Feasibility of Isotope Harvesting at a Projectile Fragmentation Facility: $^{67}$Cu

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The work presented here describes a proof-of-principle experiment for the chemical extraction of $^{67}$Cu from an aqueous beam stop at the National Superconducting Cyclotron Laboratory (NSCL). A 76 MeV/A $^{67}$Cu beam was stopped in water, successfully isolated from the aqueous solution through a series of chemical separations involving a chelating disk and anion exchange chromatography, then bound to NOTA-conjugated Herceptin antibodies, and the bound activity was validated using instant thin-layer chromatography (ITLC). The chemical extraction efficiency was found to be 88$^{+6}_{-3}$% and the radiochemical yield was 95%. These results show that extraction of radioisotopes from an aqueous projectile-fragment beam dump is a feasible method for obtaining radiochemically pure isotopes.

There are many isotopes that could be useful in a range of disciplines from medicine to stockpile stewardship, geology, nuclear astrophysics and biology that are not available in the quantities needed from conventional production methods based on small medical cyclotrons or reactors$^1$-$^7$. The construction of the Facility for Rare Isotope Beams (FRIB) will allow "harvesting" usable quantities of many of these isotopes, concurrent with secondary-beam operations for basic nuclear science experiments. The FRIB design has provisions for delivering research quantities ($\mu$Ci to Ci) of radioisotopes as an ancillary service to the on-line experimental program by collecting radioisotopes produced or stopped in an aqueous beam dump$^8$-$^9$ in the primary target facility while secondary beams are produced for on-line experiments. Ideally this collection or "harvesting" of isotopes at FRIB could provide isotopic material for which there is currently no comparable source.

One such radioisotope is $^{67}$Cu which can be used in medicine as a therapeutic isotope$^6$,$^{10}$-$^{11}$. Its relatively long 2.58 day half-life is ideal for labeling antibodies that also have several-day biological half-lives. Obtaining therapeutic doses that are typically on the order of hundreds of mCi/patient$^{12}$ has proved to be quite difficult due to inconsistent production and no reliable continuous supply$^6$-$^{13}$-$^{14}$. $^{67}$Cu can be made via several nuclear reactions: $^{68}$Zn(p,2p)$^{67}$Cu, $^{70}$Zn(p,α)$^{67}$Cu, $^{67}$Zn(n,p)$^{67}$Cu, and $^{68}$Zn(γ,p)$^{67}$Cu; however each reaction has practical drawbacks. For example, $^{68}$Zn(p,2p)$^{67}$Cu has a low but broad (in energy) cross section and in order to efficiently produce the large quantities needed for therapeutic doses thick targets and high-energy proton accelerators such as those at Brookhaven$^{15}$-$^{16}$ or Los Alamos National Laboratories$^{17}$ need to be employed. These are multiuser facilities that cannot routinely dedicate proton beam to produce a continuous supply of $^{67}$Cu$^{12}$. Studies at several facilities of the $^{68}$Zn(p,α)$^{67}$Cu reaction have obtained varying yields and the production of the large quantities needed for therapeutic studies is challenging with this method$^{18}$-$^{20}$. Production via the $^{67}$Zn(n,p)$^{67}$Cu and $^{68}$Zn(γ,p)$^{67}$Cu reactions have undesirable side reactions and concerns about waste products create challenges for their large scale use$^{21}$. It is estimated that FRIB will be able to produce a saturated activity of $^{67}$Cu as high as ~2 Ci depending on the primary beam$^{22}$ so that weekly harvesting of $^{67}$Cu could provide a more consistent supply of this isotope. Therefore, given the difficulty of other production methods, the favorable half-life and well-understood chemistry of $^{67}$Cu, this isotope was selected for a proof-of-principle test of isotope harvesting from an aqueous beam stop for projectile fragments. The work reported here can be extended to other projectile fragments collected in an aqueous beam stop.

Previously, a liquid-water target system/beam stop was designed and tested at the NSCL for a first attempt to harvest useful radioisotopes from a beam dump similar to what will exist at FRIB$^{22}$. This system was used successfully to collect several samples of $^{24}$Na from a projectile fragment beam at $\sim$85 MeV/u and an intensity of $\sim$2 × 10$^8$ pps. In the present work, the liquid water target system described in ref [8] was used to collect several samples of $^{67}$Cu that were also produced as a secondary beam at the NSCL. The aqueous samples were then...
transported to Washington University in St Louis, MO and Hope College in Holland, MI for chemical separation, antibody labeling, and offline counting. We report here the results of these studies and the observation of relatively high chemical extraction efficiency.

**Results**

**Beam Calibration and Collection.** A 76 MeV/A $^{67}$Cu secondary beam of 77% purity was selected from a fragmented $^{76}$Ge primary beam using the A1900 fragment separator$^{22}$ and delivered to the S1 vault where the liquid water target station was set up to collect the incoming beam. In order to quantify the amount of $^{67}$Cu delivered to the target, calibration curves had to be developed to convert the signals from the non-intercepting beam monitors that were continuously operating during beam collections and the fully-intersecting Faraday-cups or scintillators that could not be in place during the sample collections.

Two separate calibration methods were used to determine the number of beam particles delivered to the liquid water target station during two calibration runs performed approximately 12 hours apart. One calibration run was prior to most of the collections and the other was post most of the collections. In the first method a calibration curve was established to normalize the proportional data from the non-intercepting beam monitors (Monitors A and B in Figure 1) to the direct measurements of the primary beam current (in nA) with a Faraday cup. The proportional rates from A and B were integrated over the course of each run and then multiplied by the conversion factor of $4.1 \pm 0.2 \times 10^5$ $^{67}$Cu ions per second per particle-nA of primary beam (determined by the A1900 operations group) to give the rate of $^{67}$Cu atoms delivered. The second method established calibration curves of two non-intersecting beam monitors A and B with the focal-plane plastic scintillator that was used to measure the rate of secondary beam arriving at the A1900 focal plane in particles per second, and these values were integrated over the course of each run and multiplied by the purity factor of 77% to obtain the total atoms of $^{67}$Cu delivered to the beam stop. Due to the different working ranges of the detectors used to measure the beam current, two separate calibration curves had to be used. The first curve converted the high-intensity beam measured with the non-intersecting beam monitors A and B to a medium intensity non-intersecting beam monitor C and the second curve converted the medium intensity non-intersecting beam monitor C to the low intensity focal-plane scintillator values in particles per second. The data for both methods for the second calibration run carried out post-collection are shown in Figure 1. Linear functions were found to provide excellent descriptions of all of the data.

Five collections were performed, each lasting approximately four hours, and the water from each run was collected as an individual sample for each run. The numbers of $^{67}$Cu ions delivered to the liquid water target system in each run, calculated using the two methods, along with the values obtained by measuring the gamma-ray activity for aliquots taken from the samples using an HPGe detector are listed in Table 1. The values obtained from the two calibration curves were

![Figure 1](http://www.nature.com/scientificreports)
transport efficiency of the 66Cu secondary beam into the liquid water target system from the A1900 separator to the water target system was found to be 84 ± 5%. The modest intensity reduction occurred between the focal plane and the end station or on the collimator immediately in front of the liquid water target station entrance window.

Chemistry. To plan the chemical separation procedure, modeling of the radioisotopes delivered to the water cell and their decay products was carried out using the LISE++ code with the EPAX3 cross sections and the Nucleonica® decay engine, respectively, as was done for the previous 24Na work. The theoretical beam components with half-lives greater than one minute predicted by the LISE++ code to enter the target water are given in Table 2. Note that the projectile fragment separator tends to deliver more isotonic than isotopic contaminants and the primary beam is completely removed.

All of the different metals were effectively separated from the 66Cu collections using the methods described below. The chemical separation relied on absorption to a metal-chelation disk, elution, and separation by anion-exchange chromatography. All methods were validated prior to the 66Cu separations using either non-radioactive species or radioactive surrogate species as tracers. An experiment measuring the efficiency of removing germanium (primary beam) contamination that would be present in the primary beam dump in future work at FRIB was determined using PIXE (Particle Induced X-ray Emission) analysis of 0.04–0.5 ppm germanium that was passed through the chelating disk. The results showed average germanium retention of only 2.7 ± 0.9% but demonstrated that a small amount germanium binds to the chelating disk. In order to test the separation strategy for copper and gallium, 66Ge (t1/2 = 68 min) obtained from a 66Ge/67Ga generator (Eckert and Ziegler, Germany) and 64Cu (t1/2 = 68 min) produced at Washington University in St. Louis were used as radioactive tracers. Passage of 66Ga and 66Cu solutions through the chelating disk found that both metals remained bound within the disk with greater than 99% efficiency; however, 10 mL of 6 M HCl was able to elute, 98 ± 1% of 66Cu from the disk, while 97 ± 2% of 66Ga remained fixed to the disk. Subsequent separation of copper from zinc and nickel was obtained using anion-exchange chromatography and verified by ion chromatography. The percent of the initial nickel and zinc concentrations were found to be 4 ± 2% and 6 ± 4%, respectively in the 0.5 M HCl fraction. The average copper recovery from this method was shown to be 86 ± 4%. The resulting separation process is shown schematically in Figure 2.

Four of the 66Cu samples were shipped to Washington University in St. Louis and processed as described below. Table 3 contains the values for the recovery of 66Cu from the aqueous solutions containing the projectile fragments from the NSCL. The average recovery of 66Cu was found to be 88 ± 3%, a value in excellent agreement with the tracer studies. The separated (final) 66Cu fractions were analyzed using an HPGe detector (Canberra, USA) calibrated using a 2 mL mixed source (Eckert and Ziegler, Germany) and no radioactive impurities were observed. The gallium species had completely decayed from the sample before processing due to its short half-life and the decontamination factors for nickel and zinc were 20 and 12.5 respectively.

Test labeling of an antibody, Trastuzumab, was performed to demonstrate that 66Cu separated from the water beam dump would be chemically active. Bicinchoninic acid (BCA) assay showed the final concentration of the conjugated NOTA-Bz-NCS-Trastuzumab to be 7.9 mg/mL. The radiochemical yield of 66Cu-NOTA-Bz-NCS-Trastuzumab of 0.5 μCi/μg was found to be greater than 95% by radio instant thin-layer chromatography (ITLC). Figure 3 shows the results of fast-protein liquid chromatography of NOTA-Bz-NCS-Trastuzumab, radio ITLC of 66Cu-NOTA-Bz-NCS-Trastuzumab, radio ITLC of 66Cu-EDTA along with an illustration of the NOTA chelate bound to Trastuzumab.

The production of 7Be from reactions of beam on water. One concern with using an aqueous beam dump to collect high-energy fragments is the buildup of long-lived radioactivity from nuclear reactions with the water. 7Be (t1/2 = 53.3 d) is produced in reactions between essentially all fast ion beams and water. While 7Be can be easily separated from the 66Cu in this experiment, there may be instances where this contaminant could interfere with the harvesting process. The predicted production rate of 7Be with an incident medium mass beam (A = 67) is 6.4 × 10−4/incident ion. The 7Be concentrations in the present samples were measured using HPGe spectroscopy and showed that the production of 7Be per incident ion on water was 5 ± 2 × 10−4.

In addition to 7Be, 11C and 13N were observed byproducts of the interaction of the secondary beam on water. Due to the short half-life of these isotopes they were not observed on the HPGe detector, however their decay was observed using a CsI detector that monitored the collection vials upon completion of a four-hour collection. Future experiments will be designed in order to quantify the amount of these short-lived byproducts that are produced.

Discussion

A liquid water target station previously designed to collect fast, secondary beams from the National Superconducting Cyclotron Laboratory was used to collect five samples of 66Cu. The five collections lasted for four hours each, and the samples were then shipped to Hope College and Washington University in St. Louis for further analysis.
analysis and chemical separation. $^{67}$Cu was effectively separated from the beam contaminants with an average recovery of $88 \pm 3\%$. The resulting $^{67}$Cu was then bound to NOTA-Bz-NCS-Trastuzumab with a radiochemical yield of $>95\%$. These results demonstrate the feasibility of harvesting useful radioisotopes from the aqueous beam dump at the Facility for Rare Isotope Beams (FRIB) now under construction at Michigan State University. Future experiments will be performed to collect and separate $^{67}$Cu from an unanalyzed, i.e., mixed secondary beam to better mimic the conditions that would be present in the actual FRIB beam dump.

The overall production of $^{67}$Cu at the NSCL was estimated to be $7.33 \times 10^3$ pps for the $^{76}$Ge beam and experimental conditions reported here$^{22}$. An observed rate of $1.13 \times 10^7$ pps of $^{67}$Cu was measured during this experiment which closely matches the predicted yield for the NSCL. Since the same codes are used to estimate yields at FRIB, this bodes well for an expected production rate of $\sim 2$ Ci of $^{67}$Cu in the primary beam dump at saturation during regular operations. Similarly the production of $^{7}\text{Be}$ was also measured to be $5.7 \times 10^3$ pps, which also compare favorably with the predicted production rate of $4.7 \times 10^3$ pps.

While this proof-of-principle experiment made use of a water target cell to collect the beam in a small volume of non-circulating water, in the future at the Facility for Rare Isotope Beams there will be ion exchangers present which could act as potential sources for isotopetagged water, in the future at the Facility for Rare Isotope Beams (FRIB) now under construction at Michigan State University. Future experiments will be performed to collect and separate $^{67}$Cu from an unanalyzed, i.e., mixed secondary beam to better mimic the conditions that would be present in the actual FRIB beam dump.

The most promising radionuclides for isotope harvesting from FRIB would be those which cannot be produced easily by other means, such as with a reactor or small medical cyclotron. Additionally, isotopic availability may depend on the needs of the primary user, how pure the isotopes need to be and the frequency of production. While this is a very promising source of isotopes for many fields, it should be noted that isotopes obtained in this manner will likely be available sporadically. Thus, due to the nature of this technique, it is likely that medium- to long-lived research isotopes will be the most promising targets for isotope harvesting from heavy-ion fragmentation facilities.

**Methods**

**Beam Collection and Calibration.** A 25 particle-nA 130 MeV/u $^{76}$Ge beam was fragmented using a 510.8 mg/cm$^2$ thick beryllium production target in the A1900 projectile-fragment separator$^{22}$. After fragmentation of the primary beam, $^{67}$Cu was selected using the A1900 with a 236.7 mg/cm$^2$ aluminum wedge in the center, the focal plane (FP) mass-selection aperture set to 10 mm, and the momentum acceptance slits open to 2% (full momentum acceptance). Table 4 lists the exact experimental parameters used in the separator and beam line. The beam intensity and purity were measured using the standard delta-E/Time-of-Flight technique with a silicon-PIN diode in the A1900 focal plane and cyclotron RF signal prior to delivery of the beam to the experimental end station$^{22}$. The secondary beam was collected for four hours in the liquid water target system before being transferred to a collection vessel. Four additional collections each lasting four hours were made before shipping samples to Washington University and Hope College for chemical separation and analysis.

At the NSCL there are several non-intercepting beam monitors, Faraday cups, and a BC-400 plastic scintillator that can be used to measure beam current. The Faraday cups and scintillator can be used to determine particles per second, however, only the non-intercepting beam monitors can be used to measure the beam at full intensity during the sample collections. Calibration curves had to be established comparing the non-intercepting beam monitors with the Faraday cups and beam line. The beam beam intensity and purity were measured using the standard delta-E/Time-of-Flight technique with a silicon-PIN diode in the A1900 focal plane and cyclotron RF signal prior to delivery of the beam to the experimental end station$^{22}$. The secondary beam was collected for four hours in the liquid water target system before being transferred to a collection vessel. Four additional collections each lasting four hours were made before shipping samples to Washington University and Hope College for chemical separation and analysis.

**Separations Involving Chelating Disk.** All separations using the high-performance extraction disk (3 M Empore, MN) were carried out with 100 mL 1.25 M ammonium acetate (trace metals grade) and pH adjusted to 5 by adding acetic acid (trace metals grade). The solution was then drawn through a 25 mm diameter extraction disk in a glass microanalysis vacuum filter holder (Millipore, MA) with vacuum after the disk was pretreated by rinsing with 20 mL milli-Q water, 20 mL 3 M nitric acid (trace metals grade), 2–50 mL milli-Q water, 50 mL 0.1 M ammonium acetate, followed by two additional rinses of 25 mL milli-Q water, allowing the disk to dry between each rinse. When passing the 100 mL sample through the extraction disk a new vacuum flask was used to save the eluent so that it could be analyzed by HPGe detector for radioactivity that did not bind to the extraction disk.

| Table 3 | Chemical recovery of $^{67}$Cu from the aqueous solutions produced at the NSCL |
|---------|---------------------------------|
|         | Run2 [kBq] | Run3 [kBq] | Run4 [kBq] |
| Starting Activity | 452 [9] | 425 [8] | 403 [8] |
| Recovered Activity | 388 [8] | 380 [8] | 353 [7] |
| Percent Recovery | 86 [2] | 89 [3] | 88 [2] |
Germanium Separation Validation. Several concentrations of aqueous germanium ranging from 0.04–0.5 ppm in 1.25 M ammonium acetate were passed through the extraction disk using the method described above. The chelation disk was analyzed using particle-induced x-ray emission (PIXE) by irradiating the chelation disk with 3.4 MeV protons for five minutes at beam intensities between 2.25–2.80 nA. A Si(Li) detector was used to measure the resulting x-rays. The characteristic K, x-rays for germanium were analyzed to determine the amount of germanium bound to the chelation disk.

$^{68}$Ga and $^{64}$Cu Separation Validation. 200 $\mu$Ci of $^{68}$Ga obtained from a $^{68}$Ge/$^{68}$Ga generator and 200 $\mu$Ci of $^{64}$Cu produced at Washington University in St. Louis were added to 100 mL of 1.25 M ammonium acetate and passed through the extraction disk using the method given above. The extraction disk was surveyed by HPGe for $^{68}$Ga and $^{64}$Cu using the characteristic gamma rays, 1077.3 keV (3.22%) for $^{68}$Ga and 1345.8 keV (0.475%) for $^{64}$Cu. The extraction disk was returned to the filter holder and 10 mL of 6 M hydrochloric acid (trace metals grade) was added to remove $^{64}$Cu from the extraction disk. Both the extraction disk and the filtrate were analyzed by HPGe to measure amounts of $^{68}$Ga and $^{64}$Cu present in each.

Copper, Nickel, and Zinc Separation Validation. Aqueous solutions containing 100 mg of copper, nickel and zinc (1000 mg/L AAS Standards, Sigma Aldrich, USA) were added to 100 mL 1.25 M ammonium acetate solution at pH 5 and passed through the chelation disk as described above. 10 mL of 6 M hydrochloric acid (trace metals grade) was then added to remove the nickel, copper, and zinc that were bound to the chelation disk. This solution was then passed through an anion-exchange column using 2.5 g AG1-8X resin (Bio-Rad, USA) and washed with an additional 10 mL of 6 M HCl to remove the nickel followed by 10 mL of 0.5 M HCl to remove the copper. Each 10 mL fraction was brought to dryness by evaporation through overnight heating at 80°C and reconstituted in 1 mL Ultratrace water (Sigma Aldrich, USA) and analyzed by an HPLC trace metal ion chromatography method.

$^{67}$Cu Separation. First, each 100 mL sample was turned into a 1.25 M solution of NH$_4$OAc by adding 9.6 g trace metals grade ammonium acetate and pH adjusted to 5 by adding trace metals grade acetic acid. The solution was then passed through the chelation disk as described above. The filter holder was transferred to a clean vacuum flask and 10 mL of 6 M trace metals grade hydrochloric acid was added to remove $^{67}$Cu along with the nickel and zinc contaminants. The 6 M HCl elution was then transferred to an anion-exchange column with AG1-X8 resin, the 10 mL eluate was collected and then an additional 10 mL of 6 M hydrochloric acid was passed through the column and collected. Finally 10 mL of 0.5 M hydrochloric acid was added to the resin and 1 mL fractions of the eluate were collected. All eluates were analyzed for radiochemical purity by observing the characteristic gamma rays in an HPGe detector.

| Table 4 | Experimental parameters for the $^{67}$Cu secondary beam at the NSCL |
| Block | Description | Value |
| Primary Beam Target | Beryllium | 510.797 mg/cm² |
| D1 Bp | Dipole magnet | 3.2822 Tm |
| I1 Slits | Momentum acceptance (dp/p) | 0.2% |
| D2 Bp | Dipole magnet | 3.2822 Tm |
| I2 Slits | Momentum acceptance (dp/p) | 2% |
| I2 Wedge | Aluminum | 236.685 mg/cm² |
| D3 Bp | Dipole magnet | 2.9147 Tm |
| D4 Bp | Dipole magnet | 2.9147 Tm |
| CRADO6 | BC-400 scintillator (removable) | 155 μm |
| D5 Bp | Dipole steering magnet | 2.9027 Tm |
| D6 Bp | Dipole steering magnet | 2.9027 Tm |
| Exit Window | Zirconium | 75 μm |
| Air Gap | Air | 89 μm |
| Water Target Window | Kapton | 8 μm |
| Water Target | Liquid water | 73 mm |

Figure 3 | (A) Structure of the NOTA-Bz-NCS conjugated to lysine residues on Trastuzumab. (B) Fast Protein Liquid Chromatography of NOTA-Bz-NCS-Trastuzumab indicating an intact conjugated antibody. (C) Radio instant thin layer chromatography of $^{67}$Cu-NOTA-Bz-NCS-Trastuzumab challenged with EDTA. The sharp peak at 60 mm and the absence of a later peak signifies a fully labeled antibody. (D) Similar to (C) where a broad peak between 100–130 mm signifies $^{68}$Cu-EDTA and serves as a negative control.
Concentration of the NOTA-Bz-NCS-Trastuzumab was measured using the bicinchoninic acid (BCA) assay.

The two 1 mL 0.5 M hydrochloric acid samples with the largest amounts of $^{67}$Cu were subjected to dryness and reconstituted using 50 μL of 0.1 M ammonium acetate at pH 5.5. 5 μL was transferred to a new tube and 5 μL 50 mmol EDTA was added. The remaining 45 μL was added to a microtube along with 2 μL NOTA-Bz-NCS-Trastuzumab and brought to 50 μL by adding 0.1 M ammonium acetate at pH 5.5. Tubes were placed in a shaking incubator at 37°C for 1 hr. A Zeba spin desalting column (40 K, 0.5 mL) was used to buffer exchange into 0.1 M ammonium acetate buffer at pH 5.5. The solution containing $^{67}$Cu-NOTA-Bz-NCS-Trastuzumab was then used to incubate 1 μL NOTA-NCS stock solution and 96 μL 0.1 M sodium carbonate pH 9.0 and incubated for 1 hr at 37°C on a shaking incubator. A Zeba spin desalting column (40 K, 0.5 mL) was used to buffer exchange into 0.1 M ammonium acetate buffer at pH 5.5. The two 1 mL 0.5 M hydrochloric acid samples with the largest amounts of $^{67}$Cu were subjected to dryness and reconstituted using 50 μL of 0.1 M ammonium acetate at pH 5.5. 5 μL was transferred to a new tube and 5 μL 50 mmol EDTA was added. The remaining 45 μL was added to a microtube along with 2 μL NOTA-Bz-NCS-Trastuzumab and brought to 50 μL by adding 0.1 M ammonium acetate at pH 5.5. Tubes were placed in a shaking incubator at 37°C for 1 hr. A Zeba spin desalting column (40 K, 0.5 mL) was used to buffer exchange into 0.1 M ammonium acetate buffer at pH 5.5. The solution containing $^{67}$Cu-NOTA-Bz-NCS-Trastuzumab was then used to incubate 1 μL NOTA-NCS stock solution and 96 μL 0.1 M sodium carbonate pH 9.0 and incubated for 1 hr at 37°C on a shaking incubator. A Zeba spin desalting column (40 K, 0.5 mL) was used to buffer exchange into 0.1 M ammonium acetate buffer at pH 5.5.

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Additional information
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