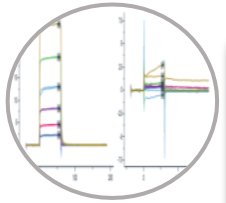
CACHE#3 Compounds Advancing to Round #2 (hit expansion)

HTS flowchart

Final hit rate: 1.6%

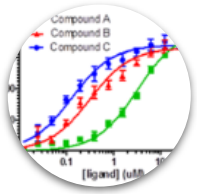
5.



SPR Counter screening: 18 confirmed hits were tested by SPR against NSP3_SARS2 and PARP14 proteins with a 6-points concentration range starting from 100/50/30 µM in duplicates. Another 18 HTRF hits that misbehaved with 2% DMSO in the SPR buffer were re-tested with 4% DMSO.

28 final hits confirmed to bind NSP3 by HTRF and SPR are advancing to Round #2, regardless of their PARP14 binding status

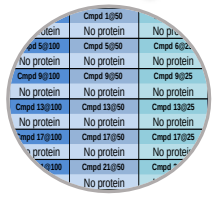
4.



SPR Dose response: 166 compounds selected from the HTRF hit confirmation step were tested by SPR against NSP3_SARS2 protein with a 6-points concentration range (top concentration selected according to DLS aggregation/solubility data at 100 and 50 µM) in duplicate.

Hit criteria: (KD<100 uM, % of binding >30, chi2 < 10% Rmax) – 16 hits and 2 possible hits (KD<100 uM, % of binding >15)

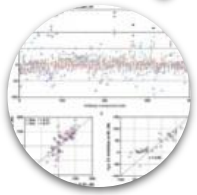
3.



HTRF Hit confirmation for 302 compounds selected from primary screen: 3 concentration points 100, 50 and 25 µM were tested by HTRF for each compound in duplicate. In parallel compound-only controls (without protein) were tested at the same concentrations in duplicate.

Confirmed hit criteria: dose dependency trend with >30% inhibition @100 µM and no fluorescence interference by control – 150 compounds with dose-dependency and 16 additional could be hits

2.

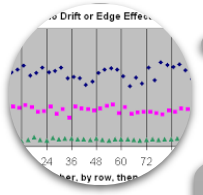


HTRF Primary HTS: 1738 compounds were tested in duplicates as 2 independent runs @100 µM by HTRF.

Initial hit criteria: > 30 % inhibition in a single run - 281 compounds selected

Criteria#2: compounds demonstrating “overflow” signal in 2 runs – 21 compounds selected

1.



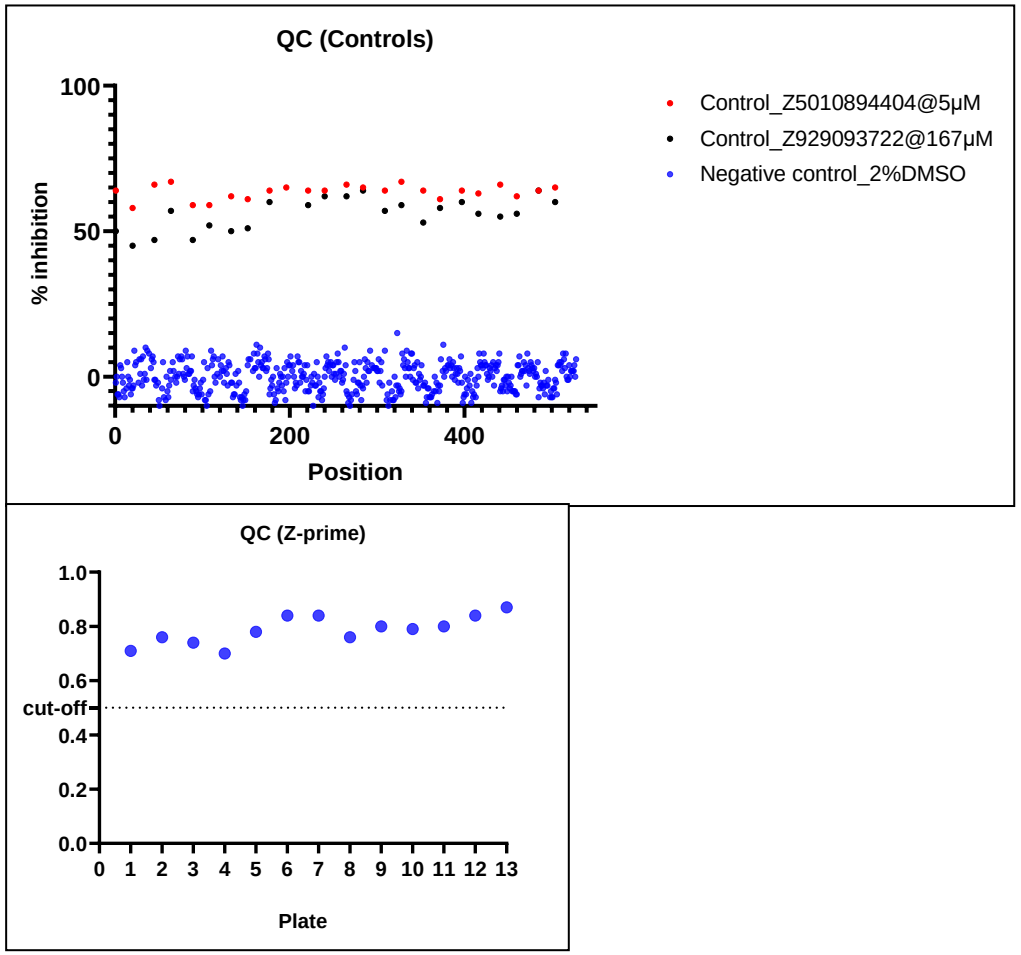
Assay setup and validation: 8 previously published reference compounds (doi.org/10.1073/pnas.2212931120) were used for HTRF and SPR assays optimization and for Uniformity test (http://www.ncbi.nlm.nih.gov/books/NBK83783/)

SGC

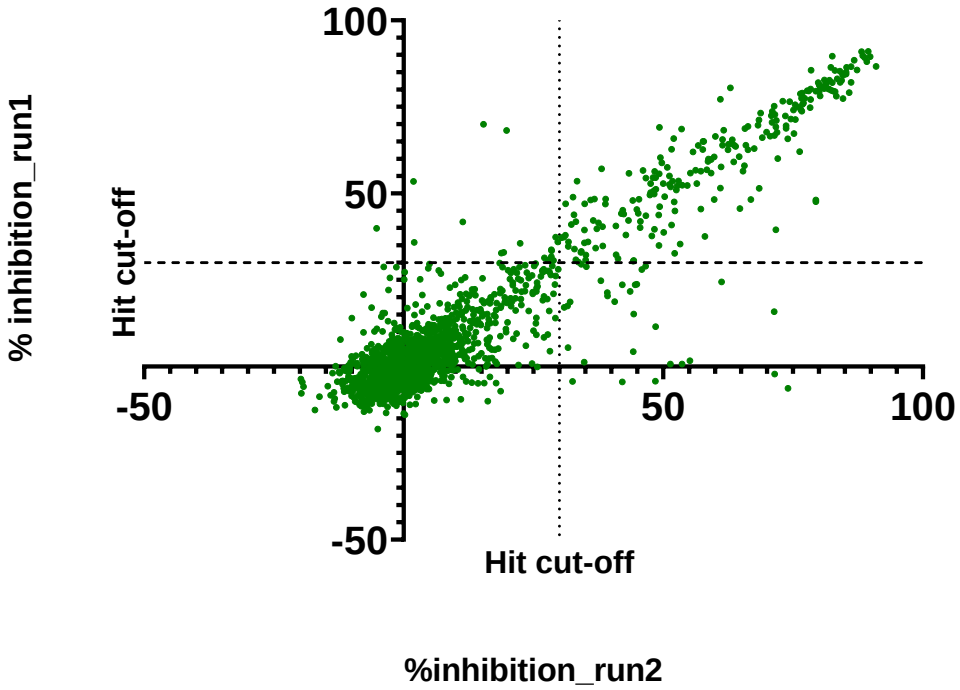
Molecular Biophysics

Primary screen (HTRF)

QC plots

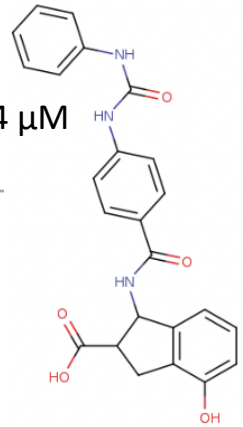


Single point (100 uM compound concentration) in duplicate; correlation between runs



SPR QC data

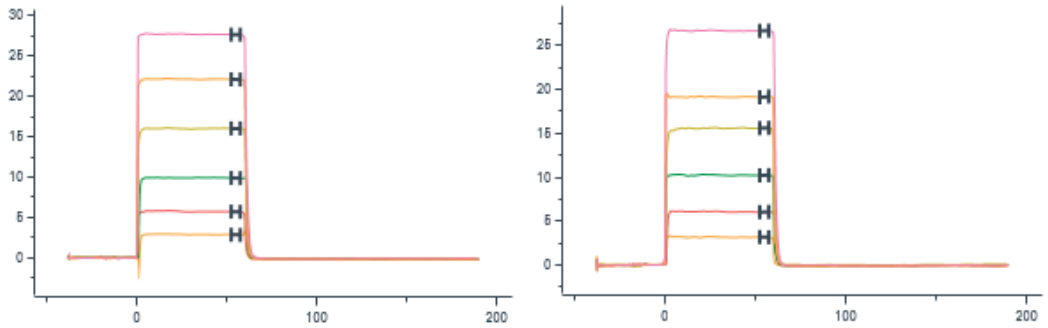
published Ki: 0.4 µM



QC data (Positive Controls)

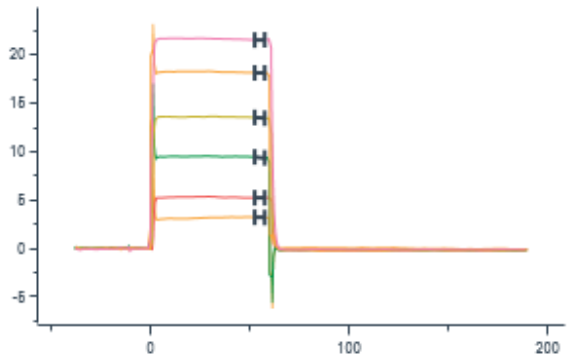
ADP-ribose

NSP3_SARS2



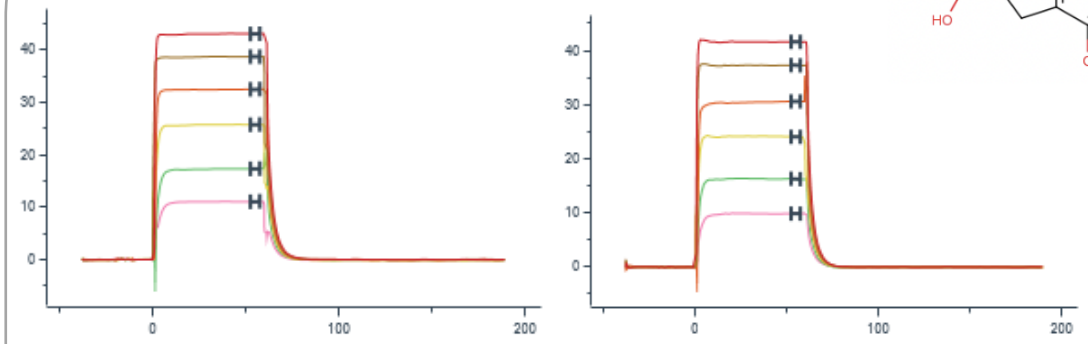
Run first: K_D = 9.5 µM, 100% binding
Run last: K_D = 10 µM, 92% binding
K_{disp}/IC50 (HTRF) = 12 µM

PARP14a

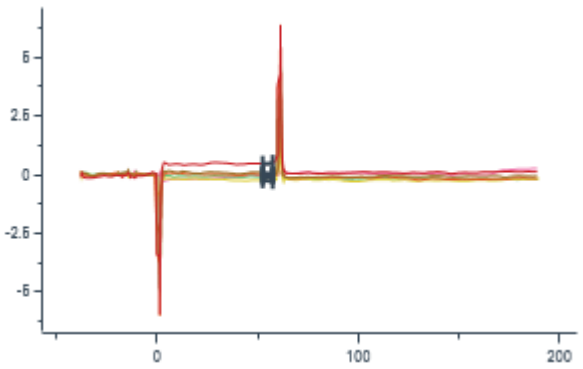


K_D = 7.2 µM
Binding = 88%

Z5010894404



Run first: K_D = 1.2 µM, 124% binding
Run last: K_D = 1.3 µM, 121% binding
K_{disp}/IC50 (HTRF) = 2.5-5 µM

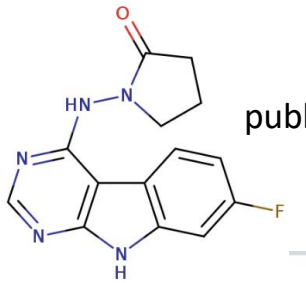


K_D = NA
No binding

SGC

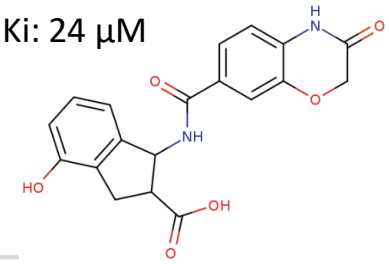
Molecular Biophysics

SPR QC data



published Ki: 1.5 µM

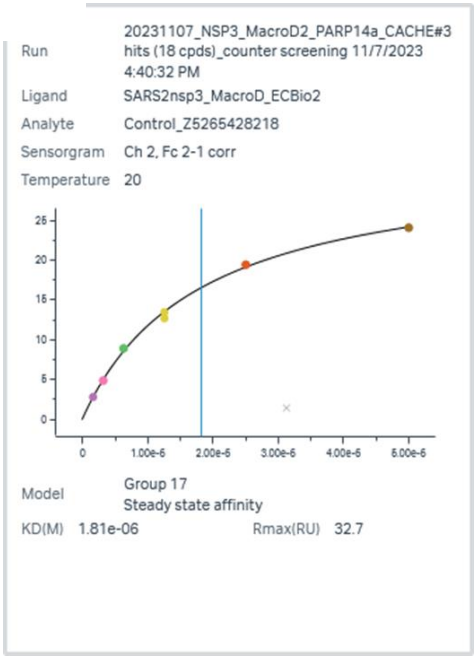
published Ki: 24 µM

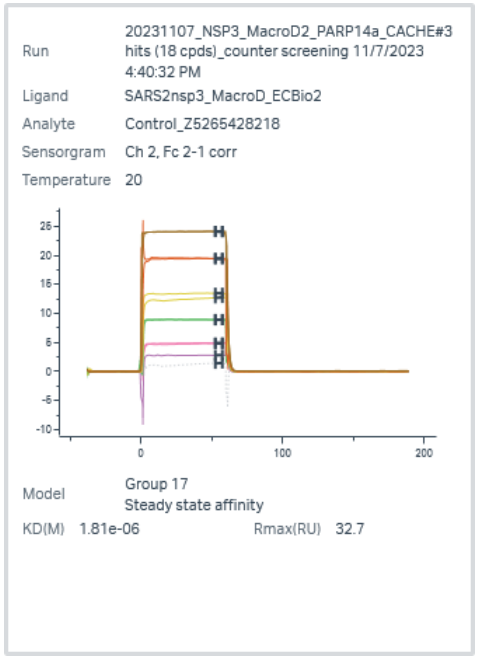


QC data (Additional Positive Controls)

NSP3_SARS2

Z5265428218

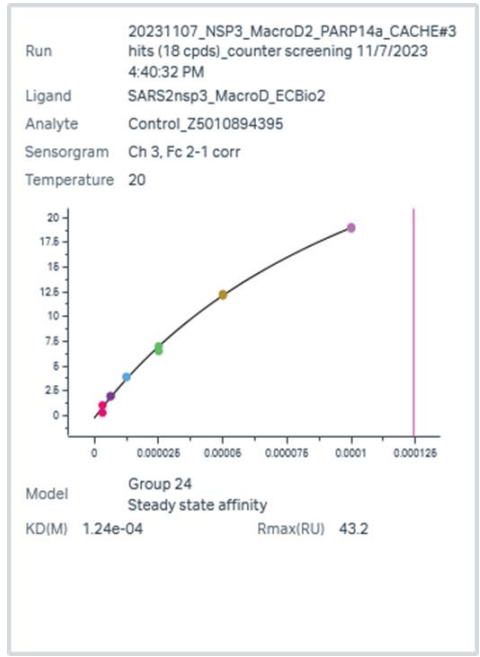


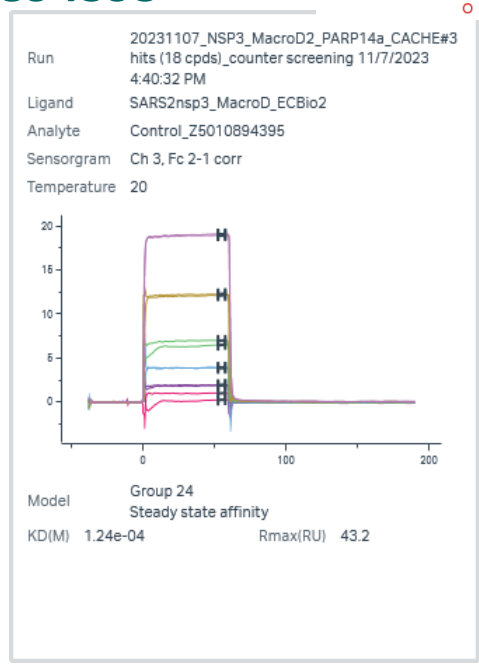


Binding = 125%
K_D = 1.8 µM

K_{disp}/IC_{50} (HTRF) = 5.7 µM

Z5010894395





Binding = 130%
K_D = 124 µM

K_{disp}/IC_{50} (HTRF) = 240 µM

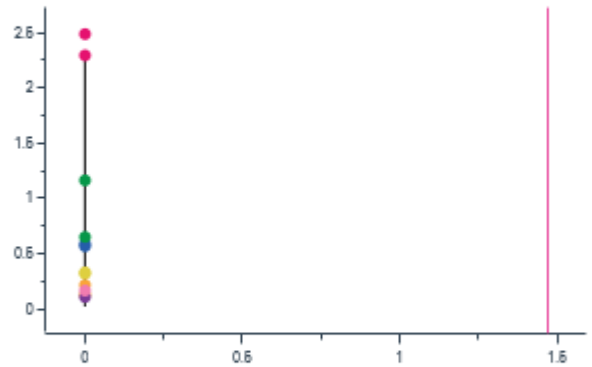
SGC

Molecular Biophysics

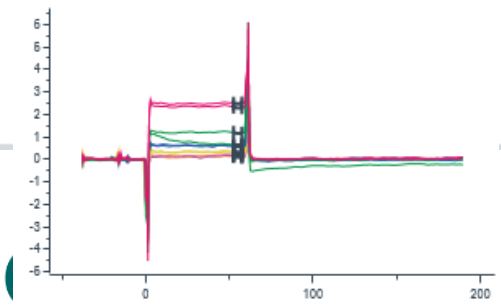
CACHE3HI_1690_45

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000405a_PARP14a
Sensorgram	Ch 6, Fc 2-1 corr
Temperature	20

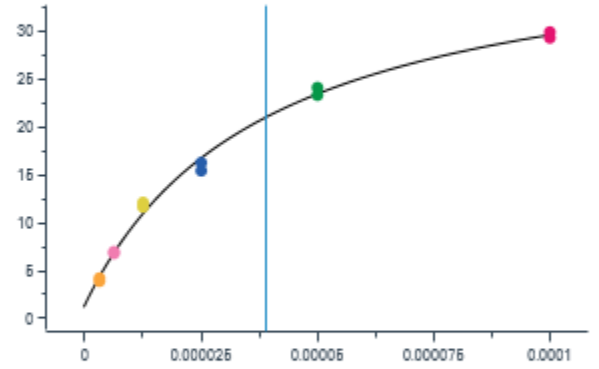


Model	Group 29 Steady state affinity
KD(M)	1.47e+00
Rmax(RU)	33117.6

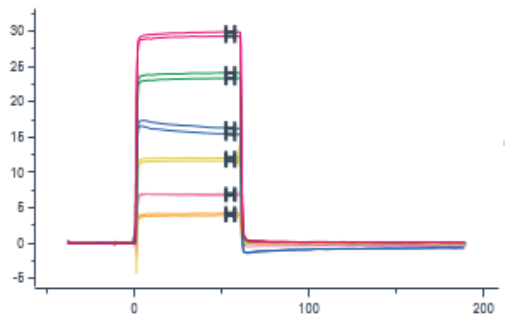


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000405a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 7 Steady state affinity
KD(M)	3.88e-05
Rmax(RU)	39.1



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 60.1
%inh@50 µM = 39.6
%inh@25 µM = 27.6

SPR confirmation/selectivity test

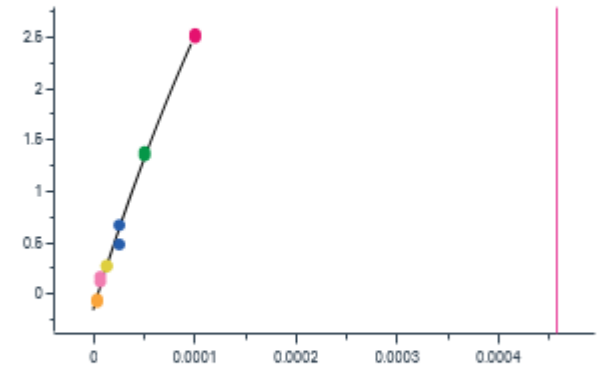
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	39	35	111	38.8
PARP14a	1470774.4	29	NA	no binding

Molecular Biophysics

CACHE3HI_1690_48

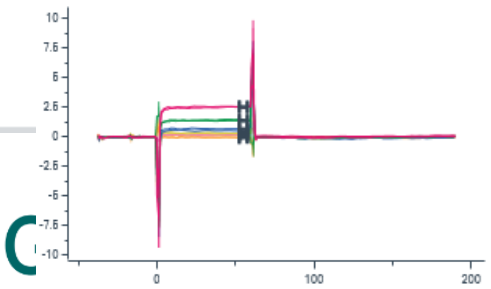
PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000983a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20



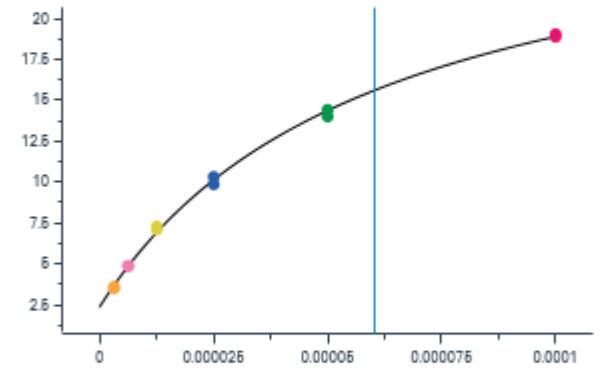
Model Group 42
Steady state affinity

KD(M) 4.58e-04 Rmax(RU) 14.7



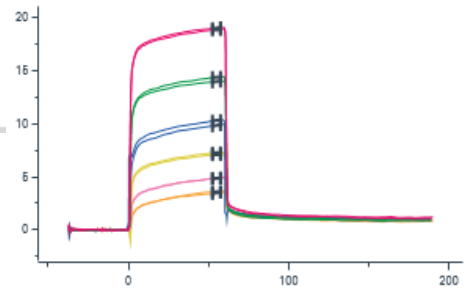
NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000983a
Sensorgram	Ch 4, Fc 2-1 corr
Temperature	20



Model Group 20
Steady state affinity

KD(M) 6.04e-05 Rmax(RU) 26.5



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 46.5
%inh@50 µM = 34.0
%inh@25 µM = 18.4

SPR confirmation/selectivity test

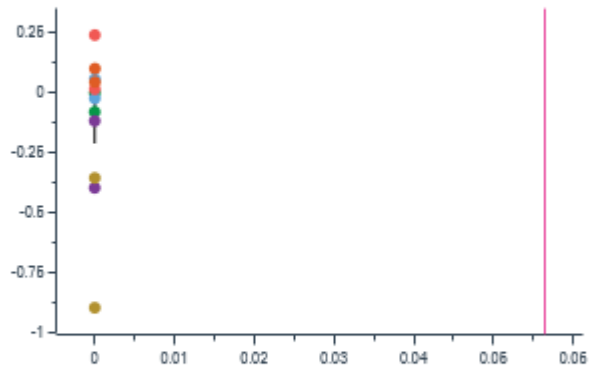
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	27	30	87	60.4
PARP14a	14.7	28	53	no binding

Molecular Biophysics

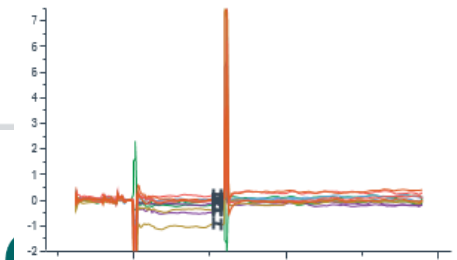
CACHE3HI_1690_63

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000761a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

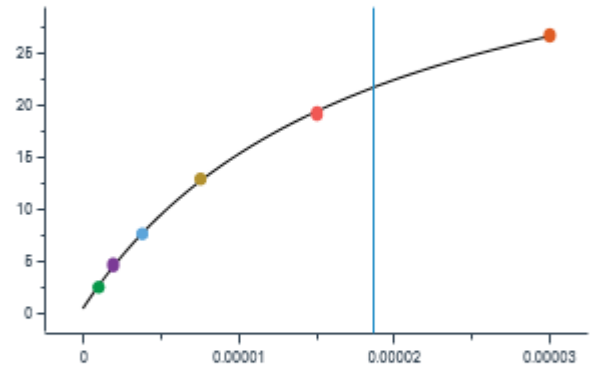


Model	Group 36 Steady state affinity
KD(M)	5.66e-02
Rmax(RU)	534.2

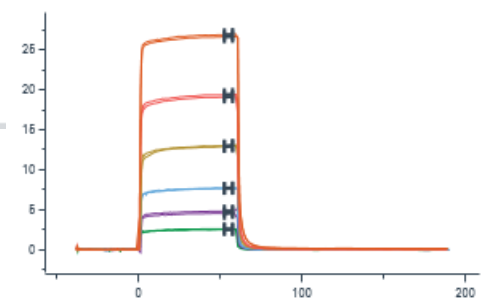


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000761a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model	Group 14 Steady state affinity
KD(M)	1.87e-05
Rmax(RU)	42.2



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 86.9
%inh@50 µM = 42.5
%inh@25 µM = 20.2

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	42	43	98	18.7
PARP14a	534.2	37	NA	no binding

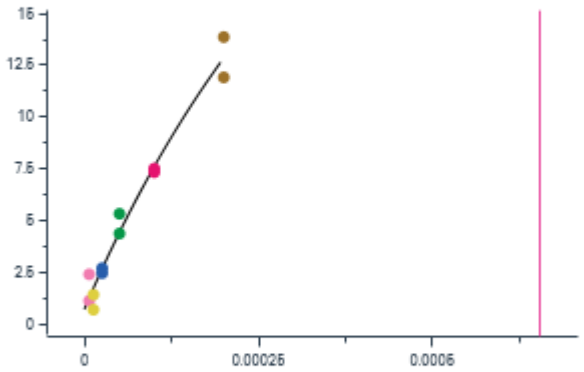
SGC

Molecular Biophysics

CACHE3HI_1690_87

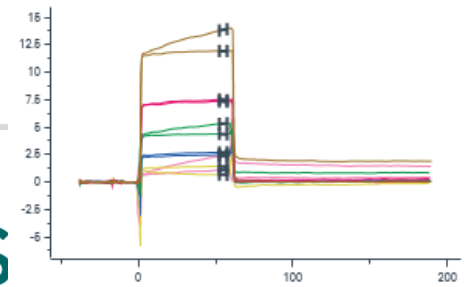
PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR001560a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20



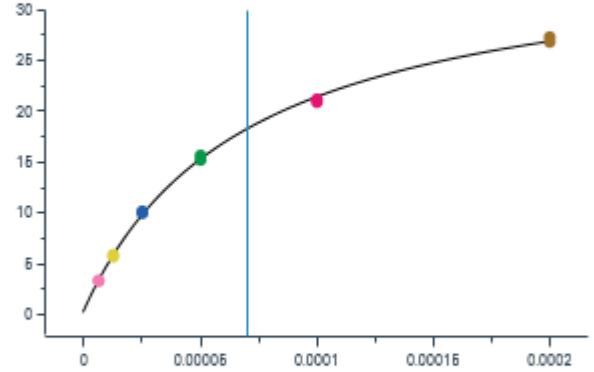
Model Group 44
Steady state affinity

KD(M) 6.56e-04 Rmax(RU) 51.8



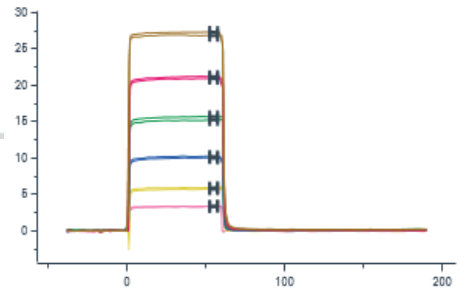
NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001560a
Sensorgram	Ch 4, Fc 2-1 corr
Temperature	20



Model Group 22
Steady state affinity

KD(M) 7.03e-05 Rmax(RU) 35.7



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 47.6
%inh@50 µM = 35.6
%inh@25 µM = 20.4

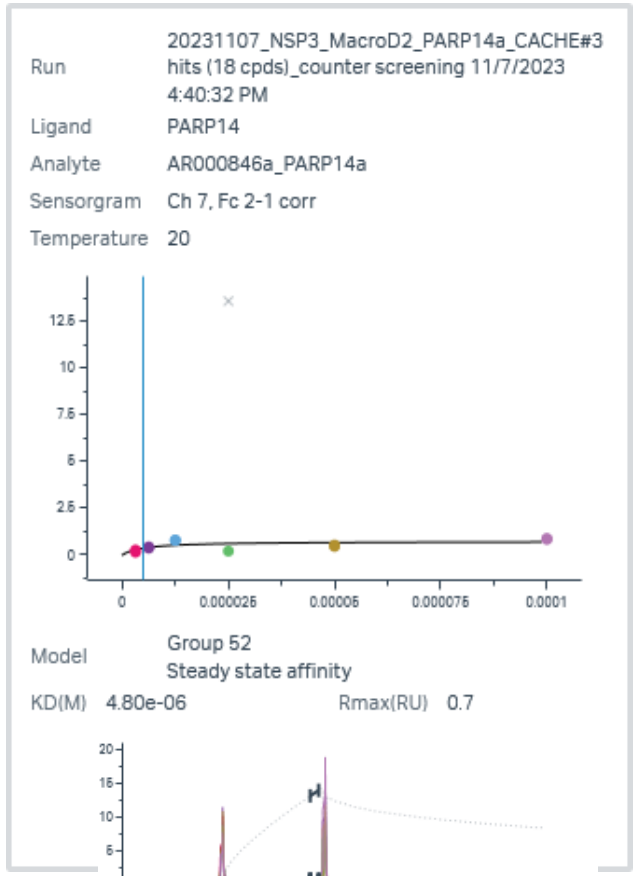
SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	36	34	105	70
PARP14a	52	31	167	Linear

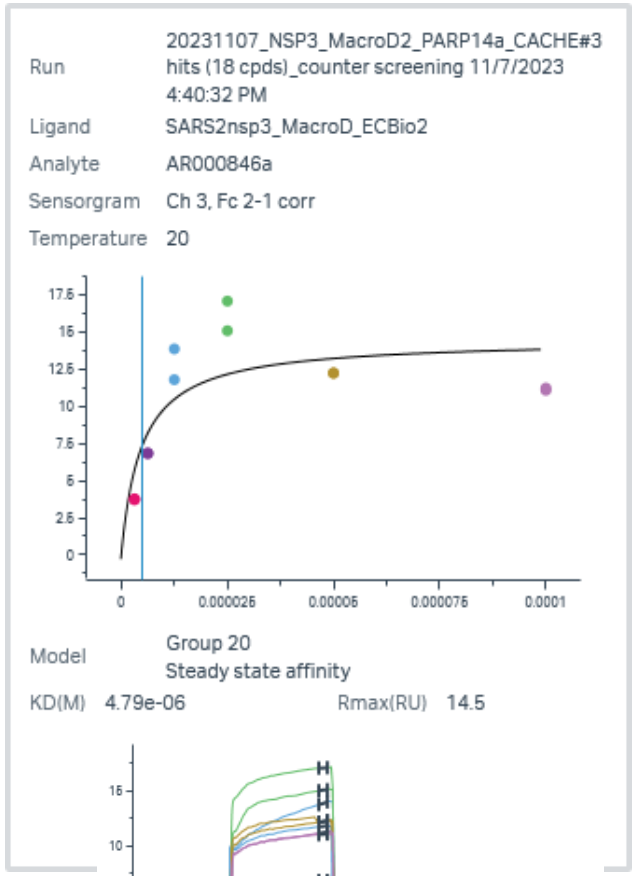
Molecular Biophysics

CACHE3HI_1690_36

PARP14a



NSP3_SARS2



Previous data:

HTRF_displacement hit confirmation:

%inh@100 uM = 83

%inh@50 uM = 78

%inh@25 uM = 67

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	14.5	31.0	47	48
PARP14a	0.7	24.5	3.0	NB

SGC

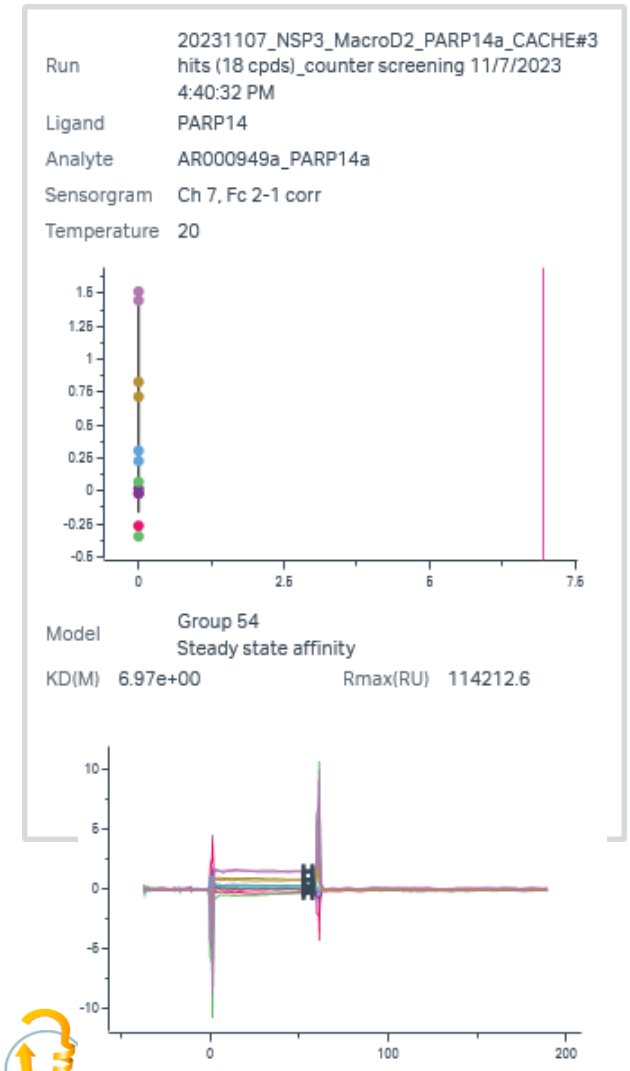
Molecular Biophysics

CACHE3HI_1690_92

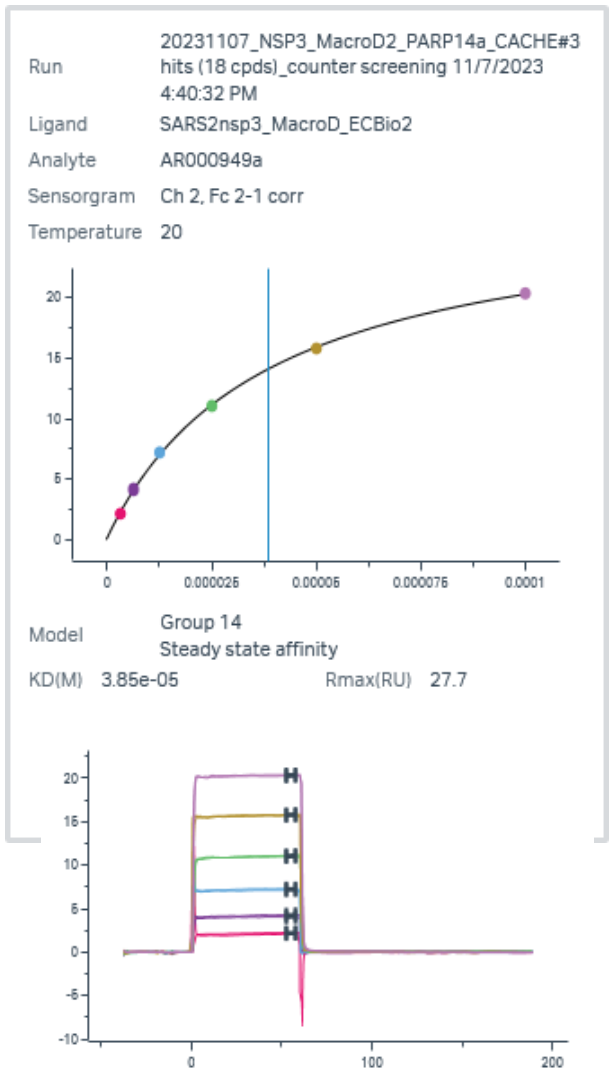
Previous data:

HTRF_displacement hit confirmation:
%inh@100 uM = 67
%inh@50 uM = 50
%inh@25 uM = 29

PARP14a



NSP3_SARS2



SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	27.7	23.9	116	38.5
PARP14a	114212.6	18.5	NA	NB

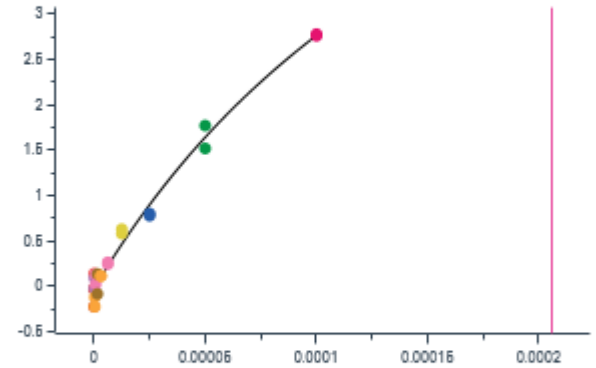
SGC

Molecular Biophysics

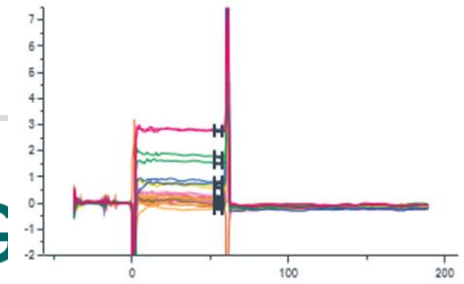
CACHE3HI_1696_50

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000657a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20

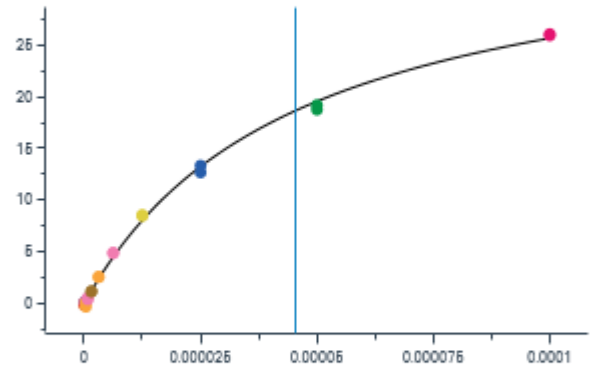


Model	Group 40 Steady state affinity		
KD(M)	2.06e-04	Rmax(RU)	8.5

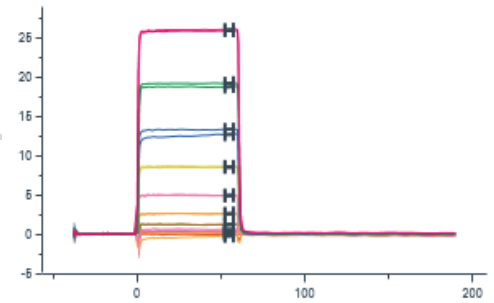


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000657a
Sensorgram	Ch 4, Fc 2-1 corr
Temperature	20



Model	Group 18 Steady state affinity		
KD(M)	4.53e-05	Rmax(RU)	37.2



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 56.5
%inh@50 µM = 40.0
%inh@25 µM = 25.7

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	37	36	104	45
PARP14a	8.5	33	26	no binding

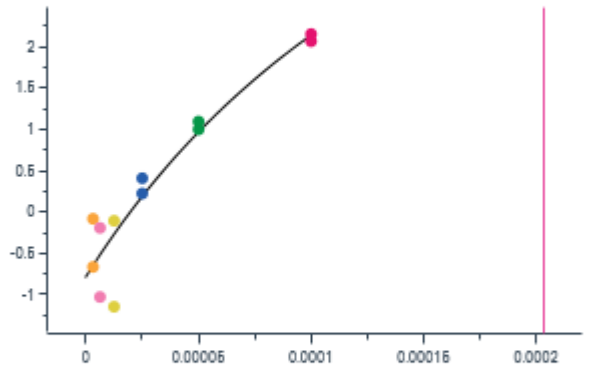
SG

Molecular Biophysics

CACHE3HI_1696_6

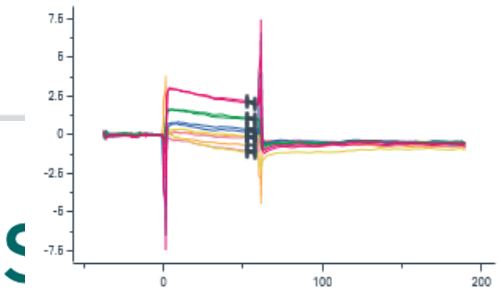
PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000475a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20



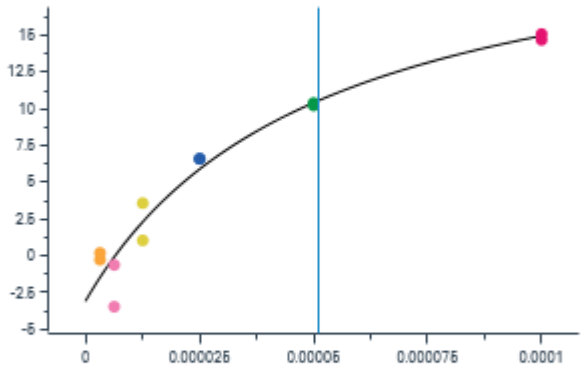
Model: Group 35 Steady state affinity

KD(M) 2.04e-04 Rmax(RU) 8.9



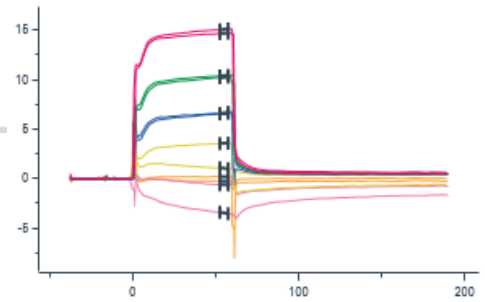
NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000475a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model: Group 13 Steady state affinity

KD(M) 5.10e-05 Rmax(RU) 27.2



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 44.6
%inh@50 µM = 25.0
%inh@25 µM = 10.3

SPR confirmation/selectivity test

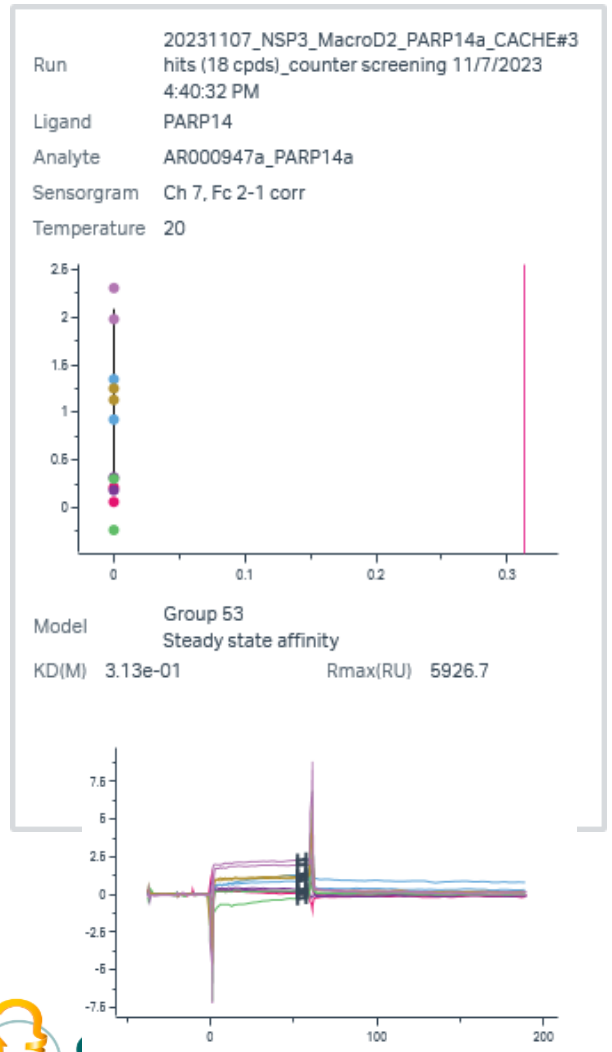
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	27	37	73	51
PARP14a	9	32	28	no binding

Molecular Biophysics

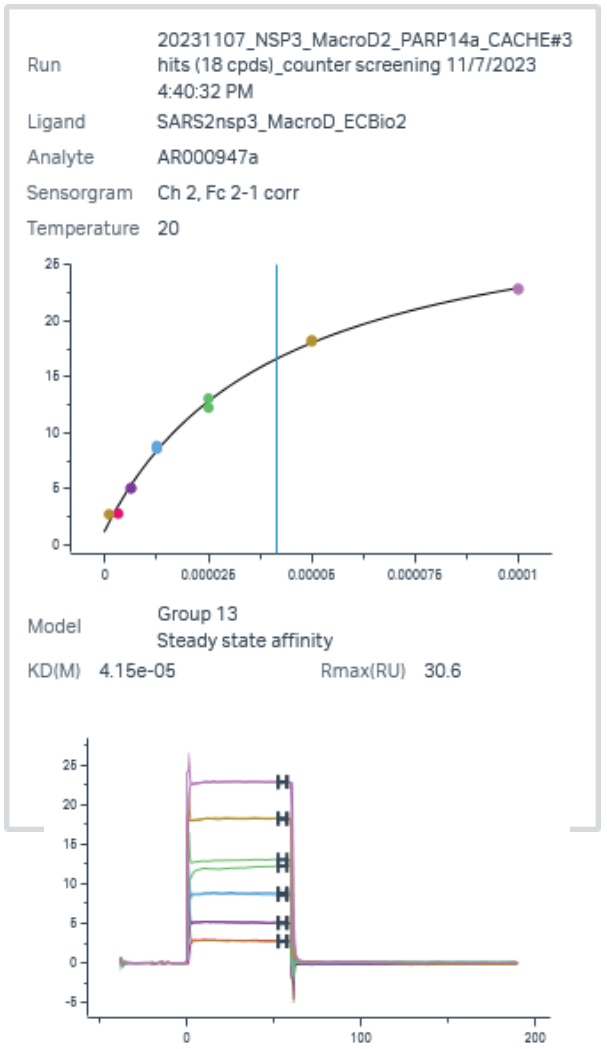
CACHE3HI_1696_67

4% DMSO

PARP14a



NSP3_SARS2



Previous data:

<u>HTRF_displacement hit confirmation:</u>

%inh@100 uM = 56

%inh@50 uM = 40

%inh@25 uM = 26

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	30.6	29.7	103	41.5
PARP14a	5926.7	23	NA	NB

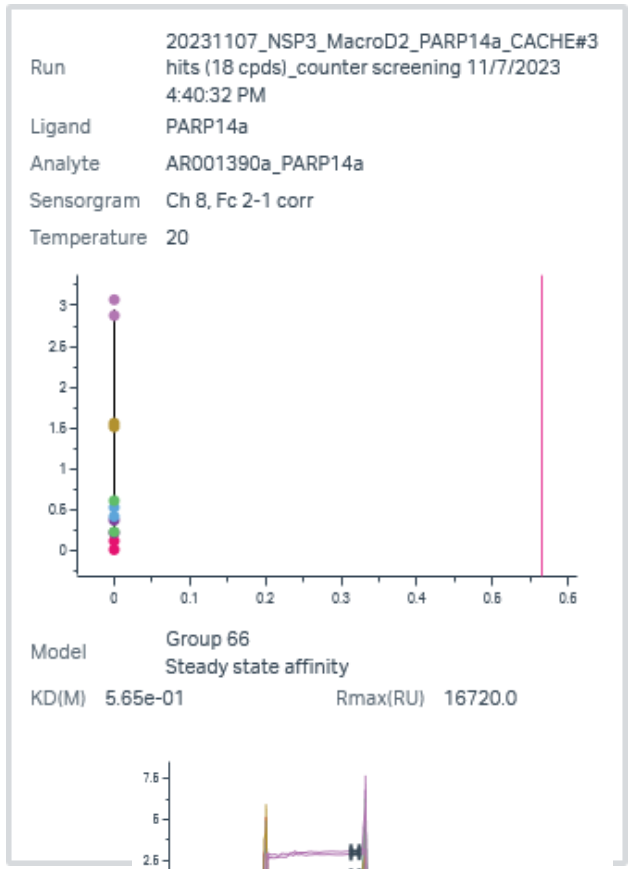
SGC

Molecular Biophysics

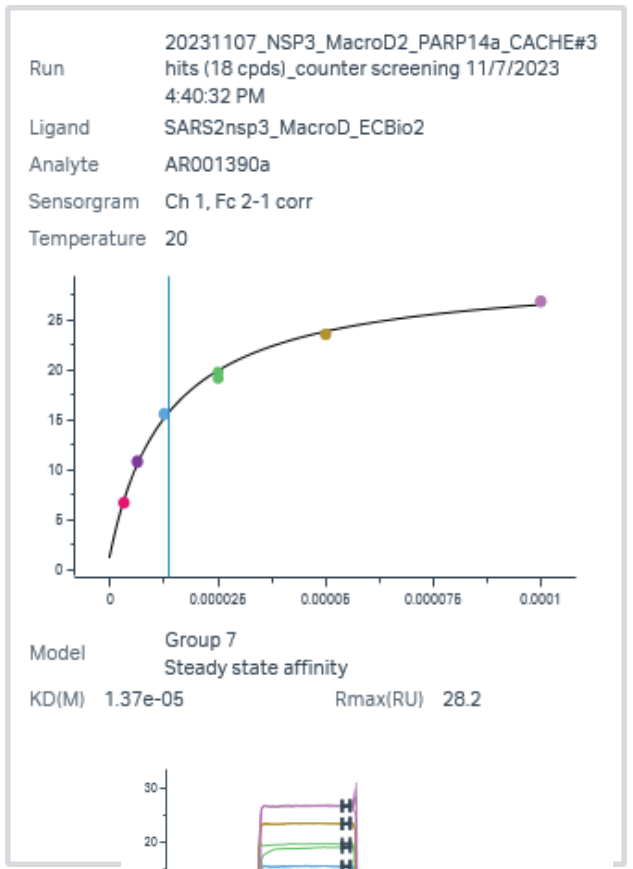
CACHE3HI_1696_78

4% DMSO

PARP14a



NSP3_SARS2



Previous data:

HTRF_displacement hit confirmation:

%inh@100 uM = 78

%inh@50 uM = 66

%inh@25 uM = 48

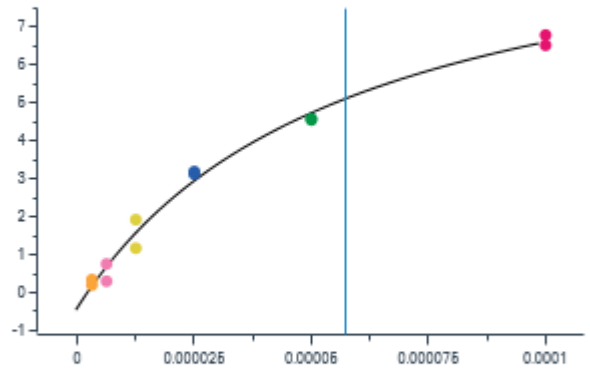
SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	28.2	34	83	13.7
PARP14a	16720.0	25.6	NA	NB

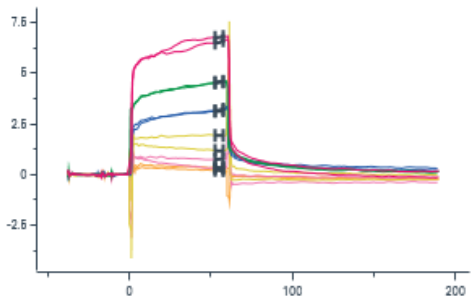
CACHE3HI_1700_52

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000861a_PARP14a
Sensorgram	Ch 6, Fc 2-1 corr
Temperature	20

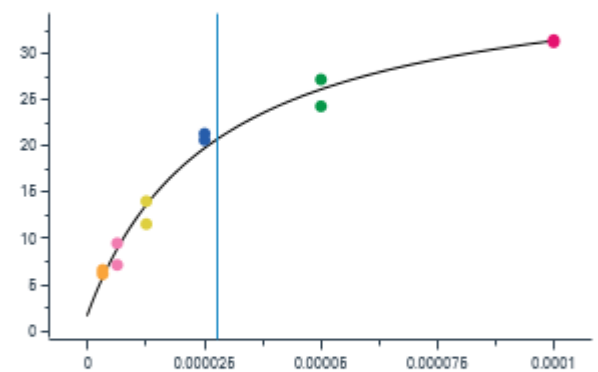


Model	Group 30 Steady state affinity		
KD(M)	5.73e-05	Rmax(RU)	11.0

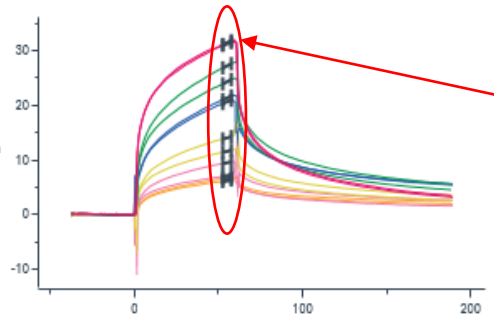


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000861a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 8 Steady state affinity		
KD(M)	2.78e-05	Rmax(RU)	37.6



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 81.1
%inh@50 µM = 69.0
%inh@25 µM = 31.7

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	38	40	94	28
PARP14a	11	32	34	57

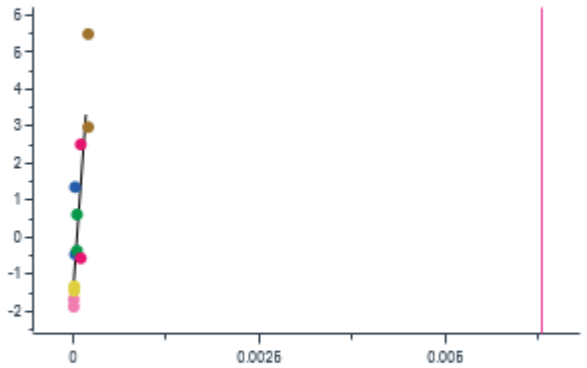
Estimated KD is not reliable, binding profile does not show steady state

Molecular Biophysics

CACHE3HI_1705_4

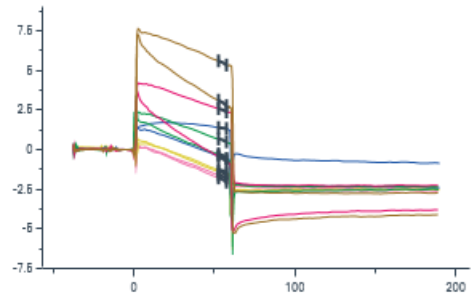
PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000437a_PARP14a
Sensorgram	Ch 5, Fc 2-1 corr
Temperature	20



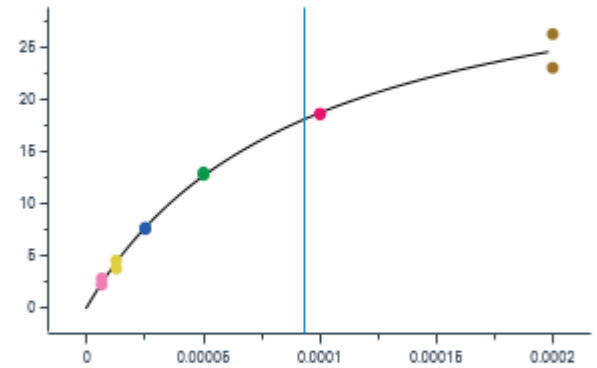
Model Group 25
Steady state affinity

KD(M) 6.29e-03 Rmax(RU) 180.0



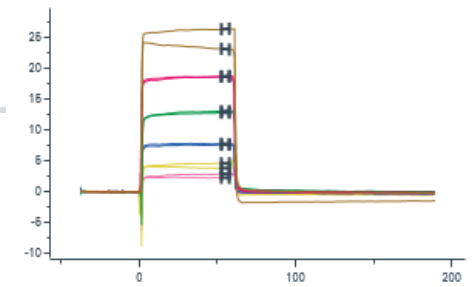
NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000437a
Sensorgram	Ch 1, Fc 2-1 corr
Temperature	20



Model Group 3
Steady state affinity

KD(M) 9.33e-05 Rmax(RU) 35.9



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 37.5
%inh@50 µM = 20.3
%inh@25 µM = 9.9

SPR confirmation/selectivity test

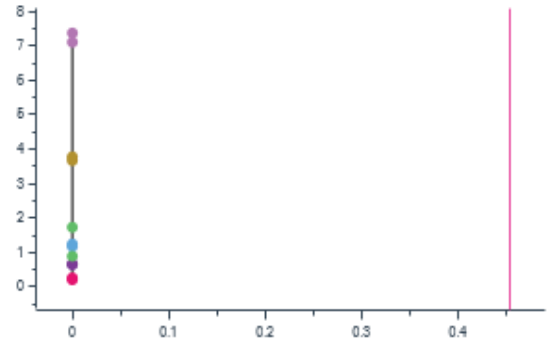
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	36	33	109	93
PARP14a	180	29	NA	weak binding

Molecular Biophysics

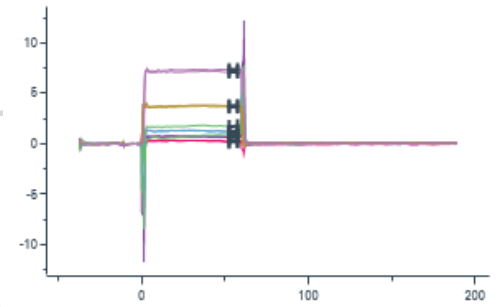
CACHE3HI_1706_56

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14
Analyte	AR001356a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

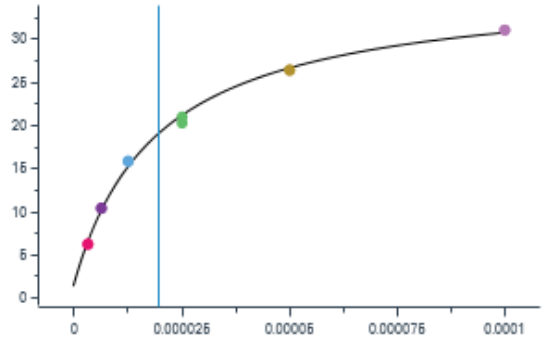


Model	Group 55 Steady state affinity
KD(M)	4.53e-01
Rmax(RU)	32392.5

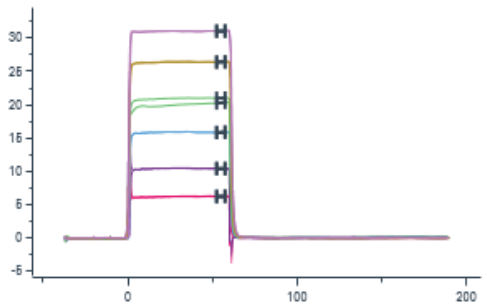


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001356a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 15 Steady state affinity
KD(M)	1.97e-05
Rmax(RU)	34.6



Previous data:

HTRF_displacement hit confirmation:

%inh@100 uM = 84

%inh@50 uM = 72

%inh@25 uM = 54

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	34.6	39.1	89	19.7
PARP14a	32392.5	23.9	NA	Weak

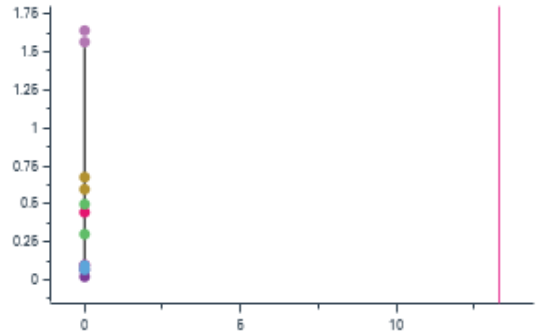
SGC

Molecular Biophysics

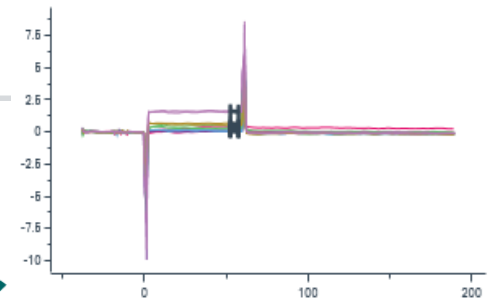
CACHE3HI_1708_42

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14a
Analyte	AR000138a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20

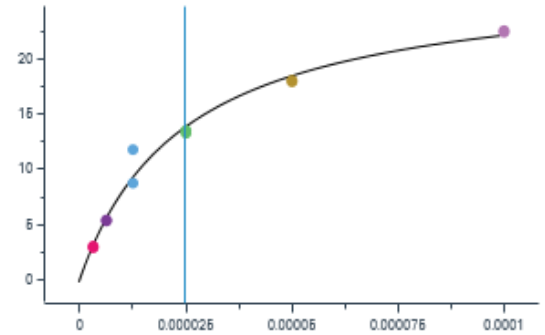


Model	Group 59 Steady state affinity		
KD(M)	1.33e+01	Rmax(RU)	202915.0

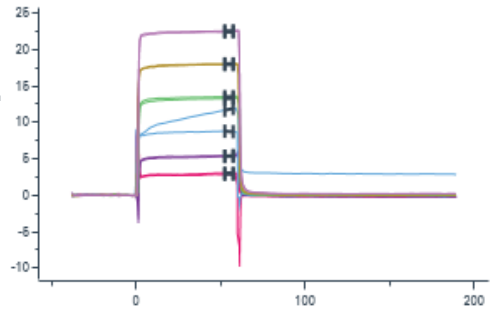


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000138a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 11 Steady state affinity		
KD(M)	2.49e-05	Rmax(RU)	27.5



Previous data:

HTRF_displacement hit confirmation:

%inh@100 uM = 66

%inh@50 uM = 46

%inh@25 uM = 34

SPR confirmation/selectivity test

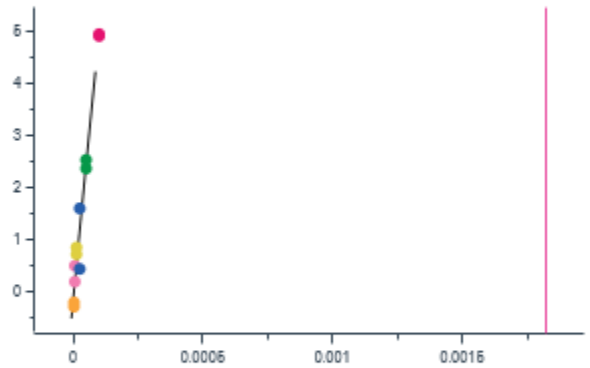
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	27.5	24.1	114	25
PARP14a	202915.0	19	NA	NB

Molecular Biophysics

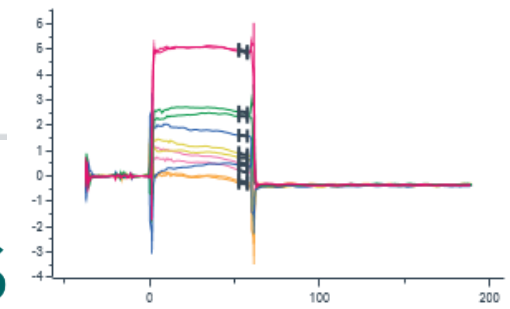
CACHE3HI_1708_87

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000081a_PARP14a
Sensorgram	Ch 5, Fc 2-1 corr
Temperature	20

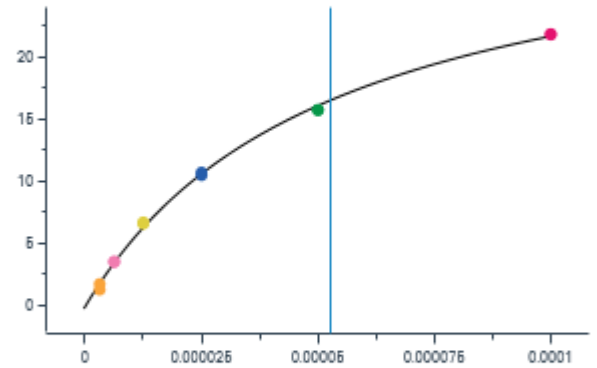


Model: Group 24
Steady state affinity
KD(M) 1.82e-03 Rmax(RU) 97.4

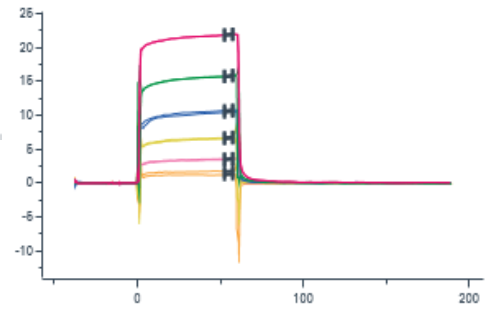


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000081a
Sensorgram	Ch 1, Fc 2-1 corr
Temperature	20



Model: Group 2
Steady state affinity
KD(M) 5.27e-05 Rmax(RU) 33.3



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 51.6
%inh@50 µM = 38.2
%inh@25 µM = 20.3

SPR confirmation/selectivity test

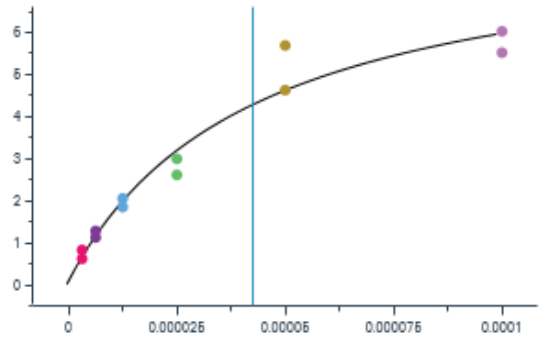
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	33	32	106	53
PARP14a	1819.9	28	NA	weak binding

Molecular Biophysics

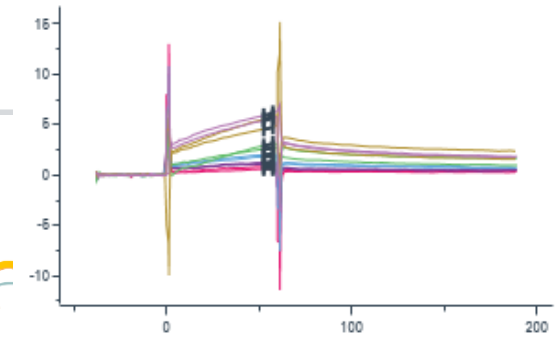
CACHE3HI_1714_34

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14
Analyte	AR001714a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

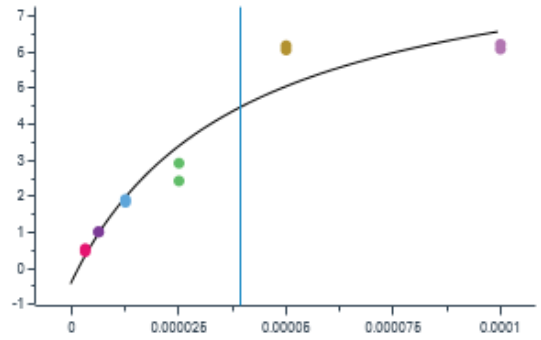


Model	Group 57 Steady state affinity
KD(M)	4.26e-05
Rmax(RU)	8.4

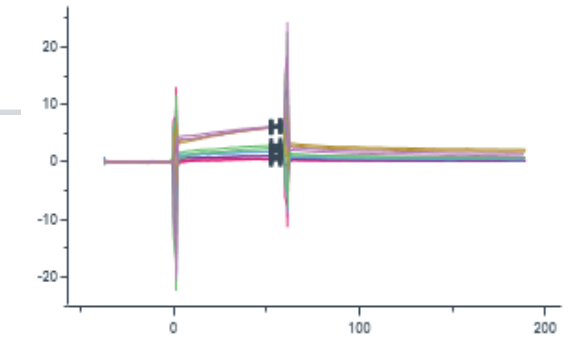


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001714a
Sensorgram	Ch 1, Fc 2-1 corr
Temperature	20



Model	Group 8 Steady state affinity
KD(M)	3.93e-05
Rmax(RU)	9.7



Previous data:

<u>HTRF_displacement hit confirmation:</u>
%inh@100 uM = 40
%inh@50 uM = 15
%inh@25 uM = 1

SPR confirmation/selectivity test

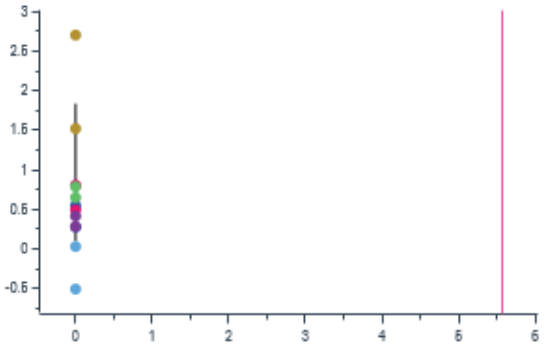
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	9.7	37.7	25.6	39.3
PARP14a	8.4	30.8	27.2	43

Molecular Biophysics

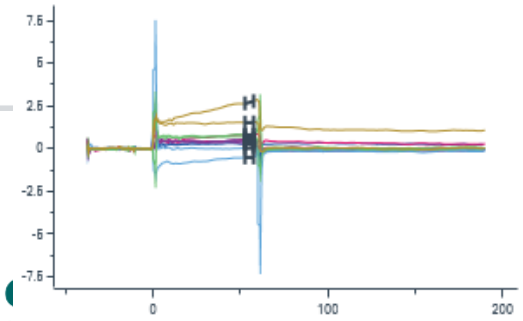
CACHE3HI_1715_71

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14
Analyte	AR000614a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

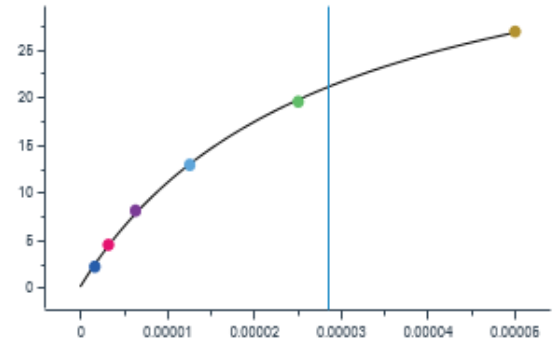


Model	Group 51 Steady state affinity
KD(M)	5.58e+00
Rmax(RU)	194269.8

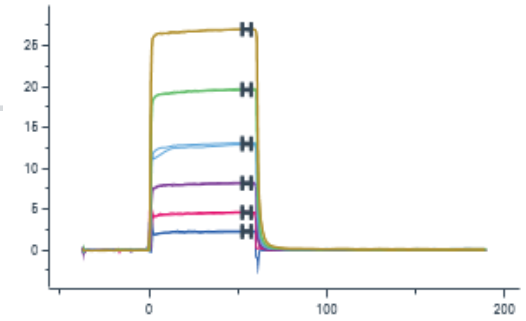


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000614a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model	Group 19 Steady state affinity
KD(M)	2.84e-05
Rmax(RU)	41.6



Previous data:

HTRF_displacement hit confirmation:

%inh@100 uM = 66

%inh@50 uM = 93

%inh@25 uM = 23

SPR confirmation/selectivity test

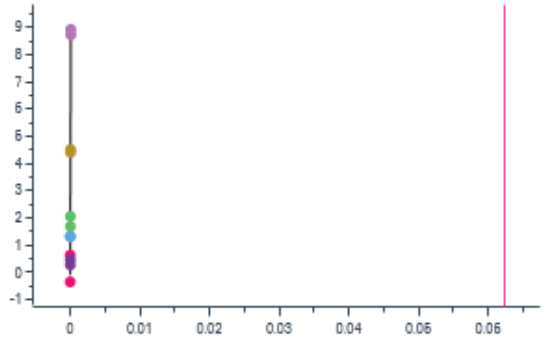
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	41.6	32.6	127.5	28.4
PARP14a	194269.8	25.8	NA	NB

Molecular Biophysics

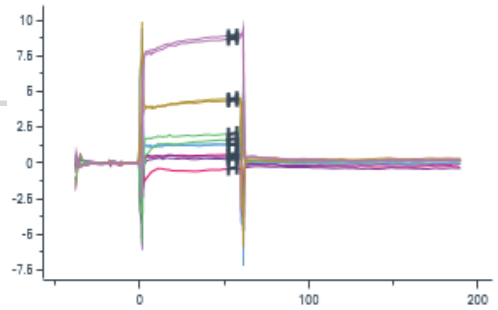
CACHE3HI_1715_85

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14a
Analyte	AR001421a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20

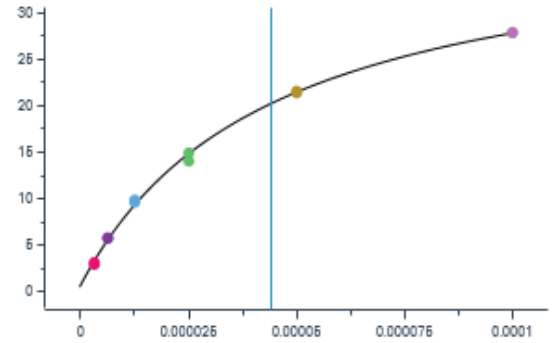


Model	Group 67 Steady state affinity
KD(M)	6.23e-02
Rmax(RU)	5571.6

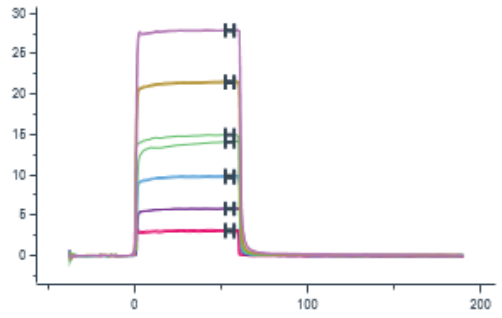


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001421a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 16 Steady state affinity
KD(M)	4.42e-05
Rmax(RU)	39.0



Previous data:

<u>HTRF displacement hit confirmation:</u>
%inh@100 uM = 67
%inh@50 uM = 47
%inh@25 uM = 32

SPR confirmation/selectivity test

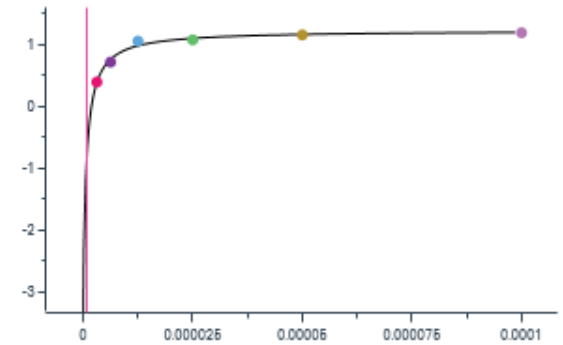
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	39.0	31.3	124.4	44
PARP14a	5571.6	24.7	NA	weak

Molecular Biophysics

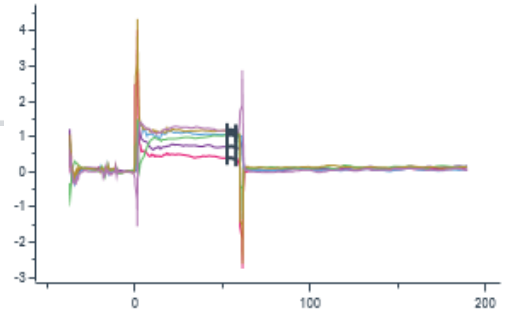
CACHE3HI_1715_88

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14a
Analyte	AR000892a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20

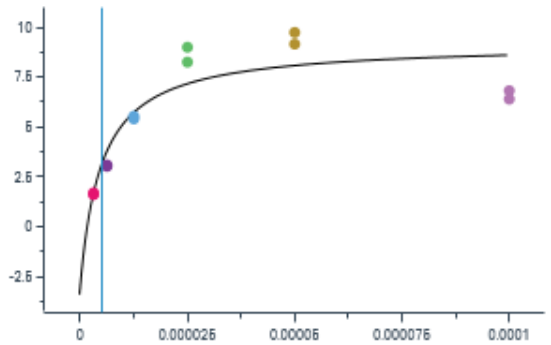


Model	Group 62 Steady state affinity
KD(M)	7.90e-07
Rmax(RU)	4.2

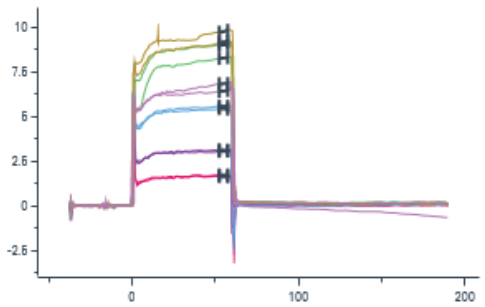


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000892a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model	Group 21 Steady state affinity
KD(M)	4.91e-06
Rmax(RU)	12.2



Previous data:

HTRF displacement hit confirmation:

%inh@100 uM = 80

%inh@50 uM = 65

%inh@25 uM = 22

SPR confirmation/selectivity test

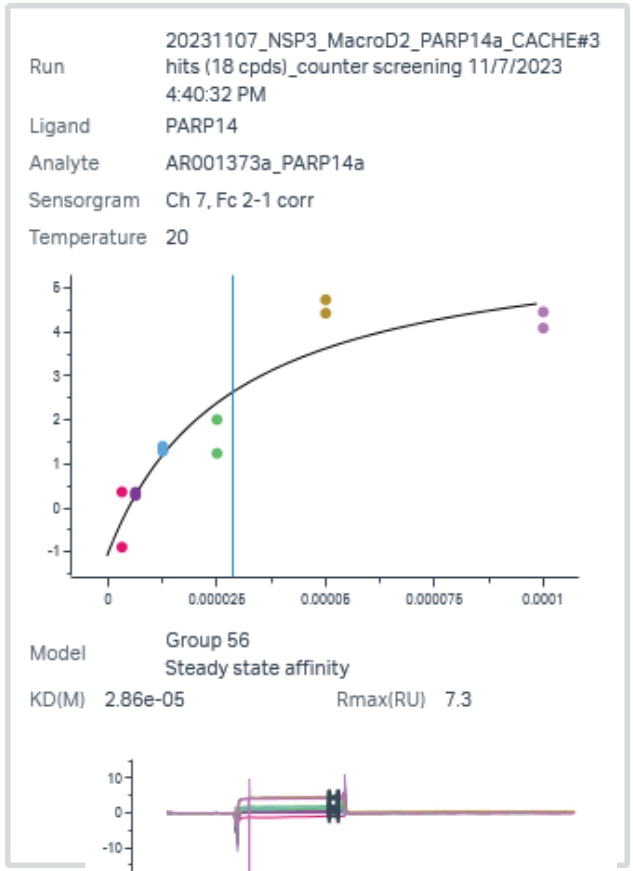
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	12.2	35.3	34.6	49
PARP14a	1.3	22.7	5.7	NB

SGC

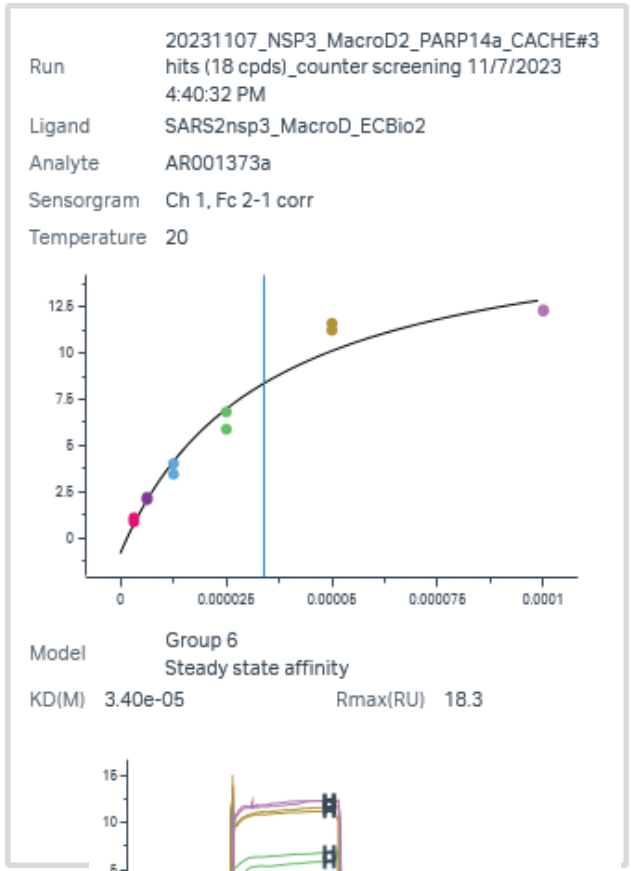
Molecular Biophysics

CACHE3HI_1715_92

PARP14a



NSP3_SARS2



Previous data:

<u>HTRF displacemen hit confirmation:</u>
%inh@100 uM = 47
%inh@50 uM = 27
%inh@25 uM = 16

SPR confirmation/selectivity test

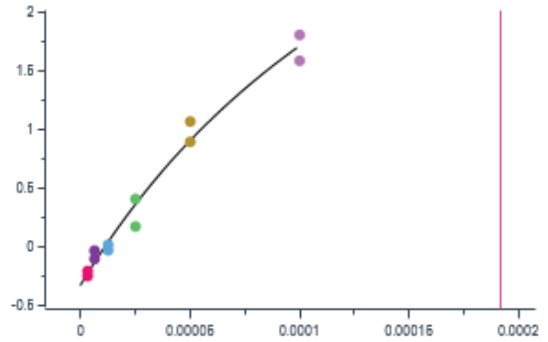
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	28.5	18.3	64	34
PARP14a	7.3	23	31	29

Molecular Biophysics

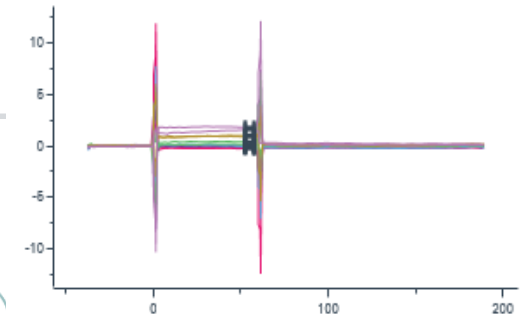
CACHE3HI_1715_95

PARP14a

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	PARP14
Analyte	AR000398a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

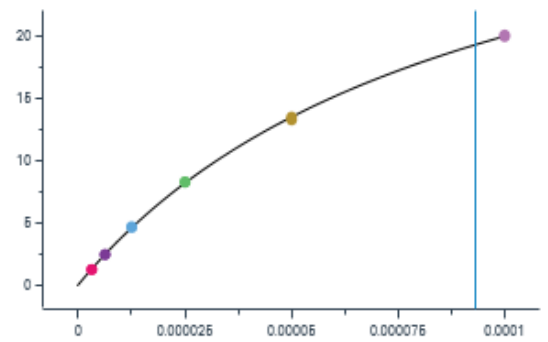


Model	Group 50 Steady state affinity
KD(M)	1.92e-04
Rmax(RU)	5.9

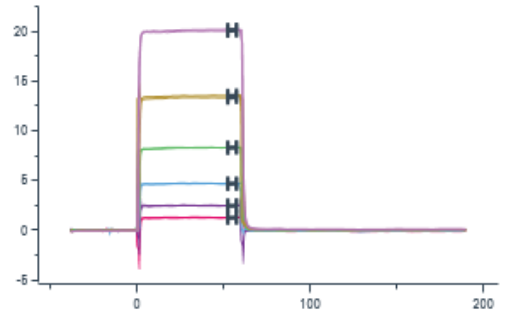


NSP3_SARS2

Run	20231107_NSP3_MacroD2_PARP14a_CACHE#3 hits (18 cpds)_counter screening 11/7/2023 4:40:32 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000398a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model	Group 18 Steady state affinity
KD(M)	9.31e-05
Rmax(RU)	38.6



Previous data:

HTRF_ displacement hit confirmation:

%inh@100 uM = 35

%inh@50 uM = 21

%inh@25 uM = 11

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	38.6	29.8	129.4	93
PARP14a	5.9	23.6	25.2	NB

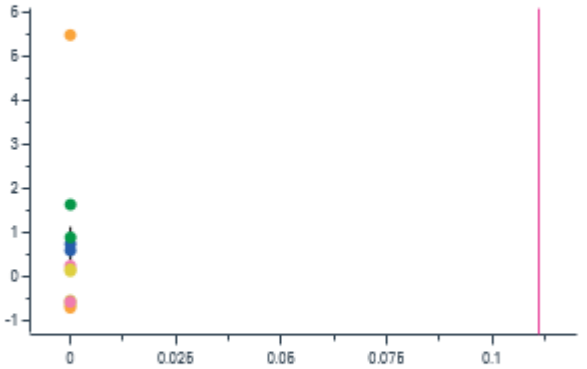
SGC

Molecular Biophysics

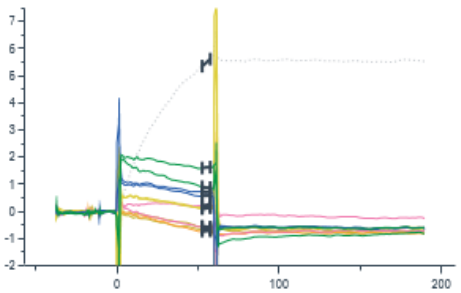
CACHE3HI_1715_91

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000917a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20

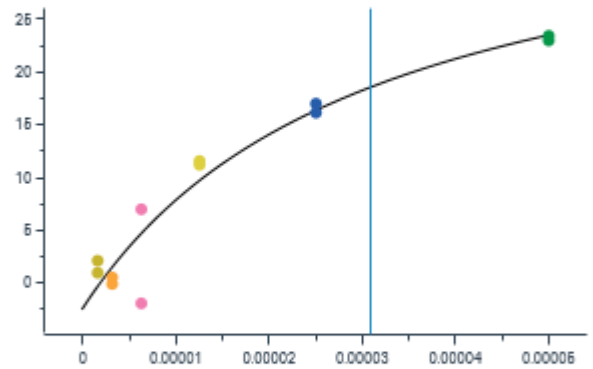


Model	Group 41 Steady state affinity
KD(M)	1.11e-01
Rmax(RU)	1704.2

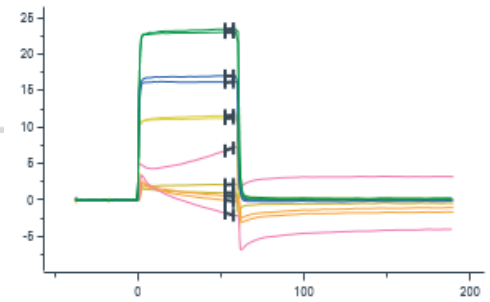


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000917a
Sensorgram	Ch 4, Fc 2-1 corr
Temperature	20



Model	Group 19 Steady state affinity
KD(M)	3.08e-05
Rmax(RU)	41.8



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 56.8
%inh@50 µM = 40.2
%inh@25 µM = 23.2

SPR confirmation/selectivity test

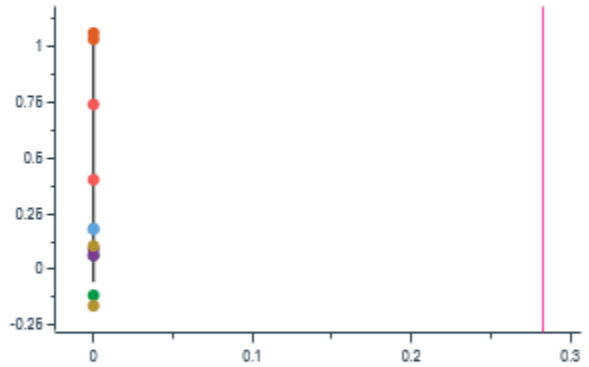
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	31	34	124	31
PARP14a	111033.2	31	NA	no binding

Molecular Biophysics

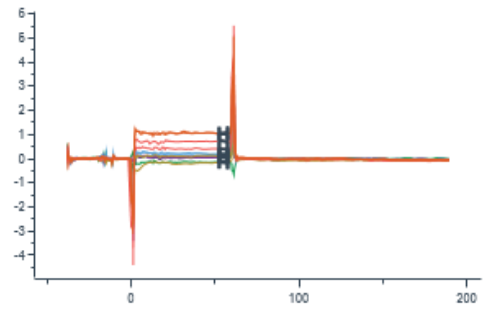
CACHE3HI_1715_76

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR001223a_PARP14a
Sensorgram	Ch 6, Fc 2-1 corr
Temperature	20

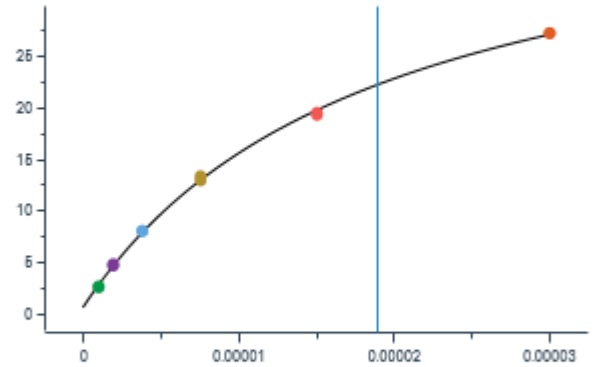


Model	Group 32 Steady state affinity
KD(M)	2.83e-01
Rmax(RU)	10363.9

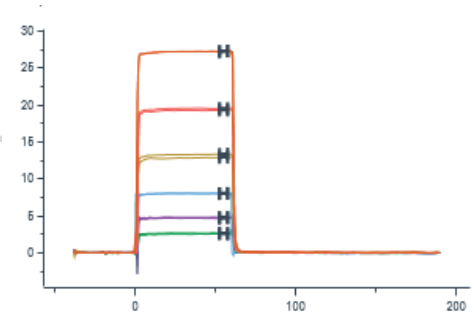


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001223a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 10 Steady state affinity
KD(M)	1.89e-05
Rmax(RU)	43.0



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 48.0
%inh@50 µM = 36.5
%inh@25 µM = 23.5

SPR confirmation/selectivity test

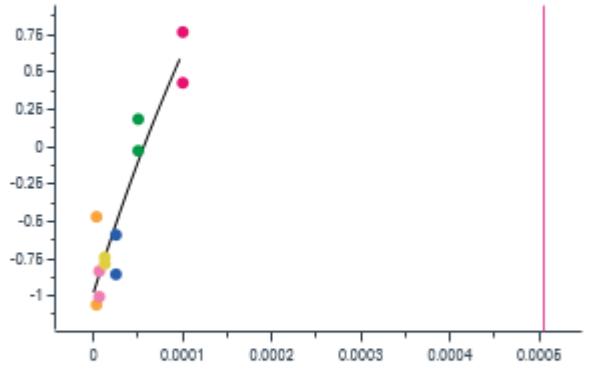
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	43	42	103	19
PARP14a	283427.3	34	NA	no binding

Molecular Biophysics

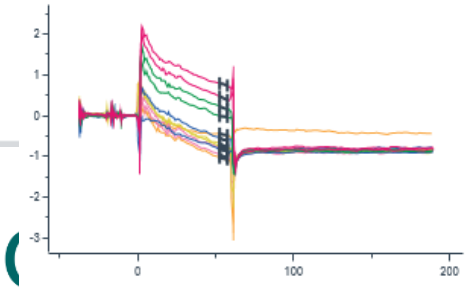
CACHE3HI_1715_89

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR001694a_PARP14a
Sensorgram	Ch 6, Fc 2-1 corr
Temperature	20

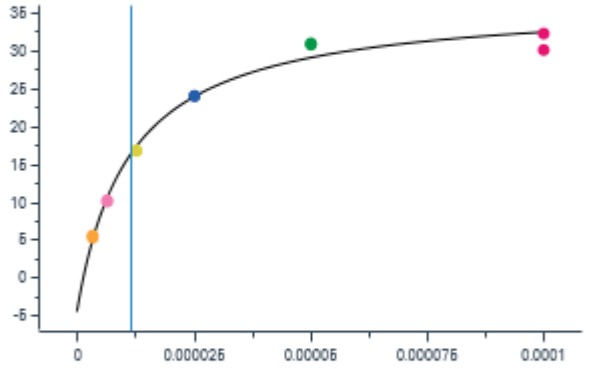


Model	Group 33 Steady state affinity
KD(M)	5.06e-04
Rmax(RU)	9.8

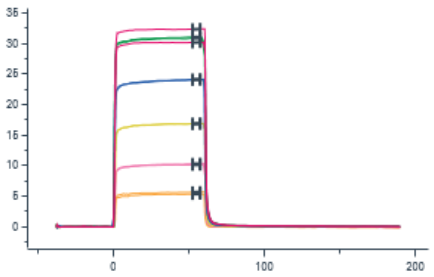


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001694a
Sensorgram	Ch 2, Fc 2-1 corr
Temperature	20



Model	Group 11 Steady state affinity
KD(M)	1.15e-05
Rmax(RU)	40.2



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 81.9
%inh@50 µM = 48.4
%inh@25 µM = 30.5

SPR confirmation/selectivity test

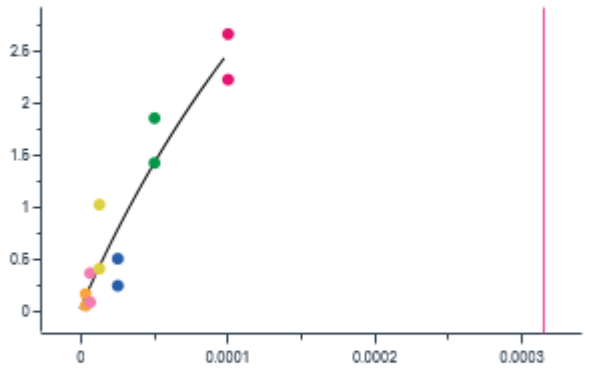
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	40	46	87	11.5
PARP14a	9.8	38	26	no binding

Molecular Biophysics

CACHE3HI_1715_83

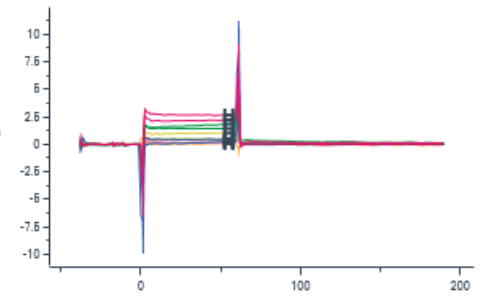
PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000711a_PARP14a
Sensorgram	Ch 5, Fc 2-1 corr
Temperature	20



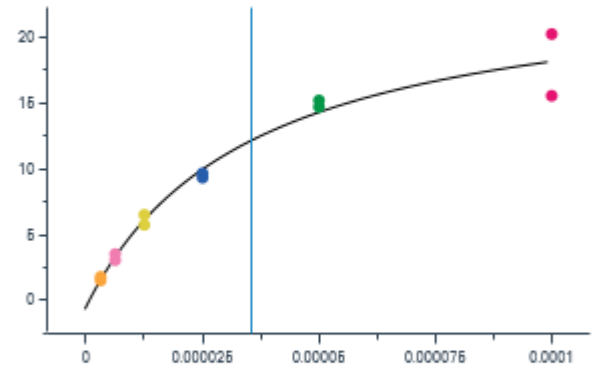
Model: Group 27
Steady state affinity

KD(M) 3.15e-04 Rmax(RU) 10.1



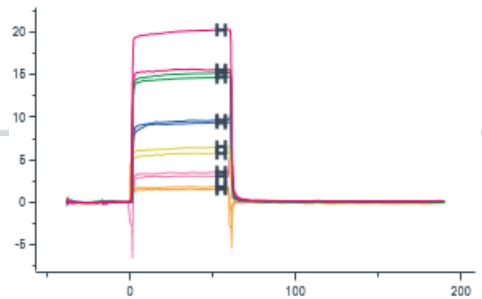
NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000711a
Sensorgram	Ch 1, Fc 2-1 corr
Temperature	20



Model: Group 5
Steady state affinity

KD(M) 3.54e-05 Rmax(RU) 25.3



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 81.6
%inh@50 µM = 37.4
%inh@25 µM = 14.8

SPR confirmation/selectivity test

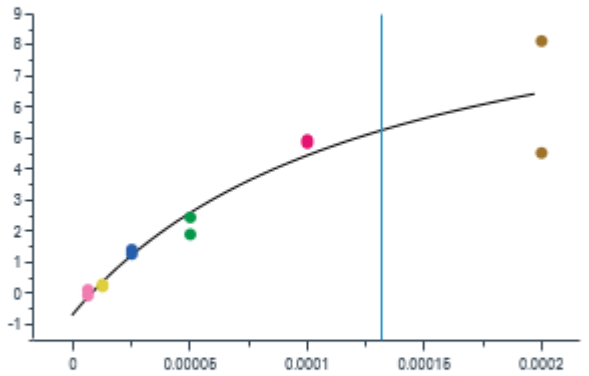
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	25	41	61	35.4
PARP14a	10	36	28	no binding

Molecular Biophysics

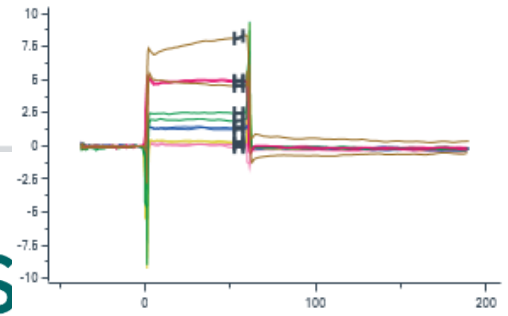
CACHE3HI_1715_82

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000961a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

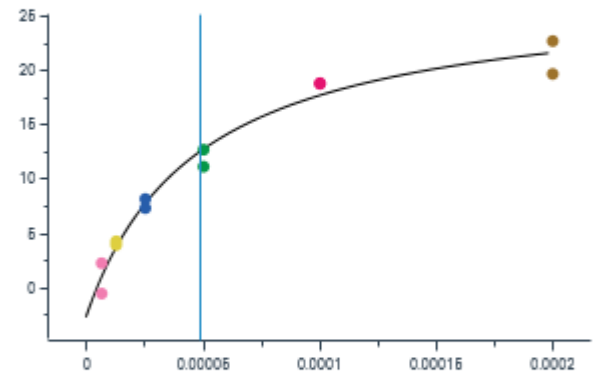


Model	Group 38 Steady state affinity
KD(M)	1.31e-04
Rmax(RU)	11.8

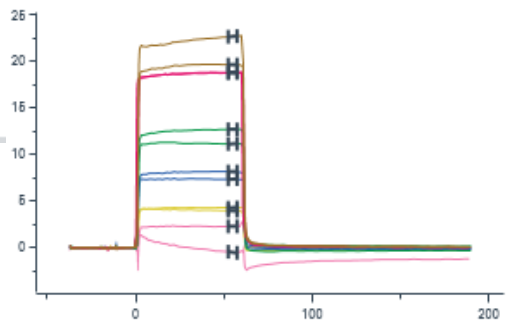


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000961a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model	Group 16 Steady state affinity
KD(M)	4.85e-05
Rmax(RU)	29.8



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 68.2
%inh@50 µM = 24.8
%inh@25 µM = 8.6

SPR confirmation/selectivity test

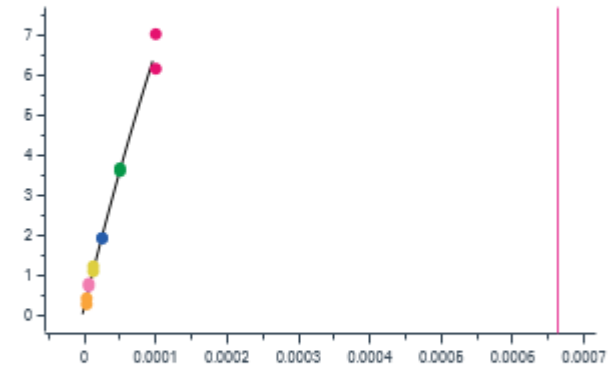
Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	30	46	64	49
PARP14a	12	40	30	131

Molecular Biophysics

CACHE3HI_1718_58

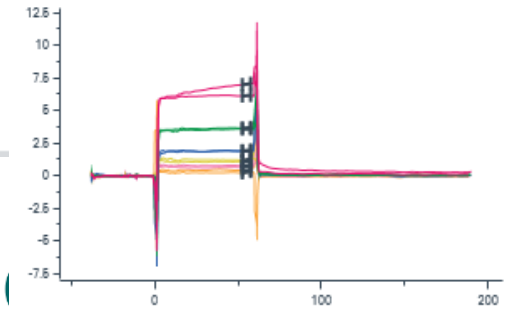
PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR001533a_PARP14a
Sensorgram	Ch 8, Fc 2-1 corr
Temperature	20



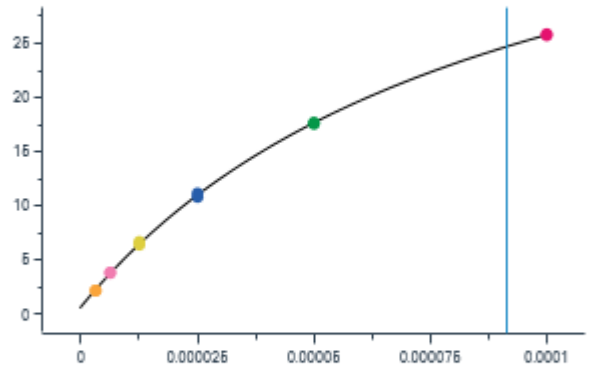
Model: Group 43
Steady state affinity

KD(M) 6.62e-04 Rmax(RU) 48.6



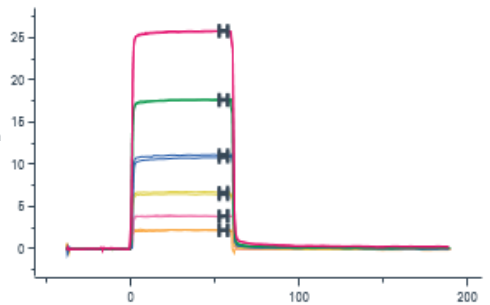
NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR001533a
Sensorgram	Ch 4, Fc 2-1 corr
Temperature	20



Model: Group 21
Steady state affinity

KD(M) 9.15e-05 Rmax(RU) 48.0



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 35.8
%inh@50 µM = 17.1
%inh@25 µM = 9.7

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	48	35	139	91.5
PARP14a	49	32	153	weak binding

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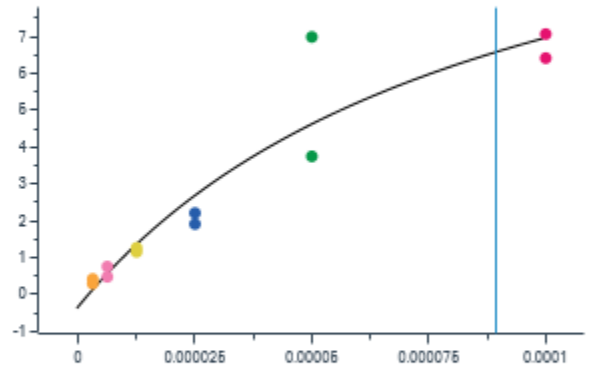
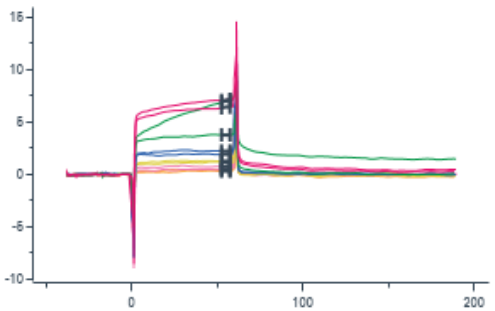
CACHE3HI_1718_59

PARP14a

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	PARP14a
Analyte	AR000775a_PARP14a
Sensorgram	Ch 7, Fc 2-1 corr
Temperature	20

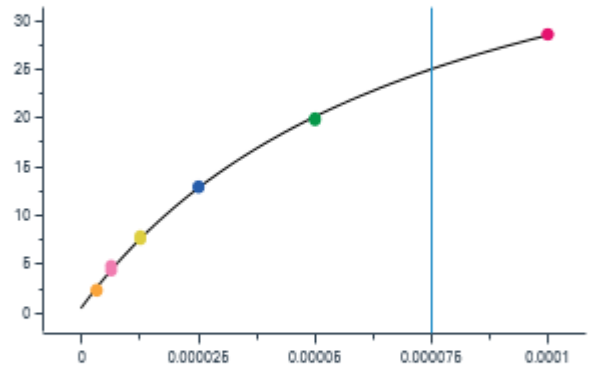


Model	Group 37
	Steady state affinity
KD(M)	8.95e-05
Rmax(RU)	13.8

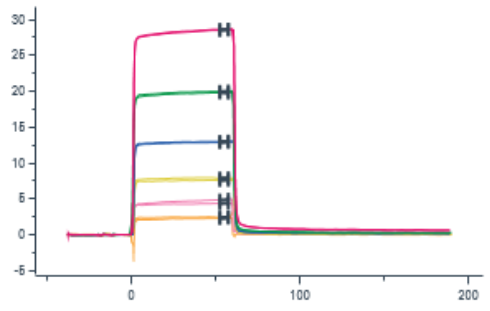


NSP3_SARS2

Run	20231215_NSP3_PARP14a_CACHE#3 hits (18 cpds)_counter screening_2 12/15/2023 5:31:29 PM
Ligand	SARS2nsp3_MacroD_ECBio2
Analyte	AR000775a
Sensorgram	Ch 3, Fc 2-1 corr
Temperature	20



Model	Group 15
	Steady state affinity
KD(M)	7.49e-05
Rmax(RU)	48.6



Previous data:

HTRF_displacement results confirmation:
%inh@100 µM = 43.5
%inh@50 µM = 18.7
%inh@25 µM = 9.1

SPR confirmation/selectivity test

Protein	RUmax_ experiment	RUmax_ expected	% binding	K_D _µM, n=2
NSP3_SARS2	49	39	125	75
PARP14a	14	34	41	90

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