Supporting Information

REMD Simulations Reveal the Dynamic Profile and Mechanism of Action of Deleterious, Rescuing and Stabilizing Perturbations to NBD1 from CFTR

Authors: Michael Zhenin, Efrat Noy, Hanoch Senderowitz*

Affiliations: Department of Chemistry, Bar Ilan University, Ramat-Gan 52900, Israel

*Corresponding author, e-mail: hsenderowitz@gmail.com

Table S1. System size and acceptance exchange rates between neighboring replicas for all constructs considered in this work.

Structure	# Atoms	# Water Molecules	0 - 1	1 - 2	2 - 3	3 - 4	4 – 5	5 - 6
wt – NBD1	3,644	11,455	0.23	0.21	0.24	0.22	0.24	0.24
F508del – NBD1	3,631	11,887	0.25	0.23	0.25	0.24	0.23	0.25
V510D – NBD1	3,640	11,890	0.22	0.23	0.23	0.22	0.24	0.22
V510D/F508del – NBD1	3,627	11,466	0.23	0.22	0.26	0.24	0.26	0.25
3M – NBD1	3,643	11,890	0.23	0.21	0.24	0.24	0.23	0.23
3M/F508del – NBD1	3,630	11,462	0.25	0.24	0.24	0.23	0.21	0.24
F508del/BIA – NBD1	3,652	11,449	0.23	0.23	0.25	0.25	0.23	0.25

Structure	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17
wt – NBD1	0.23	0.27	0.24	0.22	0.24	0.23	0.23	0.24	0.25	0.24	0.25
F508del – NBD1	0.24	0.25	0.24	0.22	0.24	0.23	0.25	0.26	0.25	0.26	0.26
V510D – NBD1	0.24	0.24	0.23	0.21	0.23	0.23	0.23	0.24	0.24	0.22	0.25
V510D/F508del – NBD1	0.25	0.25	0.25	0.23	0.26	0.24	0.26	0.27	0.26	0.24	0.27
3M – NBD1	0.25	0.25	0.23	0.22	0.25	0.24	0.24	0.24	0.21	0.23	0.25
3M/F508del – NBD1	0.26	0.24	0.26	0.23	0.24	0.24	0.25	0.26	0.24	0.25	0.24
F508del/BIA – NBD1	0.24	0.24	0.26	0.21	0.24	0.26	0.24	0.26	0.24	0.26	0.26

Structure	17 - 18	18 - 19	19 - 20	20 - 21	21 - 22	22 -23	23 - 24	24 - 25	25 - 26	26 - 27
wt – NBD1	0.23	0.25	0.23	0.24	0.24	0.24	0.24	0.26	0.27	0.25
F508del – NBD1	0.26	0.26	0.24	0.25	0.26	0.25	0.23	0.26	0.27	0.24
V510D – NBD1	0.25	0.23	0.24	0.25	0.24	0.25	0.24	0.25	0.25	0.24
V510D/F508del – NBD1	0.26	0.26	0.26	0.24	0.25	0.25	0.27	0.24	0.27	0.26
3M – NBD1	0.23	0.25	0.22	0.24	0.25	0.24	0.25	0.25	0.25	0.26
3M/F508del – NBD1	0.26	0.24	0.26	0.25	0.27	0.27	0.25	0.26	0.26	0.26
F508del/BIA – NBD1	0.27	0.26	0.25	0.25	0.25	0.25	0.25	0.28	0.26	0.24

Structure	27 - 28	28 - 29	29 - 30	30 - 31
wt – NBD1	0.25	0.25	0.25	0.25
F508del – NBD1	0.25	0.27	0.26	0.27
V510D – NBD1	0.24	0.25	0.23	0.24
V510D/F508del – NBD1	0.25	0.26	0.26	0.25
3M – NBD1	0.25	0.25	0.26	0.26
3M/F508del – NBD1	0.26	0.26	0.27	0.27
F508del/BIA – NBD1	0.26	0.25	0.27	0.26

Table S2. Averaged solvent accessible surface area (SASA; $Å^2$) for all residues of all constructs considered in this work.

Residue Number	SASA wt-NBD1	SD	SASA F508del-NBD1	SD	SASA V510D-NBD1	SD	SASA V510D/F508del-NBD1	SD	SASA 3M-NBD1	SD	SASA 3M/F508del-NBD1	SD	F508del/BIA-NBD1	SD
385	94	2	95	1	91	2	91	3	93	3	92	1	95	2
386	97	2	95	2	95	4	94	3	98	3	95	2	95	3
387	158	2	145	5	153	4	141	7	151	2	144	2	145	4
388	92	1	101	2	92	4	105	2	96	1	104	0	101	4
389	74	0	76	2	75	3	81	1	75	1	80	2	82	3
390	48	1	52	1	48	3	49	1	50	2	49	2	50	2
391	47	3	40	2	44	1	41	4	43	3	41	2	42	4
392	6	0	5	0	5	1	6	0	6	1	5	0	6	0
393	41	1	43	1	39	1	41	5	40	1	41	2	40	2
394	7	1	7	0	7	1	7	2	6	0	7	1	7	1
395	85	1	90	2	85	3	87	1	86	1	85	1	86	3
396	71	1	73	2	71	2	71	1	71	1	72	1	72	2
397	2	0	2	0	3	0	2	0	2	0	3	1	2	0
398	32	1	33	0	33	1	32	1	31	1	33	1	32	1
399	1	0	1	0	1	0	1	0	1	0	2	0	1	0
400	82	2	86	2	80	2	80	2	77	1	79	1	83	0
401	141	3	133	3	138	2	142	5	136	4	137	3	136	3
402	118	2	132	3	119	2	112	0	117	1	116	2	118	4
403	150	1	145	1	149	0	149	0	151	1	151	1	149	2
404	70	2	62	2	69	1	62	0	66	2	57	4	67	2
437	47	3	57	2	48	2	45	1	49	3	50	4	48	2
438	104	1	97	2	103	1	103	2	103	2	102	1	102	2
439	43	2	43	1	43	1	41	1	43	1	44	0	43	1
440	43	1	41	2	44	2	42	1	41	1	43	0	42	1
441	3	0	3	0	3	0	3	0	3	0	3	0	3	0
442	87	1	80	3	88	2	83	2	87	2	85	4	84	1
443	86	2	89	1	85	2	86	2	85	2	87	2	88	3
444	7	1	8	0	6	0	8	0	7	0	7	0	8	1
445	73	2	76	1	72	1	76	1	74	2	73	2	74	6
446	14	2	16	1	13	0	16	1	14	1	15	0	15	4
447	105	1	103	1	103	1	101	1	98	2	102	2	99	5
448	8	1	7	0	8	0	7	0	8	1	8	1	7	1
449	69	1	66	3	68	1	67	3	62	1	68	2	69	3
450	138	3	132	3	137	3	133	1	138	4	134	1	133	8
451	21	0	21	1	21	1	22	1	22	1	20	1	19	0
452	45	1	44	2	43	1	43	1	44	1	48	2	44	7
453	13	1	16	1	13	1	17	1	12	0	17	0	16	1
454	5	0	6	0	5	0	6	0	6	0	7	1	6	1
455	5	0	4	0	5	0	4	0	3	0	4	0	4	0
456	3	1	4	0	3	0	3	0	3	0	4	0	3	0
457	3	1	4	0	3	0	3	1	3	1	4	0	4	0
458	9	1	10	1	10	1	9	1	8	1	11	1	8	1

459	54	1	53	3	55	2	52	4	56	3	55	3	51	1
460	125	1	124	1	125	1	125	0	125	1	124	1	125	0
461	72	1	72	1	72	0	72	1	73	1	72	0	72	1
462	13	1	13	1	13	1	14	2	17	0	13	0	14	1
463	14	1	13	1	15	1	14	2	14	1	14	1	13	1
464	40	1	41	2	39	0	39	1	30	1	42	1	39	1
465	50	2	45	4	50	1	32	1	50	2	43	2	33	1
466	18	1	16	0	19	1	17	1	17	1	16	0	17	0
467	6	1	6	0	5	0	6	1	5	0	6	0	6	1
468	4	0	4	1	4	0	4	0	4	0	4	0	4	0
469	54	1	51	2	54	1	50	0	51	1	51	1	50	1
470	10	0	11	0	10	1	11	1	11	0	11	0	11	0
471	22	1	20	1	22	2	19	1	19	1	17	1	19	1
472	81	1	81	1	82	1	82	0	80	1	79	0	81	1
473	52	0	53	0	53	0	52	0	53	0	53	0	53	0
474	110	0	109	2	109	1	109	1	109	0	108	0	109	0
475	18	1	19	1	17	0	21	2	17	1	21	2	19	1
476	127	1	128	1	127	2	128	0	125	2	127	0	127	1
477	42	1	43	2	42	2	43	1	44	1	41	0	43	1
478	70	1	69	1	69	1	70	1	70	1	70	1	69	1
479	116	1	116	1	116	0	116	1	116	0	116	1	116	0
480	33	1	34	1	33	1	34	1	34	1	33	0	35	0
481	126	3	131	2	127	1	130	2	130	1	129	1	130	0
482	55	2	55	1	55	1	55	1	56	1	54	1	54	1
483	122	3	122	3	120	2	118	3	120	3	123	4	121	4
484	61	2	64	1	58	2	66	4	66	1	67	0	64	1
485	91	6	83	1	88	3	81	7	81	4	83	3	88	2
486	40	1	41	1	44	2	40	2	41	2	38	1	38	2
487	121	1	121	3	122	0	123	3	118	1	121	0	121	3
488	55	2	51	3	54	2	48	1	54	2	51	0	51	1
489	13	2	11	1	12	1	10	1	16	1	11	0	10	1
490	70	1	63	2	68	2	59	2	66	1	62	0	62	2
491	23	2	19	2	23	0	20	1	18	1	20	1	21	1
492	44	2	41	1	45	4	41	3	44	4	38	4	37	4
493	84	3	90	8	81	2	104	3	89	3	93	3	103	2
494	141	4	145	10	140	4	150	2	143	5	162	4	152	8
495	38	2	41	2	41	2	44	3	44	3	47	4	36	2
496	126	4	154	1	126	6	151	4	133	3	155	3	148	2
497	16	2	12	1	16	1	12	1	22	2	13	3	11	1
498	66	5	89	3	69	1	88	2	71	2	94	4	87	2
499	79	1	76	1	81	3	77	0	81	2	78	0	78	0

500	23	1	25	1	24	1	24	1	24	1	25	0	24	0
501	32	1	35	3	33	0	33	1	35	0	34	1	32	1
502	16	3	20	1	18	1	20	1	16	1	15	4	19	3
503	68	3	65	2	67	3	63	1	69	2	63	4	63	0
504	95	2	98	2	97	1	96	2	97	1	96	2	95	3
505	5	0	15	1	7	1	15	1	7	0	17	1	15	1
506	5	0	8	0	6	0	7	0	5	0	8	1	7	0
507	27	1	42	4	30	1	41	3	28	2	35	2	35	3
508	101	3			110	4			101	2				
509	71	0	48	3	71	1	56	4	72	2	45	2	44	1
510	69	2	131	6	68	3	119	2	71	5	137	4	140	2
511	82	1	70	1	73	0	68	1	83	1	71	0	74	1
512	103	2	106	5	105	2	102	4	100	2	102	0	107	6
513	66	1	64	1	64	1	65	2	65	2	63	0	67	1
514	83	2	84	2	83	1	82	1	82	1	81	0	82	2
515	171	2	170	0	168	3	170	1	171	0	171	0	170	1
516	97	1	101	3	84	1	97	2	97	1	102	1	108	3
517	15	4	15	1	17	1	18	2	12	1	13	4	14	2
518	107	5	107	1	108	4	109	4	102	2	103	0	110	4
519	44	1	45	1	42	1	48	2	43	0	46	1	42	1
520	13	1	13	0	13	1	13	1	12	1	13	0	11	0
521	12	1	13	0	15	1	14	1	10	1	12	1	14	1
522	115	1	117	2	117	2	117	2	118	1	116	1	118	2
523	11	1	12	1	11	1	12	0	10	0	12	0	12	1
524	2	0	2	1	2	0	2	0	3	0	2	0	2	0
525	53	2	53	1	53	1	55	2	50	1	55	1	56	3
526	11	0	13	0	12	0	13	1	9	0	10	1	14	2
527	64	2	60	4	63	2	62	2	61	2	65	3	67	4
528	113	5	118	1	115	1	116	4	107	2	116	3	117	2
529	46	3	50	3	47	2	49	3	39	2	40	3	50	3
530	26	2	28	1	26	1	28	2	25	1	26	2	28	3
531	70	1	67	3	67	0	65	1	67	1	69	2	71	3
532	144	3	143	2	144	3	142	3	145	2	141	2	145	2
533	85	4	92	6	88	2	98	2	84	2	87	3	94	5
534	98	0	97	1	97	3	98	1	98	1	97	1	98	0
535	103	2	103	1	100	3	98	2	99	2	101	2	102	5
536	115	4	111	2	114	2	113	5	111	2	117	0	114	2
537	45	8	50	1	56	5	57	7	47	4	47	9	47	3
538	83	4	85	2	82	0	83	2	86	2	80	5	80	2
539	48	1	48	2	44	1	44	5	49	5	45	1	46	2
540	104	1	104	1	102	1	103	1	101	0	103	1	104	1

541	29	3	26	2	28	1	29	2	24	1	22	1	30	1
542	36	1	33	2	37	1	35	1	32	1	28	3	36	2
543	130	3	113	3	127	4	113	1	127	1	112	2	112	1
544	36	0	15	1	32	1	16	1	41	2	24	2	15	2
545	16	1	8	1	14	2	9	1	20	1	11	3	9	0
546	138	2	131	1	136	1	132	2	138	3	136	4	133	3
547	97	4	107	5	106	1	108	2	94	3	94	4	108	5
548	36	3	42	3	38	3	40	3	38	2	42	1	42	1
549	73	1	73	1	76	2	72	2	60	1	61	1	70	2
550	39	3	40	3	47	1	40	2	108	1	105	1	36	3
551	19	2	18	2	18	2	19	1	19	2	20	1	17	1
552	37	4	46	2	36	1	44	3	45	1	50	3	46	4
553	67	4	66	5	74	2	67	2	39	2	35	2	60	4
554	15	3	18	3	17	2	14	1	10	1	20	1	17	1
555	29	2	32	1	28	0	32	2	26	2	27	1	33	1
556	8	1	11	0	8	1	11	1	8	0	9	0	12	1
557	19	0	24	3	20	2	27	2	21	2	23	2	26	1
558	23	1	18	1	22	1	16	2	19	3	19	2	16	2
559	1	0	1	0	1	0	1	0	1	1	1	0	1	0
560	41	2	98	3	47	5	104	2	48	3	95	2	101	4
561	17	1	14	0	15	1	14	1	21	1	13	0	12	1
562	22	1	22	1	21	1	22	1	23	0	23	2	19	0
563	14	1	26	1	14	1	25	1	15	1	26	1	28	2
564	97	2	106	3	88	2	105	3	99	2	108	1	108	1
565	84	1	88	1	89	1	88	1	81	1	87	1	85	2
566	7	1	6	0	8	1	6	1	5	0	5	0	6	0
567	55	1	48	2	56	1	47	3	51	1	48	3	47	2
568	5	1	6	0	4	0	6	1	6	0	6	0	6	1
569	17	1	16	1	18	1	16	1	19	1	16	1	14	0
570	2	1	2	0	2	0	2	0	2	1	2	0	2	0
571	18	0	16	1	18	1	16	1	17	0	17	1	16	2
572	13	2	22	2	12	1	23	2	21	1	13	3	18	3
573	34	5	36	1	37	4	37	1	25	3	42	0	45	4
574	26	1	21	2	27	0	22	1	17	2	25	5	24	3
575	45	4	42	3	44	2	42	2	52	1	42	1	48	4
576	64	2	62	1	65	2	65	0	62	1	64	0	64	0
577	127	9	141	4	130	10	138	4	124	7	141	6	152	8
578	30	3	35	3	30	4	33	3	30	2	37	1	36	3
579	70	2	75	2	65	5	76	4	79	3	77	1	77	4
580	112	1	107	2	113	1	106	0	105	1	105	1	105	2
581	129	2	122	1	129	1	122	1	127	1	121	0	120	1

582	34	1	34	2	32	1	34	2	41	0	34	1	34	2
583	29	2	30	2	33	3	28	1	25	1	30	2	29	2
584	117	2	121	3	119	1	121	5	114	2	119	0	118	1
585	83	2	84	2	83	2	83	2	88	1	84	2	82	0
586	9	1	8	1	9	1	7	0	7	1	9	0	8	1
587	18	3	24	1	18	2	23	2	19	2	26	1	24	2
588	75	1	87	3	74	2	88	0	76	2	86	3	84	1
589	36	2	34	0	36	1	35	2	33	1	34	0	34	0
590	5	0	4	0	5	0	5	0	5	0	5	1	5	0
591	15	1	13	1	14	0	12	1	12	1	14	0	13	1
592	37	1	41	0	38	1	42	1	36	0	42	1	40	1
593	141	1	138	1	141	1	138	1	141	0	139	1	140	0
594	81	2	83	5	82	2	87	1	81	1	86	1	84	1
595	18	1	17	1	18	0	17	0	18	1	17	1	23	1
596	48	4	50	2	48	2	50	2	45	4	53	4	54	1
597	122	2	118	3	122	1	114	2	121	1	116	0	121	2
598	68	2	64	1	71	1	62	2	66	2	65	1	67	3
599	10	0	8	0	9	1	8	1	10	0	8	0	8	1
600	19	2	20	1	18	0	20	0	18	1	21	1	20	1
601	5	0	6	0	4	0	6	1	5	1	6	0	6	0
602	10	1	10	1	11	1	11	1	12	0	11	0	11	3
603	13	2	18	3	13	1	20	1	20	2	17	1	22	2
604	16	1	18	1	16	1	19	0	19	0	20	0	18	1
605	50	5	47	3	47	1	45	0	52	4	44	3	47	3
606	95	3	88	2	96	1	84	2	88	1	84	1	84	2
607	77	3	69	2	76	5	71	2	72	2	73	5	71	4
608	99	2	99	2	99	2	98	3	95	3	100	1	97	0
609	20	4	22	0	20	2	23	1	23	0	23	2	22	2
610	6	0	5	0	6	1	5	0	6	0	5	1	5	0
611	109	3	111	1	110	1	114	2	109	1	113	0	115	1
612	105	3	110	1	105	1	110	2	105	1	114	2	107	2
613	23	1	21	0	22	0	21	0	22	0	22	1	21	1
614	72	1	75	1	71	1	74	1	72	2	73	0	75	2
615	53	2	57	1	53	1	56	1	51	1	57	1	56	1
616	4	0	4	0	4	0	4	0	4	0	4	0	4	0
617	7	0	8	1	7	1	8	1	8	0	7	0	8	0
618	11	2	8	1	10	1	8	1	10	3	8	1	8	1
619	6	0	5	0	5	0	5	0	6	0	5	0	5	1
620	79	3	75	2	78	2	73	2	80	1	72	4	70	2
621	131	2	126	2	132	2	128	2	133	2	128	1	129	1
622	7	0	6	0	7	1	6	1	7	1	6	1	7	0

623	42	1	40	1	43	1	42	1	43	0	42	1	41	1
624	55	1	56	1	54	1	55	1	55	1	53	0	54	2
625	105	5	104	0	102	3	97	4	103	2	99	3	97	2
626	40	3	37	2	36	1	37	3	36	1	36	1	37	2
627	84	2	82	1	85	1	82	2	88	0	81	0	81	2
628	18	0	16	1	18	0	17	1	19	1	17	0	17	1
629	59	1	58	0	58	1	58	1	60	1	58	0	59	1
630	54	1	53	1	58	3	55	3	61	0	54	0	53	2
631	81	1	80	1	81	1	81	0	82	1	81	0	81	0
632	96	4	93	1	94	2	95	2	92	1	94	0	95	1
633	27	4	20	2	24	1	23	3	25	3	21	1	21	2
634	111	2	106	2	110	2	111	6	113	1	108	0	110	1
635	111	2	111	1	112	0	114	1	111	0	113	1	113	0
636	101	8	93	7	88	5	86	10	91	4	90	1	89	2
637	128	8	119	3	130	4	121	5	125	2	124	0	124	5
638	95	6	82	3	99	4	90	5	91	4	92	3	90	3
639	88	6	93	3	86	3	97	6	87	6	97	8	99	2
640	121	10	110	7	108	4	102	7	94	5	105	14	97	1
641	38	5	31	4	38	5	28	5	29	6	30	4	27	3
642	55	6	57	0	60	2	65	3	61	4	64	8	66	1
643	136	2	134	2	129	6	135	3	138	5	129	10	133	2
644	68	20	40	4	59	4	32	3	57	8	37	15	31	2
645	36	4	36	2	38	4	33	3	34	3	34	1	33	1
646	45	2	43	2	49	3	42	1	48	3	43	2	41	2

Table S3. Hydrogen bonds between ATP and its binding site residues averaged over all simulations for all constructs considered in this work. Numbers give the fraction of simulation time (±SD) each hydrogen bond was maintained.

Donor	Acceptor	wt-NBD1	F508del-NBD1	V510D-NBD1	V510D/F508del-NBD1	3M-NBD1	3M/F508del-NBD1	F508del/BIA
ALA462-Main-N	ATP-O1B	0.002±0.001	0.007±0.008	0.001±0.000	0.017±0.023	0.043±0.034	0.002±0.001	0.011±0.008
ALA462-Main-N	ATP-O2B		0.001±0.000				0.002±0.000	
ATP-N6	GLU402-Main-O		0.001±0.000					
ATP-N6	GLU402-OE1	0.012±0.011		0.004±0.001		0.001±0.000	0.003±0.000	
ATP-N6	GLU402-OE2	0.001±0.000		0.003±0.000	0.001±0.000	0.002±0.000	0.001±0.001	
ATP-N6	GLU403-Main-O		0.014±0.018					
ATP-N6	GLU403-OE1							0.001±0.000
ATP-N6	GLU474-Main-O					0.001±0.000		
ATP-N6	GLU621-Main-O				0.008±0.000	0.005±0.006	0.003±0.000	0.001±0.000
ATP-N6	GLU621-OE1							0.002±0.000
ATP-N6	GLU621-OE2							0.001±0.000
ATP-N6	GLY404-Main-O						0.010±0.000	
ATP-N6	GLY461-Main-O				0.001±0.000			
ATP-N6	GLY622-Main-N							0.001±0.000
ATP-N6	PRO439-Main-O					0.001±0.000		
ATP-N6	SER466-OG					0.002±0.000		
ATP-N6	SER492-OG				0.001±0.000			0.001±0.000
ATP-N6	THR438-Main-O	0.001±0.000	0.003±0.000	0.002±0.001	0.007±0.004	0.006±0.006	0.003±0.001	0.006±0.000
ATP-N6	TRP401-Main-O	0.001±0.000					0.001±0.000	
ATP-N6	TRP401-NE1					0.001±0.000		
ATP-N6	VAL440-Main-O				0.003±0.000	0.003±0.000		0.001±0.000
ATP-O2'	GLU402-OE1		0.018±0.013					0.003±0.000
ATP-O2'	GLU402-OE2		0.007±0.004					0.001±0.000
ATP-O2'	GLU403-CD		0.001±0.000					
ATP-O2'	GLU403-OE1		0.017±0.021					
ATP-O2'	GLU403-OE2		0.001±0.000					
ATP-O3'	GLU402-OE1		0.020±0.011					0.004±0.000
ATP-O3'	GLU402-OE2		0.006±0.001					
ATP-O3'	GLU403-OE1		0.019±0.025					
ATP-O3'	GLY461-Main-O	0.093±0.006	0.007±0.006	0.109±0.034	0.003±0.001	0.032±0.011	0.004±0.003	0.003±0.002
ATP-O3'	SER466-OG				0.004±0.000	0.001±0.000		
ATP-O3'	THR460-OG1							0.001±0.000
ATP-O3'	VAL440-Main-O	0.002±0.000						
GLN493-NE2	ATP-N7							0.001±0.000
GLN493-NE2	ATP-O1A				0.002±0.000	0.001±0.000		0.002±0.001

GLN493-NE2	ATP-O1G	0.021±004	0.090±0.063	0.013±0.000	0.017±0.008	0.011±0.012	0.177±0.012	0.028±0.023
GLN493-NE2	ATP-O2A					0.003±0.000		
GLN493-NE2	ATP-O2G	0.006±0.003	0.002±0.001	0.008±0.003	0.002±0.000	0.011±0.002	0.001±0.000	0.001±0.000
GLN493-NE2	ATP-O3G				0.003±0.000	0.018±0.015		
GLU402-Main-N	ATP-N6						0.001±0.000	
GLY461-Main-N	ATP-O1A					0.002±0.001		
GLY461-Main-N	ATP-O1B	0.036±0.049	0.016±0.010	0.012±0.000	0.005±0.003	0.130±0.032	0.027±0.029	0.021±0.024
GLY461-Main-N	ATP-O1G					0.024±0.000		
GLY461-Main-N	ATP-O2B		0.002±0.000					
GLY461-Main-N	ATP-O3A					0.010±0.008		
GLY461-Main-N	ATP-O3B	0.321±0.034	0.326±0.040	0.326±0.029	0.330±0.017	0.047±0.016	0.302±0.019	0.351±0.022
GLY463-Main-N	ATP-O1B	0.220±0.037	0.079±0.046	0.250±0.004	0.106±0.010	0.150±0.018	0.036±0.019	0.108±0.033
GLY463-Main-N	ATP-O2B	0.042±0.000	0.009±0.011	0.010±0.000		0.004±0.004	0.018±0.000	0.023±0.000
GLY463-Main-N	ATP-O3A		0.001±0.000			0.001±0.000	0.001±0.000	
GLY463-Main-N	ATP-O5'					0.001±0.001		
LYS464-Main-N	ATP-O1B	0.474±0.096	0.150±0.028	0.570±0.035	0.164±0.012	0.152±0.020	0.133±0.021	0.206±0.037
LYS464-Main-N	ATP-O2A				0.047±0.000			
LYS464-Main-N	ATP-O2B	0.040±0.000	0.003±0.002	0.008±0.000		0.013±0.013	0.018±0.000	0.018±0.000
LYS464-Main-N	ATP-O3A		0.001±0.000			0.001±0.000		
LYS464-NZ	ATP-O1B	0.536±0.037	0.614±0.026	0.543±0.019	0.618±0.057	0.462±0.006	0.584±0.049	0.637±0.024
LYS464-NZ	ATP-O1G	0.628±0.189	0.019±0.024	0.745±0.027	0.020±0.027	0.308±0.039	0.002±0.000	0.037±0.022
LYS464-NZ	ATP-O2B	0.101±0.000	0.038±0.000	0.025±0.000	0.032±0.000	0.018±0.013	0.059±0.000	0.019±0.000
LYS464-NZ	ATP-O3B					0.008±0.0001		
SER466-Main-N	ATP-O2A	0.268±0.042	0.386±0.059	0.270±0.012	0.334±0.045	0.136±0.042	0.460±0.025	0.350±0.030
SER466-OG	ATP-O2A	0.816±0.010	0.811±0.037	0.832±0.019	0.770±0.077	0.354±0.084	0.829±0.010	0.765±0.044
SER466-OG	ATP-O3'					0.006±0.000		
SER466-OG	ATP-O4'	0.007±0.000						
SER573-OG	ATP-O2G					0.001±0.000		
SER605-OG	ATP-O1G					0.002±0.000		
THR438-OG1	ATP-N1	0.002±0.001	0.007±0.000	0.002±0.001		0.001±0.000	0.002±0.001	0.001±0.000
THR460-OG1	ATP-O1A					0.005±0.001		
THR460-OG1	ATP-O1B	0.001±0.000		0.001±0.000	0.020±0.000	0.001±0.000		
THR460-OG1	ATP-O1G	0.114±0.092	0.568±0.015	0.014±0.010	0.584±0.053	0.119±0.063	0.557±0.031	0.536±0.046
THR460-OG1	ATP-O2B				0.001±0.000			
THR460-OG1	ATP-O3B				0.001±0.000	0.003±0.000		
THR460-OG1	ATP-O3G	0.642±0.187	0.051±0.042	0.706±0.071	0.058±0.036	0.392±0.060	0.011±0.011	0.114±0.060
THR465-Main-N	ATP-O1A			0.009±0.000				
THR465-Main-N	ATP-O2A	0.003±0.000	0.004±0.002	0.001±0.000	0.017±0.025	0.003±0.001	0.004±0.003	0.004±0.001
THR465-Main-N	ATP-O2B	0.099±0.023	0.005±0.005	0.103±0.012	0.006±0.007	0.420±0.097	0.002±0.000	0.007±0.005
THR465-Main-N	ATP-O3A					0.038±0.020		
THR465-OG1	ATP-O1A					0.023±0.010		

THR465-OG1	ATP-O1B					0.006±0.000		
THR465-OG1	ATP-O2A				0.004±0.000	0.010±0.010		
THR465-OG1	ATP-O2B					0.284±0.060		
TRP401-NE1	ATP-N3		0.001±0.000					0.001±0.000
TRP401-NE1	ATP-N7		0.002±0.000	0.002±0.001		0.006±0.004	0.001±0.000	
TRP401-NE1	ATP-O3'				0.001±0.000	0.004±0.000		
TRP401-NE1	ATP-O4'							0.001±0.000

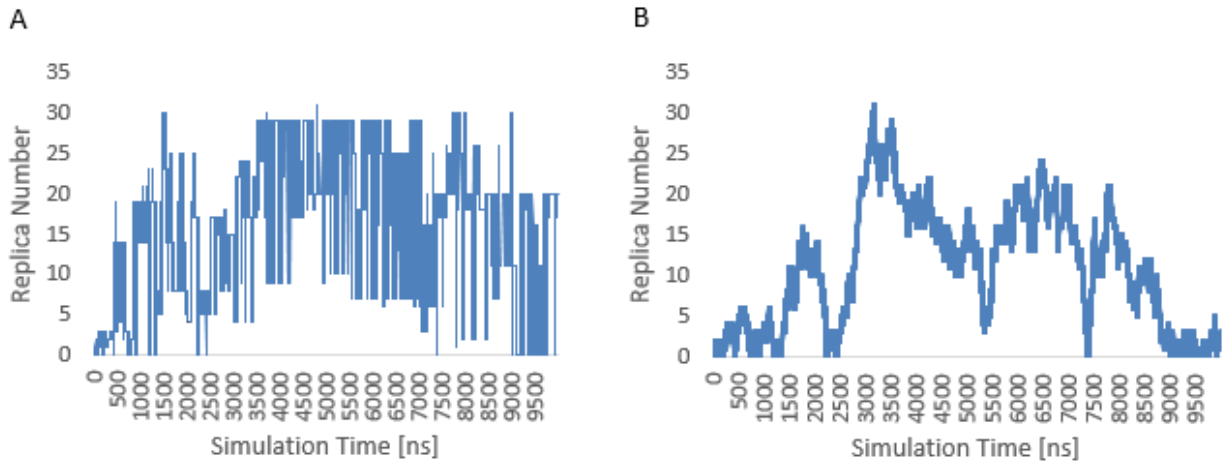

Figure S1. Ground state replicas contribution and round trip of wt-NBD1 (A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

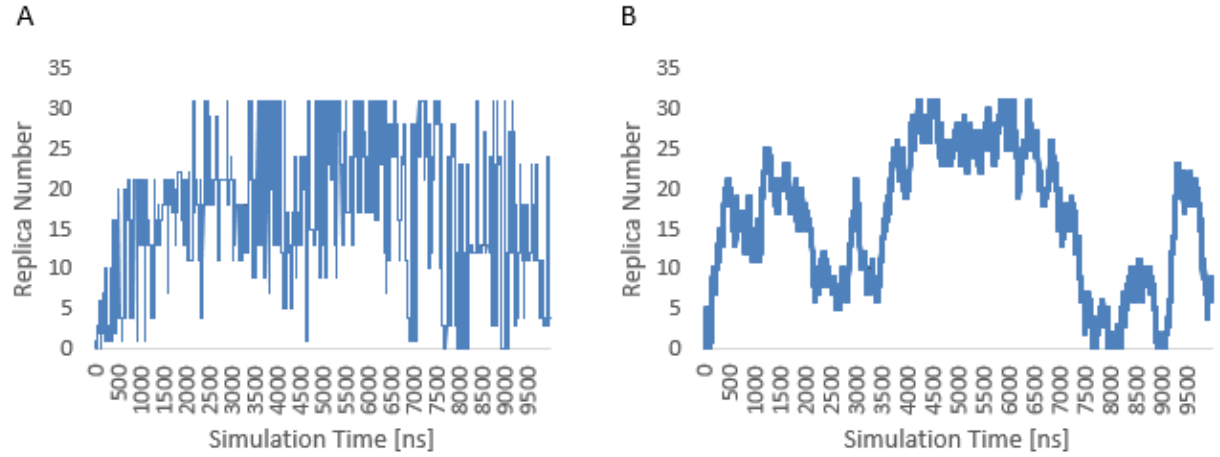

Figure S2. Ground state replicas contribution and round trip of F508del-NBD1 (A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

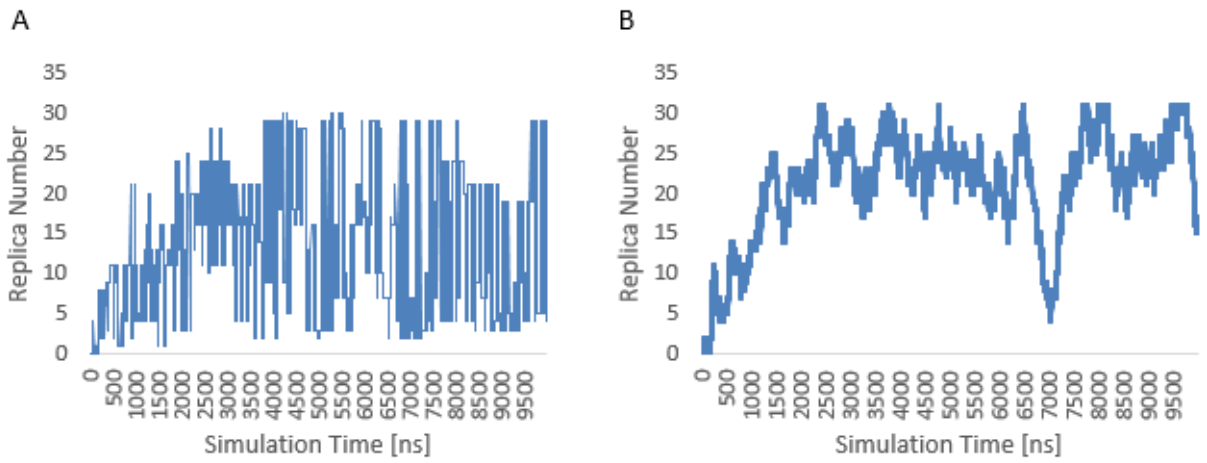

Figure S3. Ground state replicas contribution and round trip of V510D-NBD1 (A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

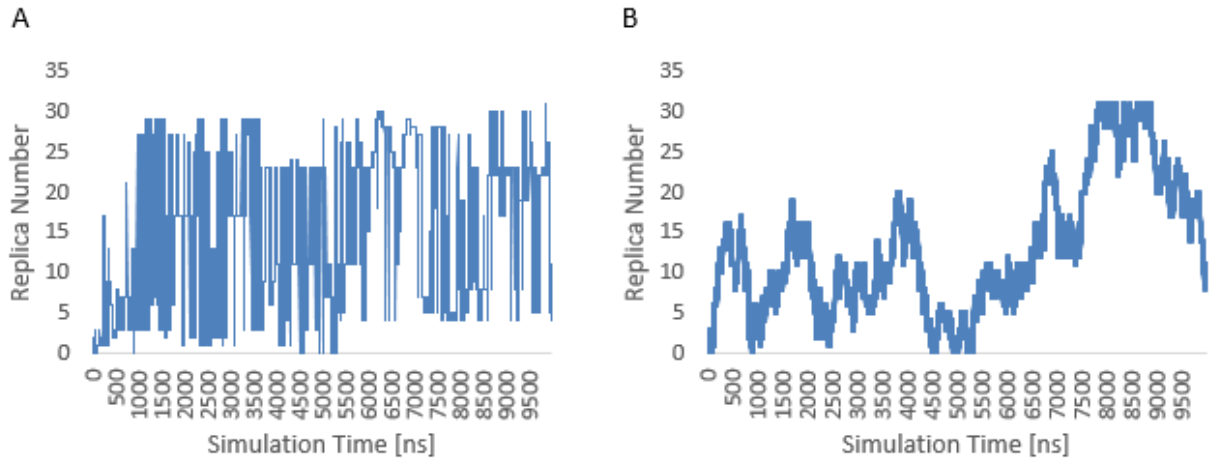

Figure S4. Ground state replicas contribution and round trip of V510D/F508del-NBD1 (A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

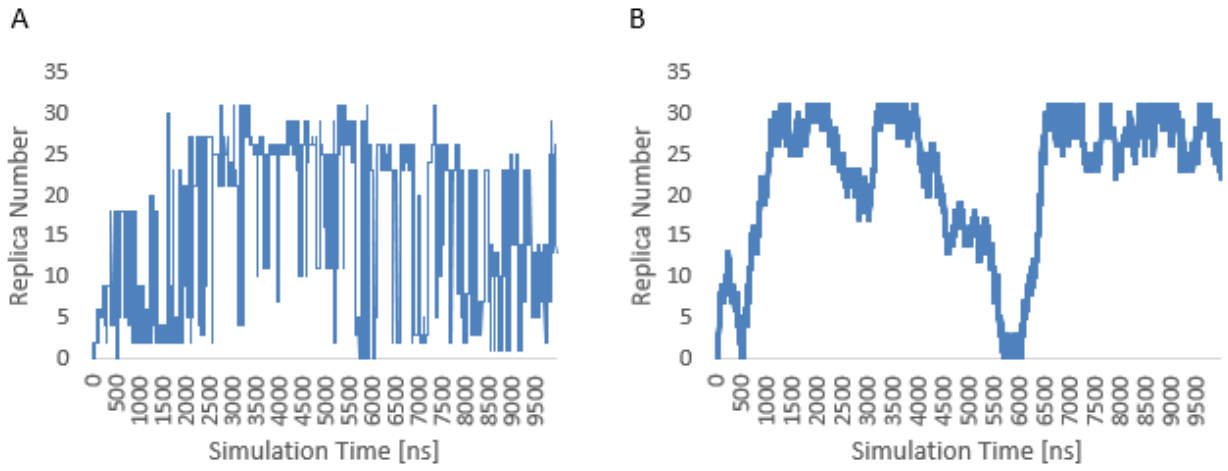

Figure S5. Ground state replicas contribution and round trip of 3M-NBD1 (A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

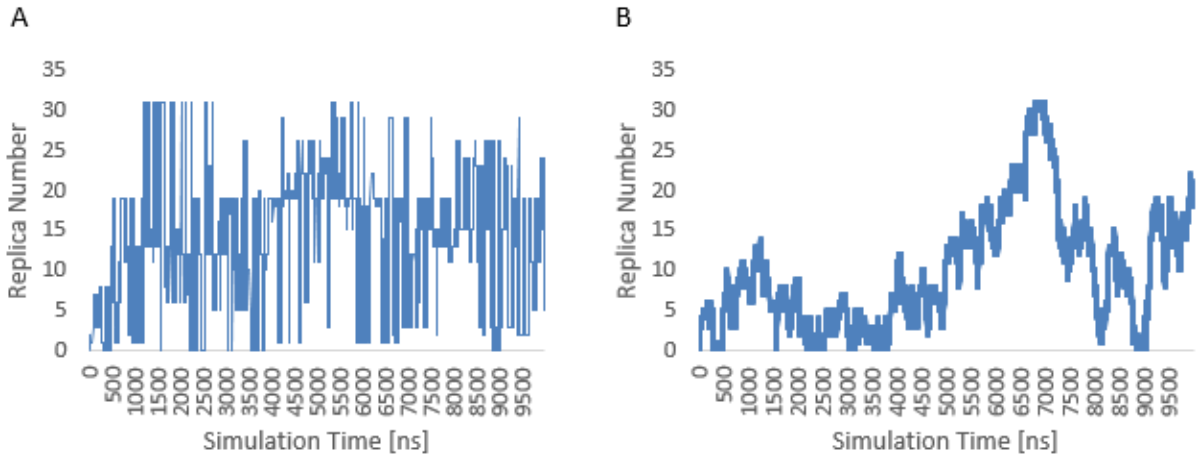

Figure S6. Ground state replicas contribution and round trip of 3M/F508del-NBD1 (A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

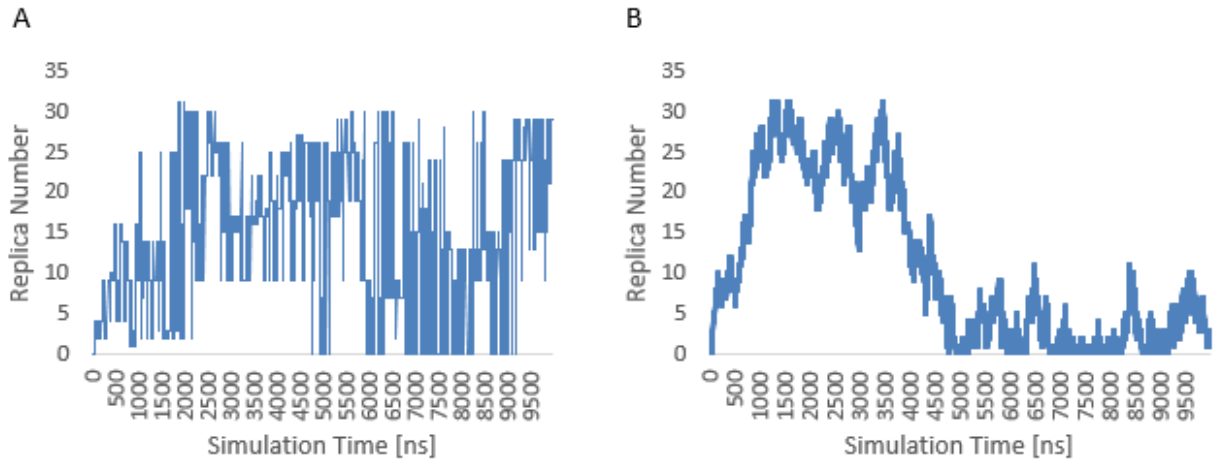

Figure S7. Ground state replicas contribution and round trip of F508del/BIA-NBD1
(A) Contribution of replicas to the analyzed, lowest temperature trajectory. (B) Progress of the lowest temperature replica throughout the simulation.

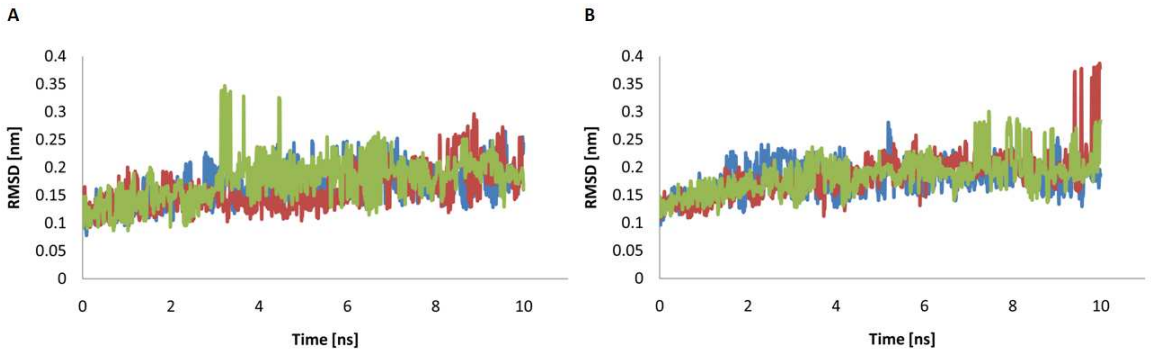

Figure S8. RMSD profiles of wt and F508del-NBD1 (A) REMD-derived RMSD profiles of wt–NBD1 (three repeats). The average RMSD over all simulations, relative to the crystal structure, was 1.68±0.39 Å. (B) REMD-derived RMSD profiles of F508del–NBD1 (three repeats). The average RMSD over all simulations, relative to the crystal structure, was 1.84±0.34 Å

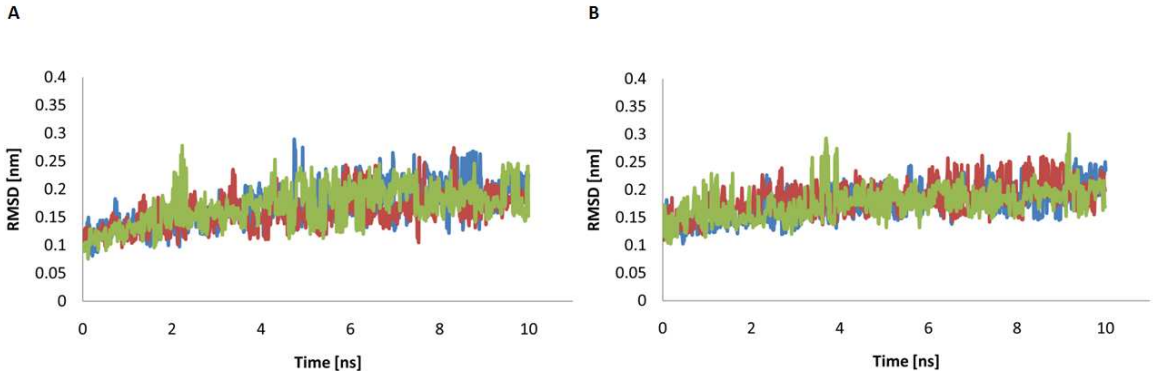

Figure S9. RMSD profiles of V510D and F508del/V510D-NBD1 (A) REMD-derived RMSD profiles for V510D–NBD1 (three repeats). The average RMSD over all simulations, relative to the initial (e.g., crystal structure after the introduction of the V510D mutation; see methods section) structure, was 1.66±0.34 Å. (B) REMD-derived RMSD profiles for F508del/V510D–NBD1 (three repeats). The average RMSD over all simulations, relative to the initial structure, was 1.79±0.29 Å

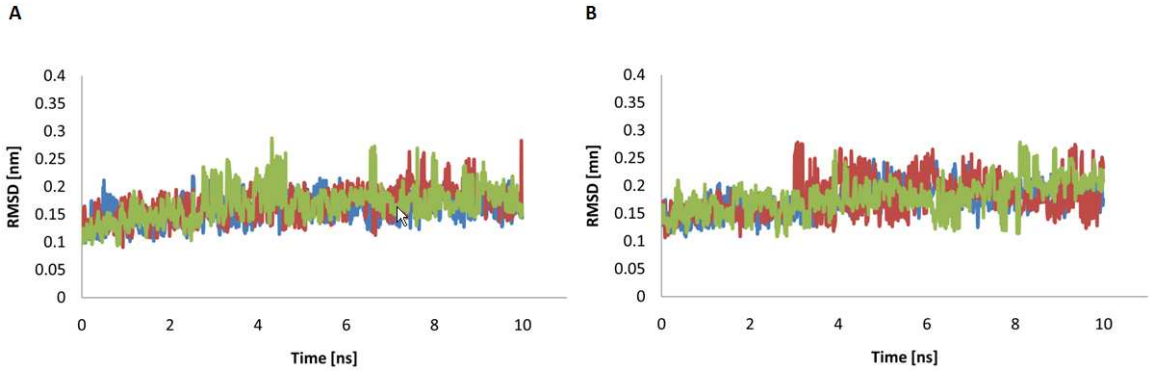

Figure S10. RMSD profiles of 3M and F508del/3M-NBD1. (A) REMD-derived RMSD profiles for 3M–NBD1 (three repeats). The average RMSD over all simulations, relative to the initial (e.g., crystal structure after the introduction of the 3M mutations; see methods section) structure, was 1.65±0.29 Å. (B) REMD-derived RMSD profiles for F508del/3M–NBD1 (three repeats). The average RMSD over all simulations, relative to the initial structure, was 1.77±0.30 Å

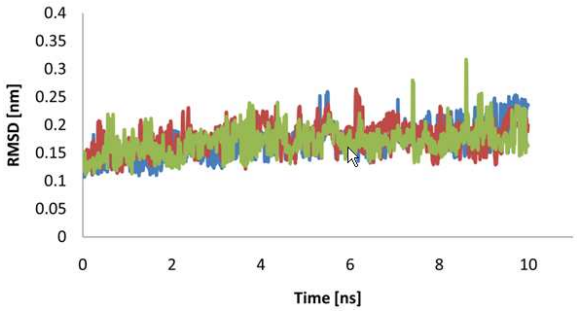

Figure S11. RMSD profile of F508del/BIA-NBD1. REMD-derived RMSD profile for F508del/BIA–NBD1 (three repeats). The average RMSD over all simulations, relative to the initial (e.g., crystal structure after the insertion of BIA; see methods section) structure, was 1.71±0.28 Å.
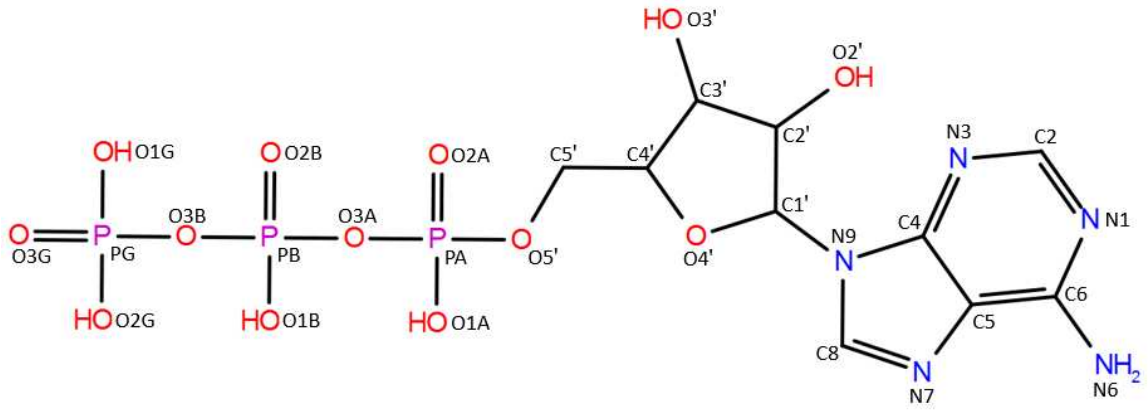
Figure S12. Numbering system of ATP.