Purification and physicochemical studies of metalloprotease, cotinifolin from *Euphorbia cotinifolia*

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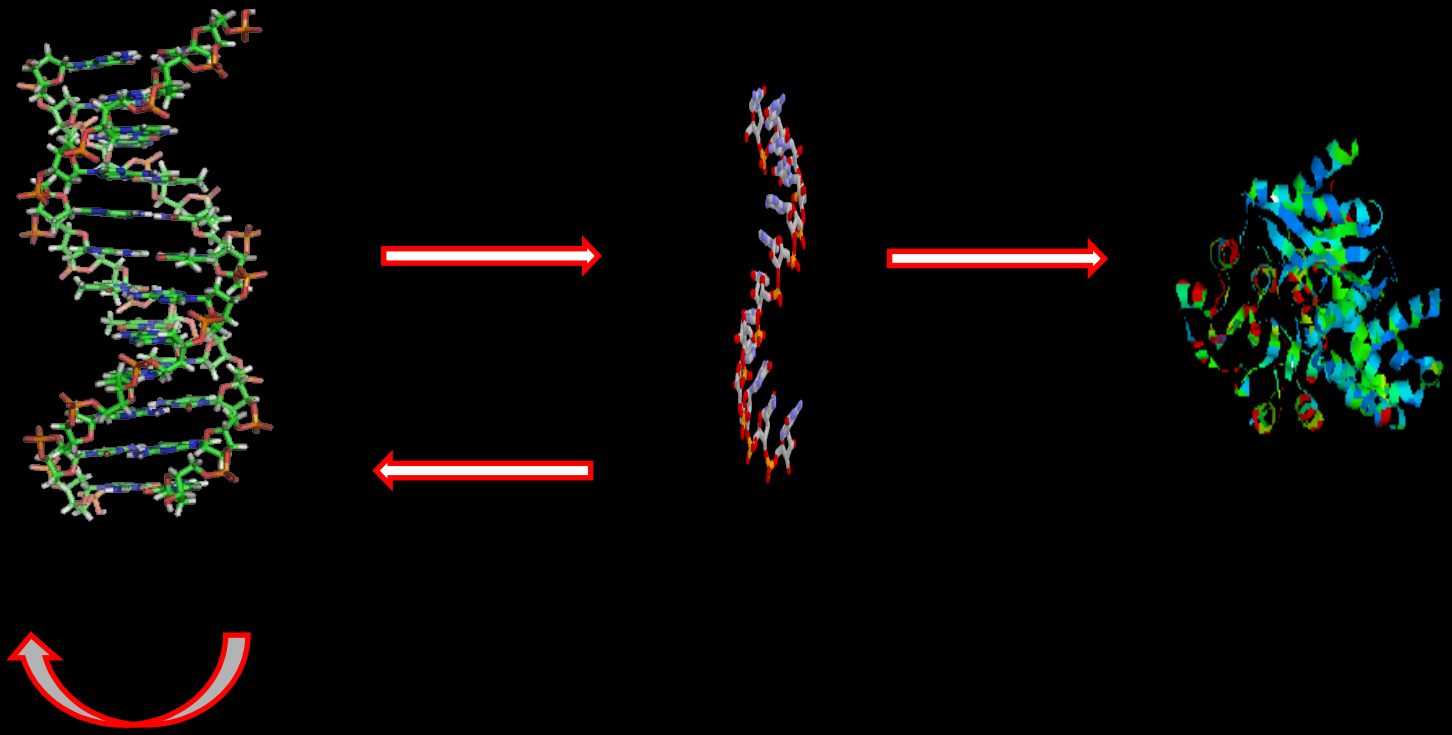
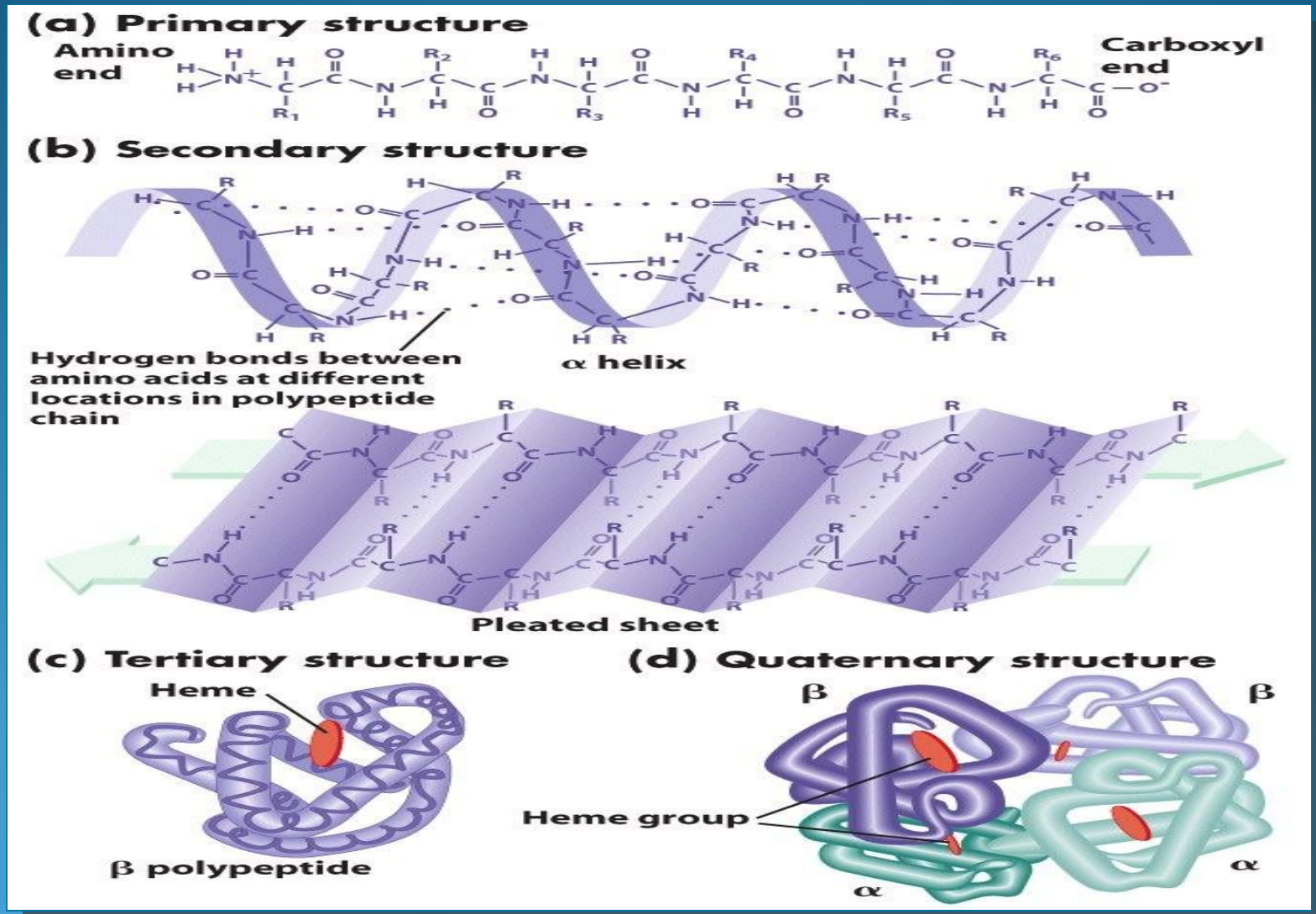
Introduction of proteins and protein folding

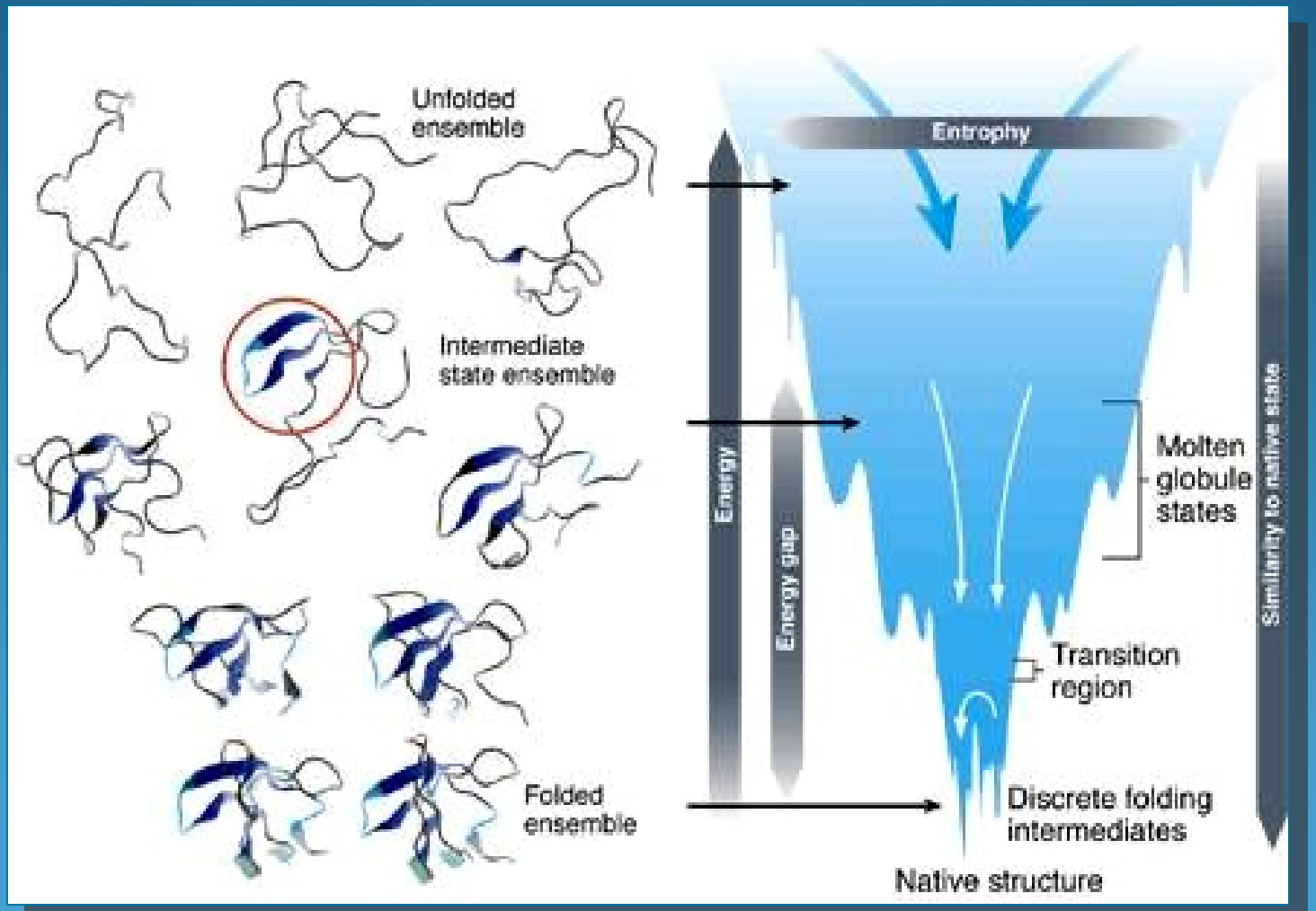


Protein, An introduction!!

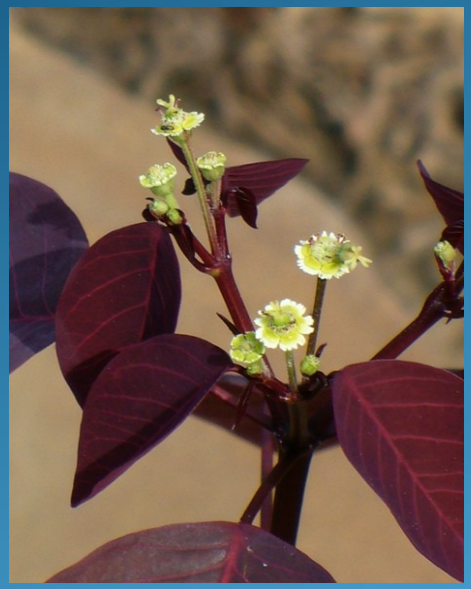


Nature Precedings : doi:10.1038/npre.2011.6397.1 : Posted 12 Sep 2011

Protein folding funnel



Biochemical and spectroscopic characterization of a novel metalloprotease, cotinifolin from an antiviral plant shrub: *Euphorbia cotinifolia*



Euphorbia cotinifolia **plant with medicinal implications**

⬇

Latex extracted from stem

⬇

Using anion exchange chromatography and Hydrophobic interaction chromatography

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Purification of Metalloprotease (Cotinifolin)

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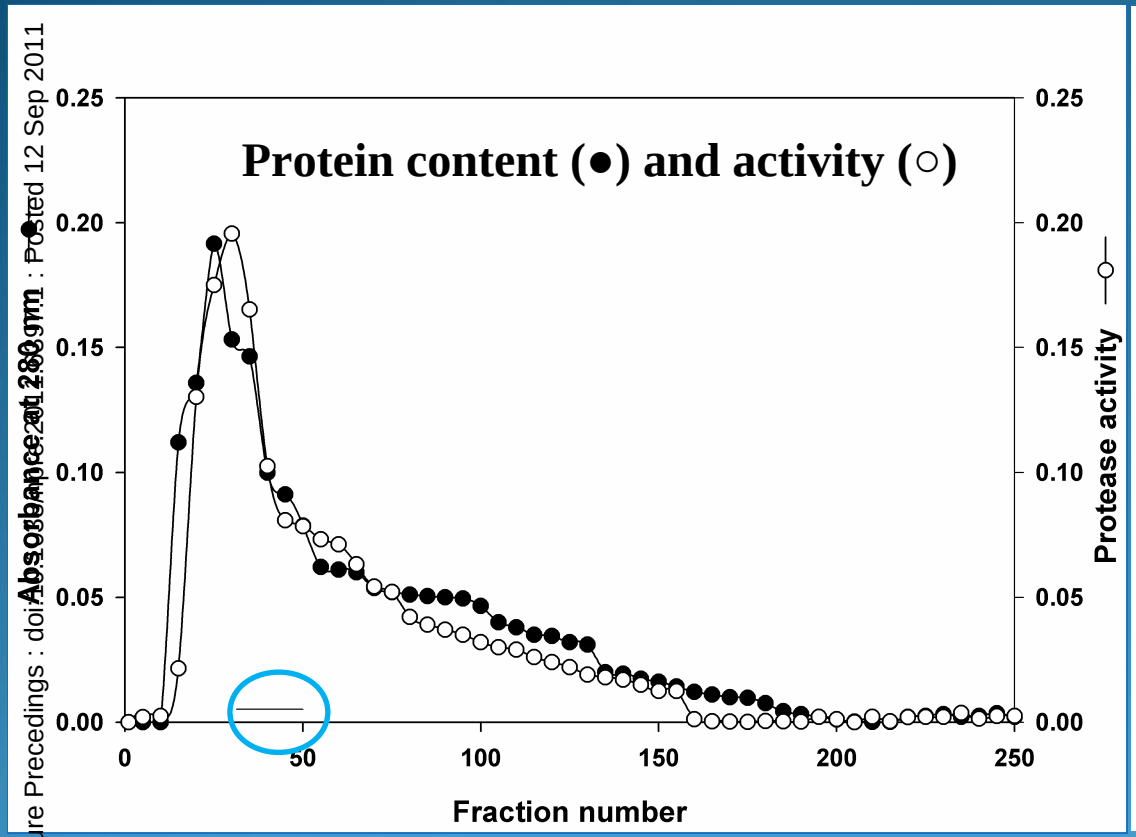
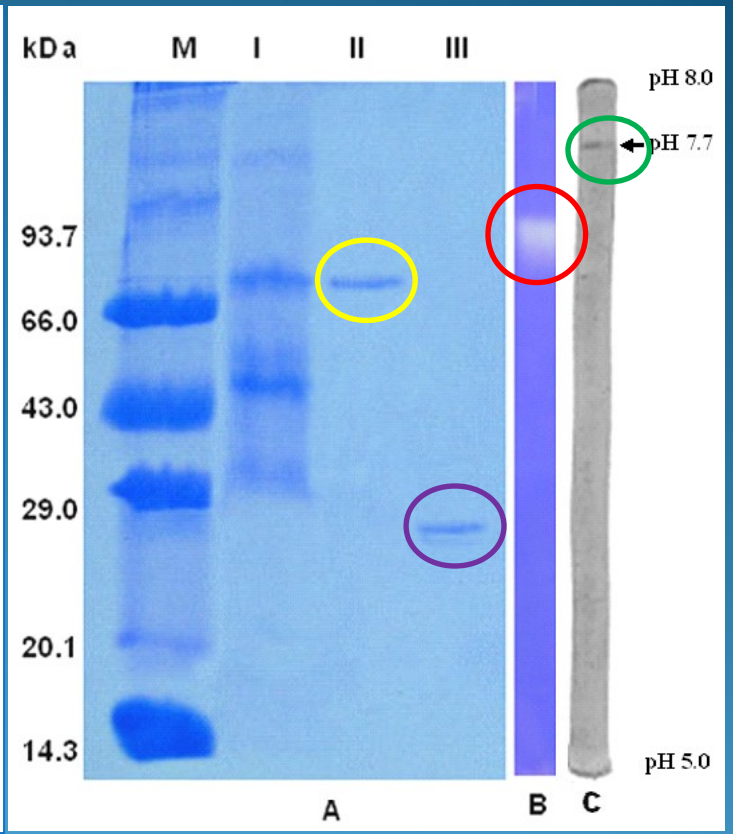
- **pH, Temperature optima and Stability**
- **Effect of inhibitors**
- **Effect of metal ions**
- **Effect of substrate concentration on Reaction Velocity**
- **Effect of Chaotrops, Organic Solvents, detergents**
- **Autodigestion study**
- **Spectroscopic Studies: Absorbance, Fluorescence Circular Dichroism**

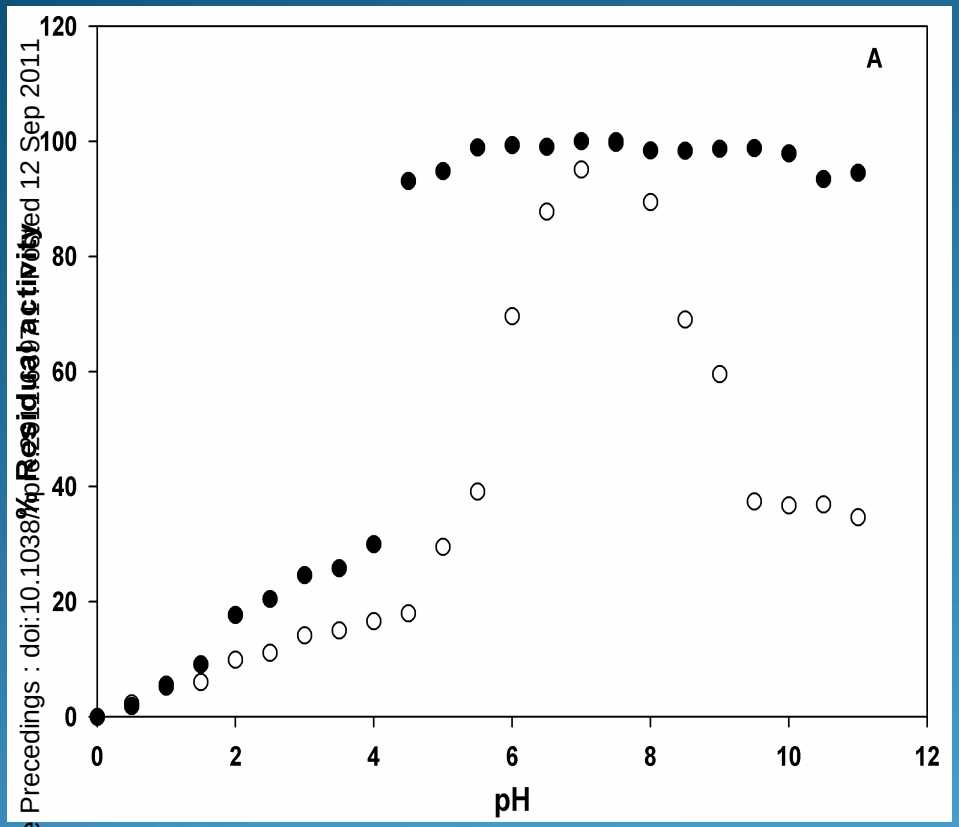
Purification of metalloprotease, Cotinifolin

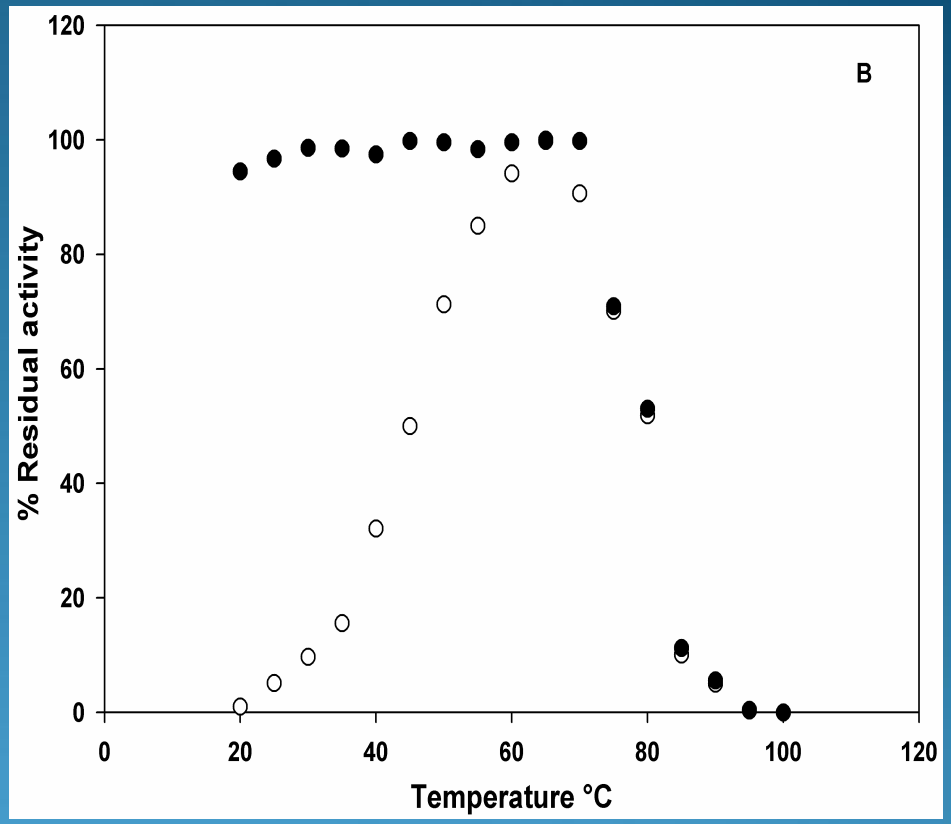




Steps	Total protein (mg)	Total activity (units)	Specific activity (units.mg^{-1})	Yield (%)
Crude extract	315	6160	19.55	100
DEAE Sepharose	115	2324	20.20	37.7
HIC column	18	418	23.22	6.78

pH, Temperature optima and Stability





Effects of pH Activity (○) Stability (●)

pH Optima 7.5

PH Stability 4 - 11

Effect of temperature Activity (○) Stability (●)

Temperature Optima 65 °C

Temperature Stability 20 – 75 °C

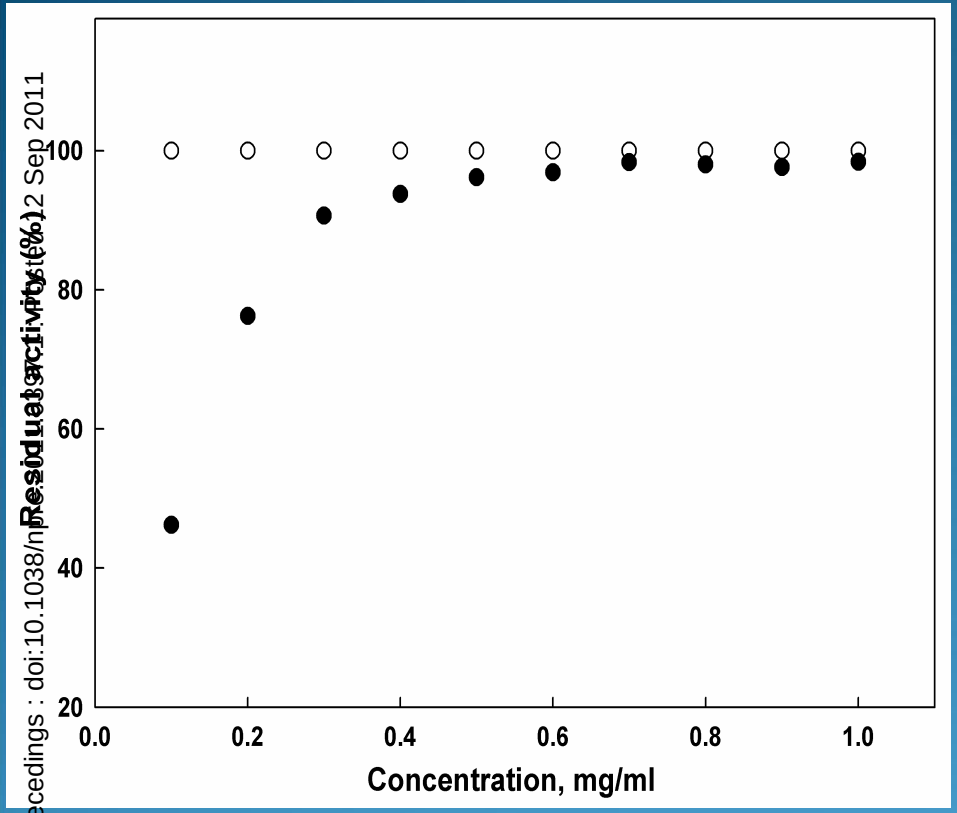
Chaotroph & Organic solvent	Concentration (M)	Residual activity (%)
Ethanol	50%	30
Methanol	50%	85
Iso propanol	50%	95
Butanol	50%	50
N-Hexane	50%	100
Acetonitrile	50%	100
Dioxane	50%	50
Trition X-100	3.5%	85
SDS	0.1%	50
Exilin	1%	50
GuHCl	1.5M	90
Urea	8M	90

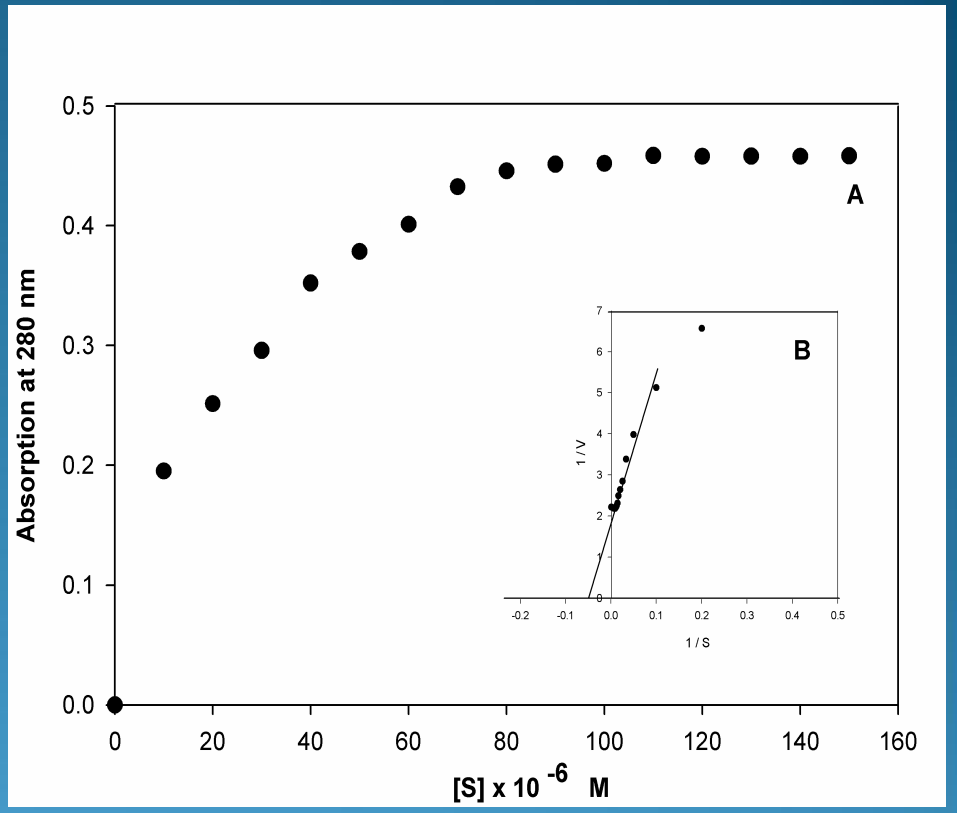
Inhibitors	Concentration (M)	Residual activity (%)
IAA	10^{-3}	80
PMSF	10^{-3}	85
SBTI	10^{-3}	90
o-phenanthroline	10^{-3}	0
	10^{-4}	5
	10^{-5}	10
EGTA	10^{-3}	60
	10^{-4}	80
	10^{-5}	100
EDTA	10^{-3}	40
	10^{-4}	55
	10^{-5}	70

Metal ions	Concentration (M)	Protease activity (%)
Hg^{2+}	10^{-3}	100
Mg^{2+}	10^{-3}	75
Ca^{2+}	10^{-3}	80
Ba^{2+}	10^{-3}	95
Cs^{2+}	10^{-3}	75
Ni^{2+}	10^{-3}	125
Mn^{2+}	10^{-3}	95
Co^{2+}	10^{-3}	15
Zn^{2+}	10^{-3}	50
Cu^{2+}	10^{-3}	60

Enhanced by : Ni^{2+}

Inhibited by: Co^{2+}





Autodigestion of Cotinifolin

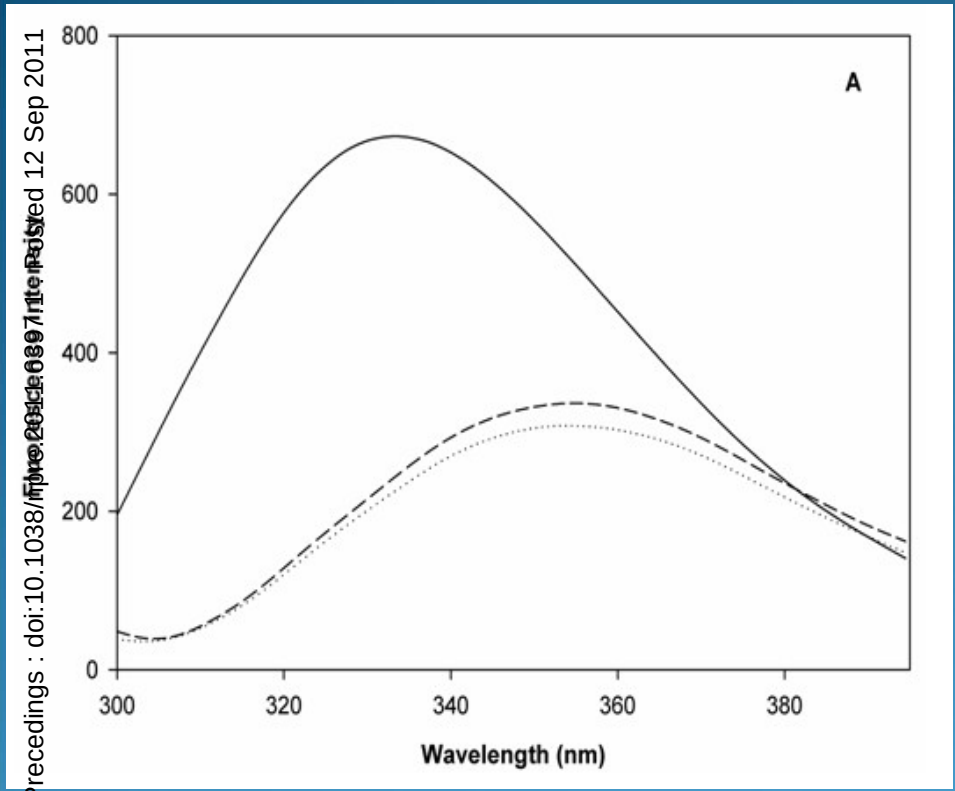
- Residual activity (●)
- Activity after 5 min (○) of incubation, taken as 100% activity

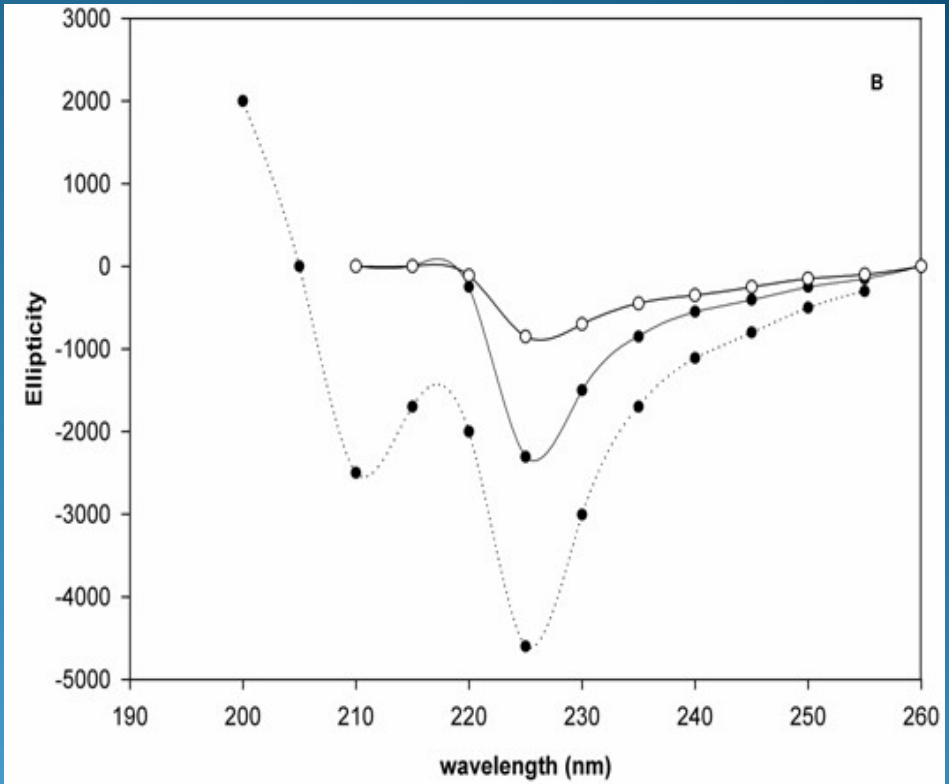
Effect of substrate concentration on reaction velocity

A. Michaelis–Menten equation
B. Lineweaver–Burk plot

***K*m = 20 µM ± 0.5.**

Spectroscopic studies of metalloprotease, cotinifolin





A. Intrinsic fluorescence spetra

Native (—)

Denatured conditions

3 M GuHCl (– –)

6 M GuHCl(·····)

B. Circular dichroism spectrum

Native (---●---)

Denatured conditions

3 M GuHCl (–●–)

6 M GuHCl (–○–)

Summary

- Adequate amount of latex
- Easy economic purification
- Broad substrate specificity
- Stability against different temperature, pH
- Stability against different organic solvents
- Excellent model system to study structure-function relationship of other plant metalloprotease
- Crucial for food and biotechnological industries as well as protein folding studies.

Thank You