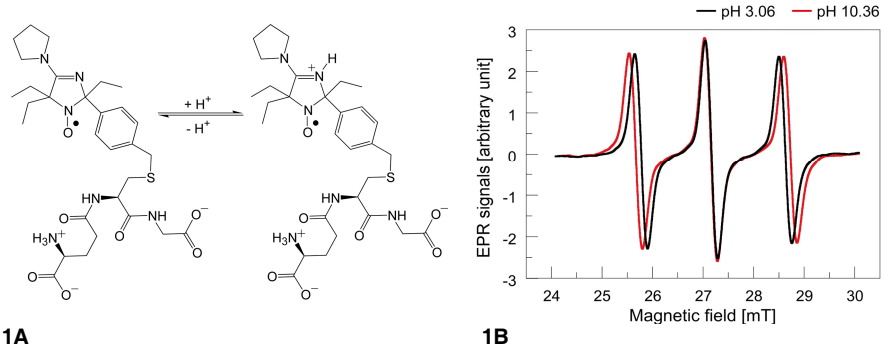
Electron paramagnetic resonance spectroscopy for in vivo measurement of tumour extracellular pH- the effect of X-ray irradiation

Jonathan Goodwin[1,2], Katsuya Yachi[3], Masaki Nagane[4], Hironobu Yasui[4], Yusuke Miyaki[3], Osamu Inanami[4], Andrey Bobko[5], Valery Khramstov[5], and Hiroshi Hirata[3]
[1]Department of Radiology, Hokkaido University Hospital, Sapporo, Hokkaido, Japan, [2]Division of Ultrahigh Field MRI, Iwate Medical University, Yahaba, Iwate, Japan, [3]Division of Bioengineering and Bioinformatics, Hokkaido University, Sapporo, Japan, [4]Laboratory of Radiation Biology, Hokkaido University, Sapporo, Japan, [5]Division of Pulmonary, Allergy, Critical Care & Sleep Medicine, The Ohio State University, Columbus, Ohio, United States

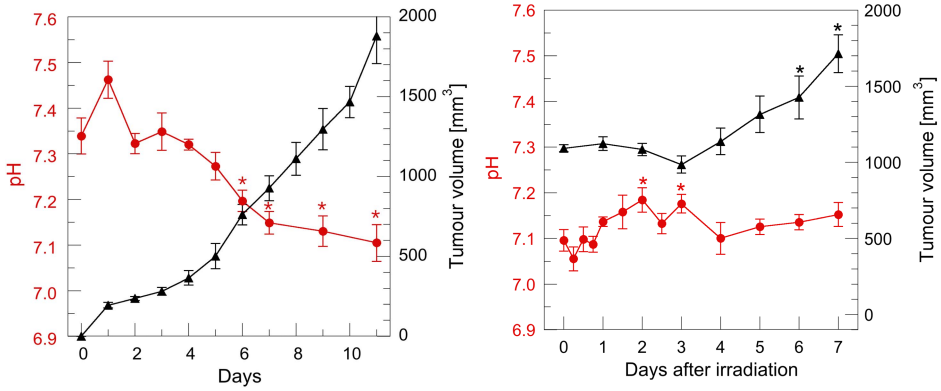
Target audience – NMR scientists in biomedicine, oncologists and drug development scientists.
Purpose - Changes in extracellular pH (pH_e) in tumour may provide a useful biomarker for tumour cell metabolism[1-4]. In this study, we assess the viability of continuous-wave electron paramagnetic resonance (CW-EPR) spectroscopy with a pH-sensitive nitroxide to measure pHe in the mouse model, before and after X-ray irradiation.



1A 1B

Figure 1. (A) Molecular configuration of protonated and non-protonated forms of pH sensitive spin probe RSG:2-(4-((2-(4-Amino-4-carboxybutanamido)-3-(carboxymethylamino)-3-oxoproylthio)methyl)phenyl)-4-pyrrolidino-2,5,5-triethyl-2,5-dihydro-1□-imidazol-1-oxyl (this compound is abbreviated as R-SG). **(B)** pH dependent spectra of R-SG. Hyperfine coupling constant (HFC), defined as half the distance between first and third spectra, varies according to local pH.

Methods - Intravenous injection of pH-sensitive nitroxide (R-SG) (Fig.1A) was used with 750 MHz CW-EPR to measure the hyperfine coupling constant (HFC, Fig.1B) in C3H HeJ mice hind leg squamous cell tumour. Repeated measurements were obtained during normal tumour growth, and in response to a single 10 Gy dose of X-ray irradiation. pHe was determined using an R-SG titration curve relating HFC to pH.



2A 2B

Figure 2. (A) Relationship between tumour volume and pH_e during normal tumour growth. Each data point represents the mean of 6 mice with SEM displayed. Day 0 - prior to squamous cell injection into the right hind leg. An inverse relationship between tumour volume and pH_e was observed in all mice from Day 3. Significant difference in pH_e between Day 0 and Day N is shown * ($p < 0.05$). **(B)** Tumour regrowth and pH_e in irradiated mice. The mean tumour volume before irradiation was approximately equal to the non-irradiated group at Day 9 in Fig. 2A. Data points represent the mean of 6 mice with standard error of the mean displayed. Significant difference in pH_e and tumour volume between Day 0 (measured before irradiation) and Day N is shown * ($p < 0.05$).

Results - An inverse relationship was observed between tumour volume and pH_e, whereby during normal tumour growth a constant reduction in pH_e was observed after Day 3 (Fig. 2A). This relationship was disrupted by X-ray irradiation, and from 2-3 days post exposure, a transitory increase in pH_e was observed (Fig. 2B).

Conclusion - In this work we demonstrate the viability of CW-EPR spectroscopy with R-SG nitroxide to obtain high sensitivity pH_e measurements in mouse tumour model with an accuracy of < 0.1 pH units. The ability to measure pH_e change in response to X-ray irradiation, suggests this may offer an alternative technique for assessing treatment response to existing and novel cancer therapies.

References – 1.) Bobko AA, Eubank TD, et al. In vivo monitoring of pH, redox status, and glutathione using L-band EPR for assessment of therapeutic effectiveness in solid tumors. Magnetic resonance in medicine 2012;67(6):1827-1836. 2.) Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nature reviews Cancer 2004;4(11):891-899. 3) Gerweck LE, Seetharaman K. Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. Cancer research 1996;56(6):1194-1198. 4.) Hashim AI, Zhang X, et al. Imaging pH and metastasis. NMR in biomedicine 2011;24(6):582-591.