*Review*

# Targeting HIF-1α Function in Cancer through the Chaperone Action of NQO1

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**Abstract:** HIF-1α is a master regulator of oxygen homeostasis involved in different stages of cancer development. Thus, HIF-1α inhibition represents an interesting target for anti-cancer therapy. It was recently shown that HIF-1α interaction with NQO1 inhibits its proteasomal degradation, thus suggesting that targeting the stability of NQO1 could led to destabilization of HIF-1α as a therapeutic approach. Since the molecular interactions of NQO1 with HIF-1α are beginning to be unraveled, we review here our current knowledge on the intracellular functions and stability of NQO1, its pharmacological modulation by small ligands, and the molecular determinants of its roles as a chaperone of many different proteins including cancer-associated factors such as p53 and p73α. This knowledge is then discussed in the context of potentially targeting the intracellular stability of HIF-1α by acting on its chaperone, NQO1. This could result in novel anti-cancer therapies.

**Keywords:** HIF-1α;NQO1; hypoxia; angiogenesis; cancer; protein:protein interactions; ligand binding; proteasomal degradation

## 1. HIF-1α: structure, function, regulation and disease

Oxygen homeostasis is one of the main principles of life for most organisms, especially metazoa. The availability of oxygen regulates many physiological processes that are strictly controlled by a complex network of molecules in the organism. In metazoa, the master transcription factor that supervises this regulatory network is the Hypoxia-induced factor 1 (HIF-1). HIF-1 regulates the expression of a multitude of genes in response to oxygen availability and plays a fundamental role in many physiological and pathological processes [1, 2].

### 1.1. HIF-1α structure and function

HIF-1 is a DNA-binding complex composed of two subunits (α and β), both basic helix-loop-helix (bHLH) proteins of the PER-ARNT-SIM (PAS) family. HIF-1β (initially named aryl hydrocarbon nuclear receptor translocator, ARNT) is constitutively expressed, and thus, its expression and activity are oxygen-independent. In contrast, HIF-1α is strongly repressed in normoxia and induced in hypoxia [3]. In response to low oxygen, both subunits of HIF-1α dimerize, and HIF-1 binds to the hypoxia response element (HRE) of the promoter or enhancer region of the target genes to induce transcription. HIF-1 induces the transcription of numerous genes [4, 5]

involved in many biological processes, such as angiogenesis, erythropoiesis, anaerobic metabolism, cell survival, and cell proliferation. Thus, it is crucial for cancer development (see Section 1.3).
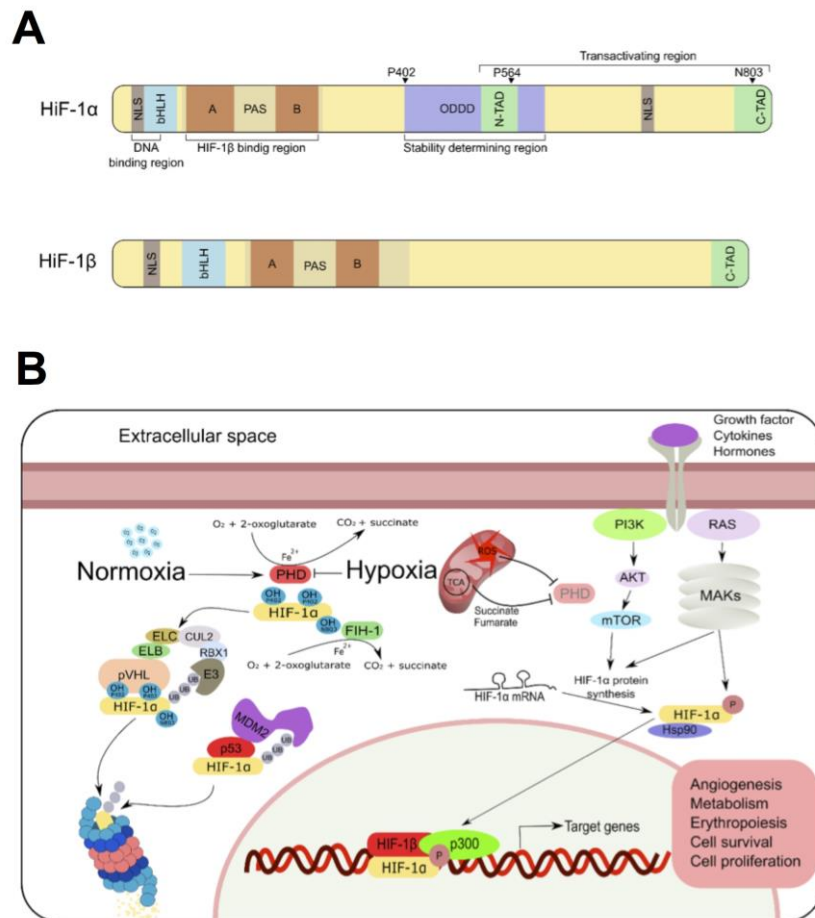


**Figure 1.** Structure and regulation of HIF-1$\alpha$. (**A**) Schematic representation of human HIF-1$\alpha$ and H1$\beta$ structure. Both proteins form part to HLH–PAS transcription factor family and contain a N-terminal bHLH domain (implicated in DNA binding) and two PAS domains (responsible of its dimerization). HIF-1$\alpha$ contains an oxygen-dependent degradation domain (ODDD) that mediates oxygen-regulated stability, and two transactivation domains (TAD), that mediate its transcriptional activity and its stability. (**B**) In normoxia, HIF-1$\alpha$ is subjected to oxygen-dependent hydroxylation by PHD hydroxylases, conducting HIF-1$\alpha$ to ubiquitination by the pVHL and proteasomal degradation. MDM2/p53 are also involved in ubiquitination and degradation of HIF1A protein in the VHL-independent manner. Under low oxygen levels (as well as some metabolites of TCA cycle) PHD is inhibited, HIF-1$\alpha$ translocates to the nucleus and promotes the activation of target genes. Transactivation of HIF-1$\alpha$ could be also induced by PI3K/MAPKs signaling pathways upon activation of some growth factor, hormones and cytokines.

The basic domain and the carboxy-terminus of PAS are specifically required for DNA binding of HIF-1 to the HRE DNA sequence 5V-RCGTG-3V, whereas the HLH domain and the amino-terminus of the two subunits of PAS proteins mediate their dimerization [6]. In addition to ubiquitous HIF-1$\alpha$ and HIF-1$\beta$, both subunits exist as a series of isoforms encoded by distinct genetic loci with different splice variants [7, 8, 9, 10]. HIF-1$\alpha$ constitutes the most prominent member of the three HIF-1$\alpha$ subunits in the human genome. The most important structural elements of HIF-1$\alpha$ are summarized in Figure 1A. HIF-1 has two nuclear location sequences (NLS) at the amino (residues 17-74) and carboxyl (residues 718-721) ends of the protein [11]. The stability of the protein is mainly regulated at the oxygen dependent degradation domain (ODDD). This domain contains two peptide sequences rich in proline (P), glutamic acid (E), serine (S), and threonine (T) at

residues 499-518 and 581-600 [3]. PEST-like motifs are present in short-lived proteins rapidly targeted for intracellular degradation. They mediate HIF-1 degradation by the ubiquitin-proteasome pathway [12, 13]. HIF-1$\alpha$ also contains two transactivation domains, N-TAD (residues 531-575) and C-TAD (residues 786-826) [14]. Under hypoxia several co-activators such as CBP/p300/SRC-1 interact with the C-TAD inducing HIF-1$\alpha$ transcriptional activity, while N-TAD is responsible for stabilizing HIF-1$\alpha$ against degradation [15].

*1.2. Regulation of HIF-1$\alpha$ expression, protein levels and transcriptional activity*

Although HIF-1$\alpha$ is tightly regulated at the transcriptional, translational and posttranslational levels [4], protein degradation plays a dominant role in regulating its activity.

### 1.2.1. Oxygen-dependent regulation

Most of HIF-1 regulation is mediated by the HIF-1$\alpha$ subunit. Under normoxic conditions, HIF-1$\alpha$ protein is constitutively synthesized and rapidly targeted for ubiquitination and proteasomal degradation, thus keeping minimal steady-state levels. In this scenario, HIF-1$\alpha$ becomes hydroxylated by members of the prolyl hydroxylase domain (PHD) family on two specific prolines (P402 and P564) of the ODDD and NTAD domains, respectively [16, 17, 18]. Hydroxylation of these proline residues generates a binding site for an E3-ubiquitin ligase complex that contains the von Hippel Lindau protein (pVHL), elongin B (ELB), elongin C (ELC), cullin 2 (CUL2), and ring box protein 1 (RBX1). As a result, HIF$\alpha$ is polyubiquitinated and degraded by the proteasome [19, 20, 21, 22] (Figure 1A).

In addition to this oxygen-dependent regulation of HIF-1$\alpha$ stability, oxygen affects DNA binding and transcriptional activity as well. This effect is mediated by the factor inhibiting HIF-1$\alpha$ (FIH-1), an asparagyl hydroxylase that catalyzes the hydroxylation of Q803 within the C-TAD domain of HIF-1$\alpha$. Hydroxylation of Q803 leads to a steric clash that prevents the binding of the p300 and CBP coactivators to HIF-1$\alpha$, abolishing their transactivation [23].

In hypoxia, impaired hydroxylation of HIF-1$\alpha$ allows dimerization of the transcriptional complex and the subsequent expression of its target genes. The mitochondrial electron transport chain (MEC) consumes the vast majority of cellular oxygen and may play an important role in HIF-1$\alpha$ regulation by determining the amount of oxygen available to PHD enzymes [24, 25, 26]. In this sense, pharmacological or genetic inhibition of mitochondrial respiration may prevent the accumulation of HIF-1$\alpha$ in hypoxia [27, 28]. However, there are other ways in which mitochondria affect the stability of HIF-1$\alpha$ (Figure 1B). Thus, in addition to requiring 2-oxoglutarate as a co-substrate, several metabolites of the tricarboxylic acic (TCA) cycle, especially succinate and fumarate, inhibit the HIF hydroxylases [26, 29, 30, 31]. In addition, reactive oxygen species produced by MEC in hypoxia also increase the stability of HIF-1$\alpha$ [27, 32, 33, 34, 35].

### 1.2.2. Growth factor signaling pathways

HIF1 is induced by numerous stimuli other than hypoxia, including growth factors, cytokines and hormones [36, 37, 38, 39, 40, 41]. These signals lead to the activation of mTOR (by PI3K/AKT) and the mitogen activated protein kinases (MAPK) (by the RAS/RAF/MEK/ERK kinases cascade) which can phosphorylate 4E-BP1 favoring cap-dependent translation of HIF-1$\alpha$ mRNA [42, 43, 44]. MAPKs also play an important role in the regulation of the transactivation activity of HIF-1. Once activated, members of this family phosphorylate HIF1$\alpha$, enhancing the binding of the coactivator p300/CBP [43, 45].

### 1.2.3. The Mdm2 pathway

A complex interplay exists between p53 and HIF-1$\alpha$. On one hand, HIF-1$\alpha$ binds to Mdm2 (the mouse's double minute 2 homologue), inhibiting Mdm2-dependent degradation of p53 [46]. On the other hand, p53 also mediates HIF-1$\alpha$ activity. In this sense, at moderate expression levels, p53 competes with HIF-1$\alpha$ for p300, consequently decreasing the transcriptional activity of HIF-1$\alpha$.

Moreover, high levels of p53 expression lead to HIF-1$\alpha$ degradation [47]. This is due to binding of p53 to HIF-1$\alpha$ that allows Mdm2-mediated ubiquitination of HIF-1$\alpha$ and its proteasomal degradation [48] (Figure 1B).

### 1.2.4 Heat shock protein 90 (Hsp90)

HIF$\alpha$ stability is also regulated by other signaling pathways, such as Hsp90 inhibitors that promote HIF$\alpha$ degradation in a pVHL-independent manner [49, 50]. In addition, Hsp90 is known to bind directly with HIF-1$\alpha$ inducing some conformational changes in its structure to fit and couple with HIF-1$\beta$ initiating its transactivation [51] (Figure 1b).

### *1.3. HIF-1$\alpha$ in cancer*

Cancer is defined as the autonomous growth and spread of a clone of somatic cells, under selective pressure, which is able to use multiple cellular pathways to modify its environment in favor of its own proliferation and against the needs of the organism, escaping physiological constraints on cell growth, including immune surveillance [52].

As the cell clone grows, its core becomes further removed from the blood supply and changes in oxygen availability are key for tumor cells to reorganize their metabolism in order to match oxygen supply with cellular demands. Thus, hypoxia becomes one of the key features of tumor microenvironment [53], a fact first reported in early studies where hypoxic cells were shown to surround the necrotic center in histological sections of lung carcinomas [54] and later demonstrated in essentially all solid tumors (reviewed in [53]). Intratumoral hypoxia is widespread and almost independent of tumor size, grade, stage, or histology [55]. Numerous studies have elucidated the molecular mechanisms involved in the cellular response to hypoxia, and three leaders in the field have been awarded recently the Nobel Prize in Medicine for their achievements, including their seminal work on HIF-1$\alpha$, a critical transcription factor for adaptation to hypoxia [56, 57] (https://www.nobelprize.org/prizes/medicine/2019/summary/).

HIF-1$\alpha$ regulates a number of physiological pathways involved in cancer, such as cell proliferation, survival, apoptosis, angiogenesis, glucose metabolism, immune cell activation and stem cell regulation [56, 58]. In particular, HIF-1$\alpha$ plays an important role in at least two of the main biological capabilities acquired during the multistep process of tumor development (the "hallmarks" of cancer, [52]): reprogramming cell metabolism and inducing angiogenesis.

### 1.3.1 Role of HIF in reprogramming cancer cell metabolism

When oxygen is available to mammalian cells, MEC and oxidative phosphorylation allow the highest yield of ATP from glucose, but many tumor cells choose oxygen-independent glycolysis even in normoxia conditions ("aerobic glycolysis" or Warburg effect) to extract smaller amounts of ATP but also valuable metabolites essential for the rapid growth of neoplasms [59]. Reaching this "sweet spot" of optimal utilization of glucose that maximizes cell growth requires a well-coordinated rearrangement of cell metabolism. Although HIF-1$\alpha$ has long been recognized as a key player in response to hypoxia, hypoxia-independent mechanisms for HIF-1$\alpha$ signaling have also been recently reported when oxygen is available to the cell ("pseudohypoxic" conditions) [60].

Reviewing the number of cancer types where HIF-1$\alpha$ is part of the oncogenic process exceeds the aim of this article. In the recent Pancancer analysis of more than 2500 cancer genomes, VHL, a tumor suppressor gene (TSG) and the key regulator of HIF-1$\alpha$, was among the most commonly mutated TSGs, following the highly prevalent TP53, CDKN2A/B, PTEN and SMAD4 [61]. Given its historical relevance, we will center this review on renal cancer, a type of malignancy that was central to the understanding of the role of hypoxia, angiogenesis and metabolism in cancer [62]. The study of hereditary cancer syndromes was, like in other examples of TSGs, crucial to the discovery of the mechanism of disease involving VHL. VHL (von Hippel-Lindau) family members carrying loss-of-function (LoF) mutations at the VHL gene are predisposed to kidney cancers. In particular, carriers display an autosomal dominant risk for clear cell–type renal cell carcinoma (ccRCC) in

addition to other health problems [63]. Moreover, another type of RCC, named papillary-type 2 RCC, where VHL mutations are not involved, is known to harbor mutations in the gene for Fumarate Hydroxylase (FH), an enzyme of the TCA cycle. These mutations deregulate HIF expression also, underscoring the important role of angiogenic and metabolic responses in renal carcinoma.

LoF mutations at VHL are observed in a majority of ccRCC, interfering with HIF-1$\alpha$ degradation and promoting a pseudohypoxic metabolic reprogramming of cancer cells. Stabilized HIF-1$\alpha$ translocates to the nucleus, where it dimerizes with HIF$\beta$ and upregulates transcription of genes related to cellular metabolism, reprograming it towards aerobic glycolysis by increasing conversion from pyruvate to lactate (via upregulation of LDHA) and by blocking pyruvate conversion to acetyl-CoA by PDH (via upregulation of PDK1). Stable HIF also increases transcription of transporters for nutrients such as glucose and glutamine, ultimately enhancing the glycolytic and reductive carboxylation pathways, respectively [64]. In addition, HIF suppresses aerobic respiration by increasing levels of BCL2-related BNIP3 and BNIP3L, which leads to mitochondrial autophagy [65, 66]. HIF also interferes with aerobic respiration via microRNAs, such as miR-210, which disrupts Fe-S clusters necessary for the MCE [67]. Another important consequence of HIF stabilization in cancer is that, by upregulating the expression of glycolytic enzyme pyruvate kinase M2 (PKM2), it amplifies its own transcriptional activity, resulting in a positive-feedback mechanism [68].

### 1.3.2 Role of HIF in cancer neoangiogenesis

The capability to induce abnormal vascular growth that ensures abundant supply of nutrients is another important hallmark of cancer and it has been one of the most successful targets of modern cancer therapy [52]. Neoangiogenesis is particularly conspicuous in renal cell carcinoma and prominent vascular remodeling as been linked to HIF disregulation as a consequence of VHL mutations [69]. The key molecular event in tumor angiogenesis is the increase in Vascular Endothelial Growth Factor (VEGF)-A induced by the hypoxia resulting from tumor growth but also from mutations in the HIF pathway [70]. Many other factors are involved in blood vessel growth in tumors [71]. These complex angiogenesis patterns and disregulation of vessel stabilization and maturation have been also well documented in renal carcinoma [72].

### 1.3.3 Association of HIF-1$\alpha$ expression and cancer prognosis

Although the complexities of cancer prognosis (the high variability of tumor mutations, histological types, grades and stages) makes it too simplistic to think that the levels of expression of a single gene such as *HIF1A* might have significant impact on prognosis, increased HIF-1$\alpha$ and HIF-2$\alpha$ protein levels in diagnostic tumor biopsies have been correlated with poor prognosis in other types of cancer also, such as brain, breast, colon, cervix, endometrium, head/neck, lung, ovary, pancreas, prostate, rectum and stomach (reviewed in [1]). More recently, high HIF-1$\alpha$ expression has been studied in triple-negative breast cancer (TNBC) [73]. TNBC are a heterogeneous group of poorly differentiated, highly aggressive and metastatic breast cancer type. They lack specific molecular-targeted therapy and there is an urgent need to find effective biomarkers rather than relying of their lack of hormone receptors and Her2 expression to define this subgroup. These authors [73] found that HIF-1$\alpha$ and c-Myc expression detected by immunohistochemistry could add to the development of a prognostic nomogram including histological grade and stage of cancer (based on tumor size (T), lymph node involvement (N) and distant metastases (M), known as TNM status). In an effort to validate the proposed nomogram in our institution, we are currently evaluating the feasibility of including HIF-1$\alpha$ immunohistochemistry in the biomarker profile (Figure 2). As a transcription factor induced in tumor cells by hypoxic environmental stress, HIF-1$\alpha$ could regulate a variety of downstream genes, thereby increasing the invasive ability of tumors and their resistance against standard radiotherapy and chemotherapy.
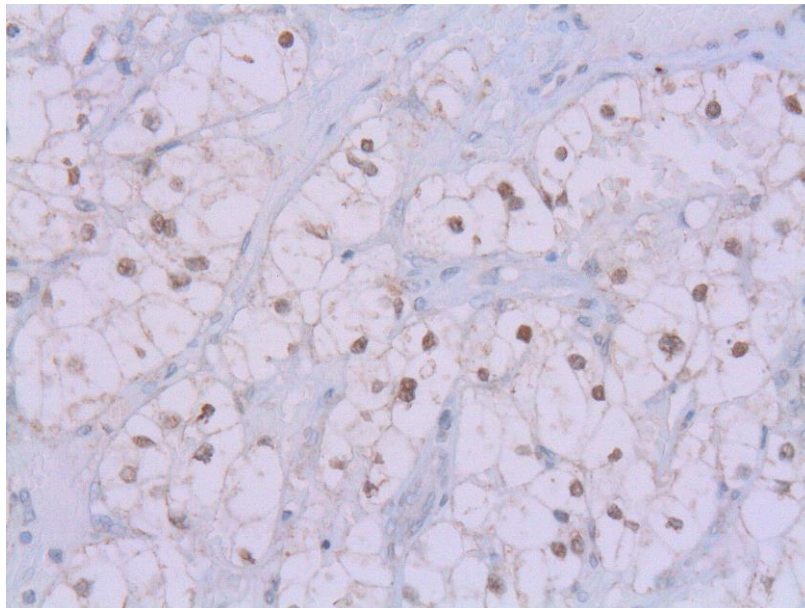
**Figure 2.** Nuclear HIF-1α expression (brown staining) in clear cell renal carcinoma. Common deletions of the VHL gene result in lack of HIF1 degradation, with the subsequent activation of HIF1 as a nuclear transcription factor that drives tumor cell proliferation.

## 2. NQO1 as a protein chaperone: current knowledge and potential application to target HIF-1α stability

The recent description of NQO1 as a protein chaperone protecting HIF-1α against degradation [74] supports that targeting this protein-protein interaction (PPI) could allow inactivation of HIF-1α. Since our knowledge on this particular PPI is very limited, in this section we will describe a wealth of information available for the activity and stability of NQO1, focusing on what we know on other PPI by which NQO1 acts as a chaperones and how small ligands and missense mutations are capable of modulating these PPI.

### 2.1. Overview of NQO1 expression, regulation and functions: on the potential roles of O₂ levels and HIF-1α

NAD(P)H quinone oxidoreductase 1 (NQO1; DT-diaphorase; EC 1.6.5.2) functions as a homodimer of 31 kDa subunits [75, 76]. Each subunit contains two domains: an N-terminal domain (NTD, comprising approximately residues 1-225), that binds tightly one molecule of FAD and is capable of folding and assembly into dimers autonomously, and a C-terminal domain (CTD, comprising the last 40-50 residues) that completes the monomer:monomer interface and the binding sites of NAD(P)H and the substrates (Figure 3A) [75, 77, 78, 79, 80, 81]. Its main enzymatic function is to catalyse the two electron reduction of quinones, thus avoiding the production of reactive, and potentially cytotoxic, semiquinones (Table A1) [82]. Since one of its substrates is menadione (vitamin K3) it can function in blood clotting, although its role appears to be minor compared to vitamin K oxidoreductase [83]. Other substrates include α-tocopherol-quinone and ubiquinone, which are maintained in their reduced (antioxidant) forms by the enzyme [84, 85]. NQO1 can also catalyse the reduction of superoxide radicals and thus plays a role in directly protecting the cell from reactive oxygen species [86]. The enzymatic cycle of NQO1 follows a ping-pong mechanism with two main steps or semireactions (Figure 3B): in a first one, NAD(P)H binds to the holo-form (NQO1$_{holo}$) of the enzyme (FAD is not released during the cycle), consequently reducing the flavin to FADH$_2$ and releasing NAD(P)$^+$. This reductive half-reaction is extremely fast, with a rate constant in the range of $10^9$ M$^{-1} \cdot$s$^{-1}$. Importantly, the rate of this reductive half-reaction is strictly NADH-dependent, and thus, should be accelerated due to the HIF-mediated enhancement of aerobic glycolysis (raising cytosolic NADH levels, section 1.3.1); in a second and slower step (with a rate constant of $10^5$-$10^6$ M$^{-1} \cdot$s$^{-1}$), the oxidative half-reaction, the substrate binds and is reduced by the

FADH$_2$, thus releasing the reduced substrate and regenerating the holo-enzyme [76, 79]. Dicoumarol, a potent anticoagulant and mitochondrial uncoupling agent, acts as a competitive inhibitor of NAD(P)H during the NQO1 cycle (Table A1) [87].  The high level of NQO1 expression in some cancer cells, coupled with its role in defence against ROS, has led to the suggestion that NQO1 inhibition may be a novel therapy for the disease. Dicoumarol is an effective inhibitor of pancreatic cancer cells in culture, but no NQO1 inhibitors are yet in clinical use [88].
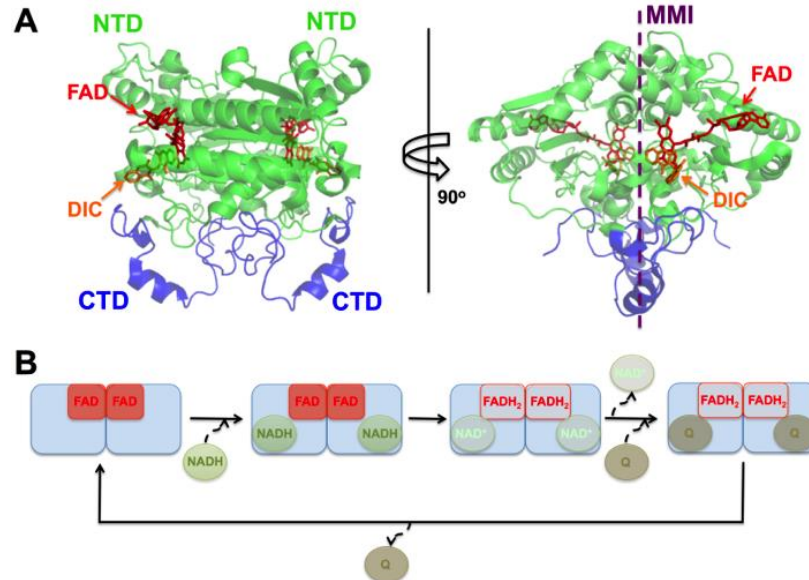


**Figure 3.** Structure and enzymatic cycle of NQO1. (**A**) Three-dimensional structure of NQO1$_{dic}$ (with FAD and dicoumarol bound)(PDB ID 2F1O). The N-terminal (NTD) and C-terminal (CTD) domains are indicated. MMI stands for the monomer:monomer interface. (**B**) Schematic representation of the catalytic cycle of NQO1.

The NQO1 protein has also non-enzymatic functions in which it mostly participates in PPI, mediating the stability of its binding partners (Figure 4 and Table A2). Indeed, and as we will further discuss in sections 2.2 and 2.3, several of the ligation states populated of NQO1 during the catalytic cycle (NQO1$_{holo}$, either oxidized and reduced, or the dead-end complex of the holo-enzyme with dicoumarol, NQO1$_{dic}$) are very relevant to the discussion of PPIs developed by NQO1.

NQO1 is also known to undergo different post-translational modifications (PTMs), some of which have been characterized in a site-specific manner revealing that these modulate NQO1 function and stability in different manners. Phosphorylation of NQO1 at T128 by the kinase Akt results in polyubiquitination and degradation of NQO1 via the proteasome [89]. A phosphomimetic mutation on S82 showed reduced FAD binding affinity and consequent loss of intracellular protein stability, although the kinase involved in this phosphorylation event has not been identified yet [90]. Consequently, phosphorylation of NQO1 at different sites may have an impact on the intracellular stability of its protein partners through destabilization of NQO1.

NQO1 is expressed in most human cell types (https://www.genecards.org/cgi-bin/carddisp.pl?gene=NQO1) and its expression can be upregulated in response to stress. Two control elements are located upstream of the human NQO1 gene – the antioxidant response element (ARE) and the xenobiotic response element (XRE) [91, 92, 93, 94]. A key regulator of the ARE is nuclear factor erythroid 2-related factor 2 (Nrf2). This transcription factor from the basic leucine zipper family is located in the cytosol under non-stressed conditions. Here it interacts with Kelch like-ECH-associated protein 1 (KEAP1) that targets it for ubiquitin-mediated proteasomal degradation [95, 96].  Oxidative stress is sensed by the oxidation of disulphide bonds in KEAP1 [97]. This releases Nrf2, which translocates to the nucleus and, together with members of the small musculoaponeurotic fibrosarcoma protein family (sMaF), binds to AREs [98]. This results in the recruitment of the Mediator complex, the acetylation of Nrf2 and the activation of a range of genes involved in antioxidant defense, including NQO1 [99, 100, 101].

Nrf2 also upregulates genes for NADPH generation, thus ensuring that enzymes like NQO1 have sufficient reduced cofactor [102, 103]. The ARE also responds to some antioxidants (e.g. resveratrol and quercetin), by upregulating the expression of NQO1 and other antioxidant enzymes [104, 105]. Interestingly, the ARE that controls the expression of NQO1, is also activated by hypoxic conditions [106]. Thus, NQO1 protein levels increase under hypoxic conditions [107]. Consequently, NQO1 is overexpressed in tumours due to their hypoxic environment.
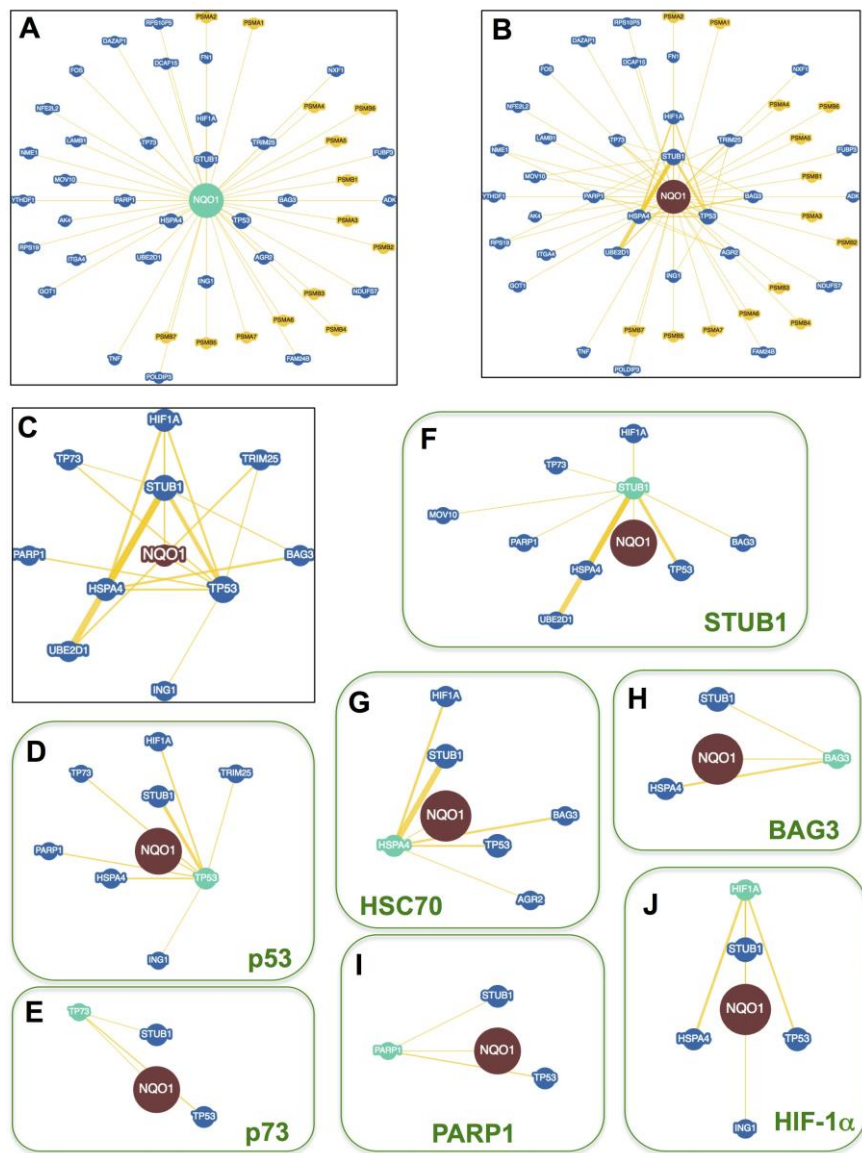


**Figure 4.** The NQO1 interactome. (**A**) NQO1 protein partners described in at least one report. (**B**) Connectivity between NQO1 partners. The shorter the radial distance between a partner and NQO1 indicates a higher interconnectivity. Partners in yellow are from mouse and in blue from human. (**C**) A zoom from panel B shows the most highly interconnected partners of NQO1. (**F-J**) Interactions between NQO1 partners in panel C and other proteins to highlight their different interconnectivity. The thickness of connecting yellow lines is related to the number of reports describing the interaction. Data were retrieved from the BioGRID database (https://thebiogrid.org/108072) [108].

The XRE is activated by the aryl hydrocarbon receptor (AhR), a basic helix-loop-helix transcription factor. The protein binds to a wide range of hydrophobic xenobiotics and translocates to the nucleus in response. Here it heterodimerises with aryl hydrocarbon nuclear translocator (ARNT or HIF1β) and binds the XRE [109, 110]. In the case of the NQO1 gene, AhR at the XRE and Nrf2 functionally cooperate to regulate its expression [111]. Like the ARE, the XRE can be induced

by hypoxia  [112].   Although HIF-1$\alpha$ also partners with HIF1$\beta$ to regulate transcription, siRNA silencing of the HIF1$\alpha$ gene does not affect NQO1 expression  [113]. This suggests that HIF1$\alpha$ is not directly involved in regulating NQO1 expression.

*2.2. NQO1 macromolecular interactions*

To date, there are 47 protein partners of NQO1 compiled in the BioGRID database (https://thebiogrid.org/108072)  [108]. This set includes 33 human proteins (actually one is a pseudo-gene product). The remaining 14 are from rodents and belong to different subunits of the 20S proteasome (Figure 4A). In addition, NQO1 can interact specifically with certain RNAs modulating their expression  [114]. Focusing on protein partners, there is certain evidence within the NQO1 interactome supporting that different partners (i.e. nodes) of this network can interact not only with NQO1 but also among each other, Consequently, this could generate regulatory circuits upon interaction of different partners in two- or multiple-way manners (i.e. binary, ternary, quaternary….complexes; Figure 4B). This also suggests that we may affect the properties (e.g. intracellular activity or stability) of one node by targeting different adjacent nodes in the network, an effect that could be amplified particularly in nodes with high connectivity. The most representative and consistently reported nodes with such a high connectivity for NQO1 are shown in Figure 4C and Table A2. Importantly, HIF-1$\alpha$ belongs to this group.

A critical reason for better understanding the interactome of NQO1 emerges from a well-known consequence on its interaction with partners: it leads to changes in the stability of the partner against (Ub-dependent or –independent) proteasomal degradation. Thus effects on the intracellular levels of NQO1 or in the strength of its interaction with partners may translate into altered stability of the nodes in the interactomic network. For instance, reducing the availability of the FAD precursor riboflavin leads to increased population of the flavin-free NQO1$_{apo}$ state, which is very sensitive to proteasomal degradation through ubiquitin-dependent and -independent mechanisms  [115, 116]. This is in part caused by the destabilization of the CTD of NQO1 in the absence of bound FAD that promotes its ubiquitination and degradation  [116]. Remarkably, addition of the inhibitor dicoumarol does not stabilize the NQO1 protein intracellularly but seems to reduce its ability to interact with and stabilize these protein partners (see Table A2). Binding of NAD(P)H, which reduces the bound flavin cofactor, seems to enhance the interaction of NQO1 with these protein partners ( [117], Table A2). A recent study also suggested that NQO1 with a reduced flavin cofactor could be a physiologically important state in the cell  [118], and thus very relevant to understand the interactome dynamics of NQO1 (note that the population of this state can be promoted due to HIF-mediated increased levels of cytosolic NADH, section 1.3.1). Additional factors modulating NQO1 intracellular stability, such as protein phosphorylation events, small ion binding or single amino acid changes  [119, 120, 121, 122, 123], may thus propagate and amplify these effects to the stability of the NQO1 protein partners.
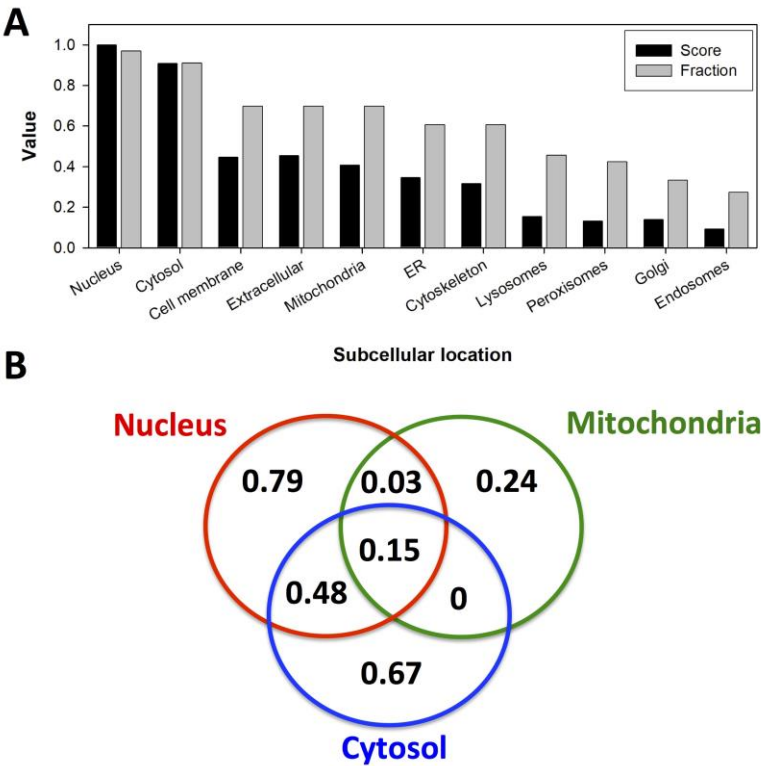
**Figure 5.** Subcellular location of NQO1 interacting human partners based on data from GeneCards®. For all the interactors, the subcellular compartment (location) and its confidence (1-5, from the lowest to the highest) were retrieved from GeneCards®. (**A**) Accumulated score for each organelle as the sum of the numerical degree of confidence for all partners found in a given compartment. The highest accumulated score (i.e. for the nucleus) was used to normalize yielding the *Score*. As *Fraction*, we refer to the fraction of all the partners found in a given organelle. Note that the ratio Score:Fraction gives a measure of the degree of confidence for finding a given partner in a given subcellular location. (**B**) Subcellular location of NQO1 partners in the three subcellular locations of NQO1 reported with confidence (i.e. equal or higher than 3) as the fraction of the total of NQO1 partners. Overlapping regions in the Venn diagram reveal the presence of an NQO1 partner in at least two subcellular locations.

Another critical issue for understanding the interaction of NQO1 with its partners and subsequent effects of these interactions on the stability of its interactome is the subcellular location of NQO1 and its partners. It is generally accepted that NQO1 subcellular location is primarily cytosolic, where it can fulfill its roles as antioxidant/detoxifiying enzyme and is able to interact with protein and RNA partners. However, there is also certain evidence supporting its location in other subcellular compartments, mostly mitochondria and the nucleus (with low confidence in other organelles)(Figure 5A; GeneCards®; https://www.genecards.org/cgi-bin/carddisp.pl?gene=NQO1&keywords=NQO1). Importantly, most of the protein partners of NQO1 also seem to primarily localize in the nucleus and the cytosol, and to a moderate extent in the mitochondria, cell membrane and extracellularly, apparently coexisting between more than one subcellular location (Figure 5A-B). In the case of the cytosol and the nucleus, about 50% of NQO1 partners may operate in any of these locations and translocate between these two to fulfill their roles in cytosolic and nuclear processes such as control of DNA expression (p53, HIF-1α    c-FOS, NRF-2, NME1, FUBP3), regulation of RNA and translation (POLDIP3, YTHDF1, NXF1, MOV10, DAZAP1, RPS19), regulation of nuclear protein function (PARP1 and TRIM25), protein folding and degradation (BAG-3, eIF4G1, DCAF15, HSPA4, STUB1, UBE2D1). In addition, a number of metabolic enzymes in the cytosol and mitochondria (ODC, NDUSF7, NME1, ADK, GOT1, AK4) could also interact with NQO1 in these compartments. Indeed, NQO1

overexpression in mice is known to enhance glycolytic and mitochondrial respiration activities and enhance metabolic flexibility, mimicking the beneficial effects of caloric restriction [124]. It is worth noting that the proteasomal protein degradation machinery may operate through rather similar mechanisms in the cytosol and the nucleus [125, 126], and thus, the well-known chaperone role of NQO1 for different protein partners (see Table A2), may indeed operate in the cytosol thus increasing the levels of cytosolic proteins amenable to import to other organelles (such as nucleus or mitochondria; [74, 127]) and plausibly by stabilizing these proteins upon import of both the partner and NQO1 in these organelles. Noteworthy, although more rarely described, the presence of NQO1 in other subcellular locations such as cytoskeleton may explain other roles of the multifunctional NQO1 protein. For instance, recent studies have supported that NQO1 may provide an adequate supply of NAD$^+$ for deacetylase activity of different sirtuins associated with microtubule dynamics [118, 124, 128]. These studies also highlight the potential plasticity of NQO1 subcellular location during different cellular conditions or stages (e.g. the recruitment of cytosolic NQO1 to cytoskeletal structures during cell division [118]).
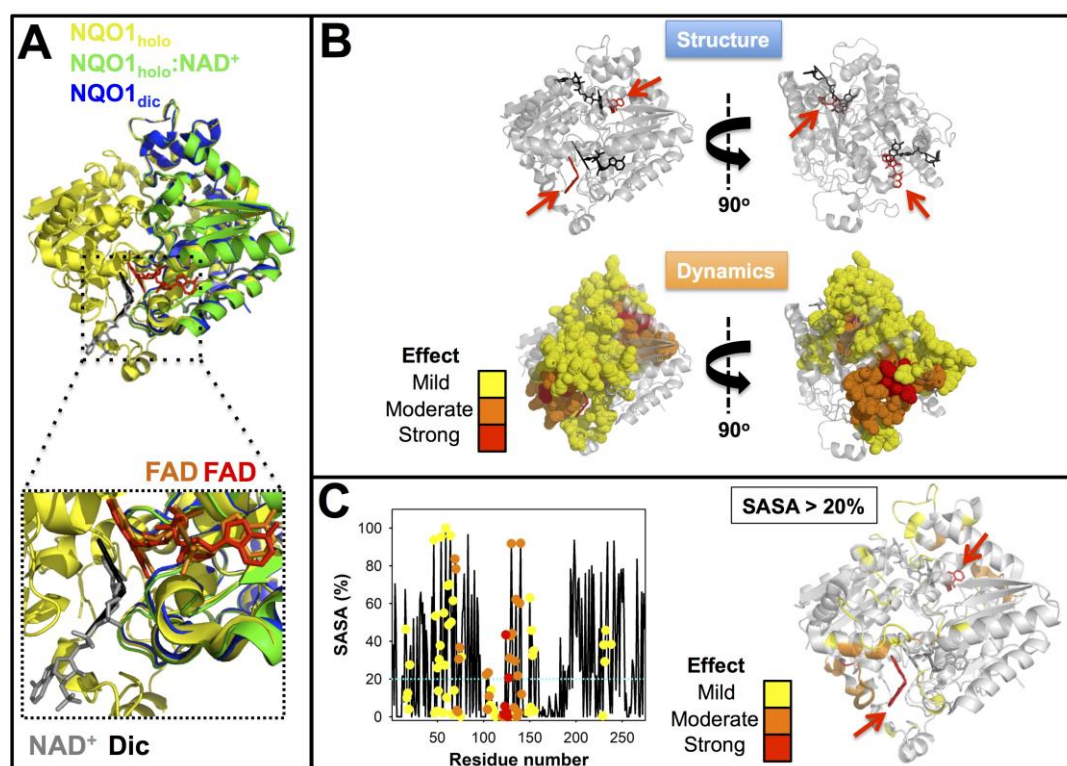


**Figure 6.** Structure and dynamics of NQO1 upon binding different ligands. (**A**) Structural overlay of the X-ray structures of NQO1$_{holo}$ (1D4A), NQO1$_{holo}$:NAD$^+$ (kindly supplied by Profs. Mario Bianchet and Mario Amzel, John Hopkins University Medical School, Baltimore, USA) and NQO1$_{dic}$ (2F1O). The lower panel shows a zoom highlighting the position of the FAD (orange, NQO1$_{dic}$ and red, NQO1$_{holo}$:NAD$^+$), NAD$^+$ and dicoumarol (Dic). (**B**) Dicoumarol binding causes long-range effects on the structural dynamics of NQO1 WT. Residues shown in dot representation are those for which the structural dynamics is reduced according to HDXMS [129]. (**C**) Most of the residues whose dynamics is reduced upon dicoumarol binding are solvent-exposed. The plot in the right shows the solvent accessible surface area (SASA) for the each residue as calculated in [129] and colour circles indicate the magnitude of the change in structural dynamics. The figure on the left shows the structural location of solvent-exposed residues (SASA > 20%). The color scales in panels B and C reflect the magnitude of the changes in protein dynamics according to [129] and red arrows indicate the position of dicoumarol.

*2.3. Changes in NQO1 stability, structure and dynamics upon ligand binding: implications for the stability of its protein partners*

The functional chemistry of NQO1 is remarkably complex. At least four different ligation states (with small molecules bound) are relevant to understand the intracellular stability and enzymatic activity of NQO1 as well as its effects on the function and stability of its macromolecular partners: i) $NQO1_{apo}$, which has no ligand bound; ii) $NQO1_{holo}$, which contains a molecule of oxidized FAD per NQO1 monomer; iii) $NQO1_{holo-red}$, containing the FAD cofactor reduced upon interaction with NAD(P)H and hydride transfer; and iv) $NQO1_{dic}$, a ternary complex of $NQO1_{holo}$ with the inhibitor dicoumarol bound. These different ligation states interact differently with NQO1 protein partners [78, 117, 127]. High-resolution structural models for these complexes are, to the best of our knowledge, not reported. Thus, we will focus in this section on the structural and dynamic consequences of ligand binding to NQO1 in an attempt to obtain some insight into their regulatory effects on NQO1 interactions with protein partners (always from an NQO1 point of view).

$NQO1_{apo}$ exists as a stable and expanded dimer characterized by significantly large conformational flexibility [76, 78, 79, 80, 119, 130, 131, 132]. Although no high resolution structural model is available for this state, recent kinetic studies using hydrogen-deuterium exchange mass spectrometry (HDXMS) have identified a minimal stable core that holds the protein dimer while most of the protein exists forming a highly dynamic structural ensemble, including the FAD and substrate binding sites in non-competent states for binding [129]. This remarkable conformational flexibility makes $NQO1_{apo}$ likely the most relevant state to understand the intracellular stability of NQO1, since the flexible CTD acts as initiation site for rapid degradation through ubiquitin-dependent and -independent proteasomal pathways [90, 116]. It is plausible that this unstable and highly dynamic $NQO1_{apo}$ state is not capable of interacting with NQO1 protein partners, or at least, does so with lower affinity [78].

Binding of FAD triggers a large conformational change leading to compaction of the protein dimer, increase in ordered secondary structure, overall conformational stabilization and a large decrease of protein dynamics that is sensed in almost the entire protein structure [78, 79, 80, 119, 129, 130, 131, 133]. This $NQO1_{holo}$ state is amenable for high-resolution structural studies [81](Figure 6A). This state is also known to be intracellularly stable and likely to interact with protein partners quite efficiently [115, 116, 117, 119, 123].

Binding of NAD(P)H results in FAD reduction, leading to a state we named $NQO1_{holo-red}$. This reaction is extraordinarily fast (close to the diffusion limit) and this state is likely unstable unless strongly reducing and/or anaerobic conditions are used (at least *in vitro*, [79]). Consequently, detailed structural analyses on this state are non-existant. However, it is worth commenting on some studies carried out under less stringent (i.e. aerobic) conditions, particularly because a wealth of work support that generally, this state may strongly interact with NQO1 protein partners [117, 127]. Using biochemical *in vitro* assays with NADP(H), it has been reported that $NQO1_{holo-red}$ influences the conformation of the CTD affecting the interaction with antibodies raised against it [118] and apparently increasing the thermodynamic stability of this domain [80, 118]. Intriguingly, the changes in conformation and stability of the CTD reported for $NQO1_{dic}$ and $NQO1_{holo-red}$ are strikingly similar, suggesting again that subtle differences in structure and dynamics between these two states are likely responsible for their opposing effects on the interaction of NQO1 with protein partners.

Binding of dicoumarol (or $NAD^+$) causes small structural rearrangements in the protein structure [75, 78, 81, 131, 134](Figure 6A). Therefore, from NQO1 structural point of view, it is not straightforward to understand how $NQO1_{dic}$ prevents binding to or largely decrease binding affinity for macromolecular partners with subsequent effects on the stability of these partners [78, 127]. An alternative explanation has emerged from studies on NQO1 protein dynamics by HDXMS [129]. Binding of dicoumarol to WT NQO1 causes strong effects on NQO1 protein dynamics, affecting the local stability of the protein core and these effects propagate through long distances in the protein structure (Figure 6B). Some of these effects are sensed by the CTD (Figure 6B), which may explain how dicoumarol binding mildly increases the thermodynamic stability of this domain by about 1.5

kJ·mol$^{-1}$ [80, 135]. Interestingly, a significant fraction of the residues whose stabilities are affected by dicoumarol binding appear on the protein surface and far from the ligand binding site (Figure 6C). This may imply that NQO1$_{dic}$ interacts differently with macromolecular partners through changes in the local stability and dynamics of the(se) binding site(s), which are likely located on NQO1 protein surface.

*2.4. Mutations and polymorphisms in NQO1. Disease and protein interactions*

The association of NQO1 activity with several human diseases with a huge social impact, particularly cancer, HIV infection and neurological and cardiovascular diseases, has attracted the attention towards the effects of naturally-occurring single amino acid changes on NQO1 and the predisposition provided by these changes towards disease [76, 79, 116, 119, 122, 123, 130, 133, 136, 137, 138, 139, 140, 141]. There are 106 missense variants described in human population (ExAC and gnomAD databases; https://gnomad.broadinstitute.org/gene/ENSG00000181019?dataset=gnomad_r2_1) and 47 missense variants in the COSMIC database (https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=NQO1#variants). By far, the two most common single amino acid variants are p.P187S and p.R139W, with allelic frequencies in human population of about 0.25 and 0.03, respectively. Consequently, these two polymorphisms have been characterized in some detail. In addition, and to the best of our knowledge, only one other naturally-occurring single amino acid variant, p.K240Q (found in gnomAD and COSMIC databases), has been characterized. Since the studies aimed at characterizing the effects of these single amino acid variants have been recently reviewed [76, 132], we will just outline in this section some of the key features described for them focusing on their potential impact on the interaction with protein partners and with those ligands known to modulate NQO1:protein interactions.

The intracellular effects of p.P187S can be ascribed to changes in its stability and activity, respectively. This variant decreases by 10- to 40-fold the affinity for FAD thus promoting the population of NQO1$_{apo}$ [79, 120, 122, 133, 136, 142]. Unlike WT NQO1, p.P187S in both NQO1$_{holo}$ and NQO1$_{apo}$ are degraded similarly, due to a strong thermodynamic destabilization of the CTD in NQO1$_{holo}$ that triggers ubiquitination and degradation [78, 79, 116, 119, 120, 122]. Remarkably, the crystal structure of p.P187S in the NQO1$_{dic}$ state is virtually identical to that of the WT enzyme [79]. This seems to be paradoxical, because P187 is fully buried in the protein structure, close to the NQO1 dimer interface and belongs to the minimally stable core of NQO1$_{apo}$, and thus, a mutation to serine should have catastrophic effects on protein structure and function [129, 136]. Detailed biochemical, biophysical, computational and mutational studies have shown that the effects of p.P187S are pleiotropic in the structure, function and stability of NQO1$_{apo}$ and NQO1$_{holo}$, highlighting the critical role of propagation of the local stability effects due to p.P187S to long distances in the protein structure and affecting the dynamics and stability of critical regions of NQO1 function [78, 79, 90, 119, 120, 122, 131, 133, 136, 137]. These studies on p.P187S have also supported that different functional sites located far (over 10-20 Å) in the structure are functionally and energetically coupled, and thus, we might modify the interaction of NQO1 with its partners by using an allosteric site (i.e. a ligand binding or mutated site) far from the protein:protein binding site. p.P187S is known to affect the interaction with NQO1 partners and lead to their destabilization, although it is not clear whether the origin of these alteration resides mainly in altered protein:protein interactions (i.e. binding affinity) or in the decreased intracellular stability of this variant that is reflected in those of the partners [78, 115, 116, 119, 120, 123, 143].

The polymorphism p.R139W represents a beautiful example in which a single nucleotide change may affect protein functionality at post-transcriptional and translational levels. In the one hand, it affects normal processing of NQO1 mRNA leading to exon 4 skipping, that produces a shorter version of the NQO1 protein that is extremely unstable inside cells due to the lack of part of the catalytic site (residues 102-139) critical for protein folding and stability [144]. On the other hand, full-length NQO1 is also translated containing the single amino acid exchange p.R139W, which has only small effects on protein stability and function [119, 130, 133]. Consequently, the former effect is

likely the prevalent one to explain the loss of intracellular NQO1 activity due to this polymorphism [119, 130, 133, 144]. In addition, it must be expected that the decrease in full-length NQO1 protein caused by this polymorphism may negatively impact the stability of protein partners, although to our knowledge, no experimental evidence for this is available.

The rare mutation p.K240Q was investigated to determine whether variable structural perturbations (mutations to glutamine as well as more perturbating variants such as changes to glycine and glutamate) in the CTD could propagate through the NQO1 structure to affect different functional sites [122, 136]. K240 is a solvent-exposed residue involved in a highly-stabilizing electrostatic network in the CTD [122]. This site accepted different types of mutations without compromising protein levels, activity, stability and solubility upon expression in *E. coli*. [122, 136]. However, detailed biophysical and biochemical experimentation, actually revealed that the p.K240Q mutation affected locally the stability of the CTD, and this effect was more pronounced in the more perturbing in the mutations p.K240G and p.K240E [122, 136]. A similar gradual effect was observed on the affinity for FAD, a remarkable result considering that the FAD binding site is located at 20 Å from the altered site [122, 136]. Studies with this mutation thus highlighted the plasticity of the NQO1 protein in different ligation states to transmit the effects of single amino acid changes and ligand binding. Due to this plasticity, we may anticipate that rare mutations found in cancer cell lines (COSMIC) or whole-genome sequence studies (gnomAD) such as p.K240Q would have certain effects on NQO1:protein interactions and consequently the stability of its partners. This hypothesis awaits to be tested experimentally.

## 3. Targeting the chaperone role of NQO1 to inactivate HIF-1α: future perspectives

The chaperone role of NQO1 on HIF-1α has been recently addressed in some detail in relation with hypoxia and normoxia [74]. While hypoxic and normoxic conditions provided somewhat different effects, the overall consequence of NQO1:HIF-1α interaction was the stabilization of HIF-1α upon interaction. The expression levels of NQO1 consistently modulated the levels of HIF-1α likely due to the specific interaction of NQO1 with the ODD domain HIF-1α in the cytosol, rather than to changes in HIF-1α mRNA at the transcriptional level [74]. This interaction enhances the intracellular stability of HIF-1α by suppressing its ubiquitination in the cytosol, and thus promoting its nuclear import and transcriptional activity [74]. This chaperone effect is enhanced under hypoxic conditions due to increased expression of NQO1 [74, 107]. Intriguingly, the presence of the p.P187S polymorphism did not prevent the chaperone action of NQO1, although it is well known to reduce NQO1 protein levels due to intracellular destabilization (see Section 2.4).

We could envision different strategies to disrupt NQO1 interaction with HIF-1α leading to increased degradation of the latter:

i) To prevent PPI by targeting the PP binding site or an allosteric site. We must note that allosterism is likely a critical feature for the multifunctionality of both NQO1 (sections 2.3 and 2.4) and HIF-1α [145, 146]. Allosterism in HIF-1α is dramatically exemplified by the negative effector CITED2, that competes with HIF-1α for the same binding site on CBP/p300 attenuating the transcriptional activity of HIF-1α [145, 146]. Remarkably, CITED2 and HIF-1α show the same binding affinity for CBP/p300, although the former is much more efficient in displacing the latter from binary complexes with CBP/p300 due to enhanced HIF-1α release linked to the intrinsic disorder of the C-TAD domain. We must note that an important caveat to rational screening for ligands is the lack of high-resolution structural models neither for a complex of NQO1 with a partner nor for the ODD of HIF-1α. There is also no detailed biochemical mapping of the interaction site or plausible molecular models. In addition to identifying non-covalent binders, potential, specific covalent modifiers should also be considered (*cf* the cysteine modification of *Giardia lamblia* triose phosphate isomerase by omeprazole which destabilises the dimer [147]).

ii) A high-throughput screen for ligands that target the formation of the NQO1: HIF-1α complex. Both (i) and (ii) would require a rapid, reproducible, robust assay for the interaction *in vitro*, for example labeling of the proteins with a fluorophore and a quencher. A cell-based assay would also

be required to test hits from the screen under *in vivo* conditions, for example proteins labeled with FRET donors and acceptors, co-immunoprecipitation or indirectly by measuring amounts of HIF1$\alpha$. Although challenging due to several highly disordered regions in the HIF-1$\alpha$ protein, such screening would be still feasible as recently reported for inhibiting PPI in the cancer-associated and intrinsically disordered protein NUPR1  [148, 149].

iii)  To use dicoumarol-like molecules that may target that interaction with lower second-site effects  [150, 151, 152]. It must be noted regarding this approach that the effect of NADH or dicoumarol have not been tested for the interaction of NQO1 with HIF-1$\alpha$. Many dicoumarol analogues that function as NQO1 inhibitors have been reported. It is reasonable to assume that they would also antagonize the protein's interactions with binding partners in a similar way to dicoumarol. As such, they represent immediately available compounds, which could be tested for their ability to antagonize the NQO1/HIF1$\alpha$ interaction. However they are likely to inhibit or antagonize many of NQO1's functions and may, therefore, cause significant off-target effects. Some of these may be undesirable in the context of cancer therapy, e.g. the antagonism of the NQO1/p53 interaction and consequent down-regulation of p53-mediated apoptosis  [153, 154].

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**Appendix A**

Tables A1 and A2 compile information on known NQO1 substrates, inhibitors and protein partners.

| *Compound* | *Comments* | *References* |
|:---:|:---:|:---:|
| ***Substrates*** | | |
| *Dichlorophenolindolphenol (DCPIP)* | *Non-physiological, but often used in in vitro assays* | *[155, 156]* |
| *Menadione (Vitamin K$_3$)* | *Reaction occurs in vivo but enzyme likely to play only a minor role in blood clotting* | *[83, 156]* |
| *Coenzyme Q$_{10}$ (ubiquinone)* | *NQO1 maintains this, and related compounds, in the reduced form* | *[157, 158]* |
| *Superoxide ions* | *Likely physiological role in directly combatting oxidative stress in vivo.* | *[86]* |
| *Fe(III) ions* | *Probably non-physiological* | *[159]* |
| *Idebenone* | *Important in the metabolism of this drug (a coenzyme Q$_{10}$ mimic)* | *[160]* |
| *(3-hydroxymethyl-5-aziridinyl-1-methyl-2-(H-indole-4, 7-indione)-propenol) EO9* | *Reduction activates this anticancer compound* | *[161]* |
| *Quinone epoxides* | *Potentially important if members of this group of compounds used as drugs.* | *[162]* |
| *β-lapachone* | *Futile cycling involving NQO1 results in reduced cellular concentrations of NAD(P)H contributing to cell death* | *[163, 164]* |
| *Mitomycin C* | *Reduction activates this akylating cytotoxic drug* | *[165, 166]* |

| | | |
|---|---|---|
| *Tirapazamine and other heteroaromatic N-oxides* | *Slow reaction.   Superoxide also produced* | *[167]* |
| *Benzofuroxans* | *Reduction by NQO1 may play a minor role in cytotoxicity* | *[168]* |
| *Nitroaromatics* | *Reactivity correlates with electrode potential* | *[169, 170]* |
| *Aminochrome* | *Reaction is important in protection against Parkinson's Disease and other neurological diseases* | *[171, 172]* |
| ***Inhibitors*** | | |
| *Dicoumarol and derivatives thereof* | *High affinity; competes with NAD(P)H; negatively cooperative; often used in experimental studies; derivatives may be anticancer lead compounds; dissociates NQO1-p53 complexes resulting in increased p53 degradation and inhibition of apoptosis* | *[143, 151, 154, 156, 173, 174]* |
| *Curcumin* | *May dissociate NQO1-p53 complexes resulting in increased p53 degradation and inhibition of apoptosis.   Other studies suggest it may enhance the NQO1-p53 interaction in vivo* | *[175, 176]* |
| *Resveratrol* | *Potent inhibitor of the related protein NQO2; only weakly inhibits NQO1* | *[177]* |
| *Warfarin* | *NQO1 is a secondary target for this anticoagulant* | *[174, 178]* |

**Table A1.**   Selected substrates and inhibitors of NQO1.

| Macromolecule | Effect on partner stability | Effect of NAD(P)H | Effect of dicoumarol | General comments | References |
|---|---|---|---|---|---|
| p53 | *Binds to, and stabilises, the full-length protein, protecting it from proteasomal degradation* | *Increases affinity of interaction.* | *Antagonises interaction.* | *Interaction promotes p53-mediated apoptosis. Dicoumarol down-regulates this by promoting release of p53 from NQO1 and consequent degradation of p53.* | [117, 143, 153, 179, 180] |
| p73α | *Binds to, and stabilises, the full-length protein, protecting it from proteasomal degradation.* | *Increases affinity of interaction; effect not observed with NAD+.* | *Antagonises interaction.* | *Interaction promotes p73α-mediated apoptosis. Dicoumarol down-regulates this by promoting release of p73α from NQO1 and consequent degradation of p73α. No interaction with p73β which lacks a SAM domain at the C-terminus. The SAM in p73α is responsible for the interaction.* | [78, 117] |
| Ornithine decarboxylase (ODC) | *NQO1 binds to, and stabilises ODC, preventing proteasomal degradation* | *Not known.* | *Antagonises interaction* | *ODC monomer (inactive) degradation is enhanced by binding antizyme 1 (AZ1) which targets the ODC/AZ1 complex to the proteasome. NQO1 protects monomeric ODC by binding the ODC/AZ1 heterodimer.* | [181, 182] |
| mRNA encoding SERPINA1 (α-1-antitrypsin) | *No effect on stability* | *Not known* | *Not known* | *Does not affect the amount of mRNA, but does enhance the translation by binding to 3′-UTR. This results in more protein* | [114] |
| 20S proteasomal subunit | *Not known* | *No effect* | *Not known* | *NQO1 interacts with the proteasome and negatively regulates proteolytic activity. NQO1-apo is degraded by the proteasome.* | [115] |
| HIF-1α | *NQO1 binds HIF-1α, stabilises it and prevent proteasomal degradation* | *Not known* | *Not known* | *Interaction occurs in cytoplasm. NQO1 enhances transcription of HIF-1α regulated genes, presumably by increasing the amount of HIF-1α. See also main text.* | [74] |
| Hsp70/HSPA4 | *Interaction most likely occurs* | *Not known* | *Not known* | *NQO1-p.P187S only interacts very weakly.* | [183] |

| | | | | |
|---|---|---|---|---|
| | *with newly synthesised NQO1, presumably stabilising it and assisting folding.* | | | |
| *STUB1/CHIP* | *Not known* | *Not known* | *Not known* | *NQO1 ubiquitination is mediated upon binding to STUB1 which triggers NQO1 degradation.*<br>*NQO1-p.P187S and apo-NQO1 are degraded efficiently by this mechanism.*<br>*STUB1's TPR domain is required for the interaction.* | *[116, 184]* |
| *c-FOS* | *NQO1 binding to c-FOS protects the former from 20S proteasomal degradation* | *Not known* | *Not known* | *NQO1 localises c-Fos (at least partly) to the cytoplasm. Free c-Fos or c-Fos in complex with other transcription factors, is localised to the nucleus.* | *[185]* |
| *Bcl2-associated Athanogene 3 (BAG3)* | *Not known* | *Not known* | *Not known* | *BAG3 regulates the proteasome. siRNA knockdown of BAG3 reduces proteasomal activity.* | *[186]* |
| *ING1B (p33)* | *NQO1 binds to p33^{ING1b} tumour suppressor protecting the former from 20S proteasomal degradation.* | *Increases affinity of interaction* | *Not known* | *NQO1 binds preferentially to Ser-126 phosphorylated p33^{ING1b}. This phosphorylation is induced by genotoxic stress and increases the in vivo half-life of the protein from 5.7 h to 16.8 h.* | *[187, 188]* |
| *HIV-1 Tat* | *HIV-1 Tat binds to NQO1 stabilizing the former* | *Not known, but assumed to promote interaction.* | *Antagonises interaction.* | *HIV Rev downregulates NQO1 destabilizing HIV/Tat complex and thus resulting in increased Tat degradation. Dicoumarol inhibits HIV replication.* | *[138]* |
| *eIF4GI* | *eIF4GI binds to NQO1 leading to protection of the former against proteasomal degradation* | *Not known.* | *Antagonises interaction.* | *This interaction modulates mRNA translation. Dicoumarol downregulates translation. Interaction does not require RNA binding by eIF4GI. Oxidative stress reduces the amount of cellular eIF4GI.* | *[189]* |
| *Homocysteine-induced endoplasmic reticulum* | *Stabilises Herp by protecting it from protasomal degradation* | *Not known* | *Antagonises interaction* | *Herp is up-regulated in the unfolded protein response (UPR). Herp's cellular half-life is increased by NQO1.* | *[190, 191]* |

| | | | | | |
|---|---|---|---|---|---|
| *protein (Herp)* | | | | | |
| *PGC-1α* | *Stabilises this intrinsically disordered protein and protects it from proteasomal degradation.* | *Enhances interaction* | *Antagonises interaction* | *Cellular levels of NQO1 and PGC-1α are correlated. Stabilisation of PGC-1α by NQO1 leads to induction of genes encoding enzymes of gluconeogénesis.* | *[192]* |
| *RIL (reversion-induced LIM domain protein; PDLIM4)* | *NQO1 binds and stabilises unstructured C-terminal región of one alternately spliced isoform (RILaltCterm) protecting it from proteasomal degradation.* | *Not known* | *Increases RIL proteasomal degradation, presumably by antagonising RIL/NQO1 interaction* | *RILaltCterm accumulates in response to oxidative stress and stimulates actin cytoskeleton rearrangement.* | *[193]* |
| *TAp63γ* | *Stabilises TAp63γ and protects it from proteasomal degradation.* | *Not known* | *Not known* | *NQO1-p.P187S does not interact. Interaction occurs in response to genotoxic stress.* | *[194]* |

**Table A2.**   Macromolecular binding partners of NQO1 and effects of ligand binding.

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