#### A challenging EXAFS analysis problem

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# Transport of metal contaminants in the environment

There are numerous natural and man-made point sources of toxic metals which find their way into water systems used for human and agricultural applications.



# The safe use of water requires monitoring and eventual remediation of bioavailable metal species.

image from 'a http://lightsources.org, Credit: Argonne National Laboratory

# Real-time, field-ready sensors

Sophisticated laboratory and synchrotron methods exist to detect and speciate water contaminants at very low concentrations. The real-world task of environmental monitoring requires a fast, flexible, sensitive, selective method of detecting contaminants *in the field*.

Fast Obtain results while still in the field

Flexible Easy to carry and easy to use in the field

Sensitive Detect contaminant concentrations below regulated human health hazard levels

Selective Respond to the target metal but not to other metals

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We want Spock's tricorder!

Fast Obtain results while still in the field

- Flexible Easy to carry and easy to use in the field
- Sensitive Detect contaminant concentrations below regulated human health hazard levels
- Selective Respond to the target metal but not to other metals

# Catalytic DNA-based sensors



The sensor has a receptor, a cleavage site, and paired fluorophore and quencher.

J. Liu, et al. A catalytic beacon sensor for uranium with parts-per-trillion sensitivity and millionfold selectivity PNAS, 104:7 (2007) 2056-2061 To DOI: 10.1073/pnas.0607875104

# Building a sensor device

These DNA sensors can be incorporated into a hand-held device. Water is dropped onto an array of sensors and read using photodiodes.



Photosensor array



Wells containing selective DNAzyme sensors

# **DNA-based Hg sensor**

U.S. EPA limit on Hg in water is 10 nM (2 ppb)

The DNA-based sensor for Hg has a detection limit of 2.4 nM

Questions:

- How and where does the metal bind?
- Under what conditions does the metal remain bound to the DNA?
- How many binding sites are there on a sensor?
- Do different metals behave differently?
- Can DNAzymes be designed more rationally?
- And, of course, what can XAS tell us about any of these questions (keeping in mind the very local nature of the XAS measurement)?

J. Liu and Y. Lu. Rational Design of "Turn-On" Allosteric DNAzyme Catalytic Beacons for Aqueous Mercury Ions with Ultrahigh Sensitivity and Selectivity, Angew. Chemie, 46:40 (2007) 7587–7590 \* DOI: 10.1002/anie.200702006

### XAS measurements

Solutions:

- 50 mM cacodylic acid as a buffer
- 100 mM NaClO<sub>4</sub> to maintain pH=6.10
- glycerol to promote glassification upon freezing

Samples:

Control 15 mM Hg Sample 3 mM Hg with 3 mM DNA Sample with excess Hg 6 mM Hg with 3 mM DNA

#### Measure EXAFS at 10 K

Experiment			
	-		

# Cryostat



Displex cryostat at APS 20BM.

- He exchange gas
- 10 mm wide opening for beam
- $\sim 12 \text{ mm}$  wide inner shroud
- Fluorescence measured through hole on side with a Ge detector
- At that time, 20BM did *not* have a focusing mirror

#### Here is the fluorescence spectrum:

🔁 MCA Display v6a			_ [0] ×
Live Time	1.26+4-		ROI Events/Live Time
Real Time	1.1E+4-		Hg 151
85.68 sec	1.0E+4-	1	AS 1340
Spectrum Events	9.0E+3-		Fe ka
1326096	8.0E+3-		<u>0</u>
Events/Live Time	7.0E+3-		
15813.56	6.0E+3-		2n <b>31</b>
Main Peak Energy 60.0 eV	5.06+3-		Pb 1904
Energy 60.0 eV Chan. # 3	4.0E+3-		TVBa
	3.0E+3-		Ba/Or S
Element 7 V << >>	2.06+3-	111	0. <b>33</b>
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The Hg L $\alpha$  peak is the tiny thing near the green line.

The neighboring peak is vastly larger!

#### Here is the fluorescence spectrum:



The Hg L $\alpha$  peak is the tiny thing near the green line.

The neighboring peak is vastly larger!

#### What's cacodylic acid?

MCA Display v6a			_ 🗆 ×
Live Time	1.26+4-	1	ROE Events/Live Time
Real Time	1.1E++-		Hg 151
85.68 sec	1.0E+4-	Λ.	As 1340
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Element 7 🔻 << >>	2.06+3-	لم )	Qu 🚺
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#### Here is the fluorescence spectrum:

The Hg L $\alpha$  peak is the tiny thing near the green line.

The neighboring peak is vastly larger!



The big peak is As  $K\alpha$  (~10.5 keV), our Hg L $\alpha$  (~10 keV) peak is on its shoulder.



The samples were packaged back at the University of Illinois and were about 15 mm by 3 mm.

We had to put the samples in the cryostat upright and slit the beam down to  $\sim 1$  mm.



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We had to put the samples in the cryostat upright and slit the beam down to  $\sim 1$  mm.

#### Plan ahead!

Forgetting about the details leads to much worse data!

#### Our main sample



This poor data is due to low concentration, small beam, and large background from the As.

We measured 42 scans, taking about 22 hours.



### Sample and control



Chemistry has certainly happened.

The control is clearly Hg in some kind of aqueous form.

The sample with DNA is clearly different from the control.

#### Is all Hg taken up by the DNA?

To answer this, we measured a sample with excess Hg.



Let's go do some linear combination fitting. (Note the isosbestic points.)

#### First question

#### Is all Hg taken up by the DNA?

To answer this, we measured a sample with excess Hg.



Let's go do some linear combination fitting. (Note the isosbestic points.)



Yes, all the Hg is taken up by the DNA. 47(1)% sample + 53(1)% control



# 2D and 3D representations

The 2D figures on the previous page were generated from the canonical SMILES strings:

 Adenisine
 c1=Nc2=c(c(=N1)N)N=CN2C3C(c(c(03)COP(=0)(0)0)0)0

 Thymidine
 cc1=cN(c(=0)Nc1=0)c2Cc(c(02)COP(=0)(0)0)0)0

 Guanosine
 c1=Nc2=c(N1C3C(c(c(03)COP(=0)(0)0)0)Nc(=Nc2=0)N

 Cytidine
 c1=cN(c(=0)N=C1N)c2c(c(c(02)COP(=0)(0)0)0)0

#### Neat! But we need 3D structures to run FEFF...

#### Structure from PubChem

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H-0 0	BioActivity Data Links (a) This Compound with Similar Compounds with Similar Conformers
0 <sup>20</sup> 0-H	Related Compounds  Same, Connectity (14) Same, Stereochemistry (2) Same, Isotopes (13) Similar Compounds (1048) Similar Conformers (077) Vew
Identification	

#### http://pubchem.ncbi.nlm.nih.gov/

#### Cartesian coordinates: 3D SDF file

#### 9700

-OEChem-05141416293D

36 37 0	1 0 0	0 0 0	999	V20	00										
-3.5515	-1.5175	0.1599	P	0	0	0	0	0	0	0	0	0	0	0	0
-0.4389	1.3396	1.0202	2 0	0	0	0	0	0	0	0	0	0	0	0	0
-0.9101	4.1569	-0.0812	2 0	0	0	0	0	0	0	0	0	0	0	0	0
-2.7552	-0.1874	0.6247	0	0	0	0	0	0	0	0	0	0	0	0	0
3.6173	1.7470	0.3907	0	0	0	0	0	0	0	0	0	0	0	0	0
3.8378	-2.8022	-0.2452	2 0	0	0	0	0	0	0	0	0	0	0	0	0
-2.5475	-2.2163	-0.8977	0	0	0	0	0	0	0	0	0	0	0	0	0
-4.7267	-0.9241	-0.7790	0	0	0	0	0	0	0	0	0	0	0	0	0
-4.0197	-2.4002	1.2798	0	0	0	0	0	0	0	0	0	0	0	0	0
1.6113	0.5684	0.1973	N	0	0	0	0	0	0	0	0	0	0	0	0
3.7127	-0.5224	0.0726	N	0	0	0	0	0	0	0	0	0	0	0	0
-1.0101	2.8736	-0.6948	C	0	0	2	0	0	0	0	0	0	0	0	0
-1.5699	1.8660	0.2995	C	0	0	1	0	0	0	0	0	0	0	0	0
0.3733	2.3378	-0.9829	C	0	0	0	0	0	0	0	0	0	0	0	0
0.7701	1.7196	0.3478	C	0	0	1	0	0	0	0	0	0	0	0	0
-2.2796	0.6993	-0.3750	C	0	0	0	0	0	0	0	0	0	0	0	0
1.0112	-0.6708	0.0146	C	0	0	0	0	0	0	0	0	0	0	0	0
3.0176	0.6816	0.2323	C	0	0	0	0	0	0	0	0	0	0	0	0
1.6792	-1.8209	-0.1381	C	0	0	0	0	0	0	0	0	0	0	0	0
3.1656	-1.7831	-0.1119	C	0	0	0	0	0	0	0	0	0	0	0	0
1.0130	-3.1449	-0.3336	C	0	0	0	0	0	0	0	0	0	0	0	0
-1.6278	2.9841	-1.5911	Н	0	0	0	0	0	0	0	0	0	0	0	0
-2.2303	2.3332	1.0386	Н	0	0	0	0	0	0	0	0	0	0	0	0

(+ several more hydrogen atoms + bonding information)

Here is the "SDF" file for thymidine monophosphate from PubChem.

Along with lots of stuff not relevant to the EXAFS analysis, we find the Cartesian coordinates of all the atoms in thymidine monophosphate!

SDF = Structure data file

#### Cartesian coordinates: Feff input file

TITLE Hg decorating thymidine monophosphate

HOLE	4	1.0	*	Hg	L3	edge	(12284	eV),	S0^2
*	mph	ase,mp	oath,	mf	eff	,mchi			
CONTROL	1	1		1		1			
PRINT	1	0		0		0			
RMAX	6.0								

#### POTENTIALS

ipot element Hg 0 50 1 8 0 2 7 Ν з 6 С 4 15 Ρ 5 1 н

#### ATOMS

*	x	У	z	ipot
	-3.5515	-1.5175	0.1599	4
	-0.4389	1.3396	1.0202	1
	-0.9101	4.1569	-0.0812	1
	-2.7552	-0.1874	0.6247	1
	3.6173	1.7470	0.3907	1
	3.8378	-2.8022	-0.2452	1
	-2.5475	-2.2163	-0.8977	1
	-4.7267	-0.9241	-0.7790	1
	-4.0197	-2.4002	1.2798	1
	1.6113	0.5684	0.1973	2
	3.7127	-0.5224	0.0726	2
	-1.0101	2.8736	-0.6948	3
	-1.5699	1.8660	0.2995	3
	0.3733	2.3378	-0.9829	3
*	(and so	on)		

- Do some cutting and pasting
- Add some boilerplate for the header
- Make a sensible POTENTIALS list

What about the Hg atom?

# What is the likely location of the Hg atom?

- Thymine forms its hydrogen bond with adenisine via the N atom
- The engineered DNA sensor is known to have a T-T mismatch
- Earlier NMR work was interpreted at having the Hg bridging the T-T mismatch.

That said, I don't know much about this chemistry.



Y. Miyake, et al., Mercury<sup>II</sup>-Mediated Formation of Thymine-Hg<sup>II</sup>-Thymine Base Pairs in DNA Duplexes. J. Am. Chem. Soc. (2006) v.128, 2172-2173 **\*** DOI: 10.1021/ja056354d



challenging EXAFS analysis problem





Not a great fit, but it tells us that the Hg atom is about 2.05 Å away from it's neighbor. Using the known nucleotide structures, I wrote a small program to solve some trigonometry:

The Hg atom is ...

- … 2.05 Å away from its neighbor
- In the same plane as the neighboring atoms
- In equidistant from the second neighbors (6- and 5-member ring options)
- In collinear with the 1<sup>st</sup> and 2<sup>nd</sup> neighbors (monodentate option)

Finally, write out 'feff.inp' files with Hg as the absorber.

# 5-member ring option: coordinates

TITL	E Hg o	decor	ating	thymi							
HOLE	1	4	1.0	* Hg	L3 ec	ige	(1228	4 e'	V),	S0^2	2
CONT	ROL	1	1	1	1						
PRIN	T	1	0	0	0						
RMAX		6.0									
POTE	INTIAL:	S									
*	ipot	Z	elem	ent							
	0	50	Hg								
	1	8	0								
	2	7	N								
	3	6	С								
	4	15	Р								
ATOM	IS										
*	x		У	z		ot					
	0.49			63093		853			Hg		0.0000.0
	-3.718			00000		.249			0		6.44507
	-3.91			59800		. 290			Ρ		5.56632
	-5.35		-1.5	58600		. 600			0		6.65531
	-3.020			44600		. 110			0		4.98947
	-3.35			12200		459			0		4.59727
	-1.95	500	0.0	01100	0	. 305	500		С	3	3.59210
	-1.48	700		40500	0	.637	700	3 (	С	3	3.07534
	-0.110	000	1.3	31500	1	.031	100	1 1	0		2.05000
	-1.523	300	2.	38600	-0	.517	700	3 (	С	4	4.30462
	-1.77			69900		.006			0		4.77194
	-0.154			19400		. 161		-	С		4.35705
	0.734			75700		.001		-	С		3.07532
	1.68			62300		. 156			N		3.23452
	1.54	600	-0.3	35900	-1	. 107	00	3 (	С	4	4.21393
	0.64	900	-0.3	39000	-1	.930	000	1 1	0		4.89315
	2.52	400	-1.3	32100	-1	.063	300	2 1	N		4.82117
	3.58	700	-1.4	40200	-0	. 181	100	3 (	С	4	4.78223
	4.40	700	-2.3	31400	-0	. 224	100	1 1	0	5	5.77995
	3.65	500	-0.3	33500	0	785	500	3 (	С	3	3.89431
	4.864	400	-0.	10800	1	628	300	3 (	С	4	4.59276
	2.71	300	0.	59300	0	.750	000	3 (	С	3	3.05336



# 5-member ring option: paths

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Scatte 0001 0002 0003 0004 0005 0006 0007 0008 0009 0011	Degen 1.000 3.000 1.000 4.000 2.000 1.000 4.000 1.000 1.000	Reff 2.0396 3.0680 3.2345 3.2670 3.4587 3.5921 3.7677 3.8641 3.8943	(00) (00) (00) (00) (00) (00) (00) (00)				100.00 90.99 28.47 51.62 16.35 19.42 6.38 14.82 15.29	2 2 3 4 2 3 3 3 3 2	single scattering single scattering obtue triangle dog-leg single scattering other double scat other double scat single scattering other double scat
Scatte 0000 0001 0002 0003 0004 0005 0006 0007 0008 0009	Degen 1.000 3.000 1.000 4.000 2.000 1.000 2.000 1.000 2.000 1.000 2.000	Reff 2.0396 3.0680 3.2345 3.2670 3.4587 3.5921 3.7677 3.8943 4.0024	(() 0 (() 0 (() 0 () 0 (() 0 () 0 (() 0 () 0		© 0 0 0 0	0	100.00 90.99 28.47 51.62 16.35 19.42 6.38 14.82 15.29 5.98	2 2 3 4 2 3 3 3 2 3 3	single scattering single scattering obtuse triangle dog-leg single scattering other double scat single scattering other double scat
Concellent Concellent	Degen 1.000 3.000 1.000 4.000 2.000 1.000 2.000 1.000 2.000 1.000 1.000 1.000	Reff 2.0396 3.0680 3.2345 3.2670 3.4587 3.5921 3.7677 3.8641 3.8943 4.0024 4.0792	(00) (00) (00) (00) (00) (00) (00) (00)	0 0 0 0 0 0 0 0 0 0 0		0	100.00 90.99 28.47 51.62 16.35 19.42 6.38 14.82 15.29 5.98 6.62	2 2 3 4 2 3 3 3 2 3 4 4	single scattering single scattering obtue triangle dog-leg single scattering other double scat other double scat single scattering other double scat
Scatte 00001 0002 0003 0004 0005 0006 0007 0008 0009 0011 0012	Degen 1.000 3.000 1.000 4.000 2.000 1.000 2.000 1.000 2.000 1.000 2.000 1.000 2.000	Reff 2.0396 3.0680 3.2345 3.2670 3.4587 3.5921 3.7677 3.8641 3.8943 4.0024 4.0024 4.0792 4.0875	(00) (00) (00) (00) (00) (00) (00) (00)	0 0 0 0 0 0 0 0 0 0 0		0	100.00 90.99 28.47 51.62 16.35 19.42 6.38 14.82 15.29 5.98 6.62 5.04	2 2 3 4 2 3 3 3 2 3 4 3 4 3	single scattering single scattering obtuse triangle dog-leg single scattering other double scat other double scat rattle other double scat rattle other double scat other double scat
Scatte 0001 0002 0003 0004 0005 0006 0007 0008 0009 0011 0012 0013	Degen 1.000 3.000 1.000 4.000 2.000 1.000 2.000 1.000 2.000 1.000 2.000 2.000 2.000	Reff 2.0396 3.0680 3.2345 3.2670 3.4587 3.5921 3.7677 3.8641 3.8943 4.0024 4.0024 4.0792 4.0875 4.1352	(00) (00) (00) (00) (00) (00) (00) (00)			0	100.90 90.99 28.47 51.62 16.35 19.42 6.38 14.82 15.29 5.98 6.62 5.04 9.04	2 2 3 4 2 3 3 2 3 4 3 4 3 3 4 3 3	single scattering single scattering obcuse triangle dog-leg single scattering other double scat ather double scat single scattering other double scat rattle other double scat obcuse triangle

Run FEFF, drag-n-drop first 6 paths, transfer them to the plotting list, plot in R:



This looks sort of promising ... or does it?

A challenging EXAFS analysis problem

# 5-member ring option: VPath

We fit a sum of paths to the data, so let's examine the sum of these paths. In ARTEMIS, this is called a "VPath."



#### Not so promising, after all.

A challenging EXAFS analysis probler

#### Monodentate option: coordinates

TITLE Hg	decor	ating	thymic	line mono	ophospha	ate	
HOLE	4	1.0	* Hg	L3 edge	(12284	eV),	S0^2
CONTROL	1	1	1	1			
PRINT	1	0	0	0			
RMAX	6.0						
POTENTIAL							
* ipot		elem	ent				
0	50	Hg					
1	8	0					
2	7	N					
3	6	С					
4	15	Р					
ATOMS							
* x		У	z	ipot			
5.74			80032	-0.294		Hg	0.00000
-3.71			00000	-1.249		0	9.67837
-3.91			59800	0.290		Р	9.91863
-5.35			58600	0.600		0	11.35435
-3.02			44600	1.110		0	8.97789
-3.35			12200	0.459		0	9.83988
-1.95	500	0.	01100	0.305	500 3	С	8.61105
-1.48	700	1.	40500	0.63	700 3	С	8.95772
-0.11	000	1.	31500	1.03	100 1	0	7.88572
-1.52	300	2.	38600	-0.517	700 3	С	9.54572
-1.77			69900	-0.006		0	10.62446
-0.15			19400	-1.16		С	8.45356
0.73			75700	0.00		С	7.48765
1.68			62300	-0.156		N	6.00394
1.54			35900	-1.107		С	5.48832
0.64			39000	-1.930		0	6.34502
2.52	400	-1.	32100	-1.063	300 2	N	4.13555
3.58	700	-1.	40200	-0.18	100 3	С	3.22719
4.40	700	-2.	31400	-0.224		0	2.05000
3.65			33500	0.78		С	4.18739
4.86			10800	1.628		С	4.25452
2.71	300	0.	59300	0.750	000 3	С	5.43826



#### Monodentate option: VPath

Same exercise – run feff, drag-n-drop the first few paths, make a VPath, plot with the data.



Better than the 5-member ring option, but still not so great.

A challenging EXAFS analysis problem

# 6-member ring option: coordinates

TITLE Hg decorating thymidine monophosphate							
HOLE	4	1.0	* Hg	L3 edge	(12284	eV),	S0^2
CONTROL	1	1	1	1			
PRINT	1	0	0	0			
RMAX	6.0						
POTENTIAL	S						
* ipot		elem	ent				
0	50	Hg					
1	8	0					
2	7	N					
3	6	С					
4	15	Р					
ATOMS							
* x		У	z	ipot			
2.40			80748	-2.458		Hg	0.00000
-3.71			00000	-1.249		0	6.29242
-3.91			59800	0.290		Р	6.99112
-5.35			58600	0.600		0	8.43040
-3.02			44600	1.110		0	6.50160
-3.35			12200	0.459		0	6.98896
-1.95	500	0.	01100	0.305	500 3	С	5.87972
-1.48	700	1.	40500	0.63	700 3	С	6.51567
-0.11	000	1.	31500	1.03	100 1	0	5.95606
-1.52	300	2.	38600	-0.517	700 3	С	6.79387
-1.77			69900	-0.006		0	8.11301
-0.15			19400	-1.16		С	5.76519
0.73			75700	0.00		С	5.44613
1.68			62300	-0.156		N	4.19199
1.54			35900	-1.107		С	2.92421
0.64			39000	-1.930		0	3.03360
2.52			32100	-1.063		N	2.05000
3.58			40200	-0.18		С	2.92356
4.40	700	-2.	31400	-0.224		0	3.03859
3.65			33500	0.78		С	4.26357
4.86			10800	1.628		С	5.47827
2.71	300	0.	59300	0.750	000 3	С	4.68340



# 6-member ring option: VPath

Again – run FEFF, drag-n-drop the first few paths, make a VPath, plot with the data.



I actually like this one quite a bit! The amplitude is off by about a factor of 2, but the phase is quite close.

# Metal sensors Experiment DNA Model building The fit Post mortem

Number of independent pointsk-range:  $2 Å^{-1}$  to  $8.8 Å^{-1}$ R-range: 1 Å to 2.8 Å $N_{idp} = 2\Delta k \Delta R / \pi \approx 7.8$ 

0	$E_0$ and amp are variables $\ldots \ldots \ldots$	(1,2)
2	Hg-N distance and $\sigma^2$ are variables $\ldots$	(3,4)
3	Hg-O distance and $\sigma^2$ are variables $\ldots$	(5,6)
4	Assume that the ring is <b>completely rigid</b> , this allows us to approximate contributions of various single and multiple scattering paths without	the

introducing any more variables.

# Metal sensors Experiment DNA Model building The fit Post mortem Trigonometry



Here's a formula for a triangle in a plane:

$$D(Hg - C) = \frac{a - b}{\cos(\theta)} \cos(\varphi/2)$$
$$\tan(\theta) = \frac{a + b}{a - b} \tan(\varphi/2)$$

$$arphi$$
 =116.25°  
b =1.378 Å

**a** and  $\sigma^2_{Hq\cdot N}$  are variables of the fit.

Assuming the ring is **rigid**, then we approximate  $\sigma^2_{\text{Hg}}$ . (and others) by scaling geometrically from  $\sigma^2_{\text{Hg}}$ .N

Metal sensors	Experiment	DNA	Model building	The fit	Post mortem	
Paths						
Path 1 O = C - N O = C - N $H_{g}$ $\Delta R_1 \text{ and } \sigma_1^2$	$\stackrel{\scriptscriptstyle N}{\underset{\scriptstyle}{\overset{\scriptstyle}{\overset{\scriptstyle}{\overset{\scriptstyle}}}}} \times 1$	Path 2 (SS $0 = \sum_{H_g}^{C} \sum_{H_g}^{N} C_s$ $\Delta R_2$ computed with $\sigma_2^2 \propto \sigma_1^2$	×2 ≈₀	Path 3 (SS) $C \qquad N \qquad \times$ $C \qquad N \qquad X \qquad X$	<2	
Path 4	(MS)	Path 5 (MS	S)	Path 6 (MS)		
$\sigma_{4}^{\text{C}} := \sigma_{2}^{\text{C}}$	$\mathbb{N}$ ×4 $\mathbb{C}$ $\mathbb{N}$ $\mathbb{C}$ $\mathbb{N}$ $\mathbb{N}$ $\mathbb{C}$ $\mathbb{N}$ \mathbb{N} $\mathbb{N}$ $\mathbb{N}$ $\mathbb{N}$ $\mathbb{N}$ $\mathbb{N}$ $\mathbb{N}$ $\mathbb{N}$	$\begin{array}{c} & & \\ & & \\ & & \\ 0 \end{array} \xrightarrow{C} \\ & & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & $	×2 ≈ <sub>0</sub>	$\begin{array}{c} & & & \\ & & \\ & & \\ & & \\ & & \\ & \\ & $	< <b>4</b> and 3	

Metal sensors	Experiment	DNA	Model building	The fit	Post mortem
		Fittin	g result		
			amp	$1.86\pm0.44$	
4	3mM 3mM		$E_0$	$1.41 \pm 1.91$	

Λ

Λ



amp	$1.86\pm0.44$
$E_0$	$1.41 \pm 1.91$
$\Delta R(N)$	$0.006\pm0.028$
$\Delta R(O)$	$-0.058\pm0.063$
$\sigma^2(N)$	$0.0046 \pm 0.0045$
$\sigma^2(O)$	$0.0096 \pm 0.0081$

#### Why is amp near 2?

The Hg atom bridges 2 thymines. Our FEFF model had Hg bound to 1 thymine. So  $S_0^2$  is really 0.93(44)!

ivietal sensors	Expertment	DINA	Model building	The fit	Post mortem
			tainties		
		Oncer	tatilites		

- The data are short i.e. little information content and noisy
- The uncertainties are all quite large, although the best fit values all make sense
- $S_0^2$  came out right, although with large uncertainty
- The  $\sigma^2$  approximations are sensible, but certainly not correct
- The assumption that the ring is rigid is sensible, but certainly not correct
- The assumption that the Hg atom sits in the plane of the ring is sensible, but certainly not correct

Our data are  $\ensuremath{\textit{consistent}}$  with the Hg atom bound to the N atom in the 6-member nitrogenous base

- The As in the cacodylic acid hurt. Use a different buffer.
- The sample geometry hurt. Use better packaging or a focusing mirror.

Those two things could have increased efficiency by about an order of magnitude. Another couple inverse Ångstroms would have made a huge difference!

#### What have we learned?

- The science question required interpretation of both XANES and EXAFS
- Quick first shell fit to approximate the first shell distance
- Made input for FEFF from published structural data and a sensible guess for the location of the Hg atom
- Tried several possible coordination geometries, but only pursued the one that looked promising
- Dealt with limited information by applying interesting constraints
- We didn't exactly *solve* the structure, but we demonstrated that the EXAFS data are consistent with the assumption from NMR