Adenosine nucleotide binding sites on Complex V from diverse organisms

Aabhinav Parashar & Kelath Murali Manoj**

Satyamjayatu: The Science & Ethics Foundation,
Shoranur-2 (PO), Palakkad District, Kerala, India-679122.
Email: murman@satyamjayatu.com (ORCID: 0000-0003-4515-994X)
Email: parashar.abhinav.2008@gmail.com (ORCID: 0000-0003-4229-0248)

Abstract: Using in silico docking approaches, we scan the various subunits of Complex V (F_0F_1 ATPase) for putative adenosine nucleotide binding sites. We find that multiple generic ADP/ATP binding sites are present on the alpha-beta binding sites and a conserved ATP binding site is present on the epsilon subunit. These findings support the murburn model of Complex V.

Keywords: ATP, ADP, Complex V, ATP synthesis, ATP hydrolysis, binding site

Boyer's model proposes a complex reversible and deterministic scheme for ATP synthesis and hydrolysis, mediated by Complex V (Lehninger et al., 2002). We had proposed an irreversible and stochastic murburn model for this enzyme's function (Manoj et al., 2021). Herein, using molecular docking of ligands on the available pdb files of diverse Complex V molecules, we explore the binding profiles of the bioenergetically relevant protein.

Figure 1 shows the approach we adopted (as detailed in Parashar & Manoj, 2021) to screen for putative ANBS on the surface of Complex V.

Figure 1: The structural details of Complex V. A. An overview of important motifs and chains is presented using the crystal structure of F-ATPase from *Bos taurus* (PDB ID: 2WSS) (Rees et al., 2009). The position of DELSEED (black) is on the β -bulb close to γ -shaft. Walker motifs are shown in blue and green on α -bulb (sky blue) and β -bulb (pale yellow). The extension of γ -shaft is presented in red and also with a distinct ribbon structure. The other two associated chains with the head of γ -shaft are shown in pale tan (for δ) and magenta (for ϵ , another known site of ATP binding). Both α and β -bulbs are shown from the inner core side by hiding other four bulbs to provide a clarity on the positioning of the above mentioned motifs. B. Represents the grid boxes taken to scan for potential ADP and ATP binding sites. The grid covers the Walker motif regions on both α - and β -bulbs. Four bulbs (two of α and two of β) are faded to depict the grid prominently.

Figure 2 shows the salient results we obtained, as highlighted from the details given in Table 1. We ratified that non-specific ANBS are present on both alpha and beta monomers. The Walker-motif bearing sites were not necessarily the ones giving higher affinity ANBS. There was no consensus in the alpha-beta monomers as to which site was conserved to be high affinity ATP-site or ADP-site. There exists no conserved consensus either on the ATP/ADP binding preference for the various sites across the diverse species either. Therefore, the mechanism of cooperative conformation change mechanism can be safely jettisoned. (There can always be the

induced fit argument, but that would be a non-evidence proposal! If that were to be true, one would need to imagine that these multitudes of sites undergo periodic changes in affinities or else, they exist only to mess with its own fundamental purpose! It is quite possible that some of the surface residues would be high energy and they could change conformations in physiology. If so, one would not expect them to deterministically affect the selectivity strongly.) The pore valve (epsilon-delta protein) is clearly an ATP binding locus, as the ATP binding is higher than ADP in all four of the F-ATPases explored. The conserved Walker motifs signifies a high phosphate concentration at the alpha-beta bulb and the generic ANBS (adjacent to the DELSEED loop and Walker motifs) reactions shown in Figure 1.

Figure 2: NBS on alpha-beta bulb and epsilon subunit in F-ATPases. **A.** Nucleotide binding site on epsilon unit. Epsilon (ϵ) motif (magenta) shows interaction with both ATP (red) and ADP (bright yellow), with the former preferred over the latter. gamma-shaft is presented in light pink whereas α and β -bulbs are presented in blue and pale yellow respectively. **B.** ADP/ATP binding sites on yeast F-ATPase (PDB ID: 2HLD) is presented. α - and β -bulbs are shown in sky blue and pale yellow, respectively. Walker motifs on α - and β -monomers are represented as blue ribbon and green, respectively and are enclosed in a rectangle. Both ADP and ATP binds to Walker motif present in the β -bulb. Also, two murburn sites (selected based on highest binding energies among the murburn sites) are shown in ovals. For clarity, ADP is coloured in bright yellow and ATP in red. Same colour codes are maintained for other subunits as well. Four bulbs (two of α and two of β) are faded to maintain clarity.

References

Lehninger A, D.L.N. and Cox, M.M., 2004. Lehninger Principles of Biochemistry, 4th edn, Macmillan.

Parashar, A. and Manoj, K.M., 2021. Murburn precepts for cytochrome P450 mediated drug/xenobiotic metabolism and homeostasis. *Current Drug Metabolism*. 22, p. 315-326.

Figure S2. The functional perceptions of the working modalities of F and V ATPases. A (Nakanishi-Matsui *et al.*, 2010), B (Lehninger textbook, 2013), C (Ueno *et al.*, 2018), and D (Nishi & Forgac, 2003) are various depictions from the last two decades of available literature. Unmarked arrows in the V-ATPase in panel A show structural variability in the monomers. Refer text/source for details.

Nakanishi-Matsui M, Sekiya M, Nakamoto RK, Futai M. The mechanism of rotating proton pumping ATPases. *Biochim Biophys Acta*. 2010 Aug;1797(8):1343-52. doi: 10.1016/j.bbabi.2010.02.014.

Ueno H, Suzuki K, Murata T. Structure and dynamics of rotary V₁ motor. *Cell Mol Life Sci*. 2018;75(10):1789-1802. doi:10.1007/s00018-018-2758-3

Nishi T, Forgac M. The vacuolar (H⁺)-ATPases--nature's most versatile proton pumps. *Nat Rev Mol Cell Biol*. 2002 Feb;3(2):94-103. doi: 10.1038/nrm729.

Table S1: Docking interactions of Adenosine nucleotides with various ‘rotary ATPases’ (Complex V)

No.	Protein (pdb)	Ligand	ΔG (kcal/mol); K_d (μM); monomer	Interacting amino acids	Generic remarks (Walker/Murburn)
1.	Mammalian (Bos) Mitochondrial F-ATPase (1H8E/2WSS [*])	ADP	-4.55; 463; ϵ^*	R5, Y11, S15 [*]	Although more preferred at δ , ADP binds at the reported conserved site at ϵ also.
			-5.88; 68; δ^*	K74, L100, L103 [*]	Interaction with Walker motif in β -bulb.
			-7.88; 2; β	G157, G159, V160, G161, K162, T163, V164	Interaction with Walker motif in α -bulb.
			-6.30; 24; α	Q172, T173, G174, K175, T176	Murburn site on α -bulb.
			-5.39; 112; α	D36, G37, V75, N78, D79, A240, Q280, M281	Second murburn site on α -bulb.
			-5.18; 159; α	R373, S376, A377, K391	Second murburn site on β -bulb.
			-3.97; 1230; β	I130, V132, K175, H177, L434, K435	
		ATP	-4.84; 282; ϵ^*	V1, R5	As observed with ADP, ATP also prefers to bind at δ than its conserved site at ϵ .
			-6.88; 9; δ^*	K74, Y75, V98, L100, L103	Interaction with Walker motif in α -bulb.
			-7.52; 3; α	R171, Q172, K175, T176, S177, E328	Murburn site on β -bulb.
2.	Plant (<i>Spinacia</i>) Chloroplast F-ATPase (1FX0/6VON [#])	ADP	-5.90; 47; β	I116, H117, E119, R231, T284, T291, R295	Murburn site on β -bulb.
			-5.75; 61; α	S372, R373, S376, K391	Murburn site on α -bulb
			-4.23; 791; β	N172, K175, A176, L434	Second murburn site on β -bulb.
			-3.25; 4140; $\epsilon^{\#}$	K112, L117 [#]	Not very efficient binding, although highest affinity at ϵ site.
			-4.39; 610; β	F139, L142, D143, T144	Two dominant binding cluster sites (one in α - and another in β -bulb), but none of them are Walker motifs.
			-4.29; 711; α	I61, M77, K114	Second murburn site on α -bulb.
			-4.19; 844; α	R94, Q97	Third murburn site on α -bulb.
		ATP	-3.90; 1370; α	N110, K114, A226, T228	Murburn site on β -bulb.
			-3.22; 4350; β	T144, L146	Second murburn site on β -bulb.
			-2.55; 13450; β	G173, D332, L352, R354, G355	
3.	Yeast (<i>Saccharomyces</i>) F-ATPase (2HLD)	ADP	-4.37; 621; $\epsilon^{\#}$	K109, K112 [#]	Relatively better affinity than ADP.
			-5.85; 52; α	Q173, T174, G175, T177, T181, Q201, D263, E321, Q425	Interaction with Walker motif in α -bulb.
			-4.48; 517; β	R277, Q280, S283, Y298, Y328, A331	Murburn site on β -bulb.
			-4.17; 874; α	M77, D79, N110, K114	Second murburn site on α -bulb.
			-3.62; 2230; α	R94, Q97, L112	Third murburn site on α -bulb.
			-3.42; 3090; ϵ	F43, T45	Weak binding as observed with the ϵ site of plants.
			-5.87; 50; β	G157, K163, R190, N257, R260	Interaction with Walker motif in β -bulb.
		ATP	-5.47; 98; β	V161, Y345, Q416	Murburn site on β -bulb.
			-5.38; 115; α	Q149, W191, K199, G489	Murburn site on α -bulb.
			-4.75; 332; α	I367, N368, V369, Y399, Q428	Second murburn site on α -bulb.
			-4.04; 1090; ϵ	I8	Relatively better affinity than ADP.
			-6.99; 8; β	G157, G158, V310, V312, L335, R337	Interaction with Walker motif in β -bulb.
			-6.00; 40; α	P147, Q149, K199, T440, E441, R486	Murburn site for ATP binding is less preferred on α -bulb unlike

4.	Bacterial (<i>Caldalkalibacillus</i>) F-ATPase (5HKK)	ADP	-5.25; 141; α	A379, Q381, K386	the ones observed in mammals and plants.
			-4.95; 236; β	H455, Y458, K469	Murburn site on α -bulb.
			-4.56; 456; ϵ	R92, R99, H100, R123, R127	Murburn site on β -bulb.
			-6.72; 12; β	I111, H112, Q280, R284	Moderate binding at the conserved ϵ site.
			-6.22; 28; β	G154, V155, G156, K157, T158	Cluster 1 (interacting with Walker motif) displays weaker binding compared to Murburn site (cluster 2).
			-6.00; 40; α	D170, Q172, T173, G174, K175, T176	Both preferred ADP binding sites observed on β -bulb alone.
			-5.36; 118; α	S264, A268, R271, E284	A third cluster site is observed with relative binding energy at the Walker motif of α -bulb same as that of ATP binding site.
			-4.16; 894; β	Q64, Y270, R279, D289, R296	Murburn site on α -bulb.
			-6.27; 25; ϵ	R92, R99, H100, R123, R127	Murburn site on β -bulb.
			-6.97; 8; β	I111, H112, R113, Q280, R284	Good affinity binding at the ϵ site.
5.	Bacterial (<i>Thermusthermophilus</i>) V-ATPase (3W3A)	ADP	-5.65; 72; α	Q172, T173, G174, K175, T176, T177, E320, R354	Walker motif interacts with ATP in the α -bulb, but is considerably weaker than ATP binding on β -bulb where interaction is at a Murburn site rather than a Walker motif.
			-4.79; 306; β	G154, V155, G156, K157, T158	Walker motif site on β -bulb.
			-4.08; 1020; β	A259, G262, R263	Second murburn site on β -bulb.
			-3.30; 3780; β	R34, G37, T39, S60, N212	Third murburn site on β -bulb.
			-3.13; 5090; α	E96, L111, G112, Q113, I121, E122, T123, Y126	Murburn site on α -bulb.
			-5.44; 103; α	I288, A289	Murburn site on α -bulb.
			-5.17; 161; α	I490	Second murburn site on α -bulb.
			-4.90; 255; α	W407, I422	Third murburn site on β -bulb.
			-4.13; 946; β	I167	Murburn site on β -bulb.
			-3.95; 1280; β	N363, V366, K368, K370	Walker motif interacts with ATP in the β -bulb.
6.	Archaea (<i>Nanoarchaeum</i>) V/A-ATPase (5BN4)	ATP	-5.33; 124; α	T237, R408, A421	Walker motif interacts with ATP in the α -bulb.
			-4.38; 615; β	K376, Q377, R453	Murburn site on β -bulb.
			-3.15; 4920; α	L215, W424, S427, I490	Murburn site on α -bulb.
			-2.89; 7560; β	N362, N363, V366, K368, K370	Walker motif interacts with ATP in the β -bulb.
		ADP	-8.51; 1; α	P224, G226, S227, G228, K229, T230, V231	Strong binding of ADP with the Walker motif in α -bulb.
			-7.48; 3; β	A121, H122, S325, R326	Murburn site on β -bulb.
			-5.45; 101; β	R248, R249, V290	Second murburn site on β -bulb.
			-5.21; 152; β	N285, D286, I287, K322	Third murburn site on β -bulb.
			-3.86; 1470; α	E263, A493, E496	Murburn site on α -bulb.
		ATP	-8.16; 1; β	A121, H122, L324, S325, R326, K329	ATP doesn't bind to Walker motif. Binds to two different
			-5.66; 71; β	R248, R249, P252, A253, V290, D293	Murburn sites on β -bulb.
			-4.39; 602; α	K407, K411, Y419, E455, S456	Murburn site on α -bulb.
			-4.35; 645; β	N285, D286, I287, L305, Q307	Third murburn site on β -bulb.
			-4.19; 845; β	R248, R249, T288, V290	Fourth murburn site on β -bulb.