HILLMARK
smart lead design and consulting

AI-Powered Structural Bioinformatics and Computational Technologies for Drug Discovery

LEIDEN
DRUG DEVELOPMENT
CONFERENCE

3rd Leiden Drug Development Conference (LDDC-3)
19 September 2024

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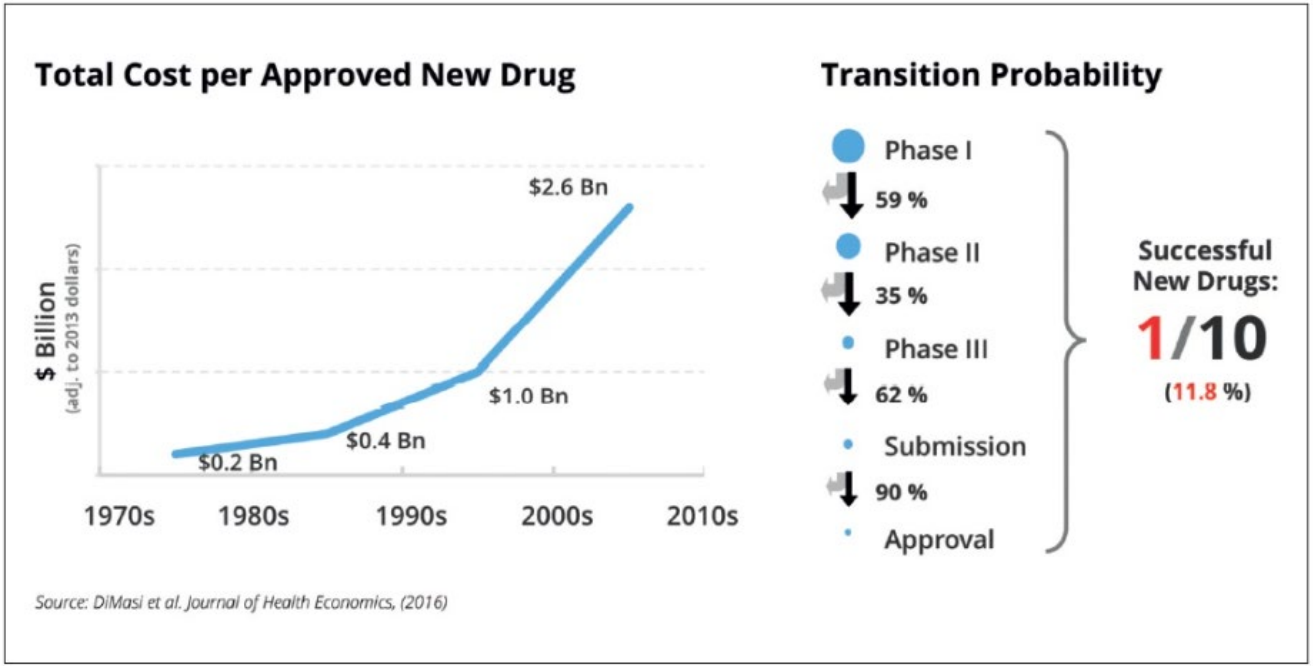
Hillmark B.V.

The problem

Companies spend more than ever on new drugs
but few make it to the market



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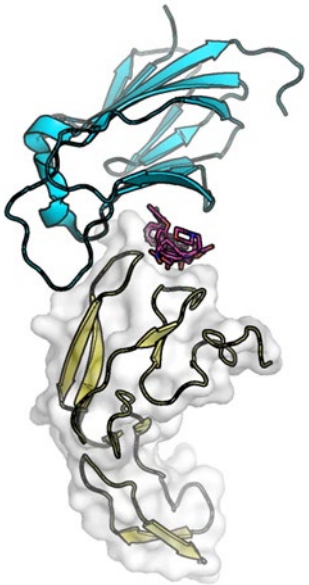
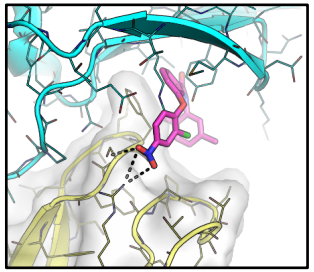
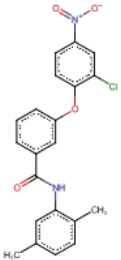
The opportunity



Hillmark promises smarter discovery and optimization through computational rationalized, **green** and **sustainable** approaches



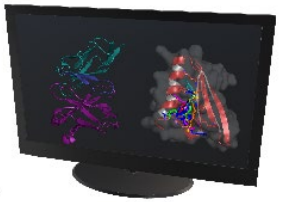


Computational structure-based molecular design and lead finding

- *accelerated discovery & development*
- *focused discovery through application of in silico ADME-Tox filters*
- ***reduction in time and cost***
- *more focused in vitro testing avoiding unnecessary testing and reduction of waste → lowering of environmental fingerprint*

Development of Bioactive Compounds

In Silico Methods



- Structure-Based Virtual Screening
- Rational Molecular Design
- Molecular Docking
- MD simulations
- BFE calculation
- AI/ML

Experiments

- Protein Purification
- In vitro Assays
- Cell-based Assays
- Direct Binding Measurement (SPR and ITC)
- In vivo

Small Compounds

Therapeutic Proteins

Peptides

Molecular Degraders

Drug Targets:
- Enzymatic Proteins
- Receptors
- Protein-Protein Interactions
- IDPs/IDPRs (Histones)
- Both intracellular and extracellular

Drug Targets in:
- Inflammatory Diseases
- Cardiovascular Diseases
- Sepsis
- Cancer
- Viral Diseases

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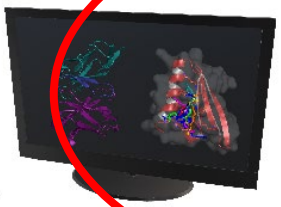
How do we do it?

In Silico Methods



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Showcase Projects

TECHNOLOGY

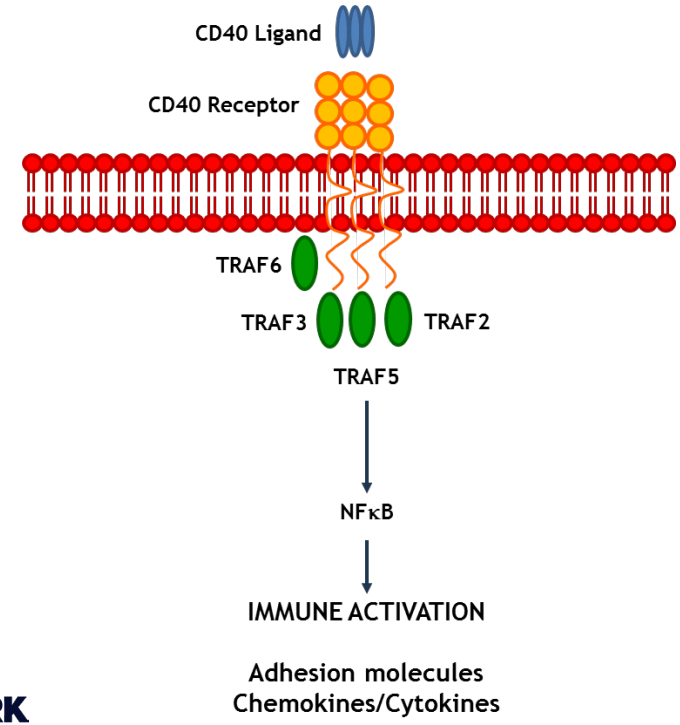
Showcase 1: Development of Small Molecule Inhibitors

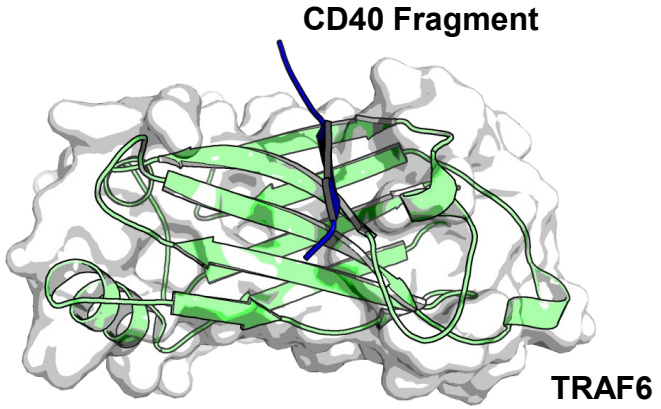
Structure-Based Virtual Screening (SBVS) to Identify Novel TRAF6 Inhibitors

Other Successful Study Targets: coagulation factors V and VIII, Dengue Protease, AMPK, Myt1 Kinase, Activated Protein C, SecA, Streptokinase, TRAF2, SARS-CoV-2 RdRp, SMPD3

TNF Receptor Associated Factor - TRAF

Adapter proteins in CD40 signaling





PDB ID 1LB6: TRAF6-CD40 Complex

- **CD40-TRAF6 interactions drive atherosclerosis**

AIM: To develop inhibitors to disrupt CD40-TRAF6 interactions

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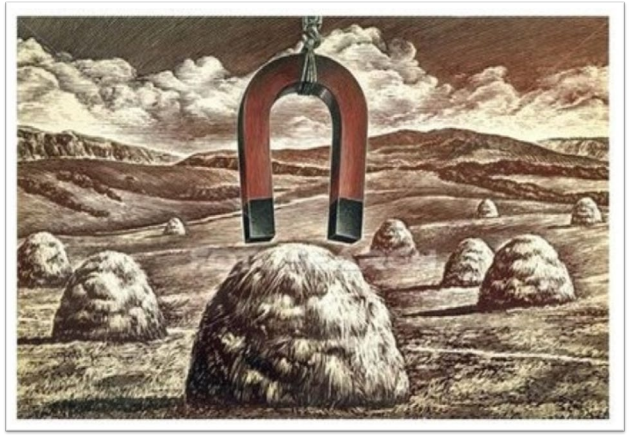
Lutgens E et al., ***J. Exp. Med.***, 2010

Find a New Compound – Finding a Needle in a Haystack

https://zinc.docking.org/

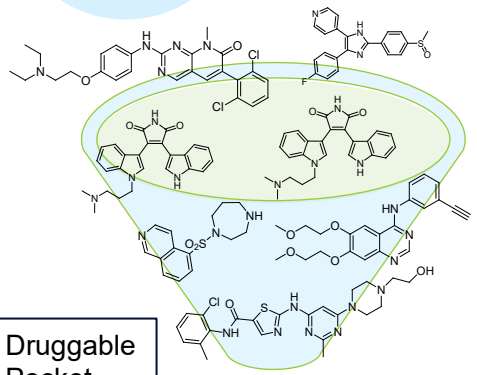
ZINC database – a DB of commercially-available compounds

- contains **over 750 million purchasable compounds**

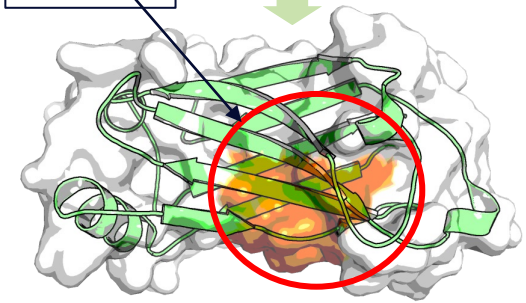


HOW?

Structure-Based Virtual Screening (SBVS)



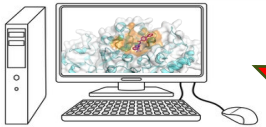
Druggable Pocket



ChemBridge Collection
~ 400,000 unique compounds

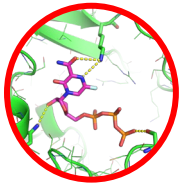
ADME-Tox Filtering
Drug-like Properties

Filtered Database
~270,000 compounds



Several Steps of
Molecular Docking
(AI-Powered Docking)

Post-Docking Analysis
800 compounds

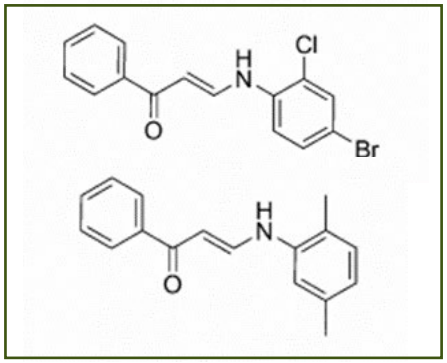


Protein-Ligand Interactions

Score & Ranking

Experiments

> 50 Hits



- **Higher hit rate than HTS**
- **IC_{50} at low µM level**
- **Inhibitory Activity in both *in vitro* & *in vivo* experiments**

Chatzigeorgiou, Seijkens et al ***PNAS*** *2014; Zarzyka et al.* ***J Chem Inf Mod*** *2015; Seijkens et al,* ***J Am Coll Cardiol****. 2018*

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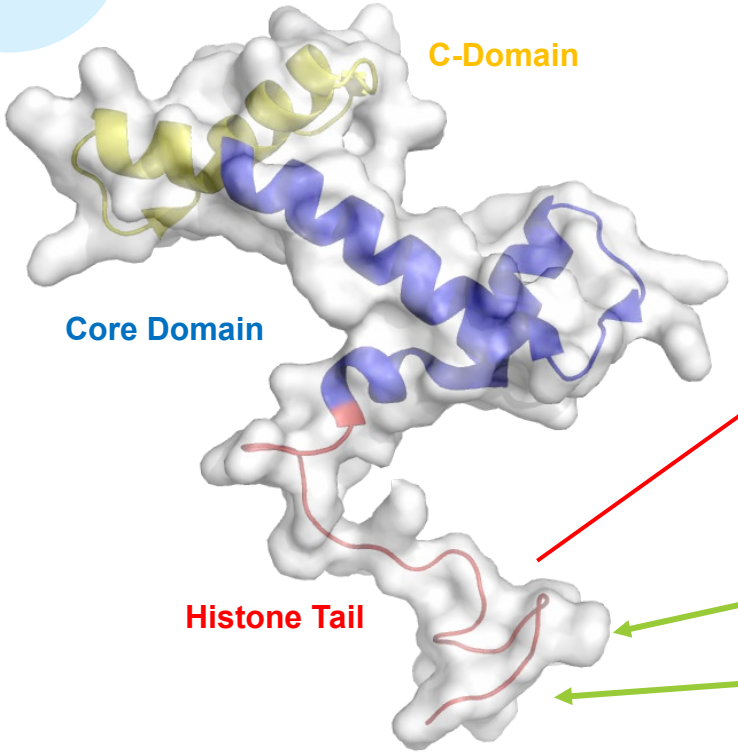
Showcase 2: Design and Development of Peptidic Inhibitors

Structure-Based Methods to Develop Peptidic Inhibitors Targeting Extracellular Histones

Other Successful Study Targets: CCL5-HNP1, GPIbα-VWF A1, CXCR4-CXCL12

Targeting Extracellular Histones



Histones: Sepsis, COVID-19, Thrombosis Atherosclerosis etc.

N-terminal Histone - the major domain activating cell death
- Intrinsically Disordered Region (IDR)
- unstructured proteins, lacking stable secondary or tertiary structures
- no druggable pocket

Peptides

Engineered Proteins

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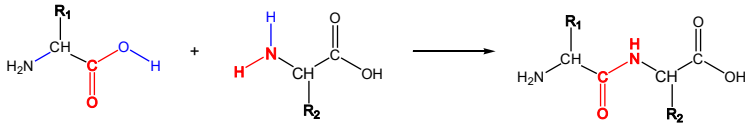
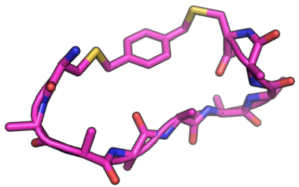
Wildhagen K et al, ***Thromb Res.*** *2015, Huckriede J et al,* ***Front Cell Infect Microbiol.*** *2021, Nicolaes GAF and Soehnlein O.,* ***Trends Pharmacol Sci.*** *2024, Keulen GM et al.,* ***Curr. Opin. Hematol.*** *2024*

Peptide Design

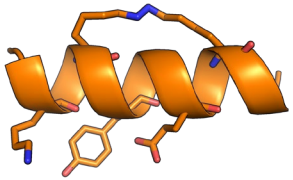
Peptide - A compound consisting of two or more amino acids linked by a peptide bond



Linear Peptide



Cyclic Peptides



Stapled Peptides

other types of peptidic inhibitors (or peptidomimetics):
D-Amino Acid peptides, Backbone extension, Cα-replacement etc.

Peptide design is challenging

Peptide with 2 AA	= 20 X 20	= 400
Peptide with 3 AA	= 20 X 20 X 20	= 8,000
Peptide with 4 AA	= 20 X 20 X 20 X 20	= 160,000
.		
.		
.		
Peptide with 10 AA	= 20 X 20 X 20 X 20 X 20 X 20 X 20 X 20 X 20 X 20	**= 10,240,000,000,000**
.		
.		

More than 10,000 billion possibilities

How to find one potential peptidic inhibitor from these 10,000 billion candidates?

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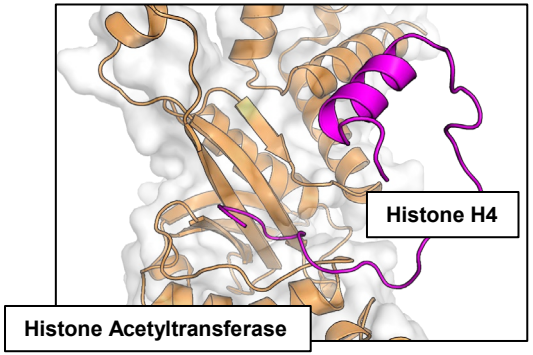
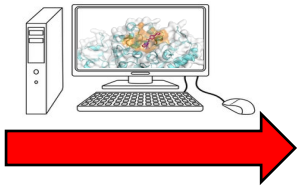
Structure-Based Peptide Design: Histone H4 Inhibitors

1. Identification of (related) Protein-Protein Complex

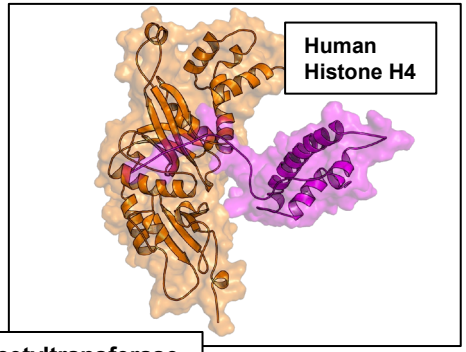


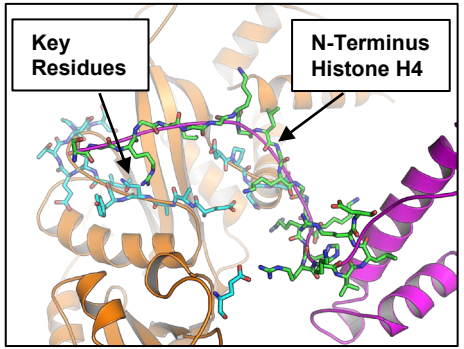
PDB 4PSW: histone acetyltransferase in complex with *Ophiophagus Hannah* histone H4

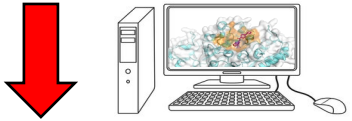


Protein-Protein Docking
MD Simulations

2. Determination of histone H4-binding protein complex



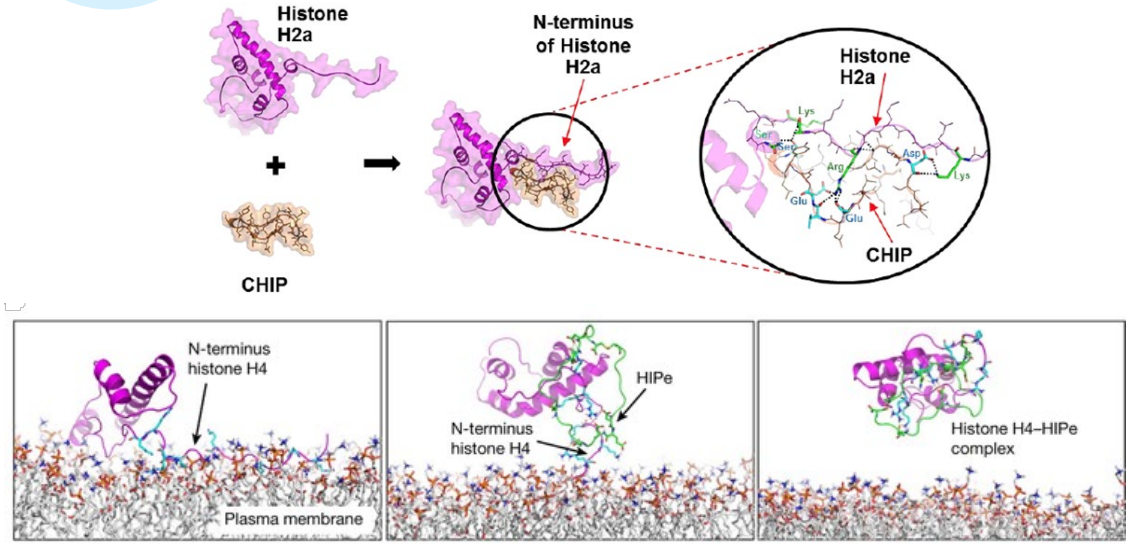




3. *In Silico* Peptide Design

4. Binding Free Energy (BFE) Calculations

Anti-Histone Peptide Inhibitors



Peptide contains 28 AA
= 20^{28} possibilities

***in silico:* 9 cyclic peptides were designed**

↓

***in vitro*: 4 peptides were selected for testing.**

↓

***in vivo*: 1 potent peptide shows therapeutic benefits.**

Newly Developed Histone Inhibitors

- demonstrate inhibitory activity *(in vitro* and functional assays)
- exhibit potential therapeutic benefits in mouse models

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Silvestre-Roig et al, ***Nature****, 2019; Schumski et al,* ***Circulation****, 2021; Wichapong et al,* ***Comput Struct Biotechnol J.****, 2021*

Showcase 3: Design and Development of Therapeutic Proteins

Engineered Therapeutic Proteins: Novel Activated Protein C (APC) variants

Other Successful Study Targets: Factor V, Factor VIII, ADAMTS13, Prothrombin, Protein S, Annexin A5

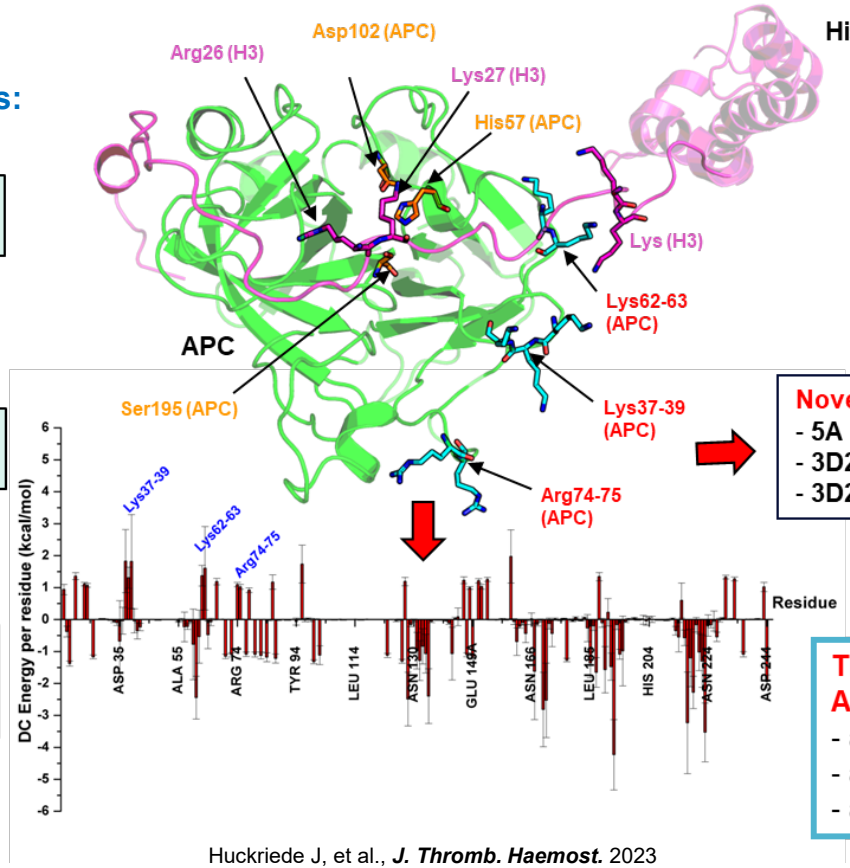
Design of Novel APC Variants

APC contains 461 residues:
***possibilities* = 20^{461}**

1. Protein-Protein Docking (APC-Histone H3 complex)

2. MD simulations of the selected docking pose

3. BFE calculation and per-residue energy decomposition (DC) analysis



4. In silico mutation of selected residues

Novel APC variants:
- **5A** (K37-39A + R74-75A)
- **3D2D** (K37-39D + K62-63D)
- **3D2D2A** (K37-39D + K62-63D + R74-75A)

Protein Purification & Experiments

The novel 3D2D- and 3D2D2A-APC variants show
- a decreased anticoagulant activity
- an improved binding to histone H3
- an ability to proteolyze histone H3

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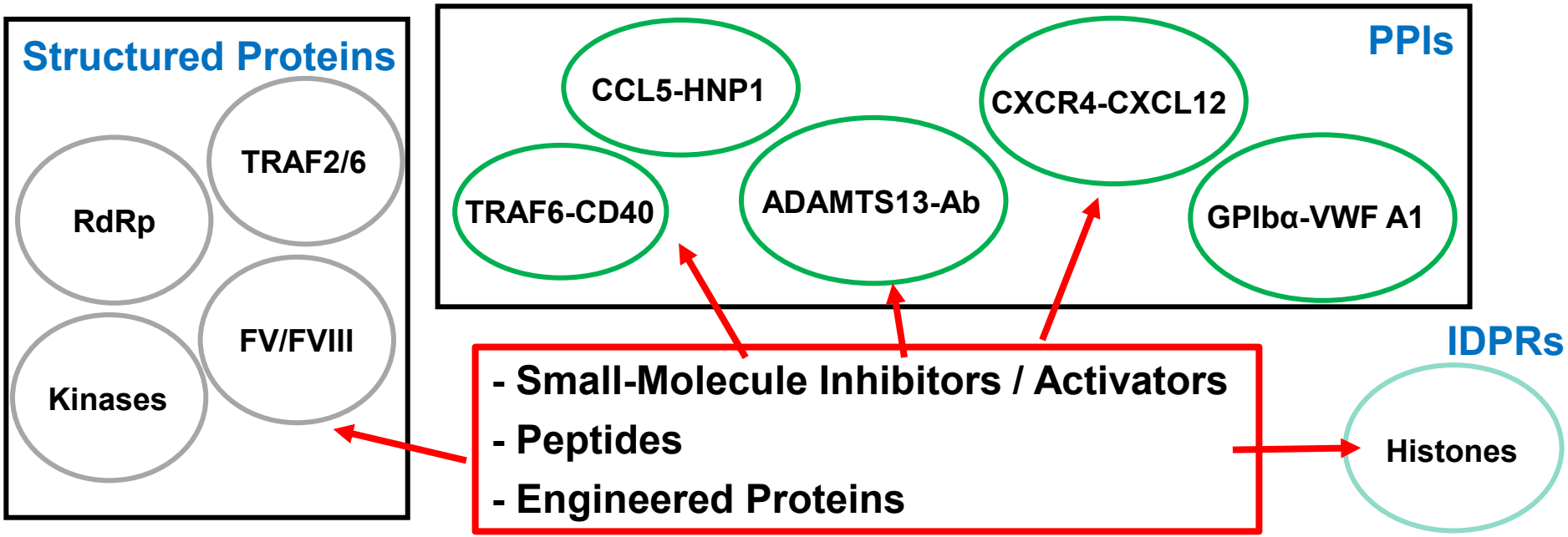
Huckriede J, et al., ***J. Thromb. Haemost.*** 2023

Conclusion

By use of computer-based methods (*in silico*),

- we can significantly reduce numbers of compounds subjected to experimental testing (*in vitro & in vivo*)
- we have developed lead compounds:



Acknowledgment

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https://www.3dstructure-function.nl/
https://www.hillmarkbiopharma.com/

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Thank you for your Attention