A Quest for Potent Allosteric Inhibitors of AAA ATPase p97

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AAA ATPase

- **A**TPases **A**ssociated with diverse cellular **A**ctivities
  - Characterized by 1 or 2 conserved ATP-binding domains (AAA domains)

- **Structure:** Ring-shaped with pore in center, typically hexomeric complex formed by six identical promoters.

- **Roles:** Protein degradation, protein refolding, membrane fusion, DNA replication, microtubule dynamics, intracellular transport, transcriptional activation, disassembly of protein complexes and aggregates.

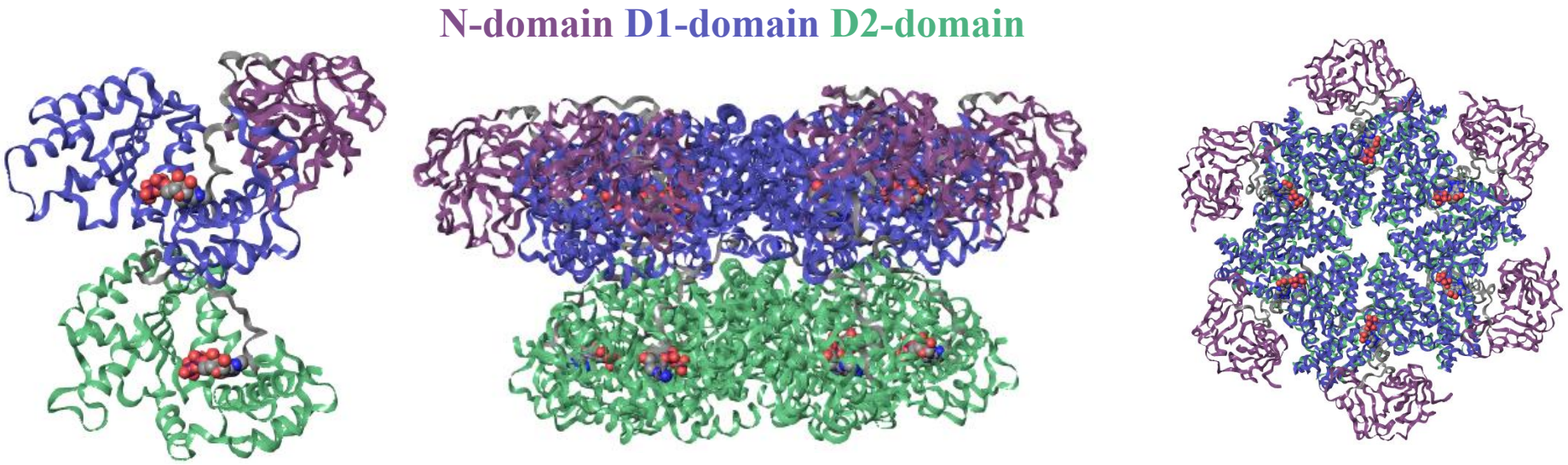
- **MOA:** Operate by ATP hydrolysis which generates a conformational change. This produces a mechanical force, allowing for substrate remodeling and other cellular functions.

Wilkinson, A. et al. *Genes to Cells,* ***2001, 6, 575***

p97

- Also know as Valosin Containing Protein (VCP) or Cdc48 (in yeast).
  - Type II AAA ATPase (contains 2 AAA domains) with a homohexamierc ring structure.

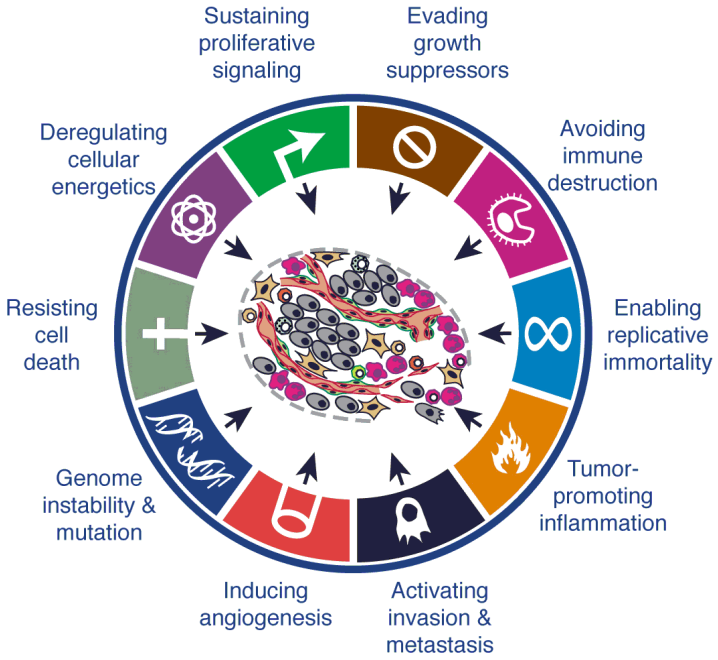
- Linked to many roles within the cell, primarily protein remodeling and quality control but it is also associated with autophagy, signaling and chromatin function.
  - Numerous cellular activities moderated by binding of cofactors and adaptors.



1) NCB, 2012, 14, 117 2) Celeste Alverez

p97 and Cancer

- P97 has been found to be upregulated in many cancers such as:
  - Colorectal
  - Lung
  - Liver
  - Prostate
  - Pancreatic
  - Breast



- Increased expression has been linked to poor prognosis.

- Currently **no** FDA approved therapeutics that target p97.

Why continue to target cancer?

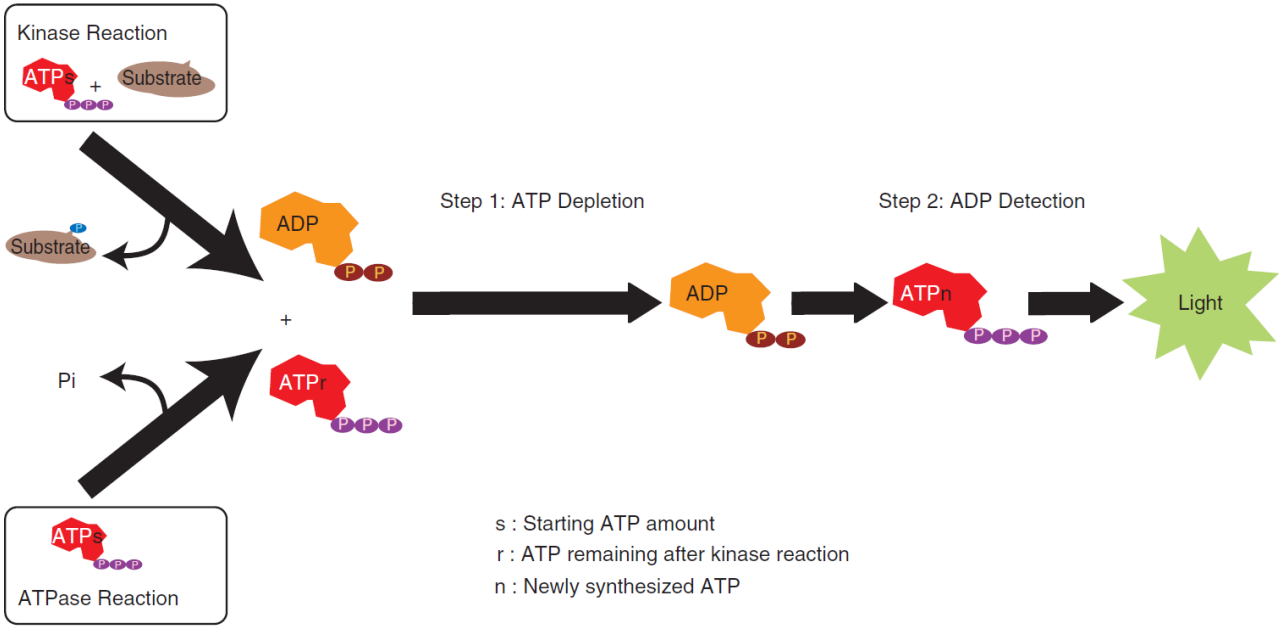
Some facts about cancer…

- 7.6 million deaths attributed to cancer (2008).
- $895 billion dollars lost due to cancer, in other words, 1.5% of the worlds GDP (2008).
- 1 in 2 men and 1 in 3 in the US will develop cancer at some point in their life (2015).
- 5 year survival rate in the US is 68% (2015).
- Currently no definitive cure exists.

In *vitro* assay: 1

ADPGlo Biochemical assay

- Luminescent kinase assay that measures ADP formed from an ATPase or kinase reaction.
- ADP is converted into ATP, which is converted into light by Luciferase.



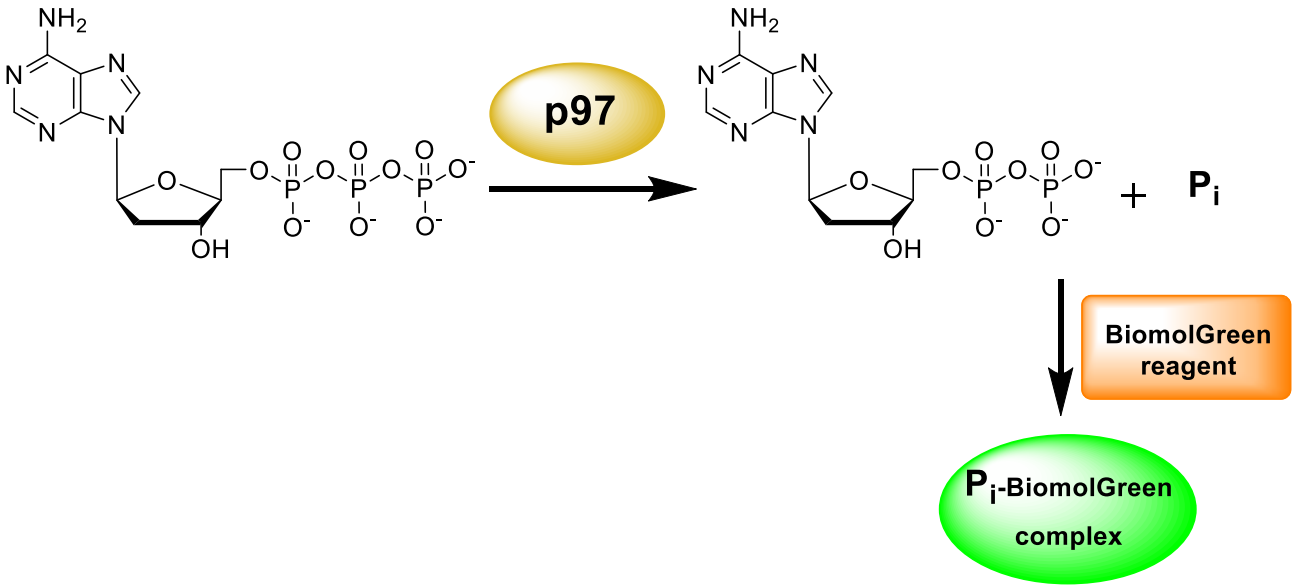
Adapted from: *ASSAY and Drug Development Technologies*. **2010**, 7: 560.

In *vitro* assay 2

BIOMOL Green ATPase Biochemical Assay

- ATPase cleaves phosphate to generate ADP and inorganic phosphate (P_i).

- BiomolGreen reagent complexes with P_i to produce light.

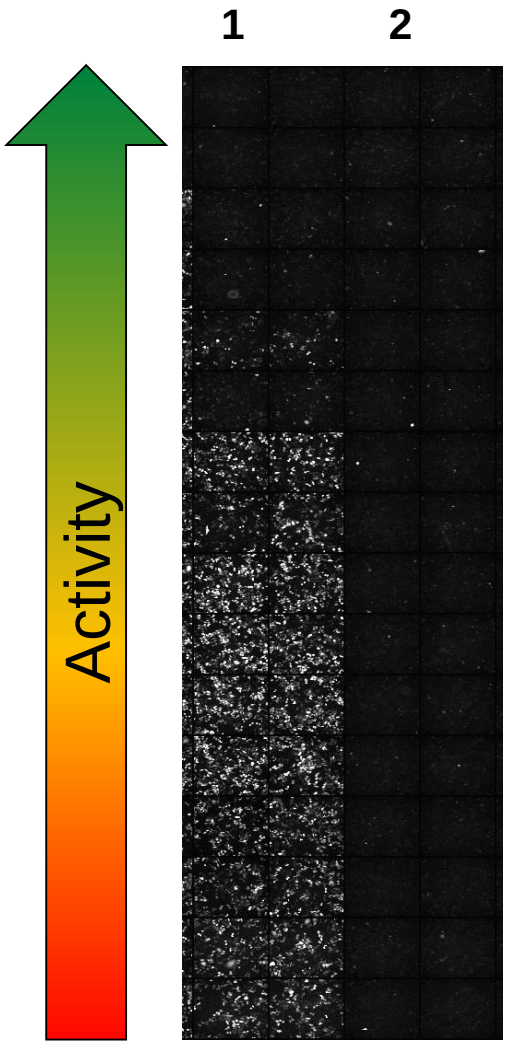


In *vivo* assay

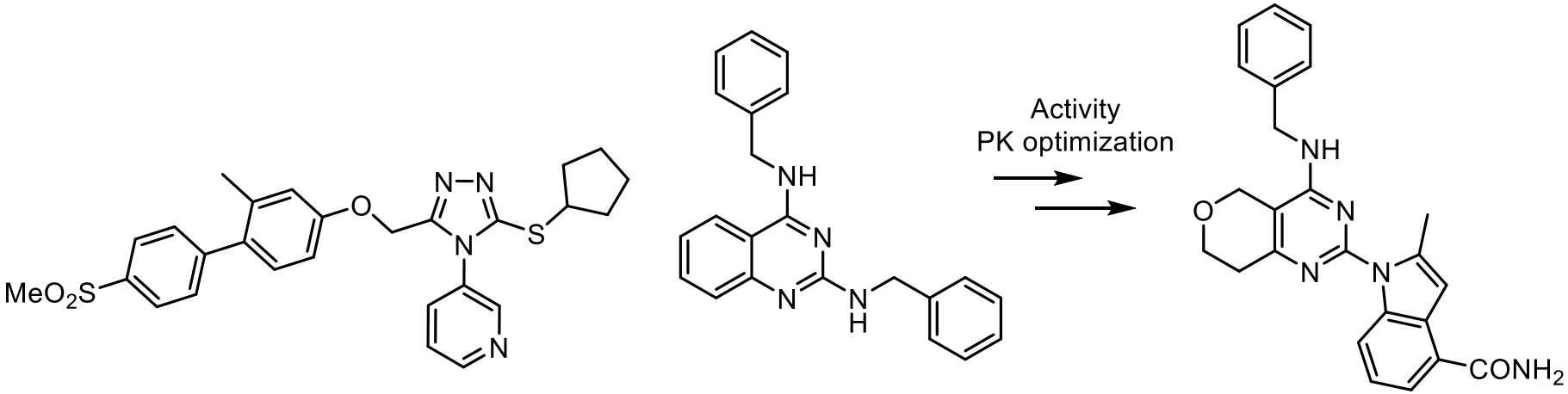
Ubiquitin accumulation assay

- If the Ubiquitin Proteasome System (UPS) is blocked then $Ub^{G76V}GFP$ accumulates within the cells. $Ub^{G76V}GFP$ is expressed in a Hela cell line.

- Assay measures the capacity of UPS based on a GFP-fusion UPS reporter.



Literature inhibitors of p97



NMS-873
IC_{50} (p97) = 26 nM
IC_{50} (HeLa) = 1.5 μM
Allosteric non competitive inhibitor
Binds D1-D2 linker
Poor solubility

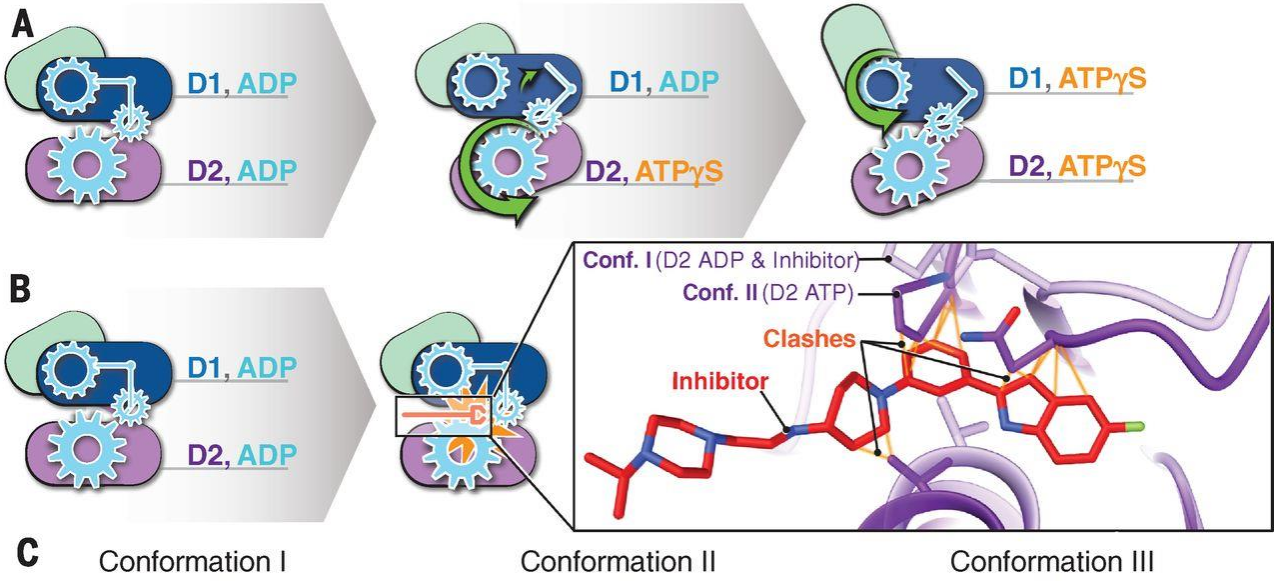
DBeQ
IC_{50} (p97) = 1.6 μM
GI_{50} (HeLa) = 3.1 μM
ATP competitive
Binds both D1 and D2
Selective

CB-5083
IC_{50} (p97) = 14 nM
EC_{50} (HeLa) = 333 nM
ATP competitive
D2 Selective
Orally bioavailable
Phase 1 clinical trials

Proc. Natt. Acad., Sci USA, ***2011,*** *108, 4834.*
Nat. Chem. Biol., ***2013****, 9, 548.*

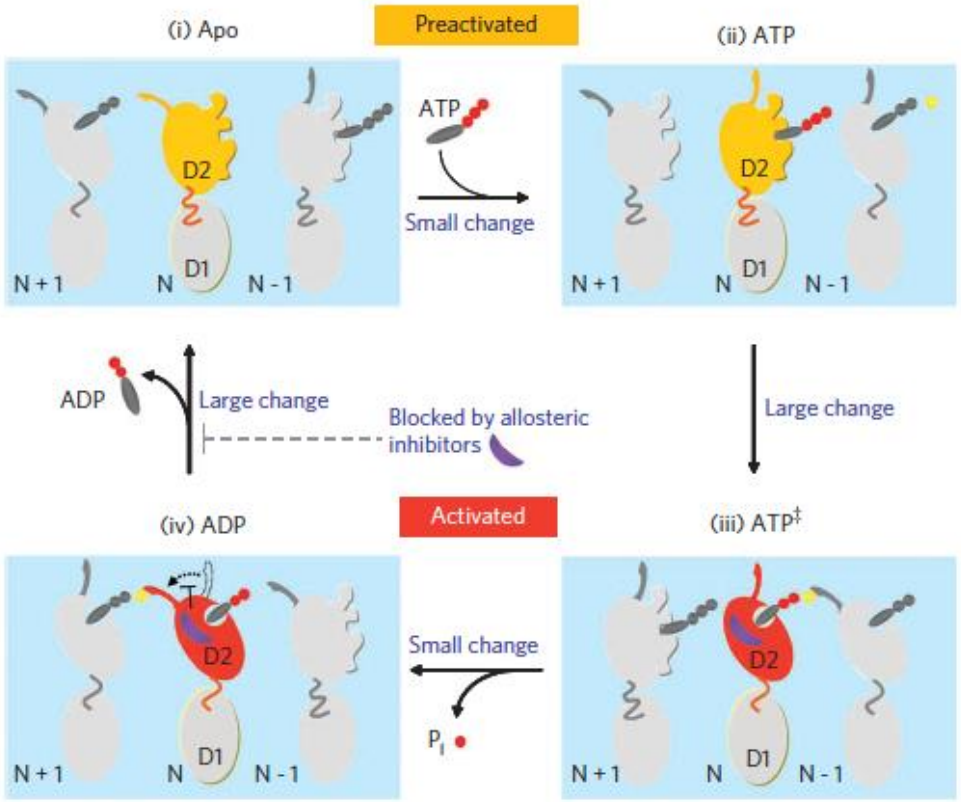
Mechanism of Inhibition

- ATP binds to D2 then D1 domain resulting in a confirmation change.
- Allosteric inhibitor binds to D2 domain resulting steric clashes upon confirmation change.
- This “freezes” the enzyme, rendering it inert.

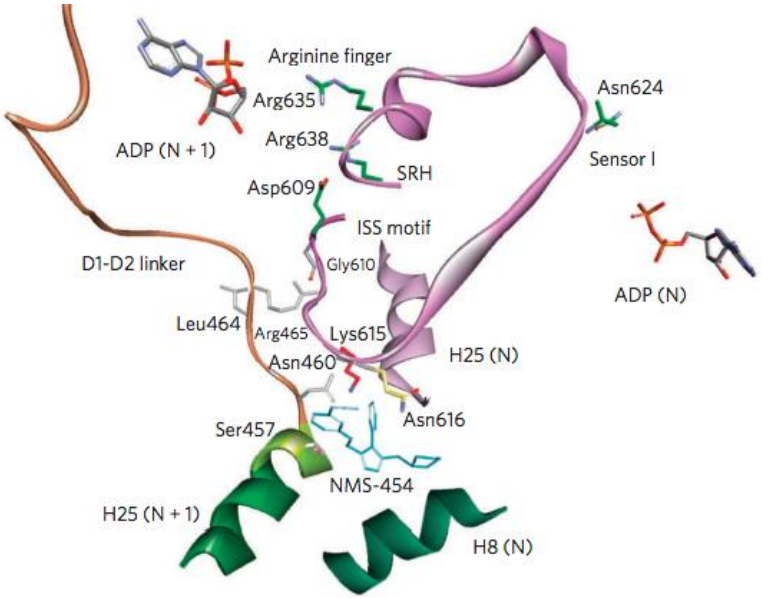


Science **2016**, 351, 871

Inhibitor Binding Site



Cross-linking results



Binding of allosteric inhibitor prevents the movement of the arginine finger toward the γ-phosphate, preventing ATP hydrolysis and freezing D2 in the ADP-bound state.

Madhaghi et al. *Nat. Chem. Biol.*, ***2013, 9, 548***.

Future objectives

- Continue to improve existing scaffold by further SAR exploration.
  - Lower molecular weight.
  - Improve solubility.
  - Improve metabolic stability.
  - Retain or improve activity.

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