

Kinetic Isotope Effects from QM/MM Subset Hessians: “Cut-Off” Analysis for S_N2 Methyl Transfer in Solution

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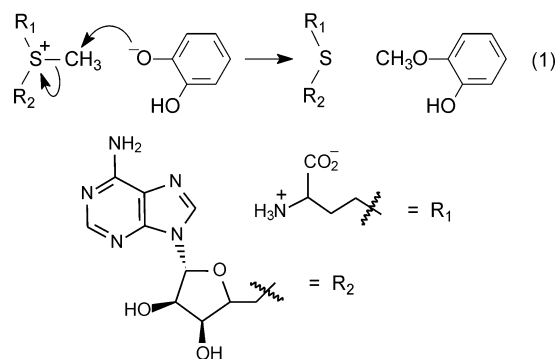
ABSTRACT: Isotopic partition-function ratios and kinetic isotope effects for reaction of *S*-adenosylmethionine with catechol in water are evaluated using a subset of 324 atoms within its surrounding aqueous environment at the AM1/TIP3P level. Two alternative methods for treating motion in the six librational degrees of freedom of the subset atoms relative to their environment are compared. A series of successively smaller subset Hessians are generated by cumulative deletion of rows and columns from the initial 972×972 Hessian. We find that it is better to treat these librations as vibrations than as translations and rotations and that there is no need to invoke the Teller–Redlich product rule. The validity of “cut-off” procedures for computation of isotope effects with truncated atomic subsets is assessed: to ensure errors in $\ln(\text{KIE}) < 1\%$ (or 2% for the quantum-corrected KIE) for all isotopic substitutions considered, it is necessary to use a less-restrictive procedure than is suggested by the familiar two-bond cutoff rule.

INTRODUCTION

One of the most powerful experimental techniques for probing the nature of chemical reaction mechanism is the measurement of a kinetic isotope effect (KIE), a rate constant ratio that arises from isotopic substitution at a particular position in a molecule. The magnitude and direction of a KIE contains information about the mechanistic events in a chemical reaction, reflecting differences in bonding between the reactants and the transition state.¹ Hybrid quantum mechanical/molecular mechanical (QM/MM) methods are now commonly used for calculations of KIEs for reactions occurring in solution^{2,3} or within enzyme active sites.^{4,5} Systems as large as many thousands of atoms are nowadays treated in geometry optimizations and molecular dynamics simulations, but usually only a subset of atoms is considered in KIE calculations,^{3,5} for which vibrational frequencies are computed by means of a subset Hessian. The purpose of this paper is to perform a critical analysis of the methods used to obtain KIEs from subset Hessians.

In the early days of KIE calculations, a standard procedure to simplify the computations was to employ the cutoff rule first introduced by Stern and Wolfsberg.⁶ They had noted that it was possible to leave out parts of a “large” molecule without significantly affecting the value of a calculated KIE, provided that (a) it was around room temperature, (b) the omitted atoms were more than two bonds distant from the position of isotopic substitution where force constants changed from the reactant structure to the transition structure, and (c) the force constants for that portion of the molecule retained were correct (i.e., the same as they would be in the whole molecule without the cutoff).⁷ Melander and Saunders discussed the practical usefulness of this simplification, as a means by which to reduce the cost of KIE calculations, but added a wise precautionary note that the cutoff procedure would be valid only if it were justifiable to ignore the influence of medium effects on the isotope effect.⁸ Procedures⁹ employing the cutoff rule have been used extensively in enzymology, where KIE calculations involving whole enzyme–substrate complexes are not tractable.

A cutoff procedure is invoked implicitly whenever a subset Hessian is used, and it is timely now to enquire about the validity of this approach. Whereas the original cutoff rule was formulated for molecules in which the omitted atoms were covalently linked to the retained atoms, the systems now typically studied by QM/MM methods involve noncovalent solvent–solute or enzyme–substrate interactions: What form of cutoff rule is appropriate for such systems? We considered this question a few years ago in the context of a study of the chorismate–prephenate rearrangement in water and in the active site of chorismate mutase,¹⁰ but now we present the results of a more comprehensive analysis for S_N2 methyl transfer (eq 1) from *S*-adenosylmethionine (AdoMet) to catechol in aqueous solution. In addition to the question of how many atoms should be included in the subset Hessian, we also assess two different procedures for evaluating the KIE in terms of isotopic partition-function ratios (IPFRs) determined for reactant-state (RS) and transition-state (TS) structures of subsets. Although subset (or partial) Hessians have been discussed by others (see below), a thorough and critical analysis in their use in isotope effect has previously been lacking.



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THEORY

KIEs and IPFRs for Whole Molecules with Separable Translations and Rotations. The conventional TS theory treatment of KIEs with the Born–Oppenheimer, rigid-rotor and harmonic oscillator approximations (and neglecting tunnelling and transmission-factor contributions) considers the rate-constant ratio k/k' (where the prime denotes the rate constant for the heavier isotopologue) as the product of three factors (eq 2) involving translational, rotational, and vibrational partition functions for the isotopologous RS and TS species.^{7,8} The first is the mass and moments-of-inertia factor (MMI), where $|I|$ is the determinant of the moment-of-inertia tensor (i.e., the product of the three principal moments of inertia) of a nonlinear N -atomic molecule of molecular mass M ; rotational symmetry factors are omitted here since they are all equal to unity for the applications to be presented below. The second is the Boltzmann excitational (EXC) factor corresponding to the relative populations of the higher vibrational quantum states, and the third is the zero-point energy (ZPE) factor. EXC involves a product, and ZPE a summation, over $3N - 6$ and $3N - 7$ real vibrational frequencies of RS and TS, respectively, where $u = hc\nu/k_B T$ with frequency (s^{-1}) replaced by wavenumber ν (cm^{-1}) and h , k_B , c , and T are the Planck and Boltzmann constants, the velocity of light, and the absolute temperature. Inspection of the forms of the MMI, EXC, and ZPE factors shows that the KIE may be rewritten simply as a quotient of IPFRs for RS and TS (eq 3):

$$k/k' = \frac{\left[\frac{|I'|}{|I|} \right]_{RS}^{1/2} \left[\frac{M'}{M} \right]_{RS}^{3/2} \prod_i^{3N-6} \left[\frac{1 - \exp(-u_i)}{1 - \exp(-u'_i)} \frac{\exp(u_i/2)}{\exp(u'_i/2)} \right]_{RS}}{\left[\frac{|I'|}{|I|} \right]_{TS}^{1/2} \left[\frac{M'}{M} \right]_{TS}^{3/2} \prod_i^{3N-7} \left[\frac{1 - \exp(-u_i)}{1 - \exp(-u'_i)} \frac{\exp(u_i/2)}{\exp(u'_i/2)} \right]_{TS}} \quad (2)$$

$$KIE = \underbrace{\text{MMI}}_{\text{MMI}} \times \underbrace{\text{EXC}}_{\text{EXC}} \times \underbrace{\text{ZPE}}_{\text{ZPE}} \quad (3)$$

It is common to replace MMI in eq 2 by an equivalent factor containing only vibrational frequencies. According to the Teller–Redlich product rule,¹¹ the masses and moments of inertia for a pair of isotopologues are related to the vibrational frequencies: The equality expressed by eq 4 assumes separability of translational and rotational motions from vibrational motions within the harmonic approximation. Substituting the vibrational product (VP) for each of RS and TS in eq 2, the KIE may be written as eq 5, the Bigeleisen equation, where VPR is the vibrational product ratio.^{6,12} Two points should be noted: (i) The product of ratios of atomic masses m that appears eq 4 vanishes from the KIE because it is identical for both RS and TS; and (ii) it is conventional to consider the ratio of imaginary transition frequencies for the TS as a separate factor, so that (in eqs 5 and 6) the VPR and EXC products and the ZPE summation are all taken over $3N - 7$ real TS frequencies.

$$\begin{aligned} \prod_i^{3N-6} \frac{\nu'_i}{\nu_i} &= VP \\ &= MI \\ &= \left[\frac{|I'|}{|I|} \right]^{1/2} \left[\frac{M'}{M} \prod_j^N \frac{m_j}{m'_j} \right]^{3/2} \end{aligned} \quad (4)$$

$$k/k' = (\nu_{\ddagger}'/\nu_{\ddagger}) \times VPR \times EXC \times ZPE \quad (5)$$

$$VPR = \prod_i^{3N-6} \left[\frac{\nu'_i}{\nu_i} \right]_{RS} / \prod_i^{3N-7} \left[\frac{\nu'_i}{\nu_i} \right]_{TS} \quad (6)$$

Use of the Bigeleisen equation enables the determination of KIEs from normal-mode frequencies without the need to consider the MMI factor explicitly.¹³ However, some time ago I pointed out¹⁴ that there was some practical merit in separately determining the VP and mass moment-of-inertia terms for a pair of isotopologues, rather than relying upon their equality, particularly when the vibrational frequencies had been obtained using Hessians in Cartesian coordinates from standard packages for ab initio or semiempirical molecular orbital calculations. Older implementations involving numerical second derivatives tended to produce Hessians which, upon mass weighting and diagonalization,¹⁵ yielded eigenvectors corresponding to translation and rotation of the whole molecule which did not have zero eigenvalues. This meant that the Teller–Redlich product rule was not exactly satisfied and consequently that application of the Bigeleisen equation incurred some error.

KIEs and IPFRs for Subsets. The relationships outlined above assume that each molecular system, either RS or TS, corresponds to a zero-gradient stationary point on a potential energy surface. Reliable optimized geometries and Hessians are readily available from modern quantum chemical packages employing second-derivative methods, provided that convergence thresholds are set suitably tightly. However, during the past 15 years it has become common to perform simulations for very large molecular systems (e.g., molecules in solution or within enzyme active sites) but to compute explicit Hessians for only a subset of the total number of atoms in the system. For example, relaxation of a specified subset of atoms to a local minimum (or saddle point) may be performed within a frozen environment of the remaining atoms. In the context of QM/MM methods, the mobile subset and the frozen environment may be the same as the QM and MM regions, although different selections may also be made. In these circumstances the N_s subset atoms do not in themselves constitute a stationary structure in which vibrational degrees of freedom are separable from translations and rotation. The constraining influence of the environment means that diagonalization of the mass-weighted $3N_s \times 3N_s$ Hessian in Cartesian coordinates generally yields $3N_s$ nonzero eigenvalues which include six corresponding to libration of the whole subset with respect to its environment: “translation” and “rotation” of the subset as a whole are not free or separable motions but are coupled with the internal vibrational degrees of freedom.

An approximate way to calculate KIEs and IPFRs for a subset of N_s atoms (within a larger environment of $N - N_s$ atoms) is to project out the six librational degrees of freedom and to treat them instead as translations and rotations, as we have previously described.^{10,14} In practice, the $3N_s \times 3N_s$ Hessian in Cartesian coordinates is first transformed into a $(3N_s - 6) \times (3N_s - 6)$ Hessian in a set of internal coordinates and then back transformed, whereupon $3N_s - 6$ pure vibrational eigenvalues and 6 zero eigenvalues for translational and rotational motion are obtained. This procedure is equivalent to treating the subset as if it were a freely translating and freely rotating cluster isolated from its environment, but whose internal vibrations are governed by force constants whose values are determined by the influence of the environment. The

Table 1. IPFRs, KIEs, and Contributing Factors at 298.15 K Evaluated Using the Largest AM1/TIP3P Subset Hessian Corresponding to 324 Atoms

isotopic substitution		all frequencies method (eqs 12 and 13)				translation/rotation method with products taken (eqs 7 and 8)						% error in $\ln(f)$ or \ln KIE
		VP	EX	ZP	$(f)_{af}$	VP	TR	MI	EX	ZP	$(f)_{tr}$	
α -D ₃	RS	0.044347	1.722473	21938.08	1675.782	0.044452	1.000000	0.044452	1.717391	21966.50	1676.968	0.01
	TS	0.044347	1.542157	29289.28	2003.105	0.044450	0.999936	0.044453	1.537843	29325.92	2004.779	0.01
	KIE	1	1.116925	0.749014	0.836592			0.999982	1.116753	0.749047	0.836485	−0.07
α - ¹³ C	RS	0.886520	1.049739	1.196608	1.113582	0.887196	1.000000	0.887196	1.048837	1.196748	1.113603	0.02
	TS	0.886520	1.038529	1.138539	1.048227	0.887190	0.999982	0.887205	1.037667	1.138663	1.048280	0.11
	KIE	1	1.010795	1.051003	1.062348			0.999990	1.010765	1.051011	1.062314	−0.05
³⁴ S _{lg}	RS	0.913174	1.044557	1.081951	1.032033	0.914565	1.000000	0.914565	1.042908	1.082018	1.032037	0.01
	TS	0.913174	1.045113	1.076553	1.027430	0.914581	1.000030	0.914554	1.043437	1.076623	1.027398	−0.11
	KIE	1	0.999468	1.005015	1.004480			1.000012	0.999493	1.005012	1.004515	0.77
¹⁸ O _{nu}	RS	0.838239	1.079712	1.242839	1.124840	0.839791	1.000000	0.839791	1.077759	1.242803	1.124851	0.01
	TS	0.838239	1.068512	1.248618	1.118348	0.840227	1.000576	0.839743	1.066168	1.248390	1.117693	−0.52
	KIE	1	1.010482	0.995372	1.005805			1.000056	1.010872	0.995524	1.006404	10.3

subset KIEs and IPFRs may then be determined by replacing N by N_s in eq 2 and by using the equality in eq 4, leading to eqs 7 and 8 with pure vibrational frequencies; we call this the “translation/rotation” method. The MI, EX, and ZP factors are labeled to assist interpretation of Tables 1 and 4.

$$(f_{RS})_{tr} = \underbrace{\left[\frac{|I'|}{|I|} \right]_{RS}^{1/2} \left[\frac{M'}{M} \prod_j \frac{m_j}{m_j'} \right]_{RS}^{1/2}}_{\text{MI}} \underbrace{\prod_i \left[\frac{1 - \exp(-u_i)}{1 - \exp(-u_i')} \right]}_{\text{EX}} \underbrace{\prod_i \left[\frac{\exp(u_i/2)}{\exp(u_i'/2)} \right]}_{\text{ZP}} \quad (7)$$

$$(f_{TS})_{tr} = \underbrace{\left[\frac{|I'|}{|I|} \right]_{TS}^{1/2} \left[\frac{M'}{M} \prod_j \frac{m_j}{m_j'} \right]_{TS}^{1/2}}_{\text{MI}} \underbrace{\prod_i \left[\frac{1 - \exp(-u_i)}{1 - \exp(-u_i')} \right]}_{\text{EX}} \underbrace{\prod_i \left[\frac{\exp(u_i/2)}{\exp(u_i'/2)} \right]}_{\text{ZP}} \quad (8)$$

Is there a form, analogous to the Bigeleisen equation, that expresses the KIE and IPFRs in terms only of vibrational frequencies for a subset? The product of the $3N_s$ eigenvalues of the vibrational secular matrix is equal (eq 9) to the product of determinants of the Wilson **F** and **G** matrices for potential and kinetic energies,¹⁶ respectively, which in turn is equal to the product of determinants of the potential energy matrix **V** and kinetic energy matrix **T** in mass-weighted Cartesian coordinates.¹⁵ There is a problem when there are zero eigenvalues, as for translation and rotation, since then eq 9 is not valid; the solution is the Teller–Redlich product rule for molecules whose $3N$ degrees of freedom are separable into 3 translations, 3 rotations, and $3N - 6$ vibrations.

$$\prod_i^{3N} \lambda_i = |\mathbf{F}||\mathbf{G}| = |\mathbf{V}||\mathbf{T}| \quad (9)$$

However, using the Cartesian formulation of the vibrational problem, it is easy to note that since the frequencies are proportional to the square roots of the vibrational eigenvalues, and **T** is the unit matrix, the product of the ratios of frequencies for isotopologues with the same Hessian (within the Born–Oppenheimer approximation) reduces simply (eq 10) to the ratio of products of atomic masses used to mass weight the Hessian prior to diagonalization. Furthermore, the atomic masses are the same for both isotopologues, except for the isotopically substituted atom(s) alone.

$$\prod_i^{3N} \frac{\nu_i'}{\nu_i} = \left[\frac{|\mathbf{V}'|}{|\mathbf{V}|} \right]^{1/2} = \left[\prod_j^N \frac{m_j}{m_j'} \right]^{3/2} \quad (10)$$

With a subset Hessian there are, in general, $3N_s$ nonzero vibrational frequencies, and so there is no difficulty in evaluating the product on the left-hand side of eq 10 over $3N_s$ ratios ν_i'/ν_i for an isotopologous pair. Thus there is no need to invoke the Teller–Redlich product rule when a subset Hessian is used. The product of the isotopic ratios for all $3N_s$ frequencies is equal to the three-halves power of the product of the ratios of atomic masses for the specific isotopic substitution. In the context of a typical calculation, satisfaction of this rule requires nothing more than that the matrix diagonalization is performed correctly. Nonetheless, the extent to which it is indeed borne out in practice is demonstrated by the results presented below.

In the light of the above, an alternative way to calculate KIEs and IPFRs for subsets of atoms is simply to replace the MMI factor in eq 2 by extending the products for EXC and the summations for ZPE over $3N_s$ (for RS) or $3N_s - 1$ (for TS) vibrations and librations, as in eqs 11–13, in which the contributing factors VP, EX, and ZP (cf. Tables 1 and 3) are identified for clarity. Note that VP may be replaced by the MI factor, which differs from unity only for isotopically substituted atoms within the subset N_s ; however, these factors cancel from the KIE since the same isotopic substitution applies to both RS and TS.

$$k/k' = (f_{RS})_{af} / (f_{TS})_{af} \quad (11)$$

$$(f_{RS})_{af} = \underbrace{\left[\prod_i^{3N_s} \frac{\nu_i'}{\nu_i} \right]_{RS}}_{\text{VP}} \underbrace{\prod_i \left[\frac{1 - \exp(-u_i)}{1 - \exp(-u_i')} \right]}_{\text{EX}} \underbrace{\prod_i \left[\frac{\exp(u_i/2)}{\exp(u_i'/2)} \right]}_{\text{ZP}} \quad (12)$$

$$= \left[\prod_j^{N_s} \frac{m_j}{m_j'} \right]^{3/2} \prod_i^{3N_s} \left[\frac{\sinh(u_i/2)}{\sinh(u_i'/2)} \right]_{RS}$$

$$\begin{aligned}
 (f_{\text{TS}})_{\text{af}} &= \frac{v_{\ddagger}'}{v_{\ddagger}} \left[\prod_i^{3N_{\ddagger}-1} \underbrace{\frac{v_i'}{v_i}}_{\text{VP}} \underbrace{\frac{[1 - \exp(-u_i)]}{[1 - \exp(-u_i')]}}_{\text{EX}} \underbrace{\frac{\exp(u_i/2)}{\exp(u_i'/2)}}_{\text{ZP}} \right]_{\text{TS}} \\
 &= \left[\prod_j^{N_{\ddagger}} \frac{m_j}{m_j'} \right]^{\frac{3}{2}} \prod_i^{3N_{\ddagger}-1} \left[\frac{\sinh(u_i/2)}{\sinh(u_i'/2)} \right]_{\text{TS}}
 \end{aligned} \quad (13)$$

The high-temperature limit of a subset KIE evaluated as either $(f_{\text{RS}})_{\text{tr}}/(f_{\text{TS}})_{\text{tr}}$ or $(f_{\text{RS}})_{\text{af}}/(f_{\text{TS}})_{\text{af}}$ is the ratio of transition frequencies, $\nu_{\ddagger}/\nu_{\ddagger}'$, since motion along the transition vector is considered to be classical in transition-state theory. Within the inherent assumption of separability in this treatment of KIEs from subset Hessians, the quantum correction $\Gamma_{\ddagger}/\Gamma_{\ddagger}'$ for motion along the transition vector may be approximated by Bell's expression¹⁷ for an inverted parabola, eq 14.

$$\frac{\Gamma_{\ddagger}}{\Gamma_{\ddagger}'} = \frac{\nu_{\ddagger}}{\nu_{\ddagger}'} \frac{\sin(u_{\ddagger}'/2)}{\sin(u_{\ddagger}/2)} \quad (14)$$

COMPUTATIONAL METHODS

QM/MM calculations were performed for a complex of cationic AdoMet and anionic catechololate (described by AM1)¹⁸ within a sphere containing about 500 water molecules described by the flexible TIP3P MM potential¹⁹ within CHARMM.²⁰ Geometry optimizations were carried out for a minimum energy structure RS and for a first-order saddle point TS for eq 1 using the GRACE²¹ suite of utilities as previously reported²² for this reaction both in water and in an enzyme active site. Numerical differentiation of analytical gradients of the QM/MM potentials yielded large Hessians of dimension 972×972 for subsets of both RS (Figure 1) and TS

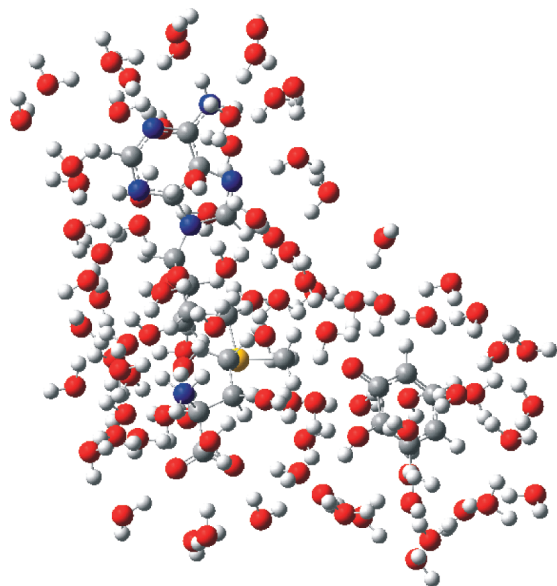


Figure 1. The 324-atom “large” subset (cutoff model 1 with 87 waters) for RS selected from AdoMet, catechololate, and ~500 waters.

involving the 63 QM atoms together with 87 MM water molecules whose oxygen is within 14 Å of the transferring methyl carbon atom. Isotopic partition-function ratios and KIEs ($k_{\alpha\text{-H}_3}/k_{\alpha\text{-D}_3}$, $k_{\alpha\text{-}^{12}\text{C}}/k_{\alpha\text{-}^{13}\text{C}}$, $k^{32}\text{S}_{\text{ig}}/k^{34}\text{S}_{\text{ig}}$, $k^{16}\text{O}_{\text{nu}}/k^{18}\text{O}_{\text{nu}}$) were computed by means of the equations above in which the six external degrees of freedom of the 324-atom subset were treated either

as vibrations only (eqs 12 and 13) or as translations and rotations (eqs 7 and 8).

A series of calculations was then carried out in which first water molecules and subsequently atoms of AdoMet and catechololate furthest from the positions of isotopic substitution were removed from the kinetic energy term for the normal modes of vibration: this was performed by deletion of the corresponding rows and columns of the large Hessians. This process was continued until only the six atoms subject to isotopic substitution were included in “cut-off” Hessians of dimension 18×18 . It is important to understand that the retained elements of each Hessian were unchanged from their values in the large Hessian. Although the calculations were actually carried out in mass-weighted Cartesian coordinates (for which the kinetic energy is represented simply by the unit matrix), this procedure is equivalent to keeping the potential energy matrix **F** unchanged while eliminating atoms from the kinetic energy matrix **G** (cf. eq 9).

Note that the large subset Hessians are themselves determined by means of QM/MM calculations in which the subset atoms experience the influence of the environment of the surrounding atoms of the full system. Even at the final stage of the cutoff procedure followed here, the six remaining atoms feel this influence through the values of the Hessian elements which are quite different from those that would be obtained by calculating a Hessian for only six atoms.

RESULTS AND DISCUSSION

Large Subset Hessians. Table 1 contains values of $(f_{\text{RS}})_{\text{af}}$, $(f_{\text{TS}})_{\text{af}}$, $(f_{\text{RS}})_{\text{tr}}$, and $(f_{\text{TS}})_{\text{tr}}$ evaluated at 298.15 K using the full 972×972 subset Hessians determined with the AM1/TIP3P method, along with the various factors contributing to each IPFR and also their ratios contributing to the KIEs. Note that it is not the purpose of this paper to present quantitatively accurate results for either the IPFRs or the KIEs; thus it is irrelevant that a different choice of method to describe either the QM or the MM regions would possibly give more reliable numerical values. Furthermore, the optimized structures for RS and TS are neither unique nor necessarily the most representative (cf. comments made previously²² concerning the need to take averages over many configurations in order to obtain KIEs that may be meaningfully compared with experiment, regardless of the choice of QM/MM method). The point of this study is to examine how the values of the IPFRs and KIEs depend upon: (a) whether they are evaluated by treating the six external degrees of freedom of the subset atoms (with respect to their environment within the larger solvated system) as vibrational frequencies or as translational and rotational motions, and (b) the extent of the “cut-off” achieved by progressively omitting more and more atoms from the subset Hessians. In order to discuss the magnitude of errors in these successive approximations, it is necessary first to establish a reference point. It seems reasonable to choose the largest possible system, in which the cutoff is most distant from the positions of isotopic substitution.

IPFRs defined as “heavy/light” always have values >1 , becoming larger at lower temperatures but tending toward unity as the temperature increases. The value of f_{RS} for $\alpha\text{-D}_3/\alpha\text{-H}_3$ is about 1676, reflecting the much larger number of effectively accessible microstates for the heavier isotopologue. The product VP of the frequency ratios is <1 because the heavier isotopologue has lower frequencies, but the ratio of zero-point energies ZP is much greater than 1 because the

frequencies for the heavier isotopologue enter the bottom of the ratio in eqs 7, 8, 12, and 13 and the “light/heavy” ratio is accentuated by the exponential.

The VP term evaluated over $3N_s - 6 = 966$ internal vibrational frequencies is exactly equal to the MI term. The quotient $TR = VP/MI = 1$ (to 5 parts in 10^8) shows that the Teller–Redlich product rule is satisfied perfectly for practical purposes. The exceedingly small remaining discrepancy arises from the fact that the projected Hessian was transferred from the program that performed the projection to the one that determined the IPFRs and associated quantities by means of a formatted file that retained only 8 decimal places for the Cartesian force constants, and in consequence, the vibrational frequencies contained very small errors as evidenced by frequencies for the six translational and rotational modes having nonzero values $|L| < 0.2 \text{ cm}^{-1}$.

The six lowest frequencies from the “all frequencies” method have values $< 50 \text{ cm}^{-1}$, but it is not possible to associate these with the six librations without inspection of the corresponding normal modes, since there is coupling between the internal and external degrees of freedom. Note also that the frequencies entering the EXC and ZPE factors in eqs 12 and 13 are not the same as, nor can they be associated simply with, the $3N_s - 6$ pure internal vibrational frequencies involved in eqs 7 and 8. It is illuminating to observe that the vibrational products VP taken over all $3N_s = 972$ vibrational frequencies of the $\alpha\text{-D}_3/\alpha\text{-H}_3$ isotopologues of both RS and TS are virtually identical, and each agree with the three-halves power of the product of ratios of isotopic masses $[(1.007825/2.014102)^3]^{3/2}$ to about 1 part in 10^9 , which is obviously greater than the number of significant figures in the masses themselves. This provides a convincing test of eq 10 and suggests that there is negligible error involved in evaluating IPFRs and KIE directly by the all frequencies method with subset Hessians. The Teller–Redlich product rule is therefore unnecessary and superfluous when working with subset Hessians.

The factors contributing to the IPFR for the $\alpha\text{-D}_3/\alpha\text{-H}_3$ isotopologues of the TS behave in a similar manner but give $f_{TS} \approx 2003$. Relatively more microstates are effectively accessible to the heavier isotopologue in the TS than in the RS, leading to an inverse $KIE = f_{RS}/f_{TS} \approx 0.836$. This result is, of course, in accord with the well-known mnemonic that ‘the lighter isotope prefers the situation with looser bonding’ which, in this case, is RS. The VP evaluated for the $3N_s - 6$ internal vibrational frequencies of the TS (including ν_z) leads to the quotient $TR = VP/MI$ deviating from unity by 6 parts in 10^5 , showing that the Teller–Redlich product rule is satisfied quite well. The larger discrepancy for TS than for RS is probably due to inaccuracies in the construction of the **B** matrix that transforms from Cartesians into internal valence coordinates. GRACE contains a utility to generate a set of valence coordinates automatically, which is very useful for very large systems (such as this with 966 valence coordinates), but the resulting set is neither unique nor always best suited for the required purpose when, for example, three atoms approach collinearity.

The differences in f_{RS} and f_{TS} as between the two methods for treating the external modes may be expressed as percentage errors with respect to the values for the all frequencies method: errors $< 0.1\%$ are satisfyingly small. As noted above, it is likely that treating the external modes as translations and rotations overestimates their freedom. The dimensionless Gibbs free energy $(G - \varepsilon)/RT$ with respect to the classical potential energy minimum²³ for the light (unsubstituted) isotopologue

of RS at 298.15 K and 1 atm is 2626 with all degrees of freedom treated as vibrations and 2589 with the external motions treated as translations and rotations, of which 2833 and 2831, respectively, are the ZPE contributions; slightly larger when the extra six low-valued frequencies are included. Replacing these modes by translational and rotational motions slightly reduces the excitational contribution from -207 to -199 but increases the translational + rotational contribution by much more, from 0 to -43 . The correct free energy of the subset within its environment would require an anharmonic treatment of the external modes for its evaluation, but it is likely that the true value is closer to that obtained within the harmonic approximation for six quantized librations than the ideal-gas, rigid-rotor approximation for six classical translations and rotations. For this reason, the all frequencies method, utilizing the Teller–Redlich product rule, is taken as the point of reference against which all other results in this study are compared.

The error in the $\alpha\text{-D}_3$ KIE from the translation/rotation method appears to be very small (0.01%) when expressed as a percentage of the ratio $(f_{RS}/f_{TS})_{af}$. However, it is usual to interpret KIEs in terms of the extent to which they differ from unity: of course, the natural logarithm of the KIE is proportional to the free-energy difference arising from isotopic substitution as well as being approximately equal to $1 - KIE$ for small differences from unity. Arguably, therefore, it is more appropriate to consider the error in $\ln(KIE)$; at 0.07% , this is still very small but reflects a larger error in the deviation of the $\alpha\text{-D}_3$ KIE from unity. The errors in $\ln(f_{RS})$ and $\ln(f_{TS})$ are each $\sim 0.01\%$.

The results for the other isotopologous pairs ($\alpha\text{-}^{12}\text{C}/\alpha\text{-}^{13}\text{C}$, $^{32}\text{S}_{lg}/^{34}\text{S}_{lg}$, $^{16}\text{O}_{nu}/^{18}\text{O}_{nu}$), although much smaller in magnitude, are similar in kind to those just discussed above and will not be discussed in detail. The errors in $\ln(f_{RS})$, $\ln(f_{TS})$, and $\ln KIE$ for $\alpha\text{-}^{12}\text{C}/\alpha\text{-}^{13}\text{C}$ are very small, but the error in KIE is larger (0.77%) for $^{32}\text{S}_{lg}/^{34}\text{S}_{lg}$ and very much larger (10.3%) for $^{16}\text{O}_{nu}/^{18}\text{O}_{nu}$. The latter KIE is very close to unity, and so the multiplicative effect of small errors in each of the contributing factors happens to create a large error in the final result; this could be significant if calculated the KIE were to be compared with an experimental value.

Cut-off Models. Table 2 contains the specification for each cutoff model of the subset Hessians for RS and TS and may be understood in conjunction with Figure 2. Models 1–5 include cut-offs involving only hydrogen bonds and other noncovalent interactions between water molecules and between solvent and solute; models 6–9 include cut-offs involving covalent bonds of the reacting solutes. Table 3 contains the EX and ZP factors contributing to the IPFRs for both RS and TS, along with the ratio (ν_z/ν_z^*) of isotopic transition frequencies and the KIE, evaluated at 298.15 K using the cutoff AM1/TIP3P subset Hessians with external modes treated as vibrations; eq 12 is used for $(f_{RS})_{af}$ (with products taken over $3N_s$ frequencies), and eq 13 is used for $(f_{TS})_{af}$ (with products taken over $3N_s - 1$ frequencies). The VP factors for RS and TS are omitted from Table 3 because they have constant values equal to $[(1.007825/2.014102)^3]^{3/2}$ for the H_3/D_3 isotopologous pair and $(12/13.003354826)^{3/2}$, $(31.9720707/33.96786665)^{3/2}$, and $(15.99491463/17.991603)^{3/2}$, respectively, for the $^{12}\text{C}/^{13}\text{C}$, $^{32}\text{S}/^{34}\text{S}$, and $^{16}\text{O}/^{18}\text{O}$ pairs. Similarly, Table 4 contains the MI, EX, and ZP factors contributing to the IPFRs for both RS and TS, along with the KIE, evaluated at 298.15 K using the

Table 2. Specification of Subset Hessians

cutoff model	number of atoms, N_s		description	comment
	RS	TS		
1	324	324	all atoms of AdoMet, catecholate, and 87 waters	all atoms in Figure 1
2	231	237	delete waters with O atom >7.5 Å from any isotope	
3	176	170	delete waters with O atom >6.0 Å from any isotope	
4	111	105	delete waters with O atom >4.5 Å from any isotope	all atoms in Figure 2
5	63	63	delete all waters	
6	27	27	delete atoms >3 bonds from any isotope	includes gold, green, blue, and lilac circles in Figure 2
7	17	17	delete atoms >2 bonds from any isotope	includes green, blue, and lilac circles in Figure 2
8	9	9	delete atoms >1 bonds from any isotope	includes blue and lilac circles in Figure 2
9	6	6	include only isotopically substituted atoms	includes lilac circles in Figure 2

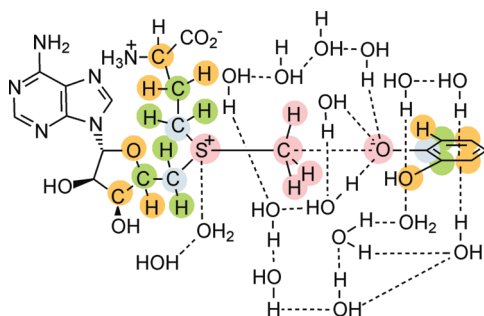


Figure 2. Atoms included in RS cutoff model 4, together with the limits for the 3-bond (model 6, gold), 2-bond (model 7, green), 1-bond (model 8, blue) and 0-bond (model 9, lilac) cut-offs.

cutoff AM1/TIP3P subset Hessians with external modes treated as translations and rotations; eq 7 is used for $(f_{RS})_{tr}$ (with products taken over $3N_s - 6$ frequencies), and eq 8 is used for $(f_{TS})_{tr}$ (with products taken over $3N_s - 7$ frequencies). The errors in these results for the two methods are presented together in Table 5 in order to facilitate their comparison. Finally, the approximate quantum corrections (eq 14) for motion along the transition vector are given in Table 6.

Consider first the results for treating the external modes as vibrations (Tables 3 and 5). Although the total zero-point energy for each species decreases markedly as the number of atoms N_s retained in the cutoff subset Hessian decreases, the isotopic sensitivity as expressed by the ratio ZP increases. This is partly compensated by a diminution in value of the EX factor as the extent of the cutoff increases, but the resultant is a small increase in both $(f_{RS})_{af}$ and $(f_{TS})_{af}$ as the size of the Hessian is decreased. It is important to note that this is purely a kinetic energy effect; the elements of the Hessian corresponding to particular subset atoms are unchanged in each cutoff model. It is remarkable that the errors in $\ln(f_{RS})_{af}$ and $\ln(f_{TS})_{af}$ for the H_3/D_3 and $^{12}C/^{13}C$ substitutions do not exceed 0.15% for even the more severe cutoff model 9. Larger errors (up to 16%) are found in the extreme for the $^{32}S/^{34}S$ and $^{16}O/^{18}O$ pairs; this is to be expected as these are terminal atoms are directly connected to atoms deleted in model 9. Overall, the “2-bond”

cutoff (model 7) recommended by Stern and Wolfsberg² involves errors in $\ln(f)$ consistently less (and usually much less) than 1% when all $3N_s$ degrees of freedom of the subset are treated as harmonic vibrations. The errors (not shown) in $KIE = (f_{RS}/f_{TS})_{af}$ may appear to be quite modest; however, the errors in $\ln(KIE)$ are significantly larger. Although less than 0.1% for $\alpha-H_3/\alpha-D_3$ and $^{32}S_{lg}/^{34}S_{lg}$ with the 2-bond cutoff model 7, this model gives $\ln(KIE)$ errors of -4.9 and $+4.1\%$, respectively, for $\alpha-^{12}C/\alpha-^{13}C$ and $^{16}O_{nu}/^{18}O_{nu}$ substitutions and even larger errors for the more extreme cut-offs. These $\ln(KIE)$ errors more truly reflect the error in the actual isotope effect, which is usually only a small difference from unity. In order to ensure that the error in $\ln(KIE)$ remained less than 1% for all four isotopic substitutions considered here, it would be necessary to work with cutoff model 4, which retains all solvent water molecules whose O atom is less than 4.5 Å from any isotopically substituted atom.

Now consider the results for treating the external modes as translations and rotations (Tables 4 and 5). The MI factor is not constant in value and so is shown, but otherwise the trends in EX, ZP, and $(f)_{tr}$ for both RS and TS are generally similar to those for the “all-frequencies” method. Note that the trends in ZP and $(f)_{tr}$ are not monotonic for all cutoff models. The results for models 8 and 9 might seem to be anomalous but are in fact correct. The changes in the total zero-point energy of the $3N_s - 6$ internal vibrations for each isotopologue of RS and TS are indeed monotonic, but as the absolute magnitude of the zero-point energy decreases, the ratios that determine the ZP factors vary unpredictably for the most extreme cut-offs. As if this were not warning enough in regard to the reliability of this method, the errors in $\ln(f_{RS})_{tr}$ and $\ln(f_{TS})_{tr}$ can reach nearly 500%! In general, treating the external modes as translations and rotations leads to errors in $\ln(f_{RS})_{tr}$ and $\ln(f_{TS})_{tr}$ very much larger than when those modes are treated as vibrations; for the 2-bond cutoff model the difference is a factor of about 10^4 . Note that the Teller–Redlich product rule is satisfied very well in every case: The magnitude of $\ln(VP/MI)$, which expresses the deviation from unity of the ratio, is typically in the range 10^{-5} to 10^{-8} . The trends in the $\ln(KIE)$ errors are not monotonic, and even the larger subset Hessians may lead to errors of a few percent in an unpredictable manner. The Stern and Wolfsberg² recommended 2-bond cutoff (model 7) gives errors in $\ln(KIE)$ ranging from -6.7 to $+12\%$, depending upon the nature of the isotopic substitution.

The imaginary transition frequencies TF are surprisingly sensitive to the size of the subset Hessian for both methods. The errors for the heavy isotopologues are shown in Table 5 with respect to the magnitude of this frequency for the largest Hessian (model 1). The all-frequencies method gives errors smaller by a factor of about 2 for the most extreme cut-offs as compared to the translation/rotation method, but less severe cut-offs give similar values for each method. Consequently several cutoff models show quite similar differences in the ratios $\nu_{\ddagger}/\nu_{\ddagger}'$ as between the two methods, although the largest errors again occur with the translation/rotation method.

Finally, the quantum corrections $\Gamma_{\ddagger}/\Gamma_{\ddagger}'$ to $\nu_{\ddagger}/\nu_{\ddagger}'$ (eq 14) are rather small (Table 6) but show significant dependence on the size of the subset Hessian: Errors in $\ln(\Gamma_{\ddagger}/\Gamma_{\ddagger}')$ are more than 100% for some cutoff models with the translation/rotation method. Including the quantum correction in the $\alpha-H_3/\alpha-D_3$, KIE involves a relatively small error (0.5% or less for even the most extreme cutoff) with the all-frequencies method but much larger for smaller Hessians with the translation/rotation

Table 3. IPFRs, KIEs, and Contributing Factors at 298.15 K Evaluated Using the Cut-Off AM1/TIP3P Subset Hessians with External Modes Treated As Vibrations (the All-Frequencies Method)

isotopic substitution	cutoff model	RS			TS			$\nu_{\ddagger}/\nu'_{\ddagger}$	KIE
		products taken over $3N_s$ frequencies (eq 12)			products taken over $3N_s - 1$ frequencies (eq 13)				
		EX	ZP	$(f_{RS})_{af}$	EX	ZP	$(f_{TS})_{af}$		
$\alpha\text{-D}_3$	1	1.722473	21938.08	1675.782	1.542157	29289.28	2005.348	1.001120	0.836592
	2	1.722294	21940.40	1675.785	1.542034	29291.63	2005.356	1.001124	0.836593
	3	1.722158	21942.29	1675.797	1.541932	29293.53	2005.384	1.001139	0.836600
	4	1.721562	21950.50	1675.844	1.541448	29302.73	2005.452	1.001173	0.836624
	5	1.714194	22058.25	1676.863	1.536587	29408.41	2006.467	1.001237	0.836763
	6	1.710417	22107.19	1676.880	1.529184	29554.40	2006.911	1.001336	0.836669
	7	1.707843	22141.15	1676.928	1.525860	29619.05	2007.126	1.001434	0.836686
	8	1.700505	22241.27	1677.274	1.522178	29690.96	2007.766	1.001745	0.836850
	9	1.698610	22271.21	1677.660	1.520399	29730.60	2008.286	1.001839	0.836905
$\alpha\text{-}^{13}\text{C}$	1	1.049739	1.196608	1.113582	1.038529	1.138539	1.084455	1.034562	1.062348
	2	1.049712	1.196639	1.113582	1.038503	1.138515	1.084429	1.034585	1.062397
	3	1.049695	1.196660	1.113583	1.038484	1.138352	1.084333	1.034659	1.062569
	4	1.049626	1.196741	1.113585	1.038412	1.137947	1.084108	1.034885	1.063023
	5	1.049121	1.197332	1.113600	1.037840	1.137488	1.083704	1.035487	1.064053
	6	1.048464	1.198084	1.113601	1.036214	1.138321	1.083836	1.036480	1.064944
	7	1.048087	1.198517	1.113603	1.035605	1.138409	1.083893	1.037062	1.065489
	8	1.046878	1.199942	1.113640	1.034707	1.138134	1.083744	1.038072	1.066708
	9	1.046528	1.200431	1.113722	1.034360	1.138191	1.083718	1.038343	1.067091
$^{34}\text{S}_{lg}$	1	1.044557	1.081951	1.032033	1.045113	1.076553	1.028206	1.000755	1.004480
	2	1.044516	1.081993	1.032033	1.044993	1.076675	1.028204	1.000754	1.004481
	3	1.044493	1.082017	1.032033	1.044976	1.076692	1.028201	1.000752	1.004482
	4	1.044303	1.082215	1.032034	1.044902	1.076765	1.028195	1.000749	1.004486
	5	1.044132	1.082394	1.032035	1.044747	1.076914	1.028181	1.000745	1.004496
	6	1.042626	1.083957	1.032035	1.043232	1.078477	1.028174	1.000739	1.004497
	7	1.040808	1.085876	1.032059	1.041526	1.080285	1.028178	1.000705	1.004482
	8	1.032384	1.096403	1.033631	1.032782	1.091202	1.029703	1.000563	1.004380
	9	1.030151	1.099562	1.034366	1.029835	1.095430	1.030798	1.000616	1.004079
$^{18}\text{O}_{nu}$	1	1.079712	1.242839	1.124840	1.068512	1.248618	1.122388	1.003613	1.005805
	2	1.079692	1.242864	1.124842	1.068497	1.248639	1.122388	1.003609	1.005804
	3	1.079569	1.243015	1.124851	1.068478	1.248675	1.122388	1.003598	1.005801
	4	1.079213	1.243438	1.124862	1.068278	1.248917	1.122383	1.003587	1.005804
	5	1.076410	1.246979	1.125136	1.064616	1.253518	1.122660	1.003590	1.005804
	6	1.070406	1.254141	1.125286	1.060111	1.259185	1.122606	1.003270	1.005666
	7	1.067263	1.258216	1.125627	1.057689	1.262580	1.122774	1.003016	1.005564
	8	1.062819	1.266644	1.128449	1.054130	1.270311	1.125203	1.002440	1.005332
	9	1.052333	1.298877	1.145749	1.047078	1.293405	1.137848	1.002312	1.009272

method. However, when the semiclassical KIE has a small magnitude, as for $^{12}\text{C}/^{13}\text{C}$, the effect of the quantum correction is much greater with both methods. To ensure a <2% error in $\ln(\text{KIE}_{\text{corr}})$ for all four isotopic substitutions considered here, it would be necessary to work with cutoff model 4, which retains all solvent water molecules whose O atom is less than 4.5 Å from any isotopically substituted atom.

Relation to Previous Work. Li and Jensen described²⁴ a method for ‘partial Hessian vibrational analysis’ (PHVA) of large systems which, as we already noted, was apparently similar to the procedure in our previous work.¹⁰ Their method divides the whole N -atomic system into two blocks (in our terminology an N_s -atomic subset and its environment) and computes the subset Hessian explicitly, while setting the diagonal elements for the environment equal to a very small value. The full $3N \times 3N$ Hessian is subjected to the projection procedure of Miller et al.,²⁵ mass-weighted and diagonalized, and yields six zero frequencies for overall translation and rotation of the whole system, $3(N - N_s) - 6$ small ($<1 \text{ cm}^{-1}$) frequencies

corresponding essentially to vibrational motion within the environment, 3 frequencies (typically $<10 \text{ cm}^{-1}$) for motion of the subset relative to its environment, and $3N_s - 3$ frequencies corresponding mainly to vibrational motion within the subset but also including motion coupled with the environment. This method differs from ours in that it treats the coupling of the subset with the environment neither as six librations nor as six translations and rotations. At the extra expense of projecting and diagonalizing a $3N \times 3N$ matrix rather than a $3N_s \times 3N_s$ one, it includes the dynamical influence of the environment treated, in effect, as having atoms with near-infinite masses. However, for the purpose of calculating KIEs (which Li and Jensen²⁴ did not report), it is unlikely that isotopically sensitive frequencies would be affected by inclusion of the environment in this way, provided that the subset is not too small.

Ghysels et al.²⁶ have discussed a variety of partial-Hessian-based methods for normal-mode analysis that have been proposed by themselves and others over recent years to obtain vibrational spectra for large systems. Their mobile block

Table 4. IPFRs, KIEs, and Contributing Factors at 298.15 K Evaluated Using the Cut-Off AM1/TIP3P Subset Hessians with External Modes Treated As Translations and Rotations (the Translation/Rotation Method)

isotopic substitution	cutoff model	RS				TS				
		products taken over $3N_s - 6$ frequencies (eq 7)				products taken over $3N_s - 7$ frequencies (eq 8)				
		MI	EX	ZP	$(f_{RS})_{tr}$	MI	EX	ZP	$(f_{TS})_{tr}$	KIE
$\alpha\text{-D}_3$	1	0.044452	1.717391	21966.50	1676.968	0.044453	1.537843	29325.92	2004.78	0.836485
	2	0.044485	1.715420	21983.62	1677.584	0.044483	1.536362	29343.43	2005.39	0.836537
	3	0.044527	1.713325	21996.60	1678.101	0.044536	1.534361	29358.34	2006.20	0.836458
	4	0.044624	1.708041	22034.99	1679.516	0.044661	1.529415	29396.36	2007.92	0.836445
	5	0.044838	1.694889	22113.05	1680.489	0.044864	1.521216	29428.92	2008.47	0.836701
	6	0.045379	1.548531	29846.38	2097.310	0.045421	1.427099	38129.52	2471.56	0.848577
	7	0.046171	1.378118	44781.98	2849.434	0.046194	1.308680	55674.92	3365.76	0.846595
	8	0.047959	1.110456	173157.52	9221.797	0.047989	1.081960	226729.51	11772.28	0.783349
	9	0.063023	1.096119	77948.68	5384.735	0.068125	1.061559	87250.10	6309.80	0.853393
$\alpha\text{-}^{13}\text{C}$	1	0.887196	1.048837	1.196748	1.113603	0.887205	1.037667	1.138663	1.048280	0.941341
	2	0.887404	1.048528	1.196832	1.113614	0.887392	1.037388	1.138650	1.048207	0.941266
	3	0.887662	1.048182	1.196887	1.113621	0.887724	1.036991	1.138521	1.048079	0.941145
	4	0.888257	1.047386	1.197022	1.113647	0.888495	1.036082	1.138074	1.047657	0.940744
	5	0.889539	1.045854	1.196985	1.113588	0.889754	1.034752	1.136728	1.046557	0.939806
	6	0.892410	1.035190	1.210004	1.117819	0.892563	1.026400	1.145762	1.049663	0.939028
	7	0.895869	1.026008	1.221044	1.122346	0.895796	1.019497	1.154160	1.054049	0.939148
	8	0.901255	1.014967	1.237245	1.131763	0.900838	1.011378	1.165879	1.062218	0.938552
	9	0.915390	1.010508	1.219523	1.128070	0.909280	1.008690	1.157326	1.061478	0.940968
$^{34}\text{S}_{ig}$	1	0.914565	1.042908	1.082018	1.032037	0.914554	1.043437	1.076623	1.027398	0.995506
	2	0.915081	1.042277	1.082064	1.032038	0.915018	1.042732	1.076665	1.027265	0.995375
	3	0.915622	1.041670	1.082057	1.032040	0.915683	1.042136	1.076673	1.027432	0.995536
	4	0.916908	1.040200	1.082070	1.032043	0.917009	1.040701	1.076598	1.027433	0.995533
	5	0.919033	1.038073	1.081781	1.032045	0.918947	1.038774	1.076313	1.027425	0.995523
	6	0.927199	1.027413	1.084214	1.032840	0.926684	1.028751	1.078421	1.028088	0.995399
	7	0.937006	1.018739	1.082880	1.033679	0.936140	1.020319	1.077065	1.028771	0.995251
	8	0.952228	1.008389	1.077309	1.034449	0.951533	1.009190	1.071799	1.029225	0.994950
	9	0.979198	1.003247	1.035669	1.017419	0.980262	1.003491	1.025640	1.008906	0.991633
$^{18}\text{O}_{nu}$	1	0.839791	1.077759	1.242803	1.124851	0.839743	1.066168	1.248390	1.117693	0.993637
	2	0.840239	1.077156	1.242842	1.124858	0.840140	1.065550	1.248362	1.117547	0.993501
	3	0.840972	1.076186	1.242893	1.124871	0.841005	1.065017	1.248626	1.118375	0.994225
	4	0.842670	1.073981	1.242957	1.124890	0.843226	1.062394	1.248438	1.118399	0.994230
	5	0.846872	1.067983	1.243650	1.124814	0.847118	1.056673	1.249091	1.118095	0.994026
	6	0.853422	1.056330	1.250094	1.126954	0.855407	1.045103	1.252700	1.119898	0.993739
	7	0.865037	1.045375	1.250027	1.130385	0.866833	1.035139	1.250813	1.122346	0.992889
	8	0.892312	1.025577	1.237661	1.132626	0.892147	1.018970	1.231671	1.119676	0.988567
	9	0.946619	1.012936	1.094623	1.049595	0.938559	1.011476	1.098737	1.043064	0.993778

Hessian (MBH) method has been applied in calculations of partition functions within the transition-state theory of reaction rates but not to KIEs; their procedures seem to treat librational degrees of freedom as both vibrations and as translations/rotations at the same time.²⁷ An implementation of the MBH method with QM/MM Hessians has recently been reported, and it has been suggested that it could be applied usefully in KIE calculations; however, it was incorrectly asserted that IPFRs (rather than partition functions per se) are governed by the low-frequency modes.²⁸

Świderek et al.²⁹ have described a procedure that first uses a low-level QM method to identify which normal modes and frequencies contribute most to an IPFR and then generates a Hessian with a higher-level method in the reduced dimensionality space spanned by these eigenvectors. This method converges from below toward the correct value of an IPFR and gives small (and possibly acceptable) errors using only about one-third of the total number of frequencies for the full system. However, the resulting errors on KIEs (or equilibrium

isotope effects) were not presented; moreover, with refreshing candour these authors also reported that the partial Hessian approach was superior to their scheme, giving accurate IPFRs with only a very small number of subset atoms. Świderek and Paneth³⁰ recently reported equilibrium isotope effects upon inhibitor binding to an enzyme calculated by a QM/MM method with explicit water using variously sized models, which they claimed did not give meaningfully different results; however, their version of the Bigeleisen equation for these binding isotope effects involves products over $3N - 6$ frequencies for each species, and so it is not clear how the six librational modes were treated.

Schaad et al. investigated³¹ the consequences of introducing small random errors into Cartesian force constants or frequencies into KIE calculations using either eq 2 or 5; these authors concluded that results from eq 5 containing the VP factor were less affected than by these errors than those from eq 2 containing the MMI factor instead. In this context it is of interest to read Wolfsberg's comments regarding the

Table 5. Errors in IPFRs, KIEs, Product Rule, And Transition Frequencies at 298.15 K Evaluated Using Cut-Off AM1/TIP3P Subset Hessians with the All Frequencies (af) and Translation/Rotation (tr) Methods

	subset	% error in ln(IPFR)			% error in ln(KIE)			ln(VP/MMI) _{tr}			% error in $\ln \mu_{\text{tr}}^{\text{af}}$			% error in $\ln \mu_{\text{tr}}^{\text{tr}}$		
		$(f_{\text{RS}})_{\text{af}}$	$(f_{\text{TS}})_{\text{af}}$	$(f_{\text{TS}})_{\text{tr}}$	$(\text{KIE})_{\text{af}}$	$(\text{KIE})_{\text{tr}}$	RS	TS	af	tr	af	tr	af	tr	af	tr
$\alpha\text{-D}_3$	1	0	0	0.071	0	0	-5.5×10^{-8}	-6.4×10^{-5}	0	0	0	0	0	0	0	0
	2	2.0×10^{-4}	4.0×10^{-4}	0.11	-0.0011	-0.035	1.1×10^{-7}	1.4×10^{-5}	-0.14	-0.11	0.3	-0.5	0.3	-0.5	0.3	-0.5
	3	9.0×10^{-4}	1.8×10^{-3}	0.14	-0.0056	0.018	1.7×10^{-7}	2.4×10^{-7}	-0.63	-0.61	1.6	0.8	-0.63	-0.61	1.6	0.8
	4	3.7×10^{-3}	5.2×10^{-3}	0.22	0.13	0.026	3.2×10^{-7}	1.3×10^{-6}	-1.8	-1.8	4.7	3.9	-1.8	-1.8	4.7	3.9
	5	0.065	0.056	0.28	0.16	-0.14	-1.7×10^{-7}	1.4×10^{-6}	-4.2	-4.2	10	9.2	-4.2	-4.2	10	9.2
	6	0.066	0.078	25	23	-8.0	-8.6×10^{-8}	-2.9×10^{-5}	-5.2	-5.3	19	-15	-5.2	-5.3	19	-15
	7	0.068	0.089	70	68	-6.7	-2.4×10^{-8}	3.9×10^{-8}	-5.7	-6.6	28	-34	-5.7	-6.6	28	-34
	8	0.089	0.12	450	490	37	-7.7×10^{-9}	-1.9×10^{-9}	-7.5	-13	56	192	-7.5	-13	56	192
	9	0.11	0.15	220	210	-11	-2.0×10^{-8}	-4.8×10^{-9}	-8.8	-16	64	456	-8.8	-16	64	456
$\alpha\text{-}^{13}\text{C}$	1	0	0	1.9×10^{-3}	0	0	-3.5×10^{-8}	-1.8×10^{-5}	0	0	0	0	0	0	0	0
	2	-4.2×10^{-9}	-2.4×10^{-3}	2.8×10^{-3}	0.076	0.13	-8.9×10^{-9}	1.1×10^{-5}	-0.15	-3.3	0.1	0.0	-0.15	-3.3	0.1	0.0
	3	1.0×10^{-4}	-0.011	3.5×10^{-3}	0.34	0.34	-8.9×10^{-9}	3.6×10^{-8}	-0.63	-3.8	0.3	0.2	-0.63	-3.8	0.3	0.2
	4	3.0×10^{-4}	-0.032	5.9×10^{-3}	1.0	1.0	-5.3×10^{-8}	5.3×10^{-8}	-1.8	-5.0	0.9	0.8	-1.8	-5.0	0.9	0.8
	5	1.6×10^{-3}	-0.069	5.7×10^{-4}	2.7	2.7	-3.6×10^{-8}	1.3×10^{-7}	-4.3	-7.4	2.6	2.6	-4.3	-7.4	2.6	2.6
	6	1.7×10^{-3}	-0.057	0.38	4.0	4.1	7.1×10^{-8}	-1.9×10^{-6}	-5.3	-8.5	5.5	3.6	-5.3	-8.5	5.5	3.6
	7	1.9×10^{-3}	-0.052	0.79	4.9	3.9	0.0	0.0	-5.9	-9.7	7.1	2.3	-5.9	-9.7	7.1	2.3
	8	5.2×10^{-3}	-0.066	1.6	6.8	4.9	0.0	0.0	-7.8	-16	10	-1.5	-7.8	-16	10	-1.5
	9	0.013	-0.068	1.3	7.4	0.66	9.2×10^{-9}	0.0	-9.0	-18	11	-6.1	-9.0	-18	11	-6.1
$^{34}\text{S}_{\text{lg}}$	1	0	0	3.7×10^{-4}	0	0	-2.3×10^{-7}	3.0×10^{-5}	0	0	0	0	0	0	0	0
	2	2.9×10^{-9}	-2.0×10^{-4}	4.8×10^{-4}	0.022	2.9	-2.6×10^{-7}	1.6×10^{-4}	-0.14	-0.072	-0.1	0.9	-0.14	-0.072	-0.1	0.9
	3	2.9×10^{-9}	-5.0×10^{-4}	6.6×10^{-4}	0.045	-0.67	-2.1×10^{-7}	-2.4×10^{-7}	-0.62	-0.58	-0.4	0.1	-0.62	-0.58	-0.4	0.1
	4	1.0×10^{-4}	-1.1×10^{-3}	9.9×10^{-4}	0.13	-0.61	-6.2×10^{-7}	-4.1×10^{-7}	-1.8	-1.7	-0.9	-0.4	-1.8	-1.7	-0.9	-0.4
	5	2.0×10^{-4}	-2.4×10^{-3}	1.2×10^{-3}	0.36	-0.39	0.0	-7.3×10^{-7}	-4.2	-4.2	-1.3	-0.7	-4.2	-4.2	-1.3	-0.7
	6	2.0×10^{-4}	-3.1×10^{-3}	0.078	0.38	2.4	-1.3×10^{-7}	0.0	-5.1	-5.3	-2.1	14	-5.1	-5.3	-2.1	14
	7	2.5×10^{-3}	-2.7×10^{-3}	0.16	0.045	5.7	9.4×10^{-9}	0.0	-5.7	-6.6	-6.6	41	-5.7	-6.6	-6.6	41
	8	0.15	0.15	0.23	-2.2	12	0.0	0.0	-7.4	-13	-25	149	-7.4	-13	-25	149
	9	0.23	0.251	-1.4	-8.9	87	0.0	9.8×10^{-9}	-8.7	-16	-19	241	-8.7	-16	-19	241
$^{18}\text{O}_{\text{nu}}$	1	0	0	8.6×10^{-3}	0	0	8.4×10^{-8}	5.8×10^{-4}	0	0	0	0	0	0	0	0
	2	1.7×10^{-3}	4.1×10^{-9}	1.4×10^{-3}	-0.017	2.1	1.3×10^{-7}	7.1×10^{-4}	-0.14	-0.35	-0.1	-0.5	-0.14	-0.35	-0.1	-0.5
	3	8.5×10^{-3}	4.1×10^{-9}	0.024	-0.069	-9.3	9.3×10^{-8}	-2.8×10^{-7}	-0.62	-0.86	-0.4	-0.1	-0.62	-0.86	-0.4	-0.1
	4	0.017	-4.0×10^{-4}	0.038	-0.017	-9.3	3.7×10^{-7}	7.5×10^{-7}	-1.8	-2.0	-0.7	-0.4	-1.8	-2.0	-0.7	-0.4
	5	0.22	0.024	-0.02	-0.017	-6.1	-2.5×10^{-7}	1.2×10^{-6}	-4.2	-4.5	-0.6	-0.6	-4.2	-4.5	-0.6	-0.6
	6	0.34	0.019	1.6	-2.4	-1.6	3.0×10^{-7}	-1.7×10^{-6}	-5.1	-5.6	-9.5	-2.1	-5.1	-5.6	-9.5	-2.1
	7	0.60	0.034	4.2	-4.1	12	8.7×10^{-9}	-4.3×10^{-8}	-5.6	-6.9	-17	4.5	-5.6	-6.9	-17	4.5
	8	2.7	0.25	5.9	-8.1	80	8.9×10^{-9}	0.0	-7.4	-13	-32	38	-7.4	-13	-32	38
	9	1.4	-59	-7.1	59	-2.2	0.0	0.0	-8.6	-16	-36	61	-8.6	-16	-36	61

Table 6. Errors in Quantum-Corrected KIEs at 298.15 K Evaluated Using Cut-Off AM1/TIP3P Subset Hessians

	subset	all frequencies				translation/rotation			
		Γ/Γ^*	$\ln(\Gamma/\Gamma^*)$	KIE_{corr}	$\ln(\text{KIE}_{\text{corr}})$	Γ/Γ^*	$\ln(\Gamma/\Gamma^*)$	KIE_{corr}	$\ln(\text{KIE}_{\text{corr}})$
$\alpha\text{-D}_3$	1	1.000530	0	0.837035	0	1.000528	0	0.836927	0
	2	1.000534	0.7	0.837040	0.00	1.000529	−0.2	0.836981	0.04
	3	1.000547	3.2	0.837058	−0.02	1.000543	2.3	0.836912	0.08
	4	1.000579	9.3	0.837108	−0.05	1.000575	8.4	0.836926	0.07
	5	1.000645	22	0.837302	−0.18	1.000638	20	0.837234	−0.13
	6	1.000710	34	0.837263	−0.15	1.000507	−4.3	0.849007	−8.0
	7	1.000771	45	0.837331	−0.20	1.000407	−23	0.846940	−6.6
	8	1.000971	83	0.837663	−0.42	1.002009	279	0.784922	36
	9	1.001047	97	0.837781	−0.50	1.003983	650	0.856791	−13
$\alpha\text{-}^{13}\text{C}$	1	1.016871	0	1.080272	0	1.016863	0	1.080228	0
	2	1.016938	0.4	1.080392	0.1	1.016921	0.3	1.080376	0.1
	3	1.017163	1.7	1.080806	0.6	1.017146	1.6	1.080753	0.6
	4	1.017726	5.0	1.081866	1.9	1.017706	4.9	1.081810	1.8
	5	1.018974	12	1.084242	4.8	1.018970	12	1.084234	4.7
	6	1.019869	18	1.086103	7.0	1.019569	16	1.085770	6.6
	7	1.020411	21	1.087237	8.3	1.019802	17	1.085880	6.7
	8	1.021664	28	1.089816	11	1.021186	25	1.088045	9.3
	9	1.022291	32	1.090877	13	1.020905	24	1.084952	5.6
$^{34}\text{S}_{\text{lg}}$	1	1.000358	0	1.004839	0	1.000360	0	1.004876	0
	2	1.000358	0.3	1.004841	0.04	1.000362	1.2	1.005010	3.5
	3	1.000362	1.2	1.004845	0.1	1.000363	1.6	1.004849	0.2
	4	1.000370	3.4	1.004858	0.4	1.000372	3.9	1.004860	0.4
	5	1.000389	8.8	1.004887	1.0	1.000391	9.4	1.004890	1.0
	6	1.000393	9.9	1.004892	1.1	1.000460	29	1.005084	5.1
	7	1.000379	6.1	1.004863	0.5	1.000583	63	1.005357	11
	8	1.000313	−12	1.004694	−3.0	1.001152	222	1.006234	29
	9	1.000350	−2.0	1.004431	−8.4	1.001643	359	1.010094	108
$^{18}\text{O}_{\text{nu}}$	1	1.001715	0	1.007529	0	1.001713	0	1.008127	0
	2	1.001719	0.3	1.007533	0.05	1.001710	−0.3	1.008263	9.7
	3	1.001734	1.1	1.007545	0.2	1.001739	1.4	1.007558	0.4
	4	1.001777	3.6	1.007591	0.8	1.001781	3.9	1.007595	0.9
	5	1.001876	9.4	1.007691	2.1	1.001875	9.4	1.007896	4.9
	6	1.001741	1.5	1.007417	−1.5	1.001890	10	1.008202	8.9
	7	1.001624	−5.3	1.007197	−4.4	1.002072	21	1.009249	23
	8	1.001359	−21	1.006698	−11	1.003064	79	1.014665	94
	9	1.001317	−23	1.010601	41	1.003708	116	1.009993	33

presence of nonzero translational/rotational frequencies in GAUSSIAN03 calculations and their effect upon the reliability of the Teller–Redlich product rule.³²

There was a clear advantage in using the Bigeleisen equation for equilibrium isotope effects back in the time when vibrational frequencies were obtained experimentally and with greater precision than the moments of inertia were known for molecules of interest. However, since nowadays it is just as simple for a computer to determine the MMI factor as it is to evaluate VPR and $\nu_{\text{z}}/\nu_{\text{z}}'$, the reason why most KIE programs coupled with quantum chemistry packages use the Bigeleisen equation seems to be that it is easier to deliberately omit low frequencies of uncertain reliability without introducing large errors.

CONCLUSIONS

A Hessian computed for a subset of N_{s} atoms within a larger system generally yields $3N_{\text{s}}$ nonzero vibrational eigenvalues corresponding to the six external degrees of freedom for the subset vibrating with respect to its environment. In the case of an isotopologous pair of subset atoms, the product of the isotopic ratios for all $3N_{\text{s}}$ frequencies is equal to the three-

halves power of the product of the ratios of atomic masses for the specific isotopic substitution. There is no need to invoke the Teller–Redlich product rule when a subset Hessian is used, since this is necessary only when there are zero eigenvalues for separable translation and rotation degrees of freedom. Consequently, for the evaluation of IPFRs and KIEs, there is no need to use the Bigeleisen equation in its conventional form, where products are taken over $3N_{\text{s}} - 6$ or $3N_{\text{s}} - 7$ vibrational frequencies for minima and saddle points, respectively. It is more appropriate to use a simpler form (eqs 11–13) in which products are taken over $3N_{\text{s}}$ or $3N_{\text{s}} - 1$ frequencies.

The $\text{S}_{\text{N}}2$ methyl transfer from *S*-adenosylmethionine to catecholate in water has been used as a model system. IPFRs and KIEs evaluated using a large AM1/TIP3P subset Hessian (corresponding to 324 atoms) have been compared for two alternative methods for treating the six external modes. It is found that it is better to treat these as harmonic vibrations (the all-frequency method) rather than to treat them as free translations and rotations along with a projected Hessian from which the translational and rotational contributions have been eliminated (the translation/rotation method).

A series of smaller subset Hessians, generated by deletion of rows and columns corresponding to particular atoms being removed, has allowed the validity of “cut-off” procedures for computation of kinetic isotope effects to be assessed. These calculations leave the elements of the Hessian corresponding to retained atoms unchanged for each successive cutoff and are equivalent to removing atoms from the kinetic energy part of the molecular vibrational problem. Errors in IPFRs and KIEs have been evaluated using a range of cutoff models with AM1/TIP3P subset Hessians down to as small as only the six atoms involved in the isotopic substitutions considered here ($\alpha\text{-H}_3/\alpha\text{-D}_3$, $\alpha\text{-}^{12}\text{C}/\alpha\text{-}^{13}\text{C}$, $^{32}\text{S}_{\text{lg}}/^{34}\text{S}_{\text{lg}}$, and $^{16}\text{O}_{\text{nu}}/^{18}\text{O}_{\text{nu}}$). Again, the all-frequency method is demonstrated to be superior to the translation/rotation method, giving considerably smaller errors in general. The “two-bond cut-off” rule proposed by Stern and Wolfsberg gives respective errors in the natural logarithms of the KIEs (without tunnelling) of -0.06 , 5 , 0.05 , and -4% for these isotopic substitutions with the all-frequency method but -7 , 4 , 6 , and 12% for the same substitutions with the translation/rotation method. However, to ensure that the error in $\ln(\text{KIE})$ remains less than 1% (or 2% for the quantum-corrected KIE) for all four isotopic substitutions considered here, it is necessary to use a less-restrictive cutoff procedure which retains all covalently bonded atoms to a distance of at least three-bonds together with solvent water molecules whose O atom is less than 4.5 \AA from any isotopically substituted atom.

To the extent that KIEs (and also equilibrium isotope effects) may be treated to a satisfactory degree of approximation by means of a “cut-off” procedure, it implies that these ratios of rate (or equilibrium) constants are essentially local properties of the system, reflecting changes in curvature of the potential energy surface in the immediate vicinity of the position(s) of isotopic substitution. However, the surprising degree of sensitivity of KIE values to the extent of cutoff implies that these are not entirely local properties of the system. These findings have important practical implications for reliable computational simulation of KIEs for large systems, for example, in solution or within an enzyme active site. To obtain reliable KIEs requires not only an appropriate choice of (say) QM/MM method but also an adequate description of the environment in regard to both its potential and kinetic energies influence upon the positions of isotopic substitution. The bottom line is this: Regardless of considerations of the choice of QM/MM method, for accurate calculations of KIEs in large systems it is recommended to select a subset of atoms including all covalently bonded atoms to a distance of at least three-bonds from any isotopically substituted atom, together with any solvent atoms within 4.5 \AA .

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Notes

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