Computational assessment of TRPA1 interaction with 4-hydroxynonenal

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Introduction:

TRPA1 is a transient receptor potential (TRP) cation channel superfamily member. These receptors have a role in many processes such as inflammation, metabolism, sensation, and tumorigenesis. Previous studies showed that TRPA1 can form adducts with reactive aldehydes, which trigger the release of pro-inflammatory mediators. The reactive aldehydes can be exogenous, from alcohol or tobacco consumption, or can result from lipid peroxidation under oxidative stress conditions.

We show in our preliminary studies, using Ca^{2+} microfluorimetry, that 4-hydroxynonenal (4-HNE) stimulates Ca^{2+} uptake by TRPA1. 4-HNE is an endogenous aldehyde with an essential role in both physiological and pathological processes. To further elucidate this effect, we used molecular docking methods to investigate the interaction of human TRPA1 with 4-HNE.

Molecular docking methods were used to: (a) confirm the affinity of 4-HNE for the expected binding site represented by the cysteine pocket, including Cys621, the main residue involved in the binding of covalent ligands and (b) to refine the position of 4-HNE into the binding site.

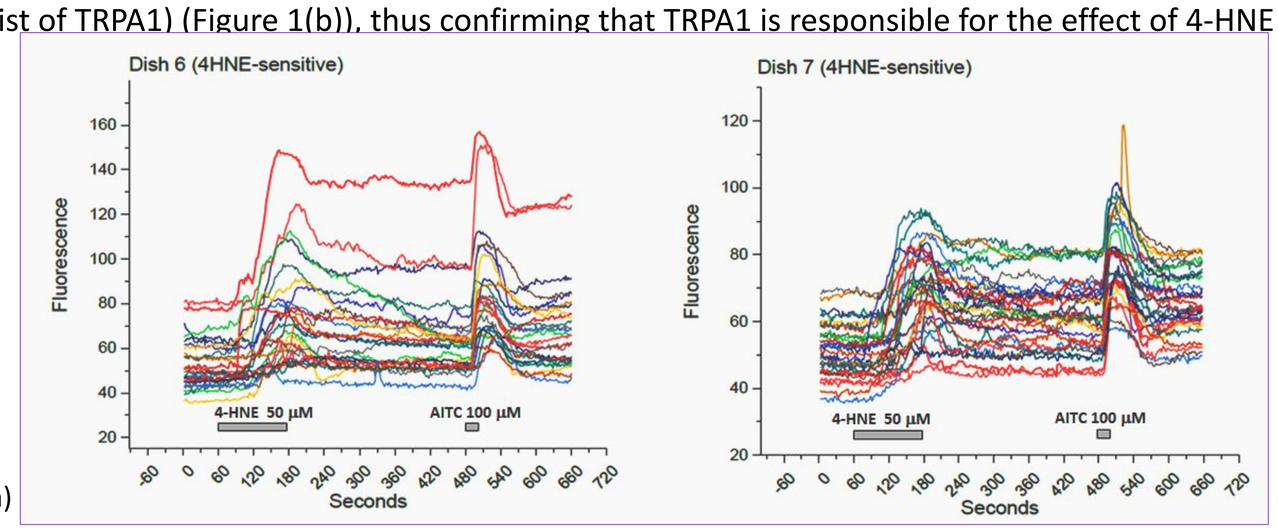
Preliminary results:

-the effect of 4-HNE on TRPA1 was measured by calcium microfluorimetry on Panc-1 cells (the protocol is given in (1))

-4-HNE stimulated Ca^{2+} uptake – Figure 1 (a)

-following the stimulation with 4-HNE, Ca^{2+} uptake can be inhibited with A-967079 (a specific antagonist of TRPA1) and recovered by the treatment with AITC (the specific agonist of TRPA1) (Figure 1(b)), thus confirming that TRPA1 is responsible for the effect of 4-HNE

(a)



(b)

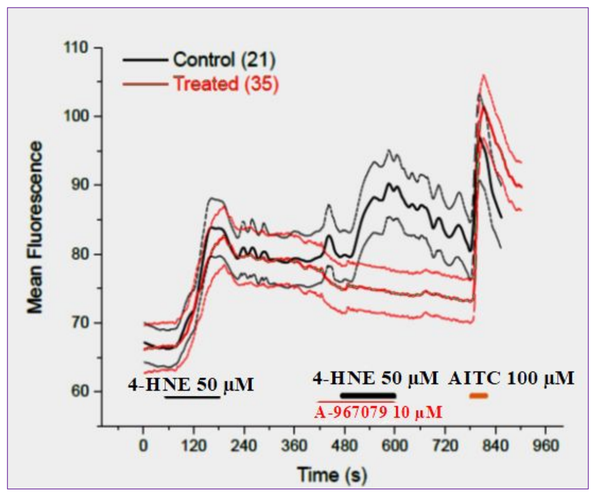


Figure 1. (a) Calcium microfluorimetry sequence on Panc-1 cells treated with 4-HNE and AITC. (b) Mean fluorescence calculated for cells treated with the 4-HNE, followed by the treatment with A-967079 (Treated) or 4-HNE (Control) and a last treatment with AITC.

Materials and methods:

-Molecular docking was performed using AutoDock4 (2) with AutoDock Tools4 (3)

-Calculation involved the crystal structure of TRPA1 with a bound covalent ligand – PDB id 6V9X (4) (Figure 2)

-Initial prospective docking was performed on the tetrameric structure stripped of ligands; the grid size was chosen to fit a full subunit

-the position of 4-HNE in the binding site was optimized by covalent docking

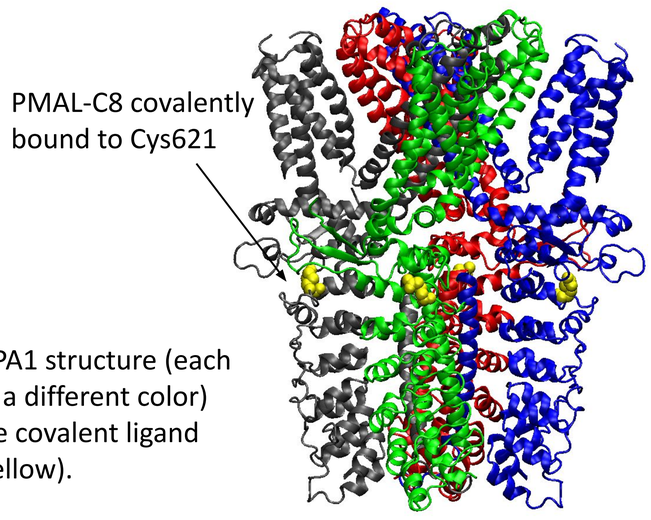


Figure 2. TRPA1 structure (each subunit is in a different color) bound to the covalent ligand PMAL-C8 (yellow).

Results and discussions:

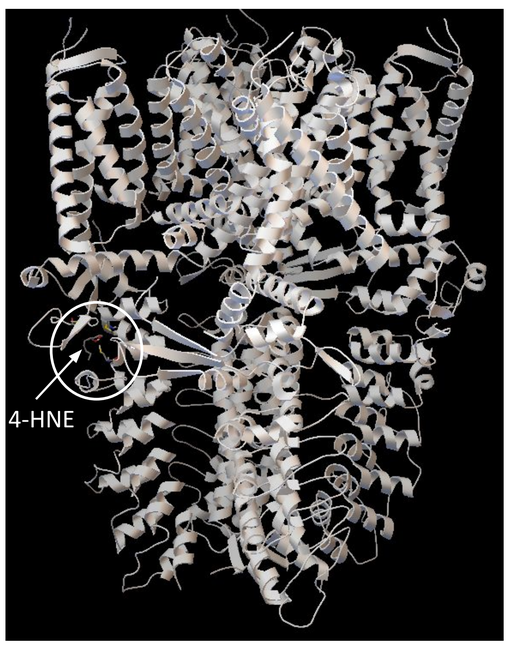


Figure 3. TRPA1 structure with docked 4-HNE (the binding site is circled).

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Estimated Free Energy of Binding    =   -2.85 kcal/mol  [=(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, Ki   =    8.15 mM (millimolar)

(1) Final Intermolecular Energy     =   -4.94 kcal/mol
    vdW + Hbond + desolv Energy     =   -4.84 kcal/mol
    Electrostatic Energy            =   -0.09 kcal/mol
(2) Final Total Internal Energy     =   -0.43 kcal/mol
(3) Torsional Free Energy           =   +2.09 kcal/mol
(4) Unbound System's Energy  [=(2)] =   -0.43 kcal/mol
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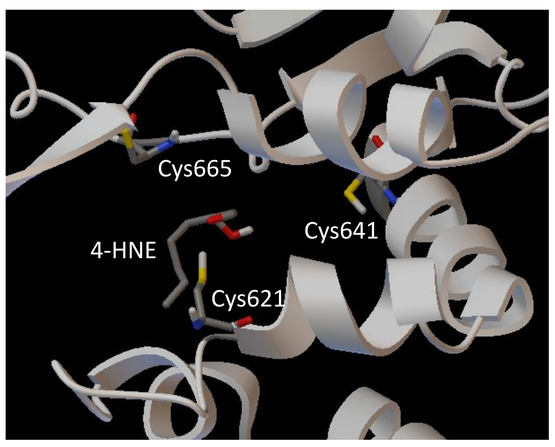

Figure 4. Detail on 4-HNE binding site. The Cys residues are labeled.

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Estimated Free Energy of Binding    =   -3.20 kcal/mol  [=(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, Ki   =    4.48 mM (millimolar)
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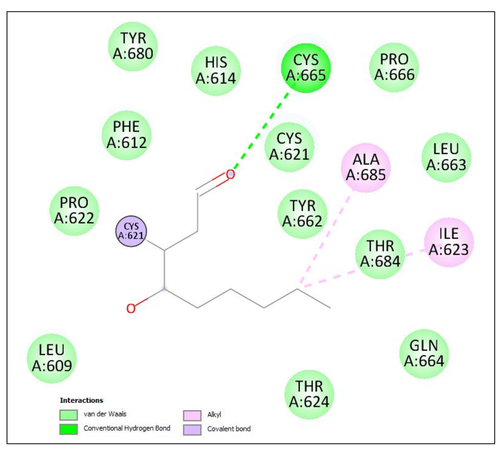

Figure 5. 2D-interaction map of 4-HNE with the receptor in the most favorable pose after covalent docking.

-4-HNE was docked to TRPA1 and a favorable binding site is represented by the Cys pocket (Figure 3)

-docked 4-HNE is close to Cys621, the residue expected to be involved in the covalent biding of 4-HNE (Figure 4)

-covalent docking improved the interaction energy of 4-HNE with the receptor

-in the best pose identified by covalent docking, 4-HNE interacts with several residues, including Cys665 (Figure 5)

Conclusions:

Our preliminary results show that 4-HNE stimulates the uptake of Ca^{2+} trough TRPA1 channels. 4-HNE has affinity for the known binding site comprising Cys residues. Covalent docking helped identify a position with an even more favorable energy, in which 4-HNE is bound to Cys621 and forms a hydrogen bond with Cys665. The obtained data would provide insight into possible future pharmacological strategies for targeting the interaction between TRP channels and reactive aldehydes in pathological conditions.

References:

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