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Sensitivity of immune response quality to influenza helix 190 antigen structure displayed on a modular virus-like particle

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ABSTRACT

Biomolecular engineering enables synthesis of improved proteins through synergistic fusion of modules from unrelated biomolecules. Modularization of peptide antigen from an unrelated pathogen for presentation on a modular virus-like particle (VLP) represents a new and promising approach to synthesize safe and efficacious vaccines. Addressing a key knowledge gap in modular VLP engineering, this study investigates the underlying fundamentals affecting the ability of induced antibodies to recognize the native pathogen. Specifically, this quality of immune response is correlated to the peptide antigen module structure. We modularized a helical peptide antigen element, helix 190 (H190) from the influenza hemagglutinin (HA) receptor binding region, for presentation on murine polyomavirus VLP, using two strategies aimed to promote H190 helicity on the VLP. In the first strategy, H190 was flanked by GCN4 structure-promoting elements within the antigen module; in the second, dual H190 copies were arrayed as tandem repeats in the module. Molecular dynamics simulation predicted that tandem repeat arraying would minimize secondary structural deviation of modularized H190 from its native conformation. *In vivo* testing supported this finding, showing that although both modularization strategies conferred high H190-specific immunogenicity, tandem repeat arraying of H190 led to a strikingly higher immune response quality, as measured by ability to generate antibodies recognizing a recombinant HA domain and split influenza virion. These findings provide new insights into the rational engineering of VLP vaccines, and could ultimately enable safe and efficacious vaccine design as an alternative to conventional approaches necessitating pathogen cultivation.

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1. Introduction

Synthetic biomolecular engineering has enhanced understanding of biological processes and design of novel proteins [1,2]. Fusion of different protein domains to realize a synergic combination of modules [3] has been used extensively to create hybrid enzymes [4] and humanized antibodies [5]. This approach has yet to impact vaccines significantly, but rational vaccine design based on presentation of protective antigen modules [6,7] is emerging.

VLPs are protein assemblies that mimic viral structure [8–14]; modular VLPs combine a VLP with an antigen module from an unrelated pathogen [9,15–17]. Two types of modules are commonly used: (i) short peptides expected to have minimal structure [18–21]; and (ii) larger domains with expected tertiary structures [22–25]. There remains a significant knowledge gap about the display of peptide antigen with specific secondary structure *e.g.* α-helix, on an unrelated VLP. Here we address this knowledge deficit using influenza as a model pathogen.

Hemagglutinin (HA) is an influenza surface glycoprotein comprising globular HA1 and stalk HA2 domain. HA mediates viral entry in two steps: (i) viral attachment to the sialic acid receptor; and (ii) fusion of the viral and endosomal membrane [26,27]. Antibodies that bind within the receptor binding region on HA1 block viral attachment and are neutralizing [28,29]. The receptor binding site within HA1 determines viral specificity [30] and is bordered by hypervariable regions including helix 190 [31]. Helix 190 (H190; Fig. 1A) possesses a defined helicity and contains the immunodominant antigenic site Sb [32,33]; monoclonal antibodies against this site have been shown to be protective in mice [34]. The immunogenicity, function and defined secondary structure of H190 suggest it is an interesting candidate for structural display on an unrelated VLP.

We chose to explore murine polyomavirus (MuPyV) VLP for modularization with H190. High-efficiency microbial expression

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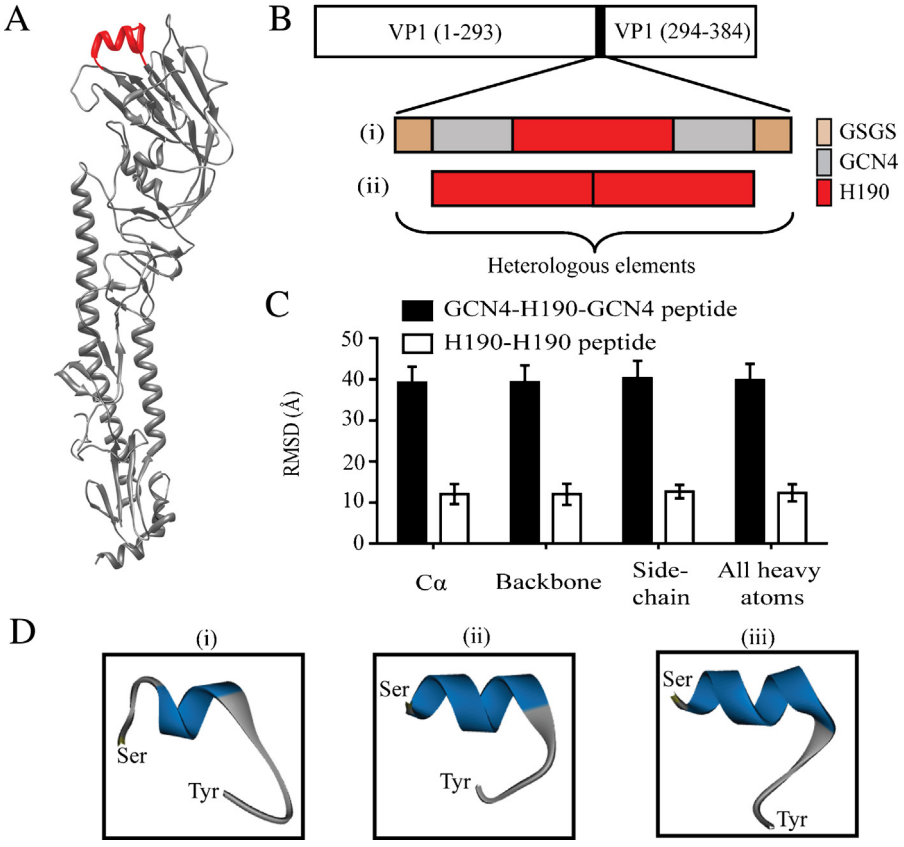

Fig. 1. (A) Crystal structure of H1N1 HA with H190 highlighted in red. Data for A/California/04/2009 HA (3AL4.pdb), bearing one amino acid difference from A/California/07/2009 HA, is shown here. The picture was created using UCSF Chimera 1.5.2 [65], (B) modular MuPyV VP1 designs showing the heterologous elements in (i) VP1-GCN4-H190-GCN4; and (ii) VP1-H190-H190. Numbers refer to residues of VP1, (C) root-mean-square-deviation (RMSD) of C^{α}, backbone, side chain, and all heavy atoms of simulated GCN4-H190-GCN4 and H190-H190 peptides with reference to H190 on HA (3MLH.pdb), and (D) final conformation of H190 element in simulated peptides (i) GCN4-H190-GCN4; and (ii) H190-H190, in comparison to (iii) native H190 structure in HA. α-Helical structures are highlighted in blue. The image was created using Accelrys Discovery Studio® 3.0. Positions of Ser1 and Tyr16 on the peptides are as indicated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and purification processes for modular MuPyV VLPs have been reported [9]. We explore whether the helicity of different H190 antigen module designs affect the quality, *i.e.* native protein recognition, of the antibodies raised by vaccination with the modular VLPs.

Two display strategies were compared: (i) display of H190 using flanking helix promoting elements; and (ii) tandem repeat arraying of H190. The first strategy explores GCN4 as flanking structure-promoting elements within the antigen module. GCN4 is well characterized and has been used as an α-helical scaffold [35,36] or as a helix promoter [37,38]. We previously reported the inclusion of GCN4 in an antigenic module designed to display Group A *Streptococcal* antigen on a VLP [9]. However, these studies have not established whether the GCN4 elements have any positive effect on the immune response quality. The second strategy explores the use of tandem repeats, which are ubiquitous in proteins, and form domains with extensive secondary structures including helices [39]. The use of this strategy has been restricted to present conformational proline-rich peptide to effect the necessary structure [40,41], or to simply increase immunogenicity [42–44].

Modularized VLPs were assessed computationally and *in vivo*. Molecular dynamics (MD) simulation predicted that modularization using tandem repeats would provide a closer structural match of modularized H190 and the equivalent native region in HA. *In vivo* testing confirmed this strategy was superior as it induced antibodies able to bind recombinant HA1 protein and split influenza virion. These findings illustrate, for the first time, the importance of VLP antigen module design on the quality of an immune response, providing new insights into the design rules for synthetic biomolecular engineering of VLP vaccines.

2. Materials and methods

2.1. Simulation of peptides GCN4-H190-GCN4 and H190-H190

Peptides GCN4-H190-GCN4 and H190-H190 were simulated using GROMACS version 3.3.3 in water containing 137 mM NaCl for 20 ns, 3 times for each peptide, as described in Supplementary information.

2.2. Cloning, purification and assembly of modular VLPs

E. coli codon-optimized DNA oligomers corresponding to the antigen modules containing heterologous elements (Fig. 1B) were cloned into pGEX-VP1 vectors as described previously [9]. Modular proteins VP1-GCN4-H190-GCN4 and VP1-H190-H190 were recombinantly produced and prepared as described [45,46]. After endotoxin removal to below 5 EU/ml, purified modular capsomeres were assembled *in vitro* into modular VLPs and dialyzed against PBS [9]. The endotoxin levels of the capsomeres were below the suggested limit for a recombinant protein based vaccine, which is 20 EU/ml [47].

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