Supporting information for: DNA Polymerase λ Active Site Favors a Mutagenic Mispair between the Enol Form of Deoxyguanosine Triphosphate Substrate and the Keto Form of Thymidine Template: A Free Energy Perturbation Study

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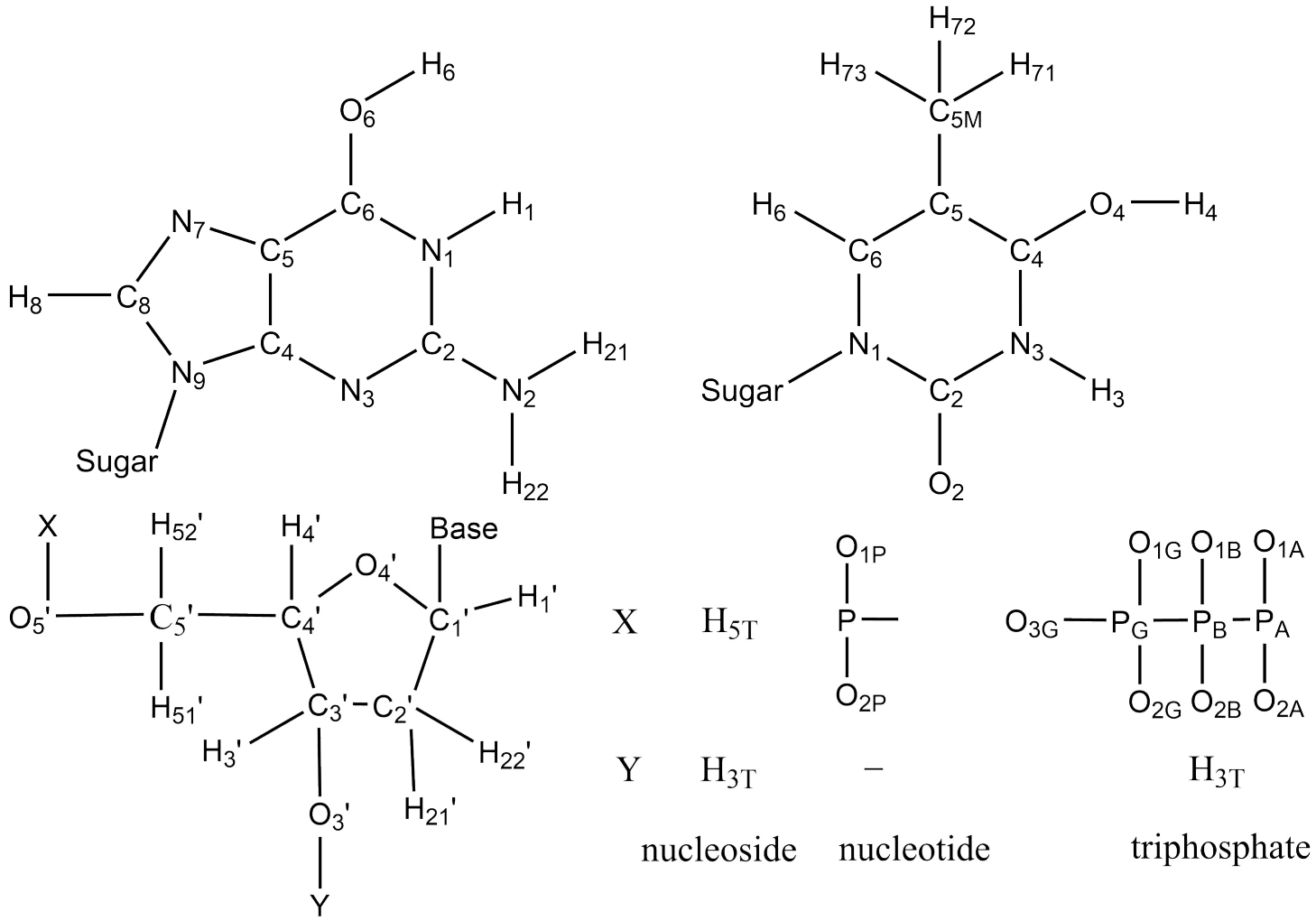

Figure S1: Atom names in nucleosides, nucleotides, and nucleoside triphosphates. The nucleobase moieties for thymine and guanine are shown at the top right and top left corners, respectively. The atom names and atom types in the sugar moiety for the nucleosides, nucleotide residues in DNA, and nucleoside triphosphates are shown at the bottom.

Table S1: Atom types and RESP charges for G, G^*, and G^- nucleosides.

	G		G^*		G^-	
Atom Name	Type	Charge	Type	Charge	Type	Charge
C2	cd	0.766339	ca	0.954444	cd	0.97237
C4	CB	0.177772	CB	0.253992	CB	0.281372
C5	cd	0.176533	ca	0.104201	cd	0.036986
C6	c	0.535321	ca	0.629525	c	0.736036
C8	CK	0.088502	CK	0.098874	CK	0.02945
H1	hn	0.366849	x	0	x	0
H21	hn	0.39767	hn	0.397724	hn	0.361166
H22	hn	0.39767	hn	0.397724	hn	0.361166
H6	x	0	ho	0.452243	x	0
H8	h5	0.201908	h5	0.201736	h5	0.16066
N1	n	−0.570311	nb	−0.805337	nc	−0.874386
N2	nh	−0.916206	nh	−0.952239	nh	−0.977511
N3	nc	−0.634038	nb	−0.720743	nc	−0.84771
N7	nd	−0.566948	nd	−0.554651	nd	−0.561674
N9	N*	0.025302	N*	0.029884	N*	0.014147
O6	o	−0.548128	oh	−0.584266	o	−0.688423
C1’	CT	0.171375	CT	0.142135	CT	0.080896
C2’	CT	−0.039375	CT	−0.047802	CT	−0.113163
C3’	C3	0.065026	C3	0.077455	C3	−0.001311
C4’	CT	0.138705	CT	0.102089	CT	0.115804
C5’	CI	−0.052613	CI	−0.035301	CI	−0.087521
H1’	H2	0.091967	H2	0.112161	H2	0.156467
H2’1	HC	0.045188	HC	0.048172	HC	0.064089
H2’2	HC	0.045188	HC	0.048172	HC	0.064089
H3’	H1	0.172164	H1	0.168975	H1	0.196718
H3T	HO	0.442251	HO	0.441429	HO	0.434045
H4’	H1	0.082871	H1	0.093973	H1	0.066167
H5’1	H1	0.096884	H1	0.09406	H1	0.120463
H5’2	H1	0.096884	H1	0.09406	H1	0.120463
H5T	HO	0.448218	HO	0.444481	HO	0.443971
O3’	OH	−0.657251	OH	−0.656429	OH	−0.649045
O4’	OS	−0.407499	OS	−0.39626	OS	−0.381811
O5’	OH	−0.638218	OH	−0.634481	OH	−0.633971

Table S2: Atom types and RESP charges for G, G^*, and G^- nucleotide residues in DNA.

	G		G^*		G^-	
Atom Name	Type	Charge	Type	Charge	Type	Charge
C2	cd	0.766339	ca	0.954444	cd	0.97237
C4	CB	0.177772	CB	0.253992	CB	0.281372
C5	cd	0.176533	ca	0.104201	cd	0.036986
C6	c	0.535321	ca	0.629525	c	0.736036
C8	CK	0.088502	CK	0.098874	CK	0.02945
H1	hn	0.366849	x	0	x	0
H21	hn	0.39767	hn	0.397724	hn	0.361166
H22	hn	0.39767	hn	0.397724	hn	0.361166
H6	x	0	ho	0.452243	x	0
H8	h5	0.201908	h5	0.201736	h5	0.16066
N1	n	−0.570311	nb	−0.805337	nc	−0.874386
N2	nh	−0.916206	nh	−0.952239	nh	−0.977511
N3	nc	−0.634038	nb	−0.720743	nc	−0.84771
N7	nd	−0.566948	nd	−0.554651	nd	−0.561674
N9	N*	0.025302	N*	0.029884	N*	0.014147
O6	o	−0.548128	oh	−0.584266	o	−0.688423
C1’	CT	0.171375	CT	0.142135	CT	0.080896
C2’	CT	−0.039375	CT	−0.047802	CT	−0.113163
C3’	C3	0.065026	C3	0.077455	C3	−0.001311
C4’	CT	0.138705	CT	0.102089	CT	0.115804
C5’	CI	−0.052613	CI	−0.035301	CI	−0.087521
H1’	H2	0.091967	H2	0.112161	H2	0.156467
H2’1	HC	0.045188	HC	0.048172	HC	0.064089
H2’2	HC	0.045188	HC	0.048172	HC	0.064089
H3’	H1	0.172164	H1	0.168975	H1	0.196718
H4’	H1	0.082871	H1	0.093973	H1	0.066167
H5’1	H1	0.096884	H1	0.09406	H1	0.120463
H5’2	H1	0.096884	H1	0.09406	H1	0.120463
O3’	OS	−0.5232	OS	−0.5232	OS	−0.5232
O4’	OS	−0.407499	OS	−0.39626	OS	−0.381811
O5’	OS	−0.4954	OS	−0.4954	OS	−0.4954
O1P	O2	−0.7761	O2	−0.7761	O2	−0.7761
O2P	O2	−0.7761	O2	−0.7761	O2	−0.7761
P	P	1.1659	P	1.1659	P	1.1659

Table S3: Atom types and RESP charges for G, G^*, and G^- nucleoside triphosphates.

	GTP		G^*TP		G^-TP	
Atom Name	Type	Charge	Type	Charge	Type	Charge
C2	cd	0.766339	ca	0.954444	cd	0.97237
C4	CB	0.177772	CB	0.253992	CB	0.281372
C5	cd	0.176533	ca	0.104201	cd	0.036986
C6	c	0.535321	ca	0.629525	c	0.736036
C8	CK	0.088502	CK	0.098874	CK	0.02945
H1	hn	0.366849	x	0	x	0
H21	hn	0.39767	hn	0.397724	hn	0.361166
H22	hn	0.39767	hn	0.397724	hn	0.361166
H6	x	0	ho	0.452243	x	0
H8	h5	0.201908	h5	0.201736	h5	0.16066
N1	n	−0.570311	nb	−0.805337	nc	−0.874386
N2	nh	−0.916206	nh	−0.952239	nh	−0.977511
N3	nc	−0.634038	nb	−0.720743	nc	−0.84771
N7	nd	−0.566948	nd	−0.554651	nd	−0.561674
N9	N*	0.025302	N*	0.029884	N*	0.014147
O6	o	−0.548128	oh	−0.584266	o	−0.688423
C1'	CT	0.171375	CT	0.142135	CT	0.080896
C2'	CT	−0.039375	CT	−0.047802	CT	−0.113163
C3'	C3	0.065026	C3	0.077455	C3	−0.001311
C4'	CT	0.138705	CT	0.102089	CT	0.115804
C5'	CI	−0.052613	CI	−0.035301	CI	−0.087521
H1'	H2	0.091967	H2	0.112161	H2	0.156467
H2'1	HC	0.045188	HC	0.048172	HC	0.064089
H2'2	HC	0.045188	HC	0.048172	HC	0.064089
H3'	H1	0.172164	H1	0.168975	H1	0.196718
H3T	HO	0.442251	HO	0.441429	HO	0.434045
H4'	H1	0.082871	H1	0.093973	H1	0.066167
H5'1	H1	0.096884	H1	0.09406	H1	0.120463
H5'2	H1	0.096884	H1	0.09406	H1	0.120463
O3'	OH	−0.657251	OH	−0.656429	OH	−0.649045
O4'	OS	−0.407499	OS	−0.39626	OS	−0.381811
O5'	OS	−0.5959	OS	−0.5959	OS	−0.5959
O1A	O2	−0.8799	O2	−0.8799	O2	−0.8799
O1B	O2	−0.8894	O2	−0.8894	O2	−0.8894
O1G	O3	−0.9526	O3	−0.9526	O3	−0.9526
O2A	O2	−0.8799	O2	−0.8799	O2	−0.8799
O2B	O2	−0.8894	O2	−0.8894	O2	−0.8894
O2G	O3	−0.9526	O3	−0.9526	O3	−0.9526
O3A	OS	−0.5689	OS	−0.5689	OS	−0.5689
O3B	OS	−0.5322	OS	−0.5322	OS	−0.5322
O3G	O3	−0.9526	O3	−0.9526	O3	−0.9526
PA	P	1.2532	P	1.2532	P	1.2532
PB	P	1.3852	P	1.3852	P	1.3852
PG	P	1.265	P	1.265	P	1.265

Table S4: Atom types and RESP charges for T, T^*, and T^- nucleosides.

	T		T^*		T^-	
Atom Name	Type	Charge	Type	Charge	Type	Charge
C2	C	0.609813	C	0.861849	C	0.845287
C4	c	0.605625	cd	0.72646	c	0.831097
C5	cc	−0.016757	cd	−0.088752	cc	−0.07027
C5M	c3	−0.226956	c3	−0.211258	c3	−0.267118
C6	CM	−0.256891	CM	−0.189642	CM	−0.263794
H3	hn	0.346267	x	0	x	0
H4	x	0	ho	0.454188	x	0
H6	h4	0.275253	h4	0.283199	h4	0.220855
H71	hc	0.078287	hc	0.075959	hc	0.067931
H72	hc	0.078287	hc	0.075959	hc	0.067931
H73	hc	0.078287	hc	0.075959	hc	0.067931
N1	N*	0.005248	N*	−0.063053	N*	−0.088568
N3	n	−0.508718	nc	−0.808953	n	−0.910954
O2	o	−0.598371	o	−0.643527	o	−0.746896
O4	o	−0.561431	oh	−0.60667	o	−0.706966
C1’	CT	0.048992	CT	−0.001216	CT	−0.020932
C2’	CT	−0.08295	CT	−0.069095	CT	−0.047544
C3’	C3	0.081394	C3	0.083399	C3	−0.009845
C4’	CT	0.17055	CT	0.192492	CT	0.089044
C5’	CI	−0.037817	CI	−0.090148	CI	−0.102829
H1’	H2	0.186339	H2	0.165739	H2	0.200996
H2’1	HC	0.060231	HC	0.066365	HC	0.048456
H2’2	HC	0.060231	HC	0.066365	HC	0.048456
H3’	H1	0.106307	H1	0.160804	H1	0.186994
H3T	HO	0.439073	HO	0.447269	HO	0.426464
H4’	H1	0.113518	H1	0.076024	H1	0.074051
H5’1	H1	0.089481	H1	0.104115	H1	0.127716
H5’2	H1	0.089481	H1	0.104115	H1	0.127716
H5T	HO	0.443101	HO	0.451756	HO	0.436489
O3’	OH	−0.654073	OH	−0.662269	OH	−0.641464
O4’	OS	−0.3887	OS	−0.395678	OS	−0.363745
O5’	OH	−0.633101	OH	−0.641756	OH	−0.626489

Table S5: Atom types and RESP charges for T, T^*, and T^- nucleotide residues in DNA.

	T		T^*		T^-	
Atom Name	Type	Charge	Type	Charge	Type	Charge
C2	C	0.609813	C	0.861849	C	0.845287
C4	c	0.605625	cd	0.72646	c	0.831097
C5	cc	−0.016757	cd	−0.088752	cc	−0.07027
C5M	c3	−0.226956	c3	−0.211258	c3	−0.267118
C6	CM	−0.256891	CM	−0.189642	CM	−0.263794
H3	hn	0.346267	x	0	x	0
H4	x	0	ho	0.454188	x	0
H6	h4	0.275253	h4	0.283199	h4	0.220855
H71	hc	0.078287	hc	0.075959	hc	0.067931
H72	hc	0.078287	hc	0.075959	hc	0.067931
H73	hc	0.078287	hc	0.075959	hc	0.067931
N1	N*	0.005248	N*	−0.063053	N*	−0.088568
N3	n	−0.508718	nc	−0.808953	n	−0.910954
O2	o	−0.598371	o	−0.643527	o	−0.746896
O4	o	−0.561431	oh	−0.60667	o	−0.706966
C1’	CT	0.048992	CT	−0.001216	CT	−0.020932
C2’	CT	−0.08295	CT	−0.069095	CT	−0.047544
C3’	C3	0.081394	C3	0.083399	C3	−0.009845
C4’	CT	0.17055	CT	0.192492	CT	0.089044
C5’	CI	−0.037817	CI	−0.090148	CI	−0.102829
H1’	H2	0.186339	H2	0.165739	H2	0.200996
H2’1	HC	0.060231	HC	0.066365	HC	0.048456
H2’2	HC	0.060231	HC	0.066365	HC	0.048456
H3’	H1	0.106307	H1	0.160804	H1	0.186994
H4’	H1	0.113518	H1	0.076024	H1	0.074051
H5’1	H1	0.089481	H1	0.104115	H1	0.127716
H5’2	H1	0.089481	H1	0.104115	H1	0.127716
O3’	OS	−0.5232	OS	−0.5232	OS	−0.5232
O4’	OS	−0.3887	OS	−0.395678	OS	−0.363745
O5’	OS	−0.4954	OS	−0.4954	OS	−0.4954
O1P	O2	−0.7761	O2	−0.7761	O2	−0.7761
O2P	O2	−0.7761	O2	−0.7761	O2	−0.7761
P	P	1.1659	P	1.1659	P	1.1659

Table S6: Atom types and RESP charges for T, T^*, and T^- nucleoside triphosphates.

	TTP		T^*TP		T^-TP	
Atom Name	Type	Charge	Type	Charge	Type	Charge
C2	C	0.609813	C	0.861849	C	0.845287
C4	c	0.605625	cd	0.72646	c	0.831097
C5	cc	−0.016757	cd	−0.088752	cc	−0.07027
C5M	c3	−0.226956	c3	−0.211258	c3	−0.267118
C6	CM	−0.256891	CM	−0.189642	CM	−0.263794
H3	hn	0.346267	x	0	x	0
H4	x	0	ho	0.454188	x	0
H6	h4	0.275253	h4	0.283199	h4	0.220855
H71	hc	0.078287	hc	0.075959	hc	0.067931
H72	hc	0.078287	hc	0.075959	hc	0.067931
H73	hc	0.078287	hc	0.075959	hc	0.067931
N1	N*	0.005248	N*	−0.063053	N*	−0.088568
N3	n	−0.508718	nc	−0.808953	n	−0.910954
O2	o	−0.598371	o	−0.643527	o	−0.746896
O4	o	−0.561431	oh	−0.60667	o	−0.706966
C1'	CT	0.048992	CT	−0.001216	CT	−0.020932
C2'	CT	−0.08295	CT	−0.069095	CT	−0.047544
C3'	C3	0.081394	C3	0.083399	C3	−0.009845
C4'	CT	0.17055	CT	0.192492	CT	0.089044
C5'	CI	−0.037817	CI	−0.090148	CI	−0.102829
H1'	H2	0.186339	H2	0.165739	H2	0.200996
H2'1	HC	0.060231	HC	0.066365	HC	0.048456
H2'2	HC	0.060231	HC	0.066365	HC	0.048456
H3'	H1	0.106307	H1	0.160804	H1	0.186994
H3T	HO	0.439073	HO	0.447269	HO	0.426464
H4'	H1	0.113518	H1	0.076024	H1	0.074051
H5'1	H1	0.089481	H1	0.104115	H1	0.127716
H5'2	H1	0.089481	H1	0.104115	H1	0.127716
O3'	OH	−0.654073	OH	−0.662269	OH	−0.641464
O4'	OS	−0.3887	OS	−0.395678	OS	−0.363745
O5'	OS	−0.5959	OS	−0.5959	OS	−0.5959
O1A	O2	−0.8799	O2	−0.8799	O2	−0.8799
O1B	O2	−0.8894	O2	−0.8894	O2	−0.8894
O1G	O3	−0.9526	O3	−0.9526	O3	−0.9526
O2A	O2	−0.8799	O2	−0.8799	O2	−0.8799
O2B	O2	−0.8894	O2	−0.8894	O2	−0.8894
O2G	O3	−0.9526	O3	−0.9526	O3	−0.9526
O3A	OS	−0.5689	OS	−0.5689	OS	−0.5689
O3B	OS	−0.5322	OS	−0.5322	OS	−0.5322
O3G	O3	−0.9526	O3	−0.9526	O3	−0.9526
PA	P	1.2532	P	1.2532	P	1.2532
PB	P	1.3852	P	1.3852	P	1.3852
PG	P	1.265	P	1.265	P	1.265

Table S7: Charge states of residues in the pol λ/DL complex with the gapped DNA. AMBER nomenclature for the residue is used.[S1] GLU0, ASP0, LYS0, ARG0, DG0, DC0, DA0, DC50, DG30, DG50 stand for GLU, ASP, LYS, ARG, DG, DC, DA, DC5, DG3, DG5 whose charges are neutralized.

Protein sequence in pol λ/DL.

NALA-1	THR-2	ASN-3	HIE-4	ASN-5	LEU-6	HIE-7	ILE-8	THR-9	GLU0-10
LYS0-11	LEU-12	GLU-13	VAL-14	LEU-15	ALA-16	LYS-17	ALA-18	TYR-19	SER-20
VAL-21	GLN-22	GLY-23	ASP0-24	LYS-25	TRP-26	ARG0-27	ALA-28	LEU-29	GLY-30
TYR-31	ALA-32	LYS-33	ALA-34	ILE-35	ASN-36	ALA-37	LEU-38	LYS-39	SER-40
PHE-41	HID-42	LYS0-43	PRO-44	VAL-45	THR-46	SER-47	TYR-48	GLN-49	GLU0-50
ALA-51	CYS-52	SER-53	ILE-54	PRO-55	GLY-56	ILE-57	GLY-58	LYS0-59	ARG0-60
MET-61	ALA-62	GLU0-63	LYS0-64	ILE-65	ILE-66	GLU0-67	ILE-68	LEU-69	GLU0-70
SER-71	GLY-72	HIE-73	LEU-74	ARG0-75	LYS0-76	LEU-77	ASP0-78	HID-79	ILE-80
SER-81	GLU0-82	SER-83	VAL-84	PRO-85	VAL-86	LEU-87	GLU-88	LEU-89	PHE-90
SER-91	ASN-92	ILE-93	TRP-94	GLY-95	ALA-96	GLY-97	THR-98	LYS0-99	THR-100
ALA-101	GLN-102	MET-103	TRP-104	TYR-105	GLN-106	GLN-107	GLY-108	PHE-109	ARG0-110
SER-111	LEU-112	GLU0-113	ASP0-114	ILE-115	ARG0-116	SER-117	GLN-118	ALA-119	SER-120
LEU-121	THR-122	THR-123	GLN-124	GLN-125	ALA-126	ILE-127	GLY-128	LEU-129	LYS-130
HIP-131	TYR-132	SER-133	ASP-134	PHE-135	LEU-136	GLU-137	ARG-138	MET-139	PRO-140
ARG-141	GLU-142	GLU-143	ALA-144	THR-145	GLU-146	ILE-147	GLU-148	GLN-149	THR-150
VAL-151	GLN-152	LYS0-153	ALA-154	ALA-155	GLN-156	ALA-157	PHE-158	ASN-159	SER-160
GLY-161	LEU-162	LEU-163	CYS-164	VAL-165	ALA-166	CYS-167	GLY-168	SER-169	TYR-170
ARG-171	ARG-172	GLY-173	LYS-174	ALA-175	THR-176	CYS-177	GLY-178	ASP-179	VAL-180
ASP-181	VAL-182	LEU-183	ILE-184	THR-185	HIE-186	PRO-187	ASP0-188	GLY-189	ARG0-190
SER-191	HIE-192	ARG0-193	GLY-194	ILE-195	PHE-196	SER-197	ARG0-198	LEU-199	LEU-200
ASP0-201	SER-202	LEU-203	ARG-204	GLN-205	GLU0-206	GLY-207	PHE-208	LEU-209	THR-210
ASP-211	ASP-212	LEU-213	VAL-214	LYS-215	GLY-216	GLU-217	THR-218	LYS-219	TYR-220
LEU-221	GLY-222	VAL-223	CYS-224	ARG0-225	LEU-226	PRO-227	GLY-228	PRO-229	GLY-230
ARG-231	ARG-232	HIE-233	ARG-234	ARG-235	LEU-236	ASP-237	ILE-238	ILE-239	VAL-240
VAL-241	PRO-242	TYR-243	SER-244	GLU-245	PHE-246	ALA-247	CYS-248	ALA-249	LEU-250
LEU-251	TYR-252	PHE-253	THR-254	GLY-255	SER-256	ALA-257	HIE-258	PHE-259	ASN-260
ARG-261	SER-262	MET-263	ARG-264	ALA-265	LEU-266	ALA-267	LYS0-268	THR-269	LYS0-270
GLY-271	MET-272	SER-273	LEU-274	SER-275	GLU-276	HIP-277	ALA-278	LEU-279	SER-280
THR-281	ALA-282	VAL-283	VAL-284	ARG0-285	ASN-286	THR-287	HID-288	GLY-289	CYS-290
LYS0-291	VAL-292	GLY-293	PRO-294	GLY-295	ARG0-296	VAL-297	LEU-298	PRO-299	THR-300
PRO-301	THR-302	GLU-303	LYS-304	ASP0-305	VAL-306	PHE-307	ARG0-308	LEU-309	LEU-310
GLY-311	LEU-312	PRO-313	TYR-314	ARG-315	GLU-316	PRO-317	ALA-318	GLU-319	ARG-320
ASP-321	CTRP-322								

Template and primer strands in the gapped DNA.

DC50-1	DG0-2	DG0-3	DC0-4	DT-5	DG-6	DT-7	DA-8	DC-9	DT-10	DG30-11
DG50-12	DC0-13	DC0-14	DG30-15	···	DC50-16	DA0-17	DG0-18	DT-19	DA-20	DC3-21

Table S8: Contributions to $\Delta\Delta G_{w\to p}$ (in kcal $\cdot$ mol^{-1}) along the block III and the other blocks of FEP paths in fig. 4.

Block	III	I+II+IV+V
$G \cdot T \to G^- \cdot T$	2.22	−0.38
$G \cdot T \leftarrow G^- \cdot T$	3.45	0.44
$G \cdot T \to G^* \cdot T$	−7.54	0.04
$G \cdot T \leftarrow G^* \cdot T$	4.65	0.41
$G \cdot T \to G \cdot T^-$	8.36	0.10
$G \cdot T \leftarrow G \cdot T^-$	−10.74	−0.08
$G \cdot T \to G \cdot T^*$	−8.64	−0.31
$G \cdot T \leftarrow G \cdot T^*$	9.76	0.42
$G \cdot T^- \to G \cdot T^*$	−18.82	−0.51
$G \cdot T^- \leftarrow G \cdot T^*$	20.23	0.15
$G^* \cdot T \to G^- \cdot T$	7.43	−0.09
$G^* \cdot T \leftarrow G^- \cdot T$	−6.73	0.60

References

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