Cite this: *Med. Chem. Commun.*,
2018, 9, 1577

Received 21st May 2018,
Accepted 17th August 2018

DOI: 10.1039/c8md00263k

rsc.li/medchemcomm

Porphyrins as ligands for ^{64}Cu copper: background and trends

Edgar Aguilar-Ortíz,^{*a} Amir R. Jalilian^b and Miguel A. Ávila-Rodríguez ^{*a}

Porphyrins and ^{64}Cu have emerged as a novel synergic option for applications in PET molecular imaging. Both the characteristics and photophysical properties of macrocyclic porphyrins and the relatively long half-life of the copper isotope, in addition to the increased tumor-specific uptake of porphyrins compared to normal cells, make this complex an attractive option not only for diagnosis but also for therapeutic applications. Herein, we present an overview of the latest results on the development of PET agents based on porphyrins and ^{64}Cu , including methods used to improve the selectivity of these macrocycles when conjugated with biological units such as monoclonal antibodies, peptides or proteins.

1. Introduction

The use of porphyrins in medical applications has captured the attention of the scientific community since they have shown great advantages over other common methods used in

the diagnosis and treatment of diseases. It is well known that typical cancer therapies (chemotherapy, surgery and radiation therapy) could generate extensive damage to patients. The way in which porphyrins act on biological tissues relies on singlet oxygen generation, which is the main key in the cytotoxic role of these agents. In the diagnosis field, these macrocyclic compounds could be a great choice due to their ability to form stable complexes with several metallic species including copper, gallium, zinc, technetium, and palladium, among other radionuclides that are used in nuclear medicine.^{1–3} The advantage of using porphyrins is not just because of the

^a Unidad Radiofarmacia-Ciclotrón, División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510 Cd. Mx., Mexico.
E-mail: edgaraguilar08@gmail.com, avilarod@uwalumni.com

^b Department of Nuclear Sciences and Applications, International Atomic Energy Agency (IAEA), Vienna, Austria

Edgar Aguilar-Ortíz

Dr. Edgar A. Aguilar-Ortiz is a postdoctoral research fellow at the National Autonomous University of Mexico (UNAM) in the Faculty of Medicine. He received his BS in Chemistry from the Faculty of Chemistry and his MSc and PhD degrees from the Material Research Institute (UNAM). During his doctoral studies, he was a visiting scholar at the University of Montreal in the Polymers Department (2014–2015). His areas of interest include the study of optical properties of porphyrins for photodynamic therapy, synthesis of macrocyclic compounds for biological applications, radiolabeling of organic macrocycles using metallic radionuclides, and functionalization of organic compounds with biological moieties using click chemistry techniques.

Amir R. Jalilian

Prof. Amir R. Jalilian is a Technical Officer at the Radioisotope Products and Radiation Technology Section at the International Atomic Energy Agency (IAEA). He performed his Pharm Doctoral thesis under the supervision of Prof. Abbas Shafiee in 1995. After his graduation, he obtained a PhD degree in Radiopharmacy in 2000. He continued his profession at the University of Alberta, Canada and Yale School of Medicine, USA, as a post-doctoral fellow. Prof. Jalilian's research interests cover applied targeted tumor diagnosis and therapy using radiolabeled compounds. During the last 18 years, he has been active in the development of radiopharmaceuticals in developing countries leading to completion of clinical trials and the marketing of some radiopharmaceuticals in some countries.

complex's stability, but also due to its easy modification, either before or after the macrocycle formation, by adding biological moieties or other species of pharmacological interest.⁴

Another interesting point on the use of porphyrins as probes relies on the photophysical properties of these macrocycles to generate, as mentioned before, singlet oxygen. These species have found applications in multiple fields including organic synthesis,⁵ wastewater treatment,^{6,7} sterilization processes,⁸ and medicine, especially in photodynamic therapy (PDT).^{9,10} The use of photosensitized dyes like porphyrins has captured the attention of the scientific community mainly due to their minimum secondary effects compared with conventional therapies.⁹

2. Porphyrins

2.1 Synthetic methods

The word porphyrin is derived from the Greek word *porphyria*, meaning “purple”, as these systems exhibit intense absorption in the UV and visible (UV-vis) region given their highly conjugated structures. Porphyrins are macrocyclic systems made by condensation of the pyrrole ring and aldehyde units conjugated by methane bridges; as a result, the final structure of porphyrin systems bears a highly conjugated structure.

The first experiments for porphyrin synthesis were conducted by Rothmund,^{11,12} who in the 1930s reacted pyrrole and formaldehyde under reflux at 90–95 °C in an inert atmosphere for thirty hours. Then the mixture was cooled and extracted with pyridine and, after the purification process, the yield was 1 mg of porphyrin for each pyrrole gram. The final product was soluble in pyridine and chloroform and slightly soluble in ether and methanol. The extreme synthesis conditions make this method unfavorable for employing sensitive aldehydes, and given its poor yield, industrial applications of this methodology are not economically viable.

Miguel A. Ávila-Rodríguez is a tenured Associate Professor and Head of the Cyclotron and Radiopharmaceutical Labs at the National Autonomous University of Mexico (UNAM). He received his PhD degree in Medical Physics from the University of Wisconsin-Madison (USA). After that, he was a postdoctoral researcher fellow at the University of Alberta in the Edmonton PET Centre (Canada), and at the University of Turku in the Turku PET Centre (Finland). His area of expertise is in the production of non-conventional radionuclides for medical applications and radiopharmaceuticals for PET molecular imaging. He is well-known for his contributions in the production of metallic radionuclides.

Dr. Miguel A. Avila-Rodriguez is a tenured Associate Professor and Head of the Cyclotron and Radiopharmaceutical Labs at the National Autonomous University of Mexico (UNAM). He received his PhD degree in Medical Physics from the University of Wisconsin-Madison (USA). After that, he was a postdoctoral researcher fellow at the University of Alberta in the Edmonton PET Centre (Canada), and at the University of Turku in the Turku PET Centre (Finland). His area of expertise is in the production of non-conventional radionuclides for medical applications and radiopharmaceuticals for PET molecular imaging. He is well-known for his contributions in the production of metallic radionuclides.

Later on during the 1960s, Adler and Longo used the same starting materials (pyrrole and benzaldehyde) under propionic acid reflux at 141 °C;¹³ they observed that under these conditions, it was possible to use a wider selection of substituted aldehydes, which increased the synthesis yield to 20%.¹⁴ The main problem that this methodology faces is that it is unfavorable with aldehydes that bear sensitive groups; another drawback was the level of impurities, making the purification process difficult, mainly with porphyrins that could not precipitate or crystallize, without mentioning the poor reproducibility.

More recently in the 1980s, the strategy reported by Lindsey *et al.*,¹⁵ using chloroform or dichloromethane, acid catalysis (CF_3COOH or BF_3OEt_2) and oxidant media (DDQ or *p*-chloranil), was found to be more reliable and reproducible. The advantages of this methodology come from its relatively mild conditions, making it possible to use a broad variety of aldehydes, easy purification process, and relatively high yield (30–40%).

2.2 Photochemical behavior

Porphyrins bear 26 π delocalized electrons responsible of their optical, electronic, magnetic and redox characteristics. These features can be easily modulated by changing the structure of the aldehyde or by adding metals at the macrocyclic center, which have captured the attention of several research groups. Due to their high number of delocalized electrons, porphyrins can absorb several wavelengths in the UV-vis region, showing a well-defined spectrum composed of two bands: the first one is denoted as the Soret band located between 400–500 nm, which is attributed to the $S_0 \rightarrow S_2$ transition; the second group of bands is named Q-bands (four bands in free base porphyrins) located between 500–700 nm, attributed to the $S_0 \rightarrow S_1$ transition. In the case of metallated porphyrins, the four Q-bands turned into two bands due to the increase of symmetry in the macrocycle. Porphyrins can also absorb photons resulting in photo-excited states, which have a relatively long lifetime; as a consequence of this absorption, they exhibit strong fluorescence maxima located at 600–800 nm due to $S_1 \rightarrow S_0$ transitions and the quantum yield rises by 10%.¹⁶

2.3 Porphyrins as singlet oxygen generators and their applications in PDT

Singlet oxygen was first described by Khan and Kasha in 1963; they interpreted the chemiluminescence generated by the reaction of hypochlorite–peroxide to be due to singlet oxygen generation.¹⁷

The electronic structure of oxygen is composed of a ground state $^3\Sigma_g^-$ (spin triplet) and two lowest-energy excited electronic states (singlet excited states), $^1\Delta_g$ and $^1\Sigma_g^+$, with energy states of 95 and 158 kJ, respectively.¹⁸ Singlet species have different stabilities that are based on their aqueous lifetimes which are 10^{-6} – 10^{-3} s for $^1\Delta_g$, and 10^{-11} – 10^{-9} s for $^1\Sigma_g^+$, correspondingly.¹⁹ Although these species are named singlet,

their reactivity is not the same; while $^1\Sigma_g^+$ has a higher energy and is not known to react with other molecules,^{20,21} $^1\Delta_g$ has a different behavior, as this species is the one known as “singlet oxygen”.^{22,23}

The mechanism by which photosensitized molecules generate singlet oxygen has been well described^{24–26} and follows the following steps: once the photosensitizer absorbs photons, transition between the ground state (S_0) to the singlet excited state (S_n) yields a lowest excited singlet state (S_1), which through intersystem crossing generates a triplet state (T_1). This species is capable of reacting in two ways: type I and type II mechanisms (Fig. 1).

The type I mechanism involves free radicals, which are derived from electron transfer or hydrogen-atom abstraction between the sensitizer and other organic molecules (eqn (1)). Once these radicals are generated, they can react with oxygen, which creates a superoxide radical anion. In the type II mechanism, singlet oxygen is generated by the energy transfer from the photosensitizer (in the triplet state) to the oxygen molecule (eqn (2)). Before degradation by photobleaching, each photosensitizer molecule can generate 10^3 – 10^5 molecules of singlet oxygen.

An important characteristic of singlet oxygen lies on its lifetime and capability to react with several molecules, among them are biomolecules (unsaturated lipids and α -amino-acid residues), and its capability to diffuse (45 nm),²⁷ making it possible to diffuse across a single cell length.

Several porphyrin analogues have been developed for PDT; among them, HPPH (Fig. 2) has been demonstrated to have excellent results and limited skin phototoxicity.^{1,28,29} The clinical trials of HPPH have shown its efficacy against lung, esophagus, head and neck cancer.^{30–32}

2.4 Porphyrins as chelating agents

Porphyrins bear a vacant center, which is an ideal site to host different metallic cations.^{33–35} It is well known that porphy-

R=hexyl:HPPH

Fig. 2 2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a, brand name Photoclor.

rins act as tetradentate ligands, making it possible to coordinate species that have a minimum coordination number of 4; higher coordination numbers are possible through ligands that can be either neutral or anionic.³⁶ In the case of divalent metals like Zn^{2+} , Mg^{2+} , Cu^{2+} , Fe^{2+} , Co^{2+} and Ni^{2+} , it is possible to obtain electronically neutral compounds. The structure of these complexes could be either square-planar or octahedral. For trivalent metals like Fe^{3+} and Mn^{3+} , an anionic axial ligand is necessary to form an electrically neutral complex, whose geometry could be pentacoordinate square-pyramidal or hexacoordinate complexes. In the case of an eight-coordinate species, it forms a sandwich compound that has a square antiprism geometry.³⁷ Structurally, the interaction between metals and porphyrin has been well established and it is formed by two main types of interactions: σ -interaction is established by the nitrogen lone pairs directed to the center of the macrocycle, and π -interaction is made by p_π and/or d_π with nitrogen p_π orbitals.³⁸

Since the conditions to incorporate a metal center into the porphyrin core are quite facile, and given that the photo-physical properties of these metallated compounds change, compared with the free base porphyrin, several types of metalloporphyrins have been developed and have applications in fields such as catalysis,^{39,40} dye sensitized solar cells (DSSCs),⁴¹ and sensors,⁴² and in the development of novel drugs.⁴³

3. Metallic radionuclides

Given the short half-life of traditional PET isotopes (^{11}C , ^{13}N , ^{15}O and ^{18}F), they are only useful to investigate some biological processes that happen in short times.⁴⁴ Although these radioisotopes are the most commonly used, it is necessary to produce other radionuclides with a longer half-life that better match the biokinetics of new molecules like proteins, peptides, antibodies and nanoparticles, which are biological targets of interest. There is a broad variety of metallic radionuclides that meet this characteristic, including isotopes of zirconium, yttrium, indium, gallium, cobalt, and copper,

Fig. 1 Singlet oxygen diagram generation. Figure adapted from ref. 19.

some of them listed in Table 1, which have the potential to be used in the development of new agents in the field of radiopharmaceutical research.^{45–49}

The research in this field has not just been focused on the development of radionuclides, but also on the design of novel ligands that could chelate and transport these metals to the target tissue, including two main types of chelating agents: open-chain chelators and macrocyclic chelators.^{50,51}

3.1 From the copper age to nuclear medicine

3.1.1 Nature of copper. The first records on the use of copper date back to 5000 B.C. The name is derived from the Latin word *cuprum*; some authors believe that the origin of the name is from *aes cyprum* (the metal of Cyprus), as this metal was mainly mined in Cyprus during the Roman era. The early uses of this metal were in the manufacture of weapons and tools. Eventually, the interest in copper increased mainly due to the advances on metallurgy and as result, alloys like bronze (copper with tin) and brass (copper and zinc) expanded the use of copper. With the birth of electricity in the nineteenth century, the uses of this metal increased due to its good electrical properties, among them its high electrical conductivity ($59.6 \times 10^7 \text{ S m}^{-1}$). More recently, copper found new applications in superconductivity research, giving origin to high-temperature copper-oxide-based semiconductors.⁵²

Copper is a chemical element with atomic number 29, located at the position of transition metals on the periodic table. It is a soft, malleable and ductile metal. It is documented that copper is distributed on Earth but in different quanti-

ties, approximately 68 ppm in the Earth's crust, 0.003 ppm in seawater, and as an important point, 1.0 ppm is distributed in the human body. One of the most accessible oxidation states of copper is Cu(II); meanwhile, Cu(I) and Cu(III) are species present just using some specific donors that stabilize the oxidation state. Cu(II) is known for its borderline hardness, its ionic radius is 57–73 pm, and it bears a coordination number of 4–6, making it suitable to form five membered chelate rings. One interesting aspect is that once copper forms complexes with macrocyclic chelators, it shows better kinetic inertness and *in vivo* stability, compared with those of a linear chelate. This is mainly due to the macrocyclic effect.⁵³

3.1.2 Copper and biological aspects. Copper has been applied in the medical field because of the role it plays in many physiological functions such as neurotransmitter biosynthesis, tissue formation and enzyme functions.^{54,55} This metal has important functions not only in some synthetic pathways, it is important to mention that irregularities in its levels may also lead to some diseases like Menkes and Wilson's syndrome, which are the consequence of a deficiency (caused by mutations in the copper-transporter gene ATP7A-chromosome) or an overload in concentration of copper (the result of a mutation in the ATP7A gene-chromosome), respectively.⁵⁶

Given the important role that copper plays in tissue formation, some authors proposed that its presence is critical in the process of angiogenesis and therefore in tumor growth;^{57,58} taking into account that tumor cells need high concentrations of copper to grow, in contrast to normal cells, novel types of therapies have been targeted to modify the supply of this metal and as a result the process of vasculature formation could be changed, altering the progress of tumor growth.^{56,59} Several chelating agents have been developed in order to assess their ability to inhibit endothelial cell proliferation. The earlier assays in this kind of therapy were focused on the treatment of Wilson's disease, using penicillamine and triethylenetetramine,^{60,61} but due to the high toxicity of these chelates, derivatives of *cis*, *cis*-1,3,4-triamino-cyclohexane (TACH) have been developed (Fig. 3).

The other treatment approach uses the REDOX properties of Cu(I), as it can be oxidized by H_2O_2 generating a hydroxyl radical by a Fenton or even by Haber–Weiss reaction. Evidence suggests that inhibition of some antioxidant enzymes and production of radicals could be classified as a treatment for cancer;^{62,63} compounds like Casiopeina⁶⁴ and thiosemicarbazones⁶¹ have been evaluated to inhibit tumor cell proliferation following this rationale (Fig. 4).

Table 1 Main characteristics of metallic radionuclides

Radionuclide	Half-life (h)	Production	Decay mode	$E_{\beta^+ \text{ max}}$ (keV)
⁶⁰ Cu	0.4	Cyclotron ⁶⁰ Ni(p,n) ⁶⁰ Cu	β^+ (92.5%) EC (7.5%)	3920
⁶¹ Cu	3.3	Cyclotron ⁶¹ Ni(p,n) ⁶¹ Cu	β^+ (61.5%) EC (38.5%)	1220
⁶² Cu	0.16	Generator ⁶² Zn/ ⁶² Cu	β^+ (98%) EC (2%)	2910
⁶⁴ Cu	12.7	Cyclotron ⁶⁴ Ni(p,n) ⁶⁴ Cu ⁶⁴ Ni(d,2n) ⁶⁴ Cu	β^+ (17.6%) EC (43.9%) β^- (38.5%)	656
⁶⁷ Cu	61.83	Cyclotron ⁶⁸ Zn(p,2p) ⁶⁷ Cu ⁷⁰ Zn(p, α) ⁶⁷ Cu	β^- (100%)	575
⁵⁵ Co	17.5	Cyclotron ⁵⁸ Ni(p, α) ⁵⁵ Co	β^+ (76%) EC (24%)	570
⁶⁶ Ga	9.5	Cyclotron ⁶³ Zn(p,n) ⁶⁶ Ga	β^+ (57%) EC (43%)	4153
⁶⁸ Ga	1.1	Cyclotron ⁶⁸ Zn(p,n) ⁶⁸ Ga Generator ⁶⁸ Ge/ ⁶⁸ Ga	β^+ (89%) EC (11%)	1880
⁸⁶ Y	14.7	Cyclotron ⁸⁶ Sr(p,n) ⁸⁶ Y	β^+ (31.95%) EC (68.05%)	2335
⁸⁹ Zr	78.4	Cyclotron ⁸⁹ Y(p,n) ⁸⁹ Zr	β^+ (22.75%) EC (77.25%)	1744

Fig. 3 TACH derivatives.

Finally, taking into account the cellular homeostasis, regulated by several proteins, researchers have demonstrated that proteasome plays a crucial role in the timely degradation of proteins, making it possible to maintain the cellular equilibrium by processes like protein quality control, antigen processing, apoptosis, *etc.*^{65–67} It is important to emphasize that cancer cells are more sensitive to proteasome inhibition than normal cells (Fig. 5).

3.2 The use of copper in positron emission tomography (PET)

Copper has two stable isotopes (^{63}Cu and ^{65}Cu , relative abundances of 69.17% and 30.83%, respectively) and 27 known radioisotopes, but just four of them have been employed in molecular imaging (^{60}Cu , ^{61}Cu , ^{62}Cu , and ^{64}Cu).⁶⁸ The interest in these radionuclides lies in their decay modes and broad range of half-lives (Table 1).^{48,69} However, in recent years, the interest of the global research community has been focused on the properties of ^{64}Cu , from its production to its application in the medical field. As an example of the increasing interest in this radionuclide, it is worth mentioning that the International Atomic Energy Agency (IAEA) recently launched a Coordinated Research Project (CRP) entitled “Copper-64 radiopharmaceuticals for theranostic applications”⁷⁰ with the main objective of formulating guidelines to enhance and strengthen the expertise and capability of member states in the production, quality control, preclinical and possible clinical application of copper-64 radiopharmaceuticals with theranostic properties.^{71–75}

When talking about ^{64}Cu , one important topic is its production, which can either proceed using a reactor or a cyclotron; the different methods of production lead to products with varying quality in terms of radiochemical and chemical impurities, in addition to yield and specific activity. Production by a reactor can lead to high or low specific activity, depending on whether the production is by direct activation (n,γ) on a Cu target, or indirect activation through a (n,p) reaction on a Zn target, respectively. The neutron-induced reaction on a ^{64}Zn target has a good yield, but the co-production of ^{65}Zn ($t_{1/2} = 245$ days) as a contaminant species is a great disadvantage.^{48,76} As an alternative, the use of a medical cyclotron has shown better results in the production of ^{64}Cu , in terms of both yield and specific activity.^{77–81} A high spe-

Fig. 5 Proteasome inhibitors.

cific activity ^{64}Cu is best achieved using isotopically enriched targets *via* the $^{64}\text{Ni}(p,n)$ reaction.⁸²

As previously mentioned, the great interest in ^{64}Cu lies in two main aspects: its characteristics of radioactive decay and coordination chemistry. I) Radioactive decay: this radionuclide bears a $T_{1/2} = 12.7$ h, and decays 17.4% β^+ , 39% β^- and 43.6% E.C. The low positron energy ($E_{\text{max}} = 0.65$ MeV) and the lack of abundant high energy γ -emissions make this radionuclide suitable not just as a diagnosis probe but also as a therapeutic agent.^{47,83,84} II) Coordination chemistry: the chemistry of copper compounds has been limited to three oxidation states (I–III). However, most Cu(I) complexes show high lability, limiting their applications for radiopharmaceutical production, while Cu(III) is a rare species that is possible to attain only with the use of strong π -donating ligands. Cu(II), on the other hand, due to its softness, can form complexes with species like amines, imines, and bidentate ligands (such as bipyridine).⁸⁵ These complexes can have a broad variety of geometries including square planar, distorted square planar, trigonal pyramidal, square pyramidal and distorted octahedral. As a consequence of this “rich chemistry”, several types of chelators have been developed in order to transport or stabilize Cu(II) species, *e.g.* cyclam and cyclen moieties, sarcophagine chelators, and cross-bridged tetraamine ligands⁸⁶ (Fig. 6).

4. Porphyrins as [^{64}Cu]Cu chelates

The use of porphyrins as ^{64}Cu chelates has demonstrated several advantages over their counterparts based on cyclam and cyclen moieties.⁸⁷ Among these, it is worth mentioning that:

1) The multiple pathways of tumor accumulation.⁸⁸ Porphyrins may interact with tumor cells in different ways; one of them involves the interaction with low-density lipoproteins (LDLs). Porphyrin-LDL particles are internalized by endocytosis and taking into account that tumor cells have a hyperproliferation metabolism, their need for this kind of molecule is increased. As a result, the cellular uptake of LDL-bound porphyrins is facilitated,^{89–91} and upon degradation of LDLs by lysosomes, porphyrins are released intracellularly. In the same way, the increased expression of transferrin receptors in cancer tissues could be a strategy to internalize porphyrins and form a complex based on transferrin-bound

Fig. 4 Casiopeina and thiosemicarbazone derivatives.

Fig. 7 Porphyrins structures studied earlier with PET.

Fig. 6 Most known bifunctional chelates and their derivatives, adapted from ref. 48.

porphyrins and as a result, the macrocycle could be accumulated in the tumor stroma.^{92,93} The second process that is responsible for porphyrin tumor accumulation involves the ability of this macrocycle to form aggregates, which could be phagocytosed by tumor-associated macrophages that have the potential to reduce tumor growth and destroy neoplastic tissues.^{94–96}

2) Another advantage that makes porphyrins excellent options to be used as ^{64}Cu chelates is their complex stability under physiological conditions. Novel protocols that improve the conditions, yield and labeling time of these macrocycles have been recently described, making it possible to explore the development of a radiopharmaceutical-kit that could be used at room temperature.⁹⁷

The first report on the radiolabeling of porphyrins using ^{64}Cu was documented in 1957, and was evaluated in neoplastic tissues.⁹¹ Later on, in the 1980s Wilson *et al.* used ^{64}Cu -labelled porphyrins to detect brain tumors, but the results were not convincing because PET technology was on its earliest stages of development and the spatial resolution was poor⁹⁸ (Fig. 7).

The group of Fazaeli *et al.*⁹⁹ labelled a penta-fluoro phenyl porphyrin using ^{64}Cu ; however due to the harsh reaction conditions used to incorporate the copper nuclei (reflux at 100 °C, 1 h) it is difficult to use this strategy with biological moieties. The specific activity was on the order of 14–16 GB $mmol^{-1}$ and the radiochemical purity was >97%. Bio-distribution results showed that after 2 h of injection most of

the activity was concentrated in the kidney and liver, with the urinary tract being the main route of excretion, because of the fluoride properties.^{100,101}

Other kinds of modified porphyrins have been developed in order to improve either uptake or clearance. Fazaeli *et al.*¹⁰² developed a complex based on a fluoro phenyl porphyrin grafted on nanoporous MCM-41 silica in order to obtain a carrier system for delivery of PET tracers. Bio-distribution studies show major uptake by the kidney, stomach and tumor, resulting in a promising compound that has high tumor targeting efficiency and fast clearance. On the other hand, Huang *et al.*¹⁰³ and Cheng *et al.*¹⁰⁴ have synthesized a series of porphyrin-PEG polymers labeled with ^{64}Cu that could help to evaluate renal function; PEG incorporation to the porphyrin core improves its stability, and the results of renal function evaluation showed that the clearance reached 60% of the injected dose (collected in urine).

The group of Luo and Wu¹⁰⁵ developed a multifunctional nanoparticle system based on poly(vinyl alcohol)-porphyrin (PPNs), which was labeled with ^{64}Cu ; the advantage of these molecules is that they integrate PET, PDT, PTT (photothermal therapy), and even drug delivery functions. Upon irradiation by near infrared light, PPNs could be activated to release reactive oxygen species, heat, and drugs simultaneously in the tumor sites.

The group of Li *et al.*¹⁰⁶ developed a theranostic strategy based on nanoporphyrins labeled with ^{64}Cu (^{64}Cu Nps); this compound initiated tumor accumulation 4 h after injection, and reached the maximum level of uptake at 16 h. After 24 h, the radiolabelled compound was found primarily at the implanted tumors with low-background signaling in the rest of the body.

5. Conjugated based on ^{64}Cu -porphyrins and biological molecules

The main goal of functionalizing porphyrins with biological units is to enhance the tumor uptake and therefore decrease

side effects. In this aspect, porphyrins have been successfully functionalized under mild conditions, making it possible to keep the biological moiety intact and hence retain the high-target specificity. Reports following this approach include macromolecules from peptides to proteins and antibodies.^{1,107–109}

Roberts *et al.*¹¹⁰ reported the use of *N*-4-nitrobenzyl-5-(4-carboxyphenyl)-10,15,20-tris(4-sulfophenyl) porphine conjugated with polyclonal rabbit immunoglobulin G (RIgG), and the conjugate porphyrin-RIgG was radiolabeled with ⁶⁴Cu (Fig. 8), obtaining a radiolabeling yield of 60%, probably due to the presence of the antibody that could reduce the metallation process by steric hindrance. The specific activity was 18 MBq mg⁻¹ suggesting an average of two porphyrins per antibody unit. On the other hand, coupling was not specific for any region of the antibody as a consequence of the free amino groups present on the antibody. This poor specificity on conjugation resulted in a reduced immunoreactivity of around 60%, compared with that of the unmodified antibody.^{111–114} The advantage of using these kinds of conjugates is that it is possible to combine the properties of porphyrin (as a chelating agent and its photophysical properties) with the high specificity reaction of the antibody-antigen, which explains why the use of monoclonal antibodies (mABs) has emerged as a novel therapeutic tool against several diseases, among them cancer.¹¹⁵

Fawwaz *et al.*¹¹⁶ evaluated the activity of a porphyrin conjugated with an antibody (*via* esterification), using a molar ratio of 1 : 10, respectively; after purification they determined a ratio of 2 porphyrins per antibody. The conjugate was labeled with ⁶⁴CuCl, and an ELISA assay was conducted, resulting in 72% immunoreactivity.

In 2013 Mukai *et al.* evaluated a conjugate based on ⁶⁴Cu-porphyrin-peptide (Fig. 9). The percentage of conjugation between the porphyrin and peptide was 11%. The radiolabeling was performed using mild conditions (pH 6, 50 °C and two hours of reaction) in order to avoid radiolysis, obtaining specific activities of 96.2 MBq nmol⁻¹ for [⁶⁴Cu]PPIX-PEG6-BBN and 1440 MBq nmol⁻¹ for [⁶⁴Cu]Cu-PPIX. In order to estab-

Fig. 9 [⁶⁴Cu] PPIX-PEG6-BBN.

lish a specific comparison, similar studies were carried out using [⁶⁴Cu]Cu-DOTA-PEG6-BBN, which was obtained with a specific activity of 3–6 MBq nmol⁻¹ and a radiochemical purity of 97%, while the radiochemical purity of [⁶⁴Cu]Cu-PPIX-PEG6-BBN was 96.7%. The PC-3 human prostatic carcinoma cell line was used to evaluate the specificity, and [⁶⁴Cu]Cu-PPIX-PEG6-BBN showed a higher uptake than [⁶⁴Cu]Cu-PPIX, suggesting a synergic role between hydrophobicity by PPIX and the association effect between GRP and BBN.¹¹⁷

The use of a porphyrin unit, folate (for target delivery to FR-expressing tumor cells), and a peptide linker was reported by Shi *et al.*¹¹⁸ (Fig. 10). The radiolabeling yield for this compound was almost quantitative (99.9%), obtaining a radiochemical purity of 98% and a specific activity of 2.66 × 10⁶ GBq mol⁻¹. The labeling conditions (60 °C, 10–20 minutes of reaction in DMSO) made this strategy less convenient to human use; however, it is worth mentioning that the metalloporphyrin showed high stability in physiological media.

Liu *et al.*^{119–121} have used nanoparticles based on lipids or phospholipids conjugated with porphyrin. Porphysomes could act as drug-delivery systems (due to the properties of liposomes); on the other hand, they exhibited the photonic properties of the porphyrin. Once radiolabeling was performed they obtained radiochemical purities higher than 98% and a radiochemical yield (decay-uncorrected) of 95%, while the specific activity was 36 mCi μmol⁻¹ (2800 Ci μmol⁻¹ per nanoparticle). It is worth noting that porphysomes were cleared by the hepatobiliary route, and as a result, high

Fig. 8 Conjugate based on porphyrin-monoclonal antibody.

Fig. 10 Porphyrin-folate conjugate.

concentrations in the liver and spleen were detected and none in the bladder. This is a great advantage because a high concentration of the radiotracer in the bladder results in a darkening of the image, making it difficult to obtain a diagnosis. These ^{64}Cu -porphyrins were evaluated in order to compare their response in prostate cancer and compared with ^{18}F -FDG, resulting in a superior tumor-to-prostate selectivity.¹²²

Finally, the strategy "Multimodal Image-Guided Surgical" used by Muhanna *et al.*¹²³ has demonstrated excellent results in head and neck cancer, by using a porphyrin lipoprotein (PLP) labeled with ^{64}Cu (mimicking nanoparticles) that was able to detect the primary tumor and metastatic nodes. The high accuracy of detection of the PLP relies on the fact that both preoperative PET and intraoperative fluorescence imaging are feasible for the identification of unknown primaries and recurrent tumors.

Conclusions

Positron emission tomography has emerged as a multidisciplinary technique in which people with specialization (physicists, chemists, engineers, pharmacologists, medical doctors, veterinary doctors, computer scientists and mathematicians) generate each day advances that result in better diagnosis giving the patient a chance for a better quality of life. As a clear example: the progress and improvements in the development of metallic radionuclides (as PET tracers) that have arisen as an option that could overcome the common disadvantages of conventional PET radionuclides of the main sequence CNOF (^{11}C , ^{13}N , ^{15}O and ^{18}F) given their short half-lives.

The use of ^{64}Cu and porphyrins has been demonstrated to be an excellent approach to obtain a theranostic couple (PET diagnosis from ^{64}Cu and photodynamic therapy from porphyrins). It is clear that there is a long way to go when talking about the design and improvement of molecules with theranostic capabilities, and the synthesis of novel porphyrin-conjugates that could behave in a selective way against some diseases or disorders (using peptides, monoclonal antibodies or lipids) seems to be the right approach. More importantly, having the capability to chelate longer half-life metallic radionuclides will make it possible for the PET technique be available for a greater number of people even in places where cyclotrons are not yet available.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was supported by UNAM-DGAPA Grant PAPIIT-IT202518, CONACYT Grand APN-2017-4837, and the International Atomic Energy Agency CRP code F22067 and RC 20569. E. Aguilar-Ortiz received a Postdoctoral fellowship from Programa de Becas Posdoctorales-DGAPA-UNAM. The authors

want to thank Mrs. Josefina Bolado, Head of the Scientific Paper Translation Department, from División de Investigación at Facultad de Medicina, UNAM, for editing the English-language version of this manuscript.

References

- 1 N. Vahidfar and A. R. Jalilian, An Overview of Labeled Porphyrin Molecules in Medical Imaging, *Recent Patents and Topics on Imaging*, 2005, 5, 3–12.
- 2 P. Hambright, R. Fawwaz, P. Valk, J. McRae and A. J. Bearden, The distribution of various water soluble radioactive metalloporphyrins in tumor bearing mice, *Bioinorg. Chem.*, 1975, 5, 87–92.
- 3 K. D. Lakouas, D. Huglo, S. Mordon and M. Vermandel, Nuclear medicine for photodynamic therapy in cancer: Planning, monitoring and nuclear PDT, *Photodiagn. Photodyn. Ther.*, 2017, 18, 236–243.
- 4 D. T. Mody, Pharmaceutical development and medical applications of porphyrin-type macrocycles, *J. Porphyrins Phthalocyanines*, 2000, 4, 362–367.
- 5 M. Nowakowska and M. Kępczyński, Polymeric photosensitizers 2. Photosensitized oxidation of phenol in aqueous solution, *J. Photochem. Photobiol., A*, 1998, 116, 251–256.
- 6 R. Gerdes, O. Bartels, G. Schneider, D. Wöhrle and G. Schulz-Ekloff, Photooxidations of Phenol, cyclopentadiene and citronellol with photosensitizers ionically bound at a polymeric ion exchanger, *Polym. Adv. Technol.*, 2001, 12, 152–160.
- 7 R. Gerdes, *et al.*, Photo-oxidation of phenol and monochlorophenols in oxygen-saturated aqueous solutions by different photosensitizers, *J. Photochem. Photobiol., A*, 1997, 111, 65–74.
- 8 N. L. Oleinick, A. R. Antunez, M. E. Clay, B. D. Richter and M. E. Kenney, New phthalocyanine photosensitizers for photodynamic therapy, *Photochem. Photobiol.*, 1993, 57, 242–247.
- 9 M. Ethirajan, Y. Chen, P. Joshi and R. K. Pandey, The role of porphyrin chemistry in tumor imaging and photodynamic therapy, *Chem. Soc. Rev.*, 2011, 40, 340–362.
- 10 T. J. Dougherty, Photosensitizers: therapy and detection of malignant tumors, *Photochem. Photobiol.*, 1987, 45, 879–889.
- 11 P. Rothmund, A New Porphyrin Synthesis. The Synthesis of Porphin¹, *J. Am. Chem. Soc.*, 1936, 58, 625–627.
- 12 P. Rothmund, Formation of Porphyrins from Pyrrole and Aldehydes, *J. Am. Chem. Soc.*, 1935, 57, 2010–2011.
- 13 A. D. Adler, F. R. Longo, J. D. Finareli, J. Goldsmacher, J. Assour and L. Korsakoff, A Simplified Synthesis for Meso-Tetraphenylporphine, *J. Org. Chem.*, 1967, 32, 476.
- 14 A. Adler, F. Longo and W. Shergalis, Mechanistic Investigations of Porphyrin Syntheses. I. Preliminary Studies on ms-Tetraphenylporphin¹, *J. Am. Chem. Soc.*, 1964, 86, 3145–3149.

- 15 J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney and A. M. Marguerettaz, *Rothemund and Adler-Longo Reactions Revisited: Synthesis of Tetraphenylporphyrins under Equilibrium Conditions*, *J. Org. Chem.*, 1987, **52**, 827–836.
- 16 P. J. Spellane, M. Gouterman, A. Antipas, S. Kim and Y. C. Liu, Porphyrins. 40.1 Electronic Spectra and Four-Orbital Energies of Free-Base, Zinc, Copper, and Palladium Tetrakis(Perfluorophenyl)Porphyrins, *Inorg. Chem.*, 1980, **19**, 386–391.
- 17 A. U. Khan and M. Kasha, Red Chemiluminescence of Molecular Oxygen in Aqueous Solution, *J. Chem. Phys.*, 1963, **39**, 2105–2106.
- 18 G. Herzberg, *Molecular Spectra Vol I*, D. Van Nostrand Company Inc., 1950.
- 19 C. A. Long and D. R. Kearns, Radiationless Decay of Singlet Molecular Oxygen in Solution. II. Temperature Dependence and Solvent Effects, *J. Am. Chem. Soc.*, 1975, **97**, 2018–2020.
- 20 R. D. Scurlock, B. Wang and P. R. Ogilby, Chemical Reactivity of Singlet Sigma Oxygen ($b^1\Sigma_g^+$) in Solution, *J. Am. Chem. Soc.*, 1996, **118**, 388–392.
- 21 M. Bodesheim and R. Schmidt, Chemical Reactivity of Sigma Singlet Oxygen O_2 ($^1\Sigma_g^+$), *J. Phys. Chem. A*, 1997, **101**, 5672–5677.
- 22 C. S. Foote, Photosensitized Oxygenations and the Role of Singlet Oxygen, *Acc. Chem. Res.*, 1968, **1**, 104–110.
- 23 E. L. Clennan and A. Pace, Advances in Singlet Oxygen Chemistry, *ChemInform*, 2005, **36**, 6665–6691.
- 24 R. Bonnett, Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy, *Chem. Soc. Rev.*, 1995, **24**, 19–33.
- 25 M. C. DeRosa and R. J. Crutchley, Photosensitized singlet oxygen and its applications, *Coord. Chem. Rev.*, 2002, **233–234**, 351–371.
- 26 P. R. Ogilby, Singlet oxygen: there is indeed something new under the sun, *Chem. Soc. Rev.*, 2010, **39**, 3181–3209.
- 27 W. M. Sharman, C. M. Allen and J. E. van Lier, Role of activated oxygen species in photodynamic therapy, *Methods Enzymol.*, 2000, **319**, 376–400.
- 28 G. M. Loewen, R. Pandey, D. Bellnier, B. Henderson and T. Dougherty, Endobronchial photodynamic therapy for lung cancer, *Lasers Surg. Med.*, 2006, **38**, 364–370.
- 29 D. A. Bellnier, W. R. Greco, G. M. Loewen, H. Nava, A. R. Oseroff and T. J. Dougherty, Clinical pharmacokinetics of the PDT photosensitizers porfimer sodium (Photofrin), 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (Photochlor) and 5-ALA-induced protoporphyrin IX, *Lasers Surg. Med.*, 2006, **38**, 439–444.
- 30 D. A. Bellnier, W. R. Greco, G. M. Loewen, H. Nava, A. R. Oseroff and T. J. Dougherty, Mild skin photosensitivity in cancer patients following injection of Photochlor (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a; HPPH) for photodynamic therapy, *Cancer Chemother. Pharmacol.*, 2006, **57**, 40–45.
- 31 Y. Chen, R. Miclea, T. Srikrishnan, S. Balasubramanian, T. J. Dougherty and R. K. Pandey, Investigation of human serum albumin (HSA) binding specificity of certain photosensitizers related to pyropheophorbide-a and bacteriopurpurinimide by circular dichroism spectroscopy and its correlation with in vivo photosensitizing efficacy, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3189–3192.
- 32 M. L. Magne, C. O. Rodriguez, S. A. Autry, B. F. Edwards, A. P. Theon and B. R. Madewell, Photodynamic therapy of facial squamous cell carcinoma in cats using a new photosensitizer, *Lasers Surg. Med.*, 1997, **20**, 202–209.
- 33 C. M. Lemon, P. J. Brothers and B. Boitrel, Porphyrin complexes of the period 6 main group and late transition metals, *Dalton Trans.*, 2011, **40**, 6591.
- 34 M. John Plater, S. Aiken and G. Bourhill, Metallated porphyrins containing lead(II), copper(II) or zinc(II), *Tetrahedron*, 2002, **58**, 2415–2422.
- 35 E. B. Fleischer, Structure of porphyrins and metalloporphyrins, *Acc. Chem. Res.*, 1970, **3**, 105–112.
- 36 R. Rossin, D. Pan, K. Qi, J. L. Turner, X. Sun, K. L. Wooley and M. J. Welch, ^{64}Cu -labeled folate-conjugated shell cross-linked nanoparticles for tumor imaging and radiotherapy: synthesis, radiolabeling, and biologic evaluation, *J. Nucl. Med.*, 2005, **46**, 1210–1218.
- 37 W. R. Scheidt, Trends in metalloporphyrin stereochemistry, *Acc. Chem. Res.*, 1977, **10**, 339–345.
- 38 K. Tatsumi and R. Hoffmann, Metalloporphyrins with Unusual Geometries. 1. Mono-, Di-, Triatom-Bridged Porphyrin Dimers, *J. Am. Chem. Soc.*, 1981, **103**, 3328–3341.
- 39 J. Olguín, H. Müller-Bunz and M. Albrecht, Springloaded porphyrin NHC hybrid rhodium(III) complexes: carbene dissociation and oxidation catalysis, *Chem. Commun.*, 2014, **50**, 3488–3490.
- 40 B. M. J. M. Suijkerbuijk and R. J. M. Klein Gebbink, Merging porphyrins with organometallics: synthesis and applications, *Angew. Chem., Int. Ed.*, 2008, **47**, 7396–7421.
- 41 M. Urbani, M. Grätzel, M. K. Nazeeruddin and T. Torres, Meso-substituted porphyrins for dye-sensitized solar cells, *Chem. Rev.*, 2014, **114**, 12330–12396.
- 42 S. Ishihara, J. Labuta, W. Van Rossom, D. Ishikawa, K. Minami and J. P. Hill, Porphyrin-based sensor nanoarchitectonics in diverse physical detection modes, *Phys. Chem. Chem. Phys.*, 2014, **16**, 9713–9746.
- 43 C. T. Lum, A. S.-T. Wong, M. C. Lin, C.-M. Che and R. W.-Y. Sun, A gold(III) porphyrin complex as an anti-cancer candidate to inhibit growth of cancer-stem cells, *Chem. Commun.*, 2013, **49**, 4364–4366.
- 44 P. W. Miller, N. J. Long, R. Vilar and A. D. Gee, Synthesis of ^{11}C , ^{18}F , ^{15}O , and ^{13}N radiolabels for positron emission tomography, *Angew. Chem., Int. Ed.*, 2008, **47**, 8998–9033.
- 45 C. F. Ramogida and C. Orvig, Tumour targeting with radiometals for diagnosis and therapy, *Chem. Commun.*, 2013, **49**, 4720–4739.
- 46 C. S. Cutler, H. M. Hennkens, N. Sisay, S. Huclier-Markai and S. S. Jurisson, Radiometals for combined imaging and therapy, *Chem. Rev.*, 2013, **113**, 858–883.
- 47 S. Bhattacharyya and M. Dixit, Metallic radionuclides in the development of diagnostic and therapeutic radiopharmaceuticals, *Dalton Trans.*, 2011, **40**, 6112–6128.

- 48 T. J. Wadas, E. H. Wong, G. R. Weisman and C. J. Anderson, Copper chelation chemistry and its role in copper radiopharmaceuticals, *Curr. Pharm. Des.*, 2007, **13**, 3–16.
- 49 O. F. Ikotun and S. E. Lapi, The rise of metal radionuclides in medical imaging: copper-64, zirconium-89 and yttrium-86, *Future Med. Chem.*, 2011, **3**, 599–621.
- 50 E. W. Price and C. Orvig, Matching chelators to radiometals for radiopharmaceuticals, *Chem. Soc. Rev.*, 2014, **43**, 260–290.
- 51 E. Boros, Y.-H. L. Scott Lin, C. O. Ferreira, B. O. Patrick, U. J. Hafeli, M. Adam and C. Orvig, One to chelate them all: investigation of a versatile, bifunctional chelator for ^{64}Cu , $^{99\text{m}}\text{Tc}$, Re and Co, *Dalton Trans.*, 2011, **40**, 6253–6259.
- 52 R. R. Conry, Copper: Inorganic and Coordination Chemistry Based in part on the article Copper: Inorganic and Coordination Chemistry by Rebecca R. Conry and Kenneth D. Karlin which appeared in the Encyclopedia of Inorganic Chemistry, in *Encyclopedia of Inorganic Chemistry*, 1st edn, 2006.
- 53 D. S. Matteson and G. L. Larson, Boron-tin and boron-lead tetrametallomethanes, *J. Am. Chem. Soc.*, 1969, **91**, 6541–6542.
- 54 R. A. Festa and D. J. Thiele, Copper: an essential metal in biology, *Curr. Biol.*, 2011, **21**, R877–R883.
- 55 S. Lutsenko, Human copper homeostasis: a network of interconnected pathways, *Curr. Opin. Chem. Biol.*, 2010, **14**, 211–217.
- 56 C. Santini, *et al.*, Advances in copper complexes as anticancer agents, *Chem. Rev.*, 2014, **114**, 815–862.
- 57 M. M. Eatock, A. Schätzlein and S. B. Kaye, Tumour vasculature as a target for anticancer therapy, *Cancer Treat. Rev.*, 2000, **26**, 191–204.
- 58 H. Xie and Y. J. Kang, Role of copper in angiogenesis and its medicinal implications, *Curr. Med. Chem.*, 2009, **16**, 1304–1314.
- 59 F. Wang, P. Jiao, M. Qi, M. Frezza, Q. P. Dou and B. Yan, Turning tumor-promoting copper into an anti-cancer weapon via high-throughput chemistry, *Curr. Med. Chem.*, 2010, **17**, 2685–2698.
- 60 J. M. Walshe, Treatment of Wilson's disease with trientine (triethylene tetramine) dihydrochloride, *Lancet*, 1982, **1**, 643–647.
- 61 M.-C. Liu, T.-S. Lin, J. G. Cory, A. H. Cory and A. C. Sartorelli, Synthesis and Biological Activity of 3- and 5-Amino Derivatives of Pyridine-2-carboxaldehyde Thiosemicarbazone, *J. Med. Chem.*, 1996, **39**, 2586–2593.
- 62 Q. Kong, J. A. Beel and K. O. Lillehei, A threshold concept for cancer therapy, *Med. Hypotheses*, 2000, **55**, 29–35.
- 63 J. Fang, H. Nakamura and A. K. Iyer, Tumor-targeted induction of oxytress for cancer therapy, *J. Drug Targeting*, 2007, **15**, 475–486.
- 64 C. Trejo-Solís, G. Palencia, S. Zuñiga, A. Rodríguez-Ropon, L. Osorio-Rico, S. Torres Luvia, I. Gracia-Mora, L. Marquez-Rosado, A. Sánchez, M. E. Moreno-Gracia, A. Cruz, M. E. Bravo-Gómez, L. Ruiz-Ramírez, S. Rodríguez-Enriquez and J. Sotelo, Cas Ilgly Induces Apoptosis in Glioma C6 Cells In Vitro and In Vivo through Caspase-Dependent and Caspase-Independent Mechanisms, *Neoplasia*, 2005, **7**, 563–574.
- 65 E. Eytan, D. Ganuth, T. Armon and A. Hershko, ATP-dependent incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin, *Proc. Natl. Acad. Sci. U. S. A.*, 1989, **86**, 7751–7755.
- 66 R. Hough, G. Pratt and M. Rechsteiner, Purification of two high molecular weight proteases from rabbit reticulocyte lysate, *J. Biol. Chem.*, 1987, **262**, 8303–8313.
- 67 D. Voges, P. Zwickl and W. Baumeister, The 26S proteasome: a molecular machine designed for controlled proteolysis, *Annu. Rev. Biochem.*, 1999, **68**, 1015–1068.
- 68 P. J. Blower, J. S. Lewis and J. Zweit, Copper radionuclides and radiopharmaceuticals in nuclear medicine, *Nucl. Med. Biol.*, 1996, **23**, 957–980.
- 69 A. Niccoli Asabella, G. L. Cascini, C. Altini, D. Paparella and A. Notaristefano, The copper radioisotopes: a systematic review with special interest to ^{64}Cu , *BioMed Res. Int.*, 2014, **2014**, 786463.
- 70 Copper-64 Radiopharmaceuticals for Theranostic Applications. (2017). Available at: <https://www.iaea.org/projects/crp/f22067>.
- 71 A. Boschi, P. Martini, E. Janevik-Ivanovska and A. Duatti, The emerging role of copper-64 radiopharmaceuticals as cancer theranostics, *Drug Discovery Today*, 2018, 1489–1501.
- 72 C. Ferrari, A. Niccoli Asabella, C. Villano, B. Giacobbi, D. Coccetti, P. Panichelli and G. Rubini, Copper-64 Dichloride as Theranostic Agent for Glioblastoma Multiforme: A Preclinical Study, *BioMed Res. Int.*, 2015, **2015**, 129764.
- 73 A. R. Jalilian and J. Osso Jr, The current status and future of theranostic Copper-64 radiopharmaceuticals, *Iran. J. Nucl. Med.*, 2017, **25**, 1–10.
- 74 C. Biggin, M. Harris, A. Hedt and C. Jeffery, Radiological properties of Next Generation Theranostics (Cu-64/Cu-67), *J. Nucl. Med.*, 2017, **58**, 1014–1014.
- 75 M. A. Avila-Rodriguez, C. Rios, J. Carrasco-Hernandez, J. C. Manrique-Arias, R. Matinez-Hernandez, F. O. Gracia-Pérez, A. R. Jalilian, E. Martinez-Rodriguez, M. E. Romero-Piña and A. Diaz-Ruiz, Biodistribution and radiation dosimetry of [^{64}Cu]copper dichloride: first-in-human study in healthy volunteers, *EJNMMI Res.*, 2017, **7**, 98.
- 76 K. R. Zinn, T. R. Chaudhuri, T.-P. Cheng, J. Steven Morris and W. A. Meyer, Production of no-carrier-added ^{64}Cu from zinc metal irradiated under boron shielding, *Cancer*, 1994, **73**, 774–778.
- 77 D. W. McCarthy, R. E. Shefer, R. E. Klinkowstein, L. A. Bass, W. H. Margeneau, C. S. Cutler, C. J. Anderson and M. J. Welch, Efficient production of high specific activity ^{64}Cu using a biomedical cyclotron, *Nucl. Med. Biol.*, 1997, **24**, 35–43.
- 78 F. Szelecsényi, G. Blessing and S. M. Qaim, Excitation functions of proton induced nuclear reactions on enriched ^{61}Ni and ^{64}Ni : Possibility of production of no-carrier-added ^{61}Cu and ^{64}Cu at a small cyclotron, *Appl. Radiat. Isot.*, 1993, **44**, 575–580.

- 79 M. A. Avila-Rodriguez, J. A. Nye and R. J. Nickles, Simultaneous production of high specific activity ^{64}Cu and ^{61}Co with 11.4 MeV protons on enriched ^{64}Ni nuclei, *Appl. Radiat. Isot.*, 2007, **65**, 1115–1120.
- 80 J. C. Manrique-Arias and M. A. Avila-Rodriguez, A simple and efficient method of nickel electrodeposition for the cyclotron production of (^{64}Cu), *Appl. Radiat. Isot.*, 2014, **89**, 37–41.
- 81 Q. Xie, H. Zhu, F. Wang, X. Meng, Q. Ren, C. Xia and Z. Yang, Establishing Reliable Cu-64 Production Process: From Target Plating to Molecular Specific Tumor Micro-PET Imaging, *Molecules*, 2017, **22**, 641.
- 82 X. Sun and J. C. Anderson, Production and Applications of Copper-64 Radiopharmaceuticals, *Methods Enzymol.*, 2004, **386**, 237–261.
- 83 A. Obata, S. Kasamatsu, J. S. Lewis, T. Furukawa, S. Takamatsu, J. Toyohara, T. Asai, M. J. Welch, S. G. Adamas, H. Saji, Y. Yonekura and Y. Fujibayashi, Basic characterization of ^{64}Cu -ATSM as a radiotherapy agent, *Nucl. Med. Biol.*, 2005, **32**, 21–28.
- 84 J. M. Connett, C. J. Anderson, L. W. Gou, S. W. Schwarz, K. R. Zinn, B. E. Rogers, B. A. Siegel, G. W. Philpott and M. J. Welch, Radioimmunotherapy with a ^{64}Cu -labeled monoclonal antibody: a comparison with ^{67}Cu , *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 6814–6818.
- 85 R. B. Martin, *Hard and Soft Acids and Bases. in Encyclopedia of Inorganic Chemistry*, ed. R. B. King, R. H. Crabtree, C. M. Lukehart, D. A. Atwood and R. A. Scott, John Wiley and Sons, Ltd, 2006.
- 86 C. J. Anderson and R. Ferdani, Copper-64 radiopharmaceuticals for PET imaging of cancer: advances in preclinical and clinical research, *Cancer Biother. Radiopharm.*, 2009, **24**, 379–393.
- 87 F. Bryden and R. W. Boyle, Metalloporphyrins for Medical Imaging Applications, *Adv. Inorg. Chem.*, 2016, **68**, 141–221.
- 88 J. Osterloh and M. G. H. Vicente, Mechanisms of porphyrinoid localization in tumors, *J. Porphyrins Phthalocyanines*, 2002, **06**, 305–324.
- 89 G. Jori and E. Reddi, The role of lipoproteins in the delivery of tumour-targeting photosensitizers, *Int. J. Biochem.*, 1993, **25**, 1369–1375.
- 90 D. Kessel, Porphyrin-lipoprotein association as a factor in porphyrin localization, *Cancer Lett.*, 1986, **33**, 183–188.
- 91 R. Bases, S. S. Brodie and S. Rubinfeld, Attempts at tumor localization using $\text{Cu } 64$ -labeled copper porphyrins, *Cancer*, 1958, **11**, 259–263.
- 92 S. Nakajima, T. Moriyama, H. Hayashi, I. Sakata, Y. Nakae and T. Takemura, Hemopexin as a carrier protein of tumor-localizing Ga-metalloporphyrin-ATN-2, *Cancer Lett.*, 2000, **149**, 221–226.
- 93 M. R. Hamblin and E. L. Newman, Photosensitizer targeting in photodynamic therapy. I. Conjugates of haematoporphyrin with albumin and transferrin, *J. Photochem. Photobiol., B*, 1994, **26**, 45–56.
- 94 C. E. Lewis, R. Leek, A. Harris and J. O. McGee, Cytokine regulation of angiogenesis in breast cancer: the role of tumor-associated macrophages, *J. Leukocyte Biol.*, 1995, **57**, 747–751.
- 95 K. D. Elgert, D. G. Allea and D. W. Mullins, Tumor-induced immune dysfunction: the macrophage connection, *J. Leukocyte Biol.*, 1998, **64**, 275–290.
- 96 M. Korbelik, G. Kros and D. J. Chaplin, Photofrin uptake by murine macrophages, *Cancer Res.*, 1991, **51**, 2251–2255.
- 97 K. Kilian, M. Pęgieć and K. Pyrzyńska, The fast method of Cu-porphyrin complex synthesis for potential use in positron emission tomography imaging, *Spectrochim. Acta, Part A*, 2016, **159**, 123–127.
- 98 G. Firnaeu, D. Maass, B. C. Wilson and W. P. Jeeves, ^{64}Cu labelling of hematoporphyrin derivative for non-invasive in vivo measurements of tumour uptake, *Prog. Clin. Biol. Res.*, 1984, **170**, 629–636.
- 99 Y. Fazaeli, *et al.*, Preparation, nano purification, quality control and labeling optimization of [^{64}Cu]-5,10,15,20-tetrakis (penta fluoro phenyl) porphyrin complex as a possible imaging agent, *J. Radioanal. Nucl. Chem.*, 2013, **295**, 255–263.
- 100 P. Shah and A. D. Westwell, The role of fluorine in medicinal chemistry, *J. Enzyme Inhib. Med. Chem.*, 2007, **22**, 527–540.
- 101 S. Swallow, Fluorine in medicinal chemistry, *Prog. Med. Chem.*, 2015, **54**, 65–133.
- 102 Y. Fazaeli, S. Feizi, R. A. Jalilian and A. Hejrani, Grafting of [^{64}Cu]-TPPF20 porphyrin complex on Functionalized nanoporous, *Appl. Rad. Isot.*, 2016, **112**, 13–19.
- 103 H. Huang, R. Hernandez, J. Geng, H. Sun, W. Song, F. Cheng, A. S. Graves, R. Nickles, C. Cheng and W. Cai, Lovell F. J. A porphyrin-PEG polymer with rapid renal clearance, *Biomaterials*, 2016, **76**, 25–32.
- 104 L. Cheng, D. Jiang, A. Kamkaew, F. H. Valdovinos, H.-Y. Im, L. Feng, G. C. Englang, S. Goel, E. T. Barnhart, Z. Liu and W. Cai, *et al.* Renal-Clearable PEGylated Porphyrin Nanoparticles for Image-Guided Photodynamic Cancer Therapy, *Adv. Funct. Mater.*, 2017, **27**, 1–10.
- 105 Y. Luo, H. Wu, C. Feng, K. Xiao, X. Yang, Q. Liu, T.-Y. Lin, H. Zhang, H. J. Walton, Y. Ajena, Y. Hu, S. K. Lam and Y. Li, 'One-Pot' fabrication of highly versatile and biocompatible poly(vinyl alcohol)-porphyrin-based nanotheranostics, *Theranostics*, 2017, **7**, 3901–3914.
- 106 Y. Li, T.-Y. Lin, Y. Luo, Q. Liu, W. Xiao, W. Guo, D. Lac, H. Zhang, C. Feng, S. Wachsmann-Hogiu, H. J. Walton, R. S. Cherry, J. D. Rowland, D. Kukis, C. Pan and S. K. Lam, A smart and versatile theranostic nanomedicine platform based on nanoporphyrin, *Nat. Commun.*, 2012, **5**, 1–15.
- 107 B. L. Josefsen and W. R. Boyle, Unique Diagnostic and Therapeutic Roles of Porphyrins and Phthalocyanines in Photodynamic Therapy, Imaging and Theranostics, *Theranostics*, 2012, **9**, 916–966.
- 108 P. A. Waghorn, Radiolabelled porphyrins in nuclear medicine, *J. Labelled Compd. Radiopharm.*, 2014, **57**, 304–309.

- 109 Y. Zhang and J. F. Lovell, Porphyrins as Theranostic Agents from Prehistoric to Modern Times, *Theranostics*, 2012, 2, 905–915.
- 110 J. C. Roberts, S. L. Newmyer, J. A. Mercer-Smith, S. A. Schreyer and D. K. Lavalley, Labeling antibodies with copper radionuclides using N-4-nitrobenzyl-5-(4-carboxy-phenyl)-10,15,20-tris(4-sulfophenyl) porphine, *Appl. Radiat. Isot.*, 1989, 40, 775–781.
- 111 A. J. Bullous, C. M. A. Alonso and R. W. Boyle, Photosensitizer–antibody conjugates for photodynamic therapy, *Photochem. Photobiol. Sci.*, 2011, 10, 721.
- 112 D. Mew, C. K. Wat, G. H. Towers and J. G. Levy, Photoimmunotherapy: treatment of animal tumors with tumor-specific monoclonal antibody-hematoporphyrin conjugates, *J. Immunol.*, 1983, 130, 1473–1477.
- 113 M. Del Governatore, M. R. Hamblin, E. E. Piccinini, G. Ugolini and T. Hasan, Targeted photodestruction of human colon cancer cells using charged 17.1A chlorin e6 immunoconjugates, *Br. J. Cancer*, 2000, 82, 56–64.
- 114 L. R. Milgrom and F. O. Neill, Towards Synthetic-Porphyrin/Monoclonal Antibody Conjugates Scheme S-mAb, *Science*, 1995, 51, 2137–2144.
- 115 A. García Merino, Monoclonal antibodies, Basic features, *Neurologia*, 2011, 26, 301–306.
- 116 R. Fawwaz, P. Bohdiewicz, D. Lavalley, T. Wang, S. Oluwale, J. Newhouse and P. Alderson, Use of metalloporphyrins in diagnostic imaging, *Nucl. Med. Biol.*, 1990, 17, 65–72.
- 117 H. Mukai, Y. Wada and Y. Watanabe, The synthesis of ⁶⁴Cu-chelated porphyrin photosensitizers and their tumor-targeting peptide conjugates for the evaluation of target cell uptake and PET image-based pharmacokinetics of targeted photodynamic therapy agents, *Ann. Nucl. Med.*, 2013, 27, 625–639.
- 118 J. Shi, W. B. T. Liu, J. Chen, D. Green, D. Jaffray, C. B. Wilson, F. Wang and G. Zheng, Transforming a Targeted Porphyrin Theranostic Agent into a PET Imaging Probe for Cancer, *Theranostics*, 2011, 1, 363–370.
- 119 T. W. Liu, T. D. MacDonald, J. Shi, B. C. Wilson and G. Zheng, Intrinsically copper-64-labeled organic nanoparticles as radiotracers, *Angew. Chem., Int. Ed.*, 2012, 51, 13128–13131.
- 120 D. Luo, S. Goel, H.-J. Liu, A. K. Carter, D. Jiang, J. Geng, J. C. Kuttyreff, W. J. Engle, W.-C. Huang, S. Shao, C. Fang, W. Cai and F. J. Lovell, Intrabilayer ⁶⁴Cu Labeling of Photoactivatable, Doxorubicin-Loaded Stealth Liposomes, *ACS Nano*, 2017, 11, 12482–12491.
- 121 C. S. Jin, L. Cui, F. Wang, J. Chen and G. Zheng, Targeting-triggered porphyrin nanostructure disruption for activatable photodynamic therapy, *Adv. Healthcare Mater.*, 2014, 3, 1240–1249.
- 122 C. S. Jin, M. Overchuk, L. Cui, C. B. Wilson, G. R. Bristow, J. Chen and G. Zheng, Nanoparticle-Enabled Selective Destruction of Prostate Tumor Using MRI-Guided Focal Photothermal Therapy, *Prostate*, 2016, 76, 1169–1181.
- 123 N. Muhanna, L. Cui, H. Chan, L. Burgess, S. C. Jin, D. T. MacDonald, E. Huynh, F. Wang, J. Chen, C. J. Irish and G. Zheng, Multimodal image-guided surgical and photodynamic interventions in head and neck cancer: From primary tumor to metastatic drainage, *Clin. Cancer Res.*, 2016, 22, 961–970.