Development of OmniUltra™: A Transgenic Chicken System for the Generation of Ultralong CDRH3 Antibodies, Mini-Proteins, and Structured Peptides

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Background

- Ultralong CDRH3 domains, naturally produced in bovine species, have a distinctive stalk-knob structure. At just ~4–6 kDa, this picobody™ is the smallest functional antibody fragment.
- Ultralong CDRH3 may target novel or cryptic epitopes owing to their unique structure.
- We present OmniUltra™, a transgenic chicken expressing ultralong CDRH3 on a human VH framework.
- OmniUltra chickens combine the host recognition of an evolutionary-distant species with the ultralong CDRH3 antigen-binding unit.

Design & Evaluation

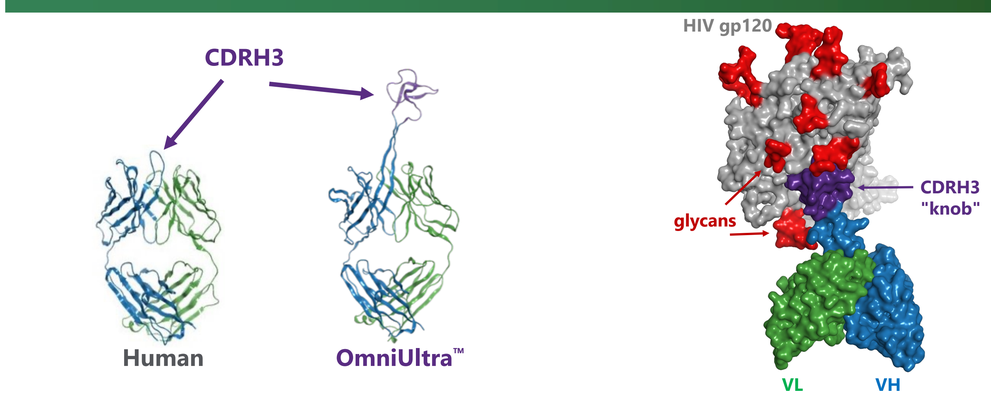

Figure 1. Structural comparison of conventional (heavy and light) antibodies to ultralong CDRH3 "knob" antibodies. OmniUltra chickens are designed to express ultralong CDRH3 in the functional V region with ultralong diversity in the pseudogenes for gene conversion.
Structural figures modified from Stanfield et al, Sci Adv, 2020.

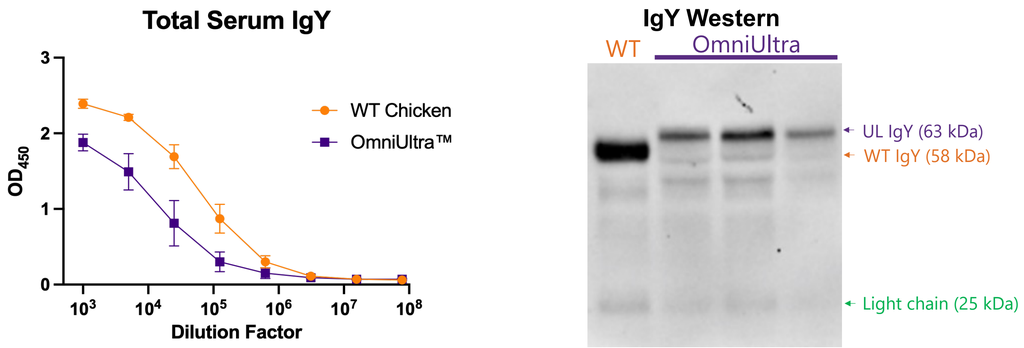

Figure 2. OmniUltra chickens express serum IgY. Western blot analysis of the serum IgY exhibits larger sizes indicative of ultralong CDRH3.

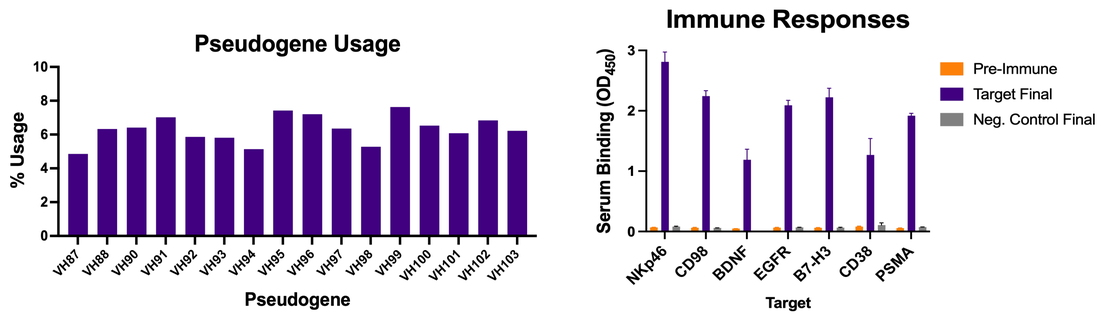

Figure 3. OmniUltra chickens utilize all pseudogenes for diversification of the ultralong CDRH3 repertoire and are immunoresponsive to a variety of antigen targets.

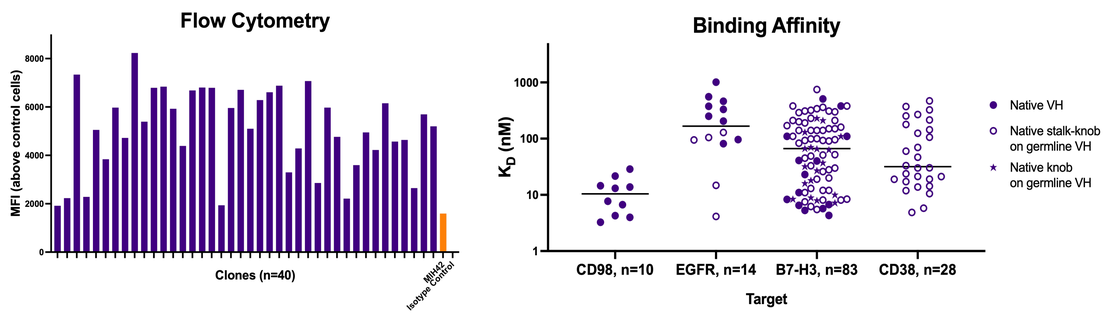

Figure 4. OmniUltra antigen-specific IgG clones bind to native protein in flow cytometry (B7-H3 shown; controls in orange) and also exhibit a broad range of affinities, based on SPR kinetic analysis, either in native VH form or on a germlined framework.

Clone Characterization

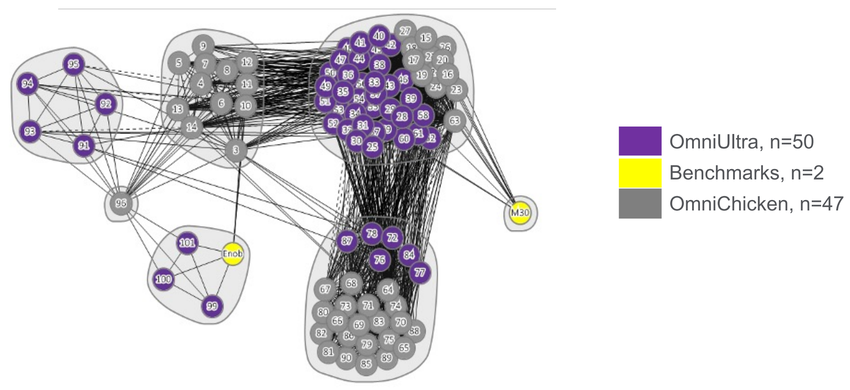

Figure 5. OmniUltra antibody clones (to B7-H3) bind to overlapping epitope communities as OmniChicken, as well as novel epitopes. Benchmarks represent literature/clinical antibodies produced by mouse immunization. Binning was performed on the 2Ig-B7H3 (monomeric) isoform while immunizations of OmniUltra were performed with the 4Ig-B7H3 (dimeric) isoform.

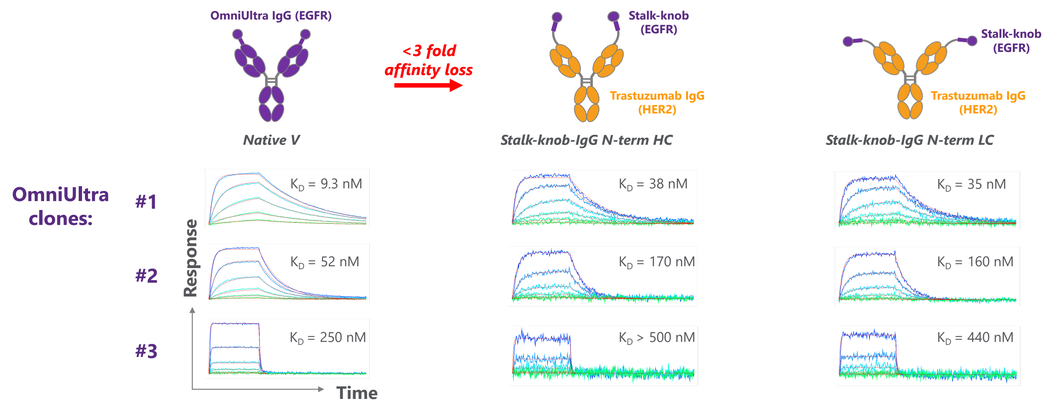

Figure 6. OmniUltra EGFR stalk-knobs tethered to HER2 IgG to produce EGFR x HER2 bispecific antibodies in tetravalent 2+2 Morrison formats exhibit minimal loss of affinity.

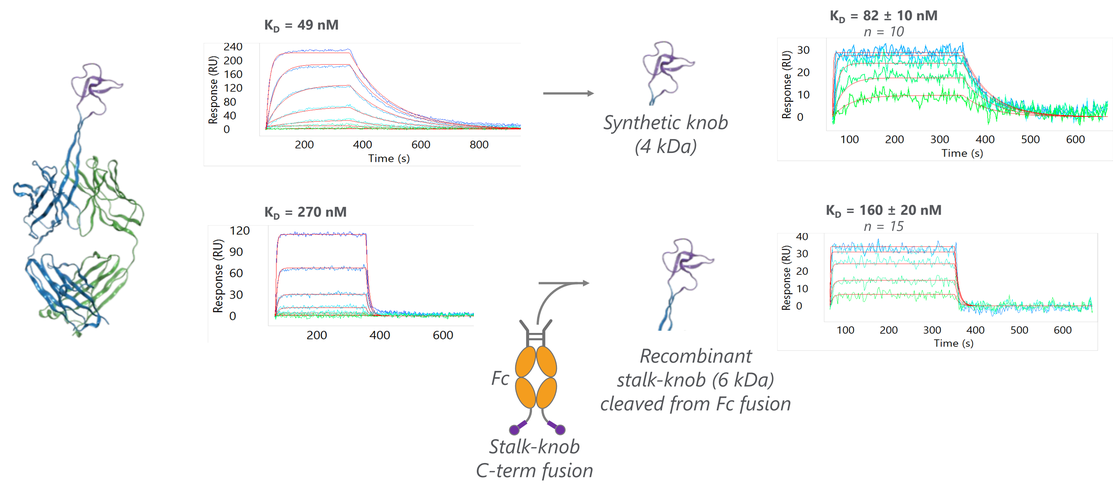

Figure 7. OmniUltra CDRH3 knobs (picobodies™) can serve as autonomous binding units with maintained affinity. They can be produced by chemical synthesis or isolated by cleavage from Fc-fusions in mammalian expression systems (HEK).

Conclusions

- OmniUltra chickens express ultralong CDRH3 in the context of human V frameworks.
- OmniUltra chickens are immunoresponsive and produce ultralong CDRH3 antibodies to a variety of immunogen targets.
- Pseudogene-driven gene conversion contributes to the diversification of ultralong CDRH3.
- Ultralong CDRH3 antibodies can target epitopes that overlap with and extend beyond those from other chicken host platforms.
- Ultralong CDRH3 can be isolated as autonomous binding units (picobodies™) or tethered for bispecifics with minimal loss to affinity.
- Picobodies™ (structured peptides) can be chemically synthesized or isolated by cleavage from recombinant Fc fusions produced in mammalian cell expression systems.