Supplementary Information

Materials

Purified HSV samples (HSV-1-MacIntyre Strain, HSV-2-G Strain) were purchased from Advanced Biotechnologies Inc (Maryland, USA). The Ph.D.-12™ phage display library (~$1.2x10^9$ independent clones) was purchased from New England Biolabs (Hitchin, Hertfordshire, UK). Escherichia coli ER2738 (F+ strain) was used for M13 phage propagation and was cultured at 37 °C on Luria–Bertani agar or broth, supplemented with tetracycline. Mouse monoclonal HRP labelled anti-M13 antibody was purchased from GE Healthcare (Buckinghamshire, UK). Bovine serum albumin and 1-Step ultra TMB (3,3',5,5,'–tetramentylbenzidine) were from Fisher Scientific (Dublin, Ireland). DNA extraction was performed using a QIAprep spin M13 kit from Qiagen (West Sussex, UK) and sequencing was performed using the 96 gIII primers (5´ d(CCCTCATAGTTAGCGTAACG)3') from New England Biolabs (Hertfordshire, UK). Monoclonal anti-HSV-1 antibody was purchased from Abcam (Cambridge,UK). α-t-butyloxycarbonylamino-ω-carboxy succinimidyl ester polyethylene glycol (BOC-NH-PEG-NHS) MW 3000, and methoxyl succinimidyl ester polyethylene glycol (CH_3O-PEG-NHS) MW 2000 were purchased from Rapp Polymere (Germany). Bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), polyethylenimine 50 wt. % (MW 1300), potassium carbonate, 2-(N-morpholino)ethanesulfonic acid (MES), 2-mercaptoethanol (BME), potassium sulphate, sodium dodecylsulphate (SDS), trifluoroacetic acid, Tween-20, and ultrasensitive streptavidin coupled to peroxidase were all purchased from Sigma Aldrich. Biotin-BSA (BBSA), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), excellulose desalting columns, sulfo n-hydroxylsucciminide (sulfo-NHS), N-hydroxysulfosuccinimide (NHS), and, N-succinimidyl S-Acetylthioacetate (SATA) and sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC) were purchased from Thermo Scientific. The particles used in this study included 2.8 µm carboxylic acid beads, streptavidin beads, and protein A beads (Dynabeads, Invitrogen) that have a reported coefficient of variance of 3%. All chemicals and proteins were used as purchased without further purification. Buffers used include phosphate buffer saline (PBS) (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4), phosphate buffer saline tween (PBST) (PBS with 0.05 (v/v)% Tween(R)), carbonate buffer (50mM sodium carbonate, pH 8.2), MES buffer (50mM MES, pH 6.0).

Isolation of HSV-binding peptides The detailed biopanning procedures have been described in our previous article.[32] Briefly, purified herpes simplex virus-1 (Advanced Biotechnologies, Maryland, USA) was immobilized overnight in microtiter wells before addition of approximately $2x10^9$ phage particles to the wells. Non-binding phage were discarded. Bound phages were eluted in 100 µL of a low-pH glycine buffer (200 mM, pH 2.2) for 15 min at room temperature, followed by neutralization (15 µL of 1 M Tris buffer, pH 9.1). Eluted phage were amplified and resubmitted to another 2 rounds of selection as reported previously.[1] In total, three rounds of selection were performed before DNA extraction was performed using a QIAprep spin M13 kit. Sequencing was performed by Entelechon (Germany).

Phage pool immunoscreening

After each round of selection, the phage pool was submitted to binding experiments by direct ELISA with all three amplified eluates (phage pool) from the previous rounds of selection. BSA and wild-type M13 were used as negative controls. Briefly, 100 µL of herpes simplex virus (100 µg.mL, in PBS) was immobilized onto a 96-well microtiter plate and incubated overnight at 4^0C, before blocking with 2% BSA in PBS for 90 min at room temperature. Each well was washed five times using PBST (0.1 %) before addition of a sample of eluate from each round in PBST. The relative amount of phage was measured by incubated in each well with100 µL of HRP-conjugated anti-M13 pVIII monoclonal antibody (1:2500) for 60 min. The wells were washed five times as before, followed by the addition of 100 µL of TMB substrate to each well. The reaction was terminated by the addition of 30 µL of 1 M sulphuric acid. Absorbance was measured at 450 nm on a microtiter plate reader (Biotek EL808, Winooski, USA).

Surface Plasmon Resonance (SPR)

The BIAcore T200 instrument and all the reagents for analysis were obtained from GE Healthcare Ltd. (Buckinghamshire, UK). For antibody kinetics, soluble purified HSV-1 was immobilized (approximately 500 RU) on a carboxymethyldextran CM5 sensor chip activated with a 1:1 mix of N-hydroxysuccinimide (50 mM) and N-ethyl-N-(dimethylaminopropyl)- carbodiimide (200 mM) by a 7 min pulse. Anti-HSV monoclonal antibodies were then passed over the HSV-1 surface in HBS-EP buffer [0.01 mM Hepes (pH 7.4), 0.15 mMNaCl, 0.005% polysorbate 20 (v/v) at a flow rate of 20 µL.min-1 at 25 ºC. Glycine-HCl (10 mM, pH 2.0) was injected for 30 sec at 20 µL.min-1 to regenerate the sensor chip between successive samples. As shown in Figure S3A the antibody dissociation constant K_D was derived from kinetic constants ($K_D = k_{off} / k_{on}$). Affinity constants for peptide interactions were calculated from steady state binding levels (R_{eq}) at different analyte concentrations (C). Fitting was performed after generation of a plot of R_{eq} against C. Towards this,biotinylated synthetic peptides were immobilized on SA sensor chips at ~150 RU in HBS-P buffer. Increasing concentrations of purified HSV-1 were then passed over the surface in PBS-P buffer at a flow rate of 20 µL.min-1 at 25 ºC. Regeneration was performed using Glycine as before.

Transmission Electron Microscopy (TEM) All images were taken by a Tecnai G2 20 TWIN Transmission Electron Microscope (FEI Company). The sample was prepared by depositing HSV-1 or magnetic particles on a 300-mesh carbon-coated copper grid. Negative staining was obtained by adding 2% uranyl acetate solution on the grid. The images for particles were performed at 200 keV and the images for virus were performed at 200 keV and 80 keV.

Particle Characterization

All zeta potential measurements were performed using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, UK), using the DTS-1060 cells. 20 µl of particles at 1 mg/ml, were placed into a 1.5ml centrifuge tube and washed three times with 1ml solution of 1mM KCl solution at the required pH, before being suspended in appropriate buffer and pH.

Influence of reaction time, magnetic force, bead size, and

binding capacity on the streptavidin-biotin MBA assay

Figure S4 presents the percent of dimers produced by the reaction of 3 µm SPMs with BBSA as the density of streptavidin and bead concentrations were varied. Curves 1 and 2 present the results for a SPM bead concentration of 50 fM with streptavidin coverage's of $2x10^6$ and $2x10^5$ molecules per bead, respectively. Curve 3 presents the results for a SPM bead concentration of 5 fM and streptavidin coverage of $2x10^6$ molecules per bead. Three observations can be made about these results. First, decreasing the streptavidin density on the beads resulted in a significant decrease in the fraction of dimers formed at any given BBSA concentration. This resulted in a corresponding decrease in sensitivity for the lower coverage beads. Second, decreasing the number of beads whilst maintaining a high streptavidin coverage, resulted in a higher sensitivity of the assay and an earlier on-set off the hook effect. Third, decreasing the number of beads did not decrease the sensitivity of the assay, which confirms that the rate of convective transport of BBSA to the beads is not the rate-limiting step in the formation of dimers.[2,3]

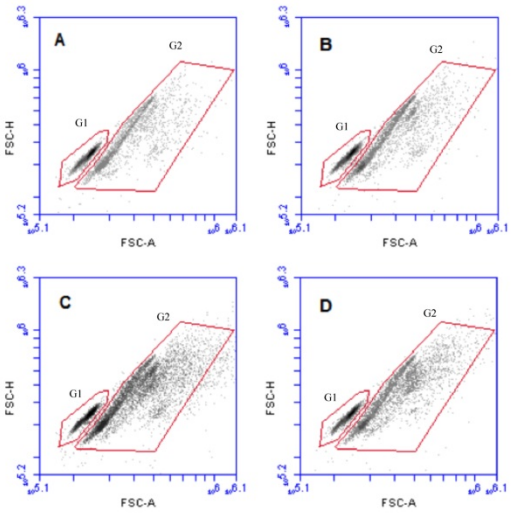

Figure S1. Flow cytometry analysis of HSV-1 assays using SPM-G1 beads, HSV-1 in the concentrations of A) 2.1 x 10^2, B) 2.1 x 10^4, C) 2.1 x 10^6, D) 2.1 x 10^8 vp/mL. The monomer and aggregate gating areas have been identified as G1 and G2, respectively.

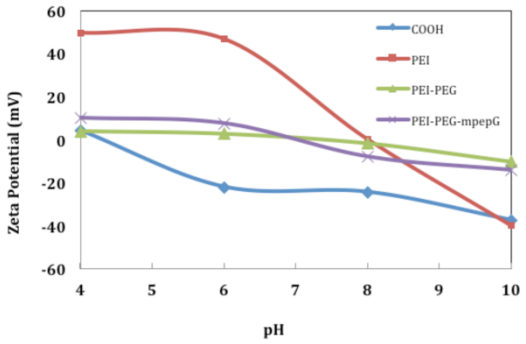

Figure S2. Zeta Potential measured as a function of pH for SPMs.

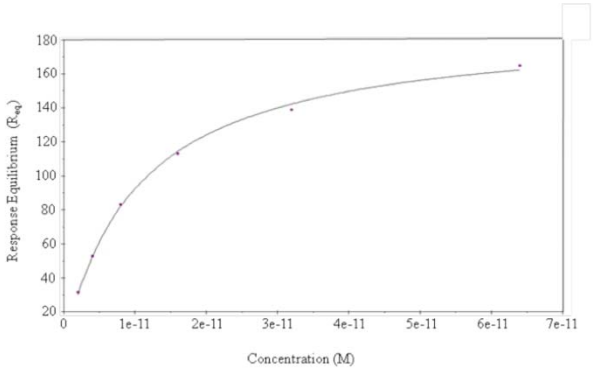

Figure S3. Affinity analysis of steady state data for MAP-G. Steady state binding levels were determined over a range of HSV-1 concentrations; (2pM, 4pM, 8pM, 16pM, 32pM and 64pM). Values for steady state binding for each of the concentrations tested were plotted using R_{eq} versus concentration (M). Subsequent fitting to a steady state model found the K_D to be 1.04e-11M, with a theoretical Rmax value of 189.

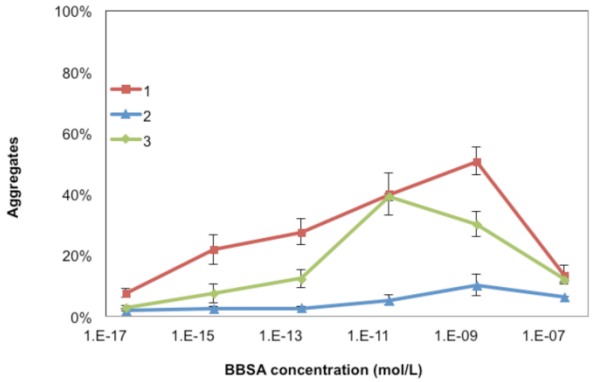

Figure S4. Effects of varying the bead concentration on the distribution of 3 µm bead aggregates. Curves 1 - 50 fM bead concentration, 75 nM binding capacity. Curves 2 - 50 fM bead concentration, binding capacity of 8 nM. Curves 3 - 5 fM bead concentration, binding capacity of 7 nM.

Table S1. Reaction rates and time constants of the streptavidin-biotin MBA assay

Receptor concentration (M)	Da_{II} [1]	k_{S-b} (1/s) [2]	k_{S-b} (m/s) [3]	τ *(sec)*
10^{-8}	0.39	1	1.5×10^{-6}	780[3]
10^{-9}	0.039	0.1	1.5×10^{-7}	7,800
10^{-10}	0.0039	0.01	1.5×10^{-8}	78,000

[1] Second Damkohler number $Da_{II} = \frac{kC_o^{n-1}L^2}{D}$ where k is the reaction rate, C_o is the initial concentration of the ligand, L is a critical dimension (1.5 microns), and n is the order of the reaction (Cussler, E.L., Diffusion: Mass Transfer in Fluid Systems, Cambridge University Press, 1984). The Da_{II} was calculated based on a streptavidin-BBSA reaction rate of k_{S-b} of 10^8 $M^{-1}s^{-1}$,

which is the upper limit for a diffusion limited, homogeneous protein reaction.

[2] $k_{S\text{-}b}$ is the reaction rate of free BBSA with strepavidin on the bead for a defined concentration of streptavidin

[3] $k_{S\text{-}b}$ is the reaction rate expressed in term of a surface concentration.

[4] τ has been calculated for 50 fM of 3 micron beads

References

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2. L.Cohen-Tannoudji, E. Bertrand, J. Baudry, C. Robic, C. Goubault, M. Pellissier, F. Thalmann, N.K. Lee, C.M. Marques, and J. Bibette, *Phys. Rev. Lett.*, 2008, **100**, 108301-1-4.

3. E. R. Billinge, J. Muzard and M. Platt, *Nanomater. Nanosci.* 2013, **1**:1. http://dx.doi.org/10.7243/2053-0927-1-1.