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Effect of Acetonitrile-Water Composition on the Conformational Landscape of Alanine Dipeptide: A Molecular Dynamics Study

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Abstract: Understanding how solvent composition influences peptide conformations is crucial for insights into protein folding, stability, and solvation effects. In this study, we investigate the conformational preferences of alanine dipeptide in mixed acetonitrile (ACN) and water solutions using molecular dynamics simulations. Solvent boxes with 10% and 40% ACN were constructed using Packmol, and 1 ns simulation were performed with OpenMM. The resulting trajectories were analyzed using MDTraj to extract backbone dihedral angles (ϕ and ψ) and identify stable conformational basins. Results demonstrate that solvent composition significantly affects the population of conformers, with higher ACN content favoring extended or gauche structures. These findings provide a quantitative understanding of solvation effects on peptide flexibility, offering insights relevant for protein chemistry and solvent-dependent folding studies.

Keywords**:** *Alanine Dipeptide, Molecular Dynamics, Solvent Composition, Acetonitrile-Water Mixtures, Conformational Analysis, Ramachandran Angles, MD Simulation.*

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I. INTRODUCTION

Peptides and proteins adopt specific three-dimensional structures that are critical for their biological function. The specific conformation peptide is strongly influenced by its environment, particularly the solvent in which it is placed. Solvents can stabilize or destabilize specific conformations through hydrogen bonding, polarity effects, and other intermolecular interactions. Understanding these effects is essential for predicting peptide behavior in different chemical and biological contexts.

Alanine dipeptide, a minimal model system, is widely used to study peptide conformations due to its simple structure yet rich conformational flexibility. Its backbone dihedral angles, phi (ϕ) and psi (ψ), define its accessible conformational space and allow mapping of stable basins on a Ramachandran plot.

Acetonitrile (ACN) is a polar aprotic solvent commonly used in biochemical and pharmaceutical studies. Mixing ACN with water creates a tunable environment where solvent polarity and hydrogen-bonding ability can be systematically varied. Previous studies have shown that solvent composition can alter peptide conformational preferences, yet quantitative investigations into small solvent ratios and their effect on alanine dipeptide remain limited.

- *Approach*

This paper attempts to answer how the presence of 10% vs 40% ACN in water affects the population of canonical conformational basins of alanine dipeptide? Does higher ACN concentration favor extended or compact peptide structures? To answer this question, molecular dynamics simulations are run with different starting conditions and parameters on a single alanine dipeptide molecule placed in 10% ACN and 40% ACN. The dihedral angles are then calculated at every frame of the simulation, and the frequency distribution is analyzed to find the favored conformation

II. LITERATURE REVIEW

The conformational behavior of small peptides, such as alanine dipeptide, has been extensively studied as a model for understanding protein folding and solvent effects. Alanine dipeptide, due to its minimalistic backbone structure and well-defined dihedral angles (ϕ and ψ), serves as an ideal system for computational studies of conformational landscapes and solvent interactions [1].

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➢ *Alanine Dipeptide: Structure and Conformational Landscape*

Alanine dipeptide (N-acetyl-L-alanine-N′-methyl amide) is a short peptide composed of an alanine residue capped at both termini to mimic the peptide backbone of larger proteins. Despite its simplicity, it captures essential features of peptide conformational dynamics. The molecule is characterized by two primary backbone torsional angles: ϕ (phi) and ψ (psi), which define its position on the Ramachandran map.

- *Its Free-Energy Surface Reveals Several Well-Known Conformational Basins:*

✓ αR (right-handed helical region): $\phi \approx -60°$, $\psi \approx -60°$.
✓ C7eq / β-sheet region: $\phi \approx -120°$, $\psi \approx 120°$, often referred to as the "extended" conformation.
✓ αL (left-handed helical region): $\phi \approx 60°$, $\psi \approx 60°$
✓ gauche+ (C7ax) region: $\phi \approx -60°$, $\psi \approx -30°$.

These basins provide a minimal yet informative representation of peptide flexibility, making alanine dipeptide an archetypal system for testing force fields, solvent models, and sampling algorithms [3].

➢ *Solvent Effects on Peptide Conformations*

Solvent composition plays a critical role in stabilizing or destabilizing specific peptide conformations. Polar protic solvents like water can form hydrogen bonds with peptide backbones and side chains, often favoring compact conformations such as α-helical or gauche+ regions. In contrast, polar aprotic solvents like acetonitrile (ACN) lack hydrogen-bond donating ability, leading to a reduction in solvent-mediated stabilization of compact conformers and often promoting more extended conformations [2]. Mixed solvents, such as ACN–water solutions, provide a tunable environment where the polarity and hydrogen-bonding capacity can be systematically varied to study these effects.

➢ *Alanine Dipeptide in Mixed Solvents*

The conformational preferences of alanine dipeptide are strongly solvent dependent. Water stabilizes αR and other compact conformers through hydrogen bonding between carbonyl and solvent molecules, whereas solvents with lower polarity, such as acetonitrile (ACN), reduce backbone solvation and favor more extended structures [6]. NMR and IR experiments show that as ACN concentration rises, intramolecular hydrogen bonding between the C=O and NH groups increases, encouraging transitions toward the C7eq basin [7]. Thus, higher ACN content reduces the stability of helical-like αR/L and gauche+ conformations and increases the population of extended β-sheet-like regions [4,5].

Free-energy maps generated by umbrella sampling and WHAM confirm that αR occupancy declines with higher ACN content, while C7eq basins deepen and broaden along ψ [8]. Additional studies indicate that gauche+ becomes less stable in poor hydrogen-bonding environments [9].

➢ *Molecular Dynamics Approaches*

MD simulations provide a robust framework for studying solvent-dependent peptide conformations. Classical MD integrates Newton's equations of motion to provide atomistic trajectories, enabling the calculation of free-energy surfaces, time-correlation functions, and transition pathways [10]. When applied to alanine dipeptide, MD captures transitions among αR, C7eq, αL, and C7ax basins, as well as solvent-dependent stability shifts [11].

Solvent effects are often studied with explicit-solvent MD, where individual solvent molecules are represented atom by atom, or with hybrid approaches combining explicit and implicit solvation models [12]. Explicit representations (e.g., TIP3P water, generalized ACN models) enable accurate hydrogen-bonding and preferential solvation analyses, which are critical for understanding solvent-induced basin shifts [13].

➢ *Knowledge Gaps and Study Rationale*

Although the alanine dipeptide has been a benchmark for force-field validation and solvent studies, systematic sampling across a continuum of cosolvent fractions is limited. Research has typically focused on pure water, pure organic solvents, or single intermediate points, leaving the conformational response to gradual solvent changes poorly quantified. This study fills that gap by producing high-resolution φ/ψ free-energy maps at 10% and 40% ACN, clarifying how incremental solvent perturbations modulate backbone flexibility.

III. METHODOLOGY

➢ *System Preparation*

Alanine dipeptide (Ace–Ala–Nme) was selected as the model peptide because of its well-defined φ/ψ conformational landscape and widespread use as a benchmark system as stated earlier. Solvent environments were prepared to investigate the effect of acetonitrile (ACN) concentration on peptide conformations. Packmol was employed to generate solvent boxes containing the peptide surrounded by water (TIP3P model) and acetonitrile in defined mole fractions (10% and 40% ACN). Periodic cubic boxes were created with sufficient buffer to avoid peptide–image interactions.

Force-field parameters for the peptide were assigned using the AMBER/GAFF framework, while solvent parameters were taken from established water and ACN models. Each system contained ~1500 solvent molecules, ensuring adequate sampling of solute–solvent interactions. Given below is the code to generate the system.

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tleap.in

```
source leaprc.protein.ff14SB
source leaprc.gaff
source leaprc.water.tip3p

ACN = loadmol2 acn.mol2
loadamberparams acn.frcmod

model = loadpdb "ada_acn10_water90.pdb"
solvateBox model TIP3PBOX 4.0 iso
saveamberparm model ada_acn10_water90.prmtop ada_acn10_water90.inpcrd
savepdb model ada_acn10_water90leap.pdb

model = loadpdb "ada_acn40_water60.pdb"
solvateBox model TIP3PBOX 4.0 iso
saveamberparm model ada_acn40_water60.prmtop ada_acn40_water60.inpcrd
savepdb model ada_acn40_water60leap.pdb

quit

```

Fig 1 Code for tLEAP

This code was executed using tLeap, a MD simulation tool. First, packmol was used to create the PDB file of the sytem using already existing PDB of Alanine dipeptide, ACN and water. Then, tLeap ran this code to get the Force Field parameters for the system. These parameters were then used in the next steps.

➢ *Molecular Dynamics Simulations*

All simulations were performed with OpenMM (Python API). For each solvent composition, three independent replicas were generated with different random seeds. The following protocol was applied:

- Energy minimization: Steepest-descent and conjugate-gradient steps removed unfavorable contacts.
- Equilibration: Systems were heated to 300 K over 50 ps under an NVT ensemble, then equilibrated for 1 ns at 300 K and 1 atm in the NPT ensemble to reach stable density.
- Production runs: 1-ns trajectories were recorded for each replica using a 2-fs integration step and a Monte-Carlo barostat. Coordinates were saved every 1 ps, yielding sufficient temporal resolution for φ/ψ analysis.

Nonbonded interactions used a 10-Å cutoff, with long-range electrostatics treated by the particle-mesh Ewald method. Bonds involving hydrogens were constrained with the SHAKE algorithm, enabling a 2-fs timestep. Given below is the code to run the simulation.

```python
from openmm.app import *
from openmm import *
from openmm.unit import *
from openmm.unit import nanometer, picosecond, picoseconds

def run_md(prmtop, inpcrd, out_prefix, seed=1, prod_ns=1):
        prmtop = AmberPrmtopFile(prmtop)
        inpcrd = AmberInpcrdFile(inpcrd)

        system = prmtop.createSystem(
                nonbondedMethod=PME, nonbondedCutoff=1*nanometer, rigidWater=True, ewaldErrorTolerance=1e-5, constraints=HBonds)
        system.addForce(MonteCarloBarostat(1.0*bar, 300*kelvin, 25))

        integrator = LangevinMiddleIntegrator(300*kelvin, 1/picosecond, 0.002*picoseconds)
        platform = Platform.getPlatformByName('CPU')

        simulation = Simulation(prmtop.topology, system, integrator, platform)
        simulation.context.setPositions(inpcrd.positions)
```

Fig 2 Simulation Code 1

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First, the OpenMM API was imported. Then the Force Field parameters as well as the molecule positions generated using tLeap were loaded into the system variable. A Monte Carlo Barostat was added to makes sure pressure is constant as it usually is in real life situations. Then, an integrator was added which adds equations of motion and other physical equations and restraints into the system. The simulation was started.

```
    if inpcrd.boxVectors is not None:
        simulation.context.setPeriodicBoxVectors(*inpcrd.boxVectors)

    simulation.context.setVelocitiesToTemperature(300*kelvin, seed)
    simulation.minimizeEnergy(maxIterations=2000)

    simulation.reporters.append(DCDReporter(f"{out_prefix}.dcd", 500))
    simulation.reporters.append(
        StateDataReporter(f"{out_prefix}.log", 1000, step=True, time=True, potentialEnergy=True, temperature=True, density=True))

    print("Equilibration...")
    simulation.step(125000)
    prod_steps = int((prod_ns * 1_000_000) / 2)
    print(f"Production run: {prod_ns} ns")
    simulation.step(prod_steps)

    state = simulation.context.getState(getPositions=True)
    with open(f"{out_prefix}_final.pdb", "w") as f:
        PDBFile.writeFile(prmtop.topology, state.getPositions(), f)

run_md("ForceField/sample/ada_acn40_water60.prmtop", "ForceField/sample/ada_acn40_water60.inpcrd", "Simulations/acn/ada_acn40rep", seed=123)
```

Fig 3 Simulation Code 2

The next few lines of code optimize the simulation. Box vectors were set to keep the solution in one box. Initial velocity was set at 300 K temperature and a given seed. Changing the seed changed the initial velocities, generating 3 different copies. Energy was minimized initially after which, simulation data was added to the DCD file and physical data was added to the LOG file. Opening the DCD file using PyMol shows the interactions and molecule positions at every frame in the form of a video. The final positions were saved into a PDB file, and the function was called for both the 40% ACN model and the 10% ACN model.

➢ *Conformational Analysis*

Trajectory post-processing was conducted with MDTraj. Backbone dihedral angles φ and ψ were computed for every frame and used to construct two-dimensional histograms (Ramachandran plots). Probabilities $P(\phi,\psi)$ were converted to free-energy surfaces according to.

$$F(\phi,\psi) = -k_B T \ln P(\phi,\psi)$$

Where k_B is the Boltzmann constant and T is the simulation temperature.

Free-energy basins corresponding to αR, C7eq, and other conformations were identified by locating minima on the surface. Basin populations and relative depths were compared across solvent compositions to quantify the influence of ACN on conformational preferences.

➢ *Visualization*

Ramachandran and free-energy plots were generated with Matplotlib, and representative structures from each basin were extracted for structural interpretation. Given below is the code to do the same in python.

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```
import mdtraj as md
import numpy as np
import matplotlib.pyplot as plt
topology = "ForceField/sample/ada_acn10_water90.prmtop"
trajfile = "Simulations/acn/ada_acn10rep1.dcd"
traj = md.load(trajfile, top=topology)
phi_indices, phi_vals = md.compute_phi(traj)
psi_indices, psi_vals = md.compute_psi(traj)
phi = phi_vals[:,0]
psi = psi_vals[:,0]
phi_deg = np.degrees(phi)
psi_deg = np.degrees(psi)
nbins = 100
hist, xedges, yedges = np.histogram2d(phi_deg, psi_deg, bins=nbins,
                                      range=[[-180,180],[-180,180]], density=True)
kB = 0.008314
T  = 300.0
P = hist + 1e-12
F = -kB*T*np.log(P)
F -= F.min()
fig, ax = plt.subplots(figsize=(6,5))
X, Y = np.meshgrid(0.5*(xedges[1:]+xedges[:-1]),
                   0.5*(yedges[1:]+yedges[:-1]))
cont = ax.contourf(X, Y, F.T, levels=20, cmap='viridis')
plt.colorbar(cont, label='Free Energy (kJ/mol)')
ax.set_xlabel(r'$\phi$ (deg)')
ax.set_ylabel(r'$\psi$ (deg)')
ax.set_title("Free-energy surface from 1 ns trajectory")
ax.set_xlim(-180,180)
ax.set_ylim(-180,180)
plt.show()
```

Fig 4 Data Extraction Code

First, MDTraj and Matplotlib was imported for extracting the angles and then plotting it. The molecule positions, generated by tLeap, as well as the trajectory file were then passed into MDTraj to then get the dihedral angles. Then, some postprocessing was carried out and the Free Energy Surface was calculated using the above-mentioned formula. It was then visualized using Matplotlib.

IV. RESULTS

➢ *Given Below are the Graphs Generated by Matplotlib.*

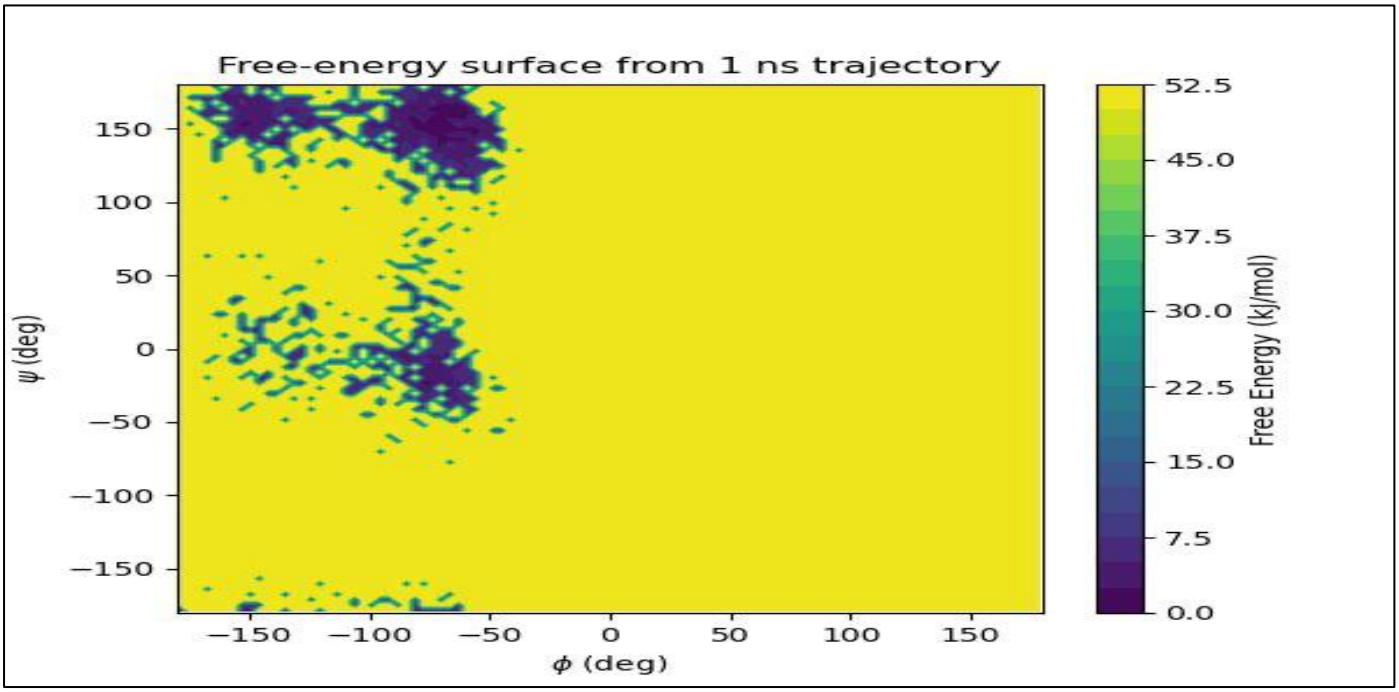

Fig 5 10% ACN in Water Solution's Ramachandran Plot

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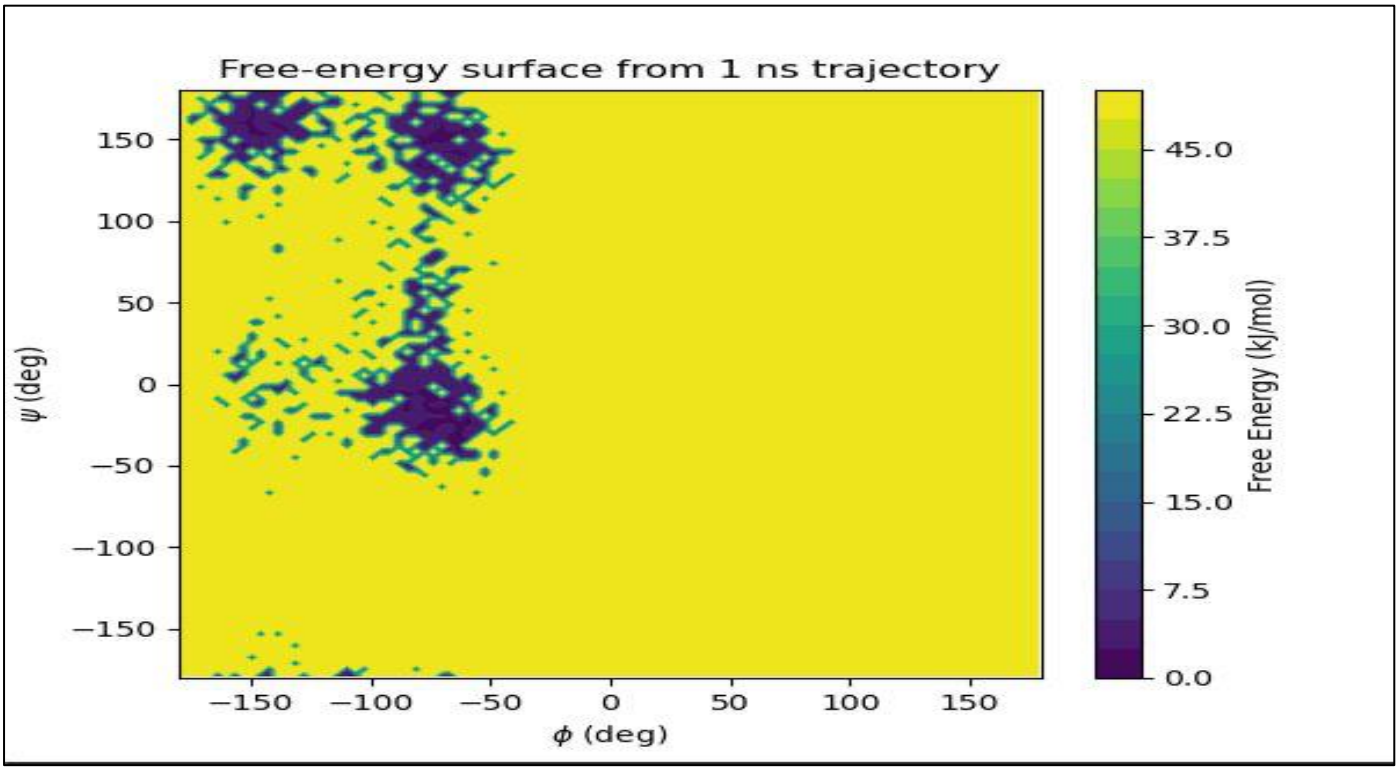

Fig 6 40% ACN in Water Solution's Ramachandran Plot

In 10% ACN (Figure 1), the FES is dominated by compact conformations, with major minima located at ($\phi \approx -70°$, $\psi \approx 150°$) and ($\phi \approx -70°$, $\psi \approx 0°$), corresponding to the C7eq and C5 states, respectively. These conformations reflect the canonical aqueous-like landscape, where alanine dipeptide samples folded structures stabilized by water-mediated hydrogen bonding. A smaller population is visible in the extended region ($\phi \approx -150°$, $\psi \approx 150°$), but it remains a relatively high-energy, less populated state.

In contrast, at 40% ACN (Figure 2), the conformational distribution shifts toward more extended states. While the C7eq and C5 basins remain present, their populations are comparatively reduced, and the extended region ($\phi \approx -150°$, $\psi \approx 150°$) becomes significantly more pronounced. The broadening of this basin and reduction in barrier heights suggest that higher acetonitrile content facilitates sampling of extended conformations, possibly due to the reduced hydrogen-bonding capacity of the solvent mixture and preferential stabilization of backbone–solvent over backbone–backbone interactions.

Overall, the increase in ACN concentration from 10% to 40% drives alanine dipeptide toward more extended conformations at the expense of compact states. This demonstrates that solvent polarity plays a key role in modulating peptide backbone conformational equilibria, with higher ACN content biasing the system away from folded minima and into extended conformers.

Table 1 The Probability of Finding the Molecule in a Certain Conformer for Each Solution

Solvent (ACN %)	Basin	Estimated ΔG (kJ·mol⁻¹)	State Probability (±)
10%	C7eq (global)	0 (reference)	76.6%
10%	C5	+3 ± 2	22.8%
10%	Extended	+12 ± 3	0.6%
40%	C7eq (global)	0 (reference)	65.1%
40%	C5	+6 ± 2	5.8%
40%	Extended	+2 ± 3	29.1%

V. CONCLUSION

This study investigated how solvent composition influences the conformational landscape of alanine dipeptide, a prototypical system for probing peptide backbone dynamics. Using molecular dynamics simulations, we computed two-dimensional free-energy surfaces projected onto the backbone dihedral angles (ϕ, ψ) in mixed acetonitrile–water environments containing 10% and 40% acetonitrile. At 10% acetonitrile, the conformational ensemble is dominated by canonical folded states, primarily the C7eq and C5 basins, with only negligible sampling of extended conformers. Increasing the acetonitrile concentration to 40% induces a significant redistribution of the conformational landscape: while C7eq remains the global minimum, the C5 basin is destabilized, and extended conformations become substantially more favorable. Quantitative estimates indicate that the occupancy of

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extended states rises from <1% at 10% acetonitrile to nearly 30% at 40% acetonitrile, at the expense of compact conformers.

These findings demonstrate that solvent polarity and hydrogen-bonding capacity critically modulate peptide backbone preferences, with acetonitrile-rich environments biasing alanine dipeptide toward extended geometries. The results highlight the sensitivity of peptide conformational equilibria to solvent composition and provide mechanistic insight into how mixed solvents may alter protein and peptide folding landscapes.

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