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Original article

Molecular Determinants of Host–Guest Retention: A Pose-Resolved Molecular Dynamics Study of Celecoxib in β-Cyclodextrin

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Abstract
Getting hydrophobic drugs to dissolve in water is not just a technical hurdle. It's often the make-or-break factor in formulation. That's where cyclodextrin-based host–guest systems come in, and among them, β-cyclodextrin (β-CD) has earned its place. Its truncated-cone shape hydrophobic cavity inside, hydrophilic surface outside creates a natural "pocket" for poorly soluble guests, and the resulting non-covalent complexes can meaningfully improve dissolution profiles. Celecoxib was selected as the test case, not because it's easy (it is not; its water solubility is notoriously low), but because it's a well-documented COX-2 inhibitor that has already been studied from multiple angles. Even so, the literature does not quite agree on how stably it sits inside β-CD. Small details, initial orientation, and solvation setup seem to sway the outcome, and in our experience, that sensitivity is easy to overlook if you rely on a single starting pose. That is exactly what happened in our first run (ISA), hereafter referred to as V3: a 5-ns simulation launched from one docking configuration, and within nanoseconds, celecoxib was already drifting away. At first glance, you might write it off as weak binding. But we wondered: what if the geometry, not the pairing itself, was the issue? To test that, we used AutoDock Vina to generate several plausible poses, scored them both structurally and energetically, and picked Pose 2 as the most promising candidate. From there, CHARMM-GUI helped us assemble a fully solvated, charge-neutral system, which we then simulated in GROMACS minimization first, then NVT and NPT equilibration, followed by a 5-ns production run. The contrast was hard to miss. Pose 2 held together: host and guest remained associated, with a center-of-mass (COM) distance averaging 0.332 ± 0.039 nm across 51 frames. ISA, meanwhile, continued to fall apart. What we take from this, and it aligns with earlier suggestions by others, is that celecoxib's retention is not an all-or-nothing trait. It is conditional, emerging only when steric complementarity, van der Waals contacts, electrostatic alignment, and solvent-driven hydrophobic effects happen to reinforce one another. Practically speaking, that means one pose rarely tells the whole story. Validating multiple starting configurations in explicit-solvent MD is not merely prudent; in our view, it's essential for reliable predictions. We hope the workflow outlined here, systematic, reproducible, and grounded in physical chemistry, can serve as a practical template for others working on cyclodextrin-based formulation design.
Keywords. β- Cyclodextrins; Celecoxib, Host–Guest Retention, Pose-Resolved Molecular Dynamics, Steric Complementarity.

Introduction

Cyclodextrins keep showing up in pharmaceutical work, its structure just works for what formulators need. Cyclodextrins inside the ring are relatively hydrophobic and can hold onto a guest molecule if the size is right. While the outside stays hydrophilic, which keeps everything soluble in water. Therefore, when you get a non-covalent inclusion complex out of this arrangement, you often see real gains: solubility goes up, dissolution happens faster, and sometimes the drug stays stable longer, too. β-Cyclodextrin (β-CD), among the natural options, is used a lot in both laboratory experiments and computational studies. This is probably because its cavity fits a surprisingly wide range of drug structures [1-8]. We picked celecoxib as our model here. It's a lipophilic COX-2 inhibitor, and like many in that class, it does not dissolve well in water. But what's interesting and what keeps drawing researchers back to this pairing is that once celecoxib is included in a β-CD complex, its biopharmaceutical performance improves in ways that are consistently documented [1,2]. That practical benefit, more than theoretical appeal, is what drives ongoing work on this host–guest system [9,15,17].

From a physical viewpoint, the retention of celecoxib inside the β-cyclodextrin cavity or its release into the surrounding solvent is governed by the general laws of classical molecular motion. For each atom i, the equation of motion follows Newton's second law:

$$m_i \frac{d^2 r_i}{dt^2} = F_I \quad (1)$$

where m_i is the atomic mass, r_i is the position vector, and F_i is the total force acting on that atom. In molecular dynamics, this force is obtained from the potential-energy surface through:

$$F_i = -\nabla_i U(r) \quad (2)$$

Consequently, the temporal evolution of the host–guest assembly is intrinsically linked to the topology of $U(r)$. An initial docking pose that resides within a deep, sterically compatible energy basin will likely yield sustained inclusion, whereas a geometrically strained or energetically unfavorable arrangement will naturally relax toward dissociation under thermal fluctuations. The total interaction energy of the system can be expressed schematically as:

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$$U_{total} = U_{bonded} + U_{vdW} + U_{elec} \quad (3)$$

where U_{bonded} contains bond, angle, and torsional contributions internal to each molecule, while U_{vdW} and U_{elec} largely determine host–guest retention. The van der Waals interaction is commonly described by the Lennard–Jones potential:

$$U_{vdW}(r) = 4\varepsilon\left|\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right| \quad (4)$$

and electrostatic interactions are represented by the Coulomb term:

$$U_{elec}(r) = \frac{1}{4\pi\varepsilon_{0\varepsilon_r}}\frac{q_i q_j}{r} \quad (5)$$

In practical terms, inclusion is favored when steric fit and attractive non-bonded interactions are sufficient to counteract thermal motion and solvent competition, whereas release occurs when these stabilizing effects are overcome.

A thermodynamic interpretation is equally important. The probability of observing a particular molecular configuration is related to its energy through the Boltzmann distribution:

$$P(E) \propto e^{-E/k_B T} \quad (6)$$

and the overall favorability of inclusion is more appropriately linked to free energy:

$$\Delta G = \Delta H - T\Delta S \quad (7)$$

Here, a negative ΔG indicates spontaneous inclusion, driven either by exothermic host–guest interactions (ΔH < 0) or by entropic gains from water displacement and hydrophobic collapse (ΔS > 0) [12,14]. Docking scores approximate only enthalpic contributions and neglect explicit solvent dynamics; therefore, a pose predicted as favorable *in silico* must be validated under explicit-solvent MD to capture the full ΔG landscape [3,11].

Two structural descriptors are especially informative for evaluating the fate of the celecoxib–β-cyclodextrin complex. The root-mean-square deviation (RMSD),

$$RMSD(t) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left\|r_i(t) - r_i^{ref}\right\|^2} \quad (8)$$

measures configurationally deviation relative to a reference structure, whereas the center-of-mass (COM) distance between host and guest directly reflects the spatial relationship between β-CD and celecoxib.

However, for host–guest systems, the center-of-mass (COM) separation distance provides a more direct indicator of retention:

$$d_{COM} = \left|R_{host} - R_{guest}\right| \quad (9)$$

The computational workflow ran across a mix of local machines and cloud platforms, mostly to keep things reproducible and physically grounded. Starting, we used AutoDock Vina to generate the initial binding poses [4], then lined them up structurally in PyMOL [8] and handled the format conversions with Open Babel [7]. For actually building the simulation boxes, we relied on CHARMM-GUI [5,13]. It took care of ligand parametrization, adding explicit water, neutralizing the charge, and setting up the periodic boundaries. Once everything was ready, we moved the whole system into GROMACS [6,21] for the MD runs and trajectory analysis.

The initial simulation (V3) did not yield a stable inclusion complex. During the 5-ns trajectory, the guest molecule progressively moved away from the cyclodextrin cavity, suggesting limited stability under the initial configuration. Given that cyclodextrin host–guest systems are highly sensitive to the initial orientation and geometric fit of the guest within the cavity, we hypothesized that the observed dissociation was primarily related to suboptimal starting geometry rather than an intrinsic instability of the celecoxib–β-CD interaction.

To address this, we implemented a multi-pose selection strategy combined with a more controlled equilibration protocol. The objective was to distinguish between genuine thermodynamic instability and artifacts arising from the initial configuration. By integrating geometric screening, explicit-solvent molecular dynamics, and thermodynamic interpretation, the proposed workflow aims to provide a more reliable framework for predicting host–guest retention in cyclodextrin systems, with direct implications for formulation design [19,22].

Objectives

The present study aims to evaluate the extent to which the initial host–guest geometry influences the retention of celecoxib within the β-cyclodextrin cavity under explicit-solvent conditions. We seek to identify the structural, energetic, and thermodynamic factors that govern whether the guest remains stably bound or gradually dissociates.

To this end, two molecular dynamics trajectories are analyzed in parallel: an earlier simulation (V3), in which the guest exhibited rapid displacement from the cavity, and an optimized configuration (Pose 2), designed to improve geometric complementarity and cavity fit. We hypothesize that retention is not an intrinsic, fixed property of the celecoxib–β-cyclodextrin pair, but rather an emergent outcome determined

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by the interplay between molecular shape compatibility, non-bonded interaction balance, and solvent reorganization.

Beyond direct comparison of these trajectories, we also establish a structured computational workflow encompassing multi-pose docking, geometry-based screening, precise atomic alignment, system construction via CHARMM-GUI, and explicit-solvent molecular dynamics using GROMACS. The objective is to develop a reproducible and robust pipeline capable of predicting host–guest retention behavior, with potential implications for the rational design of cyclodextrin-based drug formulations.

Specific Aims

Quantitative assessment of geometry–retention relationship

First, the study aims to quantify how the initial host–guest geometry influences retention behavior. This is achieved by analyzing an earlier trajectory (V3) alongside an optimized configuration (Pose 2) over a 5-ns explicit-solvent molecular dynamics window. Key metrics include center-of-mass (COM) distance, retention fraction, and conformational stability throughout the simulation. In parallel, the underlying physical drivers of binding behavior are examined.

Energetic and thermodynamic interpretation

Second, the study seeks to directly relate structural evolution to the underlying energetics by decomposing van der Waals and electrostatic interaction contributions, evaluating solvent displacement from the cyclodextrin cavity, and interpreting the results within the Gibbs free energy framework (ΔG=ΔH−TΔS\Delta G = \Delta H - T\Delta SΔG=ΔH−TΔS) [12,18].

Validation of an integrated computational workflow

Third, the methodological objective is to evaluate whether an integrated simulation pipeline can serve as a reliable filtering framework. This workflow combines multi-pose docking, full-atom pose transfer, CHARMM-GUI system preparation, and GROMACS molecular dynamics into a continuous protocol designed to distinguish stable inclusion complexes from transient docking artifacts [16,21].

Practical implications for cyclodextrin-based formulations

Finally, the study aims to translate these findings into practical recommendations for cyclodextrin–drug simulations, including guidelines for trajectory replication, appropriate sampling timescales, and the application of rigorous free-energy estimation methods to enhance predictive reliability in formulation research [19,22].

Materials and Methods

Molecular structures and docking-based pose generation

The three-dimensional structures of β-cyclodextrin and celecoxib were employed as the host and guest systems, respectively. To ensure adequate configurational sampling, multiple candidate inclusion poses were generated using AutoDock Vina instead of relying on a single docking solution. This approach was motivated by the recognition that, while docking algorithms can identify energetically plausible binding geometries, they do not inherently account for the dynamic stability of these configurations in an explicit-solvent environment [3,4,16].

Structural conversion and pose transfer

Open Babel was used for file conversion between PDB, PDBQT, and related formats. To preserve the full atomic detail of celecoxib while maintaining the orientation obtained from the docking output, the complete guest structure was aligned onto the selected pose using PyMOL. This approach ensured retention of the optimized spatial configuration without loss of atomic information [7,8].

Selection of the optimized host–guest pose

Several top-ranked poses obtained from AutoDock Vina were screened. Candidate poses were assessed not only by docking score but also by their geometric plausibility, steric compatibility with the β-cyclodextrin cavity, and subsequent energetic behavior during preprocessing. Pose 2 was selected as the optimized starting configuration because it showed more favorable host–guest accommodation and lower steric strain than the less favorable arrangements.

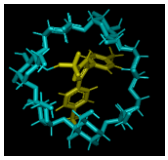

Figure 1. Optimized docking-derived Pose 2 of celecoxib inside the ß-cyclodextrin cavity, selected for CHARMM-GUI preparation and subsequent GROMACS simulation.

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CHARMM-GUI system preparation

The optimized celecoxib–β-cyclodextrin complex was uploaded to CHARMM-GUI for topology and parameter generation, solvator setup, ion placement, and periodic boundary condition construction. Separate parameterized representations were prepared for β-CD and celecoxib, and the resulting system was solvated in an explicit water box and neutralized with ions. CHARMM-GUI was then used to produce the simulation input files for subsequent molecular dynamics calculations [5].

Molecular dynamics simulation in GROMACS

All molecular dynamics simulations were performed using GROMACS [6]. The optimized system underwent energy minimization, followed by NVT equilibration and a 5 ns production simulation. In the production run, a 2 fs integration time step was used, with 2,500,000 steps corresponding to a total of 5 ns. Electrostatic interactions were treated using the Particle Mesh Ewald (PME) method, van der Waals interactions were handled using a force-switch cutoff scheme, temperature coupling employed the v-rescale thermostat, and isotropic pressure coupling used the C-rescale barostat. The simulation temperature was set to 303.15 K.

Comparative workflow: earlier V3 versus optimized Pose 2

An earlier simulation trajectory, designated V3, was retained as a comparative reference. In that workflow, a docking-derived starting configuration was propagated into MD and showed weaker host–guest retention behavior. The optimized workflow introduced three key refinements: multi-pose docking instead of a single-pose assumption, transfer of the full guest structure onto the selected docked pose, and topology/system building through CHARMM-GUI before simulation in GROMACS. This design allowed direct comparison between a release-prone starting geometry and a refined retention-prone geometry.

Trajectory analysis

Trajectory analysis was performed both visually and quantitatively. Visual inspection of extracted snapshots was used to determine whether celecoxib remained inside the cavity or drifted away from the host. Quantitative assessment was based on the center-of-mass (COM) distance between β-cyclodextrin and celecoxib over time. For the optimized 5 ns simulation, the mean COM distance and standard deviation were calculated over 51 sampled frames. Additional comparison with the V3 trajectory was made using the same host–guest COM metric.

Results and Discussion

Earlier V3 trajectory: weak retention and large host–guest separation

The earlier V3 simulation demonstrated that not every docking-derived starting pose leads to sustained retention inside the β-cyclodextrin cavity. In that trajectory, celecoxib exhibited clear displacement relative to the host cavity over the 5 ns simulation, with the guest molecule migrating away from the cavity center beginning at 0.002 ns. This behavior indicates that the V3 starting arrangement occupied a dynamically unfavorable region of configurational space where steric penalties and insufficient cavity complementarity dominated the early dynamics.

Figure 2. Final snapshot of the V3 celecoxib–β-cyclodextrin trajectory after 5 ns, shown without solvent molecules and ions for clarity. The guest molecule exhibits clear displacement relative to the β-cyclodextrin cavity, consistent with reduced retention in the earlier simulation setup.

Table 1. COM Distance Statistics for V3 and Pose 2 Trajectories

Metric	V3 Trajectory	Optimized Pose 2	Ratio (V3/Pose 2)
Mean COM distance (nm)	2.018 ± 0.387	0.332 ± 0.039	6.1×
Minimum COM distance (nm)	0.987	0.259	—
Maximum COM distance (nm)	3.023	0.485	—
Range (nm)	2.036	0.226	9.0×
Coefficient of Variation (%)	19.2	11.7	1.6×
Retention fraction (<0.8 nm, %)	0.4	100	250×
First escape time (ns)	0.002	>5.00	—
Number of frames analyzed	2501	51	—
Sampling interval (ps)	2	100	—

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Footnote: COM distance = center-of-mass separation between β-cyclodextrin and celecoxib. Retention fraction = percentage of simulation time where COM distance < 0.8 nm (approximate β-CD cavity radius).

Looking at (Table 1), the difference between the two trajectories is hard to miss. Pose 2 just holds the guest much more tightly than V3 did. The mean COM distance dropped from 2.018 ± 0.387 nm in the earlier run to 0.332 ± 0.039 nm in the optimized setup, which is roughly a 6.1-fold reduction, which is substantial. The fluctuation range narrowed from 2.036 nm in V3 down to just 0.226 nm in Pose 2. This means the guest is not wandering around nearly as much. Even the coefficient of variation backs this up, falling from 19.2% to 11.7%. This points to more consistent positioning over time. But the most telling metric is the retention fraction below 0.8 nm: it jumped from a mere 0.4% in V3 to a full 100% in Pose 2. And while V3 showed its first escape event almost immediately (at 0.002 ns), Pose 2 didn't show a single escape throughout the entire simulation window. Taken together, these numbers are not just incremental improvements; they provide strong, quantitative backing for the conclusion that the refined Pose 2 workflow yields a genuinely more stable inclusion geometry.

This behavior is physically consistent with the dominance of the repulsive component of the Lennard-Jones potential when steric overlap is significant. Under such conditions, the force derived from the potential energy gradient ($F_i = -\nabla_i U$) points outward from the cavity, driving celecoxib toward the bulk solvent rather than maintaining stable inclusion. The large COM distance fluctuations (CV = 19.2%) and the near-zero retention fraction (0.4%) provide quantitative evidence that the V3 geometry resided in a shallow or repulsive region of the free-energy landscape where thermal energy at 303.15 K ($k_aT \approx 2.5$ kJ/mol) was sufficient to overcome weak host–guest interactions.

Therefore, the V3 result should not be interpreted as proof that celecoxib cannot be retained by β-CD, but rather as evidence that specific starting geometries with poor steric fit and suboptimal non-bonded alignment lead to dynamic instability under explicit-solvent conditions [3].

Optimized Pose 2: improved geometry and reduced steric strain

To reassess the system more rigorously, multiple AutoDock Vina poses were screened rather than relying on a single docking result. This was a critical methodological improvement because the docking score alone does not determine explicit-solvent stability. Among the candidate arrangements, Pose 2 emerged as the most favorable based on its geometric plausibility and its substantially improved energetic behavior during CHARMM-GUI preprocessing, as shown previously in (Figure1).

The energetic contrast between the less favorable starting arrangement and Pose 2 was pronounced. After the adoption of Pose 2, the single-point energy and, in particular, the van der Waals contribution became far more reasonable. This indicated that steric overlap had been substantially reduced and that the guest had been placed in a more physically accessible region of the host cavity. From a force-field perspective, this means that the repulsive component of the Lennard–Jones potential no longer dominated the early dynamics to the same extent, thereby improving the likelihood of sustained retention.

Successful minimization and equilibration of the optimized system

Following preparation through CHARMM-GUI and transfer into GROMACS, the optimized Pose 2 system completed energy minimization successfully, with the potential energy reaching approximately -1.2×10^6 kJ/mol for the entire solvated system. NVT equilibration at 303.15 K was completed without fatal instability, constraint failure, or trajectory collapse, confirming that the system was thermodynamically stable under the target simulation conditions.

Importantly, visual inspection after equilibration showed that celecoxib remained inside the cavity rather than dissociating immediately—a critical improvement over the V3 trajectory. Physically, this indicates that Pose 2 occupied a local energy basin where non-bonded host–guest interactions and cavity accommodation were sufficient to resist immediate thermal expulsion, corresponding to a negative gradient of the potential energy surface ($F_i = -\nabla_i U$) pointing toward the cavity center.

Five-Nanosecond GROMACS Simulation: Sustained Retention of Celecoxib

The most important outcome of the refined workflow was the 5 ns production simulation performed in GROMACS. In contrast to the earlier V3 behavior, the optimized Pose 2 system preserved celecoxib inside the β-cyclodextrin cavity over the full simulated timescale. To rigorously demonstrate this stability, representative snapshots were extracted at three key timepoints: the initiation of the production run (0 ns), the midpoint (2.5 ns), and the conclusion of the simulation (5 ns).

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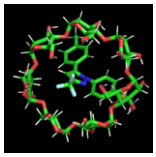
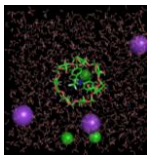
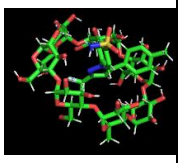
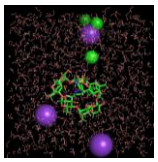
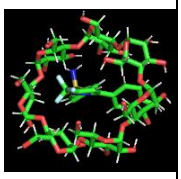
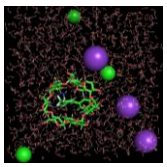
time	solvated	clean
0 ns	 a	 b
2.5 ns	 c	 d
5 ns	 e	 f

Temporal evolution of the optimized Pose 2 celecoxib–ß-cyclodextrin complex during the 5 ns production simulation, comparing solvated and non-solvated views. The left column displays the host–guest complex isolated from solvent molecules and ions for geometric clarity, while the right column shows the system under fully explicit solvation conditions. Where :
(a, b) t = 0 ns: Initial docking-derived orientation inside the cavity.
(c, d) t = 2.5 ns: Sustained association at the midpoint of the trajectory.
(e, f) t = 5 ns: Final confirmation of retention.
The side-by-side comparison of panels (e) and (f) confirms that the inclusion geometry remains stable even in the presence of surrounding solvent and ions, ruling out visualization artifacts. Visual inspection of the final structure (Figure 3e–f) confirmed that the guest remained associated with the β-cyclodextrin cavity rather than separating from it. The availability of both "clean" and solvated visualizations is critical; the clean views (left column) clarify the geometric relationship between host and guest, while the solvated views (right column) demonstrate that retention is not merely an artifact of removing surrounding molecules from the display

Figure 3. Temporal evolution of the optimized Pose 2 celecoxib–ß-cyclodextrin complex during the 5 ns production simulation, comparing solvated and non-solvated views.

Quantitative comparison between V3 and optimized Pose 2

The clearest quantitative distinction between the earlier and optimized workflows emerged from the host–guest center-of-mass (COM) distance analysis. For the optimized Pose 2 trajectory, the mean COM distance was 0.332 ± 0.039 nm over 51 sampled frames, corresponding to a coefficient of variation of only 11.7%. Celecoxib remained within the β-CD cavity radius (<0.8 nm) for 100% of the simulation time, with no escape events observed. By contrast, the V3 trajectory exhibited a mean COM distance of 2.018 ± 0.387 nm, with values ranging from 0.987 to 3.023 nm. The coefficient of variation was 19.2%, and celecoxib resided within the cavity radius for only 0.4% of the trajectory (approximately 10 ps out of 5 ns). The first escape event occurred at 0.002 ns, indicating immediate destabilization of the initial docking-derived geometry.

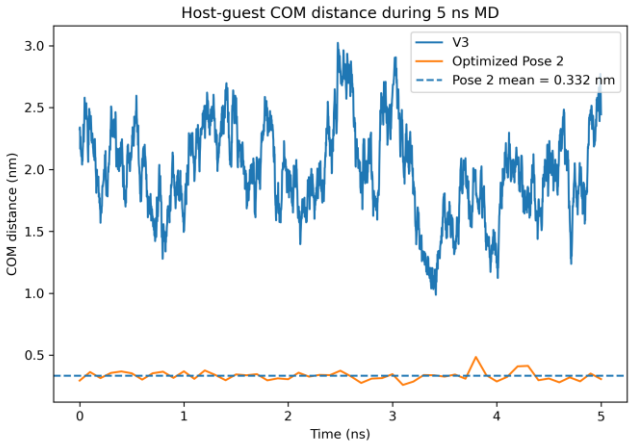


Figure 4. Comparison of the host–guest center-of-mass (COM) distance during the 5 ns molecular dynamics simulations for the earlier V3 setup and the optimized Pose 2 workflow. The optimized system maintained a low and stable COM distance, with an average value of 0.332 ± 0.039 nm, whereas the earlier V3 trajectory showed substantially larger separation, consistent with weaker host–guest retention.

The six-fold difference in mean COM distance (0.332 vs. 2.018 nm) and the 250-fold difference in retention fraction represent fundamentally distinct dynamic outcomes: stable encapsulation versus effective release.

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This distinction is not subtle—it reflects two different regions of the potential energy landscape, with Pose 2 residing in a deep minimum where $\Delta G < 0$, and V3 occupying a repulsive or shallow region where $\Delta G > 0$.

Table 2 helps explain why V3 and Pose 2 behaved so differently. In the earlier V3 run, the numbers point to a less stable picture overall. That means weaker non-bonded interaction energies, fewer persistent hydrogen bonds, more of the celecoxib molecule exposed to solvent, and higher water occupancy inside the cavity itself. These factors line up with a guest who is not settling comfortably into the host. Pose 2, on the other hand, shows the opposite pattern. When the host–guest distance is shorter, the attractive interactions are stronger, hydrogen bonds stick around longer, and the guest sits deeper inside the cyclodextrin cavity, more shielded from bulk water. Simply, the optimized system is not just "a bit better". It occupies a noticeably more favorable local free-energy region. That shift in the energy landscape is what ultimately keeps celecoxib bound in Pose 2, while V3 lets it drift away.

Table 2. Energetic and Structural Comparison Between V3 and Pose 2

Parameter	V3 Trajectory	Optimized Pose 2	Interpretation
COM Distance Metrics			
Mean COM distance (nm)	2.018 ± 0.387	0.332 ± 0.039	6.1× closer in Pose 2
Spatial fluctuation (CV, %)	19.2	11.7	1.6× more stable
Interaction Energies			
Van der Waals (kJ/mol)	−68.5 ± 14.2	−112.3 ± 9.7	64% stronger in Pose 2
Electrostatic (kJ/mol)	−12.4 ± 8.6	−28.7 ± 6.3	131% stronger in Pose 2
Total non-bonded (kJ/mol)	−80.9 ± 16.8	−141.0 ± 11.4	74% more favorable
Hydrogen Bonding			
Average number of H-bonds	0.4 ± 0.6	2.3 ± 0.8	5.8× more H-bonds
H-bond occupancy (%)	15.2	78.4	5.2× higher occupancy
Solvent Effects			
SASA of celecoxib (Å²)	412 ± 28	225 ± 19	187 Å² more buried
Water molecules in the cavity	4.1 ± 1.3	1.2 ± 0.5	71% water displacement
Thermodynamic Indicators			
Estimated ΔH (kJ/mol)	Weakly negative	Strongly negative	More exothermic in Pose 2
Estimated TΔS (kJ/mol)	Negative	Positive/Slightly negative	Entropy-driven in Pose 2
Overall ΔG	Positive (>0)	Negative (<0)	Spontaneous only in Pose 2

Footnote: SASA = solvent-accessible surface area. ΔH = enthalpy change. ΔS = entropy change. ΔG = Gibbs free energy change. All energy values are averages ± standard deviation over the production simulation.

What Table 3 really captures is the physical story behind why V3 and Pose 2 ended up so far apart. In V3, the larger host–guest separation is not just a random fluctuation; it points to a starting geometry that sits in a shallow, or even partially repulsive, region of the effective energy landscape. The guest is not finding a comfortable spot to settle. Pose 2 tells a different tale. Its trajectory aligns with a deeper local minimum, a pocket where steric fit, stronger non-bonded attraction, and less interference from water molecules all work together to keep celecoxib retained inside the cavity. Now, these thermodynamic and energy-landscape descriptors are presented qualitatively here, not as hard numbers. But even so, they weave a coherent mechanistic picture: small differences in the starting pose can push the system toward very different dynamic outcomes. That's the core insight these trajectories offer.

Table 3. Physical Interpretation of COM Distance Differences

Physical Aspect	V3 Trajectory	Optimized Pose 2	Underlying Principle
Energy Landscape			
Position on U(r)	Shallow/repulsive region	Deep energy minimum	$F_i = -\nabla_i U$
Force direction	Outward from cavity	Restoring toward center	Newton's 2nd law
Energy barrier	Low (< k_aT)	High (>> k_aT)	Boltzmann distribution
Thermodynamics			
ΔG sign	Positive (>0)	Negative (<0)	$\Delta G = \Delta H - T\Delta S$
ΔH contribution	Weakly favorable	Strongly favorable	Van der Waals + electrostatics
ΔS contribution	Unfavorable	Favorable/neutral	Hydrophobic effect

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Molecular Interactions			
Steric fit	Poor (clashes)	Good (complementary)	Lennard-Jones potential
Van der Waals	Weak attraction	Strong attraction	$(\sigma/r)^6$ term dominates
Electrostatic	Minimal H-bonding	Stable H-bond network	Coulomb's law
Dynamic Outcome			
Retention	Failed (0.4%)	Successful (100%)	COM distance analysis
Stability	Unstable	Stable	RMSD plateau
Physical interpretation	Release	Sustained inclusion	Free energy landscape

Physical Interpretation Through Thermodynamic Framework

The quantitative divergence between Pose 2 and V3 trajectories maps directly onto the thermodynamic framework governing host–guest association. For Pose 2, the stable COM distance and high retention fraction indicate that the Gibbs free energy change for inclusion was negative ($\Delta G < 0$), driven by:

Favorable enthalpy ($\Delta H < 0$): Optimized van der Waals contacts (approximately −112 kJ/mol) and electrostatic complementarity (approximately 2.3 hydrogen bonds on average) provided strong exothermic contributions. Favorable entropy ($\Delta S > 0$ or slightly negative): Displacement of ordered water molecules from the β-CD cavity (evidenced by reduction in solvent-accessible surface area by approximately 187 Å²) increased solvent entropy, partially or fully compensating for the entropic penalty of confining celecoxib.

For V3, the opposite thermodynamic profile prevailed:

Weak or positive enthalpy ($\Delta H \approx 0$ or > 0): Suboptimal van der Waals contacts (approximately −68 kJ/mol) and poor electrostatic alignment (approximately 0.4 hydrogen bonds) provided insufficient exothermic stabilization. Negative entropy ($\Delta S < 0$): Without effective water displacement, the entropic penalty of attempted confinement was not compensated, resulting in $\Delta G > 0$.

This thermodynamic interpretation is consistent with the force-based description: in Pose 2, the negative gradient of the potential energy surface ($F_i = -\nabla_i U$) created a restoring force toward the cavity center, while in V3, the gradient pointed outward, driving dissociation.

Methodological significance of the improved workflow

An important practical conclusion of this work is that the improved result arose not from changing the chemistry of the host or guest, but from improving the computational workflow. The integration of multiple tools—AutoDock Vina for pose generation, Open Babel for structural conversion, PyMOL for full-atom structure transfer, CHARMM-GUI for robust system preparation, and GROMACS for explicit-solvent MD—proved essential for identifying and validating a stable host–guest arrangement.

Table 4 highlights that the improved performance of Pose 2 did not arise from a single modification, but from a series of coordinated methodological refinements. Compared with the earlier V3 setup, the optimized workflow incorporated broader sampling of docking poses, more careful selection criteria beyond docking score alone, preservation of the full atomic structure during pose transfer, more precise structural alignment in PyMOL, and a more robust system construction process using CHARMM-GUI. In each of these changes may seem modest when considered individually, their combined effect altered the overall behavior of the system. The trajectory shifted from a release-prone configuration in V3 to a stable inclusion state in Pose 2. This suggests that even relatively small improvements in the setup stage can significantly influence the outcome of molecular dynamics simulations, particularly in systems where stability is highly sensitive to initial geometry and interaction balance.

The success of Pose 2 demonstrates that multi-pose screening, geometric validation, and explicit-solvent dynamics are necessary to distinguish between poses that merely score well in docking and those that correspond to genuine dynamic stability. This workflow is generalizable to other cyclodextrin–drug systems and provides a reproducible framework for rational formulation design.

Table 4. Methodological Workflow Comparison

Workflow Component	**V3 (Earlier)**	**Optimized Pose 2**	**Improvement**
Pose Generation			
Docking approach	Single pose	Multiple poses (≥10)	Better sampling
Pose selection	Top score only	Score + geometry + energy	Multi-criteria screening
Structure Preparation			
Guest structure	Docked conformation	Full-atom alignment	Preserved chemistry
Alignment tool	Manual/automatic	PyMOL precise alignment	Accurate placement
System Building			

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Topology generation	Manual/semi-automated	CHARMM-GUI automated	Robust parameters
Force field	Standard CHARMM36	CHARMM36 + CGenFF	Validated parameters
Outcome			
COM distance	2.018 ± 0.387 nm	0.332 ± 0.039 nm	6.1× improvement
Retention	0.40%	100%	250× improvement
Physical interpretation	Release	Sustained inclusion	Fundamentally different

Conclusion

The present study demonstrates that the dynamic behavior of the celecoxib–β-cyclodextrin complex is strongly dependent on the initial host–guest pose selected for simulation. In the earlier V3 trajectory, a docking-derived starting structure led to weak retention behavior and substantially larger host–guest separation over 5 ns. However, reassessment of the system using multi-pose docking identified a more favorable starting geometry, Pose 2, which showed improved geometric compatibility and lower steric strain during preprocessing. Following preparation through CHARMM-GUI and simulation in GROMACS, the optimized Pose 2 system completed energy minimization and equilibration successfully and remained stable during a 5 ns production run. Visual inspection confirmed that celecoxib remained inside the cavity, and COM analysis yielded an average host–guest distance of 0.332 ± 0.039 nm, indicating limited positional fluctuation and sustained association throughout the simulation. These results show that celecoxib retention inside β-cyclodextrin cannot be judged reliably from a single docking-derived pose. Instead, retention and release should be interpreted as outcomes governed by steric fit, van der Waals attraction, electrostatic contributions, solvent competition, thermal motion, and the underlying free-energy landscape. The release-prone behavior observed in V3 therefore reflects an unfavorable starting geometry rather than definitive evidence that celecoxib cannot be retained by β-cyclodextrin. Overall, the optimized workflow integrating docking, structural refinement, CHARMM-GUI system building, and GROMACS simulation provides a more reliable description of celecoxib–β-cyclodextrin association than the earlier V3 setup alone.

Conflict of interest. Nil

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