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Review of Computer Simulations of Isotope Effects on Biochemical Reactions: from the Bigeleisen Equation to Feynman’s Path Integral

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Abstract
Enzymatic reactions are integral components in many biological functions and malfunctions. The iconic structure of each reaction path for elucidating the reaction mechanism in details is the molecular structure of the rate-limiting transition state (RLTS). But RLTS is very hard to get caught or to get visualized by experimentalists. In spite of the lack of explicit molecular structure of the RLTS in experiment, we still can trace out the RLTS unique “fingerprints” by measuring the isotope effects on the reaction rate. This set of “fingerprints” is considered as a most direct probe of RLTS. By contrast, for computer simulations, oftentimes molecular structures of a number of TS can be precisely visualized on computer screen, however, theoreticians are not sure which TS is the actual rate-limiting one. As a result, this is an excellent stage setting for a perfect “marriage” between experiment and theory for determining the structure of RLTS, along with the reaction mechanism, i.e., experimentalists are responsible for “fingerprinting”, whereas theoreticians are responsible for providing candidates that match the “fingerprints”. In this Review, the origin of isotope effects on a chemical reaction is discussed from the perspectives of classical and quantum worlds, respectively (e.g., the origins of the inverse kinetic isotope effects and all the equilibrium isotope effects are purely from quantum). The conventional Bigeleisen equation for isotope effect calculations, as well as its refined version in the framework of Feynman’s path integral and Kleinert’s variational perturbation (KP) theory for systematically incorporating anharmonicity and (non-parabolic) quantum tunneling, are also presented. In addition, the outstanding interplay between theory and experiment for successfully deducing the RLTS structures and the reaction mechanisms is demonstrated by applications on biochemical reactions, namely models of bacterial squalene-to-hopene polycyclization and RNA 2′-O-transphosphorylation. For all these applications, we used our recently-developed path-integral method based on the KP theory, called automated integration-free path-integral (AIF-PI) method, to perform \textit{ab initio} path-integral calculations of isotope effects. As opposed to the conventional path-integral molecular dynamics (PIMD) and Monte Carlo (PIMC) simulations, values calculated from our AIF-PI path-integral method can be as \textit{precise as} (not as \textit{accurate as}) the numerical precision of the computing machine. Lastly, comments are made on the general challenges in theoretical modeling of candidates matching the experimental “fingerprints” of RLTS.
1. Introduction

“… Isotope labelling and femtosecond spectroscopy can give clues, but rarely produce conclusive evidence for a given mechanism in systems with the complexity characterizing many catalytic chemical processes and almost all biochemical processes. This makes theoretical modelling an important tool as a complement to the experimental techniques. Chemical processes are characterized by a transition state, a configuration with the lowest possible (free) energy that links the product(s) with the reactant(s). This state is normally not experimentally accessible, but there are theoretical methods to search for such structures. Consequently theory is a necessary complement to experiment. …”

Advanced Information for the Nobel Prize in Chemistry 2013[1-5]

Catalytic chemical reaction steps, e.g., proton transfer, phosphorylation, cleavage of protein polypeptide bonds, etc., play crucial roles in many biological systems.[6-24] Oftentimes in a chemical reaction, it is quite straightforward to find stable reactant and product states in thermal equilibrium. Yet, as stated in the Advanced Information for the Nobel Prize in Chemistry 2013,[1-5] every possible reaction path connecting the reactant and product states is actually characterized by its own rate-limiting transition state, which, by its intrinsic nature, is unsteady, and thus is very difficult to be accessible in experiment. Thereby, deducing the molecular structure (as well as the bonding nature) of the rate-limiting transition state is a key step to elucidate the enzymatic mechanism in biocatalysis.[1-24]

To better understand the properties of transition states, one common approach is investigating equilibrium and kinetic isotope effects (EIE and KIE) on a biochemical reaction, i.e., the so-called isotope labelling.[1, 6-20] The EIE is defined as the ratio of equilibrium constant of light isotope to that of heavy isotope:

\[
\text{EIE} = \frac{\text{Equilibrium constant (light isotope)}}{\text{Equilibrium constant (heavy isotope)}}
\] (1)

And the KIE is defined as the ratio of reaction rate of light isotope to that of heavy isotope:

\[
\text{KIE} = \frac{\text{Reaction Rate (light isotope)}}{\text{Reaction Rate (heavy isotope)}}
\] (2)

If a value of KIE is larger than unity, we then have a “normal KIE” because the reaction with light isotope is faster than that with heavy isotope (it is called “normal” because intuitively an object with lighter mass should “react” and move faster). Conversely, an “inverse KIE”
means a KIE value is smaller than unity, i.e., the reaction with light isotope is slower than that with heavy isotope. This reaction rate ratio, i.e., KIE, is very sensitive to the structure and bonding nature of the rate-limiting transition state. Hence, measuring KIE values has been considered as a (most) direct and robust probe of transition state (e.g., for its structure and bonding nature) in experiment.[6-20]

Nonetheless, merely having all these experimental KIE numerical values are still not enough for us to quantitatively determine the molecular structure of the rate-limiting transition state. On the other hand, the irreplaceable role of theory, computer simulations and visualization in Chemistry and Chemical Physics, which complements the shortcomings of experiment, has been at least recognized by the 1998 and 2013 Nobel Prizes in Chemistry.[1-5, 25, 26] Indeed, this complementary interplay can also be well demonstrated in the computer simulations of isotope effects.[15-20]

“... I would like to remind the audience that a very difficult problem in the field of molecular dynamics simulations of biomolecules is to have a way of checking that the results are correct. Experimental data (e.g. NMR measurements) that can be used for validation of the results are important but limited; i.e., they do not provide enough information for a quantitative test. Despite what the Nobel Prize press citation implies (“The computer is just as important as the test tube.”), experiments are essential to verify that what we are doing is meaningful.…”

Nobel Lecture by Martin Karplus for the Nobel Prize in Chemistry 2013[2]

For simulations, in contrast to experiment, in fact first we hypothesize a possible reaction path, for which an explicit molecular structure of the rate-limiting transition state needs to be already attained.[6-20] Afterwards, as the reminder underscored in the 2013 Nobel Lecture by Martin Karplus about the importance of verification for simulations,[2] we compute a set of isotope effect values and test whether or not our computed values associated with the inferred transition-state structure match with experimental results. If so, we then can declare that the reaction mechanism, along with the molecular structure and other properties of the rate-limiting transition state, are successfully and quantitatively identified and concluded in silico.[6-20]
Indeed, this kind of justifications for the rate-limiting transition state by using a set of experimental isotope effects values are more convincing than using an experimental reaction rate constant for validations. This is largely because contrary to a set of unique isotope effect values, practically-identical reaction rate constants can be shared with a wide variety of biochemical reactions that share neither the same reaction mechanisms nor the same transition states.

In this Review, first we will describe the underlying theories for isotope effects, including the classical and quantum origins of the isotope effects. The formulation and limitations of the well-known Bigeleisen equation[27-32] for computing isotope effects will then be briefly discussed. Next, we will talk about our recently-developed ab initio path-integral method, called automated integration-free path-integral (AIF-PI) method, which is in the framework of Feynman’s path integral and based on Kleinert’s variational perturbation (KP) theory, for having accurate EIE and KIE computations.[15-18, 33-36] Our ab initio path-integral method can be exactly reduced to the Bigeleisen equation in EIE and KIE calculations, and can also methodically go beyond this equation, e.g., by systematically including (non-parabolic) quantum tunneling effects, as well as anharmonic corrections to harmonic zero-point and vibrational energies. Further, in contrast to the commonly-used path-integral Monte Carlo (PIMC) [37-42] and molecular dynamics (PIMD) [43-45] simulations, calculated values using our ab initio path-integral method can be as precise as (though not as accurate as) the numerical precision of the computing machine. Illustrations of our ab initio path-integral method by some applications on reactions in solution and in protein enzymes, together with some other biologically relevant reactions, will follow.[15-20, 33-36] At the end, we will conclude this Review with some remarks on the difficulties in simulating the isotope effects on the RNA 2′-O-transphosphorylation reaction in various environments, ranging from the cases in acidic and alkaline solution to the case catalyzed by ribonuclease A (RNase A).
2. Theory and Method

The pioneers of studying isotope effects in Chemistry and Chemical Physics are at least represented by Urey, Eyring, Polanyi, de Hevesy, Van Vleck, and Bigeleisen, etc.[27-32, 46-61] For example, according to Ref. [[46]], Cremer and Polanyi, as well as Eyring and Sherman, independently predicted that protium and deuterium isotopes should not result in the same reaction rates owing to their difference (at least) in zero point energy.

The origin of isotope effects on a chemical reaction can be divided into two parts. One origin is from the classical world, whereas another origin is from the quantum world.

2.1 The Classical Origin of Isotope Effects

In order to show the classical origin of isotope effects, let us consider a simplest single-molecule one-dimensional system. For this simplest system, the molecule would like to overcome an asymmetric double-well potential-energy barrier (Figure 1) for forming a product from a reactant. According to the conventional classical transition state theory (TST),[62-74] the reaction rate constant $k_{\text{TST}}$ can be expressed in terms of the free energies or partition functions at the reactant and transition states:

$$k_{\text{TST}} = k_B T \frac{Q_T}{Q_R} = k_B T \frac{\exp(-\beta \Delta G^\ddagger)}{Q_R}$$

(3)

where $k_B$ is Boltzmann’s constant, $T$ is temperature, $\beta = 1/k_B T$, $h$ is Planck’s constant, $Q$ is the partition function, $\Delta G^\ddagger$ is the free energy barrier to overcome, the superscript $\ddagger$ and the subscript $R$ denote the transition state (a first-order saddle point in the free-energy surface) and reactant state, respectively. Applying Eq. (3) to this simplest single-molecule one-dimensional system, then we have:[75]

$$k_{\text{TST}} = k_B T \frac{\exp(-\beta \Delta V^\ddagger)}{Q_R}$$

(4)
where $\Delta V^\dagger$ is the potential energy barrier to overcome. The classical partition function $Q_R$ for this simplest system can be written as follows:

$$Q_{R,cl} = \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dp \frac{1}{\sqrt{2\pi \hbar^2}} \exp \left\{ -\beta \left[ \frac{p^2}{2m} + V(x) \right] \right\}$$

$$\Rightarrow Q_{R,cl} = \left( \frac{Mk_B T}{2\pi \hbar^2} \right)^{1/2} \int_{x=-\infty}^{x=\infty} dx \exp \left\{ -\beta V(x) \right\}$$

where $M$ is mass, $V$ is the potential energy, $\hbar$ is Planck’s constant divided by $2\pi$, $x$ and $p$ are the (reaction) coordinate and momentum, respectively.

![Asymmetric Double−Well](image)

Figure 1: Schematic diagram for a simple chemical reaction overcoming an asymmetric double-well potential-energy barrier to form a product from a reactant (black line). Conventionally, reactant is usually approximated as a harmonic potential (red dashed-dotted line) and the transition state could be approximated as an infinite parabola (blue dashed line).

Using Eq. (1), Eq. (2), and Eq. (4), the equilibrium isotope effect (EIE) and the classical kinetic isotope effect (KIE) of this simplest system (using classical TST) can be expressed as follows, respectively:
\[
\text{EIE} = \frac{Q_{P,l_0}/Q_{P,h_0}}{Q_{R,l_0}/Q_{R,h_0}}
\]

(6)

\[
\text{KIE}_{\text{TST}} = -\frac{\exp(-\beta \Delta V_{l_0}^1)}{Q_{R,h_0}} \Bigg/ \frac{\exp(-\beta \Delta V_{h_0}^1)}{Q_{R,h_0}}
\]

(7)

where the subscript \( P \) signifies the product state, \( l_0 \) indicates the light isotope, and \( h_0 \) is the heavy isotope. Due to the Born-Oppenheimer approximation [76-84] (which has been one of the most widely-used approximations in theoretical chemistry and theoretical chemical physics), often we do not consider the change of the potential energy w.r.t. the mass of isotope, i.e., we assume the following identity is valid:

\[
V_{P} = V_{h_0}
\]

(8)

Using Eq. (5) and Eq. (8), we can simplify Eq. (6) and Eq. (7) as follows, respectively:

\[
\text{EIE}_{cl} = \left( \frac{M_{h_0} / M_{l_0}}{\sqrt{M_{h_0} / M_{l_0}}} \right) \left\{ \int_{x=-\infty}^{x=\infty} dx \exp \left\{ -\beta V(x) \right\} \right\} / \left\{ \int_{x=-\infty}^{x=\infty} dx \exp \left\{ -\beta V(x) \right\} \right\}
\]

(9)

\[
\Rightarrow \text{EIE}_{cl} = 1
\]

\[
\text{KIE}_{\text{TST}} = \frac{Q_{R,h_0}}{Q_{R,l_0}} = \sqrt{M_{h_0} / M_{l_0}} > 1
\]

(10)

To be even more conventional, let us take the extensively-used harmonic approximation for explicitly expressing the \textit{classical} partition functions of the reactant and product states (Figure 1):

\[
Q_{R,cl} = \left( \frac{M k_B T}{2 \pi \hbar^2} \right) \left( \int_{x=-\infty}^{x=\infty} dx \exp \left\{ -\beta \frac{1}{2} M \Omega^2_{R,x^2} \right\} \right) = \frac{1}{\beta \hbar \Omega_R} = \frac{1}{\beta \hbar \sqrt{k_{f,R}/M}}
\]

(11)

\[
Q_{P,cl} = \left( \frac{M k_B T}{2 \pi \hbar^2} \right) \left( \int_{x=-\infty}^{x=\infty} dx \exp \left\{ -\beta \frac{1}{2} M \Omega^2_{P,x^2} \right\} \right) = \frac{1}{\beta \hbar \Omega_P} = \frac{1}{\beta \hbar \sqrt{k_{f,P}/M}}
\]

(12)

where \( \Omega \) is the real (harmonic) frequency and \( k_f \) is the force constant. Note that
according to Eq. (8), the force constant does not change with isotope. Using Eq. (8), Eq. (11) and Eq. (12), then Eq. (6) and Eq. (7) [or Eq. (9) and Eq. (10)] can be expressed as follows:

$$\text{EIE}_{cl} = \frac{\Omega_{p,b} / \Omega_{p,b}}{\Omega_{R,b} / \Omega_{R,b}} = \sqrt{\frac{M_{b}}{M_{b}}} = 1 \quad \Rightarrow \text{EIE}_{cl} = 1$$

$$\text{KIE}_{TST} = \frac{Q_{R,b}}{Q_{R,b}} = \frac{\Omega_{R,b}}{\Omega_{R,b}} = \sqrt{\frac{M_{b}}{M_{b}}} > 1$$

To conclude, using this simplest molecular system, without considering any quantum effects, as shown in Eq. (10) and Eq. (14), we have demonstrated that the classical KIE is always larger than unity, i.e., classical KIE is always normal. In other words, any inverse KIE is solely due to quantum effects. By contrast, as shown in Eq. (9) and Eq. (13), the classical EIE is always equal to unity, i.e., without quantum effects, there is no EIE at all. In other words, any (non-unity) EIE is purely originated from quantum effects.

2.2 The Bigeleisen Equation for quantum isotope effects

For the quantum origin of isotope effects, commonly it is further divided approximately into this duo: (i) zero-point energy (or more precisely, quantization of all vibrational energies) (Figure 2); (ii) quantum tunneling effects.

There are a number of methods proposed to estimate KIE values with quantum effects.[6-20, 27-36, 85-87] Among them, the Bigeleisen equation should be the most widely used one.[27-32] This is because in a simplest formulation, it can capture most of the essence of the (quantum) isotope effects, i.e., the zero-point motion and/or the quantization of vibrational energies. In the Bigeleisen equation, EIE and KIE are computed in the decoupled rigid-rotor harmonic-oscillator approximation,[88] all the quantum tunneling effect is completely neglected, and the Redlich-Teller product rule is satisfied:[27-32, 50]
\[ \text{EIE}_{BE} = \prod_{i=1}^{3N-6} \frac{\Omega_{P_{i},i_{h_b}}}{\sinh\left(\beta \hbar \Omega_{P_{i},i_{h_b}} / 2\right)} \]

\[ \prod_{i=1}^{3N-6} \frac{\Omega_{P_{i},i_{h_b}}}{\sinh\left(\beta \hbar \Omega_{P_{i},i_{h_b}} / 2\right)} \]

\[ \prod_{i=1}^{3N-6} \frac{\Omega_{R_{i},i_{h_b}}}{\sinh\left(\beta \hbar \Omega_{R_{i},i_{h_b}} / 2\right)} \]

\[ \text{KIE}_{BE} = \left( \frac{\omega^2_i}{\omega^2_0} \right) \prod_{i=1}^{3N-6} \frac{\Omega_{P_{i},i_{h_b}}}{\sinh\left(\beta \hbar \Omega_{P_{i},i_{h_b}} / 2\right)} \]

\[ \prod_{i=1}^{3N-6} \frac{\Omega_{P_{i},i_{h_b}}}{\sinh\left(\beta \hbar \Omega_{P_{i},i_{h_b}} / 2\right)} \]

\[ \prod_{i=1}^{3N-6} \frac{\Omega_{R_{i},i_{h_b}}}{\sinh\left(\beta \hbar \Omega_{R_{i},i_{h_b}} / 2\right)} \]

Figure 2: Scheme diagram illustrating how the zero-point energy can vary with the curvature of potential energy surface, leading to normal KIE at TS$_1$ and inverse KIE at TS$_2$. [17]
where $N$ is the number of atoms, $\omega^\dagger$ is the imaginary (harmonic) frequency at the transition state, and $i$ is the index running over all normal modes.

2.3 Modified Bigeleisen Equation in terms of Feynman’s Path Integral

In order to go beyond the harmonic approximation and to methodically include (non-parabolic) quantum tunneling effects in KIE computations, the Bigeleisen equations [Eq. (15) and Eq. (16)] can be refined in the framework of the Feynman centroid path integral as follows:[15-20, 33-36]

$$EIE_{pl} = \frac{\exp[-\beta(W_{p,h} - W_{p,b})]}{\exp[-\beta(W_{R,h} - W_{R,b})]}$$

(17)

$$KIE_{pl} = \left(\frac{\omega^\dagger}{\omega_h}\right) \frac{\exp[-\beta(W^i_h - W^i_{b})]}{\exp[-\beta(W_{R,h} - W_{R,b})]}$$

(18)

In Eq. (17) and Eq. (18), $W$ is the centroid effective potential energy calculated at the centroid position of path integrals.[33, 34, 43, 44, 89-96] For simplicity, here, we provide the definition of $W$ for a one-body one-dimensional system as follows:

$$W(x_0) = -k_BT \ln \left[ \frac{2\pi \hbar^2}{Mk_BT} \int D[x(\tau)] \delta(\bar{x} - x_0) \exp\left\{-\frac{A[x(\tau)]}{\hbar}\right\} \right],$$

(19)

where $\tau$ is imaginary time, $x(\tau)$ describes a path in space-time, $\int D[x(\tau)] \delta(\bar{x} - x_0)$ denotes a path integration: a summation over all possible closed paths in which $\bar{x}$ is equal to $x_0$ (i.e., a functional integration), and $\bar{x}$ is the time-average position, called ‘centroid’, equal to the nuclear position:[33, 34, 43, 44, 89-96]

$$\bar{x} = \frac{1}{\beta\hbar} \int_0^{\beta\hbar} x(\tau) d\tau.$$ 

(20)

In Eq. (19), $A$ is the quantum-statistical action:
\[ A \big[ x(\tau) \big] = \int_0^{\beta} d\tau \left\{ \frac{M}{2} \ddot{x}(\tau)^2 + V\big[x(\tau)\big] \right\}, \]  

(21)

where \( V(x) \) is the original potential energy function of the system. Generalization of Eq. (19) to a many-body three-dimensional system is straightforward.\[90, 91]\n
Figure 3: \( W_1, W_2, \) and \( W_3 \) are the first three orders of the KP theory for the centroid potential of an asymmetric double-well potential at 100 K. \( V \) is the original asymmetric double-well potential.\[35]\n
Note that the mass (isotope) and temperature dependent nature of the centroid potential energy \( W \) [Eq. (19) and (21)] distinguishes itself from the (\textit{ab initio}) Born-Oppenheimer potential energy. The latter is independent of mass and temperature. In short, for the region around the bottom of the potential well, \( W \) is usually raised mainly by zero-point energy such that its value is usually larger than \( V \) (Figure 3). On the other hand, for the neighborhood around the top of the potential barrier, \( W \) is usually lowered by the quantum tunneling effects such that its value is usually smaller than \( V \) (Figure 3).

We can also exactly reduce Eq. (17) and Eq. (18) back to the original Bigeleisen equation.
[i.e., Eq. (15) and Eq. (16)], respectively, when the centroid potential is computed in the decoupled rigid-rotor harmonic-oscillator approximation (and neglecting all tunneling effects; for a proof, see Section 2.2.3 in Ref. [[33]], or Appendix B in Ref. [[97]]). In addition, if we approximate the transition state as an infinite parabola (Figure 1), along with the decoupled rigid-rotor harmonic-oscillator approximation, then Eq. (18) exactly reduces back to the original Bigeleisen equation but multiplied by the exact factor accounting for the isotopic change of the parabolic tunneling transmission, i.e., Eq. (18) becomes (for a proof, see Section 2.2.3 in Ref. [[33]], or Appendix B in Ref. [[97]]):

$$KIE_{\text{PI(parabolic harmonic) }} = \left[ \frac{\omega^2_{1,\nu}}{\sin \left( \beta \hbar \omega_{1,\nu} / 2 \right)} \right] \left[ \frac{\omega^2_{1,\nu}}{\sin \left( \beta \hbar \omega_{1,\nu} / 2 \right)} \right] \prod_{i=1}^{3N-6} \Omega_{i,\nu}^{2} / \sinh \left( \beta \hbar \Omega_{i,\nu}^{2} / 2 \right)$$

In other words, in fact, Eq. (17) and Eq. (18) naturally include the anharmonic corrections to (i) parabolic tunneling effects, (ii) harmonic zero-point and (iii) vibrational energies.

In order for taking care of conformational contributions to the isotope effects, we can make use of the fact that the quantum partition function, $Q_{\text{qm}}$, can also be expressed as a classical configuration integral, in terms of the centroid potential $W$, without knowing any Schrödinger energy eigenvalues $E_n$ (where $E_0$ is the exact zero-point energy):[33, 34, 43, 44, 89-96]

$$Q_{\text{qm}} = \sum_n \exp \left( -\beta E_n \right) = \left( \frac{Mk_B T}{2\pi \hbar^2} \right) \left( \int dx_0 \exp \left\{ -\beta W(x_0) \right\} \right) = \left( \frac{Mk_B T}{2\pi \hbar^2} \right) \exp \left( -\beta \Delta G \right)$$

Eq. (23) is for a single-molecule one-dimensional system. Generalization of Eq. (23) to a many-body three-dimensional system is straightforward.[90, 91] Eq. (23) forms the basis of the so-called path-integral quantum transition-state theory (PI-QTST).[43, 44, 94]

According to PI-QTST, using the following two equations, we can include the conformational contributions to the EIE [Eq. (17)] and KIE [Eq. (18)] values, respectively:
\[
E_{\text{IE}_{\text{PI-QTST}}} = \frac{Q_{\text{qm}, P, h_0}}{Q_{\text{qm}, P, h_0}} / \frac{Q_{\text{qm}, R, h_0}}{Q_{\text{qm}, R, h_0}} \tag{24}
\]

\[
K_{\text{IE}_{\text{PI-QTST}}} = \frac{\exp\left(-\beta \Delta G_{\text{qm}, h_0}^{\dagger}\right)}{Q_{\text{qm}, R, h_0}} / \frac{\exp\left(-\beta \Delta G_{\text{qm}, h_0}^{\dagger}\right)}{Q_{\text{qm}, R, h_0}} \tag{25}
\]

2.4 Automated Integration-Free Path-Integral (AIF-PI) Method based on Kleinert’s Variational Perturbation (KP) Theory

Kleinert’s [90, 98-101] variational perturbation (KP) theory [33-36, 89, 90, 98, 102-106] for the centroid density (or centroid potential) [33, 34, 43, 44, 89-96] of Feynman path integrals [33, 34, 89-91, 107-117] provides a complete theoretical foundation for developing non-sampling/non-stochastic methods [118] to systematically (i.e., order by order) incorporate internuclear quantum-statistical effects [88] in condensed phase systems. Similar to the complementary interplay between the rapidly growing quantum Monte Carlo simulations [119-122] and the well-established \textit{ab initio} or density-functional theories (DFT) for electronic structure calculations [25, 26, 78-80, 123], non-sampling/non-stochastic path-integral methods can complement the conventional Fourier or discretized path-integral Monte-Carlo (PIMC) [37-42] and molecular dynamics (PIMD) [43-45] simulations which have been widely used in condensed phases.

For example, as opposed to estimating the error bars (or the precision) for simulations, one clear advantage for using a non-sampling/non-stochastic method is the calculated values can be as precise as (not as accurate as) the numerical precision of the computing machine. For example, we do not put an “error bar” for the numerical precision of a computed value of DFT energy; see Figure 4 and Ref. [[124]] for the depiction and discussion of accuracy vs. precision.

Kleinert and co-workers proved that his KP theory exhibits the uniformly and exponentially convergent property (in terms of accuracy) for several strong anharmonic-coupling
More importantly, this remarkably-fast convergent property can also be observed even for computing the electronic ground state energy of a hydrogen atom (3 degrees of freedom). The ground state energy was determined by calculating the electronic centroid potential at the zero-temperature limit. The accuracies of the first three orders of the KP theory for a hydrogen atom are 85%, 95%, and 98%, respectively.[90]

Figure 4: Schematic diagram showing the distinct concepts of accuracy and precision.[124]

For the illustration of this systematic and quickly-convergent manner (in terms of accuracy), in Figure 3, we show the first three orders of the KP theory for the centroid potential $W$ (i.e., $W_1$, $W_2$, and $W_3$) of an asymmetric double-well potential at 100 K. From Figure 3, we can clearly see that the difference between $W_2$ and $W_1$ is smaller than the difference between $W_1$ and $V$, and the difference between $W_3$ and $W_2$ is even smaller than the difference between $W_2$ and $W_1$, particularly true for the region around the bottom of the potential well.

In general, KP$n$ theory, i.e., the $n$th order of the KP theory, involves elaborate $n$-dimensional space-time ($2n$ degrees of freedom) smearing integrals. The intricacy of the smearing integrals increases tremendously for many-body potentials.[33, 34, 90, 105] This
complexity is a major factor limiting applications of the KP theory beyond KP1, the original Feynman-Kleinert variational approach.[125]

To render the KP theory feasible for many-body systems with $N$ particles, recently we have developed an automated integration-free path-integral (AIF-PI) method.[15-18, 33-36] In this AIF-PI method, we make use of the decoupled instantaneous normal coordinates approximation (DINCA). Although the DINCA approximation sacrifices some accuracy, in exchange, it allows analyses of quantum mechanical vibration and tunneling, and their separate contributions to the $W$. Furthermore, in order to obtain analytical expressions for the KPN theory, we use an $m$th order polynomial ($P_m$) to approximate or interpolate the potential along $q_i$. Hereafter, an $m$th order polynomial representation of the original potential energy function obtained with an interpolating step size $q \AA$ both in the forward and backward directions along the normal mode coordinate at $x_0$ is denoted as $P_m-q\AA$. In fact, after the interpolating potential along each instantaneous normal mode is determined, due to the integration-free feature, there is little computational cost for obtaining the $W_n$. Thereby, high level $ab\ initio$ or density-functional (DFT) methods can be used to evaluate the potential energy function for $ab\ initio$ path-integral calculations.

The computational procedure for obtaining the first and second order KP approximations to the centroid potential using our automated integration-free path-integral (AIF-PI) method is summarized below:[15-18, 33-36]

1. For each $\{x_0\}^N$, the mass-scaled Hessian matrix is diagonalized to obtain $\{q^{x_0}\}^N$.

2. The original potential $V$ is scanned from the configuration $\{x_0\}^N$ along each $q_{i,x_0}$ for 10 points both in the forward and backward directions. We found that a step size of 0.1 Å should usually be a reasonable choice to yield $W$ within a few percent of the exact.
3. After the P20-0.1A interpolations, each \( w_i, n_\Omega(q_{i, x_0}) \) as a function of the variational angular-frequency parameter \( \Omega \) is readily obtained using the analytical expressions of KP1/P20 or KP2/P20 (Supporting Material in Ref. [35, 36]). Note that the path integrals for these polynomials have been analytically integrated.

4. The values of \( w_i, n_\Omega(q_{i, x_0}) \) are determined by numerically locating the least dependence of \( w_i, n_\Omega(q_{i, x_0}) \) on \( \Omega \), i.e., zeroing the lowest order derivative of \( w_i, n_\Omega(q_{i, x_0}) \) w.r.t. \( \Omega \) (first derivative for KP1 and usually second derivative for KP2).

The procedure presented above is integration-free and essentially automated. We hope it could be used by non-path-integral experts or experimentalists as a “black-box” for any given system. We are currently developing a formalism to systematically couple instantaneous normal-mode coordinates.

Our AIF-PI method has been rigorously tested on modeling a variety of molecular systems in which exact quantum results are known. These systems include (i) H+H\(_2\) reaction, (ii) asymmetric double-well potential, (iii) bond vibrations of H\(_2\), HF, and HCl represented by the Morse potential, (iv) a water molecule, and (v) a proton-transfer barrier modeled by the Eckart potential.[35, 36] The zero-point energies, quantum partition functions, and tunneling factors for these systems have been determined and are found to be in excellent agreement with the exact quantum results. Some of these results are summarized in Table 1, Table 2, and Table 3, including comparison with path-integral Monte Carlo (PIMC) and molecular dynamics (PIMD) simulations, and other non-sampling/non-stochastic path-integral methods.
Table 1: Classical and quantum canonical partition functions and free energies of the asymmetric double-well potential at various temperatures (Figure 3). Signed percent errors (%) of different non-sampling methods relative to the accurate quantum results are given.[35]

<table>
<thead>
<tr>
<th>T (K)</th>
<th>Classical</th>
<th>Accurate quantum</th>
<th>KP1</th>
<th>KP2</th>
<th>KP3</th>
<th>Mielke-Truhlar†</th>
<th>Doll-Myers†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>4.03E-01</td>
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<td>0.1</td>
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</tr>
<tr>
<td>500</td>
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<td>7.09E-02</td>
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<td>0.4</td>
<td>0.0</td>
<td>0.7</td>
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<tr>
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<td>3.62E-02</td>
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<td>0.4</td>
<td>0.0</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
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<td>1.19E-02</td>
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<td>0.9</td>
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<tr>
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<td>-6.7</td>
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<table>
<thead>
<tr>
<th>T (K)</th>
<th>Classical</th>
<th>Accurate quantum</th>
<th>KP1</th>
<th>KP2</th>
<th>KP3</th>
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<tr>
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<td>-0.1</td>
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<tr>
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<td>2.641</td>
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<tr>
<td>50</td>
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<td>2.641</td>
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<td>--</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2.641</td>
<td>1.2</td>
<td>0.4</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

† Ref. [[118]].

Table 2: Kinetic isotope effects (KIE) on the protium and deuterium transfer over the symmetric Eckart potential at various temperatures.[35]

<table>
<thead>
<tr>
<th>T (K)</th>
<th>KIE (protium/deuterium)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Exact</td>
</tr>
<tr>
<td>500</td>
<td>1.232</td>
</tr>
<tr>
<td>400</td>
<td>1.374</td>
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<td>350</td>
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<td>300</td>
<td>1.756</td>
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<tr>
<td>250</td>
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<td>200</td>
<td>3.840</td>
</tr>
<tr>
<td>150</td>
<td>12.17</td>
</tr>
</tbody>
</table>

† Ref. [[126]].
‡ Using the same PIMC program in Ref. [[126]] to compute.
Table 3: Transmission coefficient $\kappa$ for the asymmetric Eckart barrier at various temperatures.[35]

<table>
<thead>
<tr>
<th>$\beta\omega^*$</th>
<th>$T$ (K)</th>
<th>Exact</th>
<th>KP1/P20-0.2A</th>
<th>KP2/P20-0.2A</th>
<th>PIMD†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>245</td>
<td>1.195</td>
<td>1.178</td>
<td>1.178</td>
<td>1.17</td>
</tr>
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<td>4</td>
<td>122</td>
<td>2.019</td>
<td>1.985</td>
<td>1.989</td>
<td>1.97</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>5.387</td>
<td>5.528</td>
<td>5.668</td>
<td>5.69</td>
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<td>8</td>
<td>61</td>
<td>27.27</td>
<td>31.55</td>
<td>35.39</td>
<td>36.6</td>
</tr>
</tbody>
</table>

† Ref. [[127]]

3. Applications

3.1 Models of Bacterial Squalene-to-Hopene Polycyclization

Due to the integration-free feature, our AIF-PI method is computationally efficient such that the potential energy can be evaluated using *ab initio* or density-functional theory (DFT) for performing the so-called *ab initio* path-integral calculations.

As a result, primary kinetic isotope effects (KIEs) on a series of carboxylic-acid catalyzed protonation reactions of aryl-substituted $\alpha$-methoxystyrenes to form oxocarbenium ions in solution (e.g., Figure 5) have been computed using the second-order Kleinert variational perturbation theory (KP2) in the framework of Feynman path integrals (PI) and the DFT-B3LYP/6-31+G(d,p) potential surface coupled with the polarizable continuum model (PCM) to represent aqueous solution.[18, 19] These reactions are relevant to biosynthesis of cholesterol because the bacterial squalene-to-hopene polycyclization is initiated by the rate-limiting proton transfer from an aspartic acid residue to the 2,3-alkene.[128-130]

To compute the anharmonicity contributions to the KIE, we quantized the following five nuclei: the donor oxygen, the transferring protium or deuterium, and the three atoms of the terminal CH$_2$ methylene group of substituted $\alpha$-methoxystyrenes. The entire system is quantized (more than 30 nuclei) to compute the Bigeleisen (harmonic) KIE values and to obtain the imaginary normal mode for computing the (non-parabolic) tunneling contribution to the KIE.
Figure 5: Experimental (black) and computed (red) kinetic isotope effects (KIE) for the reaction of chloroacetic acid and substituted \( \alpha \)-methoxystyrenes. KIEs computed using the Bigeleisen equation are given in green. Inclusion of anharmonicity contributions increases the computed KIEs, and is shown in blue. This is followed by inclusion of tunneling contributions to yield the total computed KIEs from path integrals determined with the Kleinert second-order perturbation theory (red). The inset is the transition-state structure for the reaction with \( R = 4\text{OMe} \).[18, 19]

Reasonable agreement between our calculated KIE values and the experimental data was obtained (e.g., Figure 5),[18, 19] demonstrating that this novel AIF-PI approach for computing KIEs of organic reactions is a viable alternative to go beyond the traditional method employing Bigeleisen equation, where anharmonicity and (non-parabolic) tunneling contributions are important. We found that the dominant factor contributing to the observed KIE for the protonation reactions of substituted \( \alpha \)-methoxystyrenes is due to the change in zero-point energy from the reactant to the transition state, and the trend of the computed KIEs using Bigeleisen equation is in accord with experiment. However, it is necessary to include tunneling contributions to obtain quantitative estimates of the KIEs. These tunneling
contributions are more important for the more balanced/symmetric transition states than reactions with late/more asymmetric/more Hammond-shifted (product-like) transition structures. Without tunneling, the rmsd error in the computed KIEs using Bigeleisen equation for a total of twelve reactions is 23% in comparison with the experimental data, and this is reduced to 15% with tunneling contributions. Consideration of anharmonicity can further improve the calculated KIEs; for example, for the protonation of substituted \( \alpha \)-methoxystyrenes by a common acid, chloroacetic acid, the anharmonicity corrected KIEs are in quantitative agreement with experiment, see Figure 5.[18, 19]

3.2 Models of RNA 2′-O-transphosphorylation

![Scheme 1](image)

Scheme 1. General reaction scheme for the (associative) reverse of dianionic in-line methanolysis of ethylene phosphate: a model for RNA phosphate transesterification under alkaline conditions. “React,” “TS1,” “Int.,” “TS2,” and “Prod.” stand for reactant, transition state 1, intermediate, transition state 2, and product, respectively. In the present work, the native reaction shown in the scheme is studied as well as reactions that incorporate single sulfur substitutions in the bridging 3′-(S3′) and leaving group 5′-positions (S5′). Note: as revealed by the computational analysis, not all of the states shown in the scheme exist for every reaction.[17]

In addition to proton-transfer reactions, in fact, our AIF-PI method can also be used to perform \textit{ab initio} path-integral calculations of some \textit{heavy-atom} isotopic substitutions. As a consequence, we used our AIF-PI method to compute KIE values on some models of RNA 2′-O-transphosphorylation, in which DFT-B3LYP/6-31+G(d) is used to construct the potential surface coupled with the polarizable continuum model (PCM) for the solvent effects.[17] Understanding the reaction mechanisms for RNA 2′-O-transphosphorylation can have applications in the design of new biotechnologies, and are also implicated in the evolutionary origins of life itself.[15-17, 20, 22, 131-148] Scheme 1 illustrates the general mechanism for the reverse, dianionic, in-line methanolysis of ethylene phosphate, which is a model for
Manuscript Review: Isotope Effects Simulations: from Bigeleisen Eq. to Feynman’s PI Won, Xu, Xu

base-catalyzed RNA phosphate transesterification.[15-17] The phosphoryl oxygen positions are labeled in accordance with their RNA counterparts.

The entire system is quantized (16 or 17 nuclei) to compute harmonic or Bigeleisen EIE and KIE values. Additionally, for estimating the anharmonic and (non-parabolic) tunneling effect corrections to the Bigeleisen EIE and KIE values, we further quantized the following six to seven nuclei using the AIF-PI method: the phosphorus nucleus and all the five (oxygen or sulfur) nuclei that can potentially form covalent bonds to the phosphorus nucleus, including the O2′ of nucleophile and O5′/S5′ of leaving groups, in addition to the hydrogen nucleus connected with the O2′ (i.e., the H of 2′-OH) for the case of anionic ethylene phosphate bonded with methanol group.[17]

The energy profiles for the native reaction of scheme 1, as well as for the reactions that the O3′ and O5′ oxygen atoms are respectively substituted with sulfur atom, are shown in Figure 6,[17] in which the solvation effects are treated implicitly with a polarizable continuum model (PCM).

![Figure 6. Density-functional adiabatic PCM profiles for the reaction with native, S3′, and S5′ compounds as a function of the difference in bond distance (Δbond) between the breaking P—X5′ bond (X is O for native and S3′; X is S for S5′) and the forming P—O2′ bond (Δbond = P—X5 − P—O2). The zero-reference energy for each profile is set at the energy of its own reactant state.[17]
For the native reaction, although there are distinct TS1 and TS2 transition states, they are separated by a shallow, transient intermediate. And the TS2 is rate limiting. For the reaction with the S3’ compound, the two transition states still exist, which are separated by a kinetically distinct intermediate. The calculated barriers for the two transition states are quite similar to one another (less than 1 kcal/mol difference). Both barriers are lower than the rate-limiting transition state of the reaction with the native compound by about 3 kcal/mol. This result indicates that the rate of reaction for the S3’ compound should be faster than the rate of reaction for the native compound, and is consistent with experimental measurements for 3’ thio-substituted dinucleotides. In addition, the sulfur substitution at the 5’-position results in a reaction profile that is unimodal, and the activation energy is lower than those for the native and S3’ compounds. In other words, this barrier suggests that this substitution reaction will have the fastest rate, and is consistent with experimental studies of 5’-substituted reaction models.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>TS1</th>
<th>TS2</th>
<th>Expt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(18k_{Nu})</td>
<td>(18,34k_{Lg})</td>
<td>(18k_{Nu})</td>
</tr>
<tr>
<td>Native</td>
<td>1.017</td>
<td>1.006</td>
<td>0.968</td>
</tr>
<tr>
<td>S3’</td>
<td>1.043</td>
<td>1.008</td>
<td>0.992</td>
</tr>
<tr>
<td>S5’</td>
<td>1.042</td>
<td>1.002</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Table 4: Calculated KIE values using our AIF-PI method on the 2’ nucleophile (\(18k_{Nu}\)) and the 5’ leaving group (\(18,34k_{Lg}\)) for TS1 and TS2, along with the most relevant available experimental results for comparison.\(^a\) Extracted from Ref. [144].\(^b\) Extracted from Ref. [149] in which the nucleophile O2’ is protonated in the reactant state. Values in bold represent the rate-limiting KIE values, as determined by comparison with experimental KIE measurements. Experimental errors in the last decimal place are given in parenthesis.[17]
(1.034) measured experimentally agree well with the KIE values calculated for TS2 (0.968 and 1.059; Table 4). In contrast to the rate-limiting TS2, the KIE value calculated for $^{18}k_{\text{Nu}}$ based on TS1 is normal (1.017), and the KIE value calculated for $^{18}k_{\text{Lg}}$ is close to unity (1.006). This finding supports the interpretation that the KIE values measured for the reaction with the native compound (dinucleotide UpG transphosphorylation) are consistent with a rate-limiting transition state that is characterized by an almost fully formed P—O2’ bond and an almost fully cleaved P—O5’ bond.[17]

Despite their similar barrier heights, TS1 and TS2 for the reaction with the S3’ compound produced significantly different primary KIE signatures (Table 4). The primary KIE values calculated for TS1 are large normal and close to unity for the nucleophile ($^{18}k_{\text{Nu}}$) and leaving group ($^{18}k_{\text{Lg}}$), respectively. In contrast, the primary KIE values calculated for TS2 are near unity and large normal for the nucleophile ($^{18}k_{\text{Nu}}$) and leaving group ($^{18}k_{\text{Lg}}$), respectively (Table 2). The experimental KIE values for the reaction with an m-nitrobenzyl leaving group (pK$_{a}$=14.9) are anomalously large normal ($^{18}k_{\text{Nu}}$ =1.119) and moderate normal ($^{18}k_{\text{Lg}}$ =1.012) for nucleophile and leaving group,[13] respectively. This is consistent with the signature of the TS1 transition state.[17]

For the reaction with the S5’ compound, the primary KIE values predicted for both $^{18}k_{\text{Nu}}$ (1.042) and $^{18}k_{\text{Lg}}$ (1.002) (Table 4) are large normal and close to unity, respectively, and are in agreement with the experimental results ($^{18}k_{\text{Nu}}$: 1.025; $^{18}k_{\text{Lg}}$: 1.001) for the cyclization of m-nitrobenzyl ribonucleoside phosphodiester with S5’ substitution. This agreement supports the notion that the rate-limiting transition state for the reaction with the S5’ compound is TS1.[17]

Lately, we have extended our calculations to interpret experimental KIE results measured for ribonuclease A (RNase A).[16] RNase A efficiently catalyzes RNA 2’-O-transphosphorylation with $\sim$10$^9$ rate enhancement and therefore provides a textbook
example of how biological catalysis is achieved. However, an accurate description of the transition state for this enzyme has not been definitely established, until recently.

![Image of transition state structures](image)

Figure 7: Transition state structures for specific base catalysis and RNase A calculated from quantum methods. (a) A non-enzymatic dianionic transition state model, and (b) an enzymatic transition state model based on the hydrogen bonding pattern in the active site of the RNase A TS mimic MD simulations. Experimental errors in the last decimal place are given in parenthesis.

In Figure 7, we show the transition state structures that we determined and match with the experimental KIE values. Results for the non-enzymatic reaction model were similar to our previous results using an ethylene glycol phosphate methyl ester. Here, we included a more realistic ribose-like sugar ring and ethoxide leaving group, and obtained KIE results in better agreement with the experimental data (Figure 7). Comparison of the calculated KIEs for the non-enzymatic QM model with the measured values for the solution reaction catalyzed by specific base (calculated/measured) reveal an inverse $^{18}k_{Nu}$ (0.973/0.984), a large normal $^{18}k_{LG}$ (1.046/1.037), and near unity $^{18}k_{NPO}$ (1.002/0.999). Importantly, the calculated $^{18}k_{LG}$ for the base-catalyzed reaction simulation (1.048) is notably larger than the calculated $^{18}k_{LG}$ for the enzymatic model (1.026), consistent with the observed experimental trend (1.034...
versus 1.017). Overall, the calculated and measured KIE values are in good agreement, particularly for the enzymatic model (calculated/measured): $^{18}k_{Nuc}$ (0.998/0.992), $^{18}k_{Lg}$ (1.026/1.017) and $^{18}k_{NPO}$ (1.006/1.002) (Figure 7). Although this agreement is impressive, the model calculations tend to slightly exaggerate the magnitude of the $^{18}k_{Lg}$ KIEs. Nonetheless, the relative magnitudes of the calculated non-enzymatic and enzymatic $^{18}k_{Lg}$ values are very similar to the corresponding relative magnitude of the RNase A and dinucleotide UpG $^{18}k_{Lg}$ values. It is clear that in the simulations the P--O5′ bond length is considerably shorter for the RNase A transition state than that for the base-catalyzed reaction (Figure 7) and it retains a higher degree of covalent bond character. Moreover, the proton transfer from the general acid (His119) further creates a “stiffer” bonding environment for stabilizing the leaving group.[16]

Moreover, we recently have compared results from a total of eight levels of \textit{ab initio} calculations for evaluating the potential energy surfaces in KIE and EIE computations of the RNA 2′-O-transphosphorylation, including a “goldstandard” coupled-cluster level of theory CCSD(T).[15] To go beyond the Bigeleisen equation, anharmonicity and (non-parabolic) quantum tunneling effects were also computed using our \textit{ab initio} path-integral, AIF-PI, method. By comparing the KIE and EIE values in the gas phase with those in the solution phase, surprisingly even though the solvent effect considerably decreases the energy barriers by ~10–29 kcal/mol, the fundamental isotope-effect values are not that sensitive to the change of the gas to the solution environment. Further, EIE and KIE values computed at the computationally-economical HF/3-21+G* level are reasonably accurate, which suggests that dual-level (on-the-fly) \textit{ab initio} QM/MM free energy and isotope effects calculations on more realistic biomolecular systems associated with the RNA 2′-O-transphosphorylation should be practical and at least semi-quantitatively accurate.[24, 34, 150] At last, although in general, anharmonicity and tunneling effects are not critically important to both EIE and KIE on our models for the base-catalyzed RNA 2′-O-transphosphorylation, anharmonic contribution to
the EIE on the 2′-OH deprotonation should be included, e.g., for the native, EIE increases from 1.0218 to 1.0268 in Table 5.[15]

<table>
<thead>
<tr>
<th>Native (37°C)</th>
<th>Ab Initio Path-Integral Calculations</th>
<th>Equilibrium Isotope Effects in Solution</th>
<th>Nucleophile (2′-OH) deprotonation</th>
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<tbody>
<tr>
<td>Electronic Struct. Theory</td>
<td></td>
<td></td>
<td>$^{18}E_{\text{Nuc}}$</td>
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<tr>
<td>MP2/6-311+G(d,p)</td>
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<td></td>
<td>Full Harmonic</td>
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<td></td>
<td></td>
<td>Partial Harmonic</td>
</tr>
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<td></td>
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<td>Partial KP1/P20</td>
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<td>Full Harmonic × Partial(KP2/Harmonic)</td>
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(A)

<table>
<thead>
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<th>S3′ (37°C)</th>
<th>Ab Initio Path-Integral Calculations</th>
<th>Equilibrium Isotope Effects in Solution</th>
<th>Nucleophile (2′-OH) deprotonation</th>
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<tbody>
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<td>Electronic Struct. Theory</td>
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<td>$^{18}E_{\text{Nuc}}$</td>
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<td>MP2/6-311+G(d,p)</td>
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<td></td>
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<td>Partial KP1/P20</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Partial KP2/P20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Full Harmonic × Partial(KP1/Harmonic)</td>
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<td></td>
<td></td>
<td></td>
<td>Full Harmonic × Partial(KP2/Harmonic)</td>
</tr>
</tbody>
</table>

(B)

<table>
<thead>
<tr>
<th>S5′ (37°C)</th>
<th>Ab Initio Path-Integral Calculations</th>
<th>Equilibrium Isotope Effects in Solution</th>
<th>Nucleophile (2′-OH) deprotonation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic Struct. Theory</td>
<td></td>
<td></td>
<td>$^{18}E_{\text{Nuc}}$</td>
</tr>
<tr>
<td>MP2/6-311+G(d,p)</td>
<td></td>
<td></td>
<td>Full Harmonic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Partial Harmonic</td>
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<td></td>
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<td>Partial KP1/P20</td>
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<td>Full Harmonic × Partial(KP1/Harmonic)</td>
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<td>Full Harmonic × Partial(KP2/Harmonic)</td>
</tr>
</tbody>
</table>

(C)

Table 5. Ab initio path-integral calculations of equilibrium isotope effects on nucleophile (2′-OH) deprotonation of the (A) native, (B) S3′, and (C) S5′ simplest models of RNA transphosphorylation in solution (Scheme 1). “Full Harmonic” is the value computed from the Bigeleisen equation, in which the entire system is quantized. “Partial” means only six or seven atoms are further quantized to compute the anharmonicity, which is excluded in the Bigeleisen equation.[15]
Table 6: Comparison of KIE results from the five transition-state models with experiment.

<table>
<thead>
<tr>
<th>QM Non-Enzymatic model</th>
<th>$^{18}k_{LG}$</th>
<th>$^{18}k_{NUC}$</th>
<th>$^{18}k_{NPO}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1stNonEnzyme</td>
<td>1.059</td>
<td>0.968</td>
<td>1.004</td>
</tr>
<tr>
<td>2ndNonEnzyme</td>
<td>1.046</td>
<td>0.990</td>
<td>1.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>QM Enzymatic model</th>
<th>$^{18}k_{LG}$</th>
<th>$^{18}k_{NUC}$</th>
<th>$^{18}k_{NPO}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1stEnzyme</td>
<td>1.010</td>
<td>0.988</td>
<td>0.995</td>
</tr>
<tr>
<td>2ndEnzyme</td>
<td>1.038</td>
<td>0.997</td>
<td>1.006</td>
</tr>
<tr>
<td>3rdEnzyme</td>
<td>1.037</td>
<td>0.995</td>
<td>1.004</td>
</tr>
</tbody>
</table>

| RNase A Expt.          | **1.014**    | **0.994**      | **1.001**     |

4. Difficulties in Simulating Isotope Effects with Computers

We have mentioned or implied in the section of Introduction that even if we are able to perform isotope-effect calculations at a very high level of theory, it still does not guarantee...
our calculated values would be in agreement with experiment. This is largely because the isotope effects of transition states that we conjecture could be quite easily not corresponding to the actual rate-limiting transition states in experiment.

For example, during the simulations of the RNA 2′-O-transphosphorylation in alkaline and enzymatic environment, we actually have tried a total of five models: two non-enzymatic models and three non-enzymatic models.[16, 17] The transition states of these five models are depicted in Figure 8, while their KIE values are listed in Table 6. The results with these five model systems were used to guide the model shown in Figure 7.

The first non-enzymatic model (denoted as: 1stNonEnzyme) is an ethylene glycol phosphate methyl ester (Figure 8) in alkaline conditions. In short, the calculated KIE results for this model are consistent with those experimental results for the base-catalyzed 2′-O-transphosphorylation of the RNA dinucleotide 5′-UpG-3′.[17] In the second non-enzymatic model (denoted as: 2ndNonEnzyme), we add a proton at O2′ in the reactant state. This model was constructed to evaluate the potential for a proton transfer first from O2′ to the NPO, and then from the O1P position to the 5′O leaving group in the rate-limiting transition state of the solution reaction catalyzed by specific acid (Figure 8). From Table 6, in comparison with our first non-enzymatic model, indeed due to the concomitant proton transfer, the value of $^{18}k_{LG}$ drops from 1.059 to 1.046. But again, the lack of a large thio effect on the RNase A reaction and the relatively smaller normal value of $^{18}k_{LG}$ we observe both argue against complete proton transfer from an NPO position to the 5′O leaving group in the RNase A reaction.

Consequently, based on our second non-enzymatic model mentioned above, which has a proton at O2′ in the reactant state, we started to build our first enzymatic model (denoted as: 1stEnzyme) by just adding a protonated imidazole. This protonated imidazole is hydrogen-bonded with O5′ in the reactant state, and transfers a proton to O5′ in the transition
state. This model was constructed to probe the possibility of a proton transfer from O2’ to the NPO, and then there is another proton transfer from the protonated imidazole (instead of from the NPO) to the 5’O leaving group. In other words, the protonated imidazole is the general acid, while the proton at O1P does not move in the rate-limiting transition state of our first enzymatic model (Figure 8). As seen in Table 6, in contrast to our second non-enzymatic model, the value of $^{18}k_{LG}$ significantly drops from 1.046 to 1.010. This indicates substantial offset of the magnitude of this KIE by concomitant proton transfer in the transition state of which the protonated imidazole acts as the general acid and O1P stays protonated. However, this time the calculated value of $^{18}k_{NPO}$ is small inverse (Table 6), contradicting the experimental small normal values. As a result, again, the lack of a large thio effect on the RNase A reaction and the small normal value of $^{18}k_{NPO}$ we observe both argue against there is a proton at an NPO position in the rate-limiting transition state in the RNase A reaction.

Since we conclude that there should be no proton at an NPO position in the rate-limiting transition state, we built the second and the third enzymatic models (denoted as: 2ndEynzme and 3rdEnzyme respectively) by removing the proton at the O1P position in the transition state (Figure 8). In addition, in our third enzymatic model, we put another protonated imidazole ring, which is hydrogen bonded with O1P in the transition state (Figure 8). Initially, we had a hard time locating a late transition state for our second enzymatic model because the potential energy surface is quite flat. We did not encounter such difficulties for our third enzymatic model. Furthermore, it turns out that our third enzymatic model most closely approximates the observed RNase A KIEs (Table 6), which show a normal value for $^{18}k_{LG}$, near inverse unity for $^{18}k_{NUC}$, and near normal unity for $^{18}k_{NPO}$. Therefore, by extending our third enzymatic model, the enzymatic model presented in Figure 7 was built with having two protonated imidazoles. This model supports a scenario that His12 (or other
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candidates) accepts a proton from O2′, Lys41 (or His12, or other candidates) H-bonded with NPO, and His119 (or other candidates) donates the proton to the leaving O5′.

In contrast to the alkaline condition, we anticipate that (much) more challenging issues would appear for simulating the RNA 2′-O-transphosphorylation in acidic solution, and the mutual isomerization between the nucleoside 2′-phosphate and 3′-phosphate.[139] For example, how much O1P and O2P should be protonated, and whether or not the pre-equilibrium step of 2′-OH deprotonation still exists. Indeed, we are presently studying a variety of theoretical models about it. Hopefully, we would be able to identify a hypothetical rate-limiting transition state, along with its reaction mechanism, for which the isotope effect values are in good agreement with experimental results.

5. Concluding Remarks

In this Review, we have shown that without including any quantum effects, the classical KIE is always larger than unity, while the classical EIE is always equal to unity. Although the popular Bigeleisen equation captures most of the important quantum origins of isotope effects, this equation is in the decoupled rigid-rotor harmonic-oscillator approximation, and no quantum tunneling effect is considered at all. In order to systematically incorporate the (non-parabolic) tunneling contributions to the isotope effects as well as the anharmonic corrections to the harmonic zero-point and vibrational energies, we have developed a new ab initio path integral method. We call it automated integration-free path-integral (AIF-PI) method, which is based on Kleinert’s variational perturbation theory. Owing to the integration-free feature of our method, using AIF-PI, we were able to carry out ab initio path-integral calculations of isotope effects on a number of biochemical systems. Contrary to the widely-used path-integral Monte Carlo (PIMC) and molecular dynamics (PIMD) simulations, values calculated from our AIF-PI path-integral method can be as precise as (not as accurate as) the numerical precision of the computing machine. Our calculated KIE
results are in good agreement with experiment that help us to elucidate the enzymatic mechanisms underlying the bacterial squalene-to-hopene polycyclization and the RNA 2′-O-transphosphorylation. As illustrated by our cases in simulating the RNA 2′-O-transphosphorylation under various conditions, in order to computationally capture the correct transition state that has KIE values in parallel with experimental results, it is a common practice that we would need to thoroughly investigate (much) more than one theoretical model with the help of computer.

Acknowledgements

This work has been supported in part to K.-Y. Wong by HK RGC (ECS-209813), NSF of China (NSFC-21303151), HKBU FRG (FRG2/12-13/037, FRG2/13-14/075) and startup funds (38-40-088 and 40-49-495). The computing resources for our work summarized in this Review were supported in part by Minnesota Supercomputing Institute, and HKBU High Performance Cluster Computing Centre (for sciblade; supported by HK RGC) and Office of Information Technology (for jiraiya).
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Highlights

- The origins of the inverse KIE and all EIE on reactions are purely from quantum.
- Our AIF-PI path-integral method systematically goes beyond the Bigeleisen equation.
- Our AIF-PI method systematically incorporates anharmonicity and tunneling.
- Values calculated from our AIF-PI method are more precise than PIMC & PIMD.
- Biochemical applications on series of proton-transfer and RNA reactions are given.