Supporting Information for:

Folding and binding of an intrinsically disordered protein: fast, but not 'diffusion-limited'

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SUPPLEMENTARY METHODS

Pseudo-First Order kinetics

Due to the problem of PUMA oligomerization (see main text results), Mcl-1 was used in excess. All traces were fit to a single exponential (eq S1).[1]

$$F = F_{\infty} + \Delta F \exp(-k_{obs} t) \quad \textbf{(S1)}$$

where $k_{obs} = k_{+}$[Mcl-1], F is fluorescence, k_{+} is the association rate constant, t is time, ΔF is the fluorescence amplitude of the reaction and F_{∞} the final fluorescence.

Low concentration (nM) association

Association was followed by manual mixing of Mcl-1 and PUMA solutions at nM concentrations, monitored using intrinsic (tryptophan) fluorescence, using a Varian Cary Eclipse (Agilent Technologies). Excitation at 280 nm and emission at 340 nm, both slit widths were set to 20 nm. Traces were fit to eq 3 in the main text or the following reversible eq S2 [2]:

$$b = -\left(\frac{k_-}{k_+}\right) - 2[A]_0;$$

$$z = \sqrt{\left(\frac{k_-}{k_+}\right)^2 + 4\left(\frac{k_-}{k_+}\right)[A]_0}$$

$$F = F_0 + \Delta F\left(\frac{(b-z)(1-\exp(zk_+t)}{2\left(-1+\left(\frac{b-z}{b+z}\right)\exp(zk_+t)\right)}\right) \quad \textbf{(S2)}$$

where k_+ is the association rate constant (fixed at 1.59×10^7 M^{-1} s^{-1}, obtained from higher concentration irreversible association, see main text), k_- is the dissociation rate constant, t is time, ΔF is the fluorescence amplitude of the reaction and F_0 the initial fluorescence.

Viscosity determination

The viscosity of solutions at different temperatures and concentrations of urea were calculated using published empirical formulae[3,4]. Viscosity of glucose solutions were measured using BS/U-TUBE A viscometer tubing (Cannon) at 25˚C.

Estimation of R_h for PUMA and Mcl-1 constructs

The radius of gyration (R_g) for Mcl-1 was estimated at 1.56 nm using the NMR structure (pdb 1WSX), using the program VMD (http://www.ks.uiuc.edu). This was converted to an estimate for the hydrodynamic radius (R_h) using the empirical relationships $R_h = 1.29R_g$ [5] to give R_h = 2.01 nm. R_h for PUMA estimated at 1.34 nm[6]. Using eq 1 in the main text this gives a k_+=7.73x10^9 M^{-1} s^{-1} (at 25 °C in H_2O).

Effect of temperature on R_h

As temperature is increased the PUMA peptide looses helicity and its R_h may increase. However, the estimate for the association rate constant is relatively insensitive to the sizes of the proteins and doubling R_h for PUMA (2.68 nm) gives, using eq 1, k_+=7.59x10^9 M^{-1} s^{-1}, only 2% lower. A faster 'diffusion-limited' rate is observed when there is a discrepancy between the two sizes of the proteins, one can diffuse faster while the other acts as a larger target. As PUMA's R_h gets larger with temperature this should *slow* the 'diffusion-limited' reaction rate as it gets more similar in size to Mcl-1.

SUPPLEMENTARY FIGURES

Figure S1. Mcl-1 undergoes only small conformational changes upon PUMA binding. The NMR structure of Mcl-1 in the absence of PUMA (pdb 1WSX, magenta) is overlaid with the NMR structure of Mcl-1 (pdb 2ROC, grey) with PUMA (pdb 2ROC, blue) bound (RMSD of 1.78 Å). Apparently the α-helices that make up the binding site separate slightly upon binding the PUMA peptide.

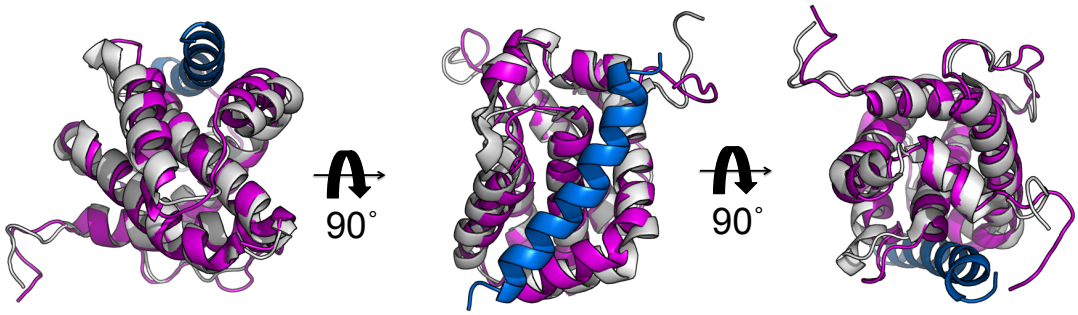

Figure S2. Relevant protein sequences for this study. (**a**) Sequence of the 27 aa PUMA peptide used in the NMR structure pdb 2ROC (top) compared to the 34 aa PUMA peptide used in this study (bottom). (**b**) Sequence of full-length PUMA (Uniprot Q99ML1) (top) compared to the PUMA peptide used in this study (bottom). Note M144I mutation chosen to match the peptide to that in the NMR structure (pdb 2ROC). (**c**) Sequence of full length Mcl-1 (UniProt P97287) (top) compared with the truncated version used in this study (bottom). Truncation chosen to match with NMR structure (pdb 2ROC), the N-terminal GS is an artifact from the protease cleavage during purification. (**d**) Sequence of GB1-PUMA construct (top) compared to the folded GB1 sequence (pdb 3GB1) followed by the PUMA peptide used in this study (both shown on bottom).

a

$^{+}H_3N$-EEEWAREIGAQLRRIADDLNAQYERRM-COO^-

Ac-VEEEEWAREIGAQLRRIADDLNAQYERRRQEEQH-NH_2

b

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MARARQEGSSPEPVEGLARDSPRPFPLGRLMPSAVSCSLCEPGLPAAPAAPALLPAA
------------------------------------------------------------

YLCAPTAPPAVTAALGGPRWPGGHRSRPRGPRPDGPQPSLSPAQQHLESPVPSAPEA
------------------------------------------------------------

                               144
LAGGPTQAAPGVRVEEEEWAREIGAQLRRMADDLNAQYERRRQEEQHRHRPSPWRVM
-------------VEEEEWAREIGAQLRRIADDLNAQYERRRQEEQH----------

YNLFMGLLPLPRDPGAPEMEPN
-----------------------
```

c

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MFGLRRNAVIGLNLYCGGASLGAGGGSPAGARLVAEEAKARREGGGEAALLPGARVV
------------------------------------------------------------

ARPPPVGAEDPDVTASAERRLHKSPGLLAVPPEEMAASAAAAIVSPEEELDGCEPEA
------------------------------------------------------------

IGKRPAVLPLLERVSEAAKSSGADGSLPSTPPPPEEEEDDLYRQSLEIISRYLREQA
-----------------------------------GSEDDLYRQSLEIISRYLREQA

TGSKDSKPLGEAGAAGRRALETLRRVGDGVQRNHETAFQGMLRKLDIKNEGDVKSFS
TGSKDSKPLGEAGAAGRRALETLRRVGDGVQRNHETAFQGMLRKLDIKNEGDVKSFS

RVMVHVFKDGVTNWGRIVTLISFGAFVAKHLKSVNQESFIEPLAETITDVLVRTKRD
RVMVHVFKDGVTNWGRIVTLISFGAFVAKHLKSVNQESFIEPLAETITDVLVRTKRD

WLVKQRGWDGFVEFFHVQDLEGGIRNVLLAFAGVAGVGAGLAYLIR
WLVKQRGWDGFVEFFHVQDLEGG-----------------------
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d

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MRGSHHHHHHGLVPRGSTYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDG
----------------MTYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDG

EWTYDDATKTFTVTENYDIPTSHGIEGRVEEEEWAREIGAQLRRIADDLNAQYERRR
EWTYDDATKTFTVTE-------------VEEEEWAREIGAQLRRIADDLNAQYERRR

QEEQH
QEEQH
```

Figure S3. PUMA peptide undergoes reversible oligomerization to an α-helical structure. (**a**) The Circular dichroism spectra of the PUMA peptide becomes more α-helical at higher concentrations, consistent with the formation of an α-helical oligomer. (**b**) Oligomers are visible on denaturing SDS-PAGE gel of PUMA peptide at ~10-100 μM concentrations. White numbers refer to elution volumes from FPLC trace (**c**). (**c**) FPLC Superdex G30 trace shows the earlier elution (and therefore larger size) of PUMA oligomers (i), for comparison a trace for a similar size monomeric peptide is shown (ii).

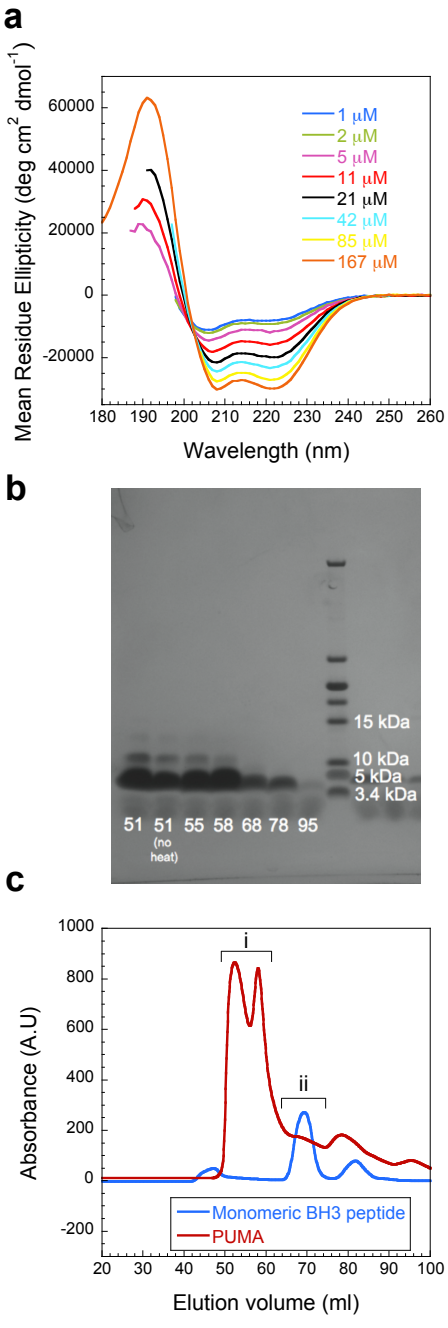


Figure S4. Fluorescence spectra of Mcl-1 and PUMA peptide show Mcl-1 is folded, PUMA is unfolded and binding occurs. Mcl-1 (magenta) shows an intrinsic fluorescence spectrum characteristic of buried tryptophans whereas PUMA (blue) shows the spectrum of a solvent exposed tryptophan. An increase in fluorescence is seen upon mixing 1 μM: 1 μM of Mcl-1 and PUMA peptide. 1:1 complex (black solid line) has a greater signal than the spectrum predicted for no interaction (dashed line), which is the sum of the PUMA alone (blue) and Mcl-1 alone (magenta) spectra.

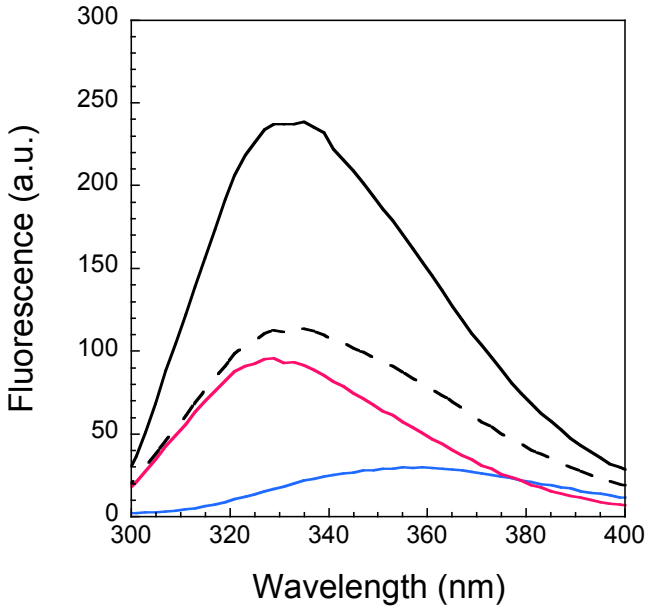


Figure S5. Pseudo-first-order results. Plot of the observed association rate constant after fitting kinetics traces, under pseudo-first order conditions with Mcl-1 in excess, to a single exponential function (Supplementary Methods). k_{obs} is linear with concentration of Mcl-1 to give a concentration-independent estimate of k_{+}.

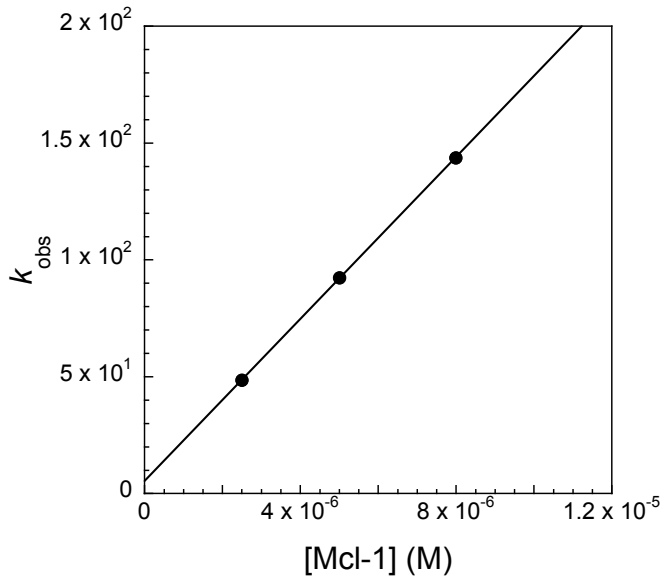


Figure S6. Low concentration association kinetics can yield k_- and therefore K_d values. (**a**) Association kinetics of 7.1 nM Mcl-1 manually mixed with 7.1 nM PUMA, fluorescence data (blue) fit well to the reversible model (black, see Supplementary Methods) (**b**) As the concentration is low (7.1 nM), and close to the K_d, significant back reaction is expected during association and consequently data (blue) fit poorly to an irreversible model (black, see main text, Figure 1D and Methods). (**c**) Association kinetics of 14.1 nM Mcl-1 manually mixed with 14.1 nM PUMA fit well to the reversible model (**d**) Association kinetics at 14.1 nM fit poorly to an irreversible model.

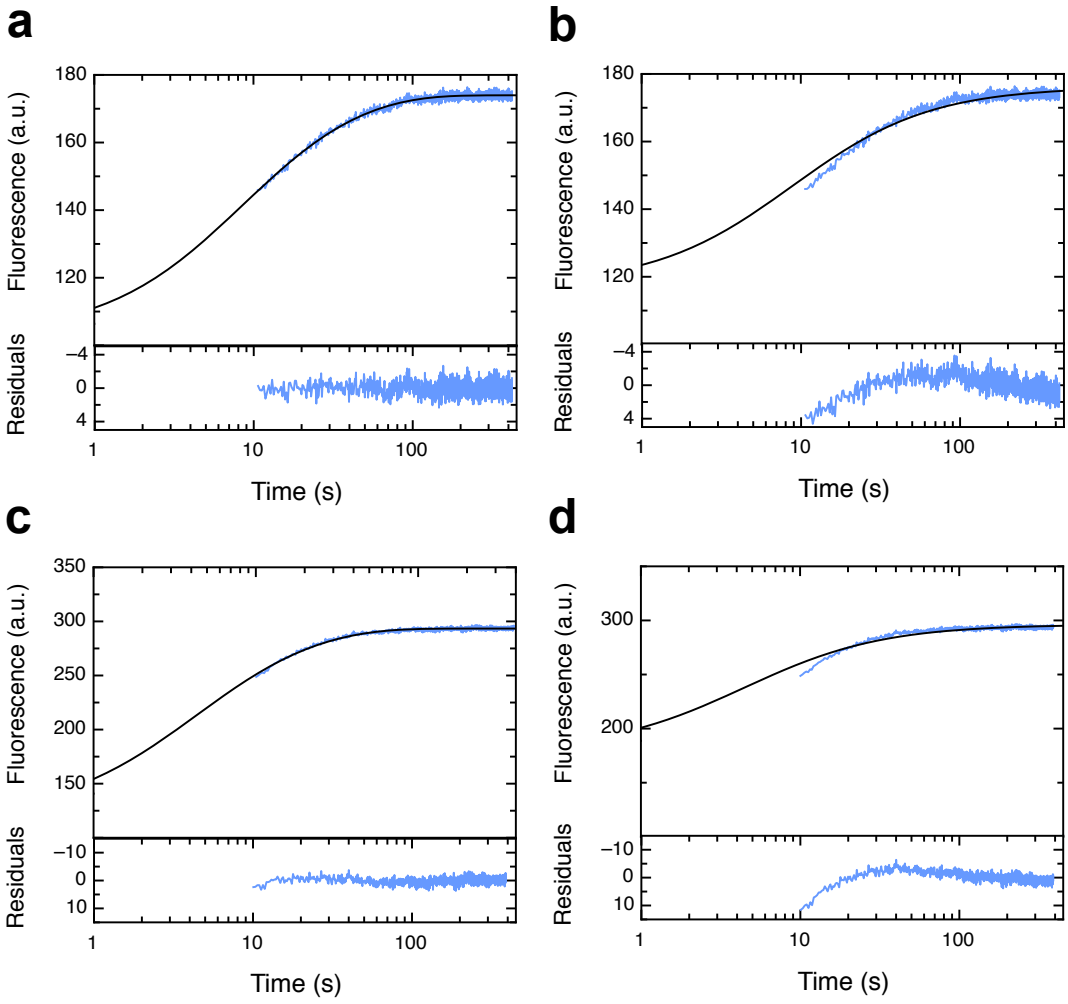


Figure S7. Ionic strength dependence of k_+, linear plot of k_+ with $1/(1+\kappa a)$. According to the empirical Debye-Hückel-like approximation (see main text Methods) $\ln(k_+)$ should vary with the ionic strength according to eq 4 where κ is the inverse of the Debye length and a the minimal distance of approach (see main text Methods). A linear dependence is observed and fit to a straight line to obtain the values for k_{+basal} (quoted in the main text) and electrostatic energy of interaction[7] for PUMA U = -3.4 (± 0.2) kcal mol^{-1} and for GB1-PUMA U = -4.2 (± 0.2) kcal mol^{-1}.

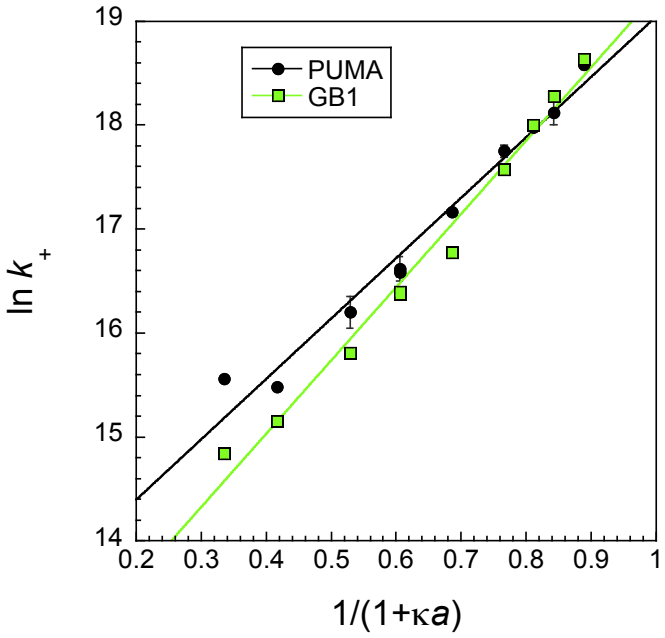


Figure S8. Mcl-1 remains folded up to 3.5 M urea. Mcl-1 fraction folded calculated from the intrinsic tryptophan fluorescence of Mcl-1 (1 μM Mcl-1, 25 °C, 50 mM PO_4 pH 7.0, excitation at 280 nm, 10 nm pathlength, emission at 360 nm, 5nm pathlength).

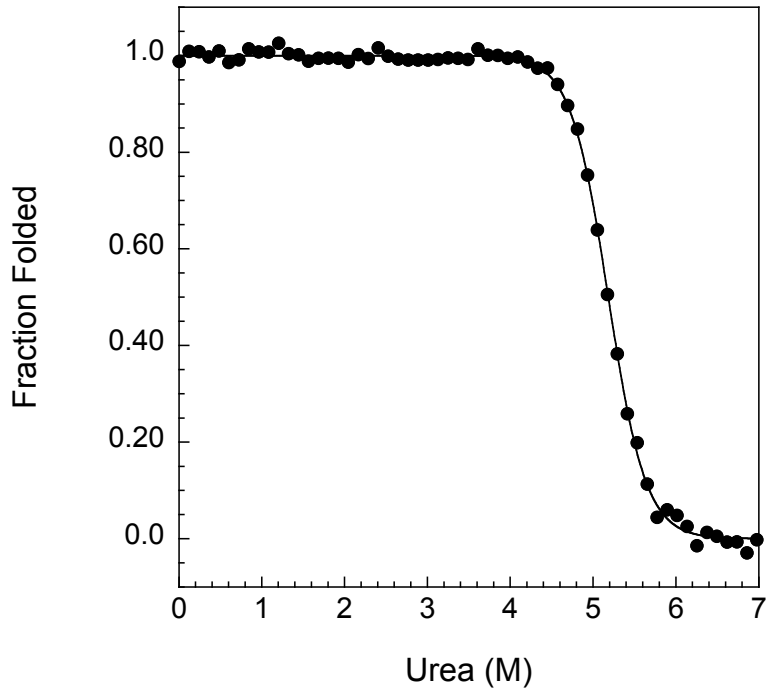
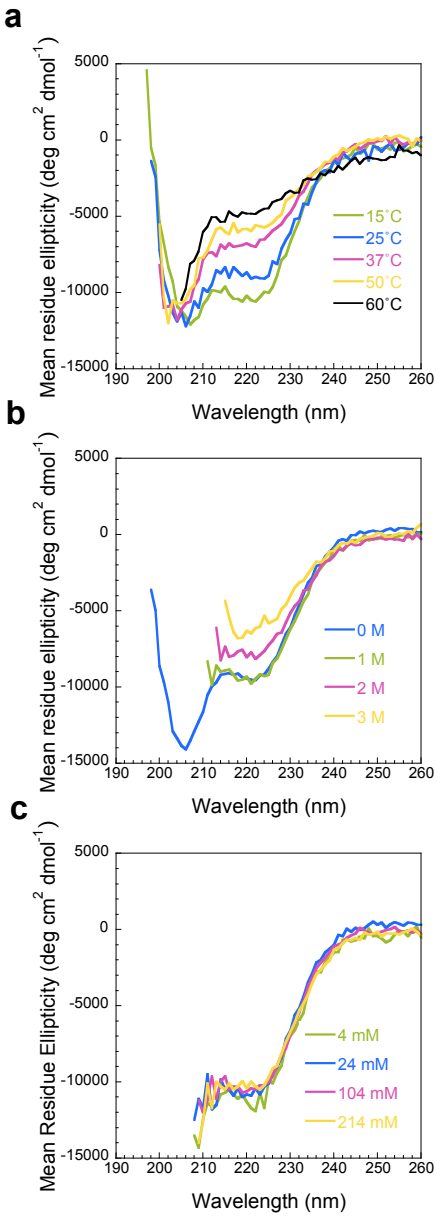


Figure S9. Changes in helicity of PUMA peptide with solvent and temperature. Due to the oligomerization of the PUMA peptide circular dichroism (CD) was recorded at the low concentration of 1 μM with a 10 mm pathlength cuvette to maximize signal. Errors due to buffer subtraction and high absorbances prevent quantitative analysis of these spectra. However, each individual figure was recorded on the same day so qualitative conclusions can be drawn. (**a**) The helicity of the PUMA peptide is highly temperature dependent, with lower temperatures favoring helical conformations. (**b**) Increasing concentration of the denaturant urea results in the disfavoring of helical conformations. (**c**) Changing the ionic strength of the solution did not result in any significant change in CD signal.



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