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3-AMINO-1,2,4-TRIAZOLE AT THE CHITOSAN–COLLAGEN INTERFACE: A MECHANISTIC FRAMEWORK FOR BRIDGING, CAPPING AND LATTICE INCLUSION

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Abstract

Chitosan–collagen hybrids derive their behaviour from a thin interfacial region where a cationic polysaccharide, a hydrated triple helix and ordered water compete for one hydrogen-bond inventory. This review asks what 3-amino-1,2,4-triazole (3-AT), an 84 Da hydrogen-bonding heterocycle, could do there. A structured search yielded 177 records (Supplementary Table S2); no eligible study combining all three components was identified within the defined search strategy, so every ternary statement is inference paired with a named falsifiable observable. With a conjugate-acid pK_a of 4.04, 3-AT is 72% neutral at pH 4.5 and 96% at pH 5.5: it cannot serve as a counter-ion for chitosan ammonium or compete for collagen carboxylate, and being monovalent it cannot crosslink. Three non-covalent roles remain – interstitial bridging, capping of the hydrogen-bond ladder and inclusion in the hydrated chitosan lattice – behind two confounders to be excluded first: retitration of the casting sol by 3-AT itself, and covalent capture of its exocyclic amine by crosslinkers. The roles predict divergent diffraction, calorimetric, mechanical and degradation signatures, so they are experimentally separable; the necessary control set is specified.

Keywords: chitosan, collagen, 3-amino-1,2,4-triazole, supramolecular interface, hydrogen bonding, hydration, network stability, biomaterials

Introduction

Chitosan and collagen are combined because their deficiencies are complementary: collagen gives a cell-instructive triple helix but little strength, chitosan a cationic film-forming backbone without fibrillar hierarchy (Rinaudo, 2006). Their properties are not additive; association depends on the state of collagen (Taravel &

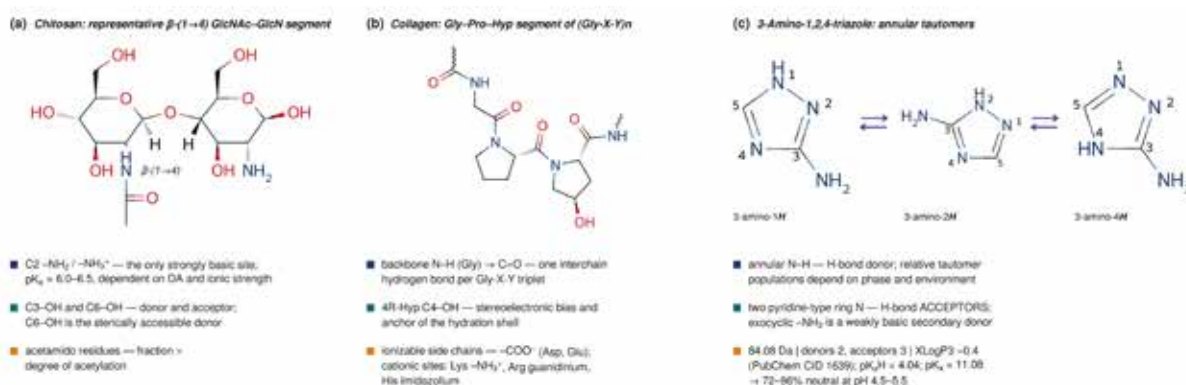
Domard, 1993) and on a contact map set by chitosan acetylation and collagen hydroxylation (Przybyłek et al., 2022). The interface is thus the property-determining element, and it is crowded: every additive must displace something already bound (Kaczmarek-Szczepańska et al., 2025). 3-AT is a suitable probe: small enough to enter that region without templating a phase of its

own, hydrophilic, and presenting an annular N–H donor, two ring-nitrogen acceptors and an exocyclic amine on a rigid scaffold. It is essentially uncharged where chitosan is charged, a regime no polymeric additive occupies. Reviews do not close the gap: chitosan–collagen work compresses the interface into one ammonium–carboxylate statement (Devernois & Coradin, 2023), and triazole–biomaterial work is dominated by the click-derived 1,2,3-isomer, where the ring is a covalent junction, not a guest (Erdmann & Wennemers, 2013).

Molecular architecture of the three components

Chitosan's interfacial inventory is three-fold: the C2 amine, its only strongly basic site; the C3 and C6 hydroxyls, C6–OH being the accessible donor; and residual acetamido groups. Two free-amine estimates circulate and are not interchangeable: dissolution in acetic acid requires a degree of protonation of only about 0.5 (Rinaudo et al., 1999), whereas Henderson–Hasselbalch with $pK_a \approx 6.0$ – 6.5 predicts 76–99%. They reconcile only through the charge-dependent depression of a polyelectrolyte's apparent pK , so the free-amine fraction must be measured.

Figure 1. Interaction-relevant chemistry of the three components



Note. (a) Chitosan repeating unit; (b) a Gly–Pro–Hyp segment of the triple helix; (c) the annular tautomers of 3-amino-1,2,4-triazole. No covalent linkage between the components is implied

Chitosan's solid-state hydrogen bonding is polymorph-dependent, structural water being integral to the hydrated form, and small guests can enter that lattice: monocarboxylic acids form a distinct complex phase whose removal drives a crystal transition (Naito et al., 2015). The triple helix carries one interchain N–H $\cdots$ O=C bond per Gly-X-Y triplet inside a hydration cylinder in which water bridges peptide acceptors (Bella et al., 1994). Two caveats follow. First-shell residence is nanosecond to sub-nanosecond (Melacini et al., 2000), so a guest competes on occupancy, not by displacement. And where water was seen to replace a direct interchain bond, it was at a glycine substitution mimicking a disease mutation and came with local untwisting: a precedent for the geometry of bridging, and equally evidence that

bridging in the helix core is a defect rather than a design option.

3-AT is $\text{C}_2\text{H}_4\text{N}_4$, 84.08 Da, with two donors, three acceptors and a negative computed partition coefficient placing it in the aqueous phase. Potentiometry gives $pK_a = 4.04$ for the conjugate acid and 11.08 for annular N–H deprotonation (Kröger & Freiberg, 1965), so the protonated fraction is about 28% at pH 4.5, 11% at pH 5.0 and 4% at pH 5.5. Which face it presents is set by environment-dependent annular tautomerism; in matrix-isolated complexes the annular N–H, not the exocyclic amine, donates (Mucha et al., 2023). It decomposes in two stages, the first with an activation energy of $124 \pm 8 \text{ kJ mol}^{-1}$ (Sánchez-Soto et al., 1995). Table 1 collects these values.

Table 1. Interaction-relevant chemistry of chitosan, collagen and 3-amino-1,2,4-triazole

Feature	Chitosan	Collagen (type I)	3-Amino-1,2,4-triazole
H-bond donors	C3–OH, C6–OH, C2–NH ₂ , acetamido N–H	backbone N–H, Hyp O–H, side-chain N–H/O–H	annular N–H (primary), exocyclic NH ₂ (secondary)
H-bond acceptors	ring and hydroxyl O, amine N	peptide C=O, carboxylate O, Hyp O	two pyridine-type ring N; amine lone pair
Ionisable site, pKa	C2–NH ₃ ⁺ ; pK _a ≈ 6.0–6.5, DA- and I-dependent	Asp/Glu –COOH, Lys/Arg/His; pI ≈ 8	conjugate acid 4.04; annular N–H 11.08
Charge at pH 4.5–5.5	≈ 50% protonated at typical acid stoichiometry	net cationic (below pI)	72% neutral at pH 4.5, 96% at pH 5.5
Valency toward a partner chain	multivalent polyelectrolyte	multivalent	monovalent – cannot crosslink
Ordered water	integral to the hydrated polymorph	cylinder of hydration, sub-nanosecond residence	hydrophilic; partitions to the aqueous phase
Reactive handle	C2–NH ₂ toward aldehyde / genipin / carbodiimide	Lys ε–NH ₂ toward the same	exocyclic NH ₂ – competes

Note. Component-level measurements on the isolated species; none has been measured in a ternary chitosan–collagen–3-AT system

Interactions at the native interface

Pairing of chitosan –NH₃⁺ with collagen –COO[–] is invoked most often and demonstrated least directly; with an isoelectric point near 8 both polymers are net cationic across the casting window, so attraction must arise locally against a repulsive background. In chitosan polyelectrolyte complexes the main infrared bands do not shift at all, complexation producing new bands instead (Lawrie et al., 2007). Band appearance, not band shift, is therefore the reporter, yet much of this literature reads shifts of five to ten wavenumbers in a window holding collagen amide II, chitosan –NH₂ scissoring and the ammonium mode together.

Three candidate roles for 3-amino-1,2,4-triazole

Two routes must be excluded before any direct role is claimed. Because pKaH lies inside the casting window, added 3-AT is itself a buffer: it consumes acid, raises sol pH and lowers chitosan's protonation, so a property change may arise with no guest–polymer contact at all. Separately, the exocyclic amine

is a competent nucleophile toward the aldehyde, genipin and carbodiimide chemistries used on these blends. What follows is restricted to crosslinker-free constructs at matched pH and ionic strength.

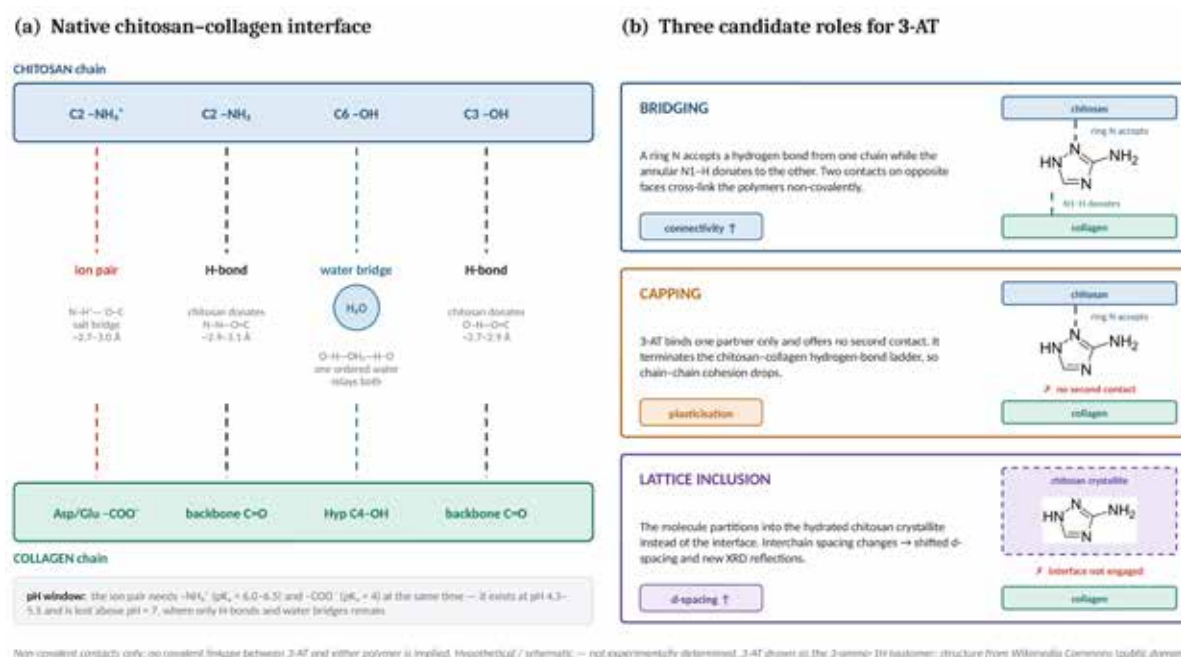
Within that restriction 3-AT is neutral and monovalent, so three non-covalent roles remain. In bridging it inserts interstitially, accepting from one polymer at a ring nitrogen and donating from its annular N–H to the other, raising inter-chain connectivity. In capping it binds one partner preferentially and terminates connectivity, acting as a chain-stopper and plasticiser. In lattice inclusion it partitions into the hydrated chitosan crystallite as a distinct complex phase.

These are dominant-expression limits, not exclusive alternatives; mixed states give intermediate signatures. Because the chitosan–collagen contact is multivalent and 3-AT is not, kinetic site occupancy during solvent removal is likelier than equilibrium displacement, which does not separate bridging from capping. No bridging by 3-AT has been reported in any protein–polysaccharide system, and the only reported chitosan/3-AT

material is a covalently grafted cotton-fabric

finish with no collagen and no network data (Aboelnaga et al., 2017).

Figure 2. The native interface and the three candidate roles for 3-amino-1,2,4-triazole



Note. Contacts are non-covalent throughout; broken lines denote hydrogen bonds or local ion pairs. Schematic, not experimentally determined

Discriminating the roles

The roles predict different signatures (Table 2). Diffraction discriminates most sharply: inclusion produces new Bragg reflections rather than broadening alone (Naito et al., 2015), bridging and capping none. A collagen-free control is mandatory, since collagen alone already abolishes the low-angle hydrated-chitosan reflection (Becerra

et al., 2022); without that reflection, inclusion is unavailable and the scheme reduces to two roles. Calorimetry supplies the water axis, but bound water and equilibrium swelling must be reported separately, because in crosslinked chitosan membranes the two do not co-vary (Ostrowska-Czubenko & Gierszewska-Drużyńska, 2009).

Table 2. Divergent signatures of the three candidate roles

Observable	Bridging	Capping	Lattice inclusion
Wide-angle X-ray diffraction	no new reflections	no new reflections	new Bragg reflections
Non-freezing water (DSC)	decreases	increases	crystal transition rather than monotonic change
Equilibrium swelling	measure separately from bound water	measure separately from bound water	little change
Modulus / elongation	modulus $\uparrow$, elongation $\downarrow$	modulus $\downarrow$, elongation $\uparrow$	modulus $\approx$, brittleness $\uparrow$
Enzymatic degradation	slowed by steric shielding	accelerated	largely unchanged

Observable	Bridging	Capping	Lattice inclusion
Raman I ₁₂₄₈ /I ₁₂₇₁	perturbed if the helix is reached	perturbed if the helix is reached	unchanged
Functional outcome	stable films, slow resorption	compliant, fast-swelling matrices	brittle, processing-sensitive

Note. Predicted signatures, not measured data. Diffraction separates inclusion from the other two; mechanics and degradation separate bridging from capping. Every row requires a crosslinker-free blank, a collagen-free binary and a charge-matched control

Operational definitions differ between studies: in one collagen/chitosan/silk-fibroin series, swelling in phosphate-buffered saline ran from $1302 \pm 30\%$ to $7580 \pm 50\%$ while liquid uptake in the same work was 10–15% (Grabska-Zielińska et al., 2021). Vibrational spectroscopy is best used as a helix reporter: the Raman amide III doublet near 1271 and 1248 cm^{-1} resolves triple-helical content (Frushour & Koenig, 1975) against a far weaker water background than infrared. No band position is established for free 3-AT in a biopolymer phase, so finding a marker band in an empty window is a prerequisite. Mechanically the roles predict opposite signs, but only a single-laboratory composition series under one protocol is defensible.

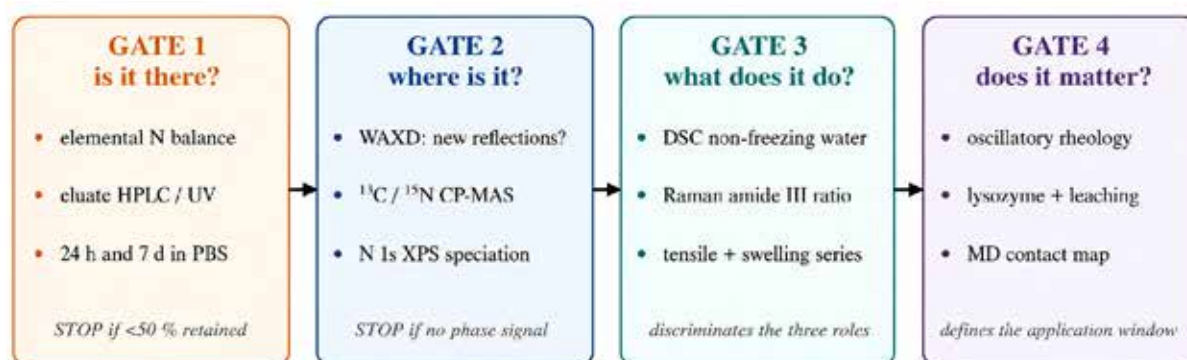
transition falls from 107 °C to 64 °C at a 1:1 ratio (Thongchai et al., 2020), so a modifier effect must be substantial to be interpretable and measured at matched water content. In thermogravimetry an extra mass-loss step matching the first decomposition stage of 3-AT would betray a segregated crystalline fraction, though its absence is informative only above the loading at which such a fraction resolves. Degradation must be phase-resolved: lysozyme attacks the chitosan phase and leaves the triple helix, which needs collagenase; dual-crosslinked collagen–chitosan films retained 85–89% of their mass after 14 days in lysozyme and lipase (Shah et al., 2019). Each assay must quantify modifier leaching, since mass loss and modifier loss are otherwise indistinguishable.

Thermal behaviour, degradation and safety

Blending is itself a large thermal effect: in one chitosan–collagen series the glass

Experimental roadmap

Figure 3. Gated experimental sequence for testing the ternary system



Retention must be quantified before any property is attributed to the modifier: a highly soluble guest will otherwise be credited with effects it has already left behind.

Note. Retention is quantified before phase location, phase location before mechanism, and mechanism before application. Each gate requires the controls in Table 2

A toxicological constraint precedes any application. 3-AT is amitrole, a regulated herbicide with documented developmental toxicity (Landauer et al., 1971), and its protein adduct forms through a peroxide-derived enzyme intermediate (Margoliash et al., 1960); the assumption of non-covalent behaviour therefore fails in peroxide- and catalase-rich environments such as inflamed wound beds. Collagen offers no comparable activating centre, so this concerns the biological environment rather than a predicted collagen reaction; but a weakly bound fraction elutes freely, making leaching an exposure route before it is a measurement artefact. No biomedical claim should be advanced without eluate cytotoxicity data.

The decisive measurements are neither expensive nor novel, but their order matters. Every stage requires three controls – a crosslinker-free blank, a collagen-free chitosan/3-AT binary at matched processing, and a charge-matched additive-free sol – without which no observation is attributable to the guest.

Conclusion

3-Amino-1,2,4-triazole is chemically well suited to the chitosan–collagen interface and entirely untested there. Its pKa removes electrostatic competition and its monovalency removes crosslinking, leaving three non-covalent roles whose diffraction, calorimetric, mechanical and degradation signatures point in different directions. They are distinguishable in principle, provided the control set above is applied and retention is quantified before any property is attributed to the modifier. Until then, 3-AT at this interface remains a testable hypothesis.

Supplementary Table S1 summarises the search strategy, databases, terms and screening criteria; Table S2 lists the 177 retained records with search category, evidence tier and screening decision; Table S3 gives their distribution across evidence domains. Twenty-three records are cited here, and none describes a material containing chitosan, collagen and 3-AT together. The dataset is archived at Zenodo (Sharopov, 2026).

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