Structural Basis of Human NOX5 Activation

Supplementary Information

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Supplementary Figure 1 Cryo-EM image analysis of NOX5 in pre-reaction state and representative cryo-EM density maps. **a.** Cryo-EM data processing flow of NOX5 in pre-reaction state (with NADPH and EGTA). **b.** The cryo-EM densities and models of transmembrane helices

and small molecules. **c.** Fourier shell correlation (FSC) curves between model and map of NOX5 in pre-reaction state.







b



TM4





TM5



inner heme

outer heme





FAD





NADPH

NADPH

С





Supplementary Figure 2 Cryo-EM image analysis of NOX5 in intermediate states and representative cryo-EM density maps. **a.** Cryo-EM data processing flow of NOX5 in intermediate states (with NADPH and Ca²⁺). **b.** The cryo-EM densities and models of transmembrane helices and small molecules (intermediate state 3). **c.** Fourier shell correlation (FSC) curves between model and map of NOX5 in intermediate state 3.

post-reaction state dataset process







TM4

b

TM5 TM6





inner heme

outer heme







NADP+

FAD

Supplementary Figure 3 Cryo-EM image analysis of NOX5 in post-reaction state and representative cryo-EM density maps. **a.** Cryo-EM data processing flow of NOX5 in post-reaction state (with NADP+ and Ca²⁺). **b.** The cryo-EM densities and models of transmembrane helices and small molecules. **c.** Fourier shell correlation (FSC) curves between model and map of NOX5 in post-reaction state.



Supplementary Figure 4 Structural features of NOX5. **a.** Domain architecture of human NOX5. **b.** Protomer structure of human NOX5. **c.** Structural comparison between human NOX5 and DUOX1 catalytic cores. NOX5 is colored and DUOX1 is in grey. **d.** The transmembrane domain of human NOX5 and the heme-coordinating residues. **e.** The electron transfer pathway in NOX5: NADPH – FAD – inner heme – outer heme - oxygen. Side chains of residues between two hemes are shown. **f.** The cryo-EM densities of lipid and NADPH for pre-reaction state, intermediate state

(consensus model) and post-reaction state. Lipid or alkyl chains are colored in red. NADPH or NADP+ is colored in green. **g.** The lipid-binding pocket of *cs*NOX5 and mouse DUOX1. Lipid or alkyl chains are colored in red. **h.** Structural comparison of preTM1 orientation between *cs*NOX5, mouse DUOX1 and human NOX5. *cs*NOX5 is colored in pale green. Mouse DUOX1 is colored in blue. Pre-reaction and post-reaction states of human NOX5 are colored in magenta and orange, respectively. PreTM1 is indicated with red arrow. **i.** Unique sequence insertions in the DH domain. **j.** Structural details of the cytosolic DH domain.

а				TA	11 2 linkor				_
	preTM1		TM1			T	M2	S2-3a	
$n0x5 \sqrt{2}$	I TRAYWHNHR	SOL ECLATYA	GLHVLLEGLA	ASAHRD	I GAS	VMVAKGCGOC	I NEDCSELAV		250
DUOX1	OFKRELENYR	RHIGCVAVEY	ALAGGLELER	AYYYAFAAHH	TGITDTTRVG	LILSEGTAAS	ISEMESYLLL	TMCRNLITEL	1112
DUOX2	QYKREVENYR	RHIVCVALES	ALCVGVFADR	AYYYGFASPP	SDIAQTTLVG	LILSRGTAAS	VSEMESYILL	TMCRNLITEL	1109
nox1	GNWVVNHW	FSVLFLVVWL	GLNVELEVDA	FLKYEKADKY	YYTRKILGST	LACARASALC	LNENSTLILL	PVCRNLLSFL	79
nox2	GNWAVNEG	LSIEVILVWL	GLNVFLFVWY	YRVYDIPPKF	FYTRKLLGSA	LALARAPAAC	LNENCMLILL	PVCRNLLSFL	79
nox3	- MGCWILNEG	LSTILVLSWL	GINFYLFIDT	FYWYEEEESF	HYTRVILGST	LAWARASALC	LNFNCMLILI	PVSRNLISFI	80
nox4	-WRSWLANEG	VKHLCLFIWL	SMN VL L FWK T	FLLYNQGPEY	HYLHQMLGLG	LCLSRASASV	LNLNCSLILL	PMCRTLLAYL	83
				7140			TM3-4 linke	r	
	-(S2-3b)			TM3					•
nox5_v2	RATWLAQVLP	LDQNIQ	FHQLMGYVVV	GLSLVHTVAH	TVNFVLQAQA	EASPFQFWEL	LLTTRPGIGW	VHGS	320
DUOX1	RETFLNRYVP	FDAAVD	FHRLIASTAI	VLTVLHSVGH	VVNVYLFSIS	PLSVLSCLFP	GLFHDDGSEL	PQKYY	1183
DUOX2	RETFLNRYVP	FDAAVD	FHRWIAMAAV	VLAILHSAGH	AVNVYIFSVS	PLSLLACIFP	NVFVNDGSKL	PQKFY	1180
nox1	RGICSFCSRI	LRKQLDHNLT	FHKLVAYMIC	LHIAIHIIAH	LENEDCYSRS	RQAIDGSLAS	ILSSLSHDEK	KGGSWLNPTQ	159
nox2	RGSSACCSTR	VRRQLDRNLT	FHKMVAWMTA	LHSAIHIIAH	LENVEWCVNA	RVNNSDPYSV	ALSELG DR	QNESYLNFAR	157
nox3	RGISICCRGP	WRRQLDKNLR	FHKLVAYGIA	VNATTHIVAH	FFNLERYHWS	QSEEAQGLLA	ALSKLGNI	PNESYLNPVR	158
nox4	RGSQKVPSRR		FHITCGVIIC	TESGVHVAAH	LVNALNFSVN	YSEDFVELNA	ARYRDEDPRK.	TM5-6 linker	153
	11/13-4 11	iker	TM4			TM5		TWD-0 IIIIKEI	•
nox5 v2		ASP	TGVALLLLL	LMFICSSSCI	RRSGHEEVEY	WTHLSYLLVW	LLLIFHG		370
DUOX1		WWFFQTVPGL	TGVVLLLILA	IMYVFASHHF	RRR - SFRGFW	LTHHLYILLY	VLLIIHGSFA	L 10	1245
DUOX2		WWFFQTVPGM	TGVLLLLVLA	IMYVFASHHF	RRR-SFRGFW	LTHHLYILLY	ALLIHGSYA	L 10	1242
nox1	SRNTTVE	YVTFTSIAGL	TGVIMTIALI	LMVTSATEFI	RRS-YFEVFW	YTHHLFIFYI	LGLGIHGIGG	IVRGQTEESM	235
nox2	KRIKNPEGGL	YLAVTLLAGI	TGVVITLCLI	LIITSSTKTI	RRS-YFEVFW	YTHHLEVIEE	IGLAIHGAER	IVRGQTAESL	236
nox3	TFPTNTTTEL	LRTIAGV	TGLVISLALV	LIMTSSTEFI	RQA-SYELFW	YTHHVFIVFF	LSLAIHGTGR	IVRGQTQDSL	234
nox4		- LLFTTVPGL	TGVCMVVVLF	LMITASTYAI	RVS-NYDIFW	YTHNLFFVFY	MLLTLHVSGG	LLKYQTNLDT	221
		TN	45-6 linker						
						_	IN	б	
nox5_v2						P	NFWKWLLVPG	ILFFLEKAIG	391
DUOX1						LP	RFHIFFLVPA	IIYGGDKLVS	1267
DUOX2						LP	TFHIYFLVPA	ITYGGDKLVS	1264
nox1	NESHPRKCAE			SF	EMWDDRDSHC	RRPKFEGHPP	ESWKWILAPV	ILYICERILR	287
nox2	AVHNITVCEQ			KI	SEWG-KIKEC	PIPQFAGNPP	MTWKWIVGPM	FLYLCERLVR	287
nox3	SLHNITFCRD			RY	AEWQ-TVAQC	PVPQFSGKEP	SAWKWILGPV	VLYACERIIR	285
nox4	HPPGCISLNR	ISSQNISLPE	YFSEHFHEPF	PEGESKPAEF	TQHKEVKICM	EEPRFQANFP	QTWLWISGPL	CLYCAERLYR	301
nox5_v2	LAVSRMAAVC	IMEVNLLPSK	VTHLLIKRPP	FFHYRPGDYL	YLNIPTIARY	EWHPFTISSA	P-EQKDTIWL	HIRSQGQWTN	470
DUOX1	LSRKKVE - IS	VVKAELLPSG	VTHLRFQRPQ	GFEYKSGQWV	RIACLALGTT	EYHPFTLTSA	PHED TLSL	HIRAAGPWTT	1344
DUOX2	LSRKKVE - IS	VVKAELLPSG	VTYLQFQRPQ	GFEYKSGQWV	RIACLALGTT	EYHPFTLTSA	PHED TLSL	HIRAVGPWTT	1341
nox1	FYRSQQK - VV	ITKVVMHPSK	VLELQMNK-R	GFSMEVGQYI	FVNCPSISLL	EWHPFTLTSA	PEED FFS I	HIRAAGDWTE	363
nox2	FWRSQQK - VV	ITKVVTHPFK	TIELQMKK-K	GFKMEVGQYI	FVKCPKVSKL	EWHPFTLTSA	PEED FFSI	HIRIVGDWTE	363
nox3	FWRFQQE - VV	ITKVVSHPSG	VLELHMKK-R	GFKMAPGQYI	LVQCPAISSL	EWHPFTLTSA	PQED FFSV	HIRAAGDWTA	361
nox4	YIRSNKP-VT	IISVMSHPSD	VME I RMVK - E	NFKARPGQYI	TLHCPSVSAL	ENHPFTLTMC	PTETKATFGV	HLKIVGDWTE	379
			INIS1		Phos				
			DOVITMOKOOD	O OKOO E LL LE	KUKECNUKOV	IDODVOTOTO	DIEACEUAV		FFO
nox5_v2	RLTESFRASU	PLGRGSKRLS	RSVINKKSQR	SSKGSETLLE	KHKFCNIKCT	I DOPTOTPTR	RIFASERAVL	VCCCLCVTPF	550
DUOXI	REREITSAP-			IGDRCAR-	TPKLT	LDGPFGEGHQ	EWHKFEVSVL	VGGGTGVTPF	1395
000/2	REREITSSP-			KGNGCAG-	TPRLT	VDCDFCTACE	EWHKFEVSVL	VGGGTGVTPF	1392
noxi	NL TRAFE			KOEFOD AWK	IPRIE	VDGPFGTASE	DVFQTEVAVL	VGAGIGVIPF	410
nox2	GLENACGOD-			COAL OF DWG	LPRIA	VDGPFGTASE	DVFSTEVVML	VGAGIGVIPF	410
noxs	ALLEAFGAE-			GUAL GEPWS-		UDGPEGERLE	DVFHTPVCVC	VAGGLOVTPE	414
1084	KFRULLEPP-			SSQUSEILPF	TUSKNTPKLT	IDGPFGSFFE	ESLINTEVSLU	VAGGIGVIPF	430
			NS2						
nox5 v2	ASILQSIMYR	HQKRKHTCPS	CQHSWIEGVQ	DNMKLHKVDF	IWINRDQRSF	EWFVSLLTKL	EMDQAEEAQY	GRFLELHMYM	630
DUOX1	ASILKDLVFK	- \$\$	VS	CQVFCKKIYF	IWVTRTQRQF	EWLADII	REVEENDH	QDLVSVHIYI	1454
DUOX2	ASILKDLVFK	- SS	LG	SQMLCKKIYF	IWVTRTQRQF	EWLADII	QEVEENDH	QDLVSVHIYV	1451
nox1	ASILKSIWYK	FQC	AD	HNLKTKKIYF	YWICRETGAF	SWFNNLLTSL	E-QEMEELGK	VGFLNYRLFL	474
nox2	ASILKSVWYK	YCN	NA	TNLKLKKIYF	YWLCRDTHAF	EWFADLLQLL	E-SQMQERNN	AGFLSYNIYL	480
nox3	AALLKSIWYK	CSE	AQ	TPLKLSKVYF	YWICRDARAF	EWFADLLLSL	E - TRMSEQGK	THFLSYHIFL	478
nox4	ASILNTLLDD	WK		- PYKLRRLYF	IWVCRDIQSF	RWFADLLCML	H-NKFWQENR	PDYVNIQLYL	498
		IN	IS3 REFE						
nox5_v2	TSALGKNDMK	AIGLQMALDL	LANKEKKDST	IGLQTRTQPG	RPDWSKVFQK	VAAE KKGK	VQVFFCGSPA	LAKVLKGHCE	708
DUOX1	TQLAEKFDLR	TIMLYICERH	FQKVLNRSLF	IGLESTIFFG	RPPFEPFFNS	LQEVHPQVRK	IGVESCGPPG	MIKNVEKACQ	1534
DUOX2	TQLAEKFDLR	TIMLYTCERH	FQKVLNRSLF	TOLKOKTOFO	RPPFEPFFNS	LQEVHPQVRK	TGVFSCGPPG	MIKNVEKACQ	1531
noxi	TOWDERCOMMU	AALNED	KAIDIV	TGLKQKTSFG	RPMWDNEFST	TATSHPKSV-	VGVFLCGPRI	LAKSLRKCCH	545
nox2	TOWDESQANH	FAVHHD	EEKDVI	TOLKOKTEYO	RPNWUNEFKT	ASUMPNIR-	IGVELCOPEA	LAETLSKQST	551
nox3	GWDENQALH	TALHWD	ENIDVI	IGL KQK I FYG	RENWINEEKQ	AYNHPSSS-	TGVFFCGPKA	LSKILQKMCH	549
nox4	SQIDGIQKII	GERYH		- ALNSKLFIG	RPRWKLLFDE	TAK - YNRGKT	VEVECCOPNS	LSKILHKLSN	201
				-					
nox5 v2	KFG	- FRFFQENF -	719	b					
DUOX1	LINRQD RT	HFSHHYENF -	1551		480 DPLGR	GSKRLSRSVTMRK	SORSSKGSEILLEK	HKF 514	
DUOX2	LVNRQD RA	HEMHHYENE -	1548						
nox1	RYSSLDPRKV	QFYFNKENF -	564		Inot ·		J	. 7+	
nox2	SNSESGPRGV	HFIFNKENF-	570			nannnnnHHH		: Jnet	
nox3	LYSSADPRGV	HFYYNKESF-	568		jnmm :	нннннннннн	1	:jhmm	
nox4	QNNSY GT	RFEYNKESFS	578		jpssm :	ННННННННН		: jpssm	

Supplementary Figure 5 Sequence analysis of NOX5. **a.** Sequence alignment of NOX5 paralogs. Transmembrane helices are labeled as TM1-6. Sequence in green boxes are unique insertions (INS1-3) in the DH domain. PhosR and REFBD are highlighted. Dimer interface residues are indicated by red triangles and last residue of NOX4 is indicated by a red color. **b.** Secondary structure predication of the PhosR segment by the Jpred server¹.

nox5_v2 DUOX1 DUOX2	LLFNLEEERQ LLFSSEEERG	ALVENLRGAL AFVQQLWDFC	KESGLSIQEW VRWALGLHVA	ELREQELMRA EMSEKELFRK	AVT <mark>REQRRHL</mark> AVTKQQRER I	LETFFRHLFS LEIFFRHLFA	QVLDINQADA QVLDINQADA	EF1A RWLRWVTQQF 17 GTLPLDSSQK 793 GTLPLDSSQK 797	3
	— ——	EF1B	EF2	A	EF2	B —	EF3A		
nox5_v2	KT I AGEDGE I	SLQEFKAALH	VKESFFAERF	FALFDSDRSG	TITLQELQEA	LTLLIHGSPM	DKLKFLFQVY	DIDGSGSIDP 97	
DUOX1	VREALTCELS	RAEFAESLGL	KPQDMFVESM	FSLADKDGNG	YLSFREFLDI	LVVFMKGSPE	EKSRLMFRMY	DFDGNGLISK 873	3
DUOX2	VREALTCELS	RAEFAESLGL	KPQDMFVESM	FSLADKDGNG	YLSFREFLDI	LVVFMKGSPE	DKSRLMFTMY	DLDENGFLSK 877	ŗ
	EF3B		EF4A		EF4B				
nox5_v2	DELRTVLQSC	LRESAISLPD	EKLDQLTLAL	FESADADGNG	AITFEELRDE	LQRFPGVMEN	LTISA	162	2
DUOX1	DEFIRMLRSF	IEISNNCLSK	AQLAEVVESM	FRESGFQDKE	ELTWEDFHFM	LRDHNSELRF	TQLCVKGVEV	P-EVIKDLCR 952	2
DUOX2	DEFFTMMRSF	IEISNNCLSK	AQLAEVVESM	FRESGFQDKE	ELTWEDFHFM	LRDHDSELRF	TQLCVKGGGG	GGNGIRDIFK 957	t





NOX5 (pre-reaction vs IS3)

а





REFBD-like motif (α4 of DH domain in DUOX1)







intermediate state 3

Supplementary Figure 6 Conformational changes in EFD. **a.** Sequence alignment of the EFD. The EF hands are labeled as EF1A-EF1B to EF4A-EF4B. **b.** Canonical sequence of an EF hand. Helix A and Helix B are shown. Residue numbers of loop are indicated with 1, 3, 5, 7, 9 and 12. **c.** Cryo-EM densities of EFD with pre-reaction state and intermediate state 3. **d-e.** Comparison of the EFD between NOX5 and DUOX1 upon Ca²⁺ binding. **f.** The movement of REFBD upon activation in NOX5 and DUOX1. **g.** Impact of EFD on the enzymatic activity of NOX5. Curved are plotted (average +/-SD) with 5 technical repeats. Y-axis indicates the generation rate of O_2^- (μ M/ μ M (NOX5)), x-axis indicates the time course (min). Source data are provided as a Source Data file. **h.** Conserved aspartates (D638 and D658) locate in REFBD with intermediate state 3.



Supplementary Figure 7 Dimer interface of NOX5. **a.** Structural details of NOX5 dimer interfaces #1 and #2. **b**. The cryo-EM density of the dimer interface #2 near the zinc-binding site. Top panel and bottom panel show the cryo-EM densities of interface #2 near the zinc-binding site with level threshold values of 0.58 and 0.88 (in ChimeraX), respectively. Zinc density is indicated with red arrow. **c.** Gel filtration curves of NOX5 and interface mutants (R426A, R530A and R531A) using a Superose 6 increase column by monitoring Trp fluorescence. SDS-PAGE of the purified constructs are shown. We couldn't purify the R530A/R531A double mutation. Source data are provided as a Source Data file. **d.** Gel filtration curves of NOX5 and putative zinc-binding cysteine

mutants (C571S and C568S/C571S) using a Superose 6 increase column by monitoring Trp fluorescence. We couldn't purify the C568S mutation. Source data are provided as a Source Data file.



Supplementary Figure 8 Consensus structure of the intermediate state. **a.** Cryo-EM map (left panel) and atomic model (right panel) of the NOX5 intermediate state (consensus model, with C2 symmetry) are shown. **b.** Structural comparison of NOX5 protomers between post-reaction (colored) state and intermediate state 3 (grey).



Supplementary Figure 9 Structural comparison in the catalytic core of NOX5 before and after Ca²⁺ activation. **a.** Distance between NBD and FBD in human NOX5 and DUOX1 structures. Pre-reaction state and intermediate 3 of NOX5 are colored in grey and green, respectively. Ca²⁺-free and Ca²⁺-bound structures of DUOX1 are colored in grey and green, respectively. **b.** The movement of preTM1 densities between pre-reaction state and intermediate states. Pre-reaction state, intermediate state 2 and intermediate state 3 are colored in grey, cyan and yellow, respectively. **c.** Structural alignment of the NOX5 catalytic cores with pre-reaction state and intermediate state 3. The NADPH-binding site is zoomed in to show the movement of preTM1 relative to the rest. **d.** The subtle movement of NBD in response to the REFBD displacement. **e.** The local cryo-EM density near NADPH of pre-reaction state. **f.** The local cryo-EM density near

NADPH of intermediate state 3. **g.** The local cryo-EM density near NADPH of intermediate state (consensus model).



Supplementary Figure 10 Electrostatic surface representation of NOX5 in pre-reaction state and intermediate state 3. The PhosR segment is indicated by a black dashed cycle.



Supplementary Figure 11 Fractions of MD simulation. **a.** The RMSD of chemical groups from NADPH and FAD in pre-reaction state and intermediate state 3 at every 100 ns up to 500 ns, showing data convergence. The minima, maxima, centre, 25% percentile and 75% percentile of groups are provided with Supplementary Table 3. The figure of 0-500 ns is the same as Figure 3e and the minima, maxima, centre, 25% percentile and 75% percentile values are described in legend. The bar covers 25% and 75% of the data. All data are plotted on the violin plot. Pre and IS3 represent pre-reaction state and intermediate state 3, respectively. Data in all triplicates are combined to a single histogram. **b.** Distance distribution between FAD and NADPH in pre-reaction state and intermediate state 3 at every 100 ns up to 500 ns, showing data convergence. Data in all triplicates are combined to a single histogram. Pre and IS3 represent pre-reaction state and intermediate state 3 (red) over 500 ns (n=6). **d.** The root mean square fluctuation (RMSF) analysis of NOX5 amino acid side chains with intermediate state 3 (red) over 500 ns (n=6). **d.** The root mean square fluctuation (RMSF) analysis of NOX5 amino acid side chains with pre-reaction state (black) over 500 ns (n=6). The shaded region shows the standard deviation. Data in all triplicates are combined to a single histogram.

Supplementary Tables

Supplementary Table 1 Cryo-EM data collection, refinement and validation statistics.

	NOX5	NOX5 NOX5 (Ca-NADPH)					
	(EGTA-	Consensus	Ca state	Ca state	Ca state 3	(EMDB-42013)	
	NADPH)	(EMDB-	1	2	(EMDB-	(PDB 8U7Y)	
	(EMDB- 42014)	42015)			42016)		
	(PDB 8U85)	(PDB 8U86)			(PDB 8U87)		
Data collection and					,		
processing							
Magnification	105,000	105,000				130,000	
Voltage (kV)	300		30	00		300	
Electron exposure (e–/Å ²)	82.6		81	.8		76.886	
Defocus range (µm)	0.8~1.6		0.8	~1.6		1~2.6	
Pixel size (Å)	0.826		0.8	326		0.6485	
Symmetry imposed	C2	C2	C1	C1	C1	C2	
Initial particle images (no.)	1,373,175		2,00	1,493		2,058,434	
Final particle images (no.)	83,823	201,424	60,435	66,111	89,751	220,000	
Map resolution (Å)	3.2	3.3	4.31	4.03	3.86	4.06	
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	
Map resolution range (Å)	2.5-30	2.5-30	3.5-30	3-30	3-30	3-30	
Refinement							
Initial model used	500T						
(PDB code)	500X						
Model resolution (Å)	3.3	3.5	N/A	N/A	3.8	4.3	
FSC threshold	0.5	0.5			0.5	0.5	
Model resolution range (Å)	3.3-30	3.5-30			3.8-30	4.3-30	
Map sharpening <i>B</i> factor ($Å^2$)	-118	-140	-136	-160	-135	-172	
Model composition							
Non-hvdrogen atoms	10203	7623			8575	8803	
Protein residues	719	719			719	719	
Ligands	HEB (2),	HEB (2), NDP,		HEB	(2), NDP,	HEC (2), NAP,	
e	NDP,	FAD, D12	2, D10,	FAD	, D12, ZN	FAD, D12, D10,	
	FAD, PX2, ZN	ZN	, -,		, ,	ZN	
<i>B</i> factors (Å ²)							
Protein	65.32	27.45			80.59	80.96	
Ligand	37.37	20.23			68.86	52.80	
R.m.s. deviations							
Bond lengths (Å)	0.009	0.006	N/A	N/A	0.005	0.003	
Bond angles (°)	0.788	0.704			0.643	0.639	
Validation							
MolProbity score	1.94	2.03	N/A	N/A	1.98	1.80	
Clashscore	11.24	11.72			12.80	7.36	
Poor rotamers (%)	0.61	0.79			0.00	0.00	
Ramachandran plot							
Favored (%)	94.51	92.92	N/A	N/A	94.73	94.19	
Allowed (%)	5.49	7.08			5.27	5.81	
Disallowed (%)	0.00	0.00			0.00	0.00	

Supplementary Table 1 Cryo-EM data collection, refinement and validation statistics.

Supplementary Table 2 System set-up detail.

System	Box dimension	NOX5	Heme- B	NADPH	FAD	Zn	POPC	Na ⁺	Cl-	TIP3P water
Pre-reaction state	15.8 x 15.8 x 13.9 nm ³	2	4	2	2	1	735	220	216	78739
Intermediate state 3	15.8 x 15.8 x 13.7 nm ³	2	4	2	2	1	733	218	214	77602

Supplementary Table 2 System set-up detail.

Supplementary Table 3 Maxima, Minima and percentile values of Supplementary Figure 11a.

	Intermediate state 3						Pre-reaction state				
Group	Centre	Maxima	Minima	75% percentile	25% percentile	Centre	Maxima	Minima	75% percentile	25% percentile	
0-100 ns											
Nicotinamide	4.42	11.91	0.00	5.76	2.90	3.42	12.96	0.03	7.24	2.15	
Phospho- ADP-ribose	3.07	6.64	0.00	3.67	2.46	2.70	6.22	0.03	3.17	2.29	
Flavin	1.97	4.64	0.00	2.43	1.57	1.83	3.66	0.04	2.20	1.41	
D-ribitol	2.71	6.65	0.00	3.38	2.18	2.34	6.00	0.03	2.94	1.79	
ADP	3.66	9.33	0.00	4.79	2.89	3.88	8.53	0.04	5.11	3.10	
0-200 ns											
Nicotinamide	5.13	19.67	0.00	6.65	3.50	5.10	12.96	0.03	7.45	2.84	
Phospho- ADP-ribose	3.15	6.64	0.00	3.71	2.61	2.89	7.44	0.03	3.41	2.44	
Flavin	2.11	4.64	0.00	2.52	1.71	1.91	4.00	0.04	2.33	1.50	
D-ribitol	2.98	7.43	0.00	3.77	2.41	2.76	6.00	0.03	3.38	1.98	
ADP	4.16	9.84	0.00	5.57	3.23	4.91	11.33	0.04	6.04	3.56	
0-300 ns											
Nicotinamide	5.90	21.11	0.00	9.10	4.14	5.38	12.96	0.03	7.43	3.30	
Phospho- ADP-ribose	3.35	7.88	0.00	4.01	2.76	3.00	7.44	0.03	3.62	2.50	
Flavin	2.26	5.49	0.00	2.77	1.80	2.08	6.13	0.04	2.65	1.62	
D-ribitol	3.31	9.17	0.00	4.33	2.50	3.13	8.56	0.03	3.92	2.20	
ADP	4.50	13.74	0.00	6.60	3.39	5.10	13.98	0.04	6.54	3.85	
0-400 ns											
Nicotinamide	6.29	22.51	0.00	9.95	4.22	5.58	15.07	0.03	7.85	3.64	
Phospho- ADP-ribose	3.55	8.13	0.00	4.40	2.87	3.07	7.44	0.03	3.76	2.60	
Flavin	2.32	5.68	0.00	2.95	1.78	2.16	6.13	0.04	2.86	1.64	
D-ribitol	3.47	9.17	0.00	4.59	2.58	3.37	8.96	0.03	4.28	2.44	
ADP	5.07	13.74	0.00	6.91	3.48	5.55	13.98	0.04	7.10	4.20	

Supplementary Table 3 Maxima, Minima and percentile values of Supplementary Figure 11a.

Supplementary References

1 Drozdetskiy, A., Cole, C., Procter, J. & Barton, G. J. JPred4: a protein secondary structure prediction server. Nucleic acids research 43, W389-W394 (2015).