

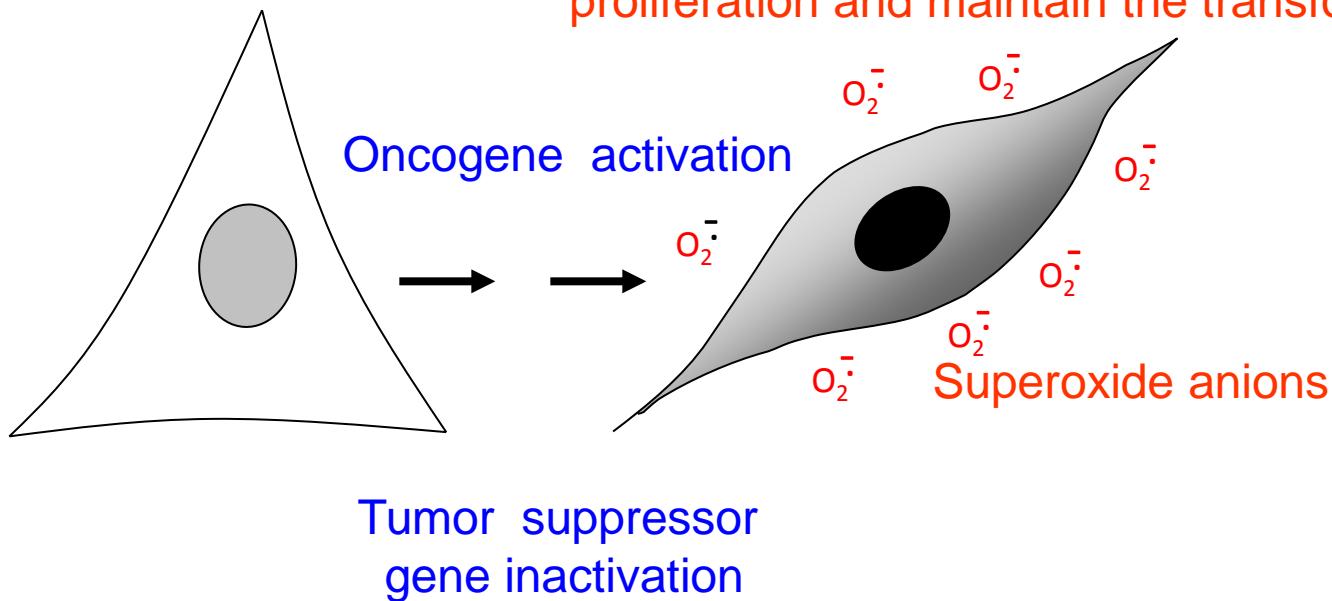
Reactive oxygen species-mediated
intercellular signaling of malignant cells:
Control of multistep oncogenesis and a
chance for novel therapeutic approaches.

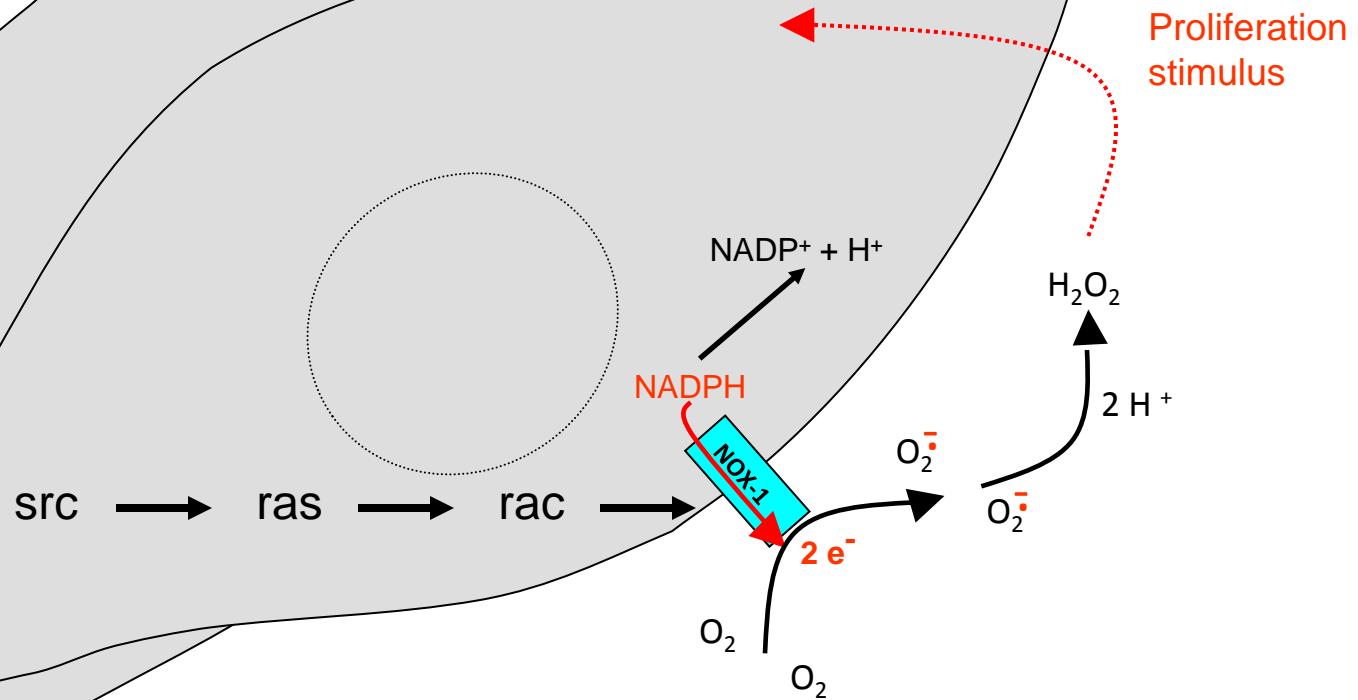
Georg Bauer
University of Freiburg, Germany

Nontransformed
cell

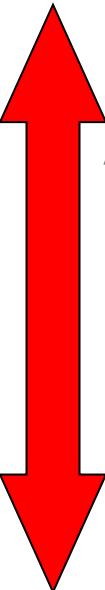
Transformed
cell

Extracellular reactive oxygen species drive proliferation and maintain the transformed state!





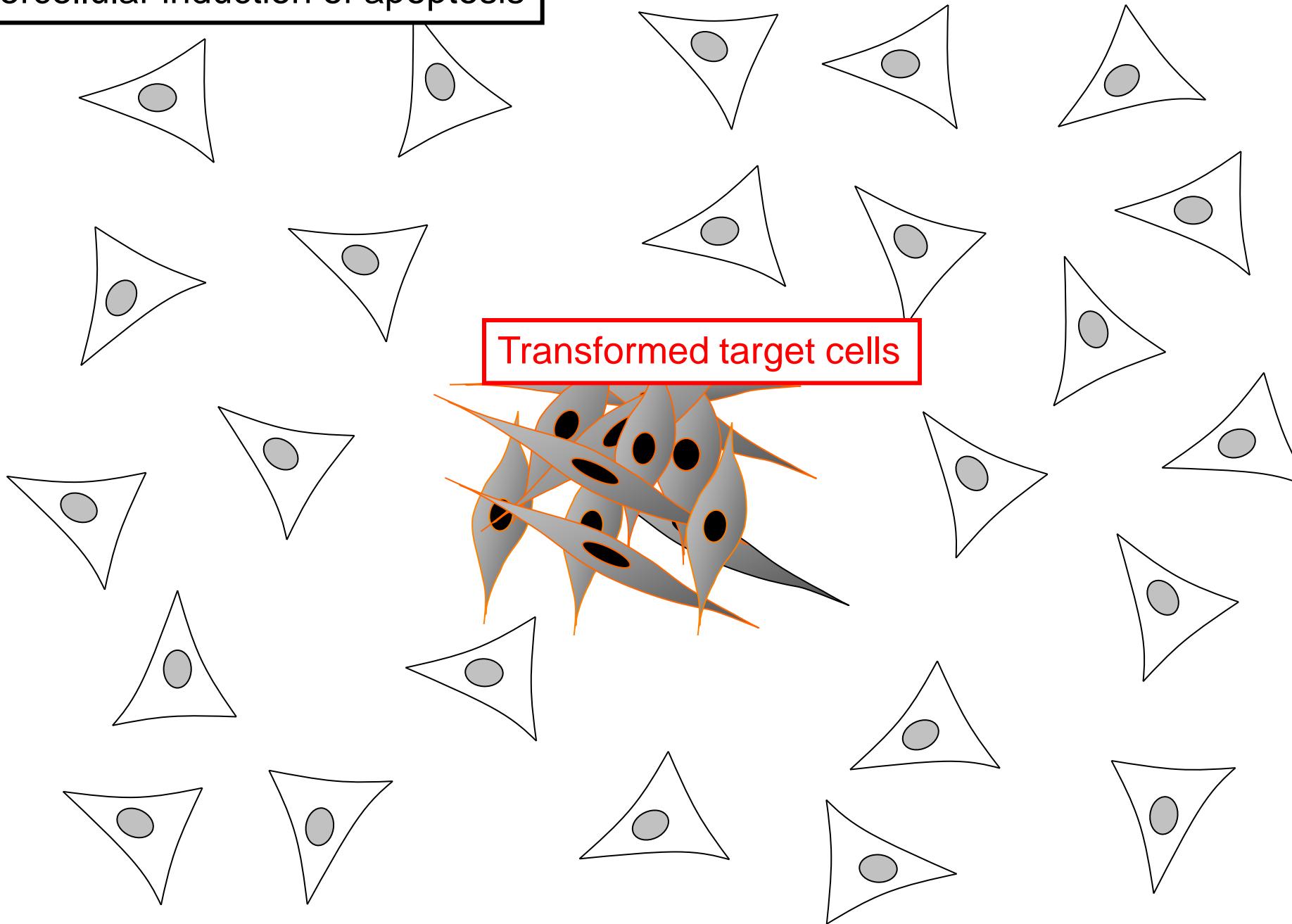
Procarcinogenic effects of ROS:

- 
- A) initiation of transformation through mutagenesis,
 - B) maintenance of the transformed state through extracellular superoxide anions

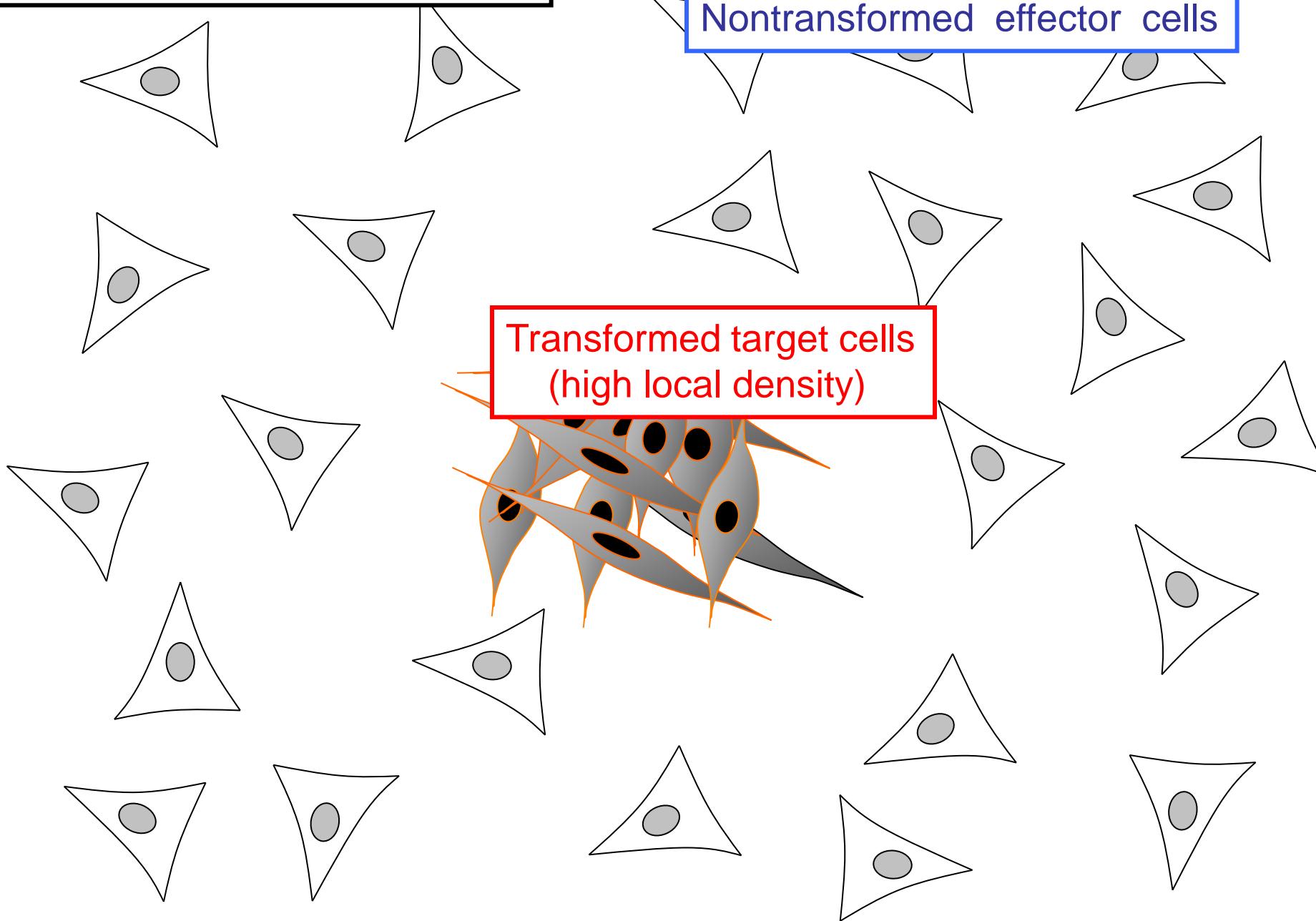
Anticarcinogenic effects of ROS:

Elimination of transformed cells through intercellular ROS signaling and apoptosis induction

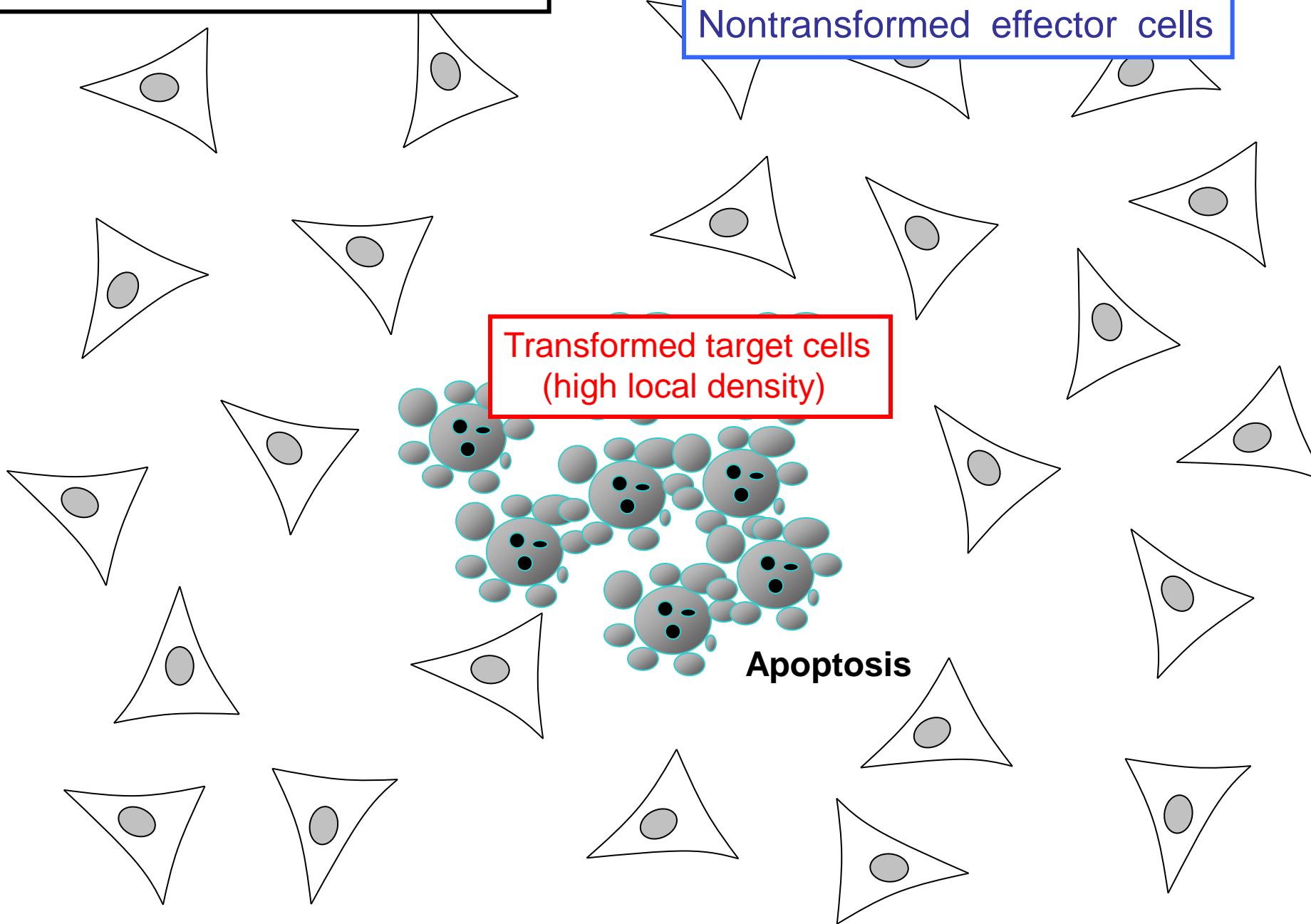
Intercellular induction of apoptosis



Intercellular induction of apoptosis

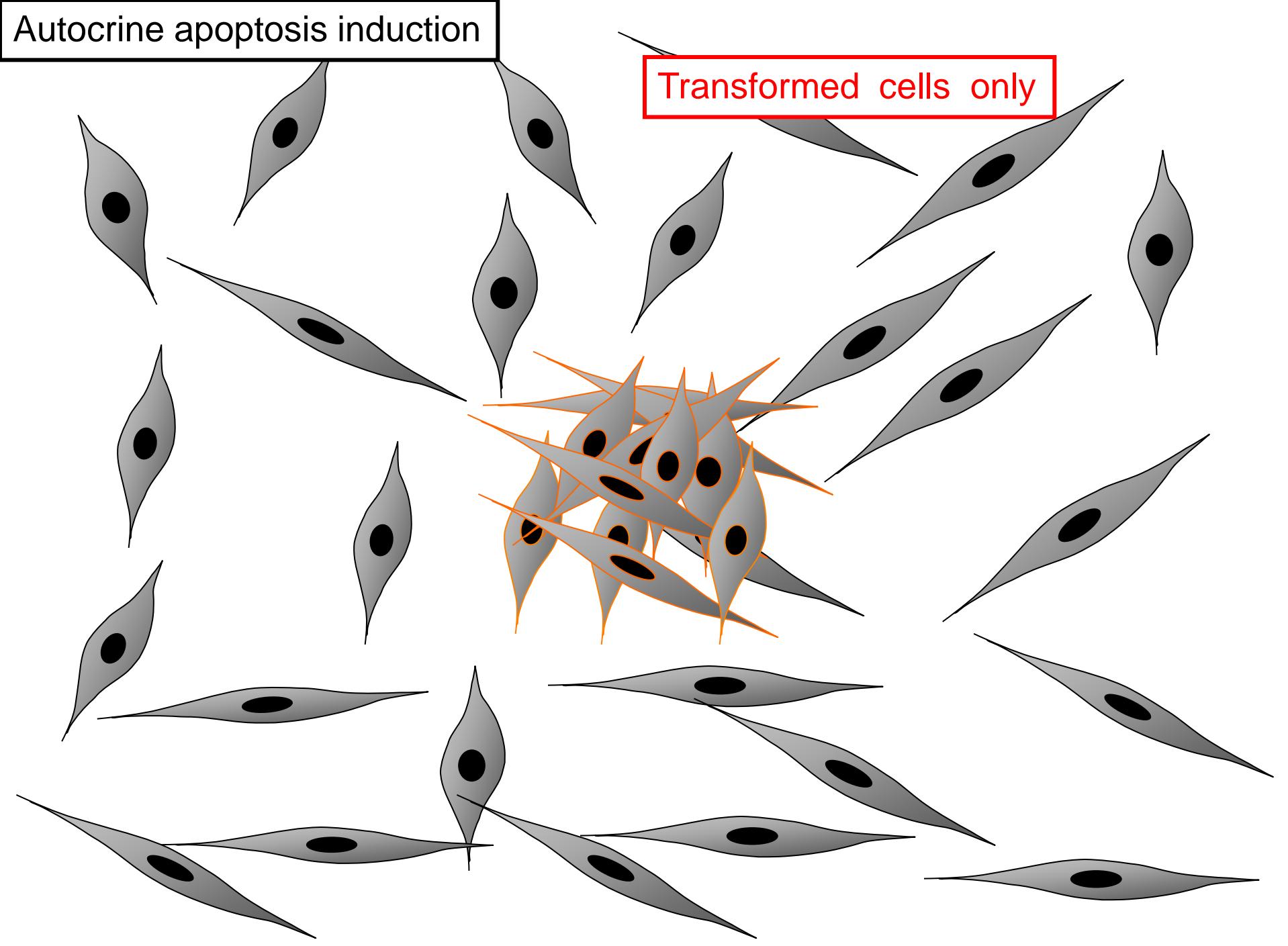


Intercellular induction of apoptosis



Autocrine apoptosis induction

Transformed cells only

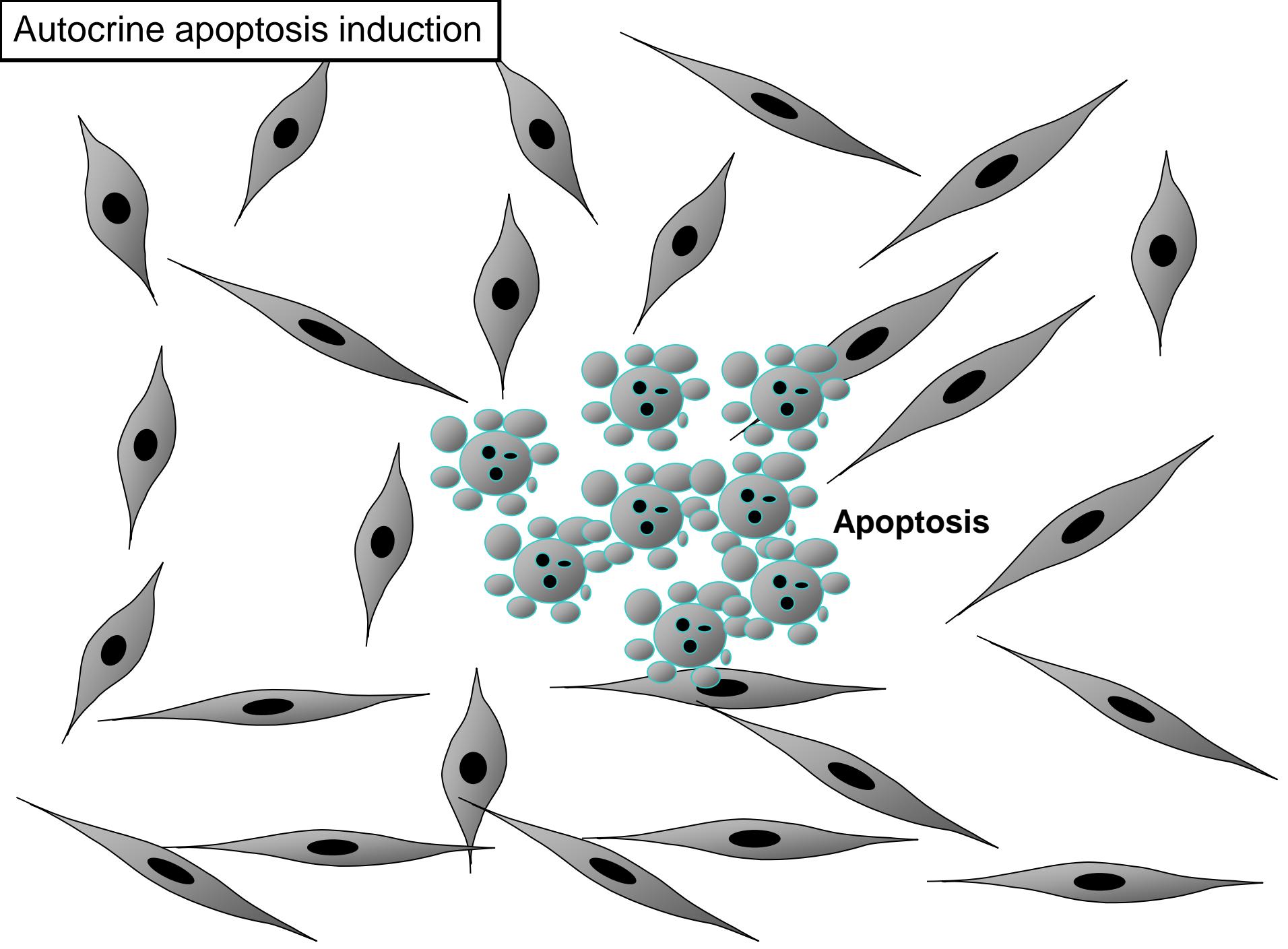


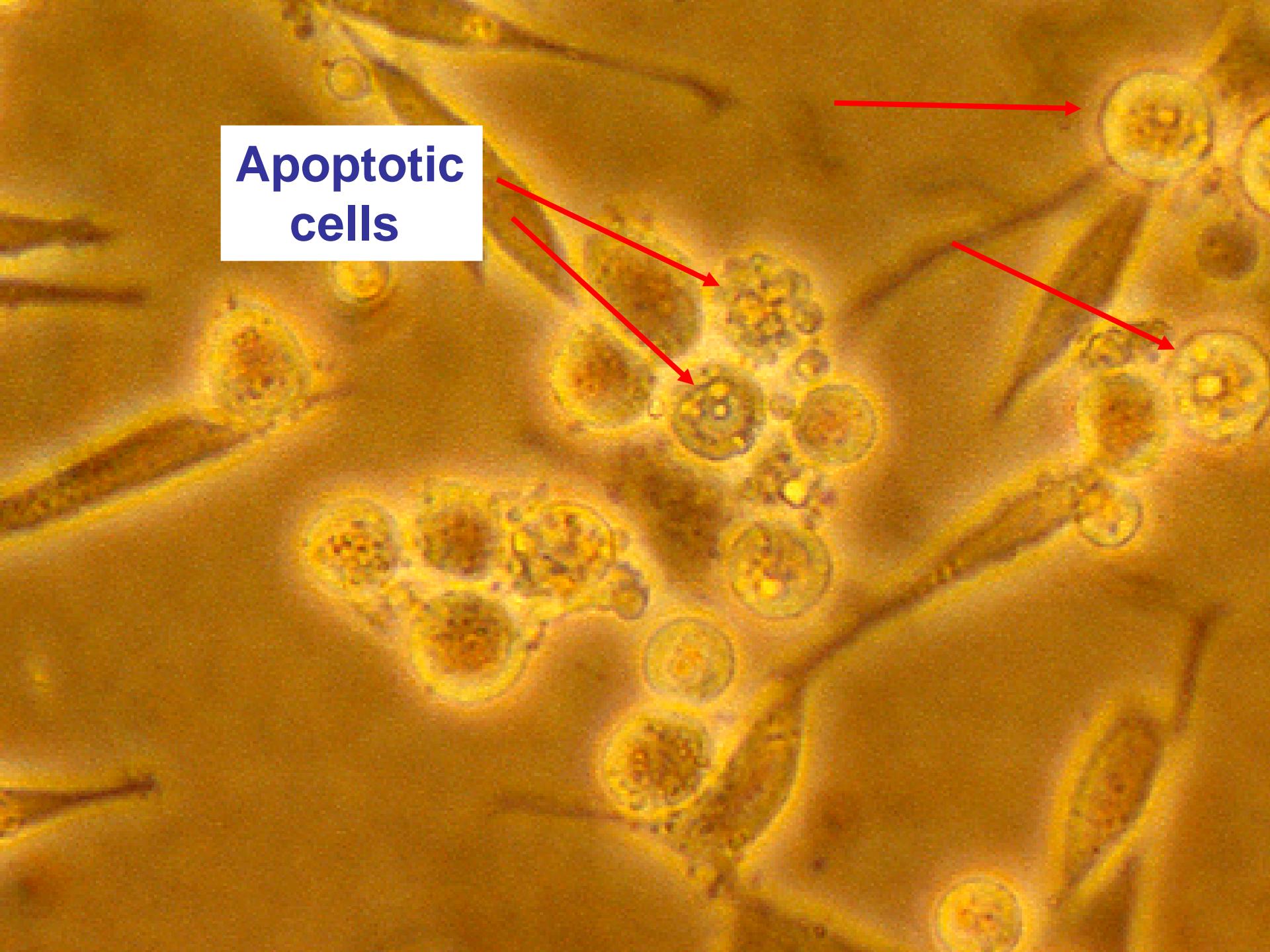
Autocrine apoptosis induction

Effector cell function

Target cell function
(high local cell density
required)

Autocrine apoptosis induction

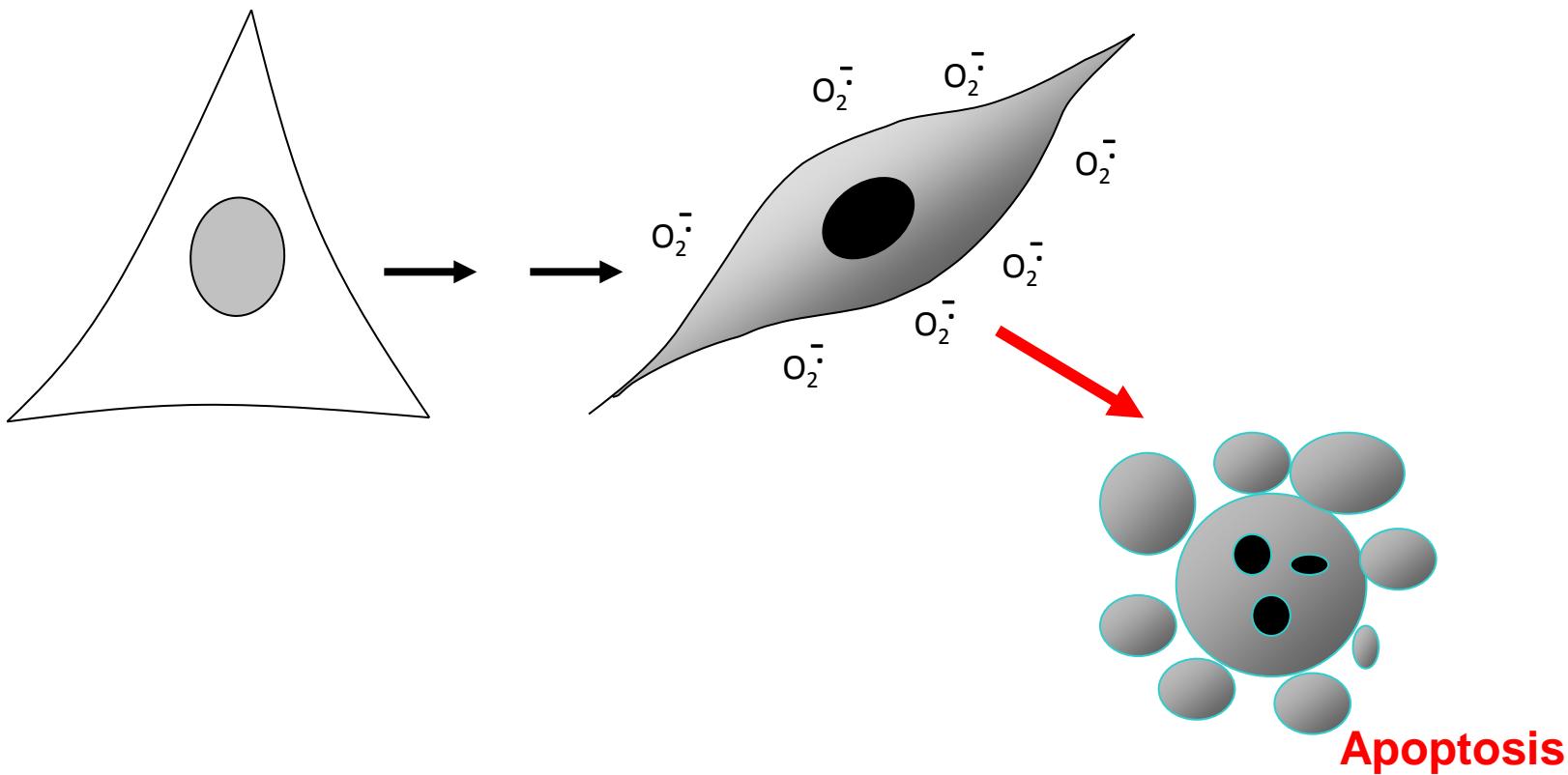




**Apoptotic
cells**

**Nontransformed
cell**

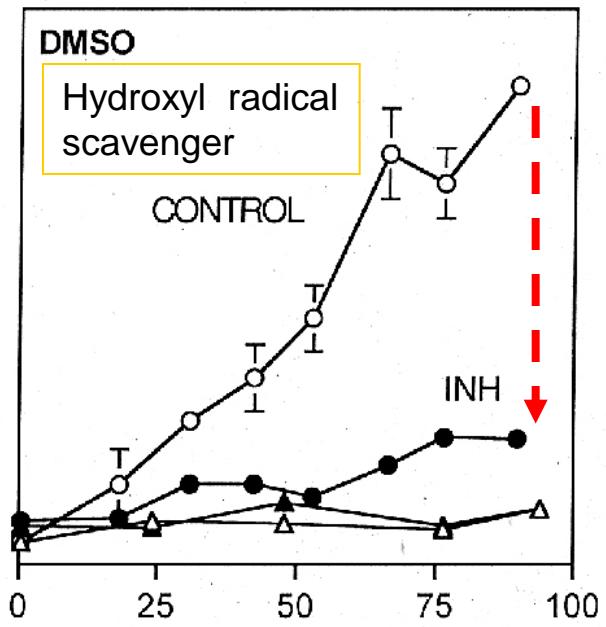
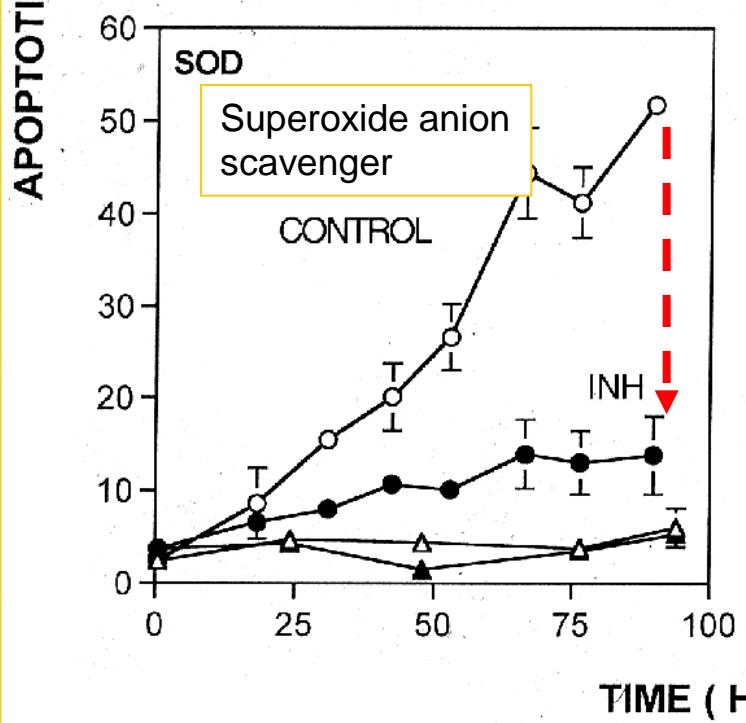
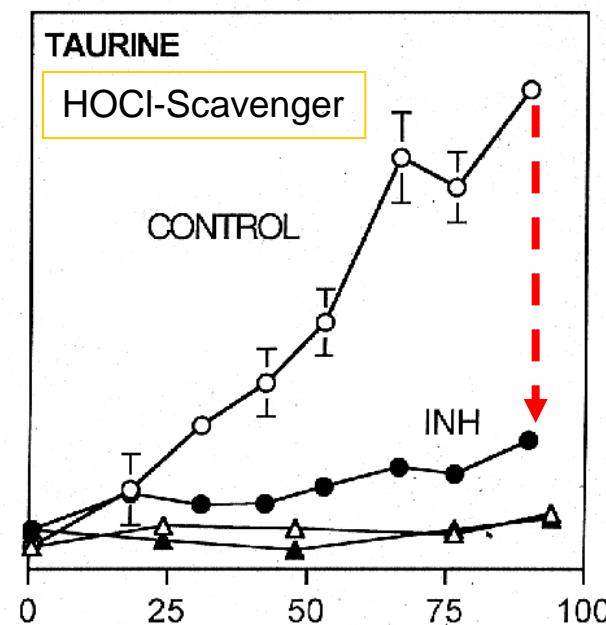
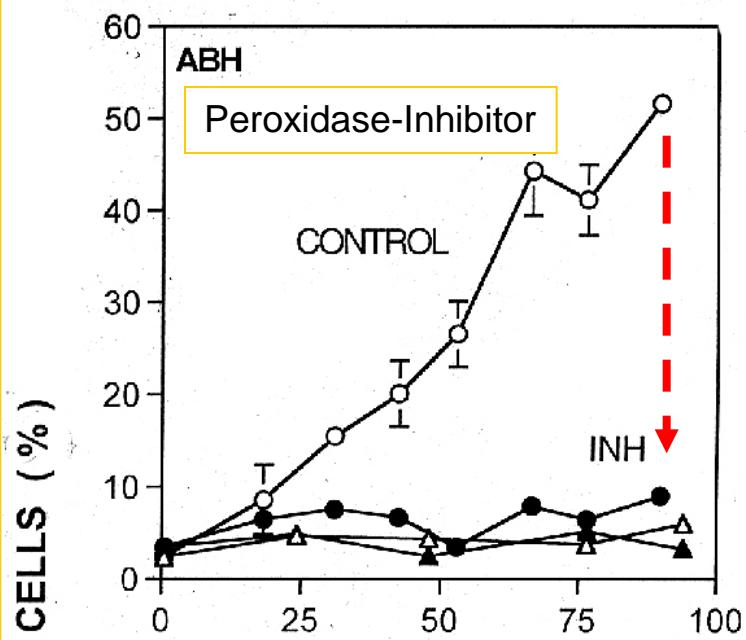
**Transformed
cell**

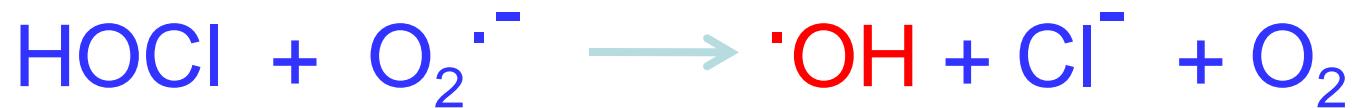


Elucidation of intercellular ROS signaling:

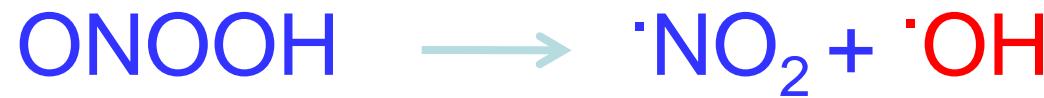
- 1) Inhibitor studies
- 2) Reconstitution experiments
- 3) Verification of reconstitution experiments
with inhibitors
- 4) siRNA-based analysis

Inhibitor studies





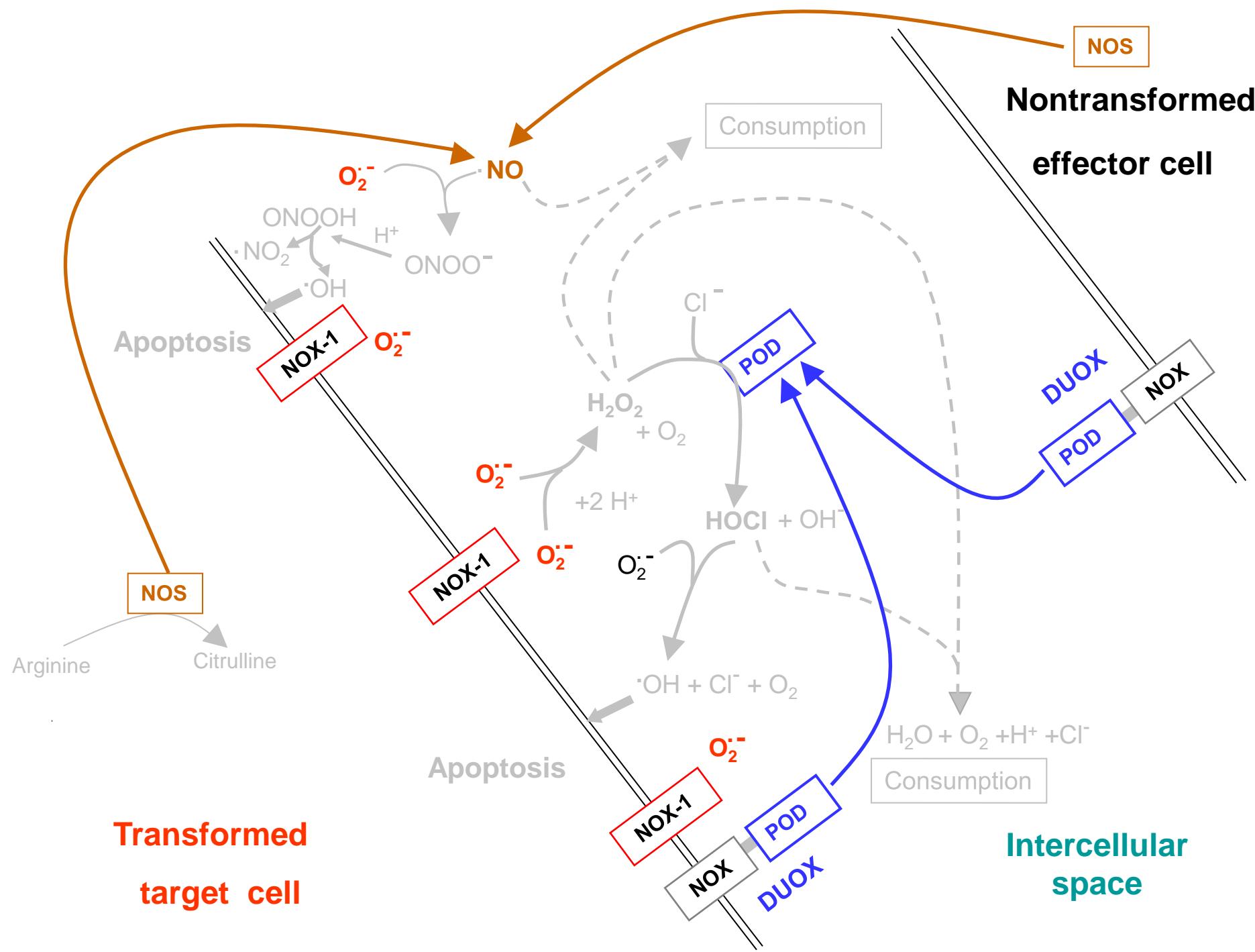
HOCl signaling pathway

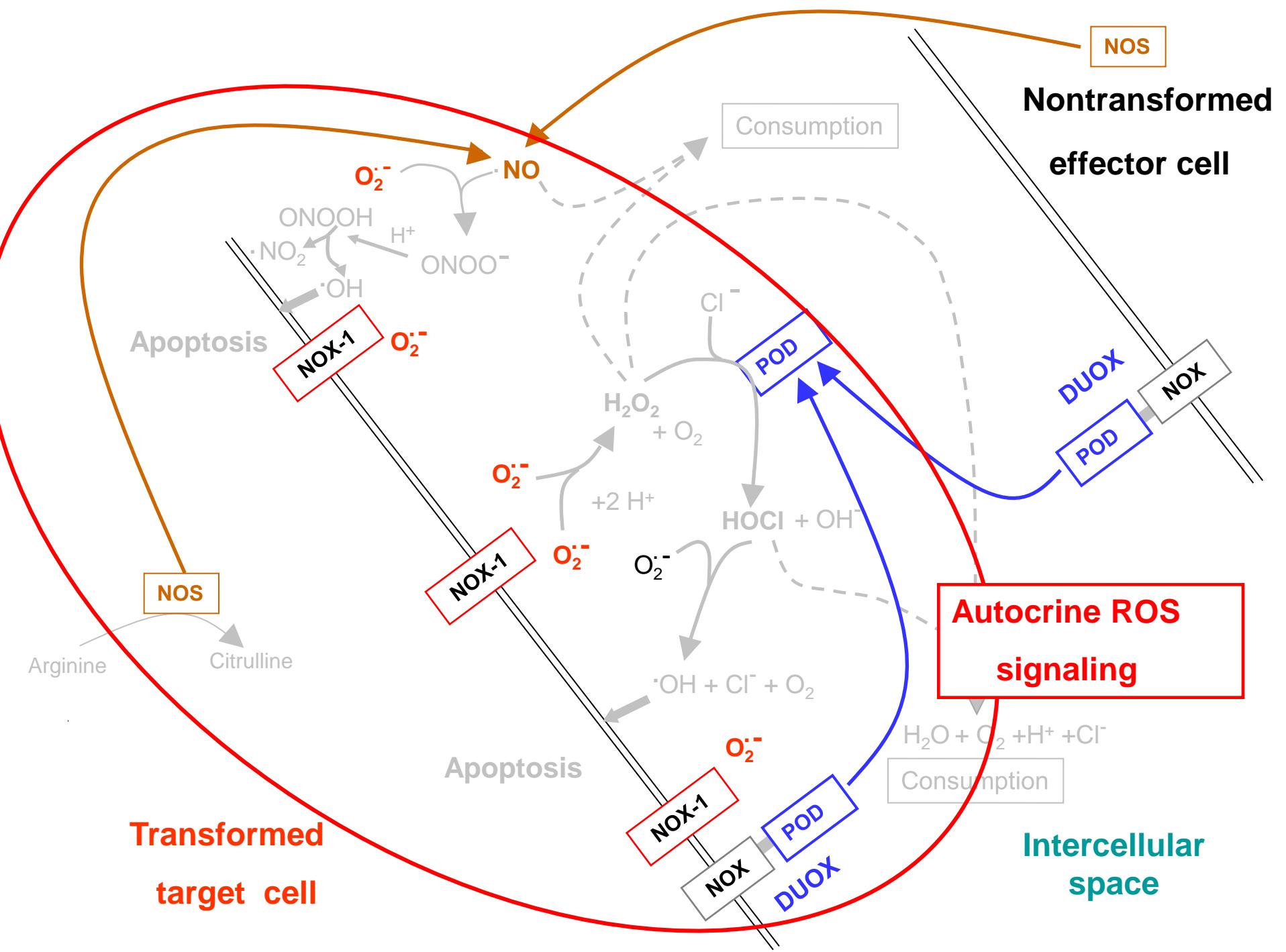


NO/peroxynitrite signaling pathway

Signaling pathways

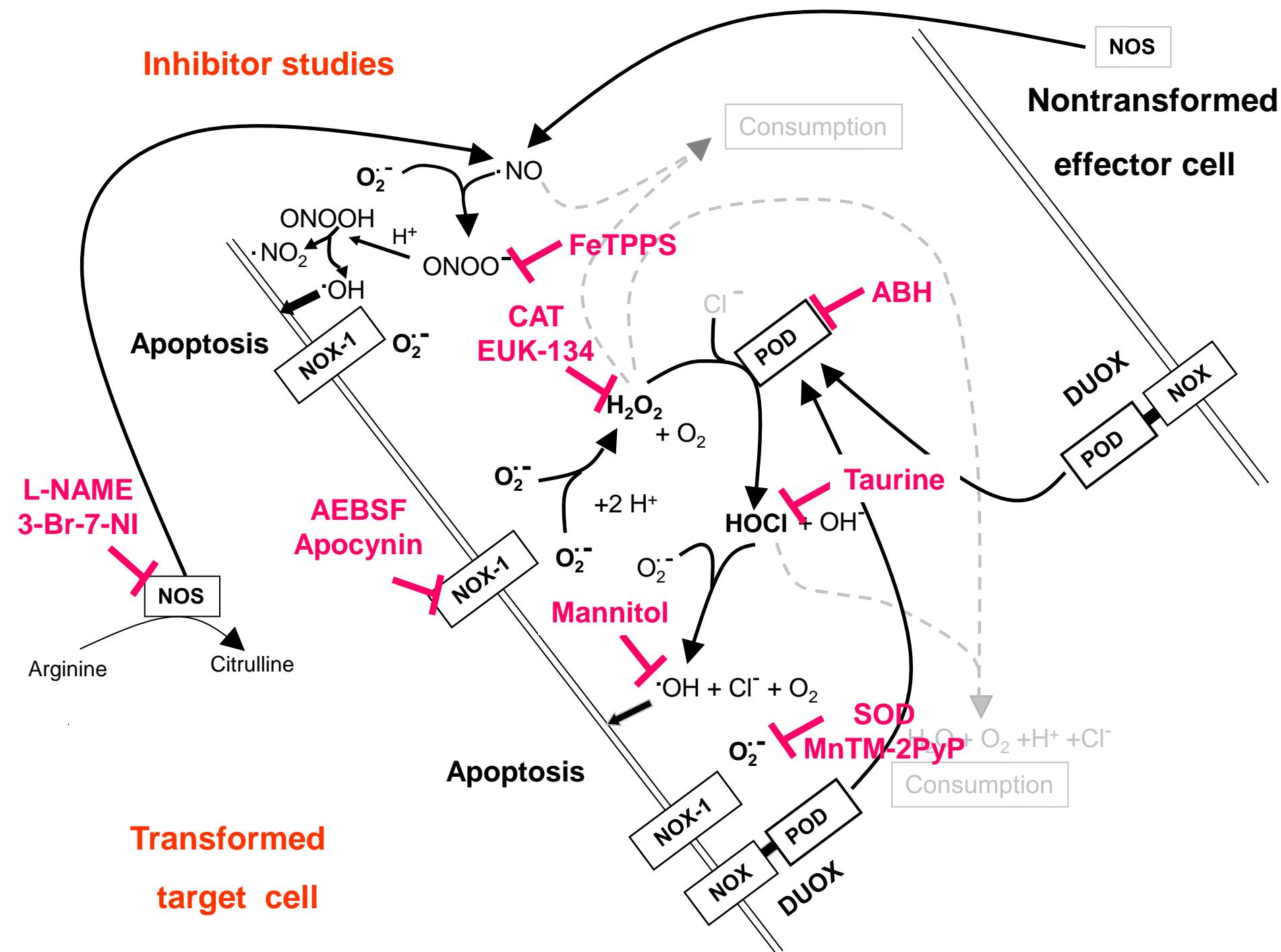
Basic features



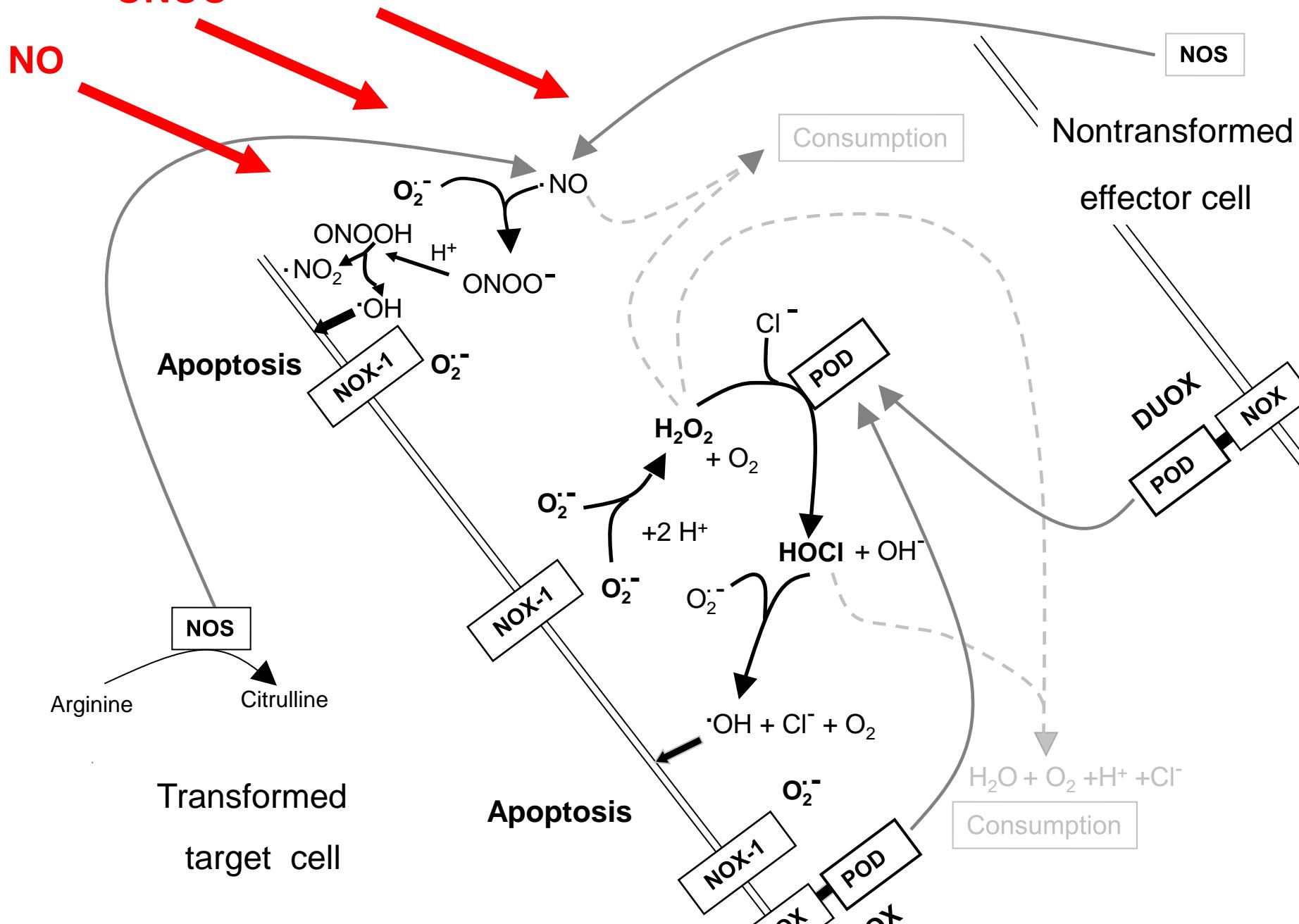


Further elucidation of ROS signaling pathways

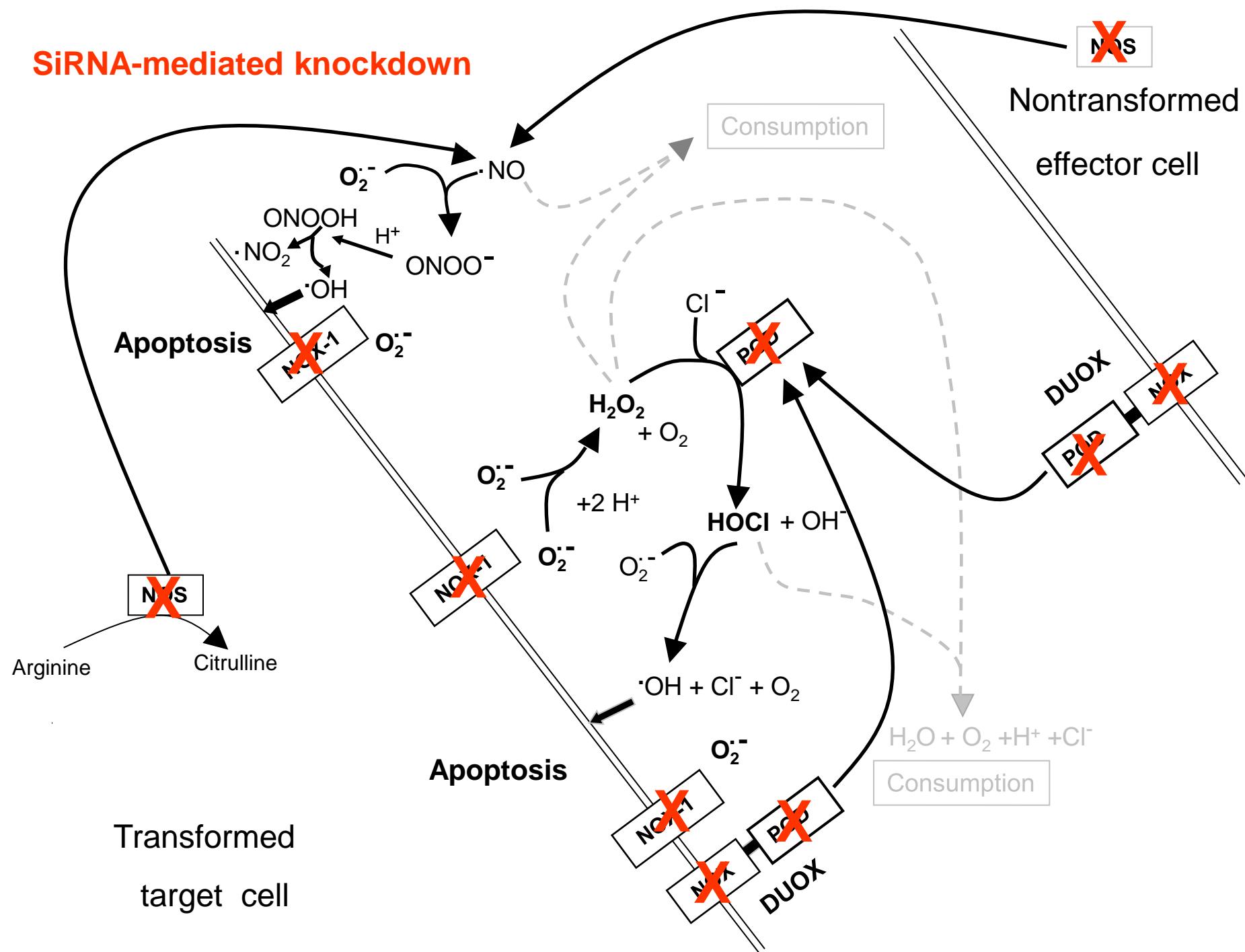
Inhibitor studies



Addition of signaling molecules (Reconstitution experiments)

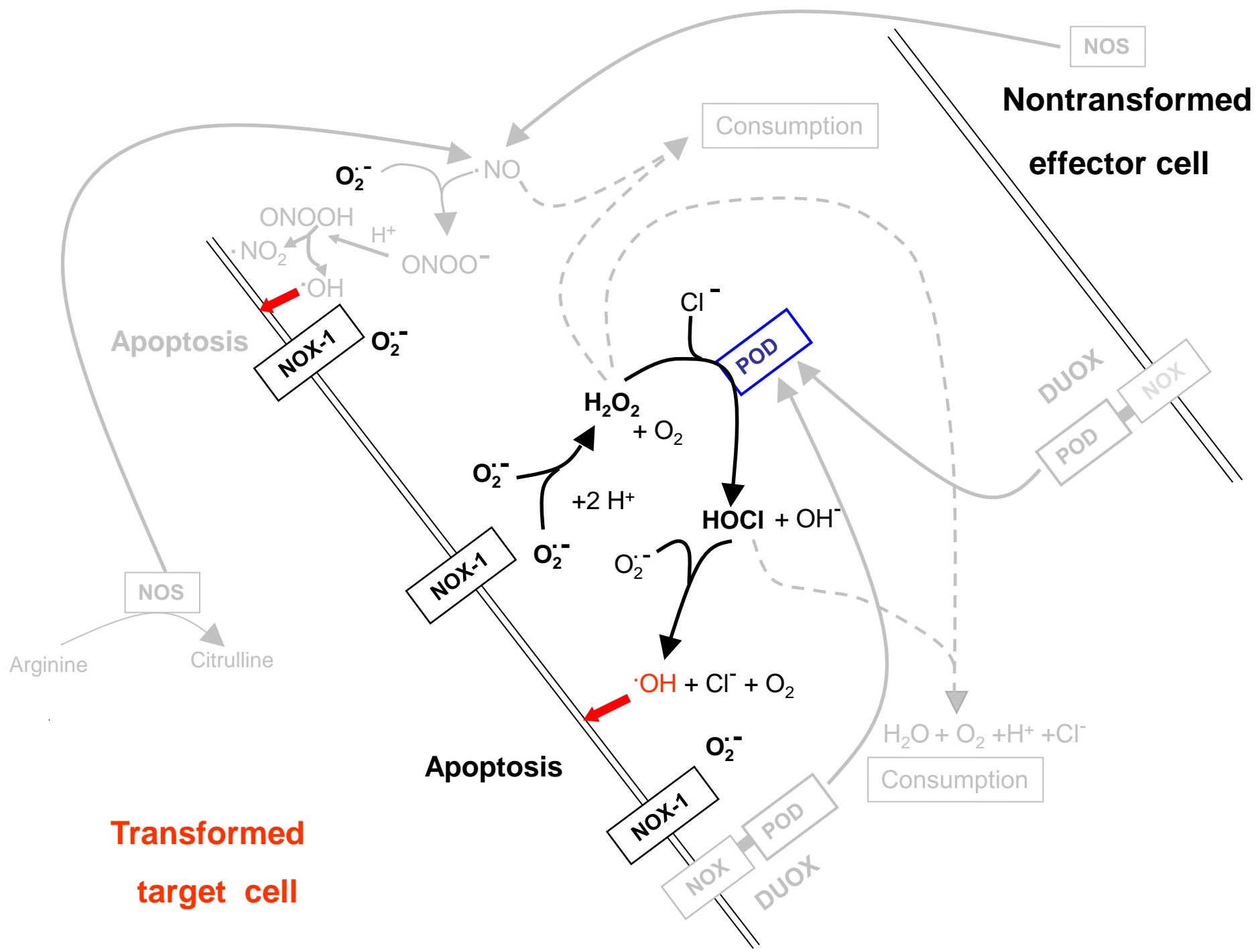


SiRNA-mediated knockdown

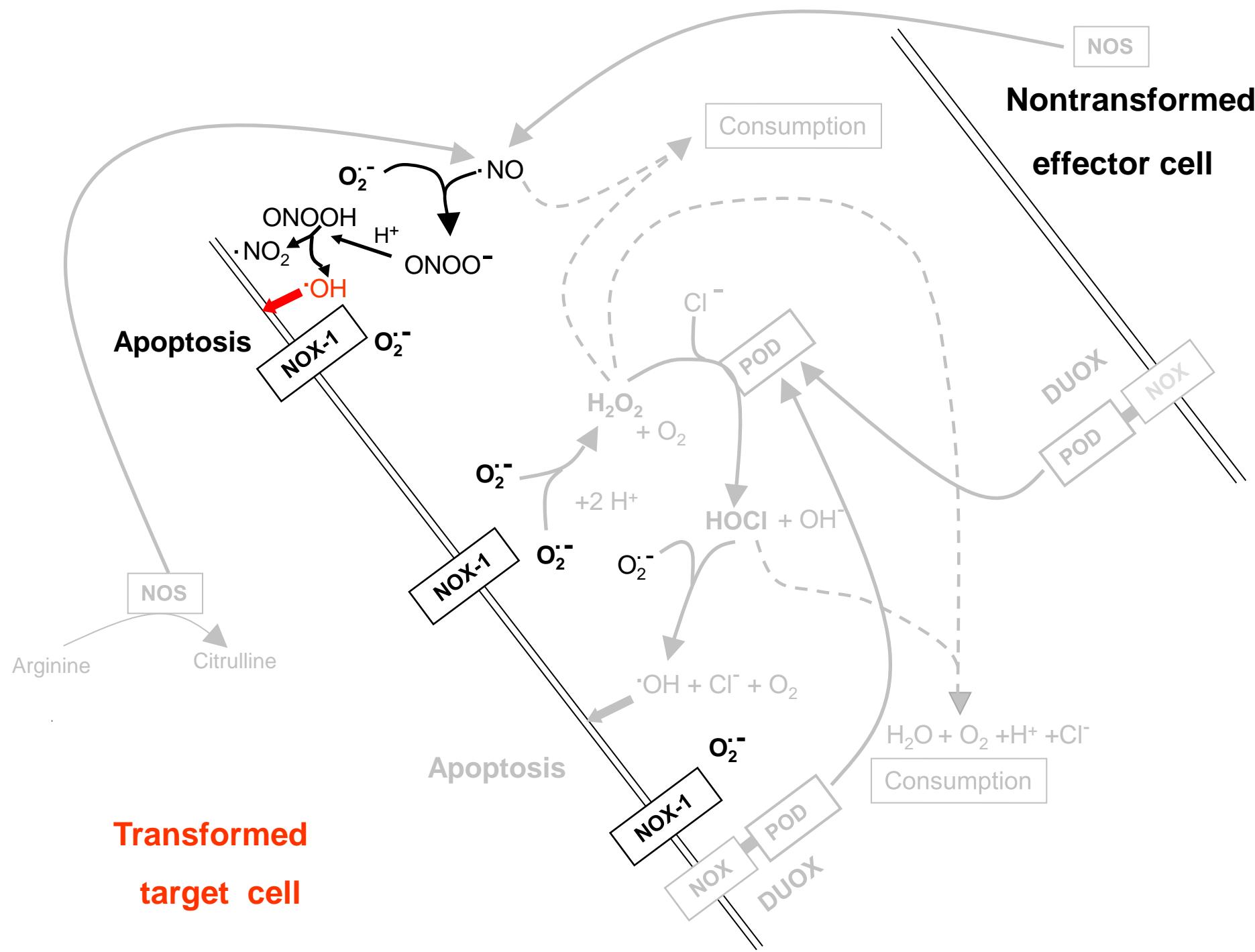


HOCl signaling pathway

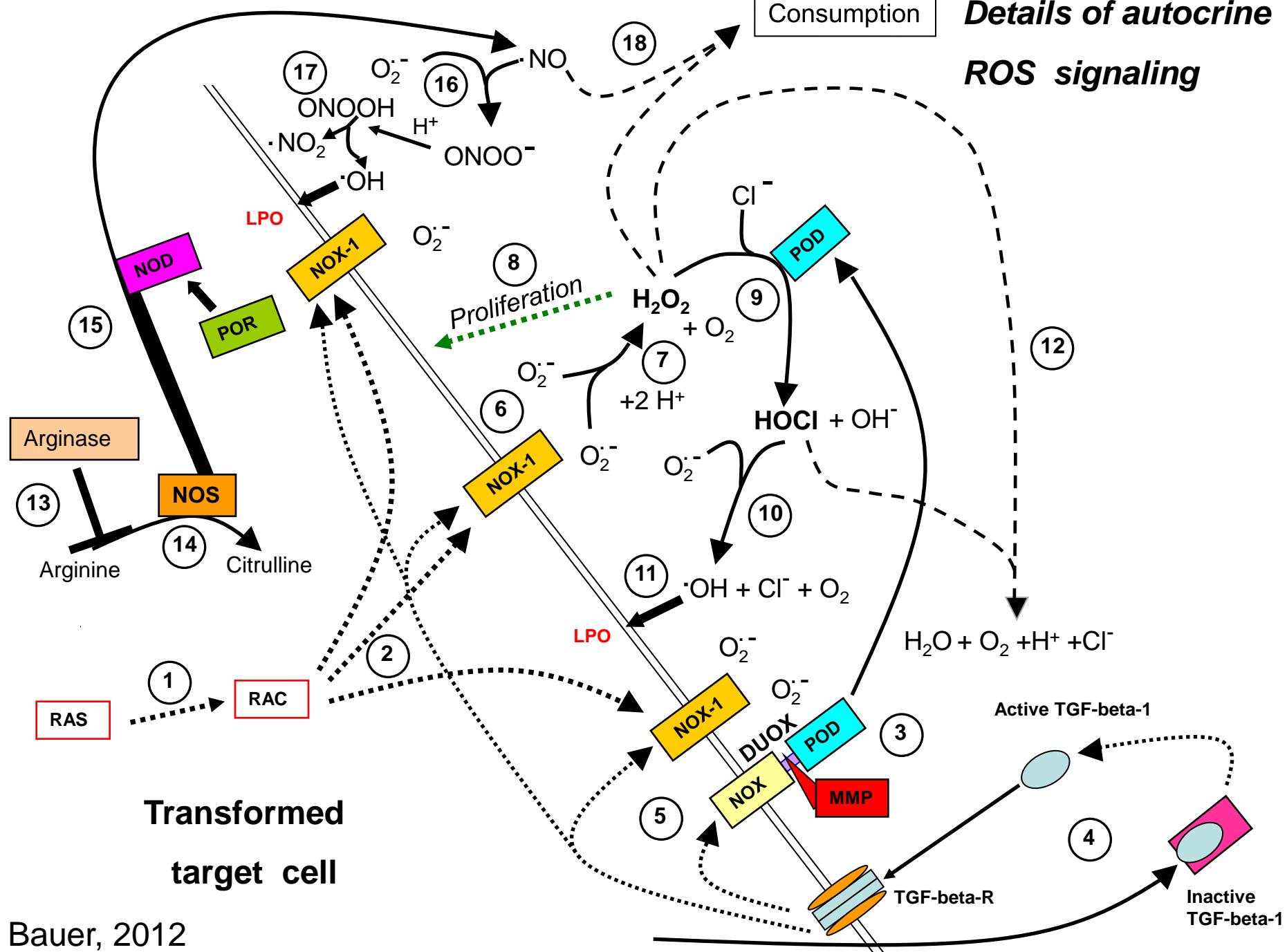
Transformed target cell

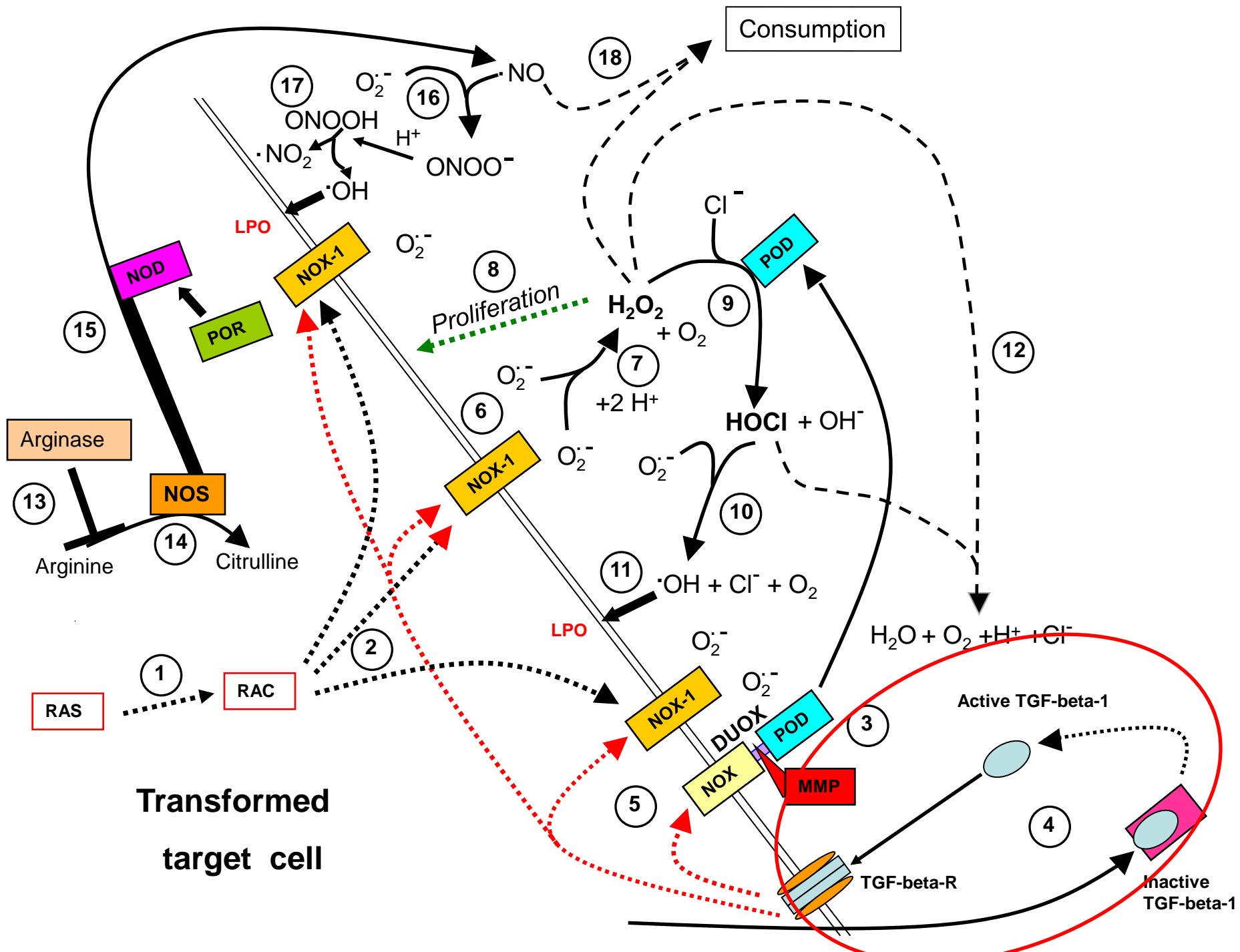


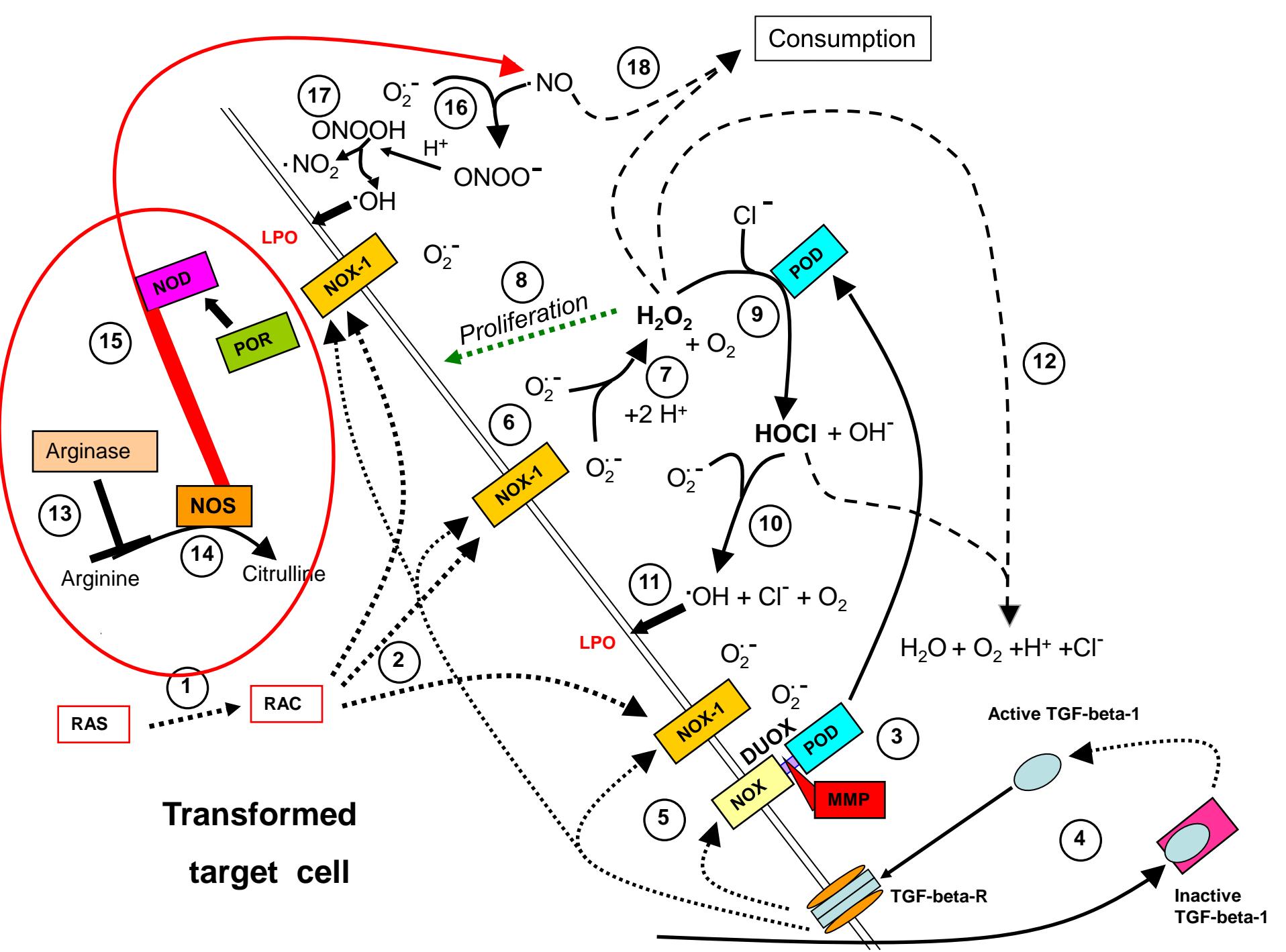
NO/peroxynitrite signaling pathway



Details of autocrine ROS signaling

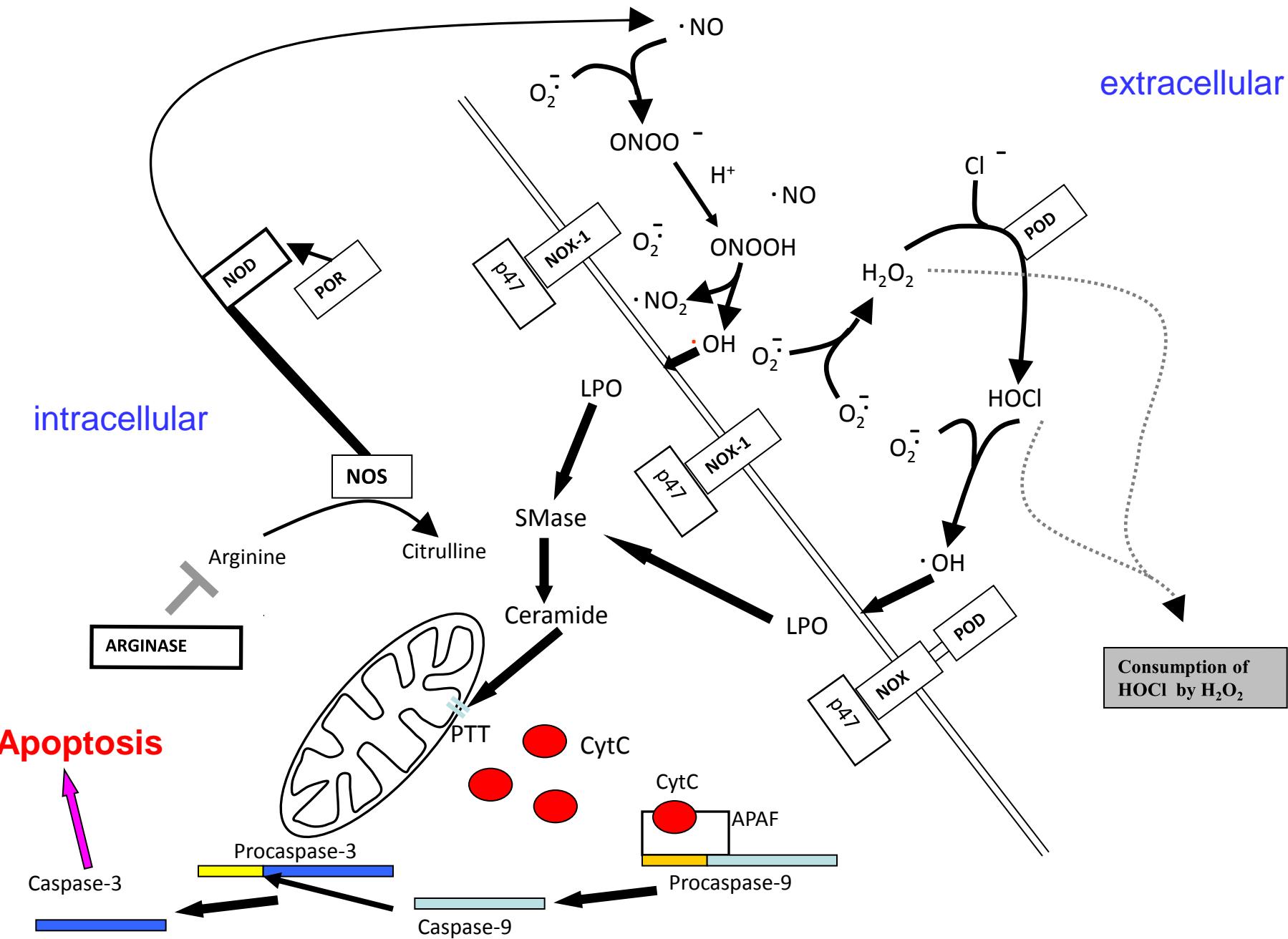






Intracellular pathways
activated by intercellular
ROS-mediated signaling

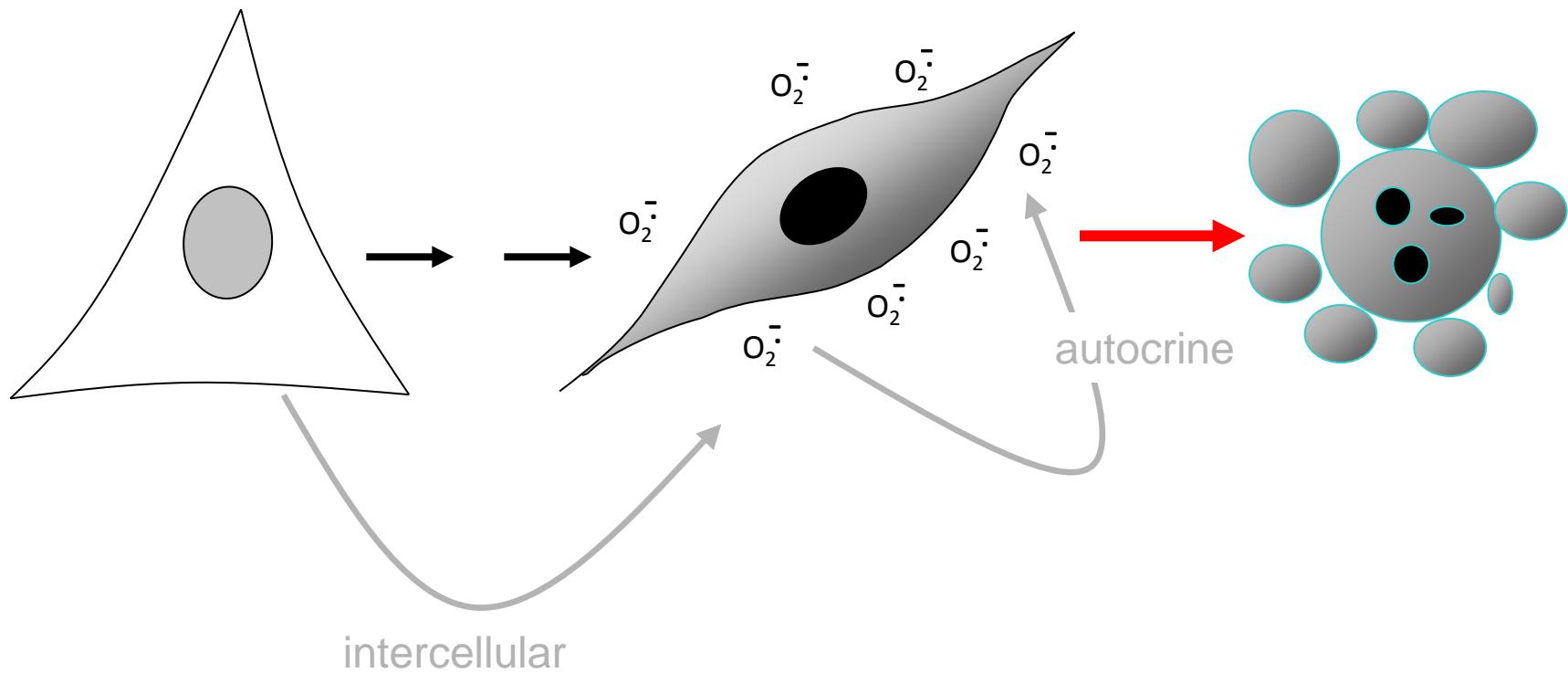
Intracellular signaling pathways after intercellular ROS-mediated signaling



**Nontransformed
cell**

**Transformed
cell**

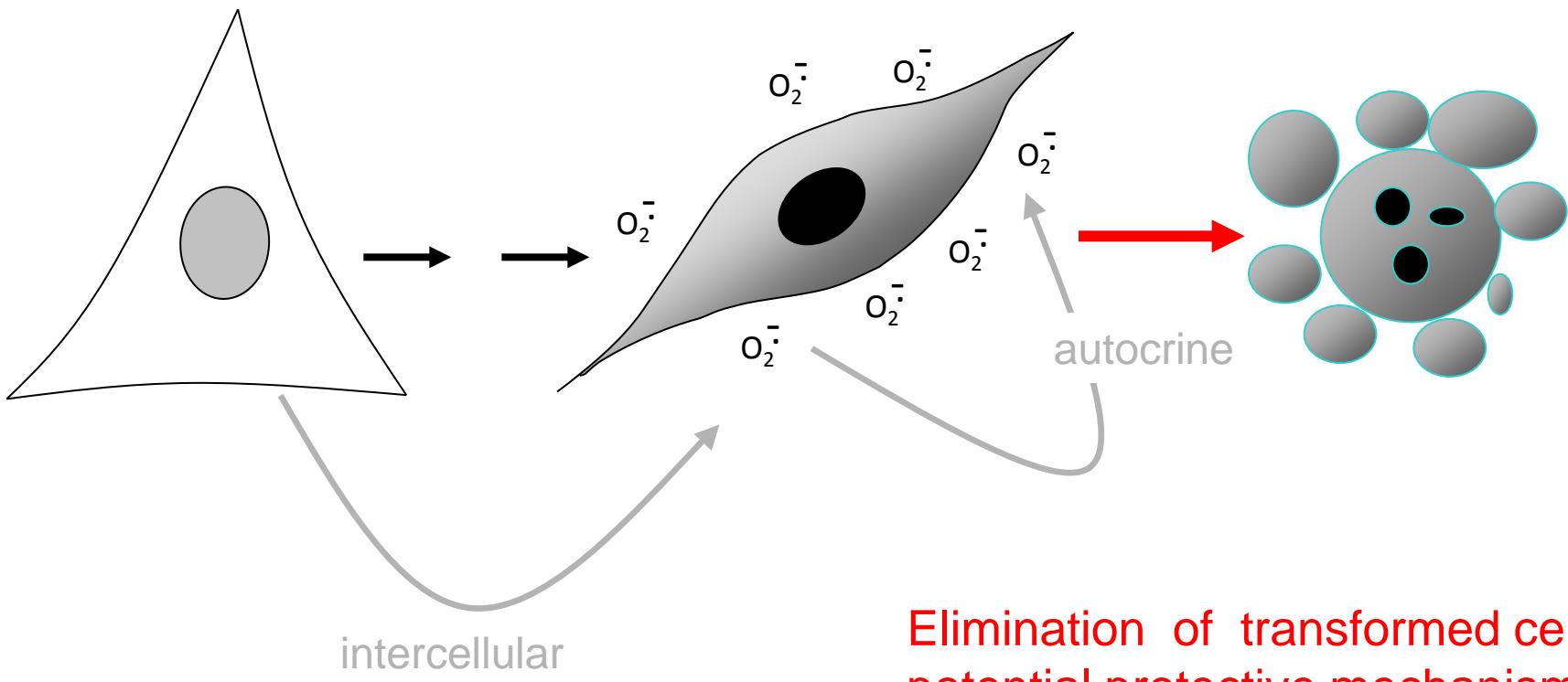
Apoptosis



Nontransformed
cell

Transformed
cell

Apoptosis



Elimination of transformed cells:
potential protective mechanism
against tumor formation?

Requirements for tumor progression:

Maintaining proliferation stimulus of ROS

and

Protection against apoptosis induction

by ROS

Mechanism of tumor cell resistance

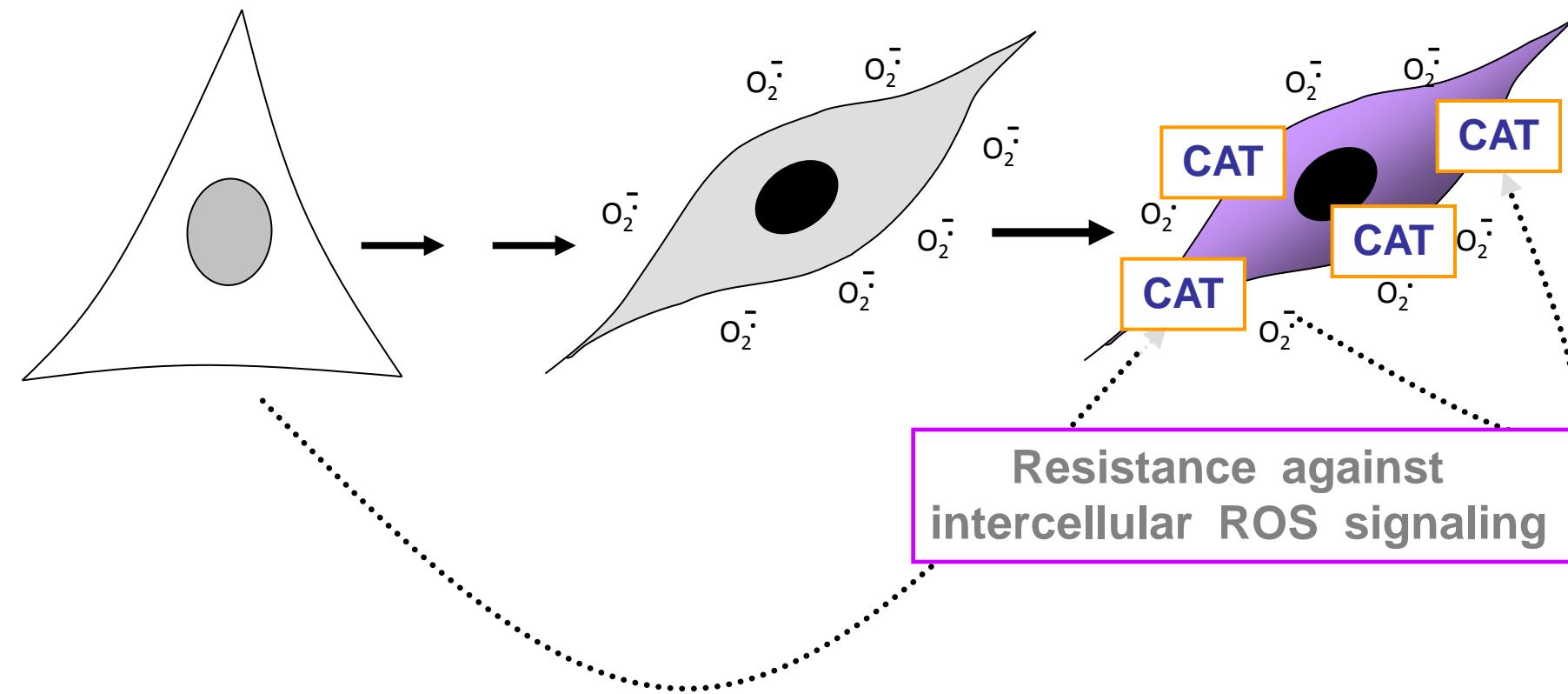
Nontransformed
cell

Transformed
cell

Tumor
cell

Membrane-associated catalase

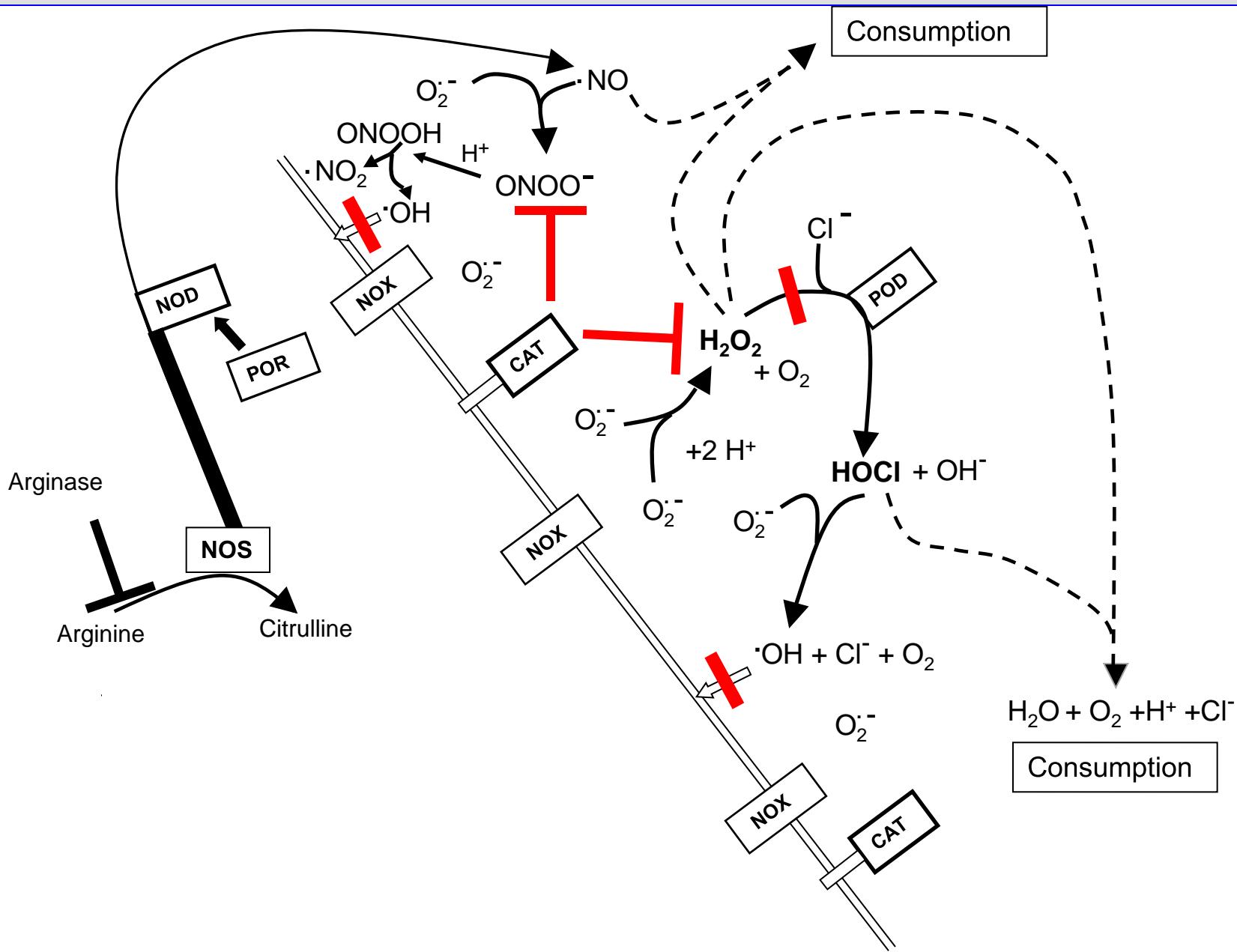
Resistance against
intercellular ROS signaling

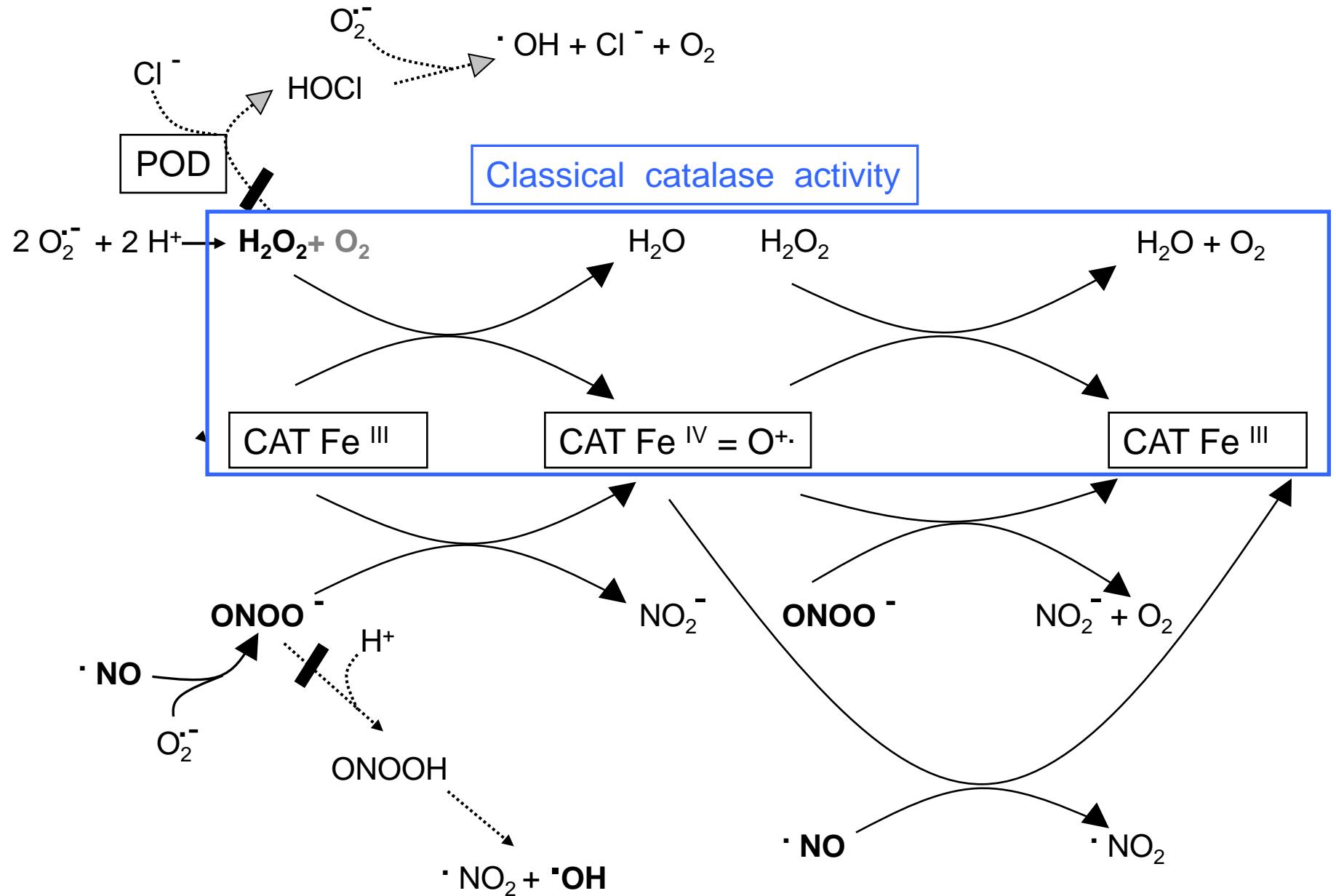


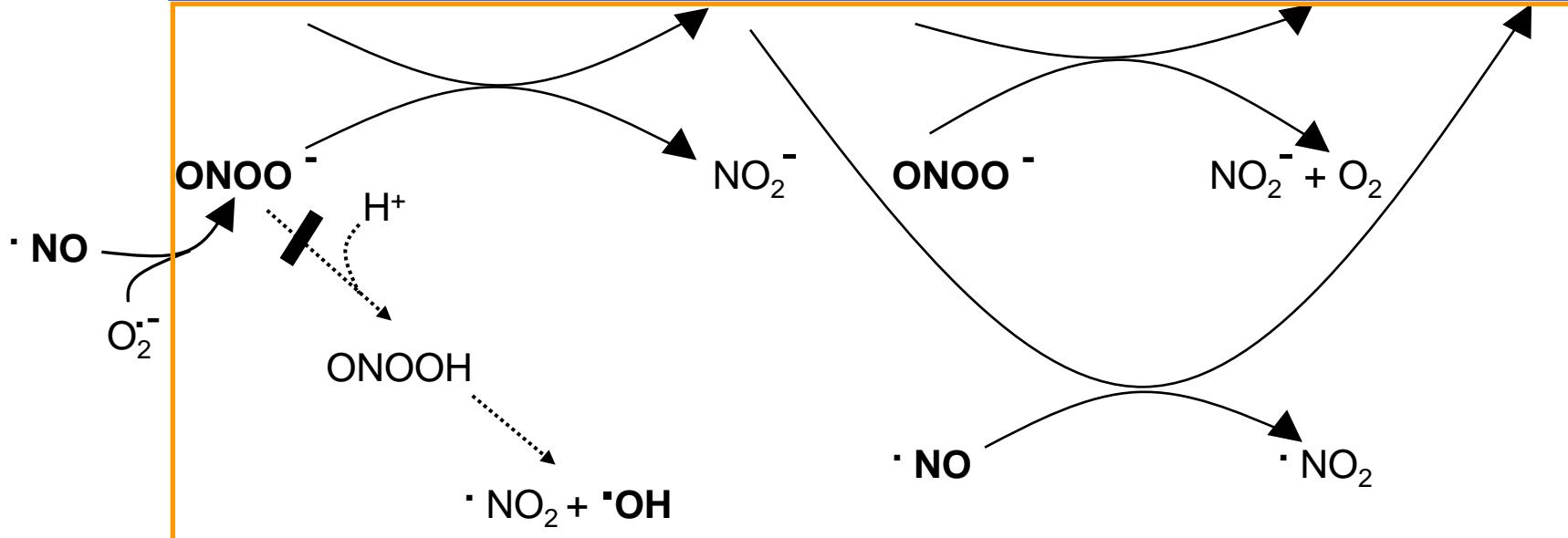
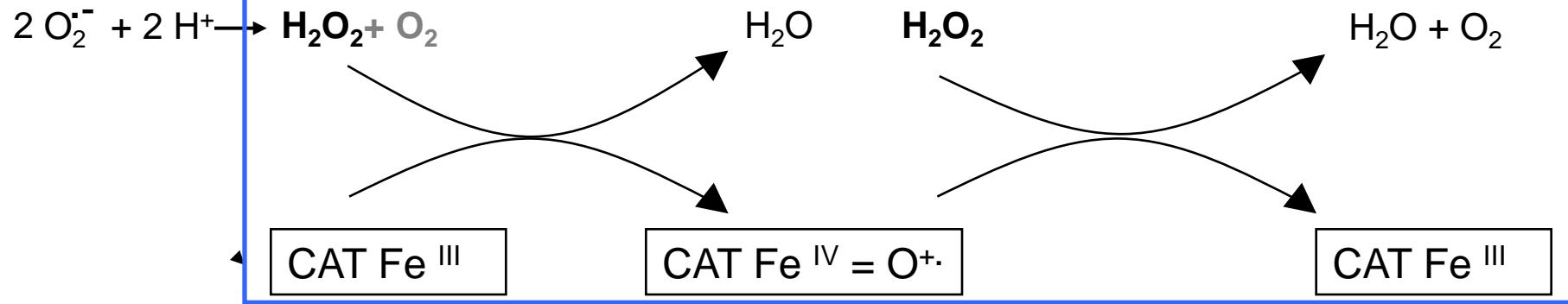
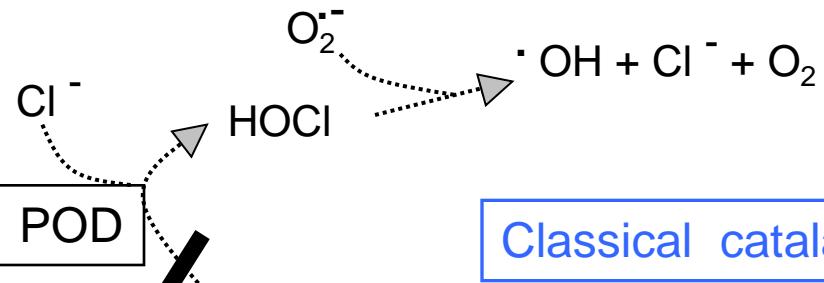
Role of membrane-associated catalase
for the protection of tumor cells
against ROS signaling.

How can catalase protect against
multiple signaling pathways?

Catalase protects tumor cells against HOCl and NO/peroxynitrite signaling



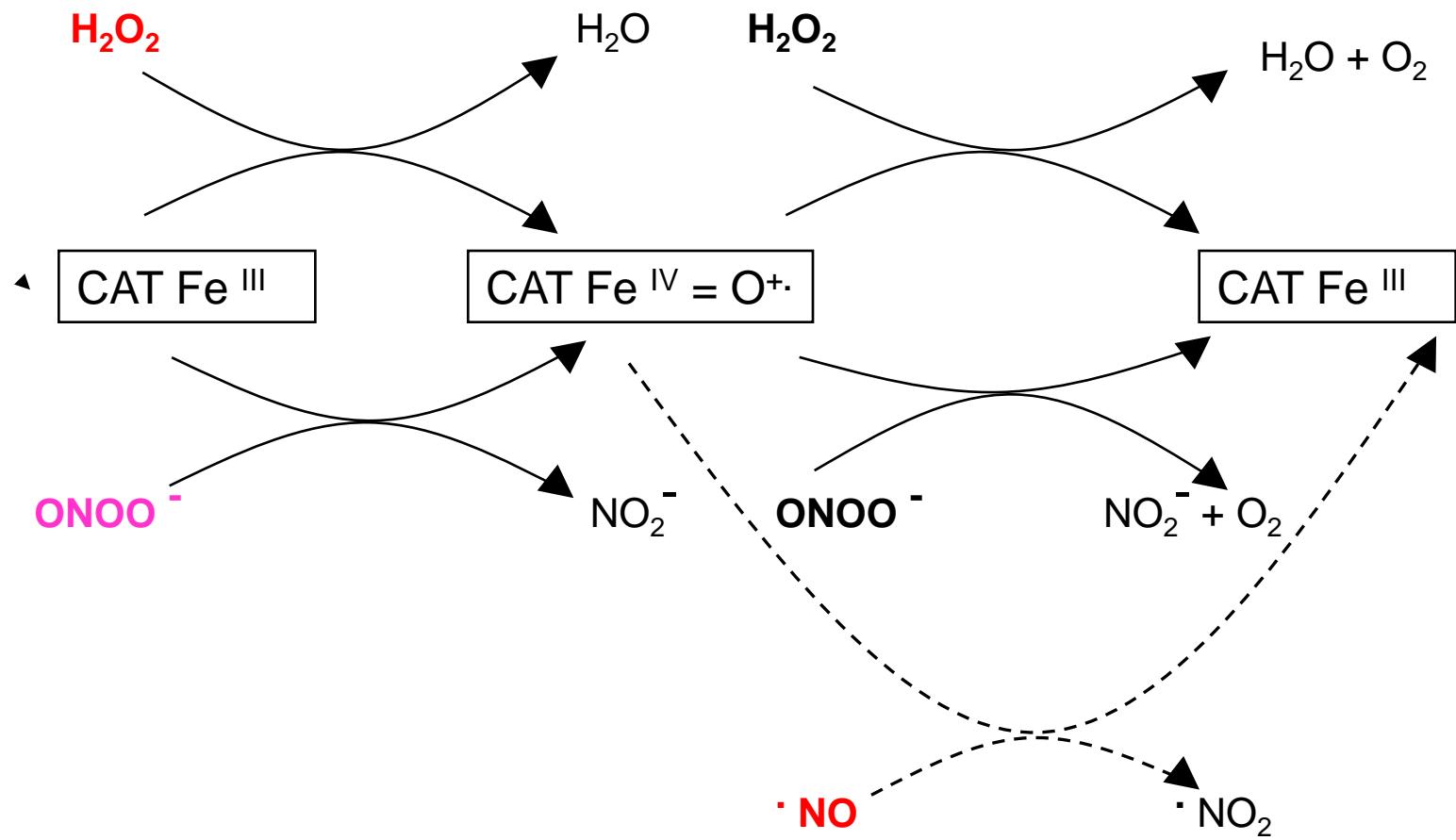




Additional catalase functions

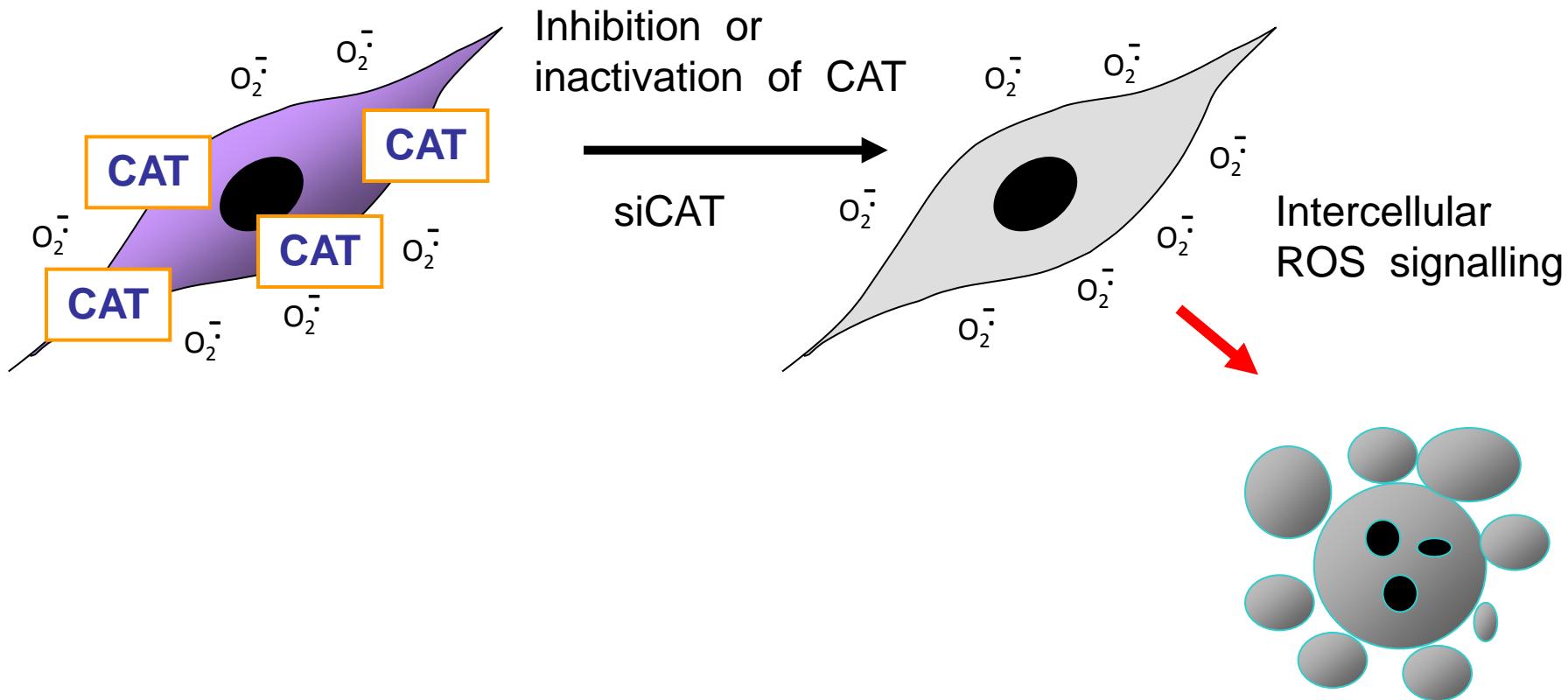
Heinzelmann and Bauer, 2010

Catalase is multifunctional!

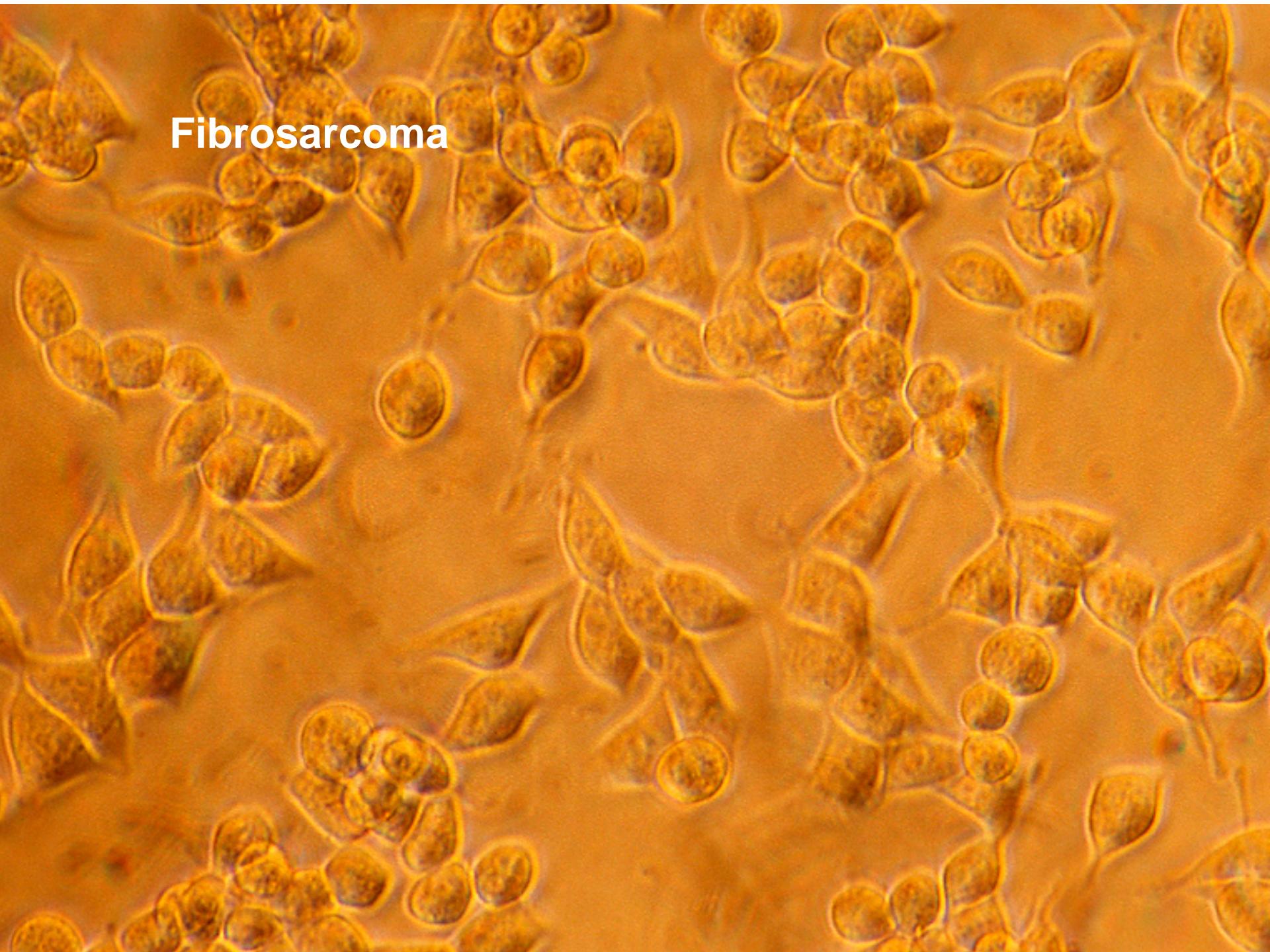


Utilization of ROS signaling
chemistry for novel,
mechanism-based
antitumor strategies

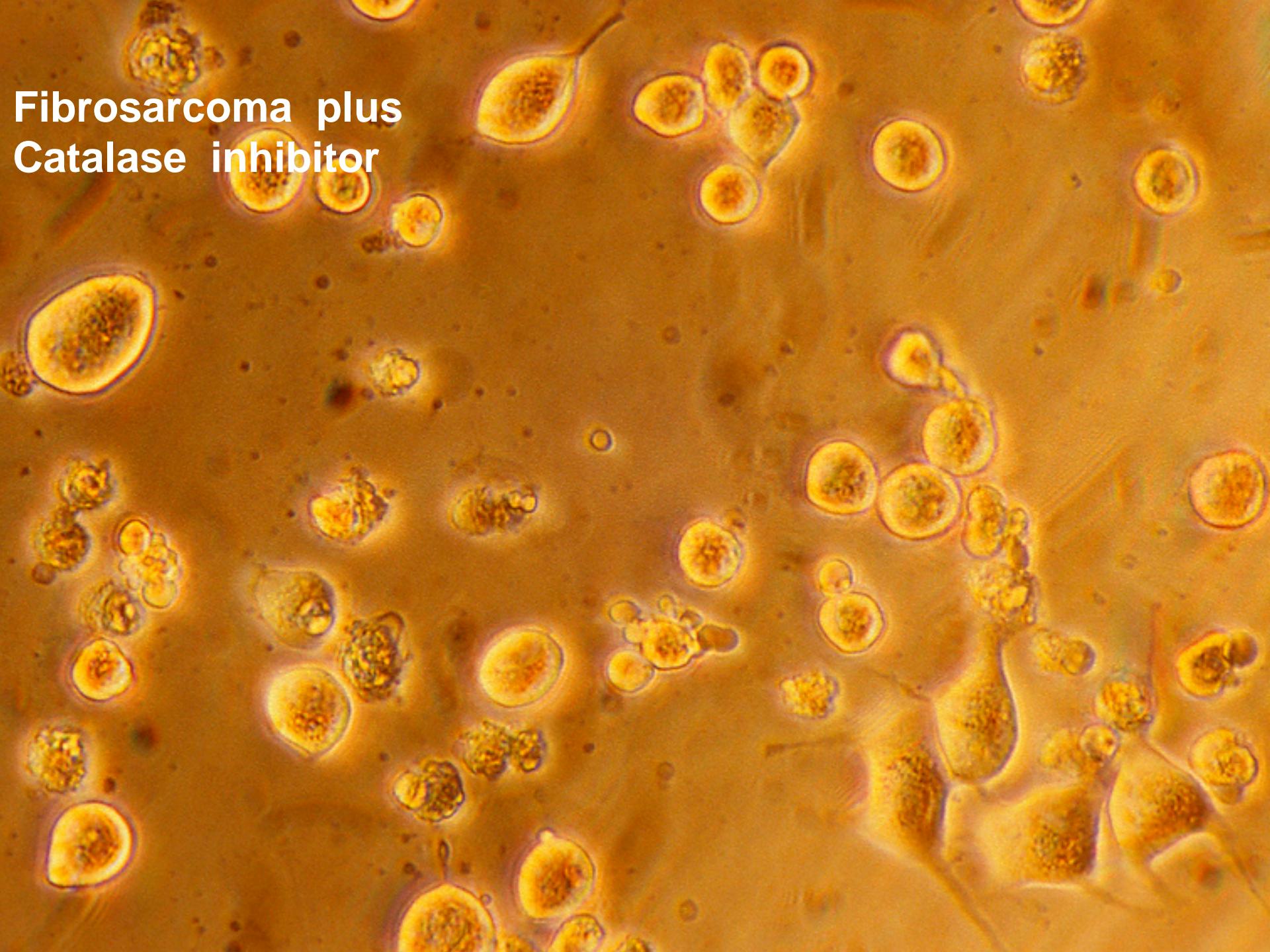
Tumor cell



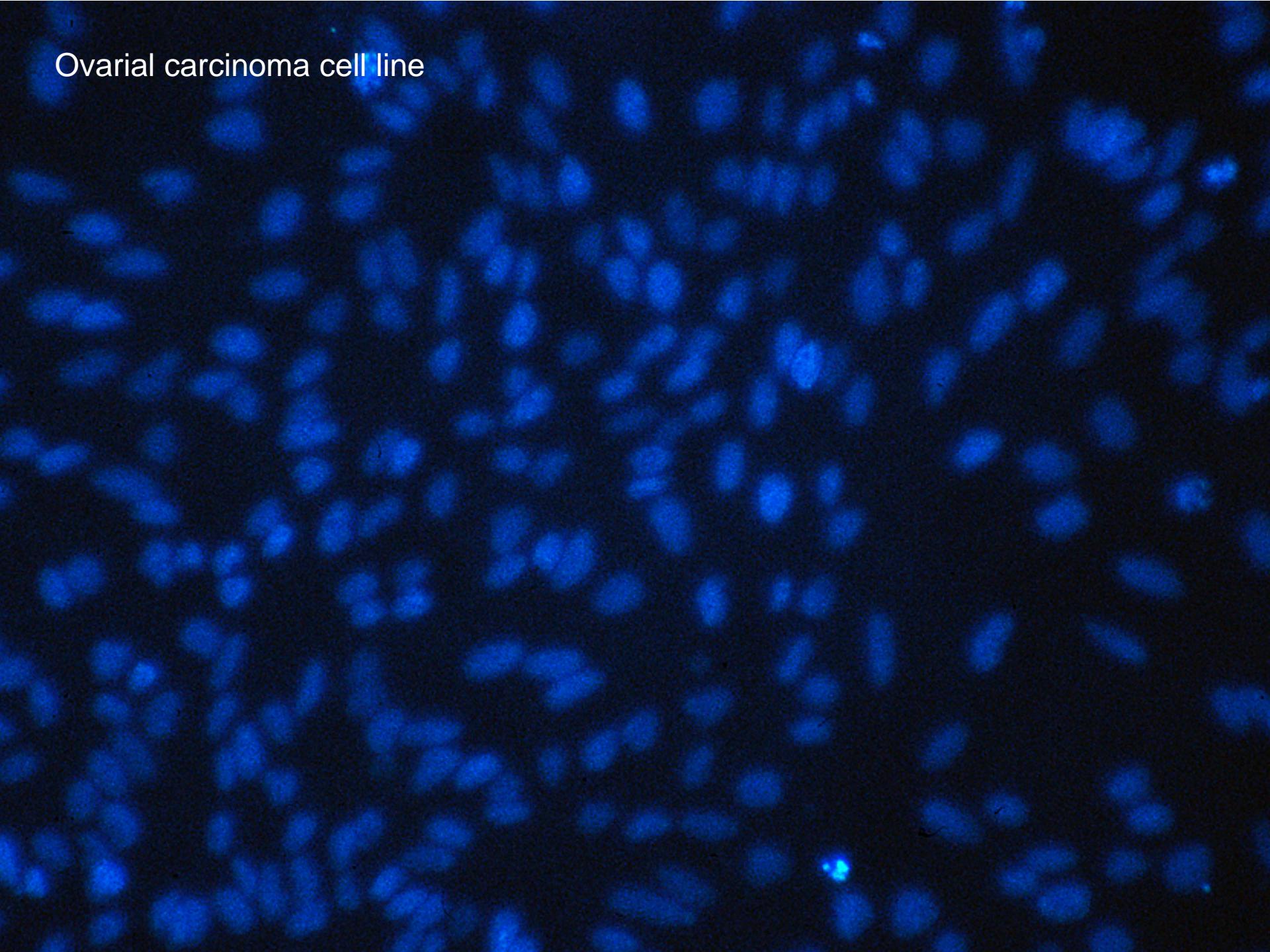
Fibrosarcoma



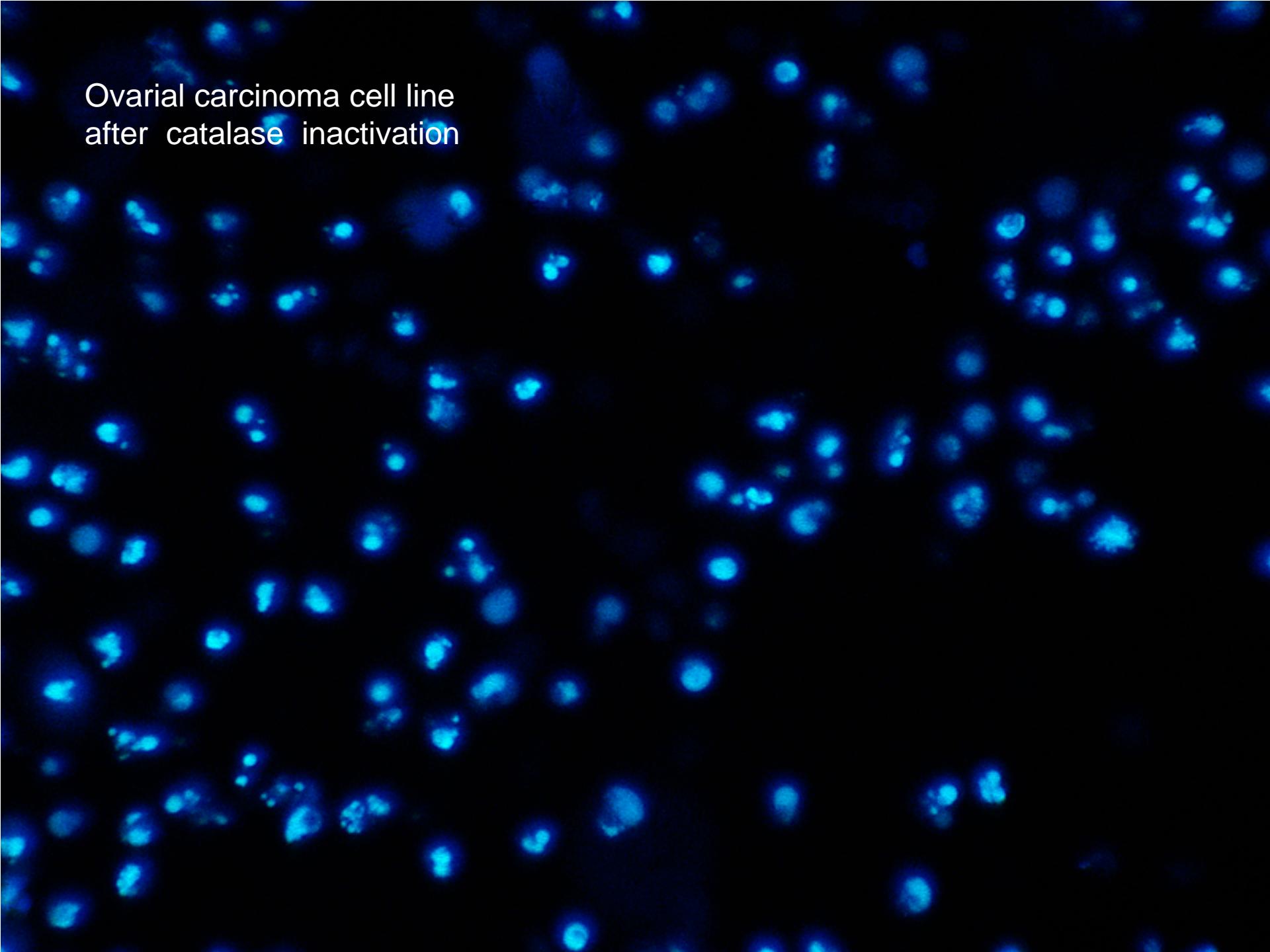
Fibrosarcoma plus
Catalase inhibitor



Ovarial carcinoma cell line

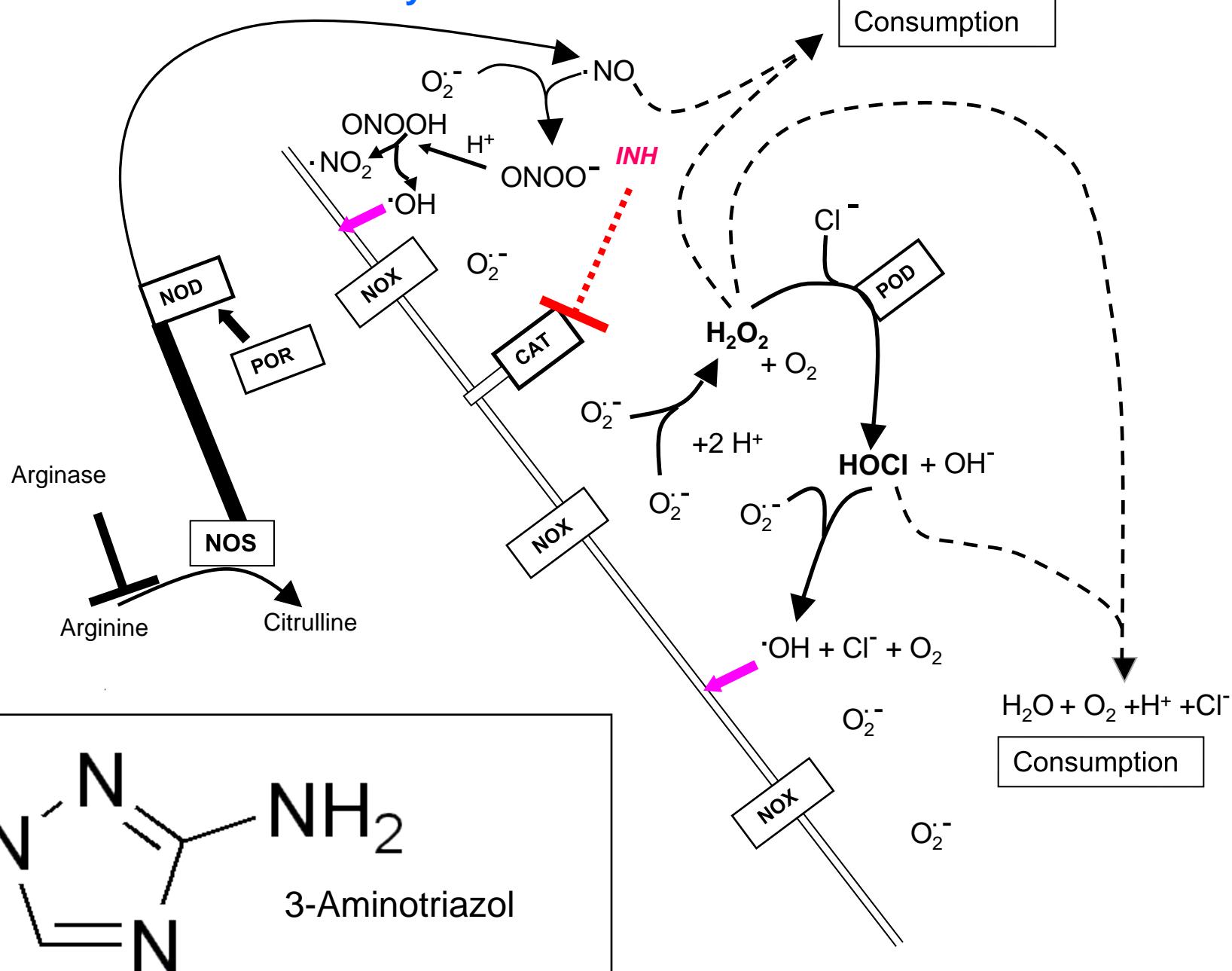


Ovarial carcinoma cell line
after catalase inactivation

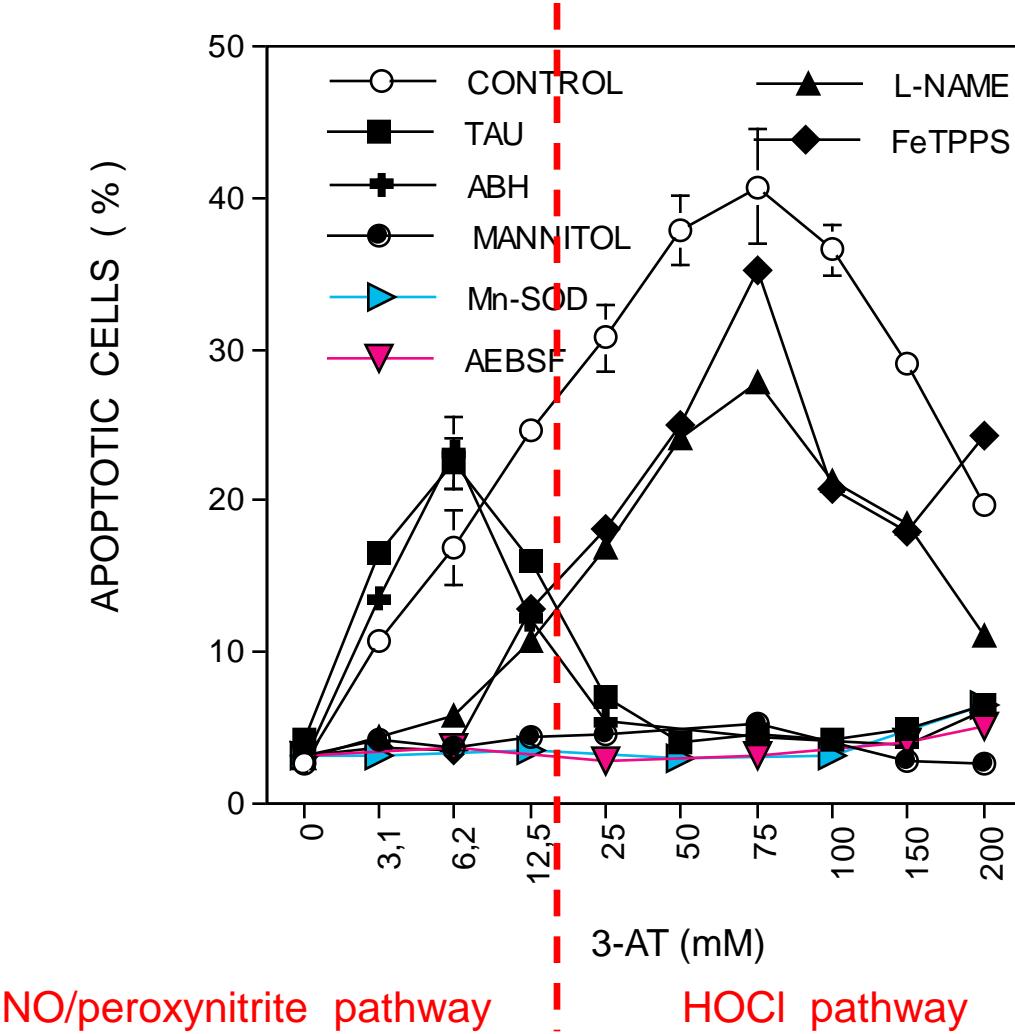


Distinct ways to sensitize
tumor cells for
apoptosis induction

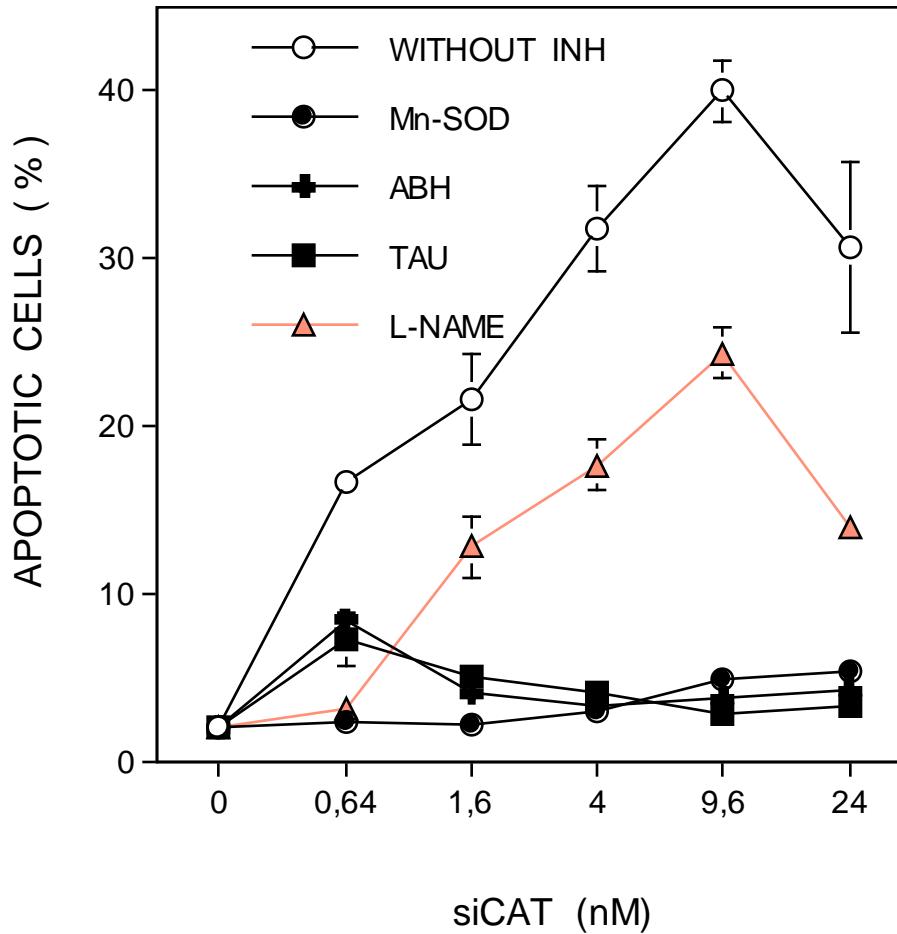
1. Inhibition of catalase by 3-aminotriazole



*Scavenger for superoxide anions
and inhibitor of NADPH oxidase*

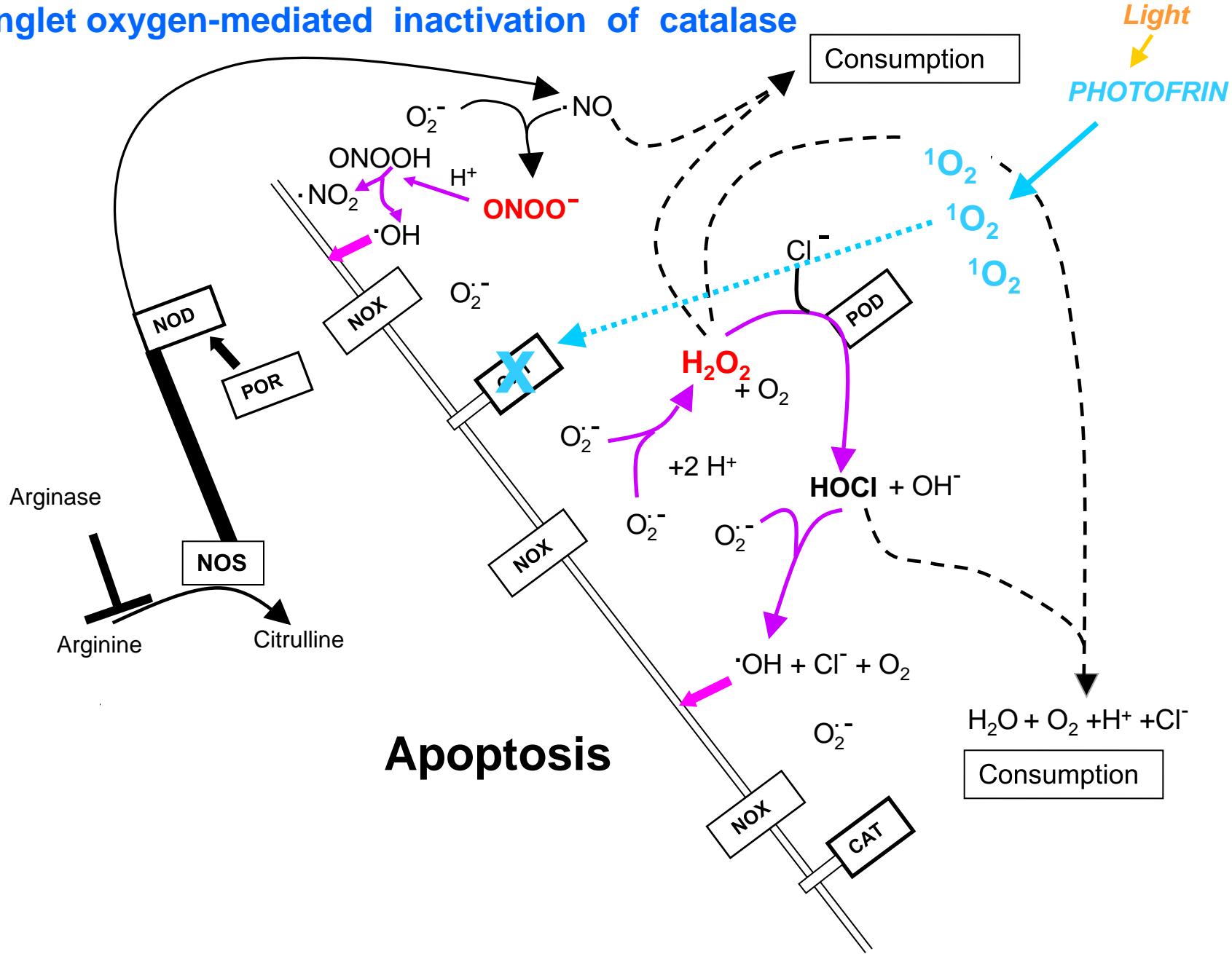


2. SiRNA-mediated knockdown of catalase



siRNA-mediated knockdown of catalase sensitizes tumor cells for ROS signaling

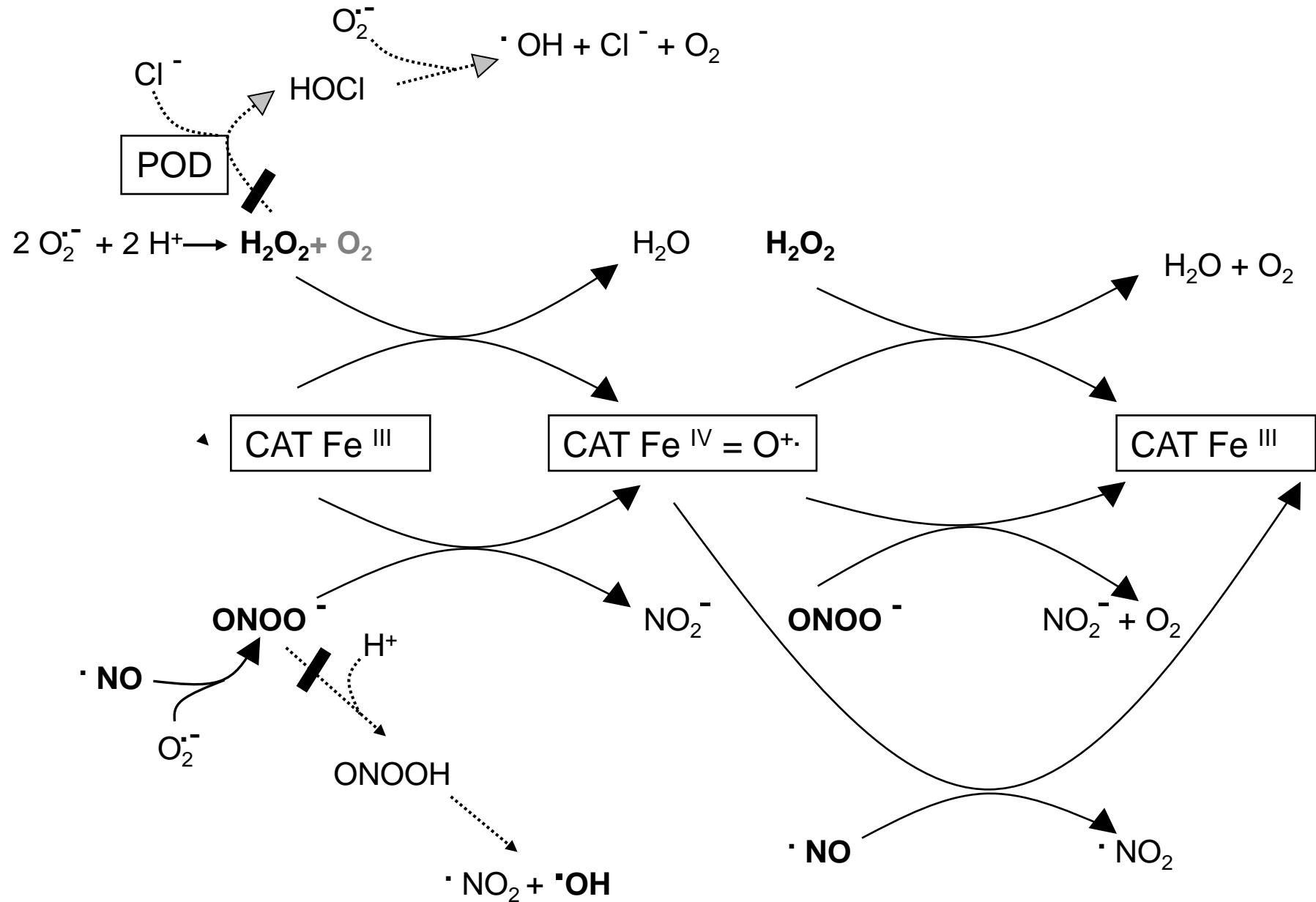
3. Singlet oxygen-mediated inactivation of catalase

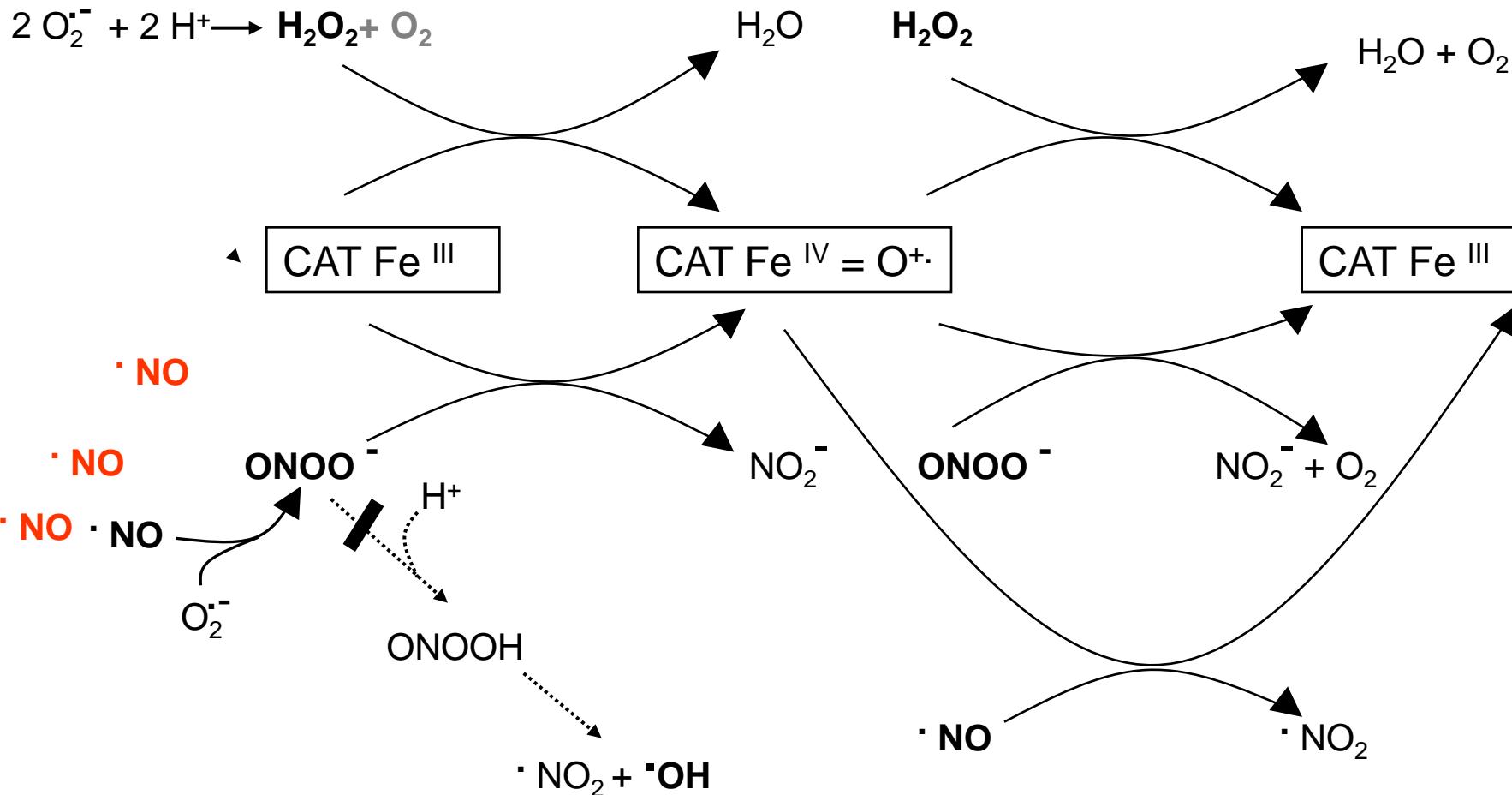
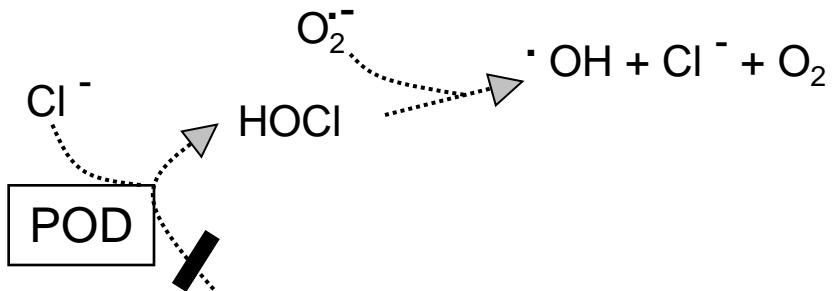


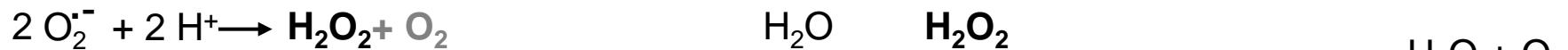
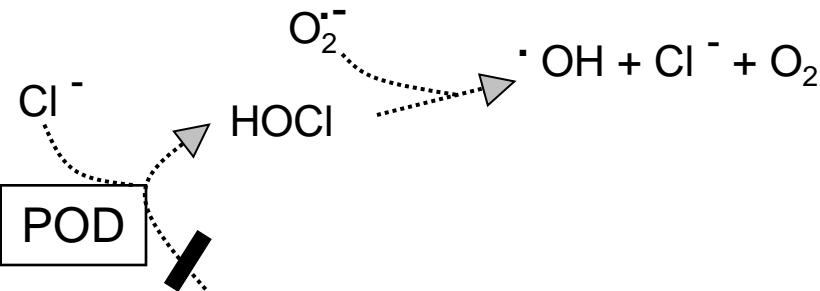
4. Inactivation of catalase by *tumor cell-derived* singlet oxygen

Increase in the cellular NO concentration (through addition of arginine, inhibition of arginase or inhibition of NO dioxygenase) causes generation of extracellular singlet oxygen of the tumor cells. Singlet oxygen inactivates catalase and thus restores apoptotic ROS signaling specifically in tumor cells.

This process requires amplification through APO/FAS receptor mediated stimulation of NOX1 and NOS.







CAT Fe ^{III} NO

CAT Fe ^{III}

CAT Fe ^{IV} = O⁺

CAT Fe ^{III}

·NO

·NO

·NO ·NO

O²⁻

ONOO⁻

H⁺

ONO₂H

NO₂⁻

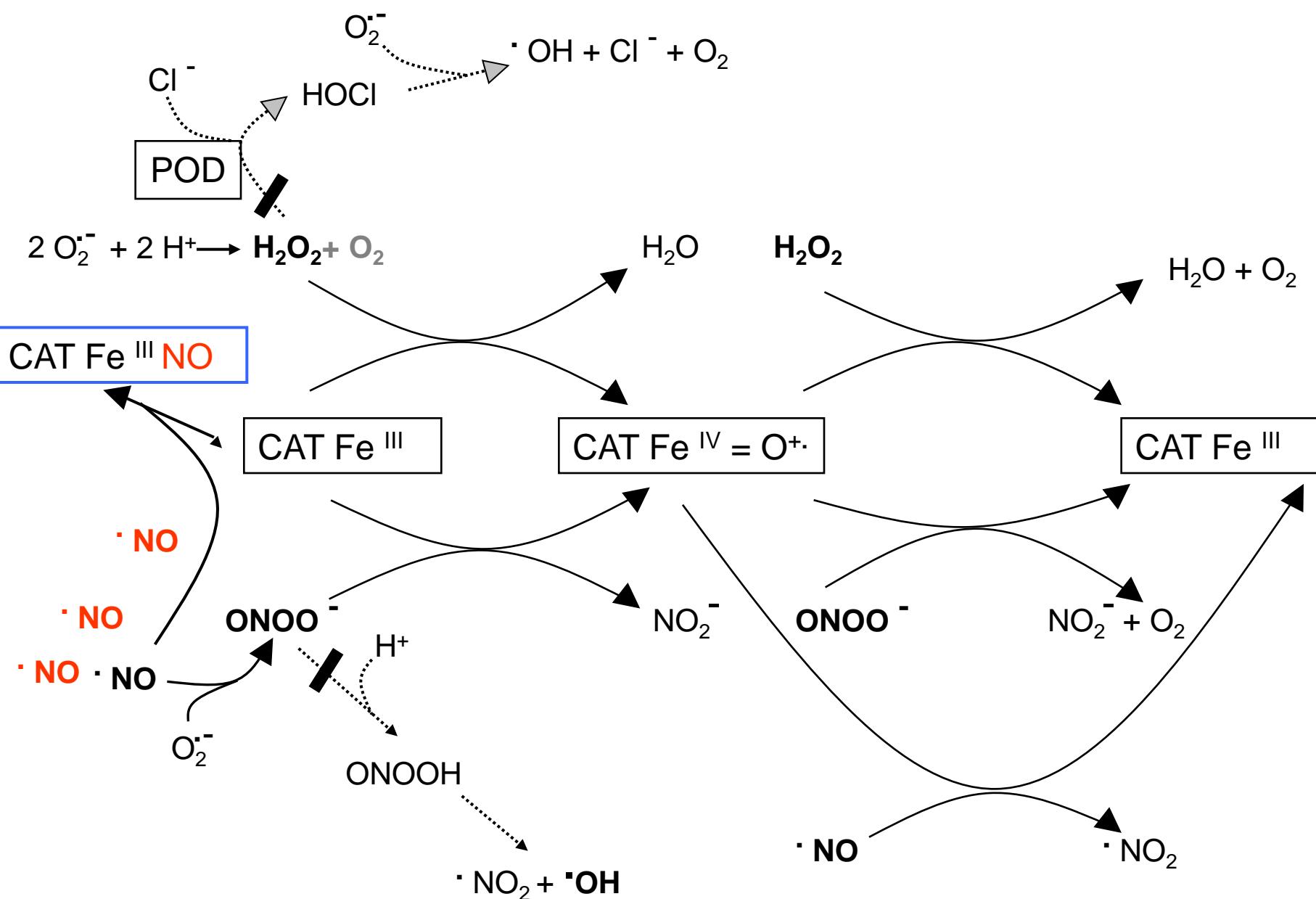
ONOO⁻

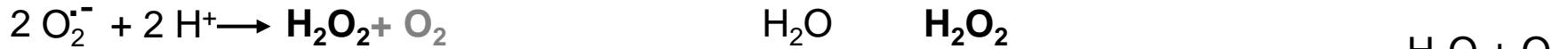
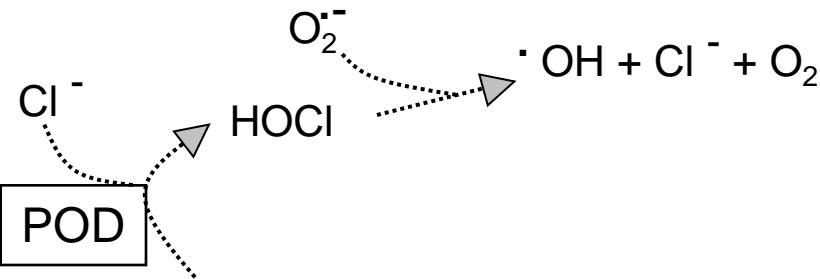
NO₂ + O₂

·NO₂

·NO

·NO₂ + ·OH





CAT Fe III NO

CAT Fe III

CAT Fe IV = O⁺

CAT Fe III

·NO

·NO

·NO ·NO

O₂⁻

ONOO

H⁺

ONO₂

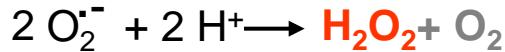
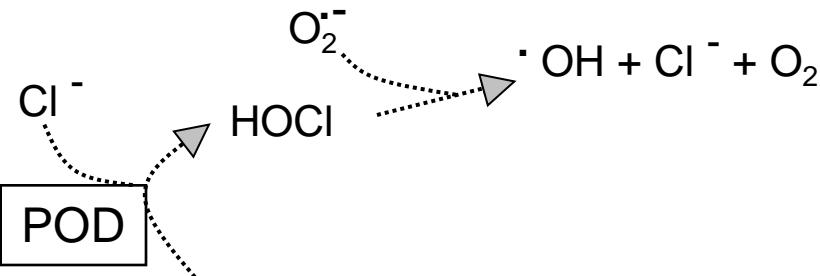
ONO₂

NO₂⁻

ONOO⁻

NO₂⁻ + O₂

·NO₂ + ·OH

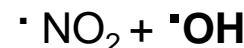
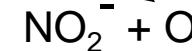
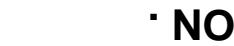


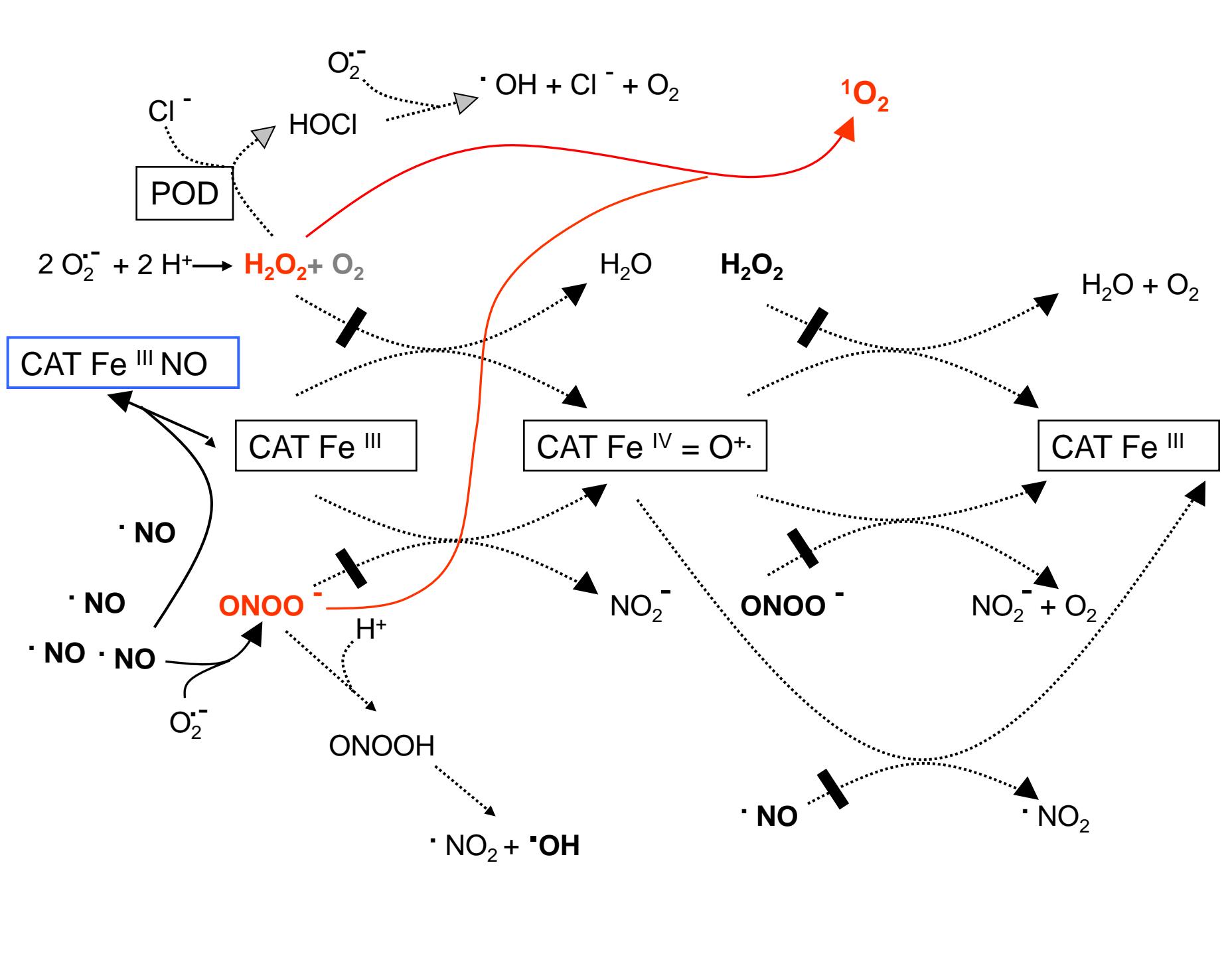
CAT Fe III NO

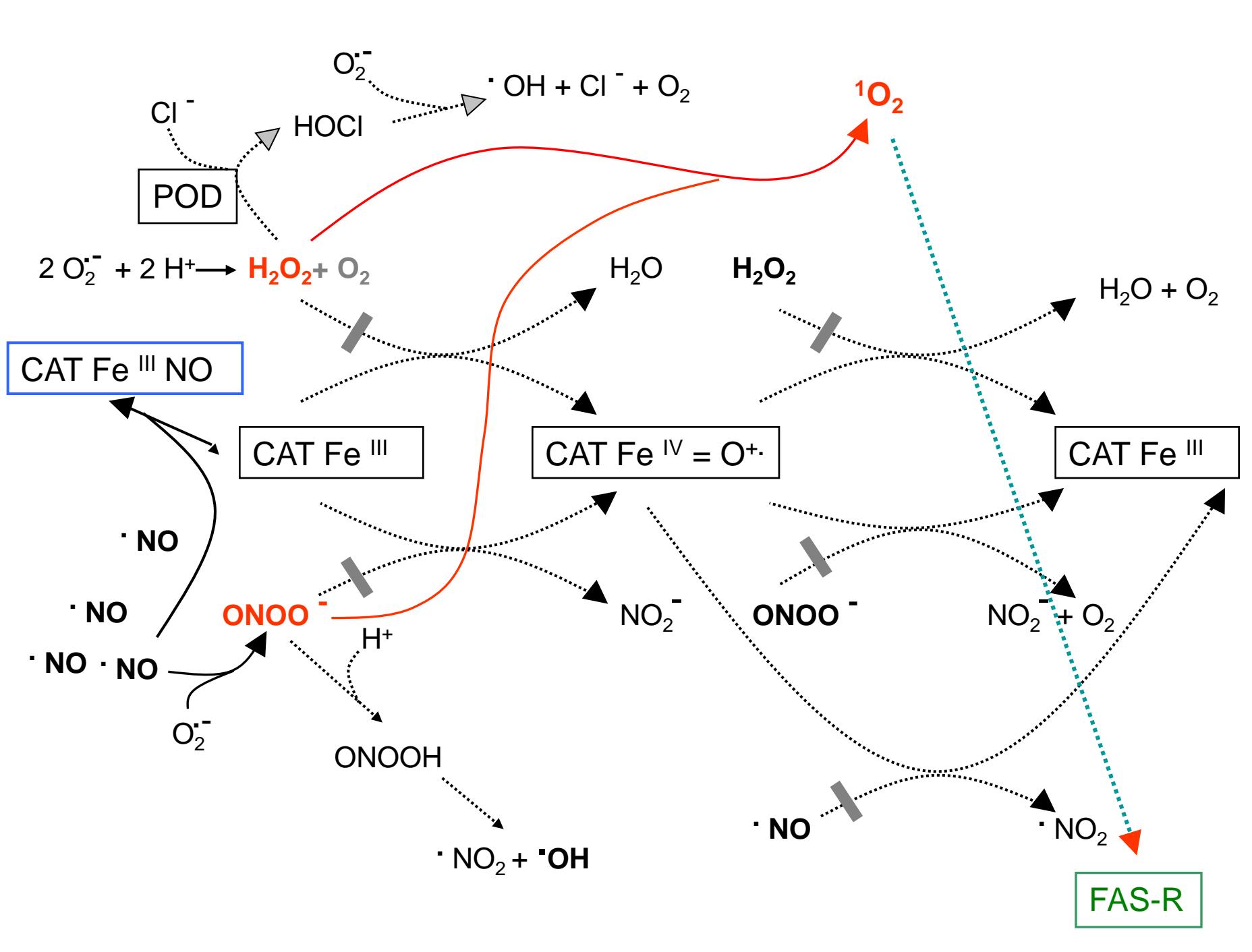
CAT Fe III

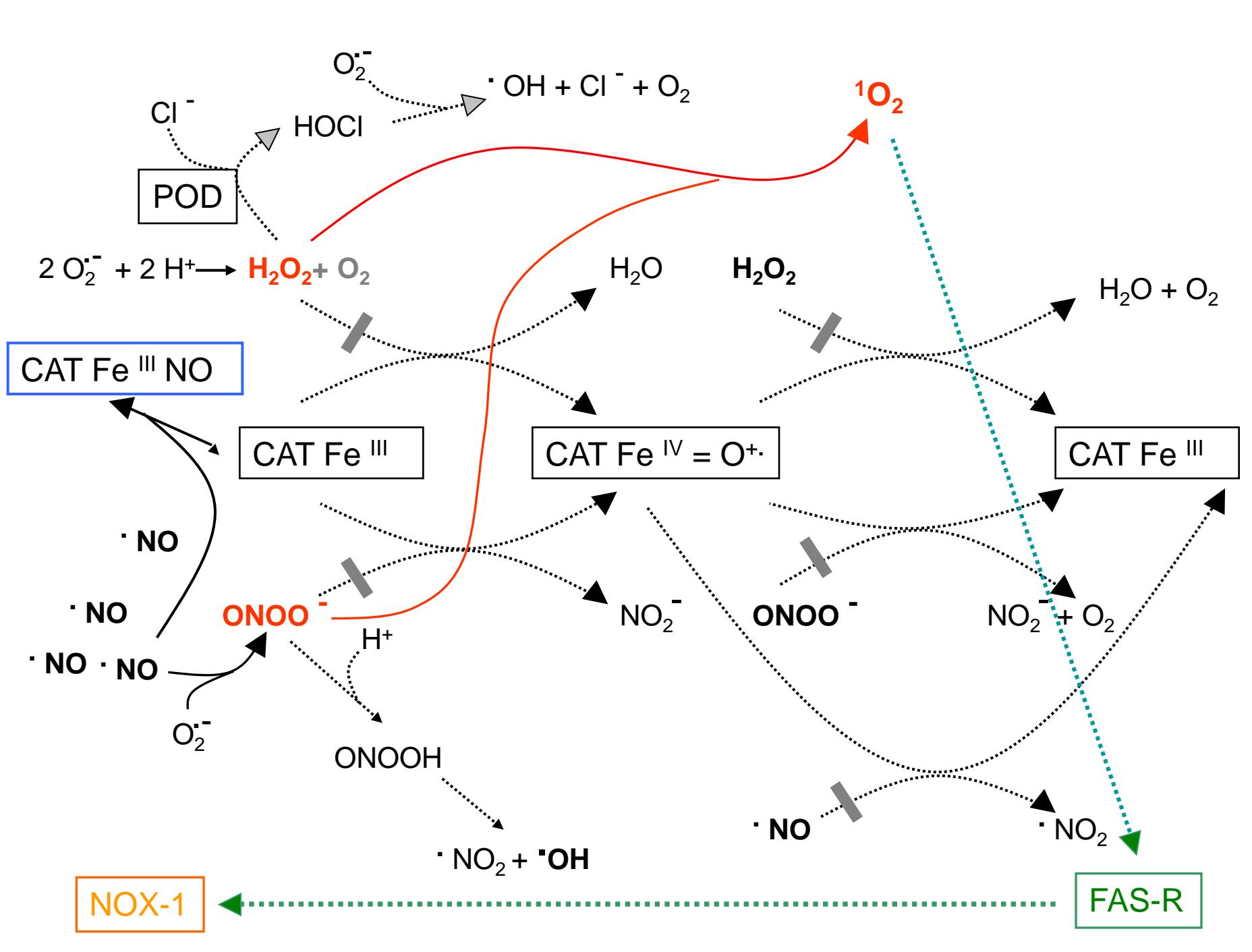
CAT Fe IV = O⁺

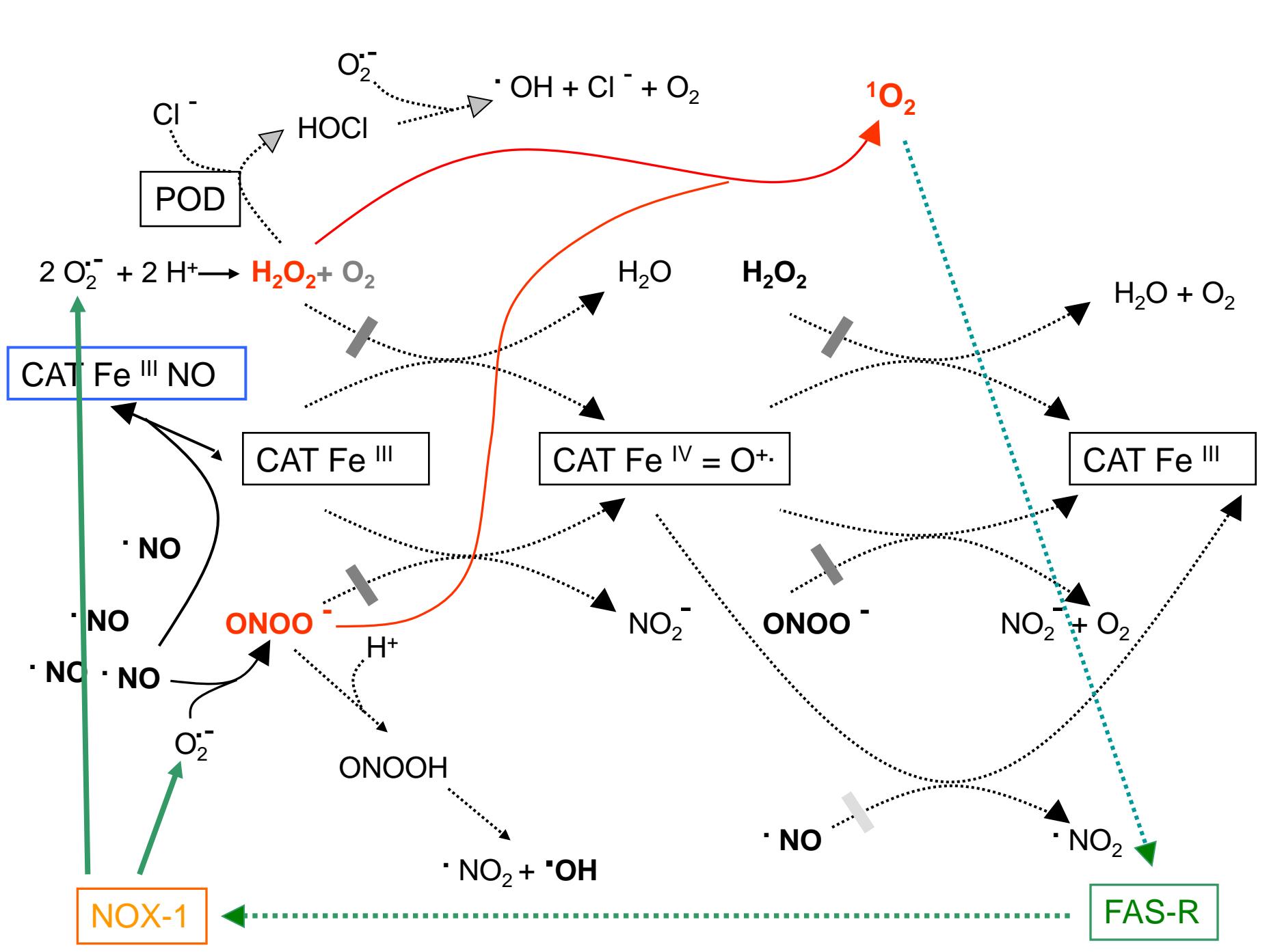
CAT Fe III

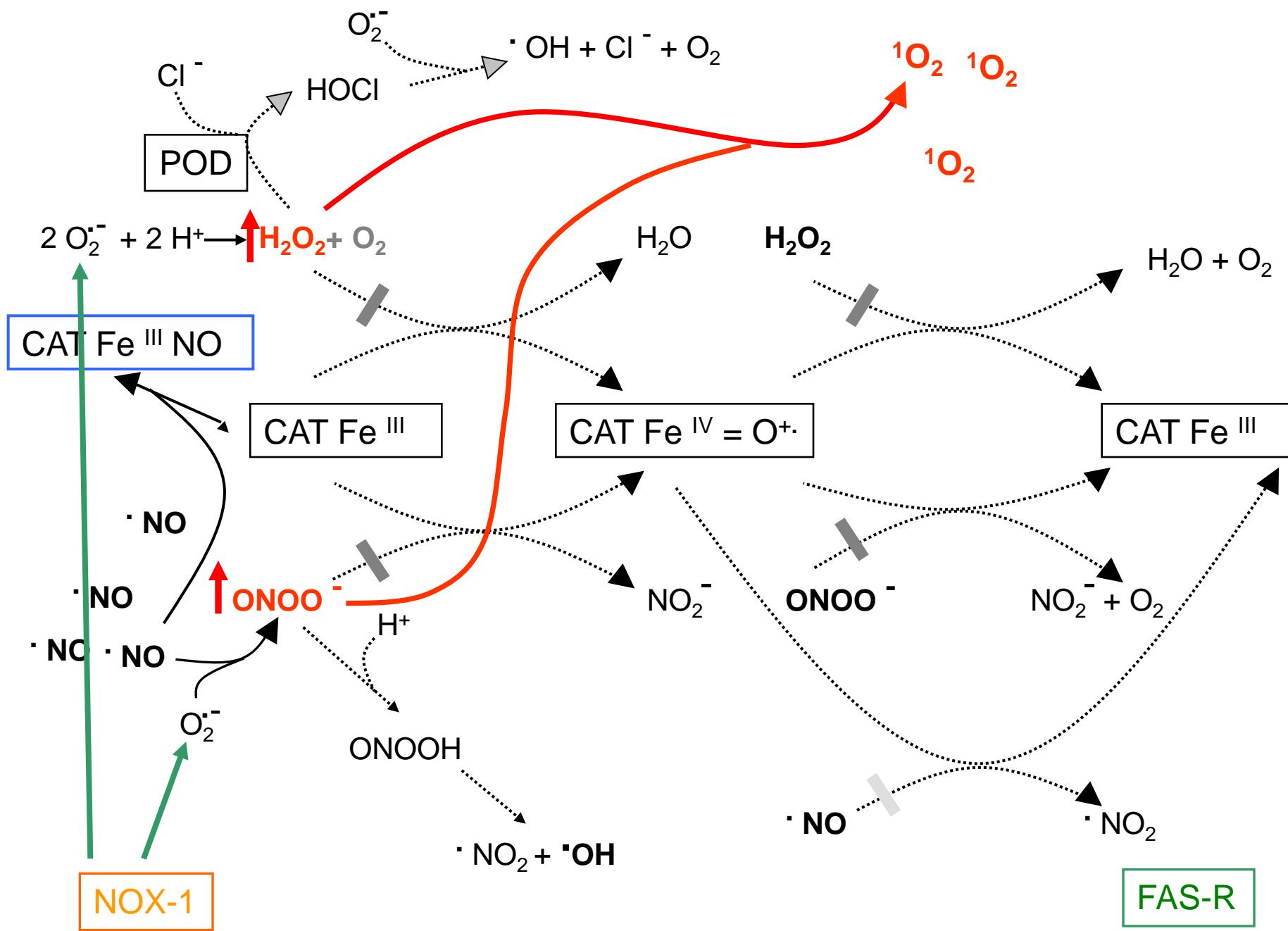


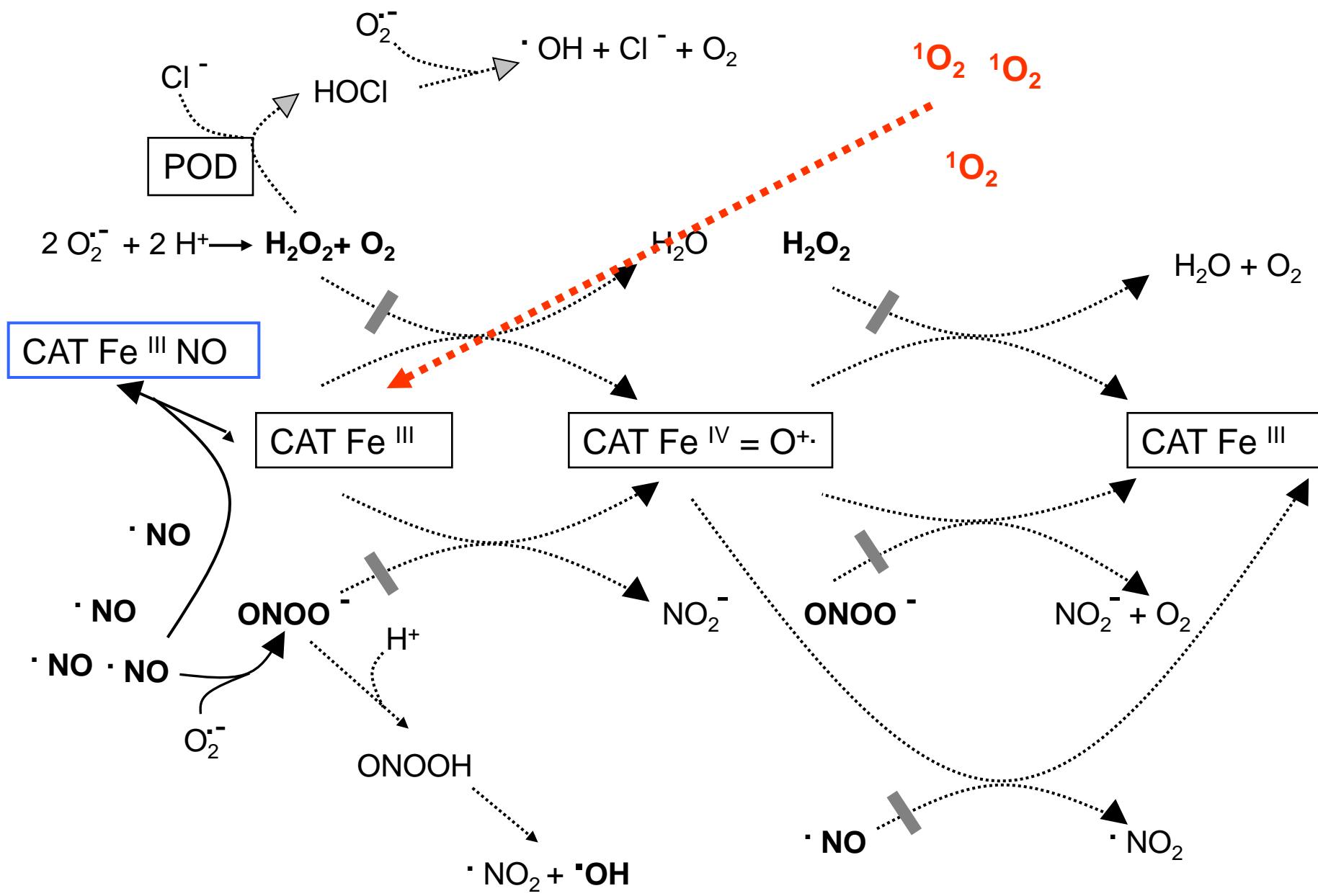


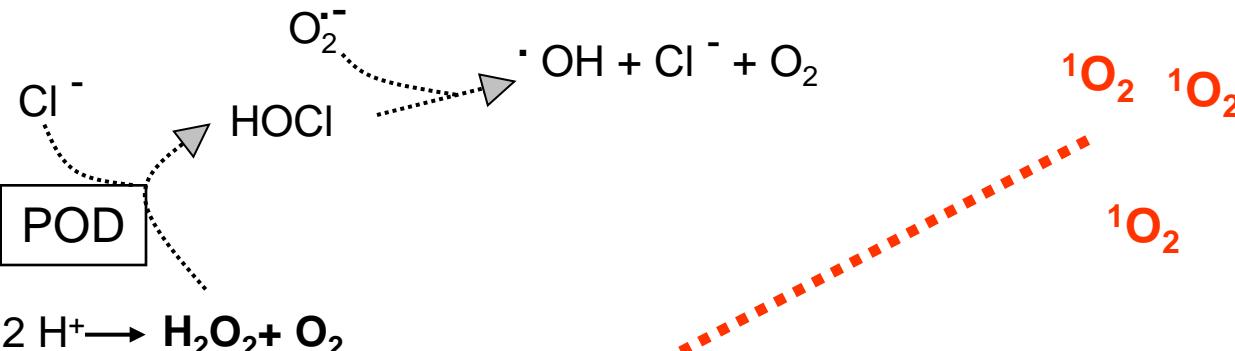






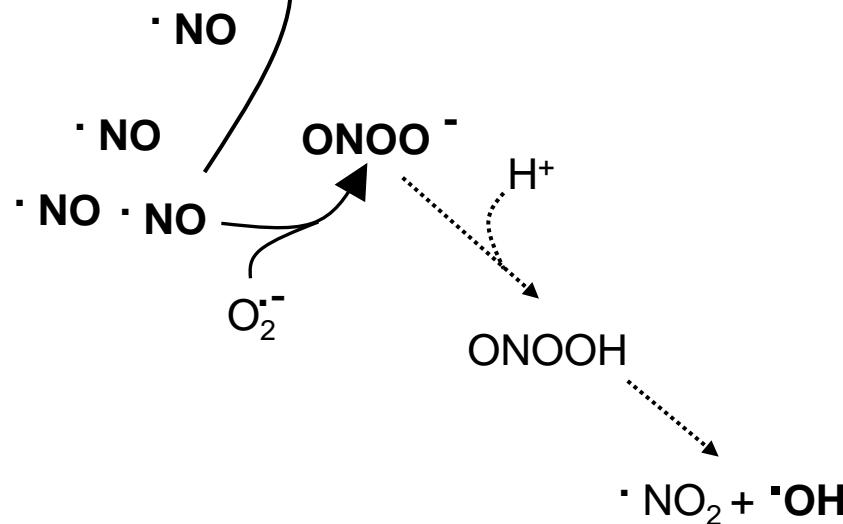


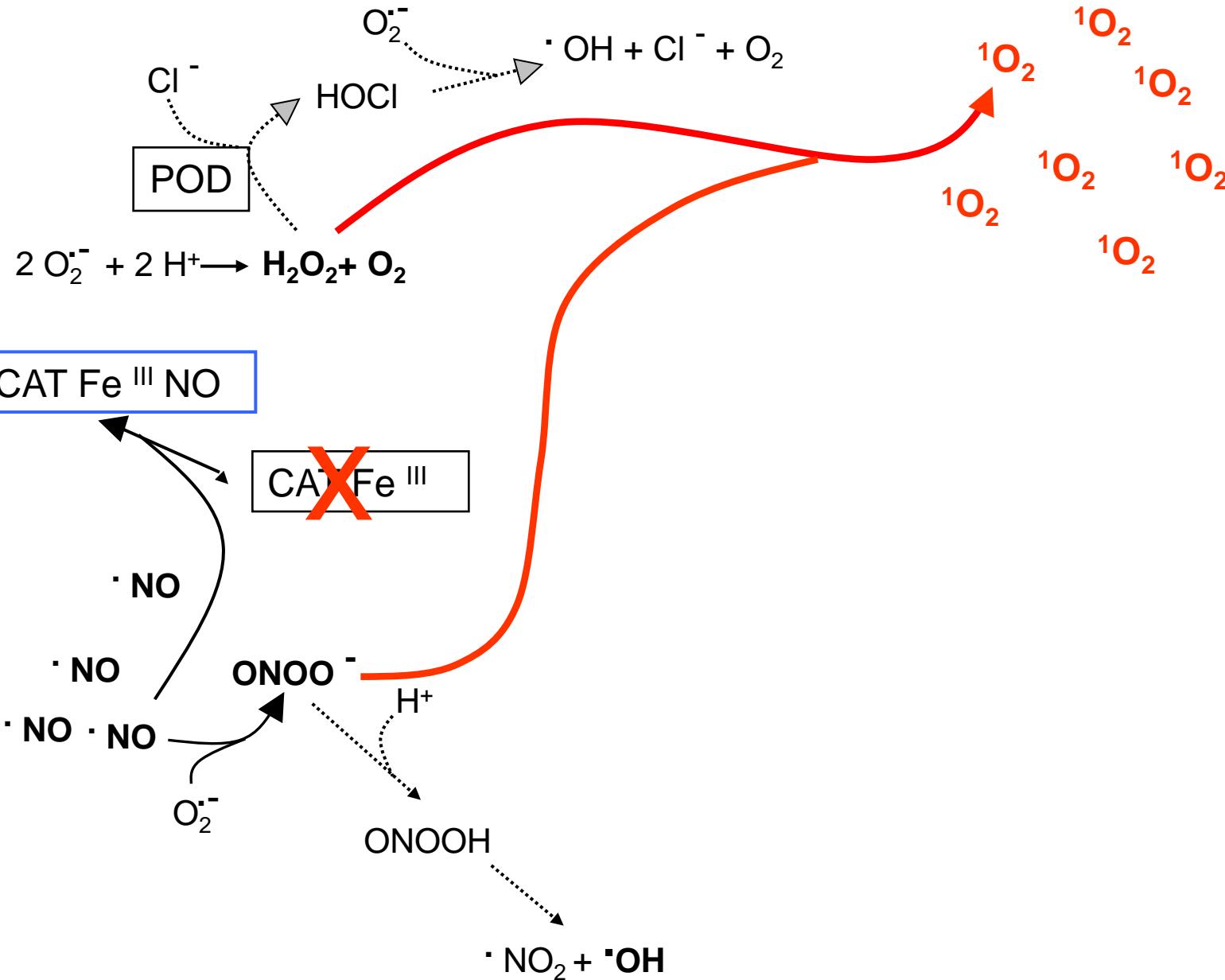


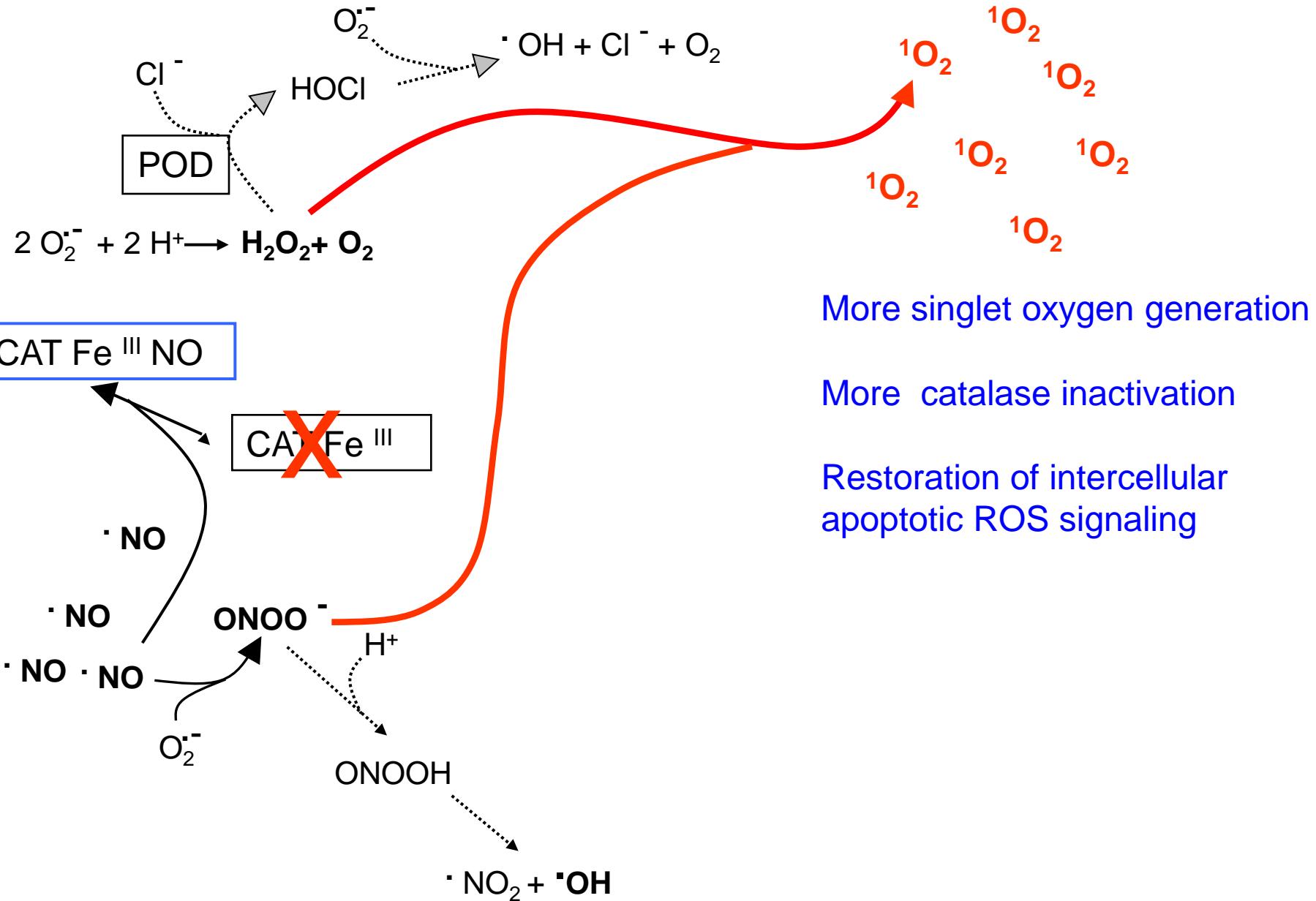


CAT Fe III NO

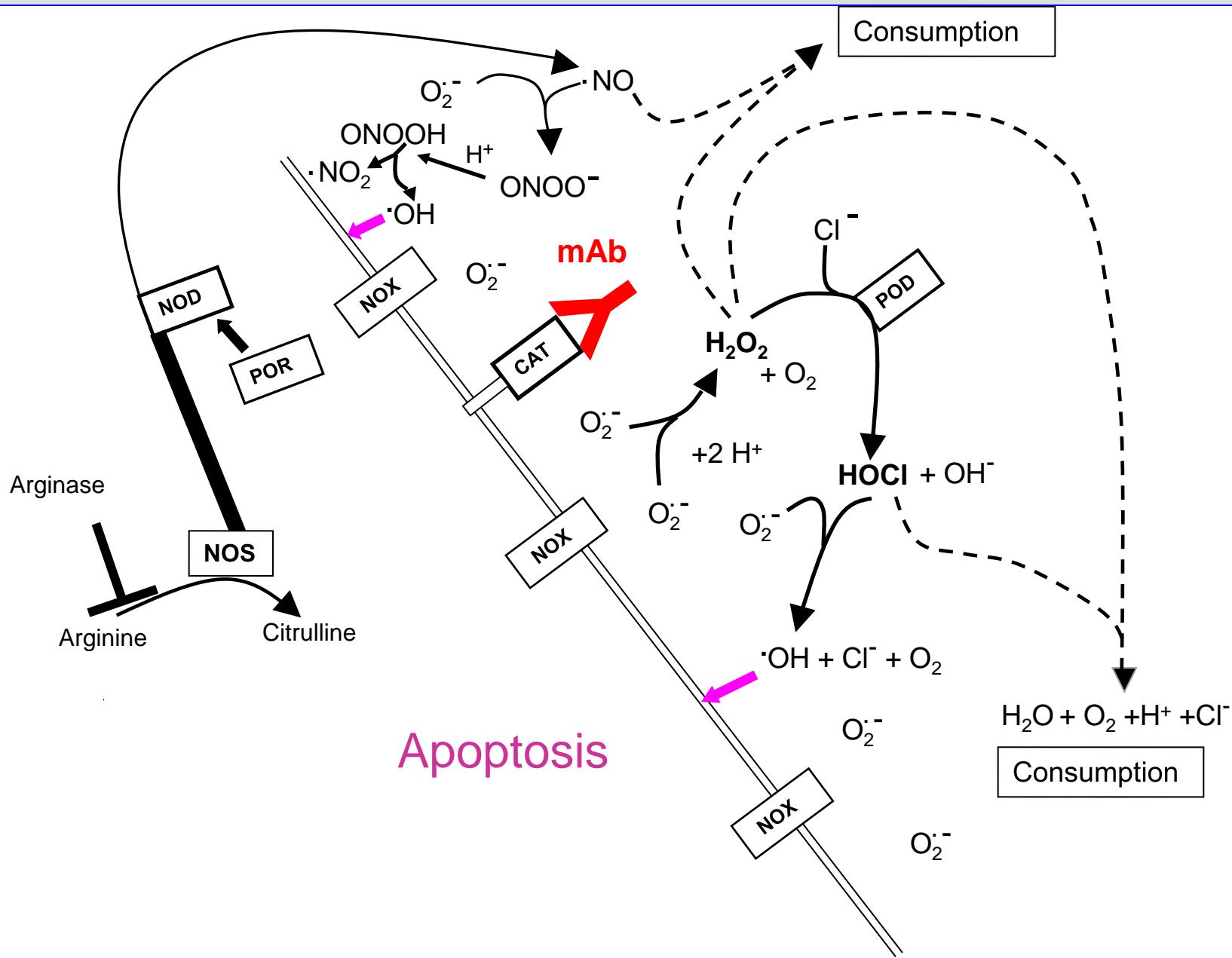
~~CAT Fe III~~



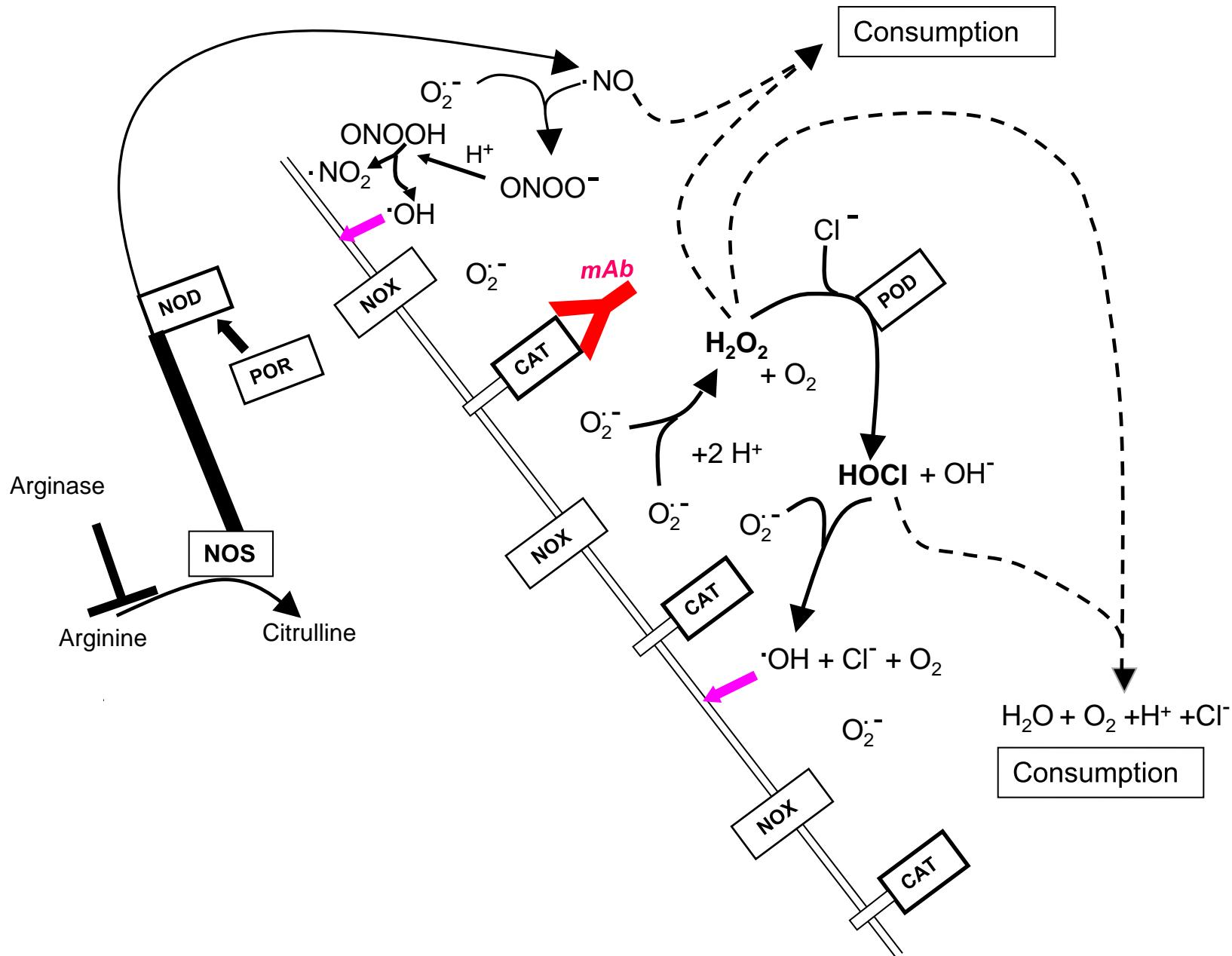


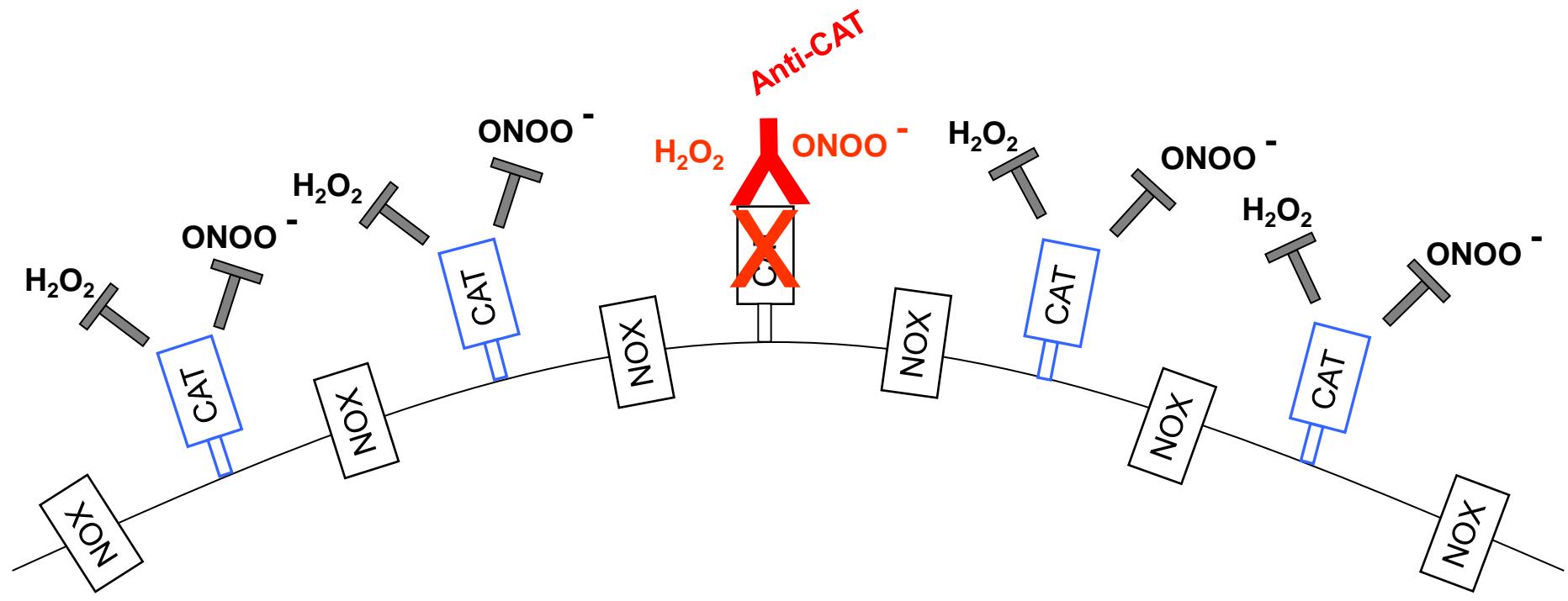


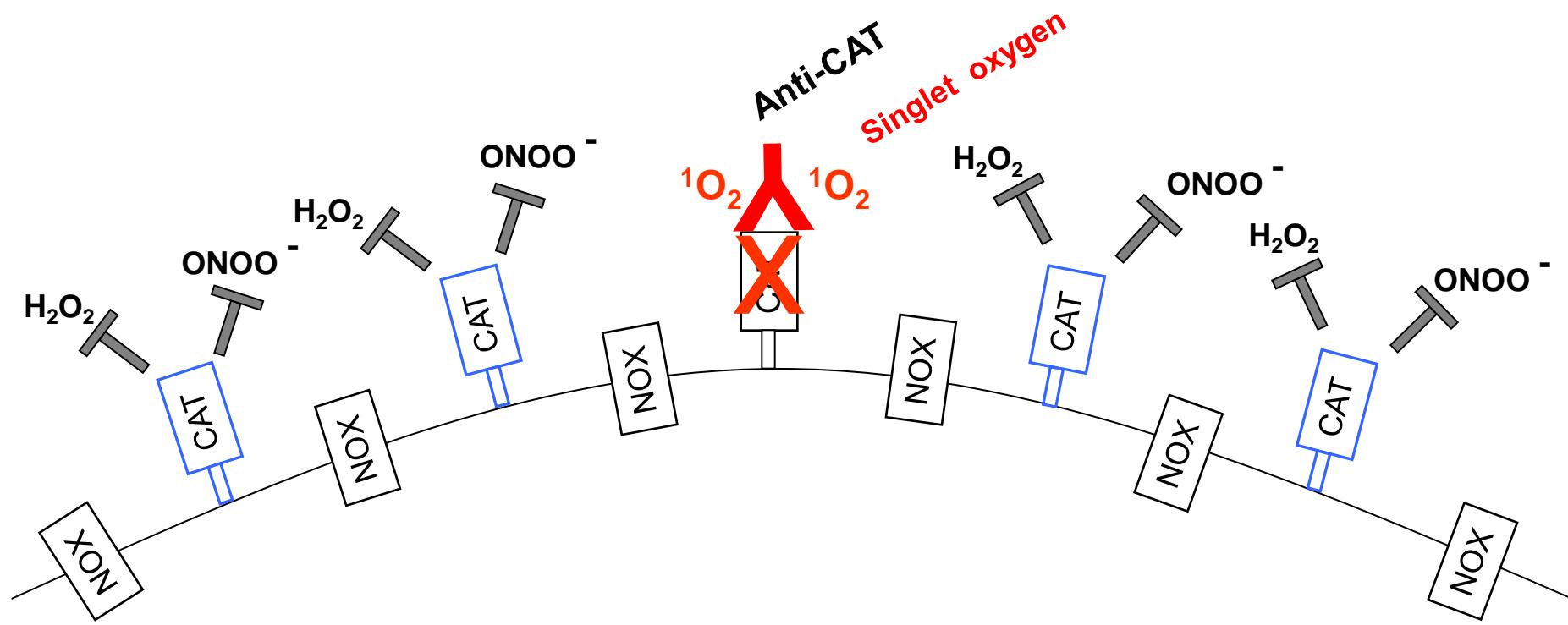
5. Inhibition of catalase by mAb sensitizes tumor cells for ROS signaling

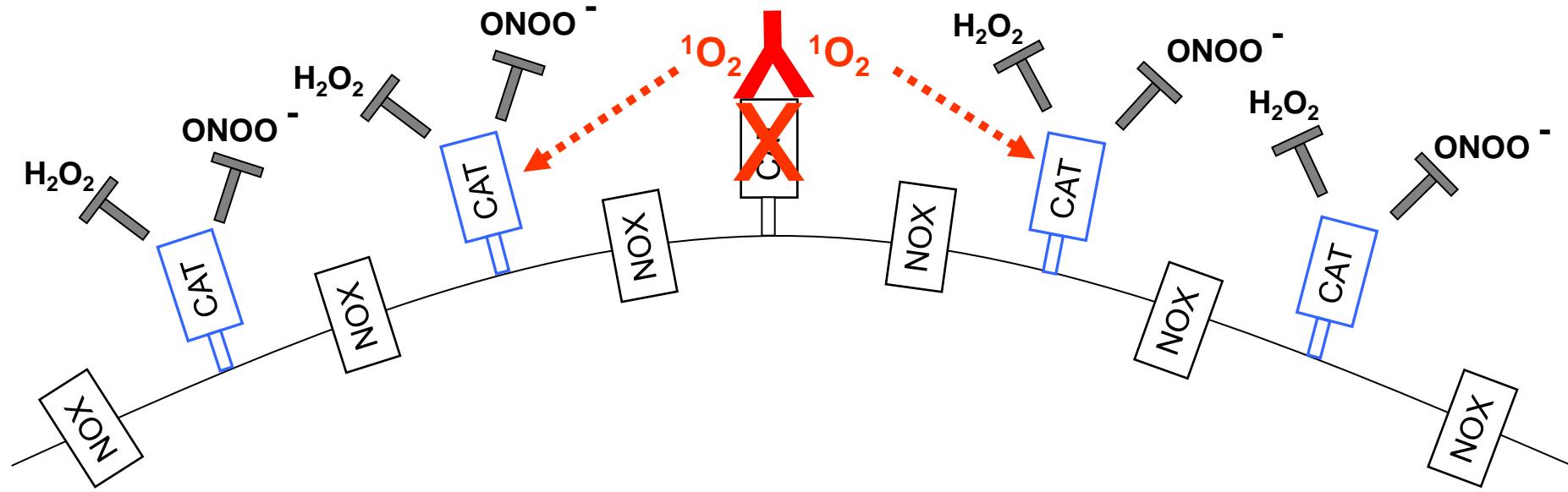


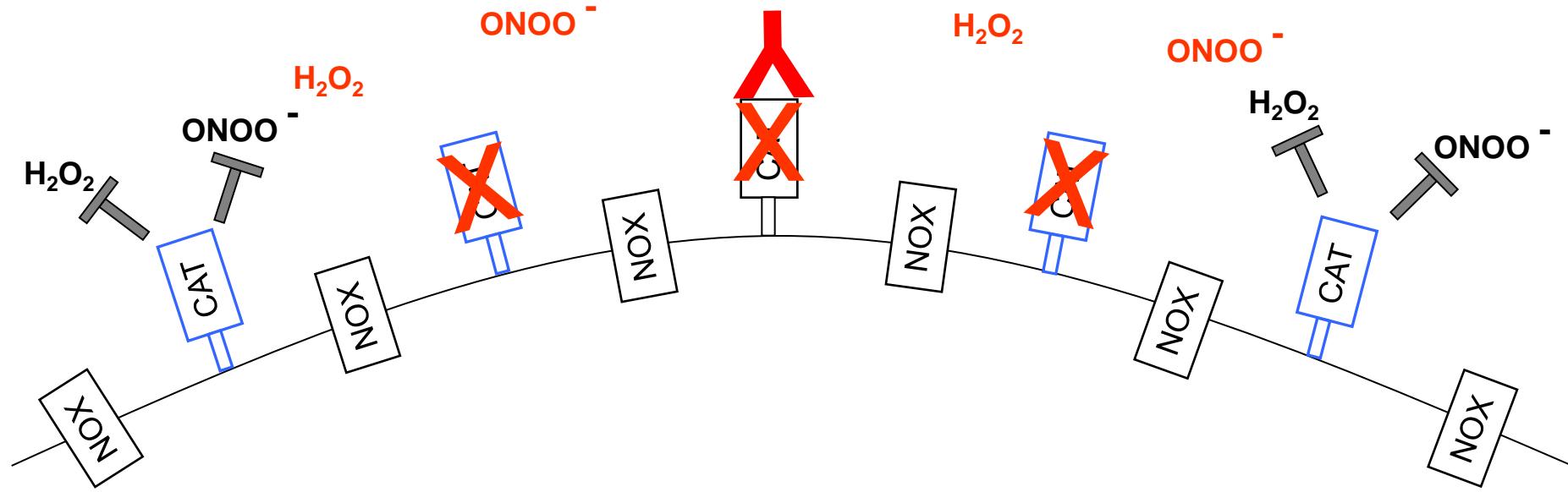
In addition to restoration of ROS signaling, anti-CAT triggers dynamic, automplificatory inactivation of catalase by singlet oxygen (next page)

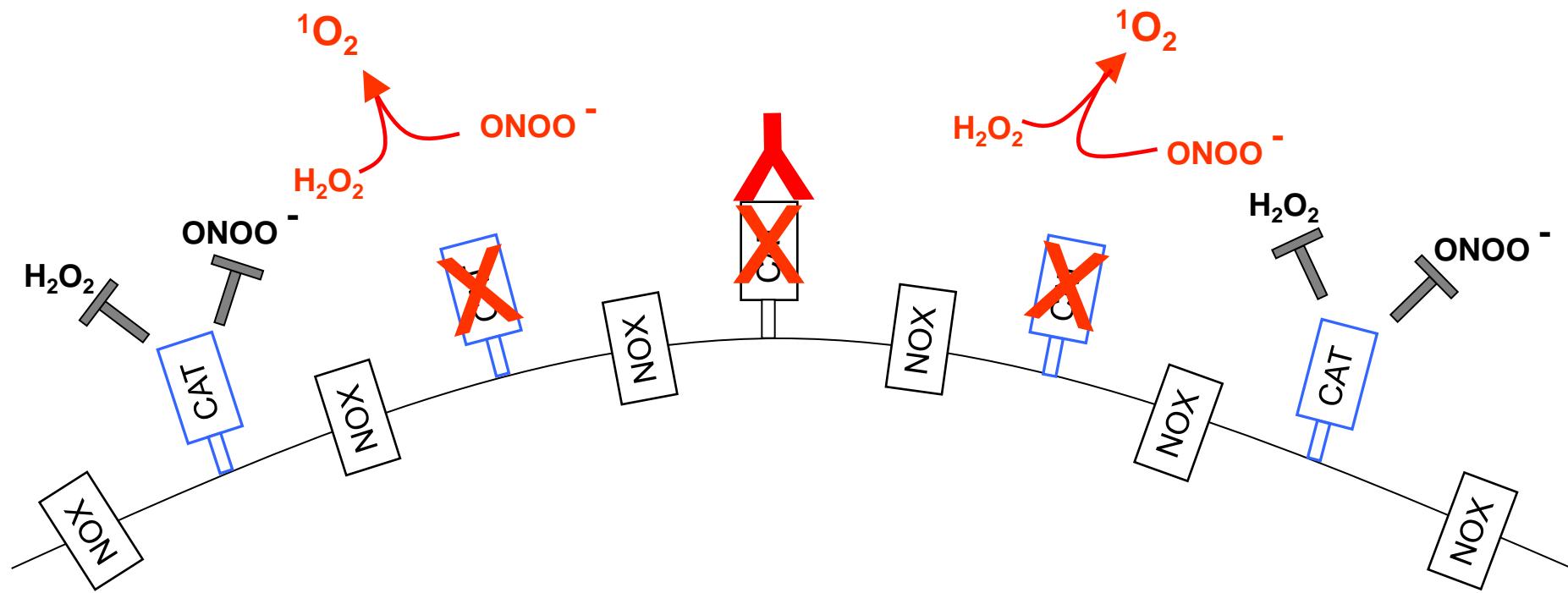


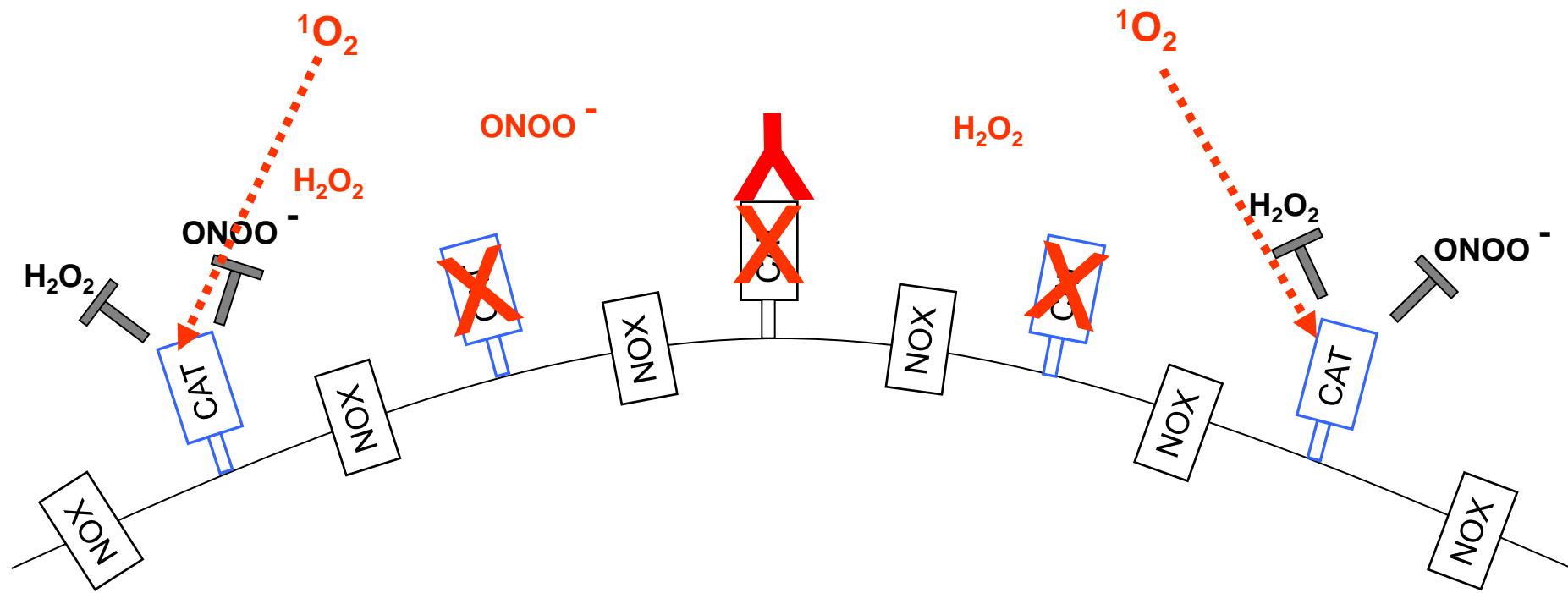


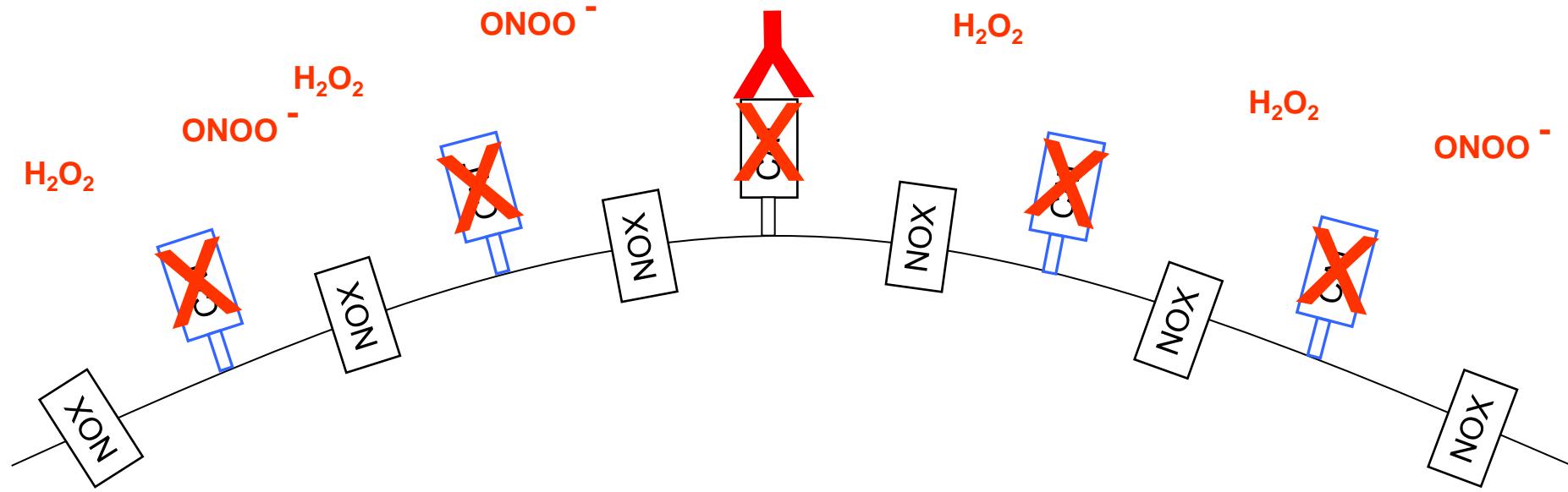


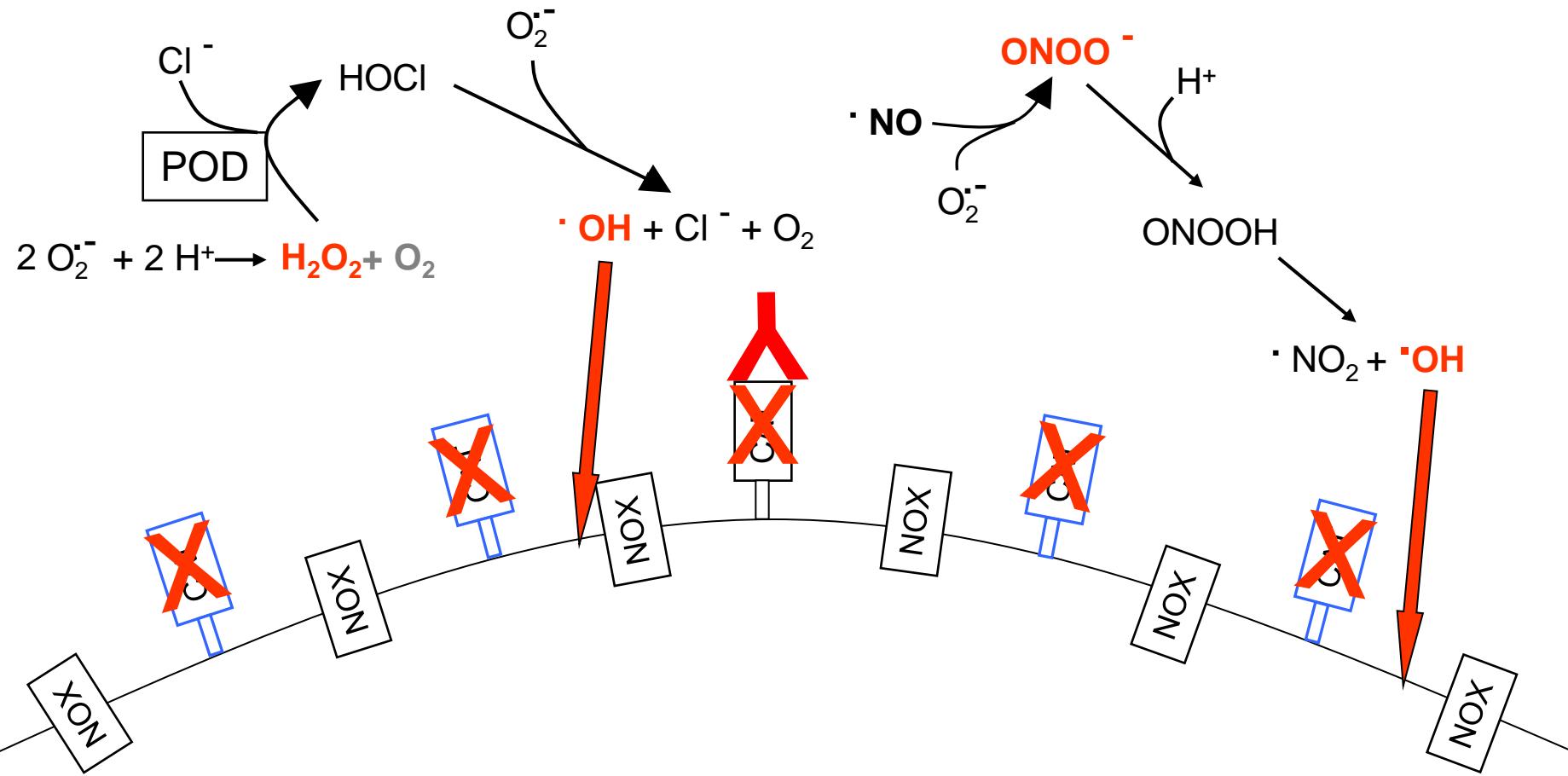


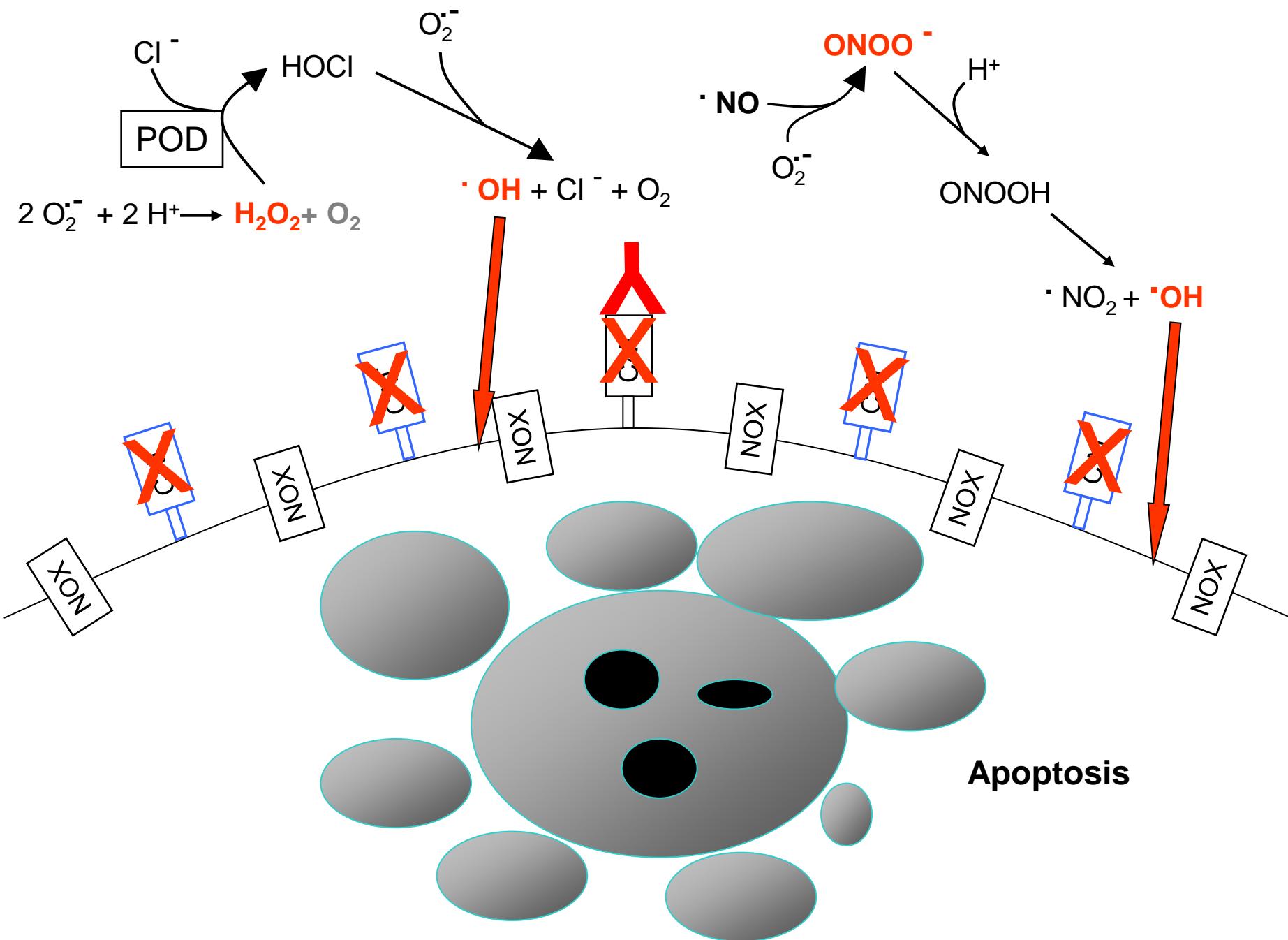












For potential future therapeutic applications we focus on

- i) recombinant neutralizing antibodies,
- ii) compounds that trigger extracellular singlet oxygen generation by tumor cells;
- iii) the utilization of synergy effects between these compounds.

These approaches are highly specific for the ROS-relevant phenotype of tumor cells (NOX1 activation combined with expression of membrane-associated catalase) and do not affect intracellular catalase of nonmalignant cells.

All three approaches have been extensively evaluated *in vitro*.

They have been proven valid for tumor cells derived from the most frequent as well as the most aggressive tumors.

Our concepts for novel tumor therapy, based on restoration of intercellular ROS signaling, are protected by several patents and patent applications.

First proof-of-concept experiments *in vivo* have been successful.

Summary:

Transformed cells generate extracellular superoxide anions.

Extracellular superoxide anions and their dismutation product H_2O_2 drive the proliferation of malignant cells.

They are also the basis for the HOCl and the NO/peroxynitrite signaling pathway that selectively eliminate transformed cells.

Tumor progression requires resistance against apoptosis-inducing ROS signaling.

Resistance of tumor cells is acquired through expression of membrane-associated catalase.

Catalase inhibits HOCl signaling through decomposition of H_2O_2 and interferes with NO/peroxynitrite signaling through oxidation of NO and decomposition of peroxynitrite.

Inhibition of membrane-associated catalase of tumor cells with monoclonal antibodies or its inactivation through singlet oxygen causes ROS-mediated apoptotic selfdestruction of the tumor cells.

This efficient and highly selective principle is a promising approach for novel strategies for tumor therapy.

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