

Hetero-Trimerization Domain of Type IX Collagen: A Unique Tool for Hetero-Specific Combinatorial Libraries

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Collagens and collagen-like proteins are elongated molecules whose structure is distinguished by the presence of a triple helical domain that frequently constitutes the major portion of the protein. For the proper folding of the triple helix collagens contain trimerization domains. These domains ensure a single starting point for triple helix formation and are also responsible for the chain selection in hetero-trimeric collagens. These domains are not only crucial for biological functions, but they are also attractive tools for protein engineering and biomaterial design. Recently, two homo-trimerization domains of type XV and XVIII collagens have been successfully used to produce and test a new class of multivalent and multispecific antibody-based reagents for therapy [1,2].

Here we report the crystal structure of the NC2 domain (only 35 residues) of human hetero-trimeric collagen IX with all three distinct chains. Naturally located in the middle of the triple-helical domain, the isolated form of the NC2 domain provides all N- and C-termini for additional fusion payloads. Three distinct chains can be produced individually and then reconstituted into the hetero-trimer bearing six different extensions. This system opens doors for combinatorial libraries of targeted delivery, as a limited number of different targeting payloads (N variants) can be specifically combined in the trimer (N³ variants) and screened for best multi-specific molecules.

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2. Cuesta AM, Sánchez-Martín D, Blanco-Toribio A, Villate M, Enciso-Álvarez K, Alvarez-Cienfuegos A, Sainz-Pastor N, Sanz L, Blanco FJ, Alvarez-Vallina L. Improved stability of multivalent antibodies containing the human collagen XV trimerization domain. *MAbs*. 2012 4(2):226-32.