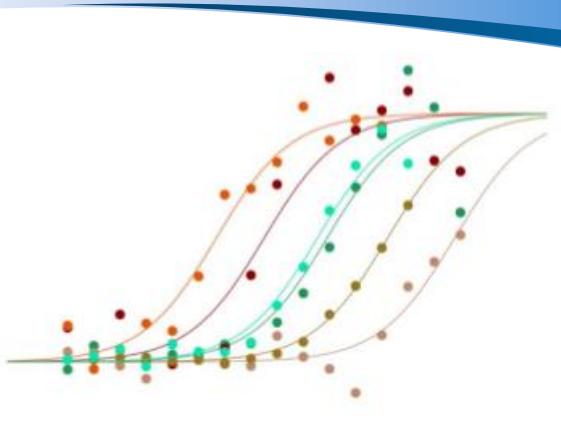
JEDI Program/JDI01

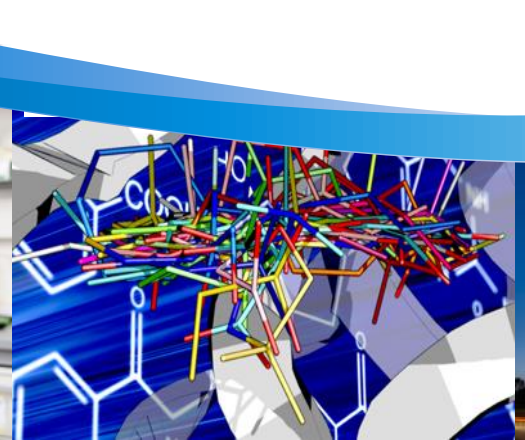
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

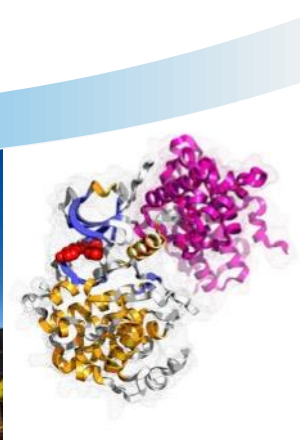
SARS-CoV-2 Nucleocapsid protein

nanoDSF / MST/ TRIC measurements

May 27, 2021





Status

- nanoDSF:
  - The nucleocapsid shows a well defined melting transition and good thermal stability (Tm of 45.4°C).
  - However, a second very broad melting transition is observed around 65°C, indicating sample heterogeneity.
  - The nucleocapsid is not significantly affected by detergents or reducing agents except DTT.

  - VHH E4-3 (nanobody for the NTD) shows good thermal stability and a single melting transition.
  - However, VHH H3-3 (nanobody against CTD) shows two melting events, suggesting sample heterogeneity.
  - Moreover, both nanobodies were sensitive towards buffer exchange: about 50% of protein was lost during buffer exchange.

Status

- TRIC (Dianthus):
  - Nucleocapsid was successfully labelled on the His-tag with RED-Tris-NTA dyes.
  - In a first experiment, nucleocapsid bound VHH E4-3 (nanobody against NTD) with a determind K_D of 5.6 nM.
  - However, this result was not reproducible in terms of affinity and signal-to-noise ratio and showed variation between nanobodies stored at 4°C and -80°C.
  - No binding of VHH H3-3 (nanobody against CTD) to nucleocapsid was observed.

- Labelled MST :
  - For comparison, the interactions were also measured on the NT.115 instrument and yielded similar results. The same issues with the nanobodies and low reproducibility were observed.

➢ Overall, the QC of the nanobodies needs to be reviewed and labelled MST requires further assay optimization.

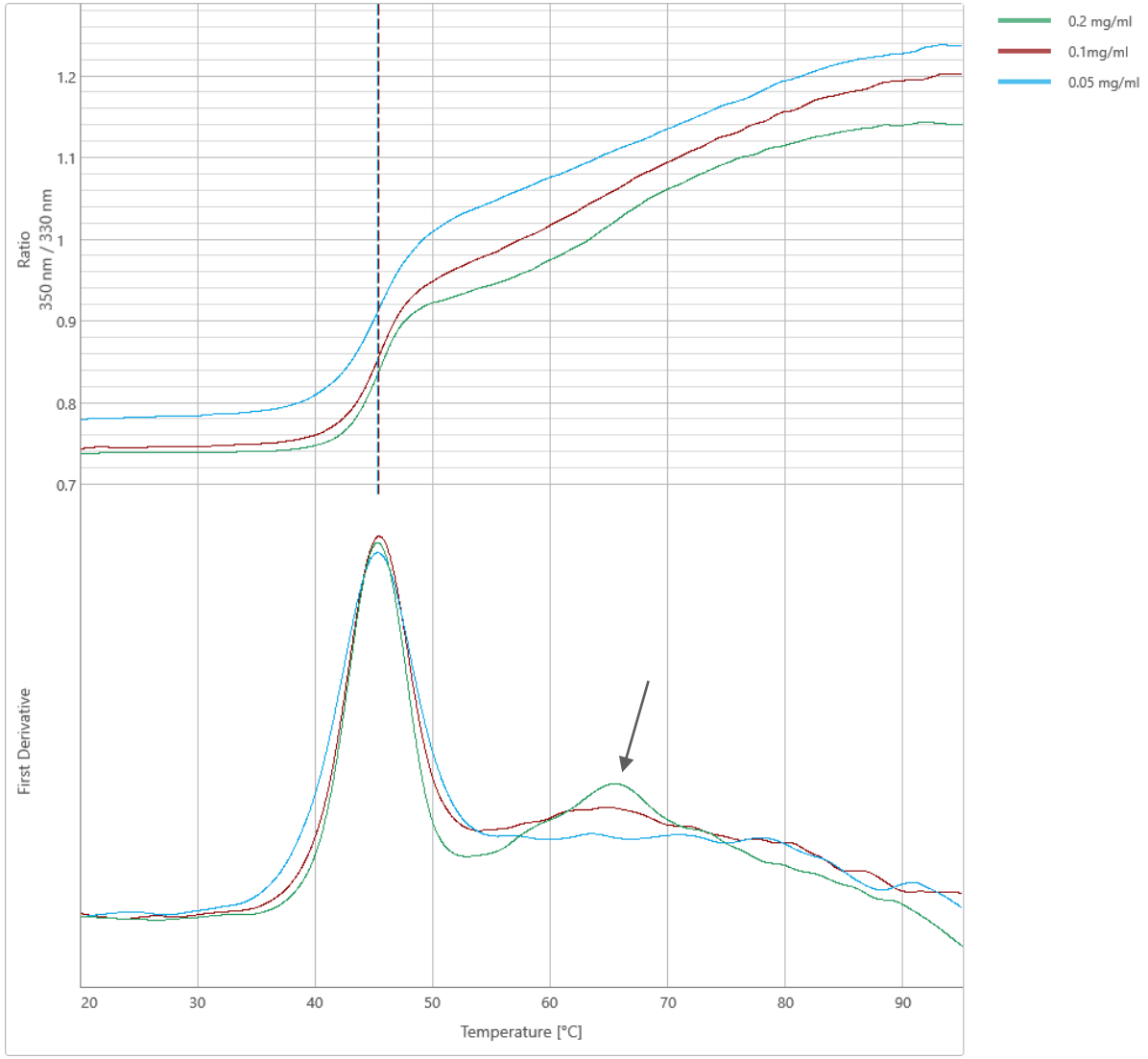
nanoDSF

SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)

nanoDSF Assay Conditions

Protein	0.05/ 0.1/ 0.2 mg/ml SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)
Assay Buffer	50 mM TRIS pH 7.5, 150 mM NaCl
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 25 % Temperature ramp: 2°C/min

SARS-CoV-2 Nucleocapsid protein thermal stability



Condition	Ø T_m [°C] [1]	Analysis mode
0.2 mg/ml	45.3	ratio
0.1 mg/ml	45.5	ratio
0.05 mg/ml	45.4	ratio

[1] determined in singlicate

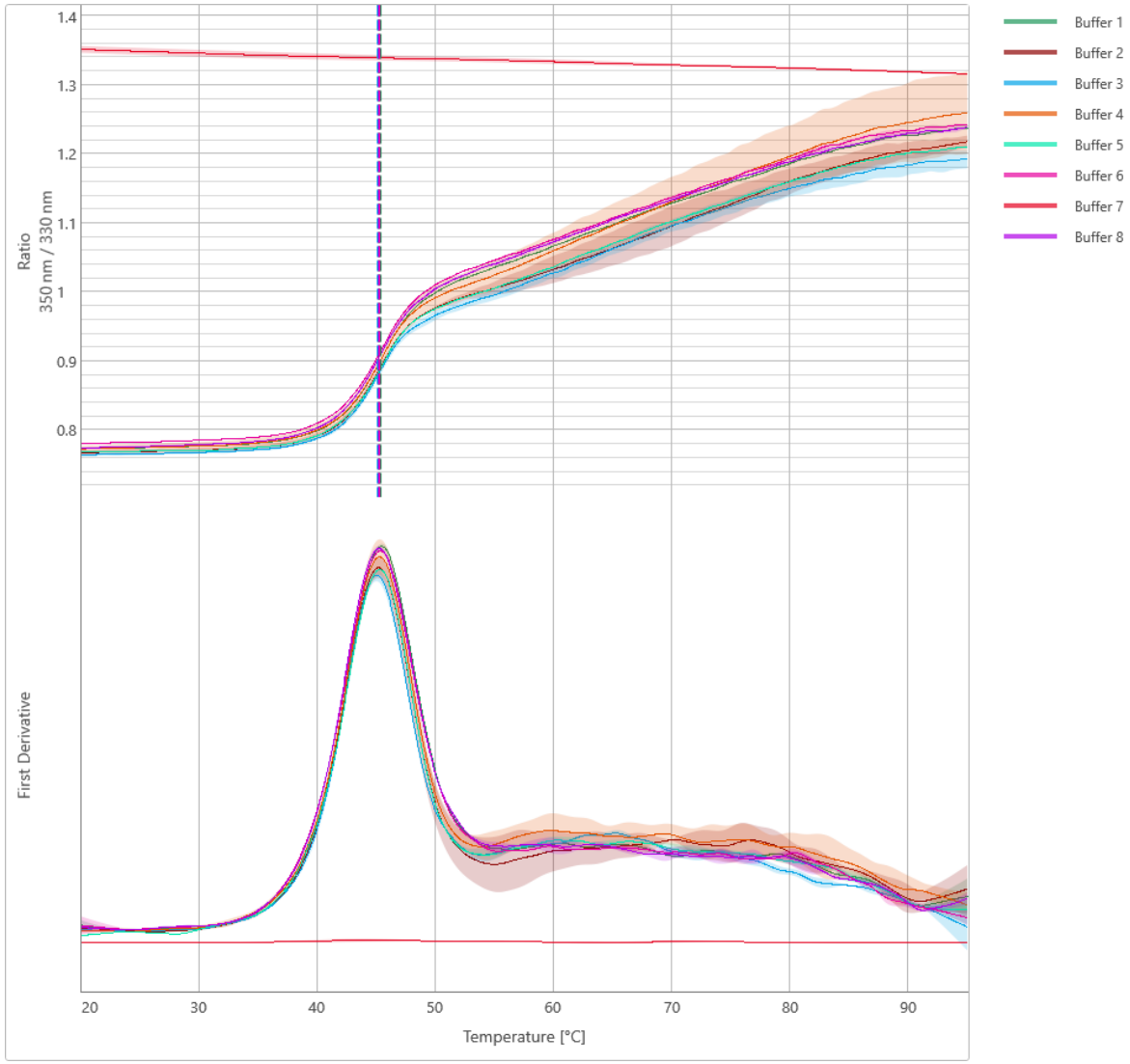
- The nucleocapsid shows a well defined melting transition with Tm of 45.4°C. A second very broad melting transition is observed around 65°C (⟶), suggesting sample heterogeneity.
- A concentration of 0.05 mg/ml was selected for further measurements.

nanoDSF Assay Conditions

Protein	0.05 mg/ml SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)
Assay Buffer	8 Buffers (see table)
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 70 % Temperature ramp: 2°C/min

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

Effect of reducing agents and detergents on SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) thermal stability



Condition	Ø T_m [°C] [1]	s.d. [°C]	Analysis mode
Buffer 1	45.5	0.0	ratio
Buffer 2	45.2	0.1	ratio
Buffer 3	45.1	0.0	ratio
Buffer 4	45.3	0.0	ratio
Buffer 5	45.3	0.0	ratio
Buffer 6	45.4	0.0	ratio
Buffer 7	-	-	ratio
Buffer 8	45.3	0.0	ratio

[1] determined in duplicate

- No significant impact of detergents or reducing agents on protein thermal stability was observed, except for buffer 7 (DTT), in which the protein is completely unfolded.

nanoDSF

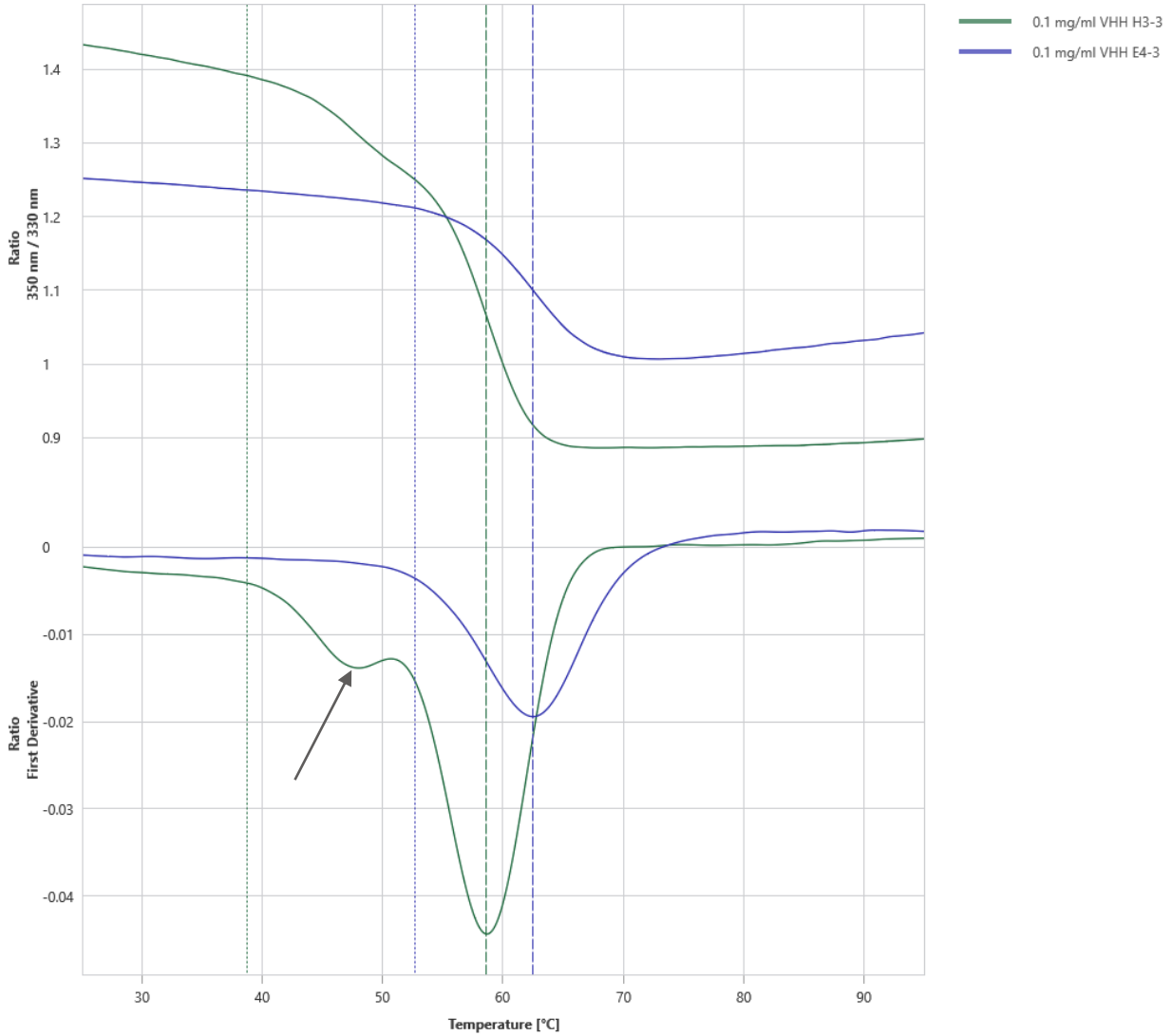
VHH H3-3 EEG1 (PD14991-1) CTD nanobody

VHH E4-3 EEF1 (PD14989-1) NTD nanobody

nanoDSF Assay Conditions

Protein	0.1 mg/ml VHH H3-3 EEG1 (PD14991-1) (nanobody against CTD) 0.1 mg/ml VHH E4-3 EEF1 (PD14989-1) (nanobody against NTD)
Assay Buffer	PBS
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 100 % Temperature ramp: 2°C/min

Thermal stability of NTD and CTD nanobodys



Condition	Ø T_m [°C] [1]	s.d. [°C]	Analysis mode
0.1 mg/ml VHH-H3-3	58.6	0.0	ratio
0.1 mg/ml VHH-E4-3	62.5	0.0	

[1] determined in duplicate

- VHH-H3-3 (CTD nanobody) shows a main melting transition at 58.6°C and a minor melting transition at ~48°C (⟶), suggesting sample heterogeneity.
- VHH E4-3 (NTD nanobody) shows a well defined melting transition with Tm of 62.5°C.

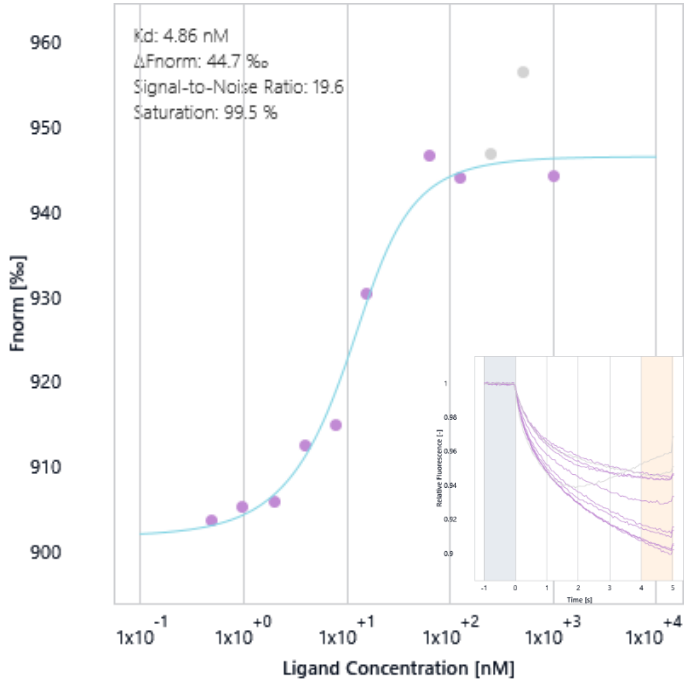
TRIC (Dianthus)

SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)

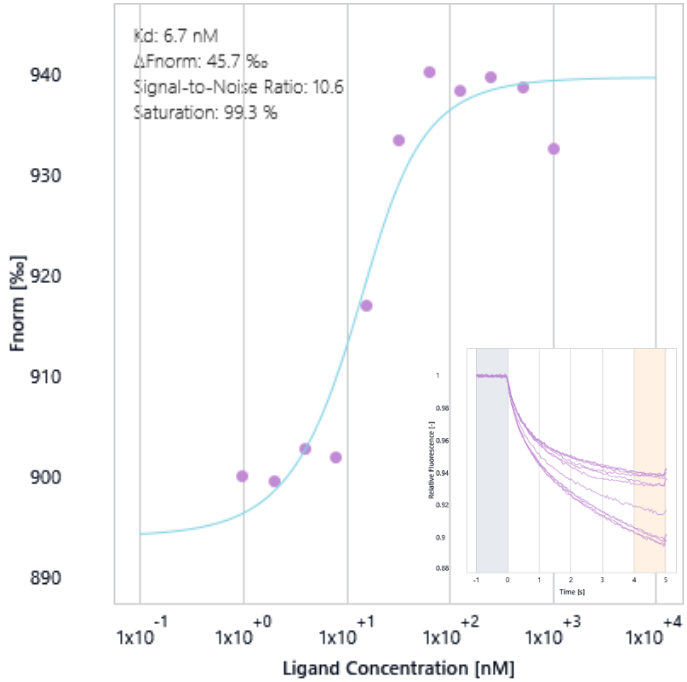
TRIC Assay Conditions

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: 13 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Singlicates	
Assay buffer	50 mM HEPES pH 7.5, 150 mM NaCl, 0.05% Tween20	
Titrant	SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. vs. SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)



RED-tris-NTA 1st gen.



RED-tris-NTA 2nd gen.

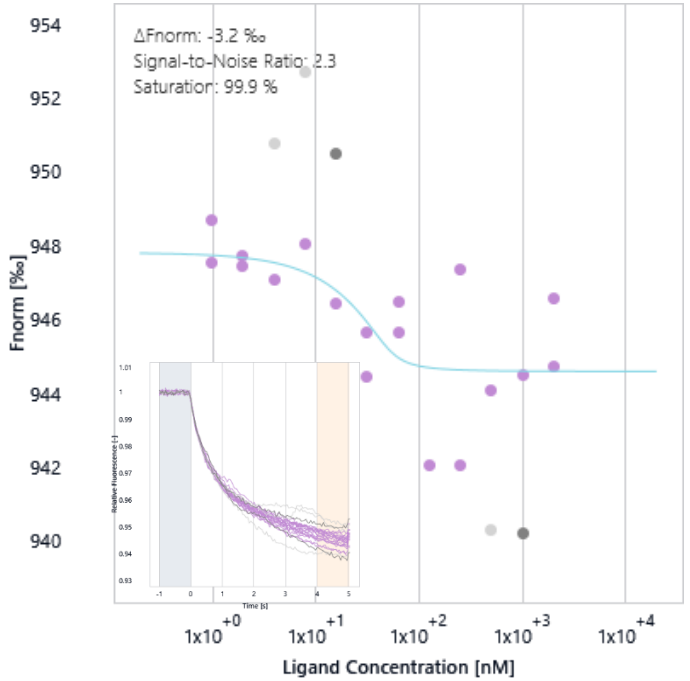
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 1st gen.	RED-tris-NTA 1st gen.	Nucleocapsid	4.9E-09	2.3E-09	1.0E-08	44.7	19.6	5	aggregation
RED-tris-NTA 2nd gen.	RED-tris-NTA 2nd gen.	Nucleocapsid	6.7E-09	2.3E-09	2.0E-08	45.7	10.6	5	

- RED-tris-NTA 1st gen. binds with a determined K_D of 4.9 nM the protein, some aggregation was observed.
- RED-tris-NTA 2nd gen. binds with a determined K_D of 6.7 nM the protein.
- Both dyes can be used to non-covalently label the nucleocapsid.

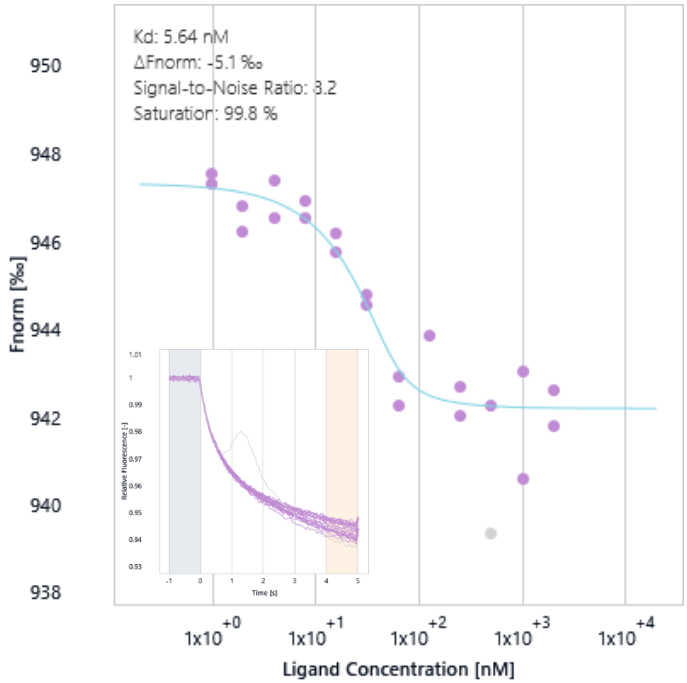
TRIC Assay Conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)		
Fluorophore	RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 25 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 26 % (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, 0.05% Tween20 (56% Protein lose at buffer exchange of VHH E4-3) Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 (54% Protein lose at buffer exchange of VHH E4-3) Buffer 3: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Pluronic (59% Protein lose at buffer exchange of VHH E4-3) Buffer 4: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.1% Pluronic (54% Protein lose at buffer exchange of VHH E4-3)		
Titrant	VHH E4-3 (nanobody against NTD)	EEF1 (PD14989-1)	2 µM – 0.98 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 (NTD nanobody)



Buffer 1

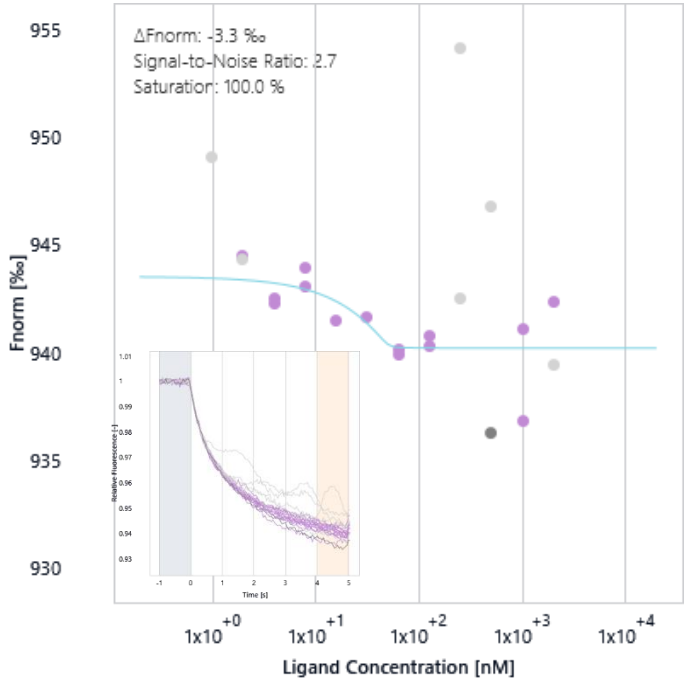


Buffer 2

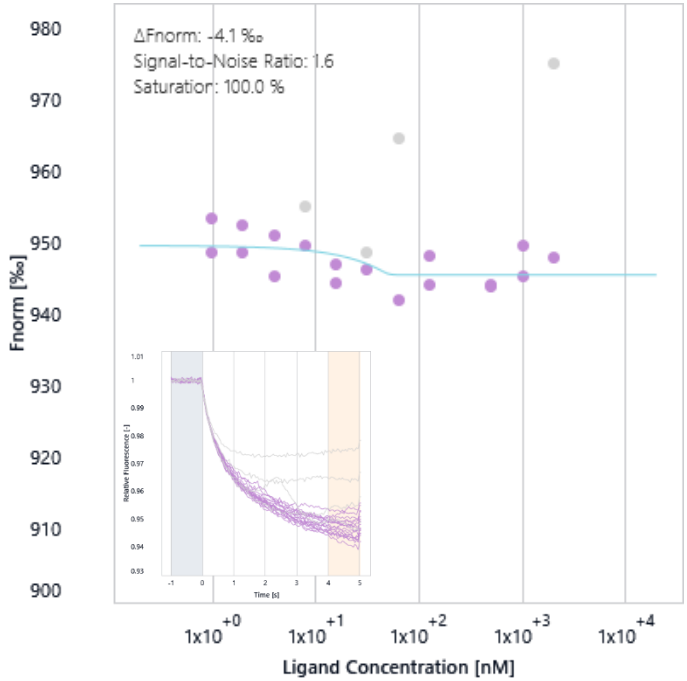
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	-	-	-	-	-	5	Buffer 1, Aggregation
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	5.6E-09	1.0E-9	3.0E-08	- 5.1	8.2	5	Buffer 2

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 1, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 5.6 nM in buffer 2.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 (NTD nanobody)



Buffer 3



Buffer 4

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.	Nucleocapsid	VHH E4-3	-	-	-	-	-	5	Buffer 3, Aggregation
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	-	-	-	-	-	5	Buffer 4, Aggregation

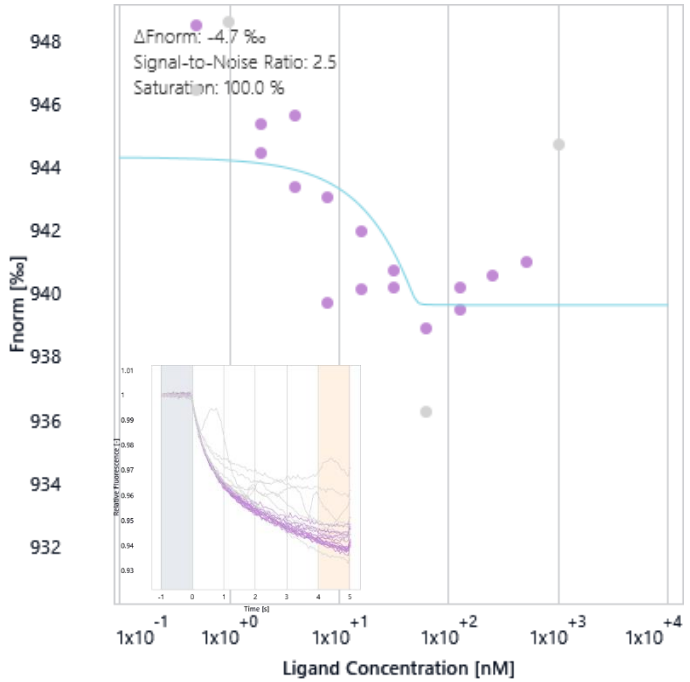
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 3, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 4, aggregation was observed.

TRIC Assay Conditions

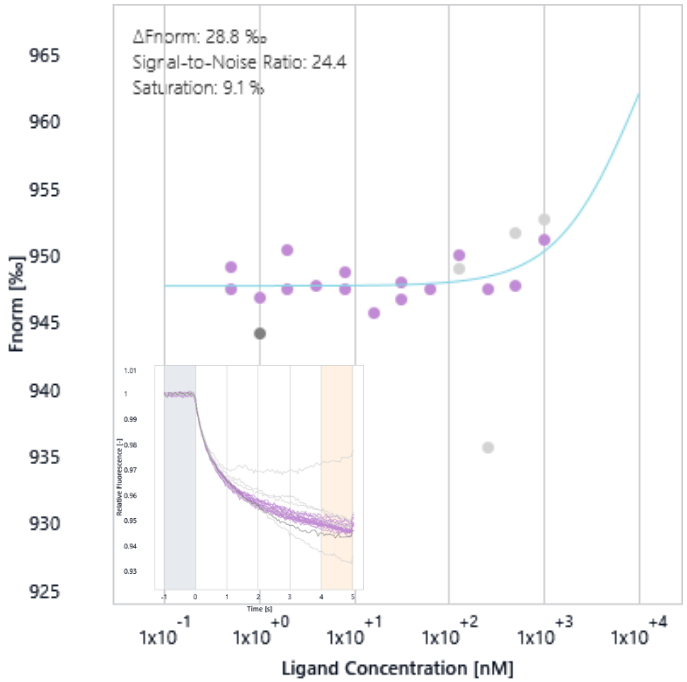
Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)		
Fluorophore	RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 25 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 26 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20		
Titrant	VHH E4-3 (nanobody against NTD) VHH H3-3 (nanobody against CTD)	EEF1 (PD14989-1) EEG1 (PD14991-1)	1 µM – 0.49 nM (12 conc.)

Titrant	Buffer	Protein loss during buffer exchange
VHH E4-3	Buffer1	41%
VHH H3-3	Buffer1	45%

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 and VHH H3-3



VHH E4-3 (nanobody against NTD)

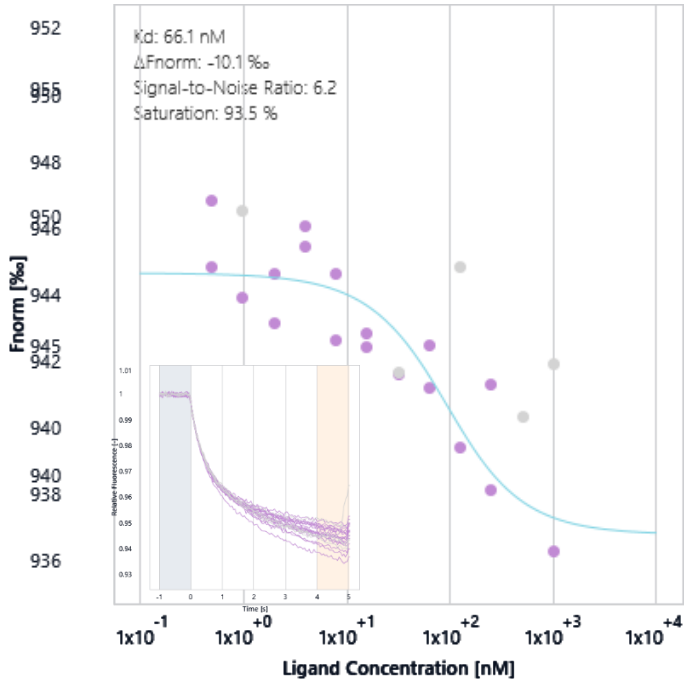


VHH H3-3 (nanobody against CTD)

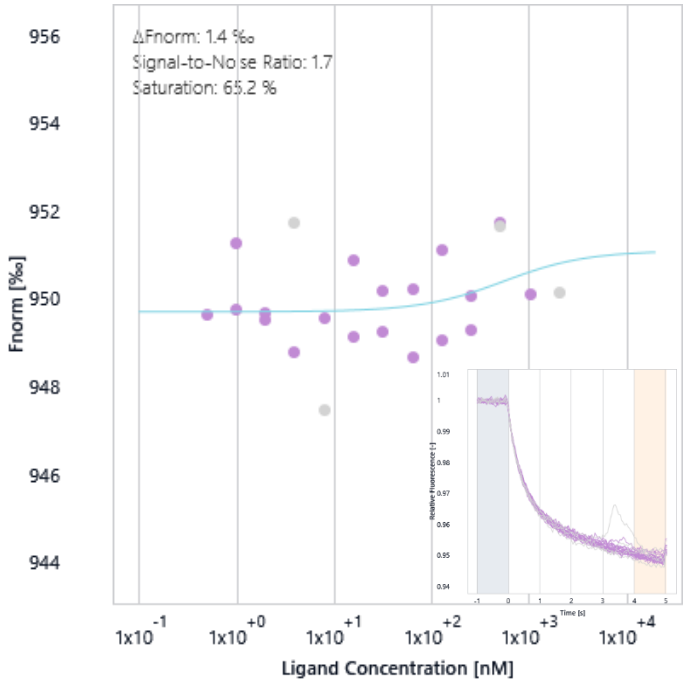
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3 (stored at 4°C)	-	-	-	-	-	5	Aggregation
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3 (stored at 4°C)	-	-	-	-	-	5	Aggregation

- RED-tris-NTA 2nd gen. labelled Nucleocapsid shows strong aggregation and no clear binding to VHH E4-3 (stored at 4°C) using the previously optimized assay buffer.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3, aggregation was observed.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 and VHH H3-3



VHH E4-3 (nanobody against NTD)



VHH H3-3 (nanobody against CTD)

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.	Nucleocapsid	VHH E4-3 (stored at -80°C)	~6.6E-08	-	-	-	-	5	Aggregation, potential binding
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3 (stored at -80°C)	-	-	-	-	-	5	Aggregation

- RED-tris-NTA 2nd gen. labelled Nucleocapsid shows aggregation and only potential binding to VHH E4-3 (stored at -80°C) with reduced affinity compared to the previous measurement.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3, aggregation was observed.

TRIC Summary:

Binding affinity of 2 nanobodies to SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)

Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
VHH E4-3	-	-	-	-	-	5	Buffer 1, Aggregation
VHH E4-3	5.6E-09	1.0E-9	3.0E-08	- 5.1	8.2	5	Buffer 2
VHH E4-3	-	-	-	-	-	5	Buffer 3, Aggregation
VHH E4-3	-	-	-	-	-	5	Buffer 4, Aggregation
VHH E4-3	-	-	-	-	-	5	Buffer 2, aggregation (4°C stored nanobody, after 2 days)
VHH H3-3	-	-	-	-	-	5	Buffer 2, aggregation (4°C stored nanobody, after 2 days)
VHH E4-3 (stored at -80°C)	~6.6E-08	-	-	-	-	5	Buffer 2, Aggregation (-80°C stored nanobody, after 2 days)
VHH H3-3 (stored at -80°C)	-	-	-	-	-	5	Buffer 2, Aggregation (-80°C stored nanobody, after 2 days)

Labelled MST

SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)		
Fluorophore	RED-tris-NTA 2nd gen.		
Labelling conditions	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: 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, 0.05% Tween20 (56% Protein lose at buffer exchange of VHH E4-3) Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 (54% Protein lose at buffer exchange of VHH E4-3) Buffer 3: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Pluronic (59% Protein lose at buffer exchange of VHH E4-3) Buffer 4: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.1% Pluronic (54% Protein lose at buffer exchange of VHH E4-3)		
Titrant	VHH E4-3 (NTD nanobody)	EEF1 (PD14989-1) (stored at 4°C)	2 µM – 0.98 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 (NTD nanobody)

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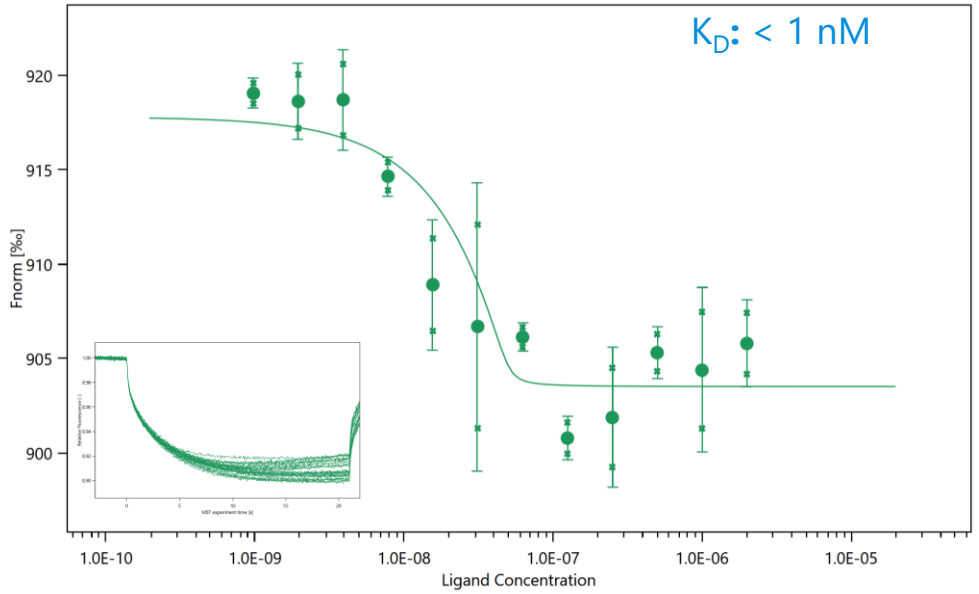
Buffer 1



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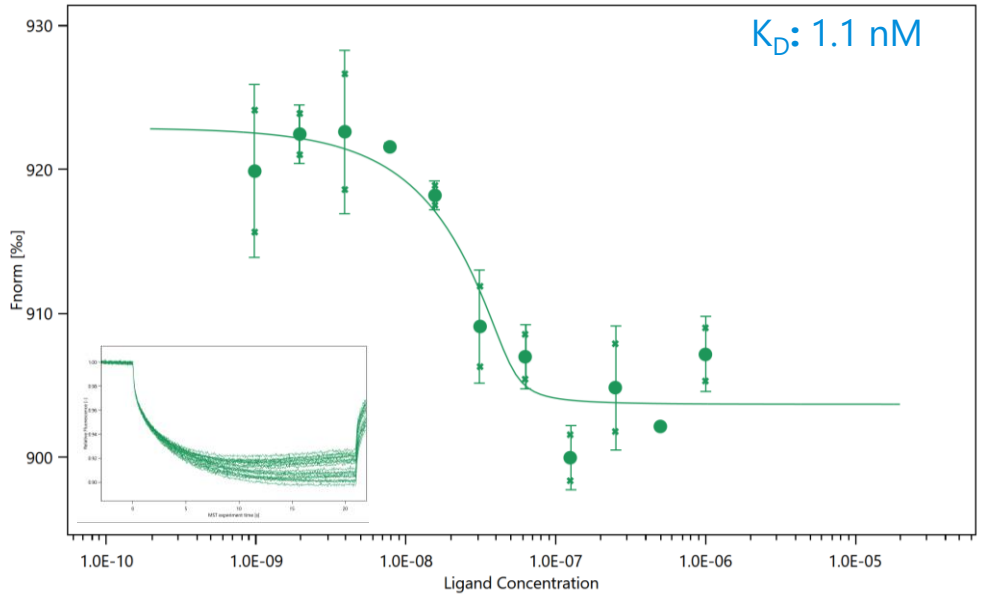

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	<1.0E-09	2.7E-18 – 0.04	14.3	6.1	20	Buffer 1
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	1.1E-09	4.0E-12 – 3.4E-7	19.2	8.3	20	Buffer 2

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds with a determined K_D <1 nM VHH-E4-3 in buffer 1. Due to the high protein concentration, the affinity cannot be accurately determined.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds with a determined K_D of 1.1 nM VHH-E4-3 in buffer 2.

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RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 (NTD nanobody)

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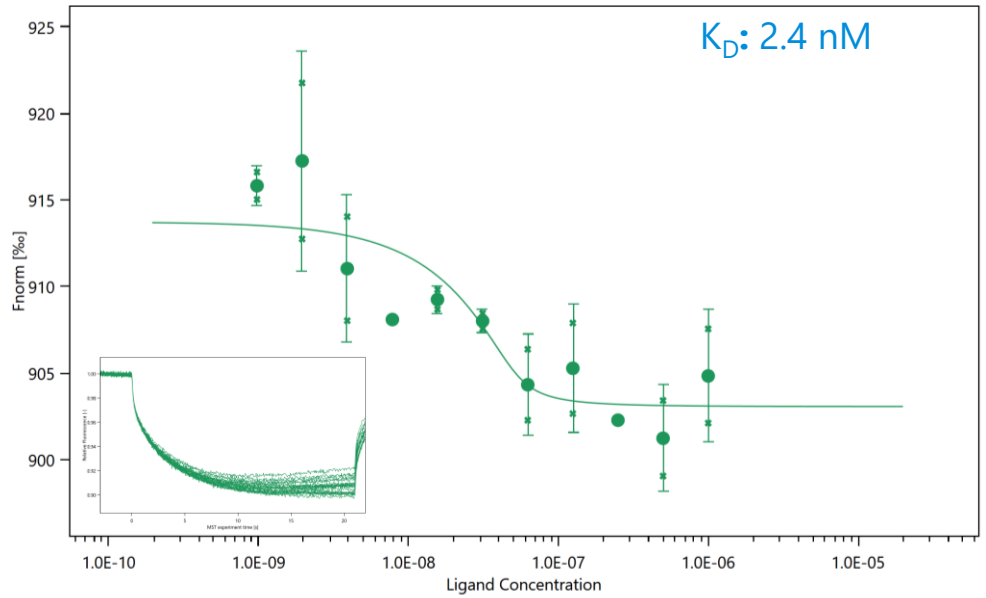
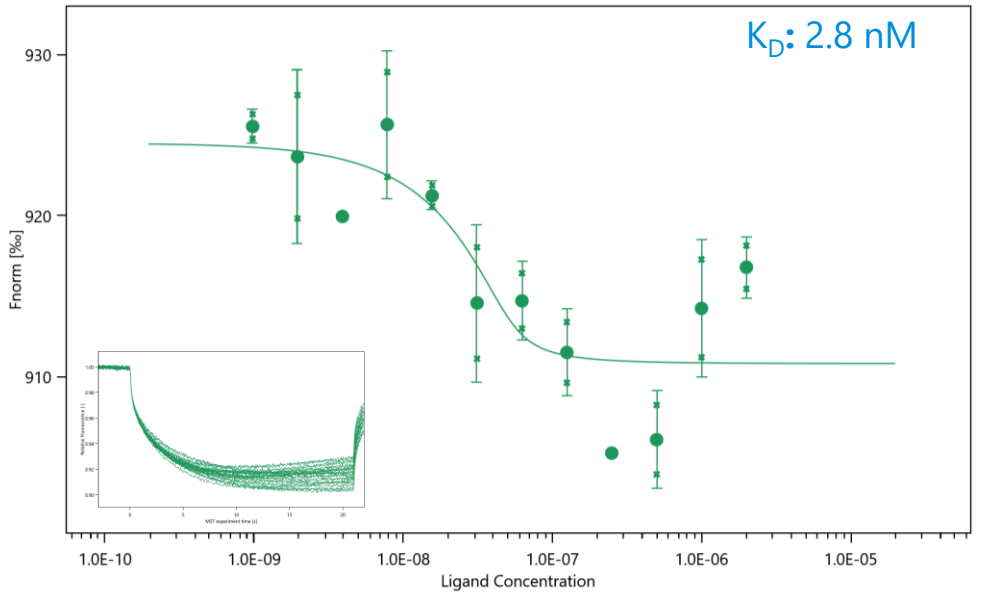
Buffer 3



Buffer 4



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	2.4E-09	3.0E-12 – 1.8E-06	10.6	4.6	20	Buffer 3
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	2.8E-09	4.0E-12 – 2.3E-06	13.7	4.0	20	Buffer 4

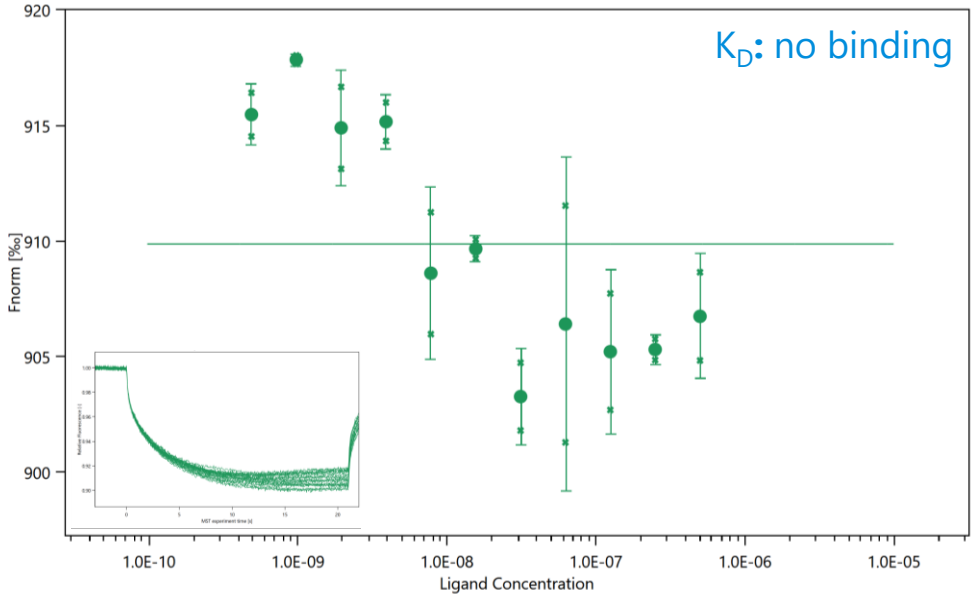
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds with a determined K_D of 2.4 nM VHH-E4-3 in buffer 3.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds with a determined K_D of 2.8 nM VHH-E4-3 in buffer 4.
- Signal-to-noise ratio is low in both buffers.

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)		
Fluorophore	RED-tris-NTA 2nd gen.		
Labelling conditions	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: 80 % MST Power: 40 % and 60 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20		
Titrant	VHH E4-3 (nanobody against NTD) VHH H3-3 (nanobody against CTD)	EEF1 (PD14989-1) EEG1 (PD14991-1)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 (NTD nanobody)

Medium MST



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 (stored at 4°C)	-	-	-	-	20	Medium MST, low S/N

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH-E4-3 stored at 4°C in the previously optimized assay buffer.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH E4-3 (NTD nanobody)

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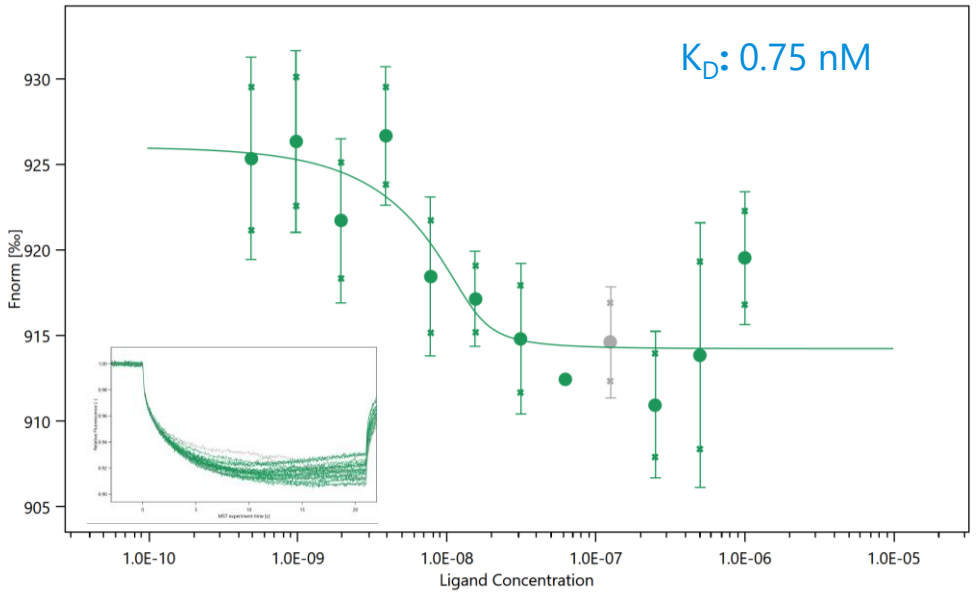
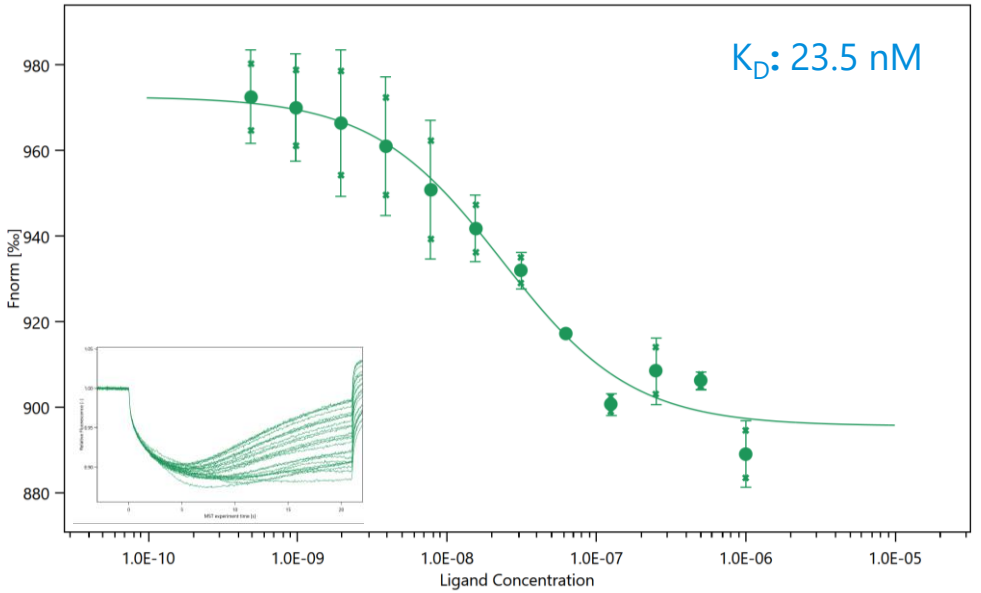
Medium MST



High MST



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 (stored at -80°C)	7.5E-10	2.6E-14 – 2.1E-05	11.8	4.5	20	Medium MST, low S/N
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3 (stored at -80°C)	2.4E-08	-	77.1	16.7	20	High MST

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH-E4-3 stored at -80°C with a determined K_D of 0.75 nM, but the signal-to-noise ratio is low (MST medium) .
- At high MST power, RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH-E4-3 with a determined K_D of 23.5 nM.

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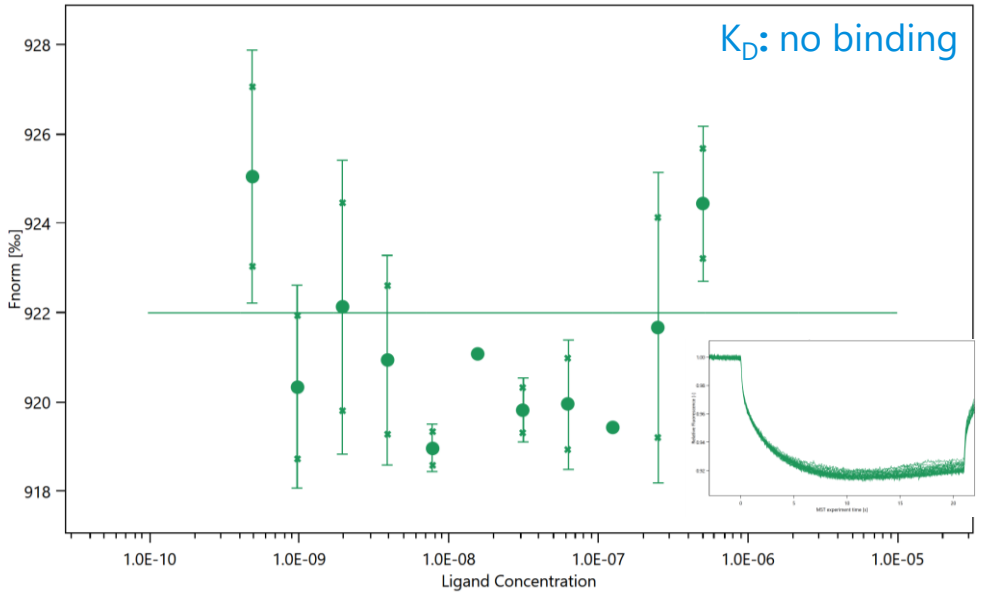
RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH H3-3 (CTD nanobody)

Medium MST



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 H3-3 (stored at 4°C)	-	-	-	-	20	Medium MST

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH-H3-3 stored at 4°C in the previously optimized assay buffer.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH H3-3 (CTD nanobody)

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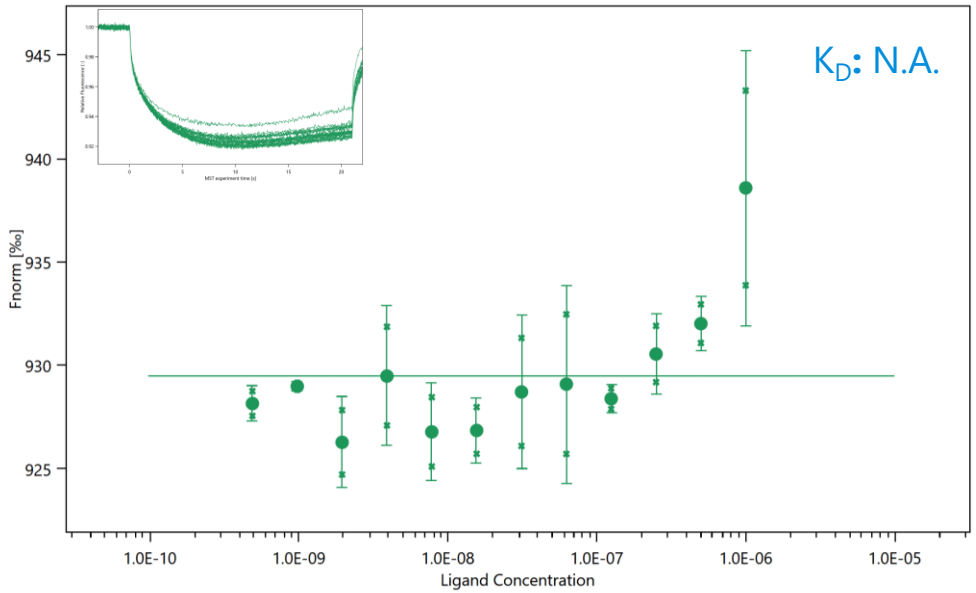
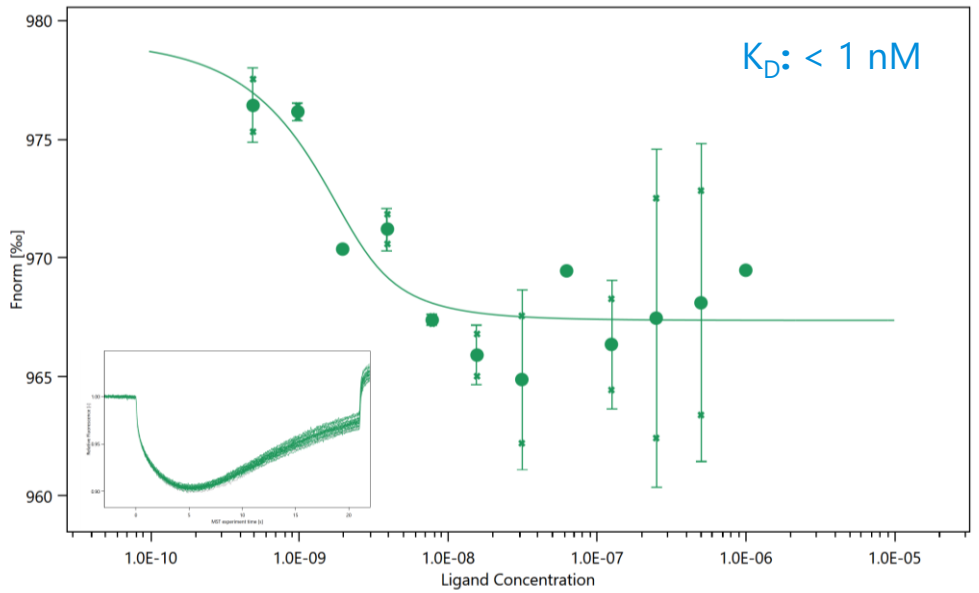
Medium MST



High MST



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 H3-3 (stored at -80°C)	-	-	-	-	20	Medium MST
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3 (stored at -80°C)	<1.0E-09	7.0E-12 – 2.1E-8	11.8	7.3	20	High MST

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH-H3-3 stored at -80°C (MST medium).
- At high MST power, RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH-H3-3 with a determined K_D < 1 nM. Due to the high protein concentration, the affinity cannot be accurately determined.

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MST summary:

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Binding affinity of 2 nanobodys to RED-tris-NTA 2nd labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)

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 (NTD nanobody)	7.5E-10	2.6E-14 – 2.1E-05	11.8	4.5	20	Medium MST, low S/N
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3 (NTD nanobody)	2.4E-08	-	77.1	16.7	20	High MST
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3 (CTD nanobody)	-	-	-	-	20	Medium MST
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3 (CTD nanobody)	<1.0E-09	7.0E-12 – 2.1E-8	11.8	7.3	20	High MST

Next steps

- Discuss nanobody QC
- Further TRIC/MST assay optimization (additional Nucleocapsid protein required)

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