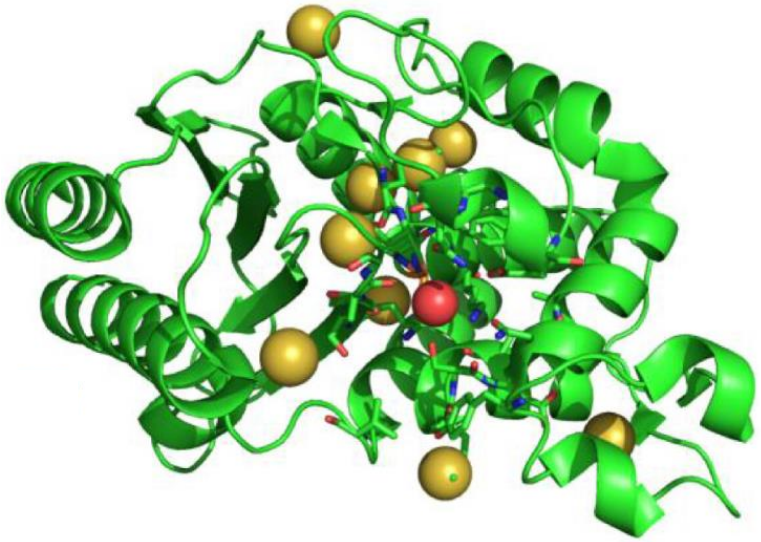
# Applications of PAC spectroscopy to proteins

Lars Hemmingsen (lhe@chem.ku.dk), ISOLDE workshop 2023



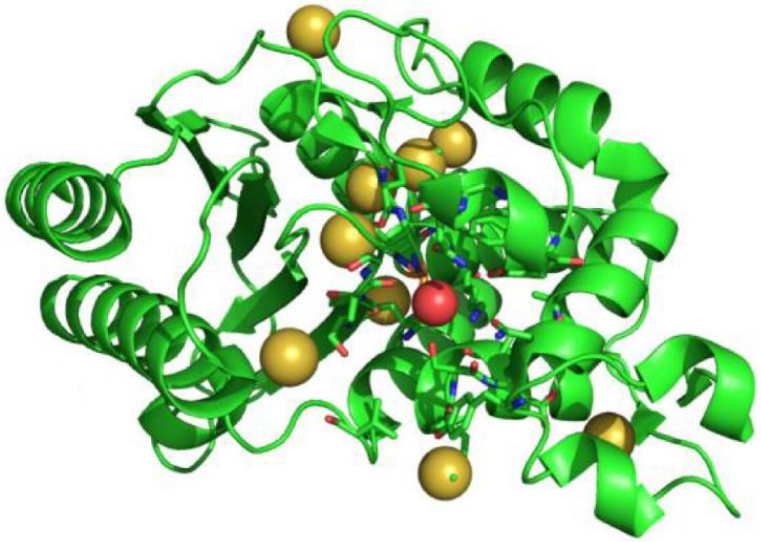
## Recent Bio-PAC @ ISOLDE

- Bacterial resistance against antibiotics (β-lactamases)[1]
- $^{111}$Ag chelators for radiopharmacy[2]
- $^{199m}$Hg and $^{111}$Ag reference compounds[3]
- Hg(II) toxicity: Hg(II) binding to Zn-hooks[4]
- Metal ion sensor proteins[5,6]

1. Nafaee *et al.*, Protein Science, 2023, 32, e4809
2. Tosato *et al.*, Applied Radiation and Isotopes, 2022, 190, 110508
3. Jancso *et al.*, Nuclear Instr. Methods in Physics Research A 2021, 1002, 165154
4. Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738
5. Balogh *et al.*, Hyperfine Interactions, submitted
6. Tóth *et al.*, J. Am Chem. Soc., submitted

# Applications of PAC spectroscopy to proteins

Lars Hemmingsen (lhe@chem.ku.dk), ISOLDE workshop 2023



Bela Gyurcsik, Uni. of Szeged, Hungary

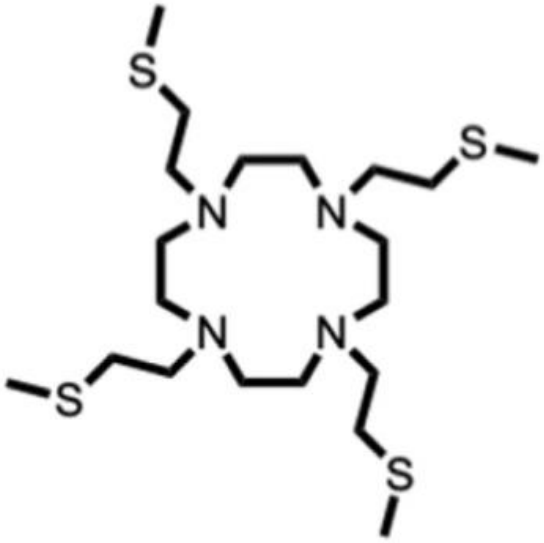
## Recent Bio-PAC @ ISOLDE

- Bacterial resistance against antibiotics (β-lactamases)[1]
- $^{111}$Ag chelators for radiopharmacy[2]
- $^{199m}$Hg and $^{111}$Ag reference compounds[3]
- Hg(II) toxicity: Hg(II) binding to Zn-hooks[4]
- Metal ion sensor proteins[5,6]

1. Nafaee *et al.*, Protein Science, 2023, 32, e4809
2. Tosato *et al.*, Applied Radiation and Isotopes, 2022, 190, 110508
3. Jancso *et al.*, Nuclear Instr. Methods in Physics Research A 2021, 1002, 165154
4. Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738
5. Balogh *et al.*, Hyperfine Interactions, submitted
6. Tóth *et al.*, J. Am Chem. Soc., submitted

# Applications of PAC spectroscopy to proteins

Lars Hemmingsen (lhe@chem.ku.dk), ISOLDE workshop 2023



Marianna Tosato and Valerio di Marco, Uni. Of Padova
Mattia Asti, AUSL-IRCCS di Reggio Emilia
Italy

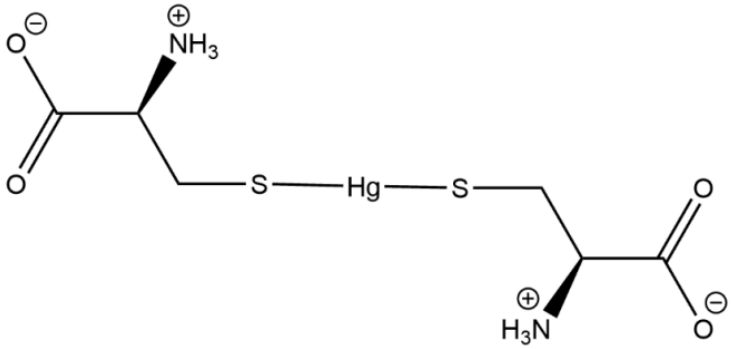
## Recent Bio-PAC @ ISOLDE

- Bacterial resistance against antibiotics (β-lactamases)[1]
- $^{111}$Ag chelators for radiopharmacy[2]
- $^{199m}$Hg and $^{111}$Ag reference compounds[3]
- Hg(II) toxicity: Hg(II) binding to Zn-hooks[4]
- Metal ion sensor proteins[5,6]

1. Nafaee *et al.*, Protein Science, 2023, 32, e4809
2. Tosato *et al.*, Applied Radiation and Isotopes, 2022, 190, 110508
3. Jancso *et al.*, Nuclear Instr. Methods in Physics Research A 2021, 1002, 165154
4. Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738
5. Balogh *et al.*, Hyperfine Interactions, submitted
6. Tóth *et al.*, J. Am Chem. Soc., submitted

# Applications of PAC spectroscopy to proteins

Lars Hemmingsen (lhe@chem.ku.dk), ISOLDE workshop 2023



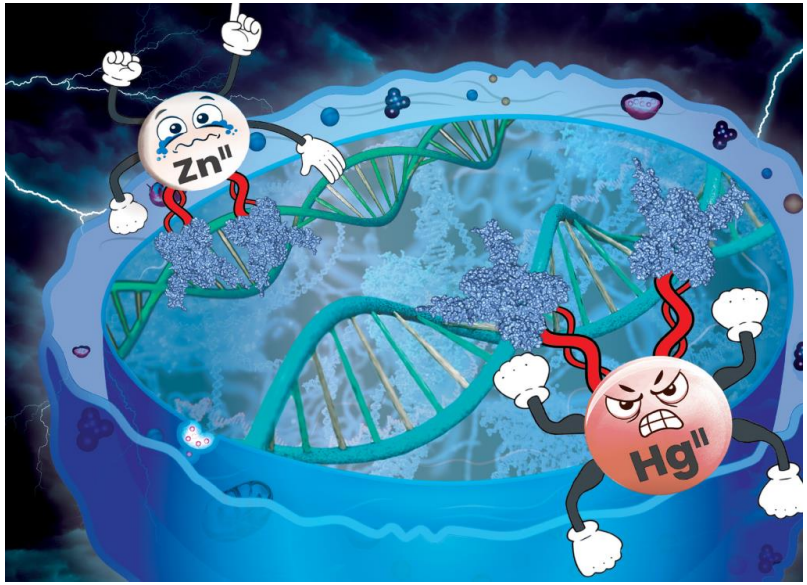

## Recent Bio-PAC @ ISOLDE

- Bacterial resistance against antibiotics (β-lactamases)[1]
- $^{111}$Ag chelators for radiopharmacy[2]
- $^{199m}$Hg and $^{111}$Ag reference compounds[3]
- Hg(II) toxicity: Hg(II) binding to Zn-hooks[4]
- Metal ion sensor proteins[5,6]

1. Nafaee *et al.*, Protein Science, 2023, 32, e4809
2. Tosato *et al.*, Applied Radiation and Isotopes, 2022, 190, 110508
3. Jancso *et al.*, Nuclear Instr. Methods in Physics Research A 2021, 1002, 165154
4. Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738
5. Balogh *et al.*, Hyperfine Interactions, submitted
6. Tóth *et al.*, J. Am Chem. Soc., submitted

# Applications of PAC spectroscopy to proteins

Lars Hemmingsen (lhe@chem.ku.dk), ISOLDE workshop 2023



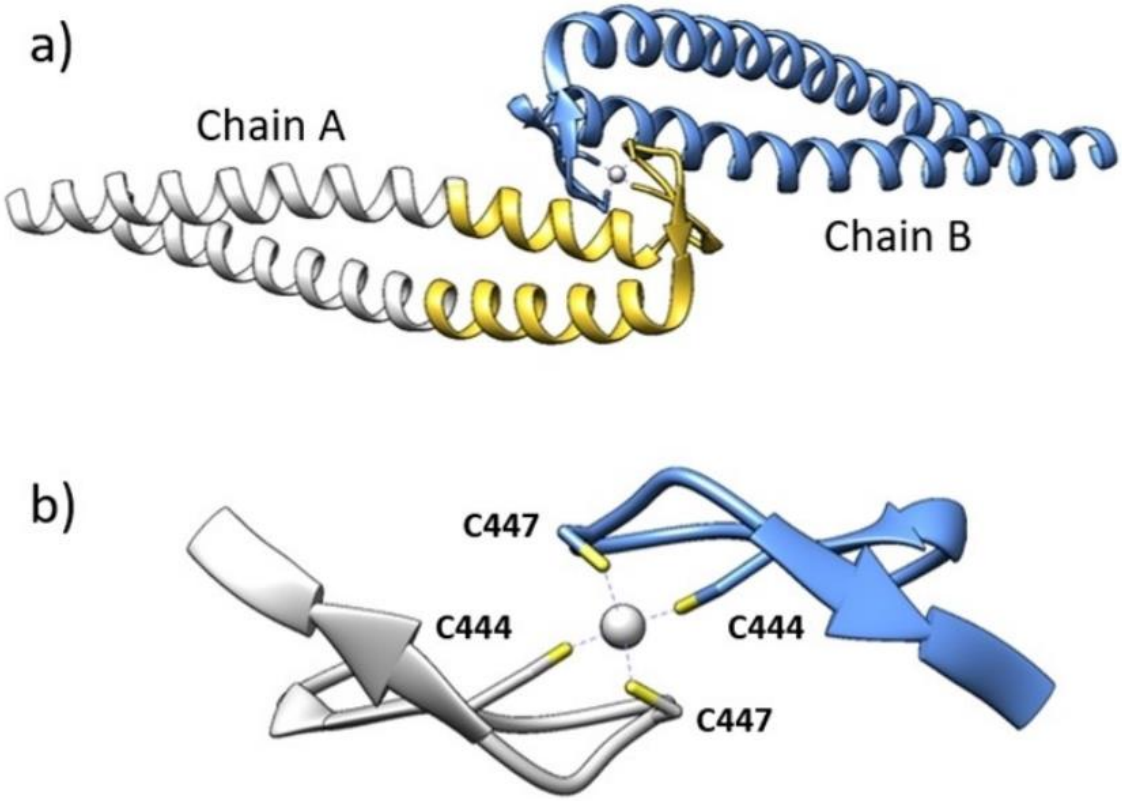

Artur Krezel, Uni. of Wroclaw, Poland

## Recent Bio-PAC @ ISOLDE

- Bacterial resistance against antibiotics (β-lactamases)[1]
- $^{111}$Ag chelators for radiopharmacy[2]
- $^{199m}$Hg and $^{111}$Ag reference compounds[3]
- Hg(II) toxicity: Hg(II) binding to Zn-hooks[4]
- Metal ion sensor proteins[5,6]

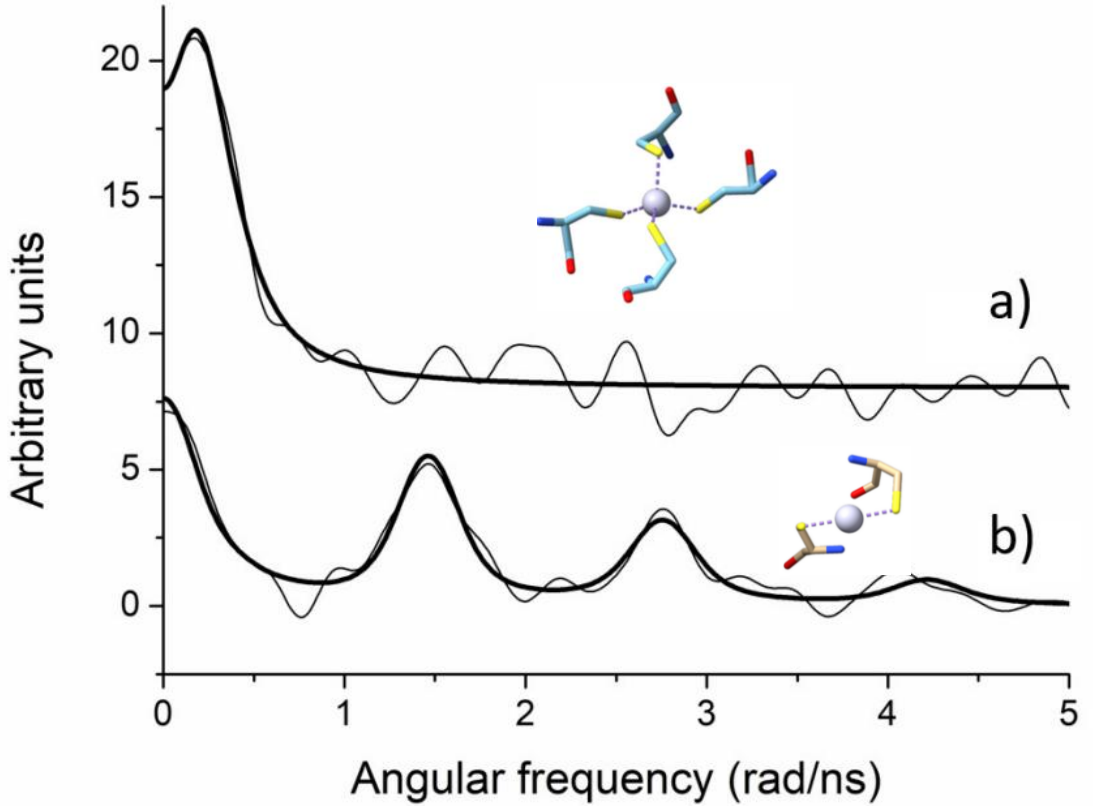
1. Nafaee *et al.*, Protein Science, 2023, 32, e4809
2. Tosato *et al.*, Applied Radiation and Isotopes, 2022, 190, 110508
3. Jancso *et al.*, Nuclear Instr. Methods in Physics Research A 2021, 1002, 165154
4. Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738
5. Balogh *et al.*, Hyperfine Interactions, submitted
6. Tóth *et al.*, J. Am Chem. Soc., submitted

# Coordination geometry of Zn(II) in the Zn-hook




Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738

# Coordination geometries of Hg(II) in the Zn-hook: $^{199m}$Hg PAC data




**1:2 Hg(II):Hook**
**$HgS_4$**

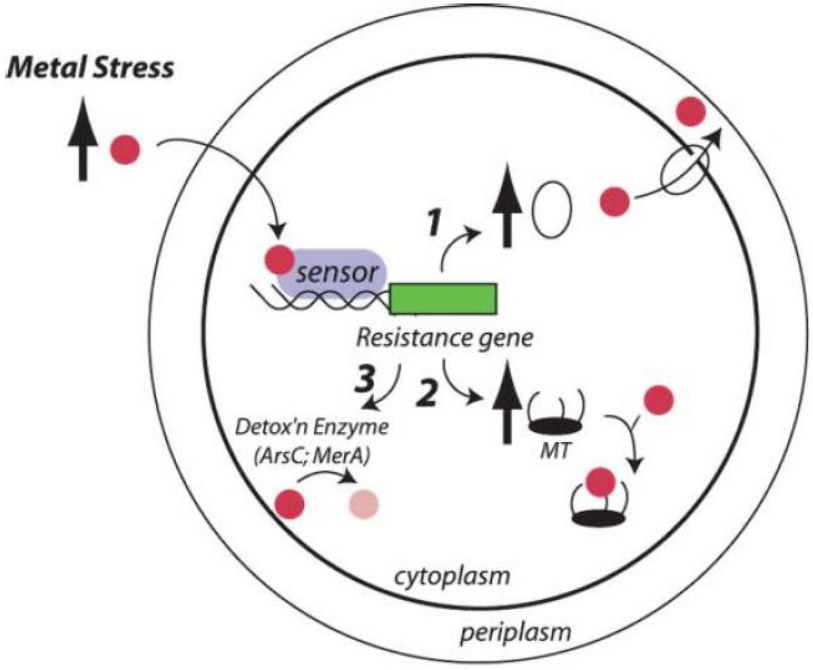
**1:1 Hg(II):Hook**
**$HgS_2$**

**Surprising Hg(II) preference for $HgS_4$ and most stable metal ion-protein complex reported**

Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738

# Applications of PAC spectroscopy to proteins

Lars Hemmingsen (lhe@chem.ku.dk), ISOLDE workshop 2023



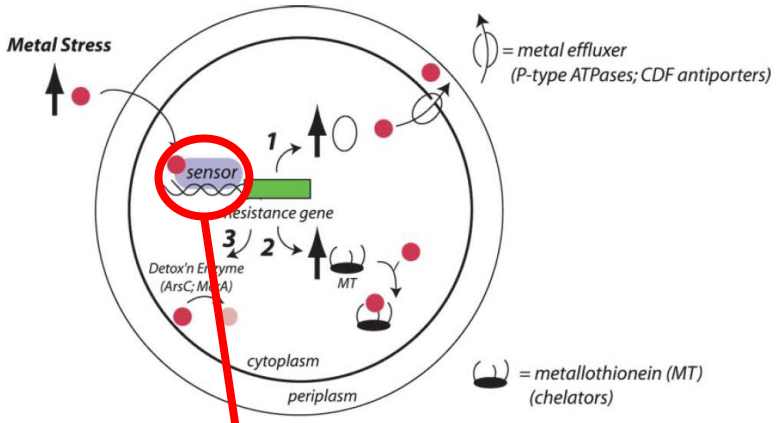

Giedroc and Arunkumar Dalton Trans. 2007, 3107

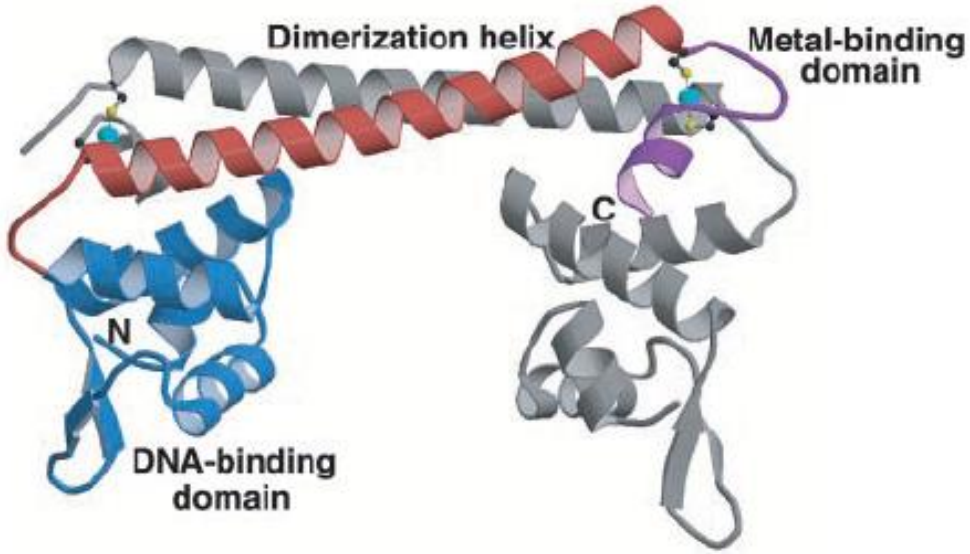
## Recent Bio-PAC @ ISOLDE

- Bacterial resistance against antibiotics (β-lactamases)[1]
- $^{111}$Ag chelators for radiopharmacy[2]
- $^{199m}$Hg and $^{111}$Ag reference compounds[3]
- Hg(II) toxicity: Hg(II) binding to Zn-hooks[4]
- Metal ion sensor proteins[5,6]

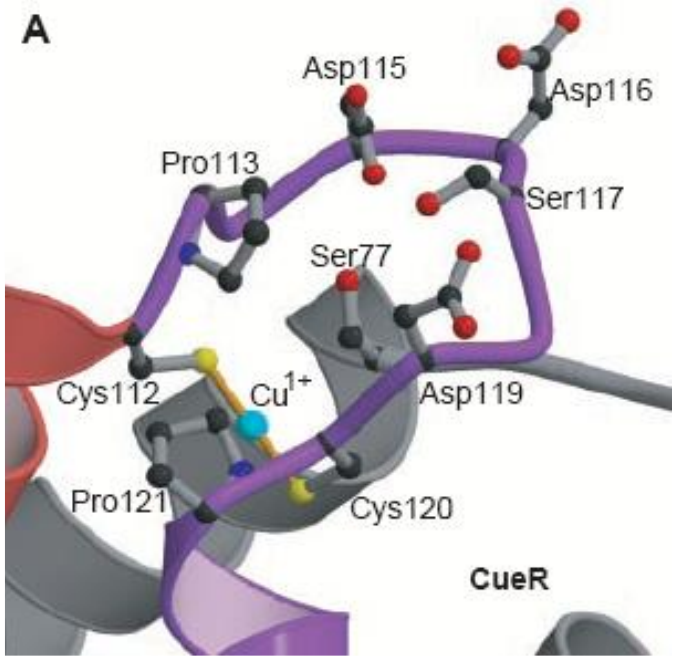
1. Nafaee *et al.*, Protein Science, 2023, 32, e4809
2. Tosato *et al.*, Applied Radiation and Isotopes, 2022, 190, 110508
3. Jancso *et al.*, Nuclear Instr. Methods in Physics Research A 2021, 1002, 165154
4. Luczkowski *et al.*, Chem. Eur. J., 2022, 26, e202202738
5. Balogh *et al.*, Hyperfine Interactions, submitted
6. Tóth *et al.*, J. Am Chem. Soc., submitted

# In *E. coli* $Cu^{I}$ and $Ag^{I}$ is controlled by CueR



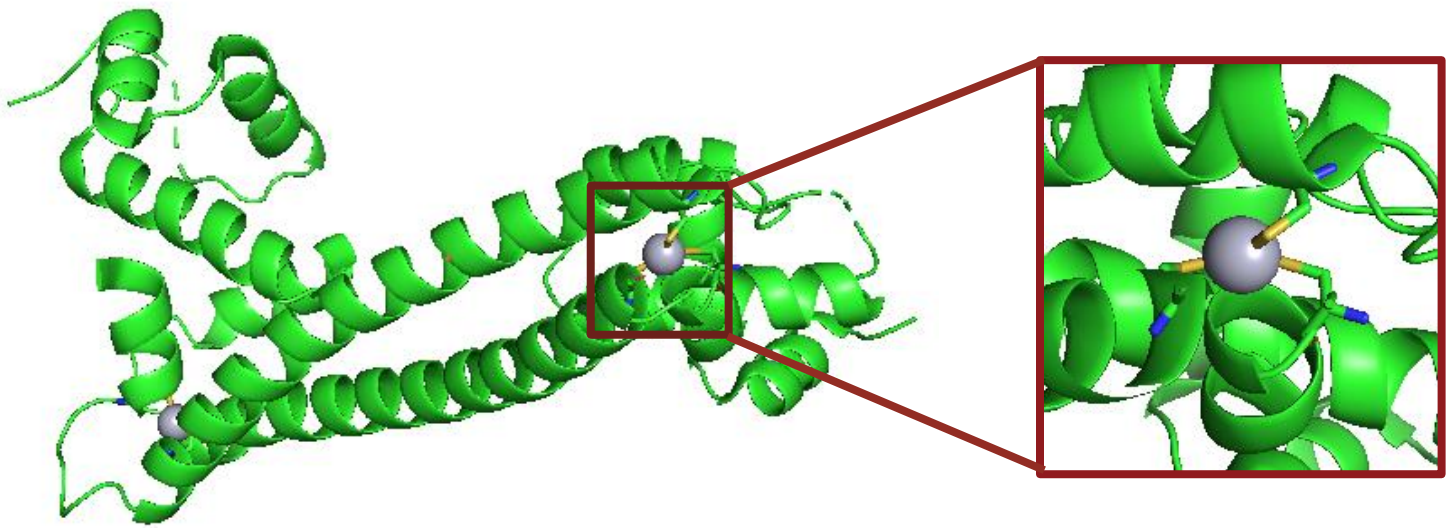

**With a distorted linear $CuS_2$ metal site**




Changela et al., Science, 2003, 301, 1383; Philips et al. Science, 2015, 349, 877

# What controls the metal ion selectivity?

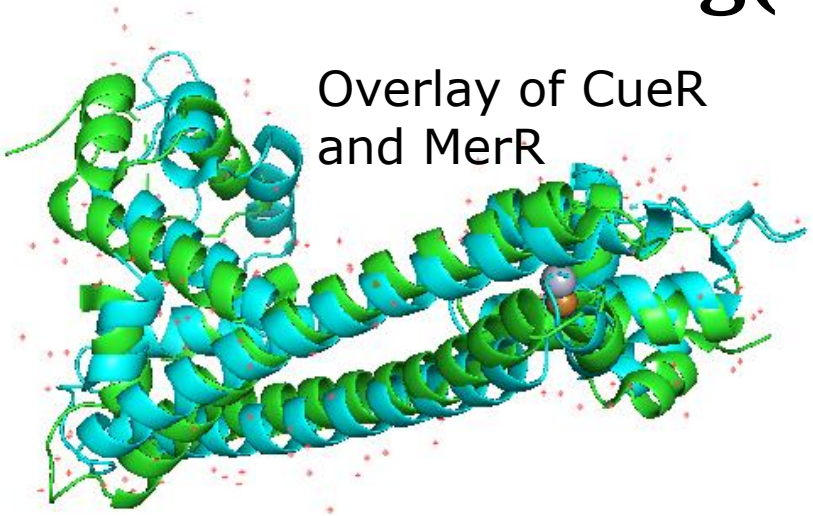
## The MerR protein is a Hg(II) sensor



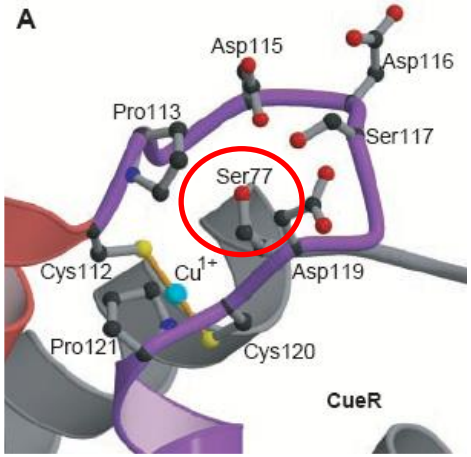
**With a distorted trigonal planar $HgS_3$ metal site**

Wang *et al.* Sci Rep., 2016, 6, 33391; Balogh *et al.,* Hyperfine Interactions, submitted

# Re-designing the CueR protein to become a Hg(II) sensor



Overlay of CueR and MerR




**S77C-CueR**

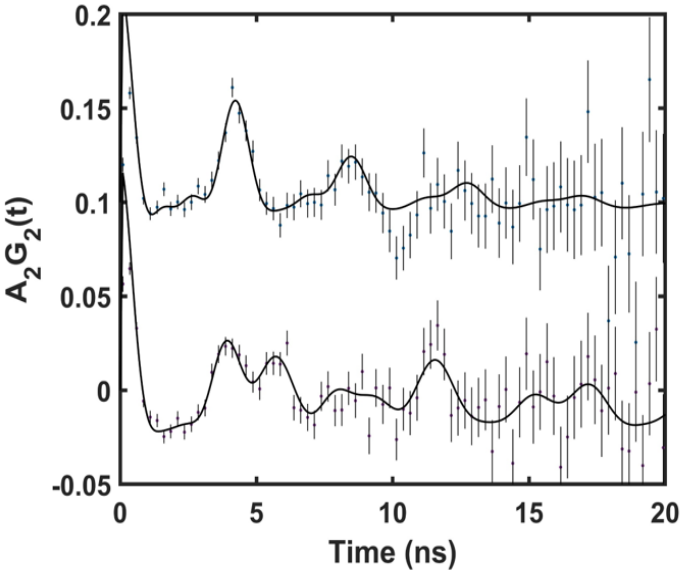
(actually S77C-ΔC7-CueR)

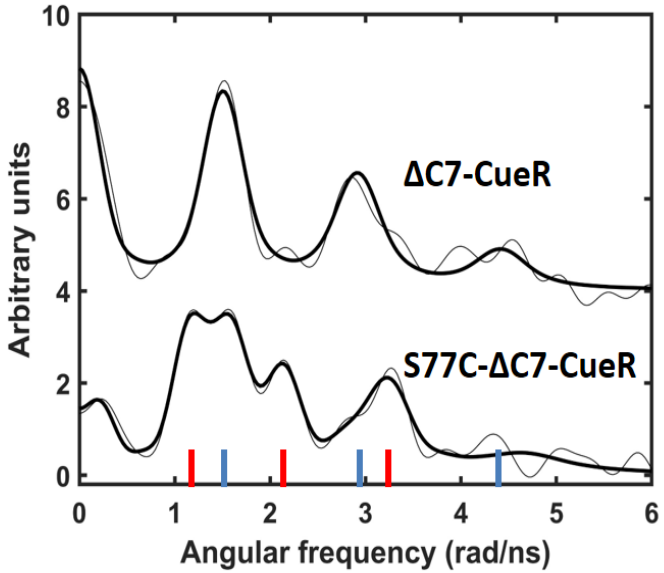
```
Cu,Ag,Au(I)   CueR(E coli)              72  DPQ.RHSADVKRRTLEKVAEIERHIEELQSMRDQLLALANACPG.DDSA..DCPIIENLS.GCCHHRAG.
              CueR(P putida)            72  DRQ.RASADVKALARQHIDELNQKIRELGELRDTLQDLVEHCSG.DHRP..DCPILKELASGCCAQPARA
              CueR(Y pestis)            72  NPA.RHSADVKAATLLKVAEIEQHINDLNQMRMRLLALAEECPG.DEGA..DCPIINSLA.GCCHSSSSL
              CueR(V cholerae)          72  DPN.RTSAAVRARAQEKWQEISRKLSELTMIKQQLEEWIASCPG.DQGS..DCPIIEQLKGHCCSNNKTK
              CueR(S typhi)             72  DPR.RHSADVKKRTLEKVAEIERHISELQSMRDQLLAMAESCPG.DDSA..DCPIIDNLS.GCCHHKAQK
              HmrR(S meliloti)          72  DRS.RASSDVKAVALEHIAELERKIAAIQDMTRTLKHLASHCHG.DGRP..DCPIIEEMAKGGGAAKTEI
              HmrR(R leguminosarum)     71  DKD.RASADVRDIAQTKLTEIDRKIRELTELRRTLEHLVHACHGND.RP..DCPILEELSDGA.......
              PmtR(P mirabilis)         72  NRE.RTSADVKAIALSHIDELNRKITQLQRMTQTLSHLAQECQG.DNNP..DCPIIAKLVEPQTGTEH..

Hg(II)        MerR(Tn501)               78  DGTH..CEEASSLAEHKLKDVREKMADLARMEAVLSELVCACHA..RRGNVSCPLIASLQGGASLAGSAM
              MerR(Tn21)                78  DGTH..CEEASSLAEHKLKDVREKMADLARMETVLSELVCACHA..RKGNVSCPLIASLQGEAGLARSAM
              MerR(S marcescens)        78  DGTH..CEEASSLAEHKLQDVREKMTDLARMETVLSELVFACHA..RQGNVSCPLIASLQGEKEPRGADA
              MerR(S aureus)            73  DQDGERCKDMYAFTVQKTKEIERKVQGLLRIQRLLEELKEKCP..DEKAMYTCPIIETLMGGPDK.....
              MerR(B sp. RC607)         73  DRDEAKCRDMYDFTILKIEDIQRKIEDLKRIERMLMDLKERCP..ENKDIYECPIIETLMKK........
```

Wang *et al.* Sci Rep., 2016, 6, 33391; Changela *et al.,* Science, 2003, 301, 1383; Balogh *et al.,* Hyperfine Interactions, submitted

# Re-designing the CueR protein to become a Hg(II) sensor: $^{199m}$Hg PAC data



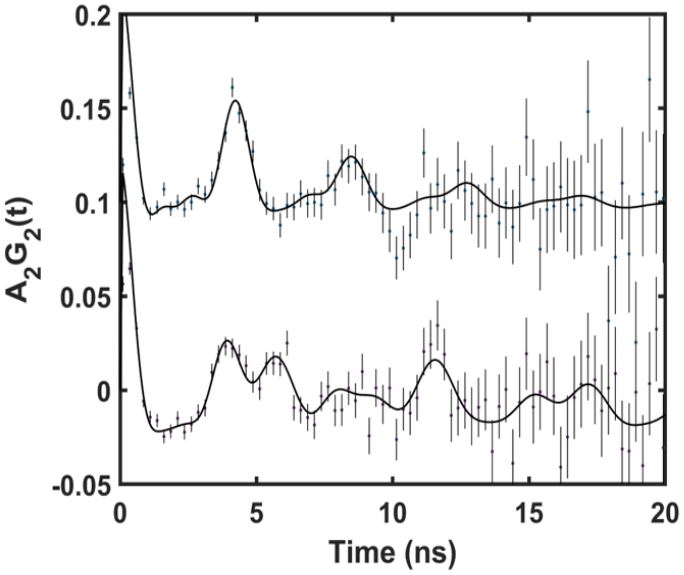



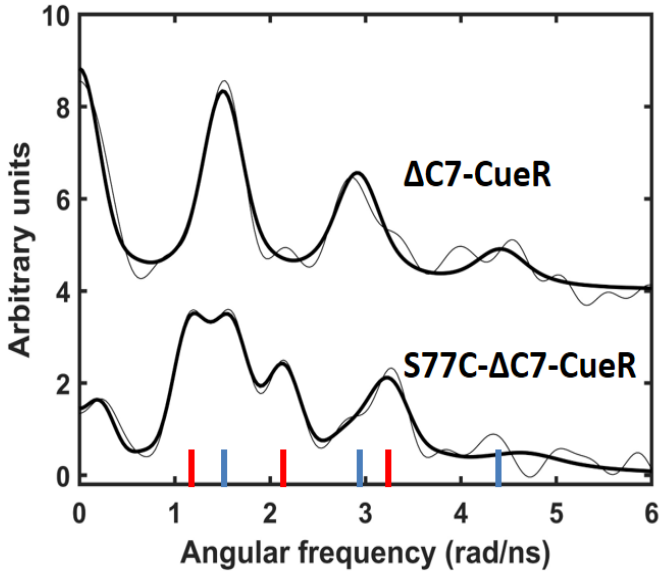

$HgS_2$

$HgS_2$ and $HgS_3$

Balogh *et al.*, Hyperfine Interactions, submitted

# Re-designing the CueR protein to become a Hg(II) sensor: $^{199m}$Hg PAC data




$HgS_2$

$HgS_2$ and $HgS_3$

**The effort to create a $HgS_3$ site was (only) partly successful**

**=> changes beyond the first coordination sphere are required**

Balogh *et al.*, Hyperfine Interactions, submitted

# Collaborations

University of Szeged, Hungary

Attila Jancso

Rasmus Fromsejer

Kurt V. Mikkelsen  Niels J.Christensen

Béla Gyurcsik

Peter W. Thulstrup  Marianne L. Jensen

Ria K. Balogh

Eva Freisinger

Karl Johnston

Joao G.M. Correia

Juliana Schell

Marek Łuczkowski

Michał Padjasek

Józef Ba Tran

Olga Kerber

Jelena Habjanič

Artur Krężel

# Funding


CERN


ISOLDE


Bundesministerium
für Bildung
und Forschung

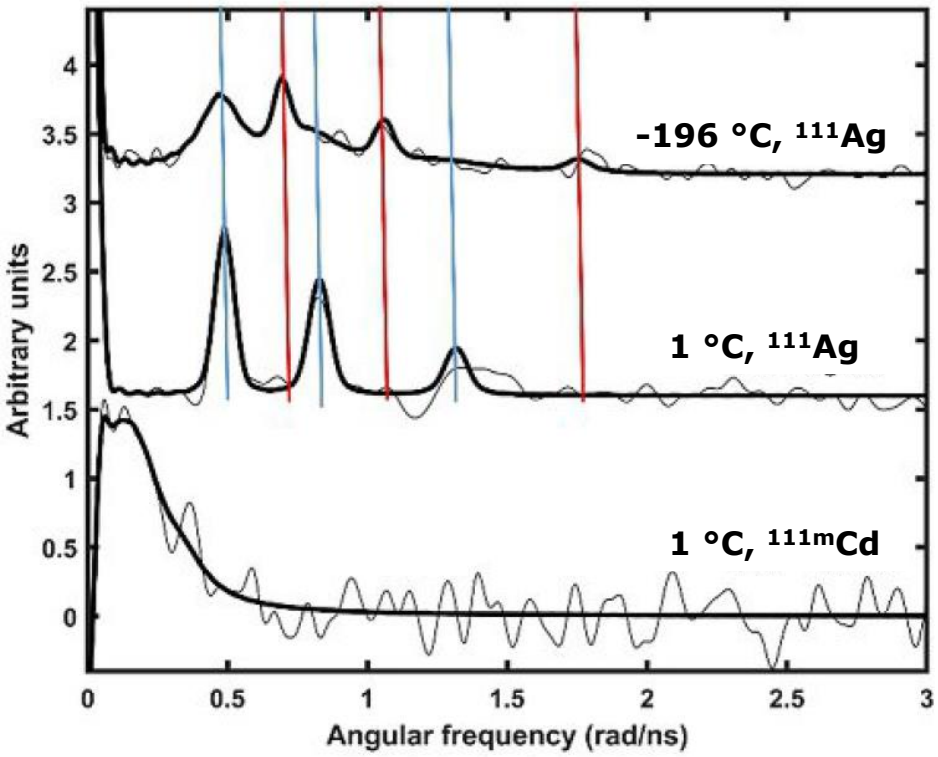

Danish Agency for Science
Technology and Innovation
Ministry of Science
Technology and Innovation

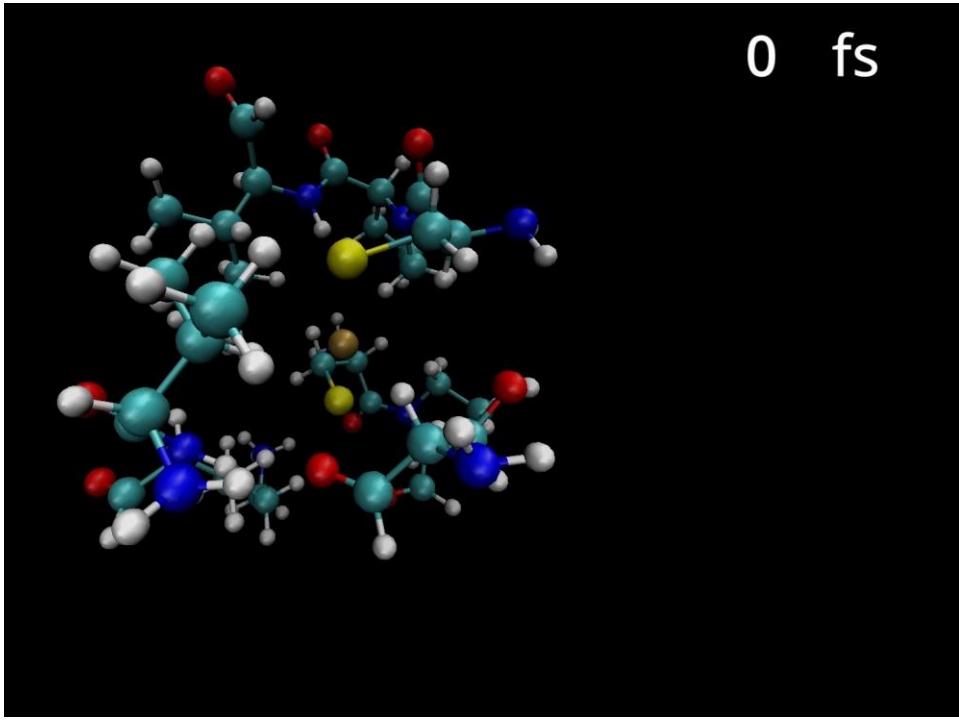

INDEPENDENT RESEARCH
FUND DENMARK

# $^{111}$Ag PAC applied to the CueR protein

## Relaxation phenomena following nuclear decay

$$^{111}Ag^{I} \rightarrow {}^{111}Cd^{II} + e^{-} + \bar{\nu}_e$$




Balogh *et al.,* Chem. Eur. J., 2020, 26, 7451; Fromsejer *et al.,* PCCP, 2021, 23, 25689; Balogh *et al.,* Hyperfine Interactions, submitted