Structural analysis of the zebrafish fhl1 protein focusing on the zinc fingers

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Background

- Mutations of *FHL1* gene cause some muscle diseases such as reducing body myopathy (RBM) [1] and rigid spine syndrome (RSS) [2].
- The FHL1 protein encoded by the *FHL1* gene consists of four and a half LIM domains, and each LIM domain has two zinc finders [3] (Fig. 1).
- Zinc finger consists of zinc-binding amino acids, and each zinc finger has a β-hairpin and an α-helix.
- Although the overall structure of the human FHL1 protein is unknown, the conformation of each LIM domain LIM1, 2, 3, 4 is known.

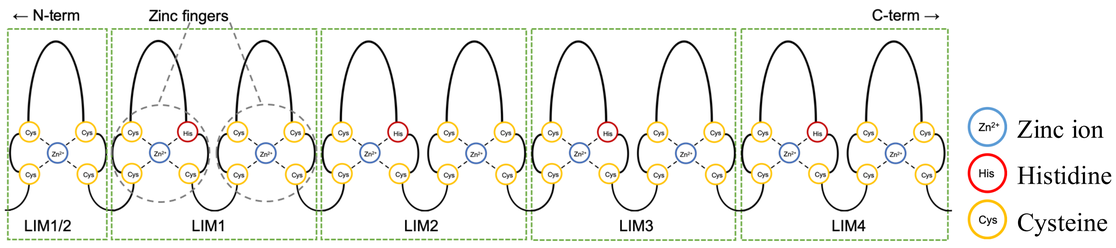


Fig. 1. Schematic diagram of the overall structure of the FHL1 protein (created based on [3])

- In zebrafish, a model organism, it has also been reported that mutation in the *fhl1* gene causes symptoms of muscle disease [4].
- However, it is unknown how mutation of the *fhl1* gene affects the conformation of the fhl1 protein.

Objective

To clarify the stable three-dimensional structure of the zebrafish fhl1 protein *in silico*, we perform MD simulations of the wild-type zebrafish fhl1 protein.

Methods

Molecular Dynamics simulation

The system was set up and filled with water and counterions. After energy minimization, equilibration by both *NVT* and *NPT* ensemble were performed for 100 ps each, followed by MD simulation. Each trial was restarted by adding counterions, and 4 trials were performed.

Simulation conditions

The three-dimensional structure was generated from the amino acid sequence using AlphaFlod2. By adding zinc atoms to this structure, the three-dimensional structure of the zebrafish fhl1 protein (Fig. 2) [5] was created. This structure was used as the initial structure, and MD simulations were performed under the following conditions.

- Software：GROMACS 2021.5
- Boundary Condition：Periodic boundary conditions
- Box size (nm)：20 × 20 × 20
- Force field：Amber ff99SB-ILDN, ZAFF
- Water model：TIP3P
- Time (ns)：1000 × 4 trials
- Temperature (K)：300 (constant)
- Pressure (bar)：1 (constant)
- Number of molecules
  fhl1：1
  Cl^-：1
  Water：262,200

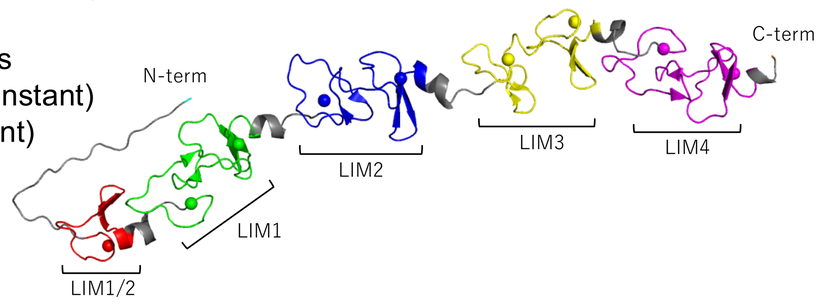


Fig. 2. The three-dimensional structure of the zebrafish fhl1 protein [5]

Analysis

RMSD (Root mean square deviation)

$$\mathrm{RMSD}(t) = \sqrt{\frac{\sum_{i=1}^{N} m_i \|\boldsymbol{r}_i(t) - \boldsymbol{r}_i(t_0)\|^2}{\sum_{i=1}^{N} m_i}}$$

$i = 1, 2, \ldots, N$
m_i：mass of atom i
$\boldsymbol{r}_i(t)$：position of atom i at time t
$\boldsymbol{r}_i(t_0)$：position of atom i at time t_0
$\boldsymbol{r}_\mathrm{G}(t)$：coordinate center of mass for molecule at time t

R_g (Radius of gyration)

$$R_\mathrm{g}(t) = \sqrt{\frac{\sum_{i=1}^{N} m_i \|\boldsymbol{r}_i(t) - \boldsymbol{r}_\mathrm{G}(t)\|^2}{\sum_{i=1}^{N} m_i}}$$

Analysis of the twist between two zinc fingers

To quantify the twist between two zinc fingers, the changes in the dihedral angles θ were calculated (Fig. 3). The residue furthest from the zinc atom among the residues that make up the β-hairpin fold was taken as the top (Table 1).

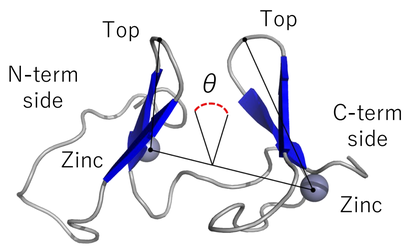


Fig. 3. Dihedral angle between two zinc fingers

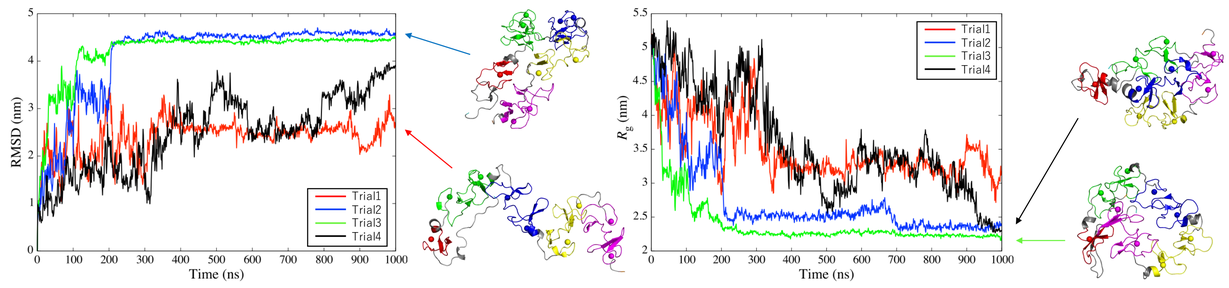
Table 1. Dihedral angle in the initial structure of each LIM domain and residues defined as tops

	Dihedral angle (θ)	Residues as top
LIM1	−45°	K73, D101
LIM2	−33°	K135, G162
LIM3	−57°	Q194, E221
LIM4	−49°	E258, G285

Result

RMSD, R_g of the whole fhl1 protein

- The RMSD of the whole fhl1 protein (Fig. 4a) showed large fluctuations of 1–4 nm, but remained stable after 200 ns in trial 2 and 3.
- In all trials, the structure changed to smaller R_g (Fig. 4b).
- Trial 4 showed a more complex folded structure than trial 2 and 3.

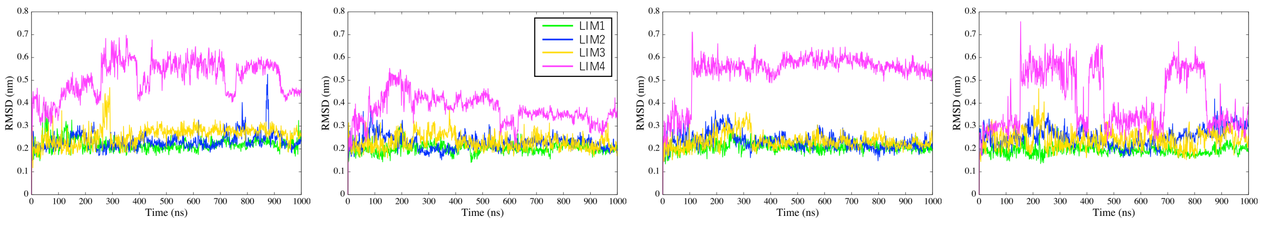


(a) RMSD (b) R_g

Fig. 4. Time evolution of RMSD and R_g of the whole fhl1 protein

RMSD of each LIM domain

- The RMSD of each LIM domain (Fig. 5) showed large fluctuations in LIM4 domain at all trials, showing a different trend form among LIM 1–3 domains.



(a) Trial 1 (b) Trial 2 (c) Trial 3 (d) Trial 4

Fig. 5. Time evolution of RMSD of each LIM domain

Changes in the twist of each LIM domain

- In the LIM 1–3 domain, the dihedral angles did not change significantly from the initial values (Fig. 6a–c).
- In the LIM4 domain, there was a change in the twist between the two zinc fingers (Fig. 6d).

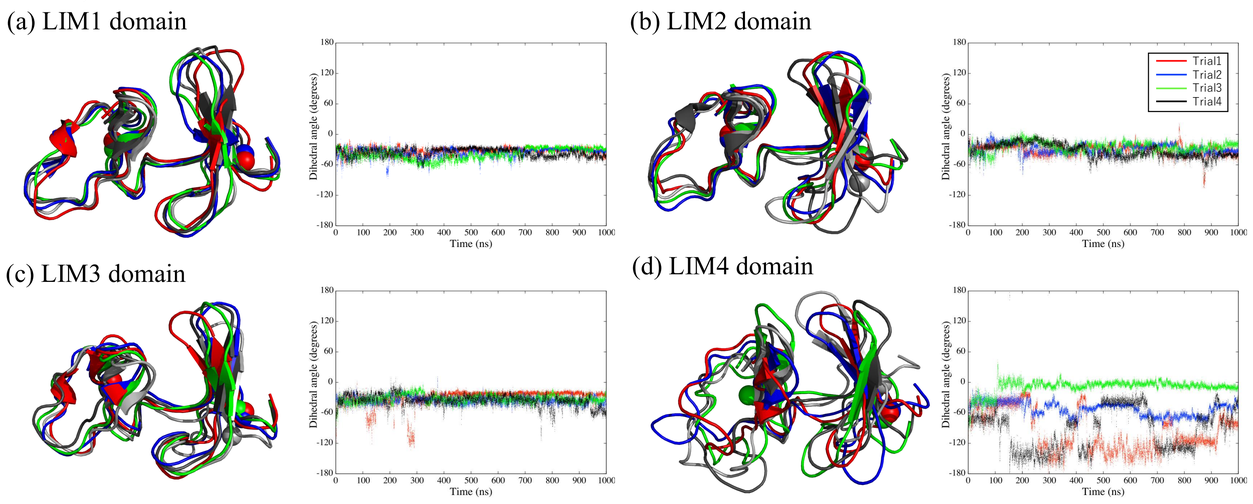
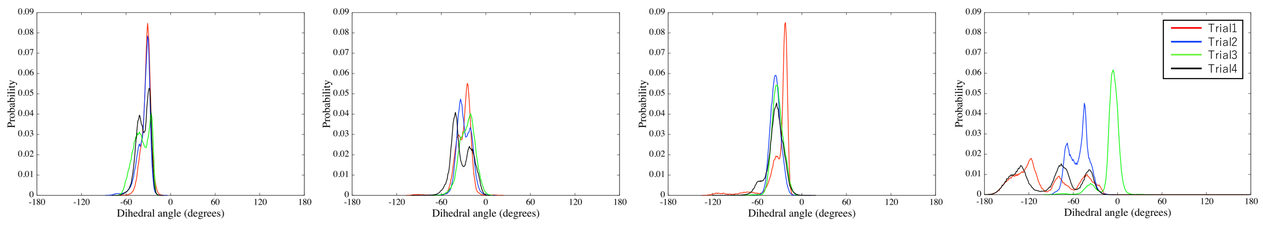


Fig. 6. Structure changes and time evolution of the dihedral angles of each LIM domain

Distribution of possible dihedral angles for each LIM domain

- The dihedral angles of the LIM4 domain (Fig. 7d) had a wide range, but those of the LIM1–3 domains (Fig. 7a–c) remained within the range of −60° to 0°, showing little change from the initial structure.
- In trials 1 and 4, three angles with high probability of existence were observed in the dihedral angles of the LIM4 domain (Fig. 7d).

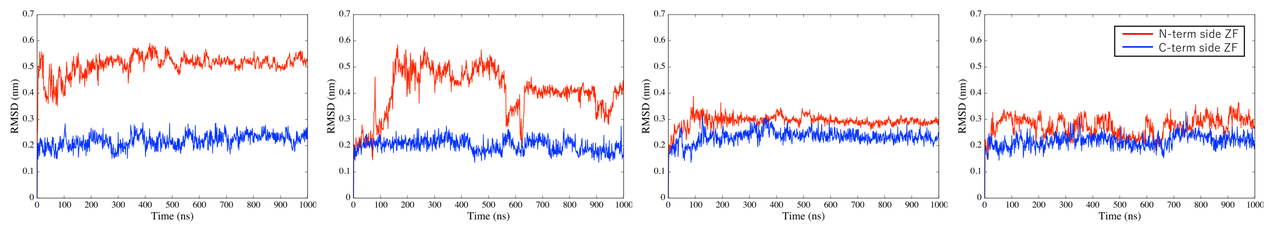


(a) LIM1 domain (b) LIM2 domain (c) LIM3 domain (d) LIM4 domain

Fig. 7. Probability of dihedral angle in two zinc fingers of each LIM domain

RMSD of each zinc finger in the LIM4 domain

- In the LIM4 domain, which showed the largest structural change, the zinc finger at the N-term side had the largest RMSD (Fig. 8).



(a) Trial 1 (b) Trial 2 (c) Trial 3 (d) Trial 4

Fig. 8. Time evolution of RMSD of each zinc finer in LIM4 domain

Summary

- The whole fhl1 protein underwent a structural change, becoming smaller and rounder.
- The LIM4 domain showed larger structural changes than the LIM1–3 domains.
- In the LIM4 domain, a twist was observed between two zinc fingers.
- Of the two zinc fingers in the LIM4 domain, the zinc finger at the N-terminus showed the largest change in RMSD.

Future outlook

- We would like to analyze the transition of structural changes in the LIM4 domain.
- Since the structures obtained in this simulation are all different, the more extensive structural search will be conducted to derive the more stable structure.
- Analysis of the interactions among nine zinc fingers is necessary.

Acknowledgment

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References

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