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SOD mimics: From the tool box of the chemists to cellular studies

Clotilde Policar, Jean Bouvet, H el ene C. Bertrand and Nicolas Delsuc



Abstract

Superoxide dismutases (SODs) are metalloproteins that protect cells against oxidative stress by controlling the concentration of superoxide (O_2^-) through catalysis of its dismutation. The activity of superoxide dismutases can be mimicked by low-molecular-weight complexes having potential therapeutic applications. This review presents recent strategies for designing efficient SOD mimics, from molecular metal complexes to nanomaterials. Studies of these systems in cells reveal that some SOD mimics, designed to react directly with superoxide, may also indirectly enhance the cellular antioxidant arsenal. Finally, a good understanding of the bioactivity requires information on the cell-penetration, speciation, and subcellular location of the SOD mimics: we will describe recent studies and new techniques that open opportunities for characterizing SOD mimics in biological environments.

Addresses

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Keywords

SOD mimics or mimetics, Design of metallodrugs, X-fluorescence imaging or X-fluorescence microscopy XFM, Cellular studies.

Abbreviations

BNP/SNP, Boehmite nanoparticles/silica nanoparticles; COVID, Coronavirus disease; cyt c, Ferricytochrome c; DMSO, Dimethyl sulfoxide; EDTA, Ethylenediaminetetraacetic acid or 2,2',2'',2'''-(ethane-1,2-diyl-dinitrilo)tetraacetic acid; EPR, Electron paramagnetic resonance; H,P, and T, HEPES, phosphate and TRIS buffers; ICP-MS, Inductively-coupled plasma mass spectrometry; IECs, Intestinal epithelial cells; Keap1-nrf2, Kelch-like ECH-associated protein 1 - nuclear factor erythroid 2-related factor 2; LC-MS/MS, Liquid Chromatography coupled with tandem mass spectrometry; MCF, McCord and Fridovich assay; MCM-41, Mesoporous MCM-41 silica; MnPs, Mn-porphyrins; MOF, Metal-organic frameworks; NBT / XTT, Nitro blue tetrazolium / 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxianilide;

NHE, Normal Hydrogen Electrode; PAM, Poly-aza-macrocycles; PFA, Paraformaldehyde; PR, Pulsed radiolysis; RNS/ROS, Reactive nitrogen species/reactive oxygen species; SBA-15, Mesoporous SBA-15 silica; SF, Stopped-flow; SOD, Superoxide dismutase; XAS/XFM, X-Ray absorption spectroscopy/X-Ray fluorescence microscopy.

Introduction

Superoxide dismutases (SODs) are metalloproteins involved in the cellular protection against superoxide, a reactive species derived from dioxygen by one-electron reduction. Superoxide (O_2^- [1]) is a metastable radical anion endowed with both signaling and deleterious properties [2,3]. SODs participate in the control of its cellular concentration (about 10 pM in mammalian cells [4]) by catalyzing its dismutation (1), resulting from two mono-electronic processes (2) and (3), with (1) = (2) + (3)



SODs are remarkably efficient proteins that catalyze superoxide dismutation with kinetics only limited by diffusion (ca. $10^9 \text{ mol}^{-1}\text{Ls}^{-1}$). Four families of SODs use different metallic redox couples, Cu(II/I)–Zn(II), Cu(II/I), Fe(III/II), Mn(III/II) or Ni(III/II) [2,5], with a redox potential in a very narrow range (ca. 0.30 V/NHE) [2,6]. Optimal kinetics is expected for a potential at 0.36 V/NHE [6,7], the mid-point potential [8,9] of the two couples involved in superoxide dismutation ($E_{\text{pH7}}^{\circ}(\text{O}_{2\text{aq}}/\text{O}_2^-) = -0.18 \text{ V/NHE}$ and $E_{\text{pH7}}^{\circ}(\text{O}_2^-/\text{H}_2\text{O}_2) = 0.91 \text{ V/NHE}$, apparent redox potentials at pH7 and taking into account dioxygen low solubility in water [10]). This pinpoints the redox potential as a key parameter, but electrostatic attraction by a positively charged funnel described for all SODs, except Cu-only SOD in which the Cu active site is open to solvent [11], also contributes to the fast, only diffusion-limited, reaction with superoxide [2]. Oxidative stress, described as an imbalance between the flow of reactive species and protective pathways, is associated with a range of physiological conditions. Chemists can take inspiration

from nature to design complexes or materials reproducing SOD activity [6], and that could be used for therapeutic applications [12–14]. These aspects have been extensively reviewed [6,13,15–19] along with the therapeutic applications of SOD mimics [12,14,20,21]. Note that the SOD mimics discussed here are presented in both the **Scheme 1** (molecular structures and abbreviations) and **Table 1** (physicochemical properties and references), Cp standing for compound and Pep for peptides.

The redox dependence mentioned earlier was not always experimentally observed for SOD mimics, suggesting that other parameters could be key players. Various studies on a range of SOD mimics, with Mn^{II} (Cp22–23, Cp24–28), Cu^{II} (Cp11,14), or Ni^{II} (Pep18), have shown the importance of a coordinated water molecule [8,22–25]. This can be rationalized either by a fast exchange with superoxide, in an inner-sphere electron transfer requiring that superoxide enters the coordination sphere of the metal ion [25] involving a M-OO intermediate, or through the effect of proton donation to the reduced peroxide to lead to H₂O₂ release [26]. Second coordination sphere effects have also been described, revealing a possible fine modulation of the Mn-OO bond's strength upon variation of electronic effects on a Schiff-base ligand (Cp38–43) [27]. The dimerization of monometallic Mn(II) complexes upon reaction with superoxide was also observed [27], a known process leading to Mn(III)–Mn(IV) μ -oxo complexes [6,8] that are 'dead-end' for superoxide dismutation activity. This dimerization process was shown to be prevented by a sterically hindered metal ion environment [8]. Bulky substituents can also favor catalysis by modulating the stability of intermediates of the catalytic cycle (M–OO). Indeed, isopropyl moieties (Cp9) were shown to preclude hydrogen bonds that stabilize a Cu-peroxo intermediate (with Cp10) [28]. This was in favor of catalysis, as a too stable intermediate would disfavor the release of the product (H₂O₂). Several studies of Cu(II) or Mn(II) complexes with cyclic polyamines also suggested structural effects that could finely modulate the SOD-like activity: flexibility of the ligand that would permit molecular rearrangements of the complex along the catalytic pathway [29], or that would favor Mn(II) oxidation to the Jahn-Teller distorted Mn(III) complex [30], in relation to redox tuning.

Below, we describe selected recent strategies for designing efficient SOD mimics and discuss the importance of cell studies to understand, and possibly improve, bioactivity, including the investigation of cellular pathways and of SOD mimics cellular fate with innovative imaging approaches.

New directions in the design of SOD mimics: from low molecular-weight complexes to nanomaterials

Peptide-based complexes

Peptides have been used as ligands to generate metal complexes mimicking the different SOD enzymes.

Linear short sequences (Pep4–17) engaged in complexes with Cu^{II} [31,32] or Ni^{II} [33] cations have shown promising intrinsic catalytic activities. To circumvent the high conformational flexibility of peptides that entropically disfavors coordination [34], a cyclic peptide (Pep3) [35] or preorganized peptide scaffolds as *de novo* mini-proteins [36] have been used. A strategy involving the generation of a library of peptides screened with metal ions through an activity-based assay has led to the characterization of a copper complex mimicking SOD (Pep1), efficient even in cellular models of oxidative stress [37]. This strategy has been successfully extended to the discovery of the first peptidyl dicopper(II) complex (Pep2) mimicking the catalase enzyme [38]. Interestingly, depending on the activity assay used for the screening, complexes possessing only the desired activity were selected—desired activity, namely, monoelectronic SOD activity with a monometallic peptide complex or bi-electronic catalase activity with a dicopper complex [37,38].

Redox-ligand based SOD mimics

So far, there are no known SODs using an organic redox cofactor, probably because monoelectronic redox processes could lead to reactive radical intermediates. However, Goldsmith et al. have prepared a Zn(II)-complex with a di-hydroxybenzyl ligand (Cp34) Zn^{II}-OPh-OH that could potentially lead to a Zn^{II}-phenoxyl-radical ($[Zn^{II-\circ}OPh-O^- \leftrightarrow Zn^{II-}OPh-O^\circ]$) [39,40]. This Zn^{II}-phenoxyl radical acted as a SOD mimic, cycling between a Zn^{II}-*para*-quinone and a Zn^{II}-phenoxyl radical. This reactivity is associated with the di-hydroxybenzyl moiety, with its rich monoelectronic chemistry involving radical-phenoxyl and quinone. Whereas Zn^{II} analogs of some Mn^{II} SOD mimics with a bound phenol (Cp29) have been shown to be redox inactive [41–44], the Zn^{II}-OPh-OH complex (Cp34) was found electroactive, with a quasi-reversible wave at 0.35 V/NHE, very close to the optimal redox potential for superoxide dismutation (0.36 V/NHE). Zn^{II} was suggested to play a double role, attracting the anion substrate [45] and facilitating the cycling between the phenoxyl radical and quinone. In 2021, the mechanism of superoxide dismutation was investigated for Mn(II) analogs (Cp35–36) using cryospray-ionization mass spectrometry (CSI-MS), and an intricate mechanism involving quinone intermediates, but also a quinone-Mn^{IV}=O, was beautifully characterized [46].

Nanoparticles

Nanoparticles have been developed, either through the incorporation of known discrete SOD mimics in non-active materials (silica, alumina) or by the use of metal-based materials, including metallic nanoparticles or metal–organic frameworks, in which the basic structural unit can be responsible for antioxidant

Table 1

Names, abbreviations, redox potentials, and catalytic activities of SOD mimics mentioned here.

SOD mimics	Abbreviation	$E_{1/2}$ (mV vs. NHE)	$\log k_{cat}(\text{O}_2^-)$: method _{detector} (pH) = $\log k_{cat}(\text{buffer})$	References
Porphyrins				
Mn ^{III} TE-2-PyP ⁵⁺	Cp1	+228	MCF _{cyt c} (7.8) = 7.76 (P)	[20,86]
Mn ^{III} TnHex-2-PyP ⁵⁺	Cp2	+314	MCF _{cyt c} (7.8) = 7.48 (P)	[20]
Mn ^{III} TnBuOE-2-PyP ⁵⁺	Cp3	+277	MCF _{cyt c} (7.8) = 7.83 (P)	[12]
Mn ^{III} TBAP ³⁻	Cp4	-194	MCF _{cyt c} (7.8) = 3.16 (P)	[20,86]
Fe ^{III} (P ²⁺) (H ₂ O) (OH) ⁴⁺	Cp5	+298	–	[26]
Mn ^{III} (P ²⁺) (H ₂ O) ₂ ⁵⁺	Cp6	+270	–	[26]
Fe ^{III} (P ⁶⁺) (H ₂ O) (OH) ⁸⁺	Cp7	+249	–	[26]
Mn ^{III} (P ⁶⁺) (H ₂ O) ₂ ⁹⁺	Cp8	+200	–	[26]
PAM				
[Cu ^{II} L1(OH ₂) ₂] ²⁺	Cp9	–	MCF _{NBT} (7.4) = 7.14 (T)	[28]
[Cu ^{II} L2(OH ₂) ₂] ²⁺	Cp10	–	MCF _{NBT} (7.4) = 6.86 (T)	[28]
Cu ^{II} ₂ -L3	Cp11	+57	MCF _{NBT} (7.4) = 6.73 (H)	[23]
Cu ^{II} ₂ -BNP-L3	Cp12	–	MCF _{NBT} (7.4) = 7.63 (H)	[23]
Cu ^{II} ₂ -SNP-L3	Cp13	–	MCF _{NBT} (7.4) = 6.09 (H)	[23]
Cu ^{II} ₂ -L4	Cp14	-33	MCF _{NBT} (7.4) = 7.07 (H)	[23]
Cu ^{II} ₂ -BNP-L4	Cp15	-78	MCF _{NBT} (7.4) = 7.56 (H)	[23]
Cu ^{II} ₂ -SNP-L4	Cp16	–	MCF _{NBT} (7.4) = 6.98 (H)	[23]
Mn ^{II} pyane	Cp17	–	SF(7.4) = 7.44 (H)/6.92 (P)	[79]
Mn ^{II} pyane-pyrene	Cp18	–	SF(7.4) = 6.98 (H)/6.46 (P)	[79]
Mn ^{II} pyane-rhodB	Cp19	–	SF(7.4) = 7.08 (H)/6.54 (P)	[79]
Mn ^{II} M40403 (Imisopasem)	Cp20	+525	PR(7.4) = 6.55 (P)	[30]
Mn ^{II} GC4419 (Avasopasem)	Cp21	–	–	–
Mn ^{II} 3,6-PC2A	Cp22	+925	MCF _{NBT} (7.8) = 5.01 (P)	[22]
Mn ^{II} 3,9-PC2A	Cp23	+794	MCF _{NBT} (7.8) = 4.29 (P)	[22]
Amine Centered				
[Mn ^{II} (TMIMA) ₂] ²⁺	Cp24	+704	MCF _{cyt c} (7.8) = 6.56 (P)	[8]
[Mn ^{II} (BMPG)] ⁺	Cp25	+549	MCF _{cyt c} (7.8) = 6.68 (P)	[8]
[Mn ^{II} (IPG)] ⁺	Cp26	+759	MCF _{cyt c} (7.8) = 6.28 (P)	[8]
[Mn ^{II} (BIG)] ⁺	Cp27	+789	PR(7.8) = 6.58 (P)	[8]
[Mn ^{III} (PI) ₂] ⁺	Cp28	+514	MCF _{cyt c} (7.8) = 6.18 (P)	[8]
[Mn ^{III} (PI) ₂] ⁺	Cp28	+514	PR(7.8) = 6.15 (P)	[87]
[Mn ^{III} (PI) ₂] ⁺	Cp28	+514	MCF _{cyt c} (7.8) = 6.82 (P)	[8]
Salans				
Mn ^{II} 1 ⁺	Cp29	+440	MCF _{cyt c} (7.8) = 6.85 (P)	[42]
Mn ^{II} 1-Re ⁺	Cp30	+487	SF(7.4) = 6.70(H)	[88]
Mn ^{II} 1-Re ⁺	Cp30	+487	MCF _{cyt c} (7.8) = 6.81 (P)	[42]
Mn ^{II} 1-R ₉ ¹⁰⁺	Cp31	+419	SF(7.8) = 6.81 (M)	[43]
Mn ^{II} 1-RW ₉ ⁷⁺	Cp32	–	SF(7.4) = 6.70 (H)	[43]
Mn ^{II} 1-MPP ³⁺	Cp33	+404	SF(7.4) = 7.24 (H)	[43]
[Zn ^{II} (Hqp1)] ⁺	Cp34	+305	SF(7.4) = 7.34 (H)	[43]
[Mn ^{II} (ptp1) (H ₂ O)] ⁺	Cp35	+305	SF(7.4) = 6.53 (H)/7.28 (P)	[39]
[Mn ^{II} (Hqp1) (H ₂ O)] ⁺	Cp36	–	SF(7.4) = 6.56 (H)/6.04 (P)	[46]
[Mn ^{II} (H ₂ qp2) (H ₂ O)]	Cp37	–	SF(7.4) = 7.99 (H)/6.90 (P)	[46]
[Mn ^{II} (H ₃ qp2) (H ₂ O)] ⁺	Cp37	–	SF(7.4) = 7.08 (H)/7.00 (P)	[46]
Salans				
–	Cp38	+91	MCF _{NBT} (7.8) = 6.19 (P)	[27]
–	Cp39	+159	MCF _{NBT} (7.8) = 6.18 (P)	[27]
–	Cp40	+274	MCF _{NBT} (7.8) = 5.97 (P)	[27]
–	Cp41	+315	MCF _{NBT} (7.8) = 6.00 (P)	[27]
–	Cp42	+454	MCF _{NBT} (7.8) = 5.81 (P)	[27]
–	Cp43	+720	MCF _{NBT} (7.8) = 6.07 (P)	[27]
Bimetallic Cu–Zn and Cu–Cu				
[Cu ^{II} Zn ^{II} (dien) ₂ (μ-Im)](ClO ₄) ₃	Cp44	–	MCF _{NBT} (7.8) = 6.81 (P)	[89]
[Cu ^{II} Zn ^{II} (dien) ₂ (μ-Im)](ClO ₄) ₃ @MCM-41	Cp45	–	MCF _{NBT} (7.8) = 7.79 (P)	[47]
[Cu ^{II} Zn ^{II} (dien) ₂ (μ-Im)](ClO ₄) ₃ @SBA-15	Cp46	–	MCF _{NBT} (7.8) = 7.40 (P)	[48]
[Cu ^{II} ₂ (dien) ₂ (μ-Im)](ClO ₄) ₃	Cp47	–	MCF _{NBT} (7.8) = 6.89 (P)	[89]
[Cu ^{II} ₂ (dien) ₂ (μ-Im)](ClO ₄) ₃ @MCM-41	Cp48	–	MCF _{NBT} (7.8) = 7.71 (P)	[47]
[Cu ^{II} ₂ (dien) ₂ (μ-Im)](ClO ₄) ₃ @SBA-15	Cp49	–	MCF _{NBT} (7.8) = 7.37 (P)	[48]
Peptides				

(continued on next page)

Table 1 (continued)

SOD mimics	Abbreviation	$E_{1/2}$ (mV vs. NHE)	$\log k_{cat}(\text{O}_2^-)$: method _{detector} (pH) = $\log k_{cat}(\text{buffer})$	References
OCP1- Cu ^{II} Ac-PDHKHHKH-NH ₂	Pep1	+250	MCF _{XTT} (7.4) = 7.38 (H)	[37]
CATm1-Cu ^{II} ₂ Ac-PHYKHLH-OH	Pep2	+345	—(no SOD activity, but a catalase activity)	[38]
Cu ^{II} /Cu ^{II} ₂ /Cu ^{II} Zn ^{II} cyclic(HKHGPG) ₂	Pep3	—	MCF _{NBT} (7.4) = 6.67/6.71/6.92 (T)	[35]
Ac-HAAHVH-NH ₂ [Cu ^{II} H ₋₁ L5] ⁺	Pep4	+202	MCF _{NBT} (7.4) = 7.59 (P)	[31]
Ac-HAAHGH-NH ₂ [Cu ^{II} H ₋₁ L6] ⁺	Pep5	+214	MCF _{NBT} (7.4) = 7.79 (P)	[31]
Ac-HGGHGH-NH ₂ [Cu ^{II} H ₋₁ L7] ⁺	Pep6	+230	—	[31]
Ac-HGGGGHGH-NH ₂ [Cu ^{II} H ₋₁ L8] ⁺ [Cu ^{II} H ₋₂ L8]	Pep7	+247	MCF _{NBT} (7.4) = 7.56 (P)	[31]
Ac-HHGH-NH ₂ [Cu ^{II} L9] ²⁺ [Cu ^{II} H ₋₁ L9] ⁺	Pep8	+313/+264	—	[31]
Ac-S2H3-NH ₂ [Cu ^{II} L10] ²⁺ [Cu ^{II} H ₋₁ L10] ⁺	Pep9	+293	MCF _{NBT} (7.4) = 7.30 (P)	[31]
Ac-S3H4-NH ₂ [Cu ^{II} L11] ²⁺	Pep10	—	MCF _{NBT} (7.4) = 7.81 (P)	[31]
Cu ^{II} Ac-HKHKH-NH ₂	Pep11	—	—	[32]
Cu ^{II} Ac-HHKHKKH-NH ₂	Pep12	—	—	[32]
Cu ^{II} Ac-KAHEFGHSLGLDHSK-NH ₂	Pep13	—	—	[32]
Cu ^{II} HADHDHKK-NH ₂	Pep14	—	—	[32]
Cu ^{II} Ac-RHQAGPPSHR-NH ₂	Pep15	—	—	[32]
Ni ^{II} HHDLPQGVY-NH ₂	Pep16	—	MCF _{NBT} (7.8) = 4.85 (P)	[33]
Ni ^{II} HCDLPHGVY-NH ₂	Pep17	—	MCF _{NBT} (7.8) = 3.89 (P)	[33]
[Ni ^{II} L ^{3S} (OH ₂) ⁻]	Pep18	Epa = +724 (100 mV/s)	SF(7.75) = 5.26(H)	[24]
Nanoparticles				
CeO ₂ NP	—	—	MCF _{cyt c} (7.2) = 9.56 (T) ^a	[51]
MnO NP (8 nm size)	—	—	MCF _{cyt c} (7.4) = 9.38 (P) ^a	[54]
Mn ₃ O ₄ NP	—	—	20 μg/mL eq. 1U/mL SOD ^{a,b}	[56]
Cu-TBAP nanodots	—	—	12.5% of SOD activity ^{a,c}	[62]

Half-wave redox potentials ($E_{1/2}$) have been converted and expressed in mV vs. NHE (Normal Hydrogen Electrode) for comparison, with $E_{1/2} = (E_{\text{anodic peak}} + E_{\text{cathodic peak}})/2$. The intrinsic activity of the SOD mimics $\log k_{cat}(\text{O}_2^-)$; hence, the kinetics of reaction with superoxide out of any cellular context [6], is displayed under the form $\text{method}_{\text{detector}}(\text{pH}) = X$ (Buffer) indicating method used (MCF, SF, PR, see below), buffer, pH. When several buffers are mentioned: P corresponds to a phosphate buffer whereas H is for HEPES, T is for TRIS buffer, and M for MOPS buffer, concentrations are not shown.

'MCF' refers to the rate of the constant back-calculated from the McCord and Fridovich assay [90]. This assay is a kinetic competitive assay between the SOD mimic studied and a detector. In this assay, superoxide is produced by xanthine oxidase as a slow flow. Several UV-visible detectors have been used in the literature, including nitro blue tetrazolium 'NBT', ferricytochrome *c* 'cyt *c*', 2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2*H*-tetrazolium-5-carboxianilide 'XTT' as a detector. The IC_{50} is the concentration of the SOD mimic that reduces the reaction rate of the detector with superoxide by a factor of 2. Then $k_{cat} = k_{\text{detector}} \times [\text{detector}] / \text{IC}_{50}$ [6,8,91]. Back-calculating the k_{cat} was only possible when the authors explicitly gave the concentration used for the detector.

'PR' stands for pulsed radiolysis and 'SF' for Stopped-Flow (see Ref. [92] for a comprehensive description of the methodology). Both in PR and SF, a high concentration of superoxide is provided in one go at the beginning of the kinetics measurement.

In these direct methods for the kinetics evaluation (PR or SF), superoxide can be given in excess to the catalyst, ensuring several turnovers are possible. In the MCF assay, which is an indirect assay, the superoxide is provided at a continuous slow rate close to that encountered in biological systems. Indirect and direct methods are thus complementary.

^a $\log(k_{cat})$ determined per mole NPs.

^b Method using the production of superoxide with xanthine oxidase and a fluorescent or EPR-based detection, with respectively hydroethidine or 5,5-dimethyl-1-pyridine N-oxide.

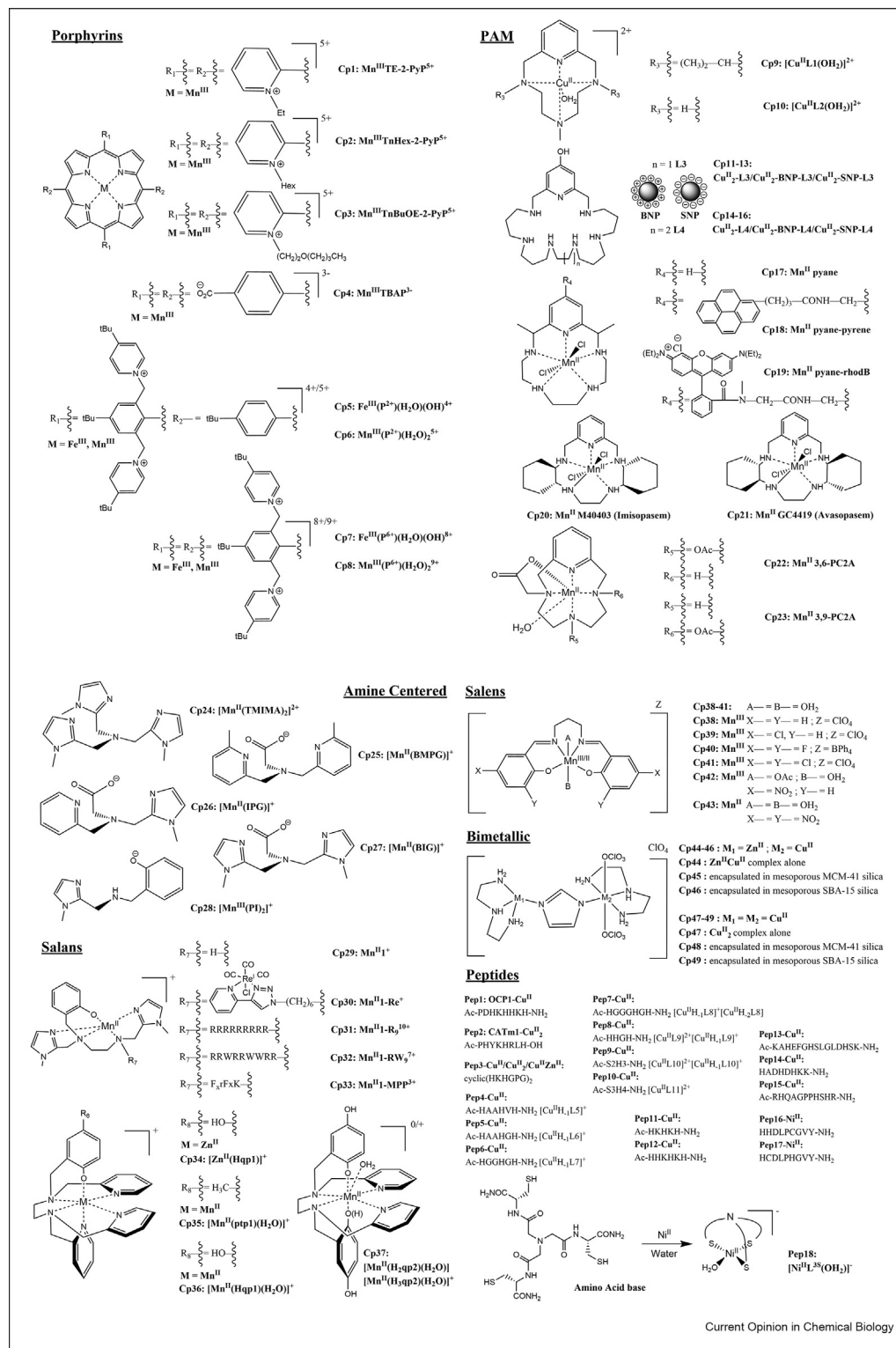
^c MCF-type assay, using superoxide generated upon the UV irradiation of riboflavin and methionine and NBT as a detector.

activity. These two different approaches are described in the sections below.

Loading Cu-M imidazolate-bridged complexes ($M = \text{Cu}^{\text{II}}$ or Zn^{II} , Cp44 and 47) in silica nanopores of different sizes (MCM-41 [47] or SBA-15 [48], Cp45,46 and 48,49) led to improved catalysis of superoxide dismutation more pronounced in the case of the smallest pores (MCM-41 [47]; see Table 1). The matrix was suggested to maintain Cu and H-Im-Zn(II) in close vicinity in the course of the catalytic cycle

[47], during which the imidazolate has been described to decoordinate from Cu(II) upon reduction to Cu(I). Garcia-Espana et al. have grafted hepta-azacyclophane ligands (Cp11,14) in a positively charged boehmite, with a significant enhancement of the antisuperoxide activity [49], as opposed to the negatively charged silica that produced no enhancement (see Table 1). This effect was attributed to the positively charged scaffold, which should help in driving O_2^- to the active site, mimicking the positively charged funnel of native enzymes [23].

Scheme 1



Structures of the SOD mimics mentioned here. Cp stands for compound, Pep for peptides, BNP for boehmite nanoparticles, SNP for silica nanoparticles, S for sarcosyl, MCM-41 for mesoporous MCM-41 silica, SBA-15 for mesoporous SBA-15. Core structures of five SOD mimic families—porphyrins, poly-aza-macrocycles (PAM), ligands amine centered, salan-type ligands, salen-type ligands, bimetallic—are given with variable side chains (R_x). For some of the molecules, the name/code from the original papers is also reminded.

Metal-based nanomaterials display a high density in metal centers that can act as catalytically active redox sites for catalysis [50]. The SOD-like activities of these materials were evaluated qualitatively or reported *per mole of NPs*, as k_{cat} or in the percentage of SOD activity (see Table 1). However, these values are difficult to compare with those determined for molecular catalysts, as there is a high number of accessible catalytic sites per NPs. Nonetheless, these materials display valuable antioxidant activity, approaching those of SOD on a *per mole NPs* basis. After the pioneering work of Self et al. on ceria nanoparticles [51], later encapsulated in various protein scaffolds [52,53], Mn-based nanoparticles (e.g. Mn_3O_4) [54–56] or Fe-based Prussian blue [57–59] were recently characterized for their multiple catalytic activities—SOD, catalase and GSH-peroxidase—possibly retained in cells or *in vivo* [55]. Interestingly, Singh et al. have evidenced that the supernatants of Mn_3O_4 NPs were not active, nicely ruling out the possible involvement of leached ion in the activity [55]. The activities were dependent on the shape and size of the particles [55]. In these condensed systems, the clustering effect of the substrate, proposed in another context [45], could be at stake. Metal–organic frameworks (MOF) have also been used as a scaffold to build metalloenzyme mimics [50], and a few MOF have been designed, either with dispersed copper [61] or using porphyrin (TBAP, see Scheme 1 for the porphyrine acronym) and copper(II) as building blocks [62] with activity reported to 12.5% that of SOD. These MOF are endowed with multiantioxidant activities, but also Fenton pro-oxidant effect [61,62]. Some of these materials have been applied as antioxidants or anti-inflammatory agents [54–57,59,62], others as anticancer agents, their anticancer activity being related to toxicity associated with pro-oxidant effect due to the overproduction of H_2O_2 and/or of HO° [60,61]. Note that similar anticancer activity has also been described for Mn-porphyrins and has already been extensively reviewed [21,63].

Studies in cells: pathways beyond direct antioxidant effects and physicochemical characterization in cells

An indirect mode of action enhancing the antioxidant cellular defenses

Recently, an increasing interest has grown about potential ‘side reactions’ performed more specifically by Mn-porphyrins (MnPs) with reactive oxygen species (ROS) or cellular compounds (ONOO^- , H_2O_2 , H_2S , $\text{CO}_3^{\bullet-}$, ascorbate, and GSH) [13] and their mechanism of action in cells [7,18,63]. As early as 2004, an increase in extracellular SOD and MnSOD was reported [64] after incubating cells with MnPs, suggesting that these compounds may act not solely as a direct antioxidant by reducing ROS concentration but also indirectly: they may induce antioxidant cell feedback through a pro-oxidant effect, enhancing the protective cell arsenal.

Batinic et al. have reported such a pro-oxidant effect of MnPs in 2012 [65,66]. They have extensively shown then that besides their reactions against ROS and reactive nitrogen species (RNS), MnPs display an effect on thiols regulation and cellular sulfur metabolism [7,18,63,67], which activates the Nrf2 [68] pathway. This is interesting as the Keap1-Nrf2 pathway [69,70] constitutes the major defense system against electrophilic and oxidative stress: Keap1 acts as a redox sensor through the oxidation or modification of its cysteine residues upon oxidative/electrophilic stress, which results in the Nrf2-induced up-regulation of several antioxidant enzymes (see Figure 1). It shows that compounds endowed with an overall antioxidant cellular activity may actually be active by the induction of cellular antioxidant feedback via the Keap1-Nrf2 pathway through a pro-oxidant effect. Interestingly, this activation would constitute a late but long-lasting effect, as opposed to an immediate catalytic antioxidant direct effect, but possibly lasting shorter if the antioxidant catalyst is metabolized.

Examples of SOD-mimics inducing this Nrf2 pathway, or not

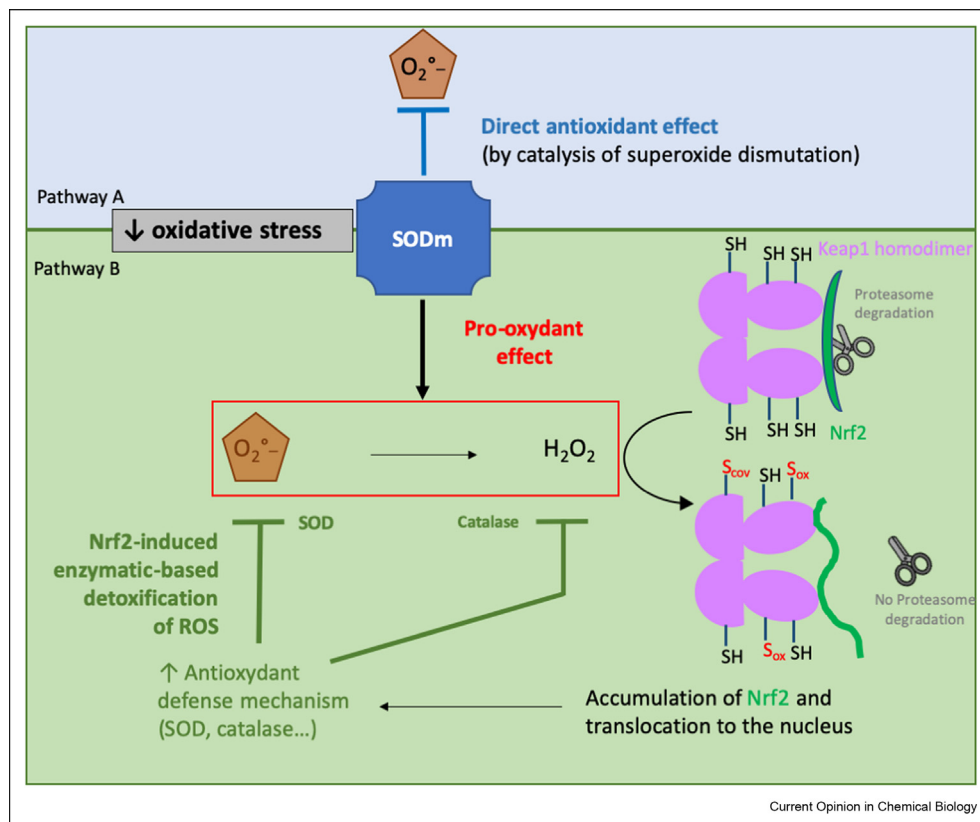
$\text{MnTnBuOE-2-PyP}^{5+}$ (Cp3) is an example of a SOD mimics inducing such an activity. This Mn-porphyrin has been shown to upregulate the expression of enzymes under the control of Nrf2, including NQO1, catalase and MnSOD, through Nrf2 activation in hematopoietic stem cells in transgenic mice [67]. The activation of Nrf2 is suggested to occur via Keap1’s S-glutathionylation. Similarly, a polyazamacrocyclic MnSOD mimic, M40403 (Cp20), was shown to increase the expression level of Nrf2 in the skin of diabetic rats [71]. But this scheme is not general. In contrast, the salan-type SOD mimics Cp29-31 reduce the overexpression of mitochondrial MnSOD associated with lipopolysaccharide-induced oxidative stress in intestinal epithelial cells (IECs), indicating that these SOD mimics can complement mitochondrial MnSOD. This result pinpoints a direct antioxidant effect as opposed to an activation of the Nrf2 pathway. No effect on cytosolic CuSOD or catalase was recorded, showing the importance of mitochondria and the specific management of superoxide [41–44].

Detection in cells

The biological efficiency of SOD mimics is linked to their intrinsic SOD activity and also to other parameters such as cellular uptake, subcellular distribution, and speciation in biological environments that are intricate and abound with Lewis bases [6].

Overall cellular uptake of metal complexes can be quantified using techniques such as inductive-coupled plasma mass spectrometry ICP-MS [42,44] whatever the metal ion (Mn, Fe, Cu, Zn, Ni), or specific

Figure 1



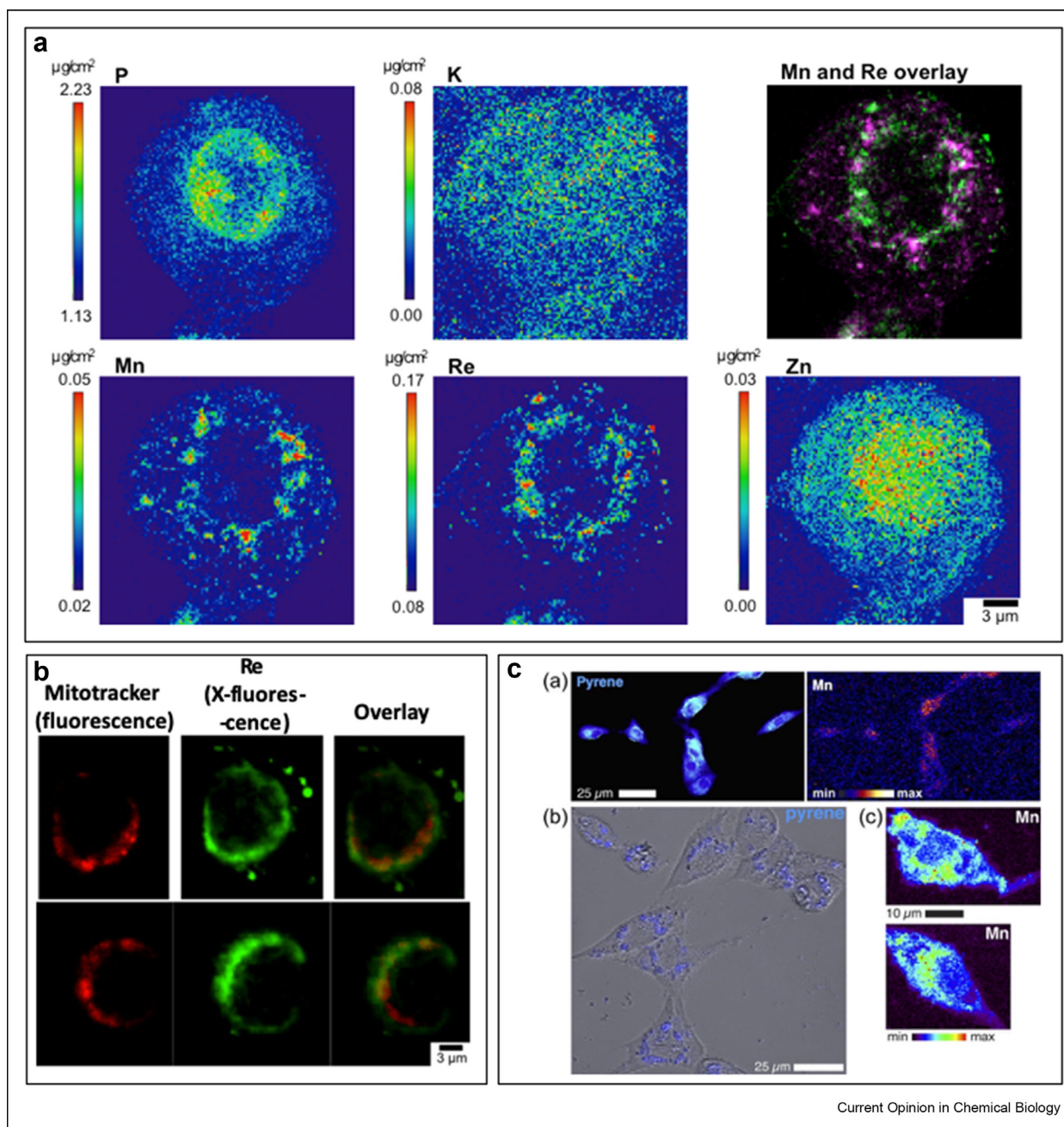
Schematic representation of the two possible pathways from the literature for the SOD mimics cellular activity. Pathway A corresponds to the direct effect of SOD mimics, with their ability to catalytically dismutate superoxide. Pathway B is associated with a pro-oxidant effect of SOD mimics: as they produce H₂O₂, they can activate the Nrf2-signaling pathway. Nrf2 is continuously produced in cells, and Keap1, by binding Nrf2 in a particular conformation, promotes its degradation by the proteasome. Oxidative or chemical stress is sensed by modification of Keap1 cysteines. Their covalent modification/oxidation by electrophiles or oxidants (S_{cov} or S_{ox}) leads to an inhibition of Nrf2 degradation by the proteasome. Nrf2 then accumulates in the cytosol and translocate to the nucleus, where it activates genes of a series of proteins, including antioxidant proteins such as catalase or SODs. The balance between these two pathways depends on the speed of dismutation (k_{cat}) in comparison with the cellular capacity to detoxify H₂O₂ and also on the H₂O₂ content of the cell. Pathway A may occur as soon as the SOD mimics enter the cells or the mitochondria but is also prone to decrease in time due to the SOD mimic degradation. In contrast, Pathway B requires time to activate protein synthesis (from 30 min to several hours), but this has a long-lasting effect as it works by boosting the cellular antioxidant feedback.

techniques such as EPR, available for instance in the case of $S = 5/2$ Mn^{II} [41–43,72], EPR and ICP-MS estimates being shown to be consistent [42]. In the case of MnPs, UV–vis spectroscopy of cell lysates [44,63,73] also gave estimates similar to ICP-MS [44]. Quantification of MnP in mice tissues, after injection or oral gavage, was performed using LC-MS/MS [74,75], and the trend of their penetration in the brain matched with the lipophilicity, the more lipophilic compounds (MnTnBuOE-2-PyP⁵⁺ (Cp3), MnTnHex-2-PyP⁵⁺ (Cp2)) being more concentrated in brain tissues such as cortex and hippocampus, whereas MnTE-2-PyP⁵⁺ (Cp1) was not detected in the brain. The Mn oxidation state in MnPs was investigated using resonant Raman spectroscopy in the 1250–1550 cm⁻¹ range in whole endothelial cells: MnTnBuOE-2-PyP⁵⁺ (Cp3) and MnTnHex-2-PyP⁵⁺ (Cp2) were predominantly in their

reduced Mn(II) form and present at the mitochondria, as seen by cellular fractionation [76]. The weaker stability of the PMn(II) redox state could make it prone to exchanges with Zn(II) [77,78], which does not seem to occur in cells, probably due to the low bioavailability of Zn(II) being sequestered at the active site of enzymes and mainly in the nucleus. Last but not least, X-ray absorption spectroscopy (XAS) can be used to quantify the relative content of metal complexes directly in cells prepared for X-ray fluorescence microscopy (XFM) (see below) [41,79,80] or in whole-cell pellets (freeze-dried) [79]. Note that applications of XAS and XFM in bio-inorganic research have been reviewed recently [81].

Distribution in cells is also a key to the understanding of bioactivity. A first paper showed different cellular distributions for two MnPs. MnTE-2-PyP⁵⁺ (Cp1) was

Figure 2



Imaging SOD mimics in cells. 2a. Elemental distribution of P, K, Mn, Re, and Zn in an HT29-MD2 cell incubated with Cp30. The phosphorus (P) and zinc (Zn, K-lines) maps were used to identify the nucleus area. The overlay (top right) corresponds to the Mn (magenta) and Re (L-lines) (green) maps. The regions corresponding to an overlap of both elements are displayed in white. Intestinal epithelial cells HT29-MD2 were incubated for 2 h with Cp30 (100 μM , 0.02% DMSO) before cryofixation and freeze-drying. Images were recorded on the 2-ID-D beamline of the APS synchrotron (excitation at 12.0 keV chosen to excite both Re and Mn; integration time, 2 s per pixel; pixel size, 200 nm). Scale bar, 3 μm . This figure shows the un-even and punctuated distribution in cells of Cp30. It was colocalized with a Mito Tracker previously mapped in classical fluorescence (not shown). The merging of Re and Mn maps shows areas where Re does not colocalize with Mn, revealing that partial decoordination occurred. **2b.** Confocal fluorescence images of HT29-MD2 cells incubated with a mitochondrial marker (MitotrackerTM, fluorescence) and X-fluorescence maps of Cp30 (Re, X-fluorescence). Intestinal epithelial cells HT29-MD2 were incubated for 1h30 with Cp30 (100 μM , 0.02% DMSO) before the addition of MitotrackerTM deep red (200 nM, 30 min), washing with 50 mM EDTA, cryofixation, and freeze-drying. Confocal fluorescence images of MitotrackerTM deep red (ex 633 nm, em 645–750 nm) were recorded with an open pinhole. X-fluorescence (Re) images were recorded on the Nanoscopium beamline of SOLEIL synchrotron (excitation at 14.1 keV; integration time, 2.4 s/pixel; pixel size, 300 nm). Scale bar, 3 μm . The overlay shows that part of the Re signal overlaps with the MitotrackerTM, suggesting that in these areas, Cp30 is localized in the mitochondria. This correlates with EPR titration that shows an accumulation of Mn in these organelles as compared to control cells, but to a lesser extent than Mn1 (Cp29). **2c.** Distributions of the pyrene tag and manganese, which are similar in SH-SY5Y cells treated with 200 μM Cp18 for 24 h. (a) Fixed cells imaged by both fluorescence microscopy (pyrene tag, left) and low-resolution XFM (manganese, right). (b) Confocal fluorescence microscopy image of the pyrene tag (blue) in living cells (bright field) treated with Cp18. (c) High-resolution elemental maps of manganese in chemically fixed (PFA) cells. Figure 2a and b from Ref. [42] and Figure 2c from Ref. [79].

found evenly distributed, while MnTnHex-2-PyP⁵⁺ (Cp2), known to be accumulated five times more than MnTE-2-PyP⁵⁺ in the mitochondria *in vivo* [17,77], displayed a perinuclear distribution within A549 cells [80]. Mathieu et al. have imaged SOD mimics Cp29-33 in freeze-dried HT29-MD2 cells. Whereas Cp29 was homogeneously distributed in cells [41], Cp30 showed a punctuated distribution localized around the nucleus (Figure 2a) [42]. Using quantification (ICP-MS) in a mitochondria-enriched fraction and by colocalization studies with a fluorescent mitochondria-tracker imaged prior to XFM (Figure 2b), Cp30 was found co-localized with this organelle [42]. In a comprehensive paper, Weekley et al. followed a range of Mn-PAMs (Cp17-19), functionalized with a fluorescent probe or not, using XAS of whole cells frozen pellets (see above) and XFM (Figure 2c). Some of the Mn-PAMs were shown to decompose into Mn(II)-phosphate [79]. In both studies [41,79], integration over the cell to determine a range of intracellular concentrations was used, from which a relative scaling for the penetration of various compounds, including MnCl₂, was proposed. Mathieu et al. suggested an indirect way to question the speciation of a Mn-complex (Cp30) using XFM imaging, tagging the ligand with heavy metal (by conjugation to a Re-trisCO probe [82]). The Re(ligand) and Mn X-ray fluorescence maps, recorded above the edges of both Re and Mn, were overlapping, but only partly: a few areas showed only Re, revealing some dissociation in cells [42]. In this study, the distribution was also studied both by cell fractionation and XFM, XFM presenting the advantage of fewer steps for sample preparation, which can minimize risks of artefactual contamination or relocalization. Weekley et al. have also studied the distribution of the Mn-PAMs functionalized by a fluorescent probe using both XFM [79] and classical fluorescence (Figure 2c), showing that the fluorescent probes could be cleaved in cells. They conclude with the importance of studying the fate of the complexes in cells.

Conclusion

All these studies highlight the importance of studying SOD mimics in biological environments. Cellular and *in vivo* studies are key to the understanding of their bioactivity, which is a prelude to their therapeutic applications [7,12,14,18,63]. Note that Cp29 was shown to alleviate inflammation in a colitis mice model [41] or reduce the side effects of a treatment by oxaliplatin [83]. Interestingly, Mn-porphyrins (MnTE-2-PyP⁵⁺ (Cp1) and MnTnBuOE-2-PyP⁵⁺ (Cp3)) [7] and Mn-PAMs (Cp20-21) [7,84] have advanced to several clinical trials, for anticancer (as sensitizer or radio-protector) or anti-inflammatory applications. Some recent applications of SOD mimics in the context of

COVID were investigated to reduce inflammation and cytokine storm [85], undergoing clinical trials (NCT04555096). Overall, the story of SOD mimics and recent developments show that both tool box of the chemists and the knowledge of the cellular and biological pathways are important in the development of compounds mimicking SODs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- * of special interest
- ** of outstanding interest

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Spectroscopic and magnetic analyses of these materials confirmed the metal centers of the complexes have kept the coordination sphere after insertion into the MCM-41 silica matrix. These complexes@MCM-41 showed a SOD activity increased by one order of magnitude in comparison to the nonencapsulated complexes (see ref. 89). This enhancement SOD was attributed to a matrix effect, the close-fitting of the complexes into the nanochannels of MCM-41 silica favoring the Cu active site and HlmZn(or Cu) group to stay in close proximity during catalysis.

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nanozymes produced a higher flow in H₂O₂. In the tumor environment, where the level of H₂O₂ is close to the toxic threshold, this can lead to toxicity, whereas no toxicity is seen in noncancerous cells, which have a lower level of H₂O₂. The nanozymes showed toxicity for breast cancer cell, with increase in lipid peroxidation, but had no effect on the proliferation of normal cells (HUVEC and LO₂). These nanozymes derived from MOF are thus promising for tumor chemotherapy.

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