

Predicted Configuration and Stability of the ATAD2/SOX10 Complex Using Molecular Dynamics Simulations

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Abstract. *Background/Aim:* ATAD2, a melanoma competence factor, forms a protein complex with SOX10 that facilitates expression of SOX10 developmental target genes. The complex enables a strong transcriptional response to oncogenes such as BRAF^{V600E} and is sufficient to endow oncogenic competence to melanocytes. The elucidation of the ADAT2/SOX10 complex structure may facilitate the development of drugs that can block formation of the complex. *Materials and Methods:* We used the ClusPro web server for protein-protein docking to visualize and analyze the complex and GROMACS to perform molecular dynamics simulations. *Results:* ClusPro protein docking analysis demonstrated the central position of ADAT2 in the ADAT2/SOX10 complex. Molecular dynamics simulations of ATAD2 docked to SOX10 suggest that ATAD2/SOX10 is not a stable structure. *Conclusion:* The central position of ADAT2 in the complex suggested that ADAT2 complexed to SOX10 may have the capability to modify multiple functions of the latter, one of which allows BRAF^{V600E} to impart increased oncogenic function to melanocytes. The results of the molecular dynamics simulations imply that the ADAT2/SOX10 complex is not stable and might be disrupted by a therapeutic molecule, reducing the risk of melanoma. Knowledge of the ADAT2/SOX10 complex structure may facilitate the development of drugs that can block complex formation.

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Melanoma, the most dangerous form of skin cancer, begins in melanocytes. The specific etiology of melanomas is unknown; however, UV radiation from the sun and tanning lights increases the risk of acquiring melanoma (1). A genetic component is also present.

Sry-related HMg-Box gene 10 (SOX10) is a key transcription factor (2). Adenosine triphosphatase family AAA domain-containing protein (ATAD2), a melanoma competence factor, forms a protein complex with SOX10 that promotes expression of SOX10 developmental target genes, as well as genes involved in the mitogen activated protein kinase (MAPK) pathway (2). The complex is sufficient to give oncogenic competence to melanocytes by facilitating a high transcriptional response to oncogenes like BRAF^{V600E} (2). Melanoma can arise in normally resistant melanocytes because of the complex. We have used the ClusPro web server for protein-protein docking to visualize and analyze the complex and GROMACS to perform molecular dynamics simulations (3).

Materials and Methods

The ClusPro server (<https://cluspro.org>) is a widely used tool for protein-protein docking (4). The server provides a simple home page for basic use, requiring only two files in Protein Data Bank (PDB) format (4). The quality of automated docking by ClusPro is very close to that of the best human predictor groups (5).

PDB entries were searched on the RCSB PDB (<https://www.rcsb.org/>). We identified one that allowed us to examine the relationship of ATAD2 to SOX10: 6CPS, referring to the crystal structure of the bromodomain of the human ATAD2 with a disulfide bridge. The PDB entry had been solved using x-ray diffraction, with a resolution of 1.93 Å (6). SOX10 had no entry in the RCSB Protein Data Bank. However, we obtained a protein structure prediction for human SOX10 from AlphaFold, an artificial intelligence (AI) system developed by DeepMind that predicts a protein's 3D structure from its amino acid sequence. AlphaFold regularly achieves accuracy competitive to experimental data (7, 8).

We then used GROMACS (v. 2021.3) to perform molecular dynamics simulations of ATAD2 docked to SOX10. GROMACS is a molecular dynamics package mainly designed for simulations of proteins, lipids, and nucleic acids (3). The all-atom OPLS-AA/L force field and SPC/E water model were used for simulations.

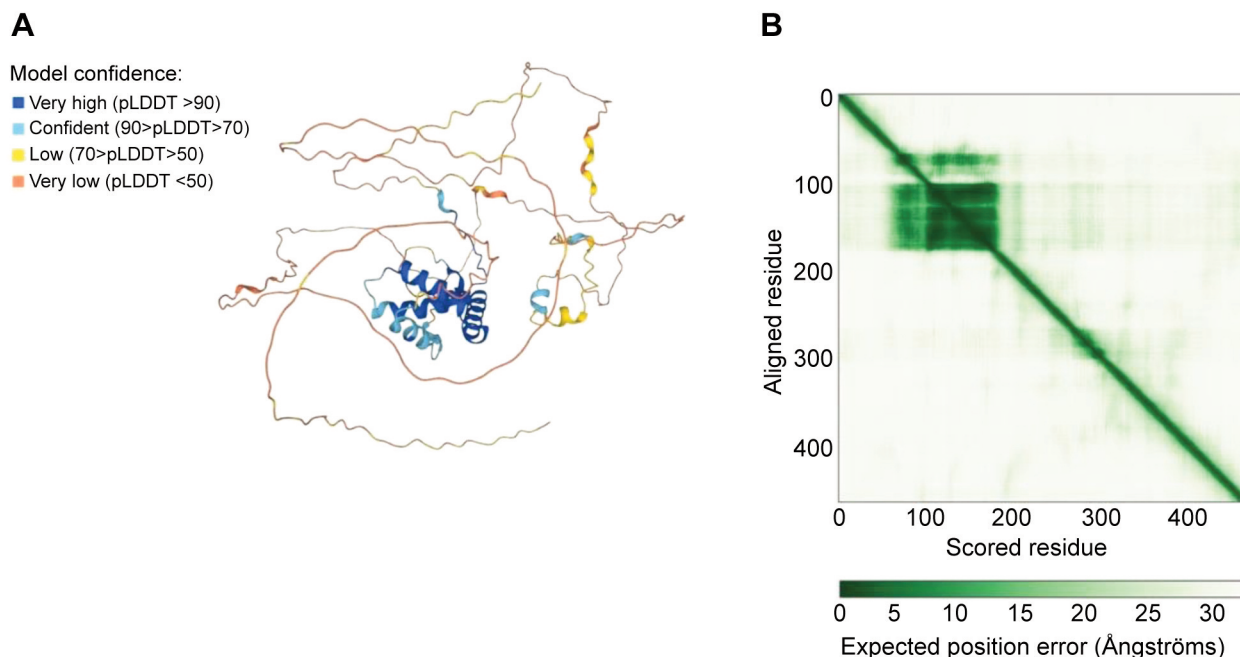


Figure 1. (A) SOX10 in AlphaFold, which produces a per-residue confidence score (pLDDT) between 0 and 100 (upper left). (B) Predicted aligned error for SOX10 10, indicative of AlphaFold's inter-domain accuracy. SOX10 has one clearly defined domain (dark green square upper left). The color at position (x, y) indicates AlphaFold's expected position error at residue x, when the predicted and true structures are aligned on residue y. Dark green represents low and light green high error. We are confident in the relative position of aligned residues 100-180.

Energy minimization was performed using the steepest descent method. System Equilibration was done in two phases. The first phase was conducted under an NVT ensemble (constant Number of particles, Volume, and system Temperature). The second phase was conducted under an NPT ensemble, wherein the Number of particles, Pressure, and Temperature are all constant. This ensemble is also called the "isothermal-isobaric" ensemble, and most closely resembles experimental conditions.

Results

The small predicted AlphaFold aligned error of SOX10 is shown in Figure 1. The predicted aligned error is useful for assessing AlphaFold's inter-domain accuracy. Figure 2 shows the top six ClusPro ATAD2/SOX10 protein-protein docked models. The sorting order is balanced, rather than electrostatic-favored, hydrophobic-favored, van der Waals interaction energy (E_{vdw}) or electrostatic energy (E_{elec}) favored. In the docked models, ATAD2 is at the center or near the center of SOX10. Figure 3 shows a detailed view of the top ClusPro ATAD2/SOX10 docked model (model 0). ATAD2 is near the center of SOX10.

When docking ATAD2 to SOX10, the ClusPro results display the model scores for the balanced coefficient set. The clusters of docked structures are listed in Table I in order of cluster size together with the actual weighting factors for the energy terms. The table lists each cluster's size (*i.e.*, the

Table I. ClusPro results showing the top 6 model scores for the balanced coefficient set when docking ATAD2 to SOX10. The table shows the actual weighting coefficients of the energy terms and lists the clusters of docked structures in order of cluster size. For each cluster, the table shows the size (*i.e.*, the number of docked structures or members), the weighted energy score of the cluster center (*i.e.*, the structure that has the highest number of neighboring structures in the cluster), and the weighted energy score of the lowest energy structure in the cluster.

Cluster	Members	Representative	Weighted energy score
0	84	Center	-999.2
0	84	Lowest energy	-1,085.1
1	69	Center	-832.9
1	69	Lowest energy	-952
2	61	Center	-900.8
2	61	Lowest energy	-1,079.1
3	51	Center	-861.5
3	51	Lowest energy	-987.9
4	43	Center	-953.8
4	43	Lowest energy	-1,000.7
5	41	Center	-869.7
5	41	Lowest energy	-924.6

number of docked structures or members), the cluster center's energy (*i.e.*, the structure with the most structures nearby it), and the cluster's lowest-energy structure's energy (4).

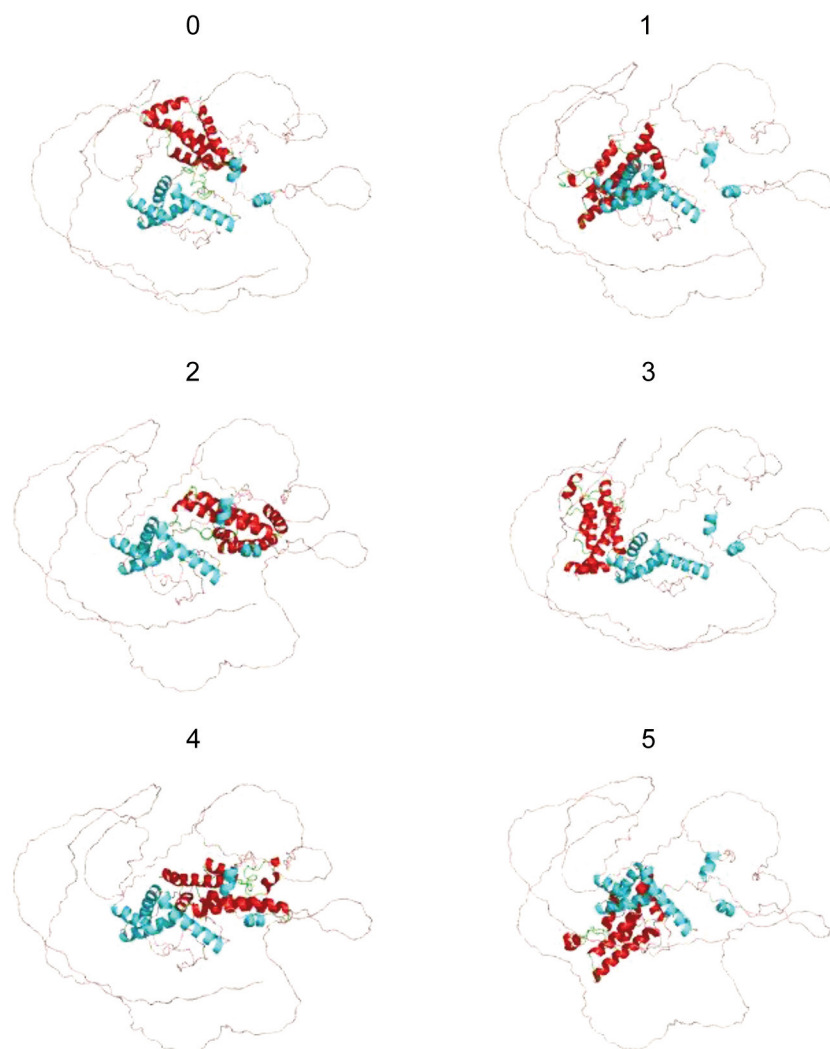


Figure 2. The top six ClusPro ATAD2/SOX10 docked models. The sorting order is balanced, rather than by electrostatic-favored, hydrophobic-favored, van der Waals interaction energy (E_{vdw}) or electrostatic energy (E_{elec}). Note that in the models ATAD2 (red) is at the center or near the center of SOX10.

Molecular dynamics simulations of ATAD2 docked to SOX10 are shown in Figure 4. The time series (Figure 4A) indicates that the structure does not stabilize appreciably. The RMSF (root mean square fluctuations) captures, for each atom, the fluctuation about its average position, indicating considerable flexibility in regions of the peptide. The constantly diminishing radius of gyration values (Figure 4B) do not reach an equilibrium. These results suggest that ATAD2 docked to SOX10 is not a stable structure.

Discussion

The SOX10 gene is a member of a gene family that is involved in the genesis of tissues and organs during embryonic

development (9). The SOX gene family maintains normal function of specific cells after birth (10). SOX proteins are well known transcription factors that help control the activation of genes by binding to key areas closely. The SOX10 gene is active in neural crest cells during embryonic development (11). These cells travel from the developing spinal cord to certain embryonic areas, where they give rise to a variety of cell types. The SOX10 gene produces a protein that controls the activation of other genes that signal neural crest cells to differentiate into specific cell types. The SOX10 protein is required for the formation of intestinal neurons (enteric nerves) and the production of melanocytes (12). Melanocytes create melanin, a pigment that contributes to the color of the skin, hair, and eyes. Melanin is also important to inner ear function (13).

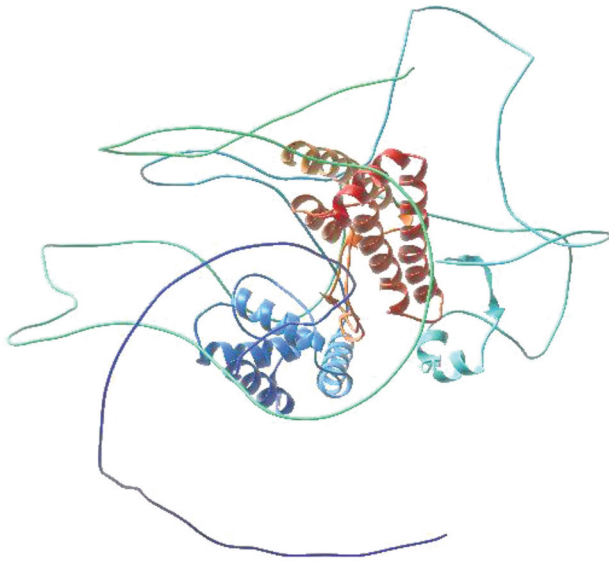


Figure 3. Detailed view of top ClusPro ATAD2/SOX10 docked model (model 0, rotated vertically). ATAD2 (red, orange) is near the center of SOX10.

Melanoma is a malignancy that develops from the melanocytic lineage of the neural crest. Melanocytes are responsible for skin pigmentation, as well as protecting epidermal keratinocytes from DNA damage caused by ultraviolet (UV) irradiation. Melanoma is mostly found on the skin, although it can also be found in other places, such as the eye or mucosal surfaces. A founder mutation is thought to be essential for melanomagenesis, as it causes uncontrolled proliferation of the afflicted cells (14). Surprisingly, similar changes, known as driver mutations, are also found in normal, healthy skin. But it is uncertain why some cells can form tumors after obtaining an oncogenic driver mutation, while others remain mostly dormant. The study by Baggiolini *et al.* provided evidence that supported the concept of oncogenic competence, which states that oncogenes must have a permissive chromatin landscape to alter cells (2).

Despite the existence of a driver mutation like BRAF^{V600E}, which replaces Val600 with Glu, melanocytes have a high threshold for progressing to melanoma. Melanomagenesis is enabled by the chromatin-modifying enzyme ATAD2, which is expressed in lower levels in melanocytes than in melanoblasts and neural crest cells (15, 16).

The central position of ADAT2 in the ADAT2/SOX10 complex suggests that complexed ADAT2 may have the capability to modify multiple SOX10 functions, one of which allows BRAF^{V600E} to impart increased oncogenic function to melanocytes. The results of molecular dynamics simulation imply that the ADAT2/SOX10 complex is not

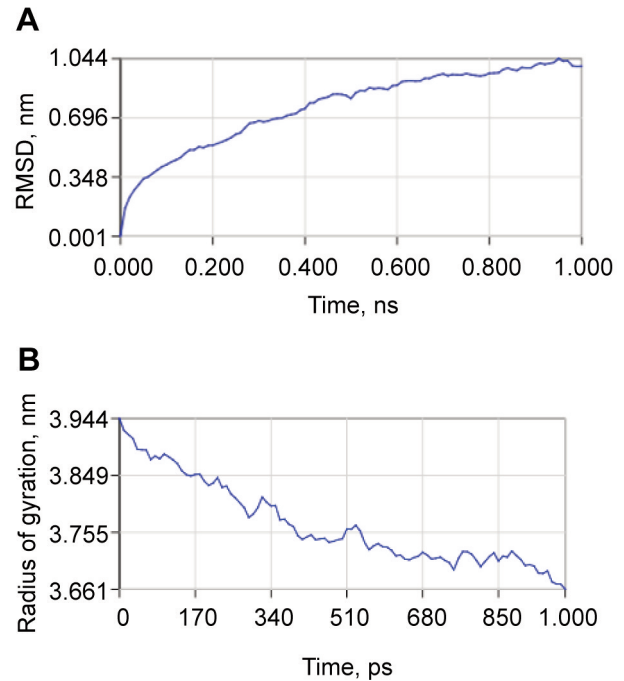


Figure 4. (A) Molecular dynamics simulations of ATAD2 docked to SOX10. Time series shows the RMSD levels continually increasing, indicating that the structure does not stabilize appreciably. (B) The constantly diminishing radius of gyration (Rg) values that do not stabilize or reach an equilibrium suggest that ATAD2 docked to SOX10 is not a stable structure.

stable and might be disrupted by a therapeutic molecule, reducing the risk of melanoma.

Our analysis has weaknesses. We do not have conclusive evidence supporting an interaction of the bromodomain of ADAT2 with SOX10. Bromodomains recognize and bind to acetylated lysine (17), and we cannot ascertain whether SOX10 is acetylated at any surface residues. If so, AlphaFold would not show post-translational modifications in the model, so this would need to be added before the docking experiment is carried out. Moreover, bromodomains are not known to mediate protein-protein interactions through mechanisms outside acetylated lysine recognition and we are uncertain of which protein domains are involved in the molecular interactions between SOX10 and ATAD2; therefore, we cannot say for certain that the interaction is through the bromodomain. A weakness in our Gromacs simulation is that it was carried out for only 1ns, a relatively short time, although other published simulations do utilize this time interval (18).

Conclusion

The central position of ADAT2 in the complex suggested that ADAT2 complexed to SOX10 may have the capability to

modify multiple functions of the latter, one of which allows BRAF^{V600E} to impart increased oncogenic function to melanocytes. The results of the molecular dynamics simulations imply that the ADAT2/SOX10 complex is not stable and might be disrupted by a therapeutic molecule, reducing the risk of melanoma. Knowledge of the ADAT2/SOX10 complex structure may facilitate the development of drugs that can block or disrupt complex formation and prevent melanoma. Further studies are warranted.

Conflicts of Interest

None.

Authors' Contributions

SL and PHR contributed equally to the conception, writing, and data analysis of this study.

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