
A Universally Applicable ^{68}Ga -Labeling Technique for Proteins

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Although protein-based PET imaging agents are projected to become important tracer molecules in the future, the labeling of complex biomolecules with PET radionuclides is inexpedient and, most of the time, challenging. **Methods:** Here we present a straightforward labeling chemistry to attach the versatile radionuclide ^{68}Ga to proteins. Introducing the ^{68}Ga chelating agent NODA-GA-T (2,2'-(7-(1-carboxy-4-(2-mercaptoethylamino)-4-oxobutyl)-1,4,7-triazonane-1,4-diy)diacetic acid) by reaction with proteins chemically processed with sulfo-SMCC (4-(*N*-maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-*N*-hydroxy-succinimide ester sodium salt) results in labeling precursors, enabling a simple and rapid kit-labeling procedure that requires no workup of the radiolabeled proteins. Various ^{68}Ga -proteins were labeled using this method, and the radiochemical yields and specific activities of the labeled proteins were determined. To show that the radiotracers are applicable for in vivo studies, proof-of-concept small-animal PET images were acquired in healthy rats using ^{68}Ga rat serum albumin for blood-pool imaging and ^{68}Ga -annexin V for apoptosis imaging in mice with a left ventricular myocardial infarction. **Results:** The proteins could be modified, yielding 1.2–1.7 ^{68}Ga -labeling sites per protein molecule. All investigated proteins could be labeled in high radiochemical yields of 95% or more in less than 10 min in 1 step, using acetate-buffered medium (pH 3.5–4.0) at room temperature without any further purification. The labeled proteins displayed specific activities of 20–45 GBq/ μmol (540–1,200 Ci/mmol). In the proof-of-concept in vivo studies, ^{68}Ga rat serum albumin and ^{68}Ga -annexin V were successfully used for in vivo imaging. Both radiotracers showed a favorable biodistribution in the animal models, thus demonstrating the usefulness of the developed approach for the kit ^{68}Ga labeling of proteins. **Conclusion:** The preprocessing of proteins proceeds in high chemical yields and with high protein recovery rates after purification. These precursors can be stored for several months at -20°C without degradation, and ^{68}Ga labeling can be performed in a 1-step kit-labeling reaction in high radiochemical yields. Two of the derivatized model proteins were successfully used in proof-of-

concept in vivo imaging studies to prove the applicability of this kit ^{68}Ga -labeling technique.

Key Words: ^{68}Ga -labeling; kit; proteins; PET; rat serum albumin; annexin V

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In the context of molecular and nuclear in vivo imaging, the radioactive labeling of proteins that bind to specific targets in the human body and therefore represent promising compounds for application as radiotracers can suffer from complicated and inefficient labeling procedures. Among in vivo imaging modalities, PET is one of the most sophisticated imaging methods available (1). Therefore, bioactive molecules labeled with PET radionuclides are of increasing interest for molecular imaging purposes (2,3). Although the commonly used radionuclides for PET are mainly ^{18}F (half-life 110 min) and ^{11}C (half-life 20 min), ^{68}Ga is increasingly gaining popularity because it can be obtained from a commercially available $^{68}\text{Ge}/^{68}\text{Ga}$ generator system that delivers the ^{68}Ga nuclide reliably for up to a year, independent of a cyclotron. It has already been demonstrated that ^{68}Ga -labeled peptides display favorable properties for in vivo tumor imaging with PET (4,5). However, few examples of ^{68}Ga -labeled proteins or other large biomolecules can be found in the literature, despite many promising tracer candidates for different biologic target applications, such as labeled serum proteins, annexin V, and antibody fragments, that could find application in oncology, neurology, and cardiology. This situation is mainly due to the fact that the chelator DOTA, which is predominantly used for radiometal nuclide complexation in nuclear medicine, requires high temperatures or long reaction times to form a complex with ^{68}Ga (6). Because proteins are susceptible to heat-induced disintegration, chelators requiring a heating step for complexation are not suitable for such a radiolabeling approach. Thus, the envisioned ^{68}Ga -labeling procedure for proteins should comprise only 1 simple, fast, and preferably quantitative labeling step at room temperature without the need for further purification.

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In this regard, NOTA (2,2',2''-(1,4,7-triazonane-1,4,7-triyl)triacetic acid) and HBED (*N,N'*-bis[2-hydroxy-5-(carboxyethyl)benzyl] ethylenediamine-*N,N'*-diacetic acid) derivatives have been shown to be introducible into proteins, forming stable complexes with ^{68}Ga (7–10). Technical shortcomings of the chelator derivatives used so far are the relatively long reaction time that is needed for complexation of the radiometal, a factor not affecting gallium isotopes displaying long half-lives but strongly hampering the labeling with the short-lived ^{68}Ga (half-life 68 min); intricate multistep syntheses of the chelator derivatives; ^{68}Ga incorporation rates that result in an inevitable final purification step that runs counter to the intended kit radiolabeling of the proteins; high immunogenicity of the chelator; and inability to determine the number of introduced chelators per macromolecule (protein) by a simple procedure (11–14). This last shortcoming is an important criterion, because it was shown that a high number of derivatization sites per protein molecule can lead to a dramatic loss of the biologic activity of the modified biomolecule (15–17). In the case of the isothiocyanate derivative of 2,2',2''-(1,4,7-triazonane-1,4,7-triyl)triacetic acid, the number of derivatization sites per protein molecule was determined using a ^{14}C -labeled chelator tagging, a highly complicated and impractical method (11,18). Alternatively, the number of introduced chelators can also be determined using an isotopic dilution titration assay or a mass spectrometry analysis of protein fragments—approaches that are also time consuming.

Hence, the synthesis of a different, unprotected chelator derivative is needed that allows an easy quantification of protein modification sites, exhibiting efficient coupling properties to chemical moieties present in proteins and quantitative ^{68}Ga -labeling capabilities under mild conditions for the labeling of proteins. This synthesis would introduce an efficient tool for protein-based PET tracer development. The quantitative ^{68}Ga -labeling reaction of this technique would furthermore result in tracers requiring no further purification, enabling a true kit-labeling procedure for proteins intended for in vivo imaging application.

We demonstrate here a technique for the kit radiolabeling of proteins with ^{68}Ga for many exemplary proteins that differ in structure and molecular weight (MW), demonstrating that proteins can be labeled quantitatively with ^{68}Ga after simply derivatizing the protein with 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-*N*-hydroxysuccinimide ester sodium salt (sulfo-SMCC) and the sulfhydryl-derivatized chelator 2,2'-(7-(1-carboxy-4-(2-mercaptoethylamino)-4-oxobutyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA-GA-T). All chemical procedures for protein modification display an efficiency between 85% and 98% using only minute amounts of protein, making it possible to label even costly starting materials such as annexin V for imaging of apoptosis. These derivatized proteins can be labeled with ^{68}Ga in short reaction times of only 7 min and high radiochemical yields of 95% or more, producing radiotracers that can be applied without sub-

sequent purification. Thus, this technique is the first that allows a true kit labeling of proteins with ^{68}Ga .

MATERIALS AND METHODS

All commercially available chemicals were of analytic grade and used without further purification. 4-(4,7-bis(2-*tert*-butoxy-2-oxoethyl)-1,4,7-triazonane-1-yl)-5-*tert*-butoxy-5-oxopentanoic acid (NODA-GA(*t*Bu)₃) was purchased from Chematech. Sulfo-SMCC was purchased from SigmaAldrich. NAP-5 columns, Vivaspin 6 concentrators, and the in situ apoptosis kit ApopTag were purchased from GE Healthcare, Sartorius, and Millipore, respectively. The $^{68}\text{Ge}/^{68}\text{Ga}$ generator used for radiolabeling was an IGG100 system purchased from Eckert & Ziegler. An Agilent 1200 system equipped with a Raytest Gabi Star radioactivity detector was used for analytic and semipreparative high-performance liquid chromatography (HPLC). Chromolith Performance (RP-18e, 100–4.6 mm; Merck) and Chromolith SemiPrep (RP-18e, 100–10 mm; Merck) columns, operated with a flow of 4 and 8 mL \times min⁻¹, respectively, were used for chromatography. All experiments involving animals were approved by the local animal use committee. A detailed description of animal models and experiments is provided in the supplemental material (available online only at <http://jnm.snmjournals.org>).

Preparation of NODA-GA-T

Tert-butyl-2,2'-(7-(1-*tert*-butoxy-1,5-dioxo-5-(2-(tritylthio)ethylamino)pentan-2-yl)-1,4,7-triazonane-1,4-diyl) diacetate (*S*-trityl-tris-*tert*-butyl-mercaptoethylamino-NODA-GA-T). A solution of *N,N'*-dicyclohexyl-carbodiimide (189 mg; 919 μmol) in 5 mL of pyridine was added dropwise at room temperature to a solution of NODA-GA(*t*Bu)₃ (500 mg; 919 μmol) and *S*-trityl-mercaptoethanolamine trifluoroacetate (293 mg; 919 μmol) in 40 mL of water:MeCN 1:1. After reaction overnight, the solvents were evaporated, and the product was purified via semipreparative HPLC with 0%–30% MeCN + 0.1% trifluoroacetic acid (TFA) in 5 min as the gradient (retention time 3.9 min). The product was isolated as a white solid (343 mg; 406 μmol ; 44%), and the chemical identity was confirmed by ^1H - and ^{13}C -nuclear magnetic resonance and high-resolution mass spectrometry.

Meraptoethylamino-NODA-GA (NODA-GA-T). *S*-Trityl-tris-*tert*-butyl-mercaptoethylamino-NODA-GA (343 mg; 406 μmol) was dissolved in a mixture of 5 mL of TFA and 200 μL of triisopropylsilane and reacted for 3 h at room temperature. The volatile components were evaporated, and the product was purified using semipreparative HPLC with 0%–20% MeCN + 0.1% TFA in 5 min as the gradient (retention time 2.3 min). The product was isolated as a yellow hardening oil (90 mg; 207 μmol ; 51%), and the chemical identity was confirmed by ^1H - and ^{13}C -nuclear magnetic resonance as well as high-resolution mass spectrometry.

Sulfo-SMCC Derivatization of Proteins

A freshly prepared solution of sulfo-SMCC (92 μg ; 210 nmol) in 150 μL of dimethylformamide:water 1:1 was added to a solution of the protein (30 nmol) in 500 μL of phosphate buffer (0.1 M, pH 7.2). After 1 h of incubation, the derivatized protein was purified via size-exclusion chromatography using a NAP-5 column. The derivatized protein was recovered in high yields of 95%–98%. The number of maleimide functions per protein molecule was determined by back-titration with Ellman's assay using sodium 2-mercaptoethanesulfonate and was found to be between 1.2 and 1.7.

Conjugation of NODA-GA-T to Sulfo-SMCC-Derivatized Proteins

A freshly prepared solution of NODA-GA-T (131 μg ; 300 nmol) in phosphate buffer (0.1 M, pH 7.2) was added to the solution of the sulfo-SMCC-derivatized protein (20 nmol) and reacted for 30 min. Subsequently, the protein solution was diluted with *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES) buffer (0.025 M, pH 4.0) and purified by ultrafiltration using Vivaspin 6 centrifugal concentrators (MW cutoff, 10 kDa for annexin V, human serum albumin, bovine serum albumin, rat serum albumin (RSA) and DT CRM197 (nontoxic diphtheria toxin, immunologically crossreacting mutant); MW cutoff, 100 kDa for humanized monoclonal antibody 425). The ultrafiltration was repeated several times to reduce the amount of small impurities to about $1/50,000$. The protein was recovered in yields of 85%–95%.

Kit Labeling of Derivatized Proteins with ^{68}Ga

$^{68}\text{Ga}^{3+}$ (240–340 MBq) for the radiolabeling reaction was obtained by fractionated elution of a commercially available $^{68}\text{Ge}/^{68}\text{Ga}$ generator in 1 mL of 0.1 M HCl. The pH of the solution was adjusted to 3.5–4.0 by adding approximately 85 μL of a 1.25 M sodium acetate solution. Subsequently, a solution of the NODA-GA-T-derivatized protein (6.9–10 nmol) in HEPES buffer (0.025 M, pH 4.0) was added and incubated for 7 min at room temperature. After radiolabeling, HEPES buffer (2 M, ~ 150 μL) was added to this mixture to adjust the pH to 7.0, and the solution was filtered sterile. The radiolabeled proteins were analyzed by analytical radio-HPLC (gradient of 0%–100% MeCN + 0.1% TFA in 5 min) and found to be 95%–99% pure. The specific activities of the proteins were between 20 and 45 GBq/ μmol .

RESULTS

Preprocessing of Various Proteins

To enable a broad dissemination of ^{68}Ga -labeled proteins by providing a kit-labeling technique, we contrived a labeling procedure for proteins consisting of 2 preprocessing steps and a final labeling step. The chemical derivatization steps do not need to be performed directly before the actual radiolabeling, because the derivatized precursor protein can be stored for several months at -20°C without altering the radiolabeling characteristics. Furthermore, these preprocessing steps can be performed in large scale (and potentially outsourced), yielding many labeling kits. The radiolabeling itself can be performed within minutes, providing directly applicable radiotracers.

The protein preprocessing comprises the derivatization of about 30–600 nmol of protein first with sulfo-SMCC, a maleimide-introducing cross-linker, yielding a small number of maleimido functionalities per protein for further modification (Fig. 1). The inserted amount of maleimido groups—intended for the subsequent reaction with the sulfhydryl group of NODA-GA-T—can easily be quantified using Ellman's assay. In the case of the tested proteins, a 7-fold excess of sulfo-SMCC per protein yielded between 1.2 and 1.7 maleimides per molecule, resulting in a minor structural alteration that is an essential prerequisite for a preserved biologic activity (15–17). The modified proteins were purified using size-exclusion gel chromatography, with high recovery rates of 95%–98%.

The sulfo-SMCC-derivatized proteins were subsequently reacted with an excess of the chelating agent NODA-GA-T (Fig. 1) in aqueous medium at pH 7.2 for 30 min and purified by ultrafiltration to produce the final labeling precursors in yields between 85% and 95%.

To show the applicability of our approach for several proteins and to cover a wide range of compounds characterized by strongly varying MWs and structures, the following proteins were modified with sulfo-SMCC and subsequently conjugated to NODA-GA-T: a humanized monoclonal antibody (IgG; MW, 150 kDa), holo-transferrin (MW, 80 kDa), rat serum albumin (MW, 69 kDa), human serum albumin (MW, 68 kDa), bovine serum albumin (MW, 66 kDa), DT CRM197 (nontoxic diphtheria toxin, immunologically crossreacting mutant) (MW, 63 kDa), annexin V (MW, 36 kDa), and SDF-1 α (MW, 11 kDa). All derivatized proteins displayed a shelf-life of several months at -20°C , making them particularly suitable for long-term storage.

Radioactive Labeling of Proteins with ^{68}Ga

To demonstrate the applicability of our ^{68}Ga -labeling strategy, we reacted 6.9–10 nmol of each modified protein—obtained by derivatization with sulfo-SMCC and subsequent conjugation to NODA-GA-T—with 240–340 MBq of $^{68}\text{Ga}^{3+}$ in acetate-buffered aqueous medium at pH 3.5–4.0 for 7 min at ambient temperature. It was evident that after this reaction time, 95% or more (in many cases up to 99%) of $^{68}\text{Ga}^{3+}$ was incorporated into the proteins investigated (Supplemental Fig. 1). The achieved specific activities were high (20–45 GBq/ μmol [540–1,200 Ci/mmol]) as a result of the low amounts of proteins used. The labeling reactions were performed with either freshly derivatized proteins or proteins being stored for weeks or months, and no influence on the labeling yield was observed.

To show the *in vivo* applicability of the ^{68}Ga -labeled proteins obtained using this labeling technique, we performed proof-of-concept studies for 2 of the proteins: ^{68}Ga -RSA and ^{68}Ga -annexin V.

^{68}Ga -Labeled RSA for Blood-Pool Imaging

Blood-pool imaging by planar imaging or SPECT can accurately determine left and right ventricular volumes and ejection fractions in both humans and mice (19,20) and can thus be used to assess different kinds of cardiac or cardiotoxic oncologic diseases and therapies. For blood-pool imaging, ^{68}Ga -RSA obtained by the described kit-labeling technique was applied to healthy rats, and the biodistribution was studied (Supplemental Fig. 2). For myocardial landmarking, ^{18}F -FDG was used in the same animals. The blood pool in the heart could be well delineated from the accumulation of ^{18}F -FDG in the myocardium (Fig. 2).

^{68}Ga -Labeled Annexin V for Apoptosis Imaging

Biomarkers for the quantification of apoptosis, which is involved in a great variety of cardiovascular diseases such as heart failure, myocardial infarction, and atherosclerosis, are of particular interest because they allow the discrimination between newly infarcted apoptotic and scarred

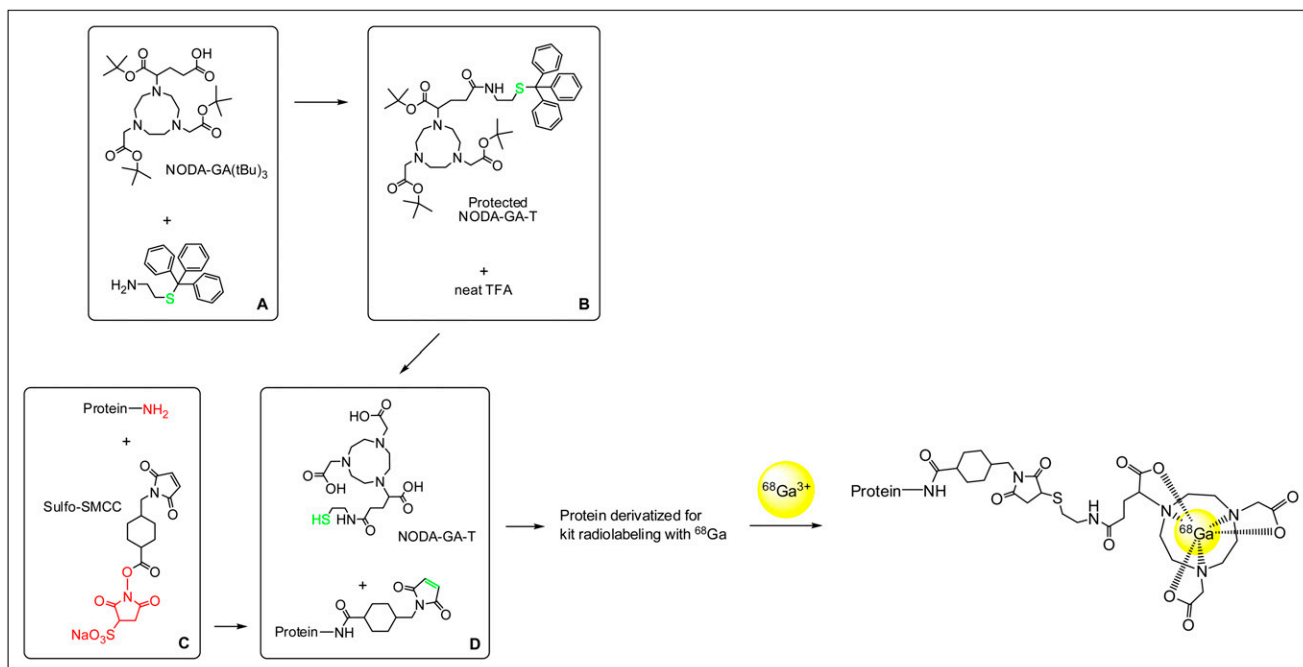


FIGURE 1. Synthesis of ^{68}Ga chelator NODA-GA-T: (A) commercially available NODA-GA($t\text{Bu}$)₃ is reacted with S-Trityl-mercaptoethanolamine trifluoroacetate using *N,N'*-dicyclohexyl-carbodiimide to yield S-Trityl-tris-*tert*-butyl-protected NODA-GA-T. (B) This intermediate is deprotected using neat TFA to yield NODA-GA-T. (C) Derivatization of protein with sulfo-SMCC (amino function [red]) reacts with sodium-sulfo-succinimidyl ester [red]). (D) Coupling of NODA-GA-T (sulfhydryl function [green]) reacts with maleimido group [green]); radiolabeling of protein with $^{68}\text{Ga}^{3+}$ (yellow).

dysfunctional tissues (21). Furthermore, they should be valuable tools to investigate antiapoptotic effects of modern therapies and concurrently to enable therapy monitoring at earlier stages of myocardial infarction. Because annexin V specifically binds to externalized phosphatidylserine of apoptotic cells, it should be a useful biomarker for the assessment of cardiovascular damage, and its application for in vivo imaging of apoptosis is intriguing (22–24).

In contrast to the highly demanding ^{18}F labeling of annexin V (a process generating the radiolabeled product in low yields because of the complicated and time-consum-

ing multistep syntheses), our ^{68}Ga -labeling technique provides the ^{68}Ga -labeled annexin V in a straightforward synthesis within a short preparation time of only 15 min (start of synthesis to injectable solution). To show the applicability and attained biologic activity of ^{68}Ga -annexin V produced by this method, the tracer was administered to mice with left ventricular myocardial infarctions. A well-definable ^{68}Ga -annexin V accumulation could be observed after 60 min, clearly delineating the infarct area. These findings could be verified by a massively decreased uptake of ^{18}F -FDG in the same area (Supplemental Fig. 3 and Fig. 3).

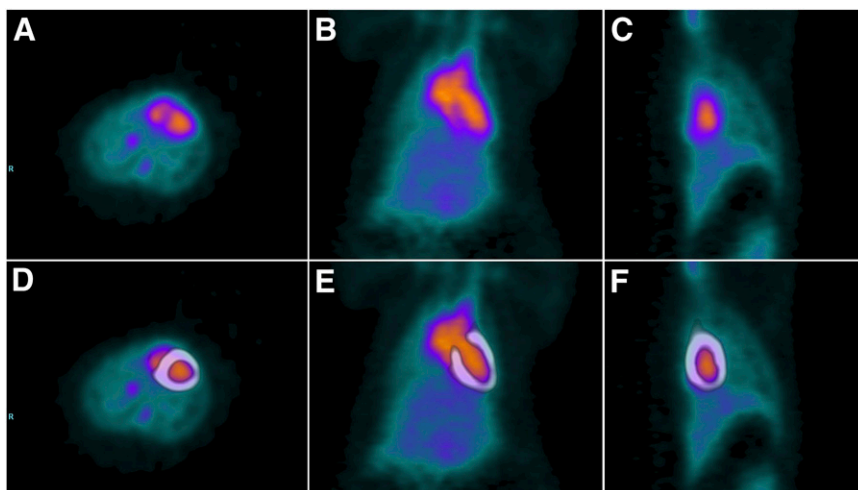


FIGURE 2. In vivo blood-pool imaging in healthy rat using ^{68}Ga -albumin. (A–C) ^{68}Ga -albumin scan of heart region in axial (A), coronal (B), and sagittal views (C). (D–F) Overlay of ^{68}Ga -albumin scan (color images) with ^{18}F -FDG scan (gray scale images) in same animal. ^{18}F -FDG as biomarker for myocardial glucose metabolism indicates left ventricular myocardial borders.

To determine whether the observed uptake of ^{68}Ga -annexin V in the heart tissue during the *in vivo* PET scans is related to apoptosis that is induced by the myocardial infarction, *ex vivo* autoradiography and apoptosis staining experiments of the excised hearts were performed. The ^{68}Ga radioactivity distribution in the heart tissue corresponds to the apoptotic area, which was confirmed via terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay—an established method to detect DNA fragmentation that is a result of the apoptotic signaling cascade (Supplemental Fig. 4) (25). The commercially available assay is capable of selectively identifying apoptosis over necrosis, which is also characterized by severe DNA damage.

DISCUSSION

The radioactive labeling of proteins for *in vivo* imaging of various biologic targets known to be involved in human diseases such as cancer and cardiovascular diseases is developing into an indispensable medical tool in nuclear imaging.

So far, ^{18}F has been in the focus for the synthesis of radiolabeled proteins applicable in PET. However, despite many advancements, none of the developed strategies has been translated into protein-based imaging agents for routine PET application—a shortcoming that can be attributed to the intricate radiosyntheses required. As a result of this and the so-far unsatisfactory strategies to label proteins with ^{68}Ga , more convenient ways to introduce the promising radionuclide ^{68}Ga into proteins are highly sought after.

Our procedure, involving 2 protein derivatization steps (which do not need to be performed directly before radiolabeling but can be performed weeks to months before) and 1 fast and convenient ^{68}Ga -labeling step, provides a universally applicable tool to label proteins with ^{68}Ga . All investigated proteins—varying in MW and structure—were amenable toward a chemical modification using our techni-

que. Both protein derivatization steps proceeded in high yields and with high protein recovery rates between 85% and 98%.

The ^{68}Ga labeling of proteins for routine PET applications should meet the following requirements: high incorporation yields of ^{68}Ga , fast radiolabeling reactions of the chelator-modified proteins with ^{68}Ga , low amounts of precursor protein to achieve high specific activities and reduce costs, and no necessary final purification, yielding radiotracers ready for *in vivo* application after the labeling reaction and enabling a kit ^{68}Ga labeling of proteins. Our technique meets these requirements because it is capable of labeling proteins with ^{68}Ga within 7 min in high radiochemical yields of 95% or more. Thus, no purification is required after the labeling reactions of the modified proteins with ^{68}Ga before the final PET application. After neutralization and sterile filtration, the ^{68}Ga -labeled proteins are directly applicable for PET, qualifying this method as a true kit-labeling technique for the ^{68}Ga labeling of proteins.

The simplicity of our method provides a convenient kit ^{68}Ga -labeling tool for proteins that can easily be integrated into routine PET tracer production at low costs (Supplemental Fig. 5). The practical value of proteins labeled by this strategy has been demonstrated in proof-of-concept studies using 2 of the ^{68}Ga -labeled proteins, rat serum albumin and annexin V, for *in vivo* blood-pool and apoptosis imaging with small-animal PET. Both compounds were found eligible for the intended imaging purpose. ^{68}Ga -RSA visualized the *in vivo* blood pool of a rat heart accurately, whereas ^{18}F -FDG as a metabolic tracer delineated the healthy myocardium (Fig. 2). These findings can be easily transferred to a human application in which gated blood-pool imaging of the heart enables the determination of left ventricular functional parameters such as the left ventricular ejection fraction volume. The low number of derivatization or labeling sites per protein molecule permits preserved biologic properties and an almost unaffected biodistribution, as shown for ^{68}Ga -annexin V through *in vivo* imaging, autoradiography, and TUNEL assay data. ^{68}Ga -annexin V—obtained using our kit ^{68}Ga -labeling procedure—was used for apoptosis imaging *in vivo* in a mouse model with a left ventricular infarction (Fig. 3). In the infarct area, a considerable uptake was observed after 60 min. The specific uptake of ^{68}Ga -annexin V in apoptotic areas was confirmed by autoradiography and the TUNEL assay that was used for apoptosis staining (Supplemental Fig. 4).

We are confident that ^{68}Ga -labeled proteins have the potential to become routine PET tracers in clinical nuclear medicine. The ^{68}Ga -labeling reaction proceeds at room temperature within 7 min without forming any side products, providing the radiolabeled protein in a minimum radiochemical yield of 95% or more, making purification dispensable. Furthermore, because the procedure is simple, it requires only a minimum of lead-shielded laboratory space and can even be performed by nonspecialized personnel.

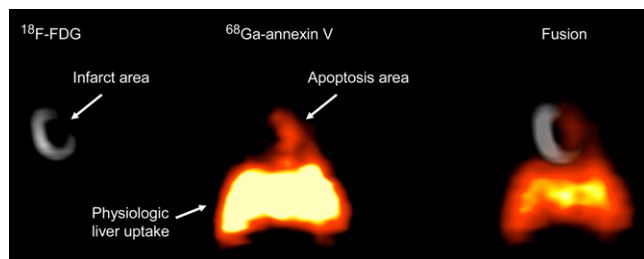


FIGURE 3. Imaging apoptosis *in vivo* in mouse 2 d after occlusion of left anterior descending artery (all images coronal view). (Left) ^{18}F -FDG scan shows massively decreased uptake in anterior wall, whereas ^{18}F -FDG uptake in remaining myocardial areas is preserved. (Middle) Sum image of last 30 min of 90-min ^{68}Ga -annexin V scan in same animal, showing specific uptake in area of decreased metabolism, indicating present apoptosis. (Right) Fusion of ^{18}F -FDG and ^{68}Ga -annexin V scans.

CONCLUSION

Our ^{68}Ga kit with the generator-produced PET isotope ^{68}Ga provides an efficient tool to label proteins for in vivo imaging applications with PET. The number of labeling sites per protein is low and can easily be determined. A variety of proteins could easily be derivatized in a 2-step procedure involving the chelator derivative NODA-GA-T. The radiolabeling with ^{68}Ga proceeds quickly (15 min from start of synthesis to injectable tracer solution), with a high labeling efficiency of at least 95%, making purification dispensable. As proof of concept, 2 of the ^{68}Ga -labeled proteins, RSA and annexin V, were demonstrated to be eligible for in vivo blood-pool and apoptosis imaging in animal models, respectively. Our kit-labeling procedure for the synthesis of protein-based ^{68}Ga PET agents gives chemists and clinicians a versatile universal tool to obtain ^{68}Ga protein-based radiotracers in a research or clinical setting.

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