SUPPLEMENTAL INFORMATION

Charge-mediated Fab-Fc interactions in an IgG1 mAb induce reversible self-association, antibody cluster formation, and elevated viscosity

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SUPPLEMENTAL RESULTS

Post-lyophilization biophysical characterization suggests no change in mAb-J structure and aggregation profile

Lyophilization can introduce unwanted stress and cause structural destabilization of proteins leading to protein aggregation. [1, 2] A combination of circular dichroism (CD), size exclusion chromatography (SEC), and viscosity measurements was used to ensure preservation of mAb-J structural integrity post lyophilization. Comparison of CD spectra of non-lyophilized mAb-J control at 5 and 60 g/L (diluted to 0.3 g/L for CD) and reconstituted mAb-J samples revealed no changes in the secondary structure caused by the lyophilization/reconstitution process (see Figure S1A). All samples of lyophilized mAb-J were diluted to 0.5 g/L for SEC. Figure S1B compares SEC profiles of non-lyophilized mAb-J control and lyophilized mAb-J samples. No increase in soluble aggregate or fragment population was observed in lyophilized samples after reconstitution, although a slight decrease in the monomer content was observed. Monomer loss is attributed to a small increase in insoluble aggregate content (Table S3). Since all samples were centrifuged prior to analysis, the insoluble aggregate did not alter the HX measurements. To make sure that mAb-J samples undergo reversible self-association after lyophilization/reconstitution process, the viscosity of reconstituted mAb-J samples at 5 and 60 g/L was compared to non-lyophilized control. There were no differences in viscosity between the two set of samples (Figure S1C). Post-lyophilization biophysical characterization confirmed that our optimized lyophilization cycle does not introduce unwanted structural artifacts in mAb-J samples and it can therefore be utilized for HX-MS experiments.

REFERENCES

1. Carpenter J, Prestrelski S, Arakawa T. Separation of freezing-and drying-induced denaturation of lyophilized proteins using stress-specific stabilization: I. Enzyme activity and calorimetric studies. Arch Biochem Biophys 1993; 303:456-64.

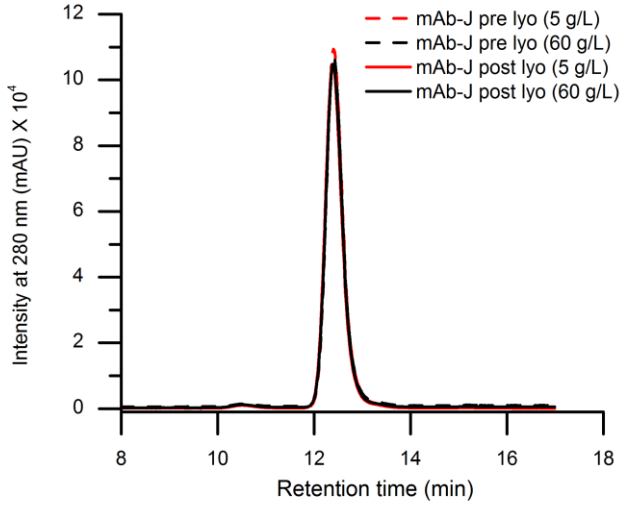
2. Carpenter JF, Chang BS, Garzon-Rodriguez W, Randolph TW. Rational design of stable lyophilized protein formulations: theory and practice. Springer, 2002.

3. Kavan D, Man P. MSTools—Web based application for visualization and presentation of HXMS data. Int J Mass Spectrom 2011; 302:53-8.

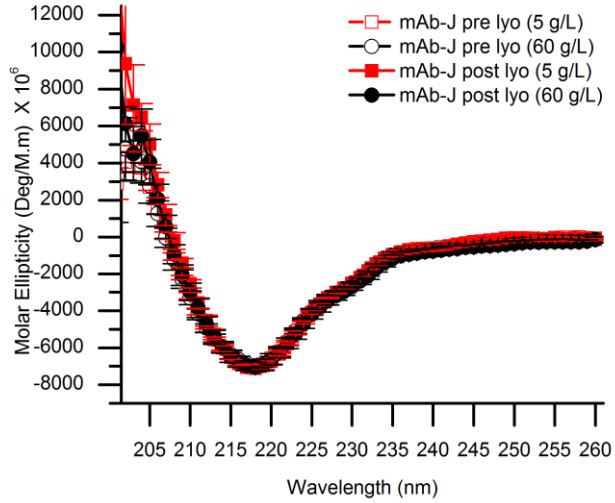
SUPPLEMENTAL FIGURE CAPTIONS

Figure S1: Post-lyophilization biophysical characterization of mAb-J. (A) Circular dichroism (CD) spectra showing the effect of lyophilization process on secondary structure of mAb-J. (B) Size exclusion chromatography (SEC) chromatograms comparing pre and post lyophilization mAb-J samples. (C) Solution dynamic viscosity measurements comparing pre- and post-lyophilization mAb-J samples to test the effect of lyophilization on mAb-J protein-protein interactions. Samples of mAb-J were prepared in control buffer containing 10% (w/v) trehalose. Error bars in panel B and C represent one standard deviation from three independent measurements.

A


mAb-J pre lyo (5 g/L)
mAb-J pre lyo (60 g/L)
mAb-J post lyo (5 g/L)
mAb-J post lyo (60 g/L)
Intensity at 280 nm (mAU) X 10^4
Retention time (min)

B


mAb-J pre lyo (5 g/L)
mAb-J pre lyo (60 g/L)
mAb-J post lyo (5 g/L)
mAb-J post lyo (60 g/L)
Molar Ellipticity (Deg/M.m) X 10^6
Wavelength (nm)

C

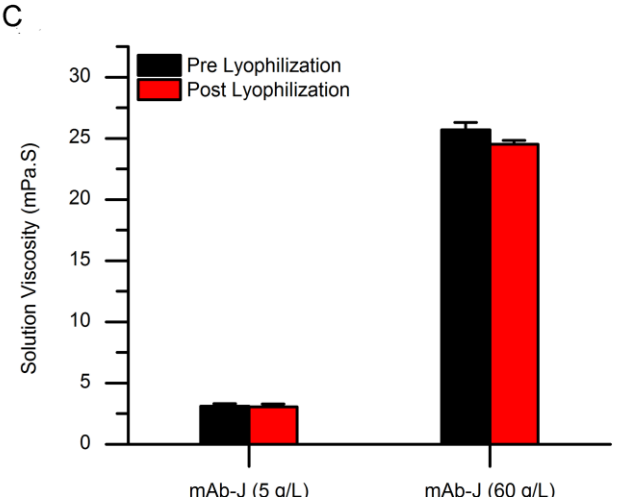
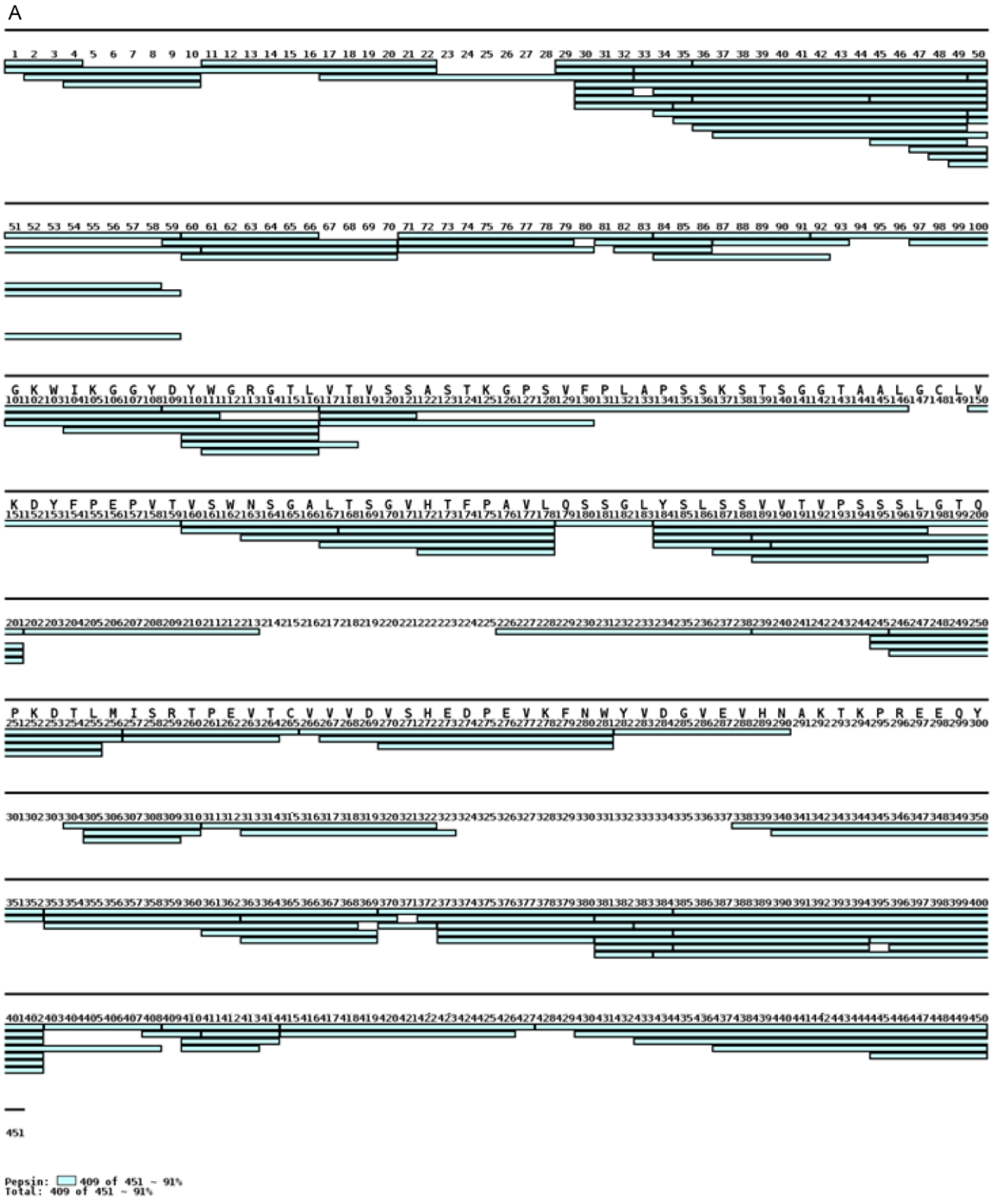

Pre Lyophilization
Post Lyophilization
Solution Viscosity (mPa.S)
mAb-J (5 g/L)
mAb-J (60 g/L)

Figure S2: Peptic peptide coverage map of mAb-J (A) heavy chain and (B) light chain. 182 peptide segments of mAb-J covered 91% of heavy chain and 93% of light chain primary sequence. [3]



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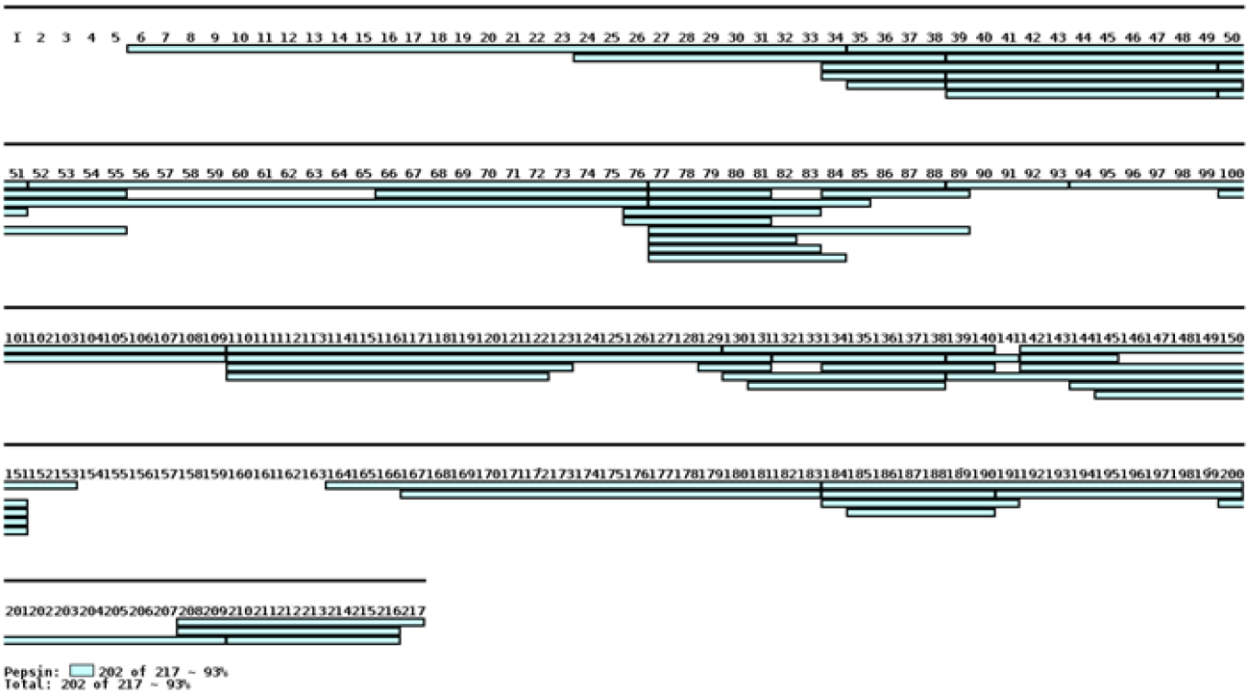
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101102103104105106107108109110111112113114115116117118119120121122123124125126127128129130131132133134135136137138139140141142143144145146147148149150
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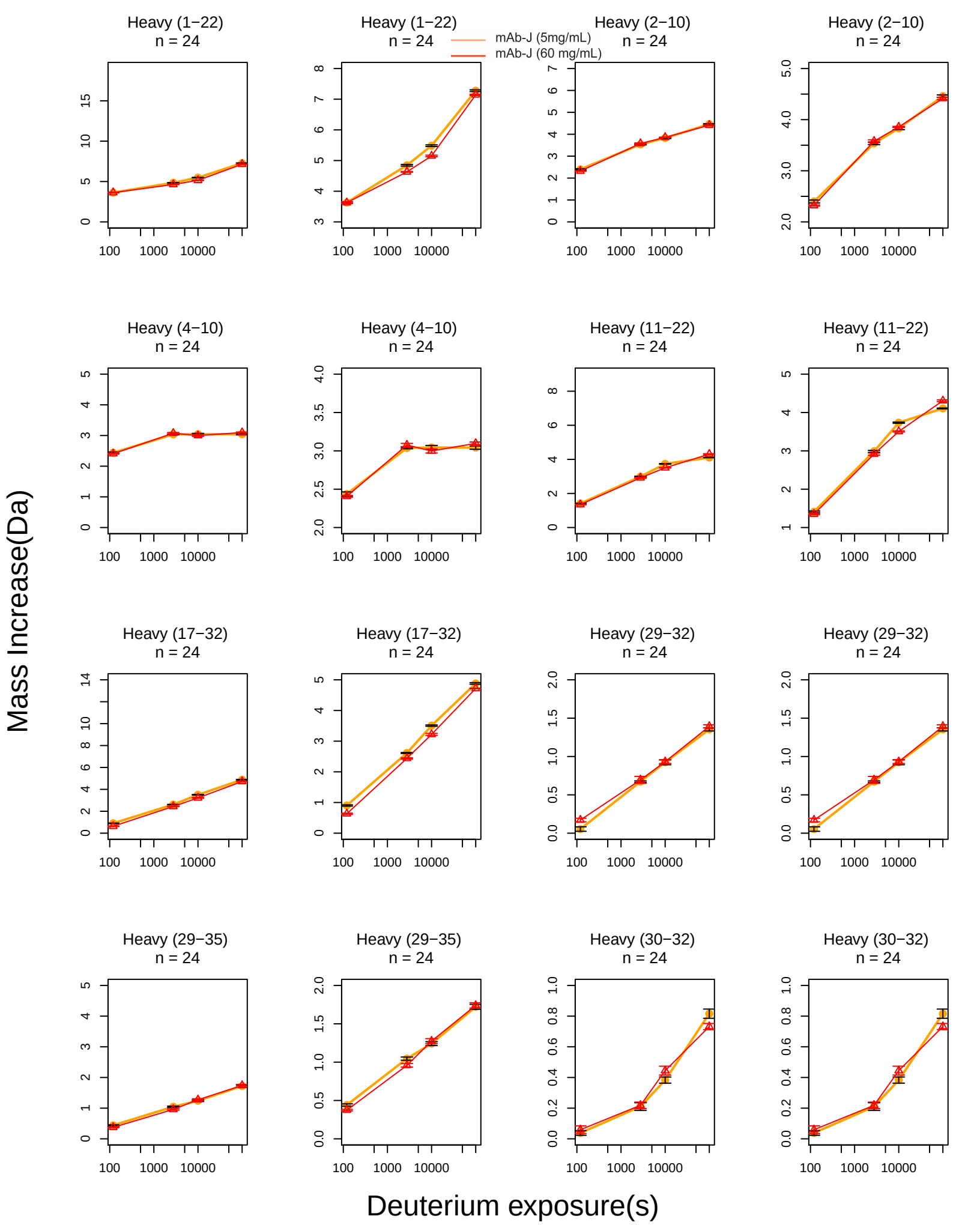
201202203204205206207208209210211212213214215216217
Pepsin: 202 of 217 ~ 93%
Total: 202 of 217 ~ 93%

Figure S3: Deuterium uptake plots for 182 peptide segments of mAb-J comparing HX kinetics between 5 and 60 g/L mAb-J samples (orange and red, respectively). Domain location of the peptide is given in parentheses. Error bars represent one standard deviation from three independent HX experiments. The number of independent measurements for each peptide is denoted by n.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Heavy (1−22)
n = 24
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mAb-J (60 mg/mL)
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Heavy (2−10)
n = 24
Heavy (4−10)
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Heavy (4−10)
n = 24
Heavy (11−22)
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Heavy (29−35)
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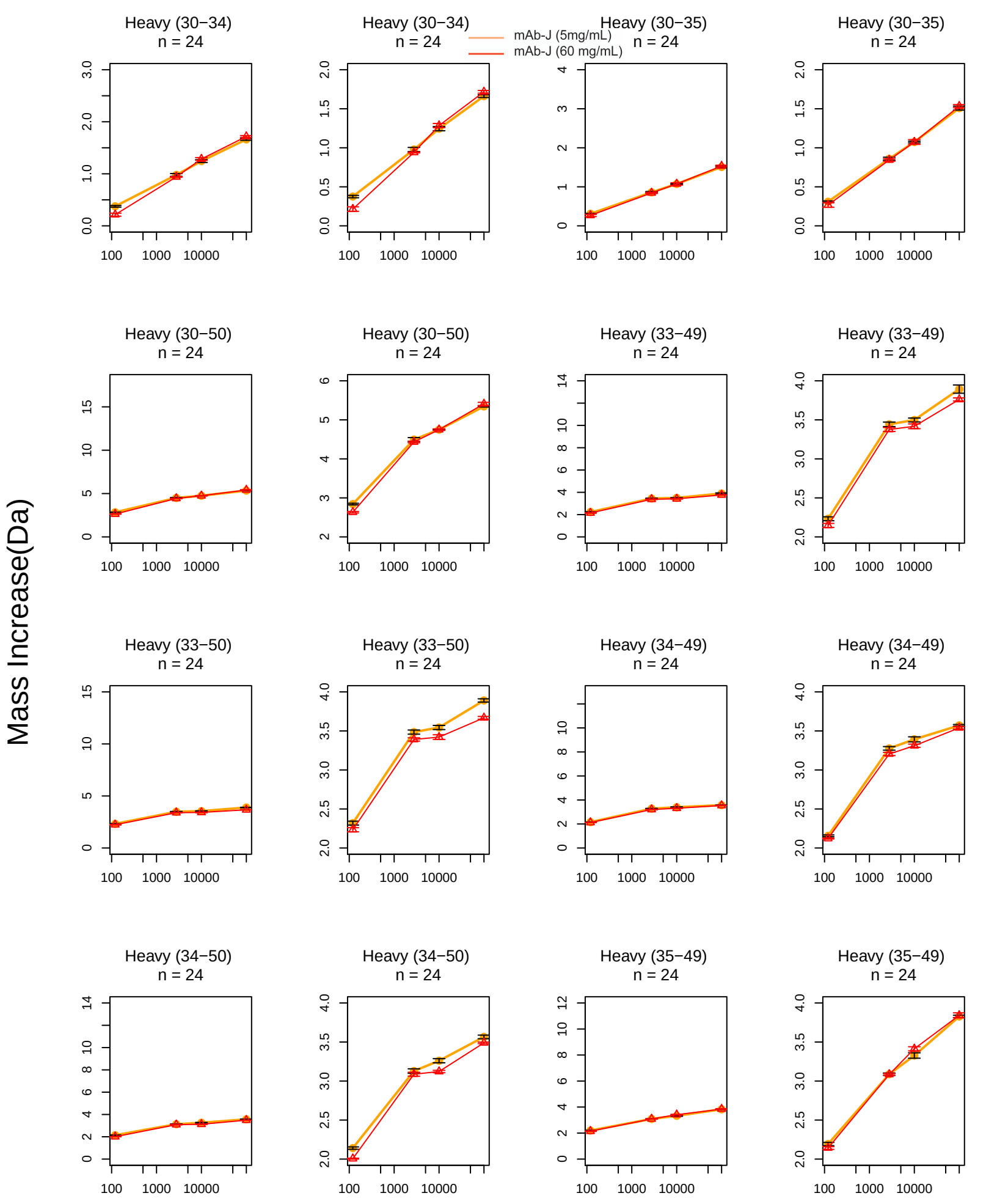
Mass Increase(Da)
Deuterium exposure(s)


mAb-J (5mg/mL)
mAb-J (60 mg/mL)
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Heavy (30−34) n = 24
Heavy (30−35) n = 24
Heavy (30−35) n = 24
Heavy (30−50) n = 24
Heavy (30−50) n = 24
Heavy (33−49) n = 24
Heavy (33−49) n = 24
Heavy (33−50) n = 24
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Heavy (34−49) n = 24
Heavy (34−50) n = 24
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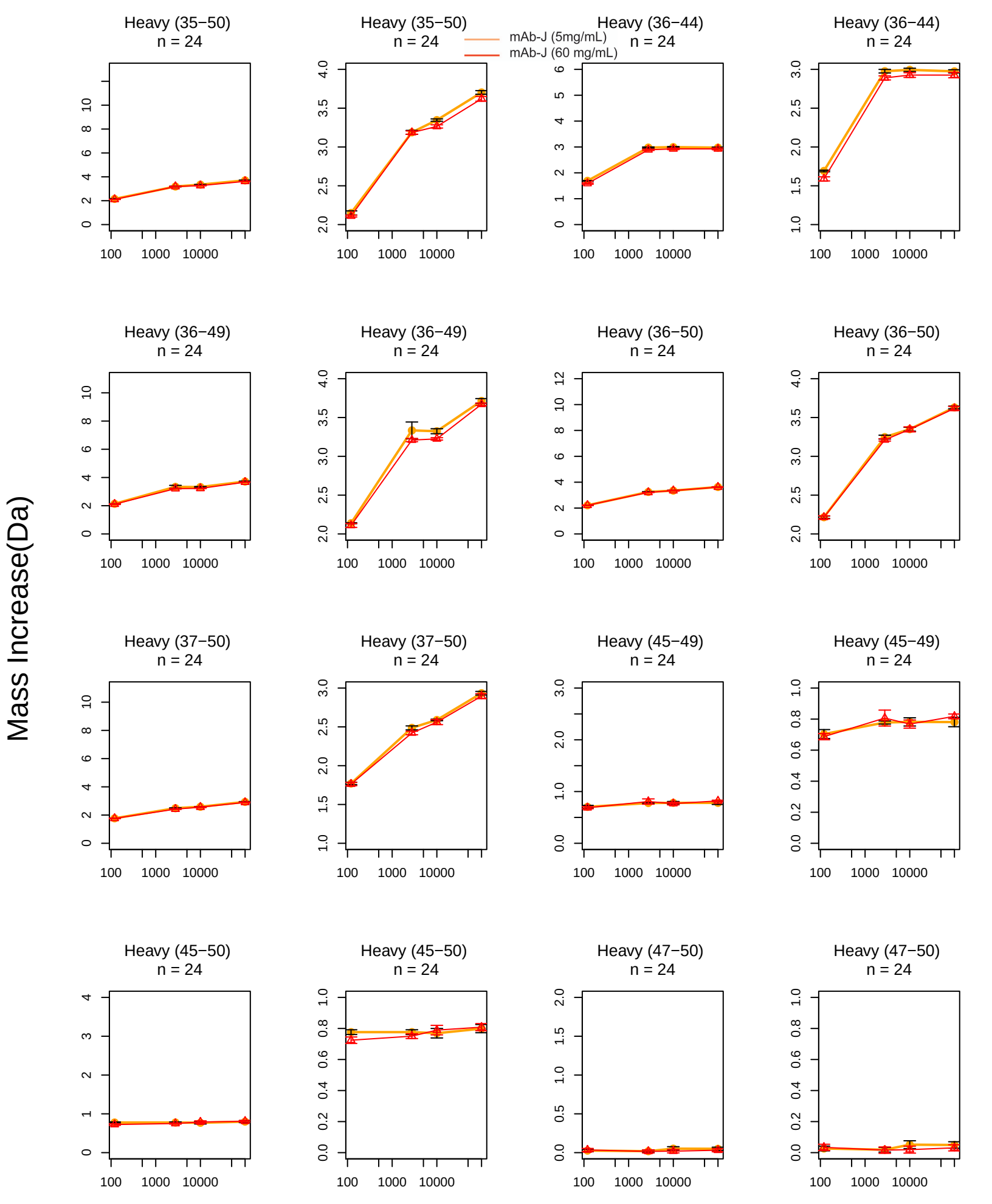
Heavy (35−49) n = 24
Heavy (35−49) n = 24
Mass Increase(Da)
Deuterium exposure(s)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mAb-J (60 mg/mL)
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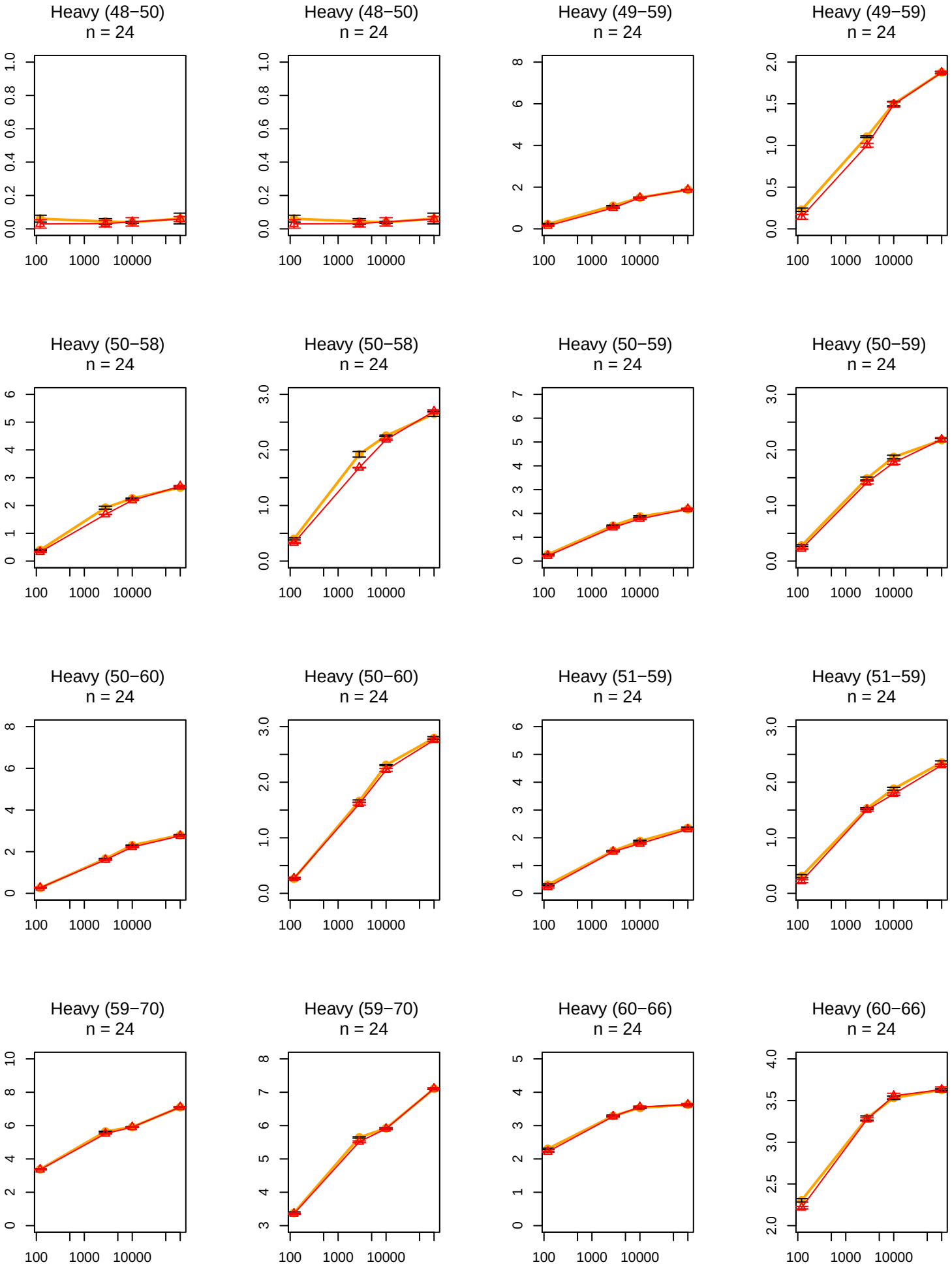
Mass Increase(Da)
Deuterium exposure(s)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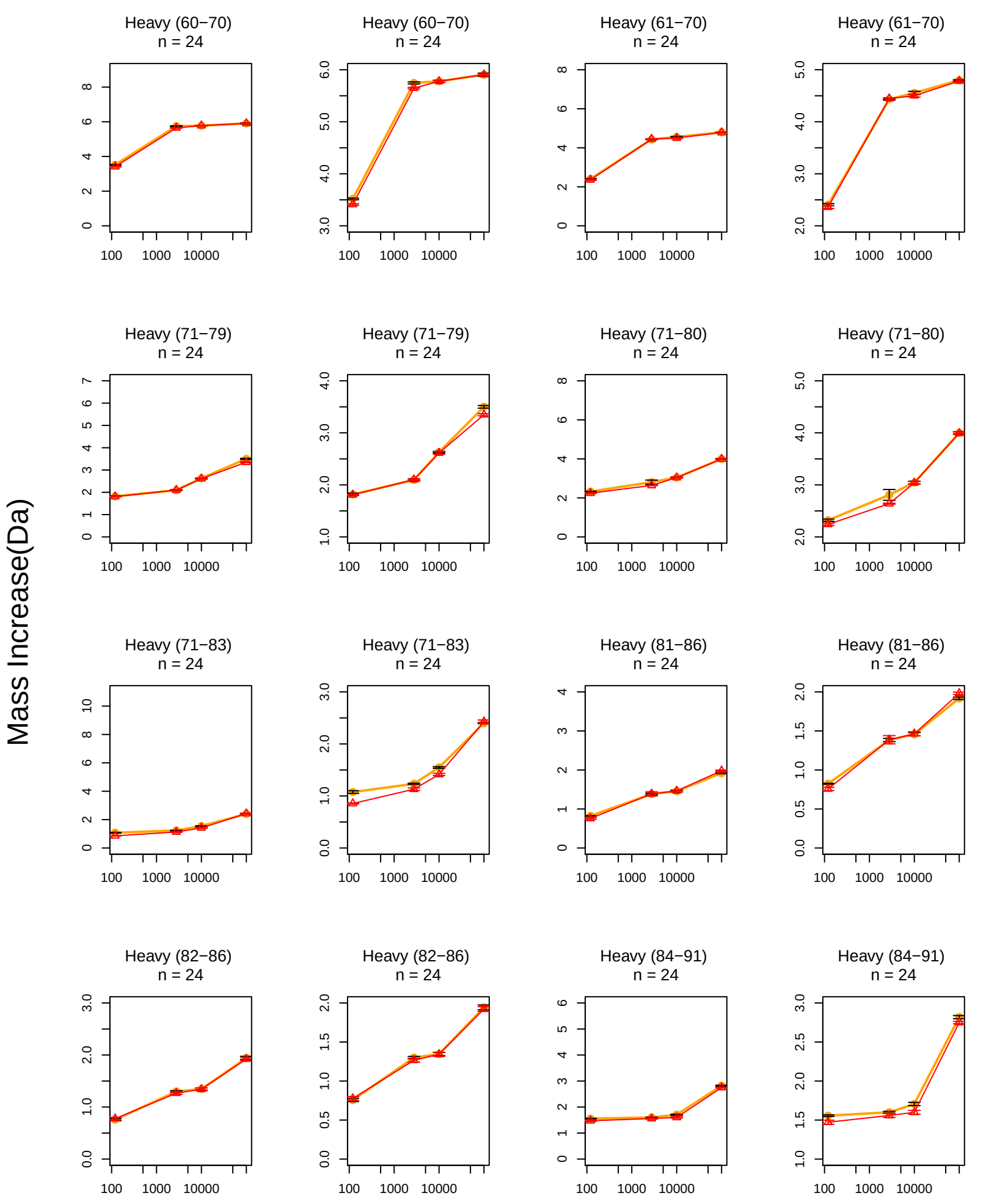
Mass Increase(Da)
Deuterium exposure(s)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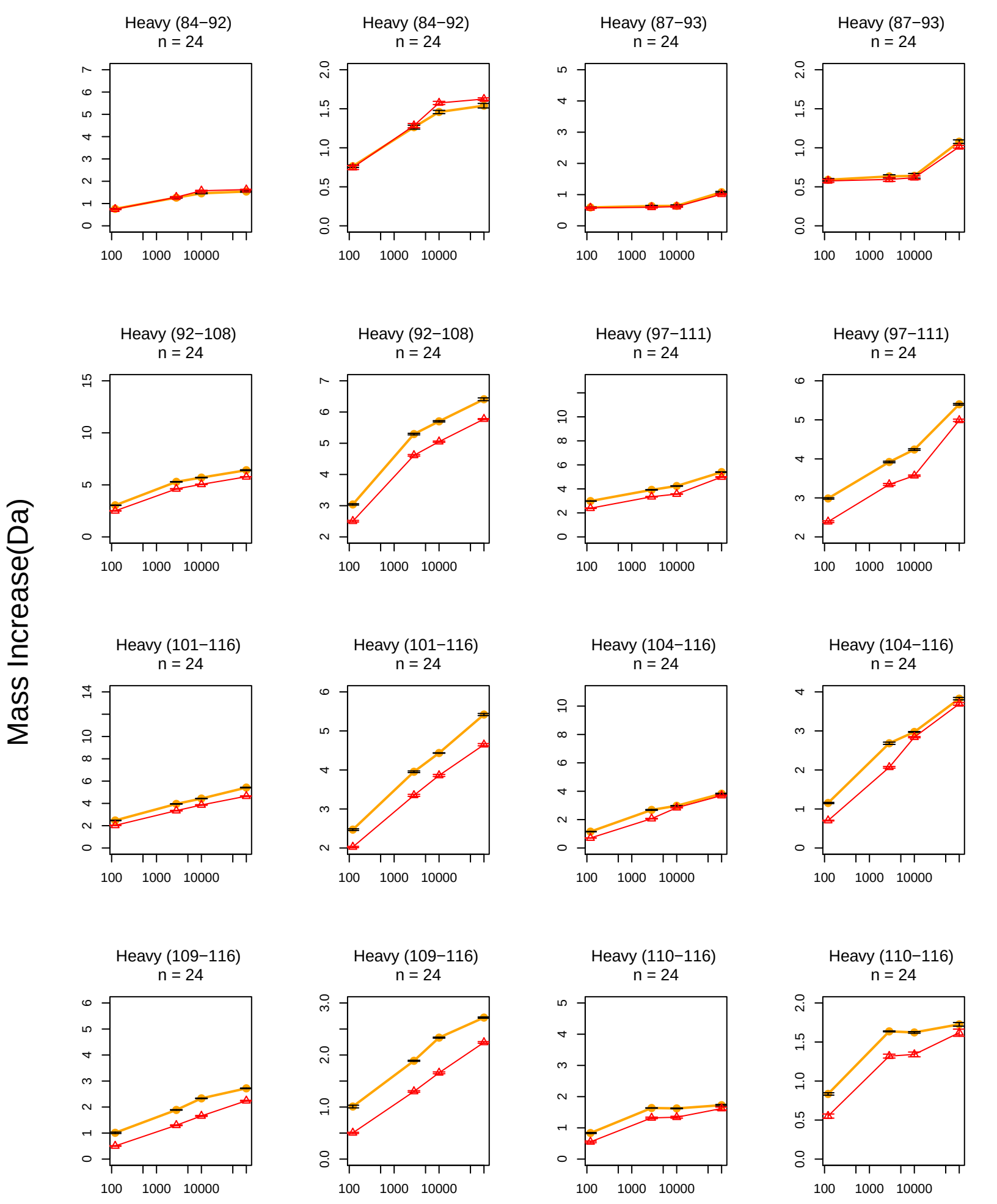
Mass Increase(Da)
Deuterium exposure(s)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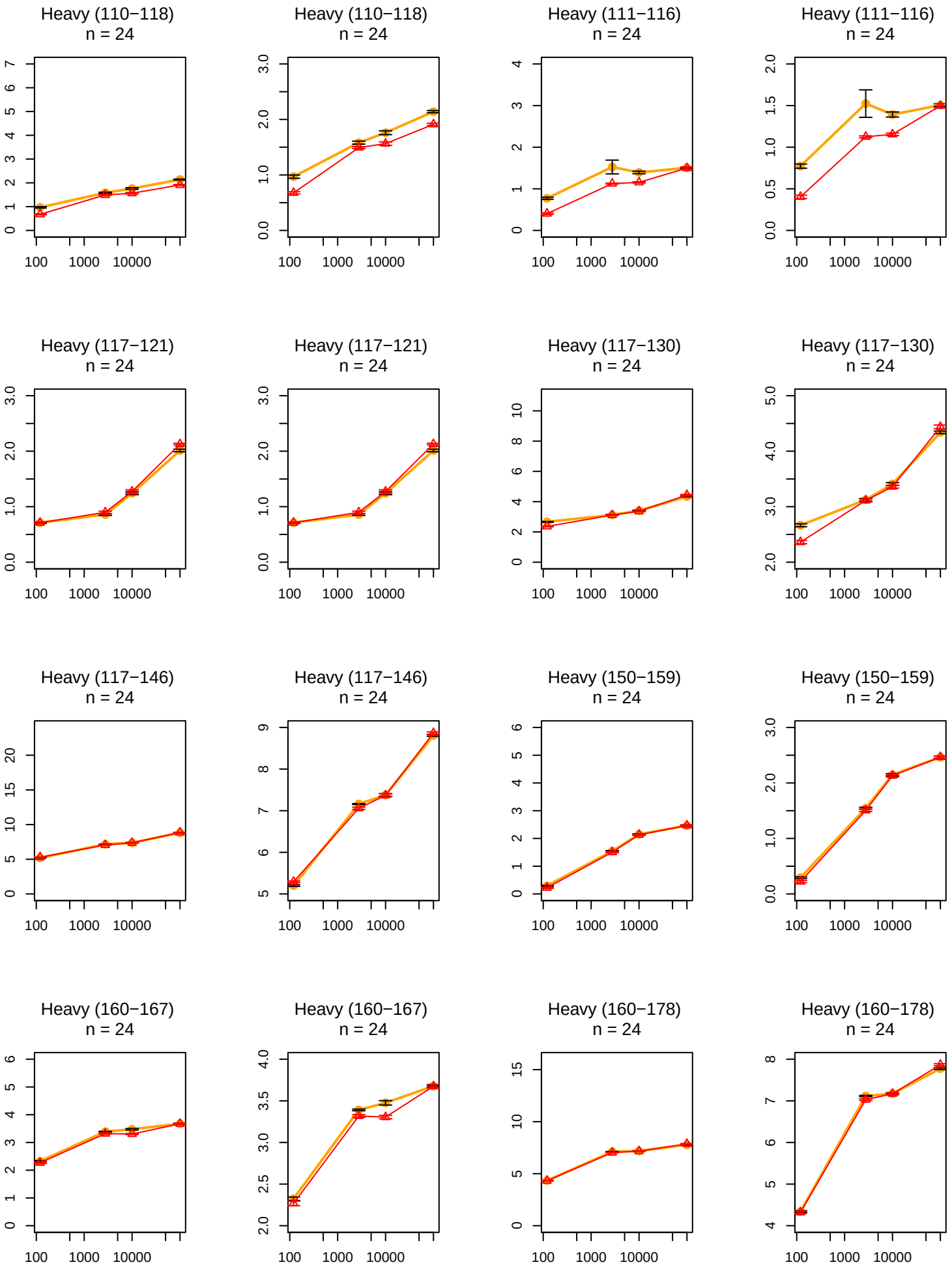
Mass Increase(Da)
Deuterium exposure(s)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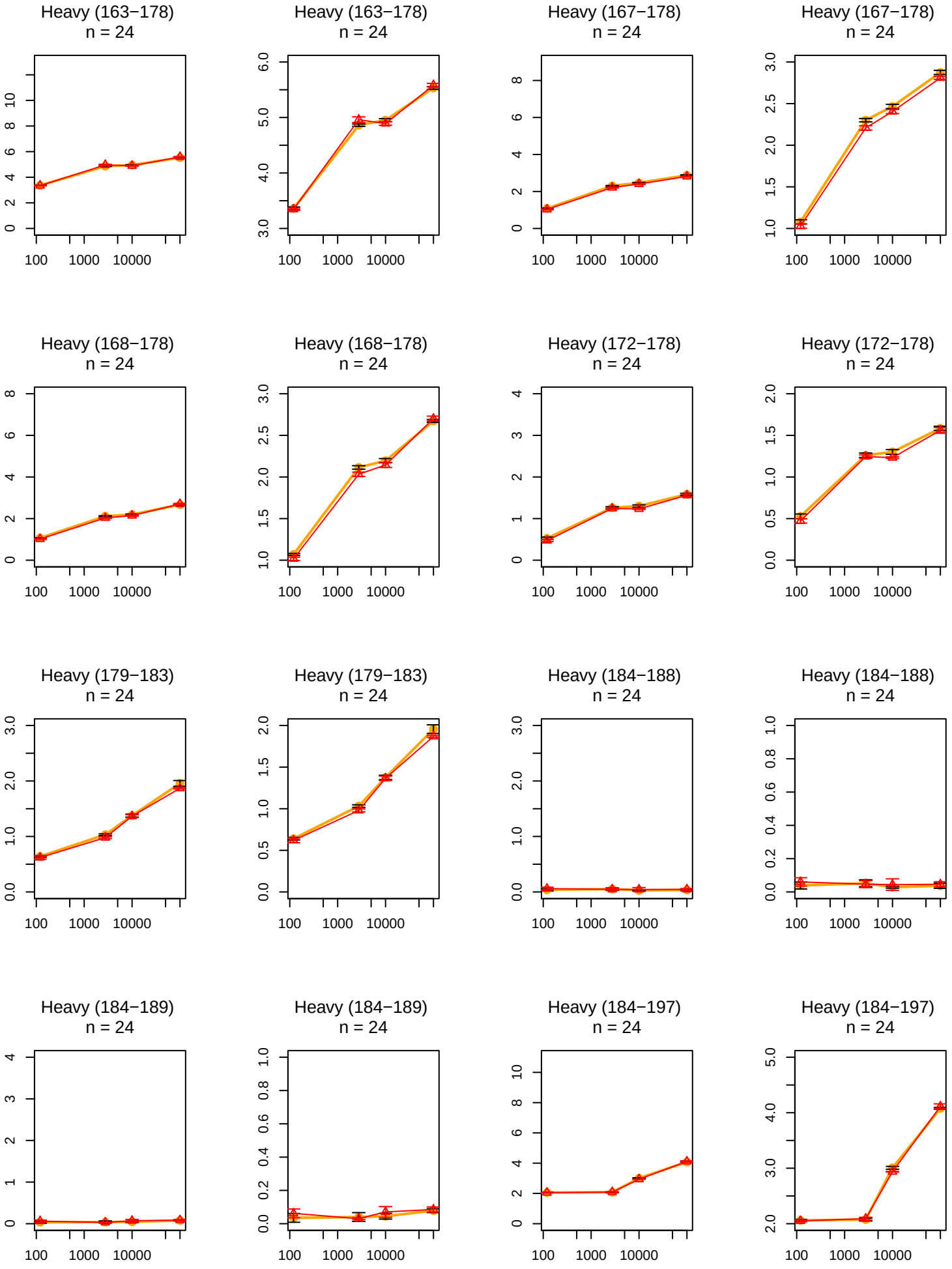
Mass Increase(Da)
Deuterium exposure(s)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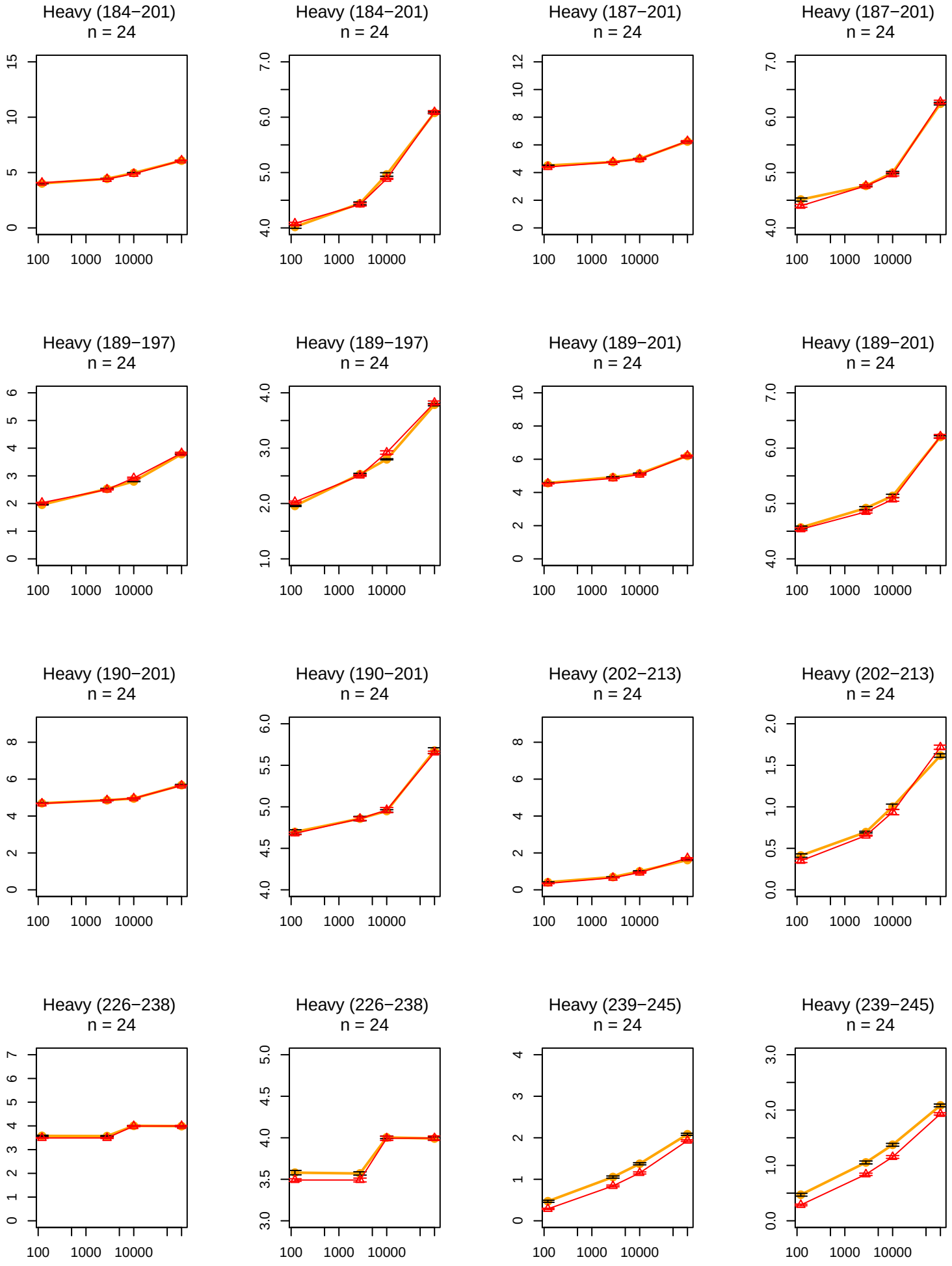
Mass Increase(Da)
Deuterium exposure(s)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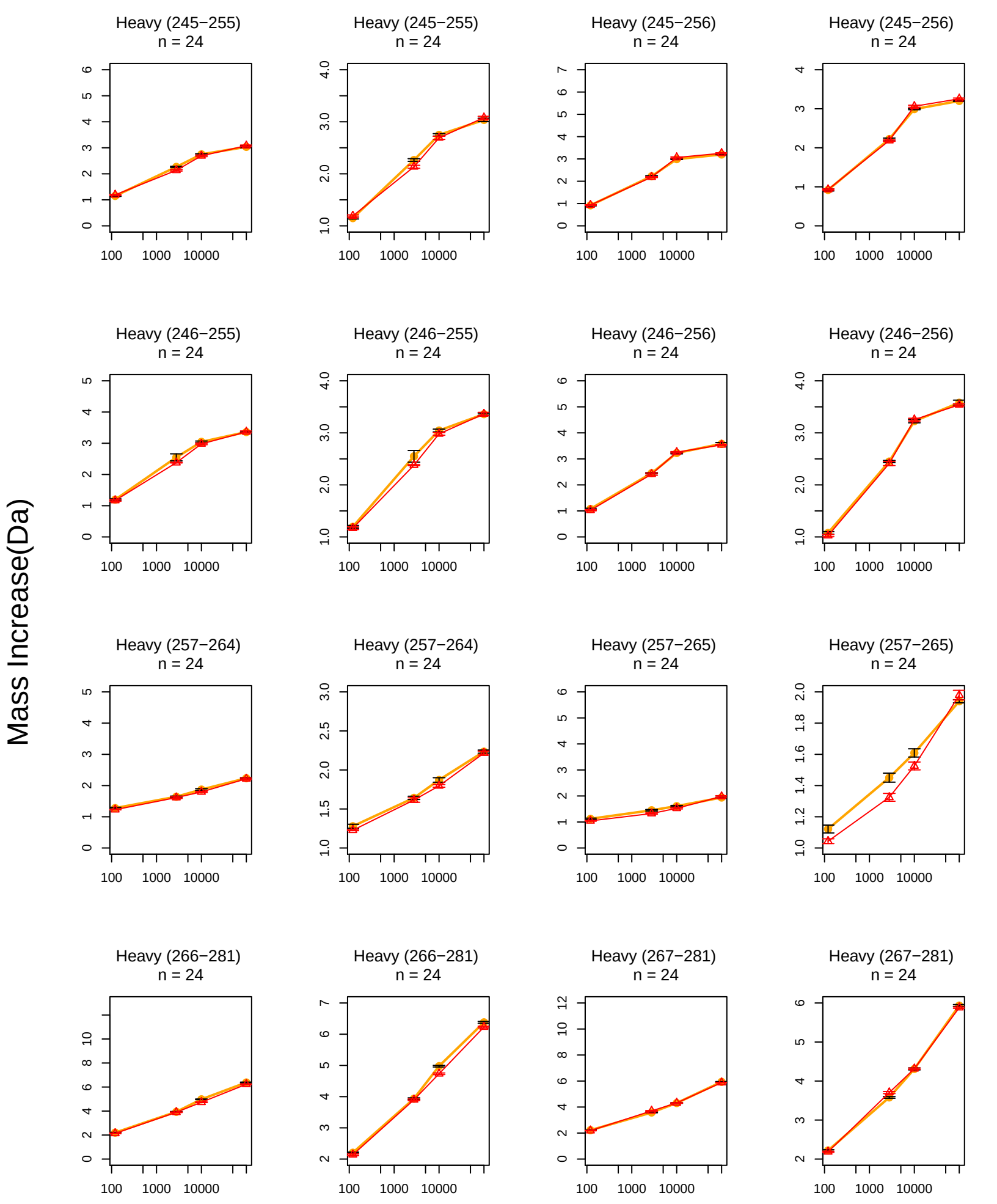
Mass Increase(Da)
Deuterium exposure(s)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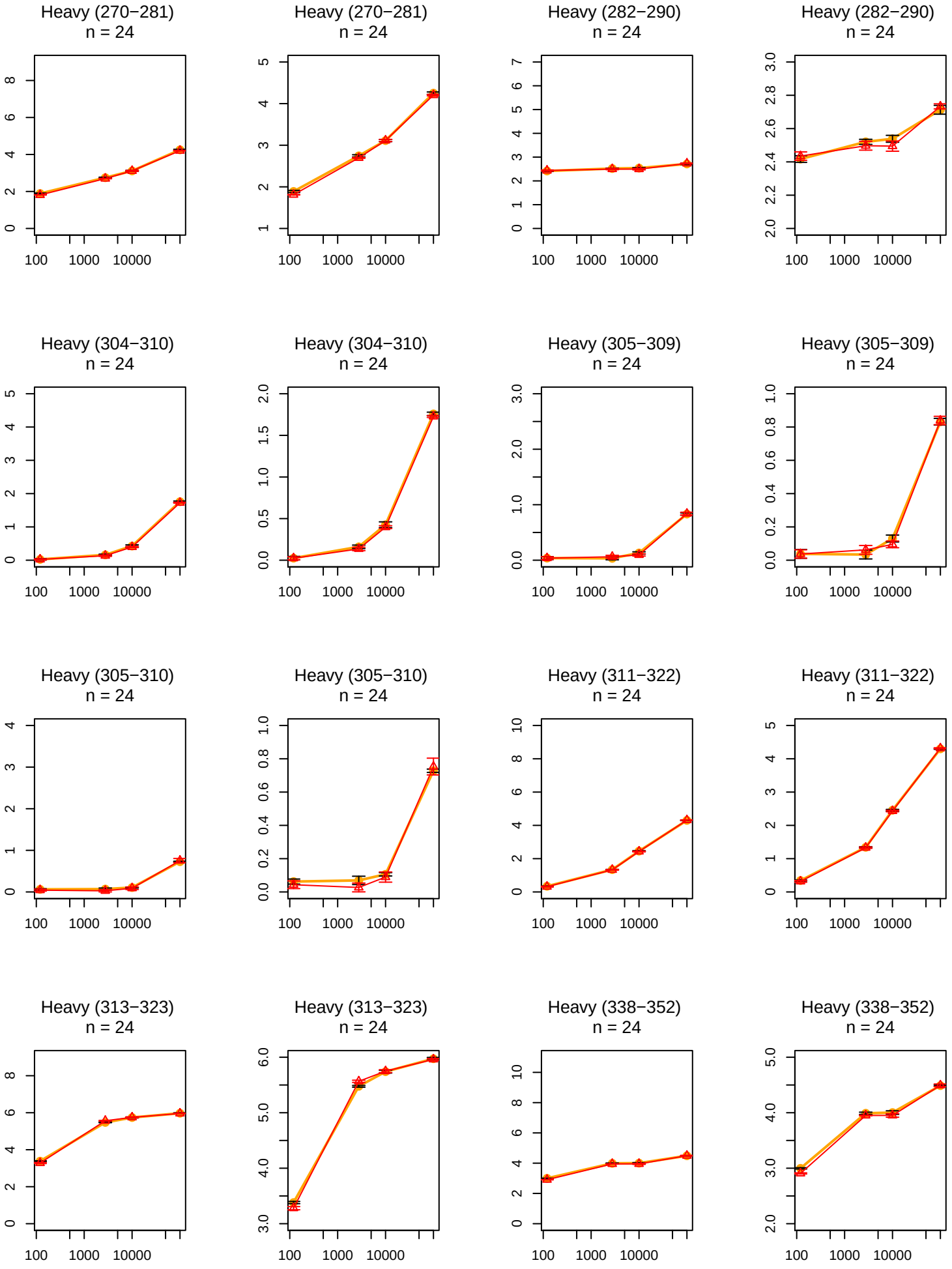
Mass Increase(Da)
Deuterium exposure(s)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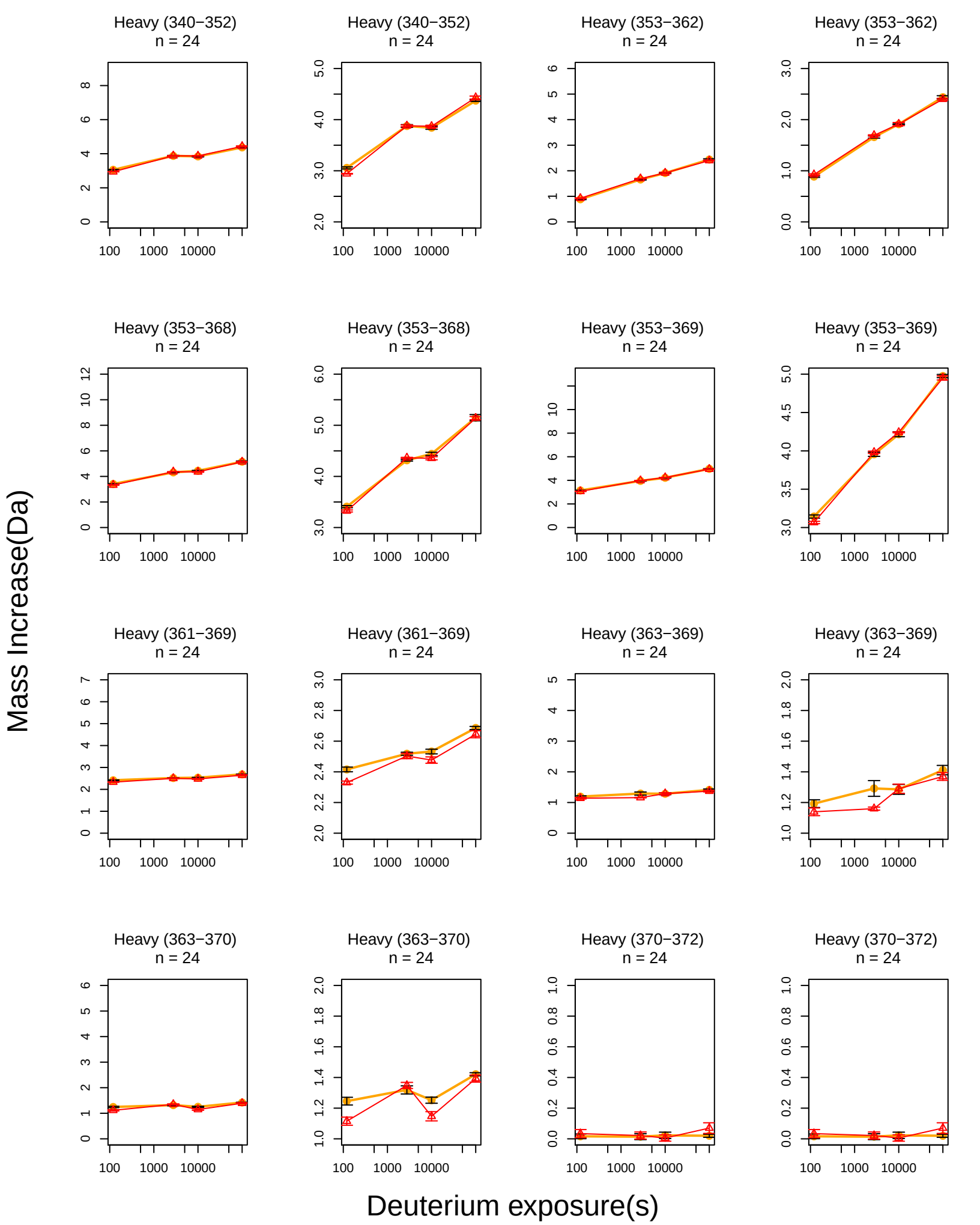
Mass Increase(Da)
Deuterium exposure(s)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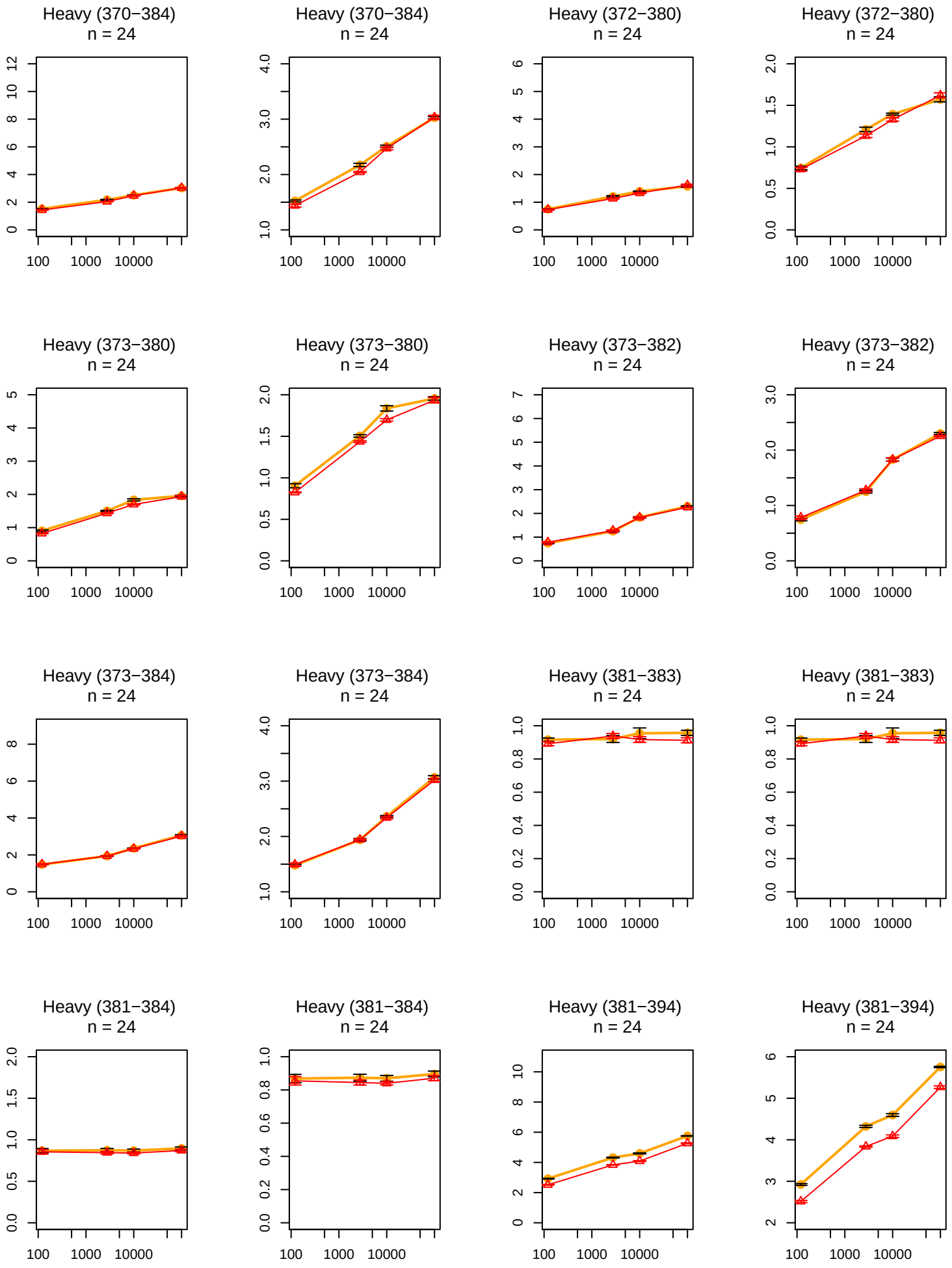
Mass Increase(Da)
Deuterium exposure(s)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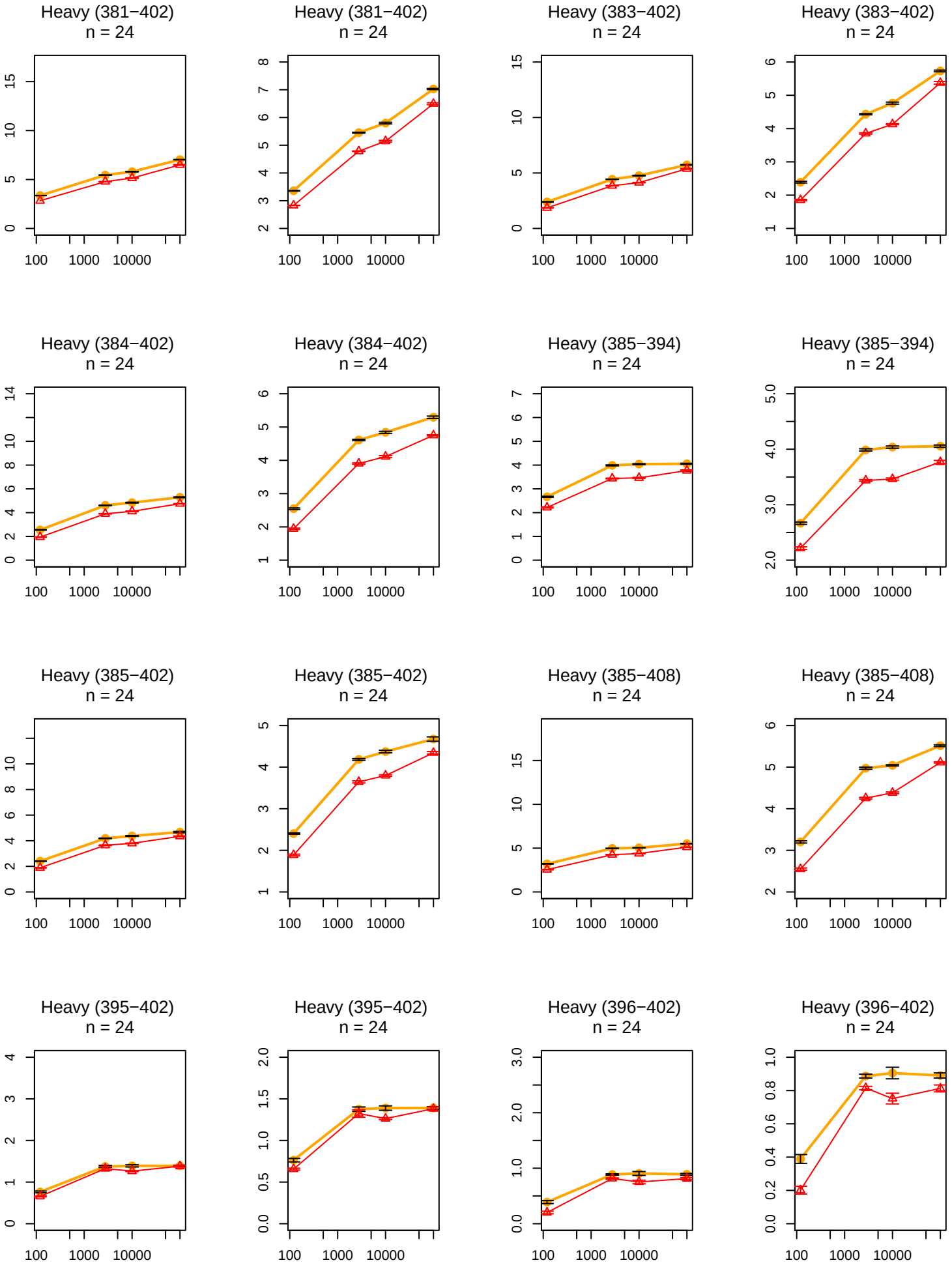
Mass Increase(Da)
Deuterium exposure(s)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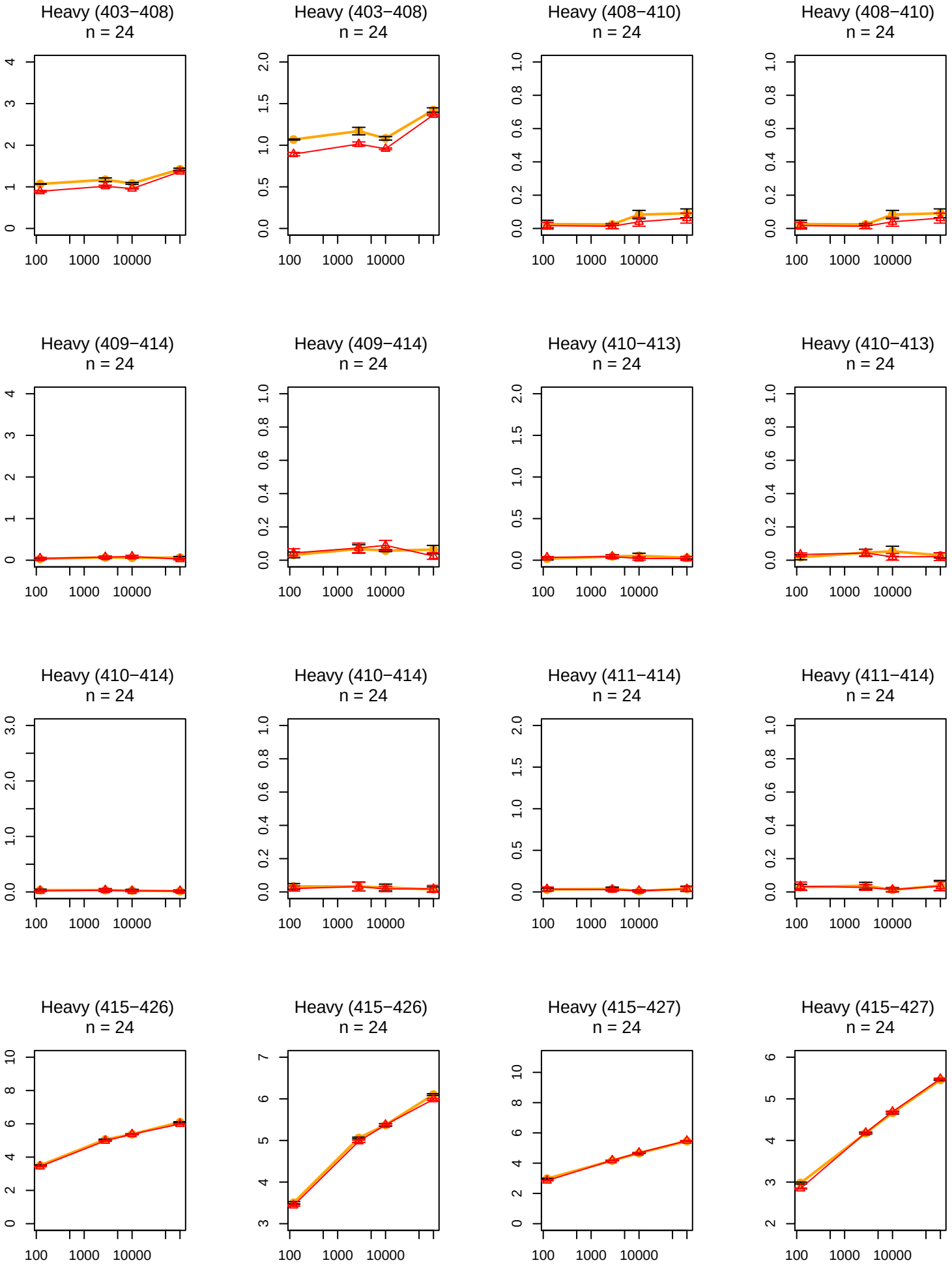
Mass Increase(Da)
Deuterium exposure(s)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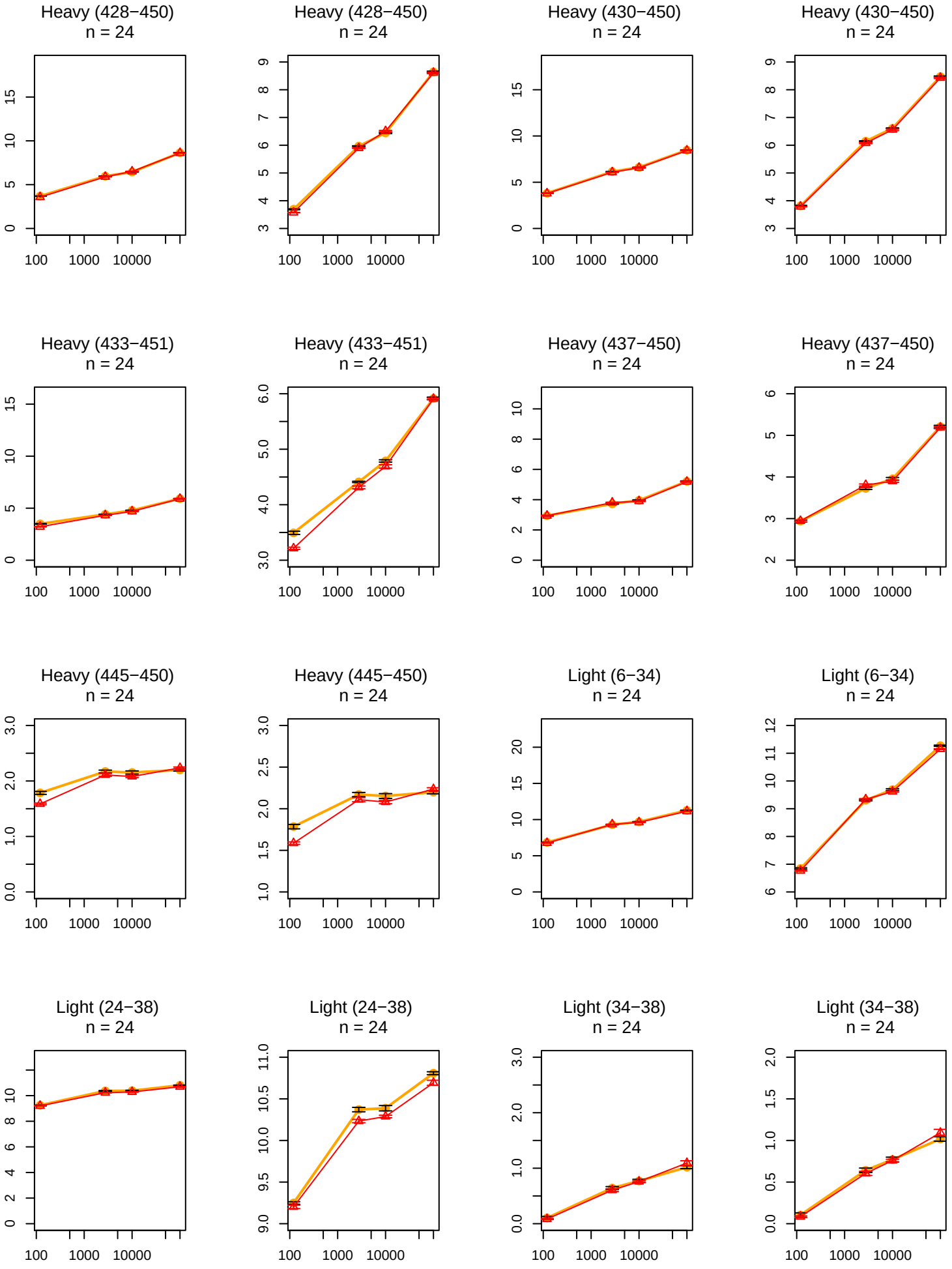
Deuterium exposure(s)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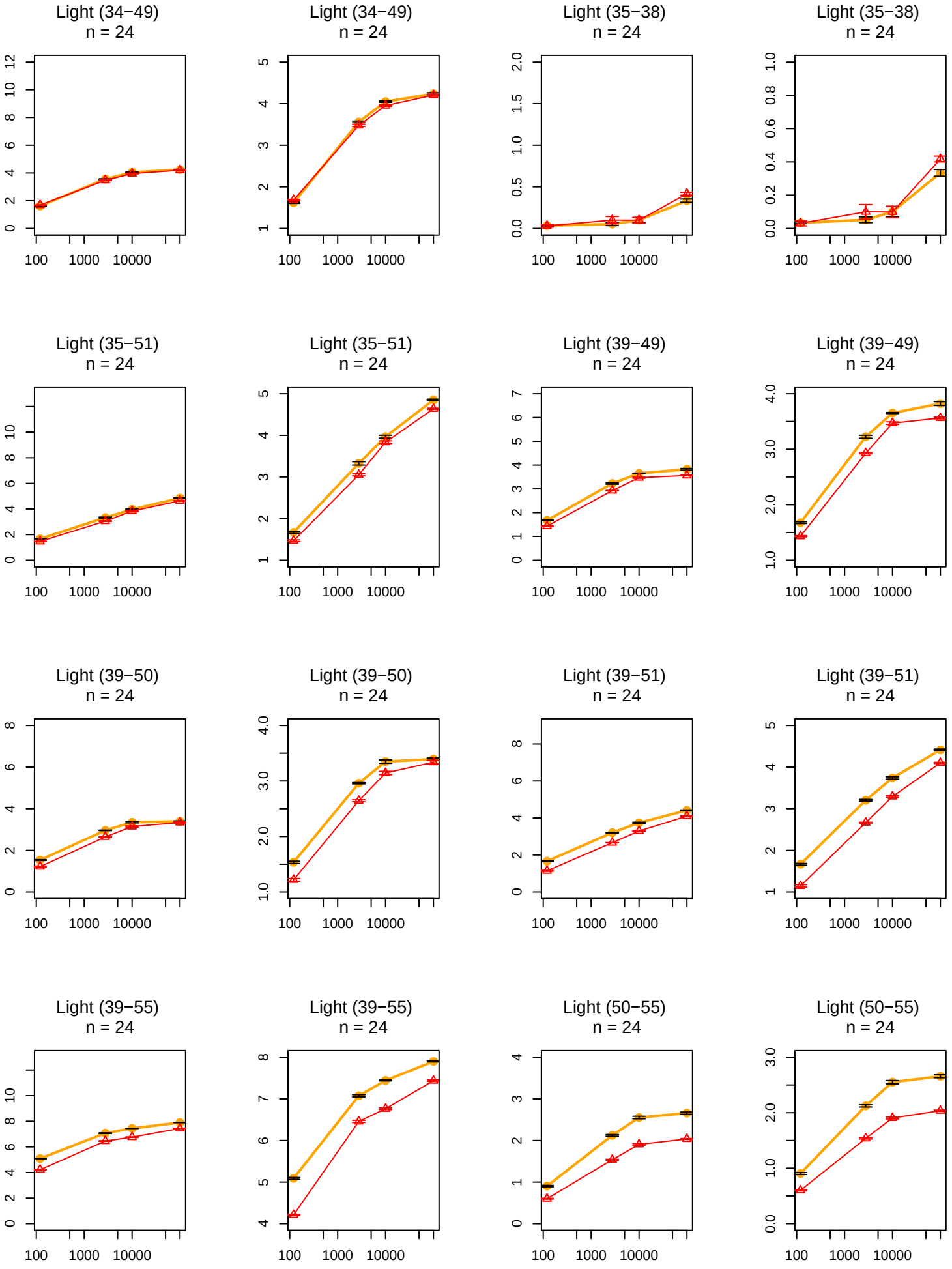
Mass Increase(Da)
Deuterium exposure(s)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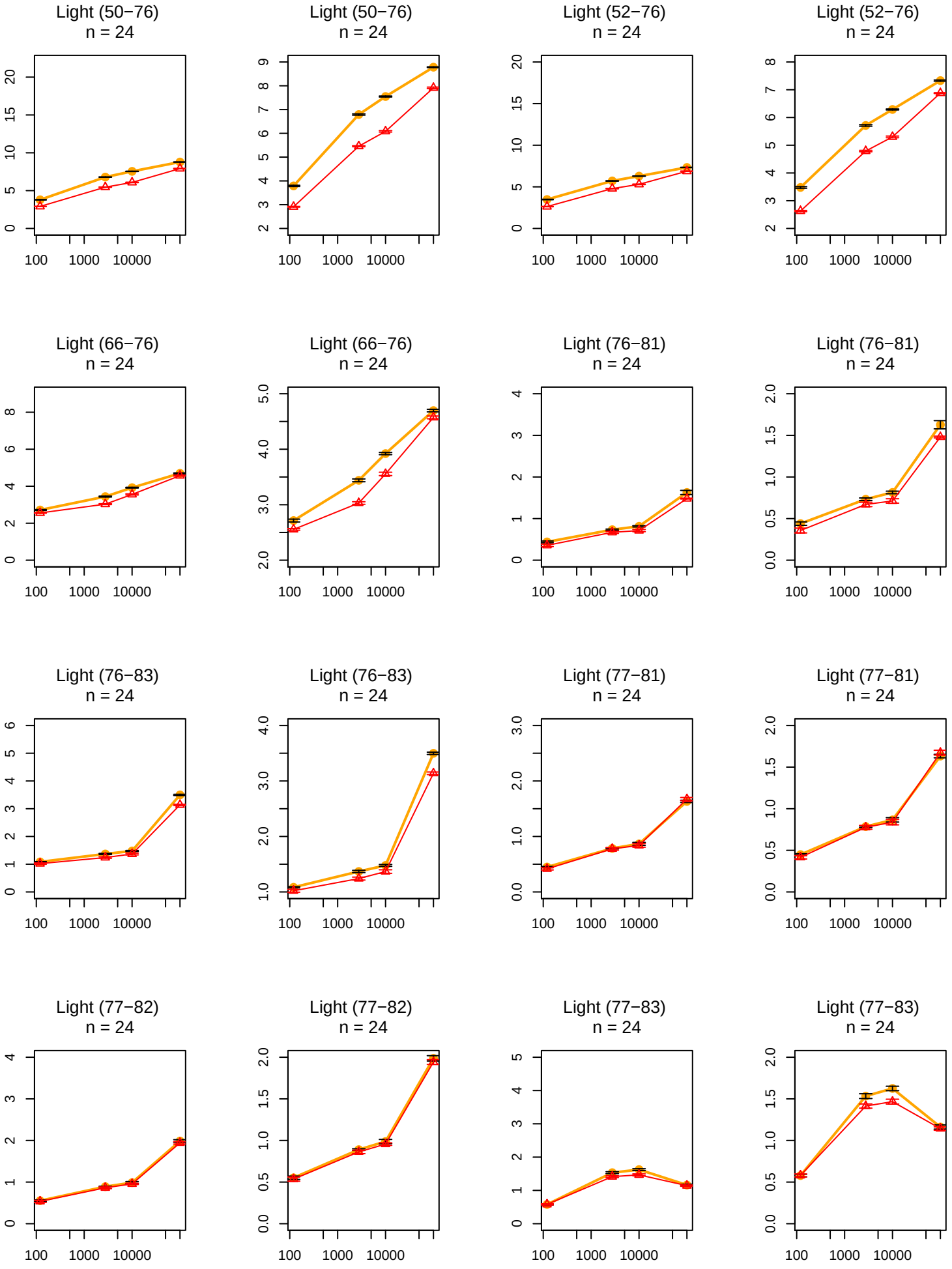
Mass Increase(Da)
Deuterium exposure(s)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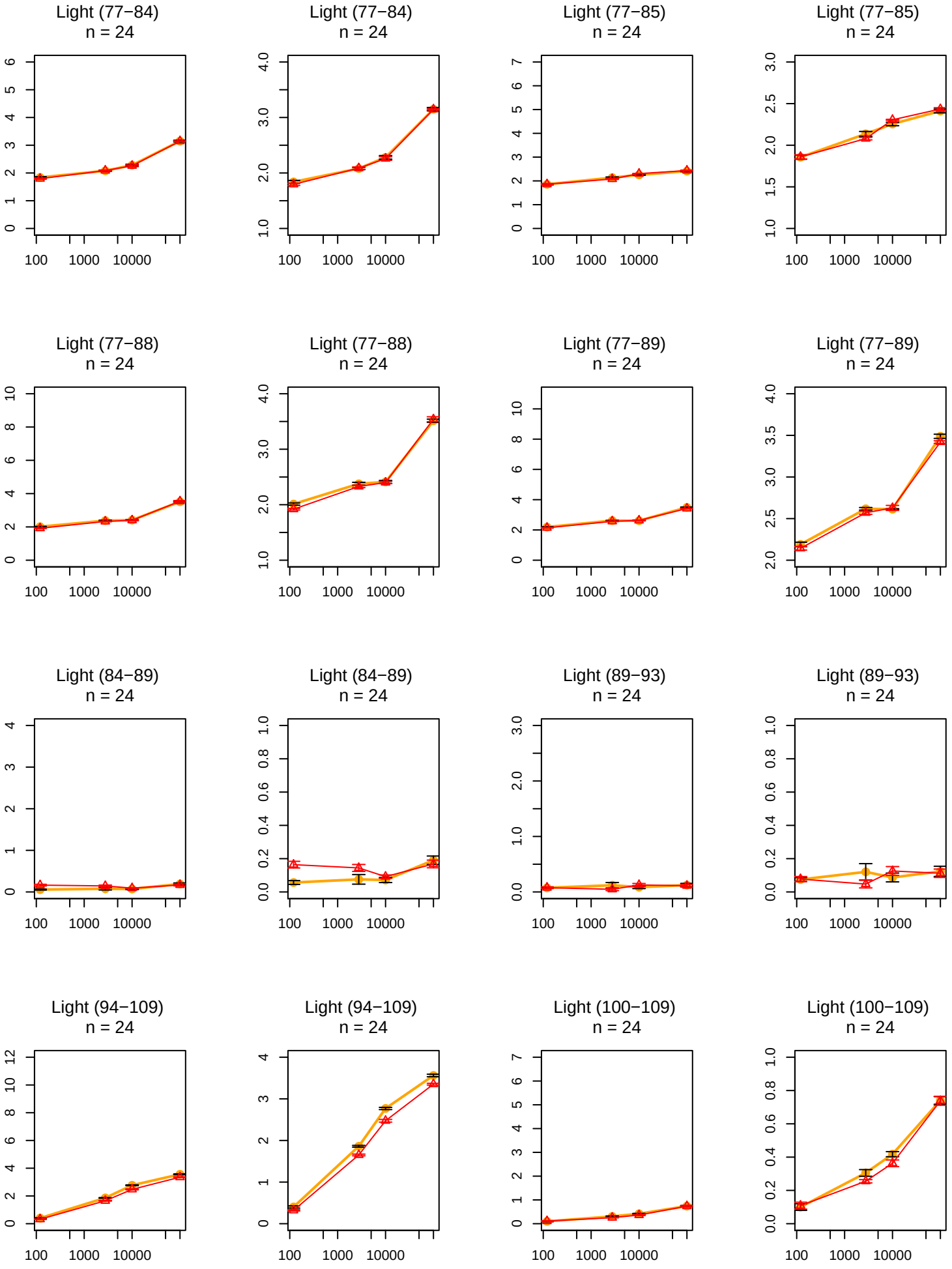
1000
10000
Mass Increase(Da)
Deuterium exposure(s)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Light (94−109)
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Light (94−109)
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Light (100−109)
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Light (100−109)
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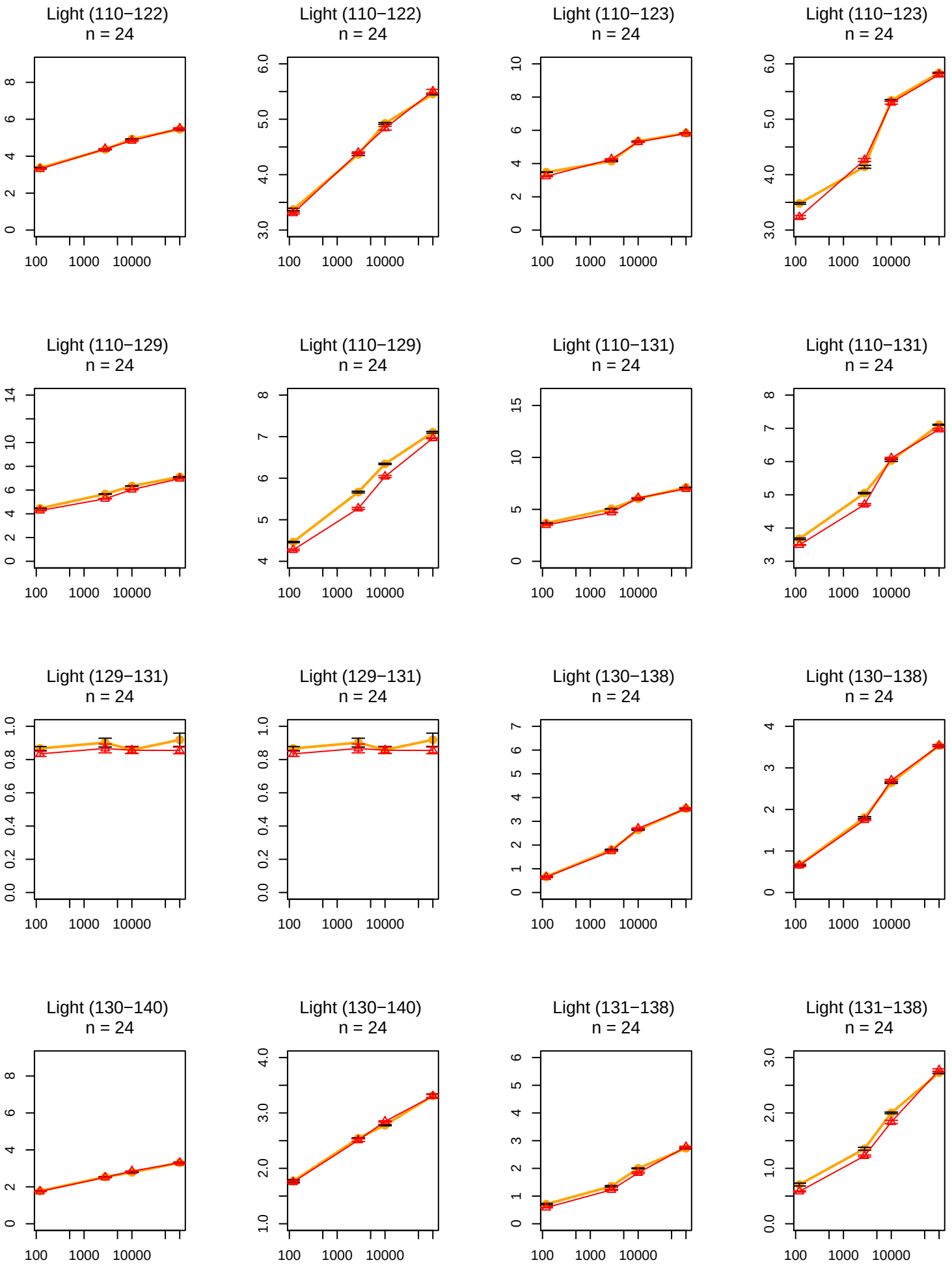
Mass Increase(Da)
Deuterium exposure(s)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Light (130−140)
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Light (130−140)
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Light (131−138)
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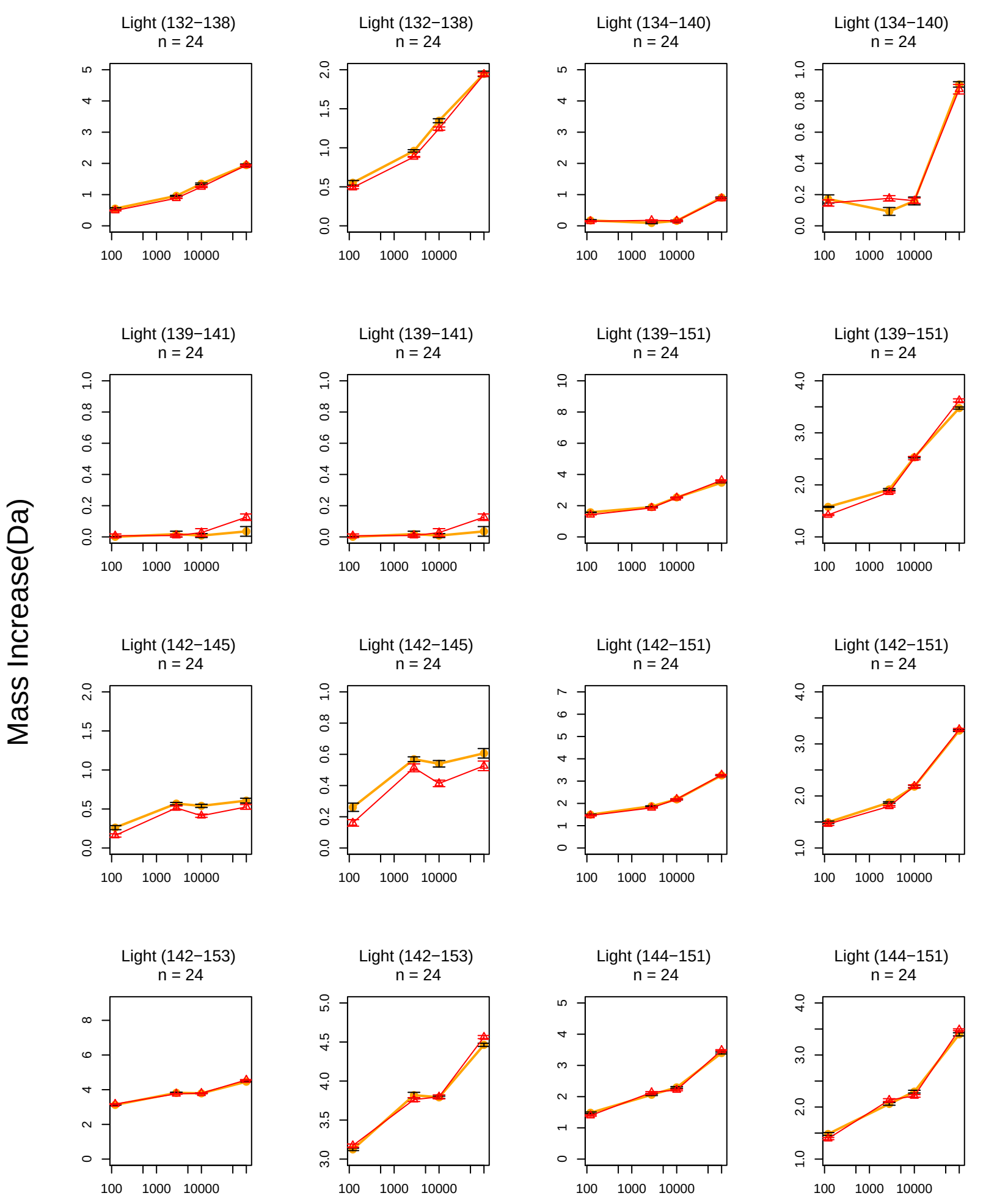
Light (131−138)
n = 24
Mass Increase(Da)
Deuterium exposure(s)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Light (142−145)
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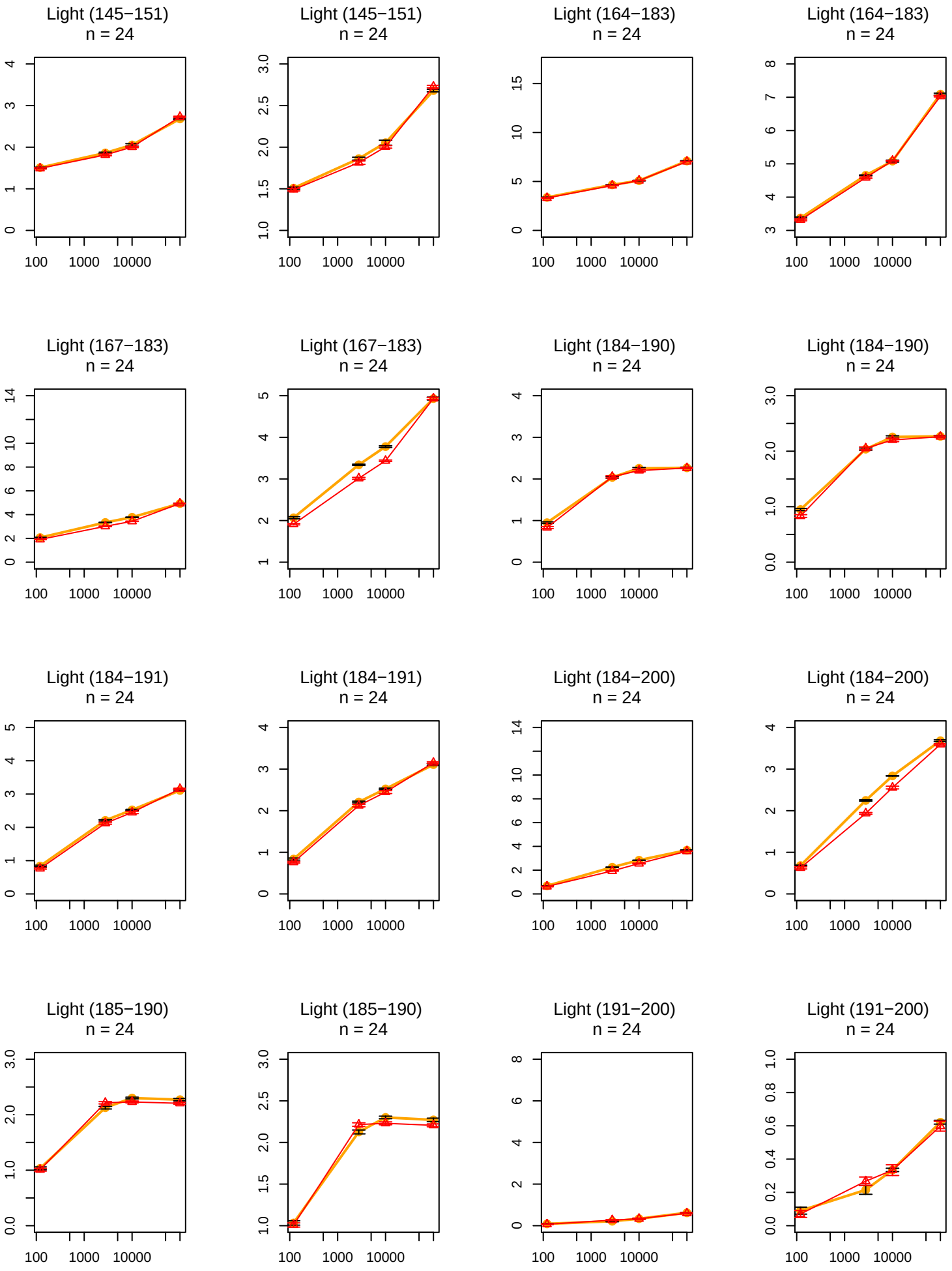
n = 24
Light (142−153)
n = 24
Light (142−153)
n = 24
Light (144−151)
n = 24
Light (144−151)
n = 24
Mass Increase(Da)
Deuterium exposure(s)


Light (145−151)
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Light (184−190)
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Light (184−191)
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Light (184−200)
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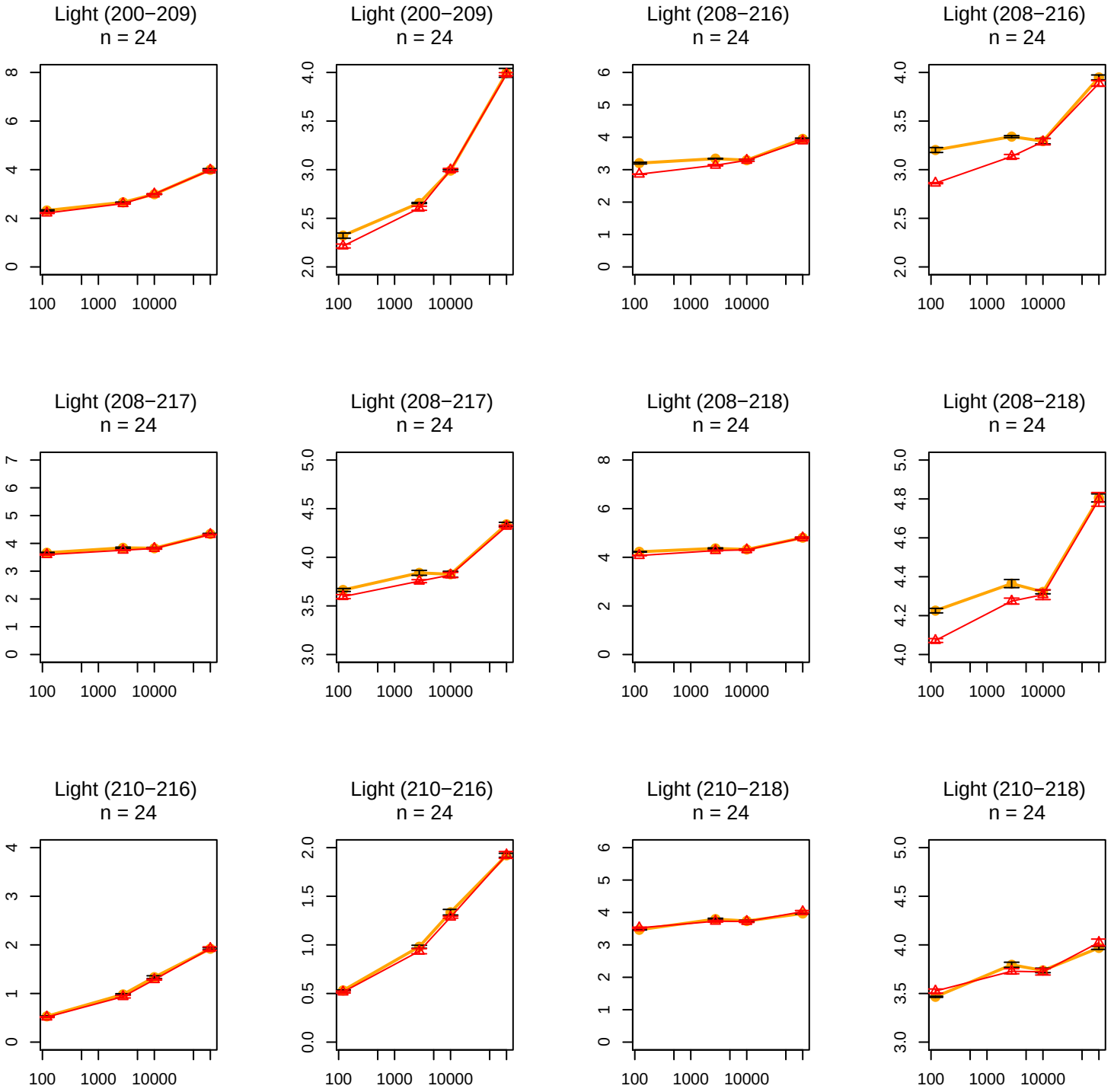
Light (184−200)
n = 24
Light (185−190)
n = 24
Light (185−190)
n = 24
Light (191−200)
n = 24
Light (191−200)
n = 24
Mass Increase(Da)
Deuterium exposure(s)



Mass Increase(Da)

Deuterium exposure(s)

Figure S4: Difference plots showing HX differences after 2760 seconds of hydrogen exchange for 182 peptides. The plots compare mAb-J samples at 60 g/L in control containing 10% (w/v) trehalose with mAb-J samples at 60 g/L containing additional (A) 100 mM arginine and (B) 100 mM NaCl, $\Delta m(t) = m_{60,\text{no additive}}(t) - m_{60,\text{arginine/NaCl}}(t)$. (Refer to the caption for Figure 5 for a more detailed description of difference plots).

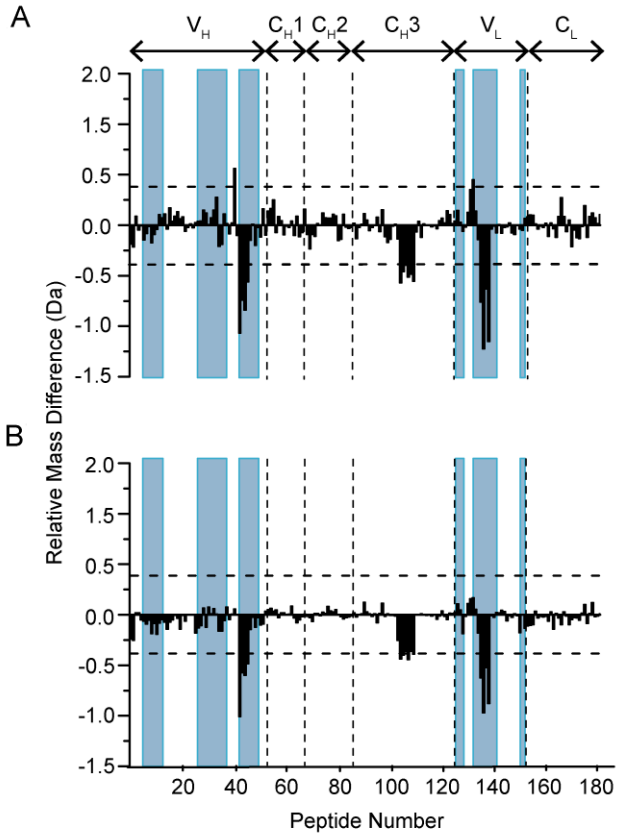
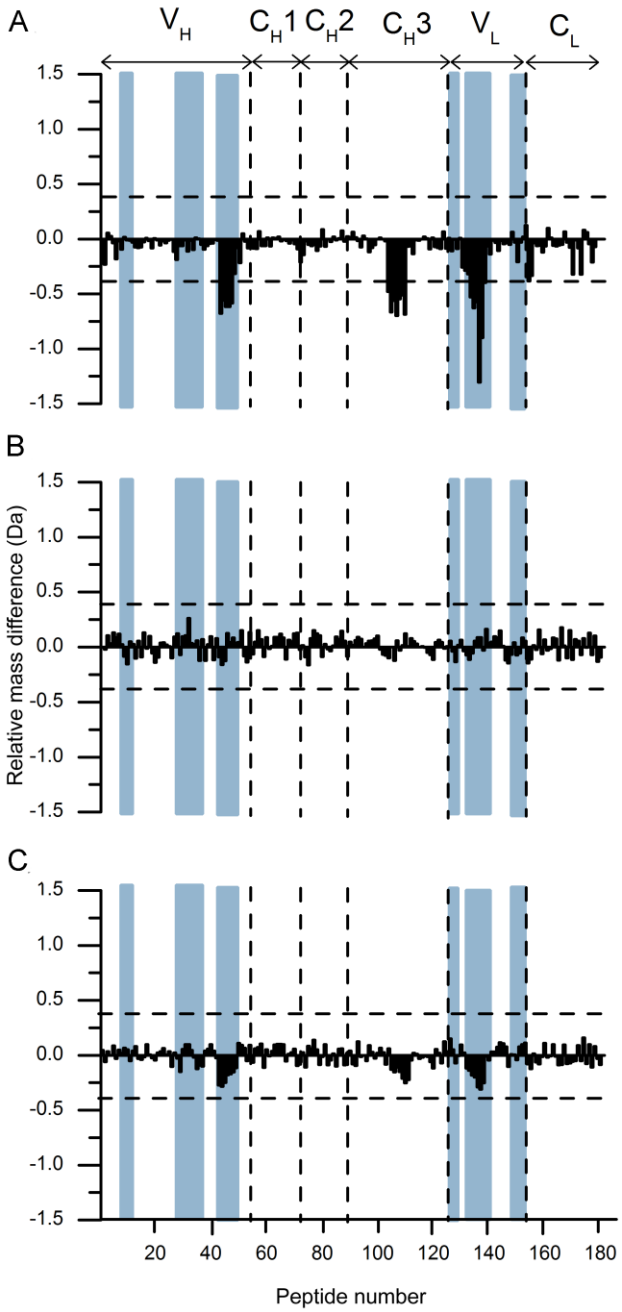


Figure S5: Difference plots showing relative mass differences at 2760 second exchange time-point for 182 peptides. The plots compare mAb-J samples at 60 g/L in control containing (A) no additional charged additives, (B) 100 mM arginine and (C) 100 mM NaCl with mAb-J samples at 5 g/L containing no additional charged additives, $\Delta m(t) = m_{60,\text{arginine/NaCl}}(t) - m_{5,\text{no additives}}(t)$. (Refer to the caption for Figure 5 for a more detailed description of difference plots).



SUPPLEMENTAL TABLES

Table S1. Protein-protein interaction equilibrium models based on best-fit of the static light scattering data of mAb-J. Light scattering experiments were conducted at 25°C. Antibody samples were prepared in the “control buffer” containing 10% (w/v) trehalose at pH 6.0.

Antibody Association Model	Chi^2
Monomer-Dimer	3.2e-2
Monomer-Trimer	7.1e0
Monomer-Trimer-Hexamer	2.4e-1
Monomer-Trimer-Hexamer-Isodesmic	4.7e-0
Monomer-Dimer-Tetramer	5.3e-3

Table S2. Osmotic second virial coefficient values for different mAb-J solution conditions tested. Light scattering experiments were conducted at 25°C. Antibody samples were prepared in the “control” containing control buffer plus 10% (w/v) trehalose at pH 6.0.

mAb-J Solution Composition	A_2 (osmotic second virial coefficient)
Control	-9.6×10^{-5} mol mL g^{-2}
Control + 100 mM NaCl	2.0×10^{-5} mol mL g^{-2}
Control + 100 mM arginine	9.0×10^{-5} mol mL g^{-2}

Table S3. Evaluation of lyophilization process on mAb-J aggregation as measured by size exclusion chromatography (SEC). Antibody samples were prepared at 5 and 60 g/L in control buffer. Lyophilized samples were reconstituted with D_2O. Experimental data are the mean and standard deviation calculated from three independent measurements on three separate lyophilized vials of mAb-J at 5 and 60 g/L.

mAb-J Sample	% Monomer	% Soluble Aggregates	% Insoluble Aggregates	% Fragments
5 g/L, Pre Lyophilization	99.6 ± 0.1	0.3 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
5 g/L, Post Lyophilization	98.1 ± 0.8	0.4 ± 0.1	1.5 ± 0.8	0.1 ± 0.1
60 g/L, Pre Lyophilization	99.5 ± 0.1	0.4 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
60 g/L, Post Lyophilization	97.7 ± 0.7	0.4 ± 0.1	1.8 ± 0.8	0.1 ± 0.1

Table S4. Peptic peptides of mAb-J (from HX-MS analysis) with their location on the primary structure and ordinal peptide numbers.

Peptide Number	Location
1	mAb-J Heavy 1-22 (VH)
2	mAb-J Heavy 2-10 (VH)
3	mAb-J Heavy 4-10 (VH)
4	mAb-J Heavy 11-22 (VH)
5	mAb-J Heavy 17-32 (VH)
6	mAb-J Heavy 29-32 (VH)
7	mAb-J Heavy 29-35 (VH)
8	mAb-J Heavy 30-32 (VH)
9	mAb-J Heavy 30-34 (VH)
10	mAb-J Heavy 30-35 (VH)
11	mAb-J Heavy 30-50 (VH)
12	mAb-J Heavy 33-49 (VH)
13	mAb-J Heavy 33-50 (VH)
14	mAb-J Heavy 34-49 (VH)
15	mAb-J Heavy 34-50 (VH)
16	mAb-J Heavy 35-49 (VH)
17	mAb-J Heavy 35-50 (VH)
18	mAb-J Heavy 36-44 (VH)
19	mAb-J Heavy 36-49 (VH)
20	mAb-J Heavy 36-50 (VH)
21	mAb-J Heavy 37-50 (VH)
22	mAb-J Heavy 45-50 (VH)
23	mAb-J Heavy 47-50 (VH)
24	mAb-J Heavy 48-50 (VH)
25	mAb-J Heavy 49-59 (VH)
26	mAb-J Heavy 50-58 (VH)
27	mAb-J Heavy 50-59 (VH)
28	mAb-J Heavy 50-60 (VH)
29	mAb-J Heavy 51-59 (VH)
30	mAb-J Heavy 59-70 (VH)
31	mAb-J Heavy 60-66 (VH)
32	mAb-J Heavy 60-70 (VH)
33	mAb-J Heavy 61-70 (VH)
34	mAb-J Heavy 71-79 (VH)

Peptide Number	Location
35	mAb-J Heavy 71-80 (VH)
36	mAb-J Heavy 71-83 (VH)
37	mAb-J Heavy 81-86 (VH)
38	mAb-J Heavy 82-86 (VH)
39	mAb-J Heavy 84-91 (VH)
40	mAb-J Heavy 84-92 (VH)
41	mAb-J Heavy 87-93 (VH)
42	mAb-J Heavy 92-108 (VH)
43	mAb-J Heavy 97-111 (VH)
44	mAb-J Heavy 101-116 (VH)
45	mAb-J Heavy 104-116 (VH)
46	mAb-J Heavy 109-116 (VH)
47	mAb-J Heavy 110-116 (VH)
48	mAb-J Heavy 110-118 (VH)
49	mAb-J Heavy 111-116 (VH)
50	mAb-J Heavy 117-121 (VH)
51	mAb-J Heavy 117-130 (VH)
52	mAb-J Heavy 117-146 (CH1)
53	mAb-J Heavy 150-159 (CH1)
54	mAb-J Heavy 160-167 (CH1)
55	mAb-J Heavy 160-178 (CH1)
56	mAb-J Heavy 163-178 (CH1)
57	mAb-J Heavy 167-178 (CH1)
58	mAb-J Heavy 168-178 (CH1)
59	mAb-J Heavy 172-178 (CH1)
60	mAb-J Heavy 179-183 (CH1)
61	mAb-J Heavy 184-188 (CH1)
62	mAb-J Heavy 184-189 (CH1)
63	mAb-J Heavy 184-197 (CH1)
64	mAb-J Heavy 184-201 (CH1)
65	mAb-J Heavy 187-201 (CH1)
66	mAb-J Heavy 189-197 (CH1)
67	mAb-J Heavy 189-201 (CH1)
68	mAb-J Heavy 190-201 (CH1)
69	mAb-J Heavy 202-213 (CH1)
70	mAb-J Heavy 229-245 (CH2)
71	mAb-J Heavy 239-244 (CH2)
72	mAb-J Heavy 239-245 (CH2)
73	mAb-J Heavy 239-255 (CH2)

Peptide Number	Location
74	mAb-J Heavy 239-256 (CH2)
75	mAb-J Heavy 245-255 (CH2)
76	mAb-J Heavy 245-256 (CH2)
77	mAb-J Heavy 246-255 (CH2)
78	mAb-J Heavy 246-256 (CH2)
79	mAb-J Heavy 257-264 (CH2)
80	mAb-J Heavy 257-265 (CH2)
81	mAb-J Heavy 266-281 (CH2)
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83	mAb-J Heavy 270-281 (CH2)
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89	mAb-J Heavy 313-323 (CH2)
90	mAb-J Heavy 338-352 (CH2)
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92	mAb-J Heavy 353-362 (CH3)
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106	mAb-J Heavy 381-394 (CH3)
107	mAb-J Heavy 381-402 (CH3)
108	mAb-J Heavy 383-402 (CH3)
109	mAb-J Heavy 384-402 (CH3)
110	mAb-J Heavy 385-394 (CH3)
111	mAb-J Heavy 385-402 (CH3)
112	mAb-J Heavy 385-408 (CH3)

Peptide Number	Location
113	mAb-J Heavy 395-402 (CH3)
114	mAb-J Heavy 396-402 (CH3)
115	mAb-J Heavy 403-408 (CH3)
116	mAb-J Heavy 408-410 (CH3)
117	mAb-J Heavy 409-414 (CH3)
118	mAb-J Heavy 410-413 (CH3)
119	mAb-J Heavy 410-414 (CH3)
120	mAb-J Heavy 411-414 (CH3)
121	mAb-J Heavy 415-426 (CH3)
122	mAb-J Heavy 415-427 (CH3)
123	mAb-J Heavy 428-450 (CH3)
124	mAb-J Heavy 430-450 (CH3)
125	mAb-J Heavy 433-451 (CH3)
126	mAb-J Heavy 437-450 (CH3)
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129	mAb-J Light 24-38 (VL)
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136	mAb-J Light 39-51 (VL)
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141	mAb-J Light 66-76 (VL)
142	mAb-J Light 76-81 (VL)
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145	mAb-J Light 77-82 (VL)
146	mAb-J Light 77-83 (VL)
147	mAb-J Light 77-84 (VL)
148	mAb-J Light 77-85 (VL)
149	mAb-J Light 77-88 (VL)
150	mAb-J Light 77-89 (VL)
151	mAb-J Light 84-89 (VL)

Peptide Number	Location
152	mAb-J Light 89-93 (VL)
153	mAb-J Light 94-109 (VL)
154	mAb-J Light 100-109 (VL)
155	mAb-J Light 110-122 (CL)
156	mAb-J Light 110-123 (CL)
157	mAb-J Light 110-129 (CL)
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161	mAb-J Light 130-140 (CL)
162	mAb-J Light 131-138 (CL)
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165	mAb-J Light 139-141 (CL)
166	mAb-J Light 139-151 (CL)
167	mAb-J Light 142-145 (CL)
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169	mAb-J Light 142-153 (CL)
170	mAb-J Light 144-151 (CL)
171	mAb-J Light 145-151 (CL)
172	mAb-J Light 164-183 (CL)
173	mAb-J Light 167-183 (CL)
174	mAb-J Light 184-190 (CL)
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176	mAb-J Light 184-200 (CL)
177	mAb-J Light 185-190 (CL)
178	mAb-J Light 191-200 (CL)
179	mAb-J Light 200-209 (CL)
180	mAb-J Light 208-216 (CL)
181	mAb-J Light 208-217 (CL)
182	mAb-J Light 210-216 (CL)