

Understanding the Translational Bottleneck: How Nuclear Magnetic Resonance Spectroscopy, Cryo-Electron Microscopy, and Artificial-Intelligence-Driven In Silico Modeling Transform Lethal Venoms into Medicinal Therapies

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ABSTRACT

While venom peptides hold immense potential for therapeutic drug development, characterization of these proteins has historically been constrained by limitations of traditional wet-lab bioassay guided fractionation. This “translational bottleneck” stems from a central question found in the study of venomics: how can researchers efficiently map the complex, highly flexible disulfide-dense frameworks of biotoxins to create medicinal therapies with extreme cellular receptor specificity? Historically, this bottleneck persisted because traditional structural methods, like X-ray crystallography, regularly failed due to low yields of venom and the physical resistance of small, flexible proteins to uniform crystallization. To address this challenge, this review synthesizes evidence from Nuclear Magnetic Resonance (NMR) spectroscopy, Cryo-Electron Microscopy (Cryo-EM) and Artificial Intelligence (AI)-driven *in silico* deep learning pipelines, to evaluate how these tools expedite traditional drug discovery timelines. Our analysis reveals that modern venomics operates as a highly coordinated funnel, efficiently utilizing both simulated and physical testing. Within this system, AI serves as an initial filter to screen millions of sequences while high-resolution empirical testing through NMR and Cryo-EM provides the necessary validation for targeted clinical pathways. The real-world scalability of this funnel is demonstrated through successful venom-derived therapies, including non-opioid pain analgesics (Ziconotide), targeted autoimmune immunomodulators (Dalazatide), and anti-platelet therapies (Eptifibatide and Tirofiban). Ultimately, these findings show how the multi-modal usage of the technologies optimizes and accelerates clinical translation, while highlighting where persistent challenges, such as the environmental blindness of static machine learning folds, require ongoing refinement.

INTRODUCTION

Over millions of years, predatory species, like the cone snail and viper, have evolved complex chemical weapons designed to hijack the physiological systems of their prey with nanometer precision (Terlau & Olivera, 2004). These substances, which are studied under the field of venomomics, represent a large reservoir of bioactive peptides that have been naturally refined for high stability and extreme target specificity. These therapies are unlike traditional synthetic drugs, which often result in systemic side effects because their targeted cellular receptors are replicated across multiple tissue systems. Venom-derived biotoxins possess a refined structure that allows them to distinguish between closely related ion channels and receptors with near accuracy (Vetter & Lewis, 2021; Lazarovici et al., 2019).

Despite this potential, venomomics is currently dealing with a problematic translational bottleneck in the structural characterization of venom peptides for therapeutic drug development. Traditionally, identifying therapeutic leads based on venoms relied on a procedure called bioassay-guided fractionation. This procedure involves taking the venom and applying it to human cells to elicit a physiological change. If a response does occur, researchers break down the venom to its core peptide sequences and re-test each sequence to pinpoint which exact peptide sequence is actively causing the reaction (Escobas & King, 2014). For perspective, the UniProt Knowledgebase contains over 229 million peptide sequences, yet this is only a tiny fraction of the number of toxins available for pharmacological development (UniProt Consortium, 2023).

This bottleneck exists because venom peptides are extremely difficult to characterize due to their high density of disulfide bonds, which cause extremely flexible structures that resist uniform alignment. For instance, X-ray crystallography requires the growth of high-quality, rigid crystallized structures, yet, many venom toxins are too small, too flexible, or too scarce to crystallize successfully. Consequently, characterizing venom proteins by understanding the 3D shape of the protein product and determining the function of the peptide is the primary hurdle in the venomomics pipeline. Without this structural clarity, designing new drugs, such as engineering the ShK toxin to ignore healthy tissue while targeting diseased T-cells, remains extremely difficult (Pennington et al., 2009). Fortunately, recent advances in technologies are fundamentally transforming the study of venomomics by providing insights and aiming to close the gap caused by the translational bottleneck.

This paper explores the modern technological advancements that are currently solving this bottleneck: Nuclear Magnetic Resonance (NMR) spectroscopy, Cryo-Electron Microscopy (Cryo-EM), and Artificial Intelligence (AI)-driven *in silico* modeling. By examining how these tools provide a bridge between natural venom-derived peptides and clinical application, this review will showcase how these tools have turned deadly toxins into medicinal therapies in three clinical areas: chronic pain, autoimmune disorders, and cardiovascular disease.

METHODOLOGY

This review was conducted using a systematic literature search aimed at identifying technological advances and venom-based therapies to combat the translational bottleneck. A total of 36 review articles, experimental studies, preprints, and other scholarly sources were chosen to be analyzed in this review. Primary research articles and review papers were sourced from databases including PubMed, Science Direct, and Google Scholar.

The selection criteria focused on three distinct pillars:

1. **Venomics Technologies:** Sources were selected based on their contribution to the understanding of NMR, Cryo-EM, and deep-learning models and how they would impact the translational bottleneck in venomics (e.g., Chari & Stark, 2023; Guan et al., 2024).
2. **Clinical Examples and Validation:** Only venoms with established clinical pathways were included to ensure the research remained grounded in medical necessity. The clinical pathways focused on were those targeting chronic pain (Conotoxins), autoimmune regulation (ShK), and cardiovascular disease (Disintegrins).
3. **Historical and Statistical Context:** Statistics on disease prevalence and the opioid crisis were pulled from the American Heart Association (Tsao et al., 2023) and the CDC (Rudd et al., 2016) to further demonstrate the importance of venom-based therapies and the need to address the translational bottleneck.

The search was conducted using keywords, such as “NMR spectroscopy venomics,” to isolate papers that specifically addressed the methods of characterization and clinical outcomes. To ensure the review remained current, a publication date filter was primarily set to the last 20 years, though landmark structural papers from the early 2000s or late 1990s were retained if they provided FDA-approved leads. Keywords were used to filter out sources to give the most accurate sources for each technology and medical condition discussed in the paper:

Category	Official MeSH Terms Used	Specific Keywords/Text Words	Databases Targeted
NMR Spectroscopy	Nuclear Magnetic Resonance Spectroscopy	“high-throughput NMR,” “peptide structure,” “venomics NMR spectroscopy,” “NMR spectroscopy + bottleneck venomics.”	PubMed, ScienceDirect, Google Scholar
Cryo-EM	Cryoelectron Microscopy	“cryo-electron microscopy,” “cryo-em	PubMed, ScienceDirect, Google

		venomics,” “large protein complexes in venomics,” “structural biology venomics,” “venomics bottleneck technology.”	Scholar
<i>in silico</i> modeling (AI)	Artificial Intelligence, Computer Simulation, Models, Molecular Computation	“Deep Learning architectures,” “in silico venom modeling,” “AI venomics,” “machine learning venomics,” “venomics technology AI,” “AI venomics limitations.”	PubMed, ScienceDirect, Google Scholar
Chronic Pain	Conotoxins, Analgesics, Spinal Cord, Nociceptors	“Opioid crisis,” “non-opioid analgesics,” “Conotoxins,” “cone snail pharmacology,” “Intrathecal drug delivery,” “nociceptive signaling pathways.”	Google Scholar, PubMed
Autoimmune Disorders	Multiple Sclerosis, T-Lymphocytes, Memory, Sea Anemones	Multiple sclerosis,” “effector memory T-cells,” “Stichodactyla helianthus,” “selective immunosuppression.”	PubMed, ScienceDirect
Cardiovascular Disease	Disintegrins, Thrombosis, Platelet Aggregation Inhibitors	“Disintegrins,” “integrin antagonism,” “thrombosis,” “platelet aggregation inhibitors.”	PubMed, Google Scholar, ScienceDirect

Table 1: Systematic Literature Review Strategy with Keywords and Databases

INTRODUCING THE TECHNOLOGIES

I. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy remains a foundational technology in structural venomics due to its unique ability to characterize proteins in a liquid environment in ways that mimic cell environments (Reumont et al., 2022). Unlike other structural methods, NMR observes the behavior of atomic nuclei (typically ^1H , ^{13}C , and ^{15}N) under the influence of a high-frequency magnetic field, allowing researchers to capture the dynamic flexibility of venom peptides, which is not usually captured in crystallography. NMR's ability to reduce the venomics bottleneck lies in its primary strength to characterize small, flexible toxins in extremely small quantities. This low material requirement makes the technology highly useful for venomics, where obtaining enough biomass via the physical milking of rare predatory species is not only extremely difficult, but incredibly dangerous to the creature. In an experiment aimed to identify spider venom and turn them into medicinal therapies, 2D and ^{31}P -NMR were essential technologies for visualizing how spider-derived peptides physically anchor into and deteriorate bacterial membranes (Mironov et al., 2025). The ability to see the mechanism in action, especially in a fluid environment that mimics real cells, is invaluable to any other technologies currently being used.

However, just as any other technology, NMR does have its drawbacks. Firstly, NMR has significant sensitivity limits regarding size and affinity. Dalvit et al. (2023) highlight a titration “blind spot” where NMR fails to accurately measure dissociation constants (K_D) for strong ligands (those with $K_D < 1\mu\text{M}$). Because high-affinity binding often results in a rectangular titration curve, it is mathematically impossible to distinguish between a potent binder and a moderate one without complex displacement assays. Furthermore, NMR struggles to characterize proteins exceeding 50-100 kDa. Larger proteins tumble more slowly in solution, leading to signal broadening and overlap that typically limits the technology to smaller proteins (Dalvit et al., 2023; Lucas, 2023). The signal broadening is a direct consequence of the physical behavior of proteins in a magnetic field, specifically the rotational correlation time (Levitt, 2008). As a protein's molecular weight increases, its ability to tumble rapidly in aqueous solutions decreases. Due to this, the slower rotating molecules cause nuclear spins to dephase more quickly, causing sharp peaks to turn into broad humps on the spectrum. For venomics, this creates a significant hurdle for studying protein complexes with multiple domains. NMR becomes increasingly limited in its mapping abilities as the target protein increases in size. This makes it impossible to resolve the precise atomic interactions without the help of other imaging methods. Lastly, while NMR does minimize the consumption of biological venom samples, it requires multi-million dollar high-field spectrometers, continuous liquid helium refrigeration, and highly specialized computation physics staff, restricting its utility to well-funded research institutions (Dalvit et al., 2023; Lucas, 2023). As a result, while NMR is the gold standard for measuring the kinetics of small peptides, it serves more as a specialized tool, rather than a universal solution, necessitating other technologies and models to be used for larger clinical targets.

II. Cryo-Electron Microscopy (Cryo-EM)

Cryo-EM has emerged as a promising tool for resolving high-molecular-weight molecules relevant to venomics, such as transmembrane ion channels and multi-domain enzyme complexes (Zhang et al., 2025). The process of using this technology involves several steps: first, a biological sample must undergo vitrification, or flash-freezing it in a thin layer of non-crystalline ice. This is followed by high-energy electron bombardment to create thousands of two-dimensional projections that are computationally reconstructed into a 3D map (Chari & Stark, 2023). Cryo-EM's primary strength is its ability to achieve near-atomic resolution for incredibly large macromolecules that undergo significant conformational changes and are otherwise impossible to stabilize for traditional high-resolution imaging.

While traditional X-ray crystallography requires the growth of perfect, rigid crystal lattices, Cryo-EM can achieve resolutions often $<4 \text{ \AA}$ from proteins in a near-native, membrane-like state (Lucas, 2023). This is highly relevant to venomics as the vast majority of therapeutic venom targets are complex, membrane-bound receptors that often denature or lose their functionality almost immediately when removed from their surrounding lipid environment for crystallization. Cryo-EM completely bypasses this problem by allowing channels to be imaged inside their protective lipid environments. This was recently demonstrated in a study, where Cryo-EM achieved a $3.3 \text{ \AA}$ resolution structure of the human prothrombinase complex, mapping a massive protein-protein interface of $3,142 \text{ \AA}^2$ that was previously too flexible for high-resolution imaging (Üstok et al., 2025).

Despite these breakthroughs, Cryo-EM faces significant technical and physical challenges. The technology typically exhibits a size limitation, struggling to properly map out small venom peptides (usually around $<50 \text{ kDa}$) because they lack the mass necessary for accurate image alignment (Chari & Stark, 2023; Lucas, 2023). Furthermore, to reach atomic resolution, researchers must collect and process between 100,000 and 1,000,000 individual particles, demonstrating the massive amount of computational power and time required (Chari & Stark, 2023). Additionally, the air-water interface during sample preparation can cause delicate toxins to denature or gain biased orientations (Lucas, 2023). The issue of biased orientations is created by the vitrification process itself. While vitrification seeks to capture multiple snapshots of a protein's 3D structure in non-crystalline ice, the flexibility of the venom peptides allows them to exist as multiple shapes rather than a simple, rigid form. For example, if the flash-freezing process captures 100,000 particles in multiple shapes, the computational reconstruction must sort through lots of images to achieve the atomic resolution, like the one cited by Chari and Stark (2023). Overall, while Cryo-EM is an effective tool for validating large-scale therapeutic targets, it remains a high-cost, high-effort method that lacks the throughput required for initial discovery.

III. *in silico* Modeling using AI

The most recent development to target the venomics bottleneck pipeline is the move from physical testing to *in silico* discovery through Deep Learning. *in silico* modeling refers to the advanced computational architectures that use simulations and machine-learning algorithms to model structures in a visual space before conducting a traditional wet-lab experiment. These modern computational methods have

effectively allowed researchers to screen millions of sequences in days rather than years. The power of this throughput was evidenced in a recent study, where a Venomics AI pipeline was used to screen 16,123 venom proteins, generating an impressive 40,626,260 venom-encrypted peptides (Guan et al., 2024). The efficiency and scale of observation allows for the discovery of venom-based therapeutic leads that traditional proteomics might miss. Unlike historical homology modeling, which required a known protein template to make a prediction (Gupta, 2014), modern deep-learning architectures can predict the folds of entirely unique toxins with high confidence, effectively bridging the “structural gap” between genetic sequencing and physical reality.

However, the central question remains: can pattern recognition replace physical testing? The consensus among current research is that AI serves as a filter rather than final proof. A significant drawback was found in the research using the Venomics AI pipeline. Of the 40 million peptides screened by the deep learning model, only 386 high-potential candidates were identified, and experimental validation was still required to confirm antimicrobial efficacy (Guan et al., 2024). This low hit rate necessitates massive secondary screening, demonstrating why AI might not be the best at fully characterizing venom peptides. Furthermore, AI models are often environmentally “blind”, as they fail to predict how a peptide’s shape might transition when moving from an aqueous environment to a bacterial cell wall (Guan et al., 2024; Lucas, 2023). This often lies in the complexity of disulfide frameworks (Craik et al., 2001). Venom peptides are frequently stabilized by dense cysteine “knots,” which are covalent bonds that lock the protein into its natural shape. Modern deep-learning models are efficient at predicting common protein folds, yet they struggle to calculate the exact formation of these knots in a biological setting. Unlike larger proteins that fold based on hydrophobic interactions, venom toxins rely on precise chemical links to determine their shape. If an *in silico* model fails at predicting the location of a single disulfide bridge, the entire lead is rendered non-functional in a clinical environment. Therefore, while AI is able to allow venom identification and discovery for the bottleneck, it still requires experimental verification through NMR (for small peptides) or Cryo-EM (for target-binding) to confirm the accuracy and mechanism of the identified peptides.

CLINICAL APPLICATIONS

By utilizing NMR, Cryo-EM, and AI/*in silico* modeling, researchers have been able to characterize and engineer venom peptides to address global health problems in chronic pain, autoimmune disorders, and cardiovascular disease.

I. Chronic Pain and the Opioid Crisis

There is a high necessity for non-opioid analgesics for clinical treatment of chronic pain. This demand has reached a critical peak due to the escalating opioid epidemic. In fact, in 2015 alone, the United States recorded over 33,000 opioid-involved overdose deaths, a crisis heavily driven by the addictive potential and side effects of traditional narcotics (Rudd et al., 2016). Venom-derived peptides offer a solution.

The primary success story in this field is Ziconotide (Prialt), a synthetic version of the ω -conotoxin MVIIA derived from the fish-hunting cone snail, *Conus magus* (McGivern, 2022). Unlike opioids, which target mu-opioid receptors throughout the central nervous system, Ziconotide acts as a selective inhibitor of N-type voltage-gated calcium channels ($Ca_v2.2$) (McGivern, 2022). By blocking these channels at the presynaptic terminals of nociceptive neurons in the spinal cord, the toxin effectively inhibits the release of pro-nociceptive neurotransmitters like glutamate, the primary transmitter responsible for sending fast pain signals to the brain, and Substance P, a specialized neuropeptide that amplifies pain signaling and enables faster and stronger neuron firings.

The structure of cone snail venom contains thousands of such inhibitory peptides, each evolved for high structural stability and target specificity (Terlau & Olivera, 2004). However, the issue for these drugs remains the way they are administered to patients. For instance, Ziconotide cannot cross the blood-brain barrier, and instead requires intrathecal administration via a pump due to its highly polar and hydrophilic peptide structure (McGivern, 2022; Bottros & Christo, 2014). While this inability to cross the blood-brain barrier is technically a benefit (prevents the drug from interacting with the upper brain, eliminating the risks of respiratory depression, euphoria, and substance addiction), the administration of the drug is a downside that requires invasive intrathecal delivery via a surgically implanted infusion pump directly into the spinal fluid (McGivern, 2022; Bottros & Christo, 2014). Current research is utilizing structural data to engineer ways to orally take drugs like Ziconotide, so that the stability of the natural toxin is maintained while overcoming the challenges associated with its delivery (Vetter & Lewis, 2021).

II. Autoimmune Disorders and Selective Immunomodulation

There is a high demand for targeted immunomodulators in the clinical treatments of neurodegenerative autoimmune diseases, like multiple sclerosis (MS). In MS, the immune system's effector T-cells mistakenly attack the myelin sheath of the neurons in the central nervous system (Dobson & Giovannoni, 2018). Traditional treatments often involve broad immunosuppression, which leaves patients vulnerable to infections, systemic toxicity, and secondary malignancies (Dobson & Giovannoni, 2018). Venomics has provided an alternative treatment method that avoids the negative effects of immunosuppression by targeting the Kv1.3 potassium channel, which is a unique cell receptor that is on the surface of activated T-cells causing the disease (Chandy et al., 2004).

The lead candidate for this therapy is the ShK toxin (ShK-168, or Dalazatide), derived from the sea anemone *Stichodactyla helianthus* (Pennington et al., 2009). Unlike traditional biologics, which cause broad systemic suppression, ShK-168 is incredibly target-sensitive and is highly inhibitory of the Kv1.3 voltage-gated potassium channel (Chandy et al., 2004). Since T-cells, specifically the encephalitogenic effector memory T-cells, upregulate expression of these Kv1.3 channels upon activation, blocking them effectively stops the intracellular calcium signaling cascades required for T-cell proliferation and localized neuro-inflammatory responses without affecting other vital immune cells (Chandy et al., 2004).

Early NMR studies identified the precise structure and ionization behavior of ShK, which provided a blueprint for how the toxin plugs the potassium channel pore (Tudor et al., 1998). A problem encountered with the toxin was that it lacked selectivity, also blocking Kv1.1 channels in the nervous system. This blockage triggered unintended effects, such as neurological tremors, seizures, and cardiac arrhythmias (Pennington et al., 2009; Chandy et al., 2004). Using structure-based engineering and *in silico* modeling, researchers developed Dalazatide. By attaching a p-phosphono-L-phenylalanine group to the N-terminus, they created a 100-fold increase in selectivity for the T-cell's Kv1.3 channel (Pennington et al., 2009). This structural modification allows the drug to suppress MS and, additionally, Type-1 Diabetes patients without causing neurological damage or toxicity.

III. Cardiovascular Health and Anti-Platelet Therapy

Cardiovascular disease remains the leading cause of death globally, accounting for approximately 19 million deaths per year (Tsao et al., 2023). A significant portion of these deaths results from thrombosis: the formation of blood clots that trigger heart attacks and strokes. Snake venoms, particularly those from vipers, contain disintegrins, which are evolved to induce systemic bleeding in prey by inhibiting platelet aggregation (Selistre-de-Araujo et al., 2010).

Disintegrins function by utilizing a specific structural loop containing an RGD (Arg-Gly-Asp) motif. This motif functions as a structural mimic that binds with high affinity to the $\alpha_{IIb}\beta_3$ integrin (glycoprotein GP IIb/IIIa) on the surface of human platelets (Lazarovici et al., 2019). By occupying these receptors, disintegrins prevent fibrinogen from cross-linking platelets, thereby reducing thrombus formation.

The successful transition from toxin to therapy is exemplified by two major FDA approved drugs, Integrilin (Eptifibatide) and Aggrastat (Tirofiban) (Lazarovici et al., 2019). Both drugs are administered intravenously during active myocardial infarction and percutaneous coronary interventions (angioplasty) to prevent the blood vessels from closing in on themselves (re-occlusion).

The engineering of these drugs relied on structural discovery. Researchers utilized NMR spectroscopy to map the exact spatial geometry of the native venom loop found in disintegrins from the Southern pygmy rattlesnake (*Sistrurus miliarius barbouri*), the Barbourin (a cyclic heptapeptide). This work revealed that modifying the venom's natural RGD sequence to a KGD sequence (Lys-Gly-Asp) drastically eliminated reactions with non-platelet integrins. This insight allowed chemists to compress the unstable snake protein into the highly stable Integrilin that mimics the targeting antagonistic behavior of the platelet glycoprotein IIb/IIIa receptor pocket. Early landmark clinical cases, such as those evaluated by Ohman et al. (1997), proved that combining this venom mimic with accelerated tissue plasminogen activator (t-PA) protocols safely enhanced early coronary artery patency, successfully restoring blood during active myocardial infarctions.

Similarly, Aggrastat was synthesized as a highly specialized, non-peptide molecule modeled after the 3D structure of Echistatin from the saw-scaled viper (*Echis carinatus*). Because Tirofiban functions as a highly specific and completely reversible competitive inhibitor, it alters emergency cardiac care.

Demonstrated in an evaluation by Bizzarri et al. (2001), patients receiving continuous Tirofiban infusions could safely undergo emergency open-heart coronary artery bypass grafting (CABG) directly after an acute coronary crisis. Because the molecule has an incredibly short metabolic half-life, it provides a protective effect, namely “platelet anesthesia,” during the CABG procedure. Clinical data further supports this by revealing that patients treated with Tirofiban actually preserved normal platelet counts postoperation ($199.5 \pm 70.4 \times 10^3/\mu\text{L}$) compared to untreated control patients ($150.6 \pm 33.4 \times 10^3/\mu\text{L}$; $P < 0.01$), and required significantly fewer blood and plasma transfusions. This proves that structural streamlining can successfully isolate a toxin’s therapeutic benefit from its lethal characteristics.

By utilizing NMR and Cryo-EM to visualize these protein-protein interfaces, modern researchers have moved beyond just mimicking the peptides to now designing synthetic disintegrins. Using *in silico* molecular pipelines, scientists are also able to computationally derive RGD sequences in venoms and mutate them into alternate sequences to intentionally change the drug’s target away from blood platelets and toward the $\alpha_v\beta_3$ integrins found on malignant tumor cells. Overall, these technologies have guided structural alterations to allow for the design of novel therapies to inhibit angiogenesis (a tumor forces the body to grow new blood vessels to feed it) and tumor metastasis (Selistre-de-Araujo et al., 2010).

SUMMARY OF THE RESULTS OF STRUCTURAL VENOMICS TECHNOLOGIES AND CLINICAL PIPELINES

Technology	Primary Structural Strength	Critical Limitation	Key Application Clinical for Scalability
NMR Spectroscopy	Captures venom peptides in liquid environments that mimic cells. Ideal for small peptides	It has a size limit of around 50-100 kDa due to signal broadening. Also has a titration limitation for high-affinity binders ($K_D < 1\mu\text{M}$).	The ShK Toxin from the sea anemone <i>Stichodactyla helianthus</i> . This toxin helps target T-cells in MS while sparing healthy tissue.
Cryo-EM	Achieves atomic resolution less than 4 Å. Ideal for large molecular complexes and membrane channels.	It has a mass constraint of around 30 to 50 kDa. Small toxins lack mass for particle alignment. Also has a high cost for computation calculations.	Human prothrombinase complex (reconstructed to map interfaces involved in blood coagulation and thrombosis)
<i>in silico</i> Modeling (AI)	Can screen millions of sequences and predict structural folds of	It struggles to accurately apply the predicted venom	The Venomics AI Pipeline reports high false-positive rates,

	venom peptides within days.	peptides to cell environments, such as calculating how complex disulfide cysteine knots react in response to certain biological settings.	thus needing secondary validation through other methods, like NMR or Cryo-EM.
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Table 2: Results Summary for Each Technology

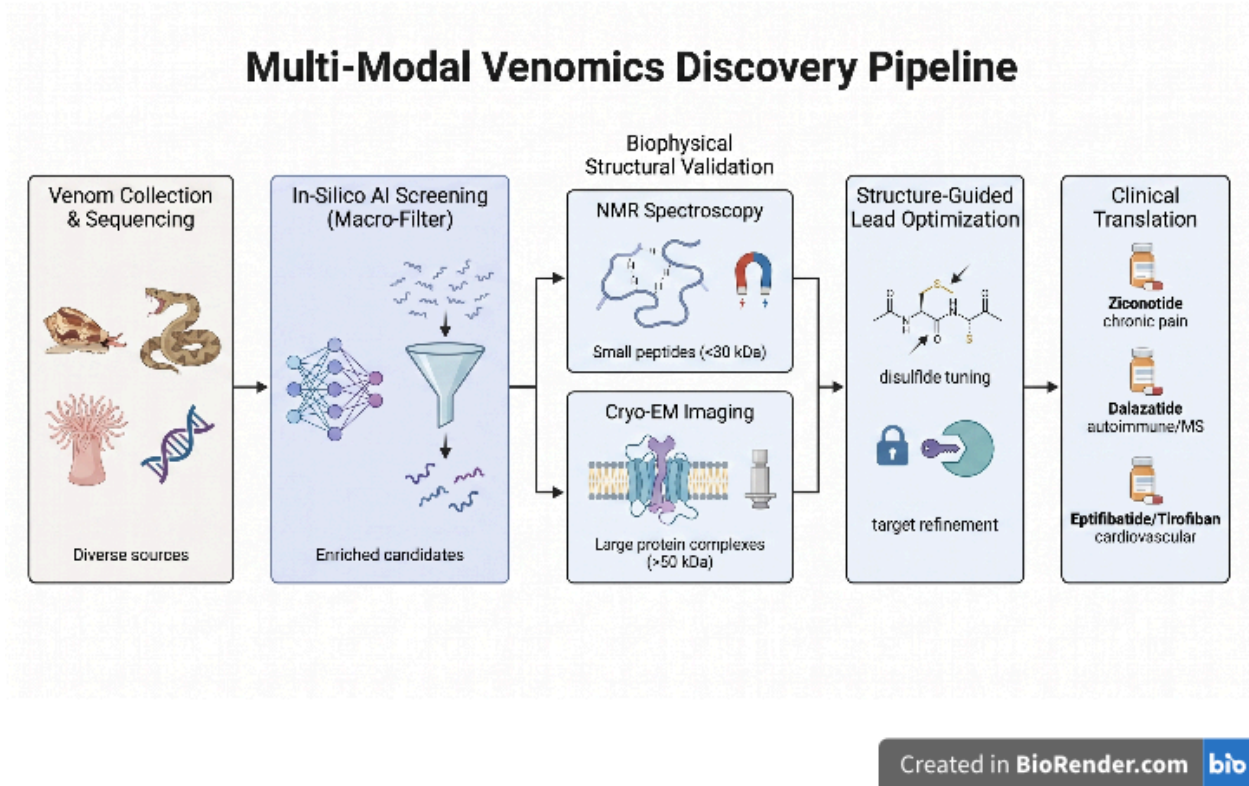


Figure 1: The Multi-Modal Venomics Discovery Pipeline. Conceptual schematic illustrating the integration of high-throughput technologies in venom drug discovery. Venom peptides are initially screened via *in silico* AI deep-learning pipelines as an initial macro-filter. High resolution empirical validation is provided by NMR spectroscopy (for small, flexible peptides) and Cryo-EM (for large membrane-bound protein-receptor complexes), followed by structure-guided optimization and clinical translation into targeted therapeutics.

DISCUSSION

Rather than functioning as isolated workflows, *in silico* modeling, NMR spectroscopy, and Cryo-EM form an interdependent drug discovery pipeline. Machine learning models act as an initial macro-filter, rapidly screening vast databases of venom peptides to predict bioactivity and binding affinity. High-resolution NMR spectroscopy then serves as a validator, determining structural fluctuations, ligand interaction, and disulfide connectivity for smaller peptide fragments less than 30 kDa in solution. For high-molecular-weight targets (e.g., membrane-bound G-protein coupled receptors, ion channels), Cryo-EM provides the 3D structural map of the peptide-receptor complex. This integrated workflow and technological progression are summarized in Figure 1, illustrating the path from venom collection to clinical application. Integrating these technologies creates a complementary workflow in which AI rapidly prioritizes promising venom peptides, NMR experimentally validates the structures of smaller toxins in solution, and Cryo-EM resolves larger peptide-receptor complexes that fall outside NMR's practical range. Together, these methods compensate for one another's limitations rather than functioning as competing approaches.

The findings of this review describe a significant shift in the venomics therapeutic development pipeline. For decades, drug discovery prioritized determining the exact structure of venom proteins before establishing their function. This is colloquially referred to as structure-function analysis. Today, this process has streamlined to be more efficient. As evidenced by the work of Guan et al. (2024), AI can now generate millions of peptides that have predicted functions and folding patterns in a matter of days. However, the analysis reveals that computational prediction does not replace the need for physical testing; rather, it changes its role. Instead of serving as the primary tool for initial discovery, physical biophysical testing can now function as a strict validator and macroscopic filter. AI operates as a computational funnel to compress millions of data points into high-probability leads, but empirical lab validation remains the final call required to translate the peptide into a viable, clinical therapy.

A critical challenge in venomics analysis is the size selectivity of these technologies. NMR remains the standard for smaller peptides, such as the ShK-186 peptide from sea anemones, since it can detect the subtle side-chain interactions that define target specificity and selectivity (Pennington et al., 2009). However, as venom-based targets become more complex, such as the massive prothrombinase complexes analyzed by Üstok et al. (2025), NMR reaches a size limit. This is where Cryo-EM transitions in to further analyze these complexes and characterize them. This multi-method approach is what allows lethal venoms to become usable, safe therapies for a variety of diseases.

While the integration of AI and high-resolution imaging has accelerated venom discovery, there are several foundational limitations that require further research to safely provide clinically viable therapies. The three primary limitations are the inability to replicate physiologic conditions with the current *in silico*

discovery models, the resolution and sensitivity limits of the NMR and Cryo-EM technologies, and the environmental impact posed by the extensive data mining of computational venomomics.

I. Environmental “Blindness” in Computational Models

The primary limitation of current *in silico* models, including the Venomics AI pipeline (Guan et al., 2024), is the lack of environmental context for the target molecules. Proteins do not exist in a vacuum. Their folding and final 3D structure are heavily influenced by their surroundings, such as pH, salinity, and the presence of lipid membranes. Many venom toxins are highly flexible, or disordered, in aqueous solutions and are only lethal upon physical contact with a target cell membrane (Mironov et al., 2025).

Because most AI models are trained on static crystal structures from the Protein Data Bank, they often fail to predict these conditional conformational shifts. This blind spot leads to higher rates of false positives, where a peptide predicted to be a qualified candidate in a computer simulation fails to fold correctly or function in a living biological system. This is why AI and other computational models require physical testing to confidently confirm the actual peptide function.

While static structural prediction platforms face documented limitations in modeling loop regions and complex cysteine knots, computational algorithms address this issue. Emerging hybrid frameworks that couple deep learning architectures with Molecular Dynamics (MD) simulations offer dynamic sampling over extended time scales. Furthermore, these machine learning models trained on specialized peptide datasets are increasingly capable of accounting for solvent dynamics, slowly bridging the gap between predictive modeling and empirical testing.

II. Resolution and Sensitivity Limits in NMR and Cryo-EM

In NMR spectroscopy, a significant limitation is a titration blind spot in which measurements of high affinity ligands ($K_D < 1\mu\text{M}$) provide limited information about the peptides’ actual nature in a biological system. As noted by Dalvit et al. (2023), for the most potent venom-derived drugs, the binding curve becomes rectangular, making it mathematically impossible to distinguish between a high-affinity candidate and a moderate one without secondary, time-consuming displacement assays.

In clinical pharmacology, distinguishing between these high affinity tiers is vital to human safety. A high-affinity drug binds tightly and has selectivity to its target, meaning that a patient requires only a small dose to achieve a therapeutic effect, drastically minimizing systemic side effects. On the other hand, a moderate-affinity drug binds loosely, demanding a much higher dose to remain effective. This increase in volume significantly increases the risk of off-target cross-reactivity, systemic toxicity, and liver or kidney stress (Kenakin, 2009).

Cryo-EM currently faces a size limitation with smaller venom peptides (typically less than 30-50 kDa). Small venom peptides lack the molecular mass necessary for the computational alignment of particles for the machine. As a result, this significantly impacts the translational bottleneck, where medium-sized

toxins that are too large for high-resolution NMR but too small for standard Cryo-EM are unable to be identified.

III. The Environmental and Resource Burden of Computational Venomics

Furthermore, while transitioning from traditional wet-lab assays to *in silico* deep learning solves the translational bottleneck of venom scarcity, it introduces another challenge: an immense computational resource load. Mining massive datasets, executing millions of molecular docking simulations, and training multi-layered neural networks to predict disulfide bonding patterns requires immense computational processing power.

The operation of massive server farms and high-performance Graphics Processing Units (GPUs) required to run pipelines like the Venomics AI network demands vast amounts of electricity and continuous air cooling. This shift transforms a portion of the venomics translational bottleneck from a material constraint into an environmental concern. The significant carbon footprint and environmental toll generated by these data-mining centers represent a constraint that reveals how the digital optimization of medicine is not resource-free and poses a question on what should be prioritized: the biological discoveries venoms have to offer versus the expenditure of vast resources and energy.

CONCLUSION

The transformation of nature's most toxic biological venoms into life-saving therapies is the pinnacle of modern structural biology. As this review has demonstrated, by integrating the use of NMR spectroscopy, Cryo-EM, and AI-driven *in silico* modeling, researchers are becoming increasingly effective at dismantling the bottleneck that has long hindered the clinical transition of venom peptides. The clinical successes of FDA-approved therapeutics, such as Ziconotide, Dalazatide, and Eptifibatide, serve as proof for the immense pharmacological potential locked within the venoms of predatory species. However, as analyzed in the discussion of current limitations, no single technology can completely solve the translational bottleneck. The environmental blindness of AI and deep learning models and the size and resolution constraints of imaging techniques necessitate a multi-method approach. No single technology can completely resolve the complexity of venom; rather, the integration of these techniques provides the most viable path forward.

High-throughput structural characterization and structural determination represent significant milestones in target validation. However, structural insight alone does not guarantee clinical translation. Transitioning a candidate venom peptide into a medicinal therapy requires overcoming pharmacological and manufacturing barriers due to the complex chemical synthesis required. Peptide therapeutics have drawbacks such as short biological half-lives and potential immunogenicity. Additionally, delivery of these venom-based therapies remains a primary obstacle. An example is Ziconotide, which requires

intrathecal administration due to the limited blood-brain barrier permeability. Future research must prioritize optimized delivery mechanisms, cost-effective and scalable manufacturing, and production strategies alongside structural target identification. Beyond these scientific advances, candidate therapeutics must also undergo extensive preclinical toxicology studies, phased clinical trials, and rigorous regulatory review before they can achieve widespread clinical use.

Looking toward the next decade, the clinical and public health implications of structural venomics remain substantial and continue to expand. In a world facing an escalating opioid crisis and a rising rate of cardiovascular and autoimmune diseases, the need for highly selective, non-addictive, and low-toxicity drugs has never been greater. The blueprints for these medicines already exist in the venom glands of the cone snail, the viper, the sea anemone, and many more. The transition from lethal toxin to clinical therapy is no longer a biological curiosity, but rather a cornerstone of future precision medicine.

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