Supplementary Table 1

Identified INTRA-peptide BS^3 and CBDPS cross-links in isolated lipid-free, monomeric apoA-I samples derived from mixed ^{14}N and ^{15}N labeled proteins

Cross-link	Peptides involved[a]	Mod.[b]	X-Linker	Peptide mass[c]		Span
				^{14}N	^{15}N	
				Da		
S87-K88	84-QEM**SK**DLEEVK-94	XL	BS^3	1472.72	1486.68	Intra
		--[d]	--[d]			Intra
K12-K23	11-V**K**DLATVYVDVL**K**DSGR-27	XL	BS^3	2015.14	2037.08	Intra
		XL	CBDPS	2386.15	2408.09	Intra
S55-K59	46-LLDNWDSVT**S**TFS**K**LR-61	XL	BS^3	2019.05	2040.98	Intra
		XL	CBDPS	2390.09	2412.04	Intra
K94-K96	89-DLEEV**K**A**K**VQPYLDDFQK-106	XL	BS^3	2302.20	2325.13	Intra
		XL	CBDPS	2673.24	2696.18	Intra
K133-K140	132-Q**K**LHELQE**K**LSPLGEEMR-149	XL	BS^3	2302.22	2329.14	Intra
		XL	CBDPS	2673.26	2700.18	Intra
K206-K208	196-ATEHLSTLSE**K**A**K**PALEDLR-215	XL	BS^3	2346.26	2373.18	Intra
		XL	CBDPS	2717.30	2744.22	Intra
K140-S142	132-QKLHELQE**K**L**S**PLGEEMR-149	XL,H	BS^3	2458.30	2485.23	Intra
		--[d]	--[d]			--[d]
S36-K40	28-DYVSQFEG**S**ALG**K**QLNLK-45	--[d]	--[d]			--[d]
		XL	CBDPS	2505.15	2528.11	Intra
K106-K107	97-VQPYLDDFQ**KK**WQEEMELYR-116	XL	BS^3	2782.36	2811.28	Intra
		--[d]	--[d]			--[d]
K52-K59		--[d]	--[d]			--[d]
	46-LLDNWD**S**VTSTFS**K**LR-61	XL	CBDPS	2390.09	2412.04	Intra

[a] Lysines or serines involved in cross-links are in bold.

[b] Chemical modifications: XL = 1 complete cross-link (BS^3 +138.06808 Da, CBDPS +509.09682 Da), H = 1 hydrolyzed cross-linker (BS^3: +156.07864 Da, CBDPS: +527.10738 Da).

[c] Experimentally derived monoisotopic mass for each peptide with each isotope and the combinations.

[d] Not detected. These ions were detectable in one sample set; for example CBDPS but not BS^3.

Supplementary Table 2

Identified INTER-peptide BS^3 and CBDPS cross-links in isolated lipid-free, monomeric apoA-I samples derived from mixed ^{14}N and ^{15}N labeled proteins

Cross-link	Peptides involved[a]	Mod.[b]	X-Linker	Peptide mass[c] ^{14}N	Peptide mass[c] ^{15}N	Span
				Da		
K118-K239	117-Q**K**VEPLR-123	XL	BS^3	1608.93	1628.87	Intra
	239-**K**LNTQ-243	XL	CBDPS	1319.98	1999.93	Intra
K94-K239	89-DLEEV**K**AK-96	XL	BS^3	1670.93	1688.88	Intra
	239-**K**LNTQ-243	XL	CBDPS	2041.96	2059.91	Intra
K133-K239	132-Q**K**LHELQEK-140	XL	BS^3	1892.06	1914.99	Intra
	239-**K**LNTQ-243	XL	CBDPS	2263.09	2286.03	Intra
K182-K239	178-LEAL**K**ENGGAR-188	XL	BS^3	1897.04	1920.97	Intra
	239-**K**LNTQ-243	XL	CBDPS	2268.08	2292.01	Intra
NT-239	-1-**G**DEPPQSPWDR-10	XL	BS^3	2022.98	2046.92	Intra
	239-**K**LNTQ-243	XL	CBDPS	2394.02	2417.96	Intra
K118-S142	117-Q**K**VEPLR-123	XL	BS^3	2037.11	2061.04	Intra
	141-L**S**PLGEEMR-149	XL	CBDPS	2408.15	2432.08	Intra
S167-K239	161-THLAPY**S**DELR-171	XL	BS^3	2041.07	2065.00	Intra
	239-**K**LNTQ-243	XL	CBDPS	2412.13	2432.09	Intra
S87-K239	84-QEM**S**KDLEEVK-94	XL	BS^3	2075.06	2097.00	Intra
	239-**K**LNTQ-243	XL	CBDPS	2446.10	2468.04	Intra
K107-K239	107-**K**WQEEMELYR-116	XL	BS^3	2151.09	2175.02	Intra
	239-**K**LNTQ-243	XL	CBDPS	2522.13	2546.06	Intra
K118-K133	117-Q**K**VEPLR-123	XL	BS^3	2158.23	2185.15	Intra
	132-Q**K**LHELQEK-140	XL	CBDPS	2529.28	2556.20	Intra
K118-K182	117-Q**K**VEPLR-123	XL	BS^3	2163.23	2191.15	Intra
	178-LEAL**K**ENGGAR-188	XL	CBDPS	2534.26	2562.18	Intra
K96-K239	95-A**K**VQPYLDDFQK-106	XL	BS^3	2191.17	2215.10	Intra
	239-**K**LNTQ-243	XL	CBDPS	2562.21	2586.14	Intra
K12-K239	11-V**K**DLATVYVDVLK-23	XL	BS^3	2202.27	2225.21	Intra
	239-**K**LNTQ-243	--[d]	CBDPS	--[d]	--[d]	--[d]
NT-K118	-1-**G**DEPPQSPWDR-10	XL	BS^3	2289.16	2317.08	Intra
	117-Q**K**VEPLR-123	XL	CBDPS	2660.20	2688.12	Intra
K182-K208	178-LEAL**K**ENGGAR-188	XL	BS^3	2306.28	2335.20	Intra
	207-A**K**PALEDLR-215	XL	CBDPS	2677.28	2706.20	Intra
NT-K94	-1-**G**DEPPQSPWDR-10	XL	BS^3	2351.15	2377.07	Intra
	89-DLEEV**K**AK-96	XL	CBDPS	2722.19	2748.13	Intra
S201-K208	196-ATEHL**S**TLSEK-206	XL	BS^3	2364.25	2391.17	Intra
	207-A**K**PALEDLR-215	XL	CBDPS	2735.28	2762.21	Intra
K107-K118	107-**K**WQEEMELYR-116	XL	BS^3	2417.26	2445.18	Intra
	117-Q**K**VEPLR-123	XL	CBDPS	2788.30	2816.22	Intra
NT-K208	-1-**G**DEPPQSPWDR-10	XL	BS^3	2432.22	2461.13	Intra
	207-A**K**PALEDLR-215	XL	CBDPS	2803.25	2832.17	Intra
K107-K208	107-**K**WQEEMELYR-116	XL	BS^3	2560.31	2589.23	Intra
	207-A**K**PALEDLR-215	XL	CBDPS	2931.36	2960.27	Intra
NT-K133	-1-**G**DEPPQSPWDR-10	XL	BS^3	2572.27	2603.19	Intra
	132-Q**K**LHELQEK-140	XL	CBDPS	2943.32	2974.23	Intra
NT-K182	-1-**G**DEPPQSPWDR-10	XL	BS^3	2577.26	2609.17	Intra
	178-LEAL**K**ENGGAR-188	XL	CBDPS	2948.31	2980.21	Intra
K96-K208	95-A**K**VQPYLDDFQK-106	XL	BS^3	2600.40	2629.32	Intra
	207-A**K**PALEDLR-215	XL	CBDPS	2971.45	3000.37	Intra
S58-K239	46-LLDNWDSVTSTF**S**KLR-61	XL	BS^3	2621.39	2651.30	Intra
	239-**K**LNTQ-243	XL	CBDPS	2992.43	3022.35	Intra
S59-K239	46-LLDNWDSVTSTFS**K**LR-61	XL	BS^3	2621.39	2651.30	Intra
	239-**K**LNTQ-243	XL	CBDPS	2992.44	3022.35	Intra
K140-K239	134-LHELQE**K**LSPLGEEMR-149	XL	BS^3	2648.40	2679.31	Intra
	239-**K**LNTQ-243	XL	CBDPS	3019.45	3050.35	Intra
K133-S142	132-Q**K**LHELQEK-140	--[d]	BS^3	--[d]	--[d]	--[d]
	141-L**S**PLGEEMR-149	XL	CBDPS	2691.27	2718.19	Intra
K107-K182	107-**K**WQEEMELYR-116	XL	BS^3	2705.36	2737.28	Intra
	178-LEAL**K**ENGGAR-188	XL	CBDPS	3076.39	3108.31	Intra
K40-K239	28-DYVSQFEGSALG**K**QLNLK-45	XL	BS^3	2736.45	2767.36	Intra
	239-**K**LNTQ-243	--[d]	CBDPS	--[d]	--[d]	--[d]

K96-K182	95-A**K**VQPYLDDFQK-106 178-LEAL**K**ENGGAR-188	XL --[d]	BS[3] CBDPS	2745.44 --[d]	2777.37 --[d]	Intra --[d]
NT-K88	-1-**G**DEPPQSPWDR-10 84-QEMS**K**DLEEVK-94	XL XL	BS[3] CBDPS	2755.28 3126.32	2785.20 3156.25	Intra Intra
K12-K182	11-V**K**DLATVYVDVLK-23 178-LEAL**K**ENGGAR-188	XL XL	BS[3] CBDPS	2756.55 3127.61	2787.46 3158.53	Intra Intra
K107-S167	107-**K**WQEEMELYR-116 161-THLAPY**S**DELR-171	--[d] XL	BS[3] CBDPS	--[d] 3220.44	--[d] 3252.35	--[d] Intra
NT-K96	-1-**G**DEPPQSPWDR-10 95-A**K**VQPYLDDFQK-106	XL XL	BS[3] CBDPS	2871.39 3242.44	2903.29 3274.30	Intra Intra
NT-K12	-1-**G**DEPPQSPWDR-10 11-V**K**DLATVYVDVLK-23	XL XL	BS[3] CBDPS	2882.49 3253.54	2913.40 3284.45	Intra Intra
K96-S167	95-A**K**VQPYLDDFQK-106 161-THLAPY**S**DELR-171	XL XL	BS[3] CBDPS	2889.47 3260.52	2921.38 3292.43	Intra Intra
K118-K140	117-Q**K**VEPLR-123 134-LHELQE**K**LSPLGEEMR-149	XL XL	BS[3] CBDPS	2914.59 3285.62	2949.48 3320.52	Intra Intra
K88-K96	84-QEMS**K**DLEEVK-94 95-A**K**VQPYLDDFQK-106	XL XL	BS[3] CBDPS	2923.47 3294.51	2953.38 3324.46	Intra Intra
S167-K182	161-THLAPY**S**DELR-171 178-LEAL**K**ENGGAR-188	--[d] XL	BS[3] CBDPS	--[d] 2966.39	--[d] 2998.30	--[d] Intra
K96-K107	95-A**K**VQPYLDDFQK-106 107-**K**WQEEMELYR-116	XL XL	BS[3] CBDPS	2999.50 3370.54	3031.40 3402.45	Intra Intra
K40-K118	28-DYVSQFEGSALG**K**QLNLK-45 117-Q**K**VEPLR-123	XL --[d]	BS[3] CBDPS	3002.64 --[d]	3037.52 --[d]	Intra --[d]
K208-K239	196-ATEHLSTLSEKA**K**PALEDLR-215 239-**K**LNTQ-243	XL,H --[d]	BS[3] CBDPS	3104.66 --[d]	3139.56 --[d]	Intra --[d]
K195-K208	189-LAEYHA**K**ATEHLSTLSEK-206 207-A**K**PALEDLR-215	XL XL	BS[3] CBDPS	3176.69 3547.74	3213.59 3584.64	Intra Intra
K40-K133	28-DYVSQFEGSALG**K**QLNLK-45 132-Q**K**LHELQEK-140	XL --[d]	BS[3] CBDPS	3285.75 --[d]	3323.64 --[d]	Intra --[d]
NT-K59	-1-**G**DEPPQSPWDR-10 46-LLDNWDSVTSTFS**K**LR-61	XL XL	BS[3] CBDPS	3301.62 3672.66	3339.50 3710.55	Intra Intra
NT-K140	-1-**G**DEPPQSPWDR-10 134-LHELQE**K**LSPLGEEMR-149	XL,H --[d]	BS[3] CBDPS	3740.87 --[d]	3783.75 --[d]	Intra --[d]
S204-K208	189-LAEYHAKATEHLSTL**S**EK-206 207-A**K**PALEDLR-215	XL,H --[d]	BS[3] CBDPS	3332.78 --[d]	3369.67 --[d]	Intra Intra
K226-K239	216-QGLLPVLESF**K**VSFLSALEEYTK-238 239-**K**LNTQ-243	XL --[d]	BS[3] CBDPS	3337.83 --[d]	3371.75 --[d]	Intra --[d]
K77-K239	62-EQLGPVTQEFWDNLE**K**ETEGLR-83 239-**K**LNTQ-243	XL XL	BS[3] CBDPS	3357.70 3728.74	3395.59 3766.62	Intra Intra
NT-K23	-1-**G**DEPPQSPWDR-10 11-VKDLATVYVDVL**K**DSGR-27	XL,H --[d]	BS[3] CBDPS	3453.76 --[d]	3491.66 --[d]	Intra --[d]
K118-K226	117-Q**K**VEPLR-123 216-QGLLPVLESF**K**VSFLSALEEYTK-238	XL --[d]	BS[3] CBDPS	3604.01 --[d]	3641.90 --[d]	Intra --[d]
K77-K182	62-EQLGPVTQEFWDNLE**K**ETEGLR-83 178-LEAL**K**ENGGAR-188	XL XL	BS[3] CBDPS	3911.98 4283.03	3957.85 4328.88	Intra Intra
NT-K77	-1-**G**DEPPQSPWDR-10 62-EQLGPVTQEFWDNLE**K**ETEGLR-83	XL XL	BS[3] CBDPS	4037.93 4408.97	4083.79 4454.83	Intra Intra
K77-K88	62-EQLGPVTQEFWDNLE**K**ETEGLR-83 84-QEMS**K**DLEEVK-94	XL XL	BS[3] CBDPS	4090.01 4461.03	4133.88 4504.91	Intra Intra
K40-K140	28-DYVSQFEGSALG**K**QLNLK-45 132-QKLHELQE**K**LSPLGEEMR-149	XL,H --[d]	BS[3] CBDPS	4454.36 --[d]	4504.22 --[d]	Intra --[d]

[a] Lysines involved in cross-links are in bold.

[b] Chemical modifications: XL = 1 complete cross-link (BS^3 +138.06808 Da, CBDPS +509.09682 Da), H = 1 hydrolyzed cross-linker (BS^3: +156.07864 Da, CBDPS: +527.10738 Da).

[c] Experimentally derived monoisotopic mass for each peptide with each isotope and the combinations.

[d] Not detected. These ions were detectable in one sample set; for example CBDPS but not BS^3.

Supplementary Table 3

Experimental parameters from SAXS sampling of monomeric apoA-I cross-linked with CBDPS or BS3

ApoA-I Sample	**I(O)[a] (Guinier)**	**R_g[b] (Guinier)**	**Real Space R_g**	**D_{max}[c]**	**Volume**	**DAMMIF NSD[d]**
	cm^{-1}	**Å**	**Å**	**Å**	**$Å^3$**	**Å**
CBDPS						
4.0 mg/ml	603	26	25.29	85	70165	
2.0 mg/ml	301	25.8	25.24	83	68091	0.579 ± 0.032
1.0 mg/ml	149	25.1	25.53	83	70191	
BS^3						
4.0 mg/ml	569	25.95	25.54	88	77337	
2.0 mg/ml	294	25.58	25.25	81	77334	0.596 ± 0.024
1.0 mg/ml	140	27.67	25.24	81	83946	

Supplementary Table 4

Universal cross-linking list across four studies on lipid-free monomeric apoA-I

Residue		Reported[a]				Cross-Linker[b]			Distance[c]
1	2	Silva et al	Pollard et al	Segrest et al	Melchior	MDA	BS3	CBDPS	(Å)
1	12		X	X	X	X	X	X	23
1	23				X		X		28
1	59		X		X		X	X	28
1	77				X		X	X	28
1	88				X		X	X	28
1	94				X		X	X	28
1	96	X			X		X	X	28
1	118		X		X		X	X	28
1	133				X		X	X	28
1	140				X		X		28
1	182				X		X	X	28
1	195			X		X			23
1	208				X		X	X	28
1	239				X		X	X	28
12	23	X	X	X	X	X	X	X	23
12	182				X		X	X	28
12	195		X						28
12	226			X		X			23
12	239				X		X		28
23	59	X	X	X	X	X			23
23	239			X		X			23
36	40				X		X	X	28
40	45	X	X	X		X			23
40	59		X						28
40	94			X		X			23
40	118		X		X		X		28
40	133		X	X	X	X	X		23
40	140		X		X		X		28
40	182		X						28
40	239		X	X	X	X	X		23
45	59		X						28
52	59				X		X	X	28
55	59				X		X	X	28
58	239				X		X	X	28
59	195			X		X			23
59	239			X	X	X	X	X	23
77	88				X		X	X	28
77	182				X		X	X	28
77	195			X		X			23
77	208			X		X			23
77	239				X		X	X	28
87	88				X		X		28
87	239				X		X	X	28
88	94	X	X						28
88	96				X		X	X	28
88	118		X						28
88	195			X		X			23
94	96	X	X	X	X	X	X	X	23
94	226			X		X			23
94	239		X	X	X	X	X	X	23
96	106	X	X						28
96	107				X		X	X	28
96	167				X		X	X	28
96	182				X		X		28
96	195	X		X		X			23
96	208	X		X	X	X	X	X	23
96	226	X							28

96	239				X	X	X	X	X	23
106	107	X			X	X	X	X	X	23
107	118					X		X	X	28
107	167					X			X	28
107	182					X		X	X	28
107	208					X		X	X	28
107	239				X	X	X	X	X	23
118	133			X	X	X	X	X	X	23
118	140	X		X	X	X	X	X	X	23
118	142					X		X	X	28
118	182					X		X	X	28
118	195				X		X			23
118	208				X		X			23
118	226				X	X	X	X		23
118	239				X	X	X	X	X	23
133	140	X		X	X	X	X	X	X	23
133	142					X		X		28
133	239				X	X	X	X	X	23
140	142					X		X	X	28
140	239				X	X	X	X	X	23
167	182					X			X	28
167	239					X		X	X	28
182	195				X		X			23
182	208					X		X	X	28
182	226				X		X			23
182	239					X		X	X	28
195	206				X		X			23
195	208					X		X	X	28
195	226				X		X			23
195	239				X		X			23
201	208					X		X	X	28
204	208					X		X		28
206	208	X		X	X	X	X	X	X	23
206	226				X		X			23
208	239					X		X		28
226	238	X								28
226	239				X	X	X	X		23
238	239	X		X	X		X			23

[a] Cross-linked residues reported in the current study or Silva et al [1], Pollard et al [2], or Segrest et al [3].
[b] Cross-link found with cross-linking reagent
[c] Upper-limit Cα-Cα distance used as cross-linking constraint for consensus model

Supplementary Table 5

Reported structural features of lipid-free monomeric apoA-I determined by far circular dichroism

Author	Year	A-I Source	[PRO] mg/ml	Temp (°C)	Alpha	Beta-Sheet	Coil/ Turns	Ref
Davidson et al	1996	Plasma	0.05-0.1	25	57.0%		43.0%	[4]
Bergeron et al	1997	Native His-apoA-I	0.067	24	56.0% 50.0%		44.0% 50.0%	[5] **a**
Rogers et al	1998	Plasma	0.01-0.56	25	68.0%		32.0%	[6] **b**
Sparks et al	1999	Plasma	0.067	24	49.0%		51.0%	[7] **c**
Suurkuusk et al	1999	Plasma	0.1	25	44.0%		56.0%	[8] **d**
Davidson et al	1999	Pro-apoA-I	0.05-0.1	25	56.0%		44.0%	[9] **d**
Huang et al	2000	Pro-apoA-I	0.1	25	45.0%		55.0%	[10]
Panzenbock et al	2000	Plasma	0.1	25	52.0%	7.9%	37.3%	[11]
Weinberg et al	2000	Plasma	0.08	25	54.0%		46.0%	[12]
Sigalov et al	2001	Plasma	0.1	25	62.0%		38.0%	[13]
Gorshkova et al	2002	Pro-apoA-I	0.025-0.1	25	58.0%		42.0%	[14] **d**
Reschly et al	2002	Plasma	0.1	25	40.0%		60.0%	[15]
Saito et al	2003	Plasma r-apoA-I	0.025-0.05	25	49.0% 46.0%		51.0% 54.0%	[16] **e**
Fang et al	2003	Plasma r-apoA-I	0.06-0.1	25	60.0% 55.0%		40.0% 45.0%	[17] **f**
Saito et al	2004	Plasma r-apoA-I	0.025	25	46.0% 44.0%		54.0% 56.0%	[18] **e**
Han et al	2005	r-apoA-I	0.07	25	52.0%		48.0%	[19] **d**
Silva et al	2005	Plasma	0.03	25	55.0%	8.0%	37%	[1]
Zhu et al	2005	His-apoA-I	0.06-0.1	25	54.0%		46.0%	[20] **a**
Arnulphi et al	2005	Plasma	0.054	15 37	48.0% 50.0%		52.0% 50.0%	[21]
Gorshkova et al	2006	Pro-apoA-I	0.025-0.08	25	58.0% 59.0%		42.0% 41.0%	[22] **g**
Tanaka et al	2008	r-apoA-I	0.05	25	44.0%		56.0%	[23] **d**
Fukuda et al	2008	Plasma	0.06	37	44.0%		56.0%	[24]
Kono et al	2009	r-apoA-I	0.03-0.05		46.0%		54.0%	[25] **d**
Chetty et al	2009	Plasma	0.05	25	49.0%		51.0%	[26]
Jayaraman et al	2012	Plasma	0.02	25	57.0%		43.0%	[27]
Zehender et al	2012	r-apoA-I	0.1	25	45.0%		55.0%	[28] **h**
Della-Riva et al	2013	r-apoA-I	0.1	25	56.0%		44.0%	[29] **i**

[a] Studies performed on recombinant human apoA-I which contains Met-Arg-Gly-Ser-$(His)_6$ on the NT

[b] CD was obtained on a range of values that both fall within and exceed concentrations reported for monomeric apoA-I .

[c] The source of apoA-I is unclear. "Lyophilized apoA-I (purity > 96%) were purchased from Biogenesis."

[d] Studies performed on recombinant human proapoA-I which contains a hexapeptide on the NT normally cleaved when secreted into plasma.

[e] Studies performed on recombinant human apoA-I which containing a Gly-Ser, on the NT.

[f] Studies performed on recombinant human apoA-I containing a Gly-Ala-Met-Gly-Ser, on the NT.

[g] Studies performed on recombinant human proapoA-I containing a hexapeptide on the NT normally cleaved when secreted into plasma. Protein was expressed in either a baculovirus (top) or adenovirus (bottom) system.
[h] Studies performed on recombinant human apoA-I containing a Gly-Gly on the NT.
[i] Studies performed on recombinant human apoA-I which contains a point mutation, E2D at the N-terminus.

Supplementary Table 6

Reported cleavage sites on lipid-free monomeric apoA-I determined by limited proteolysis

Author	Year	A-I Source	[PRO] mg/ml	Temp (°C)	Proteolytic Enzyme	Cleavage Sites	Ref
Roberts et al	1997	Plasma	0.1	37	a,b	Y115, E125, E136, E191, Y192, L200, L211, E212, E223, F225, E235	30
Rogers et al	1997	Plasma	0.1	37	a	F57, L122, Y192	31

[a] Chymotrypsin: Cleaves at the carboxy-terminal side of Tyrosine (Tyr, Y), Phenalanine (Phe, F), Tryptophan (Trp,W), and Leucine (Leu, L).
[b] *S. Aureus* V8 protease: Cleaves at the carboxy-terminal side of glutamic acid (Glu, E) residues

Supplementary Table 7

Reported molecular dimensions for lipid-free monomeric apoA-I measured by sedimentation velocity

Author	Year	A-I Source	[PRO] mg/ml	Length (Å)	Width (Å)	Axial Ratio	Ref
Barbeau et al	1979	Plasma	0.05-0.7	151.2	25.2	6.0	[32]
Edelstein et al	1980	Plasma	0.05	164	25	6.6	[33]
Rogers et al	1998	Plasma	0.1-0.2	168	24	7.2	[31]