Computational Analysis of nAChR α4 and β2 Subunit Stability and NMR Study of Protein Anesthetic Interaction

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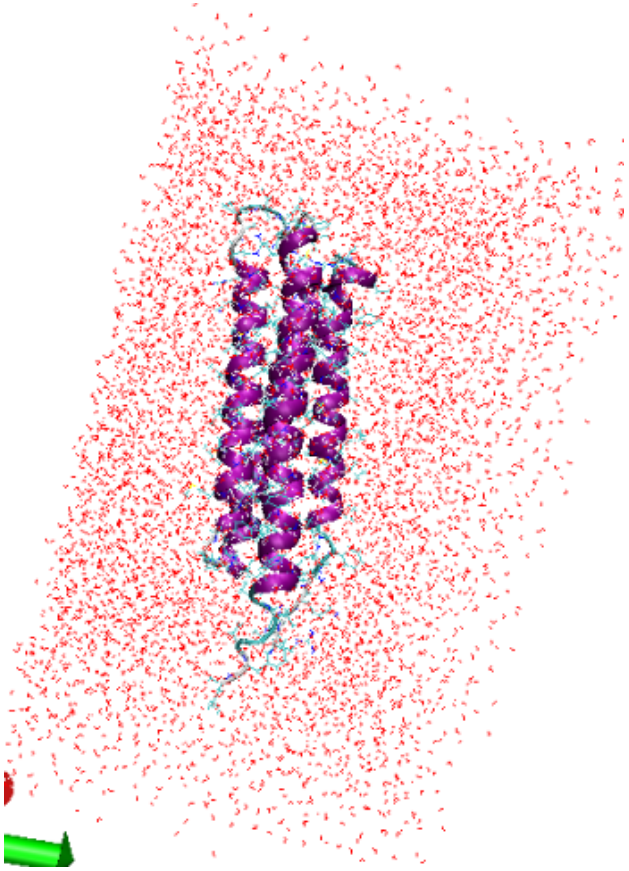
General Anesthetics

- Induce unconsciousness and prevent painful stimuli from being recognized
- Modify flow of sodium ions into neurons
- Both ion-gated channels/cys-loop regions of membrane-bound proteins implicated in activity
- How is anesthetic effect accomplished?

α4/β2 Subunit Characteristics



1) α4=136 aa long, β2=142
2) Transmembrane proteins
3) High pI naturally (α4=7.64, β2=8.97) =low stability in NMR solution with low pH
4) Form heteropentameric nAChR (3 α4, 2 β2)

Challenges of NMR

- Difficult to perform NMR on membrane-bound proteins
  - Unstable sample
  - Poor protein folding
  - Variable flexibility
  - Size limitations

Increasing Stability

- Native $\alpha 4$ and $\beta 2$ sequences unstable in solution suitable for NMR (low pH)
- Sequence mutations act to increase stability (mutants stable for >1 week, native <1 day)
- Because $\beta 2$ mutant still tends to aggregate, further mutation necessary to give $\beta 2$ the same level of stability as $\alpha 4$

NAMD Simulations

- Examine stability of $\alpha 4$ and $\beta 2$ nAChR subunits
- Dimerize subunits and calculate dimer stability
- Model cell membrane properties and repeat simulations

Simulations (Contd.)

- Repeat dynamics simulations using mutant sequences instead of original pdb sequence
- Observe stability of $\alpha 4 \beta 2$ heteropentamer
- Stability measured by rmsd calculations

NMR Spectroscopy

- Titration experiment: chemical shifts caused by anesthetic with concentration varying over time
- Concentration lowered by running sample at high temp for long periods of time
- Tryptophan signals unique due to ring contribution in signal; anesthetic interaction with trp observed

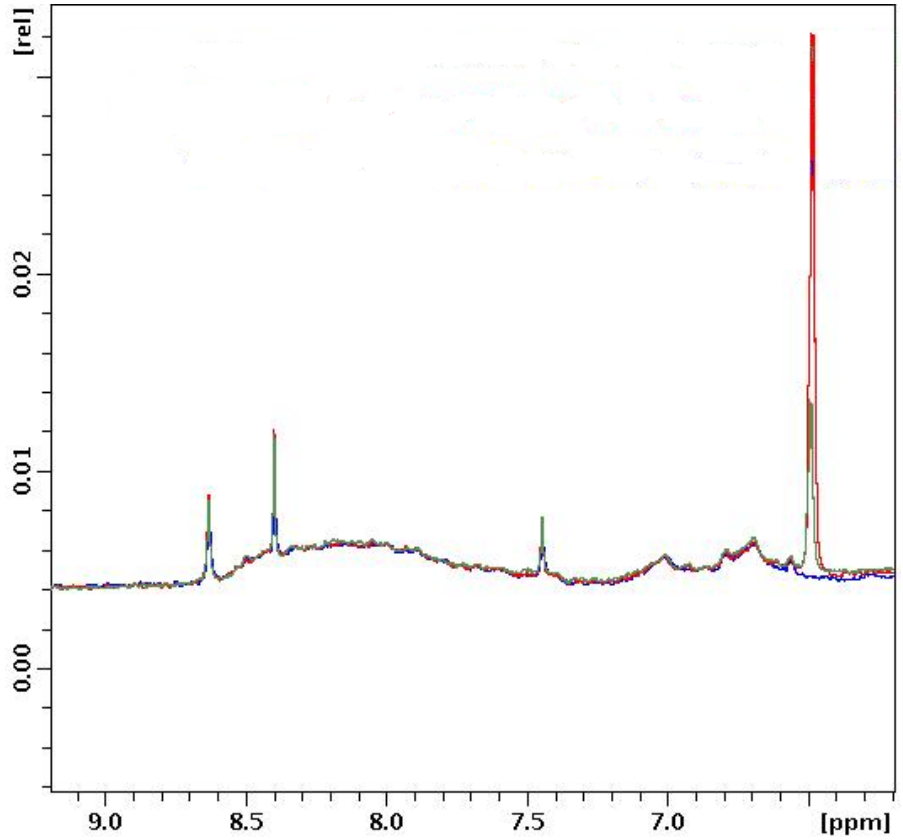
Experimental Methods

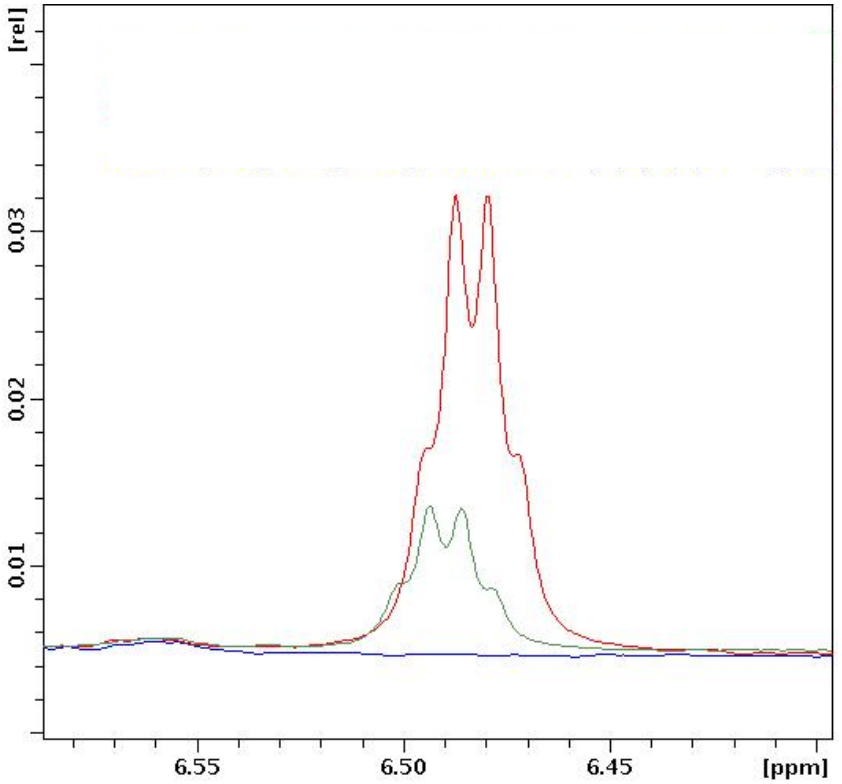
- NMR sample
  - 250 μl α4
  - 80 mM LDAO detergent
  - pH 4.7
  - ^{15}N labeled
- NMR Spectrometer
  - 700 MHz
  - 45°C
  - p3919gp (water suppression, 1D spectra)
  - TROSY (2D spectra with sharper peaks)

Halothane Concentration

Blue - no halothane Red - 4.0 mM halothane

Green - 1.7 mM halothane



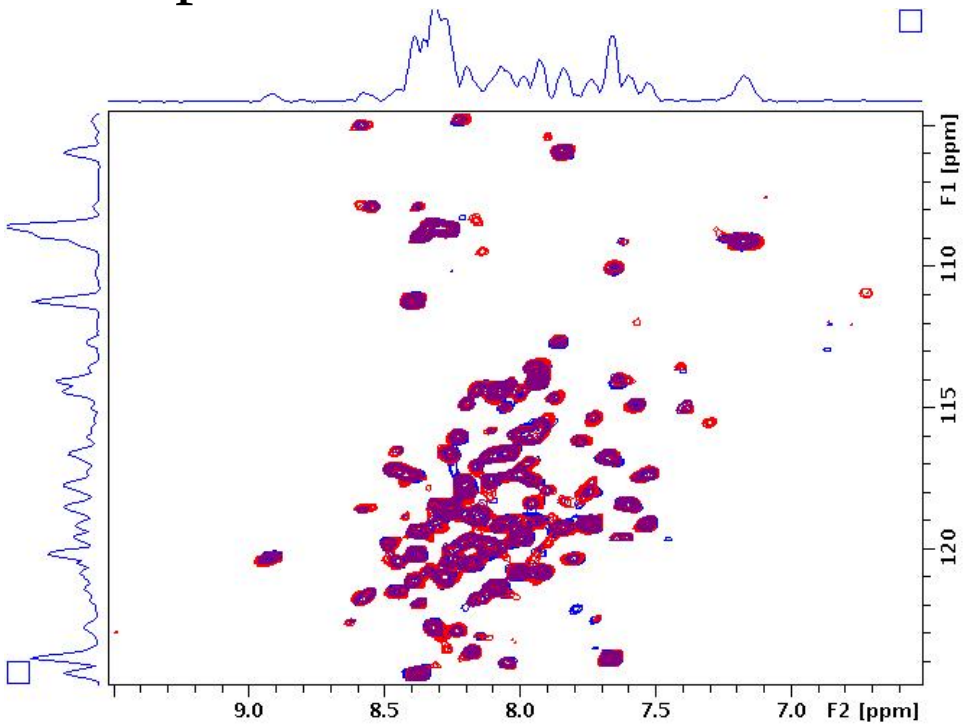


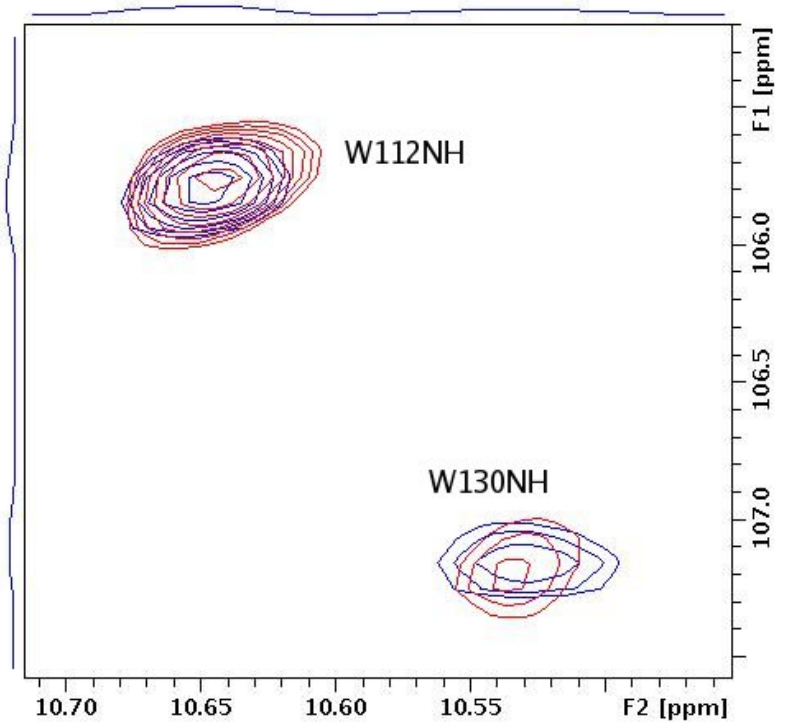
p3919gp spectra: NS=16 D_1=1s Sw=16 ppm TD=16k

Effects of Halothane on α4

Blue - no halothane Red - 4.0 mM halothane

Purple - 1.7 mM halothane



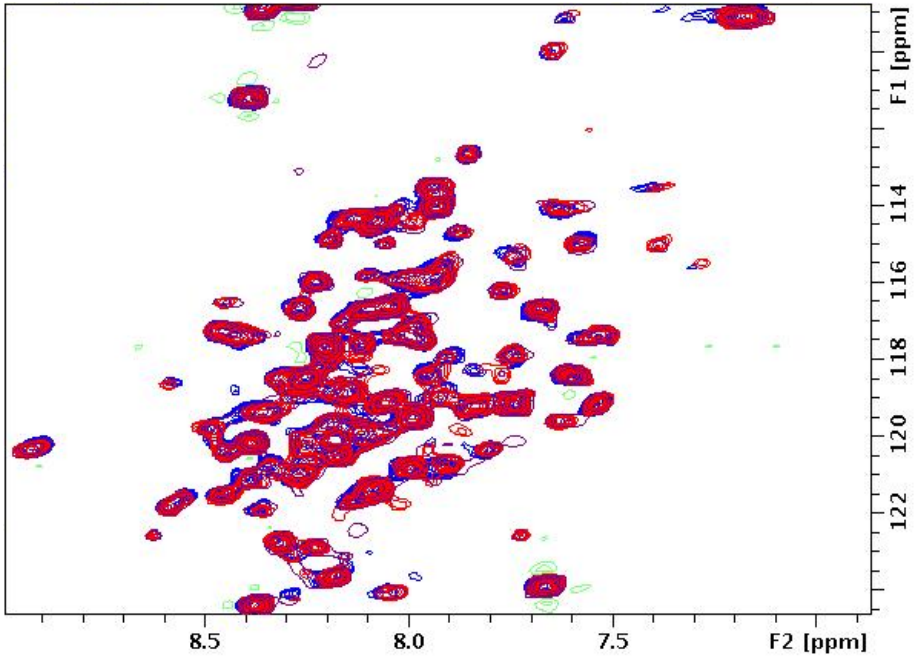


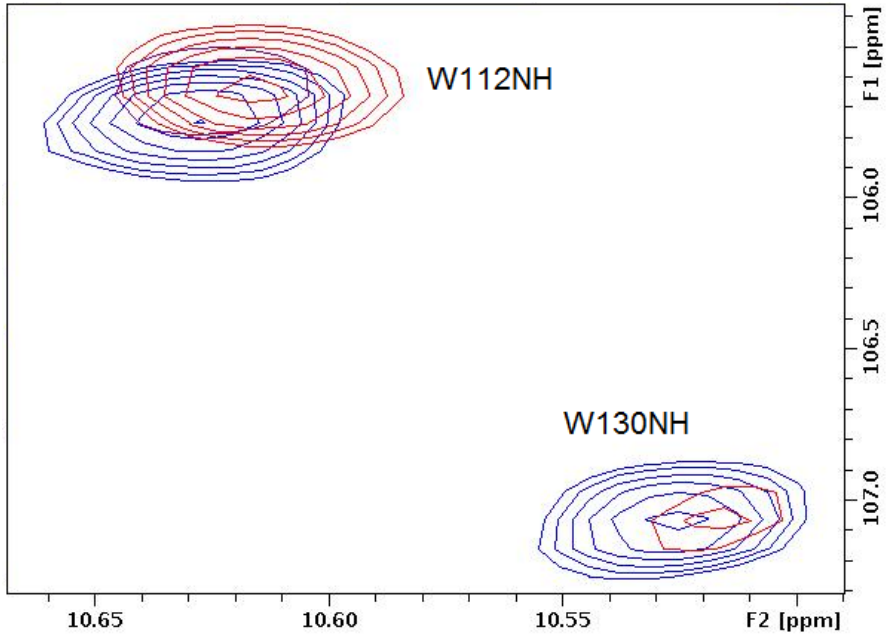
2D TROSY-HSQC NS=64 D_1=1s Sw=13 ppm TD=1K (^{1}H), 128 (^{15}N)

Effects of Isoflurane on α4

Blue - no isoflurane Red - 5.0 mM isoflurane

Purple - 1.83 mM isoflurane



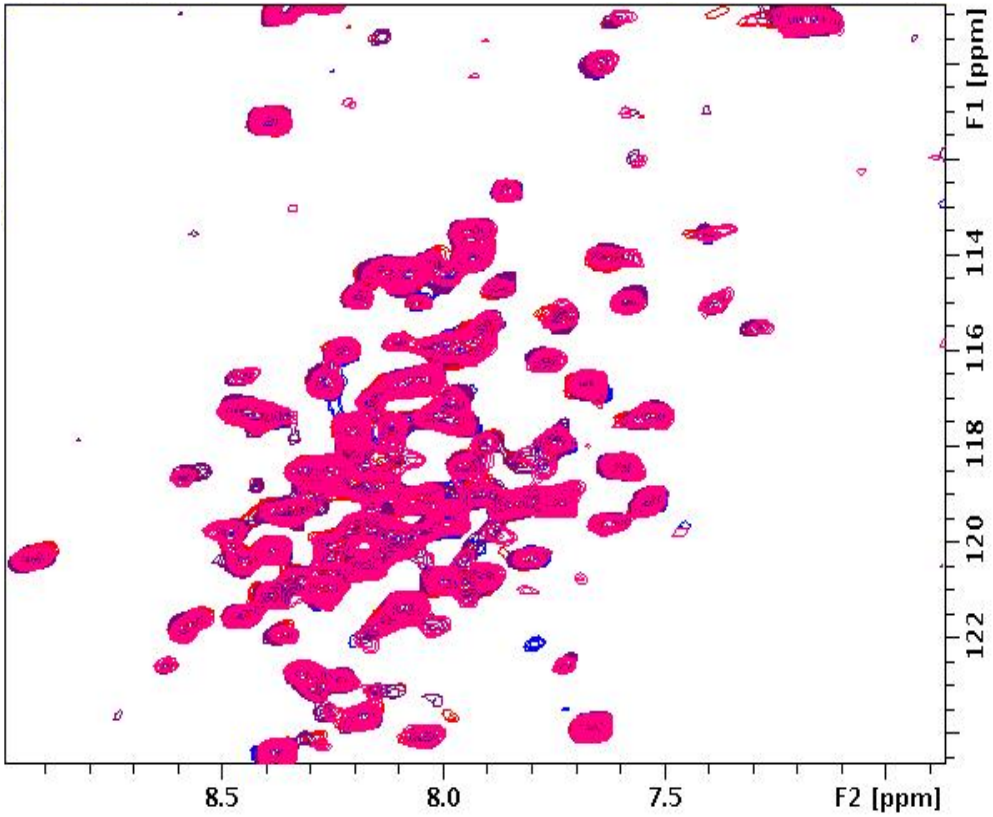


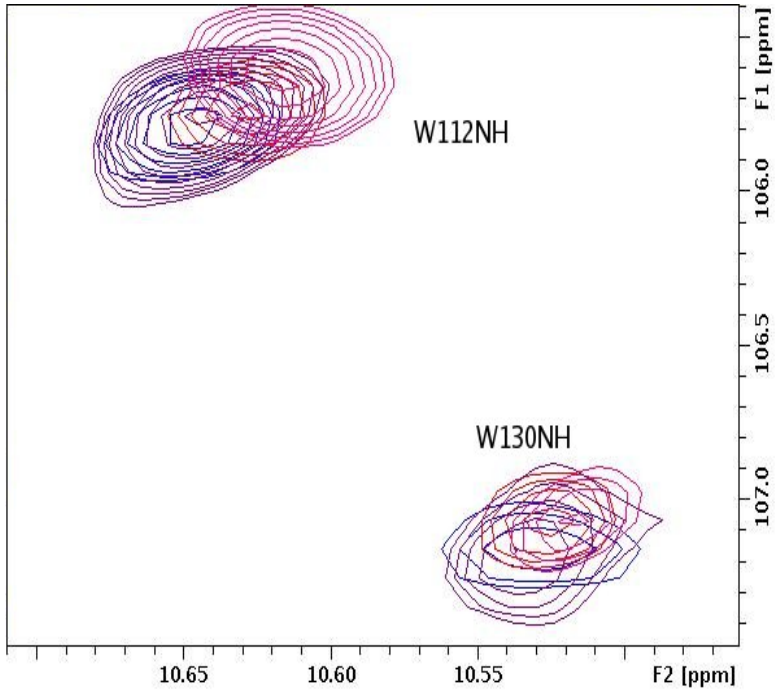
2D TROSY-HSQC NS=64 D_1=1s Sw=13 ppm TD=1K (^{1}H), 128 (^{15}N)

Halo Effects vs. IsoF Effects

Blue - no halo Purple - 4.0 mM halo

Red - halo removed Pink - 5.0 mM isoF





2D TROSY-HSQC NS=64 D_1=1s Sw=13 ppm TD=1K (^{1}H), 128 (^{15}N)

Conclusions

- Isoflurane = stronger anesthetic (caused greater chemical shifts)
- W130NH = more reactive tryptophan (near end of loop 4, not concealed within helix)

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References

1. C.G. Canlas, T. Cui, L. Li, Y. Xu, and P. Tang. 2008. *J. Phys. Chem. B.* 112: 14312–14318.

2. P. Tang and Y. Xu. 2002. *Proc Natl Acad Sci.* 99: 16035–16040.