

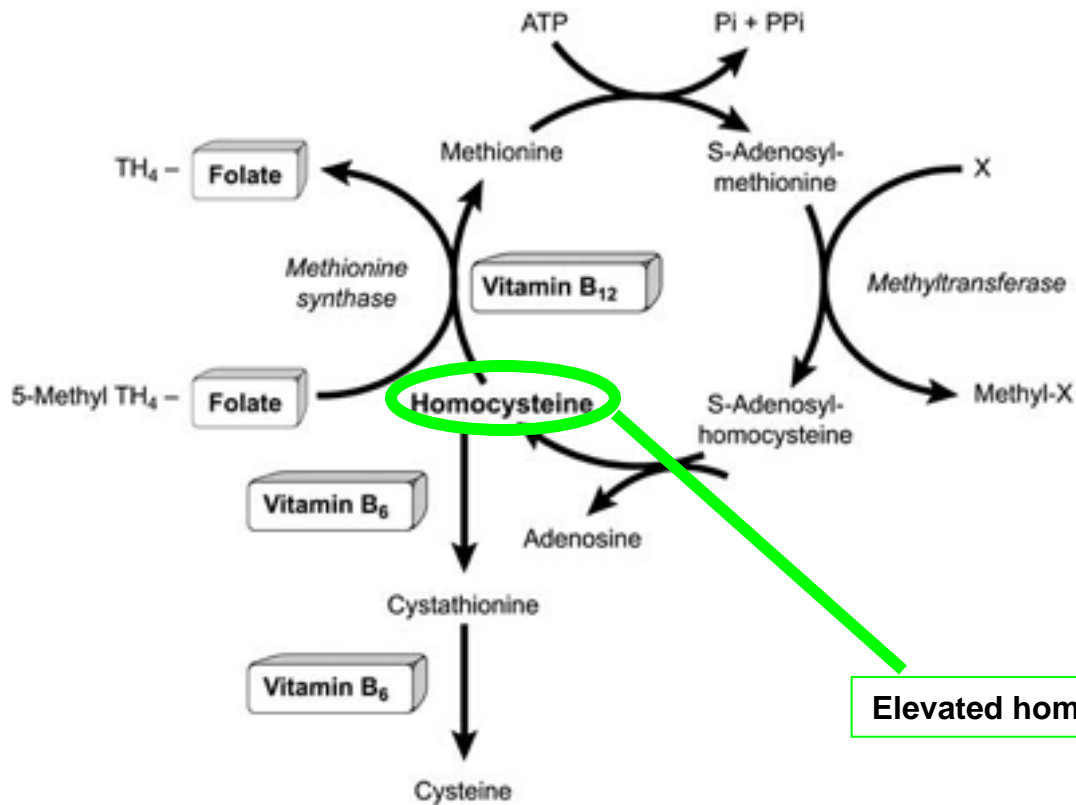


Laurentian University  
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## Development of a diagnostic kit for homocysteine, a biomarker for Cardiovascular Diseases

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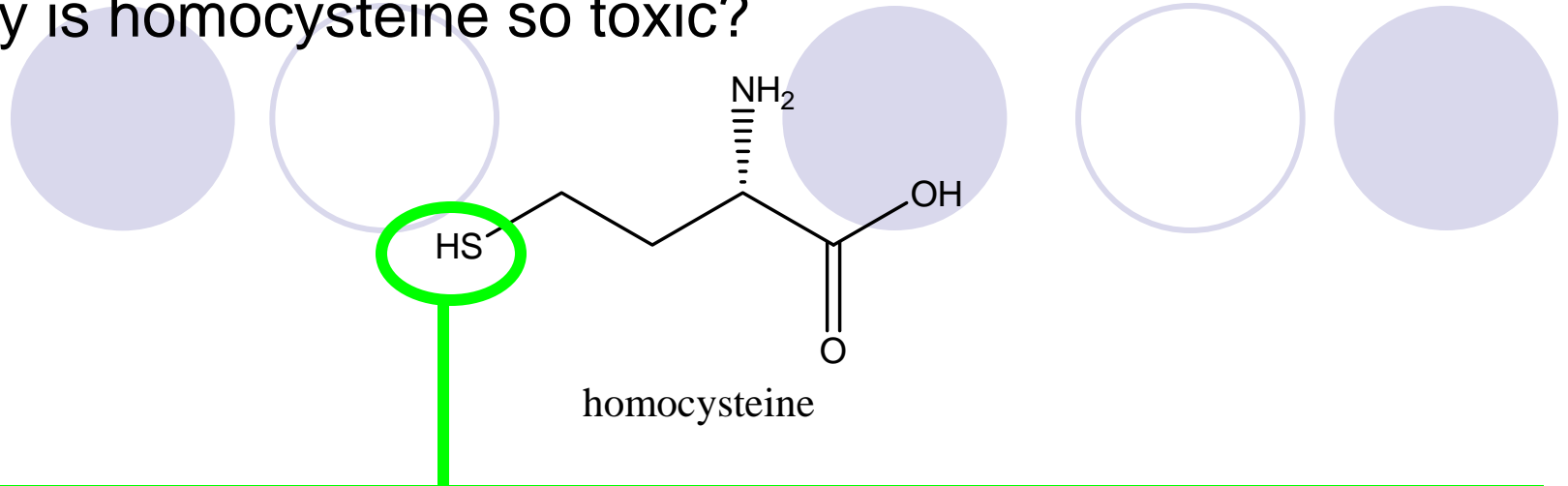
# What is homocysteine?



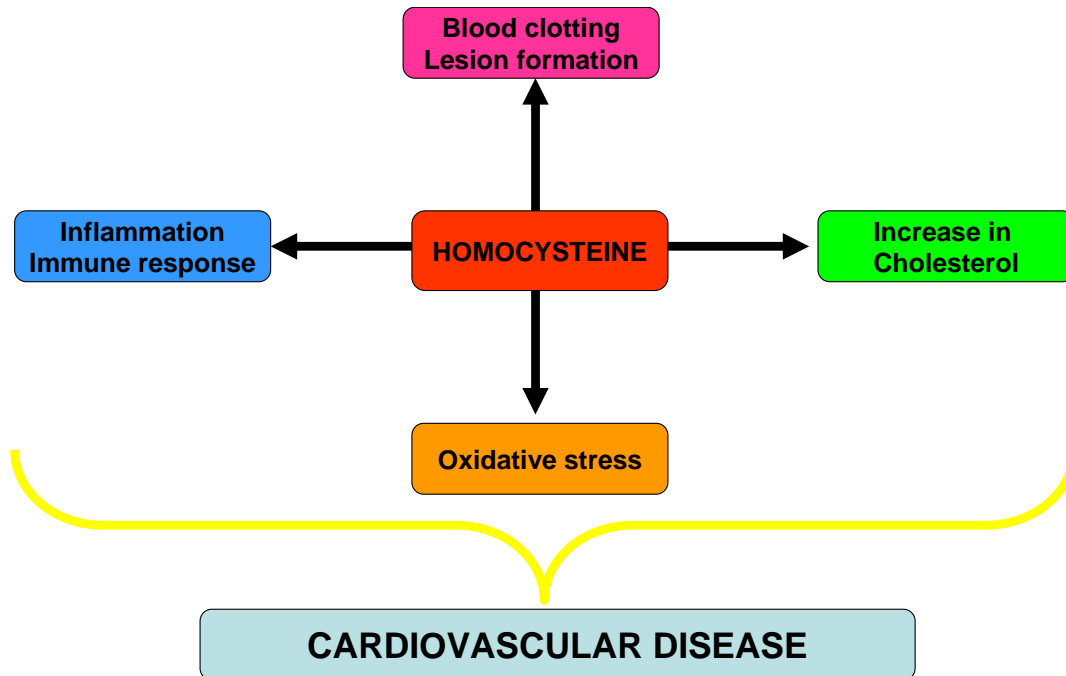
- Homocysteine is a sulfur-containing amino acid
- Produced following the methylation of target proteins
- Homocysteine is metabolized in the human liver.
  - Methylation
  - Trans-sulfuration

Elevated homocysteine levels can be quite toxic!

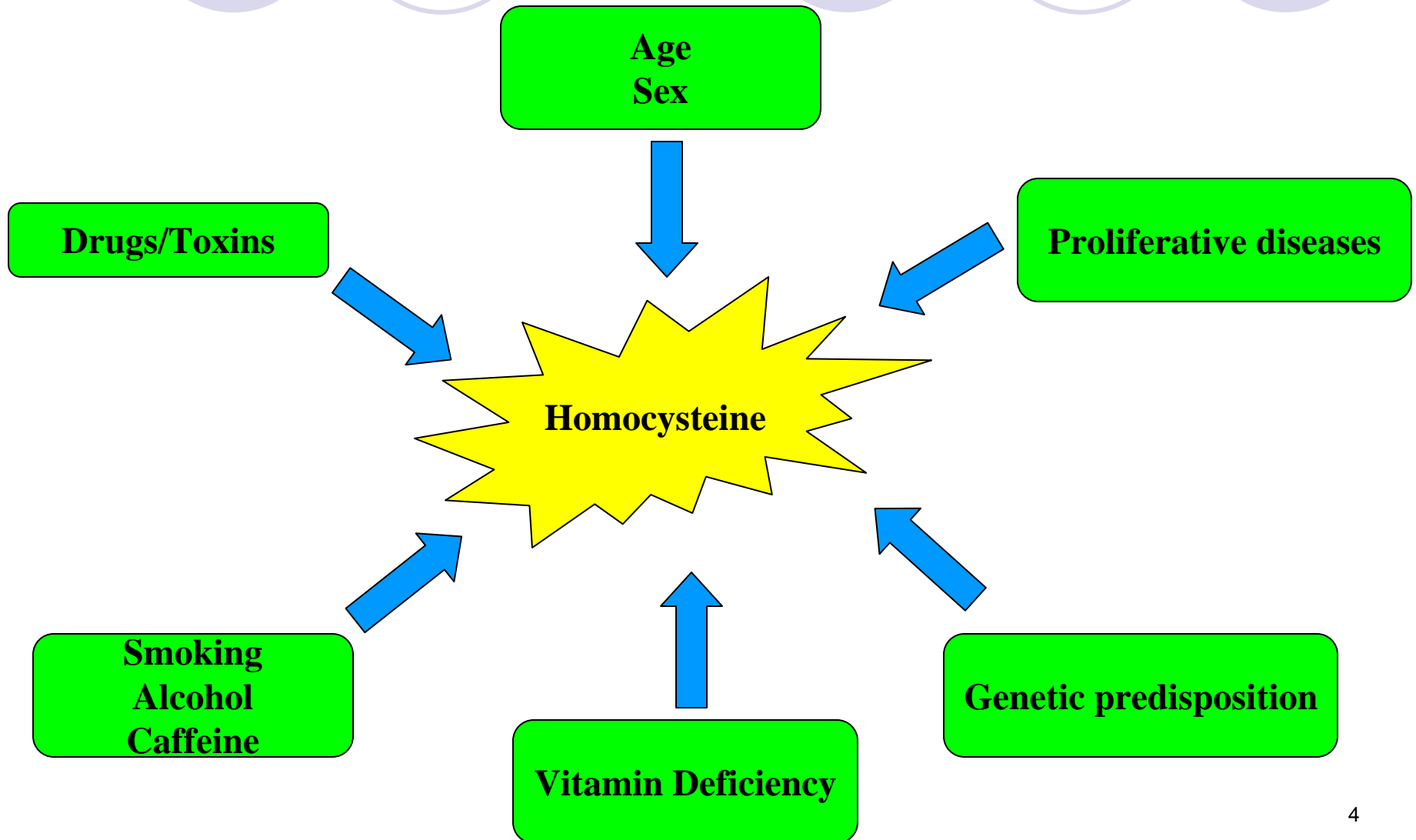
# Why is homocysteine so toxic?



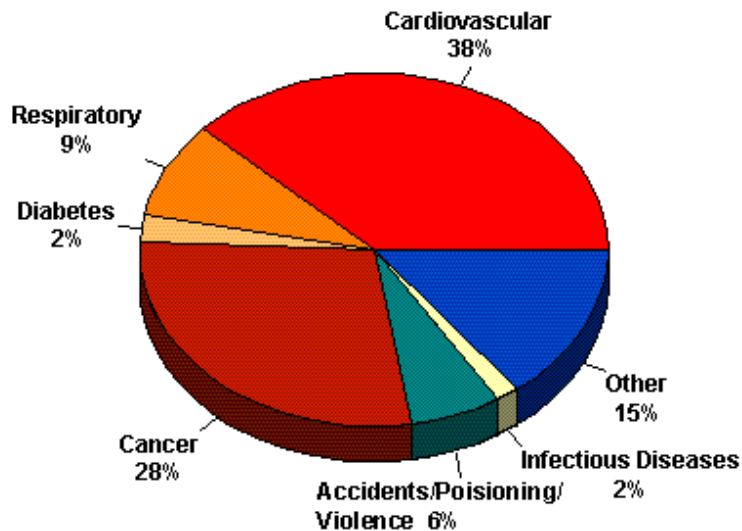
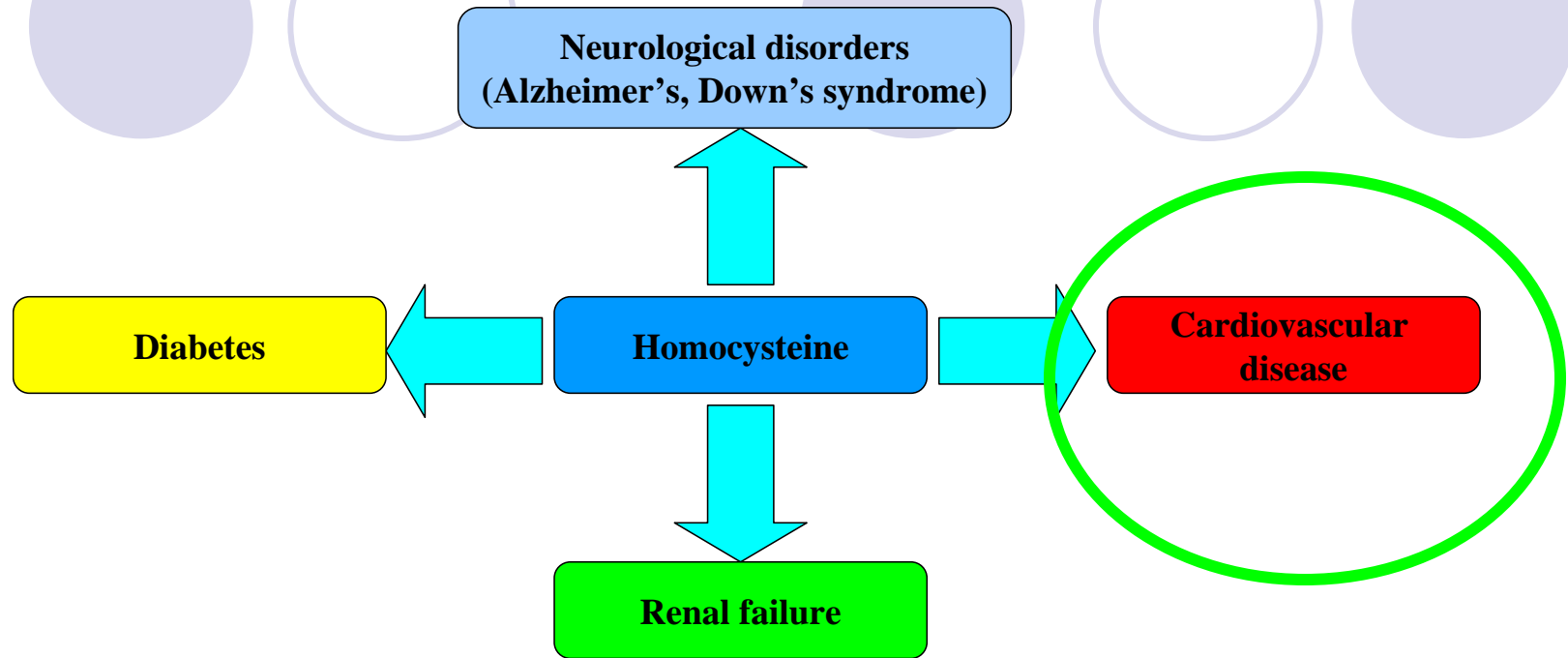
- Highly reactive thiol (low redox potential) capable of covalently modifying various proteins and compounds involved in numerous biological processes.



# Causes of elevated homocysteine levels

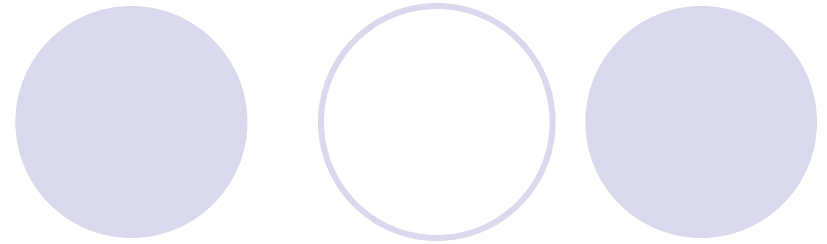


# Diseases associated with homocysteine



Disease distribution in Canada

# CVD biomarkers



<b>Biomarker</b>	<b>Drawback</b>
<b>Homocysteine</b>	<b>Cost and Time</b>
<b>Lipoproteins (cholesterol)</b>	<b>Differentiation between good cholesterol and bad cholesterol</b>
<b>c-reactive protein</b>	<b>Indicator of infection and inflammation</b>
<b>Ascorbic acid</b>	<b>Fluctuates according to diet</b>
<b>Lactate dehydrogenase</b>	<b>Circulating lactate dehydrogenase is also an indicator of liver failure, kidney disease, and heart failure</b>

High homocysteine prompts the release of c-reactive protein and the oxidation of LDL

# Current assays for measuring the blood levels of homocysteine

Assay	Cost	Drawback
HPLC	65\$ per assay	Sophisticated equipment Highly trained staff Expensive Not high throughput
Immunoassays	\$600 per kit	Reagents are expensive Highly trained staff Sophisticated equipment
Inosine dehydrogenase	FDA approved	4 enzyme steps Requires a number of cofactors
Lactate dehydrogenase	FDA approved	4 enzyme steps Requires a number of cofactors

Enzymatic assays →

**Although homocysteine detection can be used as a biomarker for CVD and other diseases, a cost effective and accurate method of deciphering homocysteine levels in the blood is still required.**

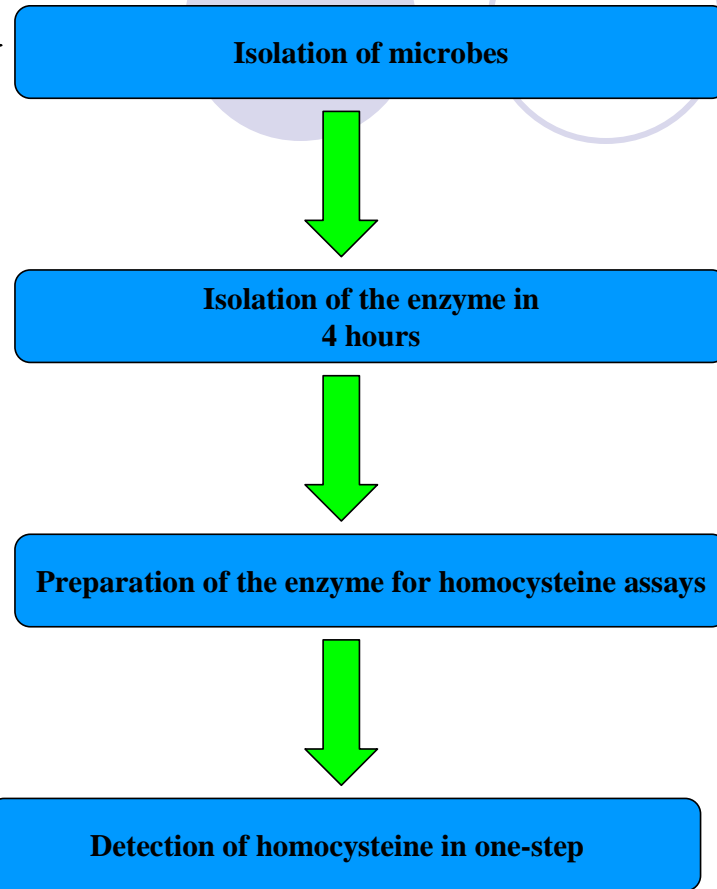


**Strategy: Discovering a one-step enzymatic method  
for detecting homocysteine**

# The isolation of the homocysteine-metabolizing enzyme



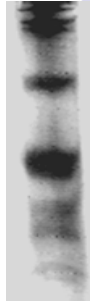
**Microbial consortium growing in a nutrient medium**



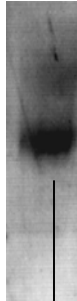
- Amount of protein isolated from one 200mL microbial culture is 30mg/mL which can run up to 150 assays
- Only a crude protein fraction is required for the accurate measurement of homocysteine.
- Total time required to prepare the enzyme extract is 7 hours,

# Enzyme identification

Total protein stain



Activity stain

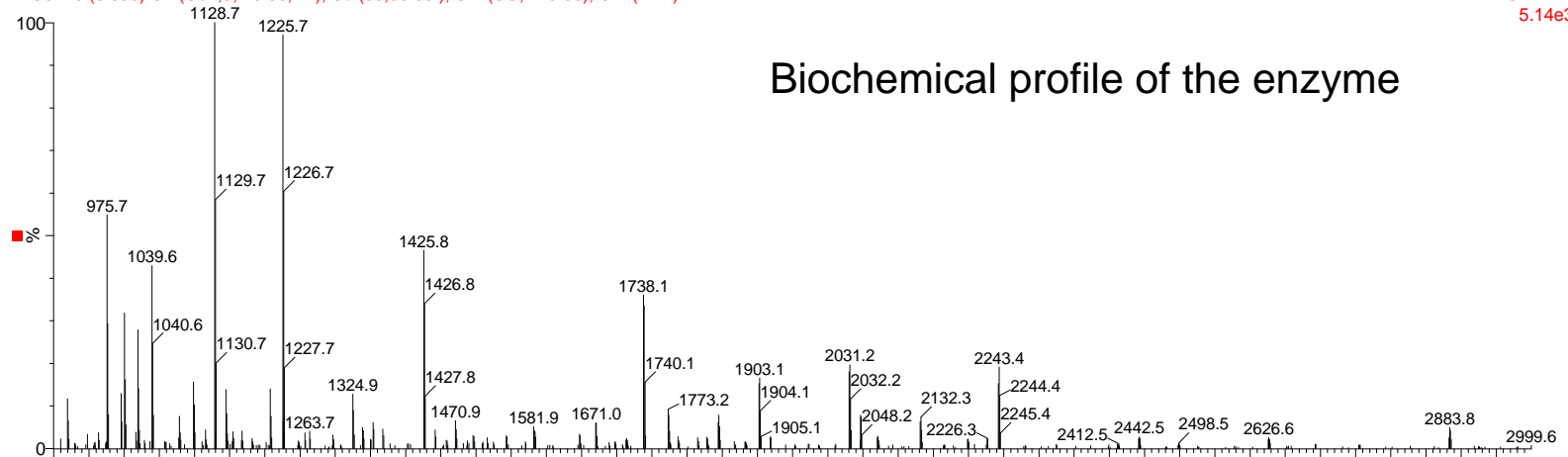


This activity assay depends on the conversion of NAD into NADH which can be detected easily by spectrophotometry

← Homocysteine-metabolizing enzyme

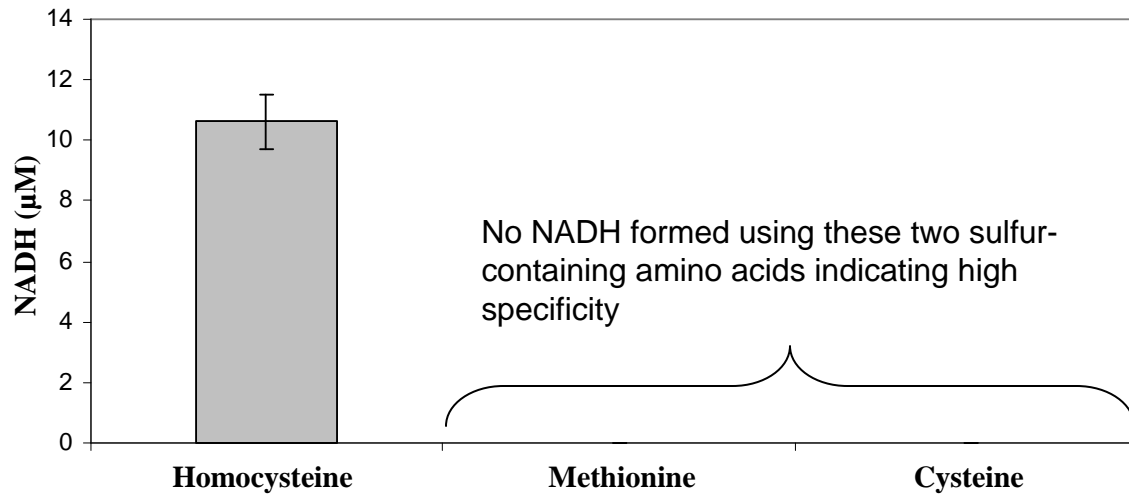
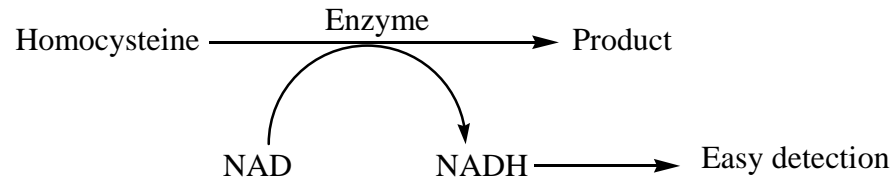
MALDI-TOF for enzyme identification

WG3 18 (0.599) Cn (Cen,3, 75.00, Ht); Sb (99,90.00 ); Sm (SG, 2x3.00); Cm (7:21)



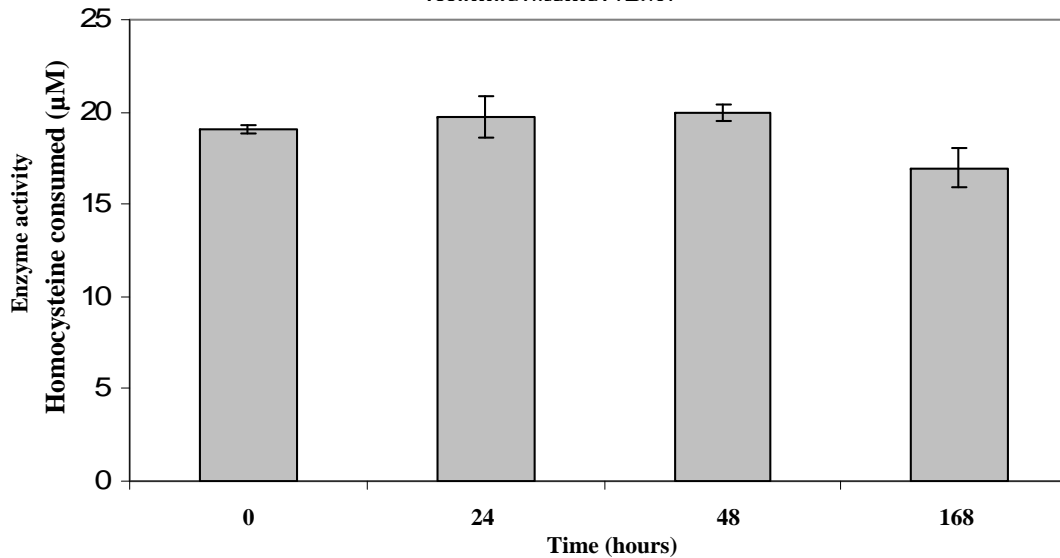
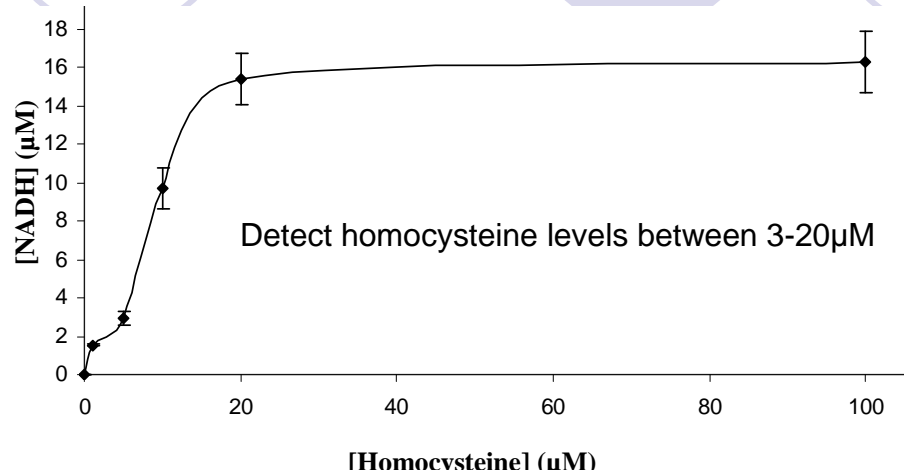
Biochemical profile of the enzyme

# Detection of homocysteine levels by spectrophotometry



The enzymatic detection of homocysteine in this one-step method is performed by monitoring the formation of NADH, a by-product of homocysteine metabolism, with a spectrophotometer. This instrument is very cheap and found almost every clinical setting.

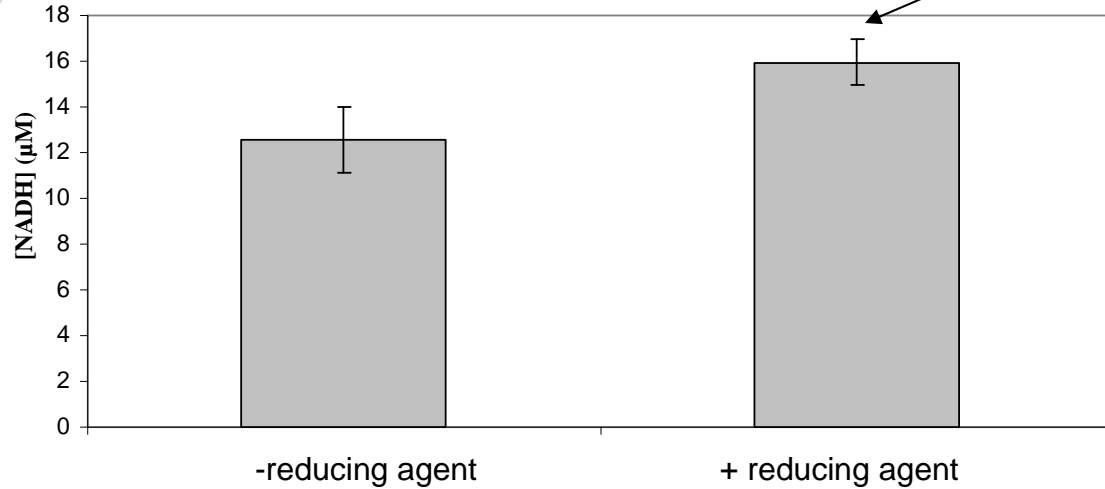
# Enzyme sensitivity and stability



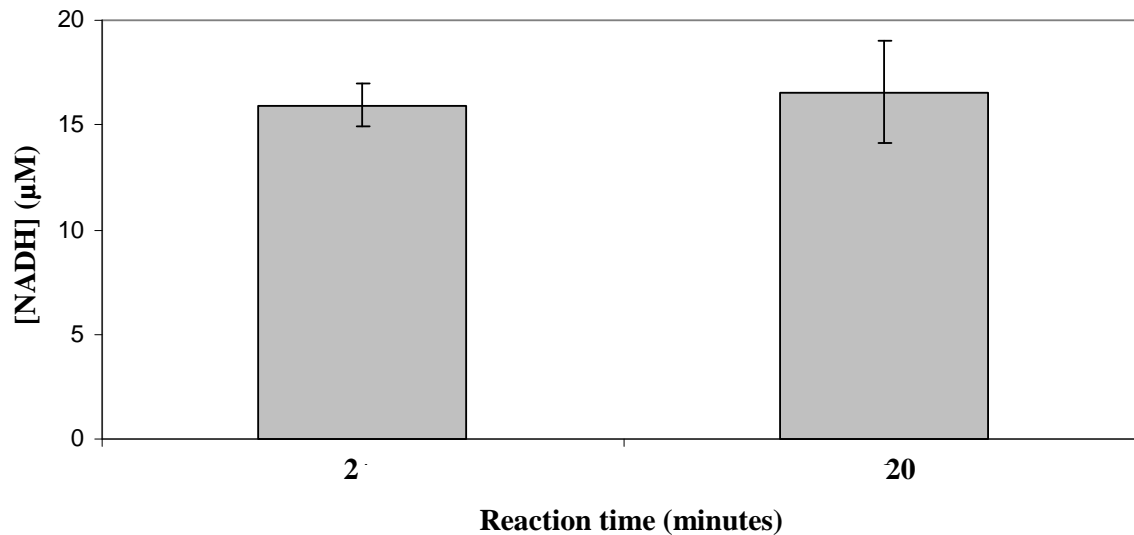
The powdered form of the enzyme is stable for 1 week

# Assay optimization

Reducing agents increased accuracy of the detection assay

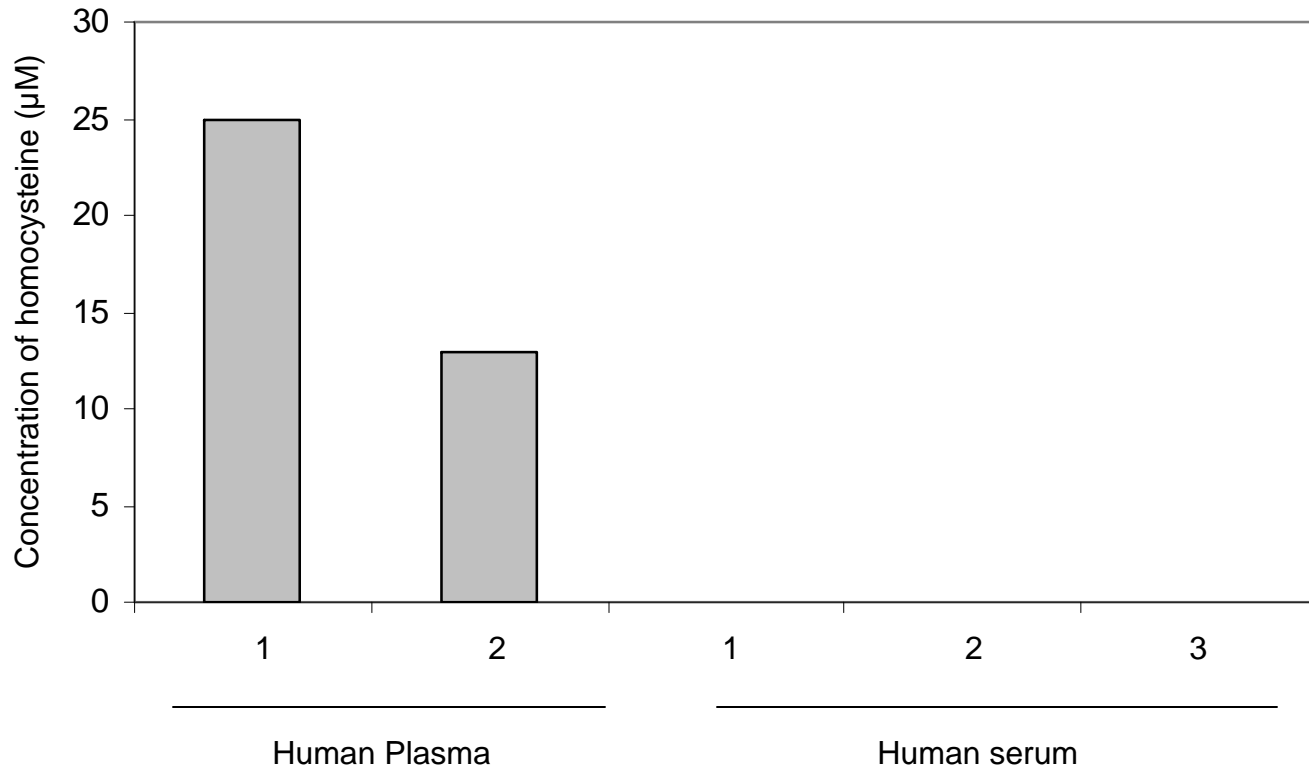


Reactions can be performed for up to 20 minutes



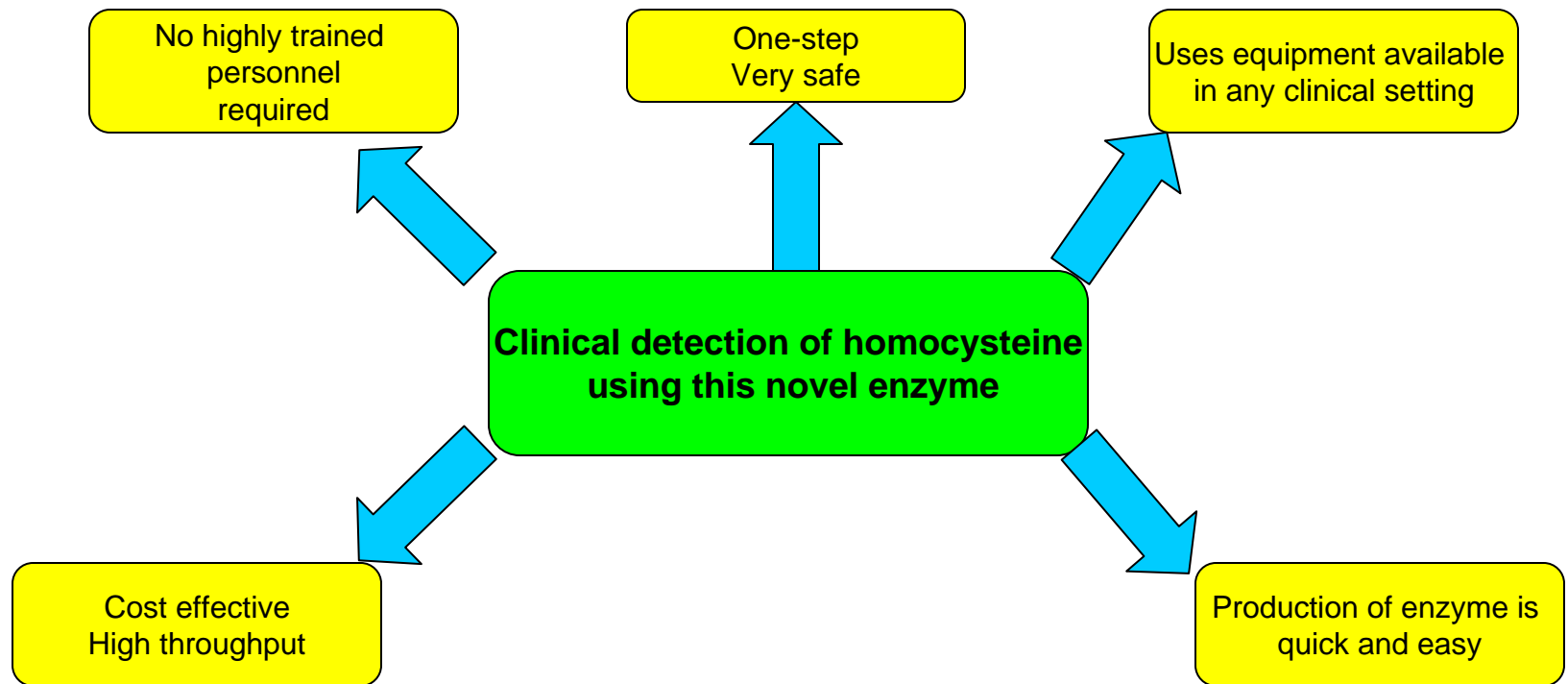
# Detection of homocysteine in human blood

Human plasma samples are required for the assay.

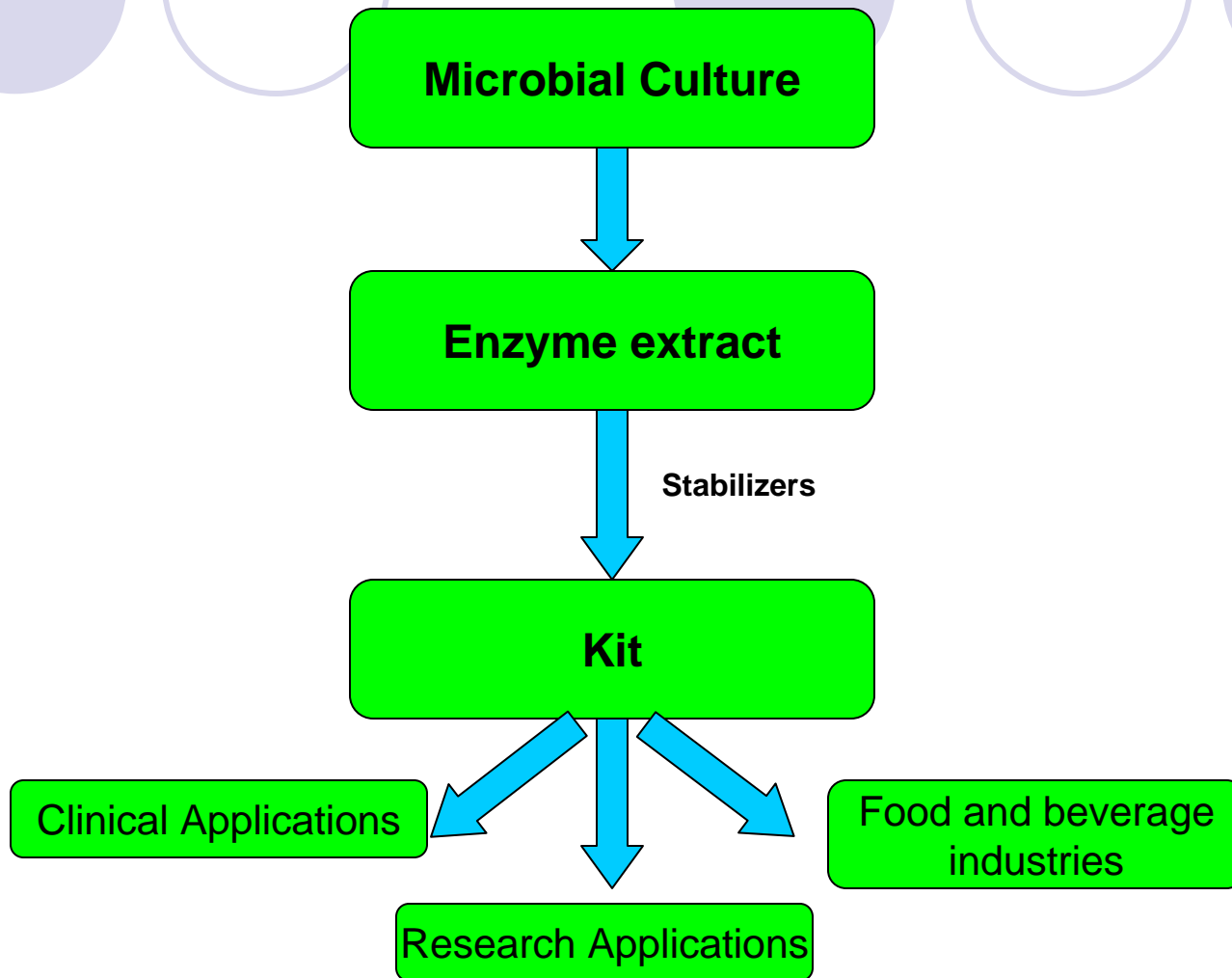


Most of the protein is removed for clinical analysis.

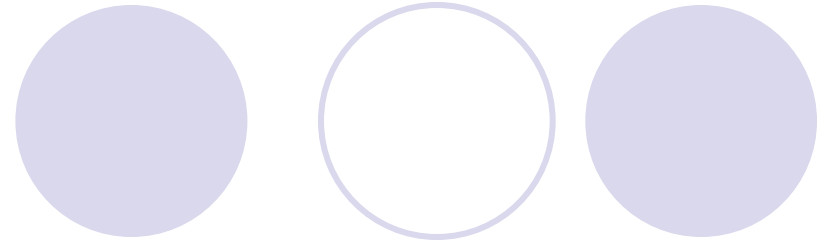
# Use of this enzyme in the clinical detection of homocysteine



Concluding remarks:  
Genesis of a facile homocysteine kit



# Research Team



Dr. Vasu Appanna  
Project leader



Dr. Ryan Mailloux  
Research associate

