The tenacious recognition of yeast telomere sequence by Cdc13 is fully exerted by a single OB-fold domain

Supplemental Text and Figures

Methods

Protein expression, purification, and ssDNA binding assays were performed as described in the main text. Limited trypsinolysis of Cdc13-MOB-DBD was performed at a protein:trypsin ratio of 1000:1 in 50 mM potassium phosphate, pH 8, 300 mM NaCl, 100 mM Na_2SO_4, 5 mM βME at 25°C over a time course of 2 hours with a final overnight time point. 20 μL samples were removed from the digestion reaction at intermittent time points, added to 5 μL 5X SDS sample buffer, boiled, and held on ice until separated by SDS-PAGE. Protein bands were visualized by Coomassie blue staining.

The NMR sample was expressed and purified as described in the main text, using minimal expression media as previously described (1), with some modification. The free ^{15}N-MOB (344-496) was concentrated to 350 μM, and the ^{1}H-^{15}N HSQC data were collected at 20°C on a Varian Unity Inova 600 MHz spectrometer. After this spectrum was obtained Tel11 was titrated into the NMR sample to 1.5-2-fold molar excess (525-700 μM), and the equivalent experiment was then repeated.

Tandem size exclusion chromatography-multiangle light scattering (SEC-MALS) was performed as follows. 25-50 mg of purified recombinant protein was injected onto either a Shodex KW-802.5 column (BSA and Cdc13-MOB) or Shodex KW-803 column (BSA, Cdc13, and Cdc13-L91R) pre-equilibrated in 50 mM Tris, pH 7.8, 300 mM NaCl. Refractive index (Optilab DSP Interferometric Refractometer, Wyatt Technologies), multiangle light scattering (Dawn EOS MALS photometer, Wyatt Technologies), and UV absorbance (Spectrasystems UV3000) were monitored. Debye plots for determining molar mass were generated with Astra 4.8 (Wyatt Technologies) using the Zimm transformation for the extrapolation of the intensity of scattered light (Kc/R_θ) at an incident angle of 0° ($\theta = 0$). A specific refractive index increment (dn/dc) of 0.185 mL/g was used for calculation of the optical constant term (K) of the scattered light intensity. Data were plotted using Kaleidagraph and Adobe Illustrator.

Supplemental Table 1. $K_{D,app}$ values for the Cdc13 specificity profile.
Nucleotide substitutions are shown in bold, using IUPAC abbreviations (H = A,C,T; V = A,C,G). Errors are reported as the standard error of the mean (n = 3).

Oligo	$K_{D,app,HS}$ (nM)	$K_{D,rel}$	ΔΔG (kcal/mol)
GTGTGGGTGTG	0.30 +/- 0.03	1	-
HTGTGGGTGTG	64 +/- 5	220	3.0 +/- 0.10
G**V**GTGGGTGTG	6.8 +/- 0.7	25	1.7 +/- 0.1
GT**H**TGGGTGTG	92 +/- 28	390	3.1 +/- 0.2
GTG**V**GGGTGTG	53 +/- 10	160	2.8 +/- 0.2
GTGT**H**GGTGTG	0.40 +/- 0.03	1.4	0.16 +/- 0.1
GTGTG**H**GTGTG	0.73 +/- 0.05	2.5	0.49 +/- 0.1
GTGTGG**H**TGTG	8.9 +/- 0.6	31	1.9 +/- 0.1
GTGTGGG**V**GTG	0.43 +/- 0.03	1.7	0.2 +/- 0.1
GTGTGGGT**H**TG	6.0 +/- 0.9	20	1.7 +/- 0.2
GTGTGGGTG**V**G	1.5 +/- 0.08	5.4	0.9 +/- 0.1
GTGTGGGTGT**H**	0.51 +/- 0.05	1.8	0.3 +/- 0.1

Supplemental Figure Legends

Supplemental Figure 1. SEC-MALS analysis of full-length Cdc13. The molecular mass of Cdc13 was determined in solution by SEC-MALS. LS, light scatter; UV, ultraviolet light absorbance; RI, refractive index; MM, molar mass. The calculated molecular mass of wildtype *S. cerevisiae* Cdc13 was 206 kDa, indicating that the Sf9-produced Cdc13 protein is a homodimer.

Supplemental Figure 2. Comparison of Cdc13 and Cdc13-DBD binding behavior. (A) The specificity profiles of Cdc13 (this work) and Cdc13-DBD (2) are plotted together. Values were calculated as $\Delta\Delta G = RT \ln (K_{D1} / K_{D2})$, where K_{D1} is the apparent binding constant for the substituted oligonucleotide and K_{D2} is the apparent binding constant for the cognate Tel11 oligonucleotide. Errors are reported as standard error of the mean. (B) The changes in free energy of binding for alanine mutations within Cdc13 (this work) and Cdc13-DBD (1) are plotted. Values were calculated as above, with K_{D1} as the apparent binding constant for the alanine mutant and K_{D2} as the apparent binding constant for wildtype protein.

Supplemental Figure 3. Characterization of Cdc13-MOB-DBD. (A) Purified recombinant Cdc13-MOB-DBD, Cdc13-DBD, and Cdc13-MOB proteins, visualized by SDS-PAGE and Coomassie blue staining. (B) Limited trypsinolysis of Cdc13-MOB-DBD, visualized by SDS-PAGE and Coomassie blue staining. The two trypsinolysis products have the same mobility as Cdc13-MOB and Cdc13-DBD, as labeled. (C)

Purified recombinant Cdc13-DBD and Cdc13-MOB-DBD were analyzed by low-salt EMSA (circles) and high-salt double-filter binding (squares). Quality data were obtained for a titration from three logs below to three logs above the $K_{D,app}$.

Supplemental Figure 4. Cdc13-MOB exists in a monomer/dimer equilibrium and does not bind Tel11. (A) SEC-MALS elution profile of Cdc13-MOB. LS, light scatter; UV, ultraviolet light absorbance; RI, refractive index; MM, molar mass. Two elution peaks were observed for *S. cerevisiae* Cdc13-MOB. At 8 mL elution volume is a peak corresponding to a ~51 kDa species, consistent with a Cdc13-MOB homodimer, while a larger peak that eluted at 8.8 mL was calculated to be a 21 kDa species, consistent with a Cdc13-MOB monomer. These data indicate that concentrated Cdc13-MOB exists in a monomer/dimer equilibrium in solution in 300 mM NaCl, consistent with previous report (3). (B) EMSA of Cdc13-MOB-DBD incubated with radiolabeled Tel11. (C) HSQC of Cdc13-MOB-DBD in the absence (red) and presence (blue) of unlabeled Tel11. Blue peaks (+Tel11) that do not superimpose with red peaks (-Tel11) are the result of protein degradation during the timecourse of data collection, which has also been observed with Cdc13-DBD (D.S.W., unpublished observations).

Supplemental Figure 5. Cdc13 and Cdc13-5 exhibit indistinguishable ssDNA binding activity. Cdc13 and Cdc13-5 were purified as previously described (4) and evaluated for Tel11 binding in 5 mM HEPES, pH 7.8, 75 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM Na_2EDTA, 0.1 mg/mL BSA, 1 mM DTT and separated on precast 4-20 % gradient ReadyGels (BioRad). Representative curves are shown. There was no observable difference between Cdc13 and Cdc13-5 in either $K_{D,app}$ (top) or specificity (bottom) within the critical 5' GxGT motif in the substrate.

Supplemental Figure 6. SEC-MALS analysis of Cdc13-L91R. The molecular mass of Cdc13-L91R was determined in solution by SEC-MALS. LS, light scatter; UV, ultraviolet light absorbance; RI, refractive index; MM, molar mass. The calculated molecular mass of *S. cerevisiae* Cdc13 was 105 kDa, indicating that Cdc13-L91R is a monomer in solution.

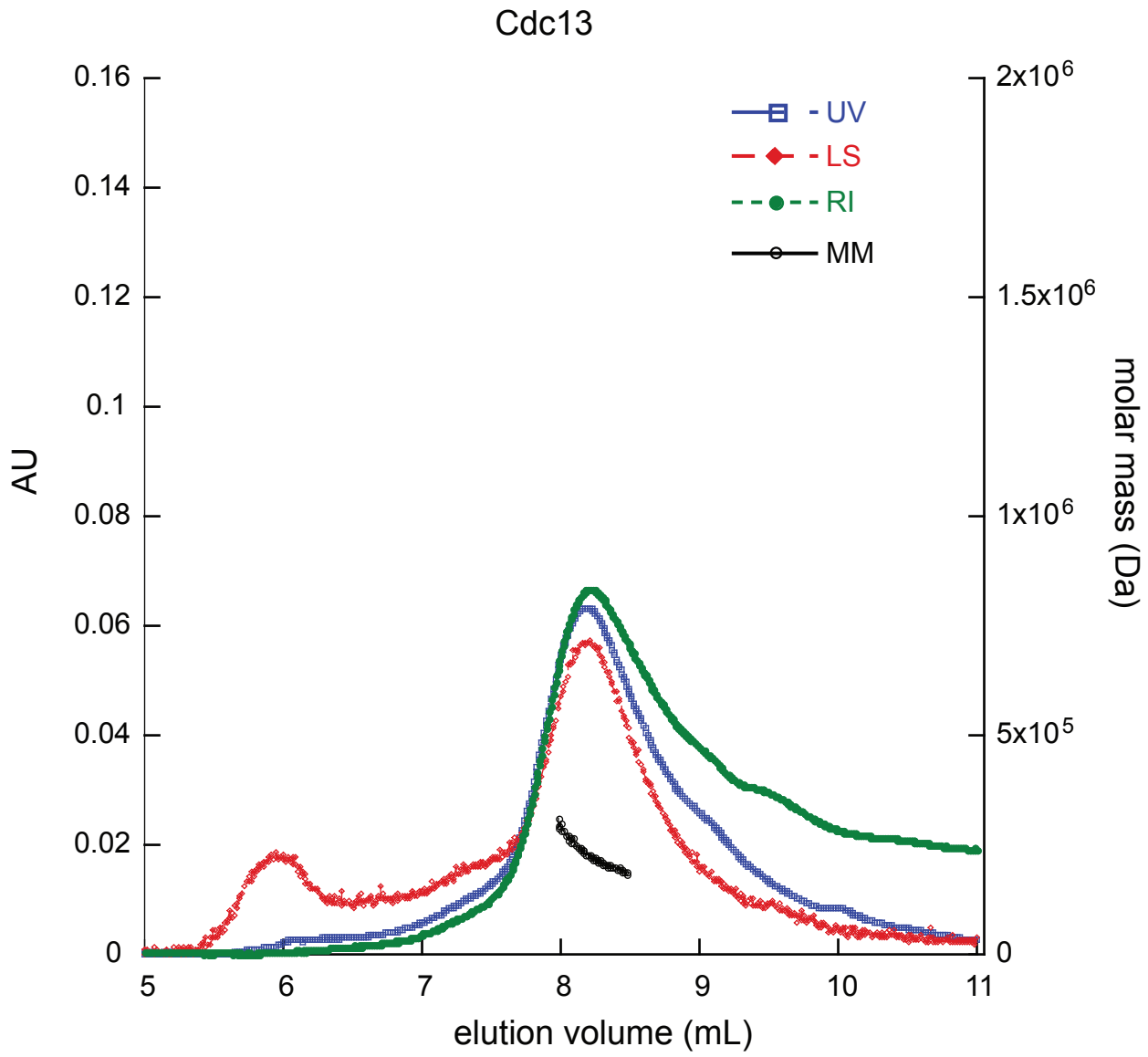
References

1. Anderson,E.M., Halsey,W.A. and Wuttke,D.S. (2002) Delineation of the high-affinity single-stranded telomeric DNA-binding domain of *Saccharomyces cerevisiae* Cdc13. *Nucleic Acids Research*, **30**, 4305–4313.

2. Eldridge,A., Halsey,W. and Wuttke,D. (2006) Identification of the determinants for the specific recognition of single-strand telomeric DNA by Cdc13. *Biochemistry*, **45**, 871–879.

3. Mason,M., Wanat,J.J., Harper,S., Schultz,D.C., Speicher,D.W., Johnson,F.B. and Skordalakes,E. (2012) Cdc13 OB2 Dimerization Required for Productive Stn1 Binding and Efficient Telomere Maintenance. *Structure*, 10.1016/j.str.2012.10.012.

4. Zappulla,D.C., Roberts,J.N., Goodrich,K.J., Cech,T.R. and Wuttke,D.S. (2009) Inhibition of yeast telomerase action by the telomeric ssDNA-binding protein, Cdc13p. *Nucleic Acids Research*, **37**, 354–367.



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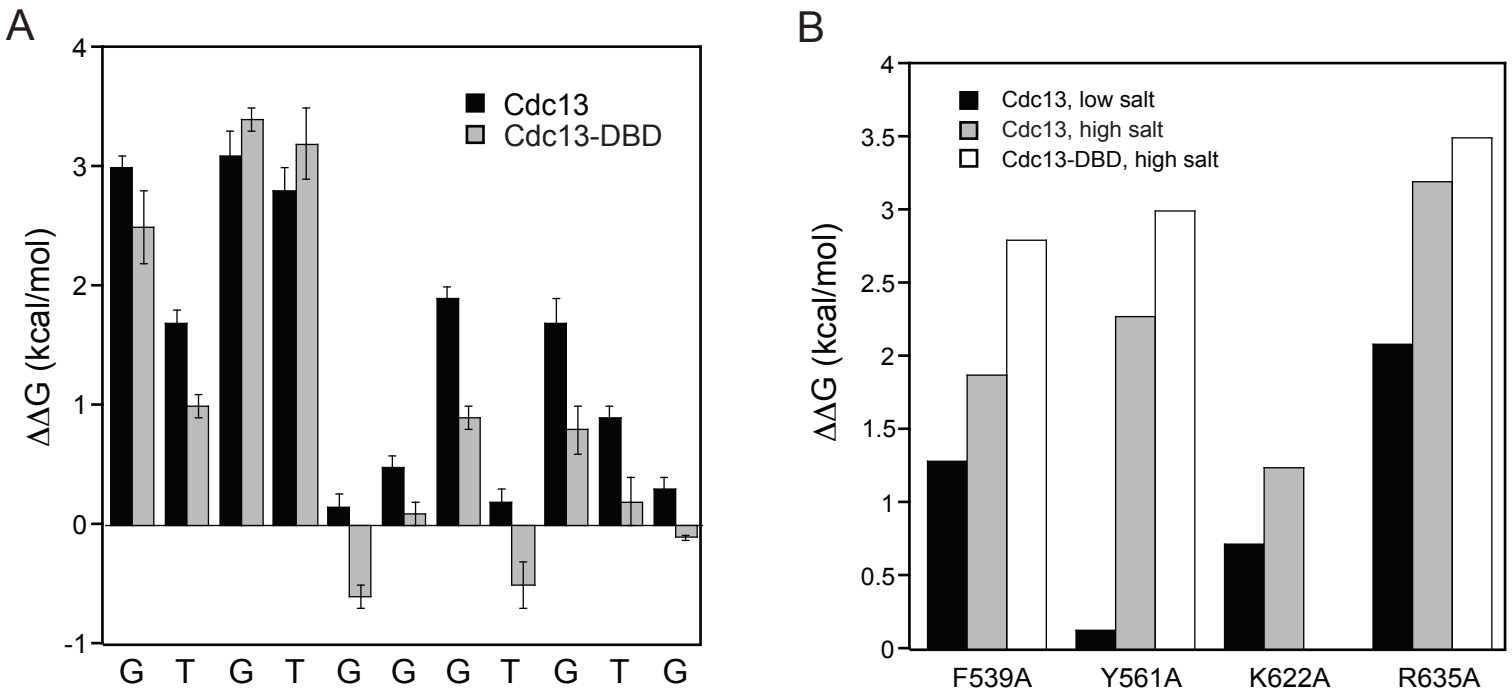

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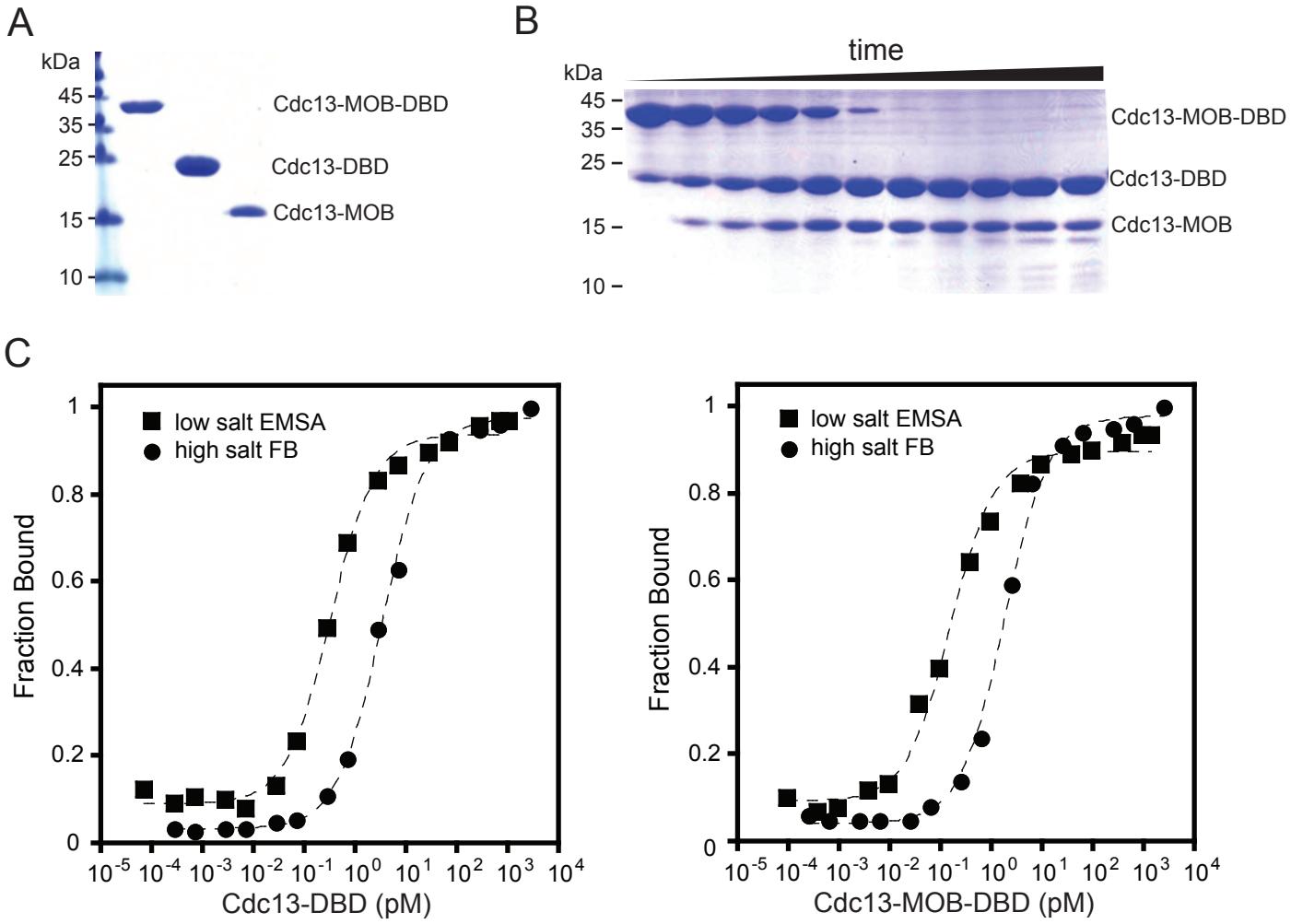

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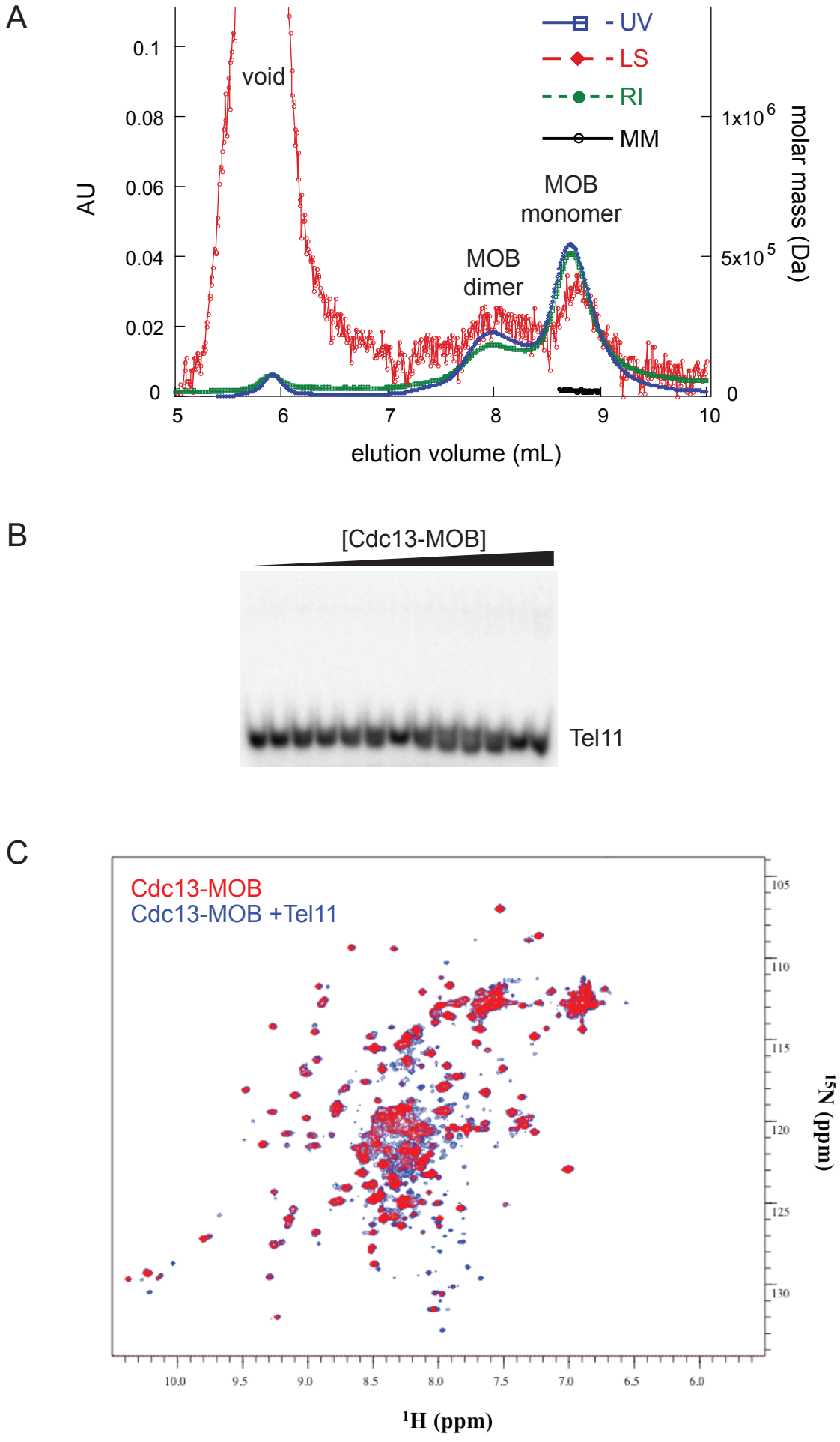

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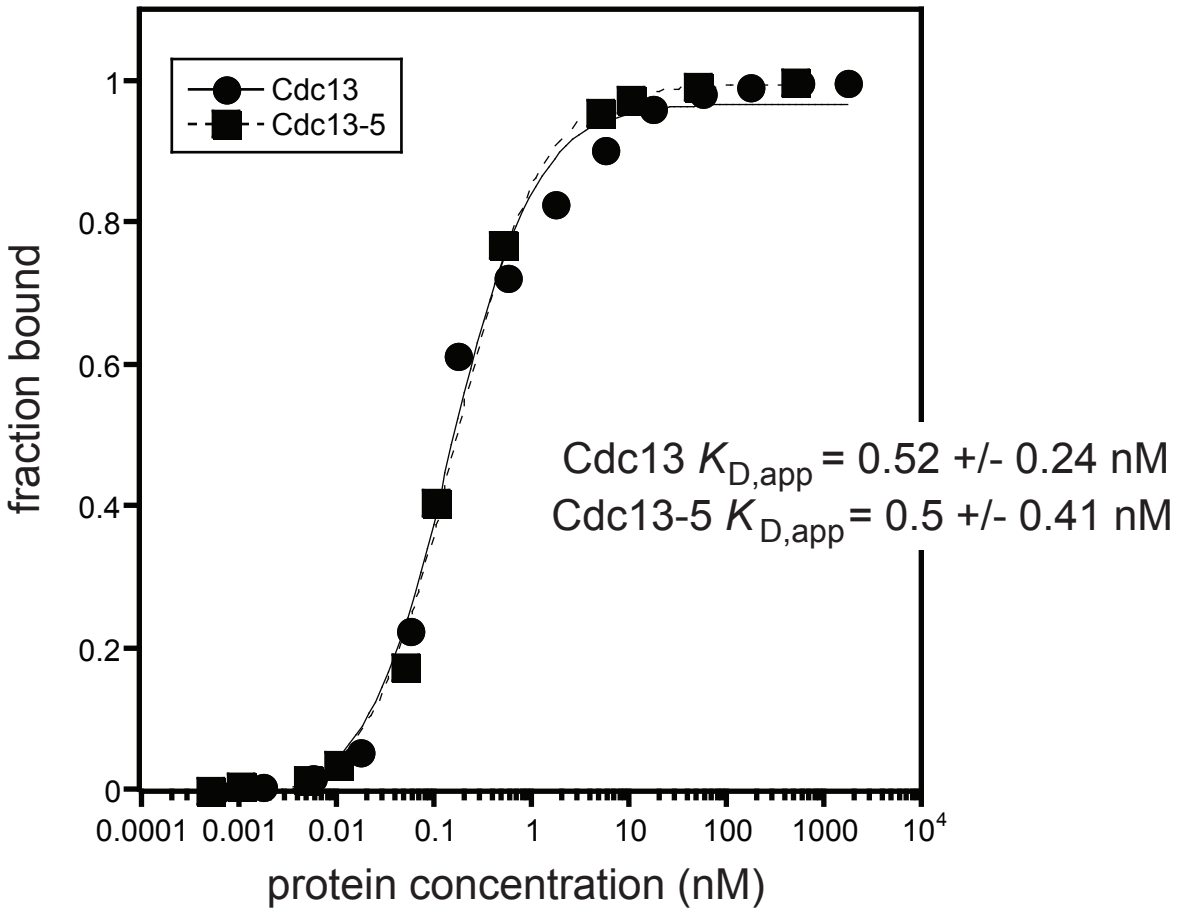

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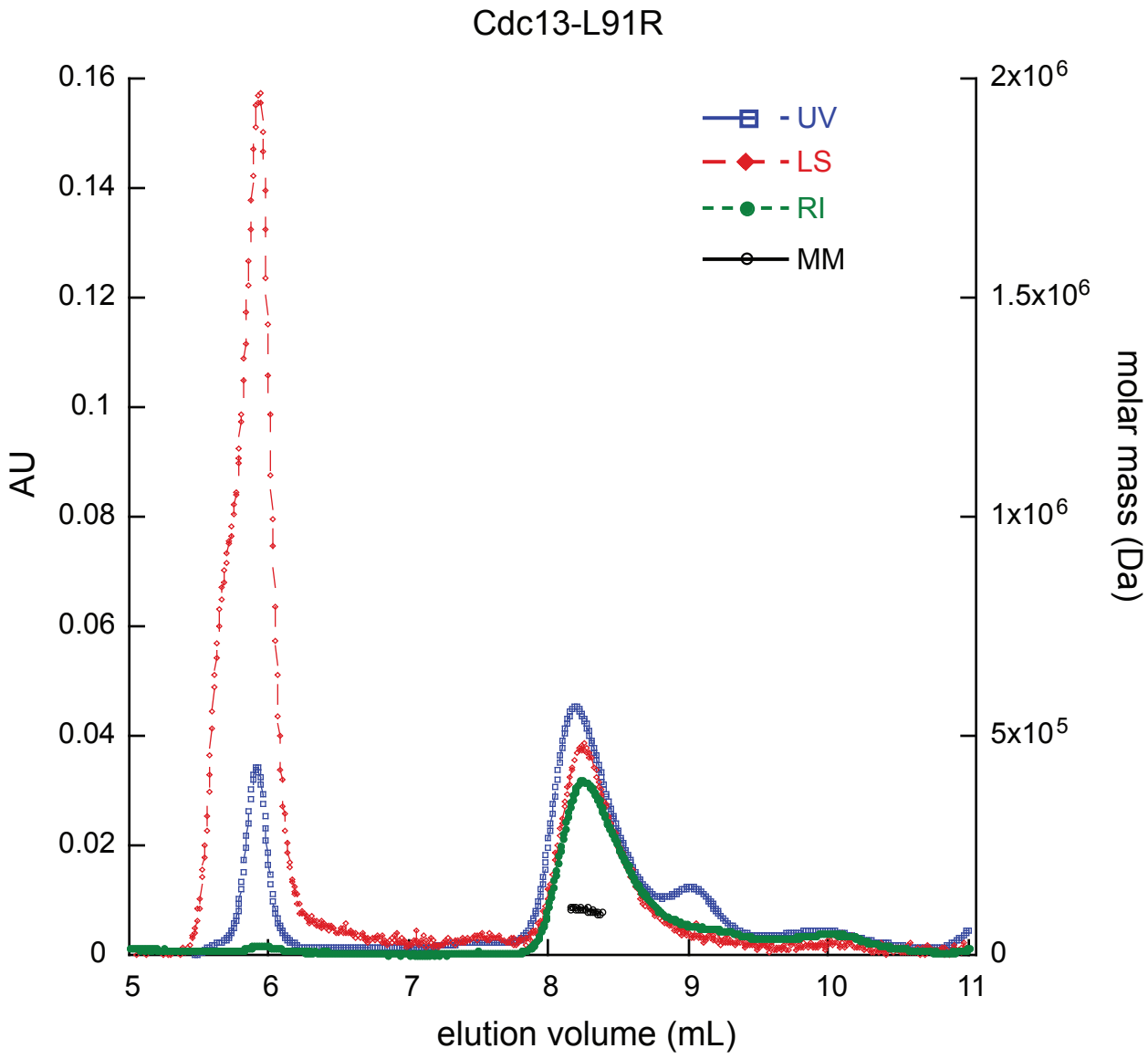

Supplemental Figure 6.