Study of NOX5 stability by Molecular Dynamics methods

Baptiste ETCHEVERRY, Fabien CAILLIEZ, Aurélien DE LA LANDE, Marc BAADEN

Introduction :

NOX - NADPH Oxydase (for Nicotinamide Adenine Dinucleotide Phosphate Oxydases) are transmembrane proteins involved in the production of reactive oxygen species in eukaryotic organisms. In humans there are 7 isoforms of NOX.

Common characteristics - NOX are composed of two differents domains. A cytosolic dehydrogenase domain in red where a Flavin Adenine Dinucleotide (FAD) cofactor is bound non-covalently. The transmembrane domain, illustrated here in blue, chelates 2 heme cofactors by histidine ligands at the heart of the 6 forming it. (**Fig 1**)

Electron pathway - The electron transfer is carried out in several successive steps. Electrons are initially transmitted by NADPH to the flavin cofactor in the cytosolic part of the protein. They are then transported to the internal and external heme in the transmembrane area, to finally reach the extra-cellular pocket where the dioxygen is reduced to superoxide ion. (**Fig 1, right**)

Fig 1: NOX5 representation, TM in blue, DH in red (A). Plausible pathway of electron transfer in B. [2]

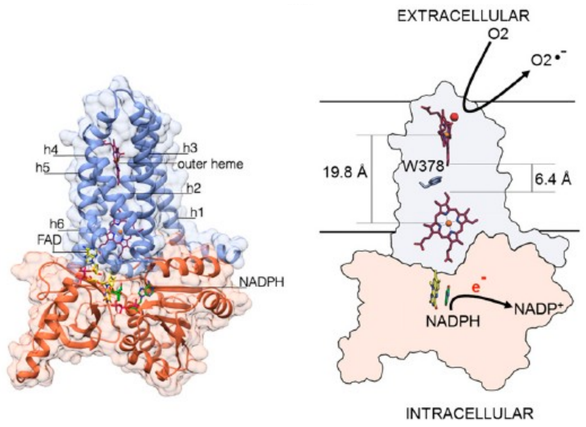


Fig 2a : Cofactor configuration in Magnani experimental model **[A]** [1]

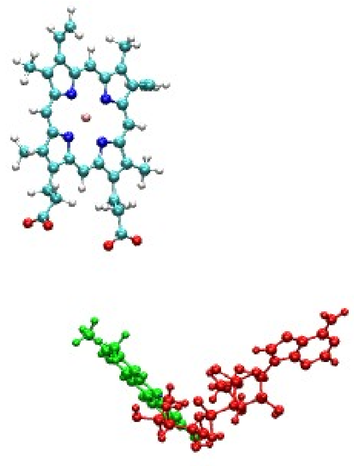
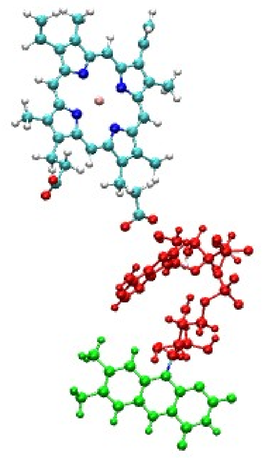


Fig 2b : Cofactor configuration in Sun experimental model **[B]**



Structural data – Two experimental structues of the NOX5 isoform have currently been obtained. One X-Ray structure, solved by F.Magnani *et al.* in 2017 [1], and a second by cryo-electron microscopy method determined very recently by Ji Sun *et al.* (**Fig 2**). The main structural difference is located at the level of the flavin cofactor of the NOX complex. The orientation of the cofactor towards the inner heme is radically different. In the X-Ray structure (**Fig 2a**), the isoalloxazine group in green is directed directly towards the inner heme (electron acceptor). In the cryo-EM structure (**Fig 2b**), the adenosine group in red is intercalated between the isoalloxazine and the heme, leading to the hypothesis of an electronic relay role.
What can be the impact of this configurational difference on the electron transfer kinetics (and therefore the rate of superoxyde production)? To tackle this issue we have been looking at the stability of FAD and its interactions with the NOX complex using Molecular Dynamics simulations.

Methods :

- System building using CHARMM-GUI [3]
- Models characteristics
  - Membrane composed of POPE (neutral) and POPG (negatively charged) lipids :
    - POPE 20 % / POPG 80 % **[mb1]**
    - POPE 80 % / POPG 20 % **[mb2]**
  - 2 Sets of FAD charges pre-determined by DFT calculations **[para1]**[4]**[para2]**[5]
  - Redox states of the system (inner/outer Heme) :
    - Initial state Ferrous (Fe^{2+}) / Ferric (Fe^{3+}) **[st1]**
    - Final state Ferric (Fe^{3+}) / Ferrous (Fe^{2+}) **[st2]**

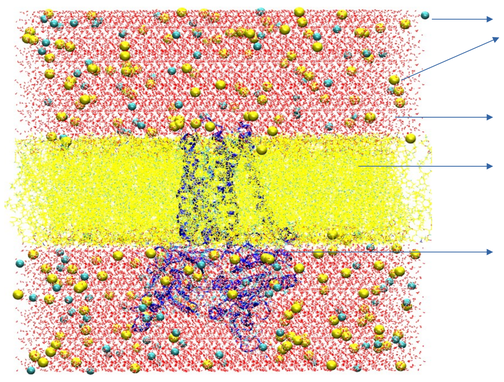


Fig 3a : Example of a NOX5 model built with membrane, water and ions, approximately 180 000 atoms

- Molecular Dynamics parameters
  - CHARMM forcefield [6] (to define interactions in the system) :

$$E_{tot} = \sum_i \frac{k_i}{2}(l_i - l_{i,0})^2 + \sum_i \frac{k_i}{2}(\theta_i - \theta_{i,0})^2 + \sum \frac{v_n}{2}(1+\cos[n\omega - y]) + \sum_{i,j} \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} + \sum_{i,j} \varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - 2\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]$$

E_{bonds} E_{angles} $E_{torsions}$ E_{VDW} E_{elstat}

Equilibration phase (1 ns order duration)
- Harmonics constraints on protein backbone, lipids and cofactors / 6 cycles of approximatively 200 ps of relaxation
- Supplementary constraints to preserve interactions between FAD and NOX5 then relaxation (**Fig 3b**)

  - Production phase (about 45 ns for each trajectory)

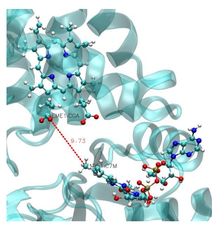


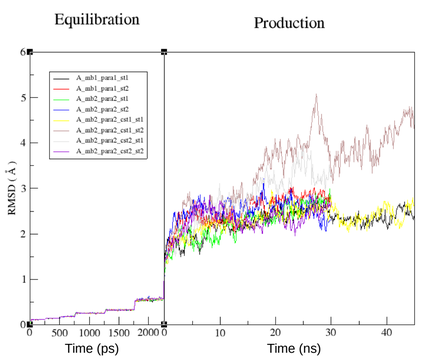
Fig 3b : Supplementary constraint to force FAD-inner Heme proximity

- Analysis methods
  - Membrane stability measures:
    - Thickness
    - Area per lipid
  - Protein stability measures:
    - RMSD (Root Mean Square Deviation) of protein backbone
    - RMSD hemes and flavine cofactors

$$RMSD(v,w) = \sqrt{\frac{1}{n}\sum_{i=1}^{n} ||v_i - w_i||^2}$$

Analysis of FAD/protein interaction

Stability of the structure in the simulations



Definition of system names :

Membrane type — Redox state of the system

A_mb1_para1_st1

Experimental structure — FAD set of charges

Fig 4a : RMSD of protein backbone.
Constraints during equilibrium phase relaxed during 6 steps.
Stabilization for almost all simulations over the production phase

Characterization of FAD environment

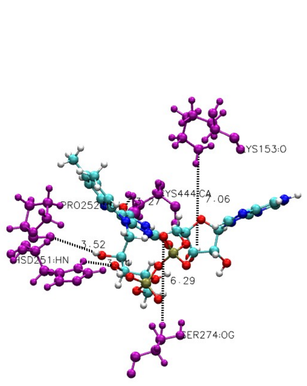


Fig 4b : Major interactions of FAD with its environment

Fig 4c — **Fig 4d**

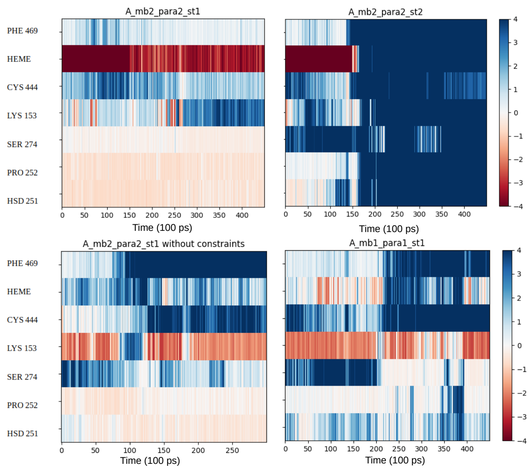


Fig 4e — **Fig 4f**

Fig 4-c-d-e-f : Analysis of the evolution of interaction between FAD and its environment compared to initial structures.

Fig 4c: $d_{FAD\text{-}Heme}$ remains stable as π-stacking interaction between isoalloxazine group and Phenylalanine 469 C-ter of NOX5.

Fig 4d: FAD is not stable in its cavity and moves away of inner Heme when π-stacking is broken

Fig 4ef : π-staking breaks early in the simulation when no restraint is added to favor it during equilibration phase.

Fig 4f : FAD gets unstable just after the loss of π-stacking interaction.

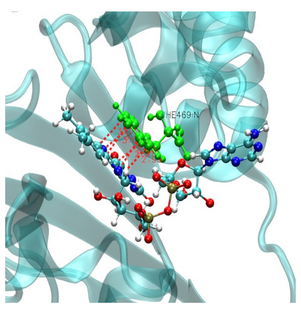


Fig 5 : Representation of π-stacking between C-ter phenilalanine residue (green) and FAD.

Conclusion - We showed by multiple analysis of trajectories that π-stacking interaction between the C-terminal residue of the protein and the isoalloxazine group of the Flavin (**Fig 5**) is a necessary factor of FAD stability in its cavity. It seems primordial to keep a conformation *a priori* favorable to the electron transfer with the inner heme.

Perspectives - The conditions now etablished to obtain a simulation favorable to the electron transfer, its study is in progress.
The influence of the differents parameters of simulation such as the membrane composition on the electron transfer processes will be studied

Building a complete structure of NOX5 from cryo-EM data

Artificial intelligence software Colabfold [7] is used to rebuild a complete structure of NOX5.
The prediction (blue) reveals a structural deformation compare to the experimental template (red). (**fig 6b**)
To keep original atomic positions both structure are aligned several times around each missing residues region (**fig 6c**).
New coordinates of Colabfold structure are pasted on the initial strucutre of Sun to give a complete structure of NOX5

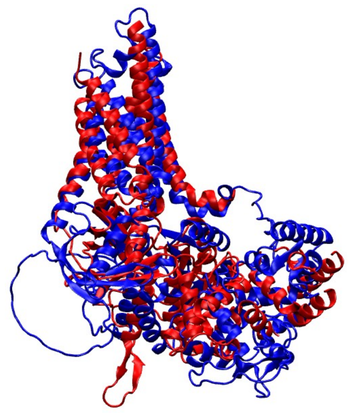


Fig 6b : Colabfold prediction of NOX5 structure from original sequence and experimental template. In Red the experimental structure is shown and in blue the Colabfold prediction structure.

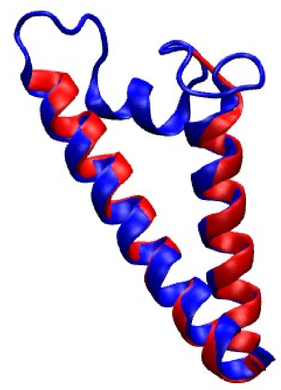


Fig 6c : Example of the reconstruction of one part of the chain by alignement of the two structures on the 2 helixes around the missing residues.

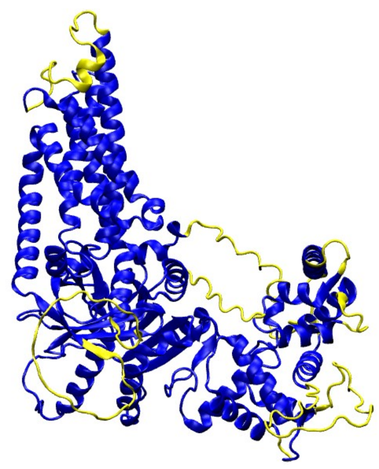


Fig 7a : Final structure of NOX5 Sun model

Conclusion – We have built a complete NOX5 structure (**Fig 7a**). In blue the experimental coordinates of Ji Sun & collaborators and in yellow all the missing residues rebuilt and pasted to the final structure.
First MD simulations show a stability of the system on the time scale of 15 ns (**Fig 7b**).

Perspectives - Molecular dynamics are now possible on time scale around 100 of ns.
Studies of the impact of the FAD configuration on electron transfer is the next step to follow.

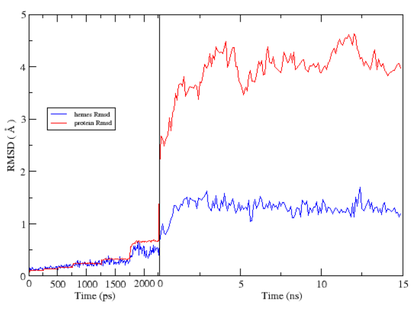


Fig 7b : Stability of protein and hemes RMSD in our first MD simulation based on cryo-EM structure

References :

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Acknowledgements :

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