Engineering Thermophilic Microorganisms for Mineral Recovery

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My laboratory’s research

Interfacing living cells & materials

TerAvest, CAF. *Biotechnology & Bioengineering* (2016).
introduction

Need robust, selective metal binding under hot, salty conditions

<table>
<thead>
<tr>
<th>Metal</th>
<th>~Conc. (mg/kg H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>71,000</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>11,000</td>
</tr>
<tr>
<td>K⁺</td>
<td>4,500</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>2,000</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>100</td>
</tr>
<tr>
<td>Gd³⁺</td>
<td>10x10⁻³</td>
</tr>
</tbody>
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Can we combine robustness with selectivity?


Introduction

Thermophilic S-layers provide a robust display approach

Strategy: S-layer scaffolded metal binding domains

Thermophilic host: *Geobacillus stearothermophilus*

Robust scaffold: SbsB s-layer

Selective binding: Metal-binding domain
We identified criteria to get 90% pure Zn$^{2+}$, Gd$^{3+}$

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$K_d$ for Zn$^{2+}$ $<$ 30 µM

$K_d$ for Gd$^{3+}$ $<$ 10 nM

$\frac{K_d\text{ Zn}^{2+}}{K_d\text{ Co}^{2+}} > 10$

$\frac{K_d\text{ Gd}^{3+}}{K_d\text{ Ca}^{2+}} > 10^5$

Charrier, CAF, in prep.
results

We designed thermophilic S-layers to bind specific metals

1. Zn domain.
2. Double gadolinium domain.

Charrier, CAF, in prep.
results

Engineered S-layers self-assemble into nanosheets

Native s-layer nanosheet

Engineered s-layer nanosheet

Charrier, CAF, in prep.
Engineered S-layers self-assemble into nanosheets

Native s-layer nanosheet

Engineered s-layer nanosheet

Charrier, CAF, in prep.
Engineered nanosheets bind $\text{Zn}^{2+}$ with higher affinity than native

Isolated binding domain:
1 $\text{Zn}^{2+}$ per binding domain
$K_d = 0.14 \text{ nM}$

Charrier, CAF, in prep.
results

At 40°C, engineered nanosheets bind Zn\(^{2+}\) with higher affinity

Displayed binding domain:
~ 4 Zn\(^{2+}\) per binding domain

Charrier, CAF, in prep.
Engineered nanosheets bind Zn\(^{2+}\) more selectively

Analog brine solution: KCl, CaCl, & NaCl

Displayed binding domain: ~1.6 Zn\(^{2+}\) per binding domain

Charrier, CAF, in prep.
At 40ºC, engineered nanosheets bind Gd³⁺ more selectively

Displayed binding domain: ~0.7 Gd³⁺ per binding domain

Charrier, CAF, in prep.
Surface-layer proteins can be used to create robust materials that bind with biological-specificity.

Engineered nanosheets have higher affinity, selectivity for their metal of interest than native nanosheets.
On-going work

In vivo expression for geothermal applications

Thermophilic host: *Geobacillus stearothermophilus*

Robust scaffold: SbsB s-layer

Selective binding: Metal-binding domain
Future work

Differing melting temperatures could provide a unique level of control.
summary

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