

CHEMBIOCHEM

Supporting Information

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CHEM**BIO**CHEM

Supporting Information

for

Isofagomine-Induced Stabilization of Glucocerebrosidase

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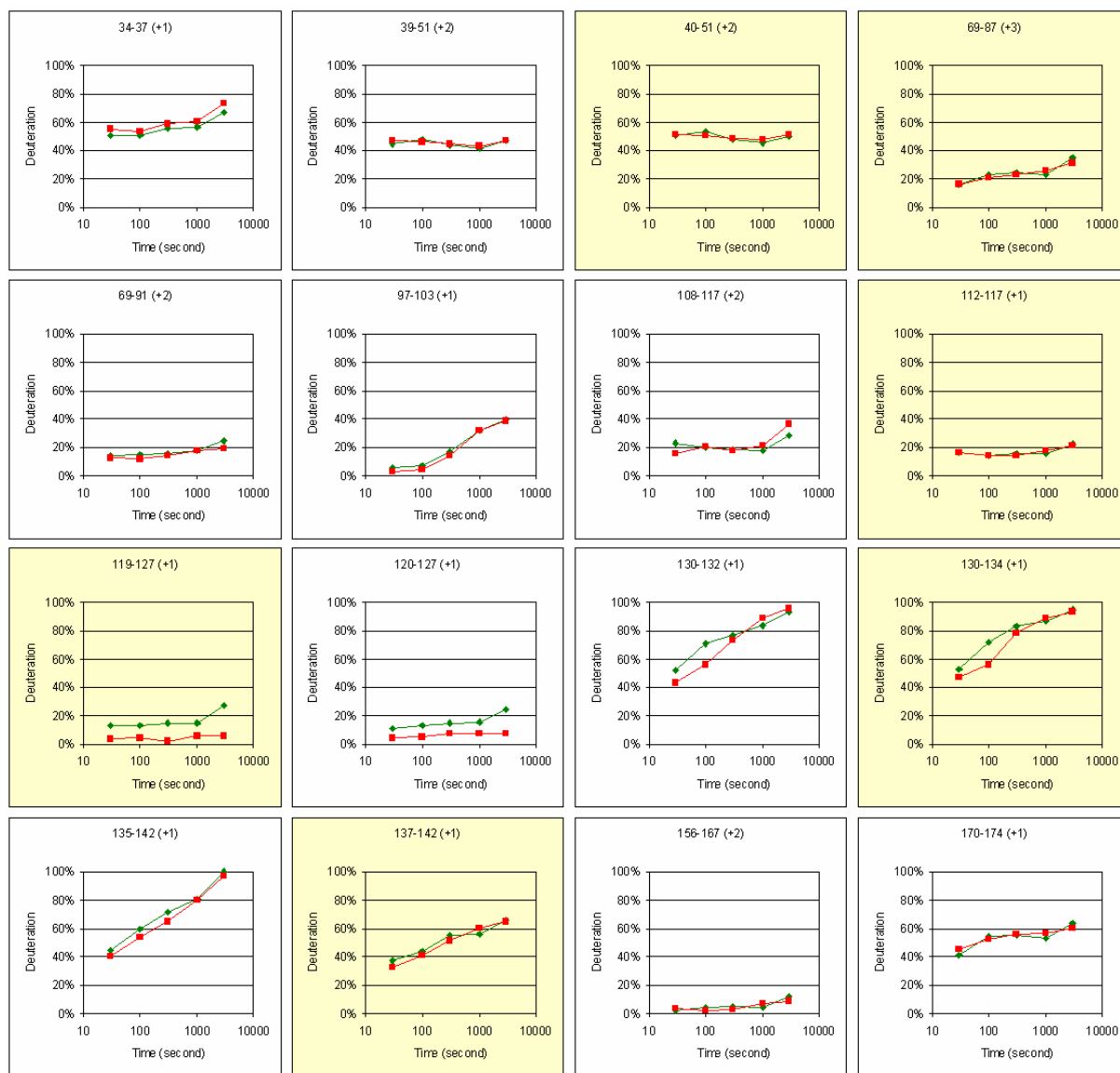
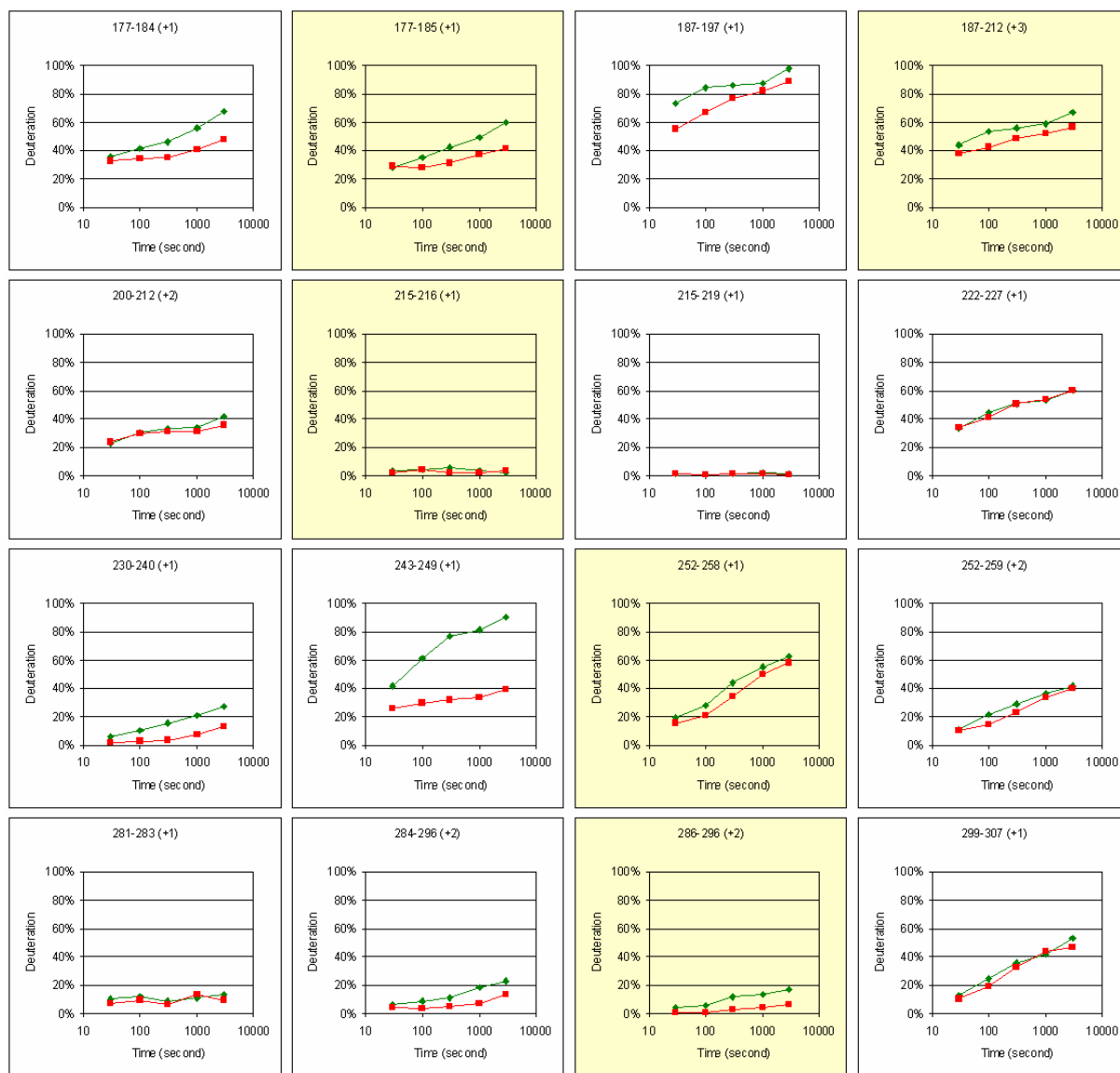
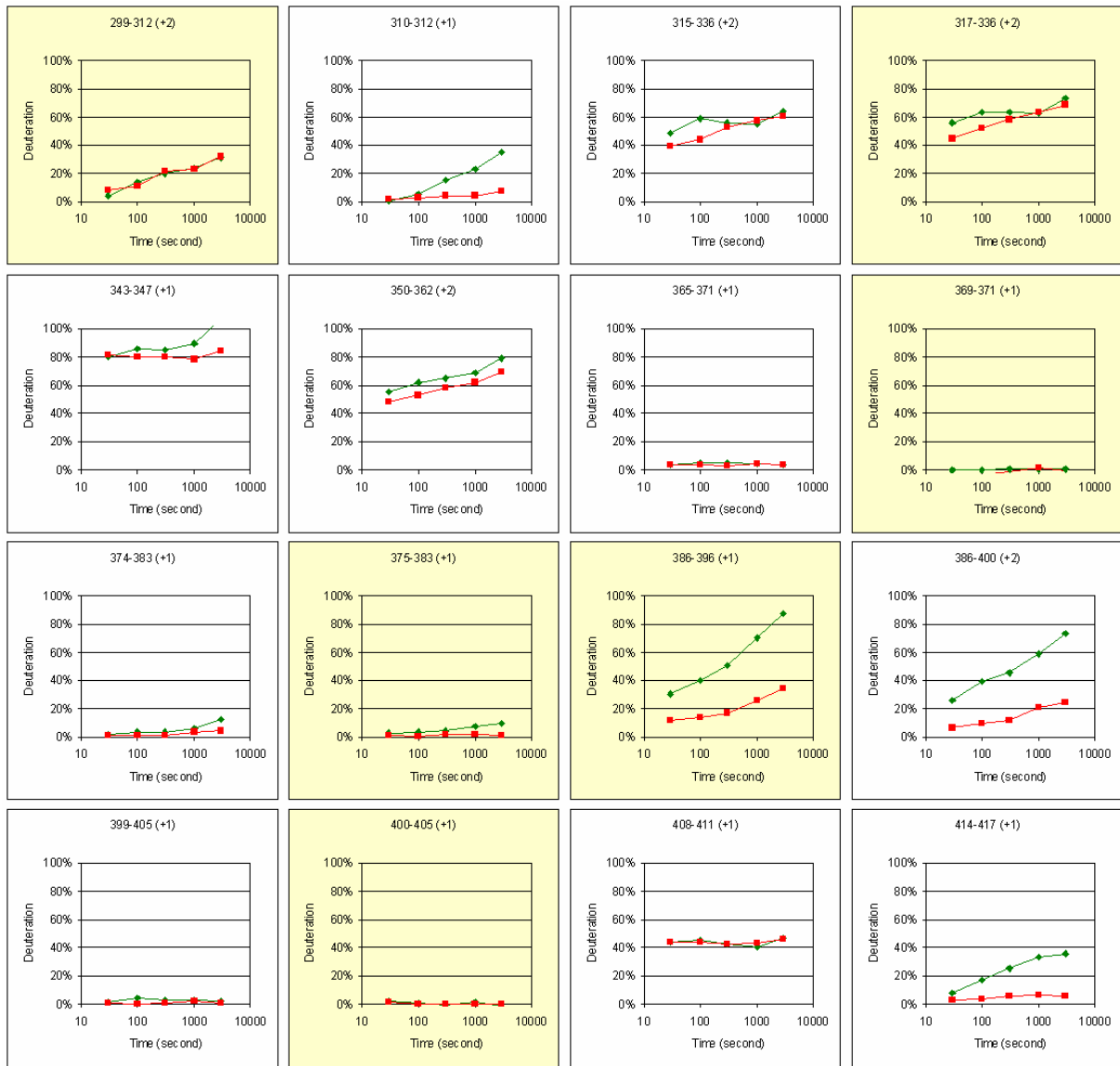


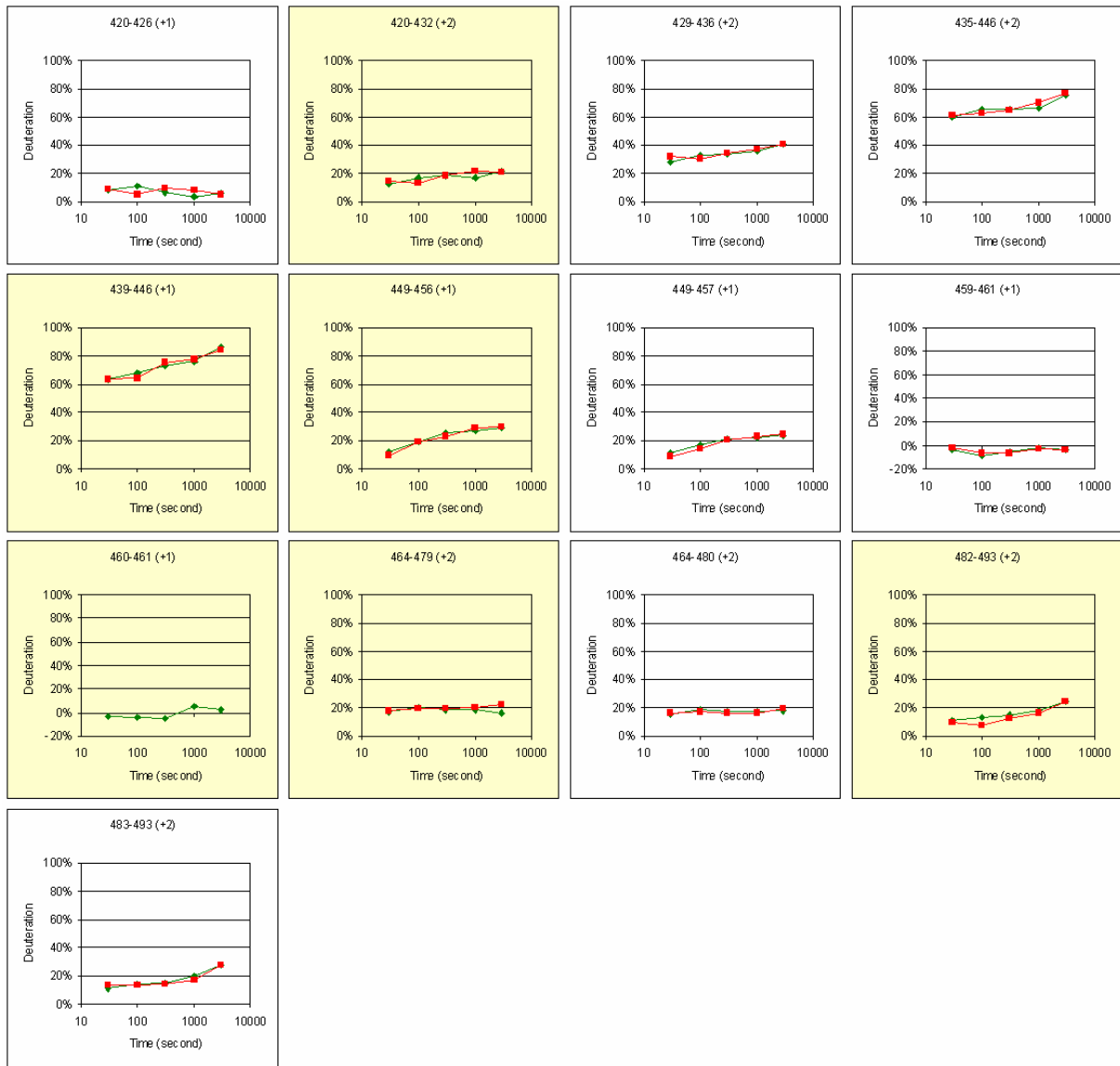
Figure S1. A) Deuteration level at 30, 100, 300, 1000 and 3000 s of 61 GCaSe peptides as a result of amide hydrogen/deuterium exchange in the presence of a 59-fold molar excess of isofagomine (red) or in its absence (green). White panels correspond to data illustrated in Figures 2 – 5. Yellow panels correspond to backup segments which give higher confidence in deuteration level of each region (e.g., the deuteration level of segment 40-51 is very similar to that of segment 39-51).



B) Deuteration level at 30, 100, 300, 1000 and 3000 s of 61 GCcase peptides as a result of amide hydrogen/deuterium exchange in the presence of a 59-fold molar excess of isofagomine (red) or in its absence (green). White panels correspond to data illustrated in Figures 2 – 5. Yellow panels correspond to backup segments which give higher confidence in deuterium level of each region (e.g., the deuterium level of segment 40-51 is very similar to that of segment 39-51).



C) Deuterium level at 30, 100, 300, 1000 and 3000 s of 61 GCcase peptides as a result of amide hydrogen/deuterium exchange in the presence of a 59-fold molar excess of isofagomine (red) or in its absence (green). White panels correspond to data illustrated in Figures 2 – 5. Yellow panels correspond to backup segments which give higher confidence in deuteration level of each region (e.g., the deuteration level of segment 40-51 is very similar to that of segment 39-51).



D) Deuteration level at 30, 100, 300, 1000 and 3000 s of 61 GCASE peptides as a result of amide hydrogen/deuterium exchange in the presence of a 59-fold molar excess of isofagomine (red) or in its absence (green). White panels correspond to data illustrated in Figures 2 – 5. Yellow panels correspond to backup segments which give higher confidence in deuterium level of each region (e.g., the deuterium level of segment 40-51 is very similar to that of segment 39-51).

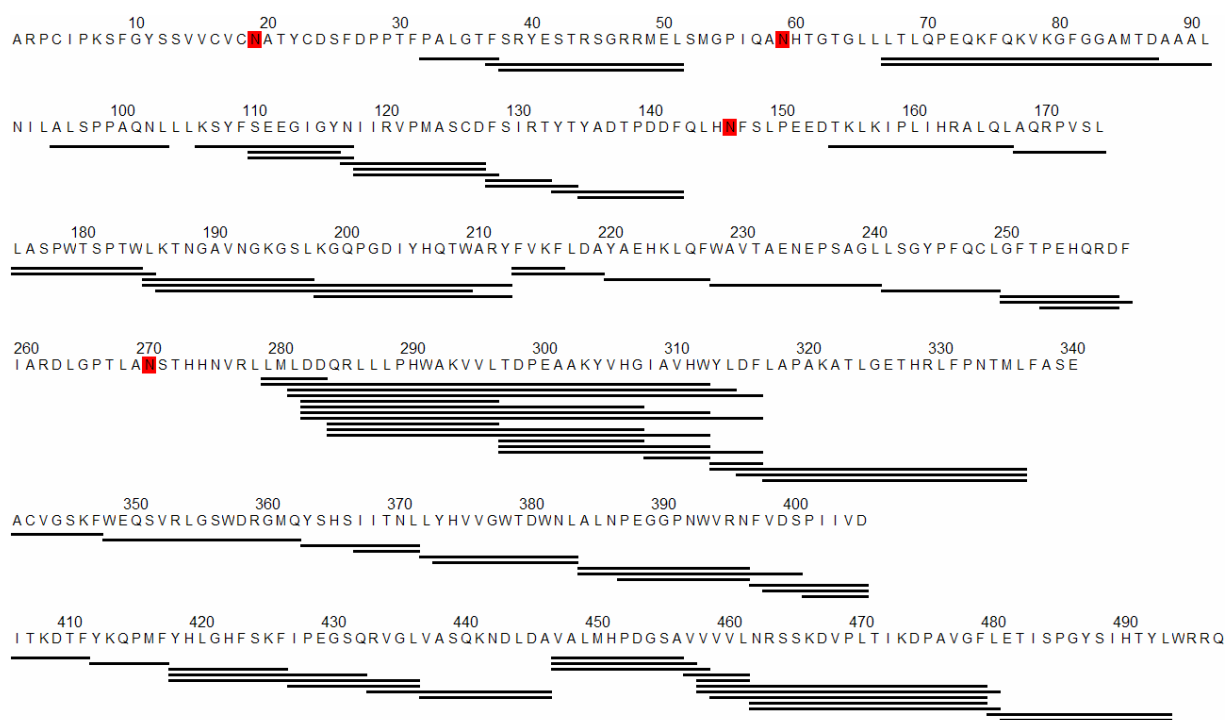


Figure S2. Pepsin digest peptide coverage map of acid-β-glucosidase (GCase). Bold lines indicate identified peptides. Coverage amounts to 82% (408/497 residues). The first 39 residues correspond to the propeptide and are therefore not included in the sequence coverage calculation or illustration. Known N-linked glycosylation sites are highlighted in red.

Table S1. Deuteration levels of indicated GCase segment (\pm IFG) as a function of five on-exchange periods.

Segment	ligand-free						with isofagomine					
	30 s	100 s	300 s	1000 s	3000 s	Av.	30 s	100 s	300 s	1000 s	3000 s	Av.
34-37	51%	51%	56%	57%	67%	56%	55%	53%	59%	60%	73%	60%
39-51	45%	47%	44%	42%	47%	45%	47%	46%	45%	44%	47%	46%
40-51	50%	54%	48%	45%	50%	49%	52%	51%	48%	48%	51%	50%
69-87	16%	23%	24%	23%	35%	24%	16%	21%	23%	26%	31%	23%
69-91	14%	15%	15%	18%	24%	17%	13%	12%	14%	18%	19%	15%
97-103	6%	8%	18%	34%	42%	22%	4%	5%	15%	34%	41%	20%
108-117	23%	20%	19%	18%	29%	22%	16%	21%	18%	22%	37%	23%
112-117	17%	15%	16%	16%	23%	17%	16%	14%	15%	18%	22%	17%
119-127	14%	13%	15%	15%	28%	17%	4%	5%	2%	6%	6%	5%
120-127	11%	14%	15%	16%	26%	16%	4%	5%	7%	8%	7%	6%
130-132	52%	71%	77%	84%	93%	75%	44%	56%	73%	89%	96%	72%
130-134	53%	72%	83%	86%	95%	78%	47%	56%	79%	89%	93%	73%
135-142	45%	60%	71%	80%	100%	71%	41%	54%	65%	79%	97%	67%
137-142	38%	45%	57%	58%	68%	53%	34%	42%	53%	62%	67%	52%
156-167	3%	4%	5%	4%	12%	6%	4%	2%	3%	7%	9%	5%
170-174	41%	55%	55%	53%	63%	53%	45%	52%	56%	57%	60%	54%
177-184	35%	42%	46%	56%	68%	49%	33%	34%	35%	41%	48%	38%
177-185	29%	35%	43%	49%	60%	43%	29%	29%	31%	37%	42%	34%
187-197	73%	85%	85%	87%	97%	85%	56%	67%	77%	82%	89%	74%

187-212	44%	54%	56%	59%	67%	56%	38%	43%	49%	53%	57%	48%
200-212	23%	30%	34%	34%	42%	33%	24%	30%	31%	31%	36%	30%
215-216	4%	5%	6%	4%	2%	4%	2%	4%	3%	3%	3%	3%
215-219	1%	1%	1%	3%	1%	1%	2%	1%	2%	1%	1%	1%
222-227	34%	45%	50%	53%	61%	49%	34%	41%	51%	54%	60%	48%
230-240	6%	11%	16%	22%	28%	17%	2%	3%	4%	8%	14%	6%
243-249	41%	61%	77%	82%	91%	70%	26%	30%	32%	34%	39%	32%
252-258	20%	28%	44%	55%	63%	42%	16%	21%	34%	50%	58%	36%
252-259	12%	22%	30%	38%	43%	29%	11%	16%	24%	35%	42%	26%
281-283	11%	12%	9%	11%	13%	11%	8%	10%	6%	14%	9%	9%
284-296	7%	9%	11%	19%	24%	14%	5%	4%	5%	8%	14%	7%
286-296	4%	6%	12%	13%	17%	10%	1%	1%	3%	4%	6%	3%
299-307	13%	25%	37%	43%	55%	35%	11%	20%	33%	45%	48%	31%
299-312	5%	14%	21%	25%	32%	19%	8%	11%	23%	24%	33%	20%
310-312	1%	5%	16%	24%	36%	16%	2%	2%	5%	4%	9%	4%
315-336	49%	61%	57%	57%	66%	58%	41%	46%	55%	59%	62%	53%
317-336	56%	63%	63%	63%	73%	64%	45%	52%	58%	63%	69%	57%
343-347	82%	88%	87%	92%	109%	92%	83%	82%	82%	81%	87%	83%
350-362	57%	63%	66%	71%	82%	68%	50%	54%	59%	63%	71%	59%
365-371	4%	5%	5%	5%	4%	5%	4%	3%	3%	4%	4%	4%
369-371	0%	0%	1%	0%	1%	0%	-2%	-3%	-2%	2%	-1%	-1%
374-383	2%	4%	3%	6%	13%	6%	2%	2%	2%	4%	4%	3%
375-383	3%	4%	5%	7%	10%	6%	2%	1%	2%	2%	2%	2%
386-396	31%	42%	52%	72%	90%	57%	13%	15%	17%	27%	35%	21%
386-400	27%	40%	47%	61%	75%	50%	7%	10%	13%	22%	25%	15%
399-405	2%	4%	3%	3%	3%	3%	1%	0%	1%	2%	1%	1%
400-405	2%	0%	-1%	1%	-1%	0%	1%	0%	0%	0%	0%	0%
408-411	44%	45%	43%	40%	47%	44%	44%	44%	43%	43%	47%	44%
414-417	9%	18%	26%	35%	37%	25%	3%	4%	6%	7%	6%	5%
420-426	8%	11%	7%	3%	6%	7%	9%	5%	9%	8%	6%	7%
420-432	13%	17%	19%	17%	22%	18%	15%	13%	19%	21%	21%	18%
429-436	28%	33%	34%	36%	41%	34%	32%	30%	35%	37%	41%	35%
435-446	59%	66%	66%	66%	75%	66%	61%	62%	65%	70%	77%	67%
439-446	64%	68%	73%	76%	86%	73%	64%	64%	75%	77%	84%	73%
449-456	12%	19%	25%	27%	29%	22%	10%	19%	23%	29%	30%	22%
449-457	11%	17%	22%	22%	24%	19%	9%	14%	21%	23%	25%	18%
459-461	-4%	-8%	-6%	-2%	-4%	-5%	-2%	-7%	-6%	-3%	-4%	-4%
460-461	-3%	-4%	-5%	5%	3%	-1%	0%	1%	3%	1%	2%	1%
464-479	18%	20%	19%	19%	17%	19%	19%	20%	20%	21%	23%	21%
464-480	16%	19%	18%	17%	19%	18%	17%	17%	17%	17%	20%	18%
482-493	11%	13%	15%	18%	25%	16%	9%	7%	13%	16%	25%	14%
483-493	12%	14%	15%	20%	28%	18%	13%	13%	14%	17%	28%	17%

Table S2. Deuteration level differences of indicated GCase segments, in response to 59-fold molar excess of isofagomine.

Segment	30 s	100 s	300 s	1000 s	3000 s	Av.
34-37	4%	2%	3%	3%	6%	4%
39-51	2%	-1%	0%	1%	0%	0%
40-51	1%	-3%	0%	3%	1%	0%
69-87	0%	-2%	-1%	3%	-4%	-1%
69-91	-1%	-3%	-1%	0%	-5%	-2%
97-103	-2%	-3%	-3%	0%	-1%	-2%
108-117	-7%	1%	-1%	4%	8%	1%

112-117	0%	0%	-1%	2%	-1%	0%
119-127	-10%	-8%	-13%	-9%	-22%	-12%
120-127	-7%	-8%	-8%	-8%	-18%	-10%
130-132	-8%	-15%	-4%	5%	2%	-4%
130-134	-6%	-16%	-4%	2%	-2%	-5%
135-142	-4%	-6%	-6%	-1%	-3%	-4%
137-142	-4%	-3%	-4%	4%	-1%	-2%
156-167	1%	-2%	-2%	3%	-3%	0%
170-174	4%	-3%	1%	3%	-3%	1%
177-184	-3%	-8%	-11%	-15%	-20%	-11%
177-185	1%	-7%	-11%	-12%	-18%	-9%
187-197	-18%	-18%	-9%	-6%	-9%	-12%
187-212	-6%	-11%	-7%	-6%	-10%	-8%
200-212	1%	-1%	-3%	-3%	-6%	-2%
215-216	-1%	0%	-3%	-1%	1%	-1%
215-219	0%	0%	0%	-1%	0%	0%
222-227	0%	-4%	1%	1%	-1%	-1%
230-240	-3%	-8%	-12%	-14%	-14%	-10%
243-249	-15%	-31%	-45%	-48%	-51%	-38%
252-258	-4%	-7%	-9%	-5%	-4%	-6%
252-259	-1%	-6%	-6%	-3%	-1%	-3%
281-283	-3%	-3%	-3%	3%	-4%	-2%
284-296	-2%	-5%	-6%	-11%	-10%	-7%
286-296	-3%	-5%	-8%	-9%	-10%	-7%
299-307	-2%	-5%	-3%	2%	-6%	-3%
299-312	3%	-3%	2%	-1%	1%	0%
310-312	1%	-2%	-11%	-19%	-27%	-12%
315-336	-8%	-15%	-3%	2%	-4%	-5%
317-336	-11%	-11%	-5%	0%	-4%	-6%
343-347	1%	-6%	-5%	-11%	-22%	-9%
350-362	-7%	-9%	-7%	-7%	-10%	-8%
365-371	0%	-2%	-2%	0%	0%	-1%
369-371	-2%	-3%	-2%	1%	-1%	-2%
374-383	0%	-2%	-2%	-2%	-9%	-3%
375-383	-1%	-3%	-2%	-5%	-8%	-4%
386-396	-18%	-26%	-34%	-44%	-53%	-35%
386-400	-19%	-30%	-33%	-38%	-48%	-34%
399-405	-1%	-4%	-2%	-1%	-2%	-2%
400-405	-1%	-1%	1%	-1%	1%	0%
408-411	0%	-1%	0%	3%	0%	0%
414-417	-5%	-13%	-20%	-27%	-30%	-19%
420-426	1%	-6%	3%	5%	0%	1%
420-432	2%	-4%	0%	4%	-1%	0%
429-436	4%	-2%	1%	1%	0%	1%
435-446	2%	-3%	-1%	3%	1%	1%
439-446	0%	-4%	2%	1%	-2%	-1%
449-456	-2%	0%	-2%	2%	1%	0%
449-457	-3%	-3%	-1%	1%	0%	-1%
459-461	2%	2%	0%	-1%	1%	1%
460-461	3%	5%	8%	-4%	0%	2%
464-479	1%	-1%	1%	2%	6%	2%
464-480	1%	-2%	-1%	-1%	1%	0%
482-493	-2%	-6%	-2%	-2%	0%	-2%
483-493	2%	-1%	0%	-3%	0%	0%