Electronic Supplementary Information for

Identifying the Protonation Site and the Scope of Non-Proline Cis-Peptide Bond Conformations: A First-Principles Study on Protonated Oligopeptides

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Table S1 The relative electronic energies, relative zero-point vibrational energy correction (ΔZPVE), relative Gibbs free energy correction (ΔG) and the corrected electronic and Gibbs free energies (in kcal mol^{-1}) of the most stable conformers of different GGGGH isomers at 298 K, with optimization at the B3LYP-D3(BJ)/6-311++G (d, p) level and single-point energy calculations by the MP2/6-311++G (2df, 2p) and advanced DSD-PBEP86-D3(BJ)/aug-cc-pVTZ methods.

Conf.	Relative electronic energy		ΔZPVE	ΔG	Corrected relative electronic energy		Corrected relative Gibbs free energy	
	E^{mp2}	E^{dsd}			E^{mp2}	E^{dsd}	G^{mp2}	G^{dsd}
$GGGGH_{a1}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$GGGGH_{a2}$	-0.16	0.38	0.32	0.22	0.16	0.71	0.06	0.60
$GGGGH_{a3}^{*}$	1.46	1.22	-0.23	-1.26	1.23	0.99	0.21	-0.04
$GGGGH_{a4}$	0.82	1.67	-0.33	-0.07	0.49	1.34	0.75	1.60
$GGGGH_{a5}$	0.51	1.60	0.18	0.37	0.69	1.78	0.88	1.97
$GGGGH_{a6}$	1.48	2.00	-0.20	0.50	1.28	1.80	1.98	2.50
$GGGGH_{a7}^{*}$	2.70	2.81	-0.34	0.14	2.36	2.47	2.85	2.95
$GGGGH_{a8}^{*}$	2.75	3.11	-0.46	0.14	2.30	2.65	2.90	3.25
$GGGGH_{a9}$	2.12	2.58	0.09	0.25	2.21	2.67	2.37	2.83
$GGGGH_{a10}^{*}$	3.45	3.20	-0.10	-0.01	3.35	3.11	3.43	3.19
$GGGGH_{b1}^{*}$	10.62	9.31	-2.12	-3.92	8.51	7.19	6.70	5.38
$GGGGH_{b2}$	13.22	10.86	-2.88	-6.19	10.34	7.99	7.03	4.68
$GGGGH_{b3}$	13.30	10.93	-2.90	-6.23	10.40	8.04	7.07	4.70
$GGGGH_{b4}$	12.36	10.82	-2.14	-4.26	10.22	8.69	8.09	6.56
$GGGGH_{b5}^{*}$	12.82	11.22	-1.80	-3.91	11.01	9.42	8.91	7.31
$GGGGH_{c1}^{*}$	8.2	7.32	-1.07	0.02	7.13	6.24	8.23	7.34
$GGGGH_{c2}$	13.5	11.12	-3.8	-7.34	9.71	7.33	6.16	3.78
$GGGGH_{c3}$	13.29	11.31	-2.43	-5.03	10.87	8.89	8.26	6.29
$GGGGH_{c4}$	13.57	11.45	-2.52	-5.17	11.05	8.93	8.39	6.27
$GGGGH_{c5}$	13.63	11.67	-2.36	-5.09	11.27	9.31	8.54	6.58
$GGGGH_{d1}$	11.63	10.15	-2.3	-4.05	9.32	7.84	7.58	6.1
$GGGGH_{d2}$	12.32	10.57	-2.6	-4.94	9.72	7.97	7.38	5.63
$GGGGH_{d3}$	13.27	12.03	-2.31	-4.31	10.95	9.71	8.96	7.72
$GGGGH_{d4}$	13.96	12.48	-2.58	-5.16	11.38	9.9	8.8	7.32
$GGGGH_{d5}^{*}$	15.45	13.53	-2.74	-5.05	12.72	10.79	10.4	8.47

Table S2 The relative electronic energies, relative zero-point vibrational energy correction (ΔZPVE), relative Gibbs free energy correction (ΔG) and the corrected electronic and Gibbs free energies (in kcal mol^{-1}) of the most stable conformers of different GGGGGH isomers at 298 K, with optimization at the B3LYP-D3(BJ)/6-311++G (d, p) level and single-point energy calculations by the MP2/6-311++G (2df, 2p) and advanced DSD-PBEP86-D3(BJ)/aug-cc-pVTZ methods.

Conf.	Relative electronic energy		ΔZPVE	ΔG	Corrected relative electronic energy		Corrected relative Gibbs free energy	
	E^{mp2}	E^{dsd}			E^{mp2}	E^{dsd}	G^{mp2}	G^{dsd}
$GGGGGH_{a1}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$GGGGGH_{a2}$	1.56	0.87	-0.64	-0.82	0.92	0.23	0.74	0.05
$GGGGGH_{a3}$	0.32	0.31	0.18	0.75	0.50	0.49	1.07	1.06
$GGGGGH_{a4}$	1.10	0.76	0.22	0.77	1.32	0.99	1.86	1.53
$GGGGGH_{a5}$	0.71	0.81	0.30	0.20	1.02	1.12	0.91	1.01
$GGGGGH_{a6}$	2.41	2.33	-0.53	-1.40	1.88	1.80	1.01	0.93
$GGGGGH_{a7}^{*}$	3.90	3.19	-1.01	-3.00	2.89	2.18	0.89	0.19
$GGGGGH_{a8}^{*}$	4.37	3.46	-0.93	-3.05	3.44	2.54	1.32	0.41
$GGGGGH_{a9}$	3.67	3.18	-0.27	-0.86	3.40	2.92	2.80	2.32
$GGGGGH_{a10}$	3.95	4.06	-1.12	-3.00	2.83	2.94	0.95	1.06
$GGGGGH_{b1}$	11.36	10.33	-1.56	-3.03	9.80	8.78	8.34	7.31
$GGGGGH_{b2}$	11.80	10.90	-1.59	-1.96	10.21	9.32	9.85	8.95
$GGGGGH_{b3}$	12.36	11.28	-1.55	-3.36	10.81	9.73	9.01	7.92
$GGGGGH_{b4}$	13.01	11.43	-1.49	-3.06	11.51	9.93	9.95	8.37
$GGGGGH_{b5}$	12.80	12.11	-1.78	-3.89	11.02	10.33	8.91	8.21
$GGGGGH_{c1}^{*}$	13.15	10.97	-2.71	-3.48	10.44	8.26	9.67	7.49
$GGGGGH_{c2}^{*}$	14.84	12.4	-2.47	-3.91	12.36	9.93	10.92	8.49
$GGGGGH_{c3}^{*}$	15.25	12.84	-2.44	-3.94	12.81	10.41	11.31	8.90
$GGGGGH_{c4}^{*}$	16.20	13.88	-2.55	-3.32	13.65	11.33	12.88	10.56
$GGGGGH_{c5}$	15.89	14.16	-1.5	-2.02	14.39	12.66	13.87	12.13

Table S3 The relative electronic energies, relative zero-point vibrational energy correction (ΔZPVE), relative Gibbs free energy correction (ΔG) and the corrected electronic and Gibbs free energies (in kcal mol^{-1}) of the most stable conformers of different GAGH isomers at 298 K, with optimization at the B3LYP-D3(BJ)/6-311++G (d, p) level and single-point energy calculations by the MP2/6-311++G (2df, 2p) and advanced DSD-PBEP86-D3(BJ)/aug-cc-pVTZ methods.

Conf.	Relative electronic energy		ΔZPVE	ΔG	Corrected relative electronic energy		Corrected relative Gibbs free energy	
	E^{mp2}	E^{dsd}			E^{mp2}	E^{dsd}	G^{mp2}	G^{dsd}
$GAGH_{a1}$*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$GAGH_{a2}$	0.75	0.71	-0.17	-0.43	0.59	0.54	0.32	0.28
$GAGH_{a3}$*	1.83	1.80	-0.27	-0.83	1.56	1.53	1.00	0.97
$GAGH_{a4}$*	1.84	1.89	0.13	0.37	1.97	2.02	2.22	2.27
$GAGH_{a5}$*	1.84	1.90	0.13	0.36	1.97	2.03	2.21	2.26
$GAGH_{a6}$	2.94	2.84	-0.22	-0.43	2.72	2.62	2.52	2.41
$GAGH_{a7}$	2.70	2.89	-0.05	0.04	2.64	2.83	2.74	2.93
$GAGH_{a8}$	2.74	2.86	0.23	1.11	2.97	3.09	3.85	3.97
$GAGH_{a9}$*	3.64	3.27	-0.06	-0.87	3.58	3.21	2.78	2.40
$GAGH_{a10}$*	3.89	3.75	-0.31	-0.57	3.59	3.44	3.32	3.18
$GAGH_{b1}$	3.80	1.98	-1.95	-2.82	1.85	0.03	0.99	-0.84
$GAGH_{b2}$	4.41	2.38	-2.07	-2.85	2.34	0.30	1.56	-0.47
$GAGH_{b3}$	4.62	3.05	-1.76	-2.84	2.86	1.29	1.78	0.21
$GAGH_{b4}$	4.72	3.30	-1.72	-2.79	3.00	1.58	1.93	0.51
$GAGH_{b5}$	5.26	3.64	-1.78	-2.75	3.49	1.86	2.51	0.89
$GAGH_{c1}$	9.84	7.94	-1.62	-2.07	8.22	6.32	7.77	5.87

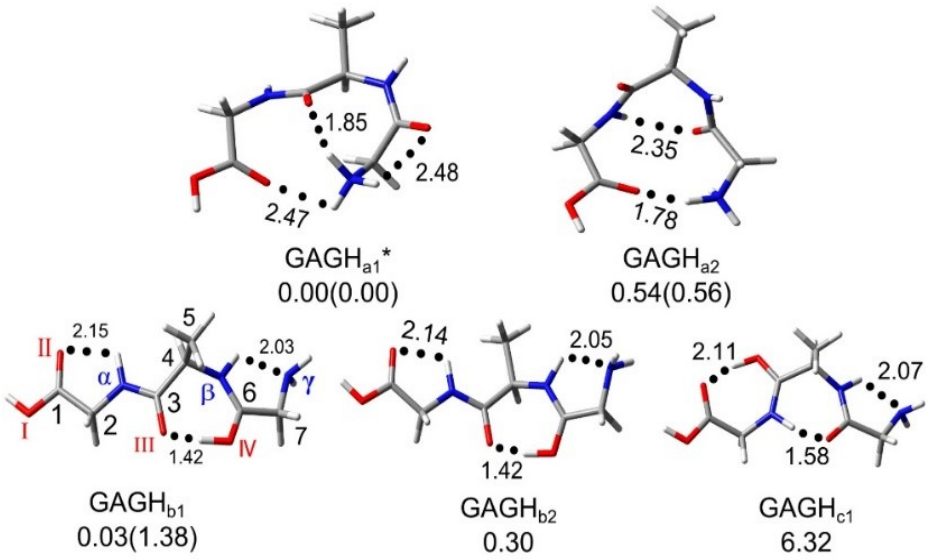

Fig. S1 Geometric structures of the most stable conformers of different isomers of GAGH and their relative energies based on temperature independent electronic energies (in kcal mol^{-1}) calculated at the DSD-PBEP86-D3(BJ)/aug-cc-pVTZ level with zero-point correction from B3LYP-D3(BJ)/6-311++G (d, p). The relative energies in parentheses were determined by further CBS-QB3 calculations.

Table S4 Theoretical values of PA and GB (in kcal mol^{-1}) for different oligopeptides GG, AAA, GAG, GGGG and GGGGG based on different protonated isomers and the corresponding neutral molecules calculated at the DSD-PBEP86-D3(BJ)/aug-cc-pVTZ level with enthalpy and Gibbs free energy corrections at the B3LYP-D3(BJ)/6-311++G (d, p) level, together with previous experimental results [a-e] for comparison.

	GG						
	Experiment			Theory			
	MIKE[a]	FT-MS[b]	FT-ICR[c]	GGH_a	GGH_a^*	GGH_b	Ave
PA	219.1±0.8	224.5	223.6	221.66	219.96	220.79	221.58
GB	-	213.5±2	215.3±2	213.38	210.96	212.04	213.25
	AAA						
	Experiment			Theory			
	Ref.[d]			$AAAH_a^*$	$AAAH_a$	$AAAH_b$	Ave
PA	-	-	-	233.16	230.83	231.49	232.94
GB	221.08	-	-	224.32	221.19	223.00	224.14
	GAG						
	Experiment			Theory			
			FT-ICR[e]	$GAGH_a^*$	$GAGH_a$	$GAGH_b$	Ave
PA		-	226.8	228.37	228.01	228.28	228.35
GB		-	218.5±2.4	219.32	219.18	220.11	220.00
	GGGG							
	Experiment			Theory				
	MIKE[a]	FT-MS[b]	FT-ICR[c]	$GGGGH_a$	$GGGGH_a^*$	$GGGGH_b$	$GGGGH_b^*$	$GGGGH_c$
PA	227.2±0.4	226	233.3	232.68	231.36	224.89	224.16	224.68
GB	-	215±2	225±2.3	221.69	221.84	216.38	217.23	218.10
	GGGGG							
	Experiment			Theory				
	MIKE[a]	FT-MS[b]	FT-ICR[c]	$GGGGGH_a$	$GGGGGH_a^*$	$GGGGGH_b$	$GGGGGH_c$	Ave
PA	231.8±0.7	228.1	233.6	235.39	232.84	226.14	226.90	234.55
GB	-	217.1±2	225.3±4	226.48	226.48	219.07	219.03	226.64

[a] MIKE (mass analyzed ion kinetic energy) experiment from ref. 52.

[b] FT-MS (Fourier transform mass spectrometry) experiment form from ref. 53.

[c] FT-ICR (Fourier transform ion cyclotron resonance mass spectrometer) experiment from ref. 54.

[d] from ref. 55.

[e] FT-ICR experiment from ref. 56.

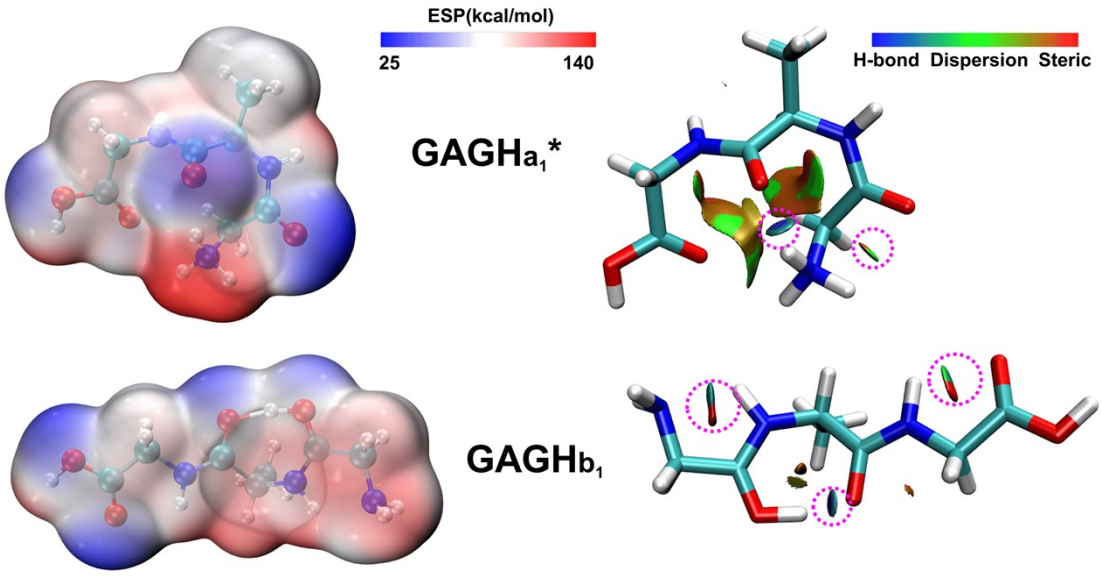

Fig. S2 The ESP distributions and the intra-molecular interactions (including the dispersion interaction) for the most stable conformers of the cis- and trans- tripeptide $GAGH_{a1}^{*}$ and $GAGH_{b1}$.

Table S5 Summary of surface analysis for the protonated oligopeptides

Conf.	**Overall surface area (Å²)**	**V_{max} (kcal/mol)**	**V_{min} (kcal/mol)**	**MPI (Kcal/mol)**
GGH_{a3}*	158.51	149.66	36.01	98.03
GGH_{a1}	168.03	151.84	32.22	93.57
$AAAH_{a1}$*	263.14	135.60	25.54	79.27
$AAAH_{a3}$	256.75	140.16	24.03	78.05
$GAGH_{a1}$*	223.34	132.17	25.31	84.44
$GAGH_{a2}$	227.04	144.50	21.22	82.73
$GGGGH_{a3}$*	253.32	119.07	20.65	79.27
$GGGGH_{a1}$	252.96	134.57	27.75	79.82
$GGGGGH_{a7}$*	298.92	116.03	18.73	73.43
$GGGGGH_{a1}$	275.28	127.35	33.92	75.99

Table S6 Representative isomers of protonated oligopeptides, together with their respective percent abundances at 298K, which were calculated by the DSD-PBEP86-D3(BJ)/aug-cc-pVTZ with Gibbs free energy corrections at the B3LYP-D3(BJ)/6-311++G (d, p) level.

	GGH_a	Percent (%)	GGH_a^*	Percent (%)	GGH_b	Percent (%)	
	GGH_{a1}	84.78	GGH_{a3}^*	43.49	GGH_{b1}	92.60	
	GGH_{a2}	14.88	GGH_{a4}^*	53.27	GGH_{b2}	1.58	
	SUM	99.66	SUM	96.76	GGH_{b3}	4.16	
					SUM	98.34	
$AAAH_a^*$	Percent (%)	$AAAH_a$	Percent (%)	$AAAH_b$	Percent (%)	$AAAH_c$	Percent (%)
$AAAH_{a1}^*$	93.74	$AAAH_{a3}$	65.61	$AAAH_{b1}$	60.88	$AAAH_{c1}$	63.40
$AAAH_{a2}^*$	4.38	$AAAH_{a6}$	19.86	$AAAH_{b2}$	26.88	$AAAH_{c2}$	36.6
SUM	98.12	$AAAH_{a7}$	4.98	$AAAH_{b3}$	10.21	SUM	100
		$AAAH_{a9}$	4.68	SUM	97.97		
		SUM	95.13				
	$GAGH_a^*$	Percent (%)	$GAGH_a$	Percent (%)	$GAGH_b$	Percent (%)	
	$GAGH_{a1}{}^*$	79.40	$GAGH_{a2}$	96.09	$GAGH_{b1}$	52.47	
	$GAGH_{a3}{}^*$	15.39	$GAGH_{a6}$	2.62	$GAGH_{b2}$	28.32	
	SUM	94.79	SUM	98.71	$GAGH_{b3}$	8.97	
					$GAGH_{b4}$	5.37	
					SUM	95.13	
	$GGGGH_a$	Percent (%)	$GGGGH_a{}^*$	Percent (%)	$GGGGH_b{}^*$	Percent (%)	
	$GGGGH_{a1}$	65.84	$GGGGH_{a3}{}^*$	98.57	$GGGGH_{b1}{}^*$	89.87	
	$GGGGH_{a2}$	23.81	$GGGGH_{a7}{}^*$	0.63	$GGGGH_{b5}{}^*$	3.46	
	$GGGGH_{a4}$	4.43	SUM	99.20	$GGGGH_{b7}{}^*$	4.41	
	SUM	94.08			SUM	97.74	
	$GGGGH_b$	Percent (%)	$GGGGH_c$	Percent (%)	$GGGGH_d$	Percent (%)	
	$GGGGH_{b2}$	49.24	$GGGGH_{c2}$	95.20	$GGGGH_{d1}$	28.99	
	$GGGGH_{b3}$	46.85	$GGGGH_{c3}$	1.39	$GGGGH_{d2}$	64.90	
	$GGGGH_{b4}$	2.05	$GGGGH_{c4}$	1.42	$GGGGH_{d4}$	3.69	
	SUM	98.14	SUM	98.01	SUM	97.58	
	$GGGGGH_a$	Percent (%)	$GGGGGH_a{}^*$	Percent (%)	$GGGGGH_b$	Percent (%)	
	$GGGGGH_{a1}$	33.42	$GGGGGH_{a7}{}^*$	59.24	$GGGGGH_{b1}$	42.82	
	$GGGGGH_{a2}$	30.53	$GGGGGH_{a8}{}^*$	40.76	$GGGGGH_{b3}$	15.18	
	$GGGGGH_{a5}$	6.07	SUM	100	$GGGGGH_{b4}$	7.14	
	$GGGGGH_{a6}$	6.93			$GGGGGH_{b5}$	9.30	
	SUM	76.95			$GGGGGH_{b10}$	6.00	
					SUM	80.44	
	$GGGGGH_b{}^*$	Percent (%)	$GGGGH_c$	Percent (%)			
	$GGGGH_{b15}{}^*$	100	$GGGGH_{c1}$	77.98			
	SUM	100	$GGGGH_{c2}$	14.40			
			$GGGGH_{c3}$	7.14			
			SUM	99.52			

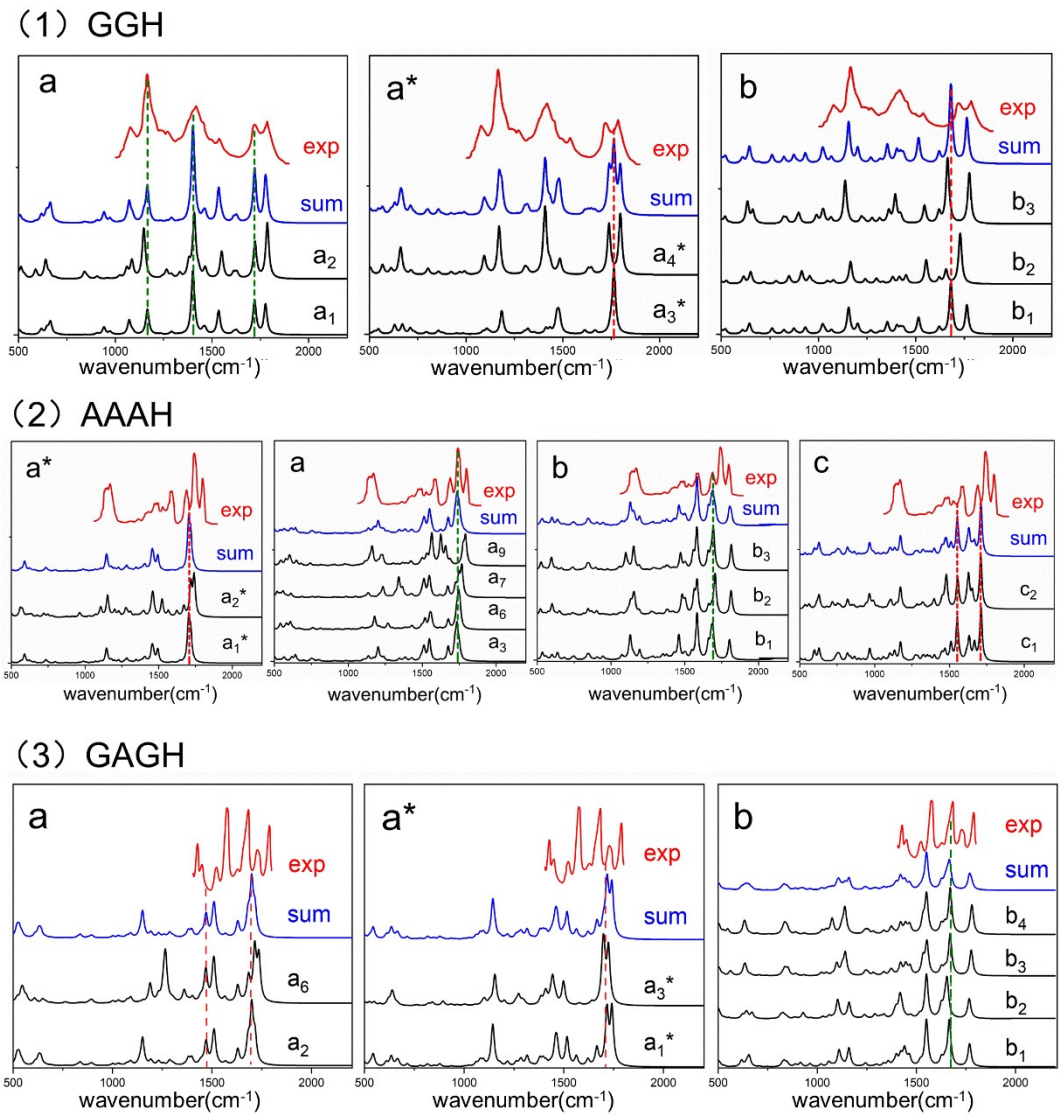

Fig. S3 Simulated IR spectra of the dominant conformers for different isomers of (1) GGH, (2) AAAH and (3) GAGH at 298 K, calculated at the B3LYP-D3(BJ)/6-311++G (d, p) level of theory, as well as their summation ("sum" in blue) calculated using the percentage abundances listed in Table S6, together with the experimental IRMPD results ("exp" in red from ref. 8, 9, 58) for comparison. A Lorentzian profile with a full width at half maximum (FWHM) of 20 cm^{-1} is used to convolute the calculated spectra.

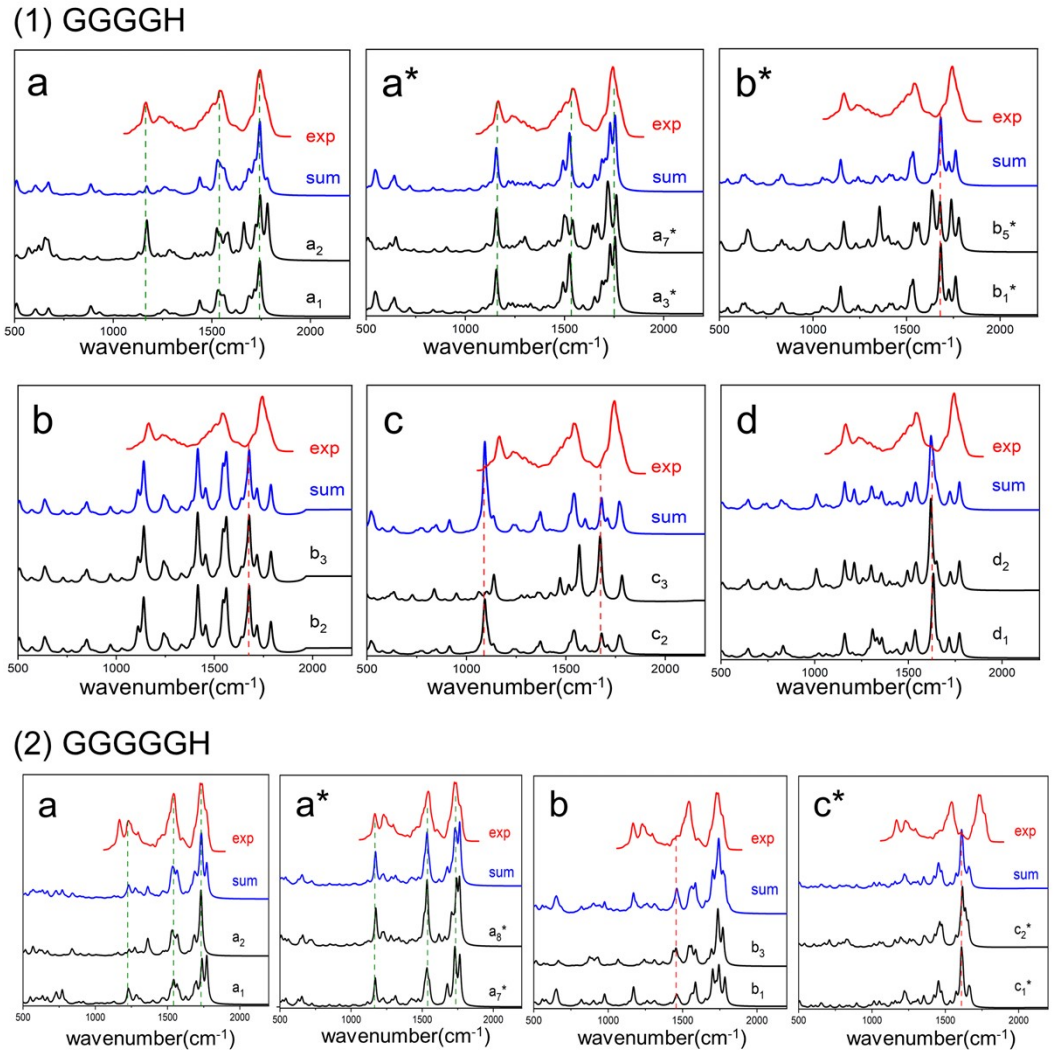

Fig. S4 Simulated IR spectra of the dominant conformers for different isomers of (1) GGGGH and (2) GGGGGH at 298K calculated at the B3LYP-D3(BJ)/6-311++G (d, p) level of theory, as well as their summation ("sum" in blue) calculated using the percentage abundances listed in Table S6, together with the experimental IRMPD results ("exp" in red from ref. 9) for comparison. A Lorentzian profile with a full width at half maximum (FWHM) of 20 cm^{-1} is used to convolute the calculated spectra.

Table S7 Scaling factors obtained according to the IR spectral comparisons between the anharmonic and harmonic frequencies calculations on the most stable conformer of each isomer at the B3LYP-D3(BJ)/6-311++G (d, p) level.

GGH	GGH_a	GGH_{a*}	GGH_b		
	0.98	0.992	0.970		
AAAH	$AAAH_{a*}$	$AAAH_a$	$AAAH_b$	$AAAH_c$	
	0.975	0.975	1	0.970	
GAGH	$GAGH_{a*}$	$GAGH_a$	$GAGH_b$	$GAGH_c$	
	0.972	0.970	0.975	0.975	
GGGH	$GGGGH_a$	$GGGGH_{a*}$	$GGGGH_{b*}$	$GGGGH_c$	$GGGGH_d$
	0.995	0.980	0.980	0.975	0.975
GGGGGH	$GGGGGH_a$	$GGGGGH_{a*}$	$GGGGGH_b$	$GGGGGH_c$	
	0.987	0.995	0.990	0.940	

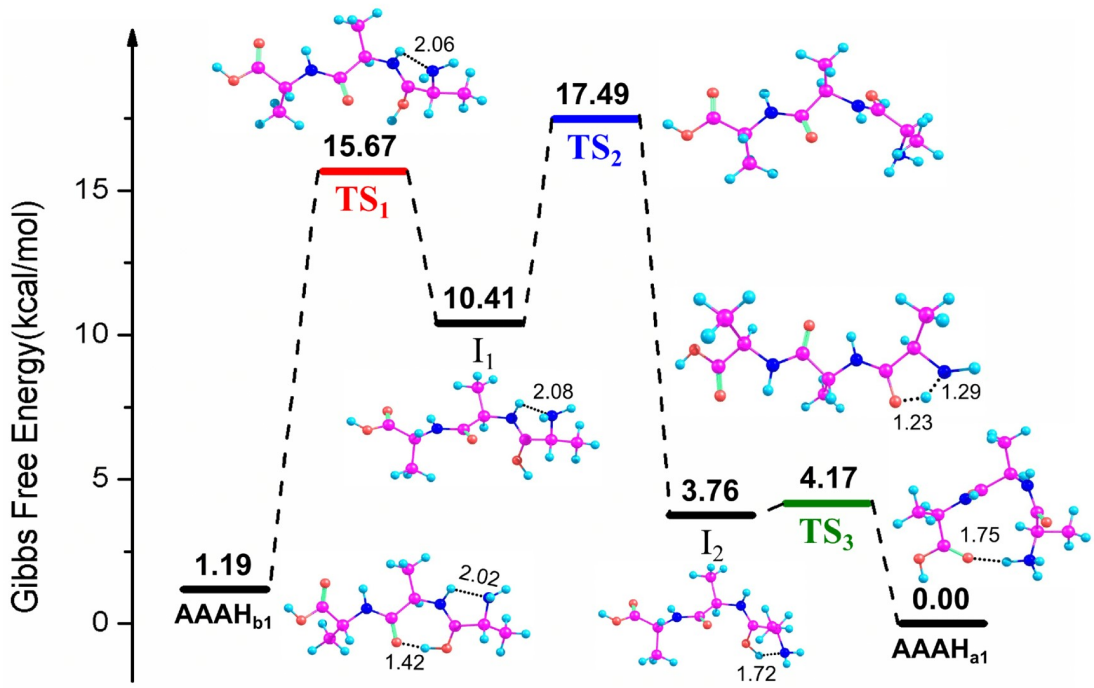

Fig. S5 Transition states (TS) for the interconversion between the most abundant conformers of $AAAH_{a1}$ and $AAAH_{b1}$ at 298K. The relative Gibbs free energy values were determined at the DSD-PBEP86-D3(BJ)/aug-cc-pVTZ level of theory with the Gibbs free energy corrections at the B3LYP-D3(BJ)/6-311++G (d, p) level. Here I_1 and I_2 are two intermediate structures. TS_1: $AAAH_{b1}$ to I_1; TS_2: I_1 to I_2; TS_3: I_2 to $AAAH_{a1}$.

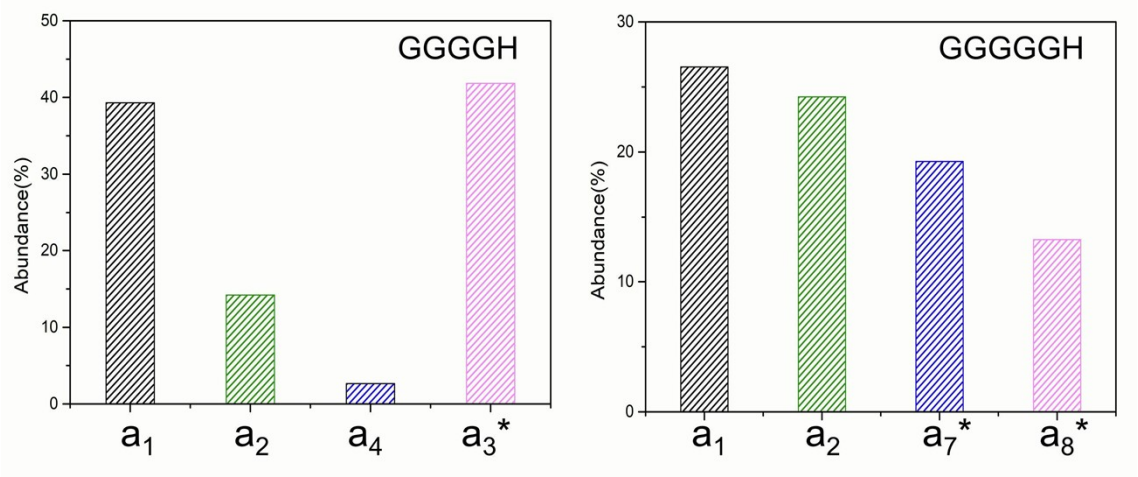

Fig. S6 Percent abundances for different isomers of protonated oligopeptide GGGGH and GGGGGH at 298 K. The abundances were calculated by DSD-PBEP86-D3(BJ)/aug-cc-pVTZ with Gibbs free energy corrections at the B3LYP-D3(BJ)/6-311++G (d, p) level of theory.

Table S8 Representative isomers of protonated oligopeptides GGH, AAAH, GAGH, GGGGH and GGGGGH, together with their respective percent abundances at 498K. The abundances were calculated by DSD-PBEP86-D3(BJ)/aug-cc-pvtz with Gibbs free energy corrections at the B3LYP-D3(BJ)/6-311++G (d, p) level of theory.

GGH	Percent (%)	AAAH	Percent (%)	GAGH	Percent (%)	GGGGH	Percent (%)	GGGGGH	Percent (%)
GGH_{a1}	56.79	$AAAH_{a1}^{*}$	48.37	$GAGH_{a1}^{*}$	7.28	$GGGGH_{a1}$	14.51	$GGGGGH_{a1}$	6.47
GGH_{a2}	22.66	$AAAH_{a2}^{*}$	4.93	$GAGH_{a2}$	6.75	$GGGGH_{a2}$	9.71	$GGGGGH_{a2}$	7.00
GGH_{a4}^{*}	3.89	$AAAH_{a3}$	1.29	$GAGH_{b1}$	31.87	$GGGGH_{a3}^{*}$	45.31	$GGGGGH_{a10}$	11.46
GGH_{b1}	11.03	$AAAH_{b1}$	15.18	$GAGH_{b2}$	20.21	$GGGGH_{a7}^{*}$	16.60	$GGGGGH_{a11}$	11.50
SUM	94.37	$AAAH_{b2}$	14.55	$GAGH_{b3}$	13.07	$GGGGH_{b1}^{*}$	0.37	$GGGGGH_{a7}^{*}$	32.60
		$AAAH_{b3}$	7.09	$GAGH_{b4}$	9.58	$GGGGH_{b2}$	2.29	$GGGGGH_{a8}^{*}$	30.93
		$AAAH_{c1}$	0.05	$GAGH_{c1}$	0.00	$GGGGH_{c2}$	7.47	$GGGGGH_{b1}$	0.01
		SUM	91.46	SUM	88.76	$GGGGH_{d2}$	0.46	$GGGGGH_{c1}$	0.00
						SUM	96.72	SUM	99.97

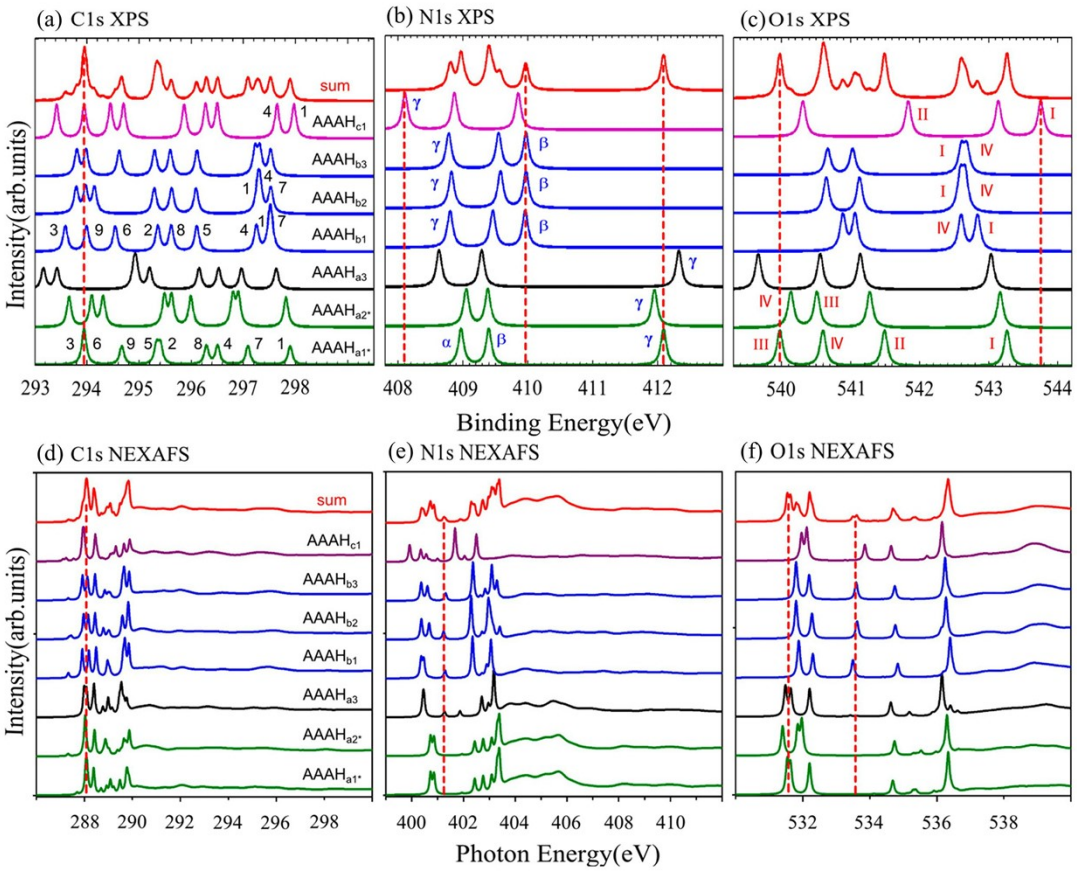

Fig. S7 Calculated (a) C1s, (b) N1s, and (c) O1s XPS spectra and (d) C1s, (d) N1s, and (f) O1s NEXAFS spectra of the low-energy conformers of AAAH as well as the averaged spectra ("sum" plotted in red) at 498 K, computed according to their equilibrium distributions. Spectra of each conformer are plotted in a colour connoting the isomer to which the conformer belongs.

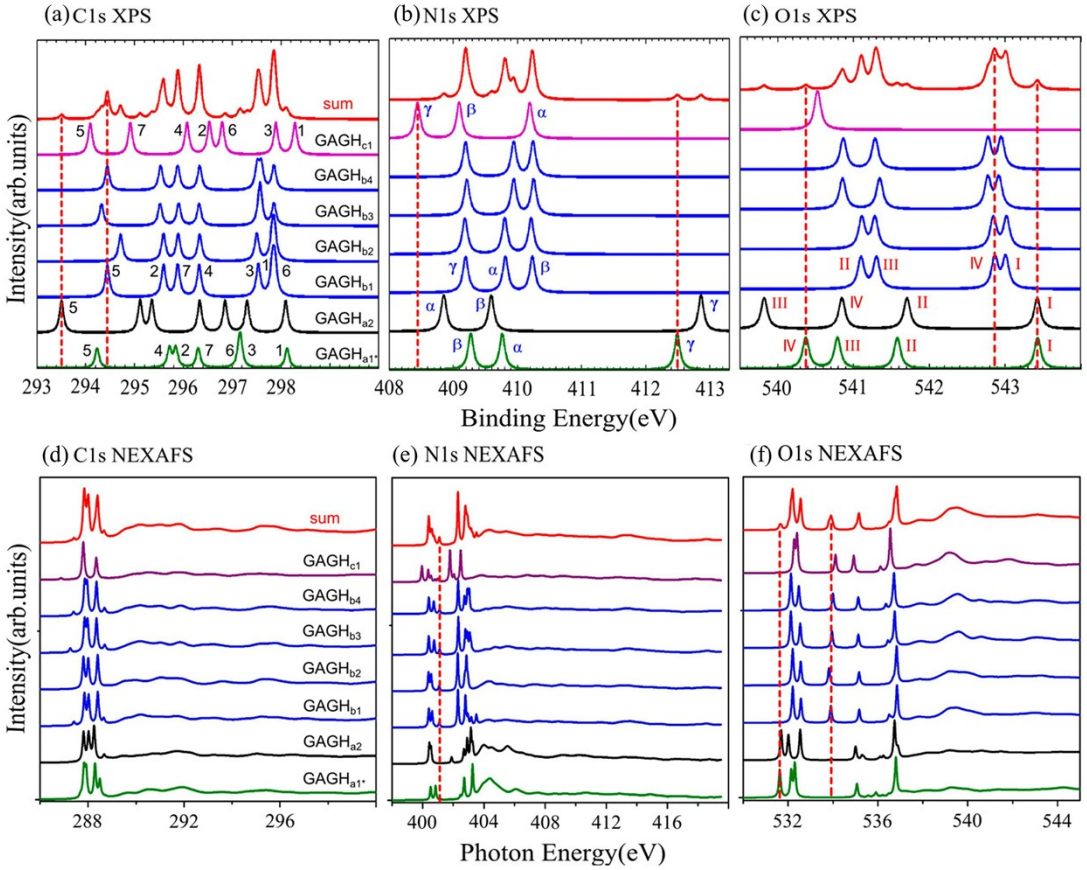

Fig. S8 Calculated (a) C1s, (b) N1s, and (c) O1s XPS spectra and (d) C1s, (d) N1s, and (f) O1s NEXAFS spectra of the lowest-energy conformers of GAGH as well as the averaged spectra ("sum" plotted in red) at 498 K, computed according to their equilibrium distributions. Spectra of each conformer are plotted in a colour connoting the isomer to which the conformer belongs.

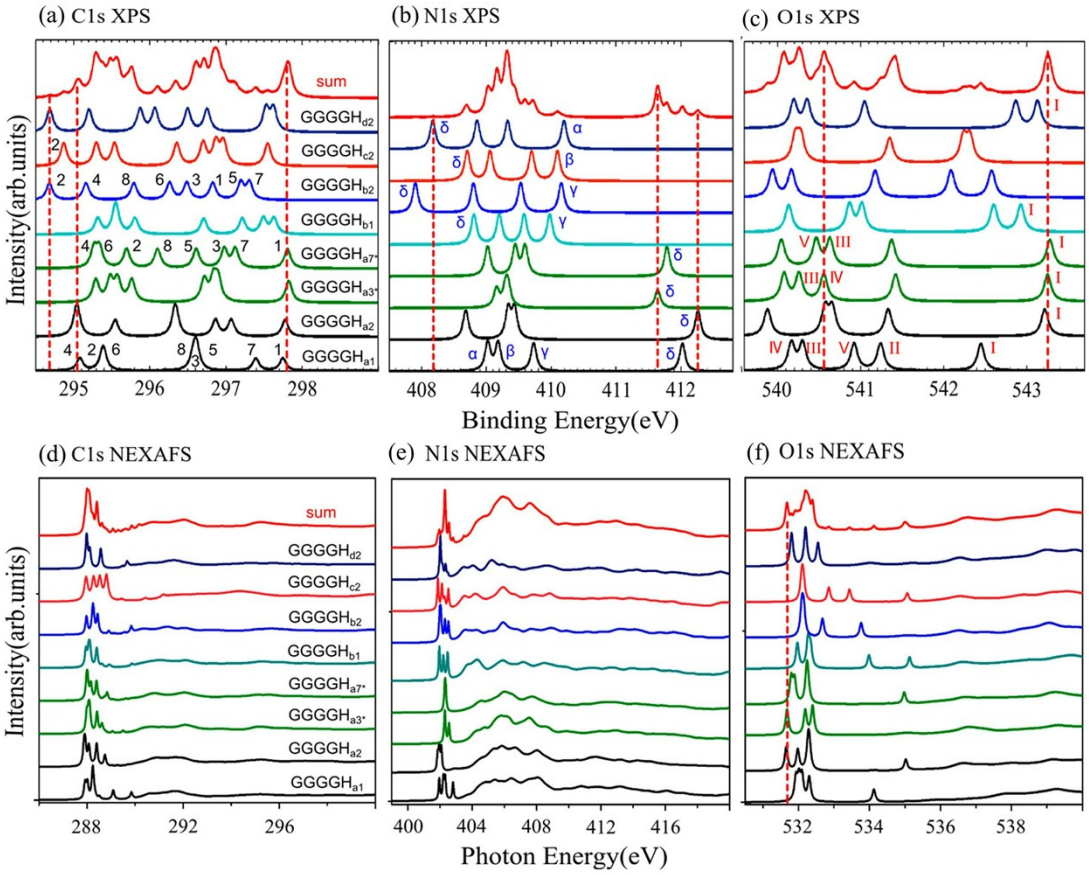

Fig. S9 Calculated (a) C1s, (b) N1s, and (c) O1s XPS spectra and (d) C1s, (d) N1s, and (f) O1s NEXAFS spectra of the lowest-energy conformers of GGGGH as well as the averaged spectra ("sum" plotted in red) at 498 K, computed according to their equilibrium distributions. Spectra of each conformer are plotted in a colour connoting the isomer to which the conformer belongs.

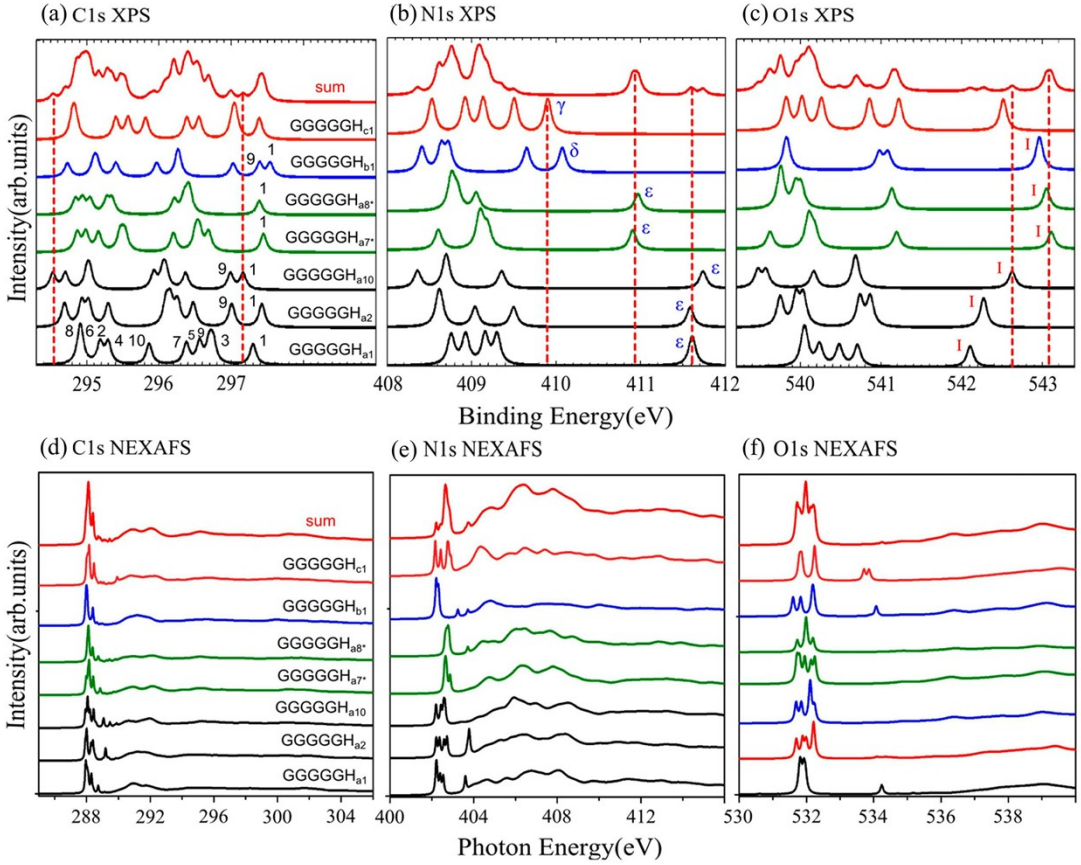

Fig. S10 Calculated (a) C1s, (b) N1s, and (c) O1s XPS spectra and (d) C1s, (d) N1s, and (f) O1s NEXAFS spectra of the lowest-energy conformers of GGGGGH as well as the averaged spectra ("sum" plotted in red) at 498 K, computed according to their equilibrium distributions. Spectra of each conformer are plotted in a colour connoting the isomer to which the conformer belongs.