

When Purification Changes Biological Activity

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Author approved release edition. Literature analysis and proposed research; no new VARUNÉ experimental results are reported.

Abstract

Purification is often described as a progression from an uncertain extract towards a better-defined active ingredient. That progression can improve chemical understanding whilst making biological interpretation more difficult. A stronger signal may follow enrichment, removal of an antagonist, altered exposure, or interference with the measurement. A weaker signal may follow loss of active material, disruption of a useful mixture, a change in physical state, or removal of an interfering constituent. This critical narrative report connects natural-products isolation and bioseparation principles to selected primary studies on polar exopolysaccharides, trace-active impurities, botanical combinations, aggregation, and reporter interference. Its argument is that chemical recovery, functional recovery, and causal attribution must be recorded separately. Activity is an endpoint-dependent response, not a conserved substance. The proposed VARUNÉ Bio contribution is a decision record linking each separation to the material removed, the material retained, the exposure delivered, and the explanation still compatible with the observations. Four equations clarify mass recovery, specific activity, trace-constituent dominance, and one reference model for combination effects. A staged research design is proposed for a single rights-accessible preparation, including mock processing, recovered-fraction recombination, independent reference material, orthogonal endpoints, and batch-level analysis. These are literature-derived research proposals. No new laboratory results, candidate efficacy, validated proprietary method, or confirmed commercial partnership is reported.

1 The research question

When biological activity changes after purification, what evidence distinguishes a change in the responsible substance from a change in its presentation or measurement?

This question matters because the same observed result can support incompatible development decisions. If activity follows a minor constituent, the major molecule may be the wrong asset. If purification removes an optical interferent, reduced apparent activity may improve the evidence. If an inactive co-constituent maintains solubility, its removal may reduce delivered exposure without changing the active molecule. If a defined combination has a reproducible useful effect, insisting on a single constituent could destroy the product being investigated. Conversely, invoking synergy whenever fractionation fails can conceal an ordinary recovery problem.

The argument developed here is that purification should be treated as a controlled perturbation of a preparation. Its analytical value comes from knowing what the perturbation changed. The word “pure” cannot substitute for that account. Neither can a single chromatographic peak, a successful structure assignment, or a repeated biological signal. Each answers a different question.

The report concentrates on causal attribution and interpretation. Companion reports address environmental sampling, protein interfaces, wet adhesion, and skin measurements in greater depth. Their underlying evidence requirements overlap, but their endpoints are not interchangeable. A polymer changing ice growth, a botanical preparation changing bacterial growth, and a reporter responding in cultured cells do not demonstrate the same kind of useful function.

2 Sources and analytical method

This is a targeted critical narrative synthesis prepared on 28 October 2026, not a systematic review. Source selection began with the uploaded third edition of *Natural Products Isolation*, particularly chapter 1 on separation objectives, fractionation, recovery, and purity; chapter 2 on extraction-related change and contamination; and selected analytical sections of *Bioseparations Science and Engineering*. The present report re-examined relevant passages rather than repeating the earlier continuous chapter readings. No additional complete-book or complete-chapter reading is claimed. [\[1-3\]](#)

The uploaded primary papers on *Polaribacter* SM1127 and *Colwellia psychrerythraea* 34H were checked at the relevant methods, results, and discussion passages. Online searches then sought cases that could challenge simple interpretations of purification. Queries included “rufomycin 0.24 impurity rufomycin”, “synergy-directed fractionation goldenseal”, “promiscuous inhibitors aggregation”, “MTT plant extracts interference”, and “PTC124 luciferase stabilization”. Primary publication records, publisher pages, PubMed records, and indexed primary full-text passages were preferred. Search-engine coverage was supplemented by a second engine for aggregation and reporter-mechanism checks. [\[4-12\]](#)

Cases were retained when they exposed a different failure of attribution or supplied a discriminating comparison. They were not selected to estimate how frequently any failure occurs. Searches were not exhaustive; no registered protocol, duplicate screening, comprehensive citation search, or formal risk-of-bias instrument was used. Direct access to some online full texts was blocked, whilst indexed primary passages remained accessible. Those cases are identified in the references. The detailed quantitative analysis below is therefore algebraic and illustrative, not a pooled estimate or reanalysis of the original raw data.

Four evidence levels remain separate throughout: a finding reported by the original researchers; a transferable methodological lesson; the author's synthesis proposed for VARUNÉ Bio; and a future experiment needed to evaluate that synthesis. The source authors retain credit for their discoveries. The organisation of those findings into an attribution-focused research plan is the analytical contribution developed in this report.

3 Purification changes more than concentration

3 1 What is being recovered

Natural-products isolation begins with a purpose. Obtaining enough material to identify a structure is different from obtaining enough to test biological function or manufacture a reproducible ingredient. The textbook explicitly identifies several explanations for lost activity during fractionation: retention on the separation medium, instability, precipitation, distribution across too many fractions, and loss of cooperating constituents. These are alternatives to investigate, not interchangeable diagnoses. Its

discussion of approximate activity recovery through dilution series is useful, but the proportionality between signal and active amount must be established in the actual assay. [1]

The extraction chapter extends the problem upstream. Collection, storage, drying, extraction, and concentration can alter the material before a first “crude extract” is tested. External substances can also enter the preparation. Its specific solvent methods concern mainly small organic metabolites and cannot be transferred mechanically to a functional protein or hydrated polysaccharide. The transferable principle is that a sample's processing history is part of its identity. [2]

Bioseparation analysis adds another distinction: identity, purity, biological activity, and physical characteristics are different attributes. A measurement can be repeatable whilst missing the decisive constituent. A purity percentage requires a defined detection method and denominator. It does not describe everything that could matter biologically. Historical textbook examples of analytical specifications are illustrative; they are not current universal release criteria for a VARUNÉ preparation. [3]

3 2 A mass balance is necessary but does not conserve activity

For a chemically defined target that does not transform during separation, an accounting identity is useful:

$$m_{T,0} = \sum_i m_{T,i} + m_{T,\text{retained}} + m_{T,\text{unaccounted}}$$

Equation 1. Accounting for a specified target across a separation.

Here $m_{T,0}$ is the target mass entering the step; $m_{T,i}$ is its mass in recovered fraction i ; $m_{T,\text{retained}}$ is target measured on the apparatus or in a recovered deposit; and $m_{T,\text{unaccounted}}$ is the unresolved balance. All terms use the same mass unit, such as milligrams. This is standard mass accounting applied to the proposed study, not a newly discovered law. It assumes valid target quantification and no transformation. If the target reacts, degrades, or changes covalent form, a reaction-aware molar or elemental balance is required. An unexplained deficit cannot simply be labelled “degradation”. [1-3]

Dry mass alone is a weak substitute. Salts, residual water, counterions, and co-extractives can change the denominator. Conversely, a polymer's mass can remain whilst its molecular-size distribution, substitution, aggregation, or hydration changes. The research record should therefore distinguish nominal mass, compositionally assigned target mass, and the relevant physical state.

Activity cannot ordinarily be placed into Equation 1. Two fractions can inhibit the same population, approach the same assay ceiling, interfere with one another, or behave nonlinearly with dose. Adding their percentage effects is not an activity balance. Even a recombined preparation need not reconstruct the original state if the original mixing history cannot be restored. This is the first central analytical conclusion: mass recovery constrains interpretation, but does not determine functional recovery.

4 Contrasting primary cases

4 1 A purified structure and a crude functional preparation

Sun and colleagues characterised an EPS from the Arctic bacterium *Polaribacter* sp. SM1127. Their paper describes additional chromatography for structural characterisation, but its methods identify the viscosity, moisture-retention, antioxidant, and fibroblast experiments as tests of crude EPS. The fibroblast experiment used cold exposure at 4 °C for 20 hours, with Annexin V and propidium iodide analysis. It was not a freeze-thaw experiment. Consequently, the structural account of the purified fraction cannot automatically be used as the identity of the preparation responsible for every functional result. The paper supports activity of specified preparations under its conditions; it does not isolate the causal contribution of every constituent. [4]

This creates a tractable question for further research: which measured properties survive movement from the original crude preparation to a reproducibly defined composition? A positive answer could support a more precise material proposition. A negative answer would require locating the missing contributor, determining whether the material changed, or revising the proposed product. Neither outcome can be inferred merely from the shared name “EPS”.

4 2 Residual debris remains an attribution question

The later study by Cirella and colleagues examined lipid A and immunostimulatory properties from the same strain. It explicitly describes an ethanol-precipitated preparation containing EPS and residual bacterial material, and includes further separation before EPS testing. The authors nevertheless state that trace bacterial debris could not be fully excluded as contributing to TLR4 signalling at 100 ng/mL. The relatively weak response compared with *E. coli* LPS is a comparison within particular cellular assays. It does not establish complete removal of immunologically active material, finished-product safety, or a purified polymer-specific mechanism. [5]

The two *Polaribacter* studies are not a controlled before-and-after purification trial. They differ in preparations, endpoints, and experimental questions. Connecting them is valuable because they expose an unresolved attribution boundary; combining them as if they demonstrated a single purification trajectory would be an analytical error.

4 3 A defined polymer with a bounded material endpoint

Casillo and colleagues purified and characterised the EPS from *Colwellia psychrerythraea* 34H and tested inhibition of ice recrystallisation with a modified splat assay. Chemical analysis, NMR, and modelling were connected to a specific material measurement. The assay measured ice-grain behaviour relative to controls; it did not establish recovery of a preserved enzyme, survival of a clinically relevant cell product, or a commercial cryopreservation process. [6]

This case is a useful counterweight to excessive scepticism about purification. Defined materials can retain measurable function. The remaining question is how far that function transfers. A later VARUNÉ study could build on the material endpoint whilst asking a different question about preservation of useful downstream activity. Such a study would need to measure that downstream activity directly and retain the comparator, storage, and reconstitution conditions.

4 4 A trace constituent can overturn the nominated active

Choules and colleagues reported that isolated rufomyzine appeared active against *Mycobacterium tuberculosis*, whereas synthesised material was inactive. Further analytical work attributed the original signal to a highly active rufomycin-related constituent present at approximately 0.24% by mass. The case demonstrates why a minor chemical contribution can have a major biological contribution, and why independently obtained material can challenge an attractive activity assignment. It does not establish a universal impurity threshold or imply that every trace constituent is consequential. [7]

4 5 A useful partner may be missed when fractions are tested alone

Junio and colleagues used synergy-directed fractionation in goldenseal. They identified flavonoids that enhanced berberine's antimicrobial activity and investigated an efflux-related explanation. Crucially, the paper distinguishes a simplified screen for enhancement from subsequent combination testing: lowering the partner's MIC at a single fraction concentration does not by itself distinguish additivity from synergy. This case supports retaining a combination-sensitive branch of analysis when justified, rather than assuming that every valuable fraction must be active alone. [8]

4 6 A signal can belong to the assay system

McGovern and colleagues linked apparently promiscuous enzyme inhibition to colloidal aggregates using biochemical and physical observations. This is a reason to consider physical state and assay dependence when fractionation changes apparent inhibition. It is not a basis for dismissing every aggregating natural material: aggregation may be intrinsic to the intended material function. The question is whether the observed effect supports the proposed mechanism and use. [9]

Bruggisser and colleagues observed conflicting cell-number and MTT results with kaempferol, and demonstrated cell-free reduction of the tetrazolium reagent by tested compounds and extracts. Removing a reducing constituent can therefore lower a viability-associated signal without reducing viable cells. That specific interference finding should not be generalised to every redox assay, but it gives a concrete reason for a no-cell control and an endpoint based on different measurement chemistry. [10]

Auld and colleagues examined PTC124-mediated luciferase inhibition and stabilisation. Their mechanistic analysis explains how interaction with a reporter can yield apparent activation in certain cellular reporter measurements. The lesson here is confined to assay interpretation: an intervention may change the reporter's abundance or detection chemistry rather than the biological process the reporter was intended to represent. No wider conclusion about a compound's clinical efficacy follows from this example. [11]

Primary case	What the evidence distinguishes	Remaining boundary
SM1127 EPS [4,5]	Structural material, crude functional preparation, and residual-cell question	Causal contributor to each endpoint remains preparation-specific
<i>Colwellia</i> EPS [6]	Characterised polymer and ice-recrystallisation endpoint	Downstream preservation utility requires its own measurement

Primary case	What the evidence distinguishes	Remaining boundary
Rufomyazine [7]	Major constituent versus potent minor contributor	Impurity relevance depends on potency and assay conditions
Goldenseal [8]	Enhancement screen versus interaction evidence	Combination conclusions depend on the declared reference model
Aggregation and reporters [9-11]	Intended target response versus physical or detection-system effects	Orthogonal evidence is needed for the nominated mechanism

5 Quantifying the interpretation

5 1 Enrichment and recovery answer different questions

For an enzyme preparation assayed in a verified linear initial-rate regime, operational quantities can be written as:

$$q_j = \frac{U_j}{m_j}, \quad F_j = \frac{q_j}{q_0}, \quad Y_{U,j} = 100 \frac{U_j}{U_0}$$

Equation 2. Specific activity, enrichment factor, and activity recovery under fixed assay conditions.

U_j is the total measured activity in preparation j , in explicitly defined units such as micromoles of product per minute under stated conditions. m_j is the chosen denominator, for example total protein mass in milligrams. q_j then has units U/mg; F_j is dimensionless; and $Y_{U,j}$ is a percentage. These standard operational definitions require comparable assays, valid dilution correction, and a clearly stated mass denominator. They are not appropriate for summing bounded percentage responses or treating inverse IC_{50} as a conserved quantity. [1,3]

An illustrative calculation, not experimental data, shows the distinction. A starting preparation containing 100 mg total protein and 100 U has 1 U/mg. A purified preparation containing 10 mg and 40 U has 4 U/mg: fourfold enrichment and 40% activity recovery. Reporting only enrichment hides the loss of measured capacity. Reporting only recovery hides the improvement in activity relative to total protein.

There is an additional trap. An apparent recovery above 100% might reflect removal of an inhibitor or altered assay accessibility. It does not violate mass conservation because U is an observed rate, not target mass. Before celebrating the increase, the study should ask whether the same active entity is present, whether the delivered concentration is comparable, and whether the original assay underestimated it.

5 2 Purity must be interpreted against potency

In a deliberately simplified low-response regime with independent, approximately linear contributions, the relative signal from a minor constituent I and nominated major constituent M is:

$$\frac{E_I}{E_M} \approx \frac{m_I s_I}{m_M s_M}$$

Equation 3. Illustrative relation between relative amount and relative response contribution.

E_I and E_M are response increments measured on the same scale; m_I and m_M are delivered masses; and s_I and s_M are local response slopes per mass. Ratios are dimensionless. The approximation requires the slopes to exist in the tested range, no important interaction, and comparable exposure. It fails at saturation, with substantial antagonism or synergy, or when nominal dose differs from available dose. It is an algebraic explanatory model, not a fitted relationship for the rufomyazine study.

For illustration, a minor-to-major mass ratio of 0.002 and a 1,000-fold slope ratio give a response ratio of two. The minor component could dominate despite representing a small mass fraction. The practical inference is that an impurity detection limit should be assessed against the biological question, rather than a purity percentage alone. An unknown constituent cannot be granted a reassuring biological limit simply because its analytical signal is small.

5 3 Recombination is not a synonym for synergy

For fractional inhibition between zero and one, one standard independence expectation is:

$$E_{AB}^{\text{ind}} = E_A + E_B - E_A E_B, \quad \Delta = E_{AB}^{\text{obs}} - E_{AB}^{\text{ind}}$$

Equation 4. A Bliss-type independence expectation and an observed deviation.

E_A and E_B are fractional effects at the component doses actually present in the combination, with the same endpoint, control, and time. All terms are dimensionless. The expression follows from multiplying unaffected fractions under independence and is associated with the classical independent-action framework. A positive Δ denotes greater inhibition than this expectation, not proof of a unique biological mechanism. Other reference models may be more appropriate; the choice must precede interpretation. The original Bliss source was checked at its bibliographic record and summary, not read in full. [12]

If A and B each give 0.20 inhibition, the illustrated independence prediction is 0.36, not 0.40. A mixture producing 0.40 therefore exceeds that reference by 0.04, but the conclusion still depends on uncertainty, dose estimation, model suitability, and a valid endpoint. An apparent difference smaller than ordinary assay or batch variation is not persuasive interaction evidence. A recovered mixture restoring the crude effect may simply restore dose, solubility, ionic conditions, or surface protection.

6 Varun analysis and the connected research argument

6 1 The correct unit of attribution is a preparation in a defined state

The literature makes it tempting to ask whether “the molecule” works. That question is incomplete until the material tested is specified. My proposed analytical position is to attach every activity claim to a preparation, processing history, physical state, exposure, and endpoint. The nominated molecule can then become the causal explanation if the evidence supports that attribution.

This does not make molecular identity unimportant. It prevents identity from doing more explanatory work than the data permit. Two preparations can contain the same major polymer but differ in salts, low-abundance constituents, size distributions, aggregation, or residual cells. Conversely, two

preparations with different minor fingerprints may deliver the same reproducible function. The useful research task is to identify which differences predict the decision-relevant outcome.

A fingerprint should therefore be evaluated as a predictor, not merely displayed as a quality symbol. If a dominant feature is stable across batches whilst function varies, the analysis must look beyond that feature or question the biological measurement. If a minor feature predicts activity, correlation should guide a targeted intervention, not be declared causation. The strongest next comparison is one that selectively changes the candidate contributor whilst checking what else changed.

6 2 Purification can reveal an asset by weakening a headline result

Maximum signal is not the same as maximum research value. An initially impressive preparation could contain both a useful constituent and an interfering one. Removing the latter may reduce the screen result whilst improving interpretation or the separation between useful and unwanted effects. That possibility is an analytical hypothesis, not an excuse for a failed purification.

The decision should be made against a predeclared objective. For an ingredient, that might be a reproducible material performance at an acceptable composition. For an enzyme, it might be useful recovered activity after the complete workflow. For a discovery compound, it might be a defined mechanism supported by appropriate selectivity evidence. The objective determines whether a lower average response is an acceptable trade for better consistency, interpretability, or compatibility. No single purity or potency figure can resolve all those objectives.

6 3 Mixture dependence should be mapped before it is commercialised

A reproducible mixture can be scientifically and commercially meaningful without every interaction being called synergy. The first question is whether the combination performs usefully and consistently. The next is whether its composition and processing can be controlled. Mechanistic interaction becomes important when it changes quality control, manufacturing, claims, or the comparison against alternatives.

The proposed sequence is therefore reconstruction before elaborate mechanism. If recombined measured fractions cannot reproduce the source preparation under comparable exposure, there is insufficient foundation for a confident interaction story. If reconstruction succeeds, selective omission and add-back can identify necessary contributors. A dose matrix and suitable reference model then test interaction. Even that result remains conditional on the preparation and endpoint.

This creates a useful negative outcome: a programme can retain a standardised mixture while withdrawing an unsupported single-molecule or synergy claim. Alternatively, it can discover that the useful constituent is easier to supply alone. Neither path should be chosen because it sounds more sophisticated. The choice should follow reproducibility, usefulness, rights, and cost.

6 4 The decisive comparison often concerns what was removed

Development work commonly concentrates attention on the retained fraction. The connected reading suggests that the discarded material may hold the explanation. A removed constituent might be the active, an antagonist, a stabiliser, a reporter interferent, or an irrelevant burden. Keeping an appropriately controlled record of those streams can turn a failed purification into a discriminating experiment.

This is also a boundary on the proposed framework. Comprehensive analysis of every stream could consume more resources than the candidate warrants. The response is not to abandon attribution, but to make the next analysis decision-specific. First locate the separation step where the outcome changes. Then test the explanations that would lead to different development decisions. A small number of well-chosen perturbations can be more valuable than a larger descriptive catalogue.

7 A proposed VARUNÉ Bio attribution study

The proposed initial project is a bounded assessment of one accessible preparation with one intended use. It requires an accountable scientific lead, material provenance, permitted controls, a funded analytical scope, and an agreed decision. It is not an instruction to launch several laboratory programmes.

The first stage would reproduce the starting response with an independent preparation and characterise the uncertainty. Technical repeats would estimate measurement variation; independently prepared batches would assess preparation variation. Those are different units of replication. The study should retain unsuccessful preparations and reasons for exclusion. A result that depends on choosing the best batch is unsuitable as evidence of dependable performance.

The second stage would compare an unprocessed aliquot, a time-matched handling control, and a mock-processed aliquot. These comparisons help distinguish separation from time, solvent exchange, concentration, or storage. Where feasible, a process blank would detect contributions from reagents and apparatus. The aim is to define the intervention before analysing a collection of fractions.

The third stage would track the nominated target and recover the streams needed to explain the changed response. Fractions would be compared both at a defined target-equivalent exposure and at a defined amount of starting-material equivalent, because the two normalisations answer different questions. Each comparison would retain vehicle, pH, ionic strength, and delivered-volume information. Apparent equivalence would need a specified tolerance rather than a non-significant statistical test alone.

Competing explanation	Most useful comparison	Interpretation that would change the next step
Physical recovery loss	Target accounting across recovered and retained streams	Recover or redesign the loss point before changing the biological thesis
Chemical or physical-state change	Identity and state before and after mock processing	Preserve or deliberately redefine the active material
Removal of a cooperating constituent	Measured recombination followed by omission and add-back	Evaluate a controlled combination without presuming synergy
Trace-active attribution	Independently sourced reference and targeted minor-constituent analysis	Reassign the active if the nominated major constituent fails

Competing explanation	Most useful comparison	Interpretation that would change the next step
Measurement interference	No-target controls and a different endpoint chemistry	Redesign the readout before ranking preparations
Unstable batch composition	Independent batches with linked composition and function	Narrow specifications or stop the candidate if the effect is uncontrollable

The fourth stage would challenge the leading interpretation using a second measurement that fails differently. Two assays using the same reporter chemistry are not strongly independent. A target-free or cell-free control may expose interference, but it cannot alone establish the intended biological effect. Likewise, an interference countermeasure must be checked for its own effect on the material or target. Removing aggregates, adding a surfactant, or changing a solvent may perturb genuine activity as well as artefact.

The final stage would compare the best-supported preparation against the relevant existing alternative in the intended workflow. The endpoint and smallest decision-relevant improvement would be agreed before confirmatory testing. Replication and sample size would follow variance estimates, the required precision, and the consequence of a wrong decision; this report supplies no universal sample number. A defensible conclusion could be continued development, a changed composition, a changed claim, or closure.

Decision	Evidence needed	Stop or redirect condition
Continue a single-constituent proposition	Independent material reproduces the effect with credible exposure and endpoint controls	Activity instead follows another constituent or a measurement artefact
Continue a mixture proposition	Controlled composition, reconstructable function, and useful batch consistency	Required composition cannot be reproduced or justified economically
Develop a purification step	Improved decision-relevant performance with measured recovery	Added cost and losses remove the intended advantage
Offer an attribution service	A programme owns a real unresolved decision and can supply suitable material	No budget, access, acceptance criterion, or reusable permitted output

8 How the work could serve existing programmes

The most useful proposed contribution is a constituent-attribution and recovery package for an existing ingredient or discovery programme. An EPS team might need to know which functional claims survive standardisation. A natural-product group might need to resolve a mismatch between

an isolated and independently prepared material. A production partner might need to understand whether a new supply route preserves the relevant composition and function.

VARUNÉ Bio could contribute the agreed comparison design, linked sample and analytical records, uncertainty analysis, and decision report. A potential paid feasibility contract would specify the preparation, endpoint, comparator, deliverables, and what decision the partner expects to make. Potential customers or collaborators remain categories to investigate, not confirmed interested organisations. No funding availability, contract, laboratory capability, material access, or ownership is established by this report.

The company value would depend on rights to reusable methods, permitted data, process improvements, or a defined development role. Providing an assessment does not confer ownership of the original molecule or organism. The research plan should also distinguish a service output from proprietary product IP. Those are different commercial positions and should be negotiated explicitly if a real programme wishes to proceed.

9 Limits and publication boundaries

The evidence is intentionally heterogeneous. It shows that several attribution failures are possible; it does not estimate their prevalence in polar biotechnology. The small-molecule examples are methodological counterexamples, not direct proof of behaviour in a polysaccharide preparation. The polar EPS papers do not establish a VARUNÉ ingredient's efficacy, human benefit, manufacturing readiness, or finished-product safety.

Some decisive online sources were available only as abstracts or indexed selected passages. Full methods, supporting information, and raw data should be reviewed before making paper-specific quantitative comparisons or using them to justify a confirmatory protocol. The algebraic examples are illustrative and contain no fitted experimental parameters. The proposed decision record has not been prospectively validated against alternative research workflows.

This report advances a critical synthesis rather than claiming a new chemical principle. Its value is the explicit connection between recovery, material identity, exposure, and observation, and the resulting choice of experiments that could change a development decision. No third-party source figures or substantial text passages are reproduced.

10 Conclusion

Purification is informative when the change in activity can be connected to the change in the preparation. Enrichment alone does not identify the active, loss alone does not prove instability, and recombination alone does not prove synergy. A credible programme keeps target recovery, functional response, delivered exposure, and measurement validity visible together. For VARUNÉ Bio, the next valuable step would be a funded comparison on one accessible preparation that resolves an actual attribution decision. The scientific contribution would be an explanation that survives challenge, including the possibility that the original interpretation must change.

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Authorship and research provenance

Varun Sharma is the sole author and approved this edition for release on 28 October 2026. No new experimental data are reported; published findings remain attributable to the cited investigators.

Competing interests. Varun Sharma founded VARUNÉ Bio, which may benefit commercially from applications discussed here. Funding and contributor declarations remain subject to author confirmation.

This report shares concepts and sources with the five companion reports dated 28 October 2026; it presents a distinct literature analysis.