# Drug discovery research for small molecule protein-protein interaction (PPI) modulators with unique *in silico* drug design strategy

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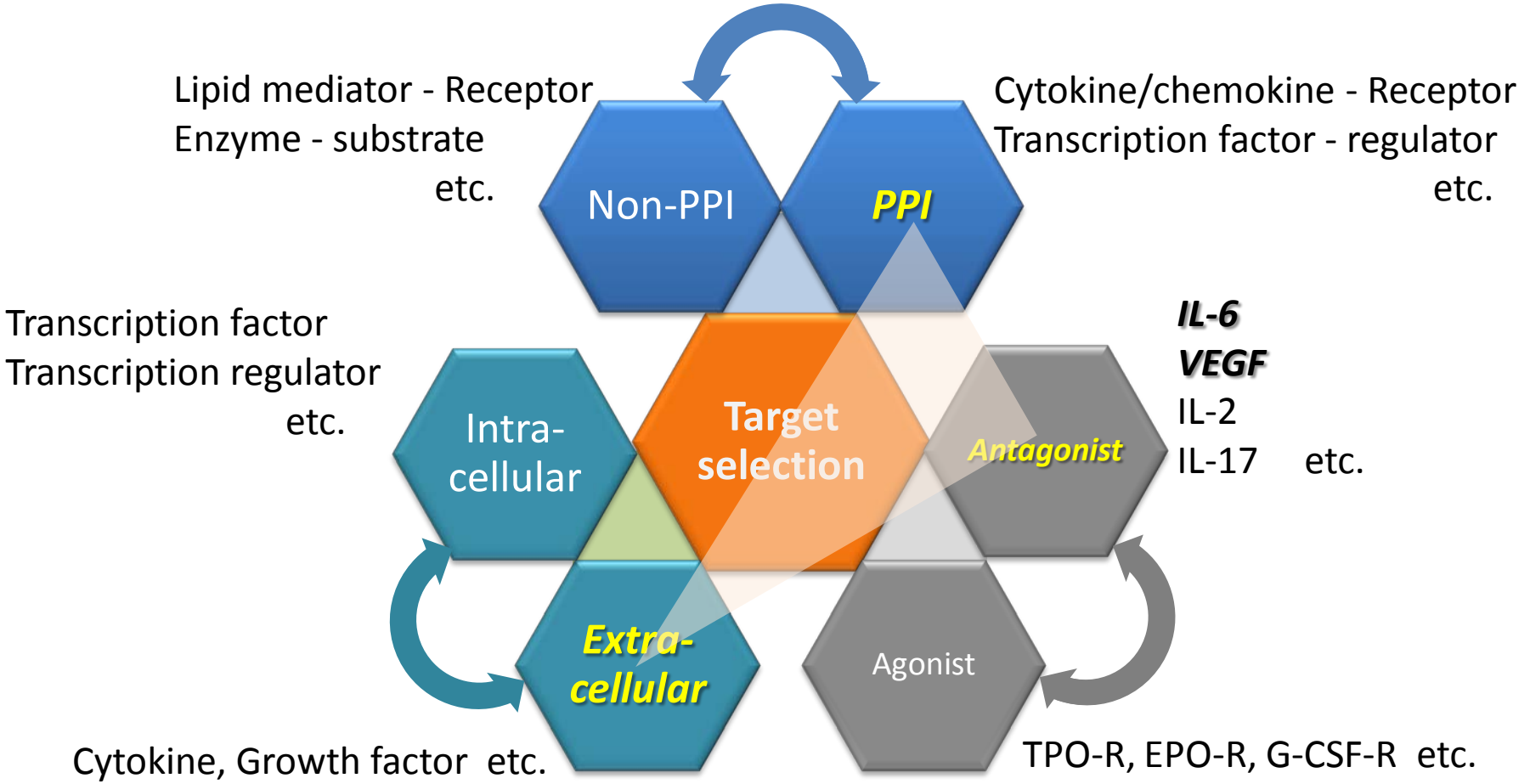
# Interprotein's targets for small molecule drug discovery




***Problem to be solved in drug discovery***

- Exhaustion of target molecules
- Decline in productivity

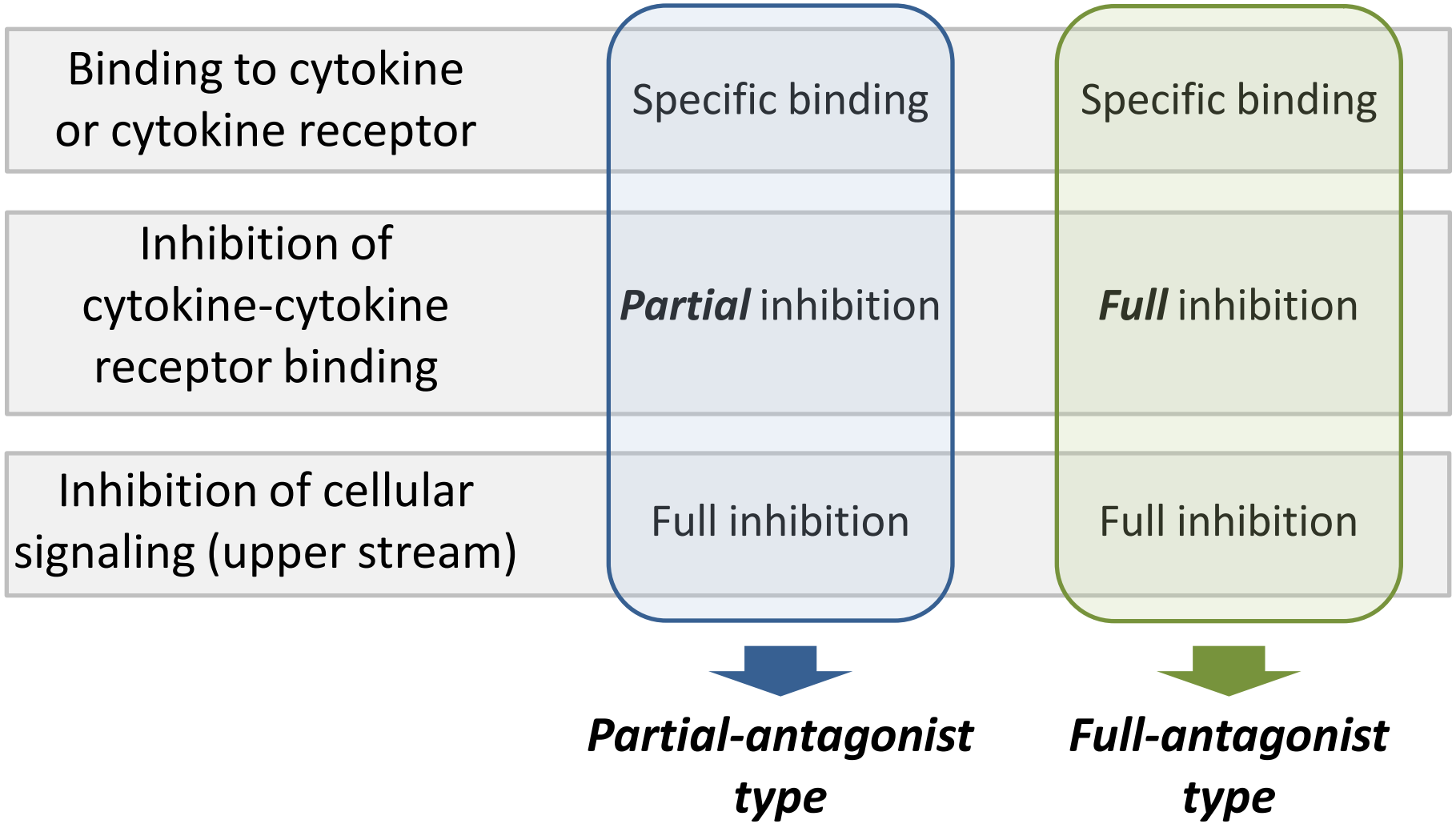
***Solution by INTERPROTEIN***

- Opening up potential target molecules
- Raising productivity

# Points of today's talk

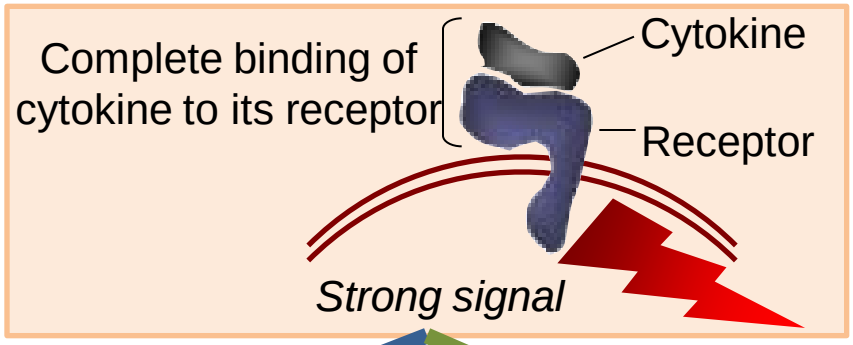
1. In-house PPI inhibitor programs
   - 1.1. Small molecule IL-6 inhibitor
   - 1.2. Small molecule VEGF inhibitor
2. Collaborative research for new drug targets with unique *in silico* molecular design strategy, INTENDD (INTerprotein's Engine for New Drug Design)
   - 2.1. Identification of small molecule binding site and *in silico* screening by SBSG (Structure-Based Scaffold Generation)
   - 2.2. Strategic evaluation of small molecule PPI inhibitors, especially cytokine-receptor interaction regulators

# *Two concepts for cytokine-cytokine receptor interaction inhibitors*

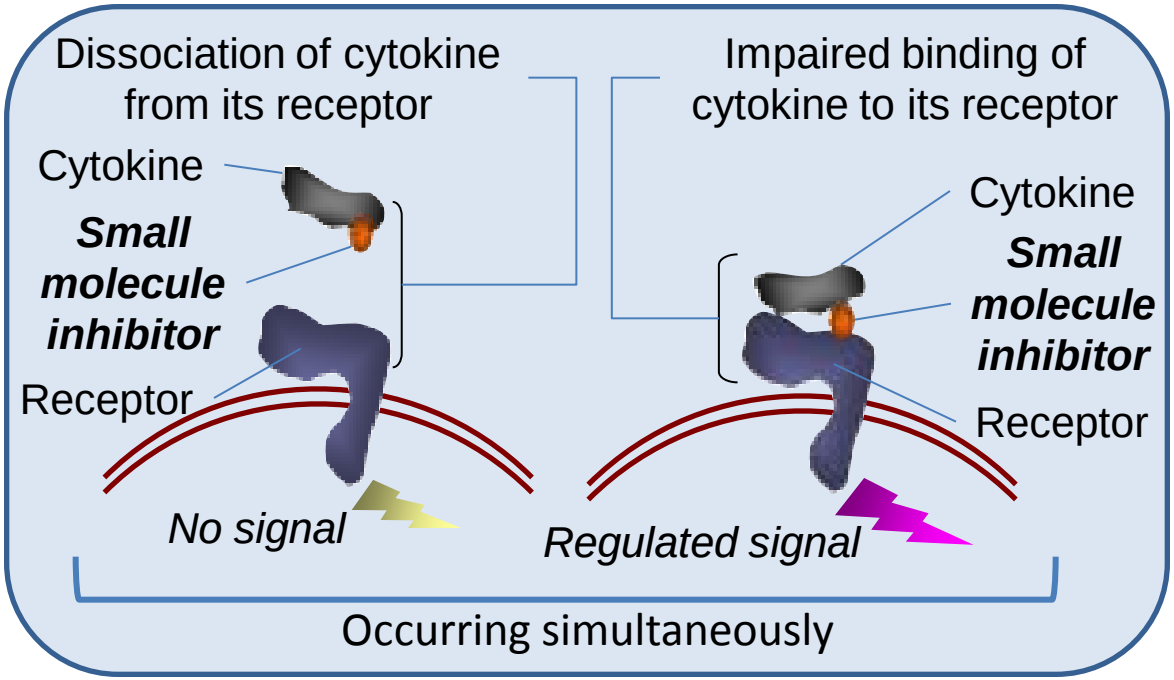


# Image of inhibition of cytokine-cytokine receptor interaction by small molecule compounds

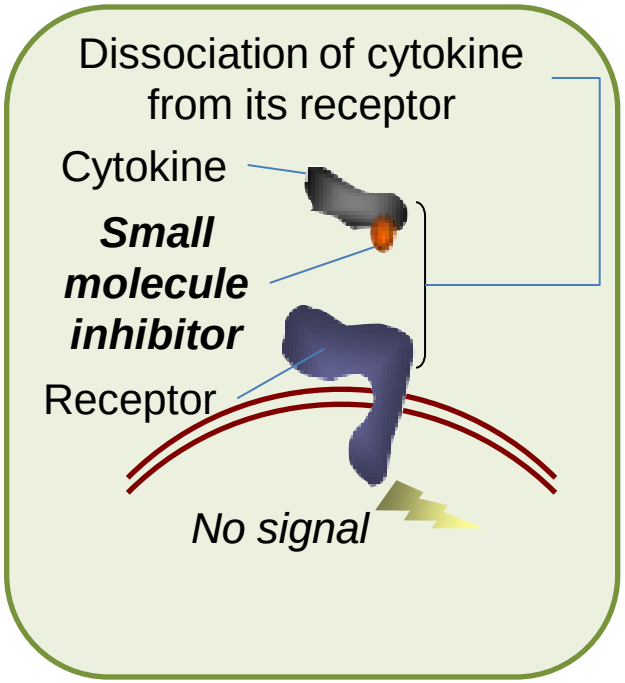
***Pathophysiological situation***




***Partial-antagonist type***



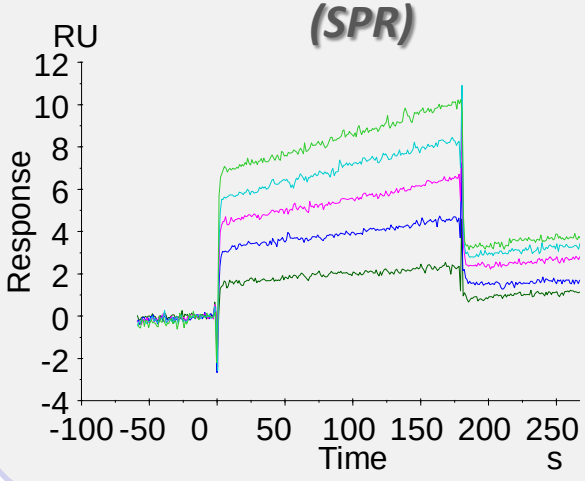

***Full-antagonist type***



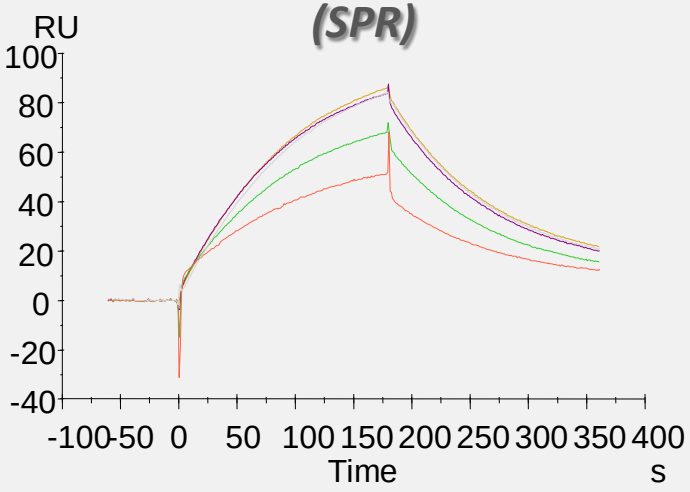
# Two types of small molecule IL-6 inhibitors

## Partial-antagonist type compound

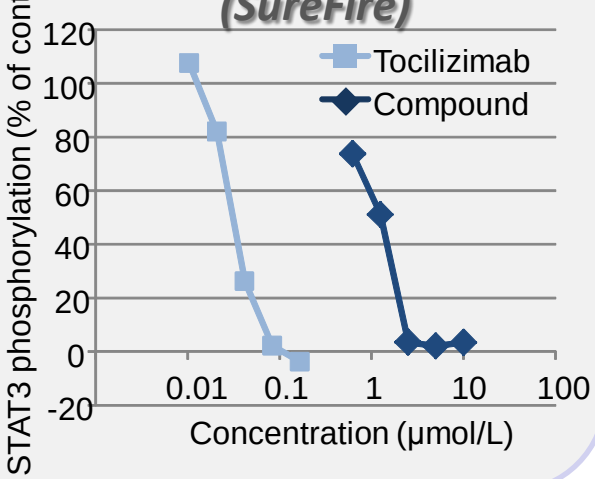
### Binding to IL-6 (SPR)




### Inhibition of IL-6 binding to IL-6R (SPR)

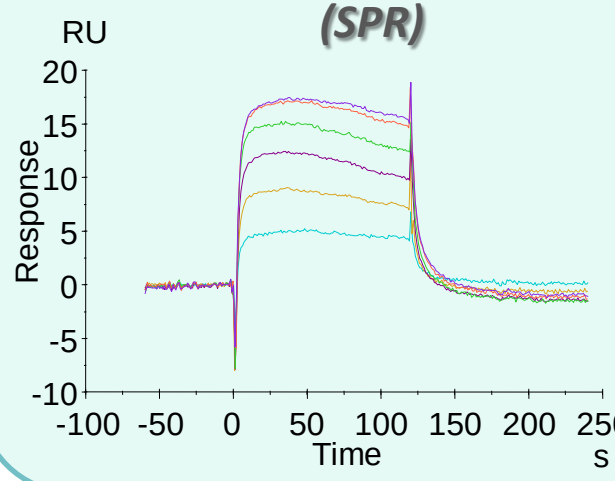



### Inhibition of p-STAT3 (SureFire)

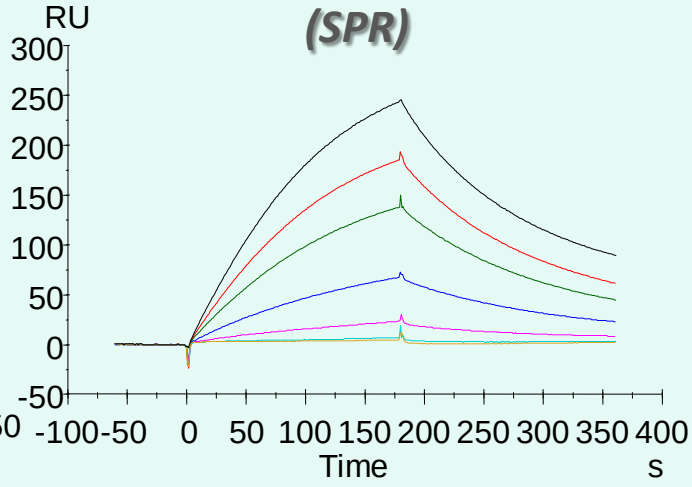



## Full-antagonist type compound

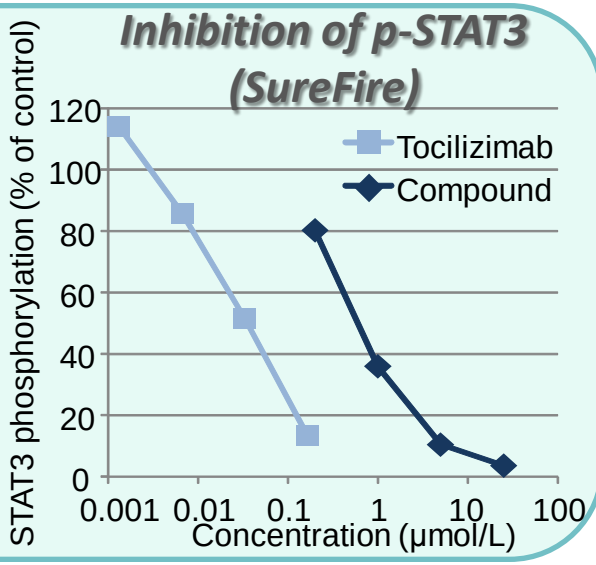
### Binding to IL-6 (SPR)




### Inhibition of IL-6 binding to IL-6R (SPR)




### Inhibition of p-STAT3 (SureFire)



# Small molecule IL-6 inhibitor (IL-6/IL-6R interaction inhibitor)

## <Concept>

- Replacement/dose reduction of tocilizumab (Actemra), and further expansion of anti-IL-6 therapies for tocilizumab-approved diseases and other autoimmune/ inflammatory diseases

## <Present status>

- Under synthesis and evaluation of compounds for optimization.
- Compound X shows good *in vivo* PK profile in mice (30 mg/kg, p.o.; F value, 26%).

| Parameters tested | Criteria | **Compound X** | Positive compounds |
|---|---|---|---|
| clogP | 2.0<clogP<6.0 | **Clear** | - |
| tPSA ($A^2$) | <140 | **Clear** | - |
| pSTAT3 ($IC_{50}$, μmol/L) | <50 | **Clear (<1)** | 39/136 (28.7%) |
| $IC_{50}$ ratio vs. tocilizumab | <1/1000 | **Clear (1/50-1/200)** | - |
| Binding to IL-6 (SRP, RU) | >5 | **Clear** | 70/88 (79.5%) |
| Inhibition of IL-6/IL-6R binding (SPR, % inhi.) | >5 | **Clear** | 18/122 (14.8%) |
| Interaction with IL-6 ($^{15}N$-NMR) | Chemical shift change at proposed binding site | **Clear (change at 7/10 residues)** | 34/54 (62.9%) |

# Small molecule VEGF inhibitor (VEGF/VEGFR interaction inhibitor)

## <Concept>

- Replacement and/or dose reduction of bevacizumab (Avastin), and further penetration of anti-VEGF therapies (acquisition of indications for which bevacizumab has been not approved; diffusion of combination therapy with small molecule VEGF inhibitor and current standard chemotherapies → ***for distinction from tyrosine kinase inhibitors, TKIs)***

## <Present status>

- Under synthesis and evaluation of compounds aiming for lead optimization.
- Compound Y shows great *in vivo* efficacy in mouse xenograft model (40 mg/kg, p.o.).

| Parameters tested | Criteria | **Compound Y** |
|---|---|---|
| VEGF-stimulated HUVEC growth | $IC_{50}$ < 100 nM | **Clear** |
| Selectivity | 10-fold $IC_{50}$ or more for:<br>EGM2-stimulated HUVEC growth<br>LS174T/fibroblast growth | **Clear** |
| In vivo efficacy | Comparable to bevacizumab | **Clear** |
| Binding to VEGF (SRP) | > 5 RU | Under assessment |
| Inhibition of VEGF/VEGFR binding (SPR) | > 5% | Under assessment |
| Interaction with VEGF ($^{15}$N-NMR) | Chemical shift change at proposed binding site | Under assessment |

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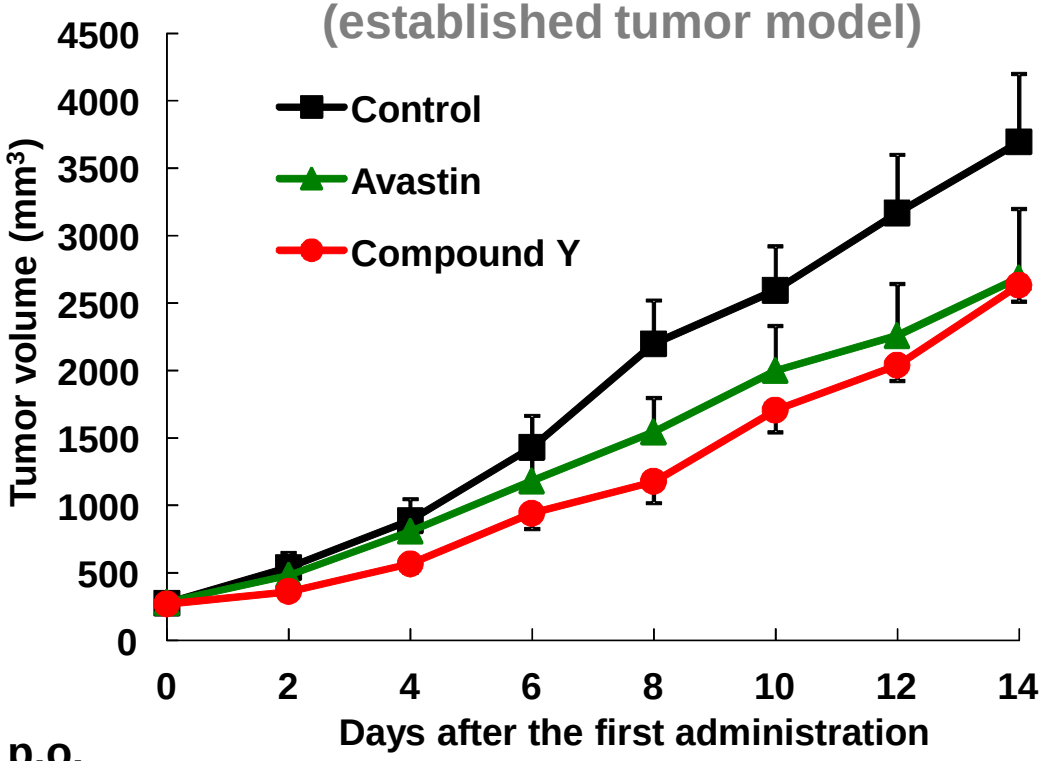
# Pharmacological profile of Small molecule VEGF inhibitor (Preceding compound, Compound Y)

## Summary of *in vitro* evaluation

| Test | IC50 (nM) |
|---|---|
| VEGF-HUVEC | < 15.6 |
| EGM2-HUVEC | 304 |
| LS174T | > 1000 |
| Fibroblast | > 1000 |

## LS174T-inoculated xenograft model in nude mice (established tumor model)




**Experimental group:**

1. **Control (PBS, days 0 - 8, i.p.)**
2. **Avastin 5 mg/kg day 0, 3, 7, i.p.**
3. **Compound Y 40 mg/kg days 0 - 8, p.o.**

**Data: mean ± S.E.M. (n = 5)**

**LS174T inoculation: s.c.**

**Tumor volume at the start of administration: 276 mm³**

# Unique SBDD strategy, INTENDD

***Essential Principle for Drug Design:*** **Shape" and "Color" have to be matched**
***("Shape" means 3D surface structure, and "color" means H-bond and so on)***

## *Components of INTENDD (INTerprotein's Engine for New Drug Design)*

1) Identification of target cavity *[1 month]* → ***Identification of Target Cavity with Precise 3D Model***

2) *In silico* screening by Structure-Based Scaffold Generation (SBSG) method (proposal of around 200 compounds) *[3 months]*


(3D models can be produced easily in 2 hours)

## *Value & Advantage of INTENDD*

A) Discover real hits compounds in high-ranked 200 comp.
B) Create new & druggable scaffolds with wide diversity at one try (also applicable to back-up compounds)
C) Acceptable structures for medicinal chemists
D) Proven MOA by SPR, NMR, X-ray, HDx in many projects
E) Not restricted by types of targets (PPI, enzyme, receptor and so on)
F) Applicable not only to hit identification but also lead generation and optimization

# Procedure of SBSG method

## SBSG: Structure-Based Scaffold Generation

Commercially available chemical libraries
(ca. 10 millions)

i. Search of ring positions at the cavity
ii. Formation of ligand skeletons（ca.50 structures manually）
iii. Search of skeletons in available compound data bases
iv. Collision check with protein atoms
v. Selection of promising compounds by clustering and filtering by binding structure-based mechanisms

Proposal of compounds for wet screening
(ca. 2 hundreds) → Wet Screening
*<not require large cost and HTS >*

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# Comparison between SBSG and current methods

| Item | SBSG | Docking |
|---|---|---|
| Coverage of search space | Complete with novel skeletons | Limited to data base cmps. |
| Examined compounds | Ca. $10^6$ data base cmps. | Ca. $10^6$ data base cmps. |
| Major driving force in search | Binding structure-based mechanism | Calculated binding energy-based |
| Scoring of cmps. | Filtering on assumed mechanism | Calculated binding energy |
| Time frame for design | 1 month x 3 iterations | 1 – 3 months |
| Required number of cmps. to obtain hit cmps. in wet screening systems | 200 – 300<br>◆ Low purchase cost of cmps.<br>◆ Easily assayed by manual screening system (no need for HTS) | A few thousands or more |
| Number of hits & diversity | 10 - 30 with broad diversity | 0 – 5 with poor diversity |

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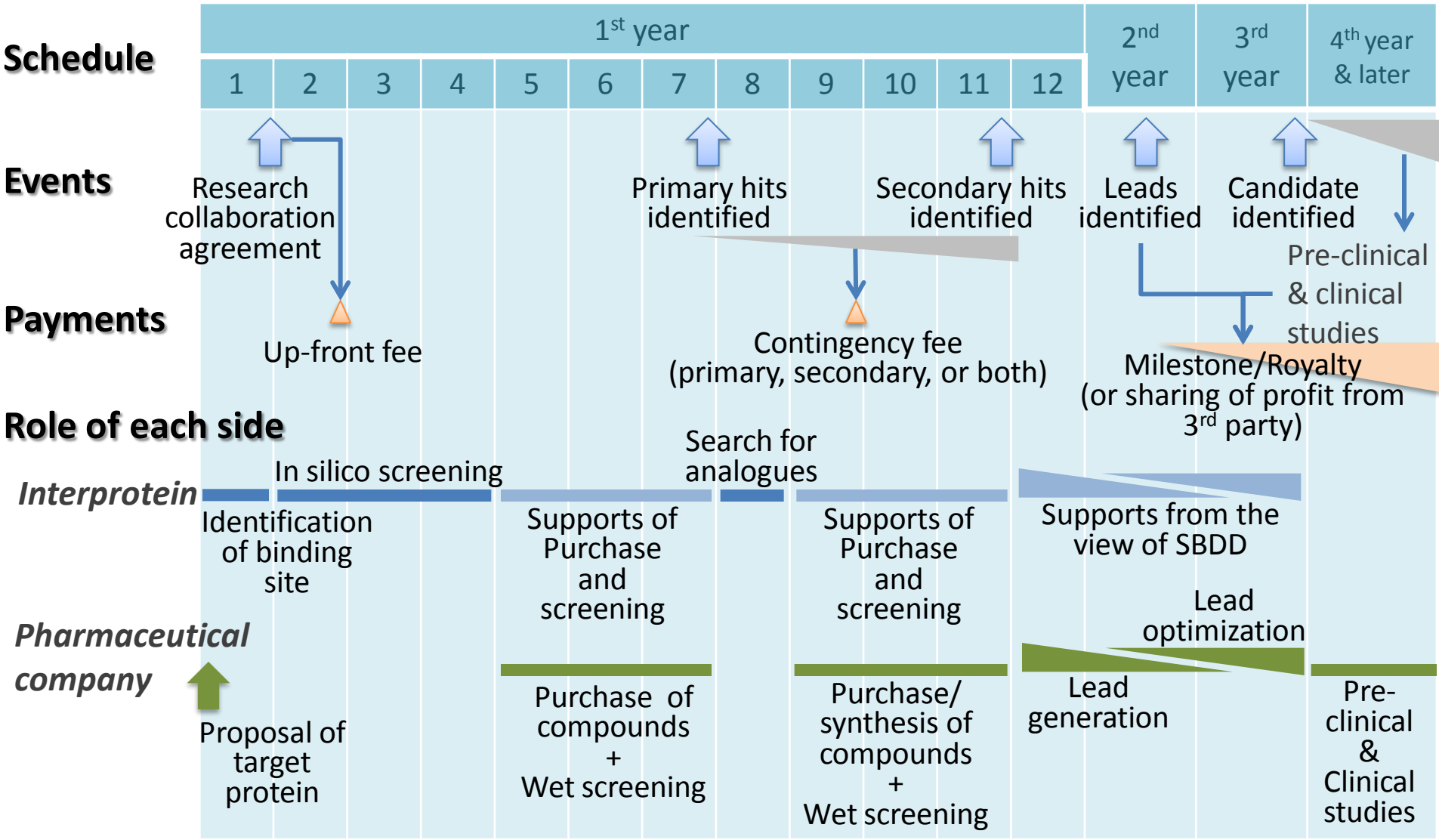
# Example of collaborative research

## - the case where information on X-ray crystal structure of target protein is existing -



# *Functions and expertise of Interprotein*

## 1. Proposal of hit candidates by INTENDD

- Real 3D model-based identification of binding site
- *In silico* screening by SBSG method

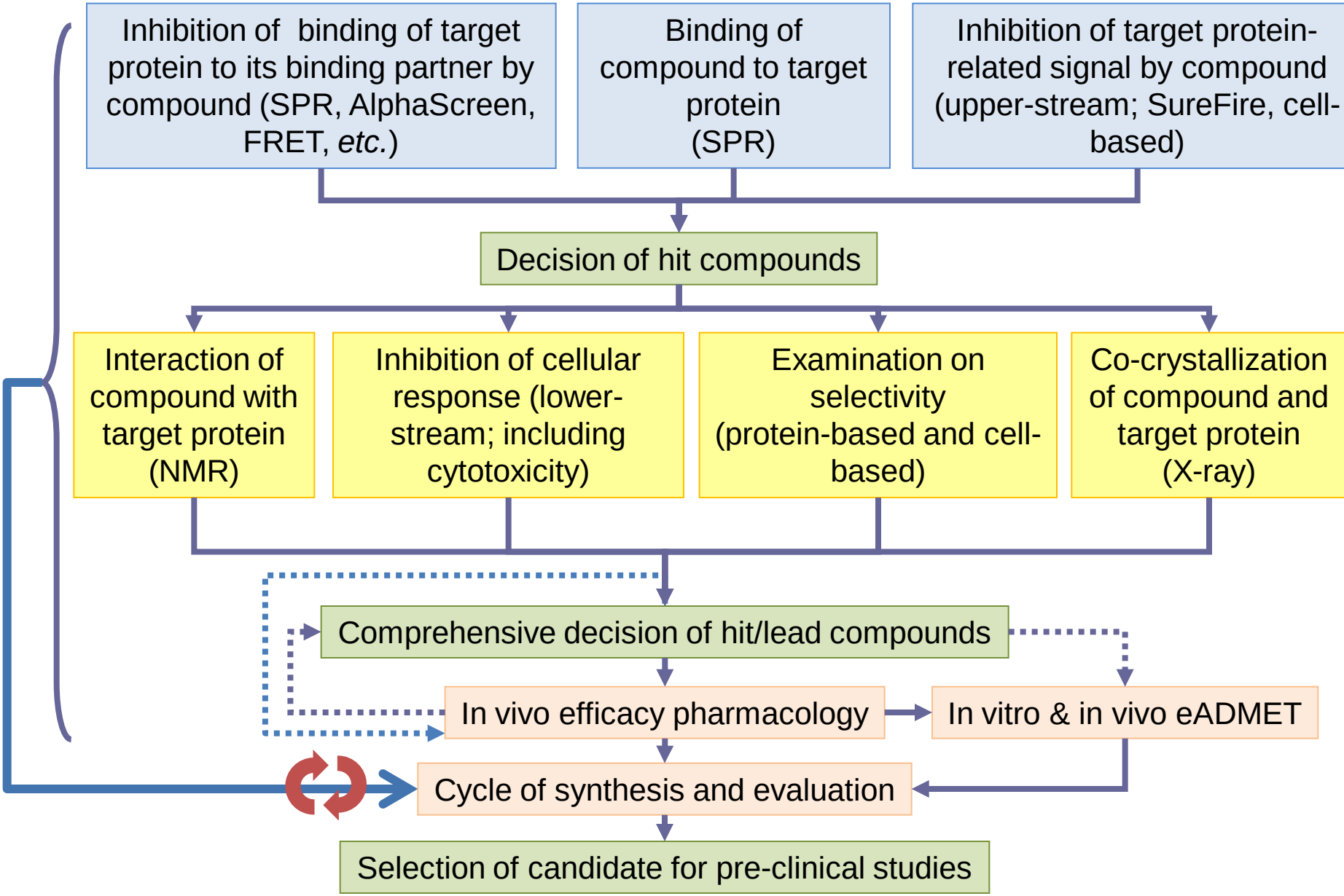
## 2. Synthesis of compounds

- Know-how of lead generation/optimization of small molecule PPI inhibitors

## 3. Evaluation of compounds

- Know-how of strategic assessment of small molecule PPI inhibitors
- Close collaboration with experts of protein/drug discovery research (NB Health Laboratory, Kyoto Sangyo Univ., Osaka Univ., MARUWA Foods & Biosciences, Japan Aerospace Exploration Agency (JAXA), RIKEN, etc.)

# Strategic evaluation of small molecule PPI inhibitors



# Outlines of R&D activities of Interprotein

## 1. In-house programs *(-> searching for license/collaboration partners)*

| Program | Stage | Mechanism of action |
|---|---|---|
| **VEGF inhibitor** | **Lead optimization** | **Inhibition of VEGF/VEGFR2 interaction; not TKI** |
| **IL-6 inhibitor** | **Lead optimization** | **Inhibition of IL-6/IL-6R interaction** |
| Tubulin inhibitor | Lead optimization | Inhibition of tubulin polymerization |
| Notch 1 inhibitor | Lead optimization | Inhibition of NICD/RBP-Jk/MAM interaction |
| IgE inhibitor | Lead generation | Inhibition of IgE/FcεRI interaction |

## 2. Drug discovery research for new targets

*(-> searching for collaborative research partners)*

| Target | Main role | |
|---|---|---|
| | Interprotein | Pharmaceutical Company |
| Proposed by the Partners<br>PPIs/non-PPIs<br>inhibitors/agonists | *In silico* screening by INTENDD<br>(support of wet screening ) | Wet screening by protein/cell-based assay systems |

***Ajinomoto Pharmaceuticals Co. Ltd.***
***Takeda Pharmaceutical Co. Ltd., etc.***

Ex. of collaboration

# *The result of collaborative research with Takeda Pharmaceutical Company Limited*
## *- News by BioCentury Publications Inc. -*

Published on Monday, December 3, 2012

***Interprotein Corp.***, Osaka, Japan
***Takeda Pharmaceutical Co. Ltd.*** (Tokyo:4502), Osaka, Japan

Interprotein said partner Takeda will continue to evaluate small molecule inhibitors of protein-protein interactions under a 2011 deal after **about 11% of the compounds tested were found to bind to the target protein**. Interprotein said it is "generally recognized" that hit rates against protein targets through computational drug design are "well below 11%." The companies partnered last December to develop the compounds using Interprotein's Engine for New Drug Design (INTENDD) technology. The companies could not be reached for comment (see BioCentury, Jan. 9).

# Partnering contact

## We are searching alliance partners for:

1. Small molecule VEGF inhibitor
2. Small molecule IL-6 inhibitor
3. Small molecule Notch1 inhibitor
4. Small molecule IgE inhibitor
5. Tubulin polymerization inhibitor

→ ***License and/or research collaboration***

6. Inhibitors/agonists for new drug targets (PPI/non-PPI)

→ ***Collaborative research***

***Contribution to raising the productivity of drug discovery research for all types of drug targets with 3D structure information***

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