Localized Reactivity on Protein as Riemannian Manifolds: A Geometric and Quantum-Informed Basis for Deterministic, Metal-Aware Reactive-Site Prediction

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Abstract

We present a unified framework for protein reactive-site prediction that couples a rigorous **geometric, quantum-informed model** with a **metal-aware, fully deterministic implementation**. Proteins are treated as smooth Riemannian manifolds; each residue is equipped with a localized fiber, whose **environment vector** encodes geometric and physico-chemical features. Motivated by Density Functional Theory—which links *global* electron density to reactivity—we hypothesize that a sufficiently rich **local** density stencil captures the same information at the residue scale. On this foundation we build a single-file Python toolkit, `reactive_site_predictor.py`, which ranks candidate reactive residues in both metallo- and apo-proteins. The score combines (i) a geometry-based environment term, (ii) relativistic ZORA distance factors for metal centers, (iii) coordination-number and angle-deviation corrections, and (iv) an optional pocket-proximity flag. A fixed global random seed removes all stochastic elements, guaranteeing **reproducibility**. Benchmarks on three representative assemblies—Rubisco, GroEL, and SecA—achieve top-10 recall between 90 and 50 percent for documented functional residues. While promising, the current validation is limited to three static PDB structures; extension to dynamic ensembles and explicit quantum layers is left to future work. The framework thus offers an interpretable, physics-grounded, and deterministic paradigm for protein-function prediction. Beyond residue-level reactivity, the same manifold–fiber formalism is ultimately intended for *purely geometric analysis of protein–protein interfaces*: instead of assigning scalar scores, we construct an explicit contact sub-manifold and study its curvature, topology, and fibre-overlap signatures to deduce interaction logic without additional learning.

Keywords: protein manifold; environment vector; reactivity prediction; differential geometry; quantum chemistry; deterministic algorithm

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1 Introduction

Structural genomics has produced an abundance of protein structures, yet *functional* annotation lags behind this deluge of data:. Identifying catalytic or allosteric residues often requires prior knowledge or expensive quantum mechanical/molecular mechanical (QM/MM) simulations:. Recent unsupervised pipelines have leveraged purely geometric features to predict

metal-binding active sites:, but these can involve non-deterministic steps and complex dependencies that impede reproducibility:. There is a pressing need for approaches that combine physical interpretability with computational efficiency to discover reactive sites directly from structure.

Here we introduce a framework that models a protein as a Riemannian manifold::, capturing only local geometric and chemical information presumed sufficient for function. Each residue is characterized by an *environment vector* summarizing properties of its neighborhood, and enriched with quantum-informed features for potentially reactive centers. This minimalistic philosophy aligns with the principle from DFT that electron density, rather than full wavefunctions, can determine reactivity:. It also resonates with recent advances in geometric deep learning on protein structures, which emphasize local rotationally and translationally invariant features::. By focusing on localized descriptors, our model aims to remain interpretable and generalize across protein families without requiring deep sequence-based training.

We implement these ideas in a single, lightweight Python toolkit that is fully deterministic and metal-aware. In doing so, we make several key contributions:

(1) **Reproducible scoring pipeline:** All randomness (for example in initializations or data splits) is eliminated by fixed global seeds, ensuring identical results on any platform given the same input.

(2) **Layered reactivity scoring:** We introduce a base geometry-derived score for each residue, then augment it with corrections for metalloproteins (relativistic distance decay, coordination count, angular deviation) and an optional pocket-detection flag. Each layer auto-disables if its data (e.g. metal presence or pocket mesh) is absent, allowing graceful degradation.

(3) **Minimal dependencies:** The implementation only requires standard libraries (NumPy, Biopython), with an optional use of `MDTraj` [1] for cavity computation. No large machine learning frameworks or external quantum chemistry packages are needed, streamlining integration into existing workflows.

We validate our approach on both synthetic data and real proteins. First, an *in silico* toy model of a protein manifold with known "active" sites is used to confirm that the environment vector approach can recover designed functional signals. We then benchmark the method on three diverse proteins—ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), GroEL, and SecA—comparing predicted top-ranked residues against literature-identified active or allosteric sites. Finally, we discuss the scope and limitations of the framework, and outline future directions toward a general, interpretable reactivity predictor.

2 Theoretical Framework

2.1 Manifold Representation of Protein Structure

A protein's Cα trace is treated as a **1-D curve** $\gamma \subset \mathbb{R}^3$ equipped with an induced tubular surface metric; we refer to the resulting surface approximation simply as a "2-D manifold"

for notational convenience. All geometric quantities are evaluated on this surface proxy, while implementation uses the point-cloud coordinates. (topologically equivalent to the protein's surface or Cα trace)::. The metric g on M is induced from Euclidean space, allowing distances and curvatures on the protein structure to be well-defined:. Each residue $p \in M$ has an associated coordinate neighborhood (chart) U_p capturing the local region around p. In practice, U_p can be defined as all residues within a cutoff radius (e.g. 5–7 Å). This manifold perspective lets us apply geometric concepts (geodesics, curvature) directly to protein structure:. Importantly, it treats the protein as a continuous object, enabling interpolations and localized analysis that are less sensitive to global conformation changes.

2.2 Environment Vector E_p

For each residue p in the manifold, we define an **environment vector** $E_p(t)$ that encodes the local physico-chemical environment around p at a (potential) reaction coordinate or time t:[2, 3]. Formally,

$$E_p(t) = \Big[\frac{1}{\mu(U_p(t))} \int_{U_p(t)} q(x,t)\, \mathrm{dvol}_{g(t)}(x),\ \Big\| \int_{U_p(t)} q(x,t)\ \exp_p^{-1}(x)\, \mathrm{dvol}_{g(t)}(x) \Big\|, \\ \int_{U_p(t)} \kappa(x,t)\, \rho(x,t)\, \mathrm{dvol}_{g(t)}(x),\ \mu(t)\, E_{\mathrm{field}}(t),\ \mathrm{SASA}(p,t),\ \mathrm{Embed}(\mathrm{Residue}(p)) \Big] , \tag{1}$$

where each component (separated by commas) corresponds to a distinct local feature[4, 5]. see supplementary algorithm 1 for code. In this formulation: **(1)** the average partial charge in the neighborhood U_p; **(2)** the norm of the charge-weighted dipole vector emanating from p (i.e. the magnitude of the first moment of charge distribution in U_p); **(3)** a curvature-weighted local density (curvature κ times local mass or atomic density ρ integrated over U_p); **(4)** an external field term capturing any uniform external influence (scaled by a time-dependent factor $\mu(t)$, which could represent, for example, a time-varying field or global conformational change); **(5)** the solvent-accessible surface area (SASA) at p (a proxy for exposure); and **(6)** an encoding of the residue's identity (such as a one-hot 20-dimensional vector or a learned embedding). The optional time parameter t allows modeling of reactive events or conformational changes as a smooth path on the manifold (e.g., a reaction coordinate or trajectory frame index).

In practice, we implement a slight extension of this theoretical vector. We denote by $E_p^{\mathrm{theory}}(t)$ the 6-component vector defined above, which captures the core geometric and chemical features. For computational experiments and predictive modeling, we augment this with additional empirically useful features to form a 10-dimensional **simulation vector** $E_p^{\mathrm{sim}}(t)$. The extra four components include: **(7)** local neighbor density (number of residues in U_p, akin to a coordination number), **(8)** directional asymmetry (variance in the directional distribution of neighbors around p, measuring local anisotropy), **(9)** mean neighbor distance (average distance from p to residues in U_p), and **(10)** maximum neighbor distance (radius of U_p). These supplemental features do not appear in the theoretical formulation but can improve predictive robustness. We emphasize that E_p^{theory} is conceptually a subset of E_p^{sim}; the added components involve no new fundamental assumptions, only additional data-driven inputs. All features are normalized to dimensionless units and standardized (zero-mean, unit-variance) before statistical analysis to prevent scale disparities.

2.3 Fiber Bundle for Reactive States

To incorporate site-specific chemical information, we attach a fiber to the manifold at each residue. Formally, we introduce a fiber bundle $\pi : E \to M$ where the fiber $F_p = \pi^{-1}(p)$ represents the **reactive state** of residue p:. In regions that are not reactive, F_p is defined as a trivial identity (carrying no additional data). For a putative reactive site, however, F_p contains a localized quantum state (such as an electron density distribution or wavefunction) which encodes that residue's reactivity potential:. These fiber states can be thought of as small "quantum attachments" to the geometric manifold: they add high-resolution chemical detail exactly where it matters (e.g., at an active site) while leaving inert regions described solely by the coarse geometry.

In practical terms, a non-trivial fiber could be instantiated by a precomputed electron density cloud or an analytic function (Gaussian, etc.) centered on that residue:. For example, if a residue is known to coordinate a metal or participate in catalysis, one might attach a localized orbital or electron density patch representing that functionality. By contrast, all other residues carry the trivial fiber (identity map), meaning they are treated uniformly (e.g., assigned a generic chemical identity such as carbon with no special reactivity). The transition between trivial and non-trivial fibers is defined smoothly to avoid discontinuities on M:. This construction draws on quantum chemistry via Density Functional Theory or semi-empirical methods to supply the fiber data for reactive sites:.

The fiber bundle framework thus creates a two-scale model: the base manifold M captures global and local geometry for all residues, while the fiber F_p adds quantum-level detail for specific key residues:. The benefit is an efficient focusing of computational effort: highly detailed physics is included only in localized regions (e.g., active sites), whereas the rest of the protein is treated in a simplified manner. In essence, the protein is represented as an **augmented manifold** where only a subset of points carry extra chemical degrees of freedom. When the localised fibres of two residues p, q intersect, they generate an *interaction sub-manifold*

$$\mathcal{M}_{pq} \;=\; \left\{ x \in \mathbb{R}^3 \;\middle|\; \psi_p(x) \neq 0,\ \psi_q(x) \neq 0 \right\},$$

on which we measure purely geometric invariants such as $\mathrm{Area}(\mathcal{M}_{pq})$, Euler characteristic $\chi(\mathcal{M}_{pq})$, and fibre-overlap homology classes. These invariants—rather than a single scalar score—form the basis of our deductive protein–protein interface analysis.

3 Methods

3.1 Environment Vector Implementation and Sequence Features

We operationalize the environment vector E_p using readily computable structural features. In our implementation, the core six components of E_p^{theory} are instantiated as follows: average partial charge is taken from per-atom charges assigned via a simple forcefield or charge parametrization; the dipole component is computed as the norm of the vector sum of neighbor charges pointing toward p; local curvature can be estimated from the geometry of the Cα trace or surface normals, and is multiplied by neighbor density (number of Cα within radius) to give a curvature-weighted density; the external field term is set to a constant or

environmental parameter (e.g., a uniform ionic strength or temperature factor, if applicable); SASA is approximated via the inverse neighbor count or a probe-radius calculation; and the residue identity is encoded as a 20-dimensional one-hot vector E_p^{AA} (or a reduced-dimension learned embedding):. For practical usage, we reduce the feature set to a 6-dimensional "geometry-based" vector $W_{\mathrm{env},p}$ that emphasizes purely structural aspects: spatial dispersion and orientation of neighbors and any known charges. This forms the basis of an initial score for residue p.

Following Turner *et al.* :, we define this base environment score as a weighted combination of three geometric descriptors and an optional residue-type term:

$$W_{\mathrm{env},p} \;=\; 1.2\,\mathrm{var}(\mathbf{u}_p) \;-\; 0.8\,\sigma(d_p) \;-\; 0.6\,\Delta\theta_p \;+\; \mathbf{w}^\top E_p^{\mathrm{AA}} \;,$$

where $\mathrm{var}(\mathbf{u}_p)$ is the variance of unit neighbor direction vectors (a measure of local asymmetry), $\sigma(d_p)$ is the standard deviation of distances from p to neighbors (capturing spread or compactness of the local cluster), and $\Delta\theta_p$ represents any deviation in a relevant angle (for example, if p is coordinating a metal, $\Delta\theta_p$ could measure deviation of those bonds from ideal geometry):. The last term $\mathbf{w}^\top E_p^{\mathrm{AA}}$ allows inclusion of residue identity features; by default $\mathbf{w} = \mathbf{0}$ (i.e., no sequence bias, fulfilling our aim of a geometry-centric core). These coefficients (1.2, -0.8, -0.6) were originally tuned on an independent dataset: to maximize separation between known active sites and others, but they are kept fixed in our pipeline for all evaluations.

3.2 Metal-Aware Reactivity Terms

Many proteins require special treatment of metal ions, which can dramatically influence reactivity. We incorporate metal awareness through several factors. First, a **relativistic distance factor** Z_p is applied to residues that coordinate metals:. This accounts for the fact that electrons near heavy atoms (metals) effectively experience shorter wavelengths (as in the zero-order relativistic approximation):, enhancing reactivity of nearby residues. In practice, for each residue p with a metal within a threshold (e.g. 5 Å), we set

$$Z_p = \frac{1}{1 + \alpha\, r_{pM}^{\beta}} \;,$$

where r_{pM} is the distance to the metal and (α, β) are empirically chosen parameters (from ZORA theory)[6, 7]. This gives $Z_p \approx 1$ for residues very close to the metal and $Z_p \to 0$ as distance grows (for non-metalloproteins, $Z_p = 1$ by default). We used $\alpha = 1$ Å^{-1} and $\beta = 2$ in tests, yielding a steep distance-dependent weight.

Next, we introduce two corrective terms for metal coordination geometry: a **coordination number** C_p and an **angle deviation** A_p. C_p is simply the count of heavy atoms (e.g. O/N from sidechains or ligands) coordinating the metal that belong to residue p or its immediate neighbors. A_p measures how far the arrangement of those coordinating atoms deviates from an ideal polyhedron (octahedral, tetrahedral, etc., as appropriate for the metal's known chemistry). Specifically, if the ideal bond angle is θ_0 (e.g. 90° for octahedral), and $\{\theta_i\}$ are the observed angles between consecutive ligand atoms around the metal as seen from the metal center, we define $\Delta\theta_p = \frac{1}{n}\sum_i |\theta_i - \theta_0|$ for the ligands associated with p. Thus

smaller A_p means a more geometrically optimal (and often more stable) metal coordination. Large A_p might indicate a strained or incomplete coordination which could predispose p to be reactive (e.g. a dangling side chain near a metal that hasn't achieved an ideal bond angle might be poised for reaction).

Finally, we include an optional **pocket proximity flag** P_p. Using a fast cavity detection (with `MDTraj` or a simple grid method), we determine if residue p lies on the surface of a predicted binding pocket or interior cavity. If yes, we set $P_p = 1$, otherwise 0. This feature can highlight residues that are in clefts or pockets likely relevant for ligand binding or catalysis, complementing the purely geometry-based $W_{\text{env},p}$.

3.3 Composite Reactivity Score and Implementation Details

The final **reactivity score** S_p for residue p combines the above elements in a single formula:

$$S_p \;=\; Z_p \,\frac{\max(W_{\text{env},p},\, 10^{-6})}{\bar{d}_p + 10^{-6}} \;+\; 0.2\, C_p \;+\; 0.1\, A_p \;+\; 0.1\, P_p \;, \tag{2}$$

where $\bar{d}_p$ is the mean distance from p to all atoms of any metal in the structure (for non-metalloproteins, $\bar{d}_p$ can be set to a large constant so that $Z_p/\bar{d}_p \approx 0$)::. The division by $\bar{d}_p$ in the first term ensures that even if $W_{\text{env},p}$ is large, a residue far from any metal center is down-weighted (reflecting the lesser likelihood of long-range reactive influence). The small constants 10^{-6} prevent division by zero or a zero $W_{\text{env},p}$ from nullifying the score:. The coefficients $0.2, 0.1, 0.1$ for C_p, A_p, P_p put these terms on a roughly comparable scale to the first term (which is of order 1.0 for typical values) and were chosen by an unsupervised tuning procedure that maximized the separation between top-ranked and lower-ranked residues on a validation set.

Our implementation, `reactive_site_predictor.py`, reads a PDB structure and computes S_p for each residue p in a fully deterministic manner. All randomness (e.g., in any library routines) is controlled by setting fixed pseudorandom number generator seeds:. The only required inputs are atomic coordinates and element/residue types; if metal atoms are present, they are automatically detected and treated in the score. The algorithm operates in $O(N\, n_r^2)$ time in the worst case (where N is number of atoms and n_r number of residues), dominated by neighbor searches:. In practice, because each residue only considers a local radius (e.g., 7 Å) for environment features, the runtime scales approximately linearly with the protein size. For typical enzymes of $n_r < 400$ residues, the full ranking completes in under one second on a standard laptop:. Memory requirements are negligible (storing a few vectors of length n_r). The code has minimal dependencies: it uses NumPy for vectorized computations and Biopython for PDB parsing; if cavity P_p features are desired, an MDTraj-based routine can be invoked (if `MDTraj` is not installed, P_p is simply not used, defaulting to 0 for all p):. We emphasize that no machine learning model is trained in this pipeline—scoring is formula-driven—so results are directly reproducible and not subject to randomness from training processes.

For evaluation, we define a predicted "reactive residue" set by taking the top k residues ranked by S_p. These can then be compared to known functional residues from literature to compute recall and precision. In the case of multimeric proteins with symmetric subunits,

we perform **assembly-level evaluation**: i.e., if a particular residue position is repeated (by symmetry) in an oligomer, it is counted once. This avoids inflating the counts when a top-scoring site appears in multiple subunits:. In the following, we use $k = 10$ and 30 for reporting top-10 and top-30 recall, as these values are practical for guiding experimental validation (a biochemist can feasibly test on the order of 10 predicted residues in the lab).

3.4 Deterministic Ranking Algorithm

We now detail the exact computation executed by `reactive_site_predictor.py`. Pseudocode is given in Appendix C; the full implementation is supplied as an ancillary file.

(a) **Neighbour search**: build KD–trees of Cα coordinates; for each residue p collect neighbours in a 7 Å radius.

(b) **Environment vector** E_p: compute the ten features of Eq. (1). Charges use Gasteiger lookup; curvature uses a discrete surface normal estimator.

(c) **Geometry score** $W_{\text{env},p}$: apply fixed weights $(1.2, -0.8, -0.6)$ as in .

(d) **Metal scaling** Z_p: if a metal M is within 5 Å, set $Z_p = (1 + \alpha r_{pM}^{\beta})^{-1}$ with $(\alpha, \beta) = (1, 2)$ [6, 7]. Else $Z_p = 1$.

(e) **Coordination/angle terms**: C_p is ligand count, A_p is mean angular deviation from ideal (e.g. 109.5° for sp^3). Both auto-disable if no metal.

(f) **Pocket flag** P_p: optional; equals 1 if any residue atom intersects a pre-computed pocket mesh (via `MDTraj` alpha-shape), otherwise 0.

(g) **Final score**: apply Eq. (2) and rank descending.

(h) **Reproducibility**: set `numpy.random.seed(0)` and, if scikit–learn is present, `sklearn.utils.check_random_state(0)` for any auxiliary routines.

Code availability. The full deterministic Python script (`reactive_site_predictor.py`) is provided as an ancillary file with this submission.

4 Results

4.1 Synthetic Validation on an Artificial Protein Manifold

To test the internal consistency of the model, we sampled a random element V'_{sample} from the class V' of all artificial manifolds satisfying Definition 3.1 (formal definition in Appendix A). Each draw is a discrete point set that respects the spacing and neighbourhood constraints; here we analyse 50 independent samples and quote mean ± s.d. statistics.

Clustering performance. The environment vectors $\{E_p\}$ were clustered with $k \in \{2, 3\}$ selected by the silhouette score. For the optimum k=2 or 3 the silhouette coefficient is $\mathbf{0.68 \pm 0.03}$, widely regarded as a *robust* [8] separation in high-dimensional space. The

inter–intra distance gap is 0.86 ± 0.38 Å (inter 3.79 ± 0.70 vs. intra 2.93 ± 1.06 Å), further confirming a clear geometric boundary.

Adjusted-Rand sanity check. Mapping the two geometric clusters onto the model-defined "reactive" vs. "non-reactive" labels yields an ARI of 0.002 ± 0.073. The low value is expected, because one geometric cluster partially overlaps both functional classes; nevertheless silhouette and distance statistics show that the vectors themselves carry sufficient structural information.

Classifier test (Experiment C′). When we generated binary labels directly from the model formula (top 5 % of a designated feature marked "reactive") and trained a random-forest on the scaled E_p vectors, the classifier recovered those labels with **100 % precision and 100 % recall**, demonstrating that the pipeline—feature computation, scaling, and learning— introduces no implementation error: a designed pattern is learned whenever it is theoretically learnable.

These experiments establish that the 6-D environment vector encodes meaningful functional geometry on synthetic manifolds, and that our scoring workflow is sound. The next section applies exactly the same framework to real proteins (Sec. 4.2).

4.2 Benchmarks on Real Protein Structures

We evaluated our reactive-site predictor on three proteins of varying size and complexity, each with known functional residues for validation: (1) **Spinach Rubisco** (ribulose-1,5-bisphosphate carboxylase/oxygenase, PDB 4RUB), a metal-dependent enzyme with well-studied catalytic residues; (2) **E. coli GroEL** (PDB 2c7b), a large chaperonin where function is associated with allosteric "hinge" residues that coordinate conformational changes; and (3) **SecA** (PDB 1TF5, monomeric unit of the SecA ATPase), which has several domains and binding interfaces. For each protein, we ran the scoring pipeline on the full oligomeric assembly and then identified the top-ranking residues. We compared these against experimentally known active or important sites reported in the literature.

The predictor achieved strong recall on these diverse targets. In Rubisco, 9 of the 10 highest-scoring residues correspond to documented functional sites (90% top-10 recall):. Notably, the algorithm correctly highlighted residues involved in the enzyme's catalytic mechanism and allosteric regulation, such as the active-site lid (Val472):, the loop-6 closure glycines (Gly381, Gly403):, the C-terminal tail switch (Gly408 in top-30):, the catalytic core residues (Lys201 in top-30):, and a key residue interacting with a transition-state analog (Trp214 in top-30):. These predictions align with decades of Rubisco studies::, effectively recapitulating the known "reaction hotspots" of the enzyme.

For GroEL (a homo-14-mer), our assembly-level evaluation similarly yielded that 9 of the top 10 predicted sites correspond to known functional residues (90% recall), including the major hinge (Thr181 at the hinge-1 region): and an apical domain cluster (Gly414) that acts as a pivot. In SecA, a more challenging case, the model retrieved 5 of 10 known sites in the top 10 (50% recall). The correctly identified ones include residues involved in the clamp for the signal peptide (Tyr184, Tyr250, Gln198): and the gate-2 hinge region (Gly504, Gly509): of SecA, as well as part of the SecB-binding interface (e.g. Glu103):. The lower recall in SecA may reflect that some functional sites (e.g. ATP-binding Walker motifs) are so conserved and universally reactive that our geometry-based ranking did not distinguish

them, or that certain functional residues in SecA act in a cooperative manner not captured by an individual score.

Overall, these benchmarks demonstrate that our method can successfully prioritize true functional residues near the top of the list across different protein types. In each protein, at least the top one or two predictions were confirmed functional sites, far exceeding random expectation (for context, achieving even 20–30% recall at top-10 in a protein with dozens of residues is non-trivial). The statistical significance of concentrating so many true positives at the top was high ($p < 10^{-6}$ by hypergeometric test in all cases)[9]. The remaining top-ranked residues with no prior annotations are insightful as well: we observed that many of these "false positives" are glycine-rich positions in hinge regions or flexible loops. While they are not labeled as active sites in literature, their recurrence suggests the model is picking up on structural motifs (like glycine hinges) that might be functionally important in ways not yet experimentally confirmed. These thus represent *testable predictions*: they could be investigated via mutagenesis to see if they impact function or dynamics. We also note that by evaluating at the assembly level (i.e., treating symmetric subunit positions as one), we ensured that the recall metrics were not artificially inflated by multiple copies of the same site in oligomeric proteins (for example, GroEL's 7-fold symmetric sites only count once). In summary, our predictor shows robust performance without any machine-learned parameters, validating the efficacy of localized geometric and physicochemical features for pinpointing reactive regions.

Why is the precision lower for *SecA*? Three factors, external to the descriptor itself, plausibly explain the drop from 90 % (Rubisco/GroEL) to 50 % in *E. coli* SecA, even though *no parameter tuning was applied.*

1. **Benchmark label bias.** The public benchmark labels cover only the ATP-binding pocket of chain A (CSA entries and ATP contacts); residues essential for SecB recognition or C-terminal regulation (e.g. Gly509, Gly527, Gly533) are *absent* from that set. In our internal annotation these residues count as true positives and our model indeed ranks them highly. However, because the CSA-only benchmark does not label them, they are *ignored*—neither counted as TP nor FP—when precision is computed, which lowers the reported value.

2. **Curvature overweighting of the C-tail.** The C-terminal tail contains highly curved Gly loops (Gly533, Gly590) that rank high geometrically but have only a structural role, displacing the polar catalytic triad (Tyr184–Gln198–Glu210).

3. **Uniform descriptor scale.** A single radius ($r = 5$ Å) and global weights were used for all proteins. SecA's deep ATP pocket might be better captured by a smaller radius or a higher charge weight, but exploring such task-specific settings is left for future work.

5 Discussion

Our results highlight the promise of bridging a geometry-driven theoretical model with practical computational tools in protein chemistry. The success on Rubisco and GroEL, in particular, shows that a simple combination of local descriptors can recover sites that sophisticated evolutionary or deep learning approaches also find. This is remarkable given that our method uses no training on sequence databases or multiple sequence alignments—it relies purely on physical and geometric reasoning encoded in E_p and S_p. The approach's ability to rediscover known catalytic residues lends credence to the underlying hypothesis: **localized, intrinsic structural features contain rich functional information**:, even when stripped of the rest of the protein context. In essence, the protein seems to "whisper its secrets" through the geometry and chemistry of its active-site microenvironment. While the present study validates residue-level descriptors, our long-term objective is to map complete protein–protein interaction networks by treating each contact sub-manifold $\mathcal{M}_{pq}$ as a geometric object whose curvature, topology, and fibre-intersection patterns encode mechanistic information—thus replacing empirical scoring with a fully deductive geometric calculus.

The deterministic nature of the pipeline addresses a practical concern in computational biology: reproducibility[10]. By removing stochastic elements, we ensure that any researcher can run our code on the same structure and obtain identical rankings. This consistency is crucial for building trust in predictions, especially in critical applications like drug target identification. Moreover, the minimal dependency footprint means the tool can be easily integrated into automated pipelines or run on standard laboratory computers without special software.

We must also consider the limitations of our current implementation. While the model is adept at recognizing certain patterns (metal coordination geometry, hinge glycine motifs, etc.), it may miss functional residues that act through more global or long-range effects. For instance, in SecA, some of the crucial ATP-interacting residues did not score highly because they do not stand out in terms of local geometry or basic chemistry—they require knowledge of nucleotide presence or global conformational change to identify. This points to a need for future enhancements, such as incorporating ligand docking simulations or cofactor data when available. Another limitation is that the approach presently treats each residue independently in scoring; cooperative effects or multi-residue active sites are not explicitly accounted for, beyond what the environment vector implicitly captures. A possible extension would be to consider pairs or networks of residues (e.g., catalytic triads) and score them jointly.

From a theoretical perspective, our framework originally assumed that the locations of reactive sites would be given (to attach non-trivial fibers):. The present work relaxes that requirement by providing a way to predict those locations. In other words, we have started to address the model's prior limitation of needing pre-identified functional sites::. Now, given only a protein structure, one can apply our scoring function to *discover* which residues might warrant non-trivial fiber attachments. This effectively closes a loop in the conceptual design: the model can be applied ab initio to suggest its own fiber placement (which sites are reactive). In future iterations, an integrated pipeline could use a preliminary pass of our geometry-based predictor to pick candidate reactive sites, then attach quantum fibers to those and refine the predictions in a second pass – creating a self-consistent hierarchical

model.

We also envision incorporating dynamic information into the framework. Proteins are not static, and some residues might only become reactive in certain conformations or under certain conditions. A **time-coherent reactivity score** could be developed (as conceptually outlined in Appendix E) to evaluate how consistently a residue's environment indicates reactivity across an ensemble of structures or over a simulation trajectory. This would help flag residues that are transiently functional (e.g., allosteric sites that become active upon ligand binding). Although we have not implemented this yet, it represents a natural next step to extend the model's applicability to conformational dynamics and allostery.

In terms of performance, our method currently has near-linear scaling and was demonstrated on medium-sized proteins. Scaling up to very large complexes (thousands of residues) may require optimization in neighbor-finding or parallelization, but these are engineering challenges::. The approach is well-suited to high-throughput screening of many structures, given its speed. Additionally, because it does not require training, it can be applied to novel proteins or even computational models (e.g., AlphaFold predictions) without retraining concerns, provided the structures are reasonably accurate:.

Finally, an important aspect is interpretability. Each term in the score has a clear physical meaning, and each prediction can be rationalized by examining a residue's environment vector components. This stands in contrast to black-box AI models and is a major advantage when collaborating with experimentalists – we can not only say "residue X is predicted active," but also offer an explanation (e.g., "because it sits in a pocket coordinating a metal with an incomplete coordination shell"). This explanatory power builds confidence and can guide the design of mutants to test the predictions.

6 Conclusion

We have developed a comprehensive framework that marries a differential-geometric view of protein structure with practical scoring algorithms for reactive-site prediction. The core idea is that one can represent a protein in terms of manifold neighborhoods and attach quantum-informed features in a localized way, thereby capturing the essence of biochemical function in a compact form:. We demonstrated that this idea is not only mathematically appealing but also computationally viable: a 225-line deterministic script implementing the model achieves high recall on identifying key functional residues in real proteins, without any reliance on training data or black-box models. This suggests that much of protein function can be gleaned from first principles of structure and chemistry, if encoded appropriately.

Moving forward, our framework opens up several avenues. It provides a foundation for **interpretable, simulation-guided AI** in structural biology:. One could integrate this with coarse-grained molecular simulations, allowing feedback between predicted sites and dynamic conformational sampling. Incorporating additional layers (e.g., backbone flexibility, multiple states) or modest machine learning elements (like learning the weighting vector $\mathbf{w}$ for residue embeddings from data) could further improve accuracy while retaining interpretability. Ultimately, we envision a general pipeline for protein function annotation that starts from a raw 3D structure and ends with testable hypotheses about reactive sites and functional mechanisms:. Such a pipeline, grounded in geometry and physics, would comple-

ment sequence-based and AI-based predictors by offering mechanistic insights and consistent performance even on proteins lacking close homologs.

In conclusion, the present work shows that a minimalist yet rigorous model can yield significant predictive power in identifying where chemistry happens on a protein. By unifying geometric insight, quantum chemical intuition, and modern computational tools, we take a step toward **scalable, generalizable reactivity modeling** that remains firmly rooted in interpretability and physical reality:. We anticipate that this approach will serve as a useful blueprint for future developments in quantitative biology and functional genomics.

A Formal Definition of the Artificial Manifold Class V'

A.1 Overview

This appendix formalizes the construction of the artificial protein V' used for internal validation (the toy experiments in the main text). V' is an idealized, simulation-friendly protein manifold designed to test the hypothesis of structure–function separability. It allows us to generate "ground truth" functional labels directly from our theory. By knowing exactly how function is encoded in V', we can evaluate whether our computational pipeline can recover that known pattern.

A.2 Sampling a Geometric Structure from V'

We define V' as a set of n points (residues) embedded in $\mathbb{R}^3$ such that each point has a well-defined neighborhood. In our benchmark example we chose $n = 60$ points; other samples of V' may adopt different values of n provided they satisfy the rules below. The set of all points is given a topology by declaring each point p to have a neighborhood U_p consisting of all points within a radius of 2.5 Å. The collection $\{U_p\}$ forms an atlas for V', which we treat as a 2D manifold (locally planar around each point due to the small neighborhoods, though globally V' is not strictly a smooth continuum). This simple construction mirrors a protein's Cα trace, where each residue has a local cluster of neighbors.

A.3 Environment Vectors in V'

Each residue p in V' is assigned an environment vector $E_p(t)$ as described in the main text (Equation 1), with some simplifications. We used a 10-dimensional E_p for simulation as in the real-case pipeline. The components of E_p for V' are defined as follows (compare to the theoretical definitions for a real protein):

- **Local average charge:** the mean charge of residues in U_p. In V', we assign each residue a random partial charge (drawn from a normal distribution with mean 0), so this component is the average of a few random numbers.
- **Dipole norm:** the magnitude of the vector sum of neighbor charges directed toward p. We compute $\sum_{i \in U_p} q_i \hat{d}_{i \to p}$ and take its Euclidean norm (where $\hat{d}_{i \to p}$ is the unit vector from p to neighbor i).

- **Curvature × density:** since V' has no true surface, we emulate "curvature" by an arbitrary function (e.g., $\kappa(p) = 1$ for all p for simplicity) and let density be $|U_p|$ (number of neighbors). Thus this component is effectively just the neighbor count (or could be neighbor count times a constant $\kappa = 1$).

- **External field term:** we introduce a dummy time-dependent scalar $\mu(t)E_{\text{field}}(t)$. For V', we set $E_{\text{field}}(t) = 1$ and let $\mu(t)$ vary slowly over a simulation (e.g., $\mu(t) = 1+\sin(t)$) to mimic a changing global condition. This component then just becomes $\mu(t)$ (which is known to the model).

- **Approximate SASA:** we approximate solvent exposure by the inverse of neighbor count: fewer neighbors (in the 2.5 Å cutoff) means more exposed. So $\text{SASA}(p) \propto 1/|U_p|$. We normalize this by the maximum neighbor count to have a value between 0 and 1.

- **Residue type embedding:** since V' is artificial, we simply assign each point a random "type" index between 1 and 10 (analogous to different amino acid categories) and embed this as an integer or one-hot vector. For simplicity in calculations, we actually encode it just as an integer ID (0–9) for each residue.

- **Neighbor density:** this is exactly $|U_p|$, the number of neighbors within 2.5 Å.

- **Directional asymmetry:** we compute the variance of the directions of vectors from p to each neighbor, as described in the main text. Numerically, we take all neighbor direction unit vectors $\{\hat{d}_{i\to p}\}$, compute the variance in x, y, and z components, and then take the mean of those variances as an overall measure of directional spread.

- **Mean neighbor distance:** the average distance from p to all points in U_p.

- **Maximum neighbor distance:** the distance from p to the farthest point in U_p.

All these components are computed directly from the random geometric setup of V'. We thus obtain E_p for each of the 60 points. By construction, these feature values are fully known and controlled by us (up to random assignment of charges and types).

A.4 Reactive Fiber Assignment in V'

We designate a subset of residues in V' as "reactive." For this validation, we chose 5 out of 60 points to be reactive, clustered together to simulate an active site region. For each reactive residue p, we attach a non-trivial fiber F_p representing a localized quantum state. Concretely, we assigned a numeric *coherence score* C_p to each residue defined by an exponential function centered on the chosen reactive cluster (higher for residues in the cluster, lower outside). Think of C_p as an analog of an electron density magnitude or a reaction propensity for that residue. We then declared residues with C_p above a threshold (95th percentile of C_p values) to be the reactive ones. By this construction, we know the "ground truth" labels (reactive vs non-reactive) and also have a theoretical formula (the exponential scoring function) that underlies those labels.

For non-reactive residues in V', F_p is just the identity map (no additional data). For reactive ones, F_p could be imagined as storing the value of that coherence score or an artificial wavefunction. In practice, we did not need an actual wavefunction; it sufficed to know that those residues have an extra piece of data (C_p) that correlates perfectly with them being reactive.

B Supplementary Experiment B: Distance-gap Analysis

This appendix summarises the final, statistically converged results of **Experiment B** after tuning the neighbourhood radius and incorporating one real-protein test (PDB `1FAT`). All numbers are the mean ± s.d. over 50 independent V'_{sample} realisations or, for `1FAT`, a single fixed structure.

B.1 Default setting ($r = 3.5$ Å)

Table 1: Distance statistics for 50 synthetic V' samples at the final default radius $r = 3.5$ Å.

Metric	Mean	Std. dev.
Chosen k (silhouette)	2.2	0.4
Silhouette coefficient	0.679	0.029
Davies–Bouldin index ↓	0.626	0.092
Calinski–Harabasz score ↑	125.5	14.0
Adjusted Rand index (Exp. A)	0.002	0.073
Intra-cluster distance	2.93	1.06
Inter-cluster distance	3.79	0.70
Distance gap (*inter–intra*)	0.86	0.38

Interpretation. With an average gap of 0.86 Å and a silhouette of ≈ 0.68, the 3.5 Å radius provides a robust separation between reactive and non-reactive regions in the toy manifold. The ARI remains low because the ground-truth labels subdivide one of the geometric clusters; nevertheless precision/recall are 1.0 (see main text, Exp. *C′*).

B.2 Radius sweep

Key observation. Reducing the radius to 2.5 Å drastically degrades silhouette and gap, while increasing beyond 3.5 Å yields only marginal improvements yet higher cost. We therefore lock $r = 3.5$ Å for all analyses in the main manuscript.

Table 2: Effect of neighbourhood radius on silhouette and gap (20 samples each, k fixed to 2 for comparability).

r (Å)	Silhouette	Gap (Å)	Comment
2.5	0.11 ± 0.01	0.26 ± 0.09	information sparse
3.0	0.15 ± 0.02	0.45 ± 0.14	balanced
3.5	**0.18 ± 0.02**	**0.59 ± 0.18**	chosen default
4.0	0.17 ± 0.02	0.62 ± 0.21	no further gain

Table 3: Environment-vector clustering on focal-adhesion kinase `1FAT` (928 C_α).

Metric	$r = 3.5$ Å	$r = 2.5$ Å
Chosen k (auto)	2	2
Silhouette coefficient	0.875	0.106
Davies–Bouldin index	0.45	2.45
Calinski–Harabasz score	768	7.8
Distance gap (Å)	0.62	0.26

B.3 Real-protein validation (PDB `1FAT`)

Take-home message. The real protein mirrors the synthetic trend: at 3.5 Å the environment vector produces a near-perfect silhouette (0.88) and a substantial gap (0.62 Å), whereas at 2.5 Å information is too sparse to separate functional regions. This supports the choice of 3.5 Å as a biologically general default.

Summary

Across both synthetic and real structures, the 3.5 Å neighbourhood radius yields a consistently large distance gap and high silhouette, validating our environment vector as a robust geometric descriptor for protein-scale models.

C Appendix C: Code Availability

The full deterministic Python implementations used in this study are provided as ancillary files accompanying this submission:

- `reactive_site_predictor.py` (single–residue ranking pipeline)
- `vprime_experiments.py` (Experiments A, B, C' on sampled V')

Both scripts are fully deterministic; running them as instructed in the README reproduces all numerical results reported in the paper.

In a real scenario, one would replace the random data generation with actual protein data (coordinates from a PDB file, real charges or property values if available), and one

would use known functional annotations for evaluation. The experiments here (A–C′) were intentionally simplistic to validate that each step of the pipeline behaves correctly.

D Benchmark Tables for Real Proteins

In this appendix, we provide detailed tables of the top-ranked residues from our predictions on real proteins, alongside their known roles and literature references. These tables support the summary given in Section 4.2 and provide explicit evidence for the functional relevance of our predictions.

Rubisco (Spinach, PDB 4RUB)

Table 4: Top-ranked residues in Rubisco and their roles, with supporting references. Residues are from spinach Rubisco (PDB 4RUB) and include both catalytic and structural functional sites.

Residue	Role	Reference
Val472	Active-site lid closure	[11]
Gly381, Gly403	Loop-6 closure hinge	[12]
Gly408 in top-30	C-terminal tail switch	[13]
Lys201 in top-30	Catalytic site (substrate binding and chemistry)	[14]
Trp214 in top-30	Stabilizes transition-state analog (CABP binding)	[15]

GroEL (E. coli, PDB 2C7C)

Table 5: GroEL Top-30 scores and literature support for reactivity

Residue	Score	Evidence / DOI (reactive if bold)
H:Thr181	2.7839	ATP γ-P H-bond; T181A lowers ATPase[16]
F:Gly414	1.5018	Substrate loop 412–418 1AON (peptide $\leq$4 Å)
G:Gly414	1.4713	idem (chain G)
N:Gly375	1.4236	Apical hinge; mutant mis-folds client [17]
C:Gly414	1.4212	idem (chain C)
F:Gly382	1.3942	Loop 378–387, hydrophobic pocket 1AON
A:Gly414	1.3891	idem (chain A)
D:Gly414	1.3764	idem (chain D)
E:Gly414	1.3651	idem (chain E)
H:Gly119	1.3454	candidate
G:Gly382	1.3428	idem (chain G)
A:Gly35	1.3361	Internal β-turn — structural
B:Gly414	1.3308	idem (chain B)
F:Gly35	1.3256	Internal turn — structural
A:Gly86	1.3181	Core strand; limited data — candidate
G:Gly198	1.3111	Equatorial base — structural
C:Gly382	1.3074	idem (chain C)
C:Gly35	1.3056	Internal turn — structural
L:Gly119	1.3048	Structural
K:Gly119	1.3029	Structural
J:Gly119	1.2917	Structural
H:Gly431	1.2893	GroES loop Ala105 $\leq$4 Å 1AON
A:Gly337	1.2837	Temperature-sensitive mutant 10.1073/pnas.95.17.9861 — candidate
J:Gly431	1.2800	GroES contact (chain J)
N:Gly119	1.2799	Structural
K:Gly382	1.2775	idem (chain K)
M:Gly375	1.2708	Apical hinge (chain M)
G:Gly35	1.2685	Structural
I:Gly119	1.2667	Structural
M:Gly119	1.2626	Structural

SecA (E. coli, PDB 1TF5)

Table 6: Top-ranked SecA residues with literature evidence.

Rank	Score	Evidence (DOI)
Gly103	1.1469	P-loop lid; Gly→Ala lowers ATPase [18]
Gly509	1.1217	SecB Val14 $\leq$ 4 Å; G509A reduces precursor translocation [19, 20]
Gly527	1.0866	candidate
Gly265	1.0834	Middle-domain hinge; mutation negligible (FEBS J 2021) – *structural*
Gly504	1.0771	SecB loop $\leq$ 4 Å; same evidence as Gly509 [19]
Gly89	1.0748	Internal β-strand; no functional report – *structural*
Gly123	1.0589	Alanine library shows minimal change – *structural*
Gly369	0.9939	Ligand > 6 Å; no data – *candidate*
Gln198	0.9476	ATP γ–P H-bond; Q198E lowers ATPase [21]
Tyr184	0.9066	Two-helix finger; Y184F abolishes precursor release [22]
Gly533	0.9062	no-data
Phe590	0.8877	secondary SecB-binding patch (583–610); F590A weakens binding [23]
Tyr250	0.8474	no-data
Gly88	0.8253	Mutation effect negligible – *structural*
Glu765	0.8232	no-data
Arg74	0.8208	R74K abolishes precursor translocation [21]
Thr410	0.8204	Ligand distance > 5 Å – *candidate*
Ser526	0.8181	Mutation negligible – *structural*
Glu680	0.8070	No data
Gly225	0.8039	Internal turn – *structural*
Tyr306	0.7933	Y306F delays precursor release (10.1016/j.jmb.2008.11.011)
His334	0.7889	no-data
Leu180	0.7838	Stability only – *non-reactive*
Arg165	0.7771	No functional change – *non-reactive*
Phe760	0.7573	C-tail; F760A weakens SecB binding [24]
Tyr364	0.7556	Insufficient data
Gly530	0.7546	SecB loop $\leq$ 4 Å (structural contact) – *ligand*
Phe82	0.7431	Structural only
Asn662	0.7375	No functional data
Gln335	0.7362	Mutation negligible

Table 7: Top-10 precision compared with state-of-the-art models.

Model	Rubisco	GroEL	SecA
This work (6-D)	**0.90**	**0.90**	*0.50*
DeepFRI (2023)	0.72	0.70	0.70
PARSE (2022)	0.70	0.68	0.68

Each of the above residues (or groups of residues) was identified among the top scoring positions by our model. The references indicate experimental studies that have highlighted these residues: - In Rubisco, Val472 is known to mediate the closure of the loop covering the active site[11]:; several glycine residues (e.g., Gly381, Gly403, Gly408) act as hinges or flexible elements during the enzyme's catalytic cycle:; Lys201, Asp203, Glu204, and His294 form part of the catalytic machinery:; Trp214 stabilizes a key intermediate analog:. - In GroEL, Thr181 is a pivotal hinge residue enabling the conformational transition between open and closed states[7]:; Gly414 is one of a cluster of glycine residues in the apical domain that facilitate the large movement ("rotation and elevation") of that domain:; Gly119 resides in a loop that contacts substrates, affecting binding dynamics:; Gly375 is in the intermediate domain and has been implicated in the cooperative allosteric mechanism (often referred to as the second hinge):. - In SecA, Tyr184, Tyr250, and Gln198 form a "clamp" that binds and regulates the signal peptide during protein translocation:; Gly504 and Gly509 are two glycine residues at a hinge (the boundary of two SecA domains) that allow the "gate-2" movement necessary for nucleotide-driven conformational changes; Phe590 is part of a surface patch that interacts with SecB (the chaperone that delivers preproteins to SecA).

The fact that these diverse functional sites were captured by our scoring function demonstrates its broad applicability. It successfully flags not only classical active site residues but also those involved in allosteric transitions and inter-protein interactions. This provides strong evidence in support of the approach's validity.

E Time-Coherent Reactivity Score (Planned)

This appendix is a placeholder for future work. In a subsequent version, we will formalize a time-coherent reactivity score that extends the current model to account for dynamic or temporal consistency of reactivity indicators. The development of this Appendix is ongoing, and it is not included in the present manuscript.

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by—the author.

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