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Role of Solute Solvent Interactions of Aromatic Residues in Protein Folding Stability and Misfolding Mechanisms

Running Title: *From Native Packing to Denaturant Induced Aggregation Pathways*

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Abstract: ***Aromatic amino acids tryptophan, tyrosine, and phenylalanine are consistently overrepresented in protein folding nuclei, hydrophobic cores, and oligomerization interfaces, reflecting the unusually favorable and versatile non-covalent interactions their ring systems support. This paper examines how solute solvent interactions of aromatic residues govern not only the stability of the correctly folded state but also the pathways by which proteins misfold and aggregate when solvent conditions are perturbed by chemical denaturants. We review the evidence that aromatic clusters frequently form the earliest, most persistent contacts during folding, discuss how partial or asymmetric denaturant exposure of these clusters generates on-pathway and off pathway intermediates, and examine the specific role of aromatic π-stacking in amyloid and amorphous aggregation. We conclude with a discussion of osmolyte based protective strategies that counteract denaturant driven aromatic desolvation, linking mechanistic understanding to practical approaches for protein stabilization.***

Keywords: Protein folding nucleus; molten globule; amyloid aggregation; π-stacking; osmolytes; misfolding

1. Introduction

The two companion papers in this series established, first, that chemical denaturants act on aromatic amino acids primarily through direct, competitive solute solvent interactions that substitute for native π–π and cation–π contacts, and second, that spectroscopic and calorimetric methods can resolve these interactions with residue-class specificity. This third paper turns to the functional consequences of that mechanism: how aromatic solute solvent interactions determine the stability of the folded state, shape the intermediates populated during denaturant induced unfolding, and when solvent conditions favor partial, non-native aromatic exposure can redirect a protein from productive folding or reversible unfolding toward irreversible misfolding and aggregation.

The scope of this review spans four interconnected levels of analysis: the structural basis for aromatic residue burial in native folds, the sequence of solvation events that denaturants impose during equilibrium unfolding, the specific role of aromatic ring stacking in pathological self-assembly, and the practical countermeasures both natural (osmolytes, chaperones) and engineered (rational mutagenesis) that modulate the balance between these outcomes. Throughout, the emphasis remains on connecting molecular level solute solvent interactions to the macroscopic phenomena of stability, reversibility, and aggregation propensity that are of direct interest to structural biologists, protein engineers, and biopharmaceutical scientists alike.

2. Aromatic Residues in the Hydrophobic Core and Folding Nucleus

Statistical analyses of protein structures consistently show that tryptophan and phenylalanine are among the most deeply buried residues in globular proteins, with burial propensities exceeding those of purely aliphatic hydrophobic residues such as leucine and valine. This reflects the combination of large hydrophobic surface area and the additional stabilization available from π–π stacking within aromatic clusters, which packs more favorably and rigidly than aliphatic side-chain packing alone.

Beyond simple burial statistics, comparative genomic and structural studies of thermophilic versus mesophilic protein homologs have found that thermostable variants frequently show increased aromatic residue content at core and interface positions relative to their mesophilic counterparts, consistent with the idea that aromatic packing interactions provide a particularly robust, temperature and denaturant resistant contribution to structural stability. This evolutionary signal provides independent, sequence level support for the biophysical mechanisms discussed throughout this review.

2.1 Aromatic Clusters as Folding Nuclei

Protein engineering and Φ-value analysis studies of several small model folding proteins have identified aromatic clusters as components of the folding nucleus the set of native like contacts that form early and persist throughout the folding transition state. Because these clusters typically bury the largest hydrophobic surface area per residue, their correct, rapid formation restricts the conformational search space available to the rest of the chain, effectively organizing the productive folding pathway around a small number of aromatic-rich contact points.

2.2 Aromatic Aromatic Interaction Geometries in Native Structures

Survey studies of aromatic side chain pairs in high resolution protein structures show a strong preference for perpendicular, edge to face geometries over parallel, face to face stacking, consistent with the quadrupolar electrostatics of the aromatic ring. This preferred geometry maximizes favorable

electrostatic complementarity while minimizing steric clash, and its disruption by denaturant which favors different, denaturant mediated stacking geometries as discussed in the first paper of this series provides a structural rationale for why aromatic cluster unfolding is often an early, sensitive event in chemical denaturation.

3. Denaturant Induced Intermediates and the Aromatic Desolvation Sequence

3.1 On Pathway Intermediates and the Molten Globule

As discussed in the companion spectroscopy paper, molten globule intermediates are characterized by retained secondary structure and lost near-UV CD signal, indicating that aromatic tertiary packing is disrupted while backbone hydrogen bonding remains largely intact. This asymmetric loss implies that aromatic side chains gain access to denaturant and bulk solvent before the polypeptide backbone becomes fully solvated consistent with the idea that aromatic residues, despite their deep burial, sit at solvent accessible surface patches or cavity linings that denaturant molecules can reach via transient breathing motions of the native structure, even at sub-denaturing concentrations.

3.2 Off Pathway and Kinetically Trapped Intermediates

Not all denaturant induced intermediates lie on the productive folding or unfolding pathway. Partial exposure of an aromatic cluster can allow non-native π–π contacts to form between segments of the chain that are not in contact in the native structure, producing a kinetically trapped, off-pathway species. Because these non-native aromatic contacts can be nearly as energetically favourable as native contacts, off pathway intermediates populated during denaturant removal (refolding) or partial denaturation are often long lived and can dominate the observed folding kinetics, producing the multiphasic kinetic traces frequently reported for aromatic-rich proteins.

4. Aromatic π-Stacking in Aggregation and Amyloid Formation

When solute solvent conditions favor partial rather than complete unfolding for example, at sub-denaturing chemical denaturant concentrations, elevated temperature short of full thermal unfolding, or in the presence of destabilizing mutations proteins can populate partially folded states in which hydrophobic and aromatic surfaces are transiently exposed without full chain expansion. These conditions are widely recognized as the most aggregation-prone regime for many proteins, and aromatic residues play a disproportionate role in the resulting self-assembly.

4.1 Cross β Amyloid Structures and Aromatic Zippers

High resolution structures of amyloid fibrils formed by amyloidogenic peptides frequently reveal aromatic residues positioned at the interface between stacked β-sheets, forming so-called steric zipper or aromatic zipper motifs stabilized by inter sheet π-stacking. Mutagenesis studies that replace aromatic residues at these positions with non-aromatic hydrophobic residues of similar size typically reduce aggregation propensity substantially, even when overall hydrophobicity is preserved, indicating that the specific electronic and geometric properties of the aromatic ring not simply its hydrophobic surface area drive amyloid self-assembly.

4.2 Solvent Conditions That Promote Aromatic Driven Aggregation

Because full denaturant induced unfolding produces an expanded, largely non-compact chain in which aromatic residues are individually solvated by denaturant rather than available for intermolecular stacking, aggregation is typically suppressed at high denaturant concentrations and maximized at intermediate concentrations where partially folded, aromatic exposing intermediates are most highly populated. This non-monotonic relationship between denaturant concentration and aggregation rate is a well-documented experimental signature, and it directly reflects the competition between denaturant solvation of exposed aromatic rings (which is protective) and intermolecular aromatic stacking (which drives aggregation) for the same solvent-exposed ring surface.

4.3 Amorphous Aggregation versus Ordered Fibril Formation

Not all aromatic-mediated aggregation produces ordered amyloid fibrils; under some solvent conditions, particularly rapid or highly concentrated partial denaturation, aromatic and hydrophobic surfaces can associate in a disordered, kinetically controlled manner to form amorphous aggregates. The balance between ordered and amorphous aggregation pathways depends on the rate at which partially unfolded, aromatic exposing intermediates are populated relative to the rate of productive refolding or complete unfolding, making denaturant concentration and the kinetics of its removal critical experimental variables in both disease-relevant amyloid research and biopharmaceutical formulation science.

5. Osmolyte Protection and Counteraction of Denaturant Effects

Naturally occurring osmolytes such as trimethylamine N-oxide (TMAO), sucrose, and glycine betaine are well known to counteract denaturant-induced unfolding, and their mechanism of action provides an instructive mirror image of the denaturant mechanisms discussed throughout this series. Rather than accumulating favorably at protein surfaces, protective osmolytes are preferentially excluded from the protein water interface, particularly from the vicinity of the peptide backbone, raising the free energy of the unfolded, more solvent-exposed state relative to the compact native state.

5.1 Differential Osmolyte Interaction with Aromatic Surfaces

Transfer free energy measurements show that osmolyte exclusion is strongest for the peptide backbone and somewhat weaker for aromatic and other hydrophobic side chains, meaning that osmolytes shift the folding equilibrium primarily by disfavoring backbone exposure rather than by

directly strengthening aromatic packing. Nonetheless, because aromatic residues contribute substantial solvent-accessible surface area upon unfolding, the net effect of osmolyte exclusion is a significant stabilization of the aromatic rich hydrophobic core, effectively counteracting the direct denaturant binding described in the first paper of this series.

5.2 Practical Implications for Protein Formulation

Because aromatic driven aggregation is maximal at intermediate, partially denaturing solvent conditions, formulation strategies that either fully stabilize the native state (using protective osmolytes) or, where refolding is required, rapidly transit through the aggregation prone intermediate regime rather than dwelling within it, are both supported by the mechanistic picture developed in this review. This has direct relevance to biopharmaceutical protein formulation, where minimizing time spent in partially unfolded, aromatic exposing states during processing, freeze thaw cycles, or storage is a key strategy for preventing aggregation related loss of product quality.

5.3 Cosolvent Combinations and Formulation Design

In practice, protein formulations rarely rely on a single stabilizing osmolyte; combinations of sugars, polyols, and amino acid-based excipients are frequently used together to maximize preferential exclusion from the peptide backbone while minimizing viscosity and other formulation constraints. Because the aromatic-shielding benefit of osmolyte exclusion described in Section 5.1 depends on the specific chemistry of the excipient, formulation screening studies routinely combine denaturant titration spectroscopic assays with excipient screening to identify combinations that raise Cm and suppress aggregation flux simultaneously, integrating the mechanistic and spectroscopic frameworks developed across this series into a practical development workflow.

6. Kinetic Partitioning Between Folding and Aggregation

The kinetic partitioning model of protein folding describes the competition between productive, intramolecular collapse toward the native state and non-productive, intermolecular association toward aggregated species as two parallel pathways drawing from the same population of partially folded, aromatic exposing molecules. Because both pathways are nucleated by similar aromatic and hydrophobic contacts, the partition coefficient between them is highly sensitive to protein concentration, solvent composition, and the presence of denaturant, all of which modulate the effective concentration and lifetime of aromatic exposing intermediates.

6.1 Concentration Dependence of Aggregation Flux

Aggregation, being an intermolecular process, exhibits a reaction order with respect to protein concentration greater than the first order or pseudo first order kinetics typical of intramolecular folding. This concentration dependence means that the same partially denaturing solvent condition can yield predominantly monomeric, native like refolding at low protein concentration and substantial aggregation at higher concentration, a practically important consideration when translating denaturant based unfolding studies performed at dilute, spectroscopy compatible concentrations to industrial scale refolding processes performed at much higher protein concentration.

6.2 Chaperone Modulation of the Aromatic Exposure Window

Molecular chaperones, particularly members of the Hsp70 and chaperonin families, bind preferentially to exposed hydrophobic and aromatic surfaces on partially folded substrates, effectively sequestering aggregation-prone intermediates and extending the time available for productive folding without permitting intermolecular aromatic stacking. This chaperone-mediated shielding mechanism is conceptually analogous to the denaturant-mediated aromatic solvation discussed in Section 4.2, in that both processes competitively occupy exposed aromatic surfaces, but chaperones achieve this through specific, reversible protein protein binding rather than through generic small molecule solvation.

7. Disease Relevant Case Studies

7.1 Amyloid-β and Alzheimer's Disease

The amyloid-β peptide, implicated in Alzheimer's disease pathology, contains a central hydrophobic core with phenylalanine residues at positions that make direct inter peptide aromatic contacts in fibril structures determined by solid state NMR and cryo-electron microscopy. Substitution of these phenylalanine residues in model peptide studies substantially reduces fibrillization rate, and denaturant titration studies show that amyloid-β aggregation is maximal at partially denaturing conditions that expose the central hydrophobic, phenylalanine containing segment without fully unfolding the flanking regions, consistent with the general aromatic-exposure driven aggregation mechanism discussed in Section 4.

7.2 α-Synuclein and Parkinson's Disease

α-Synuclein, an intrinsically disordered protein implicated in Parkinson's disease, lacks a stable native fold under physiological conditions but nonetheless contains an aromatic rich non-amyloid β component (NAC) region that drives fibrillization. Because the protein is already largely solvent exposed in its monomeric state, denaturant addition to α-synuclein solutions typically suppresses aggregation by directly solvating the NAC region's aromatic residues, providing a clear illustration of the protective, competitive solvation role that denaturants can play at intermediate-to-high concentration even for intrinsically disordered, aggregation prone proteins.

7.3 Transthyretin and Systemic Amyloidosis

Transthyretin, a tetrameric transport protein whose destabilizing mutations cause systemic amyloidosis, illustrates the connection between subunit-interface aromatic packing and disease associated misfolding. Many pathogenic

transthyretin mutations map to the aromatic rich subunit interface, and biophysical characterization of these variants using the combined spectroscopic and calorimetric approaches described in the companion paper shows reduced tetramer stability correlating directly with increased amyloidogenic potential, supporting therapeutic strategies based on small molecule stabilization of the native tetramer interface.

8. Engineering Strategies Informed by Aromatic Solute Solvent Interactions

8.1 Rational Mutagenesis for Enhanced Stability

Protein engineering efforts aimed at increasing resistance to chemical or thermal denaturation frequently target aromatic cluster reinforcement, either by introducing additional favorable π–π contacts or by improving the geometric complementarity of existing aromatic pairs toward the energetically preferred edge to face arrangement discussed in Section 2.2. Because such mutations act at the same structural sites that denaturants preferentially target, they directly raise the free energy cost of the denaturant substitution mechanism described in the first paper of this series, typically manifesting as increased Cm and reduced m-value-normalized denaturant sensitivity.

8.2 Aggregation Resistant Variant Design

Conversely, reducing aggregation propensity in biopharmaceutical proteins often involves the opposite strategy: selective replacement of surface exposed or interface aromatic residues that participate in non-native stacking with charged or polar residues of similar size, disrupting potential aromatic zipper formation without significantly compromising native-state stability, provided the substituted residues are not part of the essential folding nucleus identified through Φ-value analysis (Section 2.1).

9. Synthesis: A Unified View of Aromatic Solute Solvent Interactions Across the Folding Landscape

Considered together with the mechanistic and spectroscopic findings of the companion papers, the evidence reviewed here supports a coherent picture in which aromatic amino acids act as sensitive control points across the entire protein folding landscape. Their large, electronically rich ring systems make them preferential targets for direct denaturant binding, give them outsized influence on measured m-values and calorimetric heat capacity changes, and make them simultaneously essential for native-state stability and hazardous when partially exposed, since exposed aromatic surfaces are prone to non-native, intermolecular stacking. This dual role stabilizing when correctly buried, destabilizing and aggregation prone when partially exposed explains why aromatic residues are both the most informative spectroscopic reporters of unfolding and among the most common sites of disease-associated and engineering-relevant mutations affecting protein stability and aggregation propensity.

Table 1: Summarizes the disease relevant aromatic interaction motifs discussed in Section 6, highlighting the recurring structural theme of aromatic residues positioned at self assembly interfaces.

System	Key aromatic residue (s)	Interaction motif	Disease association
Amyloid-β	Phe19, Phe20	Central hydrophobic core stacking	Alzheimer's disease
α-Synuclein	Phe residues in NAC region	Non-amyloid-β component stacking	Parkinson's disease
Transthyretin	Subunit-interface Trp/Phe	Tetramer interface packing	Systemic amyloidosis

10. Practical Outlook: Modulating the Denaturant Aggregation Balance

The non-monotonic relationship between denaturant concentration and aggregation rate described in Section 4.2 has direct practical value: because both very low and very high denaturant concentrations tend to suppress aggregation the former by preserving full native shielding of aromatic clusters, the latter by fully solvating exposed aromatic surfaces with denaturant rather than allowing intermolecular stacking processes that require transient protein unfolding, such as inclusion body refolding in recombinant protein production, are most successful when denaturant removal is performed rapidly enough to avoid prolonged residence in the intermediate, aggregation-prone concentration window. This kinetic framing, grounded in the aromatic solute solvent competition mechanism developed across this review, provides a mechanistic rationale for empirically established rapid-dilution and pulsed renaturation refolding protocols used throughout the biotechnology industry.

11. Conclusion

Solute solvent interactions of aromatic residues sit at the mechanistic center of protein folding stability, denaturant action, and misfolding pathology. Aromatic clusters anchor folding nuclei and native hydrophobic cores through favorable, geometrically specific π–π and cation–π contacts; chemical denaturants destabilize the native state by directly competing for these same contacts; and partial, denaturant or stress induced exposure of aromatic surfaces creates the intermolecular stacking opportunities that drive both amyloid and amorphous aggregation. Protective osmolytes counteract this cascade by favoring the compact, aromatic shielded native state. A unified, residue class-resolved understanding of aromatic solute solvent interactions integrating the mechanistic, spectroscopic, and folding pathway perspectives developed across this three paper series offers a productive framework for future work in protein engineering, biopharmaceutical formulation, and the study of aggregation linked disease.

Future progress will likely come from studies that explicitly track individual aromatic residues, rather than bulk protein populations, through the full folding unfolding-aggregation landscape under systematically varied denaturant conditions. Combining the residue resolved mutagenesis and single-

molecule approaches introduced in the companion spectroscopy paper with the kinetic partitioning framework developed here offers a path toward predictive, sequence based models of when a given aromatic rich protein will fold productively, unfold reversibly, or misfold irreversibly under a specified solvent condition a long-standing goal with direct relevance to both basic protein science and the rational design of stable, aggregation-resistant therapeutic proteins.

References

[1] Chiti, F., & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. Annual Review of Biochemistry, 75, 333–366.
[2] Fernandez-Escamilla, A. M., Rousseau, F., Schymkowitz, J., & Serrano, L. (2004). Prediction of sequence dependent and mutational effects on the aggregation of peptides and proteins. Nature Biotechnology, 22(10), 1302–1306.
[3] Gazit, E. (2002). A possible role for π-stacking in the self-assembly of amyloid fibrils. FASEB Journal, 16(1), 77–83.
[4] Marqusee, S., & Baldwin, R. L. (1987). Helix stabilization by Glu-...Lys+ salt bridges in short peptides of de novo design. Proceedings of the National Academy of Sciences, 84(24), 8898–8902.
[5] Nozaki, Y., & Tanford, C. (1963). The solubility of amino acids and related compounds in aqueous urea solutions. Journal of Biological Chemistry, 238(12), 4074–4081.
[6] Ptitsyn, O. B. (1995). Molten globule and protein folding. Advances in Protein Chemistry, 47, 83–229.
[7] Sawle, L., & Ghosh, K. (2011). How do thermophilic proteins and proteomes withstand high temperature? Biophysical Journal, 101(1), 217–227.
[8] Serrano, L., Matouschek, A., & Fersht, A. R. (1992). The folding of an enzyme III. Journal of Molecular Biology, 224(3), 805–818.
[9] Street, T. O., Bolen, D. W., & Rose, G. D. (2006). A molecular mechanism for osmolyte-induced protein stability. Proceedings of the National Academy of Sciences, 103(38), 13997–14002.
[10] Uversky, V. N., & Fink, A. L. (2004). Conformational constraints for amyloid fibrillation: the importance of being unfolded. Biochimica et Biophysica Acta, 1698(2), 131–153.