Sensitive Detection of Zinc(II) in the Prostate with a Gadolinium-Based MRI Contrast Agent

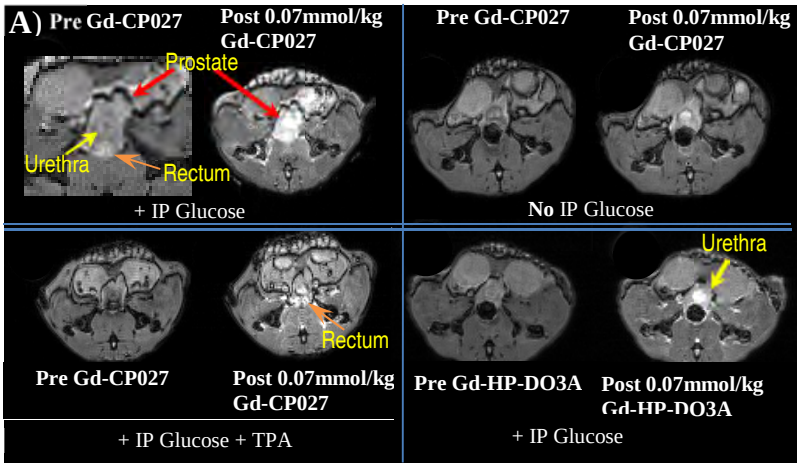
Veronica Clavijo Jordan[1,2], Christian Preihs[1], Shiuhwei Chen[3], Shanrong Zhang[1], Wen-hong Li[3], Neil Rofsky[2], and Dean Sherry[1,4]

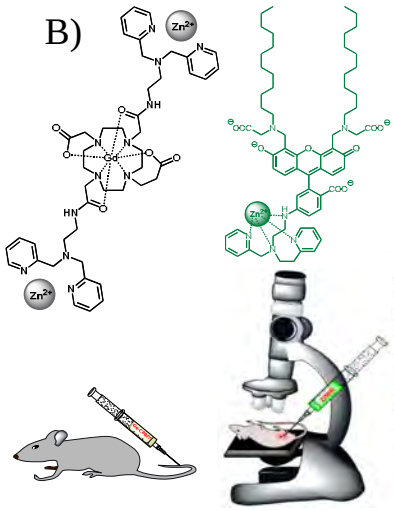
[1]*Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, Texas, United States,* [2]*Department of Radiology, UT Southwestern Medical Center, Dallas, Texas, United States,* [3]*Departments of Cell Biology and of Biochemistry, UT Southwestern Medical Center, Dallas, Texas, United States,* [4]*Department of Chemistry, UT Dallas, Texas, United States*

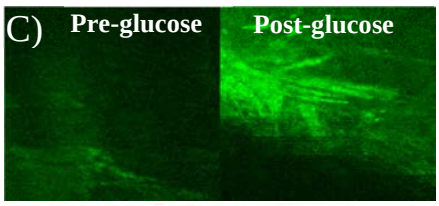
Introduction: There is an increasing need for the development of more efficient, more sensitive, and less invasive diagnostic methods for prostate cancer. Analyzing the potential markers for prostate cancer it is clear that zinc and citrate follow a general trend in terms of malignancy[1]. In prostate cancer, the citrate and zinc levels in the gland are significantly reduced, while remaining unchanged for healthy tissue and other confounding prostatic conditions such as benign prostatic hyperplasia (BPH), and prostatic intraepithelial neoplasia (PIN) [2]. Given the challenges in distinguishing between these conditions using current diagnostic methods, the development of, non-invasive zinc/citrate sensing tools could be of great advantage. Here we report a novel gadolinium-based contrast agent capable of sensitively detecting zinc using MRI. A previously designed sensor, Gd-DOTA diBPEN, has been shown to be able to bind to zinc and human serum albumin (HSA) to form a high T_1 relaxivity complex[3]. By rationally improving the chelate, it is possible to tune the water exchange rate to enhance the T_1 relaxivity while keeping its binding capabilities towards zinc and HSA. The administration of this agent *in vivo* results in the selective detection of zinc(II) present in the prostate following a bolus injection of glucose suggesting a novel mechanism of zinc(II) secretion in the prostate as a result of glucose stimulation.

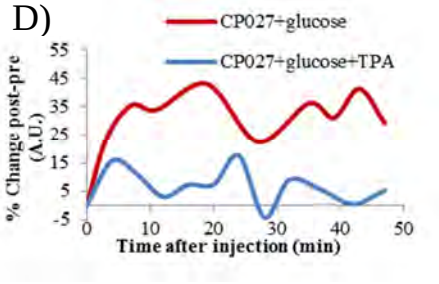
Methods: *Gd-CP027 synthesis:* The asymmetric zinc(II) sensor Gd-CP027 was prepared by coupling two equivalents of the zinc(II) binding moiety with the asymmetric cyclen core. Deprotection and complexation with Gd(III) afforded the target molecule which was then subjected to extensive preparative HPLC purification. The compound was then ready to be used for all *in vitro/vivo* studies described. *In vivo MRI:* 8-week old CB57/blk6 mice were fasted for at least 12 hours; the animals were anaesthetized with isofluorane and catheterized via the tail vein. Two pre-injection 3D T1-weighted gradient echo scans were obtained (TE/TR 1.69/3.34ms, NEX 8, Matrix 128x128x128) using a 9.4T Varian MRI scanner. After an IP injection of 50µl of 20% w/v D-glucose, 0.07mmol/Kg CP027 was injected using a syringe pump at a rate of 70µl/min. Immediately after injection, a series of 3D T_1-weighted scans were obtained to observe the dynamic glucose response and zinc(II) release in the prostate. Similarly, a series of controls were obtained by observing the response in the prostate after (1) no glucose stimulation, (2) blocking the available zinc(II) with a zinc chelator, Tris(2-pyridylmethyl)amine, (TPA), and (3) injecting a non-targeted control Gd-HP-DO3A. *In vivo confocal:* A fluorescent zinc(II) responsive probe (ZIMIR[4]) was used to test the hypothesis that glucose stimulates zinc release from the prostate. ZIMIR was synthesized following published protocols[4]. The ventral prostate of a fasted C57blk6 mouse was surgically exposed, ZIMIR was suspended in SAB buffer, 2µM DPAS, and food coloring (1:100 dil) to form a final compound concentration of 20µM. ZIMIR was injected using a glass-pulled needle directly into the lobe until a droplet (0.02-0.03µl) was observed to perfuse through the ducts of the gland. This process was repeated 5-7 times at the same location. The injection was then covered with a coverslip. Immediately after injection, the mouse was transferred to a confocal microscope. The lens was placed directly on top of the injection site and a series of baseline images were obtained based on the emission of ZIMIR-zinc(II) at 515nm. 50µl of 20%w/v D-glucose was then injected IP and images were collected after 5 minutes.

Results and Conclusion: Here we report a novel gadolinium-based contrast agent with the capability of sensitively detecting zinc(II) using MRI. We successively modified the design of the macrocyclic ligand of Gd-DOTA diBPEN[3] in an effort to tune the water exchange rate. This resulted in an enhancement of T_1 relaxivity from 5.6mM^{-1}s^{-1} to 57.3mM^{-1}s^{-1} upon binding to zinc(II) and HSA (Figure B, upper left). Our agent Gd-CP027 was then injected *in vivo* via the tail vein (Figure B, lower left), the contrast agent "turned on" in the prostate only after a bolus injection of glucose IP (Figure A top row). In order to determine if the enhancement is specific, we blocked any available zinc(II) in the circulation and inside cells by pre-injecting TPA (Figure A, bottom left); after glucose and contrast agent injection, the prostate enhancement was reduced almost 6-fold (Figure D). Also, Gd-HP-DO3A was injected and no significant enhancement was observed in the prostate but was seen in the urethra (Figure A, lower right). Our MRI results suggest that glucose stimulates the secretion of zinc from the cells into the interstitial space where it becomes available to bind to our contrast agent. In an effort to determine if this mechanism of action is indeed present, we injected a zinc(II) responsive fluorescent probe, ZIMIR, directly into the ventral lobe of the mouse prostate and imaged the live mouse using a confocal microscope (Figure B, right). This probe contains two lipophilic chains that allow it to anchor to the cell lipid bilayer (Figure B, right). Therefore, its emission at 515nm would only be possible if zinc is either already present in the interstitial space and/or if there is zinc secretion from inside to outside the epithelial cells of the prostate. Here we observed that before glucose stimulation, some non-specific fluorescence was observed as a result of free zinc(II) in the tissue. However, after glucose stimulation the fluorescence intensity increased drastically, and perhaps delineated the prostatic ducts in the ventral lobe (Figure C). This confirmed that glucose is indeed responsible for the secretion of zinc(II) from epithelial secreting cells into the prostate interstitial space. Therefore, a mechanism involving glucose metabolism can be observed, possibly stimulating citrate synthesis, and in turn zinc secretion into the prostate gland interstitial space.









We conclude that the rational design of a zinc(II) responsive contrast agent can sensitively detect zinc secretion in the non-cancerous prostate gland *in vivo*. This may be a novel and more accurate paradigm for the detection of prostate cancer (presumably non-enhancing) and thus, distinguish it non-invasively from other confounding conditions.

References: 1. Costello L., *et al.*, The Prostate, 1998.
2. Franklin R., *et al.*, Arch. of Biochem. and Biophys, 2007.
3. Esqueda AC, *et al*., JACS, 2009.
4. Li D., *et al.*, PNAS, 2011.