Applying synthetic biology in infectious disease prevention and facing biosecurity challenges

Tian Qi[a,1], Yue Fang[a,1], Wenqing Liu[a,1], Xiao He[a], Lingzhe Kong[a], Ruibing Chen[a,b,*], Lei Zhang[a,c*]

[a] Department of Pharmaceutical Botany, School of Pharmacy, Naval Medical University, Shanghai, China.

[b] State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs, Beijing, China.

[c] College of Life Sciences and Medicine, Key Laboratory of Plant Secondary Metabolism and Regulation of Zhejiang Province, Zhejiang Sci-Tech University, Hangzhou 310018, China.

[*] Corresponding author.

Ruibing Chen, rbchenstar@163.com

Lei Zhang, nmu_dpb@163.com

[1] Tian Qi & Yue Fang & Wenqing Liu contributed equally to this work.

Abstract: Current threats of infectious diseases are severe, marked by prominent challenges such as emerging and re-emerging infections and antimicrobial resistance. Traditional prevention and control methods face limitations including lengthy development cycles, relatively narrow therapeutic targets and insufficient diagnostic sensitivity. Synthetic biology, an emerging interdisciplinary field, utilizes the "Design-Build-Test-Learn" (DBTL) cycle applied in numerous research studies within the biosecurity field. This review therefore focuses on the recent innovations and advances of synthetic biology techniques making the prevention and treatment of infections more efficient. For bacterial infections, these include engineered phage therapy, synthetic antimicrobial peptide design, and quorum sensing interference. For viral infections, the review covers intelligent diagnostic technologies, mRNA vaccines development, and CAR-T cell therapy. For invasive fungal diseases, the application of synthetic biology has enabled the discovery of novel drug targets and the development of antifungal nanomaterials. At the same time, however, the rapid advances in synthetic biology pose some other significant biological safety challenges. It is imperative to establish a tripartite combination that integrates institutional, technological, and ethical measures, thereby forging a development pathway that balances innovation efficacy with safety constraints. In conclusion, applying synthetic biology in infectious disease prevention uncontroversially offers novel routes to face severe biosecurity challenges.

Keywords: Infectious diseases; Synthetic biology; Antimicrobial resistance; Diagnostics; Biosecurity

The global public health system is currently facing increasingly severe challenges posed by infectious diseases. The frequency of outbreaks of emerging and re-emerging infectious diseases has increased significantly. The COVID-19 pandemic has resulted in over 7 million deaths worldwide[1], and potential pathogens capable of causing global pandemics, such as highly pathogenic avian influenza, Ebola virus disease, and "Disease X" – repeatedly emphasized by the World Health Organization (WHO) in recent years – continue to threaten human health[2,3]. The spread of antimicrobial resistance has also become a major public health crisis. WHO data indicates that the detection rates of multidrug-resistant bacteria, such as Carbapenem-resistant *Enterobacteriaceae* (CRE) and Methicillin-resistant *Staphylococcus aureus* (MRSA), continue to climb, directly causing approximately 1.27 million deaths annually. Projections suggest that by 2050, the annual death toll related to antimicrobial resistance will exceed 10 million[4]. Furthermore, invasive fungal diseases cause over 1.5 million deaths each year, with 19 fungal pathogens, including *Cryptococcus*, *Candida auris*, and *Aspergillus fumigatus*, listed in the WHO's Fungal Priority Pathogens List[5,6]. It is undeniable that the conventional strategies such as broad-spectrum antibiotic development, attenuated/inactivated vaccine platforms, and chemical library screens for antimicrobials have exhibited great value in the development of drugs and treatment methods in the past, helping us survive in infectious diseases. However, current prevention and control measures for infectious diseases face significant challenges: the development pipeline for new antibiotics has progressed slowly over the past 30 years; vaccine development cycles struggle to keep pace with pathogen mutation rates; resistance to traditional antifungal drugs is becoming increasingly severe; and the sensitivity and accessibility of existing detection and diagnostic technologies remain relatively low[7,8].

Against this backdrop of escalating infectious disease threats and the limitations of traditional methods , the new ideas and tools stemmed from synthetic biology are highly anticipated to address these challenges. Synthetic biology is regarded as the third revolution in the life sciences following the discovery of the DNA double helix

and the Human Genome Project[9–11]. Through the "Design-Build-Test-Learn" (DBTL) cycle, it integrates core technologies such as gene editing, DNA synthesis, and biosensing to achieve precise design and reconstruction of biological systems[12]. Exemplified by engineered phages specifically targeting drug-resistant bacteria, biological components quickly adaptable to emerging viruses, and mRNA vaccines, synthetic biology enables the development of more precise and rapid strategies in the context of infectious disease prevention and control[13,14].These advancements thereby transcend the constraints of traditional methods and pioneer a strategic transformation in our overall approach to combating infectious diseases.

However, the rapid development of synthetic biology also presents significant biosecurity challenges, creating a double-edged sword effect of "technology empowerment versus risk management". Organisms altered through synthetic biology techniques may carry risks such as dangerous mutations, uncontrolled spread, and environment disruption[15,16]. These risks may lead to new and unpredictable infection crises that are difficult to control. Therefore, establishing a responsible innovation framework, where safety-by-design principles are integrated into the research and development lifecycle, is essential for harnessing the benefits of synthetic biology while mitigating its risks[17]. In summary, this review aims to systematically explore the breakthroughs in the application of synthetic biology for infectious disease prevention and control, analyze the accompanying biosecurity challenges, and provide insights to promote the innovative development in this field **(Fig. 1)**.

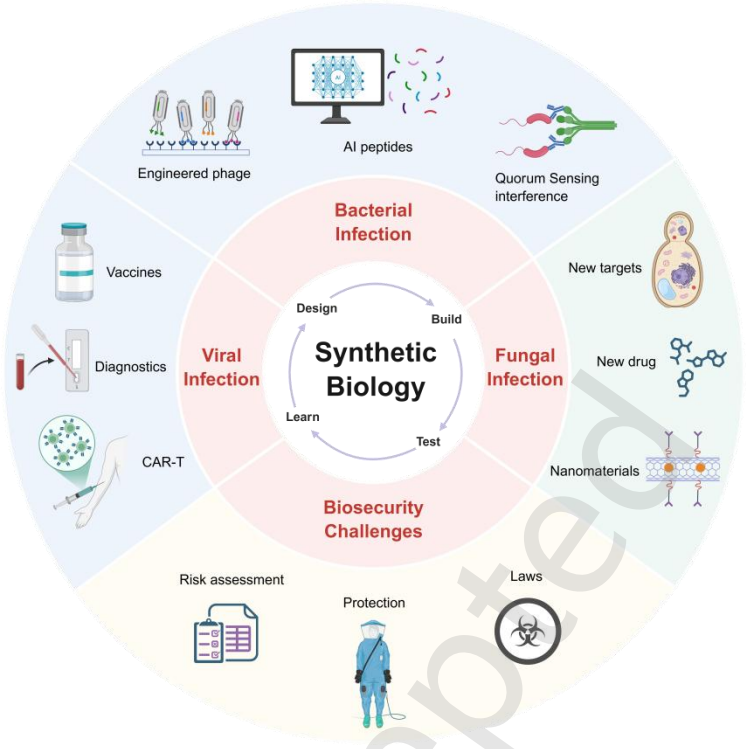

Fig 1. Schematic of synthetic biology applications in infectious disease prevention and associated biosecurity challenges. Bacterial infection: engineered phages, synthetic antimicrobial peptides, quorum sensing interference. Viral infection: intelligent diagnostic technologies, vaccine design, CAR-T cell therapy. Fungal infection: novel drug targets, antifungal nanomaterials. Biosecurity challenges: legal regulations, risk assessment, and protective measures.

1. Application of synthetic biology in the prevention and control of bacterial infectious diseases

1.1 Current status of bacterial infections

Bacterial infections refer to the process where pathogenic or conditionally pathogenic bacteria invade the host organism, proliferate, and lead to local or systemic pathological reactions. Common bacterial infectious diseases include infectious diarrhea, pneumonia, among others. Infectious diarrhea encompasses cholera, *Salmonella* infections, *E. coli* infections, etc.; pneumonia includes diseases

caused by *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, etc.; and other various bacterial infectious diseases such as gonorrhea, tuberculosis, and anthrax[18].

According to a 2022 Global Burden of Disease study led by Professor Mohsen Naghavi, common bacterial infections ranked as the second leading cause of death worldwide in 2019, resulting in 7.7 million deaths and trailing only ischemic heart disease[19]. The study further highlighted that five bacterial pathogens (*Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*) accounted for more than half of these global bacterial death-related cases. The significant health burden associated with these pathogens necessitates heightened global health attention. Concurrently, with the emergence of "superbugs" like Methicillin-resistant *Staphylococcus aureus* (MRSA), Carbapenem-resistant *Enterobacteriaceae* (CRE), and Vancomycin-resistant *Enterococci* (VRE), the efficacy of traditional antibiotic treatments has declined significantly. For instance, the clinical efficacy rate of β-lactam antibiotics against MRSA has dropped to below 30%. More critically, the pipeline for new antibiotics has nearly dried up over the past 30 years, with only 12 new antibiotics approved globally between 2010 and 2019, most being structural analogs of existing antibiotics[4].This current situation urgently requires breakthrough therapeutic strategies to address the increasingly severe bacterial resistance crisis.

1.2 Engineered phage therapy

Bacteriophages are kind of viruses that specifically infect bacteria, archaea, and algae, widely present in soil, river water, air, and living organisms. Under electron microscopy, bacteriophages are classified into three primary morphological types:tailed, spherical, and filamentous. The majority of characterized phages possess a tailed structure, consisting of a head and a tail. The chemical composition of a bacteriophage comprises a protein-encased capsid and internal nucleic acid. The nucleic acid resides in the head; the DNA of most phages is double-stranded, while proteins form both the head capsid and the tail **(Fig. 2A)**. Since their discovery in

1915, their advantages, such as relatively small genomes and fast reproduction rates, have made them important materials or tools in basic biological research on genetic regulation and genetic engineering, driving development in many fields of biology[20]. However, natural phages have limitations including a narrow host range, the development of bacterial phage tolerance, and instability of phage particles, which restrict their application[21]. Adopting synthetic biology methods and concepts for the rational design and synthesis of artificial phages can enhance, alter, or utilize specific characteristics of phages while compensating for their shortcomings. These modifications of phages primarily focus on several aspects: adding functional genes to the phage genome to enhance infectivity efficiency, reprogramming tail fiber to alter host range, and functionalizing capsid proteins to facilitate the packaging and delivery of antimicrobial cargo **(Fig. 2B** and **2C)**[22,23].

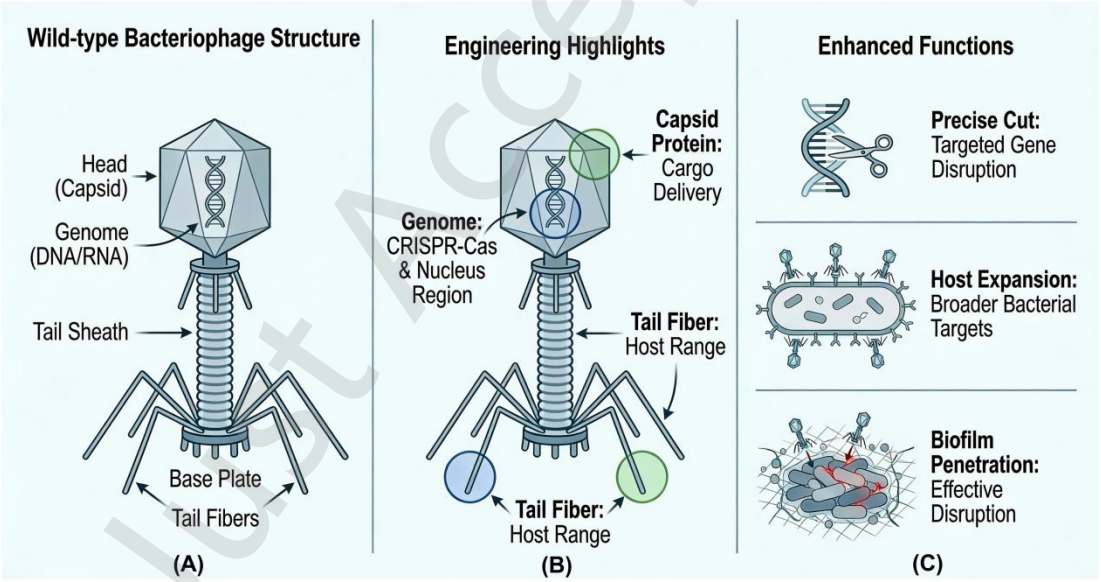

Fig 2. Synthetic biology-driven engineering of bacteriophages for antimicrobial applications. (A) Wild-type bacteriophage structure showing core components. (B) Key engineering targets: genome (CRISPR-Cas integration) and tail fiber (host range modification). (C) Enhanced functions achieved through engineering, including targeted gene disruption, host expansion, and biofilm penetration.

The Danish company SNIPR Biome has developed a CRISPR-armed phage (SNIPR001) capable of precisely targeting and clearing drug-resistant *E. coli* in the

gut by introducing the CRISPR-associated protein system. SNIPR001 consists of four engineered phages, each carrying a specific gene editing system that can precisely identify and cleave key regions of the *E. coli* genome, leading to bacterial DNA breakage and death. Unlike traditional phages, this design not only relies on the natural bacteriolytic ability of phages but also enhances bactericidal efficiency through the CRISPR system and reduces the risk of bacterial escape mutations. Furthermore, by optimizing the phage's tail fiber proteins, it can recognize a wider range of *E. coli* strains, expanding therapeutic coverage. This research has currently entered Phase I clinical trials[24]. Biofilm structures are often associated with persistent chronic bacterial infections. Composed of various macromolecules such as proteins, polysaccharides, lipids, and DNA, biofilms can limit the diffusion of molecules and particles, thereby protecting pathogenic bacteria from attack by antimicrobial agents like phages and antibiotics, posing a significant obstacle in bacterial control and making many clinical infections difficult to eradicate[25–27]. Recent research has revealed a novel anti-biofilm strategy involving the Pf4 phage in *Pseudomonas aeruginosa* biofilms: this phage edits its own genome through the PfrT reverse transcriptase and PhrD RNA, generating "Super-Infective" (SI) variants. These variants can replace the original prophage in the host chromosome, subsequently prompting the host bacteria to produce high yields of SI phages and toxins, ultimately disrupting the biofilm through bacterial lysis and explosive phage release. This endogenous editing mechanism opens new avenues for developing synthetic biology tools to target and destroy drug-resistant bacterial biofilms[28].

Currently, the rapid development of Artificial Intelligence (AI) and high -throughput sequencing technology greatly aids the design and modification of phages [29–31]. By integrating omics data research with AI, the interaction mechanisms between phages and hosts can be understood comprehensively and systematically, such as phage gene expression regulation and how phages overcome bacterial immune defense mechanisms. This enables the rapid identification of the most effective phage combinations and even the design of synthetic phages with novel

functions[32]. However, to date, the vast diversity of phage types and gene pools in nature has not been fully utilized[33]. With continuous advancements in various technologies, the artificial synthesis of phage genomes will undoubtedly better address the various limitations of large-scale phage application and promote the faster development of phage applications.

1.3 Synthetic antimicrobial peptide design

Antimicrobial peptides (AMPs) are a large class of active oligopeptides with resistant functions against pathogens such as bacteria, fungi, parasites, and viruses[34,35]. Because they generally carry a sufficient amount of positive charge and often possess hydrophobicity, they can electrostatically interact with negatively charged biological membranes, penetrate, and disrupt membrane structures, leading to cell death[36,37]. Based on their origin and structural characteristics, they can be classified into natural antimicrobial peptides and synthetic antimicrobial peptides. Natural antimicrobial peptides are peptides extracted from plants, animals, or microorganisms. Although diverse, they often suffer from drawbacks such as low content, poor stability, cumbersome extraction steps, and high costs. Synthetic antimicrobial peptides, on the other hand, are prepared through chemical synthesis or genetic engineering methods, modifying the structures of natural antimicrobial peptides to enhance their antibacterial activity or improve stability[38]. Currently, most antimicrobial peptides are biosynthesized using microbial expression systems. In prokaryotic expression systems, *E. coli* is the most commonly used due to its fast growth rate, low cultivation cost, and well-understood biological background[39]. Among eukaryotic expression systems, yeast expression systems are commonly used, including *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Chlamydomonas reinhardtii*[40]. However, using microbial expression systems to produce natural antimicrobial peptides can sometimes result in defects such as excessively low yield, high host toxicity, insufficient resistance activity, and some AMPs may exhibit significant hemolysis, making their efficient synthesis challenging[41,42].

The development of synthetic biology and the rise of AI provide powerful tools for the design of antimicrobial peptides. Researchers can modify existing antimicrobial peptides using gene editing techniques to change their amino acid sequences, thereby enhancing their antibacterial activity or reducing toxicity to host cells[43]. A research team led by Luis Pedro Coelho at Fudan University utilized machine learning to identify nearly one million potential antimicrobial peptides from the global microbiome. They tested 100 of these synthetic antimicrobial peptides against 11 clinically relevant pathogenic bacterial strains, including *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcu saureus*, Vancomycin- resistant *Enterococcus faecalis*, and Vancomycin-resistant *Enterococcus faecium*. Ultimately, they confirmed that 79 were active *in vitro*, and 63 exhibited inhibitory effects against the pathogenic strains[44]. The research team led by Wang Huaimin at Westlake University developed an AI model called TransSAFP, which for the first time accurately predicts the self-assembly behavior and biological functions of peptide molecules, with an efficiency potentially hundreds of billions of times that of humans. Using TransSAFP, the team de novo designed self-assembling antimicrobial peptides that are highly efficient, low-toxicity, and non-resistant. These peptides demonstrated highly effective, broad-spectrum bactericidal ability both *in vitro* and *in vivo*, offering a new strategy to address the global antibiotic resistance crisis[45]

1.4 Quorum Sensing interference

Quorum Sensing (QS) is an intercellular communication mechanism whereby microorganisms coordinate by secreting and perceiving specific signaling molecules. When the concentration of signaling molecules reaches a threshold, microorganisms activate or suppress the expression of specific genes, thereby regulating physiological behaviors such as biofilm formation, virulence factor secretion, and metabolite synthesis. This mechanism is widespread in bacteria and fungi and plays an important role in microbial ecological adaptation and pathogenicity[46,47]. However, traditional research has mostly been confined to laboratory cultures of single strains, making it

difficult to simulate group behaviors in complex natural environments[48]. The rise of synthetic biology provides new tools for the study and application of quorum sensing. By designing genetic circuits, optimizing signaling pathways, and constructing synthetic microbial communities, precise regulation of microbial behavior can be achieved[49,50].

Synthetic biology utilizes quorum sensing circuits to program cell behavior and repurposes modified quorum sensing systems as biosensors for detecting microbial communities, offering better signal specificity, real-time monitoring capability, and scalability. Specific amino acid sites in the quorum sensing system were rationally designed to construct a LuxR/LuxI QS system highly sensitive to 3OC6-HSL, and based on this, developed a whole-cell biosensor capable of detecting 3OC6 produced by *Yersinia pestis*[51]. Furthermore, synthetic biology can construct artificial synthetic microbial communities at the population level, establishing connections between different strains or species and regulating gene expression in multiple cell types through cell signaling mechanisms, enabling their co-survival under the same culture conditions. These play important roles in the field of metabolic engineering. Co-cultivation of synthetic microbial communities can distribute complex long synthetic pathways into different strains, achieving reduced metabolic burden, blocked side reactions, alleviated metabolic crosstalk, and significantly improving product yield and productivity[52,53]. Lignans are a class of secondary metabolites distributed in plants, which possess antibacterial, antifungal, and antiviral activities. By constructing a mutually dependent community based on met15Δ and ade2Δ auxotrophic yeast, using ferulic acid as a metabolic bridge, the de novo synthesis of lignan glycosides from glucose was achieved, simulating plant multicellular division of labor in metabolism and providing insights for the microbial manufacturing of complex natural products[54].

2. Application of synthetic biology in the prevention and control of viral infectious diseases

2.1 Current status of viral infections

Viral infection refers to the process by which viruses invade the organism through various routes and proliferate within susceptible host cells. Viral infections can cause a range of symptoms, from asymptomatic (showing no apparent symptoms) to severe disease. Infectious diseases caused by viruses seriously threaten human health and life. Particularly, some viral infections cause diseases that spread rapidly and have high fatality rates; once outbreaks occur, they can cause immeasurable harm to society. A research paper published in The Lancet in 2024 indicated that for adults globally, the impact of the COVID-19 pandemic has been more profound than any event in the past half-century, including military conflicts and natural disasters[55]. During 2020-2021, 84% of the 204 countries and regions analyzed experienced a decline in life expectancy, revealing the significant impact of this novel virus. Even now, the COVID-19 virus has not disappeared; its constant mutation and transmission characteristics continue to pose threats to global public health security. The rapid mutation and immune escape of viruses persistently challenge global public health security systems[56]. Therefore, developing efficient intelligent diagnostic systems and antiviral vaccines has become an urgent task.

2.2 Intelligent diagnostic systems

Current laboratory methods for pathogen diagnosis mainly include pathogen culture, antigen-antibody detection, real-time quantitative PCR, and next-generation sequencing. However, these detection technologies have limitations such as long pathogen culture cycles, limited application scope, and insufficient sensitivity[57]. CRISPR-Cas is an adaptive immune mechanism used by bacteria and archaea to cleave foreign invading nucleic acids, protecting themselves from viral intrusions like phages. It has not only been developed for precise gene editing but also for nucleic acid detection, demonstrating advantages such as speed, high throughput, high sensitivity, and strong specificity[58].

Aiming at the high transmissibility of SARS-CoV-2 infected persons during the asymptomatic or pre-symptomatic phase, a rapid and sensitive SARS-CoV-2 diagnostic detection method based on the CRISPR-Cas system was developed[59]. This

method utilizes customized CRISPR-Cas12a/gRNA complexes and fluorescent probes to detect target amplicons generated by standard RT-PCR or isothermal Recombinase Polymerase Amplification (RPA), enabling sensitive detection in areas lacking equipment required for qPCR diagnostics. Additionally, a universal CRISPR/Cas detection platform (g-CURS) based on a glass fiber interface was designed, enabling ultra-high sensitivity identification and portable rapid quantitative detection of various low-abundance analytes (such as nucleic acids or proteins)[60]. Platform g-CURS possesses efficient and accurate clinical detection performance, highly specific identification of SARS-CoV-2 Delta mutant cDNA and HPV16 E7 gene fragments, with a detection time of approximately 30 minutes. Meanwhile, this system can accurately identify mutation differences within 6 bases, possessing single-base mismatch resolution capability. In clinical samples of HPV16, the detection results were completely consistent with qPCR, achieving simultaneous detection of multiple substances with portable CRISPR/Cas *in vitro* diagnostic technology, providing a more convenient and economical solution for early disease diagnosis and dynamic monitoring.

2.3 Vaccine development

Vaccines are one of the most effective measures for human disease prevention and control. Over the centuries, scholars have continuously endeavored to explore the development of various vaccine types, from the earliest live attenuated vaccines and inactivated vaccines to modern technologies like genetically engineered vaccines and nano-vaccines[61]. Today, through synthetic biology, synthetic substances (i.e., vaccines) with specific antigenicity and immunogenicity can be designed, including genome codon-optimized vaccines, nucleic acid vaccines based on artificially synthesized genomes, viral vector-based vaccines, virus-like particle-based vaccines, and recombinant yeast-expressed vaccines, which can significantly induce the immune system to produce immune responses against specific pathogens[62,63]. This approach is more flexible compared to traditional vaccine preparation processes, enabling a rapid response to infectious disease outbreaks and pathogen mutations,

which is of great significance for dealing with emerging and re-emerging infectious diseases.

The "2023 Nobel Prize in Physiology or Medicine" was awarded to Professors Katalin Karikó and Drew Weissman for their discoveries concerning nucleoside base modifications. Specific chemical modifications of mRNA nucleotides can effectively reduce its immunogenicity while significantly enhancing its stability and translational efficiency, thereby establishing the molecular foundation for the feasibility and clinical application of mRNA vaccines[64]. As depicted in **Fig. 3**, using DNA as a template and RNA polymerase to directly incorporate modified nucleotides (Ψ UTP and 5mCTP) during *in vitro* transcription enables the one-step synthesis of functional mRNA molecules equipped with a 5' cap, a modified nucleotide sequence, and a 3' poly(A) tail. On January 10, 2020, shortly after the SARS-CoV-2 sequence was released, Moderna successfully created the mRNA-1273 COVID-19 vaccine in just 42 days[65]. On March 5, 2020, the U.S. Food and Drug Administration (FDA) completed its review of mRNA-1273 and approved it for clinical trials. mRNA vaccines are more suitable for responding to large-scale outbreaks compared to traditional vaccines, and advancements in technologies like synthetic biology have greatly addressed core issues such as mRNA molecule synthesis and modification and nucleic acid delivery systems, facilitating the rapid development of mRNA vaccines, accelerating their clinical application, and significantly shortening the vaccine development cycle[66]. Meanwhile, using synthetic biology technology, live attenuated vaccines with lower toxicity can be generated through genome codon deoptimization, effectively enhancing vaccine safety[67]. COVI-VAC is currently the only live SARS-CoV-2 virus vaccine, generated by recoding the SARS-CoV-2 genome according to the SAVE algorithm, systematically introducing 283 silent mutations, and deleting its furin cleavage site. Preclinical results showed that compared to the wild-type strain, the COVI-VAC candidate strain's virulence was significantly attenuated, but the antibody response induced *in vivo* was comparable to that caused by infection with the wild-type virus[68]. Furthermore, for viruses with frequent

mutations, such as HIV-1 and influenza viruses, flexible antigen design using synthetic biology technology is also very important for improving vaccine immunogenicity and breadth.

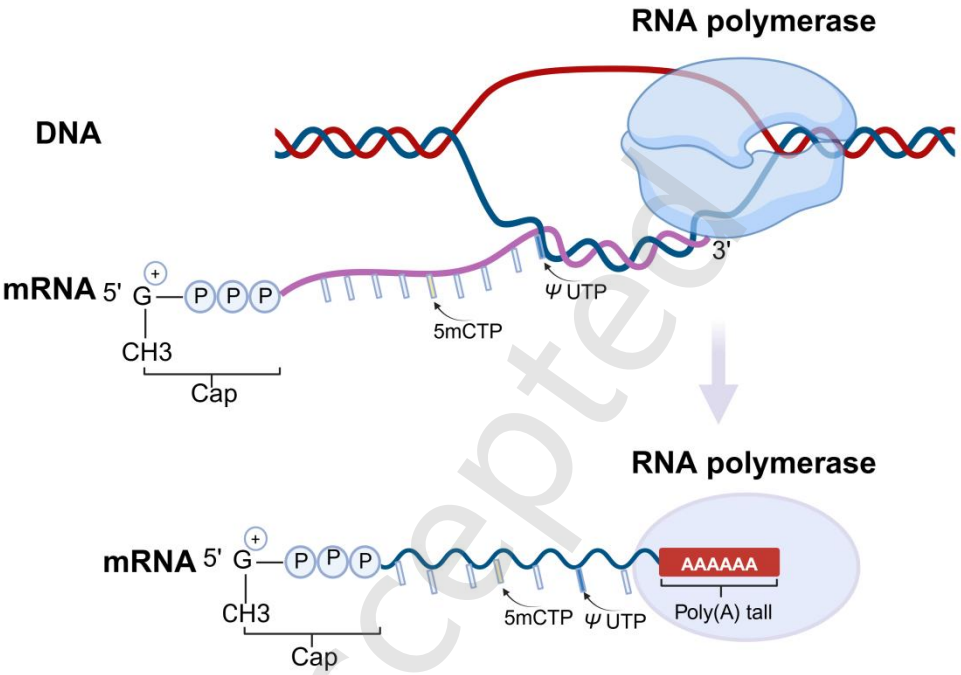

Fig 3. Schematic of nucleotide modification in mRNA vaccine design. The schematic highlights chemical modifications of nucleosides (e.g., pseudouridine) that enhance mRNA stability, reduce immunogenicity, and improve translational efficiency, contributing to rapid vaccine development against emerging viruses.

2.4 CAR-T cell therapy

Synthetic biology following the DBTL engineering framework has the ability to reprogram mammalian cells. Chimeric antigen receptor T-cell (CAR-T) therapy exemplifies this approach: through genetic engineering, T cells are modified to express artificially designed CAR molecules, transforming them into "living drugs" capable of precisely recognizing and attacking specific antigens. This technology fully embodies synthetic biology's capacity for modular deconstruction and rational reassembly of the immune system—breaking down T cell functions into

programmable units of sensing, processing, and execution, followed by systematic design and construction[69–71].

In the field of infectious disease prevention and control, CAR-T therapy offers new strategies for clearing latent viral reservoirs and drug-resistant pathogens. For instance, in Human Immunodeficiency Virus (HIV) infection, conventional antiretroviral therapy cannot eliminate the viral reservoir latent in resting $CD4^+$ T cells [72,73]. Synthetic biology strategies address this by designing CAR structures targeting HIV envelope proteins (such as gp120), such as those based on the CD4 extracellular domain or single-chain variable fragments (scFv) from broadly neutralizing antibodies **(Fig. 4)**[74–76]. This equips T cells with the ability to specifically recognize and eliminate HIV-infected cells. To optimize therapeutic efficacy, researchers employ synthetic biology approaches for multifaceted engineering: optimizing CAR signaling circuits by incorporating co-stimulatory domains like 4-1BB to enhance T cell persistence and expansion *in vivo*[77,78]; or utilizing "cell vaccine" strategies, such as constructing HIV-CAR T cells based on Cytomegalovirus (CMV)-specific T cells, followed by stimulating their expansion *in vivo* through CMV vaccination[79,80]. These approaches help overcome challenges such as the scarcity of target cells and low antigen density in viral reservoirs.

A large-scale long-term safety study demonstrated that among 783 individuals receiving genetically modified T-cell therapy, including HIV patients, no evidence of insertional mutagenesis-related tumorigenesis associated with vector integration was found. This supports the safety profile of this strategy in the field of infectious diseases[81]. Thus, CAR-T therapy exemplifies the comprehensive design capabilities of synthetic biology across molecular, circuit, and cellular levels, offering a highly promising engineered immunotherapy platform for achieving functional cures for HIV and addressing other refractory infections.

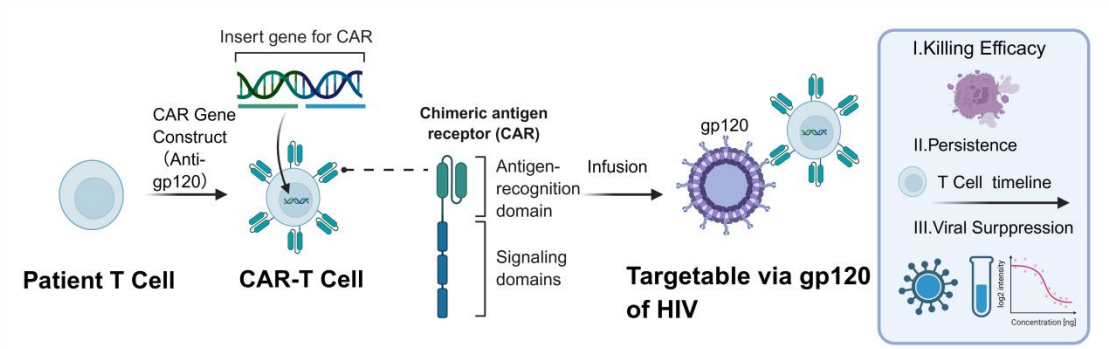

Fig 4. Schematic of Anti-gp120 CAR design and functional validation. The gene construct encoding a chimeric antigen receptor (CAR) specific to HIV gp120 is inserted into T cells. The engineered CAR consists of an anti-gp120 antigen-recognition domain linked to intracellular signaling domains. Upon encountering HIV-infected cells expressing gp120, the CAR-T cells are activated, leading to targeted killing, enhanced persistence, and sustained viral suppression *in vitro* or *in vivo*.

3. Application of synthetic biology in the prevention and control of invasive fungal diseases

3.1 Current status of invasive fungal diseases

Invasive fungal disease (IFD) refers to severe infections where pathogenic fungi invade deep tissues, blood, or internal organs of the human body, often life-threatening[82]. Currently, IFD has become a global public health problem, causing over 1.5 million deaths annually, necessitating high attention worldwide[6]. Therefore, on October 25, 2022, the WHO for the first time released the "WHO Fungal Priority Pathogens List," listing 19 fungi posing public health risks, including *Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus*, and *Candida albicans*. WHO categorized these fungal pathogens into three priority groups based on their priority: critical, high, and medium priority groups, aiming to raise public awareness of various fungal infections and their treatment[5].

Traditional antifungal drugs are mainly divided into four categories (polyenes, azoles, echinocandins, and flucytosine) and function by inhibiting nucleic acid synthesis and protein synthesis, disruption of microtubules and inhibition of mitosis, disruption of cell wall and membrane **(Fig. 5)**. However, challenges such as hepatorenal toxicity, single targets, and increasingly severe drug resistance issues pose significant challenges to the clinical application of these drugs[83]. Utilizing synthetic biology technology, on one hand, the metabolic networks of microorganisms can be reconstructed to achieve efficient biosynthesis of antifungal drugs[84,85]; on the other hand, intelligent drug delivery systems can be designed to improve treatment specificity.

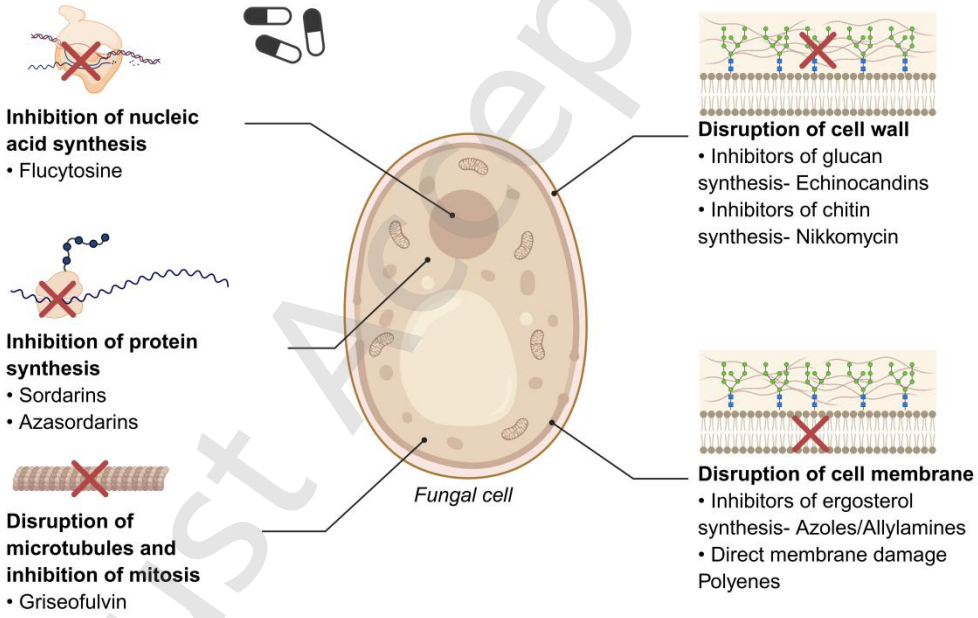

Fig 5. Classification and mechanisms of action of traditional antifungal drugs. Polyenes (amphotericin B) target ergosterol in the fungal cell membrane; azoles (fluconazole) inhibit the CYP51 enzyme in the ergosterol biosynthesis pathway; echinocandins (caspofungin) inhibit β-1,3-glucan synthase; flucytosine interferes with fungal nucleic acid synthesis.

3.2 New targets for antifungal drugs

Research on targets for antifungal drugs mainly focuses on disrupting fungal cell membranes, cell walls, and enzymes related to fungal biosynthesis and metabolic pathways. Drugs targeting these components can destroy the integrity and synthesis of fungal cell membranes and walls, thereby inhibiting fungal growth and reproduction[86]. Fungal cell membrane targets typically include ergosterol synthesis enzymes, phospholipases, cholesterol esterases, etc.[87]; the cell wall is mainly composed of polysaccharides (such as chitin, β-glucan, and mannan), proteins, and lipids[88]; simultaneously, scientists are also committed to finding new targets to develop highly effective antifungal drugs. Using synthetic biology technology, specifically modifying the genome of pathogenic fungi through gene editing allows observation of changes in fungal drug sensitivity, identifying new genes or drug targets associated with antifungal resistance[89,90]; it can also study the function of specific enzymes in fungal growth and reproduction, directly observing the impact of enzyme deficiency or reduced activity, thereby determining the role of key enzymes in the entire synthesis pathway[91].

The research team led by Wang Zongqiang efficiently discovered the novel antifungal natural product mandelamine through phylogeny-guided genome mining. To clarify its mechanism, they used homologous recombination technology to knock out the mandL and mandQ genes, which not only verified the function of the relevant gene cluster and obtained mutants lacking sugar chain modifications but also revealed the regulatory role of glycosylation on mandelamine's phospholipid targeting ability. By optimizing fermentation conditions, the team achieved high-efficiency production of mandelamine, with its water solubility increased by 9700 times compared to traditional polyene drugs, addressing the pain point of poor water solubility of traditional drugs. Using techniques like ITC and UV-vis, the team further analyzed that mandelamine functions by specifically binding to fungal cell membrane phospholipids, causing ion leakage, thereby avoiding the resistance problem associated with traditional drugs targeting ergosterol[92]. This research constructed a complete technological chain, providing a new synthetic biology paradigm for

antifungal drug development and providing important guidance for innovation in the field. Similarly, butyrolactol A (BLA) was discovered, a natural product that inhibits the flippase Apt1-Cdc50, disrupts membrane asymmetry, and restores echinocandin efficacy against resistant fungi such as *Cryptococcus* and *Candida auris*. Using synthetic biology, they developed a high-yield BLA producer and resolved its binding mode via cryo-EM, revealing a competitive inhibition mechanism. This study establishes flippases as a new antifungal target and highlights the role of synthetic biology in leveraging natural products to combat drug resistance **(Fig. 6)**[93]. By constructing knockout mutants of genes such as GCN5, RTT109, and SET1 using homologous recombination technology, combined with RNA-Seq transcriptome analysis and ChIP-qPCR epigenetic detection, it was found that the histone acetyltransferase GCN5 affects fungal drug resistance and virulence by regulating cell membrane ergosterol synthesis, drug efflux pumps, and the calcineurin signaling pathway. Furthermore, the GCN5 inhibitor CPTH2 was designed for combination with echinocandin drugs, demonstrating synergistic bactericidal effects *in vitro* and in animal models, significantly reducing the fungal load of multidrug-resistant *Candida auris*[94]. This series of technological applications revealed the potential of epigenetic regulators as new antifungal targets, providing new ideas for combination therapy based on synthetic biology strategies to overcome fungal resistance, and promoting innovation in antifungal therapies centered on gene editing, omics analysis, and targeted inhibitor development.

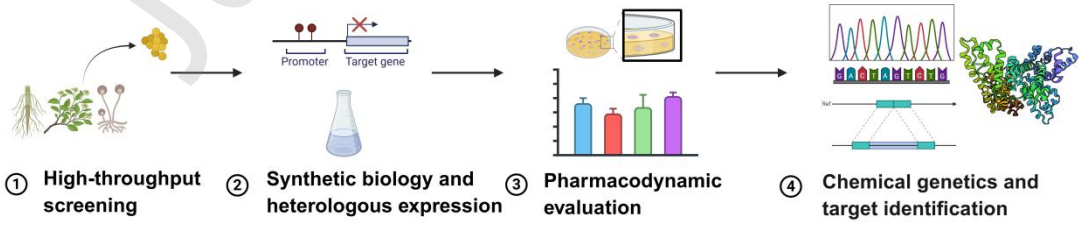

Fig 6. Schematic illustration of synthetic biology applications in antifungal drug target discovery. The diagram shows the construction of fungal mutants via gene editing techniques, combined with phenotypic screening and omics analysis, to identify new drug sensitivity-related targets.

3.3 Antifungal nanomaterials

Antifungal nanomaterials, as a novel therapeutic approach, have attracted widespread attention in the antifungal field. They refer to materials with dimensions at the nanoscale (typically 1-100 nm), including metal nanomaterials (such as silver nanoparticles), carbon-based nanomaterials (such as carbon nanotubes and graphene), quantum dots, metal-organic frameworks (MOFs),as well as mesoporous silica nanoparticles (MSNs) and lipid-based nanocarriers[95]. These materials, through careful design and preparation, possess various unique physical and chemical properties that can enhance their interaction with fungal cells, achieve stimulus-responsive drug release, and overcome the body's physiological barriers to precisely deliver drugs to the infection site[96]. Additionally, nanomaterials can effectively capture fungal toxins through hydrophobic interactions, hydrogen bonding, electrostatic interactions, covalent bonding, and π-π stacking [Fig.4C][97,98].

From a synthetic biology perspective, the functionality of these nanomaterials can be significantly enhanced by integrating biologically engineered modules.Using synthetic biology technology to construct molecular modules with specific targeting functions and integrating these modules onto the surface of nanomaterials enables specific recognition and binding to unique molecular markers on fungal cell walls or membranes. For example, by surface-modifying with Dectin-1 receptor ligands, liposomes can specifically recognize β-glucan in invasive Candida, enhancing binding ability to various fungal cells, thereby improving the efficacy and specificity of antifungal treatment[98]. Furthermore, mesoporous silica nanoparticles (MSNs) have been extensively explored as versatile platforms for antifungal drug delivery. Through chemical functionalization with biologically active molecules—such as the natural antimicrobial peptide epsilon-poly-L-lysine (EPL)—MSNs can achieve synergistic antifungal effects and enhanced solubility of lipophilic drugs like itraconazole (ITZ)[99]. Similarly, fatty acid nanoemulsion-templated silica nanoparticles have been developed as green and efficient carriers for loading hydrophobic antimycobacterial drugs, demonstrating efficacy against drug-resistant strains[100].

Utilizing synthetic biology technology to integrate biosensing elements and signal transduction pathways into the design of nanomaterials enables them to sensitively perceive specific microenvironmental signals at the fungal infection site, such as changes in local pH, changes in specific enzyme activity, and accumulation of Reactive Oxygen Species (ROS). When nanomaterials detect these specific signals, internal biological or chemical mechanisms are triggered, precisely controlling the release process of the carried antifungal drugs, achieving more precise and effective treatment[101–103]. Synthetic biology can also target different types of biological barriers, such as the skin's stratum corneum barrier, the corneal epithelial and endothelial barriers, and the tight junction structures of the blood-brain barrier, by modifying specific biological molecules on the surface of nanomaterials, enabling them to interact with receptors or transport proteins on barrier cell surfaces, promoting the endocytosis and transcellular transport processes of nanomaterials **(Fig. 7)**[104–106].

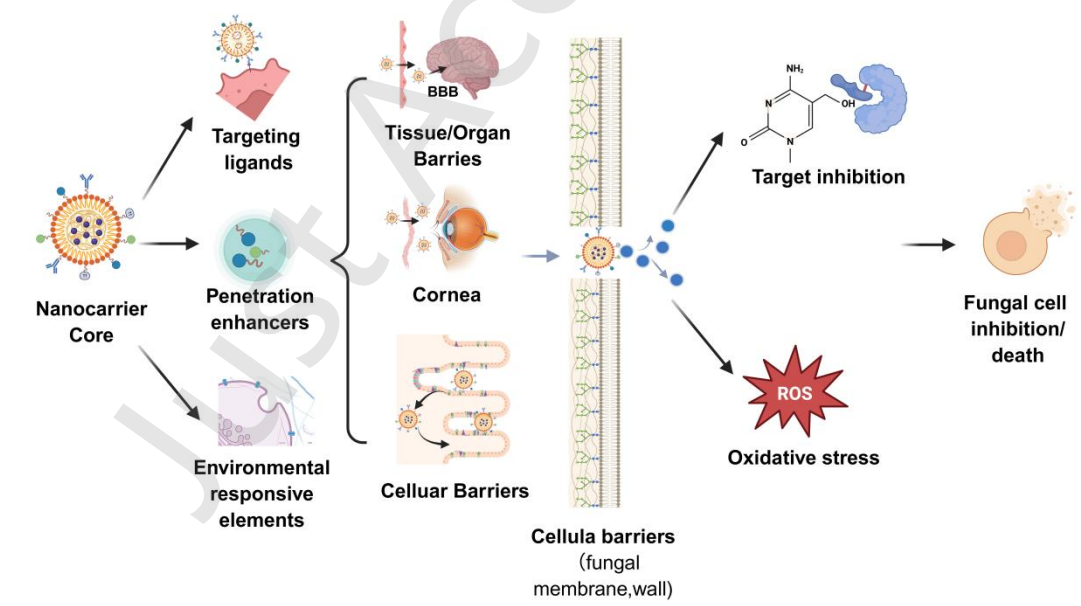

Fig 7. Schematic illustration of targeted delivery and stimulus-responsive release mechanisms of antifungal nanomaterials. The surface of nanomaterials can be modified with specific ligands to recognize fungal cell wall components. The nanomaterial core can be loaded with antifungal drugs and designed to release its payload in response to infection microenvironment signals. Furthermore, the surface

can be functionalized to overcome physiological barriers enhancing drug accumulation at deep infection sites.

4. Biosecurity challenges of synthetic biology and potential countermeasures

4.1 Biosecurity challenges of synthetic biology

According to China's "Biosafety Law of the People's Republic of China" promulgated in 2020, biosafety refers to the state where biotechnology can develop steadily and healthily, people's life and health and ecosystems are relatively free from danger and threat, and the biological field possesses the capacity to safeguard national security and sustainable development[107]. With the rapid development of synthetic biology, related biosafety risks have attracted increasing attention, mainly including risks associated with the release of synthetic pathogenic microorganisms, environmental and ecological risks caused by synthetic substances, and potential biosafety issues arising from the rapid development of AI in the field of synthetic biology[108].

Gene editing tools can be used to reconstruct highly pathogenic viruses, even designing drug-resistant, highly transmissible "super pathogens." Researchers chemically synthesized the poliovirus in test tubes in 2002[109], and in 2005, used genome editing technology to reconstruct the extinct "Spanish" influenza virus strain[110]. Subsequently, horsepox virus, Ebola virus, SARS-CoV-2, etc., have been synthesized in laboratories[111]. Gain-of-Function (GOF) research, such as genetic modification of highly pathogenic avian influenza H5N1 virus for transmissibility and host specificity, has also been conducted[68]. If such artificially synthesized pathogens are not regulated and are weaponized, they could break the limitations of traditional biological warfare, making gene editing a new type of bioweapon carrier, seriously threatening people's life and health. AI algorithms can accelerate the design of pathogenic proteins unrecognizable by the human immune system or enable the simplification of pathogen synthesis through automated platforms—non-professionals using biological printers could produce customized viruses in simple laboratories,

significantly lowering the threshold for bioweapon manufacturing[112,113]. More critically, These artificially designed organisms are very prone to triggering unpredictable leakage accidents. These risks not only threaten the stable and healthy development of biotechnology but also directly impact the capacity of the biological field to maintain national security, and even global security.

4.2 Potential countermeasures

Addressing the biosafety issues arising from synthetic biology requires the construction of multi-dimensional, dynamic comprehensive response strategies. At the institutional level, establishing a classified and graded management system is core: implement differentiated control measures based on the research object (pathogen hazard level), technical path ("dry" AI design or "wet" physical synthesis), and activity risk. High-risk activities must be conducted in BSL-4 biosafety laboratories, subject to national-level approval and filing; medium and low-risk activities require institutional or provincial-level supervision. Simultaneously, establish a dynamically updated pathogen list (integrating directories like the "Catalogue of Pathogenic Microorganisms Transmitted to Human Beings"), adjusting risk levels based on real-time risk assessments. Regarding the regulatory framework, it is necessary to improve specialized laws and regulations, refine synthetic biology safety evaluation standards under the "Biosafety Law," clarify the responsibilities of multi-departmental coordination to avoid "fragmented management," and establish an "adaptive governance" mechanism to regularly review the matching of policies and technological development.

At the technical prevention and control level, strengthen laboratory biological risk management: implement full-process risk assessment, utilize bioinformatics and big data simulation platforms to enhance the scientific nature of risk prediction; for "dry" research, establish a tripartite screening mechanism involving "AI tool providers - data platforms - users" to monitor access and use of sensitive gene sequences; for "wet" research, strictly enforce laboratory operating procedures, equip with protective facilities matching the risk level, and have contingency plans.

Simultaneously, strengthen the construction of environmental monitoring networks, introduce new biosensors and molecular typing technologies, track engineered microbial leakage or gene pollution through wastewater testing, soil testing, etc., and achieve early warning by combining pathogen transmission dynamics data.

5. Conclusion and perspective

The application of synthetic biology in the prevention and control of infectious diseases has ushered in a new era of precise intervention, providing revolutionary tools to address the global crises of emerging pathogens, antimicrobial resistance, and invasive fungal infections. Synthetic biology enables the rational engineering of phages, synthetic antimicrobial peptides, genetic circuits, intelligent diagnostic systems, and next-generation vaccines, fundamentally overcoming the bottlenecks of traditional prevention and control strategies such as long development cycles, narrow targeting, and low bioavailability. Meanwhile, the integration of synthetic biology with functional nanomaterials further extends the reach of these technologies, enabling targeted delivery, stimuli-responsive release, and synergistic fungicidal effects, especially for poorly soluble antifungal agents and drug-resistant strains. Together, these advances establish synthetic biology as a core driving force for transforming infectious disease prevention and control systems.

However, the translational application of synthetic biology in the field of infectious disease prevention and control is still hindered by a series of key technical bottlenecks. From the perspective of common challenges, the insufficient robustness and stability of engineered biological systems is a core obstacle. Threats such as recognition and clearance by the host immune mechanism, rapid degradation by metabolic pathways *in vivo*, and adaptive mutations generated by pathogens for survival compromise the functional persistence of systems like engineered phages, synthetic gene circuits, and mRNA vaccine platforms. Furthermore, the development of synthetic biology is accompanied by security challenges such as the leakage of synthetic pathogenic microorganisms and the risk of AI-driven bioweapons.

Future perspectives should therefore focus on translational, application-oriented improvements that address the limitations identified above. First, future studies should prioritize enhancing the stability and robustness of engineered systems, including engineered phages, synthetic gene circuits, and mRNA-based platforms, to reduce immune clearance, extend in vivo half-life, and minimize pathogen escape mutations. Second, greater emphasis should be placed on biosafety-by-design throughout the engineering cycle, particularly for synthetic microorganisms and engineered nanomaterials, to prevent unintended leakage, horizontal gene transfer, or ecological disruption. Third, the synergistic design between synthetic biology modules and targeted, stimuli-responsive nanodelivery systems should be further explored, especially for poorly soluble antifungal drugs, to improve solubility, controlled release, and infection-site specificity while lowering toxicity. Fourth, unified standardized evaluation systems and coordinated global regulatory frameworks should be established, covering consistent efficacy metrics, biosafety assessment protocols, scalable manufacturing standards, and biosecurity governance, to ensure reproducibility, clinical translation, and innovation of synthetic biology–based anti-infective technologies.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2023YFC3504800), the National Natural Science Foundation of China (grant nos. 82225047, 82522090, and 82474032), and the Key project at central government level (The ability establishment of sustainable use for valuable Chinese medicine resources, 2060302-2505-01).

Ethic Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Tian Qi and Ruibing Chen wrote the manuscript. Tian Qi, Yue Fang and Wenqing Liu were involved in figure preparation and discussion. Xiao He, Lingzhe Kong, Ruibing Chen revised the manuscript. Ruibing Chen and Lei Zhang supervised the project.

Reference

[1] Ruddon RW. Progress in molecular biology and translational science. Preface. *Prog Mol Biol Transl Sci*. 2010;95:xi.

[2] Mipatrini, D., Montaldo, C., Bartolini, B., Rezza, G., Iavicoli, S., Ippolito, G., Zumla, A., & Petersen, E. (2022). 'Disease X'-time to act now and prepare for the next pandemic threat. *European journal of public health*, *32*(6), 841–842.

[3] Zhao, M., Lei, L., Jiang, Y., Tian, Y., Huang, Y., & Yang, M. (2025). Unveiling the Threat of Disease X: Preparing for the Next Global Pandemic. *Journal of medical virology*, *97*(2), e70227.

[4] Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399(10325):629-655.

[5] WHO fungal priority pathogens list to guide research, development and public health action[M]. 1st ed. Geneva: World Health Organization, 2022.

[6] Almeida F, Rodrigues ML, Coelho C. The Still Underestimated Problem of Fungal Diseases Worldwide. *Front Microbiol*. 2019;10:214.

[7] Theuretzbacher U. The global resistance problem and the clinical antibacterial pipeline. *Nat Rev Microbiol*. 2025;23(8):491-508.

[8] Zumla A, Hui DS, Al-Tawfiq JA, Gautret P, McCloskey B, Memish ZA. Emerging respiratory tract infections. *Lancet Infect Dis*. 2014;14(10):910-911.

[9] Zhao, Y., Coelho, C., Hughes, A. L., Lazar-Stefanita, L., Yang, S., Brooks, A. N., Walker, R. S. K., Zhang, W., Lauer, S., Hernandez, C., Cai, J., Mitchell, L. A., Agmon, N., Shen, Y., Sall, J., Fanfani, V., Jalan, A., Rivera, J., Liang, F. X., Bader, J. S., … Boeke, J. D. (2023). Debugging and consolidating multiple synthetic chromosomes reveals combinatorial genetic interactions. *Cell*, *186*(24), 5220–5236.e16.

[10] Tian, F., Li, J., Nazir, A., & Tong, Y. (2021). Bacteriophage - A Promising Alternative Measure for Bacterial Biofilm Control. *Infection and drug resistance*, *14*, 205–217.

[11] Zhang, W., Lazar-Stefanita, L., Yamashita, H., Shen, M. J., Mitchell, L. A., Kurasawa, H., Lobzaev, E., Fanfani, V., Haase, M. A. B., Sun, X., Jiang, Q., Goldberg, G. W., Ichikawa, D. M., Lauer, S. L., McCulloch, L. H., Easo, N., Lin, S. J., Camellato, B. R., Zhu, Y., Cai, J., … Boeke, J. D. (2023). Manipulating the 3D organization of the largest synthetic yeast chromosome. *Molecular cell*, *83*(23), 4424–4437.e5.

[12] Zhang, X. E., Liu, C., Dai, J., Yuan, Y., Gao, C., Feng, Y., Wu, B., Wei, P., You, C., Wang, X., & Si, T. (2023). Enabling technology and core theory of synthetic biology. *Science China. Life sciences*, *66*(8), 1742–1785.

[13] Zhao, M., Tan, X., Liu, Z.-q, Dou, L., Liu, D., Pan, Y.-j, Ma, Y.-f, & Yu, J.-l

(2023). Engineered phage with cell-penetrating peptides for intracellular bacterial infections. *mSystems*, *8*(5), e0064623.

[14] Zhang, H., Zhu, R., Wang, Z., He, R., Zhang, Y., Luan, J., Yan, Y., Zhang, Y., & Wang, H. (2025). Programming virulent bacteriophages by developing a multiplex genome engineering method. *mBio*, *16*(6), e0358224.

[15] McMullan, L. K., Flint, M., Chakrabarti, A., Guerrero, L., Lo, M. K., Porter, D., Nichol, S. T., Spiropoulou, C. F., & Albariño, C. (2019). Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and *in vitro* analysis. *The Lancet. Infectious diseases*, *19*(9), 1023–1032.

[16] Lipsitch, M., & Inglesby, T. V. (2014). Moratorium on research intended to create novel potential pandemic pathogens. *mBio*, *5*(6), e02366-14.

[17] Watson J. (2022). Synthetic Biology: State Regulation in the Biomedical Context. *American journal of law & medicine*, *48*(4), 447–468.

[18] Doron S, Gorbach SL. Bacterial Infections: Overview. International Encyclopedia of Public Health. 2008:273–82.

[19] GBD 2019 Antimicrobial Resistance Collaborators (2022). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet (London, England)*, *400*(10369), 2221–2248.

[20] Salmond, G. P., & Fineran, P. C. (2015). A century of the phage: past, present and future. *Nature reviews. Microbiology*, *13*(12), 777–786.

[21] Duan, Y., Young, R., & Schnabl, B. (2022). Bacteriophages and their potential for treatment of gastrointestinal diseases. *Nature reviews. Gastroenterology & hepatology*, *19*(2), 135–144.

[22] Rostøl, J. T., & Marraffini, L. (2019). (Ph)ighting Phages: How Bacteria Resist Their Parasites. *Cell host & microbe*, *25*(2), 184–194.

[23] Ando, H., Lemire, S., Pires, D. P., & Lu, T. K. (2015). Engineering Modular Viral Scaffolds for Targeted Bacterial Population Editing. *Cell systems*, *1*(3), 187–196.

[24] Gencay, Y. E., Jasinskytė, D., Robert, C., Semsey, S., Martínez, V., Petersen, A. Ø., Brunner, K., de Santiago Torio, A., Salazar, A., Turcu, I. C., Eriksen, M. K., Koval, L., Takos, A., Pascal, R., Schou, T. S., Bayer, L., Bryde, T., Johansen, K. C., Bak, E. G., Smrekar, F., … Sommer, M. O. A. (2024). Engineered phage with antibacterial CRISPR-Cas selectively reduce E. coli burden in mice. *Nature biotechnology*, *42*(2), 265–274.

[25] Sahoo, K., & Meshram, S. (2024). Biofilm Formation in Chronic Infections: A Comprehensive Review of Pathogenesis, Clinical Implications, and Novel Therapeutic Approaches. *Cureus*, *16*(10), e70629.

[26] Liu, S., Lu, H., Zhang, S., Shi, Y., & Chen, Q. (2022). Phages against Pathogenic Bacterial Biofilms and Biofilm-Based Infections: A Review. *Pharmaceutics*, *14*(2), 427.

[27] Tian, F., Li, J., Nazir, A., & Tong, Y. (2021). Bacteriophage - A Promising Alternative Measure for Bacterial Biofilm Control. *Infection and drug*

resistance, *14*, 205–217.

[28] Guo, Y., Lin, S., Chen, R., Gu, J., Tang, K., Nie, Z., Huang, Z., Weng, J., Lin, J., Liu, T., Waldor, M. K., & Wang, X. (2024). A reverse transcriptase controls prophage genome reduction to promote phage dissemination in Pseudomonas aeruginosa biofilms. *Cell reports*, *43*(11), 114883.

[29] Li, S., Xu, M., Yang, D., Yang, M., Wu, H., Li, X., Yang, C., Fang, Z., Wu, Q., Tan, L., Xiao, W., & Weng, Q. (2024). Characterization and genomic analysis of a lytic *Stenotrophomonas maltophilia* short-tailed phage A1432 revealed a new genus of the family *Mesyanzhinovviridae*. *Frontiers in microbiology*, *15*, 1400700.

[30] He, P., Cao, F., Qu, Q., Geng, H., Yang, X., Xu, T., Wang, R., Jia, X., Lu, M., Zeng, P., & Luan, G. (2024). Host range expansion of Acinetobacter phage vB_Ab4_Hep4 driven by a spontaneous tail tubular mutation. *Frontiers in cellular and infection microbiology*, *14*, 1301089.

[31] King, S.H., Driscoll, C.L., Li, D.B., Guo, D., Merchant, A.T., Brixi, G., Wilkinson, M.E., & Hie, B.L. (2025). Generative design of novel bacteriophages with genome language models. *bioRxiv*.

[32] Huss, P., Chen, J., & Raman, S. (2023). High-throughput approaches to understand and engineer bacteriophages. *Trends in biochemical sciences*, *48*(2), 187–197.

[33] Potera C. (2013). Phage renaissance: new hope against antibiotic resistance. *Environmental health perspectives*, *121*(2), a48–a53.

[34] Mookherjee, N., & Hancock, R. E. (2007). Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. *Cellular and molecular life sciences : CMLS*, *64*(7-8), 922–933.

[35] Hancock R. E. (1997). Peptide antibiotics. *Lancet (London, England)*, *349*(9049), 418–422.

[36] Huan, Y., Kong, Q., Mou, H., & Yi, H. (2020). Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. *Frontiers in microbiology*, *11*, 582779.

[37] Oliveira Júnior, N. G., Souza, C. M., Buccini, D. F., Cardoso, M. H., & Franco, O. L. (2025). Antimicrobial peptides: structure, functions and translational applications. *Nature reviews. Microbiology*, *23*(11), 687–700.

[38] Niederhafner, P., Sebestík, J., & Jezek, J. (2008). Glycopeptide dendrimers. Part II. *Journal of peptide science : an official publication of the European Peptide Society*, *14*(1), 44–65.

[39] Li, C., Blencke, H. M., Paulsen, V., Haug, T., & Stensvåg, K. (2010). Powerful workhorses for antimicrobial peptide expression and characterization. *Bioengineered bugs*, *1*(3), 217–220.

[40] Schütz, M., Schöppe, J., Sedlák, E., Hillenbrand, M., Nagy-Davidescu, G., Ehrenmann, J., Klenk, C., Egloff, P., Kummer, L., & Plückthun, A. (2016). Directed evolution of G protein-coupled receptors in yeast for higher functional production in eukaryotic expression hosts. *Scientific reports*, *6*, 21508.

[41] Luong, H. X., Thanh, T. T., & Tran, T. H. (2020). Antimicrobial peptides -

Advances in development of therapeutic applications. *Life sciences*, *260*, 118407.
[42] Seo, M. D., Won, H. S., Kim, J. H., Mishig-Ochir, T., & Lee, B. J. (2012). Antimicrobial peptides for therapeutic applications: a review. *Molecules (Basel, Switzerland)*, *17*(10), 12276–12286.
[43] Cardoso, M. H., Orozco, R. Q., Rezende, S. B., Rodrigues, G., Oshiro, K. G. N., Cândido, E. S., & Franco, O. L. (2020). Computer-Aided Design of Antimicrobial Peptides: Are We Generating Effective Drug Candidates?. *Frontiers in microbiology*, *10*, 3097.
[44] Santos-Júnior, C. D., Torres, M. D. T., Duan, Y., Rodríguez Del Río, Á., Schmidt, T. S. B., Chong, H., Fullam, A., Kuhn, M., Zhu, C., Houseman, A., Somborski, J., Vines, A., Zhao, X. M., Bork, P., Huerta-Cepas, J., de la Fuente-Nunez, C., & Coelho, L. P. (2024). Discovery of antimicrobial peptides in the global microbiome with machine learning. *Cell*, *187*(14), 3761–3778.e16.
[45] Liu, H., Song, Z., Zhang, Y., Wu, B., Chen, D., Zhou, Z., Zhang, H., Li, S., Feng, X., Huang, J., & Wang, H. (2025). De novo design of self-assembling peptides with antimicrobial activity guided by deep learning. *Nature materials*, *24*(8), 1295–1306.
[46] Bassler, B. L., Wright, M., Showalter, R. E., & Silverman, M. R. (1993). Intercellular signalling in Vibrio harveyi: sequence and function of genes regulating expression of luminescence. *Molecular microbiology*, *9*(4), 773–786.
[47] Fuqua, W. C., Winans, S. C., & Greenberg, E. P. (1994). Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *Journal of bacteriology*, *176*(2), 269–275.
[48] Ruan, Q., Geng, S., Yu, J., Lu, L., Liu, Y., Chen, J., Liao, Q., & Guo, R. (2026). Microbial quorum sensing: Mechanisms, applications, and challenges. *Biotechnology advances*, *86*, 108733.
[49] Cardona-Serra, S., , Rosaleny, L. E., , Giménez-Santamarina, S., , Martínez-Gil, L., , & Gaita-Ariño, A., (2021). Towards peptide-based tunable multistate memristive materials. *Physical chemistry chemical physics : PCCP*, *23*(3), 1802–1810.
[50] Webster, L. J., Villa-Gomez, D., Brown, R., Clarke, W., & Schenk, P. M. (2024). A synthetic biology approach for the treatment of pollutants with microalgae. *Frontiers in bioengineering and biotechnology*, *12*, 1379301.
[51] Li, J., Liu, R., Chen, Y., Liu, S., Chen, C., Liu, T., Yang, S., Zhuang, Y., Yang, R., Cui, Y., Song, Y., Wang, T., & Teng, Y. (2021). Computer-Aided Rational Engineering of Signal Sensitivity of Quorum Sensing Protein LuxR in a Whole-Cell Biosensor. *Frontiers in molecular biosciences*, *8*, 729350.
[52] Wanapaisan, P., Laothamteep, N., Vejarano, F., Chakraborty, J., Shintani, M., Muangchinda, C., Morita, T., Suzuki-Minakuchi, C., Inoue, K., Nojiri, H., & Pinyakong, O. (2018). Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium. *Journal of hazardous materials*, *342*, 561–570.
[53] Chen, H., Peng, H., Ellis, T., & Ledesma-Amaro, R. (2026). Programmable cell-cell adhesion in synthetic yeast communities for improved

bioproduction. *Nature chemical biology*, 10.1038/s41589-025-02081-1.

[54] Chen, R., Chen, X., Chen, Y., Yang, J., Chen, W., Zhou, Y. J., & Zhang, L. (2025). De novo biosynthesis of plant lignans by synthetic yeast consortia. *Nature chemical biology*, *21*(10), 1487–1496.

[55] GBD 2021 Demographics Collaborators (2024). Global age-sex-specific mortality, life expectancy, and population estimates in 204 countries and territories and 811 subnational locations, 1950-2021, and the impact of the COVID-19 pandemic: a comprehensive demographic analysis for the Global Burden of Disease Study 2021. *Lancet (London, England)*, *403*(10440), 1989–2056.

[56] Jian, F., Wang, J., Yisimayi, A., Song, W., Xu, Y., Chen, X., Niu, X., Yang, S., Yu, Y., Wang, P., Sun, H., Yu, L., Wang, J., Wang, Y., An, R., Wang, W., Ma, M., Xiao, T., Gu, Q., Shao, F., … Cao, Y. (2025). Evolving antibody response to SARS-CoV-2 antigenic shift from XBB to JN.1. *Nature*, *637*(8047), 921–929.

[57] Liu, Q., Jin, X., Cheng, J., Zhou, H., Zhang, Y., & Dai, Y. (2023). Advances in the application of molecular diagnostic techniques for the detection of infectious disease pathogens (Review). *Molecular medicine reports*, *27*(5), 104.

[58] Bharathkumar, N., Sunil, A., Meera, P., Aksah, S., Kannan, M., Saravanan, K. M., & Anand, T. (2022). CRISPR/Cas-Based Modifications for Therapeutic Applications: A Review. *Molecular biotechnology*, *64*(4), 355–372.

[59] Huang, Z., Tian, D., Liu, Y., Lin, Z., Lyon, C. J., Lai, W., Fusco, D., Drouin, A., Yin, X., Hu, T., & Ning, B. (2020). Ultra-sensitive and high-throughput CRISPR-p owered COVID-19 diagnosis. *Biosensors & bioelectronics*, *164*, 112316.

[60] Yu, T., Zou, S., Long, Y., Ou, Y., Liu, S., Kang, T., Song, L., Sun, C., & Liu, G. (2025). Glass fiber-interfaced CRISPR/Cas biosensing adaptable for diverse biomarker detection. *Trends in biotechnology*, *43*(8), 2003–2028.

[61] Rauch, S., Jasny, E., Schmidt, K. E., & Petsch, B. (2018). New Vaccine Technologies to Combat Outbreak Situations. *Frontiers in immunology*, *9*, 1963.

[62] Tournier, J. N., & Kononchik, J. (2021). Virus Eradication and Synthetic Biology: Changes with SARS-CoV-2?. *Viruses*, *13*(4), 569.

[63] Stanfield, B. A., Kousoulas, K. G., Fernandez, A., & Gershburg, E. (2021). Rational Design of Live-Attenuated Vaccines against Herpes Simplex Viruses. *Viruses*, *13*(8), 1637.

[64] Li, B., Luo, X., & Dong, Y. (2016). Effects of Chemically Modified Messenger RNA on Protein Expression. *Bioconjugate chemistry*, *27*(3), 849–853.

[65] Corbett, K. S., Edwards, D. K., Leist, S. R., Abiona, O. M., Boyoglu-Barnum, S., Gillespie, R. A., Himansu, S., Schäfer, A., Ziwawo, C. T., DiPiazza, A. T., Dinnon, K. H., Elbashir, S. M., Shaw, C. A., Woods, A., Fritch, E. J., Martinez, D. R., Bock, K. W., Minai, M., Nagata, B. M., Hutchinson, G. B., … Graham, B. S. (2020). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature*, *586*(7830), 567–571.

[66] Pfeifer, B. A., Beitelshees, M., Hill, A., Bassett, J., & Jones, C. H. (2023). Harnessing synthetic biology for advancing RNA therapeutics and vaccine

design. *NPJ systems biology and applications*, *9*(1), 60.

[67] Zhang, C., Hou, J., Li, Z., Shen, Q., Bai, H., Chen, L., Shen, J., Wang, P., Su, Y., Li, J., Zhang, Q., Liu, C., Xi, X., Qi, F., Chen, Y., Xie, X., Ye, A. Y., Liu, X., Plebani, R., Church, G., … Si, L. (2025). PROTAR Vaccine 2.0 generates influenza vaccines by degrading multiple viral proteins. *Nature chemical biology*, *21*(9), 1330–1340.

[68] Wang, Y., Yang, C., Song, Y., Coleman, J. R., Stawowczyk, M., Tafrova, J., Tasker, S., Boltz, D., Baker, R., Garcia, L., Seale, O., Kushnir, A., Wimmer, E., & Mueller, S. (2021). Scalable live-attenuated SARS-CoV-2 vaccine candidate demonstrates preclinical safety and efficacy. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(29), e2102775118.

[69] Schuster S. J. (2019). CD19-directed CAR T cells gain traction. *The Lancet. Oncology*, *20*(1), 2–3.

[70] Zhang, L. N., Song, Y., & Liu, D. (2018). CD19 CAR-T cell therapy for relapsed/refractory acute lymphoblastic leukemia: factors affecting toxicities and long-term efficacies. *Journal of hematology & oncology*, *11*(1), 41.

[71] Liu D. (2019). CAR-T "the living drugs", immune checkpoint inhibitors, and precision medicine: a new era of cancer therapy. *Journal of hematology & oncology*, *12*(1), 113.

[72] Ruelas, D. S., & Greene, W. C. (2013). An integrated overview of HIV-1 latency. *Cell*, *155*(3), 519–529.

[73] Ho, Y. C., Shan, L., Hosmane, N. N., Wang, J., Laskey, S. B., Rosenbloom, D. I., Lai, J., Blankson, J. N., Siliciano, J. D., & Siliciano, R. F. (2013). Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell*, *155*(3), 540–551.

[74] Cappell, K. M., & Kochenderfer, J. N. (2023). Long-term outcomes following CAR T cell therapy: what we know so far. *Nature reviews. Clinical oncology*, *20*(6), 359–371.

[75] Liu, B., Zhang, W., Xia, B., Jing, S., Du, Y., Zou, F., Li, R., Lu, L., Chen, S., Li, Y., Hu, Q., Lin, Y., Zhang, Y., He, Z., Zhang, X., Chen, X., Peng, T., Tang, X., Cai, W., Pan, T., … Zhang, H. (2021). Broadly neutralizing antibody-derived CAR T cells reduce viral reservoir in individuals infected with HIV-1. *The Journal of clinical investigation*, *131*(19), e150211.

[76] Rothemejer, F. H., Lauritsen, N. P., Juhl, A. K., Schleimann, M. H., König, S., Søgaard, O. S., Bak, R. O., & Tolstrup, M. (2023). Development of HIV-Resistant CAR T Cells by CRISPR/Cas-Mediated CAR Integration into the *CCR5* Locus. *Viruses*, *15*(1), 202.

[77] Cappell, K. M., & Kochenderfer, J. N. (2021). A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains. *Nature reviews. Clinical oncology*, *18*(11), 715–727.

[78] van der Stegen, S. J., Hamieh, M., & Sadelain, M. (2015). The pharmacology of second-generation chimeric antigen receptors. *Nature reviews. Drug discovery*, *14*(7), 499–509.

[79] Maldini, C. R., Claiborne, D. T., Okawa, K., Chen, T., Dopkin, D. L., Shan, X.,

Power, K. A., Trifonova, R. T., Krupp, K., Phelps, M., Vrbanac, V. D., Tanno, S., Bateson, T., Leslie, G. J., Hoxie, J. A., Boutwell, C. L., Riley, J. L., & Allen, T. M. (2020). Dual CD4-based CAR T cells with distinct costimulatory domains mitigate HIV pathogenesis *in vivo*. *Nature medicine*, *26*(11), 1776–1787.

[80] Guan, M., Lim, L., Holguin, L., Han, T., Vyas, V., Urak, R., Miller, A., Browning, D. L., Echavarria, L., Li, S., Li, S., Chang, W. C., Scott, T., Yazaki, P., Morris, K. V., Cardoso, A. A., Blanchard, M. S., Le Verche, V., Forman, S. J., Zaia, J. A., … Wang, X. (2022). Pre-clinical data supporting immunotherapy for HIV using CMV-HIV-specific CAR T cells with CMV vaccine. *Molecular therapy. Methods & clinical development*, *25*, 344–359.

[81] Jadlowsky, J. K., Hexner, E. O., Marshall, A., Grupp, S. A., Frey, N. V., Riley, J. L., Veloso, E., McConville, H., Rogal, W., Czuczman, C., Hwang, W. T., Li, Y., Leskowitz, R. M., Farrelly, O., Karar, J., Christensen, S., Barber-Rotenberg, J., Gaymon, A., Aronson, N., Bernstein, W., … Fraietta, J. A. (2025). Long-term safety of lentiviral or gammaretroviral gene-modified T cell therapies. *Nature medicine*, *31*(4), 1134–1144.

[82] Bassetti, M., Azoulay, E., Kullberg, B. J., Ruhnke, M., Shoham, S., Vazquez, J., Giacobbe, D. R., & Calandra, T. (2021). EORTC/MSGERC Definitions of Invasive Fungal Diseases: Summary of Activities of the Intensive Care Unit Working Group. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, *72*(Suppl 2), S121–S127.

[83] Fisher, M. C., Alastruey-Izquierdo, A., Berman, J., Bicanic, T., Bignell, E. M., Bowyer, P., Bromley, M., Brüggemann, R., Garber, G., Cornely, O. A., Gurr, S. J., Harrison, T. S., Kuijper, E., Rhodes, J., Sheppard, D. C., Warris, A., White, P. L., Xu, J., Zwaan, B., & Verweij, P. E. (2022). Tackling the emerging threat of antifungal resistance to human health. *Nature reviews. Microbiology*, *20*(9), 557–571.

[84] Men, P., Zhou, Y., Xie, L., Zhang, X., Zhang, W., Huang, X., & Lu, X. (2023). Improving the production of the micafungin precursor FR901379 in an industrial production strain. *Microbial cell factories*, *22*(1), 44.

[85] Zhang, X., Cheng, S., Yang, J., Lu, L., Deng, Z., Bian, G., & Liu, T. (2024). Metabolic engineering of *Glarea lozoyensis* for high-level production of pneumocandin B0. *Synthetic and systems biotechnology*, *10*(2), 381–390.

[86] Childers, D. S., Avelar, G. M., Bain, J. M., Larcombe, D. E., Pradhan, A., Budge, S., Heaney, H., & Brown, A. J. P. (2020). Impact of the Environment upon the Candida albicans Cell Wall and Resultant Effects upon Immune Surveillance. *Current topics in microbiology and immunology*, *425*, 297–330.

[87] Van Daele, R., Spriet, I., Wauters, J., Maertens, J., Mercier, T., Van Hecke, S., & Brüggemann, R. (2019). Antifungal drugs: What brings the future?. *Medical mycology*, *57*(Supplement_3), S328–S343.

[88] Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., Hensel, G., Kumlehn, J., & Schweizer, P. (2010). HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis. *The Plant cell*, *22*(9), 3130–3141.

[89] Pérez-Cantero, A., Martin-Vicente, A., Guarro, J., Fortwendel, J. R., & Capilla, J. (2023). Analysis of the *cyp51* genes contribution to azole resistance in *Aspergillus* section *Nigri* with the CRISPR-Cas9 technique. *Antimicrobial agents and chemotherapy*, *65*(5), e01996-20.

[90] Demuyser, L., Palmans, I., Vandecruys, P., & Van Dijck, P. (2020). Molecular Elucidation of Riboflavin Production and Regulation in Candida albicans, toward a Novel Antifungal Drug Target. *mSphere*, *5*(4), e00714-20.

[91] Hu, X., Yang, P., Chai, C., Liu, J., Sun, H., Wu, Y., Zhang, M., Zhang, M., Liu, X., & Yu, H. (2023). Structural and mechanistic insights into fungal β-1,3-glucan synthase FKS1. *Nature*, *616*(7955), 190–198.

[92] Deng, Q., Li, Y., He, W., Chen, T., Liu, N., Ma, L., Qiu, Z., Shang, Z., & Wang, Z. (2025). A polyene macrolide targeting phospholipids in the fungal cell membrane. *Nature*, *640*(8059), 743–751.

[93] Chen, X., Duan, H. D., Hoy, M. J., Koteva, K., Spitzer, M., Guitor, A. K., Puumala, E., Fiebig, A. A., Hu, G., Yiu, B., Chou, S., Bian, Z., Choi, Y., Guo, A. B. Y., Wang, W., Sun, S., Robbins, N., Averette, A. F., Cook, M. A., Truant, R., … Wright, G. D. (2026). Butyrolactol A enhances caspofungin efficacy via flippase inhibition in drug-resistant fungi. *Cell*, *189*(2), 620–639.e28.

[94] Zhang, Y., Zeng, L., Huang, X., Wang, Y., Chen, G., Moses, M., Zou, Y., Xiong, S., Xue, W., Dong, Y., Tian, Y., Guan, M., Hu, L., Yin, Z., Zhou, D., Huang, X., & Chen, C. (2025). Targeting epigenetic regulators to overcome drug resistance in the emerging human fungal pathogen Candida auris. *Nature communications*, *16*(1), 4668.

[95] Schaefer, S., Corrigan, N., Brunke, S., Lenardon, M. D., & Boyer, C. (2024). Combatting Fungal Infections: Advances in Antifungal Polymeric Nanomaterials. *Biomacromolecules*, *25*(9), 5670–5701.

[96] Zhao, X., Zhang, S., Zhang, M., Zhang, Z., Zhou, M., & Cao, J. (2025). Antifungal Performance and Mechanisms of Carbon Quantum Dots in Cellulosic Materials. *ACS nano*, *19*(14), 14121–14136.

[97] Xie, M., Gao, M., Yun, Y., Malmsten, M., Rotello, V. M., Zboril, R., Akhavan, O., Kraskouski, A., Amalraj, J., Cai, X., Lu, J., Zheng, H., & Li, R. (2023). Antibacterial Nanomaterials: Mechanisms, Impacts on Antimicrobial Resistance and Design Principles. *Angewandte Chemie (International ed. in English)*, *62*(17), e202217345.

[98] Liu, F., Chen, Y., Huang, Y., Jin, Q., & Ji, J. (2024). Nanomaterial-based therapeutics for enhanced antifungal therapy. *Journal of materials chemistry. B*, *12*(37), 9173–9198.

[99] Song, Y., Zhu, P., Wu, Y., Tan, L., Wei, W., Liu, S., Huang, Q., & Chen, J. (2019). Epsilon-poly-l-lysine decorated ordered mesoporous silica contributes to the synergistic antifungal effect and enhanced solubility of a lipophilic drug. Materials science & engineering. C, Materials for biological applications, 99, 231–240.

[100] Zhu, P., Cai, L., Liu, Q., Feng, S., Ruan, H., Zhang, L., Zhou, L., Jiang, H., Wang, H., Wang, J., & Chen, J. (2022). One-pot synthesis of α-Linolenic acid

nanoemulsion-templated drug-loaded silica mesocomposites as efficient bactericide against drug-resistant Mycobacterium tuberculosis. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, *176*, 106261.

[101] Huang, X., He, D., Reactive-oxygen-species-scavenging nanomaterials for resolving inflammation. *Materials today. Bio*, *11*, 100124.

[102] Chai, M., Gao, Y., Liu, J., Deng, Y., Hu, D., Jin, Q., & Ji, J. (2020). Polymyxin B-Polysaccharide Polyion Nanocomplex with Improved Biocompatibility and Unaffected Antibacterial Activity for Acute Lung Infection Management. *Advanced healthcare materials*, *9*(3), e1901542.

[103] Huang, Y., Zou, L., Wang, J., Jin, Q., & Ji, J. (2022). Stimuli-responsive nanoplatforms for antibacterial applications. *Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology*, *14*(3), e1775.

[104] Chen, H., Geng, X., Ning, Q., Shi, L., Zhang, N., He, S., Zhao, M., Zhang, J., Li, Z., Shi, J., & Li, J. (2024). Biophilic Positive Carbon Dot Exerts Antifungal Activity and Augments Corneal Permeation for Fungal Keratitis. *Nano letters*, *24*(13), 4044–4053.

[105] Zhang, S., Asghar, S., Yang, L., Hu, Z., Chen, Z., Shao, F., & Xiao, Y. (2020). Borneol and poly (ethylene glycol) dual modified BSA nanoparticles as an itraconazole vehicle for brain targeting. *International journal of pharmaceutics*, *575*, 119002.

[106] Wang, B., Zhang, W., Pan, Q., Tao, J., Li, S., Jiang, T., & Zhao, X. (2023). Hyaluronic Acid-Based CuS Nanoenzyme Biodegradable Microneedles for Treating Deep Cutaneous Fungal Infection without Drug Resistance. *Nano letters*, *23*(4), 1327–1336.

[107] Standing Committee of the National People's Congress. (2020). Biosafety Law of the People's Republic of China[Z].2020-10-17.(in Chinese)

[108] Watson J. (2022). Synthetic Biology: State Regulation in the Biomedical Context. *American journal of law & medicine*, *48*(4), 447–468.

[109] Cello, J., Paul, A. V., & Wimmer, E. (2002). Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science (New York, N.Y.)*, *297*(5583), 1016–1018.

[110] Tumpey, T. M., Basler, C. F., Aguilar, P. V., Zeng, H., Solórzano, A., Swayne, D. E., Cox, N. J., Katz, J. M., Taubenberger, J. K., Palese, P., & García-Sastre, A. (2005). Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science (New York, N.Y.)*, *310*(5745), 77–80.

[111]Dando, M. (1999). The Impact of the Development of Modern Biology and Medicine on the Evolution of Offensive Biological Warfare Programs in the Twentieth Century. Defense Analysis, 15(1), 43 – 62.

[112] Vindman, C., Trump, B., Cummings, C., Smith, M., Titus, A. J., Oye, K., Prado, V., Turmus, E., & Linkov, I. (2024). npj Biomedical Innovations, 2025, 2(1): 20.

[113]Wang, D., Tan, Z., Gao, J., Zhang, S., Shen, J., & Lu, Y. (2025). AI4Protein: transforming the future of protein design. *Science China. Life sciences*, *68*(10), 2880–2890.