Controlled immobilisation of active enzymes on the Cowpea mosaic virus capsid

Electronic Supplementary Information

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Alternative Conjugation Strategies

All general reagents, HRP (type VI-A, Product code P6782), GOX (from *Aspergillus niger*), D-glucose and 5 M sodium cyanoborohydride in 1 M NaOH were purchased from Sigma-Aldrich, UK, and used without further purification. 1-ethyl-3-(3-dimethlyaminopropyl)carbodiimide hydrochloride (EDC) was purchased from Novabiochem, *N*-hydroxysuccinimide (NHS) from Fluka, streptavidin–HRP from Upstate, sulfo-succinimidyl 4-formylbenzoate sodium salt (sulfo-S-4FB) from Solulink, and *N*-succinimidyl-S-acetylthiopropionate (SATP) and EZ-Link maleimide-activated HRP from Thermo Scientific.

Allylamine (Schiff Base) linkage

A 2000 molar excess of freshly oxidised HRP was mixed with 1 mg mL^{-1} CPMV particles suspended in 0.1 M sodium phosphate buffer pH 8.5. 5 M sodium cyanoborohydride in 1 M NaOH, 10 µL per mL of the reaction solution volume, was added. The reaction was allowed to proceed for 2 hours at ambient temperature. The modified particles (^{HRP}CPMV) were purified on a Sephacryl S-500 gel filtration column, washed and concentrated on 100 kDa cut-off columns until no HRP activity was detected in the supernatant.

SATP linkage

N-Succinimidyl-S-acetylthiopropionate (SATP) was used according to the manufacturer's instruction and coupled to CPMV as has been described previously.[1] ^{SATP}CPMV (~ 5-10 mg in 1 mL) was deprotected with 100 µL of deacetylation solution (0.5 M hydroxylamine, 25 mM EDTA in PBS, pH 7.2 (pH was adjusted with NaOH)) at ambient temperature for 2 hours with gentle stirring. The deprotected-^{SATP}CPMV particles (~ 5 mg) were purified on PD-10 columns pre-equilibrated with 10 mM sodium phosphate buffer pH 7.0 and mixed immediately with a 200 molar excess of maleimide-activated HRP. The reaction was stirred gently at 4 °C overnight (15-17 hours). $^{HRP–maleimide–SATP}$CPMV was purified on PD-10 columns, the eluted fractions were collected, concentrated and layered onto 5 mL columns of 10%-50% sucrose gradient and spun at 137000g for 90 minutes. Sucrose fractions containing $^{HRP–maleimide–SATP}$CPMV were recovered and purified.

Benzaldehyde linkage

An adaptation of the method of Brunel et al.[2] Sulfo-succinimidyl 4-formylbenzoate sodium salt (sulfo-S-4FB) in Milli–Q water was added in 2000 molar excess to CPMV (10 mg mL^{-1}, 1 mL) suspended in 10 mM sodium phosphate buffer pH 7.0 and stirred for 20 hours at ambient temperature (25 °C). ^{Benzaldehyde}CPMV was purified on PD-10 columns. The eluted sample was concentrated on 100 kDa cut-off membranes before being layered onto 5 mL 10%-50% sucrose gradient. The gradients were centrifuged at 137000g for 1.5 hours at 4 °C and 300 µL fractions were collected and dialysed against 10 mM sodium phosphate buffer pH 7.0 for 15 hours using 100 kDa molecular weight cut-off membranes. The ^{Benzaldehyde}CPMV conjugate was incubated with 200 molar excess of HRP-ADH and incubated at ambient temperature (12–15 hours). Modified particles ($^{HRP–ADH–Benzaldehyde}$CPMV) were purified by gel filtration (Sephacryl S-500), fractions were collected and concentrated.

Streptavidin-biotin linkage

^{Biotin}CPMV was prepared by our standard protocol using NHS-LC-LC-biotin.[3] Commercially available streptavidin-HRP was mixed in a 200:1 molar ratio to ^{Biotin}CPMV particles in 10 mM sodium phosphate buffer pH 7.0, the reaction was stirred gently overnight at 4 °C. $^{HRP–streptavidin–biotin}$CPMV hybrid was purified on a Sephacryl S-500 column, the eluent was monitored at 280 nm and fractions were collected and concentrated.

1. N. F. Steinmetz, D. J. Evans and G. P. Lomonossoff, *ChemBioChem*, 2007, 8, 1131-1136.

2. F. M. Brunel, J. D. Lewis, G. Destito, N. F. Steinmetz, M. Manchester, H. Stuhlmann and P. E. Dawson, *Nano Lett.*, 2010, 10, 1093-1097.

3. N. F. Steinmetz, G. Calder, G. P. Lomonossoff and D. J. Evans, *Langmuir*, 2006, 22, 10032-10037.

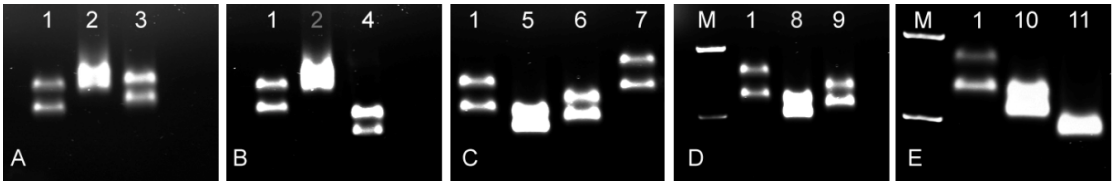

Figure S1. Wild-type CPMV and ^{Enzyme}CPMV run on a 1.2% agarose gel and stained with ethidium bromide. (A) Schiff base linkage to surface amines, (B) HRP–ADH coupled to surface carboxylates, (C) ^{SATP}CPMV reacted with maleimide-activated HRP, (D) ^{Benzaldehyde}CPMV reacted with HRP-ADH, and (E) $^{NHS\text{-}Biotin}$CPMV reacted with HRP-streptavidin. Lane 1, CPMV; 2, $^{NHS\text{–}ester}$CPMV; 3, ^{HRP}CPMV; 4, $^{HRP\text{–}ADH}$CPMV; 5, ^{SATP}CPMV; 6, ^{SATP}CPMV deprotected; 7, $^{HRP\text{–}maleimide\text{–}SATP}$CPMV; 8, ^{Benzaldehyde}CPMV; 9, $^{HRP\text{–}ADH\text{–}Benzaldehyde}$CPMV; 10, $^{NHS\text{-}biotin}$CPMV; 11, $^{HRP\text{–}streptavidin\text{–}biotin}$CPMV, and M is 1.5 kb marker.

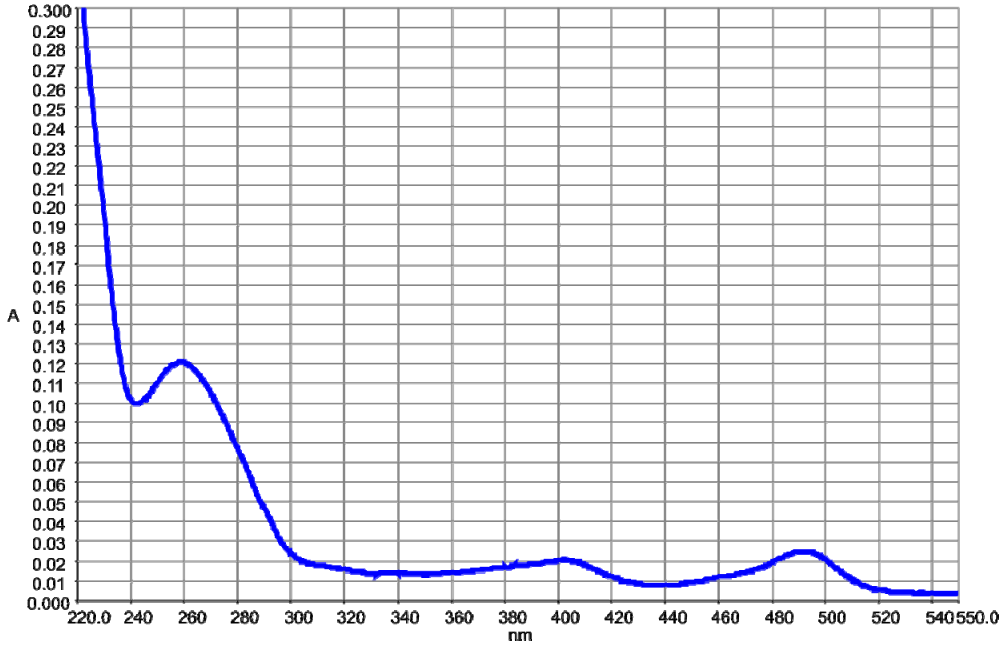

Figure S2. UV–vis spectrum for fluorescently labelled HRP conjugated to CPMV particles, $^{(DyLight488)\text{–}HRP\text{–}ADH}$CPMV. The number of dyes per virus was determined to be 32 while there are 2 ± 0.45 dyes per enzyme; this equates to 14 ± 3 enzymes per virus. The peak at 260 nm is from CPMV, at 403 nm from HRP and at 488 nm from DyLight488.

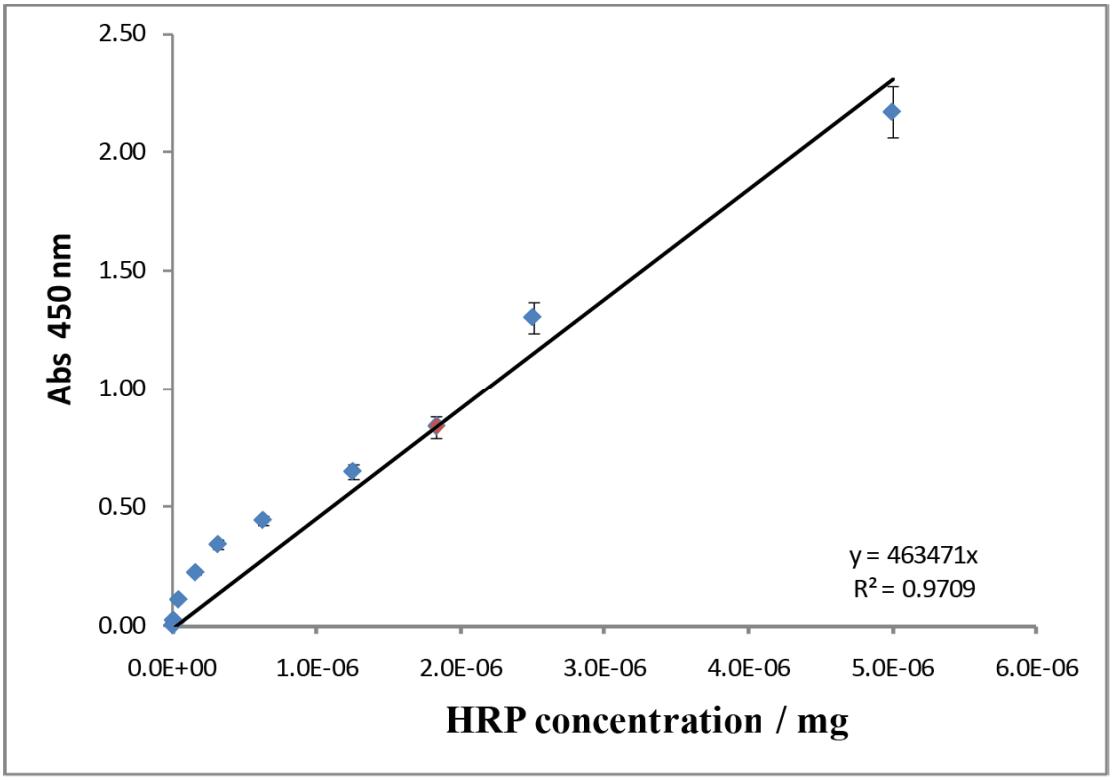

Figure S3. Calibration curve plotting known HRP concentrations against their corresponding absorbance at 450 nm. The error bars represent standard deviation. The red point is the absorbance of $^{HRP–ADH}$CPMV at 450 nm used to calculate the HRP concentration.

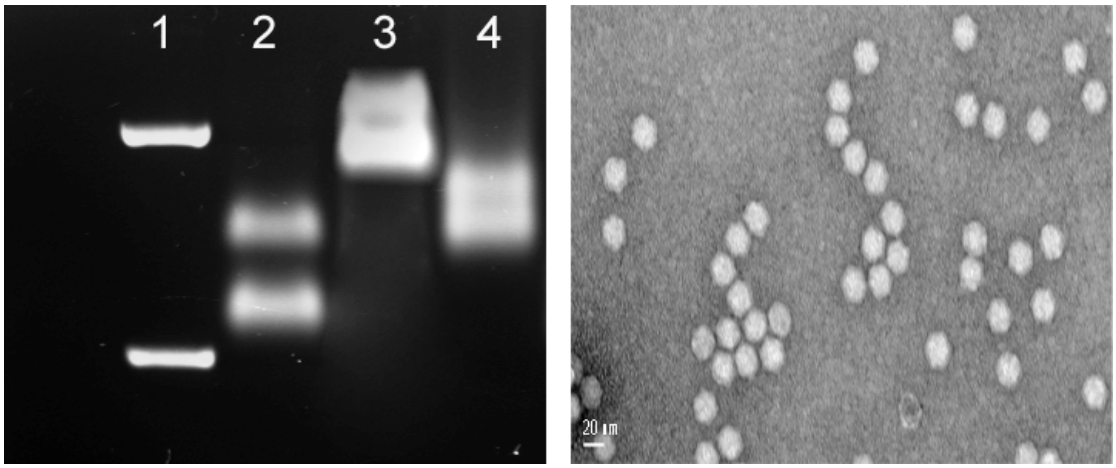

Figure S4. Right: Agarose gel (1.2%) electrophoresis of $^{GOX–ADH}$CPMV visualised by ethidium bromide staining (left). Lane1 represents a 1–kbp DNA ladder; 2, CPMVwt; 3, $^{NHS–ester}$CPMV; 4, $^{GOX–ADH}$CPMV. Left: Uranyl acetate stained TEM image of $^{GOX–ADH}$CPMV particles.

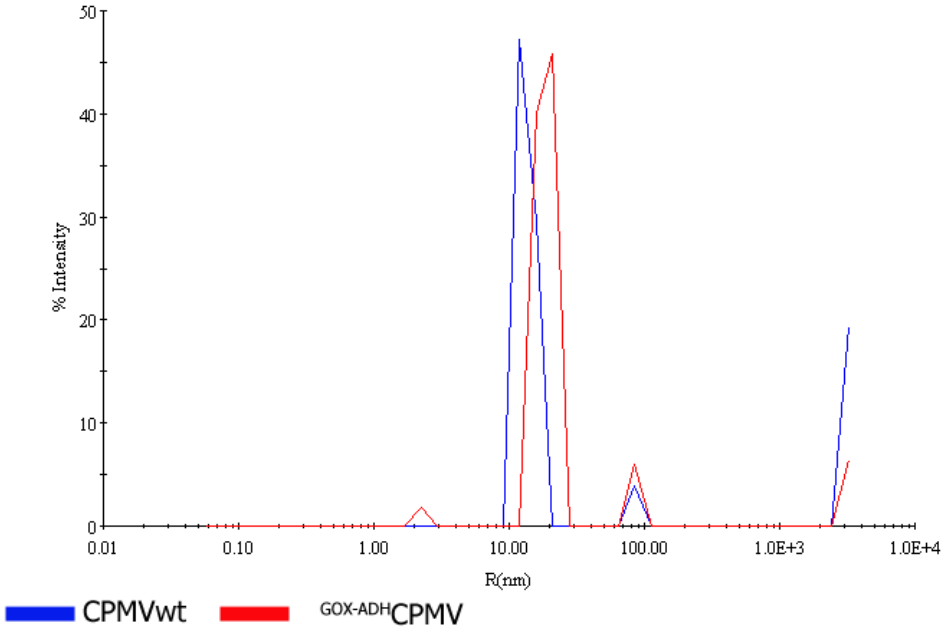

Figure S5. DLS data comparing CPMVwt and $^{GOX–ADH}$CPMV.

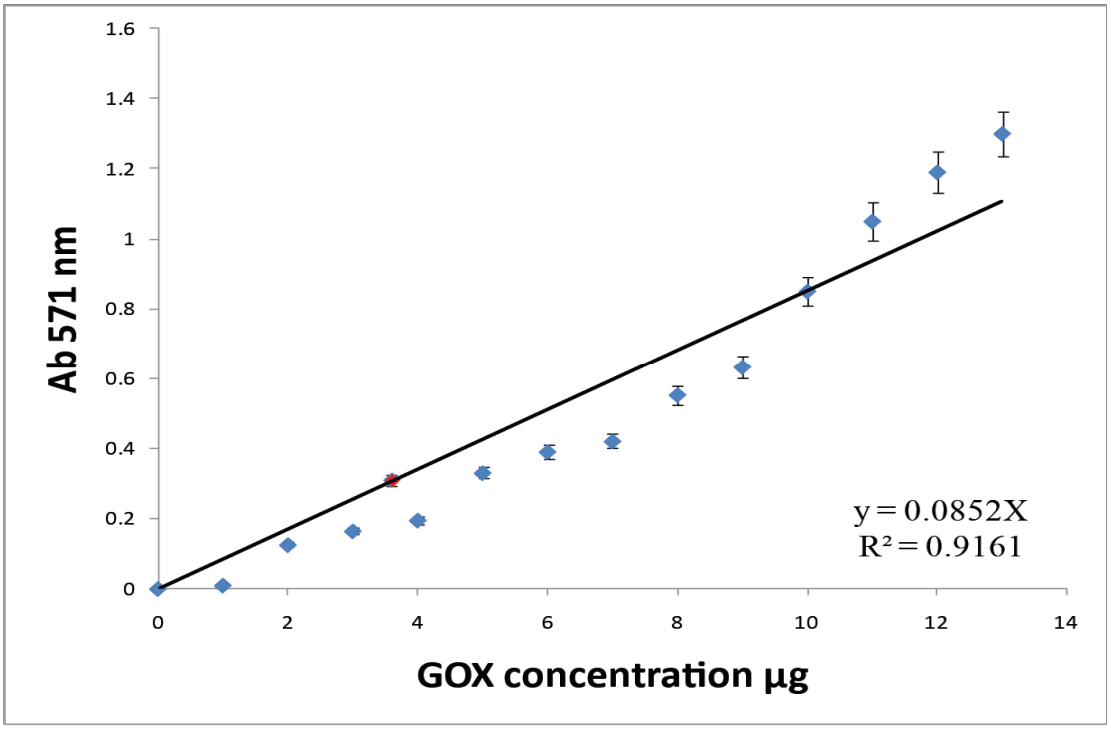

Figure S6. Calibration curve plotting known GOX concentrations against their corresponding absorbance at 571 nm. The red point is the absorbance of $^{GOX–ADH}$CPMV at 571 nm used to calculate the GOX concentration. The error bar refers to the standard deviation.

Chemical reaction	Number of enzymes per virus
Schiff Base linkage	4 ± 1
$^{\text{HRP–streptavidin–biotin}}$CPMV	3± 1
$^{\text{Benzaldehyde}}$CPMV approach	5 ± 0.5
$^{\text{SATP}}$CPMV	7 ± 1
$^{\text{HRP–ADH}}$CPMV	~ 11± 1
$^{\text{GOX–ADH}}$CPMV	2-3 ± 1

Table S1. The number of enzymes coupled to CPMV capsid external surface using different chemistry. The quantification method is based on the fluorescent labelling of enzymes.