

Bispecific Antibodies (BsAbs) Services

Protein Production & Antibody Discovery



Drive Value with
Novel Immuno-Oncology Products Based on
Proprietary Discovery Platform

Y-BIOLOGICS

How to make your bispecific antibody format?

1. What are bispecific antibodies (bsAb)?

Bispecific antibodies (bsAbs) represent a major advancement in therapeutic biotechnology. Unlike conventional antibodies that bind a single antigen, bsAbs can simultaneously bind two different targets. This dual-targeting capability enables innovative therapeutic strategies, particularly in oncology, immunotherapy, and hematologic diseases.

However, the success of a bsAb therapy depends heavily on selecting the appropriate format. Because multiple bispecific formats exist, each with unique advantages and limitations, choosing the optimal format is a critical step in drug development.

2. The two main groups of bsAb

Bispecific antibodies are a class of engineered antibodies designed to target two distinct antigens simultaneously. They possess unique structures that allow them to bind to multiple targets, enabling novel therapeutic strategies in various disease areas.

BsAbs can be broadly categorized into two main groups: those with Fc regions and those without. The Fc region is the tail region of an antibody that interacts with immune cells and mediates immune effector functions. Fc-containing bsAbs retain Fc-mediated effector functions such as ADCC and CDC. While Fc-lacking bsAbs often show improved tissue penetration and may reduced certain safety risks. Both approaches offer unique advantages depending on the therapeutic objective.

BsAbs hold immense potential for addressing unmet medical needs and driving innovation in drug development. In the following sections, we will evaluate the key factors to consider when choosing a bsAb format, empowering you to make informed decisions.

Case study

Case Study on Bispecific Antibody Production

Engineering and reformatting your antibody: from mAb to bsAb

1. Knobs-in-holes
2. CrossMab
3. IgG-scFv
4. Y-body (2 by 1 or 1 by 1)
5. T Cell Engager
6. VHH antibody

3. Five factors when choosing a BsAb format

The antibody format significantly influences efficacy, safety, pharmacokinetics, and manufacturability. Selecting the most suitable format is therefore critical for successful therapeutic development.

After considering the therapeutic objective and target antigens, it's essential to evaluate pharmacokinetics, manufacturing feasibility, and immunogenicity of the antibody under consideration.

1) Therapeutic objective

Consider the specific disease target(s) and the desired mechanism of action for the bsAb. For example, if the goal is to activate immune cells and enhance tumor cell killing, a bsAb format capable of engaging both T cells and tumor cells may be preferred.

2) Target antigens

Evaluate the expression levels and distribution of the target antigens in tissues and cells of interest. Additionally, consider the specificity and affinity required for each target antigen to ensure effective target engagement and therapeutic efficacy.

3) Pharmacokinetics and pharmacodynamics (PK/PD)

Consider factors such as serum half-life, tissue distribution, and clearance rates. Additionally, evaluate the pharmacodynamic effects of the bsAb, including receptor binding kinetics and downstream signaling pathways.

4) Immunogenicity and safety

Assessing the potential immunogenicity of the bsAb format is crucial for ensuring patient safety. Consider strategies to minimize immunogenicity, such as humanization of antibody sequences.

5) Manufacturing considerations

Evaluate the feasibility and scalability of manufacturing the chosen bsAb format. Consider factors such as expression system compatibility, purification methods, and downstream processing requirements. Choosing a format that is compatible with current manufacturing capabilities can streamline the production process and reduce development costs.

At Y-Biologics, we specialize in navigating you through the intricate process of selecting the optimal bispecific antibody format tailored to your therapeutic objectives. But we don't just provide guidance; we also handle the production of the required antibodies, ensuring the highest quality in minimal time. With our collaborative methodology and state-of-the-art technology, you can confidently propel your bsAb projects towards clinical triumph.

4. Common bispecific antibody formats

When selecting the optimal bispecific antibody format for your therapeutic goals, several commonly used architectures may be considered. These include the Y-body system (2 by 1 or 1 by 1), which facilitates the creation of bsAbs through a straightforward Fab-arm exchange. In Fab arm exchange, two antibody fragments containing different antigen-binding sites are combined under controlled laboratory conditions to allow bonds to form, creating a bispecific molecule.

Another option is the IgG-scFv format. In this format, two single-chain variable fragments (scFvs) from the same antibody are fused to the heavy or light chains of another antibody. scFvs consist of the variable regions of both heavy and light chains connected by a flexible linker, which together comprise an antigen-binding site. IgG-scFvs are tetravalent, with two binding sites per antigen (2 + 2 valence). They have variable expression and purification schemes depending on the specific scFv sequence used.

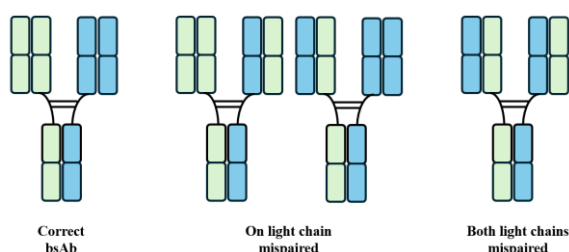
The Knobs-into-Holes (KIH) format and related techniques like CrossMab present additional methods 1 + 1 bispecific generation. In these formats, heavy chains of two different antibodies are modified to introduce distinctive binding sites—a “knob” or “hole”—to promote their heterodimerization. However, these formats can come with their own specific challenges, such as undesired homodimer formation or improper heavy/light chain pairing.

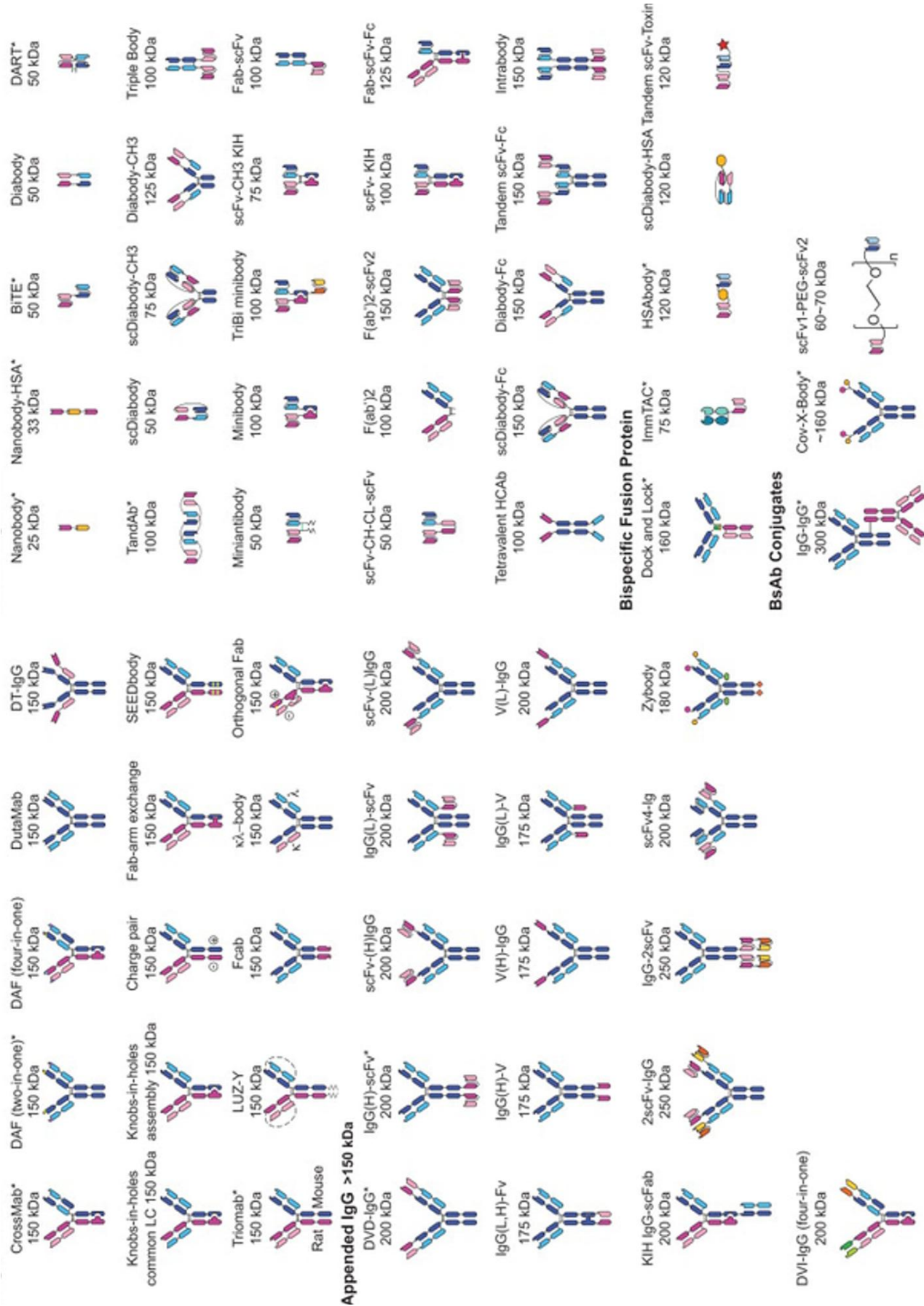
5. Bispecific antibody production with Y-Biologics

Selecting the appropriate bispecific antibody format is essential for successful therapeutic development. By collaborating with experts and utilizing advanced technologies, researchers can navigate this process effectively and accelerate bispecific antibody development.

Y-Biologics offers comprehensive support throughout the development journey, from format selection to production. Y-Biologics delivers high-quality bispecific antibody production with correct heavy-chain/light-chain pairing efficiency. This technology enables rapid transient production of variants for discovery and non-GMP development studies, accelerating the identification of lead candidates and facilitating earlier access to engineered bispecifics.

Whether you require format selection, antibody engineering, or rapid protein production, Y-Biologics provides an integrated platform to accelerate your bispecific antibody development.





	Upper hinge	Core hinge	Lower hinge	Disulfide bonds
	Papain		Pepsin	
	↓		↓	
HuIgG ₁	EPKSCDKTHT	CPPCP	APELLGGP	2
HuIgG ₂	ERK	CCVE CPPCP	APVA -GP	4
HuIgG ₄	ESKYGPP	CPSCP	APE FL GGP	2
EU:	216	226	231	238

Figure 3.2 Human antibody hinge sequences

Comparison of the sequences of the human IgG₁, IgG₂, and IgG₄ hinge regions, with separation of the upper hinge, core hinge, and lower hinge sequences. Differences in sequences in hulG₂ and hulG₄ from hulG₁ are noted in bold, and the number of disulfide bonds within each hinge is noted at the right. The left and right arrows denote the cleavage sites for papain and pepsin, respectively. Numbering based on hulG₁ according to the EU system is noted at the bottom. Note that the lower hinge is also part of the C_H2 constant domain, as shown in Figure 9.2.