The Role of GCN4 Conformational Dynamics in DNA Binding and Recognition

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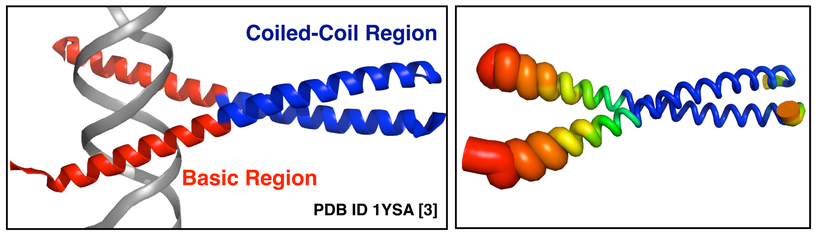
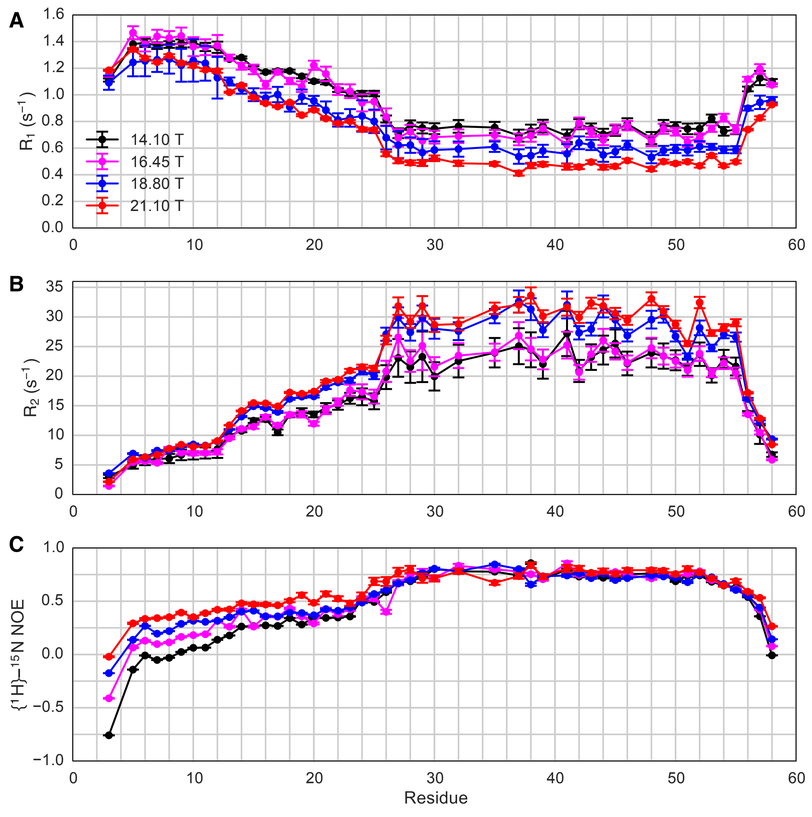
Introduction

Intrinsically disordered proteins (IDPs) are known to play important roles in regulatory and signaling pathways. A critical aspect of this function is the ability of IDPs to form highly specific complexes with target molecules. To understand the role of conformational dynamics in recognition, we have studied the basic leucine-zipper domain (bZip) of the transcription factor GCN4 from *S. cerevisiae*. GCN4 bZip is an α-helical homodimer with the C-terminal leucine zipper region forming a coiled-coil and the N-terminal basic region binding DNA. In the absence of DNA, the basic region is partially disordered. NMR spin relaxation experiments (R_1, R_2, and {^{1}H}–^{15}N heteronuclear NOE) recorded at four static magnetic fields have been used to study the conformational dynamics of *apo* GCN4. The results of these experiments confirm the residue-specific order parameters (S^2) previously determined by spectral density mapping using relaxation data recorded at a single static magnetic field [1], and in particular, support the conclusion that local regions of restriction in the basic region are associated with transient helical conformations. The relaxation data acquired at multiple static magnetic fields additionally enable characterization of internal dynamic processes of the basic region of the bZip domain because the well-ordered leucine zipper coiled-coil dominates the global diffusion tensor. The basic region exhibits internal motions on two time scales, τ_f = 40–70 ps and τ_s = 1–2 ns. The slower process may be associated with transient helix formation and could facilitate binding to DNA through a combination of conformational selection and induced fit mechanisms [2].



NMR Spin Relaxation Experiments



The NMR **(A)** longitudinal (R_1) and **(B)** transverse (R_2) relaxation rate constant and **(C)** {^{1}H}-^{15}N heteronuclear NOE were measured for *apo* GCN4 at 14.1, 16.45, 18.8, and 21.1 T at 300 K. U-[^{15}N, ^{2}H]-labeled GCN4 was 800 µM monomer in 50 mM KCl, 50 mM Na-acetate-d_3, pH 4.5, and 10% ^2H_2O. Relaxation rates and NOE ratios were determined with Relax [4,5]. Errors for R_1 and R_2 rates were determined from Monte Carlo simulations, while the error for NOE ratios was determined from the noise floor.

References
1. Bracken C., Carr P.A., Cavanagh J. & Palmer A.G. (1999) *J Mol Biol* **285**, 2133-46.
2. Robustelli P., Trbovic N., Friesner R.A. & Palmer A.G. (2013) *J Chem Theory Comput* **9**, 5190-200.
3. Ellenberger T.E., Brandl C.J., Struhl K. & Harrison S.C. (1992) *Cell* **71**, 1223-37.
4. d'Auvergne E. & Gooley P. (2008) *J Biomol NMR* **40**, 107-19.
5. d'Auvergne E. & Gooley P. (2008) *J Biomol NMR* **40**, 121-33.

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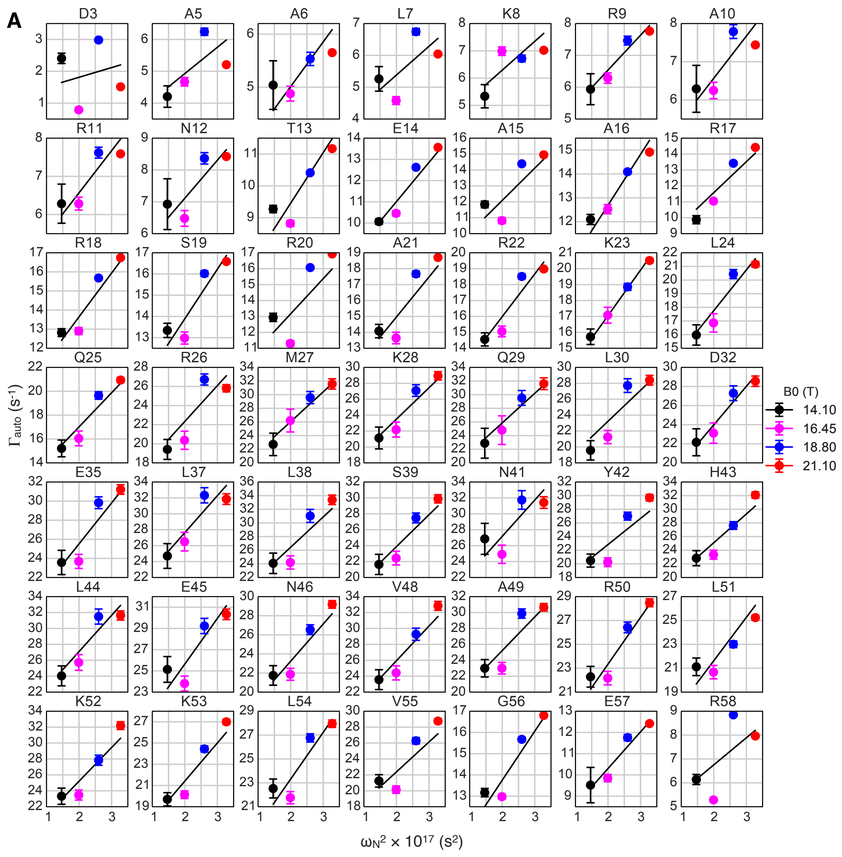
GCN4 Does Not Exhibit Conformational Exchange

$$\Gamma_{auto} = R_2 - 0.5R_1 - 0.454\sigma_{NH} \quad (1)$$

$$\sigma_{NH} = R_1(NOE - 1)\gamma_N/\gamma_H \quad (2)$$

$$\Gamma_{auto} = [(2/9)\Delta\sigma^2 J(0) + \Theta_{ex}]\omega_N^2 + 0.5d^2 J(0) \quad (3)$$



(A) The CSA dipole-dipole auto-relaxation rate constant (Γ_{auto}) was calculated at each static field using equations 1 and 2. For each residue, linear regression was performed using equation 3, both in the presence and absence of the conformational exchange contribution (Θ_{ex}). F-statistical testing with a confidence level α = 0.1 indicated no residues were undergoing conformational exchange on the appropriate timescale. **(B)** The spectral density function *J(0)* was calculated from the linear regression and is compared to that determined previously [1].

Model-Free Analysis

$$\text{Model 0}: J(\omega) = \frac{2}{5}\tau_m \quad (1)$$

$$\text{Model 1}: J(\omega) = \frac{2}{5}\tau_m\left(\frac{S^2}{1+(\omega\tau_m)^2}\right) \quad (2)$$

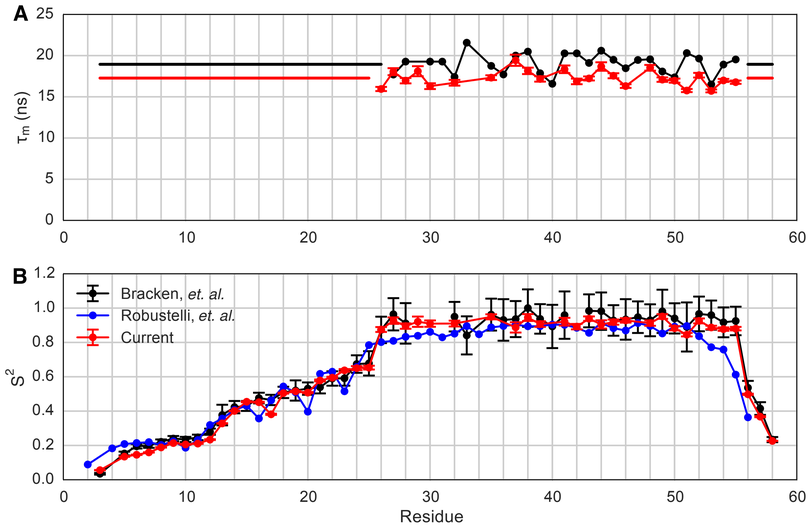
$$\text{Model 2}: J(\omega) = \frac{2}{5}\tau_m\left(\frac{S^2}{1+(\omega\tau_m)^2} + \frac{(1-S^2)(\tau_e+\tau_m)\tau_e}{(\tau_e+\tau_m)^2+(\omega\tau_e\tau_m)^2}\right) \quad (3)$$

$$\text{Model 5}: J(\omega) = \frac{2}{5}\tau_m\left(\frac{S^2}{1+(\omega\tau_m)^2} + \frac{(S_f^2-S^2)(\tau_s+\tau_m)\tau_s}{(\tau_s+\tau_m)^2+(\omega\tau_s\tau_m)^2}\right) \quad (4)$$

$$\text{Model 6}: J(\omega) = \frac{2}{5}\tau_m\left(\frac{S^2}{1+(\omega\tau_m)^2} + \frac{(1-S_f^2)(\tau_f+\tau_m)\tau_f}{(\tau_f+\tau_m)^2+(\omega\tau_f\tau_m)^2} + \frac{(S_f^2-S^2)(\tau_s+\tau_m)\tau_s}{(\tau_s+\tau_m)^2+(\omega\tau_s\tau_m)^2}\right) \quad (5)$$

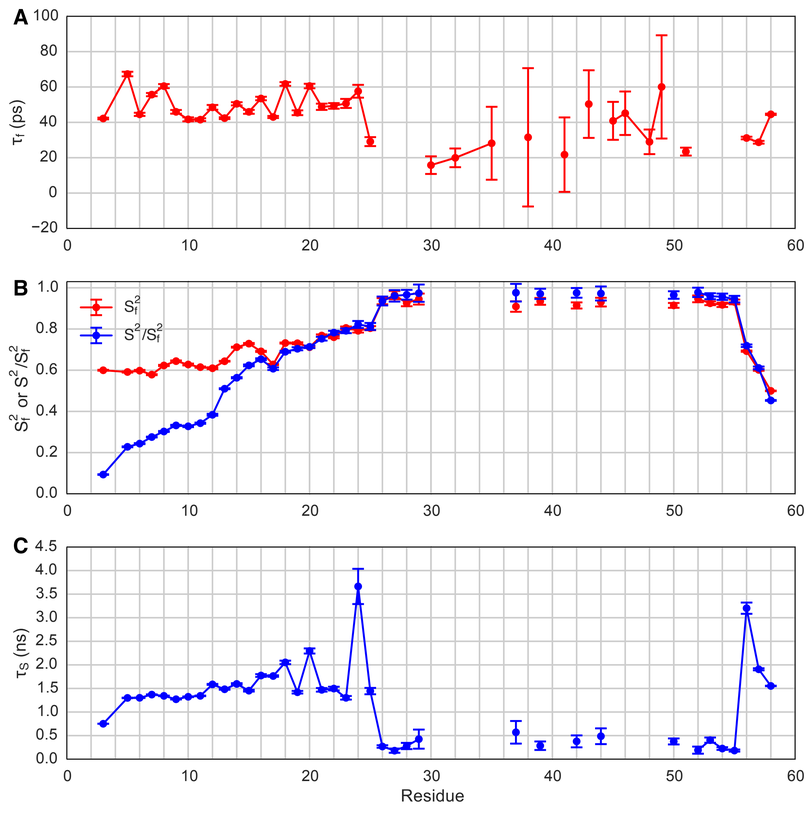
GCN4 ^{15}N dynamics were characterized using the model-free formalism with Relax [4,5]. GCN4 was analyzed in two parts: (I) a local correlation time (τ_m) was determined for the coiled-coil region (residues 26–55); (II) the average τ_m of the coiled-coil region was fixed for analysis of the basic region (residues 2–25) and C-terminus (residues 56–58). Only models that do not contain a conformational exchange term (equations 1–5) were considered. Errors were determined by Monte Carlo simulations, and Akaike information criterion was used for model selection.

Correlation with Previous Results



(A) The correlation time (τ_m) was determined for each residue in the coiled-coil region (residues 26–55). The mean value (17.3 ns) is denoted as a straight line for the basic region (residues 2–25) and C-terminus (residues 56–58). These results are compared to those of Bracken, *et. al.* [1], which were analyzed similarly (mean τ_m = 18.9 ns). The smaller average τ_m relative to that reported previously is likely due to reduced non-specific aggregation at the lower sample concentration. **(B)** The order parameter (S^2) determined for GCN4 closely matches those determined previously by NMR [1] and calculated from molecular dynamics simulations [2].

Dynamics of the Basic Zipper Region



(A) Fast internal motions (τ_f = 40–70 ps) are present for the basic region; **(B)** however, the order parameter (S^2) is dominated by internal motions on a slower timescale as seen by a comparison of S_f^2 to S^2/S_f^2. **(C)** The slower internal motions of the disordered basic region are of the timescale τ_s = 1.0–2.5 ns.

Conclusions

- Model-free analysis has been performed on *apo* GCN4 at four static magnetic fields.
- The correlation time and spectral density function *J(0)* are in excellent agreement with a previous NMR study performed at a single static field.
- The order parameters are consistent with those determined previously by both NMR and molecular dynamics simulations.
- **The current approach allows internal motions to be studied experimentally, which was not possible previously.**
- **Two internal dynamic processes are present in the basic region, and the slow process may facilitate DNA binding by the transient formation of helices.**