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Review article

Engineering of bacterial outer membrane vesicles for biotechnological applications

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ABSTRACT

Outer membrane vesicles (OMVs) are spherical, nano-sized proteoliposomes naturally secreted by Gram-negative bacteria. By incorporating parental membrane components and encapsulating diverse cellular cargos, OMVs function as potent mediators of biological communication. Despite inherent challenges, including low production yields, heterogeneous composition, and stochastic cargo loading, OMVs hold immense promise across broad biotechnological landscapes. In this review, we examine current advancements in OMV chassis engineering and functional diversification, providing a comprehensive synthetic biology perspective on their role as modular and programmable systems. We discuss five established strategies for inducing hyper-vesiculation alongside two complementary frameworks for constructing synthetic vesicles. These engineered OMVs facilitate transformative applications spanning biomedical and biotechnological domains, notably as high-fidelity platforms for studying membrane proteins within their native lipid environments. As synthetic biology increasingly converges with materials science, future innovations such as stimuli-responsive vesicles, self-assembling architectures, and hybrid bio-synthetic materials are poised to emerge. Such advancements position synthetic OMVs at a critical intersection to address complex challenges in medicine, environmental remediation, and advanced materials science, offering unprecedented opportunities for both fundamental discovery and industrial applications.

1. Introduction

Gram-negative bacteria naturally produce outer membrane vesicles (OMVs), which are nanoscale spherical proteoliposomes typically ranging from 10 to 300 nm in diameter. During biogenesis, OMVs selectively incorporate components from the parental outer membrane (OM) while encapsulating periplasmic or cytoplasmic contents within their luminal cavity. These vesicles exhibit diverse structural and biochemical compositions depending on their formation processes, including programmed OM blebbing or stress-induced explosive cell lysis, which eventually dictate their biological functions (Toyofuku et al., 2023). As potent cellular proxies, OMVs facilitate horizontal gene transfer and mediate sophisticated inter-species communication without the need for direct cellular contact or active movement. Furthermore, they play essential roles in biofilm formation, environmental adaptation, and the targeted delivery of virulence factors (Baker et al., 2014). Despite their biological importance, our current understanding of the molecular machinery governing universal biogenesis and precise cargo loading remains fragmentary, presenting a significant bottleneck for their standardized application.

In recent years, OMVs have gained substantial attention as versatile scaffolds in synthetic biology and nanomedicine. Their intrinsic ability to stabilize molecular cargo, combined with inherent biocompatibility and tunable surface properties, makes them ideal candidates for diverse applications from targeted drug delivery to multienzyme biocatalysis (Tan et al., 2018; Amalia and Tsai, 2023). In addition, OMVs have emerged as promising diagnostic and biosensing platforms because they serve as biomolecular signatures that reflect the real-time physiological

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and pathological status of their parental cells (Xie et al., 2022). Compared to synthetic lipid nanoparticles, OMVs offer several advantages, including inherent biodegradability, intrinsic, tunable immunostimulatory property, and the capacity for modular functionalization through genetic engineering of the parental strains. By bridging the gap between natural biological functions and engineered precision, OMVs are uniquely positioned at the intersection of nanotechnology, synthetic biology, and biomedical engineering, offering new opportunities for both fundamental research and biotechnological applications.

Traditional OMV research, however, faces significant challenges, including limited production yields, heterogeneous compositions, and unpredictable cargo loading (Sartorio et al., 2021). Synthetic biology offers robust solutions through modularization and compartmentalization, reducing these complexities to controllable biological variables (Stock and Gorochowski, 2024). By precisely tuning genetic and metabolic components, synthetic biology-driven vesicles address critical bottlenecks, resulting in enhanced productivity and structural uniformity. This engineered approach substantially expands the utility of OMVs to meet diverse global demands. This review provides a concise yet comprehensive overview of OMV engineering and applications from a synthetic biology perspective. We highlight current advancements in enhancing vesicle production, emerging surface decoration technologies, and their strategic relevance to biotechnological applications.

2. Engineering strategies for OMV production chassis

Translating OMVs into practical therapeutics or industrial tools necessitates the enhancement of vesicle yield, precise functionalization via cargo loading and improved safety profiles mitigating endotoxin-associated toxicity. As OMVs are derived from microbial hosts, genetic manipulation of the source organism is pivotal for optimizing these parameters. Establishing a robust production chassis is therefore crucial for advancing applications such as vaccine development, targeted drug delivery, and synthetic biology. Here, we discuss microbial engineering strategies aimed at developing tailored platforms for scalable and application-specific OMV production.

2.1. Conventional approaches to induce hyper-vesiculating phenotypes

Despite the lack of a universal biogenesis model for OMVs, even within closely related species, researchers have pioneered diverse strategies to bolster vesicle secretion. The mechanisms underlying hyper-vesiculation can be broadly categorized into five main strategies (Fig. 1 and Table 1): (1) weakening the Outer Membrane-Peptidoglycan (OM-PG) crosslinking, (2) inducing OM destabilization, (3) modulating phospholipid (PL) transport, (4) engineering lipopolysaccharides (LPS) biosynthesis/remodeling, and (5) applying exogenous environmental stress factors.

2.1.1. Weakening the outer membrane crosslinks

The complex architecture of the bacterial envelope requires the liberation of the OM from the OM-PG-inner membrane (IM) network as a critical prerequisite for OMV budding. Understanding this delicate balance provides both mechanistic insights and strategic frameworks for enhancing vesicle production.

The Tol-Pal complex plays a crucial role in OM invagination during cell division and maintains membrane integrity through lipid homeostasis (Tan and Chng, 2025). This highly conserved complex comprises the integral IM subcomplex (TolQ, TolR, TolA), the periplasmic protein TolB, and the OM lipoprotein Pal (Fig. 1A). Non-covalent interactions between Pal and TolB facilitate OM-PG tethering, thereby stabilizing the cell envelope. Disrupting these interactions through the deletion of core complex genes promotes membrane blebbing and hyper-vesiculation across various species, achieving up to a 50-fold increase in OMV

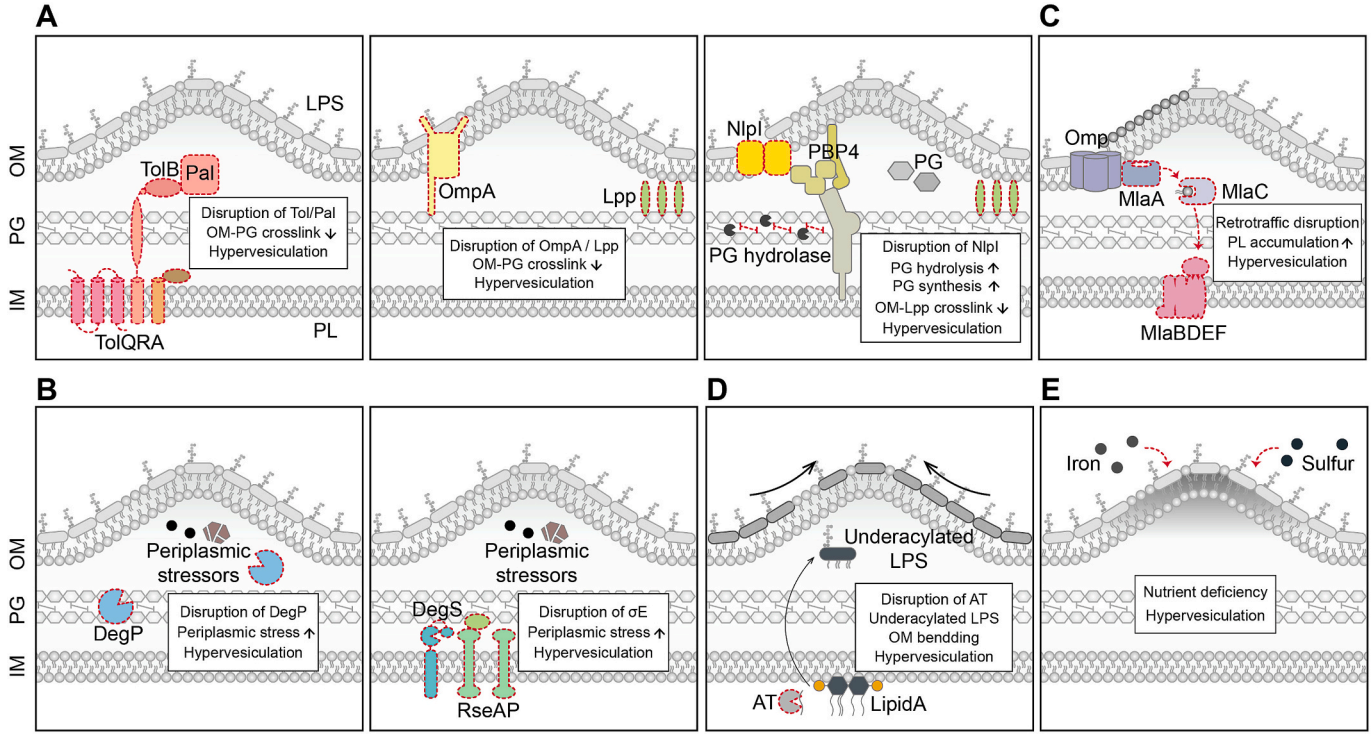


Fig. 1. The envelope architecture of Gram-negative bacteria and strategies for enhancing OMV production. The envelope of Gram-negative bacteria is composed of two membranes, the OM and IM, separated by the periplasmic space. The OM has an asymmetric organization with LPS and PLs, whereas the IM consists of a PL bilayer. The periplasmic space contains repeating disaccharide chains that form a net-like structure, the PG layer, and diverse periplasmic proteins. A variety of OM proteins, lipoproteins, and periplasmic components dynamically interact with each other to maintain envelope stability. Five engineering strategies for hyper-vesiculation are demonstrated: (A) Reduced OM-PG crosslinks via disruption of Tol/Pal complex, OmpA, Lpp and NlpI. (B) Increased envelope stress via disruption of DegP, and σ^E pathway. (C) Accumulation of mis-localized PL. (D) Perturbation of LPS homeostasis. (E) Extracellular stress such as nutrient depletion.

Table 1
Strategies for enhancing OMV production.

Principle	Organism	Variant	OMV production	Reported trade-off	Reference
Weakening OM-PG crosslinking	*E. coli* MG1655	Δ*tolQRA*	50-fold	Reduced OM integrity Cell division defect OM lipid dyshomeostasis Clumping	(Tan and Chng, 2025)
	Helicobacter pylori 251	Δ*tolB*	2-fold	Shorter morphology Flagella defect Reduced membrane integrity Blebbing	(Turner et al., 2015)
	Shigella flexneri	Δ*tolR*	4-fold	Reduced membrane integrity	(Pastor et al., 2018)
	Pseudomonas aeruginosa	Δ*oprF* Δ*oprI*	8-fold 3-fold	– –	(Wessel et al., 2013)
	Vibrio cholerae	Δ*ompA*	3-fold	–	(Valeru et al., 2014)
	Geobacter sulfurreducens	Δ*ompA*	1.21-fold	–	(Fang et al., 2024)
	E. coli BW25113	Δ*nlpI*	>100-fold	–	(Schwechheimer et al., 2015)
Inducing OM destabilization	*E. coli* BW25113	Δ*degP*	41.95-fold	Growth defect	(Schwechheimer and Kuehn, 2013)
Modulating PL transport	*Haemophilus influenzae*	Δ*vacJ* Δ*yrbE*	1.6-fold 2.2-fold	– –	(Roier et al., 2016)
	Vibrio cholerae	Δ*vacJ* Δ*yrbE*	3.9-fold 4.3-fold	– –	
Engineering LPS dysbiosis	*Salmonella enterica* serovar Typhimurium	*pagL* OE	4-fold	–	(Elhenawy et al., 2016)
	Neisseria meningitidis	Sulfur source	>2-fold	–	(Gerritzen et al., 2019)
Applying environmental stress	*Shigella flexneri*	WT, Heat	4.24-fold	–	(Pastor et al., 2018)
		Δ*tolR*, Heat	1.86-fold	Blebbing Reduced membrane integrity	

production (Table 1) (Bernadac et al., 1998; Berlanda Scorza et al., 2012; Turner et al., 2015; Pastor et al., 2018; de Jonge et al., 2022).

Mutations in *lpp* and *ompA* similarly compromise membrane stability. Lpp, the most abundant lipoprotein in *Escherichia coli* (approximately 750,000 copies per cell), anchors to the OM via its lipid moiety and exists in two forms, which are solely anchored to the OM or covalently bound to PG (Nikaido and Nakae, 1979). Strains lacking *lpp* exhibit diminished structural integrity and consequently overproduce vesicles in many organisms (Fig. 1A) (Wessel et al., 2013; Valeru et al., 2014; Nevermann et al., 2019; Fang et al., 2024). Likewise, OmpA establishes a robust membrane structure by non-covalently tethering its C-terminal domain to the PG layer. Its loss accelerates OM blebbing (Fig. 1A). Notably, while the complete loss of the Tol-Pal complex disturbs lipid homeostasis and can hinder growth, partial knock-downs are often more desirable for engineering scalable production platforms (Tan and Chng, 2025).

Furthermore, OM crosslinking can be indirectly modulated by altered PG dynamics. In *E. coli*, the lipoprotein NlpI coordinates PG remodeling by recruiting the endopeptidase Spr or PG synthase PBP4 in a phase-dependent manner (Fig. 1A). In *nlpI*-deficient cells, accelerated PG synthesis attempts to compensate for uncontrolled PG hydrolase activity. These altered PG dynamics lead to defective Lpp-OM crosslinking, subsequently boosting OMV formation by more than 100-fold compared to the wild type strain (Table 1) (Schwechheimer et al., 2015).

2.1.2. Inducing outer membrane destabilization

A critical biological function of OMVs is the maintenance of envelope homeostasis by expelling periplasmic stressors. As the cell envelope functions as the primary barrier for sensing and responding to environmental changes, Gram-negative bacteria have evolved sophisticated protective mechanisms (Mitchell and Silhavy, 2019). In *E. coli*, DegP plays a central role in this process, functioning as a periplasmic chaperone at lower temperatures and switching to protease activity above 37 °C via the σ^E envelope-stress response (Mitchell and Silhavy, 2019). Under thermal stress, DegP deficiency leads to the accumulation of misfolded proteins, aberrant LPS, or fragmented PG within the periplasm, triggering an approximately 40-fold increase in OMV production as a stress-resistance mechanism (Fig. 1B and Table 1) (Button et al., 2007; Schwechheimer and Kuehn, 2013).

This relationship is further supported by the σ^E mutation model. The σ^E factor, encoded by the *rpoE* pathway, represents the only known essential envelope stress response in *E. coli*. Dysfunction in σ^E results in hyper-vesiculation, which effectively removes toxic cargo from the cells (McBroom and Kuehn, 2007). This phenotype is also observed in *Salmonella* and *Pseudomonas*, underscoring the conserved role of σ^E across Gram-negative bacteria (Fig. 1B) (Macdonald and Kuehn, 2013).

2.1.3. Modulating phospholipid transport

In Gram-negative bacteria, the OM is externally coated with LPS, while its inner leaflet consists of phospholipids (PL). This asymmetric composition establishes a robust structural barrier against various antibiotics (Di Lorenzo et al., 2022). Such asymmetry is strictly maintained by a retrograde PL transport system. When PLs abnormally accumulate in the outer leaflet of the OM, this trafficking system removes and redirects them to the inner membrane (IM), a homeostatic mechanism conserved across diverse Gram-negative bacteria (Guest et al., 2023).

In human pathogens *Helicobacter pylori* and *Vibrio cholerae*, disruption of this retrograde trafficking system enhances OMV production by 2–4 folds without compromising OM integrity (Fig. 1C and Table 1) (Roier et al., 2016). This extraction of mis-localized PLs is mediated by the *vacJ/yrbABCDE* locus, where VacJ serves as an OM lipoprotein and YrbABCDE functions as an IM ABC-type transporter. Deletion of *vacJ* or *yrb* genes in *V. cholerae* halts this trafficking, leading to PL retention in the outer leaflet and a significant surge in vesiculation (Zingl et al., 2020). The elevated PL content observed in these OMVs further validates the link between PL transport and vesiculation. Furthermore, this mechanism is regulated by iron availability in a Fur-dependent manner and remains highly conserved (Roier et al., 2016).

2.1.4. Engineering LPS dysbiosis

LPS, the primary constituent of the OM, possesses specific acylation patterns within its lipid A domain that determine the physical properties of the OM. According to lipid packing theory, the ratio of cross-sectional areas between lipid headgroups and acyl chains determine membrane curvature (Goldfine, 1984). While wild-type lipid A typically contains six acyl chains, underacylated lipid A, resulting from *pagL* overexpression in *Salmonella,* alters the membrane's intrinsic curvature. This modification induces a significant 4-fold increase in OMV production (Fig. 1D and Table 1) (Elhenawy et al., 2016).

2.1.5. Applying environmental stress

Environmental stressors significantly modulate vesicle production across various bacterial species (Fig. 1E). In *Neisseria* spp., nutrient depletion, specifically sulfur limitation, triggers PL overproduction. This leads to the outward bulging of the OM and the subsequent release of OMVs with distinct biochemical compositions (Gerritzen et al., 2019). Similarly, fluctuations in iron concentrations affect not only vesiculation rates but also increase the heterogeneity in size, flexibility, and proteomic profiles of the released OMVs (Płaczkiewicz et al., 2023).

2.2. Systematic approaches to engineer OMV production hosts

While individual case studies have demonstrated that single-gene deletions can enhance OMV production by more than 100-fold (Table 1), OMV yields are heavily affected by environmental factors. Consequently, the reproducibility and scalability of such enhancements remain limited (Kulp and Kuehn, 2010). Furthermore, inconsistencies in OMV quantification methods across studies hinder direct comparisons between genotypes, complicating the development of a robust OMV production chassis. Even within the same study, disparate measurement techniques can yield conflicting results. For instance, ELISA-based quantification using OMV-specific antibodies reported 600-fold and 22-fold increases in OMV production for Δ*tolB* and Δ*pal* strains of *Helicobacter pylori*, respectively. In contrast, electron microscopy analysis revealed only a 2-fold increase in the Δ*tolB* strain and no significant change in the Δ*pal* strain (Table 1) (Turner et al., 2015). Since genetic perturbations can alter both OMV composition and abundance, cross-study comparisons involving different analytical approaches must be interpreted with caution (Roier et al., 2016).

2.2.1. Genome-scale screening of genes affecting OMV biogenesis

To isolate the effects of individual factors and develop a robust production chassis, a systematic genotype–phenotype mapping of OMV biogenesis is essential. For example, high-throughput screening studies using a single-gene knockout collection and transposon (Tn) mutagenesis have identified various genes that modulate vesiculation (McBroom et al., 2006; Kulp et al., 2015; Roier et al., 2016; Nevermann et al., 2019). In *E. coli*, screening approximately 4000 Tn-insertion mutants in 96-well plates identified 20 genes affecting vesiculation (McBroom et al., 2006). Similarly, Tn-mutagenesis identified 9 and 20 genes affecting OMV production in *Salmonella enterica* serovar Typhi (*S.* Typhi) and *H. influenzae*, respectively (Roier et al., 2016, Nevermann et al., 2019).

Furthermore, the effects of gene disruptions in *E. coli* were systematically evaluated using the Keio knockout collection, identifying 171 strains with significant changes in vesiculation without growth defects (Kulp et al., 2015). This growth-coupled, genome-scale analysis provides critical insights into engineering principles for enhanced OMV yield. Interestingly, these investigations suggest a degree of conservation in OMV biogenesis across Gram-negative bacteria. For instance, all nine genes identified in *S.* Typhi also affected vesiculation in other species (Fig. 2A and B). Moreover, most gene disruption mutants that positively affected OMV production in *E. coli* DH5α, *S.* Typhi and *H. influenzae* maintained or even enhanced OMV production in *E. coli* BW25113 (Fig. 2C) (McBroom et al., 2006, Kulp et al., 2015, Roier et al., 2016, Nevermann et al., 2019). These findings indicate that system-wide investigations can serve as a rational basis for engineering OMV production in less-studied species of interest.

Currently, engineering approaches for enhancing OMV production are largely limited to the perturbation of individual genes. However, combinatorial genetic engineering holds significant promise for the development of robust chassis for OMV production. For example, Δ*nlpI* and Δ*mlaE* single knockout mutants exhibited 7- and 5-fold increases in OMV production, respectively, while a Δ*mlaE*Δ*nlpI* double knockout mutant showed a synergistic effect, achieving up to a 30-fold increase compared to the wild type (Ojima et al., 2020). Nevertheless, combinatorial engineering requires careful consideration, as multiple gene deletions may result in significant growth defects.

2.2.2. Engineering microbial hosts for tailored immunogenic properties

Beyond improving yield, OMV detoxification is a critical milestone for biomedical applications. The native OM components in OMVs act as a double-edged sword. They provide potent innate immune activation but can also induce fatal septic shock syndrome if not properly controlled. Here, we discuss techniques for tailoring OMV

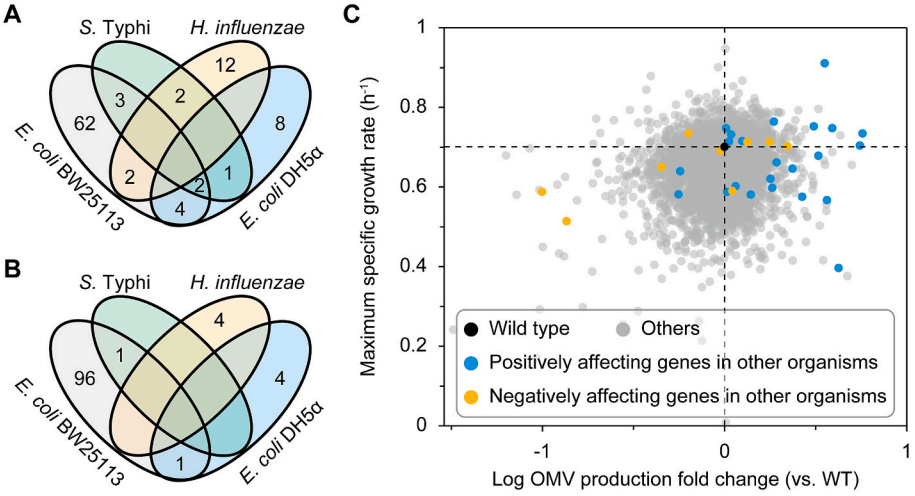


Fig. 2. System-wide analysis of genes affecting OMV biogenesis. Venn diagrams showing genes with (A) positive and (B) negative effects on OMV production identified in *E. coli* BW25113, *E. coli* DH5α, *H. influenzae* and *S.* Typhi. (C) Assessment of individual gene disruption mutants on growth and OMV production in *E. coli* BW25113 (Kulp et al., 2015).

immunogenicity to create tolerable OMVs required for biomedical applications.

A primary method for OMV detoxification is detergent-based extraction, producing dOMV. Treating bacterial cells with detergents such as sodium deoxycholate or sodium dodecyl sulfate (SDS) solubilizes LPS via hydrophobic interactions, releasing vesicle-like aggregates (Gnopo et al., 2017). Although traditionally used for meningococcal vaccines to reduce LPS-mediated reactogenicity, detergent treatment also strips away loosely associated outer membrane proteins (OMPs), which may be essential vaccine antigens (van der Ley and van den Dobbelsteen, 2011). Furthermore, dOMVs are prone to contamination with cytoplasmic components and often form irregular aggregates that complicate sterilization and filtration.

To overcome these hurdles, microbial hosts can be genetically engineered to reduce intrinsic endotoxicity. Lipid A, the hydrophobic moiety of LPS, is the primary driver of immunologic activity and host receptor interaction (Park and Lee, 2013). Modifying the acyl chain patterns or phosphate groups of lipid A can significantly reduce endotoxicity by hindering its binding to host immune receptors (e.g., TLR4). For example, OMVs derived from LPS-engineered *E. coli* and *Neisseria* strains retain high immunogenicity while minimizing harmful inflammatory responses, enhancing their clinical suitability as vaccine platforms (Gujrati et al., 2014).

3. Synthetic vesicles

Synthetic biology approaches significantly expand the functional scope and application potential of bacterial vesicles (Baker et al., 2014). The robust structure of the OM provides an ideal scaffold for incorporating functional modules, facilitated by the relative ease of bacterial genome modification. These customized OMVs, engineered to either encapsulate specific payloads within their lumen or display functional components on their surface, can be produced at scale using conventional fermentation and purification processes. Here, we discuss two complementary engineering strategies for synthetic vesicle construction: 'genetic loading' and 'post-production functionalization,' along with their respective advantages and limitations.

3.1. Genetic loading strategy

Functional modules can be incorporated into vesicles through membrane anchoring or lumenal encapsulation (Fig. 3). Anchoring proteins of interest (POI) onto OMVs via surface display allows these modules to be naturally incorporated during vesicle formation (Table 2). This concept emerged from earlier studies identifying cytolysin A (ClyA) as an effective anchoring motif for OMV decoration (Kim et al., 2008; Cheng et al., 2021). In *E. coli*, ClyA secretion utilizes type zero secretion systems associated with OMVs (Guerrero-Mandujano et al., 2017). Unlike most secreted proteins, ClyA lacks a classical N-terminal signal peptide and is released via OMVs rather than standard secretion pathways. It translocates across the IM through a poorly understood 'non-classical' mechanism, possibly involving cytoplasmic chaperones, before OMV incorporation. ClyA maintains its anchoring ability whether passengers are fused to its N- or C-terminus, though substrate and product permeability must be considered for catalytic applications (Kim et al., 2008).

The ice-nucleation protein (INP) from *Pseudomonas* sp. represents another robust candidate for surface display. Its native function facilitates ice crystal formation at sub-zero temperatures to access nutrients. INP comprises three main domains, which are an N-terminus, a central repeating domain (CRD), and a C-terminus. While the CRD is crucial for ice nucleation, it is not essential for anchoring. INP's structural adaptability provides a flexible platform, though its truncated form – lacking the CRD while retaining both terminal regions – is most widely used (Shimazu et al., 2003).

The chimeric Lpp-OmpA complex combines the signal sequence and first nine amino acids of the LPP lipoprotein with a transmembrane domain of OmpA (Francisco et al., 1992; Weyant et al., 2023; Laotee and Arunmanee, 2024), efficiently anchoring POIs to the OMV exterior. Autotransporters, consisting of a signal peptide, passenger domain, and translocon domain, also enable surface display of heterologous proteins (Rutherford and Mourez, 2006; Jong et al., 2010; Daleke-Schermerhorn et al., 2014). The classical autotransporter hemoglobin protease (Hbp) allows POIs to be anchored onto OMVs either individually or in combination.

Alternatively, genetic loading involves lumenal encapsulation of functional modules via periplasmic expression (Fantappiè et al., 2014). This method is relatively straightforward compared to membrane anchoring and is achieved by adding a leader sequence to the N-terminus of passengers. Notably, proteins fused to the OmpA signal peptide have been successfully localized into OMVs, with reports of encapsulating proteins as large as 170 kDa (Table 2) (Fantappiè et al., 2014).

The genetic loading strategy enables functional modules to naturally integrate into OMVs during vesiculation, producing vesicles uniformly in a stable, high-throughput manner. However, limitations exist regarding cargo size; most reported applications involve proteins below 70 kDa, and surface display of LacZ has been shown to fail (Table 2) (Kim et al., 2008). Nevertheless, a recent report of successful ClyA-fused dCas9 localization suggests that multiple factors beyond size alone influence efficiency (Wang et al., 2025). Other constraints include protein folding issues and a lack of prediction tools for membrane expression capability, alongside the experimental burden of strain re-engineering for each modification.

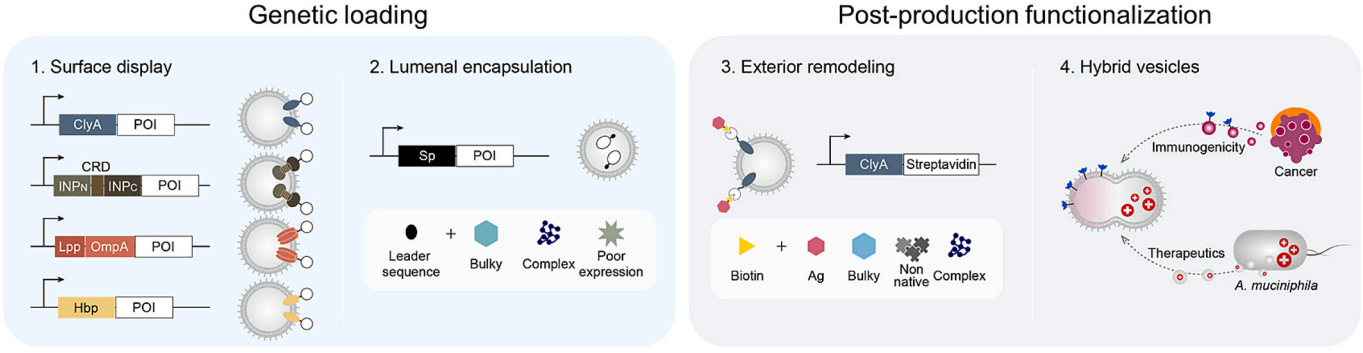


Fig. 3. Engineering strategies for synthetic vesicle construction: 'Genetic loading' and 'Post-production functionalization' approaches. The genetic loading approach includes surface display and lumenal encapsulation, achieved via strain engineering to express desired cargoes that are naturally loaded into OMVs during vesiculation. The post-production functionalization approach involves exterior remodeling and vesicle hybridization, in which functional modules are chemically or physically conjugated to OMVs.

Table 2
Overview of engineered fusion proteins for OMV localization.

Strategy	Fusion partner	Organism	Protein of interest	Size (kDa)	Reference
Genetic loading	ClyA	*E. coli* JC8031	scFv	~25	(Kim et al., 2008)
			GFP	~27	
			Bla	~29	
			OPH	~37	
			$OVA_{257–264}$	~5	
		E. coli Rosetta (DE3)	SnC	~12	(Cheng et al., 2021)
			SpC	~13	
			Luc	~61	
	INP	*P. putida* KT2440	OPH	~37	(Shimazu et al., 2003)
	Lpp-OmpA	*E. coli* BL21(DE3)	SpC	~13	(Laotee and Arunmanee, 2024)
		E. coli KPM404 ΔnlpI	eMA	~15	(Weyant et al., 2023)
	Hbp	*E. coli* JC8031	ESAT6	~9.9	(Daleke-Schermerhorn et al., 2014)
			$Ag85B_{C+N}$-ESAT6	~40	
			$Ag85B_{C+N}$-ESAT6-Rv2660c	~48	
		S. Typhimurium SL3261	MOMPIV-MOMPII	~10	
			ESAT6	~9.9	
			$Ag85B_{C+N}$-ESAT6	~40	
			$Ag85B_{C+N}$-ESAT6-Rv2660c	~48	
	OmpA signal peptide	*E. coli* BL21(DE3) ΔompA	Bla	~29	(Fantappiè et al., 2014)
			Spy0269	~40	
			Streptolysin O	~65	
			SAM_1372	~65	
	ClyA-SpC/ ClyA-SnC dual system	*E. coli* Rosetta (DE3)	SpyCEP	~170	(Cheng et al., 2021)
			SpT-$OVA_{257–264}$	~3	
			SpT-HA	~3	
			SnT-HA	~3	
			SnT-$TRP2_{180–188}$	~3	
			SpT-$OVA_{257–264}$/SnT-$OVA_{223–339}$	~3/~4	
			SpT-$OVA_{257–264}$/SnT-$TRP2_{180–188}$	~3/~3	
Post-production functionalization	Hbp-SpT	*S.* Typhimurium ΔtolRA	SpT-Adpgk	~5	(van den Berg van Saparoea et al., 2018)
			GFPnb-SpC	~24.2	
			SpC-PspAα'-SnT	~39	
			SpC-SP1690-SnT	~61	
			SpC-PspAα'-SP1690-SnT	~87	
	Lpp-OmpA-SpC	*E. coli* BL21(DE3)	SpT-scFv	~29.4	(Laotee and Arunmanee, 2024)
	Lpp-OmpA-eMA	*E. coli* KPM404 ΔnlpI	biotin-B16-M30	~2	(Weyant et al., 2023)
			biotin-3XB16-M30	~6	
			biotin-Pfs25	~25	
			biotin-GFP	~27	
			biotin-Pfs230	~34	
			biotin-CRM197 (FtO-PS glycoconjugate)	~60	
			biotin-Sx-Cm-MOMP	~100	

3.2. *Post-production functionalization strategy*

Extracellular remodeling offers modularity by separately producing OMVs and POIs, then linking them in a standardized manner (Fig. 3 and Table 2). This approach circumvents challenges associated with expressing and localizing POIs to the OM. A recent study proposed a universal platform for assembling antigens onto OMVs through interactions between biotinylated antigens and ready-made OMVs displaying streptavidin (Weyant et al., 2023). This versatile tethering platform enables rapid development cycles for antigen-studded OMVs (Weyant et al., 2023).

The SpyCatcher-SpyTag protein ligation system and its orthogonal counterpart SnoopTag-SnoopCatcher provide stable, irreversible conjugation for displaying heterologous proteins (van den Berg van Saparoea et al., 2018). Derived from the *Streptococcus pyogenes* CnaB2 domain, this system involves a SpyCatcher domain and a SpyTag peptide that form a covalent isopeptide bond with high affinity (Zakeri et al., 2012). This strategy has allowed the modification of vesicles with over 20 different antigens in one step (Weyant et al., 2023). Such modular "plug-and-display" systems enable simultaneous presentation of multiple antigens, advancing on-demand tumor vaccine development (van den Berg van Saparoea et al., 2018; Cheng et al., 2021).

Hybrid vesicle systems are also emerging, particularly for tumor therapy, by combining biological specificity with synthetic versatility. Fusing tumor-derived vesicles with therapeutic OMVs (e.g., from *Akkermansia muciniphila*) provides a new paradigm for cancer vaccination (Tong et al., 2023). For example, hybrid membranes that integrate OMVs with tumor-cell membranes (e.g., CCM–OMV@Lip-ICG) endow pathogen absorption and adjuvanticity with autologous tumor antigens (Zhao et al., 2024). Bio-orthogonal click chemistry facilitates this hybridization simply and conveniently (Bong et al., 2024). Furthermore, OMV-functionalized nanoparticles (OMV-NPs) offer enhanced cell penetration and biocompatibility. For example, OMVs displaying azide-incorporated OmpA can be covalently coupled with alkyne-functionalized NPs to create new hybrid nano-objects.

While focused on biomedical applications, this hybrid strategy offers a powerful approach for purpose-specific vesicle fusion. Post-production functionalization provides flexibility without genetic redesign, enabling rapid prototyping and the incorporation of synthetic or non-biological modules. However, post-processing steps require further optimization, including incubation times and component ratios, to minimize unreacted substrates. Taken together, engineerable vesicles developed using synthetic biology toolboxes can significantly expand applications, though careful design and methodological selection remain essential as these choices substantially influence functional outcomes.

3.3. Potential of synthetic biology in OMV engineering

Synthetic biology offers a conceptual framework for transitioning OMV engineering from empirical, trial-and-error approaches toward systematic, modular, and predictive design. By integrating principles such as standardized biological parts, rational strain engineering, and iterative design–build–test–learn (DBTL) cycles, OMV platforms can be developed into programmable systems for both biotechnological and translational applications. In this context, OMVs serve as emerging biological chassis whose properties, governing biogenesis, cargo loading and functional output can be precisely tuned through genetic modules.

Current studies increasingly use engineered or detoxified strains, but these efforts remain largely case-specific (Table 2) (Weyant et al., 2023; Su et al., 2024). In principle, a synthetic biology DBTL cycle, combined with automated biofoundry platforms, could enable systematic scaling from single-gene screening to multi-gene combinatorial engineering, yielding universal OMV production hosts with tailored immunogenicity (Fig. 4) (Rosch et al., 2024). Adopting a systematic, design-driven approach to strain engineering, paired with scalable, high-throughput validation, would significantly accelerate the translational development of OMV-based platforms.

From a synthetic biology perspective, OMV cargo loading strategies can be decomposed into modular genetic elements that dictate protein localization and incorporation (Table 2). These include signal peptides for periplasmic targeting, membrane anchoring motifs, and vesicle-associated scaffold proteins such as ClyA- and OmpA-derived fusion partners. Together, these elements represent candidate "bioparts" that bias cargo distribution toward either the lumen or the vesicle surface. On the other hand, the 'post-production functionalization' strategy offers modularity and scalability in formulating synthetic vesicles. While OMVs are inherently heterogeneous at the single-particle level, this approach enables scalable cargo loading through dose-dependent binding (Schwechheimer and Kuehn, 2015; Weyant et al., 2023). However, these potential 'bioparts' have not been systematically standardized or benchmarked across studies, limiting their rational selection and interchangeability. Establishing a curated and comparable set of such standardized parts remains a critical future direction.

Another promising avenue in OMV engineering is the integration of genetic circuits to enable dynamic control over OMV production and cargo loading (Fig. 4). In principle, inducible promoters, quorum sensing modules, or stress-responsive regulatory systems could be coupled to vesiculation pathways to modulate OMV output in response to environmental or cellular signals. This approach is especially relevant for applications such as engineered living therapeutics, where temporal and condition-dependent release of functional OMVs is desirable. The integration of disease-responsive biosensors into circuit-defined OMV biogenesis could provide unprecedented opportunities for translational applications (Zhu et al., 2025). Overall, synthetic biology promises transformative opportunities for OMV engineering, and while its full potential is yet to be realized in practical applications, it remains a frontier for intensive applied development.

4. Biomedical and biotechnological applications of OMV

The applications of OMVs, initially restricted to the biomedical field due to their native properties, are rapidly expanding into diverse sectors through advancements in synthetic biology. Here, we discuss two main categories for their applications: biomedical and biotechnological.

4.1. Biomedical applications

4.1.1. Vaccines and adjuvants

Native OMVs represent promising vaccine platforms since they naturally mimic parental OM components, functioning as immunogenic facsimiles without replication capability. Surface-exposed pathogen-associated molecular patterns (PAMPs), including flagella, lipoproteins, and LPS, provide intrinsic adjuvant properties that robustly stimulate host innate and adaptive immune responses. Furthermore, their

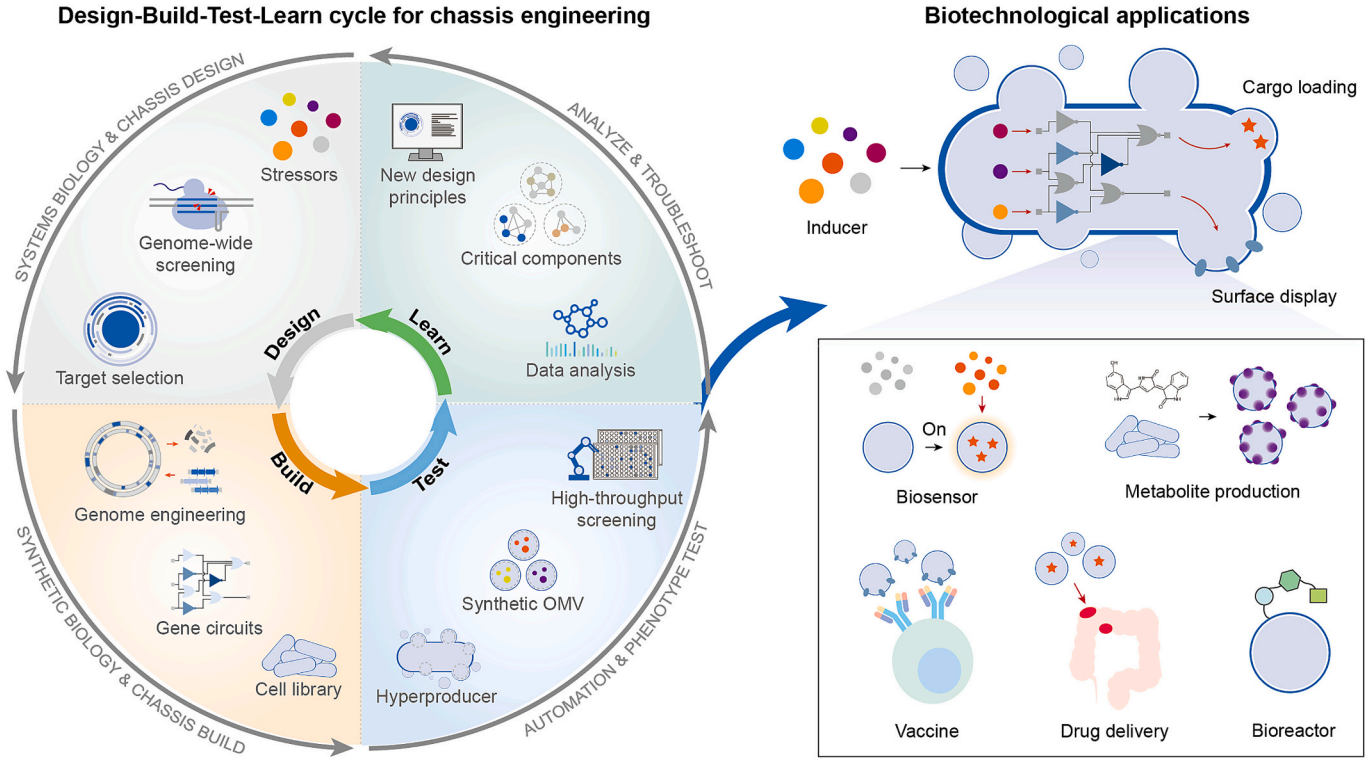


Fig. 4. Synthetic biology framework for OMV engineering and applications. Through iterative DBTL cycles, OMV production can be progressively enhanced, while enabling the on-demand tailoring of immunogenic properties. By integrating genetic circuits, synthetic biology approaches expand the functional potential of OMVs and accelerate their translational development, ranging from clinical therapeutics to biomanufacturing platforms.

nanoscale dimensions are optimal for lymph node trafficking and uptake by antigen-presenting cells (APCs), a prerequisite for eliciting antigen-specific immunity. These inherent advantages have culminated in several clinically approved OMV-based vaccines, such as Bexsero® for meningococcal prevention and PedvaxHIB® against *Haemophilus influenzae* type B (Table 3) (Petousis-Harris, 2018; Petousis-Harris and Radcliff, 2019).

Beyond native forms, bioengineered OMVs offer ideal platforms for developing multivalent vaccines with enhanced immunogenicity in a readily producible format. Generalized modules for membrane antigens (GMMA) represent an engineered OMV technology designed to streamline vaccine development, notably for shigellosis (Table 3) (Mancini et al., 2021). The GMMA platform typically incorporates three key elements: hyper-vesiculation for high-yield production, purpose-specific tailoring (including the overexpression of target antigens and elimination of off-target molecules), and detoxification. The first GMMA-based vaccine to enter clinical trials, 1790GAHB, utilizes genetically modified *S. sonnei* harboring penta-acylated LPS to minimize reactogenicity, further stabilized with Alhydrogel.

Collectively, the physicochemical properties of OMVs and GMMA technology enable these platforms to address global health concerns as affordable vaccine options, particularly for low- and middle-income countries. However, challenges remain in overcoming their potent reactogenicity and understanding their precise mechanisms of action to improve safety and tolerability.

4.1.2. Drug delivery

Recent shifts in cancer therapy toward immunotherapy have highlighted OMVs as efficient drug delivery vehicles due to their native immunogenicity (Table 3). By encapsulating biomaterials, OMVs protect payloads from degradation while their nanoscale size facilitates tissue penetration via the enhanced permeability and retention (EPR) effects. Through surface PAMPs, OMVs stimulate diverse immune cell types, including dendritic cells and macrophages, initiating potent downstream responses (Xiang et al., 2024). This capacity can be synergistically enhanced through targeted vesicle modifications.

In 2014, target-specific tumor suppression was demonstrated using siRNA-packaged OMVs displaying HER2-specific affibodies and utilizing under-acylated LPS for enhanced tolerability (Gujrati et al., 2014). Additionally, integrating OMVs into nanorobot fabrication replaces bio-incompatible materials, expanding robotic applications in oncology (Tang et al., 2024). Significant potential was also shown in skin melanoma models, where OMVs modified with $\alpha_v\beta_3$ integrin ligands and indocyanine green (ICG) enabled effective transdermal delivery and TRAIL-mediated apoptotic responses (Peng et al., 2020). Such specific delivery mechanisms mitigate the systemic toxicity often associated with conventional anticancer therapies.

Engineered living therapeutics represent an emerging class of microbial-based treatments. Despite progress, challenges regarding bacterial density and biocontainment persist. A recent study addresses these issues using commensal-derived OMVs from *E. coli* Nissle 1917 (EcN), which protect therapeutic payloads under harsh in vivo conditions and penetrate gut barriers via bacterial translocation (Gong et al., 2025). Furthermore, OMVs are gaining attention as "bactofection" platforms, providing a non-viral paradigm for nucleic acid transfer into mammalian cells with lower toxicity and the capacity to carry large DNA fragments (Heggie et al., 2025).

4.1.3. Challenges and opportunities in clinical translation

Since OMVs inherently contain LPS and other bioactive metabolites, the vesicle itself is pharmacologically active. Thus, clinical safety must be viewed as an intrinsic property of the platform rather than a mere impurity issue (He et al., 2020). In vaccine applications, the immunogenic properties are favorable since dosing is local and immunization is the intended mechanism (Keiser et al., 2010; Lee et al., 2023). However, OMV-based non-vaccine applications would be restricted under far tighter pyrogen and endotoxin expectations. Consequently, detoxification strategies are central to reducing pyrogenicity, though these often involve trade-offs in self-adjuvanticity or altered biodistribution (Keiser et al., 2011; Chen et al., 2025). For example, a recent study showed that LPS removal increased maximal tolerated dose by more than 25-fold, while also changing physicochemical and immune-stimulatory properties, highlighting the trade-off between safety and the native adjuvant effect (Chen et al., 2025).

In addition to immunogenicity, OMV populations exhibit higher heterogeneity in size and composition compared to synthetic vesicles

Table 3
Biomedical applications of OMVs.

Biomedical applications					
Application area	Name	Origin	Target	Formulation	References
Vaccine development	VA-MENGOC-BC™	*N. meningitidis*	MenB/C	dOMVs with PorA	(Pérez Rodríguez et al., 2011, Ochoa-Azze, 2018)
	MeNZB™	*N. meningitidis*	MenB	dOMVs with PorA P.1.7–2,4	(Petousis-Harris, 2018, Petousis-Harris and Radcliff, 2019)
	Bexsero®	*N. meningitidis*	MenB	dOMVs with PorA P1.4/rfHbp/ rNHBA/ rNadA	(Petousis-Harris, 2018, Studholme et al., 2019)
	Avacc10®	*N. meningitidis*	SARS-CoV-2	OMVs with Spike protein	(van der Ley et al., 2021)
	S. sonnei 1790 GAHB	*S. sonnei*	Shigellosis	GMMA with O-antigen	(Gerke et al., 2015)
	altSonflex1–2-3	*S. sonnei* *S. flexneri*	Shigellosis	GMMA with O-antigen	(Leroux-Roels et al., 2024)
	Salmonella OMV-Sf2a	*S. enterica*	Shigellosis	GMMA with O-antigen	(Tian et al., 2021)
	O-antigen-CRM197	*S.* Typhimurium 2189 *S. enteritidis* 618	Salmonellosis	GMMA with O-antigen	(Micoli et al., 2018)
	Salmonella enterica serovar Paratyphi A	*S.* Paratyphi A NVGH2041	Salmonellosis	GMMA with Vi/O:2	(Gasperini et al., 2021)
	PedvaxHIB®	*N. meningitidis* B11	HIB	PRP-OMPC conjugate	(Ahonkhai et al., 1991)
Drug delivery	$Affi_{HER2}OMV^{siRNA}$	*E. coli*	Tumor	siRNA packaged OMVs with HER2 affibody	(Gujrati et al., 2014)
	OMV-siR robot	*E. coli*	Tumor	siRNA packaged OMV with CPP	(Tang et al., 2024)
	I-P-OMVs	*E. coli*	Melanoma	OMVs with $\alpha_v\beta_3$/ ICG	(Peng et al., 2020)
	EcN OMVs	*E. coli* Nissle 1917	Detoxification	Enzyme packaged OMVs	(Gong et al., 2025)

(Note: MenB; *Neisseria meningitidis* serogroup B, MenC; *N. meningitidis* serogroup C, dOMVs; detergent-based extracted OMVs, PorA; *N. meningitidis* Porin A, rfHbp; recombinant factor H binding protein, rNHBA; recombinant Neisserial heparin-binding antigen, rNadA; recombinant Neisserial adhesion A, Shigellosis; an infectious disease caused by a group of *Shigella*, GMMA; Generalized modules for membrane antigens, PRP; Polyribosyl ribitol phosphate, OMPC; Outer membrane protein complex, HIB; *Haemophilus influenzae* type B, HER2; Human epidermal growth factor receptor 2, CPP; Cell-penetrating peptide, ICG: Indocyanine green).

(Schwechheimer and Kuehn, 2015). OMV particle abundance and content vary with production conditions, including growth medium and culture duration, complicating dose selection and comparability across studies (Yu et al., 2024). Such compositional drift raises significant analytical burdens for clinical translation. For example, the release specifications for Bexsero® are extensive, encompassing purity, protein concentration, endotoxin levels, and process related impurities (Deghmane and Taha, 2022).

While bacterial fermentation is generally cost-effective, the clinical translation of OMVs is frequently constrained by low yields and high purification costs of downstream processing (Torres-Vanegas et al., 2024). These factors can erode the cost advantage of upstream manufacturing. From a regulatory standpoint, OMVs currently benefit more from "vaccine" precedent. Non-vaccine applications will likely face rigorous review under General Biologics Guidances from the EMA or the FDA (Table 3). Ultimately, successful translation will require positioning OMVs as a distinct platform with inherent immune-activating properties, ensuring sufficient analytical transparency to distinguish beneficial adjuvanticity from uncontrolled reactogenicity.

4.2. Biotechnological applications

4.2.1. Bioreactors

Functionalized OMVs equipped with catalytic modules serve as nanoscale bioreactors, facilitating specialized biochemical reactions (Table 4). Compared to free enzymes in solution, OMV-immobilized enzymes demonstrate superior robustness and resistance to environmental changes (Homaei et al., 2013). Additionally, this immobilized strategy enables streamlined recovery of both enzymes and products, supports continuous operation, allows for rapid reaction termination, and offers greater flexibility in bioreactor design (Di Lorenzo et al., 2022). By leveraging these advantages while bypassing the metabolic constraints associated with living cells, functionalized OMVs have become increasingly attractive for diverse biocatalytic applications (Amalia and Tsai, 2023).

In *E. coli*, researchers have demonstrated OMV-mediated bioremediation for the degradation of β-lactam antibiotics (Woo et al., 2022). Synthetic OMVs expressing β-lactamase exhibited significantly enhanced catalytic activity, hydrolyzing the β-lactam rings of nitrocefin and meropenem at rates 100- and 600-fold higher than those of whole-cell biocatalysts, respectively. In aqueous environments, these OMV-encapsulated enzymes showed markedly improved stability against thermal and acidic stresses compared to their free-form counterparts.

Another critical advantage of OMV-based catalysis is the precise spatial organization of functional enzymes (Park et al., 2014). In enzymatic cascade reactions, proximity effects accelerate overall reaction rates by situating intermediates closer to subsequent enzymes, thereby increasing collision frequency and catalytic efficiency. This concept was effectively validated in OMVs through the sequential assembly of three cellulases, emphasizing the importance of enzyme proximity and cooperative action in complex cascades (Park et al., 2014). Similarly, multi-step catalysis for fatty acid conversion has been developed in engineered OMVs, further demonstrating their utility in sophisticated cascade reactions (Song et al., 2021).

Overall, synthetic OMVs offer substantial catalytic potential by providing physical protection, enhanced stability, and insulation from cellular metabolic interference. Their tolerance to toxic conditions further expands their applicability in environmental waste degradation and biomass conversion.

4.2.2. Bioimaging and biosensing

Extracellular vesicles provide biocompatible and scalable alternatives to synthetic nanoparticles for diagnostic and therapeutic applications. Bacterial OMVs represent an optimal choice due to their long-term stability, biosafety, genetic customizability, and suitability for large-scale production (Table 4). A recent study explored OMVs for

Table 4
Biotechnological applications of OMVs.

Biotechnological applications				
Application area	Key example	Technical principles	Advantages	References
Bioreactors	Antibiotic degradation	Safeguards for enzymes	Physical protection	(Woo et al., 2022)
	Cellulose degradation	Spatial organization of enzymes	Increased reaction rate	(Park et al., 2014)
	Fatty acid conversion	Spatial organization of enzymes	Increased reaction rate Biocompatibility	(Song et al., 2021)
Bioimaging and biosensing	Theranostics	Encapsulation of biopolymer-melanin	Deep-tissue optoacoustic imaging Photothermal therapy	(Gujrati et al., 2019)
	Biosensors	Encapsulation of Nluc	Good LOD (0.5 nM thrombin) Design flexibility	(Bitto et al., 2017)
	Immunoassay	Co-expression of enzyme reporter and IgG-binding domain	Good LOD (5 ng/mL IgG) Cost-effective	(Huang et al., 2019)
	Biomarkers	Polymyxin B-FITC probe	High specificity Real-time diagnosis	(Li et al., 2025)
	Radiolabeled OMVs	Functionalization with radiolabeling	Quantitative molecular imaging	(Szöllősi et al., 2023)
Membrane research	Membrane protein research	OMP enriched vesicles	Proper folding and functionality in native context	(Thoma et al., 2018)
	Drug target discovery	Proteomic analysis of OMVs	Drug accessibility to potential targets	(Altindis et al., 2014)
	Host-microbe interaction research	Proteomic analysis of OMVs	Reduced proteomic complexity	(Bhar et al., 2021)
Synthetic biology and metabolic engineering	Bioengineering	Functionalization with heterologous proteins	Intact functionality Application potentials	(Kim et al., 2008)
	Proteome-minimized OMVs	proteome-reduced OMV chassis	Improved antigen payload	(Zanella et al., 2021)
	Recombinant protein production	Recombinant-protein-filled vesicles	High-yield protein production	(Eastwood et al., 2023)
	β-carotene production	Reshaping of microbial morphology	Improved productivity	(Wu et al., 2017)
	β-carotene production	Reshaping of microbial morphology	Improved productivity	(Wu et al., 2019)
	Hydrophobic natural product production	Reshaping of microbial morphology	Improved productivity	(Yang et al., 2021)
Environmental applications	Bioremediation	Tolerability of OMV-encapsulated enzymes	Thermostability Freeze-thaw stability Field-deployable	(Alves et al., 2016, Su et al., 2017, Thakur et al., 2022)

(Note: LOD; Limit of detection).

optoacoustic imaging and theranostics by packaging naturally occurring melanin into vesicles (OMV^{Mel}) (Gujrati et al., 2019). Melanin enhances contrast in multi-spectral optoacoustic tomography (MSOT) and enables photothermal therapy by converting light into localized heat to target tumor cells. These melanin-loaded OMVs exhibit approximately 40% photothermal conversion efficiency in the near-infrared spectrum, comparable to gold nanorods, while remaining fully biodegradable. These findings establish OMV^{Mel} as a promising theranostic agent, with potential for future applications using other naturally derived cargos in imaging-based cancer therapy.

Moreover, fluorescence-labeled OMVs function effectively as in vivo biosensors. The encapsulation of nano-luciferase (Nluc) within the OMV lumen enables highly sensitive luminescence signals, and these reporting moieties can be flexibly engineered for diverse diagnostic purposes (Chen et al., 2017). By displaying IgG-binding domains on the OMV surface while localizing luminescent enzymes internally, researchers have developed low-cost immunoassays that circumvent the need for complex chemical conjugation (Huang et al., 2019). Furthermore, OMVs labeled with fluorescently conjugated polymyxin B derivatives, antibiotics known for selective binding to bacterial LPS, have been engineered for the real-time diagnosis and differentiation of bacterial infections in clinical samples, advancing their role as promising biomarkers (Li et al., 2025).

Recently, radiolabeled OMVs have been developed by conjugating vesicles with radiometal chelators, achieving high-yield labeling for positron emission tomography (PET) (Szöllősi et al., 2023). Such advancements expand the versatility of OMVs in molecular imaging and underscore their emerging value in clinical diagnostics. With further refinements in immunogenicity, tissue penetration, and biodegradability, OMVs are poised to become highly versatile platforms for next-generation bioimaging and biosensing.

4.2.3. Membrane research

OMVs provide an excellent platform for studying OMPs by mimicking native membrane environments with enriched protein content. As frontline barriers, OMs dynamically shape and protect cellular compartments. Despite their significance, in-depth studies of these components remain challenging due to a lack of appropriate tools. The primary challenge involves preserving native structural integrity and composition during the solubilization and reconstitution processes commonly used in membrane protein studies. In bacteria, OMPs function in a strictly lipid-dependent manner, necessitating complete mimicry of the native environment (Serdiuk et al., 2015).

OMVs preserve an authentic native environment, maintaining precise lipid composition, asymmetry, and protein orientations. This native context guarantees the functional integrity of target OMPs. Furthermore, OMVs can be selectively enriched with specific OMPs, enabling focused biophysical characterization and more rapid preparation compared to conventional detergent-based methods **(**Table 4**)** (Thoma et al., 2018). High-resolution studies in *E. coli* have revealed distinct unfolding pathways for OmpG, FhuA, Tsx, and BamA within OMVs, highlighting the critical importance of structure-function relationships in membrane proteomes (Thoma et al., 2018).

OMVs derived from pathogenic bacteria contribute to a systemic understanding of surface architecture and core proteins involved in host colonization, thereby supporting drug development efforts **(**Table 4**)** (Altindis et al., 2014). Pathogens employ diverse surface-mediated strategies, balancing adhesion, immune evasion, and cellular invasion, to interact with hosts. These interactions are critical for establishing infection and overcoming host defenses. Therefore, OMVs provide comprehensive insights into virulence factors as reliable "surface proxies." For commensal bacteria, microbiota-derived OMVs facilitate the exploration of host-microbiome interactions through the characterization of membrane-bound transporters, receptors, and signaling channels (Bhar et al., 2021).

4.2.4. Synthetic biology and metabolic engineering

Early work demonstrated that engineering OMVs by fusing ClyA with heterologous proteins such as β-lactamase, organophosphorus hydrolase, GFP, and an anti-digoxin scFv drives their selective packaging into OMVs in *E. coli* (Kim et al., 2008). These surface-displayed enzymes fully retained their catalytic activity, highlighting that direct localization of POIs to OMVs can be achieved while preserving functional integrity. Moreover, these findings suggest that synthetic vesicles can expand non-native functionalities, including fluorescence and antigen binding, potentially serving as reusable nano-biocatalysts for targeted delivery and catalysis.

Another synthetic biology-driven approach utilized "proteome-minimized OMVs," in which non-essential proteins are systematically removed to expand the vesicles' capacity for antigen payloads while simplifying downstream processing (Zanella et al., 2021). In parallel, high-level recombinant protein encapsulation in *E. coli*-derived OMVs has been shown to substantially boost yields and streamline purification steps, providing a robust pathway for repurposing OMVs in biomanufacturing (Eastwood et al., 2023).

Reshaping microbial morphology, such as increasing membrane surface area or inducing intracellular droplet production, can provide critical storage space for hydrophilic compounds. Several studies have suggested that the limited spatial capacity of microorganisms is directly linked to the poor titer of hydrophobic compounds (Elhadi et al., 2016; Liu et al., 2021). In this regard, OMVs may serve as efficient scaffolds for metabolic engineering **(**Table 4**)**. For instance, β-carotene, a carotenoid of significant commercial interest in the pharmaceutical and cosmetic industries, cannot be effectively secreted by natural transport systems due to its size and hydrophobicity. This often leads to membrane accumulation, which hinders further production and imposes a metabolic burden on host cells. In *E. coli*, inducing OMV formation has proven to be an effective morphological reshaping strategy, which facilitates the excretion of β-carotene through OMVs, thereby expanding product reservoirs and relieving intracellular metabolic stress (Wu et al., 2019).

A recent study further demonstrated that metabolically engineered *E. coli* strains can produce seven natural colorants spanning the rainbow spectrum (Yang et al., 2021). In this work, the production of these colorants was significantly improved through the integration of systemic metabolic engineering, morphological reshaping via the formation of both OMVs and inner membrane vesicles (IMVs), along with the optimization of fermentation processes (Yang et al., 2021).

4.2.5. Environmental applications

The environmental implications of OMVs are significant, particularly in the hydrolysis of organophosphate pollutants widely used as agricultural pesticides **(**Table 4**)**. OMVs, within which enzymes are encapsulated or displayed, preserve catalytic activity under conditions that would otherwise inactivate free enzymes, providing robust strategies amenable to field use. This safeguarding effect was demonstrated by fusing di-isopropyl-fluorophosphatase (DFPase) to the Lpp anchor, ensuring the selective recruitment of enzymes into *E. coli* OMVs (Thakur et al., 2022). These OMV-encapsulated DFPase effectively hydrolyze paraoxon and maintained 50–70% activity after room-temperature storage and repeated freeze-thaw cycles, whereas free enzymes retained only 20% activity. Notably, the activity of OMV-encapsulated DFPase remained stable even at extreme temperatures (up to 80 °C), which are highly relevant for field conditions.

In another study, organophosphorus hydrolase (OPH) was anchored to OMVs via an INP fusion combined with a cellulose-binding domain (CBD), which enabled over 15 repeated reaction cycles with negligible activity decay (<20%), improved turnover rates, broader pH tolerance (pH 5–11), and enhanced thermal stability (up to 70 °C) compared to the free enzyme. Shelf-stable OMV-packaged phosphotriesterase (PTE) was also developed using a split SpyCatcher/SpyTag system, which maintained 100-fold higher activity after two weeks at 37 °C against inactivation from repeated freeze-thaw cycles and lyophilization (Alves et al.,

2016).

Collectively, functionalized OMVs serve as "plug-and-play" nanoreactors that protect delicate enzymes from harsh environmental stressors, such as extreme temperatures, desiccation, and proteolytic degradation, while simplifying processing and enzyme recovery. By bypassing the need for inefficient cold-chain logistics and eliminating the risks associated with dead cells in immobilized biocatalysts, these cell-free green routes establish OMVs as a flexible platform for on-site pollutant treatment. The inherent biodegradability of OMV-mediated bioremediation further makes it an ideal platform for sustainable, real-world applications.

5. Future perspectives

Advances in synthetic biology are rapidly accelerating the development and application of synthetic vesicles. In this context, OMVs are emerging as versatile, modular bio-parts that enable interchangeable design and high-throughput engineering across multiple disciplines (Fig. 3). With their naturally spherical backbone, researchers can precisely determine which functions to incorporate, define the spatial localization of functional modules, and select methods that maximize delivery or catalytic efficiency. Artificial intelligence tools for prediction and simulation are expected to revolutionize high-throughput prototyping, allowing for rapid design optimization with minimal experimental overhead. Currently, structure-prediction pipelines such as AlphaFold-Multimer enable in silico screening of autotransporter fusion orientations prior to cloning, significantly streamlining OMV-display design (Schiffrin et al., 2024).

Synthetic OMVs also offer powerful advantages for investigating protein-protein interactions (PPIs). Unlike traditional methods, they mimic native membrane environments without requiring complex protein purification, all while maintaining remarkable engineering flexibility. OMV-based PPI analysis facilitates the straightforward validation of functional interfaces in artificial domains and signaling pathways that often resist conventional purification techniques. This approach further enables high-throughput screening to selectively target domain-domain interactions, providing more physiologically relevant drug target validation than traditional in vitro assays. When integrated with droplet-based microfluidics, researchers can observe these interactions in near-natural conditions using minimal sample volumes, thereby reducing costs. Furthermore, incorporating high-content imaging into these platforms promises to transform our understanding of membrane protein dynamics at unprecedented resolution.

Synthetic biology is yielding OMVs with novel biophysical properties. We anticipate the development of smart vesicles that release cargo in response to specific environmental triggers as well as self-assembling structures that mimic complex cellular architectures. Hybrid materials integrating biological and synthetic components will likely enhance stability and functional diversity. These innovations could lead to programmable vesicles capable of navigating biological systems to target specific cell types and deliver therapies with minimal off-target effects.

OMVs functionalized with regulatory genetic circuits offer enhanced control over system behavior, especially for biomedical applications. These engineered feedback mechanisms could create responsive drug delivery systems that adjust their release profiles based on real-time physiological conditions or local biomarkers. Additionally, size-controllable platforms will extend the utility of OMVs by allowing the selective encapsulation of molecules with varying molecular weights and chemical properties.

Optimizing downstream processing for quality-controlled OMV isolation remains crucial for industrial scalability. Various techniques based on density, size, precipitation, or affinity have been established, and the choice of methodology significantly impacts OMV purity, yield, and scalability (Xie et al., 2022; Torres-Vanegas et al., 2024). Continuous advancements in purification, combined with the development of production chassis tailored to specific application requirements, will be essential for achieving peak performance. As our fundamental knowledge of vesicle biology matures alongside our engineering capabilities, synthetic OMVs are positioned to transform diverse fields at the intersection of biology, medicine, and materials science, offering unprecedented opportunities to address complex challenges.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Byung-Kwan Cho and Suhyung Cho report financial support was provided by National Research Foundation of Korea. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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