CLAIMS:

1. A method for producing purified recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:
   a. in a bioreactor, culturing *E. coli* cells that produce rhARG;
   b. lysing the *E. coli* cells;
   c. removing cell debris from the lysate;
   d. loading the cell lysate on a cation exchange column;
   e. eluting the rhARG with a high salt solution;
   f. incubating the eluted rhARG with a cobalt salt to form cobalt-substituted rhARG (Co-rhARG);
   g. applying said Co-rhARG to an anion exchange column and collecting the flow-through;
   h. applying the flow-through onto a third chromatography column; and
   i. eluting the Co-rhARG from the third chromatography column with a high salt solution.
2. The method of claim 1, wherein up to 60 grams of rhARG is loaded onto the cation exchange column per liter of cation exchange resin.
3. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a high salt solution of up to 0.5 M salt concentration.
4. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a high salt solution of 0.1 M salt concentration.
5. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a gradient of 0.0 to 0.5 M salt concentration.
6. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a gradient of 0.0 to 0.2M salt concentration.
7. The method of any one of claims 1-6, wherein the cobalt salt comprises a Co^{2+} salt.
8. The method of any one of claims 1-7, wherein the cobalt salt comprises $CoCl_2$.
9. The method of any one of claims 1-8, wherein the third chromatography column comprises a multimodal chromatography (MMC) column.
10. The method of any one of claims 1-9, further comprising reacting the rhARG or Co-rhARG with a PEGylation reactant to provide a PEGylated protein.
11. The method of claim 10, wherein the PEGylated protein comprises one or more of PEGylated amino acid residues at K16, K32, K38, K40, K47, K67, K74, K82, L87, K88, K152, K154, K171, K222, K223, K312 and K321.

12. The method of claim 11, wherein the PEGylated protein comprises one of more of 15% to 60% of K16, 35% to 80% of K32, 20% to 85% of K38, 10% to 60% of K40, 10% to 60% of K47, 40% to 90% of K67, 30% to 95% of K74, 30% to 98% of K82, 15% to 65% of K87, 25% to 70% of K88, 25% to 85% of K152, 15% to 65% of K154, 20% to 75% of K171, 0% to 30% of K222, 0% to 35% of K223, 0% to 45% of K312, and 0% to 45% of K321 is PEGylated.

13. The method of any one of claims 10-12, wherein the PEGylated protein comprises PEGylated amino acid residues at least at K16, K32, K38, K40, K47, K67, K74, K82, L87, K88, K152, K154, K171, K312 and K321.

14. The method of any one of claims 10-13, wherein the PEGylated protein does not have PEGylated amino acid residues at K3, K149, K190, K195, K29, K265 and K283.

15. A method for producing purified PEGylated recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:

a. in a bioreactor, culturing *E. coli* that produce rhARG;
b. lysing the *E. coli* cells;
c. removing cell debris from the lysate;
d. loading the cell lysate on a cation exchange column;
e. eluting the rhARG with a high salt solution;
f. incubating the eluted rhARG with 10 mM $CoCl_2$ to form cobalt-substituted rhARG (Co-rhARG);
g. applying the Co-rhARG to an anion column and collecting the flow-through;
h. applying the flow-through onto a multimodal chromatography (MMC) column;
i. eluting the Co-rhARG from the MMC column with a high salt solution;
j. adding a molar excess of methoxy PEG succinimidyl carboxymethyl ester;
k. removing excess PEG.

16. A method for producing purified recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:

a. in a bioreactor, culturing *E. coli* cells that produce rhARG at 36°C to 38°C , and pH of between 7.0 and 7.4, with agitation and aeration;
    i. adjusting the temperature of the bioreactor to 29°C;
    ii. inducing *E. coli* cells to produce for rhARG;
    iii. culturing the *E. coli* cells for 18 hours;
    iv. harvesting the *E. coli* cells by centrifugation;
b. lysing the *E. coli* cells by high pressure homogenization in 25 mM HEPES at a pH of between 7.2 to 7.6, and at or below 15°C ;

c. removing cell debris from the lysate by centrifugation at or below 15°C , then filtering the lysate with a 0.8 micron filter then filtering with a 0.5 micron filter;

d. loading the cell lysate on a cation exchange column then washing the column with 25 mM HEPES, pH 7.2-7.6;

e. eluting the rhARG at room temperature with a high salt solution comprising 25mM HEPES, 0.1M NaCl, pH 7.2-7.6;

f. incubating the eluted rhARG with 10 mM $CoCl_2$ at room temperature for 2 to 8 hours to form cobalt-substituted rhARG (Co-rhARG);

i. exchanging Co-rhARG into 50mM Tris, pH 8.1-8.5;

g. applying the Co-rhARG to an anion exchange column and collecting the flow-through;

h. applying the flow-through onto a Capto multimodal chromatography (MMC) column;

i. eluting the Co-rhARG from the MMC column with a high salt solution comprising 50mM Tris, 250mM NaCl, pH 8.1-8.5.

17. A method for producing purified recombinant PEGylated recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:

a. in a bioreactor, culturing *E. coli* that produce rhARG at 36°C and 38°C, and pH of between 7.0 and 7.4, with agitation and aeration;

i. adjusting the temperature of the bioreactor to 29°C;

ii. inducing *E. coli* to produce for rhARG;

iii. culturing the *E. coli* for 18 hours;

iv. harvesting the *E. coli* by centrifugation;

b. lysing the *E. coli* cells by high pressure homogenization in 25 mM HEPES at a pH of 7.2 to 7.6, and at or below 15°C;

c. removing cell debris from the lysate by centrifugation at or below 15°C, then filtering the lysate with an 0.8 micron filter then filtering with an 0.5 micron filter;

d. loading the cell lysate on a cation exchange column then washing the column with 25 mM HEPES, pH 7.2-7.6;

e. eluting the rhARG at room temperature with a high salt solution comprising 25mM HEPES, 0.1M NaCl, pH 7.2-7.6;

f. incubating the eluted rhARG with 10 mM CoCl2 at room temperature for 2 to 8 hours to form cobalt-substituted rhARG (Co-rhARG);

i. exchanging Co-rhARG into 50mM Tris, pH 8.1-8.5;

g. applying the Co-rhARG to an anion exchange column and collecting the flow-through;

h. applying the flow-through onto a Capto multimodal chromatography (MMC) column;

i. eluting the Co-rhARG from the MMC column with a high salt solution comprising 50mM Tris, 250mM NaCl, pH 8.1-8.5 (MMC Buffer);

    i. exchanging the MMC Buffer for 20mM Sodium Phosphate, 50mM NaCl, 1.5% Glycerol, pH 7.4 (Buffer 1), and adjusting the concentration of protein to 5.0 mg/mL;

    ii. exchanging Buffer 1 for 0.1M sodium phosphate, pH 8.1-8.5 (Buffer 2), and concentrating the protein concentration to 10.0 mg/mL;

j. adding a molar excess of methoxy PEG succinimidyl carboxymethyl ester 5,000 Da approximate molecular weight, wherein 19 moles of methoxy PEG succinimidyl carboxymethyl ester is added for each mole of protein and incubating this mixture for 30 minutes to 4 hours at approximately pH 8.4;

k. removing excess PEG by exchanging Buffer 2 for 20mM Sodium Phosphate, 50mM NaCl, 1.5% Glycerol, pH 7.4.