药明康德 WuXi AppTec

a WuXi AppTec company

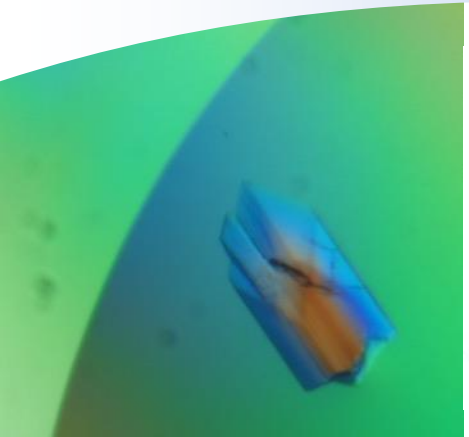
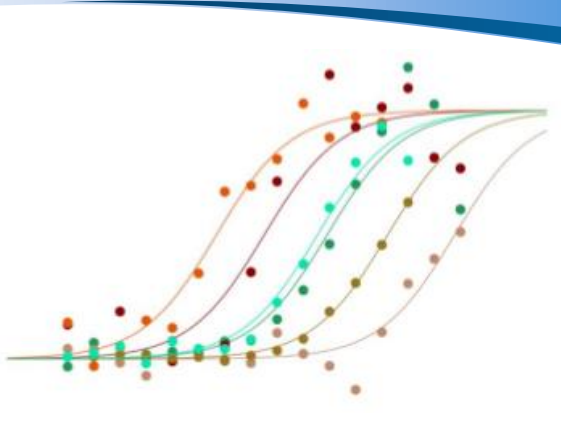
JEDI Program/JDI01

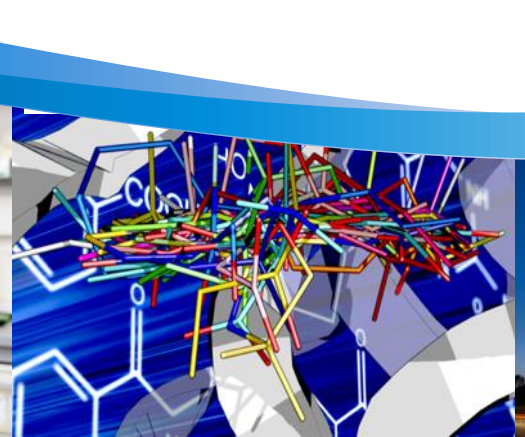
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

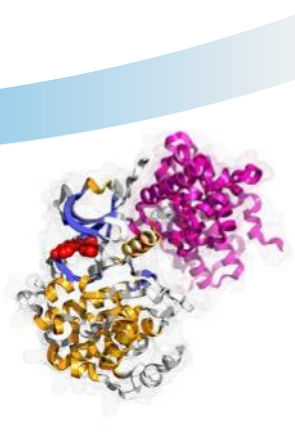
NanoDSF/MST measurements

SARS-CoV-2-Nucleocapsid protein

September 16, 2021





Status

- nanoDSF:
  - The effect of 2 nanobodies on nucleocapsid thermal stability was tested.
    - Only VHH E4-3 (NTD nanobody) significantly stabilized the nucleocapsid: ΔTm = +6°C

- Labelled MST (NT.115/NT.A):
  - Using the previously established assay conditions, RED-Tris-NTA labelled nucleocapsid was tested for reproducibility of VHH H3-3 and VHH E4-3 binding on two instruments and with both dye generations.
    - ➢ Except for one experiment with VHH E4-3 (K_D < 1 nM), no reproducible binding was observed
  - Following the nanoDSF results, further buffer optimization was performed using VHH E4-3.
    - ➢ Binding with large ΔFnorm, signal-to-noise ratio and high reproducibility was observed for VHH E4-3 in buffers containing DTT or GSH: K_D of 2-5 nM

➢ An MST assay was successfully established for nucleocapsid, and compound screening is on-going

Labelled MST

SARS-CoV-2 Nucleocapsid protein (ECJ1, 15199-2)

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM 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.A		
Capillary type	Monolith™ NT.Automated MST Premium Coated 24-Capillary Chips		
Measurement parameter	LED Power: 12 % 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, 0.1% PEG 8000, 0.05% Tween20 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD) VHH E4-3 (NTD nanobody) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG 8000	EEG1 (PD14991-1) (stored at 4°C) EEF1 (PD14989-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3 and VHH H3-3

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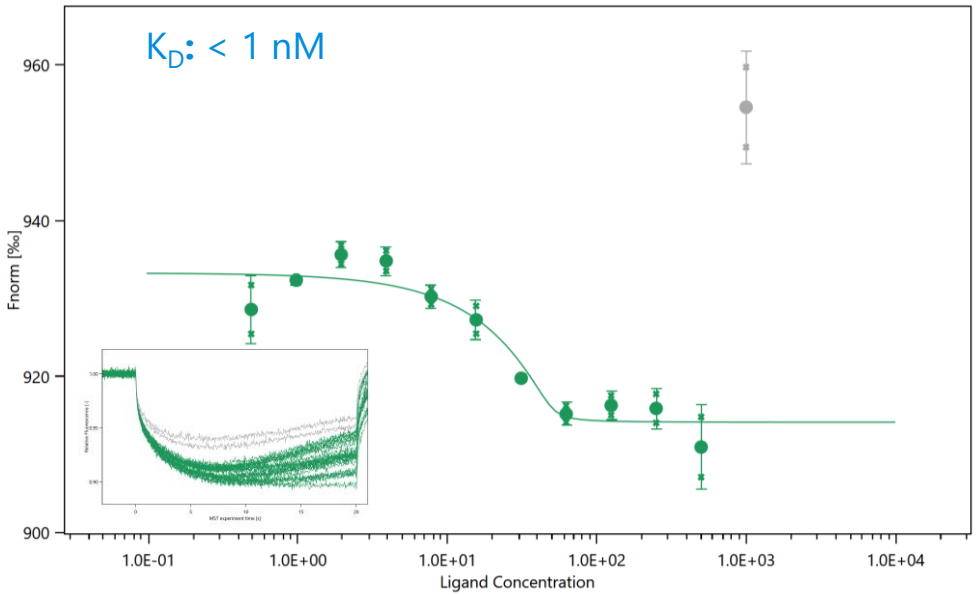
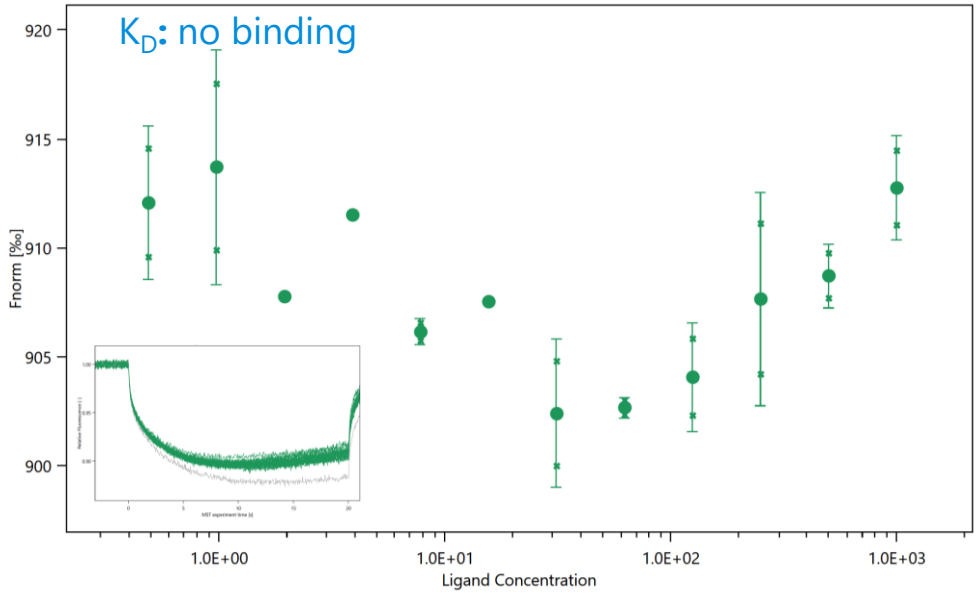
VHH E4-3



VHH H3-3



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	-	19.1	7.8	20	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	20	

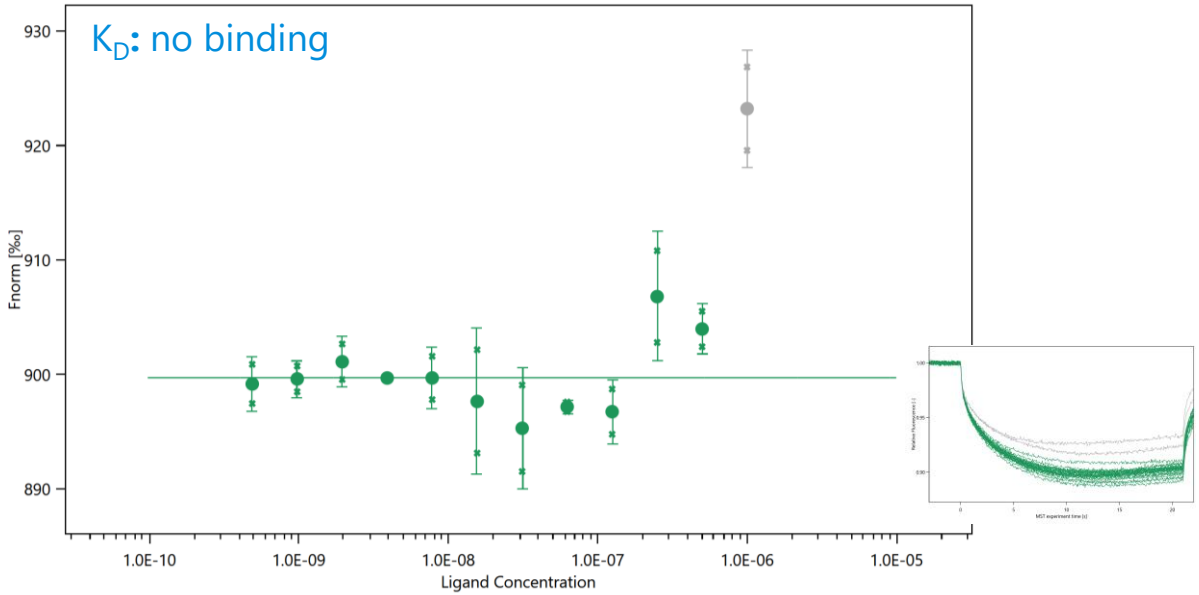
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with an estimated K_D < 1 nM.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3.

MST labelled assay conditions

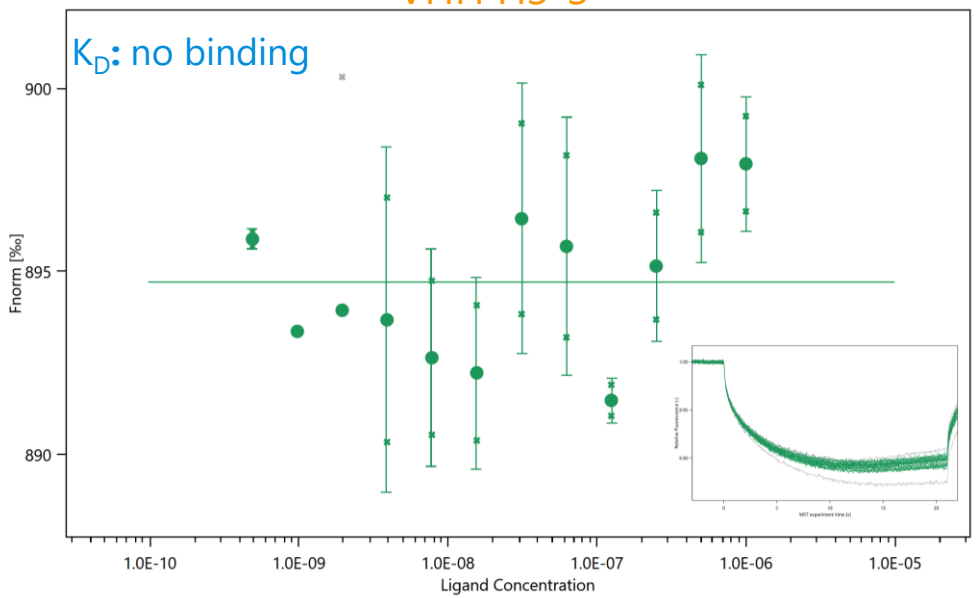
Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 25 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (03)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 90 % 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, 0.1% PEG 8000, 0.05% Tween20 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD) VHH E4-3 (NTD nanobody) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG 8000	EEG1 (PD14991-1) (stored at 4°C) EEF1 (PD14989-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3 and VHH H3-3

VHH E4-3



VHH H3-3



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	-	-	-	-	10	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	10	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 and VHH H3-3

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	100 nM protein / 25 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.A		
Capillary type	Monolith™ NT.Automated MST Premium Coated 24-Capillary Chips		
Measurement parameter	LED Power: 8 % 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, 0.1% PEG 8000, 0.05% Tween20 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD) VHH E4-3 (NTD nanobody) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG 8000	EEG1 (PD14991-1) (stored at 4°C) EEF1 (PD14989-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

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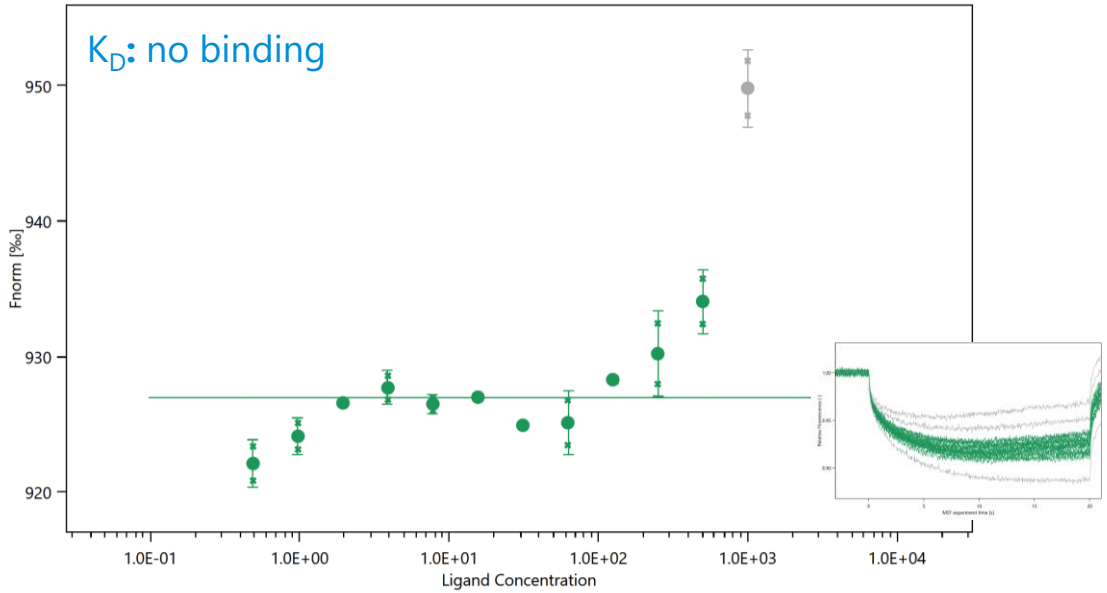
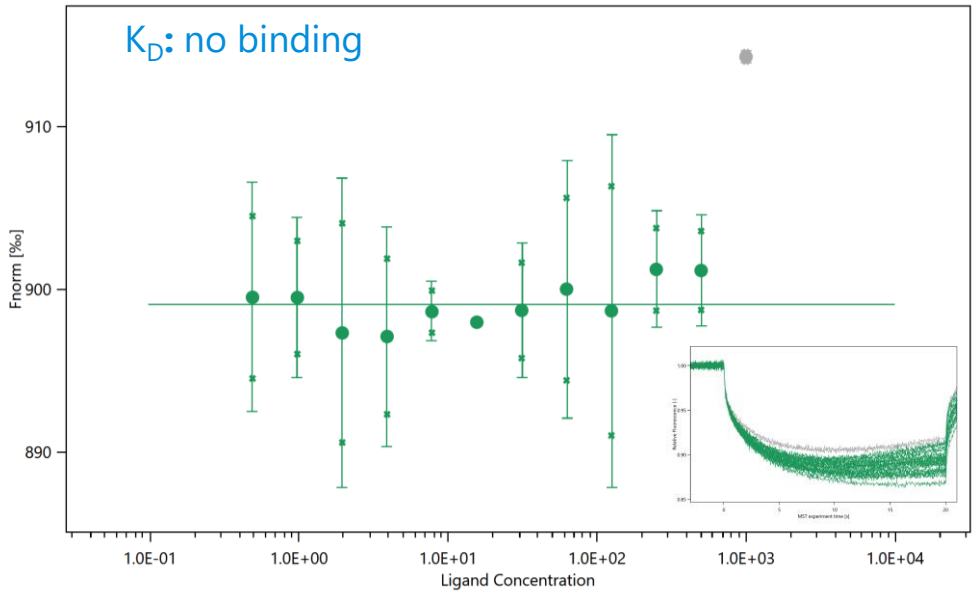
VHH E4-3, 1st gen.



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

- RED-tris-NTA 1st and 2nd gen. labelled Nucleocapsid does not bind VHH E4-3.

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RED-tris-NTA 1st and 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH H3-3

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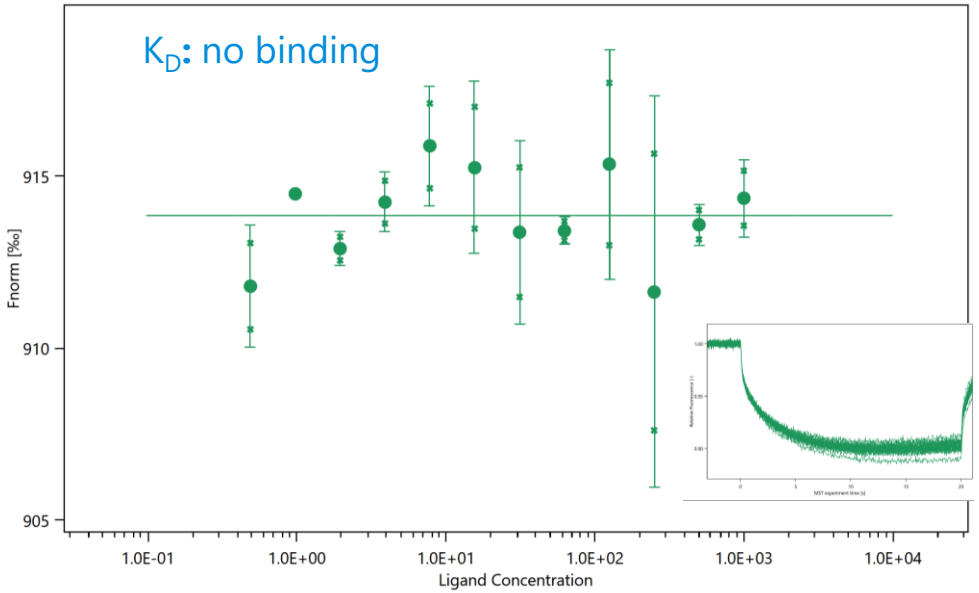
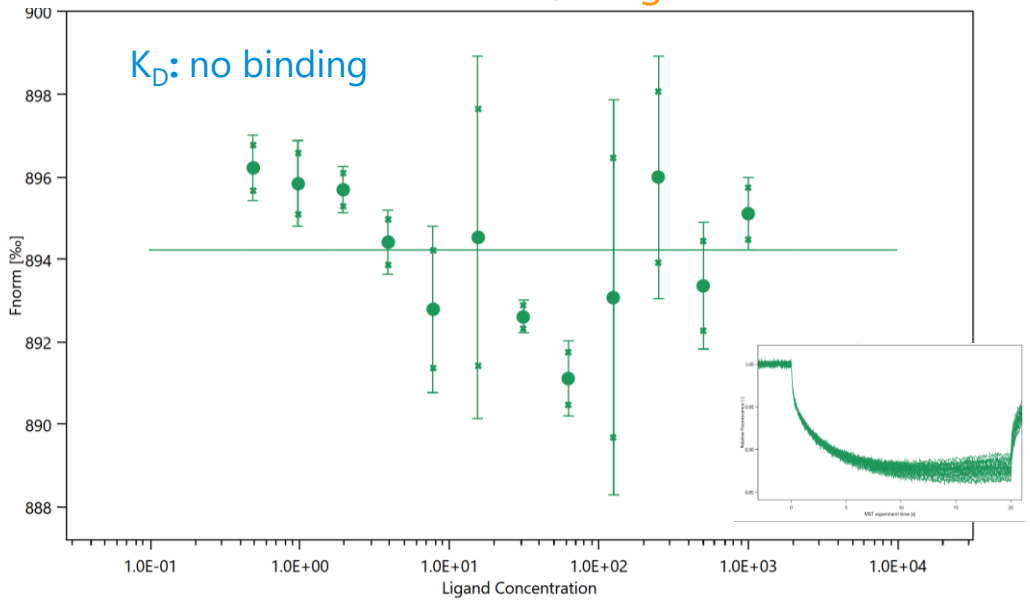
VHH H3-3, 1st gen.



VHH H3-3, 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.	Nucleocapsid	VHH H3-3	-	-	-	-	5	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	5	

- RED-tris-NTA 1st and 2nd gen. labelled Nucleocapsid does not bind VHH H3-3.

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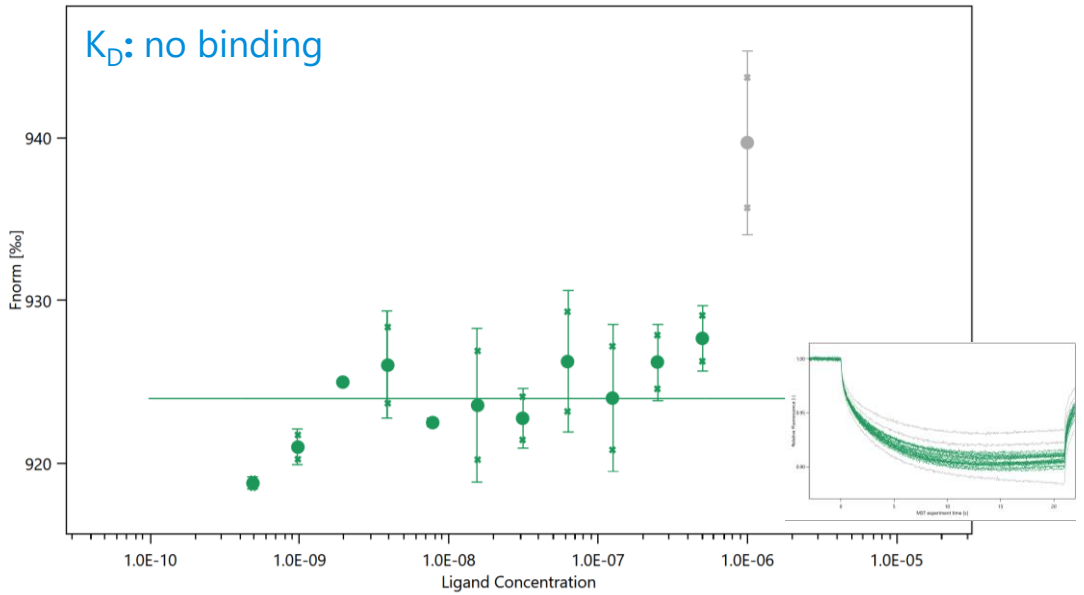
MST labelled assay conditions

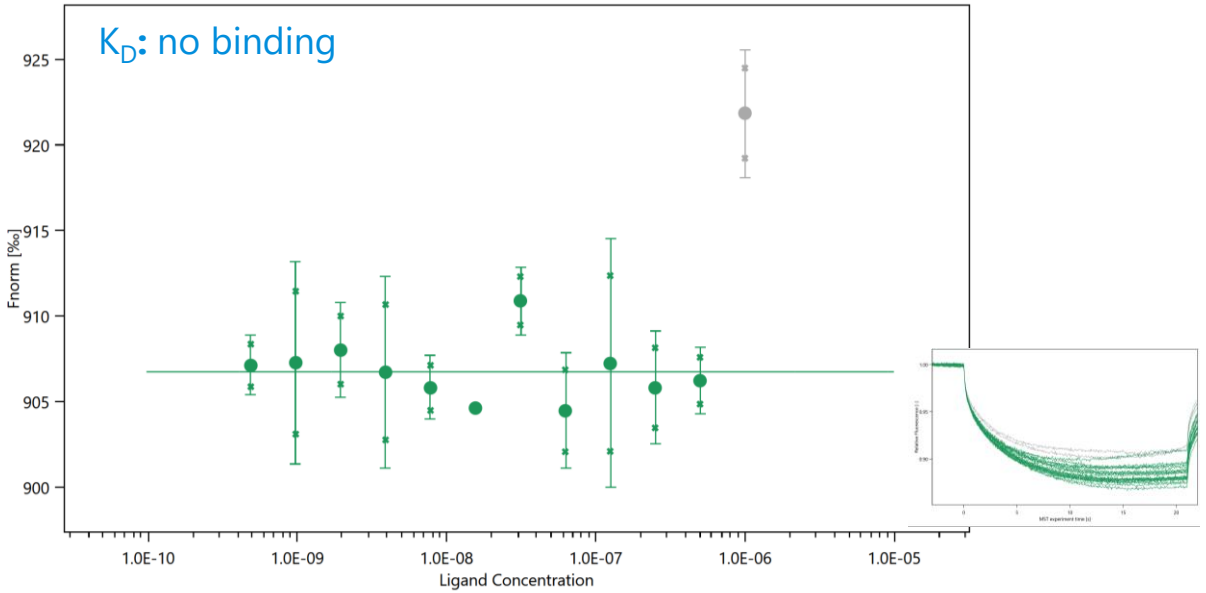
Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM RED-tris-NTA 1st and 2nd gen.		
Labelling conditions	100 nM protein / 25 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (03)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 80 % MST Power: 40 % 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 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD) VHH E4-3 (NTD nanobody) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG 8000	EEG1 (PD14991-1) (stored at 4°C) EEF1 (PD14989-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 1st and 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

VHH E4-3, 1st gen.



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

- RED-tris-NTA 1st gen. labelled Nucleocapsid does not bind VHH E4-3.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3.

RED-tris-NTA 1st and 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH H3-3

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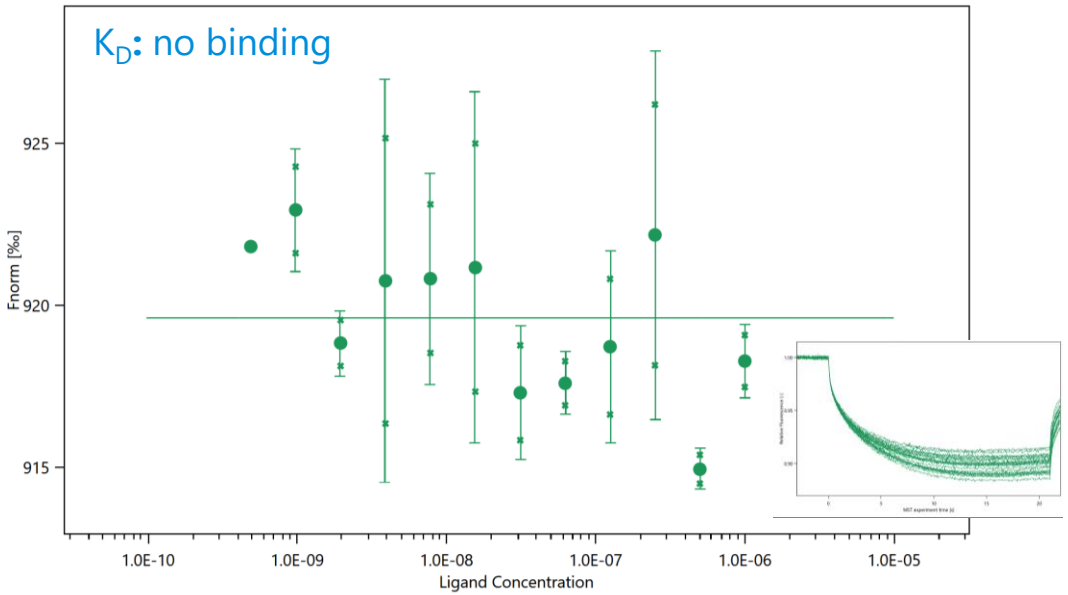
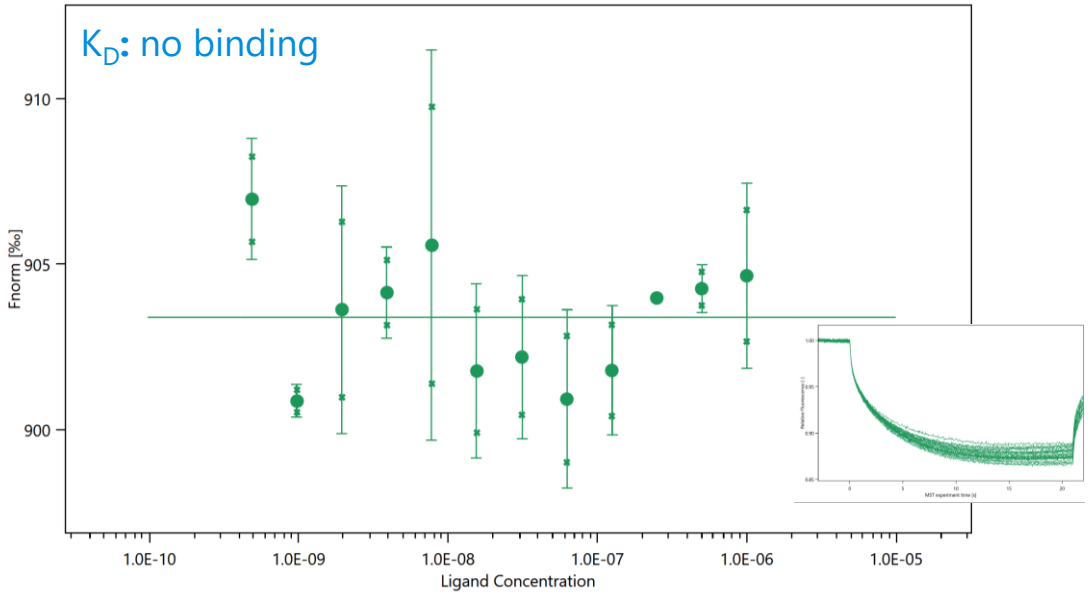
VHH H3-3, 1st gen.



VHH H3-3, 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.	Nucleocapsid	VHH H3-3	-	-	-	-	5	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	5	

- RED-tris-NTA 1st and 2nd gen. labelled Nucleocapsid does not bind VHH H3-3.

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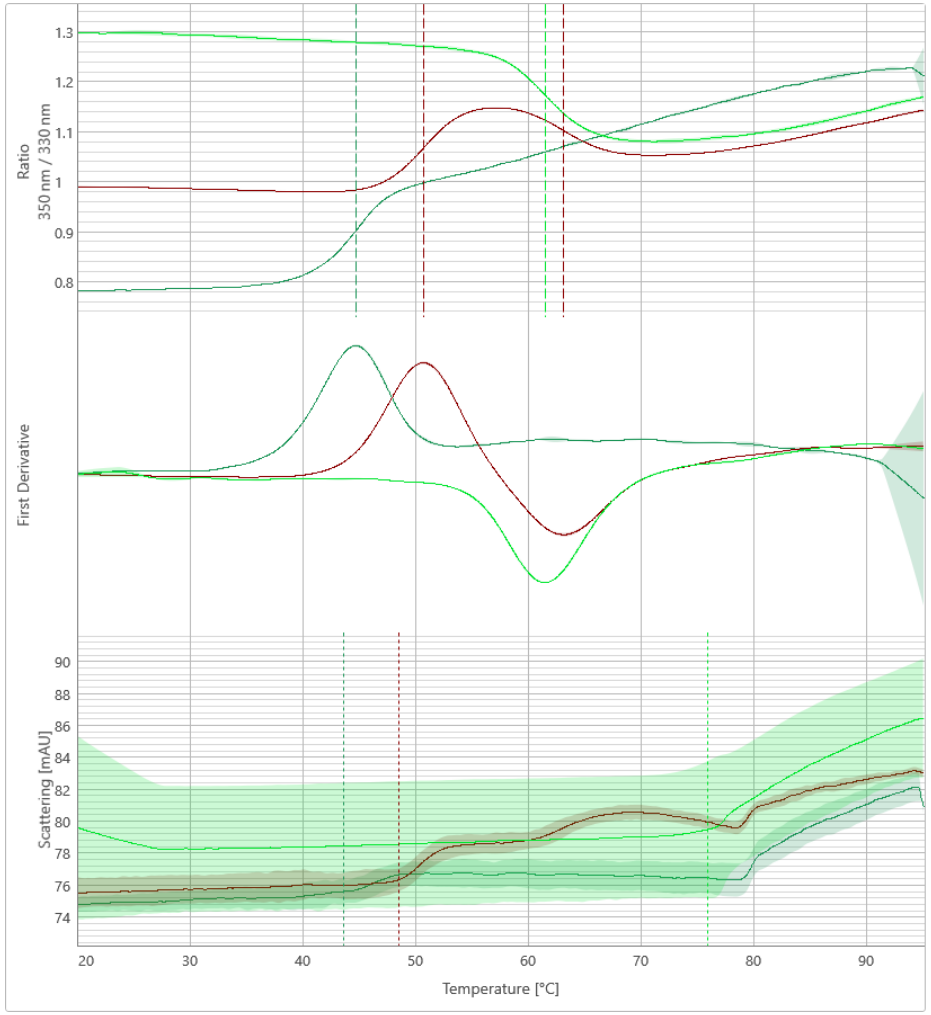
nanoDSF

SARS-CoV-2 Nucleocapsid protein (ECJ1, 15199-2)

nanoDSF Assay Conditions

Protein	0.1 mg/ml SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)
Assay Buffer	20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG-8k DMSO: 0%
compounds	4 μM VHH H3-3 (nanobody against CTD) 4 μM VHH E4-3 (NTD nanobody)
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 40°C Temperature ramp: 2°C/min

Effect of VHH E4-3 on the SARS-CoV-2 Nucleocapsid protein thermal stability

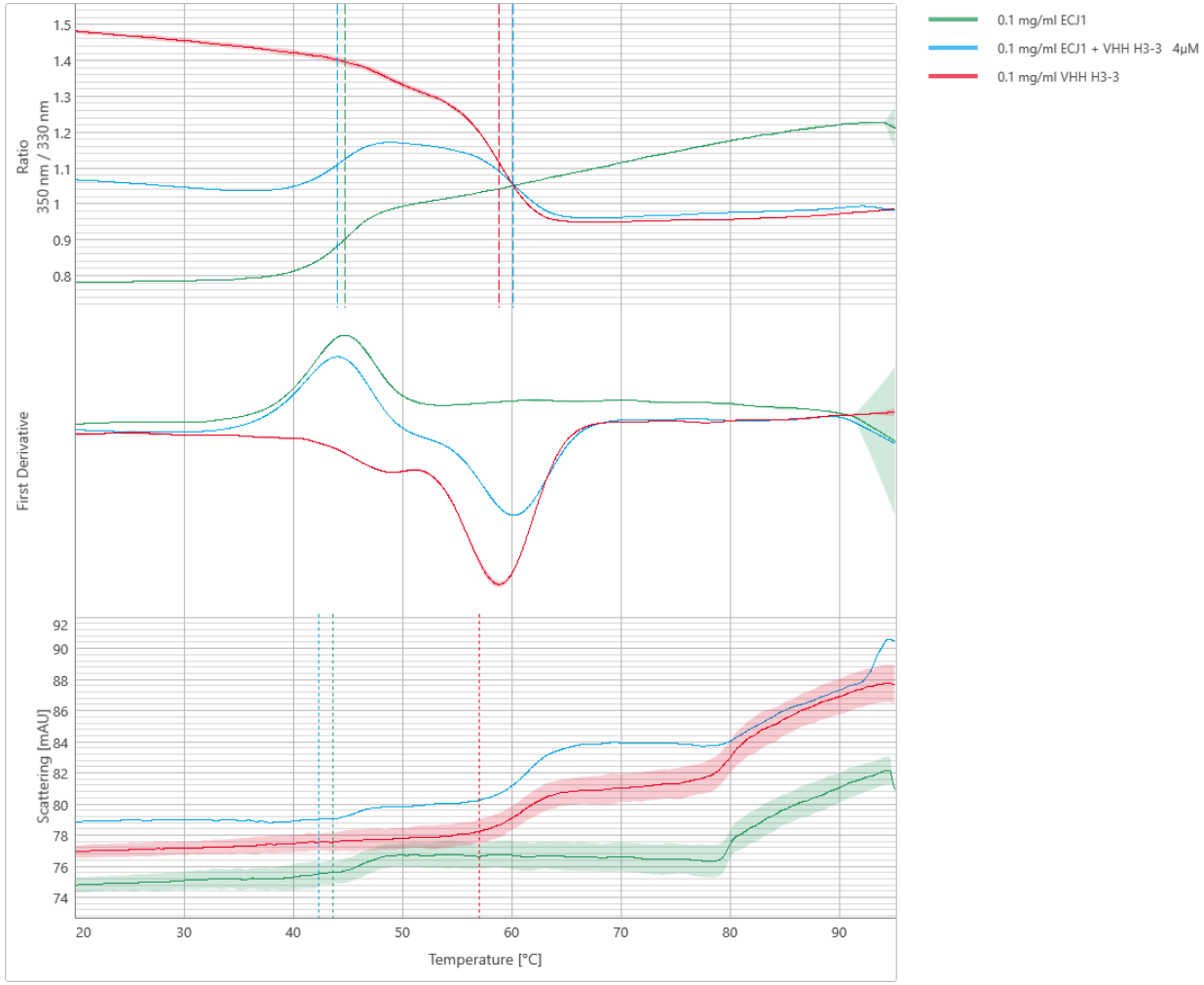


Titrant	Ø T_m [°C] [1]	s.d. [°C]	ΔT_m [°C] [2]	Ø T_{m2} [°C] [1]	s.d.2 [°C]	Ø T_{agg} [°C]	s.d. [°C]	Analysis mode
Protein	44.7	0.1	-	-	-	43.6	2.4	ratio
Protein +4 µM VHH E4-3	50.7	0.0	6.0	63.2	0.0	48.5	0.2	ratio
4 µM VHH E4-3	61.5	0.1	-	-	-	75.9	0.3	ratio

[1] determined in duplicate
[2] referenced to

- VHH E4-3 increases the protein thermal stability significantly
  - ➢ ΔT_m = 6.0

Effect of VHH H3-3 on the SARS-CoV-2 Nucleocapsid protein thermal stability



Titrant	Ø T_m [°C] [1]	s.d. [°C]	ΔT_m [°C] [2]	Ø T_{m2} [°C] [1]	s.d.2 [°C]	Ø T_{agg} [°C]	s.d. [°C]	Analysis mode
Protein	44.7	0.1	-	-	-	43.6	2.4	ratio
Protein +4 µM VHH H3-3	44.0	-	-0.7	60.2	-	42.4	-	ratio
4 µM VHH H3-3	58.8	0.0	-	-	-	57.1	0.3	ratio

[1] determined in duplicate
[2] referenced to

- VHH H3-3 has no significant impact on nucleocapsid thermal stability.

Labelled MST

SARS-CoV-2 Nucleocapsid protein (ECJ1, 15199-2)

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM 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 (02)		
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, 0.1% PEG 8000, 0.05% Tween20, 5% Glycerol Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 2 mM DTT Buffer 3: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 2 mM GSH Buffer 4: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 0.5 mM TCEP Buffer 5: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 10 mM MgCl2 DMSO: 0%		
Titrant	VHH E4-3 (NTD nanobody) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG 8000	EEF1 (PD14989-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

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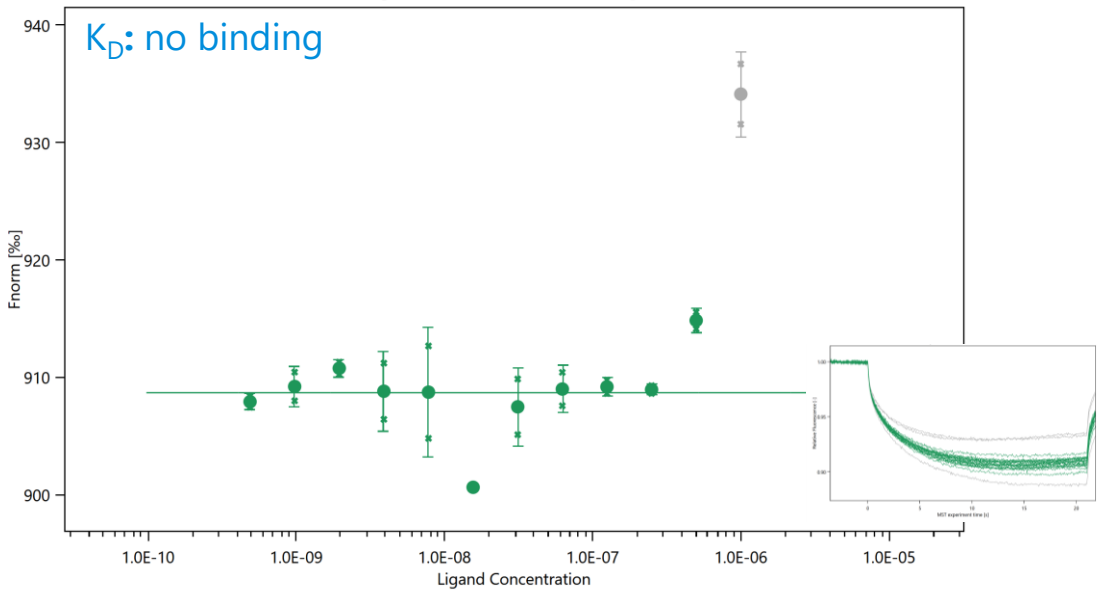
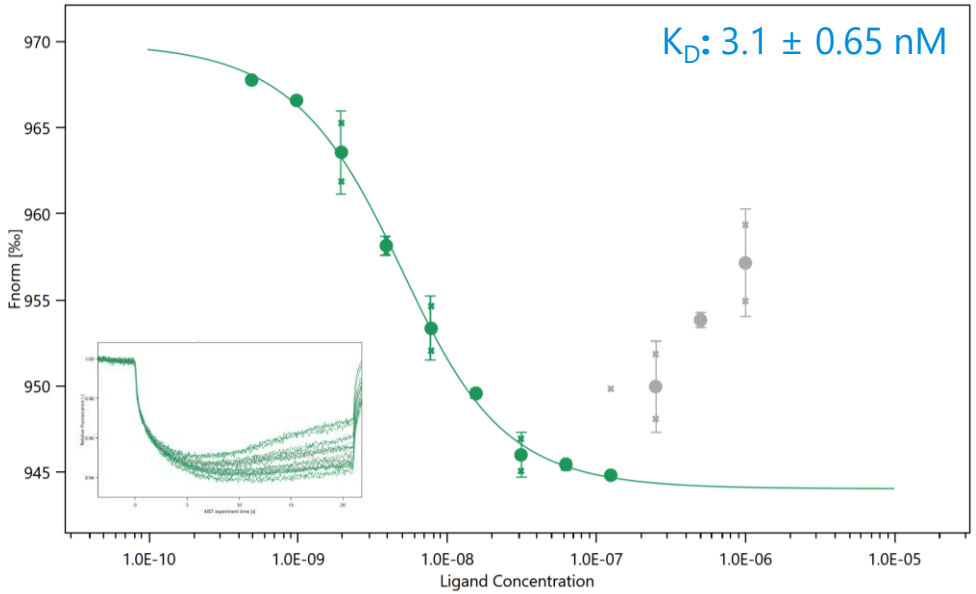
Buffer 1



Buffer 2

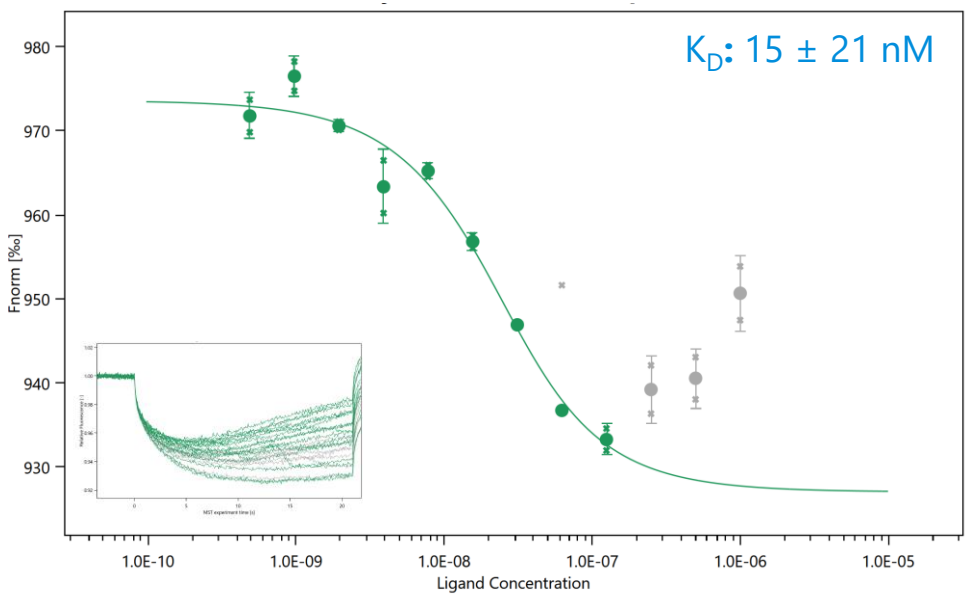


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	-	-	-	-	20	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	3.1E-09	6.5E-10	25.9	69.6	20	

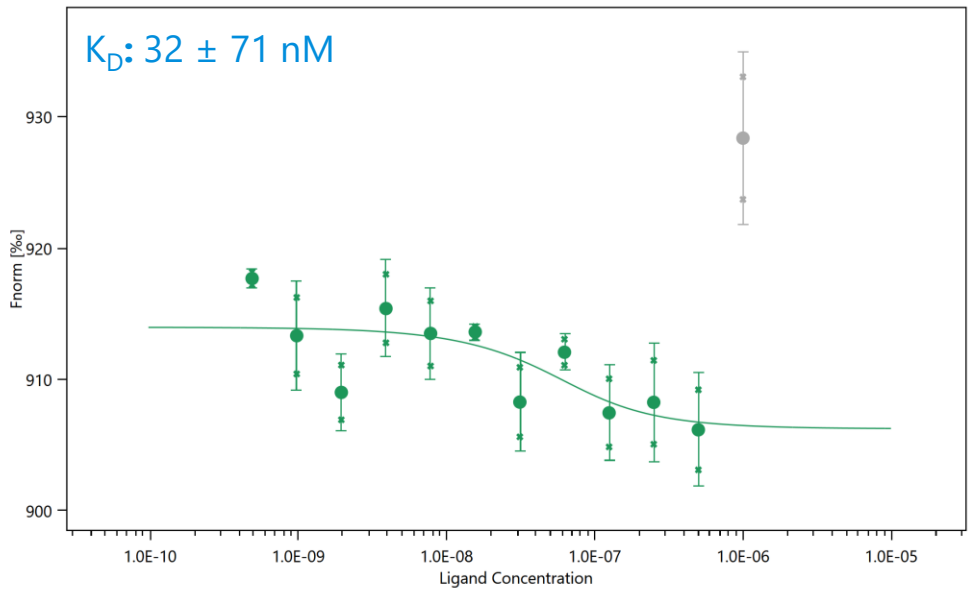
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 1.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 3.1 nM in buffer 2.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

Buffer 3



Buffer 4

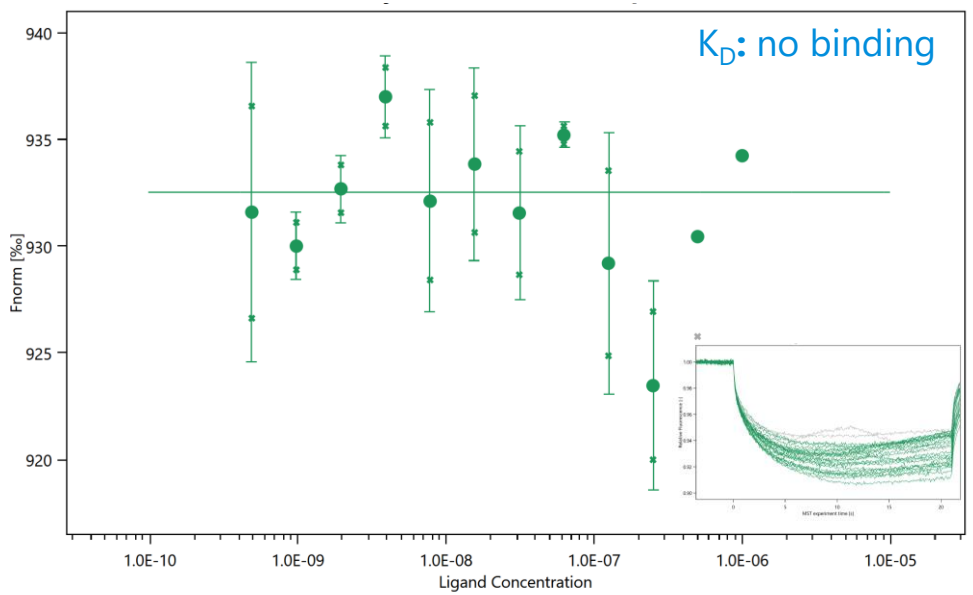


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	1.5E-08	2.1E-08	46.5	18.7	20	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	3.2E-08	7.1E-08	7.7	3.2	20	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 15 nM in buffer 3.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid potentially binds VHH E4-3 with a determined K_D of 32 nM and low signal-to-noise ratio in buffer 4.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

Buffer 5



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	-	-	-	-	20	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 5.

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM 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 (02)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 100 % MST Power: 60 % 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, 0.1% PEG 8000, 0.05% Tween20, 5% Glycerol Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 2 mM DTT Buffer 3: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 2 mM GSH Buffer 4: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 0.5 mM TCEP Buffer 5: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20, 10 mM MgCl2 DMSO: 0%		
Titrant	VHH E4-3 (NTD nanobody) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG 8000	EEF1 (PD14989-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

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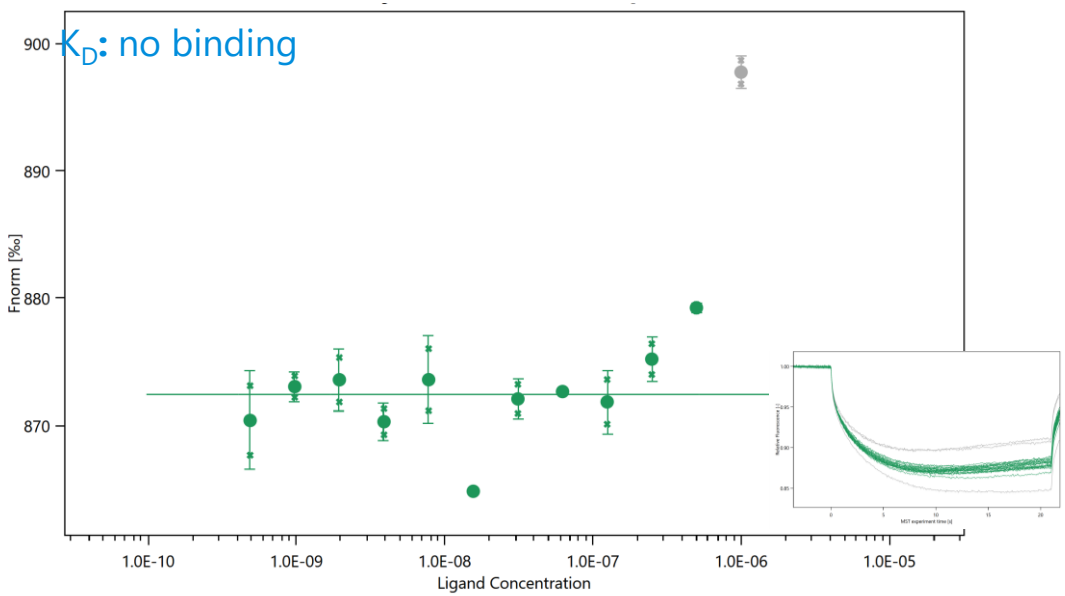
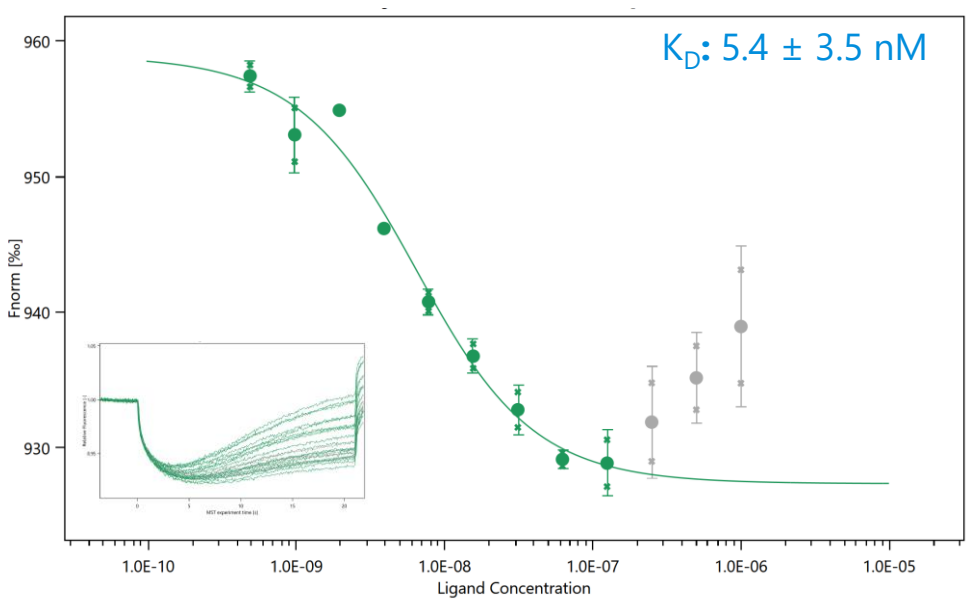
Buffer 1



Buffer 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	-	-	-	-	10	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	5.4E-09	3.5E-09	31.6	22.4	10	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 1.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 5.4 nM in buffer 2.

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RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

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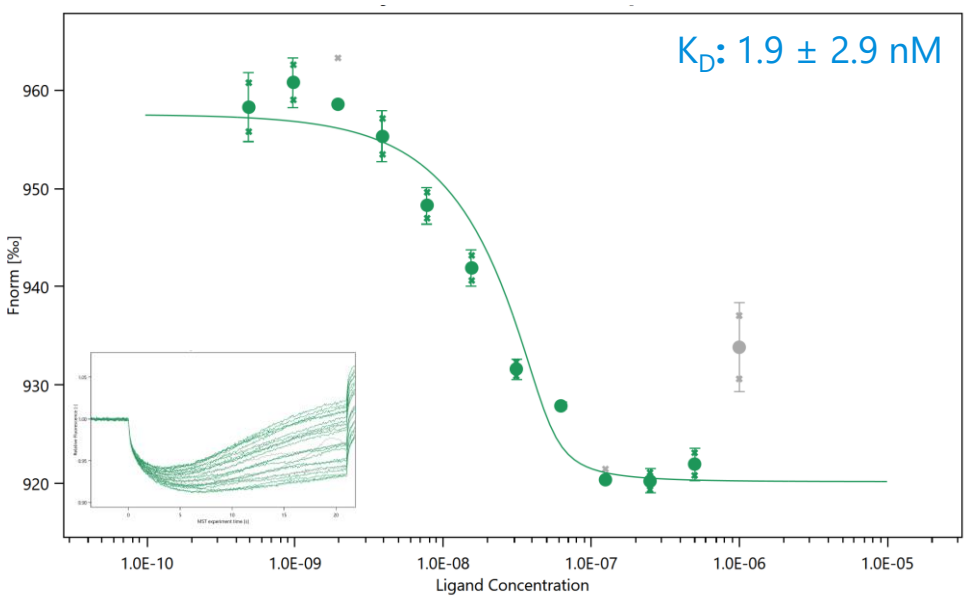
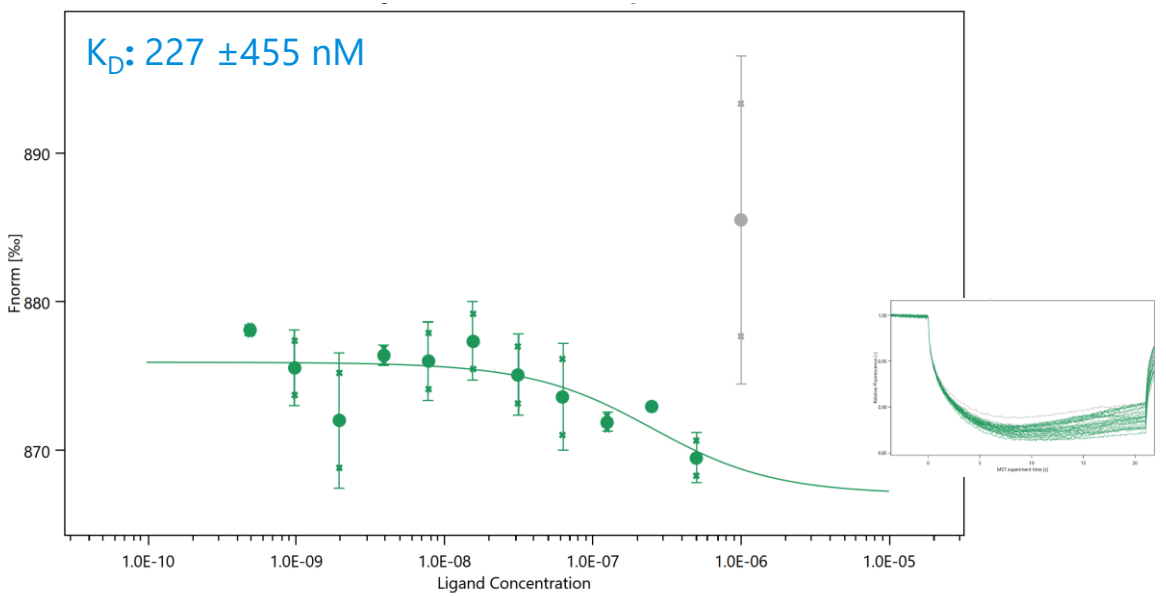
Buffer 3



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	1.9E-09	2.9E-09	37.3	12.0	10	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	2.3E-07	4.6E-07	8.9	5.3	10	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 1.9 nM in buffer 3.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 227 nM in buffer 4.

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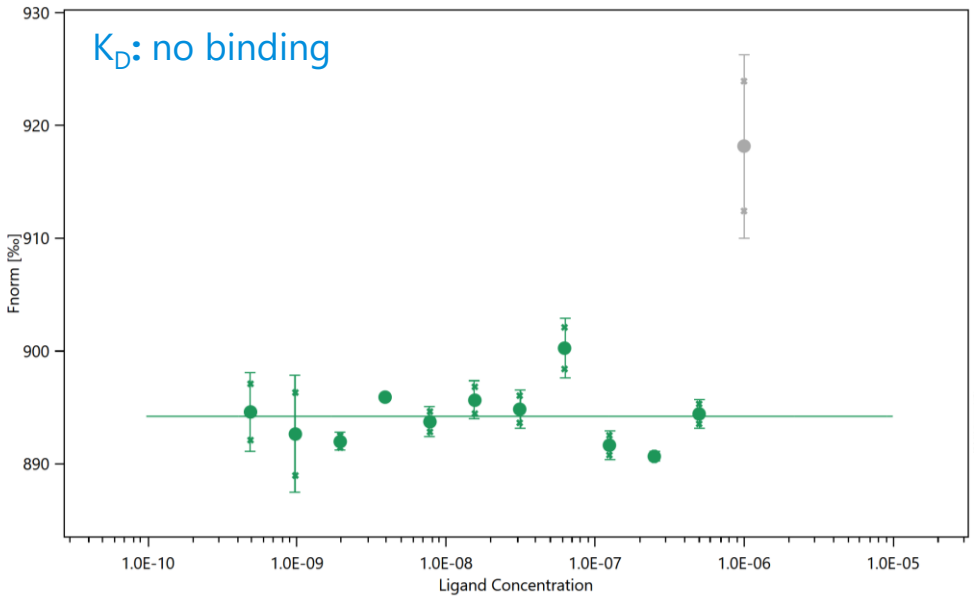
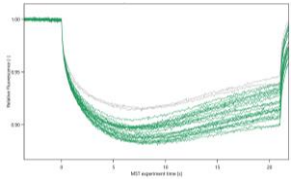
RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

Buffer 5





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	-	-	-	-	10	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH E4-3 in buffer 5.

MST labelled assay conditions

Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2)		
Fluorophore	12.5 nM 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 (02)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 100 % 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, 2 mM DTT DMSO: 2.5%		
Titrant	VHH E4-3 (NTD nanobody) - No dialysis	EEF1 (PD14989-1) (stored at 4°C)	500 nM – 0.23 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD15199-2) vs. VHH E4-3

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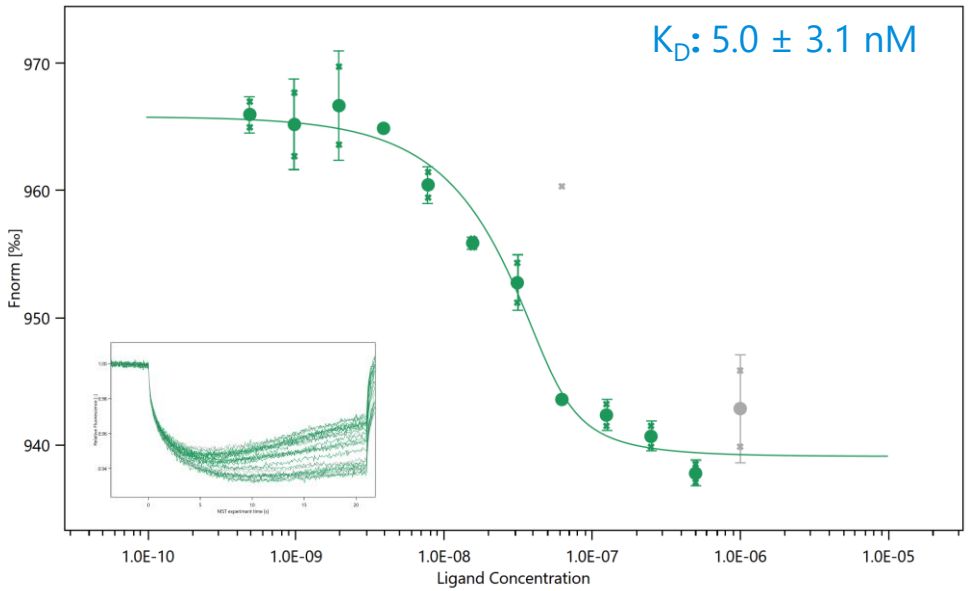
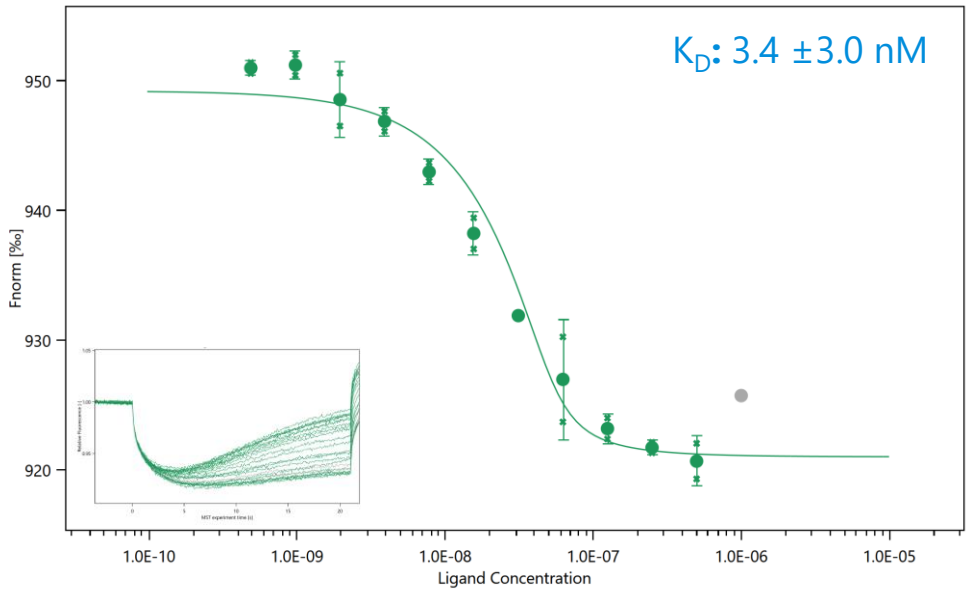
Medium MST power



High MST power



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	5.0E-09	3.1E-09	26.7	18.5	20	
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	3.4E-09	3.0E-09	28.2	15.6	10	

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 5.0 nM with medium MST power and with a determined K_D of 3.4 nM with high MST power. Larger signal-to-noise ratio is observed with high MST power, which was chosen for the following compound screening.

Next steps

- 8 pt screening of 119 cpds in singlicate using high MST power and VHH E4-3 (NTD nanobody) as a positive control (on-going)

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