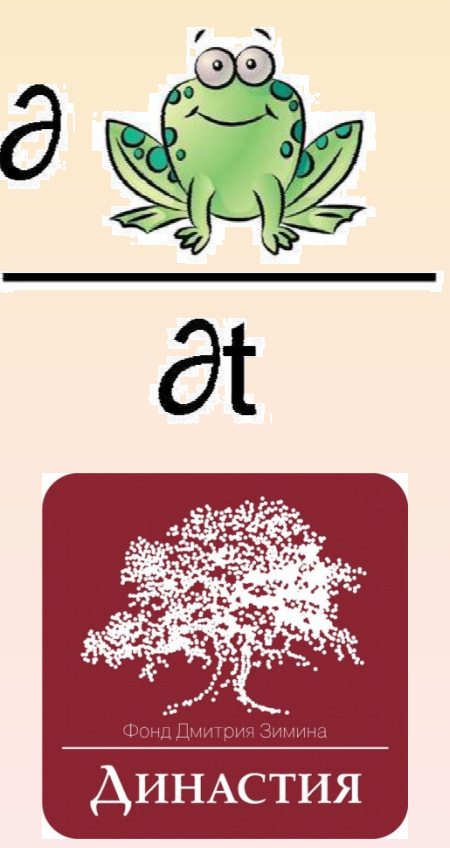


Laboratory of biophysics

Trypsin inhibitors from *Brassica rapa*



Belashkin I.^{1,8}, Bystrova A.², Gitzelzon P.³, Zakirova A.^{4,5}, Shestakova V.^{6,7}

¹ Lomonosov Moscow State University, Moscow, Russia

² Riga Technical University, Riga, Latvia

³ Pirogov Russian National Research Medical University, Moscow, Russia

⁴ South Ural State Medical University, Chelyabinsk, Russia

⁵ MBOU «Lyceum № 39», Ozersk, Russia

⁶ National Research Nuclear University MEPhI, Moscow, Russia

⁷ KOGOAU Gymnasium №1, Kirovo-Chepetsk, Russia

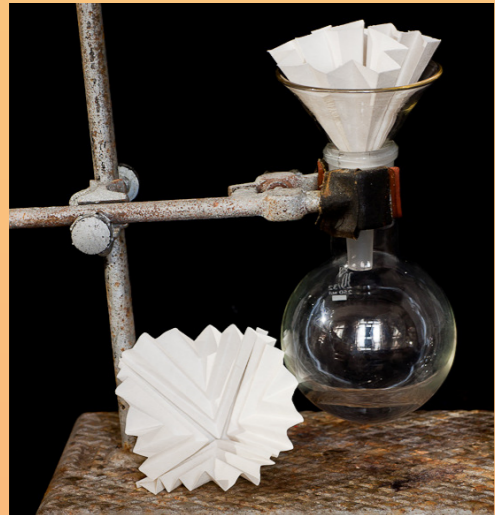
⁸ Advanced Science and Education Center, department of MSU, Kolmogorov school, Moscow, Russia



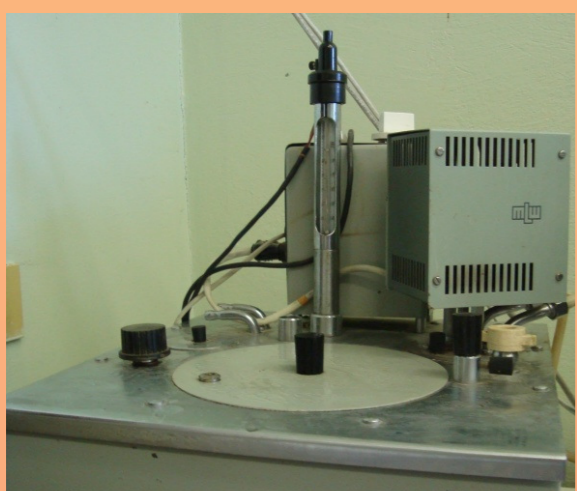
Homogenization



Extraction, filtration



Thermocoagulation



Concentration



1 2 3 4 5 6 MW 7 8 9 10



1 – first extract

2 – extract after thermocoagulation

3 – lower fraction after 3kDa filter

4 – protein concentrate

5 – washing 1

6 – washing 2

7 – washing 3

8 – eluate 1

9 – eluate 2

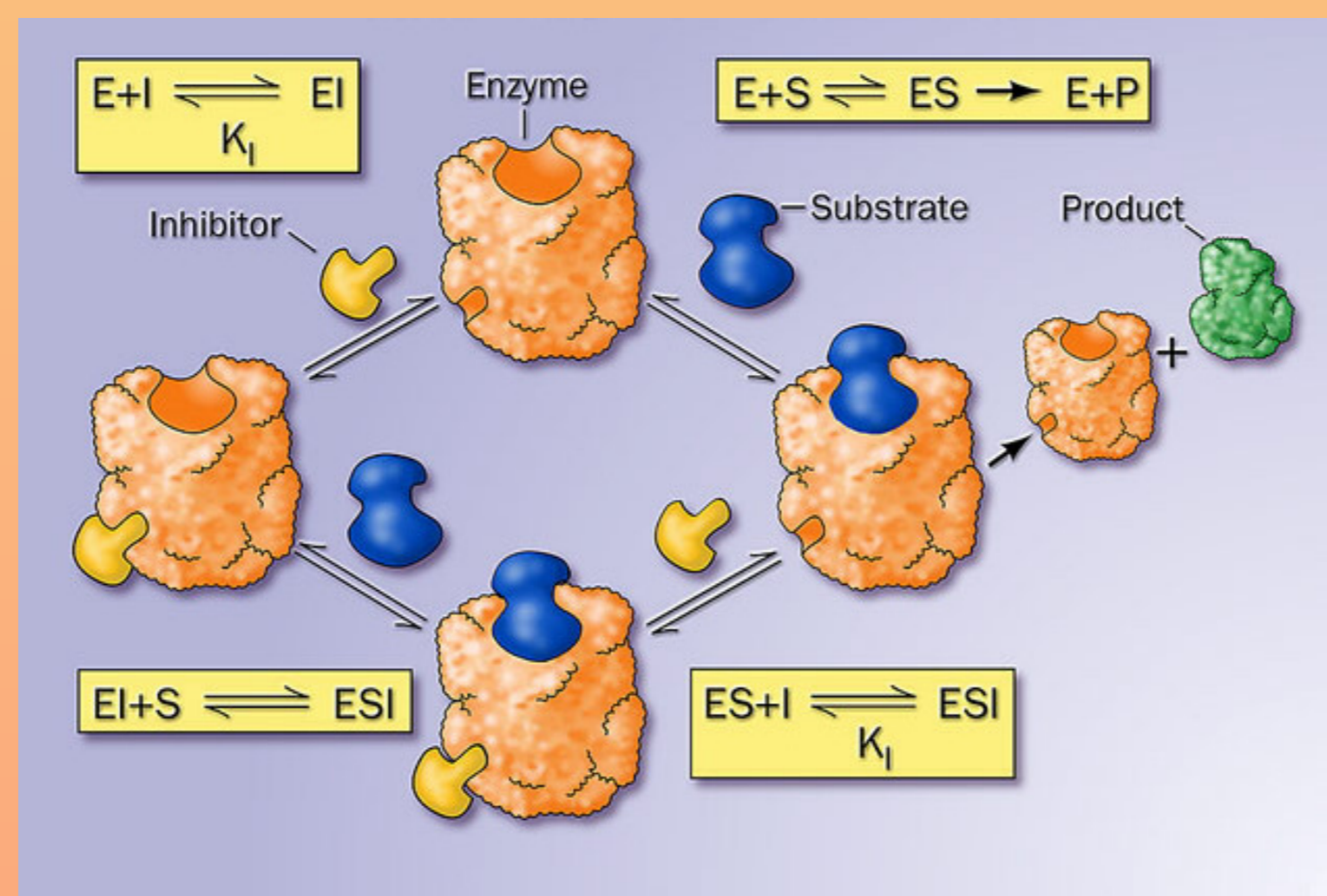
10 – eluate 3

Conclusions: we obtained trypsin inhibitor solution from *Brassica rapa* of 300 µg/ml concentration and measured its inhibition constants $K_i = 34$ nM и $K_i^{\square} = 490$ nM.

Purpose: isolation and purification of trypsin inhibitors from *Brassica rapa* and studying their inhibition mechanisms.

Results:

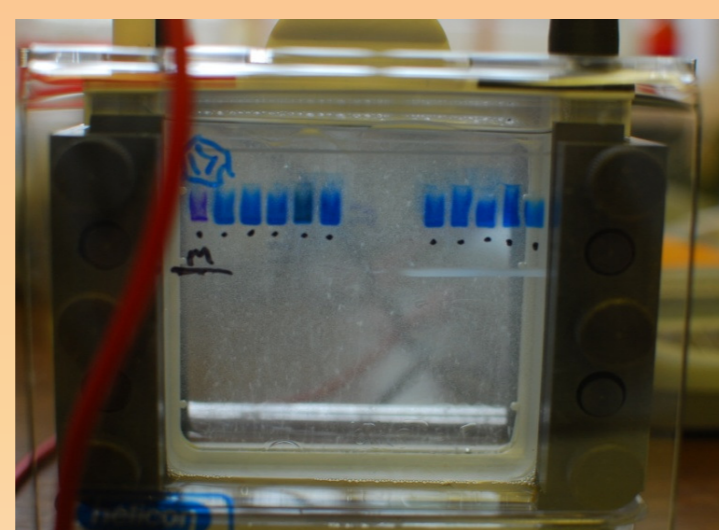
- 1) We isolated trypsin from the model pest,
- 2) isolated pure protein from *Brassica rapa* using affinity chromatography,
- 3) measured its concentration with Bradford and BCA methods,
- 4) tested inhibitor activity,
- 5) and determined inhibition type.



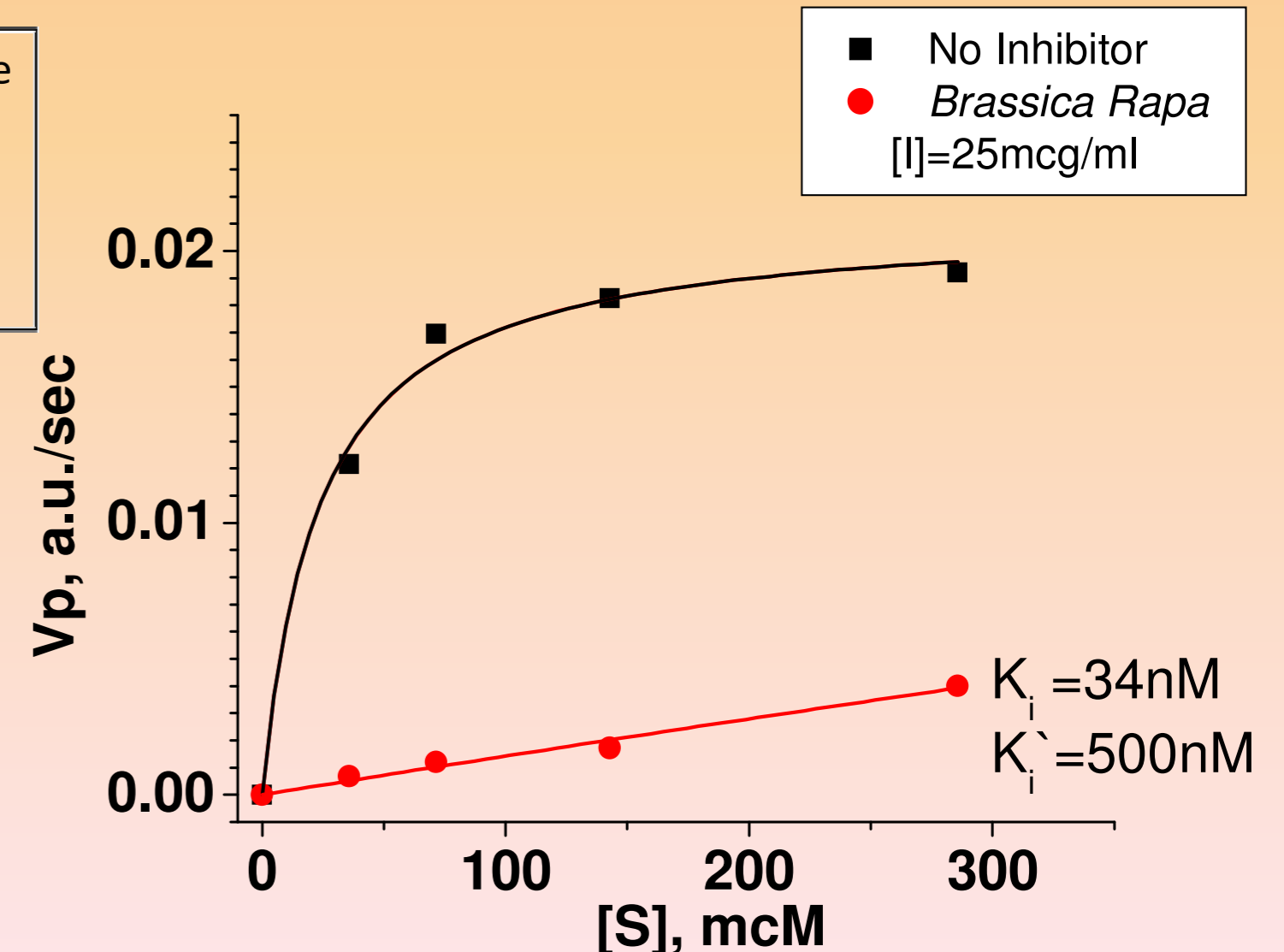
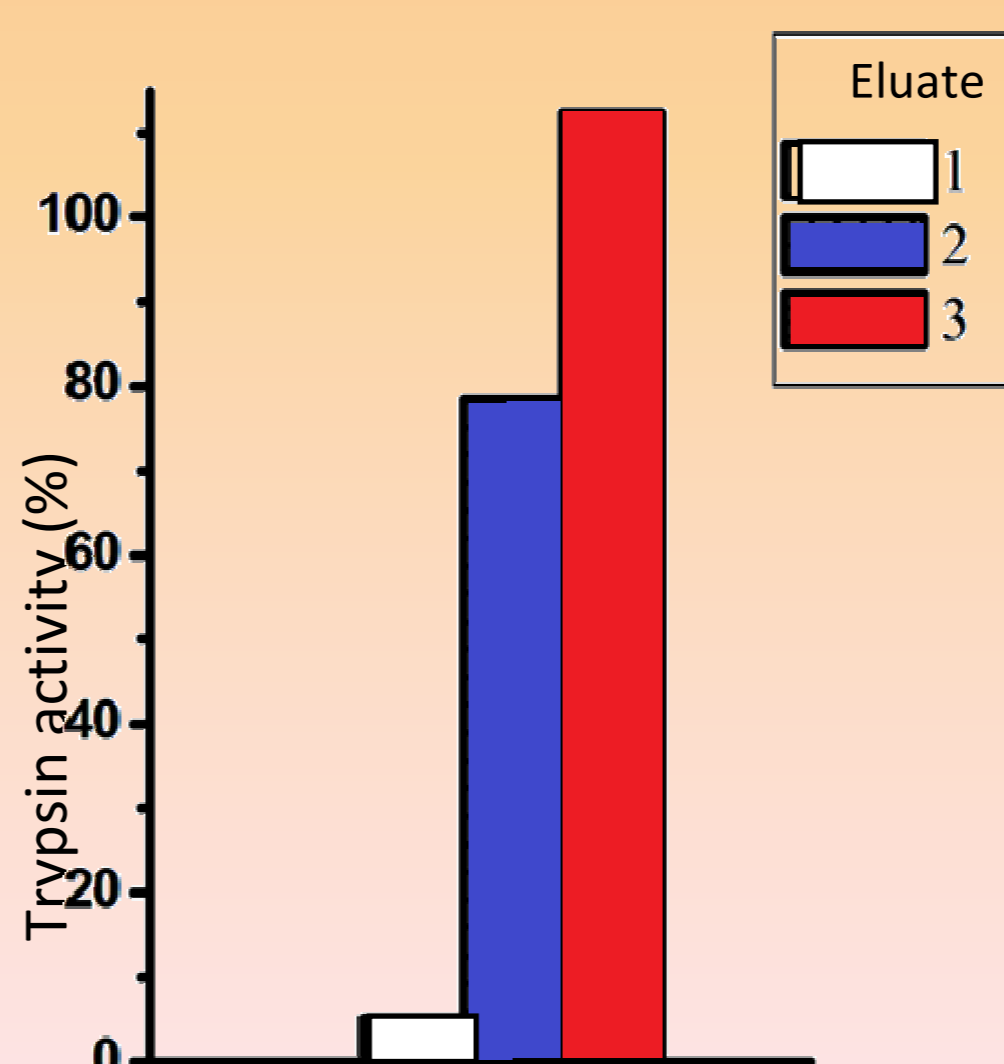
Affinity chromatography



Electrophoresis



Activity test



Homogenization



Extraction, filtration



Concentration



Ion exchange chromatography

