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Non-natural amino acid peptide microarrays to discover Ebola virus glycoprotein ligands

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Supporting Information

rVSV-zEBOV Screening on Peptide Microarray: Peptide microarrays were prepared by printing a library of 10 000 peptides, 20 aa in length on polymer-coated microscope slides according to our previously developed methods[1]. Pseudotyped rVSV-EBOV viruses were provided by Dr. Bertram Jacobs (Arizona State University) and screened directly on the peptide microarrays. The GP specific monoclonal antibody, 2G4 (kindly provided by Dr. Larry Zeitlin, Mapp Biopharma) was used to detect rVSV-EBOV binding to peptide microarrays. Samples of rVSV-zEBOV plus primary antibody (1 nM) and secondary antibody (1 nM), anti-human IgG-AlexaFluor 647, were incubated for one hour at room temperature. Samples were then applied to replicate peptide arrays, incubated for 1 hour at room temperature, washed and scanned on an Agilent Microarray scanner. Relative fluorescence for each peptide was imported into JMP Pro 13 and hierarchical clustering of the raw data from replicate arrays was used to identify peptide ligands. Peptides were selected that had high rVSV-zEBOV binding and low antibody control binding.

Synbody Library Conjugation: Peptides were purchased at > 90% purity (WatsonBio, The Woodlands, Texas, USA) for use in synbody conjugation. Stock solutions of each peptide were prepared in ddH_2O. From these stocks, peptide working solutions (1.7 mM) were prepared in 1x phosphate-buffered saline (PBS) pH 7.4 as was a working solution of the Sc0 scaffold (3.3 mM). For each synbody reaction, 175 μL of each peptide was added to a 1.5 mL microcentrifuge tube followed by 150 μL of the scaffold working solution. Acetonitrile (50 μL) was added to each solution to aid synbody solubility, and the reaction mixtures were incubated overnight with shaking at 42°C. After conjugation, each reaction was purified by HPLC using an Agilent 1260 preprative scale HPLC with a C18 column (Phenomenex Luna C18 250 mm x 10 mm – 5 micron) with a 25 minute linear gradient of 0.2% trifluoracetic acid

(TFA) in acetonitrile from 5% to 60%. Synbody containing fractions were confirmed by MALDI-MS (Bruker Microflex, Bruker Daltonics, Bilerica, MA) and lyophilized prior to use. The identity of each synbody was confirmed by MALDI-TOF-mass spectrometry, and the conjugation reaction yields were determined by integration of the appropriate HPLC peaks.

Synbody rVSV-zEBOV Screening by ELISA: Nunc MaxiSorp flat bottom 96 well ELISA plates were coated with 100 μL of 10^5 pfu per well of rVSV-zEBOV in pH 8 PBS and sealed overnight at 4^oC. The plates were washed twice with 1X PBS with 0.05% Tween-20 (PBST), and then blocked with StartingBlock (PBS) Blocking Buffer (200 μL) (ThermoScientific) at room temperature for 2 hours. The plates were washed twice with 1X PBST, and biotin-labeled synbodies were added in ELISA dilution buffer (0.5 X StartingBlock + 0.5 X PBST) with a 1 hour incubation time at room temperature. The plates were then washed twice, and 1:100 000 streptavidin-HRP (100 μL in ELISA dilution buffer) was added at room temperature and incubated for 1 hour. The plates were washed once with shaking and twice without shaking. 3,3', 5,5'-tetramethylbenzidine (TMB) (100 μL) was added and plates were incubated in the dark for 10 minutes at room temperature. Hydrochloric acid (HCl) (100 μL, 0.5 M) was added to quench the reaction. The plates were read immediately at 450 nm using Spectramax 190 microplate reader. G1.1 NoroVirus (Noro) (100 μL, 0.8 μg/well) in PBS was used to coat negative control wells, and ELISA dilution buffer was used as a control on both coated wells. Anti-Ebola antibody 13F6 (kindly provided by Dr. Charles Arntzen and Dr. Hugh Mason, Arizona State University) was used as a positive control on rVSV-zEBOV coated wells and as a negative control for norovirus coated wells.

Synbody Binding to GP by SPR: Synbodies were screened using the 4500 Series SPR system (Biosensing Instrument, Tempe, Arizona, USA). An amine immobilization was performed with pH 5.5 sodium acetate as the immobilization buffer on a CM Dextran chip (Biosensing Instruments) at 25^oC. Flow cells 1-3 were activated by an injection of freshly prepared EDC/NHS (200 μL, 200 mM/50 mM) at a flow rate of 60 μL/min. The recombinant EBOV subtype Zaire, strain H.sapiens-wt/GIN/2014/Kissidougou-C15 glycoprotein (GP) (Sino Biological Inc, cat no. 40442-V08H2)

was immobilized on flow cells 1 and 2 by injecting a solution of zGP (150 μL, 20 μg/mL) in pH 5.5 sodium acetate at a flow rate of 60 μL/min. Residual active sites were quenched by a 70 μL injection of ethanolamine with a flow rate of 60 μL/min. Synbody samples (150 μL) were injected at a flow rate of 60 μL/min at 5 concentrations (10, 5, 2.5, and 1.25 μM). The surface was regenerated using pH 2.5 glycine (90 μL). The 2G4 antibody (14 nM) was used as a positive control. Flow cell 3 was used as a subtractive control from flow cells 1 and 2. The data was analyzed using Biosensing Instrument 4500 Series Data Analysis software. SPR data was exported into GraphPad Prism and dissociation rates were determined using a one phase decay model.

Synbody Binding to EBOV GP Validation by SPR: Synbody binding kinetics for GP was measured with a Biacore T200 SPR. Synbody 1-5 was captured on one flow cell of a SA chip (GE healthcare) by flowing 4 μM synbody 1-5 at a rate of 30 μL/min with a contact time of 240 s and a dissociation time of 60 s, leaving the other surface blank for a subtractive control. Solutions of GP (0, 20, 40, 80, 160, and 320 nM) were prepared in 1X HBS-EP (GE Healthcare) (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20) running buffer and injected over the surface at a rate of 30 μL/min with a contact time of 90 s and a dissociation time ot 120 s. The surface was regenerated with a 30 s contact of a 500 mM NaCl / 10 mM NaOH solution. Kinetics were determined using Biacore 4000 Evaluation Software. The same procedure was used to determine the binding kinetics for EBOV 2-5, however, the synbody was captured on a neutravidin surface. Neutravidin in pH 5.5 acetate (40 μg/mL) was immobilized on two flow cells of a CM5 chip at 25°C with 4.5 pH sodium acetate as the immobilization buffer using standard amine immobilization. The surface was activated by the injection of freshly prepared EDC/NHS in water (200 μL, 200 mM/50 mM), and residual active sites were quenched by a solution of ethanolamine (1 M, pH 8.5). Synbody 2-5 (4 μM) was captured on one of the flow cells at a flow rate of 30 uL/min, a contact time of 240 s, and a dissociation time of 60 s. zGP binding kinetics were then determined as before.

References:

1. V. Domenyuk, A. Loskutov, S. A. Johnston and C. W. Diehnelt, *PLoS One*, 2013, **8**, e54162.

Figure S1. Analysis of the net charge of each peptide in the 10,000 D/L peptide library.

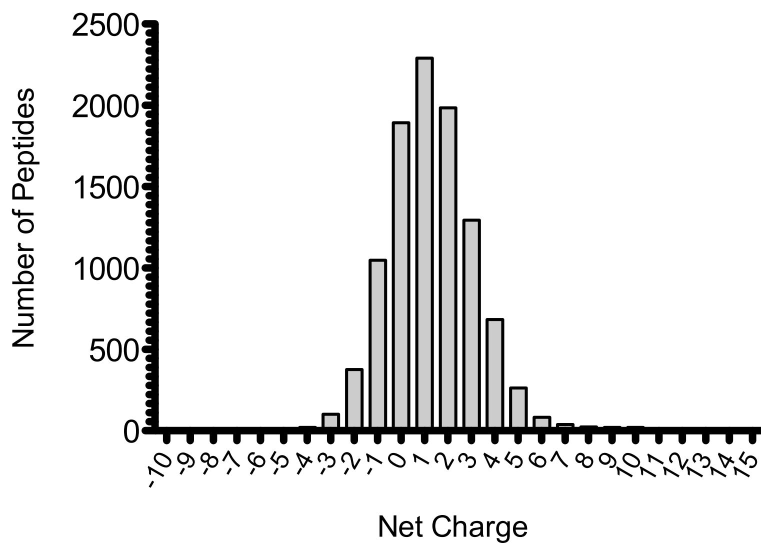

Figure S2. Results of hierarchical clustering of peptides from peptide microarray screening. **A)** Line plot of binding (y-axis)for each micrroarry (x-axis) for each cluster. **B)** Average intensity for rVSV-EBOV (red) and 2G4 mAb control (black) for each cluster. **C)** Number of peptides in each cluster (y-axis) as a function of cluster number.

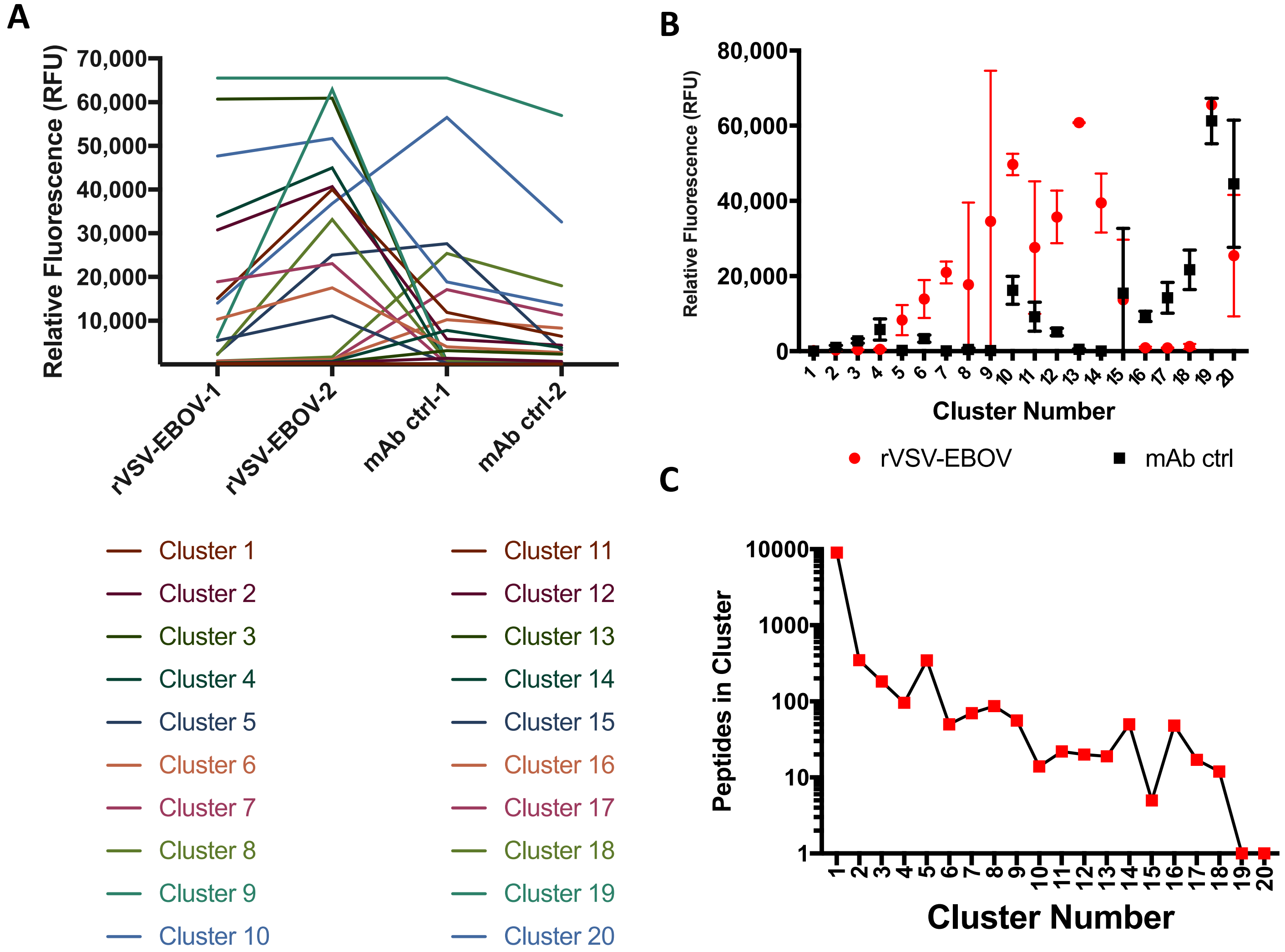

Figure S3. Line graphs for 10 selected EBOV binding peptides for each peptide microarray. The relative fluorescence (RFU) is plotted on the y-axis for each microarray.

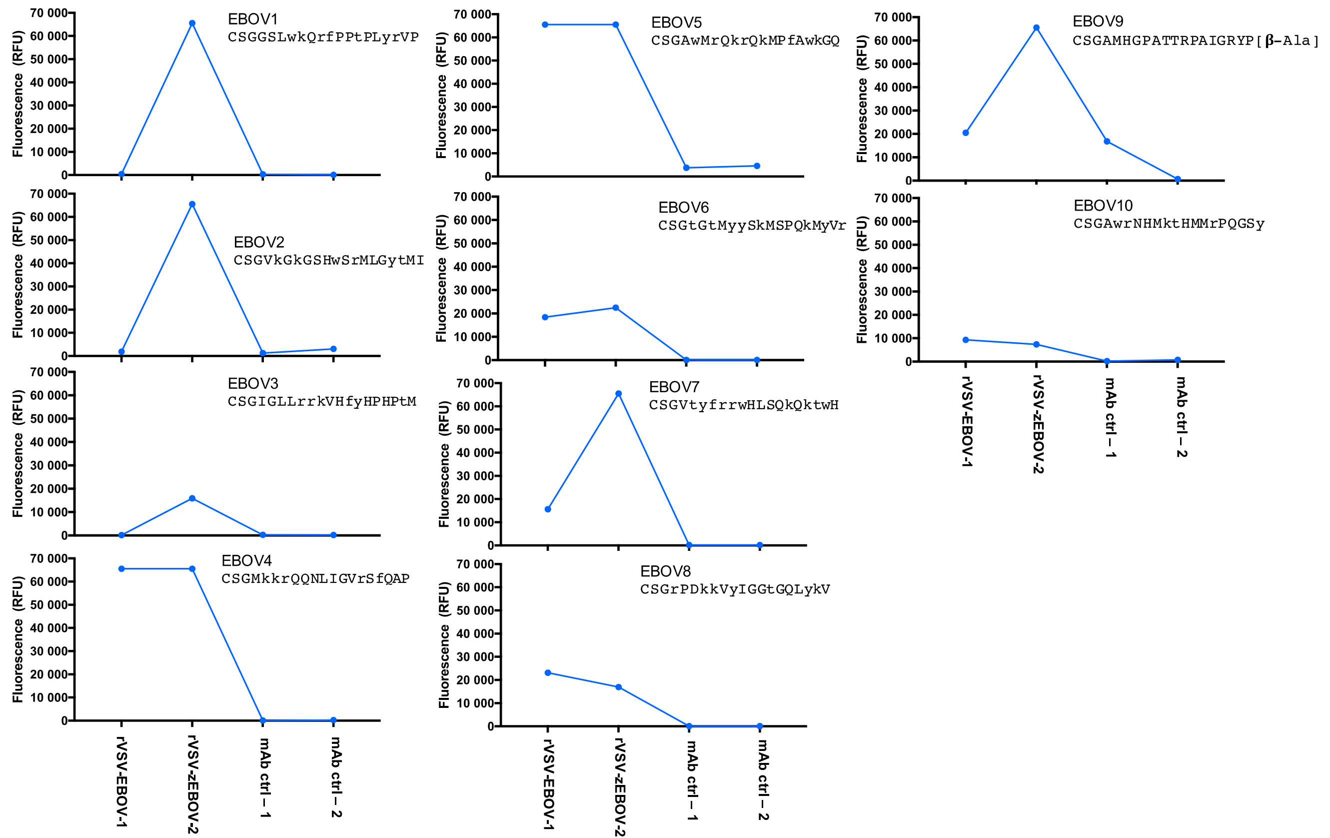

Figure S4. Reaction scheme for synbody library production. The amount of each synbody recovered is indicated in the dot plot.

Table S1. List of selected EBOV peptides .

Peptide Name	Sequence	Molecular Weight	pI	Net Charge
EBOV 1	CSGGSLwkQrfPPtPLyrVP	2289.66	10.6	3
EBOV 2	CSGVkGkGSHwSrMLGytMI	2198.60	10.4	3
EBOV 3	CSGIGLLrrkVHfyHPHPtM	2349.79	10.6	3
EBOV 4	CSGMkkrQQNLIGVrSfQAP	2248.64	11.4	4
EBOV 5	CSGAwMrQkrQkMPfAwkGQ	2424.88	11.5	5
EBOV 6	CSGtGtMyySkMSPQkMyVr	2318.72	10.0	3
EBOV 7	CSGVtyfrrwHLSQkQktwH	2548.88	10.9	4
EBOV 8	CSGrPDkkVyIGGtGQLykV	2169.51	10.1	3
EBOV 9	CSGAMHGPATTRPAIGRYP[β-Ala]	2014.30	10.1	2
EBOV 10	CSGAwrNHMktHMMrPQGSy	2378.75	10.6	3

D-amino acids indicated by lower case letters

Figure S5. ELISA signal from synbody binding to NVLP G1.1 subtracted from signal from binding to rVSV-zEBOV for each synbody. Synbodies showing signal exceeding the threshold (represented by dotted red line) were selected for further screening.

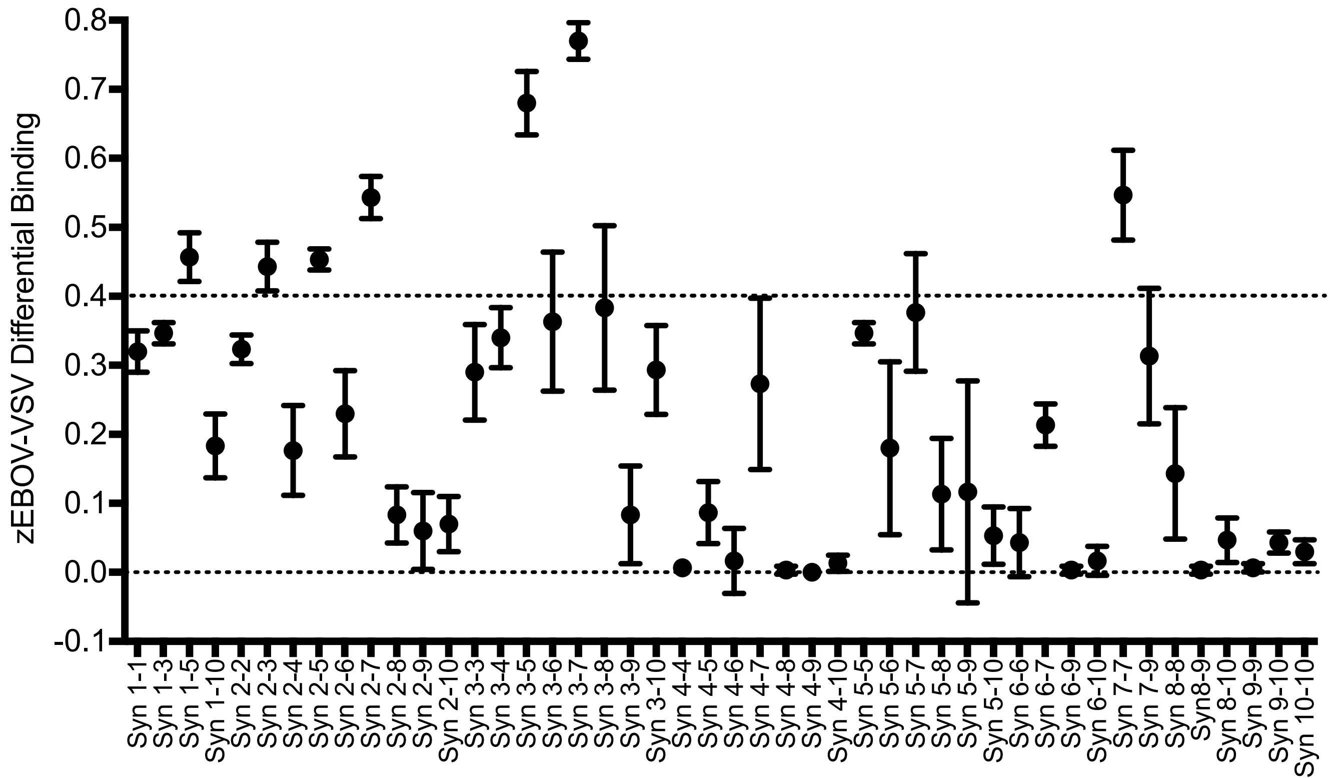

Figure S6. Sensorgrams from 10 μM A) EBOV 1-5, B) EBOV 1-10, C) EBOV 2-3, D) EBOV 2-5, E) EBOV 3-7, and F) EBOV 7-7 injected over immobilized zGP on a Biosensing SPR System.

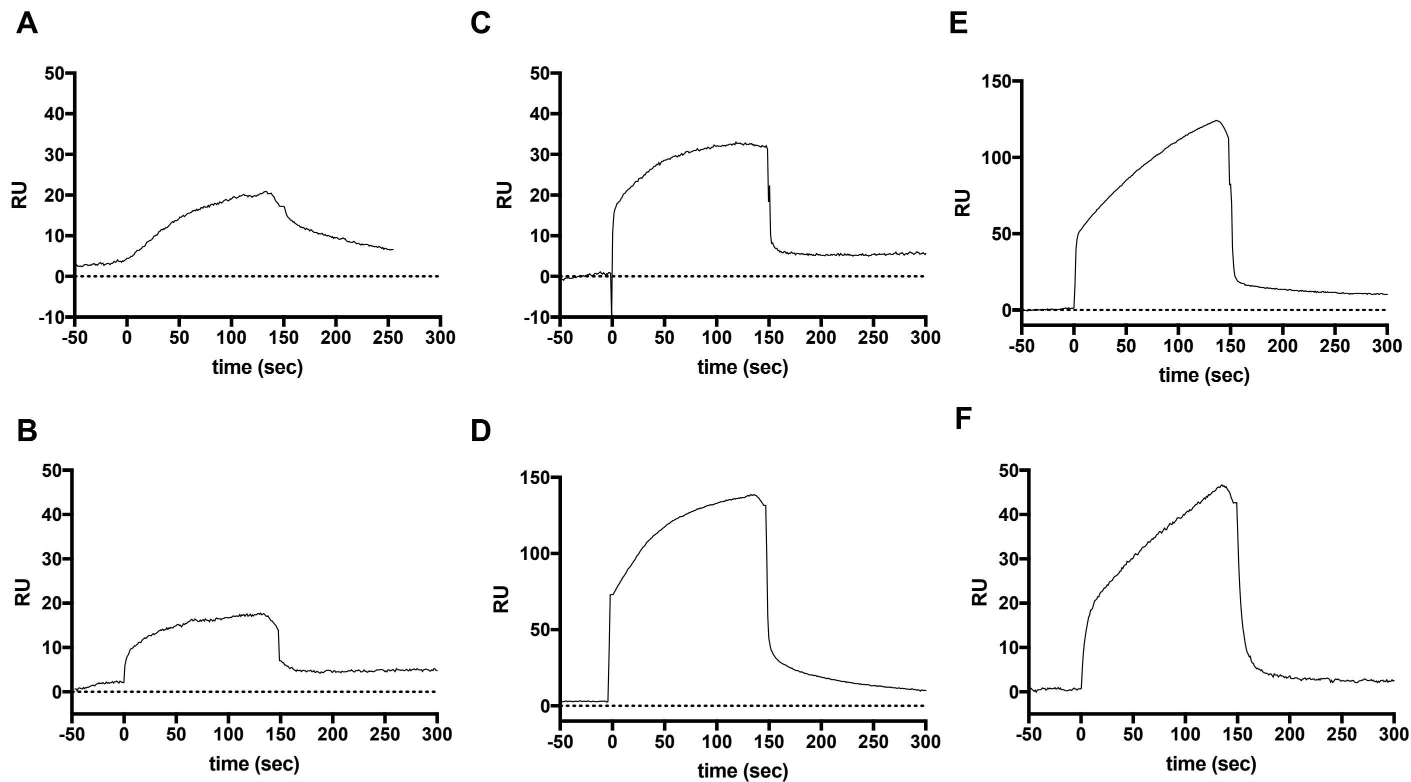

Figure S7. Average dissociation rate (k_{off}) for each synbody when screened on Biosensing 4500 SPR and Biacore T-200 against immobilized zGP at two different immobilization levels.