Supplementary Information

Controlled Surface Immobilization of Virus *via* Site-Specific Enzymatic Modification

Eun-A Kwak^{*a*} and Justyn Jaworski^{**a,b*}

Received (in XXX, XXX) Xth XXXXXXXX 20XX, Accepted Xth XXXXXXXX 20XX DOI: 10.1039/b000000x

Supplementary Figures

fd-tet p3

AA: V K K L L F A I P L V V P F Y S H S A / E T V E S C L A K P H T E N S F T N... [Leader sequence cleaved prior to packaging of mature p3] / [p3-D1, membrane penetration domain of p3 protein coat]

fd-p3lctpsr p3

DNA: GTGAAAAAATTATTATTATTCGCAATTCCTTTAGTTGTTCCTATTCTCATTCCACTCCGCT / gcgcccgcactgtgcaccccggcgcgc /GAAACTGTTGAAAGTTGTTTAGCAAAACCTCATACAGAAAATTCATTTACTAAC...

AA: V K K L L F A I P L V V P F Y S H S A / A A A L C T P S R G / E T V E S C L A K P H T E N S F T N... [Leader sequence cleaved prior to packaging of mature p3] / FGE recognition domain / [p3-D1, membrane penetration domain of p3 protein coat]

Supplementary Figure 1. DNA sequencing results (and translated amino acid below) of the p3 domain of fd-tet and fd-

p3lctpsr showing the insertion of the FGE recognition domain, containing the conserved CXPXR motif, between the

cleaved leader sequence and the membrane penetration domain.

Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B This journal is $\ensuremath{\mathbb{C}}$ The Royal Society of Chemistry 2013



Supplementary Figure 2. SDS-PAGE of fractions collected during purification of Formylglycine Generating Enzyme (FGE) by Ni-NTA column chromatography showing the pure fractions that were collected for preparing FGE stock.

Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B This journal is $\ensuremath{\mathbb{O}}$ The Royal Society of Chemistry 2013



Supplementary Figure 3. MALDI-TOF mass spectrometry of (a) unmodified LCTPSR peptide, (b) LCTPSR peptide after conversion of sulfhydryl to aldehyde, and (c) proportion of converted peptide after 1 hour reaction with FGE at 37 C. (d) Schematic structure of LCTPSR peptide before and after conversion by FGE (red circle highlights the transformation of the sulfhydryl on cysteine to an aldehyde group).



Supplementary Figure 4. Kaiser tests performed on polymer support (Rink Amide Resin) bearing Fmoc terminal blocking groups (left) and bearing freely exposed primary amines (right) on their surfaces. Dark blue color indicates the presence of exposed amine groups on the surface of the polymeric support. Colorless solution indicates no exposed amine groups were present on the Fmoc protected polymeric support.



Supplementary Figure 5. After immobilization of modified *fd-p3lctpsr* virus onto streptavidin magnetic beads, trypsin was added to the beads to liberate the virus and subsequently infect TG1 *E. coli*. The following figure reveals TG1 *E. coli* colonies growing on LB plates containing tetracycline antibiotic. The presence of the colonies indicates that the *fd-p3lctpsr* virus, that carries the resistance for tetracycline, was able to infect the host *E. coli* after being released from the beads. This confirms that the after immobilization and controlled release of virus the stability and infectivity of the virus is maintained.



Supplementary Figure 6. Standard curve for sulfhydryl content based on Ellman's test with cysteine indicating a relation of absorbance at 412nm to be approximately 0.47 times the sulfhydryl concentration (mM). (n>3)



Supplementary Figure 7. Example phage ELISA results from exposure of FGE modified phage to Fmoc and amine surfaces revealing selective coupling to amine containing surfaces. (a) Blue color appears after exposure of antibody bound virus to TMB substrate. (b) After addition of sulfuric acid, reaction is stopped to provide a yellow color which was then quantified of absorbance at 455nm by spectrophotometer to indicated the amount of bound virus.



Supplementary Figure 8. Phage ELISA results from exposure of FGE reacted phage to streptavidin magnetic beads revealing that the virus *fd-tet* does not undergo site-specific coupling to the amine containing surfaces as it does not contain the FGE recognition sequence CXPXR, while the virus *fd-p3LCTPSR* does bind selectively to the amine surface since the FGE can react with the engineered LCTPSR sequence presented on the p3 coat resulting in a reactive aldehyde group for immobilization.