Supplementary Information

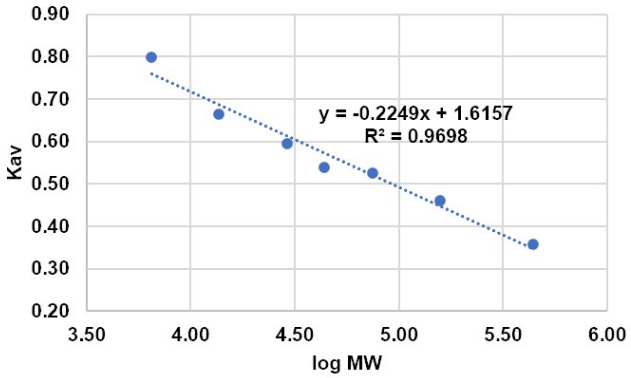
Zinc-Mediated Multimerization of the N-terminal CCHC Zinc Finger Domain of BCL11B: A Key to Stability and a Potential Therapeutic Target

Anne Susemihl[a,b], Felix Nagel[a], Piotr Grabarczyk[b], Christian Andreas Schmidt[b], Mihaela Delcea[a*]

[a] University of Greifswald, Institute of Biochemistry, Greifswald, Germany

[b] University Medicine Greifswald, Internal Medicine C, Greifswald, Germany

[*] Corresponding Author



Protein	MW [Da]	log MW	K_{av}	Volume [mL]
Ferritin	440000	5.64	0.36	1.48
Aldolase	158000	5.20	0.46	1.63
Conalbumin	75000	4.88	0.52	1.72
Ovalbumin	44000	4.64	0.54	1.741
Carbonic Anhydrase	29000	4.46	0.60	1.821
Ribunuclease A	13700	4.14	0.67	1.921
Aprotinin	6500	3.81	0.80	2.111

$BCL11B_{42-94}$	AE EV [mL]	AE MW [kDa]	WI EV [mL]	WI MW [kDa]	ZA EV [mL]	ZA MW [kDa]
WT	1.76	53.5	1.84	30.1	1.76	53.5
I70A	1.89	21.1	1.93	15.8	1.90	19.6
H76C	1.88	22.6	1.93	15.8	1.89	21.1
C81H	1.78	46.3	1.89	21.1	1.80	40.1

AE - After Expression (with Zn^{2+}), EV – Elution Volume, MW – Molecular Weight, WI – Without Ions (no Zn^{2+}), ZA – Zinc added (+ Zn^{2+})

Figure S1. Calibration curve prepared for Superdex 200 Increase 3.2/300 column installed on Äktamicro platform. Column volume is 2.4 mL, and column void volume is 0.97 mL. Calibration curve was prepared with calibration kits and following the instructions provided by Cytiva (Freiburg, Germany).

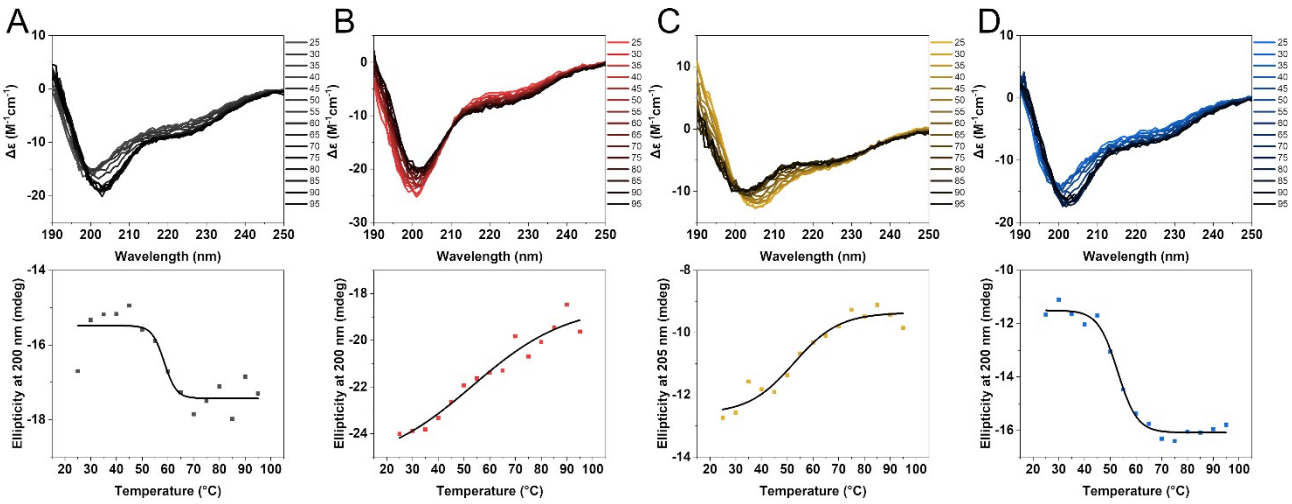


Figure S2. Circular dichroism spectra at different temperatures to determine thermal stability of $BCL11B_{42-94}$ species (A) WT, (B) I70A, (C) H76C, and (D) C81H, each without Zn^{2+}. Determined melting temperatures are displayed in Table S1.

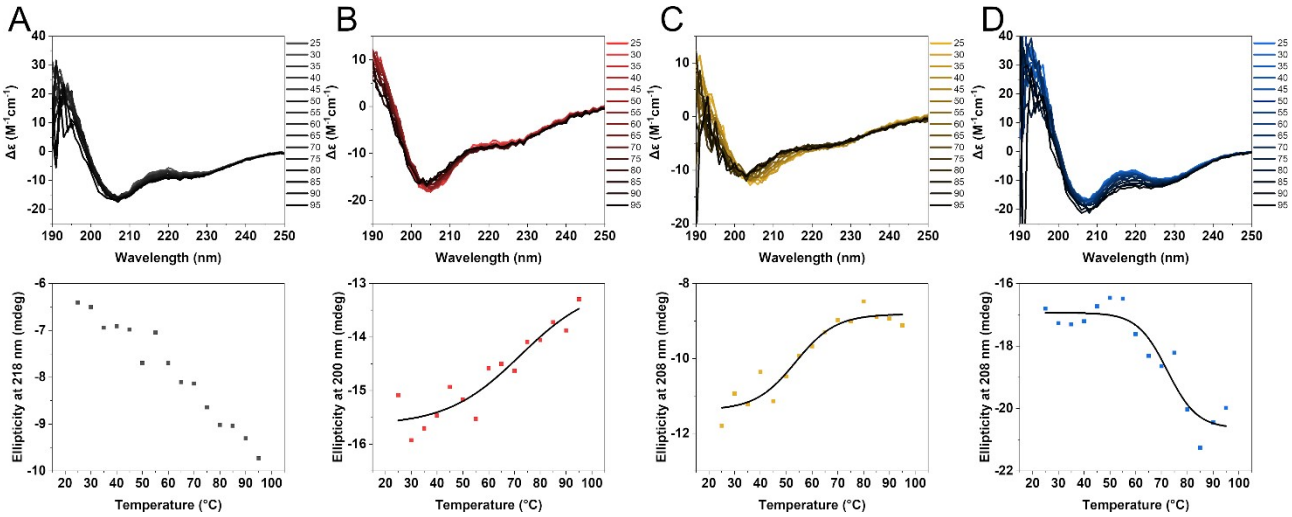
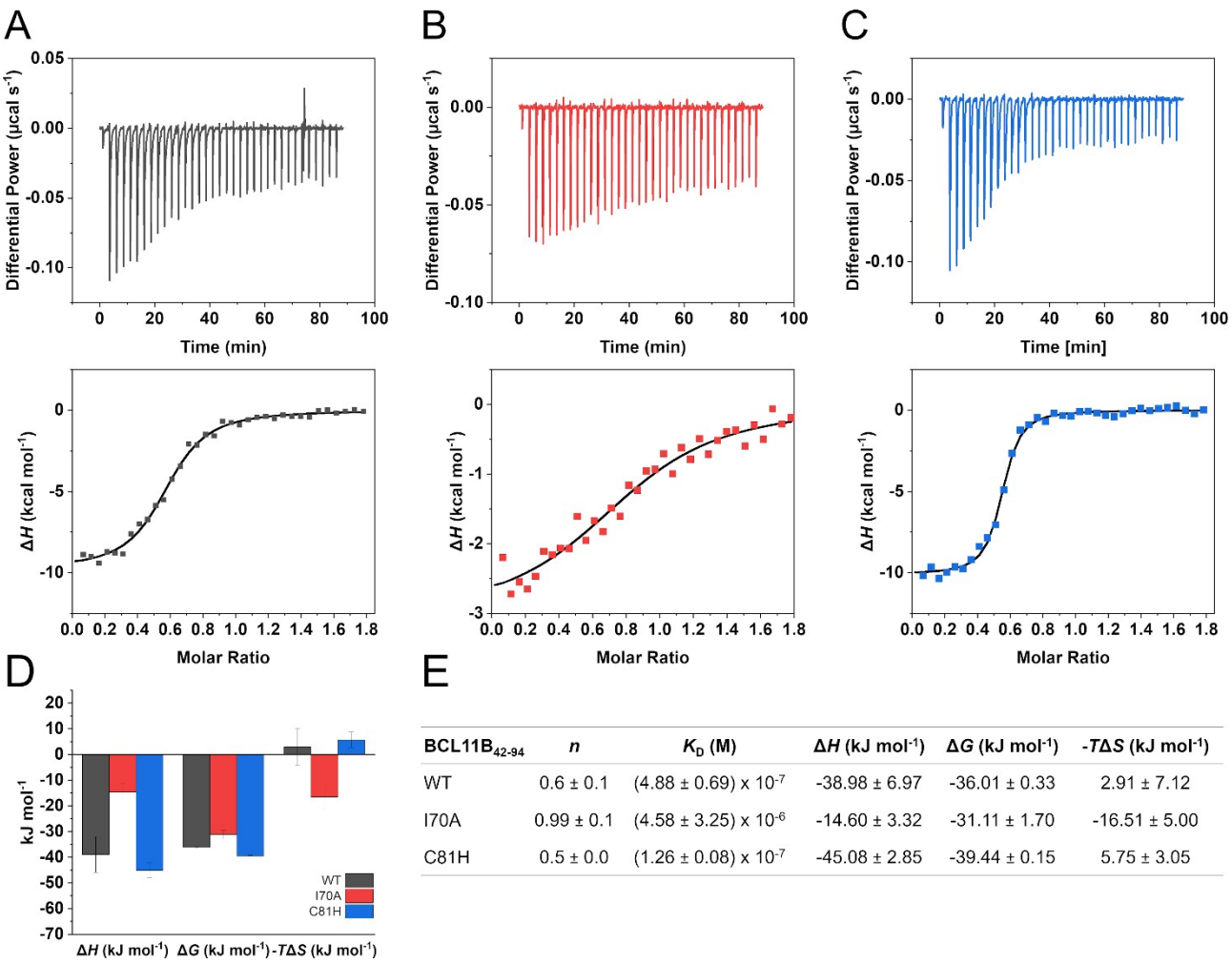


Figure S3. Circular dichroism spectra at different temperatures to determine thermal stability of $BCL11B_{42-94}$ species (A) WT, (B) I70A, (C) H76C, and (D) C81H, each saturated with Zn^{2+}. Determined melting temperatures are displayed in Table S1.

Table S1. Deconvolution data for CD spectra of $BCL11B_{42-94}$ WT, I70A, H76C, and C81H each in the presence ($+Zn^{2+}$) and in the absence ($-Zn^{2+}$) of zinc ions. Calculated secondary structure elements are depicted in %. Deconvolution was carried out using *BeStsel.com*. Melting temperatures for $BCL11B_{42-94}$ WT, I70A, H76C, and C81H each with ($+Zn^{2+}$) and without ($-Zn^{2+}$) zinc ions determined using circular dichroism spectroscopy.

Secondary Structure	WT $+Zn^{2+}$	WT $-Zn^{2+}$	I70A $+Zn^{2+}$	I70A $-Zn^{2+}$	H76C $+Zn^{2+}$	H76C $-Zn^{2+}$	C81H $+Zn^{2+}$	C81H $-Zn^{2+}$
Helix	10.5 ± 0.2	1.4 ± 0.8	9.6 ± 0.2	4.7 ± 0.4	10.3 ± 0.3	2.1 ± 1.0	10.1 ± 1.2	1.1 ± 0.1
Antiparallel	21.6 ± 0.9	34.8 ± 0.8	25.1 ± 0.3	29.2 ± 0.8	24.9 ± 1.6	34.3 ± 0.7	18.4 ± 1.3	35.3 ± 0.2
Parallel	4.1 ± 0.4	0.0	3.3 ± 0.9	0.0	6.2 ± 1.5	0.0	3.5 ± 0.5	0.0
Turn	15.5 ± 0.2	14.8 ± 0.1	15.0 ± 0.2	16.1 ± 0.2	13.6 ± 0.1	15.1 ± 0.4	17.2 ± 0.3	14.9 ± 0.1
Others	48.3 ± 0.3	49.0 ± 0.3	47.1 ± 0.5	50.0 ± 0.3	44.9 ± 0.1	48.5 ± 0.1	50.8 ± 0.3	48.6 ± 0.2
T_m (°C)	--	56.4 ± 3.8	65.1 ± 5.5	53.4 ± 0.3	53.2 ± 2.1	52.5 ± 5.5	78.7 ± 5.3	51.9 ± 0.7



$BCL11B_{42-94}$	*n*	K_D (M)	Δ*H* (kJ mol^{-1})	Δ*G* (kJ mol^{-1})	-*T*Δ*S* (kJ mol^{-1})
WT	0.6 ± 0.1	(4.88 ± 0.69) x 10^{-7}	-38.98 ± 6.97	-36.01 ± 0.33	2.91 ± 7.12
I70A	0.99 ± 0.1	(4.58 ± 3.25) x 10^{-6}	-14.60 ± 3.32	-31.11 ± 1.70	-16.51 ± 5.00
C81H	0.5 ± 0.0	(1.26 ± 0.08) x 10^{-7}	-45.08 ± 2.85	-39.44 ± 0.15	5.75 ± 3.05

Figure S4. Isothermal titration calorimetry data of $BCL11B_{42-94}$ (A) WT, (B) I70A, (C) H76C, (D) C81H and their interaction with Co^{2+}. Experimental data were fitted with a 1:1 binding site model. (E) Thermodynamic parameters of $BCL11B_{42-94}$ variants. (F) Summary of kinetic and thermodynamic parameters determined using ITC. All values represent mean ± standard deviations of at least three independent experiments.