Construction of high-yield daptomycin engineering strains and process design for the topical gel formulation workshop

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Abstract. In response to the industrial pain points of low yield of wild strains, high production costs, and single dosage form in clinical application in daptomycin industrial production, and to implement the goal of cultivating the ability to solve complex engineering problems in the undergraduate engineering education accreditation of the pharmaceutical engineering major, this study carried out research on the construction of high-yield daptomycin engineering strains and the process design of a 10 million-dose annual topical gel formulation workshop. Through genetic engineering technology, the pSET152-cumate-atrA recombinant plasmid was constructed and the cumate-induced expression system was established; with batch production as the core mode, formulation screening, process design, material balance, equipment selection, and GMP-compliant workshop layout design were completed, and a comprehensive technical and economic analysis was conducted. The results showed that the recombinant plasmid was successfully constructed and positive clones were verified by colony PCR and plasmid PCR, providing technical support for high-yield industrial strain construction; the optimal prescription and production process of daptomycin topical gel were determined, and the workshop layout meeting D and C grade clean area standards was completed with matched core equipment; the project achieved favorable after-tax annual net profit, cost-profit ratio and economic safety rate, showing good industrial feasibility and economic benefits. This study completed the practical teaching closed loop of the entire chain of "strain modification - formulation research and development - engineering design - industrial implementation" for the pharmaceutical engineering major, which can provide a reference model for undergraduate graduation design teaching and engineering practice ability cultivation of similar majors.

Keywords: Daptomycin；Genetically engineered bacteria；Cumate-induced expression system；Topical gel；Pharmaceutical workshop design； Pharmaceutical engineering education

1. Introduction

Daptomycin is a novel cyclic lipopeptide antibiotic produced by the metabolism of “Streptomyces roseosporus”. As the first marketed cyclic lipopeptide antibiotic, it demonstrates excellent antibacterial activity against multidrug-resistant Gram-positive bacteria, such as methicillin-resistant “Staphylococcus aureus” (MRSA) and vancomycin-resistant enterococci (VRE), due to its unique mechanism of action on the cell membrane. It also has a low rate of clinical resistance and is a first-line drug for treating complex skin and soft tissue infections, infective endocarditis, and other diseases [1-2].

The biosynthesis of daptomycin is encoded by the “dpt” gene cluster on the chromosome of “S. roseosporus”. This gene cluster is approximately 128 kb in length and comprises three core nonribosomal peptide synthetase (NRPS) genes, “dptA”, “dptBC”, and “dptD”, along with four functional modules including precursor synthesis genes, pathway-specific regulatory genes, and self-resistance genes. Its synthesis begins with decanoic acid, derived from fatty acid metabolism, as the lipid chain starter unit. The 13 amino acid substrates are sequentially activated by the adenylation domains of the NRPS system and undergo a series of post-translational modifications, including condensation, isomerization, and cyclization, to assemble the linear lipopeptide chain. Finally, the molecule is secreted extracellularly via a transmembrane transport system, forming the mature daptomycin molecule with complete antibacterial activity. The full biosynthetic process is shown in Figure 1.

https://doi.org/10.58244/joerai.263723

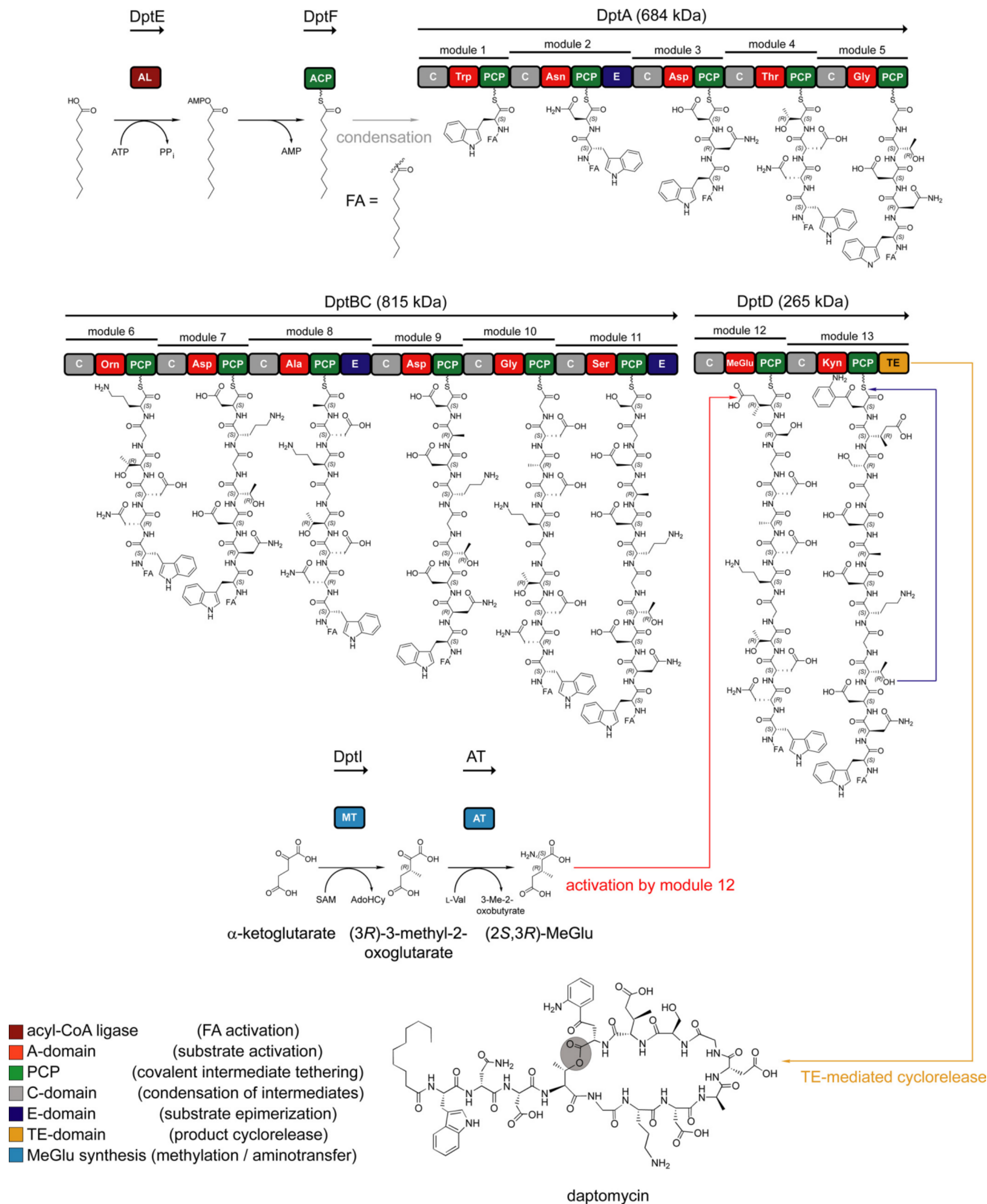

Figure 1. The synthesis process of Daptomycin

The industrialization of daptomycin currently still faces two major core bottlenecks: first, the fermentation yield of wild-type Streptomyces roseosporus is low, and the regulatory mechanisms of synthesis are complex, resulting in high production costs for the active pharmaceutical ingredient; second, the clinical dosage forms are mainly powder injections, with limited administration routes. Since skin infections are a common scenario for drug-resistant bacterial infections, developing a topical gel formulation could achieve precise local drug delivery, reduce systemic side effects, and have broad clinical and market prospects.

The undergraduate graduation project in Pharmaceutical Engineering is a core teaching component in engineering education accreditation, aimed at cultivating students' ability to solve complex pharmaceutical engineering problems and achieve multidisciplinary knowledge integration. This study, guided by the real industrial demand for daptomycin, combines synthetic biology strain modification with the full-process design of pharmaceutical engineering. It not only addresses the key technical bottlenecks of daptomycin industrialization but also establishes a practical teaching system

https://doi.org/10.58244/joerai.263723

spanning the full chain of 'molecular biology–genetic engineering–industrial pharmaceutics –pharmaceutical technology–pharmaceutical plant design–engineering economics,' providing a replicable implementation path for undergraduate practical teaching in pharmaceutical engineering. This paper systematically describes the methods for constructing high-yield engineered daptomycin strains, the full-process workshop design for topical gel formulations, and analyzes the practical value of this research in pharmaceutical engineering education.

2. Construction of High-Yield Daptomycin Engineered Strain

2.1 Experimental materials

The strains and primers used in the experiment are detailed in Tables 1 and 2; the experimental instruments include a fungus incubator, high-speed centrifuge, incubator shaker with temperature control, thermal cycler, electrophoresis apparatus, etc.; the experimental reagents include restriction endonucleases, PCR amplification kits, DNA ligation kits, plasmid extraction and gel recovery kits, as well as common biochemical reagents required for culture media preparation such as peptone and yeast extract.

Table 1 Strains Used in the Experiment

Experimental Strain	Description	Source
pSET152	Streptomyces integration vector	BGI
pSET152-cumate-atrA	Target gene template	BGI
TOP10 Competent Cells	Competent cells	Sino Biological Inc. (Shanghai)

Table 2 Primers Used in the Experiment

Primer Name	Primer Sequence
cumate-atrA-XbaI-F	GCTCTAGATCACCGCTTGAACTTGGC
cumate-atrA-EcoRI-R	CGGAATTCTCACGCCGGCCGTGAC

2.2 Experimental Method

1) Plasmid Extraction and Target Gene Amplification: Use a column-based plasmid extraction kit to extract the pSET152 vector plasmid and the template plasmid of the target gene; using cumate-atrA-XbaI-F/cumate-atrA-EcoRI-R as primers, optimize the PCR amplification program to amplify the target gene fragment, and purify the target fragment using a PCR product recovery kit.
2) Digestion and ligation: By optimizing a single-enzyme digestion time gradient, the optimal digestion conditions for Xba I and EcoR I were determined. The target gene and the pSET152 vector were then double-digested, and after gel extraction and recovery, the ligation reaction was carried out using T4 DNA ligase at vector-to-insert molar ratios of 1:3 and 1:5.
3) Transformation and Screening Verification: The ligation product was transformed into TOP10 competent cells, and positive single colonies were obtained through blue-white screening. The recombinant plasmid construction was confirmed using both colony PCR and plasmid PCR.

2.3 Experimental Results and Analysis

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1) Optimization of PCR amplification conditions: Comparing the amplification effects of two-step and three-step PCR, the results showed that the two-step PCR produced scattered bands that did not match the size of the target gene fragment (2700 bp); in contrast, the three-step PCR produced a single, bright, and clear band, with the optimal amplification achieved when the annealing time was 5 seconds, meeting the requirements for subsequent digestion and ligation experiments.
2) Optimization of enzyme digestion conditions: Through time gradient optimization, the optimal restriction conditions for the target gene were determined to be: XbaI digestion for 25 minutes and EcoRI digestion for 10 minutes; the optimal restriction conditions for the pSET152 vector were: XbaI digestion for 20 minutes and EcoRI digestion for 25 minutes. Under these conditions, the digestion products showed a single band, corresponding to the size of the target fragment, with no obvious degradation.
3) Construction and verification of recombinant plasmids: White positive colonies were obtained through blue-white screening. Both colony PCR and plasmid PCR verification results showed that the amplified product bands were around 2700 bp, consistent with the size of the target gene fragment, confirming the successful construction of the recombinant plasmid pSET152-cumate-atrA.

2.4 Function and Inducible Regulation Mechanism of atrA Gene

atrA is a pathway-specific positive regulatory gene of the dpt gene cluster responsible for daptomycin synthesis. It can directly bind to the promoters of dpt structural genes, upregulating the transcription levels of key biosynthetic genes, and is the core target determining the fermentation yield of the strain. In this study, the constructed recombinant plasmid places atrA under the strict control of a cumate-inducible expression system: in the absence of the cumate inducer, the CymR repressor protein binds to the operator sequence to inhibit atrA transcription; upon addition of cumate, the inducer binds to CymR, causing it to detach from the operator sequence and initiating high-level expression of atrA. Moreover, the expression level is positively correlated with the inducer concentration, allowing precise and dynamic regulation of the daptomycin biosynthetic pathway. The functional model of this inducible regulatory system is shown in Figure 2.

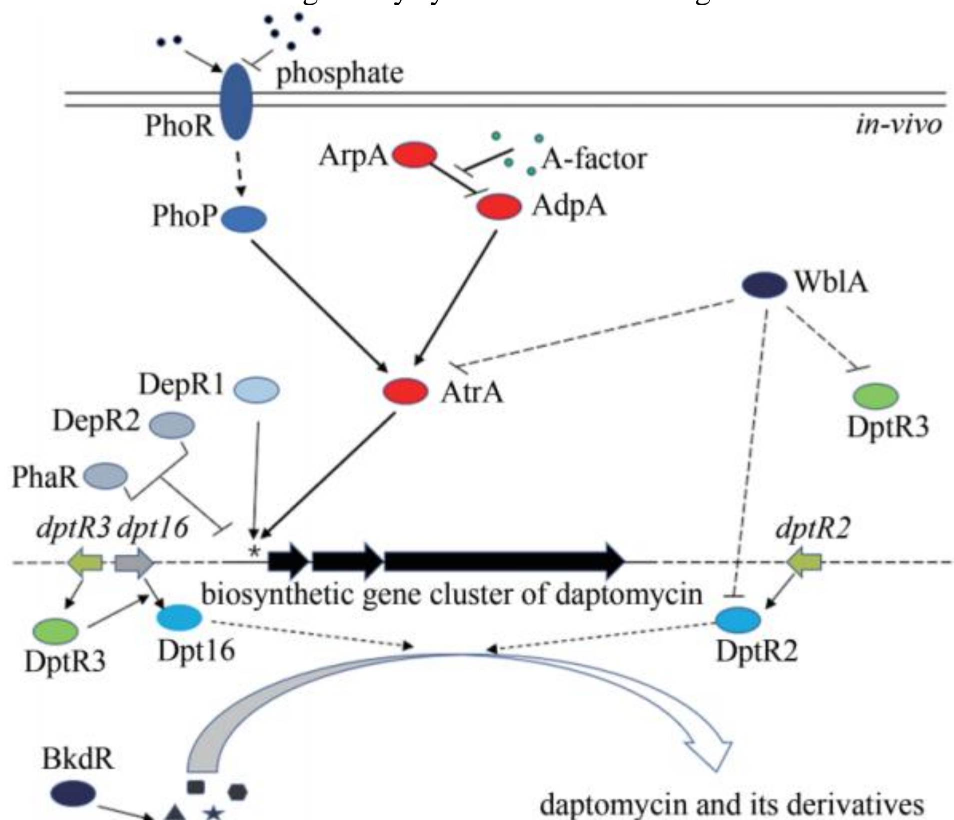

Figure 2. Regulatory Model of Daptomycin

https://doi.org/10.58244/joerai.263723

3. Process Design for Daptomycin Topical Gel Workshop

3.1 Design Fundamentals and Production Guidelines

The design site for this factory is selected in the High-tech Development Zone of Xuzhou City, Jiangsu Province. This area has well-established support for the biopharmaceutical industry, a comprehensive transportation network, and stable public utility guarantees, meeting the site selection requirements for a pharmaceutical factory. Core production plan: The designed annual output is 10 million units of daptomycin topical gel (specification 10g/unit), with 300 production days per year, operating on a two-shift intermittent production system, producing 2 batches per day, and 600 batches per year. The theoretical output per batch is 166.7 kg, with 16 hours of effective daily production and a total annual production duration of 4,800 hours. The workshop cleanliness is designed in strict accordance with the "Good Manufacturing Practice (GMP) for Pharmaceutical Products". The weighing and dissolution processes are set in a Grade D clean area, while the filling and primary packaging processes are conducted in a local Grade A environment under a Grade C clean area.

3.2 Prescription and Production Process

1) Formulation prescription: Through prescription screening, the optimal formulation (w/w) of daptomycin topical gel was determined, as detailed in Table 3. This formulation uses Carbomer 940 as the gel matrix, propylene glycol as a solubilizer and penetration enhancer, and triethanolamine as a pH adjuster. The system's pH is maintained between 6.8 and 7.2, meeting the safety and stability requirements for topical use.

Table 3 Formulation of Daptomycin Topical Gel

Component	Mass Fraction	Functional Role
Daptomycin	1%	Active Ingredient
Carbomer	1%	Gel Base
Glycerin	5%	Moisturizer
Propylene	5%	Solvent/Permeation Enhancer
Triethanolamine	1.35%	pH Adjuster
Phenoxyethanol	1%	Preservative
Disodium	0.1%	Chelating Agent/Stabilizer
Purified Water	85.55%	Solvent

2) Core Production Process: The core production process is: substrate swelling → active ingredient pretreatment → material mixing → neutralization reaction → vacuum degassing → intermediate inspection → filling and capping → outer packaging → finished product inspection → storage. The core yield of each process is: raw material pretreatment 99%, excipient/substrate dissolution 98%, mixing and homogenization 98%, degassing 98%, filling 98%, outer packaging 100%.

3.3 Material Balance

Based on the production program, formulas, and process yields, a material balance for a single batch was completed, with the results shown in Table 4. The total annual material input is 108,387.4 kg,

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with a total process loss of 8,387.4 kg. The results of the material balance provide a core basis for equipment selection, workshop material flow design, and cost accounting.

Table 4 Single-Batch Material Balance Results

Material Name	Single Batch Input/kg	Single Batch Loss/kg
Carbomer 940	1.8252	0.1585
Daptomycin	1.8252	0.1585
Glycerin	9.1260	0.7927
Propylene	9.1260	0.7927
Triethanolamine	2.4640	0.2140
Phenoxyethanol	1.8252	0.1585
Disodium	0.1825	0.0158
Purified	154.2717	11.6884
Total	180.6458	13.9791

3.4 Equipment Selection

Following the principles of GMP compliance, production compatibility, and economic practicality, and based on material balance results and production process requirements, the selection of core production equipment has been completed. A summary of the main equipment is shown in Table 5. All selected equipment meets the requirements for clean production, with parts that come into contact with materials made of 316L stainless steel. Core process equipment is integrated with CIP/SIP cleaning systems and complies with pharmaceutical production equipment management standards.

Table 5 Overview of Main Production Equipment in the Workshop

No.	Equipmen t Model	Equipment Name	Core Specifications	Quantity
1	XH-CL-3 0	Matrix Dissolving Tank	30L stainless steel reactor, stirring speed 330rpm	1
2	S212-100 L	Raw Material and Auxiliary Material Dissolving Tank	100L double-layer glass reactor, stirring speed 600rpm	1

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Table 5 (Cont.) Overview of Main Production Equipment in the Workshop

No.	Equipment Model	Equipment Name	Core Specifications	Quantity
3	SMT-B300L	Vacuum Emulsifying Stirring Tank	300L stainless steel reactor, integrated homogenization and degassing functions	1
4	RGPL-1580A	Hose Filling, Sealing, and Cartoning Production Line	Capacity 1800-4800 units/hour, filling accuracy ±1%	1

3.5 Workshop Layout Design

The workshop adopts a single-story reinforced concrete structure with a rectangular centralized layout, totaling a construction area of 1,296㎡ (48m × 27m). The column grid follows a standard 6m × 6m design, divided into four functional zones: general production area, D-level clean area, C-level clean area, and non-production auxiliary area. Core principles of layout design: strictly separate personnel and material flow paths to prevent cross-contamination; arrange clean areas sequentially according to the process flow to minimize material backtracking; locate auxiliary production and office areas upwind of production areas to reduce contamination risks; set up explosion-proof compartments for high-risk processes to meet safety production requirements. Personnel entering clean areas must go through purification procedures including changing clothes, hand washing and disinfection, buffering, and airlocks. Materials enter the clean area's temporary storage room through external cleaning, disinfection, and transfer windows, achieving complete separation of personnel and material flows.

3.6 Non-Process Design and Techno-Economic Analysis

1) Non-Process Design Key Points: Non-process design includes modules such as fire and explosion protection, safety and hygiene, water supply and drainage, electrical systems, and environmental protection. The workshop is designed as a Class C factory building with a Level II fire resistance rating, and the clean area uses Class A fire-resistant materials, equipped with a complete fire protection system and combustible gas alarm devices. The water supply and drainage system uses a purified water heat circulation network, and wastewater is treated according to standards before discharge. Exhaust gas is treated with condensation recovery and RTO incineration, solid waste is handled by qualified units, and factory boundary noise and pollutant emissions comply with national standards.

2) Techno-Economic Analysis: After calculation, the total annual cost of raw and auxiliary materials and packaging for the project is 266 million yuan, and the comprehensive costs including energy, labor, and plant depreciation are approximately 8 million yuan per year, making the total annual production cost about 274 million yuan. Based on a product ex-factory price of 50 yuan per unit, the annual sales revenue is 500 million yuan, with an after-tax annual net profit of 153 million yuan, a cost-profit ratio of 55.97%, and an economic safety margin of 35.87%. The project has strong profitability and risk resistance, and its industrial implementation is highly feasible.

4. The Practical Value of This Research in Pharmaceutical Engineering Education

This study is guided by the real needs of the industry and completes the design and research of the entire process from upstream strain modification for drug synthesis to downstream industrial production of formulations. It fully aligns with the core concepts of engineering education

accreditation for pharmaceutical engineering and has significant practical value in undergraduate teaching. First, a practice-based teaching system integrating multidisciplinary knowledge has been established. This study integrates key knowledge points from core pharmaceutical engineering courses, including molecular biology, genetic engineering, industrial pharmaceutics, pharmaceutical process engineering, plant design, engineering economics, and drug GMP regulations. It breaks down the traditional knowledge barriers between courses, enabling students to transform theoretical knowledge into engineering applications and effectively cultivating their ability to solve complex pharmaceutical engineering problems.

Second, it implements dual cultivation of innovation ability and engineering thinking. Focusing on the technical challenges in the industrialization of daptomycin, students are guided to conduct innovative research on genetic engineering strain modification, fostering their scientific and innovative thinking. Meanwhile, the full-process plant design, from process selection to compliance design and economic feasibility analysis, fully reproduces the real development process of pharmaceutical engineering projects, helping students establish an engineering mindset encompassing the complete life cycle of 'technology-compliance-economics-environmental protection.'

Third, it provides a replicable model for undergraduate graduation projects. This study forms a complete implementation path of 'industry problem-scientific research-engineering design-application verification' for graduation projects, ensuring both the academic rigor and practical applicability of the research content. It also aligns with the knowledge level and capability boundaries of undergraduates, providing a reference for project selection, teaching implementation, and process guidance for graduation designs in pharmaceutical engineering, bioengineering, and related majors.

5. Conclusion and Outlook

This study successfully constructed the pSET152-cumate-atrA recombinant plasmid and the cumate-inducible expression system, providing core technical support for the development of industrial high-yield engineered strains of daptomycin. It also completed the full-process engineering design for a workshop producing 10 million units of daptomycin topical gel per year, determining a compliant, efficient, and economically feasible production plan. Techno-economic analysis indicates that the project has good industrialization prospects.

At the same time, this study used the undergraduate graduation project of Pharmaceutical Engineering as a platform to achieve a deep integration of scientific research innovation and engineering practice, fully realizing the competency development objectives of engineering education accreditation. It not only provides a theoretical and practical foundation for the industrial application of daptomycin but also offers an implementable model for the reform of undergraduate practical teaching in Pharmaceutical Engineering. Subsequent studies can further carry out fermentation validation of the engineered strains and pilot-scale research on formulation prescriptions, refine the construction drawing depth of the workshop design, and continuously enhance the practical engineering conversion capability of the plan.

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