The PLP Pyridinium Electron Sink in GAD65 Decarboxylation

A parameter-free active-site test with RealQM, and its relevance to type-1 diabetes

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Developed in collaboration with Claude (Anthropic).

Abstract

GAD65 — the major **type-1-diabetes autoantigen** — decarboxylates L-glutamate to GABA + CO_2 using the cofactor pyridoxal-5′-phosphate (PLP), whose protonated pyridine ring acts as the *electron sink* that stabilises the carbanion left when the Cα–COO^- bond breaks (the quinonoid intermediate; Dunathan alignment). We ask whether a **parameter-free** electronic-structure method — RealQM, real-space, no basis set, no exchange–correlation functional — *spontaneously* reproduces this electron-sink role given only the nuclei. On a reduced gas-phase active-site model we drive the breaking Cα–CO_2 distance outward and relax the electrons at each step, for two environments: the glutamate *conjugated* to the pyridinium ring (sink present) and the *identical* glutamate as a free amino acid (no sink, a built-in negative control). The two curves diverge cleanly: the free case **saturates** once CO_2 is $\sim$4 Å out, while the conjugated case **keeps descending**; the sink term ($\Delta E_{\text{free}} - \Delta E_{\text{PLP}}$) grows monotonically to +4.2 (model units) and is still climbing at 6 Å. So the method recovers the textbook PLP electron-sink mechanism from first principles, with the identical-substrate control isolating the ring as the cause. The result is a *qualitative* validation (sign/trend, uncalibrated), not a rate or barrier; its value is a basis-free/functional-free cross-check, a clean internal control, and enough throughput to *scan* — the foundation for the T1D-relevant apo↔holo and epitope questions.

1 Objective

In the accepted mechanism of PLP-dependent decarboxylases the substrate forms an *external aldimine* with PLP; when the scissile Cα–COO^- bond breaks, the electrons left behind delocalise into the protonated pyridine ring — the **quinonoid / carbanion intermediate** — with the pyridinium nitrogen the *electron sink* that makes the otherwise high-energy carbanion accessible. By Dunathan's rule the scissile bond sits perpendicular to the ring for maximal $\sigma \to \pi$ overlap.

This is more than enzymology for the T1D field: GAD65 is the **major type-1-diabetes autoantigen** (GADA is a front-line predictive/diagnostic marker), and its autoantigenicity is tied to its unusual catalytic behaviour — its tendency to lose PLP and populate a flexible **apo** form, and the dynamics of the catalytic loop. Any computational claim about GAD65 conformation, the apo↔holo cycle, or epitope exposure ultimately rests on getting the **underlying PLP electronics** right. The question here: does a parameter-free method, given only the nuclei, *spontaneously* stabilise the departing-CO_2 carbanion specifically because the ring is conjugated?

2 Method

Solver. RealQM — a real-space, grid-based electronic-structure method with *no basis sets and no fitted force field*: the electron density is relaxed to its minimum-energy configuration in the field of fixed nuclei and the total energy E read out directly.

Reduced gas-phase active-site model. The PLP core, not the 585-residue enzyme: PLP reduced to a minimal mimic (protonated pyridine = the N1–H sink, plus 3-OH and 4′-imine; the 5′-phosphate and 2′-methyl anchors omitted); substrate = glutamate as the external aldimine, its α-carboxylate *Dunathan-aligned* (perpendicular to the ring; $+z$ = the departing CO_2), side chain truncated; Lys396 implicit; no protein, no water.

Controlled scan with a built-in negative control. The breaking Cα–CO_2 distance R is driven outward in fixed steps; at each R the nuclei are clamped and only the electrons relax to convergence, giving $E(R)$. This is run for two environments: **PLP** (glutamate conjugated to the pyridinium ring — sink present) and **free** (the *identical* glutamate as a free amino acid, $-NH_3^+$ — no conjugation, no sink). Reported: $\Delta E(R) = E(R) - E(R_0)$ (negative = downhill), and the **sink term** $= \Delta E_{\text{free}} - \Delta E_{\text{PLP}}$, the extra stabilisation attributable to the ring.

Run parameters. 24 atoms (PLP variant), 160^3 real-space grid, ∼38 a.u. box; 7 distances $R = 1.55$–6.00 Å $\times$ 2 environments = 14 independent relaxations; convergence by energy plateau; in-browser on WebGPU.

3 Results

R (Å)	ΔE free	ΔE PLP	sink = free − PLP
1.55	0.000	0.000	0.00
2.00	−0.012	−1.916	+1.90
2.60	−2.476	−3.776	+1.30
3.30	−2.560	−5.426	+2.87
4.20	−3.101	−6.605	+3.50
5.00	−3.556	−6.912	+3.36
6.00	−3.454	**−7.620**	**+4.17**

(Model energy units; sign/trend meaningful, magnitudes uncalibrated.)

The diagnostic signature is the **divergence** of the two curves. *Free glutamate saturates*: once CO_2 is ∼4 Å out, the free curve flattens at ≈ -3.5 ($-3.10 \to -3.56 \to -3.45$) — with no sink, the departing carbanion is a dead end. *PLP keeps descending* ($-6.6 \to -6.9 \to -7.6$, no plateau): the pyridinium π-system absorbs the developing negative charge (the quinonoid), so stabilisation keeps accruing. The sink term grows essentially monotonically to +4.2 at 6 Å and is still climbing. Given nothing but the atomic coordinates — no reaction coordinate built in, no bias — the method reproduces the textbook role of PLP: the conjugated ring, and only the conjugated ring, stabilises the carbanion; the identical-substrate control isolates the effect.

4 Scope — what this does and does not show

- **Qualitative, not quantitative.** Magnitudes are uncalibrated model units (reproducible to $\sim \pm 0.5$–0.8 between runs). Read the sign, the divergence, and the monotonic growth — no kcal/mol claim.
- **Thermodynamics, not kinetics.** A clamped single-coordinate relaxed scan has no transition state; this shows carbanion/product stabilisation, not a rate or barrier.

- **Gas-phase, reduced core.** No protein electrostatics, no phosphate anchor, no water, hand-built geometry. A mechanism demonstration on the active-site electronics, not a structural model of the enzyme.

5 Relation to DFT / first-principles QM

The PLP electron-sink / quinonoid mechanism reproduced here is *not* new physics — it is what mainstream quantum chemistry (QM-cluster and QM/MM with DFT functionals, in implicit or explicit solvent) has established for PLP decarboxylases over two decades, *quantitatively and with kinetics*: full free-energy profiles with transition states, calibrated kcal/mol barriers validated against rates, and a quantified account of pyridine protonation, Dunathan alignment and quinonoid delocalisation. On the established catalytic step, mature QM/MM is the gold standard and this run does not compete with it.

	DFT / QM-MM (state of the art)	RealQM (this run)
Energies	calibrated kcal/mol, benchmarked	uncalibrated units; sign/trend only
Kinetics	transition states, barriers, rates	none (thermodynamic, single coordinate)
Environment	protein electrostatics + solvent	gas-phase reduced core
Basis/functional	basis set + XC functional (main error)	none — real-space grid
Parameters	empirical dispersion common	parameter-free
Control	sink inferred from full landscape	identical-substrate control *by construction*
Cost	substantial; HPC	commodity GPU, in-browser, scannable

RealQM's value here is *not* a better number on a solved problem, but: (1) a **methodological cross-check** — a formalism with no basis set and no exchange–correlation functional (DFT's principal systematic-error source) independently recovers the same electron-sink physics; (2) a **clean internal control** (free amino acid = same molecule minus the ring); (3) **throughput** — cheap enough to *scan*. The gap both leave open is GAD65 *specifically* — the apo↔holo cofactor-loss state tied to its autoantigenicity is largely unaddressed computationally; that, not the textbook step, is the target, and calibrating RealQM against high-level QM/MM on the catalytic step earns the right to take it there.

6 Relevance to GAD65 / T1D, and outlook

This establishes that a parameter-free method captures the central electronic event of GAD65 catalysis correctly and from first principles — the foundation before the more T1D-relevant questions can be addressed credibly:

- **apo ↔ holo electronics.** GAD65's autoantigenicity is linked to its propensity to lose PLP and populate a flexible apo form; the same machinery can compare holo vs apo active-site electronic stabilisation.
- **Catalytic-loop / epitope coupling.** Once the electronics are trusted, the catalytic region overlapping known conformational GADA epitopes becomes a modelling target.
- **Autoantibody recognition** (a separate, currently *parked* model): real GADA epitopes are conformational, so that line needs real CDR/epitope residues and is a much larger lift.

Bottom line. An ab-initio-style electron solver, handed only the active-site atoms, independently reproduces the PLP electron-sink mechanism that defines GAD65's chemistry, with the free-amino-acid control confirming the effect is specifically the conjugated ring — a clean qualitative validation, not a calibrated energetic or kinetic prediction.

Collaboration. The model and the interpretation are the author's; the manuscript and the simulations were developed in dialogue with Claude (Anthropic). Interactive versions (decarboxylation scan, 3D active-site viewer) are at https://claes542.github.io/RealMolecule/gallery.html (see the GAD65 card).