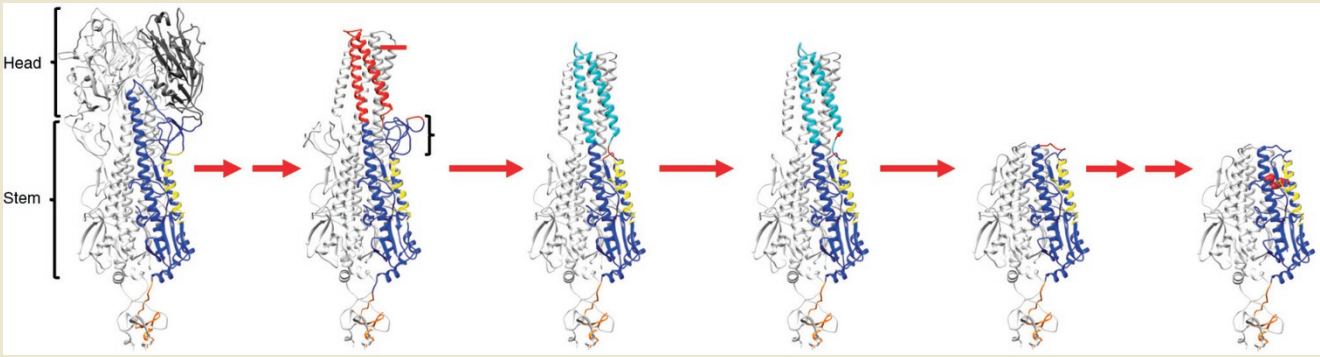

amino acids to increase solubility and a cysteine bridge to increase stability. To preserve trimerization, they added the GCN4 leucine zipper sequence to the N-terminus of the protein. At the next stage, soluble versions were created by removing the transmembrane domain and various parts of the cytoplasmic domain. At stage III, a semi-rational library was screened to optimize the preservation of the relevant epitopes. At stage IV, the position of the GCN4 trimerization motif was optimized for epitope preservation and structural stability. Finally, at the last design stage, the stability of the trimeric construct was further increased by the introduction of intermolecular cysteine bridges. Only the final construct preserved the trimeric structure of HA in solution, whereas the best construct from stage IV primarily formed dimers and the other constructs, monomers.

When tested in mice, the fully trimeric construct provided almost complete protection from a lethal challenge with a heterologous H1N1 virus, even after only one immunization. The other HA versions offered only partial protection after two or three inoculations. In a heterosubtypic setting, only constructs that were derived from the library optimization step (stage III and later) provided any protection, which increased with the multimerization level. Again, the fully trimeric form offered full protection from lethal challenge with an H5N1 virus. The trimeric HA stalk vaccine was also tested in cynomolgus monkeys and, similarly to the seasonal flu vaccine, led to a substantial reduction in the disease symptoms of an H1N1 infection.

In vitro assays showed that the antibodies produced in animals were able to both neutralize the virus directly (although this was tested only in H5N1-derived pseudoparticles) and induce antibody-dependent cellular cytotoxicity, which has been shown to be an important contributor to flu vaccine efficacy.

Yassine *et al.*[2] follow a similar strategy of structural optimization and selection of the best stalk constructs at each stage by broadly neutralizing antibody binding. They start with the ectodomain of the H1N1 A/New Caledonia/20/1999 virus they fused to the foldon trimerization domain. In subsequent generations, they engineer the following parts of the HA protein: the head domain is replaced by a short glycine-rich linker (1st generation); the membrane distal region is replaced by the HIV glycoprotein 41 (gp41) trimerization domain (2nd generation); the stem regions are further truncated (3rd generation); and finally linkers between HA and gp41 are optimized (4th generation). To avoid irrelevant immunogenicity, they subsequently replace the gp41 domains with linkers and add further core-stabilizing mutations (generations 5 and 6). Finally, to further increase the immunogenicity of the most promising constructs, they fused the ferritin subunit of *Helicobacter pylori* to the engineered HA to create self-assembling nanoparticles.

When mice and ferrets were immunized with these nanoparticles, a broad antibody response was elicited against various H1, H2, H3, H5, H7 and H9 influenza strains; however, *in vitro* neutralization activity was observed only for H1 strains. Nevertheless, the vaccine offered complete (mice) or partial (ferrets) protection from a lethal challenge with a heterosubtypic H5 virus. The molecular basis for this protection in the absence of direct virus neutralization activity is puzzling; the authors speculate that either antibody-dependent cell-mediated cytotoxicity or antibody-dependent, complement-mediated lysis might play a role, although these pathways have not been experimentally verified.

Together these papers show that carefully optimized HA stalk constructs can be stable and immunogenic enough to elicit a strong antibody response to various HA serotypes, providing protection in three different animal species. How these concepts will translate to humans, of course, remains to be seen.

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2. Yassine, H.M. *et al. Nat. Med.* **21**, 1065–1070 (2015).
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4. Pica, N. *et al. Proc. Natl. Acad. Sci. USA* **109**, 2573–2578 (2012).

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