

# Diphtheria toxin time-resolved absorption and resonance ft-ir and raman biospectroscopy and density functional theory (dft) investigation of vibronic-mode coupling structure in vibrational spectra analysis: a spectroscopic study on an anti-cancer drug

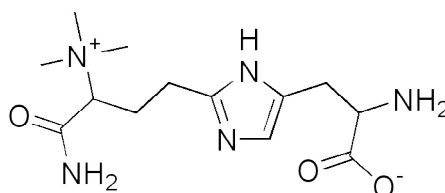
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## Abstract

Diphtheria toxin is an exotoxin secreted by *Corynebacterium diphtheriae*, the pathogenic bacterium that causes diphtheria. Unusually, the toxin gene is encoded by a bacteriophage (a virus that infects bacteria). The toxin causes the disease in humans by gaining entry into the cell cytoplasm and inhibiting protein synthesis. Parameters such as FT-IR and Raman vibrational wavelengths and intensities for single crystal Diphtheria Toxin are calculated using density functional theory and were compared with empirical results. The investigation about vibrational spectrum of cycle dimers in crystal with carboxyl groups from each molecule of acid was shown that it leads to create Hydrogen bonds for adjacent molecules. The current study aimed to investigate the possibility of simulating the empirical values. Analysis of vibrational spectrum of Diphtheria Toxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G<sup>\*</sup>, HF/6-31++G<sup>\*\*</sup>, MP2/6-31G, MP2/6-31++G<sup>\*\*</sup>, BLYP/6-31G, BLYP/6-31++G<sup>\*\*</sup>, B3LYP/6-31G and B3LYP6-31-HEG<sup>\*\*</sup>. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated. The obtained values confirm high accuracy and validity of results obtained from calculations.



Molecular structure of Diphtheria Toxin [1-42].

## Introduction

Diphtheria toxin is an exotoxin secreted by *Corynebacterium diphtheriae*, the pathogenic bacterium that causes diphtheria. Unusually, the toxin gene is encoded by a bacteriophage (a virus that infects bacteria). The toxin causes the disease in humans by gaining entry into the cell cytoplasm and inhibiting protein synthesis. Density Functional Theory (DFT) is one of the most powerful calculation methods for electronic structures [5-7]. Numerous results have been previously studied and indicate successful use of these methods [8-10]. The theory is one of the most appropriate methods for simulating the vibrational wavenumbers, molecular structure as well as total energy. It may be useful to initially consider the calculated results by density functional theory using HF/6-31G<sup>\*</sup>, HF/6-31++G<sup>\*\*</sup>, MP2/6-31G, MP2/6-31++G<sup>\*\*</sup>, BLYP/6-31G, BLYP/6-31++G<sup>\*\*</sup>, B3LYP/6-31G and B3LYP6-31-HEG<sup>\*\*</sup> approach [11-16]. It should be noted that calculations are performed by considering one degree of quantum interference as well as polarization effects of 2d orbitals in interaction [17-364].

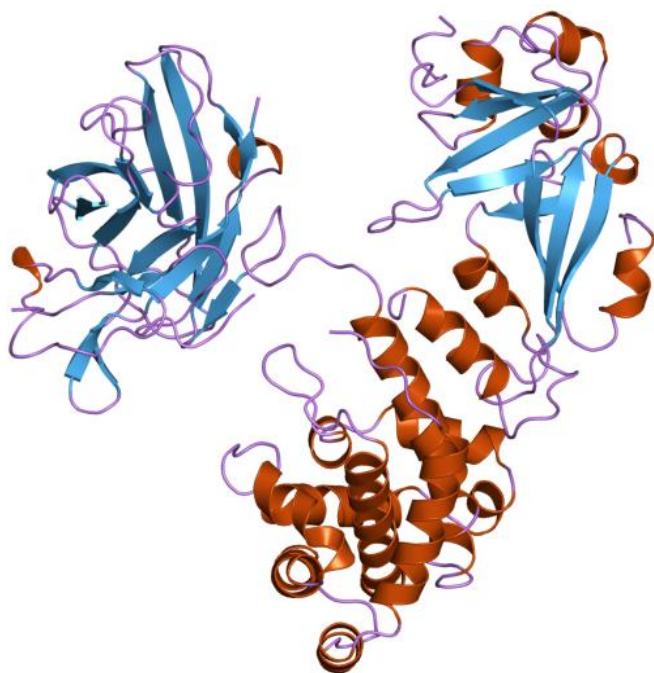
## Details of Calculations

All calculations of molecular orbital in the base of ab are performed by Gaussian 09. In calculation process, the structure of Diphtheria Toxin molecule (Figure 1) is optimized and FT-IR and Raman wavenumbers are calculated using HF/6-31G<sup>\*</sup>, HF/6-31++G<sup>\*\*</sup>, MP2/6-31G, MP2/6-31++G<sup>\*\*</sup>, BLYP/6-31G, BLYP/6-31++G<sup>\*\*</sup>, B3LYP/6-31G and B3LYP6-31-HEG<sup>\*\*</sup> base. All optimized

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**Key words:** Vibronic structure, vibrational spectra analysis, density functional theory (dft), diphtheria toxin, non-focal functions of becke, correlation functions of lee-yang-parr, time-resolved absorption and resonance, ft-ir and raman biospectroscopy, anti-cancer drug

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**Figure 1.** Section of the diphtheria toxin

structures are adjusted with minimum energy. Harmonic vibrational wavenumbers are calculated using second degree of derivation to adjust convergence on potential surface as good as possible and to evaluate vibrational energies at zero point. In optimized structures considered in the current study, virtual frequency modes are not observed which indicates that the minimum potential energy surface is correctly chosen. The optimized geometry is calculated by minimizing the energy relative to all geometrical quantities without forcing any constraint on molecular symmetry. Calculations were performed by Gaussian 09. The current calculation is aimed to maximize structural optimization using density functional theory. The calculations of density functional theory are performed by HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\* function in which non-focal functions of Becke and correlation functions of Lee-Yang-Parr beyond the Franck-Condon approximation are used. After completion of optimization process, the second order derivation of energy is calculated as a function of core coordination and is investigated to evaluate whether the structure is accurately minimized. Vibrational frequencies used to simulate spectrums presented in the current study are derived from these second order derivatives. All calculations are performed for room temperature of 373 (K).

## Vibration Analysis

Analysis of vibrational spectrum of Diphtheria Toxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\*. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated.

C-H stretching vibrations in single replacement of benzene cycles are usually seen in band range of 4000-4250  $\text{cm}^{-1}$ . Weak Raman bands are at 3989  $\text{cm}^{-1}$  and 4002  $\text{cm}^{-1}$ . C-C stretching mode is a strong Raman

mode at 1999  $\text{cm}^{-1}$ . Raman weak band is seen at 2473  $\text{cm}^{-1}$ , too. Bending mode of C-H is emerged as a weak mode at 2198  $\text{cm}^{-1}$  and 2197  $\text{cm}^{-1}$  and a strong band at 2081  $\text{cm}^{-1}$  in Raman spectrum. Raman is considerably active in the range of 2000-2250  $\text{cm}^{-1}$  which 1993  $\text{cm}^{-1}$  indicates this issue.

C-H skew-symmetric stretching mode of methylene group is expected at 3985  $\text{cm}^{-1}$  and its symmetric mode is expected at 3799  $\text{cm}^{-1}$ . Skew-symmetric stretching mode of  $\text{CH}_2$  in Diphtheria Toxin has a mode in mid-range of Raman spectrum at 3900-4020  $\text{cm}^{-1}$ . When this mode is symmetric, it is at 3895  $\text{cm}^{-1}$  and is sharp. The calculated wavenumbers of higher modes are at 3863  $\text{cm}^{-1}$  and 3893  $\text{cm}^{-1}$  for symmetric and skew-symmetric stretching mode of methylene, respectively.

Scissoring vibrations of  $\text{CH}_2$  are usually seen at the range of 2327-2381  $\text{cm}^{-1}$  which often includes mid-range bands. Weak bands at 2340  $\text{cm}^{-1}$  are scissoring modes of  $\text{CH}_2$  in Raman spectrum. Moving vibrations of methylene are usually seen at 2269  $\text{cm}^{-1}$ . For the investigated chemical in the current study, these vibrations are at 2139  $\text{cm}^{-1}$  were calculated using density functional theory. Twisting and rocking vibrations of  $\text{CH}_2$  are seen in Raman spectrum at 1715  $\text{cm}^{-1}$  and 1989  $\text{cm}^{-1}$ , respectively, which are in good accordance with the results at 1699  $\text{cm}^{-1}$  and 1964  $\text{cm}^{-1}$ , respectively.

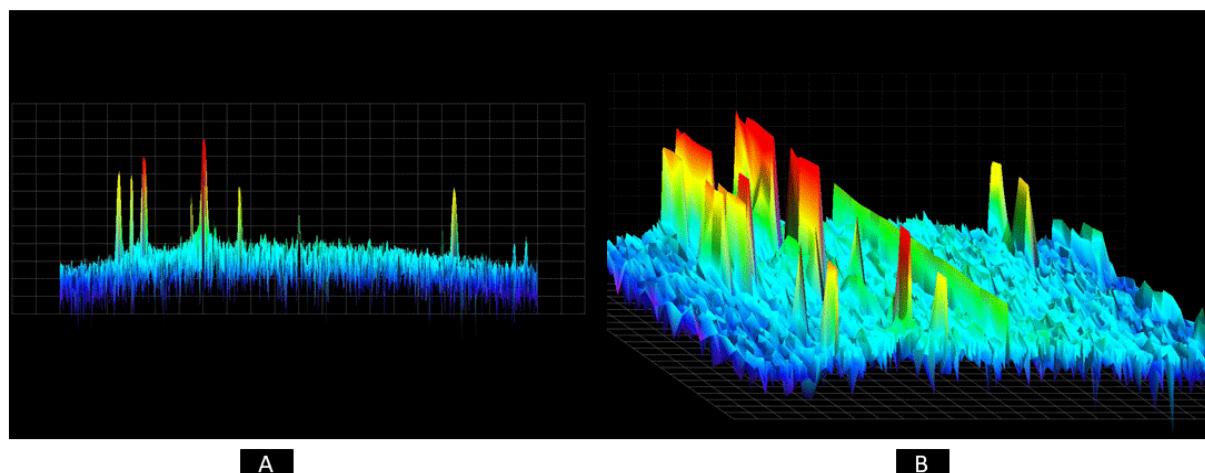
In a non-ionized carboxyl group ( $\text{COOH}$ ), stretching vibrations of carbonyl [ $\text{C=O}$ ] are mainly observed at the range of 2640-2688  $\text{cm}^{-1}$ . If dimer is considered as an intact constituent, two stretching vibrations of carbonyl for symmetric stretching are at 2540-2585  $\text{cm}^{-1}$  in Raman spectrum. In the current paper, stretching vibration of carbonyl mode is at 2597  $\text{cm}^{-1}$  which is a mid-range value.

Stretching and bending bands of hydroxyl can be identified by width and band intensity which in turn is dependent on bond length of Hydrogen. In dimer form of Hydrogen bond, stretching band of O-H is of a strong Raman peak at 2167  $\text{cm}^{-1}$  which is due to in-plane metamorphosis mode. Out-of-plane mode of O-H group is a very strong mode of peak at 1849  $\text{cm}^{-1}$  of Raman spectrum. The stretching mode of C-O (H) emerges as a mid-band of Raman spectrum at 2047  $\text{cm}^{-1}$ .

Lattice vibrations are usually seen at the range of 0-1350  $\text{cm}^{-1}$ . These modes are induced by rotary and transferring vibrations of molecules and vibrations and are including Hydrogen bond. Bands with low wavenumbers of Hydrogen bond vibrations in FT-IR and Raman spectrum (Figure 2) are frequently weak, width and unsymmetrical. Rotary lattice vibrations are frequently stronger than transferring ones. Intra-molecular vibrations with low wavenumbers involving two-bands O-H ...O dimer at 888  $\text{cm}^{-1}$ , 993  $\text{cm}^{-1}$  and 1049  $\text{cm}^{-1}$  are attributed to a rotary moving of two molecules involving in-plane rotation of molecules against each other.

## Conclusion and Summary

Calculations of density functional theory using HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\* levels were used to obtain vibrational wavenumbers and intensities in single crystal of Diphtheria Toxin. Investigation and consideration of vibrational spectrum confirm the formation of dimer cycles in the investigated crystal with carboxyl groups from each Hydrogen molecule of acid protected from adjacent molecules. The calculated vibrational spectrum which obtains from calculations of density functional theory is in good accordance with recorded empirical values which indicates successful simulation of the



**Figure 2.** 3D Simulation of (a) FT-IR spectrum and (b) Raman spectrum of Diphtheria Toxin

problem. The obtained results indicate that the results obtained from theoretical calculations are valid through comparing with empirical recorded results.

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