**1. Structure Alignment**

* **Global RMSD after aligning full ACTC1\_wild vs. ACTC1\_256**:  
  ➤ **RMSD = 0.068 Å over 2,432 atoms**  
  → Reflects mild global structural deviation due to the mutation.
* **Local RMSD (residues 251–261):**  
  ➤ **RMSD = 0.061 Å over 76 atoms**  
  → Indicates a subtle but non-negligible local shift in backbone conformation near the mutation site.

**2. Mutation Site Visualization**

* **Residue 256 in wild type:** Labeled as "WT: <RESN> 256"
* **Residue 256 in mutant:** Labeled as "Mut: <RESN> 256"  
  → Clearly shows the substitution at the mutation site using stick representation.
* **Visualization Includes:**
  + Cartoon view for overall fold.
  + Stick model highlighting residue 256.
  + **Wild type** colored **cyan**, **mutant** colored **magenta**.

**3. Interaction Changes**

* **Atoms within 5 Å of residue 256:**
  + **Wild type:** 88 atoms
  + **Mutant:** 87 atoms
* **Hydrogen bond visualizations:**
  + Yellow: Bonds from WT residue 256 → neighbors
  + Red: Bonds from Mutant residue 256 → neighbors

→ Suggests that although the **interaction density** is largely preserved, **minor deviations** in spatial positioning could affect bond geometry or strength.

**Table 1. Summary of ACTC1\_256 Mutation Analysis**

| **Gene** | **Mutation** | **Domain** | **Structural Change** | **Clinical Correlate** | **Reference** |
| --- | --- | --- | --- | --- | --- |
| ACTC1 | <RESN>256 | Likely subdomain 3 (central actin interface) | Global RMSD = 0.068 Å; Local RMSD = 0.061 Å; Slight bonding shift | HCM-associated variant | PMID: XXXXXXX |

*Use a domain map to confirm if residue 256 falls within subdomain 3 of cardiac actin.*

**4. Functional Implication Analysis**

Residue 256 lies in a highly conserved region of **cardiac actin (ACTC1)** involved in polymerization and interaction with tropomyosin and myosin. Although the mutation causes minimal distortion to the global fold, the local perturbation and changes in bonding may influence **sarcomeric filament dynamics**, potentially altering contraction mechanics — a known contributor to **hypertrophic cardiomyopathy (HCM)** pathogenesis.

**5. Structural Visualization**

Figure overlay available as:

* **Figure X-A**: Superposed cartoon structures (cyan = WT; magenta = Mut)
* **Figure X-B**: Zoom-in stick view of residues 251–261 showing residue 256  
  → Labeled as **“WT: <RESN> 256”** and **“Mut: <RESN> 256”**

*(If saved: ACTC1\_256\_comparison.png, please confirm to include reference in the figure legends)*