



Bound Water Can Change the Ranking of Natural Product Ligands in Structure-Based Screening, Free-Energy Estimation, and Mechanistic Binding Analysis

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ABSTRACT

Water molecules buried or partially confined at protein–ligand interfaces are often treated as structural details to be retained, deleted, or optimized during receptor preparation. That simplification is consequential when alternative water states change hydrogen-bond topology, ligand desolvation, pocket reorganization, or the convergence of binding free-energy calculations. This computational pharmacology perspective examines how structural waters, hydration sites, water-displacement states, and water-mediated interaction networks can influence the prioritization and mechanistic interpretation of natural-product ligands. The analysis distinguishes experimentally observed waters from ensemble hydration sites and thermodynamic water states, then connects those distinctions to docking, scoring, free-energy estimation, and ligand-ranking uncertainty. Natural products are not assumed to be intrinsically more water dependent than synthetic ligands; rather, selected natural-product and natural-product-like chemotypes may create difficult cases because dense polar functionality, stereochemical constraint, macrocyclic architecture, and solvent-responsive conformations can support competing direct and water-mediated binding microstates. The principal contribution is a proposed water-aware prioritization perspective in which robustness across defensible hydration hypotheses becomes an additional criterion for interpreting ligand rank. This approach does not presume that explicit water universally improves prediction or that water-state changes always reverse ranking. Its value instead lies in identifying cases in which hydration assumptions become decision-relevant and warrant higher-resolution sampling or experimental interrogation. The perspective is bounded by force-field error, incomplete protein and ligand-state sampling, uncertainty in experimental water assignment, target dependence, and the absence of prospective validation across broad natural-product chemical space.

Key Words: Bound water, Natural products, Molecular docking, Hydration thermodynamics, Free-energy calculations, Ligand ranking

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INTRODUCTION

In structure-based discovery, water is frequently handled as a preparation choice: crystallographic molecules may be removed wholesale, selected waters may be retained, or solvent may be introduced only after a protein–ligand pose has already been chosen. This workflow can obscure a

more fundamental point. Protein-associated water can mediate hydrogen bonding, occupy cavities that a ligand must displace, stabilize local conformations, reorganize around substituent changes, and contribute favorably or unfavorably to the thermodynamic balance of binding [1]. The relevant question is therefore not simply whether water is present in a binding site, but whether the modeled

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water state is part of the molecular hypothesis being ranked.

High-resolution structural evidence reinforces this interpretation. Neutron crystallography of trypsin complexes has shown that interfacial waters can participate directly in hydrogen-bond networks whose detailed protonation and orientation are not reducible to a ligand–protein heavy-atom contact map [2]. Such observations do not establish the free-energy contribution of every resolved water, but they show why a nominally similar ligand pose can represent a different recognition state when bridging solvent is altered. Water is thus mechanistically relevant even before scoring is considered.

The thermodynamic consequences can also be substantial in defined systems. Perturbation of protein–ligand water networks has been associated with marked changes in binding affinity, demonstrating that local solvent organization can transmit the effect of structural modification rather than merely accommodate it [3]. For ligand-ranking problems, this creates an important possibility: two compounds that appear similarly complementary in a dry pocket may not remain equivalent once the energetic cost of retaining, reorganizing, or displacing interfacial water is represented.

Water-mediated recognition is also not confined to a static first hydration shell. Simulation studies indicate that second-shell hydration and solvent reorganization can influence recognition and binding kinetics [4]. This broader view matters for natural products because the article’s argument cannot be reduced to “keep crystallographic waters.” Instead, the relevant computational object may be a distribution of occupancies, hydrogen-bonding alternatives, and coupled ligand–water–protein states. The perspective developed here therefore treats water representation as a source of model uncertainty capable, in selected systems, of changing not only mechanistic interpretation but relative ligand priority.

Structural waters, hydration sites, and binding-site thermodynamics

A structural water is an experimentally modeled solvent molecule at a particular position and under a particular measurement condition; a hydration site is an ensemble concept describing recurrent solvent occupancy in a region of space. The distinction is consequential because ligand binding can reorganize pocket hydration, and molecular-dynamics-derived water distributions need not map one-to-one onto ordered crystallographic waters [5]. A resolved water should therefore be interpreted as evidence for an accessible hydrated state, not automatically as proof of a unique or invariant thermodynamic state.

Modern methods increasingly add thermodynamic information to spatial localization. Accelerated hydration-

site approaches can identify likely pocket waters while estimating features relevant to their energetic favorability [6]. The practical implication is that receptor preparation need not be limited to a binary retain/delete decision. Hydration can instead be represented as a set of candidate states with differing support and energetic plausibility, although predicted sites remain model outputs rather than experimental observations.

WaterKit similarly frames hydration analysis as thermodynamic profiling rather than coordinate placement alone [7]. This matters because two waters occupying geometrically comparable positions can differ in their interaction environment, exchange behavior, and consequences for ligand desolvation or displacement. For screening, a coordinate-only description can therefore miss the distinction between a water that plausibly supports a ligand-mediated network and one whose removal may be favorable.

Taken together, molecular-dynamics hydration mapping, accelerated site localization, and thermodynamic profiling interrogate complementary representations of pocket solvent rather than interchangeable definitions of “the water structure” [5–7]. The manuscript consequently uses three levels of description: experimentally observed structural waters, ensemble hydration sites, and thermodynamically characterized water states. None is treated as automatically superior; the relevant representation depends on whether the computational question concerns pose plausibility, network topology, displacement energetics, or relative affinity.

Displaceable, bridging, and conserved water molecules

The common labels “bridging,” “conserved,” and “displaceable” are useful only when their evidential basis is explicit. Analyses of experimentally characterized drug–protein complexes show that organized water networks frequently occupy ligand-binding interfaces and can form recurring interaction patterns [8]. Yet recurrence in a deposited structure set does not by itself establish that an individual water is obligatory for binding. A bridging water describes a particular interaction topology; conservation describes persistence across a defined comparison; neither term independently specifies the free-energy cost of removal.

Energetic analyses further complicate geometric classification. Binding-site waters can occupy markedly different enthalpic environments, indicating that molecules with similar apparent positions need not be equivalent from the standpoint of stabilization or displacement [9]. Enthalpy alone is not a complete displacement free energy because entropy and coupled reorganization also matter. Still, the finding is sufficient to reject a homogeneous

treatment in which all observed waters receive the same mechanistic status.

Recent experimental work has moved water displaceability beyond purely computational inference by developing empirical proxies for whether binding-site waters are readily replaced [10]. This strengthens a state-based interpretation: displaceability is better viewed as a property of a water–pocket–ligand context than as a permanent label attached to one coordinate. A water may bridge one ligand, be displaced by another, or change occupancy when protein conformation, temperature, protonation, or local hydrogen-bond competition changes.

For ligand ranking, these distinctions imply that “retain conserved waters” is not sufficiently precise. The computationally relevant question is which hydration states remain defensible for each ligand and whether alternative states alter predicted interaction topology or energetic ordering. That criterion becomes especially important when chemically related ligands differ in the placement or shielding of polar functionality.

Natural product functional groups and water-mediated recognition

Natural products span a chemically diverse space that differs from a simple collection of conventional drug-like scaffolds in stereochemistry, ring architecture, functional-group patterning, and molecular complexity [11]. This diversity is relevant to hydration modeling, but it does not justify the stronger claim that natural products are inherently more water dependent. A defensible water-aware analysis must operate at the chemotype and ligand level, asking which structural features create plausible competition between direct protein contacts, intramolecular hydrogen bonds, retained interfacial waters, and water displacement.

Macrocyclic systems illustrate why this competition can become difficult. Simulations of macrocycles show solvent-dependent conformational behavior in which polar groups may become exposed or internally satisfied as the environment changes [12]. For a natural-product-like macrocycle, binding-site hydration can therefore couple to conformational selection: a polar group that appears geometrically positioned for direct contact in one conformation may instead participate in an intramolecular or water-mediated network in another. The uncertainty is not simply “where is the water?” but which ligand conformation and hydration state are jointly populated.

Contemporary exploration of natural-product-like polyketide macrolides further demonstrates the stereochemical and architectural breadth available within such chemical space [13]. That evidence establishes a realistic class of structurally complex ligands for water-aware investigation, but it does not prove that their biological activity is water mediated. Mechanistic attribution must remain target- and structure-specific.

This perspective therefore proposes a narrower hypothesis: natural-product ligands containing densely arranged hydrogen-bond donors and acceptors, constrained three-dimensional polar surfaces, or solvent-responsive macrocyclic architectures may be especially informative stress tests for alternative hydration hypotheses. The hypothesis is chemical and testable, not categorical. It predicts that uncertainty will be greatest when small structural differences change whether a polar group forms a direct contact, recruits a bridging water, displaces a preorganized water, or is internally satisfied by ligand conformation. **Table 1** aligns these mechanistic possibilities with the evidence available at this stage of the argument.

Table 1. Evidence-aligned hydration mechanisms relevant to natural-product ligand interpretation

Mechanistic situation	Evidence-aligned interpretation	Consequence for structure-based analysis	Evidence status
Ordered interfacial water	A resolved water supports at least one experimentally accessible hydrated binding geometry [2].	Do not equate a dry contact map with the observed recognition state.	Evidence-supported; system specific
Ligand-dependent hydration network	Binding can reorganize local solvent structure [5].	Compare ligand-specific hydration hypotheses rather than a single receptor water template.	Evidence-supported
Thermodynamically heterogeneous pocket water	Similar water positions may differ energetically [9].	Geometric conservation alone is insufficient to infer displacement cost.	Evidence-supported; enthalpy is incomplete
Solvent-responsive macrocycle	Polar-group presentation can change with environment [12].	Evaluate ligand conformation and hydration as coupled alternatives.	Evidence-supported for studied macrocycles

Dense or constrained natural-product polarity	Competing direct, intramolecular, and water-mediated contacts may arise.	Treat water-state sensitivity as a ligand-level question rather than a class assumption.	Proposed synthesis
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Note: “Evidence-supported” refers only to the cited mechanistic premise. The screening consequence and natural-product-specific integration are interpretive or proposed where stated.

Docking and scoring under alternative water treatments

Docking converts a structural hypothesis into a ranked set of ligand poses, so the treatment of binding-site water can alter the hypothesis before any score is interpreted. Explicit-water machine-learning scoring demonstrates that mediating waters and ligand conformational stability can be represented as model features rather than discarded during preprocessing [14]. This does not establish that explicit solvent is always advantageous; it establishes that water treatment can change the information supplied to a scoring function and therefore belongs among the assumptions of a ranking protocol.

The same issue arises at pose generation. GOLEM incorporates explicit waters into cryo-EM-guided ligand docking, showing that solvent and ligand placement can be considered jointly in structurally demanding settings [15]. Such methods expand the available hypothesis space, but a larger hypothesis space also raises model-selection questions. A retained water may stabilize a plausible pose, compensate for an incorrect ligand orientation, or reflect a structural state that is irrelevant under the assay condition.

Water-aware docking therefore needs comparison among defensible states rather than preferential acceptance of the most hydrated pose.

Affinity scoring adds another layer because cavity-water solvation and desolvation can be represented explicitly in predictive models [16]. A ligand that gains a direct protein contact may still pay for desolvating itself or reorganizing a favorable pocket network; another ligand may retain a bridging water and preserve a lower-reorganization pathway. These mechanisms cannot be inferred reliably from a dry interaction count alone.

The boundary conditions derived from this section are summarized in **Table 2**. The central implication is methodological: deleting all waters, retaining selected waters, predicting hydration sites, or allowing explicit solvent rearrangement are alternative model specifications. None is a universal default. Their relevance depends on pocket accessibility, structural confidence, ligand chemistry, the anticipated displacement event, and whether the calculation can actually sample the assumed water state.

Table 2. Boundary conditions for interpreting ligand scores under alternative binding-site water treatments

Water treatment	What it can represent	Principal boundary condition	Interpretation rule
Dry receptor	Direct protein–ligand complementarity without retained pocket waters	Misses bridging or occupancy-dependent states when these are relevant	Treat as one structural hypothesis, not a solvent-neutral reference
Selected explicit waters	Experimentally or computationally supported mediating waters [14]	Retained waters may be state- or ligand-specific	Require a reason for retention; do not freeze one network across all ligands
Joint ligand–water docking	Coupled pose and solvent placement [15]	Performance depends on structural data and search/model quality	Compare physical plausibility across alternative hydrated poses
Water-aware affinity term	Solvation/desolvation consequences of cavity waters [16]	Approximate energetics remain model and benchmark dependent	Interpret score shifts as model-dependent evidence, not measured affinity
Alternative water states	Competing retained, displaced, or reorganized networks	State choice can interact with ligand chemistry and protein conformation	Escalate when ligand order depends materially on the chosen state

free-energy methods and hydration thermodynamics

Free-energy calculations are often treated as the higher-resolution step after docking, but explicit solvent alone does not guarantee adequate water-state sampling. A buried water may exchange with bulk solvent on a timescale that ordinary molecular dynamics does not traverse during an alchemical calculation. Grand canonical Monte Carlo can enhance insertion and deletion of such waters and thereby address a convergence bottleneck that

would otherwise be hidden inside a formally explicit-solvent calculation [17]. The distinction is crucial: representing water and equilibrating water are different computational problems.

Comparative work on ligand-binding rehydration shows that enhanced-sampling techniques do not behave identically across hydration challenges [18]. Accessibility of the cavity, occupancy barriers, ligand transformation, protein motions, and the location of water sites can

determine which approach is efficient. Consequently, the manuscript does not define one enhanced-water method as a mandatory standard. Instead, difficulty in establishing stable hydration occupancy is treated as evidence that a ligand comparison requires additional sampling scrutiny. A complementary strategy is to decompose transformations involving trapped waters into more explicit state descriptions. Separation-of-states approaches can improve relative binding free-energy treatment when water states can be meaningfully specified, but the method carries prerequisites and does not convert an unknown hydration landscape into a solved problem automatically [19]. State definition itself may therefore be part of the uncertainty.

For natural-product prioritization, this matters most when a substituent change, stereochemical difference, or conformational shift opens or closes a hydrated subpocket. Apparent convergence of ligand coordinates or accumulated free-energy averages should not be interpreted as evidence of hydration convergence unless water occupancy and state transitions have also been interrogated. The proposed perspective therefore treats unexplained sensitivity to initial water placement, slow hydration exchange, or persistent alternative water states as diagnostic signals rather than mere simulation inconvenience.

Ranking instability caused by water-state assumptions

Direct benchmarking provides the strongest basis for connecting hydration treatment to ligand prioritization. Relative binding free-energy calculations designed around binding-site water displacement have shown substantial hysteresis and dependence on initial hydration state in difficult transformations [20]. These cases do not prove that water routinely reverses medicinal-chemistry

rankings. They do show that an apparently quantitative relative-affinity protocol can remain conditional on an incompletely equilibrated solvent state.

Water-sensitive ranking is not confined to alchemical calculations. A water-network-augmented two-state affinity model demonstrates that introducing explicit water-network information can change predicted binding behavior relative to conventional representations [21]. The relevance of that change remains benchmark dependent, but it establishes water-network representation as a model component capable of affecting relative predictions.

Recent fragment-docking analysis makes the boundary still clearer: alternative solvent-site prediction and treatment strategies can produce different docking and scoring outcomes across targets, without one solvent model being uniformly best [22]. Water treatment therefore behaves like a target-dependent modeling variable rather than a universally beneficial correction.

Here, ranking instability is proposed to mean a change in relative ligand prioritization across scientifically defensible water-state representations that cannot reasonably be explained by ordinary numerical uncertainty alone. Evidence from explicit-water scoring, water-displacement free-energy benchmarks, and solvent-site docking supports the premise that such sensitivity can occur, while also showing its system dependence [14, 20, 22]. The definition deliberately excludes trivial score fluctuations and does not require a complete rank reversal: instability may also appear as loss of separation between candidates or dependence of the preferred pose on hydration state.

Table 3 translates this definition into validation readiness, while **Figure 1** visualizes the manuscript-derived scales at which a water assumption can propagate into rank interpretation.

Table 3. Validation readiness of a claimed water-state-dependent ligand ranking

Claim level	Minimum evidence needed before interpretation	Failure signal	Readiness status
Pose sensitivity	Alternative defensible water states produce distinct physically plausible poses	Difference disappears after basic pose-quality or structural checks	Exploratory
Score sensitivity	Ligand score separation changes across water representations	Change is comparable with scoring noise or model instability [21]	Hypothesis-generating
Free-energy sensitivity	Hydration state alters relative free-energy behavior with adequate replicate/convergence analysis	Hysteresis or persistent state dependence remains [20]	Mechanistically unresolved
Cross-method rank sensitivity	Ordering changes across justified water treatments in more than one computational representation	Result depends on a single solvent-site or scoring implementation [22]	Requires corroboration
Decision-relevant instability	Candidate priority changes after uncertainty is considered and hydration alternatives remain plausible	No independent structural, thermodynamic, or experimental discriminator	Proposed prioritization trigger

Figure 1 shows why a water-sensitive rank should not be interpreted as a single computational event.

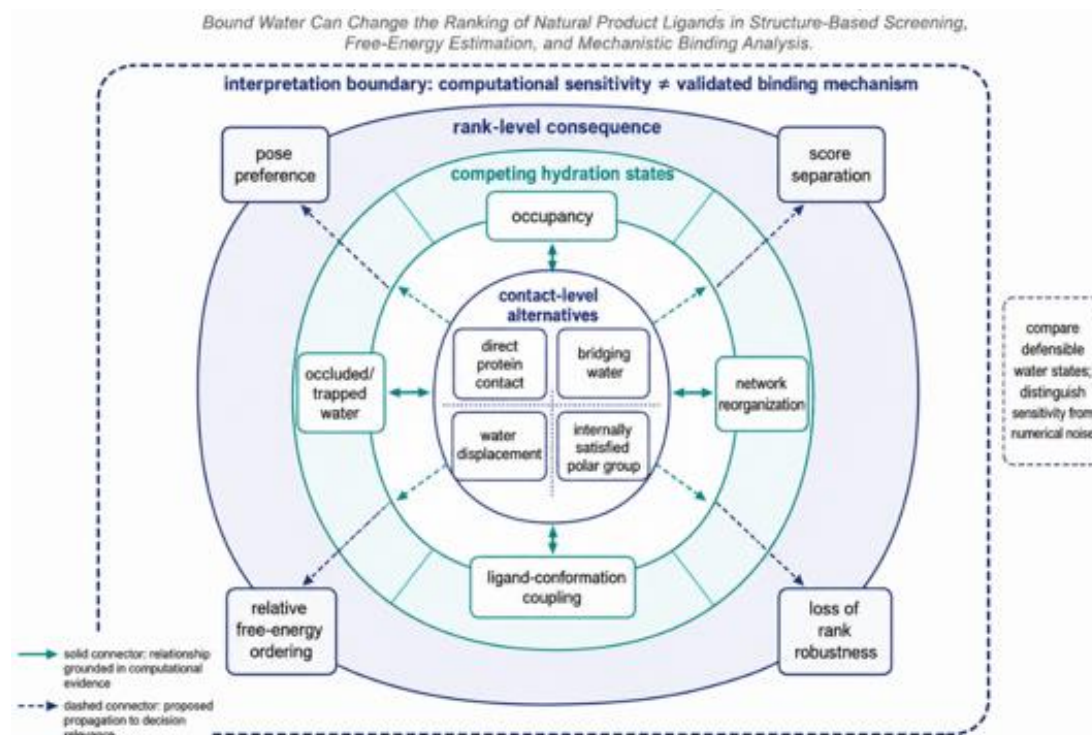


Figure 1. Where water-state assumptions enter and delimit ligand-ranking interpretation

A water-aware structure-based prioritization perspective

The proposed perspective begins by changing the decision question. Rather than asking whether a docking or free-energy workflow “includes water,” it asks whether the priority assigned to a ligand is robust across hydration states that are structurally or thermodynamically defensible. Enhanced grand-canonical sampling provides one route for interrogating occluded water states when ordinary exchange is inadequate [23]. Its role in the perspective is therefore selective escalation, not routine maximal simulation for every screened compound.

Water-site identification can also be coupled to pose and relative-affinity analysis. Methods that characterize ligand-binding-site water networks and examine their influence on orientations and relative binding affinities provide a basis for testing whether a candidate’s ranking depends on one particular hydration topology [24]. This creates an intermediate level between a dry docking screen and a fully exhaustive solvent treatment.

Lower-cost hydration and desolvation profiling offers another entry point. Statistical hydration-site methods can identify regions where water occupancy or displacement may plausibly become decision-relevant [25]. Used cautiously, such information can prioritize which ligand–target combinations deserve alternative water-state calculations rather than functioning as an independent affinity oracle.

The resulting framework is explicitly proposed: begin with the least complex representation adequate to the structural question; branch to alternative hydration hypotheses when

a ligand perturbs a supported water site or when competing contact topologies are plausible; escalate to enhanced sampling when rank depends on poorly exchanging or occluded waters; and report water-state sensitivity alongside conventional ranking uncertainty. A stable order across defensible states increases confidence in prioritization, whereas an unstable order identifies a decision that should not be hidden behind a single score. The escalation criterion should also remain comparative rather than threshold based. A large hydration penalty predicted by one method is not, by itself, a reason to promote or reject a ligand. More informative is whether the same candidate remains favored when reasonable alternatives are tested: retained versus displaced water, alternative ligand conformers, plausible protonation states, and—where justified—enhanced water exchange. This reframes additional computation as a sensitivity analysis around a decision rather than an attempt to obtain a single supposedly definitive solvent correction. It also prevents computational effort from being distributed uniformly across a screening set. Compounds whose ordering is insensitive to hydration assumptions may proceed without elaborate water treatment, whereas close competitors whose rank depends on one uncertain network become higher-priority cases for sampling or experiment. The framework therefore uses hydration complexity selectively, according to its capacity to change a practical prioritization decision.

Table 4 specifies the data-quality conditions that determine what such sensitivity can legitimately mean.

Table 4. Data quality required to convert water-state sensitivity into a prioritization consequence

Input or diagnostic	Adequate basis	Poor-quality signal	Consequence in the proposed perspective
Pocket hydration evidence	Supported structural or computational hydration hypothesis [25]	Arbitrary water deletion or unverified single template	Do not assign mechanistic meaning to rank differences
Water-state sampling	Exchange or state exploration appropriate to cavity accessibility [23]	Persistent occupancy dependence on initialization	Escalate sampling or classify ranking as unresolved
Ligand-pose consistency	Plausible poses across relevant hydration alternatives [24]	Rank driven by one strained or water-compensated pose	Reassess pose before affinity interpretation
Rank robustness	Ordering remains distinguishable after water-state and numerical uncertainty are considered	Candidate order changes with defensible hydration state	Flag water-state-sensitive prioritization
Mechanistic attribution	Independent structural, thermodynamic, or experimental evidence	Computational sensitivity alone	Describe as proposed explanation, not established mechanism

Experimental validation and mechanistic interpretation

A water-aware calculation becomes mechanistically informative only when its structural assumptions can be challenged experimentally. Temperature-dependent crystallographic work shows that protein–ligand water networks can repopulate as temperature changes [26]. Consequently, a water resolved in one crystal condition should not automatically be treated as a fixed physiological occupancy, and absence from a structure should not be equated with thermodynamic exclusion. Structural water is evidence about a state sampled under measurement conditions.

Direct computational–experimental comparison of water networks strengthens this caution. Predictions of water-mediated effects can agree or disagree with experimentally characterized binding behavior depending on the system and method [27]. Validation should therefore target the specific mechanism being proposed: whether a water persists, whether displacement accompanies a ligand modification, whether a network reorganizes, and whether the associated affinity trend is experimentally observed. Room-temperature serial crystallography provides a complementary route for interrogating ligand-bound states without assuming that cryogenic structures exhaust the relevant ensemble [28]. Together, temperature-sensitive structural measurements, direct comparison of predicted and experimental water behavior, and room-temperature ligand screening show why hydration assignments and their mechanistic interpretation are condition dependent [26–28].

For the proposed framework, the strongest validation would combine matched ligand series, alternative water-state calculations, replicate affinity measurements, and structural interrogation under conditions capable of resolving the disputed hydration state. A computational rank change alone is insufficient. Mechanistic confidence should increase only when the predicted distinction

survives plausible protein and ligand-state alternatives and is corroborated by orthogonal evidence.

Uncertainty and transferability boundaries

Water-state sensitivity must be separated from ordinary computational uncertainty. Alchemical free-energy calculations require explicit uncertainty quantification because finite sampling can make a numerically precise-looking estimate less reliable than its reported value suggests [29]. Replication and convergence analysis can characterize statistical uncertainty, but neither automatically detects a systematically incorrect force field, protonation state, protein conformation, or hydration hypothesis.

Large-scale assessment of rigorous protein–ligand free-energy calculations likewise shows that contemporary methods have finite empirical accuracy [30]. Water-aware treatment should therefore not be presented as removing the broader error budget. It addresses one class of hidden state dependence while leaving parameterization, structural preparation, assay uncertainty, and chemical-space transferability intact.

Pose generation introduces an upstream boundary. Benchmarking with PoseBusters shows that apparently successful docking predictions may violate physical constraints or generalize poorly to novel protein sequences [31]. A water-mediated explanation built on an implausible pose is therefore not rescued by sophisticated hydration thermodynamics.

This perspective consequently distinguishes four uncertainty sources: ligand/protein-state uncertainty, hydration-state uncertainty, numerical/sampling uncertainty, and transferability uncertainty. The taxonomy is proposed as an interpretive discipline rather than a validated error-decomposition model. For natural products, the categories may interact strongly because stereochemistry, macrocyclic conformational equilibria, protonation, intramolecular hydrogen bonding, and pocket

hydration can shift together. Water-state robustness should therefore increase confidence only conditionally, never substitute for broader validation.

Limitations

The perspective is constrained first by the evidence base. Most water-aware docking and free-energy studies examine selected proteins, congeneric series, fragments, or methodological benchmarks rather than broad natural-product libraries. Machine-learning scoring functions can also lose performance under more demanding generalization regimes [32]; adding hydration descriptors does not remove dataset shift or benchmark bias. No prospective multi-target study currently establishes that the proposed prioritization strategy improves natural-product hit enrichment.

Second, natural-product-like macrocycles create representation challenges independent of bound water. Force-field validation against NMR data shows that macrocyclic conformational behavior can depend on both force field and solvent [33]. An apparent hydration effect may therefore originate partly from an incorrect ligand ensemble. Similar ambiguity can arise from protein conformational state, protonation, tautomerism, uncertain water occupancy, or assay conditions.

Finally, the proposed ranking-instability construct has no validated numerical threshold. Its purpose is diagnostic: to identify when a scientific decision changes under defensible hydration assumptions. Prospective benchmarks must determine whether that diagnostic adds predictive value, which targets and chemotypes benefit, and when its computational cost is justified.

CONCLUSION

Bound water should be treated as part of the molecular hypothesis underlying structure-based ligand prioritization rather than as a uniform preprocessing detail. Structural waters, ensemble hydration sites, and thermodynamic water states encode different information, and their treatment can influence pose selection, scoring, free-energy convergence, and mechanistic interpretation.

For natural products, the relevant issue is not categorical water dependence. It is the possibility that chemically complex or conformationally adaptive ligands support competing direct, intramolecular, and water-mediated binding states whose relative plausibility changes with the hydration model. The proposed water-aware prioritization perspective therefore asks whether ligand order is robust across defensible water states and whether instability survives ordinary numerical uncertainty.

A water-sensitive rank is not proof of a water-mediated binding mechanism, nor does explicit solvent guarantee predictive improvement. Hydration hypotheses remain entangled with protein and ligand states, force fields, sampling, structural measurement conditions, and assay uncertainty. The practical contribution is consequently narrower but testable: expose hydration-dependent decisions rather than conceal them behind one receptor preparation or one score, escalate difficult cases selectively, and require experimental discrimination before converting computational water sensitivity into mechanistic certainty.

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