SUPPLEMENTARY INFORMATION

Structural implications for the formation and function of the complement effector protein iC3b

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Supplementary Table and Figure Legends:

Table S1: Average difference in peptide deuteration levels (%) between complement proteins iC3b and C3b.

Fig. S1: D-uptake plots of C3b and iC3b peptides.

Fig. S2: anti-iC3b mAb reactivity using direct ELISA.

Fig. S3: Binding profile of different C3 fragments to the captured anti-iC3b mAb.

References

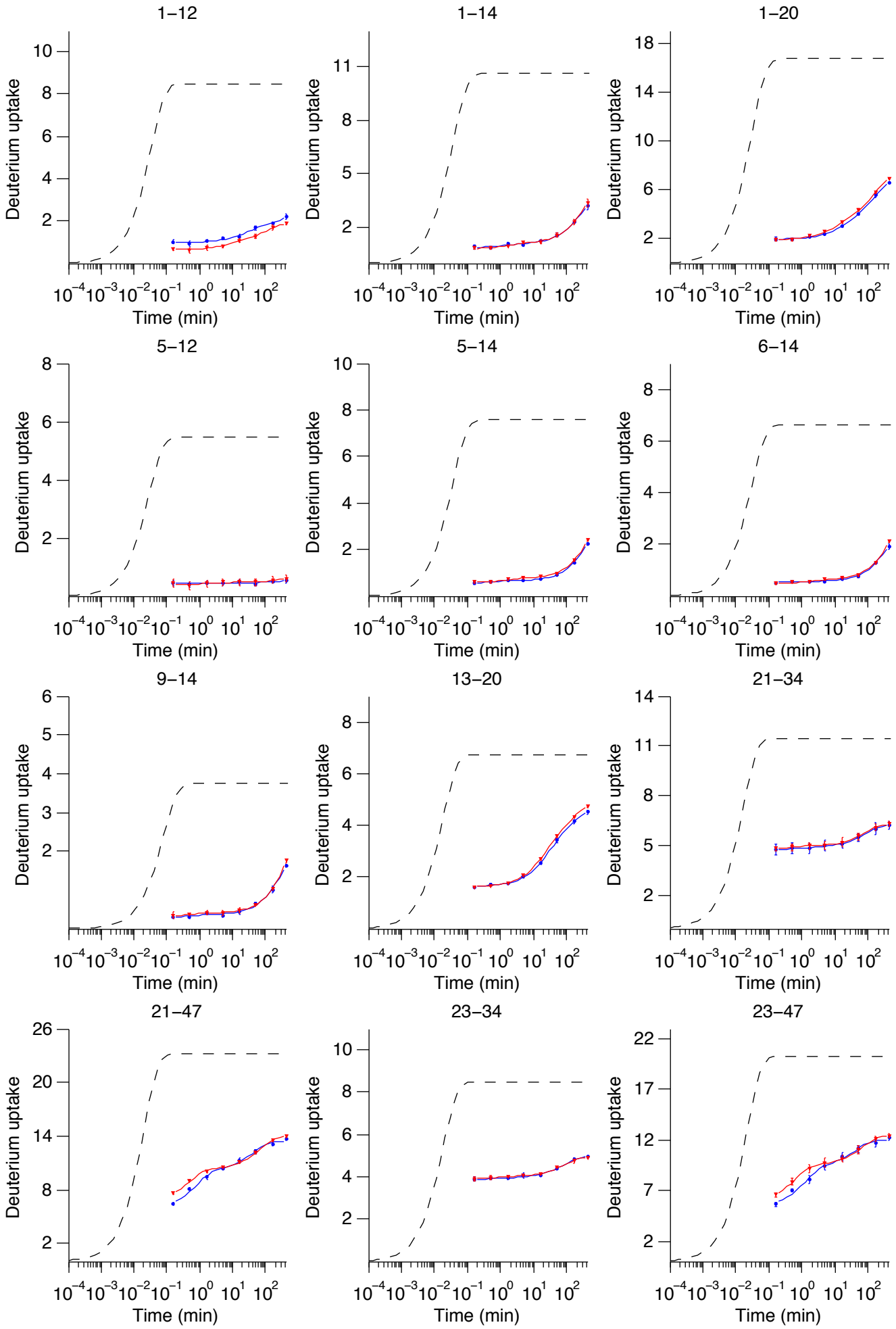
1. Behrendt, N. 1985. Human complement component C3: Characterization of active C3 S and C3 F, the two common genetic variants. *Mol Immunol* 22: 1005-1008.
2. Bai, Y., J. S. Milne, L. Mayne, and S. W. Englander. 1993. Primary structure effects on peptide group hydrogen exchange. *Proteins* 17: 75-86.
3. Morris, G. E. 1996. Epitope mapping of protein antigens by competition ELISA. In *The Protein Protocols Handbook*. J. Walker, ed. Humana Press. 595-600.
4. Hammel, M., G. Sfyroera, D. Ricklin, P. Magotti, J. D. Lambris, and B. V. Geisbrecht. 2007. A structural basis for complement inhibition by *Staphylococcus aureus*. *Nat Immunol* 8: 430-437.

Table S1: Average difference in peptide deuteration levels (%) between complement proteins iC3b and C3b

Peptide	Start	End	Domain	Average D-uptake difference (%)							
				10 sec	30 sec	100 sec	300 sec	1000 sec	3000 sec	10000 sec	25200 sec
VTVHDFPGKKLVL	35	47	MG1	10.8	9.0	4.7	1.8	-0.4	0.3	2.4	2.3
FKSEKGRNKFVTVQ ($C3_{80R}$)	74	87	MG1	11.1	9.6	11.7	7.4	6.7	5.2	9.5	12.1
VVEKVVL	94	100	MG1	2.2	6.4	11.5	20.8	21.2	10.3	2.7	1.6
VNIENPEGIPVKQDSL	143	158	MG2	-0.2	2.3	5.5	6.3	9.3	12.3	5.3	3.9
VNIENPEGIPVKQDSLSSQNQL	143	164	MG2	3.8	4.2	5.2	7.5	8.4	5.1	5.9	3.3
SSQNQLGVLPLSWDIPEL	159	176	MG2	1.4	6.7	8.4	3.7	2.7	2.5	2.4	0.9
GVLPLSWDIPEL	165	176	MG2	4.3	8.8	10.3	4.7	3.6	3.8	2.1	-0.2
VNMGQWKIRA	177	186	MG2	1.9	4.5	4.2	3.2	4.7	0.9	7.9	9.7
YYENSPQQVF	187	196	MG2	1.8	4.2	4.4	4.4	4.8	9.0	7.3	4.7
YYENSPQQVFSTE	187	199	MG2	1.0	2.9	6.0	8.7	7.9	5.0	5.7	4.1
VRTLDPERLGREGVQ	922	936	CUB^{g}	68.7	65.8	60.9	54.3	42.8	33.0	22.5	19.2
VRTLDPERLGREGVQKEDIPPADL	922	945	CUB^{g}	64.5	60.6	56.4	51.7	45.0	38.3	29.5	25.5
SDQVPDTESE	946	955	CUB^{g}	74.9	68.7	62.6	57.1	52.3	46.7	37.0	31.8
SDQVPDTESET	946	956	CUB^{g}	76.5	70.3	66.8	64.1	60.1	52.7	40.7	34.3
SDQVPDTESETRILL	946	960	CUB^{g}	81.9	78.8	76.8	74.2	74.6	69.1	62.3	58.0
SDQVPDTESETRILLQGTPVAQM	946	968	CUB^{g}/TED	79.2	74.3	70.2	67.8	61.5	56.4	48.9	43.6
TRILLQGTPVAQM	956	968	CUB^{g}/TED	67.6	63.9	57.7	51.9	45.6	38.8	33.6	29.8
TRILLQGTPVAQMTED	956	971	CUB^{g}/TED	66.6	61.1	53.7	46.7	37.2	29.9	26.5	27.7
RILLQGTPVAQM	957	968	CUB^{g}/TED	68.5	66.3	60.8	54.4	48.2	40.0	34.3	31.5
AVDAERLKHLIVTPSGCGEqNM	972	993	TED	50.1	53.5	51.9	48.3	42.4	36.7	33.3	31.1
AERLKHLIVT	975	984	TED	74.2	77.2	73.9	73.8	72.4	68.3	67.3	63.4
AERLKHLIVTPSGCGEqNM	975	993	TED	45.1	47.7	45.1	42.3	38.0	34.3	32.8	31.1
IGMTPTVIAVHYLDE	994	1008	TED	-0.4	-1.0	1.6	2.8	8.8	11.5	16.5	16.9
IGMTPTVIAVHYLDETEQ	994	1011	TED	-0.4	-0.2	1.0	3.9	9.6	15.6	18.3	17.1
IAVHYLDETEQ	1001	1011	TED	-1.1	1.1	1.8	4.0	8.0	15.2	16.7	14.5
AVHYLDETEQ	1002	1011	TED	0.0	0.1	2.3	5.0	10.0	14.7	14.3	14.6
VHYLDETEQ	1003	1011	TED	-0.2	0.8	2.4	3.5	8.5	14.0	15.7	14.3
WEKFGLEKRQGAL	1012	1024	TED	13.3	17.4	20.1	18.2	10.8	4.3	3.6	1.9
WEKFGLEKRQGALE	1012	1025	TED	10.9	14.0	17.1	19.9	11.4	5.1	1.6	2.1
WEKFGLEKRQGALEL	1012	1026	TED	9.8	13.3	14.9	15.1	8.9	3.1	3.2	4.5
VVKVFSLAVNL	1060	1070	TED	5.8	8.9	12.4	8.9	12.5	13.5	5.8	0.6
QRYYGGGYGSTQ	1237	1248	TED	29.6	26.5	20.4	15.3	11.5	4.4	1.5	1.8
QRYYGGGYGSTQATF	1237	1251	TED	23.2	22.1	17.3	14.0	9.0	7.1	7.6	5.8
MVFQALAQYQKDAPDHQELNL	1252	1272	TED/CUB^{f}	22.6	22.7	20.5	19.0	19.0	20.4	23.3	23.1
QALAQYQKDAPDHQELNL	1255	1272	TED/CUB^{f}	25.8	27.2	24.2	20.6	19.6	22.7	24.9	23.8
AQYQKDAPDHQELNL	1258	1272	TED/CUB^{f}	31.3	33.1	29.2	24.2	23.4	25.2	27.6	26.4
YQKDAPDHQELNL	1260	1272	TED/CUB^{f}	42.2	44.0	38.5	31.4	28.1	27.2	25.0	20.4
LDVSLQLPSRSSKITHRIHWESAS (C3b)	1272	1295	CUB^{f}	49.4	56.0	61.9	66.8	72.6	77.1	79.2	82.5
DVSLQLPSR (iC3b)	1273	1281	CUB^{f}	81.6	96.2	99.5	100.0	99.0	99.6	99.6	99.3
DVSLQLPSRSSKI (C3b)	1273	1285	CUB^{f}	34.4	33.9	37.6	43.7	54.2	61.9	67.5	68.6
DVSLQLPSRSSKITHRIHWESASL (C3b)	1273	1296	CUB^{f}	48.0	55.8	61.7	66.3	73.3	76.3	78.4	82.3
SLQLPSRSSKITHRIHWESASL (C3b)	1275	1296	CUB^{f}	49.4	59.7	61.6	66.8	71.7	75.0	82.1	86.6
LQLPSR (iC3b)	1276	1281	CUB^{f}	77.6	92.5	98.9	99.1	98.8	99.4	99.1	100.0
LQLPSRSSKIT (C3b)	1276	1286	CUB^{f}	13.0	18.5	24.5	28.5	35.5	45.3	52.3	55.2
LRSEETKENEG (C3b)	1297	1307	CUB^{f}	31.5	34.0	43.0	57.0	73.6	86.8	93.3	99.1
LRSEETKENEGF (C3b)	1297	1308	CUB^{f}	30.7	34.8	40.9	52.5	69.9	81.2	86.8	90.2
LRSEETKENEGFTVTAEGKGQGTL (C3b)	1297	1320	CUB^{f}	13.3	14.1	18.0	23.4	32.0	40.4	46.7	50.0
SEETKENEGFTVT (iC3b)	1299	1311	CUB^{f}	96.4	97.6	96.7	98.5	100.0	98.9	96.9	98.1
SEETKENEGFTVTAEGKGQGTL (iC3b)	1299	1320	CUB^{f}	99.6	100.0	99.3	99.2	98.8	98.8	98.7	100.0
TVTAEGKGQGTL	1309	1320	CUB^{f}	86.3	86.8	85.0	82.3	78.0	73.8	70.9	66.7
TVTAEGKGQGTLSV	1309	1322	CUB^{f}	93.5	93.2	92.1	90.4	88.1	82.9	79.5	75.8
VTMYHAKAKDQLTCNKFDL	1323	1341	CUB^{f}/MG8	30.8	29.1	26.2	24.3	20.9	20.9	18.6	18.2
YHPEKEDGKL	1471	1480	anchor/CTC	-18.2	-26.7	-41.8	-39.3	-21.4	-0.6	3.0	3.3

[1]Overall, 53 peptides with significant differences (>7%) during the course of the experiment were detected. Positive values indicate higher D-uptake levels and negative values lower levels for iC3b peptides. For peptides in CUB^{f} that are unique in iC3b or C3b (in parentheses), average deuteration levels (%) are given. Peptide 74-87 is characteristic of the $C3_{80R}$ form (1). Deamidated glutamine residues, where detected, are indicated with a 'q' in the respective peptide sequences.

Figure S1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9–14
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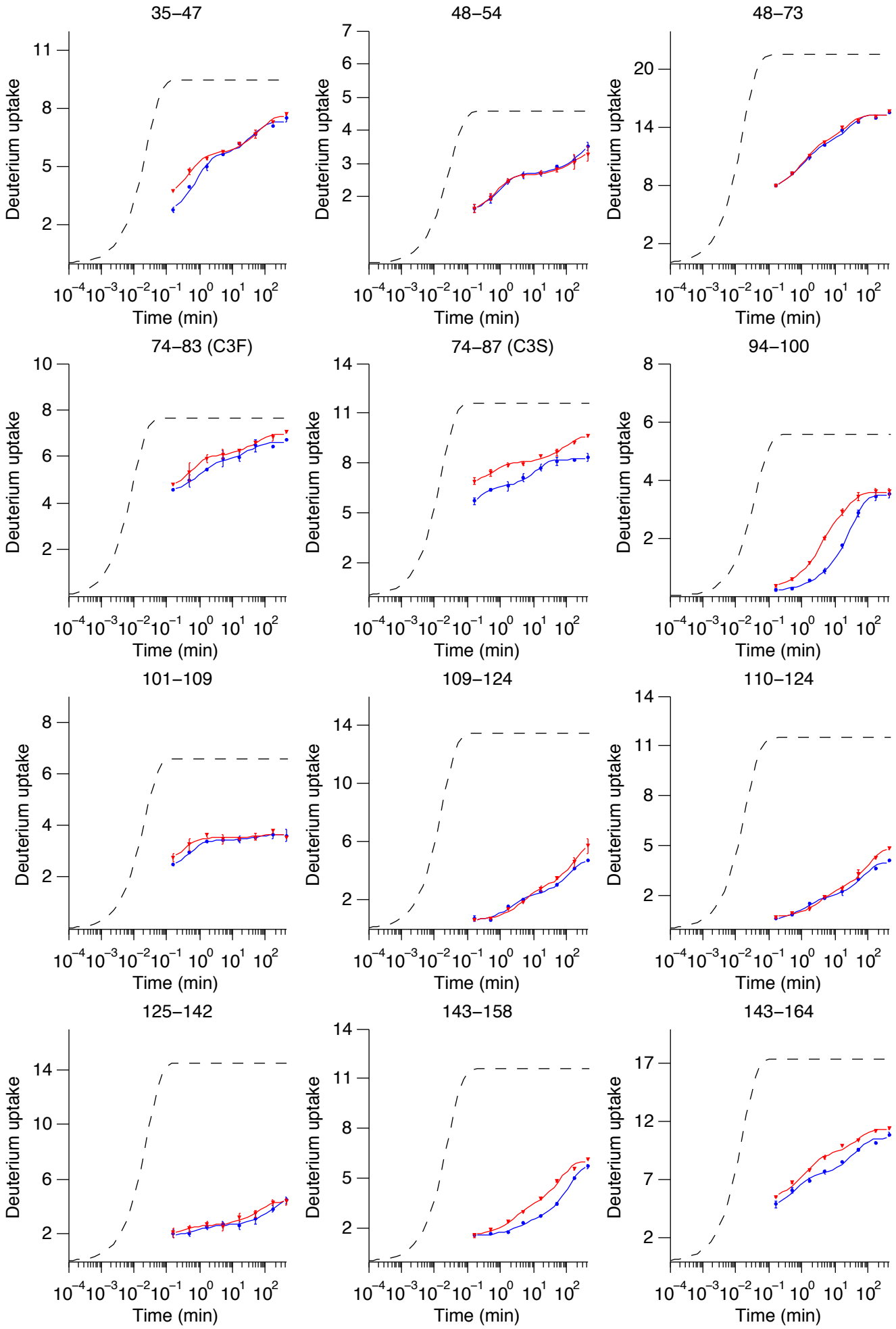
23–34
23–47
Deuterium uptake
Time (min)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48–73
74–83 (C3F)
74–87 (C3S)
94–100
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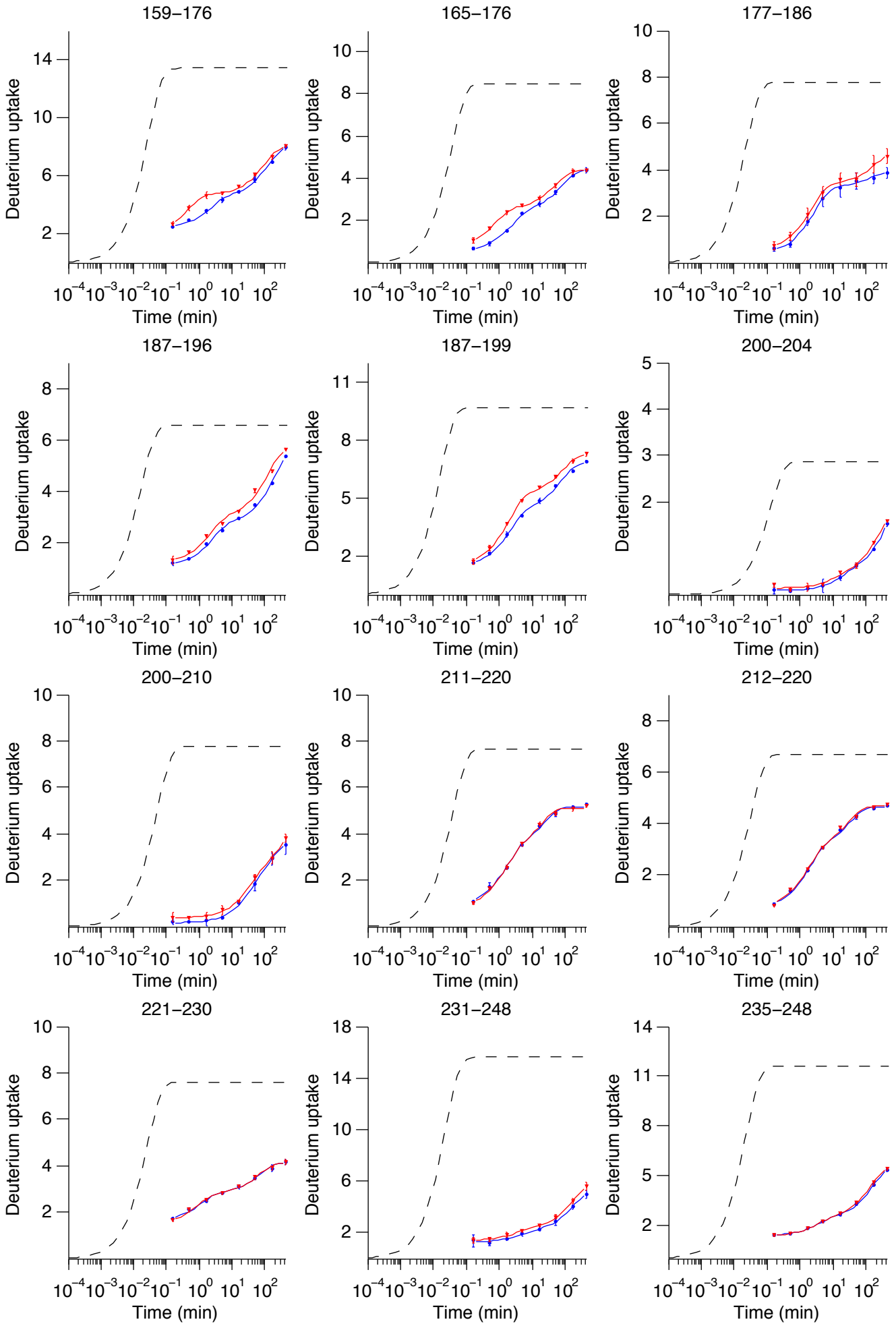
143–158
143–164
Deuterium uptake
Time (min)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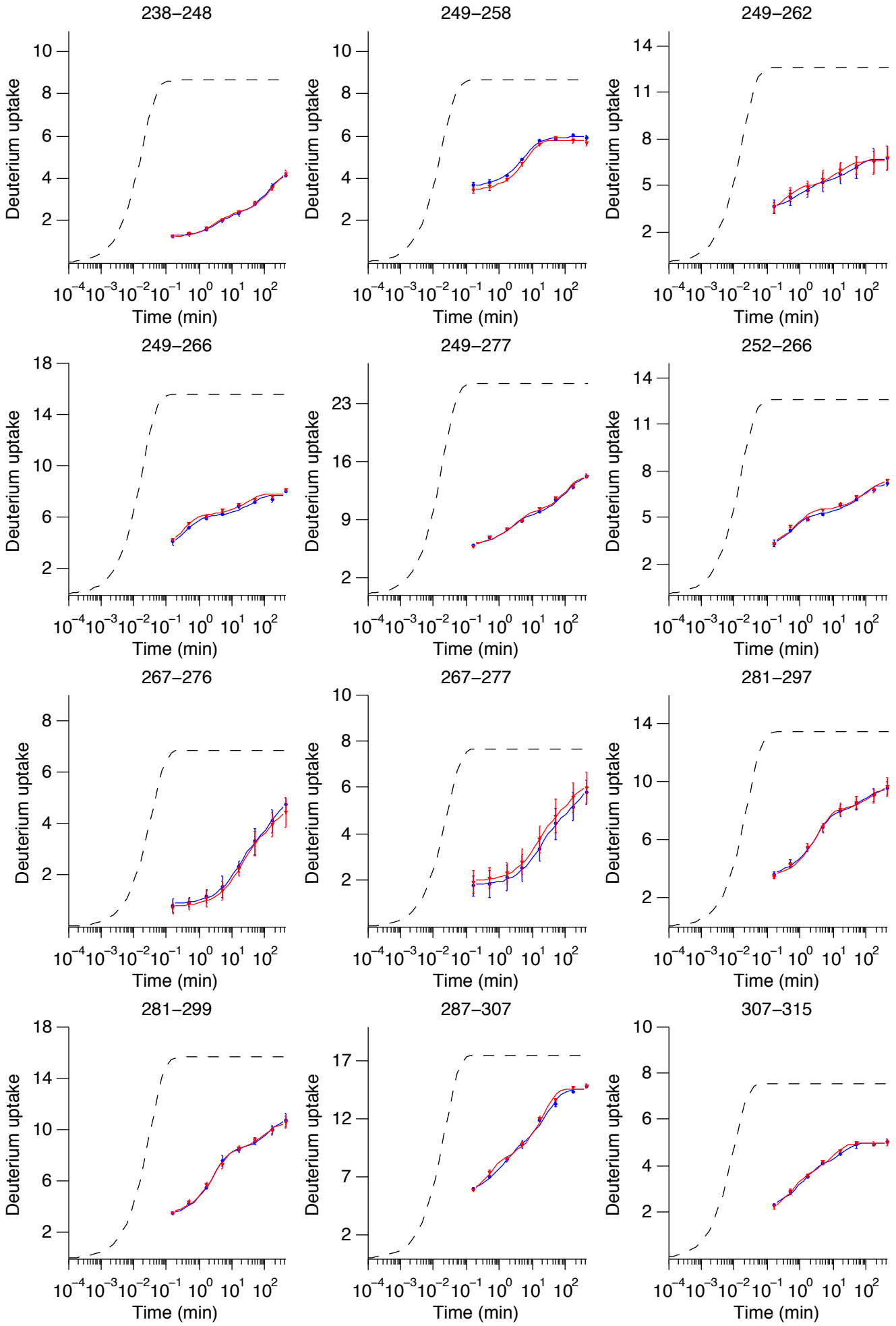
Deuterium uptake
Time (min)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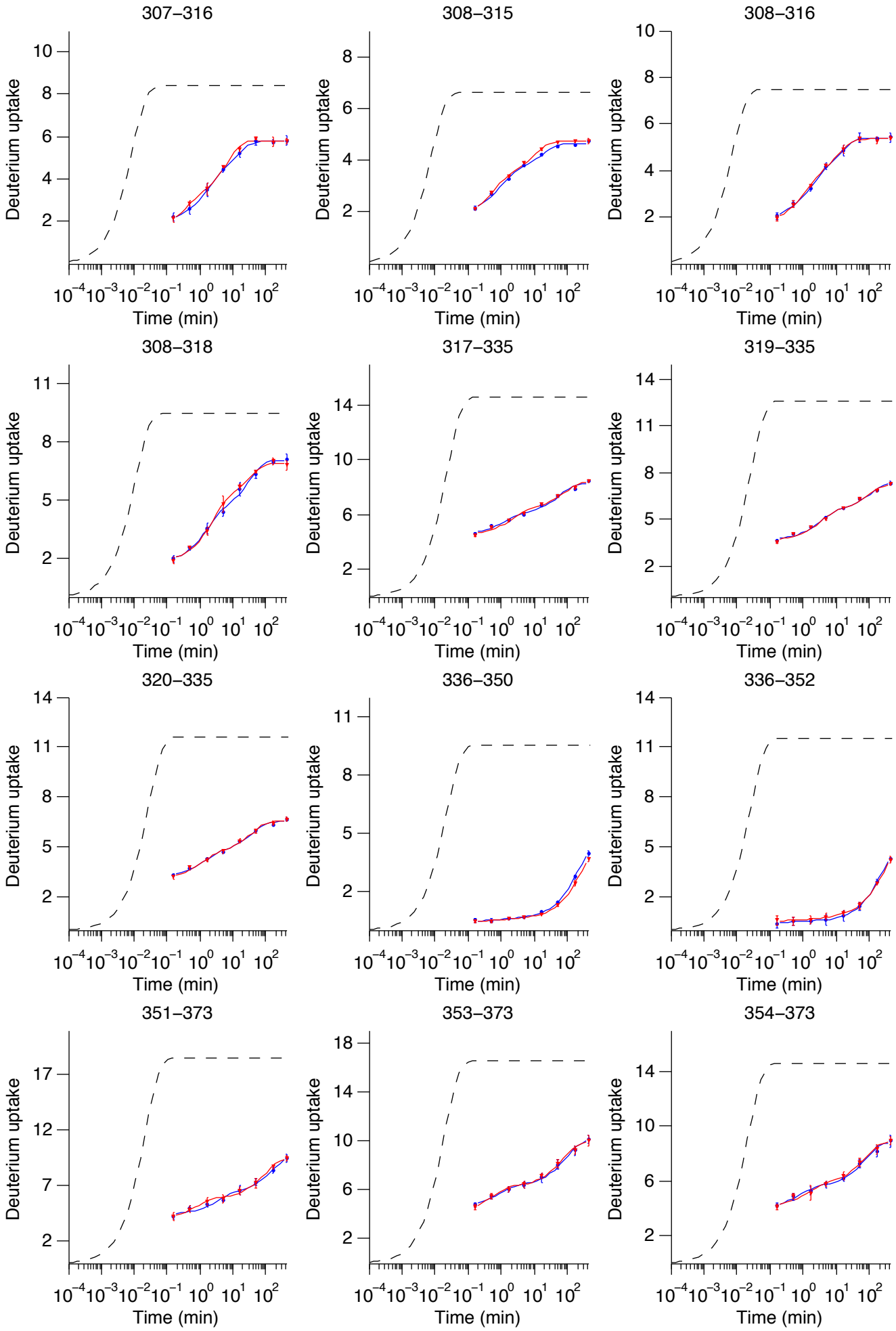
Deuterium uptake
Time (min)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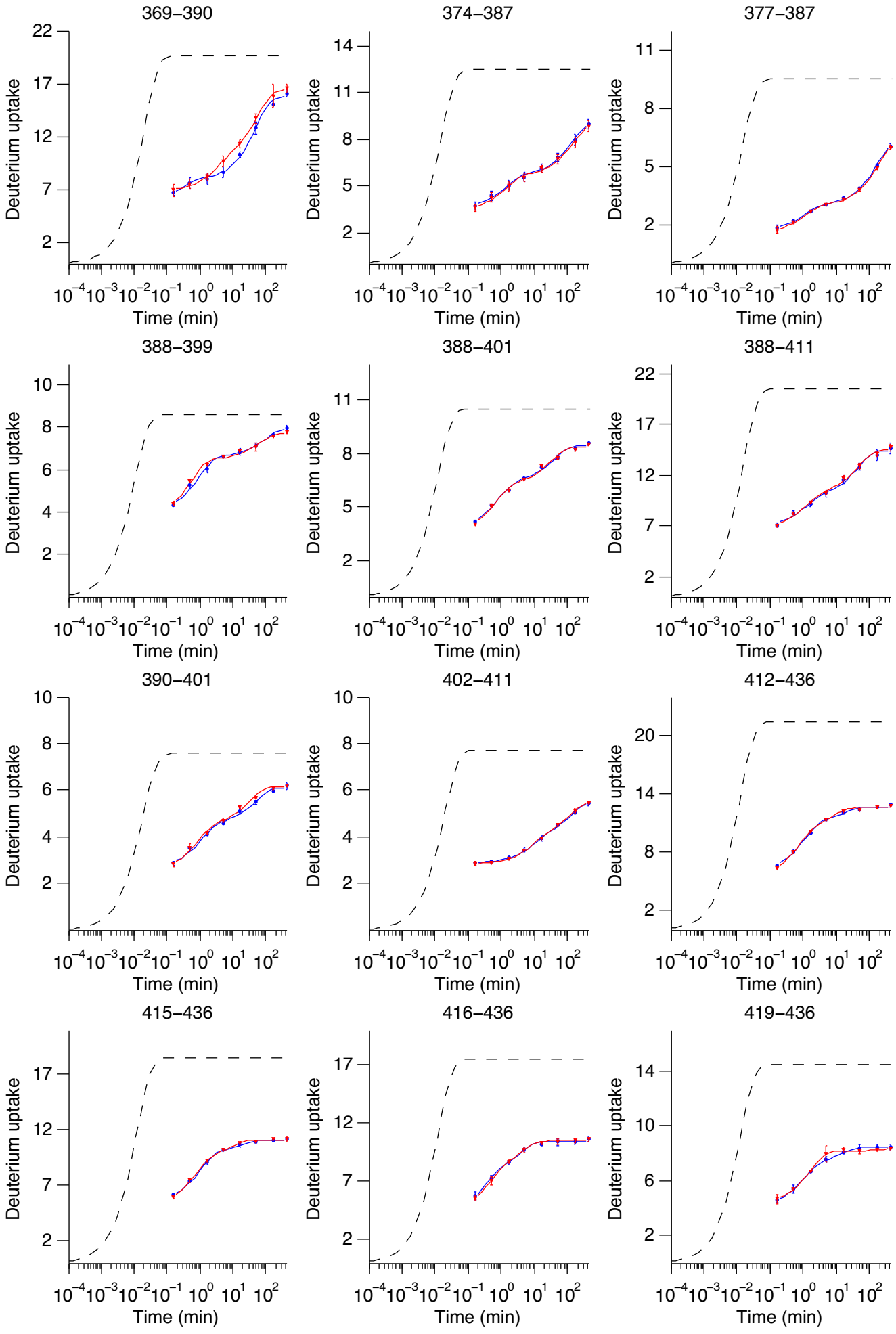
Deuterium uptake
Time (min)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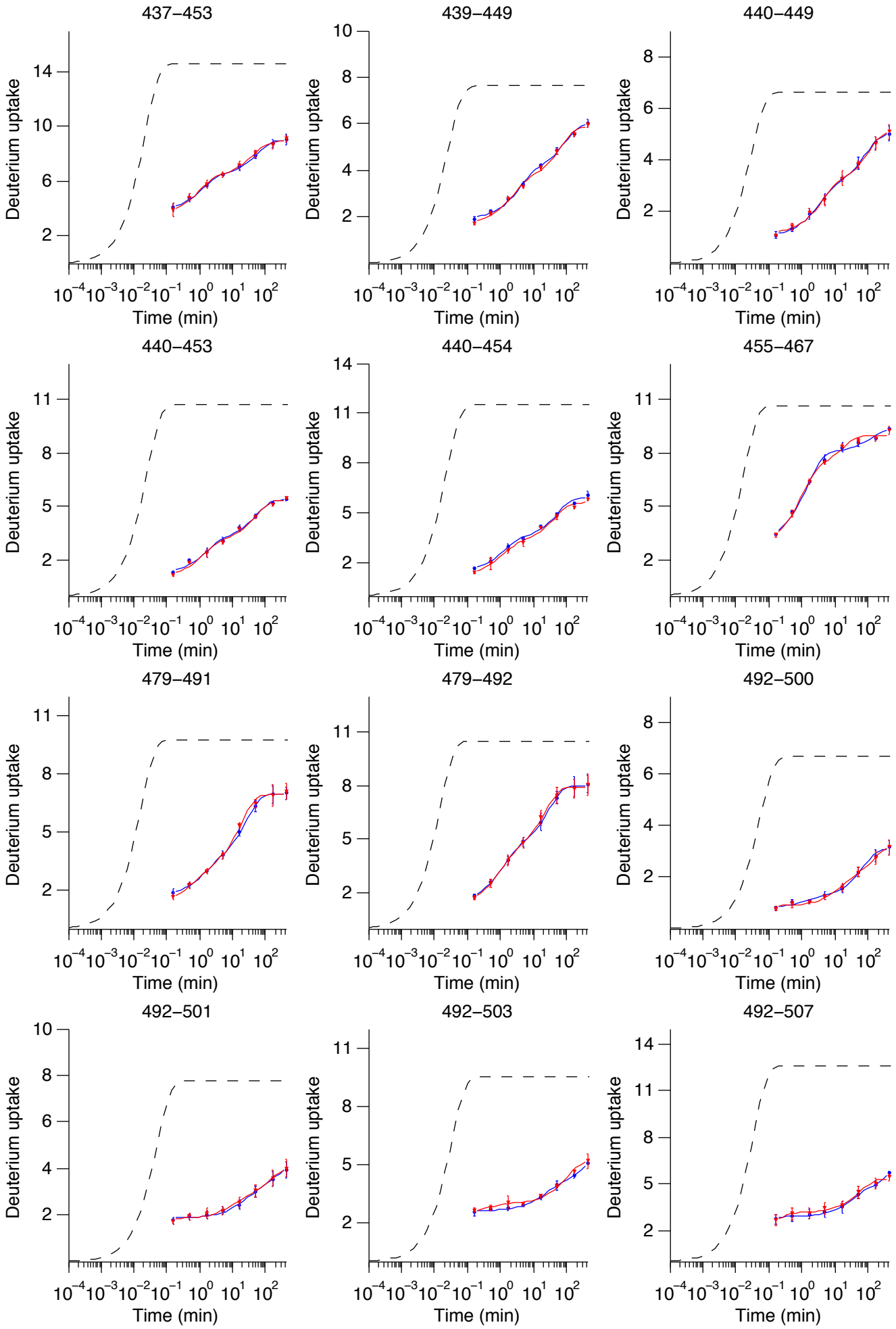
Deuterium uptake
Time (min)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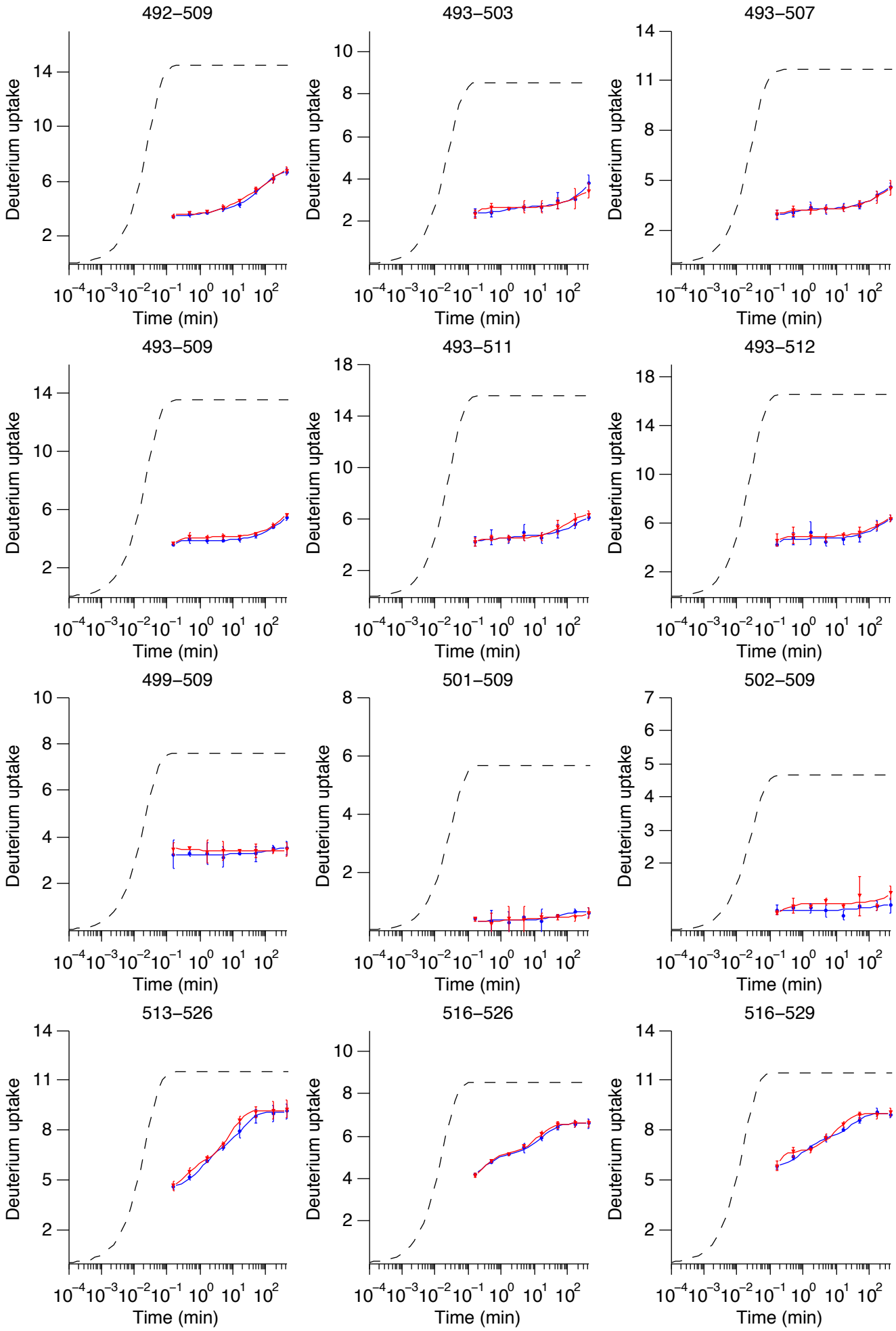
492–503
492–507
Deuterium uptake
Time (min)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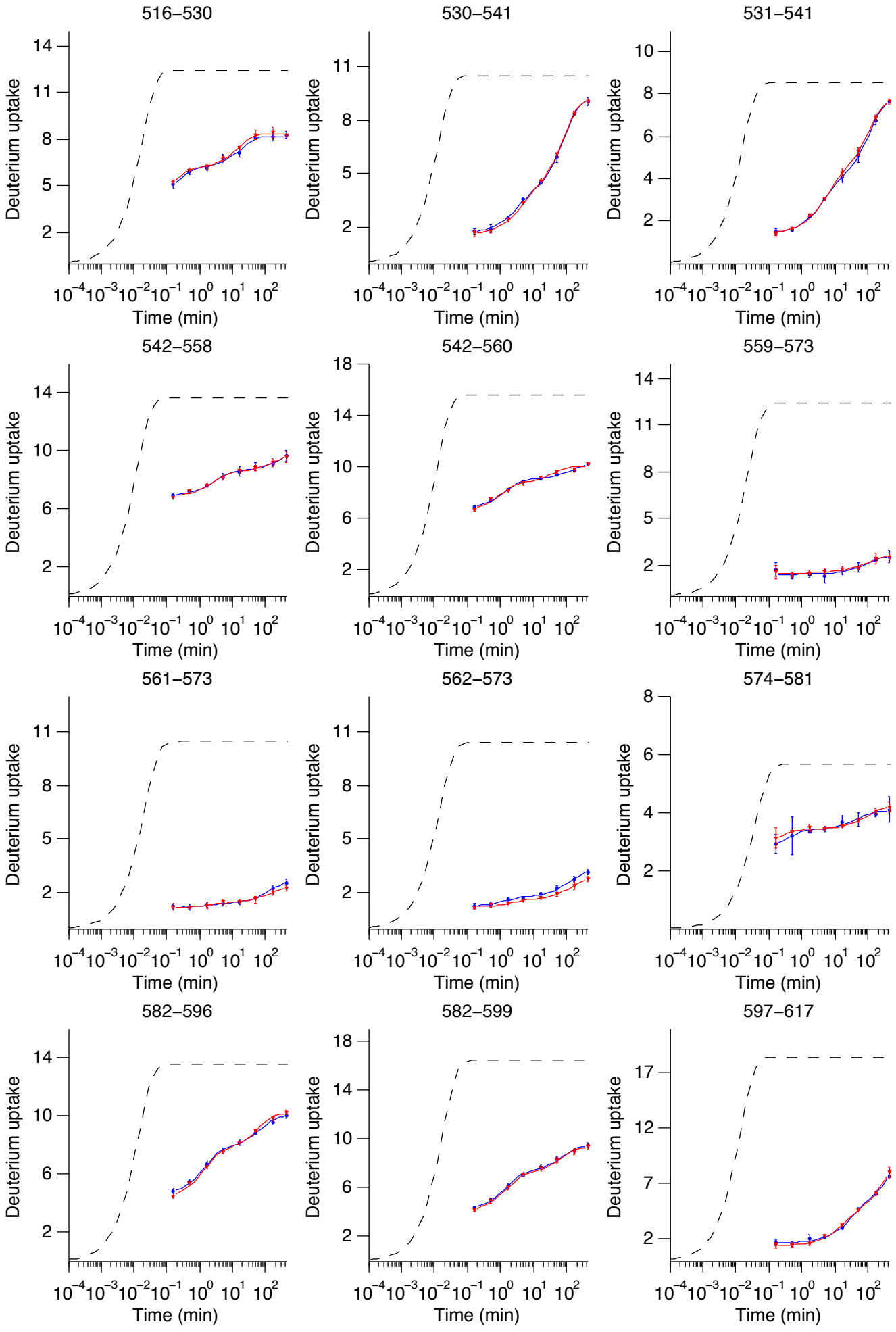
516–529
Deuterium uptake
Time (min)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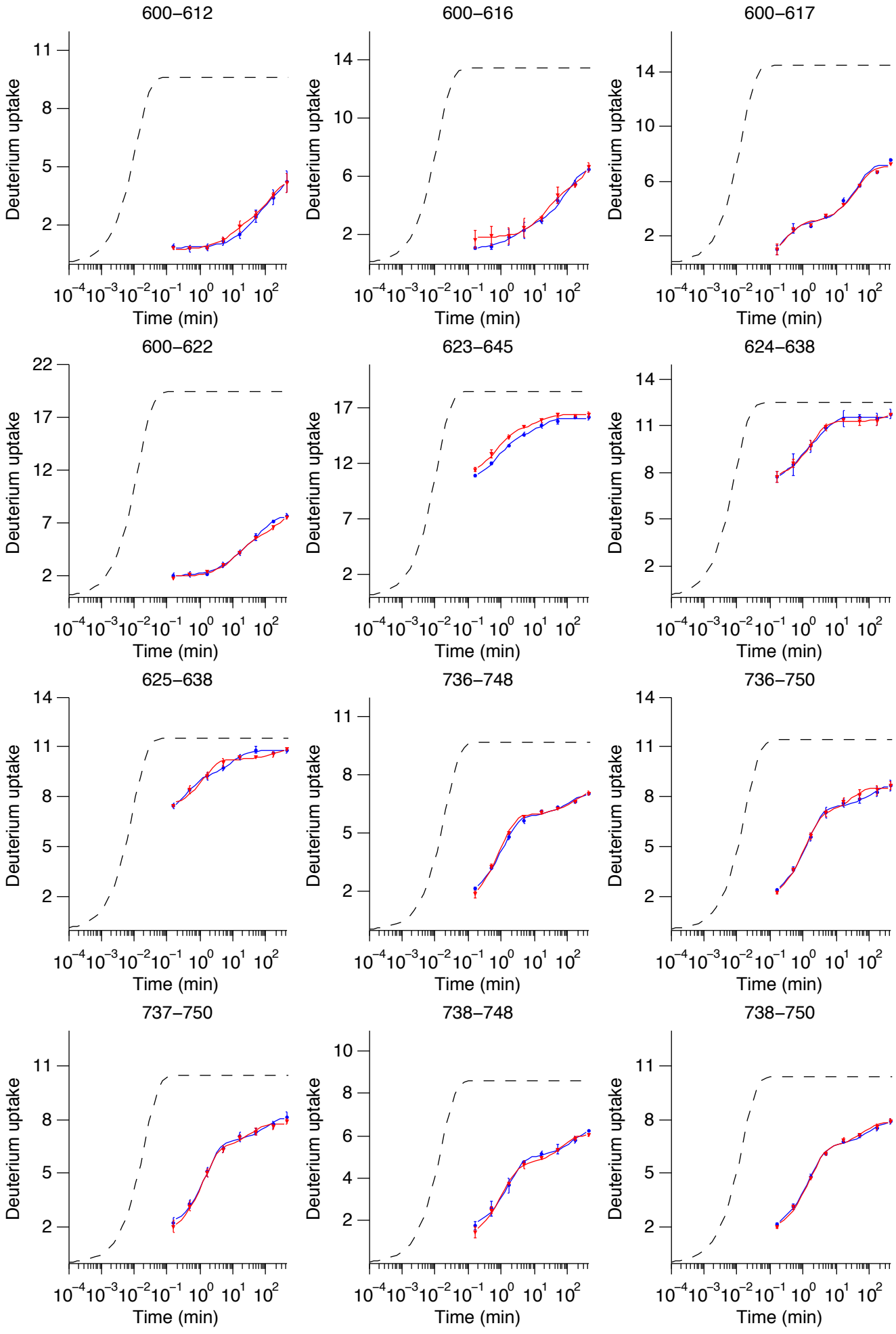
597–617
Deuterium uptake
Time (min)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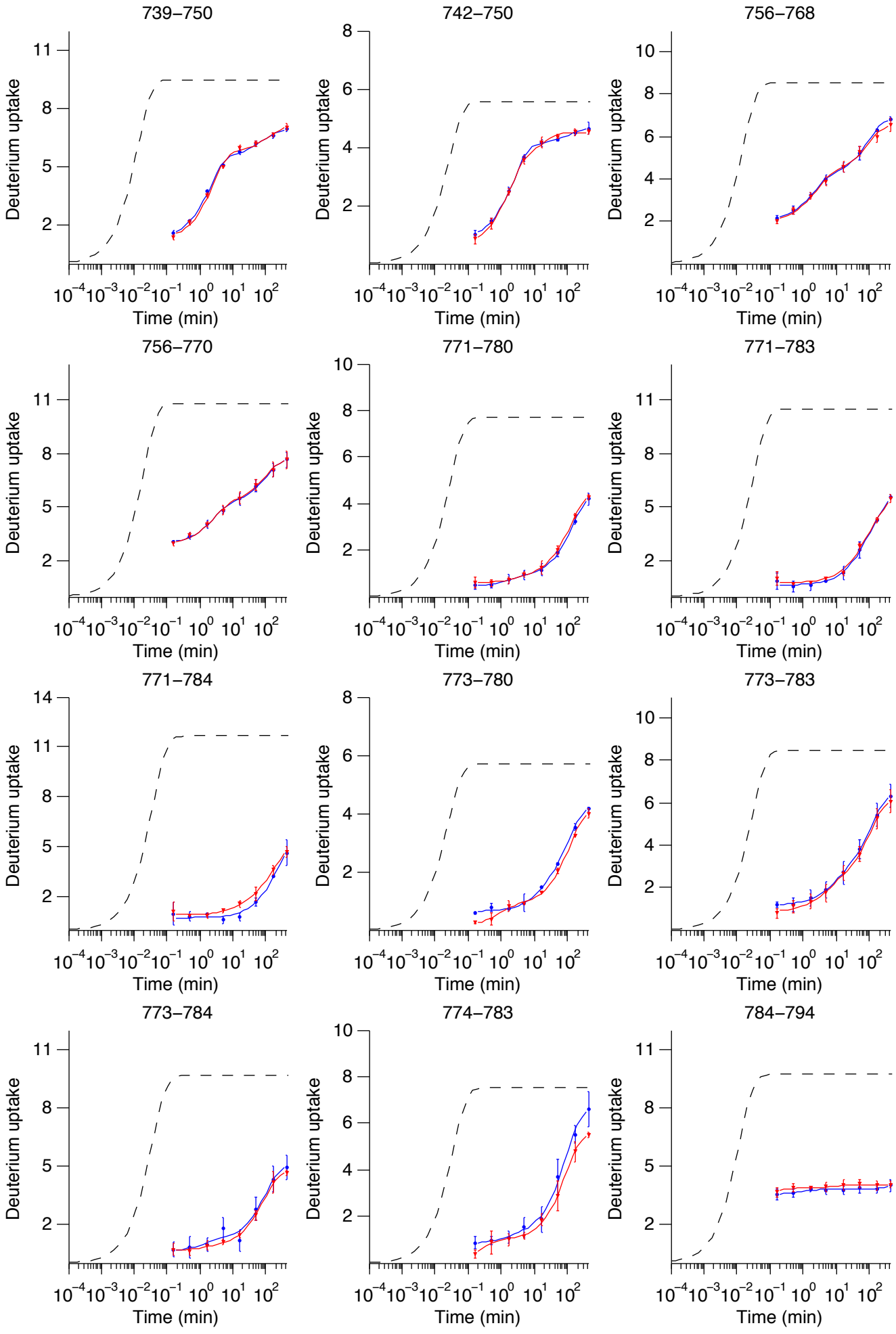
738–750
Deuterium uptake
Time (min)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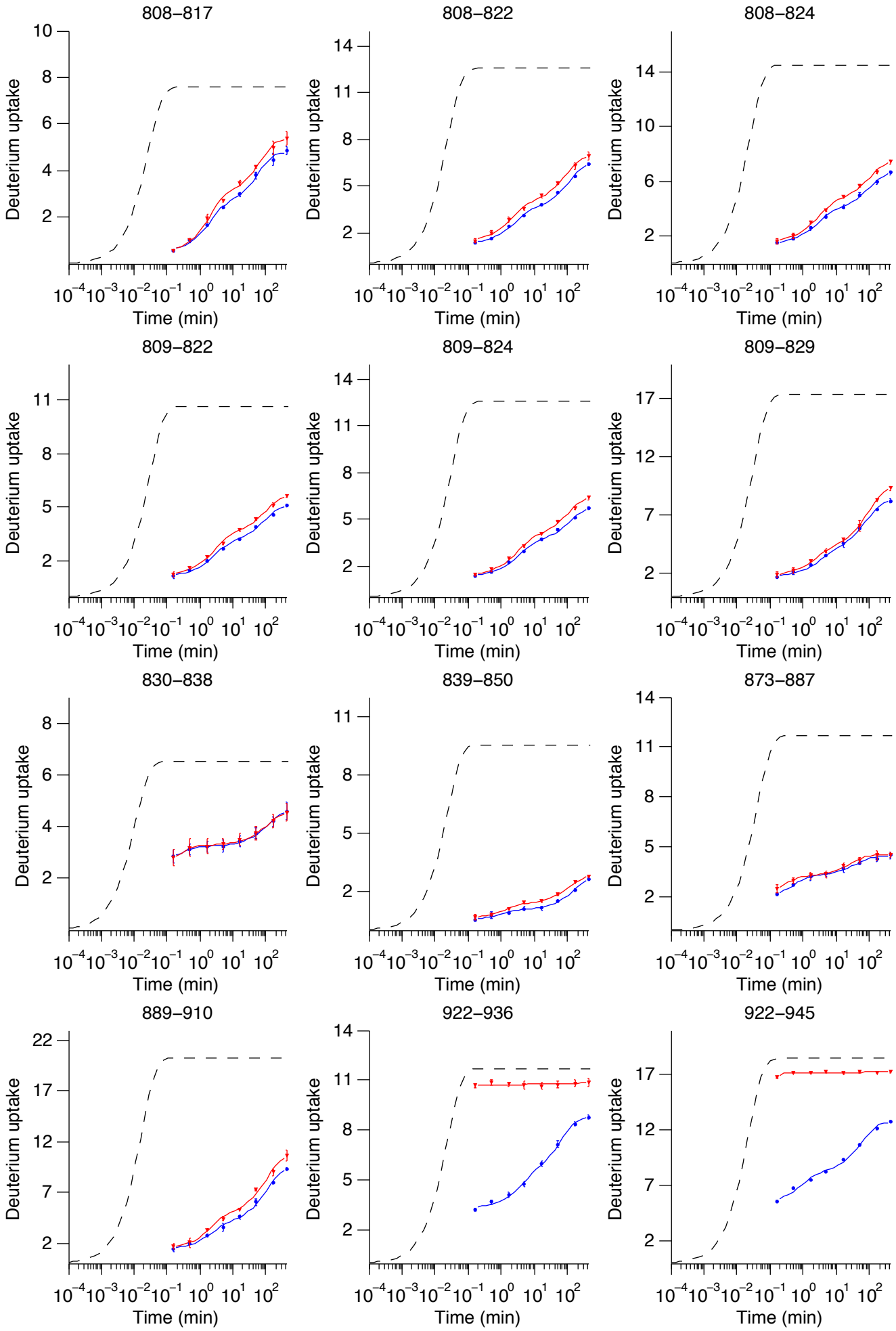
784–794
Deuterium uptake
Time (min)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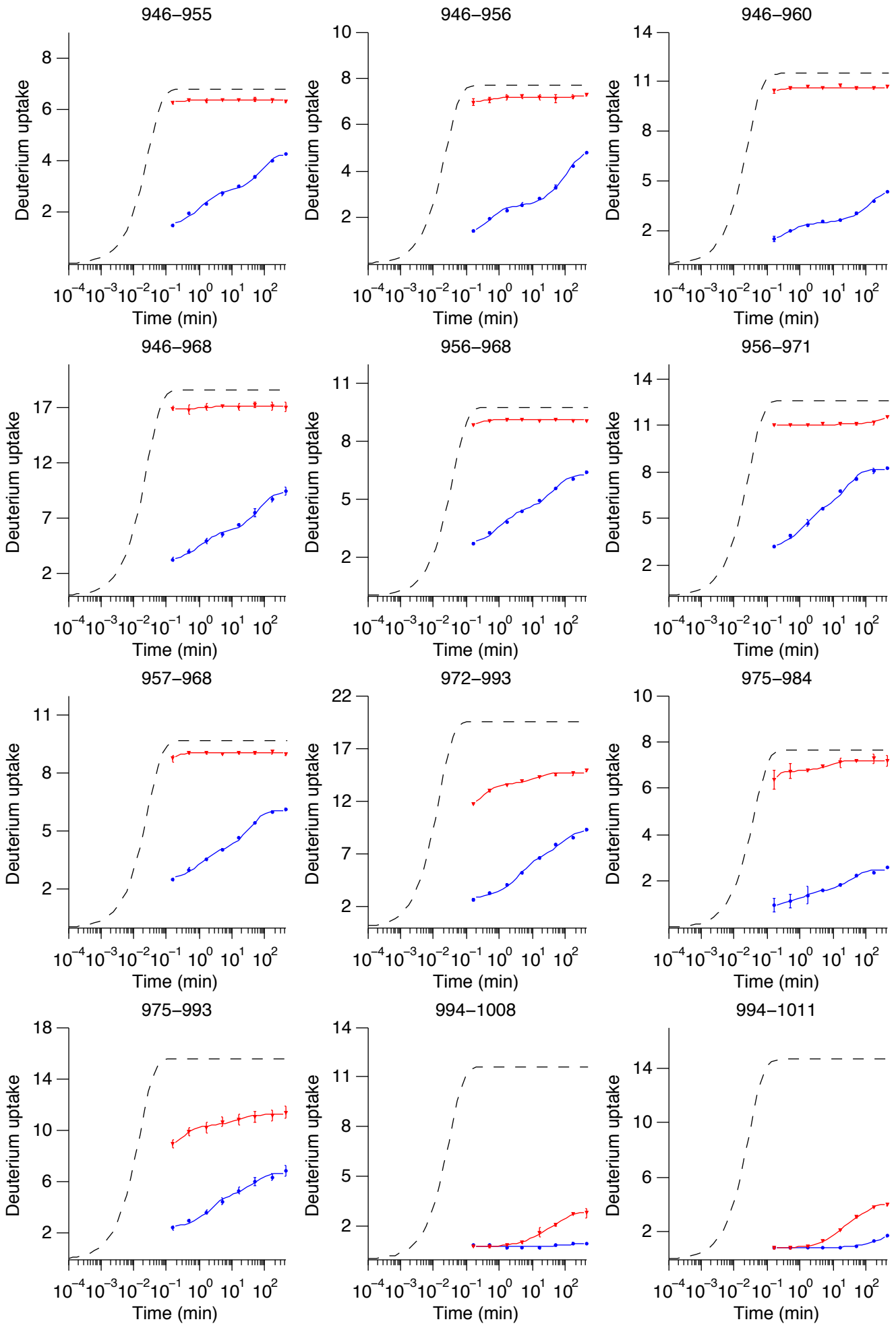
922–945
Deuterium uptake
Time (min)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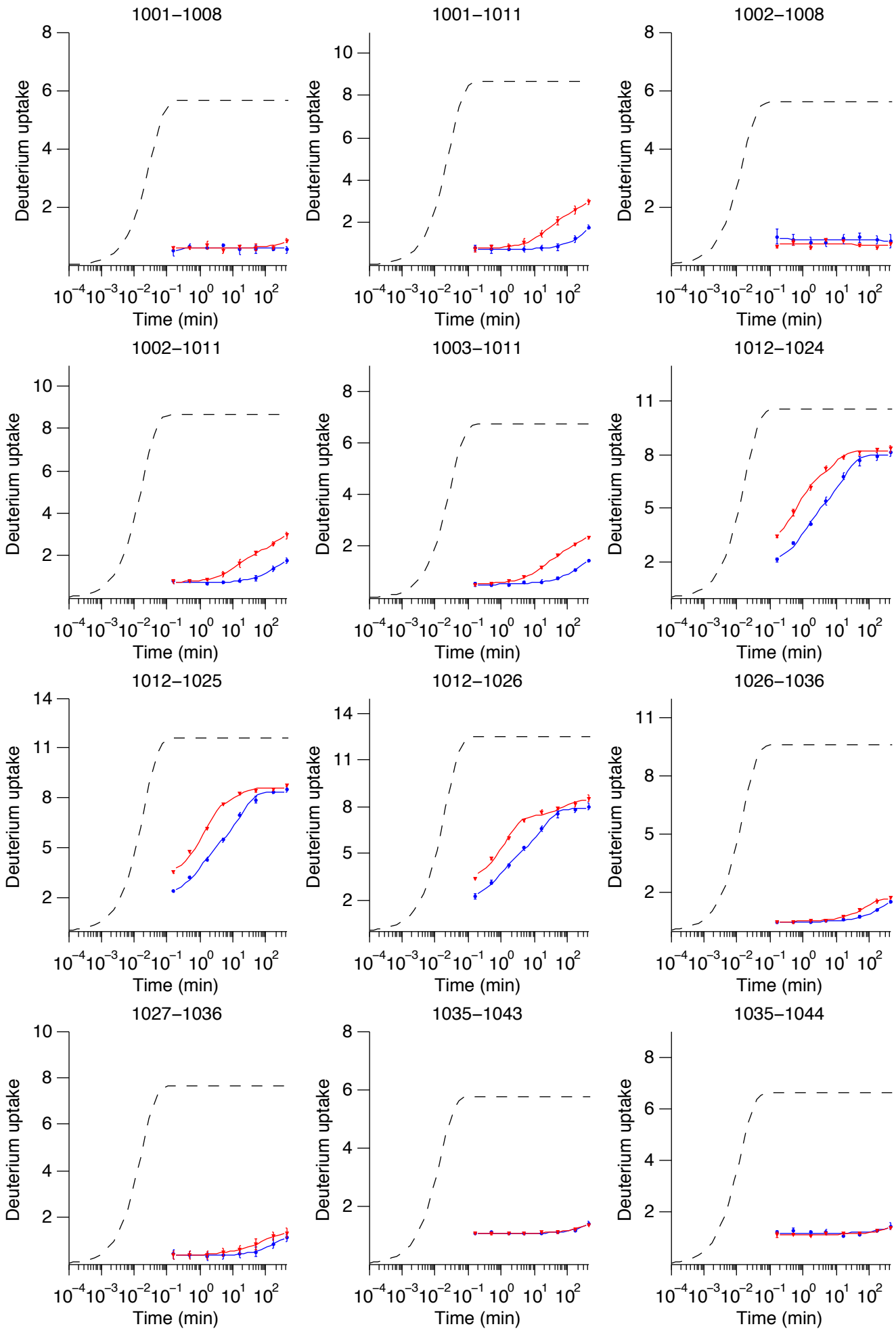
994–1008
994–1011
Deuterium uptake
Time (min)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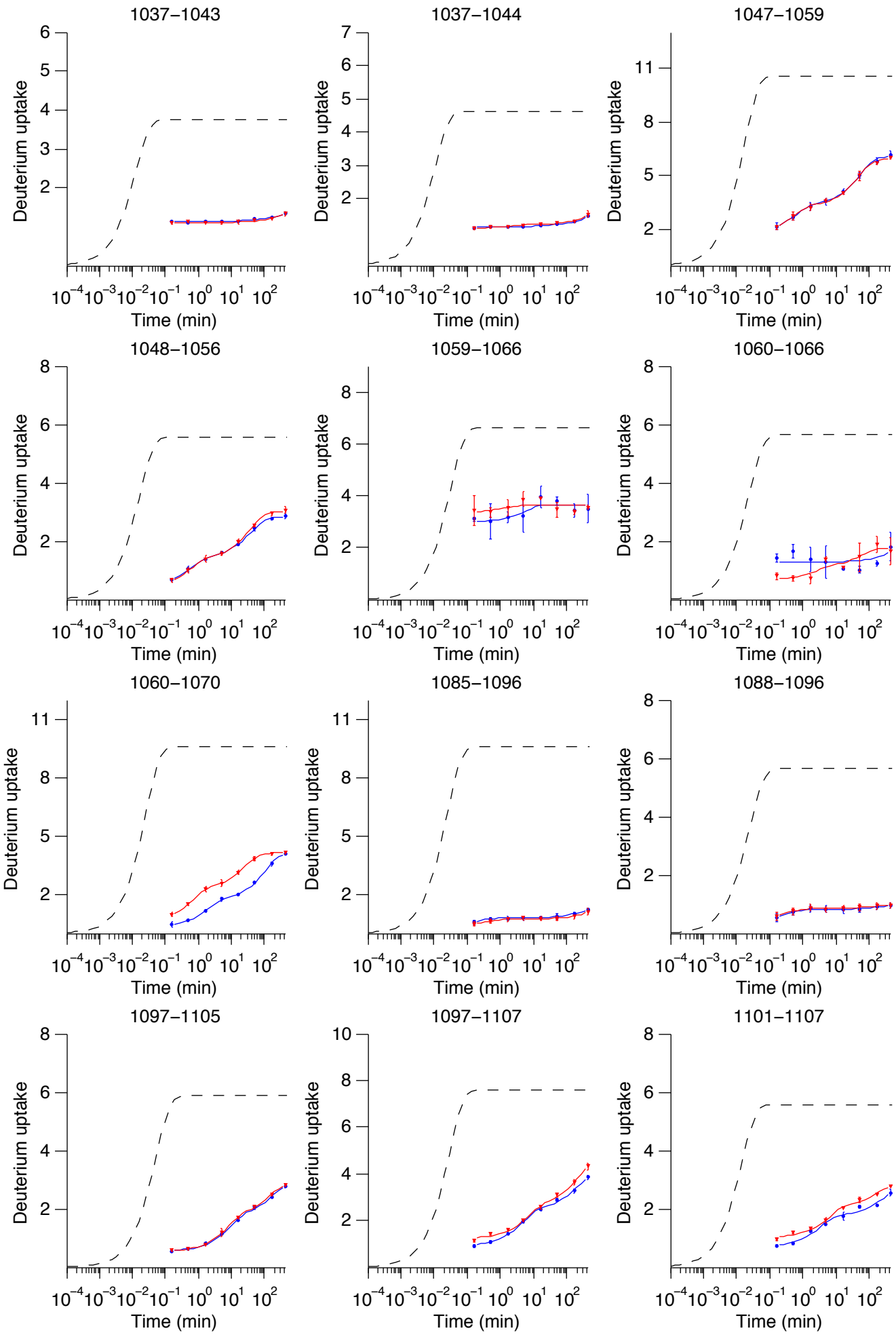
Deuterium uptake
Time (min)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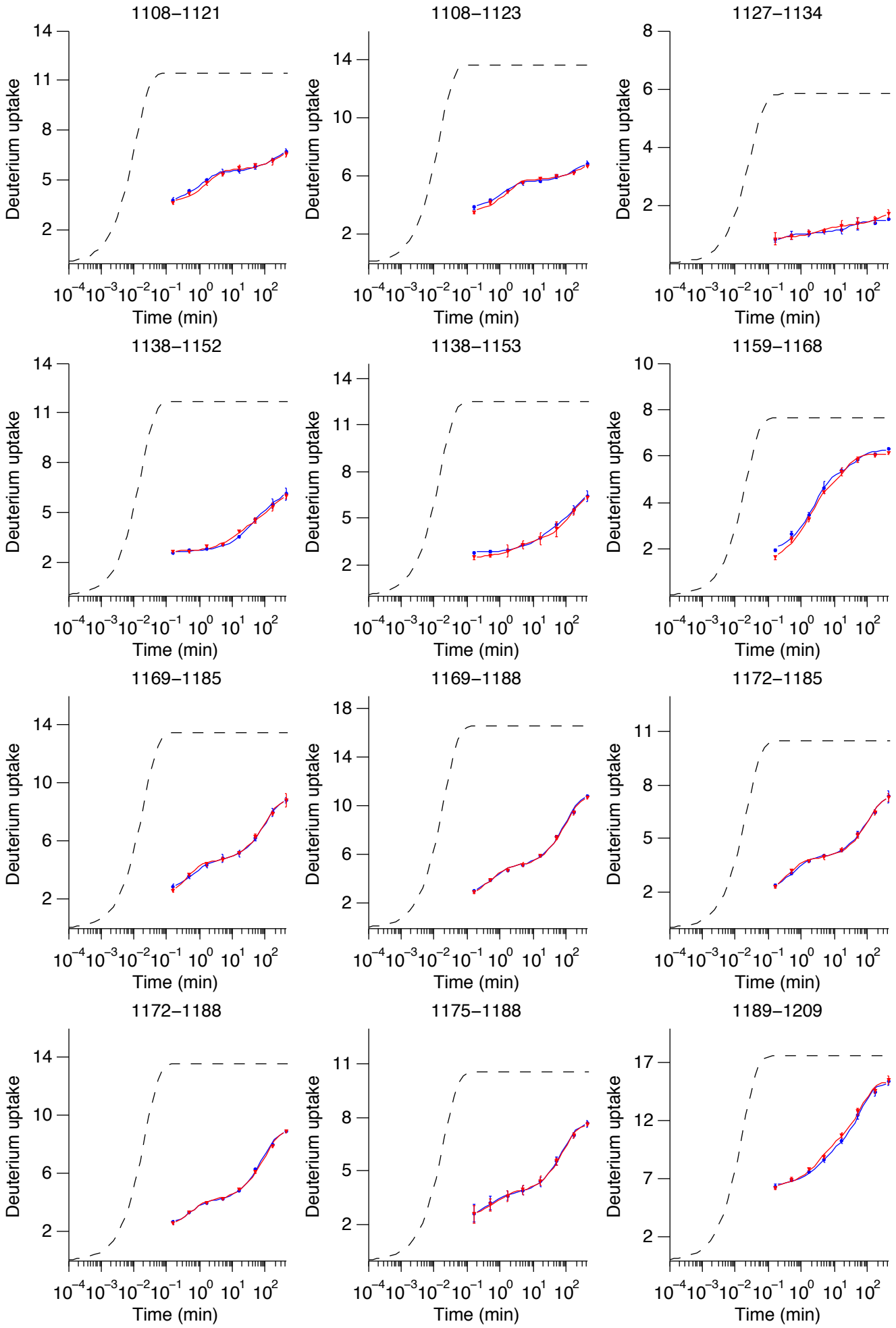
1101–1107
Deuterium uptake
Time (min)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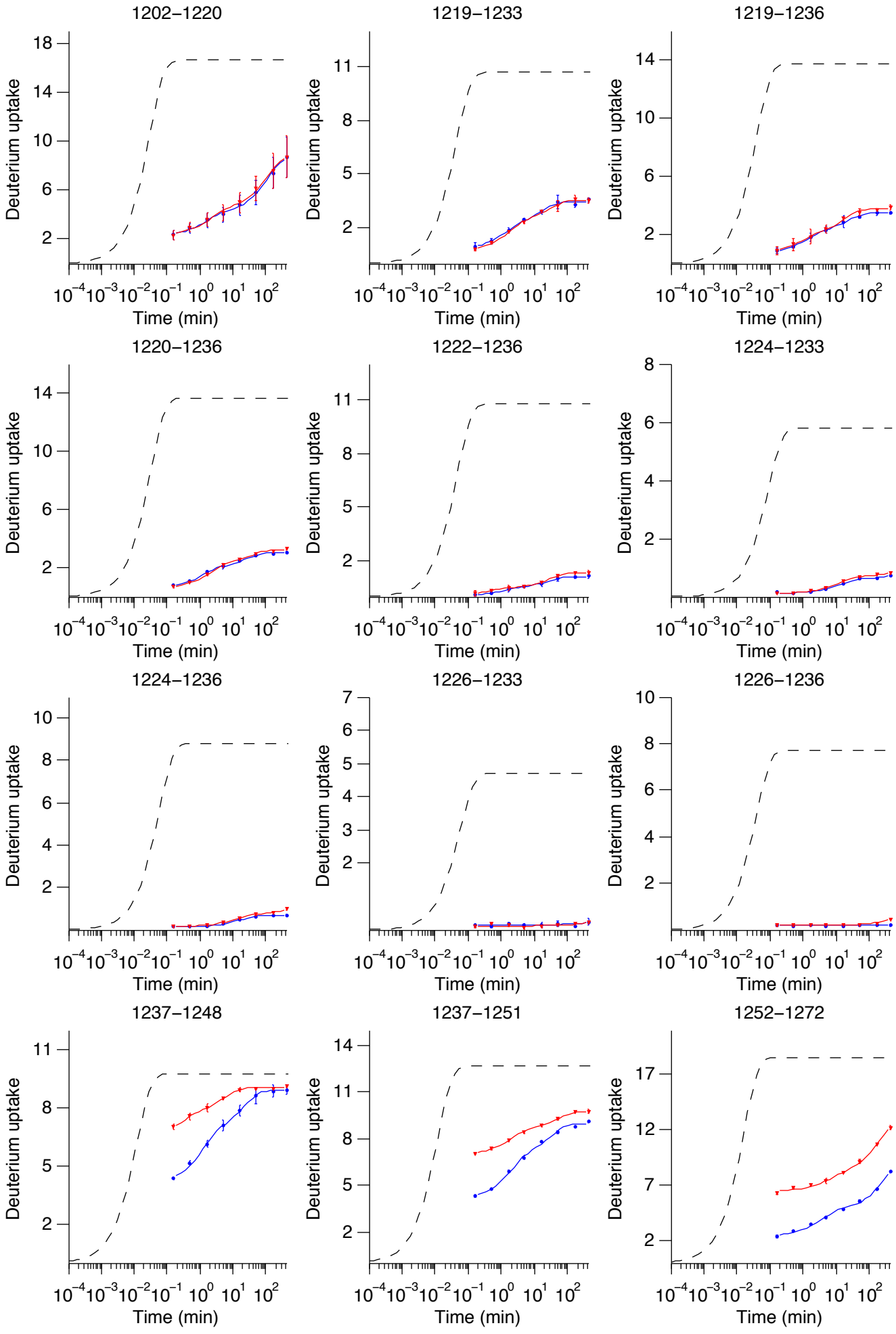
1175–1188
1189–1209
Deuterium uptake
Time (min)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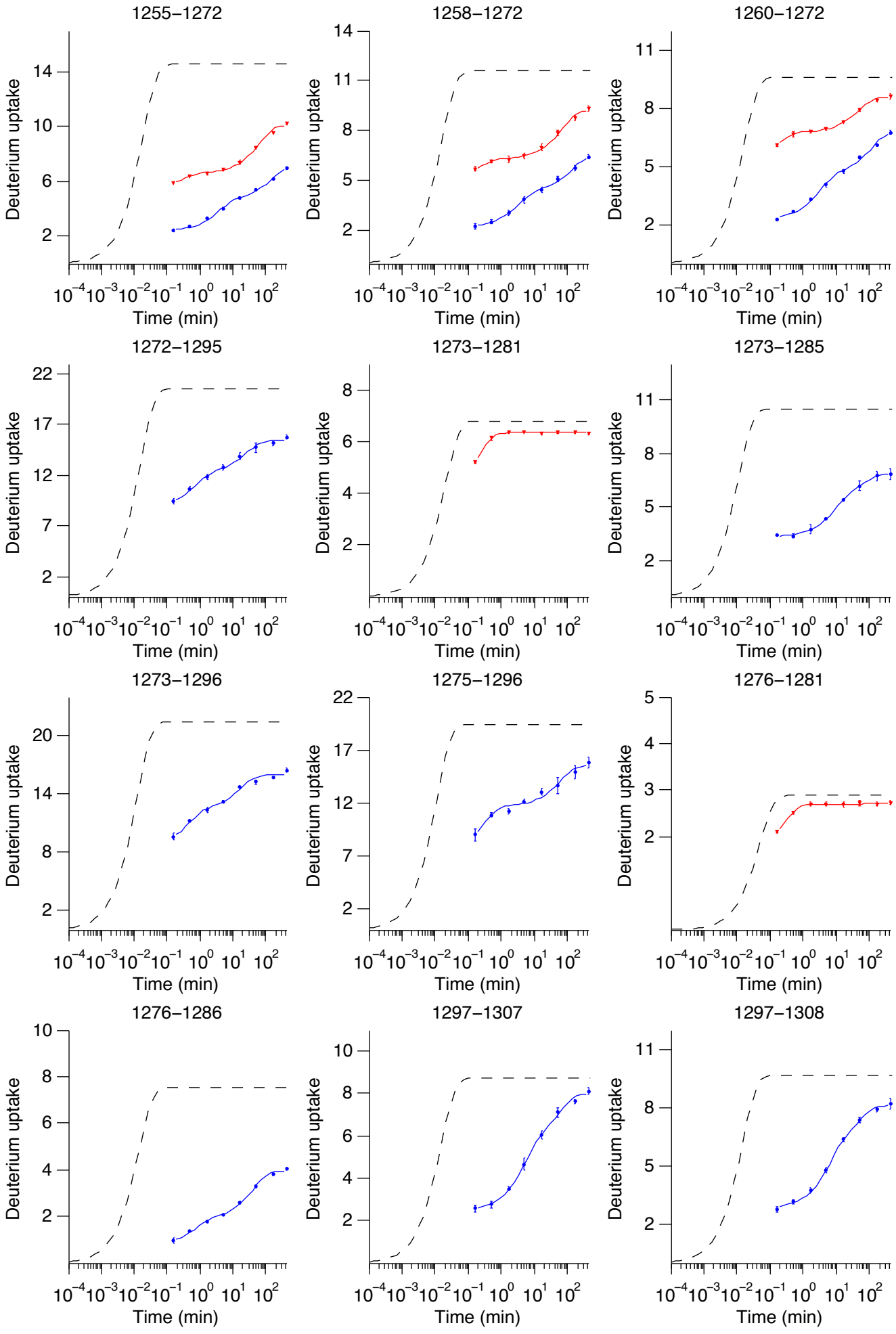
Deuterium uptake
Time (min)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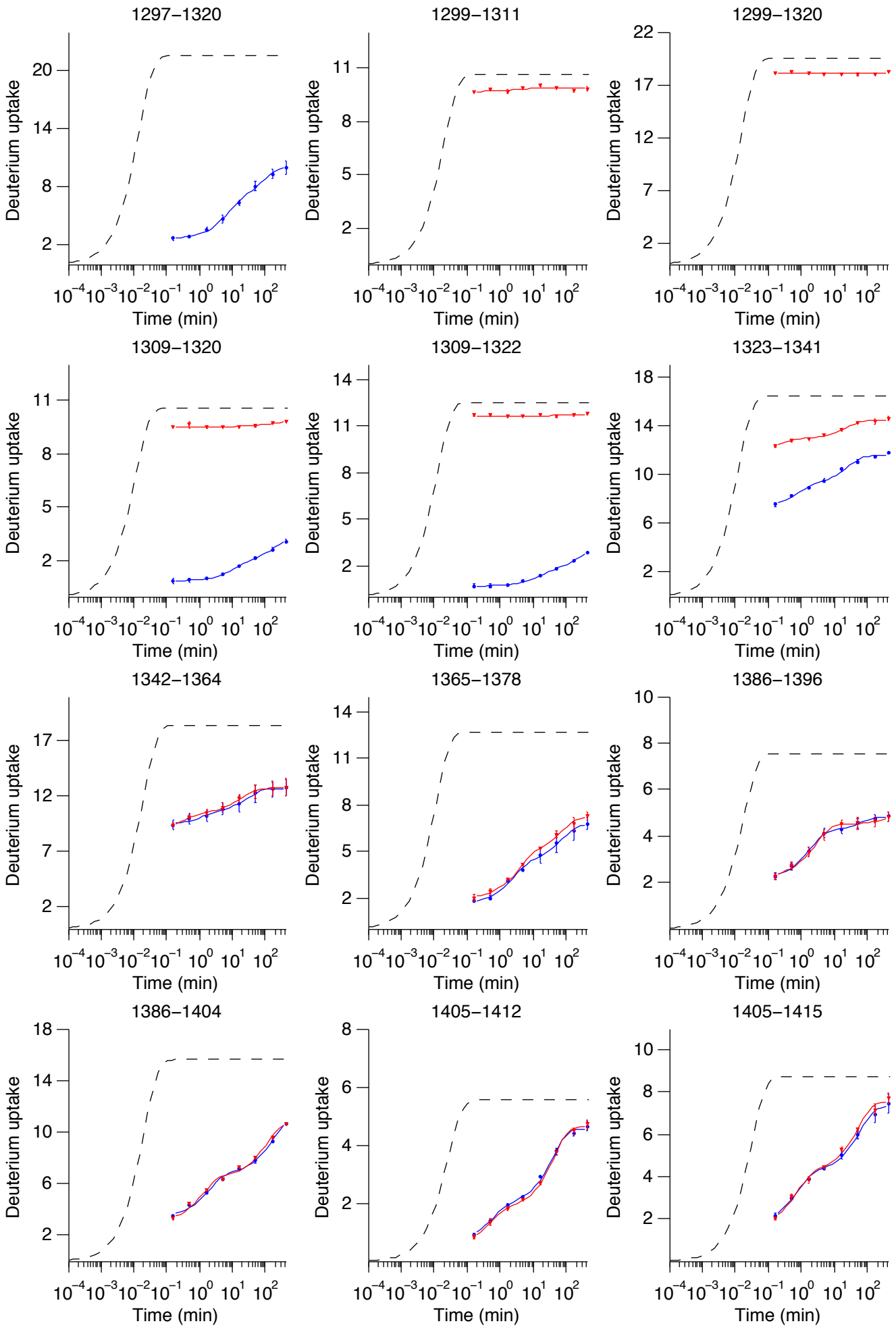
Deuterium uptake
Time (min)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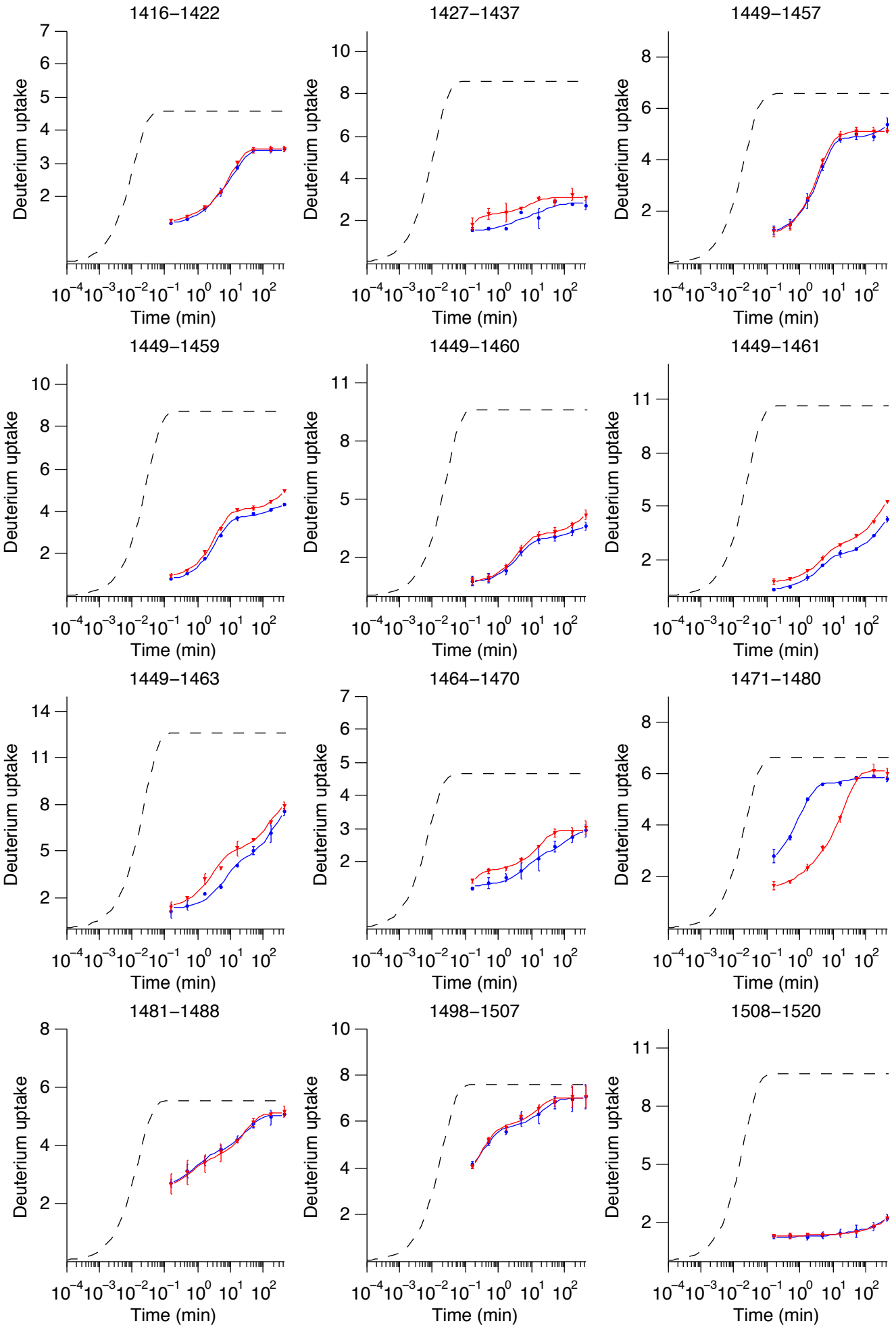
Deuterium uptake
Time (min)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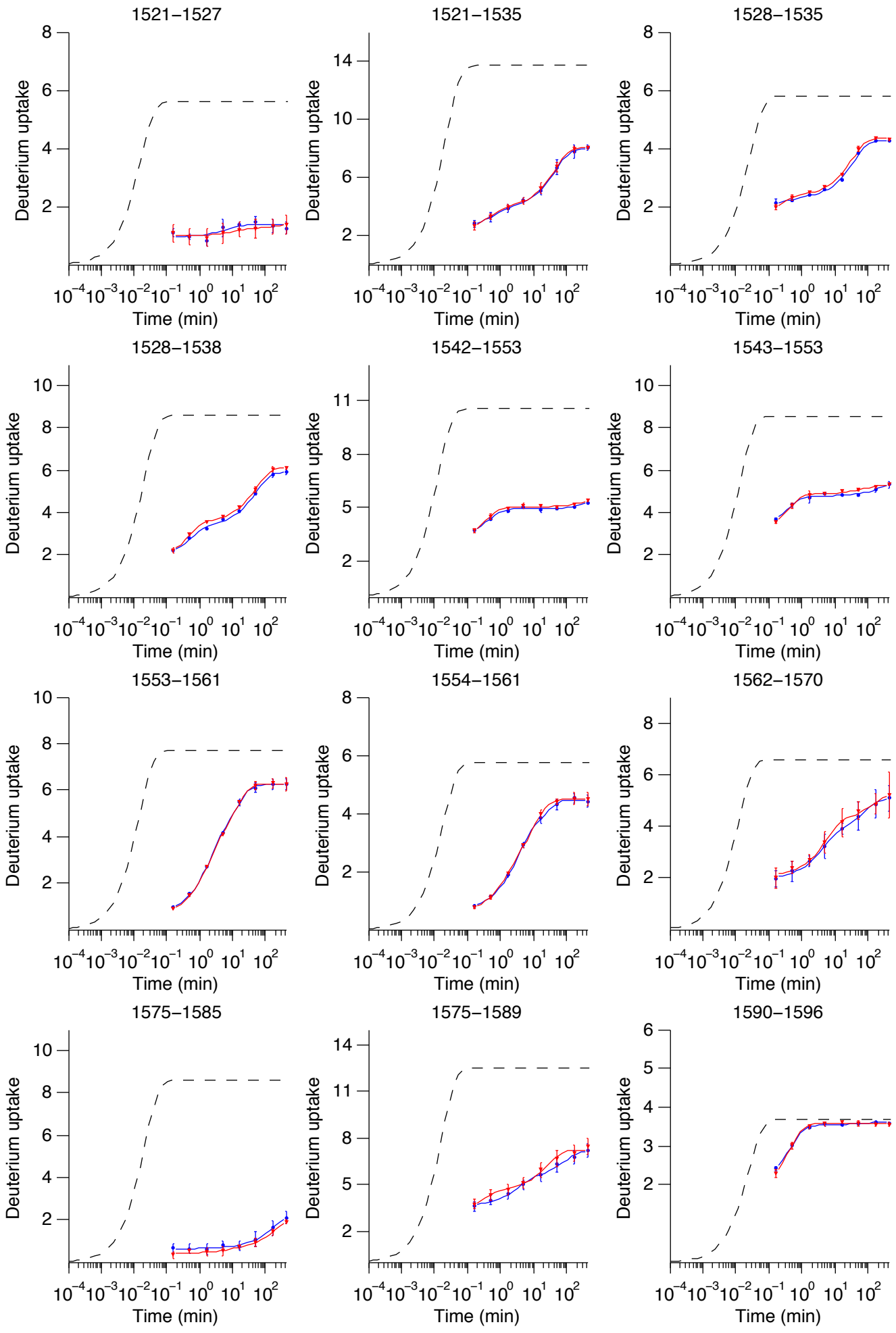
1508–1520
Deuterium uptake
Time (min)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1543–1553
1553–1561
1554–1561
1562–1570
1575–1585
1575–1589
1590–1596
Deuterium uptake
Time (min)

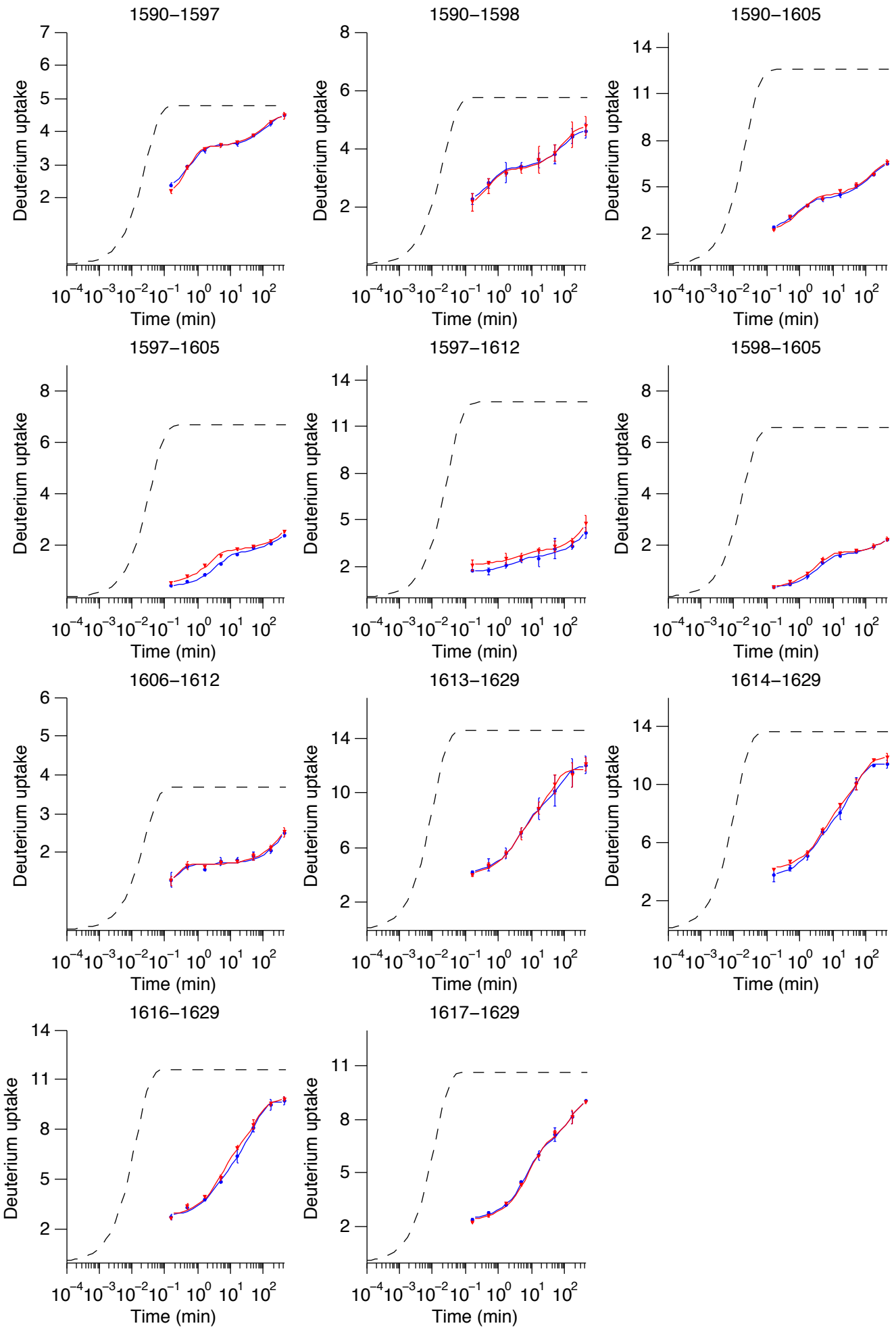
1590–1597
1590–1598
1590–1605
1597–1605
1597–1612
1598–1605
1606–1612
1613–1629
1614–1629
1616–1629
1617–1629
Deuterium uptake
Time (min)

Fig. S1: D-uptake plots of C3b and iC3b peptides.

HX plots of C3b (blue curve) and iC3b (red curve) peptides generated by pepsin digestion, compared to respective reference curves (dashed lines) calculated for the case of no protection (2). Deuterium incorporation was measured in duplicate at 0.16, 0.5, 1.67, 5, 16.67, 50, 166.67 and 420 min; error bars are indicated. Peptide residue numbers are shown at the top of each plot.

Figure S2

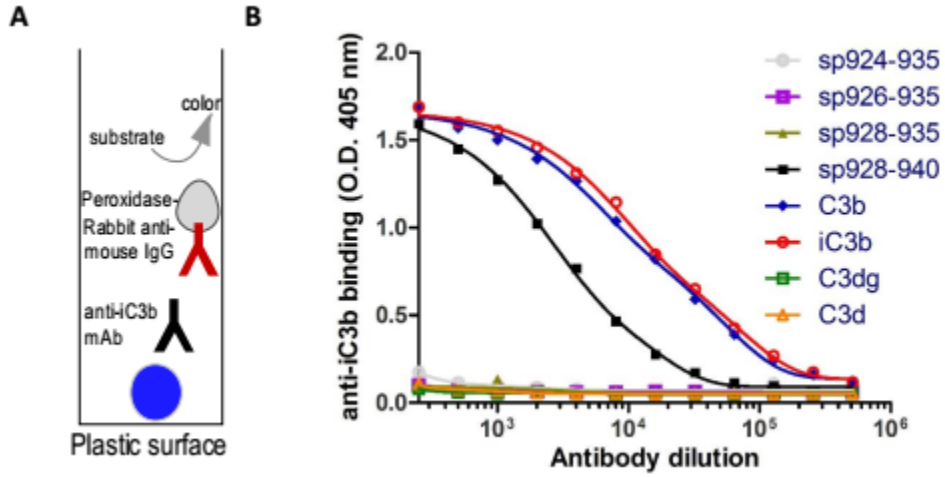

Fig. S2: Anti-iC3b mAb reactivity using direct ELISA.

(A) A direct ELISA was developed for detecting the binding activity of each synthetic peptide or C3 fragment to the anti-iC3b mAb. **(B)** A strong interaction between C3b/iC3b and the anti-iC3b mAb is observed, whereas the antibody does not react with C3dg/C3d; the affinity of the anti-iC3b mAb for C3b is likely caused by partial denaturation of C3b when directly coated on plastic surfaces (3), corroborated further by western blotting performed under denaturing conditions (Fig. 4C). Amongst the synthetic peptides tested, only sp928-940 is shown to bind to the anti-iC3b mAb indicating that this region contains the epitope.

Figure S3

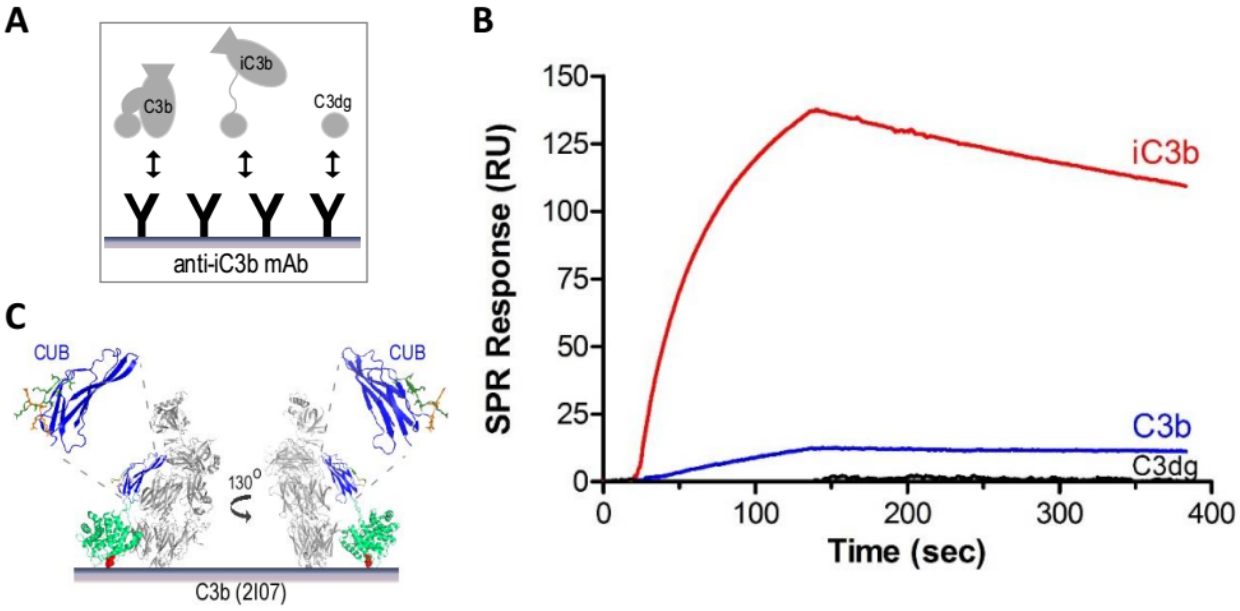

Fig. S3: Binding profile of different C3 fragments to the captured anti-iC3b mAb.

(**A, B)** Anti-iC3b mAb was captured on a SPR sensor chip via its Fc segment and C3 fragments (C3b, iC3b, C3dg) were injected at a single concentration (200 nM). The SPR signals were normalized by the molecular weight of each fragment. iC3b showed a >10-fold higher affinity for anti-iC3b mAb than did C3b, and no binding was observed for C3dg at this concentration. **(C)** The observed residual activity of the anti-iC3b mAb for C3b may be attributed to at least partial accessibility of the epitope in fluid-phase C3b.