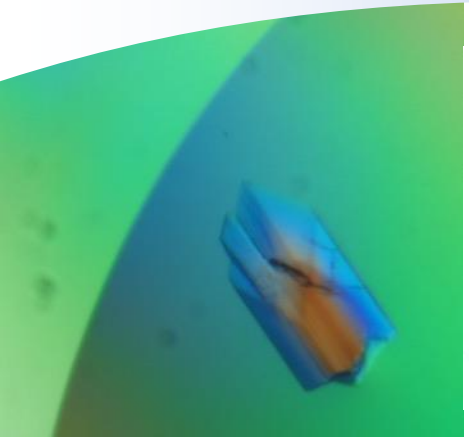
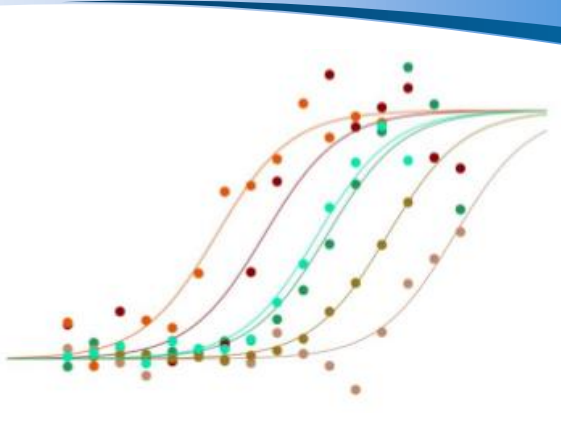
JEDI

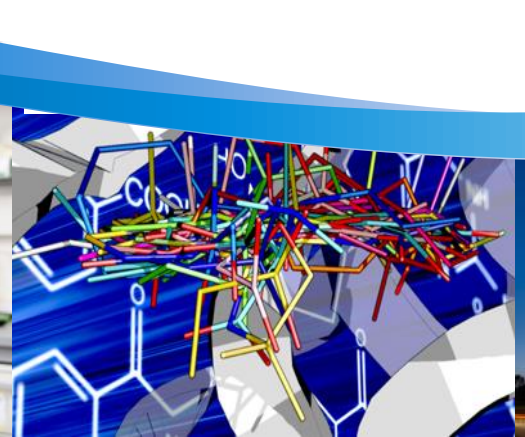
JDI01

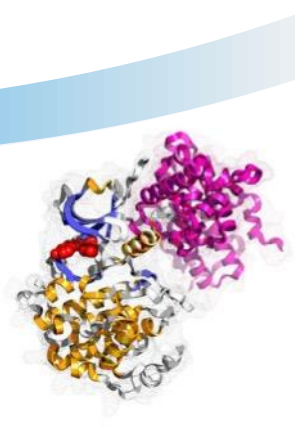
SARS-CoV-2 Nucleocapsid protein

MST/ TRIC measurements

August 5, 2021





Status

- TRIC (Dianthus) and Labelled MST (NT.115):
  - Binding of PolyT hexamers to Nucleocapsid was tested on the Dianthus and Monolith NT.115 in buffers with different salt concentrations.
    - At 150 mM NaCl, weak binding without saturation was observed on both instruments. Aggregation was observed on the Dianthus.
    - At lower salt concentrations (10 and 50 mM), no binding and aggregation was observed on both instruments.
  - Nanobodies were prepared with dialysis (instead of gel filtration), and binding of nanobodies to Nucleocapsid was tested and optimized on Dianthus/NT.115.
    - On the Dianthus, binding of both nanobodies to Nucleocapsid was observed with high affinity. However, strong aggregation was observed and K_D values could not be fitted.
    - Buffer optimization was attempted in PBS buffers (w/o buffer exchange) and Hepes buffers (w/ dialysis). No improvement was observed, aggregation could not be prevented.
    - On the NT.115 instrument (MST), better data quality with less aggregation was observed:
      - RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH H3-3 with a determined K_D of 10 nM.
      - RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 93 nM.
  - We recommend to use MST with one nanobody as tool compound for the compound screening.

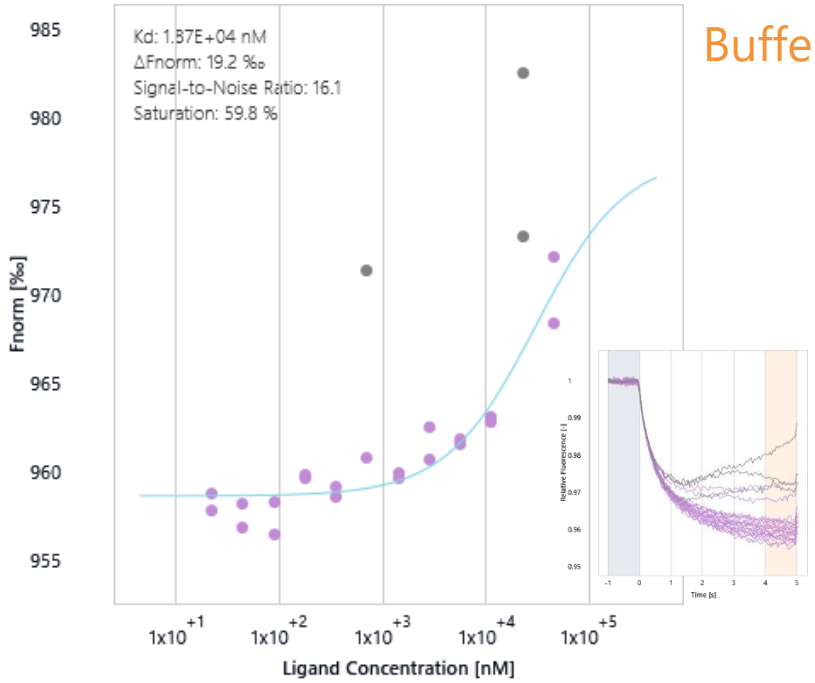
TRIC (Dianthus)

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

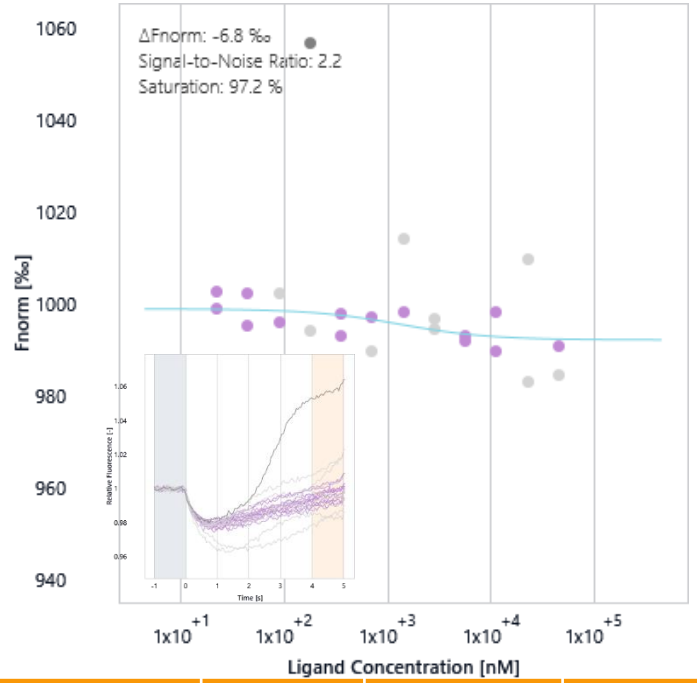
TRIC Assay Conditions

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: 37 % (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**, 0.1% PEG 8000, 0.05% Tween20 Buffer 2: 20 mM HEPES pH 7.5, **50 mM NaCl**, 0.1% PEG 8000, 0.05% Tween20 Buffer 3: 10 mM Na-phosphate pH 7.5, **10 mM NaCl**, 0.1% PEG 8000, 0.05% Tween20 DMSO: 0%		
Titrant	PolyT	JDI-884	45 µM – 22 nM (12 conc.), 1:2

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



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	PolyT	>1.9E-05	-	-	19.2	16.1	5	Buffer 1, aggregation, no saturation
RED-tris-NTA 2nd gen.	Nucleocapsid	PolyT	-	-	-	-	-	5	Buffer 2, aggregation

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds PolyT with an estimated K_D > 19 µM in buffer 1 without reaching saturation. In addition, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind PolyT in buffer 2, aggregation was observed.

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

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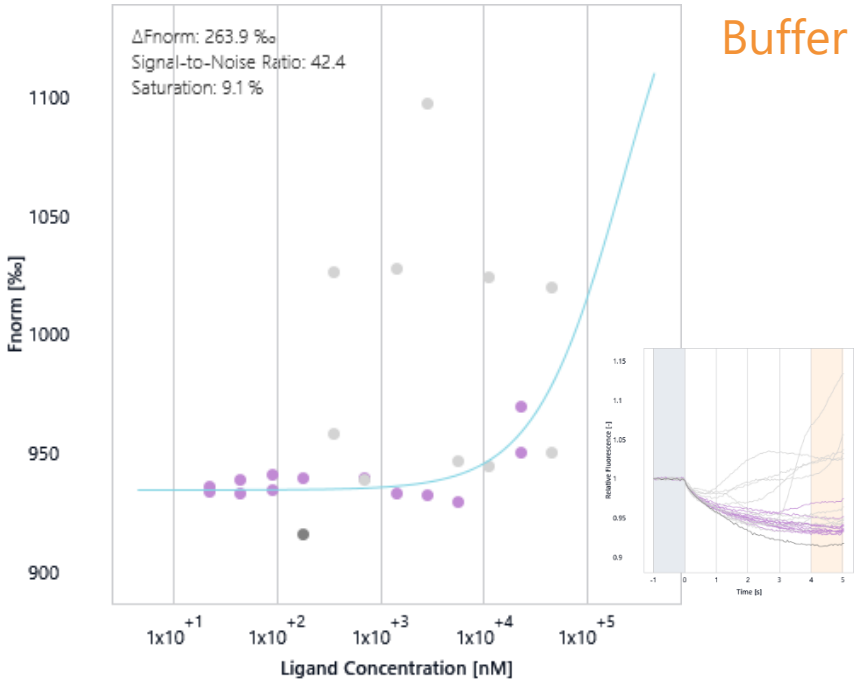
Buffer 3



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	PolyT	-	-	-	-	-	5	Buffer 3, aggregation

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

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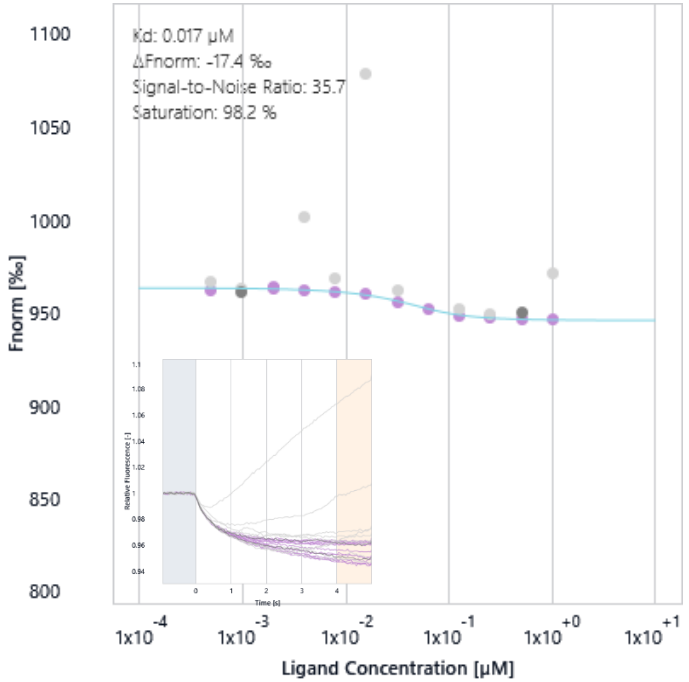
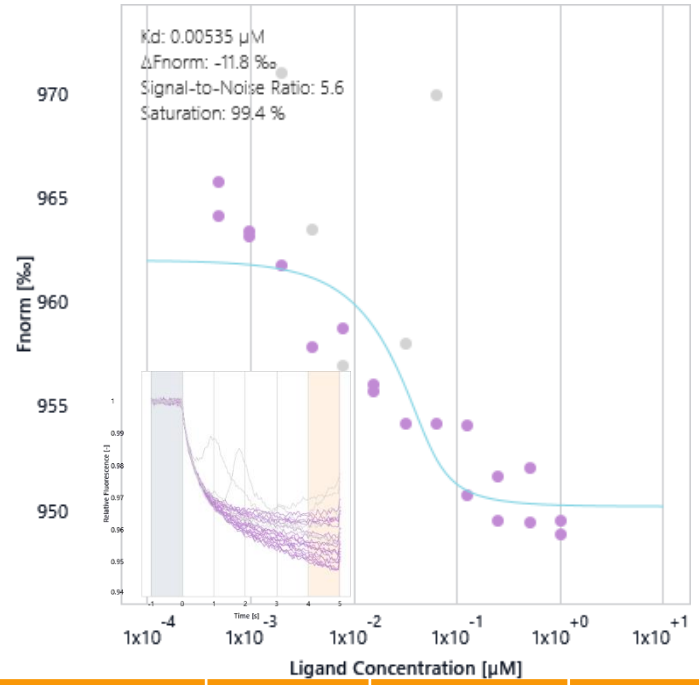
TRIC Assay Conditions

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: 28 % (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, 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, PD14785-1) vs. VHH H3-3 and VHH E4-3



VHH H3-3 (EEG1)



VHH E4-3 (EEF1)

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 H3-3	-	-	-	-	-	5	aggregation
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	-	-	-	-	-	5	aggregation

- RED-tris-NTA 2nd gen. labelled Nucleocapsid shows binding to VHH H3-3 and VHH E4-3. However, due to aggregation, no K_D value can be determined.

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: 41 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	Buffer 1: **PBS**, 0.1% PEG 8000, 0.05% Tween20 Buffer 2: **PBS**, 5% Glycerol, 0.05% Tween20 Buffer 3: **PBS**, 0.1% PEG 8000, 5% Glycerol, 0.05% Tween20 Buffer 4: **PBS**, 0.1% BSA, 0.05% Tween20 Buffer 5: **PBS**, 0.1% PEG 8000, 2mM DTT, 0.05% Tween20 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD)	EEG1 (PD14991-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

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

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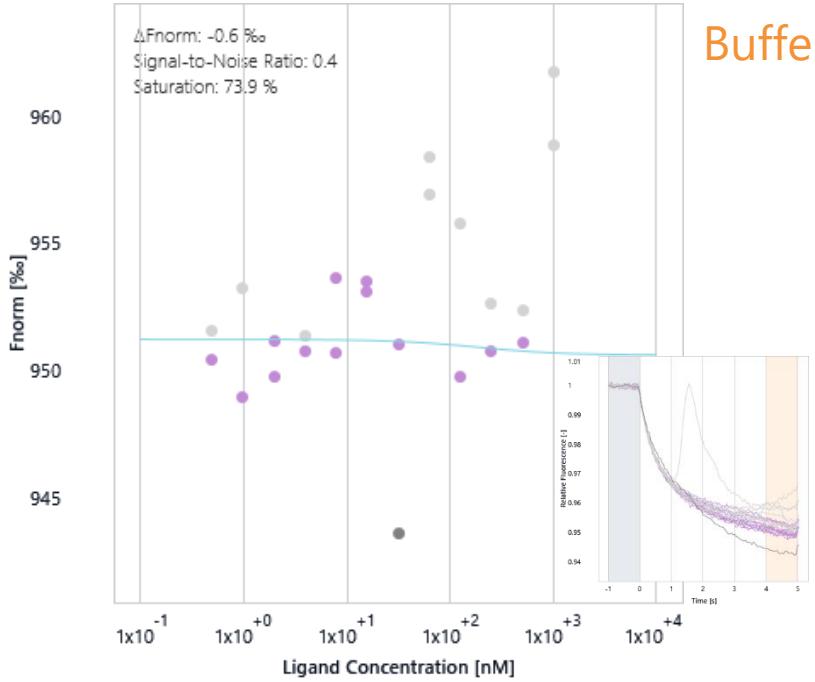
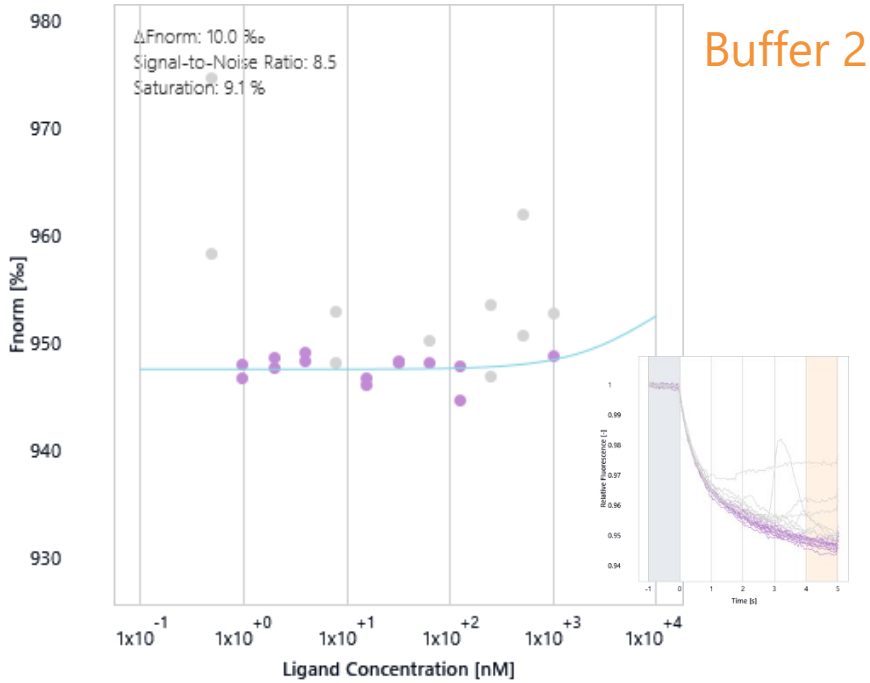
Buffer 1



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 H3-3	-	-	-	-	-	5	Buffer 1
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	-	5	Buffer 2

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 1, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 2, aggregation was observed.

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

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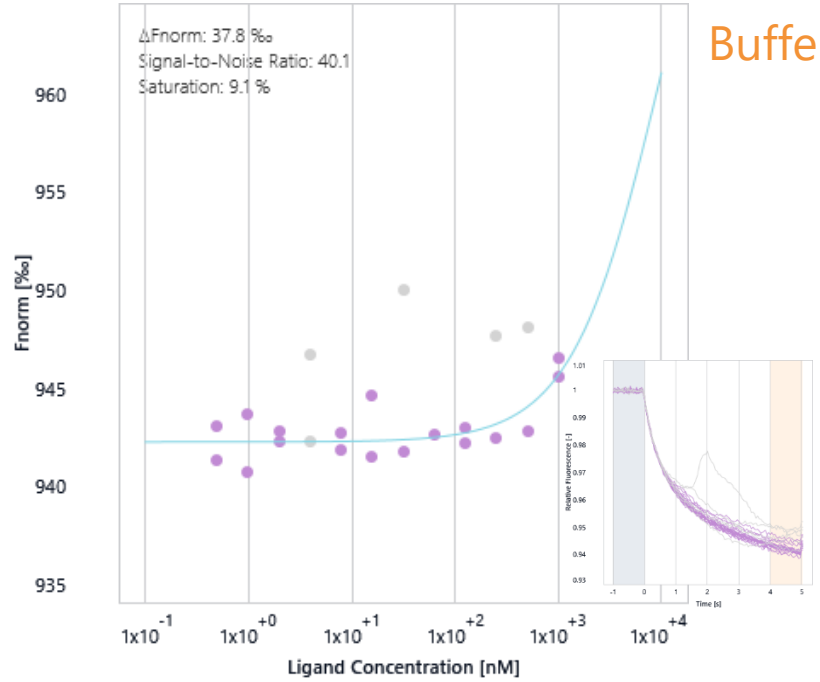
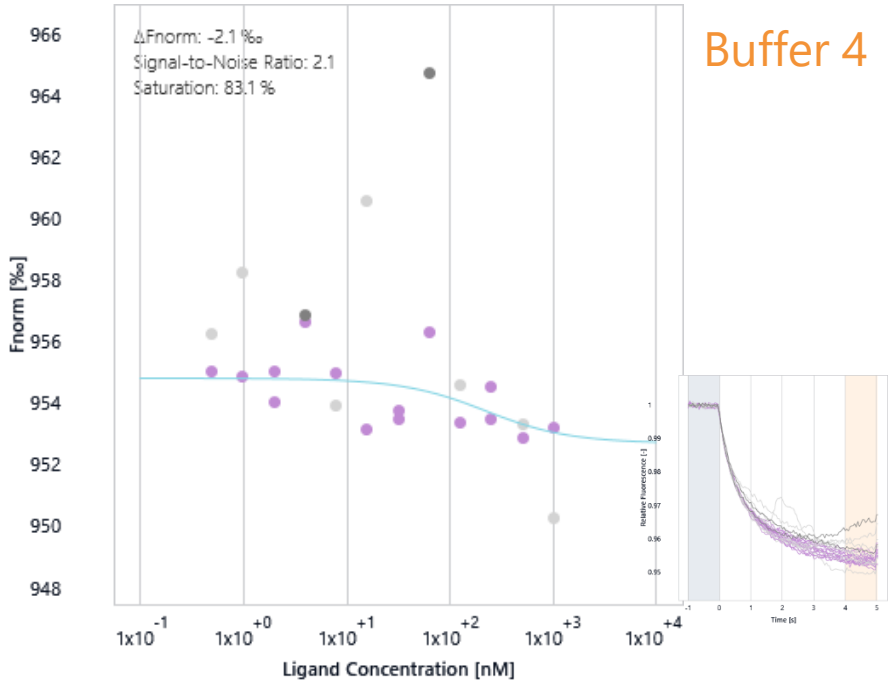
Buffer 3



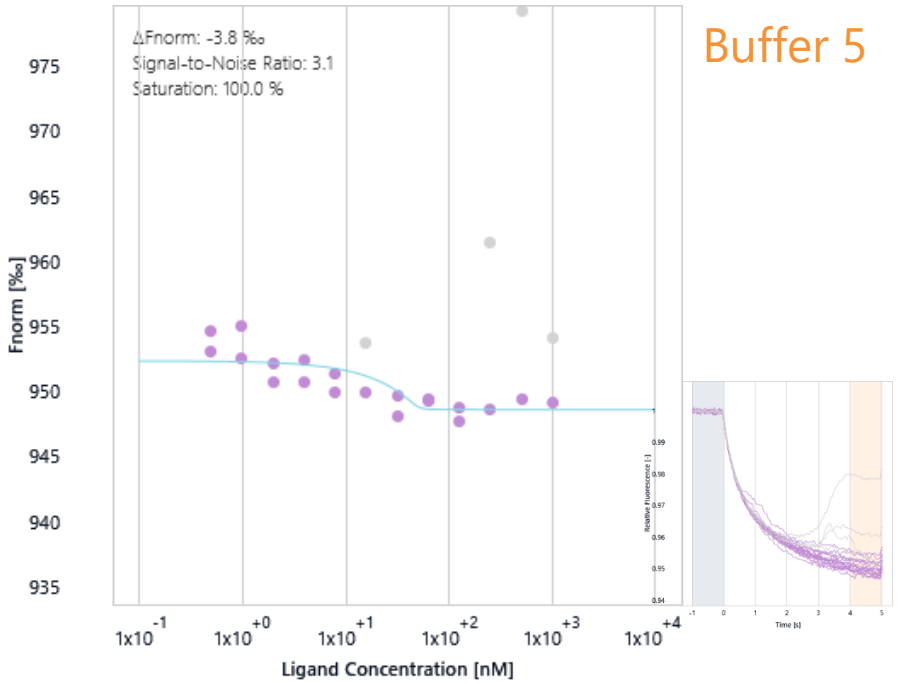
Buffer 4



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 H3-3	-	-	-	-	-	5	Buffer 3
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	-	5	Buffer 4

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 3, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 4, aggregation was observed.

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



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 H3-3	-	-	-	-	-	5	Buffer 5

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 5, 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: 28 % (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, 0.1% PEG 8000, 0.005% Tween20 Buffer 2: 20 mM **HEPES** pH 7.5, 150 mM NaCl, 5% Glycerol, 0.005% Tween20 Buffer 3: 20 mM **HEPES** pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 5% Glycerol, 0.005% Tween20 Buffer 4: 20 mM **HEPES** pH 7.5, 150 mM NaCl, 0.1% BSA, 0.005% Tween20 Buffer 5: 20 mM **HEPES** pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 2mM DTT, 0.005% Tween20 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.005% Tween	EEG1 (PD14991-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

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

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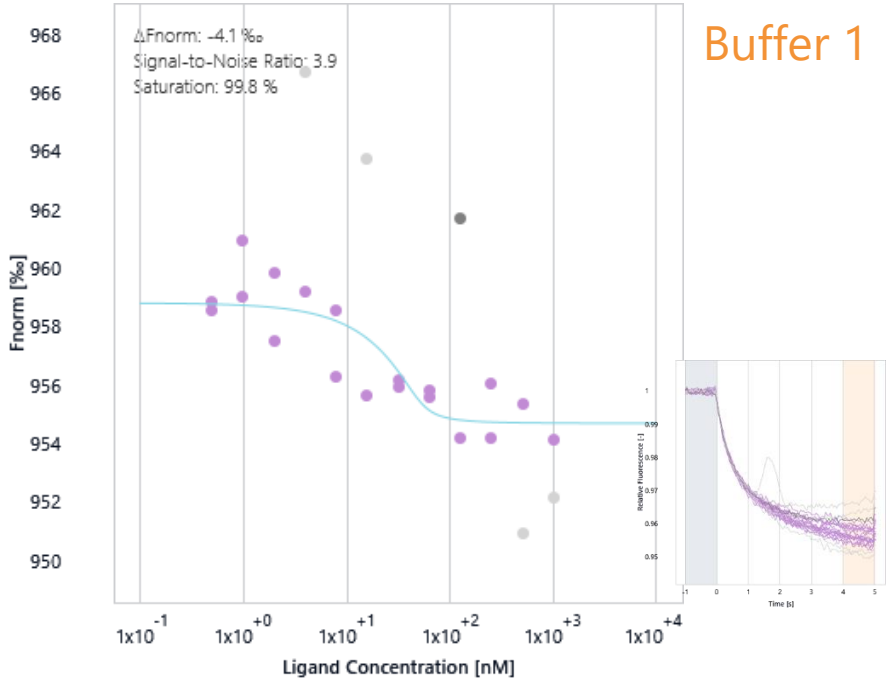
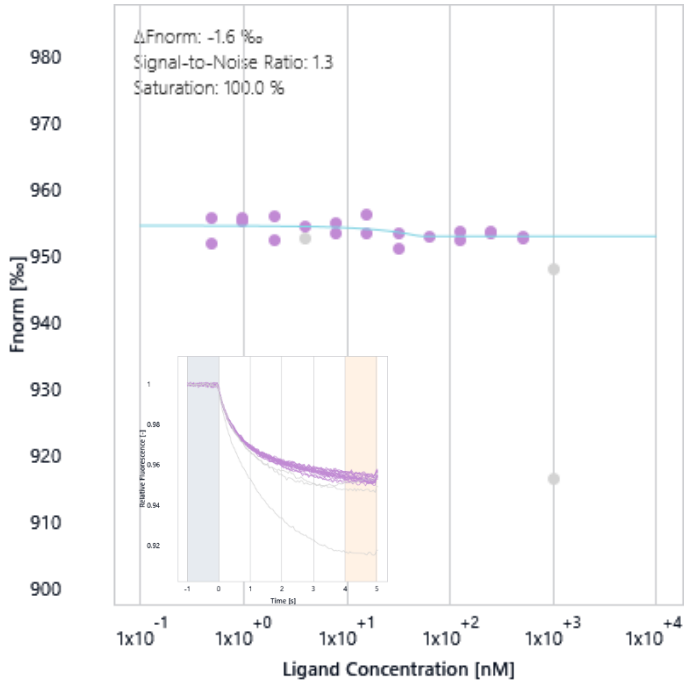
Buffer 1



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 H3-3	-	-	-	-	-	5	Buffer 1
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	-	5	Buffer 2

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 1, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 2, aggregation was observed.

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

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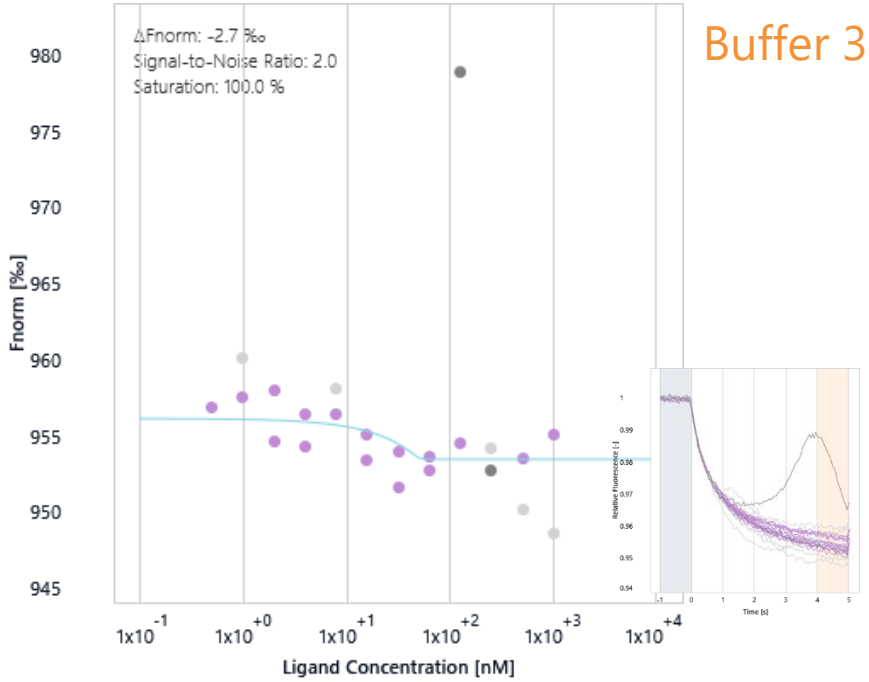
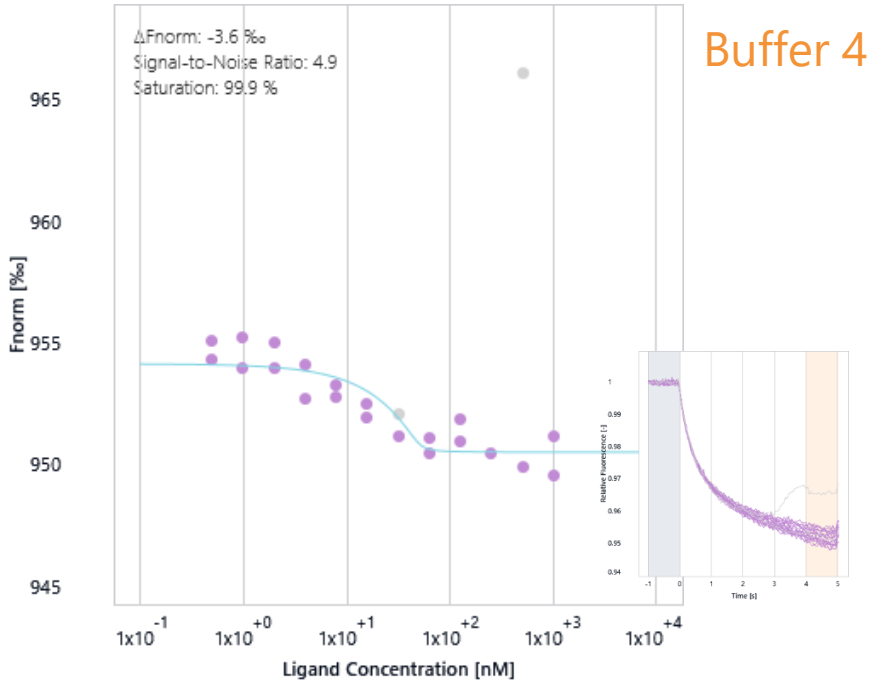
Buffer 3



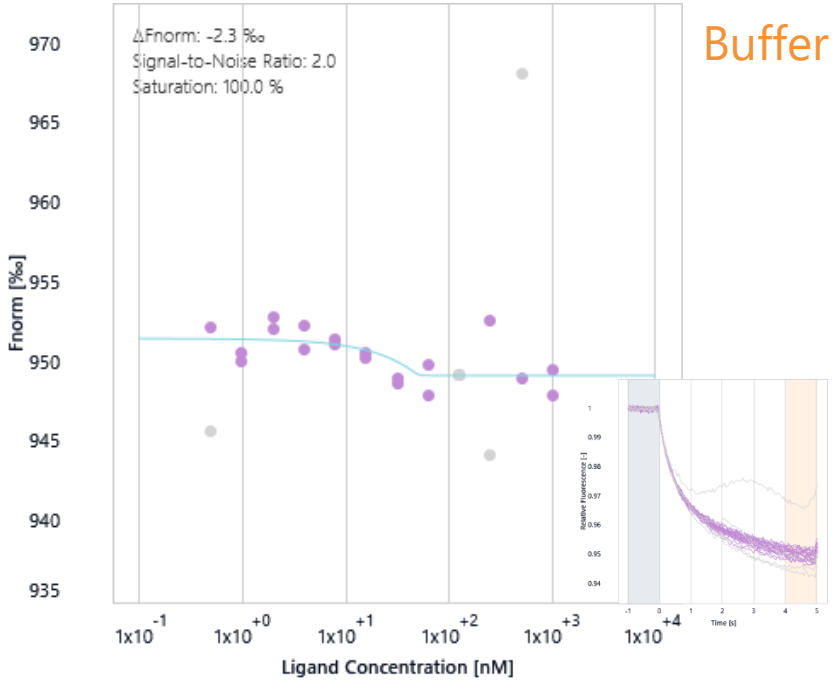
Buffer 4



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 H3-3	-	-	-	-	-	5	Buffer 3
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	-	5	Buffer 4

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 3, aggregation was observed.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 4, aggregation was observed.

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



Buffer 5

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 H3-3	-	-	-	-	-	5	Buffer 5

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 5, aggregation was observed.

Labelled MST

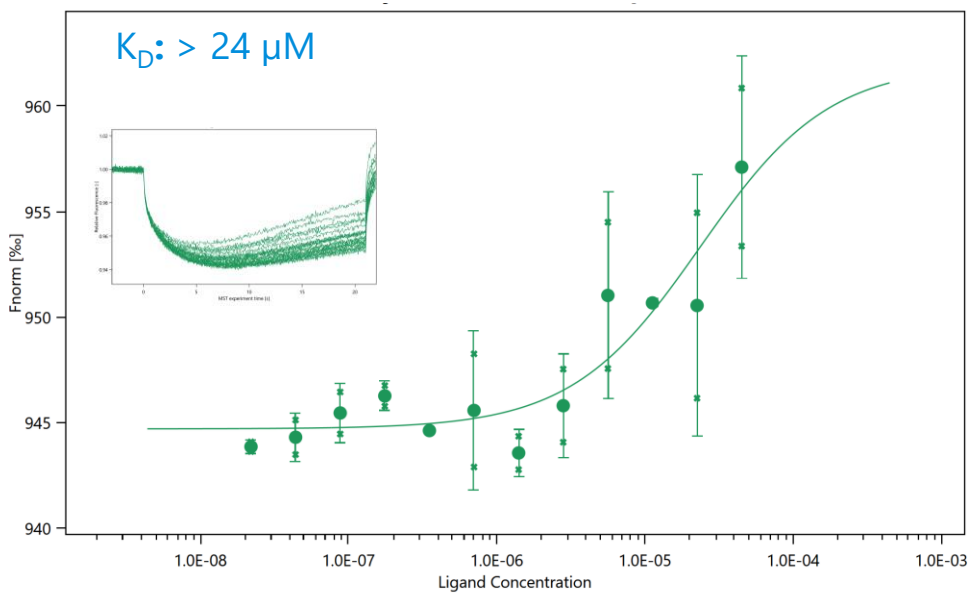
SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)

MST labelled assay conditions

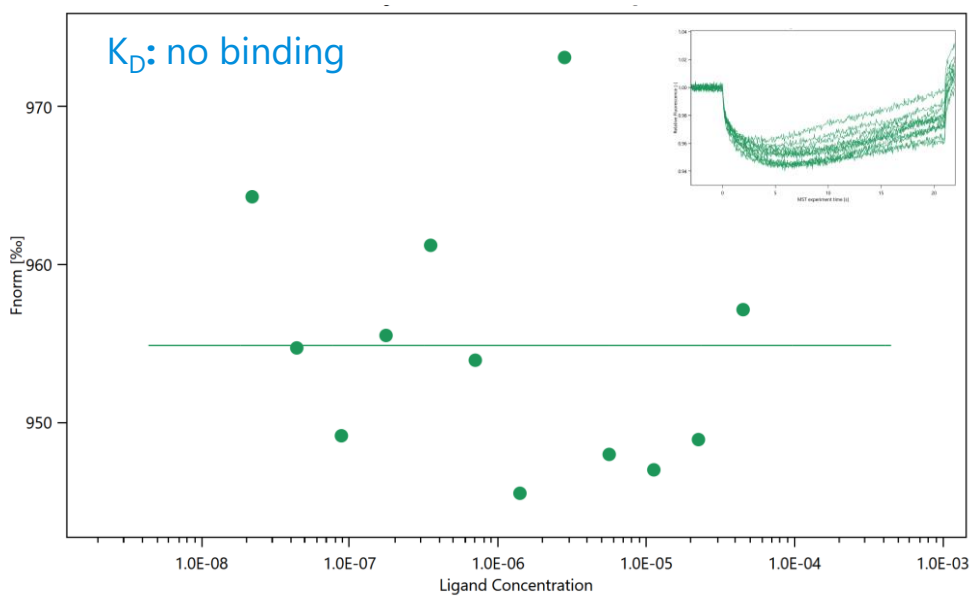
Fluor. Molecule	50 nM SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1)		
Fluorophore	RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 25 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (03)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 100 % MST Power: 40 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Singlicate and Duplicate		
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.05% Tween20 Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 Buffer 3: 10 mM Na-phosphate pH 7.5, 10 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 DMSO: 0%		
Titrant	PolyT	JDI-884	45 µM – 22 nM (12 conc.), 1:2

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

Buffer 1



Buffer 2

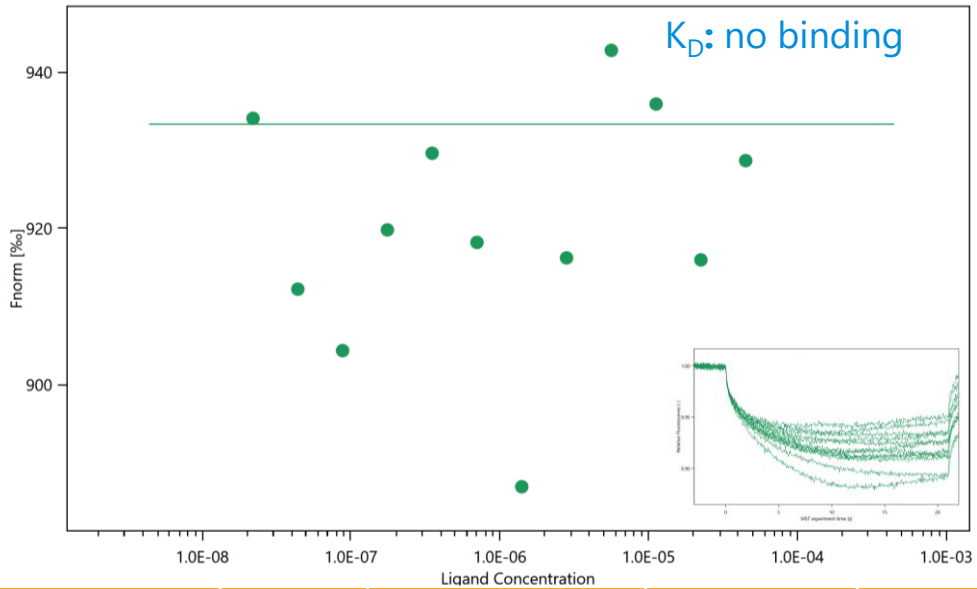


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	PolyT	> 2.4E-05	-	17.3	11.3	10	Buffer 1
RED-tris-NTA 2nd gen.	Nucleocapsid	PolyT	-	-	-	-	10	Buffer 2, aggregation

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds PolyT with an estimated K_D > 24 µM without reaching saturation in buffer 1.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind PolyT in buffer 2 and aggregation was observed.

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

Buffer 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	PolyT	-	-	-	-	10	Buffer 3

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind PolyT in buffer 3 and aggregation was observed.

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 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Singlicate		
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, PD14785-1) vs. VHH H3-3 and VHH E4-3

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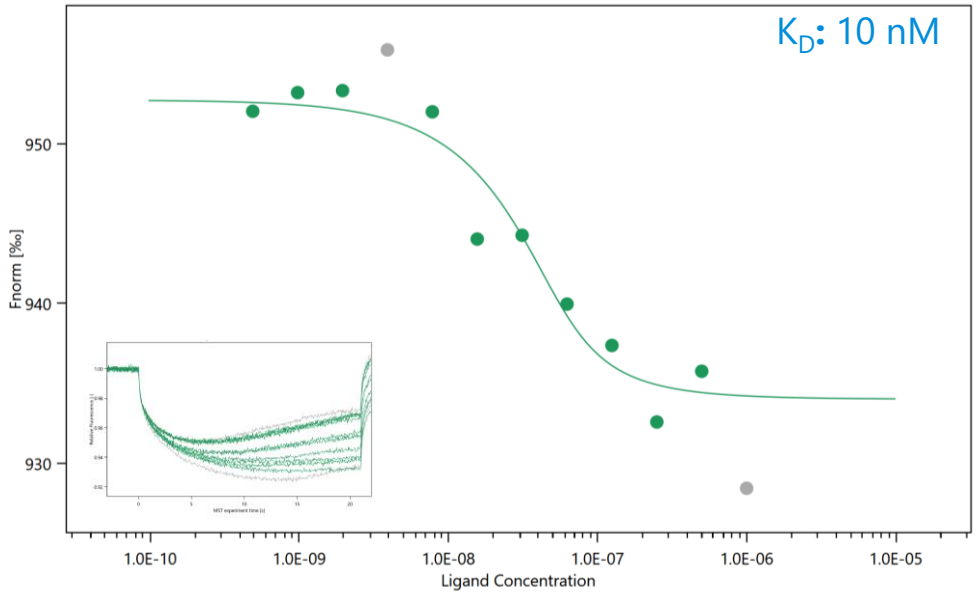
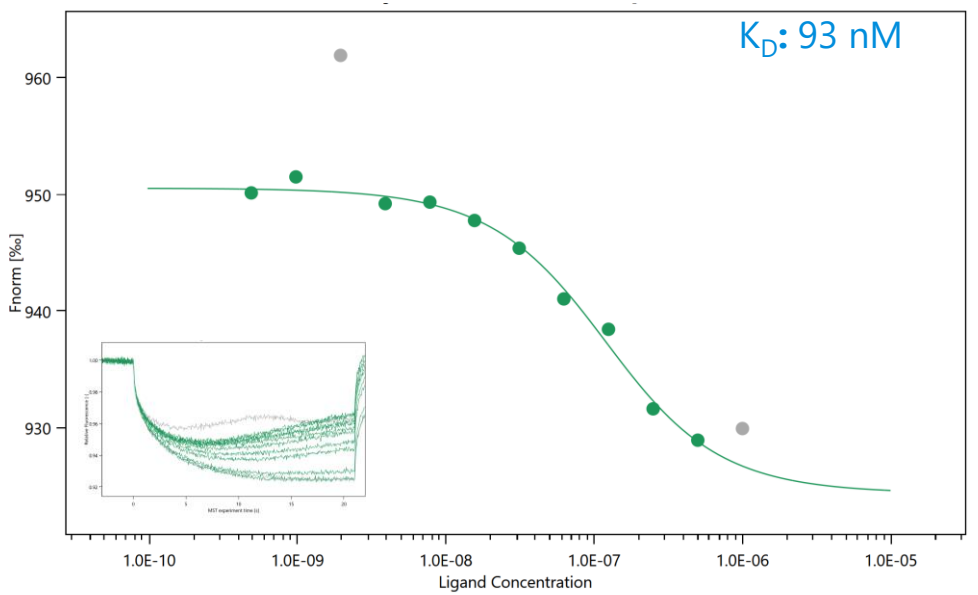
VHH H3-3



VHH E4-3



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 H3-3	1.0E-08	1.7E-09 – 6.2E-08	18.7	10.1	10	VHH H3-3
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH E4-3	9.3E-08	6.0E-08 – 1.5E-07	26.2	35.1	10	VHH E4-3

- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH H3-3 with a determined K_D of 10 nM.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid binds VHH E4-3 with a determined K_D of 93 nM.
- Low amount of aggregation was observed for both nanobodies.

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MST labelled assay conditions

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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 % MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Singlicate and Duplicate		
Assay buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 Buffer 3: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 5% Glycerol, 0.05% Tween20 Buffer 4: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% BSA, 0.05% Tween20 Buffer 5: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 2mM DTT, 0.05% Tween20 DMSO: 0%		
Titrant	VHH H3-3 (nanobody against CTD) Dialyzed into: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.005% Tween	EEG1 (PD14991-1) (stored at 4°C)	1 µM – 0.49 nM (12 conc.)

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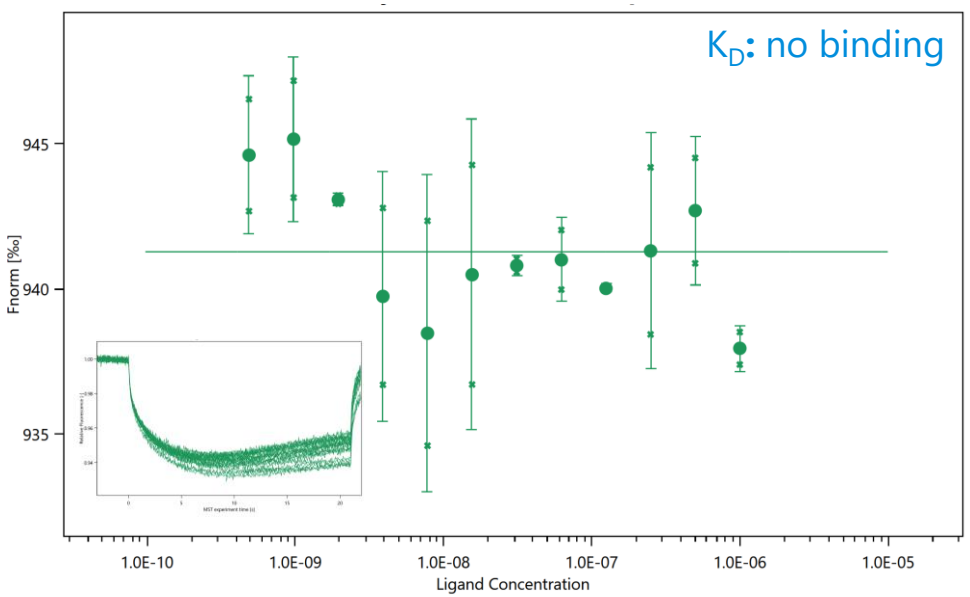
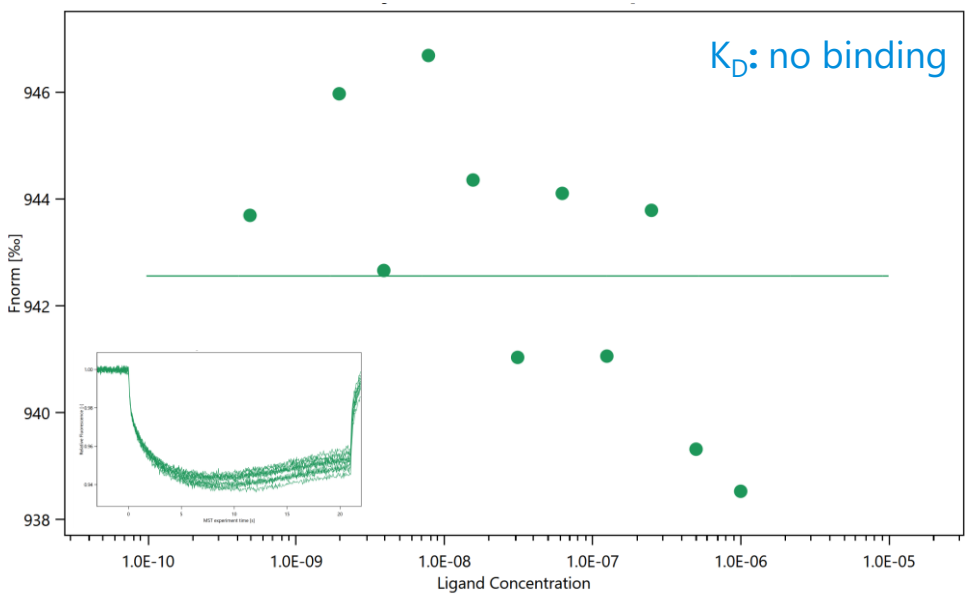
RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Nucleocapsid protein (ECJ1, PD14785-1) vs. VHH H3-3

Buffer 1



Buffer 3

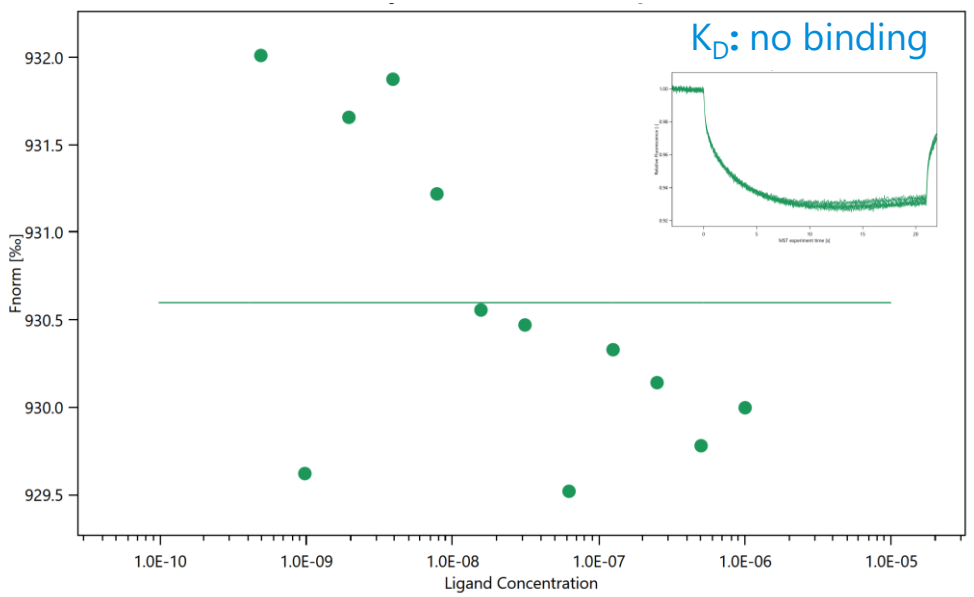


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	10	Buffer 1
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	10	Buffer 3

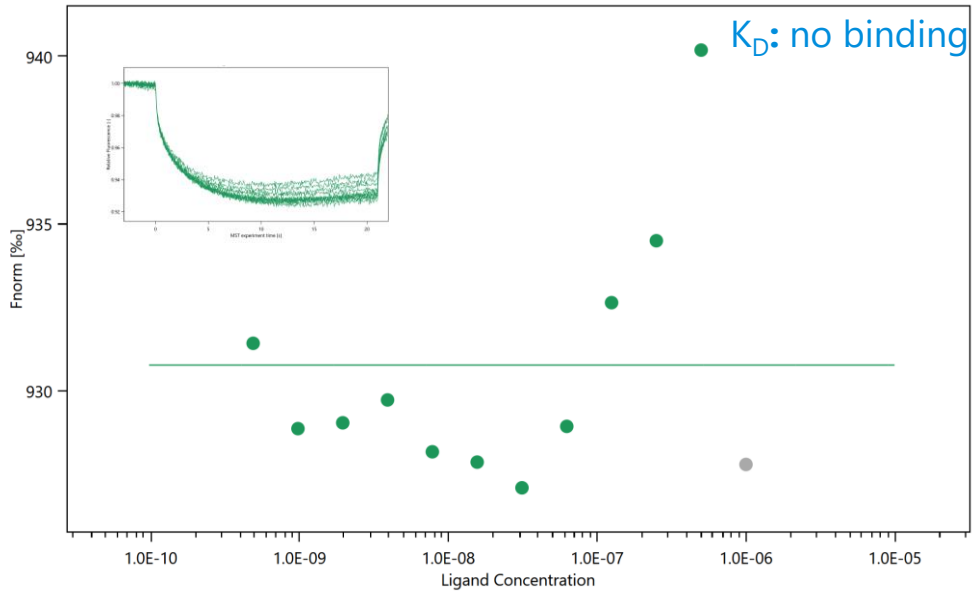
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 1.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 3.

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

Buffer 4



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 H3-3	-	-	-	-	10	Buffer 4
RED-tris-NTA 2nd gen.	Nucleocapsid	VHH H3-3	-	-	-	-	10	Buffer 5

- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 4.
- RED-tris-NTA 2nd gen. labelled Nucleocapsid does not bind VHH H3-3 in buffer 5.

Next steps

- We recommend to test the robustness of MST in the best assay buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20) using VHH H3-3 or VHH E4-3 nanobody as tool protein (prepared by dialysis).
  - If suitable, screen the compounds for Nucleocapsid binding using MST instead of Dianthus.
  - If necessary, perform additional MST buffer optimization.
- Potentially: Further Dianthus assay optimization, which we estimate to be very challenging with this protein batch

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