Human Vitamin K Epoxide Reductase as a Target of its Redox Protein

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BiMoDyM

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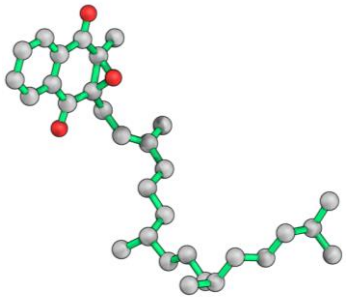
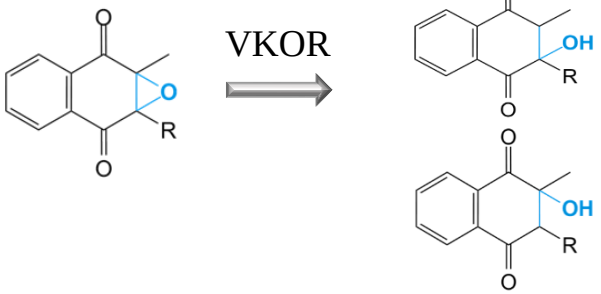
Introduction: Vitamin K epoxide reductase

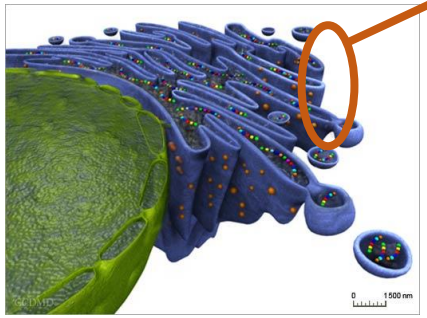
1. Vitamin K and its transformation





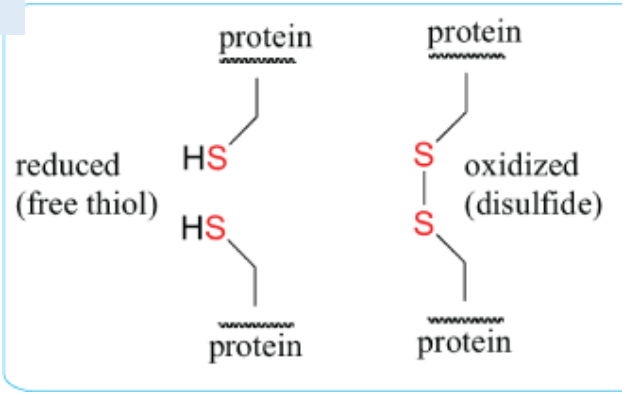
Blood coagulation co-factor

2. Vitamin K epoxide reductase



That's known ?

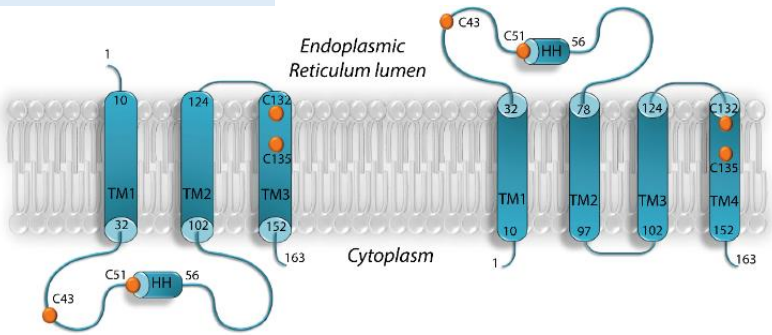
- Enzyme constituted of 163 aa_s;
- four cysteine (C) aas play a role in enzymatic mechanisms
- Cysteins can be reduced (S-H) or
- oxidised (S-S bond)
- CXXC motif forms the active site
- VKOR activation requires H-atom(s) to come from outside (redox protein)

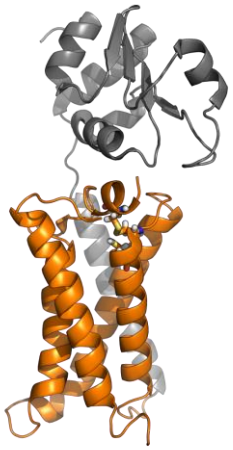


3. Challenges

What are the problems ?

- No solid empirical structural data on hVKORC1
- Redox protein is unknow





VKOR from *Synechococcus sp.* (b-VKOR)

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Identification of the functional states of human vitamin K epoxide reductase from molecular dynamics simulations†‡

N. Chatron,[abc] B. Chalmond,[a] A. Trouvé,[a] E. Benoît,[b] H. Caruel,[c] V. Lattard[b] and L. Tchertanov *[a]

In mammalians, the enzymatic activity of vitamin K epoxide reductase (VKORC1) requires a protein conformational reorganisation that includes several transient enzymatic states involving a dynamic electron transfer. Regarding the structurally non-characterised human enzyme (hVKORC1), this process remains poorly explained and the different redox states of the enzyme generated by its biochemical transformation are unknown. Here, we report a 3D model of the fully reduced hVKORC1 at the atomistic level. By exploring this model through molecular dynamics (MD) simulations, we established the most probable intermediate states of the enzyme which were used for generation of the putative functionally-related enzymatic states. Enzymatic functionality of each state was assigned by probing their recognition properties with respect to vitamin K in its quinone and hydroxyquinone forms. Two states were identified as contributing to the two-step vitamin K transformation. The state highly selective for native vitamin K was further validated through analyses of its free energy of binding with vitamin K agonists (VKAs) that showed a high correlation with the experimental inhibiting constants.

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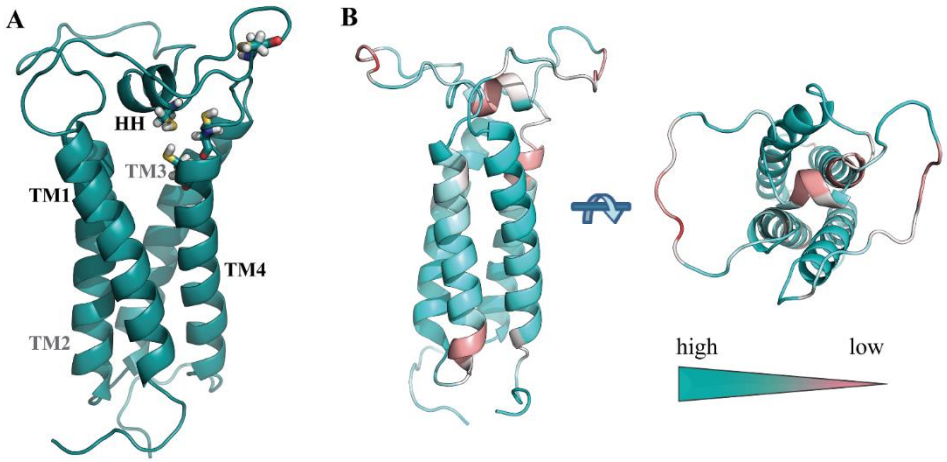
Introduction

The mammalian vitamin K epoxide reductase (VKOR) VKORC1 is an endoplasmic reticulum (ER)-resident transmembrane protein involved in γ-carboxylation of glutamate residues of vitamin K-dependent proteins (PVKD). These proteins include blood clotting factors, matrix Gla protein and osteocalcin,[1,2] species.[10–12] The function of the latter cysteine residues in human VKORC1 remain controversial[1,13,14] as the regulation of human enzyme activity differs from that of the bacterial enzyme, *i.e.* in the absence of a thioredoxin domain, hVKORC1 should use an external partner as a redox source.[15]

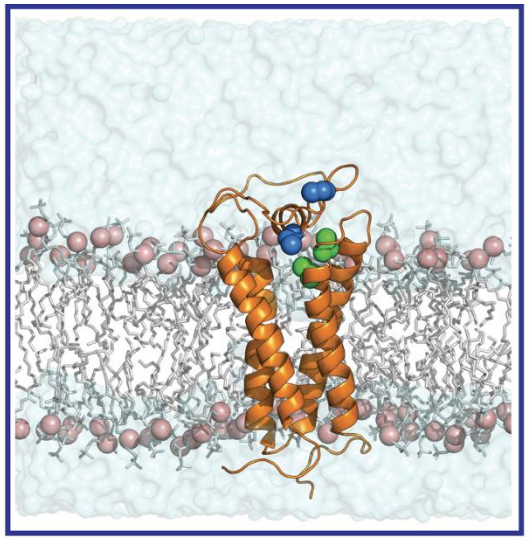
Deregulation of the catalytic activity of hVKORC1, usually prompted by naturally occurring mutations, is responsible for

- The four TM-helices 3D *in silico* model of hVKORC1 developed by Chatron *et al*. (2017) was confirmed empirically (Liu *et al.,* 2021).

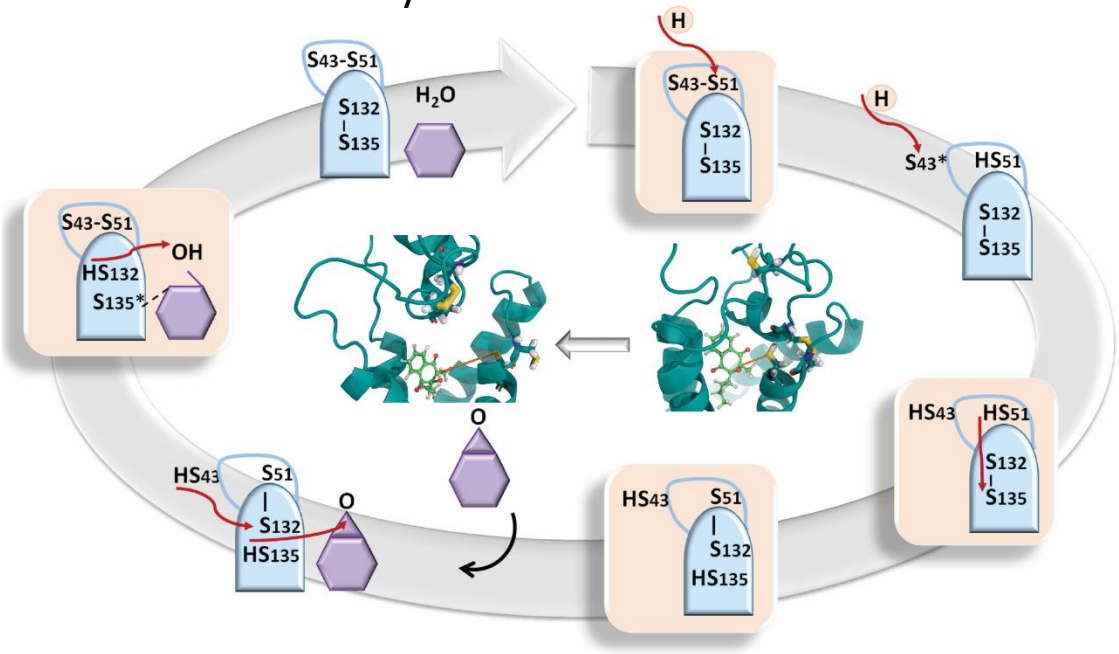


Chatron N., Chalmond B. Trouvé A., Benoît E., Caruel H., Lattard V., Tchertanov, L. Identification of the functional states of human vitamin K epoxide reductase from molecular dynamics simulations. *RSC Adv.* **2017**, *7*, 52071–52090. https://doi.org/10.1039/C7RA07463H

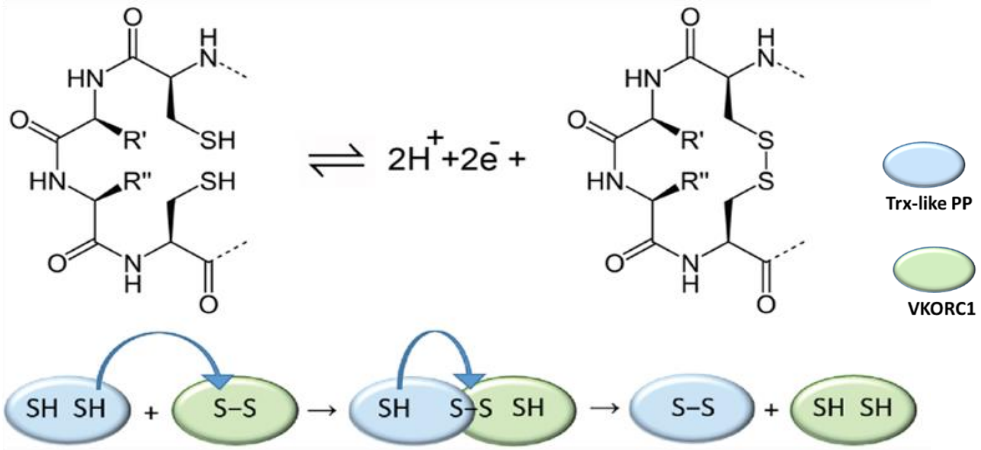
- The enzymatic-like (biologically relevant) states were identified and their role in the hVKORC1 enzymatic process was established by Chatron *et al*. 2017



MD simulation



The human VKORC1 recognition by thioredoxin-fold proteins



Activation of VKOR requires its cooperation with a redox partner delivering reducing equivalents through thiol-disulphide exchange reactions, involving a disulphide bridge from the extended luminal loop (L-loop) of VKOR. The activation process represents a first and less studied step in VKOR vital cycle.

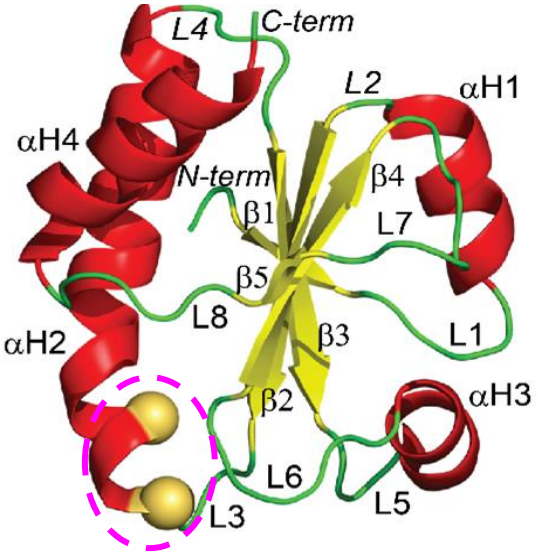
That's known ?

VKORC1 activator: **Trx-like protein with the redox activity**

Four proteins candidates: **ERp18, PDI, TMX1, TMX4**

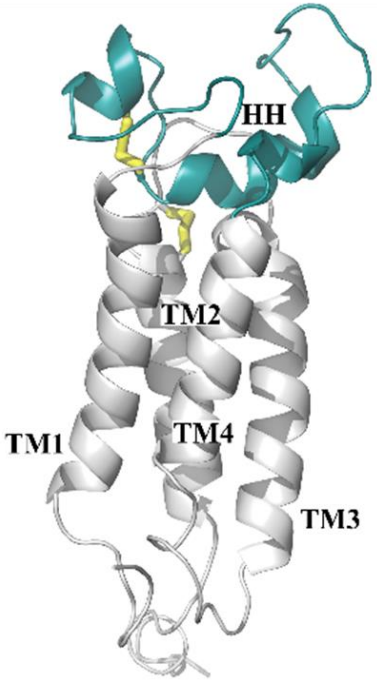
Fonction: **Maintaining of proteins in the reduced state (SH SH)**

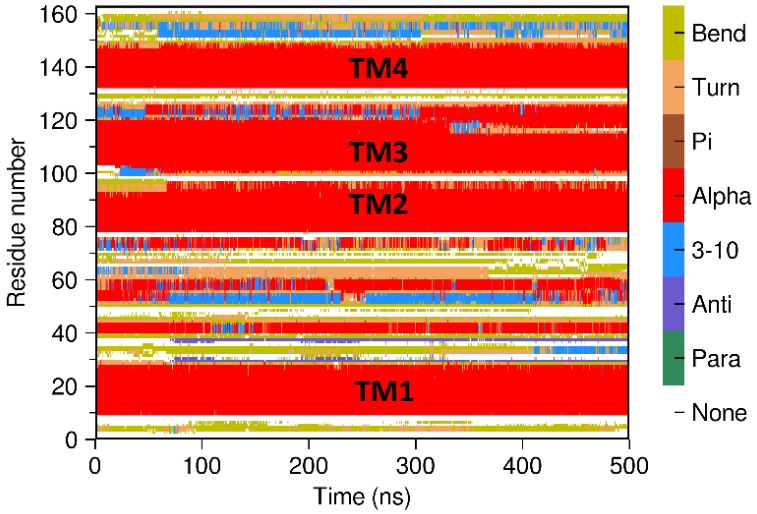
Propriety: **Contains a conserved CX_1X_2C ($X_1 \neq X_2$) motif**

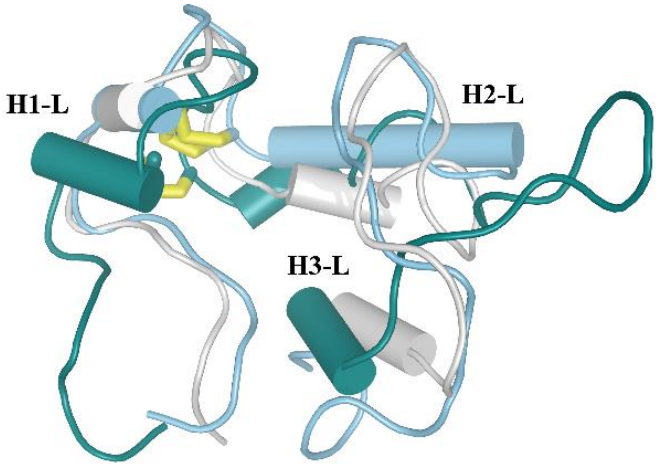


CX_1X_2C motif

VKORC1 viewed as the target of a Trx-fold protein

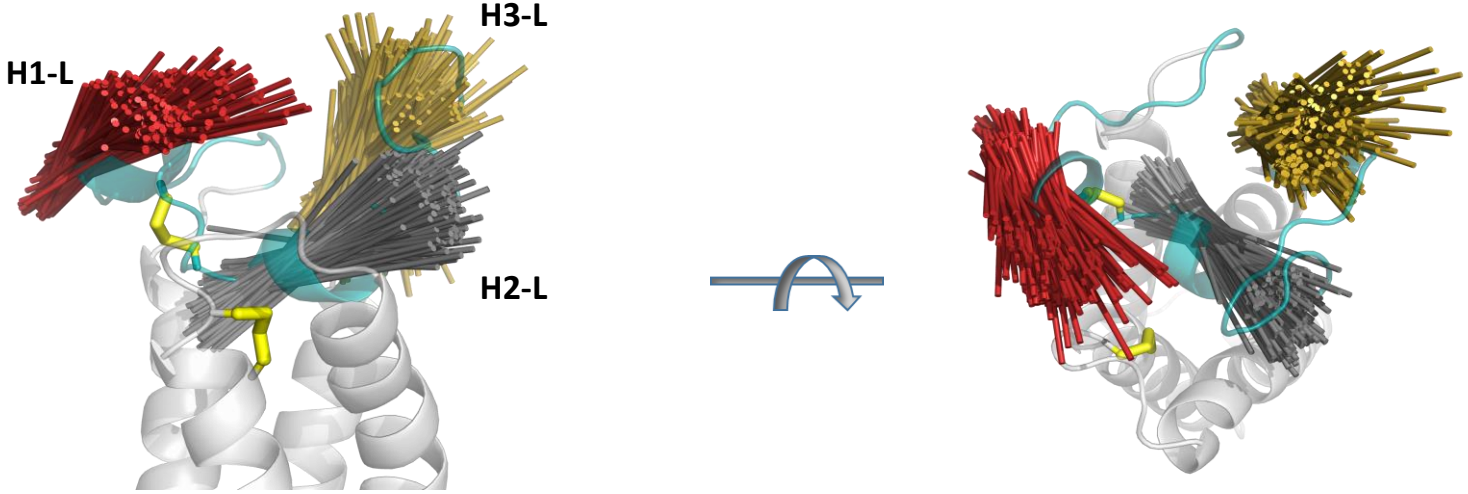


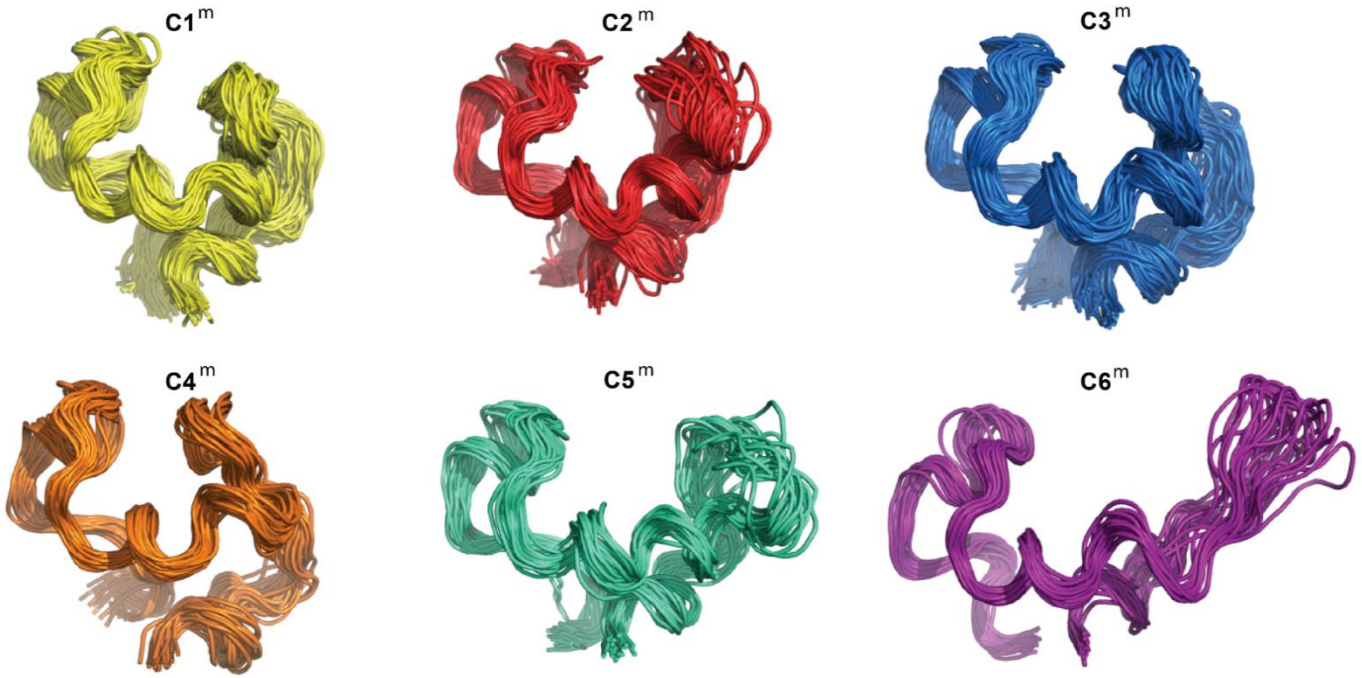




L-loop R33-N78 aas

The L-loop in the inactive (oxidised) state of hVKORC1 is noticeably less flexible than the reduced states [*Chatron et al. 2017*] and more folded. Three helices - αH1 and two transient helices, H2 and H3 - were generally maintained over the MD simulations of oxidised hVKORC1, while the length and spatial positions of the helices were highly variable.

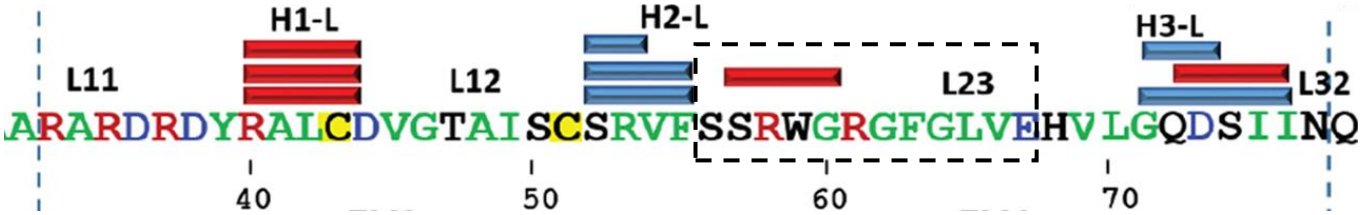




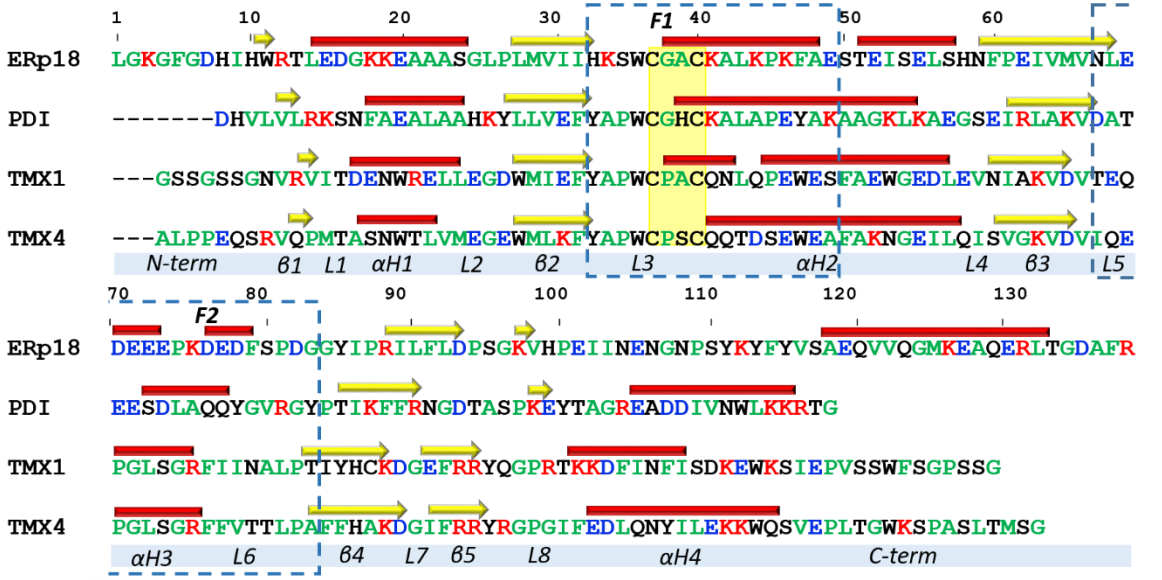
L-loop conformations vary from a compact "closed" conformation, which is prevalent, to an extended "open" conformation.

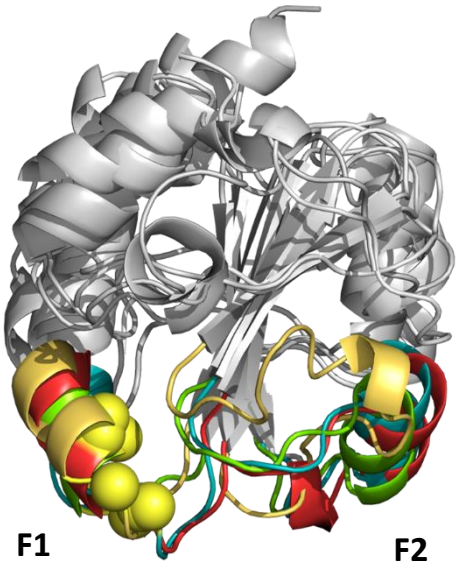
Transient helices and high conformational flexibility qualify L-loop as an **intrinsically disordered (ID) region** of hVKORC1.

- Transient H2-L helix ($\alpha H \leftrightarrow 3_{10}$ fold) controls the L-loop structural and conformational properties: its shortening promotes elongation of the coiled linker connecting H2-L and H3-L (L23). The extended L23 shows (i) a scissors-like motion concerning the H1-L helix and (ii) a large vertical displacement concerning the TMD, increasing mobility to H3-L.
- These features of H2-L and its adjacent loop L23 suggest their important functional role, such as a contribution to partner protein recognition.
- S56-H68 (especially S56-R61) fragment is suggested as a binding platform for recognising and binding a protein partner.



Which protein from four Trx-fold candidates is the most probable partner for hVKORC1?



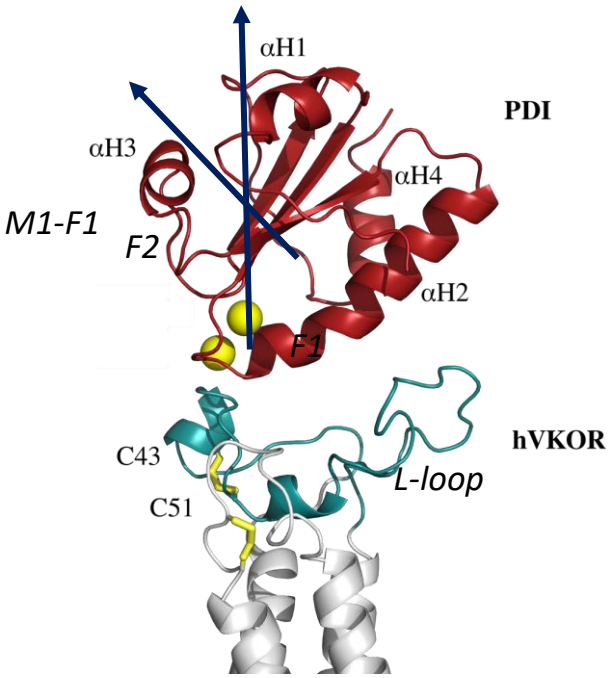


- The two fragments, **F1** and **F2**, of Trx-fold proteins were suggested as the regions that enable the intermolecular interactions through salt bridges, H-bonds and van-der-Waals interactions with a target.
- Both fragments form a frontal region exposed to the solvent in each Trx- x-fold protein, which may interact directly with a target during the electron-exchange process.

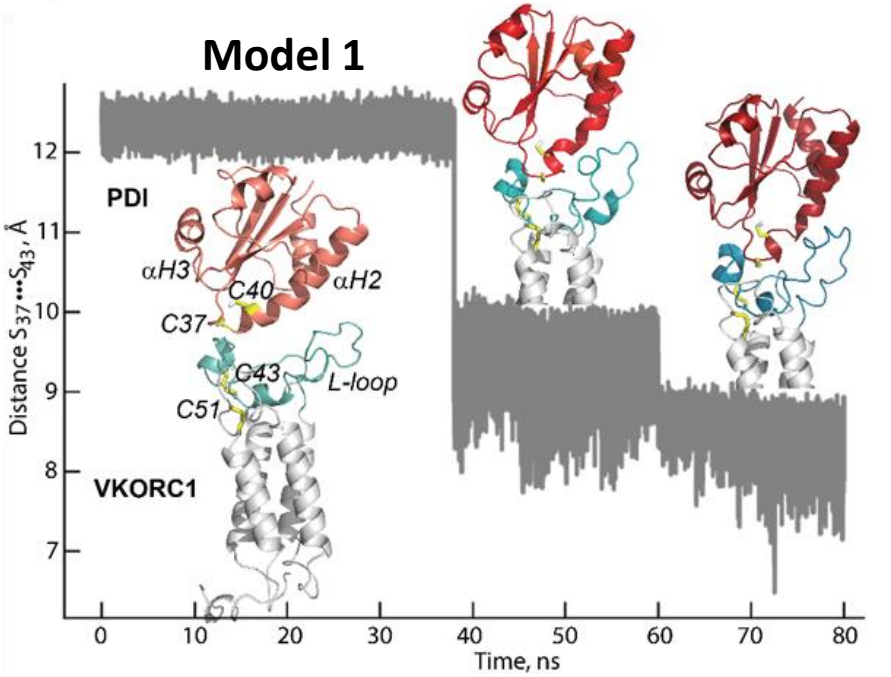
- **F1** comprises L3 and an N-extremity of αH2-helix that includes the **C1X$_1$X$_2$C2** motif; F1 in PDI is a structurally disordered, similarly to the **S56-R61** segment of VKORC1;
- **F2** is structurally adjacent to the **C1X$_1$X$_2$C2** motif and is composed of L5-αH3-L6. As Linker L5 from **F2 of PDI** shows excellent mobility and potentially enables adaptive conformational change.

PDI is the most probable (structurally) partner for hVKORC1

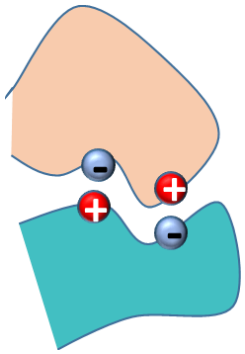
PDI-hVKORC1 Complex Modelling



Model 1



Interaction interface



M1

PDI: A42-L43-A44-P45-E46-Y47-A48-K49

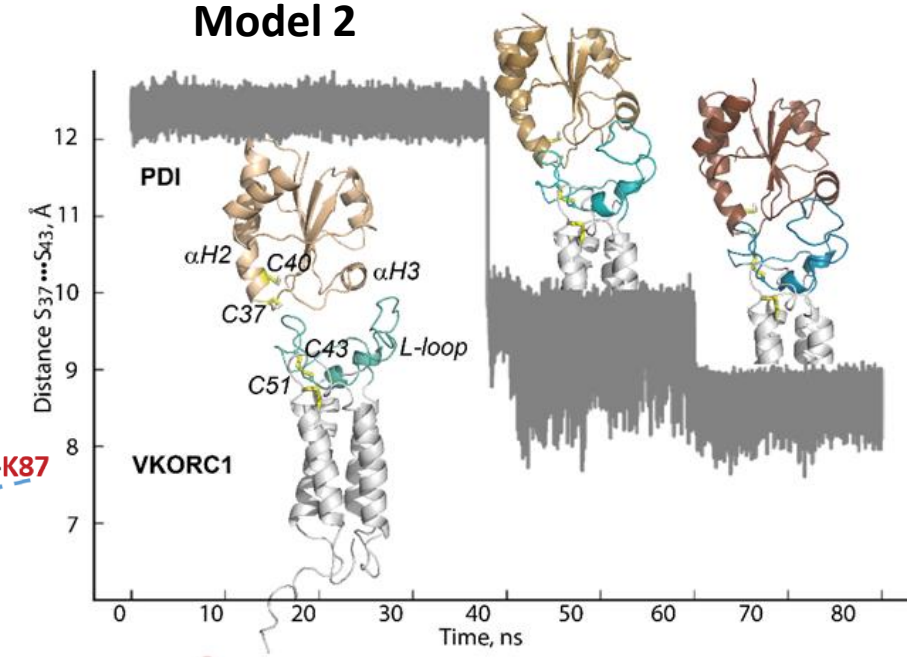
hVKORC1: R61-G62-F63-G64-L65-V66-E67

M 2

PDI: D67-A68-T69-E70-E71-S72-D73-L74-A75-Q76-Q77-Y78-G79-V80-R81-...-K87

hVKORC1: R35-...R61-G62-F63-G64-L65-V66-E67-H68-V69-L70-G71-Q72

Model 2



A first precursor complex to probe thiol-disulfide exchange reactions between PDI and hVKORC1

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Article

Identification of the Primary Factors Determining the Specificity of Human VKORC1 Recognition by Thioredoxin-Fold Proteins

Maxim Stolyarchuk †, Julie Ledoux †, Elodie Maignant, Alain Trouvé and Luba Tchertanov *

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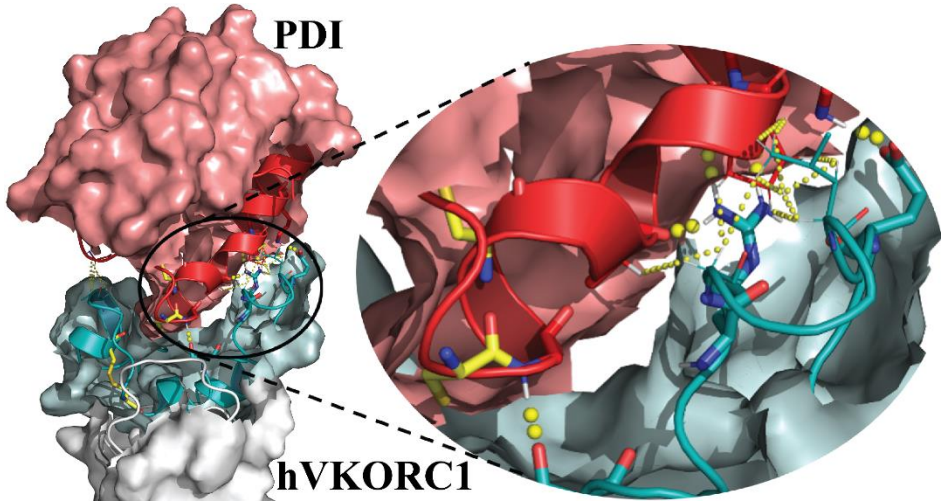
* Correspondence: Luba.Tchertanov@ens-paris-saclay.fr
† The first two authors contributed equally

Abstract: Redox (reduction–oxidation) reactions control many important biological processes in all organisms, both prokaryotes and eukaryotes. This reaction is usually accomplished by canonical disulphide-based pathways involving a donor enzyme that reduces the oxidised cysteine residues of a target protein, resulting in the cleavage of its disulphide bonds. Focusing on human vitamin K epoxide reductase (hVKORC1) as a target and on four redoxins (protein disulphide isomerase (PDI), endoplasmic reticulum oxidoreductase (ERp18), thioredoxin-related transmembrane protein 1 (Tmx1) and thioredoxin-related transmembrane protein 4 (Tmx4)) as the most probable reducers of VKORC1, a comparative in-silico analysis that concentrates on the similarity and divergence of redoxins in their sequence, secondary and tertiary structure, dynamics, intraprotein interactions and composition of the surface exposed to the target is provided. Similarly, hVKORC1 is analysed in its native state, where two pairs of cysteine residues are covalently linked, forming two disulphide bridges, as a target for Trx-fold proteins. Such analysis is used to derive the putative recognition/binding sites on each isolated protein, and PDI is suggested as the most probable hVKORC1 partner. By probing the alternative orientation of PDI with respect to hVKORC1, the functionally related noncovalent complex formed by hVKORC1 and PDI was found, which is proposed to be a first precursor to probe thiol–disulphide exchange reactions between PDI and hVKORC1.

Keywords: hVKORC1; Trx-fold proteins; protein folding; dynamics; molecular recognition; thiol–

Citation: Stolyarchuk, M.; Ledoux, J.; Maignant, E.; Trouv, A.; Tchertanov, L. Identification of the Primary Factors Determining the Specificity of the Human VKORC1 Recognition by Thioredoxin-Fold Proteins. *Int. J. Mol. Sci.* **2021**, *22*, 802. https://doi.org/10.3390/ijms22020802

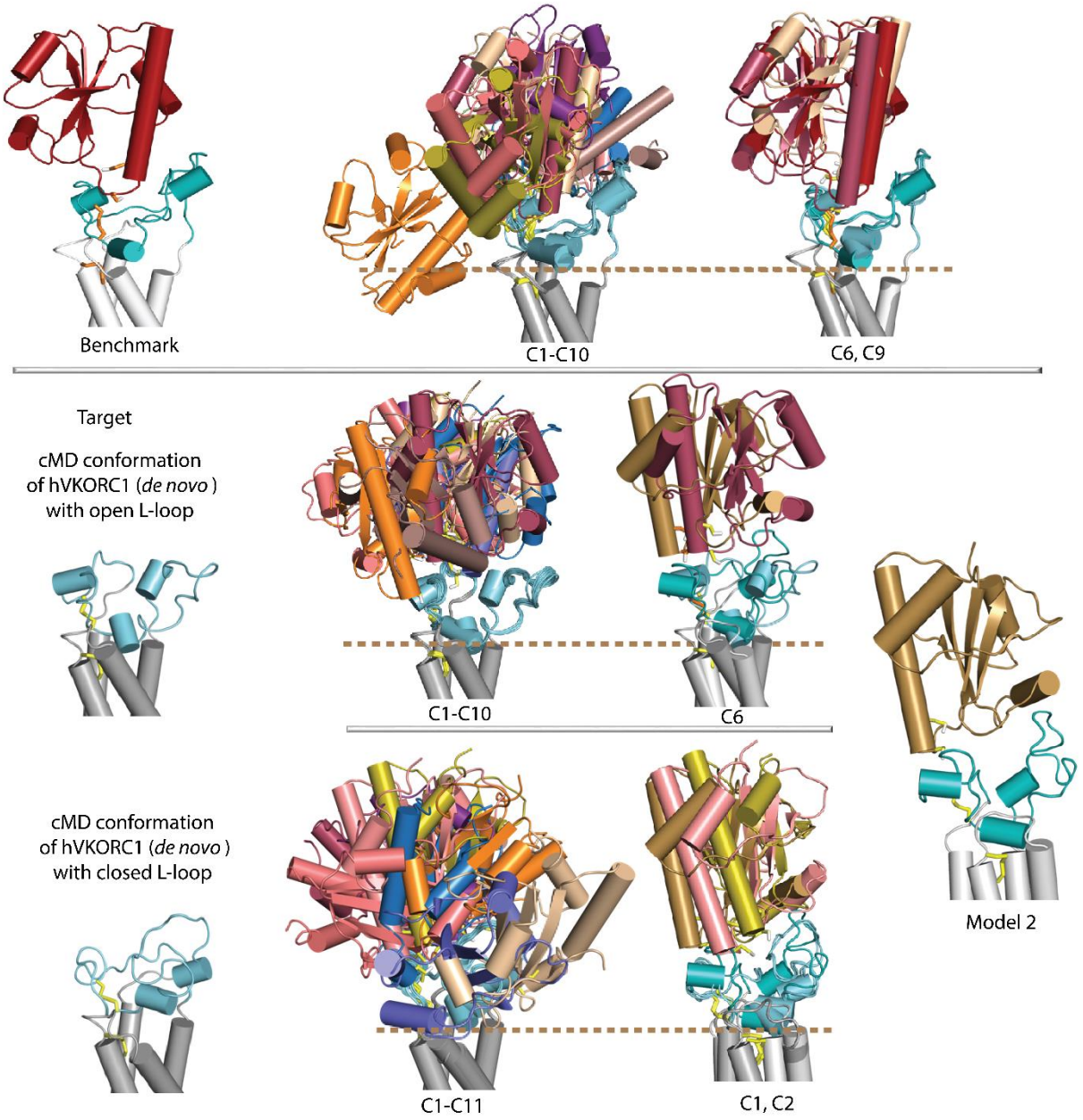
Academic Editor: Ron Orbach



- Stolyarchuk M., Ledoux J., Maignant E., Trouvé A. and Tchertanov L. (**2021**). Identification of the Primary Factors Determined the Specificity of VKOR Recognition by Thioredoxin-like Proteins. *Int. J. Mol. Sci.*, *22*(2), 802; https://doi.org/10.3390/ijms22020802

Protein-Protein Docking with HADDOCK reproduced Model 1 and Model 2

To evaluate hVKORC1 conformations as putative targets for Protein Disulfide Isomerase (PDI) suggested as a redox partner, we used High Ambiguity Driven protein-protein DOCKing (HADDOCK).



hVKORC1 is a modular protein composed of the stable transmembrane domain and intrinsically disordered L-loop.

International Journal of *Molecular Sciences*

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Article

Human Vitamin K Epoxide Reductase as a Target of Its Redox Protein

Julie Ledoux, Maxim Stolyarchuk, Enki Bachelier, Alain Trouvé and Luba Tchertanov *

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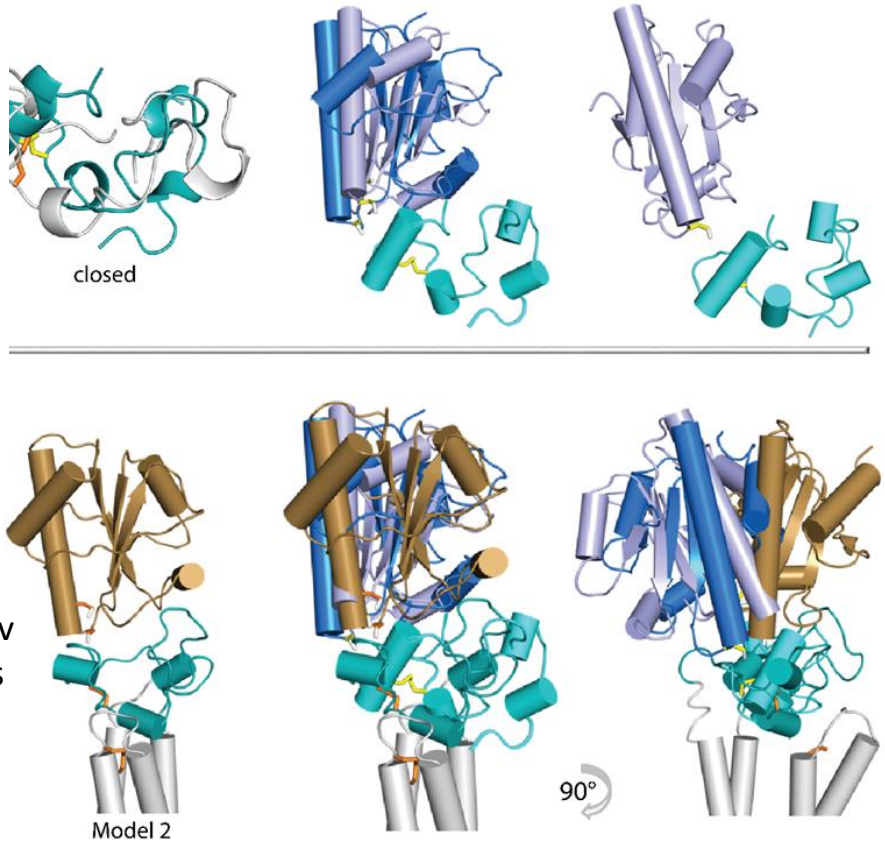
* Correspondence: luba.tchertanov@ens-paris-saclay.fr

Abstract: Human vitamin K epoxide reductase (hVKORC1) enzymatic activity requires an initial activation by a specific redox protein, a less studied step in the hVKORC1 vital cycle. Significant steric conditions must be met by enzymes, being that to adapt their configurations is mandatory for hVKORC1 activation. We studied, by molecular dynamics (MD) simulations, the folding and conformational plasticity of hVKORC1 in its inactive (fully oxidised) state using available structures, crystallographic and from *de novo* modelling. According to the obtained results, hVKORC1 is a modular protein composed of the stable transmembrane domain (TMD) and intrinsically disordered luminal (L) loop, possessing the great plasticity/adaptability required to perform various steps of the activation process. The docking (HADDOCK) of Protein Disulfide Isomerase (PDI) onto different hVKORC1 conformations clearly indicated that the most interpretable solutions were found on the target closed L-loop form, a prevalent conformation of hVKORC1's oxidised state. We also suggest that the cleaved L-loop is an appropriate entity to study hVKORC1 recognition/activation by its redox protein. Additionally, the application of hVKORC1 (membrane protein) in aqueous solution is likely to prove to be very useful in practice in either in silico studies or in vitro experiments.

Keywords: human vitamin K epoxide reductase; hVKORC1; blood coagulation; redox protein target; enzyme activation; modular protein; intrinsically disordered L-loop; conformational transition; modelling; molecular dynamics simulations; free energy landscape

Citation: Ledoux, J.; Stolyarchuk, M.; Bachelier, E.; Trouvé, A.; Tchertanov, L. Human Vitamin K Epoxide Reductase as a Target of Its Redox Protein. *Int. J. Mol. Sci.* **2022**, *23*, 3899. https://doi.org/

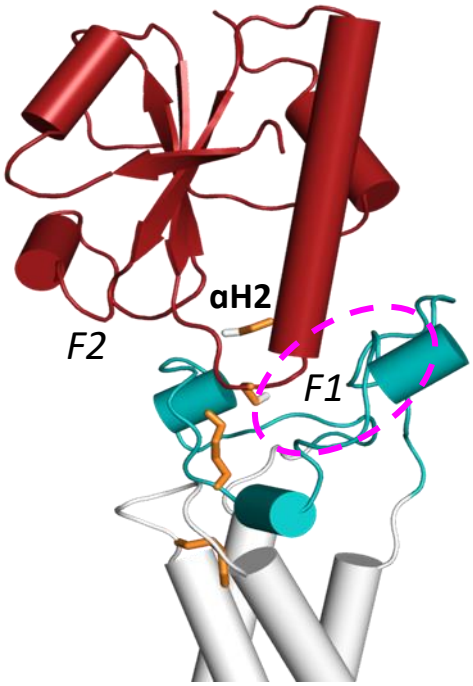
Cleaved L-loop and L-loop fused to the TMD showed comparable structural, conformational and recognition properties.



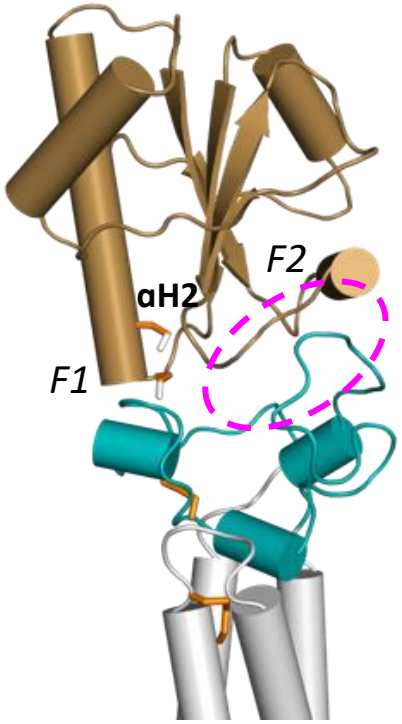
- Ledoux J., Stolyarchuk M, Bachelier E., Trouvé A. and Tchertanov L. (**2022**). Human Vitamin K Epoxide Reductase as a Target of its Redox Protein. *Int. J. Mol. Sci.* Special Issue: *"Intrinsically Disordered Proteins (IDPs) 2.0*". *Int. J. Mol. Sci.* **2022**, *23*(7), 3899; https://doi.org/10.3390/ijms230738992.

Which model of PDI-hVKORC1 complex is the functionally related ?

hVKORC1

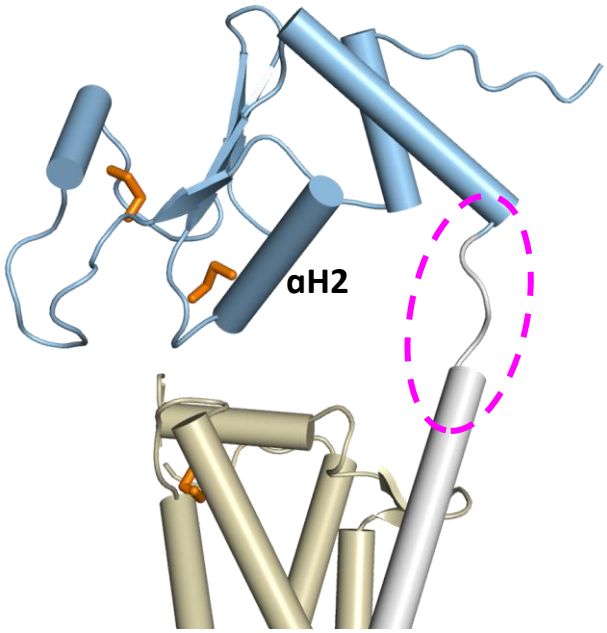


M1 (*t* = 236 ns)

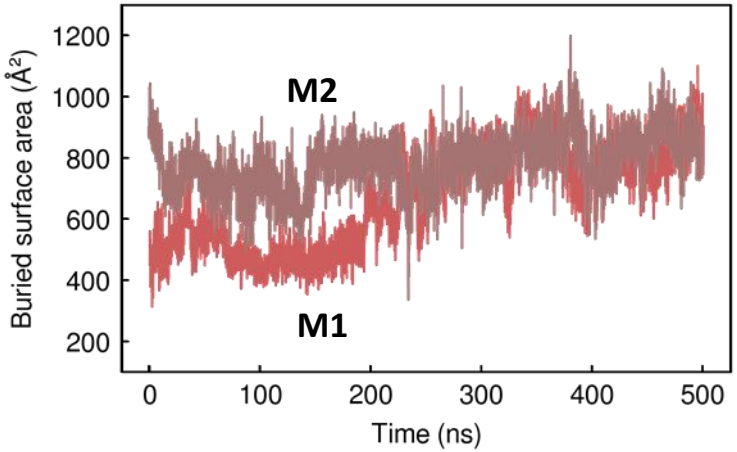


M2 (*t* = 318 ns)

b-VKOR

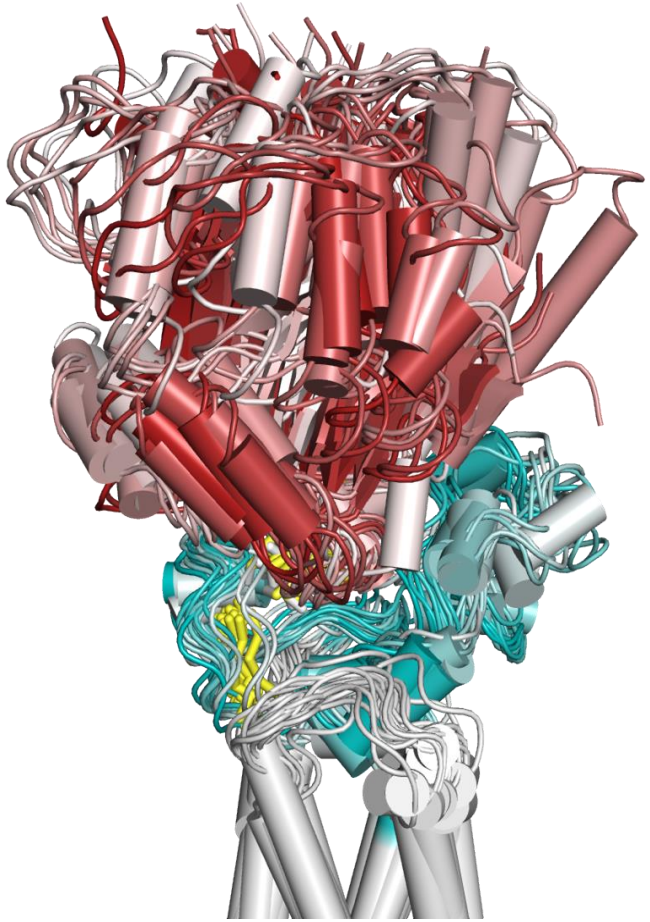


PDB ID 4NV5

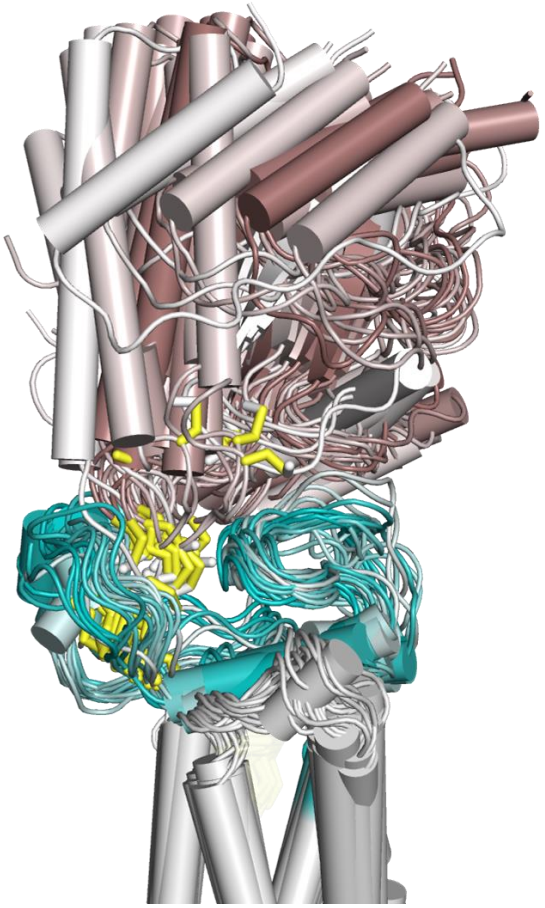


- In both models, the PDI-hVKORC1 complex is stabilised by non-covalent interactions between the L-loop of hVKORC1 and one of the putative binding PDI regions, F1 (**M1**) or F2 (**M2**). At the same time, the Trx-like domain in b-VKOR is maintained by covalent linkage with the TMD domain.
- Both types of interactions act as a hinge allowing the adaptive accommodation of Trx respective to VKOR.
- Position of PDI in **M1** is more similar to that of Trx in bVKOR, as evidenced by the parallel orientation of αH2 and other helices and the plane of the β-sheet.

Stability of the PDI-hVKORC1 models during MD simulations



20 conformations from MD trajectories from t=0 (light) to 500 ns (dark)



M1

M2

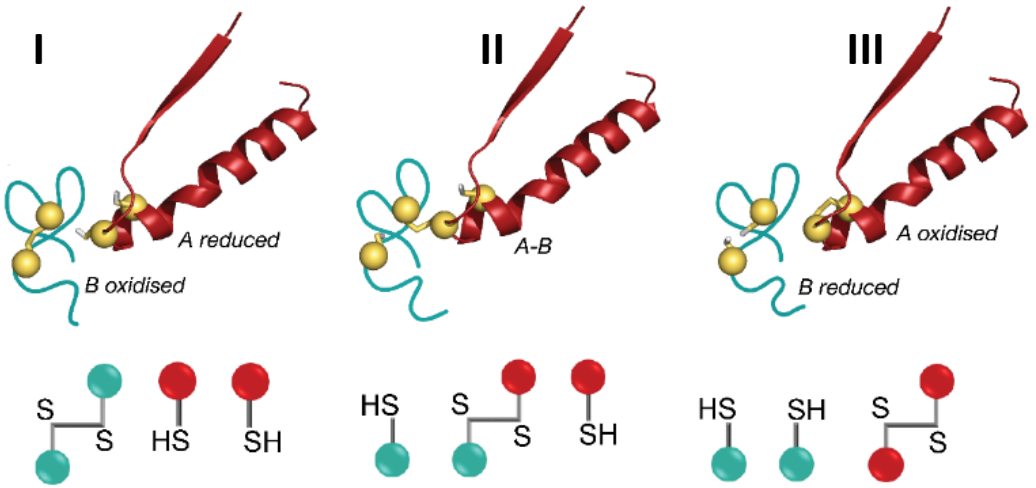
PDI: A42-L43-A44-P45-E46-Y47-A48-K49

hVKORC1: R61-G62-F63-G64-L65-V66-E67

PDI: D67-A68-T69-E70-E71-S72-...-L74-A75-...-Q77-Y78-G79-V80-R81-...-K87

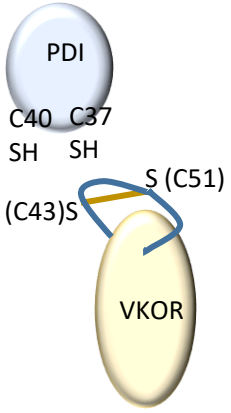
hVKORC1: R35-...R61-G62-F...64-L65-V66-E67-H...69-L70-G71-Q72

Modelling of Precursor and Successor Complexes and Transition State Assembled in a Biomolecular Proton-Electron Transfer Reaction Between PDI and hVKORC1.



I-II-III states transition: Three types of reactions are responsible for chemical transformations between the –S-S– (disulfide bond), –SH (thiol), and –S• (thiyl) functional groups: nucleophilic substitution-eliminations (specifically so-called thiol-disulfide interchange reactions), single-electron reactions of free thiols or disulfide bonds, and radical reactions.

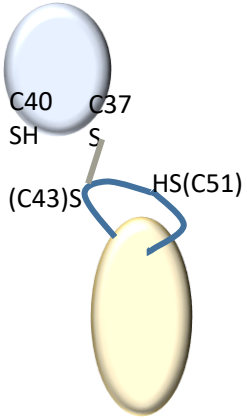
Precursor - State I



$C43^{hVKOR}$–$C51^{hVKOR}$

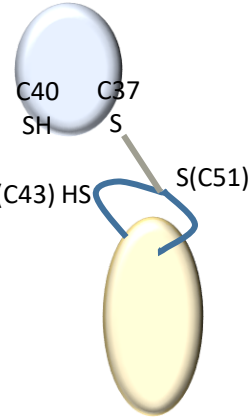
Transition State II

II-1



$C43^{hVKOR}$–$C37^{PDI}$

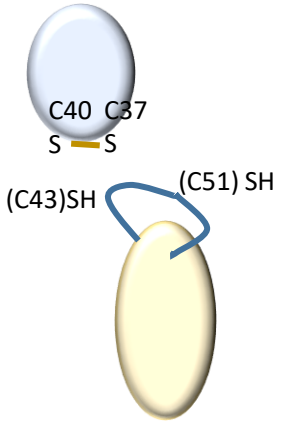
II-2



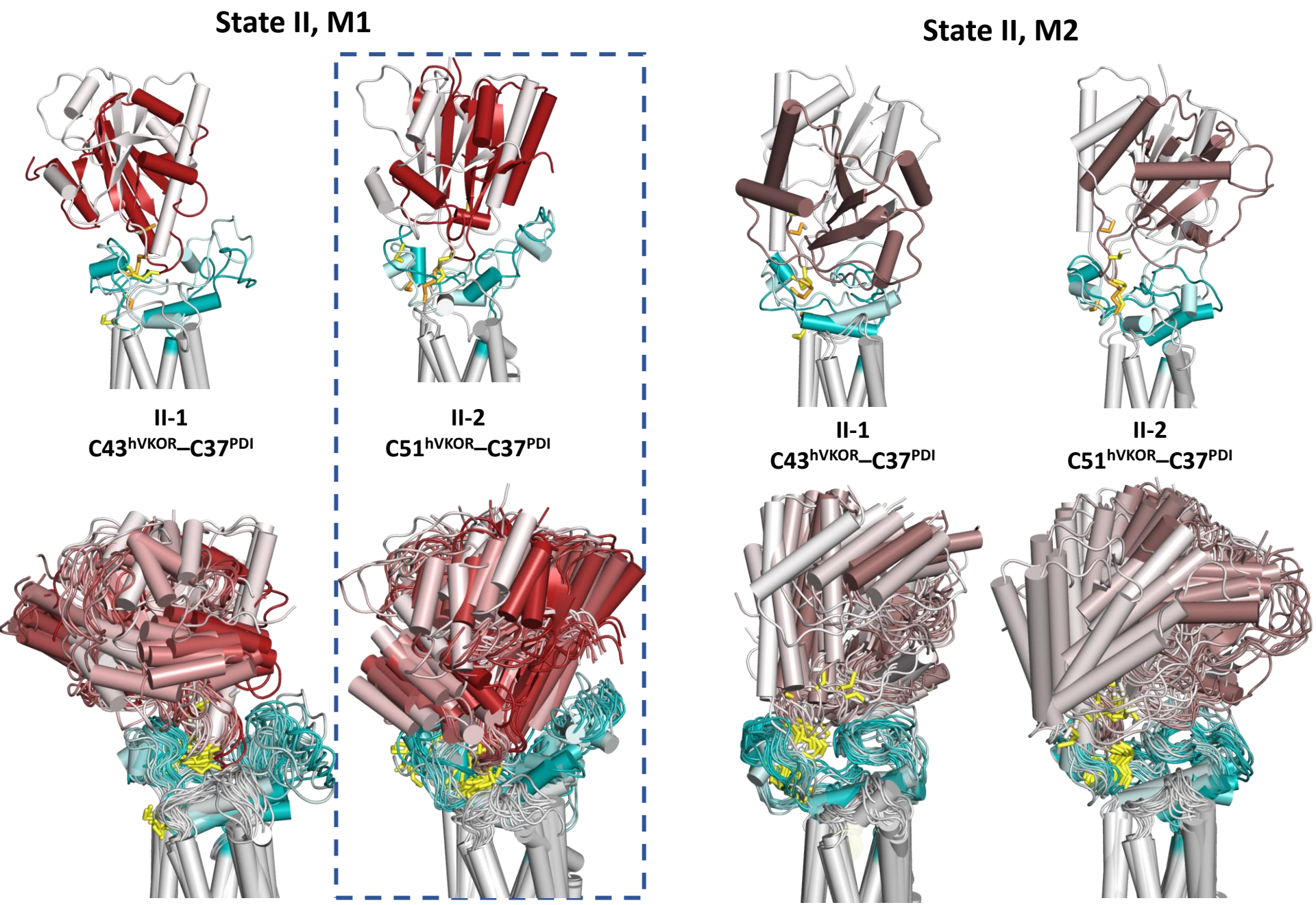
ou

$C51^{hVKOR}$–$C37^{PDI}$

Successor - State III



$C37^{PDI}$–$C40^{PDI}$



MD conformations, taken at t=0 (light) and 500 ns (dark)

CONCLUSIONS/PERSPECTIVES

- A careful *in-silico* study, based on molecular dynamic simulations of hVKORC1 in the oxidised state and a comparative analysis of four Trx proteins, viewed as the most probable reducers of hVKORC1 provided the **identification of putative recognition/binding sites** on each isolated protein.
- **PDI** was suggested as the **most probable hVKORC1 partner** (confirmed *in vivo*)
- By probing the alternative orientation of PDI concerning hVKORC1, two PDI-VKOR models were proposed, and one of them was considered a **precursor for thiol-disulphide exchange reactions**.

We focus now on the spontaneous inter-molecular thiol-disulfide exchange reactions.

- Using MD simulations coupled with an energy-based swapping criterion to simulate the dynamics of force-induced unfolding while allowing disulfide shuffling. This purely classical approach has **unique advantages** over QM methods: it does not require an a priori definition of the reacting residues but instead can predict them. It considers the large-scale protein motions and their coupling to the reaction at an atomistic level.

- **Combined QM/MM simulations:** *de novo* formation of *disulfide* bonds and *thiol*−disulfide exchange.