Discovery of selective inhibitor leads by targeting an allosteric site in Insulin-Regulated Aminopeptidase (IRAP)

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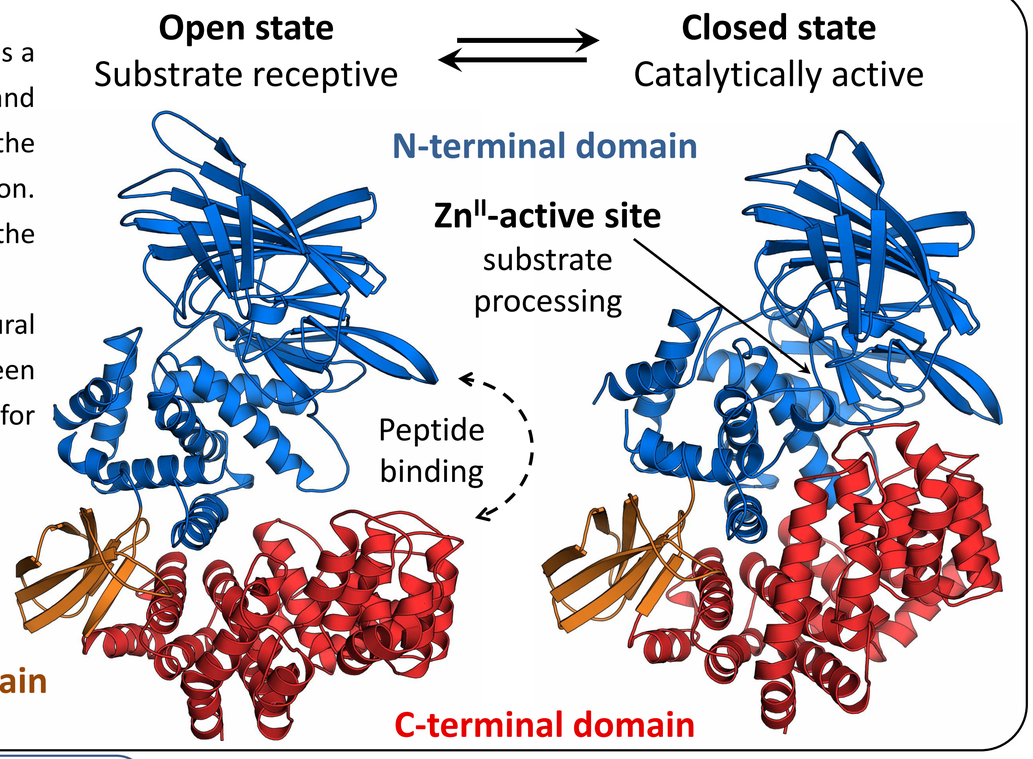
The scope

Insulin-regulated aminopeptidase (**IRAP**) **is a transmembrane, zinc-dependent aminopeptidase** that performs a variety of important biological functions. IRAP, also known as oxytocinase, leucyl-cystinyl aminopeptidase and placental leucine aminopeptidase is implicated in the **regulation of trafficking of glucose transporter type 4**, the **control of oxytocin levels in pregnancy**, as well as the **generation of antigenic peptides** for cross-presentation. IRAP is also a specific binding site for angiotensin IV, which upon binding serves as a competitive inhibitor of the enzyme. https://www.frontiersin.org/research-topics/12216/physiological-pathological-roles-and-pharmacology-of-insulin-regulated-aminopeptidase

IRAP (EC 3.4.11.3) belongs to the **M1 family of aminopeptidases** and shares high sequence and structural homology with the endoplasmic reticulum aminopeptidases ERAP1 and ERAP2. These enzymes have been shown to adopt conformations between **"open" and "closed" states**, which expose a large internal cavity for peptide entrance and mediate efficient substrate processing. https://www.frontiersin.org/articles/10.3389/fimmu.2017.00946/full

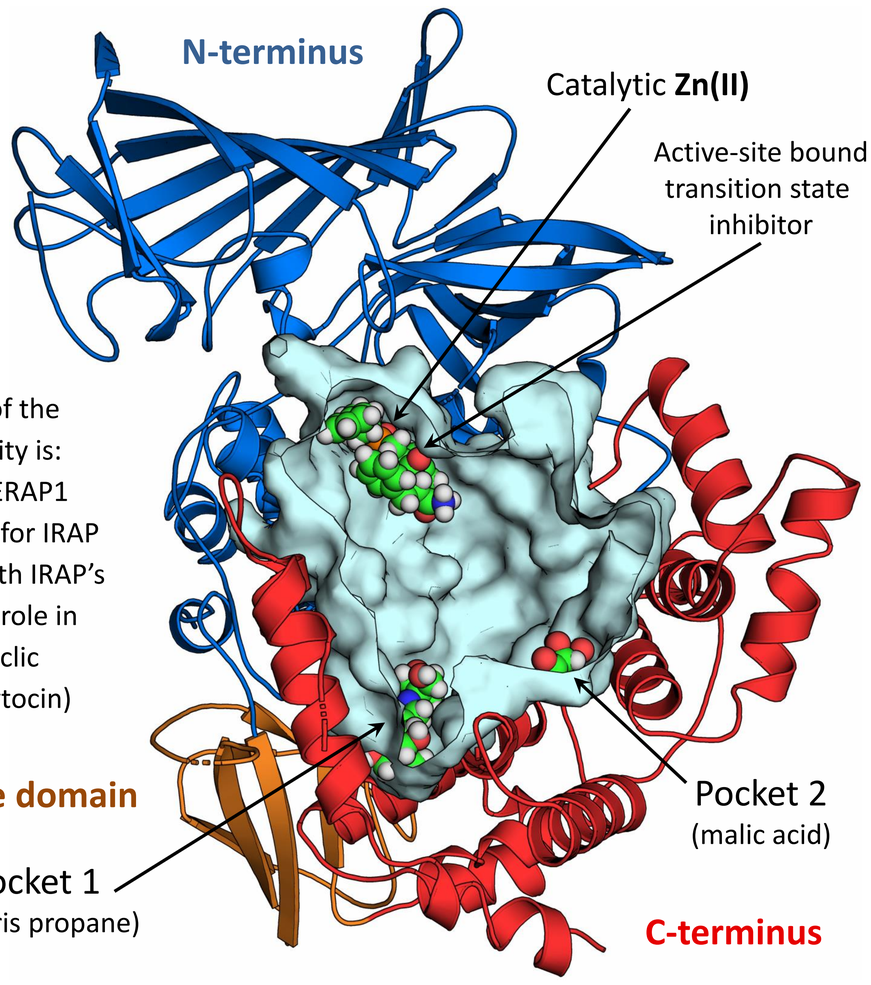
Due to its involvement in several human disease states, **IRAP is an emerging pharmaceutical target.** With the aim to discover inhibitors of IRAP that do not disrupt the physiological function of other M1 enzymes, we pursuit the discovery of **lead-like compounds that bind away from the highly homologous active site**.



The idea

A recent, high-resolution crystal structure of "closed" **ERAP1** in complex with a potent phosphinic pseudopeptide inhibitor revealed **distinct druggable pockets** occupied by two molecules from the crystallization medium.

PDB ID: 6Q4R



The volume of the substrate cavity is: 2,900 Å^3 for ERAP1 and 5,300 Å^3 for IRAP consistent with IRAP's physiological role in processing cyclic peptides (oxytocin)

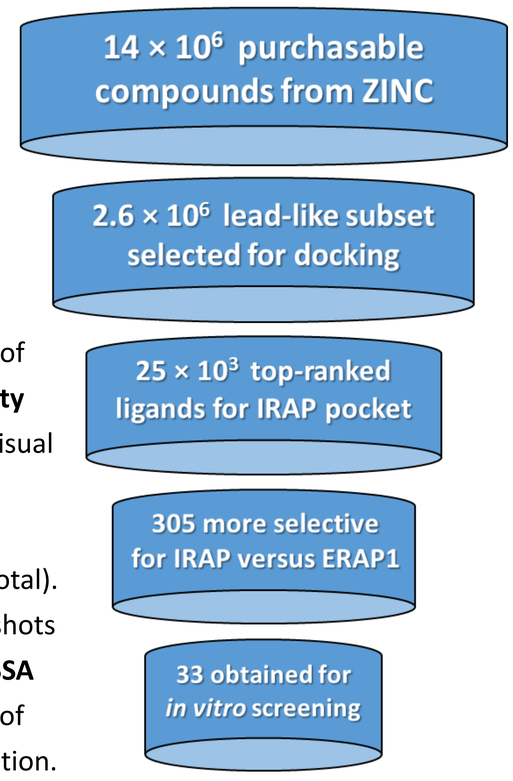
We opted to **target pocket 1** as the largest pocket and a site that comprise residues from **all three functional domains** of the enzymes, next to the hinge domain.

The method

First **we identified the corresponding pockets in the C-termial domain of IRAP** (PDB ID: 5MJ6), which interestingly, share comparably low homology with those of ERAP1 and ERAP2. To discover high-affinity compounds that bind and disrupt the physiological processing of large substrates of IRAP, we employed **structure-based virtual screening** according to the following steps:

From the purchasable chemical space of the **ZINC-15** database, we selected a subset of **lead-like** compounds (MW of 200–350 and cLogP < 3.0), clean from reactive groups or PAINS (**anodyne** subset) and **in-stock**. A total of 2.6 million compounds were docked at the selected site of IRAP using **AutoDock VINA** in order to select the **top-ranked 1%** compounds with the highest affinity.

These compounds were also docked at the related pocket of ERAP1, so as to **disfavor compounds of equally high affinity** for the enzyme with the highest homology to IRAP. After visual investigation of residue-specific interactions with the top-ranked compounds, we selected 305 compounds for **molecular dynamics simulations** of 20 ns each (> 6 μs in total). From the second half of the MDs we obtained 1,000 snapshots and carried out **free energy calculations with the MM/GBSA and MM/PBSA** methods, which guided the final selection of the most promising compounds for experimental investigation.



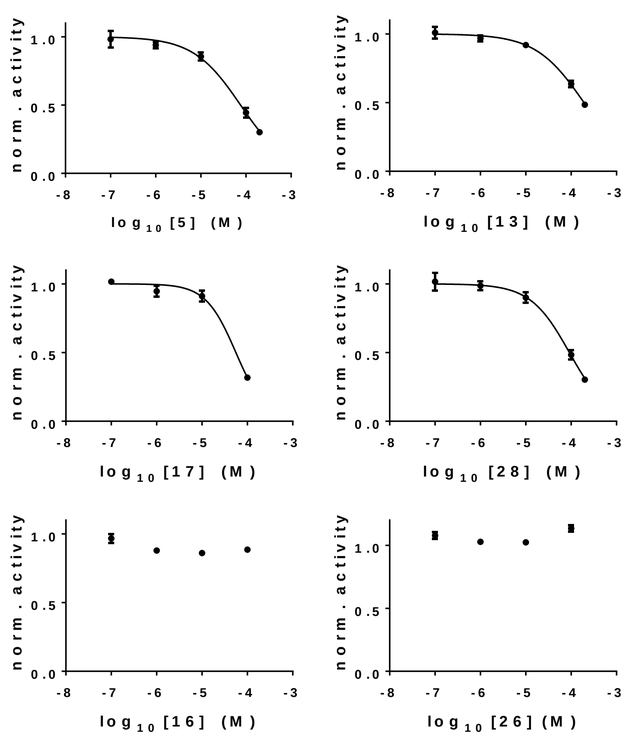
For their evaluation we employed two orthogonal functional assays: one using **a small fluorigenic substrate** (Leu-AMC), and one by **following the degradation of oxytocin by IRAP** using HPLC.

The result

Screening of the compounds using the fluorigenic Leu-AMC substrate revealed **3 compounds with IC$_{50}$ values < 100 μM** and characteristic inhibition plots shown.

ID	IC$_{50}$ (μM)	ID	IC$_{50}$ (μM)	ID	IC$_{50}$ (μM)
1	*NI*	12	*NI*	23	333
2	*NI*	13	**192**	24	337
3	192	14	*NI*	25	*NI*
4	*NI*	15	*NI*	26	***NI***
5	**76**	16	***NI***	27	*NI*
6	*NI*	17	**56**	28	**92**
7	*NI*	18	*NI*	29	*NI*
8	*NI*	19	*NI*	30	*NI*
9	*NI*	20	584	31	193
10	*NI*	21	*NI*	32	*NI*
11	*NI*	22	*NI*	33	*NI*

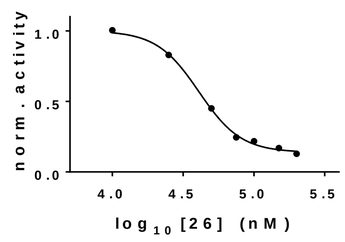
NI: no inhibition up to 1 mM compound



Interestingly, compounds that inhibit Leu-AMC with IC$_{50}$<100 μM did not display similar inhibition of the natural substrate, oxytocin. In contrast, **compound 26 that does not inhibit Leu-AMC, exhibited 69% inhibition of the cyclic substrate** at 100 μM tested. From the dose-dependent inhibition of oxytocin degradation by IRAP, followed by HPLC in the presence of compound **26**, we estimated an **IC$_{50}$ value of 40 μM** (shown below).

ID	% Inhib.	ID	% Inhib.	ID	% Inhib.
1	0	12	44	23	26
2	26	13	10	24	0
3	7	14	25	25	14
4	11	15	9	26	**69**
5	17	16	32	27	32
6	15	17	23	28	0
7	0	18	0	29	39
8	0	19	0	30	22
9	30	20	34	31	10
10	0	21	14	32	28
11	13	22	0	33	10

% estimated from HPLC peak areas



20 min incubation of 42 μM oxytocin + 20 nM IRAP

Lead compound 26 exhibits a size-specific substrate inhibition profile for IRAP, suggesting that targeting a C-domain pocket away from the active site is a viable strategy to develop selective inhibitors.

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