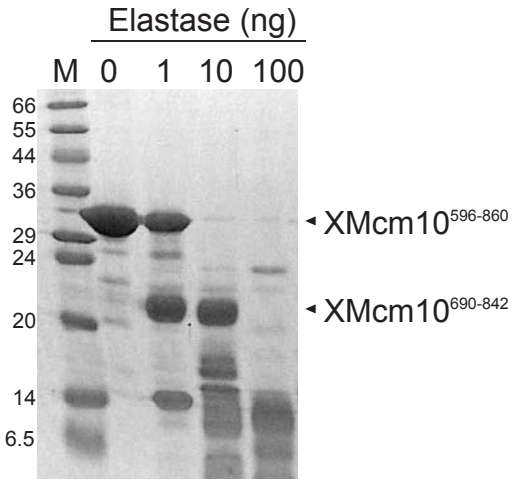
Table S1

Table S1. NMR Acquisition Parameters

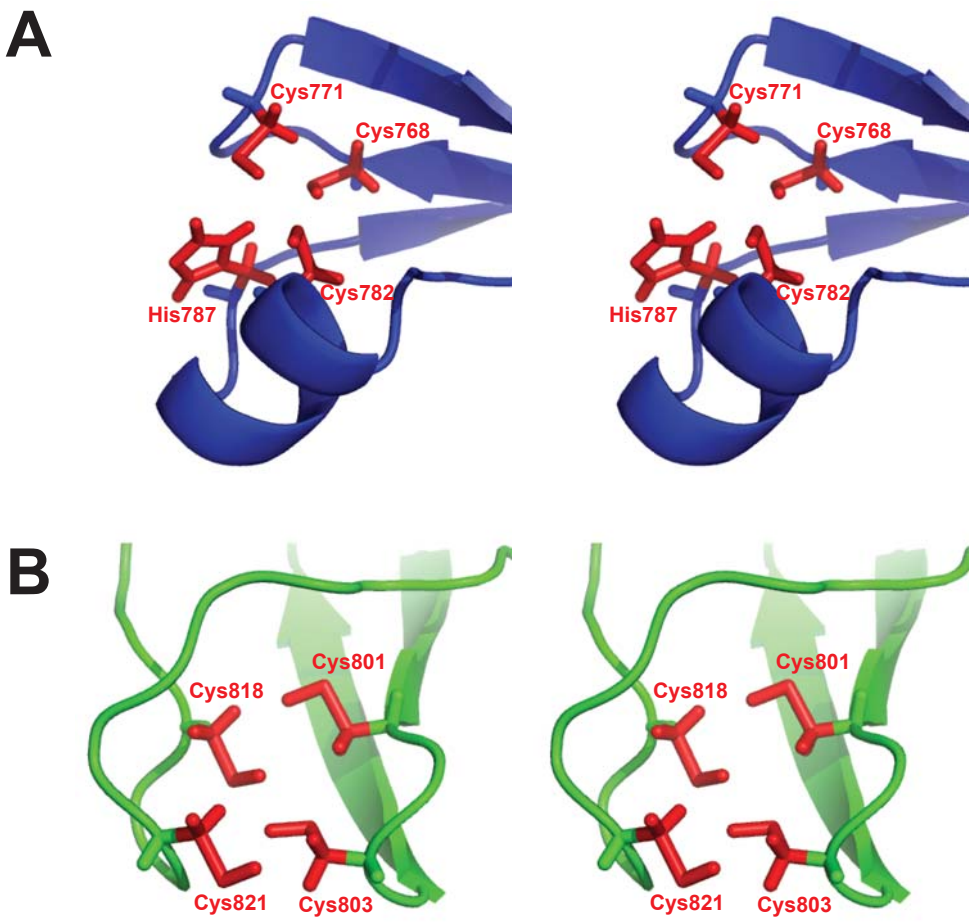
Experiment Name	Construct	Dimensions	Scans	Increments (t1×t2×t3)	Magnetic Field (MHz)	Carrier Frequency (ppm)
^{15}N HSQC†	XMcm10$^{755-842}$	2D	2	2048×256	600	4.7(H); 120.25(N)
	XMcm10$^{690-842}$	2D	32	2048×128	800	4.7(H); 117(N)
	XMcm10$^{596-860}$	2D	8	2048×256	600	4.7(H); 120(N)
	XMcm10$^{230-860}$	2D	128	2048×256	800	4.7(H); 120(N)
{^{1}H}-^{15}N NOE†	XMcm10$^{755-842}$	2D	4	1024×128	600	4.7(H); 120.25(N)
	XMcm10$^{690-842}$	2D	16	1024×128	600	4.7(H); 120(N)
HNCA‡	XMcm10$^{690-842}$	3D	40	1024×40×64	600	4.7(H); 117(N); 52.5(C)
HNCA CB‡	XMcm10$^{755-842}$	3D	8	1024×70×180	600	4.7(H); 120.5(N); 48(C)
	XMcm10$^{690-842}$	3D	64	1024×70×160	600	4.7(H); 120(N); 40(C)
CBCA(CO)NH‡	XMcm10$^{755-842}$	3D	8	1024×70×120	600	4.7(H); 120.25(N); 41(C)
	XMcm10$^{690-842}$	3D	8	1024×70×140	600	4.7(H); 120(N); 39(C)
(H)C(CO)NH-TOCSY‡	XMcm10$^{755-842}$	3D	16	1024×40×120	600	4.7(H); 120.25(N); 40(C)
	XMcm10$^{690-842}$	3D	16	1024×44×140	600	4.7(H); 120(N); 35(C)
H(CC)(CO)NH‡	XMcm10$^{755-842}$	3D	8	1024×42×180	600	4.7(H); 120.25(N); 41(C)
	XMcm10$^{690-842}$	3D	16	1024×44×140	600	4.7(H); 120(N); 41(C)
HNCO‡	XMcm10$^{690-842}$	3D	8	2048×1×128	600	4.7(H); 120(N); 176(C)
HNHA†	XMcm10$^{755-842}$	3D	16	1024×140×50	600	4.7(H); 120.25(N)
	XMcm10$^{690-842}$	3D	16	1024×128×60	600	4.7(H); 120(N)
HBHA NH†	XMcm10$^{755-842}$	3D	16	1024×50×150	600	4.7(H); 120.5(N); 41(C)
	XMcm10$^{690-842}$	3D	16	2048×48×128	600	4.7(H); 120(N); 39(C)
(HB)CB(CGCD)HD‡	XMcm10$^{755-842}$	3D	400	1024×64	600	4.7(H); 119.5(N); 33(C)
^{13}C-NOESY-HSQC‡	XMcm10$^{755-842}$	3D	16	1024×50×220	600	4.7(H); 120.25(N); 41(C)
	XMcm10$^{690-842}$	3D	16	2048×50×256	800	4.7(H); 116(N); 42(C)
^{15}N-NOESY-HSQC†	XMcm10$^{755-842}$	3D	16	1024×80×196	800	4.7(H); 120.25(N); 39.5(C)
	XMcm10$^{690-842}$	3D	16	2048×60×240	800	4.7(H); 120(N); 38(C)
COSY	XMcm10$^{755-842}$	2D	28	2048×512	800	4.7(H)
	XMcm10$^{690-842}$	2D	8	2048×512	600	4.7(H)
NOESY	XMcm10$^{755-842}$	2D	32	2048×1024	800	4.7(H)
TOCSY	XMcm10$^{755-842}$	2D	28	1024×512	800	4.7(H)
† ^{15}N-enriched sample						
‡ ^{13}C-^{15}N-enriched sample						

Figure S1



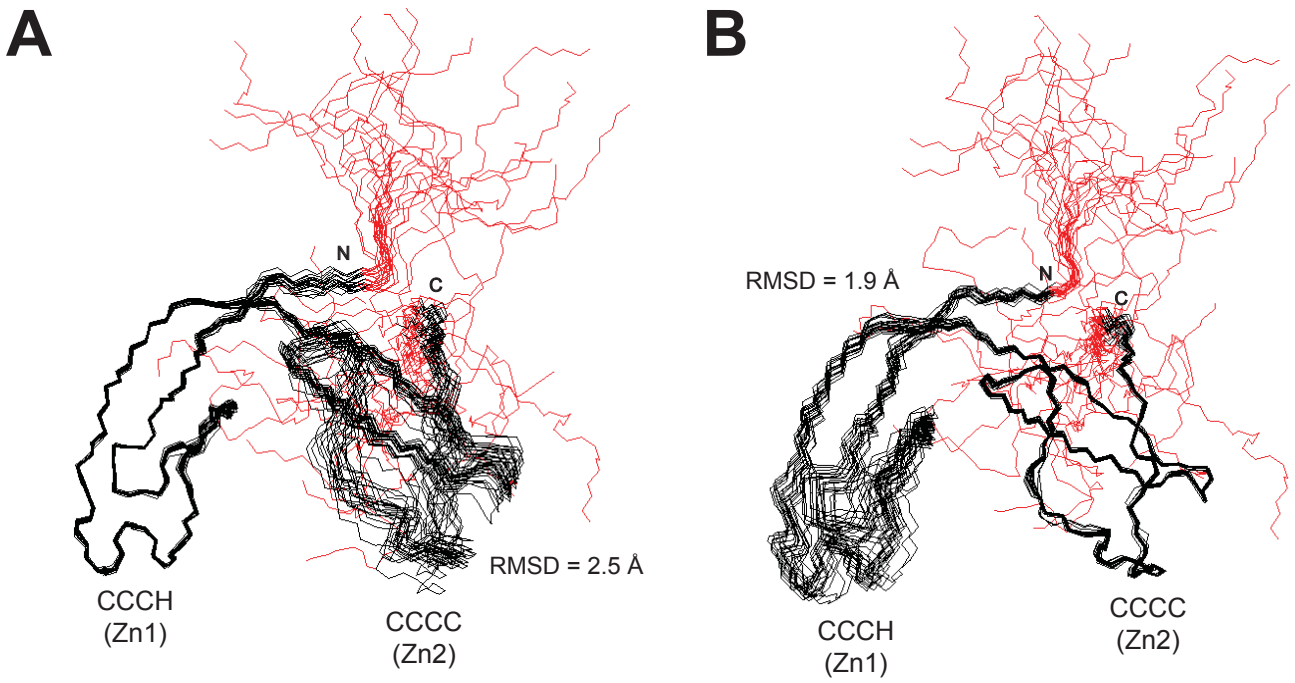
Identification of the XMcm10$^{690-842}$ CTD subdomain. Coomassie Blue stained SDS-PAGE gel of elastase-digested XMcm10$^{596-860}$. Sizes of molecular weight markers (M) in kDa are shown to the left. MALDI-TOF mass spectrometry and Edman degradation identified the promient 21-kDa molecular weight band as residues 755-842. Proteolysis reactions contained 7.5 µg XMcm10, 1–100 ng elastase, and 25 mM Tris-HCl at pH 7.5, 100 mM NaCl, and 5% glycerol. Reactions were carried out at 25 °C for 30 min and elastase inactivated by the addition of 10ul of SDS-PAGE sample buffer (63 mM Tris-HCl at pH 6.8, 700 mM 2-mercaptoethanol, 2% w/v SDS, 0.03% w/v bromophenol blue, and 10% glycerol) and heat for 5 min at 95 °C.

Figure S2



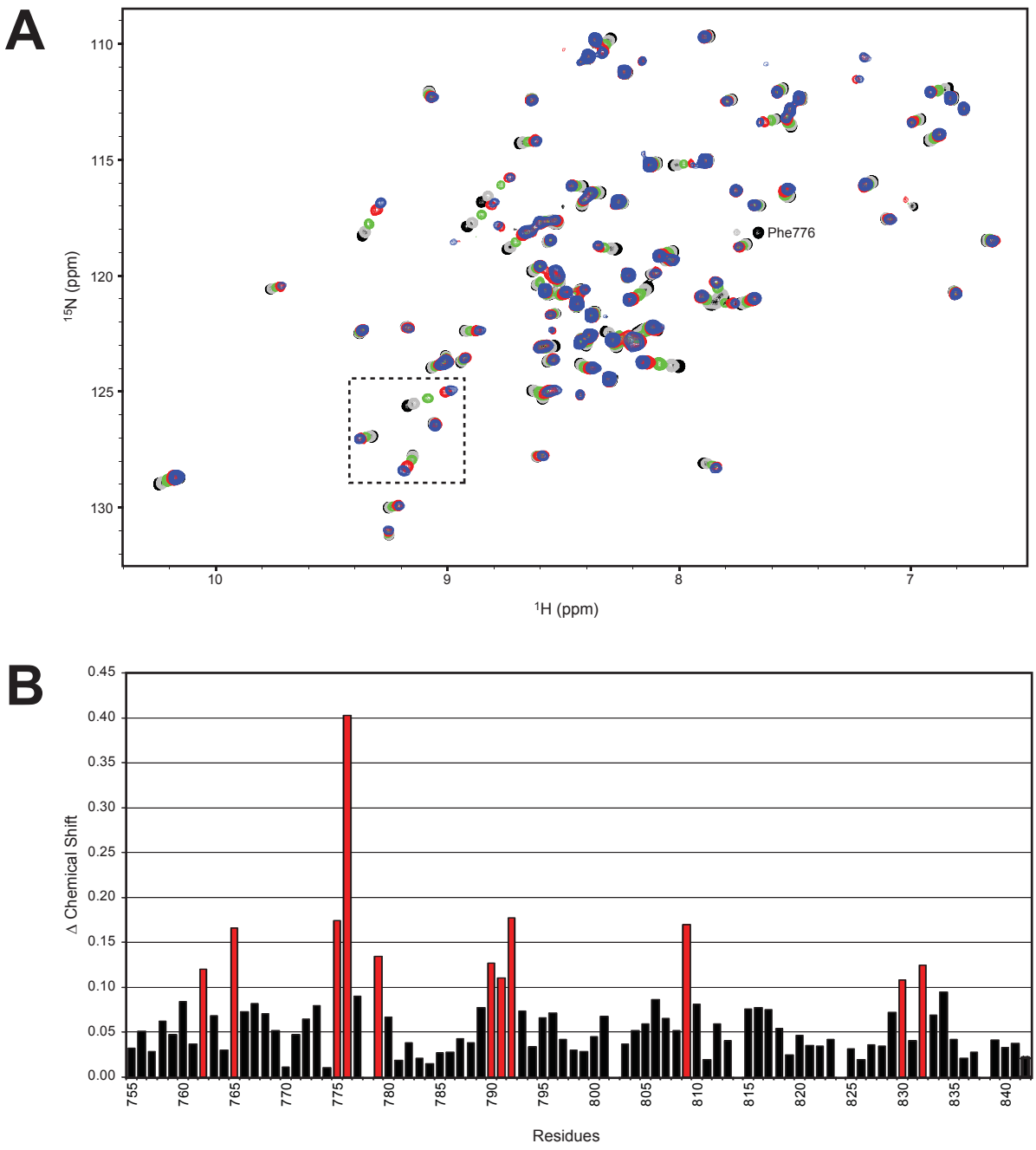
Identification of zinc coordinating residues. Shown are stereodiagrams of the CCCH (A) and CCCC (B) zinc clusters identified in XMcm10$^{755\text{-}842}$ prior to imposing restraints specific to zinc coordination. The clusters of side-chains shown in red were the result of energy minimization using only NOE distance restraints.

Figure S3



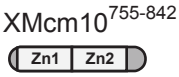
Relative motion between Mcm10-CTD zinc motifs. Backbone superposition of the ensemble of Mcm10$^{755-842}$ structures, aligned at the (A) CCCH motif (residues 765-795) and (B) CCCC motif (residues 795-830). The RMSD for backbone atoms of the unconstrained half of the model was 2.5 Å (A) and 1.9 Å (B).

Figure S4



NMR chemical shift perturbation of XMcm10$^{755-842}$ upon ssDNA binding. (A) Overlays of ^{15}N-^{1}H HSQC spectra of ^{15}N-enriched XMcm10$^{755-842}$ in the presence of 0 (black), 0.5 (grey), 1 (green), 2 (red), and 4 (blue) fold molar excess of ssDNA. Phe775, which dramatically changes resonance in response to ssDNA, is labeled. (B) Quantitation of chemical shift perturbation between the black and blue spectra shown in panel A, as defined in Experimental Procedures. Red bars represent chemical shift changes greater than one s.d. above the mean.

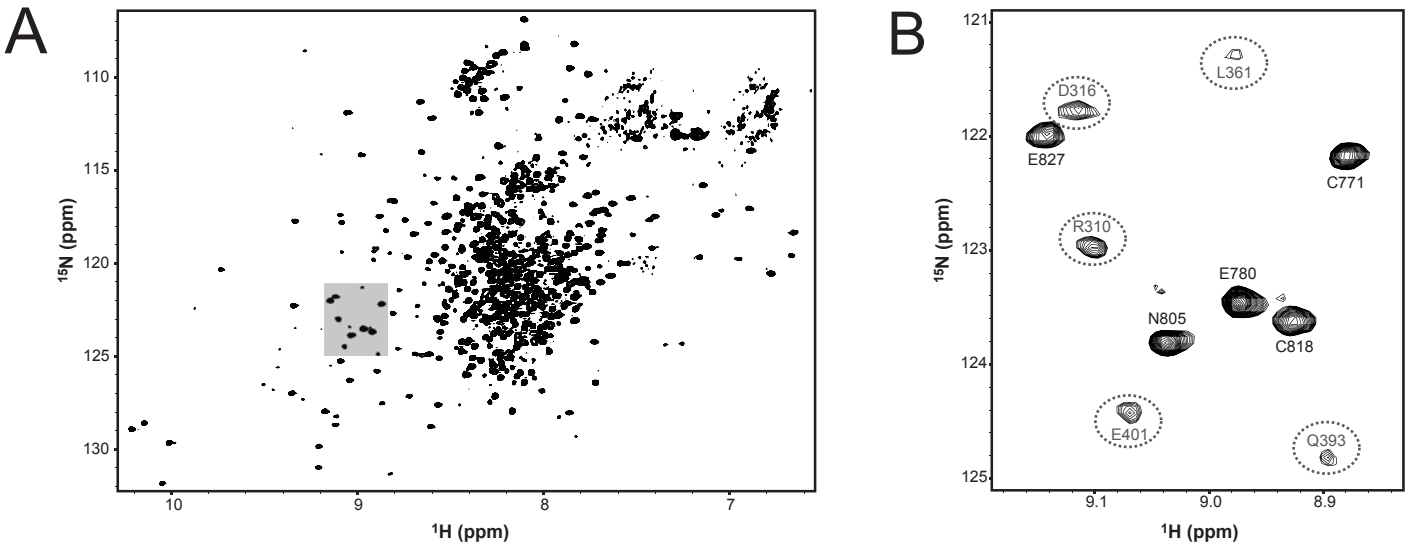
Figure S5



DNA length (nucleotides)	K_d (μM)	Sequence
25	3.3 ± 0.3	5′-ATGGTAGGCAACCATGTAGTAGTCA-FAM
20	4.1 ± 0.1	5′-AGGCAACCATGTAGTAGTCA-FAM
15	5.6 ± 0.1	5′-ACCATGTAGTAGTCA-FAM
10	17.1 ± 2.3	5′-GTAGTAGTCA-FAM
5	81.2 ± 2.1	5′-AGTCA-FAM

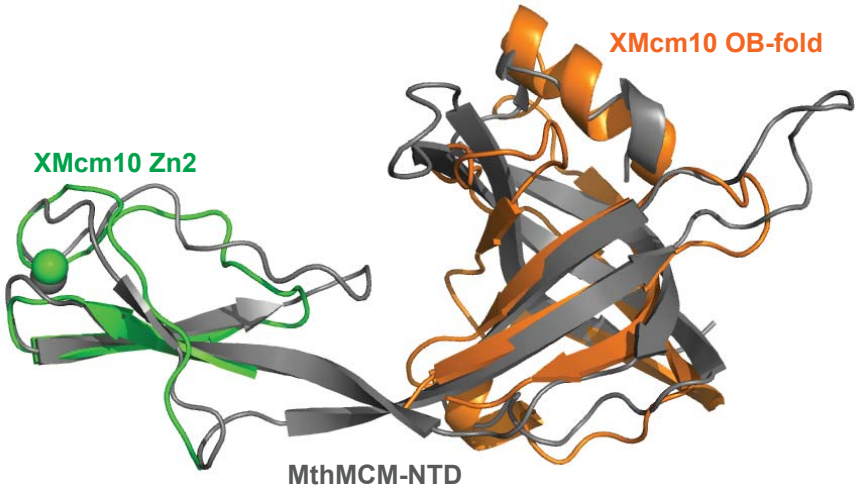
Length dependence for ssDNA binding to XMcm10$^{755-842}$. Dissociation constants (K_d) for XMcm10$^{755-842}$ binding to various lengths of ssDNA were measured by fluorescence polarization as described in Experimental Procedures. Values are the averages and standard deviations from three independent measurements. All oligonucleotides were labeled at the 3′-end with 6-carboxyfluorescein (FAM).

Figure S6



XMcm10$^{230\text{-}860}$ NMR spectrum. ^{15}N-^{1}H TROSY-HSQC spectrum of ^{15}N-enriched XMcm10$^{230\text{-}860}$ recorded at 800 MHz. (B) Expanded view of the grey shaded region of the spectrum displaying signals corresponding to residues from the ID (dotted circles) and the CTD.

Figure S7



Superposition of XMcm10-ID and -CTD onto MthMCM. Structural alignment of the CCCC zinc ribbon from XMcm10$^{755\text{-}842}$ (green), the OB-fold from XMcm10$^{230\text{-}427}$ (orange, PDB code 3EBE), and residues 94-242 of MthMCM (grey, PDB code 1LTL).