Molecular Dynamic Pharmacophore
and its application to
Design Novel MARK4 Inhibitors

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Lenka Hruba, Sony Gurska, Petr Džubák, Marian Hajduch

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Way2Drug
2022
Microtubule-Associated Protein 2 (MAP2) and MAP4

Trinczek at al., JBC, 2004

Using the process which occurs in brain as an example

The pictures were taken from Wikipedia: Alzheimer's disease
MARK4

PDB code: 5es1

Macromolecule Content
Chains: A
Sequence Length: 328
Organism: Homo sapiens

Experimental Data Snapshot
Method: X-RAY diffraction
Resolution: 2.80 Å

Sack et al., 2016

IC50, µM

<table>
<thead>
<tr>
<th></th>
<th>MARK1</th>
<th>MARK2</th>
<th>MARK3</th>
<th>MARK4</th>
</tr>
</thead>
<tbody>
<tr>
<td>5RC</td>
<td>0.0041</td>
<td>0.0025</td>
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</table>
## Initial ligands

![Initial ligands](image)

From the proprietary database

<table>
<thead>
<tr>
<th>Mol 1</th>
<th>MARK1</th>
<th>MARK2</th>
<th>MARK3</th>
<th>MARK4</th>
</tr>
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<tbody>
<tr>
<td>IC50, μM</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>0.95</td>
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<td>docking</td>
<td>-6.7</td>
<td>-7.4</td>
<td>-7.0</td>
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<td>-9.1</td>
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</tbody>
</table>

<table>
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<tr>
<th>Mol 4</th>
<th>MARK1</th>
<th>MARK2</th>
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<th>MARK4</th>
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<td>-8.1</td>
<td>-8.6</td>
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## Aim

Discover selective MARK4 inhibitors of new chemotypes to extend structural diversity of compounds for further selection and optimization
MD representative pharmacophores

Polishchuk at al., Int J Mol Sci, 2019

3D pharmacophore hashes

Kutlushina at al., Molecules, 2018

github.com/ci-lab-cz/pharmd
MD pharmacophores generation

MD snapshot timeline

150 ns

MD representative pharmacophores

Mol 1 4015
Mol 2 2532
Mol 3 5221
Mol 4 5965

MD snapshot timeline

MD pharmacophores

3D pharmacophore hashes

A B C D E F G

A B D F G

github.com/ci-lab-cz/pharmd
Validation of MD pharmacophores

MD snapshot timeline
150 ns

MD representative pharmacophores

Mol 1: 4015
Mol 2: 2532
Mol 3: 5221
Mol 4: 5965

ChEMBL + proprietary
190 inactive molecules
29 active molecules

up to 50 conformers

Virtual screening of validation set

Quality assessment of the MD representative pharmacophores

GROMACS
FAST, FLEXIBLE, FREE

ChEMBL
proprietary

NFeatures, Complex
1, mol2
2, mol1
2, mol2
2, mol3
2, mol4
3, mol1
3, mol2
3, mol3
3, mol4
4, mol1
4, mol2
4, mol3
4, mol4
5, mol1
5, mol2
5, mol3
5, mol4
6, mol3
6, mol4

Precision
Recall
Primary VS of Enamine by MD pharmacophores

MD snapshot timeline

5+ features MD representative pharmacophores

Mol 1 3938  
Mol 2 2331  
Mol 3 4989  
Mol 4 5876

RTB <= 5  
MW <= 500 Da

Virtual screening of Enamine

68337 molecules

Number of molecules

<table>
<thead>
<tr>
<th>Enamine datasets</th>
<th>Premium</th>
<th>Advanced</th>
<th>HTS</th>
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<tr>
<td>Number of molecules</td>
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<td>376097</td>
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<td>Number of Murcko scaffolds</td>
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<td>246940</td>
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</table>
Rank filtered molecules by CCA score

5+ features MD representative pharmacophores

Mol 1 3938
Mol 2 2331
Mol 3 4989
Mol 4 5876

up to 50 conformers

1783794 molecules

Virtual screening of Enamine

68337 molecules

Conformers coverage
Approach (CCA)

618 molecules

Polishchuk at al., Int J Mol Sci, 2019

github.com/ci-lab-cz/pharmd
Selecting hits by docking

MD snapshot timeline

Docking

AutoDock Vina

Virtual screening of Enamine

Conformers coverage
Approach (CCA)

5+ features MD representative pharmacophores

Mol 1 3938
Mol 2 2331
Mol 3 4989
Mol 4 5876

up to 50 conformers

1783794 molecules

68337 molecules

618 molecules

150 ns

235 molecules

24 hits

1783794 molecules

235 molecules
The found inhibitors

<table>
<thead>
<tr>
<th>Hit1</th>
<th>mol 1</th>
<th>mol 2</th>
<th>mol 3</th>
<th>mol 4</th>
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<tbody>
<tr>
<td>Tanimoto</td>
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<td>0.32</td>
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<td>0.3</td>
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<tr>
<td>IC50, μM</td>
<td>&gt;50</td>
<td>14.72</td>
<td>0.0088</td>
<td>0.032</td>
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<td>-8.026</td>
<td>-7.754</td>
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<table>
<thead>
<tr>
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<tr>
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<td>&gt;50</td>
<td>19.79</td>
<td>&gt;50</td>
<td>12.01</td>
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The found inhibitors

MARK4 and MARK3 associate with early tau phosphorylation in Alzheimer’s disease granulovacuolar degeneration bodies

Harald Lund, Elin Gustafsson, Anne Svensson, Maria Nilsson, Margareta Berg, Dan Sunnemark & Gabriel von Euler

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Conclusions

• A workflow for virtual screening based on our previous implementation of MD pharmacophores and molecular docking was developed and validated.

• We were able to find a novel MARK inhibitor which was selective over 1 and 2 subtypes and can be promising for further research.
Acknowledgements

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Thanks to my colleague PhD Olena Mokshyna
Thanks to our biology team Lenka Hruba, PhD Sony Gurska,
PhD Petr Džubák, doc. Marian Hajduch

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And thank you for watching... 😊  Question?