Identifying medically relevant xenon protein targets by in silico screening of the structural proteome

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Abstract

In a previous study, in silico screening of the binding of almost all proteins in the Protein Data Bank to each of the five noble gases xenon, krypton, argon, neon, and helium was reported. This massive and rich data set requires analysis to identify the gas-protein interactions that have the best binding strengths, those where the binding of the noble gas occurs at a site that can modulate the function of the protein, and where this modulation might generate clinically relevant effects. Here, we report a preliminary analysis of this data set using a rational, heuristic score based on binding strength and location. We report a partial prioritized list of xenon protein targets and describe how these data can be analyzed, using arginase and carbonic anhydrase as examples. Our aim is to make the scientific community aware of this massive, rich data set and how it can be analyzed to accelerate future discoveries of xenon-induced biological activity and, ultimately, the development of new “atomic” drugs.

Key words: binding sites; computational docking; enzymes; medical applications; protein binding; structural biology; virtual screening; xenon

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INTRODUCTION

The chemically inert noble gases, helium (He), neon (Ne), argon (Ar), krypton (Kr), and xenon (Xe) form molecular compounds only under extreme conditions. Being simple, chemically unreactive atoms, early biological studies focused mainly on their solubility in blood and oil or lipophilic media Proposed biological mechanisms of action involved polar narcosis.1 Since the first biological studies of Xe in 1951,2 research has revealed that the noble gases exhibit a broad spectrum of biological effects such as anti-addictive properties, tissue protection, anesthesia, neuroprotection, effects on memory, analgesia, and inhibition of apoptosis.3,4 The primary mechanism of action of Xe anesthesia and neuroprotection has been identified as antagonism of the N-methyl-D-aspartate receptor,5 but a full understanding has been elusive. Furthermore, it is highly likely many other types of useful biological activity of Xe are yet to be discovered.

If we could determine how these gases bind to molecular targets, we could better understand the molecular foundations of their biological and medical properties. Conspicuously, we could also discover new biological effects that are of potential clinical value, and design target-specific delivery systems that would make them valuable drugs. Given the very large number of potential protein targets for noble gases, a list that continues to grow, it is clearly infeasible to carry out massive scale pharmacological experiments to discover their full spectrum of biological activities. This intractability is compounded by the that pharmacology experiments using gaseous agents are more complex than those for small organic molecules.6

The dramatic increase in the capabilities of computational chemistry and availability of high-performance computing resources means that it is now possible to reliably study interactions between noble gases and the exponentially increasing number of experimental protein structures now available. We can now use these fast methods to infer hitherto unknown biological activities for noble gases from the binding strengths and binding sites on a very large number of proteins with experimental structures. These methods (inverse- or reverse-docking) have proven effective in finding drug binding sites in recent studies.7-9

Thus, to better understand the molecular bases for known biological activities of noble gases, and to identify new biological activities of noble gases, we performed in silico screening of ~130,000 proteins in the Protein Data Bank with each of the five nonradioactive noble gases. This larger study calculated the likely protein binding sites for the five noble gases, 650,000 protein-ligand calculations in total.10 We initially validated the computational methods using 399 experimental structures of diverse proteins bound to Ar, Kr, and Xe.11 The high accuracy with which we could recapitulate the binding position of these gases gave us confidence that a much larger screen of proteins would generate useful results. This massive and rich data set requires analysis to identify the gas-protein interactions that have the best binding strengths and where the binding of the noble gas occurs at a site capable of modulating the function of the protein. We initially prioritized
enzymes for analysis due to their importance as drug targets, the availability of commercial kits for testing their activity, and their well understood mechanisms of action. Similarly, we initially prioritized the interactions of Xe, because it generally has the strongest binding free energy to proteins and had already been shown to exhibit a range of biological activities under normobaric conditions.4

Here, we report a preliminary analysis of this massive data set, providing an initial, prioritized selection of potential Xe protein targets. We also describe a method of analysis and exemplify it using Xe interaction data for arginase and carbonic anhydrase (CA). We aim to demonstrate to the scientific community how this massive, rich data set can be analyzed, to stimulate future discoveries of Xe-induced biological activity and, ultimately, to lead to new “atomic” drugs.

**DATA AND METHODS**

**In silico screening**

We have described the methods used to conduct the computational screening of the structural proteome in detail in an earlier publication.11 In summary, we downloaded 127,854 protein structures from the Protein Data Bank (http://www.rcsb.org, January 2017). The experimental structures retrieved fell into two types: 87,848 structures contained one or several ligands in a binding pocket; 40,006 had no small-molecule ligands in the structure. Correct preparation of the protein structures prior to docking calculations was very important. Considerable effort was expended in developing scripts to remove nonessential ligands such as substrates, products, inhibitors were removed as these would interfere with the binding of the noble gases. When multiple experiment structures were available, we chose those with the highest resolution. We stored the structures of any small molecule ligands removed during protein preparation in the coordinate system of the experimental structures. This allowed us to calculate the distances between predicted binding positions of noble gases and atoms in ligands in the experimental structures. The open-access grid calculation and docking software we employed, AutoDock4 and Autogrid4 (Scripps Institute, https://autodock.scripps.edu),12,13 also required the structure files to be modified to be compatible with these packages. We omitted a small percentage of the structures in the initial list (7678 structures containing RNA/DNA) because they could not be processed properly by the scripts. For the 516 X-ray ensemble structures, only one member of the ensemble was used. 11,842 structures obtained by NMR methods were also omitted.

An underlying assumption is that removal of water (and potentially other solvents) from the experimental structures will not affect the noble gas binding. This assumption seems reasonable as 399 experimental noble gas binding sites were correctly predicted in our validation study11 using the same assumption.

Being atoms, noble gases have only a small number of physicochemical properties: lipophilicity; size, polarizability for example, and no conformational or rotational degrees of freedom. Our validation study employed modified universal force field parameters for the noble gases that accurately recapitulated the experimentally determined positions of noble gases in crystal structures of proteins containing Xe and Kr.11 Using these parameters, 100% of crystallographic Kr atoms were found within 1 Kr vdW diameter of a predicted binding site, 97% of the crystallographic Xe atoms lay within 2 vdW diameters of a predicted binding site, and 94% of the crystallographic Xe atoms lay within 1 Xe Van Der Waals diameter of a predicted binding site.

The AutoDock software12 is flexible, simple to parameterize for noble gases, easily scripted, extensively validated for a wide range of proteins and ligands, and can run many calculations in parallel when multiple CPUs or GPUs are available.14 The software generates a 0.375 Å grid around each protein and places a probe noble gas atom at each grid position outside and within the protein. The interaction energy between the probe atom and the protein is calculated and those larger than zero (repulsive binding interactions) removed. For binding sites located in the protein, the location with the most favorable (largest negative energy) was calculated in the protein coordinate frame. This was used to calculate the proximity of the noble gas binding sites and any small-molecule ligands in the experimental structure. Grid points are points in and around protein structures at which the interaction energy is calculated and groups of adjacent grid points with favorable binding energies define a binding site. Thus, most binding sites contain multiple grid points. We limited the number of binding sites per protein to 20 as a good compromise between computational expense and information generated.

**Ranking of potential protein targets for Xe**

Ranking of potential protein targets for Xe is based on the highest binding energy (as determined from the most negative grid point energy in each binding pocket), and binding in the same pocket as organic ligands in the specified protein. An empirical scoring function was developed to rank noble gas binding interactions using these two criteria. It scores highly on those tightly binding gases that overlap with the binding sites of small molecule ligands. For protein structures containing bound small molecule ligands, the following empirical scoring function was used:

\[ \text{Score} = \frac{E_{\text{bind}}}{\sqrt{r_{ij}}} \]

where \( E_{\text{bind}} \) is the most negative binding energy grid point in a binding pocket, and \( r_{ij} \) is the distance between the Xe probe atom in the binding site and the nearest atom of an organic ligand in the same binding site in the X-ray structure.

For experimental protein structures without bound small molecule ligands we used the natural unit of information, nat (nit or nepit), that relates to the probability of binding of the gas under physiological conditions. The Shannon (information) entropy is defined as

\[ S = -\sum p_i \ln(p_i) \]

If the probabilities \( p_i \) are calculated using the Boltzmann factor \( \exp\left(\frac{-E_i}{kT}\right) \), then
RESULTS
The global data has been made openly available at La Trobe University OPAL after registration. A selection of the highest-ranking targets (mainly enzymes) is given in Additional Table 1. The records contain the Protein Data Bank reference code, the name of the protein, the type (e.g., enzyme), the associated ligand, the distance to the ligand, the potential medical application, the score, the binding energy, and the structure of example ligands.

We exemplify how these results can be used to identify novel pharmacological applications for noble gases using a more detailed analysis of two targets, arginase and CA, provided below. These were chosen because they were hitherto unknown targets for noble gases, have therapeutic potential, and bind relatively strongly to the heavier noble gases at least.

Arginases

Binding of noble gases to the active site of arginases can potentially compete with the natural substrates for these enzymes and inhibit them. In humans, two isozymes of this enzyme exist; the first, arginase I, is an essential enzyme in the urea cycle. It is located primarily in the cytoplasm of hepatocytes (liver cells) and its deficiency causes severe pathologies. The second isozyme, arginase II, has been implicated in the regulation of intracellular arginine/ornithine metabolism and is upregulated in several pathologies. The isozyme is localized primarily in the kidney and prostate. Some arginase inhibitors are relatively lipophilic boronic acids (Table 1). Table 1 shows the distance between diverse ligands of different CA isoforms and Xe or Kr atoms, fitness scores and binding energies (Autogrid) for each binding event.

The noble gases tend to bind to the lipophilic regions of the binding site. Molecular modeling analysis using structures obtained from the large-scale docking experiments showed that Xe bound to the same region in the active site as the boronic acid moiety in the inhibitor X7A. If the in silico binding predictions are experimentally confirmed, binding of noble gases in the active site of arginases could compete with the natural substrate for these enzymes and potentially inhibit them.

CA

In humans, eight genetically distinct CA families (α, β, γ, δ, ζ, η, θ, and ι) and more than 15 isozymes of this enzyme exist. They are distributed in different cytosolic, mitochondrial, or cell membrane compartments. CAs (or carbonate dehydratases) catalyze interconversion between carbon dioxide and water and bicarbonate and protons. This interconversion is reversible and the enzyme catalyzes both forward and reverse reactions. The enzymes contain zinc coordinated to three histidines and a water molecule. This polarizes the hydrogen-oxygen bond and weakens it. A fourth histidine acts as a general acid – general base catalyst. The active site contains a pocket for carbon dioxide close to the hydroxide group that promotes the hydrolysis to attack the carbon dioxide, forming bicarbonate. Many CA inhibitors are relatively lipophilic sulfonamides, carboxylates or sulfonates (Table 2). The noble gases tend to bind in the lipophilic regions of the binding site.

Table 2 summarizes the distance between diverse ligands of different CA isoforms and Xe or Kr atoms, fitness scores and binding energies (Autogrid) for each binding event, and potential medical applications.

Table 1: Xe targets related to arginase modulators

| Protein Data Bank | Isoform | Distance (Å) | Medical application | Fitness score | E_{bind} (kcal/mol) | Example ligand |
|-------------------|---------|--------------|---------------------|--------------|---------------------|----------------|
| 4hze              | 2       | 0.2          | Endothelial function, diabetes, reperfusion injury, hypertension atherosclerosis | 325          | –1.65               | X7A (R)-2-amino-6-borono-2-(2-cyclohexylethyl)hexanoic acid |
| 3e6k              | 1       | 0.4          | Asthma, erectile dysfunction/sexual dysfunction, atherosclerosis | 182          | –1.5                | ABH; (2R)-2-amino-6-borono-hexanoic acid |
| 3e6v              |         | 0.6          |                      | 157          | –1.56               |                |
| 2aeb              |         | 0.4          |                      | 178          | –1.51               |                |

Note: E_{bind} : The most negative binding energy grid point in a binding pocket; Xe: xenon.
Molecular modeling using the structures obtained from the large-scale docking experiments showed Xe and to a lesser extent, Kr, bound to the same region in the active site as the sulfonamide moieties of inhibitors (Figure 2). The noble gas atoms clustered around the phenoxy amide moiety of ligand 0MN in CA II, and the chlorosulfonamide moiety of ligand E1F in CA XIII.

DISCUSSION

Here, we present a partial list of protein targets, largely enzymes, that may bind Xe to generate potentially useful biological activity. Docking methods were validated against X-ray crystallography data, and an empirical ranking method was developed to prioritize the most important protein-gas interactions. We exemplified these analyses using arginase and CA, interesting pharmaceutical targets for Xe.

Arginases are potentially valuable drug targets. Arginase II is co-expressed with nitric oxide synthase in smooth muscle tissue. Rapid relaxation of these tissues facilitates engorge-ment necessary for normal sexual response. Nitric oxide synthase and arginase compete for L-arginine, so blocking arginase by inhibitors maintains normal levels of arginine and normal muscle relaxation and sexual response. Arginase is a controlling factor in both male erectile function and female sexual arousal and is a potential target for treatment of sexual dysfunction. The potential of arginase II as a target for cardiovascular drug design has also been reviewed very recently. Arginase I expression is also upregulated in arteries of...
patients with coronary heart disease, inflammatory bowel disease, and pulmonary arterial hypertension. In patients with morbid obesity and diabetes, in vitro arginase inhibition improved NO-dependent vasorelaxation. In chronic obstructive pulmonary disease (COPD), arginase inhibition improved endothelial function in smokers, who have elevated arginase, compared to non-smokers by almost 20%. Given clear in vitro data showing favorable effects of arginase inhibitors on COPD, the role of noble gases to alleviate symptoms of COPD should be investigated. If these predictions are validated and show sufficient efficacy, they could form the basis for new therapeutic uses of noble gases for cardiovascular or lung diseases, with the latter offering the advantage of direct inhalation to the site of action.

Inhibitors of CA (EC 4.2.1.1) are useful drugs for glaucoma, epilepsy, renal impairment, edema, and cancer that have emerging applications as antifungal and antibacterial agents with novel mechanisms of action. At least three isoforms, CA II, IV, and XII, have been targeted by inhibitors (Table 2). A comprehensive review of isoform selective inhibitors of CA was published recently by Kumar et al. In glaucoma, a serious, common disease characterized by elevated intraocular pressure, topical second generation CA inhibitors dorzolamide and brinzolamide exhibit fewer side effects than first generation systemic drugs. Topiramate, reported beneficial effects of Ar on retinal ischemia, apoptosis, and inflammation with no significant adverse effects, although the mechanism of action is poorly understood.

CA I enhancing drugs have shown useful effects on cognitive deficiency and Alzheimer’s disease. Supuran et al. summarized the utility of selective CA IX inhibitors in cancer treatment, while selective inhibitors of CA XII are potentially useful for reversing multidrug resistance in cancers.

CA inhibitors, particularly acetazolamide, have long been used to prevent or reduce acute mountain sickness. In placebo-controlled trials across all dosing regimens and altitudes, acetazolamide prevented acute mountain sickness or reduced its severity by 50–60%. Noble gases could potentially be dosed into supplemental oxygen at subanesthetic concentrations to reduce CA activity and improve tolerance to hypoxia.

Several CA inhibitors, such as acetazolamide, methazolamide, topiramate, and zonisamide, are antiepileptic drugs. Anticonvulsant effects are probably due to CO₂ retention secondary to inhibition of red cells and brain enzymes. They may also block sodium channels and kainate/o-aminoo-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors and enhance GABAergic transmission. Topiramate (Figure 3), a potent CA II inhibitor with similar potency to acetazolamide, is a very effective antiepileptic. The potential of CA inhibitors for treating epilepsy and other neurological or neuromuscular disorders has not been fully exploited.

Thus, if the inhibition of arginases and CA isoforms by noble gases is validated, it could lead to novel therapies for erectile dysfunction, COPD, glaucoma, neurological conditions such as epilepsy, dementia, and mountain sickness.

The potential limitations of our computational approach to identify new noble gas biomedical properties include the use of simplified docking methods that make the calculations tractable, automated preparation of the protein structures, and not all proteins having structures in the RCSB database. For example, membrane proteins are underrepresented as they are difficult to study by X-ray crystallography. The ranking method is only based on the strength of Xe binding and proximity to a ligand binding to the protein. Other potentially relevant interactions may include effects on protein and membrane conformation and structure.

The docking predictions can form the basis for more detailed molecular dynamics calculations of the interaction of short-listed targets with noble gases. These allow protein dynamics to be studied, estimate on-off rates of binding sites, and define trajectories by which noble gases access proteins. Proof-of-concept molecular dynamics simulations of arginase were reported Mortier et al. Using microsecond simulations they studied flexibility of key regions of the active site.

Apart from molecular dynamics, validation of binding of noble gases to a short list of medically promising proteins, experimental validation is also essential. The binding characteristics of a short list of the most promising targets from computational screening can be verified using Xe nuclear magnetic resonance studies, X-ray crystallography, and in vitro screens. Due to the importance of enzymes as drug targets, the relative simplicity of enzyme assays, and the availability of robust assay kits for many enzyme systems, prioritizing enzymes with computationally predicted strong and effective binding to one or more noble gases is the most rational approach. However, particular challenges arise when using noble gases in in vitro and in vivo experiments (e.g., see Katz et al.).

For example, the high-throughput enzyme assay systems are not readily compatible with gaseous agents.

The great importance of small gaseous atoms, molecules, and ions in human and plant biology strongly suggests that further research into the biological properties of noble gases is useful and important. When coupled with new targeted delivery systems that deliver the gas to the required site much more efficiently than does inhalation, it will open up a new era of fast acting, reversible, chemically benign, and useful drug options for a wide range of untreated or poorly treated illnesses. We demonstrate how this rich and massive data set can be analyzed to seed future discoveries of Xe-induced biological activity and, ultimately, new “atomic” drugs.

**Author contributions**

DAW did the analysis; IK, GF and DAW drafted the paper, AWT and AW performed the original docking simulations. All authors read and approved the final version of the manuscript.

**Conflicts of interest**

None.

**Author statement**

The global data have been made openly available at La Trobe University OPAL with doi: 10.26181/617b2977824d2.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its supplementary information files.
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This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNon-Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

Additional Table 1: Results of a subset of docking calculations for Xe to diverse proteins, largely enzymes.

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