

# Oxidative Coupling of Peptides to a Virus Capsid Containing Unnatural Amino Acids

Zachary M. Carrico, Dante W. Romanini, Ryan A. Mehl, and Matthew B. Francis

## Supporting Information

### Table of Contents

General Procedures and Instrumentation	S2
Experimental	
Synthetic Procedures	S3
Peptide Synthesis	S4
Peptide Model Reactions	S5
MS2-pAF19 Production	S6
Oxidative Coupling Reactions	S8
Transmission Electron Microscopy	S8
Dynamic Light Scattering	S9
Data	
Peptide Model Reactions	S5
MS2 Labeling	S10
MS2 Stability Studies	S11
Timecourse Experiment	S11
Dynamic Light Scattering	S11
References	S12

## **General Procedures and Materials**

Unless otherwise noted, all chemicals and reagents were obtained from commercial sources and used without further purification. Protected amino acids were obtained from Novabiochem (San Diego, CA, USA). Water used in biological procedures or as a reaction or chromatography solvent was deionized using a NANOpure purification system (Barnstead, USA). Acetonitrile was HPLC-grade and used without purification. Dichloromethane was distilled immediately prior to use. Purifications by flash chromatography were performed using EM silica gel 60 (230-400 mesh) (Fisher, USA).

## **Instrumentation and Sample Analysis Preparations**

UV-Vis spectroscopic measurements were conducted on a Tidas-II benchtop spectrophotometer (J & M, Germany). Centrifugations were conducted with the following: 1) Sorvall Legend RT Plus (Thermo Scientific, USA); 2) Sorvall RC5C refrigerated high-speed centrifuge; or 3) 5415D Benchtop Centrifuge (Eppendorf, Germany)

Desalting of protein samples and removal of other small molecules was achieved using Microcon<sup>®</sup> YM-10 (10,000 MWCO) centrifugal concentrators (Millipore, USA).

**NMR.** <sup>1</sup>H and <sup>13</sup>C spectra were acquired with either a Bruker AVB-400 (400 MHz) or DRX-500 (500 MHz) spectrometer, as indicated. Proton chemical shifts are reported as  $\delta$  in units of parts per million (ppm) relative to chloroform-*d* ( $\delta$  7.26, s) or deuterium oxide ( $\delta$  4.79, s). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), dd (doublet of doublets), m (multiplet), br (broadened). Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (*n*) for a given resonance is indicated *n*H, and is based on spectral integration values. <sup>13</sup>C NMR spectra are reported as  $\delta$  in units of parts per million (ppm) relative to chloroform-*d* ( $\delta$  77.23, t)

**High Performance Liquid Chromatography (HPLC).** HPLC was performed on an Agilent 1100 series HPLC system (Agilent Technologies, USA). Sample analysis for all HPLC experiments was achieved with an inline diode array detector (DAD) and an inline fluorescence detector (FLD). Analytical chromatography was performed using a Jupiter 5 $\mu$  C18 300 $\text{\AA}$  reversed phase column (2.0 mm x 150 mm) (Phenomenex, USA). The mobile phases used in reversed-phase runs were A) ddH<sub>2</sub>O + 0.1% TFA; B) MeCN + 0.1% TFA. Preparative HPLC was performed on a ProStar prep HPLC system (Varian, USA) with a Dynamax Omnisphere 5 C18 column (21.4 mm x 250 mm) (Varian). Mobile phases used were A) ddH<sub>2</sub>O + 0.1% TFA; B) MeCN + 0.1% TFA at a flow rate of 10 mL/min.

**Gel Filtration Chromatography.** Analytical size-exclusion chromatography was performed on the above HPLC instrument equipped with a GF-250 column (4.6 mm x 300 mm) (Agilent, USA). A mobile phase of 10 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, was used at a flow rate of 0.5 mL/min.

**Mass Spectrometry (MS).** Fast Atom Bombardment (FAB) mass spectra were obtained at the UC Berkeley Mass Spectrometry Facility. Electrospray Ionization (ESI)

mass spectra were obtained on an API 150EX system (Applied Biosystems, USA) equipped with a Turbospray ion source and an Agilent 1100 series LC pump. Peptide chromatography was performed using a Jupiter 5 $\mu$  C18 300Å reversed phase column (2.0 mm x 150 mm) (Phenomenex, USA). Protein chromatography was performed using a Jupiter 5 $\mu$  C5 300Å reversed phase column (2.0 mm x 150 mm) (Phenomenex). A MeCN:ddH<sub>2</sub>O gradient mobile phase containing either 0.1% formic acid (positive ion mode) or 0.1% triethylamine (negative ion mode) at a flow rate of 250  $\mu$ L/min was used for all LC/MS. Protein mass reconstruction was performed on the charge ladder with Analyst software (version 1.3.1, Applied Biosystems).

**Gel Analysis.** For protein analysis, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)<sup>1</sup> was accomplished on a Mini-Protean apparatus (Bio-Rad, USA). Commercially available markers (Bio-Rad, USA) were applied to one lane of each gel for calculation of apparent molecular weights. Visualization of protein bands was accomplished by staining with Coomassie<sup>®</sup> Brilliant Blue R-250 (Fisher, USA). Gel imaging was performed on an EpiChem3 Darkroom system (UVP, USA). Relative protein amounts were determined from densitometry of gel bands with ImageJ software (NIH, USA).

**Transmission Electron Microscopy (TEM).** TEM was achieved using the UC-Berkeley Electron Microscope Lab's FEI Tecnai 12 transmission electron microscope at 100 kV accelerating voltage.

**Dynamic Light Scattering (DLS).** DLS measurements were obtained on a Zetasizer<sup>®</sup> Nano Series (Malvern Instruments, UK). Measurements were taken in triplicate in 10 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, at 30 °C.

## **Experimental**

**4-(4-diethylamino-phenylcarbonyl)-butyric acid succinimidyl ester (1).** To a flame-dried round bottom flask equipped with a Teflon stirbar was added 4-(*N,N*-diethylamino)aniline hydrochloride (500 mg, 2.49 mmol) and glutaric anhydride (284 mg, 2.49 mmol), followed by dichloromethane (25 mL). To this mixture was added triethylamine (252 mg, 2.49 mmol) and the resulting black solution was stirred at room temperature for 20 minutes. *N,N'*-dicyclohexylcarbodiimide (479 mg, 2.49 mmol) and *N*-hydroxysuccinimide (287 mg, 2.49 mmol) were then added and stirring was continued for an additional hour. The reaction was then filtered to remove precipitate and concentrated under reduced pressure. The residue was taken up in dichloromethane, filtered again, and then applied to a silica gel column (25-100% EtOAc / Hexanes). Fractions were collected and concentrated to provide a green oil, 390 mg (42 %) that was dissolved in dimethylsulfoxide, aliquotted into 0.25 mmol fractions of 1 mL each, and frozen for storage until use. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.67 (s, 1H),  $\delta$  7.32 (d, 2H, J = 9 Hz),  $\delta$  6.62 (d, 2H, J = 9 Hz),  $\delta$  3.31 (q, 4H, J = 7 Hz),  $\delta$  2.87 (br-s, 4H),  $\delta$  2.72 (t, 2H, J = 7 Hz),  $\delta$  2.43 (t, 2H, J = 7 Hz),  $\delta$  2.18 (p, 2H, J = 7 Hz),  $\delta$  1.12 (t, 6 H, J = 7 Hz). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  169.6, 169.5, 168.7, 145.3, 126.8, 122.3, 112.4, 44.7, 35.3, 30.0, 25.8, 21.3, 12.6. HRMS (ESI) calc'd for C<sub>19</sub>H<sub>26</sub>N<sub>3</sub>O<sub>5</sub> (M+H<sup>+</sup>) 376.1872, found 376.1874.

## Peptide Synthesis

### *General Procedures for Peptide Synthesis*

Peptides were synthesized using standard Fmoc-based chemistry. Side chain protecting groups used were: Asn(Trt), Asp(tBu), Arg(Pbf), Cys(Trt), Gln(Trt), Glu(tBu), His(Trt), Lys(Boc), Ser(tBu), Thr(tBu), Trp(Boc), Tyr(tBu). Resin linkers used were either benzyloxybenzyl alcohol (Wang) polystyrene or Rink amide resin (Novabiochem). Synthesis was accomplished through one of the following methods: 1) Manually, using 5 equivalents of amino acid in dimethylformamide with *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) (5 eq) as the coupling reagent with 1-hydroxybenzotriazole (HOBT) (5 eq) and *N,N*-diisopropylethylamine (DIPEA) (10 eq) as additives. 2) On a Liberty Automatic Microwave Peptide Synthesizer (CEM, North Carolina, USA) using 10 eq of amino acid in DMF with HBTU (10 eq) as the coupling reagent with HOBT (10 eq) and DIPEA (20 eq) as additives.

Peptides were cleaved from resin using either a cocktail of 94% trifluoroacetic acid, 5% H<sub>2</sub>O, and 1% triisopropylsilane or Reagent K (87.5% TFA, 5% thioanisole, 5% H<sub>2</sub>O, 2.5% ethanedithiol). Crude peptides were precipitated in cold *tert*-butylmethylether, purified using preparative reversed-phase HPLC, and lyophilized before use.

### *Addition of phenylene-diamine*

Once all amino acids had been coupled to a growing peptide on the resin, the N-terminal Fmoc group was removed with 20% piperidine in DMF. After washing, the resin was suspended in DMF and one thawed aliquot of NHS-ester **1** in DMSO was added. The resin was incubated with gentle shaking for 3 hours and then washed thoroughly. The peptide was then cleaved from the resin as described above.

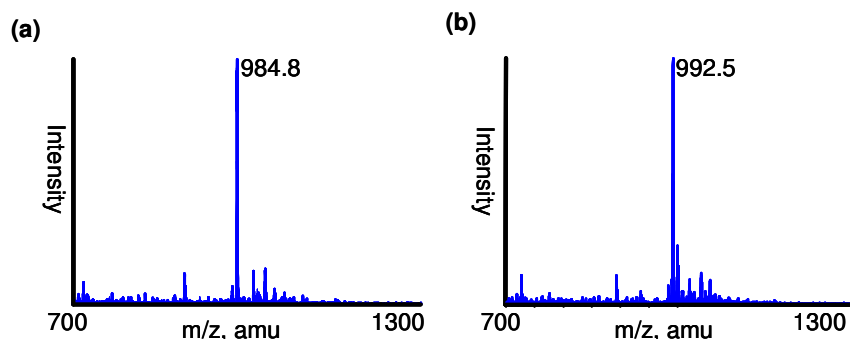
## Peptide Data Table

Peptide	Sequence	MW (calc'd)	MW (found)
A: p160	X-VPWMEPAYQRFLGGG-NH <sub>2</sub>	1967.0	1967.6
B: CTT	X-GGCTTHWGFTLCG-NH <sub>2</sub>	1597.9	1598.0
C: KR	X-CLSGRRVCG-OH	1268.5	1268.1

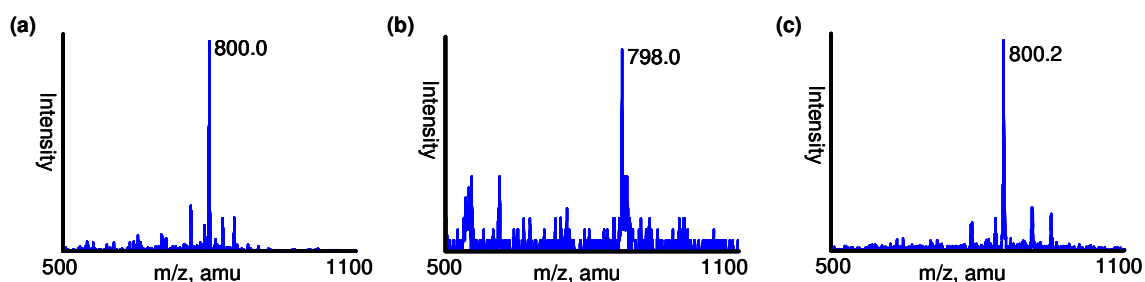
“X-“ denotes the phenylene diamine derived from compound **1**. Calculated peptide masses are monoisotopic. Peptide masses were determined using the HPLC-ESI MS instrument described. Spectra for multiply-charged species were deconvoluted to the parent mass.

## Peptide Model Studies

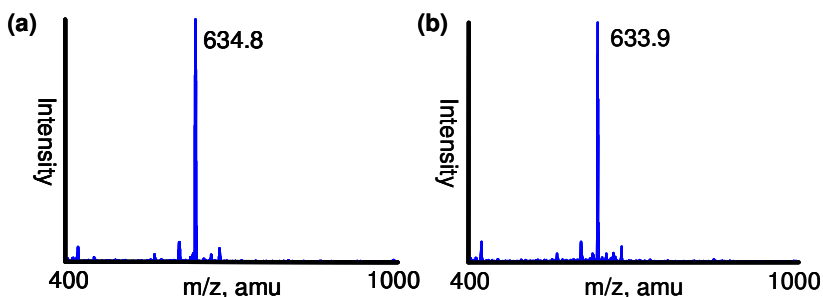
For all model studies involving peptides, lyophilized peptide was dissolved in 10 mM  $K_2HPO_4$ , pH 6.5, with MeCN as necessary to ensure complete dissolution (up to 10% vol/vol). Model reactions were run with peptides at a concentration of 0.1 mM. In cases with added oxidant, a 20 mM solution of sodium periodate was added to a final concentration of 1 mM.



**Figure S1.** (a) Peptide A. Expected  $(M+2H)2+$ : 984.5 (b) Peptide A +  $NaIO_4$ , in the presence of MS2(T19pAF). Oxidation of the methionine sidechain to a sulfoxide is observed. Expected  $(M+2H)2+$ : 992.5



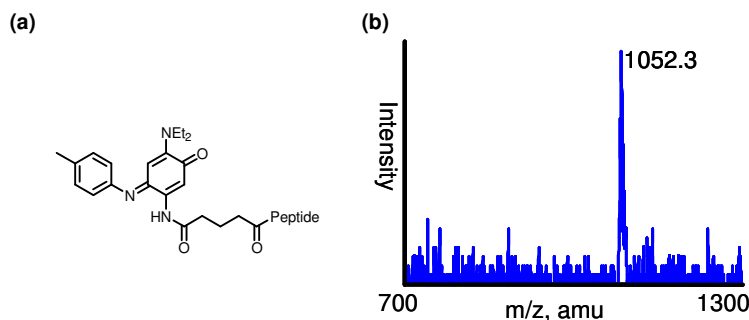
**Figure S2.** (a) Peptide B. Expected  $(M+2H)2+$ : 799.5 (b) Peptide B +  $NaIO_4$ . Disulfide formation is observed. Expected  $(M+2H)2+$ : 798.5 (c) Peptide B +  $NaIO_4$ , then DTT. Reduction of the disulfide is accomplished. Expected  $(M+2H)2+$ : 799.5



**Figure S3.** (a) Peptide C. Expected  $(M+2H)2+$ : 635.3 (b) Peptide C +  $NaIO_4$ . Disulfide formation is observed. Expected  $(M+2H)2+$ : 634.3

### Reaction of peptide A with *p*-toluidine

To a 20  $\mu$ L solution of peptide p160 (0.1 mM, **A**) was added *p*-toluidine in 0.1 M HCl to a final concentration of 1 mM. Sodium periodate was then added to a concentration of 1 mM and the reaction was allowed to proceed for 25 minutes, at which point it was analyzed by LC/MS.



**Figure S4.** (a) Proposed structure of peptide A after model oxidative coupling with *p*-toluidine. (b) ESI mass spectrum of the product of the model reaction. Expected mass  $(M+2H)^{2+}$ : 1052.0

## MS2-pAF19 production.

### Molecular Cloning

The MS2 coat protein monomer sequence was excised from a pET-20b vector and cloned into a pBAD/*Myc*-His vector (Invitrogen) as follows. To make the restriction sites of the MS2 coat protein the same as those those on the pBAD/*Myc*-His vector the NdeI restriction site on the pET-20b-MS2 vector was replaced with the NcoI restriction site using PCR. The primer 5'-AGATCGTACCATGGCTTCTAACTTTACTCAGTTC-3' and its reverse complement were used. Following PCR, the MS2 coat protein monomer sequence was cleaved from the pET vector using HindIII and NcoI restriction enzymes. The same restriction enzymes were used for pBAD-*Myc*-His. The MS2 coat protein monomer oligonucleotide and the pBAD-*Myc*-His oligonucleotide were isolated by agarose gel extraction (Qiagen Gel Extraction Kit). The cleaved pBAD vector and MS2 fragment were then ligated using T4 DNA ligase (Invitrogen). The ligated plasmid was transformed into New England Biosciences 5 $\alpha$  competent *E. coli*, and after allowing for growth the plasmid was isolated (Qiagen Spin Miniprep Kit).

Site-directed mutagenesis of pBAD-MS2 to replace Q6, D11, T15, D17, and T19 with the amber stop codon was done using the following primers:

5'-GAATTAACCATGGCTTCTAACTTTACTTAGTTCGTTCTCGTC-3' (Q6),

5'-TCAGTTCGTTCTCGTCTAGAATGGCGGAAGTGGCG-3' (D11),

5'-CGTTCTCGTCGACAATGGCGGATAGGGCGACGTGACT-3' (T15),

5'-TGGCGGAAGTGGCTAGGTGACTGTCGCC-3' (D17),

5'-CGGAAGTGGCGACGTGTAGGTGCCCCAAGCAACT-3' (T19)

and their respective reverse complements.

### *MS2 growth conditions*

The following conditions and general procedure for the production of a pAF containing protein are from a previously published protocol,<sup>2</sup> with only slight modification. The pBAD-MS2-T19pAF (ampicillin resistance) and pDULE-pAF (tetracycline resistance) plasmids were co-transformed into DH10B *E. coli* cells and plated on 2x YT-agar plates containing 50 µg/ml ampicillin and 12.5 µg/ml tetracycline. The resulting colonies were grown overnight in 5 ml of LB containing 50 µg/ml ampicillin and 12.5 µg/ml tetracycline at 37 °C and then added to 1 L of arabinose media. The 1 L culture of arabinose media was grown to an OD600 of 0.8 and then split into two 500 ml flasks. *p*-Amino-L-phenylalanine-HCl salt (pAF) (Bachem) was dissolved in water and the pH was adjusted to 7 before addition to one of the flasks to give a final concentration of 1 mM. Both 500 ml cultures were allowed to grow until the OD600 no longer increased over a two hour time span (approximately 29 h). The cells were spun down at 6000 rpm for 20 min, the supernatant was discarded, and the cell pellets were frozen at -80 °C until the protein was isolated.

The pellets were thawed and resuspended in 20 ml of 20 mM taurine buffer (pH 9) containing 6.5 mM DTT, 6 mM MgCl<sub>2</sub>, and 10 µg/ml of DNase and RNase. Following sonication for 10 min, the cells were spun down for 45 min at 11,000 rpm. Next, the supernatant was applied to a DEAE-sephadex column (GE Healthcare). Fast Performance Liquid Chromatography (FPLC) was performed on a BioRad<sup>®</sup> BioLogic<sup>™</sup> DuoFlow FPLC system. In 20 mM taurine buffer, pH 9, MS2 eluted first from the DEAE column, and was collected and precipitated using 40 % aqueous ammonium sulfate. The protein pellets were resuspended in 10 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, and applied to a Sephacryl-500 column, followed in-line by a Sephacryl-1000 column (GE Healthcare). The fractions containing MS2 were then collected and concentrated using Amicon Ultra 100 kD MWCO centrifugal concentrators (Millipore). For pBAD-MS2 lacking the stop codon, all the conditions were the same, except that no pAF was added.

#### Arabinose Media (1 L, H<sub>2</sub>O):

50 ml 10 % glycerol (autoclaved)  
2.4 ml 20 % glucose (autoclaved)  
2.5 ml 20 % arabinose (sterile filtered)  
20 ml 50x M salts (1.25 M Na<sub>2</sub>HPO<sub>4</sub>, 1.25 M KH<sub>2</sub>PO<sub>4</sub>, 2.5 M NH<sub>4</sub>Cl, 0.25 M Na<sub>2</sub>SO<sub>4</sub>; autoclaved)  
2 ml 1 M magnesium sulfate (autoclaved)  
50 ml 5% w/v L-aspartate pH 7.5 (autoclaved)  
20 ml 4 mg/ml L-leucine pH 7.5 (autoclaved)  
40 ml 25x 18 amino acid stock (5 mg/ml of every natural L -amino acid excluding tyrosine and cysteine; sterile filtered)  
1 ml 1000x heavy metal stock solution (sterile filtered)  
12.5 mg tetracycline  
50 mg ampicillin

#### 1000x heavy metal stock solution (1 L, 1 M HCl)

500 mg MoNa<sub>2</sub>O<sub>2</sub>·2H<sub>2</sub>O  
250 mg CoCl<sub>2</sub>

175 mg	CuSO <sub>4</sub> ·5H <sub>2</sub> O
1 g	MnSO <sub>4</sub> ·H <sub>2</sub> O
8.75 g	MgSO <sub>4</sub> ·7H <sub>2</sub> O
1.25 g	ZnSO <sub>4</sub> ·7H <sub>2</sub> O
1.25 g	FeCl <sub>2</sub> ·4H <sub>2</sub> O
2.5 g	CaCl <sub>2</sub> ·2H <sub>2</sub> O
1 g	H <sub>3</sub> BO <sub>3</sub>

### Oxidative Coupling Conditions

When screening oxidative coupling conditions, the reaction volume was 50  $\mu$ L and the reactions were run in 1.5 ml Eppendorf tubes. 10  $\mu$ L of 100  $\mu$ M wtMS2 or MS2-pAF19 in 10 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 7.2) was added to an amount of H<sub>2</sub>O that would produce a final volume of 50  $\mu$ L. To this was added a 2 mM solution of the phenylene-diamine peptide to give the desired final concentration. Both peptides A and B were in water containing 37.5 % acetonitrile to maintain their solubility, and the peptide C was dissolved in pure H<sub>2</sub>O. Finally, a freshly prepared solution of 100 mM NaIO<sub>4</sub> in H<sub>2</sub>O was added to the desired final concentration, and the reaction was mixed gently before standing at room temperature. After one hour the reaction was quenched with 5  $\mu$ L of 500 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (Sigma). Samples from these reactions were then analyzed by 10-20 % Tris-HCl SDS-PAGE (Bio-Rad pre-cast "Ready Gel"). Initially, it was a concern that the oxidative coupling conditions might result in disassembly of the capsids, which would not be observable by SDS-PAGE. To ensure this was not the case, even for an oxidative coupling at very high oxidant concentrations, the reactions were diluted with H<sub>2</sub>O, washed with 500  $\mu$ L of 10 mM K<sub>2</sub>HPO<sub>4</sub> pH 7.2, and concentrated over a 100 kD Microcon concentrator (Millipore). This process was repeated two additional times. The washed samples were analyzed by SDS-PAGE (Figure S5, lane 13) showing that the extent of modification before and after washing was the same and that the capsid remains intact by size exclusion chromatography and TEM (Figure S6).

A specific example of an oxidative coupling of peptide A to MS2-pAF19 is the addition of 10  $\mu$ L of 100  $\mu$ M MS2-pAF19 in 10 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, to 32.5  $\mu$ L of H<sub>2</sub>O, followed by the addition of 5  $\mu$ L of 2 mM A in 37.5 % MeCN in H<sub>2</sub>O, and finally the addition of 2.5  $\mu$ L of 100 mM NaIO<sub>4</sub> in H<sub>2</sub>O. After one hour the reaction was quenched with 5  $\mu$ L of 500 mM TCEP. The result of this reaction is shown in lane 7 of Figure S5.

Time course evaluation of the oxidative coupling of peptide A to MS2-pAF19 shows that the reaction was complete after 45 min (Figure S7).

### Transmission Electron Microscopy (TEM)

50  $\mu$ M MS2 samples in 10 mM K<sub>2</sub>HPO<sub>4</sub> pH 7.2 were applied to the copper grid, and after three minutes were gently dabbed off. A 20 mg/ml solution of uranyl acetate (UO<sub>2</sub>(OAc)<sub>2</sub>) that had been filtered (Pall Life Sciences Acrodisc<sup>®</sup> LC 13 mm syringe filter with 0.2  $\mu$ m PVDF membrane) was then applied to the copper grid. After 1.5 min the uranyl acetate was gently dabbed off and the copper grid was allowed to dry for 5 min.



### Dynamic Light Scattering (DLS)

The results obtained from the DLS measurement show that MS2-pAF19 is 27.8±0.15 nm while MS2-pAF19-peptide A is 30.6±0.07 nm. Figure S8 shows the size distribution measurements and the small deviation between runs. Figure S9 shows the heat denaturation of wtMS2, MS2-pAF19, and MS2-pAF19-peptide A, monitored at each temperature for 2 minutes. No loss of thermal stability is observed for MS2-pAF19-peptide A.

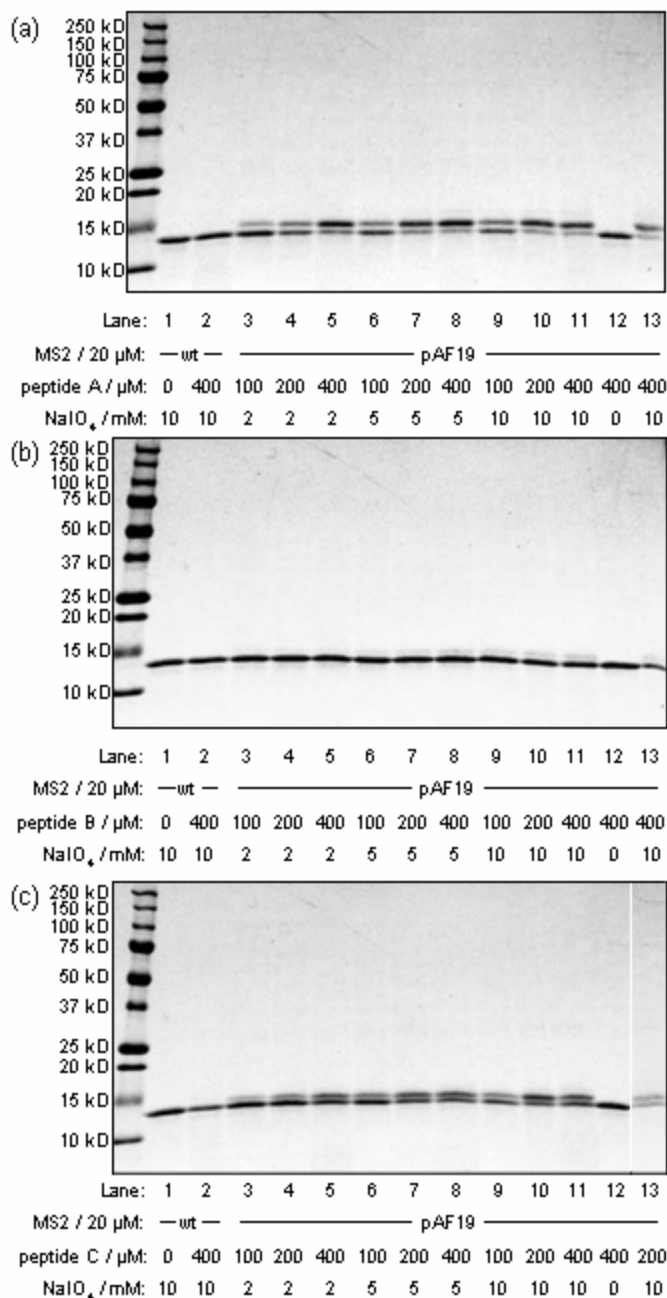


Figure S5. Oxidative coupling condition screen of (a) peptide A, (b) peptide B, and (c) peptide C. These conditions show that the extent of modification can be controlled, and that after a certain concentration, adding more peptide or oxidant does not increase coupling. The sample in Lane 13 of each gel was sequentially washed with 500 μL 10 mM K<sub>2</sub>HPO<sub>4</sub> and concentrated with a 100 kD MWCO Microcon spin concentrator three times before analysis.

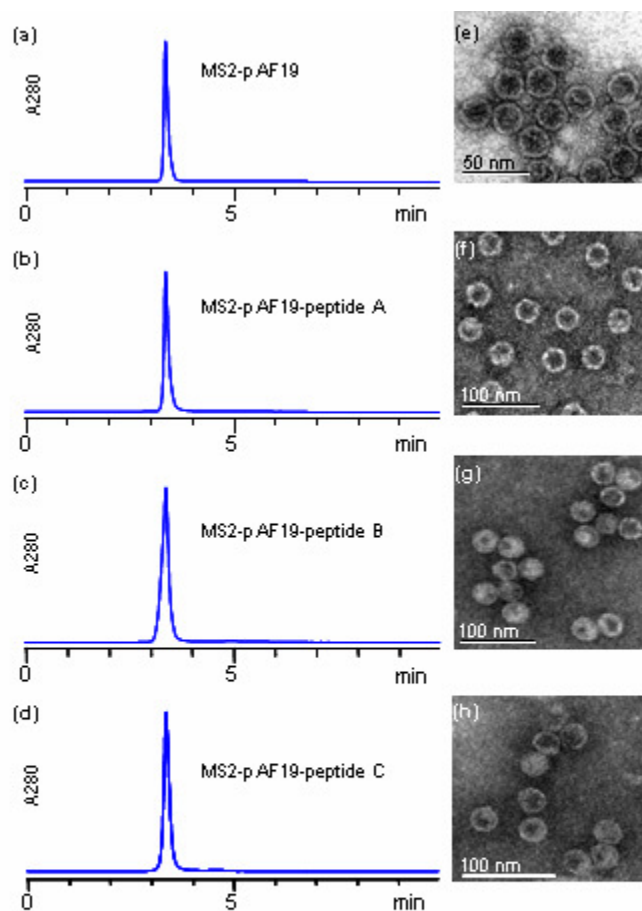


Figure S6. Abs. 280 nm trace of peptide-modified capsid from HPLC size exclusion chromatography of (a) MS2-pAF19, (b) MS2-pAF19-peptide A, (c) MS2-pAF19-peptide B, and (d) MS2-pAF19-peptide C; (b), (c) and (d) correspond to lane 13 of their respective SDS-PAGE gels in Figure S5. In all cases the capsids remain intact and no late-eluting monomer species are observed. After modification the capsids remain intact as shown by the TEM images of (e) MS2-pAF19, (f) MS2-pAF19-peptide A, (g) MS2-pAF19-peptide B, and (h) MS2-pAF19-peptide C.

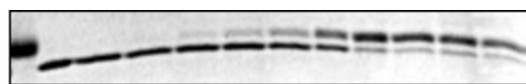
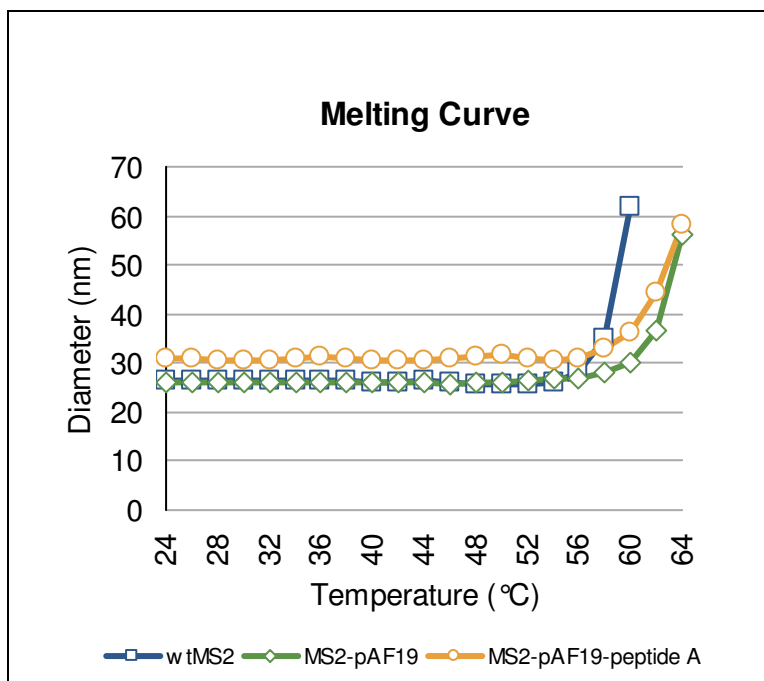


Figure S7. Time course with 20  $\mu$ M MS2-pAF19, 100  $\mu$ M peptide A, and 5 mM  $\text{NaIO}_4$ . Each time point was quenched with 5  $\mu$ L of 330 mM TCEP.

MS2-pAF19			MS2-pAF19-peptide A		
Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %
5.61	0.0	0.0	5.61	0.0	0.0
6.50	0.0	0.0	6.50	0.0	0.0
7.53	0.0	0.0	7.53	0.0	0.0
8.72	0.0	0.0	8.72	0.0	0.0
10.1	0.0	0.0	10.1	0.0	0.0
11.7	0.0	0.0	11.7	0.0	0.0
13.5	0.0	0.0	13.5	0.0	0.0
15.7	0.0	0.0	15.7	0.0	0.0
18.2	8.5	2.1	18.2	4.3	1.1
21.0	18.7	1.1	21.0	14.1	0.7
24.4	22.4	0.5	24.4	20.0	0.3
28.2	19.8	1.1	28.2	20.0	0.5
32.7	14.3	1.0	32.7	16.3	0.5
37.8	8.8	0.6	37.8	11.4	0.4
43.8	4.6	0.2	43.8	7.0	0.3
50.7	2.0	0.1	50.7	3.9	0.2
58.8	0.7	0.1	58.8	1.9	0.1
68.1	0.2	0.1	68.1	0.8	0.1
78.8	0.0	0.0	78.8	0.3	0.0
91.3	0.0	0.0	91.3	0.1	0.0
106	0.0	0.0	106	0.0	0.0
122	0.0	0.0	122	0.0	0.0

**Figure S8.** Dynamic Light Scattering (DLS) measurements of MS2-pAF19 and MS2-pAF19-peptide A taken in triplicate. These results give an average volume for MS2-pAF19 of 27.8±0.15 nm and 30.6±0.07 nm for MS2-pAF19-peptide A. The polydispersity of MS2-pAF19 is 1.168±0.008, and MS2-pAF19-peptide A has a polydispersity of 1.192±0.007.



**Figure S9.** Heat Denaturation of wtMS2, MS2-pAF19, and MS2-pAF19-peptide A as monitored by DLS. Note that the three varieties of MS2 denature at approximately the same temperature.

## References

---

<sup>1</sup> Laemmli, U. K. *Nature* 1970, **227**, 680.

<sup>2</sup> J. T. Hammill, S. Miyake-Stoner, J. L. Hazen, J. C. Jackson, and R. A. Mehl, *Nature Protocols*, 2007, **10**, 2601-2607.