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Structure and biological activity of a conformational constrained apolipoprotein A-I-derived helical peptide targeting the protein haptoglobin.

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P2a	Ac-LSPLGEEMRD RARAHVDALR THLA-amide
ApoAib	Ac-WAAUAEEURD RURAHUDAAR THAA-amide

Figure S1: Amino acid sequences of peptide P2a and ApoAib. One code letter is used (U=Aib). Conserved residues are highlighted in red. Aib is highlighted in blue.

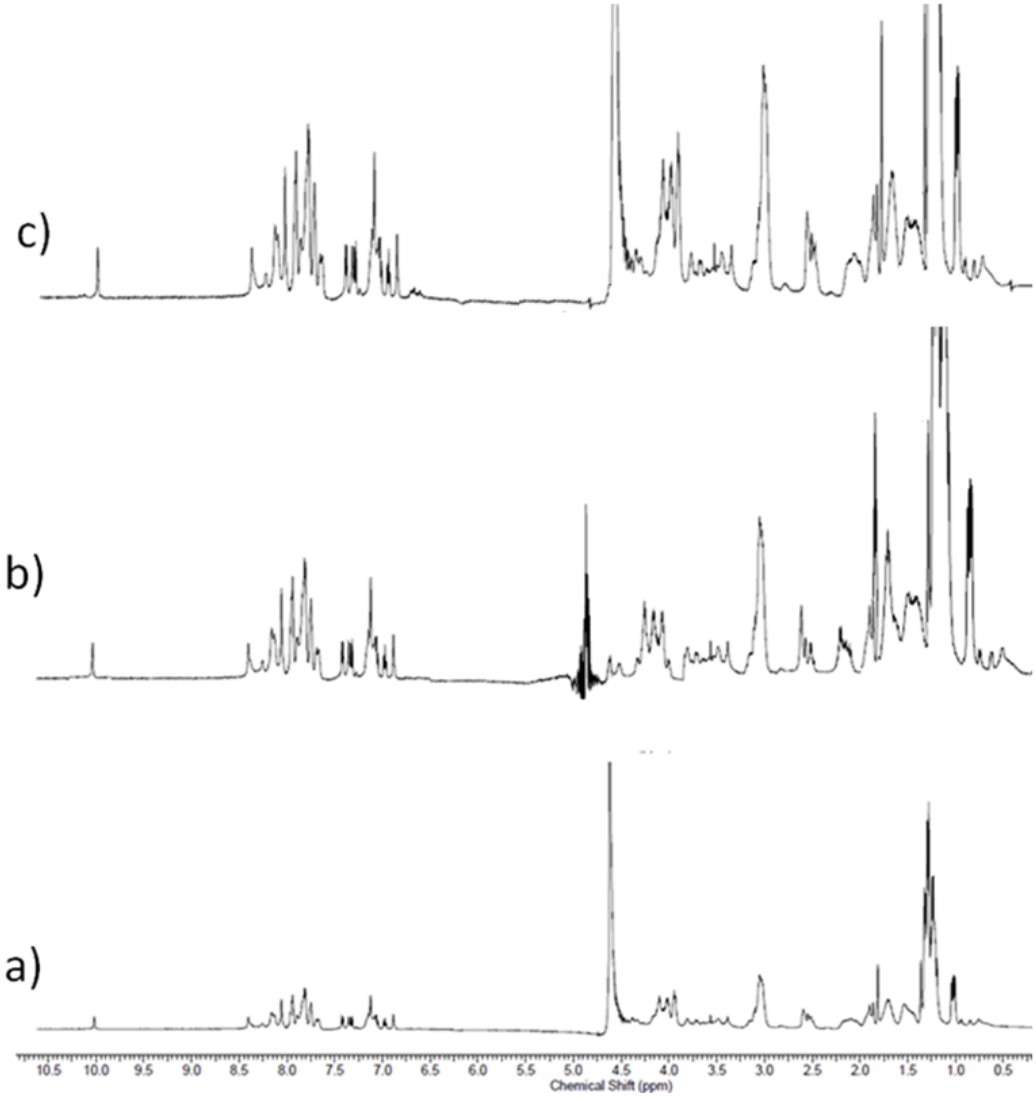

Figure S2: ^{1}H 1D spectra of ApoAib at 0.5 mM (a), 1.0 mM (b) and 3.0 mM (c) recorded at ^{1}H frequency of 600 MHz at 298 K.

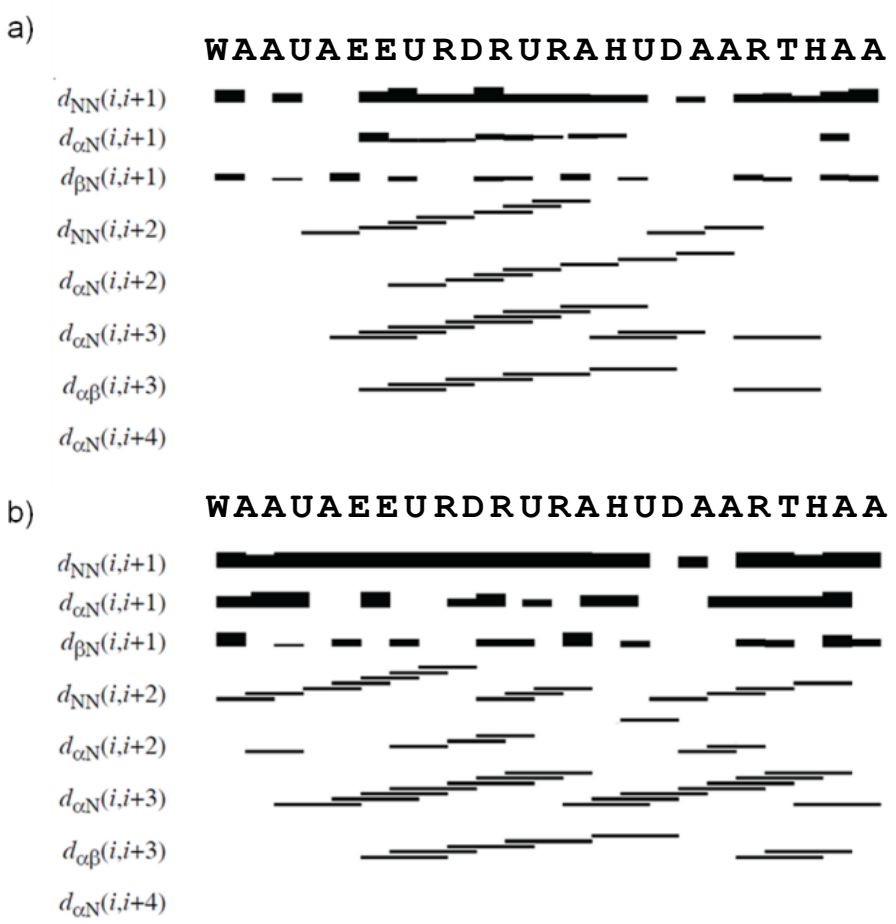

Figure S3: Sequential and medium –range NOE connectivities for the ApoAib peptide in a) free state and in b) bound state. Thick sand medium bars indicate strong and medium NOE intensities respectively. Thin bars are indicative for weak NOE intensities (U = single letter code for Aib).

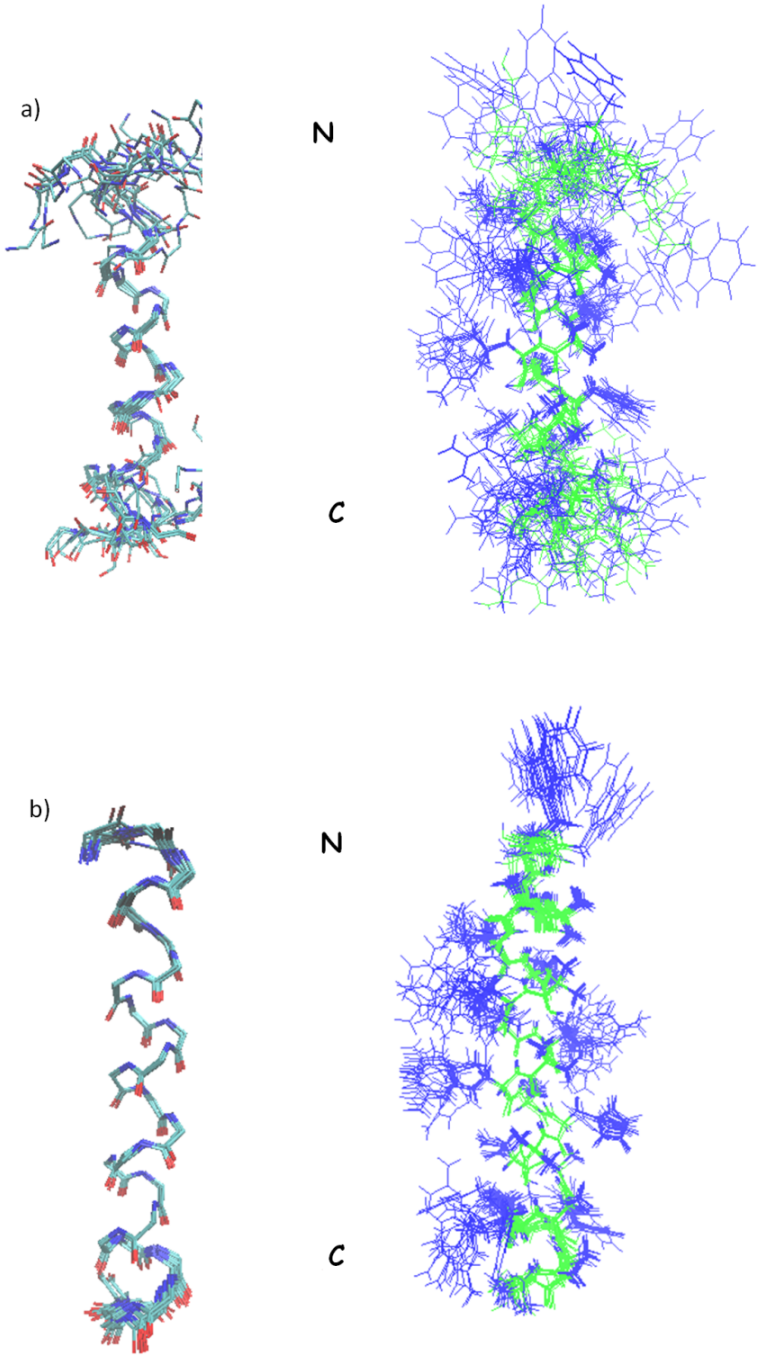

Figure S4: Superposed backbone traces for the NMR-derived structural ensemble of ApoAib a) in free and b) in bound state.

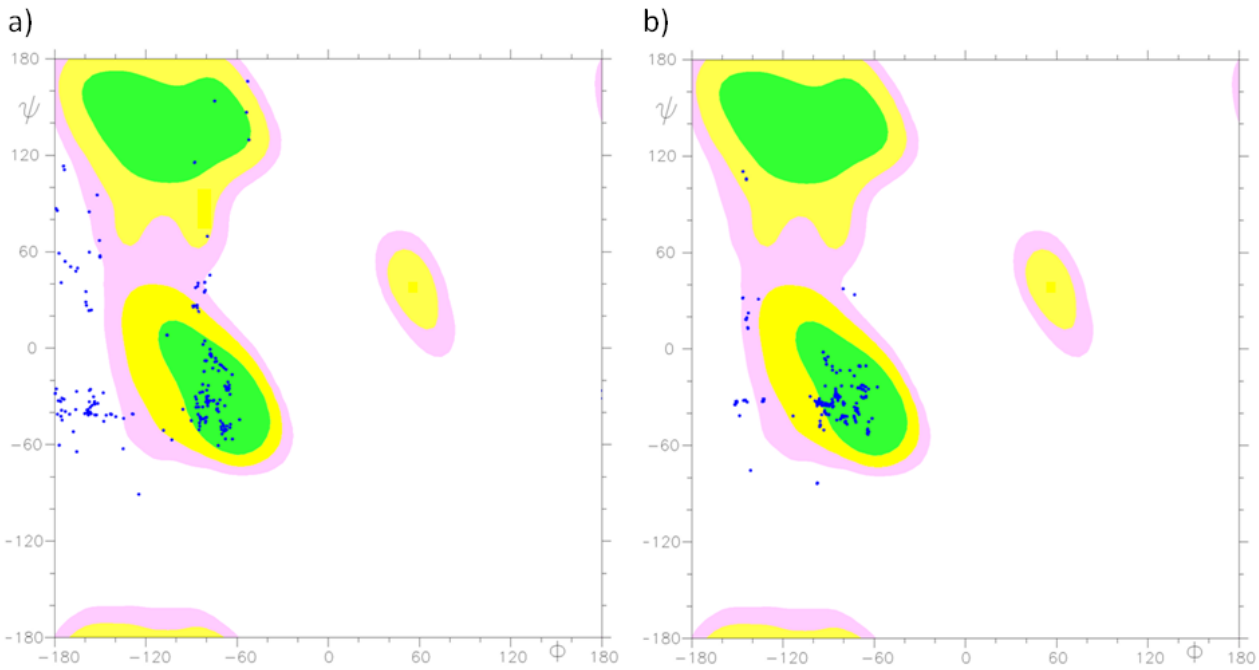

Figure S5: Ramachandran plots for peptide models in free (a) and in bound (b) state

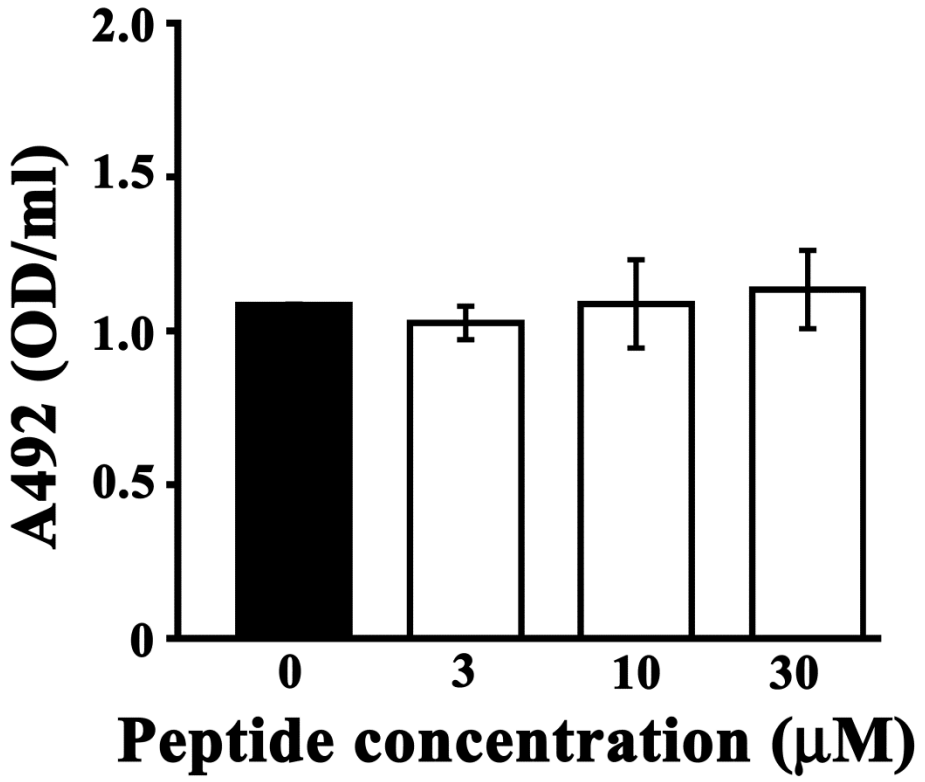

Figure S6. Competition of ApoAib with Hb for binding Hpt. ApoAib was assayed for its ability to compete with Hb for Hpt binding. Aliquots of 1 μM Hpt (50 μL), previously incubated with different amounts (3, 10, or 30 μM) of ApoAib (open bar). Hpt binding to Hb was detected using rabbit anti-Hpt IgG and GAR-HRP linked IgG, and monitoring the color development at 492 nm. The amount of Hpt bound to Hpt, in the absence of peptide (full bar) is shown as control. The samples were analyzed in triplicate, and data are expressed as means ± SEM.

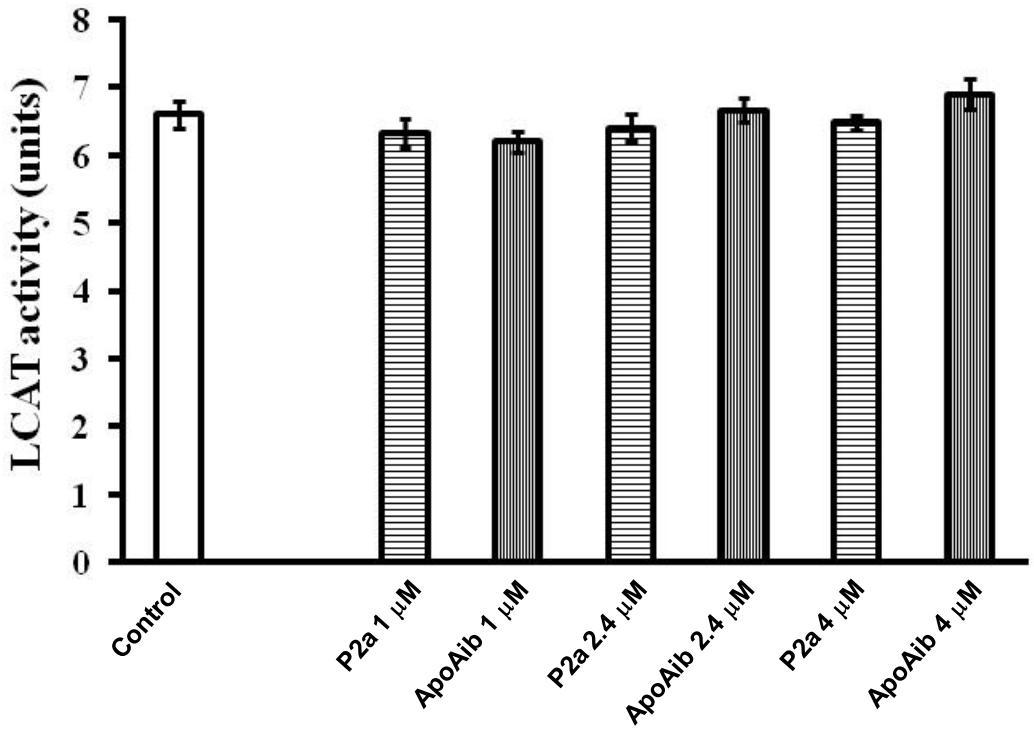

Figure S7: Effect of peptide P2a and ApoAib on LCAT activity. The LCAT activity was assayed in presence of different amounts (1, 2.4 or 4 μM) of P2a or ApoAib. A pool of plasma samples (treated with 0.65% dextran sulphate, MW = 50 kDa, in 0.2 M $CaCl_2$ to remove lipoproteins) was used as source of LCAT, while a proteoliposome (ApoA-I:lecithin:^{3}H-cholesterol, 1.5:200:18 molar ratio) was used as substrate. The LCAT activity is expressed in units corresponding to nmol of cholesterol incorporated per hour per mL of plasma. Samples were analyzed in triplicate, and data are expressed as means ± SEM.

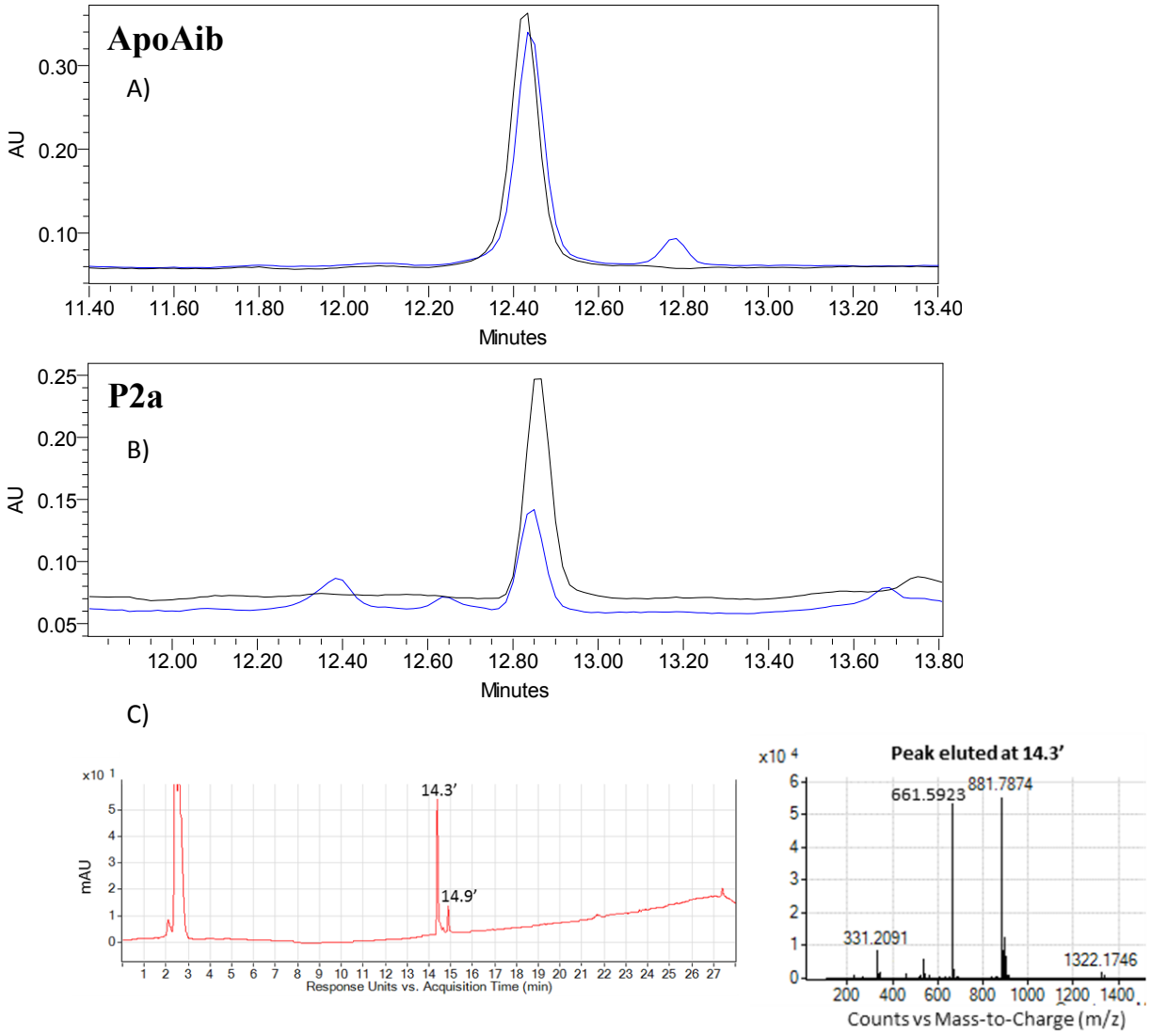

Figure S8: HPLC analysis of peptide stability in human serum. a) Chromatogram revealed at 210 nm of peptide ApoAib after 0 (black) and 4 hours (blue) incubation in human serum. b) Chromatogram revealed at 210 nm of peptide P2a after 0 (black) and 4 hours (blue) incubation in human serum. c) ESI-ToF spectrum of the ApoAib peptide after 6 hours incubation in human serum. ApoAib calculated mass peak $[M+3H]^{3+}$= 881.4444 Da; $[M+4H]^{4+}$= 661.3333 Da

Table S1: ^{1}H chemical shift assignment of the free ApoAib peptide (1mM)

Residue	δNH	δH_{α}	δH_{β}	δH_{γ}	δH_{δ}	δothers
(CH3CO)						
Trp 1	8.21	4.68	3.24;3.10			7.24;7.45; 7.40; 10.11;7.38; 7.20
Ala 2	8.15	4.22	1.29			
Ala 3	7.95	4.15	1.29			
Aib 4	8.00		1.10			
Ala 5	8.27	4,23	1.30			
Glu 6	7.81	4.15	2.04	2.30		
Glu 7	7.91	4,09	1.89; 1.93	2.25		
Aib 8	7.86		1.10			
Arg 9	7.94	4.19	1.81; 1.83	1.61	2.88	
Asp 10	8.02	4.45	2.82			
Arg 11	8.01	4.06	1.77	1.64		
Aib 12	7.96		1.07			
Arg 13	7.78	4.18	1.80; 1.75	1.62; 1.56	2.95	
Ala 14	7.86	4.13	1.28			
His 15	8.08	4.45	2.78			7.42; 6.95
Aib 16	7.91		1.02			
Asp 17	7.82	4.50	2.72			
Ala 18	8.14	4,28	1.25			
Ala 19	7.91	4.35	1.27			
Arg 20	7.70	4.35	1.66; 1.54	1.81	2.96	
Thr 21	8.10	4.33	3.91	1.39		
His 22	8.19	4.59	2,80			7.38; 6.94
Ala 23	7.84	4.27	1.31			
Ala 24	8.20	4.30	1.28			
(CONH2)						

All chemical shifts are in parts/million and are relative to water protons (4.75 ppm)table

Table S2: Observed and calculated average hydrogen bond lengths for the ApoAib in the free state

Number	**Residue**	**Atom**	**Number**	**Residue**	**Atom**	**Distance (Å)**
10	ASP	HN	6	GLU	O	2.03
11	ARG	HN	7	GLU	O	2.10
14	ALA	HN	10	ASP	O	2.09
17	ASP	HN	13	ARG	O	2.17
19	ALA	HN	15	HIS	O	1.90
20	ARG	HN	16	AIB	O	2.01
21	THR	HN	18	ALA	O	2.00
22	HIS	HN	18	ALA	O	2.03

Table S3: Observed and calculated average hydrogen bond lengths for the ApoAib in the bound state

Number	Residue	Atom	Number	Residue	Atom	Distance (Å)
7	GLU	HN	3	ALA	O	2.07
10	ASP	HN	6	GLU	O	2.00
11	ARG	HN	7	GLU	O	1.98
13	ARG+	HN	9	ARG	O	2.22
14	ALA	HN	10	ASP	O	2.00
17	ASP	HN	13	ARG	O	2.00
19	ALA	HN	15	HIS	O	1.99
20	ARG	HN	16	AIB	O	1.99
21	THR	HN	17	ASP	O	2.02
22	HIS+	HN	17	ASP	O	2.18
23	ALA	HN	19	ALA	O	2.15