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Systematic Review

Design Strategies for Heterologous Molecule Display Using *Macrobrachium rosenbergii* Nodavirus-like Particles: A Systematic Review

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Abstract

Virus-like particles (VLPs) are nanoscale platforms that can be engineered for heterologous molecule display. Among the available VLP platforms, *Macrobrachium rosenbergii* nodavirus-derived VLPs (MrNV VLPs) have emerged as a promising system for this purpose. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, 20 eligible studies were identified and qualitatively synthesized to summarize the current design strategies used for heterologous molecule display on MrNV VLPs, with a particular focus on how insert characteristics influence the selection of design strategies. The protruding (P) domain, located at the C-terminal region, serves as the main site for heterologous display. Different strategies, including genetic fusion, peptide insertion, and domain replacement, are applied depending on the size and structural complexity of the insert. Across the available studies, these approaches enabled the display of both small peptides and larger protein domains, with particle architecture maintained in studies that directly evaluated and reported VLP formation. In addition to design strategies, physicochemical properties, including structural stability, are important considerations that may influence platform performance. However, several studies did not directly report VLP assembly, and the available evidence remains heterogeneous and predominantly preclinical. Together, this review highlights the relationship between insert characteristics and design strategies in MrNV VLPs and provides insights for the rational design of VLP-based heterologous molecule display platforms for biomedical applications.

Keywords: virus-like particles; heterologous molecule display; *Macrobrachium rosenbergii* nodavirus; design strategies; biomedical applications

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1. Introduction

Virus-like particles (VLPs) have emerged as bio-macromolecule platforms for heterologous molecule display owing to their highly ordered capsid architecture and structural versatility [1]. The design of these VLP platforms requires balancing capsid structural integrity with the ability to accommodate inserts of varying size and complexity [2,3]. In many established VLP systems, this balance represents a fundamental constraint, as the incorporation of larger or conformationally complex molecules can disrupt capsid assembly or compromise functional presentation [4,5]. Therefore, understanding how capsid architecture influences the tolerance of heterologous inserts is essential for selecting appropriate design strategies and developing structurally stable VLP display platforms.

Macrobrachium rosenbergii nodavirus (MrNV), originally identified as the causative agent of white tail disease in the giant freshwater prawn *Macrobrachium rosenbergii*, has emerged as a promising VLP platform because its recombinant capsid protein readily self-assembles into virus-like particles that resemble the native virion while lacking the viral genome [6–8]. In addition to being non-infectious, MrNV VLPs retain native capsid structural organization while providing a versatile scaffold for heterologous molecule display, making them suitable for a wide range of biomedical applications. Structural studies have demonstrated that MrNV VLPs adopt a T = 3 icosahedral architecture consisting of an internal shell (S) domain that provides structural support and a protruding (P) domain connected through a flexible linker [9,10] (Figure 1, panels 1 and 2). In particular, the P-domain, located at the C-terminal region, is exposed on the particle surface and serves as the primary site for molecule display [10,11] (Figure 1, panel 3). Accordingly, MrNV VLPs have been successfully used to display a wide variety of heterologous molecules, including peptide epitopes, protein domains, and functional ligands, through direct genetic fusion, peptide insertion, and P-domain replacement [12–15].

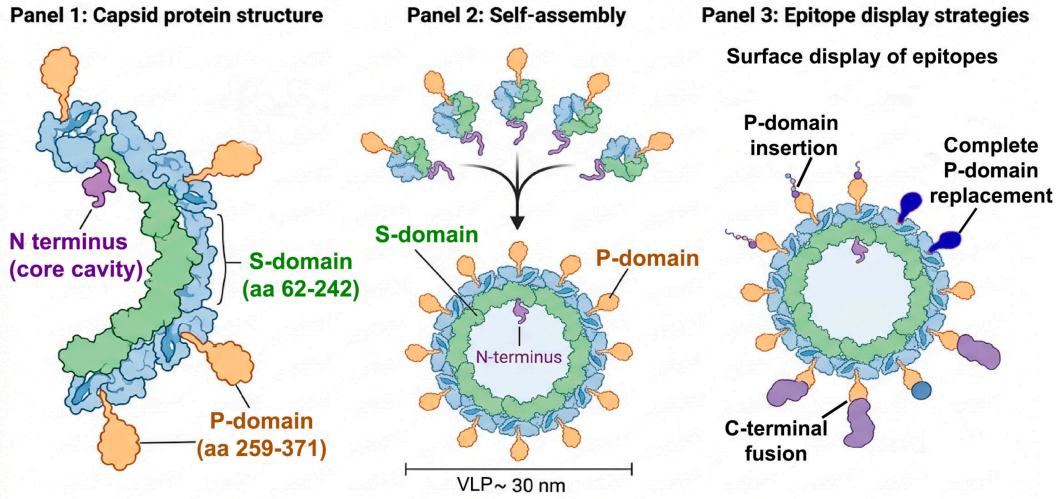


Figure 1. Schematic representation of MrNV VLP assembly and design strategies for heterologous molecule display. (**Panel 1**) illustrates the capsid protein organization, showing the N-terminus located within the inner core cavity, the central region forming the shell (S) domain (aa 62–242), and the C-terminal P-domain (aa 259–371) [9,10]. (**Panel 2**) shows the self-assembly of capsid protein monomers into a spherical VLP (~30 nm in diameter), with the S-domain forming the inner shell and the P-domain extending outward on the particle surface. (**Panel 3**) demonstrates heterologous molecule display strategies on the P-domain, including genetic fusion or insertion of small peptide epitopes and domain replacement to accommodate larger protein domains. These approaches enable surface presentation of heterologous molecules while maintaining particle integrity. This schematic illustration was created by the authors using Google Gemini (Flash Extended) based on published structural studies [9,10].

Unlike many established VLP platforms that are often optimized for specific display strategies, MrNV VLPs have demonstrated the ability to accommodate both small peptide epitopes and larger structural proteins using different design approaches. These characteristics have attracted increasing interest in the development of MrNV VLPs as a versatile platform for heterologous molecule display. Nevertheless, the size, structural characteristics, and insertion site of heterologous molecules may influence particle assembly, structural stability, surface accessibility, and their biological performance. However, the availability of evidence is fragmented because of variations in construct designs, insertion strategies, and experimental systems across studies. Consequently, it remains unclear how different design

strategies influence the structural integrity, physicochemical properties, and functional performance of MrNV VLPs.

For clarity, "displayed molecule" is used as a general term for a heterologous peptide, protein domain, antigen, or ligand presented on the VLP surface, whereas "insert" specifically refers to a sequence genetically incorporated into the capsid protein. Therefore, this review aims to systematically examine the design strategies used in MrNV VLPs for heterologous molecule display, with a particular focus on how insert characteristics influence the selection of design strategies. By synthesizing the available evidence, this review provides structural and functional insights into MrNV VLP-based molecule display and establishes a framework for the rational design of VLP-based display platforms.

2. Materials and Methods

2.1. Literature Search

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. A structured literature search was conducted to identify studies related to MrNV VLPs for heterologous molecule display and biomedical applications. Electronic databases, including MEDLINE (via PubMed) and Scopus, were searched on 6 August 2026. These databases were selected because they provide broad coverage of biomedical and life science literature and were considered appropriate for capturing the currently available studies on MrNV VLPs. The search strategies were developed using combinations of keywords related to MrNV, virus-like particles, and heterologous molecule display. The complete Boolean search strategies for each database, including the search fields, search queries, filters, and final search period, are provided in Supplementary Table S1.

2.2. Inclusion and Exclusion Criteria

Studies were included if they (i) investigated MrNV VLPs as a platform for heterologous molecule display, (ii) reported the construction, characterization, or application of engineered MrNV VLPs, (iii) were original research articles, and (iv) were published in English. Studies were excluded if they were narrative reviews, conference abstracts, editorials, book chapters, did not involve heterologous molecule display using MrNV VLPs, or did not report original experimental data.

2.3. Study Selection

Retrieved records were screened based on titles and abstracts after removal of duplicates. The title or abstract screening was performed independently by two reviewers according to the predefined eligibility criteria. Full-text articles of potentially relevant studies were then assessed for eligibility according to the predefined criteria. The full-text assessment was also performed independently by the same two reviewers. Any disagreements regarding study eligibility were resolved through discussion until consensus was reached, and no third reviewer was required. No systematic review software was used during the screening process. When duplicate publications or multiple reports describing the same study were identified, only the most complete and relevant report was included. During the final revision, the literature search was rerun using the updated search strategies, and the study selection process was repeated to identify any additional eligible studies published before the final analysis. A total of 20 studies met the inclusion criteria and were included in this review. The study selection process, including the reasons for exclusion at each screening stage, is summarized in the PRISMA 2020 flow diagram (Figure 2).

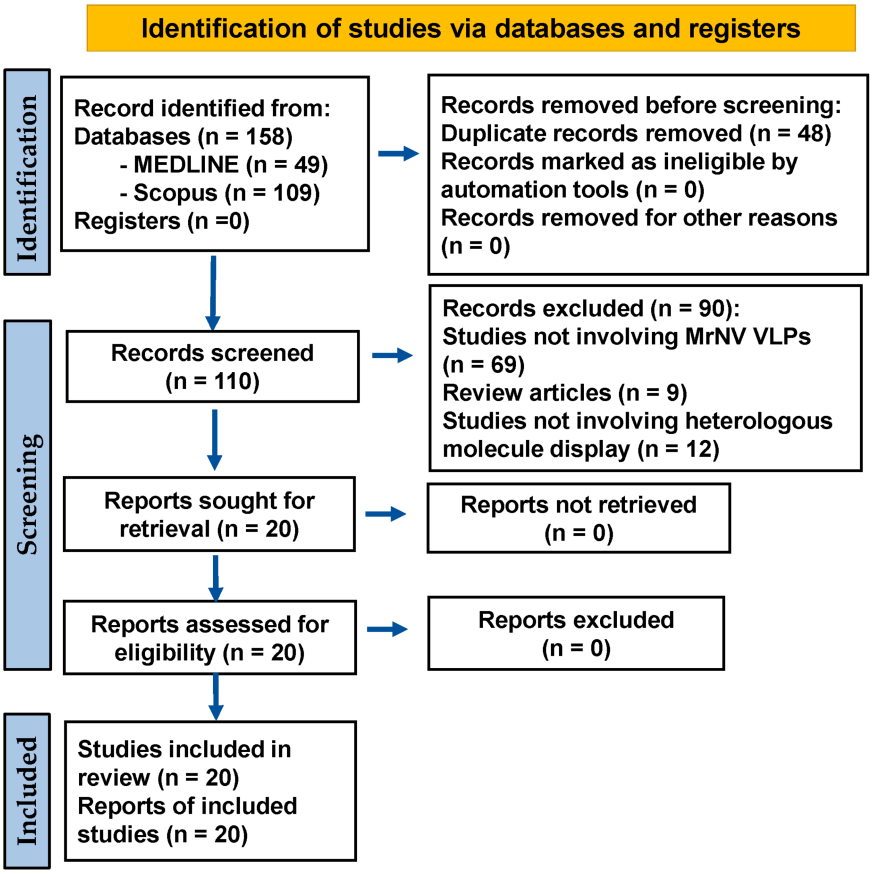


Figure 2. PRISMA 2020 flow diagram illustrating the literature search and study selection process for the systematic review of heterologous molecule display using MrNV VLPs.

2.4. Data Extraction and Synthesis

Data were extracted from the included studies, including displayed molecules, source pathogens, design strategies, expression systems, experimental models, applications, and key outcomes. Data extraction was performed independently by two reviewers. Any discrepancies in the extracted data were resolved through discussion until consensus was reached. The included studies were qualitatively analyzed and categorized based on design strategies and biomedical applications. For the purpose of this review, display strategies were classified according to the engineering approaches reported in the original studies, including C-terminal genetic fusion, genetic P-domain replacement, genetic insertion, and chemical conjugation. Peptides, protein domains, and functional ligands were categorized according to the descriptions provided by the original authors. Successful VLP assembly, structural stability, functional presentation, and particle size were extracted based on the experimental evidence reported in the respective studies.

2.5. Quality Assessment and Risk of Bias

As methodological assessment tools are generally developed for specific study designs [16] and no universally accepted quality assessment tool is currently available for systematic reviews comprising structural, in vitro, and animal studies, the Critical Appraisal of Methodological (technical) Quality, Quality of Reporting and Risk of Bias in Animal Research (CRIME-Q) framework [17] was considered appropriate for this review. The assessment consisted of 15 predefined criteria covering six domains: conflict of interest, reporting quality, internal validity, methodological rigor, external validity, and overall contribution to the research question. Each criterion was scored as 2 (fully met), 1 (partially met or unclear), or 0 (not met), while criteria that were not applicable to a particular study

design (e.g., randomization in non-interventional studies) were recorded as N/A. The predefined assessment criteria are described in Supplementary Table S2, and the complete quality assessment results for all included studies are presented in Supplementary Table S3. Quality assessment was performed independently by two reviewers. Any disagreements were resolved through discussion until consensus was reached.

3. Results

3.1. Study Selection

A total of 158 records were identified through database searching, including 49 from MEDLINE and 109 from Scopus. After removal of 48 duplicate records, 110 articles remained for title and abstract screening. Among these, 90 records were excluded based on predefined criteria, including studies not involving MrNV VLPs, narrative reviews, and studies not involving heterologous molecule display. The full texts of 20 articles were assessed for eligibility, and all were included in the final analysis [12–15,18–33]. The study selection process is illustrated in Figure 2. The characteristics and findings of the included studies are summarized in Tables 1 and 2.

Table 1. Heterologous molecules displayed on MrNV VLPs and their biological applications.

Displayed Molecule	Source Pathogen	Expression System	Experimental Model	Application	Key Biological Insight	Ref.
HBsAg "a" determinant-containing peptide	Hepatitis B virus	*E. coli*	Mouse	Vaccine	Successfully displayed antigenic domain with preserved immunogenicity	[12]
Influenza M2e peptide	Influenza A virus	*E. coli*	Mouse	Vaccine	Short conserved epitope enabled efficient antibody induction	[18]
Influenza M2e peptide	Influenza A virus	*E. coli*	Mouse	Vaccine	Repetitive display enhanced antigen-specific immune Response	[19]
HBsAg "a" determinant-containing peptide	Hepatitis B virus	Sf9	Mouse	Vaccine	Maintained structural integrity and antigenicity in eukaryotic expression system	[20]
HBsAg "a" determinant-containing peptide	Hepatitis B virus	Sf9	Mouse	Vaccine	Retained antigenicity under thermal conditions, indicating structural stability	[14]
Japanese encephalitis envelope protein (domain III)	Japanese encephalitis virus	*E. coli*	Mouse	Vaccine	Larger structured domain successfully presented while maintaining functionality	[15]
SARS-CoV-2 receptor-binding domain (RBD; β and δ variants)	SARS-CoV-2	*E. coli*	Mouse	Vaccine	Complex receptor-binding domain displayed with preserved antigenicity	[21]
RBD of SARS-CoV-2 receptor-binding domain (RBD, Wuhan strain	SARS-CoV-2	*E. coli*	Mouse	Vaccine	Structurally complex domain maintained functional presentation on VLP surface	[22]

Table 1. *Cont.*

Displayed Molecule	Source Pathogen	Expression System	Experimental Model	Application	Key Biological Insight	Ref.
Human and avian influenza A virus M2e (2× human M2e + 2× avian M2e)	Influenza A virus	*E. coli*	Chicken	Vaccine	Multivalent epitope design enabled cross-protective immunity	[23]
Human and avian influenza A virus M2e (2× human M2e + 2× avian M2e)	Influenza A virus	*E. coli*	Mouse	Vaccine	Tandem epitope display enhanced IgG response	[24]
Avian influenza M2e (H5N1, H9N2, H5N2)	Influenza A virus	*E. coli*	Chicken	Vaccine	Conserved epitope enabled subtype-specific antibody response	[13]
Influenza M2e peptide (Human and avian)	Influenza A virus	*E. coli*	Chicken	Vaccine	Multivalent display improved cross-strain protection	[25]
Human and avian influenza A virus M2e (2× human M2e + 2× avian M2e)	Influenza A virus	*E. coli*	Mouse	Vaccine	Broad protection against heterologous influenza A viruses.	[26]
Folic acid (FA)	Synthetic molecule	*E. coli*	Cancer cell lines (HT29)	Targeted drug delivery	Enabled receptor-specific targeting and enhanced cytotoxicity	[27]
GE11 peptide (EGFR-targeting peptide)	Synthetic peptide	*E. coli*	Cancer cell lines (SW480, SW620, MCF7)	Targeted drug delivery	Facilitated EGFR-specific cellular uptake	[28]
GE11 peptide (EGFR-targeting peptide)	Synthetic peptide	*E. coli*	Cancer cell lines (SW480, SW620, MCF7)	Targeted drug delivery	Enhanced cytotoxicity through receptor-mediated targeting	[29]
ACE2-targeting peptide (ACE2tp)	Synthetic peptide (angiotensin-II derived)	*E. coli*	HEK293T cells	Antiviral/viral-entry inhibition	Functioned as competitive inhibitor of viral entry	[30]
VP28 (extracellular domain)	White spot syndrome virus (WSSV)	*E. coli*	Shrimp tissue extracts, gill cryosections, and isolated hemocytes	Antiviral/viral-entry inhibition	Acted as decoy ligand to block viral attachment	[31]
tsCRD of Sambucus nigra agglutinin (SNA-I)	Plant lectin	*E. coli*	HEK293T cells	Antiviral/viral-entry inhibition	Carbohydrate-binding domain inhibited viral infection	[32]
CLEC17A carbohydrate-recognition domain (CRD)	Human protein	Insect cells (Sf9)	Sf9 insect cells	Antiviral/viral-entry inhibition	Lectin-like domain reduced viral infection via binding interference	[33]

Note: Biological applications are summarized in this table. The classification of displayed molecules according to molecular type and biological role is provided in Supplementary Table S4.

Table 2. Design strategies for heterologous molecule display using MrNV VLPs.

Insertion Site	Display Strategy	Insert Length (aa)/Copy Number	VLP Assembly	Particle Size (nm)	Ref.
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	49 aa × 1	Assembled (TEM)	~30 (TEM; approximate)	[12]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	23 aa (×1, ×3, ×5 copies)	Assembled (TEM)	~30 (TEM; approximate)	[18]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	23 aa (×3 copies)	Not directly evaluated	-	[19]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	49 aa × 1	Assembled (TEM)	~21–55 (TEM; range)	[20]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	49 aa × 1	Assembled (DLS)	56.4 (DLS; Mean)	[14]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	143 aa × 1	Assembled (TEM, DLS)	~18 (TEM; approximate), 18.2 (DLS; Mean)	[15]
C terminus of P-domain-truncated MrNV capsid protein (CΔ116-MrNV-CP)	Genetic domain replacement (P-domain replacement)	~198 aa (β-RBD)/~199 aa (δ-RBD)	Assembled (STEM, DLS)	~18–20 (STEM; range), 18.40, 18.39 (DLS; mean)	[21]
C terminus of P-domain-truncated MrNV capsid protein (CΔ116-MrNV-CP)	Genetic domain replacement (P-domain replacement)	196 aa × 1	Assembled (STEM, DLS)	~14.1 ± 1 (STEM; mean ± SD), 19.49 (DLS; mean)	[22]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	92 aa (4 × 23 aa M2e; two human + two avian copies)	Assembled (TEM, DLS)	30.32 ± 1.30 (TEM; mean ± SD); 41.04 ± 2.00 (DLS; mean ± SD)	[23]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	92 aa (4 × 23 aa M2e; two human + two avian copies)	Not reported	Not reported	[24]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	69 aa (3 × 23 aa M2e peptides)	Assembled (TEM)	~25 (TEM; approximate); ~35.4 (DLS; peak	[13]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	92 aa (4 × 23 aa M2e; two human + two avian copies)	Assembled (TEM, DLS)	Not reported	[25]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	92 aa (4 × 23 aa M2e; two human + two avian copies)	Not reported	Not reported	[26]
Surface lysine residues of full-length MrNV VLP	Chemical conjugation (post-assembly)	Not reported	Assembled (TEM)	15.54 ± 0.29 (DLS; mean Rh)	[27]
C terminus of full-length MrNV capsid protein	R-MrNV: peptide replacement; I-MrNV: peptide insertion; E-MrNV: C-terminal genetic fusion	12 aa × 1 (each construct)	R-MrNV, I-MrNV, E-MrNV: Assembled (TEM)	R-MrNV, I-MrNV, E-MrNV: 27–30 (TEM; range)	[28]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	12 aa × 1	Not reported	Not reported	[29]
C-terminal region of the P-domain	Genetic insertion (P-domain)	8 aa × 1	Assembled (TEM)	~26–28 (TEM; range)	[30]
C terminus of full-length MrNV capsid protein	C-terminal genetic fusion	~31 aa × 1	Assembled (TEM)	Not reported	[31]

Table 2. *Cont.*

Insertion Site	Display Strategy	Insert Length (aa)/Copy Number	VLP Assembly	Particle Size (nm)	Ref.
Genetic domain replacement (P-domain replacement)	Genetic domain replacement (P-domain replacement)	~146 aa × 1	Assembled (TEM)	26–29 (TEM; range)	[32]
Genetic domain replacement (P-domain replacement)	Genetic domain replacement (P-domain replacement)	~139 aa × 1	Assembled (TEM)	30–32 (TEM; range)	[33]

Note: Particle sizes are reported as described in the original studies. Microscopy-derived dimensions (TEM/STEM) and hydrodynamic diameters obtained by DLS are presented separately because these measurements are not directly interchangeable. Values are identified as means, ranges, or approximate values where reported.

3.2. Quality Assessment of the Included Studies

The methodological quality, reporting quality, and risk of bias of all 20 included studies were evaluated using the CRIME-Q framework. Overall, the included studies demonstrated good reporting quality, with all studies clearly describing their experimental methods, comprehensively reporting their results, and employing study designs appropriate for their research objectives. Most studies also clearly stated their research objectives, while appropriate statistical analyses were reported in the majority of studies. However, none of the included studies reported a priori power calculations or provided a justification for sample size adequacy. Fourteen studies were assessed using all 15 CRIME-Q criteria, whereas six studies had one non-applicable criterion (random allocation) because it was not applicable to structural characterization or in vitro studies. Accordingly, this criterion was recorded as "Not applicable" and excluded from the total score calculation. In addition, several assessment criteria, including random allocation, were considered not applicable for studies involving structural characterization or in vitro experiments due to the nature of the study design. The complete quality assessment results are presented in Supplementary Table S3.

3.3. Characteristics of Included Studies

The characteristics of the included studies are summarized in Table 1. All included studies used a MrNV capsid-derived protein as the common VLP backbone. A total of 20 studies were analyzed, covering a diverse range of molecules displayed on MrNV VLPs and their associated biological applications. The displayed molecules were classified according to their molecular type and biological role (Supplementary Table S4). Based on molecular type, peptides represented the most frequently displayed molecules, accounting for 13 of 20 studies (65.0%), with influenza A M2e being the most frequently displayed peptide. These peptides were frequently presented in multivalent formats, including tandem repeats, suggesting a preference for designs that enhance functional presentation. Protein domains were reported in 6 of 20 studies (30.0%), including structurally complex molecules such as the receptor-binding domain (RBD) of SARS-CoV-2, domain III of Japanese encephalitis virus, VP28 extracellular domain, and carbohydrate-recognition domains, demonstrating that MrNV VLPs can accommodate relatively large and structurally complex inserts. Functional ligands were identified in 1 of 20 studies (5.0%), represented by folic acid, indicating that the platform also supports the display of non-protein molecules. Based on biological role, immune activation was the most frequently reported application (13 of 20 studies, 65.0%), followed by antiviral/viral-entry inhibition (4 of 20 studies, 20.0%) and targeted drug delivery (3 of 20 studies, 15.0%). Overall, the included studies demonstrate that MrNV VLPs support the display of structurally diverse molecules, ranging from peptides to larger

protein domains and non-protein ligands. The variation in molecule types and biological roles provides a basis for selecting appropriate design strategies, which are summarized in Table 2.

Across the 20 included studies, C-terminal genetic fusion was the most frequently adopted display strategy. In contrast, genetic domain replacement (P-domain replacement), genetic peptide replacement, and chemical conjugation were employed less frequently. C-terminal genetic fusion was most commonly used for the display of short peptide epitopes, although larger inserts were also successfully displayed (Table 2). This approach enabled straightforward genetic incorporation of engineered peptides, and successful VLP assembly, as demonstrated primarily by TEM or STEM, was reported in studies that directly evaluated VLP formation. The widespread use of this strategy suggests that the C terminus of the MrNV capsid protein represents a structurally permissive site for peptide display. In contrast, P-domain replacement was predominantly applied for the display of larger protein domains, typically exceeding 100 amino acids, such as lectin-derived carbohydrate-recognition domains and viral receptor-binding domains. This strategy involves substitution of the P-domain of the capsid protein, thereby allowing the incorporation of structurally complex molecules while maintaining VLP architecture. The preferential use of P-domain replacement for larger protein domains suggests that this strategy facilitates the display of structurally complex molecules while preserving VLP assembly. Genetic peptide replacement within the P-domain was reported in only one study, indicating that this approach has been explored less frequently than other display strategies.

In addition to genetic engineering approaches, one study employed chemical conjugation (post-assembly modification) to functionalize the pre-formed VLPs with small-molecule ligands such as folic acid. This strategy bypasses genetic modification and enables the incorporation of non-protein ligands, thereby expanding the functional scope of MrNV VLPs beyond peptide or protein display. Despite the variable design strategies, successful VLP assembly was reported in studies that directly evaluated particle formation. Particle dimensions varied among engineered VLPs and were assessed using different analytical methods, including TEM, STEM, and DLS. Because microscopy-derived dimensions and DLS-derived hydrodynamic diameters are not directly interchangeable, these measurements were reported separately in Table 2 without direct cross-study comparison. Collectively, these findings demonstrate that C-terminal genetic fusion remains the most frequently used strategy for heterologous molecule display, whereas P-domain replacement and chemical conjugation expand the range of molecules that can be incorporated into MrNV VLPs, including structurally complex protein domains and non-protein ligands. This highlights the structural adaptability of MrNV VLPs, supporting their development as multifunctional nanoplatforms for diverse biomedical applications.

4. Discussion

This systematic review highlights the emerging potential of MrNV VLPs as a promising platform for heterologous molecule display. Rather than being defined by specific application aspects, the available evidence demonstrates that MrNV VLPs support a broad range of design strategies that enable the presentation of structurally diverse molecules. However, the current evidence indicates that C-terminal genetic fusion remains the predominant strategy, whereas more advanced approaches, such as P-domain replacement and chemical conjugation, have been explored in a limited number of studies. Therefore, the perceived versatility of the MrNV platform reflects its capacity to accommodate different classes of heterologous molecules rather than the widespread application of multiple design strategies.

4.1. Structural Basis and Display Strategies

Structurally, the MrNV capsid protein comprises N- and C-terminal regions that play distinct roles in domain orientation within the particles. The N-terminal region is located within the interior core cavity of the particle and contributes to cargo loading, while the shell (S-domain), which forms the structural core of the capsid, is primarily derived from the central region of the capsid protein. In contrast, the C-terminal region extends outward to form the P-domain, which is exposed on the particle surface and is involved in receptor binding and cellular internalization [6,7] (Figure 1, panels 1 and 2). Specifically, the P-domain is well-known to be the primary site for heterologous molecule display, with design strategies such as genetic fusion and peptide insertion used to present foreign epitopes on the VLP surface [28,32] (Figure 1, panel 3).

The selection of an appropriate strategy is closely related to the size and structural complexity of the inserted ligand/antigen [34]. In general, small peptide epitopes can be incorporated through direct fusion with minimal impact on particle quaternary structure. However, as the size and folding complexity of the insert increase, this method may become less effective, potentially affecting capsid stability and epitope presentation. In such cases, alternative designs, including domain replacement within the P-domain, have also been explored for the display of larger or more structured proteins. This pattern is consistent with the range of epitopes summarized in Table 1. It has been reported that small peptides, such as influenza M2e [18,19], are readily displayed using simple fusion strategies, whereas larger and more structurally complex antigens, including the SARS-CoV-2 receptor-binding domain [21,22] and Japanese encephalitis virus domain III [15], require more adaptable design approaches to maintain functional presentation. In fact, the insert size and structural complexity are important considerations when selecting an appropriate display strategy, as increasing molecular complexity may necessitate more extensive capsid modification to preserve particle assembly and antigen accessibility. Epitope display at the P-domain consistently induces antigen-specific immune responses against the corresponding viral targets, supporting its role as a preferred site for heterologous epitope presentation in MrNV VLPs. Nevertheless, direct comparisons among different display strategies remain limited because most studies evaluated individual constructs under different experimental conditions, preventing standardized assessment of assembly efficiency, display capacity, or immunological performance.

4.2. Expanding Applications Beyond Vaccines

While most studies have focused on vaccine-related applications, the use of MrNV VLPs in non-vaccine applications, particularly in cancer targeting and antiviral applications, has also emerged. In these applications, the P-domain continues to play a central role as the primary display site. For example, targeting ligands such as folic acid [27] and GE11 peptide [28,29] have been used to improve cellular uptake in receptor-positive cells, indicating that the displayed molecules retain their receptor-targeting and cellular uptake capabilities after presentation on the VLP surface. This suggests that the P-domain is not limited to antigen presentation but can also support the display of functional ligands for cell-specific targeting. However, compared with vaccine-focused studies, these applications remain relatively limited and are represented by 7 studies, most of which were conducted using in vitro models, whereas one study employed an ex vivo shrimp tissue model, which may reflect the greater complexity involved in evaluating non-immunological functions. Furthermore, the current evidence is primarily derived from proof-of-concept studies, while direct comparisons of targeting efficiency, therapeutic efficacy, or long-term stability across different ligand-display strategies remain unavailable. Consequently, the broader translational potential of MrNV VLPs beyond vaccine applications has yet to be systemat-

ically established. Taken together, the available evidence suggests that MrNV VLPs can function as a multipurpose platform for different types of molecule display (Figure 3).

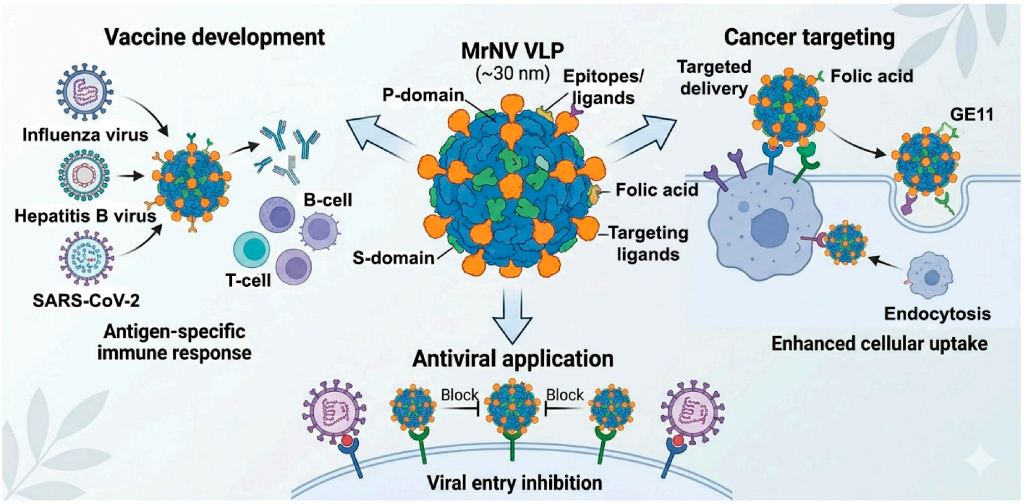


Figure 3. Multiple biomedical applications of MrNV VLPs. The central MrNV VLP (~30 nm in diameter) displays heterologous molecules or targeting ligands on the surface via the protruding (P) domain. These displayed molecules enable multiple applications. In vaccine development, VLPs presenting viral epitopes (e.g., influenza virus, hepatitis B virus, and SARS-CoV-2) induce antigen-specific immune responses. In cancer targeting, surface-displayed ligands such as folic acid and GE11 peptide facilitate receptor-mediated uptake and enhance cellular internalization. In antiviral applications, functional peptides or domains displayed on the VLP surface can inhibit viral entry into host cells. Most of these applications remain at the preclinical or proof-of-concept stage. This schematic illustration was created by the authors using Google Gemini based on the published literature.

4.3. Comparison of MrNV VLPs with Established VLP Platforms

MrNV VLPs do not rely on a single defining feature but instead offer a combination of properties that are not always present in other VLP platforms. Based on the available literature, some existing systems may exhibit a trade-off between ease of molecule display and the ability to accommodate larger or more complex proteins, as observed in commonly used peptide-display or chemically modified VLP systems [2,35]. In contrast, the available evidence suggests that MrNV VLPs can support both small peptide epitopes and larger structured proteins through different design strategies, while still maintaining functional presentation [28,30]. Rather than indicating superiority over other VLP platforms, these findings suggest that MrNV VLPs possess structural features that enable different display strategies depending on the size and complexity of the inserted molecules. In addition to structural design, physicochemical stability is an important consideration for VLP-based platforms, as the ability to maintain particle integrity under different conditions may influence their practical applications [36–38]. Platforms that can maintain their structure and function in varying environments may be more suitable for applications where both delivery and stability are important [39]. However, direct comparisons among VLP platforms should be interpreted with caution because differences in capsid architectures, expression systems, insert designs, and evaluation methods limit direct comparisons of display performance and functional outcomes. Therefore, the current evidence supports MrNV VLPs as a complementary platform with distinct structural advantages rather than a universally superior alternative to the existing VLP systems. Unlike many well-established VLP platforms derived from human, bacterial, or plant viruses, MrNV VLPs originate from an aquatic invertebrate virus and have been successfully applied in vaccine development, targeted delivery, and cancer targeting. These findings suggest that MrNV VLPs

demonstrate the potential of aquatic virus-derived VLPs as platforms for heterologous molecule display in biomedical applications. Although still in early stages of development, MrNV VLPs highlight the possibility of combining multiple design strategies with stable structural properties. For additional context, a qualitative comparison of representative VLP platforms is provided in Supplementary Table S5 [35,37,40–50].

4.4. Limitations and Future Perspectives

Despite the promising findings, several limitations should also be considered. The number of studies on MrNV VLPs is still limited, and the available evidence is largely derived from a small number of research groups using preclinical models, with limited independent validation and few studies in relevant in vivo disease or challenge models. This evidence base also includes studies conducted by members of the present review team, highlighting the importance of interpreting the current findings with appropriate caution until further independent validation becomes available. In addition, differences in experimental design, such as expression systems and evaluation methods, make it difficult to directly compare results across studies. Furthermore, the quality assessment revealed that most studies did not report a priori power calculations or justify sample size adequacy, suggesting the need for more rigorous methodological reporting in future investigations. Another limitation is the lack of direct comparison between different design strategies under similar conditions, which makes it hard to identify the most effective approach. Furthermore, unsuccessful or suboptimal construct designs are rarely reported, limiting a comprehensive assessment of the factors governing successful capsid assembly and heterologous molecule display. Future studies should focus on systematic comparisons of different design strategies under standardized experimental conditions to better define the relationships between insert characteristics, capsid architecture, and functional performance. Expanding applications beyond vaccines, particularly in targeted delivery and antiviral therapy, together with validation in relevant in vivo models, will further clarify the translational potential of MrNV VLPs. Future studies should also investigate their potential as cargo delivery platforms for nucleic acids and therapeutic molecules. In addition, improving production scalability will be essential for facilitating their broader biomedical applications.

5. Conclusions

This systematic review demonstrates that MrNV VLPs represent a promising platform for heterologous molecule display, supporting the presentation of structurally diverse molecules, including peptide epitopes, protein domains, and functional ligands, through multiple design strategies. The available evidence suggests that different display approaches are suited to different insert characteristics, providing flexibility for vaccine development as well as targeted delivery and other biomedical applications. However, the current evidence remains limited to heterogeneous preclinical and proof-of-concept studies, with substantial variation in construct design, expression systems, experimental models, and evaluation methods. These differences prevent direct comparisons among studies and limit the ability to establish standardized design recommendations. Further studies incorporating standardized experimental approaches, independent validation, and broader biological evaluation will be important to strengthen the evidence base and facilitate the future development of MrNV VLPs as a versatile molecule display platform.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/applbiosci5030077/s1, Table S1: Complete search strategies used for literature retrieval; Table S2: Predefined criteria used in the CRIME-Q quality assessment; Table S3: Quality assessment of the included studies using the CRIME-Q framework; Table S4: Classification of displayed molecules according to molecular type and biological role; Table S5: Comparison of MrNV VLPs with representative viral nanoparticle platforms and related shrimp-derived VLP systems.

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