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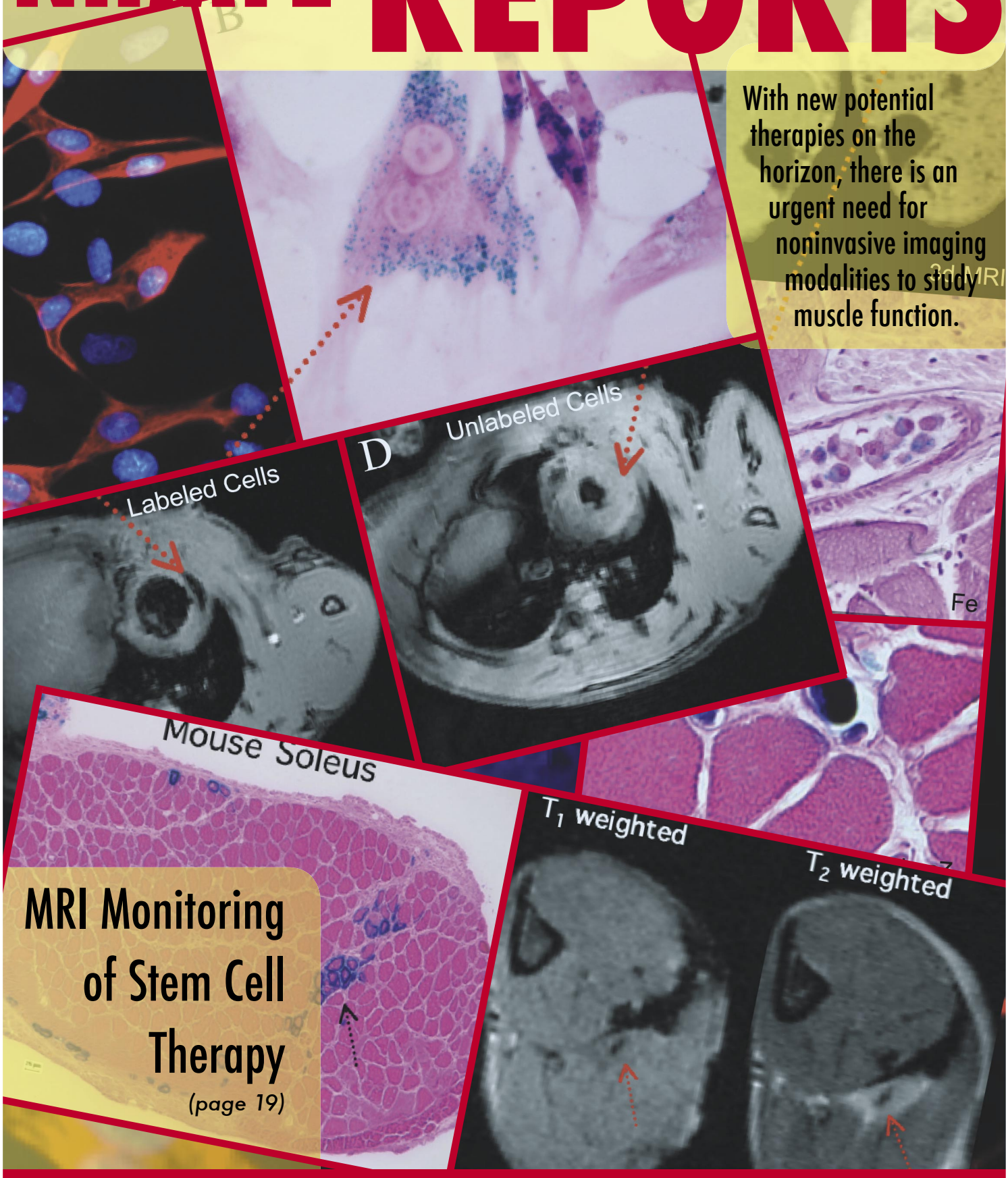
With new potential therapies on the horizon, there is an urgent need for noninvasive imaging modalities to study muscle function.



MRI Monitoring of Stem Cell Therapy

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The Role of the Turn Symmetry in the Folding and Stability of FGF-1

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The ability of proteins to fold up into a unique structure is the essence of the protein folding problem, and also is highly relevant to the area of self-assembling systems in the emerging nano-biotechnology field. In thermodynamic terms, protein folding and self-assembly is a delicate balance between enthalpy gain and entropic loss as an ensemble of conformational states accessible to a single polypeptide chain adopts a single (or severely limited number of) conformation(s). In kinetic terms, such structural changes must also occur within a time frame appropriate for living systems. In contributing to our understanding of the protein folding problem, the Logan and Blaber groups are collaborating on studies of hairpin turn regions within human acidic fibroblast growth factor (FGF-1). As outlined below, FGF is an interesting model system for protein folding studies. In addition, FGF is a potent growth factor (i.e. stimulator of cell growth). For example, FGF is known to stimulate angiogenesis (the growth of new blood vessels), and it is finding some clinical applications as a topical applied to cardiac muscle damaged by artery obstruction. It is hoped that the knowledge of FGF folding and structure coming from this research may increase its potency.

FGF is a small protein composed of primarily β-sheet and hairpin turn secondary structures (Figure 1). The FGF-1 structure is highly symmetric, with a three-fold symmetry axis being present down the barrel of the protein. This structural symmetry is not reflected in the amino acid sequence, as symmetry-related secondary structures and tertiary interactions are generated by different amino acid sequences. This raises some intriguing questions about how the symmetry of protein structure and sequence relates to the folding and stability. For example, why is the structural symmetry *not* reflected in sequence symmetry? It is possible that there is no evolutionary pressure to maintain the sequence symmetry and so there is drift in the amino acid sequence, e.g., replacing certain amino acids in the sequence does not significantly alter the stability, folding, or function of FGF-1, and so these residues would not be under evolutionary pressure to be retained. A more interesting question is whether there is evolutionary pressure to *reduce*

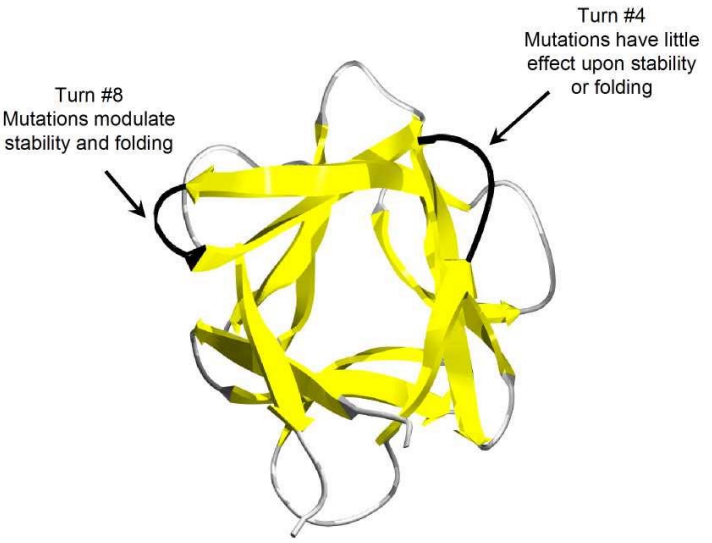

Figure 1. An illustration of the polypeptide chain conformation in FGF-1. Two turn regions are indicated in black shading: turns 4 and 8. Mutational studies indicate that changes in the amino acid sequence of turn 4 have little effect upon the stability or folding kinetics of the protein, while the amino acid sequence within the turn 8 region can have substantial effects.

the sequence symmetry. What would be the consequences for folding if the sequence were more symmetric? Would the protein fold to the same structure or would there be an increase in the percent of proteins that "misfold" by pairing incorrectly with the wrong β-strands?

Although α-helix and β-sheet secondary structures have been the subject of numerous biophysical studies, little is known regarding the thermodynamic and kinetic contribution to protein folding of hairpin turns. Turn regions are essential for the polypeptide to change direction and terminate the linear conformation of the other secondary structures. A key question to understand relates to the interplay between the energetics of the turn regions and the associated adjacent α-helical or β-sheet secondary structures. In other words, does the stability of the adjacent secondary structure drive the formation of the turn structure, or does the stability of the turn structure drive the formation of the adjacent secondary structure? The Logan and Blaber groups are investigating this question by mutating the amino acid sequence within specific turn regions in FGF-1 and determining the effects of such mutations upon the structure, thermodynamics and kinetics associated with the folding of the protein.

FGF-1 has 11 turns (12 if you consider the adjacent N- and C-termini as a discontinuous turn), and it is becoming clear that not all turns in FGF-1 are equivalent in their properties. Some turns appear to be merely connecting regions between highly stable adjacent secondary structures— substitution of the amino acids within these turns has essentially no affect upon stability, folding kinetics, or structure. In other cases, the turns appear to contribute substantially to either the stability or kinetics, and require a very specific amino acid sequence. Among these turns, turn 4 (connecting β-strands 4 and 5) and turn 8 (connecting β -strands 8 and 9) are related by three-fold symmetry of FGF. Turn 8 (residues E90-E91-N92-H93) is a type-I turn. Turn 4 (residues A49-E50-S51-V52-G53) is symmetry-related to turn 8 but forms a type II turn.

We wanted to determine why these two regions adopt different conformations, and to investigate the consequences of sequence and structure symmetry relationships between these turns. Therefore, a series of single-site mutations were designed to probe the thermodynamic importance of specific interactions that differ between these two turn regions, and also to systematically convert turn 4 into turn 8 sequence and vice versa.

Polyglycine Substitutions. Our first study systematically changed each residue in the turns 4 and 8 to the amino acid glycine. Glycine is an unusual amino acid in that it does not contain a "sidechain" group and so has fewer restrictions in the conformations that it can adopt compared to the other 19 amino acids. As shown in Table 1, substituting individual turn 4 residues into glycine did not significantly alter the thermodynamic stability or folding kinetics. Indeed, the E49G/S50G/V51G polyglycine mutant showed essentially the same stability and folding rates as wildtype. In contrast, changing His92 to Gly in turn 8 resulted in a substantial increase in stability of nearly 9 kJ/mol, due almost entirely to a 15-fold enhancement in the folding rate. Converting the remaining turn 8 residues into Gly yielded a mutant that had essentially the native state stability and folding. The dramatic effect observed in the H93G mutant reflects the steric interactions that arise from the large His sidechain at this position. Removing this sidechain by glycine replacement facilitates folding by reducing steric interactions and strain, resulting in substantial structural stabilization. Why does nature retain this His residue at this position? Functional studies reveal that this His residue is required for activity, forming electrostatic interactions with negative residues in the receptor. Thus, the stability of the protein is sacrificed for biological activity. Indeed, structural studies have shown that receptor binding is accompanied by a conformational change in turn 8; perhaps the strain introduced by the His residue "cocks the trigger" providing additional driving force for binding.

Table 1. Stability of FGF-1 mutants.

Protein	ΔΔG (kJ/mol)
E49G/S50G/V51G	-1.6
H93G	-8.9
E91G/N92G/H93G	-1.5
S50E/V51N/G52H	1.9
E91S/N92V	6.5
E91S/N92V/H93G	2.1

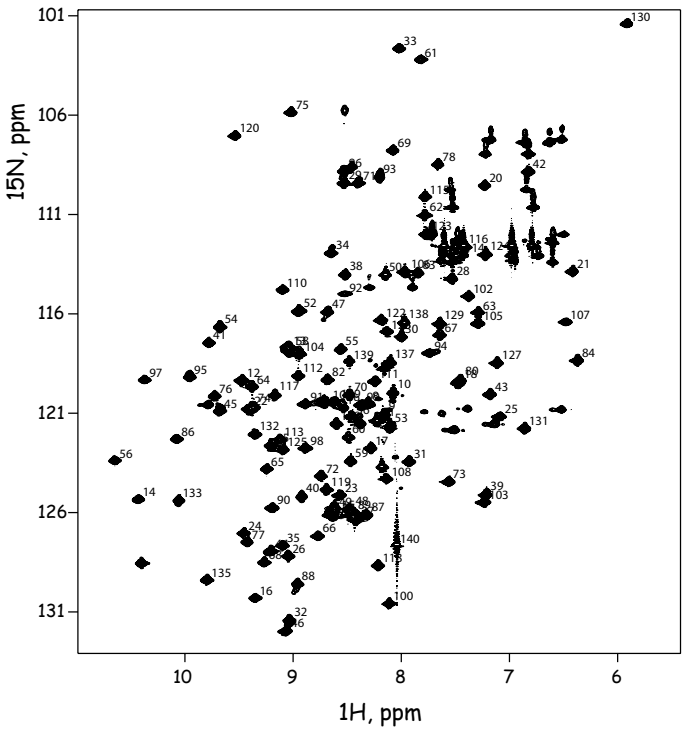

Figure 2. Two dimensional ^{1}H, ^{15}N single-quantum correlation spectrum of wildtype FGF indicating the assignments of the backbone amide resonances.

Substituting Turn 8 into Turn 4 Sequence. The polyglycine data indicated that turn 4 was more "plastic" in that it readily accommodated amino acid substitutions. In contrast, turn 8 residues showed a higher requirement for the specific amino acid sequence. In the next study, we investigated the consequences of converting turn 8 into turn 4 by creating a triple-mutant, E91S/N92V/H93G (8→4). The reverse mutant was also created, changing turn 4 into turn 8 (S50E/V51N/G52H; 4→8). As shown in Table 1 there was little change in the stability of the 4→8 mutant compared to the wildtype protein. As predicted from the polyglycine substitutions at this site, turn 4 is able to accommodate essentially any amino acid sequence at this position. The 8→4 mutant was

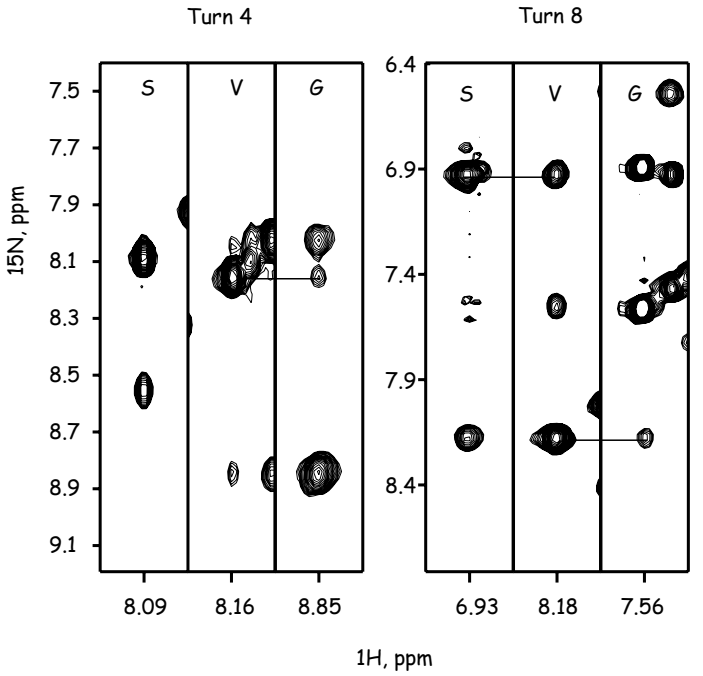

Figure 3. Composite plot of several slices taken from a 3D ^{15}N-separated NOESY-HSQC spectrum of the 8→4 mutant. The left panel shows the HN-HN NOEs observed for residues S50-V51-G52 of turn 4; the right panel shows the HN-HN NOEs observed for the same amino acid sequence in turn 8 (residues S91-V92-G93). The lines indicate the location of sequential and medium range NOEs observed for each residue.

slightly more destabilized than the other mutant. However, the majority of the destabilization arose from the E91S/N92V substitution (6.5 kJ/mol destabilizing compared to -8.9 kJ/mol stabilization from the H93G mutant). The reasons for the destabilization in the double mutant are not clear and are currently under investigation. Nevertheless, these studies further demonstrate the thermodynamic difference between turn 4 and turn 8 in that the same sequence at two symmetry-related positions has substantially different thermodynamic consequences for stability.

A key component of these studies is the structural analysis of these mutants. We have determined a high resolution structure of wildtype FGF-1 using X-ray crystallography, but the study of turn regions (and mutations therein) introduces unique problems for X-ray structure determination. In particular, it is the surface turn regions that are most often involved in crystal contacts. It is not uncommon for mutations in such regions to perturb crystal contacts and preclude crystallization. However, it is essential to understand the structural consequences of such mutations upon the turn regions. In the course of studying these turn mutations, we were unable to obtain critical structural information of some mutants using X-ray crystallography, and turned to NMR to solve this problem.

The first step was to confirm resonance assignments in wildtype FGF, for which a series of triple resonance NMR experiments were collected. The resulting assignments are indicated in Figure 2 which is a 2D ^{1}H,^{15}N single-quantum correlation experiment (HSQC). We then repeated these experiments to obtain assignments for the 8turn4 mutant (not shown). We used these assignments to characterize the turn conformation for residues in turns 4 and 8 wildtype and mutant FGF-1 proteins. Figure 3 shows that the NOE patterns obtained for the ESVG residues in turn 4 and turn 8 in the mutant protein are different. Specifically, we observe intense sequential and medium-range NOEs between the amide protons of turn 8 residues but not for the same amino acids in turn 4. These data also support the thermodynamic and kinetic investigations that point to some fundamental difference in the two turn regions. This finding confirms that the turn conformation is not merely dependent on the local amino acid sequence, but depends, in some as yet undefined manner, on the tertiary structure imposed by the folded state of the protein.

The results to date indicate the following:

1. Turn regions related by structural symmetry do not necessarily have equivalent thermodynamic and kinetic contributions towards protein stability and folding.
2. The alternative turn structures 4 and 8 in FGF-1 are not due to the local sequence within the turn, nor to structural differences in the adjoining secondary structure (i.e. anti-parallel β-strands). We are left with the conclusion that amino acid side chains, located outside of the turn region (and possibly within the adjoining secondary structure) are the prime determinants of the turn structures in turns 4 and 8.
3. Turn 8, and structural alterations within this turn, is essential for receptor binding. We conclude that the dynamics associated with this structural alteration upon receptor binding are associated with strain within this turn (at position 93 as determined by Gly mutagenesis). Again, this strain appears related to interactions with side chains outside the turn.

The focus for future work is related to elucidating which of the neighboring residues to the turn(s) are influencing structure and strain. Another area of study will be to understand the limitations upon turn identity for symmetry-related turns in structural superfolds.