Presentation Of Peng Chen`s Lab

NanJing University

Na Yang 2014-4-12

About The Team

They are a chemical biology lab focusing on **P**rotein **CH**emistry and **EN**gineering

Research

- Photo-affinity probes for studying protein-protein interactions in living cells.
- Visualization of organic hydroperoxides in living cells.
- Protein Bioorthogonal labeling in living cells.

J|A|C|S COMMUNICATIONS

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Dynamic Copper(I) Imaging in Mammalian Cells with a Genetically Encoded Fluorescent Copper(I) Sensor

Seraphine V. Wegner, Hasan Arslan, Murat Sunbul, Jun Yin, and Chuan He*

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Received November 16, 2009; E-mail: chuanhe@uchicago.edu

- They constructed a FRET reporter for copper(I),taking advantage of the conformational change induced by copper(I) binding to Amt1.

- Amt1-FRET, is highly sensitive and selective for Cu^{+} over other metal ions.

- Cu^{+} reporter shows great potential for imaging dynamic Cu+ fluctuationInside mammalian cells.

Background

ظ As one of the most important catalytic cofactors in proteins, copper is an essential element for life.

ظ Among the first-row transition metals, copper has an intrinsic high affinity for most ligands, cellular copper is associated with highaffinity copper binders.

ظ Small-molecule sensors may have issues such as water solubility, toxicity, cell permeability .

ظ Highly selective and sensitive to copper(I) and gives dynamic response to fluctuations in copper(I) availability in its biological window inside live cells is highly desirable.

The design

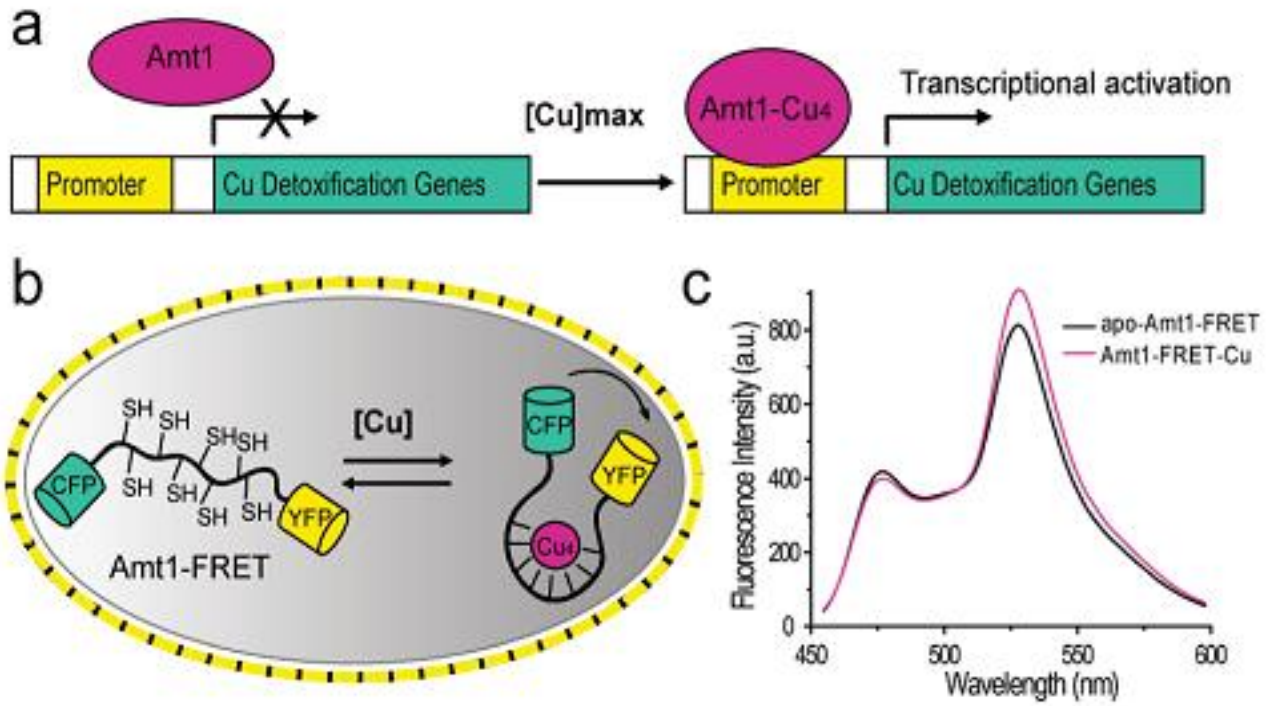


Figure 1. Design of Amt1-FRET for copper(I) imaging inside live cells.

[Cu]max indicates the upper limit of copper level sensed by Amt1 in the cell.
Amt1 consists of three distinct domains: a zinc finger domain, a copper-binding domain, and a transactivation domain

Dobi, A.; Dameron, C. T.; Hu, S.; Hamer, D.; Winge, D. R.J. Biol. Chem.1995, 270, 10171–10178

This Study about selectivity

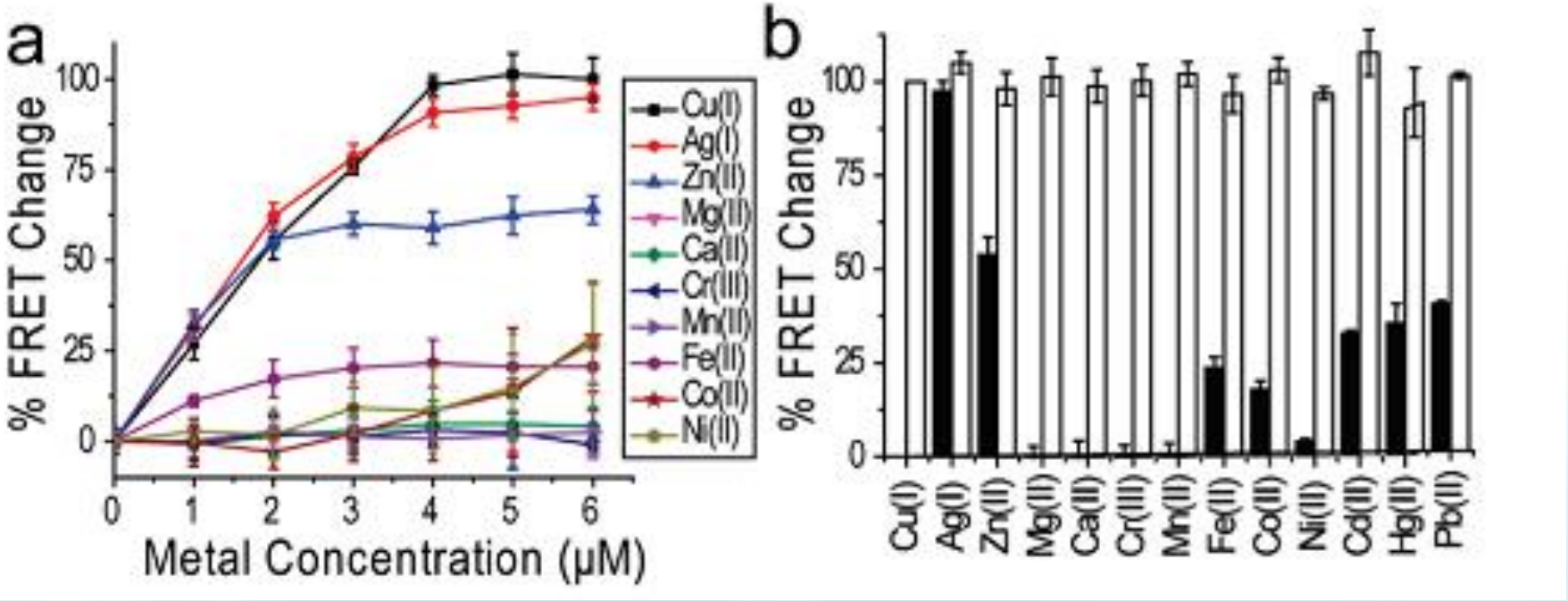
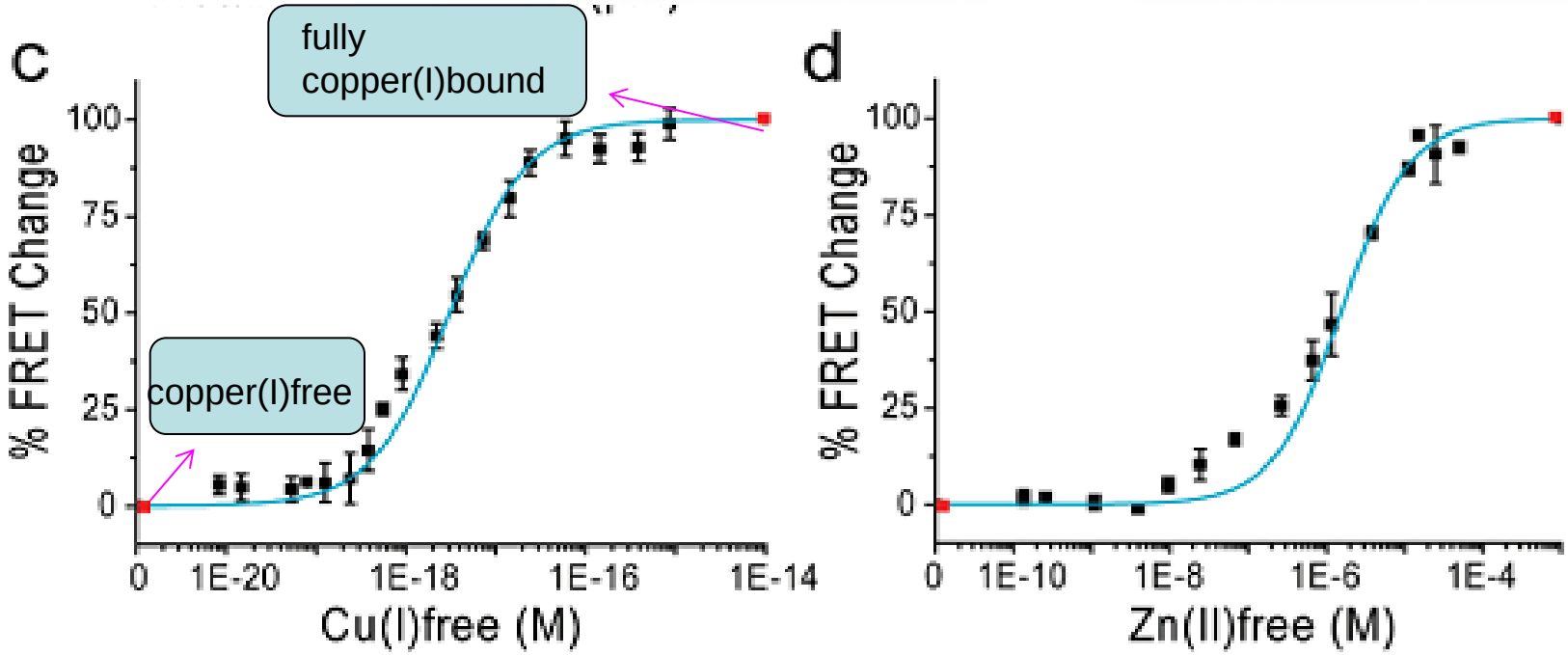


Figure 2

(a) Amt1-FRET titration with different metal ions in the presence of 4 mM DTT.

(b) Selectivity of Amt1-FRET to Cu(I). Amt1-FRET (1μM) with 5μM of the respective metal ions

This Study about affinity



Binding curve of Amt1-FRET to (c)copper(I) and (d) zinc(II)

Free Cu+ concentration : 8.6×10-16 to 8.3×10-21M using cyanide.
Free Zn2+ concentration : 5.0×10-5M to1.3×10-10M
Kd for Cu+binding: 2.5×10-18 M
Kd for Zn2+ binding: 1.4×10-6 M

Test inside mammalian cells

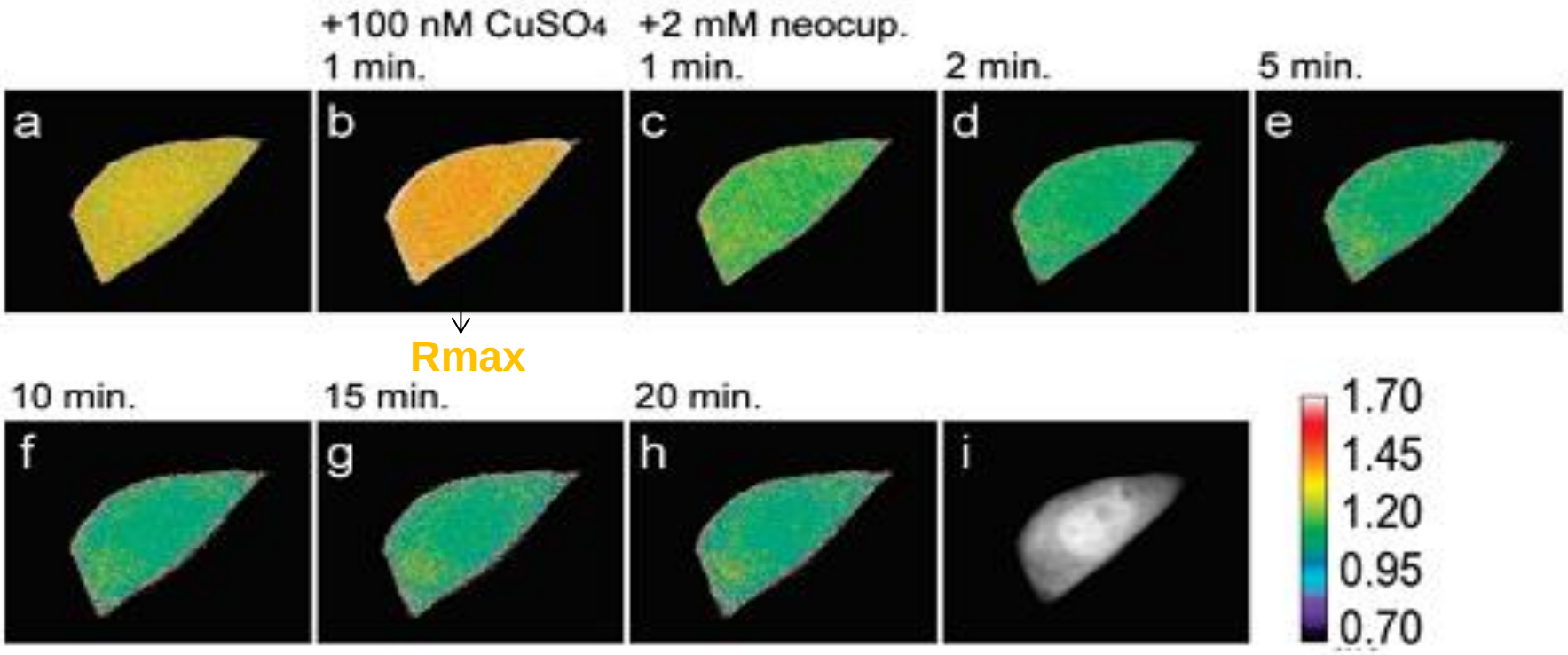


Figure 3. Imaging of available copper(I) in CHO-K1 cells by Amt1-FRET.

Neocuproine : copper(I) ligand, to find the minimum ratio (Rmin).

Elimination of interference

Addition of 1μM $Zn(NO_3)_2$ to the growth medium

2mM of a Zn^{2+} ligand EGTA was added to the cells preincubated with 10μM Zn^{2+}

no effect on the FRET signal ratio

FRET signal ratio returned to the original level but did not decrease further

initial ratio is solely due to Cu^{+} response and not to Zn^{2+}

Conclusion

- The resulting reporter, Amt1-FRET, is highly sensitive and selective for Cu^{+} over other metal ions.

- Copper imaging in CHO-K1 cells with the reporter suggests that the level of available copper inside mammalian cells may also be tightly controlled.

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

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Genetically Encoded Copper(I) Reporters with Improved Response for Use in Imaging

Jun Liu,[†] Jason Karpus,[‡] Seraphine V. Wegner,[‡,#] Peng R. Chen,[*,†,§] and Chuan He[*,†,‡]

- A genetically encoded copper(I) probe capable of monitoring copperfluctuations inside living cells.
- Insert the copper regulatory protein Ace1 into a yellowfluorescent protein

Background

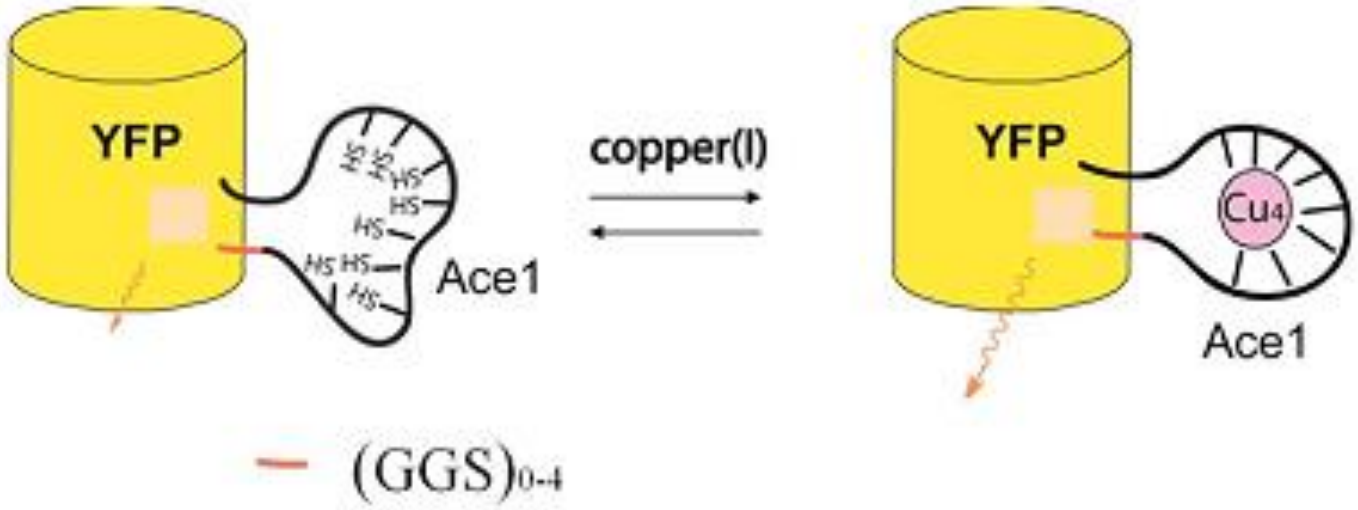
- The level of labile copper is relatively low due to the capacity of many proteins and other ligands to bind copper with high affinity.

- In Escherichia coli, the concentration of labile copper is tightly regulated by the regulatory protein CueR and has been reported to be $\sim 10^{-21}$M.

- Due to the high affinity of certain metalloregulatory proteins for copper, it has been difficult to measure and monitor changes in the concentration of copper(I) inside cells.

- Amt1-FRET made it difficult to vary the copper binding affinity of the probe.

The Design



The design of a series of YFP-Ace1, with varying linker lengths.

- The copper(I) binding domain of Ace1 was cloned between residues Y145 and H146 of YFP.
- Different lengths of GGS linkers were added to tune the binding affinity and response level.

In Vitro Characterization of YAGn Probes.

In this study, there are five reporters with different numbers of GGS linkers (0−4) . The probes were named YAGn, where ncorresponds to the number of GGS linkers inserted into the probe.

YFP-Ace1 constructs was expressed inE. Coli in the presence of 1 mM CuSO4

↓

purified via fast protein liquid chromatography (FPLC)

↓

Incubation with cyanide to regenerate the apo form

↓

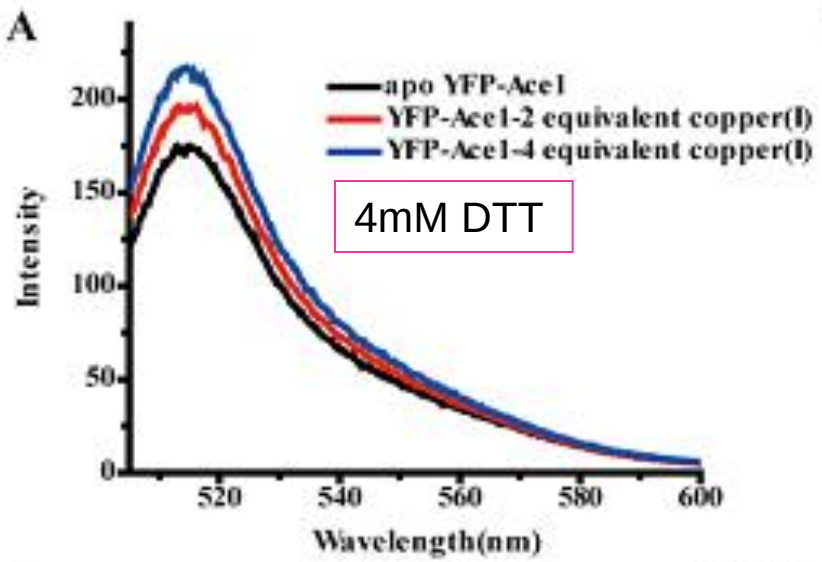
monitoring the copper(I) ,exciting the YFP at 496 nm and emission at 515 nm.

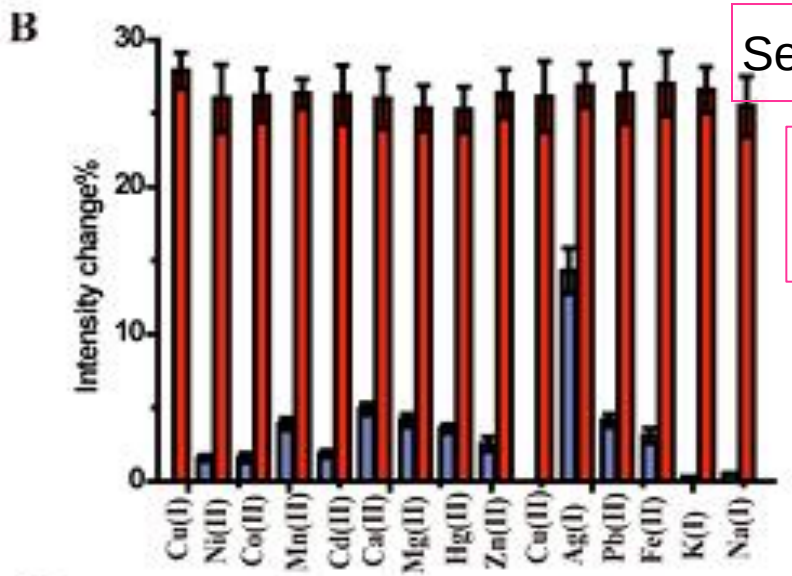
Response to Cu^+ with varying linker lengths

GGS repeats (n)	K_d (M)	response (%)
0	$(8.2 \pm 1.2) \times 10^{-18}$	38
1	$(2.0 \pm 0.8) \times 10^{-18}$	35
2	$(1.2 \pm 1.0) \times 10^{-18}$	30
3	$(4.6 \pm 1.2) \times 10^{-19}$	25
4	$(3.3 \pm 0.9) \times 10^{-19}$	25

- The addition of Cu^+ caused a significant increase in the emission for all of the YAGn probes
- Complete saturation of YAG0 with Cu^+ generated a nearly 40% increase in fluorescence emission, with similar increases observed for the other probes.

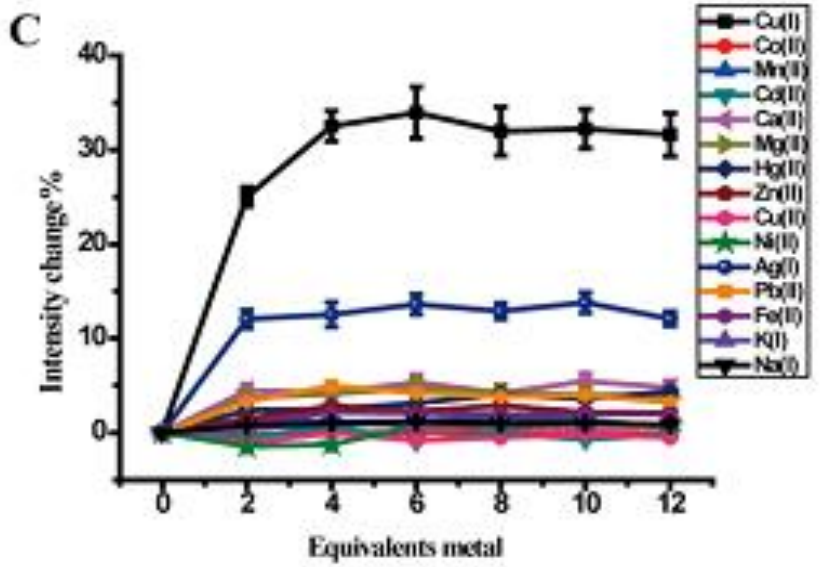
Fluorescent response of YAG2

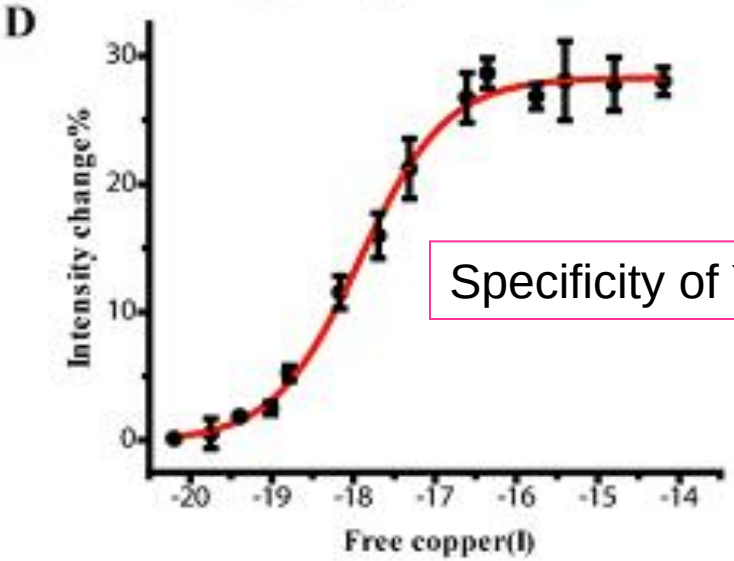




Selectivity

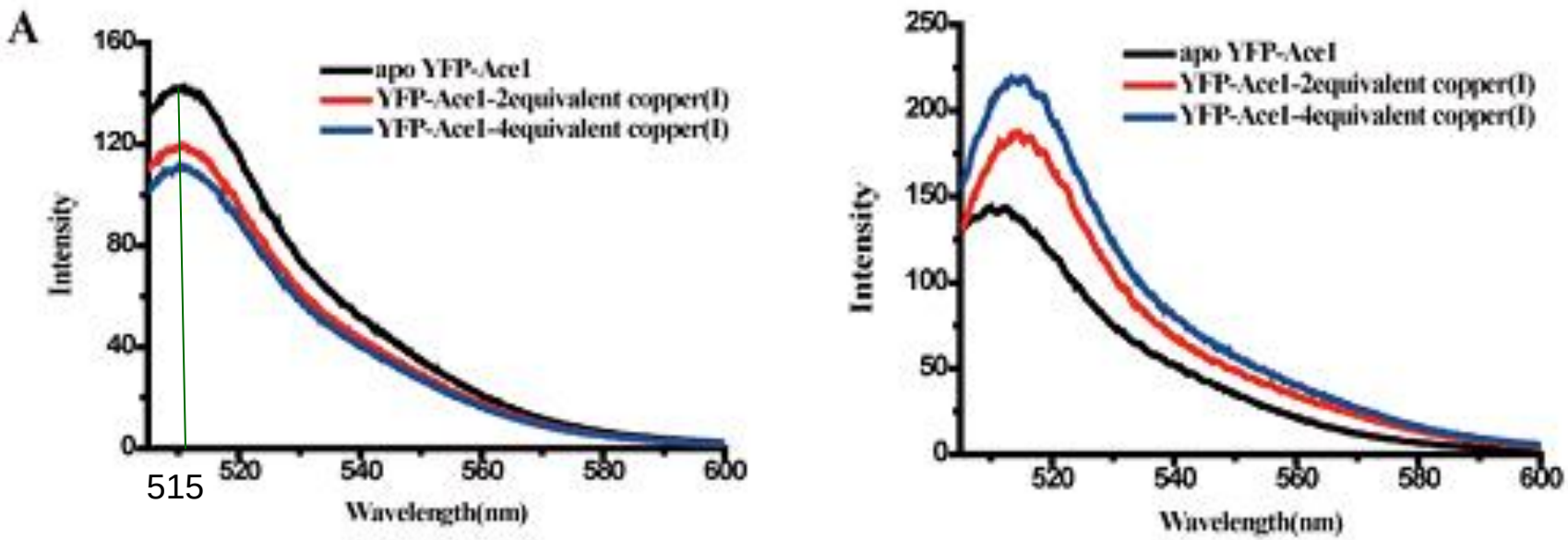
1μM
5μM
5 μM





Specificity of YAG2

Ratiometric properties

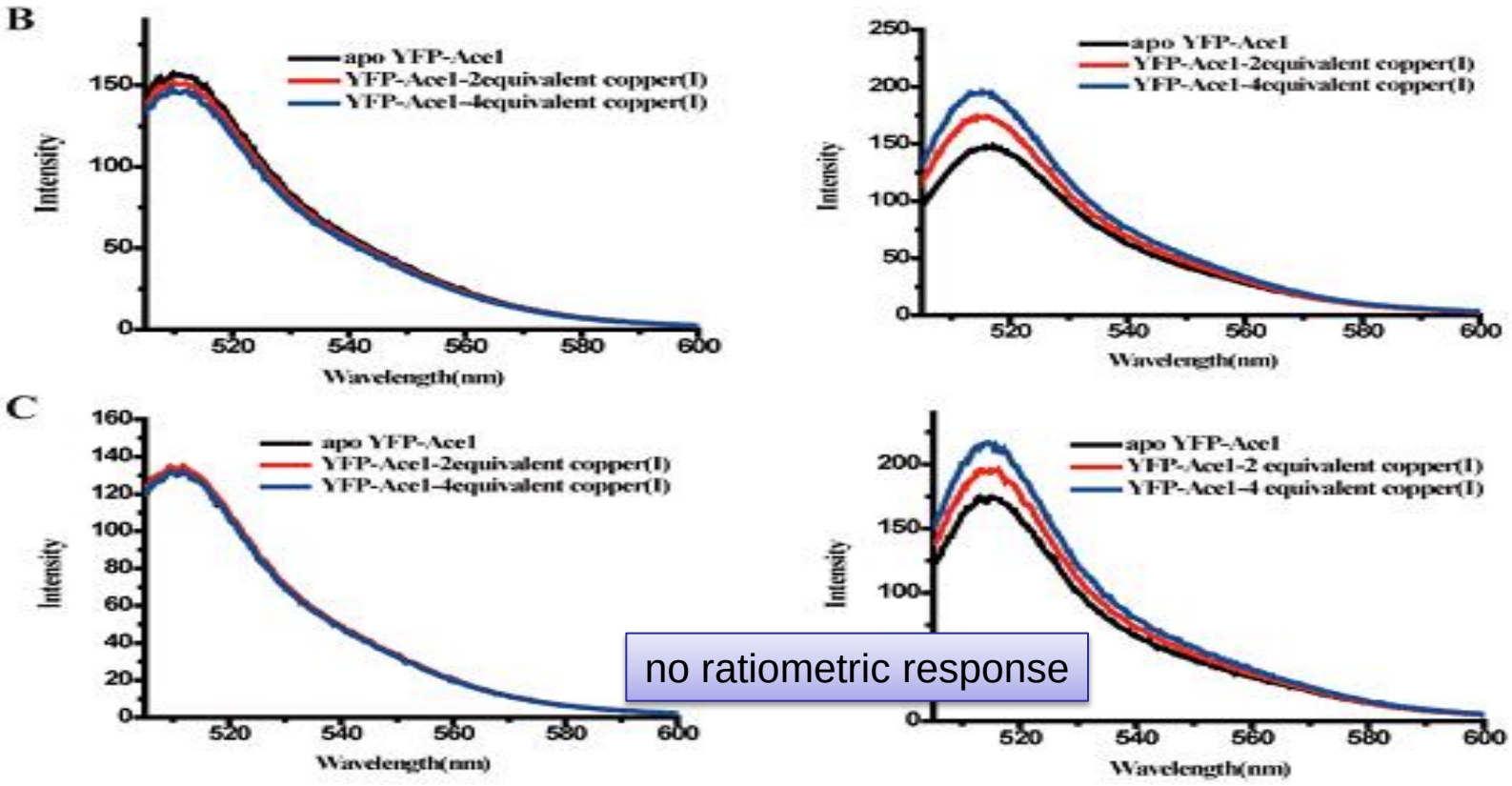


Fluorescent responses of (A) YAG0 when excited at 440nm (left) and 494 nm (right) when supplemented with additional copper(I)

Ratiometric properties

440nm

494nm



no ratiometric response

Fluorescent responses of (B) YAG1、 (C)YAG2

Selectivity of the YAGn

YAG0

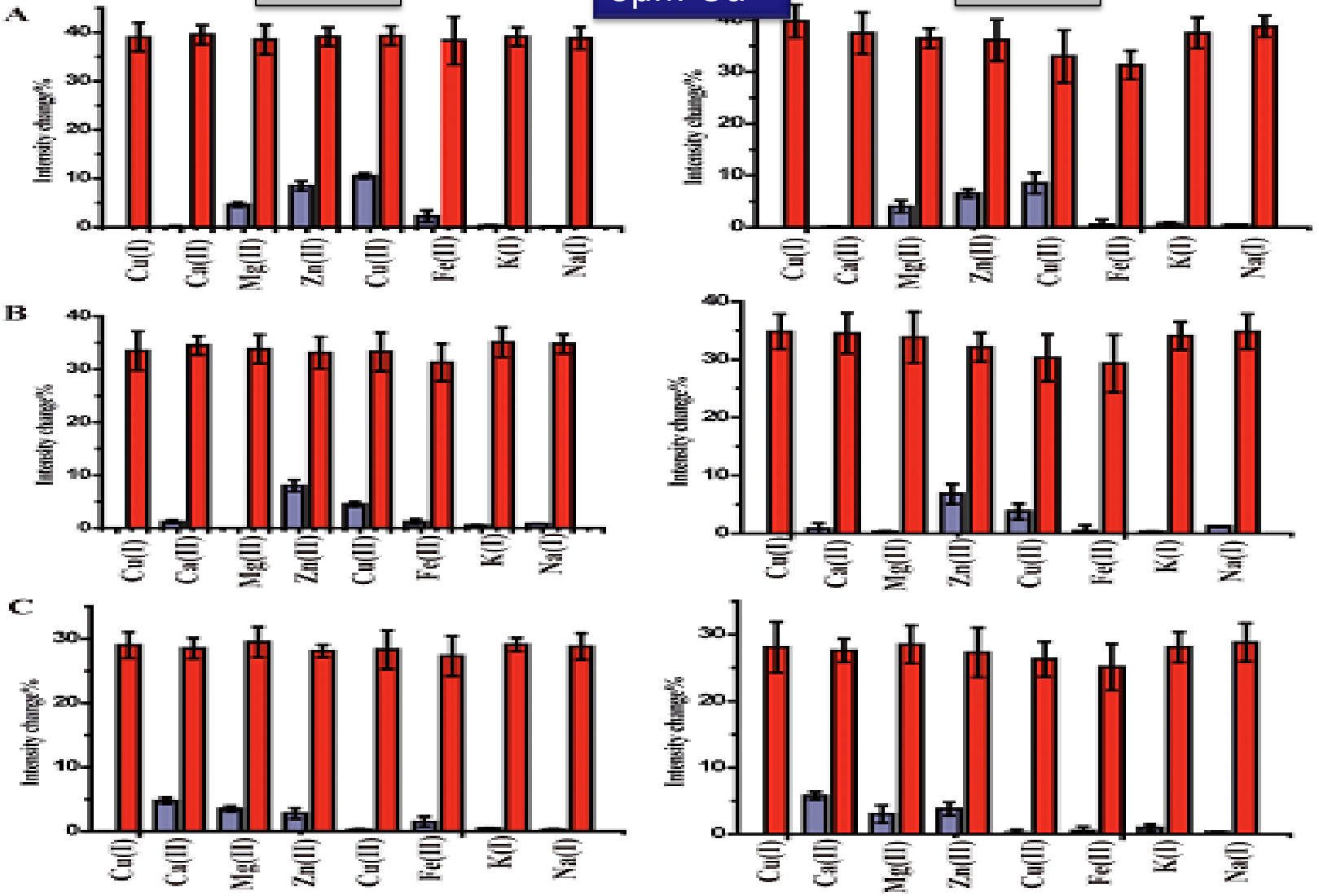
YAG1

YAG2

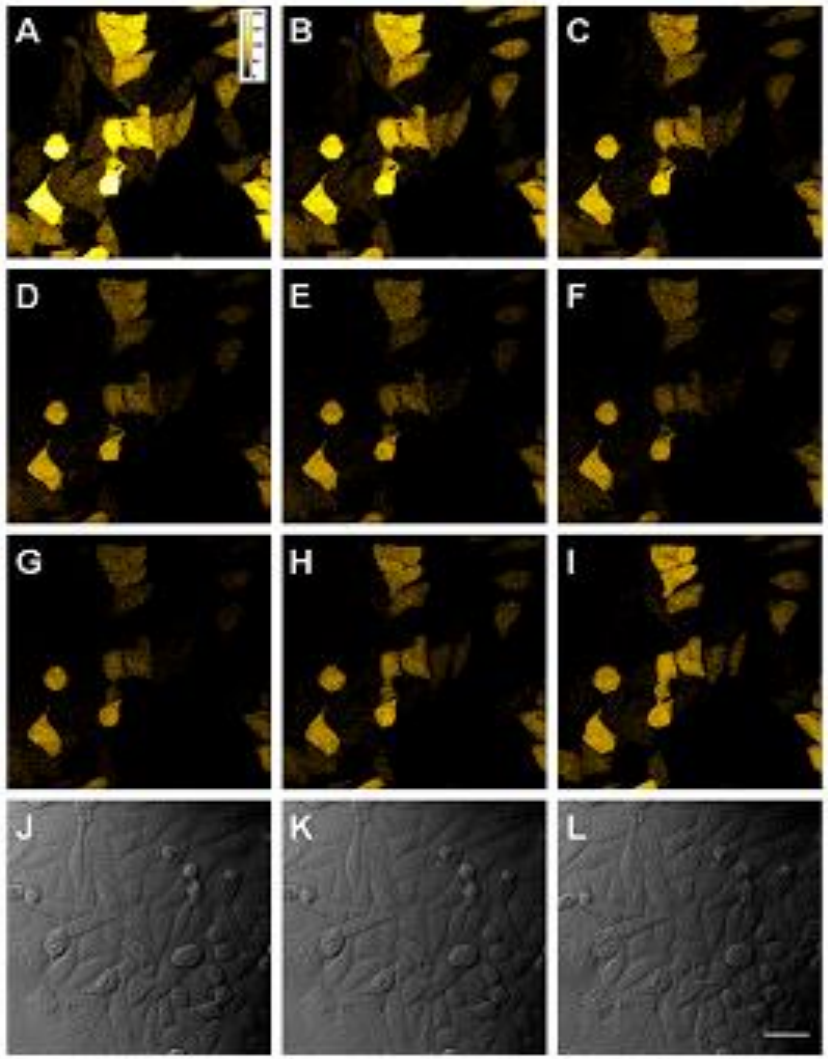
10μM

5μM Cu^{+}

50μM



In Vivo Characterization of YAG2



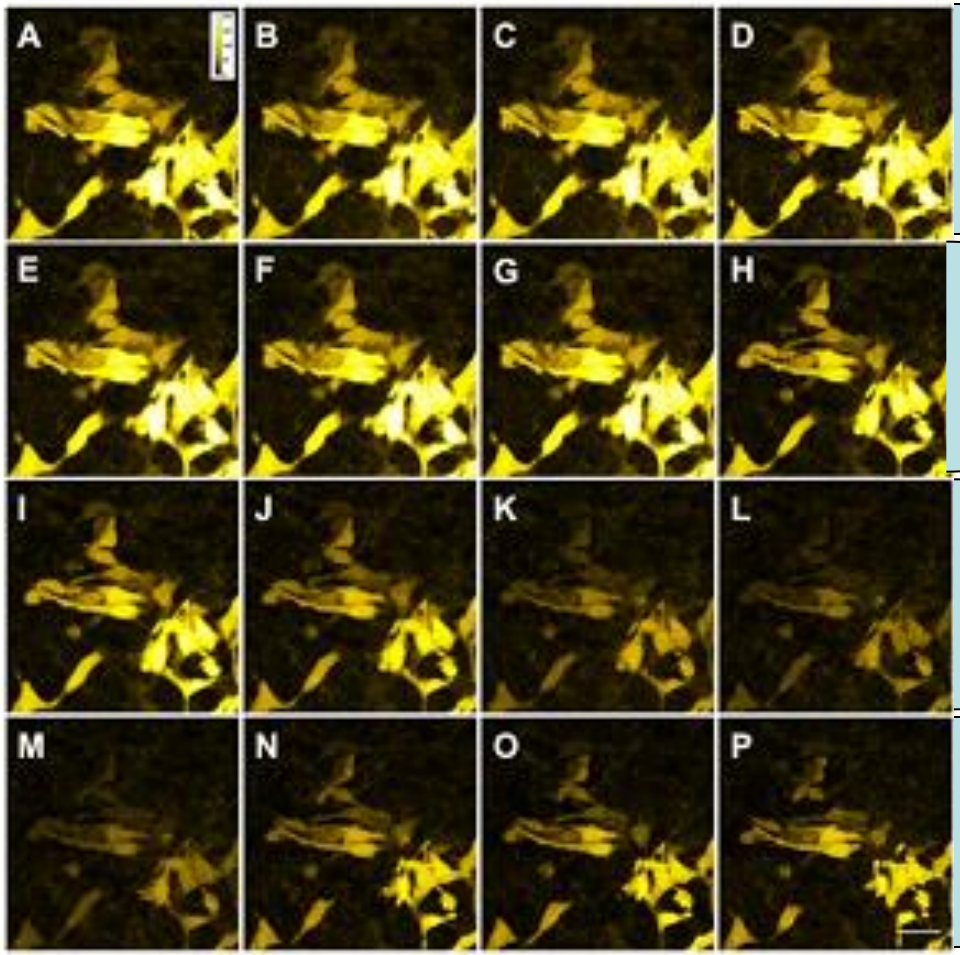
HeLa cells expressing YAG2 were incubated with 50μMCu⁺for (A) 0s, (B) 30 s, (C) 1 min, (D) 2 min, (E) 3 min, and (F) 5 min.

Subsequent to Cu⁺incubation, the known Cu⁺ chelator neocuprione was added for (G) 0min, (H) 10 min, and (I) 15 min.

DIC images (J) prior to analyte incubation, (K) after 5 min of Cu⁺ incubation(L) after 15 min of neocuprione

YAG2 response to incubation with Cu+in living HeLa cells.

Response of YAG2 in HeLa cells



Cells were incubated with 100μM zinc for (A) 0, (B) 1, (C) 2, or (D) 5 min

Add metal ion chelator EGTA for (E) 0, (F) 1, (G)5, or (H) 10 min prior to imaging

50μM copper(I) was added for (I) 0, (J) 1, (K) 2, or (L) 5 min

1 mM neocuprione was added for (M) 0, (N) 1, (O) 5, or (P) 10 min

Conclusion

- These new reporters demonstrate improved fluorescent responsesin vitro andin vivo.the newly constructed sensors also have varied binding affinities to Cu^{+}

- Shorter linkers seem to be more effective in inducing a more significant conformational change nearer to the fluorophore

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nature chemical biology

ARTICLE

PUBLISHED ONLINE: 3 NOVEMBER 2013 | DOI: 10.1038/NCHEMBIO.1380

The multiple antibiotic resistance regulator MarR is a copper sensor in *Escherichia coli*

Ziyang Hao[1,9], Hubing Lou[1,8,9], Rongfeng Zhu[2], Jiuhe Zhu[3], Dianmu Zhang[1], Boxuan Simen Zhao[1,4], Shizhe Zeng[5], Xing Chen[1], Jefferson Chan[6], Chuan He[1,4,7*] & Peng R Chen[1,2,7*]

- Here we show that copper signaling potentiates MarR derepression in E. coli

- Copper(II) oxidizes a cysteine residue (Cys80) on MarR to generate disulfide bonds between two MarR dimers, thereby inducing tetramer formation and the dissociation of MarR from its cognate promoter DNA.

Background

- MarR family of transcription factors regulates diverse genes involved in multiple antibiotic resistance.

- E. coli MarR resides in the chromosomally encoded Mar locus and negatively regulates the marRAB operon, an essential component that controls the Mar phenotype and various cellular responses

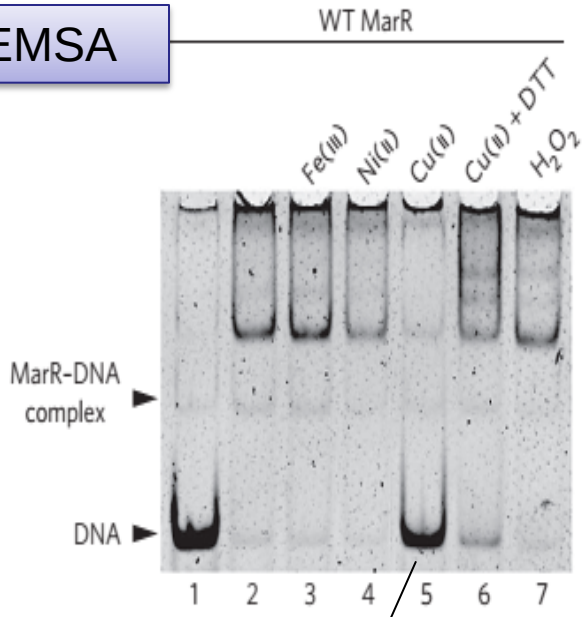
- Salicylate (SAL) has been further shown to trigger the dissociation of MarR from its promoter DNA and cause the derepression of the marRABoperon within E. colicells

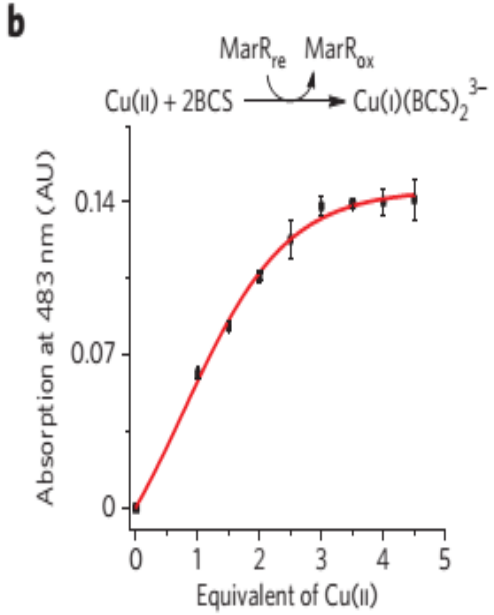
- A cellular product may function as the real signal for MarR derepression.

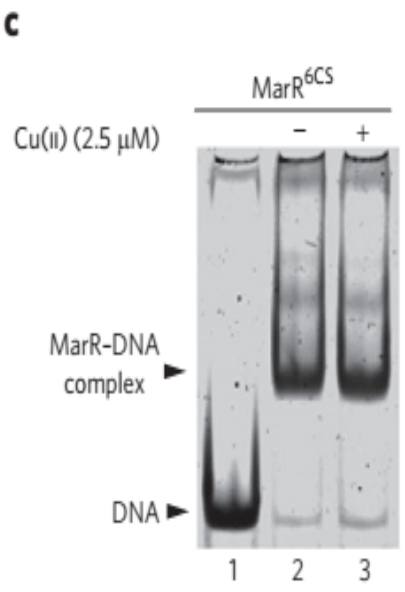
Copper(II) triggers the dissociation of MarR from DNA

Figure 1 | Copper(II) is a natural signal for MarR derepression.

EMSA





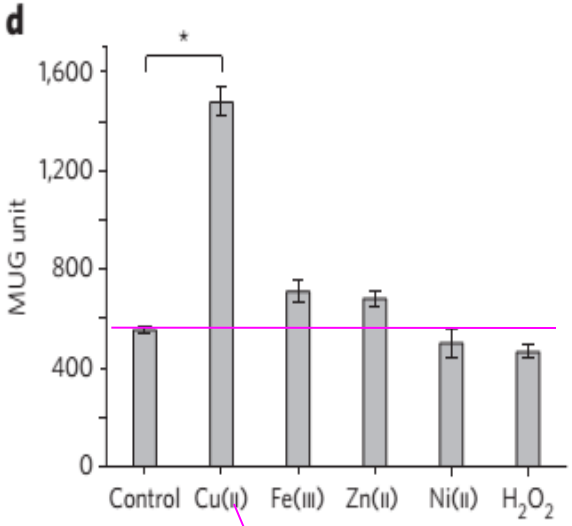


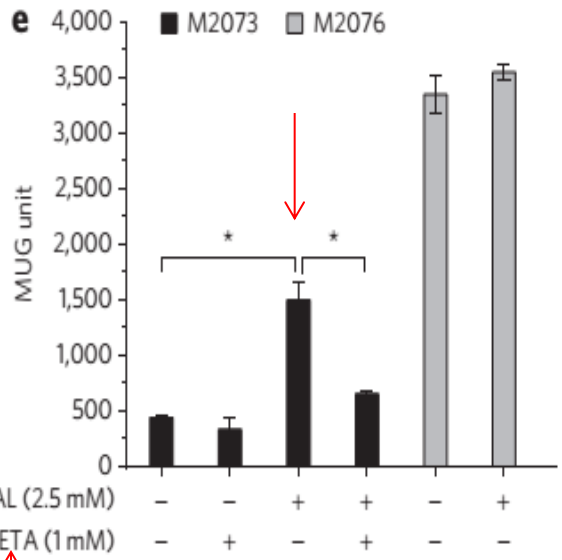
The interaction between MarR and copper(II) is redox sensitive

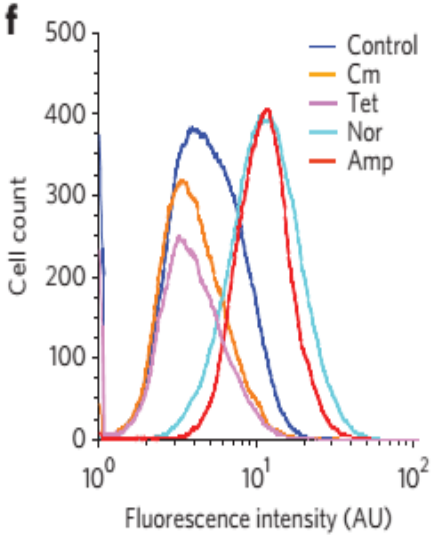
BCS: copper(I)-specific indicator

DNA-binding capability of $MarR^{6CS}$ mutant

Copper(II) is a natural inducer for MarR inside E. coli





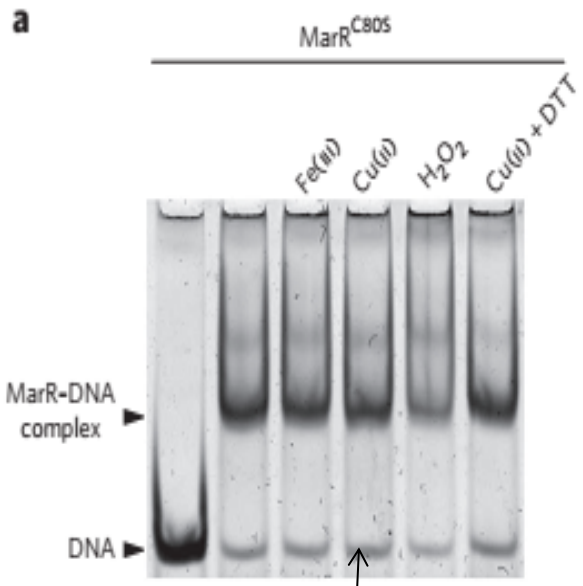


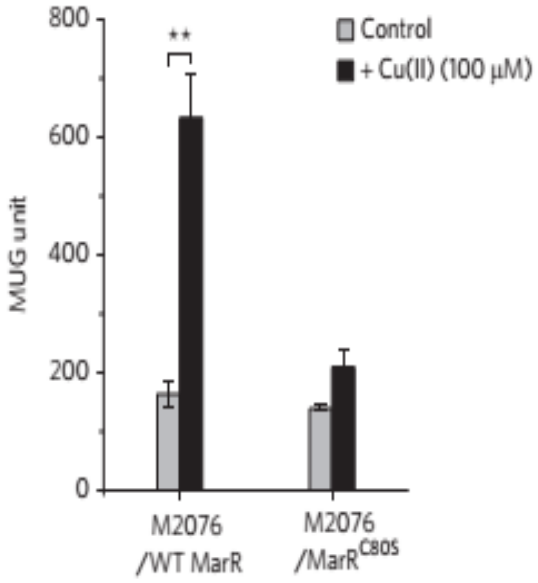
threefold increase of β-Gal activity

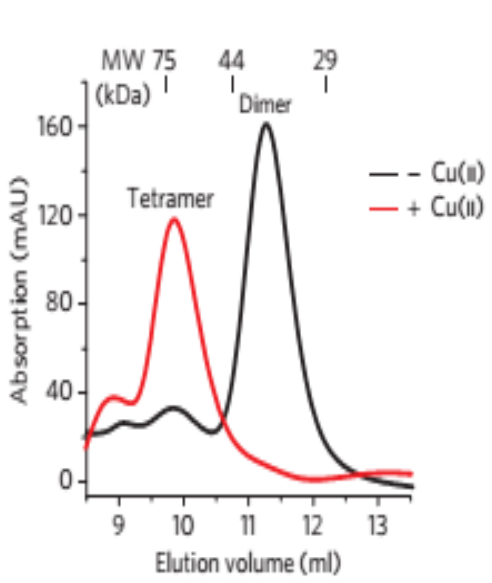
copper(II)-specific chelator

The E. coliWT (K12) strain harboring the pl(maro)-GFP reporter was treated with Tet Cm, nor or amp

Molecular mechanism of copper(II)-induced MarR activation





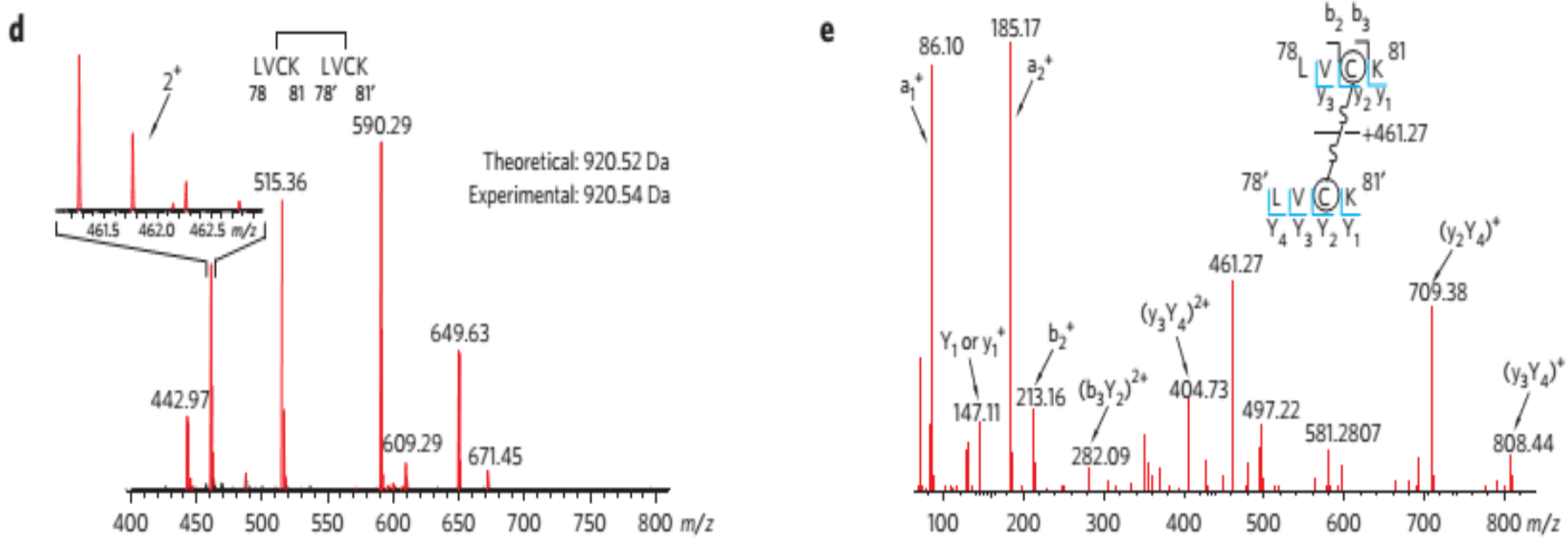


Size-exclusion chromatography

copper(II) caused the dissociation of five of the six MarR mutants , the MarR C80S mutant remained bound

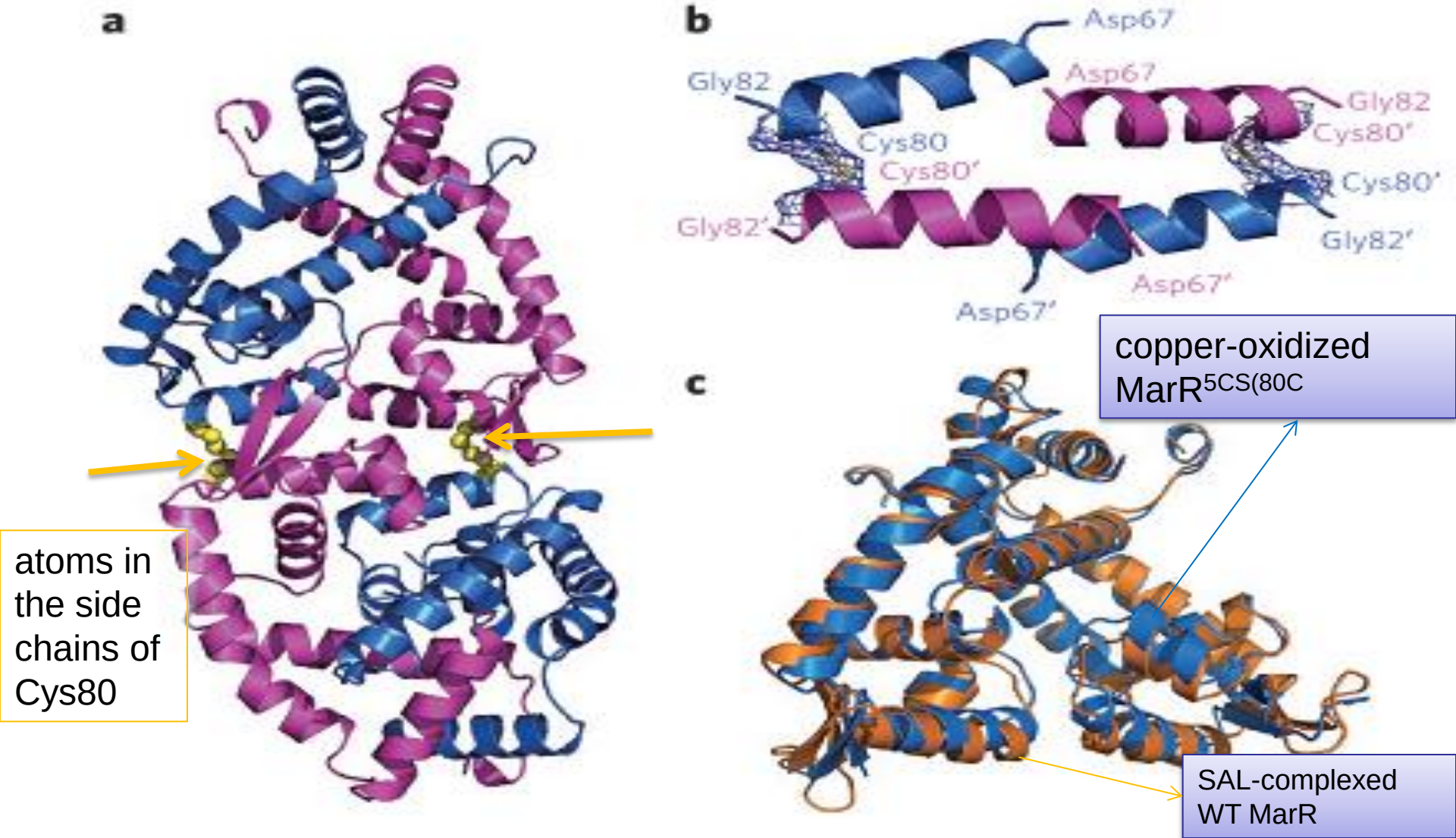
Cys80 is the key residue that is required and sufficient for copper(II)-induced MarR-DNA dissociation.

Nature of the MarR tetramer

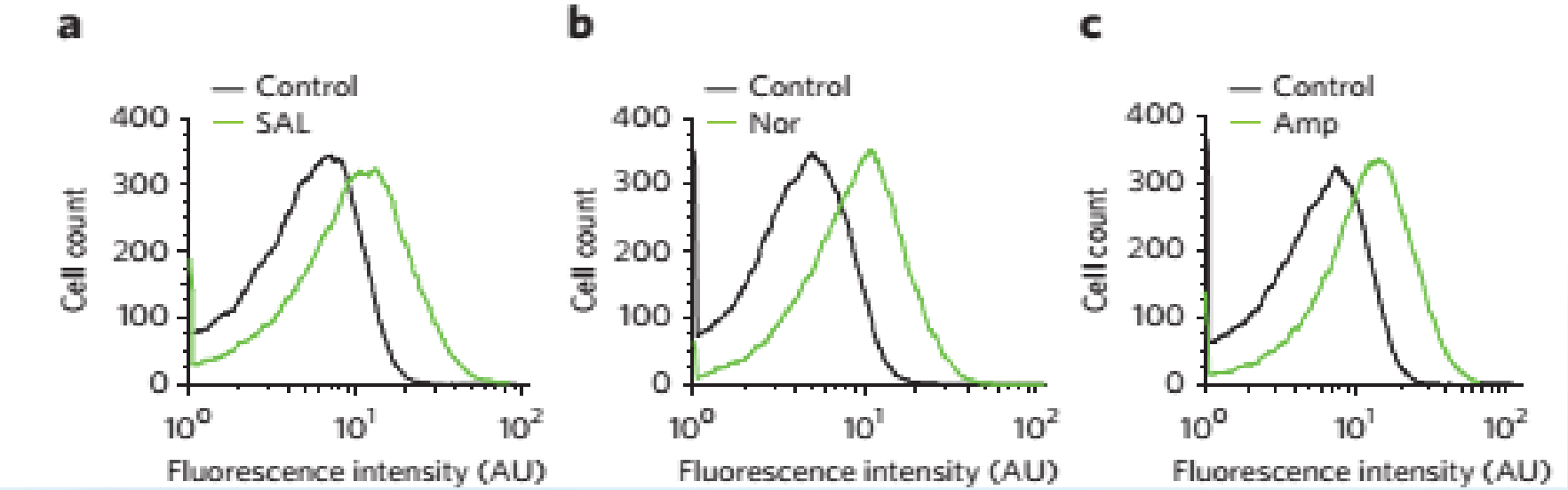


copper(II) may trigger the formation of a covalent ‘dimer-of-dimer’ of MarR via Cys80 residues in a catalytic fashion.

Crystal structure of copper(II)-oxidized MarR$^{5CS(80C}$

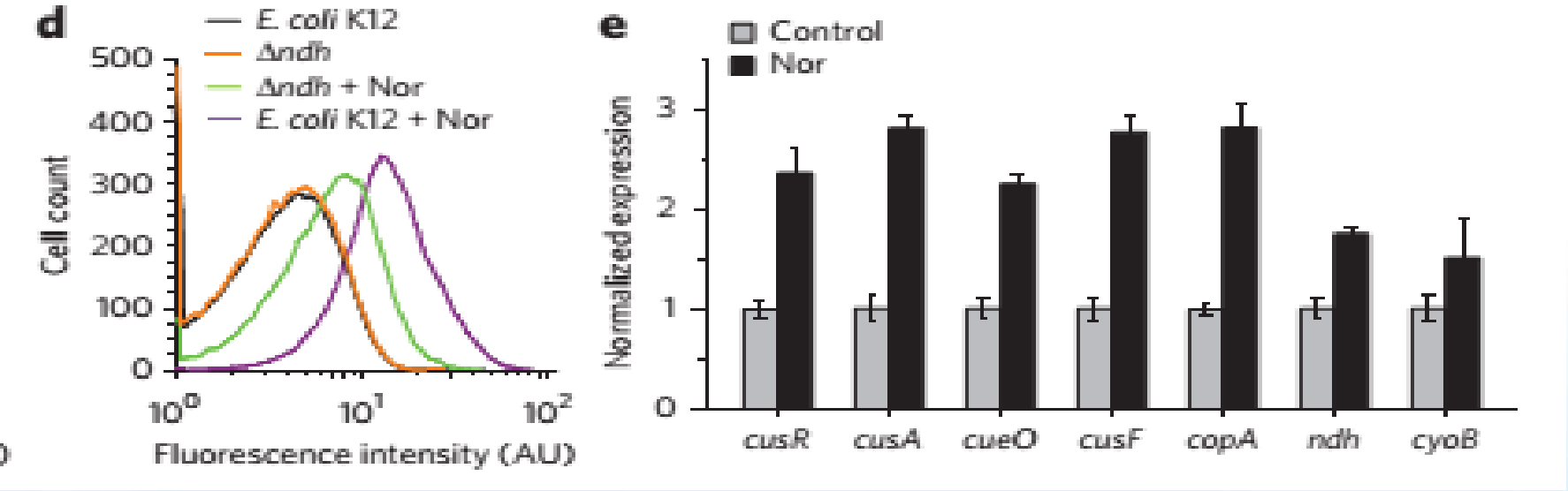


SAL- and antibiotic-triggered



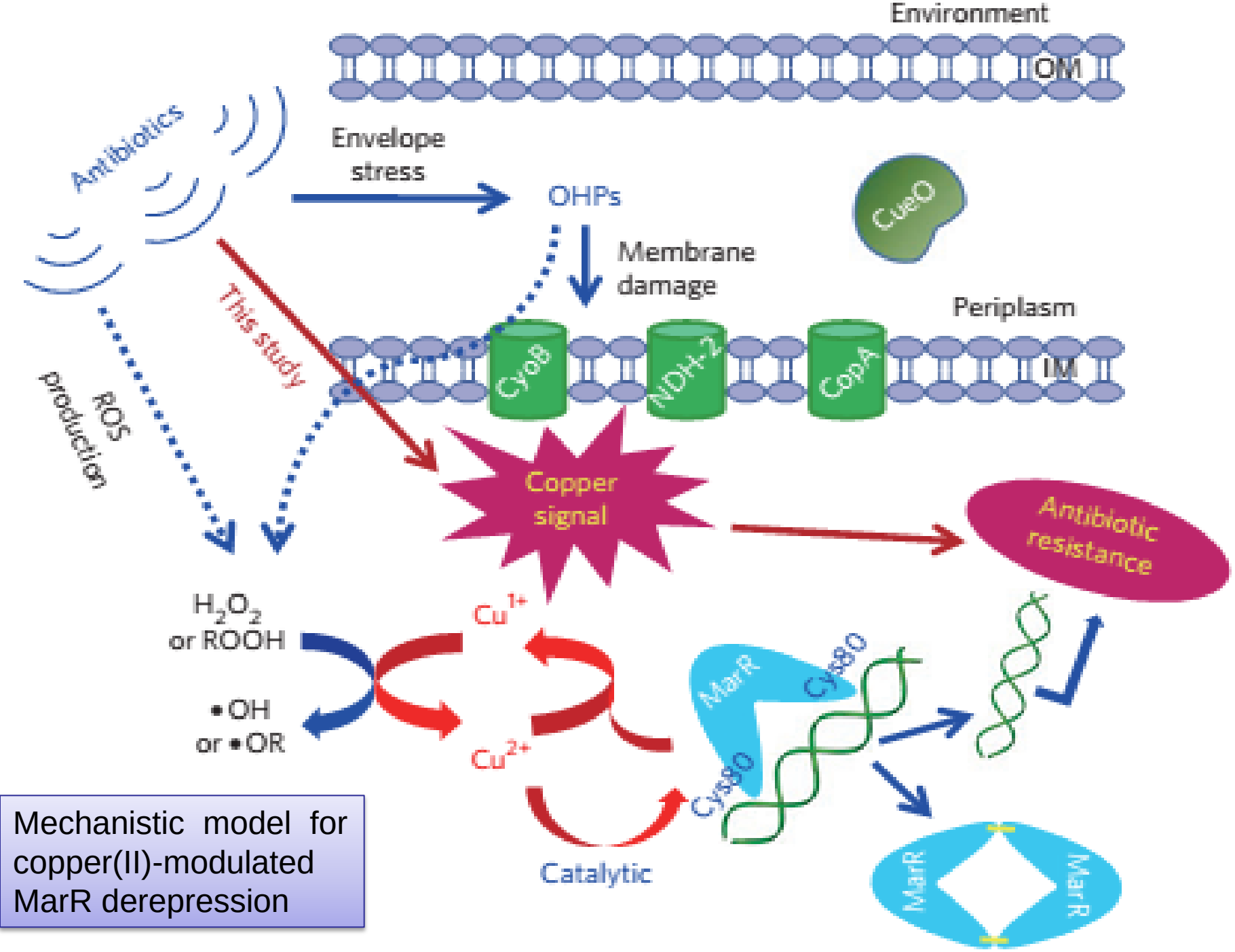
a–c Generation of copper(I) ions inside E. coliWT (K12) cells as measured by the CS-1 probe (2 μM) upon the treatment of SAl(20 mM; a), Nor (250 ng/ml; b) or Amp (2.5 μg/ml; c) for 2 h.

The Drobny Research Group

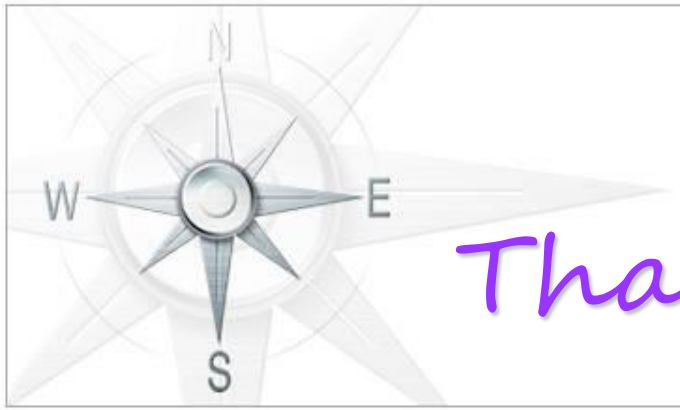


(d) Flow cytometric analysis of copper levels as measured by the CS-1 probe inside E. coliWT (K12) and ndh deletion strains after nor treatment

(e) The expression of E. colicopper-dependent genes cusR, cusA, cueO, cusF, copA, ndhand cyoBin the WT (K12) strain upon nor treatment (250 ng/ml; 2 h) as determined by qRT-PCR.



Mechanistic model for copper(II)-modulated MarR derepression



Thank your time!

Na Yang

2014-4-12