Supporting Information

Xu *et al.* 10.1073/pnas.0805293105

SI Materials and Methods

Proteins, Viruses, Antibodies, and Surrobodies. Hemagglutinin protein was purchased from Protein Sciences (H5 Protein A/Vietnam/1203/2004). Inactivated viral vaccine standards for H5N1 rgA/Vietnam/1203/2004 were kindly provided by USFDA–CBER. SLC polyclonal antibodies were generated in rabbits (Covance) immunized with peptides that were predicted to be surface exposed from human VpreB1 sequences (amino acids 61–80) or human λ5 sequences (amino acids 102 -119) conjugated to KLH (Elim Biopharmaceuticals).

Mammalian expressed surrobodies or generated by *de novo* synthesis as eukaryotic codon optimized soluble secreted genes (DNA 2.0) and then subcloned into pCI (Promega) for mammalian protein expression, sequence verified, before transfection into CHO-K1 cells (ATCC) according to manufacturers guidelines. Briefly transfections of 80% confluent cells in T-75 flasks were performed using containing equal amounts of desired surrobody chains totaling 32 μg of DNA and Lipofectamine 2000 (Invitrogen) according to manufacturers guidelines. Cells were allowed to produce proteins into 20 ml of Opti-MEM I per transfection. After 4 days the secreted surrobodies were purified from the culture supernatents using nickel chelate chromatography (Ni-NTA agarose, Qiagen). The resulting purified surrobodies were buffer exchanged into sterile PBS using centrifugal size filtration (Centricon Plus-20) and their protein concentrations determined by A280 readings, SDS gel, or Western blot analysis compared to known standards.

Hemagglutinin and Viral ELISA. Recombinant HA proteins: H5 Protein A/Vietnam/1203/2004 (Protein Sciences)-100 ng/well; FDA Influenza Virus Vaccine for H5N1 rgA/Vietnam/1203/2004 (CBER)-100 ng/well

ELISA plates were coated as indicated with either recombinant hemagglutinin protein or inactivated virus overnight at room temperature. The next day, plates were appropriately blocked (4% nonfat dry milk in PBS/0.05% Tween-20) and then 0.1 ml samples, diluted in blocking buffer, were incubated, washed, and detected using primary and secondary detection as indicated Absorbance at 450 nm was read, data recorded, and reported herein.

Phage Library Construction. ***Surrobody library cloning.*** Vectors containing either *E. coli* expression optimized Fusion 1 or Fusion 2 surrogate light chains were digested and gel purified (Qiagen). Heavy chain Vh fragments were obtained from plasmids derived from infecting XL-1Blue cells with Round 2 H5 Vietnam panned, eluted, and amplified phage. Library ligations were performed with 200 ng of Vh inserts and 1 μg of gel purified Fusion 1 or Fusion 2 vector. The ligations were incubated overnight at 14°C. Ligations were desalted using Edge BioSystem Perfroma spin columns. Six electroporations per library were done in 80 μl TG-1 aliquots, each recovered in 1 ml SOC, pooled and outgrown for 1 hour at 37°C. A sample of each was taken for plating and used to determine the total number of transformants. The remainder was transferred to 250 ml 2YT + 100 μg/ml Ampicillin + 2% glucose and grown at 37°C to midlog and then superinfected at moi 10:1 with m13K07 helperphage. After 30 min the cells were harvested and resuspended in 2YT + 100 μg/ml Ampicillin + 70 μg/ml kanamycin + 0.5 mM IPTG and grown overnight at 30°. Phage were later precipitated with standard PEG/NaCl precipitation technique, resuspended and used directly for panning. Fusion 1 libraries yielded 3.84×10^7 transformants and the Fusion 2 libraries yielded 7.8×10^7 transformants.

Panning. Essentially as described in Reference 1. Notably phage were produced in TG1 cells in overnight incubations in 2-YT media containing ampicillin (100 micrograms/ml) kanamycin (70 micrograms/ml) and 0.5 mM IPTG. Also all washes before low pH elution were repeated a total of five times.

Surrobody ELISAs. Essentially as described in ref. 1. Notably detection was accomplished as indicated in the manuscript with the following concentrations of appropriate primary or secondary antibodies. Anti-E HRP conjugated antibody 1:5,000 (Abcam), Anti-His-6 antibody 1:2,500 (Serotec), Anti-Human Ig H+L HRP conjugated antibody 1:5,000 (Jackson Immunologic), Anti-m13 antibody HRP conjgated 1:5,000 (GE Healthcare). Goat anti-mouse HRP conjugated antibodies 1:5,000 (Jackson Immunological)

Notably, for lysate-based ELISAs 1 ml deepwell cultures were grown overnight at 37°C in HB2151 *E. coli*. Cells were harvested and periplasmic lysates prepared using 0.1 volume of BBS buffer (10 mM boric acid, 150 mM NaCl, 10 mM EDTA). For *E. coli* purified proteins some clones were grown overnight at 30° with 0.5 mM IPTG induction.

For surrobody phage-based ELISAS, phage were produced in TG1 *E. coli* grown overnight at 30° with 0.5 mM IPTG induction. Before testing phage were harvested from bacterial cultures supernatants by standard PEG/NaCl precipitation.

Western Blots. Detection was accomplished as indicated in the manuscript with the following concentrations of appropriate primary or secondary antibodies. Anti-E HRP conjugated antibody 1:1,000 (Abcam), Anti-His-6 antibody 1:500 (Serotec), Anti-Human Ig H+L HRP conjugated antibody 1:1,000 (Jackson Immunologic), Goat anti-mouse HRP conjugated antibodies 1:1,000 (Jackson Immunological)

1. Barbas C, Burton, DR, Scott, JK, Silverman, GJ (2001) in *Phage Display, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Plainview, NY).

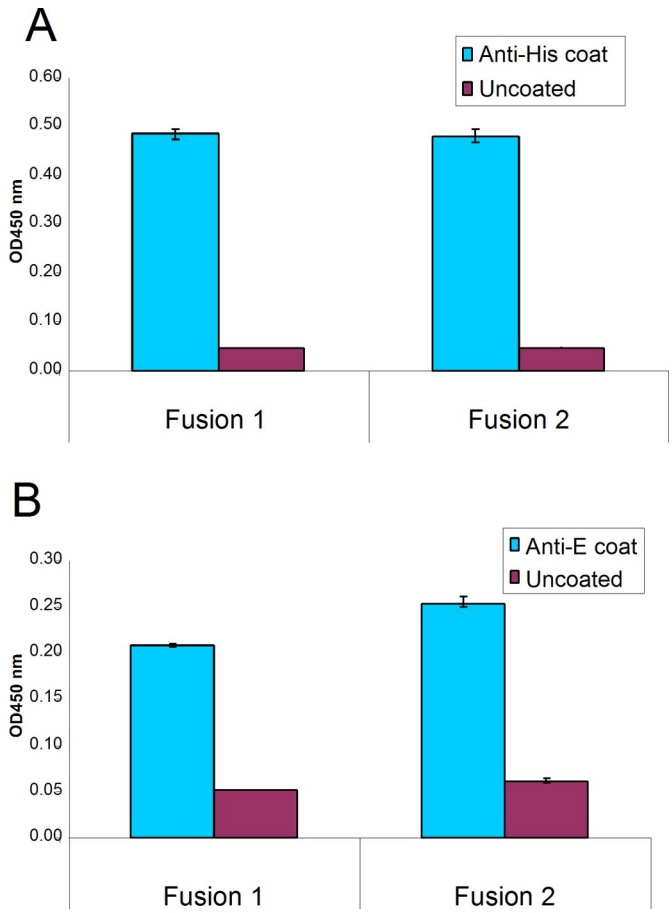


Fig. S1. Surrobody fusions Fab-like complexes are readily assembled onto filamentous phage surfaces. Surrobody Fusion phage were captured, as indicated, by either a His epitope tag present on the heavy chain *A*, or the E tag present on the surrogate light chain *B* and detected by anti-M13 HRP conjugated antibodies and quantited by ELISA.

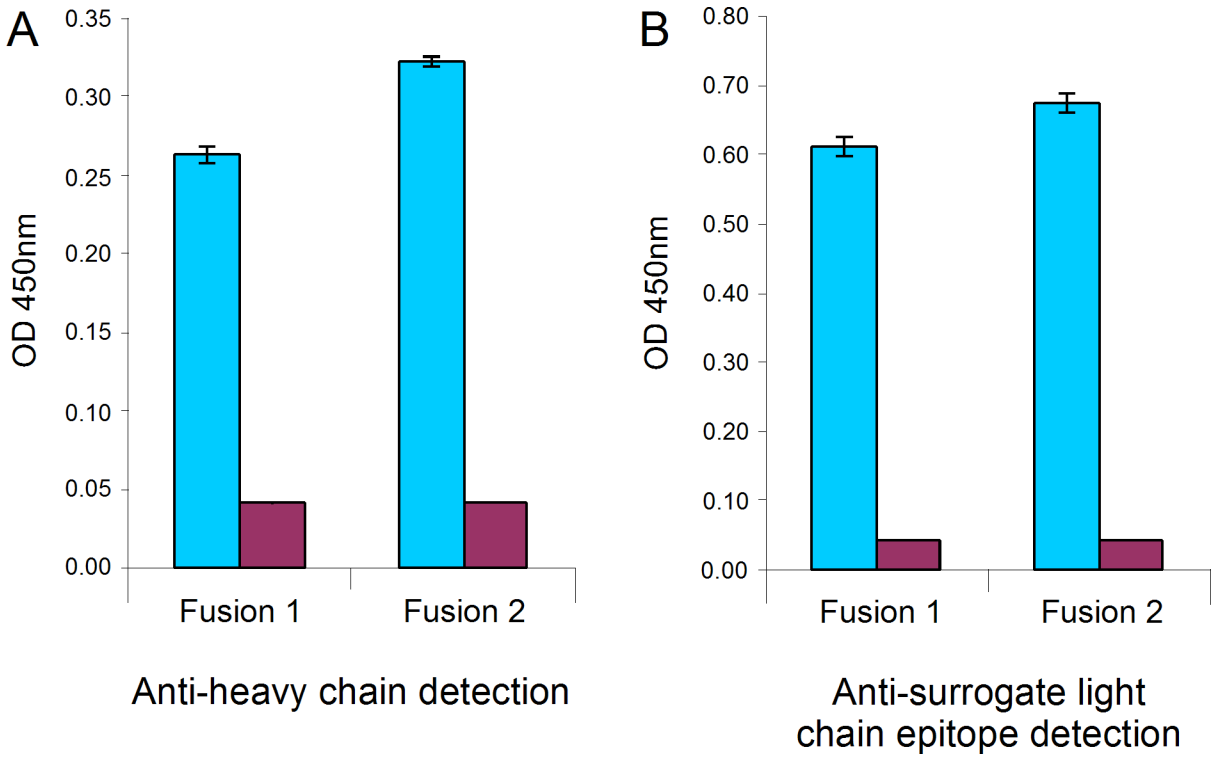
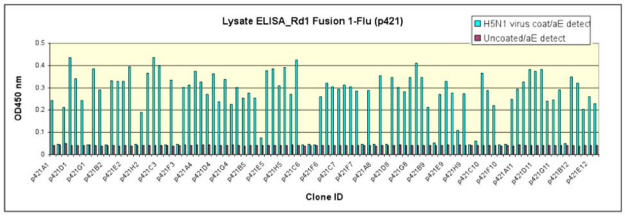
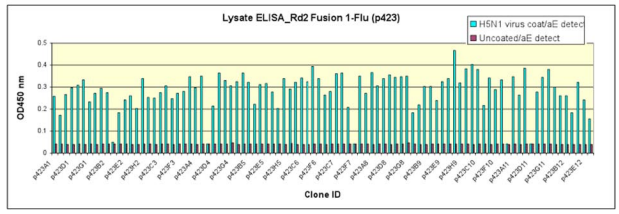


Fig. S2. Surrobody fusions Fab-like complexes isolated from *E. coli* periplasmic lysates bind antigen. Surrobody antigen binding by either Fusion 1 or Fusion 2 surrobodies were quantitated by detection with anti-His tag (*A*) and anti-E-tag antibodies (*B*). Antigen coated wells are indicated by (aqua bars) and uncoated wells are indicated by (purple bars).

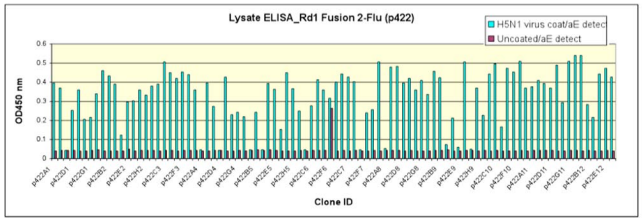
Clonal analysis of Round 1 Fusion 1 library clones



Clonal analysis of Round 2 Fusion 1 library clones



Clonal analysis of Round 1 Fusion 2 library clones



Clonal analysis of Round 2 Fusion 1 and Fusion 2 library clones

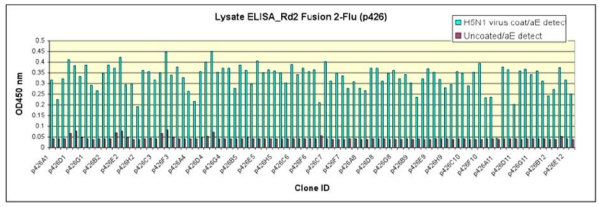
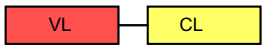
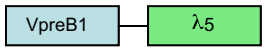
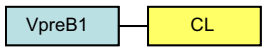
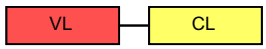
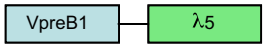
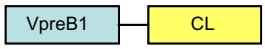


Fig. S3. Representative 96-deep well produced surrobody clonal ELISA containing heavy chain repertoires from patients, as isolated by round 1 (*Left*) or round 2 (*Right*) as indicated in their respective images.

Table S1. Surrobodies derived from known antihemagglutinin heavy chains bind antigen when paired with either SLC fusion and compare favorably to parent Fabs

Clone	Protein format	H5 affinity (Vietnam 1203/04)
F5 non-neutralizing heavy chain	Parent Fab 	1 nM
	Fusion 1 	150 – 270 nM
	Fusion 2 	250 – 400 nM
B11 neutralizing heavy chain	Parent Fab 	13 nM
	Fusion 1 	10-21 nM
	Fusion 2 	15-22 nM

Affinities were determined from purified *E. coli* produced Fab-like surrobodies using Biolayer interferometry kinetic analysis (Octet, BioForte) according to the manufacturer's recommended guidelines.

Table S2. Surrobody libraries containing heavy chain repertoires from H5N1 survivors specifically efficiently enrich unique solutions in H5N1 viral panning regardless of SLC fusion partner

Library	Number of transformants	Round of panning (fold enrichment)	Percent ELISA positive clones, %	Unique sequences
Fusion 1	3.84×10^7	1 (5×)	79	27
		2 (97×)	95	
Fusion 2	7.80×10^7	1 (20×)	88	27
		2 (48×)	99	

Unique sequences were generated from analysis of 188 clones total for each fusion library.