Engineered Metal Binding Single Chain Antibody

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Introduction

Antibody binding of antigen partners can be very specific and of high affinity. An antibody capable of binding metals and/or metal complexes would be ideal for the removal and recycling of heavy metal contaminants from waste effluent. As a model system, we have prepared a linear single chain peptide containing both the light chain and heavy chain fragments from a monoclonal antibody (AC1106) that we have previously shown to possess affinity for Ruthenium bipyridyl metal complexes (1). The construction and characterization of this initial scFv is presented. The methodology for making the immunoabsorbent material for metal cleanup is also shown. Finally, the strategy for the modification and optimization of the antibody's binding properties towards other metal complexes is described.

Experimental Plan

- Elicit antibodies to metal complex and make hybridoma (1)
- Isolate DNA sequence of heavy and light chains by RT-PCR from mRNA (2)
- Join heavy and light chain fragments via 15 aa linker by SOE-PCR
- Clone single chain antibody construct into T7 expression plasmid
- Transform plasmid into host and produce protein as inclusion bodies
- Isolate inclusion bodies, denature, affinity purify and refold to yield **pure, active metal-binding single chain antibody**
- Characterize by: Protein Gel, Sequencing, ELISA, Luminescence Activation, and Computer Models
- Clone scFv into a bacterial surface display vector (3) and grow up bacteria with metal-binding antibodies anchored to it's surface
- Modify and optimize antibody binding properties to different metal complexes
- Fix and immobilize cells to a chitosan/cellulose matrix (4) to produce a cheap material for removal and recycling of heavy metal contaminants

Antibody Sequence

CDR 1 - Heavy Chain

```
ATG CAG TCT GGA CCT GAG CTG GTG AAG CCT GGA GCT TCA ATG AAG ATA TCC TGC AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC ACC ATG AAC
 M   Q   S   G   P   E   L   V   K   P   G   A   S   M   K   I   S   C   K   A   S   G   Y   S   F   T   G   Y   T   M   N
```

CDR 2 - Heavy Chain

```
TGG GTG AAG CAG AGC CAT GGA AAG AAC CTT GAA TGG ATT GGA CTT ATT GAT CCT TAC AAT GGT GGT ACT AGC TAC AAC CAG AAG TTC AGG GGC
 W   V   K   Q   S   H   G   K   N   L   E   W   I   G   L   I   D   P   Y   N   G   G   T   S   Y   N   Q   K   F   R   G

AAG GCC ACA TTG ACT GTA GAC AAG TCA TCC AAC ACA GCC TAC ATG GAG CTC CTC AGT CTG ACA TCT AAG GAC TCT GCA GTC TAT TAC TGT GCA
 K   A   T   L   T   V   D   K   S   S   N   T   A   Y   M   E   L   L   S   L   T   S   K   D   S   A   V   Y   Y   C   A
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CDR 3 - Heavy Chain **Linker**

```
AGA GGG GGG GCT TAT GAT TAC GAC GAC CGG AAT GCT ACG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACT GTC TCT TCA GGT GGC GGT GGC TCC
 R   G   G   A   Y   D   Y   D   D   R   N   A   T   D   Y   W   G   Q   G   T   S   V   T   V   S   S   G   G   G   G   S

GGC GGT GGT GGG TCC GGT GGC GGC GGA TCA GAT GTT GTG ATG TCA CAG TCT CCA TCC TCC CTA ACT GTG TCA GTT GGA GAG AAA GTT ACT ATG
 G   G   G   G   S   G   G   G   G   S   D   V   V   M   S   Q   S   P   S   S   L   T   V   S   V   G   E   K   V   T   M
```

CDR 1 - Light Chain

```
AGC TGC AAG TCC AGT CAG AGC CTT TTA TAC ACT AGC AAT CAA AAG AAT TAC TTG GCC TGG TAC CAG CAG AAA CCA GGG CAG GCT CCT AAA CTT
 S   C   K   S   S   Q   S   L   L   Y   T   S   N   Q   K   N   Y   L   A   W   Y   Q   Q   K   P   G   Q   A   P   K   L
```

CDR 2 - Light Chain

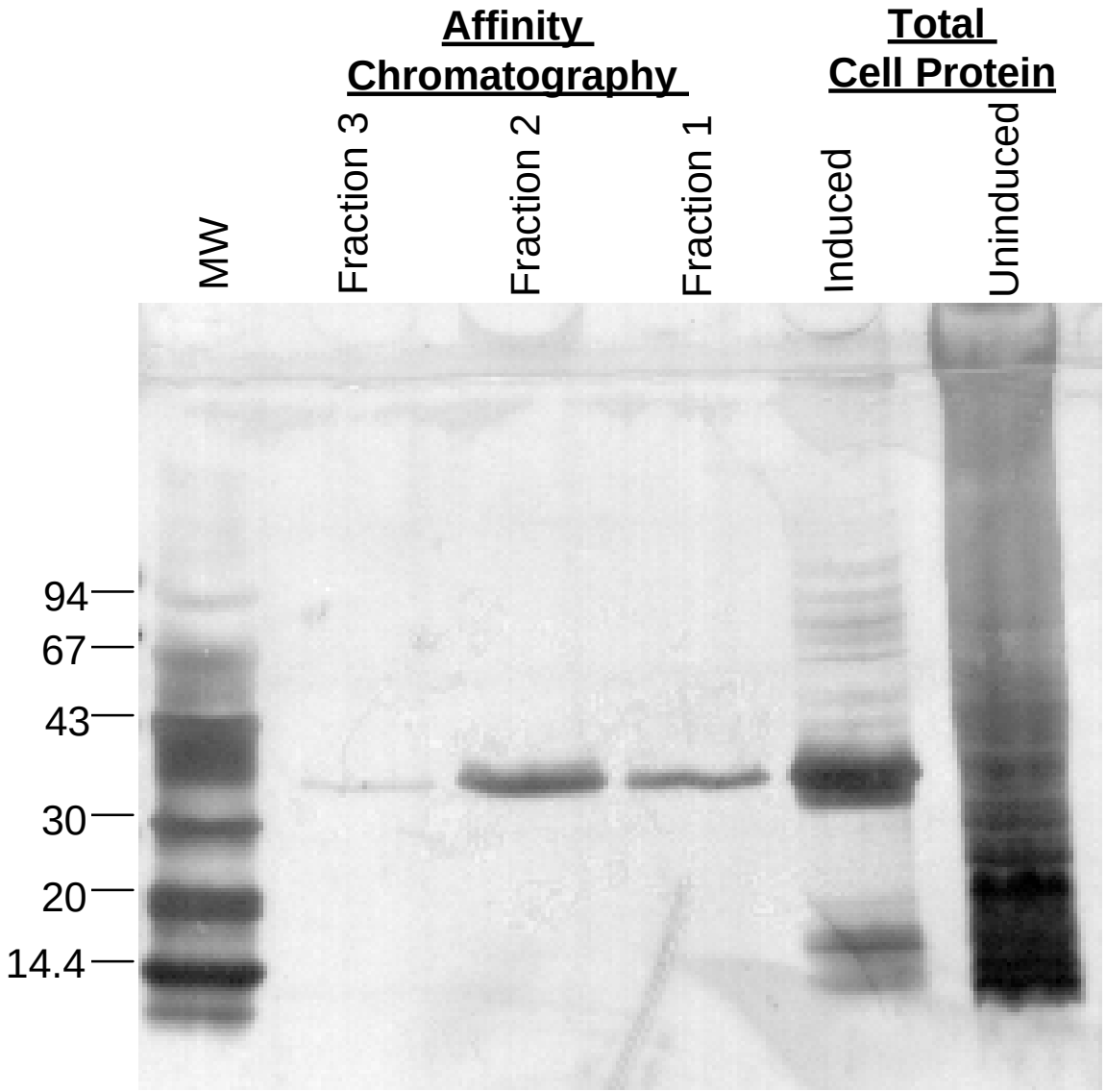
```
CTG ATT TAC TGG GCA TCC ACT CGG GAA TCT GGG GTC CCT GAT CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT
 L   I   Y   W   A   S   T   R   E   S   G   V   P   D   R   F   T   G   S   G   S   G   T   D   F   T   L   T   I   S   S
```

CDR 3 - Light Chain

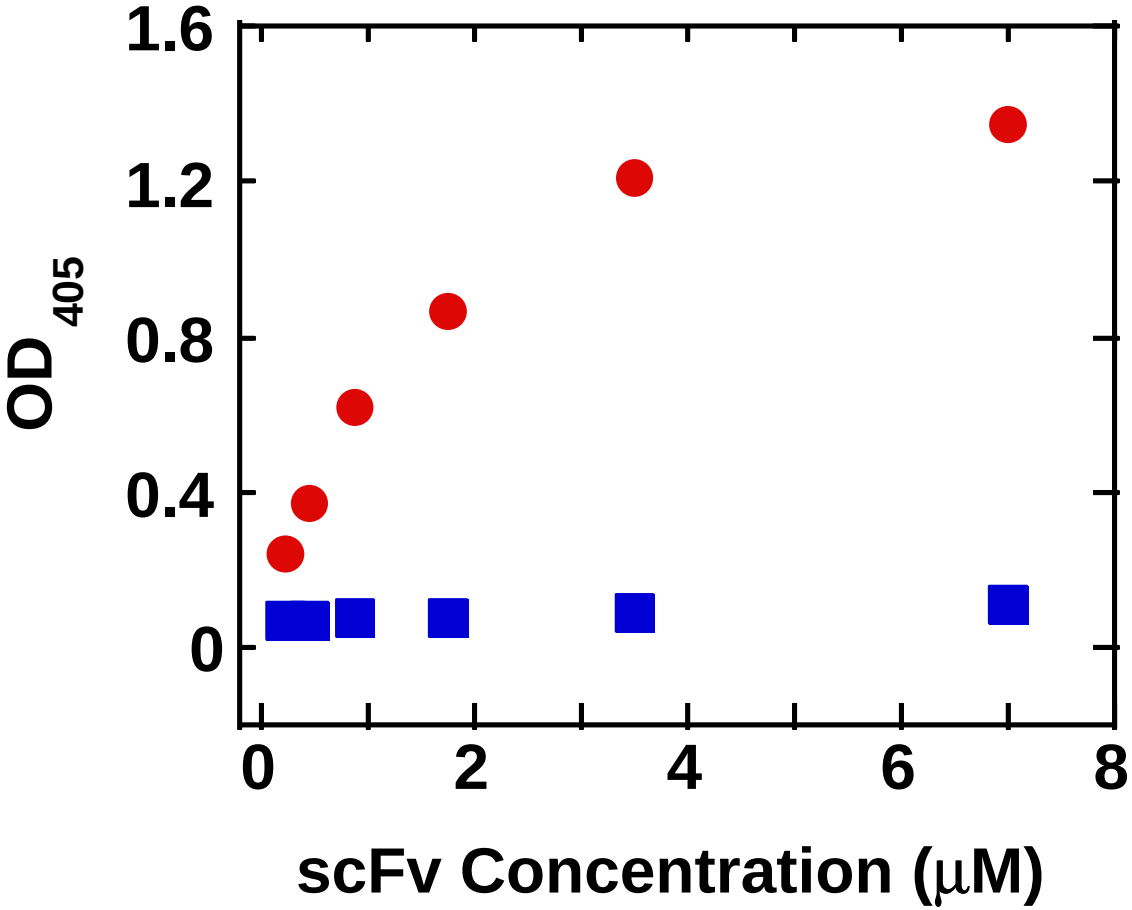
```
GTG AAG GCT GAA GAC CTG GCA GTT TAT TAC TGT CAG CAA TAT TAT AGC TAT CTC ATA TAC ACG TTC CTC GAG ATC AAA CGG GCT AGC CAG CCA
 V   K   A   E   D   L   A   V   Y   Y   C   Q   Q   Y   Y   S   Y   L   I   Y   T   F   L   E   I   K   R   A   S   Q   P

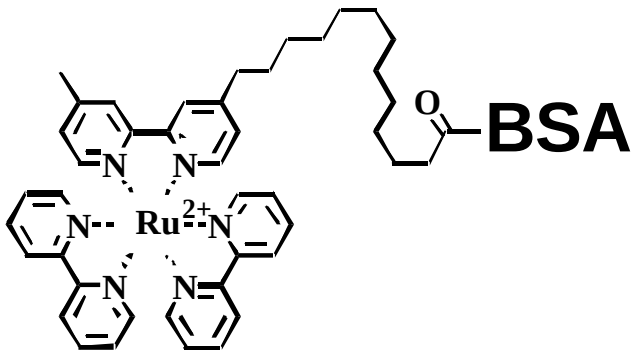
GAA CTC GCC CCG GAA GAC CCC GAG GAT GTC GAG CAC CAC CAC CAC CAC CAC TGA
 E   L   A   P   E   D   P   E   D   V   E   H   H   H   H   H   H   *
```

Affinity Purification of scFv



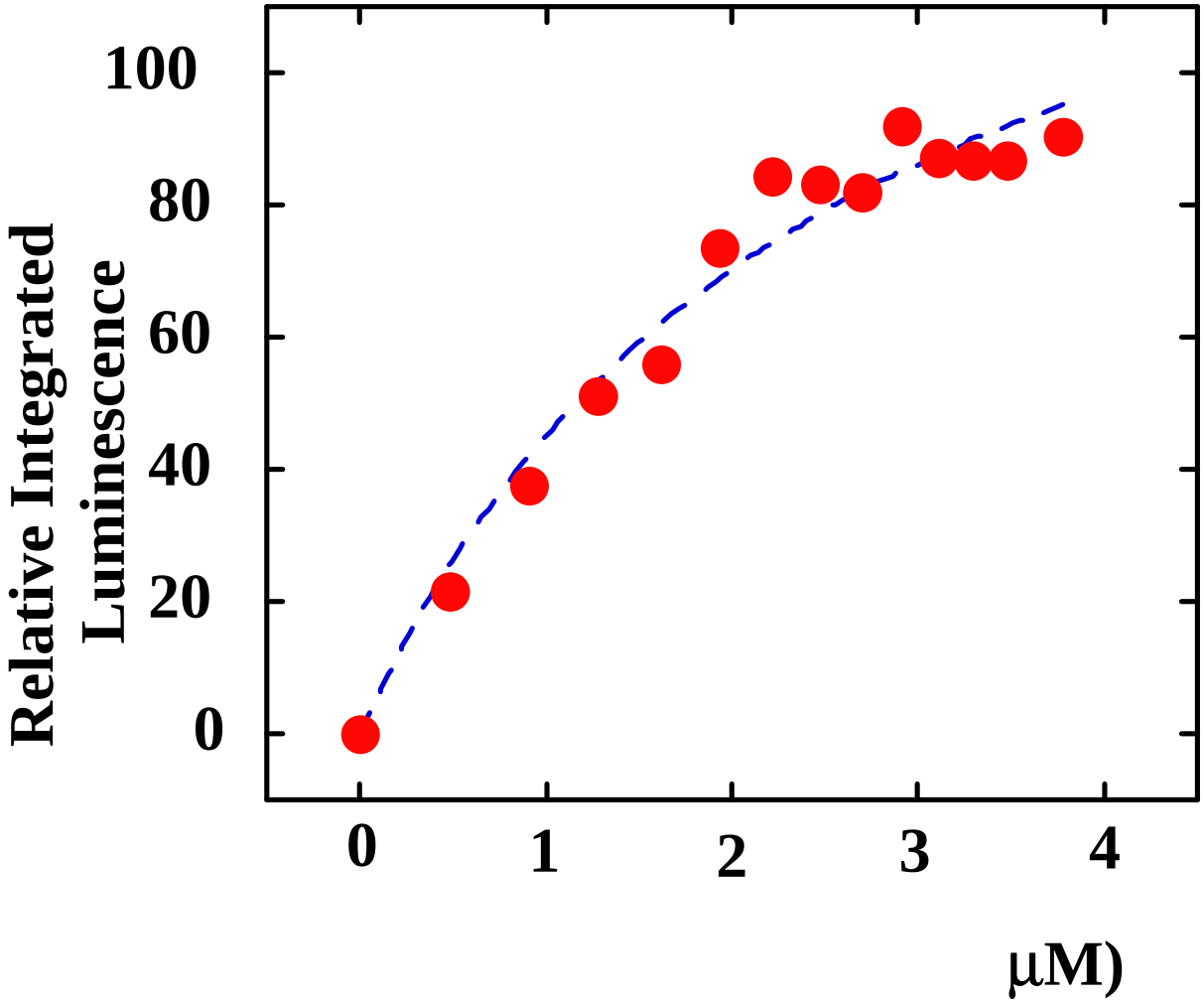
ELISA Characterization of Metal Binding Antibody

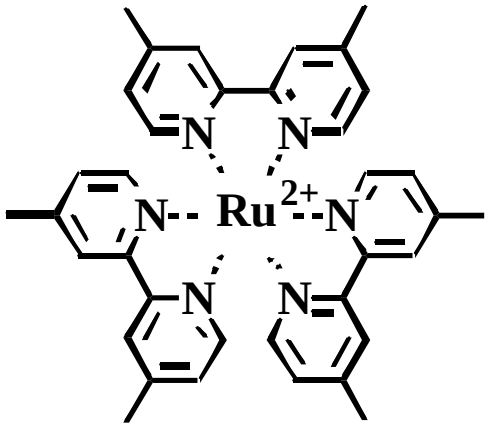




Ruthenium-BSA
BSA Control

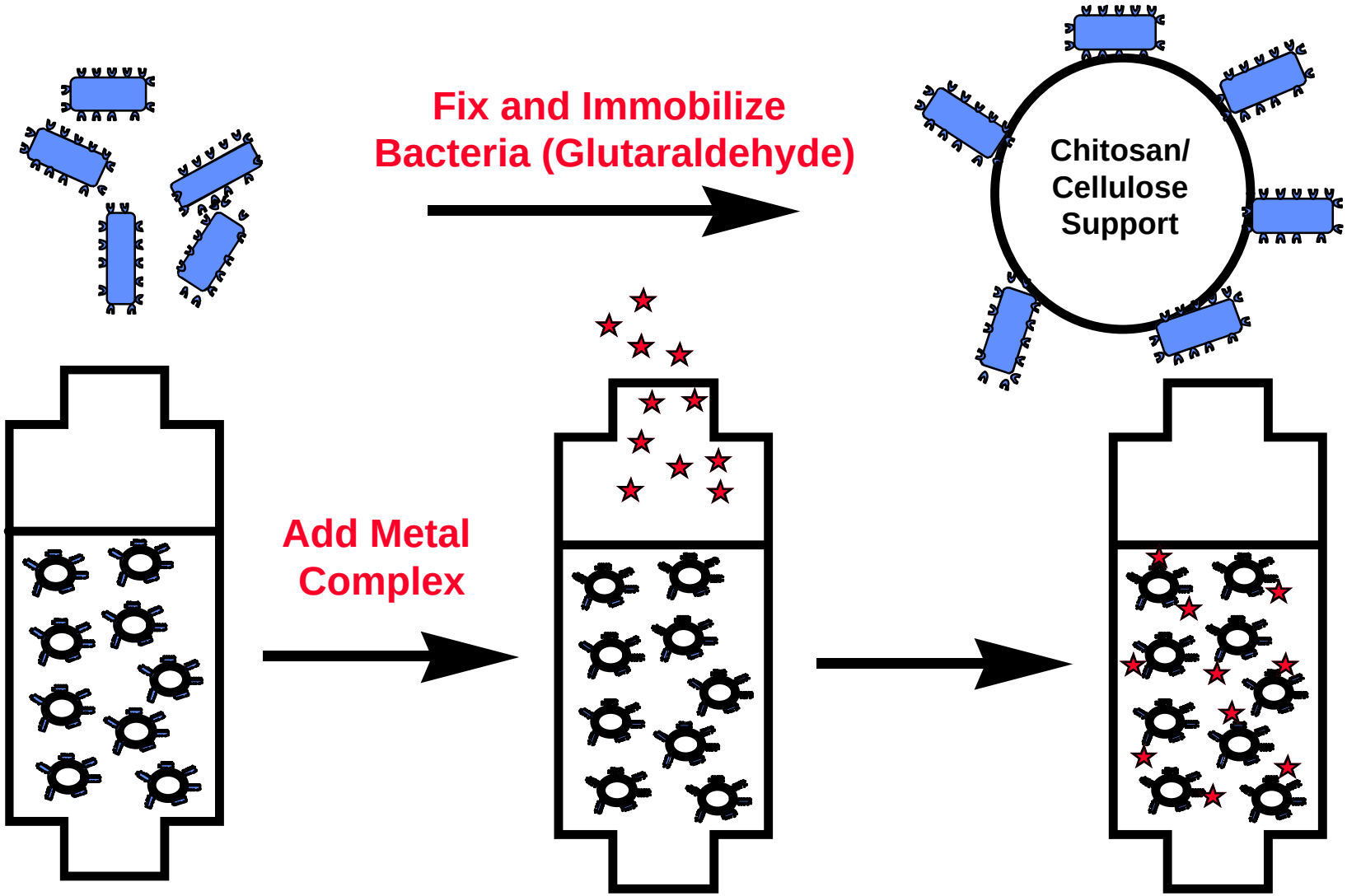
Antibody Affinity for $Ru(dmbpy)_3$ by Luminescence Activation



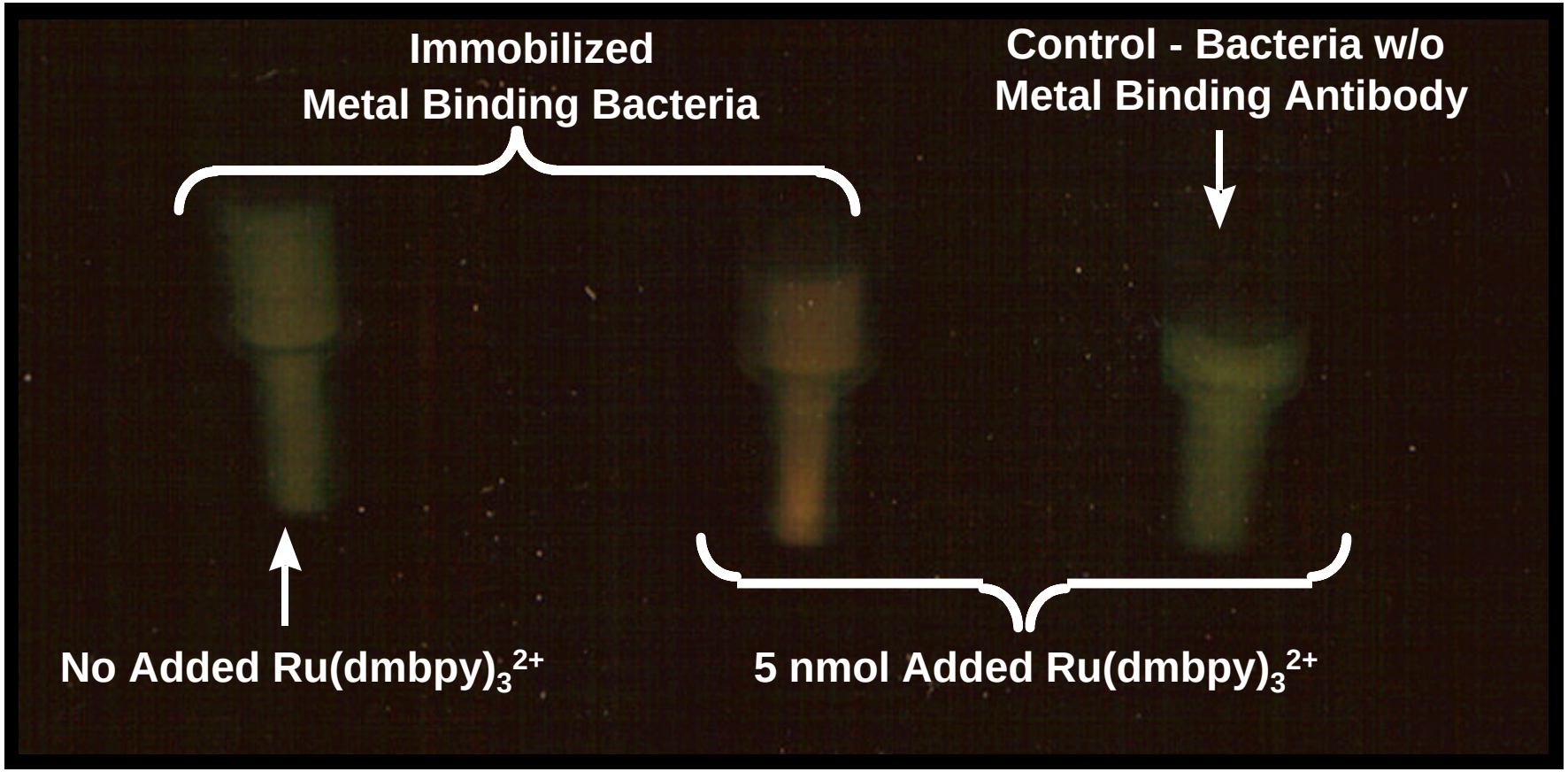


$K_a = 4 \pm 1 \times 10^5\ M^{-1}$

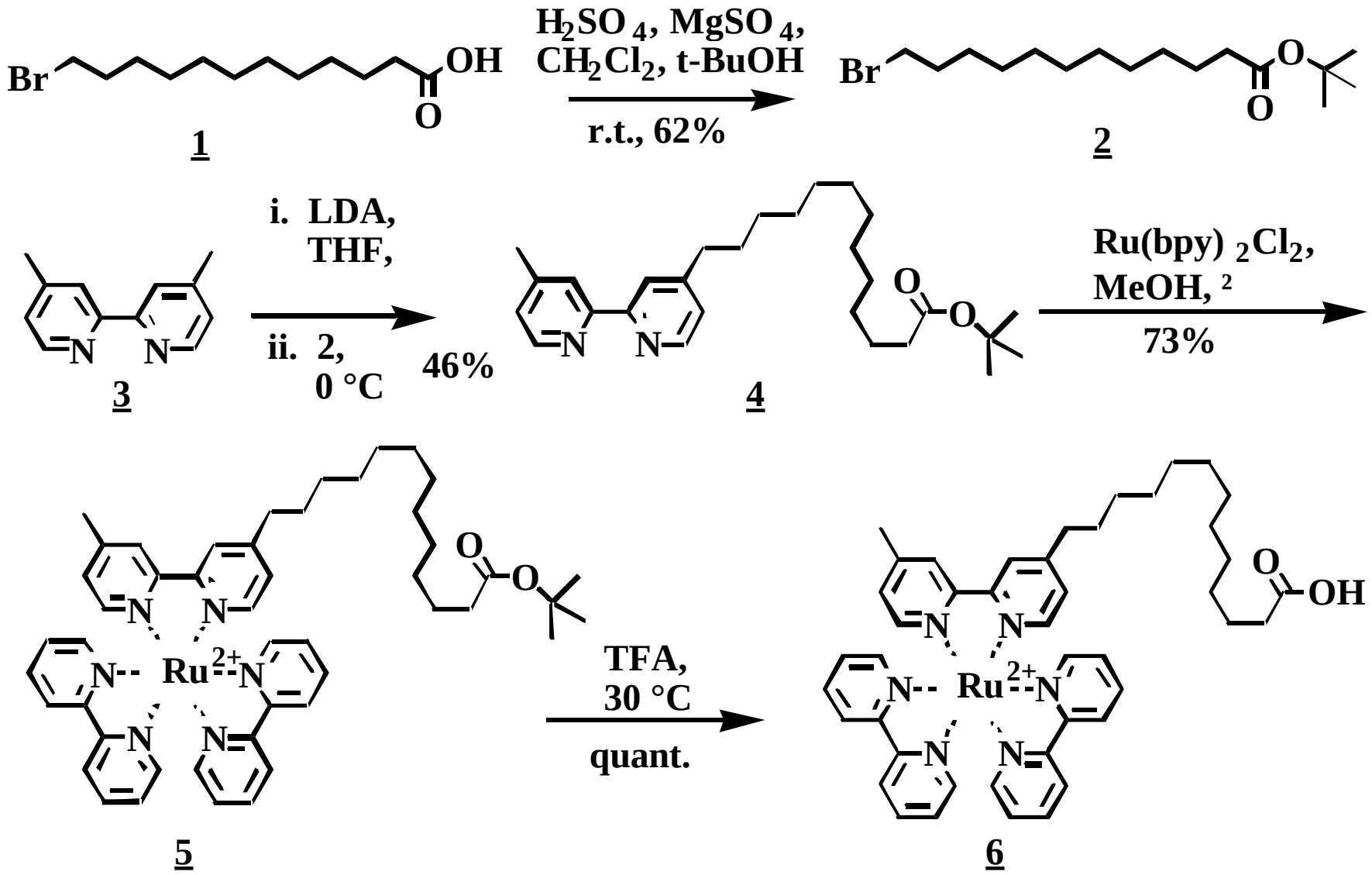
Scheme for Large Scale Metal Complex Removal

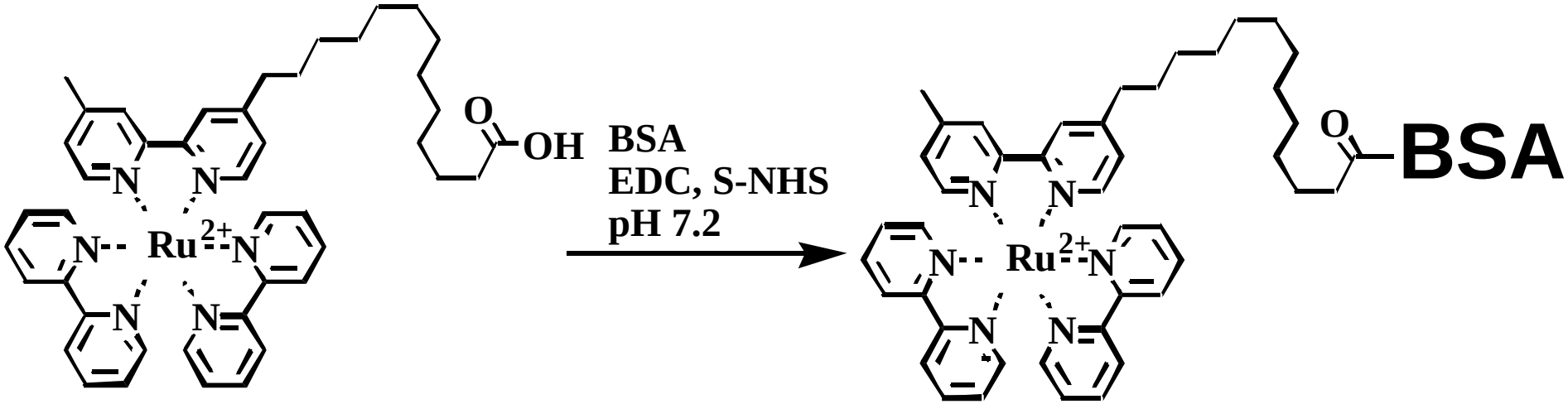


Fluorescence from Metal Bound to Immobilized Bacteria



Synthesis of Ruthenium Complex for ELISA and FACS Probe





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Summary

- Cloned gene for metal binding single chain antibody
- Expressed, purified and refolded metal binding single chain antibody
- Characterized metal binding single chain antibody
- Surfaced displayed metal binding single chain antibody anchored to the outer membrane of *E. Coli*
- Demonstrated use of immobilized *E. Coli* in an immunoabsorbent format

In progress

- Affinity maturation of the model metal binding antibody is being done using error prone PCR and mutator strain as initial randomization strategies

- Libraries generated will be screened and sorted against $Ru(dmbpy)_3^{2+}$ by flow cytometry to isolate improved antibodies

- Second generation affinity improvements will take advantage of DNA shuffling technology applied to initial positives

- A similar approach will be applied to the selection and affinity optimization of other metal complexes

References

(1) For production of the hybridoma see: Shreder, K.; Harriman, A.; Iverson, B.L. *J. Am. Chem. Soc.*, **1996**, *118*, 3192.

(2) The mRNA prep and RT-PCR were done by Gang Chen and Hua Zhang (unpublished results).

(3) For more information on display of foreign protein on the surface of bacteria see: Francisco, J.A.; Earhart, C.F.; Georgiou, G. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 2713.

(4) The fixation and immobilization protocol is based on methodology worked out by Freeman, A., Abramov, S., and Georgiou, G. (manuscript in preparation).

(5) For review on screening displayed protein libraries with cell-sorting techniques see: Georgiou, G.; Stathopoulos, C.; Daugherty, P.S.; Nayak, A.R.; Iverson, B.L.; Curtiss III, R. *Nat. Biotech.*, **1997**, *15*, 29.
For more information on techniques for generation of displayed protein libraries and more screening strategies see Marc Olsen (poster #219) and Patrick Daugherty (poster #196).