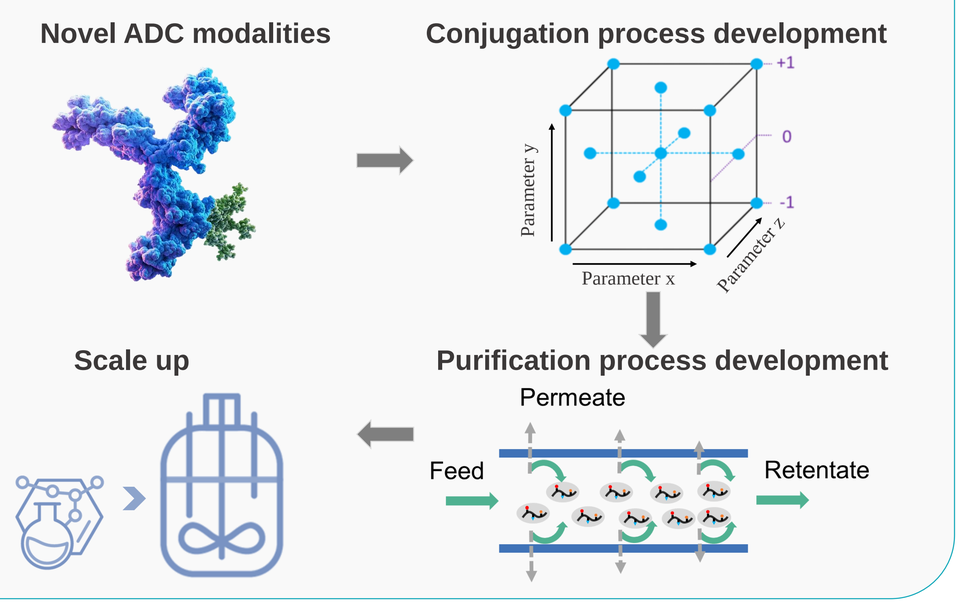
# Development of Versatile and Scalable Bioconjugation Processes for Next-Generation ADCs

Qiang Peng, Jidong Zhao, Song Liang, and Kai Gao
Shanghai Asymchem Biotechnology Co., Ltd, 4th Floor, Building No. 1, 518 Haoye Road, Jinshan Industrial Park, Jinshan, Shanghai, China

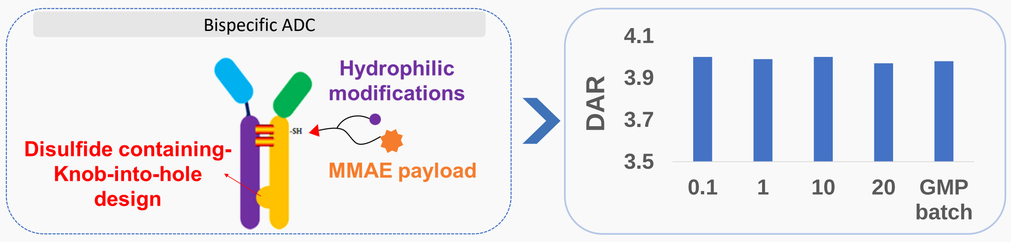
## Introduction

The development of conjugation processes for next-generation antibody-drug conjugates (ADCs), such as bispecific ADCs and dual-payload ADCs, faces significant challenges due to their complex multi-modular structures (e.g., multiple antibody-binding domains, diverse types of payloads or linkers) and novel chemical properties. As a global CDMO organization, AsymBio provides end-to-end ADC solutions to help address the CMC (chemistry manufacturing control) challenges for novel ADC modalities. In this poster, we presented several case studies to highlight our efforts in addressing the challenges in the conjugation and purification process development of next generation ADCs. Our solutions include comprehensive optimization of the conjugation process for bispecific and dual-payload ADCs, proven residual free-drug removal strategies and robust scale-up scheme. It enables the accelerated development of robust and scalable conjugation processes for next-generation ADCs, minimizing the risk in future ADC manufacturing.



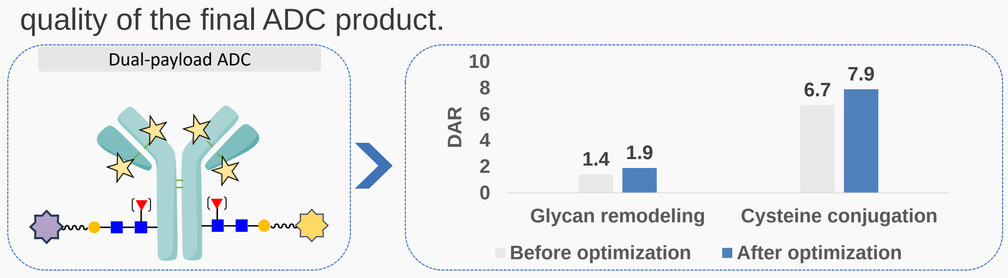

## Case Study I: Process Optimization for More Reproducible & Homogeneous Bispecific ADCs

In this case, the ADC is designed as a cysteine-conjugated nanobody-Fc fusion bispecific antibody with a targeted value of DAR 4. The payload is MMAE and the linker contains a hydrophilic moiety branch as an added modification to the molecule design. The BsAb bears a disulfide-containing knob-into-hole structure, which results in additional needs to control non-specific Cys-conjugation. We systematically optimized the reduction and conjugation process parameters to generate ADC products with DAR 4 species accounting for greater than 98%, and the DAR value remains highly consistent at different product scales.



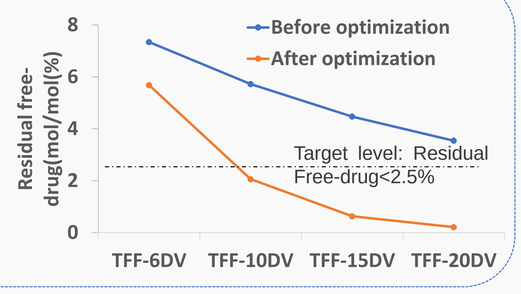

## Case Study II: Process Development for Novel Dual Payload ADCs

In this case, the ADC is designed as a dual-site DAR 2+4 dual-payload ADC. The first payload-linker is conjugated through glycosylation site conjugation, and the second payload-linker is conjugated through cysteine conjugation. Multiple purification steps are involved for the removal of process and product-related impurities. We optimized these conjugation and purification steps, and strict in-process control is implemented to significantly improve the conjugation efficiency and quality of the final ADC product.

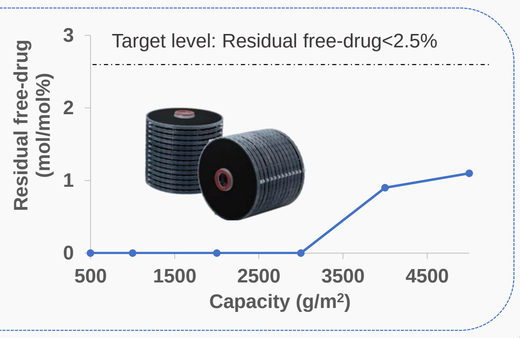



## Case Study III: Optimization on the Removal of Residual Free-drug

The hydrophilic payload-linker tends to form micelle particles, which are hard to be removed through traditional UF/DF approach. In this case, we use co-solvent in the UF/DF-1 step to remove residual free-drug to the target level, and then deploying a UF/DF-2 step to remove the added co-solvent.



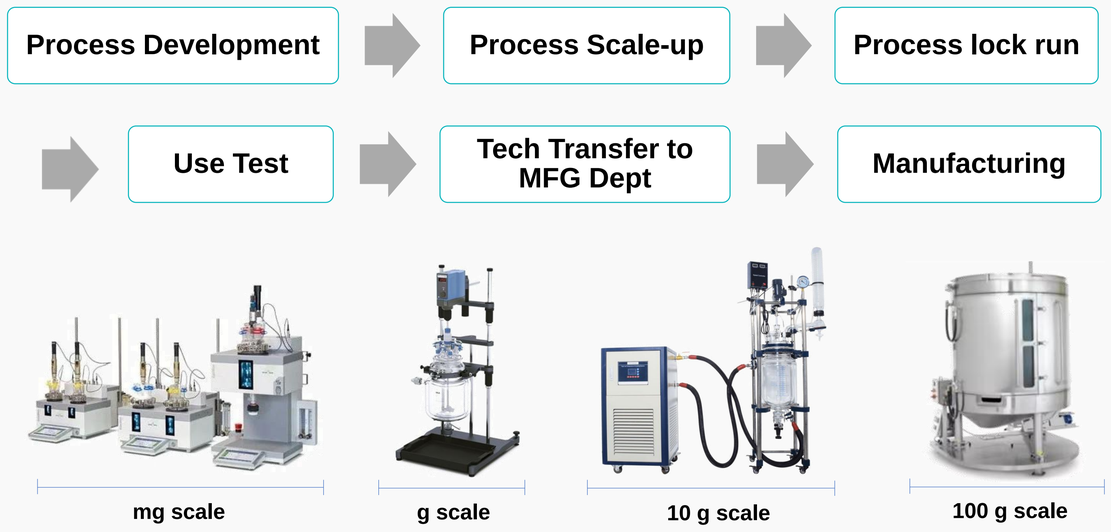

In the second example, the bulky and hydrophobic payload-linker is unable to be efficiently removed through the traditional UF/DF approach. We used activated charcoal filtration (ACF) to remove the residual free-drug to target levels with a loading capacity as high as 3000 g/m$^2$.




## Case Study IV: Robust Scale-up Strategy

AsymBio has established a comprehensive scale-up strategy by focusing on key process parameters. To ensure successful manufacturing, we confirm and lock the process parameters by performing a 20-50 g scale test run. In addition, a 2-5 gram scale of use test run using the same grade of materials as those used in manufacturing production is also carried out prior to the manufacturing kickoff.

| Process Parameter Types | Examples of Process Parameters | Scale-up Strategy |
|---|---|---|
| Volume Dependent | PL/Reducing Reagent Feeding Speed | Maintain the Same Feeding Speed |
| Volume Independent | mAb Concentration, pH, Equivalent of PL/Reducing Reagent, Reaction Time, Temperature | Optimizing the Operation Space |
| Nonlinear | Agitation Speed | CFD model and Mixing study at different scales |




## Conclusion

Case studies showcase the capability of AsymBio's bioconjugation platforms in addressing the challenges for the process development of next-generation ADCs. Our well-developed conjugation process platforms are flexible to handle different ADC molecule designs and form a solid foundation to support the process development and process transfer for pre-IND, IND and BLA projects. The conjugation process development can be expedited with a compressed timeline, typically 2-3 months, for an IND project. Under the strict compliance to EHS regulation on OEB5 level materials, we aim to safely and efficiently deliver consistent and scalable bioconjugation processes for all our clients.

Web：www.asymbio.com E-mail：MKT@asymchem.com.cn