High-Throughput Screening (HTS) by Immunoassay Tests

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High-Throughput Synthesis and Screening (HTS)

- HT Synthesis of molecules, catalysts, materials ...
- HT Screening for: molecules, macromolecules properties (biological, physical) (pure compounds or mixtures)
HTS by immunoassay tests

- HTS for enantioselective catalysts: yields & ee's
- HTS for antioxidants: protective agents against oxidative stress, selection of Norbadione A
- Total synthesis of Norbadione A

Structure of an antibody
Generation of poly- and monoclonal antibodies (Ab)

**Chemistry**
- Synthesis of the immunogen
- Synthesis of a functional derivative of H
- H₂N → BSA
- H → BSA

**Biology**
- Injection of the immunogen
- Polyclonal response
- Selection of the mouse with the best response
- Cell fusion
  - ~ 10³ hybridomas
  - selection of anti-H Ab
- Multiplication by cloning
  - ~ 10 Monoclonal Ab
  - anti-H

Competitive EIA
(Enzyme ImmunoAnalysis)

- Capacity > 1000 analysis/day
- Capture
- Wash
- S
- Color (DO): Ellman reagent
- E = Acetylcholinesterase (AChE)
Competitive EIA
(Enzyme ImmunoAnalysis)

\[ \text{Y} + \text{K}^* \rightarrow \text{K} \]
\[ B/B_0 \]

\[ \text{B/Bo} \]

DO max

\[ \text{Log } [\text{H}] \]

\[ \text{Ellman reagent} \]

\[ \text{Acetylcholinesterase (AChE)} \]

Color (DO): Ellman reagent

Principle of AChE detection with Ellman reagent

Acetylcholinesterase

Acetylcholine + Ellman reagent → YELLOW COLOR

Reading of the DO
New chemoluminescent probes

Design and synthesis of chemoluminescent probes for the detection of cholinesterase activity
P.Y. Renard

Screening for new catalysts

A Catalyst B

Classical approach

Conception of a catalyst
Synthesis
Evaluation

Combinatorial approach

Conception of a library of catalysts
High-throughput screening
Parallel Synthesis
Asymmetric catalyst

Combinatorial chemistry

\[ R\text{COOH} \rightarrow \text{Asymmetric catalyst} \rightarrow R\text{COOH} + R\text{HO}COOH \]
5 AB recognize both enantiomers of MA
5 AB are selective for MA-S
1 AB is selective for MA-R

11 Monoclonal antibodies (Ab)

Combinatorial chemistry

Asymmetric catalyst

yield

yield

ee
Conception catalysts’ library

4 M = 
(2 solvents)

[RuCl₂(p-cym)]₂
[RuCl₂(p-benz)]₂
[RhCl₃(Cp)]₂
[IrCl₂(Cp)]₂

2 Sources of hydrogen

HCOOH/TEA
KOH/iPrOH

6 Solvents

DMF, DMF/H₂O, EtOH, DMSO
DMF/EtOH, DMF/DMSO

22 Ligands

NHR₁
NHR₂

Screening results

Yields

0-10% 10-30% 30-50% 50-70% 70-90% 90-100%

ee(S)

0-10% 10-30% 30-45% 45-60% 60-75% 75-90%

High-Throughput Screening of Enantioselective Catalysts by Immunoassay.
HTS tests for the ee determination

- IR thermography (Reetz and coll. 1999)
- Capillary array electrophoresis (Reetz and coll. 2000)
- CD-HPLC (Mikami and coll. 2001)
- Electrospray ionization with isotopically labeled substrates (Reetz and coll. 1999)
- Immunoassay

HTS

- HTS for enantioselective catalysts: yields & ee’s
- HTS for antioxidants: protective agents against oxidative stress; selection of Norbadione A
- Total synthesis of Norbadione A
Protections against the oxidative stress

- Radioprotectors
- Decorporation
- Antioxidants
- UV radiations
- Free radicals: 'OH, OOH, O₂⁻
- Chelates
- Fe; Cu

Oxidative Stress

- Active Oxygen Species
- DNA strand break
- Abasic site formation
- Base degradation
- Addition of lipid peroxidation products
- PROTEIN
Antibodies’ generation

3 monoclonal antibodies

Thymidine

Antibodies’ generation

Log [Thymidine] [µM]

B/Bo (%)

0.01
0.1
1
10

10,0

25

50

75

100

mAb-62

High-throughput screening of new protective agents against oxidative stress

Fe/EDTA

137Cs

UV

H₂O

H₂O₂

HO⁻ ; HOO⁻

= Protective agent

= Degradated thymidine

- Competitive binding
- wash

- Competitive binding
- wash
Strategy for the discovery of new protective agents against oxidative stress

Lead optimisation for the synthesis of analogs

Toxicological evaluations
Physical determinations
Mechanism of action studies

Biological activities:
- anti-inflammatory
- anti-proliferative
- anti-cancer
- anti-aging
- radioprotective

Tests:
Cellular
Tissular
In vivo

different sources:
Plants, mushrooms, algaes...

Extraction
Synthesis

Libraries of Molecules

in vitro screening antioxidant activity

Mushroom extracts – UV degradation tests

Extractions:
- MeOH / Acetone / HCl
- CHCl3 then MeOH

Experimental conditions:
Thymidine (70µM) ; H2O2 (5mM) ;
irradiation at 254nm 1.75 J/cm²
Tp Phosphate 25mM pH 7.4

% of thymidine protection

Sarcodon Repandum
Cantharellus Cibarius
Pleurotus Ostreatus
Amethystus
Pisolithus Tinctorus

Norbadione A
Oxidative stress

- 70 commercial molecules
- 10 synthetic molecules
- 10 natural extracts

tested in 4h
1 highly efficient molecule found

Protection (%)
- 0-10%
- 10-30%
- 30-50%
- 50-80%
- 80-100%

Norbadione A

Antioxidative properties

Thymidine 70 μmol
UV 254nm 1.75 J/cm²
H₂O₂ 5mM in H₂O
antiox. 100 μmol

FeSO₄ 0.35 μmol +
H₂O₂ 35 μmol in H₂O

γ Ray 3h

1- Norbadione
2- Quercitine
3- Fisetin
4- Myricetin
5- Catechin
6- 4-hydroxy-4-methyl-8-nitrocoumarine
7- Trolox
DNA protection against \( \gamma \) radiations

Plasmide pUC18, phosphate buffer pH 7.4
Irradiation \(^{137}\)Cs, 30 min. (60Gy)

Norbadione Analogue

Radio- & chemoprotecting effects of Norbadione A

X Rays

Cisplatine

Survival rate of cells increased with Norbadione A

Gyotoxicity of cisplatine lowered by Norbadione A
Mushrooms and radioactivity

- In 1986, radioactive particles from the electrical plan of Tchernobyl contaminated several European countries including several French regions. Bolet bai, a commestible mushroom, contains high concentrations in cesium 137.
- In 1989, Steglich found that cesium 137 is selectively localised in the pigments present on the top of the mushroom in association with norbadione A.
- Norbadione A can be extracted in higher quantities from another mushroom Pisolithe (M. Gill).

Cesium complexation by Norbadione A: mass spectrum studies

- Speciation of norbadione in presence of Cs⁺
- The reaction of Norbadione A with cesium ions can form 1:1 and 1:2 complexes.
- The dissociation constants are: $\beta_1 = 4.9 \text{ l.mol}^{-1}$ for the 1:1 complex and $\beta_2 = 9.5 \text{ l.mol}^{-1}$ for the 1:2 complex.
Decontamination by decorporation with a chelating agent

Individual contaminated by the radioelement

Absorption of Norbadione A

Elimination of the Norbadione complexed cesium in the urine and faeces

Norbadione A, a radioprotective agent with a double mechanism of action:

1- detoxification by specific chelation and elimination of $^{137}\text{Cs}$ (decorporation)

2- antioxidant properties to capture the reactive oxygen species generated by $\gamma$ Ray emitted from $^{137}\text{Cs}$
HTS

- HTS for enantioselective catalysts: yields & ee’s
- HTS for antioxidants protective agents against oxidative stress selection of Norbadione A
- Total synthesis of Norbadione A

Norbadione A: retrosynthetic scheme

[Diagram showing the retrosynthetic scheme of Norbadione A with reactions and structural formulas]
Conclusions

- Two high-throughput screenings (HTS) by immunoassay tests for the selection of:
  - catalytic systems
  - selection of highly active antioxidant agents

- Total synthesis of Norbadione A a potent radioprotective agent selected by HTS immunoassay tests
# Acknowledgments

<table>
<thead>
<tr>
<th>SMM</th>
<th>Thierry Le Gall</th>
<th>Alain Valleix</th>
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Validation of the Enzyme Immuno Assays (EIA)

Validation on \( n = 42 \) samples

\[
y = 0.9836x - 0.464 \\
R = 0.9324
\]

\[
y = 1.0312x - 0.464 \\
R = 0.9024
\]

Antioxidant Tests

Radical sources

Target molecule

degradation products

+ Antioxidant

<table>
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<tr>
<th>idem but</th>
<th>higher conc. of unaffected target</th>
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<tbody>
<tr>
<td>conc. determination of the unaffected target</td>
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Immunoanalysis

- Concentration determination of one of the oxidized product
- idem but signal inhibition

HPLC  UV Abs.  Electrophoresis  RPE  Luminescence
Tests for the evaluation of antioxidant properties of single compounds or mixtures

- TBA method (thiobarbituric acid): inhibition of the oxidation of deoxyribose (1959)
- HPLC: inhibition of the hydroxylation aromatic compounds (1984)
- TRAP method (total peroxyl radical trapping parameter) (1987)
- RPE: inhibition of the DMPO-OH radical generation (1990)
- Randox-TEAC method (Trolox equivalent antioxidant capacity): decoloration of the ABTS radical (1993)
- Electrophoresis: inhibition of the split of DNA strand (1993)
Degradation by Radiolysis

- Irradiation: 1h – 150 Gy
- 

\[
\text{Thymidine eq. (µM)}
\]

- HPLC

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
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<tbody>
<tr>
<td>AU (267nm)</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
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Fenton type degradation

- Fenton: Fe/EDTA/H$_2$O$_2$ (1:1:100) 5min.

\[
\text{Thymidine eq. (µM)}
\]

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<td>AU (267nm)</td>
<td>0.2</td>
<td>0.15</td>
<td>0.1</td>
<td>0.05</td>
<td>0</td>
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- EIA

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<th>100</th>
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<tr>
<td>[Thymidine] eq. (µM)</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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PdCl₂(dppf), cat. Na₂CO₃ aq. THF, reflx, 3h 63%