Review

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Are Protein Cavities and Pockets Commonly Used by Redox Active Signalling Molecules?

John T. Hancock

School of Applied Sciences, University of the West of England, Bristol BS16 1QY, UK; email john.hancock@uwe.ac.uk; john.hancock@uwe.ac.uk; Tel.: +44-(0)1173282475

Abstract: It has been well known for a long time that inert gases, such as xenon (Xe), have significant biological effects. As these atoms are extremely unlikely to partake in direct chemical reactions with bio-molecules, such as proteins, lipids and nucleic acids, there must be some other mode of action to account for the effects reported. It has been shown that the topology of proteins allows for cavities and hydrophobic pockets, and it is through an interaction with such protein structures that inert gases are thought to have their action. Recently, it has been mooted that the relatively inert gas molecular hydrogen (H_2), also may have its effects through such a mechanism, and so influencing protein structures and actions. H_2 is thought to also act through interaction with redox active compounds, particularly the hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$), but not nitric oxide ($NO^{\cdot}$), superoxide anions ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2). However, instead of having a direct interaction with H_2, is there any evidence that these redox compounds can also interact with Xe pockets and cavities in proteins, so either having an independent effect on proteins or interfering with the action of inert gases. This suggestion with be explored here.

Keywords: argon; hydrogen peroxide; hydrogen sulfide; hydroxyl radicals; molecular hydrogen; nitric oxide; peroxynitrite; protein cavities; superoxide; xenon

1. Introduction

It is well recognized that under stress conditions cells produce a range of small signalling molecules [1,2]. Many of these are relatively reactive and have redox activity. Compounds which accumulate in cells include the reactive oxygen species (ROS [3]), such as hydroxyl radical ($^{\cdot}OH$), superoxide anions ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2), as well as reactive nitrogen species (RNS), such as nitric oxide ($NO^{\cdot}$ [4]) and peroxynitrite ($ONOO^-$). These compounds are produced by dedicated enzymes. This includes the NADPH oxide [5] for ROS (producing superoxide which can cascade in reactions to generate H_2O_2, for example), xanthine oxidoreductase (producing H_2O_2 or NO depending on oxygen (O_2) concentrations [6]), peroxidases metabolising H_2O_2 [7]. RNS are produced by nitric oxide synthase (NOS [8]), of which there are three isoforms in humans, and nitrate reductase (NR), which is important for plant NO generation and metabolism [9]. Hydrogen sulfide (H_2S) is also used in stress responses, and this produced by a range of enzymes in mammals: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST) [10].

Many of these signalling-active compounds are relatively toxic, so at high concentrations have a detrimental effect in cells. For example, H_2S has effects on mitochondrial function, and which partly accounts for its toxicity [11]. However, at low concentrations, these molecules have significant signalling roles, allow for stress tolerance, including during drought [12], pathogen challenge [13], salt stress [14], heavy metal stress [15], and extreme temperature tolerance [16].

The reactivity of ROS, RNS and H_2S partly accounts for the cellular responses seen. Many proteins contain one or more relatively reactive thiol groups, as side chains of cysteine residues. Such -SH groups can be deprotonated to the thiolate. If two cysteines are in the correct three-dimensional orientation they can react and combine to create a disulfide, cystine. This can add to the structural rigidity of a protein. On the other hand, thiols can be modified by a reaction with a range of reactive signalling molecules. H_2O_2 can lead to oxidation of thiol to create the sulphenic acid group [17], a modification which is reversible, so in a manner akin to phosphorylation, this oxidation can create a new protein topology, and hence activity, which can be readily reversed. Higher H_2O_2 concentrations

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can lead to higher oxidation states of the thiol, i.e., suphinic acid and sulphonic acid, and this leads to irreversible modification. NO leads to the modification of thiols to produce the -SNO group (*S*-nitrosylation [18]), again which can be reversed, in a similar manner to phosphorylation. H_2S leads to the persulfidation [19] of thiols to produce -SSH, again reversible. Such thiol modifications of proteins can account for the range of effects seen on ROS/RNS/H_2S accumulation. These reactive molecules can also react together, and create new reactive signalling molecules. Superoxide and NO can react, for example to create peroxynitrite, again a signalling molecule in its own right [20]. NO and H_2S will react to generate nitrosothiols [21], which can also act in a signalling role. Therefore, the reactivity of these molecules are important for their action in signalling.

Into this mix of reactive compounds the small relatively insert molecule H_2 needs to be added. H_2 has been shown to have effects in a range of disease conditions in humans, such as diabetes [22], degenerative disease [23] and even COVID-19 [24]. H_2 has been mooted as being reactive with hydroxyl radicals ($^{\cdot}OH$) and peroxynitrite [25]. It is thought to be unreactive with $O_2^{\cdot-}$, H_2O_2, or NO. It seems unlikely that H_2 has all its effects by interactions with $^{\cdot}OH$ or $ONOO^-$ and therefore some other mechanisms of action are probably pertinent to consider. H_2 may act through its redox action, for example, as argued previously [26]. However, it has been recently suggested that H_2 may mimic the action of other gases, i.e., it may interact with cavities and pockets in the structures of proteins [27]. By looking at how inert gases act may give an indication of how other molecules may have bio-activity. However, a question which could be posed here is: does this only apply to inert gases? Can other small signalling molecules, whether inert or not, have a similar mode of action? Here, some of the ideas and evidence are discussed.

2. Other inert gases

The classical insert gas which is known to have bio-activity is xenon (Xe). This has been known for a long time, at least since the 1940s [28]. Some of the actions of Xe include as an anaesthetic and it has cytoprotective effects [29]. There seems no doubt that Xe has physiological effects and acts on cellular function. However, if Xe is inert, how does it interact with bio-molecules?

Proteins are known to have complex, dynamic and often quite loose structures and this includes cavities and pockets in their topology. Into these cavities and pockets some small molecules may migrate. It is interesting to note that such cavities are often dubbed as "Xenon binding pockets", for example in an article by Duff and colleagues [30]. However, this does not mean that these cavities are exclusively occupied by Xe, or that the interaction of the small atom/molecule is a static event. Recent work by Turan *et al*. [31] shows that the Xe can migrate through channels in the protein, in this case myoglobin, and therefore there is a dynamic interaction taking place. Indeed, this work shows that myoglobin is in fact an allosteric protein.

The globin family has been used as a model system for studying Xe binding. This interaction has been known for a long time. For example, Schoenborn *et al*. [32] explored Xe binding to sperm whale myoglobin in the 1960s. Work continued to understand the interactions more, for example the work in the 1980s by Herman and Shankar [33] and Tilton *et al*. [34], and has continued to today, as exemplified by the work by Turan and colleagues [31]. Work with haemoglobin has a similar history, with reports spanning back to at least the 1960s [35], work taking place in the 1980s [36], and more recent work being reported [37].

However, Xe interacts with a range of proteins. Prangé *et al*. [38] list a range of proteins which were studied for the binding of Xe and Krypton (Kr). These include elastase, subtilisin, cutinase, collagenase, lysozyme, lipoamide dehydrogenase domain from the outer membrane protein P64k, urate-oxidase and the human nuclear retinoid-X receptor RXR-α. Rubin *et al*. studied maltose binding protein [39], whilst others have focused on copper amine oxidases [30,40], urate oxidase [41]. It has also been found that in humans Xe leads to an increase in erythropoietin by triggering an increased production of hypoxia-inducible factor 1α (HIF-1α) [42]. Clearly, there is a range of proteins which are able to bind inert gases with the potential for changes in their structures and therefore functions. In this vain Winkler *et al*. have reported an *in silico* screening to identify xenon protein targets [43].

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However, Xe is not the only inert gas which is known to have an influence on a cell's activity. Kr was mentioned above [38]. As with other gases this work spans back to at least the 1960s, when the solubility of Kr in biological materials was reported [44], whilst more recent work has shown that Kr nanobubbles inhibit the activity of pepsin [45]. Argon (Ar) is known to have neuroprotective [46] and organoprotective effects [47]. For example, it was shown to be neuroprotective in cerebral ischemia and brain injury in *in vitro* models [48]. Ar appears to have neuroprotection by inhibiting toll-like receptors, and it also has anti-apoptotic action [49]. Some of Ar's effects may also be through altering kinase signalling in neurons and glial cells [50]. Ar is also known to have narcotic effects. A review of argon's biological effects was published by Ye *et al*. [51], but seems certain is that Ar has effects on proteins, despite being inert.

Other inert gases worth considering here are Neon (Ne) and helium (He). Both have reported effects [52]. However, just because one gas has an effect does not mean they all do. Interestingly, in *Neurospora crassa* it was reported that the growth rate in the presence of inert gases was correlated to the molecular weight of those gases, and there was even a formula given: $R = 3.88 - 0.1785 (MW)^{½}$: where R is growth rate (in millimeters per hour at 30°C) [53]. In a neuronal injury model Xe was found to be protective, Ar and Kr had no effect, and He was detrimental effect [54], a trend also reported by Rivzi *et al*. [55]. Therefore, what is found with one gas cannot be automatically translated to the other gases. This needs to be borne in mind if we are going to extend this idea of small molecules binding to proteins across to a range of other compounds, such as ROS, RNS or H_2S.

As already mentioned, into this mix could be thrown H_2 [27]. There is no evidence that H_2 has an influence of the activity of proteins using such a mechanism. There are issues to be considered here. H_2 is extremely small, and perhaps it could be argued is too small to have such an effect. On the other hand, there is no reason to suspect that single H_2 molecules act alone, and until a thorough investigation of this possible mechanism of H_2 is carried out, there is no reason to rule this out, or indeed in, as a mode of action of H_2.

3. Do other small signalling molecule use Xe pockets?

It is clear that Xe is not the only gas which takes advantage of the cavities and channels in proteins. It is likely that other inert gases, eg Ar, Kr and He may have the same mode of action. However, the question being asked here is: can this mechanism of direct interaction with protein structures be extended to the action of small molecules which are known to have profound effects on cell signalling? Some of these candidate molecules are themselves gases, for example NO and H_2S, so perhaps this is not such as stretch to consider.

There are certainly some examples in the literature which would support small molecules interacting with Xe-binding pockets. For example, in haemoglobin, oxygen has been shown to migrate through Xe docking sites, whilst the protein is in the R-state [37]. Previously, using mutation in the protein, Scott and Gibson [56] had looked at the effects of Xe on O_2 binding to myoglobin. Furthermore, the migration and escape of both O_2 and carbon monoxide (CO) from myoglobin seems to take advantage of Xe-binding regions, and is influenced by the presence of Xe [57].

Nitrous oxide has effects on proteins and in many cases Xe is used as a model, or a method of investigation [41,58,59]. Using urate oxidase as a model Marassio *et al*. [41] report that "Xe and N_2O bind to, compete for, and expand the volume of a hydrophobic cavity" in the protein, and so this leads to inhibition of activity. Later the same group [58] argue that although both gases bind to proteins the mechanisms are not the same. With a focus on P450 monooxygenase LeBella *et al*. [59], it as shown that both Xe and nitrous oxide occupy a haem-pocket in the enzyme, and hence lead to inhibition. Therefore, here we have a gas which does not sit in the noble gas group, but does have biological activity and furthermore, seems to have a mechanism akin to that of the noble gases.

If such a mechanism can mediate the effects of nitrous oxide, is there scope to consider such a mechanism for other small signalling molecules?

In what are described as "gas pockets", Winter *et al*. explore how nitric oxide and other gases have their binding facilitated by the presence of hydrophobic cavity regions [60]. It has been suggested that NO can bind momentarily to alternate cavities in myoglobin. These cavities are some

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distance from the haem binding site and therefore not near the O_2 binding site [56,61,62]. Brunori [61] go on to say that the sites where NO bind are those which have been identified in myoglobin as binding Xe [63]. In haemoglobin it appears that NO has movement through tunnels in the protein structures. The paper discusses the presence of short and long tunnels, and emphasizes that hydrophobic residues at the entrances to such tunnels are important: Phe for the long tunnel and Ile for the short tunnel. The authors also point out that NO can "diffuse from Xe cavity to Xe cavity" [64]. NO has been shown to bind to the haem-pocket in horse radish peroxidase [65]. In a bacterial system it is thought that there is an interaction between nitrite reductase (NiR) and NO reductase (NOR), but of pertinence to the argument here, NO generated by the first enzyme (NiR) migrates to NOR, with that movement being facilitated by the NO translocating through a cavity in NiR and then a hydrophobic channel in NOR [66].

For other gases, such as carbon monoxide (CO), a similar situation has been reported. Chu *et al*. [67] asked if the binding of gases such as NO, O_2, CO or H_2 is a random event on proteins, but concluded that the migration of such ligands involved a limited number of pathways and are facilitated by the presence of specific docking sites on the protein [67]. Elber and Karplus [68] suggested that in myoglobin CO used Xe binding regions, a notion that more recently has been revisited and reported on [69]. In sperm whale myoglobin, Bossa *et al*. [70] also suggested that CO takes advantage of Xe pockets for binding and migration through the proteins, along what they describe as "additional packing defects". Others too have added to the weight of evidence that CO migrates through proteins such as myoglobin, facilitated by Xe-binding regions [71]. Using nitrogenase it was suggested that CO might migrate through the protein through a gas channel which is composed of a series of cavities [72]. Using CO as a probe in hydrogenases (*Dd*HydAB from *Desulfovibrio desulfuricans*; *Ca*HydA *Clostridium acetobutylicum*; *Cr*HydA1 from *Chlamydomonas reinhardtii*), it was shown that inhibition was dependant on the redox state of the H cluster but also the migration of the gas through the protein [73].

With the view to understanding how proteins may be useful for carbon capture – with the background of climate change – Cundari *et al*. [74] suggested that CO_2 binding to proteins is facilitated by acid/base interactions, and that β-sheet structures are better than α-helices.

But what of ROS, which are thought to have many of their effects through thiol modification? Using 4-hydroxybenzoate hydroxylase (PHBH) and phenol hydroxylase (PHHY), Hiromoto *et al*. [75] suggested that their data implied that hydrophobic pockets served as binding sites for H_2O_2. In cholesterol oxidase it was found that both O_2 and H_2O_2 are able to interact with a hydrophobic tunnel in the protein [76]. Zhao *et al*. [77] have taken this idea further and have engineered tunnels in cytochrome P450 monooxygenases which are able to accommodate H_2O_2. Superoxide anion migration was seen in, a process which was reliant on the presence of a tyrosine residue [78]. Therefore, examples of how ROS can, and need to, interact with proteins through direct physical mechanisms have been reported.

4. Conclusion and future

Many gases, such as Xe, have anaesthetic effects, and this has been known for a long time. As pointed out by Eckenhoff [79], Claude Bernard suggested in 1875 that such effects involved proteins. Eckenhoff also cite papers pointing out that the presence of hydrophobic domains in proteins where inert molecules could interact has also been known for a long time [80,81]. Such work is still continuing, as exemplified by the work of Colloc'h *et al*. [82] and Turan *et al*. [31].

There seems little doubt that inert gases such as Xe have an influence of protein activity [38], and therefore the activity of the cell, via direct interact of the gas molecules with proteins by taking advantage of the pockets and cavities which exist in protein structures. Other inert gases have similar biological effects and action, including Ar, He and Ne, whilst it has been suggested H_2 also acts in this way [27]. Certainly, for H_2 much more work focused on this potential mechanism needs to be carried out, either to confirm that this is one of the modes of action of H_2 or to rule it out. Recently work has concentrated on the interaction of H_2 with haem with the subsequent effects mediated by the removal of hydroxyl radicals [83–85]. However, such mechanisms probably do not account for all

the actions of H_2, and several modes of action probably need to be considered for get a full understanding of what H_2 is doing in cells [Hancock], including H_2 interactions with protein cavities.

Other gases such as nitrous oxide appear to use Xe binding sites for their action [41,58,59]. However, what about other small signalling molecules, which fall under the umbrella terms ROS and RNS? Although many such small reactive molecules such as H_2O_2 and NO have other mechanisms by which they interact with proteins, such as oxidation [17] and *S*-nitrosylation [18], respectively, little is known about how such molecules may interact with proteins by the exploiting physical interactions, such with hydrophobic regions, cavities and pockets, in the manner in which Xe acts. However, there is some evidence for NO, H_2O_2 and $O_2^{\cdot-}$ interacting with proteins by exploiting Xe-pockets and similar structures [75–78].

A series of questions can be mooted here. Should such interactions be more investigated? Is there competition between small signalling molecules at such interaction sites in proteins? Afterall, many of these molecules will be present, or even accumulating – such during stress responses – together in cells. Is the binding of one signalling molecule more likely than others, i.e., is there a hierarchy of binding? This seems to be the case for the noble gases [53]. Can all such molecules partake in this sort of protein interaction? Afterall, perhaps H_2 is too small? Or do these molecules manage to pack into these cavities and tunnels in some way, perhaps preventing others from interacting?

Here, there is an attempt to bring some of the relevant literature about small molecules, some of which are inert and/or gases, interacting with proteins using polypeptide cavities and tunnels. It is hoped that this inspires researchers to look outside of the normal paradigms of how ROS, RNS and H_2S may interact with proteins. The future may show that this is a fruitless exploit, but with insert gases such as Xe having profound effects on protein activity it is suggested here that such protein biochemistry is at least considered.

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References

1. Huang, H.; Ullah, F.; Zhou, D.X.; Yi, M.; Zhao, Y. Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science* **2019** *10*, 800.
2. Begara-Morales, J.C.; Chaki, M.; Valderrama, R.; Sánchez-Calvo, B.; Mata-Pérez, C.; Padilla, M.N.; Corpas, F.J.; Barroso, J.B. Nitric oxide buffering and conditional nitric oxide release in stress response. *Journal of Experimental Botany* **2018** *69*, 3425-3438.
3. Bruckdorfer, R. The basics about nitric oxide. *Molecular Aspects of Medicine* **2005** *26*, 3-31.
4. Bayr, H. Reactive oxygen species. *Critical Care Medicine* **2005** *33*, S498-S501.
5. Kleniewska, P.; Piechota, A.; Skibska, B.; Gorąca, A. The NADPH oxidase family and its inhibitors. *Archivum Immunologiae et Therapiae Experimentalis* **2012** *60*, 277-294.
6. Millar, T.M.; Stevens, C.R.; Benjamin, N.; Eisenthal, R.; Harrison, R.; Blake, D.R. Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. *FEBS Letters* **1998** *427*, 225-228.
7. Smirnoff, N. and Arnaud, D. Hydrogen peroxide metabolism and functions in plants. *New Phytologist* **2019** *221*, 1197-1214.
8. Geller, D.A.; Billiar, T.R. Molecular biology of nitric oxide synthases. *Cancer and Metastasis Reviews* **1998** *17*, 7-23.
9. Chamizo-Ampudia, A.; Sanz-Luque, E.; Llamas, A.; Galvan, A.; Fernandez, E. Nitrate reductase regulates plant nitric oxide homeostasis. *Trends in Plant Science* **2017** *22*, 163-174.
10. Paul, B.D.; Snyder, S.H. Gasotransmitter hydrogen sulfide signaling in neuronal health and disease. *Biochemical Pharmacology* **2018** *149*, 101-109.
11. Jiang, J.; Chan, A.; Ali, S.; Saha, A.; Haushalter, K.J.; Lam, W.L.M.; Glasheen, M.; Parker, J.; Brenner, M.; Mahon, S.B.; Patel, H.H. Hydrogen sulfide—mechanisms of toxicity and development of an antidote. *Scientific Reports* **2016** *6*, 20831.

12. Miller, G.A.D.; Suzuki, N.; Ciftci-Yilmaz, S.U.L.T.A.N.; Mittler, R.O.N. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment* **2010** *33*, 453-467.
13. Hancock, J.T.; Desikan, R.; Clarke, A.; Hurst, R.D.; Neill, S.J. Cell signalling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. *Plant Physiology and Biochemistry* **2002** *40*, 611-617.
14. Hasanuzzaman, M.; Raihan, M.R.H.; Masud, A.A.C.; Rahman, K.; Nowroz, F.; Rahman, M.; Nahar, K.; Fujita, M. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Molecular Sciences* **2021** *22*, 9326.
15. Berni, R.; Luyckx, M.; Xu, X.; Legay, S.; Sergeant, K.; Hausman, J.F.; Lutts, S.; Cai, G.; Guerriero, G. Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. *Environmental and Experimental Botany*, **2019** *161*, 98-106.
16. Suzuki, N.; Mittler, R. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiologia Plantarum* **2006** *126*, 45-51.
17. Schilter, D. Thiol oxidation: a slippery slope. *Nature Reviews Chemistry* **2017** *1*, 0013.
18. Astier, J.; Kulik, A.; Koen, E.; Besson-Bard, A.; Bourque, S.; Jeandroz, S.; Lamotte, O.; Wendehenne, D. Protein *S*-nitrosylation: what's going on in plants? *Free Radical Biology and Medicine* **2012** *53*, 1101-1110.
19. Filipovic, M.R.; Zivanovic, J.; Alvarez, B.; Banerjee, R. Chemical biology of H_2S signaling through persulfidation. *Chemical Reviews* **2018** *118*, 1253-1337.
20. Miles, J.A.; Egan, J.L.; Fowler, J.A.; Machattou, P.; Millard, A.D.; Perry, C.J.; Scanlan, D.J.; Taylor, P.C. The evolutionary origins of peroxynitrite signalling. *Biochemical and Biophysical Research Communications* **2021** *580*, 107-112.
21. Whiteman, M.; Li, L.; Kostetski, I.; Chu, S.H.; Siau, J.L.; Bhatia, M.; Moore, P.K. Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. *Biochemical and Biophysical Research Communications* **2006** *343*, 303-310.
22. Ming, Y.; Ma, Q.H.; Han, X.L.; Li, H.Y. Molecular hydrogen improves type 2 diabetes through inhibiting oxidative stress. *Experimental and Therapeutic Medicine* **2020** *20*, 359-366.
23. Ohno, K.; Ito, M.; Ichihara, M.; Ito, M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. *Oxidative Medicine and Cellular Longevity* **2012** 2012.
24. Alwazeer, D.; Liu, F.F.C.; Wu, X.Y.; LeBaron, T.W. Combating oxidative stress and inflammation in COVID-19 by molecular hydrogen therapy: Mechanisms and perspectives. *Oxidative Medicine and Cellular Longevity* **2021** *2021*.
25. Ohsawa, I.; Ishikawa, M.; Takahashi, K.; Watanabe, M.; Nishimaki, K.; Yamagata, K.; Katsura, K.; Katayama. Y.; Asoh, S.; Ohta, S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nature Medicine* **2007** *13*, 688.
26. Hancock, J.T.; LeBaron, T.W.; Russell, G. Molecular hydrogen: Redox reactions and possible biological interactions. *Reactive Oxygen Species (Apex)* **2021** *11*.
27. Hancock, J.T.; Russell, G.; Craig, T.J.; May, J.; Morse, H.R.; Stamler, J.S. Understanding hydrogen: Lessons to be learned from physical interactions between the inert gases and the globin superfamily. *Oxygen* **2022** *2*, 578-590.
28. Lawrence, J.H.; Loomis, W.F.; Tobias, C.A.; Turpin, F.H. Preliminary observations on the narcotic effect of xenon with a review of values for solubilities of gases in water and oils. *The Journal of Physiology* **1946** *105*, 197.
29. Jin, Z.; Piazza, O.; Ma, D.; Scarpati, G.; De Robertis, E. Xenon anesthesia and beyond: pros and cons. *Minerva Anestesiologica* **2018**, *85*, 83-89.
30. Duff, A.P.; Trambaiolo, D.M.; Cohen, A.E.; Ellis, P.J.; Juda, G.A.; Shepard, E.M.; Langley, D.B.; Dooley, D.M.; Freeman, H.C.; Guss, J.M. Using xenon as a probe for dioxygen-binding sites in copper amine oxidases. *Journal of Molecular Biology* **2004** *344*, 599-607.
31. Turan, H.T., Boittier, E. and Meuwly, M. Interaction at a distance: Xenon migration in Mb. *The Journal of Chemical Physics* **2023** *158*, 125103.
32. Schoenborn, B.P.; Watson, H.C.; Kendrew, J.C. Binding of xenon to sperm whale myoglobin. *Nature* **1965** *207*, 28-30.
33. Hermans, J.; Shankar, S. The free energy of xenon binding to myoglobin from molecular dynamics simulation. *Israel Journal of Chemistry* **1986**, *27*, 225-227.
34. Tilton Jr, R.F.; Singh, U.C.; Weiner, S.J.; Connolly, M.L.; Kuntz Jr, I.D.; Kollman, P.A.; Max, N.; Case, D.A., 1986. Computational studies of the interaction of myoglobin and xenon. *Journal of Molecular Biology* **1986**, *192*, 443-456.
35. Conn JR, H.L. Equilibrium distribution of radioxenon in tissue: Xenon-hemoglobin association curve. *Journal of Applied Physiology* **1961** *16*, 1065-1070.
36. Tilton Jr, R.F. and Kuntz Jr, I.D. Nuclear magnetic resonance studies of xenon-129 with myoglobin and hemoglobin. *Biochemistry* **1982** *21*, 6850-6857.

37. Lepeshkevich, S.V.; Gilevich, S.N.; Parkhats, M.V.; Dzhagarov, B.M. Molecular oxygen migration through the xenon docking sites of human hemoglobin in the R-state. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* **2016** *1864*, 1110-1121.
38. Prangé, T.; Schiltz, M.; Pernot, L.; Colloc'h, N.; Longhi, S.; Bourguet, W.; Fourme, R. Exploring hydrophobic sites in proteins with xenon or krypton. *Proteins: Structure, Function, and Bioinformatics* **1998** *30*, 61-73.
39. Rubin, S.M.; Lee, S.Y.; Ruiz, E.J.; Pines, A.; Wemmer, D.E. Detection and characterization of xenon-binding sites in proteins by 129Xe NMR spectroscopy. *Journal of Molecular Biology* **2002** *322*, 425-440.
40. Pirrat, P.; Smith, M.A.; Pearson, A.R.; McPherson, M.J.; Phillips, S.E. Structure of a xenon derivative of *Escherichia coli* copper amine oxidase: confirmation of the proposed oxygen-entry pathway. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications* **2008** *64*, 1105-1109.
41. Marassio, G.; Prangé, T.; David, H.N.; Santos, J.S.D.O.; Gabison, L.; Delcroix, N.; Abraini, J.H.; Colloc'h, N. Pressure-response analysis of anesthetic gases xenon and nitrous oxide on urate oxidase: a crystallographic study. *The FASEB Journal* **2011** *25*, 2266-2275.
42. Stoppe, C.; Ney, J.; Brenke, M.; Goetzenich, A.; Emontzpohl, C.; Schälte, G.; Grottke, O.; Moeller, M.; Rossaint, R.; Coburn, M. Sub-anesthetic xenon increases erythropoietin levels in humans: a randomized controlled trial. *Sports Medicine* **2016** *46*, 1753-1766.
43. Winkler, D.A.; Katz, I.; Warden, A.; Thornton, A.W.; Farjot, G. Identifying medically relevant xenon protein targets by *in silico* screening of the structural proteome. Medical Gas Research **2023** *13*, 33-38.
44. Yeh, S.Y.; Peterson, R.E. Solubility of krypton and xenon in blood, protein solutions, and tissue homogenates. *Journal of Applied Physiology* **1965** *20*, 1041-1047.
45. Wang, J.; Yuan, K.; Wang, X.; Zhang, L.; Hu, J. Influence of krypton gas nanobubbles on the activity of pepsin. *Langmuir* **2020** *36*, 14070-14075.
46. Sanders, R.D.; Ma, D.; Maze, M. Argon neuroprotection. *Critical Care* **2010** *14*:1-2.
47. Höllig, A.; Schug, A.; Fahlenkamp, A.V.; Rossaint, R.; Coburn, M. and the Argon Organo-Protective Network (AON). Argon: systematic review on neuro-and organoprotective properties of an "inert" gas. *International Journal of Molecular Sciences* **2014** *15*, 18175-18196.
48. Grottke, O.; Coburn M. Argon: neuroprotection in *in vitro* models of cerebral ischemia and traumatic brain injury. *Critical Care* **2009** *13*,1-9.
49. Ulbrich, F.; Kaufmann, K.; Roesslein, M.; Wellner, F.; Auwärter, V.; Kempf, J.; Loop, T.; Buerkle, H.; Goebel U. Argon mediates anti-apoptotic signaling and neuroprotection via inhibition of toll-like receptor 2 and 4. *PloS one* **2015** *10*, e0143887.
50. Fahlenkamp, A.V.; Rossaint, R.; Haase, H.; Al Kassam, H.; Ryang, Y.M.; Beyer, C.; Coburn, M. The noble gas argon modifies extracellular signal-regulated kinase 1/2 signaling in neurons and glial cells. *European Journal of Pharmacology* **2012** *674*, 104-111.
51. Ye, Z.; Zhang, R.; Sun, X. Bustling argon: biological effect. *Medical Gas Research* **2013** *3*, 1-4.
52. Martusevich, A.; Surovegina, A.; Popovicheva, A.; Didenko, N.; Artamonov, M.; Nazarov, V. Some beneficial effects of inert gases on blood oxidative metabolism: *In vivo* study. *BioMed Research International* **2022** *2022*.
53. Schreiner, H.R.; Gregoire, R.C.; Lawrie, J.A. New biological effect of the gases of the helium group. *Science* **1962** *136*, 653-654.
54. Jawad, N.; Rizvi, M.; Gu, J.; Adeyi, O.; Tao, G.; Maze, M.; Ma, D. Neuroprotection (and lack of neuroprotection) afforded by a series of noble gases in an *in vitro* model of neuronal injury. *Neuroscience Letters* **2009** *460*, 232-236.
55. Rizvi, M.; Jawad, N.; Li, Y.; Vizcaychipi, M.P.; Maze, M.; Ma, D., 2010. Effect of noble gases on oxygen and glucose deprived injury in human tubular kidney cells. *Experimental Biology and Medicine*, **2010** *235*, 886-891.
56. Scott, E.E.; Gibson, Q.H. Ligand migration in sperm whale myoglobin. *Biochemistry*, **1997** *36*, 11909-11917.
57. Tetreau, C.; Blouquit, Y.; Novikov, E.; Quiniou, E.; Lavalette, D. Competition with xenon elicits ligand migration and escape pathways in myoglobin. *Biophysical Journal* **2004** *86*, 435-447.
58. Abraini, J.H.; Marassio, G.; David, H.N.; Vallone, B.; Prangé, T.; Colloc'h, N. Crystallographic studies with xenon and nitrous oxide provide evidence for protein-dependent processes in the mechanisms of general anesthesia. *Anesthesiology* **2014** *121*, 1018-27.
59. LaBella, F.S.; Stein, D.; Queen G. The site of general anesthesia and cytochrome P450 monooxygenases: occupation of the enzyme heme pocket by xenon and nitrous oxide. *European Journal of Pharmacology* **1999** *381*, R1-3.
60. Winter, M.B.; Herzik Jr, M.A.; Kuriyan, J.; Marletta, M.A. Tunnels modulate ligand flux in a heme nitric oxide/oxygen binding (H-NOX) domain. *Proceedings of the National Academy of Sciences* **2011** *108*, E881-E889.
61. Brunori, M. Nitric oxide moves myoglobin centre stage. *Trends in Biochemical Sciences* **2001** *26*, 209-210.
62. Brunori, M. Structural dynamics of myoglobin. Biophys. Chem. **2000** 86, 221-230.

63. Tilton Jr, R.F., Kuntz Jr, I.D. and Petsko, G.A. Cavities in proteins: structure of a metmyoglobin xenon complex solved to 1.9. ANG. *Biochemistry* **1984** *23*, 2849-2857.
64. Daigle, R.; Rousseau, J.A.; Guertin, M.; Lagüe, P. Theoretical investigations of nitric oxide channeling in *Mycobacterium tuberculosis* truncated hemoglobin N. *Biophysical Journal* **2009** *97*, 2967-2977.
65. Ye, X.; Yu, A.; Champion, P.M. Dynamics of nitric oxide rebinding and escape in horseradish peroxidase. *Journal of the American Chemical Society* **2006** *128*, 1444-1445.
66. Terasaka, E.; Yamada, K.; Wang, P.H.; Hosokawa, K.; Yamagiwa, R.; Matsumoto, K.; Ishii, S.; Mori, T.; Yagi, K.; Sawai, H., Arai H. Dynamics of nitric oxide controlled by protein complex in bacterial system. *Proceedings of the National Academy of Sciences* **2017** *114*, 9888-9893.
67. Chu, K.; Vojtchovský, J.; McMahon, B.H.; Sweet, R.M.; Berendzen, J.; Schlichting, I. Structure of a ligand-binding intermediate in wild-type carbonmonoxy myoglobin. *Nature* **2000** *403*, 921-923.
68. Elber, R.; Karplus, M. Enhanced sampling in molecular dynamics: use of the time-dependent Hartree approximation for a simulation of carbon monoxide diffusion through myoglobin. *Journal of the American Chemical Society* **1990** *112*, 9161-9175.
69. Nishihara, Y., Sakakura, M., Kimura, Y. and Terazima, M. The escape process of carbon monoxide from myoglobin to solution at physiological temperature. *Journal of the American Chemical Society* **2004** *126*, 11877-11888.
70. Bossa, C.; Anselmi, M.; Roccatano, D.; Amadei, A.; Vallone, B.; Brunori, M.; Di Nola, A. Extended molecular dynamics simulation of the carbon monoxide migration in sperm whale myoglobin. *Biophysical Journal* **2004** *86*, 3855-3862.
71. Anselmi, M.; Di Nola, A.; Amadei, A. The kinetics of ligand migration in crystallized myoglobin as revealed by molecular dynamics simulations. *Biophysical Journal* **2008** *94*, 4277-4281.
72. Gee, L.B.; Leontyev, I.; Stuchebrukhov, A.; Scott, A.D.; Pelmenschikov, V.; Cramer, S.P. Docking and migration of carbon monoxide in nitrogenase: the case for gated pockets from infrared spectroscopy and molecular dynamics. *Biochemistry* **2015** *54*, 3314-3319.
73. Goldet, G.; Brandmayr, C.; Stripp, S.T.; Happe, T.; Cavazza, C.; Fontecilla-Camps, J.C.; Armstrong, F.A. Electrochemical kinetic investigations of the reactions of [FeFe]-hydrogenases with carbon monoxide and oxygen: comparing the importance of gas tunnels and active-site electronic/redox effects. *Journal of the American Chemical Society* **2009** *131*, 14979-14989.
74. Cundari, T.R.; Wilson, A.K.; Drummond, M.L.; Gonzalez, H.E.; Jorgensen, K.R.; Payne, S.; Braunfeld, J.; De Jesus, M.; Johnson, V.M. CO_2-formatics: how do proteins bind carbon dioxide? *Journal of Chemical Information and Modeling* **2009** *49*, 2111-2115.
75. Hiromoto, T.; Fujiwara, S.; Hosokawa, K.; Yamaguchi, H. Crystal structure of 3-hydroxybenzoate hydroxylase from *Comamonas testosteroni* has a large tunnel for substrate and oxygen access to the active site. *Journal of Molecular Biology* **2006** *364*, 878-896.
76. Chen, L.; Lyubimov, A.Y.; Brammer, L.; Vrielink, A.; Sampson, N.S. The binding and release of oxygen and hydrogen peroxide are directed by a hydrophobic tunnel in cholesterol oxidase. *Biochemistry* **2008** *47*, 5368-5377.
77. Zhao, P.; Kong, F.; Jiang, Y.; Qin, X.; Tian, X.; Cong, Z. Enabling peroxygenase activity in by engineering hydrogen peroxide tunnels. *Journal of the American Chemical Society* **2023** *145*, 5506-5511.
78. Moreno, D.M.; Martí, M.A.; De Biase, P.M.; Estrin, D.A.; Demicheli, V.; Radi, R.; Boechi, L. Exploring the molecular basis of human manganese superoxide dismutase inactivation mediated by tyrosine 34 nitration. *Archives of Biochemistry and Biophysics* **2011** *507*, 304-309.
79. Eckenhoff, R.G. Promiscuous ligands and attractive cavities. *Molecular Interventions* **2001** *1*, 258.
80. Otting, G.; Liepinsh, E.; Halle, B.; Frey, U. NMR identification of hydrophobic cavities with low water occupancies in protein structures using small gas molecules. *Nature Structural Biology* **1997** *4*, 396–404.
81. Carugo, O.; Argos, P. Accessibility to internal cavities and ligand binding sites monitored by protein crystallographic thermal factors. *Proteins* **1998**, *31*, 201–213.
82. Colloc'h, N.; Carpentier, P.; Montemiglio, L.C.; Vallone, B.; Prangé, T. Mapping hydrophobic tunnels and cavities in neuroglobin with noble gas under pressure. *Biophysical Journal* **2017** *113*, 2199-206.
83. Jin, Z.; Zhao, P.; Gong, W.; Ding, W.; He, Q. Fe-porphyrin: A redox-related biosensor of hydrogen molecule. *Nano Research* **2022** *13*, 1-6
84. Kim, S.A.; Jong, Y.C.; Kang, M.S.; Yu, C.J. Antioxidation activity of molecular hydrogen via protoheme catalysis *in vivo*; an insight from *ab initio* calculations. Research Square **2022** (pre-print).

85. Ohta, S. Molecular hydrogen may activate the transcription factor Nrf2 to alleviate oxidative stress through the hydrogen-targeted porphyrin. *Aging Pathobiology and Therapeutics* **2023** *13*, 25-32.