

HEAT SHOCK PROTEIN 90 AS POTENTIAL STRESS AND CANCER BIOMARKER

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Outline of research investigations

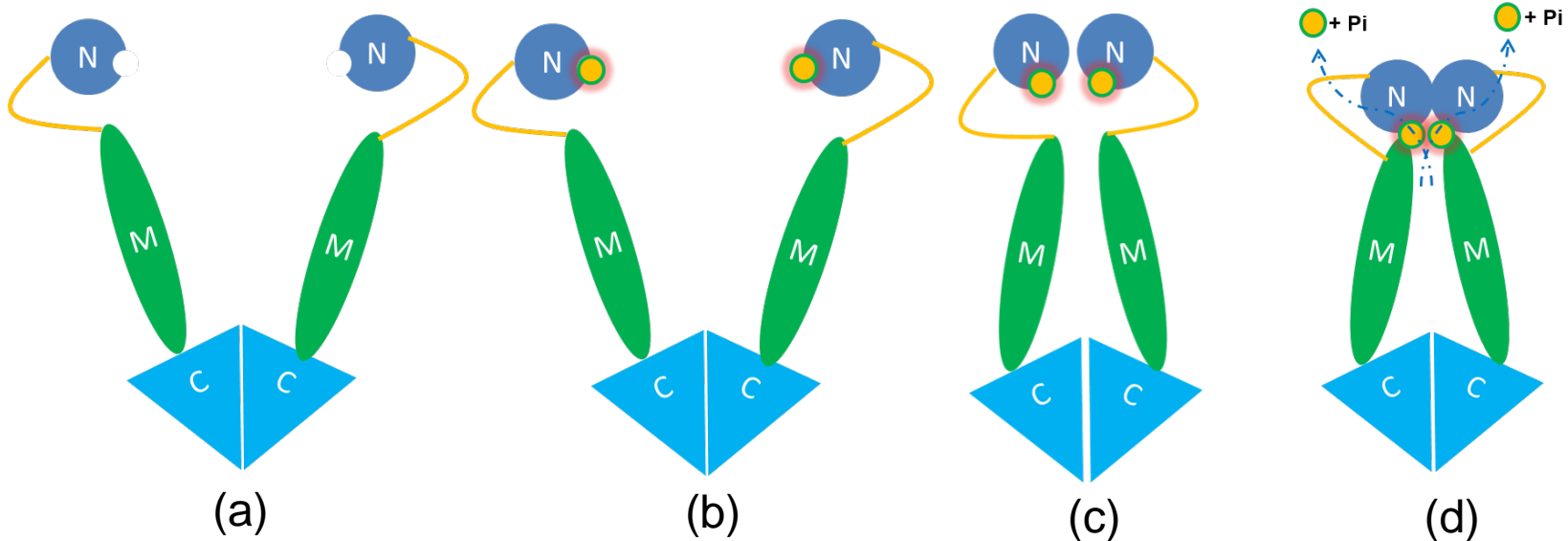
- A brief introduction: Heat shock protein 90 (Hsp90) as stress and cancer biomarker
- Luciferase based refolding assays
- Applications
- Further investigations on real samples





Heat shock protein 90 (Hsp90)

- Hsp90 is an ATPase, k_d : 400 μM), very low affinity for ATP. Hydrolysis rate: 0.1 ATP min^{-1} in humans and 1 ATP min^{-1} in yeast ([Schopf et al., 2017](#))



(a): Open V-shaped (b) Intermediate (c) Closed stage 1 (d) Closed stage 2

N: N-terminal domain ; M: Middle domain C: C-Terminal domain

 Adenosine 5'- triphosphate (ATP)  Adenosine 5'- diphosphate





Hsp90 as stress and cancer biomarker

- Hsp90 is a highly conserved and abundantly present molecular chaperone
- Assists protein folding and confers stability to maintain protein homeostasis
- Gets overexpressed during stress conditions

Heat shock response

Viral infections

Cancer

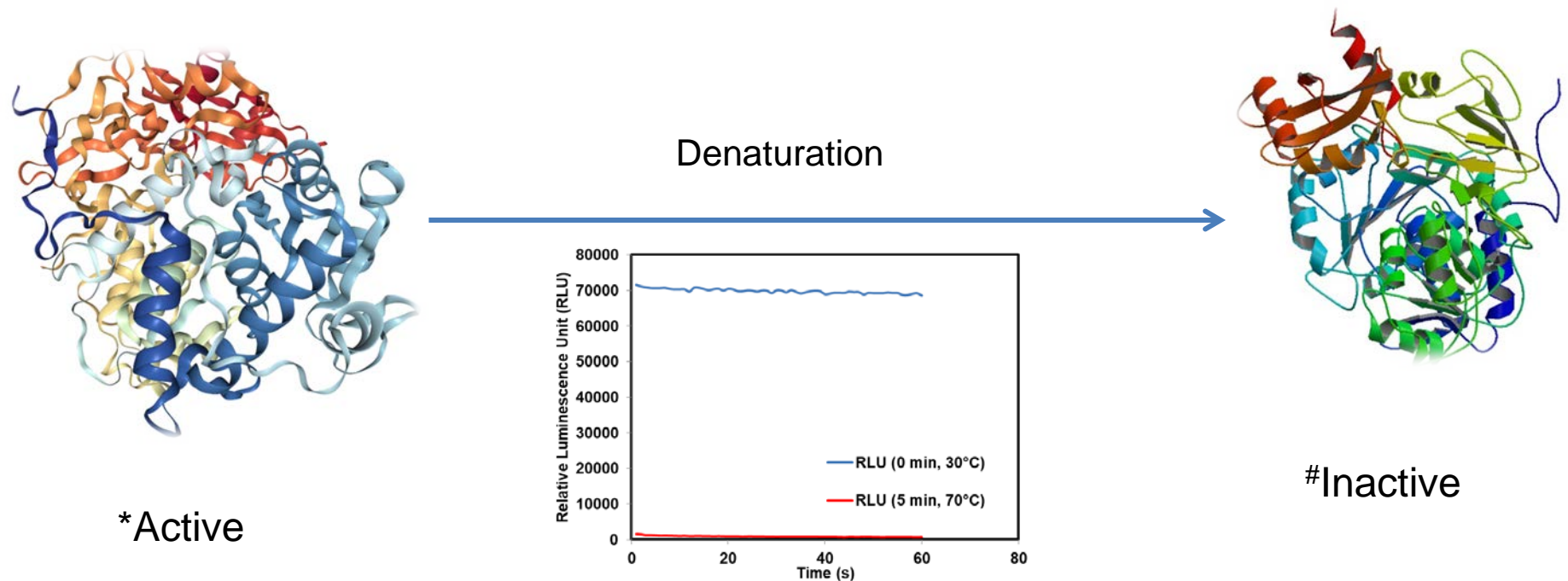
Stress

Neurodegenerative diseases



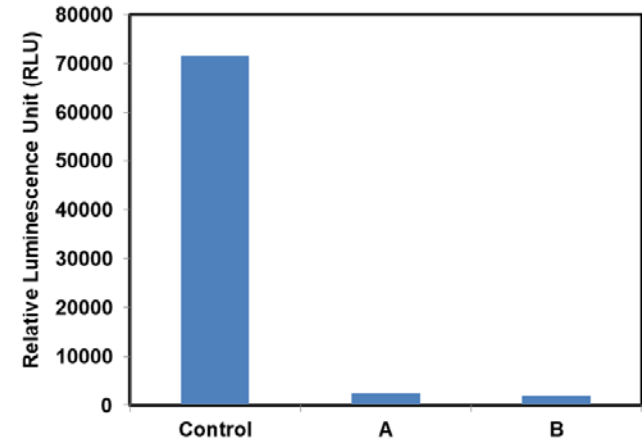
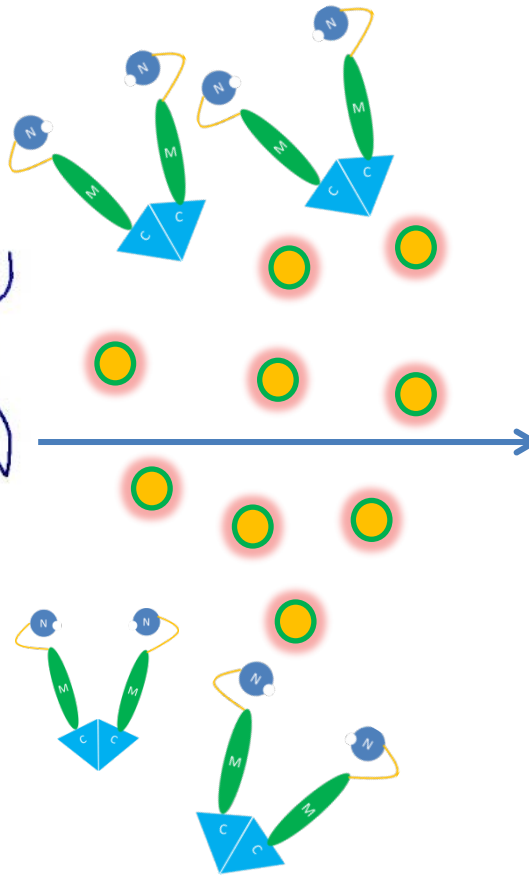
Firefly luciferase based refolding assays

- Firefly luciferase (FLuc) is a ca. 62 KDa enzyme (EC 1.13.12.7)
- Emits luminescence (520-560 nm) in presence of ATP, D-luciferin and O₂
- Rapid reaction



*#Adapted from: <http://www.rcsb.org/3d-view/1LCI/1> , <http://www.rcsb.org/structure/1BA3>





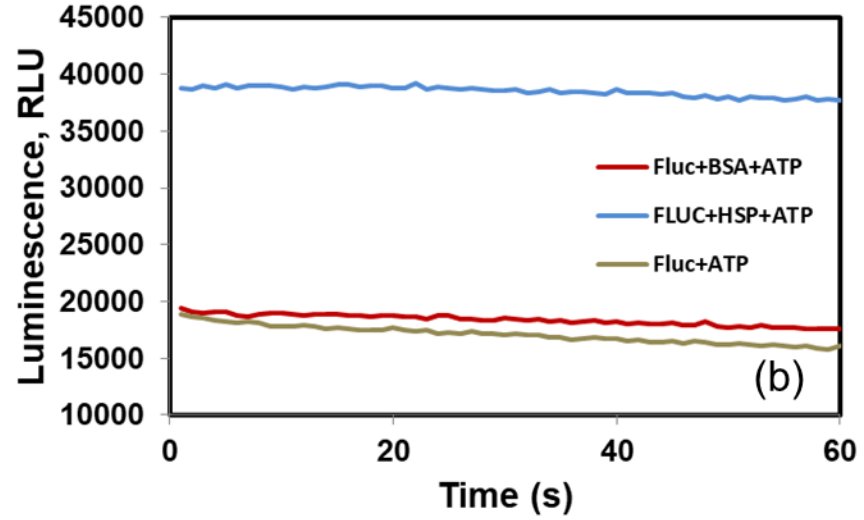
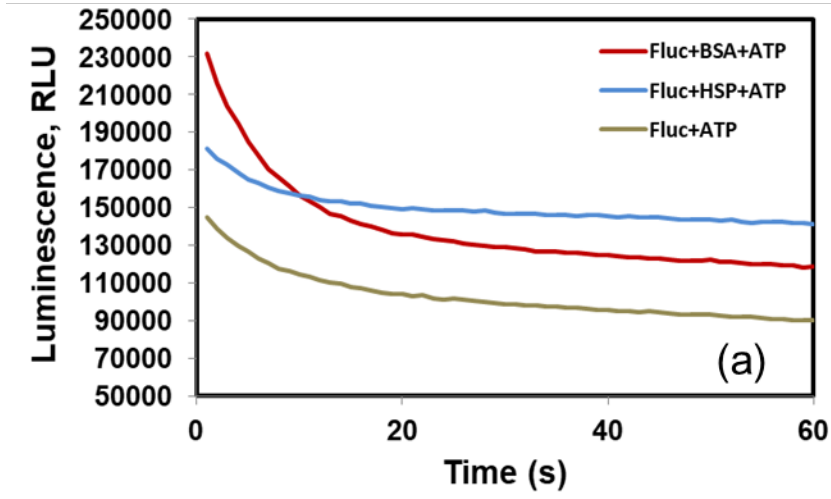
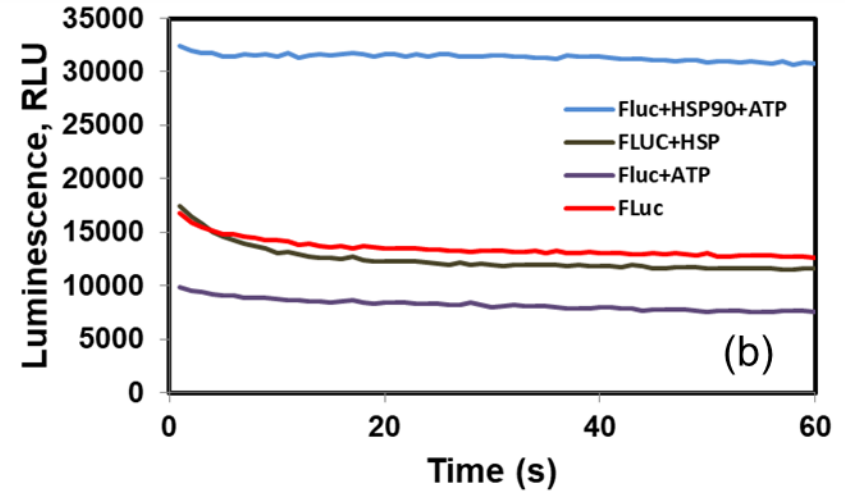
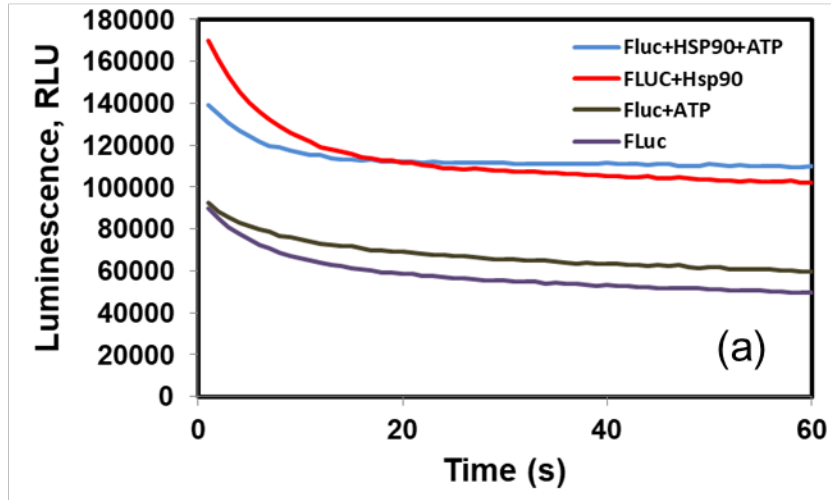
Active ?

Control: FLuc, 1.5 μM ; D-Luciferin, 0.55 μM ; 30°C; ATP, 28 nM; 0 min

A: Fluc, 1.5 μM ; D-Luciferin, 0.55 μM ; 70°C; 5 min

B: Fluc, 1.5 μM ; D-Luciferin, 0.55 μM ; 50 °C; Hsp90, 1 μM ; ATP, 28 nM (15 min incubation)





(a): Without incubation (b) 15 minutes incubation (50°C)

*Fluc, 10 nM; ATP, 1.25 mM; Hsp90, 0.5 μ M; D-Luciferin, 1.11 μ M

*Batch variations were independent of the findings





Applications

- Hsp90 activity as the indicator of stress and cancer
- FLuc based Hsp90 assay is relatively simple and can be performed on-site
- The results offer high signal to noise ratio and works in nM concentration ranges

Further investigations on real samples

- Real samples such as serum/biopsy samples needs analysis and validation
- The real samples may have multiple components which can affect the luminescence levels





Acknowledgements

- Prof. Valentina A. Kratasyuk, Head of the Chair, Department of Biophysics
- Prof. E.N. Esimbekova, Department of Biophysics
- Ms. Maria Kirillova, PhD student
- The Rector/ Vice Rectors and administrative staff, Siberian Federal University
- The International Department, Siberian Federal University
- RFBR Grant (RFFI:16-34-60100)
- The organizers of the conference (Biotechnology of New Materials–
Environment–Quality of Life)

Thank you!

