A Highly Developable Bispecific Antibody Platform Enables High-Throughput Bispecific Discovery

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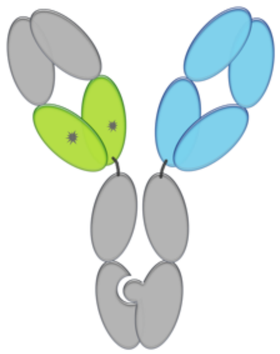
Introduction

Bispecific antibodies represent an emerging class of drug molecules that enable unique mechanisms of action relative to their monoclonal counterparts. Although two bispecific antibody molecules have gained approval and numerous others have reached the clinic, challenges remain for the creation of highly developable, human IgG-like bispecific scaffolds. Here, we describe the generation of a robust multispecific platform exhibiting biophysical properties comparable to the parent monoclonal IgGs. Through a novel domain-substitution strategy, we have enabled highly orthogonal heavy chain-light chain pairing to ensure the formation of properly paired, four-chain and five-chain multispecific molecules. The B-Body™ retains high stability and possesses transient expression titers comparable to mAbs. Furthermore, the B-Body™ is compatible with Invenra's proprietary antibody libraries to afford a truly "plug-and-play" platform for efficient high-throughput bispecific discovery. We have successfully exploited the high-throughput potential of the B-Body platform to create a series of bispecific molecules with potent biological activity acting through multiple mechanisms of action.

B-Body™ Design Strategy



Domain Substitution Strategy:

- First Fab Arm
  - Wild type Fab architecture
  - Compatible with all variable domain frameworks
- CH1/CL Domain Substitution
  - Substituted domain derived from another human antibody domain
  - Substitution enables highly specific LC/HC pairing
  - Domain substituted fab has thermostability comparable to the native Fab
  - Easy separation of initial by-products after Protein A
- Knob-in-Hole-like Mutation:
  - Enables robust HC/HC pairing
  - Mutation set is clinically validated

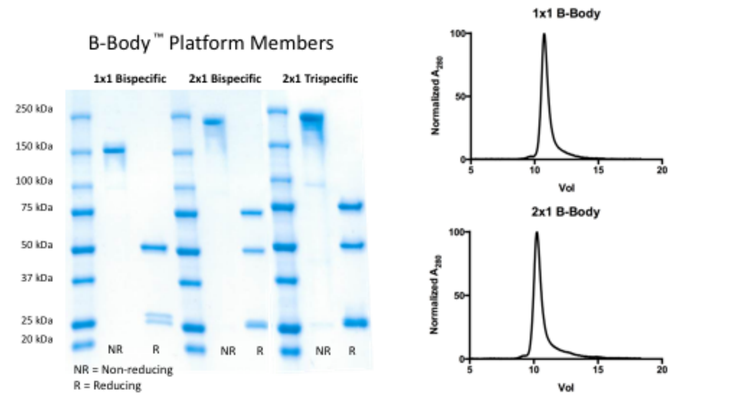
Figure 1. B-Body Platform design. The B-Body platform exploits domain substitution in the constant domain of one Fab arm to achieve highly specific heavy chain-light chain pairing. This design strategy results in highly pure bispecific antibodies after single-step purification to enable high-throughput discovery. Robust chain pairing enables facile extension to various 2x1 formats with high yield and proper assembly. Furthermore, additional sets of orthogonal mutations enable higher-order specificities, including the 2x1 trispecific B-Body.

The B-Body Possesses Superior Developability Characteristics

Parameter	Unit	Bispecific 1x1 B-Body™	Bispecific 2x1 B-Body™	Trispecific 1x2 B-Body™
Purification Yield After Protein A Purification	mg/L	300	150	100
Homogeneity After Affinity Purification	% SEC Area	98	98	85
Fab Arm Denaturation Temp (T_m)	°C	77	77	70 & 77

Table 1. Expression, purification and stability of the B-Body™ platform. Small-scale expression yields are derived from the Expi293 expression system. Thermostability of the Fab arm was determined by differential scanning fluorimetry.

B-Body™ Yields Highly Pure and Stable Bispecifics



B-Body Variant (Fab arm)	Fab T_M (°C)
BC1	69
BC5	71
BC6	77
BC15	68
BC16	66
BC28	77
BC44	76
BC45	76

Figure 2. The B-Body platform yields high-quality bispecific antibodies. A) SDS-PAGE gel of variants of the B-Body platform yield highly pure protein after single-step purification. Proper chain assembly was confirmed by LC-MS. B) Size exclusion chromatography of the 1x1 and 2x1 B-Body yield monodisperse samples after single-step purification. C) The B-Body arm was engineered to possess thermal stability comparable to the native Fab arm. D) Representative accelerated stability study of the B-Body at 8.6 mg/ml at 40 ºC in PBS validates the design principles of the platform.

HTP-Enabled Discovery Using the B-Body™ Platform

SNIPER™ Strategy

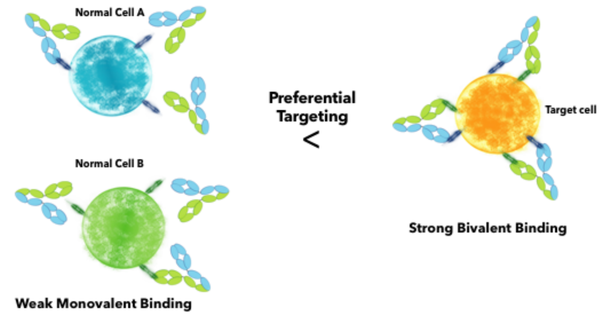


Figure 3 SNIPER™ strategy for specific cell targeting. Through the use of the B-Body™ platform, precise targeting of distinct cell types expressing two targets can be achieved through combining arms specific to each target with weak, monovalent binding. Combination in a bispecific can lead to high-affinity, specific binding.

Combinatorial Bispecific Matrices Precisely Rapidly Identify B-Body™ Candidates

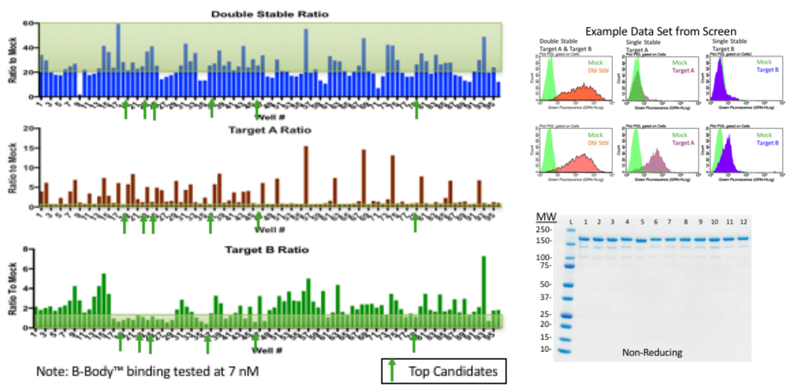


Figure 4. High-throughput identification of bispecific SNIPER™ leads. A) Flow cytometry analysis of a 96 bispecific matrix for binding to cells expressing both targets or either target alone. A number of B-Body variants possessed strong binding to the double-positive cell line while exhibiting minimal binding to either variant alone. B) Representative data for individual flow cytometry experiments demonstrating the range of characteristics of the matrix variants. C) Representative gel of the bispecific proteins purified from small-scale expression.

Conclusions

- **The B-Body platform allows for robust chain pairing and high thermo-stability necessary to produce highly developable bi- and multi-specific antibodies**
- **The B-Body platform enables high titers from small-scale expression to for high-throughput characterization**
- **We have successfully created a number of molecules using the B-Body platform, including TNFR agonists and CD3 redirected T-cell bispecfics demonstrating the "plug-and-play" potential of the B-Body**