Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Determination and Characterisation of the Surface Charge Properties of the Bacteriophage M13 to Assist Bio-nanoengineering

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Table S1. Comparison between of M13, *fd* and Pf1

	M13	Ref.	*fd*	Ref.	Pf1	Ref.
Ff filamentous phage class	I		I		II	
Genome ID	NC_003287.2		J02451.1		NC_001331.1	
Genome type	Circular ssDNA		Circular ssDNA		Circular ssDNA	
Genome length (bp)	6407		6408		7349	
Percent Identity Matrix – Clustal 2.1	100%		96.99%		50.11%	
Virion length (nm)	850-900	[1,2]	760-870	[2]	~ 2000	[3]
Virion diameter (nm)	~ 6.6	[1]	~ 5.6	[2]	~ 7	[3]
Number of major coat proteins per phage	2700	[4]	2700	[4]	7600	[4]
Coat proteins	PIII, PVI, PVII, PVIII and PIX	[2]	III, VI, VII, VIII and IX	[5]	p3, p6, p7, p8 and p9	[6]
Molecular weight (Da)	16.8×10^6	[7]	16.4×10^6	[8]	37.5×10^6	[4]

Further information on the differences between M13, *fd* and Pf1 can be found in the literature.[3,4,9–12]

Table S2. Molecular weight of M13 with calculated values

	Amino acids (aa)	Base pairs (bp)	Number of copies	MW (Da)	MW_{tot} (Da)
PIII	406	n/a	5	42 579.09	212 895.45
PVI	112		5	12 350.09	61 750.45
PVII	33		5	3 602.24	18 011.20
PIX	32		5	3 653.29	18 266.45
PVIII	50		2700	5 238.04	14 142 708.00
ssDNA	n/a	6407	1	1 976 583.36	n/a
Mass of protein capsid: 14 453 631.55 Da					
Mass of protein capsid and nucleic acid: 16 430 214.91 Da					

The molecular weight (MW) of proteins shown in **Table S2** were calculated using ExPASy Compute pI/Mw tool, available at the following link https://web.expasy.org/compute_pi/.[13–15] The MW of the ssDNA (M13 genome sequence available here https://www.ebi.ac.uk/ena/data/view/V00604&display=fasta) was calculated using DNA calculator (nucleic acid parameters: DNA; single strand; circular), available at the following link http://www.molbiotools.com/dnacalculator.html.

Table S3. Molecular weight of M13 with measured values

	Amino acids (aa)	Base pairs (bp)	Number of copies	MW (Da)	MW_{tot} (Da)	References
PIII	406	n/a	5	42 675	213 375	[16]
PVI	112		5	12 264	61 320	
PVII	33		5	3 587	17 935	
PIX	32		5	3 654	18 270	
PVIII	50		2700	5 234	14 131 800	
ssDNA	n/a	6407	1	2×10^6	n/a	[2,17]
Mass of protein capsid: 14 442 700 Da						
Mass of protein capsid and nucleic acid: 16 442 700 Da						

The total mass calculated is in agreement with the value proposed by Beck *et al.* ($16.8 \times 10^6 \pm 0.8$ Da)[7] and with the values obtained *via* combining the data in **Table S3**.

Table S4. M13 surface areas

PDB ID: 2MJZ		T	U	V	W	X	Y	Z	a	b	c	d	e	f	g	h	Average (Å²)	Subunits sum (Å²)	M13 areas (Å²)	M13 areas (nm²)	M13 areas (μm²)
Molecular surface	**Free**	5381	5379	5380	5378	5381	5380	5379	5574	5379	5380	5380	5380	5382	5377	5379	5393 ± 48				
	Assembled	5287	5285	5287	5285	5287	5284	5282	5283	5282	5283	5286	5286	5288	5283	5286	5285 ± 2	79,273	14,269,205	142692	0.143
	Core																	81,193	14,614,726	146,147	0.146
	Asbl_ext	3051	3051	3051	3050	3052	3051	3052	3050	3051	3051	3054	3054	3054	3053	3054	3052 ± 1	45,777	8,239,940	82,399	0.082
	Core_ext																	46,029	8,285,259	82,853	0.083
	Asbl_int	2232	2231	2232	2231	2231	2229	2227	2229	2227	2228	2229	2228	2230	2226	2228	2229 ± 2	33,438	6,018,801	60,188	0.060
	Core_int																	33,053	5,949,506	59,495	0.059
Solvent accessible surface	**Free**	4636	4639	4636	4636	4634	4635	4634	4807	4807	4634	4636	4634	4634	4636	4636	4658 ± 58				
	Assembled	1820	1820	1817	1817	1818	1772	1774	1775	1774	1771	1811	1809	1810	1811	1810	1801 ± 20	27,009	4,861,577	48,616	0.049
	Core																	27,021	4,863,846	48,638	0.049
	Asbl_ext	1337	1339	1335	1336	1337	1336	1337	1338	1337	1334	1374	1372	1373	1374	1373	1349 ± 17	20,233	3,641,851	36,419	0.036
	Core_ext																	20,241	3,643,327	36,433	0.036
	Asbl_int	483	482	482	482	481	436	437	438	437	436	437	437	437	437	437	452 ± 21	6779	1,220,141	12,201	0.012
	Core_int																	6776	1,219,697	12,197	0.012

Free	The area of each PVIII protein taken individually, not assembled to form the capsid
Assembled	The area of each PVIII protein assembled to form the capsid, excluding their unexposed portions due to the interactions with the surrounding PVIIIs
Core	The area of the capsid portion composed of the 15 chains named T, U, V, W, X, Y, Z, a, b, c, d, e, f, g and h from the PDB file 2MJZ, excluding their unexposed portions
Asbl_ext	The area of each assembled PVIII protein excluding the area of the residues in green (inner portion). This corresponds to the contribution of a single PVIII to the M13 external total surface. AEGDDPAKAAFNSLQASATEYIGY AWAMVVVIVGATIGIKLFKKFTSKAS
Core_ext	The area of the core excluding the area of the residues in green (inner portion). This corresponds to the contribution of 15 PVIIIs to the M13 external total surface area. AEGDDPAKAAFNSLQASATEYIGY AWAMVVVIVGATIGIKLFKKFTSKAS
Asbl_int	The area of each assembled PVIII protein excluding the area of the residues in blue (external portion). This corresponds to the contribution of a single PVIII to the M13 internal total surface. AEGDDPAKAAFNSLQASATEYIGY AWAMVVVIVGATIGIKLFKKFTSKAS
Core_int	The area of the core excluding the area of the residues in blue (external portion). This corresponds to the contribution of 15 PVIIIs to the M13 internal total surface area. AEGDDPAKAAFNSLQASATEYIGY AWAMVVVIVGATIGIKLFKKFTSKAS

The surface area in **Table S4** were calculated with pyMOL selecting individual PVIII chains (free and assembled) and the central 15 chains all together (core). The values were calculated using the molecular and solvent accessible surface parameters, respectively. Note, that the values of subunits sum of Assembled, Asbl_ext and Asbl_int correspond to the sum of the chains between T to h, while Core, Core_ext and Core_int correspond to the values of the 15 chains taken all together. The M13 areas were calculated as: (Subunits sum/15)*2700.

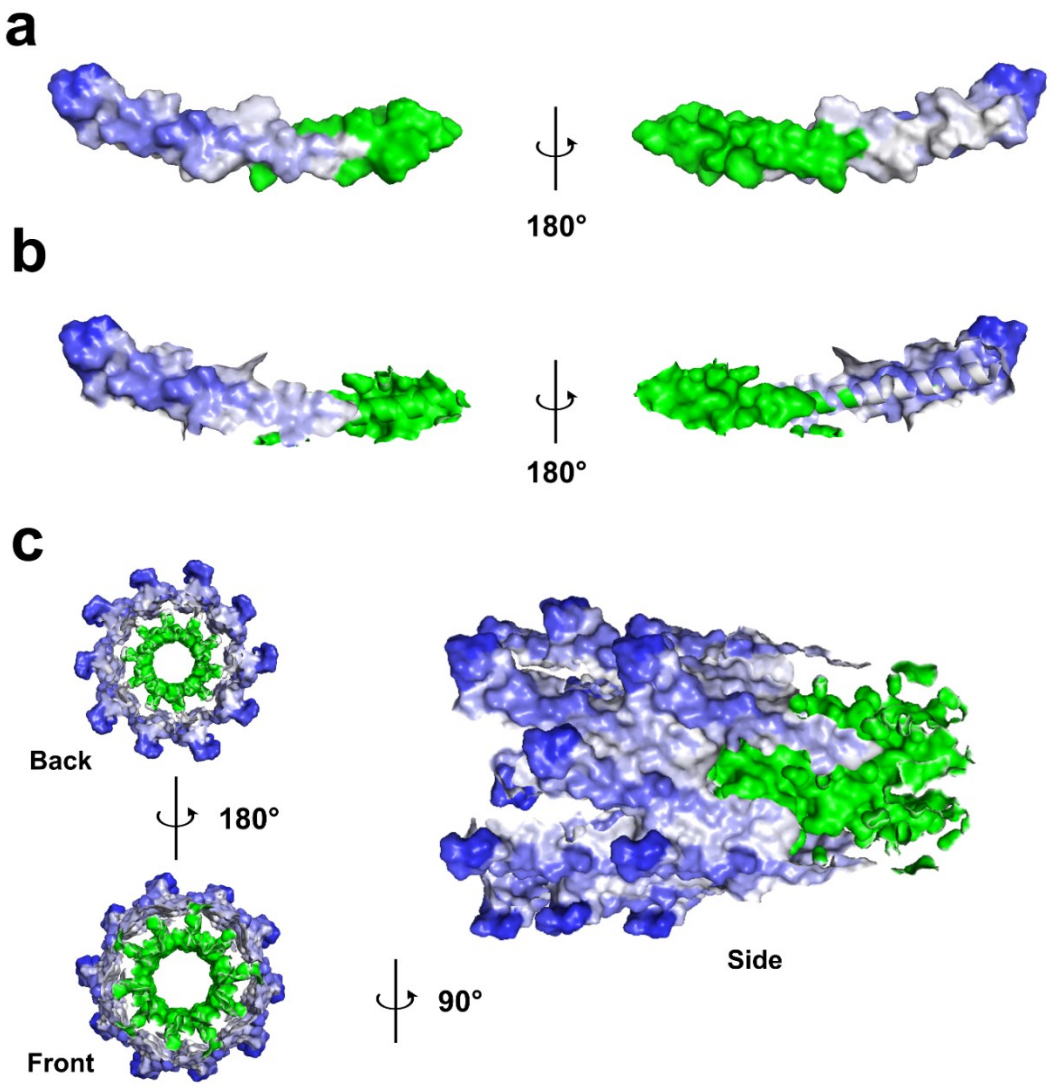
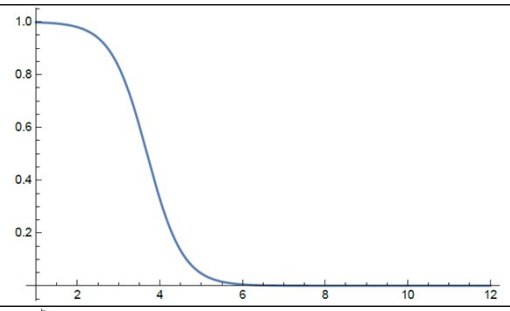
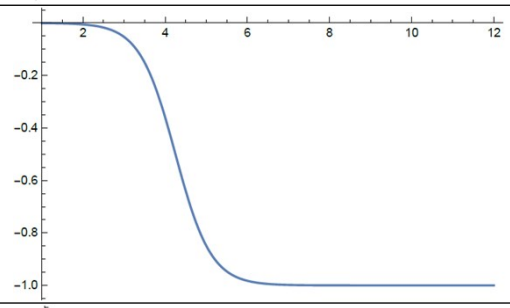
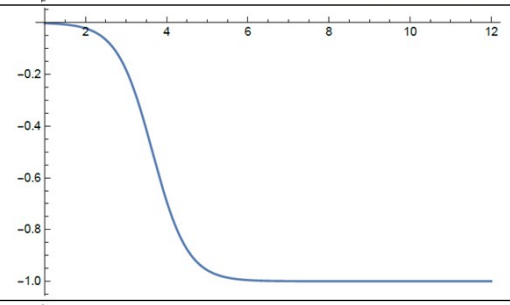
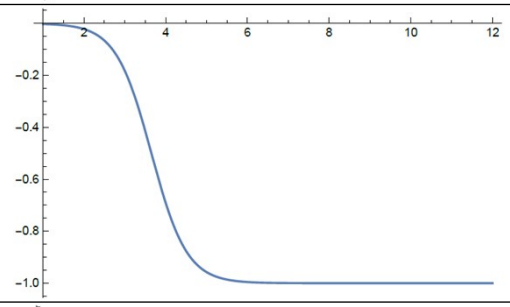
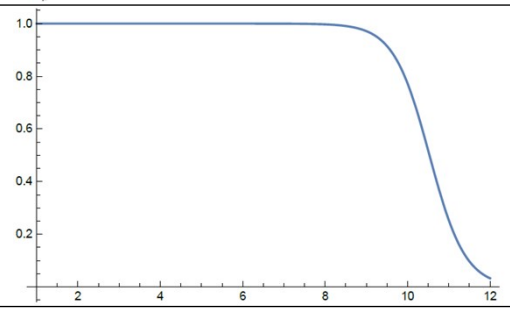


Figure S1. M13 surface areas

The surface of PVIII according to Table S4: **(a)** free, **(b)** assembled and **(c)** core.

Table S5. pKa_1 models

Title	Equation	Y axes: charge – X axes: pH
A1	(1 / (10^(ph - 3.69) + 1)) * (1)	
A2	(1 - 1 / (10^(ph - 4.25) + 1)) * (-1)	
A3	(1 - 1 / (10^(ph - 3.65) + 1)) * (-1)	
A4	(1 - 1 / (10^(ph - 3.65) + 1)) * (-1)	
A5	(1 / (10^(ph - 10.53) + 1)) * (1)	

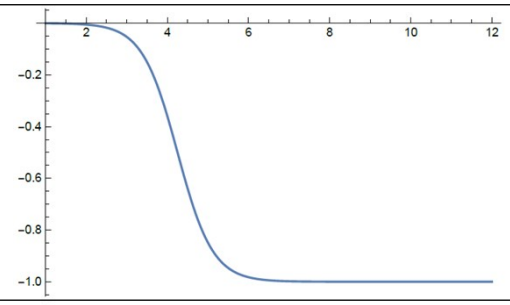
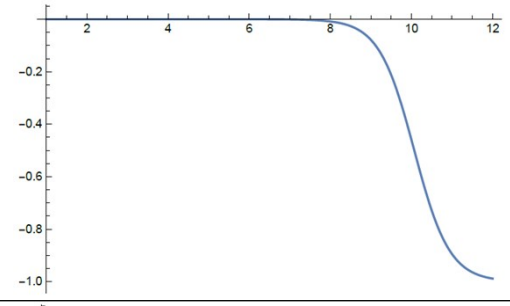
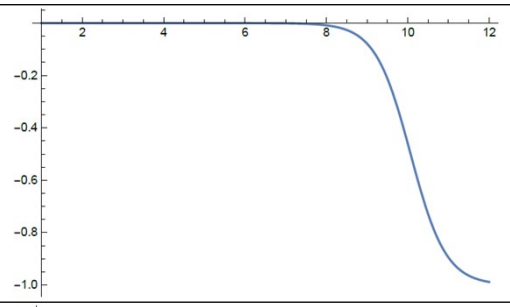
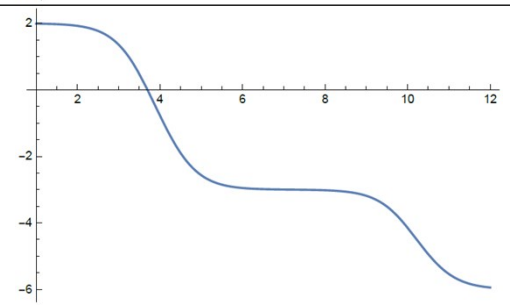
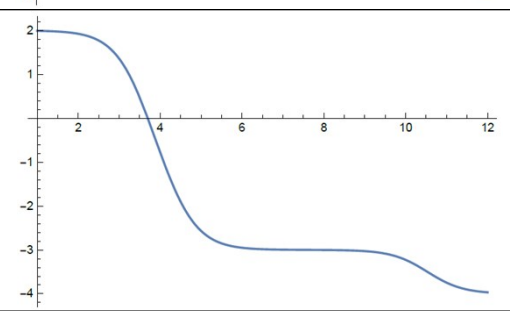
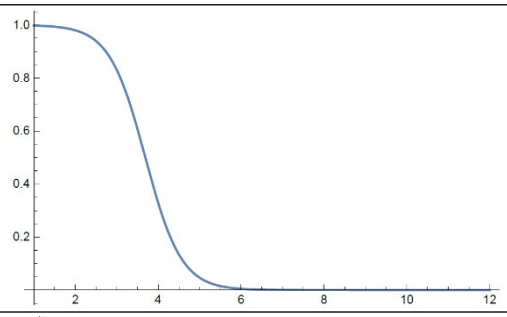
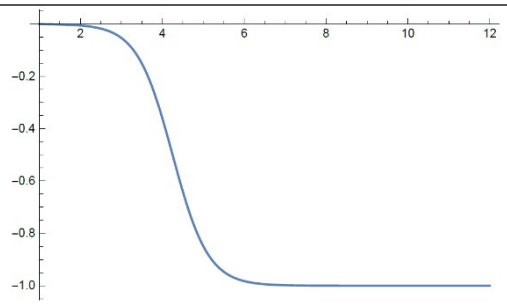
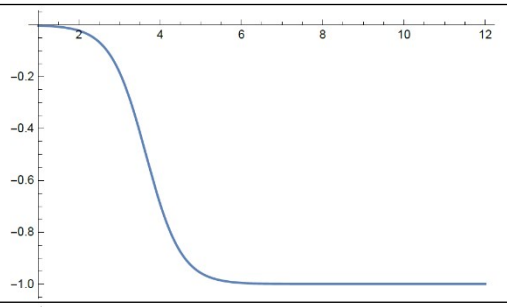
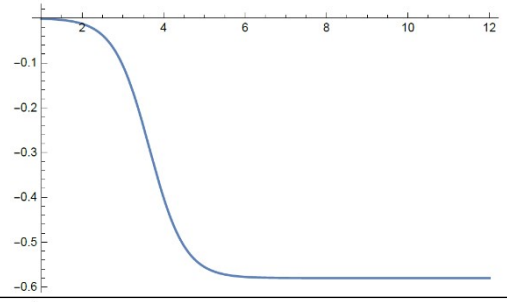
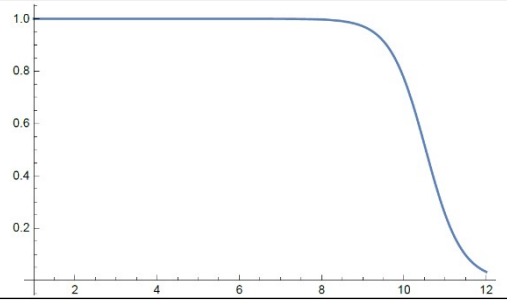
A6	(1 - 1 / (10^(ph - 4.25) + 1)) * (-1)	
A7	(1 - 1 / (10^(ph - 10.07) + 1)) * (-1)	
A8	(1 - 1 / (10^(ph - 10.07) + 1)) * (-1)	
	A_{tot} = A1 + A2 + A3 + A4 + A5 + A6 + A7 + A8	
	A_{tot} = A1 + A2 + A3 + A4 + A5 + A6	

Table S6. pKa_1 + exposure models

Title	Equation	Y axes: charge – X axes: pH
B1	(1 / (10^(ph - 3.69) + 1)) * (1)	
B2	(1 - 1 / (10^(ph - 4.25) + 1)) * (-1)	
B3	(1 - 1 / (10^(ph - 3.65) + 1)) * (-1)	
B4	(1 - 1 / (10^(ph - 3.65) + 1)) * (-0.58)	
B5	(1 / (10^(ph - 10.53) + 1)) * (1)	

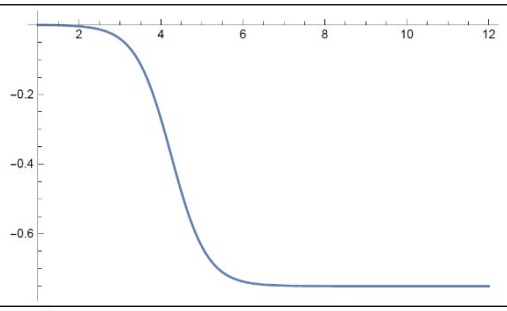
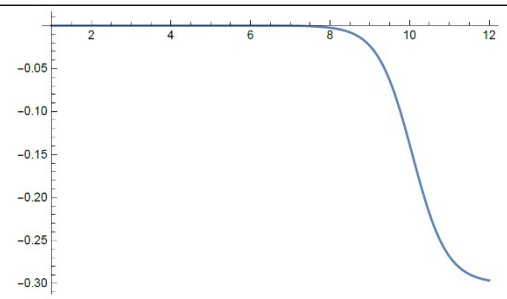
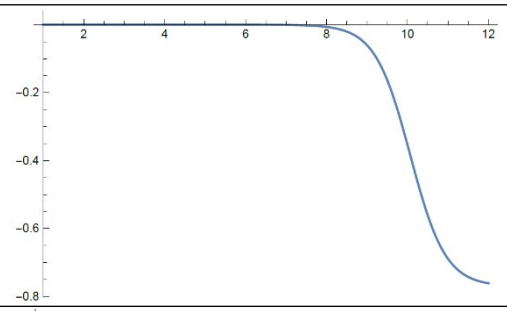
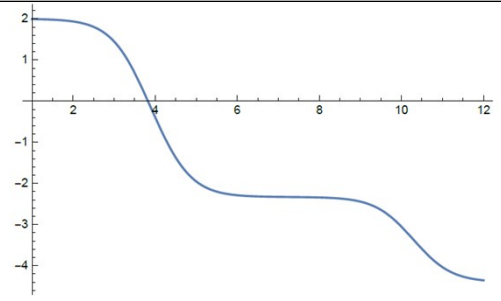
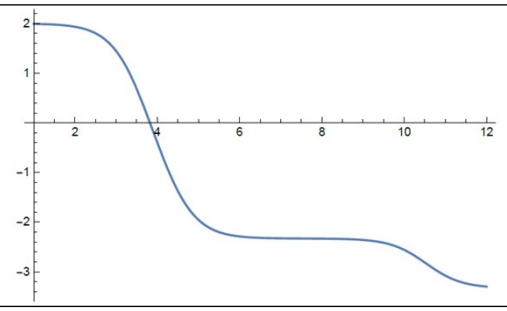
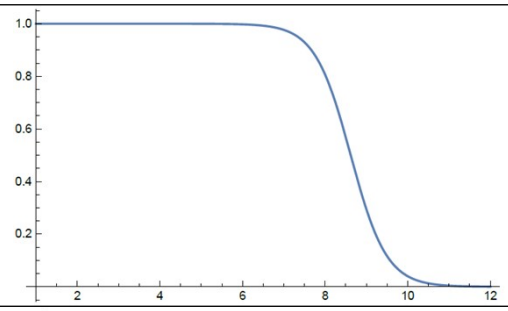
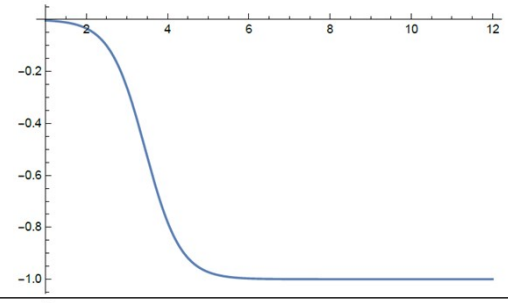
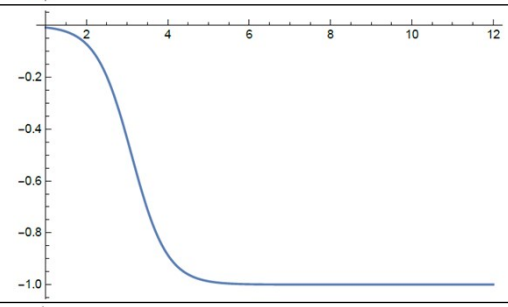
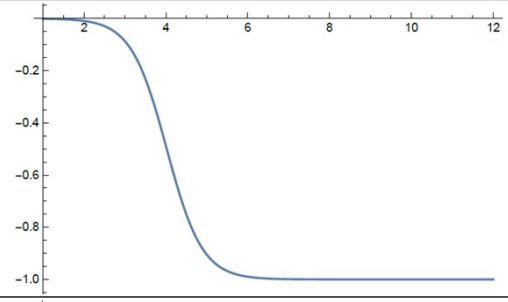
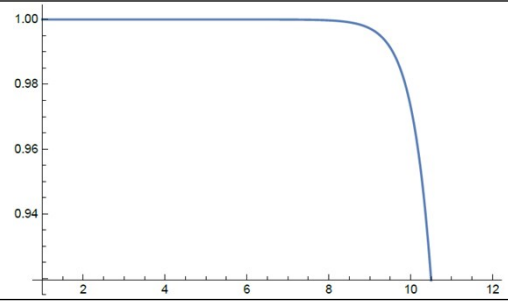
B6	(1 - 1 / (10^(ph - 4.25) + 1)) * (-0.75)	
B7	(1 - 1 / (10^(ph - 10.07) + 1)) * (-0.30)	
B8	(1 - 1 / (10^(ph - 10.07) + 1)) * (-0.77)	
	B_{tot} = B1 + B2 + B3 + B4 + B5 + B6 + B7 + B8	
	B_{tot} = B1 + B2 + B3 + B4 + B5 + B6	

Table S7. pKa_2 models

Title	Equation	Y axes: charge – X axes: pH
C1	(1 / (10^(ph - 8.62) + 1)) * (1)	
C2	(1 - 1 / (10^(ph - 3.45) + 1)) * (-1)	
C3	(1 - 1 / (10^(ph - 3.11) + 1)) * (-1)	
C4	(1 - 1 / (10^(ph - 4.02) + 1)) * (-1)	
C5	(1 / (10^(ph - 11.56) + 1)) * (1)	

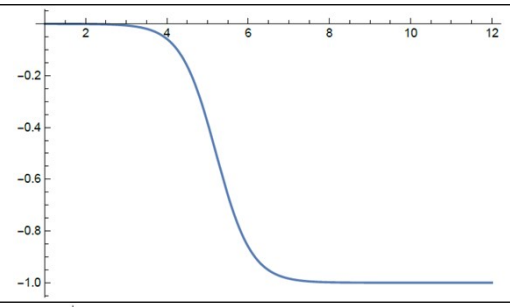
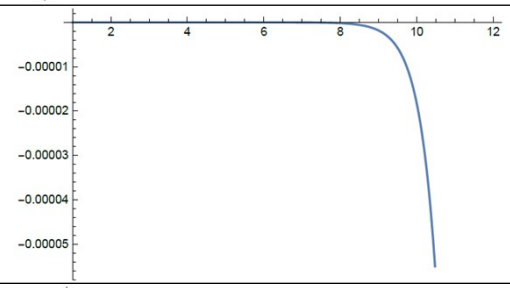
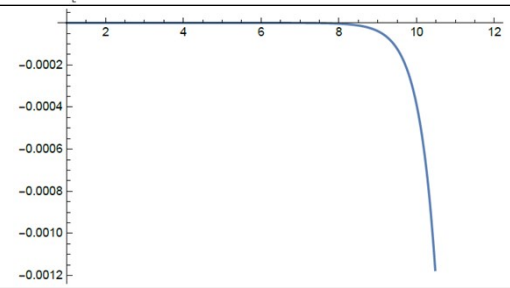
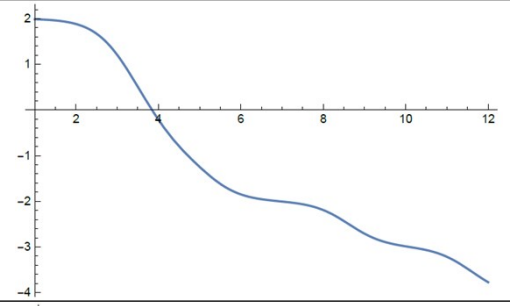
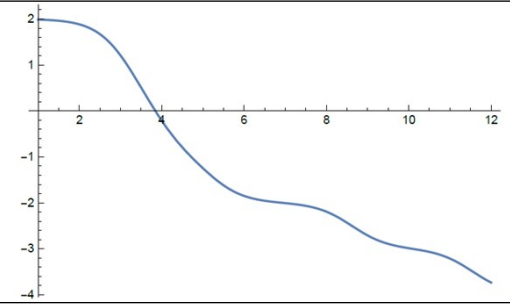
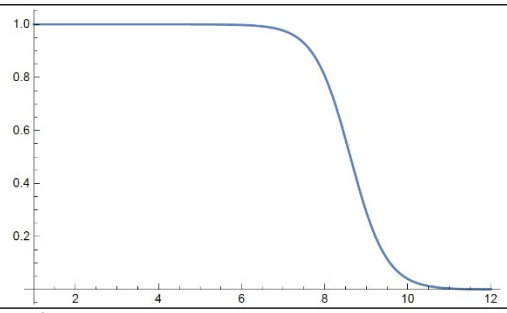
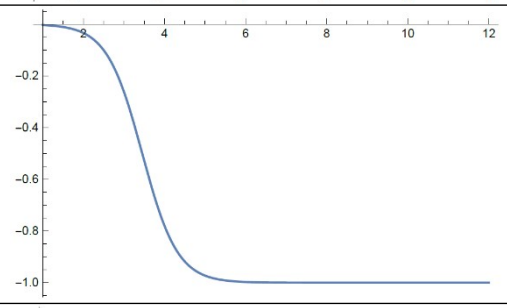
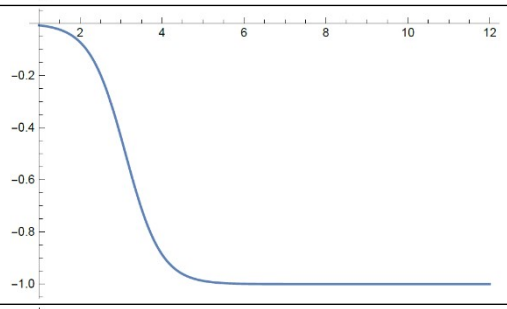
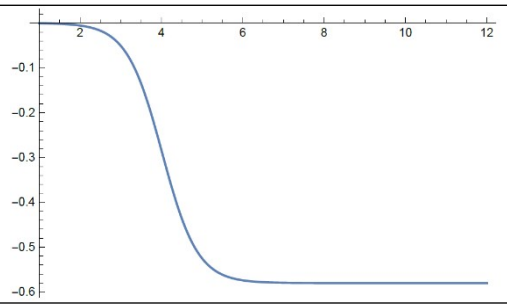
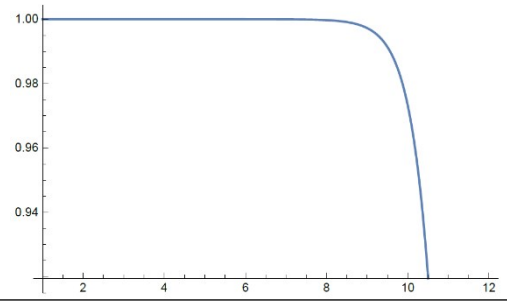
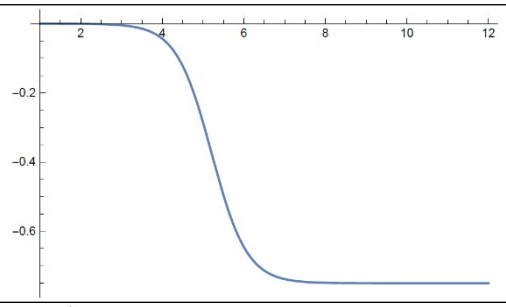
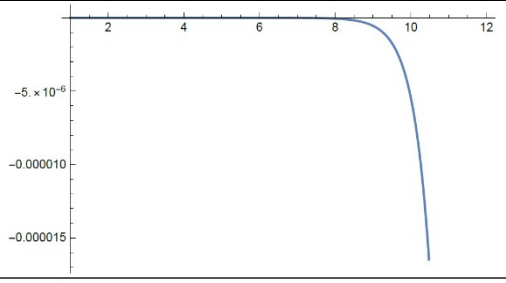
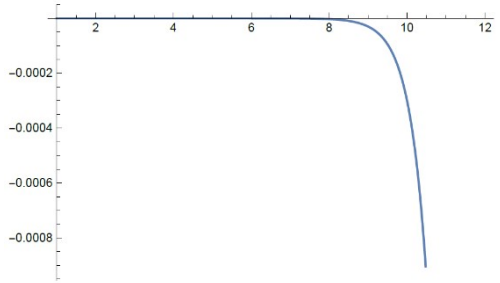
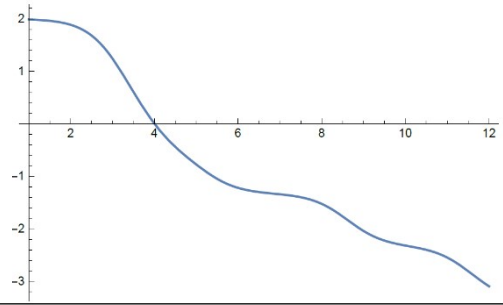
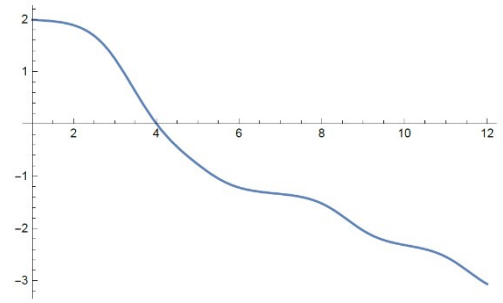
C6	(1 - 1 / (10^(ph - 5.21) + 1)) * (-1)	
C7	(1 - 1 / (10^(ph - 14.74) + 1)) * (-1)	
C8	(1 - 1 / (10^(ph - 13.14) + 1)) * (-1)	
	C_{tot} = C1 + C2 + C3 + C4 + C5 + C6 + C7 + C8	
	C_{tot} = C1 + C2 + C3 + C4 + C5 + C6	

Table S8. pKa_2 + exposure models

Title	Equation	Y axes: charge – X axes: pH
D1	(1 / (10^(ph - 8.62) + 1)) * (1)	
D2	(1 - 1 / (10^(ph - 3.45) + 1)) * (-1)	
D3	(1 - 1 / (10^(ph - 3.11) + 1)) * (-1)	
D4	(1 - 1 / (10^(ph - 4.02) + 1)) * (-0.58)	
D5	(1 / (10^(ph - 11.56) + 1)) * (1)	

D6	(1 - 1 / (10^(ph - 5.21) + 1)) * (-0.75)	
D7	(1 - 1 / (10^(ph - 14.74) + 1)) * (-0.30)	
D8	(1 - 1 / (10^(ph - 13.14) + 1)) * (-0.77)	
	D_{tot} = D1 + D2 + D3 + D4 + D5 + D6 + D7 + D8	
	D_{tot} = D1 + D2 + D3 + D4 + D5 + D6	

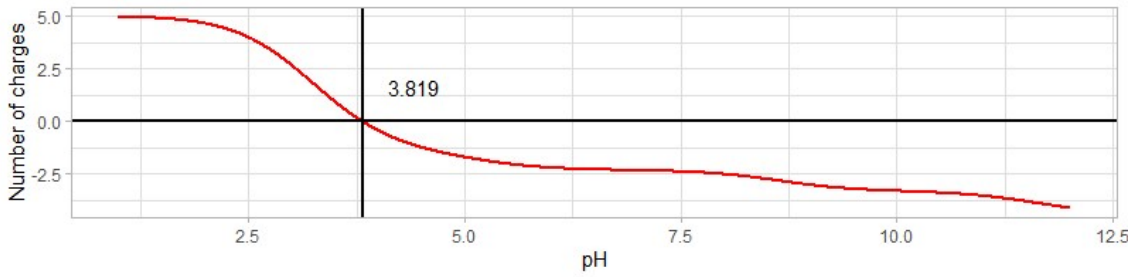
Following the code to calculate the charge of the PVIII protein based on the selected amino acids (A1, E2, D4, D5, K8, E20, Y21, Y24). The following code can be used in R to calculate the IEP of modified versions of the PVIII changing some some parameter accordingly **(Code S1)**. More information on how to use it are included in the code as well as two examples are listed below **(Code S2 and S3)**.

Code S1. IEP calculator for PVIII protein and other modified versions

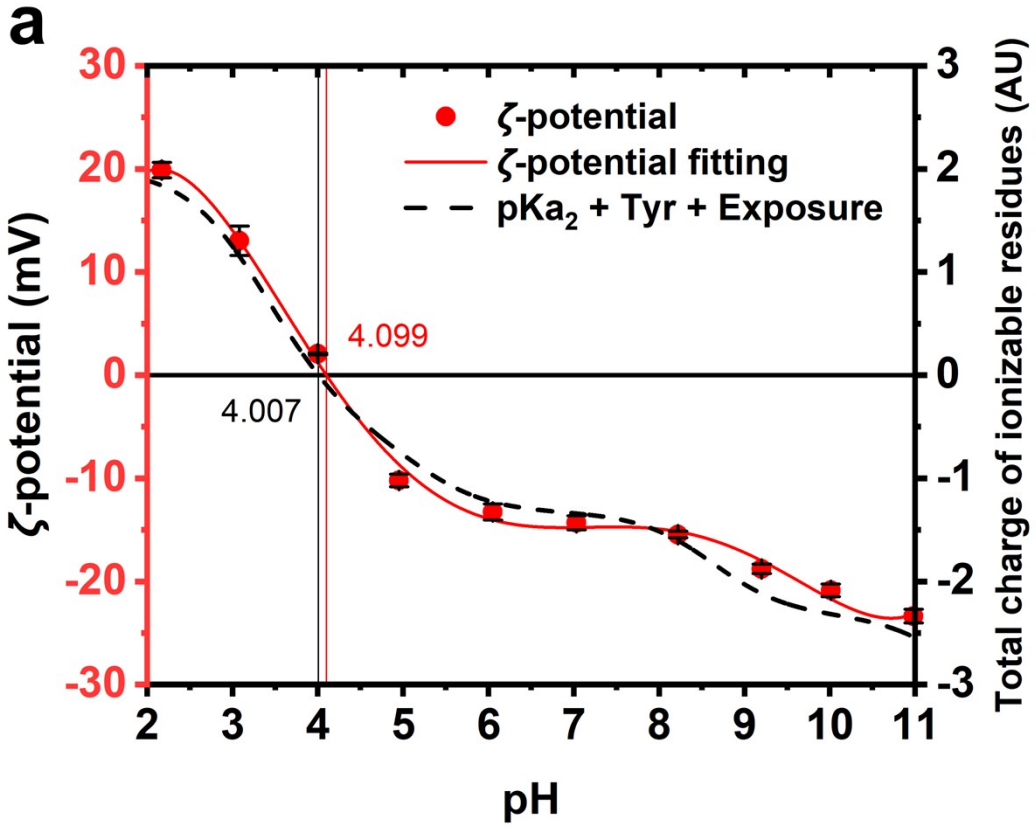
```
# Activate the necessary libraries
library(ggplot2)

# Building the X axes which is the pH scale
pH <- seq(1, 12, by=0.001)

# The amino acid listed below are the residues contributing to the total charge
of the M13 wild type
# Adjust the value row:9 column:12 accordingly with the number of residues of
your modified M13
num <- c(1:8)

# Add in brackets the amino acid name and position of your modified M13
name <- c("A1", "E2", "D4", "D5", "K8", "E20", "Y21", "Y24")

# Add the pKa of the corresponding amino acid
pka <- c(8.64, 3.45, 3.11, 4.02, 11.56, 5.21, 14.74, 13.14)

# Add 1 or 0 if the residue is positively or negatively charged
char <- c(1, 0, 0, 0, 1, 0, 0, 0)

# Add the level of exposure to the solvent of the corresponding residue
exp <- c(1, 1, 1, 0.58, 1, 0.75, 0.3, 0.77)

# Create a data frame with all data
PVIII <- data.frame(num, name, pka, char, exp)
PVIII

for(i in num){
if(PVIII$char[i] == 1){
  assign(sprintf("amino.acid_%d", i), ((1/(10^(pH - PVIII$pka[i]) + 1)) *
(PVIII$exp[i])), envir = .GlobalEnv)
  }
  else {
  assign(sprintf("amino.acid_%d", i), ((1-1/(10^(pH - PVIII$pka[i]) + 1)) * (-
PVIII$exp[i])), envir = .GlobalEnv)
  }
 rm(i)
}

# Once obtained the vectors named "amino.acis_n", if more than 8, add them to the
vector tot (Eg: amino.acid_9 + amino.acid_10)
tot <- amino.acid_1 + amino.acid_2 + amino.acid_3 + amino.acid_4 +
       amino.acid_5 + amino.acid_6 + amino.acid_7 + amino.acid_8

# IEP is the isoelectric point generated from the modified PVIII
IEP <- pH[which.min(abs(tot-0))]
# IEP2 is the closest value to zero of the generated charge values
IEP2 <- tot[which.min(abs(tot-0))]

result <- data.frame(pH, tot, IEP2)
result

plotIEP <- ggplot(result, aes(x=pH, y=tot, lable=pH)) +
           geom_point(size=0.5, shape=1, color="red") +
           geom_text(aes(label=ifelse(tot==IEP2,as.character(pH),'')),hjust=-
0.5,vjust=-1.5) +
           xlab("pH") +
           ylab("Number of charges") +
           theme_light()
plotIEP <- plotIEP + geom_hline(yintercept=0, linetype="solid", color = "black",
size=0.8)
plotIEP <- plotIEP + geom_vline(xintercept=IEP, linetype="solid", color =
"black", size=0.8)
plotIEP
```

Code S2. IEP calculator used to calculate a modified version of the PVIII

```
# Example: PVIII protein with 3 aspartic and 1 glutamic acids at the N-terminus
in position 2, 3, 4 and 5
pH <- seq(1, 12, by=0.001)
num <- c(1:12)
name <- c("A1", "D2x", "D3x", "D4x", "E5x" "E2", "D4", "D5", "K8", "E20", "Y21",
"Y24")
pka <- c(8.64, 3.11, 3.11, 3.11, 3.45, 3.45, 3.11, 4.02, 11.56, 5.21, 14.74,
13.14)
char <- c(1, 1, 1, 1, 0, 0, 0, 0, 1, 0, 0, 0)
exp <- c(1, 1, 1, 1, 1, 1, 1, 0.58, 1, 0.75, 0.3, 0.77)
PVIII <- data.frame(num, name, pka, char, exp)
PVIII
for(i in num){
  if(PVIII$char[i] == 1){
    assign(sprintf("amino.acid_%d", i), ((1/(10^(pH - PVIII$pka[i]) + 1)) *
(PVIII$exp[i])), envir = .GlobalEnv)
  }
  else {
    assign(sprintf("amino.acid_%d", i), ((1-1/(10^(pH - PVIII$pka[i]) + 1)) * (-
PVIII$exp[i])), envir = .GlobalEnv)
  }
  rm(i)
}
tot <- amino.acid_1 + amino.acid_2 + amino.acid_3 + amino.acid_4 + amino.acid_5 +
amino.acid_6 + amino.acid_7 + amino.acid_8 +
  amino.acid_9 + amino.acid_10 + amino.acid_11 + amino.acid_12
IEP <- pH[which.min(abs(tot-0))]
IEP2 <- tot[which.min(abs(tot-0))]
result <- data.frame(pH, tot, IEP2)
result
plotIEP <- ggplot(result, aes(x=pH, y=tot, lable=pH)) +
  geom_point(size=0.5, shape=1, color="red") +
  geom_text(aes(label=ifelse(tot==IEP2,as.character(pH),'')),hjust=-0.5,vjust=-
1.5) +
  xlab("pH") +
  ylab("Number of charges") +
  theme_light()
plotIEP <- plotIEP + geom_hline(yintercept=0, linetype="solid", color = "black",
size=0.8)
plotIEP <- plotIEP + geom_vline(xintercept=IEP, linetype="solid", color =
"black", size=0.8)
plotIEP
```



a



b

Equation	y = Intercept + B1*x^1 + B2*x^2 + B3*x^3 + B4*x^4 + B5*x^5	
Plot	z-potential	error
Weight	No Weighting	
Intercept	-75.82086 ± 30.16783	-4.10503 ± 12.07138
B1	116.81266 ± 30.39556	5.47588 ± 12.16251
B2	-49.08253 ± 11.21275	-2.15651 ± 4.48668
B3	8.55142 ± 1.91684	0.3817 ± 0.76701
B4	-0.67362 ± 0.15362	-0.03129 ± 0.06147
B5	0.01979 ± 0.00466	9.66421E-4 ± 0.00187
Residual Sum of Squares	5.38041	0.86147
R-Square (COD)	0.99726	0.2131
Adj. R-Square	0.99384	-0.77053

Figure S2. Determination of the IEP of M13

(a) The *ζ*-potential values were fitted with a polynomial function of order 5 and compared to the model pKa_2 which includes tyrosins and exposure level. The final IEP value was calculated *via* averaging the IEPs fom both curves. **(b)** The parameters of the fitting function.

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