Fragment screening of IL-23 by weak affinity chromatography (WAC™)

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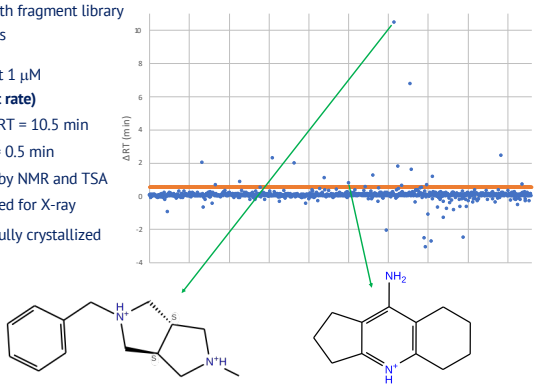
Targeting IL-23

- Interleukin 23 (IL-23) is an inflammatory heterodimeric cytokine composed of an IL-12B (p40) and an IL-23A (p19) subunit
- IL-23 signals through a receptor complex formed by IL-12Rβ1 and IL-23R
- Key cytokine for T helper type 17 cell (Th17 cell) maintenance and expansion
- Aberrant Th17 activity associated with multiple autoimmune conditions
- Clinically, antagonist antibodies targeting IL-23 have been approved (Ustekinumab) for the treatment of autoimmune diseases
- An orally administered small-molecule inhibitor of the IL-23 pathway has the potential to provide significant benefits for patients suffering from autoimmune inflammatory disorders
- Identifying small molecule inhibitors of the IL-23 pathway has proven to be a challenging process
- Fragment screening by WAC is well suited to generate hits for PPIs

WAC™ screening

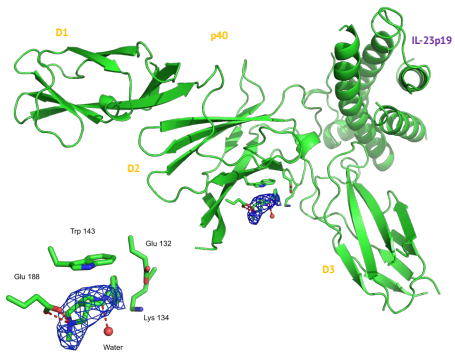
- WAC screen with fragment library
  - 1013 cmpds
  - Diverse set
  - Screened at 1 µM
- **19 hits (2% hit rate)**
- Top WAC hit ΔRT = 10.5 min
- Hit threshold = 0.5 min
- Hits validated by NMR and TSA
- 6 hits nominated for X-ray
- 2 hits successfully crystallized

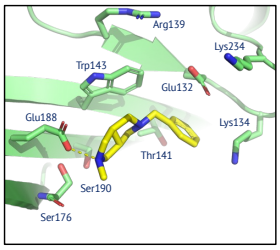


ΔRT 10.5 min
RG200734

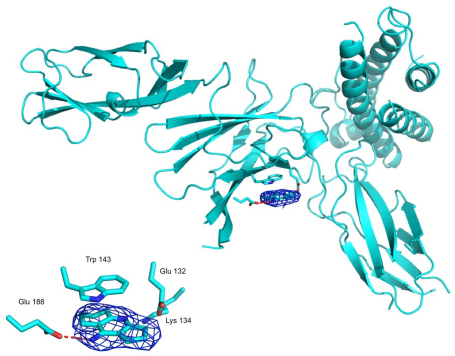
ΔRT 0.8 min
RG200616

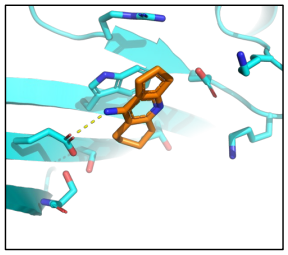
Hit-validation by X-ray crystallography





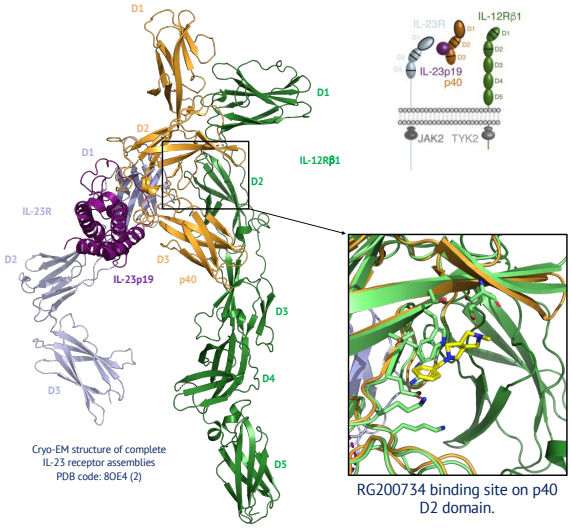
RG200734
WAC ΔRT: 10.5 min
Mw: 216 Da
Resolution: 2.70 Å





RG200616
WAC ΔRT: 0.8 min
Mw: 188 Da
Resolution: 2.95 Å

Binding site validation



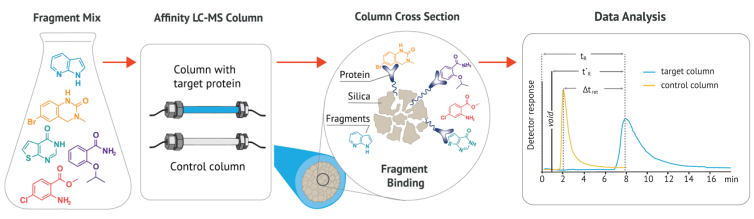
Cryo-EM structure of complete IL-23 receptor assemblies PDB code: 8OE4 (2)

RG200734 binding site on p40 D2 domain.

Conclusions

- WAC screening successfully generated fragment hits towards this challenging PPI
- Two fragment hits successfully crystallized
- Unfortunately, compounds do not block receptor interaction

Principles of WAC™



Key WAC™ features

- Affinity chromatography with immobilized target
- MS-detection enables screening at low µM, built-in QC
- Affinity range low µM to mM, direct detection with immediate K_D ranking
- High throughput (>5000 cmpds/week; cocktails of 25-100)
- Used along with TSA, NMR, X-ray for integrated hit finding, validation and progression workflow

References

1. Lupardus et. al., "*The structure of interleukin-23 reveals the molecular basis of p40 subunit sharing with interleukin-12*", J Mol Biol, 382 (2008):931-941.
2. Bloch et. al., "*Structures of complete extracellular receptor assemblies mediated by IL-12 and IL-23*", Nat Struct Mol Biol, January 29 (2024).

Contact

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