Nitrone-Modified Gold Nanoparticles: Synthesis, Characterization, and Their Potential as $^{18}$F-Labeled Positron Emission Tomography Probes via I-SPANC

Sara Ghiassian,† Lihai Yu,† Pierangelo Gobbo,‡,† Ali Nazemi,‡,⊥ Tommaso Romagnoli,‡ Wilson Luo,† Leonard G. Luyt,*‡,§,¶ and Mark S. Workentin*†

†Department of Chemistry and the Center for Materials and Biomaterials Research and §Department of Oncology, The University of Western Ontario, London N6A 5B7, Ontario, Canada

‡London Regional Cancer Program, 800 Commissioners Rd. E., London N6A 5W9, Ontario, Canada

ABSTRACT: A novel bioorthogonal gold nanoparticle (AuNP) template displaying interfacial nitrone functional groups for bioorthogonal interfacial strain-promoted alkyne—nitrone cycloaddition reactions has been synthesized. These nitrone–AuNPs were characterized in detail using $^1$H nuclear magnetic resonance spectroscopy, transmission electron microscopy, thermogravimetric analysis, and X-ray photoelectron spectroscopy, and a nanoparticle raw formula was calculated. The ability to control the conjugation of molecules of interest at the molecular level onto the nitrone–AuNP template allowed us to create a novel methodology for the synthesis of AuNP-based radiolabeled probes.

INTRODUCTION

Positron emission tomography (PET) is a noninvasive imaging technique that provides valuable real-time information on physiological, biochemical, and pharmacological processes that take place in living organisms.1–6 The engineering and synthesis of radiolabeled probes for medical imaging with PET is an area of chemistry that is extremely active and rich in challenges, which includes solubility and biological stability, control of biodistribution, and preserving molar activity. Among the different PET radionuclides, the most employed is fluorine-18 ($^{18}$F) because of its particularly favorable nuclear and chemical properties: it offers a half-life of 110 min, a $\beta^-$-branch of 97%, and a low $\beta^-$-energy of 635 keV. This allows for its facile off-site production and transportation, for relatively long imaging experiments, and the acquisition of images with higher resolution than those obtained using other radioisotopes.7

One of the most common strategies for the synthesis of robust and efficient PET probes involves the use of radiolabeled prosthetic groups that are then introduced onto the biomolecular system of interest in the last step of synthesis. This type of synthesis involves a continuous race against time and the decay of the radionuclide. In fact, the biggest challenge involves the development of synthetic steps with high reaction rates, high yields, and that take place under mild reaction conditions while using sub-micromolar amounts of radiolabeled compounds.7–18

In recent years, bioorthogonal chemistry has started to revolutionize the synthesis of radiolabeled probes, in particular for higher molecular weight biologics. In fact, bioorthogonal reactions represent ideal candidates for those critical synthetic steps because of their robustness, versatility, fast kinetics, high yields, and mild conditions. Most importantly, they have the potential to lead to easier and faster synthetic protocols for the synthesis of radioactive biologics and nanoparticles, with straightforward and approachable methods for users with basic chemistry skills.

One of the most useful bioorthogonal reactions is the strain-promoted alkyne–azole cycloaddition (SPAAC). This is a variation of the copper (I)-catalyzed Huisgen cycloaddition (CuAAC), whose driving force relies on a strained C=C triple bond imbedded in an eight-membered ring. Most importantly, the SPAAC reaction does not require the use of cytotoxic metal catalysts, overcoming the problems associated with their application, such as cytotoxicity, copper-promoted degradation of peptides and proteins, and formation of insoluble copper acetylde.19–22 The typical complementary functional group or chemical reporter of the strained alkyne is the azide because of its small dimension, stability, and biological inertness. However, utilizing more reactive alternatives to azides can greatly enhance the reaction rate of strain-promoted cyclo-
However, the former methodology has the drawback that the size and shape of the nanomaterial substrate can change during the redox reaction, causing destabilization of the nanoparticle or irreversible aggregation. As a consequence, the precise characterization of both the metallic core and organic corona becomes extremely challenging or even impossible. The latter method instead relies on a “shotgun-type” conjugation approach, which has no control over the extent of incorporation of the desired radiolabeled prosthetic group or its quantification, representing a major issue for potential therapeutic applications.

In the past few years, the Workentin research group has begun to develop a toolbox of bioorthogonal nanomaterial templates. These are stable (i.e., can be stored for indefinitely long periods of time under appropriate conditions) and biocompatible nanomaterials that display interfacial bioorthogonal moieties that are ready to react with any molecular system of interest that carries the complementary functional group or chemical reporter. The bioorthogonal nanomaterial template allows for the facile and covalent modification of the nanomaterial’s surface with reactions that are chemoselective, biocompatible, fast, high yielding, and, most importantly, orthogonal to the surface chemistry of the nanomaterial.

In this study, we further expanded the members of this bioorthogonal nanomaterial toolbox and synthesized and characterized small (∼3 nm) AuNPs functionalized with interfacial nitrone functional groups (nitrone–AuNPs) for interfacial SPANC (I-SPANC) reactions. This new bioorthogonal AuNP template is extremely resilient because the gold core is protected by a monolayer of tri- and tetra-ethylene glycol-based thiolated ligands (see Scheme 1). This allows the nitrone–AuNPs to be stored indefinitely at −20 °C and be repeatedly dried and redissolved in polar organic solvents and water with little to no aggregation. The nitrone–AuNPs could be completely characterized, thanks to the discrete size of their thiolated ligands, and the number of interfacial nitrone moieties could be determined with good precision, including the determination of a nanoparticle’s raw formula. This allowed us to investigate in detail the interfacial reactivity of the nitrone–AuNP template toward the I-SPANC reaction using model molecules. Subsequently, we determine a facile protocol for the realization of [18F]-decorated AuNPs ([18F]-AuNPs) based on an I-SPANC reaction between the bioorthogonal nitrone–AuNP template and a radiolabeled prosthetic group carrying a bicyclononyne (BCN) moiety. The [18F]-AuNPs showed a wide biodistribution profile upon in vivo experiments. This is most likely because of the small dimension of the nanomaterial and the ethylene glycol-based coating, which increases the nanomaterial biocompatibility, hinders protein absorption, and increases blood circulation time.

Normally, gold nanomaterial-based radiolabeled probes are synthesized either by deposition of a radioactive element (e.g., 64Cu, 125I, and 198Au) onto the nanostructure or by conjugating chelating agents (e.g., DOTA) or prosthetic groups carrying the radioactive element onto the nanomaterial corona. However, the former methodology has the drawback that the size and shape of the nanomaterial substrate can change during the redox reaction, causing destabilization of the nanoparticle or irreversible aggregation. As a consequence, the precise characterization of both the metallic core and organic corona becomes extremely challenging or even impossible. The latter method instead relies on a “shotgun-type” conjugation approach, which has no control over the extent of incorporation of the desired radiolabeled prosthetic group or its quantification, representing a major issue for potential therapeutic applications.

In the past few years, the Workentin research group has begun to develop a toolbox of bioorthogonal nanomaterial templates. These are stable (i.e., can be stored for indefinitely long periods of time under appropriate conditions) and biocompatible nanomaterials that display interfacial bioorthogonal moieties that are ready to react with any molecular system of interest that carries the complementary functional group or chemical reporter. The bioorthogonal nanomaterial template allows for the facile and covalent modification of the nanomaterial’s surface with reactions that are chemoselective, biocompatible, fast, high yielding, and, most importantly, orthogonal to the surface chemistry of the nanomaterial.

In this study, we further expanded the members of this bioorthogonal nanomaterial toolbox and synthesized and characterized small (∼3 nm) AuNPs functionalized with interfacial nitrone functional groups (nitrone–AuNPs) for interfacial SPANC (I-SPANC) reactions. This new bioorthogonal AuNP template is extremely resilient because the gold core is protected by a monolayer of tri- and tetra-ethylene glycol-based thiolated ligands (see Scheme 1). This allows the nitrone–AuNPs to be stored indefinitely at −20 °C and be repeatedly dried and redissolved in polar organic solvents and water with little to no aggregation. The nitrone–AuNPs could be completely characterized, thanks to the discrete size of their thiolated ligands, and the number of interfacial nitrone moieties could be determined with good precision, including the determination of a nanoparticle’s raw formula. This allowed us to investigate in detail the interfacial reactivity of the nitrone–AuNP template toward the I-SPANC reaction using model molecules. Subsequently, we determine a facile protocol for the realization of [18F]-decorated AuNPs ([18F]-AuNPs) based on an I-SPANC reaction between the bioorthogonal nitrone–AuNP template and a radiolabeled prosthetic group carrying a bicyclononyne (BCN) moiety. The [18F]-AuNPs showed a wide biodistribution profile upon in vivo experiments. This is most likely because of the small dimension of the nanomaterial and the ethylene glycol-based coating, which increases the nanomaterial biocompatibility, hinders protein absorption, and increases blood circulation time.

In the past few years, the Workentin research group has begun to develop a toolbox of bioorthogonal nanomaterial templates. These are stable (i.e., can be stored for indefinitely long periods of time under appropriate conditions) and biocompatible nanomaterials that display interfacial bioorthogonal moieties that are ready to react with any molecular system of interest that carries the complementary functional group or chemical reporter. The bioorthogonal nanomaterial template allows for the facile and covalent modification of the nanomaterial’s surface with reactions that are chemoselective, biocompatible, fast, high yielding, and, most importantly, orthogonal to the surface chemistry of the nanomaterial.
Results and Discussion

The method for the introduction of the nitrone moiety onto the AuNP’s surface requires the synthesis of the appropriate thiol-containing ligand to be used for the ligand exchange reaction with a 3 nm triethylene glycol monomethyl ether AuNP (TEG−AuNP) starting material. The TEG−AuNPs represent an ideal substrate for the synthesis of a bioorthogonal nanomaterial template because of their approachable synthesis, the possibility to synthesize them in the gram scale, and the possibility of using spectroscopic techniques [e.g., nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS)] for characterizing them and following their interfacial reactivity. The TEG−AuNP template is amphiphilic and extremely resilient. In fact, TEG−AuNPs can be repeatedly dried and redissolved in water and different polar organic solvents, dissolved in strongly acidic or basic solution,54 heated at more than 100 °C for prolonged periods of time55 with little to no aggregation, and be exposed to glutathione-rich aqueous environments with minimal evidence of ligand exchange as per 1H NMR and fluorescence spectroscopy.54 The ethylene glycol framework has also the potential function of prolonging the AuNP’s circulation half-life, and reducing the AuNP’s immunogenicity, making the nanosystem suitable for many applications in both chemical biology and nanomedicine.56–58

The desired nitrone-terminated thiol ligand 4 was designed with a tetracethylene glycol unit that separates the thiol head from the nitrone moiety (see Scheme 1). The ethylene glycol linker preserves the physical−chemical and biological properties of the AuNPs, ensures good packing of the monolayer around the gold core, and, because of the extra ethylene glycol unit, it allows the nitrone group to extend out of the corona and be more accessible to react through the I-SPANC. It is worth noting that the complementary system in which AuNPs are functionalized with strained alkynes capable of reacting with [18F]-bearing nitriles is also possible. However, this presents additional synthetic challenges relating to undesired side reactivity of the strained C=C triple bond toward the nucelophilic −SH. More importantly, we wished to preserve the generality of the [18F]-bearing prosthetic group, which can react broadly with azides and nitriles. Because of the chemical stability and ease-of-introduction of azide and nitrone groups onto diverse materials, we envisioned this represents a more general protocol for the preparation of functional PET probes.

The synthetic strategy for the synthesis of the nitrone-terminated thiol ligand 4 is reported in Scheme 1. Briefly, tetracethylene glycol-monosulfonate HO−EG4−OTos was treated with triphenylmethanethiol to prepare the protected thiol HO−EG4−SCPh3 1. The alcohol moieties of compound 1 were then oxidized to aldehyde using dimethyl sulfoxide (DMSO) and phosphorus pentaoxide. Aldehyde 2 was reacted with N-methyl hydroxylamine hydrochloride in the presence of a base to afford nitrone 3. Finally, the desired nitrone-terminated thiol ligand 4 was synthesized by deprotecting nitrone 3 in CH2Cl2/5% trifluoroacetic acid (TFA). Details of the synthetic procedure can be found in the Supporting Information. Subsequently, a ligand exchange reaction was used to incorporate the nitrone-terminated thiol 4 onto the TEG−AuNPs (see Scheme 1). The ligand exchange reaction was carried out by stirring a solution of TEG−AuNPs and ligand 4 (1:6 AuNPs to ligand 4 ratio) in CH2Cl2 for 30 min. After removing the solvent, the resulting film of AuNPs was washed with a mixture of 10:1 hexanes/isopropanol five times in order to remove any free thiol or disulfide. Finally, the nitrone−AuNPs were further purified by dialysis in Milli-Q water overnight. The resulting nitrone−AuNPs retained the
solubility properties of the TEG–AuNP starting material, being soluble in water and polar organic solvents.

Characterization of the nitrone–AuNPs was achieved using 1H NMR spectroscopy, transmission electron microscopy (TEM), thermogravimetric analysis (TGA), and XPS. Initially, the success of the exchange reaction was confirmed using 1H NMR spectroscopy, see Figure 1. The 1H NMR spectrum of the nitrone–AuNPs recorded in D2O exhibited the expected broad peaks at δH: 3.3–3.8 ppm because of the protons of the ethylene glycol units. Additionally, new signals appeared after the exchange reaction at δH: 7.4 and 4.4 ppm in a 1:2 ratio and corresponded to the proton α to the nitrogen (Hα) and the two protons of the methylene group next to the nitrone functionality (Hβ), respectively, confirming the presence of the nitrone moiety on the AuNPs’ corona. The lack of sharp signals in the 1H NMR spectrum of the nitrone–AuNPs indicates that there was no free thiol present and confirmed the efficiency of our cleaning protocol. Furthermore, the nitrone–AuNP sample was monitored over an extend period of time by 1H NMR spectroscopy and there was no evidence of free thiols, indicating very good stability of the nanoparticle interface can be easily tuned through the ligand硫化物 to nitrogen ratio it was possible to calculate that 20% of the total number of thiols, indicating very good stability of the nanoparticle.

Further information about the quantity and ratio of the thiolated ligands that compose the organic corona could be obtained from the thermogravimetric curve. The TGA showed that 33% of the mass of a nitrone–AuNP is composed by the organic corona, see Figure 1. The first derivative of the thermogravimetric curve (see Figure S2) showed two distinct components that were related to the two thiolated ligands: the nitrone-terminated ligand 4, whereas 82 ± 2% consisted of the base MeO–EG₃–S⁻ ligand.

Further information about the quantity and ratio of the thiolated ligands that compose the organic corona could be obtained from the thermogravimetric curve. The TGA showed that 33% of the mass of a nitrone–AuNP is composed by the organic corona, see Figure 1. The first derivative of the thermogravimetric curve (see Figure S2) showed two distinct components that were related to the two thiolated ligands: the nitrone-terminated ligand 4, whereas 82 ± 2% consisted of the base MeO–EG₃–S⁻ ligand.

By knowing the molecular weight of the two ligands, it was possible to calculate that 20% of the total number of ligands were nitrone–EG₃–S⁻ and 80% were MeO–EG₃–S⁻ ligands by mass. This was consistent with the analysis of the 1H NMR spectrum of the nitrone–AuNP.

XPS analysis further confirmed the successful preparation of nitrone–AuNPs and the ratio between the two different thiolated ligands (see Figures 1 and S3). The Au 4f region exhibited a pair of peaks at 84.3 and 87.6 eV assigned to the Au 4f₇/₂ and Au 4f₅/₂ peaks, respectively, which were originated by the AuNP cores and were shifted at higher binding energy (BE) because of the nanoparticle size effect.68 The S 2p core line showed the presence of two components, the S 2p₁/₂ at 162.8 eV and S 2p₃/₂ at 164.0 eV, in a 2:1 spin orbit splitting ratio related to the Au–S bonds.69 The absence of additional components in the region 163–165 and 168–170 eV confirmed that there are no free thiols and that our cleaning procedure was effective. Additionally, the XPS survey spectrum of nitrone–AuNPs clearly showed the appearance of a nitrogen peak at 399.9 eV (N 1s) with 0.6 at. %, confirming the incorporation of the nitrone group. Furthermore, from the sulphur to nitrogen ratio it was possible to calculate that 20% of the total ligands on the gold core consisted of nitrone–EG₃−SH, and this information was in line with the results obtained independently through 1H NMR spectroscopy and TGA.

Finally, TEM images of nitrone–AuNPs showed that their gold core is 2.6 ± 0.5 nm wide (Figure 1). By combining the information obtained through 1H NMR spectroscopy, TEM imaging, and TGA, and assuming that the AuNPs have a spherical shape and are perfectly monodispersed in size, it was possible to calculate a raw formula for the nitrone–AuNPs of Au₅₀₀(MeO–EG₃–S⁻)₂₀₀(nitrone–EG₃–S⁻)₄₆.6 Details of the calculations can be found in the Supporting Information.

It is worth noting that the amount of nitrone moieties at the AuNP’s interface can be easily tuned through the ligand exchange by changing the reaction time and the gold to ligand 4 ratio (see Figure S1). However, it was found that as we increased the concentration of nitrone ligand 4 in excess of 30% (by mole relative to MeO–EG₃–S⁻ in the monolayer), the resulting AuNPs were exclusively soluble in water. Although this increased hydrophilicity would be desirable for in vivo applications, because of hydrophobicity of the [¹⁹F]BCN prosthetic group, the resulting isoxazoline—formed by
experiments, we performed a proof of concept study using this and before undertaking any radiolabeled-AuNP in vivo temperature. Despite bearing the ethylene glycol linker in metric information of nitrone compound 13 strained alkyne reactivity test, we were able to employ a 1:1 equivalent of order to increase its water solubility. The detailed synthesis the strained alkyne moiety (a BCN) and the 13 was designed with a tetraethylene glycol linker in between interfacial SPANC—may display weaker water solubility. Therefore, it is of paramount importance that the nitrone–AuNPs retain their amphiphilicity in order to be used as phase-transfer agents, and covalently bind those prosthetic groups in organic solvent and carry them in water media where they can be used for their designed application.

After confirming successful incorporation of nitrone moieties, we sought to evaluate the reactivity of nitrone–AuNPs toward interfacial SPANC (I-SPANC). Because of the clean and quantitative reactivity of SPANC,\textsuperscript{23,24} the stoichiometric information of nitrone–AuNPs can be extended to establish the concentration of interfacial [19F]. In order to do this and before undertaking any radiolabeled-AuNP in vivo experiments, we performed a proof of concept study using the fluorinated (19F) strained alkyne 13 (Scheme 2). Compound 13 was designed with a tetraethylene glycol linker in between the strained alkyne moiety (a BCN) and the fluorine atom in order to increase its water solubility. The detailed synthesis and characterization of compound 13 is reported in the Supporting Information. Despite the high reactivity of BCN, compound 13 is stable for months and can be stored indefinitely at −20 °C.

Because we quantified with very good precision the amount of nitrone moieties per AuNP (0.840 μmol mg\textsuperscript{−1}), for our reactivity test, we were able to employ a 1:1 equivalent of strained alkyne 13 to interfacial nitrone. This is remarkable because it gives us unprecedented molecular level control over the synthesis of conjugated and labeled AuNPs, which will assist in the future generation of targeted agents while still controlling available nitrone sites for late-stage radiochemistry. For future investigations, this approach would also allow for the facile introduction of multiple molecular systems of interest simply by altering the molar ratio of complementary substrates. In a typical conjugation experiment, we mixed 10 mg of nitrone–AuNPs (8.4 μmol of nitrone) with 8.4 μmol of compound 13 in a mixture CH\textsubscript{3}CN/H\textsubscript{2}O 3:1 and at room temperature. Despite bearing the ethylene glycol linker in between the fluorine atom and the BCN moiety, compound 13 still required an organic solvent to be dissolved. In order to facilitate the characterization of the interfacial cycloaddition product and obtain proof of proper interfacial reactivity, we set up in parallel a control reaction using the model compound nitrone 7. The 1H NMR spectrum of the control reaction showed complete conversion to the cycloaddition product as demonstrated by the disappearance of the peaks of the starting materials, and the appearance of the NH peak at 5.2 ppm (H\textsubscript{e}), of a doublet of triplets at 4.6 ppm belonging to CH\textsubscript{3} alpha to the [19F] (H\textsubscript{f}), of the BCN’s CH\textsubscript{2} alpha to the carbamate moiety at 4.2 ppm (H\textsubscript{g}), of isoxazole’s H\textsubscript{c} at 2.7 ppm, and of BCN’s multiplets in the 0.5–2.5 ppm region. The product of the control SPANC reaction was characterized also by 19F NMR spectroscopy, which shows a fluorine peak at −223.4 ppm, and by 13C NMR spectroscopy and mass spectrometry. All the analyses confirmed a successful SPANC reaction that proceeded cleanly and quantitatively with no generation of byproducts. Proof for a successful I-SPANC reaction at the AuNP’s interface could then be obtained by comparing the 1H NMR spectrum of the control reaction product with that of the [19F]AuNPs. Figure 2 shows excellent correspondence between the typical broad peaks of the AuNP sample with those of the cycloaddition product 14. Furthermore, the complete disappearance of the nitrone protons H\textsubscript{a} and H\textsubscript{b} of the [19F] (H\textsubscript{f}), of the control reaction product with that of the [19F]AuNPs could be easily recorded in D\textsubscript{2}O, highlighting the role of the nanocarrier as a phase-transfer agent.

**Figure 2.** Characterization of [19F]AuNPs. (A) 1H and 19F NMR spectra recorded in D\textsubscript{2}O and CDCl\textsubscript{3} for [19F]AuNPs (top) and model compound 14 (bottom) and referenced against residual solvents (*); (B) typical TEM image of [19F]AuNPs (scale bar 20 nm); (C) high-resolution XPS scan of C 1s and F 1s peaks for [19F]AuNPs.
After the I-SPANC reaction was completed and before further characterization, in order to ensure complete removal of any residual free prosthetic group 13, the \(^{[19F]}\)AuNPs were purified by washing the nanoparticles with a 10:1 hexanes/isopropanol mixture and by dialysis in water overnight. The purified \(^{[19F]}\)AuNPs were then characterized by XPS and TEM. The XPS data furnished additional proof of successful I-SPANC reaction (see Figure 2). The XPS survey of \(^{[19F]}\)AuNPs shows the appearance of a peak related to the fluorine at 686.7 eV, and the high-resolution scan of the C 1s peak exhibits the appearance of a component at 288.0 and 289.2 eV related to O–C\(^\#\)=O (carbamate group) and O–C–F, respectively. The presence of the carbamate group is also confirmed by the appearance of a shoulder at 533.6 eV in the high-resolution scan of the O 1s peak. It should be noted that a new component for the C–N bond of the carbamate group should have appeared in the high-resolution scan of C 1s; however, such a component was not observed because it falls at the same BE region of the C–OH/O–C–O groups. Nonetheless, the presence of such a group is clearly shown in the high-resolution N 1s scan with a BE of ~400 eV. Finally, TEM images (see Figure 2) show no change in size, shape, or size distribution for the \(^{[19F]}\)AuNPs compared to the nitrene–AuNPs starting material. This is because of the mild reaction condition required by the I-SPANC reaction. Together, these results confirmed unequivocally that the nitrene–AuNPs were able to react with the fluorinated compound 13 through the I-SPANC to give the corresponding cycloaddition product rapidly, cleanly, and quantitatively. Because the nitrene–AuNPs reacted quantitatively, we established that each AuNP is now carrying on average 40 \(^{[19F]}\) prosthetic groups, or ∼0.84 \(\mu\)mol of compound 13 per milligram of AuNPs. This information is of great importance for the use of the final \(^{[19F]}\)AuNP as a PET contrast agent and the development of targeted agents. Most importantly, no loss of thiolated ligands was observed by 1H NMR spectroscopy (which would show appearance of sharp signals) over a period of 3 days at room temperature, indicating good stability of the nanomaterial and potential for in vivo studies.

Once we confirmed our ability to modify and quantify AuNPs with \(^{19F}\) as a model via I-SPANC, we proceeded with the synthesis of the \(^{[18F]}\) analogue of prosthetic group 13, its conjugation onto the nitrene–AuNPs, and the in vivo biodistribution investigation through PET imaging. The synthesis of \(^{[18F]}\)AuNPs is reported in Scheme 3, and it is based on the synthetic strategy for compound 13 with minor changes in order to satisfy the radiolabeling working conditions. Most importantly, from the step that introduces the fluorine atom on compound 9, all the subsequent reactions leading to the \(^{[18F]}\)AuNPs PET agent rely on excellent reaction yields and very fast reaction kinetics to minimize decay of \(^{18F}\) prior to injection. Finally, the \(^{[18F]}\)AuNPs were purified by size exclusion chromatography in order to remove any unreacted prosthetic group. The entire synthesis of the \(^{[18F]}\)AuNPs, from \(^{18F}\) delivery until end-of-synthesis, was completed in 4 h and with specific activity of 52 MBq mg\(^{-1}\) of AuNPs. Subsequently, we proceeded with the PET imaging study. The representative PET image and the region of interest (ROI) analysis reported in Figure 3 show that the \(^{[18F]}\)AuNPs displayed a broad biodistribution profile and collected mainly in the liver, lungs, heart, kidneys, and bladder. This was expected because of the small size of the nanoparticles (~3 nm) and their ethylene glycol coating that prolongs their circulation half-life.53,63 However, there are certain aspects that make these \(^{[18F]}\)AuNPs unique. More specifically, the ROI analysis confirms that the highest uptake was recorded in the liver and in the bladder.

Whereas the liver showed a high and instantaneous accumulation of AuNPs presumably because of accumulation via the reticuloendothelial system, the bladder displayed a slower and more gradual accumulation over the first 50 min after intravenous injection. This is unusual for particles of this size, which are normally excreted very quickly through the renal pathway, displaying short blood circulation half-life.53,63 The high accumulation in the lungs suggests that the nanoparticles have been trapped in the vascular bed of the lungs, which is also unusual for 3 nm AuNPs. Taken together, the biodistribution observations suggest that these 3 nm AuNPs behave in a balanced manner, with renal excretion occurring concomitantly with retention in the liver and lungs. It is possible that a level of aggregation is occurring in vivo, resulting in the unexpected liver and lung retention, as this is typically seen only for AuNPs of >5.5 nm hydrodynamic environment. Whereas a recent report indicates limited brain uptake for small AuNPs (0.07–0.13% ID/g),64 brain uptake does not appear to be significant in this instance for the

Scheme 3. Synthesis of \(^{[18F]}\)AuNPs\(^{44}\)

\[ \text{Nitrene-AuNP} \]

\[ \text{Phosphate buffer} 0.1 \text{M pH} 7.0 \]

\[ \text{r.t.}, 20 \text{ min} \]

\[ \text{[F]AuNP} \]

\[ \text{Reaction conditions: (a) } ^{18F}, \text{ K}_2\text{CO}_3, \text{ Krypobox}222, \text{ CH}_3\text{CN}, 90 \text{ °C}, 5 \text{ min}; (b) hydrazine, CH}_3\text{CN/36\% H}_2\text{O/0.1\% TFA, 60 °C, 5 min; (c) Compound 12, Et}_3\text{N, CH}_3\text{CN, room temperature, 20 min; followed by 60 °C, 5 min.} \]
incorporating a strained alkyne. The I-SPANC reaction was used to nanoparticle label AuNPs by reacting the nitrone groups present on the surface with a radiolabeled prosthetic group to prepare a radiolabeled probe. We prepared 18F-SPANC reaction and bioorthogonal nanomaterial templates with the possibility of making it water-soluble, amphiphilic, or organic solvent-soluble, depending on the desired application. Changing the amount of interfacial nitrone groups has an impact on the solubility properties of the final nanoparticle, and its ability to broadly react with azido and nitrone functionalities on other materials or biomolecules opens unprecedented opportunities for the preparation of more diverse PET imaging tools via simple “pour and mix” chemistry.

**CONCLUSIONS**

In summary, we reported a new methodology for the synthesis of nanomaterial-based PET contrast agents that is based on the use of bioorthogonal nanomaterial templates. We synthesized a new bioorthogonal AuNP template based on small (~3 nm) amphiphilic AuNPs with interfacial nitrone functionalities. The nitrene–AuNPs were fully characterized using a variety of techniques including 1H NMR spectroscopy, TGA, TEM, and XPS, and the amount of interfacial nitrone moieties was calculated with good precision using independent methods. Furthermore, the amount of interfacial nitrone moieties can be carefully tuned through the ligand exchange reaction and their number determined through the methodology reported here. Changing the amount of interfacial nitrone groups has an impact on the solubility properties of the final nanoparticle, with the possibility of making it water-soluble, amphiphilic, or organic solvent-soluble, depending on the desired application.

For the first time, we then showcased the utility of the I-SPANC reaction and bioorthogonal nanomaterial templates for the preparation of a radiolabeled probe. We prepared 18F-labeled AuNPs by reacting the nitrone groups present on the nanoparticle’s surface with a radiolabeled prosthetic group incorporating a strained alkyne. The I-SPANC reaction was quantitative when using the 19F-surrogate and the prosthetic group was covalently bound to the nanocarrier. Most importantly, the final 18F-AuNPs retained their water-solubility despite the lipophilicity of the prosthetic group, thanks to the ability of the nitrone–AuNP template to act as a phase-transfer agent. The resulting PET imaging agent was then tested in vivo, which displayed broad biodistribution, and relatively low uptake in background organs of the kidney, lung, and heart.

Importantly, through employing an AuNP nanocarrier, one can overcome the limitations of injecting “free” radiolabeled agents directly, which are often disadvantaged because of nonspecific biodistribution, and limited tunability of their structural and chemical properties. Instead, nitrene–AuNPs represent a versatile tool on which simultaneous and multivalent attachment of radiolabeled prosthetic groups, biomolecules, and drugs can be achieved with molecular level control. This allows for in vivo targeting or labeling of chemical reporters because of the bioorthogonal nature of I-SPANC. Furthermore, although 18F-labeled AuNPs are reported here, the generality of the [18F]-BCN prosthetic group and its ability to broadly react with azido and nitrone functionalities on other materials or biomolecules opens unprecedented opportunities for the preparation of more diverse PET imaging tools via simple “pour and mix” chemistry.

**MATERIALS AND METHODS**

The following reagents were used as received. Triethylene glycol monomethyl ether (MeO-EG1-OMe), tetraethylene glycol (HO-EG4-OMe), 4-dimethyaminopyridine (DMAP), potassium thioacetate, deuterated acetonitrile (CD3CN), deuterated chloroform (CDCl3), phosphorus pentoxide (P2O5), tetrachloroauric acid trihydrate (HAuCl4·3H2O), sodium borohydride (NaBH4), p-toluenesulfonyl chloride (TosCl), N-methylhydroxylamine hydrochloride (H2N(CH2)4·HCl), sodium azide (Na3N), triphenylmethane-thiol (HSCPh3), triisopropylsilane (TIPS), N,N-Diisopropylethylamine (DIPEA), phthalimide potassium, sodium iodide (NaI), hydrazine monohydrate (H2N-H2O), cesium fluoride (CsF), and O-(benzotriazole-1-y1)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Sigma-Aldrich. All common solvents, triethylenetriamine (Et3N), sodium sulfate anhydrous (Na2SO4), dry methanol (CH3OH), tert-butanol (tBuOH), hydrochloric acid (HCl), TFA, sodium hydroxide (NaOH), sodium bicarbonate (NaHCO3) and potassium carbonate (K2CO3) were purchased from Caledon. Deuterated water (D2O) was purchased from Cambridge Isotope Laboratories. Ethanol (EtOH) was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) was purchased from BDH. Dialysis membranes (MWCO 6000–8000) were purchased from Spectra/Por.

18F-Fluoride was obtained from the Nordal Cyclotron & PET Radiochemistry Facility at the Lawson Health Research Institute, London, Canada. An automated synthesis unit (TRACERlab, GE Healthcare, Schenectady, NY) was used to prepare and purify [18F] SA-64. A V-10 evaporator (Biotage, Charlotte, NC) was used to remove the solvent after high-performance liquid chromatography (HPLC) purification. A PD-10 desalting column was purchased from GE Healthcare. HPLC analysis and purification was performed on a Waters HPLC system (Milford, MA) using a dual detector system (UV and radiometric), with the mobile phase being CH3CN.
Spectra were carried out using KBr pellets on a Bruker VECTOR33 spectrometer. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02322.

Details of synthetic procedures and characterization data of compounds and details of the AuNP raw formula calculation (PDF)

**REFERENCES**

1. Phelps, M. E. Positron emission tomography provides molecular imaging of biological processes. *Proc. Natl. Acad. Sci. U.S.A.* 2000, 97, 9226–9233.

2. Massoud, T. F.; Gambhir, S. S. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. *Genes Dev.* 2003, 17, 545–580.

3. Paus, A. Positron emission tomography: the conceptual idea using a multidisciplinary approach. *Methods* 2002, 27, 195.

4. McCarthy, T. J.; Schwarz, S. W.; Welch, M. J. Nuclear medicine and positron emission tomography: an overview. *J. Chem. Educ.* 1994, 71, 830–836.

5. Czernin, J.; Phelps, M. E. Positron emission tomography scanning: Current and future applications. *Annu. Rev. Med.* 2002, 53, 89–112.

6. Fowler, J. S.; Wolf, A. P. Working against time: Rapid radiotracer synthesis and imaging the human brain. *Acc. Chem. Res.* 1997, 30, 181–188.

7. Kettenbach, K.; Schieferstein, H.; Ross, T. L. 18F-labeling using click cycloadditions. *BioMed Res. Int.* 2014, 2014, 361329.

8. Bouvet, V.; Wuest, M.; Wuest, F. Copper-free click chemistry with the short-lived positron emitter fluorine-18. *Org. Biomol. Chem.* 2011, 9, 7393–7399.

9. Campbell-Verduyn, L. S.; Mirfeizi, L.; Schoonen, A. K.; Dierckx, R. A.; Elsinga, P. H.; Feringa, B. L. Strain-promoted copper-free “click” chemistry for 18F radiolabeling of bombesin. *Org. Biomol. Chem., Int. Ed. Engl.* 2011, 50, 11117–11120.

10. Arumugam, S.; Chin, J.; Schirmacher, R.; Popik, V. V.; Kostikov, A. P. ([F]Azadibenzocyclooctyne ([F]ADIBO): A biocompatible radioactive labeling synthon for peptides using catalyst free [3+2] cycloaddition. *Bioorg. Med. Chem. Lett.* 2011, 21, 6987–6991.
(11) Boudjemelaine, M.; McNitt, C. D.; Singleton, T. A.; Popik, V. J.; Kostikov, A. P. [18F]ODIOB: a prosthetic group for bioorthogonal radiolabeling of macromolecules via strain-promoted alkyne–azide cycloaddition. Org. Biomol. Chem. 2018, 16, 363–366.

(12) Murrell, E.; Kovacs, M. S.; Luty, L. G. A Compact and Synthetically Accessible Fluorine-18 Labelled Cyclooctyne Prosthetic Group for Labelling of Biomolecules by Copper-Free Click Chemistry. ChemMedChem 2018, 13, 1625–1628.

(13) Carpenter, R. D.; Hausner, S. H.; Sutcliffe, J. L. Copper-Free Click for PET Rapid 1,3-Dipolar Cycloadditions with a Fluorine-18 Cyclooctyne. ACS Med. Chem. Lett. 2011, 2, 885–889.

(14) Hausner, S. H.; Carpenter, R. D.; Bauer, N.; Sutcliffe, J. L. Evaluation of an integrin αvβ3-specific peptide labeled with [18F] fluorne by copper-free, strain-promoted click chemistry. Nucl. Med. Biol. 2013, 40, 233–239.

(15) Zhou, Z.; Chitneni, S. K.; Devoogdt, N.; Zalutsky, M. R.; Vaidyanathan, G. Fluorine-18 labeling of an anti-HER2 VHH using a residualizing prosthetic group via a strain-promoted click reaction Chemistry and preliminary evaluation. Bioorg. Med. Chem. 2018, 26, 1939–1949.

(16) Evans, H. L.; Slade, R. L.; Carroll, L.; Smith, G.; Nguyen, Q.-D.; Idon, H.; Kamaly, N.; Stöckmann, H.; Leeper, F. P.; Abouegy, E. O.; Spivey, A. C. Copper-free click—a promising tool for pre-targeted PET imaging. Chem. Commun. 2012, 48, 991–993.

(17) Sachin, K.; Jadhav, V. H.; Kim, E.-M.; Kim, H. L.; Lee, S. B.; Jeong, H.-J.; Lim, S. T.; Sohn, M.-H.; Kim, D. W. F-18 Labeling Protocol of Peptides Based on Chemically Orthogonal Strain-Promoted Cycloaddition under Physiologically Friendly Reaction Conditions. Bioconjugate Chem. 2012, 23, 1680–1686.

(18) Kim, H. L.; Sachin, K.; Jeong, H. J.; Choi, W.; Lee, H. S.; Kim, D. W. F-18 Labeled RGD Probes Based on Bioorthogonal Strain-Promoted Click Reaction for PET Imaging. ACS Med. Chem. Lett. 2015, 6, 402–407.

(19) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. A strain-promoted [3 + 2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems. J. Am. Chem. Soc. 2004, 126, 15046–15047.

(20) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. Visualizing metabolically labeled glycoconjugates of living cells by copper-free and fast huisgen cycloadditions. Angew. Chem., Int. Ed. Engl. 2008, 47, 2253–2255.

(21) Dommerholt, J.; Schmidt, S.; Temmng, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedel, P.; van Delft, F. L. Readily accessible bicyclonynes for bioorthogonal reactions of nitrile oxide cycloadditions. Chem. Commun. 2012, 58, 7134–7136.

(22) McKay, C. S.; Moran, J.; Pezacki, J. P. Nitrones as dipoles for rapid strain-promoted 1,3-dipolar cycloadditions with cycooctynes. Chem. Commun. 2010, 46, 931–933.

(23) McKay, C. S.; Blake, J. A.; Cheng, J.; Danielson, D. C.; Pezacki, J. P. Strain-promoted cycloadditions of cyclic nitriles with cyclooctynes for labeling human cancer cells. Chem. Commun. 2011, 47, 10040–10042.

(24) Ning, X.; Temmng, R. P.; Dommerholt, J.; Guo, J.; Ania, D. B.; Debets, M. F.; Wolfert, M. A.; Boons, G.-J.; van Delft, F. L. Protein modification by strain-promoted alkynyl-nitrone cycloaddition. Angew. Chem., Int. Ed. Engl. 2010, 49, 3065–3068.

(25) Temmng, R. P.; Haasner, S.; van Eldikj, M. B.; van Hest, J. C. M.; van Delft, F. L. N-Terminal dual protein functionalization by strain-promoted alkynyl-nitrone cycloaddition. Org. Biomol. Chem. 2013, 11, 2772–2779.

(26) Ledin, P. A.; Kolishetti, N.; Boons, G.-J. Multi-Functionalization of Polymers by Strain-Promoted Cycloadditions. Macromolecules 2013, 46, 7759–7768.
interfacial Staudinger-Bertozzi ligation. *Org. Biomol. Chem.* 2015, 13, 4605-4612.

(47) Gobbo, P.; Novoa, S.; Biesinger, M. C.; Workentin, M. S. Interfacial strain-promoted alkyne-azide cycloaddition (I-SPAAC) for the synthesis of nanomaterial hybrids. *Chem. Commun.* 2013, 49, 3982-3984.

(48) Gobbo, P.; Mossman, Z.; Nazemi, A.; Niaux, A.; Biesinger, M. C.; Gillies, E. R.; Workentin, M. S. Versatile strained alkyne modified water-soluble AuNPs for interfacial strain promoted azide-alkyne cycloaddition (I-SPAAC). *J. Mat. Chem. B* 2014, 2, 1764-1769.

(49) Wang, X.; Gobbo, P.; Suchy, M.; Workentin, M. S.; Hudson, R. H. E. Peptide-decorated gold nanoparticles via strain-promoted azide-alkyne cycloaddition and post assembly deprotection. *RSC Adv.* 2014, 4, 43087-43091.

(50) Luo, W.; Gobbo, P.; Gunawardene, P. N.; Workentin, M. S. Fluorogenic Gold Nanoparticle (AuNP) Substrate: A Model for the Controlled Release of Molecules from AuNP Nanocarriers via Interfacial Staudinger-Bertozzi Ligation. *Langmuir* 2017, 33, 1908-1913.

(51) Luo, W.; Gobbo, P.; McNitt, C. D.; Sutton, D. A.; Popik, V. V.; Workentin, M. S. “Shine & Click” Photo-Induced Interfacial Unmasking of Strained Alkynes on Small Water-Soluble Gold Nanoparticles. *Chem.—Eur. J.* 2017, 23, 1052-1059.

(52) Luo, W.; Luo, J.; Popik, V. V.; Workentin, M. S. Dual-Bioorthogonal Molecular Tool: “Click-to-Release” and “Double-Click” Reactivity on Small Molecules and Material Surfaces. *Bioconjugate Chem.* 2019, 30, 1140-1149.

(53) Alkilany, A. M.; Murphy, C. J. Toxicity and cellular uptake of gold nanoparticles: what we have learned so far? *J. Nanopart. Res.* 2010, 12, 2313-2333.

(54) Weissman, M. R.; Winger, K. T.; Ghiassian, S.; Gobbo, P.; Workentin, M. S. Insights on the Application of the Retro-Michael Addition on Maleimide-Functionalized Gold Nanoparticles in Biology and Nanomedicine. *Bioconjugate Chem.* 2016, 27, 586-593.

(55) Gobbo, P.; Workentin, M. S. Improved methodology for the preparation of water-soluble maleimide-functionalized gold nanoparticles. *Langmuir* 2012, 28, 12357-12363.

(56) Lu, J.; Shi, M.; Shoichet, M. S. Click Chemistry Functionalized Polymeric Nanoparticles Target Corneal Epithelial Cells through RGD-Cell Surface Receptors. *Bioconjugate Chem.* 2009, 20, 87-94.

(57) Taylor, A.; Wilson, K. M.; Murray, C. J. Toxicity and cellular uptake of gold nanoparticles: what we have learned so far? *Chem. Soc. Rev.* 2012, 41, 2707-2717.

(58) Castner, D. G.; Hinds, K.; Grainger, D. W. X-ray photoelectron spectroscopy sulfur 2p study of organic thiol and disulfide binding interactions with gold surfaces. *Langmuir* 1996, 12, 5083-5086.

(59) Milne, M.; Gobbo, P.; McVicar, N.; Bartha, R.; Workentin, M. S.; Hudson, R. H. E. Water-soluble gold nanoparticles (AuNP) functionalized with a gadolinium(III) chelate via Michael addition for use as a MRI contrast agent. *Chem.—Eur. J.* 2013, 19, 5937-5939.

(60) Zhu, J.; Chin, J.; Wang, C.; Wängler, B.; Lennox, R. B.; Schirrmacher, R. Rapid (18)F-labeling and loading of PEGylated gold nanoparticles for in vivo applications. *Bioconjugate Chem.* 2014, 25, 1143-1150.