TOWARDS THE CREATION OF AN "ASTROPHARMACY": ON-DEMAND PEPTIDE DRUG PRODUCTION FOR USES ON EARTH AND IN SPACE

By

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Thesis

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Abstract of TOWARDS THE CREATION OF AN "ASTROPHARMACY": ON-DEMAND PEPTIDE DRUG PRODUCTION FOR USES ON EARTH AND IN SPACE*, by ARVIND VELUVALI, ScM, Brown University, MAY, 2021.*

The Astronaut Medication List, a roster detailing those drugs that astronauts take on space missions, contains several essential peptide-based drugs, which quickly degrade and must be replenished according to the astronauts' needs. However, replenishment becomes complicated on any space mission, and particularly on longer-duration missions, when resupply is difficult and it is impossible to predict the specific medication needs of the crew. Thus, it is necessary to develop a platform for on-demand peptide drug production, in order to ensure the success of long-term space exploration and colonization missions to Mars and beyond.

The Astropharmacy is a peptide drug production platform that uses genetically-engineered bacterial spores, along with a cell-free system, in order to produce drugs on-demand. Not only would this platform allow astronauts to produce drugs according to their needs, but it would also have utility on Earth: from bioterror prevention to orphan drug production, to drug production in remote areas where refrigeration is impossible.

The purpose of this study is to outline efforts to develop the Astropharmacy platform, as well as use the Astropharmacy to produce small quantities of peptide drugs. It is hoped that this thesis will have utility not only to NASA for use on long-term space missions, but also to Earth-based agencies like the Department of Defense and non-governmental organizations like the World Health Organization.

CHAPTER 1: INTRODUCTION

Cheap, easily-accessible drugs are a cornerstone of life in the developed world. Pharmaceutical drugs are used in the treatment and management of hundreds of illnesses and other ailments, from common headaches to severe cancers, with new drugs constantly in development and millions of doses of existing drugs being manufactured every day.

Pharmaceutical drugs are divided into several categories, depending on the drugs' formulation and origin. One such category is composed of peptide-based (protein-based) drugs. Peptide-based pharmaceuticals have been in use for more than 100 years, beginning with insulin in the 1920s[1]. Today, peptide-based drugs are a broad category, used to treat a variety of illnesses and symptoms. Among others, examples of such drugs include Vasopressin (used to dilate narrow blood vessels and raise blood pressure); Oxytocin (used to initiate contractions during labor, as well as to stem bleeding following childbirth); and Ziconotide (a non-opioid analgesic used in the management of chronic pain).[2]

Despite their wide utility, peptide-based drugs are more finicky than non-biological drugs like acetaminophen (Tylenol®) and diphenhydramine (Benadryl®). Peptide-based drugs require certain maintenance conditions to be met; chief among these conditions is refrigeration[3]. However, even refrigeration does not completely obviate the problem of storage; even when kept at cold temperatures, peptide-based drugs degrade much more quickly than their non-protein counterparts. For example, while insulin lasts 28 days at room temperature (15°-30° C)[4], a refrigerated, unopened vial of insulin loses its potency after one year at 2°-8° C (and manufacturers nonetheless recommend that users dispose of insulin after four weeks in the refrigerator)[5].

While the unique storage requirements of peptide-based drugs do not pose much of a problem in the developed world, where refrigeration is not only cheap but also near-universally accessible, these demands prevent peptide-based drugs from reaching all potential users. One such neglected group of users is in the developing world. According to the International Diabetes Federation, in 30 of 37 low-income countries surveyed, at least 33% of families with diabetic children lacked the refrigeration capabilities necessary to store insulin[6].

In addition to their uses on Earth, pharmaceutical drugs have found a new venue: space. Space is an incredibly fraught environment; for example, the astronauts orbiting upon the International Space Station (ISS) are exposed to a variety of space-related hazards, including low gravity, space radiation, and all of the dangers associated with life aboard the football field-sized satellite. These hazards take a toll on astronauts' health, affecting the cardiovascular, nervous, endocrine, muscular, and renal systems, among others. Indeed, astronauts have fallen ill in space, suffering through upper respiratory infections (URIs), colds, skin infections, and, in one case, a blood clot in an astronaut's jugular (which was treated successfully with blood thinners[7]). According to Dr. Jonathan Clark, a former six-time crew surgeon for NASA's space shuttle program, the poor health of crew members had an adverse impact[8] on the shuttle missions Apollo 7, Apollo 8, Apollo 9, and Apollo 13.

In order to head off concerns about astronauts' wellbeing, NASA has developed protocols to ensure that mission crews remain healthy in space. Among these protocols is the Astronaut Medication List, which details[9] 143[10] drugs that NASA astronauts bring on space missions (see Appendix 1 for a

current list). It is here that concerns about drug storage on Earth dovetail with concerns about drug storage in space; 87% of the drugs represented on the Astronaut Medication List have a shelf life of less than 24 months[11], with many of those drugs being peptide-based drugs like human granulocyte-colony stimulating factor (hG-CSF), Entolimod, Reteplase, and Teriparatide (PTH). While the storage of these drugs generally does not pose a problem on the short-term missions which NASA and other space agencies have undergone so far, NASA has stated a desire to embark upon longer missions, aiming to launch a human mission to Mars in the 2030s[12]. Indeed, a round-trip mission to Mars has been estimated to be two years in duration[13]–not including the advance manufacture of necessary components, early loading of supplies, and potential launch delays. Since drugs' shelf lives cannot be easily extended past manufacture[14], various means to obviate shelf life concerns entirely must be considered.

In recognition of this problem, NASA's Translational Research Institute for Space Health (TRISH), a cooperative agreement between the Baylor College of Medicine, the California Institute of Technology, and the Massachusetts Institute of Technology, put out a call for proposals in search of a "just-in-time" drug manufacture system. The call challenges researchers "[t]o design and develop a capability that can manufacture pharmaceuticals or biologics in less than 24 hours. This machine should (1) have the capability of reconfiguration within a couple hours and with minimal human intervention to change the drug to be manufactured and (2) use precursors that do not require refrigeration and themselves have a shelf life of more than 3 years. Mass, power and volume requirements should be reduced as much as possible, however they are not required to be mission feasible for this solicitation."[15]

Several research groups have answered TRISH's request. Karen McDonald, Professor of Chemical Engineering at UC Davis, received a TRISH grant to develop "A Plant-Based Platform for 'Just in Time' Medications", aiming to grow peptide drugs inside genetically-modified lettuce[16]. Another project, led by MIT's Robert Langer, envisions a "mother machine": a small device, ingested in the same manner as an oral drug, which resides in the user's stomach and releases the manufactured drug over a period of time[17]. These solutions and their associated advantages and disadvantages are discussed in detail in the "Discussion and Conclusions" section of this paper.

This thesis describes another "just-in-time" manufacturing system for peptide drugs. This system, developed at the NASA Ames Research Center in Mountain View, CA, is known as the "Astropharmacy". The rationale supporting the Astropharmacy, as well as the experiments undertaken in service of its development, are detailed throughout the rest of this thesis.

CHAPTER 2: HYPOTHESIS

I. Study Model

The Astropharmacy is composed of two parallel biological synthesis systems for peptide-based drugs. The first synthesis system involves cellular systems in bacteria. For this experiment, NEB 5-alpha competent *Escherichia coli* cells were used due to their reliability and wide use in the laboratory setting (we would anticipate using a more robust spore-former, such as *Bacillus subtilis*, in space).

The second synthesis system involves a cell-free system. A cell-free system uses a cell's transcriptional and translational machinery, along with a source of energy and a DNA sequence, in order to manufacture the protein encoded by that DNA sequence. These components can be housed in a variety of settings: free-floating in a test tube, for example, or even freeze-dried onto a paper cellulose backing. As opposed to a cellular system, a cell-free system carries several distinct advantages[18].

First, a cell-free system is not alive, and thus does not require the maintenance conditions necessary to keep a cell alive. Not only does this mean that a cell-free system can manufacture materials that are toxic to cells[19], but also that a cell-free system wastes no energy in the synthesis of proteins required for cell maintenance. Instead, all energy is channeled toward producing the protein of interest, leading to more robust output of the desired protein.

Second, a cell-free system is more flexible than a cellular system. Because a cell-free system can be directly manipulated, it is far easier to upscale or alter a reaction[20]. Owing to this, a cell-free system

makes it possible to toggle the quantity of drug produced, as well as to produce multiple drugs simultaneously. This flexibility is a great benefit in a resource-constrained setting like space.

This is not to say that cell-free systems do not carry disadvantages, as well. Bacteria-derived cell-free systems (such as those derived from *E. coli*) are incapable of post-translational modifications[21], which may impede a protein's normal folding characteristics and thus diminish its efficacy (yeast-based cell-free systems, however, are capable of post-translational modifications like glycosylation). Additionally, the lack of a membrane means that cell-free systems cannot synthesize membrane proteins[22]. And, finally, cell-free systems are highly susceptible to contamination during their production (which can be solved through the addition of RNAses)[23].

II. Study Scope and Limitations

Overall, this thesis aims to prove the hypothesis that peptide drugs can be quickly and effectively synthesized in cellular and cell-free systems. If so, this study will serve as a useful proof-of-concept for the Astropharmacy, and aid in the justification for its application in long-term space missions.

It is important to note that drug purification is beyond the scope of this thesis. While it is possible to purify protein products from bacteria[24] (as is commonly done in insulin manufacturing[25]) and from cell-free systems (employing the commonly-used Ni-NTA purification protocol for recombinant His-tagged proteins[26]), purification to acceptable drug standards is left as an exercise for future researchers (an examination of acceptable standards and how those might be achieved can be found in the "Discussion and Conclusions" section of this thesis).

Furthermore, while this study aligns with TRISH's goals of manufacturing peptide drugs in microgravity, it was infeasible to conduct experiments in such conditions. All experiments took place on Earth, and results observed in this paper may differ in a different gravity regime, such as the microgravity of ISS.

Finally, this thesis details the creation of a bespoke cell-free system. In replicating the experiments detailed in the subsequent section of this thesis, one might consider using a OnePot PURE system, which is less expensive, less labor-intensive, and arguably produces more consistent results[27]. OnePot PURE need not be the only commercial kit used in testing; multiple commercially-available cell-free systems could be used in parallel, in order to determine which is the most efficient and stable.

III. Drug Choice

We chose two peptide-based drugs of interest for this experiment: Teriparatide (PTH) and Human Granulocyte Colony-Stimulating Factor (hG-CSF). These peptide-based drugs were chosen from the Astronaut Medication List among other peptide-based drugs, because they share essential common characteristics with one another. Namely: their small size makes them relatively easy to produce; they are currently synthesized commercially using *E. coli*[28] [29]; and there are no post-translational modifications, which would make synthesis in an *E. coli* cell-free system difficult since laboratory *E. coli* strains lack endogenous glycosylation enzymes[30]. The applications of each drug in the space and Earth settings are described in Table 1, below.

Table 1: Drug Choice.

Drug Name	**Is it on the Astronaut Medication List?**	**Uses on Earth**	**Uses in Space**
Teriparatide	Yes	Treatment of osteoporosis	Treatment of space-induced bone density loss
Human Granulocyte Colony-Stimulating Factor (hG-CSF)	Yes	Treatment of neutropenia	Treatment of space-induced neutropenia

CHAPTER 3: MATERIALS AND METHODS

Because this thesis details the creation of two separate expression systems (a bacterial expression system and a cell-free expression system), the "Materials and Methods" section of the thesis is similarly subdivided.

I. Cellular Expression System

The aim in the Astropharmacy's cellular expression system was to transform DNA sequences encoding for peptide-based drugs into the bacterial models, using the Gibson assembly process. Gibson assembly was chosen over CRISPR because of materials available in the lab, and its relative ease-of-use.

Design of Genetic Constructs

In order to design genetic constructs, we first needed to identify the functional region of the respective drug proteins. This was done by running the two drug proteins' respective amino acid sequences through the Eukaryotic Linear Motif (ELM) database, which identifies the functional residues of eukaryotic proteins, and through the Human Protein Reference Database, which identifies functional residues in human proteins. Next, we added solubility factors in order to prevent protein clumping upon the protein's translation[31]. We added a 6x His-tag for Ni-NTA purification of protein, and finally we added a TEV protease cut site for transformation purposes.

Diagrams of assembled constructs with pET-19b and Teriparatide, and pET-19b and hG-CSF, can be found in Figs. 1 and 2, respectively.

Figure 1: pET-19b with Teriparatide Insert.

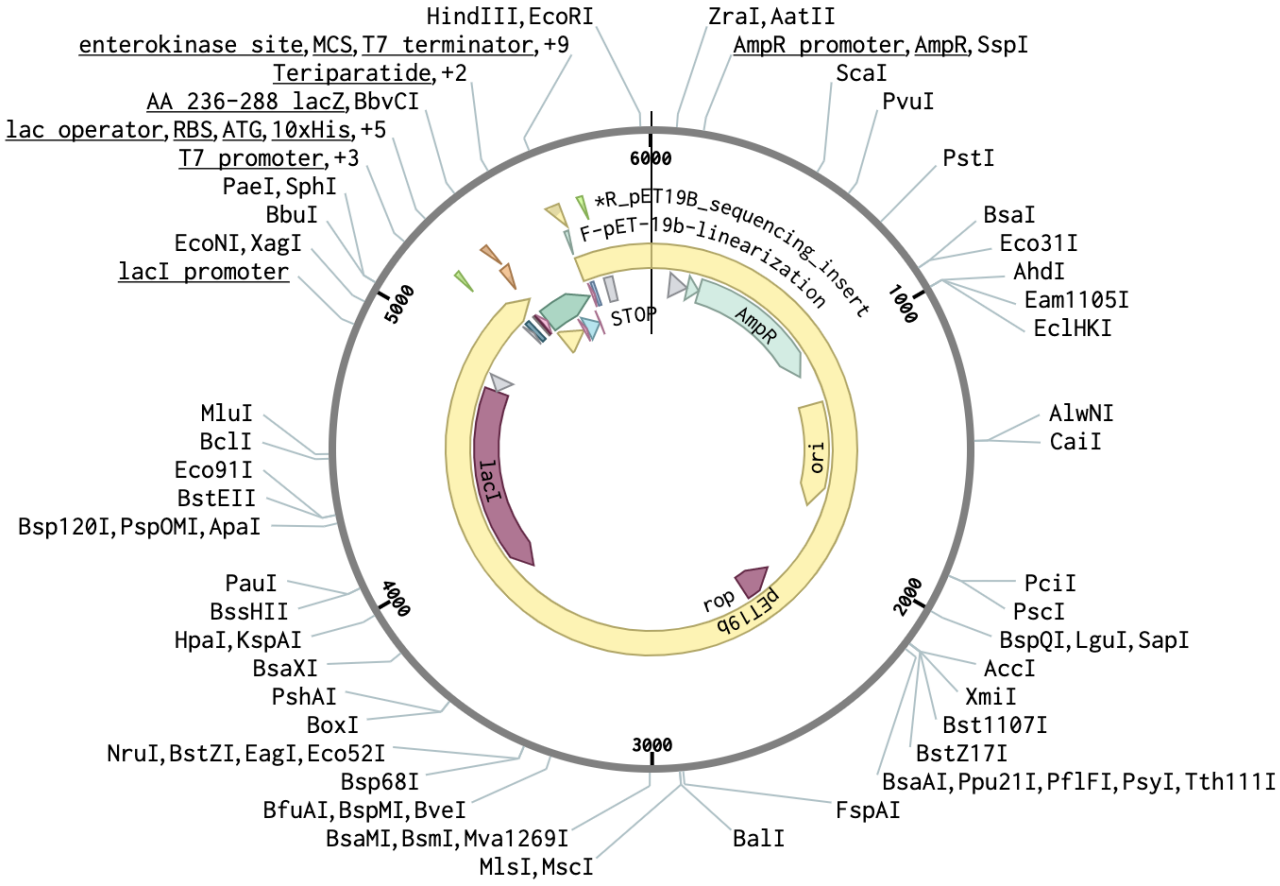
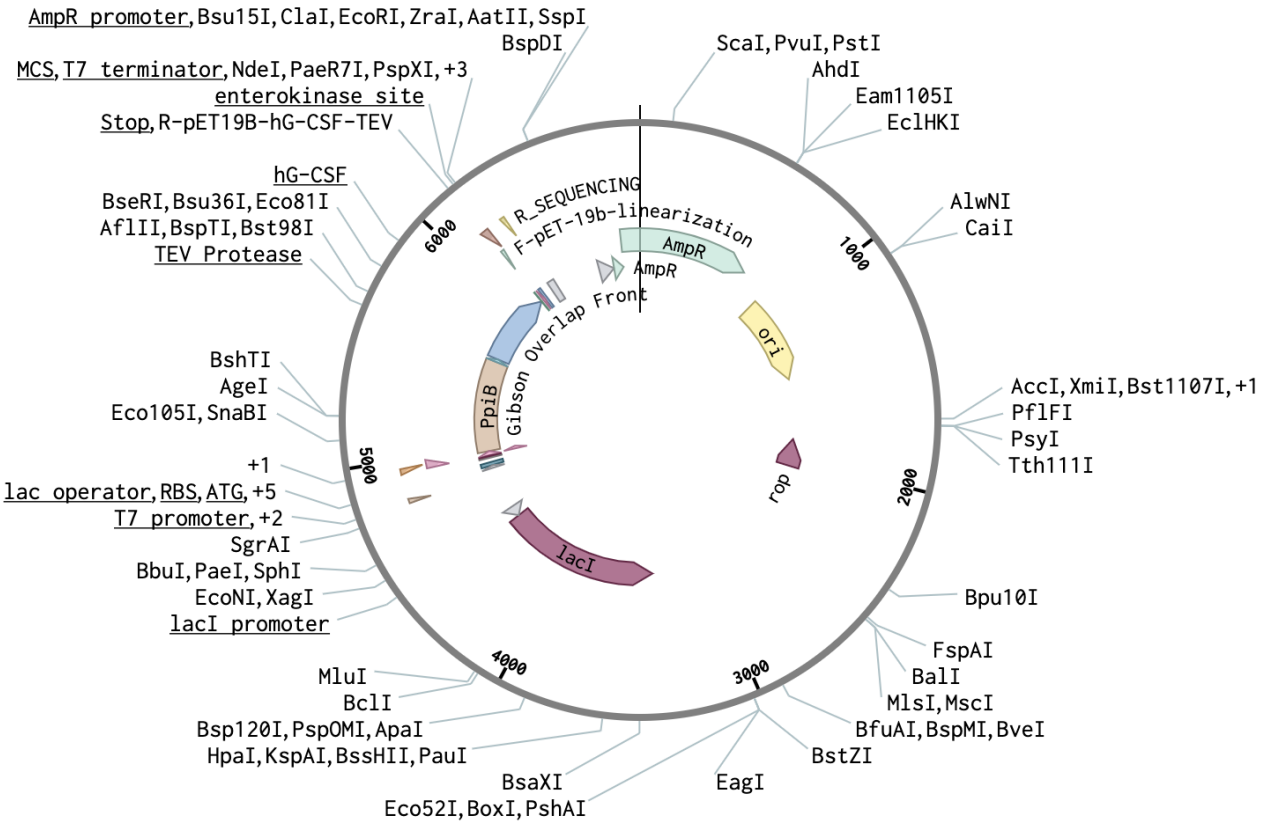


Figure 2: pET-19b With hG-CSF Insert.



Gibson Assembly

i. Materials

For Gibson assembly of our construct, we used 4 µg of lyophilized, commercially-available pET-23b[32] plasmid, because it contained the cut sites necessary for insertion of the peptide-coding DNA sequences. Additionally, we used sterile 100 µL of sterile H_2O, 3.75 µL of forward primer (designed according to the plasmid backbone and template DNA), 3.75 µL of reverse primer (designed according to the plasmid backbone and template DNA), and 37.5 µL of Q5 2X Master Mix[33]. Drug constructs

for Teriparatide and hG-CSF were ordered from AddGene. Additionally, we used a thermocycler, a thermal block, a vortex machine, a centrifuge, and eight PCR tubes.

iii. Protocol (adapted from OpenWetWare[34])

We first diluted both the forward primer and the reverse primer to a concentration of 10 μM. This was accomplished by adding sterile H_2O to the concentrated primer that arrived from AddGene, until a concentration of 10 μM was reached. Note that the total volume of the diluted forward primer and for the diluted reverse primer was 3.75 μL each, as described above.

Next, we linearized the pET-23b backbone to promote the eventual annealing of the drug constructs to the vector. This was done by adding the following into a PCR tube: 19 μL sterile H_2O; 2.5 μL forward primer (at 10 μM dilution); 2.5 μL reverse primer (at 10 μM dilution); 1 μL of template DNA; and 25 μL of Q5 2X Master Mix. This mixture was then put into a thermocycler programmed to the following order: Initial denature: 98°C for 30 seconds; Denature: 98°C for 10 seconds; Annealing: 61°C for 30 seconds; Extension: 72°C for 35 seconds. All of these steps were repeated 28 times, with a final extension at 72°C for 120 seconds. This mixture was then held at 10°C until use. We verified the linearization by sequence analysis, conducted by ElimBiopharmaceuticals, Inc.[35].

We then performed a PCR addition of overhangs to the drug constructs so that the constructs would anneal to our plasmid vector. This was done by adding the following into a PCR tube: 9.5 μL sterile H_2O; 1.25 μL forward primer (10 μM dilution); 1.25 μL reverse primer (10 μM dilution); 0.5 μL template DNA; and 12.5 μL Q5 2X Master Mix. This mixture was then put into a thermocycler

programmed to the following order: Initial denature: 98 ˚C for 30 seconds; Denature: 98 ˚C for 10 seconds; Annealing[36]: Teriparatide: 57 ˚C for 30 seconds and hG-CSF: 69 ˚C for 30 seconds; Extension: 72˚C for 15 seconds. This process was repeated 28 times, with a final extension at 72˚C for 120 seconds. This mixture was then held at 10˚C until use.

With the plasmid backbone linearized and the overhangs added onto our drug sequences, we were able to proceed with Gibson assembly. First, we preheated a thermal block to 50˚C. We suspended the vector in sterile H_2O and portioned out liquid volume of the suspension into a PCR tube corresponding with 100 ng of vector. Using New England Biolabs's Ligation Calculator[37], we calculated the amount of insert needed for the reaction, in ng, and portioned out the liquid volume of insert corresponding to the ng amount. After this, we added to the PCR tube 10 μL of Q5 2X Master Mix, and sterile H_2O such that the total volume in the PCR tube was 20 μL. This assembly process was repeated four times, so that we had 5 PCR tubes containing the necessary components for Gibson assembly. After all of the reactions were assembled, we vortexed and then centrifuged the PCR tubes at 2000 RCF for 1 minute. We incubated the PCR tubes on the preheated thermal block for 15 minutes, after which our constructs were assembled. These constructs were then stored at -20˚C until use.

Bacterial Transformation

i. Materials

For bacterial transformation, we used five centrifuge tubes; 125 μL of NEB 5-alpha competent *E. coli* cells[38]; 5 μL of assembled construct (from the previous Gibson assembly step); ice and an ice bucket;

4750 µL of SOC medium[39]; and five selection plates prepared with ampicillin. In addition, we used a thermal block, a shaking incubator, and a pipette.

ii. Protocol

First, we filled an ice bucket with ice and placed our five centrifuge tubes inside. We portioned 25 µL of NEB 5-alpha competent into each tube and let the tubes chill for 10 minutes. After this, we added 1 µL of assembled construct into each tube, and let the tubes chill for 30 minutes. Meanwhile, we preheated the thermal block to 42˚C, pouring water into each well so that each well was about halfway full. After the tubes had chilled for 30 minutes, we heat shocked the tubes on the thermal block for 30 seconds; following this, we chilled the tubes on ice for 5 minutes. After chilling, we transferred 950 µL of lukewarm SOC medium into each tube. The tubes were placed in a shaking incubator at 37˚C and 250 rpm, for 60 minutes. While the tubes were incubating, we prepared selection plates with ampicillin and incubated the plates at 37˚C. When the tubes had finished incubating, we used the pipette to mix the SOC medium and the cells, and distributed 50 µL of solution onto each plate. The plates were incubated at 37˚C for 16 hours.

Bacterial Protein Synthesis

i. Materials

For induction of bacterial protein synthesis, we used a plastic inoculation needle; 1.2 L of ampicillin-containing LB broth[40]; Isopropyl β- d-1-thiogalactopyranoside (IPTG); three 15 mL Falcon tubes, a 1 L conical flask; a 250 mL centrifuge tube; and a centrifuge. For protein extraction, we used

10 mL of ThermoFisher B-PER™ Bacterial Protein Extraction Reagent[41]; a pipette; an incubator; a spectrophotometer; and a centrifuge.

ii. Protocol

For induction of protein synthesis, we first isolated a single bacterial colony from each plate (from the previous bacterial transformation step) using the plastic inoculation needle. We suspended this colony in a 15 mL Falcon tube (since there were five plates, one could theoretically repeat this step and the following four times), along with 5 mL of Ampicillin-containing LB broth. This mixture was incubated overnight at 37°C. Additionally, we set aside 5 mL of uncontaminated Ampicillin-containing LB broth in its own Falcon tube, for use as a blank. The next morning, we placed 0.5 L antibiotic-containing LB and added 10 mL of starter culture into a 1 L conical flask. We incubated the conical flask at 37°C until $0.4 < OD < 0.6$ (optical density), using the uncontaminated LB broth as a blank and measuring OD using the spectrophotometer. After the desired OD was reached, we added IPTG to the conical flask to a concentration of 0.4 mM, and incubated the mixture at 37° C for 2 hours. After this time had elapsed, we transferred the mixture to a 250 mL centrifuge tube and spun it down at 2000 RCF until a pellet formed; we discarded the supernatant.

For protein extraction, we added 4 mL of ThermoFisher B-PER™ Bacterial Protein Extraction Reagent per gram of pellet. We mixed the solution using a pipette until homogenous, and incubated it for 15 minutes at 37℃. After this time had elapsed, we transferred the mixture to a 250 mL centrifuge tube, and spun it down at 2000 RCF until a pellet formed. Finally, we isolated the liquid fraction–this

was lysate, which contained our protein of interest–and transferred it to a clean 15 mL Falcon tube. We stored this at 20°C until use.

Protein Verification and Quantification

Following protein extraction, we verified our protein's presence via SDS-PAGE, whose material and protocol can be found online[42]. Protein quantification was conducted via the Thermo Scientific NanoDrop UV-Vis spectrophotometry system.

II. Cell-Free Expression System

As described above, a cell-free system contains all of the cellular machinery necessary for transcription and translation; a source of energy; and DNA sequence(s) coding for the protein(s) of interest. The cellular machinery is derived from cell lysate; that is, a ruptured cell whose TX/TL machinery has been extracted.

Cell lysate can be derived from many different sources. The most frequently-used sources are *E. coli*[43] cells, rabbit reticulocyte[44] cells, and wheat germ[45] cells. Construction of a cell-free system for the Astropharmacy was prototyped with *E. coli* cells because *E. coli*-based cell-free systems have higher protein yields than cell-free systems derived from other organisms, with an upper limit of 500 μg/ml of protein expression[46].

The protocol that we used to create our cell-free system is shown below. This procedure was adapted from Levine et. al[47] with the assistance of Dr. Kate Adamala[48] of the University of Minnesota, who is an expert in cell-free systems. In addition, the commercially-available cell-free system, PURExpress from New England Biolabs[49], was used as a control for the Astropharmacy; by using a commercial kit, we were able to ensure that our cell-free system constructed in the lab worked as intended. PureExpress differs from other cell-free systems[50] in that all recombinant proteins that result from this system are His-tagged, meaning that the protein can be purified simply by removing the rest of PureExpress's components. This protein can then be directly assayed without labeling and additional purification.

Finally, it is important to note that the DNA segment expressed in the cell-free system is the same segment expressed in the bacterial system.

Cell-Free Lysate Creation

i. Materials

For cell-free lysate creation, we used 50 mL of LB broth WITHOUT antibiotic; BL21 *E. coli* strain, which was purchased from ThermoFisher[51]; 10 mL of 1M Tris acetate, pH=8.2; 14 mL of 1M $Mg(CH_3COO)_2$; 5.89 g of CH_3CO_2K.; 974 mL of DI H_2O; 1 L of 2xYPTG medium, plus an additional 11 mL of 2xYPTG medium; 15 mL of 1M DTT; a biosafety hood; a 200 mL Erlenmeyer flask; a shaking incubator; a 1 L bottle; a sterile filter; three 2000 mL Erlenmeyer flasks; a spectrophotometer; a temperature gun; three ice buckets; ice; four 250 mL centrifuge containers; eight 15 mL Falcon tubes; a centrifuge; a pipette; a plastic 1000 mL tripour beaker; a Misonix XL2020 sonicator; a mini-centrifuge; 20 Eppendorf tubes; and 20 100 µL PCR tubes.

ii. Protocol

Creation of the cell-free expression system lysate was conducted over the course of 3 consecutive days.

On the first day, we turned on the biosafety hood and placed the LB broth WITHOUT antibiotic inside the hood (antibiotic is unnecessary for the BL21 *E. coli* strain[52]). We pipetted 30-50 mL of LB broth into a sterile, 200 mL Erlenmeyer flask (ample headspace is necessary for proper aeration of *E. coli*), and placed frozen BL21 *E. coli* into the Erlenmeyer flask. We incubated the Erlenmeyer flask in a shaking incubator at 37°C and 250 rpm overnight.

Additionally, we prepared Wash Buffer A. To a 1 L bottle, we added 10 mL of 1M Tris acetate, pH=8.2; 14 mL of 1M $Mg(CH_3COO)_2$; 5.89 g of CH_3CO_2K; and 974 mL of DI H_2O. The mixture was filter sterilized and stored at 4˚C until use.

On the second day, we added 333 mL of 2xYPTG media into each of three 2000 mL Erlenmeyer flasks, setting aside 1 mL of 2xYPTG media for use as a blank for the spectrophotometer. An additional 10 mL of 2xYPTG medium was set aside for dilution purposes. We then added 1mL/L of the overnight start culture to the 2000 mL Erlenmeyer flasks containing the 2xYPTG medium. The cells were grown in a shaking incubator at 30˚-34˚C and 250 rpm, until $0.4 < OD < 0.5$. Meanwhile, we filled three ice buckets with ice. Once the desired OD was reached, we removed the Erlenmeyer flasks from the incubator and put them on ice, along with four 250mL centrifuge containers and four pre-weighed 15 mL Falcon tubes. We cooled the Erlenmeyer flasks down until their contents reached 4˚C, as measured by the temperature gun. Once this temperature was reached, we divided the cell culture from the Erlenmeyer flasks evenly between the four 250mL centrifuge containers and centrifuged at 5000 RCF and 4˚C for 10 minutes. Following the centrifuge, we removed the supernatant from each centrifuge container with a pipette and discarded the supernatant. We then retrieved Wash Buffer A from the refrigerator and added 2 mL of 1M DTT. We resuspended each pellet in 100 mL of 4˚C Wash Buffer A, and centrifuged at 5000RCF and 4˚C for 15 minutes. We once more removed and discarded the supernatant from each centrifuge container, resuspended the pellets in 100 mL of 4˚C Wash Buffer A, and centrifuged at 5000 RCF and 4˚C for 15 minutes. Following this step, we removed the supernatant with a pipette and suspended the pellet in 10 mL of 4 ˚C Wash Buffer A; this mixture was transferred

to four pre-chilled, pre-weighed Falcon tubes. We centrifuged these Falcon tubes at 5000 RCF and 4˚C for 15 minutes; we removed the supernatant with a pipette, re-weighed the tubes, and calculated the mass of the pellet. Finally, the Falcon tubes (containing their pellets) were stored at -80˚C.

On the third day, we pre-cooled the centrifuge to 4˚C. We placed the frozen cells (inside the -80˚C Falcon tubes) on ice and thawed them. We resuspended the pellets by adding 1.1 mL of 4˚C Wash Buffer A added 2.2 µL DTT for every 1g of pellet weight. After this, we made an ice water bath inside the plastic 1000 mL tripour beaker. We then set a Misonix XL2020 sonicator to 20kHZ and an amplitude setting of 2; we placed the each Falcon tube inside the ice bath and programmed the sonicator to run for 5 rounds, with each round consisting of 10 seconds on and 10 seconds off. Following sonication, we centrifuged the sonicated Falcon tubes at 12000RCF and 4˚C for 10 minutes. After the centrifuge, we removed the supernatant with a pipette and portioned the supernatant into 1 mL Eppendorf tubes. We then performed a quick spin-down in a mini-centrifuge. We aliquoted 99 µL portions of the resulting supernatant (the **cell lysate**) into 100 µL PCR tubes. The lysate was flash-frozen and stored at -80˚C until use.

Cell-Free Protein Expression

i. Materials

For protein expression in our cell-free expression system, we used 10 µL Amino Acid Mix[53]; 10 µL Energy Mix[54]; 10 µL Salt Mix; 33 µL Cell Lysate (from the procedure described in the preceding section); 0.25 µL 1M DTT; 100 µg/mL T7 RNA polymerase; 3 µL RNAse inhibitor; Supplements; 13.3 µg/mL plasmid; and 30 µL of sterile H_2O. We also used a thermocycler and a PCR tube.

ii. Methods

We heated the thermocycler to 30°C. Into a PCR tube, we combined 10 µL Amino Acid Mix; 10 µL Energy Mix; 10 µL Salt Mix; 33 µL Cell Lysate (from the procedure described in the preceding section); 0.25 µL 1M DTT; 100 µg/mL T7 RNA polymerase; 3 µL RNAse inhibitor; Supplements; 13.3 µg/mL plasmid; and of sterile H_2O such that total volume was 100 µL. We incubated the PCR tube in the thermocycler for 5 hours.

Cell-Free Protein Purification and Quantification

We purified the protein using Ni-NTA purification and used SDS-PAGE to verify that the protein was being produced. Protein quantification was conducted via the Thermo Scientific NanoDrop UV-Vis spectrophotometry system.

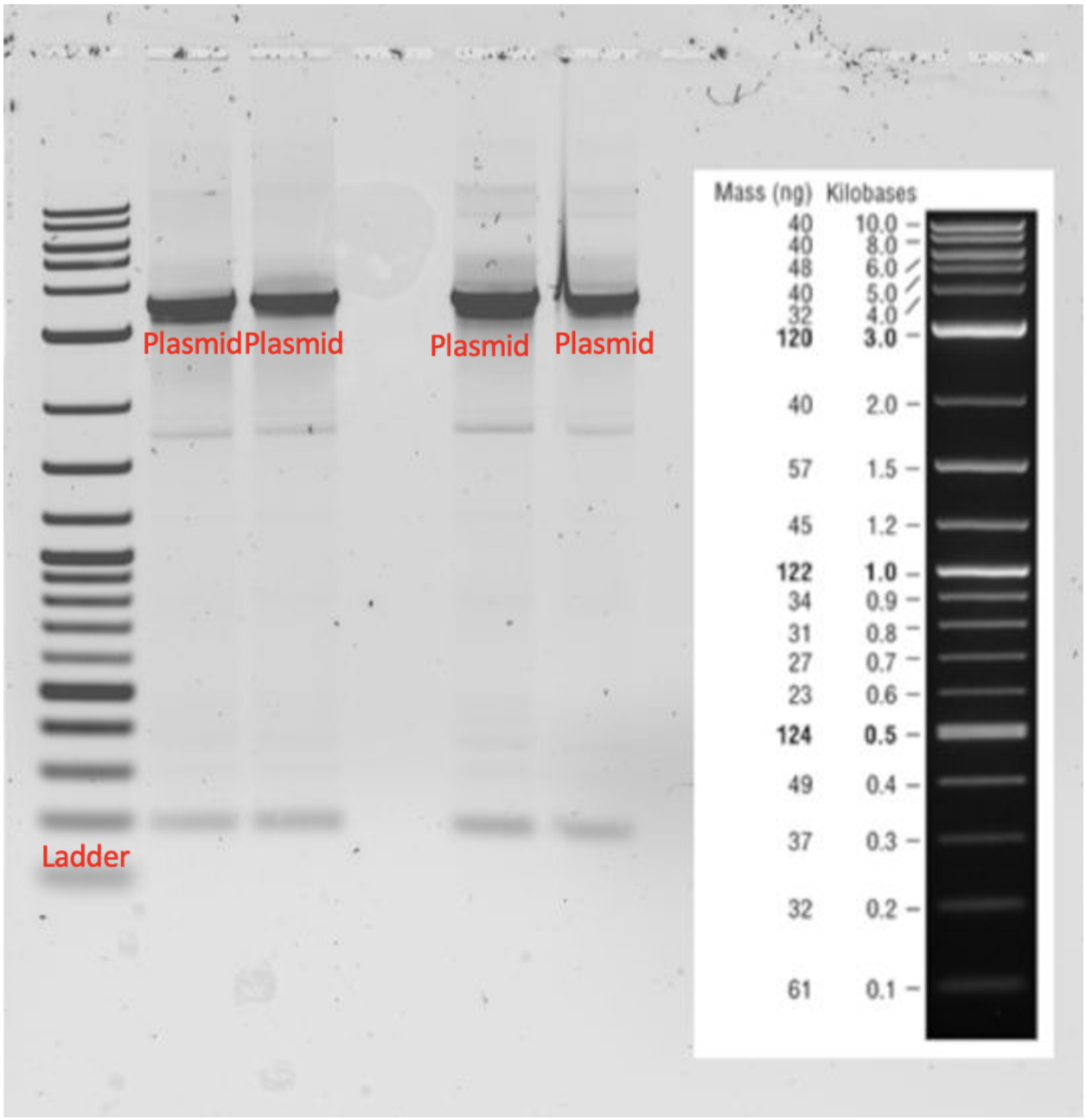
CHAPTER 4: RESULTS

I. Synthesis of Peptide Drugs in Bacteria

A therapeutic dose of a peptide-based drug can be produced by a cellular expression system within 24 hours.

We were able to successfully linearize the pET-23b plasmid backbone, which was determined through an SDS-PAGE analysis that showed that the actual size corresponded to the expected size (Fig. 3). This set us up for a successful insertion of our peptide-coding sequences into the plasmid. Indeed, we were able to insert the sequences for Teriparatide, hG-CSF, and an RFP control into the pET-23b backbone; insertion was verified by sequence analysis conducted by Elim Biopharmaceutical.

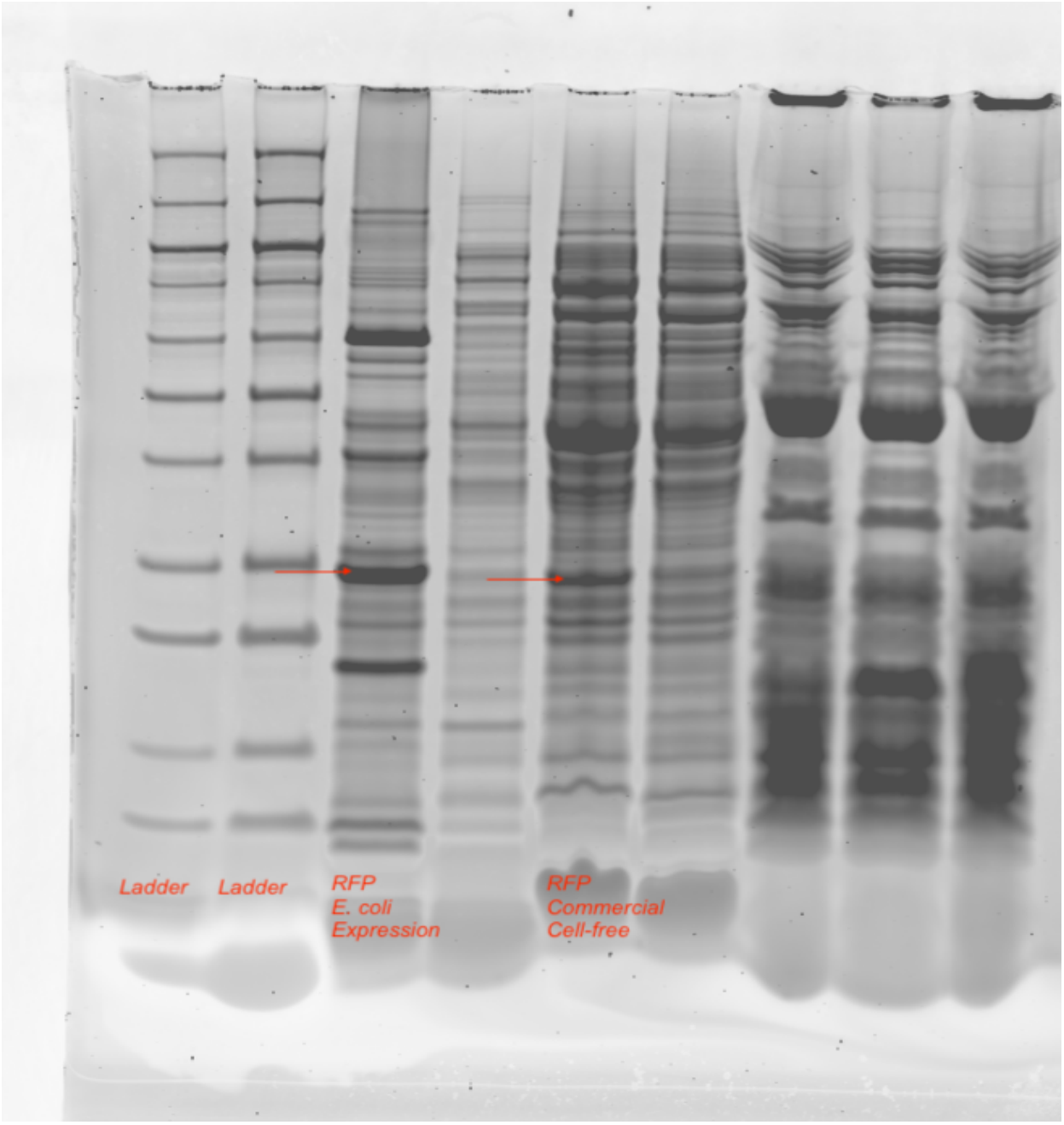
Figure 3: Successful Linearization of pET-23b. (expected size: ~3524 bp)



Following transformation, protein expression induction, and protein extraction (following centrifuge and testing of the supernatant, as described above), we assessed the protein production capabilities of our cellular expression system.

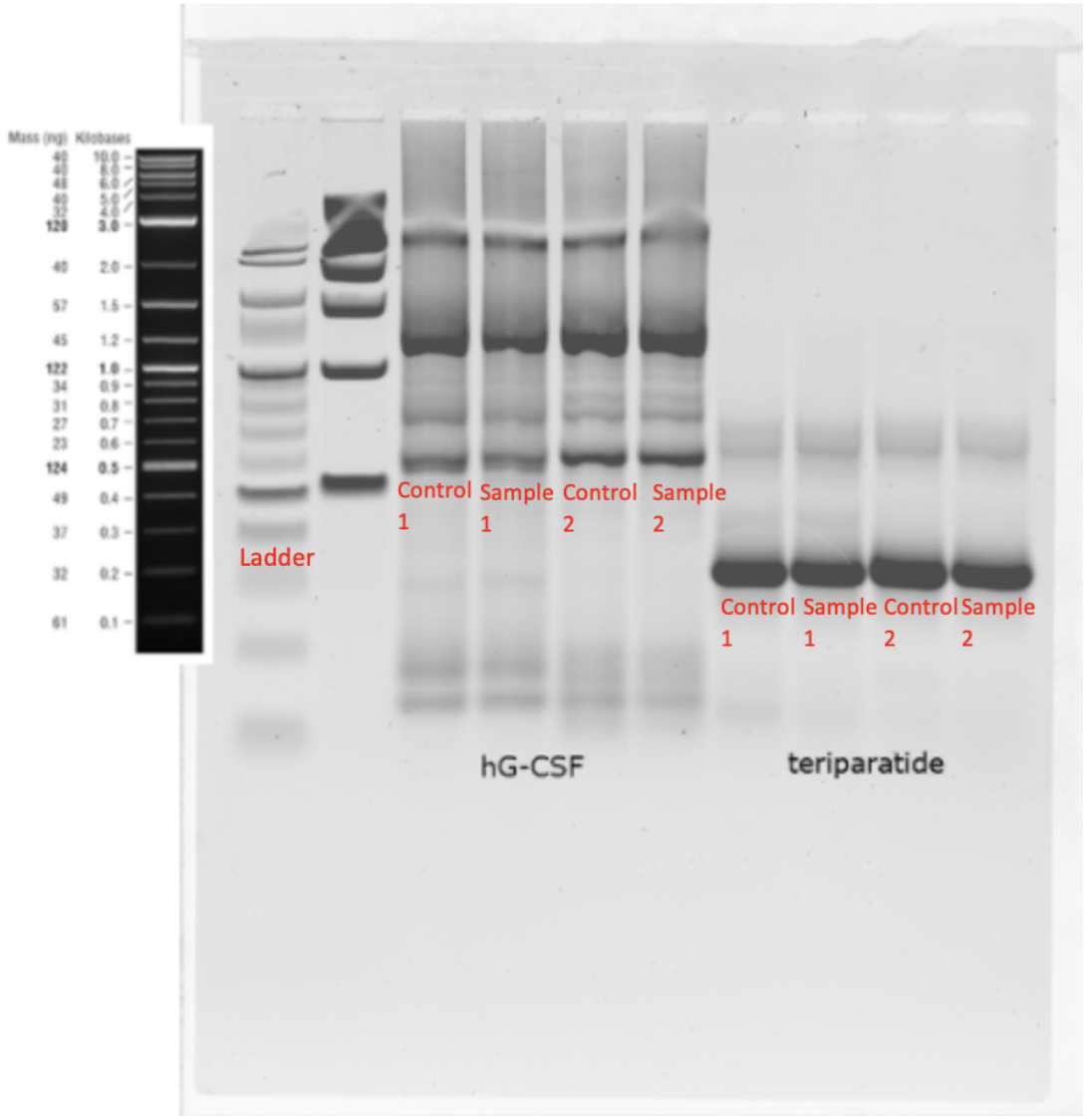
First, we established a control. We compared the RFP produced by our cellular expression system to a purified RFP protein that was available commercially. We saw that synthesis of RFP control was successful in *E. coli* (Fig. 4), which demonstrated that our cellular protein synthesis platform was functional.

Figure 4: Production of RFP control in E. coli. Purified proteins were used as controls.



With the functionality of our cellular expression system determined, we were able to proceed. We were ultimately able to demonstrate production of both Teriparatide and hG-CSF in *E. coli*; protein products were tested against purified protein controls (Fig. 5).

Figure 5: Production of Teriparatide and hG-CSF in *E. coli*. Purified proteins were used as controls.



Overall, six dose-equivalents of Teriparatide and six doses of hG-CSF (as determined by molecular weight) were produced in the cellular system within 24 hours; this far exceeded TRISH's stated goal of a single dose within that time frame, and seemed to confirm our hypothesis that a therapeutic dose of a peptide-based pharmaceutical could be produced by a cellular expression system within 24 hours.

II. Synthesis of Peptide Drugs in a Cell-Free System

An in-house cell-free system can produce a therapeutic dose of a peptide-based drug within 24 hours.

The in-house cell-free system was successful in expressing RFP, which was compared against a commercially-available purified RFP protein (Figs. 6 and 7). This demonstrated that our in-house cell-free system was capable of protein production in general.

Figure 6: RFP expression is observed under UV exposure. Red arrows correspond to RFP; the blue arrow indicates the control (no RFP).

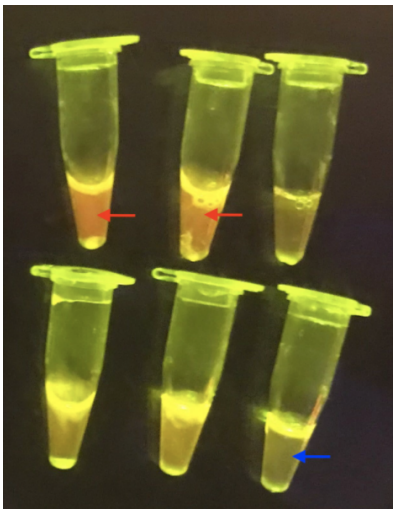
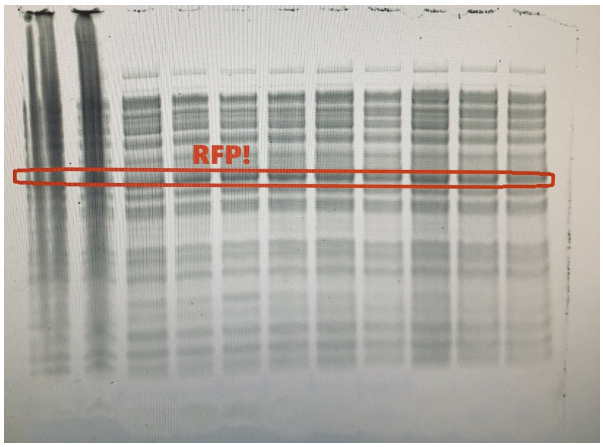


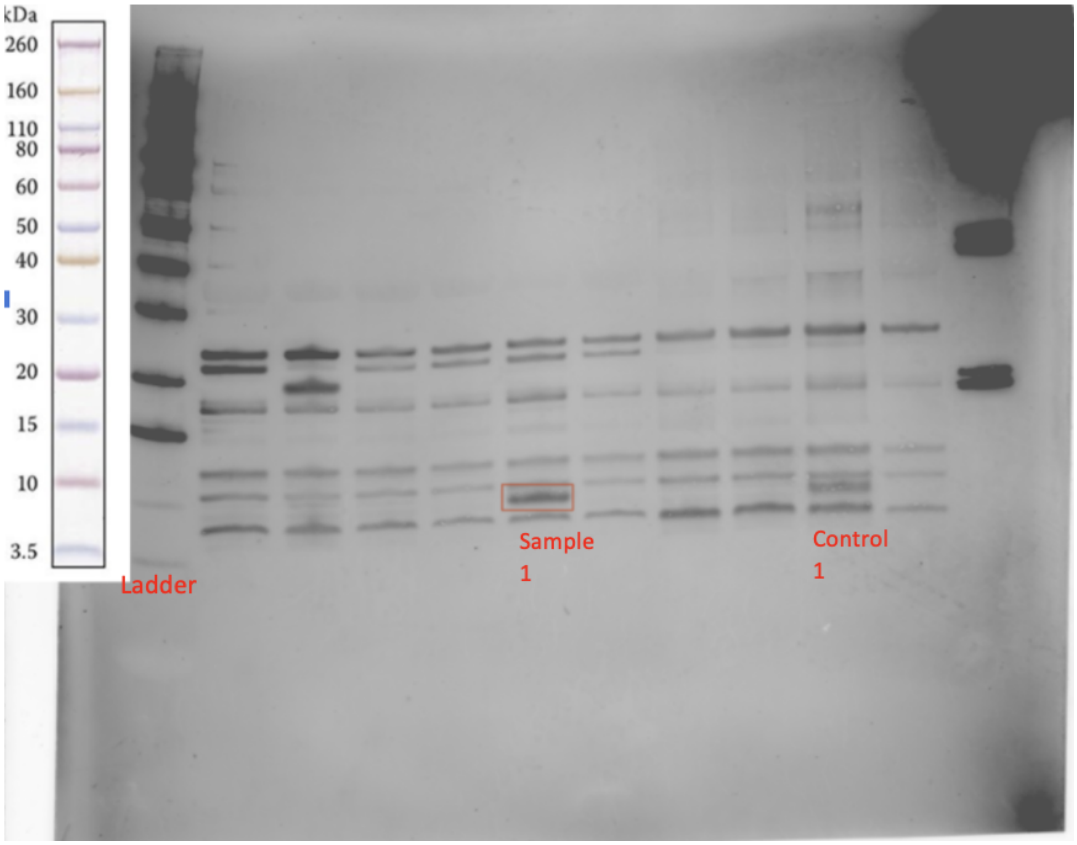
Figure 7: RFP bands are observed following SDS-PAGE analysis of resultant protein.



While expression of drug proteins in the cellular expression system was consistently successful, expression of drugs within the cell-free system showed mixed results. While Teriparatide was expressed

in the in-house cell-free system (shown against a purified protein control in Fig. 8), hG-CSF expression could not be observed under this expression system.

Figure 8: Teriparatide banding is observed in lane 6 (outlined in red) following SDS-PAGE. Purified protein was used as control.



Overall, twenty dose-equivalents of Teriparatide (as determined by molecular weight) were produced in the cell-free system within 24 hours. This seemed to confirm our hypothesis that a therapeutic dose of a peptide-based pharmaceutical could be produced by a cellular expression system within 24 hours.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

I. Discussion of Experimental Results

TRISH's call for proposals challenged researchers around the United States to design a system to manufacture pharmaceuticals or biologics within 24 hours. The Astropharmacy is a clear answer to that call; not only does it meet these goals, it exceeds them. In the experiments shown in this thesis, the Astropharmacy's cellular manufacturing system, using *E. coli*, was able to produce 6 dose-equivalents of both Teriparatide and hG-CSF–both of which appear on the Astronaut Medication List–, while the cell-free manufacturing system, using lysate derived from *E. coli*, was able to produce 20 dose-equivalents of Teriparatide. While the cell-free system did not see the same success in producing hG-CSF, this result was not due to a breakdown in the protocols developed; the same protocol was followed and the same cell lysate used for Teriparatide production, which was highly successful. In the case of hG-CSF production, which was done in a separate experiment from Teriparatide production, lab error and RNAse contamination were the likely causes of the poor outcomes. Were these experiments to be repeated, we would have taken even greater cautions to avoid contamination, including such steps as always working within a sterile laboratory hood. Indeed, we might have repeated the experiments ourselves; however, laboratory time was severely abbreviated in this experiment, owing to COVID-19 and the subsequent yearlong shutdown of the NASA Ames Research Center. Were more time available in the laboratory, successful hG-CSF production would no doubt be observed.

Overall, our bacterial expression proved to be more reliable than our cell-free expression system. This is because bacteria are generally hardier, being enclosed within a membrane and thus less susceptible to

contamination. Nevertheless, a cell-free system is still worth pursuing because of its incredible protein output potential, having produced a maximum of 20 dose-equivalents of Teriparatide to the cellular system's maximum of 6 dose-equivalents of Teriparatide.

Indeed, from a mission architecture standpoint, it makes most sense to pursue both avenues simultaneously in the space environment. As stated previously, the bacterial system is far hardier than the cell-free system (this result was also seen firsthand in this experiment), and is still incredibly low-mass. Astronauts could bring bacteria transformed with peptide drug sequences up into orbit, for use whenever those drugs were needed. At the same time, the cell-free system offers far more flexibility than the bacterial system (assuming issues of long-term stability are conquered), not requiring full transformations but mere DNA sequences input into the system (NASA[55] and other scientists[56] [57] are exploring the possibility of putting a DNA synthesizer[58] on future human space missions[59], with a variety of use-cases including pharmaceutical manufacturing). Astronauts could employ cell-free systems not only for pharmaceutical manufacturing but also for a variety of applications involving protein synthesis.

There are other proposals (including some that have been funded by TRISH[60]) that attempt to solve the problem of on-demand peptide-based pharmaceutical drug manufacturing. These proposals, and their comparisons to the Astropharmacy, will be discussed in detail below.

II. Plant-Based System

One of the proposals that has received TRISH funding for just-in-time drug manufacturing is a plant-based pharmaceutical production system in development from Dr. Karen McDonald, Professor of Chemical Engineering at the University of California, Davis. According to McDonald, "...the advantage we have is that the plant is already there and it already has all the bio-synthetic machinery to make proteins. It just doesn't have the instructions for what we want it to make yet."[61]

McDonald's idea is to employ a "gene gun" (Fig. 9), a device which shoots metal particles coated in DNA, which codes for the desired drug protein, into target plant cells (Fig. 10; viruses encoding the desired drug protein might also be used as ammunition). The host cells would then take up the protein-coding DNA and begin producing the desired drug protein. McDonald proposes to use lettuce (*Lactuca sativa*) as the plant host, as it has been grown successfully on the International Space Station[62]. The drug candidates chosen include hG-CSF (described earlier in this paper) and Teriparatide/parathyroid hormone (PTH, also described in this paper), which is used to treat bone loss that occurs as a result of reduced gravity.

Figure 9: A "Gene Gun" Schematic[63].

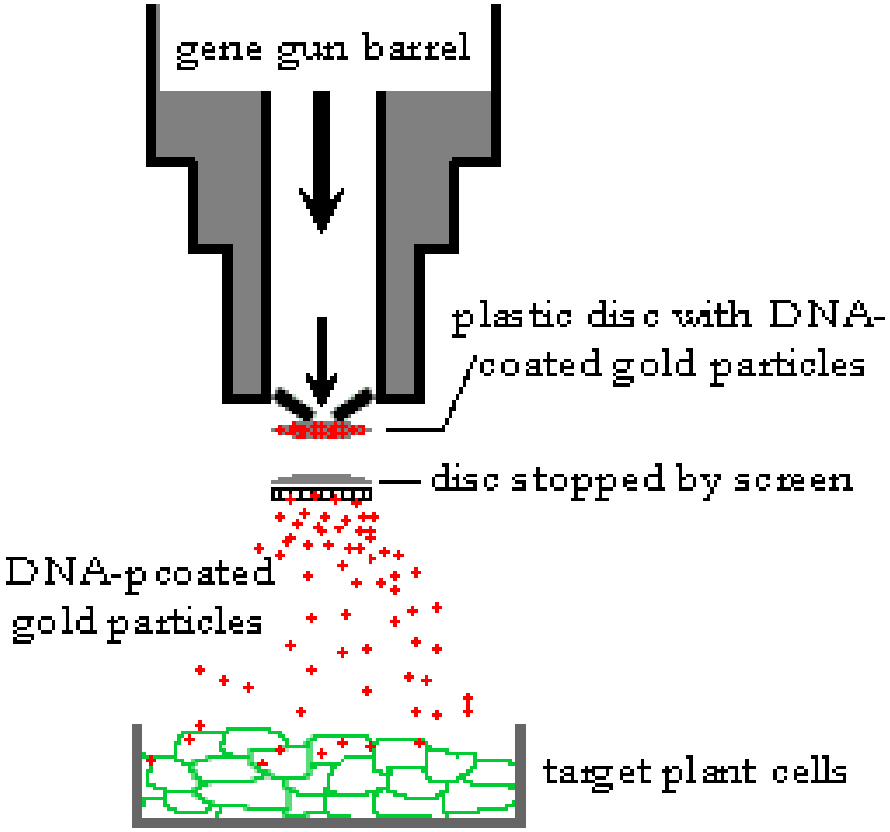


Figure 10: A "Gene Gun" in Use[64].

After the plant produces the protein of interest, the protein will be separated from the plant components with a handheld centrifuge[65]. A final purification step includes the use of a plant virus that contains a ligand to selectively bind to the drug of interest, acting as a "fishing hook"; the resulting complex is centrifuged a final time to yield the purified drug. One week after inserting PTH-coding DNA into a fully-grown lettuce plant, McDonald and her team were able to harvest lettuce containing 10 mg/kg of PTH[66] (dosage of PTH is 20 μg per day[67]).

The plant-based system contains several distinct advantages. First, plants can be shipped up to space as seeds, minimizing upmass. Second, plants do not need to be grown in a sterile environment like bacteria do (in fact, avoiding contamination proved to be a challenge for the Astropharmacy). Third, plants can perform post-translational modifications to proteins that bacteria cannot; often, post-translational modifications like glycosylation are key to a drug's function. Finally, purification of the drug protein is not, strictly speaking, completely necessary. The rationale behind using edible plants is that the drug-containing plant could simply be eaten to provide the therapeutic effect. While the Astropharmacy's purification regimen must still be developed, purification of the drugs expressed in the cellular and cell-free systems will no doubt be necessary to the point that raw outputs may not be simply consumed in the same way.

Despite the ingenuity of McDonald's idea, it will still be incredibly difficult to implement.
The "plant-based" proposal requires comparatively much more development than the Astropharmacy would. Indeed, peptide drug production on an industrial scale is already conducted using

genetically-modified bacteria[68]. While plant-based peptide drug production platforms are currently being developed[69] [70], it will be a long time before they are widely adopted; it makes more sense to adapt an existing technology (bacteria) for drug production in space, than it does to leverage an inchoate drug production system that still must be proven on Earth. With this in mind, however, it is important to note that this weakness is not necessarily disqualifying, as NASA has no plans to send humans to Mars or beyond until the 2030s or later.

Next, while drug purification may not be completely necessary (as discussed earlier), it is still an important consideration. McDonald does not propose to constantly grow lettuce cultivars that each contain different drugs; rather, the gene gun would be used to induce drug production each time a drug was needed. The drug would still need to be purified in order to be tested for safety before consumption (to ensure that the sample did not become corrupted before or during synthesis). Overall, it is far easier to purify drugs from a cell-free system or from bacteria, than it is to purify drugs from lettuce.

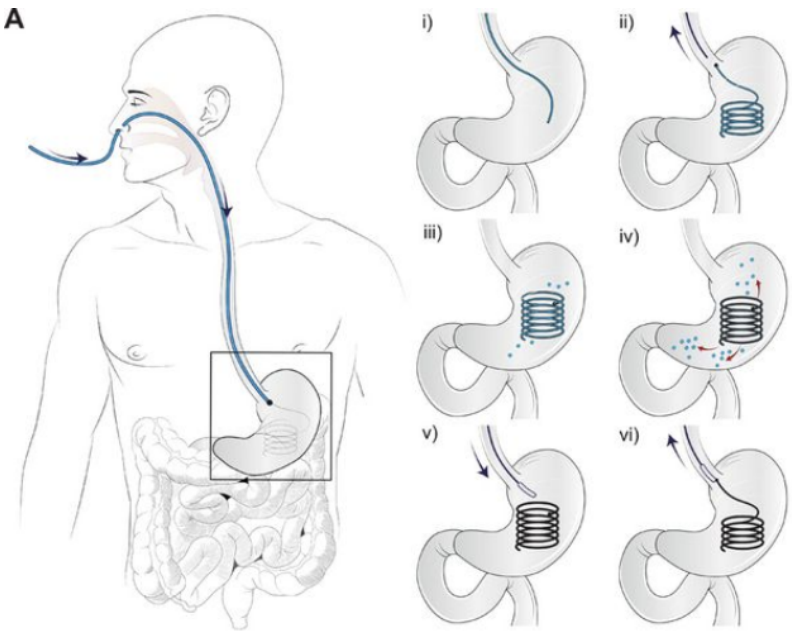
Additionally, lettuce does not grow as quickly as bacteria does. McDonald's team was able to produce a therapeutic dose of PTH within 10 days, but only when using a full-grown plant. It takes around 28 days to grow lettuce from seed to leaf in the space environment[71]; indeed, McDonald proposes to reduce upmass by launching seeds. When considering this additional time needed to produce a therapeutic dose, it seems that McDonald's solution would be less effective in fulfilling TRISH's stated goal of producing a therapeutic dose within 24 hours than the Astropharmacy would be.

Finally, the creation of DNA-coated metal nanoparticles is a complex[72] process involving numerous steps (including the creation of acid-functionalized metal nanoparticles themselves) and specialized lab equipment[73]. The relative complexity inherent to usage of a gene gun compares unfavorably to the Astropharmacy's relative ease-of-use, since cell-free systems require only DNA to work, and especially if bacteria have already been transformed with the sequences of interest before spaceflight.

III. "Mother Machine"

Dr. Robert Langer is another recipient of TRISH funding for just-in-time drug manufacturing. Langer's proposal revolves around the use of a "mother machine": a small "gastric resident" device that would be threaded through the user's nose and reside in the stomach. The "mother machine" would produce drugs to be released into the bloodstream via controlled release. This proposal is adapted from another one of Langer's projects, a similar gastric resident device for monthlong, controlled release of anti-tuberculosis drugs to improve medication regimen adherence in India[74]. Currently, Langer's proof-of-concept for space relies on *E. coli* to produce caffeine, melatonin, and acetaminophen[75] within the user's stomach, with the hope of expanding into other drugs should the technology be proven. The "mother machine" device was designed to protect delicate peptide drugs (which are usually injected) from degradation in the harsh environment of the gastrointestinal tract, and the device would be broken down and excreted safely once the drug's release was completed.

Figure 11: Description of the Mother Machine's implementation[76] [77].



No doubt, the "mother machine" proposal is ambitious. However, since this is a new product, testing and clinical trials will be necessary, as will development for new drugs like the peptide drugs taken up by astronauts. Once again, given the long runway until NASA ventures into deep space, this weakness is not necessarily disqualifying.

However, it is difficult to imagine how the "mother machine" would be toggled to accommodate the dozens of peptide drugs on the Astronaut Medical List. Flexibility is one of the key requirements in the space environment, and developing a means to easily switch between dozens of different drugs is a key step that has not yet been completed.

Finally, the potential for harm is much greater with this proposal than with the two previous (the Astropharmacy and the "plant-based" system). The "mother machine" resides inside the patient's stomach; if the device malfunctions, it could be releasing ineffective, harmful, or even deadly drugs directly into the user's bloodstream. While the "mother machine" could conceivably be made safe, the potential for harm makes it seem unnecessarily risky for use in an environment where risk minimization is the standard.

IV. Additional Uses for the Astropharmacy

One of the Astropharmacy's strengths is its wide utility, not only in the space environment, but for civilians, corporations and government agencies on Earth, as well. In addition to drug production in the Developing World (as discussed in the "Introduction" section of this thesis), there are two promising arenas in which the Astropharmacy would be useful: bioterrorism prevention and orphan drug production.

a. Bioterrorism Prevention

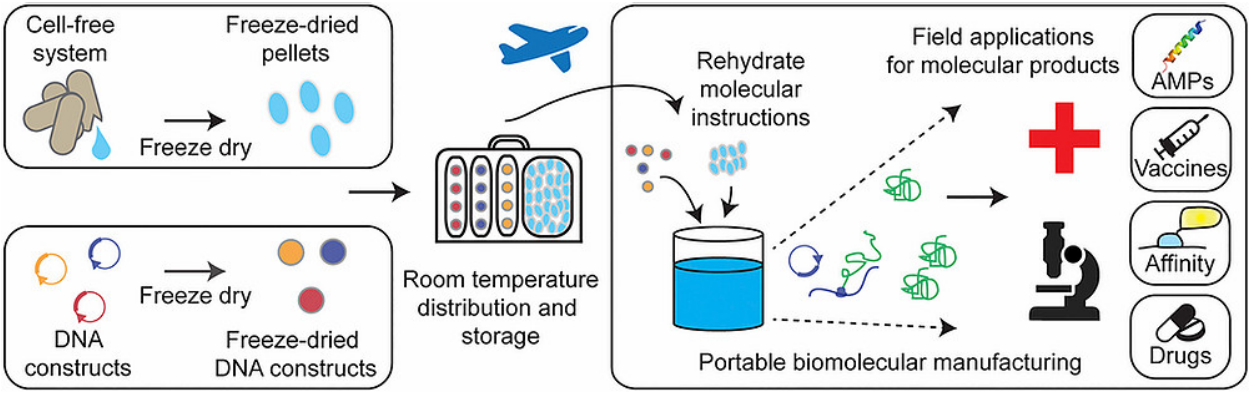
According to Dr. Kristen DeWilde, M.D[78]., Lt. Colonel in the United States Air Force, "COVID-19 has exposed how vulnerable [the United States] is to biowarfare." Given the increasing accessibility of biofabrication tools and the emergence of the field of synthetic biology, which allows humans to easily read and edit DNA, it is easier than ever to synthesize toxic or deadly biological compounds. In fact, technologies like the ability to create longer DNA sequences *de novo*[79] mean that it is easier than ever for terrorists and rogue nations to create "designer bugs" with the potential to wreak havoc on their enemies with little capital input necessary[80] [81]. This relative ease-of-use when compared to something like a nuclear attack or a drone strike, coupled with the United States's lackluster response to the COVID-19 pandemic, means that the question of a bioterror attack on the United States is not a matter of "if" but a matter of "when"[82] [83].

Because of this terrifying possibility, states need a method to adjust to designer microbes on the fly. It is evident from the coronavirus response that production and distribution of a vaccine is much more

logistically challenging than developing that vaccine. However, the Astropharmacy platform could be easily adapted to meet this challenge.

The most likely scenarios for bioterror attacks involve the creation of viral or bacterial agents[84]. Effective controls against these sorts of agents include biologics, like Pfizer/BioNTech's mRNA-based vaccine against COVID-19[85]. This is precisely the sort of functionality for which the Astropharmacy excels. By allowing users to synthesize peptide sequences quickly and easily, the Astropharmacy could produce hundreds of millions of doses of a vaccine or an antidote within a very short timeframe. Lyophilization of the cell-free machinery and/or DNA constructs onto a water-activated cellulose backing would make distribution to millions of households much easier than attempting to set up centralized vaccination or treatment centers[86], which is a slow, resource-intensive process. Indeed, a lyophilized cell-free system could be shipped easily at room temperature and rehydrated by end users for quick, cheap, and efficient distribution of vaccines and other therapeutics (Fig. 12). This application is of interest to military organizations like the Department of Defense especially, as military bases are prime targets for bioterror attacks. Rather than wait for centralized responses to chemical or biological attacks, service members might simply synthesize their own therapeutics on-demand.

Figure 12: "Just Add Water": Distribution Possibilities for Lyophilized Bacteria and Cell-Free Systems.[87]



b. Orphan Drug Production

In the United States, a disease is classified as a "rare" disease if it affects 200,000 people or fewer. There are around 7,000 rare diseases that have been identified as of the publication of this thesis[88]. Because of the limited market associated with rare diseases, governments around the world have issued incentives to convince pharmaceutical companies and manufacturers to produce drugs to serve people with rare diseases; these drugs are termed "orphan" drugs (among these incentive programs is the United States's Orphan Drug Act[89] of 1983). Almost always, these incentives include monopoly over the market for the rare disease.

Because of this, interest in pharmaceutical development for rare diseases has exploded in recent years. The number of orphan drug designations rose from 63 per year in the 1990s to 126 per year in the 2000s. The proportion of orphan drugs receiving market authorization from the FDA similarly doubled, from 17% in the 1990s to 35% in the 2000s[90] (the last available decade for which data has been compiled).

However, monopoly incentives are a double-edged sword. While the number of drugs being researched and developed for rare diseases has increased, the price of those drugs has increased, as well. Prices can be exorbitant[91], with orphan drug regimens often costing into the hundreds of thousands or millions of dollars[92]. Economists have indicated that, due to their sky-high price tag, orphan drugs may actually be less cost-effective than non-orphan drugs[93].

While some decry orphan drug prices as an example of pharmaceutical greed run amok[94], the full story is much more nuanced. A large part of the cost of orphan drugs–indeed, the entire *raison d'etre* of the Orphan Drug Act and similar legislation–revolves around manufacturing costs. A tradeoff exists for orphan drug production: manufacturers can either produce doses at scale, which is expensive and inefficient overall but leads to a lower per-dose cost; or manufacturers can produce doses individually, which is less expensive overall but leads to a higher per-dose cost. The Astropharmacy solves this problem, allowing manufacturers to have the best of both worlds: it allows for production of individual doses of drugs, at a low per-dose cost. This solution would obviate cost issues for manufacturers, potentially leading to lower-priced orphan drugs on the market and better outcomes for patients who would be more able to afford lifesaving treatments.

V. Future Directions for the Astropharmacy

There are numerous exciting directions for the Astropharmacy, all of which would provide fertile ground for future researchers. Those directions, and suggested experiments, follow below.

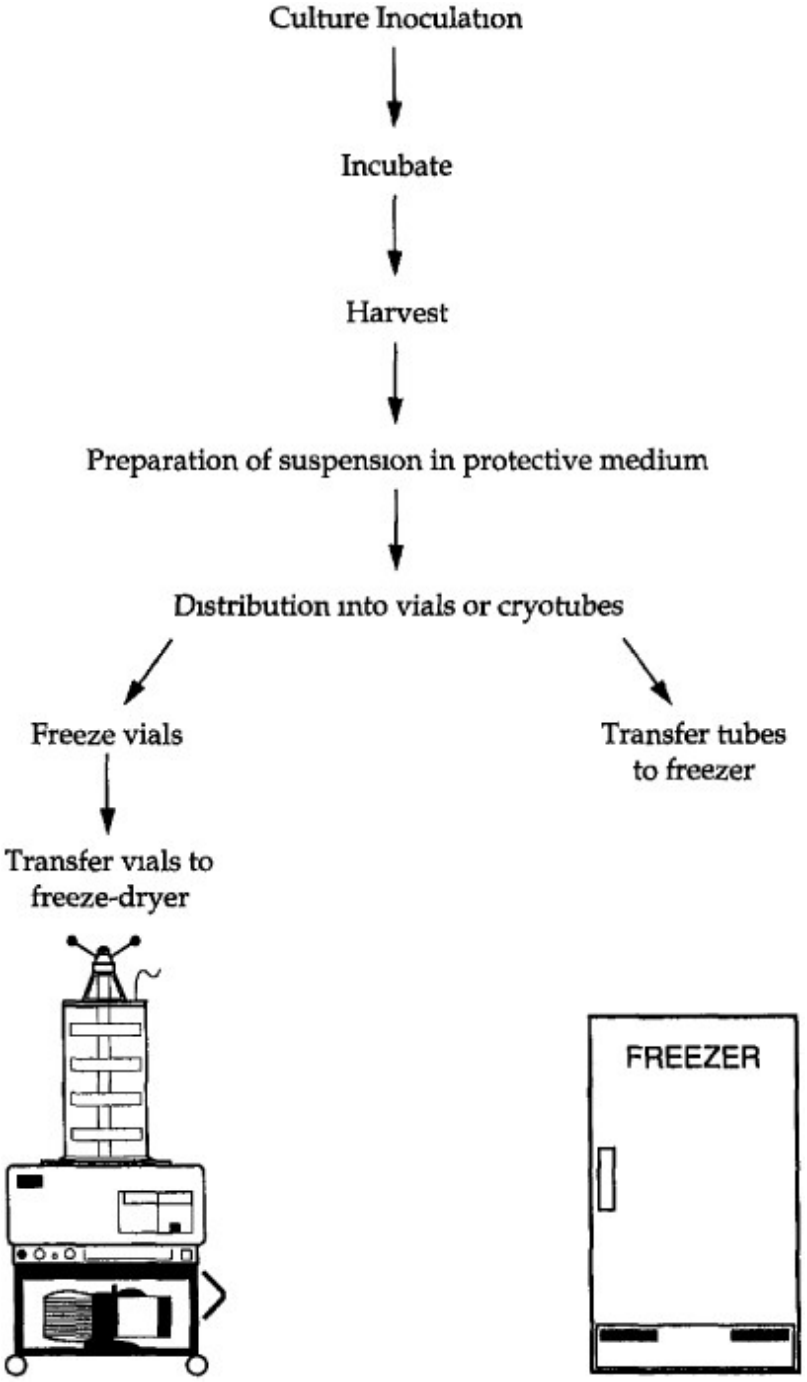
a. Lyophilization

Bacteria are advantageous for an Astropharmacy use-case because of their portability: they need only be transported in petri dishes, which are relatively low-mass. However, there is a way to reduce upmass even more, and at the same time increase both portability and stability of bacterial colonies: transporting bacteria that have been lyophilized onto paper strips. This is a relatively easy procedure[95]: bacteria are cultured, suspended in a 15% sterile glycerol solution, and freeze-dried with liquid nitrogen or frozen at -80˚C (Fig. 13). It is possible to do this aseptically, and bacteria are easily revived upon the addition of water. Indeed, *B. bacteriovorus* spores showed normal activity after more than a year of lyophilization[96]. In this way, transformed and lyophilized bacteria could be sent up on manned missions and activated only when necessary; the paper backing could be discarded to further reduce upmass.

Beyond lyophilization of bacteria, lyophilization of cell-free systems is possible, as well. Pardee et. al. were able to lyophilize a cell-free system onto a paper backing for portable, on-demand biomolecular manufacturing[97] [98]. In addition to the increased portability, lyophilization of cell-free systems on paper means that sterile and abiotic systems are able to be preserved for on-demand use[99]; this is hugely important, as contamination is a constant issue for cell-free systems (as it was for the Astropharmacy

experiments described in this paper). Overall, lyophilization would make the Astropharmacy much more effective by enhancing sterility and portability, and reducing upmass.

Figure 13: Bacterial Lyophilization Protocol[100].



b. Experiments With Other Bacterial Strains

In addition to *E. coli*, two other bacterial strains ought to be considered for use within the Astropharmacy. The first bacterial strain is *Vibrio natriegens* (Vmax™). This strain of bacteria has an extremely quick doubling time (less than 14 minutes)[101], making it ideal for the 24-hour time limit on drug production, as established by TRISH. Additionally, *V. natriegens* is a high-yield strain; researchers estimate that *V. natriegens* is able to produce at least twice[102] the protein yield of an *E. coli*-based expression system, with some researchers obtaining up to four times[103] the protein yield when compared against an *E. coli*-based expression system (Fig. 14). The incredibly high yield and fast doubling time make *V. natriegens* an incredibly compelling candidate for the Astropharmacy.

The second bacterial strain that merits consideration for use as part of the Astropharmacy is *Bacillus subtilis.* While the doubling time of *B. subtilis* is longer than that of *V. natriegens* (120 minutes[104]), *B. subtilis* is incredibly hardy, especially within a space environment, showing marked survivability inside the vacuum of space (Fig. 15). Indeed, wild-type *B. subtilis* spores were taken into space aboard several spacecrafts, including Apollo 16, Spacelab 1, and NASA's LDEF (Long-Duration Exposure Facility). After 10 days in the vacuum of space, up to 70% of *B. subtilis* spores survived; after 6 years, 1-2% of spores were still viable[105]. *B. subtilis*'s vigor means that this strain would make an ideal backup drug production system in the case of a failure elsewhere within the Astropharmacy (in keeping with NASA's principles of redundancy[106]), or indeed a reliable first-resort expression system in its own right.

Figure 14: Vmax™ Protein Expression Compared to *E. coli* Protein Expression[107]. VMax™ is capable of greater protein production within a shorter time frame.

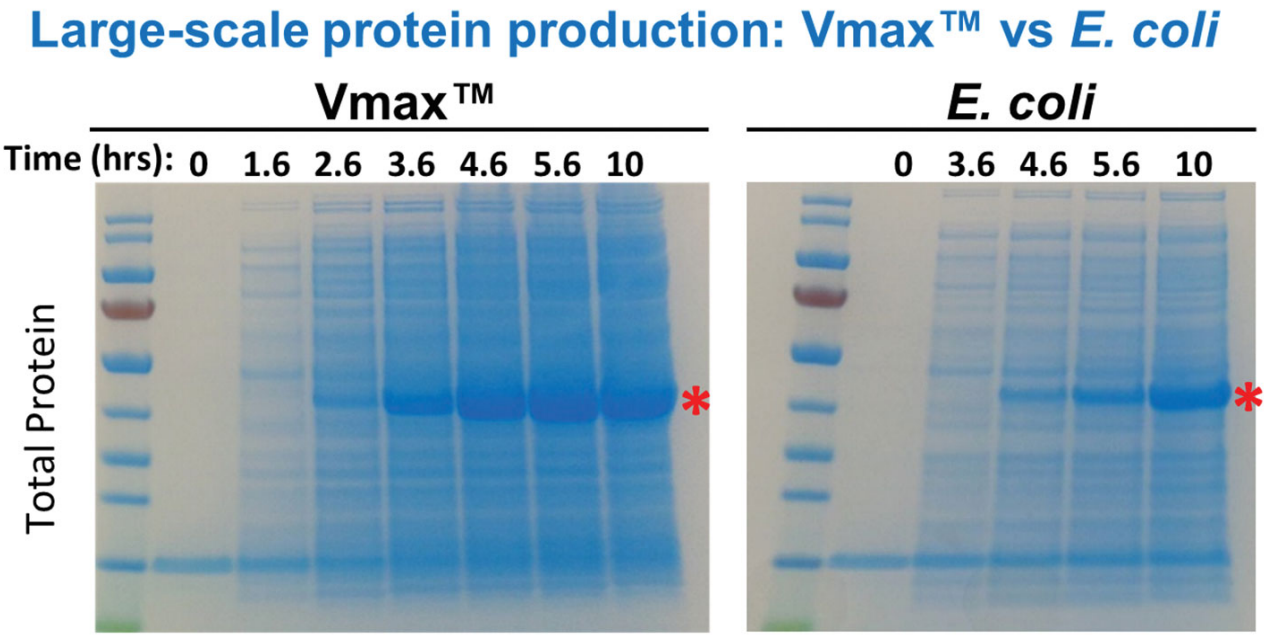
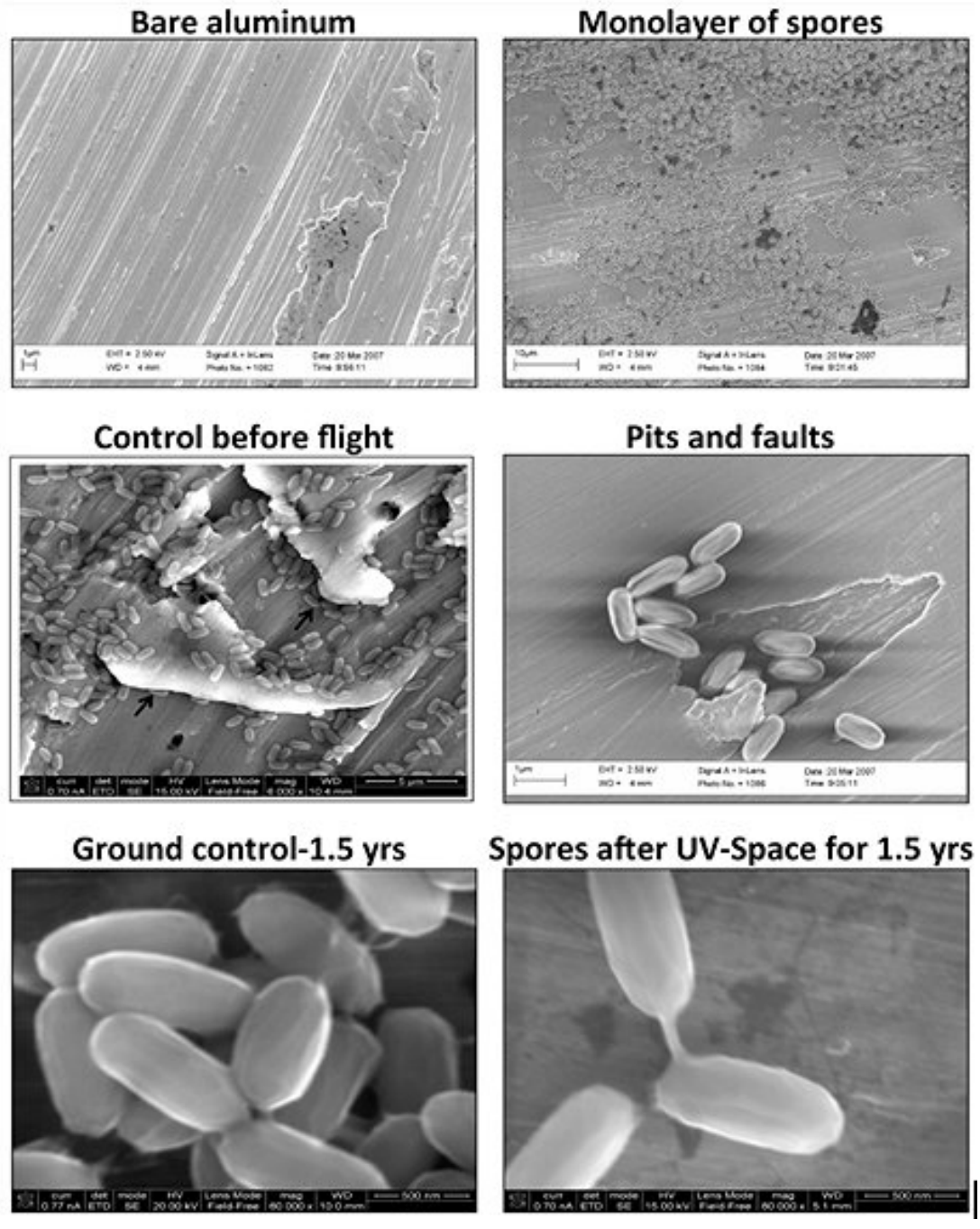


Figure 15: Survival of B. subtilis spores in open space[108]. After 1.5 years in space, the spores appear undamaged.



c. Purification

While drug purification was outside the scope of this thesis, future researchers must determine (a) whether the Astropharmacy can produce drugs to the appropriate purity; and (b) whether said drugs can be easily purified from the Astropharmacy for human consumption (other members of the Rothschild Lab at NASA Ames are currently working on the purification problem). While there are several methods[109] to purify protein products from bacteria (especially with the use of affinity tags/affinity purification[110] or fusion tags[111]), purification from a cell-free system is most commonly accomplished via the Ni-NTA purification protocol for recombinant His-tagged proteins[112].

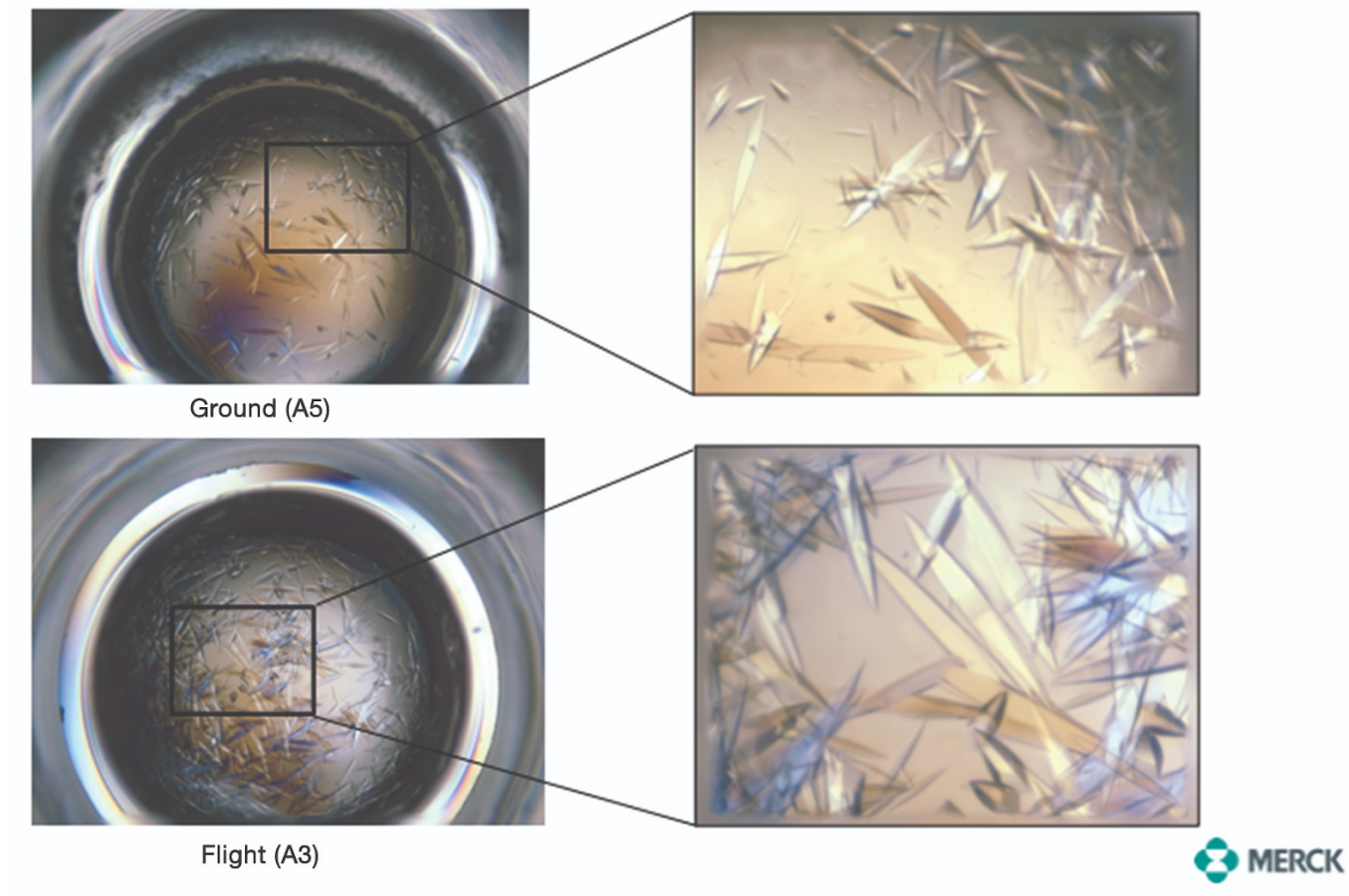
According to the World Health Organization, pharmaceutical drugs must be 99.5% pure to be considered safe for human use[113]. Purity testing may be conducted via ^{1}H-NMR[114], or, more commonly, via High-Performance Liquid Chromatography[115] (HPLC) or Ultra Performance Liquid Chromatography[116] (UPLC). In fact, UPLC can be adapted to the space environment, as proposed by Poinot et. al.[117], meaning that drug purity could be evaluated on human missions to Mars and beyond, or even in different gravity regimes like the Moon or the ISS.

d. Experiments in Microgravity

The Astropharmacy's primary use-case is in space, which is likely to involve gravity regimes that are lower than on Earth; for example, microgravity on ISS, 1/6th x g_0 on the Moon, and 1/3rd x g_0 on Mars. Bacteria have shown to exhibit different behavior in a microgravity setting than they would at normal gravity conditions[118]; additionally, proteins have demonstrated different constructions in microgravity, including drug proteins (Fig. 16)[119]. Experimentation ought to be done in microgravity

(for example, aboard the International Space Station), in order to determine whether the drug-producing capabilities of bacteria might be affected by such an environment, or whether the proteins conformations produced in space would either be different than those produced on Earth, and, if so, whether those conformational changes attenuate drug performance.

Figure 16: Differences Between Protein Crystals Grown on Earth, versus on the ISS[120]. Note the difference in shard size especially.



e. Production of Other Peptides

While the Astropharmacy was developed for drug production purposes, its potential is by no means limited to pharmaceuticals alone. Indeed, the Astropharmacy is well-suited for peptide production of all varieties, including peptides that would be of particular interest to NASA. Such peptides especially include adhesives[121] [122] and pigments. Melanin in particular has been identified as a useful pigment that might shield astronauts from harmful space radiation[123] [124], and indeed this pigment has been successfully produced by bacteria[125] in the past.

VI: Conclusion

Overall, the Astropharmacy remains a promising solution to the problem of peptide drug manufacturing in space, and problems on Earth ranging from drug accessibility in the Developing World, to bioterror prevention, to orphan drug production. In experiments conducted at NASA Ames Research Center, the Astropharmacy was able to employ *E. coli* bacteria to produce 6 dose-equivalents of Teriparatide and hG-CSF within 24 hours; and a homemade, *E. coli*-derived cell-free system to produce 20 dose-equivalents of Teriparatide within 24 hours. Its high performance in peptide drug production notwithstanding, the Astropharmacy carries several distinct advantages over currently-proposed, TRISH-funded solutions, namely the "plant-based" system and the "mother machine" system. Future research ought to be conducted into other bacterial strains including Vmax™ and *B. subtilis*, as well as into lyophilization of the bacterial and cell-free production systems, and production of other useful peptides including pigments and adhesives.

APPENDIX I: Astronaut Medication List[126]

1. Acetaminophen (Tylenol)
2. Acetazolamide (Diamox)
3. Acyclovir (Zovirax)
4. Adenosine (Adenocard)
5. Adenocard (Adenosine)
6. Adrenaline (Epinephrine)
7. Adrenaline (Epinephrine)
8. Afrin Nasal Spray
9. AK-CIDE Ophthalmic Ointment (Vasocidin Ophthalmic Ointment)
10. Albuterol (Proventil Inhaler)
11. Ambien (Zolpidem Tartrate)
12. Amikacin (Amikin)
13. Amikin (Amikacin)
14. Amoxil (Amoxicillin)
15. Amoxicillin (Amoxil)
16. Anusol HC Suppository
17. Artificial Tears (Refresh)
18. Ascriptin (Aspirin)
19. Aspirin (Ascriptin)
20. Atropine
21. Auralgan Otic Solution

22. Azithromycin (Zithromax)
23. Ayr Nasal Mist
24. Bacitracin
25. Bactrim DS (Trimethoprim/Sulfamethoxasole)
26. Bactroban (Mupirocin)
27. Benadryl (Diphenhydramine)
28. Bisacodyl (Dulcolax)
29. Carisoprodol (Soma)
30. Cefadroxil (Duricef)
31. Ceftriaxone Sodium (Rocephin)
32. Celebrex (Celecoxib)
33. Celecoxib (Celebrex)
34. Ciloxan Ophthalmic Solution (Ciprofloxacin)
35. Cimetedine (Tagamet)
36. Cipro (Ciprofloxacin)
37. Claritin
38. Clotrimazole Cream (Lotrimin Cream)
39. Cough Lozenges
40. Compazine (Prochlorperazine) Suppositories
41. Cortisporin Ophthalmic Suspension
42. Cyclogyl Ophthalmic Solution (Cyclopentolate)
43. Cyclopentolate (Cyclogyl Ophthalmic Solution)

44. Decadron (Dexamethasone)
45. Deltasone (Prednisone)
46. Demerol (Meperidine)
47. Dexamethasone (Decadron)
48. Dexedrine (Dextroamphetamine)
49. Dextroamphetamine (Dexedrine)
50. Diamox (Acetazolamide)
51. Diazepam (Valium)
52. Diclofenac Sodium (Voltaren)
53. Diflucan (Fluconazole)
54. Dilantin (Phenytoin)
55. Diphenhydramine (Benadryl)
56. Dulcolax (Bisacodyl)
57. Duricef (Cefadroxil)
58. Epinephrine (Adrenaline)
59. Epinephrine (Adrenaline)
60. Flagyl (Metronidazole)
61. Fluconazole (Diflucan)
62. Flumazenil (Romazicon)
63. Furosemide (Lasix)
64. Haldol (Haloperidol)
65. Haloperidol (Haldol)

66. Hydrocodone Bitartrate Acetaminophen (Vicodin)
67. Ibuprofen (Motrin)
68. Imipenem (Primaxin I.V.)
69. Imodium (Loperamide HCl)
70. Inderal (Propranolol Hydrochloride)
71. Isoptin (Verapamil)
72. Kenalog (Triamcinolone)
73. Kenalog in Orabase (Triamcinolone Acetonide Dental Paste)
74. Ketorolac Tromethamine (Toradol)
75. Lasix (Furosemide)
76. Lidocaine (Xylocaine with Epinephrine)
77. Lidocaine (Xylocaine) IV Cardiac
78. Loperamide HCl (Imodium)
79. Lotrimin Cream (Clotrimazole Cream)
80. Meperidine (Demerol)
81. Metronidazole (Flagyl)
82. Metoprolol Succinate (Toprol-XL)
83. Morphine Sulfate
84. Motrin (Ibuprofen)
85. Mupirocin (Bactroban)
86. MURO-128 Solution
87. Naloxone (Narcan)

88. Narcan (Naloxone)
89. Nitroglycerin Pills and Patches
90. Norethindrone/ethinyl estradiol (Ortho-Novum)
91. Nortriptyline
92. Omeprazole (Prilosec)
93. Ortho - Novum (Norethindrone/ethinyl estradiol)
94. Oseltamivir (Tamiflu)
95. Pepto-Bismol
96. Phazyme-125 (Simethicone)
97. Phenazopyridine (Pyridium)
98. Phenergan (Promethazine)
99. Phenylpropanolamine/Guaifenesin (Entex LA)
100. Phenytoin (Dilantin)
101. Polymyxin/Bacitracin (Polysporin).
102. Polysporin (Polymyxin/Bacitracin)
103. Polytrim Ophthalmic Solution (Trimethoprim Sulfate/Polymixin B)
104. Potassium Citrate (Urocit-K)
105. Prednisone (Deltasone)
106. Prednisolone (Pred Forte)
107. Pred Forte (Prednisolone)
108. Prilosec (Omeprazole)
109. Primaxin I.V. (Imipenem)

110. Prochlorperazine (Compazine) Suppositories
111. Promethazine (Phenergan) (Oral, Injectable)
112. Proparacaine Ophthalmic Solution (Eye drops)
113. Propranolol Hydrochloride (Inderal)
114. Proventil Inhaler (Albuterol)
115. Pseudoephedrine (Sudafed)
116. Pyridium (Phenazopyridine)
117. Restoril (Temazepam)
118. Rocephin (Ceftriaxone Sodium)
119. Romazicon (Flumazenil)
120. Sertraline Hydrochloride (Zoloft)
121. Silvadene Cream (Silver Sulfadiazine Cream)
122. Silver Sulfadiazine Cream (Silvadene Cream)
123. Simethicone (Phazyme-125)
124. Soma (Carisoprodol)
125. Sudafed (Pseudoephedrine)
126. Tagamet (Cimetidine)
127. Tamiflu (Oseltamivir)
128. Temazepam (Restoril)
129. Tobramycin (Tobrex)
130. Tobrex (Tobramycin)
131. Toprol-XL (Metoprolol Succinate)

132. Toradol (Ketorolac Tromethamine)
133. Triamcinolone (Kenalog)
134. Triamcinolone Acetonide Dental Paste (Kenalog in Orabase)
135. Trimethoprim/Sulfamethoxasole (Bactrim DS)
136. Trimethoprim Sulfate/Polymixin B (Polytrim Ophthalmic Solution)
137. Tylenol (Acetaminophen)
138. Urocit-K (Potassium Citrate)
139. Valium (Diazepam)
140. Vancocin (Vancomycin)
141. Vancomycin (Vancocin)
142. Vasocidin Ophthalmic Ointment (AK-CIDE Ophthalmic Ointment)
143. Verapamil (Isoptin)
144. Vicodin (Hydrocodone Bitartrate Acetaminophen)
145. Vidarabine Ophthalmic Ointment (VIRA-A)
146. VIRA-A (Vidarabine Ophthalmic Ointment)
147. Voltaren (Diclofenac Sodium)
148. Xylocaine with Epinephrine (Lidocaine with Epinephrine)
149. Xylocaine/Cardiac (Lidocaine)
150. Xylocaine Jelly
151. Zithromax (Azithromycin)
152. Zoloft (Sertraline Hydrochloride)
153. Zolpidem Tartrate (Ambien)

154. Zovirax (Acyclovir)

CHAPTER 7: ENDNOTES

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