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The variable lymphocyte receptor as an antibody alternative

Elizabeth A Waters and Eric V Shusta

Variable lymphocyte receptors (VLRs) are leucine-rich repeat proteins in jawless vertebrates that function similarly to Ig antibodies. However, VLRs possess a distinct crescent-shaped structure and modularity that results in a concave binding interface that contrasts significantly with Ig antibodies. Antigen binding interactions result in specific, high affinity VLR binding interactions with both proteins and glycans. The natural sourcing of VLRs allows for immunization strategies, while the modularity enables a whole host of protein engineering approaches including consensus scaffolds, designed libraries and directed evolution with display technologies. VLR technologies have been recently deployed for applications in cell-specific targeting, drug delivery, tumor diagnostics and even protein stabilization. It is anticipated that the VLR field will continue to emerge to provide unique solutions for targeting glycans, evolutionarily conserved proteins and cellular specificity.

Address
Department of Chemical and Biological Engineering, University of Wisconsin – Madison, Madison, WI 53706, USA

Corresponding author: Shusta, Eric V (eshusta@wisc.edu)

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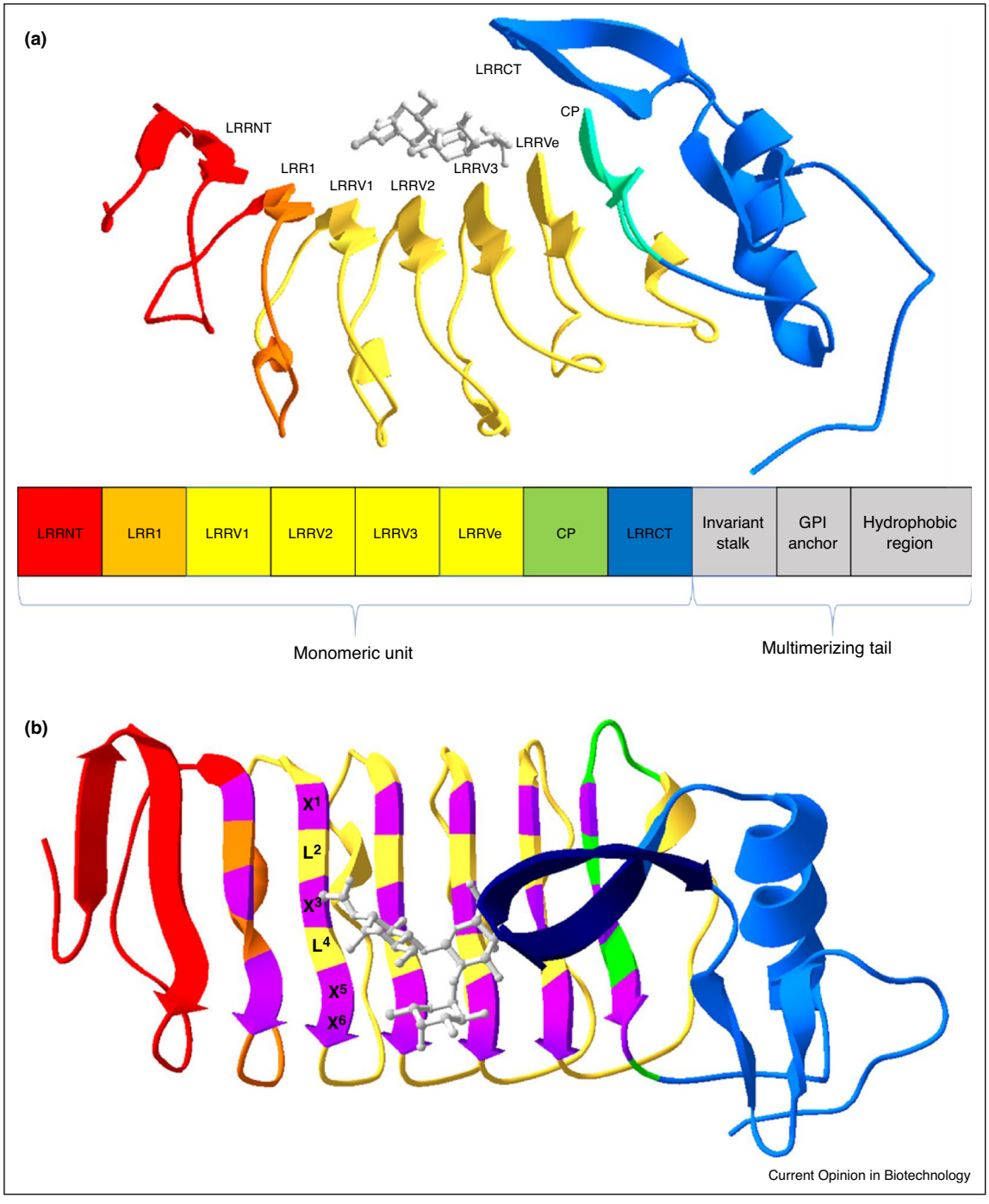
Introduction

Antibodies represent a rapidly growing therapeutic class given that their high affinity selective binding can provide neutralizing or cytotoxic therapy for many diseases. However, the antibody field has recognized that certain applications could benefit from binding scaffolds that have enhanced properties such as tissue penetration, stability or production. As such, alternative binding scaffolds have been developed, including DARPINS, affibodies, adnectins, anticalins, and cysteine knot proteins, among others [1–6]. This review will focus on a relatively new antibody alternative based on a class of natural antigen binders found in jawless vertebrates known as variable lymphocyte receptors (VLRs) and describe recent engineering and therapeutic applications.

VLR biology and structure

VLRs are part of the adaptive immune system of jawless vertebrates. Early evidence indicated that lamprey and hagfish had an adaptive immune system; and while they had cells that resemble mammalian lymphocytes, no immunoglobulin, T cell receptor, or major histocompatibility complex genes could be identified [7–9]. Instead, Pancer and colleagues identified VLRs as the antigen receptors in activated lamprey lymphocytes [10]. In contrast to the mammalian immunoglobulin fold, VLRs are leucine rich repeat (LRR) proteins that generate diversity by a combination of differential repeat number and sequence variability [10,11] (Figure 1). In a bit more detail, three different VLR genes, *VLRA*, *VLRB* and *VLRC* have been identified in jawless vertebrates, with *VLRA* and *VLRC* being expressed in lymphocytes that resemble T cells and *VLRB* expressed in lymphocytes resembling B cells [12,13]. Since VLRB is expressed in a soluble format by B cell-like lymphocytes which respond to an adaptive challenge, VLRB is the most widely used form for VLR applications. VLR diversity is generated by gene conversion-type mechanisms [10,11] which yield VLRs comprising an N-terminal cap (LRRNT), the first LRR (LRR1), up to seven variable LRR (LRRVs), an end LRR (LRRVe), a connecting peptide (CP), a C-terminal cap (LRRCT), an invariant stalk, a glycosylphosphatidylinositol (GPI) anchor, and a cysteine rich hydrophobic region that can drive VLR multimerization [14,15] (Figure 1a). These modules combine to form a crescent-shaped solenoid structure with a concave surface consisting of parallel β-sheets which forms the antigen binding site [16,17] (Figure 1). Within the antigen binding site, there exist highly variable residues with each interfacial LRRV β-sheet having six residues of the general form $X^1LX^3LX^5X^6$ [18,19]. The leucine residues (L) form the hydrophobic core and the variable amino acids (X) generate the unique antigen binding interface (Figure 1b). Soluble VLRs also multimerize by forming disulfide bonds via a cysteine-rich hydrophobic region [14], but VLRs can also be used in monomeric forms for biotechnology purposes by expression without the hydrophobic region. Thus, VLRs represent an alternative antigen receptor, with many parallels to mammalian antibodies, that can combine with antibody engineering platforms to address both traditional and unique challenges.

Figure 1



Overview of VLR structure. **(a)** Ribbon structure of a monomeric VLR in complex with an H-trisaccharide (gray) (PDB code 3E6J [16]). Below the ribbon structure is a modular rendition of a full-length VLR consisting an N-terminal cap (LRRNT), the first leucine-rich repeat (LRR) module (LRR1), three variable LRR (LRRVs), an end variable LRR (LRRVe), a connecting peptide (CP), a C-terminal cap (LRRCT), an invariant stalk, a glycosylphosphatidylinositol (GPI) anchor, and a hydrophobic region. **(b)** The VLR structure is rotated 90° with highly variable residues of the 6-residue motif ($X^1LX^3LX^5X^6$) in purple and the variable sized insert of the LRRCT in dark blue. X represents highly variable amino acids and L, leucine.