Supplementary Material

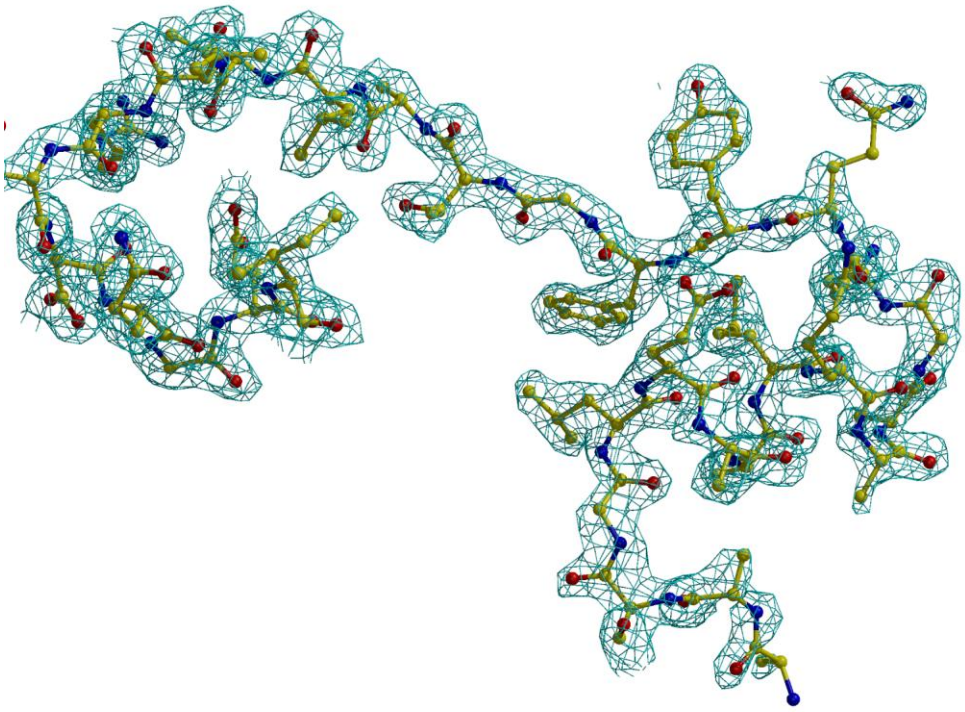

Figure S1

Schematic representation of the $2F_o$-F_c electron density map contoured at 1.0 σ level calculated in the absence of the 30 N-terminal amino acids.

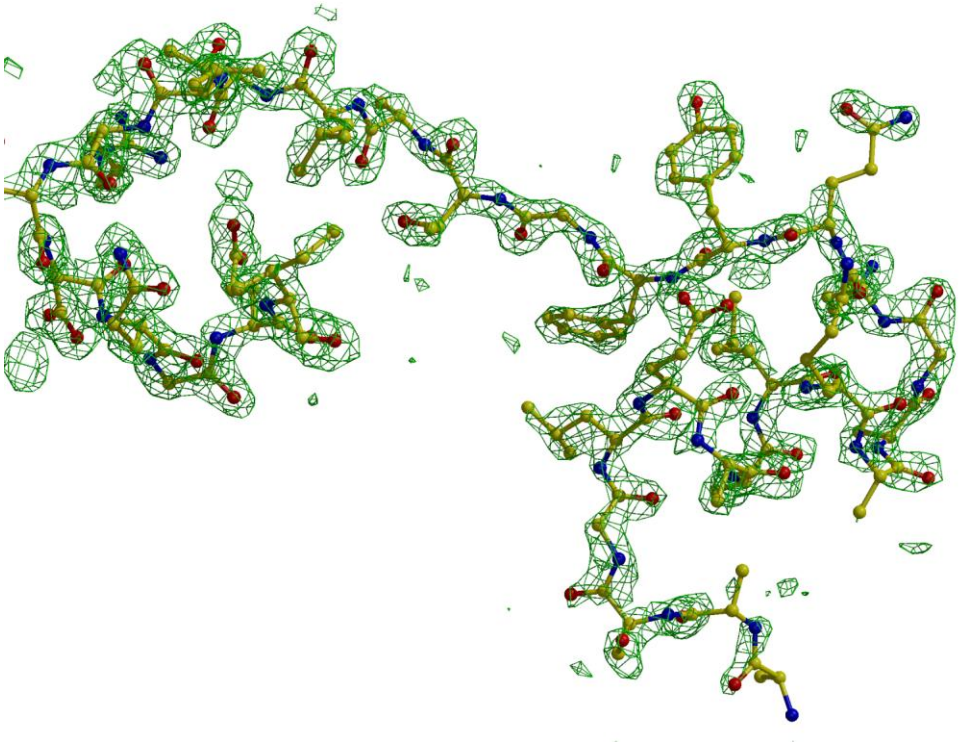

Figure S2.

Schematic representation of the F_o-F_c electron density map contoured at 3.0 σ level calculated in the absence of the 30 N-terminal amino acids.

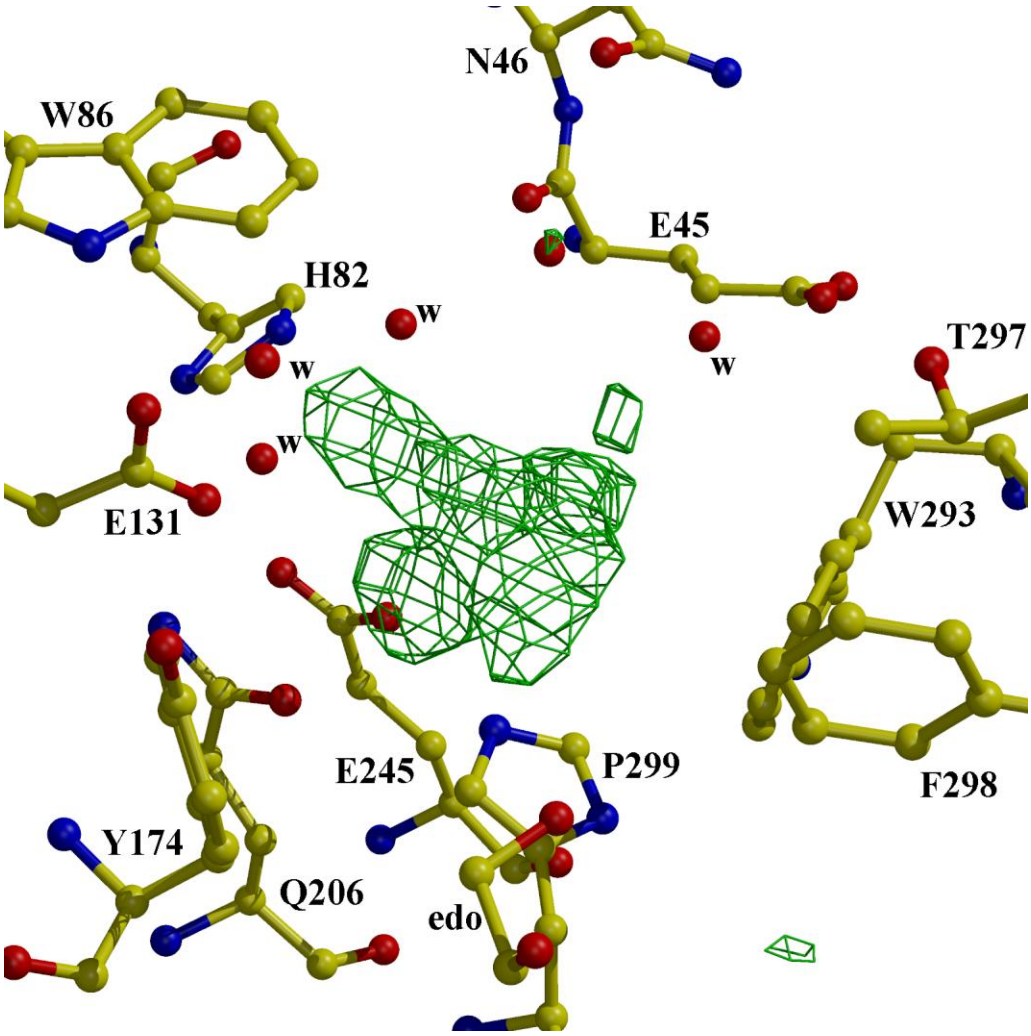

Figure S3.

Schematic representation of the F_o-F_c electron density map contoured at 3.0 σ level indicating the unassigned portion of density at the catalytic site of *Fo*Xyn10a.

Supplementary Table 1 Direct hydrogen bonds formed between the 5 monomers

Reference	**Atom**	**Target**	**atom**	**Distance (Å^2)**
Asn25 (A)	ND2	Asp309(D)	OD2	3.1
Asn56 (A)	ND2	Pro215(D)	O	2.8
Lys290 (A)	NZ	Lys290(D)	NZ	3.0
Gly296(A)	O	Lys49(D)	NZ	2.8
Asp309(A)	OD2	Asn25(D)	ND2	3.3
Phe311(A)	O	Asp31(B)	OD2	2.9
Asn25(B)	ND2	Asp309(C)	OD2	3.3
Asn56(B)	ND2	Pro215(C)	O	2.8
Pro215(B)	O	Asn56(C)	OD1	3.0
Gln257(B)	OE1	Tyr174(E)	OH	3.3
Gln257(B)	OE1	Arg140(E)	NH1	2.9
Gly296(B)	O	Asn46(C)	ND2	3.3
Gly296(B)	O	Lys49(C)	NZ	2.9
Phe311(B)	O	Asp21(A)	OD2	2.9
Asp66(C)	OD2	Ser92(E)	OG	2.6
Gly296(C)	O	Lys49(B)	NZ	2.9
Phe311(C)	O	Asp31(D)	OD2	2.9
Asn56(D)	ND2	Pro215(A)	O	2.9
Gly296(D)	O	Lys49(A)	NZ	2.9
Phe311(D)	O	Asp31(C)	OD2	2.9

Supplementary Table 2. Hydrogen bonds formed between the 5 monomers mediated by ethylene glycol and water molecules

Source Molecule → Water → EDO → Target Molecule → EDO → Water → Target Molecule						
Asn61 ND2 (A)	→	Wat133 O	→	EDO408 O2 (D)	→	Glu301 OE2 (D)
Glu301 OE2 (A)	→	EDO400 O1 (A)	→	Wat98 O	→	Asn61 ND2 (D)
Ser303 N (C)	→	EDO406 O2 (C)				
		EDO406 O1 (C)	→	Wat169 O	→	Asp25N (B)
Pro299 N (C)	→	EDO400 O1 (C)				
		EDO400 O2 (C)	→	Wat262 O	→	Asn61 ND2 (B)
Ser303 N (A)	→	EDO413 O2 (A)				
		EDO413 O1 (A)	→	Wat67 O	→	Asn25 N (D)
Asn101 N (E)	→	EDO407 O2 (E)				
		EDO407 O1 (E)	→	Wat1176 O	→	Asn322 ND2 (D)
Thr252 OG1 (A)	→	EDO409 O1 (A)				
		EDO409 O2 (A)	→	Wat1323 O	→	Val23 O (D), Glu67 OE1 (D)

Additional information

Overall, the most pronounced changes between the monomers A and E are identified mainly in the loop regions between strand β2 and helix α2a and strand β3 and α3a, located at the entrance of *Fo*Xyn10a active site. These loop regions are implicated

in intersubunit contacts formed between the monomers A, B, C and D. The contribution of ethylene glycol molecules in the close packing of the monomers is also highlighted in Supplementary Table 2. The packing of the 5 monomers in the crystal results in loss of ~15% in the accessible surface of each monomer except for E (~7%) that is more exposed to the solvent. Comparison of molecules A and E revealed a cascade of changes involving residues Lys49, Gln89 and Glu51 of monomer A to optimize the network of interactions with Gly296 and Thr297 of D. The rotameric state of Lys49 (A) changed dramatically to form favorable hydrogen bonds with Gly296 O (D) and Asn46 OD1 (A). The new position of Lys49 (A) induces further changes in Gln89 (A) (all atoms shift by ~1.2 Å) to avoid stereochemical clashes with NE1 of Gln89 (A). Gln89 OE1 (A) lies close to Glu51 OE1 (A), which also adopts a new conformation further stabilized by a water-mediated hydrogen bond with Pro55 O (A) through Wat90 O. A water molecule occupies the former position of Glu51 OE1 (Wat418 O) bridging Gln89 OE1 (A) with Thr297 O (D). In the case of monomer E, the side chain of Gln89 points towards the catalytic site of the enzyme and its conformation is stabilized through hydrogen bond interactions formed with neighbouring residues Lys49 (E), Glu51 (E). Residues such as Pro215 (D) is subjected to shifts of ~0.8 Å interacting with both EDO and residues of the opposite subunit inducing alterations in Asn56 (A) and in turn in Gln59 (A) side chains to restore the network of hydrogen bonds formed. Minor changes are also recorded upon comparison of monomers A and E, in Asn25, the “meeting point” of the 4 subunits, tightly packed in the asymmetric unit (Fig. 3, inset) (shifts by ~0.5 Å), forming a direct hydrogen bond through atom ND2 with Asp309 OD2 of their related monomers.