Development of ^{205}Tl-NMR for the Direct Study of Monovalent Metal Ions and Ligands in Nucleic Acids

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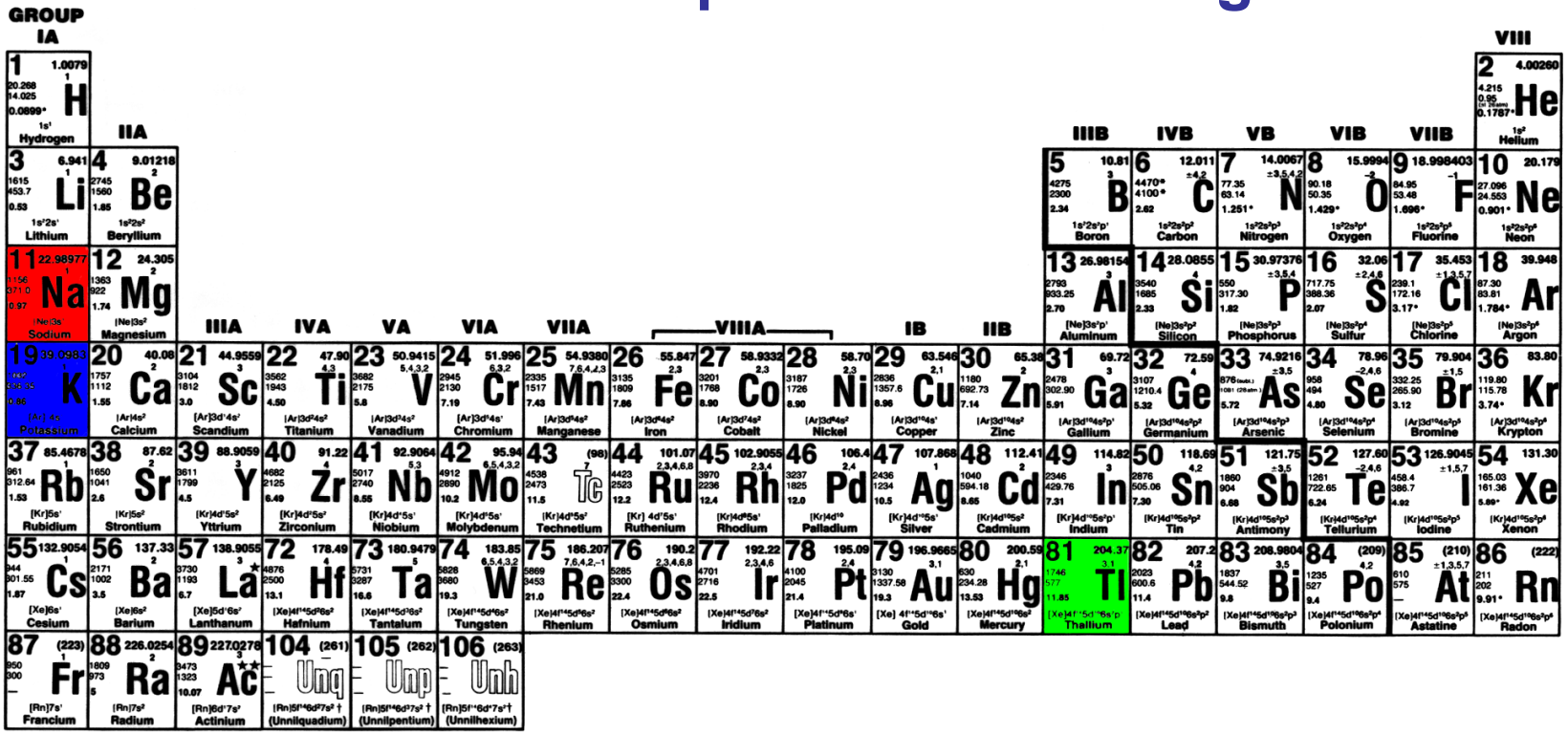
Scott A. Strobel and J. Patrick Loria Laboratories

May 30, 2006

Thallium as a potassium surrogate

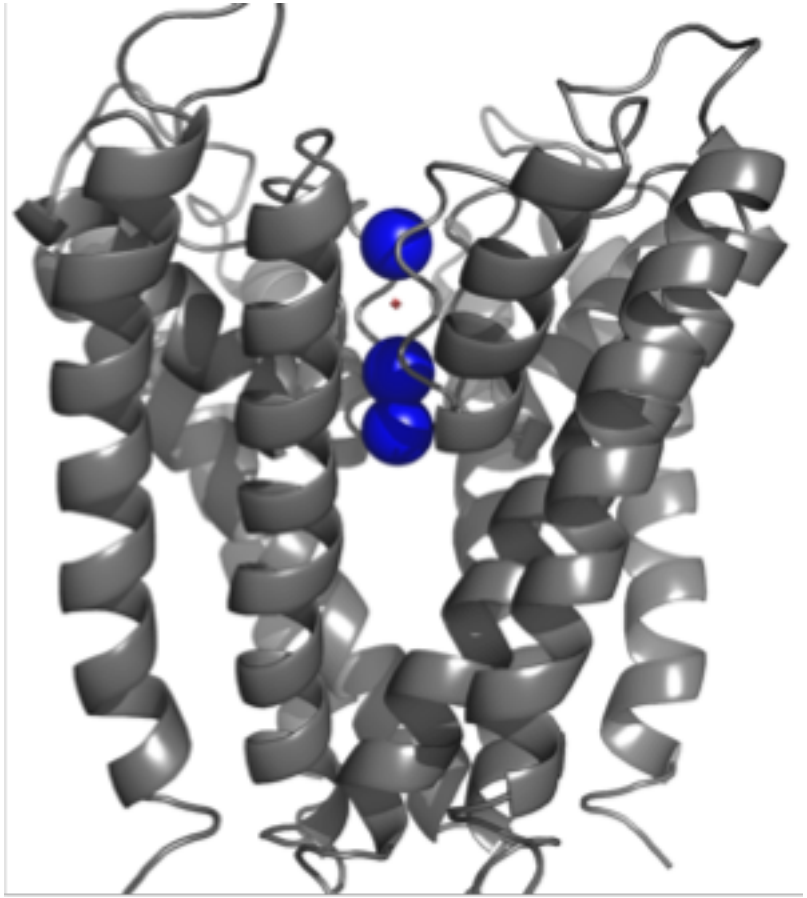


Thallium (Tl^+) and potassium (K^+) have similar

- Atomic radii—1.40 Å for Tl^+ and 1.33 Å for K^+
- Dehydration energies—77.6 kcal/mol and 76.4 kcal/mol
- Coordination geometries and bond lengths—2.4–2.7 Å

Tl^+ has been able to support enzymatic activity in many systems, including the ribosome

Importance of monovalent cations



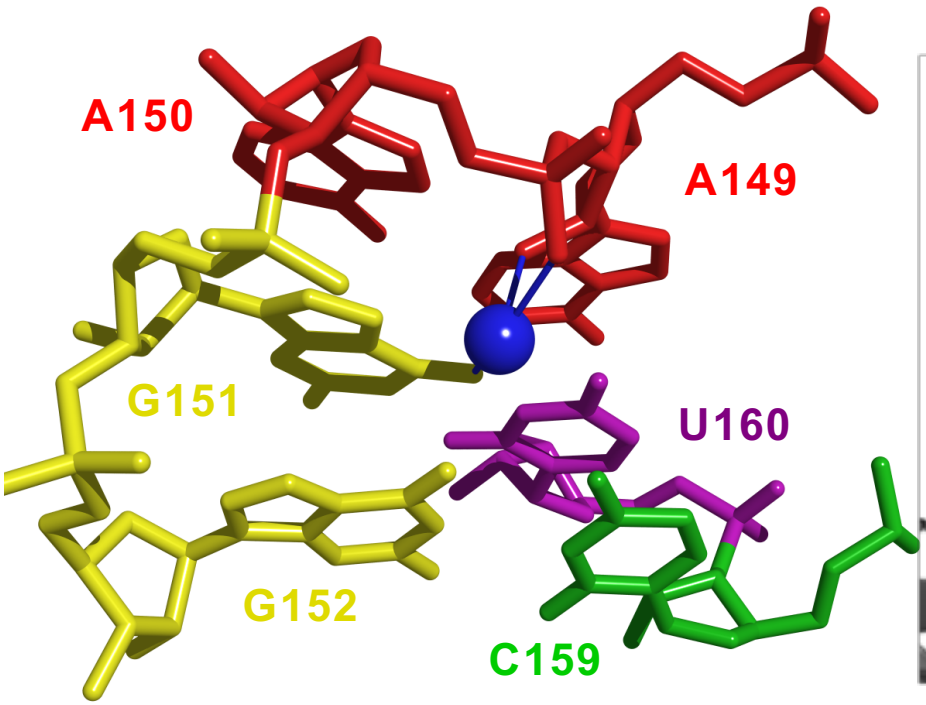
KcsA channel from *Streptomyces lividans*

Monovalent cations are found in:

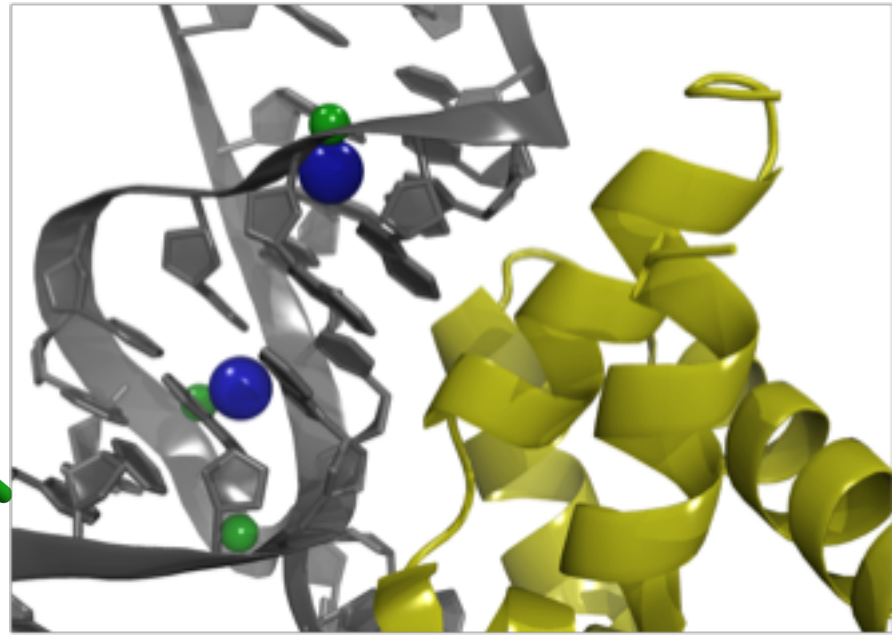
- Proteins
  potassium channel, *pyruvate kinase,*
  Na^+–K^+ ATPase
- Phospholipids
  phosphatidylinositol 4,5-bisphosphate,
  phosphatidylserine bilayers
- Carbohydrates
  proteoglycans, heparin
- Nucleic acids
  ribosome, group I intron, SRP

Doyle, D.A.; et. al. *Science.* **1998**, *280*, 69-77.

Monovalent cations in nucleic acids



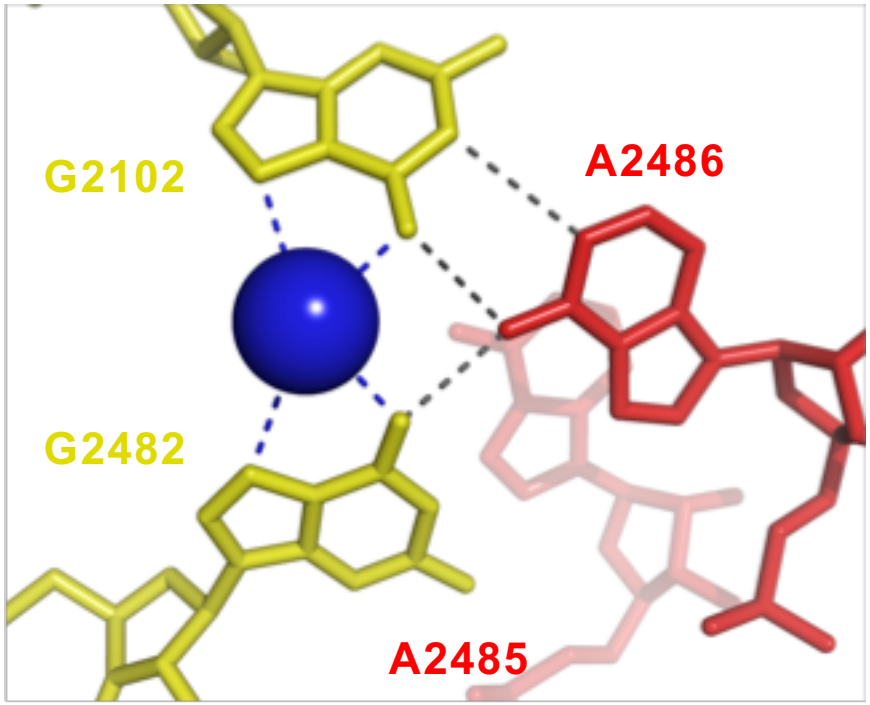
Azoarcus group I intron tetraloop receptor



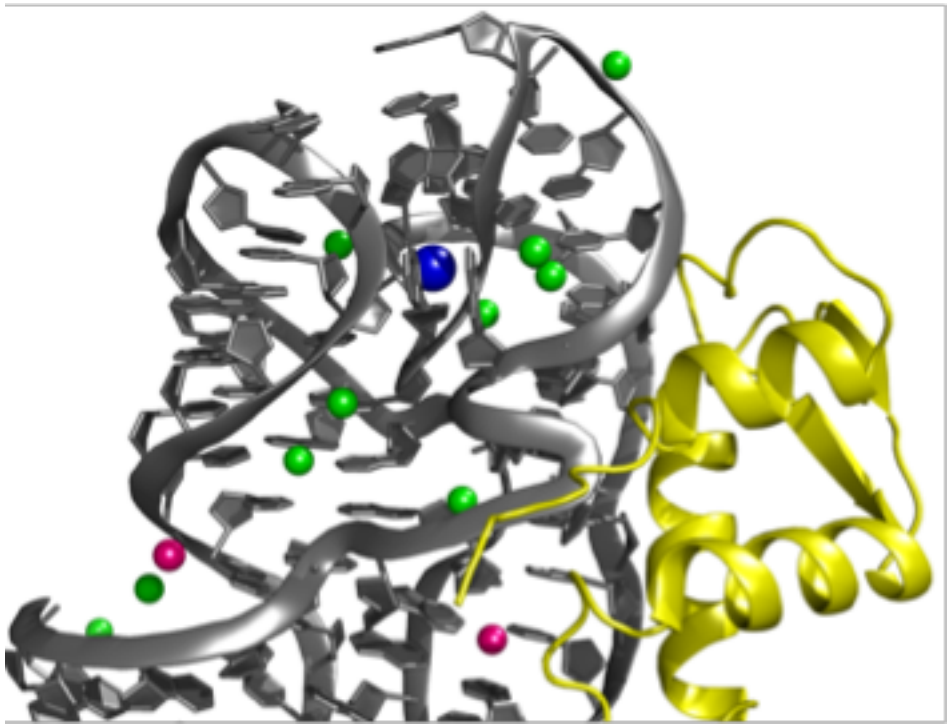
Escherichia coli signal recognition particle

Adams, P.L.; et. al. *Nature.* **2004,** *430*, 45-50.
Stahley, M. R.; Strobel, S. A. *Science.* **2005,** *309*, 1587-90.
Basu, S.; et. al. *Nat. Struct. Biol.* **1998**, *5*, 986-92.
Abramovitz, D.L.; Pyle, A.M. *J. Mol. Biol.* **1997**, *266*, 493-506.
Batey, R. T.; et. al. *Science.* **2000,** *287*, 1232-9.
Batey, R. T.; Doudna, J. D. *Biochemistry.* **2002**, *41*, 11703-10.

Monovalent cations in nucleic acids



Haloarcula marismortui 50S ribosome peptidyl transferase center



Escherichia coli L11-binding 23S rRNA

Pestka, S. *Proc. Natl. Acad. Sci. USA.* **1972**, *69*, 624-8.
Ban, N.; et. al. *Science.* **2000,** *289*, 905-20.
Nissen, P.; et. al. *Science.* **2000,** *289*, 920-30.
Conn, G. L.; et. al. *Science.* **1999,** *284*, 1171-4.
Conn, G. L.; et. al. *J. Mol. Biol.* **2002,** *318*, 963-73.

Why study monovalent metals by NMR?

- Number and position of monovalent binding sites
- Cation exchange rates and bound lifetimes
- Rapidly study effects of cation site perturbation
- Functional groups coordinating the cation(s)
- Dynamics of monovalent ligands
- Formation of single crystals not required

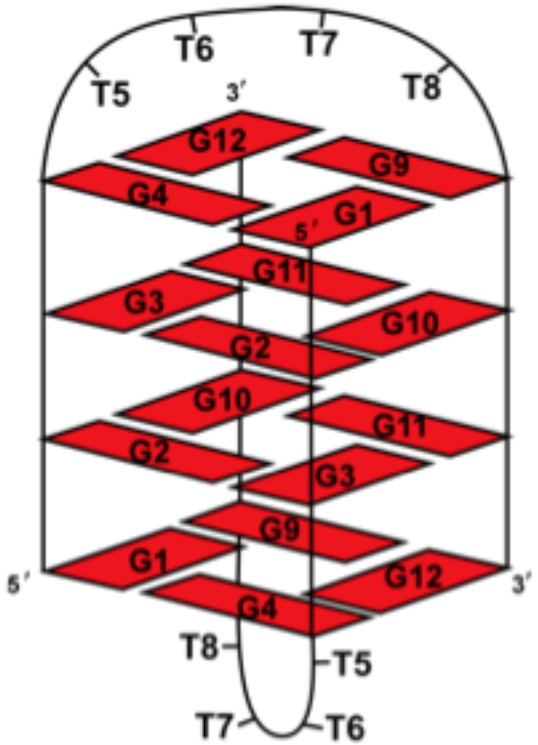
Lack of technique for direct observation has precluded the solution study of monovalent cations

Tl^+ is an excellent mimic of K^+

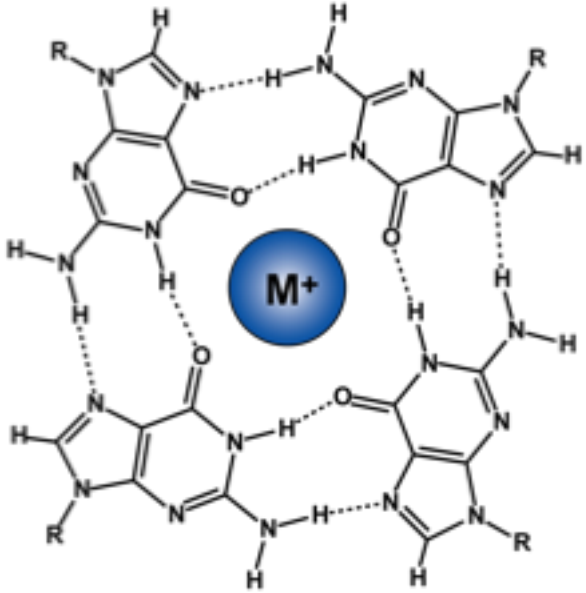
$^{205}Tl^+$ is a spin ½ nucleus with a large gyromagnetic ratio

$^{1}H > {}^{19}F > {}^{205}Tl > {}^{31}P$

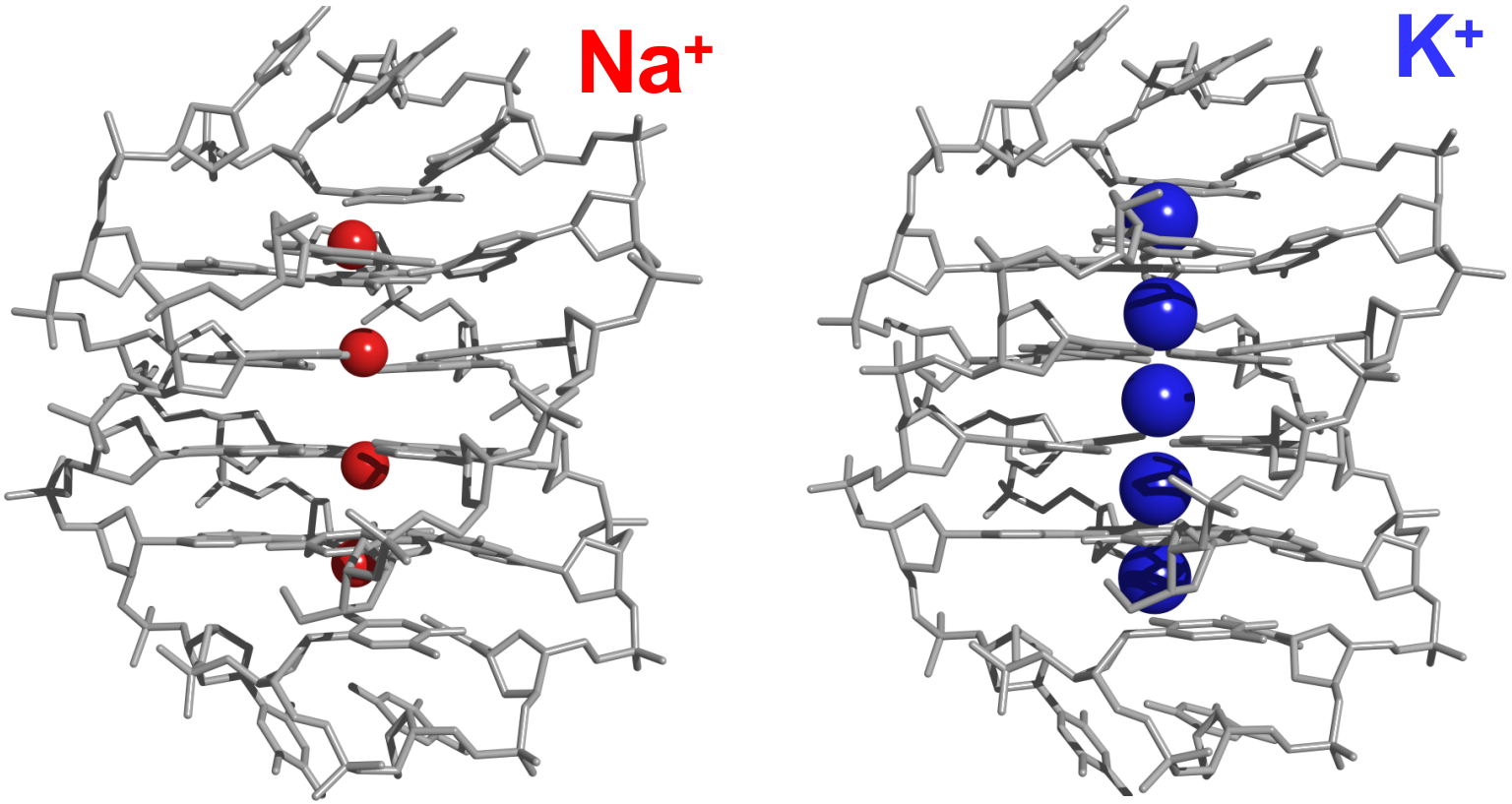
A model system for development of ^{205}Tl-NMR



- The sequence $G_4T_4G_4$ is from the telomeres of the ciliate *Oxytricha nova*
- It forms a homodimeric G-quadruplex, $d(G_4T_4G_4)_2$, *in vitro*
- G-quadruplex contains four G-quartets, each composed of four guanine bases
- Potential target for cancer therapies
- Lipophilic G-quadruplexes have been used as model systems for ion channels
- Exceptionally stable and structures have been solved by NMR and X-ray crystallography



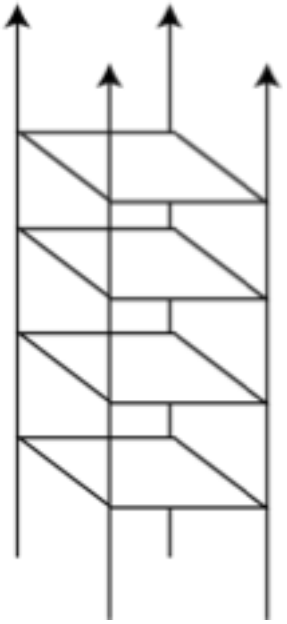
Na^+-, K^+-, and NH_4^+-forms of $d(G_4T_4G_4)_2$



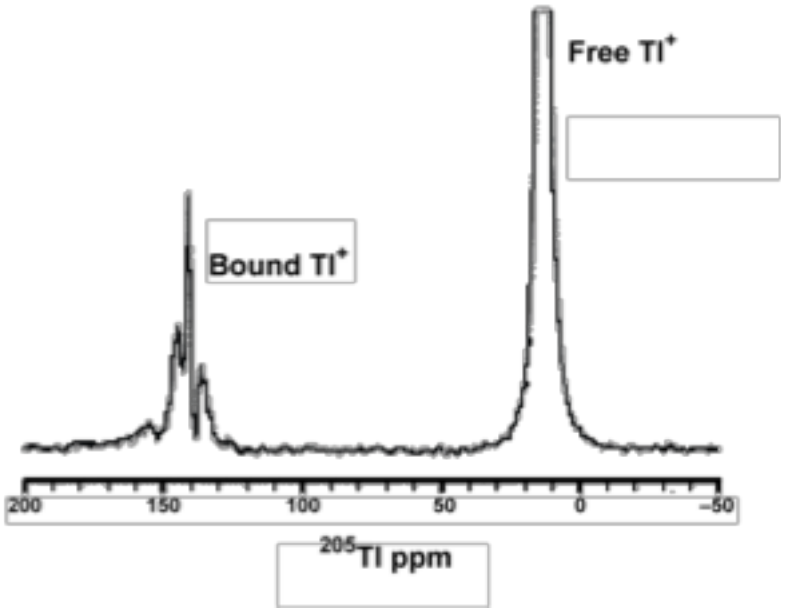
- $d(G_4T_4G_4)_2$ has been shown to bind Na^+, K^+, and NH_4^+
- Binds 3–5 monovalent cations per G-quadruplex
- Position of metal binding varies by metal type

Horvath, M.P.; Schultz, S.C. *J. Mol. Biol.* **2001**, *310*, 367-77.
Haider, S.; et. al. *J. Mol. Biol.* **2002,** *320*, 189-200.
Schultze, P.; et. al. *Nucleic Acids Res.* **1999,** *27*, 3018-28.

Previous ^{205}Tl NMR studies in nucleic acids



$d(T_2G_4T_2)_4$



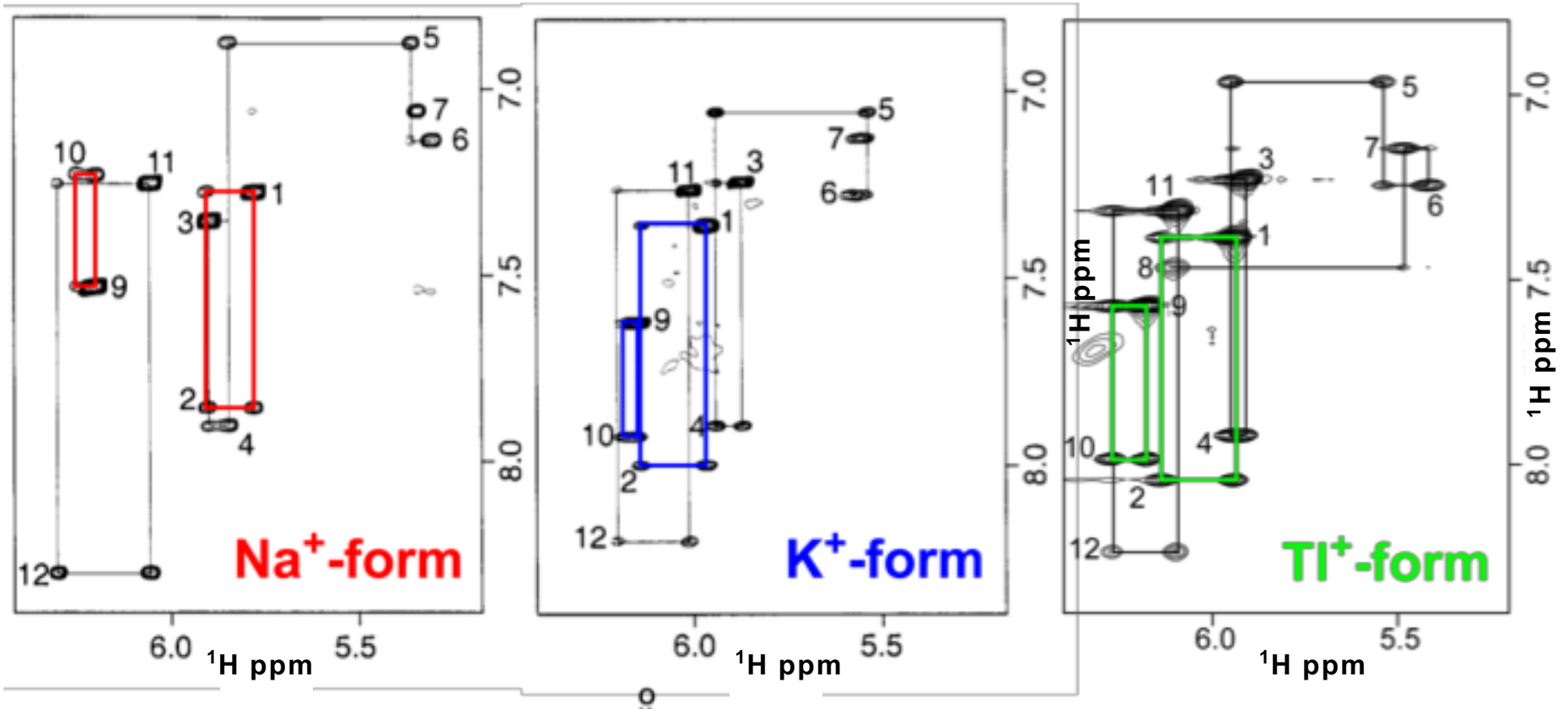
- Demonstrated that Tl^+ supports formation of the four stranded G-quadruplex, $d(T_2G_4T_2)_4$
- No specific assignment of monovalent binding sites was made
- First ^{205}Tl NMR study in nucleic acids

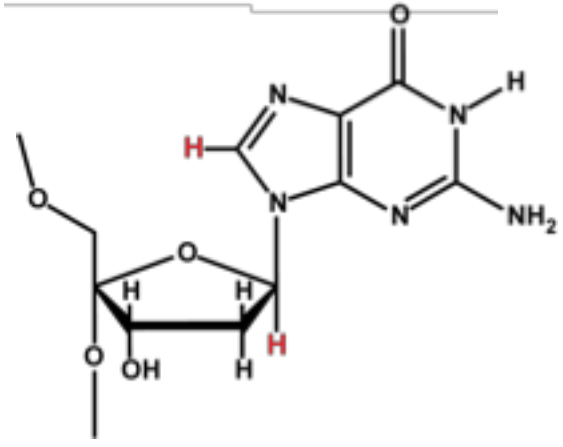
Basu, S.; et. al., *J. Am. Chem. Soc.* **2000,** *122*, 3240-1.

Solution structure of Tl^+-form of $d(G_4T_4G_4)_2$

- NMR experiments
  - ^1H–^1H NOESY (distance constraints)
  - ^1H–^1H DQF-COSY (dihedral angles)
  - ^1H–^1H TOCSY
  - ^{31}P–^1H COSY
- Structure calculation
  - Hydrogen bond, symmetry, and planarity constraints
  - *Ab initio* simulated annealing performed in CNS

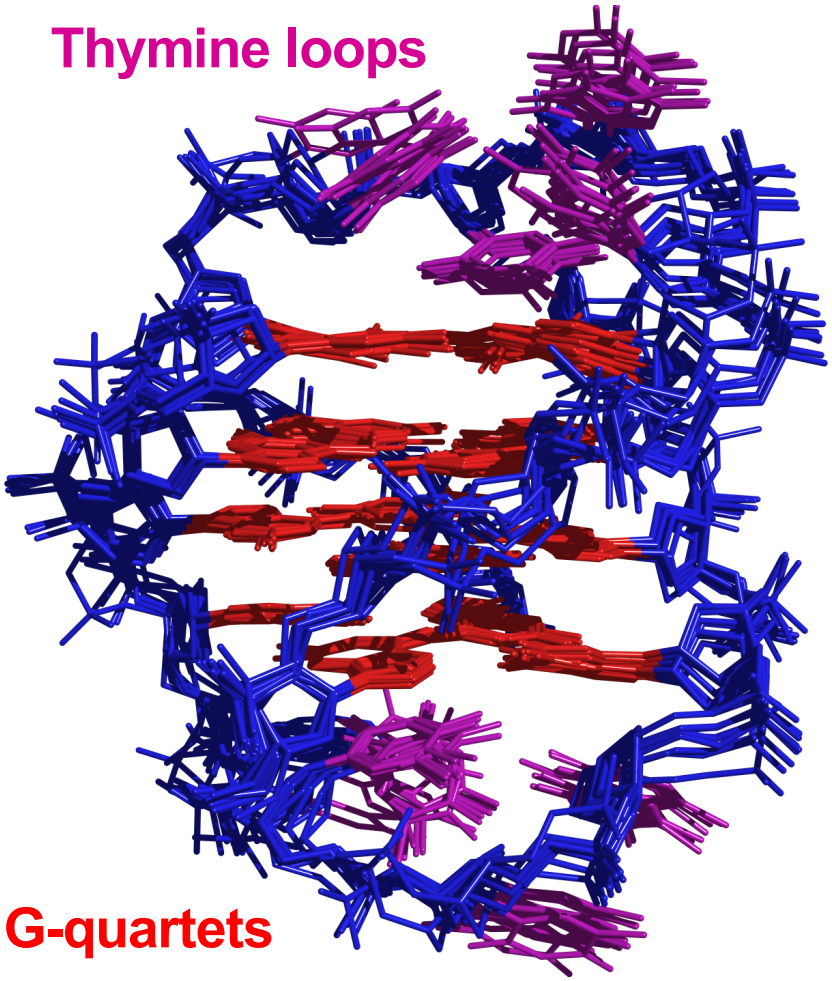
^{1}H chemical shift similarities



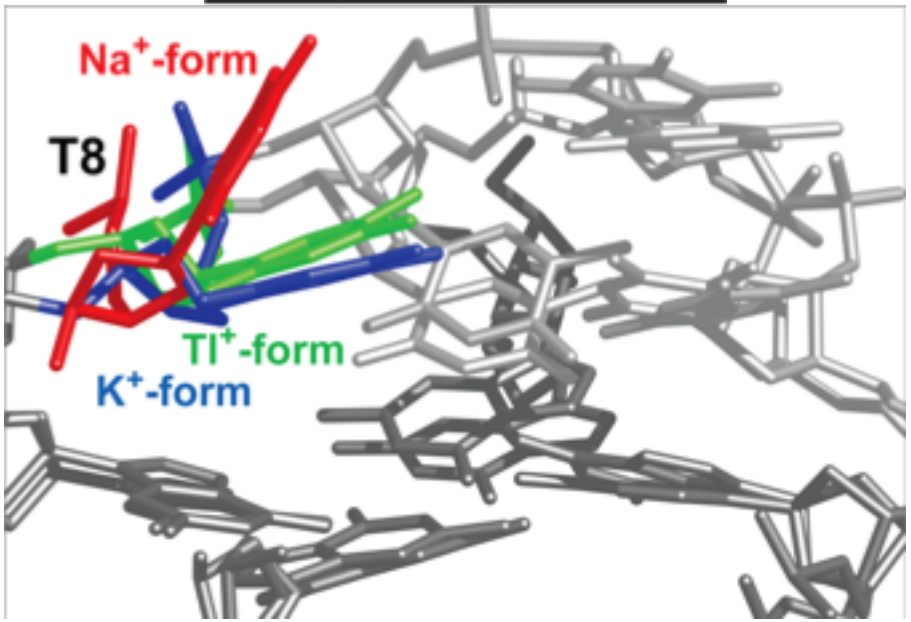


Schultze, P.; et. al., *Nucleic Acids Res* **1999,** 27, (15), 3018-28.

The Tl^+-form of $d(G_4T_4G_4)_2$ is K^+-like

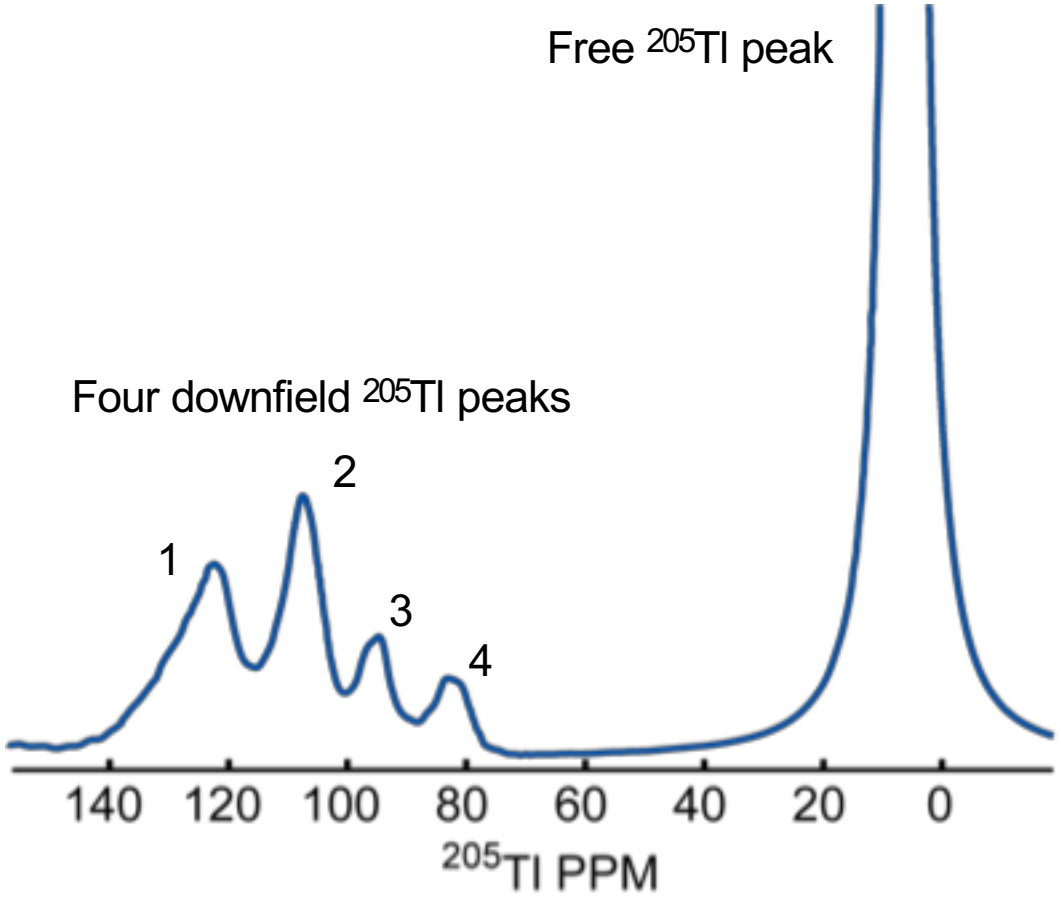


Ensemble RMSD (Å)	
All atoms (Top 10)	0.76 ± 0.16 Å
K^+-NMR Structure	1.17 ± 0.13 Å
Average violations/structure	
NOE (> 0.5 Å)	0 ± 0
Dihedrals (> 5°)	0 ± 0
NOE Restraints	
Total	395
Intraresidue	241
Interresidue	154
Long-range	38
Exchangeable	56



Smith, F.W.; Feigon, J. *Biochemistry*. **1993,** *32*, 8682-92.
Schultze, P.; et. al. *Nucleic Acids Res.* **1999,** *27*, 3018-28.

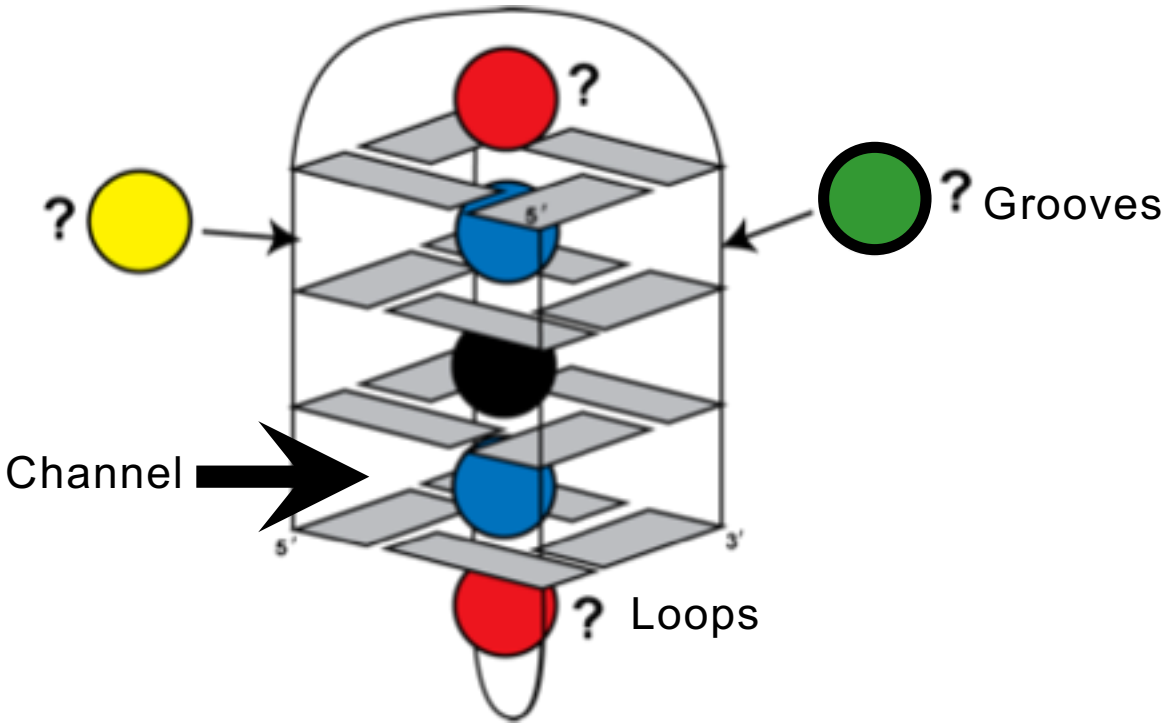
Five ^{205}Tl peaks observed by ^{205}Tl-NMR



2.5 mM $d(G_4T_4G_4)_2$, 50 mM $TlNO_3$, 10% D_2O, 298 K

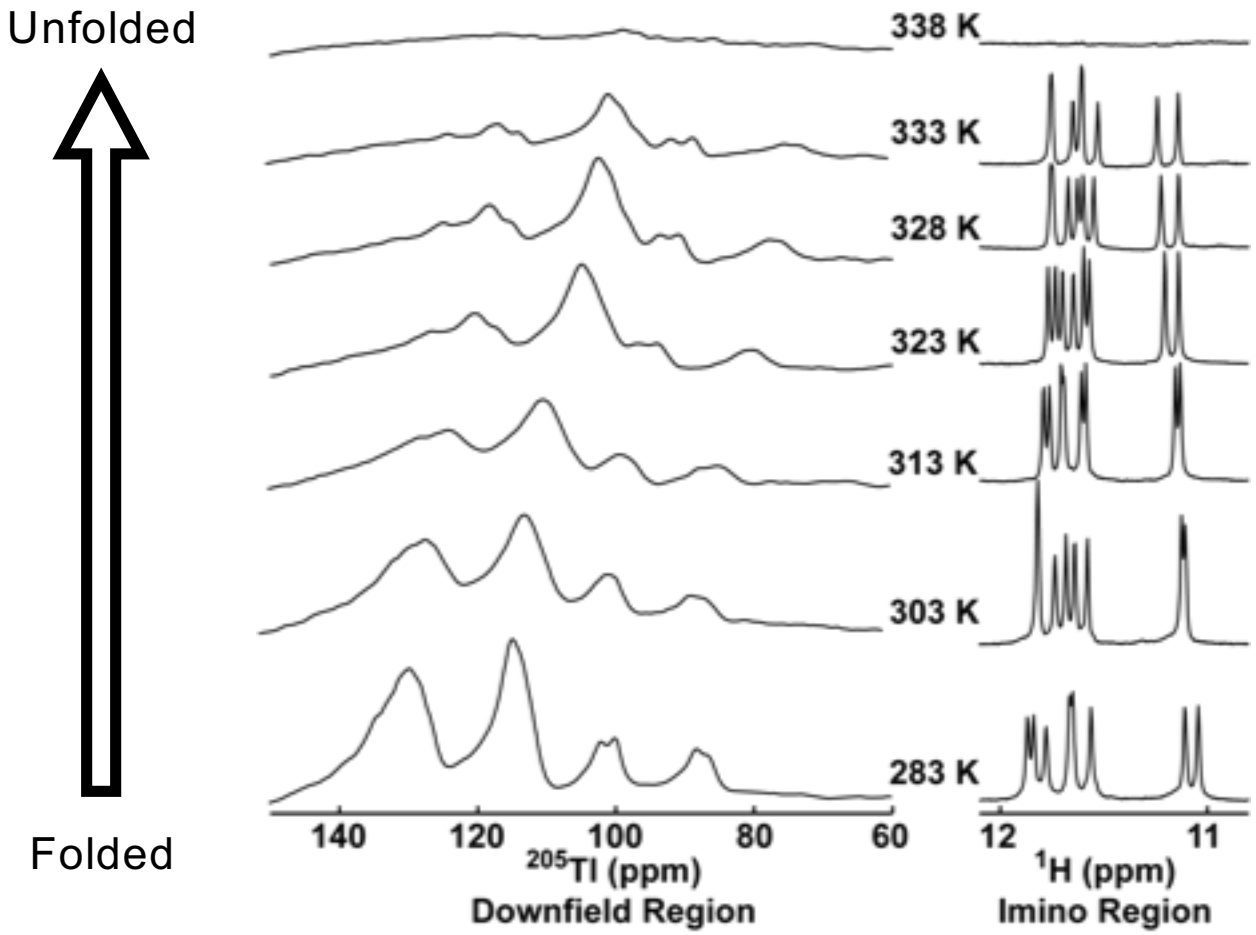
Where are each of the downfield ^{205}Tl peaks bound?

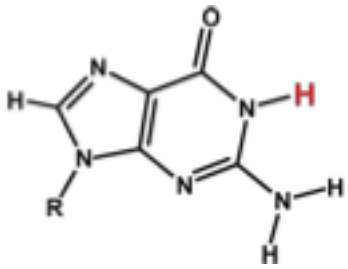
Possible Tl^+ binding sites in $d(G_4T_4G_4)_2$



- Possible monovalent binding sites include G-quadruplex channel, grooves, and thymine loops
- Groove binding sites expected to have shorter residence times and be less cation specific
- Symmetry for outer channel and loop binding sites

G-quadruplex stabilization by Tl^+



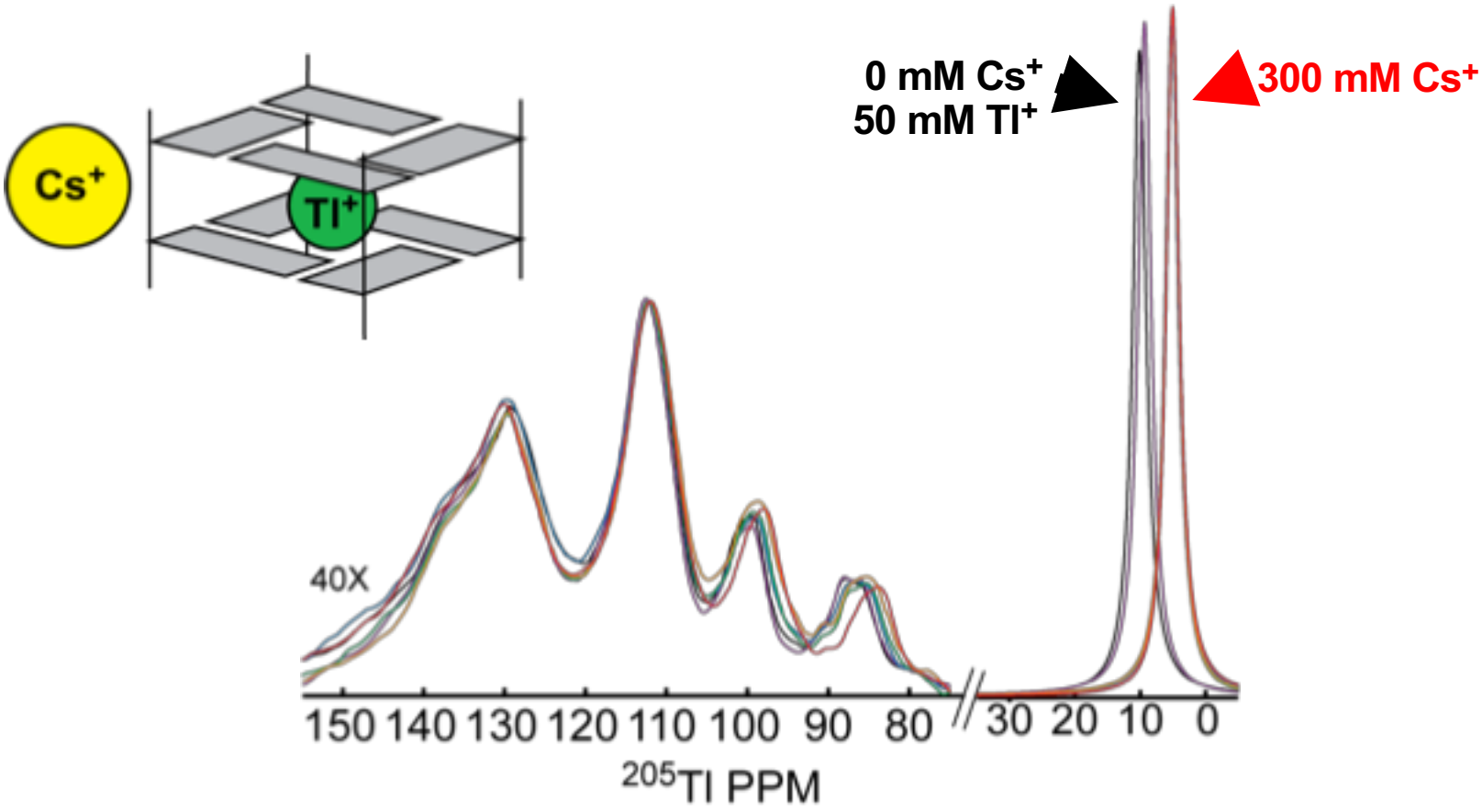


Imino Proton

- All downfield ^{205}Tl peaks have similar temperature sensitivity
- Tl^+ stabilizes $d(G_4T_4G_4)_2$ at least as well as Na^+, K^+, and NH_4^+

Dingley, A.J.; et. al. *J. Am. Chem. Soc*. **2005**, *127*, 14466-72.
Hud, N.V.; et. al. *J. Mol. Biol*. **1999**, *285*, 233-43.
Deng,H.; Braunlin, W.H. *J. Mol. Biol.* **1996**, *255*, 476-83.

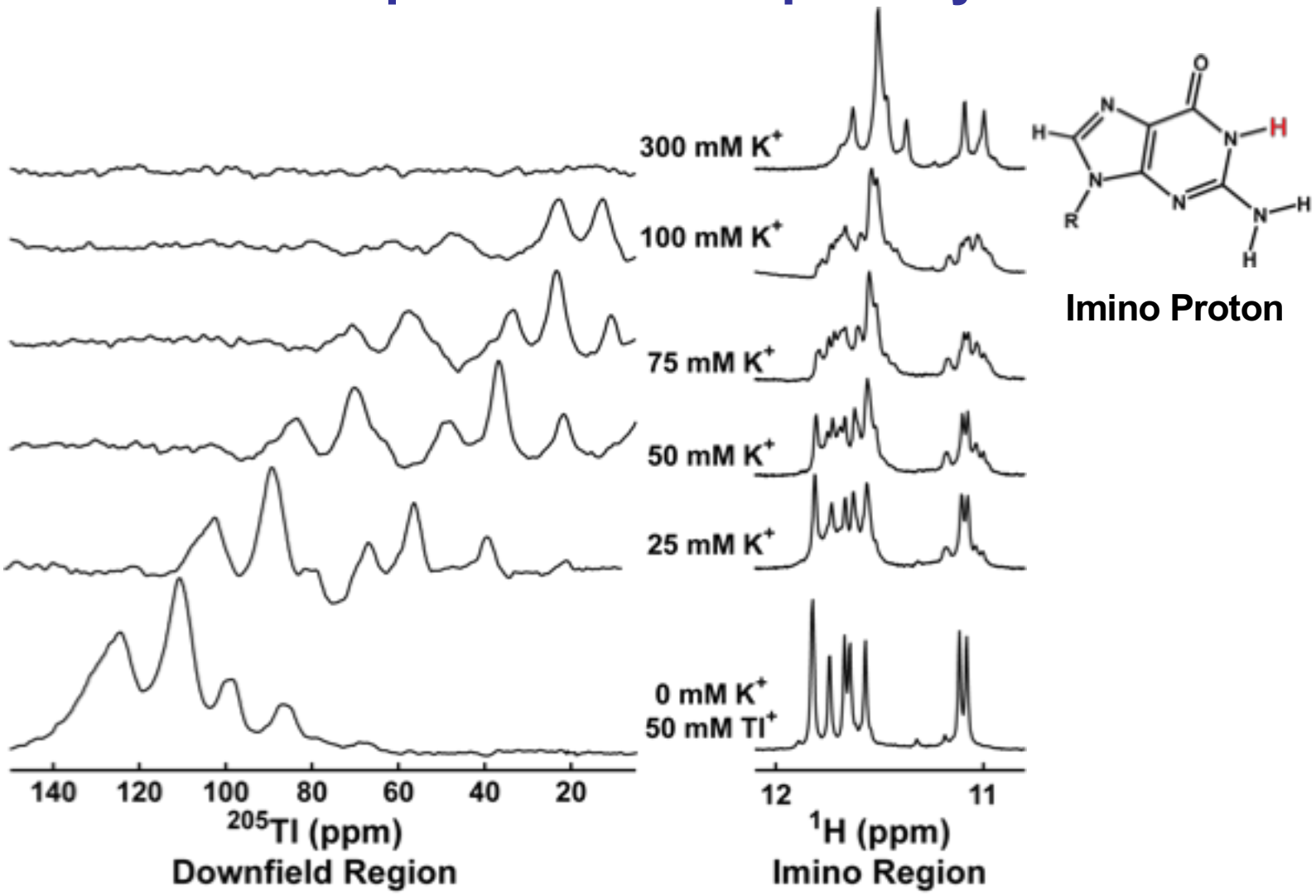
Specificity of downfield ^{205}Tl peaks



- Cs^+ is too large to bind inside G-quadruplex
  Tl^+ 1.40 Å *vs.* Cs^+ 1.69 Å
- Competes well for groove-associated sites
- No change in downfield peaks at 6X excess Cs^+

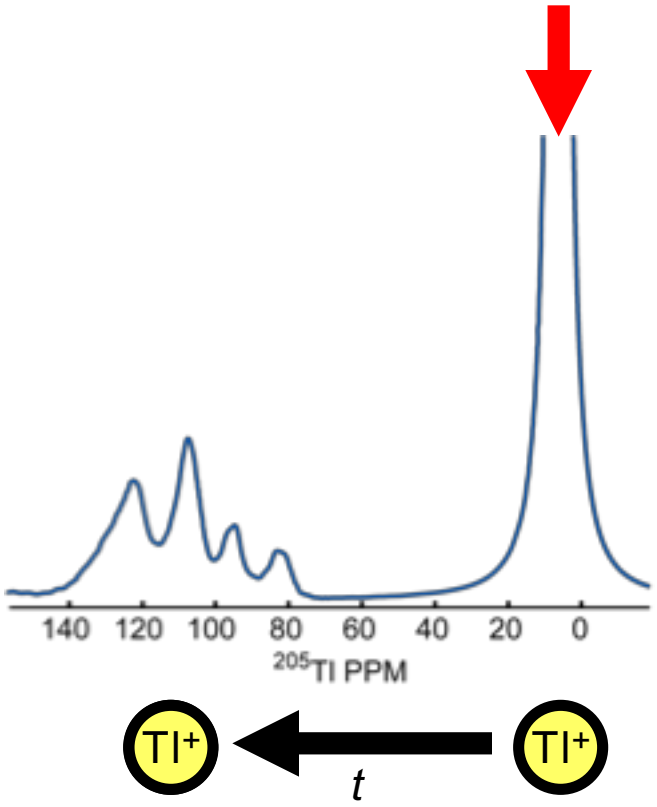
Wong, A.; Wu, G. *J. Am. Chem. Soc.* **2003,** *125*, 13895-905.

Can all $^{205}Tl^{+}$ peaks be occupied by K^{+}?



- None of the downfield ^{205}Tl peaks are from adventitious Tl^{+} binding

Measurement of bound $^{205}Tl^{+}$ lifetimes



$$M_z^A = M_0^A\left[\frac{\tau_{1A}}{\tau_A}e^{(-t/\tau_{1A})} + \frac{\tau_{1A}}{T_{1A}}\right] \qquad (1)$$

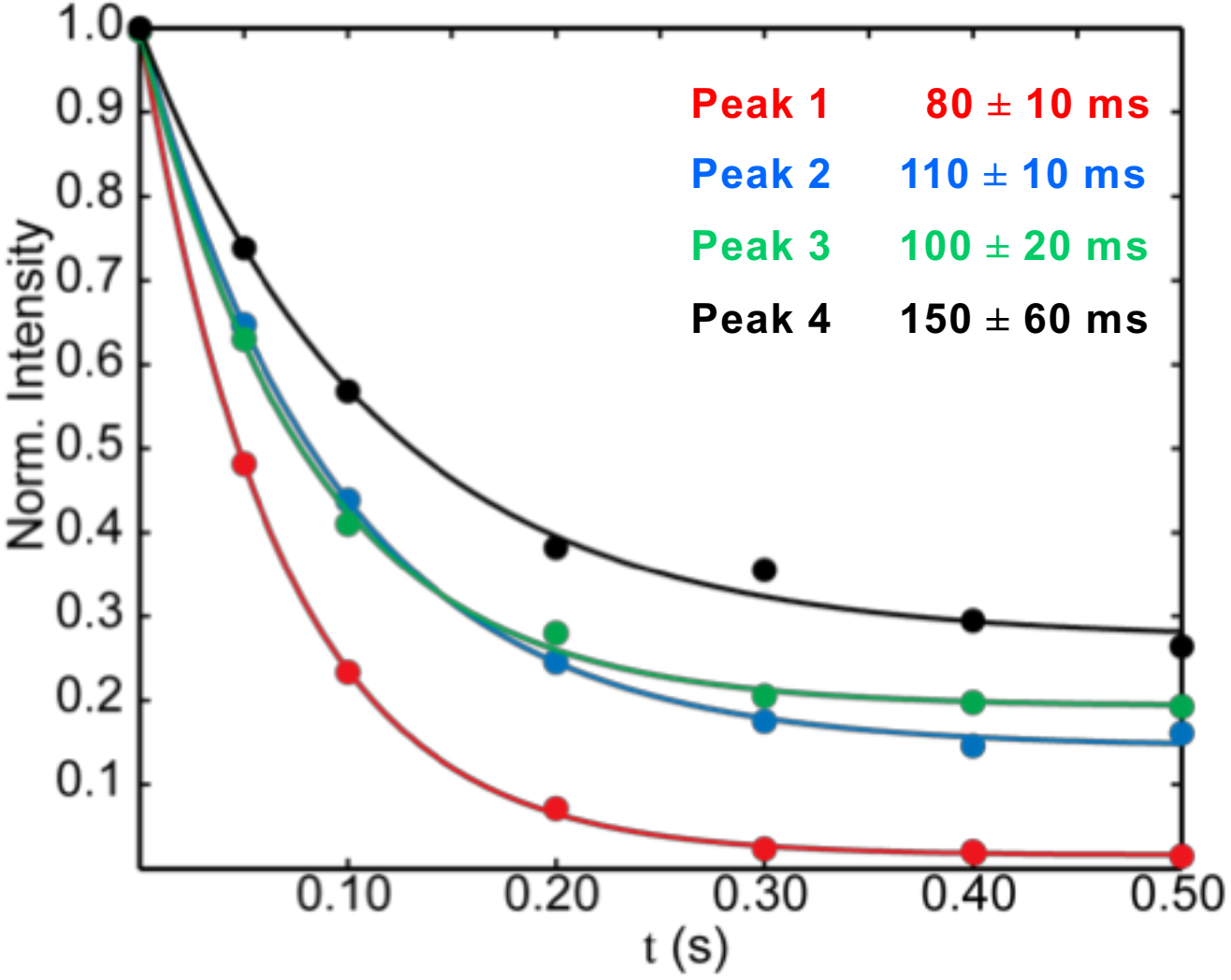
$$\frac{1}{\tau_{1A}} = \frac{1}{\tau_A} + \frac{1}{T_{1A}} \qquad (2)$$

$$\frac{M_z^A}{M_0^A} = \left[(1-\frac{\tau_{1A}}{T_{1A}})e^{(-t/\tau_{1A})} + \frac{\tau_{1A}}{T_{1A}}\right] \qquad (3)$$

- Measures exchange of $^{205}Tl^{+}$ from free to "bound" sites
- Can determine lifetimes of $^{205}Tl^{+}$ in each of these sites
- Simplified two-site exchange model assumed

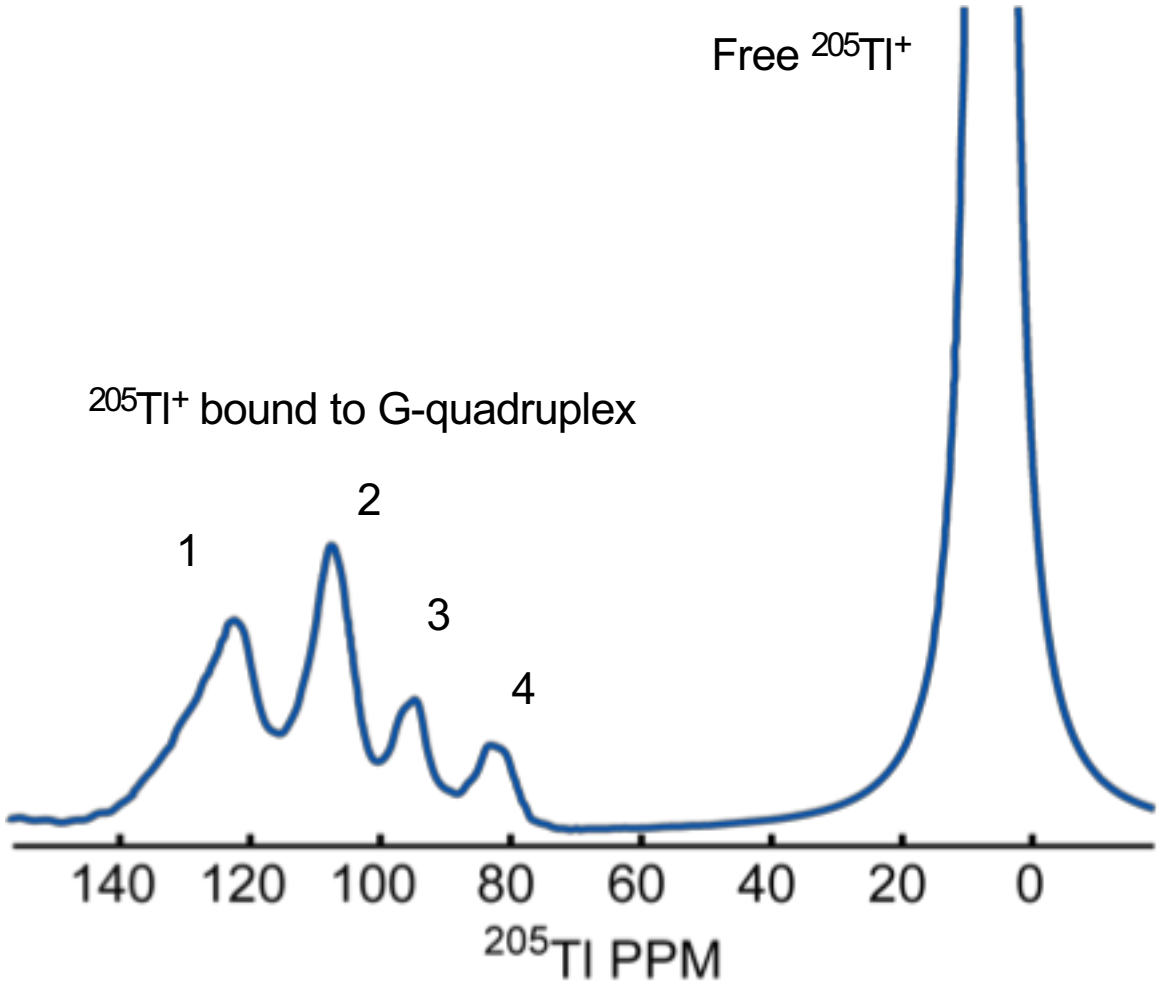
Forsén, S.; Hoffman, R.A. *J. Chem. Phys.* **1964,** *40*, 1189-96.
Forsén, S.; Hoffman, R.A. *J. Chem. Phys.* **1963,** *39*, 2892-901.

Bound lifetimes of downfield $^{205}Tl^{+}$ ions



$$\frac{M_z^A}{M_0^A} = [(1 - \frac{\tau_{1A}}{T_{1A}})e^{(-t/\tau_{1A})} + \frac{\tau_{1A}}{T_{1A}}]$$

Classification of downfield ^{205}Tl peaks

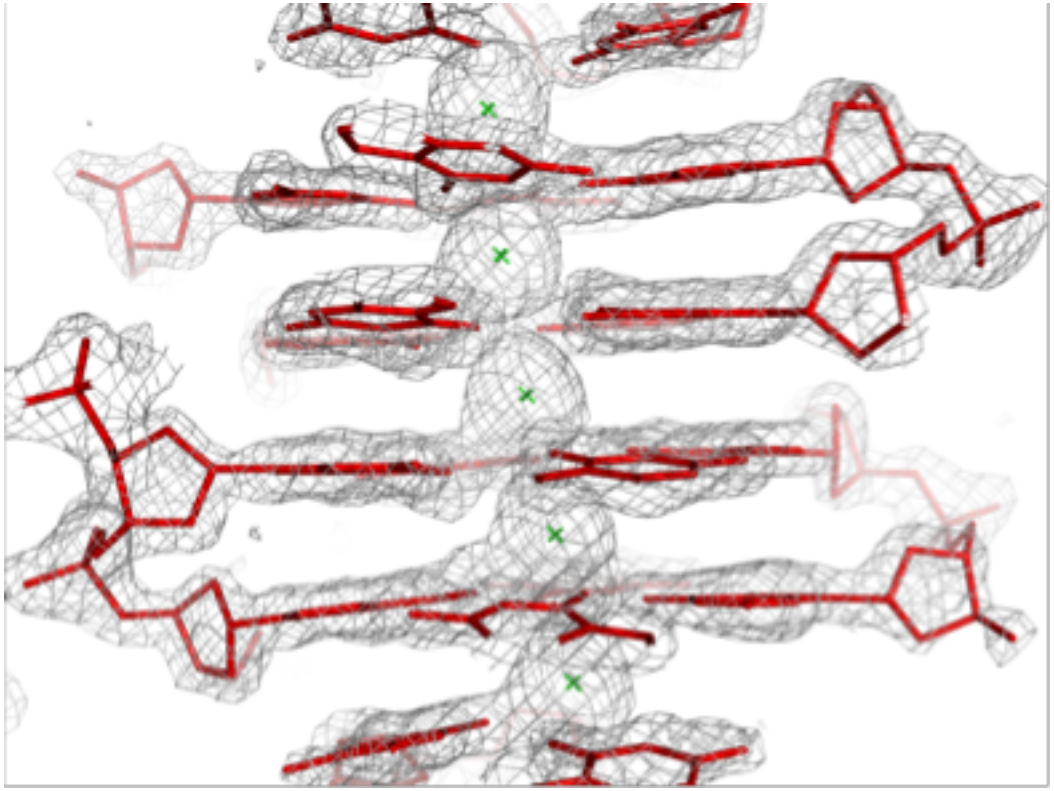


How many G-quadruplex Tl^{+} binding sites exist?

Crystallization of the Tl^+-form of $d(G_4T_4G_4)_2$

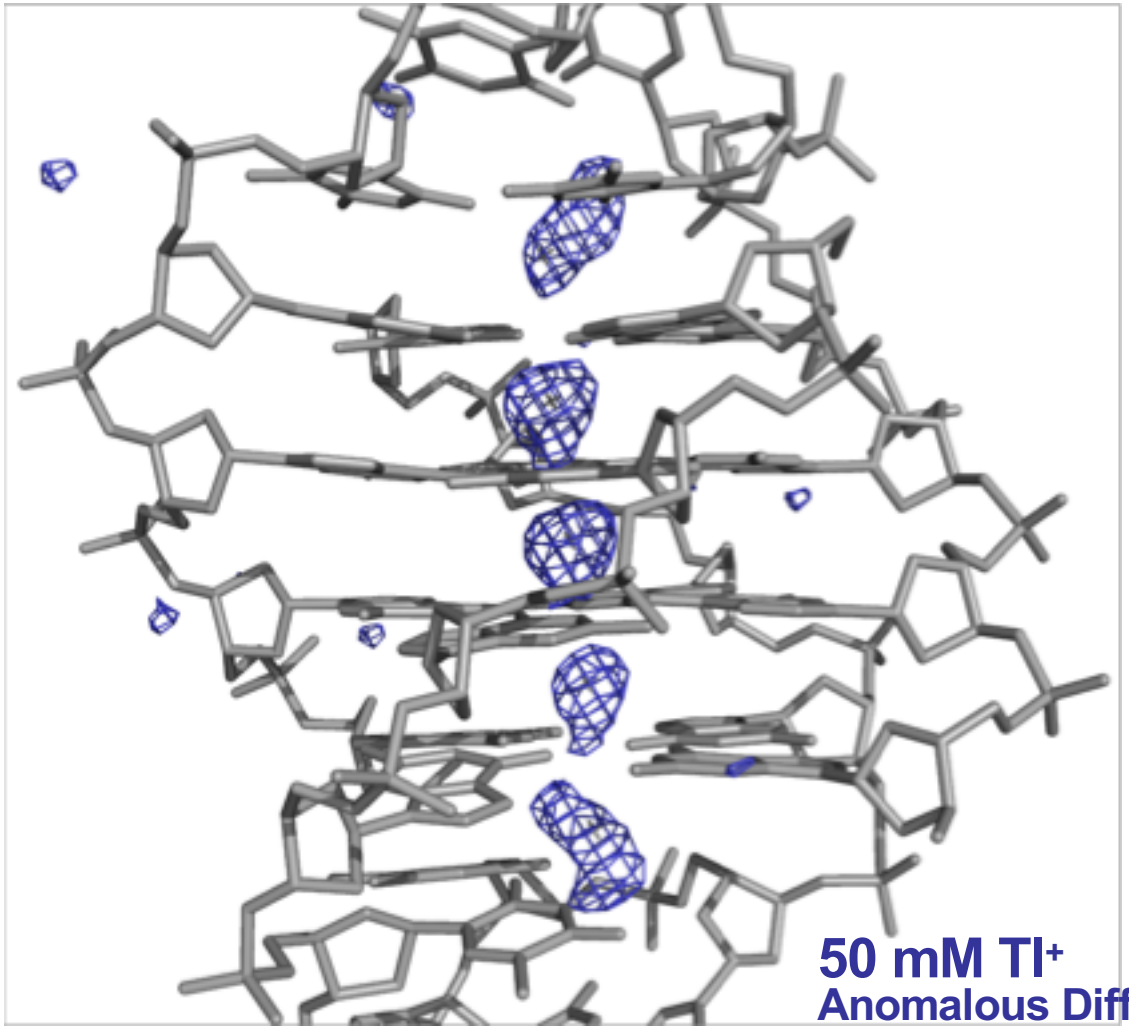
Crystallized in 85 mM K^+
Soaked in 50 mM Tl^+

Crystallographic Data	
Space group	$P2_12_12_1$
Cell dimensions (Å)	27.38, 48.21, 96.20
Wavelength (Å)	0.979
Resolution range (Å)	43.11–1.55
R-factor (%)	24.1
R_{free} (%)	25.8



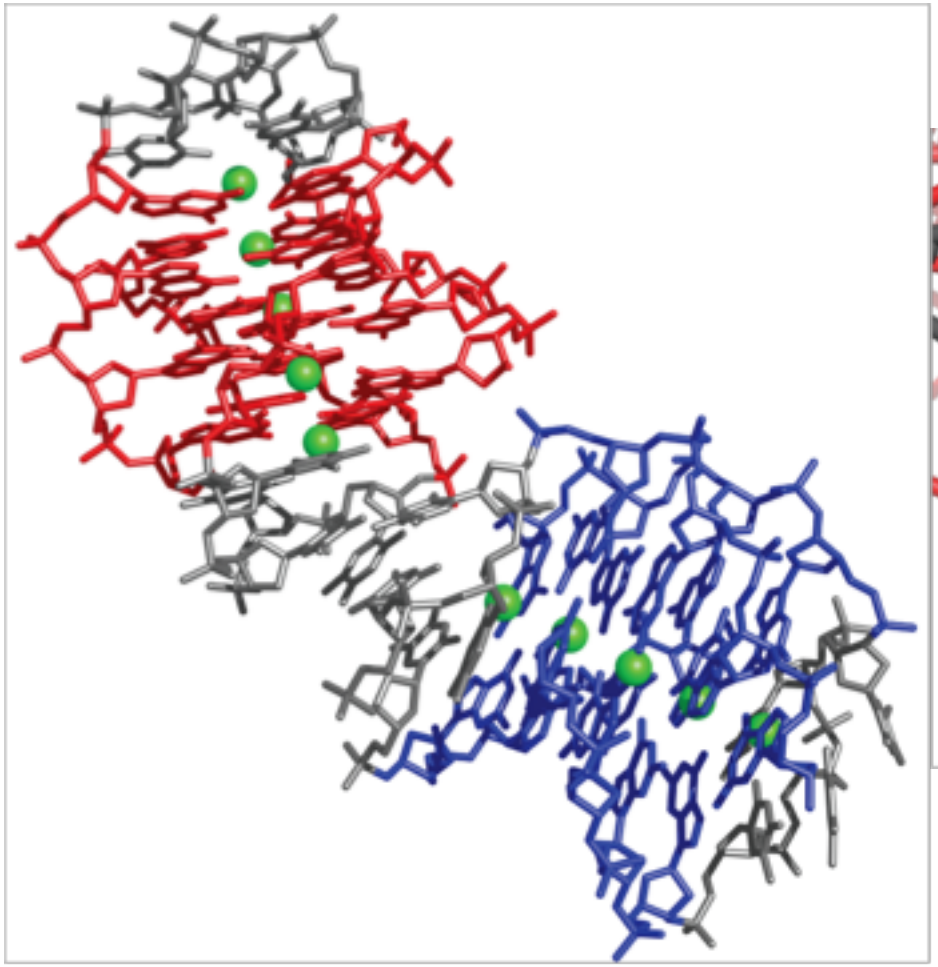
Haider, S.; et. al. *J. Mol. Biol.* **2002,** *320*, 189-200.

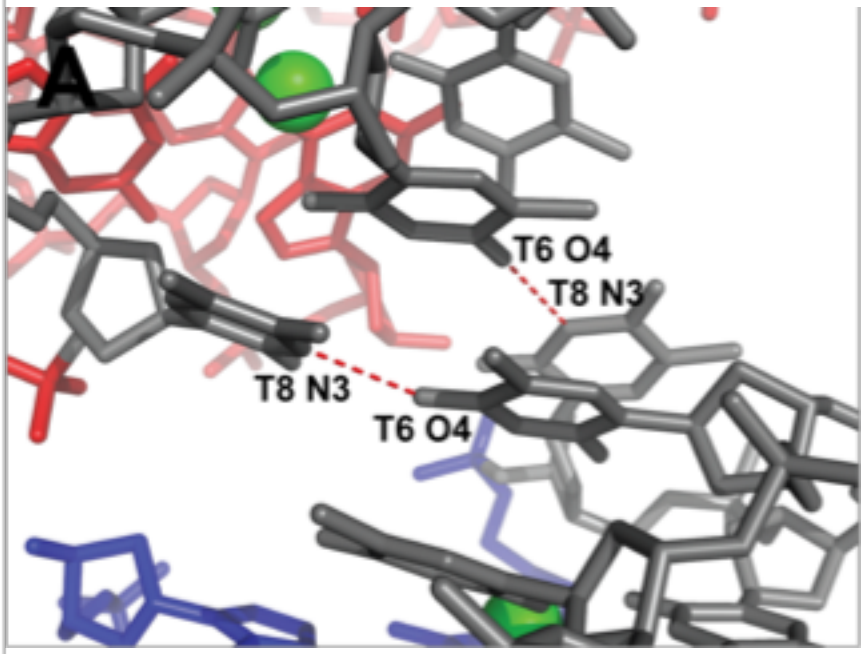
Coordination of Tl^+ ions by $d(G_4T_4G_4)_2$



- Only five ordered Tl^+ binding sites exist—three within G-quadruplex channel and two in the loops
- All metal occupancies are 100%

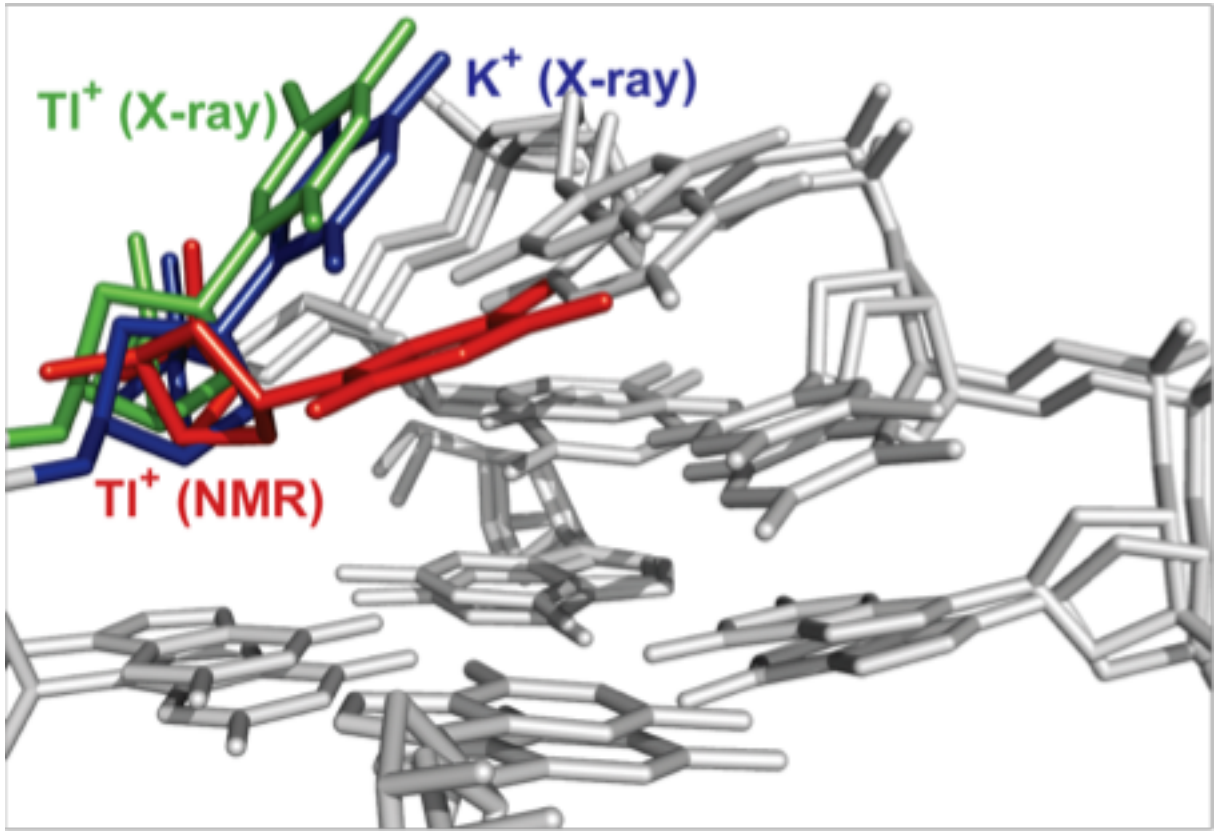
Thymine loops mediate crystal packing





- Asymmetric unit contains two G-quadruplexes
- Thymine loops (T6 and T8) facilitate packing via a pair of intermolecular hydrogen bonds

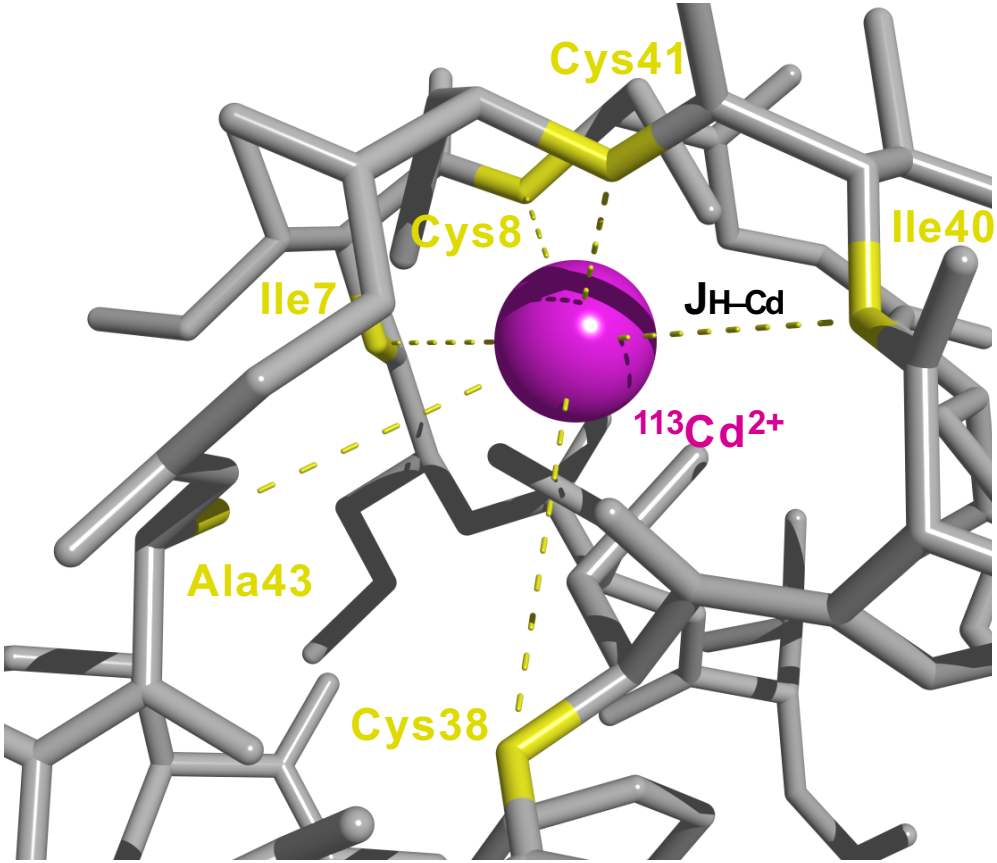
Evidence for conformational exchange in loops



- Thymine loops are in a different conformation in x-ray and solution structures
- T8 is extended in Na^+, K^+, and Tl^+ x-ray structures
- Thymine protons have faster transverse relaxation rate than those in G-quartet

Haider, S.; et. al. *J. Mol. Biol.* **2002,** *320*, 189-200.

$^{1}H–M^{2+}$ scalar couplings in proteins

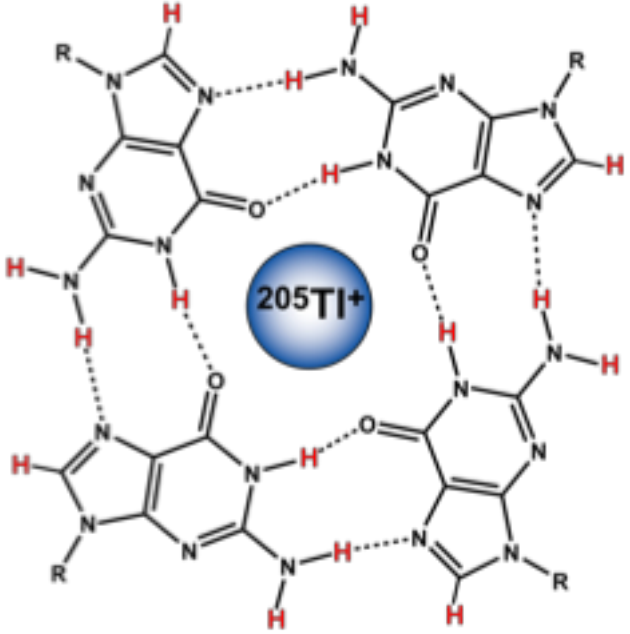


Rubredoxin from *Pyrococcus furiosus*

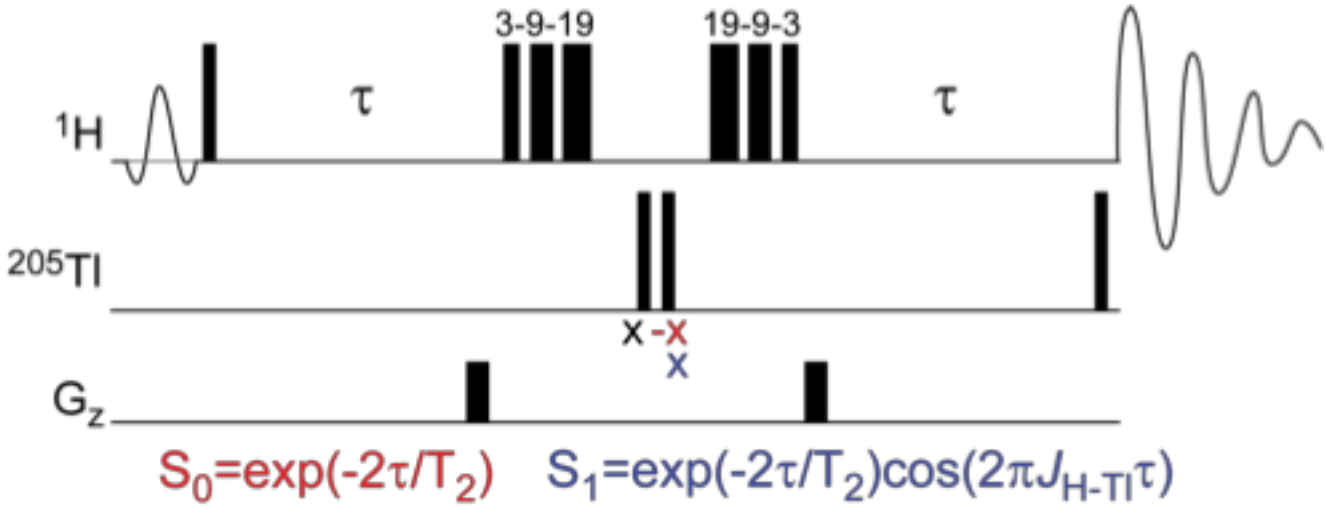
- Spin ½ divalent surrogates ($^{113}Cd^{2+}$ and $^{199}Hg^{2+}$) used to study rubredoxin, metallothionein, superoxide dismutase, and the transcription factors GAL4 and LAC9
- Spin-echo difference experiment used to detect small, metal-protein scalar couplings

Blake, P.R.; et. al., *J. Biomol. NMR.* **1992,** *2*, 527-33.

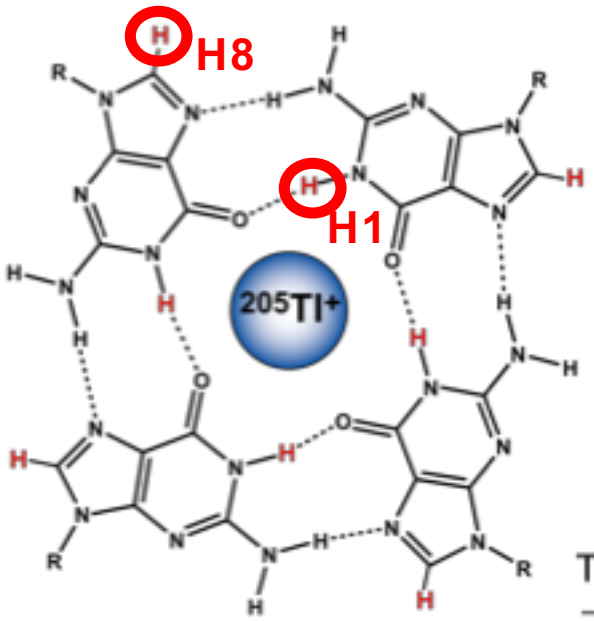
Where are the $^{205}Tl^{+}$ ions bound?

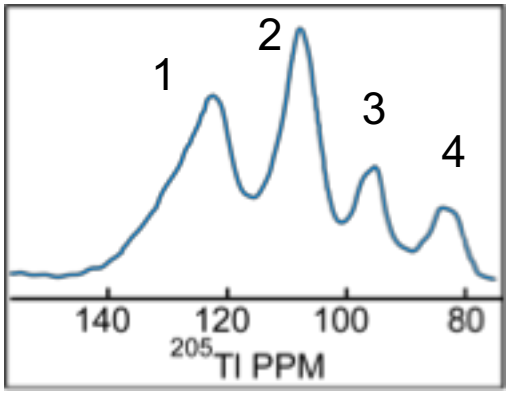


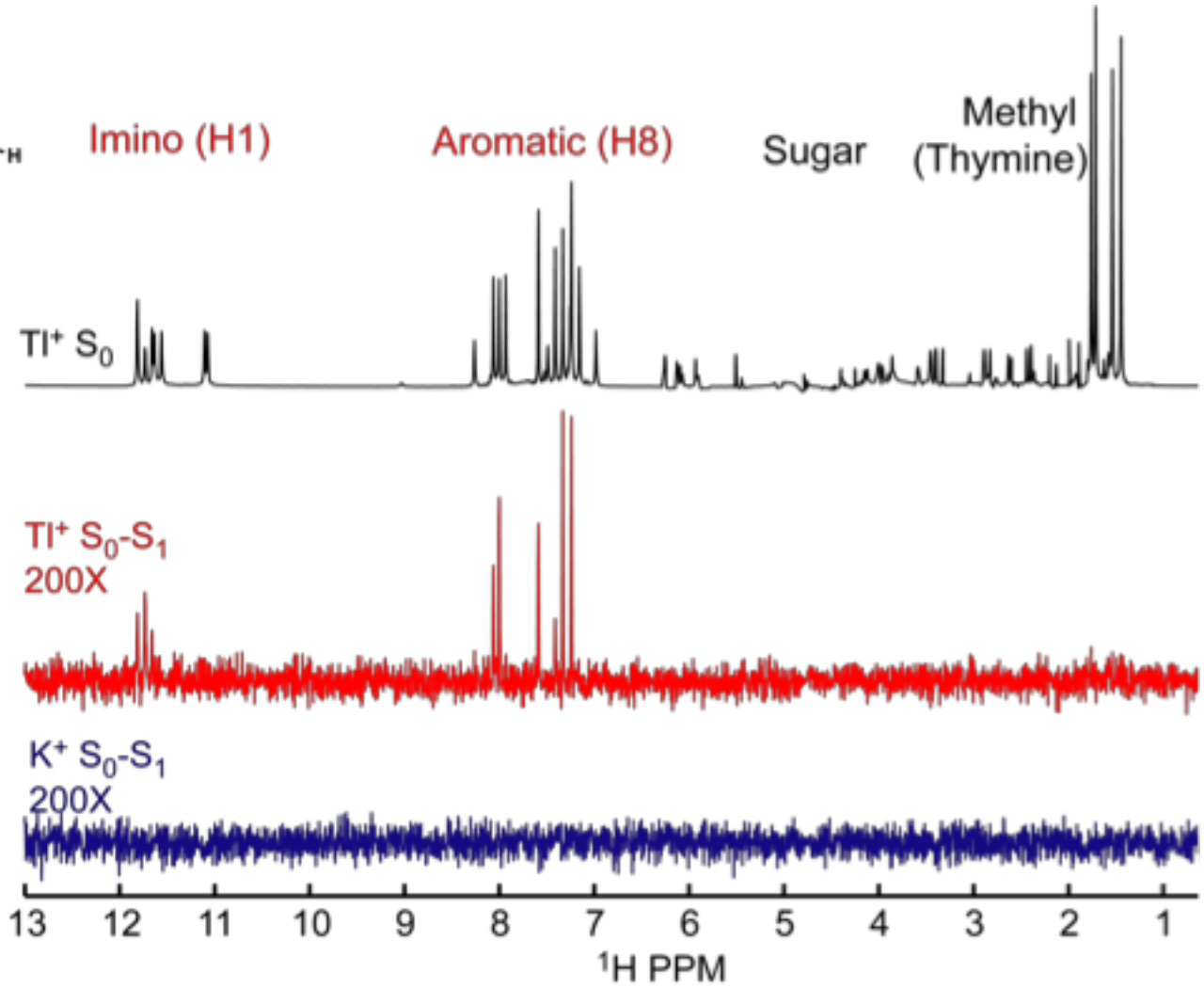
^{1}H–^{205}Tl Spin-Echo Difference Experiment



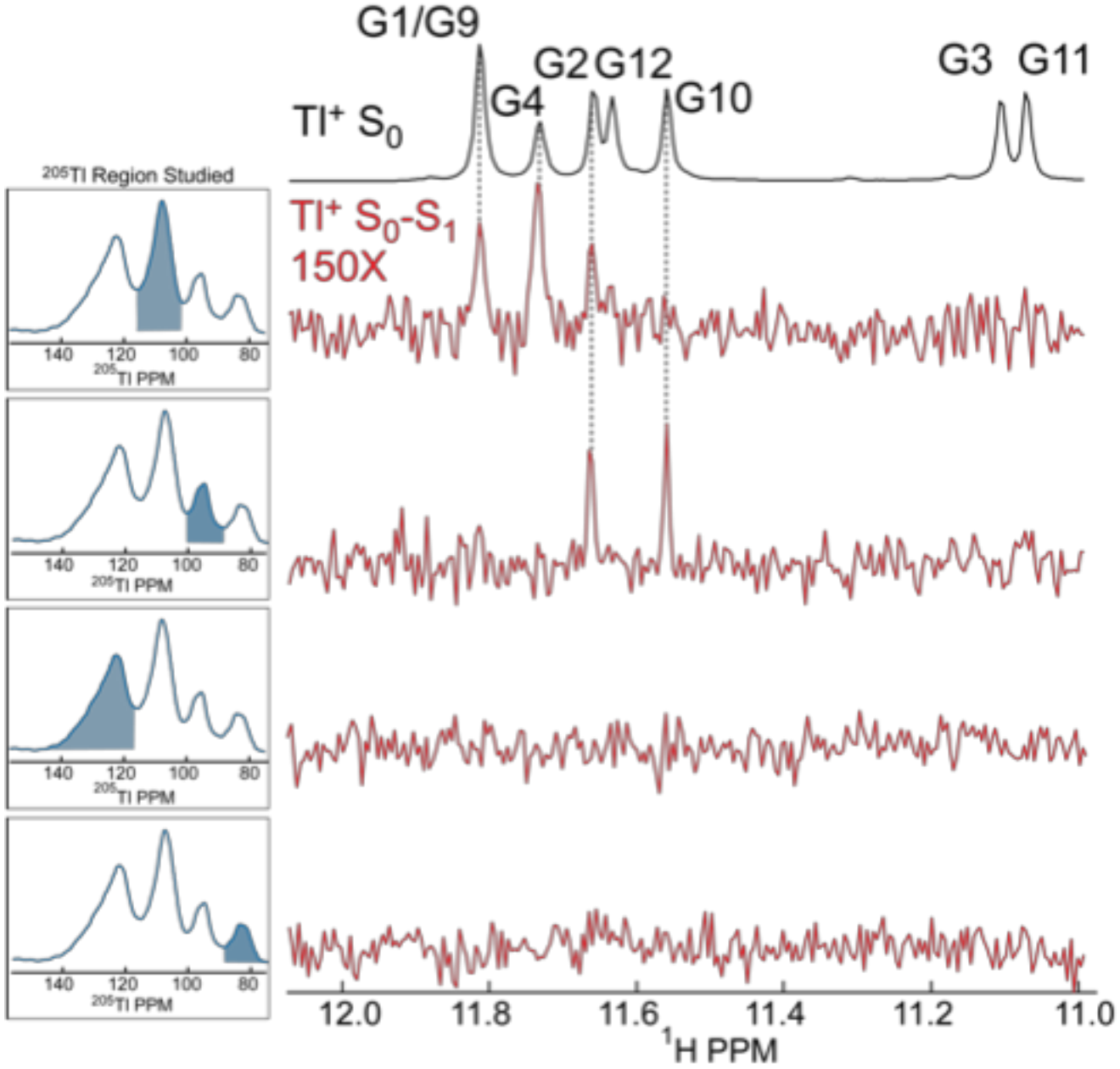
$^{205}Tl^{+}$ is scalar coupled to G H1/H8 protons

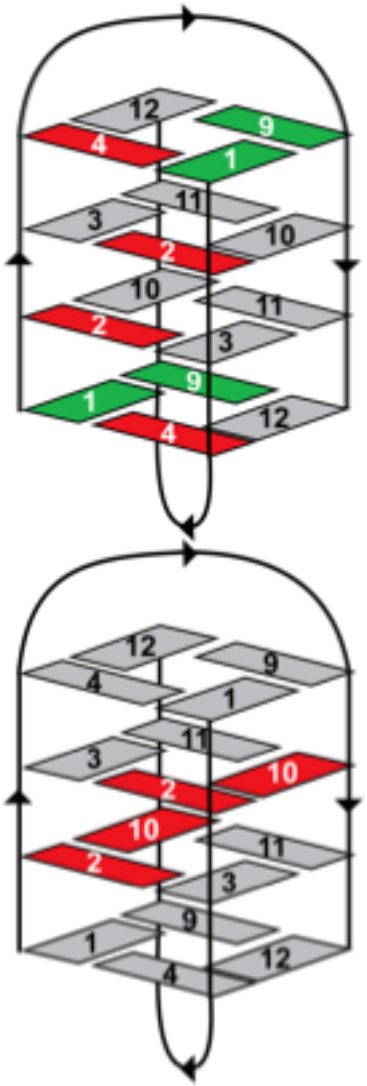




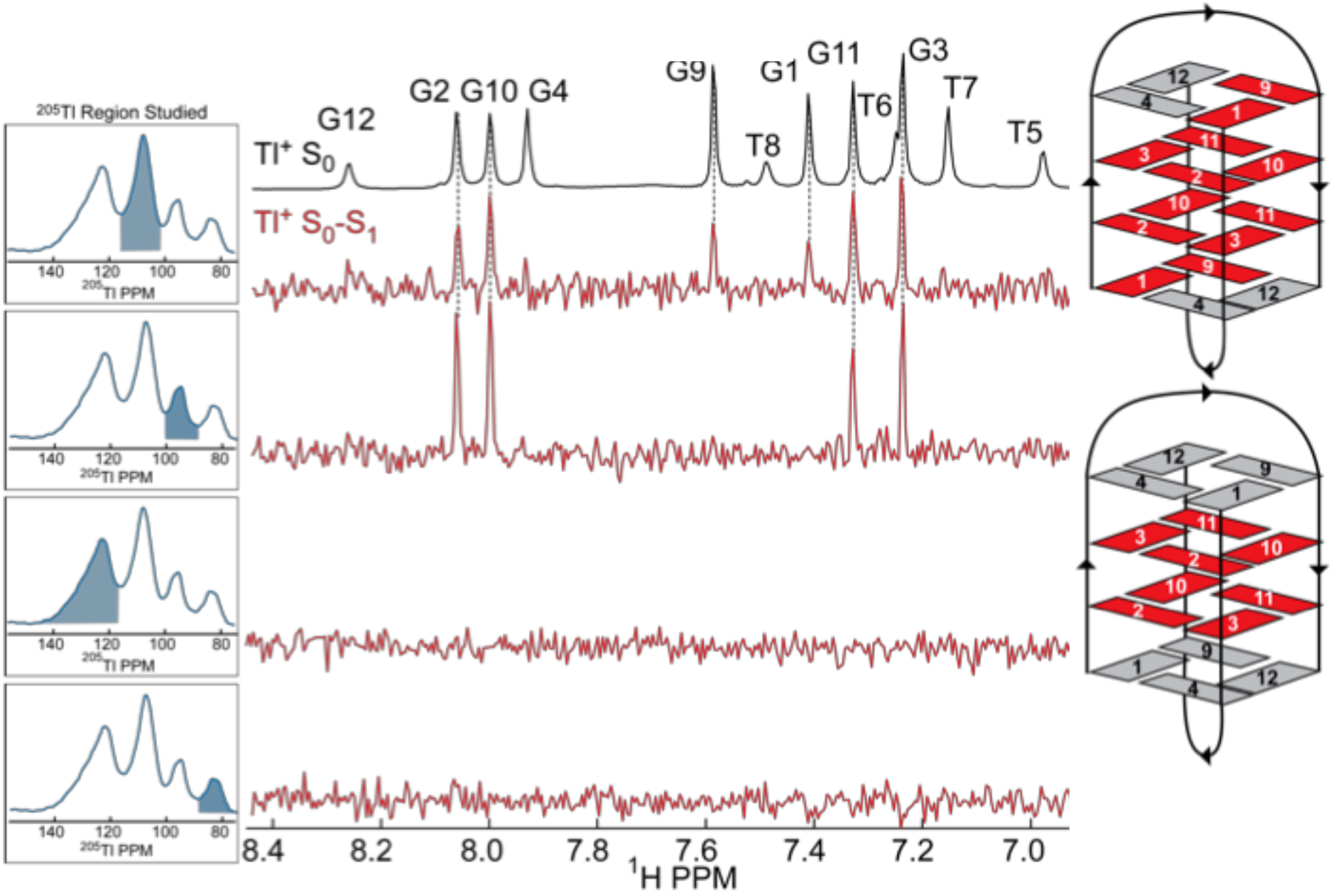


Imino (H1) scalar couplings to bound $^{205}Tl^{+}$ ions





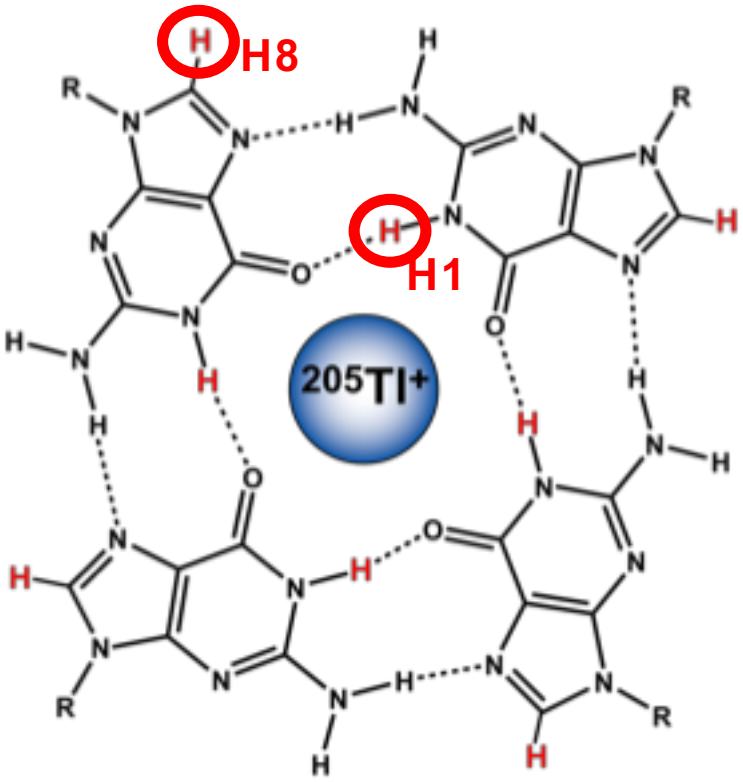
Aromatic (H8) scalar couplings to bound $^{205}Tl^{+}$ ions



Quantitation of J_{H-Tl}

	$J_{H\text{-}Tl}$ (Hz)	Peak 2	Peak 3
Imino (H1)	G1/9	0.46 ± 0.04	–
	G2	0.54 ± 0.04	0.51 ± 0.06
	G4	0.95 ± 0.06	–
	G10	–	0.44 ± 0.03
Aromatic (H8)	G1	0.34 ± 0.06	–
	G2	0.44 ± 0.05	0.52 ± 0.03
	G3	0.49 ± 0.02	0.65 ± 0.01
	G9	0.34 ± 0.04	–
	G10	0.49 ± 0.04	0.56 ± 0.02
	G11	0.47 ± 0.03	0.40 ± 0.02

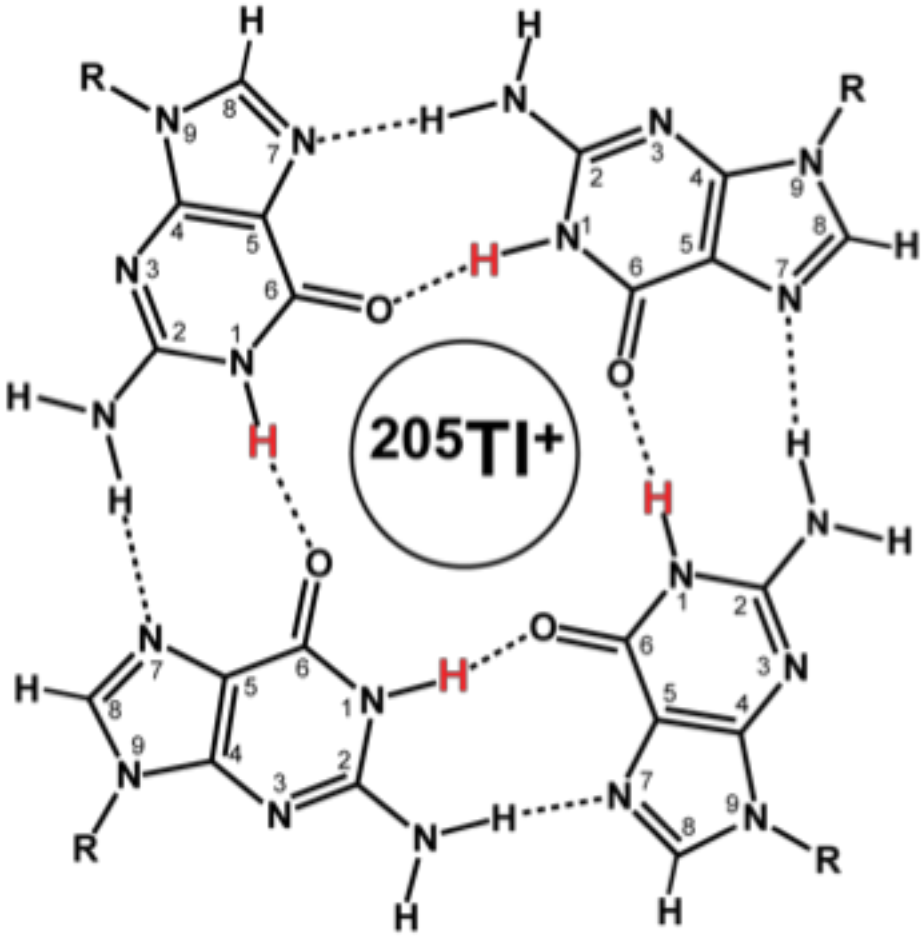
$$\frac{S_0 - S_1}{S_0} = 1 - \cos(2\pi J_{H\text{-}Tl}\tau)$$



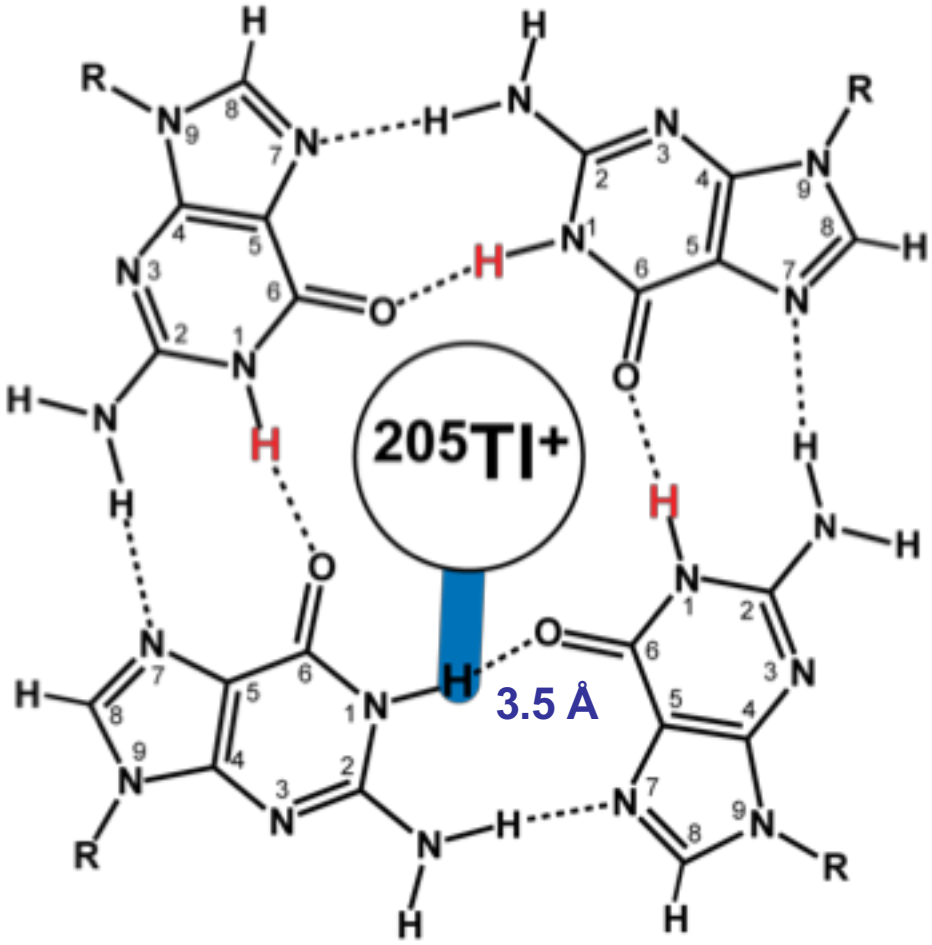
- $^{1}H–M^{2+}$ couplings as small as 0.29 ± 0.03 Hz reported for $^{113}Cd^{2+}$-substituted rubredoxin
- Scalar coupling magnitude could be used to determine ligand orientation for vicinal couplings

Blake, P.R.; et. al. *J. Biomol. NMR.* **1992,** *2*, 527-33.

Possible mechanisms for Imino ^{1}H–^{205}Tl couplings



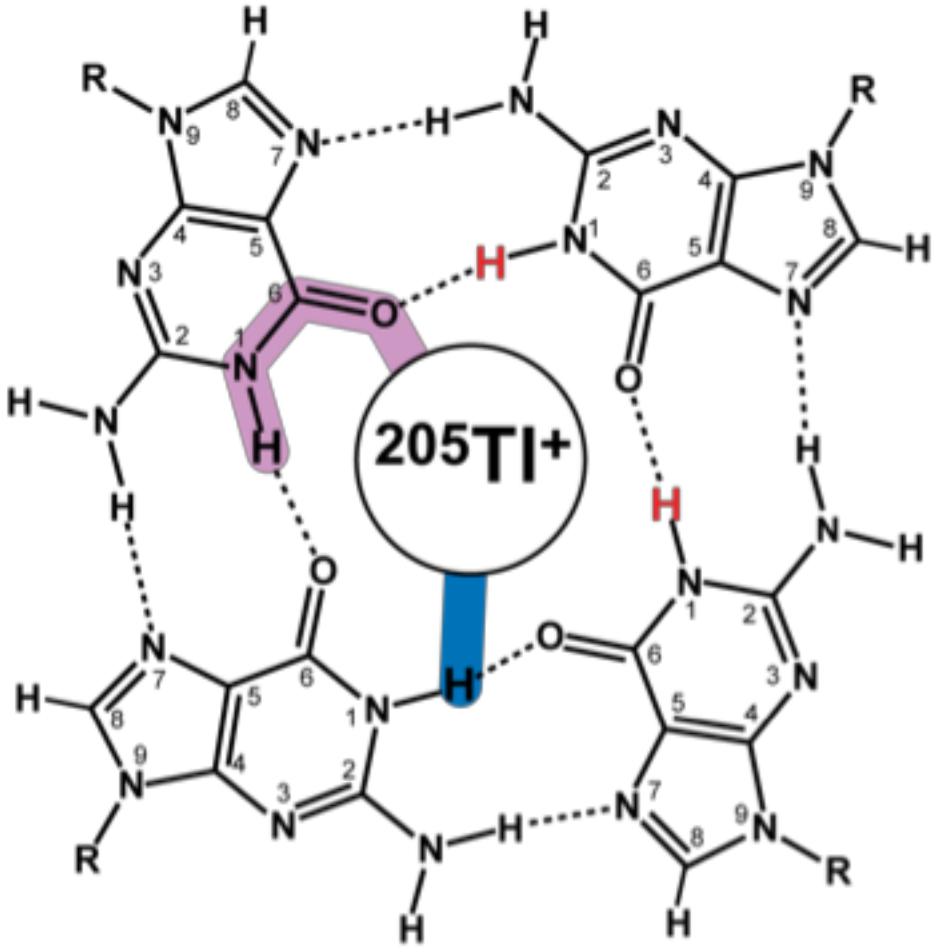
Possible mechanisms for Imino ^{1}H–^{205}Tl couplings



- Direct, though-space interaction with ^{1}H possible

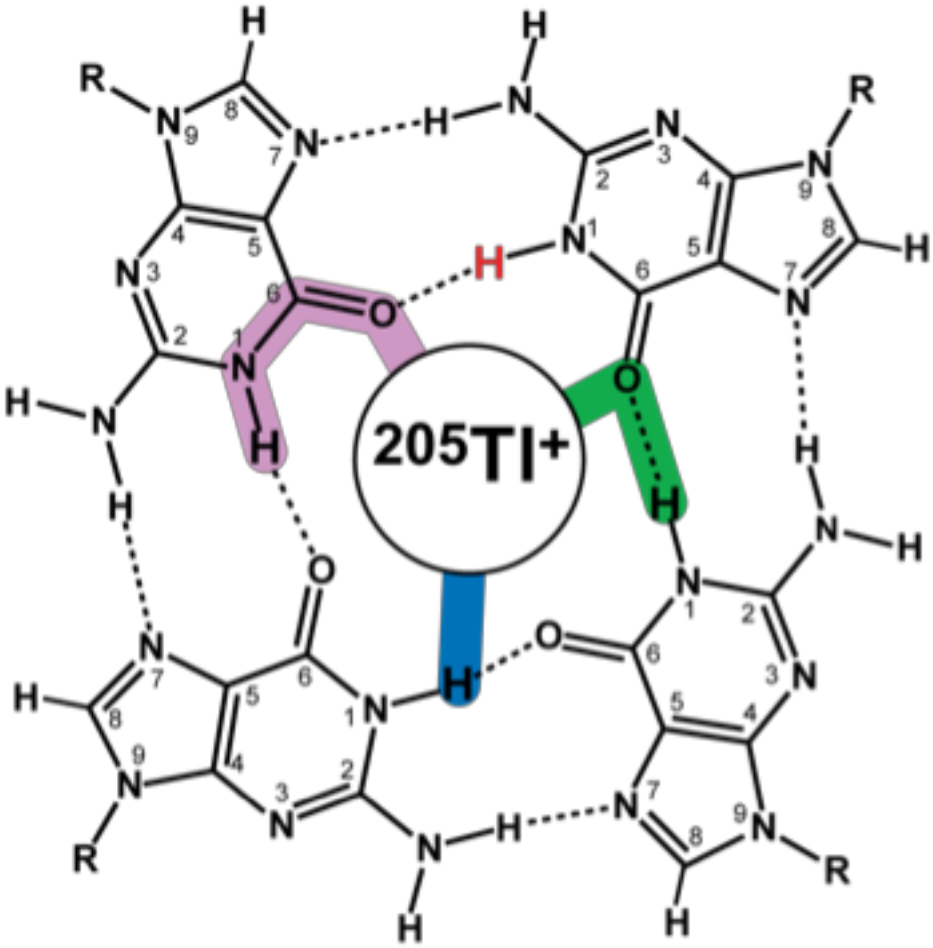
Blake, P.R.; et. al. *J. Biomol. NMR.* **1992,** *2*, 527-33.

Possible mechanisms for Imino ^{1}H–^{205}Tl couplings



^{1}H–^{205}Tl scalar coupling could be mediated by Gua O6 which coordinates $^{205}Tl^{+}$

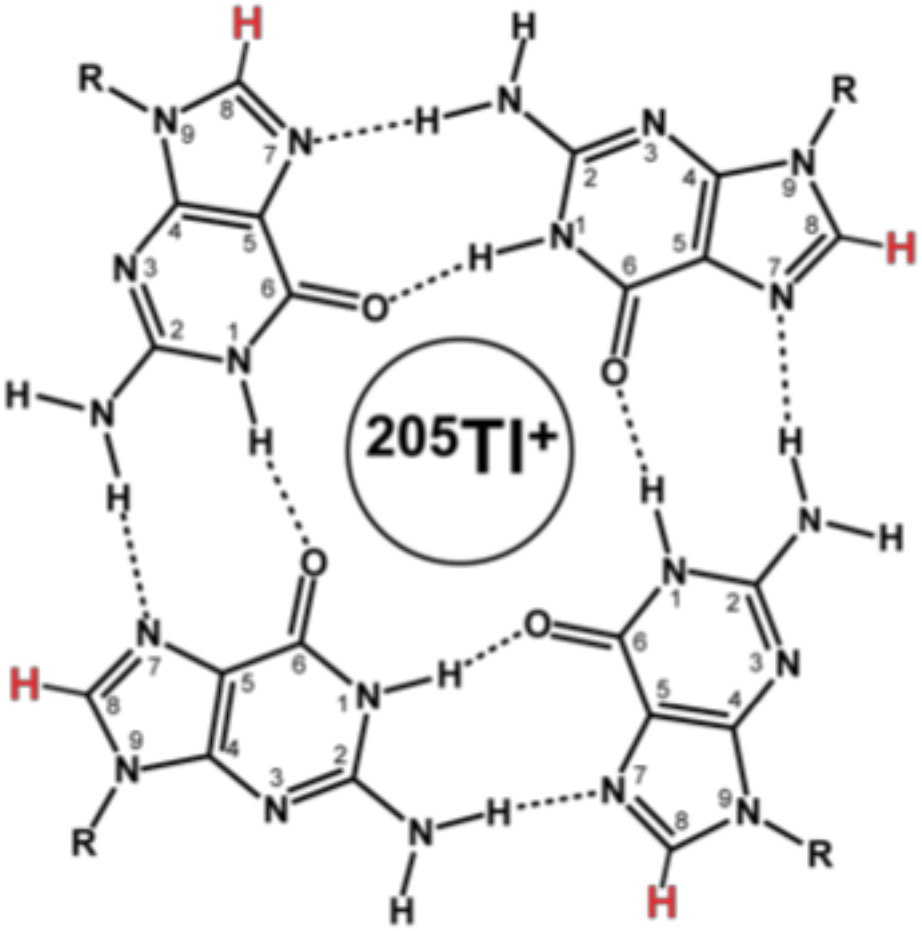
Possible mechanisms for Imino ^{1}H–^{205}Tl couplings



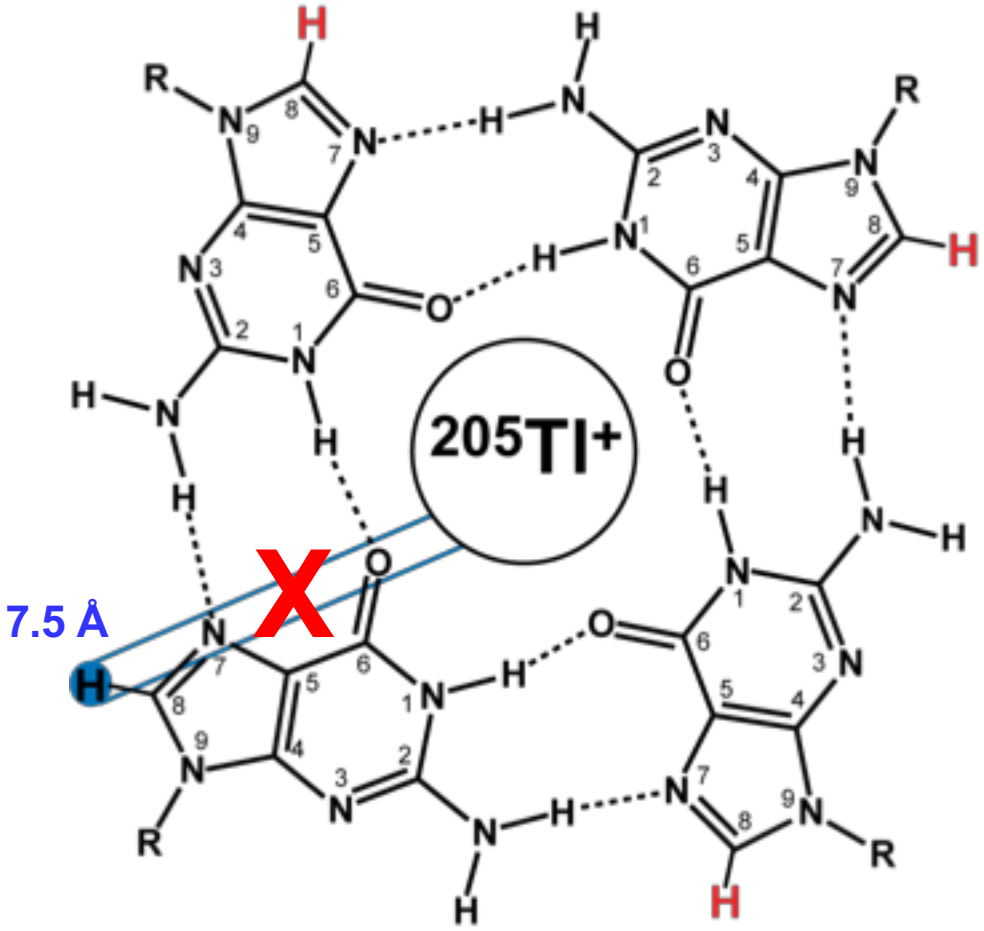
- Scalar couplings have been shown to traverse hydrogen bonds
- Multiple pathways may contribute to the observed value

Grzesiek, S.; et. al. *Methods Enzymol.* **2001,** *338*, 111-33.

Possible mechanisms for Imino ^{1}H–^{205}Tl couplings

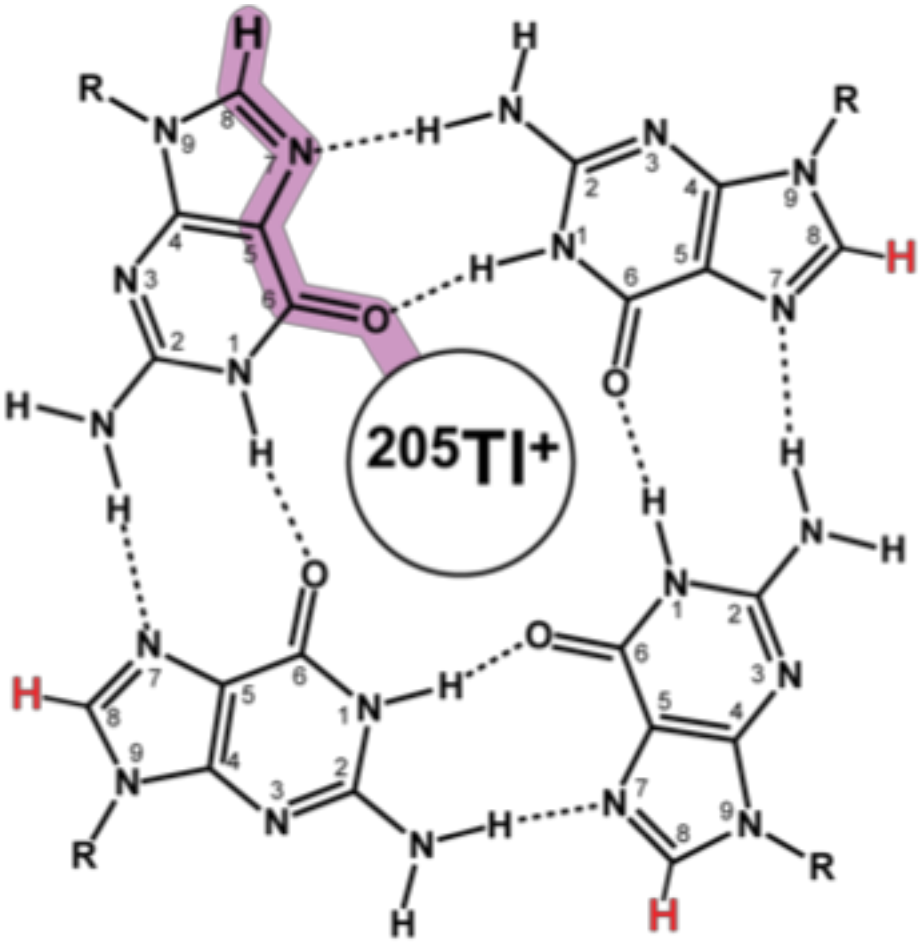


Possible mechanisms for Imino ^{1}H–^{205}Tl couplings



H8–$^{205}Tl^{+}$ distance is too long to be a direct interaction

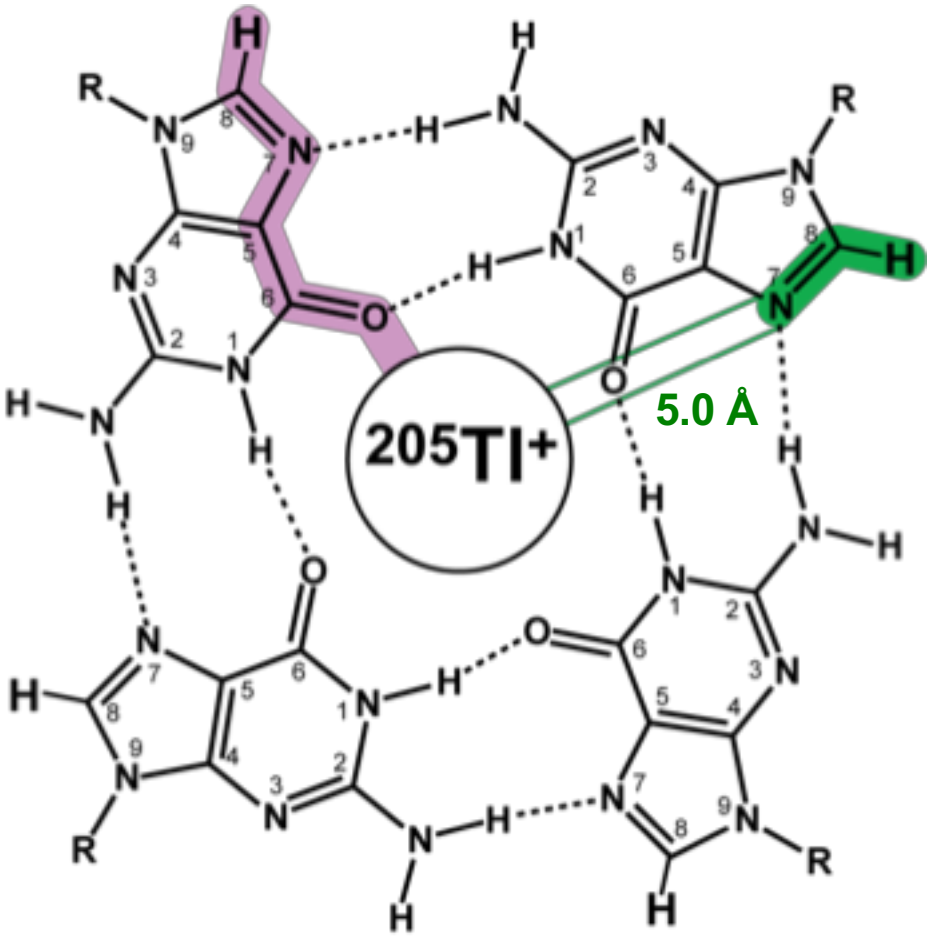
Possible mechanisms for Imino ^{1}H–^{205}Tl couplings



Five bond ^{1}H–M^{2+} scalar couplings have been reported

Blake, P.R.; et. al. *J. Biomol. NMR.* **1992,** *2*, 527-33.

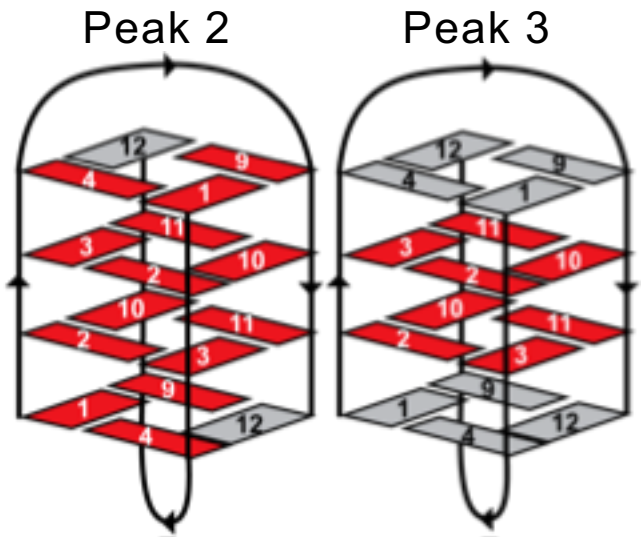
Possible mechanisms for Imino ^{1}H–^{205}Tl couplings

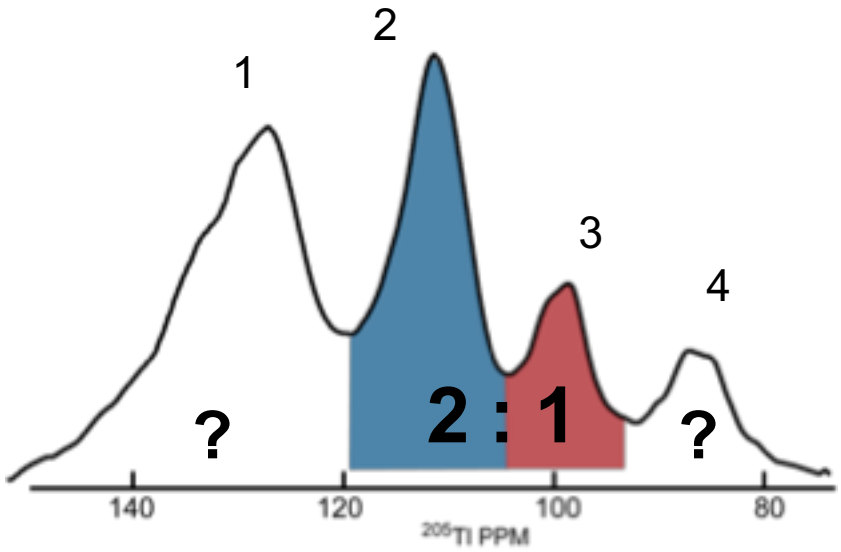


- $^{205}Tl^{+}$ has been reported to interact strongly with Gua N7
- Contributions from both pathways are possible

Taylor, E.C.; et. al. *J. Org. Chem.* **1969**, *34*, 1170.
Lee, A.G. The Chemistry of Thallium, 1971.

Assignment of bound ^{205}Tl peaks

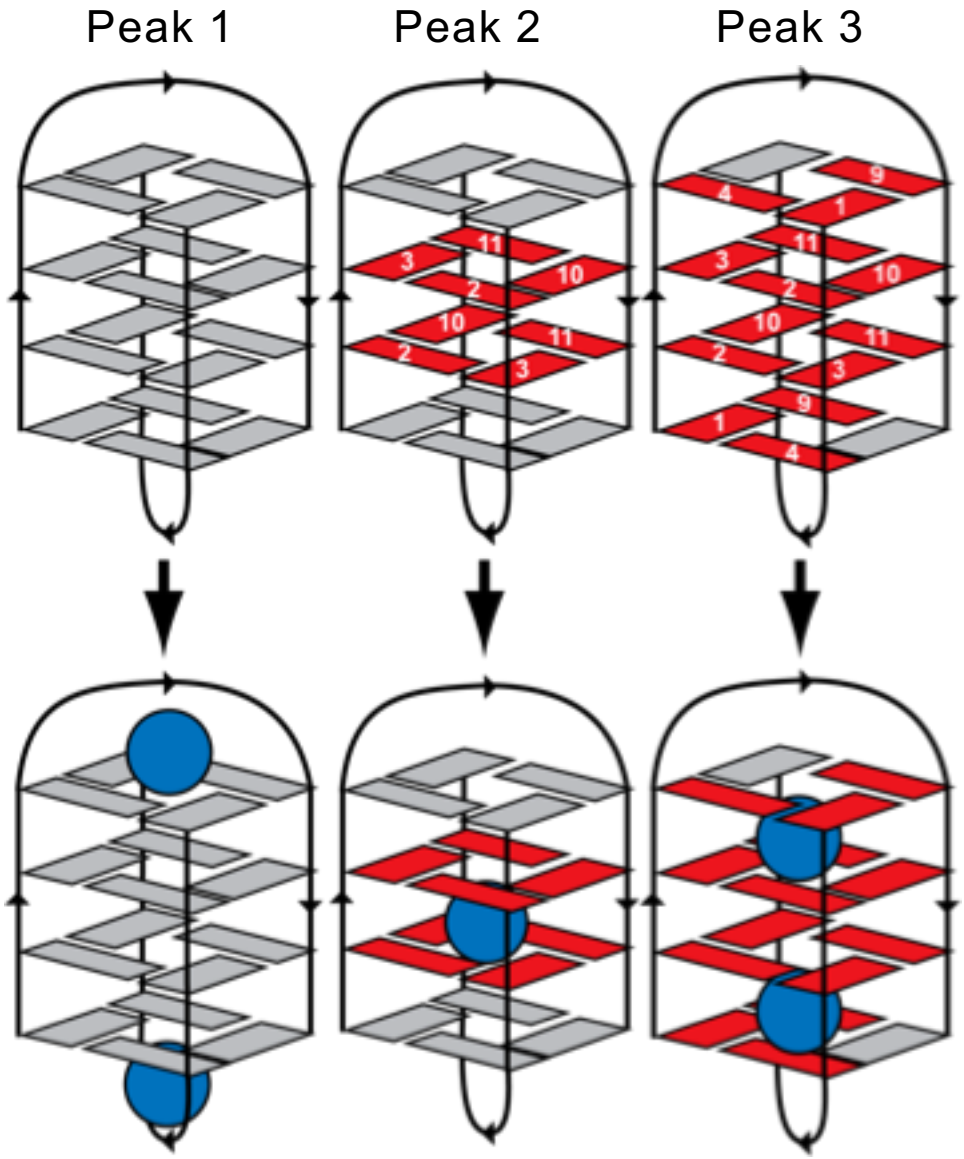




Lifetime (ms): 80 ± 10 110 ± 10 100 ± 20 150 ± 60

- What is the assignment for ^{205}Tl peaks 1 and 4?

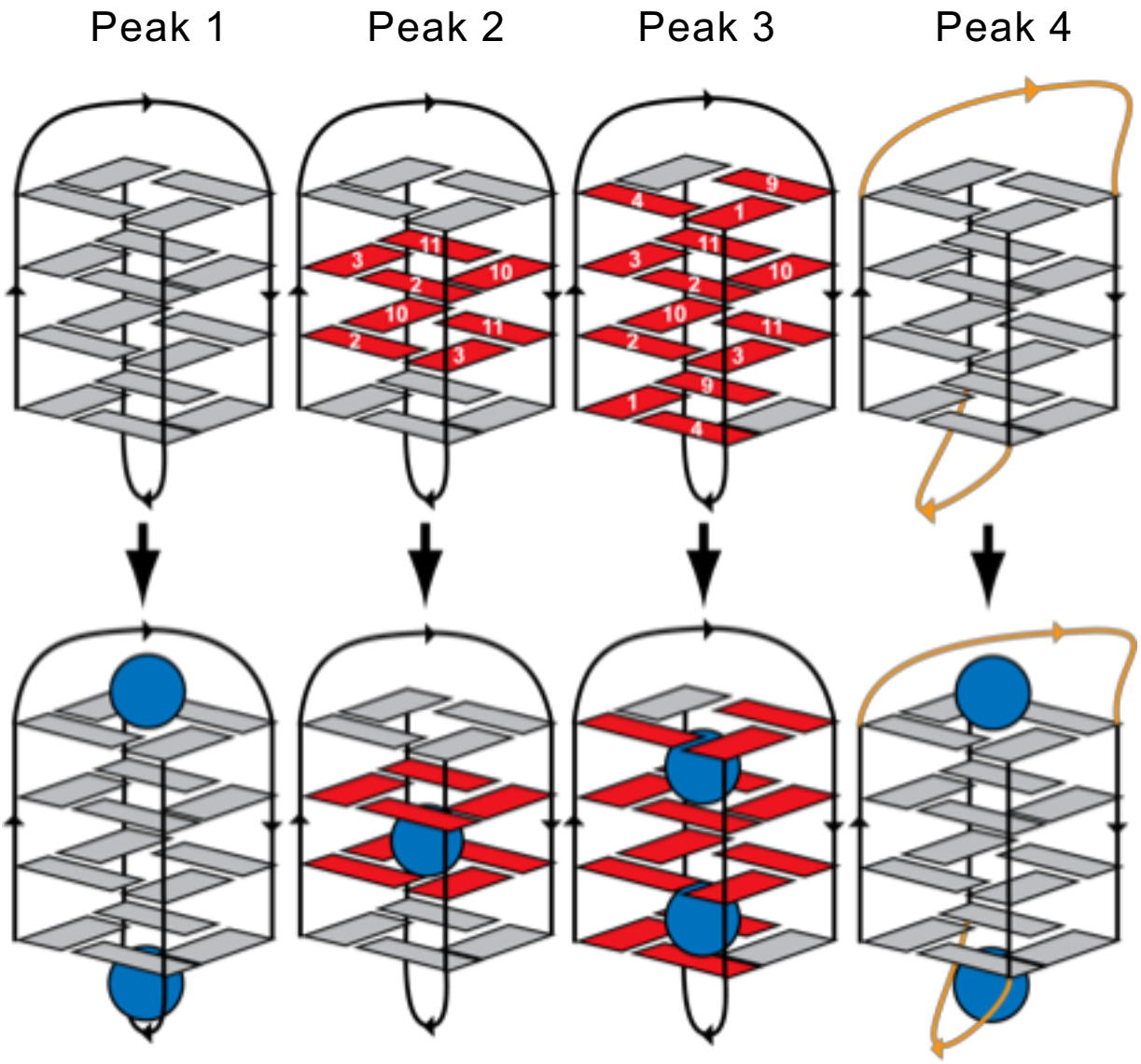
Possible assignment of ^{205}Tl peak 1



- Tl^+ binds to loops in crystal structure of $d(G_4T_4G_4)_2$
- Most likely assignment is to the thymine loops

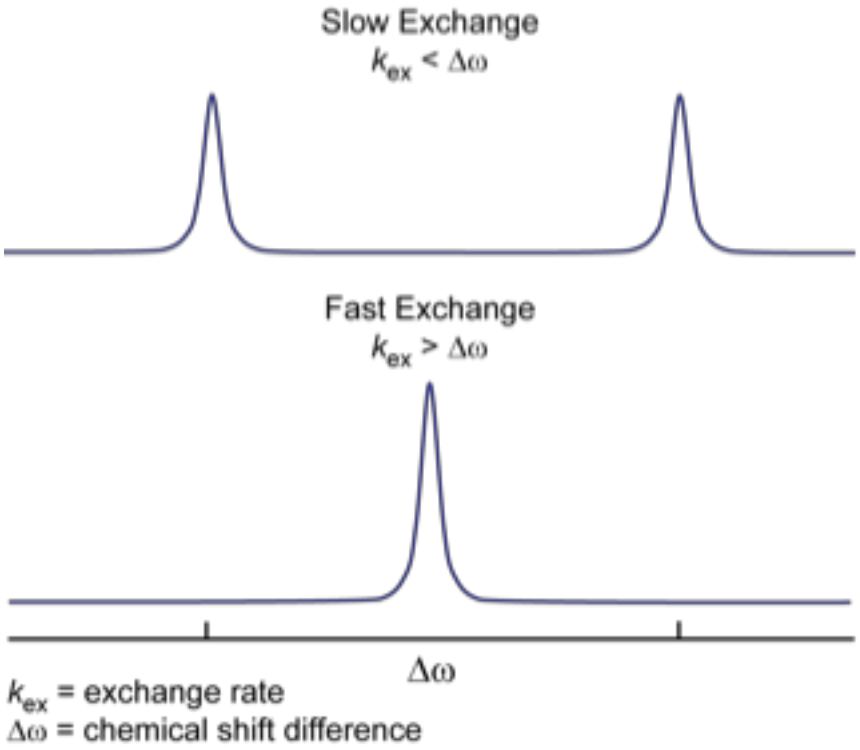
- Why aren't ^1H–^{205}Tl scalar couplings observed to this peak?
- One possible explanation: conformational exchange

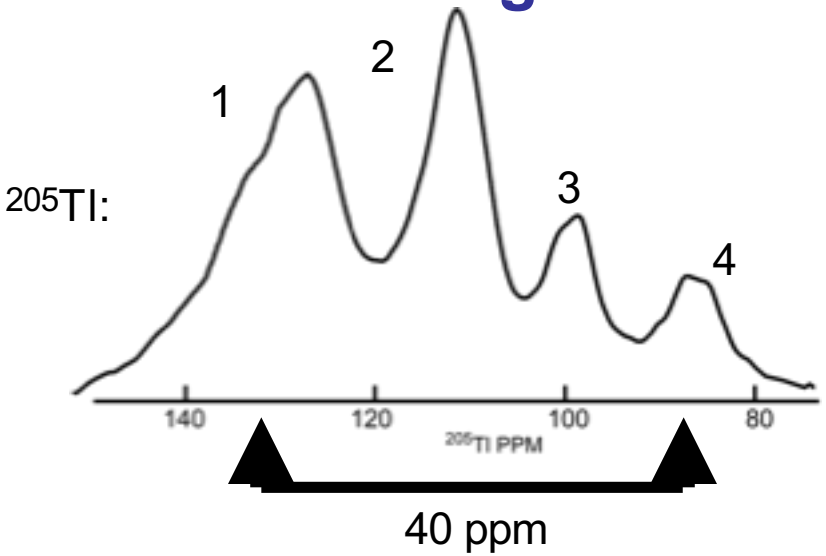
Possible assignment of ^{205}Tl peak 4

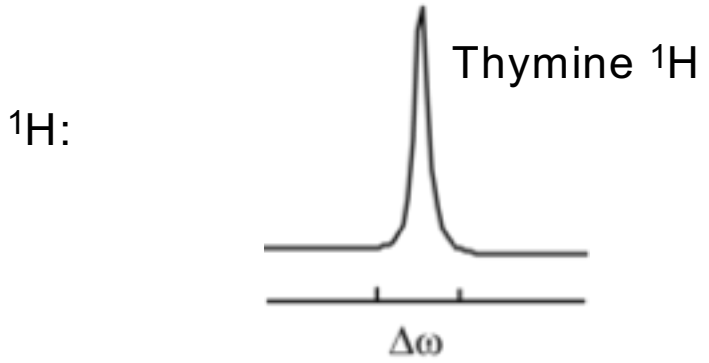


- Peak 4 could result from Tl^+ binding to loops in an alternate conformation
- Why are there two ^{205}Tl peaks but only one set of ^{1}H resonances for thymine loops?

Effect of ^{205}Tl chemical shift on exchange limit







- ^{205}Tl peaks 1 and 4 are separated by over 40 ppm (large $\Delta\omega$)
- This same $\Delta\omega$ translates to 23 ppm on ^{1}H chemical shift scale
- $\Delta\omega$ (^{1}H) << $\Delta\omega$ (^{205}Tl)
- Slow exchange limit is much larger for ^{205}Tl
- Conformational exchange is fast on ^{1}H time scale and slow on ^{205}Tl time scale

Conclusions

- Tl^+ is an excellent mimic of K^+ for NMR studies
- ^{205}Tl-NMR can be used to study bound $^{205}Tl^+$ cations
- ^{1}H–^{205}Tl scalar couplings enable assignment of ^{205}Tl peaks to monovalent binding sites
- Could provide constraints for structure determination
- The first ^{205}Tl heteronuclear NMR experiment reported
- Large ^{205}Tl chemical shift imparts generous limit on slow exchange

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