Backbone dynamics measurements on leukemia inhibitory factor, a rigid four-helical bundle cytokine

SHENGGEN YAO,^{1,2} DAVID K. SMITH,^{1,2,4} MARK G. HINDS,^{1,2} JIAN-GUO ZHANG,^{2,3}
NICOS A. NICOLA,^{2,3} AND RAYMOND S. NORTON^{1,2}

¹Biomolecular Research Institute, 343 Royal Parade, Parkville 3052, Australia

²The Cooperative Research Center for Cellular Growth Factors, PO Royal Melbourne Hospital, Melbourne 3050, Australia

³The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Melbourne 3050, Australia

(RECEIVED September 9, 1999; FINAL REVISION January 6, 2000; ACCEPTED January 18, 2000)

Abstract

The backbone dynamics of the four-helical bundle cytokine leukemia inhibitory factor (LIF) have been investigated using ¹⁵N NMR relaxation and amide proton exchange measurements on a murine–human chimera, MH35-LIF. For rapid backbone motions (on a time scale of 10 ps to 100 ns), as probed by ¹⁵N relaxation measurements, the dynamics parameters were calculated using the model-free formalism incorporating the model selection approach. The principal components of the inertia tensor of MH35-LIF, as calculated from its NMR structure, were 1:0.98:0.38. The global rotational motion of the molecule was, therefore, assumed to be axially symmetric in the analysis of its relaxation data. This yielded a diffusion anisotropy $D_{\parallel}/D_{\perp}$ of 1.31 and an effective correlation time $(4D_{\perp} + 2D_{\parallel})^{-1}$ of 8.9 ns. The average values of the order parameters (S^2) for the four helices, the long interhelical loops, and the N-terminus were 0.91, 0.84, and 0.65, respectively, indicating that LIF is fairly rigid in solution, except at the N-terminus. The S^2 values for the long interhelical loops of MH35-LIF were higher than those of their counterparts in short-chain members of the four-helical bundle cytokine family. Residues involved in LIF receptor binding showed no consistent pattern of backbone mobilities, with S^2 values ranging from 0.71 to 0.95, but residues contributing to receptor binding site III had relatively lower S^2 values, implying higher amplitude motions than for the backbone of sites I and II. In the relatively slow motion regime, backbone amide exchange measurements showed that a number of amides from the helical bundle exchanged extremely slowly, persisting for several months in ²H₂O at 37 °C. Evidence for local unfolding was considered, and correlations among various structure-related parameters and the backbone amide exchange rates were examined. Both sets of data concur in showing that LIF is one of the most rigid four-helical bundle cytokines.

Keywords: amide exchange; cytokine; leukemia inhibitory factor; ¹⁵N relaxation; NMR; protein dynamics

The cytokine leukemia inhibitory factor (LIF), a 180-residue protein of molecular mass 20 kDa, is active on a range of cell types, including adipocytes, hepatocytes, megakaryocytes, neurons, osteoblasts, embryonic stem cells, and primordial stem cells (Hilton, 1994). Many of these activities are shared with other cytokines, including interleukin-6 (IL-6), interleukin-11 (IL-11), cardiotrophin-1, and oncostatin-M (OSM) (Rose & Bruce, 1991). The solution structure of MH35-LIF, a murine–human chimera of

LIF that retains the critical receptor binding determinants (Owczarek et al., 1993; Layton et al., 1994) and structure (Maurer et al., 1994) of human LIF, has been determined by NMR (Hinds et al., 1998). MH35-LIF has a similar structure to that of murine LIF, determined by X-ray crystallography (Robinson et al., 1994). The main structural elements are a four-helical bundle (helices A, B, C, and D) in an up-up-down-down arrangement, linked by two long loops (AB and CD) and one short loop (BC), together with an N-terminal tail, as shown in Figure 1.

Along with the accumulation of three-dimensional structures for proteins of the four-helical bundle cytokine family (Sprang & Bazan, 1993), the backbone dynamics of several of these cytokines have been analyzed using ¹⁵N relaxation measurements. These include IL-3 (Feng et al., 1996), IL-4 (Redfield et al., 1992), G-CSF (Zink et al., 1994), and, most recently, murine LIF (mLIF, Purvis & Mabbitt, 1997). All exhibit relatively restricted motion in the helical bundle, but variable degrees of flexibility in the loop regions. By contrast, quantitative studies of amide proton ex-

Reprint requests to: Raymond S. Norton, Biomolecular Research Institute, 343 Royal Parade, Parkville 3052, Australia; e-mail: ray.norton@biores.com.au.

⁴Present address: Department of Biochemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Abbreviations: HSQC, heteronuclear single quantum coherence; IL, interleukin; LIF, leukemia inhibitory factor; MH35, murine-human chimera; NOE, nuclear Overhauser effect; R_1 , spin-lattice relaxation rate; R_2 , spin-spin relaxation rate; RMSD, root-mean-square deviation; SSE, sum squared error.

Fig. 1. Ribbon diagram of the NMR solution structure of MH35-LIF (residues 9–180) showing the elements of its secondary structure. These helices, in an up-up-down-down arrangement, are labeled A, B, C, and D. Diagram generated using InsightII (MSI, San Diego, California).

change behavior, which provide valuable information on protein structure, dynamics, and folding (Englander et al., 1996; Raschke & Marqusee, 1998), have not been reported for any of the four-helical bundle cytokines.

In this paper we analyze the backbone dynamics of MH35-LIF, as determined from ^{15}N relaxation parameters and amide exchange studies in $^2\text{H}_2\text{O}$. Model-free parameters for the backbone of MH35-LIF are compared with published data for other members of the four-helical bundle cytokine family, and evidence for local unfolding of MH35-LIF is examined based on the experimentally observed amide exchange rates. In addition, possible correlations among the backbone amide exchange rates and calculated structural parameters, such as solvent accessibilities and hydrogen bond energies, are discussed.

Results

^{15}N relaxation parameters and model-free parameters

The backbone ^{15}N spin-lattice and spin-spin relaxation rates, R_1 and R_2 , and the $\{^1\text{H}\}$ - ^{15}N NOE for MH35-LIF are plotted as a

function of residue number in Figure 2. R_1 and R_2 were measured for 145 of the 166 backbone NH pairs (MH35-LIF contains 13 prolines) and NOEs for 143. The average values for R_1 , R_2 , and NOE over the entire molecule were $1.3 \pm 0.1 \text{ s}^{-1}$, $12.6 \pm 2.9 \text{ s}^{-1}$, and 0.75 ± 0.11 , respectively. Initial estimation of the rotational diffusion tensor, obtained from its solution structure (Hinds et al., 1998; Protein Data Bank (PDB) access code: 1a7m model_1) and the R_2/R_1 ratios for a group of 119 residues not undergoing slow internal motion or fast chemical or conformational exchange (Brüschweiler et al., 1995; Tjandra et al., 1995), yielded a diffusion anisotropy $D_{\parallel}/D_{\perp}$ of 1.25 and an effective correlation time $(4D_{\perp} + 2D_{\parallel})^{-1}$, of 8.9 ns.

Assuming the global rotational motion of MH35-LIF was axially symmetric, the model-free parameters, S^2 , τ_f/τ_s , and R_{ex} , were obtained from fits of the relaxation data using the model selection approach (Clare et al., 1990a, 1990b; Mandel et al., 1995), as summarized in Figure 3. The data for 138 residues were fitted successfully to one of five optional combinations of model-free parameters under current fitting criteria (see Materials and methods). The extended model-free formalism (Clare et al., 1990a, 1990b) was used to fit the relaxation data for 20 residues ($\sim 14\%$ of all satisfactorily fitted residues), mainly from the N-terminus, loop regions and near the edges of the helices. The numbers of residues fitted satisfactorily to each combination of model-free parameters were 74 (S^2), 22 (S^2 and τ_f), 15 (S^2 and R_{ex}), 7 (S^2 , τ_f , and R_{ex}), and 20 (S^2 , S_s^2 , and τ_s). Final values for the rotational diffusion anisotropy $D_{\parallel}/D_{\perp}$ and effective correlation time $(4D_{\perp} + 2D_{\parallel})^{-1}$ of 1.31 and 8.9 ns, respectively, were obtained for the global rotational diffusion tensor of MH35-LIF after optimization using the combination of model-free parameters determined for each residue. In comparison, when the global rotational motion of the molecule was treated as isotropic, 135 residues were fitted satisfactorily into these five combinations of model-free parameters under the same fitting criteria and using a global correlation time τ_m of 9.75 ns, estimated from experimentally measured R_2/R_1 ratios for residues not undergoing slow internal motion or fast exchange (Kay et al., 1989; Yao et al., 1998). Corresponding numbers of residues fitted satisfactorily to each combination of model-free parameters were 67 (S^2), 14 (S^2 and τ_f), 9 (S^2 and R_{ex}), 3 (S^2 , τ_f , and R_{ex}), and 42 (S^2 , S_s^2 , and τ_s). Clearly, with the use of an axially symmetric model for global rotational motion, 22 out of 42 residues could be fitted satisfactorily under current fitting criteria without introducing a slow internal motion correlation time τ_s . Comparing these 22 residues with the 20 residues still requiring the inclusion of τ_s for satisfactory fits in the axially symmetric case; however, no obvious correlation was apparent with the orientations of their N-H bond vectors against the principle axis of the diffusion tensor, as might have been expected.

Backbone amide exchange rates

Backbone amide exchange rates were determined for 79 of the 92 backbone amide protons undergoing relatively slow exchange processes at 37°C . Of these 79 amides, 70 are located in the helices of the four-helical bundle. Figure 4 compares these experimentally determined exchange rates with calculated protection factors, hydrogen bond energies, and the distances from the backbone nitrogens to the nearest water molecule for each of the four-bundle helices of MH35-LIF. The rigidity of this protein is emphasized by the fact that, after nearly three months in $^2\text{H}_2\text{O}$, 31 amide protons

Fig. 2. Summary of backbone ^{15}N relaxation parameters (A) R_1 , (B) R_2 , and (C) steady-state $\{^1\text{H}\}-^{15}\text{N}$ NOE for MH35-LIF, recorded at 40°C and ^{15}N frequency -60.81 MHz . Residues with R_2 exceptionally higher than the averaged rate $\langle R_2 \rangle = 12.6 \pm 2.9\text{ s}^{-1}$ are indicated by + and *, respectively, for residues with and without broad peaks in a conventional HSQC spectrum.

were still evident in the ^{15}N -HSQC spectrum. Of these, most are located in the core of the four-helical bundle.

Discussion

Experimental parameters that reflect both fast and slow motions of the polypeptide backbone concur in showing that MH35-LIF is a relatively rigid protein. Here we evaluate correlations of these data with structural features of the molecule, compare the relaxation data for MH35-LIF with that for other four-helical bundle cytokines, and consider the possible functional significance of these observations.

Anisotropy of global rotational motion

Although most molecules exhibit some degree of anisotropy of their global rotational motion, ^{15}N relaxation data have been interpreted mainly under the approximation of isotropic global motion. Analyses of ^{15}N relaxation data taking account of anisotropic overall motion have been reported recently (e.g., Tjandra et al., 1995, 1996; Luginbühl et al., 1997; Cordier et al., 1998). As the principal components of the inertia tensor of MH35-LIF have a ratio of 1:0:0.98:0.38, the global rotational diffusion tensor was treated as axially symmetric in the present study. Although the combinations of model-free parameters that gave the best fit to the data for individual residues occasionally varied between the two

models, the axially symmetric rotational diffusion model resulted in only marginal changes to the order parameters S^2 compared with those derived assuming an isotropic rotational diffusion tensor. This is consistent with the results obtained for the HIV protease-inhibitor complex (Tjandra et al., 1996).

Comparison with other members of the four-helical bundle cytokine family

The backbone dynamics of several four-helical bundle cytokines have been analyzed using ^{15}N relaxation measurements, including IL-3 (Feng et al., 1996), IL-4 (Redfield et al., 1992), G-CSF (Zink et al., 1994), and, most recently, murine LIF (Purvis & Mabbitt, 1997). Good correlations between the order parameters S^2 and the secondary structural elements were observed, with greater motional flexibility in loop regions compared to the helical bundle. This is also true for MH35-LIF, with an average S^2 value of 0.91 for the four-helical bundle (Table 1). It is noteworthy that the long interhelical loops (AB and CD) of MH35-LIF and mLIF are more rigid (average S^2 values of 0.83 and 0.84, respectively, for the AB and CD loops of MH35-LIF) than their counterparts in IL-3 and IL-4 (Table 1). The poorer definition in the NMR structure of both long loops compared with the helical bundle (Hinds et al., 1998) must, therefore, reflect a combination of somewhat greater internal motion and higher surface exposure, both of which contribute to a reduction in the number of NOEs per residue in comparison with

Fig. 3. Summary of model-free parameters for MH35-LIF against the protein sequence obtained using the extended model-free formula with model selection approach. The global rotation is assumed to be axially symmetric. **A:** The order parameter S^2 ($= S_f^2 \times S_r^2$). **B:** Effective internal correlation time τ_f/τ_s (the vertical axis was broken from 190 to 300 ps as indicated by a slash). **C:** Apparent chemical and/or conformational exchange contribution R_{ex} to the transverse relaxation rate R_2 . A schematic representation of the MH35-LIF secondary structure elements is shown at the top (Hinds et al., 1998).

residues from the helical bundle (Hinds et al., 1998). The lack of definition of the N-terminus in the solution structure of MH35-LIF is due to significant internal motion, as reflected in the low order parameters for this region (see Fig. 3; Table 1).

Backbone mobilities of the binding sites of LIF

Motions at the surface of a protein could in principle either assist or interfere with its ability to form a complex with its target molecule. On the one hand, flexibility in the protein's receptor binding surface could enhance its ability to fit the binding site on its target receptor. On the other, significant internal mobility in the free ligand that became frozen out upon binding could decrease the binding affinity through an unfavorable contribution to conformational entropy.

Mutagenesis studies have identified a number of residues in human LIF that are important for binding to the LIF receptor α -chain and gp130 (the third component of the LIF signal-transducing complex). Layton et al. (1994) showed that six residues (D57, S107, H112, S113, V155, and K158) from the surface at one end of the four α -helical bundle contributed most of the binding energy involved in the interaction of human LIF and the

human LIF receptor α -chain. More recently, Hudson et al. (1996) reported three major binding sites on human LIF for human LIF receptor and gp130 binding, in which the region of human LIF most important for binding to the human LIF receptor (site III) was composed of residues mainly from the amino terminus of the D-helix and CD loop. Of these residues, F156 and K159 contribute the majority of free energy for human LIF receptor binding. Site III is located at the same end of the four α -helical bundle of LIF as the six residues identified by Layton et al. (1994). Table 2 lists S^2 values for those residues involved in binding to the LIF receptor α -chain or gp130 and the average values over each binding site. The S^2 values range from 0.71 for E154 to 0.95 for both Q25 and V121. However, it is clear that binding sites I and II, together with the six-residue site, have higher average order parameters (0.9, 0.92, and 0.88, respectively) than site III (0.79), and the lowest individual values are from site III. Except for residues H150 and E154 of binding site III, all residues in binding sites I and II and the six-residue site had relatively fast rates of internal motion ($\tau_f < 85$ ps). Among the residues in the binding sites, only S113, G124, and K158 exhibited relatively fast chemical or conformational exchange, with R_{ex} values of 2.3, 2.4, and 1.3 s^{-1} , respectively (Fig. 3). Overall, it would appear that the backbone near all of

Fig. 4. Summary of logarithm amide exchange rate (k_{ex} , ○), logarithm protection factor (P , □), hydrogen bond energy (E_h , ▲), and distance between the van der Waals' surfaces of the main-chain N nucleus and the nearest H₂O molecule (D_{N/H_2O} , ◆) vs. the sequences of the four helices of MH35-LIF. **A:** Helix-A. **B:** Helix-B. **C:** Helix-C. **D:** Helix-D. The heptad positions A and D in each helix are indicated by ▼ on the horizontal axis.

these sites is relatively ordered, although site III and the six-residue site undergo more substantial motion than sites I or II. It follows that site III and the six-residue site are the most likely to undergo conformational changes as LIF interacts with its receptor.

Recently, Feher and Cavanagh (1999) showed that regions of the *Bacillus subtilis* response regulator protein Spo0F having motion in the millisecond time scale coincided with residues and regions of the protein surface known to be important for function. In addition, millisecond-to-microsecond dynamics were found to be significant for regions of the retinoid X and retinoic acid receptors involved in dimerization (van Tilborg et al., 1999). It was proposed that this flexibility may allow these proteins to adapt to various partners with different orientations upon DNA binding. This concurs with previous suggestions that flexibility allows for diversity in binding partners (Gryk et al., 1996; Kriwacki et al., 1996; Wyss et al., 1997). Our finding that LIF is relatively rigid may reflect the fact that its biological activity is mediated solely by interactions of high affinity and high specificity with the LIF receptor chain and gp130, and therefore, it may not need the surface plasticity found in proteins that are more promiscuous in their interactions.

Our relaxation studies were carried out on a nonglycosylated form of LIF. A recent NMR study of glycosylated G-CSF suggested that the carbohydrate may increase the stability of the glycoprotein by decreasing the mobility of those residues near the site of glycosylation (Gervais et al., 1997). Thus, it is possible that glycosylation of LIF may further reduce the amplitudes or rates of internal motion of residues comprising the three binding sites on its surface.

As noted by Hinds et al. (1998), the solution structure of MH35-LIF and the crystal structure of mLIF are very similar, but there are some differences in the vicinity of site III, in particular, at the C-terminal end of the CD loop and the start of the D helix. F156, which is in the first turn of the D helix, is an important contributor to site III that differs between the solution and crystal structures. Its relaxation data, however, were fitted satisfactorily to S^2 0.92 without invoking τ_f/τ_s or R_{ex} terms, implying that the amplitude of its backbone mobility in solution is relatively restricted. Thus, internal motions on the timescale probed by our relaxation measurements do not account for the difference from the crystal structure. By contrast, K159, which is in the second turn of the helix and in closer agreement with the crystal structure, is more flexible on this timescale, with an S^2 value of 0.75.

Amide exchange

Turning to motions on a slower timescale, as probed by backbone amide exchange measurements, a fairly clear pattern of protection from exchange is evident in Figure 4, with protection factors rising to a maximum within the helices, although slightly more so toward the N-terminal ends of the A and B helices and the C-terminal ends of the C and D helices. In relation to the structure of MH35-LIF, these regions come together to form a very slowly exchanging core. This pattern is suggestive of a helix fraying or an unwinding mechanism (Wand et al., 1986) of unfolding. Within these trends, the influence of the heptad position can also be seen from Figure 4,

Table 1. Comparison of average values of S^2 and backbone pairwise RMSD of MH35-LIF with S^2 of mLIF, IL-3, and IL-4 for the common secondary structure elements

	MH35-LIF		mLIF ^b	IL-3 ^c	IL-4 ^d
	RMSD ^a (Å)	$\langle S^2 \rangle$	$\langle S^2 \rangle$	$\langle S^2 \rangle$	$\langle S^2 \rangle$
Overall sequence		0.87 ± 0.11 (138) ^e			
Helical regions	0.65 ± 0.08	0.91 ± 0.07 (92)			
A helix (24–48) ^f	0.56 ± 0.12	0.92 ± 0.06 (21)	0.83	0.91	0.89
B helix (76–104)	0.46 ± 0.09	0.92 ± 0.07 (26)	0.92	0.88	0.92
C helix (108–135)	0.42 ± 0.09	0.91 ± 0.06 (25)	0.88	0.84	0.92
D helix (154–176)	0.48 ± 0.12	0.88 ± 0.09 (20)	0.89	0.90	0.93
Loops and N-terminus		0.80 ± 0.13 (46)			
AB loop (49–75)	1.50 ± 0.47	0.83 ± 0.09 (18)	0.77	0.60 ^g	0.72
CD loop (136–153)	2.14 ± 0.84	0.84 ± 0.06 (11)	0.85	0.69	0.73
N-terminus (1–23)		0.65 ± 0.18 (10)	0.74	0.80	0.56

^aWhen the ensemble of NMR structures was superimposed over individual secondary structure elements.^bPurvis and Mabbitt (1997).^cFeng et al. (1996).^dRedfield et al. (1992).^eNumbers of residues from secondary structure elements for which S^2 values were obtained are shown in parentheses.^fLimits of secondary structure elements are for the solution structure of MH35-LIF.^gFor loop AA'.

with local protection factor maxima occurring around positions A, D, and sometimes E, and lower values in between.

A periodicity in the amide exchange rates of coiled-coil or amphipathic helical proteins correlating with the helix heptad position has been observed previously (Goodman & Kim, 1991; Radford et al., 1992; Pedersen et al., 1993). In particular, studies on GCN4 leucine zipper peptides showed that the amide protons at the A, D, and E heptad positions had much slower exchange rates than the other heptad positions (Goodman & Kim, 1991), consistent with the earlier “knobs into holes” prediction of Crick (1953) for coiled-coil packing. The present results for MH35-LIF illustrate a similar

pattern, the most strongly protected being the A position, followed by the E, B, and D positions, as shown in Figure 5. The G, C, and F positions show lower protection, with amide protons not in the helical bundle having the least protection.

Structure-related parameters, namely hydrogen bond energy, relative main-chain solvent accessibility and distance from the backbone N nucleus to the nearest H₂O molecule, were calculated from the X-ray structure of mLIF (Robinson et al., 1994). Examination for possible correlations among the measured amide exchange rates, the protection factors, and the calculated structural parameters, however, showed little correlation. The correlations of the loga-

Table 2. Summary of order parameters for residues involved in LIF binding to LIF receptor α -chain or gp130

6-Residue site ^a		Site I ^b		Site II		Site III	
Residue ^c	S^2	Residue	S^2	Residue ^c	S^2	Residue ^c	S^2
D57	0.87	K170	0.93	Q25	0.95	P51	—
S107	0.89	A(S)174	0.89	S(N)28	0.93	P106	—
H112	0.89	V175	0.88	Q32	nm ^d	T(H)150	0.75
S113	0.88			D120	0.94	K153	0.76
V155	0.90			I(V)121	0.95	D(E)154	0.71
K158	0.86			G124	0.85	F156	0.92
				S127	0.91	K158	0.86
						K159	0.75
$\langle S^2 \rangle^e = 0.88$		$\langle S^2 \rangle = 0.90$		$\langle S^2 \rangle = 0.92$		$\langle S^2 \rangle = 0.79$	

^aThe six-residue site identified by Layton et al. (1994) from studies of murine–human chimeras.^bSites I, II, and III were identified by Hudson et al. (1996) from a partial alanine scan.^cResidues in MH35-LIF are shown in the parentheses if they are different from human LIF.^dnm, not measured.^eAverage value over each binding site.

Fig. 5. Plots of negative log of amide exchange rate (●) and log of protection factor (□) vs. the heptad positions of helices. The averaged values over each heptad position are presented in solid and dashed lines, respectively. N denotes the residue N-terminal to the heptad repeat.

rithm of the amide exchange rate and the logarithm of the protection factor with hydrogen bond energy were the strongest, with correlation coefficients of only 0.55 and -0.54 , respectively.

Helix fraying

Helix fraying seems to occur in MH35-LIF, with both ends of the helical bundle having faster exchanging amides than those in the core of the bundle. As the BC loop end of the helical bundle has, in general, much faster exchanging amides than the N- and C-termini end, it is likely that the bundle starts to come apart, or possibly “unzip” from this end. The kink in the A helix, with its break in hydrogen bonding pattern, may provide a pivot point to assist this movement. Residues on the solvent-exposed faces of helices show faster exchange than those packed in the core, as has been seen by others (Goodman & Kim, 1991; Pedersen et al., 1993). This correlation can also be seen from Figure 4, for example, L90 to N96 in the B helix, but is somewhat masked in regions such as K114 to I119 in the C helix. Possibly the regions where the heptad pattern are masked are more affected by helix fraying than the flexing of the exposed residues.

Comparison of spatial distribution of rapid and slow motions

Broadly speaking, protein backbone mobilities derived from ^{15}N relaxation measurements probe rapid motion on a timescale of a few tens of picoseconds to 100 ns, whereas the amide exchange is sensitive to relatively slow motion ranging from 1 ms to a few seconds (Brüschweiler, 1994). The most striking feature of the amide exchange for MH35-LIF is the large number of extremely slowly exchanging amide protons, which includes a group of 19 amino acids that exchange less than half of their amide protons with the solvent over the entire time course of examination (greater than three months). In addition, MH35-LIF also shows large S^2

values, even for the long loop regions. The combination of these results suggests that LIF is a very stable and rigid molecule on both timescales. Figure 6 shows ribbon diagrams of MH35-LIF color coded by the order parameters (S^2 , Fig. 6A), apparent chemical or conformational exchange rates (R_{ex} , Fig. 6B) from the model-free formalism, and experimentally observed amide exchange rate constants (k_{ex} , Fig. 6C), to illustrate the contrast of the spatial distribution of mobilities of MH35-LIF on these different timescales. Clearly, the entire helical bundle of MH35-LIF is well ordered in the relatively fast motion regime. In contrast, in the slower motion regime, a gradient of stability is evident, from the core of the helix bundle toward the ends, with the core being more stable kinetically. It is interesting to note that the three residues with the largest R_{ex} values (T98, Q101, and N105) are clustered near the end of helix B, in a region that also exhibits fast amide exchange. This might be a region from which helix bundle unwinding could commence. As noted above, there is no clustering of residues with significant R_{ex} values in the receptor binding regions of LIF.

Correlation among S^2 , k_{ex} , backbone RMSD, and B-factor

It has been recognized previously that the order parameter (S^2) of the N–H vectors derived from ^{15}N relaxation measurements, which is a measure of the spatial restriction of the N–H bond, might have some degree of correlation with the backbone nitrogen RMSD from the mean NMR structure and the corresponding temperature factor of an X-ray crystal structure (Redfield et al., 1992; Powers et al., 1993; Mandel et al., 1995). Furthermore, correlation between the amide exchange rates and the temperature factors of an X-ray crystal structure has also been reported (Mori et al., 1997). Figure 7 compares backbone nitrogen RMSD, $(1 - S^2)$ and k_{ex} values of MH35-LIF and B-factors from the crystal structure of mLIF (Robinson et al., 1994). The majority of residues exhibit the same trends, with large $(1 - S^2)$ values corresponding to large

Fig. 6. Backbone ribbon diagrams of MH35-LIF (residues 9–180) illustrating the distribution of the extent of internal motion at two time scales via color coding. (A) Order parameters S^2 ; (B) apparent chemical or conformational exchange rates R_{ex} using results from the model selection approach; and (C) amide proton exchange rates k_{ex} . Color schemes: (A) red, $S^2 \leq 0.6$; yellow, $0.6 < S^2 \leq 0.8$; blue, $0.8 < S^2 \leq 1.0$; and gray, unmeasured/unfitted; (B) red, $R_{ex} > 10 \text{ s}^{-1}$; yellow, $0 \text{ s}^{-1} < R_{ex} \leq 10 \text{ s}^{-1}$; blue, $R_{ex} = 0$; and gray, unmeasured/unfitted; (C) red, not observed in the first spectrum; yellow, exchange rates were not determined due to lack of data points; cyan, $k_{ex} \geq 10^{-3} \text{ h}^{-1}$; and blue, $k_{ex} < 10^{-3} \text{ h}^{-1}$. All prolines are in black. The orientation of the structure is the same as in Figure 1. This diagram was generated using InsightII (MSI, San Diego, California).

RMSD values in the NMR structure and large B -factors in the X-ray structure. However, a residue by residue comparison shows that there is not a strict correlation among these parameters, reflecting the difference in their intrinsic motional sensitivities (Powers et al., 1993).

Conclusions

In this study, the dynamics of a murine–human chimera of LIF have been characterized by both ^{15}N relaxation and backbone amide exchange measurements. In both fast and slow motion regimes, LIF has proven to be a relatively rigid molecule. The amplitudes of the backbone mobilities show no obvious correlation with the receptor binding surfaces of the molecule, although site III appears to undergo more significant internal motion than sites I or II. It must be borne in mind, of course, that most of the contacts between LIF and the LIF receptor α -chain or gp130 are likely to be mediated by side chains rather than the backbone, and measurements of their dynamics are also important in a complete picture of the dynamics of the receptor binding surfaces and of possible changes accompanying receptor engagement. Nevertheless, the finding that LIF is a relatively rigid protein correlates well with the fact that its biological activity is mediated by receptor interactions of high affinity and high specificity. It will be interesting to determine how the backbone dynamics of LIF change upon receptor interactions and to compare these changes with those experienced by more flexible protein ligands upon interaction with their cognate receptors.

Materials and methods

Sample preparation

The cloning and expression of LIF, including various murine–human chimeras (Owczarek et al., 1993), and sample preparation

(Maurer et al., 1994; Hinds et al., 1997) have been described previously. The NMR sample used for the ^{15}N relaxation measurements was prepared by dissolving lyophilized uniformly ^{15}N -enriched protein in 90% $\text{H}_2\text{O}/10\%$ $^2\text{H}_2\text{O}$ without the addition of buffer. The concentration was $\sim 2 \text{ mM}$, and the pH was adjusted to 4.4 with NaOH or ^2HCl without correcting for the deuterium isotope effect. Similarly, for the studies of backbone amide exchange, $\sim 10 \text{ mg}$ of uniformly ^{15}N -enriched protein was dissolved in $^2\text{H}_2\text{O}$, giving a concentration $\sim 1 \text{ mM}$, and the sample pH was adjusted to 4.4. This sample was maintained at 37°C for the time period of the backbone amide exchange study.

NMR spectroscopy

All spectra for ^{15}N relaxation measurements were recorded at 40°C on a Bruker DRX-600 spectrometer. ^{15}N R_1 and R_2 data were acquired with the pulse sequences described by Stone et al. (1992), whereas the NOE data were obtained using the pulse sequence with water flip-back described by Grzesiek and Bax (1993) for minimizing possible saturation of signals from amide protons undergoing fast exchange with H_2O . All data were acquired in an interleaved manner, i.e., the pulse sequences step through the relaxation delays prior to the time increments of the indirectly detected dimension of the spectra, with quadrature detection in that dimension being achieved using time-proportional phase incrementation (Marion & Wüthrich, 1983). A spin echo time of $2 \times 1.0 \text{ ms}$ was used in the R_2 measurements to avoid sample heating. A matrix size of $4,096 \times 200$ and 32 scans per t_1 increment was used throughout, with spectral widths of 6,250 and 1,800 Hz for ^1H and ^{15}N , respectively. Twelve relaxation delays ranging from 10.0 ms to 3.02 s for R_1 and 6.1 ms to 478.9 ms for R_2 were used, including two duplicated delays for estimation of the uncertainties of peak intensities. Similarly, the measurement of $\{^1\text{H}\}\text{--}^{15}\text{N}$ NOE was carried out twice for estimation of the errors in peak intensi-

Fig. 7. Comparison of (A) RMSDs from the mean structure for the backbone N atoms, (B) “disorder” parameters ($1 - S^2$), (C) B-factors of backbone N atoms, and (D) logarithm of backbone amide exchange rates, plotted as a function of residue number. A schematic representation of the MH35-LIF secondary structure elements is shown at the top (Hinds et al., 1998).

ties. The recycle delays used were 2.1 s for the R_1 and R_2 measurements (the estimated ^1H spin-lattice relaxation times of the amide protons were ~ 0.7 s) and 5.3 s for the NOE measurement. All spectra were processed using XWINNMR, version 1.3 (Bruker AG, Karlsruhe, Germany), and analyzed with XEASY, version 1.3 (Bartels et al., 1995).

In the amide exchange study, spectra were acquired at 37°C on a Bruker AMX-500 spectrometer. A series of HSQC spectra (Bodenhausen & Ruben, 1980) was recorded with 1,024 complex data points, a spectral width of 6,024 Hz in the ^1H dimension and 128, 256, or 384 t_1 increments, respectively, for a spectral width of 1,500 Hz in the ^{15}N dimension. The residual water signal was suppressed using a 2.1 ms spin-lock purge pulse (Messerle et al., 1989). From the time of protein dissolution in $^2\text{H}_2\text{O}$ to the beginning of acquisition of the first spectrum, 6 min elapsed. After acquiring successively four spectra of four scans and four spectra of eight scans with 128 t_1 increments, an interval of 20 min was used for checking the experimental conditions. Subsequently, four spectra of 32 scans followed by four spectra of 64 scans with 256 t_1 increments were acquired. The final spectrum in this initial stage was acquired with 96 scans and 384 t_1 increments. Remaining spectra were recorded at various time intervals thereafter. In total,

24 spectra were recorded with time points from 13 min to 2,215 h (the time being taken from the midpoint of acquisition of each spectrum). All spectra were processed using UXNMR (Bruker AG).

Analysis of relaxation data

After assessing the duplicate spectra of the R_1 and R_2 data sets, a constant error of 2.5% of the maximum peak intensity (corresponding to the shortest relaxation delay) was assigned to the measured peak intensities for each residue. ^{15}N relaxation rates R_1 and R_2 were determined by fitting these measured peak intensities, respectively, to a three- and two-parameter single exponential decay curve (Stone et al., 1992) using the program *curvefit* (A.G. Palmer III, Columbia University). Errors of the relaxation rates were determined by the program *curvefit* using a Monte Carlo simulation. The steady-state $\{^1\text{H}\}-^{15}\text{N}$ NOE values were calculated from peak intensity ratios obtained from spectra acquired in the presence and absence of proton saturation, with an error of 5% for the measured intensities estimated from evaluation of the duplicate data set. For the analysis of backbone amide exchange data, cross-peak volumes rather than the inten-

sities of NH pairs were calculated by integration in the program UXNMR (Bruker AG). Decay curves were fitted to a single exponential decay using the program SigmaPlot (Jandel Corp., San Rafael, California).

Model-free formalism

The longitudinal and transverse relaxation rates and the steady-state NOE of protonated ^{15}N spins are dominated by dipolar relaxation and ^{15}N chemical shift anisotropy. The spectral density function $J(\omega)$ is usually approximated by using a model-free formalism (Lipari & Szabo, 1982a, 1982b) or an extended model-free form (Clare et al., 1990a, 1990b) if two timescales of internal motion are considered. For a molecule undergoing axially symmetric global rotational motion, the following form of the extended spectral density function is used:

$$J(\omega) = \sum_{j=1}^3 A_j \left\{ \frac{S^2 \tau_j}{[1 + (\omega \tau_j)^2]} + \frac{(1 - S_f^2) \tau_j'}{[1 + (\omega \tau_j')^2]} + \frac{(S_f^2 - S^2) \tau_j''}{[1 + (\omega \tau_j'')^2]} \right\} \quad (1)$$

where $\tau_1 = (4D_{\parallel} + 2D_{\perp})^{-1}$, $\tau_2 = (D_{\parallel} + 5D_{\perp})^{-1}$, $\tau_3 = (6D_{\perp})^{-1}$, $1/\tau_j' = 1/\tau_j + 1/\tau_f$, $1/\tau_j'' = 1/\tau_j + 1/\tau_s$ ($j = 1, 2, 3$), $D_{\parallel}$ and $D_{\perp}$ are the parallel and perpendicular rotational diffusion coefficients to the unique axis of the molecule, respectively; τ_f and τ_s are the effective correlation times for internal motion on fast and slow timescales, respectively ($\tau_f < \tau_s < \tau_m$); and $A_1 = 0.75 \sin^4 \alpha$, $A_2 = 3 \sin^2 \alpha \cos^2 \alpha$, and $A_3 = (1.5 \cos^2 \alpha - 0.5)^2$, with α being the angle between the N–H bond vector and the principal axis ($A_1 + A_2 + A_3 \equiv 1$). The square of the generalized order parameter characterizing the amplitude of internal motion S^2 is equal to $S_f^2 \times S_s^2$, where S_f^2 and S_s^2 are the squares of the generalized order parameters for internal motions on the fast and slow timescales, respectively (for simplicity, S^2 has been referred to as the order parameter throughout this paper). The order parameter specifies the degree of spatial (angular) restriction of the NH bond vector, with the value ranging from zero for isotropic internal motion to unity for completely restricted internal motion. Motions characterized by the order parameter are on the ps to ns timescale.

When $S^2 = 1$, Equation 1 reduces to (Schurr et al., 1994)

$$J(\omega) = \sum_{j=1}^3 A_j \left\{ \frac{S^2 \tau_j}{[1 + (\omega \tau_j)^2]} + \frac{(1 - S^2) \tau_j'}{[1 + (\omega \tau_j')^2]} \right\} \quad (2)$$

where $1/\tau_j' = 1/\tau_j + 1/\tau_f$, τ_f being the effective internal correlation time. Furthermore, if the internal motion is extremely fast in comparison with the global motion of the molecule ($\tau_f \rightarrow 0$), the second term in the Equation 2 can be ignored and the spectral density function reduces further to

$$J(\omega) = S^2 \sum_{j=1}^3 A_j \left\{ \frac{\tau_j}{[1 + (\omega \tau_j)^2]} \right\}. \quad (3)$$

This expression of the spectral density function is used in the initial estimation of the rotational diffusion parameters to be estimated from the experimentally measured R_2/R_1 ratios of residues with NOEs equal or larger than 0.65 and

$$(T_1/\langle T_1 \rangle - T_2/\langle T_2 \rangle) < 1.5SD \quad (4)$$

where $\langle T_1 \rangle$ and $\langle T_2 \rangle$ are the average T_1 and T_2 values over residues with NOE ≥ 0.65 , and SD is the standard deviation of $(T_1/\langle T_1 \rangle - T_2/\langle T_2 \rangle)$ (Tjandra et al., 1995). An N–H bond length of 1.02 Å and an amide ^{15}N chemical shift anisotropy of 160 ppm were assumed throughout the data analysis.

Model selection approach

Initial estimation of the rotational diffusion parameters $D_{\parallel}$ and $D_{\perp}$ and an effective correlation time $(4D_{\perp} + 2D_{\parallel})^{-1}$ of the MH35-LIF molecule were obtained using the program *R2R1_diffusion* (A.G. Palmer III, Columbia University) with the angle α between the N–H bond vector and the principal diffusion axis determined from its NMR structure (Hinds et al., 1998).

The model selection approach of Mandel et al. (1995) was followed, using the extended model-free formula for the expression of the spectral density function (Equation 1) and initial estimates of $D_{\parallel}/D_{\perp}$ and $(4D_{\perp} + 2D_{\parallel})^{-1}$. Up to three internal motion related parameters in Equation 1 were fitted, and allowance was made for possible contributions of conformational or chemical exchange to the transverse relaxation rate R_2 by including an R_{ex} term. The combinations tried were S_f^2 ; S_f^2 and τ_f ; S_f^2 and R_{ex} ; S_f^2 , S_s^2 , and τ_s ; S_f^2 , τ_f , and R_{ex} . The detailed selection criteria in the model selection approach of Mandel et al. (1995) were followed. Briefly, a combination of parameters was chosen for each residue if the sum-squared error (SSE) of the experimental relaxation data fell into a pre-selected fraction (90 or 95% confidence limits) of the back-calculated relaxation data. The SSE is defined as

$$\begin{aligned} \text{SSE} = & [(R_{1c} - R_{1e})/\sigma_{R_1}]^2 + [(R_{2c} - R_{2e})/\sigma_{R_2}]^2 \\ & + [(\text{NOE}_c - \text{NOE}_e)/\sigma_{\text{NOE}}]^2 \end{aligned} \quad (5)$$

where σ is the error of the corresponding relaxation parameter and the subscripts e and c refer to experimental and back-calculated relaxation parameters, respectively. However, if the number of fitting parameters equaled that of the experimentally measured relaxation parameters (three in the present work), a fit was not considered satisfactory unless SSE = 0. In particular, selection of a best combination of the fitting parameters was conducted for each individual residue with a 90% fitting confidence for 500 randomly distributed back-calculated data sets. For application of the original model-free formula, the slowest limit of the effective correlation time τ_f was set to 200 ps. After the best combinations of model-free parameters were determined for all residues, a final run was carried out with the fraction of the simulated data set to 95% confidence limit, and the global rotational correlation time τ_m was optimized at the same time. For those residues not fitted satisfactorily by either one of these models, the model with the SSE value having the minimum difference from the back-calculated data of the given fitting confidence limit was used in the final run.

Calculation of intrinsic exchange rates and protection factors

The intrinsic exchange rates of MH35-LIF were calculated from the equation:

$$\begin{aligned} k_{\text{intr}} = & k_{A, \text{ref}} \times (A_L \times A_R) \times [D^+] + k_{B, \text{ref}} \\ & \times (B_L \times B_R) \times [OD^+] + k_{W, \text{ref}} \times (B_L \times B_R) \end{aligned} \quad (6)$$

where A_L , A_R , B_L , and B_R are acid and base corrections for side chains, with the peptide group in question being to the left or right of the side chain and the *ref* catalysis rate constants referring to the reference peptide used after correcting for the temperature effects (Bai et al., 1993). PDLA (poly-DL-alanine), under low salt conditions, was used as the reference peptide. Reference rate constants for acid, base, and water catalysis were adjusted to 37 °C, the temperature at which backbone amide exchange in MH35-LIF was studied. A number of special considerations were as follows: because the experiments were run at pH 4.74 (after correcting for the pH meter glass electrode effect; Glasoe & Long, 1960) the base conditions for the water catalysis rate (Jeng & Englander, 1991) were used. Aspartate and glutamate correction factors were taken to be those for unprotonated side chains. Assuming normal side chain pK_a values, ~90% of the side chains of the seven Asp residues would be unprotonated as would ~75% of the side chains of the four Glu residues. However, E82 had a side chain pK_a of 3.0 (Maurer et al., 1994), so it would be largely unprotonated. H112 was taken to be unprotonated because of its pK_a of 4.1, but the other five His residues were treated as protonated (Maurer et al., 1994). The protection factor was then calculated to be the ratio of k_{intr} and k_{ex} .

The solvent accessibilities were calculated from the crystal structure of mLIF (Robinson et al., 1994) using X-PLOR (Brünger, 1992), which implements the algorithm of Lee and Richards (1971), whereas relative solvent accessibilities were determined from the values of Miller et al. (1987). Hydrogen bond energies were calculated by the formula of Kabsch and Sander (1983) implemented within the program SSTRUC (D.K. Smith, unpubl. material). Distances from amide ^{15}N nuclei to the nearest H_2O molecules were calculated from the Connolly surface (Connolly, 1983) generated by InsightII (MSI, San Diego, California).

Supplementary material in the Electronic Appendix

Supplementary material includes a table of ^{15}N relaxation parameters (R_1 , R_2 , and NOE) of MH35-LIF, a table of calculated dynamics parameters (S^2 , τ_f/τ_s , R_{ex}) and a table of amide exchange data including calculated structural parameters.

Acknowledgments

We thank Arthur Palmer for generous provision of the software package Modelfree4.0 and valuable discussions. We also thank Kevin Barnham and Paul Pallaghy for helpful discussions.

References

Bai Y, Milne JS, Mayne L, Englander SW. 1993. Primary structure effects on peptide group hydrogen exchange. *Proteins Struct Funct Genet* 17:75–86.

Bartels C, Xia T, Billeter M, Güntert P, Wüthrich K. 1995. The program XEASY for computer-supported NMR spectral analysis of biological macromolecules. *J Biomol NMR* 6:1–10.

Bodenhausen G, Ruben DJ. 1980. Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy. *Chem Phys Lett* 69:185–189.

Brünger AT. 1992. *X-PLOR, a system for crystallography and NMR*. New Haven, Connecticut: Yale University.

Brüschweiler R. 1994. Connections between NMR measurements and theoretical models of structural dynamics of biopolymers in solution. In: Tycko R, ed. *Nuclear magnetic resonance probes of molecular dynamics*. The Netherlands: Kluwer Academic Publishers. pp 301–334.

Brüschweiler R, Liao X, Wright PE. 1995. Long-range motional restrictions in multidomain zinc-finger protein from anisotropic tumbling. *Science* 268: 886–889.

Clore GM, Driscoll PC, Wingfield PT, Gronenborn AM. 1990a. Analysis of the backbone dynamics of interleukin-1 β using two-dimensional inverse detected heteronuclear ^{15}N - ^1H NMR spectroscopy. *Biochemistry* 29:7387–7401.

Clore GM, Szabo A, Bax A, Kay LE, Driscoll PC, Gronenborn AM. 1990b. Deviations from the simple two-parameter model-free approach to the interpretation of nitrogen-15 nuclear magnetic relaxation of proteins. *J Am Chem Soc* 112:4989–4991.

Connolly ML. 1983. Analytical molecular surface calculation. *J Appl Crystallogr* 16:548–558.

Cordier F, Caffrey M, Brutscher B, Cusanovich MA, Marion D, Blackledge M. 1998. Solution structure, rotational diffusion anisotropy and local backbone dynamics of *Rhodobacter capsulatus* cytochrome *c2*. *J Mol Biol* 281:341–361.

Crick FHC. 1953. The packing of α -helices: Simple coiled-coils. *Acta Crystallogr* 6:689–697.

Englander SW, Sosnick TR, Englander JJ, Mayne L. 1996. Mechanisms and uses of hydrogen exchange. *Curr Opin Struct Biol* 6:18–23.

Feher VA, Cavanagh J. 1999. Millisecond-timescale motions contribute to the function of the bacterial response regulator protein Spo0F. *Nature* 400:289–293.

Feng Y, Klein BK, McWherter CA. 1996. Three-dimensional solution structure and backbone dynamics of a variant of human interleukin-3. *J Mol Biol* 259:524–541.

Gervais V, Zerial A, Oschkinat H. 1997. NMR investigations of the role of the sugar moiety in glycosylated recombinant human granulocyte-colony-stimulating factor. *Eur J Biochem* 247:386–395.

Glasoe PK, Long FA. 1960. Use of the glass electrodes to measure acidities in deuterium oxide. *J Phys Chem* 64:188–190.

Goodman EM, Kim PS. 1991. Periodicity of amide proton exchange rates in coiled-coil leucine zipper peptide. *Biochemistry* 30:11615–11620.

Gryk MR, Jardtetzky O, Klig LS, Yanofsky C. 1996. Flexibility of DNA binding of trp repressor required for recognition of different operator sequences. *Protein Sci* 5:1195–1197.

Grzesiek S, Bax A. 1993. The importance of not saturating H_2O in protein NMR. Application to sensitivity enhancement and NOE measurements. *J Am Chem Soc* 115:12593–12594.

Hilton DJ. 1994. Leukemia inhibitory factor (LIF) In: Nicola NA, ed. *Guidebook to cytokines and their receptors*. Oxford: Smbrook and Tooze. pp 124–126.

Hinds MG, Maurer T, Zhang J-G, Nicola NA, Norton RS. 1997. Resonance assignments, secondary structure and topology of leukemia inhibitory factor in solution. *J Biomol NMR* 9:113–126.

Hinds MG, Zhang J-G, Maurer T, Nicola NA, Norton RS. 1998. Solution structure of leukemia inhibitory factor. *J Biol Chem* 273:13738–13745.

Hudson KR, Vernallis AB, Heath JK. 1996. Characterization of the receptor binding sites of human leukemia inhibitory factor and creation of antagonists. *J Biol Chem* 271:11971–11978.

Jeng M-F, Englander SW. 1991. Stable submolecular folding units in a non-compact form of cytochrome *c*. *J Mol Biol* 221:1045–1061.

Kabsch W, Sander C. 1983. Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* 22:2577–2637.

Kay LE, Torchia DA, Bax A. 1989. Backbone dynamics of proteins as studied by ^{15}N inverse detected heteronuclear NMR spectroscopy: Application to staphylococcal nuclease. *Biochemistry* 28:8972–8979.

Kriwacki RW, Hengst L, Tennant L, Reed SI, Wright PE. 1996. Structural studies of p21^{Waf1/Cip1/Sdi1} in the free and Cdk2-bound state: Conformational disorder mediates binding diversity. *Proc Natl Acad Sci USA* 93: 11504–11509.

Layton MJ, Owczarek CM, Metcalf D, Clark RL, Smith DK, Treutlein HR, Nicola NA. 1994. Conversion of the biological specificity of murine to human leukemia inhibitory factor by replacing 6 amino acid residues. *J Biol Chem* 269:29891–29896.

Lee B, Richards FM. 1971. The interpretation of protein structures: Estimation of static accessibility. *J Mol Biol* 55:379–400.

Lipari G, Szabo A. 1982a. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity. *J Am Chem Soc* 104:4546–4559.

Lipari G, Szabo A. 1982b. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 2. Analysis of experimental results. *J Am Chem Soc* 104:4559–4570.

Luginbühl P, Pervushin K, Iwai H, Wüthrich K. 1997. Anisotropic molecular rotational diffusion in ^{15}N spin relaxation studies of protein mobility. *Biochemistry* 36:7305–7312.

Mandel AM, Akke M, Palmer AG III. 1995. Backbone dynamics of *Escherichia coli* ribonuclease HI: Correlations with structure and function in an active enzyme. *J Mol Biol* 246:144–163.

- Marion D, Wüthrich K. 1983. Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of ^1H - ^1H spin-spin coupling constants in protein. *Biochem Biophys Res Commun* 113:967-974.
- Maurer T, Smith DK, Owczarek CM, Layton MJ, Zhang J-G, Nicola NA, Norton RS. 1994. NMR studies of a murine-human chimera of leukemia inhibitory factor (LIF). Comparison with human LIF. *Growth Factors* 11:271-276.
- Messerle BA, Wider G, Otting G, Weber C, Wüthrich K. 1989. Solvent suppression using a spin-lock in two- and three-dimensional NMR spectroscopy with H_2O solution. *J Magn Reson* 85:608-613.
- Miller S, Janin J, Lesk AM, Chothia C. 1987. Interior and surface of monomeric proteins. *J Mol Biol* 196:641-656.
- Mori S, Abeygunawardana C, Berg JM, van Zijl PCM. 1997. NMR study of rapidly exchanging backbone amide protons in staphylococcal nuclease and the correlation with structural and dynamics properties. *J Am Chem Soc* 119:6844-6852.
- Owczarek CM, Layton MJ, Metcalf D, Lock P, Willson TA, Gough NM, Nicola NA. 1993. Inter-species chimeras of leukemia inhibitory factor define a major human receptor-binding determinant. *EMBO J* 12:3487-3495.
- Pedersen TG, Thomsen NK, Anderson KV, Madsen JC, Poulsen FM. 1993. Determination of the rate constants k_1 and k_2 of the Linderstrom-Lang model for protein amide hydrogen exchange. *J Mol Biol* 230:651-660.
- Powers R, Clore GM, Garrett DS, Gronenborn AM. 1993. Relationships between the precision of high-resolution protein NMR structures, solution-order parameters, and crystallographic B factors. *J Magn Reson B* 101:325-327.
- Purvis DH, Mabbitt BC. 1997. Solution dynamics and secondary structure of murine leukemia inhibitory factor: A four-helix cytokine with a rigid CD loop. *Biochemistry* 36:10146-10154.
- Radford SE, Buck M, Topping KD, Dobson CM, Evans PA. 1992. Hydrogen exchange in native and denatured hen egg-white lysozyme. *Proteins Struct Funct Genet* 14:237-248.
- Raschke TM, Marqusee S. 1998. Hydrogen exchange studies of protein structure. *Curr Opin Biotechnol* 9:80-86.
- Redfield C, Boyd J, Smith LJ, Smith RAG, Dobson CM. 1992. Loop mobility in a four-helix-bundle protein: ^{15}N NMR relaxation measurements on human interleukin-4. *Biochemistry* 31:10431-10437.
- Robinson RC, Grey LM, Staunton D, Vankelecom H, Vernalis AB, Moreau J-F, Stuart DI, Heath JK, Jones EY. 1994. The crystal structure and biological function of leukemia inhibitory factor: Implications for receptor binding. *Cell* 77:1101-1116.
- Rose TM, Bruce AG. 1991. Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6. *Proc Natl Acad Sci USA* 88:8641-8645.
- Schurr JM, Babcock HP, Fujimoto BS. 1994. A test of the model-free formulas. Effects of anisotropic rotational diffusion and dimerization. *J Magn Reson B* 105:211-224.
- Sprang SR, Bazan JF. 1993. Cytokine structural taxonomy and mechanisms of receptor engagement. *Curr Opin Struct Biol* 3:815-827.
- Stone MJ, Fairbrother WJ, Palmer AG III, Reizer J, Saier MH Jr, Wright PE. 1992. Backbone dynamics of the *Bacillus subtilis* glucose permease IIA domain determined from ^{15}N NMR relaxation measurements. *Biochemistry* 31:4394-4406.
- Tjandra N, Feller SE, Pastor RW, Bax A. 1995. Rotational diffusion anisotropy of human ubiquitin from ^{15}N NMR relaxation. *J Am Chem Soc* 117:12562-12566.
- Tjandra N, Wingfield P, Stahl S, Bax A. 1996. Anisotropic rotational diffusion of perdeuterated HIV protease from ^{15}N NMR relaxation measurements at two magnetic fields. *J Biomol NMR* 8:273-284.
- van Tilborg PJA, Mulder FAA, de Backer MME, Nair M, van Heerde EC, Folkers G, van der Saag PT, Karimi-Nejad Y, Boelens R, Kaptein R. 1999. Millisecond to microsecond time scale dynamics of the retinoid X and retinoic acid receptor DNA-binding domains and dimeric complex formation. *Biochemistry* 38:1951-1956.
- Wand AJ, Roder H, Englander SW. 1986. Two-dimensional ^1H NMR studies of cytochrome *c*: Hydrogen exchange in the N-terminal helix. *Biochemistry* 25:1107-1114.
- Wyss DF, Dayie KT, Wagner G. 1997. The counterreceptor binding site of human CD2 exhibits an extended surface patch with multiple conformations fluctuating with millisecond to microsecond motions. *Protein Sci* 6:534-542.
- Yao S, Hinds MG, Norton RS. 1998. Improved estimation of protein rotational correlation times from ^{15}N relaxation measurements. *J Magn Reson* 131:347-350.
- Zink T, Ross A, Luers K, Cieslar C, Rudolph R, Holak TA. 1994. Structure and dynamics of the human granulocyte colony-stimulating factor determined by NMR spectroscopy. Loop mobility in a four-helix-bundle protein. *Biochemistry* 33:8453-8463.