



INSIGHTS INTO SUBSTRATE UNFOLDING AND ALLOSTERIC REGULATION OF INSULIN DEGRADING ENZYME: A MOLECULAR DYNAMICS STUDY

Felicia Jiang¹, Arthur O. Vale¹, Wei-Jen Tang², Esmael J. Haddadian³

¹Physical Sciences Collegiate Division, ²Ben-May Department for Cancer Research, ³Biological Sciences Collegiate Division, University of Chicago, Chicago, IL 60637, USA

Abstract

Insulin degrading enzyme (IDE) is involved in the clearance of insulin, amylin, and glucagon, peptides involved in controlling blood glucose level. IDE also degrades amyloid- β , a peptide vital for Alzheimer's disease progression. IDE has two homologous, ~50 kDa N- and C-terminal domains, IDE-N and IDE-C. For IDE to effectively degrade its substrates, both domains must come together for the catalytic cleft to assume fully closed conformation. Experimental data suggests that IDE undergoes at least two conformational switches in its catalytic cycle: one, the transition between the closed- and open-conformations, and two, the "swinging door" motion at the catalytic cleft. However, the exact mechanism of these two conformational switches is unknown. We investigated this mechanism using microsecond-long molecular dynamics simulations of IDE structures with and without substrate. We find that the N-terminus of the insulin molecule chain-A forms a cross- β -sheet with IDE secondary structure elements, placing insulin near the catalytic site. Concordantly, insulin also undergoes conformational change; the insulin chain-B central α -helix becomes less ordered, a key step in the unfolding of insulin for degradation. The presence of insulin moves IDE-N and IDE-C closer (the angle between them decreases ~5°) and causes these two domains to undergo a grinding motion. Furthermore, greater flexibility for the door, H-loop, and the hinge and interface between IDE-N and IDE-C is evident when insulin is present. These observations suggest that the presence of insulin induces both global and local conformational changes in IDE to unfold insulin for cleavage. Principal component analysis shows negatively-correlated opening and closing motions of dimeric IDE, suggesting that the capture of substrate in one monomer may allosterically induce the other to open. Our simulation and principle component analysis offer new insight into substrate unfolding inside the IDE catalytic chamber and its allosteric regulation.

Overview of Insulin Degrading Enzyme

Experimental data suggests that IDE undergoes at least two conformational switches in its catalytic cycle: one, the transition between the closed- and open-conformations, and two, the "swinging door" motion at the catalytic cleft. However, the exact mechanism of these two conformational switches is unknown.

- What are the effects of dimerization and substrate capture on IDE conformation?
- What is the mechanism of IDE transition between open and closed states?



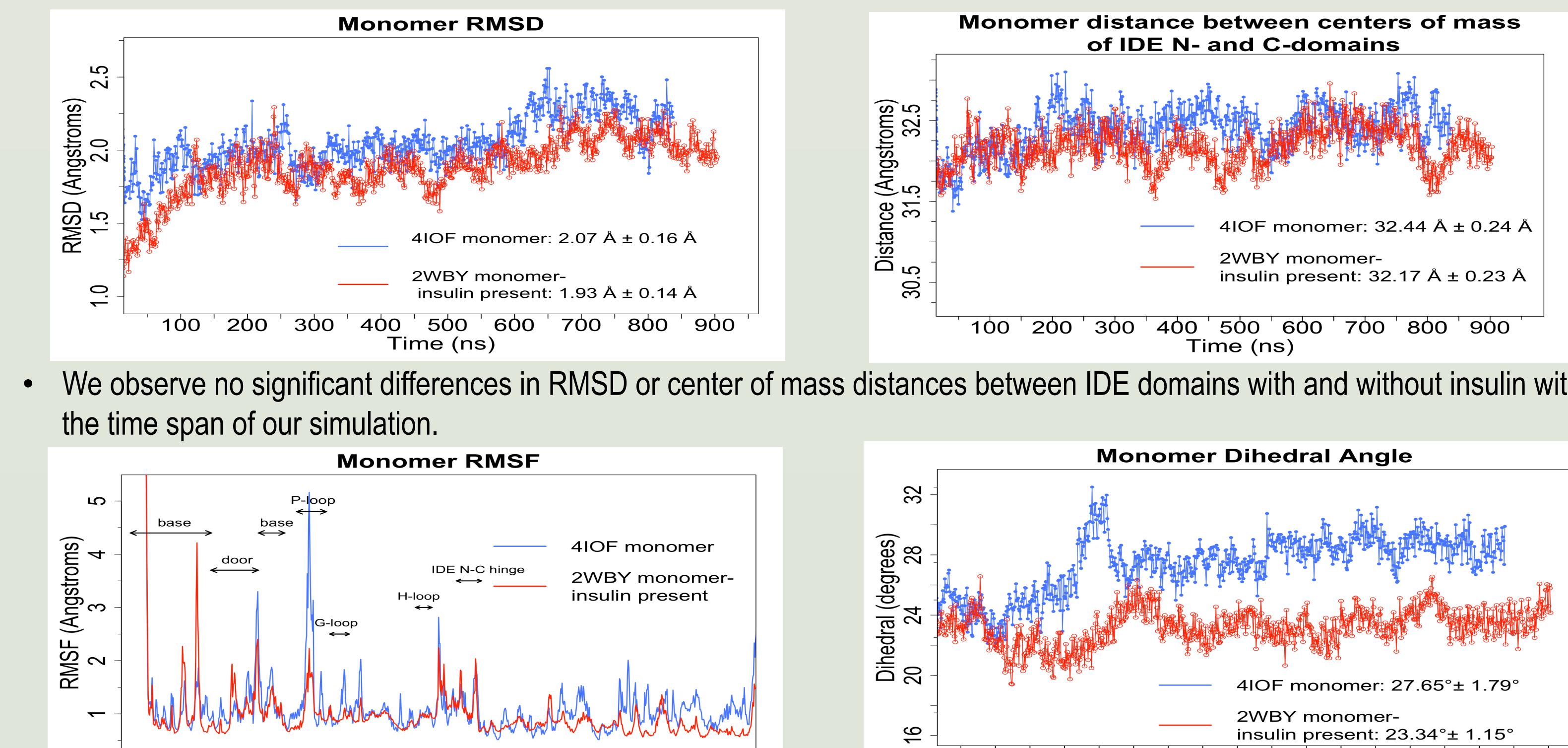
Materials and Methods

We performed all atom, explicit solvent, molecular dynamics simulations based on six structural models of IDE (table on the right). IDE monomers bind with high affinity to each other (10 nM), thus IDE exists normally as a dimer. We performed two monomer and four dimer simulations, using two IDE models: insulin-bound IDE (PDB ID 2WBY) and substrate-free IDE (4IOF). In order to study allosteric of IDE, we constructed two IDE dimers from two different initial PDB structures (chimera dimers 1 and 2).

Simulation Parameters: All simulations were performed with the NAMD molecular dynamics package, explicit TIP3P solvent and the CHARMM36 force field at 300 K and 1 Bar.

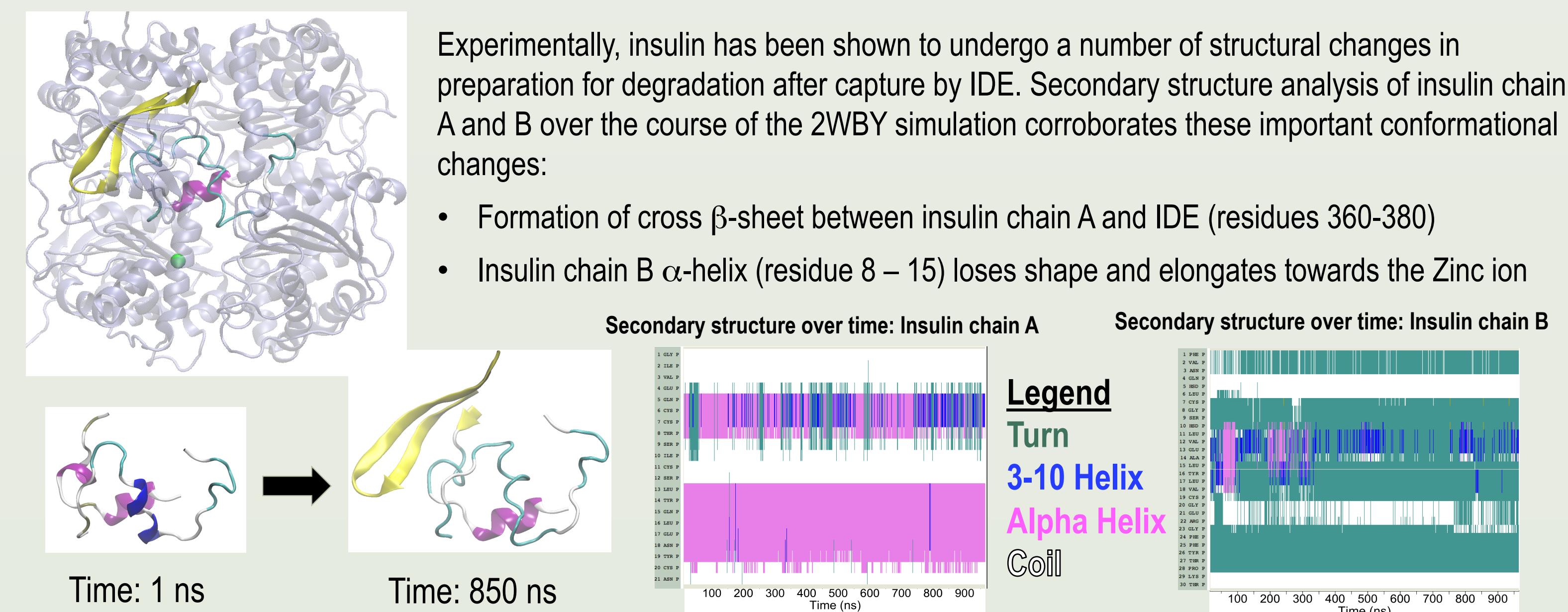
| Simulation Name | PDB code | Presence of insulin | simulation Time (ns) |
|-----------------|----------------------------|---------------------|----------------------|
| 4IOF monomer | 4IOF-chain A | none | 900 |
| 2WBY-monomer | 2WBY-chain A | present | 850 |
| 4IOF dimer | 4IOF | none | 570 |
| 2WBY dimer | 2WBY | present | 150 |
| Chimera-Dimer 1 | 4IOF-chainA & 2WBY-chain B | present (chain B) | 430 |
| Chimera-Dimer 2 | 2WBY-chainA & 4IOF-chainB | present (chain A) | 430 |

Insulin binding changes the dynamics of IDE monomers

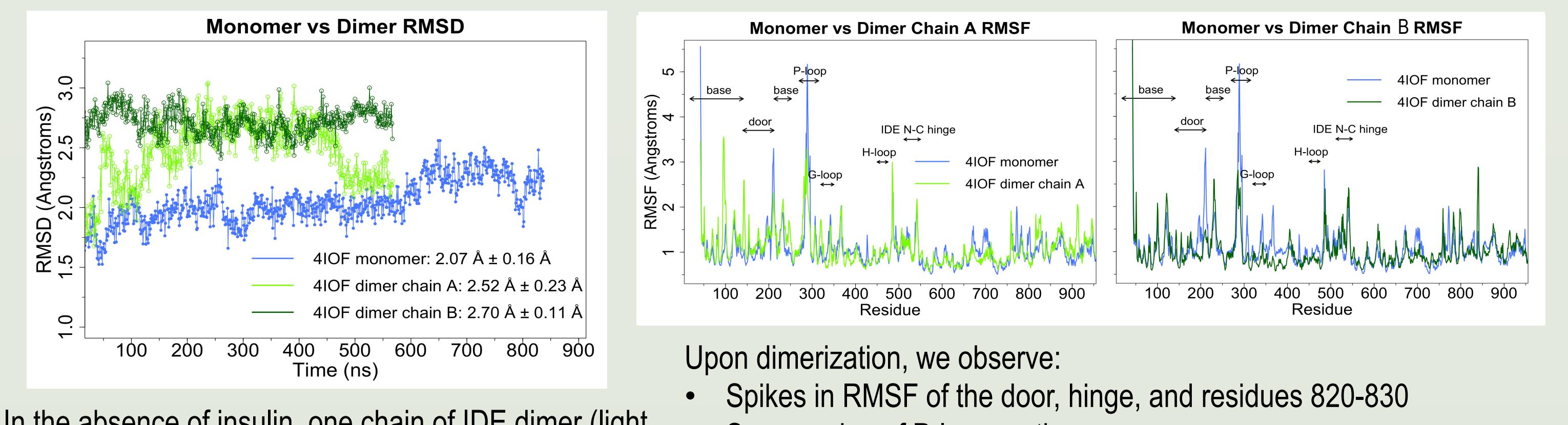


- In order to quantify the grinding motion of the IDE N- and C- domains, we measure the dihedral angle between the centers of mass of the four IDE domains. We observe a significant 3 degree decrease in dihedral angle in the monomer with insulin, suggesting a larger grinding motion between the two IDE domains.
- The presence of substrate in the IDE catalytic chamber suppresses the RMSF of the P-loop, G-loop, and H-loop, while stimulating base movement.

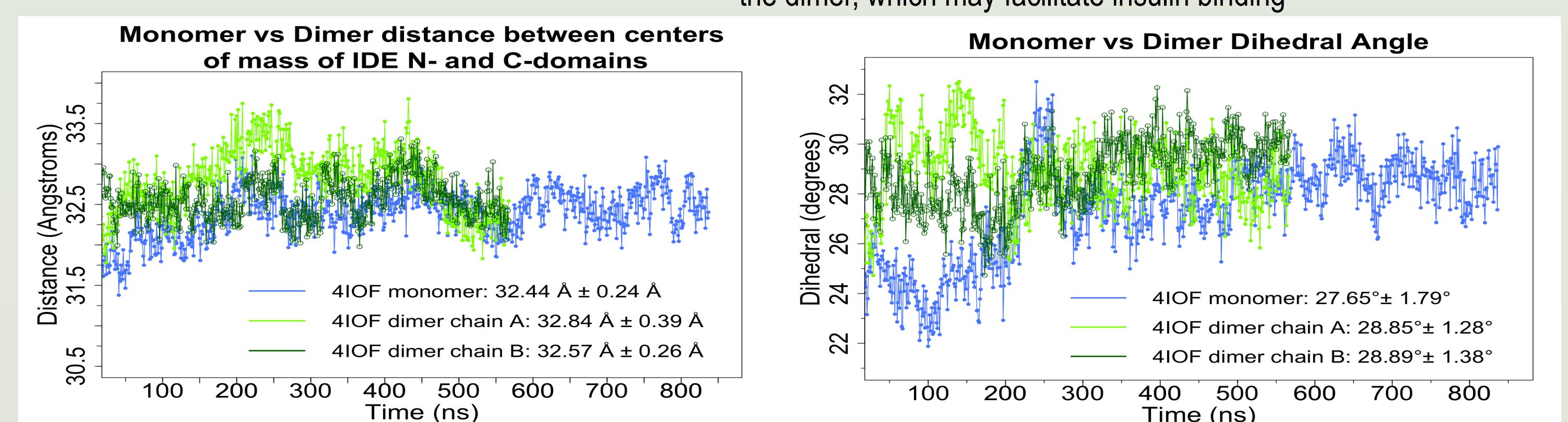
Insulin undergoes conformation changes inside IDE catalytic chamber



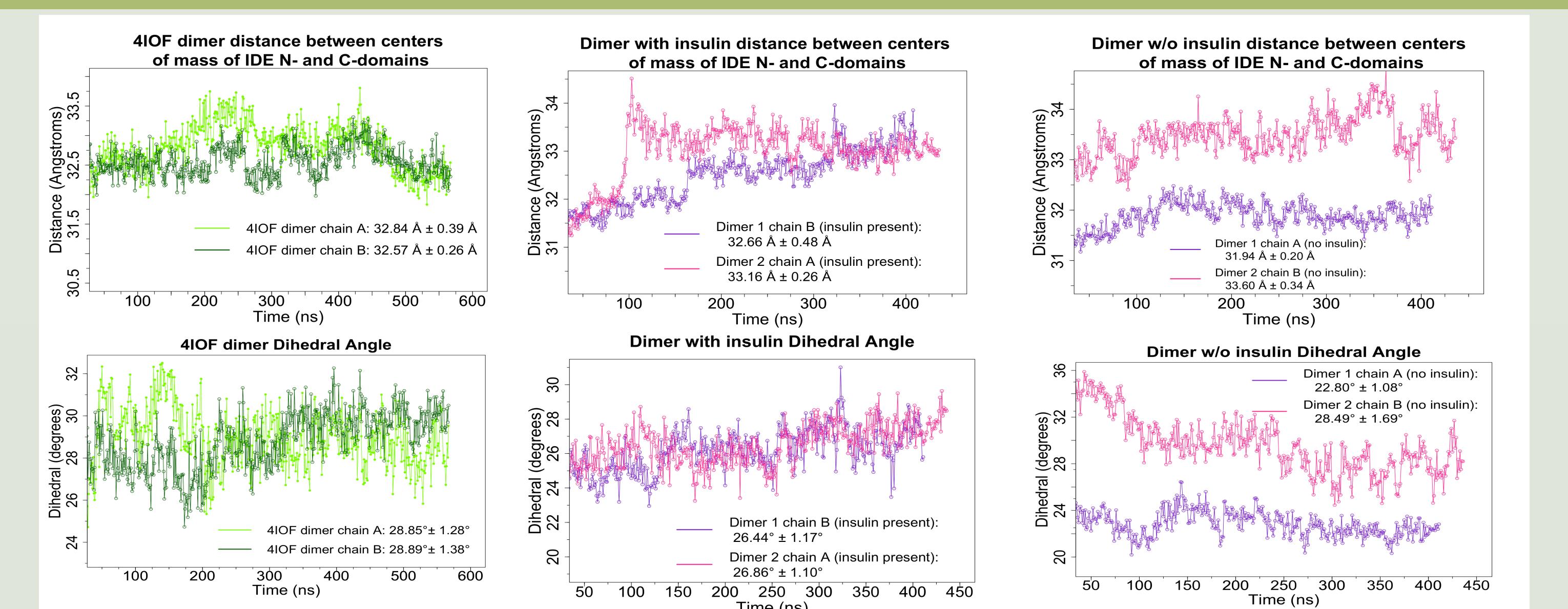
IDE dimerization in absence of insulin induces local flexibility, revealing potential mechanisms of allostery



- Upon dimerization, we observe:
- Spikes in RMSF of the door, hinge, and residues 820-830
 - Suppression of P-loop motion
 - Increase in base and door movements in one of the monomers in the dimer, which may facilitate insulin binding

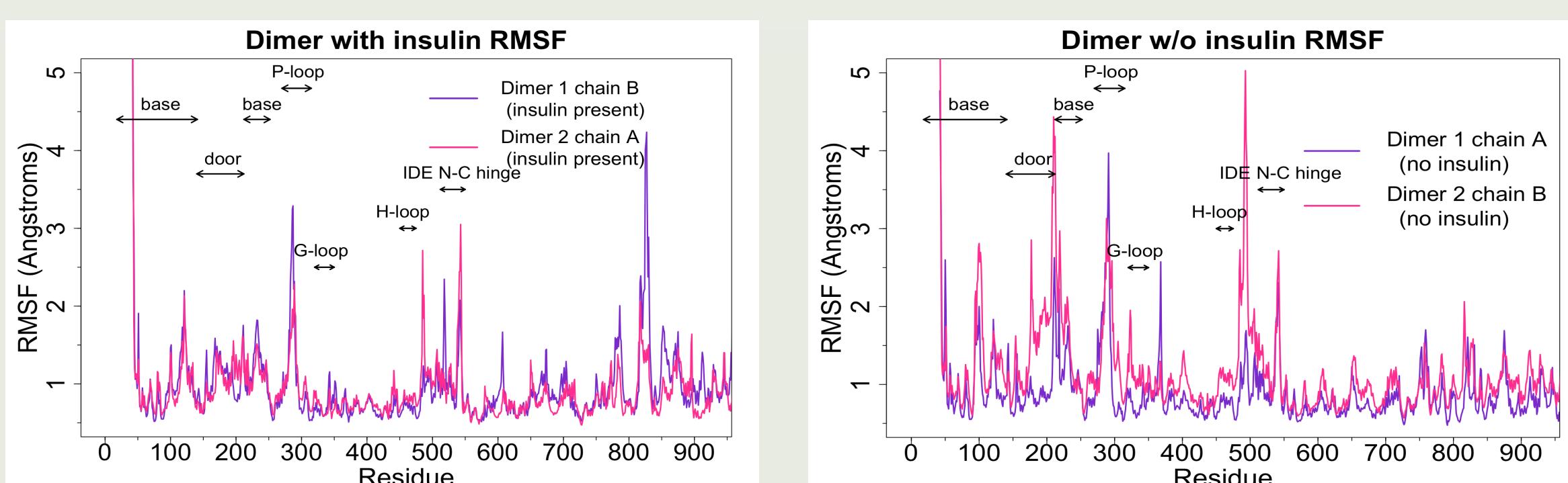


Insulin binding to one monomer of IDE dimer stabilizes its global motion, while inducing greater fluctuations in the adjacent, substrate-free monomer



- Right column: empty monomers lying adjacent to monomers containing insulin show greater fluctuations in motion
- We do not observe this allosteric effect in the substrate-free dimer, further suggesting that conformational changes in substrate-free monomers in chimera dimers is induced by presence of insulin in the adjacent monomer.

Presence of insulin in one monomer of IDE dimer stabilizes the local motion of that monomer, while causing adjacent, empty monomer to be more locally flexible

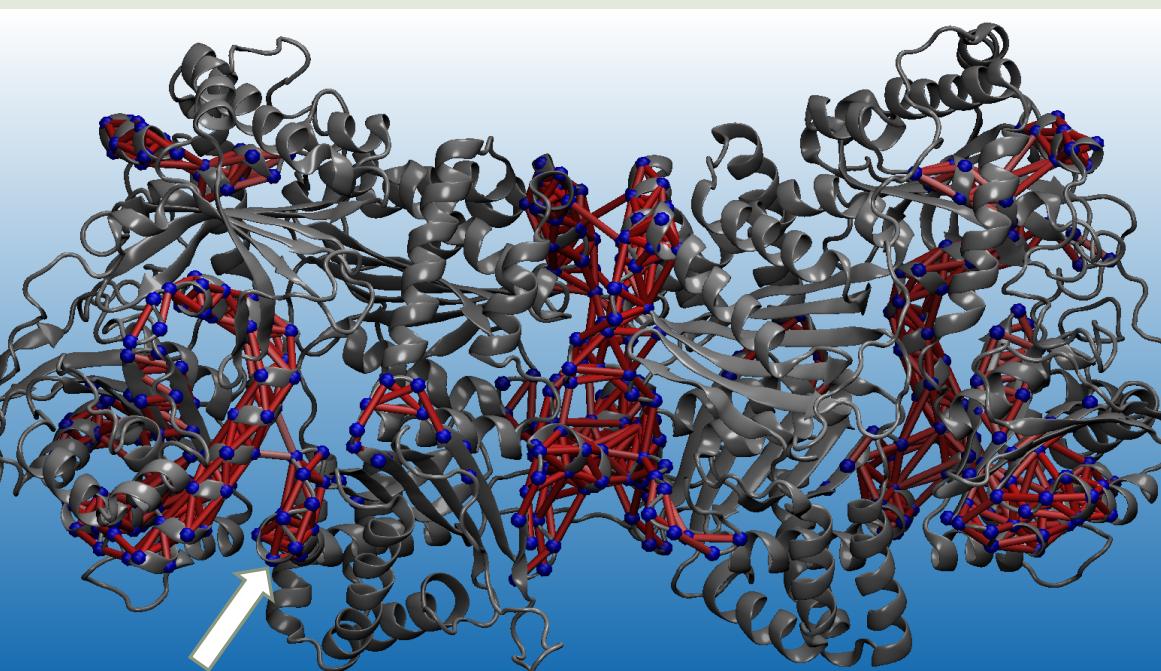
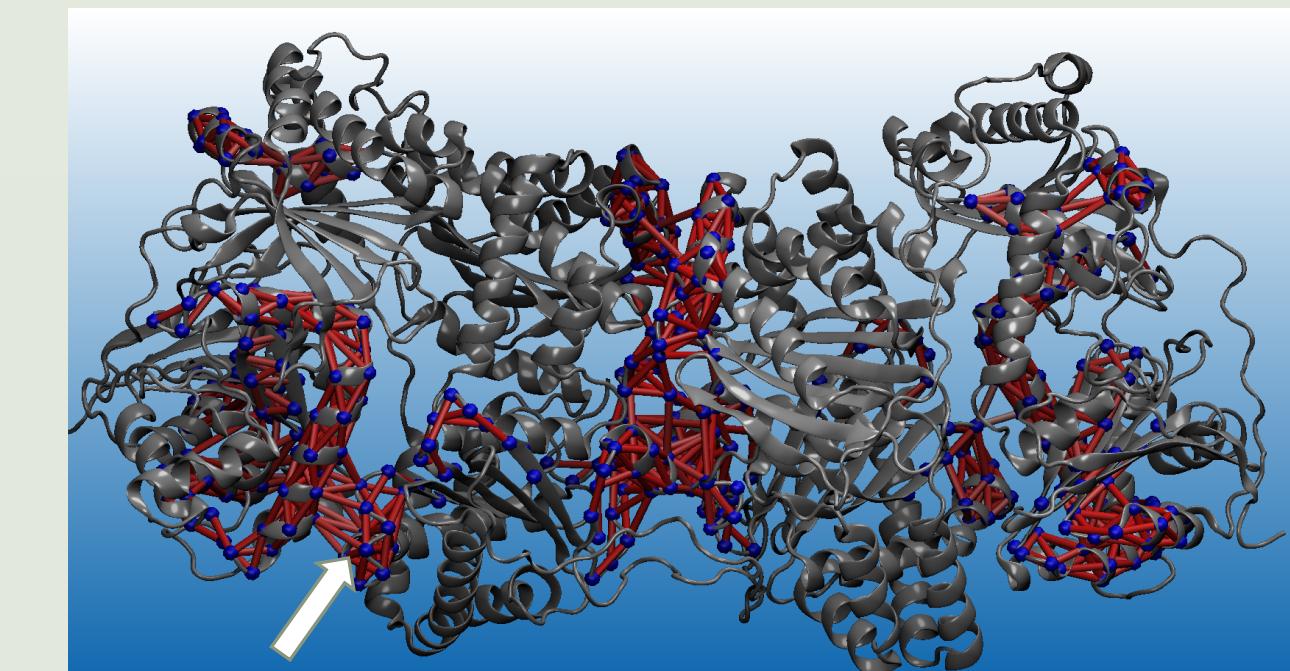


- Greater fluctuations in base, door, and G-loop motion

Network analysis reveals suppression of door domain cooperative motion in presence of insulin

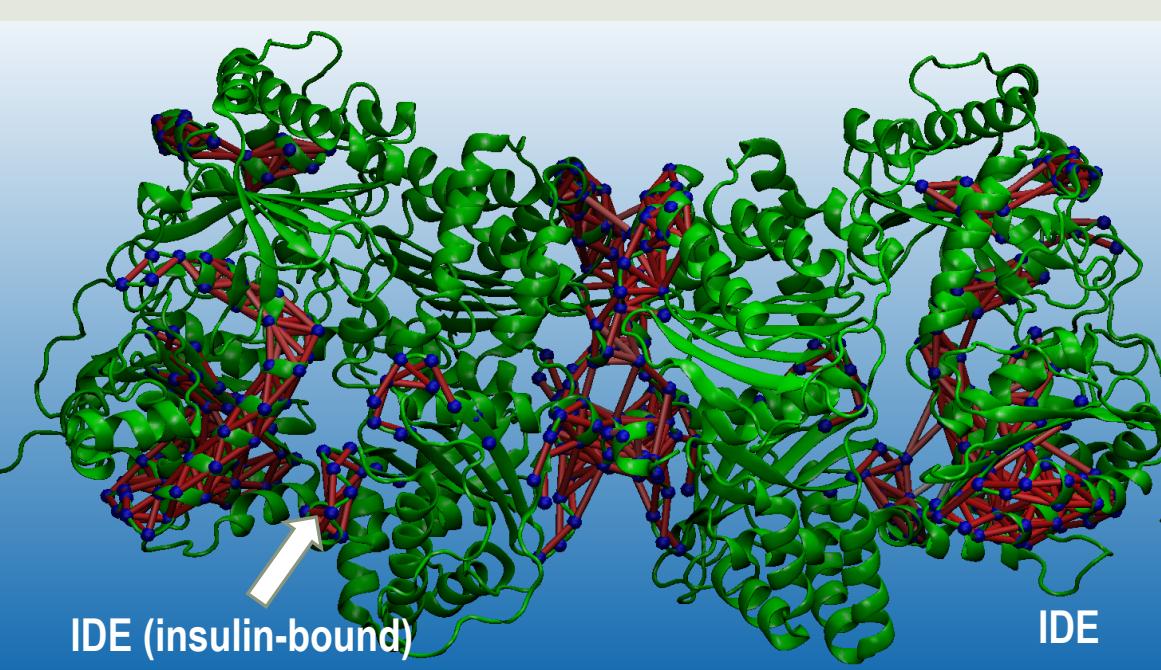
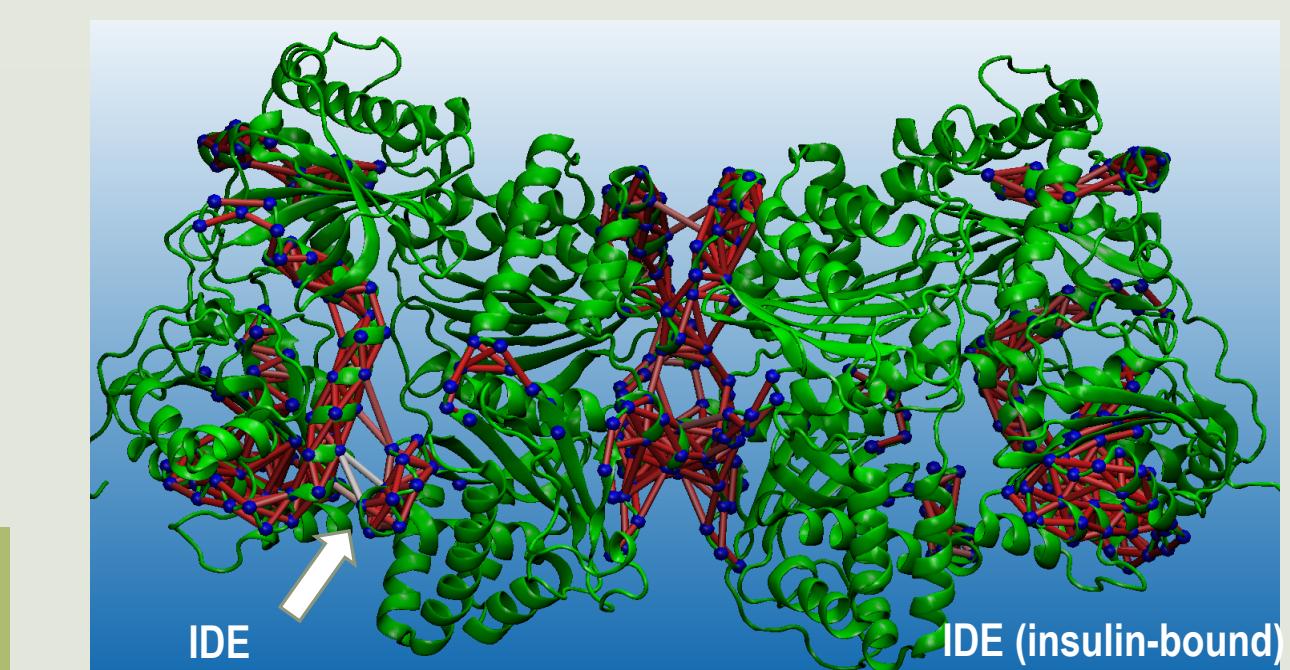
- Correlation network analysis based on the 4IOF dimer and dimer 2 trajectory reveal how residue movements correlate.
- Blue nodes represent individual residues and red edges represent correlation between movements nodes. The strength of the correlation is represented by the color gradient (white to red, low to high correlation).

Substrate-free (4IOF) dimer: Front View



- Right: strong door domain correlation with IDE C-domain residues in chain A
- Left: weak door domain correlation with IDE C-domain residues in chain B

Insulin-bound dimer: Front View



- Right: weak correlation between door and IDE C-domain movement in chain B (no insulin)
- Left: no correlation with IDE C-domain in chain A (insulin-bound)
- We observe higher door to C-domain network correlation in 4IOF dimer (no insulin) compared to dimer 2 (chain A containing insulin), suggesting that insulin-binding weakens the correlation of the door domain with IDE-C motion.

Conclusions

- Insulin-binding of IDE monomer stimulates grinding motion and local motion of residues involved in substrate recognition and catalysis.
- RMSF and network analyses reveal insulin stabilization of the door domain, reinforcing the "locked" door hypothesis of IDE substrate capture.
- The N-terminus of insulin chain A forms a cross β -sheet with IDE exosite, while chain B loses α -helix structure, allowing unfolding of insulin for its degradation.
- Dimerization of substrate-free IDE induces greater motions in residues involved in substrate capture and degradation, corroborating the observation that dimeric IDE has higher catalytic rate than monomeric IDE.
- Insulin capture by one of the two IDE monomers, as observed in physiological conditions, induces larger motion in the adjacent subunit. This may allow the adjacent subunit to better capture the substrate, while the insulin-bound monomer is degrading the substrate.
- Our analyses offer insight into substrate unfolding within the IDE catalytic chamber and allosteric regulation of IDE dimerization.