| CROSS TOKAI **Experimental Report** J-PARC | 承認日 Date of Approval 2017/4/7<br>承認者 Approver Kaoru Shibata<br>提出日 Date of Report 2017/4/7 |
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| 課題番号 Project No.<br>2015A0141<br>実験課題名 Title of experiment<br>Dynamics of the cardiomyopathy-causing mutant of troponin studied by neutron scattering<br>実験責任者名 Name of principal investigator<br>Satoru Fujiwara<br>所属 Affiliation<br>National Institutes for Quantum and Radiological Science and Technology | 装置責任者 Name of Instrument scientist<br>Kaoru Shibata<br>装置名 Name of Instrument/（BL No.）<br>BL02<br>実施日 Date of Experiment<br>16/05/19～16/05/23 |

試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。（適宜、図表添付のこと）
Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.

The following five $D_2O$ solution samples were used in this experiment.
(1) Wild-type human cardiac Troponin (MW: 52 kDa) in the $-Ca^{2+}$ state (21.2 mg/ml)
(2) Wild-type human cardiac Troponin in the $+Ca^{2+}$ state (22.1 mg/ml)
(3) K247R mutant of human cardiac Troponin in the $-Ca^{2+}$ state (21.8 mg/ml)
(4) K247R mutant of human cardiac Troponin in the $+Ca^{2+}$ state (22.7 mg/ml)
(5) $D_2O$ Buffer (50 mM HEPES (pD 8.0), 0.5 M NaCl, 5 mM $MgCl_2$, 5 mM EGTA)
Note: For the samples in the $+Ca^{2+}$ state (2 and 4), 5 mM $CaCl_2$ were added to the buffer instead of 5 mM EGTA.

2. 実験方法及び結果（実験がうまくいかなかった場合、その理由を記述してください。）
Experimental method and results. If you failed to conduct experiment as planned, please describe reasons.

Troponin (Tn) is a protein that regulates cardiac muscle contraction depending on the intracellular $Ca^{2+}$ concentration. It consists of three subunits (TnC, TnI, and TnT). Upon $Ca^{2+}$-binding to TnC, this signal is transmitted to TnI, TnT, and other regulatory proteins, leading to tension development. This regulatory function of Tn is essential for cardiac muscle contraction. Various mutations within Tn molecules, however, have been identified as a cause of inherited cardiomyopathy. Understanding how these mutations affect the physical properties of Tn is indispensable for elucidation of the molecular mechanism of the pathogenesis of inherited cardiomyopathy. In this experiment, we focused on K247R mutation of TnT, which is one of the mutants that cause inherited hypertrophic cardiomyopathy, and investigated how this mutation affects the internal dynamics of Tn at picosecond timescale by quasielastic neutron scattering (QENS) using the near-backscattering spectrometer BL02 DNA.

2. 実験方法及び結果（つづき） Experimental method and results (continued)

We used troponin core domain (Tn-CD), which consists of TnC, TnI, and TnT2 (part of TnT). All the subunits of human cardiac Tn-CD were bacterially expressed, purified, and then reconstituted into the Tn-CD. The Tn-CD containing either wild-type TnT2 (wtTn-CD) or K247R mutant of TnT2 (mtTn-CD) were dissolved in $D_2O$ buffer containing 50 mM HEPES (pD 8.0), 0.5 M NaCl, 5 mM $MgCl_2$. For the samples in the -$Ca^{2+}$ state, 5 mM EGTA was added to the buffer, while for the samples in the +$Ca^{2+}$ state, 5 mM $CaCl_2$ were added. Each of these samples (1.65 ml) was put into a cylindrical aluminum cell and then sealed with indium wire. QENS spectra were recorded for 18 hours per sample at 300 K with the energy resolution of ~12 μeV. MLF was operating at ~200 kW.

The QENS spectra of proteins were extracted by subtracting the spectra of the $D_2O$ buffer from those of the samples with an appropriate scaling factor calculated based on the scattering cross-section. The net spectra of proteins were analyzed by fitting the spectra using a sum of Lorentz functions describing macromolecular global diffusion and local diffusive motions with a Dirac delta function describing the contribution of “immobile” atoms. An example of the QENS spectra is shown in Fig. 1 with a corresponding fit (wtTn-CD in the +$Ca^{2+}$ state; Q=1.650 $Å^{-1}$).

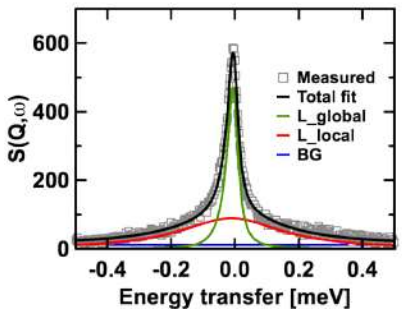


Fig. 1. Example of the QENS spectra.

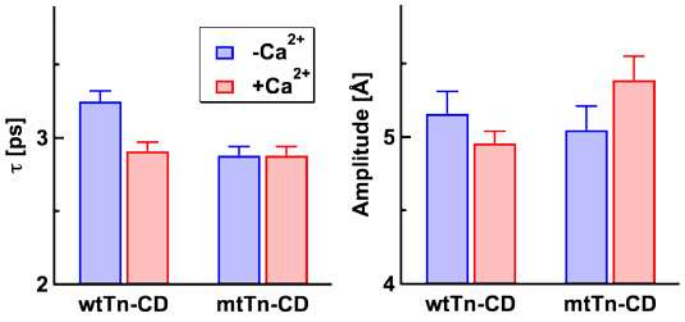


Fig. 2. Summary of the dynamics parameters.

In the current analysis, the apparent translational diffusion coefficients ($D_{app}$) of the Tn-CD molecules, the residence time (τ) and geometry of atomic local motions were evaluated (Concerning geometry of motions, average values of the amplitudes of atomic motions are presented here). Regarding the global motions, the $D_{app}$ values were found to be the same within errors for all the samples. Analysis of these $D_{app}$ values using molecular models of the Tn-CD suggests that the $D_{app}$ values include not only the global diffusion but also the segmental motions of flexible regions in the Tn-CD, the latter of which are found not to be affected by the mutation. As for the local motions, it was found that the τ values are different between the wtTn-CD and the mtTn-CD in the –$Ca^{2+}$ state (Fig. 2). In addition, whereas $Ca^{2+}$-binding to the wtTn-CD reduces both the τ values and the amplitudes, $Ca^{2+}$-binding to mtTn-CD increases the amplitudes without any change in the τ values. This experiment thus shows that the mutation changes the internal dynamics of the Tn-CD in both states. More detailed analysis and the preparation of a paper are currently underway.