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論文審査の結果の要旨および担当者

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氏　　名　SRIVASTAVA Arpita

論　文　題　目　Conformational Dynamics of Mitochondrial Import Proteins: A View from Molecular Dynamics Simulations on Experimental Data

(ミトコンドリアタンパク質輸送体サブユニットの運動と構造：
分子動力学シミュレーションで見る実験データ)

論文審査担当者

主査　名古屋大学大学院理学研究科　教授　Ph.D.　TAMA Florence
委員　名古屋大学大学院理学研究科　教授　Ph.D.　岡本　祐幸
委員　名古屋大学大学院理学研究科　教授　博士（工学）　内橋　貴之
委員　名古屋大学大学院理学研究科　准教授　博士（理学）　倭 剛久
委員　名古屋大学大学院理学研究科　准教授　博士（理学）　槇 亙介

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論 文 審 査 の 結 果 の 要 旨

The translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) complexes play a role to selectively move specific proteins across membranes into mitochondria, which is their functioning compartment within the cell. As conformational dynamics of such proteins is crucial to understand the import process, the applicant employed molecular dynamics (MD) simulations, along with experimental data, to obtain a detailed picture on the dynamics of some of the proteins forming the TOM and TIM complexes.

The applicant performed MD simulations to understand the dynamics of peptide binding to Tom20, a protein subunit of the TOM complex, and to characterize the conformational ensemble of a flexible loop in Tim21, a protein subunit of TIM complex. The applicant performed multivariate analyses to characterize the conformation ensemble and free energy calculations to identify representative conformations from the simulations.

The protein Tom20 recognizes a targeting signal in a short sequence of residues (presequence) of the protein being moved across membranes. To solve the X-ray crystal structure of the Tom20-presequence complex, a disulfide-bond tethering was required between Tom20 and the presequence. The applicant demonstrated, via MD simulations of tethered Tom20-presequence complexes in solution, minimal effects of the tethering on the conformational dynamics, validating the disulfide-bond tethering experimental design. In addition, an experimental group solved the X-ray structure of Tom20-presequence complex using a technique called crystal contact-free space (CCFS). The applicant showed for the first time, via MD simulations in crystal environment, that CCFS experiments describe the dynamics of Tom20-presequence complex observed in solution.

The protein Tim21 is also involved in protein transfer across membranes. X-ray, Nuclear Magnetic Resonance and CCFS X-ray structures of Tim21 showed different conformations of a highly flexible loop. The applicant performed MD simulations to reconcile this inconsistency in experimental data and obtain a representation of the loop conformational dynamics in solution. The new conformational ensemble in solution obtained by the applicant was found to be more consistent with the CCFS structure as compared to the other two structures.

Using MD simulations, the applicant concluded that disulfide bond tethering in X-ray experiments is useful to study the dynamics in solution for weakly binding complexes and demonstrated for the first time that the CCFS approach can reproduce protein dynamics as observed in solution. The candidate research highlighted that corroboration between experiments and simulations is critical to understand biological functions. In addition, the candidate has contributed two additional publications which showed the importance of using complementary experimental methods in conjunction with computational studies to study structure and dynamic properties of flexible structures in biomolecules. In conclusion, the candidate was found worthy of receiving the doctoral degree.