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Enormous Hydrogen Bond Strength Enhancement through π-Conjugation Gain: Implications for Enzyme Catalysis

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Supporting Information

ABSTRACT: Surprisingly large resonance-assistance effects may explain how some enzymes form extremely short, strong hydrogen bonds to stabilize reactive oxyanion intermediates and facilitate catalysis. Computational models for several enzymic residue−substrate interactions reveal that when a π-conjugated, hydrogen bond donor (XH) forms a hydrogen bond to a charged substrate (Y^-), XH can become significantly more π-electron delocalized, and this "extra" stabilization may boost the [XH···Y^-] hydrogen bond strength by ≥15 kcal/mol. This reciprocal relationship departs from the widespread pK_a concept (i.e., the idea that short, strong hydrogen bonds form when the interacting moieties have matching pK_a values), which has been the rationale for enzymic acid−base reactions. The findings presented here provide new insight into how short, strong hydrogen bonds could form in enzymes.

Short, strong hydrogen bonds have long been suggested to play important roles in enzyme catalysis,[1−8] but the nature of such bonds and the reasons for their proposed astonishing strengths (>20 kcal/mol) remain controversial. Contentious debates have centered on the possible implications of low-barrier hydrogen bonds,[6−8] especially for enzyme mechanisms involving oxyanion intermediates. For example, in the triosephosphate isomerase,[3,4] citrate synthase,[5] ketosteroid isomerase,[6,7] and photoactive yellow protein,[8] it was suggested that a catalytic base first extracts a proton from the substrate (YH = **1′−4′**) to form an oxyanion intermediate (Y^- = **1−4**), which may then be stabilized by a hydrogen bond donor (XH) (Figure 1a). It was proposed that if the XH residue and the conjugate acid of Y^- had matching pK_a values, proton transfer from XH to Y^- may proceed through a low to nonexistent barrier, resulting in extremely strong hydrogen bonding that could stabilize the emerging oxyanion on Y^-.[9−15] Indeed, low-barrier hydrogen bonds have been shown to display short heteroatom distances (≤2.6 Å) and found to be quite strong in the gas phase[16] and even in some high-dielectric media.[17]

Others argued, however, that the collective effect of many small electrostatic interactions may account for catalysis,[18] that short heteroatom distances based on X-ray data are irrelevant for hydrogen bonding in solution,[19,20] and thus there is no need to invoke the idea of low-barrier hydrogen bonding for enzyme catalysis.[18−26] Even when prefect pK_a match was achieved between a proton donor and acceptor, there appeared to be no special strengthening of a hydrogen bond.[25,26] How then might enzymes attain such enormous hydrogen bond strengths at their active sites?

Here, we show that short, strong hydrogen bonds may be achieved through π-conjugation gain in the hydrogen bond donor (XH), i.e., an idea akin to the resonance-assisted hydrogen bonding (RAHB) model.[27] RAHB describes the enhancement of hydrogen bond strengths due to increased π-electron delocalization in the interacting moieties. It is well-known that the hydrogen bond donating ability of an acid (XH) depends on the stability of its conjugate base (X^-). The more stable X^- is, the more acidic XH is. When there are delocalized π-electrons in XH, one way of stabilizing X^- is for these π-electrons to spread out even more as the negative charge develops, resulting in an increased level of π-electron delocalization across the entire π-system and more π-conjugation stabilization in the remaining X^-. We show here that the same π-conjugative effects occur when XH forms a hydrogen bond, and that this relationship may explain how short, strong hydrogen bonds might form in enzymes. In this way, a "pK_a match" between the proton-donating residue and substrate need not be necessary.[28−32]

We noted that the most representative enzyme−substrate interactions proposed to involve short, strong hydrogen bonds all employ π-conjugated residues as potent hydrogen bond donors, e.g., the imidazole (**Im**) and phenol (**Ph**) side chains of His and Tyr in the reactions catalyzed by triosephosphate isomerase, citrate synthase, ketosteroid isomerase, and photoactive yellow protein (Figure 1a). Although histidine may assume either a positively charged imidazolium form or a neutral imidazole form at physiological pH, it was proposed that in both the triosephosphate isomerase[3] and citrate synthase,[5] neutral histidine serves as a potent hydrogen bond donor in the enzymic reaction (more recent theoretical studies question the catalytic role of hisitidine in these enzymes;[4,23−35] see also ref 36). In these reactions, the **Im** or **Ph** may or may not give its imidazolic or phenolic proton to Y^- completely. However, when XH forms a hydrogen bond to Y^-, H^+ moves toward Y^-, and the emerging imidazolate or phenolate moiety (X^-) becomes more π-conjugated (i.e., more π-electron-delocalized), as shown by the resonance formalisms for **Im** and **Ph** in Figure 1b (i.e., the canonical forms on the right become more important).

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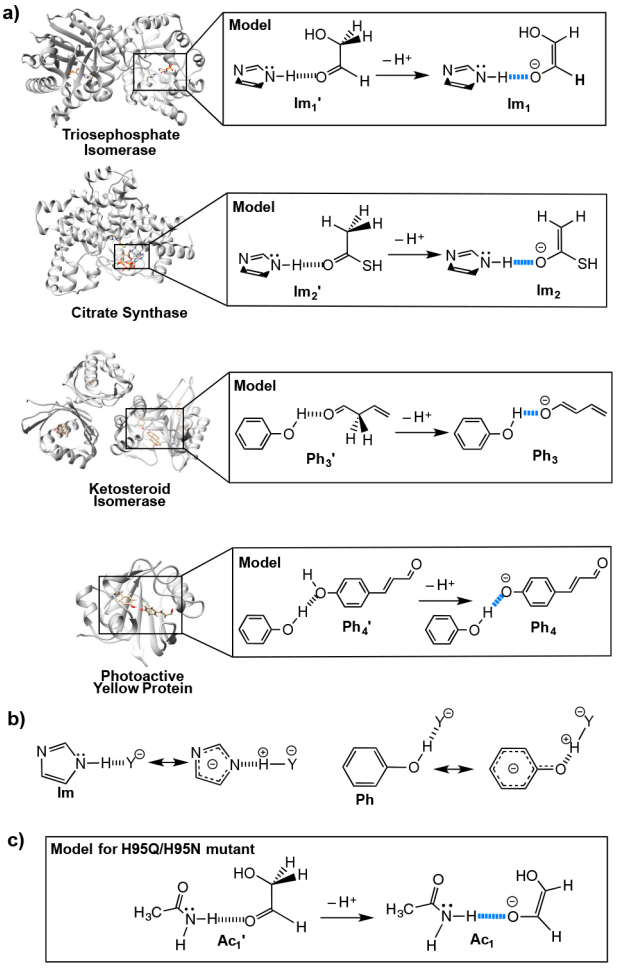

Figure 1. (a) Model acid−substrate interactions at the active sites of the triosephosphate isomerase, citrate synthase, ketosteroid isomerase, and photoactive yellow protein (short, strong hydrogen bonds colored in blue). (b) Illustration of RAHB in imidazole and phenol. (c) Model complex for the triosephosphate isomerase H95Q and H95N mutants.

Such "resonance assistance" may explain how some enzymes attain strong hydrogen bonds to stabilize reactive intermediates. We show here that for a given enzyme−substrate complex, the amount of π-conjugation gain in XH is small to negligible before the catalytic base extracts a proton from YH but becomes considerable as an oxyanionic Y^- emerges. Because the [XH···Y^-] hydrogen bond also is "charge-assisted",[37] H^+ moves more toward Y^-, and the developing X^- becomes more π-electron-polarized.[38] In this way, differential hydrogen bonding may be achieved and a large increase in hydrogen bond strength can occur as the initial enzyme−substrate complex is converted to a reactive oxyanionic intermediate. (Note that the presence and absence of π-conjugation in Y^- could further influence the XH···Y^- hydrogen bonding interaction.)

Although the RAHB concept[27] has been applied to explain many strong hydrogen bonds in chemistry (e.g., in prototropic tautomers, crystal packed structures, nucleobase pairs, and the secondary structures of proteins),[37,39,40] its implication for enzyme catalysis has largely escaped notice. In this paper, we elucidate the possible role of RAHB for several enzymic reactions, by examining a series of hydrogen-bonded [XH···Y^-] (**Im$_1$**, **Im$_2$**, **Ph$_3$**, and **Ph$_4$**) and [XH···YH] (**Im$_1$′**, **Im$_2$′**, **Ph$_3$′**, and **Ph$_4$′**) complexes that model enzymatic acid−substrate interactions.

Remarkably, large gas-phase hydrogen bonding interaction energies (ΔE_{HB}) and extremely short heteroatom distances between the hydrogen-bonded donor (D) and acceptor (A) ($d_{D\cdots A}$) were obtained for **Im$_1$**, **Im$_2$**, **Ph$_3$**, and **Ph$_4$** [ΔE_{HB} = 21.8−31.3 kcal/mol, and $d_{D\cdots A}$ = 2.484−2.671 Å (Table 1)], confirming recent experimental evidence[41] suggesting that short, strong hydrogen bonds may be available to enzymes. These values contrast with the more "normal" hydrogen bond strengths of **Im$_1$′**, **Im$_2$′**, **Ph$_3$′**, and **Ph$_4$′** [ΔE_{HB} = 5.2−6.8 kcal/mol, and $d_{D\cdots A}$ = 2.814−2.939 Å (Table 1)], which model acid residue−substrate hydrogen bonding interactions prior to the formation of an oxyanion on Y^-. Note that upon formation of hydrogen bonds to the substrate (Y = **1**−**4** or YH = **1′**−**4′**), the N−H and O−H bonds of imidazole (d_{D-H} = 1.004 Å) and phenol (d_{D-H} = 0.959 Å) are elongated by 5−12% in **Im$_1$** (1.127 Å), **Im$_2$** (1.055 Å), **Ph$_3$** (1.053 Å), and **Ph$_4$** (1.018 Å) but by only ≤1% in **Im$_1$′** (1.012 Å), **Im$_2$′** (1.015 Å), **Ph$_3$′** (0.971 Å), and **Ph$_4$′** (0.966 Å) (see Figure 2). Excellent correlation was obtained for the computed ΔE_{HB} versus Δd_{D-H} values [R^2 = 0.919 (Figure S3)], showing a relationship between a more elongated hydrogen-bonded N−H and O−H

Table 1. Computed Geometric ($d_{D\cdots A}$, d_{D-H}, and $d_{H\cdots A}$) and Energetic (ΔE_{HB},[a] $\Delta\Delta E_{HB}$,[b] and ΔDE_π[c]) Parameters for All Complexes

complex	d_{D-H} (Å)	$d_{H\cdots A}$ (Å)	$d_{D\cdots A}$ (Å)	ΔE_{HB} (kcal/mol)	$\Delta\Delta E_{HB}$ (kcal/mol)	ΔDE_π (kcal/mol)
Im$_1$	1.127	1.400	2.526	31.27	15.54	15.31
Im$_2$	1.055	1.617	2.671	23.78	10.26	10.93
Ph$_3$	1.052	1.432	2.484	26.09	8.10	8.19
Ph$_4$	1.019	1.537	2.556	21.84	6.52	6.65
Ac$_1$	1.066	1.573	2.638	25.83	8.66	7.73
Im$_1$′	1.012	2.054	2.939	5.19	2.34	2.88
Im$_2$′	1.015	1.950	2.922	6.72	2.27	4.20
Ph$_3$′	0.971	1.897	2.814	6.75	1.14	1.37
Ph$_4$′	0.966	1.946	2.890	5.75	0.73[d]	1.10
Ac$_1$′	1.010	2.102	3.024	4.29	1.18	2.78

[a] ΔE_{HB} is the computed gas-phase hydrogen bonding interaction energy at the ωB97X-D/6-311+G(2d,p) level of theory with zero-point energy (ZPE) correction. [b] $\Delta\Delta E_{HB}$ is the computed ΔE_{HB} of [XH···Y^-] or [XH···YH], where XH = **Im**, **Ph**, or **Ac**, minus that of its analogue with an unconjugated XH (XH = **In**, **Cyc**, or **Ae**; no RAHB possible). [c] ΔDE_π is the BLW-computed π-electron delocalization energy (DE_π) of XH before and after the formation of a hydrogen bond to Y at HF/6-31G(d). [d] A constrained geometry optimization was performed for the interaction between cyclohexanol and **4′** to avoid extra stacking interactions (see the footnote in Table S3).

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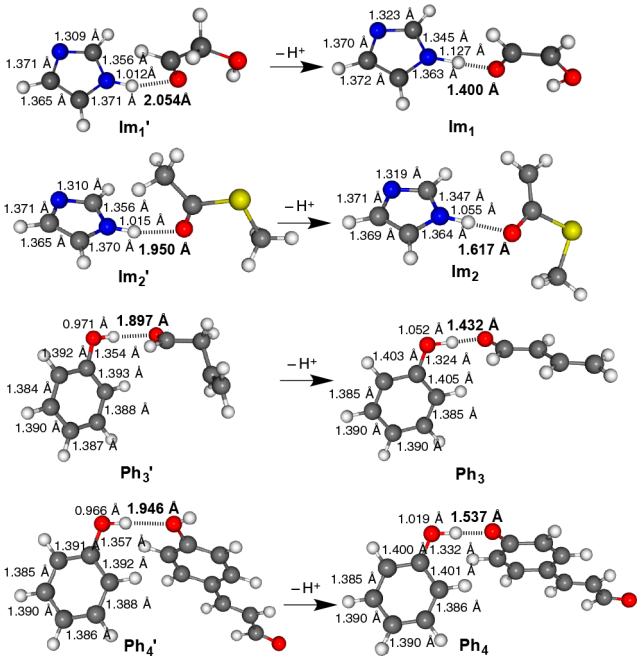


Figure 2. Optimized geometries for [XH···Y⁻] and [XH···YH] complexes at the ωB97X-D/6-311+G(2d,p) level of theory. Hydrogen bond distances are in bold.

distance and enhanced hydrogen bonding interactions. Because enzyme active sites typically have flexible loops that can fold over the bound substrate to exclude water,[42] all ΔE_{HB} values were computed[43] in the gas phase to reflect a water-occluded environment (see the full data in Table S1).

Computed block localized wave function (BLW) analyses,[44] a valence bond method designed and widely applied[45] to measure π-electron delocalization energies (DE_π) in molecules, quantified the DE_π of XH before and after hydrogen bonding to YH or Y⁻ to measure the amount of π-conjugation gain in XH [$\Delta DE_\pi = DE_\pi(XH_{hydrogen\text{-}bonded}) - DE_\pi(XH_{monomer})$]. On the basis of the BLW analysis, the computed ΔDE_π values for XH in **Im**$_1$, **Im**$_2$, **Ph**$_3$, and **Ph**$_4$ [ΔDE_π = 6.7−15.3 kcal/mol (Table 1)] are all surprisingly large (comparable to 20−50% of the ∼30 kcal/mol aromatic stabilization energy of benzene), indicating enormous π-conjugation gain in XH upon hydrogen bonding to the Y⁻ oxyanion! (Note, however, that the actual effects of π-conjugation gain in XH on the XH···Y⁻ hydrogen bond strength could vary depending on the polarity of the local enzymic environment, which has been shown to determine the distribution of negative charges among the hydrogen-bonded moieties.[46,47] Koeppe et al. demonstrated, e.g., that the hydrogen-bonded phenol−carboxylate complex exhibited a dominating phenolate−carboxylic acid form in a low-polarity solvent, but a dominating phenol−carboxylate form in a high-polarity solvent.[47]) In sharp contrast, the ΔDE_π values for XH in **Im**$_1$′, **Im**$_2$′, **Ph**$_3$′, and **Ph**$_4$′ [ΔDE_π = 1.1−4.2 kcal/mol (Table 1)] are small to negligible, indicating little π-conjugation gain in XH when it is hydrogen bonded to YH (prior to the formation of the oxyanionic Y⁻). The reliability of the BLW approach has been documented extensively by computed energetic and structural parameters consistent with experimental evidence.[45] Details and references for the BLW procedure are provided in the Supporting Information and in Table S2.

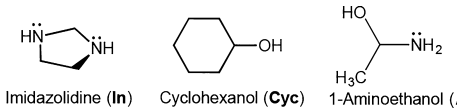
This relationship, between hydrogen bond strength and π-conjugation gain, also may explain the results of site-specific mutagenesis experiments in triosephosphate isomerase, in which replacing His95 with glutamine (Gln)[48] and asparagine (Asn)[49] reduced the catalytic activity of the resulting H95Q and H95N mutants (Figure 1c) by nearly 400- and 10^4-fold, respectively. While blends of steric and electronic factors must contribute (e.g., Cui and Karplus have shown that replacing His with Gln or Asn affected the initial rate-limiting formation of the enediolate intermediate),[4b] the reduced catalytic activity of the H95Q and H95N mutants may be better understood when the effects of RAHB are considered. Both imidazole and acetamide have side chain N−H groups, but the imidazole moiety of His is an inherently more powerful hydrogen bond donor than the acetamide (**Ac**) moiety of Gln and Asn due to a more favorable π-conjugation pattern. When His forms a hydrogen bond to **1**, for example, the increased level of cyclic six-π-electron delocalization in the imidazole moiety also enhances its aromatic character, resulting in extra π-conjugation gain in the hydrogen bond donor. When Gln or Asn forms a hydrogen bond to **1**, however, there is an increased level of four-π-electron delocalization in the acetamide moiety but no aromaticity gain.

Indeed, the computed ΔE_{HB} for **Im**$_1$ (31.3 kcal/mol) is 5.5 kcal/mol higher than that of **Ac**$_1$ (25.8 kcal/mol), reflecting a higher acidity for **Im** versus **Ac** by roughly 4 pK_a units, when hydrogen bonded to **1** (Table 1). Accordingly, the BLW-computed ΔDE_π value for imidazole in **Im**$_1$ (15.3 kcal/mol) is nearly twice that of acetamide in **Ac**$_1$ (7.7 kcal/mol), indicating more π-conjugation gain in the six-π-electron delocalized imidazole (Table 1). Computed dissected nucleus-independent chemical shifts, NICS(0)$_{πzz}$,[50] document the increased aromatic character of imidazole before (−30.9 ppm) and after (−32.8 ppm) hydrogen bonding to **1** (see refs 51 and 52).

These results show that hydrogen bond donors with particular π-conjugation patterns[53−55] may exhibit increased RAHB, and that such effects are especially pronounced, e.g., in the presence of charged hydrogen bond acceptors at the water-occluded active sites of enzymes. One might expect that all amino acids with π-conjugated side chain moieties (e.g., those with carboxylic acid groups) may display such "hydrogen bond−π-conjugation coupling", albeit to varying extents depending on their π-conjugation patterns.

Are these evaluations testable? Another way of quantifying the effect of this reciprocal π-conjugation gain is by comparing the computed ΔE_{HB} values of **Im**$_1$, **Im**$_2$, **Ph**$_3$, **Ph**$_4$, and **Ac**$_1$ and **Im**$_1$′, **Im**$_2$′, **Ph**$_3$′, **Ph**$_4$′, and **Ac**$_1$′ to those of analogous hydrogen-bonded complexes without a π-conjugated hydrogen bond donor, e.g., XH = imidazolidine (**In**), cyclohexanol (**Cyc**), and 1-aminoethanol (**Ae**) (no π-conjugation gain in XH possible, and thus no RAHB effect).

The resulting $\Delta\Delta E_{HB}$ values provide an estimate of the RAHB effect. As shown in Table 1 (column 5), the computed ΔE_{HB} values of **Im**$_1$, **Im**$_2$, **Ph**$_3$, **Ph**$_4$, and **Ac**$_1$ are all much greater than those of **In**$_1$, **In**$_2$, **Cyc**$_3$, **Cyc**$_4$, and **Ae**$_1$ ($\Delta\Delta E_{HB}$ = 6.5−15.5 kcal/mol) (see full data in Table S3). Conversely, the computed ΔE_{HB} values of **Im**$_1$′, **Im**$_2$′, **Ph**$_3$′, **Ph**$_4$′, and **Ac**$_1$′ are close to those of **In**$_1$′, **In**$_2$′, **Cyc**$_3$′, **Cyc**$_4$′, and **Ae**$_1$′ (all



Imidazolidine (**In**) Cyclohexanol (**Cyc**) 1-Aminoethanol (**Ae**)

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$\Delta\Delta E_{HB}$ values less than 2.0 kcal/mol). A linear correlation between $\Delta\Delta E_{HB}$ and ΔDE_{π} was found for all [XH···Y$^-$] and [XH···YH] complexes [R^2 = 0.976 (Figure 3)]. Evaluations based on natural bond orbital (NBO) computations (Table S4) as well as alternative unconjugated XH references (Table S5) agree and show the same excellent correlation.

Note that the studies presented here refer to anions in the gas phase and thus are crude models of the actual enzymatic environment, which require further refinements. Nevertheless, in implicit water solvation, the computed $\Delta\Delta E_{HB}$ values for **Im$_1$**, **Im$_2$**, **Ph$_3$**, **Ph$_4$**, and **Ac$_1$** [4−9 kcal/mol (see Table S6)] remain noticeable, suggesting that modest RAHB effects may still be available to enzymes with active site interiors that resemble wet polar organic solvents.

There are, of course, many ways enzymes can achieve catalysis (e.g., preorganization, transition state stabilization, ground state destabilization, proton tunneling, and cooperative hydrogen bonding), and the end result, a highly refined catalytic environment, must be the collective effect of many finely balanced interactions. We show here, however, that "resonance assistance" (which can be worth up to 50% of the benzene π-aromaticity!) may be one of the special tricks enzymes use to generate potent hydrogen bond donors and achieve hydrogen bonding interactions with astonishing strengths to facilitate catalysis. This connection improves our understanding of how enzymes work and, by implication, should extend to other biologically relevant hydrogen bond-mediated catalysis, self-assembly, and molecular recognition processes that may be "fine-tuned" through the power of π-conjugation.

COMPUTATIONAL METHODS

All geometries and hydrogen bonding interaction energies (ΔE_{HB}) were computed at the ωB97X-D/6-311+G(2d,p) level of theory employing Gaussian09 (benchmark results are included in Table S1). Geometries of the model residue−substrate complexes (**Im$_1$′**, **Im$_2$′**, **Ph$_3$′**, **Ph$_4$′**, **Im$_1$**, **Im$_2$**, **Ph$_3$**, and **Ph$_4$**) were fully optimized on the basis of initial coordinates extracted from selected Protein Data Bank files (see Supplementary Methods and Table S7). Block-localized wave function (BLW) computations at the HF/6-31G(d) level of theory quantified the π-electron delocalization energies (DE_{π}) of XH before and after the formation of a hydrogen bond to YH or Y$^-$. Nucleus-independent chemical shifts were computed at the PW91/IGLOIII level of theory.

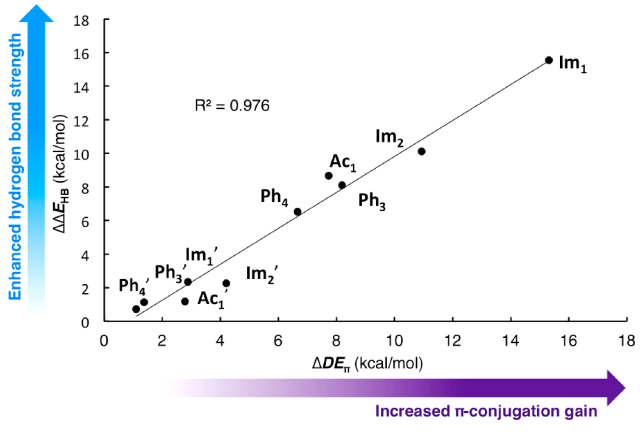

Figure 3. Plot of $\Delta\Delta E_{HB}$ vs ΔDE for all complexes.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.7b00395.

Full methods, data, and Cartesian coordinates for all structures (PDF)

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Author Contributions

J.I.W., K.I., A.M.B., and K.H.B. formulated the hypothesis of the proposed concept. J.I.W. and C.-H.W. computed, analyzed, and interpreted the results. J.I.W. wrote the manuscript.

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Notes

The authors declare no competing financial interest.

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