"New Strategies for Designing Inexpensive but Selective Bioadsorbants for Environmental Pollutants: Selection of Specific Ligands and their Cell Surface Expression."

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Period covering 9/15/96 - 9/14/97

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Technical Progress Report

Progress for the last twelve months has revolved around setting up our antibody engineering and surface display system for use with a metal-complex binding antibody.

1) We have isolated genes for the V regions of the heavy and light chains for the Ru(bpy)3 specific monoclonal AC1106 (Shreder, K S., Hariman, A., and Iverson, B.L., *J. Am. Chem. Soc*. 1996, 118, 3192-3201). This antibody binds Ru(bpy), derivatives with better than nanomolar affinity, and will serve as our generic metal-complex binding pocket. Cloning antibody genes from hybridomas is complicated by the fact that primers must be found that amplify the particular heavy and light chain genes in the hybridoma of interest. Antibody gene amplification primers are generally designed to amplify antibody repertoires from lymphocyte mRNA isolated from animals. While cloning repertoires from animals is routinely successful due to the diverse population of target m RNA, hybridomas have a single target sequence. Therefore, multiple primers and conditions must be tried before the correct primer combination is identified.

2) A -((gly),ser),- linker was added between the heavy and light chain using SOE-PCR.

3) The entire scFv construct has been ligated into pGemT using a T-vector cloning strategy.

4) More recently. the scFv has been cloned into a pET25b(+) vector for soluble expression.

5) We are in the middle of the synthesis of various metal complexes to be used for a thorough binding analysis of the soluble scFv (mentioned in 6) below).

6) We are preparing milligram quantities of anti-$Ru(bpy)_3$ scFv in preparation for the detailed characterization in solution using the metal complexes mentioned in 5) above.

7) Preliminary ELISA data has now indicated that the AC1106 scFv is active! ! ! ! !

8) We have begun cloning the AC1106 scFv into our bacterial surface expression system in preparation for antibody engineering efforts. By using iterative rounds of randomization and selection using via fluorescence activated cell sorting (FACS) the affinities for different metal ions will be evolved.

9) In advances related to the proposed work, we have been able to optimize further the efficiency of our bacterial surface expression/FACS selection system for isolating interesting new antibodies. Using the optimized protocols on an scFv that binds the heart glycoside digoxin, we were able to produce an improved scFv with subnanomolar affinity after a single round of selection! These powerful new procedures will be employed in the upcoming metal-complex binding antibody evolution studies.

Budget Information

Contract and budget period 9/14/96 to 9/14/99.

$749,392	Money received on 9/25/96	
- 161,447	In direct costs	
$587,945		
- 111,558	Iverson/Georgiou money spent as of 9/30/97 - YEAR ONE	
- 73,871	Subcontract (Univ. of Pitts.) money spent - YEAR ONE	
402,516	Balance remaining as of 9/30/97	
- 215,176	Subcontract money remaining of Univ. of Pittsburgh	
$187,340	Remaining money at UT for Iverson/Georgiou	
	YEAR TWO projected spending:	
	Georgiou summer salary	$7350
	Iverson summer salary	$5670
	Post Doc 1	$26,200
	Post Doc 2	$26,200
	Fringes	$17,663
	Supplies	$15,000
	Travel	$ 2 500
-1PQ.583	TOTAL	100.583
	YEAR THREE projected spending:	
	Georgiou summer salary	$7718
	Tverson summer salary	$5954
	Post Doc 1	$26,270
	Post Doc 2	$26,270
	Fringes	$17,877
	Supplies	$ 15.000
	Travel	$,500
-101.589	TOTAL	10 1,589
- 14,833	Balance remaining after 9/14/99 end of contract period.	