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Matching chelators to radiometals for radiopharmaceuticals

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Radiometals comprise many useful radioactive isotopes of various metallic elements. When properly harnessed, these have valuable emission properties that can be used for diagnostic imaging techniques, such as single photon emission computed tomography (SPECT, *e.g.* ^{67}Ga, ^{99m}Tc, ^{111}In, ^{177}Lu) and positron emission tomography (PET, *e.g.* ^{68}Ga, ^{64}Cu, ^{44}Sc, ^{86}Y, ^{89}Zr), as well as therapeutic applications (*e.g.* ^{47}Sc, ^{114m}In, ^{177}Lu, ^{90}Y, $^{212/213}$Bi, ^{212}Pb, ^{225}Ac, $^{186/188}$Re). A fundamental critical component of a radiometal-based radiopharmaceutical is the chelator, the ligand system that binds the radiometal ion in a tight stable coordination complex so that it can be properly directed to a desirable molecular target *in vivo*. This article is a guide for selecting the optimal match between chelator and radiometal for use in these systems. The article briefly introduces a selection of relevant and high impact radiometals, and their potential utility to the fields of radiochemistry, nuclear medicine, and molecular imaging. A description of radiometal-based radiopharmaceuticals is provided, and several key design considerations are discussed. The experimental methods by which chelators are assessed for their suitability with a variety of radiometal ions is explained, and a large selection of the most common and most promising chelators are evaluated and discussed for their potential use with a variety of radiometals. Comprehensive tables have been assembled to provide a convenient and accessible overview of the field of radiometal chelating agents.

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1. Introduction

Radiometals are radioactive isotopes that can be harnessed for applications in medical diagnosis, as well as for cancer therapy. In order to apply these isotopes to specific biological applications, the "free" radiometal ions must be sequestered from aqueous solution using chelators (ligands) to obviate transchelation and hydrolysis. Chelators used for this application are typically covalently linked to a biologically active targeting molecule, making an active radiopharmaceutical agent. The chelator is used to tightly bind a radiometal ion so that when injected into a patient, the targeting molecule can deliver the isotope without any radiometal loss from the radiopharmaceutical, effectively supplying a site-specific radioactive source *in vivo* for imaging or therapy. A rapidly expanding number of radiometals are routinely produced, with a broad variety of half-lives, emission types, energies, and branching ratios (Table 1).[1–4] The availability of a wide range of radiometal ions makes it possible to carefully pick the specific nuclear properties that are needed for a vast number of different applications. Some examples of radiometals that can be used for positron emission tomography (PET) imaging are ^{68}Ga, ^{64}Cu, ^{86}Y, ^{89}Zr, and ^{44}Sc, with PET imaging providing sensitive, quantitative, and non-invasive images of a variety of molecular processes and targets. Single photon emission computed tomography (SPECT) is an older and more ubiquitous imaging modality than PET, and, since its inception in the 1960s, ^{99m}Tc has been the workhorse isotope of SPECT. More recently, the radiometals ^{67}Ga, ^{111}In, and ^{177}Lu have been increasingly used for SPECT imaging in chelator-based radiopharmaceuticals. For therapy applications, particle emitters such as ^{111}In (Auger electron emitter), ^{90}Y and ^{177}Lu (β^-), and ^{225}Ac, ^{212}Pb, and ^{213}Bi (α), are being heavily investigated, typically in conjunction with antibody vectors (immunoconjugates) or peptides. Each radiometal ion has unique aqueous coordination chemistry properties; these must be properly attended to if these isotopes are to be safely harnessed for medical applications and use *in vivo*.

The major difference between radioactive ("hot") and non-radioactive ("cold") metal ion chemistry is that radiochemistry is typically performed under extremely dilute conditions, with radiometal ions typically being utilized at nM to pM concentrations.[5] It is also important to note that several of the elements being discussed have multiple radioactive isotopes that are useful for diagnostic or therapeutic purposes (*e.g.* $^{86/90}$Y, $^{67/68}$Ga, $^{44/47}$Sc, $^{60/61/62/64}$Cu), and all isotopes of a given element have identical chemistry.[6–11] This means that a single radiopharmaceutical agent can be radiolabeled with different isotopes

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of the same element (*e.g.* $^{86/90}$Y), and provide the same charge and physical properties, and therefore the same biological behavior and distribution *in vivo*.[6–11] This class of radiopharmaceutical that utilizes two isotopes of the same element, such as ^{86}Y for PET imaging and ^{90}Y for therapy, has been referred to as a theranostic agent.[12,13] An interesting note about ^{90}Y is that as a β^- emitter it is possible to perform biodistribution, imaging, and dosimetry studies with its bremsstrahlung X-rays, but because the spatial resolution and image quality obtained is very poor an imaging surrogate is typically used (*e.g.* ^{111}In or ^{86}Y).[14] Alternatively, ^{90}Y is unique and in some circumstances can be used directly for PET imaging because it emits a very low abundance of positrons (0.003%), which can be used to collect imaging data superior to that obtained by ^{90}Y bremsstrahlung imaging.[14]

2. Radiometal-based radiopharmaceutical design

Ligands that are typically used to construct radiometal-based radiopharmaceuticals (not always with ^{99m}Tc) are bifunctional chelators (BFCs), which are simply chelators with reactive functional groups that can be covalently coupled (conjugated) to targeting vectors (*e.g.* peptides, nucleotides, antibodies, nanoparticles). Common bioconjugation techniques utilize functional groups such as carboxylic acids or activated esters (*e.g. N*-hydroxysuccinimide NHS-ester, tetrafluorophenyl TFP-ester) for amide couplings, isothiocyanates for thiourea couplings, and maleimides for thiol couplings (Fig. 1).[17,18] Click chemistry is gaining popularity in bioconjugate chemistry, with both the traditional copper(I) catalyzed azide–alkyne Huisgen 1,3-dipolar cycloaddition "click" reaction (forming a 1,2,3-triazole-ring linkage), or newer copper-free reactions like strain-promoted azide–alkyne cycloadditions (*e.g.* dibenzocyclooctyne/azide reaction) and Diels–Alder click reactions (*e.g.* transcyclooctene/1,2,4,5-tetrazine) (Fig. 1).[19] It is interesting to note that the transcyclooctene/1,2,4,5-tetrazine copper-free click coupling displays remarkably fast reaction kinetics, allowing for novel applications like *in vivo* pre-targeting, where the click reaction can occur *in vivo* at very dilute concentrations.[20–24] The modular design of BFC systems allows for a theoretically limitless number of different vectors to be conjugated, providing molecular targeting to a constantly increasing number of biological targets.

The structure and physical properties of the radiometal–chelate complex have a large impact on the overall pharmacokinetic properties of a radiopharmaceutical, with many radiometal complexes being very hydrophilic and subsequently leading to rapid renal excretion when attached to small vectors like peptides and nucleotides (less prominent with large ~150 kDa antibodies).[6–11,179] It has been observed in peptide-conjugates that keeping the radiometal ion and peptide constant, and changing only the chelator can have drastic effects on biodistributions.[25] Radiometal-based radiopharmaceuticals contain many synthetically exchangeable components, which can be separated into different modules: the radiometal, which changes the radioactive emission properties and half-life (γ for SPECT, β^+ for PET, and β^- or α particles, or Auger electrons, for therapy); the chelator, which must be carefully matched with the radiometal for optimal stability; the BFC–vector conjugation method, for different types of bioconjugation reactions and linkages; and the vector/targeting moiety, which allows for the selection of any known molecular target for site-specific delivery of the radioactive "payload" (Fig. 2).

Eric W. Price and Chris Orvig

Eric Price received his undergraduate education at the University of Victoria in Victoria, British Columbia, Canada, where he completed his honours BSc (2009) in Chemistry as a part of the CO-OP program, performing research both in industry and academia, including work at TRIUMF and an NSERC USRA research term with Dr Matthew Moffitt. In 2009 he began his PhD studies as an NSERC scholar in a joint project between Dr Chris Orvig at the University of British Columbia and Dr Michael J. Adam at TRIUMF, partially in collaboration with Nordion, researching new radiometal-based radiopharmaceuticals of $^{67/68}$Ga, ^{64}Cu, ^{111}In, ^{177}Lu, ^{89}Zr, and ^{225}Ac. He initiated, and has been pivotal in, an international research collaboration with Dr Jason S. Lewis and Dr Brian M. Zeglis, traveling to Memorial Sloan-Kettering Cancer Center in New York, USA, and has also collaborated with the BC Cancer Agency in Vancouver, as part of his PhD work. In 2013 he won the Berson–Yalow award from the Society of Nuclear Medicine and Molecular Imaging.

Chris Orvig earned his Hons. BSc from McGill and his PhD as an NSERC of Canada scholar at MIT with Alan Davison, FRS. After postdoctoral fellowships with Kenneth N. Raymond at the University of California, Berkeley, and the late Colin J. L. Lock at McMaster University, he joined the University of British Columbia in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences. Orvig, a Fellow of the Royal Society of Canada, has received various teaching, research and service awards, and published more than 200 research papers. He is a co-inventor on many issued patents, and a certified ski instructor.

Table 1 Properties of some popular radiometal isotopes, EC = electron capture; some low abundance emissions have been omitted for brevity[1–4,15,16]

Isotope	$t_{1/2}$ (h)	Decay mode	E (keV)	Production method
^{60}Cu	0.4	β^+ (93%) EC (7%)	β^+, 3920, 3000, 2000	Cyclotron, ^{60}Ni(p,n)^{60}Cu
^{61}Cu	3.3	β^+ (62%) EC (38%)	β^+, 1220, 1150, 940, 560	Cyclotron, ^{61}Ni(p,n)^{61}Cu
^{62}Cu	0.16	β^+ (98%) EC (2%)	β^+, 2910	^{62}Zn/^{62}Cu generator
^{64}Cu	12.7	β^+ (19%) EC (41%) β^- (40%)	β^+, 656	Cyclotron, ^{64}Ni(p,n)^{64}Cu
^{66}Ga	9.5	β^+ (56%) EC (44%)	β^+, 4150, 935	Cyclotron, ^{63}Cu(α,nγ)^{66}Ga
^{67}Ga	78.2	EC (100%)	γ, 93, 184, 300	Cyclotron, ^{68}Zn(p,2n)^{67}Ga
^{68}Ga	1.1	β^+ (90%) EC (10%)	β^+, 1880	^{68}Ge/^{68}Ga generator
^{44}Sc	3.9	β^+ (94%) EC (6%)	γ, 1157 β^+, 1474	^{44}Ti/^{44}Sc generator
^{47}Sc	80.2	β^- (100%)	γ, 159 β^-, 441, 600	^{47}Ti(n,p)^{47}Sc
^{111}In	67.2	EC (100%)	γ, 245, 172	Cyclotron, ^{111}Cd(p,n)^{111m,g}In
^{114m}In	49.5 d	EC (100%)	γ, 190	Cyclotron, ^{114}Cd(p,n)^{114m}In or ^{116}Cd(p,3n)^{114m}In
^{114}In (daughter)	73 s	β^- (100%)	β^-, 1989	
^{177}Lu	159.4	β^- (100%)	γ, 112, 208 β^-, 177, 385, 498	^{176}Lu(n,γ)^{177}Lu
^{86}Y	14.7	β^+ (33%) EC (66%)	β^+, 1221	Cyclotron, ^{86}Sr(p,n)^{86}Y
^{90}Y	64.1	β^- (100%)	β^-, 2280	^{90}Zr(n,p)^{90}Y
^{89}Zr	78.5	β^+ (23%) EC (77%)	β^+, 897	Cyclotron, ^{89}Y(p,n)^{89}Zr
^{212}Bi	1.1	α (36%) β^- (64%)	α, 6050 β^-, 6089	^{228}Pb/^{212}Pb generator
^{213}Bi	0.76	α (2.2%) β^- (97.8%)	α, 5549 β^-, 5869	^{228}Th/^{212}Pb generator
^{212}Pb (daughter is ^{212}Bi)	10.6	β^- (100%)	α, 570	^{224}Ra/^{212}Pb generator
^{225}Ac	240	α (100%)	α, 5600–5830 (6)	^{226}Ra(p,2n)^{225}Ac *n*-Capture of ^{232}Th → ^{233}U → ^{225}Ac

Each radiometal ion has different chemical demands, including ligand donor atom preferences (*e.g.* N, O, S, hard/soft), coordination number, and coordination geometry; however, there are many key design considerations that can be applied universally.[26] Ligand synthesis should be relatively simple and avoid stereoisomers and non-enantio/diastereospecific reactions, modularity should be incorporated in BFC synthesis as much as possible to allow for incorporation of different bioconjugation handles, and modular synthesis also should allow for tuning of denticity and physical properties by changing the polarity and charge of the chelate (the degree of polarity can be assessed by octanol–water partition coefficients ($\log P$)), so that biodistribution properties can be adjusted.

The intention of this article is to act as a guide for researchers to find the optimal match of chelator with radiometal for radiopharmaceutical applications. The methods by which chelators are evaluated with different radiometals will be discussed, and the current "gold standard" chelators for each relevant radiometal ion will be identified. A large number of review articles with broader scopes have been written that discuss the application of radiometals in radiopharmaceuticals, covering topics ranging from chelators, coordination chemistry, synthetic

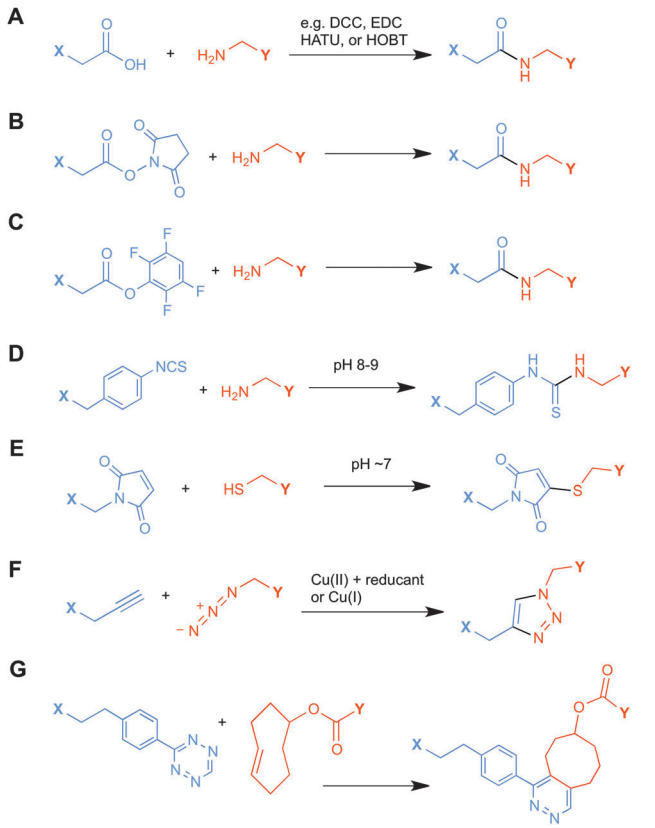

Fig. 1 Examples of bioconjugation reactions: (A) standard peptide coupling reaction between a carboxylic acid and a primary amine with a coupling reagent; (B and C) peptide coupling reactions between activated esters of tetrafluorophenyl (TFP) or *N*-hydroxysuccinimide (NHS) and a primary amine; (D) thiourea bond formation between an isothiocyanate and a primary amine; (E) thioether bond formation between a maleimide and thiol; (F) standard Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition ("click" reaction) between an azide and an alkyne; and (G) strain-promoted Diels–Alder "click" reaction between a tetrazine and transcyclooctene.

methodologies, radiometal production, radiochemistry, bioconjugation strategies, automation, molecular targeting groups (vectors), imaging modalities, and therapeutics.[3,13,15,17,18,26–51] Technetium chemistry is not covered here, because the chemistry of technetium, and the types of ligands used with it, differ drastically from those for the radiometals discussed here.[26,35,52]

2.1 Macrocyclic *versus* acyclic chelators

When designing new chelators, a historical glance at previous work reveals that macrocycles are generally more kinetically inert than acyclic chelators, even if their thermodynamic stabilities have been determined to be very similar.[53–57] Macrocyclic chelators require minimal physical manipulation during metal ion coordination, as they possess inherently constrained geometries and partially pre-organized metal ion binding sites, thereby decreasing the entropic loss experienced upon metal ion coordination.[58] To contrast this, acyclic chelators must undergo a more drastic change in physical orientation and geometry in solution in order to arrange donor atoms to coordinate with a metal ion, and subsequently they suffer a more significant decrease in entropy than do macrocycles (thermodynamically unfavorable). The thermodynamic driving force towards complex formation is therefore greater for macrocycles in general, a phenomenon referred to as the macrocycle effect.[58] A crucial property where most acyclic chelators excel and most macrocycles suffer is in the coordination kinetics and radiolabeling efficiency. The ability to quantitatively radiolabel/coordinate a radiometal in less than 15 minutes at room temperature is a common property of acyclic chelators, whereas macrocycles often require heating to 60–95 °C for extended times (30–90 minutes).[59–61] Fast room temperature radiolabeling becomes a crucial property when working with BFC-conjugates of heat sensitive molecules such as antibodies and their derivatives, or when working with short half-life isotopes such as ^{68}Ga, $^{212/213}$Bi, ^{44}Sc, and ^{62}Cu.

2.2 Matching chelators with radiometals – how are chelators evaluated?

When a new chelator is synthesized for the purpose of radiometal ion sequestration, or an old chelator is repurposed for use with a new radiometal ion, initial screening experiments are usually done by simple radiolabeling to determine a number of factors: whether the chelator can bind the radiometal ion and effectively radiolabel in high yields (quantitative is best), what temperature is required (ambient temperature is best), and what reaction time is required (faster is better). Short half-life isotopes

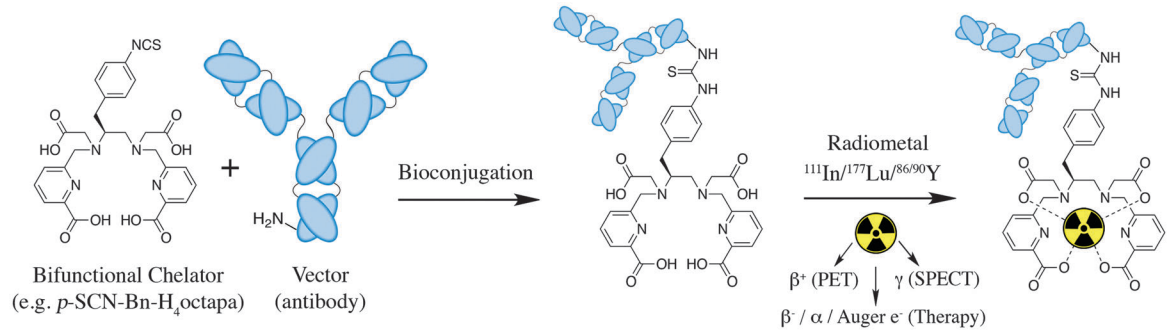

Fig. 2 Illustration of an archetypal radiometal-based radiopharmaceutical agent containing a bifunctional chelator (BFC) conjugated to a targeting vector (*e.g.* antibody, peptide, nanoparticle) using a variety of conjugation methods (*e.g.* isothiocyanate–amine coupling, peptide coupling, maleimide–thiol coupling, activated ester amide coupling, click-coupling) and then radiolabeled with a radiometal ion (*e.g.* $^{111}In^{3+}/^{177}Lu^{3+}/^{86/90}Y^{3+}$).

like ^{68}Ga are ideally matched with chelators that can radiolabel rapidly (fast radiolabeling kinetics). Longer half-life isotopes such as ^{111}In and ^{177}Lu allow for extended reaction times, but even if the half-life allows for long reaction times, completing the radiolabeling portion of radiopharmaceutical preparation is most convenient if finished in less than 10 or 15 minutes. As previously mentioned, room temperature radiolabeling is crucial for sensitive antibody vectors, which are degraded at elevated temperatures. DOTA inconveniently requires elevated temperatures for radiolabeling with essentially all radiometals (*e.g.* ^{44}Sc, ^{111}In, ^{177}Lu, $^{86/90}$Y, ^{225}Ac) but its abundant application in radiochemistry for decades, its exceptional *in vivo* stability, and the commercial availability of many different bifunctional DOTA derivatives and vector conjugates means that it is likely the most commonly used chelator to this day. Moving forward to the design and testing of new chelators, fast room temperature radiolabeling kinetics should be a priority; however, fast kinetics of radiometal incorporation (on-rate) and consequently low energetic barriers to radiometal–chelate complexation can also mean fast radiometal decorporation (off-rates) and low energetic barriers to radiometal release (Fig. 3). An arduous balancing act is required to obtain the best set of chelate properties for each application and radiometal ion, requiring the study and availability of a broad selection of different chelators with a variety of properties from which to choose.

When a chelator is identified through early screening to possess radiolabeling properties that are suitable for use with a particular radiometal ion, it must then be experimentally determined to be highly stable and inert. Further experiments are performed with the specific radiometal–chelate complex under conditions relevant to *in vivo* translation to judge its potential as the core component of a radiometal-based radiopharmaceutical. The result of radiometal loss from a radiopharmaceutical *in vivo* is the non-targeted distribution of the "free" radiometal ion in the body, and its exact fate and distribution in the body depends on the properties and biological behavior of the specific radiometal ion in question (Fig. 3). For example, ^{89}Zr and ^{68}Ga are known to accumulate in the bone when released from a BFC, where ^{64}Cu is known to accumulate in the liver. The fate of these radiometal ions can be tracked using PET/SPECT imaging in the living animal, and/or biodistribution experiments where animals are euthanized at predetermined time points, their organs harvested, and the distribution of radioactivity measured and calculated for the percentage of injected dose of radioactivity per gram of tissue (%ID/g). Each metal ion has its own unique properties that must be considered when constructing a radiometal-based imaging/therapeutic agent, such as its aqueous hydrolysis chemistry, redox chemistry, and affinity for native biological chelators.

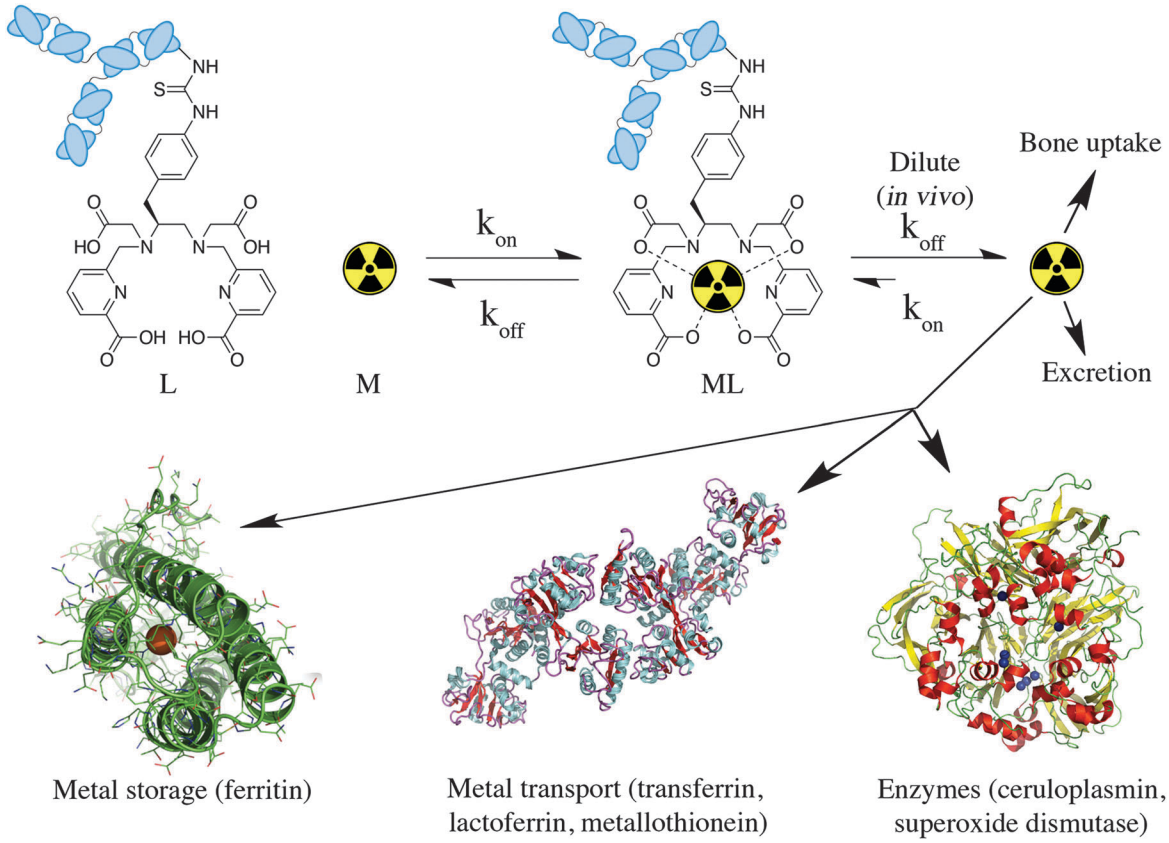

Fig. 3 Cartoon depiction of metal ion coordination kinetics, enhanced off-rate kinetics *in vivo* (extremely dilute conditions), and possible routes of radiometal ion loss *in vivo* (solid-state structures of ferritin H-chain homopolymer PDB file 1FHA, ceruloplasmin PDB file 2J5W, and *apo*-transferrin PDB file 2HAV shown).

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