Fingerprints of Conformational States of Human Hsp70 at Sub-THz Frequencies

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ABSTRACT: Large multidomain proteins occur in different conformational states to function. Detection and monitoring of these different structural states are of crucial interest for understanding the mechanics of proteins. Using computational methods, we show that different protein conformational states of the two-domain 70 kDa human Heat-shock protein (hHsp70), with similar vibrational density of states, lead to remarkably different far-IR spectra at acoustical frequencies ($\nu < 300 \text{ GHz}$). We found that the slow damped motions of the positively charged residues of hHsp70 contribute the most to collective active modes at low frequencies ($\nu < 300 \text{ GHz}$).

INTRODUCTION

Human 70 kDa heat-shock proteins (hHsp70) are highly conserved molecular chaperones essential for all living cells.\textsuperscript{1–4} They are related to many diseases and are promising targets for anticancer therapies.\textsuperscript{5,6} The structure of hHsp70 comprises two main domains: a nucleotide-binding domain (NBD) and a substrate-binding domain (SBD) connected together via a flexible linker. Each domain is divided into subdomains (Figure 1A): IA, IB, IIA, and IIB for the NBD and $\alpha$ and $\beta$ for the SBD.\textsuperscript{7,8} The nucleotide and substrate bind to a cleft at the interface between subdomains IA and IIA and to the SBD-$\beta$, respectively.\textsuperscript{9}

To perform its chaperone biological function, the hHsp70 protein occurs in two main conformational states depending on the nucleotide status of its NBD\textsuperscript{9,11–14} (Figure 1A). In the ATP-bound state of the NBD, called the open conformational state, the NBD and the SBD are docked, leading to a compact structure\textsuperscript{9,11,12} (gyration radius = 2.7 nm, largest distance = 8 nm),\textsuperscript{9} as shown in Figure 1A. In the ADP-bound state of the NBD, called the closed conformational state, the NBD and SBD domains are free to move independently and the structure is elongated (gyration radius = 3.4 nm, largest distance = 12 nm),\textsuperscript{9} as also shown in Figure 1A. Further structural differences between the two main conformations are found: the SBD-$\alpha$ lid covers the substrate-binding pocket in the closed conformation,\textsuperscript{15} whereas it is docked to subdomain IA of the NBD in the open conformation. Finally, the linker is buried into a cleft located between subdomains IA and IIA of the NBD in the open conformation, whereas it is free in the closed conformation.\textsuperscript{16,17}

There is considerable interest in hHsp70 in medicine, and detection of the exchange between the different conformations of human Hsp70 is crucial to understand the function of this key molecular chaperone. In this work, we theoretically examine the possibility of detecting these conformational changes by measuring the sub-THz vibrational modes of hHsp70 in single-molecule spectroscopy.

For 4 decades, there has been considerable theoretical interest in establishing the role of sub-THz normal modes in the protein biological function.\textsuperscript{18–37} Sub-THz vibrational modes, called acoustical modes,\textsuperscript{38} involve large-scale displacements of protein segments. The acoustical modes do not depend on the atomistic details of the molecular structure but are mainly dependent on the protein shape and on the connectivity of its main chain. The acoustical modes are thus the modes that are the most influenced by a change in the global shape/size of a protein induced by a conformational change. Low-frequency vibrational modes of proteins were measured by neutron scattering,\textsuperscript{39–49} Raman spectroscopy,\textsuperscript{50–55} and far-IR spectroscopy.\textsuperscript{56–60} The first well-resolved acoustical modes of several proteins at frequencies as low as 20–100 GHz were detected very recently using a nanobiosensing technique: extraordinary acoustic Raman (EAR) spectroscopy.\textsuperscript{61}

In EAR spectroscopy, a single molecule is confined in an optical trap and is excited by a low-frequency electromagnetic field (10–300 GHz), which interacts with the protein acoustical
modes. The low-frequency electromagnetic field is obtained by interference between two optical lasers of slightly different wavelengths. Despite its name, EAR spectroscopy differs drastically from Raman spectroscopy. The spectrum of the trapped protein is obtained by measuring the variations of its Brownian motion in the optical trap as a function of the frequency of the applied electric field. The authors suggest that the physical mechanism leading to the excitation of the acoustical modes in EAR is electrostriction, that is, the nonlinear elastic response of a material to an electric field. However, as shown here and elsewhere, the protein acoustical modes are IR active, and the linear response of proteins to an electric field must contribute significantly to the EAR spectrum. Therefore, in this work, we theoretically examine the possibility of detecting the different conformational states of the key Hsp70 chaperone by exciting its acoustical modes using a low-frequency electric field in a nanobiosensing experiment such as EAR.

RESULTS AND DISCUSSION

Because of the strong coupling between the protein and solvent, the protein and its first solvation shell can be considered an integrated system. The vibrational modes of Hsp70 surrounded by water (TIP3P model) were computed in the harmonic approximation using the all-atom CHARMM27 and the AMBER99sb-ILDN68 potential energy surfaces using the GROMACS software package. Protein structures plus water molecules of the first hydration shell (at 3 Å around the protein) were extracted from snapshots of all-atom MD simulations of Hsp70 as detailed in previous works (more details can be found in the Materials and Methods section). Vibrational modes discussed below are computed using the AMBER force-field.

Below 300 GHz ($\nu = 10$ cm$^{-1}$), there are 24 and 26 collective modes for the open and closed conformations, respectively (Figure 1B). The lowest nonzero frequency mode of Hsp70, that is, $\nu_7$, occurs at 82.8 GHz ($\nu_7 = 2.76$ cm$^{-1}$) for the open conformational state and at 43.8 GHz ($\nu_7 = 1.46$ cm$^{-1}$) for the closed conformational state. This difference $\Delta \nu$ of 39.0 GHz ($\Delta \nu = 1.3$ cm$^{-1}$) can be explained by the fact that the closed state has a more elongated structure than the open state, for which the two domains are docked. Therefore, the closed state of Hsp70 may subdivide modes of longer wavelength than the open state of Hsp70 and thus of lower frequency (Figure 1). However, these differences between the two main conformational states of Hsp70 are not visible in their density of states $D(\nu)$, as shown in Figure 1B. Therefore, at first glance, it seems not possible to identify the two main conformational states of Hsp70 based on the sole measurement of $D(\nu)$, using for example inelastic neutron scattering. However, on the basis of the experimental results of EAR spectroscopy, one may expect the acoustical modes to interact with an electric field. Each acoustical mode should have a different signature in the EAR or in the far-IR spectra of Hsp70 depending on its dipole moment. A variation of the molecular dipole moment at acoustical frequency is expected because Hsp70 has a strong dipolar character, as 81 residues are positively charged and 92 are negatively charged (Table 1).

The variation of the dipole moment of the hydrated molecule $\vec{p}_l$ induced by the $l$th vibrational mode of frequency $\nu_l$ is given by

$$\vec{p}_l = \frac{\partial \vec{p}}{\partial \nu_l} = \sum_{i=1}^{N} \vec{p}_{il} = \sum_{i=1}^{N} q_i \vec{r}_{il}$$

where $q_i$ and $m_i$ are, respectively, the charge and mass of atom $i$, $\vec{r}_{il}$ and $\vec{r}_{il}$ are the eigenvector component of atom $i$ and the normal coordinate of the $l$th mode, respectively. $N$ is the total number of atoms of the hydrated molecule (including water),
Table 1. hHsp70 Subdomains and Their Electrostatic Properties

| Subdomain | NBD | SBD |
|-----------|-----|-----|
|           | L  | α  | β  | Total |
| Number of res. | 132 | 76 | 54 | 78 | 13 | 114 | 134 | 641 |
| Total charge | −2 | +2 | −3 | +3 | −2 | −1 | −8 | −11 |
| Number of + res. | 10 | 11 | 12 | 16 | 1 | 11 | 20 | 81 |
| Number of − res. | 13 | 9 | 15 | 13 | 3 | 12 | 27 | 92 |
| Number of ARG | 4 | 3 | 5 | 10 | 4 | 5 | 31 |
| Number of LYS | 6 | 8 | 7 | 11 | 1 | 7 | 15 | 50 |
| Number of GLU | 7 | 2 | 4 | 8 | 1 | 5 | 20 | 47 |
| Number of ASP | 6 | 7 | 11 | 5 | 2 | 7 | 7 | 45 |

and \( \vec{\rho} \) is the dipole moment of the molecule induced by the elastic deformation of the molecule

\[
\vec{\rho} = \sum_{i=1}^{N} q_i \vec{\mu}_i
\]

(2)

where \( \vec{\mu}_i \) is the displacement of atom \( i \) in the harmonic approximation.

In the frequency range \([0−300] \text{ GHz}\), variations of molecular dipole moment \( |\vec{\rho}| \) of the open and closed conformations of hHsp70 were computed and are presented in Figure 2. From

![Figure 2](image)

Figure 2. Vibrational dipole moments \( |\vec{\rho}| \) as a function of frequency \( \nu \) for the open (top panel in green) and closed (bottom panel in blue) conformations of hHsp70. Vibrational dipole moments were computed using eq 1 from the normal modes computed using the AMBER99sb-ILDN force-field.

the 24 and 26 modes of the open and closed conformations of hHsp70, only a few contribute significantly to the variation of the molecular dipole moment. For example, in the open state, the mode that exhibits the largest variation of molecular dipole moment is the mode \( \nu_8 = 107.7 \text{ GHz} \) (\( \bar{\nu}_8 = 3.59 \text{ cm}^{-1} \)), whereas in the closed state, it corresponds to the mode \( \nu_9 = 61.4 \text{ GHz} \) (\( \bar{\nu}_9 = 2.05 \text{ cm}^{-1} \)). In addition, the norm of the largest variation of molecular dipole moment in the closed state of hHsp70 is larger than that in the open state. To obtain a better understanding of the dipolar active modes, we computed the dipole due to the displacement of each atom, \( \vec{\rho}_{il} = q_i \vec{u}_{il} \) in every acoustical mode \( l \). The contributions of every atom to the variation of molecular dipole moment, \( \vec{\rho}_0 \) were summed over all of the atoms of each residue (see Figure 3A,D) and over all atoms of a subdomain (see Figure 3B,E) for both conformations of hHsp70.

We deduce from Figure 3A (open state) and D (closed state) that the largest contributions to the vibrational dipole moments are due to the positively charged residues of hHsp70. Compared to negatively charged residues, ARG and LYS residues are characterized by longer side chains, that is, \( L \sim 6.4 \) Å (\( L \sim 2.6 \) Å for ASP and \( L \sim 4.0 \) Å for GLU), which leads to larger variation of the dipole moments in a vibrational mode. In detail, in mode \( \nu_8 \) of the open conformation, subdomain IIB (Figure 3B,C) has the largest dipolar contribution, followed by the two subdomains of the SBD. In fact, the global motion observed for this particular mode corresponds to a torsional motion of subdomain IIB of the NBD and of each subdomain \( \beta \) and \( \alpha \) of the SBD. A similar analysis is performed for the low-frequency mode \( \nu_9 \) of hHsp70, having the largest variation of the molecule dipolar moment in the closed conformational state. In mode \( \nu_9 \) of the closed conformation, the largest dipolar contribution arises from the deformation of the SBD-\( \alpha \) (Figure 3E,F). Particularly, positively charged residues (ARG) located in the C-terminal part of Hsp70 exhibit large dipole moments. The SBD-\( \alpha \) is the most charged subdomain of hHsp70, with a total charge of −8 (Table 1). Furthermore, compared with the open state of hHsp70, the closed conformation mode, \( \nu_9 \), shows dipole moments that are more distributed along the entire protein, and, more precisely, all of the four subdomains of the NBD exhibit large dipole moment variations (Figure 3E). This is because the two domains of the closed state can move freely and do not interact with each other, which do not block some motions compared with the open conformation. The global motion of mode \( \nu_9 \) corresponds to a stretching motion, that is, a compression/elongation of the two lobes of hHsp70, as depicted by black bold arrows in Figure 3F. In addition to the difference in shape between the open and closed conformations of hHsp70 (Figure 1A), the fact that the two main domains of the closed state do not interact with each other leads to larger vibrational dipole moments than those for the open state. These differences in the hHsp70 conformational states could serve as spectroscopic fingerprints at low frequencies (below 300 GHz).

Because the lowest frequency acoustical modes exhibit variations of molecular dipole moment as shown in Figure 3, they interact with an applied electric field \( \vec{E} \). The energy absorbed by hHsp70 from the source of a low-frequency applied electric field was calculated from the all-atom normal mode calculations using the Newton equation, leading to the following formula for the classical absorption (IR) spectrum

\[
P(\nu) = \frac{\pi}{2} \bar{E}(\nu)^2 \sum_{l=1}^{3N−6} \frac{\gamma \nu}{(\nu^2 - \nu_i^2)^2 + \nu^2 \gamma^2} |\vec{\rho}_l|^2
\]

(3)

where \( \nu \) is the frequency, \( \bar{E}(\nu) \) is the applied electric field at frequency \( \nu \), \( \gamma \) is a constant damping factor, and \( \nu_i \) is the frequency of the \( l \)th normal mode (see the Materials and Methods section for details).
Equation 3 includes the effect of damping (assumed here to be identical for all acoustical modes), which is usually ignored in the computation of the IR spectra from normal mode analysis. However, damping is a crucial parameter to interpret correctly the experimental data. One source of damping is the effect of bulk solvent, which dissipates the vibrational energy of the protein. In the present all-atom calculation, the representation of the solvent is described at two levels: explicitly by taking into account all water molecules of the hydration layer and implicitly by representing the bulk solvent by a damping factor. The calculation of the vibrational modes of a large hydrated protein such as hHsp70 is a “tour de force”, as it corresponds to the diagonalization of a large matrix ($38055 \times 38055$ for the closed state for example), and it is not possible to represent explicitly the effect of the bulk solvent. Unfortunately, the value of the damping of acoustical modes of proteins in water is not well known. No values are reported for molecular chaperones in the literature. Data for lysozyme in solution suggest that the acoustical modes of this protein are overdamped in solution with $\gamma = 594 \text{ GHz}$ ($\tilde{\nu} = 18 \text{ cm}^{-1}$),63

Figure 3. (A) Vibrational dipole moment $|\vec{p}|$ as a function of the residue index of hHsp70 in the open conformation for mode $\nu_8$. Blue and red points indicate positively and negatively charged residues, respectively. (B) Vibrational dipole moment $|\vec{p}|$ as a function of the subdomain index of hHsp70 in the open conformation for mode $\nu_8$. (C) Cartoon representation of vibrational dipole moment directions and strengths projected onto the hHsp70 open (not deformed) structure for mode $\nu_8$. The color code is the same as in Figure 1B. This figure was prepared with PyMOL. (D), (E), and (F) are the same panels as panels (A), (B), and (C), respectively, but for mode $\nu_9$ of the closed conformation of hHsp70.
whereas an undamped mode was observed at 630 GHz (\(\nu = 19.6 \text{ cm}^{-1}\)) in low-hydrated lysozyme.\(^5\) In EAR spectroscopy, the damping of a protein with a size similar to hHsp70 (conalbumin\(^6\)) is typically on the order of a few GHz. Because the value of the damping of low-frequency modes of proteins varies depending on the experimental technique, we decided to explore the range of values reported for different proteins using different techniques. In addition, as the scale of the protein motions is similar in the acoustical modes, one chooses an identical value of the damping coefficient for all of the acoustical modes computed. The IR spectra \(P(\nu)\), as given in eq 3, are thus computed by considering different values of \(\gamma\) corresponding to weakly damped to overdamped modes. As explained below, the damping has a huge impact on both intensities and positions of the peaks in the IR spectra. Figure 4 shows spectra \(P(\nu)\) of hHsp70 for the open and closed conformational states as a function of the value of the damping constant \(\gamma\) (in the computed spectra, the prefactor in eq 3, \(\frac{\pi|\vec{E}|^2}{2\hbar}\), was set arbitrarily to 1 in this work). First of all, it is clear from Figure 4 that the open and closed conformations show different spectra \(P(\nu)\), independently of the value of the damping constant \(\gamma\) and independently of the force-field used for the calculations. For example, in the case of the AMBER99SB-ILDN force-field (Figure 4A), in the spectra \(P(\nu)\) calculated for \(\gamma = 30 \text{ GHz (} \bar{\nu} = 1.0 \text{ cm}^{-1}\)), the closed conformation of hHsp70 shows an intense peak centered at \(\nu = 45 \text{ GHz (} \bar{\nu} = 1.5 \text{ cm}^{-1}\)), whereas the same peak is shifted to \(\nu = 108 \text{ GHz (} \bar{\nu} = 3.6 \text{ cm}^{-1}\)) for the open conformation. As expected, an increase in the damping constant \(\gamma\) from 3 GHz (\(\bar{\nu} = 0.1 \text{ cm}^{-1}\)) to 30 GHz (\(\bar{\nu} = 1.0 \text{ cm}^{-1}\)) goes together with an increase in the width of the peaks and with a decrease in the spectral resolution but does not change the position of the peaks because all acoustical modes have frequencies larger than \(\gamma/2\) (regime of damped modes). By increasing the damping constant from 30 GHz (\(\bar{\nu} = 1.0 \text{ cm}^{-1}\)) to 300 GHz (\(\bar{\nu} = 10 \text{ cm}^{-1}\)), another phenomenon is observed in Figure 4. There is a shift of the most intense peak of the closed conformation to a lower frequency, namely, 15 GHz (\(\bar{\nu} = 0.5 \text{ cm}^{-1}\)), because the lowest frequency \(\nu_7\) is smaller than \(\gamma/2\) (regime of overdamped modes). For a large \(\gamma = 300\) GHz, the spectra above 45 GHz of the open and closed conformational states of hHsp70 are similar with no single peak. As shown in Figure 4 for hHsp70, even for \(\gamma\) as large as 300 GHz, the two conformational states of the protein can be distinguished below 45 GHz. Note that exactly the same conclusions can be drawn from the calculations using the CHARMM27 force-field (Figure 4B). Finally, the structures of the nucleotide-free and the ADP-bound closed models of hHsp70\(^7\) are very similar, as are the spectra of their acoustical modes, as shown in Figure 5.

CONCLUSIONS
To summarize, we demonstrated that in the sub-THz frequency range the main conformations (open and closed) of hHsp70

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**Figure 4.** (A) THz spectra \(P(\nu)\) of hHsp70 for the open (green) and closed (blue) conformations computed from the normal modes calculated using the AMBER99SB-ILDN force-field. Spectra with different damping \(\gamma\) are represented: 3 GHz (top panel), 30 GHz (middle panel), and 300 GHz (bottom panel). Surface representation of open (in blue) and closed (in green) conformations of hHsp70 are shown in the inset of the top panel. (B) Same results from NMA using the CHARMM27 force-field.
could be distinguished because they exhibit different spectroscopic fingerprints at acoustical frequencies. These nonsimilar fingerprints are due to different vibrational dipole moments of the open and closed conformations of hHsp70 and due to the fact that the two main conformations are characterized by strongly different shapes. These results should prompt nanobiosensing experiments, such as EAR\textsuperscript{61} spectroscopy, for Hsp70 proteins that are of great interest in medicine. In practice, the ability to distinguish the spectra of the different conformational states of hHsp70 will depend on the time scale of the experimental method because hHsp70 occurs as a dynamical ensemble.\textsuperscript{73,74} Single-molecule Förster resonance energy transfer experiments show that the conformations of hHsp70 interconvert on a time scale of 1–4 s.\textsuperscript{73} Recording a full spectrum on a shorter time scale is however feasible in single-molecule spectroscopy.\textsuperscript{75}

**MATERIALS AND METHODS**

**Normal Mode Calculations.** The two hydrated structures of hHsp70 used to compute the normal modes in Figure 4 are the representative structures extracted from the MD of the nucleotide-free open and closed models.\textsuperscript{\textsuperscript{95,68,73}} These two structures were chosen because they were extracted from the longest MD runs and because they are the best representative relaxed structures of the open and closed conformations, respectively. The hydrated structure of ADP-bound hHsp70 used to compute the normal modes in Figure 5 was extracted from a previous MD run.\textsuperscript{73} For the all-atom calculations, only the first hydration water layer of hHsp70 was kept corresponding to 915 (open), 939 (closed), and 988 (closed and ADP-bound) water molecules, all within 3 Å from the protein atoms. Then, the hydrated hHsp70 structures were optimized using the limited memory Broyden–Fletcher–Goldfarb–Shanno algorithm implemented in the GROMACS software package\textsuperscript{71} with a force criterion of 10\textsuperscript{−7} kJ/mol/nm using the AMBER99SB-ILDN\textsuperscript{68–70} as well as CHARMM27\textsuperscript{65–67} force-fields and the TIP3P water model.\textsuperscript{54} The cutoff distance for the short-range neighbor list was 1.4 nm with the simple method and no periodic boundary condition. Switch type was used for both Coulomb and non-Coulomb potentials (with a standard cutoff of 1.0 nm and a switch cutoff of 1.2 nm). Finally, the Hessian was computed and diagonalized using the same parameters. For each system, it was verified that the first 6 eigenfrequencies were equal to zero, and there was no imaginary frequency.

**Classical Absorption Spectrum.** The classical absorption spectrum formula [eq 3] is derived as follows (adapted from a previous work on the IR response of a solid surface\textsuperscript{14}). The single hydrated protein is described as a set of uncoupled forced harmonic oscillators

\[
\ddot{\xi}_l(t) + \gamma_l \dot{\xi}_l(t) + \omega_l^2 \xi_l(t) = f_i(t) + F_l(t)
\]

where \(l = 7\) to \(3N - 6\), \(N\) being the number of atoms, \(f_i(t)\) is the effective external force driving the \(l\)th vibrational mode of the system with a frequency \(\omega_l = \frac{\hbar}{2\pi}\) and \(\xi_l\) is the normal coordinate of mode \(l\).\textsuperscript{72} Modes \(l = 1–6\) are the zero-frequency modes of a single isolated molecule and are ignored. In biomolecular force-fields used in this work to compute the normal modes of the hydrated proteins, the electric density of the protein and of the solvent is represented by fixed effective atomic charges. In this approximation, the effective force \(f_i\) due to an external uniform electric field \(E\) is given by\textsuperscript{72}

\[
f_i(t) = \bar{\rho}_i \cdot \bar{E}(t) = \sum_{\kappa=1}^{N} \frac{q_{\kappa} \bar{e}_{l\kappa}}{\sqrt{m_{\kappa}}} \bar{E}(t)
\]

where \(q_{\kappa}\) and \(m_{\kappa}\) are, respectively, the effective charge and mass of atom \(\kappa\), and \(\bar{e}_{l\kappa}\) is the eigenvector component of atom \(\kappa\) of the \(l\)th mode. Note that \(\bar{\rho}_i\) is the derivative of the molecular dipole moment relative to the normal coordinate.

In eq 4, we have introduced a phenomenological damping factor \(\gamma_l\) and a Langevin random force \(F_l(t)\). At steady state, the external force \(f_i(t)\) does work on the oscillator that is eventually dissipated as heat in the viscous fluid (the random force \(F_l(t)\) does not work on the oscillator on average). The energy gained by the \(l\)th oscillator due to the work of the external force \(f_i\) is

\[
W_l = \int_{-\infty}^{+\infty} f_i(t) \dot{\xi}_l(t) dt
\]

Using Fourier transforms in eqs 4–6 and after a little algebra,\textsuperscript{52} the energy absorbed by the set of oscillators, \(W = \sum_l W_l\) is given by

\[
W = 4\pi \sum_{l=7}^{3N-6} \int_0^{+\infty} \frac{\omega_l^2 f_l}{[\omega_l^2 - \omega^2 + \gamma_l^2 \frac{\omega_l^2}{\omega^2}]} |f_l(\omega)|^2 d\omega
\]

Finally, averaging the orientation of the molecule relative to the applied electric field\textsuperscript{52} leads to the final formula of the classical
absorption spectrum by a single molecule due to an applied electric field in the harmonic approximation

\[ P(\nu) \equiv \frac{d(W/\hbar)}{dh\nu} = \frac{\pi \hbar^2 |\tilde{E}(\nu)|^2}{2\hbar^2} \]

\[ \sum_{i=7}^{3N-6} \frac{\tilde{Y}^2}{\left(\nu_i^2 - \nu^2\right)^2 + \nu^2 \gamma_i^2} |\tilde{Y}|^2 \]

(8)

where \( \nu = \frac{\omega}{2\pi} \) is the excitation frequency of the electric field, and \( \hbar \) is the Planck constant. The classical absorption spectrum formula [eq 8] was implemented in C language and performed from GROMACS standard output files (.xvg and .top).71

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Notes
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