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► **To cite this version:**

Alessandro Paciaroni, Andrea Orecchini, Elena Cornicchi, Matteo Marconi, Caterina Petrillo, et al.. Coupled thermal fluctuations of proteins and protein hydration water on the picosecond timescale.. *Philosophical Magazine*, 2008, 88 (33-35), pp.4071-4077. <10.1080/14786430802464263>. <hal-00513973>

HAL Id: hal-00513973

<https://hal.science/hal-00513973v1>

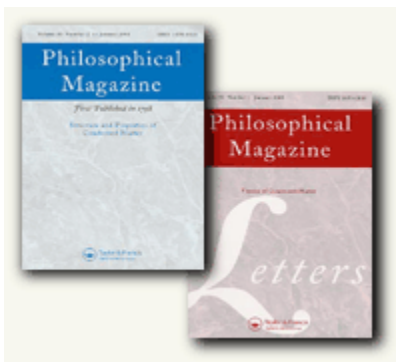
Submitted on 1 Sep 2010

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Journal:	<i>Philosophical Magazine & Philosophical Magazine Letters</i>
Manuscript ID:	TPHM-08-May-0153.R1
Journal Selection:	Philosophical Magazine
Date Submitted by the Author:	26-Jun-2008
Complete List of Authors:	paciaroni, alessandro; University of Perugia, Physics Orecchini, Andrea; University of Perugia, Physics Cornicchi, Elena; University of Perugia Marconi, Matteo; University of Perugia, Physics Petrillo, Caterina; università di perugia, physics Sacchetti, Francesco; università degli studi di perugia, dipartimento di fisica haertlein, michael; Institut Laue Langevin moulin, martine; Institut Laue Langevin
Keywords:	biomolecules, biophysics, glass transition, neutron scattering, proteins
Keywords (user supplied):	hydration water, mean square displacements, dynamical transition



Coupled thermal fluctuations of proteins and protein hydration water on the picosecond timescale.

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Abstract

The mean square displacements (MSD) of a model protein, the Maltose Binding Protein, and its hydration water have been estimated by the elastic neutron scattering intensity measured on the time-of-flight spectrometer IN5. The availability of the protein in both fully deuterated and hydrogenated form allowed us to reliably separate the contribution of the solvent interacting with biomolecule from the one of the hydrated biomolecule. The thermal fluctuations of hydration water and protein activate in the same temperature range 200-220K. This result supports a picture where the dynamical coupling between the biomolecule and the solvent is already effective in the picosecond timescale. A quantitative agreement of the MSD with the values from molecular dynamics simulations is found.

1. Introduction

Water plays a major role in determining and modulating the stability and function of biological macromolecules. This vital role is played through the peculiar action of hydration water in affecting both structure and dynamics of proteins and nucleic acids. For example, water molecules around hydrophobic and hydrophilic sites are important to settle the activity of enzyme proteins [1–3] and are part of the recognition process by other molecules or proteins. With this respect, the properties of water molecules in the immediate vicinity of the protein surface deserve particular attention as they are particularly relevant to the function [4]. Despite a large number of theoretical and experimental studies [5–13], there is no generalized picture of the dynamics of hydration water at the local molecular level yet. In fact, a detailed picture of the vibrational and diffusive motions of water molecules would shed light on the mechanisms through which they are coupled with biomolecules. Such a coupling is quite evident even in the most intriguing phenomenon which characterises the dynamics of proteins in the picosecond timescale, the so-called “dynamical transition”. This event, which consists in the activation above 200-220K of large-amplitude anharmonic protein internal motions, is well visible in hydrated biomolecules whereas it cannot be detected in dry systems [14-18]. It has been proposed that the protein dynamical transition does not correspond to a real “transition”, but it is likely the signature of the onset of the conformational mean square displacements (MSD) [19]. The existence of an intimate dynamical coupling between a biomolecule and its surrounding environment has been widely studied even for non-aqueous solvents [20-26].

In the present work we investigate the extent of thermal fluctuations in the hydration water of a fully deuterated (perdeuterated) protein, the Maltose Binding Protein (MBP), a model system often used for biotechnological purposes [27]. The exploitation of a perdeuterated protein, which has a low scattering power with respect to the hydrogen atoms of hydration water, allowed us to single-out the hydration water MSD on the spectrometer IN5 (ILL, Grenoble), i.e. on the pico- and sub-picosecond timescale, with an unprecedented accuracy. We thus provide evidence of a common dynamical onset of the thermal fluctuations in the MBP and its hydration water.

2. Materials and Methods

2.1 Samples preparation

Maltose binding protein in its hydrogenated (MBP(H)) and perdeuterated form (MBP(D)), was provided by the ILL-EMBL Deuteration Laboratory (D-Lab) in Grenoble. In the adopted protocol, MBP was produced as a histidine tagged fusion protein, which allowed its purification by immobilized metal-ions affinity chromatography (IMAC) in a one step procedure. Then, both the hydrogenated and the perdeuterated form of this protein were expressed in high-cell density cultures [28] and purified. Thanks to such a procedure a noticeable amount (~200 mg) of both MBP(H) and MBP(D) was made available. The samples were left for 3 days in solution of respectively D₂O and H₂O to exchange most of the labile hydrogen/deuterium atoms. Then, the solutions were dialysed twice against the buffer, lyophilised and finally dried under vacuum, in the presence of P₂O₅. The MBP(H) and MBP(D) powder samples were then hydrated by D₂O and H₂O vapour pressure up to a hydration degree of 0.42*h* and 0.37*h* respectively (*h* = grams of water/grams dry protein), in order to have the same number of water molecules per protein.

2.2 Incoherent neutron scattering

Thermal neutrons are powerful and widely used probes to directly obtain information on the fast motions (nano- and pico-seconds characteristic times) of biological samples. Neutrons exchange

with the sample nuclei a momentum $\hbar Q$ and an amount of energy $E=\hbar\omega$ with a probability given by the dynamical structure factor $S(Q,E)$.

Within the incoherent approximation, the signal of the MBP(H)+D₂O sample comes mainly from the non-exchangeable protein hydrogen atoms, while the spectrum of the MBP(D)+H₂O is composed respectively by the contribution from the hydration water (~72%) and the exchangeable protein hydrogens (~28%).

In order to extract the contribution coming solely from the protein hydration water, $S_{H_2O}(Q,E)$, we made the assumption that in the meV region the vibrational behaviour of the protein exchangeable hydrogen atoms is the same as the non-exchangeable ones [29]. On these bases we may write:

$$S_{H_2O}(Q,E) = S_{DH}(Q,E) - 0.26 \cdot S_{HD}(Q,E) \quad (1)$$

where $S_{DH}(Q,E)$ and $S_{HD}(Q,E)$ are the dynamical structure factors of respectively MBP(D)+H₂O and MBP(H)+D₂O, and 0.26 is the ratio between the number of the exchanged protons and the number of non-exchangeable protons.

In this work we focus on the elastic part of the spectra. An analysis of the features of the elastic scattering $S_{H_2O}(Q,E \approx 0)$ was made within the well-known Gaussian approximation [30]:

$$S_{H_2O}(Q,E \approx 0) \approx \exp(-\langle u^2 \rangle Q^2) \quad (2)$$

where $\langle u^2 \rangle$ are the mean square displacements of the water protons. We verified that Eq. (2), which holds in the small- Q region, can describe the elastic data at all the investigated temperatures.

2.3 Neutron scattering experiment

The incoherent neutron scattering measurements were performed on the high-flux time-of-flight spectrometer IN5 [31], at ILL. The incident wavelength $\lambda=5.2 \text{ \AA}$ was employed, achieving an elastic wave-vector range $0.4 \text{ \AA}^{-1} < Q < 2.2 \text{ \AA}^{-1}$, accessible energy transfers E from $-2k_B T$ to 1.5 meV and an energy resolution with a full width at half maximum of ~ 0.11 meV. The collected data were treated with a standard correction procedure, taking into account for empty cell contribution, transmission and non-uniform detector efficiency as a function of the scattering angle. The values of the transmission coefficients were 0.95 and 0.93 for respectively MBP(D)-H₂O and MBP(H)-D₂O. Multiple scattering was neglected. The samples were placed in a slab aluminium cell, at an angle of 135° with respect to the incident neutron beam, and measured from 50 to 300 K.

3. Results and Discussion

In Fig. 1 we plot the elastic intensities, integrated over a range of small- Q values ($0.4 \text{ \AA}^{-1} < Q < 1. \text{ \AA}^{-1}$), as a function of the temperature, for both MBP(H)+D₂O and the protein hydration water. In the small- Q limit ($\langle u^2(T) \rangle Q^2 \ll 1$), Eq. (2) leads to $S(Q, E \approx 0) = 1 - \langle u^2(T) \rangle Q^2$, then the departure from unity gives a measure of the mobility of the hydrogen atoms in the systems we investigated. The intensity profiles result to be noticeably dependent on the temperature, particularly above 200÷220 K, where in both cases the trend clearly deviates from the almost linear behaviour at low temperature. As we mentioned in the ‘‘Introduction’’, for the MBP(H)-D₂O system, such onset of additional mobility over the low-temperature quasi-harmonic behaviour, is the signature of the protein dynamical transition. On the other hand, the elastic intensity of the protein hydration water displays a steep decrease occurring in the same temperature range as the hydrated protein. The depletion of elastic intensity is by far more visible for protein hydration water, thus reasonably

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3 indicating that, above the protein dynamical transition, the motions of water molecules on the
4 protein surface are characterised by larger amplitudes than those of the protein atoms.

5 More quantitative information on the process taking place at 200÷220 K can be derived by
6 exploiting Eq. (2) to calculate the atomic MSD of MBP, $\langle u^2 \rangle_{MBP-IN5}$, and its hydration water,
7 $\langle u^2 \rangle_{H2O-IN5}$, which are shown in Fig. 2. As expected, $\langle u^2 \rangle_{MBP-IN5}$ and $\langle u^2 \rangle_{H2O-IN5}$ show a linear slow
8 increase in the low-temperature range and a distinct departure from this trend above 200÷220 K.
9 The characteristic times of the dynamical mechanisms which contribute to such activation must be
10 faster than around 15 ps, i.e. the inverse of the energy resolution half-width at the elastic peak (see
11 the “Neutron scattering experiment” section). In Fig. 2 we also report the MSD of the MBP
12 hydration water, measured instead on the neutron backscattering spectrometer IN16 and analysed
13 within the Gaussian approximation [32]. In this case, due to the very high energy resolution
14 ($\sim 1\mu\text{eV}$) achieved by IN16, the measured MSD arise from the average contribution of motions
15 faster than about 1.5 ns. This comparison allows us to extract the contribution to the MSD coming
16 from the picosecond time-windows of IN5 with respect to the larger nanosecond and sub-
17 nanosecond timescale accessible on IN16. Quite interestingly, if one looks at the MSD as estimated
18 in both the spectrometers, the dynamical onset of the protein hydration water motions takes place at
19 around the same temperature. This finding suggests that an important contribution to the dynamical
20 activation of protein hydration water comes from fast motions occurring already in the picosecond
21 timescale. On the other hand, above the dynamical activation, the extent of the water mobility is
22 greater when it is sampled over the wide timescale of IN16, consistently with the diffusive character
23 of the motions on the protein surface. In fact, on increasing the time-window over which the
24 motions are observed, the water molecules may explore larger and larger space regions. It is worth
25 of note that the MSD of water oxygen atoms calculated by molecular dynamics (MD) simulations
26 and estimated at $t=15\text{ ps}$, i.e. the same timescale as IN5 [32], nicely superimpose to $\langle u^2 \rangle_{H2O-IN5}$.
27 This agreement supports the exploitation of MD simulations to interpret our data. Actually, MD
28 results indicate that the dynamical onset of hydration water molecules is due to translational degrees
29 of freedom, with a diffusion rate that increases progressively with temperature. Such diffusive
30 processes sustain an increasingly faster relaxation rate of the protein-water hydrogen-bond network,
31 which would in turn be crucial to promote the so-called protein dynamical transition [33]. This
32 phenomenon, which has been put in relationship with the biological functionality [17,34,35], can be
33 depicted as the onset of large-amplitude anharmonic motions, over the prevalently harmonic low-
34 temperature protein dynamics [14,15,17]. In fact, such a dynamical transition is well visible in the
35 trend of the MSD of the hydrated protein alone (hydrogenated protein in D_2O). Above the
36 dynamical transition, the protein MSD are smaller than those of hydration water, due to the
37 structural constraints to which protein hydrogen atoms are subjected. Starting from around 180-
38 200K, the protein MSD measured on IN16 are slightly larger than those measured on IN5 (see Fig.
39 3) thus indicating that there is a not negligible contribution from fluctuations in the time-window
40 above 15 ps and below 1.5 ns. Methyl group reorientations are good candidate to explain such an
41 effect, in particular at low temperature [36].

42 In a number of previous works, hydrated protein powders were shown to exhibit a dynamical
43 transition at around 200-220 K [15,17,35]. Similar values were found for hydrated DNA [18,37].
44 Very recently, it has been suggested that the dynamical transition of hydration water is the effect of
45 a fragile-to-strong dynamical crossover, which is characterised by the change in the temperature
46 dependence of quantities such as the average translational relaxation time, and corresponds to a
47 transition in water structure from a predominantly high-density (more fluid state) to a low-density
48 (less fluid state) form [13]. However, the intrinsic origin of the dynamical crossover of protein
49 hydration water is still a matter of large debate, and as an alternative picture it has been proposed
50 that the activation at 200÷220 K is not due to a true fragile-to-strong transition but rather to a
51 vanishing of the strongly cooperative α -relaxation, occurring in coincidence of the glass transition
52 of the overall system [38]. In addition, dielectric spectroscopy data suggest that the relation between
53 the secondary relaxation of the solvent and the conformation relaxation of the hydrated protein is
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best described as symbiotic [39]. The use of high-quality neutron scattering data from hydrated perdeuterated systems can provide essential information to define this quite puzzling scenario. Our results show that the dynamics of both protein and hydration water undergoes a transition in the same temperature range, in agreement with the paper of Wood et al. [32]. We found that a large part of the contribution to the thermal fluctuations at the origin of this transition is due to dynamical processes occurring in the picosecond timescale. This result, which adds specific information concerning the characteristic times of these fluctuations, further supports the above mentioned picture of a protein dynamics slaved to the solvent [19].

4. Acknowledgements

The Institut Laue-Langevin is kindly acknowledged for providing beam time and technical and scientific support. The authors are grateful to Dr. J. Ollivier for the assistance during the experiment on the IN5 spectrometer. This work has benefited from the activities of the DLAB consortium funded by the EU under contracts HPRI-2001-50065 and RII3-CT-2003-505925, and from UK EPSRC-funded activity within the ILL-EMBL Deuteration Laboratory under grants GR/R99393/01 and EP/C015452/1.

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30 *Figure captions*

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35 **Figure 1.** Incoherent elastic intensities, normalised with respect to the lowest temperature,
36 integrated over a small Q -range ($0.4 \text{ \AA}^{-1} < Q < 1.0 \text{ \AA}^{-1}$), of MBP hydration water (full circles) and
37 MBP(H)+D₂O (empty circles).
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42 **Figure 2.** Mean square displacements vs. T of MBP hydration water, as calculated from
43 measurements on IN5 (full circles), on IN16 [32] (empty circles), and from molecular dynamics
44 simulations [32] (stars), together with MSD of MBP(H)+D₂O (full squares) measured on IN5. The
45 experimental MSD of hydration water were corrected for the contribution of exchangeable protein
46 protons [see Eq. (1)]. It is worth of note that the MSD reported on Ref. [32] were calculated with
47 the formula: $S_{H_2O}(Q, E \approx 0) \approx \exp(-\langle u^2 \rangle Q^2 / 6)$ then they have been rescaled by a factor of 6 for
48 purpose of comparison with the present results.
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52 **Figure 3.** MSD of MBP(H)+D₂O measured on IN5 (full squares) and on IN16 (empty triangles).
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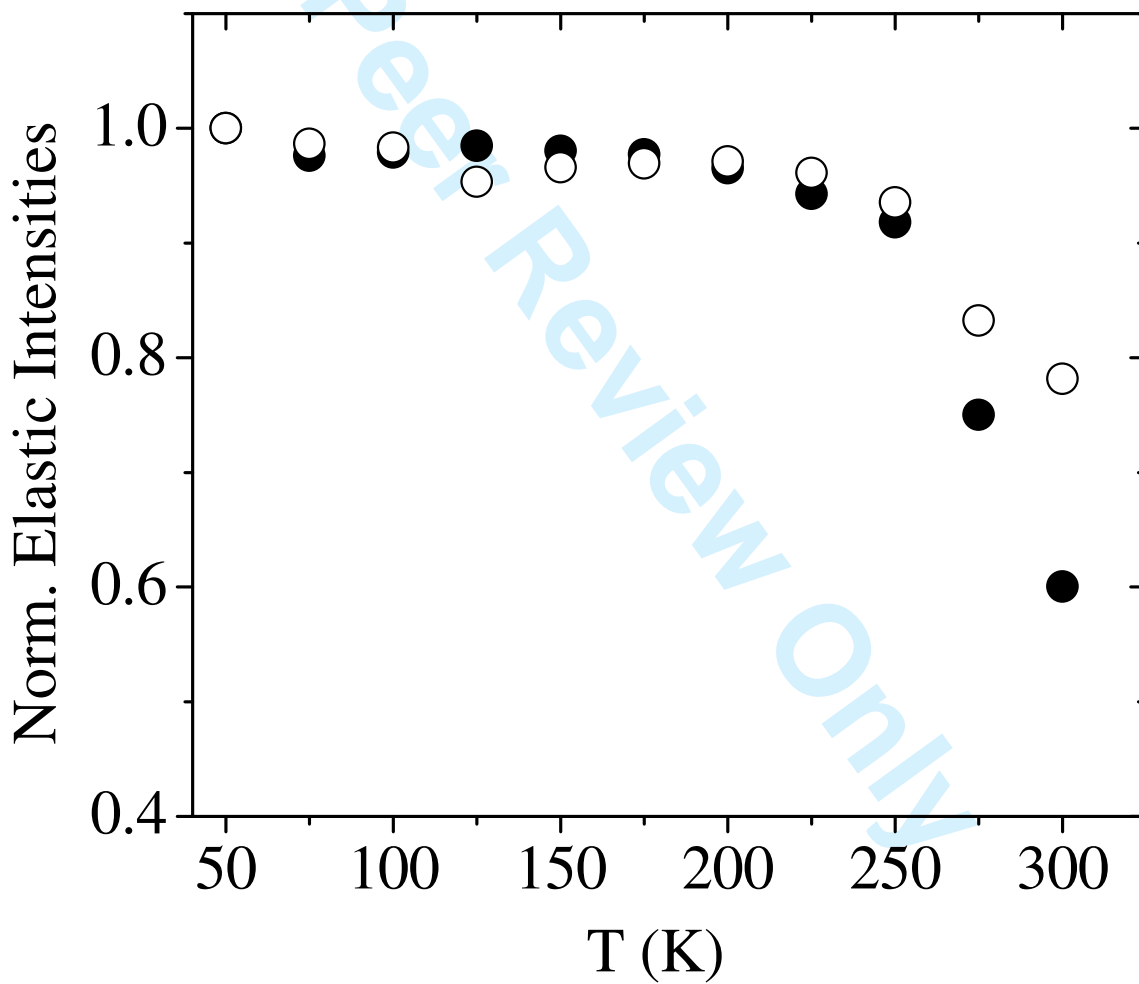


Figure 1.

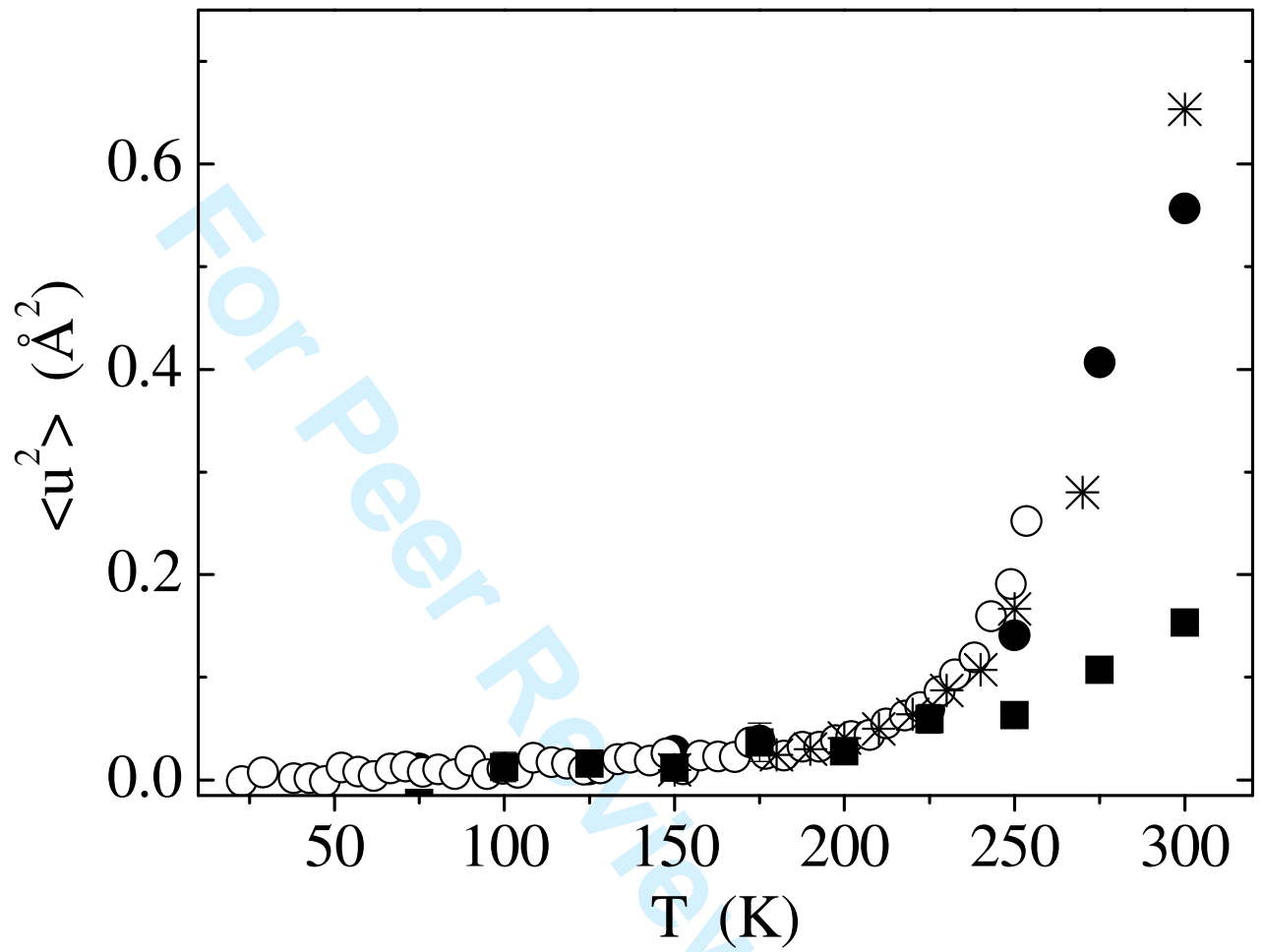


Figure 2.

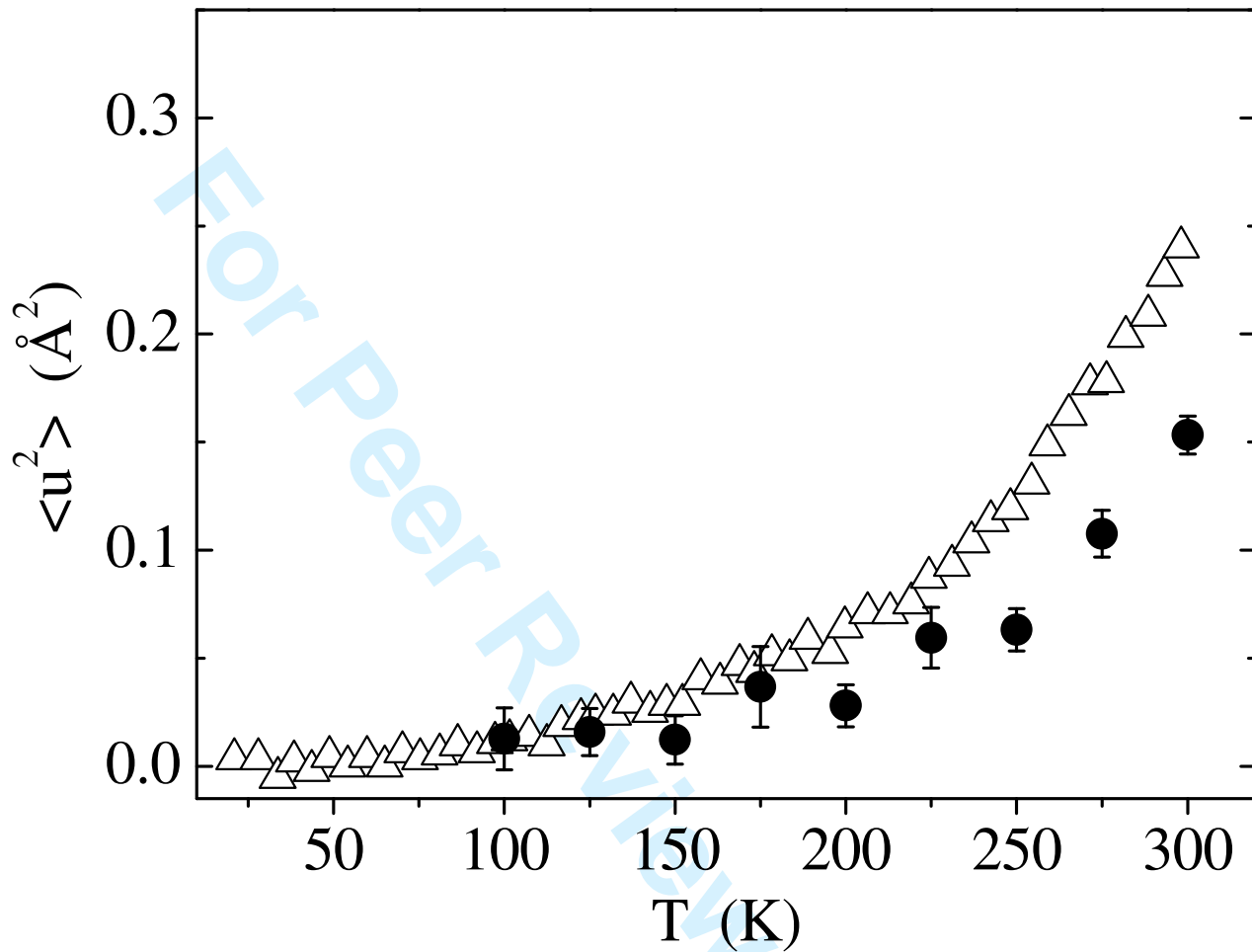


Figure 3.