Supporting Information for

A Subset of Fluorophores is Responsible for Radiation-Brightening in Viromimetic Particles

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Virus Modification and Characterization

The efficiency of labelling in the BMV-OG conjugates was estimated with the help of UV-Visible absorption spectroscopy.[1] The average number of chromophores were calculated using a molar extinction coefficient value of ε = 33,641 $cm^{-1}M^{-1}$ for Oregon green NHS ester at 496 nm in buffer at pH 4.6.

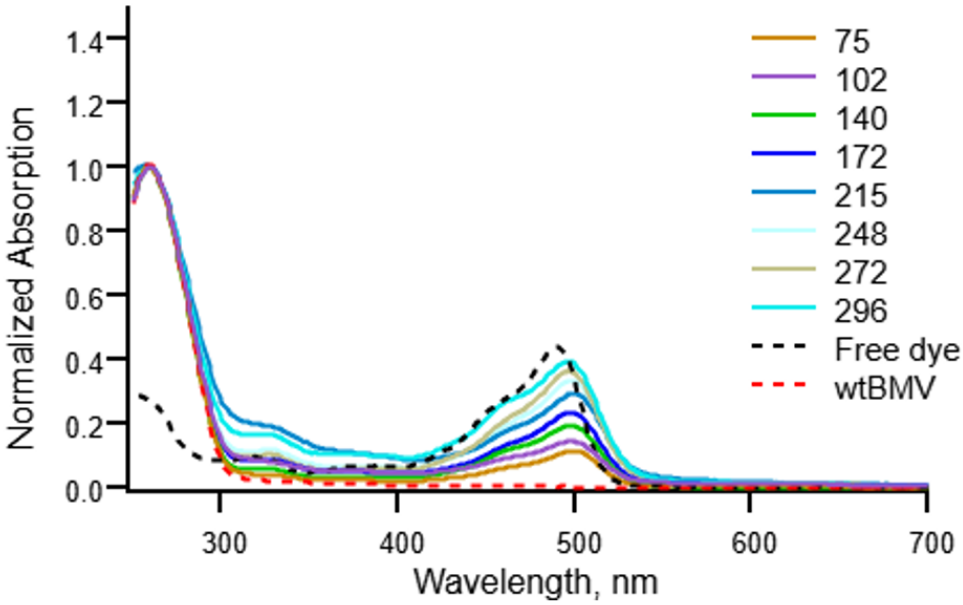

Figure S1: Steady state UV-Visible absorption spectra of BMV-OGs normalized at 260 nm. The legends show varying number of chromophores per virus in average. The absorbance spectra of free dye and wtBMV is also depicted for comparison.

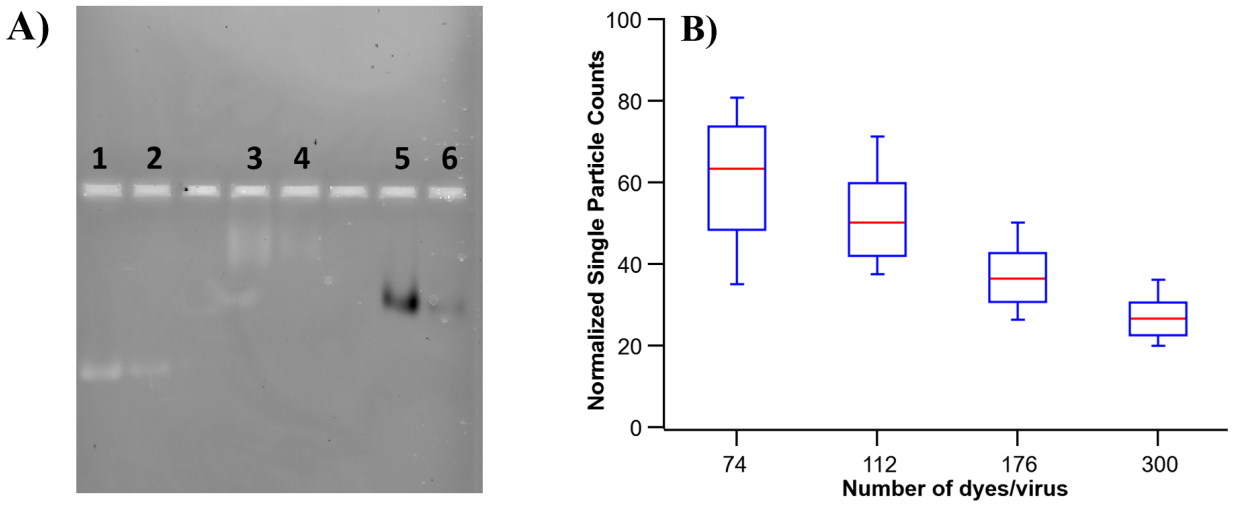

Figure S2: A) Coomassie stained native agarose gel for wtBMV (Lane 1 and 2- 0.5 and 0.25 mg/mL, respectively), BMV capsid protein (Lane 3 and 4 – 0.5 and 0.25 mg/mL) and BMV-OG with 220 dyes/virus(Lane 5 and 6 -0.3 and 0.15 mg/mL). There is a noticeable difference in electrophoretic mobility between wtBMV and OG-BMV which is interpreted as due to a difference in surface charge. B) Box and whiskers plot representing the normalized fluorescence counts to incident laser power from TIRF microscopy for BMV-OGs with 74, 112, 176 and 300 dyes/virus. The fluorescence intensity is quenched at higher dye numbers, consistent with the steady state fluorescence results from Tsvetkova *et al.*[1]

Enzymatic digestion and LC-MS/MS results

The enzymes employed for proteolytic digestion of BMV capsid protein[2] are trypsin, chymotrypsin and proteinase K(PK). The tryptic and chymotryptic cleavage sites are shown in Figure. S3 and S4. PK can digest proteins adjacent to the carboxyl group of aliphatic and aromatic amino acids.

MSTSGTG**K**MT**R**AQ**RR**AAA**RR**N**R**WTA**R**VQPVIVEPLAAGQG**K**AI**K**AIAGYSIS**K**WEAS
SDAITA**K**ATNAMSITLPHELSSE**K**N**K**EL**K**VG**R**VLLWLGLLPSVAG**R**I**K**ACVAE**K**QAQA
EAAFQVALAVADSS**K**EVVAAMYTDAF**R**GATLGDLLNLQIYLYASEAVPA**K**AVVVHLE
VEHV**R**PTFDDFFTPVY**R**

Figure S3: Tryptic cleavage sites (labelled in red) for BMV capsid protein

MSTSGTGK**M**TRAQRRAAARRNR**W**TARVQPVIVEP**L**AAGQGKAIKAIAG**Y**SISK**W**EASS
DAITAKATNA**M**SIT**L**P**H**E**L**SSEKNKE**L**KVGRV**LL**W**L**G**LL**PSVAGRIKACVAEKQAQAE
AA**F**QVA**L**AVADSSKEVVAA**MY**TDA**F**RGAT**L**GD**LL**N**L**QI**YLY**ASEAVPAKAVVV**HL**E
VE**H**VRPT**F**DD**FF**TPV**Y**R

Figure S4: Chymotryptic cleavage sites (labelled in red) for BMV capsid protein

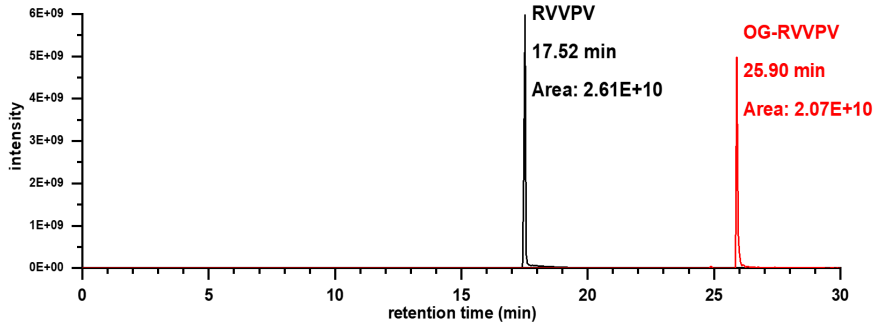

Figure S5: Extracted ion chromatogram of peptide mixture containing equal amount of RVVPV and OG488 labelled peptide. The OG488 label prolongs the peptide retention time. The peak area of labelled peptide is 20% smaller than the one of unlabeled peptide, indicating the OG488 label may change the extent of peptide ionization.

Limited Proteolysis of BMV-OGs

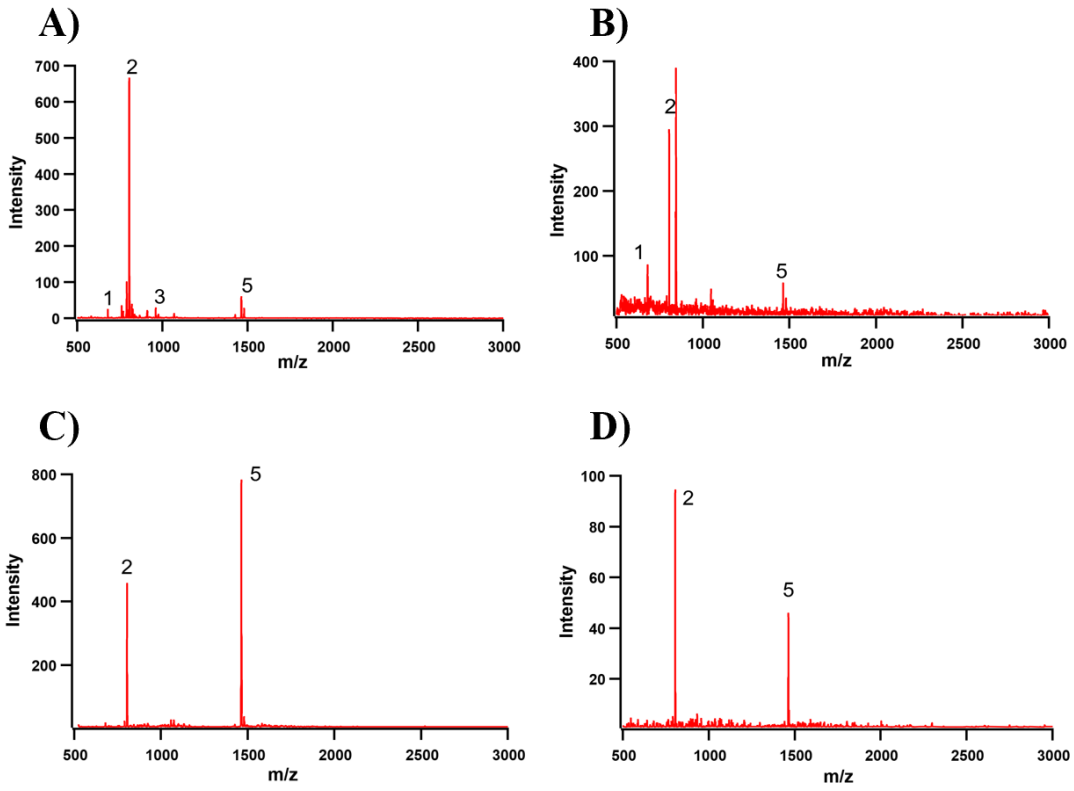

Figure S6: MALDI-TOF analysis of peptides released from BMV-OG samples after trypsin treatment for short time intervals: A) 56, B) 132, C) 231, D) 292 dyes/virus respectively. The masses and the identity of labelled ions can be seen in Table 4 of the main document

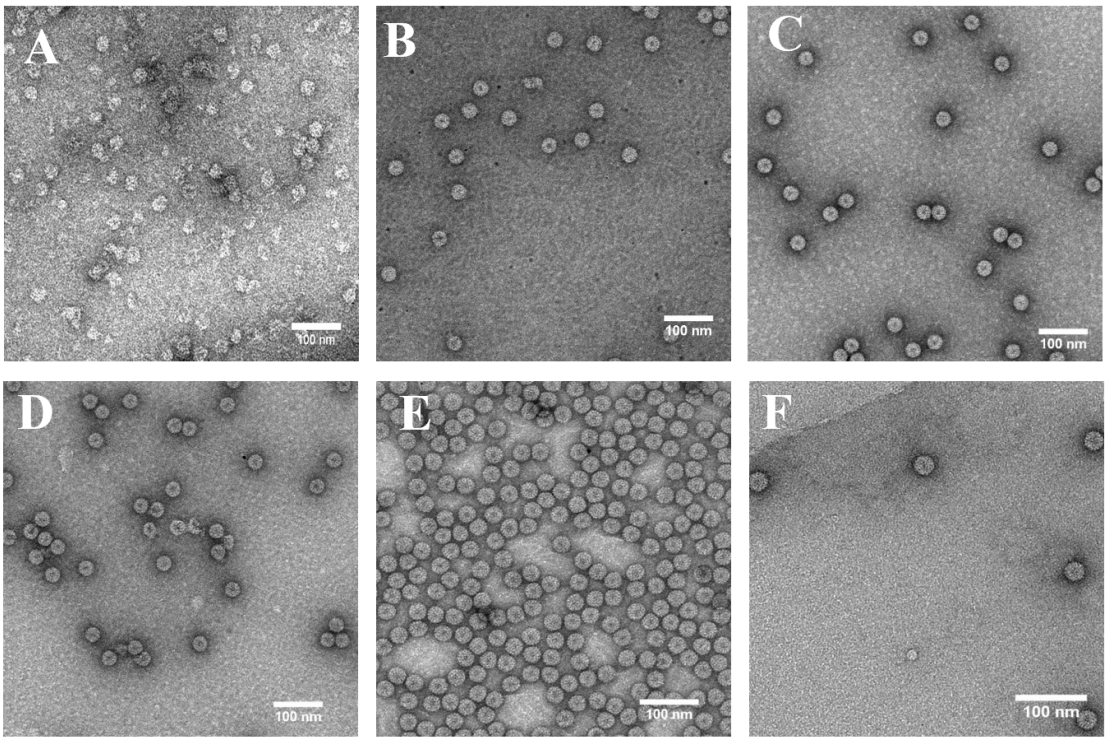

Figure S7: TEM image of particles after digestion: A) wtBMV, BMV-OGs with different dye numbers B) 56, C) 132, D) 231, E) 264, F) 292

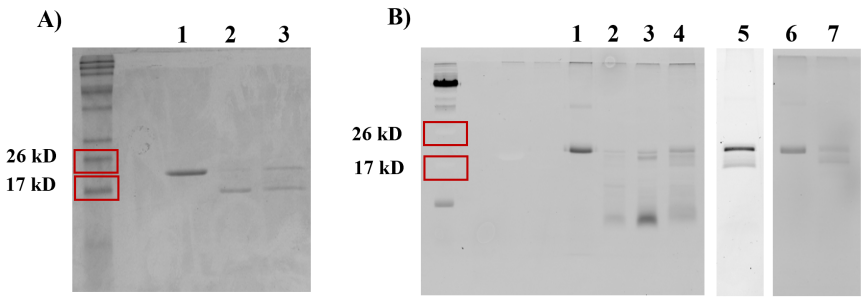

Figure S8: Coomassie-stained SDS-PAGE gel of A) wtBMV: Lane 1- Untreated; Lane 2 and 3- Trypsinated BMV for 5 and 10 mins respectively, B) BMV-OGs: Lane 1 and 6- Untreated BMV-OGs; Lane 2,3,4,5,7 - Trypsinated BMV-OGs with 56, 132, 231, 264 and 317 dyes numbers. Prestained ladder is also shown at the leftmost end for each figure.

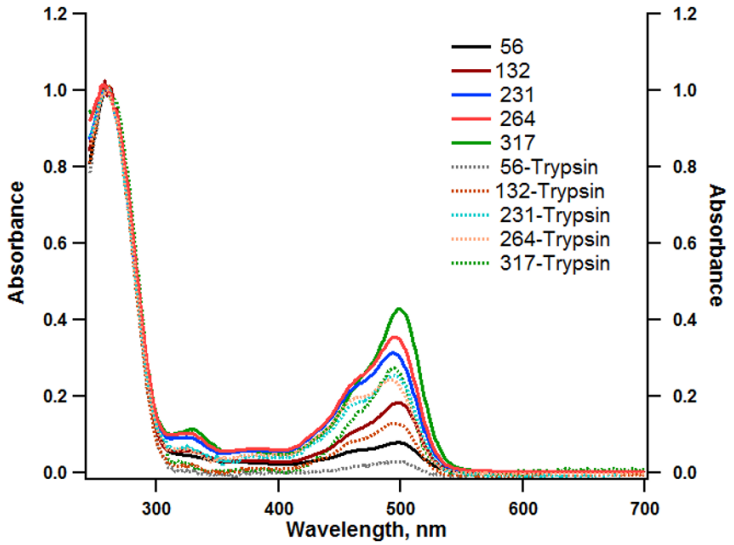

Figure S9: UV–Vis absorption spectra of BMV-OG before and after proteolytic digestion. The absorbance value at 500 nm (OG emission peak) dropped after the trypsin treatment (depicted by dashed lines). Spectra are normalized at 260 nm - the peak of protein absorption band.

cBMV mutant Modification and Characterization

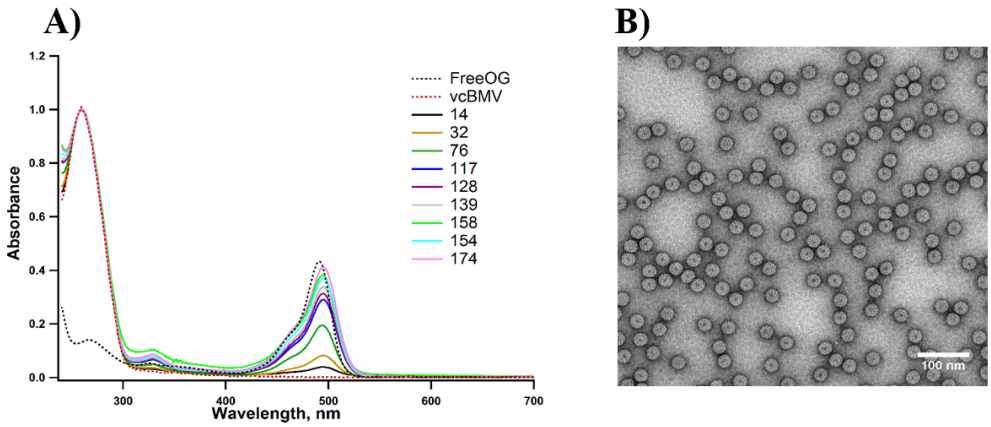

Figure S10: UV-Visible absorption spectra of cBMV-OG normalized at 260 nm. The average number of chromophores were calculated using a molar extinction coefficient value of $\varepsilon = 65{,}000\ \mathrm{cm^{-1}M^{-1}}$ for Oregon green maleimide at 496 nm, in buffer at pH 6. The legend shows the average number of chromophores per virus. B) TEM of cBMV-OG particles.

Table S1: HPLC gradient used for peptide LC-MS/MS

Time (mm:ss)	Flow Rate (nL/min)	Mobile Phase A (%)	Mobile Phase B (%)
00:00	300	98	2
00:30	300	93	7
24:30	300	62	38
25:00	300	0	100
30:00	300	0	100

Mobile phase A: 0.1% formic acid + 99.9% water

Mobile phase B: 0.1% formic acid + 19.9% water + 80% acetonitrile

Table S2: Tryptic peptides, retention time(RT) and their corresponding masses- wtBMV

Residue	Sequence	RT, min	Mass, Da
1-11	MSTSGTGKMTR	8.35-8.68	1068.52
1-8	MSTSGTGK	7.33-8.40	1358.60
27-41	VQPVIVEPLAAGQGK	17.81-18.91	1506.87
27-64	VQPVIVEPLAAGQGKAIKAIAGYSISKWEASSDAITAK	25.08-25.11	3871.14
42-64	AIKAIAGYSISKWEASSDAITAK	19.06-19.15	2384.29
45-53	AIAGYSISK	12.21-14.9	910.51
45-64	AIAGYSISKWEASSDAITAK	18.91-18.96	2071.07
27-44	VQPVIVEPLAAGQGKAIK	17.11-17.60	1820.09
54-64	WEASSDAITAK	13.80-15.63	1179.57
65-81	ATNAMSITLPHELSSEK ATNAM(oxid)SITLPHELSSEK	17.03-17.81 15.28-15.93	1829.91 1846.92
65-83	ATNAMSITLPHELSSEKNK	15.23-15.51	2074.07
65-86	ATNAMSITLPHELSSEKNKELK	15.05-15.25	2445.3
82-103	NKELKVGRVLLWLGLLPSVAGR	26.75-27.11	2420.48
84-103	ELKVGRVLLWLGLLPSVAGR	29.70-29.96	2178.35
87-103	VGRVLLWLGLLPSVAGR	29.15-29.43	1808.12
87-111	VGRVLLWLGLLPSVAGRIKACVAEK	17.81-17.85	2654.12
104-111	IKACVAEK	9.0-9.05	862.49
112-130	QAQAEAAFQVALAVADSSK	26.60-29.36	1905.97
131-142	EVVAAMYTDAFR	21.75-27.41	1373.66
166-189	AVVVHLEVEHVRPTFDDFFTPVYR	23.30-28.53	2873.48

Table S3: Chymotryptic peptides, retention time and their corresponding masses- wtBMV.

Residue	Sequence	RT, min	Mass, Da
24-35	TARVQPVIVEPL	18.38-18.53	1321.78
36-49	AAGQGKAIKAIAGY	12.70-13.06	1319.75
55-77	EASSDAITAKATNAMSITLPHEL	22.15-23.5	2374.18
86-92	KVGRVLL	10.58-10.83	785.54
120-137	QVALAVADSSKEVVAAMY	23.43-23.83	1852.96
124-137	AVADSSKEVVAAMY AVADSSKEVVAAM(Oxid)Y	17.86-19.61 14.86-14.93	1441.71 1457.71
138-146	TDAFRGATL	15.58-15.66	952.49
138-150	TDAFRGATLGDLL	24.75-25.36	1350.71
142-150	RGATLGDLL	19.91-20.58	916.53
142-152	RGATLGDLLNL	25.53-25.75	1143.66
147-155	GDLLNLQIY	28.83-29.03	1049.58
156-180	LYASEAVPAKAVVVHLEVEHVRPTF	20.05-20.23	2765.53
156-171	LYASEAVPAKAVVVHL	18.23-18.26	1667.96
158-180	ASEAVPAKAVVVHLEVEHVRPTF	17.51-17.86	2487.37
158-183	ASEAVPAKAVVVHLEVEHVRPTFDDF	20.63-22.2	2864.50
158-184	ASEAVPAKAVVVHLEVEHVRPTFDDFF	23.11-23.73	3011.56
158-171	ASEAVPAKAVVVHL	15.45-15.56	1392.82

Table S4: Proteinase K peptides, retention time and their corresponding masses- wtBMV.

Residue	Sequence	RT, min	Mass, Da
4-11	SGTGKMTR	15.88-15.96	883.43
24-35	TARVQPVIVEPL	18.61-18.81	1322.79
26-35	RVQPVIVEPL	18.06-19.93	1150.70
27-35	VQPVIVEPL	22.95-24.65	994.60
63-83	AKATNAMSITLPHELSSEKNK	8.95-9.61	2277.17
68-77	AMSITLPHEL	21.33-21.4	1112.58
69-80	MSITLPHELSSE	20.36-20.67	1344.65
69-77	M(oxid)SITLPHEL	18.46-18.66	1056.54
70-85	SITLPHELSSEKNKEL	15.03-15.13	1826.98
71-88	ITLPHELSSEKNKELKVG	14.25-14.88	2025.14
92-105	LWLGLLPSVAGRIK	24.68-24.88	1524.96
97-111	LPSVAGRIKACVAEK	20.61-20.65	1552.72
112-130	QAQAEAAFQVALAVADSSK	24.63-29.86	1905.98
113-130	AQAEAAFQVALAVADSSK	25.43-25.66	1778.92
120-137	QVALAVADSSKEVVAAMY	23.26-23.66	1852.96
121-137	VALAVADSSKEVVAAMY	22.43-22.70	1724.90
122-130	ALAVADSSK	26.21-26.43	861.46
123-130	LAVADSSK	26.3-26.65	790.43
125-130	VADSSK	26.36-26.68	606.30
158-184	ASEAVPAKAVVVHLEVEHVRPTFDDFF	23.13-23.36	3011.56
158-188	ASEAVPAKAVVVHLEVEHVRPTFDDFFTPVY	24.38-24.81	3472.80
165-184	KAVVVHLEVEHVRPTFDDFF	21.28-21.56	2385.24

Table S5: Peptides modified with OG dye generated from eznzymes A) Trypsin, B) Chymotrypsin

A)

Residue	Sequence	RT, min	Mass, Da
1-11	MSTSGTGKMTR	25.33-26.28	1462.50
27-44	VQPVIVEPLAAGQGKAIK	27.35-27.58	2213.12
42-53	AIKAIAGYSISK	26.05-29.73	1616.75
54-81	WEASSDAITAKATNAMSITLPHELSSEK WEASSDAITAKATNAM(oxid)SITLPHELSSEK	28.78-30.00 27.78-28.65	3384.51 3400.50
65-83	ATNAMSITLPHELSSEKNK	24.36-24.41	2467.09
82-89	NKELKVGR	18.08-18.41	1339.61
84-89	ELKVGR	22.65-22.81	1096.46
104-111	IKACVAEK	25.81-25.87	1256.52

B)

Residue	Sequence	RT, min	Mass, Da
24-49	TARVQPVIVEPLAAGQGKAIKAIAGY TARVQPVIVEPLAAGQGKAIKAIAGY	28.35-28.75 28.28-28.60	3017.56 3018.57
36-49	AAGQGKAIKAIAGY AAGQGKAIKAIAGY	25.41-27.06 26.05-27.11	1713.78 1714.79
86-91	KVGRVL	23.06-25.81	1066.49
157-171	YASEAVPAKAVVVHL	29.88-29.93	1948.91
158-180	ASEAVPAKAVVVHLEVEHVRPTF	27.51-27.66	2881.40
158-183	ASEAVPAKAVVVHLEVEHVRPTFDDF	29.26-29.60	3259.53
158-171	ASEAVPAKAVVVHL	28.96-29.68	1785.84

Table S6: Peptides modified with OG dye generated from Proteinase K enzyme

Residue	Sequence	RT, min	Mass, Da
24-44	TARVQPVIVEPLAAGQGKAIK	24.25-24.35	2542.31
24-49	TARVQPVIVEPLAAGQGKAIKAIAGY TARVQPVIVEPLAAGQGKAIKAIAGY	28.46-28.61	3017.55
36-49	AAGQGKAIKAIAGY	25.81-26.38	1713.78
158-175	ASEAVPAKAVVVHLEVEH	27.53-27.66	2281.06
158-171	ASEAVPAKAVVVHL	29.05-29.3	1785.84
161-170	AVPAKAVVVH	24.73-24.78	1385.64
165-189	KAVVVHLEVEHVRPTFDDFFTPVYR	27.63-27.93	3397.62

Table S7: Tryptic peptides modified with SMTA at pH6

Residue	Sequence	RT, min	Mass, Da
1-11	MSTSGTGKMTR	9.75-14.28	1109.5
27-44	VQPVIVEPLAAGQGKAIK	18.9-22.08	1861.10
27-64	VQPVIVEPLAAGQGKAIKAIAGYSISKWEASSDAITAK VQPVIVEPLAAGQGKAIKAIAGYSISKWEASSDAITAK	29.43-29.75	3912.20
42-64	AIKAIAGYSISKWEASSDAITAK	23.0-23.20	2425.30
42-53	AIKAIAGYSISK	16.86-29.18	1263.80
45-64	AIAGYSISKWEASSDAITAK	22.93-23.28	2112.10
54-81	WEASSDAITAKATNAM(oxid)SITLPHELSSEK	22.08-22.48	3048.50
65-83	ATNAMSITLPHELSSEKNK	17.68-17.86	2113.10
82-89	NKELKVGR NKELKVGR	8.06–8.81 8.95-9.11	985.60 985.60
84-89	ELKVGR	8.36-8.71	742.46
104-111	IKACVAEK	10.43-10.65	903.52

Table S8: Chymotryptic peptides modified with SMTA at pH6

Residue	Sequence	RT, min	Mass, Da
86-91	KVGRVL	9.25-9.35	713.49
124-137	AVADSSKEVVAAMY AVADSSKEVVAAM(Oxid)Y	21.33-22.78 16.88-17.03	1482.739 1498.733
158-183	ASEAVPAKAVVVHLEVEHVRPTFDDF	24.51-24.81	2906.53

Table S9: Tryptic peptides modified with SMTA at pH8

Residue	Sequence	RT, min	Mass, Da
1-11	MSTSGTGKMTR	9.01-17.15	1068.50
27-44	VQPVIVEPLAAGQGKAIK	18.9-22.08	1861.10
42-53	AIKAIAGYSISK	16.41-16.76	1263.80
45-81	AIAGYSISKWEASSDAITAKATNAMSITLPHELSSEK AIAGYSISKWEASSDAITAKATNAMSITLPHELSSEK	28.71-29.18	3924.00
45-64	AIAGYSISKWEASSDAITAK	22.93-23.28	2112.10
54-81	WEASSDAITAKATNAM(oxid)SITLPHELSSEK	22.08-22.48	3048.50
54-64	WEASSDAITAK	16.6-16.75	1220.60
65-83	ATNAMSITLPHELSSEKNK	17.68-17.86	2113.10
84-89	ELKVGR	8.36-8.71	742.46
104-111	IKACVAEK	10.43-10.65	903.52

Table S10: Chymotryptic peptides modified with SMTA at pH8

Residue	Sequence	RT, min	Mass, Da
86-91	KVGRVL	9.25-9.35	713.49
124-137	AVADSSKEVVAAMY AVADSSKEVVAAM(Oxid)Y	21.33-22.78 16.88-17.03	1482.73 1498.73
158-183	ASEAVPAKAVVVHLEVEHVRPTFDDF	24.51-24.81	2906.53
158-180	ASEAVPAKAVVVHLEVEHVRPTF	21.83-22.06	2529.40
158-184	ASEAVPAKAVVVHLEVEHVRPTFDDFF	27.58-28.25	3052.59

Computational Section Results

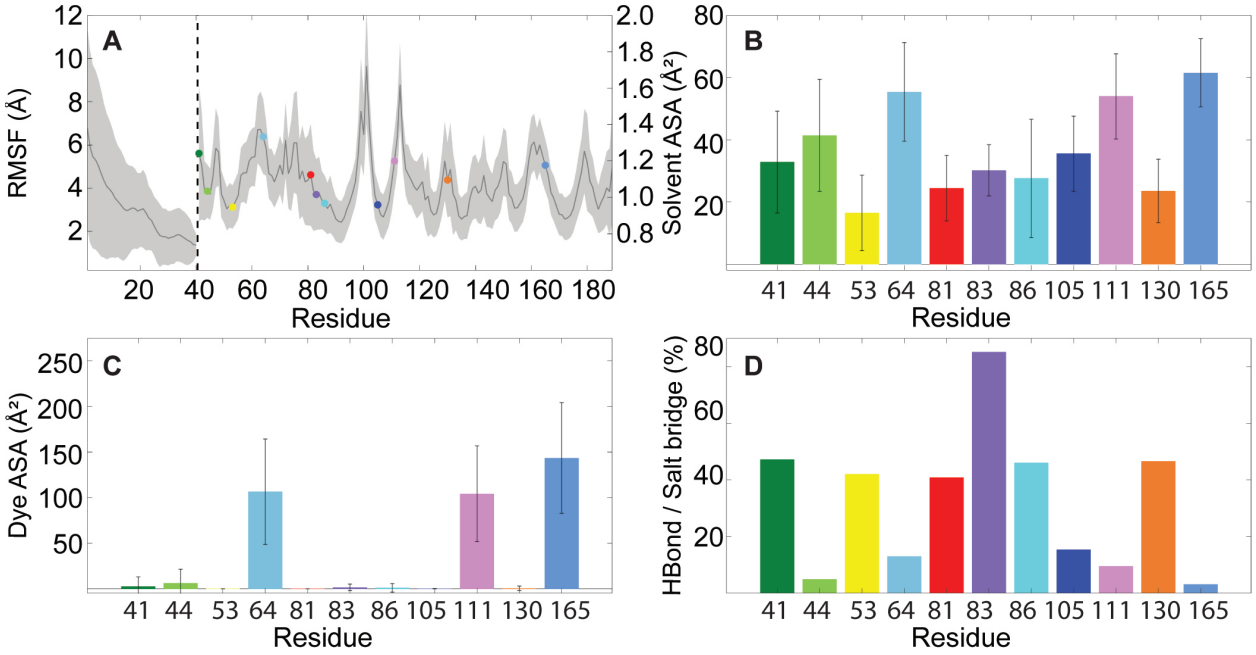

Figure S11: MD simulation results for A chains. (a) Average Cα RMSF over sixty copies of chain A. Axis shown on the left for highly flexible residues 1 to 39, and on the right for remaining residues. Lysines are highlighted according to the color scheme used in Fig. 2. (b) Average solvent accessible surface area for each lysine, calculated with a probe radius of 1.4 Å. (c) Average dye accessible surface area for each lysine, calculated with a probe radius of 5.0 Å. (d) Percentage of trajectory frames in which each lysine was involved in hydrogen bonds and/or salt bridges. Averages were calculated over 4.5 microseconds of cumulative simulation sampling. Error bars represent standard deviation.

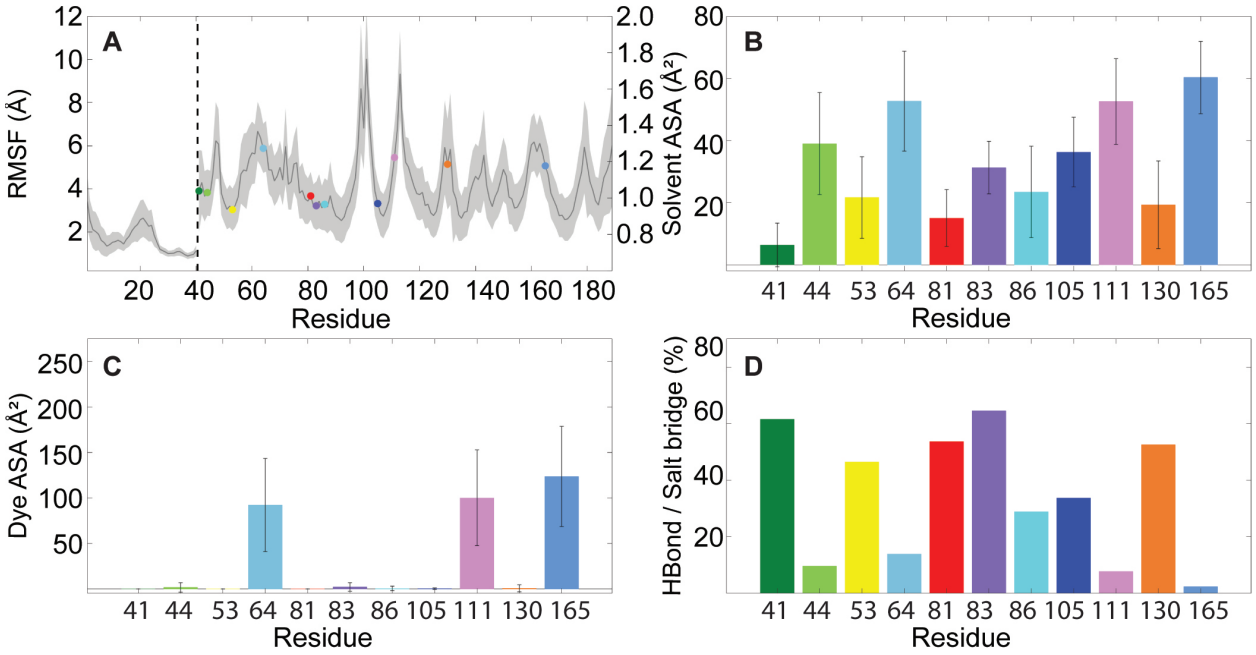

Figure S12: MD simulation results for B chains. (a) Average Cα RMSF over sixty copies of chain B. Axis shown on the left for highly flexible residues 1 to 39, and on the right for remaining residues. Lysines are highlighted according to the color scheme used in Fig. 2. (b) Average solvent accessible surface area for each lysine, calculated with a probe radius of 1.4 Å. (c) Average dye accessible surface area for each lysine, calculated with a probe radius of 5.0 Å. (d) Percentage of trajectory frames in which each lysine was involved in hydrogen bonds and/or salt bridges. Averages were calculated over 4.5 microseconds of cumulative simulation sampling. Error bars represent standard deviation.

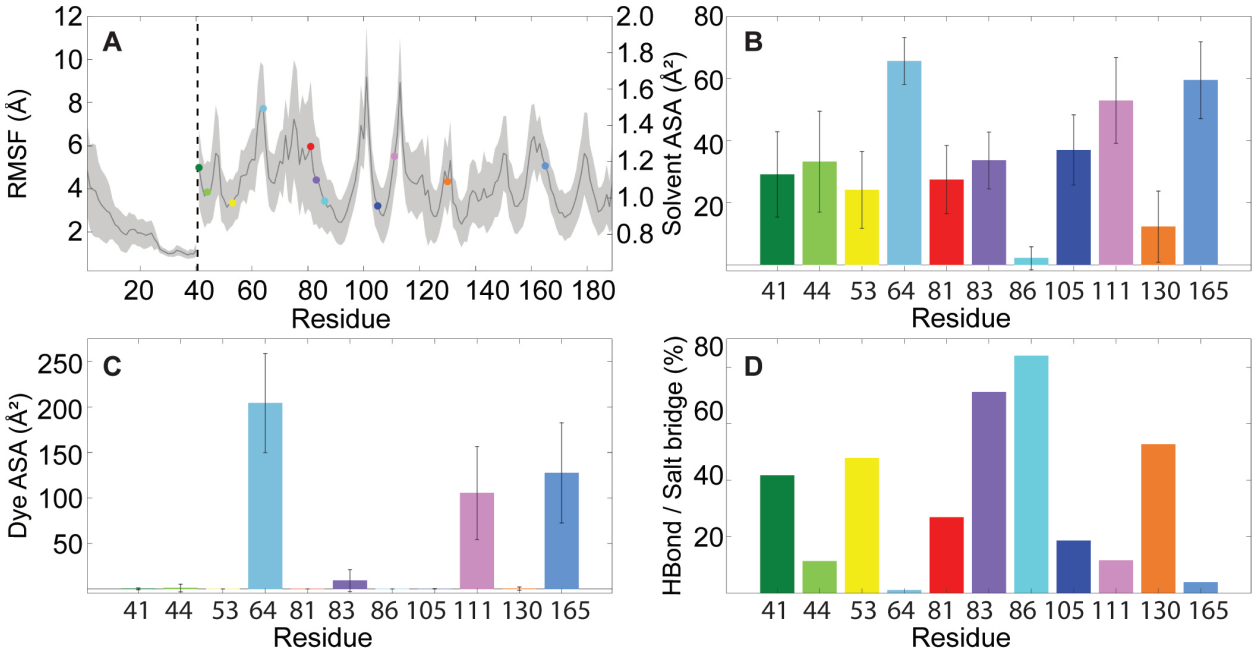

Figure S13: MD simulation results for C chains. (a) Average Cα RMSF over sixty copies of chain C. Axis shown on the left for highly flexible residues 1 to 39, and on the right for remaining residues. Lysines are highlighted according to the color scheme used in Fig. 2. (b) Average solvent accessible surface area for each lysine, calculated with a probe radius of 1.4 Å. (c) Average dye accessible surface area for each lysine, calculated with a probe radius of 5.0 Å. (d) Percentage of trajectory frames in which each lysine was involved in hydrogen bonds and/or salt bridges. Averages were calculated over 4.5 microseconds of cumulative simulation sampling. Error bars represent standard deviation.

Table S11: Lysine salt bridge interactions observed during MD simulations.

Lysine	Salt bridge partner	Type of interaction*
K41	D139	Inter
	E33	Inter
K44	D139	Inter
	D181	Inter
	D182	Inter
K53	E172	Intra
	E55	Intra
	E76	Inter
K64	E160	Intra
K81	D139	Inter
	E110	Inter
	E76	Intra
	E80	Intra
K83	D148	Inter
	E80	Intra
	E84	Intra
K86	E174	Intra
	E84	Inter/Intra
K105	D127	Intra
K111	E80	Inter
K130	D127	Intra
	E131	Inter
K165	D59	Intra
	E116	Inter

*Inter = The residues involved in the salt bridge are in **different** subunits A, B or C.
*Intra = The residues involved in the salt bridge are in **the same** subunit A, B or C.

References

(1) Tsvetkova, I. B.; Anil Sushma, A.; Wang, J. C.-Y.; Schaich, W. L.; Dragnea, B. Radiation Brightening from Virus-like Particles. *ACS nano* **2019**, *13*, 11401–11408.

(2) Lucas, R. W.; Larson, S. B.; McPherson, A. The crystallographic structure of brome mosaic virus. *Journal of molecular biology* **2002**, *317*, 95–108.