Supplementary Information

Melanin Production by Tyrosinase Activity on a Tyrosine-Rich Peptide Fragment and pH-dependent Self-Assembly of its Lipidated Analogue

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Tables

Table S1: SAXS form factor parameters of C_{16}-EELNRYY at pH 10 fit to a spherical shell i form factor model using SASfit.[1] Key: BG = background, N = scaling factor, σ = polydispersity in R_1 (Gaussian width), R_1= outer radius, R_2 = inner radius, μ = relative electron density contrast of the shell, η = relative electron density contrast of inner core.

Sample / Parameter	1 wt % C_{16}EELNRYY pH 10
BG	2.75×10^{-4}
N	1.00
σ/ Å	5.22
R_1 / Å	16.77
R_2 / Å	9.83
μ	-1.53
η	1.05×10^{-5}

Table S2. SAXS form factor parameters of EELNRYY in the pH range 3-12 fit to a generalized Gaussian coil form factor model using SASfit.[1] Key: BG = background, N= scaling factor, R_g = radius of gyration, ν = Flory exponent, I_0 = forward scattering.

Sample / Parameter	EELNRYY pH 3 (native pH)	EELNRYY pH 7	EELNRYY pH 10	EELNRYY pH 12
BG	5.5×10^{-4}	-1.8×10^{-3}	1×10^{-4}	1.4×10^{3}
N	0.988	1.27	0.643	1.54
R_g / Å	7.17	6.98	6.32	7.00
ν	0.316	0.877	0.327	0.181
I_0	7.4×10^{-3}	8.6×10^{-3}	8.8×10^{-3}	4.5×10^{-3}

Table S3. SAXS form factor parameters of C_{16}-EELNRYY at pH 10 fit to a spherical shell form factor I model using SASfit.[1] Key: BG = background, N= scaling factor, σ = Gaussian width, R_1= inner radius, R_2 = outer radius, μ = scattering contrast of inner core. η = scattering contrast of shell.

Sample / Parameter	1 wt% C_{16}EELNRYY pH 10
BG	2.75×10^{-4}
N	1.00
σ/ Å	5.22
R_1 / Å	16.77
R_2 / Å	9.83
μ	-1.53
η	1.05×10^{-5}

Table S4. SAXS fitting parameters of C_{16}-EELNRYY at pH 12 fit to a bilayer Gaussian form factor using SASfit.[1] BG = background, N = scaling factor, σ = polydispersity in t (Gaussian width), t = bilayer thickness. σ_{out} = relative electron density of outer Gaussians, b_{out} = width of outer Gaussians, σ_{core} = relative electron density of inner Gaussian, b_{core} = width of inner Gaussians, D = diameter of disc (fixed parameter)

Sample / Parameter	1 wt% C_{16}EELNRYY pH 12
BG	9.34×10^{-4}
N	1.19×10^{-8}
σ / Å	6.8
t / Å	62.7
σ_{out} / Å	0.588
b_{out}	1.36×10^{-2}
σ_{core} / Å	13.63
b_{core}	-1.55×10^{-3}
D / Å	500

Table S5. SAXS form factor parameters of C_{16}-EELNRYY at pH 12 fit to a generalized Gaussian coil form factor model using SASfit.[1] Key: BG = background, N= scaling factor, R_g = radius of gyration, ν = Flory exponent, I_0 = forward scattering.

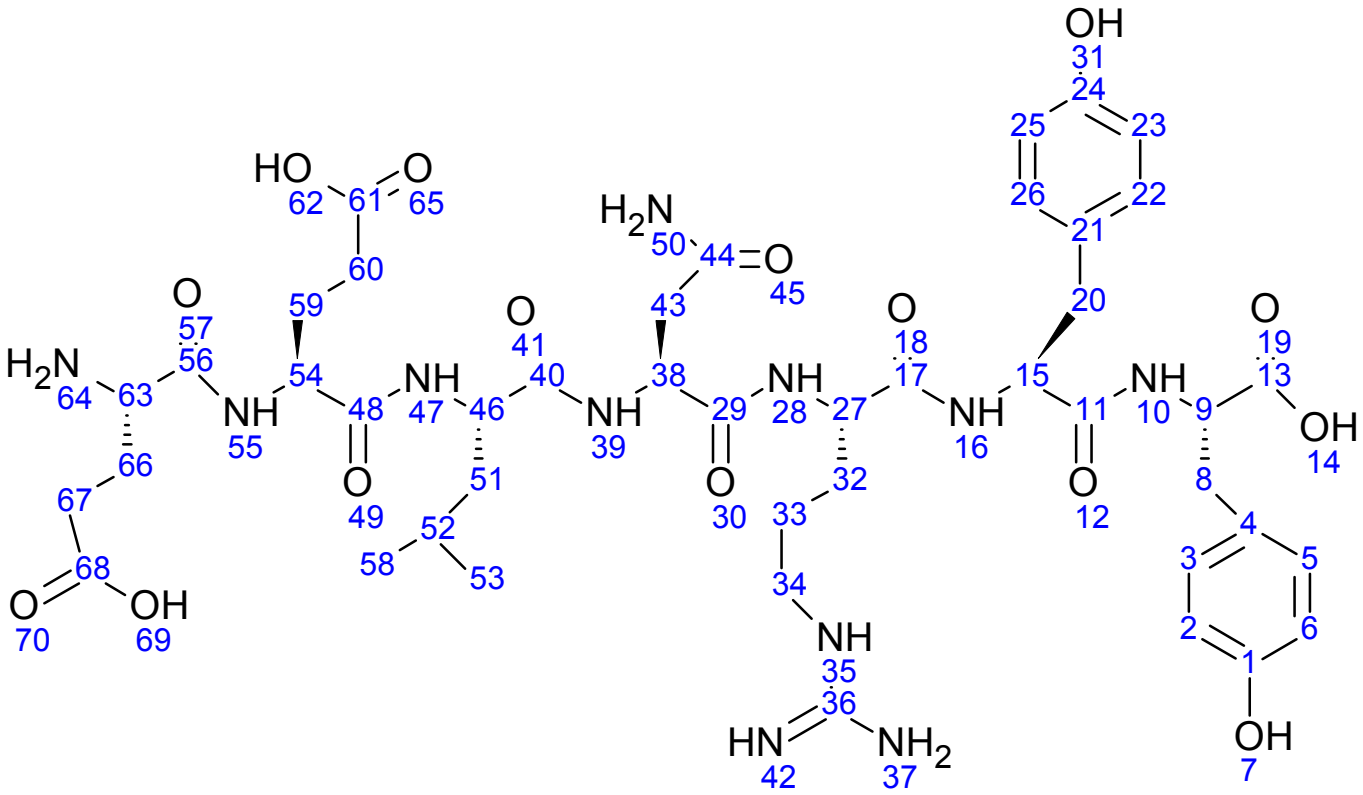
Sample / Parameter	C_{16}EELNRYY pH 12
BG	9.34×10^{-4}
N	4.83×10^{-2}
R_g / Å	19.28
ν	0.680
I_0	0.342

Table S6. SAXS form factor parameters of C_{16}-EELNRYY gel fit to a long cylindrical shell form factor model using SASfit.[1] Key: BG = background, N = scaling factor, σ = polydispertsity in R (Gaussian width), R = inner radius, DR =shell thickness, L = length of cylinder, η_{core} = relative electron density of core, η_{shell} = relative electron density of shell, η_{solv} = relative electron density of solvent.

Sample / Parameter	C_{16}EELNRYY Gel pH 7 1 wt%
BG	8.4×10^{-3}
N	2.72×10^{-9}
σ / Å	3.3
R / Å	17.4
DR / Å	14.8
L / Å	500
η_{core}	1.23×10^{-3}
η_{shell}	1.27×10^{-3}
η_{solv}	1.23×10^{-3}

Table S7. Parameters obtained from the fitting of the SAXS data in Figure S12 to a generalized Gaussian coil form factor, using SASfit.[1] Key: BG = background, R_g = radius of gyration, ν = Flory exponent, I_0 = forward scattering.

Sample / Parameter	1 wt% EELNRYY	1 wt% EELNRYY + 0.02 wt% tyrosinase
BG	2.9×10^{-4}	1.0×10^{-4}
R_g /Å	3.4	20.4
ν	0.4	0.3
I_0	3.7×10^{-3}	8.4×10^{-2}



Labelling of Carbon atoms in EELNRYY molecules, used in Tables S8-S9

Table S8. NMR resonances and their assignment to the EELNRYY molecule.

	Sample		ChemNMR estimation	
Atom	0.5 wt% EELNRYY + 0.01 wt% tyrosinase (24 hrs incubation)			
	13C	1H	13C	1H
C1	157.22 or 157.09		155.7	
C2	118.13 or 118.28	6.643 or 6.655	115.8	6.68
C3	133.56	6.912	130.2	6.96
C4	131.86		129.2	
C5	133.56	6.912	130.2	6.96
C6	118.13 or 118.28	6.643 or 6.655	115.8	6.68
O7				9.06
C8	39.43	2.734 / 2.926	36.5	2.87 / 3.12
C9	58.6	4.262	59.2	4.72
N10				8.32
C13	179.40		174.7	

O14				12.89
O19				
C11	174.73 or 174.78		171.7	
O12				
C15	57.73	4.395	58.7	4.92
N16				8.32
C20	38.97	2.668 / 2.891	37.4	3.19 / 3.44
C21	131.28		129.2	
C22	133.41	6.937	130.2	6.96
C23	118.28 or 118.13	6.643 or 6.655	115.8	6.68
C24	157.09 or 157.22		155.7	
C25	118.28 or 118.13	6.643 or 6.655	115.8	6.68
C26	133.41	6.937	130.2	6.96
O31				9.06
C17			171.0	
O18				
C27	56.02	4.060	57.1	4.44
N28				8.32
C32	31.11	1.35-1.50*	28.9	1.77
C33	26.96	1.221	24.2	1.51
C34	43.43	2.910	41.2	3.34
N35				2.5
C36	159.50		158.0	
N37				6.63
N42				7.84
C29	174.73 or 174.78 or 177.01 or 177.18		170.3	
O30				
C38	53.29	4.436	53.9	4.484
N39				8.32
C43	38.87 or 38.97	2.531	37.1	2.56 / 2.81
C44	177.01 or 177.18 or 174.73 or 174.78		172.3	
O45				
N50				7.03
C40	175.48		171.0	
O41				
C46	55.35	4.173 or 4.180	56.0	4.44
N47				8.32
C51	42.63	1.35-1.50*	41.2	1.76
C52	27.21	1.433	24.4	1.49
C53	23.65	0.701	22.5	0.9
C58	24.91	0.756	22.5	0.9
C48			171.0	
O49				
C54	56.02	4.266	56.8	4.44

N55				8.32
C59	29.48 or 29.53	1.808 / 1.926	26.9	2.06
C60	33.92	2.244 /2.244	30.0	2.33
C61	181.25		178.4	
O62				12.01
O65				
C56		172.19	171.0	
O57				
C63	55.25	3.926	52.2	3.21
N64				8.76
C66	29.40 or 29.46	1.970/1.970	26.0	2.06
C67	33.49	2.282 / 2.282	30.0	2.33
C68	180.74		178.4	
O69				12.01
O70				

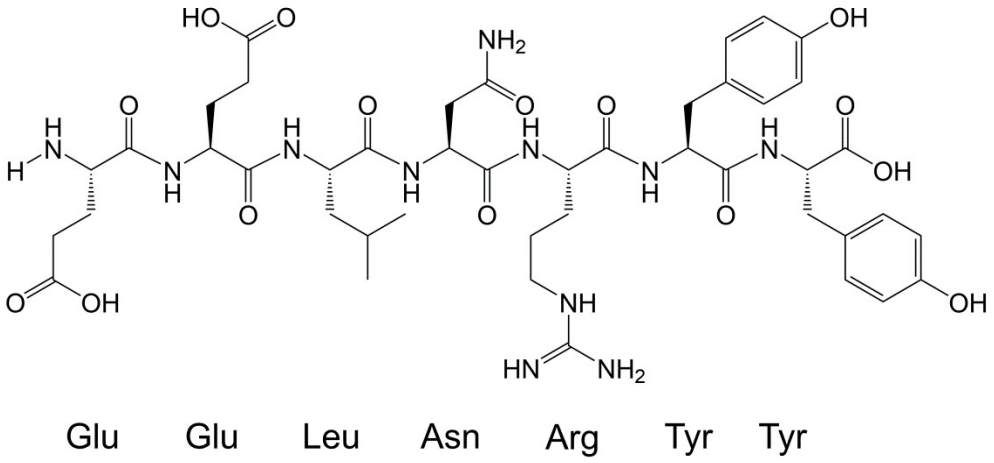
Table S9. Estimation (ChemDraw) of NMR resonances for unchanged and modified molecule.

Atom	Unchanged		-OH		=O		Experiment		
	13C	1H	13C	1H	13C	1H	13C	1H	
C1	**155.7**		**144.5**		**180.3**		146.8		-OH
C2	**115.8**	**6.68**	**145.6**		**182.0**		146.8		-OH
C3	**130.2**	**6.96**	**116.4**	**6.57**	**112.9**	**6.05**	119.7 or 119.9(HSQC)	6.60	-OH
C4	129.2		130.2		157.7		133.5 or 133.7 157.8		-OH =O
C5	130.2	6.96	122.8	6.52	152.5	7.23	124.6	6.45 or 6.49	-OH
C6	**115.8**	**6.68**	115.9	6.61	**126.6**	**5.89**	118.87 or 118.92 129.7	6.66 -	-OH =O
O7		9.06		9.48					
C8	**36.5**	**2.87 / 3.12**	36.8	2.87 / 3.12	**38.1**	**2.33 / 2.58**			
C9	**59.2**	**4.72**	59.2	4.72	**56.9**	**4.47**			
N10		8.32		8.32		8.32			
C13	**174.7**		174.7		**172.3**				
O14		12.89		12.89		11.07			
O19									
C11	**171.7**		171.7		**170.3**				
O12									
C15	**58.7**	**4.92**	58.7	4.92	**56.5**	**4.57**			
N16		8.32		8.32		8.32			
C20	**37.4**	**3.19 / 3.44**	37.7	3.19 / 3.44	**39.0**	**2.34 / 2.59**			
C21	129.2		130.2		157.7		133.5 or 133.7 157.8		-OH =O
C22	**130.2**	**6.96**	**116.4**	**6.57**	**112.9**	**6.05**	119.71 or 119.92(HSQC)	6.60	-OH
C23	**115.8**	**6.68**	**145.6**		**182.0**		146.8		-OH

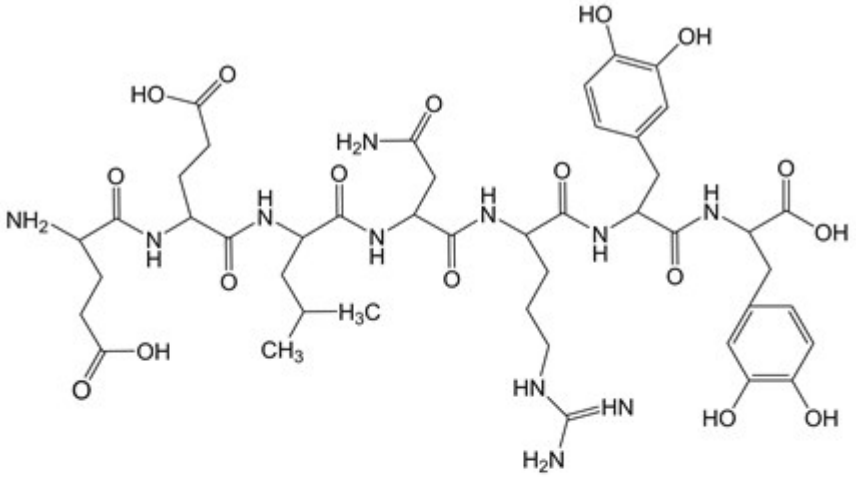
C24	**155.7**		**144.5**		**180.3**		146.8		-OH
C25	**115.8**	**6.68**	115.9	6.61	**126.6**	**5.89**	118.87 or 118.92 129.7	6.66 -	-OH =O
C26	130.2	6.96	122.8	6.52	152.5	7.23	124.6	6.45 or 6.49	
O31		9.06		9.48					
C17	171.0		171.0		171.0				
O18									
C27	57.1	4.44	57.1	4.44	57.1	4.44			
N28		8.32		8.32		8.32			
C32	28.9	1.77	28.9	1.77	28.9	1.77			
C33	24.2	1.51	24.2	1.51	24.2	1.51			
C34	41.2	3.34	41.2	3.34	41.2	3.34			
N35		2.5&		2.5&		2.5&			
C36	158.0		158.0		158.0				
N37		6.63		6.63		6.63			
N42		7.84		7.84		7.84			
C29	170.3		170.3		170.3				
O30									
C38	53.9	4.84	53.9	4.84	53.9	4.84			
N39		8.32		8.32		8.32			
C43	37.1	2.56 / 2.81	37.1	2.56 / 2.81	37.1	2.56 / 2.81			
C44	172.3		172.3		172.3				
O45									
N50		7.03		7.03		7.03			
C40	171.0		171.0		171.0				
O41									
C46	56.0	4.44	56.0	4.44	56.0	4.44			
N47		8.32		8.32		8.32			
C51	41.2	1.76	41.2	1.76	41.2	1.76			
C52	24.4	1.49	24.4	1.49	24.4	1.49			
C53	22.5	0.9	22.5	0.9	22.5	0.9			
C58	22.5	0.9	22.5	0.9	22.5	0.9			
C48	171.0		171.0		171.0				
O49									
C54	56.8	4.44	56.8	4.44	56.8	4.44			
N55		8.32		8.32		8.32			
C59	26.9	2.06	26.9	2.06	26.9	2.06			
C60	30.0	2.33	30.0	2.33	30.0	2.33			
C61	178.4		178.4		178.4				
O62		12.01		12.01		12.01			
O65									
C56	171.0		171.0		171.0				
O57									
C63	52.2	3.21	52.2	3.21	52.2	3.21			
N64		8.76		8.76		8.76			
C66	26.0	2.06	26.0	2.06	26.0	2.06			
C67	30.0	2.33	30.0	2.33	30.0	2.33			
C68	178.4		178.4		178.4				

O69		12.01		12.01		12.01			
O70									

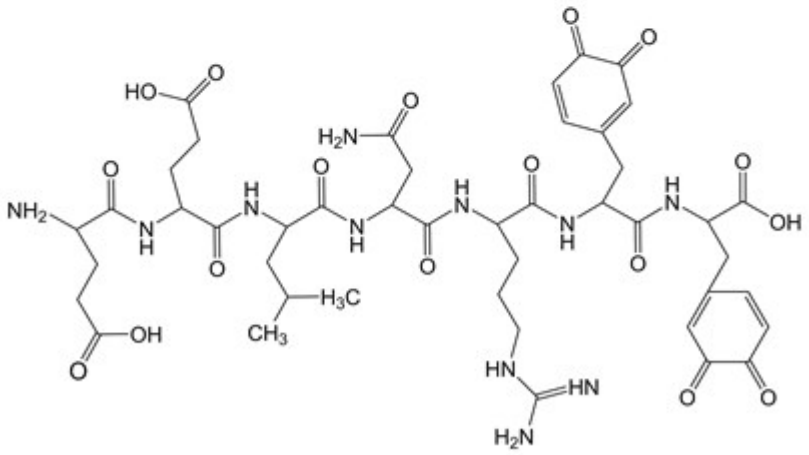
Figures and Scheme



Scheme S1. Chemical structure of EELNRYY.

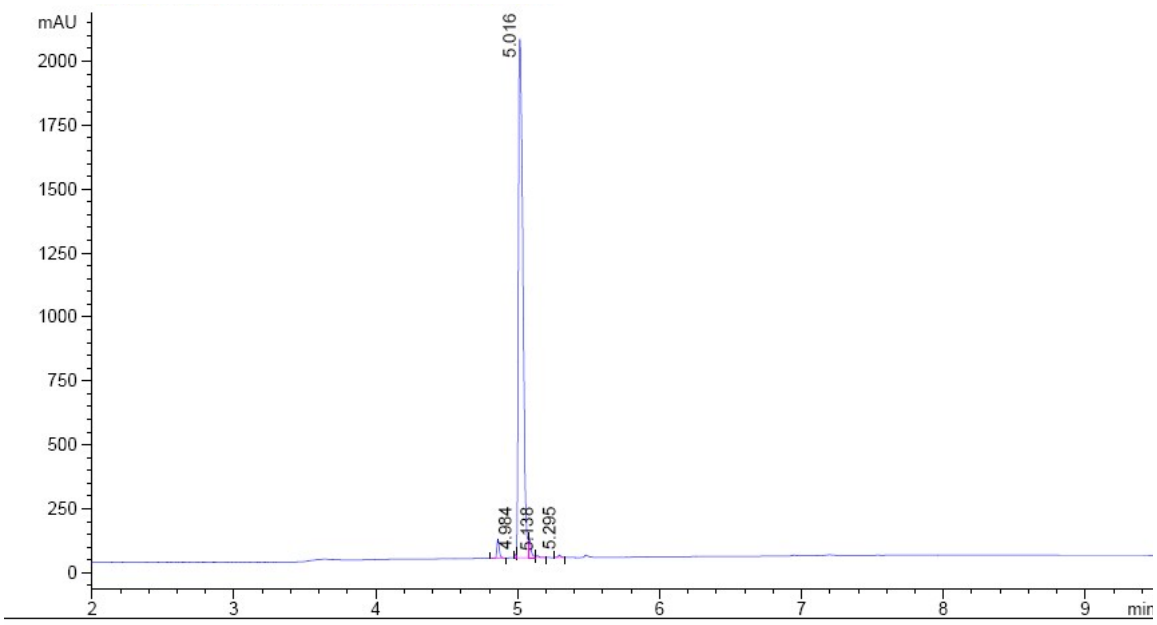


EELNRYY-DOPA



EELNRYY-DOPA-quinone

Scheme S2. Chemical structures for eumelanin precursors EELNRYY-DOPA and EELNRYY-DOPA-quinone.



#	Meas. Ret.	Area	Area %
1	4.862	85.282	1.821
2	4.984	8.838	0.189
3	5.016	4464.774	95.317
4	5.084	101.712	2.171
5	5.138	11.541	0.246
6	5.295	11.969	0.256

Figure S1. HPLC chromatogram and analysis for EELNRYY.

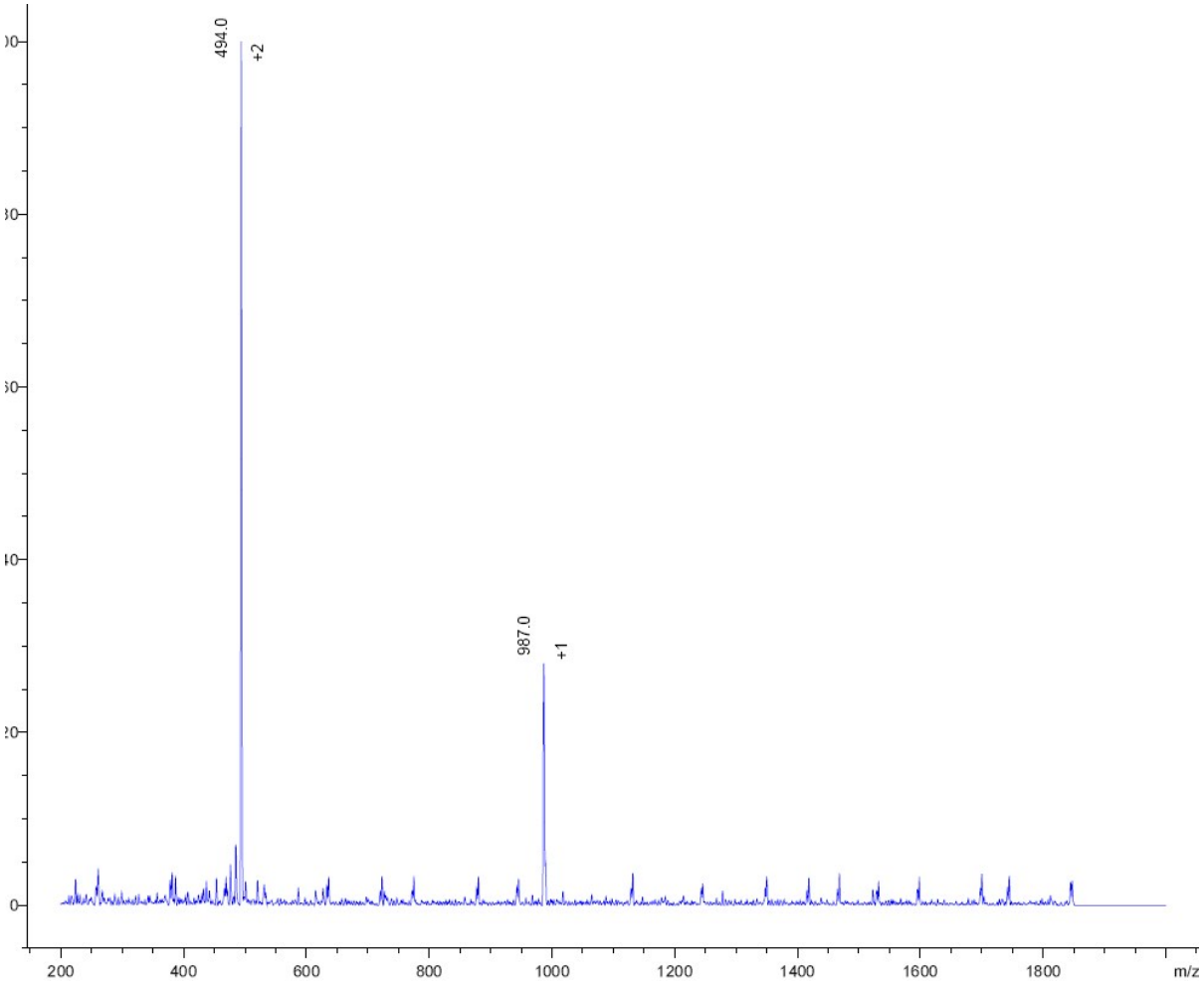
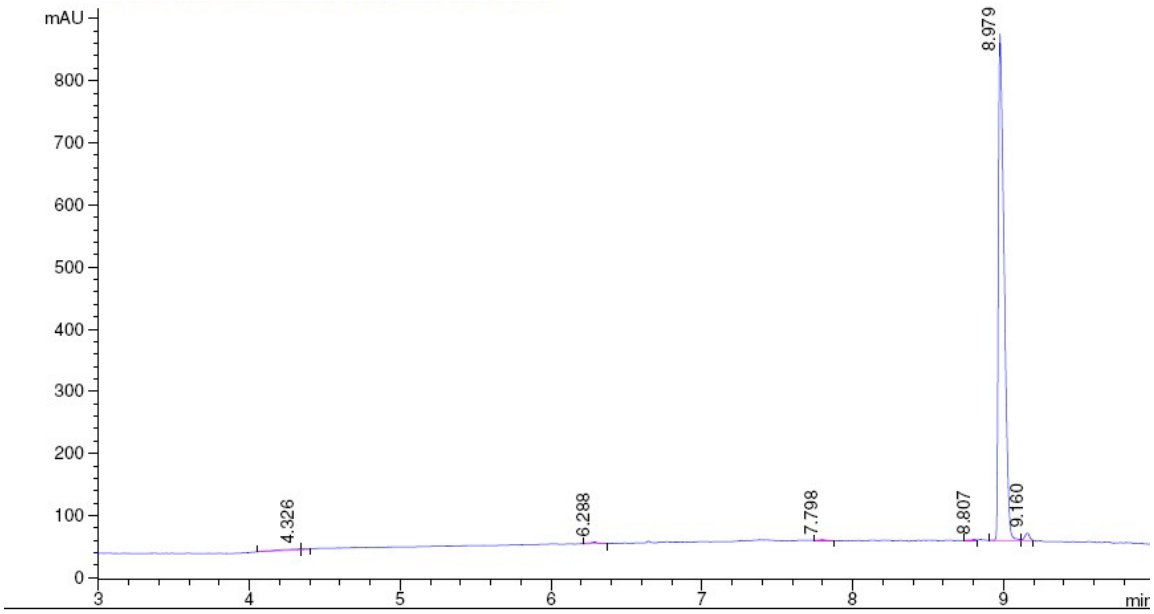


Figure S2. ESI-MS data for EELNRYY.



#	Meas. Ret.	Area	Area %
1	4.326	19.626	0.879
2	4.393	5.698	0.255
3	6.288	10.261	0.459
4	7.798	8.947	0.401
5	8.807	5.863	0.262
6	8.979	2153.696	96.413
7	9.160	29.732	1.331

Figure S3. HPLC chromatogram and analysis for C_{16}-EELNRYY.

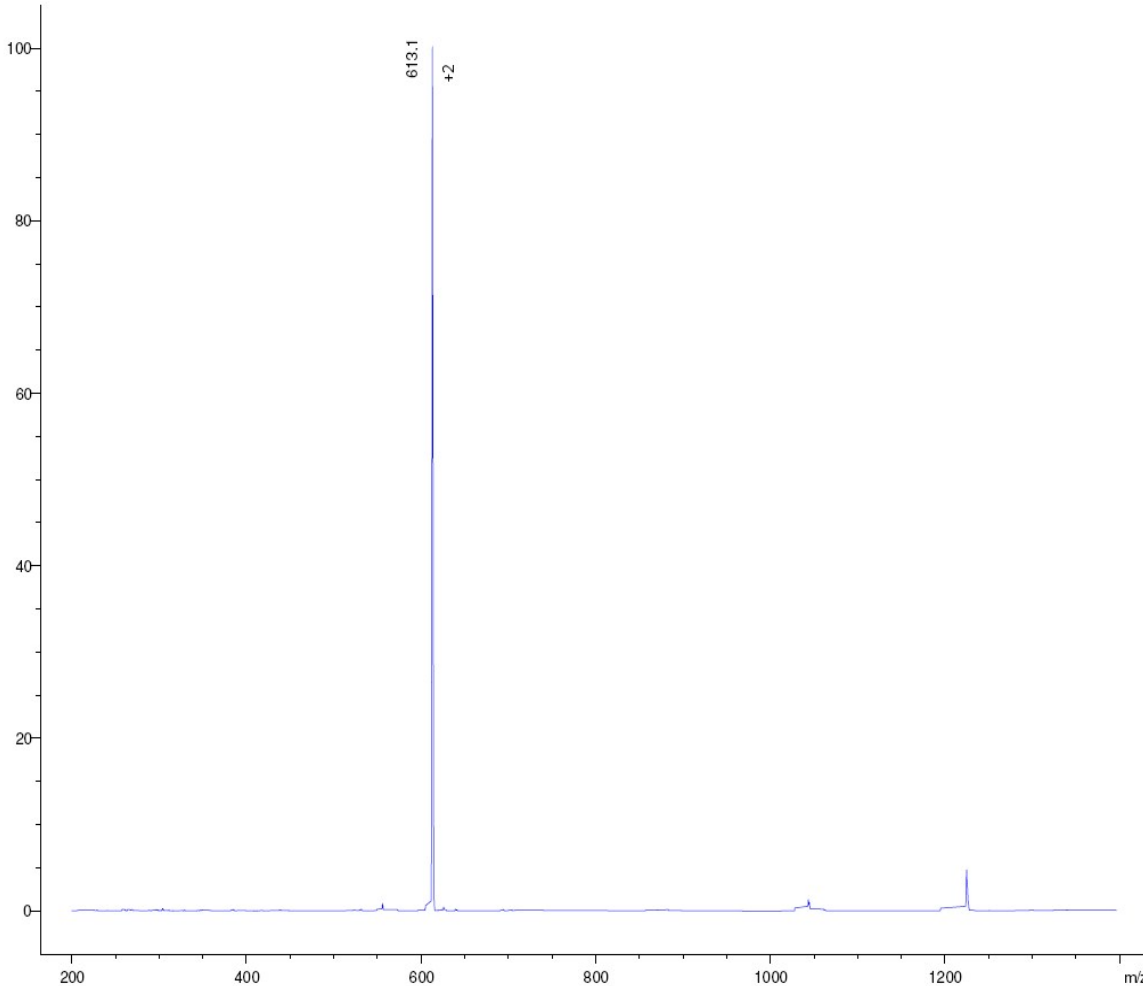


Figure S4. ESI-MS data for C_{16}-EELNRYY.

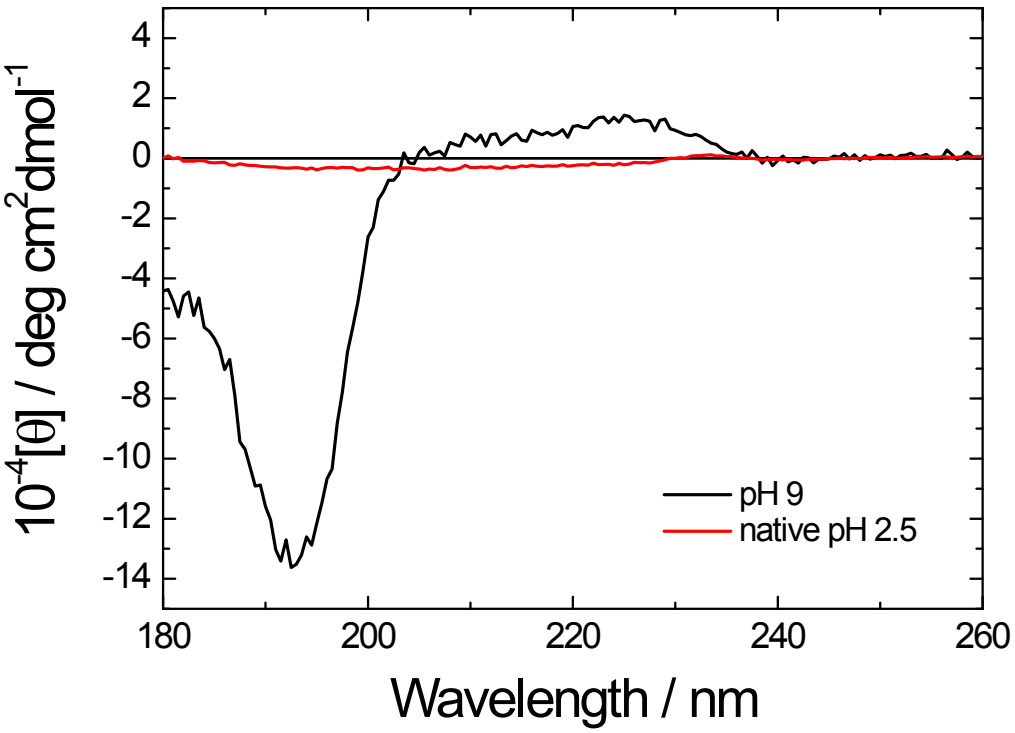
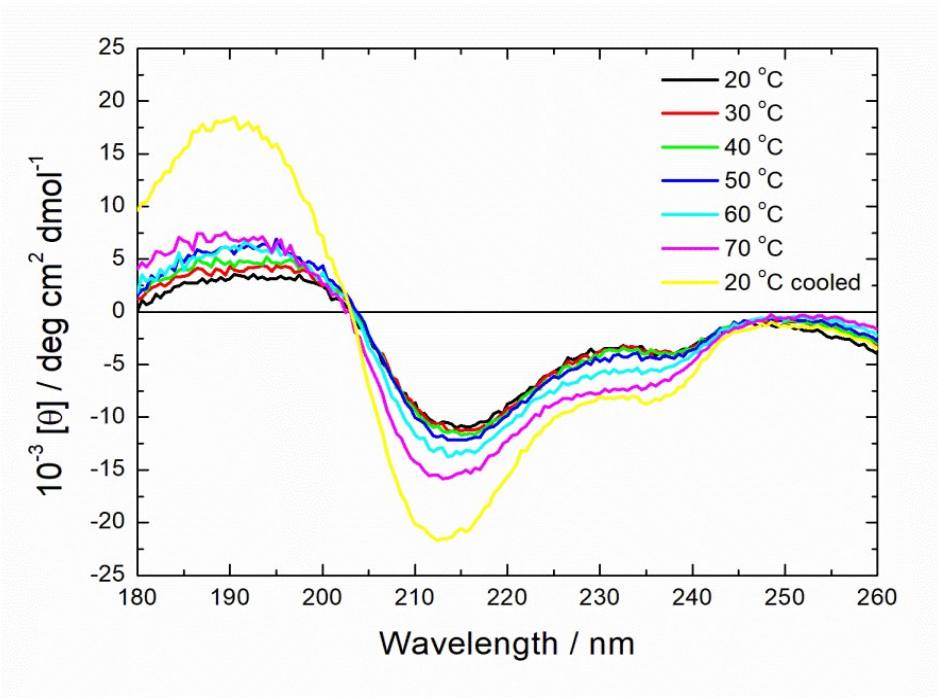


Figure S5. CD spectra for EELNRYY at pH 2.50 (native pH) and pH 9.

(a)


20 °C
30 °C
40 °C
50 °C
60 °C
70 °C
20 °C cooled
10^-3 [θ] / deg cm^2 dmol^-1
Wavelength / nm

(b)

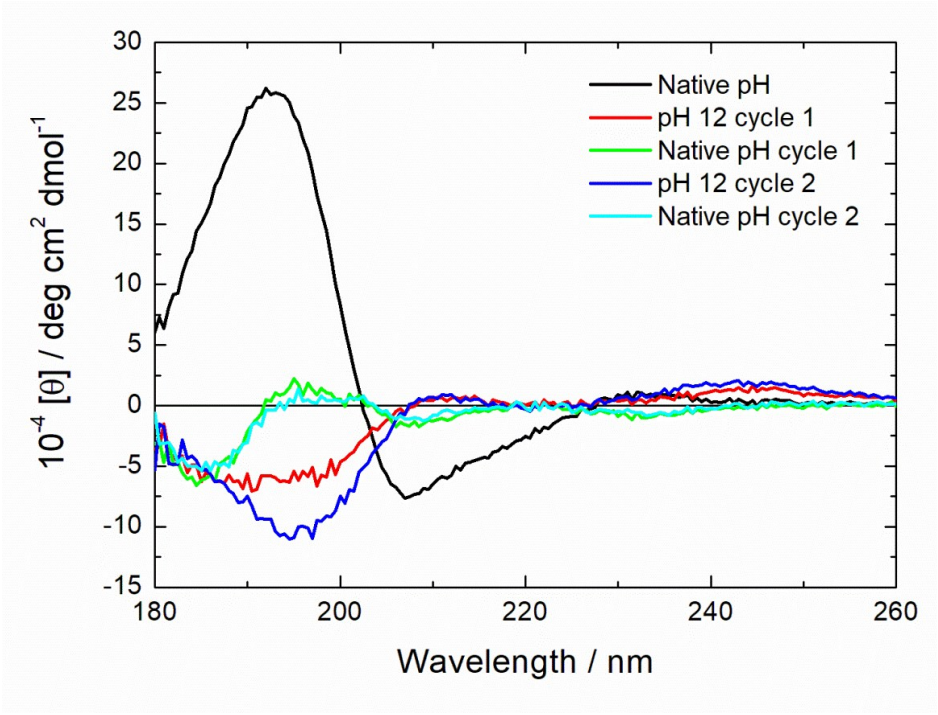
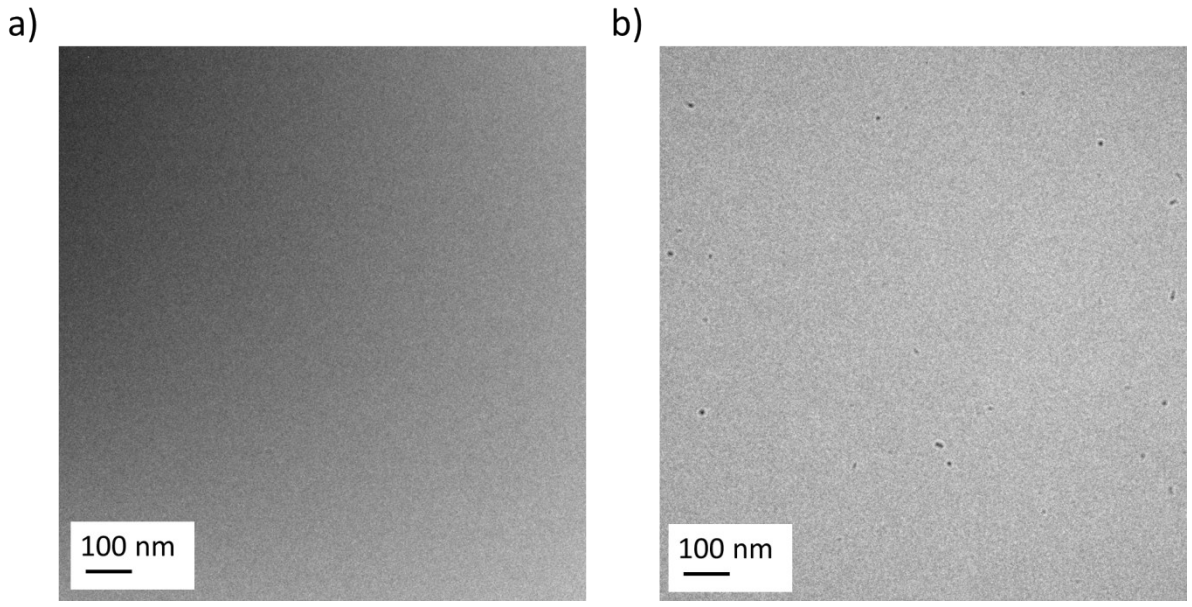

Native pH
pH 12 cycle 1
Native pH cycle 1
pH 12 cycle 2
Native pH cycle 2
10^-4 [θ] / deg cm^2 dmol^-1
Wavelength / nm

Figure S6. CD spectra from a 1 wt% C_{16}-EELNRYY solution. (a) Temperature dependence from 20 -70 ^{o}C at pH 9, (b) showing secondary structure reversibility during two pH switch cycles from native pH to pH 12 at 20 ^{o}C.



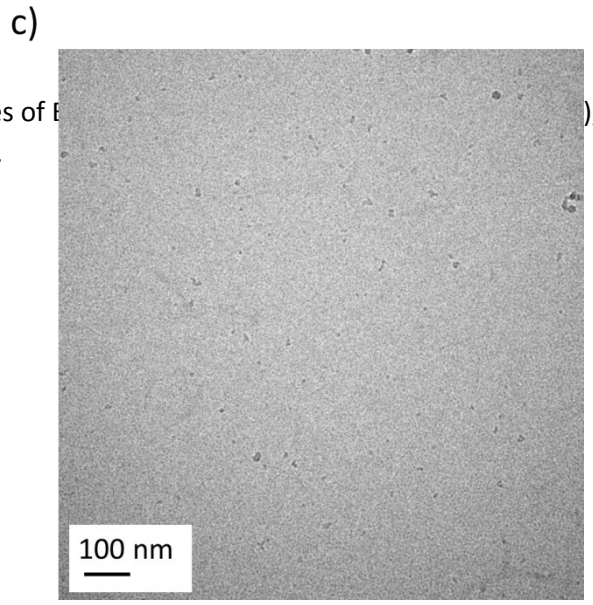


Figure S7. Cryo-TEM images of E), b) pH 10, c) pH 12. No aggregates were observed.

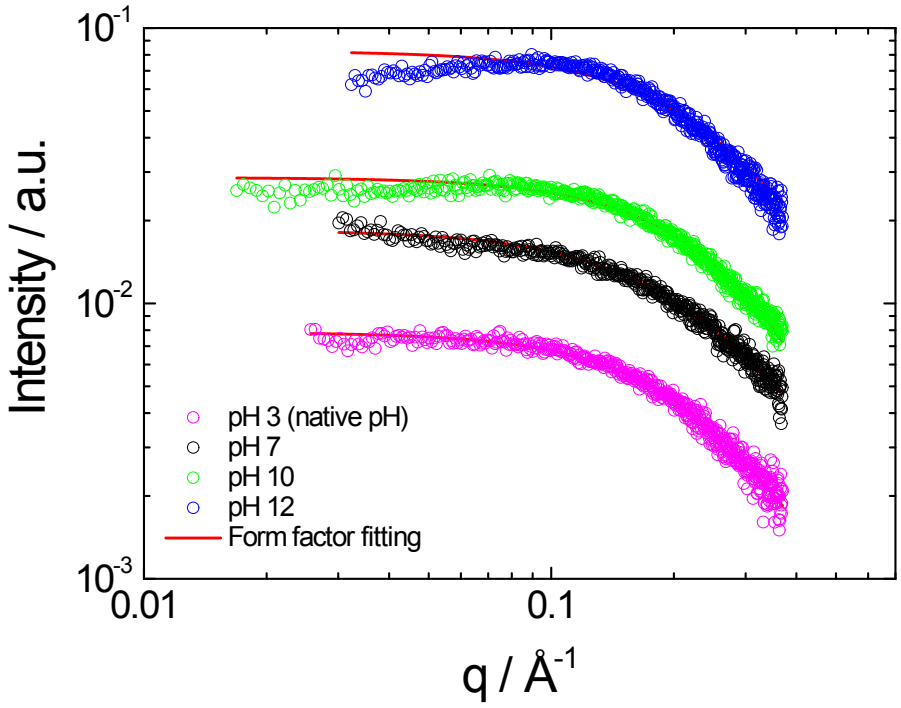


Figure S8. SAXS intensity profiles and form factor fittings of EELNRYY in the pH range 7-12. Data was fitted to a generalized Gaussian coil form factor.

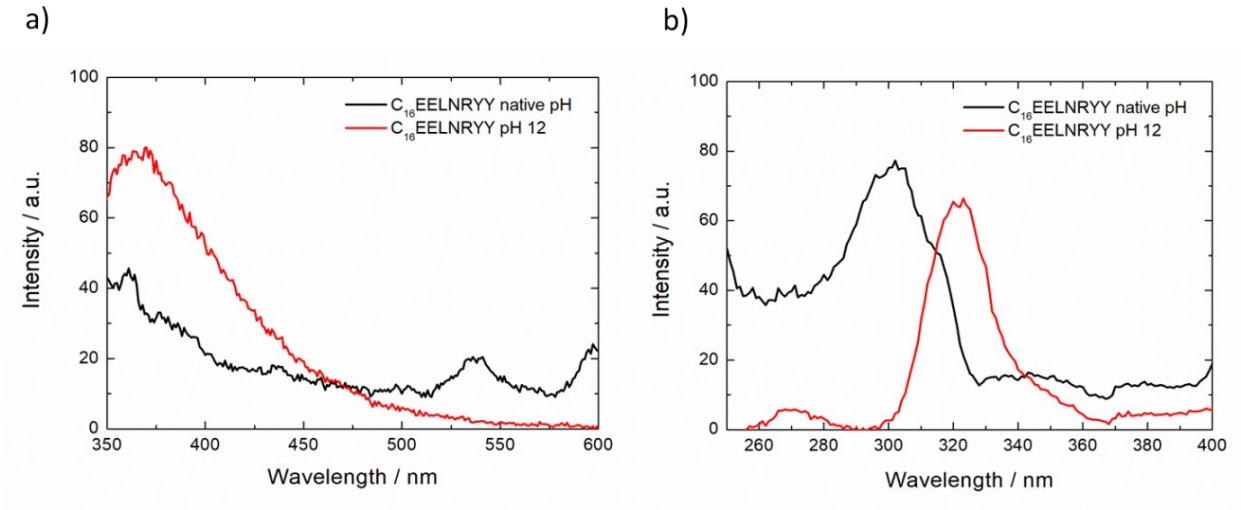


Figure S9. Fluorescence of C_{16}-EELNRYY at pH 7 (native pH in water) and pH 12 to study dityrosine formation. a) Emission spectrum at λ_{ex} = 315 nm. b) Excitation spectrum at λ_{em} = 410 nm.

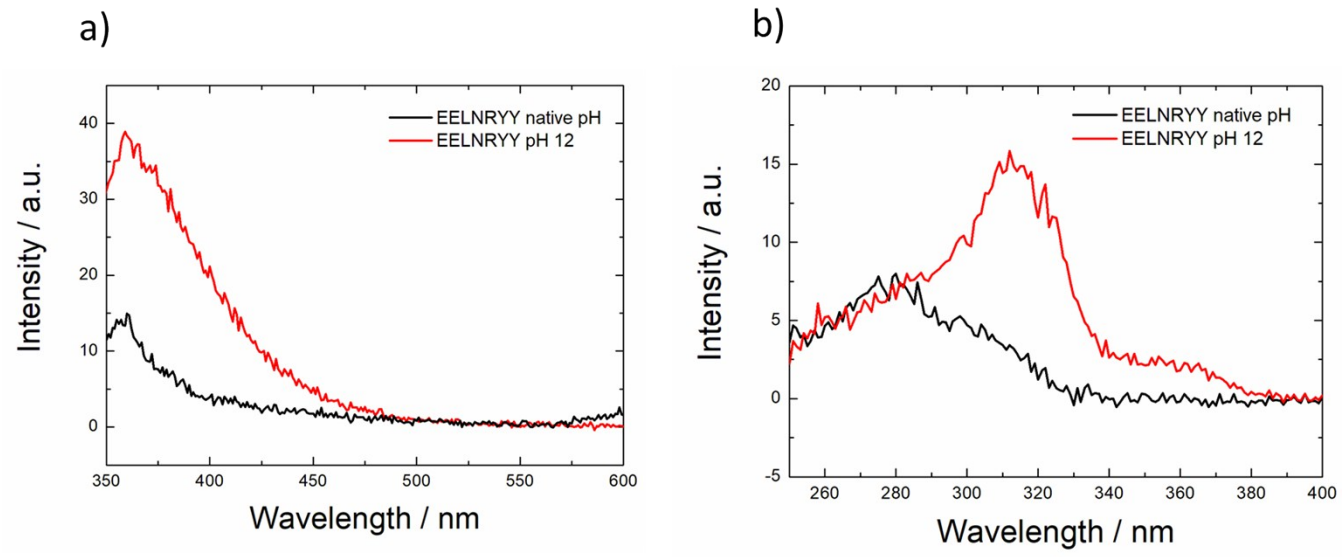


Figure S10. Fluorescence of EELNRYY at pH 2 (native pH in water) and pH 12 to study dityrosine formation. a) Emission spectrum at λ_{ex} = 315 nm. b) Excitation spectrum at λ_{em} = 410 nm.

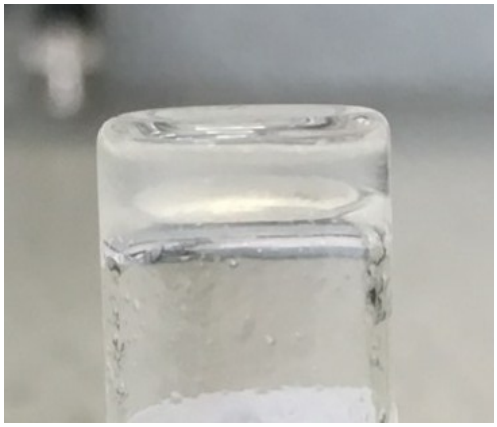


Figure S11. Gel of C_{16}-EELNRYY at 1 wt% shown by the inverted vial method.

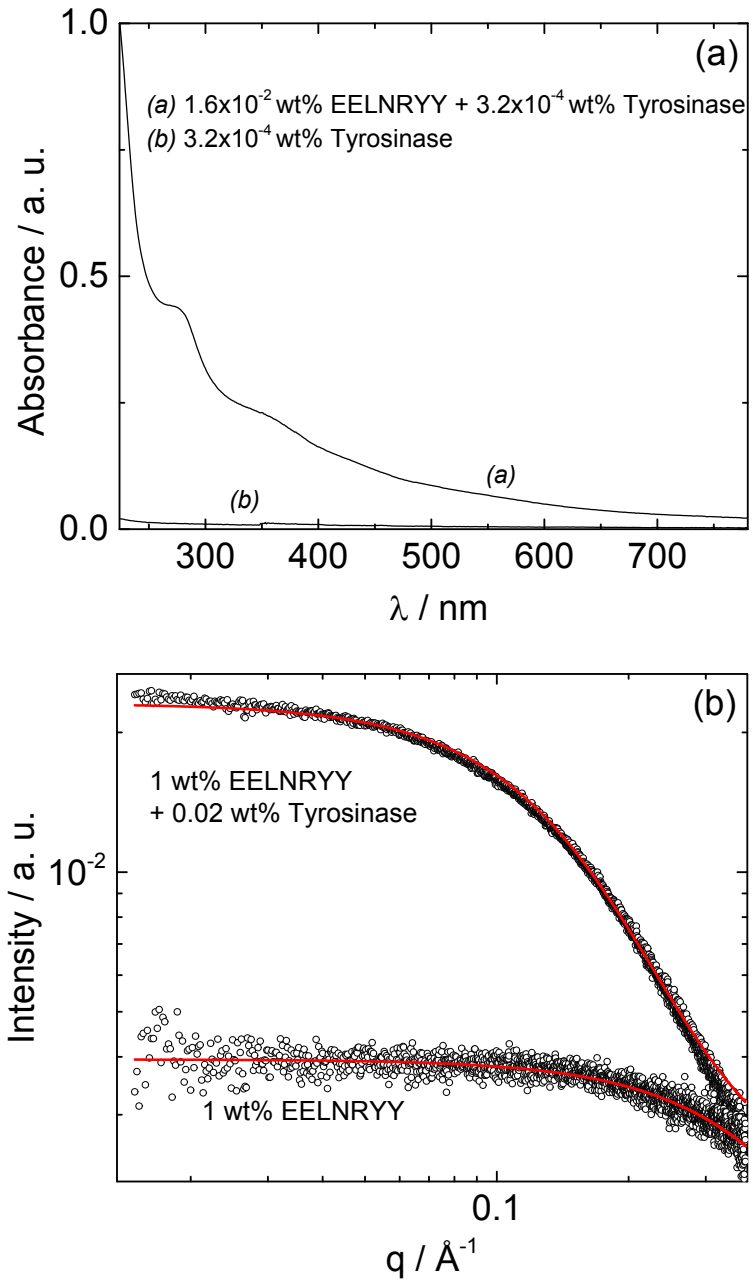


Figure S12. (a) UV-vis spectra for a sample containing 1 wt% EELNRYY + 0.02 wt% tyrosinase (Figure 7b) diluted 33x. The control Uv-vis spectrum for tyrosinase is included in the figure. (b) SAXS data corresponding to EELNRYY incubated with tyrosinase for 24 hrs. The SAXS data for the control EELNRYY solution is included in the figure. The full lines correspond to the fitting of the SAXS data according to a generalized Gaussian coil form factor. (a-b) samples are dissolved in 50 mM potassium phosphate buffer (pH 6.8).

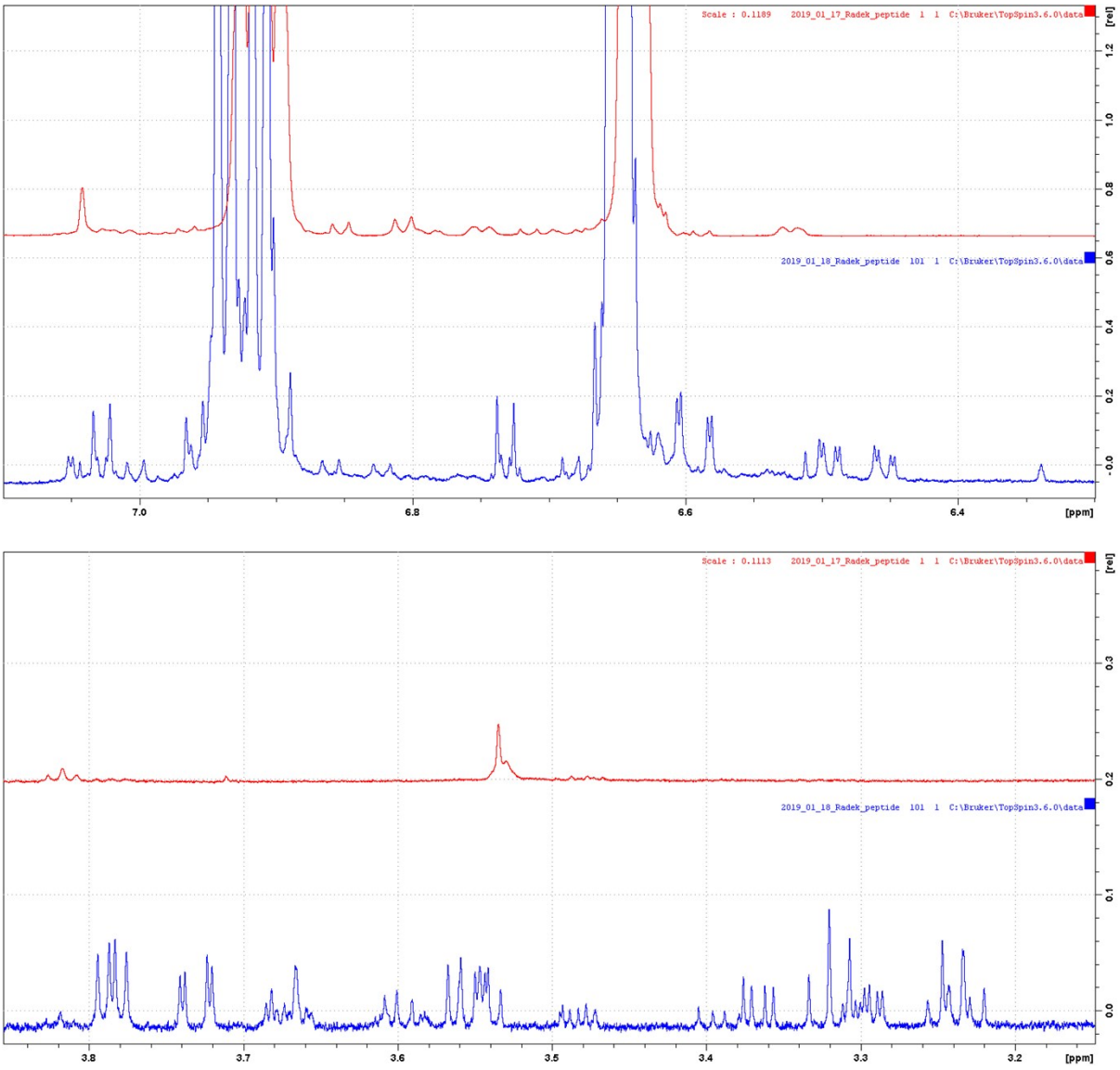


Figure S13. Comparison of the region of 1H NMR spectra of 2 wt% EELNRYY (red) and 0.5 wt% EELNRYY+ 0.01 wt% tyrosinase (blue). The additional doublets are consistent with the presence of the short versions of the peptide with tyrosine.

Reference

(1) Bressler, I.; Kohlbrecher, J.; Thünemann, A. F., SASfit: a tool for small-angle scattering data analysis using a library of analytical expressions. *J. Appl. Crystallogr.* **2015,** *48*, 1587-1598.