

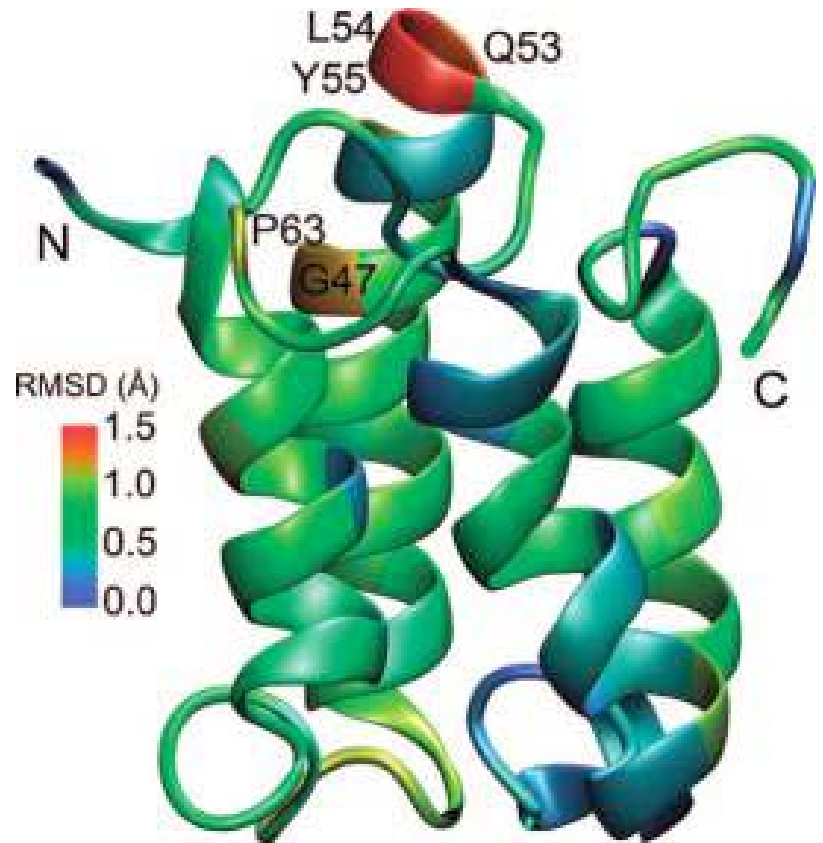
Anesthetic Modulation of Protein Dynamics: Insight from an NMR Study

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Mistic

- A 4 α -helix bundle in the transmembrane domain of Cys-loop family of receptors



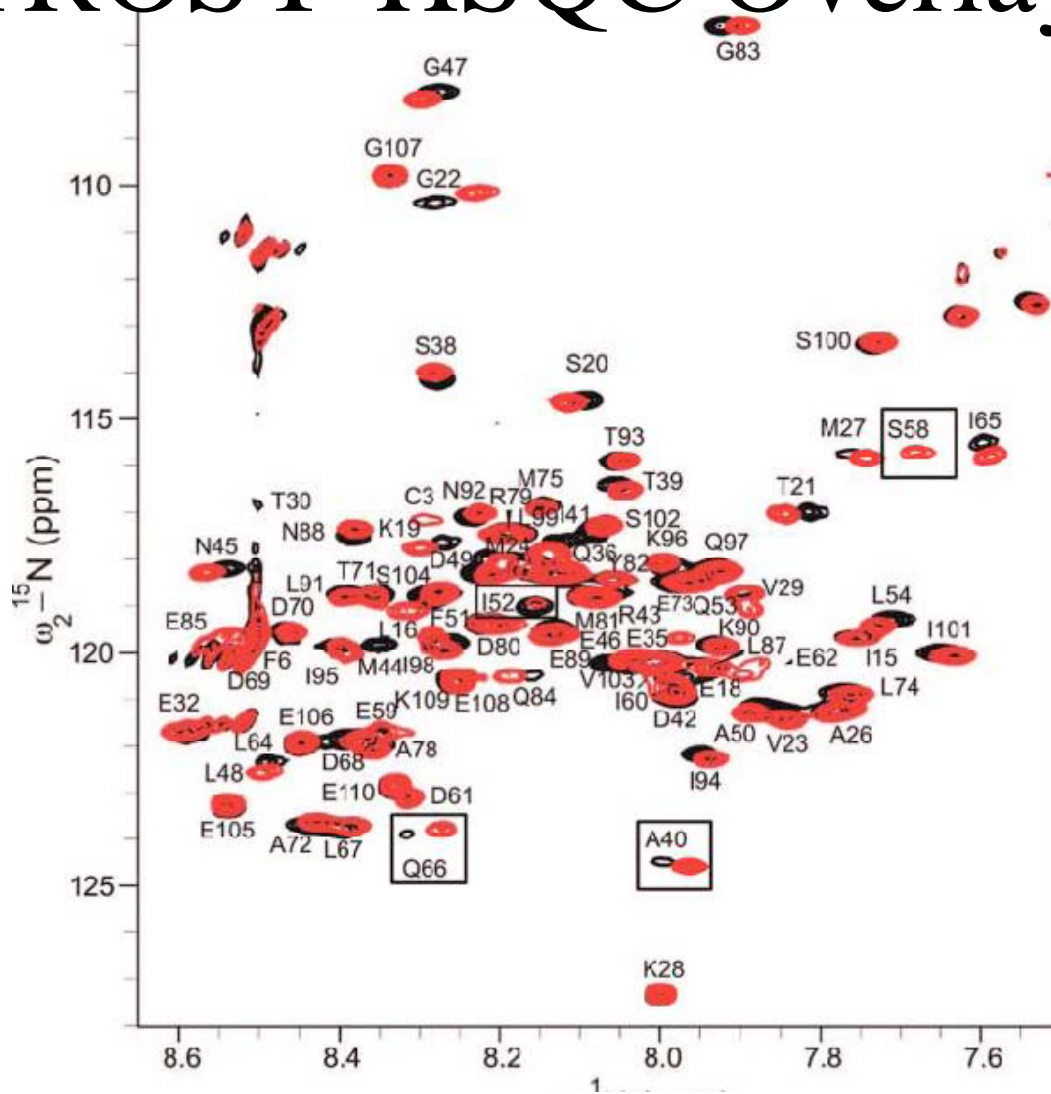
Experimental Setup

- ^1H - ^{15}N TROSY-HSQC spectra used to determine dissociation constants
- STD tests to confirm direct Mistic-anesthetic interaction
- Magnetic pulse sequences used to study protein dynamics

Sites of Halothane Interaction

- Remarkable chemical shifts after exposure to halothane
- Certain residues' signals were also either strengthened (i.e. S58) or weakened (i.e. I52)

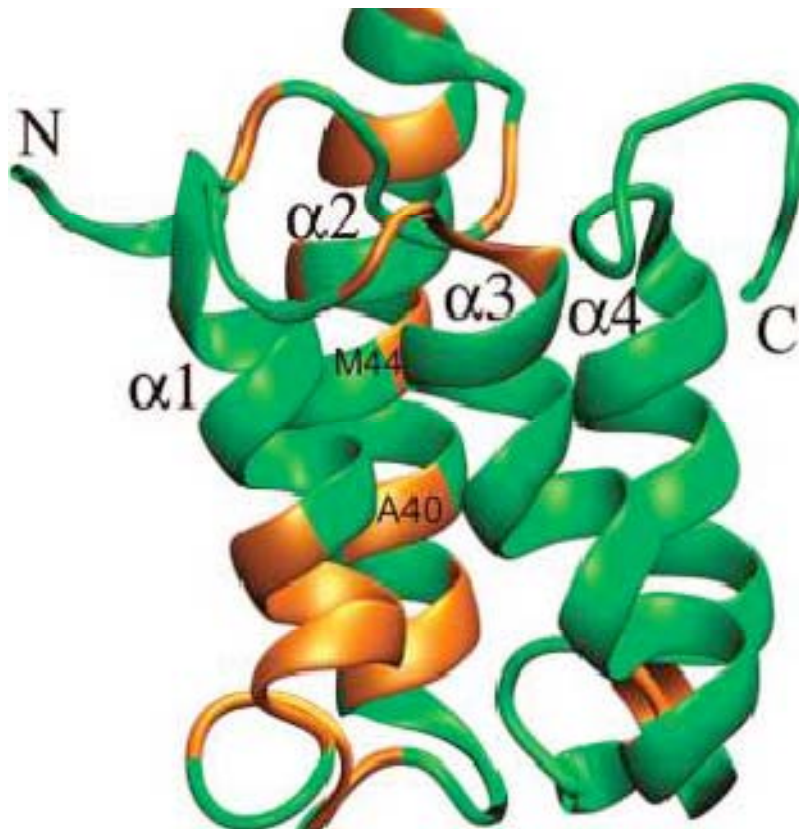
TROSY-HSQC Overlay



Sites of Interaction (Contd)

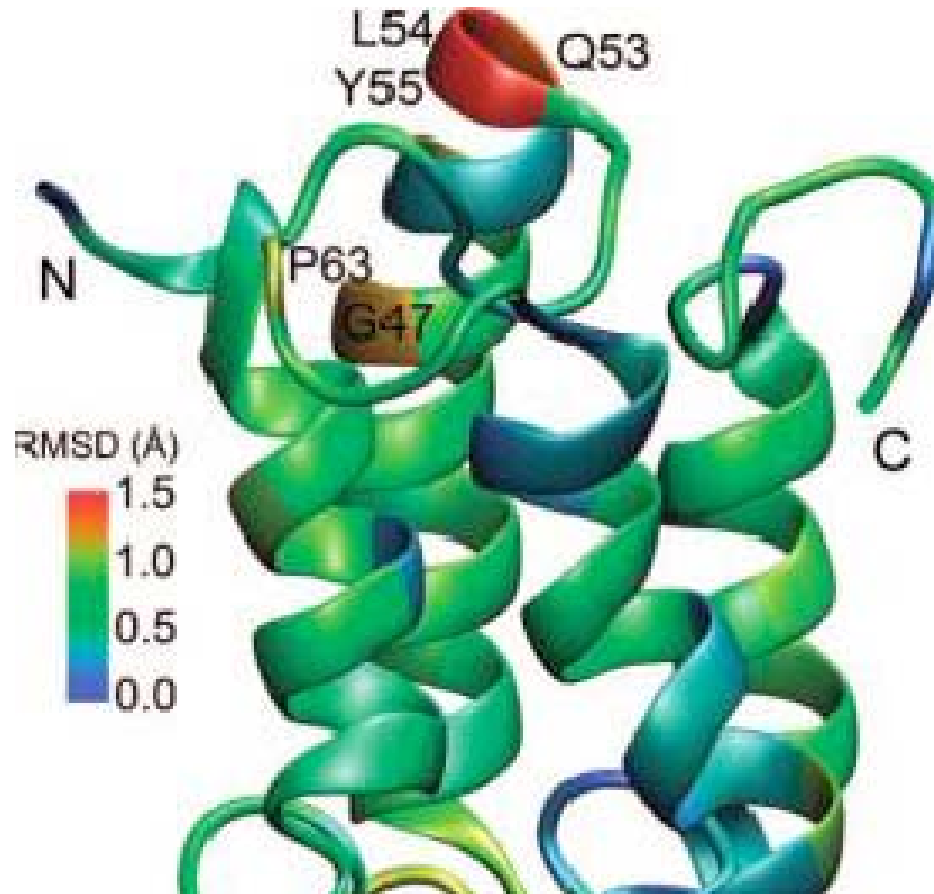
- Most helical regions unaffected while loop regions generally interacted with halothane
- Binding likely disrupts interaction between different loops or residues within the same loop

Sites of Interaction (Contd)



- Regions of significant chemical shift localized to residues near/in loops

Halothane Effects on Structure



- Relatively small rmsd values indicate conservation of structure upon halothane addition

Halothane Effects on Motion

- R_2 relaxation rates affected in loops connecting helices and along $\alpha 2$ helix (T39-N45)
- Residues T39, N45, and Y82 underwent chemical exchange on micro/millisecond time scale (- halothane) but did not undergo chemical exchange (+ halothane)

Effects on Motion (Contd)

- Two pairs of nearby residues w/ similar exchange rates affected similarly by halothane addition
- Most relaxation dispersion values decreased by halothane
- Some residues' chemical shifts not affected by halothane while dynamics were (I52, Y82)

Key Points of Study

- Residues with motion on microsecond/millisecond timescale mostly in loops between helices
- Residues near each other spatially (but not necessarily sequentially) display concerted motion

Key Points (Contd)

- Halothane seemingly capable of altering protein slow dynamics through both direct interaction with residues and allosteric modulation
- General anesthetics tend to interact preferentially with amphipathic regions of proteins