**Kinetic isotope effect**

**Definition of KIE:** "Change in the reaction rate of a chemical reaction when one of the atoms in the reactants is replaced by one of its isotopes"

\[ \text{KIE} = \frac{k_{\text{Light}}}{k_{\text{Heavy}}} \]

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**Definitions**

**Normal KIE**
The lighter isotopomer reacts faster i.e. \( \text{KIE} > 1 \)

**Reverse KIE**
The heavier isotopomer reacts faster i.e. \( \text{KIE} < 1 \)

**Primary KIE**
Most commonly, the rate-determining step requires the breaking/forming of a bond involving the isotope. [there are notable exceptions, see later (Hartwig)]

**Secondary KIE**
No bond to the isotopically labeled atom is broken or formed.

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**What is KIE used for?**
- Elucidation of reaction mechanism
- Determine which bond is broken/formed in RDS
- Transition state geometry predictions
- Selective reactions
- Protecting metabolically unstable C-H bonds

---

**Examples of primary KIE**

\[ \text{SM}_H \xrightarrow{k_H} \text{P}_H \]

\[ \text{SM}_D \xrightarrow{k_D} \text{P}_D \]

\[ \text{KIE} = \frac{k_H}{k_D} \]

\[ \text{KIE} = 7.7 \]

\[ \text{KIE} = 1.5 \]

---

**Kinetic isotope effect**

**Origin of KIE** – Mainly due to difference in zero-point energy (ZPE) between non-labelled and labelled compounds.

**What is ZPE?**

Bonds have quantized vibrational energy levels. These are approximated using the quantum harmonic oscillator model.

The $n^{th}$ discrete energy level is

$$E_n = \left( n + \frac{1}{2} \right) \hbar \nu \quad n \text{ is an integer (0, 1, 2, ...)}$$

The frequency of the oscillator is

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad k \text{ is the force constant (bond dependent)}$$

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \quad m_1 \text{ is the mass of atom '1' in the bond}$$

$$m_2 \text{ is the mass of atom '2' in the bond}$$
Kinetic isotope effect

Origin of KIE – Mainly due to difference is zero-point energy (ZPE) between non-labelled and labelled compounds.


Assumptions

1. Isotope labelling only effects mass dependent properties, most importantly vibrational frequencies (\(\nu\)).
2. Activated complex (TS) has a very low force constant (\(k\)) thus the the whole reaction profile is not lowered upon labelling (the stretching becomes translation, i.e. bond fully broken).

<table>
<thead>
<tr>
<th>frequency (cm(^{-1}))</th>
<th>ZPE (kcal/mol)</th>
<th>rel. rate (300 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C – H stretch</td>
<td>2900</td>
<td>4.15</td>
</tr>
<tr>
<td>C – D stretch</td>
<td>2100</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Takeaway: biggest KIE expected with D labelling – biggest relative change in \(m\) (H to D: \(m\) doubles, \(^{12}\)C to \(^{13}\)C: \(m\) increases only by 8%)

Case study B: elimination reactions

\[\text{Me}_2C\text{Br} + \text{NaOEt} \rightarrow \text{Me}_2C\text{=C} + \text{NaBr}\]
KIE = 6.7 consistent with E\(_2\) mechanism

\[\text{Me}_2CD\text{Br} + \text{NaOEt} \rightarrow \text{Me}_2C\text{=C}D + \text{NaBr}\]

A more nuanced analysis

Assumption 2 is an oversimplification. The bond is only partially broken in TS.

TS’s have their own ZPE! (because they retain appreciable k)

Hammond postulate [paraphrased]: TS structure resembles the molecule that it is closest in energy to.

Exothermic

Endothermic

Thermoneutral

ΔG is small
Small KIE

ΔG is small
Small KIE

ΔG is large
Large KIE

Experimental demonstration

MeNO₂ + R NH₂ ⇌ Me(NO₂)⁺ + R NH₃⁺

ΔpKₐ = 0

**Kinetic isotope effect**

**Heavy atom primary KIE**

<table>
<thead>
<tr>
<th>Nucleid substitution</th>
<th>C–H / C–D</th>
<th>C–H / C–T</th>
<th>$^{12}\text{C} / ^{13}\text{C}$</th>
<th>$^{12}\text{C} / ^{14}\text{C}$</th>
<th>$^{14}\text{N} / ^{15}\text{N}$</th>
<th>$^{16}\text{O} / ^{18}\text{O}$</th>
<th>$^{32}\text{S} / ^{34}\text{S}$</th>
<th>$^{35}\text{Cl} / ^{37}\text{Cl}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>typical $1^\circ$ KIE value</td>
<td>6-8</td>
<td>15-16</td>
<td>1.03-1.05</td>
<td>1.07</td>
<td>1.03</td>
<td>1.02</td>
<td>1.01</td>
<td>1.01</td>
</tr>
</tbody>
</table>

How to measure, for example, $^{12}\text{C} / ^{13}\text{C}$ $1^\circ$ KIE?

$$\text{SM}^{(12}\text{C}) \xrightarrow{k^{(12}\text{C})} \text{P}^{(12}\text{C}) \quad \text{KIE} = \frac{k^{(12}\text{C})}{k^{(13}\text{C})} \quad \text{SM}^{(13}\text{C}) \xrightarrow{k^{(13}\text{C})} \text{P}^{(13}\text{C})$$

Problems with absolute rates measurement:
1. Difficult and expensive synthesis of labelled compound.
2. Difficulty with obtaining accurate kinetic data (error is in the order of KIE).

How about intermolecular competition experiment?

$$\text{SM}^{(12}\text{C}) + \text{SM}^{(13}\text{C}) \xrightarrow{k^{(12}\text{C}} \xrightarrow{k^{(13}\text{C)}} \text{P}^{(12}\text{C}) + \text{P}^{(13}\text{C})$$

Advantages:
1. P and SM distribution can be accurately determined.
2. Initial enrichment can be arbitrary even natural abundance!

A general solution for $^{13}\text{C}$ and also $^2\text{H}$ (D): **Prof. Dan Singleton's experiment**

$$\frac{R}{R_0} = (1 - F) \frac{1}{\text{KIE}}$$

- $R$ recovered SM's isotope ratio
- $R_0$ original isotope ratio of SM (e.g. natural abundance)
- $F$ conversion

Example:
KIE of 1.05 at 99% conversion results in 24.5% enrichment of SM.

Reaction approaches completion ($F \to 1$)

$R/R_0$ approaches $\infty$

KIE becomes greatly magnified in the observable $R/R_0$

---

**Kinetic isotope effect**

### Case study 1: Diels-Alder reaction

- Me + Me → 98.9% conv.  
  xylenes  
  25 °C

\[
\text{KIE}_C = \frac{k^{(12)C}}{k^{(13)C}} = 1.00 \\
\text{KIE}_H = \frac{k^{(1)H}}{k^{(2)H}} = 1.00
\]

### Key observations

- KIE for C₂, C₃, H₃ are very small: expected with nonreacting centres.
- KIE difference between H₁(Z) and H₄(Z) indicate asynchronous TS in which bond forming at C₁ is more complete at the TS than the bond at C₄. [refutes previous calc. and experimental synchronous TS]

### Limitations

- Scalable (for ²H NMR), irreversible reaction.  
  [above is done on 13 mol scale!]
- Mechanism doesn't change as reaction proceeds.
- F must be high, no side reactions.

### Case study 2: Dihydroxylation

**Corey–Criegee Mechanism**

\[
\text{[3+2]} \quad \begin{align*}
\text{O=Os} & \quad \text{L}^* \\
\equiv & \quad \text{R} \\
\rightarrow & \quad \text{O=Os} \quad \text{L}^* \\
\text{O-O} & \quad \text{R}
\end{align*}
\]

\[\text{[3+2]} \quad \text{Expected KIE is normal, large, and almost equivalent on both alkene C's.}\]

**Sharpless Mechanism**

\[
\text{[2+2]} \quad \begin{align*}
\text{O=Os} & \quad \text{L}^* \\
\equiv & \quad \text{R} \\
\rightarrow & \quad \text{O=Os} \quad \text{L}^* \\
\text{O-O} & \quad \text{R}
\end{align*}
\]

\[\text{[2+2]} \quad \text{Only one C atom should display large KIE, not both.}\]

### Measured KIE's

- 1.032
- 1.034

- 1.026
- 1.045

- 1.027
- 1.028

- 1.032
- 1.034

Large, normal KIE for both C's  
Consistent with [3+2]

---

Word of caution from Hartwig – a reminder of limitations (essay)

C-H functionalisation is well set for KIE measurements, but "the interpretation of a KIE is not as simple as the measurement of a KIE."

---

Problem:
"Many recent discussions of KIE data have concluded that C–H cleavage occurs during the RDS in cases when such a conclusion cannot be drawn from the experimental data."

---

A - Parallel reactions - 2 reactions with 2 different substrates

<table>
<thead>
<tr>
<th>C-H functionalisation</th>
<th>KIE = $k_H / k_D$ (def.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_H$</td>
<td></td>
</tr>
<tr>
<td>$k_D$</td>
<td></td>
</tr>
</tbody>
</table>

Advantages
- Generally the only type that gives conclusive information on whether the C-H bond cleavage occurs during the RDS

Limitations
- Accuracy of rate constant measurement is accuracy of KIE (consider induction periods and catalyst decomposition)

---

B - Intermolecular competition - 1 reaction with 2 different substrates

<table>
<thead>
<tr>
<th>C-H or C-D functionalisation</th>
<th>KIE = $[P_H] / [P_D]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_H$</td>
<td>$P_D$</td>
</tr>
</tbody>
</table>

Advantages
- Exact same conditions - no experimental error
- Greater precision of $[P_H] / [P_D]$ than $k_H / k_D$

Limitations
- Does not provide the same information as A
- If there's no isotope effect C–H cleavage is not RDS, but presence of primary KIE doesn't mean it's the RDS!

---

C - Intramolecular competition - 1 reaction with 1 substrate

<table>
<thead>
<tr>
<th>C-H or C-D functionalisation</th>
<th>KIE = $[P_H] / [P_D]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_H$</td>
<td>$P_D$</td>
</tr>
</tbody>
</table>

Advantages
- Gives precise data
- Simple way to rule out if C–H cleavage is RDS

Limitations
- same as B

---

Mechanistic scenarios – important selected cases

N.B. "RDS can change during the reaction, and can change for the same reaction under different conditions (even T, p) without changing the elementary steps by which the reaction proceeds or the rate constants for individual steps."

C–H bond cleavage step is irreversible and is the RDS of the overall process

A: no rate change by C–H to C–D substitution
B, C: both measure product distribution that is a results from a difference in the rate of an irreversible C–H bond-cleavage step

A: no KIE. B, C: primary KIE observed.

C–H bond cleavage step is irreversible but this step occurs after an RDS

A: no rate change by C–H to C–D substitution
B: equal chance to react with C–H and C–D SM’s
C: difference in rate of C–H and C–D functionalisation

A, B: no KIE. C: primary KIE observed.

**Kinetic isotope effect**

**Model studies**

```
Model studies

PhSeCl
91%

Ph₃SnH
AIBN
95%

fredericamycin A
```

- Antitumor antibiotic agent isolated from *Steptomyces griseus*
- In vitro activity against Gram-positive bacteria
- In vivo activity against P388 tumor cell lines
- First synthesis in 1994 by Derrick L. J. Clive *et al.*

**Problem:** Facile HAT by radical intermediate

```
Problem: facile HAT by radical intermediate
```

**Solution:** Exploit primary KIE

```
Solution: exploit primary KIE
```

- Possibly first example of KIE application in total synthesis
- Final route didn’t use CD₃ as the protecting group

---

Kinetic isotope effect

![Diagram of chemical reaction]

- FR900482 (R = CHO)
- FR66979 (R = CH₂OH)

transient intermediate capable of DNA cross-linking

stabilised core [previously synthesised]

unstabilised core [unknown]

Mitomycin C
potent antitumor agent

- potent antitumor antibiotics but have serious side-effects in humans
- some members of this family held significant promise in Japanese trials

Key observation: indole CH is always exchanged

Solution: protect indole CH by exchange to CD

Kinetic isotope effect

- Strong inhibition on the growth of P-388 murine leukemia cell lines
- Overall yield of 3.5% (average of 92% yield each step) by M. Miyashita et al.

norzoekanthmine

1. DIBAL
2. Ph₃PCD₃Br
3. 9-BBN
then H₂O₂

17 steps

norzoekanthmine

6 steps

1. TPAP
NMO
2. NaClO₂

81%
+ 9% [1,5]-D shift
**Kinetic isotope effect**

1. **MeMgBr then DIBAL then LiAlD₄**
   - OTES
   - 1. MeMgBr then DIBAL then LiAlD₄
   - NaHMDS TBSCI 94%
   - DMDO 49%


---

**N-methylwelwitindolinone C**

R¹ = −NCS or −NC
R² = H or OH

- promising lead for the treatment of drug-resistant tumors
- reverses P-glycoprotein mediated multiple drug resistance to a variety of anticancer drugs in cancer cell lines

1. **1st gen. route**
   - 1. i-Bu₂AlH
   - 2. Cl₃CC(O)NCO

   - AgOTf Phl(OAc)₂
   - 33%
   - 25%

2. **2nd gen. route**
   - 1. LiEt₃BD
   - 2. Cl₃CC(O)NCO

   - AgOTf Phl(OAc)₂
   - 60%
   - 8%

Kinetic isotope effect

Advanced topics for further reading:

- Non-Linear Transition States' effect on primary KIE

\[
\begin{align*}
\text{A} & \xrightleftharpoons{+} \text{H} \xrightarrow{\text{vs.}} \text{B} \\
\end{align*}
\]

- Secondary KIE and its origin

\[
\begin{align*}
\text{OTf} \text{H} \text{H} \text{H} \text{H} \text{H} & \xrightarrow{\text{vs.}} \text{OTf} \text{D} \text{D} \text{D} \text{D} \\
\end{align*}
\]

\[
\begin{align*}
\text{AcOH} & \quad \text{KIE} = 2.06 \\
\end{align*}
\]

- Tunneling

\[
\begin{align*}
\text{H} \text{H} \text{H} \text{H} & \xrightleftharpoons{+} \text{Ph} \text{Se} \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{KIE} = 70 \\
\end{align*}
\]

- Equilibrium Isotope Effects (thermodynamic isotope effect)

\[
\begin{align*}
\text{H}_3\text{C} & \xrightleftharpoons{K = 1} \text{CH}_3 \text{CH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{K = 1.042} \\
\end{align*}
\]

- Computational KIE

Acknowledgement

I would like to thank Eugen E. Kwan for allowing me to use his lecture notes and providing me with his chemdraw files.

Further references and presentations


