Novel Antioxidant Nanozymes for Biomedical Applications

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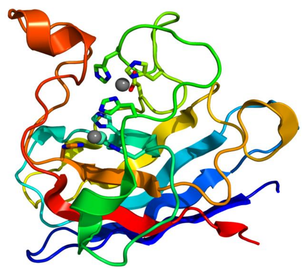
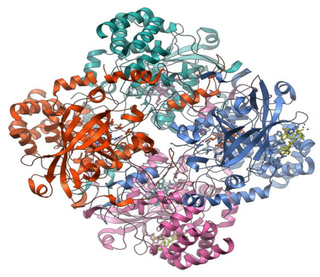


Human superoxide dismutase (SOD1)

Numerous disorders ranging from neurodegenerative till cancer are associated with overproduction of reactive oxygen species. Introduction of additional antioxidants could defense organism from oxidative stress. Superoxide dismutase (SOD) and catalase are the most efficient antioxidants in nature. Unfortunately, injection of native enzymes is useless due to their fast elimination from the body and low stability against proteases. Therefore, **development of drug delivery system** is crucial for further use.

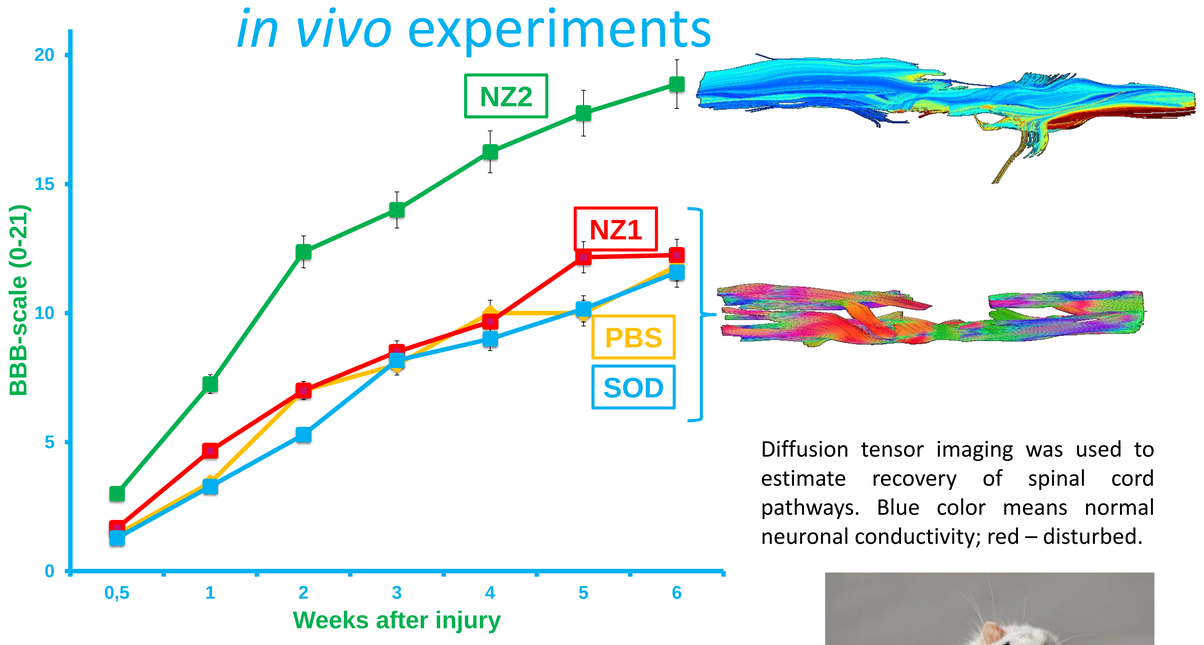


Catalase protein structure

$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$

$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$

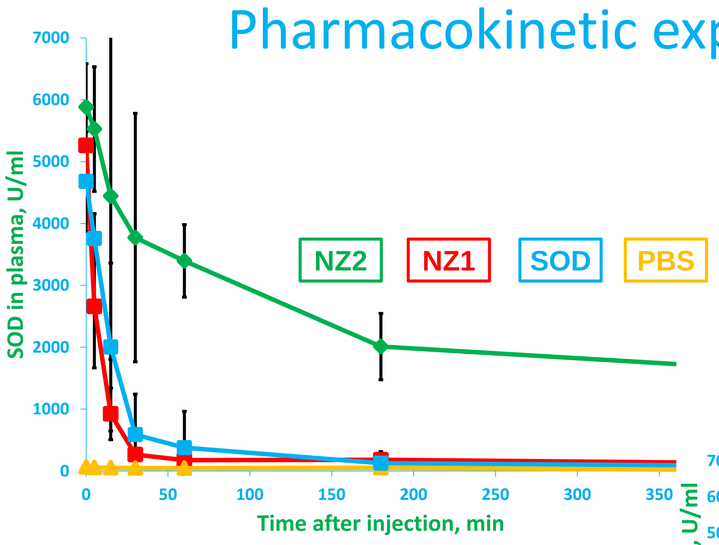
in vivo experiments



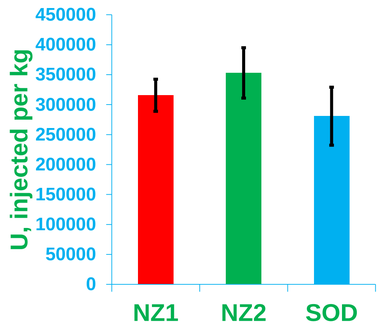
Rat spinal cord injury model was used to evaluate therapeutic efficiency of samples. Last were injected 30 minutes after injury. BBB-test and MRI were used to estimate recovery of rats. NZ2 show great therapeutic effect.

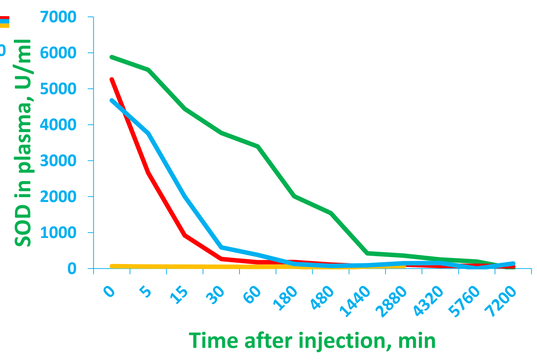
Diffusion tensor imaging was used to estimate recovery of spinal cord pathways. Blue color means normal neuronal conductivity; red – disturbed.

Pharmacokinetic experiments

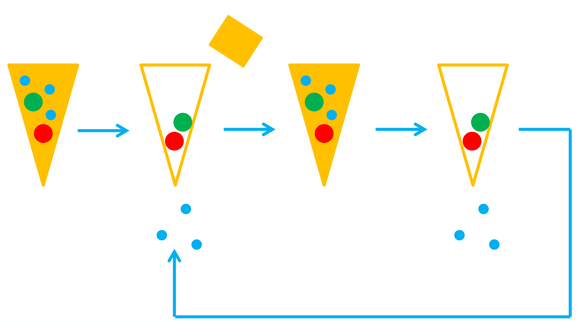


Pharmacokinetic (PK) experiments were performed on healthy Sprague Dawley rats. Equal concentrations in terms of activity were injected intravenously. SOD activity in plasma was measured with indirect enzymatic assay. NZ2 show remarkably better PK profile with enhanced $t_{1/2}$ and AUC.

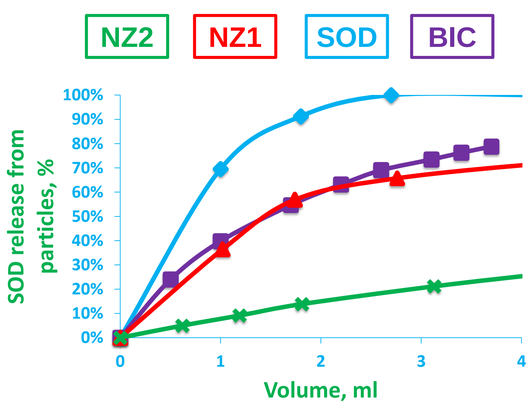




Physico-chemical characterization



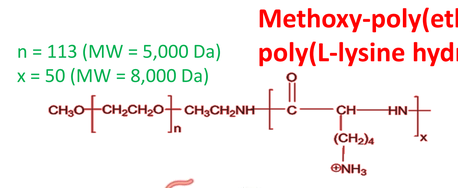
Sample	Protein yield, %	Specific activity, %	Total activity, %	Hydro-dynamic diameter, nm	PdI
BIC	25±3	16±2	4±2	30±2	0.12
NZ1	28±2	15±7	5±3	30±3	0.21
NZ2	60±5	58±6	**35±4**	40±4	0.15

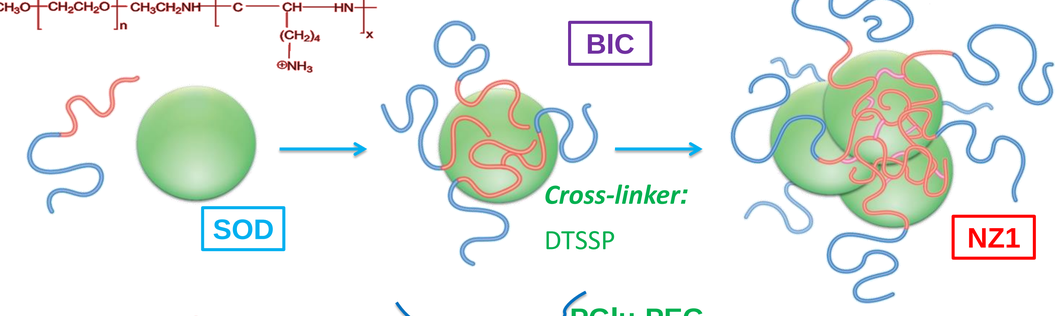


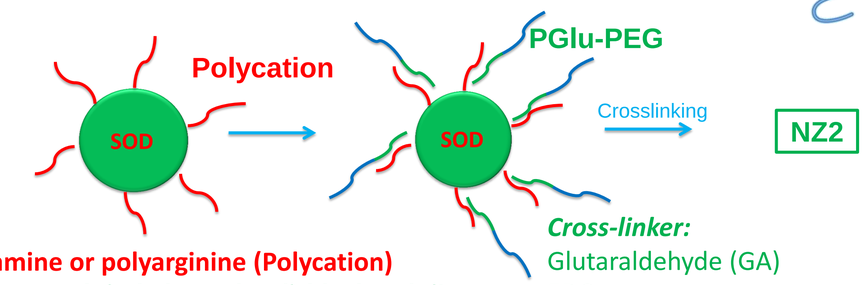
Release of enzyme from particles is crucial parameter for PK profile and therapeutic efficiency. Second layer of polymer in NZ2 allows us to use more reactive linker with no loss in activity. Such particles (NZ2) retain enzyme inside better then NZ1 and BIC. This also leads to greater protein yield and specific activity.

Methoxy-poly(ethylene glycol)-block-poly(L-lysine hydrochloride) (PLL-PEG)

n = 113 (MW = 5,000 Da)
x = 50 (MW = 8,000 Da)



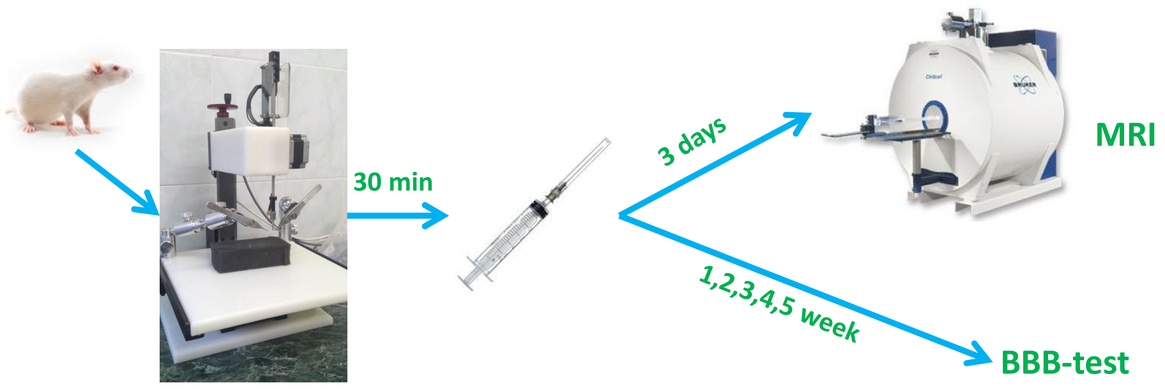




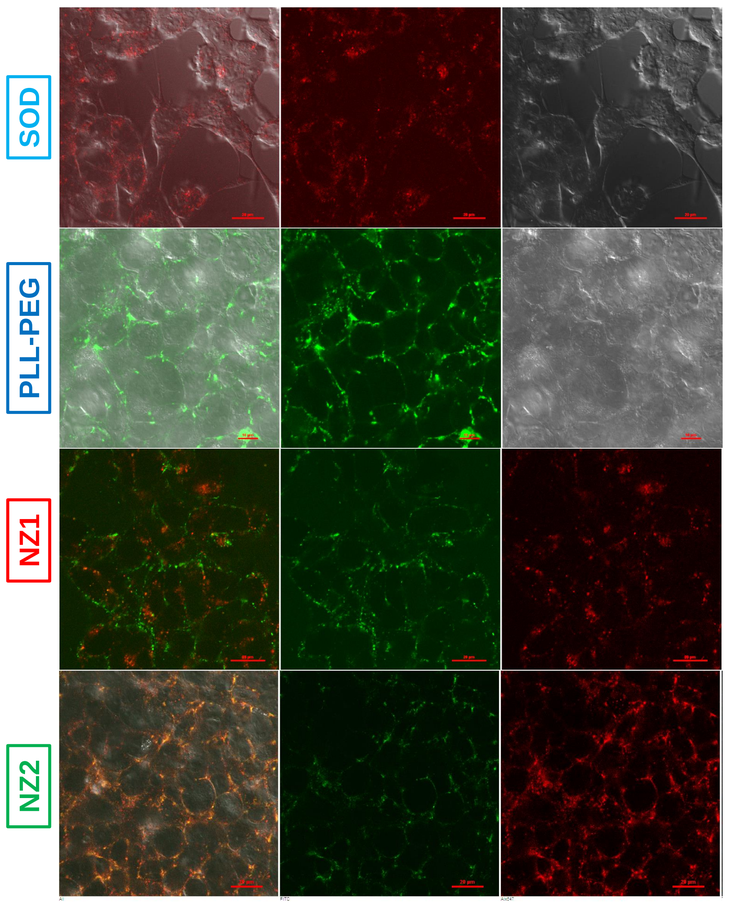
Protamine or polyarginine (Polycation)
Methoxy-poly(ethylene glycol)-block-poly(L-glutamic acid sodium salt) (PGlu-PEG)

n = 113 (MW = 5,000 Da)
x = 50 (MW = 8,000 Da)

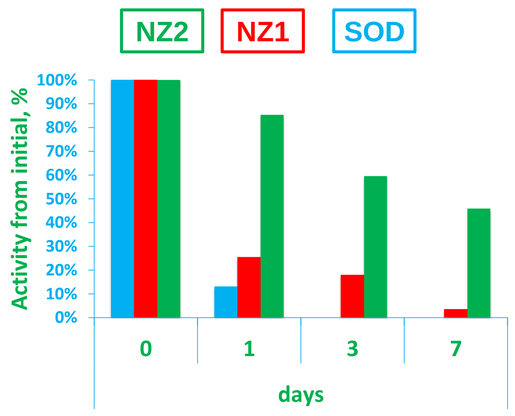
Materials & methods



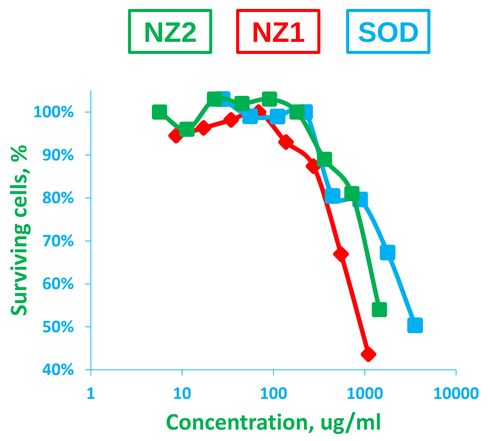
in vitro experiments



Confocal microscopy was used to investigate samples stability and internalization. HEK293 cells were incubated with samples for 30 minutes. SOD internalizes in cells while PLL-PEG is localized on membranes. NZ1 release enzyme which internalizes; polymer is on membranes. NZ2 do not release enzyme and colocalization is observed.



Stability in plasma in terms of activity was investigated by indirect enzymatic assay. Samples were incubated in plasma under 37°C during 7 days.



MTT-assay was used to evaluate cytotoxicity. Samples were incubated with HEK293 cells during 24 hours. Notably, non-toxic concentrations were used for all *in vivo* experiments .

Conclusions

- ✓ Drug delivery system for antioxidant enzymes was developed.
- ✓ Second layer of polymer defenses active site of enzyme during modification and allows us to use more active linker.
- ✓ Use of glutaraldehyde remarkably improves pharmacokinetics profile and, therefore, therapeutic efficiency of nanozymes.

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