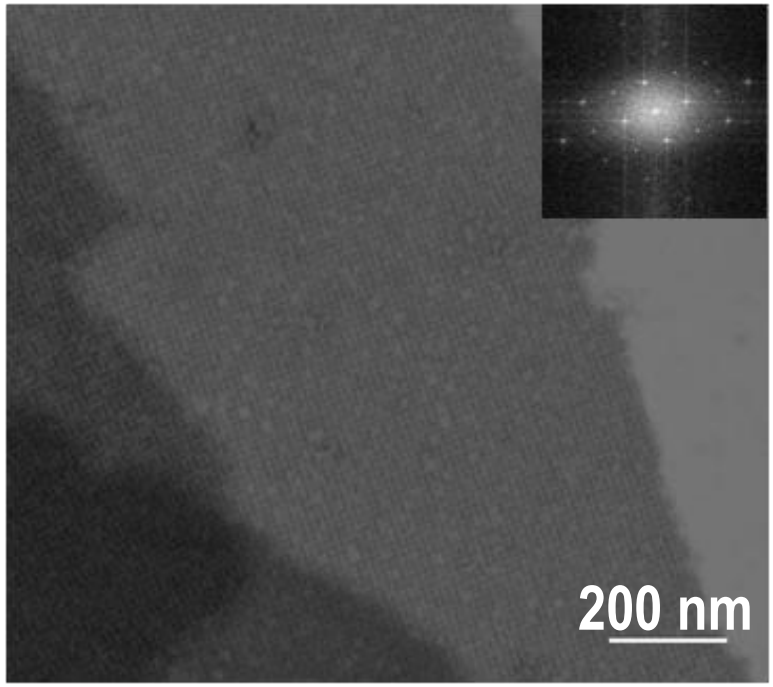


EngineeringThermophilic Microorganisms to Selectively Extract Strategic Minerals

Project Officer: Tim Reinhardt

Total Project Funding: $500k

May 14, 2015

Dr. Caroline M. Ajo-Franklin

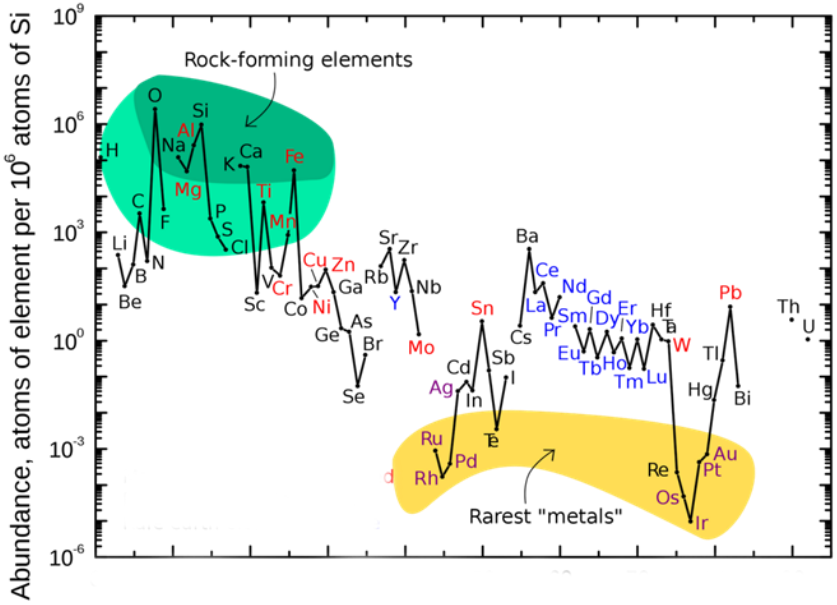
Lawrence Berkeley National Laboratory

Mineral Recovery

Relevance:

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

Mineral recovery requires tight & selective binding under hot, salty conditions



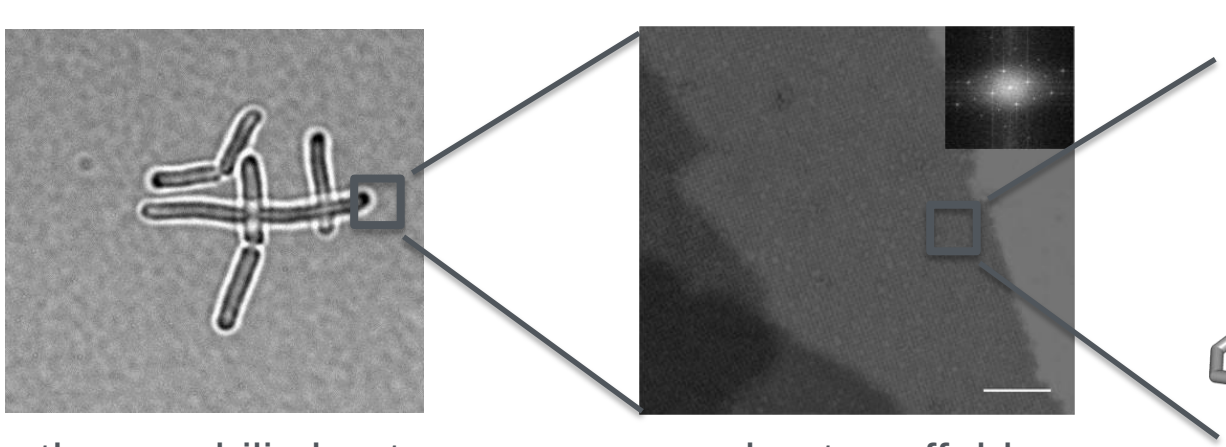
U.S. Geological Survey, Fact Sheet 087-02

Metal	~Concentration (mg/kg H_2O)
Na^+	71,000
Ca^{2+}	11,000
K^+	4,500
Mg	2,000
Zn^{2+}	100
Gd^{3+}	$10x10^{-3}$

Argonne Geothermal Geochemical Database v2.0 (2014).
Wood, S. A. Geothermal Reservior Technology Research Development and Demonstration (2001).
Gallup, DL. *Ore Geology Reviews*(1998)

Impact of Research: Robust & Selective Binding

Objective: create robust platform for high selectivity binding



thermophilic host
Geobacillus stearothermophilus

robust scaffold
S-layer

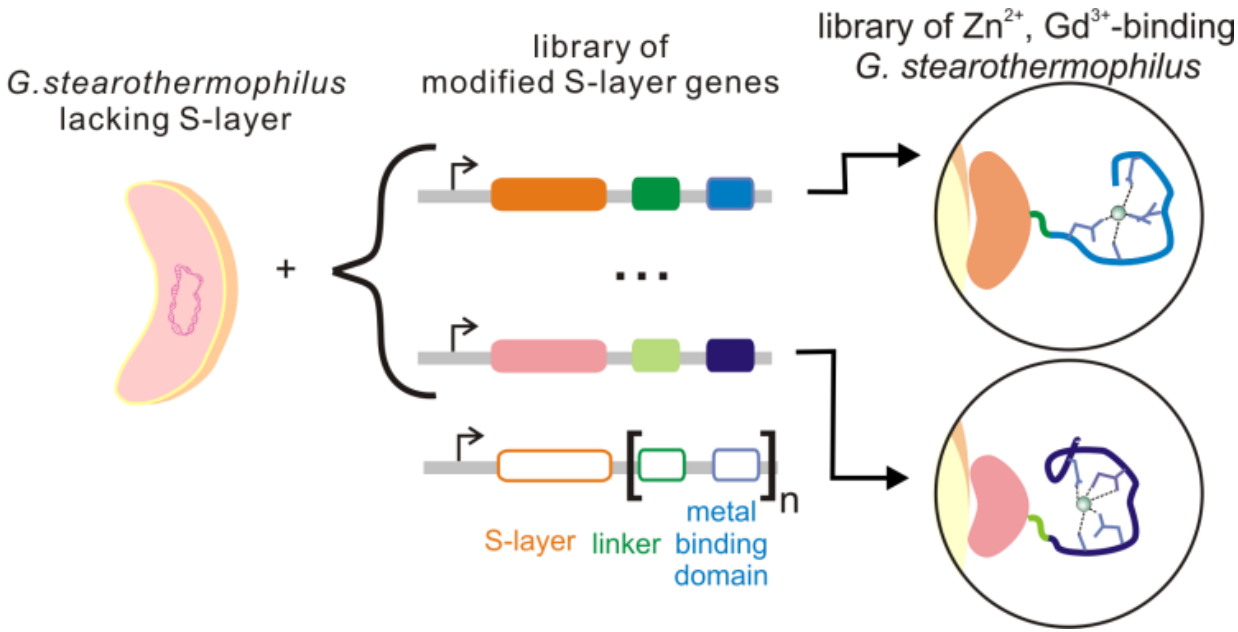
selective binding
metal binding domain

Gadd, **J Chem Technol Biotechnol** (2009).
Pazirandeh *et al.* **Appl Environ Biotechnol** (1995).

Nazina *et al* **Intl J. Syst Evol Microbiol** (2001).
Moll *et al* **PNAS** (2002).
Nitz *et al* **Angew Chem** (2004).

Approach: Make & screen many variants

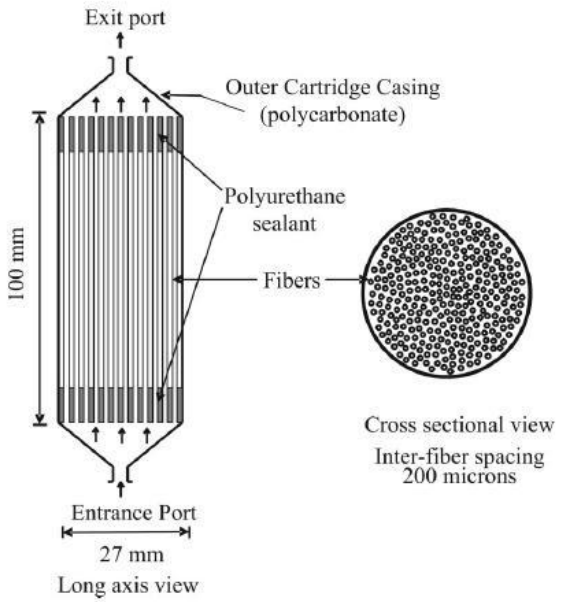
Key risk: imprecise relation between design & function

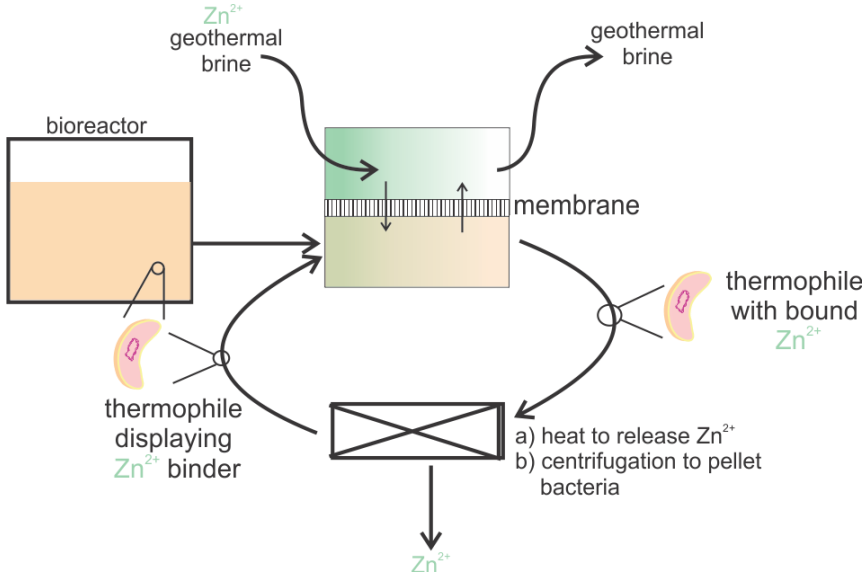


Outcome: affinity, selectivity of these variants

G. Murray, C. M. Ajo-Franklin

Approach:
Mass transport testing & Modeling





Outcome: mass transfer characteristics

B. Viani, L. Yang, J. B. Ajo-Franklin

Accomplishment: Identified criteria to get 90% pure Zn^{2+}, Gd^{3+}

Argonne Geothermal Geochemical Database, Wood report Gallup 1998

Metal	~Conc. (mg/kg H_2O)
Na^+	71,000
Ca^{2+}	11,000
K^+	4,500
Mg	2,000
Zn^{2+}	100
Gd^{3+}	$10x10^{-3}$

90% pure

K_d for Zn^{2+} < 30 µM

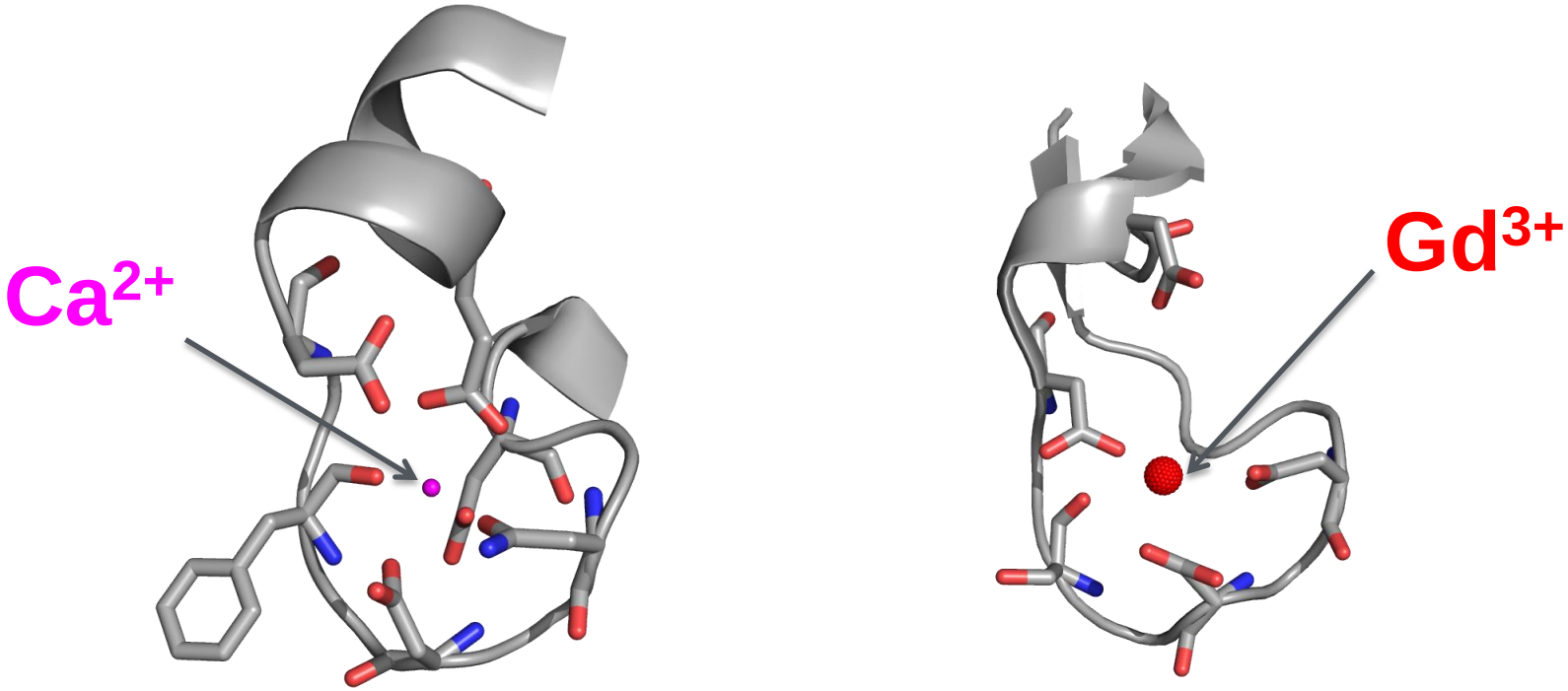
K_d Zn^{2+}/Co^{2+} >10

K_d for Gd^{3+} < 10 nM

K_d Gd^{3+}/Ca^{2+} $>10^5$

Original Planned Milestone/ Technical Accomplishment	Actual Milestone/Technical Accomplishment	Date Completed
Define metal adsorption affinities to req'd to meet targeted Zn separation factors	Define metal adsorption affinities to req'd to meet 90% pure Zn, Gd	12/31/2015

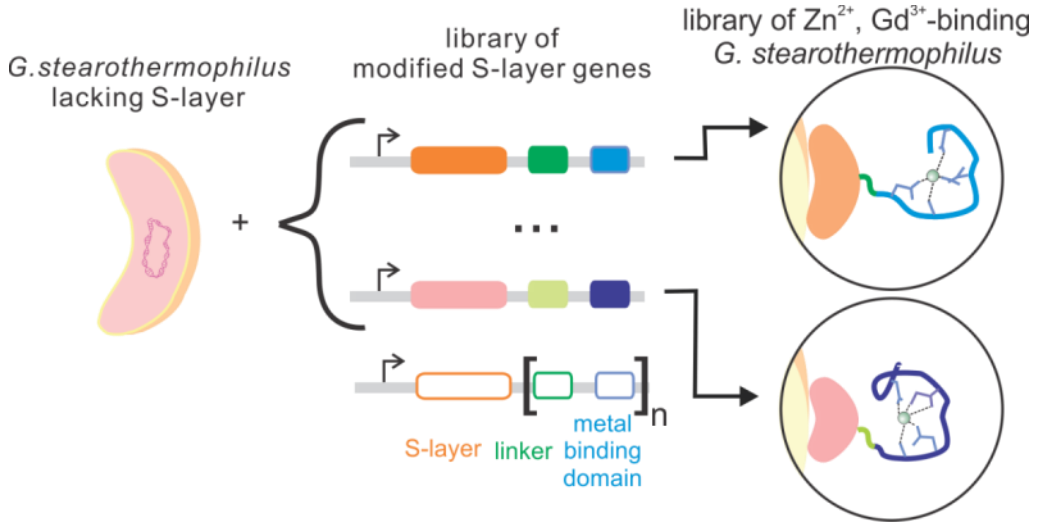
Accomplishment: Designed proteins to meet selectivity, binding criteria



Daughtry, *et al*. **ChemBioChem** (2012).

Original Planned Milestone/ Technical Accomplishment	Actual Milestone/Technical Accomplishment	Date Completed
in silico DNA construct design	*in silico* DNA construct design	12/31/2014

Accomplishment: Designed proteins to meet selectivity, binding criteria



S-layer: SbsB x **linker:** flexible, charged x **metal binding domain:** Gd^{3+}: sSE3; Gd^{3+}: dSE3; Zn^{2+}: Sp1-3; Zn^{2+}: Cp-1; Zn^{2+}: WT1-3 x **Affinity Tag:** Strep Tag, No Tag = **20 Combinations**

Original Planned Milestone/ Technical Accomplishment	Actual Milestone/Technical Accomplishment	Date Completed
in silico DNA construct design	*in silico* DNA construct design	12/31/2014

Accomplishment: *in silico* assembly strategy

J5 BioCAD generates assembly strategy & req'd DNA fragments

ID Number	Name	Size (bp)
0	pNWN	4217
1	SbsB	2784
2	(GGSG)3	36
3	DDDDK	15
4	GddSE	102
5	GdsSE	51
6	ZnCp	78
7	ZnSp	81
8	ZnWt	90
9	Strep TAA	33
10	TAA	3

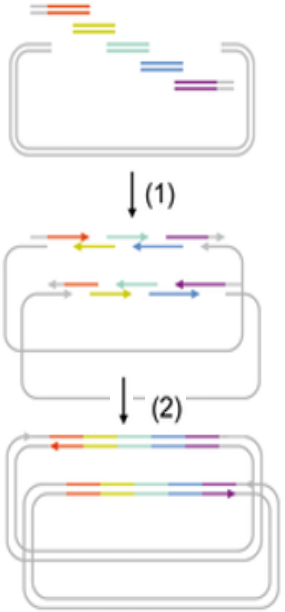
Hillson *et al.* **ACS Synth Biol** (2012).

Original Planned Milestone/ Technical Accomplishment	Actual Milestone/Technical Accomplishment	Date Completed
in silico DNA assembly strategy	*in silico* DNA assembly strategy	1/31/2015

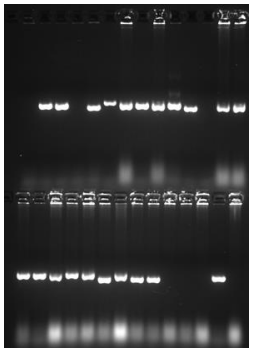
Progress: 10% of DNA constructs verified

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

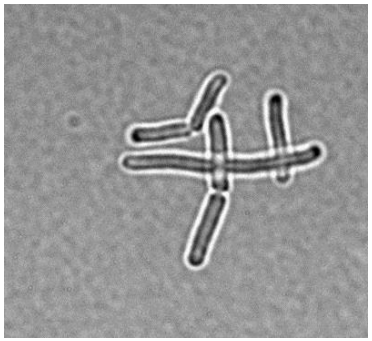
DNA assembly



Verification

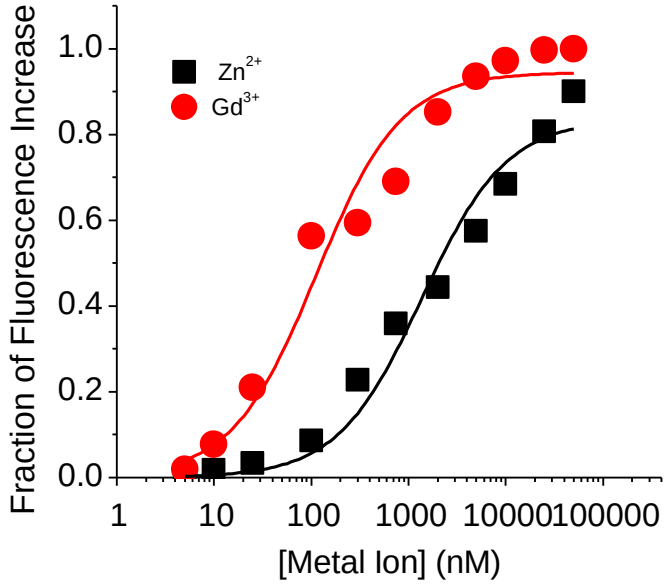


Add into Host



Original Planned Milestone/ Technical Accomplishment	Actual Milestone/Technical Accomplishment	Date Completed
Construction of ~50 eng'd strains	Construction of 20 eng'd strains	TBD

Accomplishment : High-throughput binding screen is calibrated



Midpoint of sensitivity:

Ca^{2+}: 300 µM

Zn^{2+}: 1 µM

Gd^{3+}: 0.1 µM

Original Planned Milestone/ Technical Accomplishment	Actual Milestone/Technical Accomplishment	Date Completed
Benchmark fluorescence assays for Zn^{2+} and Gd^{3+}	Benchmark fluorescence assays for Ca^{2+}, Zn^{2+} and Gd^{3+}	3/30/2015

Future Directions

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

- Critical period: construction & testing of engineered thermophiles
  - Transform into strain w/o S-layers
  - Will do initial screen with fluorescence, then follow-up with ICP-MS

- Fluorescence & ICP-MS testing
  - Will benchmark wild-type strain
  - Will begin testing 1st eng'd strains

- Ready to iterate design

Milestone or Go/No-Go	Status & Expected Completion Date
≥ 1 engineered strain demonstrates sorption **capacity** for Zn^{2+} or Gd^{3+} that exceeds wild type bacteria	On schedule, 9/31/2015
≥ 1 engineered strain demonstrates sorption **selectivity** for Zn^{2+} or Gd^{3+} that exceeds wild type bacteria	On schedule, 9/31/2015

Mandatory Summary Slide

- The primary needs for mineral recovery are selectivity & robustness.
- We identified target K_d's and selectivities to get 90% pure Zn^{2+}, Gd^{3+}.
- We have identified proteins that meet or exceed these K_ds and selectivities.
- We have designed and constructed a library of DNA constructs that will display these proteins on a robust scaffold.
- We have calibrated high-throughput fluorescence assays to rapidly screen this library.
- We are entering the critical period of testing this approach.

Additional Information

Objective: lower cost of release

- Cost of release is ≥50x cost of growing microbes, but unaddressed in literature

Release method	~Cost per 1000 kg metal	Reference
Proteolysis	$205,000	Ueda *et al.*, 2010
pH	$200	Pazirandeh *et al.*,1995.
EDTA	$51	Ueda *et al.*, 2003.
Heat	$5	proposed