

## **Supporting Material**

### **Torque Generation in $F_1$ -ATPase Devoid of the Entire Amino-Terminal Helix of the Rotor That Fills Half of the Stator Orifice**

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**TABLE S1** Amino-acid sequence of the  $\beta$ - $\gamma$  fusion mutant of TF<sub>1</sub> ( $\beta$ - $\gamma$  $\Delta$ N0) deduced from the DNA sequence\*

<b><math>\alpha</math> subunit with N-terminal His<sub>6</sub> tag<sup>†</sup></b>					
-7	-1				
<u>MHHHHHH</u>					
1	11	21	31	41	
MSIRAE EISA	LIKQQIENYE	SQIQVSDVGT	VIQVGDGIAR	AHGLDNVMSG	050
ELVEFANGVM	GMALNLEENN	VGIVILGPYT	GIKEGDEVRR	TGRIMEVPVG	100
EALIGRVVNP	LGQPV DGLGP	VETTETRPIE	SRAPGVMDRR	SVHEPLQTGI	150
KAIDALVPIG	RGQRELIIGD	RQTGKTSVAI	DTIINQKDQN	MISIVVAIGQ	200
KESTVRTVVE	TLRKHGALDY	TIVVTASASQ	PAPLLFLAPY	AGVAMGEYFM	250
YKGKHLVVVY	DDLKQAAAY	RELSLLLRRP	PGREAYPGDI	FYLHSRLLER	300
AAKLSDAKGG	GSLTALPFVE	TQAGDISAYI	PTNVISITDG	QIFLQSDLFF	350
SGVRPAINAG	LSVSRVGGAA	QIKAMKKVAG	TLRLDLAAYR	ELEAFAQFGS	400
DLDKATQAKL	ARGARTVEVL	KQDLHQPIPV	EKQVLI IYAL	TRGFLDDIPV	450
EDVRRFEKEF	YLFLDQNGQH	LLEHIRTTKD	LPNEDDLNKA	IEAFKKTFFV	500
SQ					502
<b><math>\beta</math> and <math>\gamma</math> subunits fused in the order of <math>\gamma</math>-linker peptide-<math>\beta</math><sup>‡</sup></b>					
1	11	21	31	41	
<b>MASLRDIKTR</b>	<b>INATKKTSQI</b>	<b>TKAMEMVSTS</b>	<b>KLNRAEQNAK</b>	<b>SFVPYMEKIQ</b>	050
<b>EVVANVALGA</b>	GGASHPMLVS	RPVKKTYGLV	ITSDRGLAGA	YNSNVLRLVY	100
QTIQKRHACP	DEYAIIVIGR	VGLSFFRKRN	MPVILDITRL	PDQPSFADIK	150
EIARKTVGLF	ADGTFDELYM	YNNHYVSAIQ	QEVTERKLLP	LTDLAENKQR	200
TVYEFEPSQE	ECLDVLLPQY	AESLIYGALL	DAKASEHAAR	MTAMKNATDN	250
ANELIRTLTL	SYNRARQAAI	TQEITEIVAG	ANALQ		285
<u>SSGLVPRGSH</u>	<u>MLEDP</u>				300
MTRGRVIQVM	GPVVDVKFEN	GHLPAIYNAL	KIQHKARNEN	EVDIDLTLEV	350
ALHLGDDTVR	TIAMASTDGL	IRGMEVIDTG	APISVPVGEV	TLGRVFNVLG	400
EPIDLEGDIP	ADARRDPIHR	PAPKFEELAT	EVEILETGIK	VVDLLAPYIK	450
GGKIGLFGGA	GVGKTVLIQE	LIHNIAQEHG	GISVFAGVGE	RTREGNDLYH	500
EMKDSGVISK	TAMVFGQMNE	PPGARMRVAL	TGLTMAEYFR	DEQQQDVLLF	550
IDNIFRFTQA	GSEVSALLGR	MPSAVGYQPT	LATEMGQLQE	RITSTAKGSI	600
TSIQAIYVPA	DDYTD PAPAT	TFSHLDATTN	LERKLAEMGI	YPAVDPLAST	650
SRALAPEIVG	EEHYQVARKV	QQT LQRYKEL	QDIIAILGMD	ELSDEDKLVV	700
HRARRIQFFL	SQNFHVAEQF	TGQPGSYVPV	KETVRGFKEI	LEGKYDHLPE	750
DAFRLVGRIE	EVVEKAKAMG	VEV			773

\*The F<sub>1</sub>-coding region of the expression plasmid for  $\beta$ - $\gamma$  $\Delta$ N50 was sequenced and amino acid residues deduced. Missing 50 residues have been supplemented (see below).

<sup>†</sup>Underlined portion indicates introduced N-terminal His<sub>6</sub> tag.

<sup>‡</sup>The first 285 residues correspond to wild-type  $\gamma$ , the sequence of which is slightly different from that in the original report (S1) that contained 283 rather than 285 residues. The

$\gamma$ -S107C/I210C mutations referred to in the main text are  $\gamma$ -S109C/I212C in the revised sequence above. Of the  $\gamma$  sequence shown here, 52 to 285 have been deduced from the DNA sequence of  $\beta$ - $\gamma\Delta$ N50, and the first 51 residues (shown in bold), 51 being Met-1 for  $\beta$ - $\gamma\Delta$ N50 (bold with shade), have been copied from the wild type sequence (S2). Sequence 286-300, with double underline, shows the inserted linker, and sequence 301-773 is identical to wild-type  $\beta$  without its N-terminal His<sub>10</sub> tag.

#### **SUPPORTING REREFENCES**

- S1. Ohta, S., M. Yohda, M. Ishizuka, H. Hirata, T. Hamamoto, Y. Otawara-Hamamoto, K. Matsuda, and Y. Kagawa. 1988. Sequence and over-expression of subunits of adenosine triphosphate synthase in thermophilic bacterium PS3. *Biochim. Biophys. Acta.* 933:141–155.
- S2. Shimo-Kon, R., E. Muneyuki, H. Sakai, K. Adachi, M. Yoshida, and K. Kinoshita Jr. 2010. Chemo-mechanical coupling in F<sub>1</sub>-ATPase revealed by catalytic site occupancy during catalysis. *Biophys. J.* 98:1227–1236.