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1 Mechanism of Cobalamin-Mediated Reductive
2 Dehalogenation of Chloroethylenes

3
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1 Abstract

2 Reductive dehalogenation involving cobalamin has ~~been proved to be~~ a promising strategy for
3 decontamination of polluted environments. However, cob(I)alamin can act both as a strong
4 reductant and a powerful nucleophile, ~~and~~ thus several competing dehalogenation pathways may
5 be involved. This work uses experimentally calibrated density functional theory on realistic
6 cobalamin models to resolve controversies of cobalamin-mediated reduction of chloroethylenes
7 by exploring mechanisms of electron transfer, nucleophilic substitution, and nucleophilic addition.
8 The computational results provides molecular-level insight into the competing pathways for
9 chloroethylenes reacting with cob(I)alamin: the computed ratios of inner-sphere to outer-sphere
10 pathways for perchloroethylene and trichloroethylene are 17:1 and 3.5:1 respectively in accord
11 with corresponding experimental ratios of > 10:1 and > 2.3:1, while the computed outer-sphere
12 pathway for other less-chlorinated ethylenes is hampered by high barriers (> 25 kcal/mol). Thus,
13 a new mechanistic picture ~~has been~~ obtained, ~~that~~ the highly-chlorinated ethylenes primarily
14 react via an inner-sphere nucleophilic-substitution pathway, ~~while-whereas~~ the less-chlorinated
15 ethylenes mainly react through an inner-sphere nucleophilic-addition pathway. ~~Especially, the~~
16 ~~Quantitative comparison of standard reduction potentials between-of~~ the formed chlorinated
17 cobalamin and cob(II)alamin/cob(I)alamin couple can be used to distinguish whether the inner-
18 sphere pathway ~~could-can~~ proceed or not, and ~~the~~ linear free energy relationships have been
19 developed to predict the reductive dehalogenation reactivity within a given mechanism.
20 ~~Furthermore~~ Finally, we ~~have~~ proposed new dual isotope analyses for distinguishing the ~~various~~
21 environmental dehalogenation mechanisms.

1 **KEYWORDS:** reductive dehalogenation, cobalamin, ~~competing pathways~~, density functional
2 theory, ~~standard~~ reduction potential, inner-sphere pathway, dual isotope analyses

3

4 **Introduction**

5 The widespread industrial application of halogenated compounds as solvents, chemical
6 intermediates and pesticides are of great environmental concern, ~~with due to the~~ adverse effects
7 on ecosystems and human health.¹⁻² The negative effects of halogenated compounds are generally
8 attributed to the halogen atoms; thus, the transformation of halogenated compounds to less- or
9 non-halogenated products is a promising remediation strategy.³⁻⁴ Among various dehalogenation
10 remediation strategies, cobalamin (vitamin B12) promoted dehalogenation reactions have garnered
11 considerable attention, owing to evidence that the cell component responsible for dehalogenation
12 reactions by several anaerobic bacteria is most likely this transition-metal coenzyme.⁵

13 Cobalamin is the largest by molecular mass and arguably the most complex (in terms of
14 functional groups) cofactor in biology, consisting of a cobalt atom coordinated by four nitrogen
15 atoms of the corrin ring, as shown in **Scheme 1**. Under non-reducing conditions, the cobalt atom
16 commonly exists in the +3 oxidation state (cob(III)alamin), axially coordinating two ligands
17 (methyl or cyanide group in the “upper” and 5,6-dimethylbenzimidazole (DMB) in the “lower”).⁶⁻

18 ⁷ In abiotic systems, cob(III)alamin can be reduced to 4-coordinated cob(I)alamin without axial
19 ligands in the presence of strong reducing agent in aqueous media,⁸⁻⁹ ~~while this model-These~~
20 ~~model~~ systems mimicking microbial dehalogenation ~~has have~~ been used in abiotic remediation
21 strategies for treatment of contaminated field sites.¹⁰ Compared with the rigorous selectivity of
22 enzymatic systems, *in vitro* ~~studies have shown that~~ cob(I)alamin can catalyze nonspecific
23 reductive dehalogenation of many halogenated compounds, such as chlorinated methanes, ethanes,

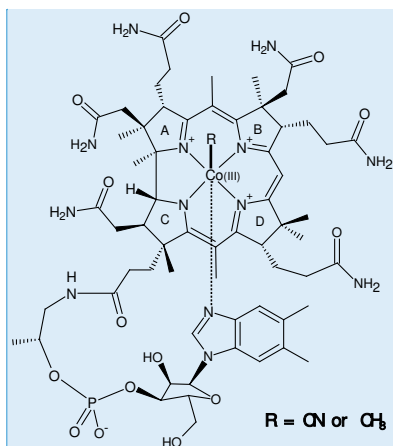
Commented [KPK1]: This refers to previous sentence, i.e. the cob(I)alamin. Is this correct? Otherwise specific which type of model systems

1 higher alkanes, ethylenes, arenes, etc.¹¹⁻¹² Thus Accordingly, the abiotically reductive
2 dehalogenation by cobalamin has substantial potential for use in remediation approaches;^{10, 13-14}
3 while some other transition metal complexes (e.g. iron porphyrin) as well as nano-scale zero-valent
4 metal systems* (e.g. iron, zinc, et al etc.) have also been reported to be potentially good useful
5 reductive dehalogenation catalysts.¹⁵⁻¹⁷ Practically, degradation of halogenated compounds by
6 reductive dehalogenation reactions may occur via numerous routes. In order to assess whether the
7 dehalogenation brings about significant detoxification, the fundamental knowledge of the
8 reductive dehalogenation mechanisms involving cobalamin is essential. However, cob(I)alamin
9 contains the unusual combination of properties that it is of being both a strong reductant,¹⁸ and one
10 of the most powerful nucleophiles that is $\sim 10^4$ times more nucleophilic than the Cl anion in S_N2
11 reactions,¹⁹ which makes the reaction modes more diverse and complex.

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12

13 **Scheme 1.** Structure of the Cobalamin



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14

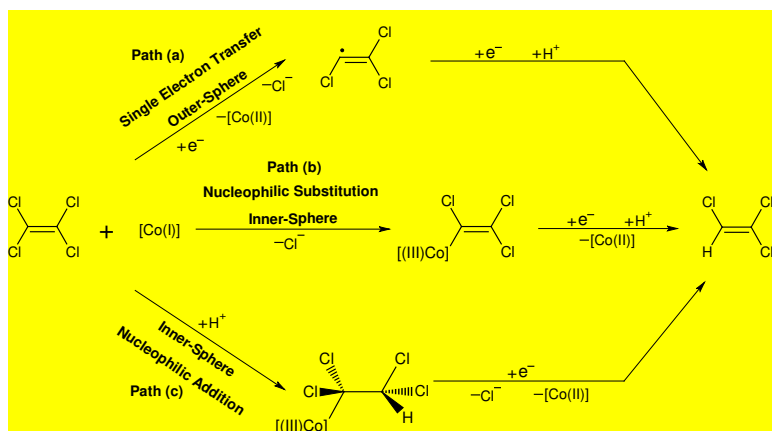
15

1 Among various halogenated compounds, the mechanistic study of cobalamin-mediated
2 reductive dehalogenation of chloroethylenes has attracted particular attention.¹¹ Cob(I)alamin has
3 been reported to participate in the sequential dehalogenation of perchloroethylene (PCE),
4 trichloroethylene (TCE), *cis*-1,2-dichloroethylene (*cis*-DCE), *trans*-1,2-dichloroethylene (*trans*-
5 DCE), and vinyl chloride (VC), resulting in the production of nontoxic ethylene.^{9, 20-25} As shown
6 in **Scheme 2**, the initial step for cob(I)alamin-catalyzed chloroethylenes can be summarized as an
7 outer-sphere (reaction occurring between chemical species with remaining in a non-connected state)
8 or an inner-sphere (with a chemical bond forming between chemical species during the reaction)
9 process, which can be categorized in more detail more specifically as single electron-transfer
10 [outer-sphere (path a)], nucleophilic substitution [inner-sphere (path b)], and nucleophilic addition
11 [inner-sphere (path c)] mechanisms. Most previous work focused on the reductive dehalogenation
12 mechanism of highly chlorinated substances, PCE and TCE.^{9, 21, 23-25} On the basis of kinetic
13 experiments, the pH-independent rate constants were observed, which may rule out inner-sphere
14 nucleophilic addition route proceeding with simultaneous protonation.²¹⁻²² Meanwhile,
15 cob(I)alamin-mediated dehalogenation of PCE and TCE with increasing amounts of *d*₇-
16 isopropanol, a D• donor, resulted in as at most 10% of the PCE-derived deuterated products and
17 30% of the TCE-derived deuterated products, in agreement with outer-sphere one-electron
18 transfer.²¹ However, this outer-sphere mechanism is not in accord with stereochemical results that
19 the dehalogenation of TCE by cob(I)alamin produces greater amount of *cis*-DCE compared to
20 *trans*-DCE (> 15:1), markedly different from the ratio obtained with identified electron-transfer
21 reagents (< 5:1).²³ Supporting for the nucleophilic substitution mechanism originated from
22 experimental observation that the of molecular mass consistent with dichlorovinylcobalamin had
23 been observed in mass spectra during the TCE dehalogenation reaction.²⁵ If this nucleophilic

1 substitution mechanism works for PCE with cob(I)alamin as well, the trichlorovinylcobalamin
2 from [the](#) PCE dehalogenation reaction should be detected, but this has not been the case.¹¹

3 The experimental work performed with isopropyl alcohol-d₇ at different concentrations has
4 showed that, in contrast to PCE and TCE, there ~~were not marked~~ [were only few](#) deuterated products
5 captured [for the cobalamin-catalyzed reductive dehalogenation of less-chlorinated substances, *cis*-](#)
6 [DCE, *trans*-DCE, and VC.](#) ~~It~~ [This](#) indicates [that](#) there is not significant quantities of free radicals
7 produced ~~in~~ [during](#) the dehalogenation process. Meanwhile, ~~the~~ kinetic experiments ~~have show~~
8 that cobalamin reductively dehalogenated ~~d~~ *cis*-DCE, *trans*-DCE, and VC in pH-dependent
9 reactions.²²

10
11 **Scheme 2.** Alternative Reaction Mechanisms for Reductive Dehalogenation of Chloroethylene
12 Catalyzed by Cobalamin^a



13
14 ^a taking PCE as an example

15
16 Isotope fractionation ~~in~~ [during](#) reductive dehalogenation of chloroethylenes with cobalamin
17 has been investigated using compound specific isotope analysis (CSIA).^{9, 26-31} CSIA ~~is able to~~

1 offers new insight into the organic pollutant degradation mechanism, especially when the
2 competing reaction pathways are unknown.³²⁻³³ In the process of cobalamin-mediated reductive
3 dehalogenation of chloroethylenes, most previous work focused on stable carbon isotopes, and
4 stable chlorine isotopes were not commonly studied so often. For example, the reported carbon
5 bulk isotope fractionation factors (ϵ_{bulkC}) are -15.8‰ for PCE²⁶ and -16.1‰ for TCE⁹; however,
6 in contrast to cobalamin-catalyzed PCE and TCE, the reported values for *cis*-DCE (-25.5‰)²⁷ and
7 VC (-31.1‰)²⁸ are much larger, which may indicate an entirely different reductive dehalogenation
8 mechanism. Until now, no systematic computational investigation of isotope fractionation within
9 the different operative pathways for reductive dehalogenation of chloroethylenes in the presence
10 of cobalamin has been undertaken although such investigation should shed light onto this ongoing
11 mechanistic debate.

12 Computational analysis of the catalytic mechanism can provide insight into the electronic
13 structure features governing reaction mechanisms,³⁴⁻⁴³ which has already been performed to
14 give Such studies have provided insight into the viability of various intermediates and pathways in
15 the reductive dehalogenation of chloroethylenes with cobalamin⁴⁴⁻⁴⁶ as well as synthetic
16 cobaloximes.⁴⁷⁻⁴⁸ More specifically, The computed electrochemical properties of the reduced
17 chloroethylenes and chlorinated-cobalamins have been useful for interpreting some experimental
18 observations, such as g. indicating that the formed chlorinated vinyl radicals during reductive
19 dehalogenation may be reduced to anionic forms competing with their rebound to cob(I)alamin to
20 produce vinylcobalamins.⁴⁴ It is noteworthy that Notably, most work has used the cobalamin
21 structure simplified through cutting off the corrin model without side chains and replacing the axial
22 DMB base with imidazole instead of DMB for studying the mechanism of reductive
23 dehalogenation, yet these substituents can affect cobalamin electronic structure substantially, so

Commented [KPK4]: I suggest citing my paper Kepp 2014 (reference 55 in the current version) where I study full cobalamins and show that these side chains are important.

1 ~~while~~ the influence of omitting the substituents and ~~substitution of~~ using the simpler axial base
2 needs to be addressed. Until now, the precise reductive dehalogenation mechanism has not been
3 established in details that would warrant explanation of all experimental observations collected so
4 far.

5 In this work we use density functional theory (DFT) on realistic full cobalamin structures to
6 address the following unsolved mechanistic questions: (i) why pH-independent rate constants
7 have only been observed for the reactions of PCE and TCE with cobalamin, but not for cobalamin-
8 catalyzed reactions of *cis*-DCE, *trans*-DCE, and VC; (ii) why there is conflicting evidence for the
9 dehalogenation processes of cobalamin-mediated PCE and TCE favoring either the outer-sphere
10 ~~pathway or the~~ inner-sphere pathway, respectively; (iii) why only dichlorovinylcobalamin has
11 been detected during upon reaction of TCE with cobalamin, but not trichlorovinylcobalamin in the
12 reaction of cobalamin-mediated PCE; (iv) whether the comparison between calculated kinetic
13 isotope effects (KIEs) and experimental apparent kinetic isotope effects (AKIEs) can be used to
14 identify the competing dehalogenation pathways.

16 Computational Methodology

17 ~~The System for Cobalamin-Mediated Chloroethylenes~~ Computational Details. All
18 calculations in this work were performed with the Gaussian 09 Revision D.01 program package.⁷⁰

19 The complete 4-coordinated cob(I)alamin species (with the nucleotide loop ~~is clipped off~~),
20 ~~was were~~ used as ~~initial structure~~ basis for our computational work. The geometry optimizations
21 and frequency analyses ~~have been were~~ carried out in the gas phase using the Perdew-Burke-
22 Ernzerhof (PBE) functional,⁴⁹⁻⁵⁰ combined with Ahlrich's TZV basis set⁵¹ for Co and 6-31G**
23 basis set⁵² for C, N, H, O and Cl (denoted ~~as~~ BSI). The PBE functional has been previously shown

Commented [KPK5]: ?

Has only dichlorovinylcobalamin been seen when TCE reacts with Cbl, and only trichlorovinylcbl with PCE? This sentence is unclear

Commented [KPK6]: Header is unclear; "cobalamin-mediated chloroethylenes" simple suggestion made

1 to accurately reproduce the experimental frequencies of the corrin-based normal modes of
2 vibration,⁸ and yields geometries in according to experimental cob(I)alamin structural parameters
3 from XAS studies,⁵³⁻⁵⁴ and accurate Co-C bond dissociation energies⁵⁵. Subsequent frequency
4 calculations were run to confirm that all ground states had only real frequencies, whereas the
5 transition states had one imaginary frequency. The computed vibrational frequencies were further
6 used to quantify the zero-point energy correction (ZPE), and enabled us to convert the electronic
7 energy to the Gibbs free energy at 298.15 K and 101.325 kPa. The intrinsic reaction coordinate
8 (IRC) calculations were performed further to verify that the transition states really-actually
9 connected the reactants and products. Based on the PBE-optimized structures in the gas phase, the
10 water solvation effects were calculated by COSMO continuum-solvation model (CPCM, dielectric
11 constant = 78.3) at the PBE/BSI level of theory. The dispersion interactions were considered
12 through doing included via single-point calculations at the PBE-D3/BSI level of theory, due to the
13 standard DFT method lacking of such interactions.⁵⁶ Unless otherwise specified, all relative free
14 energies for cobalamin-mediated chloroethylenes reactions reported were based on PBE/BSI data
15 including solvation and D3 dispersion corrections ($\Delta G + E_{\text{solv}} + E_{\text{disp}}$). We also performed PBE-
16 D3 geometry optimizations with CPCM in water solution on five structures of cob(I)alamin,
17 cob(II)alamin, base-off trichlorovinylcob(III)alamin, base-on trichlorovinylcob(III)alamin and
18 base-on trichlorovinylcob(II)alamin to verify ~~test~~ the ~~reliance~~-reliability of the above
19 ~~method approach~~, the results of which show that The results show that the effects of dispersion
20 and solution is negligible on the optimized geometries are negligible (the geometrical comparison
21 between PBE/BSI optimized structures in the gas phase and PBE-D3-CPCM/BSI optimized
22 structures is shown in **Figure S9** in of the Supporting Information).

Commented [KPK7]: If this sentence is kept XAS should be defined

Commented [KPK8]: Cite instead the 2010 reference for Grimme's D3

1 Cobalamin chemistry is generally low-spin, as implied by the ground state of cob(I)alamin
2 and cob(III)alamin with singlet state and cob(II)alamin with doublet state.^{6, 8, 57} As further proof,
3 ~~the calculations in this work at the~~Supporting this, the PBE/BSI level of theory free energy
4 including solvation and D3 dispersion corrections ~~show for~~ cob(I)alamin and cob(III)alamin (base-
5 off trichlorovinylcob(III)alamin) ~~on-reveal~~ triplet states ~~are at~~ 21.3 and 18.4 kcal/mol ~~respectively~~
6 higher energy, respectively, than their singlet state species, while the cob(II)alamin ~~on-high-spin~~
7 quartet state is 29.1 kcal/mol ~~higher than its above the low-spin~~ doublet state species; thus, in this
8 work these species were all optimized in the low-spin state.

9 Particular attention ~~has been was~~ focused on the electronic structure of cob(I)alamin. Previous
10 computational studies have investigated the electronic structure of ~~four~~4-coordinated cob(I)alamin
11 ~~without the axial DMB base and five~~5-coordinated cob(I)alamin with the axial DMB base using
12 DFT and CASSCF calculations.^{8, 19, 58} ~~The~~TD-DFT calculations on truncated ~~four~~4-coordinated
13 cob(I)alamin ~~have suggested~~suggest that ground state cob(I)alamin is purely a closed-shell singlet
14 d⁸ species.⁸ ~~Subsequent~~ CASSCF calculations ~~on the truncated cob(I)alamin~~ have shown that the
15 dominant contribution to the ground state wave function is the closed-shell singlet d⁸ Co(I)
16 configuration for ~~four~~4-coordinated cob(I)alamin,^{19, 58} and for ~~five~~5-coordinated cob(I)alamin
17 with ~~very a~~ weakly coordinated axial base,⁵⁸ while cob(I)alamin is ~~mainly dominantly the~~ open-
18 shell singlet/triplet d⁷ Co(II)-corrin (π^*)¹ diradical configuration with ~~a~~ strongly coordinated axial
19 base.⁵⁸ ~~Then, the~~ reaction mechanisms of methyl transfer between cob(I)alamin and CH₃-
20 ~~H₂Folate-H₂folate~~ catalyzed by methionine synthase ~~were revealed~~have been studied
21 computationally, with the ~~four~~4-coordinated base-off cob(I)alamin conformation for studying the
22 S_N2 pathway and the ~~five~~5-coordinated base-on cob(I)alamin conformation for studying the
23 electron-transfer pathway, giving similar barriers for these two pathways.³⁶ However, ~~while~~

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1 cob(I)alamin ~~only existing~~ in the enzyme can coordinate to the axial DMB base ~~due to the H-~~
2 ~~bonding between the base and nearby amino acid residues.~~ The X-ray absorption spectroscopy ~~ie~~
3 ~~experiments~~⁵⁴ ~~as well as~~ and DFT computations⁸ have shown that cob(I)alamin in solution is not
4 axially coordinated to DMB base at all, ~~which is proved again in this work that PBE calculations~~
5 ~~show the complete five coordinated cob(I)alamin on open shell singlet/triplet state is about 22~~
6 ~~kcal/mol higher in free energy at PBE/BSI level of theory including solvation and D3 dispersion~~
7 ~~corrections than the complete four coordinated cob(I)alamin on close shell singlet state plus DMB~~
8 ~~base in solution.~~ Supporting the strict low-spin closed-shell configurations as found in our study.
9 Furthermore, ~~in this work, the closed shell~~ the singlet stability of the ~~complete four~~4-coordinated
10 cob(I)alamin was prob~~ed~~ed by mixing ~~the highest occupied molecular orbital (HOMO) and lowest~~
11 ~~unoccupied molecular orbital (LUMO) from PBE orbitals using PBE functional, and the by~~
12 CASSCF single-point calculation on the complete ~~(not truncated) four~~4-coordinated cob(I)alamin.

13 Many studies have ~~also~~ shown the experimentally calibrated PBE and BP86 methods are
14 accurate for ground-state thermochemistry of cobalamins,⁵⁹ ~~partly because~~ and the required active
15 spaces from CASSCF e.g. CASPT2 methods are out of range to be computationally tractable, ~~and~~
16 partly because of the basis set requirements. ~~To summarize what has been mentioned~~
17 ~~above~~ Accordingly, this work focuses on the ~~four~~4-coordinated cob(I)alamin ~~on~~ with the closed-
18 shell singlet state using PBE ~~method~~ to study the reductive dehalogenation mechanisms.

19 **Electrochemical Properties of Chlorinated Cobalamin.** The aqueous-phase standard
20 reduction potentials (E^0) referenced with respect to the standard calomel electrode (SCE) (E^0 vs.
21 SCE, unit: V) of all chlorinated vinyl- and ethyl-cobalamins as well as ~~the~~
22 cob(II)alamin/cob(I)alamin couple were converted from the aqueous-phase adiabatic electron

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The new SI data are not discussed with references to Tables.

Right now only a few SI tables are mentioned.

Is it a journal policy that all SI tables and figures should be referenced in order? Some journals have that.

1 affinities (AEA), while the absolute reduction potential of SCE for cobalamin is applied was
2 4.52 V as recommended before,⁶⁰ as shown in eq 1:

$$3 \quad E^0 \text{ vs. SCE (V)} = \text{AEA} - 4.52 \quad (1)$$

4 The AEA values were in the form of free energy changes, with using the PBE single-point
5 calculations obtained from the PBE/BSI-optimized geometries using the larger 6-311+G(2d,2p)
6 basis set⁶¹ for main group atoms and TZV for cobalt (denoted as BSII), including water-solvation
7 energy and D3-dispersion corrections (BSII level of theory), and free energy corrections (BSI level
8 of theory). The vertical electron affinities (VEA) for the base-off and base-on less-chlorinated
9 ethylcobalamins were obtained from PBE/BSII/BSI single-point calculations with water solvation
10 and D3 dispersion corrections. Note for cob(II)alamin and all chlorinated-cob(II)alamins ~~on~~ with
11 doublet states, $S_{\pm 2}$ values after annihilation are range from 0.7500 to 0.7502, thus i.e. there are
12 no spin contaminations of the wavefunctions for all doublet cob(II)alamin of these species.

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13 **Electrochemical Properties and Electrophilic Reactivity of Chloroethylenes.** All
14 calculations for the electrochemical properties of chloroethylenes were performed using the
15 PBE/aug-cc-pVTZ level of theory (BSIII) with CPCM solvation model of aqueous solution
16 (dielectric constant = 78.3). VEA for all chloroethylenes are electronic energy differences in
17 aqueous phase water, while adiabatic electron affinities for all vinyl radicals are aqueous phase
18 free energy change in waters. The adiabatic electron affinities of vinyl radicals were translated into
19 aqueous-phase E^0 vs. SCE. The electrophilic index (ω), developed from based on the concept of
20 the hard and soft acids and bases (HSAB),⁶²⁻⁶⁴ were was calculated to characterize the electrophilic
21 reactivity of chloroethylenes. In order to calculate the electrophilic index (ω), firstly the highest
22 occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies
23 were calculated at the BSIII level of theory in the gas phase, thus From these, it is possible to

Commented [KPK11]: HOMO was already mentioned previously; explain at first incidence

1 quantify the three basic HSAB parameters as follows: hardness (η) as $(E_{\text{LUMO}} - E_{\text{HOMO}})/2$, softness
2 (σ), defined as the inverse of hardness ($1/\eta$), and the chemical potential (μ), as $(E_{\text{LUMO}} + E_{\text{HOMO}})/2$.
3 Then, the electrophilic index (ω) was calculated as μ^2/η .

4 **Kinetic Data.** The reaction rate constant ~~of~~ for reaction of the chloroethylenes with
5 cob(I)alamin, k , and the corresponding free energy of activation, ΔG^\ddagger , ~~can be~~ converted to each
6 ~~other according to~~ related by the Eyring equation (eq 2):

$$k = \frac{k_B T}{h} \cdot \frac{1}{c^0} \exp\left(-\frac{\Delta G^\ddagger}{RT}\right) \quad (2)$$

8 where k_B is the Boltzmann constant, h is the Planck constant, R is the gas constant, T the tempe-
9 rature in Kelvins, and c^0 is the concentration defining the standard state (typically 1 mol/L). This
10 equation was used to estimate relative rate constants from activation barriers.

11 **Isotope Effects.**⁶⁵⁻⁶⁷ The ~~obtained~~ Hessians obtained from the ~~above-mentioned~~ frequency
12 calculations after geometry optimizations were used to calculate kinetic isotope effects (KIEs) with
13 using the ISOEFF package.⁶⁸ KIEs were obtained according to the Bigeleisen equation at 298 K
14 for the transition from two separate reactants to the corresponding transition state. The apparent
15 kinetic isotope effects (AKIE) value ~~of~~ from experiments can be approximated ~~from~~ using the bulk
16 isotope fractionation factors (ϵ_{bulk}) by eq 3:

$$\text{AKIE} \approx \frac{1}{1 + n/x \cdot z \cdot \epsilon_{\text{bulk}}} \quad (3)$$

18 where n is the number of atoms of the considered element, x is the number of atoms of the
19 considered element at the reactive position, and z is number of atoms of the considered element in
20 intramolecular isotopic competition.³³ It should be noted that in this form the secondary isotope
21 effects are neglected, an assumption that ~~should be plausible~~ is reasonable for chlorine KIEs.⁶⁹

Commented [KPK12]: Just a point perhaps to mention: These calculations assume 1) Koopman's theorem applies to make HOMO /LUMO resemble IP and EA, and 2) the finite difference approximations to hardness and chemical potential viz. the Mulliken electronegativity

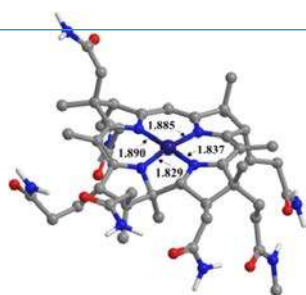
Commented [KPK13]: I assume?

1 ~~All calculations in this work were performed with the Gaussian 09 Revision D.01 program~~
2 ~~package.~~⁷⁰

4 Results and Discussion.

5 Calibration vs. Experimental Data.

6 ~~Based on DFT optimization results with the PBE functional, Our DFT computations show that~~
7 two-electron reduction of ~~the~~ complete model of cob(III)alamin to cob(I)alamin leads to both the
8 axial methyl and DMB groups leaving ~~off, providing to produce~~ the 4-coordinated cob(I)alamin
9 species ~~(, as shown in Figure 1), as expected~~. The corrin macrocycle in the complete cob(I)alamin
10 model is almost planar with averaged Co-N bond length of 1.86 Å. This is ~~in consistent with fits~~
11 the average Co-N bond lengths of 1.86 and 1.88 Å reported in two recent XAS studies.⁵³⁻⁵⁴ In
12 addition, the calculated standard reduction potential (E^0) value for the cob(II)alamin/cob(I)alamin
13 couple is -0.78 V vs. SCE, near to the corresponding experimental data of -0.85 V vs. SCE,¹⁸
14 further validating the reliability of the theory level used.



22 **Figure 1.** Chemical Structure along with Bond Lengths of Planar Co-N Bonds (Å) for
23 Cob(I)alamin.

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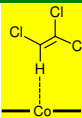
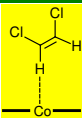
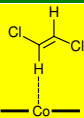
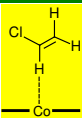
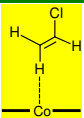
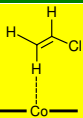
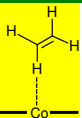
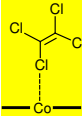
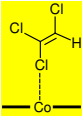
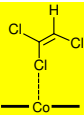
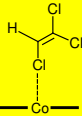
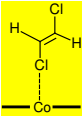
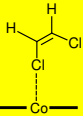
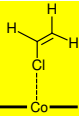
1 Recently, the dispersion-driven O-H...Pt²⁺ interaction between *trans*-[PtCl₂(NH₃)(N-
2 glycine)] and water ~~molecular~~ has been revealed experimentally at very low temperature,⁷¹ which
3 ~~supports~~ ~~indicating~~ that the d⁸ metal ions in complexes can act as H-bonding acceptors. Considering
4 ~~the fact~~ that the dominant contribution to the ground state is the closed-shell singlet d⁸ Co(I) for
5 cob(I)alamin, ~~a scientific question~~ ~~the possibility~~ arises ~~that whether that~~ the Co(I)...H interaction
6 between cob(I)alamin and a water ~~molecule~~ ~~molecular~~ can also be formed and consequently
7 influence the cob(II)alamin/cob(I)alamin reduction. ~~Then~~ ~~relevant~~ ~~computational~~
8 ~~computations~~ ~~calculations~~ ~~have been done~~,⁷² which ~~confirm~~ the possibility of forming ~~the a~~
9 Co(I)...H linkage between cob(I)alamin and water ~~molecular~~ ~~from thermodynamics~~, and ~~suggests~~
10 ~~that~~ this unusual Co(I)...H interaction may have ~~significant~~ catalytic relevance during the
11 reactivation cycle of ~~the~~ methionine synthase ~~enzyme~~.⁷³⁻⁷⁴ Therefore, the Co(I)...H as well as
12 Co(I)...Cl interactions between cob(I)alamin and chloroethylenes ~~have been~~ ~~were~~ explored in this
13 work. As shown in **Table 1**, all Co(I)...H and Co(I)...Cl bond formation processes between
14 cob(I)alamin and different chloroethylenes are endothermic in water solution based on the
15 PBE/BSI free energies including solvation and D3 dispersion corrections, which seem to be
16 nonspontaneous from thermodynamics. This is in accord with ~~previous computations suggesting~~
17 ~~the earlier computational study~~ that the Co(I)...H bond formation between cob(I)alamin and a
18 water ~~molecular~~ ~~molecule~~ ~~in water solution~~ ~~was~~ not favorable ~~in water solution where water~~
19 ~~water~~ ~~interactions~~ ~~are favored~~,⁷² and in line with previous experiments ~~indicating~~ ~~at work~~ ~~that~~
20 ~~there was~~ no such ~~observed~~ H-bonding in water solution.¹⁸ ~~Therefore, this work uses~~ ~~We thus used~~
21 the two separate reactants (i.e., cob(I)alamin and chloroethylene) as starting point to study the
22 reductive dehalogenation mechanisms in the following sections. However, ~~since~~ ~~the~~ ~~earlier~~
23 ~~computational~~ ~~computations~~ ~~work~~ showed that ~~the nonpolar solvent such as~~ chloroform (a solvent

Commented [KPK15]: Chloroform is polar just not as much as water

mimicking of with a dielectric constant similar to that of a typical protein environment) made makes the Co(I)⋯H bond formation process favorable/feasible, this unusual Co(I)⋯H or even Co(I)⋯Cl bonding needs to be considered for here in the context of reductive dehalogenation catalyzed by cob(I)alamin in enzyme.

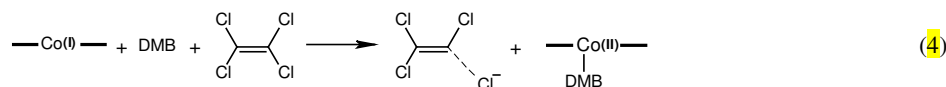
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Table 1. Computed Free Energies (kcal/mol) at the PBE/BSI level of theory with solvation and D3 dispersion corrections for Co(I)⋯H and Co(I)⋯Cl Bond Formation between Cob(I)alamin and Chloroethylenes in Water Solution

Co(I)⋯H							
ΔG	4.9	4.5	4.0	2.4	4.8	3.3	2.9
Co(I)⋯Cl							
ΔG	9.7	8.0	9.5	9.1	8.9	9.0	7.3

Outer-Sphere Electron Transfer Mechanism

We first focus on the outer-sphere electron transfer pathway of cob(I)alamin catalyzing chloroethylenes according to eq 4:



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1
2 **DMB: 5,6-dimethylbenzimidazole**

3 The free energy barriers of the outer-sphere electron transfer processes (ΔG^{\ddagger}_{ET}) can be estimated
4 from the Marcus theory⁷⁵⁻⁷⁸ (calculation details shown in the Supporting Information). The
5 obtained ΔG^{\ddagger}_{ET} , the free energy of reaction (ΔG_{ET}) ~~as well as~~ and the vertical electron affinities
6 (VEA) values for all chloroethylenes are shown in **Table 2**. Both ΔG^{\ddagger}_{ET} and ΔG_{ET} values increase
7 in the sequence PCE < TCE < *trans*-DCE < *cis*-DCE < VC, with increasing number of chlorine
8 atoms and decreasing of the ~~vertical electron affinities (VEA)~~ values. Then a linear free energy
9 relationship (LFER) between ΔG^{\ddagger}_{ET} and VEA values for the one-electron-transfer reaction was
10 ~~built~~ constructed. ~~As a result,~~ The VEA values ~~are~~ closely correlated ~~to~~ with the free energy
11 barriers with an r^2 of 0.940 ($\Delta G^{\ddagger}_{ET} = -67.02\text{VEA} + 104.11$) (in kcal/mol). Therefore, it is possible
12 to provide a computationally less demanding tool for preliminary evaluation of the free energy
13 barriers of the electron transfer process for cobalamin-mediated reductive dehalogenation of
14 halogenated compounds within one class.

15
16 **Table 2.** The Free Energies (kcal/mol) of the Electron-Transfer Reactions for Cobalamin-
17 Mediated Reductive Dehalogenation of Chloroethylenes, along with the Vertical Electron
18 Affinities (VEA, eV) of Chloroethylenes

	PCE	TCE	<i>cis</i> -DCE	<i>trans</i> -DCE	VC ^a
VEA	1.36	1.24	1.02	1.13	0.97
ΔG^{\ddagger}_{ET}	15.9	17.6	34.7	27.3	41.7
ΔG_{ET}	15.6	17.2	31.0	25.7	35.2

19 ^aVC: vinyl chloride

20
21 Note that the outer-sphere electron transfer reactions for all chloroethylenes by cob(I)alamin
22 are highly endergonic, ~~however~~ However, the calculated standard reduction potentials (E^0) of

1 vinyl radicals ~~listed in~~ **Table S3** in the Supporting Information) show that all chlorinated vinyl
2 radicals ~~with have~~ E^0 values between -0.40 V to 0.16 V, ~~i.e. they~~ can be reduced ~~at standard~~
3 ~~conditions~~ by cob(I)alamin, which could provide additional driving force for the reaction to
4 proceed. The free energy barrier ~~values~~ for PCE and TCE are considerably lower (< 20 kcal/mol),
5 ~~implicating indicating~~ that the outer-sphere electron transfer reactions for cob(I)alamin catalyzing
6 PCE and TCE could take place at normal temperatures, but such reactions are hampered by the
7 high barrier for cob(I)alamin catalyzing *trans*-DCE, *cis*-DCE and VC. These results are consistent
8 with experimental findings, that no significant amount of free vinyl radicals were produced in the
9 reactions of cob(I)alamin reducing *trans*-DCE, *cis*-DCE and VC, while significant chlorinated
10 vinyl radicals formed during the reactions of PCE and TCE catalyzed by cob(I)alamin.²² In the
11 following sections, the competition between the outer-sphere and inner-sphere reduction of
12 chloroethylenes with cob(I)alamin will be addressed.

Commented [KPK17]: Ability to reduce also depends on P and R concentrations viz. Nernst equation, but at 1:1 encounters during the reaction this would be thermodynamically favorable

14 **Inner-Sphere Nucleophilic Substitution Mechanism.**

15 **Figure 2** shows the free energy profile for the inner-sphere nucleophilic substitution of PCE
16 with cob(I)alamin, together with geometric details of the relevant molecular species. From
17 separated reactants, cob(I)alamin + PCE, the nucleophilic substitution reaction takes place via the
18 concerted transition state TS_{NS} , associated with a barrier of 14.2 kcal/mol, leading synchronously
19 to base-off trichlorovinylcob(III)alamin ~~under upon~~ loss of ~~chloride anion~~ Cl^- (the Mulliken charge
20 ~~of dissociated of Cl changes~~ from 0.11 in the reactants to -0.65 in the products), slightly exothermic
21 of -0.2 kcal/mol relative to reactants. The experimental second-order rate constant of
22 cob(I)alamin-mediated PCE varied slightly with pH ($7 \rightarrow 9$), from 125 ± 7 to 179 ± 10 $M^{-1}s^{-1}$.²¹
23 ~~Thus bringing the kinetic information into~~ Using the Eyring equation (eq 2), yields a free energy

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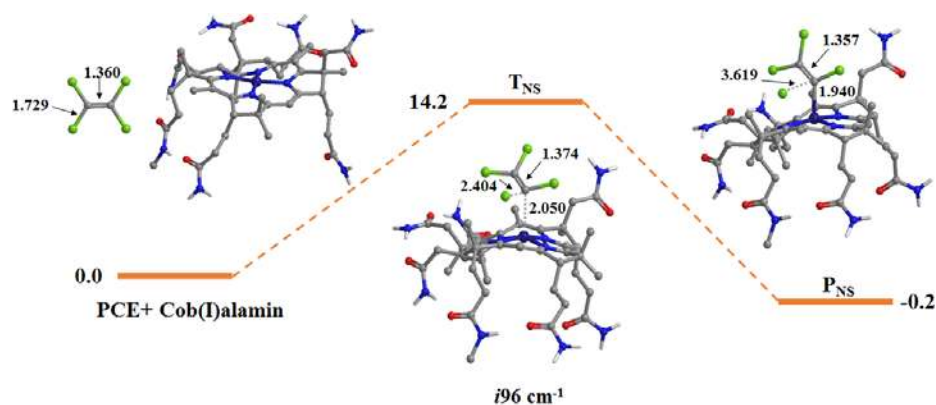
Commented [KPK18]: It is was completely lost it would be non-bonded and -1 charge since we are talking about the product?

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Commented [KPK19]: One could imagine very electronegative atoms with very negative Mulliken charge that are still nit dissociated but simply attracts electron density. Bond distance is probably more relavnt as a measure of dissociation

Commented [KPK20]: The ~means approximate, not interval

1 barrier ~~from of~~ 14.3 ~~to~~ 14.6 kcal/mol, very close to the above calculated nucleophilic substitution
2 barrier of PCE with cob(I)alamin. The ~~qualitative~~ comparison of the free energy barriers of
3 nucleophilic substitution ($\Delta G^{\ddagger}_{NS} = 14.2$ kcal/mol) and electron transfer ($\Delta G^{\ddagger}_{ET} = 15.9$ kcal/mol)
4 ~~estimates the suggests a~~ pathway ratio of nucleophilic substitution to electron transfer of ~17:1, in
5 accord with the experimental phenomenon that maximally ~~ten percent~~ 10% free radicals ~~are~~ formed
6 during dehalogenation of PCE with cob(I)alamin, ~~resulted in the i.e. a~~ ratio for inner-sphere
7 pathway to out-sphere pathway of ~~more than~~ $> 10:1$.²¹ No minima along ~~the~~ reaction path
8 corresponding to initial cob(I)alamin • PCE adduct could be located, and the IRC calculations
9 verified the reaction path leading down from the transition state to separate cob(I)alamin and PCE
10 as reactants (reverse direction) and base-off trichlorovinylcob(III)alamin as product (forward
11 direction), as shown in Figure S3 in the Supporting Information. ~~The A~~ characteristic S_N2
12 transition-state geometry, with bond making occurring simultaneously with bond breaking, is
13 easily recognized. This S_N2 transition-state geometry is rather unsymmetrical with the forming C-
14 Co bond (2.05 Å) much shorter than the breaking C-Cl bond (2.40 Å).



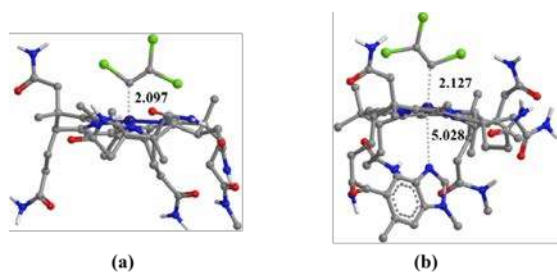
1 **Figure 2.** Free Energy Profile (kcal/mol) of Cob(I)alamin-mediated Reductive Dehalogenation of
2 PCE, along with the Optimized Geometries (Å) of the Key Species and the Imaginary Frequency
3 in Transition State in wave numbers. Relative ~~Energies-energies~~ were ~~given-in-the-Form-of-based~~
4 ~~on PBE/BSI Free-free Energies-energies~~ with ~~Singlesingle-Point-point~~ ~~Solvation-solvation~~ and D3
5 ~~Dispersion-dispersion Corrections-corrections~~ ($\Delta G + E_{\text{solv}} + E_{\text{disp}}$).

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6 Electrochemical experiments⁷⁹ and DFT calculations⁸⁰ have demonstrated that the one-
7 electron-reduced alkyl-cobalt complexes exhibit significant lowering of the Co-C bond
8 dissociation energy in comparison to their neutral precursors. Therefore, once the
9 trichlorovinylcobalamin is formed, it is essential to investigate the subsequent reductive cleavage
10 processes of Co-C bond for both the base-off and base-on trichlorovinylcobalamins, to give the
11 dehalogenation product of PCE, *i.e.* TCE. The calculated E^0 for base-off and base-on
12 trichlorovinylcobalamins are -0.63 V vs. SCE and -0.58 V vs. SCE, respectively. In combination
13 with the calculated E^0 of -0.78 V vs. SCE and experimental E^0 of -0.85 V vs. SCE¹⁸ for ~~the~~
14 cob(II)alamin/cob(I)alamin couple, it demonstrates that both the base-off and base-on
15 trichlorovinylcobalamins ~~could-can~~ be readily reduced under the reductive reaction conditions.

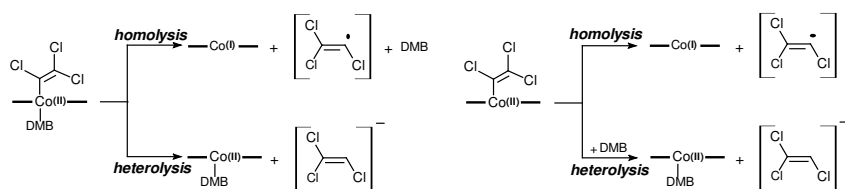
16 **Figure 3** shows the optimized structures for both the one-electron-reduced base-off and base-
17 on trichlorovinylcobalamin (trichlorovinylcob(II)alamin). It is remarkable that the DMB base
18 dissociates far away from the cobalt center with Co-N length of 5.0 Å for the base-on
19 trichlorovinylcob(II)alamin, suggesting the base-on trichlorovinylcob(II)alamin probably has both
20 ~~the~~ “base-on” and “base-off” properties. The complete base-on trichlorovinylcob(II)alamin with
21 the loose axial DMB base is quite different from the previously reported simplified base-on
22 trichlorovinylcob(II)alamin with the tight axial imidazole base,⁴⁴ partly because DMB is a weaker
23 donor ligand than imidazole and partly due to steric repulsion. Subsequently, cleavage of the Co-

1 C bond of trichlorovinylcob(II)alamin may occur homolytically to form a vinyl anion and
 2 cob(II)alamin, or heterolytically to form a vinyl radical and cob(I)alamin, as shown in **Scheme 3**.
 3 The calculated free energies for homolysis and heterolysis of the base-off form are 29.6 and 11.0
 4 kcal/mol, respectively (18.1 and 13.9 kcal/mol without dispersion correction), while the
 5 corresponding values for the base-on form are 21.8 and 3.2 kcal/mol (-3.5 and -7.7 kcal/mol
 6 without dispersion correction). Thus, the heterolytic cleavage of the Co-C bond of the base-on
 7 trichlorovinylcob(II)alamin is the most favorable pathway, which is similar to the previous
 8 theoretical examination of the Co-C cleavage of reduced *cis*-dichlorovinylcobaloxime.⁴⁸



9
 10 **Figure 3.** The Optimized Structures of Base-off Trichlorovinylcob(II)alamin (a) and Base-on
 11 Trichlorovinylcob(II)alamin (b).

12
 13 **Scheme 3.** Homolysis and Heterolysis of the Base-on and Base-off Forms of
 14 Trichlorovinylcob(II)alamin



16
 17

1 We then investigated the nucleophilic substitution mechanism of TCE, *cis*-DCE, *trans*-DCE
 2 and VC. **Table 3** summarizes the relative energies of inner-sphere nucleophilic substitution
 3 processes for cobalamin-mediated all chloroethylenes as well as ~~the electrophilic index (ω)~~ for all
 4 chloroethylenes. ~~while~~ the optimized geometries of ~~the~~ S_N2 transition-states are shown in
 5 **Figure S1** ~~in the Supporting Information~~. All the reaction paths were verified by IRC calculations
 6 ~~that the transition state leading to separate cob(D)alamin and chloroethylene as reactants in the~~
 7 ~~reverse direction and base off vinylcob(II)alamin as product in the forward direction, as shown~~
 8 ~~in Figure S4—S8 in the Supporting Information)~~. ~~And the same as the~~ Similarly, for the
 9 nucleophilic substitution mechanism of TCE with cob(I)alamin, no initial cob(I)alamin •
 10 chloroethylene adduct could be located in the reaction path. ~~It is obvious that~~ As ~~seen~~, the free
 11 energy barriers of nucleophilic substitution are generally increasing with decreasing ~~of~~ number of
 12 chlorine atoms in chloroethylenes, ~~with an exception of dehalogenation of TCE to trans-DCE with~~
 13 ~~larger barriers (minor pathway for dehalogenation of TCE)~~. Moreover, ~~quantitatively~~, increasing
 14 the electrophilic reactivity of chloroethylenes as quantified through ~~the electrophilic index (ω)~~
 15 decreases the nucleophilic substitution barrier, thus yielding a good correlation with an r^2 value of
 16 0.938 ($\Delta G^\ddagger_{NS} = -0.195\omega + 6.95$). ~~The~~ ~~This~~ result indicates the suitability of ω ~~to~~ ~~for~~ screening the
 17 reactivity of cobalamin-mediated reductive dehalogenation of halogenated compounds ~~in~~ ~~via~~ the
 18 inner-sphere nucleophilic substitution pathway.

Commented [KPK22]: All figures in the interval?

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19
 20 **Table 3.** The Relative Free Energies (kcal/mol) for Cobalamin-mediated Reductive
 21 Dehalogenation of Chloroethylenes during the Inner-Sphere Nucleophilic Substitution Processes
 22 along with the Electrophilic Index (ω) of Chloroethylenes

	PCE	TCE ^a	TCE ^b	<i>cis</i> -DCE	<i>trans</i> -DCE	VC
--	-----	------------------	------------------	-----------------	-------------------	----

ω	4.12	3.83	3.83	3.34	3.59	3.12
TS _{NS}	14.2	16.9	18.6	18.2	17.3	19.5
P _{NS}	-0.2	-7.9	-1.8	-5.1	-6.0	-12.5

^adehalogenation of TCE to produce *cis*-DCE; ^bdehalogenation of TCE to produce *trans*-DCE

It is apparent that conversion of TCE may produce *cis*-DCE and *trans*-DCE, respectively.

From the relative energies in **Table 3**, *cis*-DCE is the main product under both kinetic and thermodynamic control. The computational kinetic data from the Eyring equation (eq 2) predicts a preponderance of produced *cis*-DCE over *trans*-DCE by a factor of 18 to 1, which is in excellent agreement with the products distribution of cobalamin-mediated dehalogenation of TCE found experimentally (*cis*-DCE : *trans*-DCE ratios > 15 : 1).^{20-21, 23} The energy barrier for conversion of TCE into *cis*-DCE gives the a rate constant of 2.5 M⁻¹s⁻¹, almost the same as the experimental data from 2.4 ± 0.2 M⁻¹s⁻¹ to 3 ± 0.1 M⁻¹s⁻¹.²¹ Then, combining with Using the above obtained free energy barrier of the electron-transfer process ($\Delta G^{\ddagger}_{ET} = 17.6$ kcal/mol) for TCE with cob(I)alamin, the ratio of nucleophilic substitution pathway to electron transfer pathway is predicted to be 3.5 : 1 through based on the Eyring equation (eq 2), consistent with the experimentally determined ratio for inner sphere pathway to out sphere pathway of > 2.3 : 1.²¹

Moreover, the reaction barriers of cob(I)alamin-mediated dehalogenation of *cis*-DCE, *trans*-DCE and VC are within 20 kcal/mol, thus Accordingly, the nucleophilic substitution reactions for these less-chlorinated ethylenes by cobalamin could happen in theory in principle occur. However, the previous experimental work has shown that the increase of pH by one unit lead to a decrease of the reaction rate by roughly a factor of ten, suggesting that a proton was is involved in the rate-determining step,²² which contradicts the inner-sphere nucleophilic substitution pathway, to be discussed below.

Commented [KPK24]: Typical errors in barriers (absolute, not relative) are 5 kcal/mol. Can we explain why this is so precise? Seems more accurate than normally expected. Are we lucky or is it because this particular number has been calibrated?

1 The results ~~in former section above~~ suggest that the trichlorovinylcobalamin~~e~~ could be rapidly
 2 reduced under reductive conditions. Therefore, ~~it is necessary to we~~ investigated~~d~~ the redox
 3 potentials of other less-chlorinated vinylcobalamins to know whether they can be reduced under
 4 similar conditions. Due to the ~~preponderance~~ production of ~~more cis-DCE over than trans-DCE~~
 5 ~~for when TCE reacts with cobalamin, the following work only we~~ focused on *cis*-
 6 dichlorovinylcobalamin (~~dichlorovinylcobalamin~~). As shown in **Table 4**, the calculated E^0 values
 7 ~~are becoming become~~ more negative with ~~decreasing off fewer~~ chlorine atoms in ~~the~~ chlorinated
 8 vinylcobalamins, and the E^0 values for all base-on dichlorovinylcobalamin, *cis*- and *trans*-
 9 chlorovinylcobalamin, and vinylcobalamin are more negative than their base-off forms.
 10 ~~Especially in particular~~, the E^0 value for the base-off dichlorovinylcobalamin (-0.86 V vs. SCE) is
 11 substantially higher than for the corresponding base-on form (-1.23 V vs. SCE), while the E^0
 12 values for the base-off chlorovinylcobalamins and vinylcobalamins are only a bit more positive
 13 than for their base-on forms. ~~It is obvious that a~~ Among these less-chlorinated vinylcobalamins,
 14 only the base-off dichlorovinylcobalamin ~~would appear as is thus~~ a candidate for promotion of
 15 reduction by cob(I)alamin, although ~~the E^0 value~~ for the base-off dichlorovinylcobalamin (-0.86
 16 V vs. SCE) is a bit more negative than ~~the E^0 value off for the~~ cob(II)alamin/cob(I)alamin couple
 17 (experimental value: -0.85 V vs. SCE; calculated value: -0.78 V vs. ~~SCE~~). All other base-off and
 18 base-on chlorinated vinylcobalamins are not feasible intermediates due to their much more
 19 negative E^0 value than the cob(II)alamin/cob(I)alamin couple.

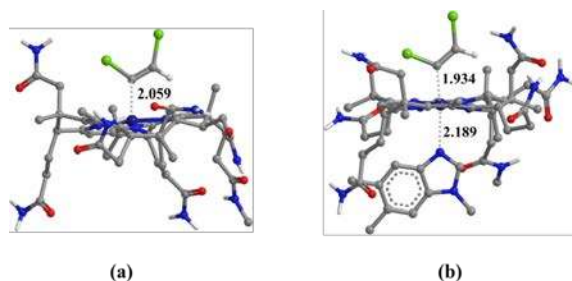
Commented [KPK25]: This is also very accurate; absolute potentials have errors that mimic the errors in functionals for IPs, i.e. 5 kcal/mol. Were they calibrated? Perhaps discuss why they agree so well

20
 21 **Table 4.** **Computed** Aqueous-Phase Standard Reduction Potentials (E^0) (V vs. SCE) for the Base-
 22 off and Base-on Vinylcobalamins

	trichlorovinyl cobalamin	dichlorovinyl cobalamin	<i>cis</i> -chlorovinyl cobalamin	<i>trans</i> - chlorovinyl cobalamin	vinyl cobalamin
--	-----------------------------	----------------------------	--------------------------------------	--	--------------------

Base off					
E⁰	-0.63	-0.86	-1.28	-1.21	-1.40
Base on					
E⁰	-0.58	-1.23	-1.39	-1.27	-1.45

1
2
3 After formation of the one-electron-reduced base-off form of dichlorovinylcobalamin
4 (dichlorovinylcob(II)alamin), the corresponding base-on form with re-coordination of the DMB
5 base to the Co center may be formed. As shown in **Figure 4**, [different from the base-on in contrast](#)
6 [to trichlorovinylcob\(II\)alamin](#), the DMB base coordinates strongly with the cobalt center [for the](#)
7 [to produce](#) base-on *cis*-dichlorovinylcob(II)alamin. The geometry difference between the base-on
8 trichlorovinylcob(II)alamin and dichlorovinylcob(II)alamin may [come-arise](#) from the much
9 stronger inductive effect of the trichlorovinyl-fragment (Mulliken charge: -0.71) compared to the
10 dichlorovinyl-fragment (Mulliken charge: -0.44) (more detailed electronic structure analysis is
11 given [in the following part below](#)). We then calculated the free energy changes of the Co-C bond
12 cleavage for both the base-off and base-on dichlorovinylcob(II)alamin. As for
13 trichlorovinylcob(II)alamin, heterolysis in the base-on forms is the thermodynamically preferred
14 mode of Co-C bond cleavage with a free energy of 7.7 kcal/mol (-7.2 kcal/mol without dispersion
15 correction) (for the detailed free energy comparisons see **Table S26** in the Supporting
16 Information).
17



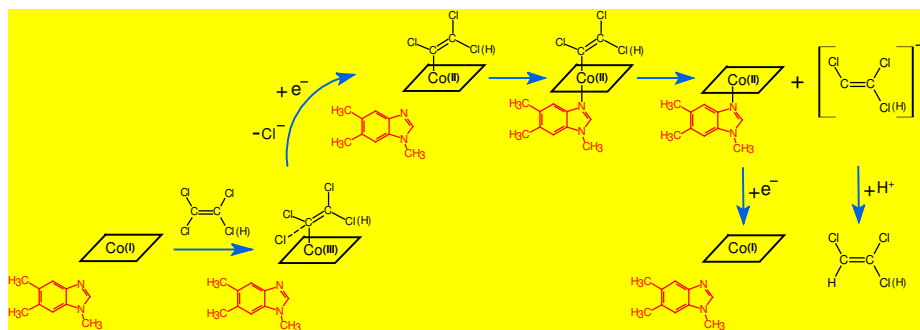
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2 **Figure 4.** The Optimized Structures of Base-off Dichlorovinylcob(II)alamin (a) and Base-on
3 Dichlorovinylcob(II)alamin (b).

4 It is noteworthy that detection of trichlorovinylcobalamin has never been successful, but
5 efforts to detect dichlorovinylcobalamin have been feasible.²⁵ This has been a long-term unsolved
6 mechanistic topic in cobalamin chemistry.¹¹ As mentioned above, the base-on
7 trichlorovinylcob(II)alamin with quite long Co-N length shown in **Figure 3** is close to its base-off
8 form, making the reduction potential of the base-on trichlorovinylcobalamin (-0.58 V vs. SCE) as
9 negative as the base-off form (-0.63 V vs. SCE), ~~so i.e.~~ the base-on trichlorovinylcobalamin is
10 easily reduced under reductive conditions. By contrast, the tight Co-N bond ~~with bond length of~~
11 2.2 \AA) in the base-on dichlorovinylcob(II)alamin shown in **Figure 4** causes the reduction potential
12 of the base-on dichlorovinylcobalamin (-1.23 V vs. SCE) to be much more negative than its base-
13 off form (-0.86 V vs. SCE), ~~resulting in implying a~~ relatively longer lifetime for the base-on
14 dichlorovinylcobalamin. Thus, it is possible to observe the mass consistent with the
15 dichlorovinylcobalamin in mass spectra of the TCE dehalogenation reaction. On the other hand,
16 although the DMB base dissociates far away from the cobalt center for the base-on
17 trichlorovinylcob(II)alamin, the ~~strong~~-destabilization ~~effect~~ by the nitrogen lone pair electrons
18 ~~from the axial of~~ DMB ~~ligand~~—results in a weaker Co-C bond for the base-on
19 trichlorovinylcob(II)alamin than its base-off form, and its Co-C bond would be more easily

1 cleaved. This proposed DMB-dependence mechanism is suggested to may be tested by distinct
2 model systems in future experimental work.

3 Thus, the study suggests that in an inner-sphere nucleophilic substitution pathway of PCE
4 and TCE with cobalamin, an “on/off” conformational ~~switch-change~~ like similar to “ping-pong”
5 playing is active (see **Scheme 4**). Specifically, the “base-off” cob(I)alamin facilitates substrate
6 reduction, after which the formed “base-off” chlorinated vinylcobalamins can be readily reduced
7 (the “base-on trichlorovinylcobalamin can be approximately taken as “base off” due to the quite
8 long Co-C bond), then re-coordination of the DMB base provides additional thermodynamic
9 driving force for the heterolytic cleavage of the Co-C bond to complete the overall reaction step.

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21 **Scheme 4.** The Proposed Reaction Pathway for Cobalamin-Mediated Reductive Dehalogenation
22 of PCE and TCE^a



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For cob(II)alamin and base-off tri- and di-chlorovinylcob(II)alamin, the spin is mainly localized on Co (spin density from 0.7 to 0.9), so the reactions for cob(II)alamin to cob(I)alamin with spin density from nearly one to zero and tri- and di-chlorovinylcob(II)alamin to tri- and di-chlorovinylcob(I)alamin with the spin density from zero to nearly one, really undergo changes of ~1 imply metal-centered reductions; Mulliken charges of dissociated Cl changes from 0.07 to 0.11 in the reactants, to (-0.46) to (-0.35) in the transition state, and to (-0.67) to (-0.65) in the product complex; Mulliken charge of The CCl₂Cl(H) fragment changes from 0.38 to 0.35 in the base-off tri-/di-chlorovinylcob(II)alamin, to 0.70 to 0.66 in the base-off tri-/di-chlorovinylcob(I)alamin, and to 0.71 to 0.44 in the base-on tri-/di-chlorovinylcob(I)alamin accordingly (see Supporting Information for more details).

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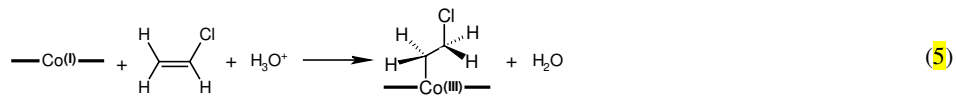
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Inner-Sphere Nucleophilic Addition Mechanism.

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The above work provides the discussion derived the reasonable mechanistic reductive dehalogenation pathway of cobalamin-mediated PCE and TCE. However, the reductive dehalogenation mechanism for cobalamin-mediated less-chlorinated ethylenes, *cis*-DCE, *trans*-DCE and VC, is still puzzling. Experimental work have has shown that *cis*-DCE, *trans*-DCE and VC were reductively dehalogenated by cob(I)alamin in a pH-dependent *mode*,²² suggesting that the initial and rate-determining step is possibly the addition of cob(I)alamin to these less-chlorinated ethylenes with simultaneous protonation. Therefore, the nucleophilic addition pathway is computed for *cis*-DCE, *trans*-DCE and VC according to eq 5 (taking VC as an example):

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25 The reaction free energies (ΔG_{NA}) for cob(I)alamin-mediated *cis*-DCE, *trans*-DCE and VC during
26 the nucleophilic addition pathway are -51.9 kcal/mol, -52.8 kcal/mol and -48.9 kcal/mol,

1 respectively. Thus, ~~the a~~ notable driving force of the nucleophilic addition pathway for all of the
2 less-chlorinated ethylenes with cob(I)alamin to produce corresponding chlorinated
3 ethylcobalamins is evident.

4 Subsequently, the E^0 and VEA of both the base-on and base-off chlorinated ethylcobalamins
5 were calculated ~~(, as is shown in Table 5)~~. Note ~~that~~ the attempts to optimize the one-electron-
6 reduced base-off dichloroethylcobalamin (dichloroethylcob(II)alamin) and chloroethylcobalamin
7 (chloroethylcob(II)alamin) lead directly to the elimination of chloride and formation of VC and
8 ethylene, respectively, so ~~it is not applicable to calculate the calculation of E^0 is not reliable value~~
9 for the base-off less-chlorinated ethylcobalamins. ~~At the same time,~~ ~~†~~The E^0 values for the base-
10 on dichloroethylcobalamin and chloroethylcobalamin are -1.19 V vs. SCE and -1.24 V vs. SCE,
11 respectively, much more negative than ~~the E^0 value~~ for the cob(II)alamin/cob(I)alamin couple, i.e.
12 they are ~~difficult-hard~~ to be reduced. However, the VEA values of the base-off forms are larger
13 than the corresponding base-on forms, so it may be inferred that the formed “base-off dichloro-
14 and chloro-ethylcob(II)alamin” would rapidly decompose ~~into the dehalogenation-dehalogenated~~
15 products. Thus, barring unexpectedly high barriers, the nucleophilic addition with simultaneous
16 protonation for cob(I)alamin-mediated less-chlorinated ethylenes would be favored even in basic
17 solutions with very low concentration of H_3O^+ .

18 ~~Experimental work~~ Kinetic studies ~~has have~~ shown that VC reacted faster with cob(I)alamin
19 than *cis*-DCE and *trans*-DCE. The significance of this ~~phenomenon~~ is difficult to evaluate, because
20 it is challenging ~~and error-prone~~ to calculate ~~the~~ acidity constants for transition-metal complexes
21 in solution, ~~thereby, to and thereby~~ obtain the free energy barriers ~~in the nucleophilic addition~~
22 ~~pathway~~ with simultaneous protonation ~~is a difficult task~~. Nevertheless, the calculated proton
23 affinity (PA) for *cis*-DCE, *trans*-DCE and VC is 5.4 eV, 5.5 eV and 5.8 eV, respectively, consistent

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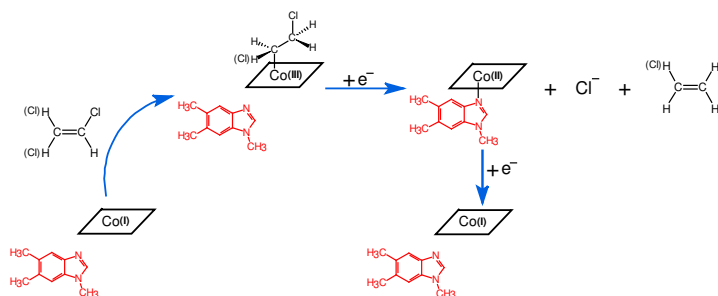
1 with the order of the experimental kinetic data (*cis*-DCE < *trans*-DCE < VC). Quantitatively, the
 2 relationships between the experimental rate constant (log k) at different pH-pH values ($\text{pH} = 7$,
 3 **8 and 9**) and PA of the less-chlorinated ethylenes reveal significant correlations between both
 4 properties (log $k_{\text{pH}k_{\text{pH}=7}}$ = 0.22PA + 5.72, $r^2 = 0.986$; log $k_{\text{pH}k_{\text{pH}=8}}$ = 0.20PA + 5.87, $r^2 = 0.994$;
 5 log $k_{\text{pH}k_{\text{pH}=9}}$ = 0.20PA + 6.04, $r^2 = 0.997$). Therefore, PA ~~would be~~ is a good probe for the kinetic
 6 information in the nucleophilic addition pathway with simultaneous protonation for cob(I)alamin-
 7 mediated halogenated compounds.

8
 9 **Table 5.** **Computed** Aqueous-Phase Standard Reduction Potentials (E^0) (V, vs. SCE) and Vertical
 10 Electron Affinities (VEA) (kcal/mol) for the Base-off and Base-on Forms of Ethylcobalamin

	Base-off	E^0	VEA	Base-on	E^0	VEA
dichloroethyl cobalamin		/	70.8		-1.19	64.8
chloroethyl cobalamin		/	65.9		-1.24	60.4

11
 12 In conclusion, the computations provide support for the mechanistic routes and indicate a
 13 distinct type of “*on/off switch*” occurring during cobalamin-mediated reductive dehalogenation of
 14 the less-chlorinated ethylenes of the nucleophilic addition pathway: ~~the~~The initial step is the
 15 addition of the “base-off” cob(I)alamin to the less-chlorinated ethylenes with simultaneous
 16 protonation. ~~Then~~ the formed base-off form of dichloro- and chloro-ethylcobalamin ~~would~~ can
 17 produce the dehalogenation products directly with formation of “base-on” cob(II)alamin under the
 18 reductive reaction conditions (see simplified sketch in **Scheme 5**).

1
 2 **Scheme 5.** The Proposed Reaction Pathway for Cobalamin-Mediated Reductive Dehalogenation
 3 of *cis*-DCE, *trans*-DCE and VC^a



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 5
 6 ^aMulliken charge of dissociated Cl changes from -0.01 ~ -0.04 in the reactants, to -0.08 ~ -0.05 in base-off
 7 dichloroethylcob(III)alamin, and to -1.0 in the product complex; Mulliken charge of CH₂Cl(H) fragment
 8 changes from -0.04 ~ -0.01 in the reactants, to -0.17 ~ -0.08 in the base-off tri-/di-chlorovinylcob(III)alamin.s
 9 can be found in Supporting Information

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11 Electronic Structure Characteristics Analysis.

12 The unique nature of the C-Co-N bonding in cobalamin, with the competing σ and π effects,
 13 has continued to be an important mechanistic subject. **Figure 5** shows the ~~importantly~~ relevant
 14 frontier molecular orbitals during the reductive processes of chlorinated vinylcobalamins (~~while~~
 15 the relevant frontier molecular orbitals for chlorinated ethylcobalamins are shown in **Figure S2** in
 16 the Supporting Information). As shown in **Figure 5 (a)**, the ~~lowest unoccupied molecular orbitals~~
 17 (LUMOs) of base-off tri- and di-chlorovinyl cob(III)alamin.s are largely associated with the σ_{Co-}
 18 $C\alpha^*$ orbital, whereas base-off non-chlorovinyl cob(III)alamin.s have LUMOs mainly coinciding
 19 with the corrin macrocycle π^* orbital, and the LUMO of base-off mono-chlorovinyl cob(III)alamin
 20 is mixed corrin macrocycle π^* (major) and $\sigma_{Co-C\alpha^*}$ (minor) ~~orbital~~. Interestingly, the mixed

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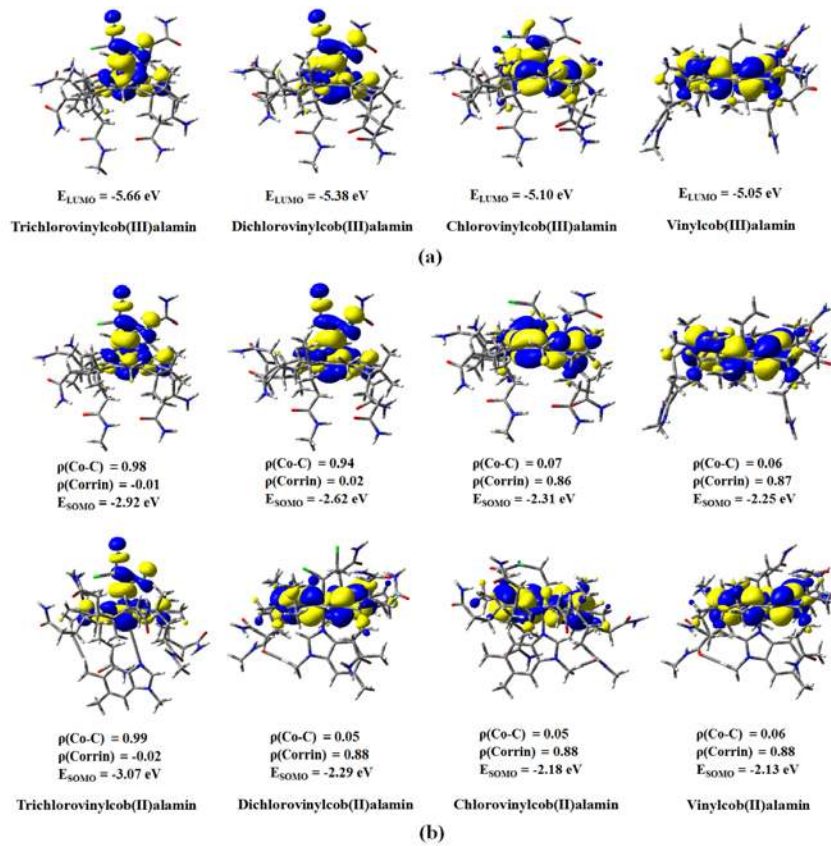
1 character ~~for~~ of the LUMOs of base-off mono-chlorovinyl cob(III)alamin resembles a transition
2 between LUMOs of base-off tri/di-chlorovinyl and non-chlorovinyl cob(III)alamins. The
3 significant difference in LUMO character is mainly due to the fact that the nitrogen atoms of the
4 corrin ring of cobalamin are more electron-rich than the mono- and non-chlorinated ethylene
5 fragments (Mulliken charge: ~~-0.25~~ ~~to~~ -0.17) compared with the tri- and di-chlorovinyl ethylene
6 fragments (Mulliken charge: ~~-0.38~~ ~~to~~ -0.33), so that the corrin ring has larger overlap with the
7 relevant d-orbital in Co than with the C_α atom in the ethylene fragment resulting in dominant π*
8 orbital for LUMOs of mono- and non-chlorinated cob(III)alamins (E_{LUMO}: -5.05 ~~to~~ -5.10 eV).
9 By contrast, the strong inductive effect of tri- and di-chlorinated ethylene fragment withdraws
10 electron density from the Co center to reduce the effective nuclear charge of the metal ion, thereby
11 lowering the energies of Co d_z² orbitals and undergoing a large stabilization of LUMOs on the σ_{Co-C_α}
12 C_α* orbital for base-off tri- and di-chlorovinyl cob(III)alamins (E_{LUMO}: -5.66 ~~to~~ -5.38 eV).
13 Generally speaking, the ~~contribution of electron-withdrawing~~ inductive effects from ~~more than or~~
14 ~~equal to two~~ ~~two or more~~ electronegative chlorine atoms ~~is able to can~~ lower the LUMOs of base-
15 off chlorinated vinylcobalamins, thus producing the corresponding lower E⁰ values shown in
16 **Table 4.**

17 These LUMOs of base-off chlorinated vinylcob(III)alamins are initially occupied upon one-
18 electron reduction to produce the corresponding single occupied molecular orbitals (SOMOs). As
19 shown in **Figure 5 (b)**, these SOMOs remain ~~the same as the localization of their precursor~~
20 ~~LUMOs unchanged as~~ reflected ~~also in from~~ the spin densities (ρ) ~~of~~ ~~that such values are~~
21 0.98 ~~to~~ 0.99 for SOMOs on the σ_{Co-C_α}* orbital of tri- and di-chlorovinyl cob(II)alamins, and ~~are~~
22 0.87 ~~to~~ 0.88 for the corrin macrocycle π* ~~orbital~~ of mono- and non-chlorinated
23 vinylcob(II)alamins. After recoordination of the DMB base, most SOMOs coincide with the corrin

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Higher potentials imply easier to reduce = lower LUMO energy.
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1 macrocycle π^* orbital of base-on cob(II)alamins; ~~particularly~~ The SOMO localization changes
2 from $\sigma_{\text{Co-C}\alpha}^*$ orbital-like ($\rho = 0.98$) in the base-off dichlorovinylcobalamin to corrin π^* orbital-like
3 ($\rho = 0.88$) in the base-on species. The reason for this major electronic structure change from base-
4 off to base-on dichlorovinylcobalamin is that an additional strong σ -antibonding between Co and
5 the DMB base in the base-on species leads to a considerable destabilization of the $\sigma_{\text{Co-C}\alpha}^*$ orbital,
6 thus resulting in SOMO with considerable corrin π orbital character. However, the strong
7 ~~withdrawing~~ inductive effect ~~from~~ of the three chlorine atoms in base-on trichlorovinylcobalamin
8 ~~is able to cancel~~ out the σ -donating effect ~~from the~~ of DMB base through still reducing the
9 effective nuclear charge of Co ~~atom~~ to lower the energy of its d_z^2 orbital, thereby retaining SOMO
10 localization on the $\sigma_{\text{Co-C}\alpha}^*$ orbital and ~~repelling~~ the DMB base. Interestingly, the distant DMB base
11 in base-on trichlorovinylcobalamin shows weak Van der Waals attraction to further lower the
12 SOMO $\sigma_{\text{Co-C}\alpha}^*$ orbital, as reflected in comparison of SOMO energies of base-off
13 trichlorovinylcobalamin ($E_{\text{SOMO}} = -2.92$ eV) and base-on trichlorovinylcobalamin ($E_{\text{SOMO}} = -3.07$
14 eV).

15



1
2 **Figure 5.** (a) Side View of the LUMOs in the Base-off Chlorinated Vinylcob(III)alamins along
3 with the LUMO Energies (E_{LUMO}); (b) Side View of the SOMOs in the Base-off and Base-on
4 Chlorinated Vinylcob(II)alamins along with the SOMO Energies (E_{SOMO}) as well as Spin Density
5 for Co-C Bond [$\rho(\text{Co-C})$] and Corrin Macrocycle [$\rho(\text{Corrin})$].

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Isotope Effects.

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1 **Table 6** summarizes the calculated average carbon KIE (KIE_C) values during the inner-sphere
 2 nucleophilic substitution pathway for cobalamin-mediated chloroethylenes, together with the
 3 experimental ϵ_{bulkC} as well as the compound average carbon AKIE ($AKIE_C$) obtained from ϵ_{bulkC}
 4 according to eq 3. The similarity of the experimental $AKIE_C$ values with and computational KIE_C
 5 values confirm that cobalamin-mediated PCE and TCE dehalogenation proceeds through the
 6 nucleophilic substitution pathway. However, the much larger experimental $AKIE_C$ values than the
 7 vs. KIE_C values from for the nucleophilic substitution pathway for *cis*-DCE and VC₇, supports the
 8 nucleophilic addition of cob(I)alamin to one of the carbon atoms of these chloroethylenes and
 9 simultaneous protonation of the other carbon atom, because only the concerted reactions could
 10 increase the kinetic isotope effects to the most-full extent.

11
 12 **Table 6.** Average Calculated Carbon Kinetic Isotope Effects (KIE_C) on the Inner-sphere
 13 Nucleophilic Substitution Pathway for Cobalamin-mediated Chloroethylenes, as well as the
 14 Experimental Carbon Bulk Isotope Fractionation Factors (ϵ_{bulkC}) and the Compound Average
 15 Carbon Apparent Kinetic Isotope Effect ($AKIE_C$)

	PCE	TCE	<i>cis</i> -DCE	<i>trans</i> -DCE	VC
KIE_C	1.028	1.032	1.026	1.030	1.027
ϵ_{bulkC}	-15.8‰	-16.1‰	-25.5‰	/	-31.1‰
$AKIE_C$	1.033	1.033	1.054	/	1.066

16
 17 Most previous work has focused on stable carbon isotopes to study the transformation process
 18 of organic pollutants. However, chlorine also has high-major relevance for as constituent of many
 19 polluting environmental compounds. In practice, only a few chlorine isotope analyses have been
 20 performed to investigate the transformation of compounds, and the firstly reported chlorine bulk
 21 isotope fractionation factor for cobalamin-mediated chloroethylene is was -4.0‰ for TCE,⁹ which

1 can be converted into $AKIE_{Cl}$ of ~ 1.012 by eq 3. The calculated average chlorine KIE for
2 cobalamin-mediated TCE in the inner-sphere nucleophilic substitution pathway is 1.009, quite
3 close to the experimental $AKIE_{Cl}$.

4 ~~As shown in this work,~~ The calculations cannot reproduce very precisely the experimental
5 data [in this instance](#), since the latter may be masked. Dual element isotope analysis has attracted
6 considerable interest, the advantage of which is that different mechanisms may be discerned
7 simply by correlating the isotope fractionation factor (ϵ) ratios for the two elements. Herein, we
8 extend the dual element isotope analysis ~~only~~-based [only](#) on experimental data ~~into~~ a new manner
9 for comparison between computations and experiments ~~through~~-by correlating the ratios of ϵ or
10 KIE for the two elements. ~~Taking As an example, we consider~~ the reaction of cobalamin-mediated
11 TCE with available dual element isotopes ~~as an example: the~~-The reverse eq 3 yields ~~the~~
12 computational ϵ_C of -15.5‰ and ϵ_{Cl} of -3.0‰ ; thus the ratio of computational ϵ_C to ϵ_{Cl} in the
13 nucleophilic substitution pathway is calculated to be 5.1 : 1.0, while the ratio of experimental ϵ_C
14 (-16.1‰) to ϵ_{Cl} (-4.0‰) is 4.0 : 1.0, ~~so there is some degree of~~[implying some](#) difference between
15 computations and experiments ~~based on the correlating ratio of ϵ~~ ; ~~In the meanwhile~~Meanwhile,
16 the calculated ratio of $AKIE_C$ to $AKIE_{Cl}$ is 1.02, while the ratio of computational KIE_C to KIE_{Cl} in
17 the nucleophilic substitution pathway is 1.02 as well. **Furthermore, the latest reported experimental**
18 **work concerning combined carbon and chlorine isotope analysis during the reductive**
19 **dehalogenation of TCE by cobalamin provides an ϵ_C value of -15.0‰ ($AKIE = 1.031$) and an ϵ_{Cl}**
20 **value of -3.2‰ ($AKIE = 1.010$),³¹ which can be converted into $AKIE_C/AKIE_{Cl}$ of 1.02 again,**
21 **although ~~there is some difference between~~the two previous studies ~~for experimental ϵ_C and ϵ_{Cl}~~**
22 **~~values differ somewhat~~.** ~~Since~~-With the most plausible nucleophilic substitution mechanism for
23 cobalamin-mediated TCE ~~is~~-outlined above, the comparison between correlating ratios of KIE for

1 the two elements ~~may~~ provides a new probe to detect the reaction mechanism. This approach holds
2 promise to bridge a gap between computations and experiments, because various factors mask the
3 AKIEs of both elements to the same extent so that correlating ratios of KIE for the two elements
4 remain the same.

5 Previous studies have indicated ~~the variability-variable in~~ isotope fractionation of PCE and
6 TCE by a ~~variety-of-different~~ microorganisms⁸¹ probably due to ~~the~~ complex enzymatic
7 environment ~~for-of~~ the dehalogenating strain, ~~thus~~ ~~Because of this~~, the elucidation of biological
8 dehalogenation mechanisms may be hampered ~~by the variability in isotope fractionation~~. In future
9 work, the reaction mechanisms of the biological dehalogenation ~~could-is envisioned to be~~
10 diagnosed ~~potentially~~ by quantum mechanical/molecular mechanical (QM/MM) or cluster
11 modelling to simulate the enzymatic environment in combination with ~~the-our~~ new proposed
12 method of ~~relating~~ calculated KIE_C/KIE_{Cl} ~~vs-and~~ experimental $AKIE_C/AKIE_{Cl}$.

14 Conclusions.

15 This work shows how computational chemistry closes some ~~key unsolved~~ research gaps ~~for~~
16 ~~relating to~~ cobalamin-mediated reduction of chloroethylenes by distinguishing different
17 mechanisms (Scheme 2). To this end, we have developed some useful quantitative methods that
18 rationalize reactivity ~~by-~~(i) serving as screening tools for predicting the reductive dehalogenation
19 reactivity ~~in-of~~ a given mechanism (e.g. *electron affinity* for electron transfer, *electrophilic index*
20 for nucleophilic substitution, and *proton affinity* for nucleophilic addition); (ii) providing *standard*
21 *reduction potentials* (E^0) of formed chlorinated-cobalamins as ~~one-an~~ important parameter ~~for~~
22 determining the feasibility of the inner-sphere pathway; (iii) ~~offering-suggesting~~ the *calculated*
23 KIE_C/KIE_{Cl} ~~vs experimental~~ $AKIE_C/AKIE_{Cl}$ as a probe for diagnosing the *overall reaction*
24 *mechanism*. ~~Thus,~~ these quantitative methods may be useful ~~for-in determination-determining of~~
25 the environmental fate and development ~~of-ing~~ remediation pathways of halogenated organic
26 pollutants.

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1 Recently, Payne *et al.* proposed a ~~third type of mechanism~~ alternative route, with the
2 cobalamin of reductive dehalogenase able to abstract bromide from 2,6-dibromophenol with
3 formation of a Co-Br bond, consequently leading to a C-Br bond cleavage concomitant with
4 protonation of the leaving group by nearby residue Tyr426.⁸² At the same time, Bommer *et al.*
5 further emphasized the role of strictly conserved Tyr246 in reductive dehalogenase pointing with
6 its phenolic group toward carbon to donate the proton to neutralize the dichlorovinyl anion formed
7 upon reaction of TCE with cobalamin cofactor.⁸³ The more detailed reaction mechanism of the
8 new proposed dehalogenation mode was computationally elucidated by Liao *et al.*⁸⁴⁻⁸⁵ This new
9 paradigm implies that the repertoire of reductive dehalogenation originating from cobalamin in
10 dehalogenase is even more diverse than previously anticipated, which may guide further
11 modification strategies for cobalamin to mimic the enzyme behavior in *in vitro* reductive
12 dehalogenations. Together with these recent mechanistic findings, our work spans the so far known
13 possible reactivity space of cobalamin in degradation of halogenated compounds, although we
14 expect that further studies are needed to define the relevance of each reaction type to specific
15 conditions and environments.

Commented [KPK33]: Route of what? Which reaction

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18 ASSOCIATED CONTENT

19 **Supporting Information.** Full citation for reference 70; estimation of activation barriers for
20 electron transfer processes by Marcus theory; computed aqueous-phase standard reduction
21 potentials of vinyl radicals; optimized geometries of S_N2 transition-state of cobalamin-mediated
22 dehalogenation of chloroethylenes; frontier molecular orbitals for less-chlorinated
23 ethylcobalamins; experimental rate constants for reaction of less-chlorinated ethylcobalamins with

1 cob(I)alamin; energies for all molecular species; Mulliken charges and spin densities; intrinsic
2 reaction coordinate (IRC) for verifying transition states; geometrical comparison ~~between-of~~
3 PBE/BSI optimized structures in the gas phase and PBE-D3-CPCM/BSI optimized structures;
4 ~~final-one_~~electron symbolic density matrix of complete cob(I)alamin from CASSCF calculations;
5 ~~eartesian-Cartesian~~ coordinates of all molecular structures ~~discussed in this work~~. This material is
6 available free of charge via the Internet at <http://pubs.acs.org>.

7

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13 Notes

14 The authors declare no competing financial interest.

15

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18 China National Supercomputing Center in Shenzhen is acknowledged for providing the Gaussian
19 09 package and the high-performance computing clusters.

20

1 ABBREVIATIONS

2 DFT, density functional theory; DMB, 5,6-dimethylbenzimidazole; PCE, perchloroethylene; TCE,
3 trichloroethylene; *cis*-DCE, *cis*-1,2-dichloroethylene; *trans*-DCE, *trans*-1,2-dichloroethylene; VC,
4 vinyl chloride; IRC, intrinsic reaction coordinate; CSIA, compound specific isotope analysis; KIEs,
5 kinetic isotope effects; AKIEs, apparent kinetic isotope effects; PBE, Perdew-Burke-Ernzerhof;
6 ZPE, zero-point energy correction; CPCM, COSMO continuum-solvation model; SCE, standard
7 calomel electrode; VEA, vertical electron affinities; HSAB, hard and soft acids and bases; HOMO,
8 highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; LFER, linear
9 free energy relationship; PA, proton affinity.

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11 REFERENCES

- 12 (1) Fenner, K.; Canonica, S.; Wackett, L. P.; Elsner, M. *Science* **2013**, 341, 752-758.
- 13 (2) Schwarzenbach, R. P.; Egli, T.; Hofstetter, T. B.; von Gunten, U.; Wehrli, B. *Annu. Rev.*
14 *Environ. Resour.* **2010**, 35, 109-136.
- 15 (3) Alonso, F.; Beletskaya, I. P.; Yus, M. *Chem. Rev.* **2002**, 102, 4009-4091.
- 16 (4) Leys, D.; Adrian, L.; Smidt, H. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2013**, 368,
17 20120316.
- 18 (5) Smidt, H.; de Vos, W. M. *Annu. Rev. Microbiol.* **2004**, 58, 43-73.
- 19 (6) Stich, T. A.; Brooks, A. J.; Buan, N. R.; Brunold, T. C. *J. Am. Chem. Soc.* **2003**, 125, 5897-
20 5914.

- 1 (7) Kozłowski, P. M.; Garabato, B. D.; Lodowski, P.; Jaworska, M. *Dalton Trans.* **2016**, 45,
2 4457-4470.
- 3 (8) Liptak, M. D.; Brunold, T. C. *J. Am. Chem. Soc.* **2006**, 128, 9144-9156.
- 4 (9) Cretnik, S.; Thoreson, K. A.; Bernstein, A.; Ebert, K.; Buchner, D.; Laskov, C.; Haderlein,
5 S.; Shouakar-Stash, O.; Kliegman, S.; McNeill, K.; Elsner, M. *Environ. Sci. Technol.* **2013**, 47,
6 6855-6863.
- 7 (10) Sorel, D.; Lesage, S.; Brown, S.; Millar, K. *Ground Water Monit. Rem.* **2001**, 21, 140-148.
- 8 (11) Kliegman, S.; McNeill, K. *Dalton Trans.* **2008**, 4191-4201.
- 9 (12) Giedyk, M.; Goliszevska, K.; Gryko, D. *Chem. Soc. Rev.* **2015**, 44, 3391-3404.
- 10 (13) Zhou, D. L.; Njue, C. K.; Rusling, J. F. *J. Am. Chem. Soc.* **1999**, 121, 2909-2914.
- 11 (14) Elsner, M.; Hofstetter, T. B., Current Perspectives on the Mechanisms of
12 Chlorohydrocarbon Degradation in Subsurface Environments: Insight from Kinetics, Product
13 Formation, Probe Molecules, and Isotope Fractionation. In *Aquatic Redox Chemistry*, Tratnyek, P.
14 G.; Grundl, T. J.; Haderlein, S. B., Eds. 2011; Vol. 1071, pp 407-439.
- 15 (15) Perlinger, J. A.; Buschmann, J.; Angst, W.; Schwarzenbach, R. P. *Environ. Sci. Technol.*
16 **1998**, 32, 2431-2437.
- 17 (16) Song, H.; Carraway, E. R. *Environ. Sci. Technol.* **2005**, 39, 6237-6245.
- 18 (17) Arnold, W. A.; Ball, W. P.; Roberts, A. L. *J. Contam. Hydrol.* **1999**, 40, 183-200.
- 19 (18) Lexa, D.; Saveant, J. M. *Acc. Chem. Res.* **1983**, 16, 235-243.

- 1 (19) Jensen, K. P. *J. Phys. Chem. B* **2005**, 109, 10505-10512.
- 2 (20) Gantzer, C. J.; Wackett, L. P. *Environ. Sci. Technol.* **1991**, 25, 715-722.
- 3 (21) Glod, G.; Angst, W.; Holliger, C.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1997**, 31,
4 253-260.
- 5 (22) Glod, G.; Brodmann, U.; Angst, W.; Holliger, C.; Schwarzenbach, R. P. *Environ. Sci.*
6 *Technol.* **1997**, 31, 3154-3160.
- 7 (23) Follett, A. D.; McNeill, K. *J. Am. Chem. Soc.* **2005**, 127, 844-845.
- 8 (24) Costentin, C.; Robert, M.; Saveant, J. M. *J. Am. Chem. Soc.* **2005**, 127, 12154-12155.
- 9 (25) Lesage, S.; Brown, S.; Millar, K. *Environ. Sci. Technol.* **1998**, 32, 2264-2272.
- 10 (26) Slater, G. F.; Lollar, B. S.; Lesage, S.; Brown, S. *Ground Water Monit. Rem.* **2003**, 23, 59-
11 67.
- 12 (27) Slater, G. F.; Lollar, B. S.; Sleep, B. E.; Edwards, E. A. *Environ. Sci. Technol.* **2001**, 35,
13 901-907.
- 14 (28) Hunkeler, D.; Aravena, R.; Cox, E. *Environ. Sci. Technol.* **2002**, 36, 3378-3384.
- 15 (29) Nijenhuis, I.; Andert, J.; Beck, K.; Kastner, M.; Diekert, G.; Richnow, H. H. *Appl. Environ.*
16 *Microbiol.* **2005**, 71, 3413-3419.
- 17 (30) Cichocka, D.; Siegert, M.; Imfeld, G.; Andert, J.; Beck, K.; Diekert, G.; Richnow, H. H.;
18 Nijenhuis, I. *FEMS Microbiol. Ecol.* **2007**, 62, 98-107.

- 1 (31) Renpenning, J.; Keller, S.; Cretnik, S.; Shouakar-Stash, O.; Elsner, M.; Schubert, T.;
2 Nijenhuis, I. *Environ. Sci. Technol.* **2014**, *48*, 11837-11845.
- 3 (32) Paneth, P. *Acc. Chem. Res.* **2003**, *36*, 120-126.
- 4 (33) Elsner, M.; Zwank, L.; Hunkeler, D.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **2005**,
5 *39*, 6896-6916.
- 6 (34) Santoro, S.; Kalek, M.; Huang, G. P.; Himo, F. *Acc. Chem. Res.* **2016**, *49*, 1006-1018.
- 7 (35) Ji, L.; Schuurmann, G. *Angew. Chem. Int. Ed.* **2013**, *52*, 744-748.
- 8 (36) Kumar, N.; Kozlowski, P. M. *J. Phys. Chem. B* **2013**, *117*, 16044-16057.
- 9 (37) Jensen, K. P.; Ryde, U. *J. Am. Chem. Soc.* **2005**, *127*, 9117-9128.
- 10 (38) Quesne, M. G.; Senthilnathan, D.; Singh, D.; Kumar, D.; Maldivi, P.; Sorokin, A. B.; de
11 Visser, S. P. *ACS Catal.* **2016**, *6*, 2230-2243.
- 12 (39) de Visser, S. P.; Quesne, M. G.; Martin, B.; Comba, P.; Ryde, U. *Chem. Commun.* **2014**,
13 *50*, 262-282.
- 14 (40) Zhang, J.; Ji, L.; Liu, W. P. *Chem. Res. Toxicol.* **2015**, *28*, 1522-1531.
- 15 (41) Leitch, J. A.; Wilson, P. B.; McMullin, C. L.; Mahon, M. F.; Bhonoah, Y.; Williams, I. H.;
16 Frost, C. G. *ACS Catal.* **2016**, *6*, 5520-5529.
- 17 (42) Swiderek, K.; Tunon, I.; Marti, S.; Moliner, V. *ACS Catal.* **2015**, *5*, 1172-1185.
- 18 (43) Swiderek, K.; Marti, S.; Moliner, V. *ACS Catal.* **2014**, *4*, 426-434.
- 19 (44) Pratt, D. A.; van der Donk, W. A. *J. Am. Chem. Soc.* **2005**, *127*, 384-396.

- 1 (45) Pratt, D. A.; van der Donk, W. A. *Chem. Commun.* **2006**, 558-560.
- 2 (46) Nonnenberg, C.; van der Donk, W. A.; Zipse, H. *J. Phys. Chem. A* **2002**, 106, 8708-8715.
- 3 (47) Buhl, M.; Golubnychiy, V. *Organometallics* **2007**, 26, 6213-6218.
- 4 (48) Follett, A. D.; McNabb, K. A.; Peterson, A. A.; Scanlon, J. D.; Cramer, C. J.; McNeill, K.
5 *Inorg. Chem.* **2007**, 46, 1645-1654.
- 6 (49) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, 58, 1200-1211.
- 7 (50) Perdew, J. P.; Burke, K.; Ernzerhof, M. *Phys. Rev. Lett.* **1996**, 77, 3865-3868.
- 8 (51) Schafer, A.; Huber, C.; Ahlrichs, R. *J. Chem. Phys.* **1994**, 100, 5829-5835.
- 9 (52) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, 56, 2257-+.
- 10 (53) Giorgetti, M.; Ascone, I.; Berrettoni, M.; Conti, P.; Zamponi, S.; Marassi, R. *J. Biol. Inorg.*
11 *Chem.* **2000**, 5, 156-166.
- 12 (54) Wirt, M. D.; Sagi, I.; Chance, M. R. *Biophys. J.* **1992**, 63, 412-417.
- 13 (55) Kepp, K. P. *J. Phys. Chem. A* **2014**, 118, 7104-7117.
- 14 (56) Grimme, S. *J. Comput. Chem.* **2006**, 27, 1787-1799.
- 15 (57) Stich, T. A.; Buan, N. R.; Brunold, T. C. *J. Am. Chem. Soc.* **2004**, 126, 9735-9749.
- 16 (58) Kumar, N.; Alfonso-Prieto, M.; Rovira, C.; Lodowski, P.; Jaworska, M.; Kozłowski, P. M.
17 *J Chem Theory Comput* **2011**, 7, 1541-1551.
- 18 (59) Jensen, K. P.; Ryde, U. *Coord. Chem. Rev.* **2009**, 253, 769-778.

- 1 (60) Kumar, M.; Galezowski, W.; Kozlowski, P. M. *Int. J. Quantum Chem* **2013**, 113, 479-488.
- 2 (61) Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, 80, 3265-3269.
- 3 (62) Pearson, R. G. *Coord. Chem. Rev.* **1990**, 100, 403-425.
- 4 (63) LoPachin, R. M.; Gavin, T.; DeCaprio, A.; Barber, D. S. *Chem. Res. Toxicol.* **2012**, 25,
- 5 239-251.
- 6 (64) Zhang, J.; Wang, C.; Ji, L.; Liu, W. *Chem. Res. Toxicol.* **2016**, 29, 841-850.
- 7 (65) Wolfsberg, M.; Van Hook, A.; Paneth, P., *Isotope Effects in the Chemical, Geological and*
- 8 *Bio Sciences*. Springer: London, 2010.
- 9 (66) Wilson, P. B.; Williams, I. H. *Angew. Chem. Int. Ed.* **2016**, 55, 3192-3195.
- 10 (67) Swiderek, K.; Tunon, I.; Marti, S.; Moliner, V.; Bertran, J. *J. Am. Chem. Soc.* **2013**, 135,
- 11 8708-8719.
- 12 (68) Anisimov, V.; Paneth, P. *J. Math. Chem.* **1999**, 26, 75-86.
- 13 (69) Paneth, P. *Environ. Chem.* **2012**, 9, 67-68.
- 14 (70) Frisch, M. J. et. al., Gaussian 09, revision D.01, Gaussian, Inc.: Wallingford, CT, 2013. see
- 15 Supporting Information.
- 16 (71) Rizzato, S.; Berges, J.; Mason, S. A.; Albinati, A.; Kozelka, J. *Angew. Chem. Int. Edit.*
- 17 **2010**, 49, 7440-7443.
- 18 (72) Kumar, M.; Kozlowski, P. M. *Angew. Chem. Int. Edit.* **2011**, 50, 8702-8705.
- 19 (73) Kumar, M.; Kumar, N.; Hirao, H.; Kozlowski, P. M. *Inorg. Chem.* **2012**, 51, 5533-5538.

- 1 (74) Kumar, M.; Kozłowski, P. M. *Coord. Chem. Rev.* **2017**, 333, 71-81.
- 2 (75) Marcus, R. A. *Angew. Chem. Int. Ed.* **1993**, 32, 1111-1121.
- 3 (76) Jones, G. O.; Liu, P.; Houk, K. N.; Buchwald, S. L. *J. Am. Chem. Soc.* **2010**, 132, 6205-
- 4 6213.
- 5 (77) Kumar, S.; Faponle, A. S.; Barman, P.; Vardhaman, A. K.; Sastri, C. V.; Kumar, D.; de
- 6 Visser, S. P. *J. Am. Chem. Soc.* **2014**, 136, 17102-17115.
- 7 (78) Lin, C. Y.; Coote, M. L.; Gennaro, A.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2008**, 130,
- 8 12762-12774.
- 9 (79) Birke, R. L.; Huang, Q.; Spataru, T.; Gosser, D. K., Jr. *J. Am. Chem. Soc.* **2006**, 128, 1922-
- 10 1936.
- 11 (80) Kozłowski, P. M.; Kuta, J.; Galezowski, W. *J. Phys. Chem. B* **2007**, 111, 7638-7645.
- 12 (81) Nijenhuis, I.; Richnow, H. H. *Curr. Opin. Biotechnol.* **2016**, 41, 108-113.
- 13 (82) Payne, K. A.; Quezada, C. P.; Fisher, K.; Dunstan, M. S.; Collins, F. A.; Sjuts, H.; Levy,
- 14 C.; Hay, S.; Rigby, S. E.; Leys, D. *Nature* **2015**, 517, 513-516.
- 15 (83) Bommer, M.; Kunze, C.; Fessler, J.; Schubert, T.; Diekert, G.; Dobbek, H. *Science* **2014**,
- 16 346, 455-458.
- 17 (84) Liao, R. Z.; Chen, S. L.; Siegbahn, P. E. M. *ACS Catal.* **2015**, 7350-7358.
- 18 (85) Liao, R. Z.; Chen, S. L.; Siegbahn, P. E. *Chem. - Eur. J.* **2016**, 22, 12391-12399.

19 **SYNOPSIS**

