Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Protein Charge Transfer Spectra in a Monomeric Protein with No Lysine

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Protein	PDB ID	Organisms	Biological role	Total aa	Charged aa	K	E	(D+R+H)
Symfoil 4P/PV2	4D8H	Synthetic	*de novo* protein folding	132	39	0	9	30
C-terminal domain of the HrcQb	1O9Y	*Pseudomonas syringae*	Structural protein	84	23	0	8	15
UIM Domain of Ataxin-3	2KLZ	*Homo sapiens*	Hydrolase	52	25	0	8	17
Intermediate' Defensin-like Peptide	2MN3	*Ornithorhynchus anatinus*	Antimicrobial protein	44	14	0	1	13

Table S1. Charged rich proteins with no lysine

Proteins rich in Arginine, Aspartate and Histidine amino acids (aa) *but* devoid of Lysine and aromatic amino acids from PDB database

	Total aa	Aromatic aa	Total charged aa	Lys (K)	Glu (E)	Arg (R)	Asp (D)	His (H)
No. of Residues	132	0	39	0	9	12	12	6
% contribution	--	0.00	29.54	0.00	6.81	9.09	9.09	4.54

Table S2. Charged amino acid composition of Symfoil PV2

Total Number of amino acids residues containing aromatic and charged amino acids are listed. Percentage contribution from the same amino acids towards total amino acids were also shown.

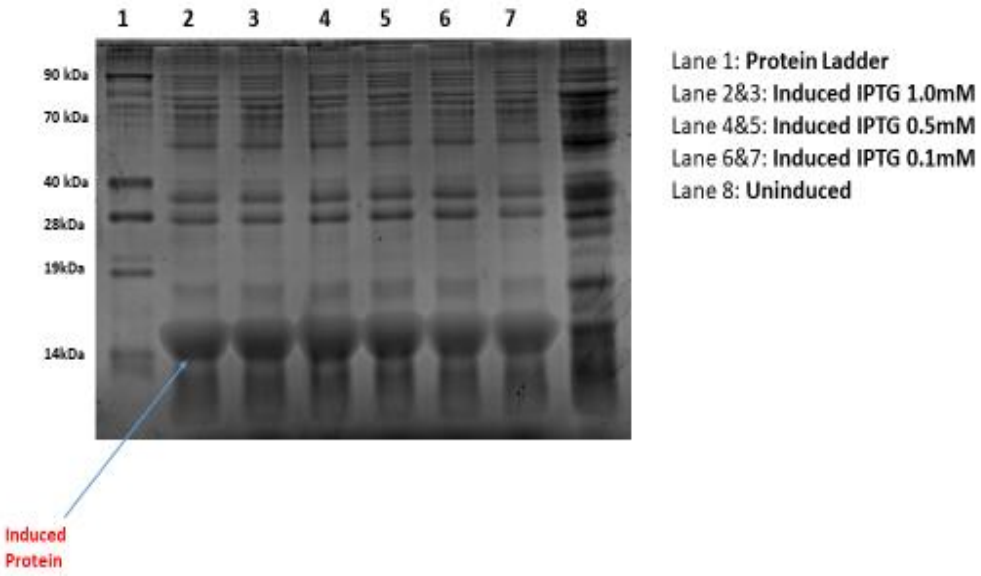

Figure S1. Expression of Symfoil PV2 in 15% SDS PAGE

Protein expression is visualized in the individual lanes for different IPTG concentrations (Lane 2-7). Lane 8 shows the uninduced fraction and Lane 1 shows the different protein standards.

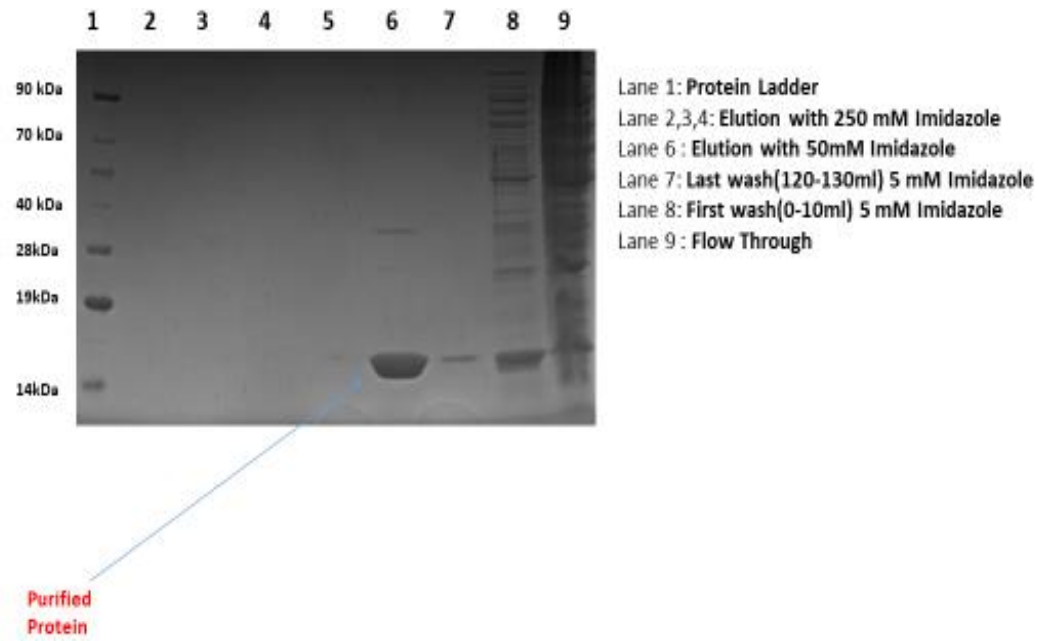

Figure S2. Purification of Symfoil PV2 in 15% SDS PAGE

Purification of protein (a clear band around 14.3 kDa) is shown in the lane 6; Lanes (2-8) show the protein fractions eluted with different Imidazole concentration. Lane 1 shows the different protein standards.

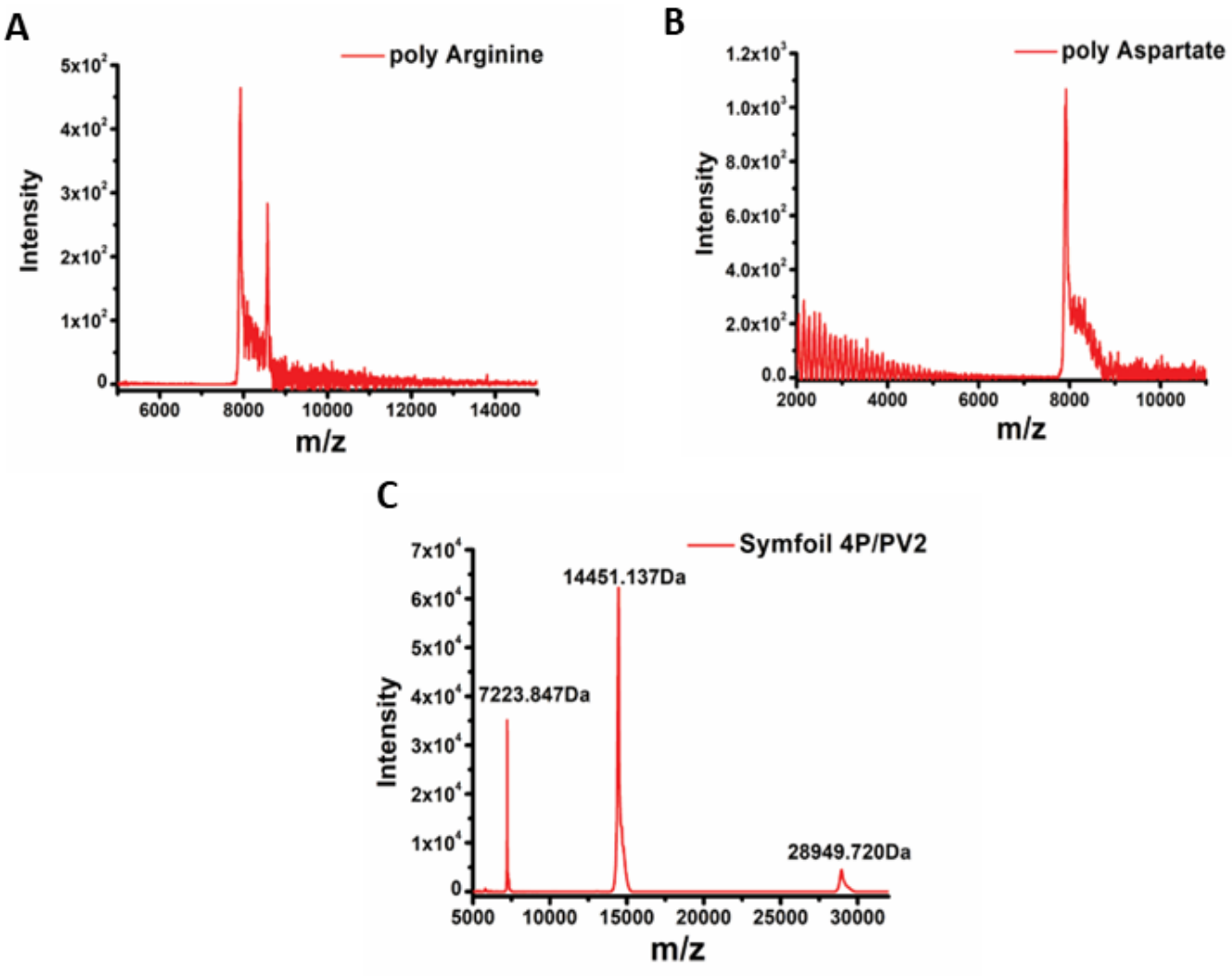

Figure S3. Molecular weight estimation by MALDI of homo-polyArginine; homo-poly Aspartate and Symfoil 4P/PV2

Mass of poly-Arginine-HCl (**A**); poly-Aspartate-NaCl (**B**); and Symfoil PV2 (**C**) were determined by using MALDI-TOF mass spectrometer. All the samples were prepared in a saturated solution of Sinapic acid in TA-30 solvent.

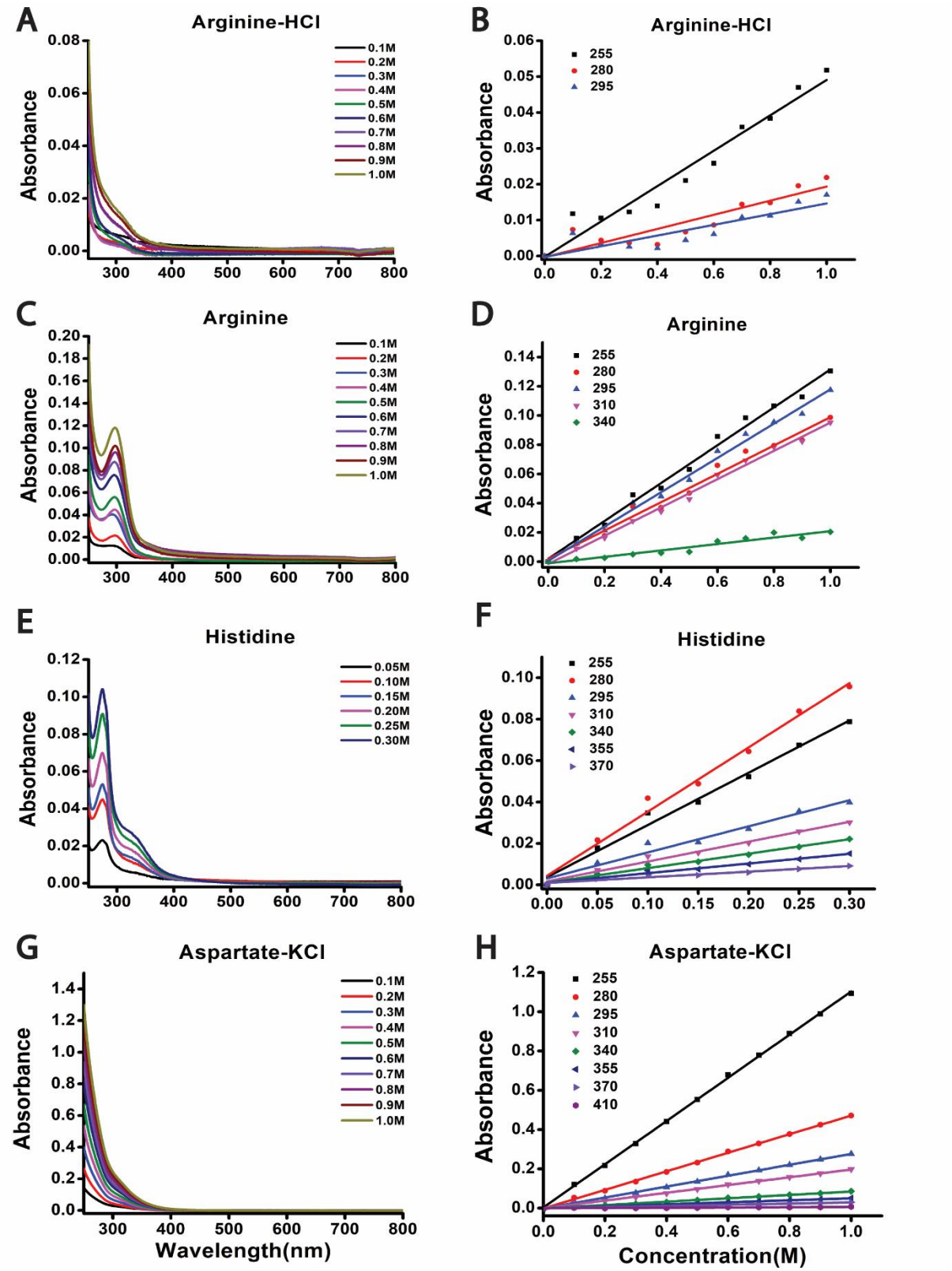

Figure S4. Linearity of the absorbance versus concentration among all amino acids

Absorbance of Arginine-HCl, Arginine, Histidine and Aspartate-KCl is shown here.10 different concentrations were used for all samples except Histidine (6 concentrations) shown in panel **A, C, E, G** and their linearity is shown at different wavelengths in panel **B, D, F, H**. All the readings were done in duplicates and in room temperature.

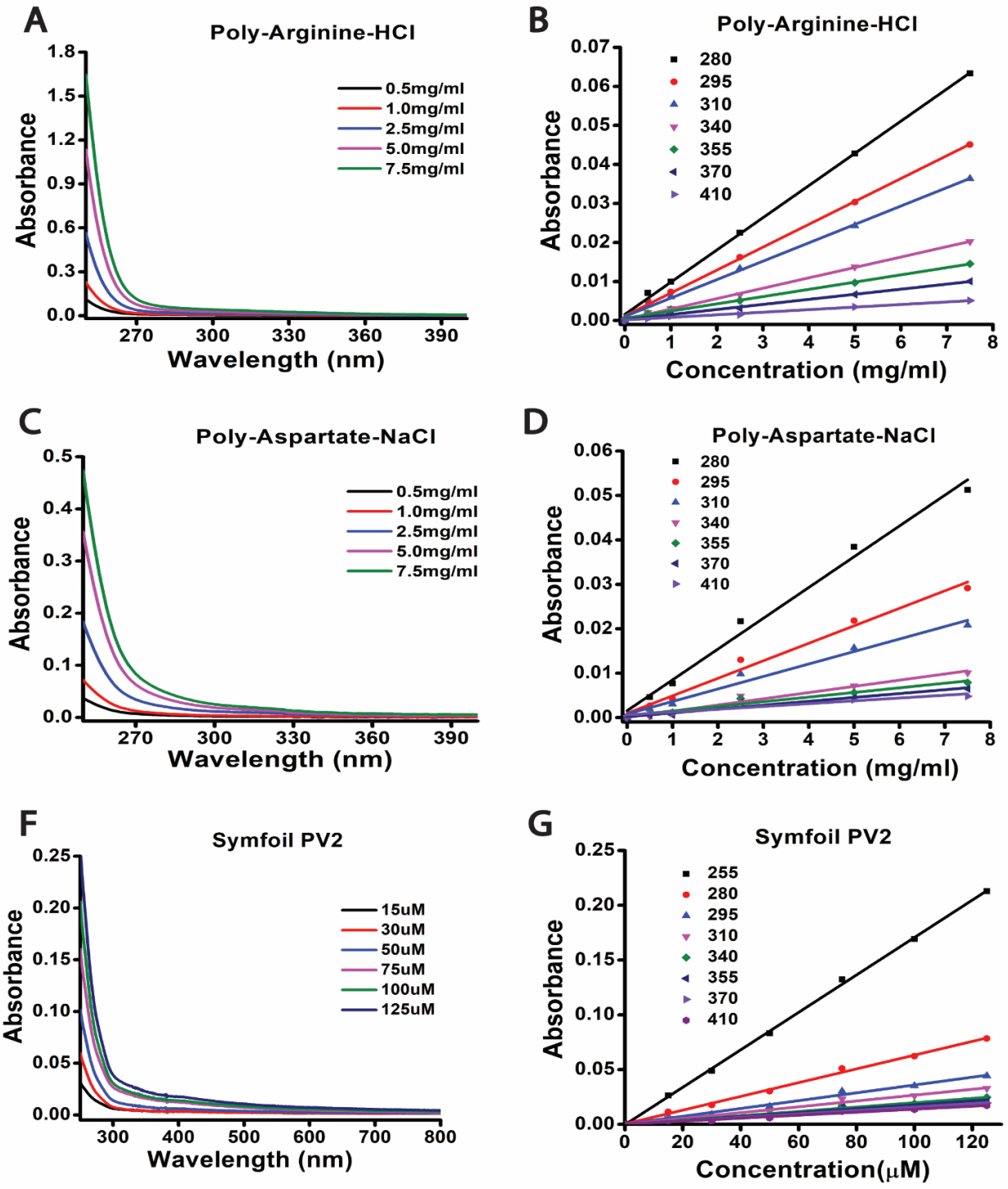

Figure S5. Linearity of the absorbance versus concentration among all peptides and proteins

Absorbance of poly-Arginine-HCl and poly-Aspartate-NaCl, Symfoil PV2 is shown here. 5 different concentrations were used for polypeptides and their linearity is shown at 7 different wavelengths in panel **A, C** and **B, D** respectively. Absorbance of Symfoil 4P/PV2 for wavelength ranging from 250-800 nm for 6 different concentration and its linear profile for 8 different wavelengths is shown in panel **F** and **G** respectively.

Wavelength (nm)	255	280	295	310	340	355	370	410	500	600	700	800
Arginine HCl	0.046 (0.006)	0.017 (0.005)	0.012 (0.004)	0.01 (0.007)	0.003 (0.0047)	0.001 (0.003)	-----	-----	------	------	-----	-----
Arginine	0.131 (0.0070)	0.098 (0.0075)	0.116 (0.0068)	0.092 (0.007)	0.018 (0.0047)	0.013 (0.004)	0.009 (0.004)	0.004 (0.004)	-------	------	------	-----
Histidine	0.264 (0.0039)	0.325 (0.0072)	0.137 (0.0041)	0.102 (0.0013)	0.073 (0.0005)	0.050 (0.0002)	0.030 (0.0003)	0.011 (0.0006)	--------	-------	------	------
Aspartate KCl	1.103 (0.0142)	0.464 (0.0124)	0.269 (0.0108)	0.191 (0.0085)	0.079 (0.0074)	0.045 (0.0068)	0.025 (0.0059)	0.002 (0.004)	-------	--------	------	------
polyArginine-HCl	1023 (9.3)	88 (21.0)	63 (14.5)	51 (11.5)	27 (5.3)	21.6 (6.4)	13.8 (3.2)	7.3 (2.1)	4.7 (2.6)	3.1 (1.9)	3.2 (2.5)	3.3 (2.9)
polyAspartate-NaCl	264.2 (17.4)	45.9 (5.5)	26.2 (3.5)	19.2 (3.0)	7 (3)	7.7 (1.8)	5.4 (1.6)	6.1 (2.3)	6.0 (3.6)	7.9 (6.8)	10 (11)	7.3 (6.2)
Symfoil PV2	1979 (130)	838 (77)	512 (74)	396 (74)	274 (73)	261 (66)	247 (73)	208 (50)	145 (50)	108 (45)	89 (44)	69 (34)

Table S3. Extinction coefficient for amino acids and protein at discrete wavelengths

Extinction coefficient of Arginine-HCl, Arginine, Histidine, Aspartate-KCl, polyArginine-HCl, polyAspartate-NaCl and Symfoil PV2 is tabulated at various discrete wavelengths. The blank boxes indicate the extinction coefficient could not be calculated precisely due to very low S/N ratio. Values in the parenthesis indicate the standard deviation for the respective extinction coefficient.

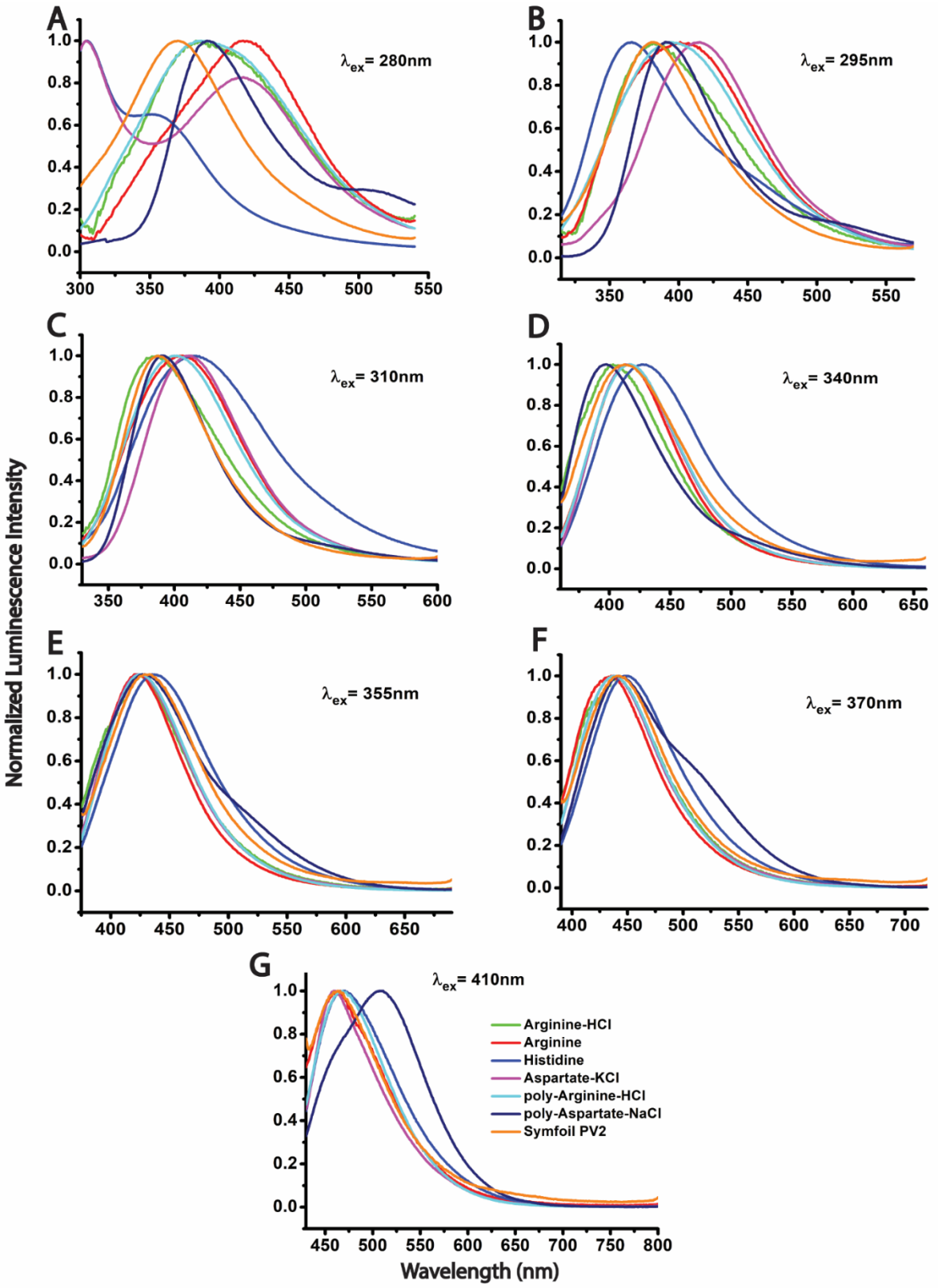

Figure S6. Normalized luminescence spectra of all the samples as a function of excitation wavelength

Peak intensity normalized luminescence is depicted as a function of excitation wavelengths: 280, 295, 310, 340, 355, 370, and 410 nm for all samples in subfigures A to G, respectively

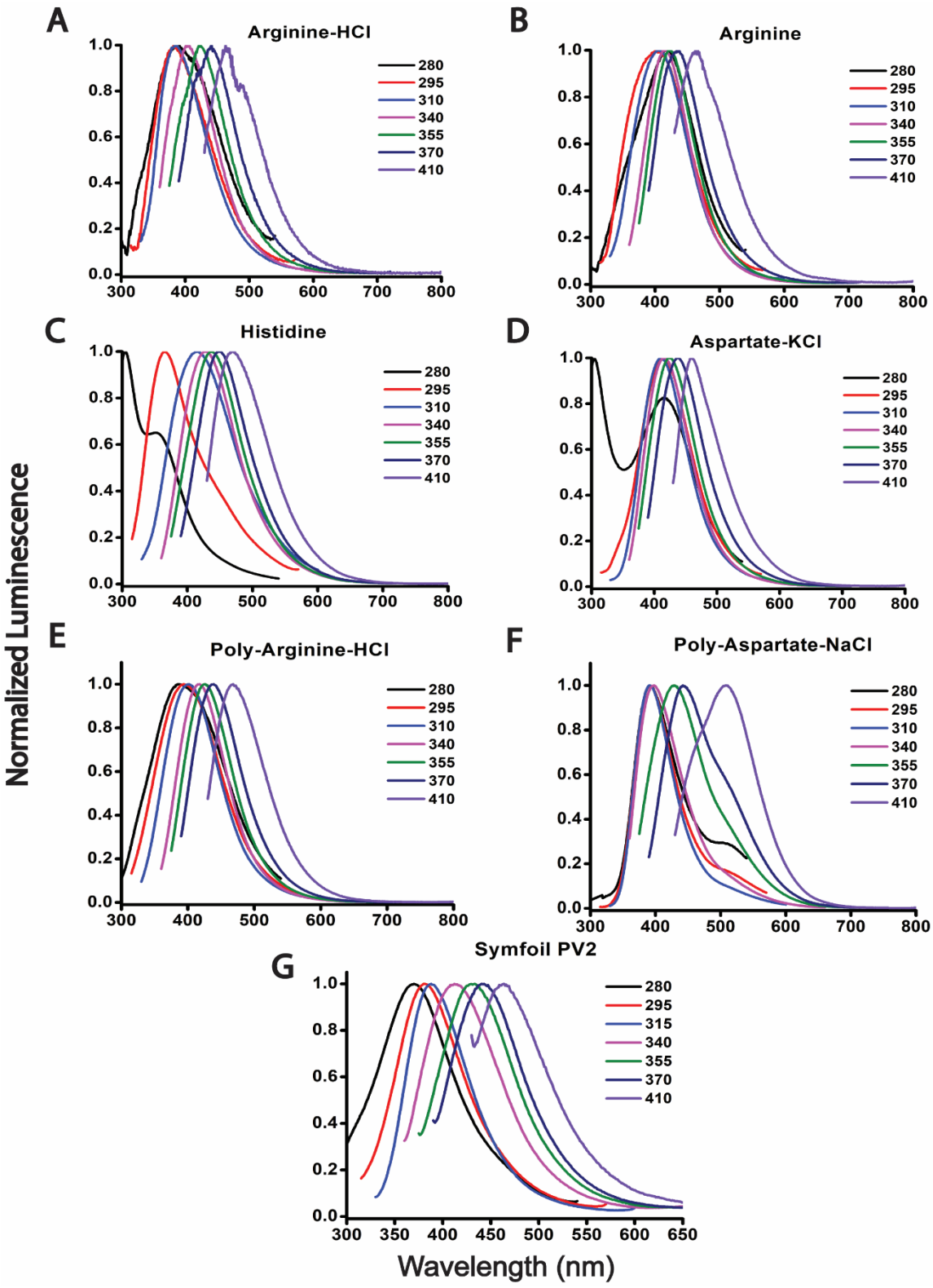

Figure S7. Normalized luminescence spectra of all the samples at different excitation wavelength

Normalized Luminescence Intensity of Arginine-HCl **(A)**; Arginine **(B)**; Histidine **(C);** Aspartate-KCl **(D)**; poly-Arginine-HCl **(E)**; poly-Aspartate-NaCl **(F)** and Symfoil PV2 **(G)** is shown here. Excited wavelengths were: 280, 295, 310, 340, 355, 370, 410 nm and their corresponding emission range were 300-540, 315-570, 330-600, 360-660, 375-720, and 430-800 nm.

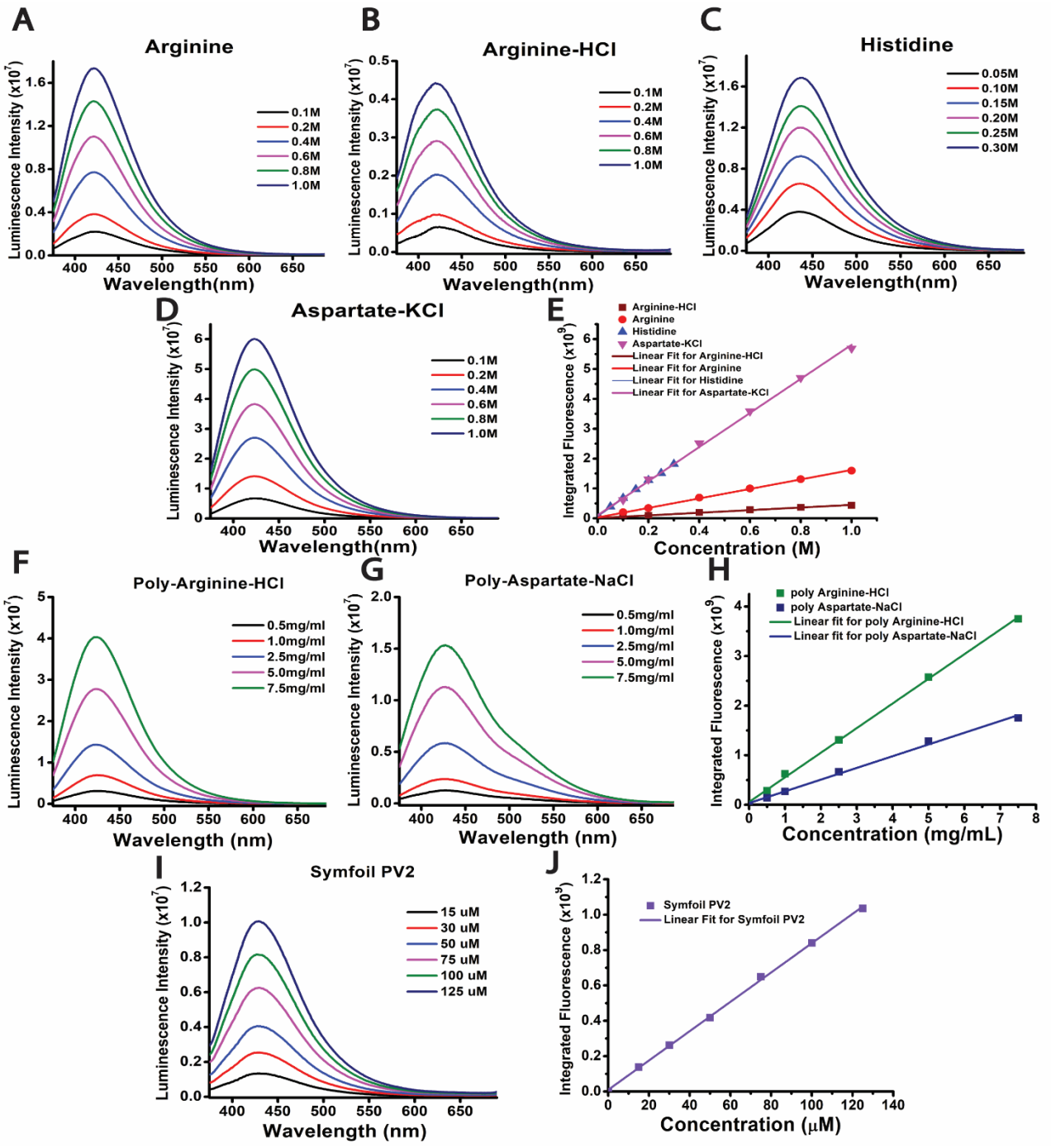

Figure S8. Linearity of the luminescence versus concentration among all the samples at 355 nm excitation

Luminescence Intensity at excitation 355 nm for various concentrations of Arginine-HCl **(A);** Arginine **(B);** Histidine **(C);** Aspartate-KCl **(D)**; polyArginine-HCl **(F);** polyAspartate-NaCl **(G)** and Symfoil PV2 **(I)** is shown here. Linearity of luminescence vs. concentration is shown in panel **E**, whereas for polypeptides and Symfoil PV2, the same are shown in panel **H** and **J,** respectively.

	ϵ_{355} ($M^{-1}cm^{-1}$)	Slope ($x10^6$) (intensity/μM)	Adj.R^2	Range of concentration	Φ_{355}	$\Phi_{355}*\epsilon_{355}$ ($M^{-1}cm^{-1}$)
Arginine-HCl	0.001	0.00044	0.9953	0-1000 mM	0.00506	0.00000506
Arginine	0.013	0.00159	0.9984	0-1000 mM	0.02586	0.00033618
Histidine	0.050	0.00592	0.9957	0-300 mM	0.01706	0.000853
Aspartate-KCl	0.045	0.00571	0.9976	0-1000 mM	0.02619	0.00117855
poly-Arginine-HCl	21.6	4.298	0.9987	0-857 μM	0.03033	0.65677
poly-Aspartate-NaCl	7.7	1.33	0.9946	0-1312.6 μM	0.03985	0.30756
Symfoil PV2	261	8.2947	0.9991	0-125 μM	0.00274	0.71514

Table S4. Correlation between Extinction coefficient and Quantum yield at 355 nm

Extinction coefficient (ε), Quantum yield (Φ), product of Φ and ε, slope of luminescence integrated intensity, concentration used for all samples are shown. Values used for the study were carried out at 355 nm excitation for each of the samples.

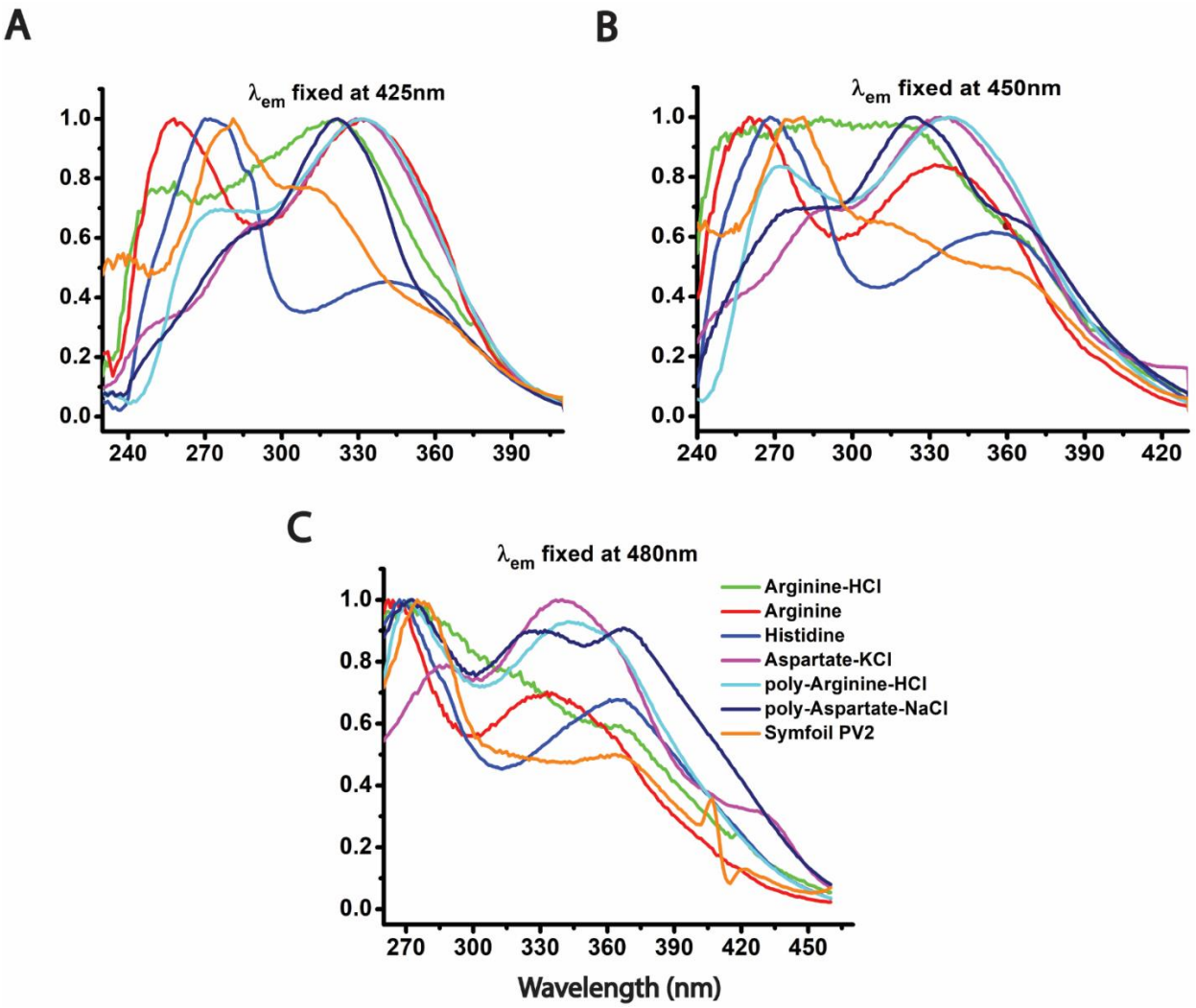

Figure S9: Luminescence Excitation Spectra at different fixed emission wavelengths

Luminescence Excitation Spectra of Arginine-HCl, Arginine, Histidine and Aspartate-KCl, poly-Arginine-HCl, poly-Aspartate-NaCl, and Symfoil PV2 for emission wavelengths: 425, 450, and 480 nm in subfigures A, B, and C, respectively.

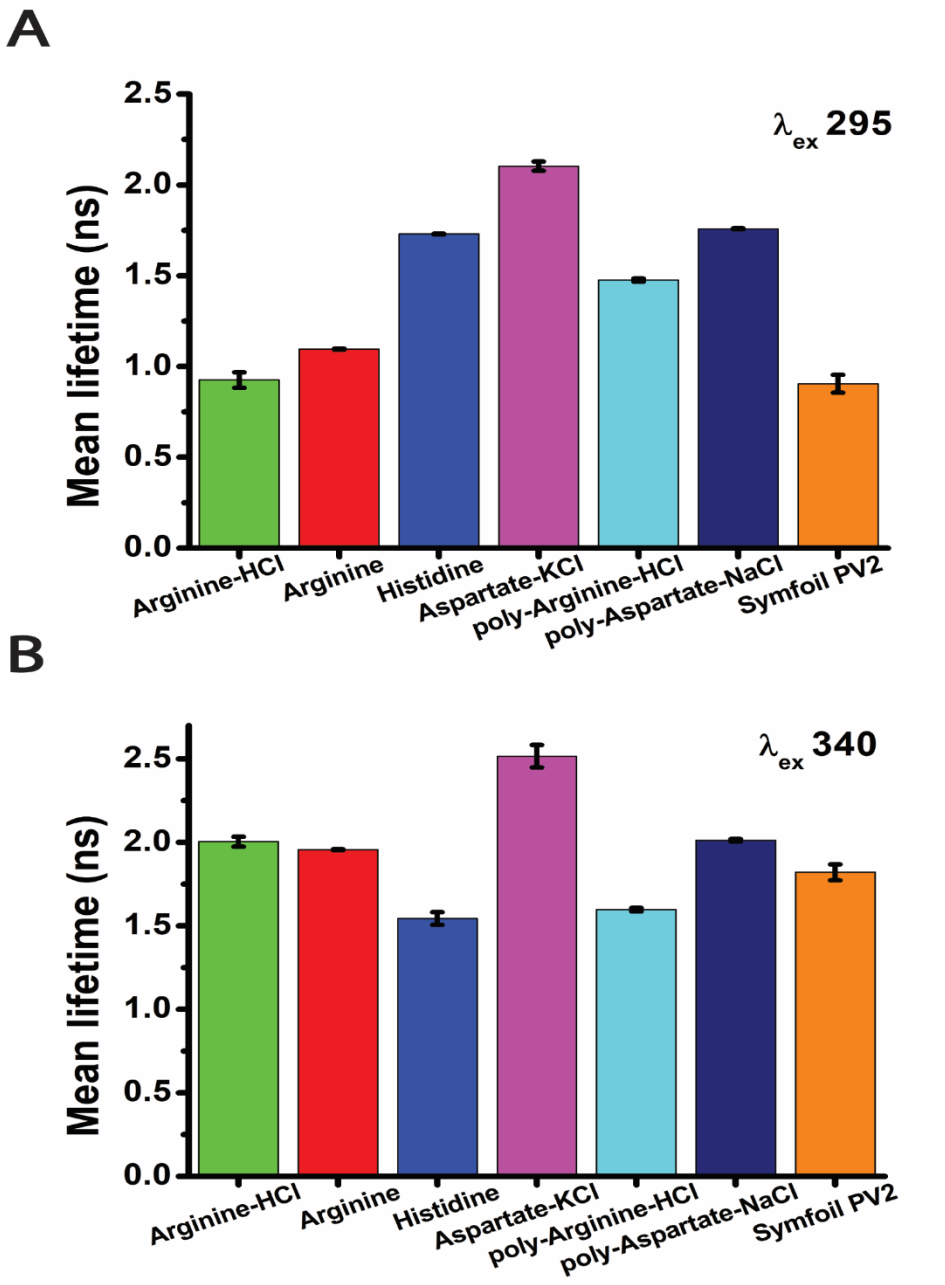

Figure S10: Mean Luminescence Lifetime at different excitation wavelengths
Subfigures A and B show the mean luminescence lifetime when excited at 295 nm and 340 nm respectively. All the lifetime values shown here are averaged from at least two independent experiments.

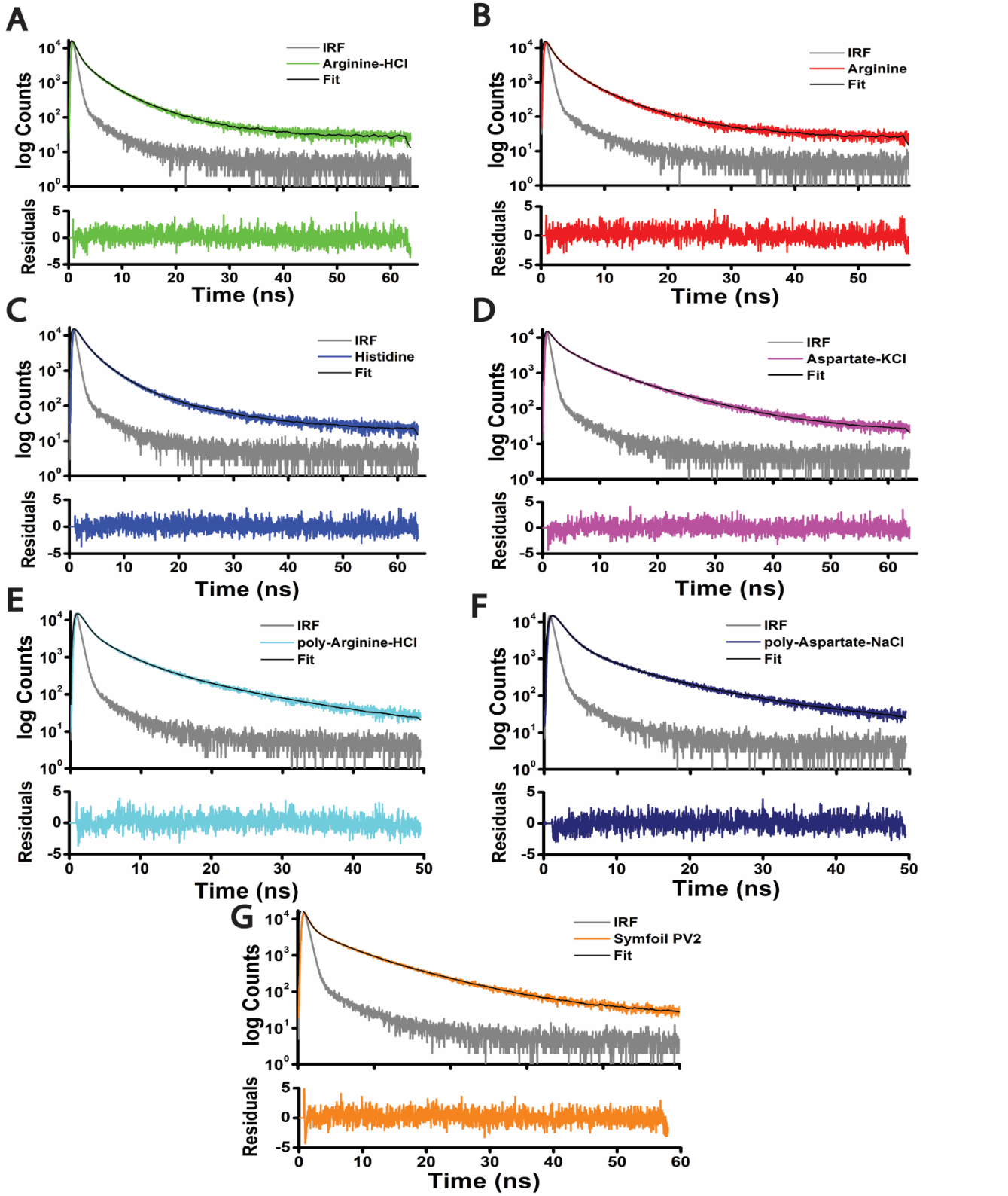

Figure S11. Luminescence intensity decay when excited at 295 nm

Luminescence Intensity Decay of Arginine-HCl **(A)**; Arginine **(B)**; Histidine **(C);** Aspartate-KCl **(D)**; poly-Arginine-HCl **(E)**;

poly-Aspartate-NaCl **(F)** and Symfoil PV2 **(G)** when excited at 295 nm is shown here along with their respective residuals.

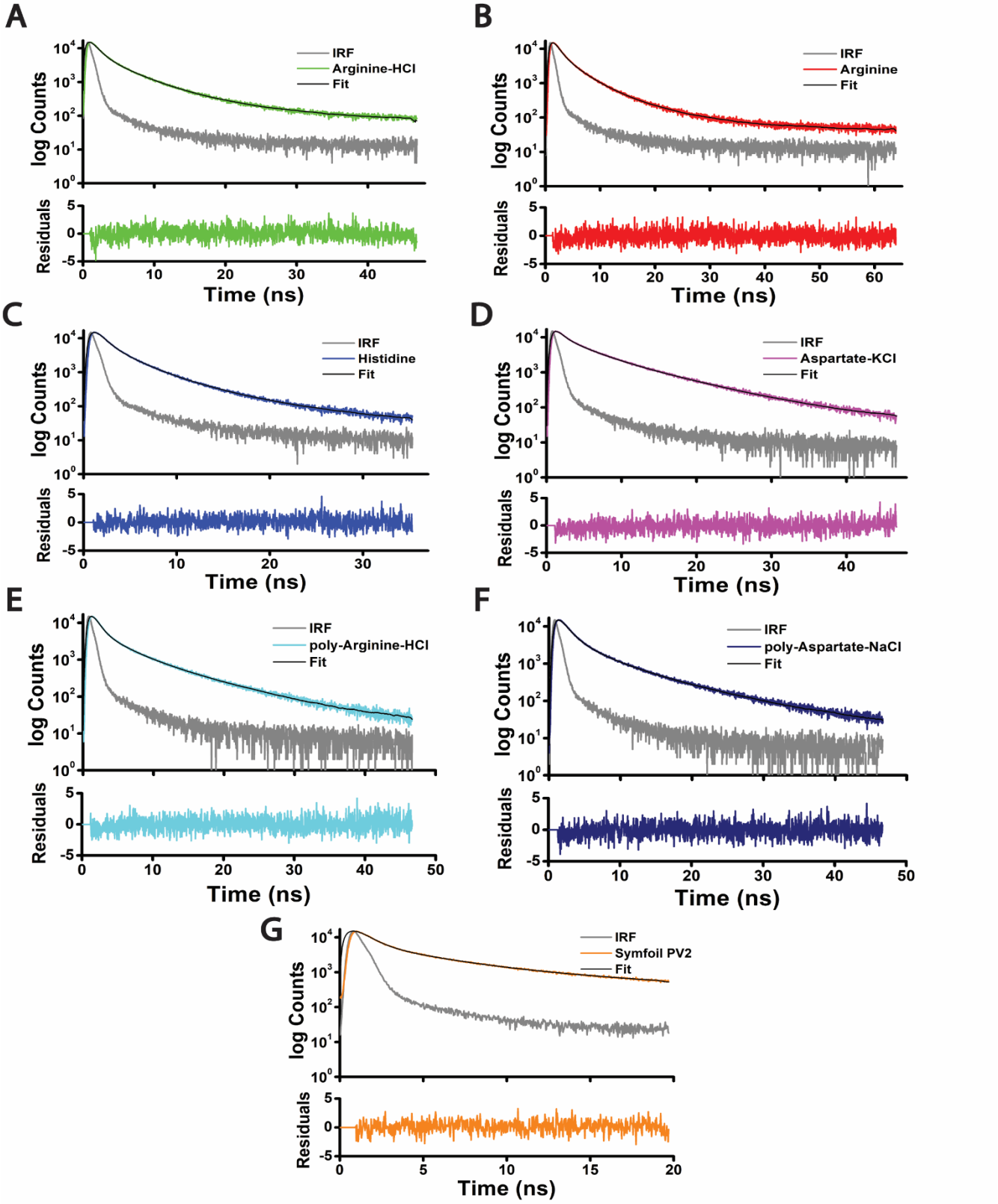

Figure S12. Luminescence intensity decay when excited at 340 nm

Luminescence Intensity Decay of Arginine-HCl **(A)**; Arginine **(B)**; Histidine **(C)**; Aspartate-KCl **(D)**; poly-Arginine-HCl **(E)**; poly-Aspartate-NaCl **(F)** and Symfoil PV2 **(G)** when excited at 340 nm is shown here along with their respective residuals.

	τ_1(ns)	τ_2(ns)	τ_3(ns)	α_1	α_2	α_3	τ_{mean}(ns)	χ^2
Arginine-HCl	0.48(0.027)	2.16(0.065)	6.26(0.050)	0.807	0.162	0.031	0.92(0.043)	1.26
Arginine	0.54(0.004)	2.28(0.022)	6.20(0.091)	0.752	0.213	0.035	1.10(0.003)	1.14
Histidine	1.00(0.010)	2.94(0.015)	10.23(0.309)	0.691	0.291	0.018	1.73(0.002)	1.07
Aspartate-KCl	0.68(0.013)	3.49(0.038)	9.22(0.054)	0.732	0.246	0.083	2.10(0.025)	1.01
Poly-Arginine-HCl	0.76(0.016)	3.23(0.055)	10.03(0.048)	0.796	0.171	0.033	1.48(0.008)	1.13
Poly-Aspartate-NaCl	1.15(0.012)	4.18(0.114)	11.43(0.108)	0.869	0.104	0.027	1.76(0.003)	1.10
Symfoil PV2	0.40(0.007)	3.03(0.113)	7.69(0.219)	0.869	0.086	0.045	0.90(0.049)	1.08

Table S5. Parameters obtained from 3 exponential fit at excitation wavelength 295 nm

Parameters obtained from 3 exponential fit for all the samples at excitation wavelength 295 nm. The individual lifetime components τ_i and mean lifetime τ_{mean} values obtained from 3 exponential fit are averaged for 2 independent measurements and shown here, while the values in the parenthesis represent their standard deviation. The amplitude values α_i along with the reduced Chi. Square shown here represent the best fit data from the 2 measurements for each sample.

	τ_1(ns)	τ_2(ns)	τ_3(ns)	α_1	α_2	α_3	τ_{mean}(ns)	χ^2
Arginine-HCl	1.05(0.009)	3.77(0.048)	10.55(0.497)	0.730	0.253	0.017	2.00(0.030)	1.08
Arginine	1.07(0.065)	3.34(0.224)	9.36(1.084)	0.664	0.298	0.038	1.96(0.003)	1.02
Histidine	0.76(0.053)	2.38(0.146)	6.18(0.228)	0.665	0.281	0.054	1.54(0.038)	1.09
Aspartate-KCl	0.65(0.058)	2.83(0.169)	7.85(0.132)	0.546	0.284	0.170	2.52(0.068)	1.07
Poly-Arginine-HCl	0.76(0.005)	2.78(0.125)	7.78(0.228)	0.751	0.176	0.073	1.60(0.011)	1.13
Poly-Aspartate-NaCl	1.11(0.018)	3.18(0.210)	8.56(0.477)	0.754	0.182	0.063	2.01(0.008)	1.06
Symfoil PV2	0.69(0.039)	3.18(0.190)	13.03(0.717)	0.763	0.186	0.051	1.82(0.048)	1.01

Table S6. Parameters obtained from 3 exponential fit at excitation wavelength 340 nm

Parameters obtained from 3 exponential fit for all the samples at excitation wavelength 340 nm. The individual lifetime components τ_i and mean lifetime τ_{mean} values obtained from 3 exponential fit are averaged for 2 independent measurements and shown here, while the values in the parenthesis represent their standard deviation. The amplitude values α_i along with the reduced Chi. Square shown here represent the best fit data from the 2 measurements for each sample.

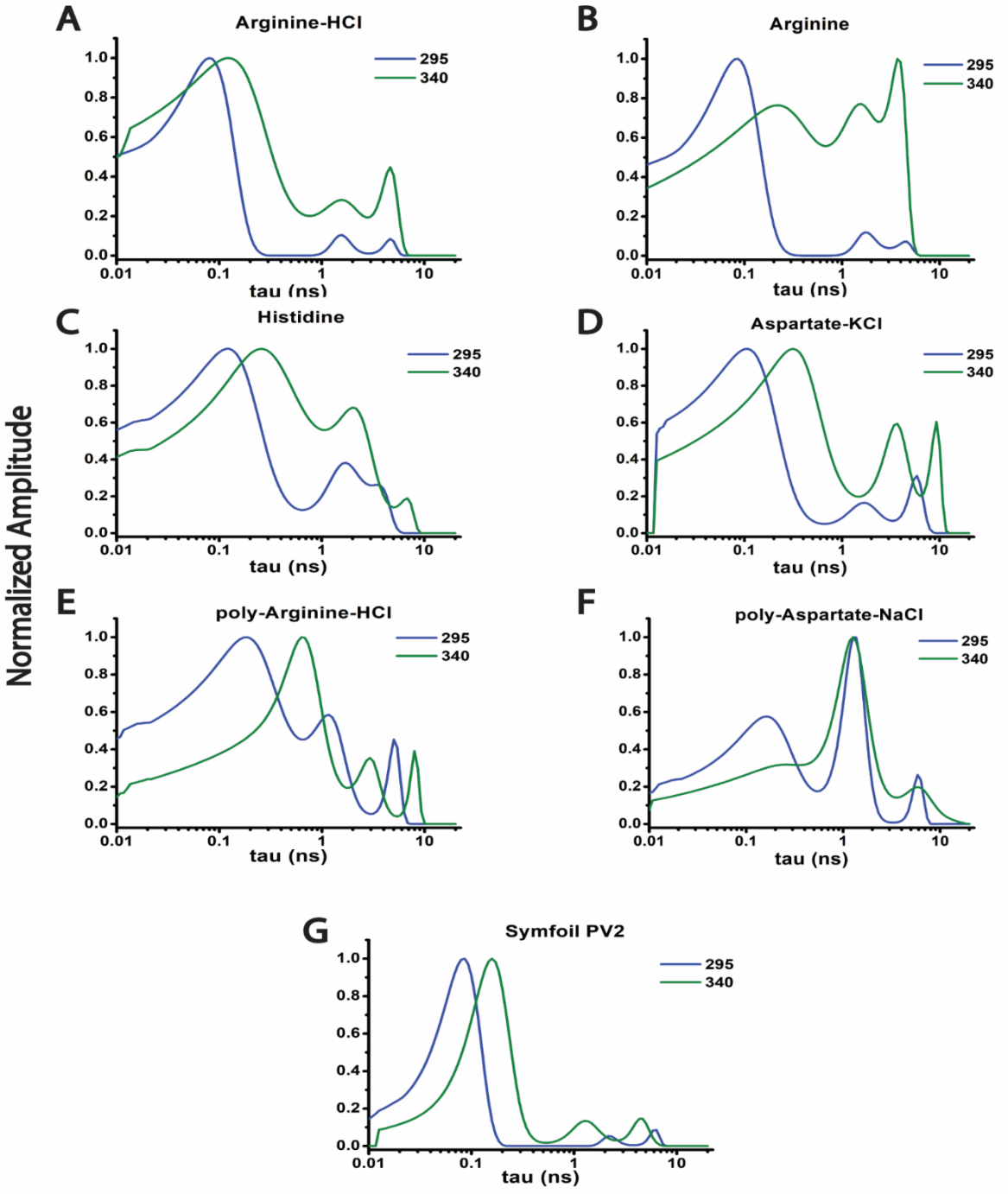

Figure S13: Luminescence Lifetime distribution from MEM analysis

MEM luminescence lifetime distribution on excitation at 295 nm and 340 nm for Arginine-HCl, Arginine, Histidine, Aspartate-KCl, poly-Arginine-HCl, poly-Aspartate-NaCl, and Symfoil PV2 are shown in subfigures A to G, respectively

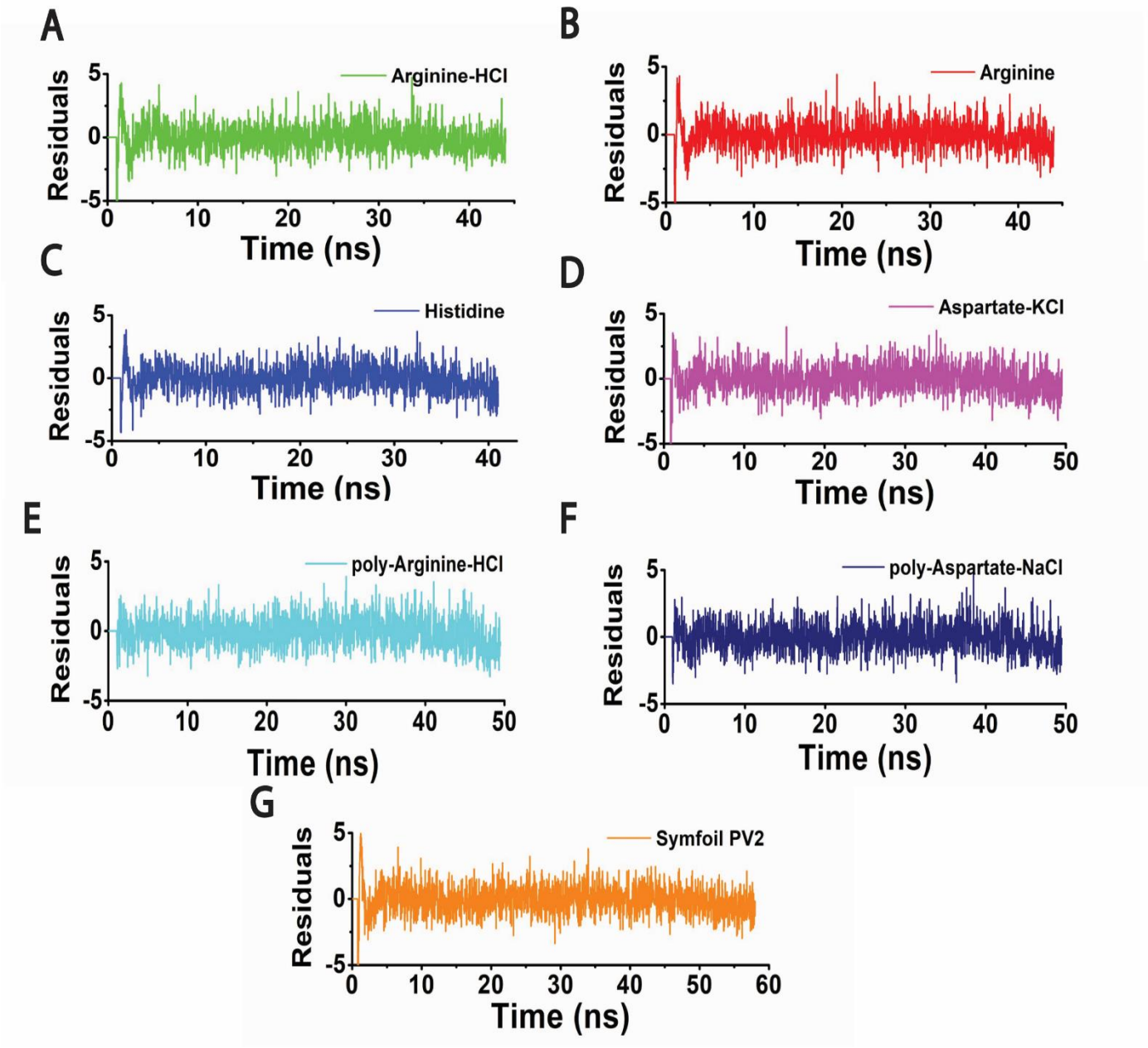

Figure S14. Residuals of MEM distribution at 295 nm excitation

Residuals of luminescence lifetime decay by MEM analysis at 295 nm excitation is shown here. Panel represent residuals for fitted data of Arginine-HCl **(A)**; Arginine **(B)**; Histidine **(C)**; Aspartate-KCl **(D)**; poly-Arginine-HCl **(E)**; poly-Aspartate-NaCl **(F)** and Symfoil PV2 **(G)**.

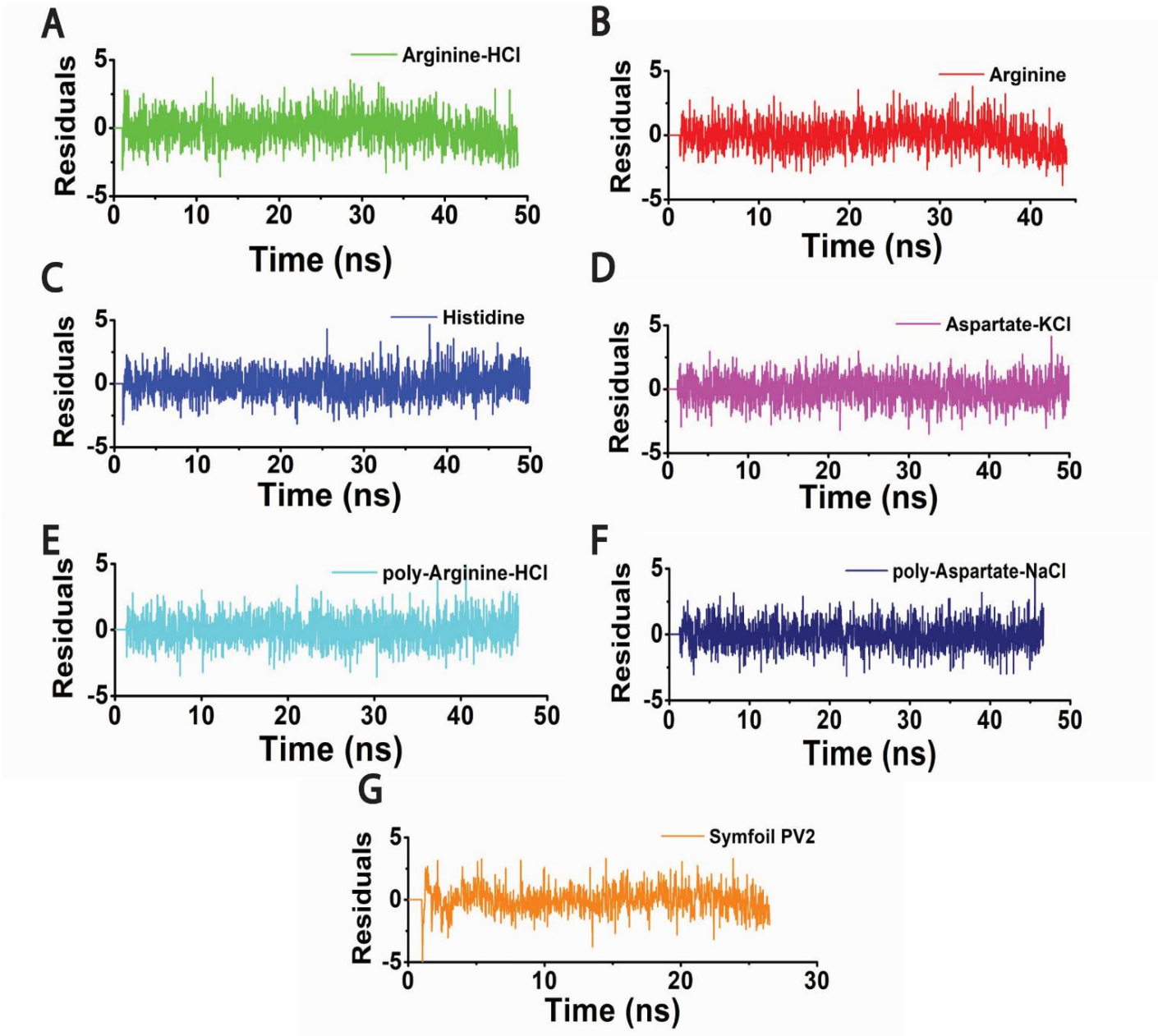

Figure S15. Residuals of MEM distribution at 340 nm excitation

Residuals of luminescence lifetime decay by MEM analysis at 340 nm excitation is shown here. Panel represent residuals for fitted data of Arginine-HCl **(A)**; Arginine **(B)**; Histidine **(C)**; Aspartate-KCl **(D)**; poly-Arginine-HCl **(E)**; poly-Aspartate-NaCl **(F)**; and Symfoil PV2 **(G)**.