Modeling and Docking Analysis of Apt2 Target Proteins

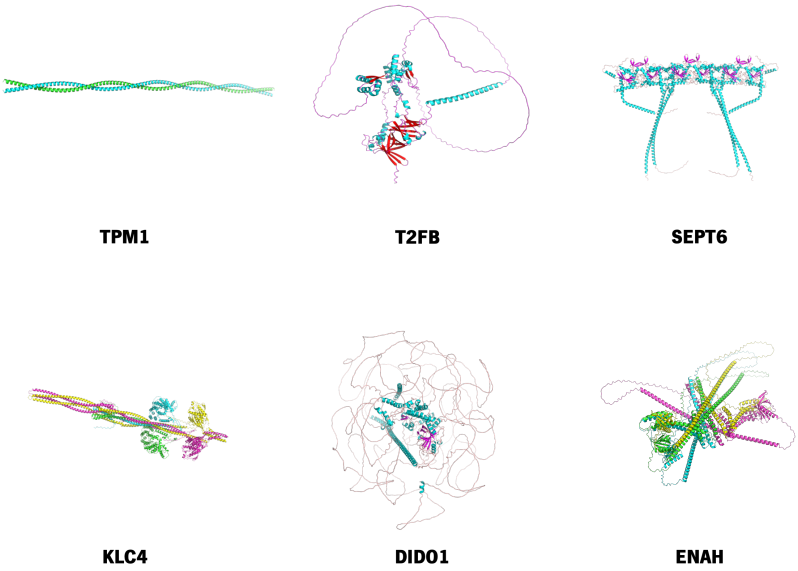

Figure 1: Structural models generated using AlphaFold

TPM1 (Tropomyosin α -1 chain, homodimer) was modeled as a coiled-coil homodimer, which is consistent with its role in stabilizing actin filaments. The model exhibits a stable α-helical core and flexible terminal regions, in agreement with structural studies on actin–tropomyosin complexes [5, 7] (UniProt ID: P09493).

T2FB (GTF2F2, heterodimer with GTF2F1/T2FA) is part of the TFIIF, a heterodimer composed of GTF2F1 (RAP74) and GTF2F2 (RAP30), which interact via a central β-barrel architecture that supports RNA polymerase II recruitment and transcription start site selection [3]. Therefore, both subunits were modeled together. (UniProt IDs: P13984 for T2FB and P35269 for T2FA).

SEPT6 (heterohexamer with SEPT2 and SEPT7) was included in a SEPT2–6–7 hexamer, reflecting its role in septin filaments [14]. Each subunit contains a GTPase domain and variable extensions, aligning with known septin stoichiometry and cryo-EM data [6, 12]. (UniProt IDs: Q14141 for SEPT6, Q15019 for SEPT2, and Q16181 for SEPT7).

DIDO1 (monomer) was modeled as a monomer, consistent with its role in transcriptional regulation and chromatin remodeling through interactions with DNA and nuclear proteins rather than multimerization [4, 2]. The predicted structure shows a globular core with extensive intrinsically disordered regions, a common feature of transcriptional regulators [17]. The absence of multimeric assemblies in structural databases supports this modeling approach (UniProt ID: Q9BTC0).

KLC4 (Kinesin light chain 4, heterotetrameric complex) is part of a kinesin-1 heterotetramer composed of two KIF5B heavy chains and two KLC4 light chains [8]. The

model highlights structured TPR domains and flexible linkers for cargo binding and regulation [13]. This stoichiometry was used to predict the model as a tetrameric assembly, reflecting the biologically relevant kinesin-1 complex (UniProt IDs: P33176 for KIF5B and Q9NSK0 for KLC4).

ENAH (homotetramer) was modeled as a homotetramer, consistent with the Ena/-VASP protein family structurally functioning via coiled-coil-mediated tetramerization [10]. In breast cancer models, particularly in MDA-MB-231 cells, Mena (ENAH) invasion isoforms promote carcinoma cell motility and metastasis [16]. (UniProt ID: Q8N8S7)

Table 1: Summary of interactions between Apt2 and each predicted protein target. Abbreviations: HBOND - hydrogen bond; VDW - Van der Waals interaction; PIPISTACK – π–π stacking interaction.

Target	Target Residue(s)	Interaction Type(s)	Aptamer Residue(s)
TPM1	LYS 140	VDW	DC 41
	GLN 144	HBOND	DC 42
	GLU 150	VDW	DC 16
T2FB	PHE 266	VDW	DG 14
	TYR 273	HBOND, PIPISTACK	DA 48, DC 49
	SER 280	VDW	DC 10
	TYR 683	VDW, VDW	DG 13, DG 14
	GLU 684	VDW	DG 14
	LYS 687	HBOND, VDW	DG 14, DG 14
SEPT6	GLN 1800	VDW	DC 21
	GLN 1804	VDW	DC 21
	LYS 1807	VDW, VDW	DT 20, DG 36
	HIS 2117	HBOND, VDW	DC 22, DG 36
KLC4	LEU 175	VDW	DA 1
	LEU 178	VDW	DA 1
	GLY 203	VDW	DC 18
DIDO1	PHE 1683	PIPISTACK, VDW	DG 6, DT 7
	THR 1684	VDW	DG 6
	PRO 1686	HBOND, VDW	DG 3
	CYS 1685	HBOND	DT 4
	HIS 1815	HBOND	DT 34
	ILE 1786	VDW	DT 47
ENAH	PRO 939	VDW	DA 31
	PRO 1504	VDW	DC 49
	PRO 1507	VDW	DC 49

To the first target, homodimer TPM1, Apt2 established Van der Waals (VDW) and hydrogen bond (HBOND) contacts with Lys140, Gln144, and Glu150, via pyrimidine-rich residues DC41, DC42, and DC16. These contacts occur near the coiled-coil surface,

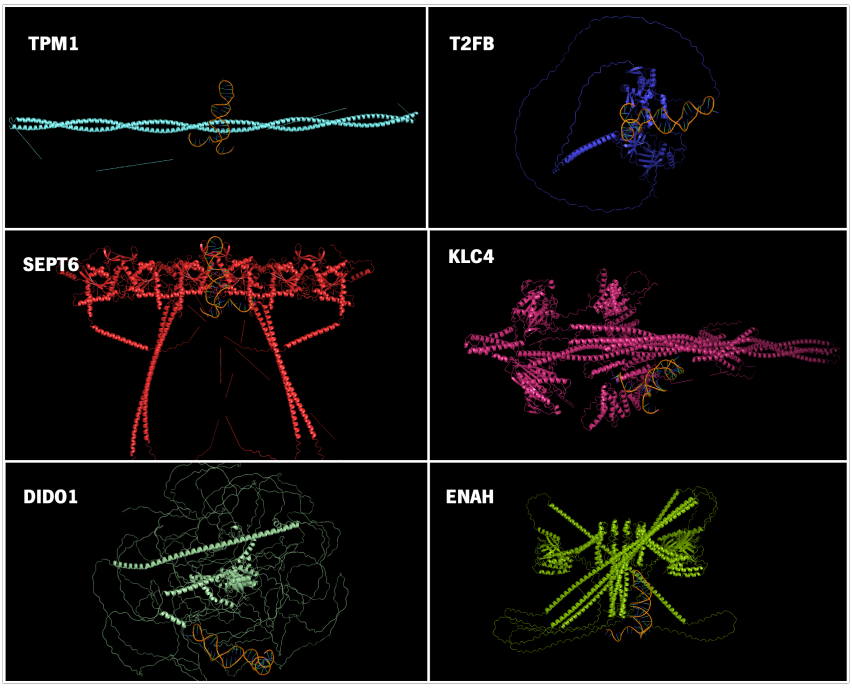

Figure 2: Representative docking poses of the Apt2 aptamer (colored in orange) with the six predicted protein targets.

suggesting accessible and stable interaction zones.

For the heterodimer composed of T2FB and T2FA proteins, the aptamer formed interactions with several residues—Phe266, Tyr273, Ser280, Tyr683, Glu684, and Lys687—via π–π stacking, Van der Waals, and hydrogen bonds, primarily involving DG13 and DG14. These occurred in a poorly structured region of the TFIIF heterodimer, likely disordered. Docking in such zones is less reliable due to structural flexibility, limiting confidence in binding predictions [15, 9]. Given the significant uncertainty associated with these interactions, they will not be considered.

Regarding the complex formed by septins 2, 6, and 7, docking revealed hydrogen bonds and Van der Waals contacts with Gln1800, Gln1804, Lys1807, and His2117, and the aptamer residues DC21, DT20, and DG36. These interactions occurred near a GTP-binding region, suggesting localized binding with potential biological significance.

To the kinesin-1 complex, Van der Waals interactions with Leu175, Leu178, and Gly203, involving DA1 and DC18, were observed. These residues lie within the TPR domain of the light chain, a known cargo-binding region, indicating a potentially functional interface [13].

For DIDO1 monomer, multiple interactions were identified involving Phe1683, Thr1684, Cys1685, Pro1686, His1815, and Ile1786. These residues contacted DG6, DT7, DT34, and DT47 via hydrogen bonds, stacking, and Van der Waals forces. Binding occurred primarily in the C-terminal segment, which is predicted to be intrinsically disordered [17, 11]. This introduces additional uncertainty, as binding predictions in these regions are inherently less reliable due to structural flexibility, a limitation that may affect docking accuracy

[15, 9]. Therefore, given the high level of uncertainty associated, these interactions will not be considered.

Finally, the ENAH homotetramer established interactions with Apt2 via three proline residues (Pro939, Pro1504, Pro1507) engaged in Van der Waals interactions with DA31 and DC49. These residues are in the disordered portion of the EVH2 domain and may mediate adaptable but low-specificity interactions, consistent with actin-regulatory behavior [1].

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