Echo dephasing and heat capacity from constrained and unconstrained dynamics of triiodothyronine nuclear receptor protein

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Abstract

The objective of this study is to observe the echo feature curves, vibrational dephasing, and heat capacity of a protein–hormone system taking thyroid hormone receptor-beta (THR- β) as an example. Constrained and unconstrained molecular dynamics simulations are performed by implementing the theory of velocity reassignments to probe the phase coherent state in terms of echo pulses. The constrained vibrations are incorporated by adjusting rigid bonds to all hydrogen atoms with an integrator parameter of 2 fs/step in order to reduce the degrees of freedom whereas 1 fs/step is used in the free vibrations of the atomic cluster. The nature of temperature auto-correlation functions changes so that echo feature curves also show a distinct nature in the cases of constrained and unconstrained vibrations. There is a large variation in kinetic temperature and internal potential energy in the echo time zone. The temperature rate of change of internal potential energy is the main contributor to the heat capacity of the native state protein–hormone system. The heat capacity of proteins estimated from this technique is in good agreement with the values from experiments. This study shows that triiodothyronine (T3) hormone makes some differences in heat capacity upon binding to the THR- β ligand binding domain (LBD). The physical properties of unliganded THR- β and T3-bound THR- β LBD in the cases of constrained and unconstrained dynamics are observed distinctly under the effect of anharmonicity on the phase coherent state of normal modes and the dephasing time lies in a range of 0.6–0.8 ps when the systems are perturbed suddenly.

Keywords Thyroid hormone receptor · Triiodothyronine · Temperature echo · Heat capacity · Dephasing · Anharmonicity

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1 Introduction

The major form of thyroid hormones secreted by the thyroid gland is 3,5,3',5'-tetraiodo-L-thyronine (thyroxine, T4). T4 is converted into 3,5,3'-triiodo-L-thyronine (T3) after intracellular activity called local deiodination [1]. T3 actively binds to the ligand binding domain (LBD) of the thyroid hormone receptor (Fig. 1) that has two subtypes: THR- α and THR- β . These are members of the nuclear receptor superfamily of ligand activated transcription factors. T3-activated nuclear receptors regulate gene expression involved in cell differentiation, growth, and homeostasis. Since the total serum concentration of T4 is 40-fold higher than T3, T4 can also bind and modulate the receptor's activity in lower affinity [2].

Due to a negative feedback mechanism, an increase in the thyroid stimulating hormone (TSH) level generally causes a decrease in T3/T4 concentrations and vice versa. The decrease in serum T3/T4 levels from the normal range is an indicator of clinical or subclinical hypothyroidism. In contrary, a decrease in TSH, i.e., an increase in T3/T4 concentration is related to hyperthyroidism [3]. THR- β is found in the liver, which is involved in the regulation of metabolic rate. The T3-bound THR- β LBD (THRT3) plays a key regulatory role on the negative feedback mechanism of circulating thyroid hormone levels for the production of the hypothalamic thyrotropin releasing hormone (TRH) and hypophyseal TSH [4]. Any mutation in THR- β can cause a resistance to thyroid hormones (RTH), which results in a disorder characterized by elevated levels of serum T3/T4 and normal or elevated thyroid stimulating hormone (TSH). The genetic disease RTH has a complex range of symptoms such as pituitary malfunction, goiter, and neurological disorder [5]. The unliganded THR- β (THR) functions as a transcriptional repressor of the target genes. The carboxyl terminal of LBD obtains the conformational change through an activation function (AF-2) when bound to T3 and this further alters the position of helix 12 [2, 6]. Even a mutation in the helix 12 region can block the recruitment of cofactors to the AF-2 domain resulting in the inhibition of both T3-mediated activation and repression of gene transcription [7]. There are seven pathways of T3 dissociation; most of which are significantly affected by THR- β heterodimerization [8]. The molecular interaction of 4-hydroxyphenyl sulphone (BPS used as a BPA alternative) toward THR- β LBD makes a structural disruption, which is a type of potential endocrine effect on the biological systems [9].

Constraints are imposed in the biomolecular systems to remove the associated rapid vibrational modes by reducing the internal degrees of freedom [10–13]. A longer time step can be used for integrating Newton's equations of motion without distorting the system. The constrained vibrations save computer time while finding the physical properties of the atomic clusters by means of computational techniques.

In the vibrational echo experiments used to study protein dynamics [14–16], the echo decay provides dephasing time under the effect of anharmonicity. The classical dynamics of atomic particles in defining the vibrational echoes can be interpreted as a probe of stability of atomic trajectories [17].

The structural and physical properties of bio-atomic clusters reflect some intrinsic features such as the temperature echo after the application of sudden perturbations and monitoring their response. We used the theory of temperature quenching and echo formation described by Xu et al. with some modifications to calculate heat capacity and diffusion and mobility constants of anharmonicity [18]. The temperature quench echo and the velocity replacement echo can be visualized through molecular dynamics (MD) simulations. In analogy with the phonons in a crystalline solid, the internal dynamics of atoms in a globular protein in a high-frequency range can also be described as the normal modes of vibration. The normal modes oscillate in a phase coherent state leading to resonances referred to as echoes in response to a suitable signal or perturbation [18, 19]. The decay of coherent states of atomic motions can be analyzed in terms of dephasing time by employing MD simulations. In the temperature echo method, the coherence of atomic motions is probed by reassigning the atomic velocities to a fixed temperature distribution ranging from zero to that of the equilibrated system.

2 Materials and methods

2.1 Theory

The time evolution of the kinetic temperature [18, 19] in a molecular system is given by

$$T(t) = \frac{2}{(3N-6)K_B} E_K(t) = \frac{2}{(3N-6)K_B} \sum_{i=1}^{(3N-6)} \frac{1}{2} m_i v_i^2 \quad (1)$$

where N refers to the number of atoms in the system and $T(t)$ acquires sharp features during the synchronization signal, probing the signal and temperature echoes. In a normal mode analysis of the protein–hormone system, the Hamiltonian associated with the i^{th} normal mode describing the echoes by kinetic energy (temperature) and potential energy is

$$H_i = \frac{p_i^2}{2m_i} + \frac{1}{2} m_i \omega_i^2 y_i^2 \quad (2)$$

The position of the i^{th} normal mode under harmonic approximation is $y_i(t) = A_i \cos(\omega_i t + \delta_i)$ and the momentum is $p_i(t) = -m_i A_i \omega_i \sin(\omega_i t + \delta_i)$. The total kinetic energy of the system is given by

$$E_K(t) = \sum_i \frac{1}{2} m_i A_i^2 \omega_i^2 \sin^2(\omega_i t + \delta_i) \quad (3)$$

Temperature autocorrelation function [19] before quenching or before velocity reassignment is given by:

$$C_{T,T}(t) = \frac{\langle E_k(t)E_k(0) \rangle - \langle E_k(0) \rangle^2}{\langle [E_k(t)]^2 \rangle - \langle E_k(0) \rangle^2} = \frac{\langle T(t)T(0) \rangle - \langle T(0) \rangle^2}{\langle [T(t)]^2 \rangle - \langle T(0) \rangle^2} = \frac{\langle \sum_i m_i^2 A_i^4 \omega_i^4 \cos(2\omega_i t) \rangle}{\langle \sum_i m_i^2 A_i^4 \omega_i^4 \rangle} \quad (4)$$

The $C_{T,T}(t)$ function can be approximated to a single exponential function with decay time τ_d , i.e., $C_{T,T}(t) \approx e^{-t/\tau_d}$.

At the first velocity reassignment, i.e., at $t=0$ (say), $v_i(0) = \lambda_1 u_i$ synchronizes the atomic oscillators or it makes them oscillate in phase. After some delay time (τ), $v_i(\tau) = \lambda_2 u_i$ probe the degree of coherence of the system. Here, λ_i are the numbers multiplied with the initial velocities u_i . The degree of coherence is characterized by time of appearance and depth of echoes. If $\lambda_1 = \lambda_2 = 0$, the system has a temperature quench echo; if $\lambda_1 = \lambda_2 = 1$, it has a constant velocity replacement echo and if $\lambda_1 \neq \lambda_2 \neq 1$, it has a velocity replacement echo. The temperature echo can be detected at $t = 2\tau$ in the first case and at $t = 3\tau/2$ in the second and third cases. In each of these cases, the depth of echo differs from its value. The largest depth of the temperature quench echo in the first case is $\Delta T = T_0/8$ where $T_1 = \lambda_1^2 T_0$ and $T_2 = \lambda_2^2 T_0$ with $\lambda_1 = \lambda_2 = 0$ having T_0 as the equilibrium temperature [18, 19].

From harmonic approximation, the temperature response function [18, 19] for the time interval, $t > \tau \gg \tau_d$, is

$$T(t) \approx T_0 \left[\frac{1 + \lambda_1^2 + 2\lambda_2^2}{4} - \frac{1 + \lambda_1^2 - 2\lambda_2^2}{4} C_{T,T}(t-\tau) - \frac{\lambda_1 \lambda_2}{2} C_{T,T} \left(\left| t - \frac{3\tau}{2} \right| \right) - \frac{1 - \lambda_1^2}{8} C_{T,T}(|t - 2\tau|) \right] \quad (5)$$

In Eq. (5), the first term on the right side represents the average temperature after the second velocity reassignment, the second term is the recovery of temperature and the third and final terms represent for $\frac{3\tau}{2}$ -echo pulse and 2τ -echo pulse, respectively. The respective echo pulses have a depth of $\Delta T(3\tau/2) = \frac{T_0}{2} \lambda_1 \lambda_2$ and of $\Delta T(2\tau) = \frac{T_0}{8} (1 - \lambda_1^2)$.

For the temperature quench echo with $T_1 = T_2 = 0$, the temperature response function deals only with the 2τ -echo pulse [18, 19], which is given by

$$T(t) \approx \frac{T_0}{4} \left[1 - C_{T,T}(t-\tau) - \frac{1}{2} C_{T,T}(|t - 2\tau|) \right] \quad (6)$$

The depth of this echo pulse under harmonic approximation is $\Delta T(2\tau) = T_0/8$.

Along with the time evolution of temperature, kinetic energy (E_K) as well as internal potential energy (U) change greatly with temperature in the echo time zone. As the total energy remains almost constant throughout the simulation in the NVE ensemble, i.e., $E_K(t) + U(t) = E_T \approx \text{constant}$ [19], we can define heat capacity in two ways. If

$$\Delta E_K(t) = \frac{(3N-6)K_B}{2} \Delta T(t) \approx -\Delta U(t)$$

then

$$C_{V1} = \left(\frac{\partial E_K}{\partial T} \right)_{N,V,E} = \frac{(3N-6)K_B}{2} \approx C_{V2} = -\frac{dU(t)}{dT(t)} = -\left(\frac{\partial U}{\partial T} \right)_{N,V,E} \quad (7)$$

where $\mathcal{C}_{V1}$ is the heat capacity in terms of kinetic energy that depends only on degrees of freedom of the system and C_{V2} is the heat capacity in terms of internal potential energies (e.g., electrostatic, van der Waal, bond, angle, dihedral, etc.) with the expense of kinetic energy (macroscopic mass motion) as indicated by the negative sign. In other words, C_{V2} is temperature dependent but not $\mathcal{C}_{V1}$.

If we implement some constrained degrees of freedom (N_C) during the simulation of the molecular system, the echo depth in kinetic energy in terms of echo depth in temperature can be expressed as

$$\Delta E_K = \left(\frac{3N - N_C - 6}{2} \right) K_B \Delta T = \mathcal{C}_{V1} \Delta T \quad (8)$$

The small fluctuation observed in the total energy of the system in its NVE ensemble is only due to fluctuations in the internal potential energy terms by means of kinetic temperature. Thus, being a function of temperature, the potential energy is the major contributor of heat capacity. It has also been verified by the results obtained after simulations. In most of the MD simulations of macromolecules, vibrations of H-atoms are constrained ($N_C \neq 0$) with their rigid bonds and a time step of 2 fs and all atomic vibrations are made free ($N_C = 0$) with the time step of 1 fs. The results obtained from these two methods are compared with the experimental values.

In case of complex thermodynamic systems like proteins, the heat capacity components can be separated regarding the different physical contributions such as internal potential energy terms (atomic and covalent bonding and all other non-covalent contributions), folding and unfolding states and interaction with solvents [20, 21]. For a native state protein in vacuo, the temperature echoes and heat capacity can be observed from the potential function describing the system [19, 20].

According to Xu et al. [18], the depth of echo decays exponentially with delay time (τ) due to the anharmonic contributions that arise from electrostatic, torsional and van der Waals interactions, i.e., $\Delta T \propto \exp(-\tau/\tau_0)$ where τ_0 is the dephasing time. So, the correct form of echo depth is given by

$$\Delta E_K = \mathcal{C}_{V1} \Delta T = \mathcal{C}_{V1} \Delta T_0 e^{-\tau/\tau_0} = \Delta E_{K0} e^{-b_0 \tau} \quad (9)$$

where b_0 is the echo damping coefficient of the protein. Friction and fluctuating forces result anharmonicity in the normal modes of vibrations. The anharmonic terms in the internal energy of the system make the normal modes dephase. In MD simulations, vibrational energy relaxation and dephasing are important to study energy transfer and reactivity in peptides and proteins [18, 22]. The diffusion coefficient (D_i) and mobility constant (μ_i) for the anharmonic interactions in the i^{th} normal mode of vibration of the system can be connected by Einstein's diffusion equation with $D_i = \mu_i K_B T$. For the process of temperature quenching and large delay time (τ), the echo depth [18] at $t = 2\tau$ can be expressed as

$$\Delta T(\tau) \approx \frac{T_0}{8} \left\langle e^{-(3\mu_i K_B T_0 \tau)} \right\rangle = \frac{T_0}{8} \left\langle e^{-(3D_i \tau)} \right\rangle = \Delta T_0 \left\langle e^{-(3D_i \tau)} \right\rangle \quad (10)$$

In the case of constant velocity reassignment echo, $\Delta T_0 = T_0/2$. If the diffusion constant (D_0) and mobility constant (μ_0) are assumed to be the same for each of the normal modes, the echo depths for the temperature and kinetic energy are related as

$$\Delta E_K(\tau) = \mathcal{C}_{v1} \Delta T(\tau) \approx \mathcal{C}_{v1} \Delta T_0 e^{-(3\mu_0 K_B T_0 \tau)} = \Delta E_{K0} e^{-(3D_0 \tau)} \quad (11)$$

Thus, the dephasing time and the anharmonic constants are

$$D_0 = \frac{1}{3\tau_0} = \frac{b_0}{3} = \mu_0 K_B T_0 \quad (12)$$

Rearranging and taking the natural log of both sides gives

$$\ln(\Delta E_K) = A - b_0 \tau \quad (13)$$

Then, we can obtain $\mathcal{C}_{v1}$ from the intercept: $A = \ln(\mathcal{C}_{v1} \Delta T_0)$; and b_0 , D_0 and τ_0 from the slope.

2.2 Simulation details

The crystal structure of T3-bound THR- β isoform (THRT3) was taken from the protein data bank (pdb) code 3GwS at 2.2-Å resolution [23]. The topologies and parameters required to prepare the complete simulated systems were used from the CHARMM force field [24–26]. As the ligand (T3) is a hetero-molecule, it was parameterized to describe its molecular geometry, partial atomic charges, atomic masses, theoretical and experimental torsion barriers [26]. All the simulations starting from the generation of the protein structure file (psf) and energy minimization, equilibration run and production run were performed with nanoscale molecular dynamics (NAMD-2.12) [27]. The simulated systems were analyzed with visual molecular dynamics (VMD-1.9.3) [28]. Both systems, i.e., THR and THRT3, were simulated by the same procedures in a water-ion environment and then in vacuo to study the physical role of T3 in its receptor proteins by the echo method.

Each of the THR and THRT3 molecules was solvated fully in a neutral water-droplet (TIP3P) of radius 37.5 Å by adding Na^+ and Cl^- ions with a concentration of 0.15 mol/l providing the cellular environment so that the complexes have 21,157 and 21,192 atoms, respectively. The prepared systems were geometrically optimized by 3000 conjugate gradient steps and equilibrated up to 7-ns coupling to the heat bath at 310 K under non-periodic boundary conditions. Furthermore, the final coordinates of each system were extracted to equilibrate separately again in vacuo up to 1 ns because the echo features are more distinct and observable in an anhydrous state rather than in a solution. In defining the force-field parameters in the configuration systems, 1–4 scaling for the electrostatic interactions was 1.0, cut-off distance for the electrostatic and van der Waal interactions was 12.0 Å with the smooth switching function starting at 10.0 Å and the pair list distance was 14.0 Å. The integrator parameter for the simulation of solvated THR and THRT3 systems was 2.0 fs/step providing a command for the rigid bonds to all H-atoms. However, all of the simulations in vacuo were performed in both cases: constrained vibrations with rigid bonds of H-atoms (2 fs per time step) and free vibrations of all atoms (1 fs per time step) and finally the results obtained were

compared. During the simulation, the Langevin temperature and damping coefficient were 310 K and 1.0/ps, respectively. The velocity Verlet algorithm [29] was used to calculate the trajectory of atomic particles by integrating Newton's equations of motion. The conformational stability of the protein–hormone systems was tested by analyzing their trajectories during the simulations and by plotting the root mean square deviation (RMSD) and radius of gyration (R_g) of the protein backbone.

After employing MD simulations for energy minimization and equilibration in the TIP3P explicit solvent and then in vacuo at 310 K, both the THR and THRT3 systems were simulated up to 5 ps removing the thermostat in the NVE ensemble. The temperature autocorrelation functions were plotted using Eq. (4) and best fitted with the single exponential function to approximate the decay time related to the systems.

To observe and compare the physical phenomena during the formation of temperature echoes in the protein–hormone systems, i.e., THR and THRT3, we used the theory of temperature quench echoes by reassigning twice the atomic velocities to zero and by using the constant velocity replacement method. We performed 15–20 different simulations for both systems in each case of 1 fs/step and 2 fs/step in the NVE ensemble with the values of delay time (τ) ranging from 0.1 to 2 ps. Using the resulting data, different graphs relating the physical variables such as temperature, energies and delay time were plotted and analyzed. The vicinity of the echo was also compared with the harmonic approximation (Eq. 6). From the different sets of numerical data for the temperature echoes, we analyzed the dephasing behavior of the systems by plotting echo depths vs. delay time.

The variations of internal potential energies in the echo time zone were plotted to analyze the impacts on bonding and non-bonding terms. Also, the plots for the number of H-bonds vs. radius of gyration were analyzed to know more about the temperature changing conformations of the protein–hormone systems. The changes in internal potential energy with respect to echo minimum temperature were used to obtain the heat capacity of the native globular proteins. Even with large fluctuations of temperature in the echo time zone, we can see the particular section of energy vs. temperature curves to obtain the heat capacity at that particular temperature. The anharmonicity coefficients such as dephasing time constant, diffusion and mobility constants were also estimated for THR and THRT3 by fitting a straight line (Eq. 13) in both cases: rigid bonds of H-atoms (2 fs/step) and free vibrations of all atoms (1 fs/step). The plotting programs used were Xmgrace, Origin-2017, and MS-Excel-2007. The validity of the results was tested by revising the simulations at least three times each.

3 Results and discussion

The THR- β isoform consists of a chain length of 259 amino acids. The unliganded THR has 3895 atoms, mass of 27,640 amu and 1952 bonds of H-atoms. The THRT3 system has 3930 atoms, mass of 28,291 amu and 1964 bonds of H-atoms. The number of atoms, charges, bonds, angles, dihedrals, impropers, cross-terms, and force field parameters yield total bonded and nonbonded potential functions in the molecular system.

RMSDs of both the THR and THRT3 systems are below 2.5 Å (Fig. 2) while simulating them separately in the explicit TIP3P water-ion environment. During geometrical optimization, equilibration and production runs, each of these molecules is conformationally stable. Although the major fold is conserved, the small peaks found in RMSD curves indicate the

Fig. 2 Root mean square deviation (RMSD) excluding H-atoms of **a** THR protein, **b** THRT3 protein, and **c** T3 hormone during the separate equilibration runs in a neutral water-ion environment at 310 K

breaking and reforming of H-bonds among the free and more stable residues, α -helix and β -sheets of the molecules during the simulations [30].

During the separate 7-ns equilibration runs of the THR and THRT3 systems solvated each in a water droplet of radius 37.48 Å, the radius of gyration (R_g) of the protein–hormone system is (18.33 ± 0.40) Å. Such a value of R_g remains almost constant (Fig. 3) indicating the continuity of conformational stability when the systems are subjected to the equilibration run in vacuo too. It is to be noted that the echo features are more distinct and observable in the native state protein in vacuo than in water because there is a higher effect of anharmonicity in a solution. As the radius of gyration indicates the protein structure compactness [31], the average values of R_g are (18.04 ± 0.09) Å, (18.35 ± 0.08) Å and (4.50 ± 0.05) Å for the THRT3, THR and T3 hormone, respectively during their equilibration runs in vacuo. The fluctuating nature of kinetic temperature of about 310 K indicates the dynamic properties of the protein atoms.

Fig. 3 Radius of gyration of both the THR and THRT3 systems during the separate 1-ns equilibration runs in the NVT ensemble in vacuo after obtaining their final coordinates from 7-ns equilibration in the neutral water-ion environment at 310 K. A small part of the graph has also been highlighted

Fig. 4 Temperature auto-correlations for THR and THRT3 simulated in the NVE ensemble **a** with 2 fs/step and rigid bonds to all H-atoms resulting in the decay time $\tau_d = 3.00 \pm 0.03$ fs and **b** with 1 fs/step and non-rigid bonds to all atoms resulting in the decay time $\tau_d = 2.80 \pm 0.15$ fs

The temperature autocorrelation function $C_{T,T}(t)$ shows the distinct nature for the simulations in the NVE ensemble with constrained rigid bonds of H-atoms (2 fs/step) and free vibration of all atoms (1 fs/step) of the THR and THRT3 molecules in vacuo. The free vibrations of H-atoms result in shoulder like features (Fig. 4-b) in the plots of autocorrelation functions with the effect of anharmonicity being clearer. The function $C_{T,T}(t)$ has been fitted with the exponential function e^{-t/τ_d} taking the data for $C_{T,T}(t)$ from 5-ps simulation in the NVE-ensemble of the equilibrated THR and THRT3 systems as shown by the dashed (red) curves in Fig. 4a, b. The decay time for both THR and THRT3 lies within the given error limit, i.e., (3.00 ± 0.03) fs for 2 fs/step and (2.80 ± 0.15) fs for 1 fs/step simulations. These values are slightly higher than the decay time (2.67 fs) of the trans-membrane protein bacteriorhodopsin found by Schulten et al. [19].

When the temperature is quenched twice to zero at a particular delay time, the echo depth as well as the nature of the echo feature curve change for the molecules THR and THRT3 and also for the cases of constrained and unconstrained normal modes of vibrations in the separate NVE simulations. Figure 5 shows the kinetic temperature distributions and the response (echo) of perturbation (quenching) in the molecular

systems that appear in the same way as explained in the theory. Here, the plots of temperature vs. time follow the same pattern as that of kinetic energy as shown in Fig. 6. The temperature $T(t)$ vs. time plots during simulations at the NVE-ensemble of the THR and THRT3 systems in equilibrium up to 5 ps show almost a constant nature about 310 K. The reassignment of atomic velocities is set to zero at $t_0 = 5$ ps and $t_1 = 5.2$ ps (delay time $\tau = t_1 - t_0 = 200$ fs). The temperature quench echo was observed at $t = t_0 + 2\tau = 5.4$ ps, i.e., at 0.4 ps after the first point of temperature quenching. At the vicinity of the echo, the mean of the temperature fluctuation is best fitted with the harmonic approximation. In the case of Figs. 5 and 6, the echo depths in terms of temperature and kinetic energy are: $\Delta T = 31.86$ K and $\Delta E_K = 311.05$ kcal/mol for THRT3. Similar calculations for THR result in $\Delta T = 33.92$ K and $\Delta E_K = 327.96$ kcal/mol. In this way, we can observe all cases of echo formation by temperature quenching and constant velocity replacement for both molecules: THR and THRT3 taking rigid bonds of H-atoms with 2 fs/step and free vibrations of all atoms with 1 fs/step simulations through the thermal equilibrium to non-equilibrium in the NVE ensembles as explained in the methodology.

The echo pulses in THRT3 are also reflected by energy profile diagrams (Figs. 6, 11 and 12) in which the total change in potential energy is equal and negative to the change in kinetic energy with the total energy remaining constant in the respective NVE ensembles of the protein–hormone system. The total energy gets a step jump along with kinetic and potential energies at the temperature quenching points, i.e., at $t_1 = 5$ ps and then $t_2 = 5.2$ ps as shown in Fig. 6. The amount of step jump in the total energy is equal to the sum of change in kinetic and potential energies and it depends on the nature of protein molecules and also on the cases of constraints imposed during the simulations.

H-bonds among N and O atoms are observed for donor-acceptor distance = 4 Å and cut-off angle = 40°. The number of H-bonds gets a step up jump and the size of the globular proteins tend to shrink at the temperature quenching points where the temperature is set to zero. Figure 7 shows the H-bond distributions for THR and THRT3 in the NVE ensembles taking rigid bonds to all H-atoms with a delay time $\tau = 2$ ps and a time step of 2 fs. The molecular

Fig. 5 Temperature echo pulse for THRT3 fitted with harmonic approximation after quenching the temperature to zero at 5.0 ps and then at 5.2 ps with the delay time $\tau = 0.2$ ps and 2 fs/step simulation in the NVE ensembles constrained with rigid bonds to all H-atoms

Fig. 6 Perturbed kinetic, internal potential, and total energy distributions parallel to the temperature distribution with delay time $\tau = 0.2$ ps and 2 fs/step simulations in the respective NVE ensembles of THRT3 keeping rigid bonds to all H-atoms

conformations change along with the formation of new H-bonds [30]. The protein becomes more globular and stable when it is perturbed under the temperature quenching and released. The THRT3 system may get jiggled to return to its original conformation at the point of second quenching (at $t = 7$ ps as shown in Fig. 8) in the presence of a heavy ligand (T3) in its LBD. The mean values of temperature, total energy, H-bonds (N_H) and radius of gyration (R_g) at the different time frames listed in Table 1 and the N_H vs. R_g plot in Fig. 9 verify that the proteins become more globular (smaller R_g) with increasing N_H while decreasing the temperature. We observe a strong negative correlation ($r = -0.86$) between R_g and N_H .

While observing the plots of individual potential energies (Fig. 10), the bigger peak (echo) in bond angle energy shows the skeletal motion of the normal modes in the proteins. In the

Fig. 7 Variations in the number of H-bonds along with time of simulations in NVE ensembles for THR and THRT3 constrained with rigid bonds to all H-atoms with a time step of 2 fs and with a delay time $\tau = 2$ ps during the formation of temperature echo by quenching

Fig. 8 Variations in the radius of gyration of THR and THRT3 at the points of temperature quenching with a step jump at the point of second quenching ($t = 7$ ps) for THRT3

case of the constant velocity replacement echo (Fig. 11), the total energy almost remains conserved even if the large fluctuation in kinetic temperature occurs in the echo time zone.

In the case of a constant velocity ($T = 310$ K) replacement echo observed in the protein–hormone systems with rigid bonds to H-atoms, the maximum energy fluctuations in the echo time zone ($t = 5.400 \pm 0.008$ ps) are ΔE_K (kinetic) = 1415.68 kcal/mol and ΔE_{BP} (bond angle potential) = 987.73 kcal/mol for THR but $\Delta E_K = 1552.84$ kcal/mol and $\Delta E_{BP} = 1025.85$ kcal/mol for THRT3. The significant change in bond angle potential suggests that the T3-hormone provides much more skeletal motion to its nuclear receptor protein.

The echo features in the internal potential energy (U) and the temperature (T) of THR and THRT3 are plotted with respect to time (t) in Fig. 12. The internal potential energies of THR and THRT3 are changing smoothly when bonds of H-atoms are made rigid resulting in a larger value of $U(t)$ for THRT3 than that of THR. However, on releasing the constrained bonds of H-atoms, the nature of $U(t)$ curves for both THR and THRT3 has changed from the constrained case and there are shoulders at many places. The shoulders appear by means of free H-atoms (larger degrees of freedom) and increasing anharmonic interactions [18]. From these echo feature curves, the heat capacity of the protein–hormone systems contributed by the internal potential energy terms can be estimated in the different temperature range. The value of the echo depth can be varied in two ways: i) by changing the delay time and ii) by changing the temperature of quenching or the velocity rescaling. The depth of the echo increases with the increase in temperature of quenching (0 to 310 K), which is verified by comparing Figs. 6 and 11. Such depth decays exponentially with delay time due to the impact of anharmonicity.

Table 1 Average values of temperature, total energy, number of H-bonds (N_H) and radius of gyration (R_g) for THR and THRT3 in NVE ensembles with rigid bonds to all H-atoms with a delay time $\tau = 2$ ps and a time step of 2 fs

Time frame	Molecule	T(K)	E_T (kcal/mol)	N_H	R_g (Å)
$0 \leq t \leq 5$ ps	THR	312.52	− 150.44	770	18.14
	THRT3	311.12	− 43.17	761	17.95
$5 \leq t \leq 7$ ps	THR	153.55	− 3331.33	821	18.00
	THRT3	160.43	− 3095.94	824	17.81
$7 \leq t \leq 13$ ps	THR	77.29	− 4854.71	852	17.91
	THRT3	78.77	− 4743.17	840	17.73

Fig. 9 Negative correlation between the number of H-bonds and radius of gyration of the protein obtained from the temperature quenching simulations of THRT3 in NVE ensembles in a time step of 2 fs

In the case of constrained or rigid bonds of H-atoms with 2 fs/step, the echo minimum temperature changes exponentially with the delay time as shown in Fig. 13. Also, the variations of kinetic energy, internal potential energy and total energy of THR and THRT3 with respect to the echo minimum temperature have been studied. In all of the cases for the constrained and free atomic vibrations of both THR and THRT3 molecules, the square of the correlation coefficient (R^2) between kinetic energy and echo minimum temperature is found to be unity, showing a very exact positive linear correlation. On the other hand, the internal potential energy has $R^2 = 0.98 \pm 0.1$ with the echo minimum temperature resulting in a negative linear correlation. The total energy almost remains constant with changing the echo minimum temperature during the dephasing of normal modes of vibrations in the molecular systems. As the kinetic energy smoothly varies with echo minimum temperature, the slightly fluctuating internal potential energy results in a temperature dependent change on heat capacity (Fig. 14). We estimated the heat capacity, dephasing time, anharmonicity diffusion and

Fig. 10 Energy distributions for THRT3 in the vicinity of the echo (temperature quenching twice to zero) with a delay time $\tau = 0.2$ ps and 1 fs/step simulation in NVE ensembles by releasing all bonds to free vibration

Fig. 11 Energy distributions for THRT3 in the vicinity of the constant velocity ($T = 310$ K) reassignment echo with a delay time $\tau = 0.2$ ps and 2 fs/step simulation in NVE ensembles constrained with rigid bonds to all H-atoms

mobility constants by analyzing the different graphs (Figs. 14 and 15) in the echo time-zone of THR and THRT3 utilizing proteins as listed in Table 2. As the heat capacity C_{V1} depends on the number of degrees of freedom, its value given by the theoretical formula from Eq. (7) and (8) is $11.60 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for THR and $11.70 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for THRT3 with unconstrained vibrations, i.e., $N_C = 0$. In the case of rigid bonds of H-atoms, $N_C = 1952$ for THR and 1964 for THRT3 and the value of C_{V1} is $9.66 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for THR and $9.75 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for THRT3. These values (within the error limit of $\pm 0.01 \text{ kcal mol}^{-1} \text{ K}^{-1}$) are the same as the slope of the straight line fitted for the kinetic energy distribution and nearly equal to the slope (C_{V2})

Fig. 12 Variations of internal potential energy (THR: curves 'f' and 'h' and THRT3: 'e' and 'g') and temperature (THR: curves 'a' and 'd' and THRT3: 'b' and 'c') in the echo time zone ($t = 5.400 \pm 0.008$ ps). These echo feature curves are related to the temperature quenching twice to zero and the delay time $\tau = 0.2$ ps with rigid bonds to all H-atoms (2 fs/time step simulations shown by *smooth lines*: 'c, d, g and h') and with non-rigid bonds to all atoms (1 fs/time step simulations shown by *dotted lines*: 'a, b, e and f')

Fig. 13 Variation of echo-minimum temperature with the delay time while dephasing THR and THRT3 after temperature quenching with the time step of 2 fs constrained with rigid bonds to all H-atoms

of the internal potential energy distribution w.r.t. the echo minimum temperature as shown in Fig. 14 during the respective dephasing simulations in the NVE ensemble of THR and THRT3. The results obtained from fitting the data after many simulations are presented in Table 2. Due to the lack of space and similarity in the graphs, a few sample graphs are shown in this paper. As the heat capacity is temperature dependent, its value has been found to increase by increasing the temperature in the echo time zone.

The average values of heat capacity C_{V2} of THR and THRT3 systems in the echo-temperature range $160 \leq T \leq 310$ K are 11.64 ± 0.49 and 12.21 ± 0.28 kcal mol⁻¹ K⁻¹, respectively, as given by

Fig. 14 Change in potential energy $U(B)$, kinetic energy $E_K(C)$ and total energy $E_T(D)$ with echo-minimum temperature during the vibrational dephasing of THRT3 after the constant velocity ($T = 310$ K) reassignment where the simulation is performed in the NVE ensemble with free vibrations of all atoms and a time step of 1 fs

Fig. 15 Linear fit in the logarithm of echo depth with the delay time during the process of the constant velocity (at $T = 310$ K) reassignment echo of the THRT3 system with 1 fs per time step simulations taking free vibrations of all atoms

the negative slope of the U vs. T straight line (Fig. 14). Such values of C_{V2} are slightly higher than the respective values 11.24 ± 0.65 and 11.62 ± 0.66 kcal mol⁻¹ K⁻¹ in the echo-temperature range $40 \leq T \leq 80$ K. These results are taken from the case of free vibrations of all atoms with 1 fs/step simulations, which are different from the case of constrained or rigid bonds of H-atoms with 2 fs/step simulations of the systems as presented in Table 2. Here, we observe that the heat capacity difference between THR and THRT3 at about 300 K is 0.57 kcal mol⁻¹ K⁻¹ and it is supplied by the T3 hormone to its nuclear receptor protein. According to Privalov and Potekhin, the partial

Table 2 Dephasing time (τ_0), damping constant (b_0), anharmonicity diffusion constant (D_0), anharmonicity mobility (μ_0) and heat capacity (C_{V2}) of unliganded thyroid hormone receptor-beta (THR) and liganded receptor-hormone system (THRT3) in the form of native globular proteins^{§§} calculated for the constrained bonds of H-atoms simulated with 2 fs/step and for the free vibrations of all atoms simulated with 1 fs/step related to this work [C_{V2} has been reported in the given echo-temperature range (ΔT) and others are at 310 K and the errors included are the standard errors of the corresponding data]

Physical parameters	Constrained vibrations with rigid bonds of H-atoms (2 fs/step)				Free vibrations of all atoms (1 fs/step)			
	THR		THRT3		THR		THRT3	
τ_0 (ps)	0.826 ± 0.034		0.625 ± 0.040		0.813 ± 0.020		0.787 ± 0.031	
b_0 (ps ⁻¹)	1.21 ± 0.05		1.60 ± 0.10		1.23 ± 0.03		1.27 ± 0.05	
D_0 (rad ps ⁻¹)	0.40 ± 0.02		0.53 ± 0.03		0.41 ± 0.01		0.42 ± 0.02	
μ_0	0.65 ± 0.03		0.86 ± 0.05		0.66 ± 0.02		0.68 ± 0.03	
μ_0 (mol kcal ⁻¹ ps ⁻¹)	0.65 ± 0.03		0.86 ± 0.05		0.66 ± 0.02		0.68 ± 0.03	
ΔT (K)	40–80	160–310	40–80	160–310	40–80	160–310	40–80	160–310
C_{V2}	8.89	10.18	9.09	10.78	11.24	11.64	11.62	12.21
(kcal mol ⁻¹ K ⁻¹)	± 0.79	± 0.46	± 0.72	± 0.60	± 0.65	± 0.49	± 0.66	± 0.28
C_{V2}	1347.97	1542.42	1345.58	1595.75	1703.03	1763.63	1720.09	1807.43
(Jkg ⁻¹ K ⁻¹)	± 119.69	± 69.69	± 106.58	± 88.81	± 98.48	± 74.24	± 97.69	± 41.44

^{§§} From Privalov and Potekhin [32], C_{V2} for different native proteins lies in the range of 1200 to 2300 Jkg⁻¹ K⁻¹ at 298 K, from Xu et al. [18], $\tau_0 = 884$ fs for pancreatic trypsin inhibitor (BPTI) and from Schulten et al. [19], $\tau_0 = 700$ fs and $b_0 = 1.43$ ps⁻¹ for bacteriorhodopsin

specific heat capacity of the different native proteins at 298 K varies within the range of 1200 to 2300 Jkg⁻¹ K⁻¹ and increases linearly with a slope of 1 Jkg⁻¹ K⁻¹ when raising the temperature [32]. In our study of the echo method in unconstrained dynamics of native globular proteins, the specific heat capacities at about 300 K are 1763.63 ± 74.24 Jkg⁻¹ K⁻¹ for THR and 1807.43 ± 41.44 Jkg⁻¹ K⁻¹ for THRT3, which lie within the range given by Privalov's study. Furthermore, the values of C_{V2} that we found in the case of constrained dynamics, 1542.42 ± 69.69 Jkg⁻¹ K⁻¹ for THR and 1595.75 ± 88.81 Jkg⁻¹ K⁻¹ for THRT3 at about 300 K are a little bit higher than the average value for the anhydrous proteins, i.e., 1250 ± 13 Jkg⁻¹ K⁻¹ at 298 K studied by Gomez et al. [21].

The dephasing times (τ_0) for THR and THRT3 in the case of free vibrations are 813 ± 20 fs and 787 ± 31 fs, respectively, which are calculated from the reciprocal of the slope of the linear fit for the logarithm of echo depth vs. delay time as shown in Fig. 15. Also, in the case of constrained vibrations, the values of τ_0 are 826 ± 34 fs for THR and 625 ± 40 fs for THRT3. Thus, the dephasing time of these systems lies in a range of 0.6–0.8 ps, which is a valid range for the echo dephasing of the native globular proteins as observed in the previous studies [18, 19]. In the study performed by Xu et al. [18] τ_0 is 884 fs for pancreatic trypsin inhibitor (BPTI) and for a similar study carried out by K. Schulten et al. 1997 for bacteriorhodopsin, it is 700 fs. In addition, the diffusion and mobility constants of anharmonicity related to τ_0 are listed in Table 2. As observed in Table 2, the dephasing time for THRT3 is a little bit smaller than that of THR. The faster dephasing of hormone binding nuclear receptors means the higher effect of anharmonicity on the normal modes of vibrations when the systems are perturbed suddenly. These parameters help to explore the energy transfer mechanism through the atoms of THRT3 to TREs probing T3-stimulated gene transcription.

4 Conclusions

We used the theory of velocity reassignments and temperature echo to study the physical properties of a native globular protein including thyroid hormone responsive features when bounded to thyroid hormone receptors. Performing MD simulations, unliganded THR- β (THR) and T3-bound THR- β LBD (THRT3) were equilibrated up to 7 ns in a cellular environment and then to 1 ns in vacuo at 310 K for the purpose of observing echo features and energy changing patterns with time and temperature in NVE ensembles. The production runs were performed in the microcanonical ensembles under constrained and unconstrained atomic vibrations to explore thermophysical properties such as temperature auto-correlations, temperature dependent hydrogen bonding and radius of gyration, heat capacity, dephasing time constant and constants of diffusion and mobility of anharmonicity. We also evaluated the simulation discrepancies by comparing the results between (a) case of constrained vibrations of H-atoms with 2 fs/step and (b) case of free vibrations of all atoms with 1 fs/step. The estimated decay times of temperature auto-correlation for THR and THRT3 are 3.00 ± 0.03 fs for case (a) and 2.80 ± 0.15 fs for case (b), which are slightly higher than 2.67 fs for bacteriorhodopsin in K. Schulten et al. 1997 [19]. The number of H-bonds of the protein gets a step up jump along with a decrease in radius of gyration at the points of temperature quenching twice to 0 K. The nature of the temperature auto-correlation function as well as echo feature curves is significantly different in cases (a) and (b). The curves are smoother in case (a) and more distinct with shoulders in case (b). Here, we focus on the analysis of changes in temperature and energy because these samples of the molecular systems transform energy from NVT to NVE

ensembles. Due to the change in internal potential energy being a function of temperature, the small fluctuation found in the total energy of the system in the NVE ensemble is only due to the fluctuating values in potential energy terms but not from kinetic energy during the conservation of total energy. It has been verified from graphical analysis that the temperature rate of change of kinetic energy, i.e., heat capacity defined in terms of it, is only the function of degrees of freedom but not of temperature in the molecular system. Thus, the potential energy is the major contributor of heat capacity. In the case of constrained or rigid bonds of H-atoms, the potentials due to bonded terms of H-atoms are not added to the net potential of the system. As a result, the heat capacity found by the temperature echo method is significantly different between cases (a) and (b) that are listed in Table 2. Since the calculations in the case of free vibration of all atoms with 1 fs/step (case-b) are more accurate, the heat capacities (C_{V2}) for THR and THRT3 are observed as $11.64 \pm 0.49 \text{ kcal mol}^{-1} \text{ K}^{-1} = 1763.63 \pm 74.24 \text{ J kg}^{-1} \text{ K}^{-1}$ and $12.21 \pm 0.28 \text{ kcal mol}^{-1} \text{ K}^{-1} = 1807.43 \pm 41.44 \text{ J kg}^{-1} \text{ K}^{-1}$, respectively at about 300 K. These values lie within the range of experimentally observed heat capacities, i.e., 1200 to 2300 $\text{J kg}^{-1} \text{ K}^{-1}$ for the native proteins studied by Privalov and Potekhin [32] and a little bit higher than $0.298 \pm 0.003 \text{ cal g}^{-1} \text{ K}^{-1}$ for the anhydrous proteins studied by Gomez et al. [21]. The results of the heat capacity (C_{V1}) obtained from the theory and the simulations match each other with a very strong positive correlation between kinetic energy and temperature ($R^2 = 1$). The dephasing time of the echo pulses has an inverse relation with diffusion and mobility of anharmonicity of the atomic vibrations and ranges from 0.6 to 0.8 ps for THR and THRT3. These parameters are valuable tools for the analytical study of biophysical phenomena associated with echo formation and dephasing, which is important in order to understand the functions of hormone binding nuclear receptors towards the regulation of gene expression.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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