



Chemoproteomic Strategies for Natural Product Target Identification Between 2017 and 2025 and Their Contribution to Mechanistic Drug Discovery

Mark Anderson^{1*}, Lisa Wong², Sarah Lee¹

¹Department of Chemoproteomics and Target Identification, College of Pharmacy, University of Florida, Gainesville, United States.

²Department of Natural Product Mechanism Discovery, Faculty of Pharmacy, National University of Singapore, Singapore.

ABSTRACT

Natural products frequently enter drug discovery through phenotypic activity, yet translating an active molecule into a defensible molecular mechanism requires more than identifying proteins that bind, enrich, label, or change stability. Chemoproteomics has expanded the experimental routes available for natural-product target identification, but different platforms interrogate different forms of molecular engagement and impose distinct interpretive limitations. This systematic review examined peer-reviewed literature published from 2017 through 2025 concerning natural-product target identification by affinity capture, photoaffinity labeling, covalent and activity-based protein profiling, thermal and other probe-free proteome-wide approaches, and integrative validation. Evidence was organized according to target nomination, competition or specificity testing, site-level information, orthogonal target-engagement validation, functional dependence, and mechanistic interpretation. Methodological quality was assessed using a proposed qualitative appraisal appropriate to heterogeneous chemoproteomic designs rather than a numerical certainty score. The literature shows a transition from enrichment-centered target fishing toward increasingly proteome-wide, site-aware, probe-free, and perturbation-enabled strategies. Across platforms, target nomination was substantially easier to establish than functional mechanism. Probe derivatization, electrophile selectivity, indirect thermal effects, target-class rather than single-target engagement, and natural-product polypharmacology repeatedly complicated interpretation. Mechanistically stronger studies combined chemoproteomic discovery with independent biochemical, structural, genetic, pharmacological, or phenotypic evidence. Chemoproteomics contributes most strongly to mechanistic drug discovery when treated as an evidence-generating system rather than a target-listing technology. A proposed evidence-convergence interpretation is developed while preserving uncertainty, method-specific observability, and the distinction between engagement and causal mechanism.

Key Words: Natural products, Chemoproteomics, Target identification, Activity-based protein profiling, Target engagement, Mechanism of action

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INTRODUCTION

Natural products remain a major source of pharmacologically useful chemical matter and have contributed directly or indirectly to numerous therapeutic agents [1]. Their value, however, creates a recurrent mechanistic problem: a compound can produce a robust

cellular or organismal phenotype while the molecular events responsible for that phenotype remain uncertain. Phenotypic drug discovery is especially compatible with this sequence because active chemistry may be recognized before a molecular target is known, making downstream

Corresponding author: Mark Anderson

Address: Department of Chemoproteomics and Target Identification, College of Pharmacy, University of Florida, Gainesville, United States.

E-mail: ✉ mark.anderson@ufl.edu

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target deconvolution an important but technically difficult stage of mechanism development [2].

Recent natural-product target-identification research therefore extends beyond asking which protein can be captured by a compound. It includes labeled and label-free strategies, proteome-wide interaction measurements, target-site mapping, and combinations of chemical and biological validation intended to distinguish candidate interactors from mechanistically relevant targets [3]. These approaches do not produce interchangeable forms of evidence. Probe-based experiments can enrich or covalently record interactions but may modify the molecule being studied, whereas label-free strategies preserve the parent compound while often inferring engagement indirectly from changes in protein stability or proteolytic behavior [4].

The central problem addressed in this review is consequently evidential rather than merely technical. Chemoproteomic detection can nominate proteins, rank candidate interactions, reveal reactive sites, or demonstrate context-dependent engagement, but none of these observations should automatically be equated with functional mechanism. The review therefore examines how chemoproteomic strategies used between 2017 and 2025 contribute to progressively stronger target attribution, where their measurements remain ambiguous, and how target-deconvolution evidence can become mechanistically useful for contemporary drug discovery.

Review questions and analytical scope

The review asks four linked questions. First, which chemoproteomic strategies have been used to identify

molecular targets of natural products, and what molecular event does each strategy actually measure? Second, how are candidate targets distinguished from nonspecific enrichment, probe-dependent capture, indirect stability effects, or chemically favored reactive proteins? Third, what forms of orthogonal evidence move an observation from target nomination toward credible engagement and functional mechanism? Fourth, how has such target information contributed to subsequent drug-discovery reasoning rather than merely expanding lists of interacting proteins?

The analytical scope therefore separates measurement mode from mechanistic confidence. Labeled affinity or photoaffinity approaches, covalent and activity-based profiling, thermal or proteolysis-based methods, and degradation-enabled strategies are treated as experimentally distinct routes into target space rather than as competing versions of one assay [3]. Label-free and probe-dependent approaches are likewise compared according to the biological information they preserve or perturb, not according to a presumed universal hierarchy [4].

For synthesis, the review distinguishes target nomination, specificity or competition evidence, direct engagement, site-level information, functional dependence, and downstream phenotype. This staged interpretation is proposed here as an organizing framework; it is not a validated scoring system and does not assume that every credible target requires every validation modality.

The search and eligibility logic derived from these questions is summarized in **Table 1**.

Table 1. Search and eligibility architecture for evidence on natural-product chemoproteomic target identification

Review component	Operational focus	Evidence eligible for synthesis	Principal exclusion boundary
Chemical scope	Natural products or directly derived target-identification constructs	Parent natural products, natural-product probes, or derivatized constructs used to interrogate target biology	Generic small-molecule studies without a direct natural-product role
Target-identification scope	Molecular target nomination or engagement	Affinity capture, photoaffinity labeling, ABPP, covalent profiling, CETSA/TPP, proteolysis-based profiling, or related chemoproteomic strategies [3]	Bioactivity studies without target-identification evidence
Interpretive scope	Evidence beyond protein detection	Competition, direct binding, site mapping, perturbation, structural evidence, pathway response, or phenotype linkage	Enrichment interpreted as mechanism without additional support
Measurement distinction	Probe-dependent versus parent-compound measurements	Labeled and label-free strategies retained as analytically distinct evidence classes [4]	Assumption that different platforms measure equivalent biological events
Publication eligibility	Contemporary peer-reviewed evidence	Journal articles published from 2017 through 2025 with verifiable DOI and eligible journal status	Preprints, conference-only reports, non-journal material, duplicate versions
Review reporting	Transparent systematic-review documentation	Search, eligibility, selection, extraction, appraisal, and synthesis procedures	Unverifiable or reconstructed quantitative selection claims

Note: The table describes the review's operational search and eligibility logic. The distinctions between nomination, engagement, and mechanism are proposed analytical categories rather than validated evidence grades.

The evidence-selection audit is shown in **Figure 1**. Because the preserved search record does not contain auditable database-level raw yields and duplicate-removal

counts, the figure deliberately separates reconstructable review information from counts that cannot be reported without estimation

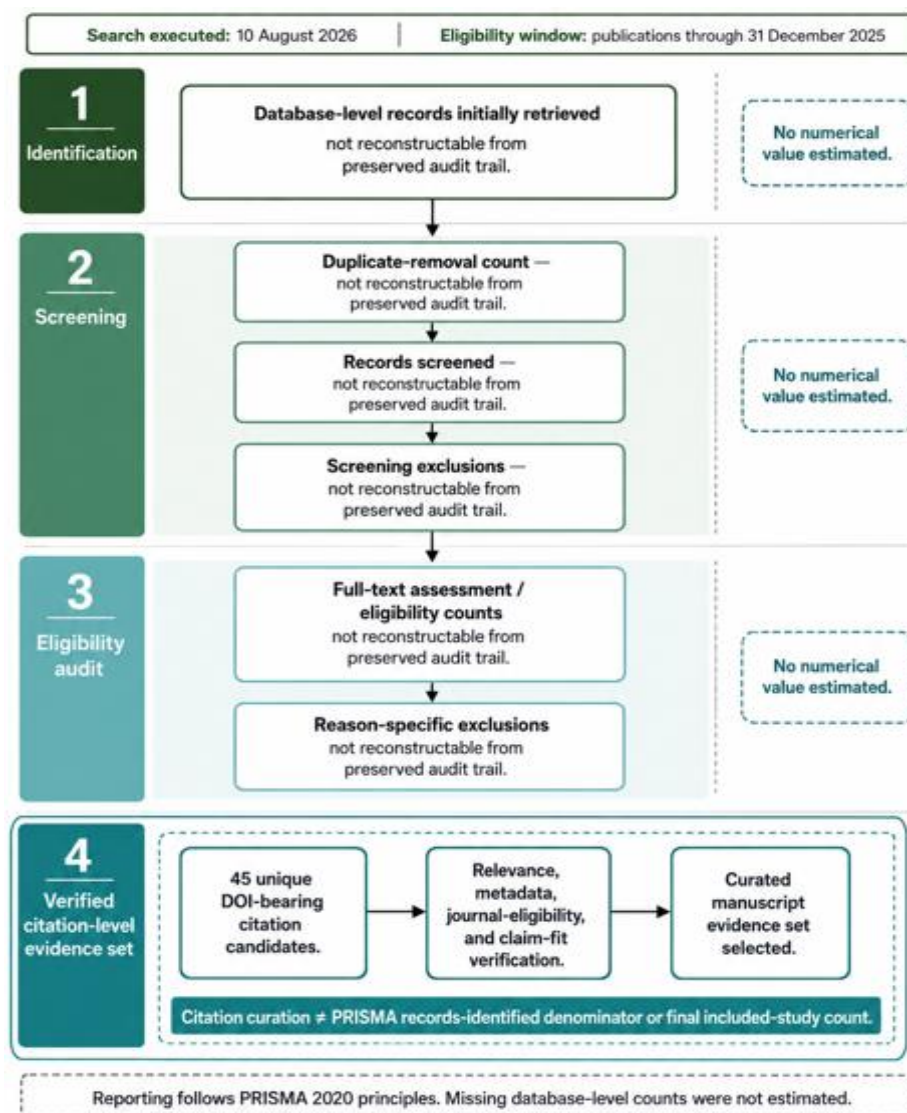


Figure 1. Auditable PRISMA 2020 evidence-selection status for the natural-product chemoproteomics review

MATERIALS AND METHODS

protocol and prisma 2020 reporting framework

The review was designed as a systematic review of chemoproteomic strategies used for natural-product target identification and mechanistic interpretation. Reporting was organized according to PRISMA 2020 principles [5]. No prospective registration record was available for the review; accordingly, no registration identifier is reported. The eligible publication interval was 1 January 2017 through 31 December 2025. Literature searching and metadata verification were conducted on 10 August 2026. The distinction between the publication cutoff and actual search date was retained to avoid retrospectively assigning a search date that did not occur. Search, eligibility,

extraction, appraisal, and synthesis decisions were prospectively separated within the review workflow so that a reference used for methodological context was not automatically treated as a primary chemoproteomic study. The review did not plan quantitative meta-analysis because the relevant studies interrogated heterogeneous compounds, proteins, biological systems, capture chemistries, competition designs, mass-spectrometric workflows, and validation endpoints. Instead, the protocol specified structured narrative synthesis around what each method measured and how subsequent evidence strengthened or weakened target attribution. Target enrichment, thermal stabilization or destabilization, covalent labeling, site identification, direct binding, functional perturbation, and phenotype concordance were

therefore retained as distinct evidential observations rather than compressed into a common numerical score.

Where the preserved review audit did not support an exact selection count, the value was not reconstructed by inference. This particularly affected the upstream PRISMA record-identification denominator and prevents numerical interpretation of **Figure 1** as a conventional complete database-flow count.

Eligibility criteria

Eligible articles were peer-reviewed journal publications from 2017–2025 with a verifiable DOI and direct relevance to natural-product target identification, chemoproteomic methodology, target engagement, site mapping, mechanistic validation, or an indispensable review-method decision. Primary synthesis emphasized studies in which a natural product or directly derived probe was used to interrogate protein targets or target-associated biology.

Sources were excluded when they fell outside the publication window, lacked eligible journal status or DOI verification, duplicated another publication version, or addressed an adjacent chemical-biology problem without a direct role in the review question. Studies were not considered mechanistically definitive merely because a protein was enriched or detected. Review articles and methodological papers could support definitions or analytical boundaries but were distinguished from primary target-identification evidence.

Information sources and search strategy

Searches used PubMed-indexed literature and publisher records for article discovery and bibliographic verification, supplemented by journal-quartile screening in SCImago-indexed profiles. Search concepts combined terms for natural products with *chemoproteomics*, *chemical proteomics*, *target identification*, *target deconvolution*, *affinity capture*, *photoaffinity*, *activity-based protein profiling*, *covalent profiling*, *CETSA*, *thermal proteome profiling*, *limited proteolysis*, *target engagement*, *site mapping*, *mechanism*, *PROTAC*, and *protein degradation*. Search sensitivity was increased by combining method terms with validation concepts including competition, direct binding, mutagenesis, structural analysis, genetic perturbation, pathway response, and phenotype. Exact-title and DOI searches were then used to resolve metadata for candidate publications. Because the search environment did not preserve exportable database-level result histories, source-specific raw yields are not presented as reconstructed counts.

Study selection

Selection proceeded conceptually from topical discovery to DOI-level deduplication, relevance assessment, and

claim-specific eligibility review. Candidate records were examined for correspondence with at least one predefined analytical domain: chemoproteomic strategy, target nomination, specificity or competition, target-site information, orthogonal engagement validation, functional mechanism, methodological limitation, or drug-discovery consequence.

Bibliographic selection and systematic-study eligibility were treated separately. A publication could be suitable methodological context without representing a primary included chemoproteomic experiment, while a scientifically relevant primary study could not legitimately be declared ineligible solely to satisfy a bibliography-size constraint. Consequently, citation curation was not substituted for a PRISMA included-study denominator. When the preserved audit record was insufficient to reconstruct a complete screening or full-text denominator, that limitation was retained rather than converted into an estimated exclusion count.

Data extraction and coding

For each selected source, extraction captured publication type, natural-product or probe identity, biological system, chemoproteomic modality, target or target class, competition or specificity design, direct-binding evidence, site-level evidence, structural or biochemical validation, genetic or pharmacological perturbation, phenotypic consequence, reported limitations, and transferability boundaries. Quantitative values were recorded only when explicitly reported and necessary for interpretation; missing characteristics were not inferred.

Primary studies were additionally coded according to the biological level reached by their evidence: target nomination, target engagement, mapped interaction site, functional dependence, pathway consequence, or organismal phenotype. The coding deliberately permitted several levels to coexist within one study. It also preserved indirect mechanisms in which an engaged protein altered a downstream functional effector rather than assuming that the phenotypic endpoint must itself be the directly bound target.

Quality and risk-of-bias appraisal

The heterogeneous chemoproteomic designs did not support use of a single conventional intervention-focused risk-of-bias instrument. A qualitative appraisal was therefore proposed for this review rather than presented as a validated chemoproteomic risk-of-bias scale. Appraisal domains considered specificity controls; parent-compound competition or dose response; independent direct-binding evidence; site identification where mechanistically relevant; functional perturbation or rescue; structural or orthogonal convergence; phenotype and biological-context

concordance; probe fidelity; replication and statistical transparency; and data availability.

These domains were used to qualify interpretation rather than generate a numerical certainty score. Absence of one modality was not treated automatically as high risk because feasible validation depends on target class, compound chemistry, assay context, and experimental question. The principal appraisal question was instead whether the strength of the manuscript's mechanistic language remained proportionate to the evidence actually generated by each study.

Synthesis approach

Because the evidence used heterogeneous compounds, proteomic readouts, target classes, and validation designs, synthesis was qualitative rather than meta-analytic. Affinity enrichment, photocrosslinking, residue-directed competition, thermal stability, limited proteolysis, and induced degradation were treated as different measurement operations rather than interchangeable target tests. PRISMA 2020 principles guided reporting [5].

The synthesis followed an evidence path from target nomination to specificity, engagement, site information, functional dependence, and phenotype. These are proposed analytical distinctions, not validated grades. A study could be strong at one level while leaving another unresolved; direct engagement, for example, can remain mechanistically incomplete when target perturbation does not explain phenotype. Conversely, a weak enrichment signal may become informative if competition, independent binding, structural localization, or functional perturbation converges on the same target. The synthesis therefore prioritized evidential concordance and claim calibration over target-list size or nominal platform novelty.

PRISMA evidence-selection results

The preserved audit confirms a publication window ending 31 December 2025 and a search executed on 10 August 2026. Database-level retrieval totals, duplicate-removal counts, screening exclusions, full-text denominators, and reason-specific exclusions were not reconstructable and therefore were not estimated, consistent with PRISMA 2020 reporting principles [5]. **Figure 1** consequently represents the auditable selection state rather than a fabricated numerical flow. Bibliography size is not interpreted as the number of systematically included primary studies, and no prevalence statement is derived from citation counts.

Characteristics of included chemoproteomic studies

The primary evidence spans non-covalent ligands, electrophilic metabolites, antibiotics, terpenoids, alkaloids,

and natural-product-derived degraders across purified proteins, cellular proteomes, microbial systems, disease models, and whole-animal phenotypes. This heterogeneity matters because target observability depends jointly on compound chemistry, probe design, protein state, proteomic workflow, and biological context [3]. Labeled and label-free methods therefore contribute complementary rather than interchangeable evidence [4]. Validation depth also varied substantially. Some studies primarily nominated candidate targets, whereas others combined proteomics with competition, direct-binding assays, mutation, structure, enzyme activity, genetic or pharmacological perturbation, pathway measurements, or in-vivo phenotype. The review therefore does not interpret a larger number of identified proteins as stronger mechanistic evidence. Instead, study characteristics are read in relation to what was measured, how alternative explanations were controlled, and whether the proposed target remained explanatory beyond the discovery assay.

Evolution of natural product chemoproteomics, 2017–2025

Chemoproteomics expanded from enrichment-centered target fishing toward proteome-wide engagement, ligandability, site mapping, and integrative deconvolution [6]. Probe synthesis and affinity capture remained foundational [7], while ABPP extended analysis toward reactive or functional protein states [8]. CETSA and thermal proteome profiling enabled parent compounds to be studied without covalent tagging by detecting compound-associated thermal changes [9].

The period is therefore better interpreted as diversification of observability than simple replacement of older methods. Affinity capture can provide physical enrichment, ABPP can localize reactivity or functional-state engagement, and thermal methods can preserve the parent structure. Each advantage is paired with a different interpretive vulnerability, so methodological evolution does not by itself imply progressively stronger causal evidence.

Affinity-based target capture

Affinity capture is useful when derivatization or immobilization preserves the interaction of interest. Diazirine photo-immobilization showed that structurally diverse natural products can be profiled while reducing some conventional linker-placement constraints [10]. Nevertheless, immobilization alters molecular presentation and accessibility. Enrichment should therefore be treated primarily as target nomination until specificity controls, parent competition, or independent binding evidence strengthen attribution. For complex natural products, the relevant question is not merely whether a target can be

recovered, but whether the immobilized construct still reports the biology of the freely diffusible parent.

Photoaffinity and covalent probe strategies

A clickable betulinic-acid photoaffinity probe identified tropomyosin as a target [11]. Honokiol-derived probes similarly illustrate why probe activity and parent-compound competition matter [12]. Artemisinin photoaffinity chemoproteomics nominated microsomal prostaglandin E synthase-2 and linked its inhibition to altered PGE2 biosynthesis and cell growth in HCT116 cells [13].

These studies show the dual role of probe chemistry: it creates analytical access while potentially changing affinity, permeability, localization, or target spectrum. Photocrosslinking can stabilize transient interactions, whereas covalent chemistry can provide residue-level information, but both can overrepresent interactions favored by probe placement or local chemical reactivity. Mechanistic interpretation therefore depends on probe-fidelity testing and an independent line of evidence linking the nominated protein to parent-compound action.

Activity-based protein profiling

Cysteine-focused competitive chemoproteomics linked myrocin G engagement with inhibition of nonhomologous end joining [14]. Holomycin profiling supported disruption of metal homeostasis and metalloprotein function rather than a simple single-target model [15]. Salinilactone ABPP identified protein disulfide-isomerases as a target class [16], whereas withaferin A profiling revealed both covalent and non-covalent interactions [17].

ABPP therefore provides chemistry-defined visibility rather than an unbiased census of all possible targets. It is particularly informative when a natural product's reactivity is mechanistically relevant and competition can distinguish ligand-sensitive residues from broadly reactive background sites. Its limitation is equally important: absence from a residue-focused profile cannot be interpreted as absence of interaction, and a highly reactive site is not automatically the dominant functional mediator.

Probe-free and competitive proteome-wide approaches

CETSA-MS profiled quercetin-associated thermal-stability changes without derivatization [18]. Tutin target identification was connected to calcineurin activation and seizure phenotype in mice [19]. Thermostability-assisted limited proteolysis extended probe-free profiling toward target-site hypotheses [20]. For PF403, TPP nomination of NAMPT was followed by biophysical, enzymatic, structural, mutational, and phenotypic validation [21].

Probe-free approaches preserve parent chemistry, but thermal or proteolytic shifts can arise indirectly through complexes, conformational state changes, altered metabolites, or pathway perturbation. Their principal strength is therefore not freedom from interpretation, but freedom from one specific source of perturbation: probe derivatization. The inferential burden moves toward establishing whether the shifted protein is directly engaged and whether that engagement matters functionally.

Table 2 summarizes the study-level measurement differences discussed to this point, while **Figure 2** integrates how those observables relate to mechanistic interpretation

Table 2. Chemoproteomic study characteristics across probe-dependent and probe-free target-identification strategies

Example	Strategy	Primary readout	Main qualification
Photo-immobilized natural products [10]	Affinity capture	Enriched interactions	Controlled target profiling
Betulinic acid [11]	Photoaffinity labeling	Photocaptured target	Target-focused follow-up
Honokiol [12]	Chemical probe profiling	Probe-dependent interactors	Parent/probe comparison
Artemisinin [13]	Photoaffinity chemoproteomics	Target nomination	Pathway/phenotype linkage
Myrocin G [14]	Cysteine competition	Reactive-site engagement	DNA-repair consequence
Holomycin [15]	Chemoproteomics	Metalloprotein disruption	Network interpretation
Salinilactones [16]	ABPP	Reactive target class	PDI assignment
Withaferin A [17]	Mixed-mode profiling	Covalent + non-covalent targets	Interaction-mode plurality
Quercetin [18]	CETSA-MS	Thermal shifts	Probe-free prioritization
Tutin [19]	Target identification	Calcineurin-associated mechanism	In-vivo phenotype
PF403 [21]	TPP	Thermal nomination	Multi-modal validation

Note: The table summarizes observability and validation, not comparative method performance.

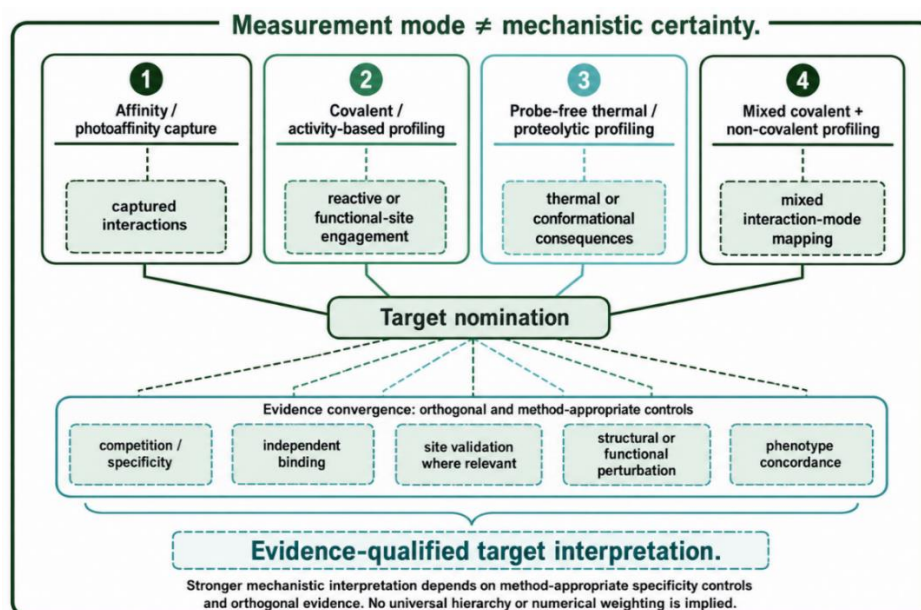


Figure 2. From chemoproteomic observability to evidence-qualified target interpretation

From protein enrichment to credible target assignment

Affinity-based protein profiling identified ACAT1 as a principal chlorogenic-acid target and strengthened the assignment through orthogonal interaction and mechanistic validation [22]. Rhodojaponin VI illustrates a different problem: N-ethylmaleimide-sensitive fusion protein mediated downstream Cav2.2 modulation, so the functional endpoint should not be mistaken for the directly engaged protein [23].

Across recent studies, assignment becomes more persuasive when nomination is followed by independent interaction and functional, structural, genetic, enzymatic, or in-vivo evidence [19, 21, 22]. “Credible target assignment” is therefore proposed here as an evidence-convergence judgment, not a fixed score. This interpretation also allows difficult cases in which several proteins remain plausible contributors rather than forcing a single-target conclusion unsupported by perturbational evidence.

Target engagement versus functional mechanism

Callyaerins target the *Mycobacterium tuberculosis* protein Rv2113 even though it is non-essential, showing that engagement does not automatically explain phenotype [24]. Ophiobolin A connected covalent targeting of mitochondrial complex IV with metabolic collapse in cancer cells [25]. Dihydrocaffeic-acid chemoproteomics linked transaldolase 1 engagement and site evidence to PERK–NF-κB signaling and anti-inflammatory effects [26]. These cases support separating molecular contact, target dependence, pathway consequence, and phenotype. Mechanistic language should stop at the deepest level

directly supported rather than treating engagement as a surrogate for downstream causality.

How probe design alters the biology being measured

Artemisinin-derived PROTACs used natural-product-derived recognition chemistry to induce degradation and identify PCLAF-associated biology [27]. Such constructs create new proximity and degradation behavior and therefore are not neutral reporters of native artemisinin pharmacology. More broadly, immobilization, derivatization, and chemistry-selective profiling can reshape the observable interactome, while electrophile-centered workflows may emphasize only part of a compound’s binding landscape [10, 12, 17].

Probe fidelity should therefore be tested rather than assumed. Retained phenotypic activity, parent competition, concentration dependence, localization, and orthogonal target engagement can each address different aspects of fidelity, but no single control guarantees that the probe recapitulates the parent compound across all cellular contexts.

Integration with genetics, structural biology, and phenotypic validation

Degradation-based celastrol profiling adds a functional perturbation layer by converting ligand recognition into induced protein loss [28]. Because degradation remains construct- and cellular-context-dependent, it complements rather than replaces genetics, direct binding, structure, mutagenesis, enzymology, or phenotype-rescue designs. PF403/NAMPT exemplifies convergence across several of these modalities [21].

Orthogonality is therefore best treated as a principle of confidence rather than a mandatory universal sequence. Structural evidence can localize an interaction, mutation can test residue dependence, and genetic or pharmacological perturbation can assess functional necessity; none alone answers every mechanistic question, but concordant results narrow plausible alternatives.

Recurring failure modes in natural product target deconvolution

Recurring errors include equating enrichment with binding, treating thermal shifts as necessarily direct, overlooking probe-induced changes, and confusing a downstream functional effector with the bound target. Polypharmacology further challenges single-protein narratives, as illustrated by holomycin [15]. Probe immobilization or derivatization can reshape the observable interactome, while electrophile-centered workflows may emphasize only part of the binding landscape [10, 12, 17].

Additional vulnerabilities include selective reporting of top-ranked targets, insufficient parent-compound competition, and mechanistic claims that extend beyond the biological system tested. These are method-specific risks requiring corresponding controls, not reasons to reject the platforms themselves.

Mechanistic contributions to contemporary drug discovery

Chemoproteomic identification of nimbolide engagement enabled exploitation of RNF114 as an E3-ligase recruiter for targeted degradation [29]. This illustrates how target deconvolution can reveal actionable protein chemistry beyond retrospective mechanism explanation. Natural-product-derived degradation strategies more broadly show how recognition can be converted into programmable protein loss [27-29].

The translational value of such findings is therefore mechanistic and enabling rather than automatically therapeutic. A newly exposed binding site, reactive residue, target class, or ligase relationship can create medicinal-chemistry opportunities, but usefulness remains conditional on construct behavior, target biology, selectivity, pharmacology, and validation in the intended biological context.

Evidence gaps and review limitations

Cross-study comparison remains constrained by differences in probe architecture, competition schemes, proteomic depth, statistical filtering, site assignment, validation modalities, and biological context [4]. A general quantitative hierarchy of chemoproteomic causality is not established [6]. The proposed evidence-convergence

framework should therefore be evaluated prospectively rather than treated as a validated scoring rule.

Reporting priorities follow directly from these gaps. Studies should distinguish discovery signal from validated engagement, describe whether the parent compound competes with the probe, report site evidence separately from protein-level nomination, and state what perturbational evidence links a target to phenotype. Negative or unresolved validation should also be visible, because failure of orthogonal confirmation is informative for judging target specificity.

RESULTS AND DISCUSSION

The reviewed literature is better understood as an expansion of experimentally accessible evidence than as a progression toward one superior platform. Probe-based methods provide capture and site-resolution opportunities; activity-based strategies interrogate reactive states; probe-free methods preserve parent chemistry but often measure indirect biophysical consequences; degradation-enabled constructs add perturbational information. Their outputs are therefore complementary but not interchangeable. This discipline is especially important for natural products because structural complexity, electrophilicity, stereochemistry, and context-dependent polypharmacology can make a technically reproducible interaction mechanistically incomplete.

The central mechanistic implication is that target identification is not a single event. Nomination can be followed by specificity testing, direct engagement, site definition, functional dependence, and phenotype concordance, but different targets and compounds may require different routes through that evidential space. The proposed synthesis does not demand maximal experimental complexity. It demands that the strength of the claim remain proportionate to independent evidence and that plausible alternatives—probe artifacts, indirect effects, or polypharmacology—remain visible.

Review limitations

The review does not estimate method sensitivity, specificity, or success rates because comparable denominators and standardized outcomes were unavailable. Journal-level eligibility also cannot substitute for study-level quality, and the selected literature spans markedly different biological and chemical contexts. Some conclusions therefore describe recurring interpretive patterns rather than universal properties of chemoproteomic platforms.

In addition, the preserved audit supports citation-level evidence curation but not a complete database-level PRISMA denominator, duplicate-removal history, or full-

text exclusion count. These values were deliberately not reconstructed. The review should consequently be interpreted as an evidence-bounded systematic synthesis with explicit selection-record limitations, not as a bibliometric estimate of the prevalence of particular target-identification strategies.

CONCLUSION

Natural-product chemoproteomics now provides diverse routes for observing molecular interactions, reactive sites, stability changes, and perturbational consequences. Its strongest contribution to mechanistic drug discovery lies not in producing longer target lists but in generating testable, progressively qualified target hypotheses. The evidence supports a disciplined distinction between target nomination, engagement, functional dependence, and phenotype. No single platform resolves all of these levels, and probe-free measurements are not intrinsically more mechanistic than probe-based ones. The proposed evidence-convergence framework therefore emphasizes complementary validation, explicit uncertainty, and claim calibration. Future progress depends on transparent probe-fidelity assessment, specificity controls, orthogonal engagement testing, and experiments that determine whether an engaged target actually explains the biological effect.

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