Recent Progress of Interprotein's Research Activities

- INTENDD® and AI-guided INTENDD®-

Interprotein®

Interprotein Corporation

Interprotein Corporation

- Location: Osaka, Japan
- Year Established: 2001
- CEO & President: Masato Hosoda
- www.interprotein.com
- @interprotein

- Expertise: Development of PPI (protein-protein interaction) modulators with small molecules or peptide
- Business Model:
  - Strategic Alliance: Target discovery and Lead optimization
  - Licensing of a pipeline

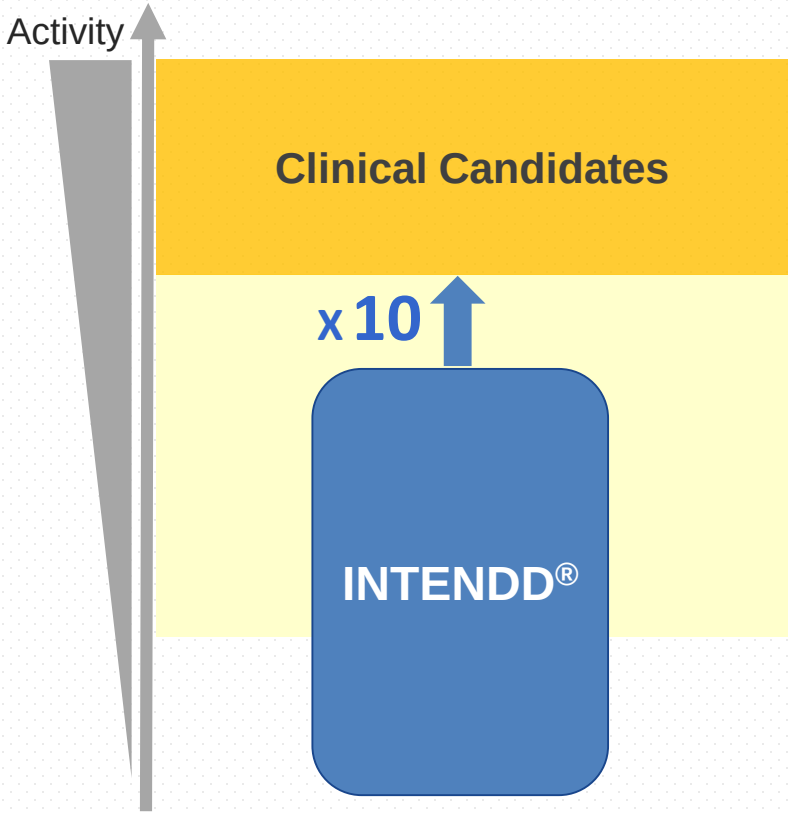
Platform Technology

- INTENDD® / SBSG®,
- A proprietary *in silico* drug design suite
  - Structure-Based Molecular Design
  - Prediction with Entropic Contribution
- Deep Learning for AI Drug Discovery

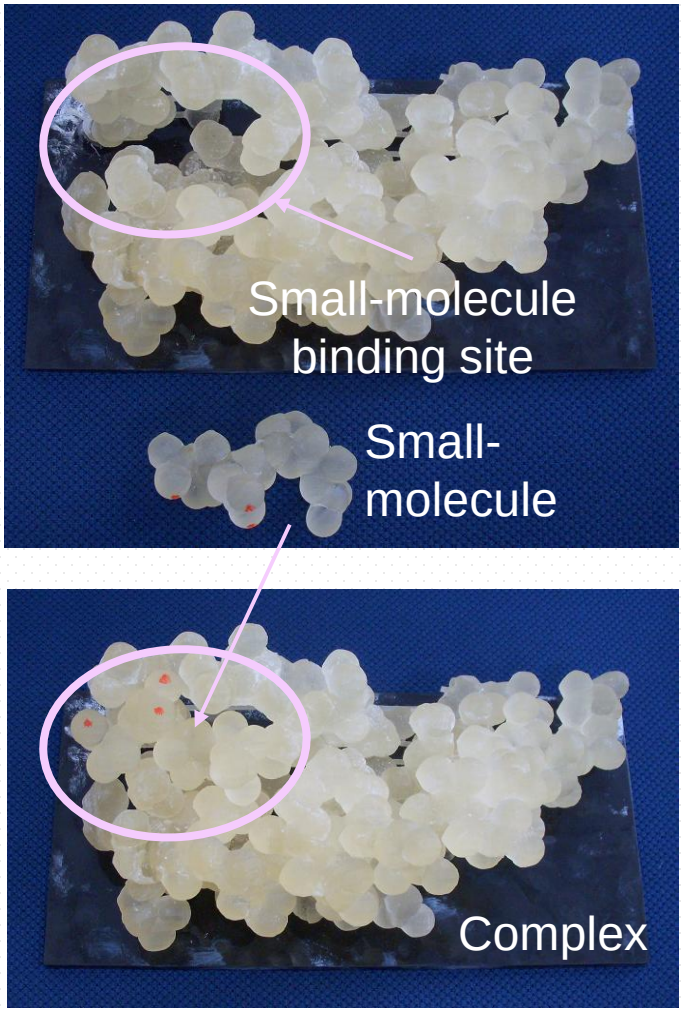
The Quality of INTENDD®

- **INTENDD®** (**INT**erprotein's **E**ngine for **N**ew **D**rug **D**esign) is our proprietary *in silico* drug discovery platform specializing in protein-protein interaction (PPI) modulators.

- **INTENDD®** enables the discovery of compounds with significantly higher **activity** at initial screening so that the hit compounds can be developed into potential clinical candidates with just 10-fold activity gain.



200 Candidates for Wet-Screening in 4 Months



3D-printed models

Schedule

1 Month

3 Months

INTENDD®

Target pocket identification

SBSG® (Structure-Based Scaffold Generation) Method

Clustering and filtering through binding structure-based mechanism

200 comps for screening

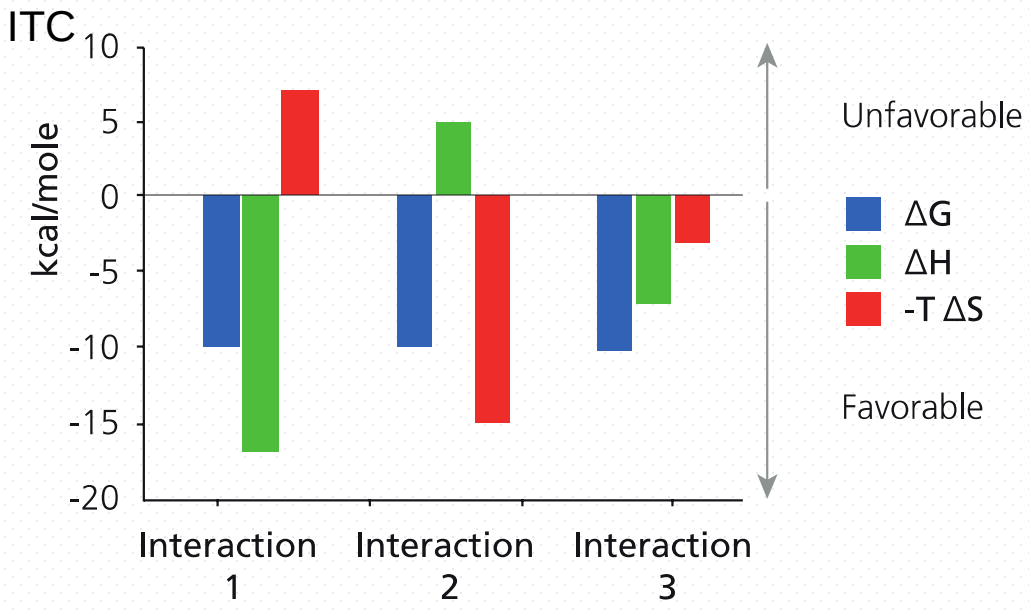
Use 3D-printed models

Molecular design of candidate comps

INTENDD® output

Protein + comp 3D structural interactions

Typical Thermodynamic Profile of Compounds



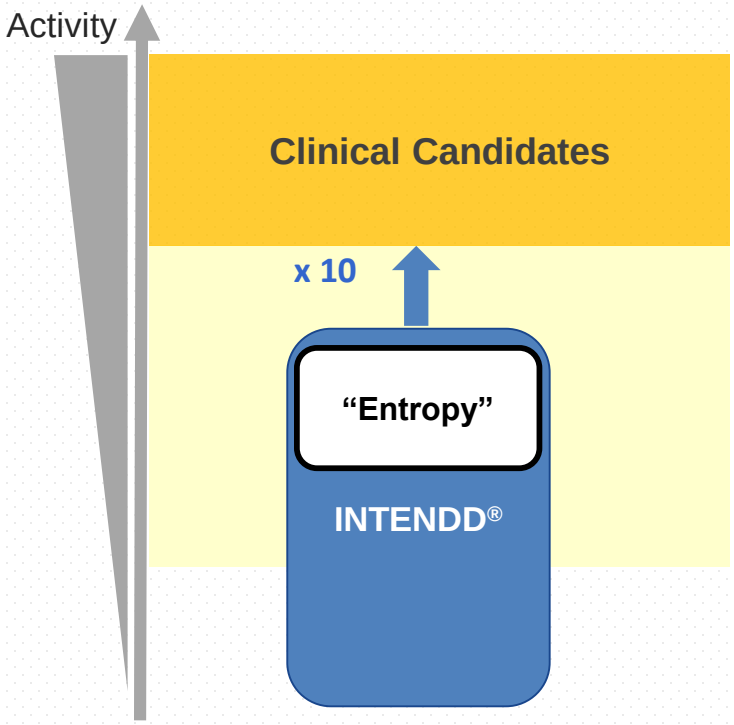
Shown are thermodynamic signatures of three interactions that have the same binding energy (ΔG). The binding energy is related to the affinity. Binding affinity is a combined function of the binding enthalpy (ΔH) and the binding entropy (ΔS). Binding enthalpy reflects the strength of the interaction due to hydrogen bond formation and van der Waals interactions. Binding entropy is a combination of the change in entropy from desolvation and conformational changes upon complex formation.

Understanding biomolecular interactions, Malvern, 2016

- Interaction 1; This type of compound would form noncovalent bonds, mostly hydrogen bonds, and tend to have higher flexibility and polarity.
- Interaction 2; This type of compound would form hydrophobic contacts and tend to have lower solubility and flexibility.
- Interaction 3; This type of compound have both favorable enthalpy and entropy gain. This profile is ideal for drug candidates. **Interprotein** assume that large favorable entropy gain is critical factor for PPI inhibition.

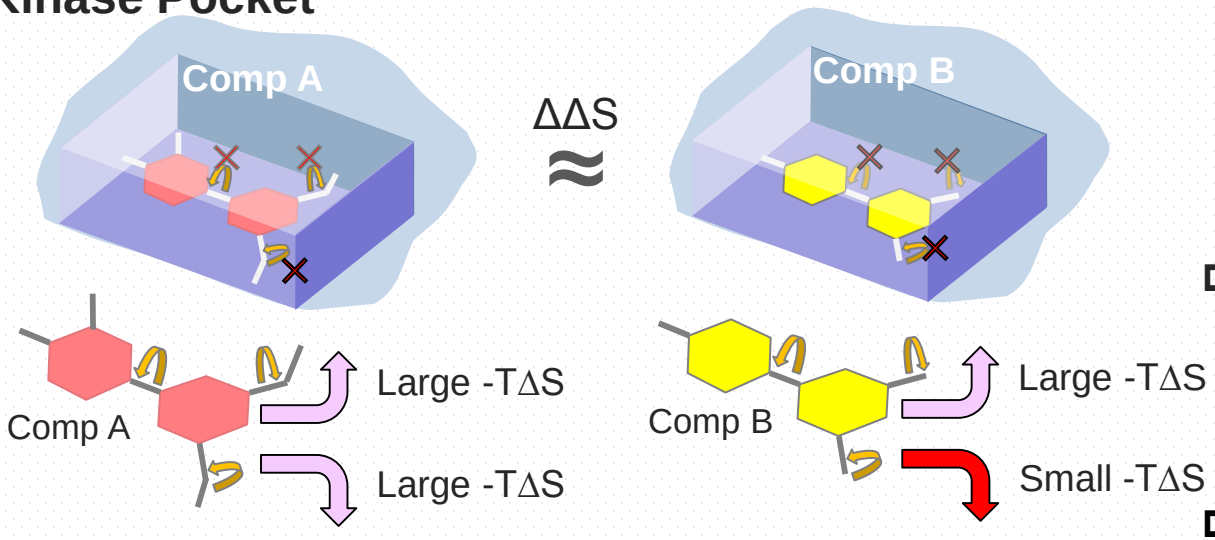
The Key Ingredient of INTENDD® | Entropy

- **INTENDD®** considers entropy contribution for binding and enables us to design hit candidate compounds of -8 ~ -12 kcal/mol range [nanomolar (10^{-7}) range in activity].



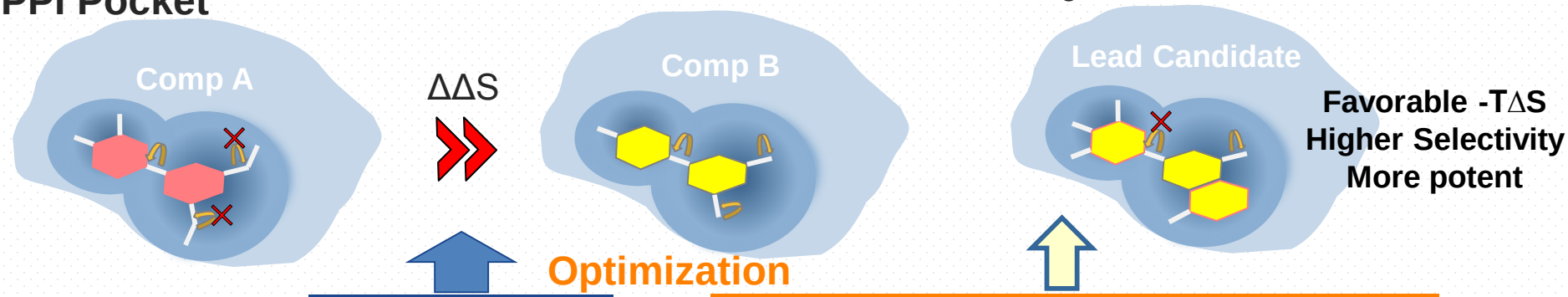
Consideration of Entropy for PPI Inhibition

Kinase Pocket



- Upon binding to kinase pocket, both compounds A & B have similar entropic penalty due to conformational restrictions. Thus, enthalpy gain contributes to higher activity.
- On PPI pocket, compound B has smaller entropic penalty than A. **INTENDD®** can pick out B as the promising compound for PPI inhibition.
- Compound A can also be optimized by **Interprotein** & pharma to generate lead candidates.

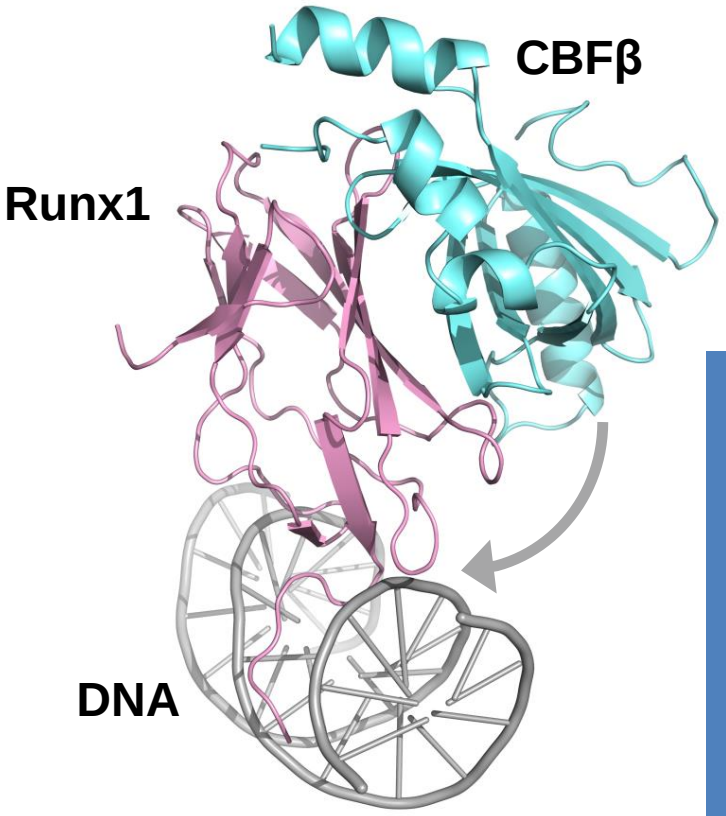
PPI Pocket



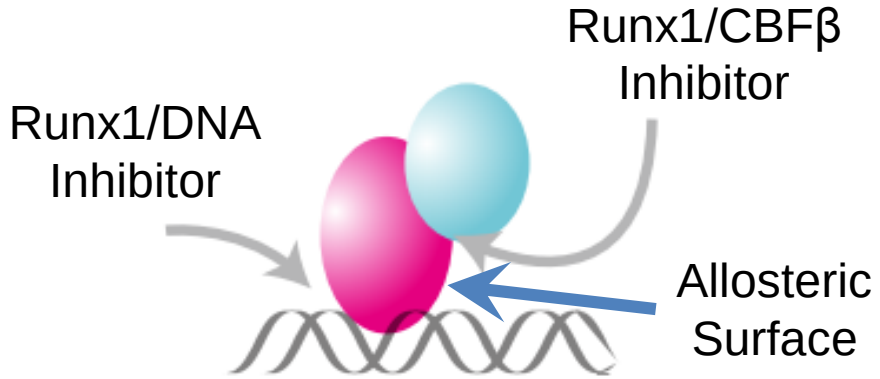
✕: conformational restriction

INTENDD® Consideration → **Interprotein (Binding Mechanism) & Pharma (Knowledge & Technique)**

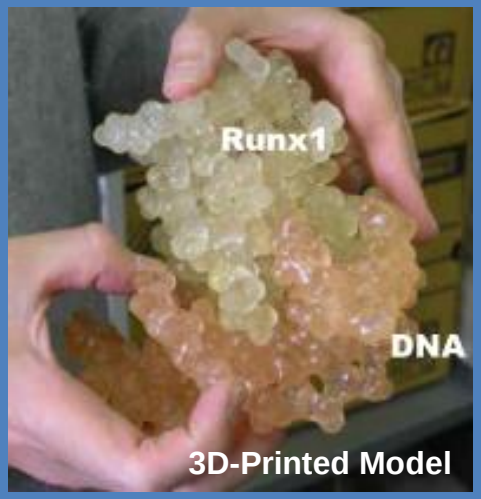
Strategy for Runx1 Inhibitor Design



CBFβ promotes DNA binding to Runx1 (Allosteric Modulation)



INTENDD®



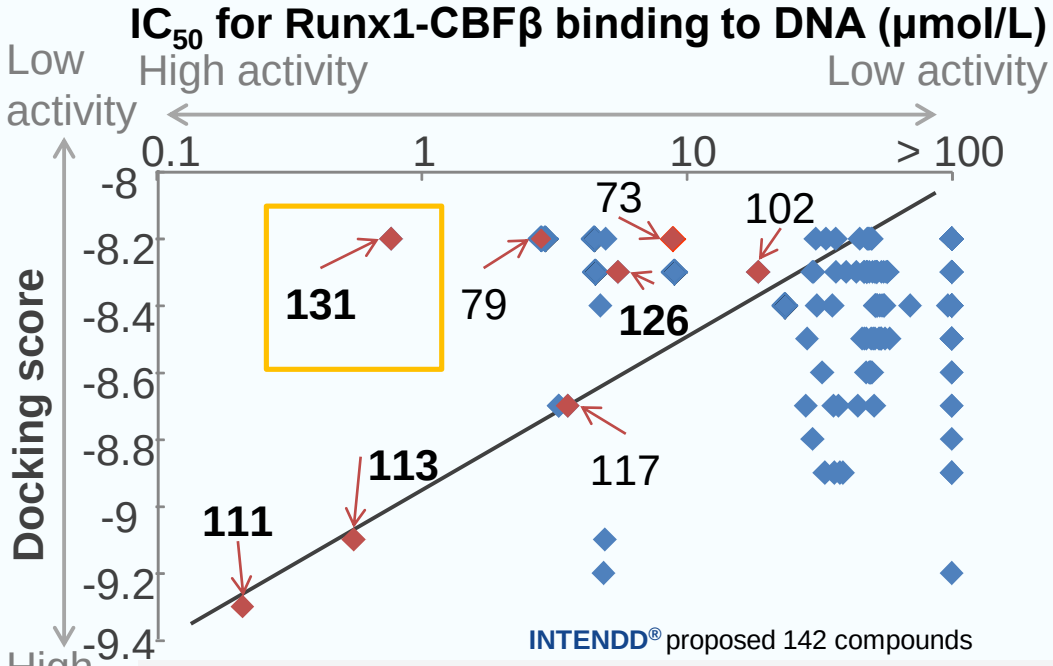
No suitable pocket for inhibition on allosteric surface

⬇

Considering protein flexibility to gain higher entropy effect

Runx1 Inhibitor Program

- Relationship between Docking Score and Activity -



◆, Hit compounds Identified based on activities and structures. **Bold** denotes compounds with K_D values less than 10 μM.

Comps	kcal /mol	IC_{50} (μM)	K_D (μM)
73	-8.2	8.9	ND
79	-8.2	2.9	35
102	-8.3	19	ND
111	-9.3	0.21	5.2
113	-9.1	0.55	1.6
117	-8.7	3.6	ND
126	-8.3	5.5	0.065
131	-8.2	0.77	9.5

ND: Not determined

- There is no linear relationship between MD-based docking score and activity. **INTENDD®** detected compound **131**, which exhibited high activity and low docking score, from 200 hit candidate pool. It would be difficult to find it with docking method in the same condition.
- **131** analogs showed favorable SAR, resulting in the identification of many highly active comps (IC50 < 1μM) in secondary screening.

Runx1-CBFβ/DNA Interaction Inhibitors

Number of hit compounds that inhibit Runx1-CBFβ/DNA binding over 50%

- 26 of 142* (hit rate: 18%) at the concentration of 100 μM
- 7 of 142 (hit rate: 5%) at the concentration of 10 μM
- 3 of 142 (hit rate: 2%) at the concentration of 1 μM

* Number of compounds tested following proposal by INTENDD®/SBSG®

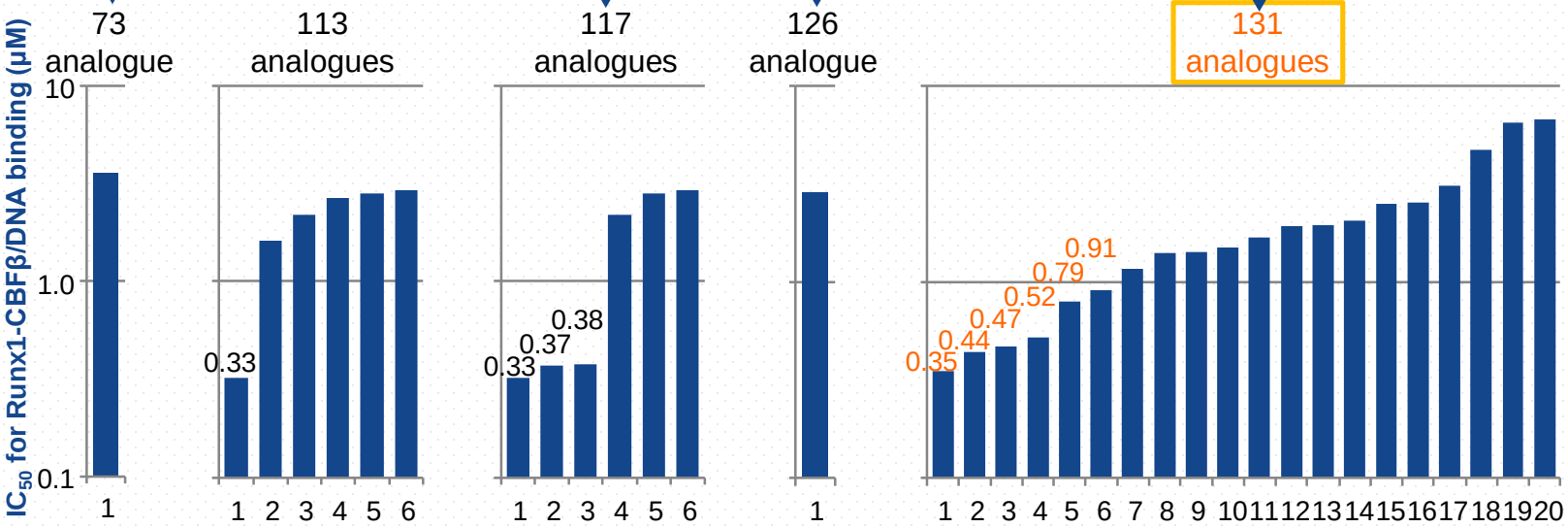
1) Inhibition of Runx1-CBFβ/DNA binding by compounds was assessed by SPR.
2) Binding affinity of compounds to Runx1 was determined by MST.
3) ND: not determined.

Primary hits (IC_{50} < 100 μM)

Compound	73	79	102	111	113	117	126	131
IC_{50} for Runx1-CBFβ/DNA binding (μM)[1)]	8.9	2.9	19	0.21	0.55	3.6	5.5	0.77
Binding affinity to Runx1 (K_D, μM)[2)]	ND[3)]	35	ND	5.2	1.6	ND	0.065	9.5

Number of tested compounds in secondary screening	1	0	2	2	6	10	17	35	Total 73
Number of secondary hit compounds	1	-	0	0	6	6	1	20	34
Hit rate in secondary screening (%)	100	-	0	0	100	60	5.9	57	46

Secondary hits (IC_{50} < 10 μM)

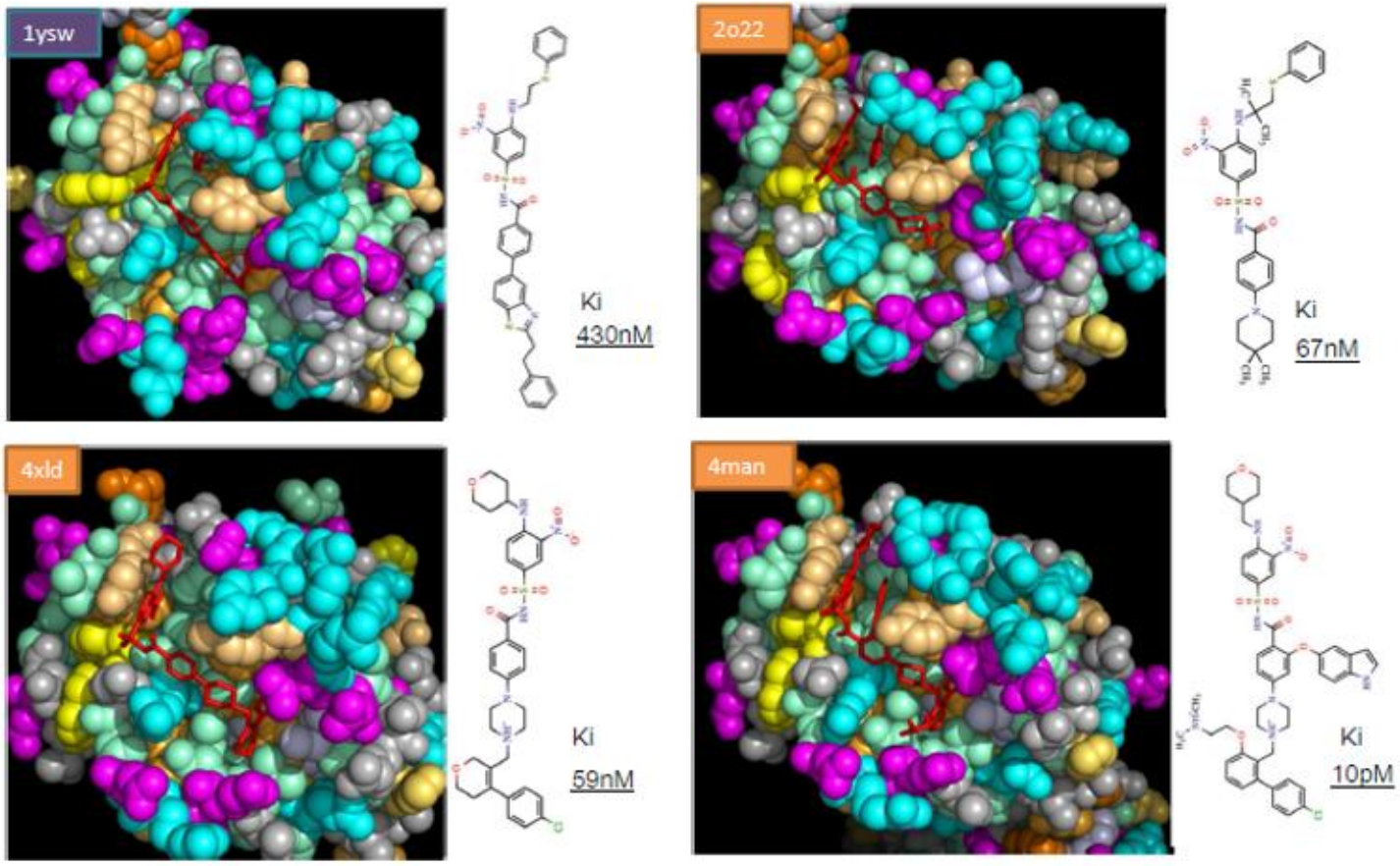


The Vision of AI-guided INTENDD®

Interprotein®

Interprotein Corporation

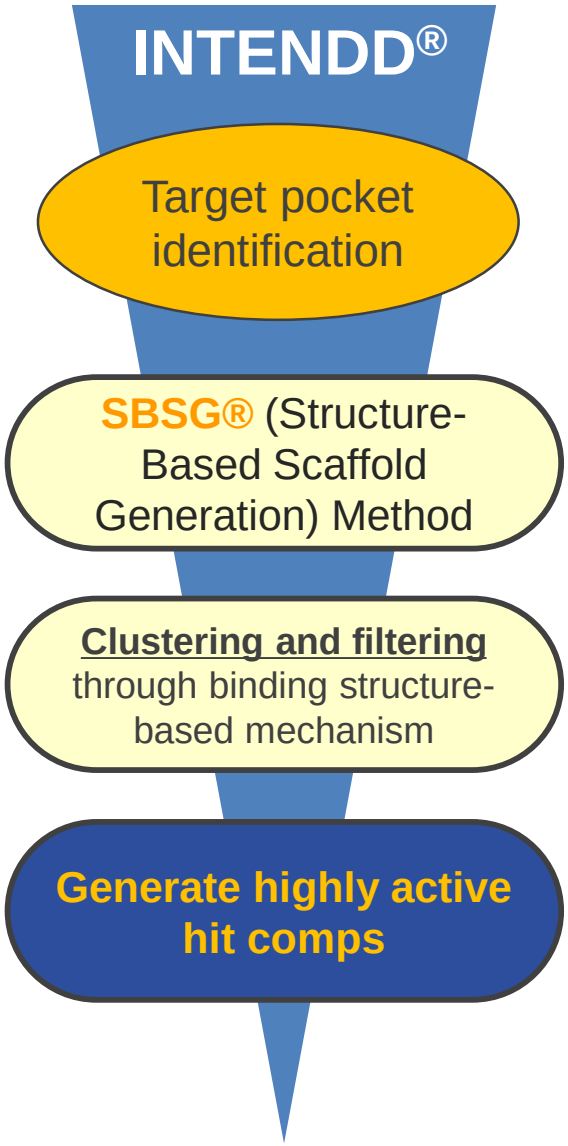
No one can explain the relationship between binding conformation and activity!

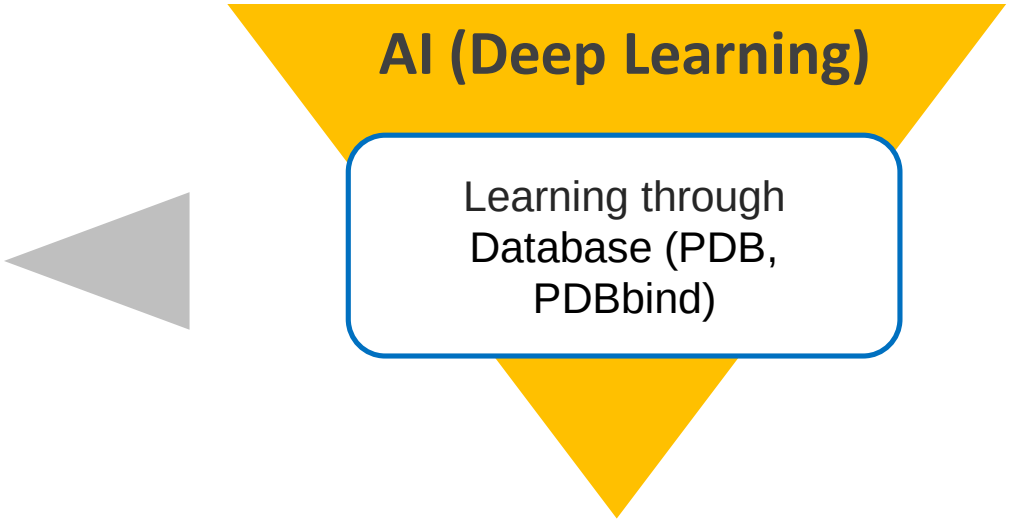


Example | BCL-2 inhibitors

Combining the ability of INTENDD® and AI, Interprotein aims to develop an AI-powered novel platform that enables precise prediction of the activity of PPI inhibitors.

AI-guided INTENDD®



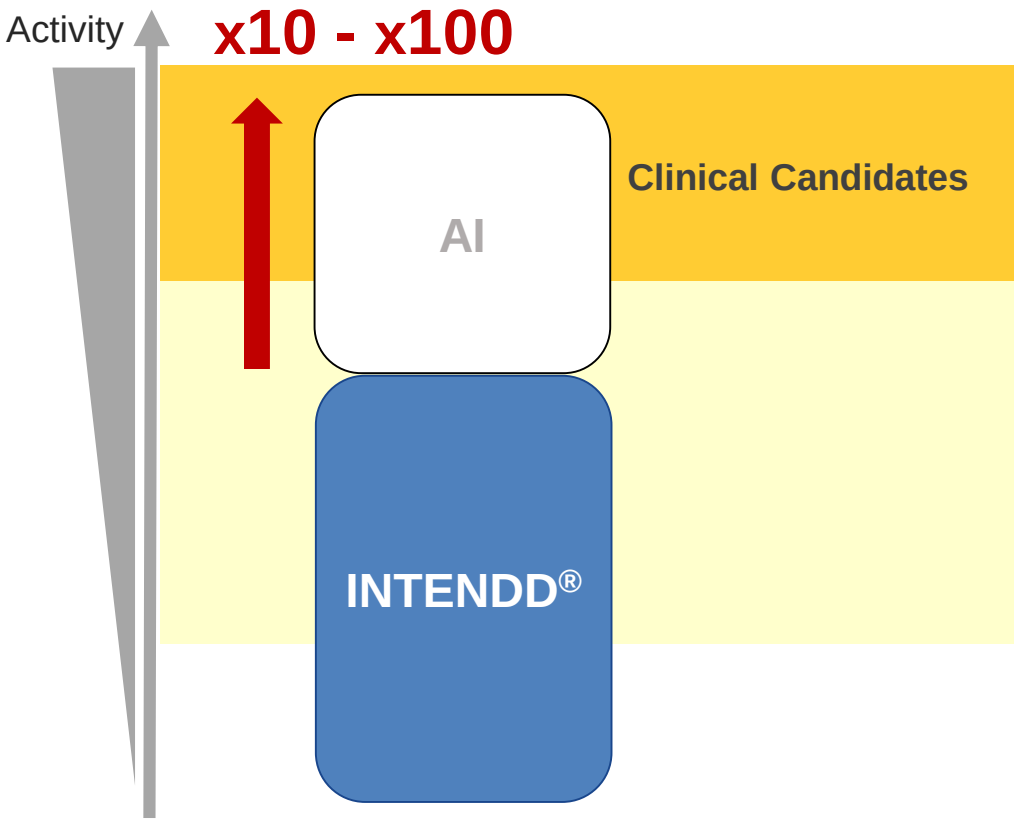


Goals of AI-guided INTENDD®

- Prediction of the activity based on the binding mechanism
- Dramatically increase initial hit rate
- Generate clinically active compounds

Vision of AI-guided INTENDD®

Compounds designed by AI-guided **INTENDD®** would have activities high enough to proceed to clinical stages.



In house project (Small Molecules)

Immunology, Allergy

Project	Stage	Note
IL-6 inhibitor	Lead generation	We have identified compounds that modulate IL-6/IL-6R interaction in a system (MOA has been almost fully verified).
IgE inhibitor	Hit validation	We have identified compounds that inhibit IgE-induced degranulation of has been partly verified.
GP130 inhibitor	Discovery	We have selected hit candidates for small molecule gp130/IL-6 interaction modulator, and also designed peptide gp130/IL-6 binding (inter-trimer)

Oncology, Hematology

Project	Stage	Note
Runx1/CBFβ	Hit validation	We have identified several compounds that bind to RUNX1 with high affinity 65 nM) and inhibit RUNX1/CBFβ binding to DNA. We are currently specificity of these compounds.
Notch1	Lead generation	We have identified compounds that inhibit Notch1-relevant transcription and tumor growth in a xenograft model. MOA has been partly verified.
Tubulin polymerization Inhibitor	Lead generation	We have identified compounds that inhibit tubulin polymerization in a cell- and suppress tumor growth in xenograft models. MOA has been almost

Chemical Targets and Stage of Development of Internal Program

Small Molecule Drug Discovery

Project	Domain	Development Stage
• IL-6 Inhibitor	Autoimmune, Inflammation, Oncology	Lead Generation
• gp130 Inhibitor	Autoimmune, Inflammation, Oncology	Hit Identification
• TNFα Inhibitor	Autoimmune	Lead Generation
• Runx1 Inhibitor	Hematology	Lead Generation
• Tumor Angiogenesis Inhibitor	Oncology	Lead Optimization
• IgE Inhibitor	Allergy	Lead Generation
• Notch 1 Inhibitor	Oncology, Hematology	Lead Generation
• Tim3 Inhibitor	Oncology (Immune Check Point)	Hit Identification
• Tubulin Polymerization Inhibitor	Oncology, Hematology	Lead Generation
• Mutant CALR Inhibitor	Hematology (Ultra rare disease)	Hit Identification
• Smurf1 inhibitor	Cardiovascular	Hit Identification

Peptide Drug Discovery

Project	Domain	Development Stage
• gp130 Inhibitor	Autoimmune, Inflammation, Oncology	Rational Design
• TIM-3 Inhibitor	Oncology (Immune Check Point)	Rational Design/ Phage Library Screening
• KIR Inhibitor	Oncology (Immune Check Point)	Rational Design
• NKG2A Inhibitor	Oncology (Immune Check Point)	Rational Design
• VIP Inhibitor	Oncology (Immune Check Point)	Rational Design
• Mutant CALR	Hematology (Ultra rare disease)	Rational Design Phage Library Screening