Imaging Tumor Metabolism and pH Using Hyperpolarized ^{13}C Biosensors and PET/MRI (A7)

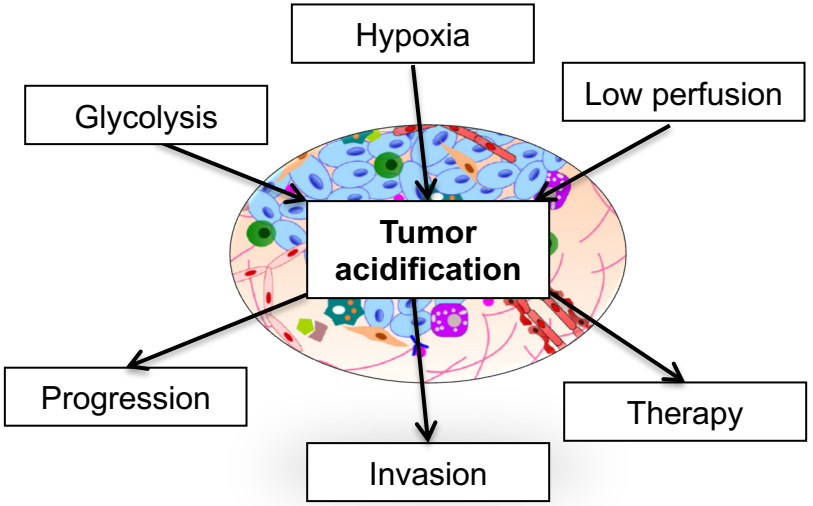
PIs: Axel Haase[1], Franz Schilling[1,2]

Martin Grashei[2], Christian Hundshammer[2], Stephan Düwel[2], Pascal Wodtke[2], Sandra Sühnel[2], Geoffrey Topping[2]

[1]Munich School of Bioengineering
[2]Department of Nuclear Medicine, TUM

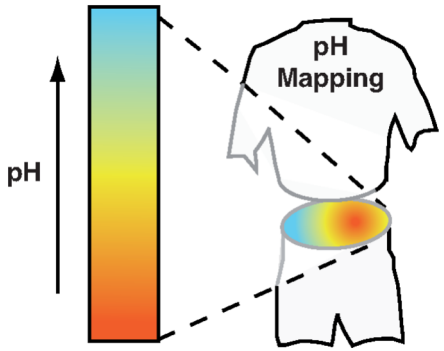
MOTIVATION: Several pathologies perturb extracellular pH (pH_e) regulatory mechanisms

- ischemia
- inflammation
- **tumors**

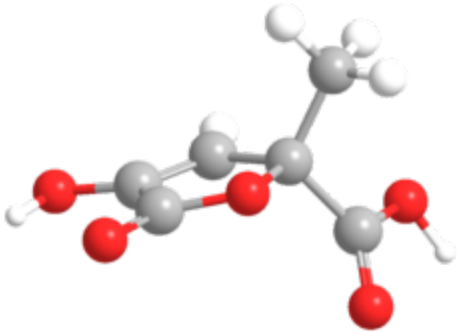


AIM: Establish non-invasive MRI-based pH imaging for preclinical and clinical applications in oncology

- tumor phenotyping
- characterizing tumor heterogeneity
- therapy monitoring and evaluation



Outline



1. **Methods Development**: Hyperpolarized MRI pH sensors

2. **Novel Contrasts**: Intratumoral pH heterogeneity

3. **pH imaging:**

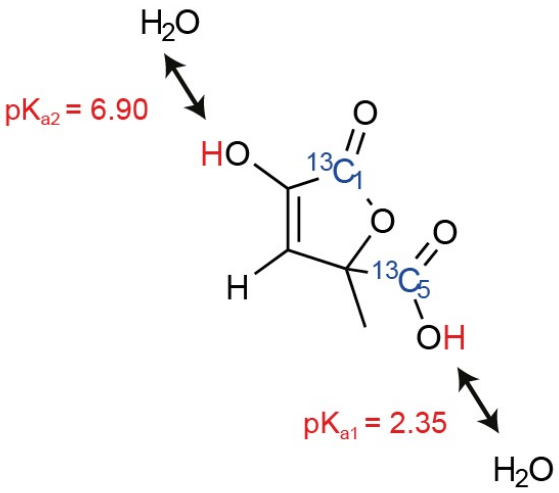
   "Imaging for selection, monitoring and individualization of cancer therapies"

   a) **Selection**: Metabolic differentiation in patient-derived glioblastoma in mice treated with NIS-expressing MSCs (with B2 and C8)
   b) **Monitoring**: T-cell activity in human myeloid sarcoma xenografts in mice (C10)
   c) **Individualization**: Metabolic heterogeneity in feline fibrosarcoma (Z2)

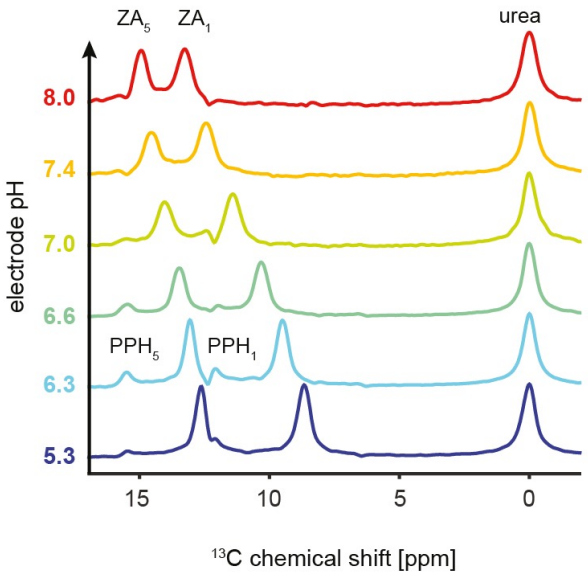
[1,5-$^{13}C_2$]zymonic acid as MRI pH_e-Sensor

Chemical structure

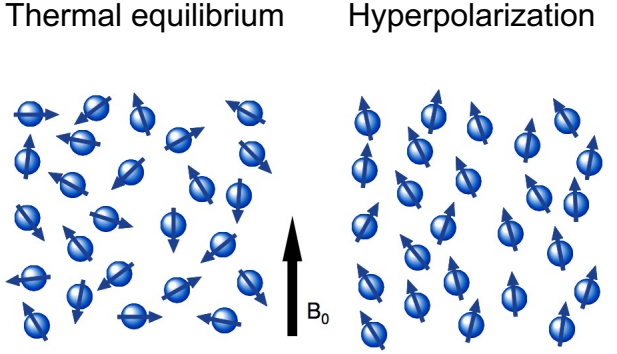
pK_a = 6.90



pH-sensitive ^{13}C NMR resonances



Hyperpolarization

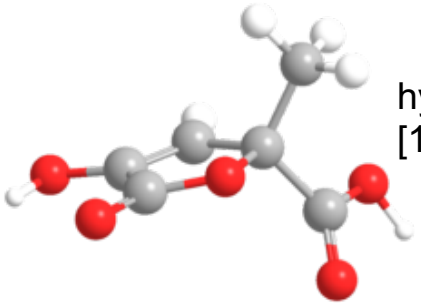


Enhancement 10.000 – 100.000 - fold

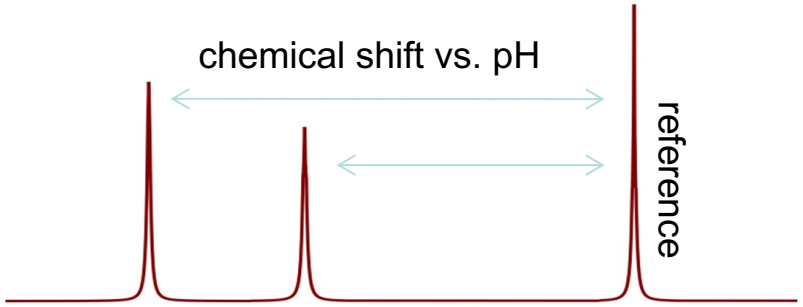
Acquisition duration for a 1D spectrum:

3 years **1 s**

[1,5-$^{13}C_2$]zymonic acid as MRI pH_e-Sensor

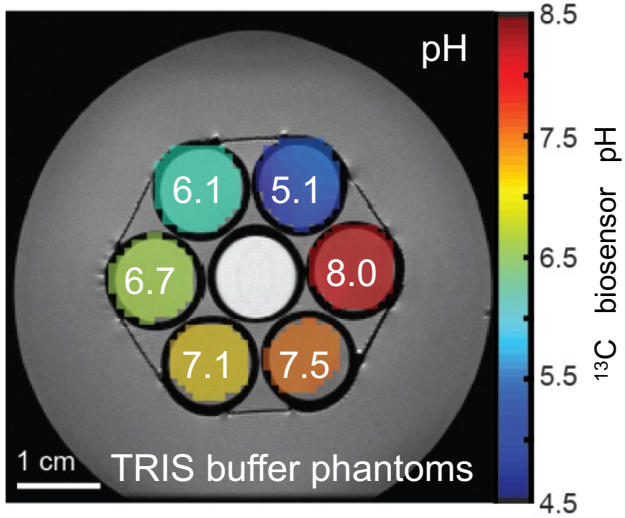


hyperpolarized
[1,5-^{13}C]zymonic acid



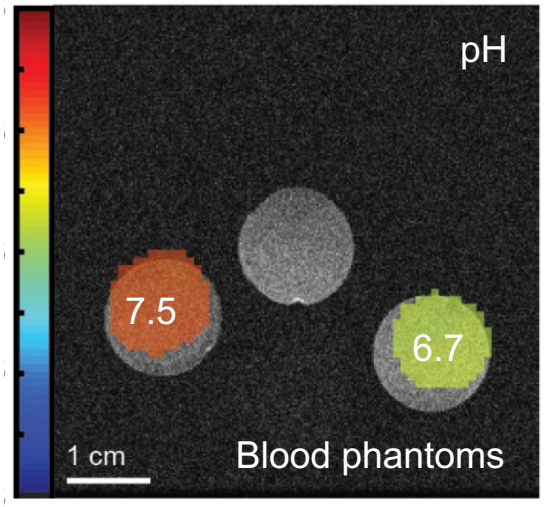
pH-phantoms

ca. 5 mM urea and zymonic acid in 80 mM TRIS-buffer



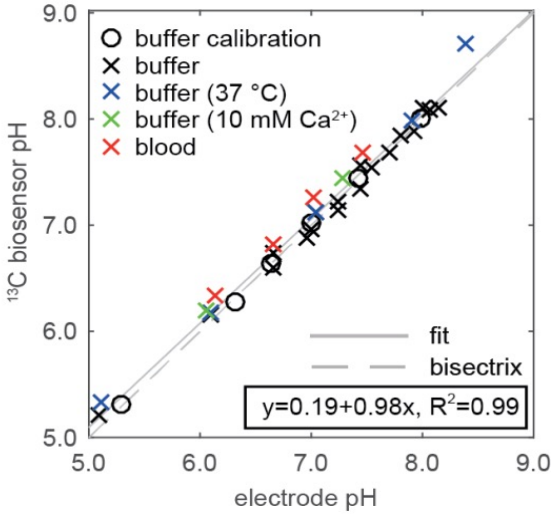
Blood-phantoms

ca. 5 mM urea and zymonic acid in titrated blood



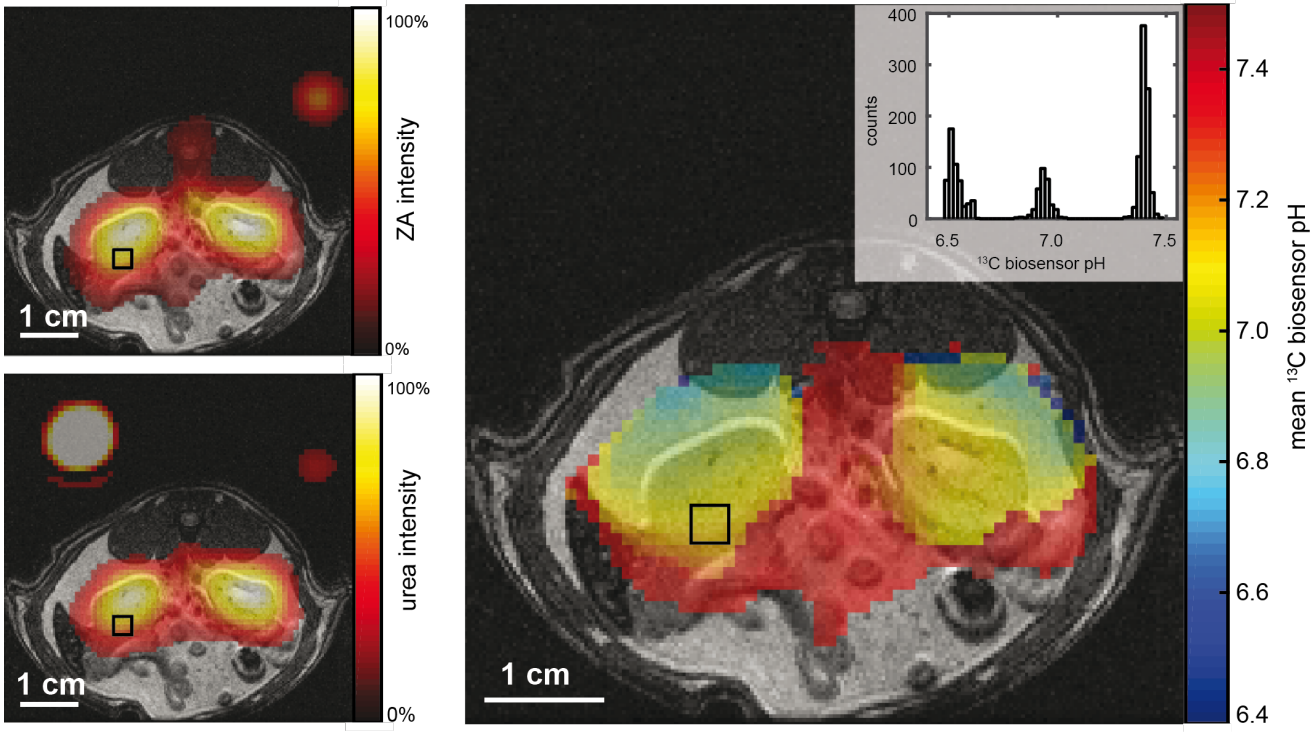
Validation with pH electrode

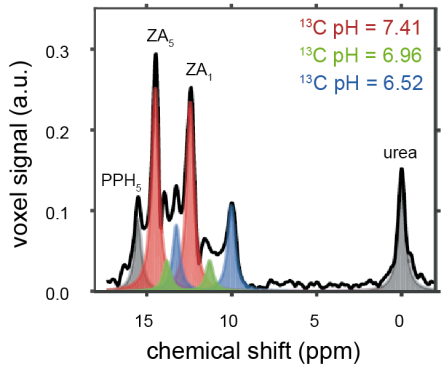
^{13}C-biosensor pH-measurements are robust to external influences

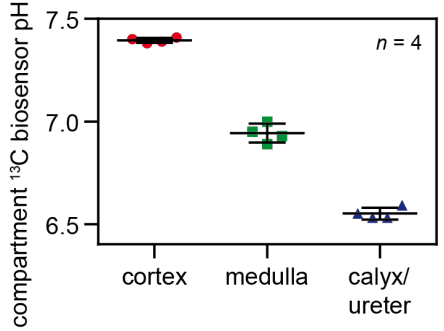


Imaging renal pH compartments

- → Zymonic acid reveals three pH compartments in rat kidneys



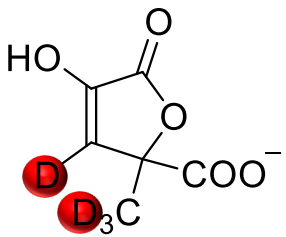


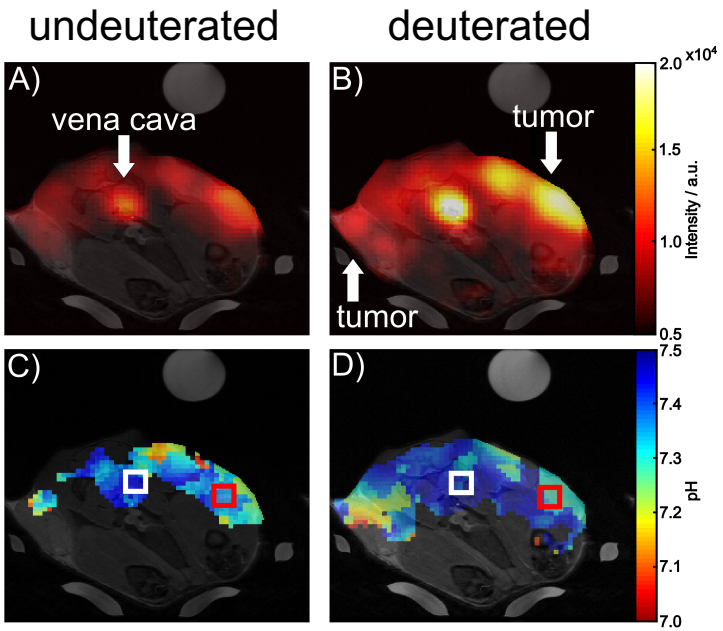


Düwel, Hundshammer, Haase, Schwaiger, Schilling et al., *Nature Communications* 8:15126 (2017)

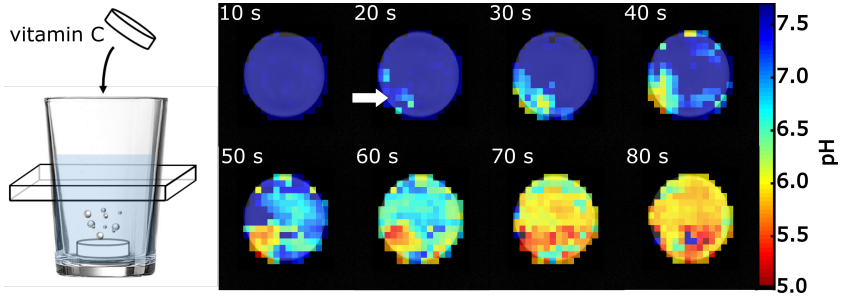
Sensitivity enhancement through deuteration

- Deuterated zymonic acid increases SNR by 50 % *in vivo*





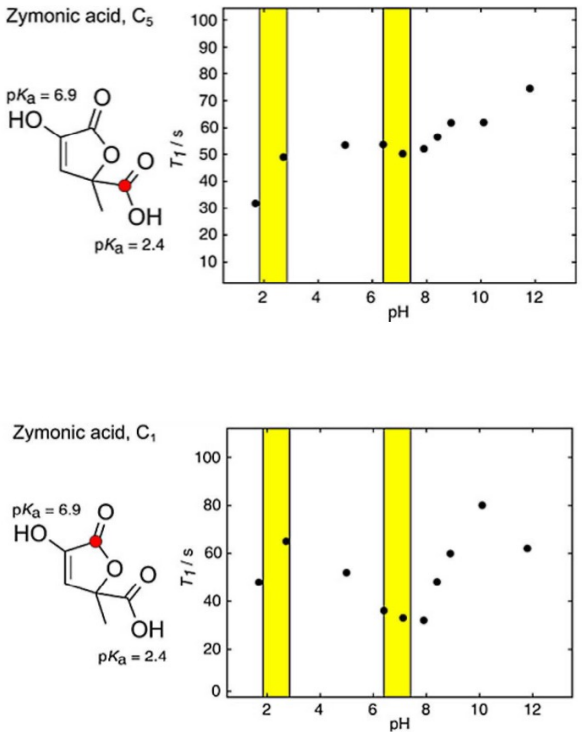
- Imaging of dynamic pH changes in *vitro*



Hundshammer et al. *ChemPhysChem* (2017) 18:2422

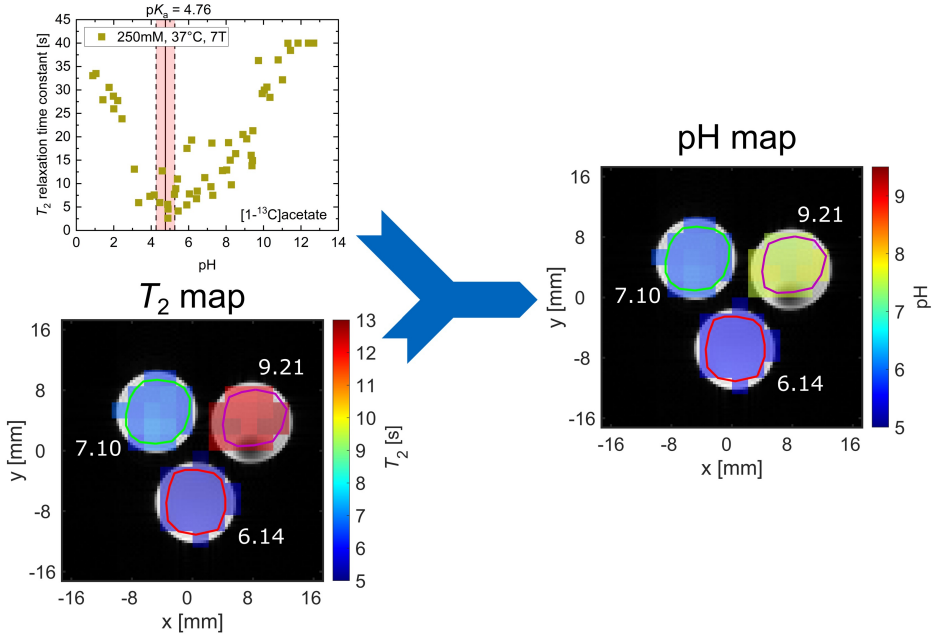
Characterization of pH effects on relaxation

- pH dependance of T_1 relaxation
- proton exchange at pK_a values reduces T_1



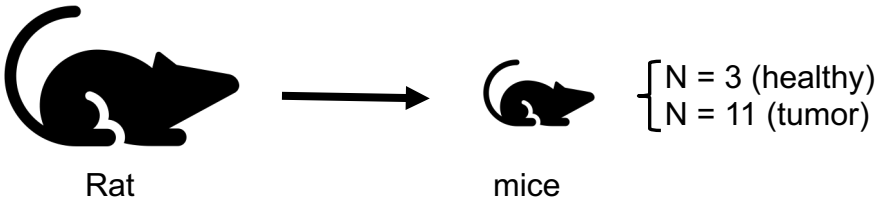
Hundshammer et al. *ChemPhysChem* (2019) 20:798

- pH dependence of T_2 relaxation
- Again proton exchange at pK_a values is a major contributor to T_2 reduction

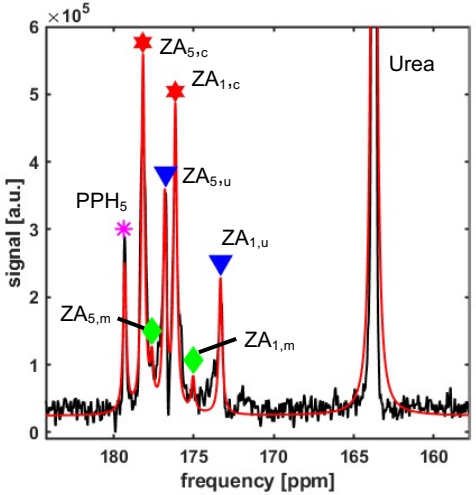


Grashei et al. *Pharmaceuticals* (2021) 14:327

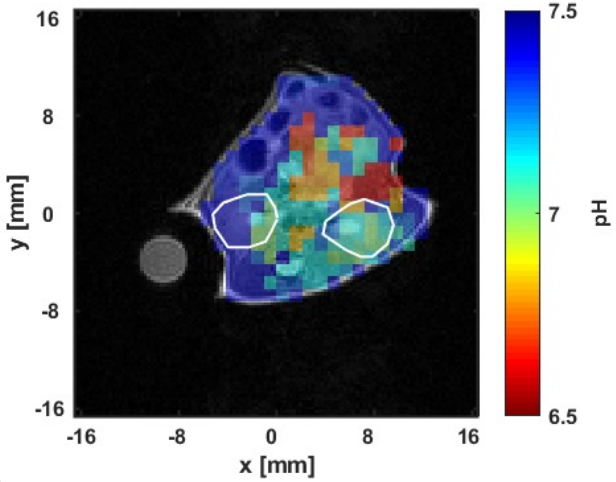
Establishing pH imaging methods in mice



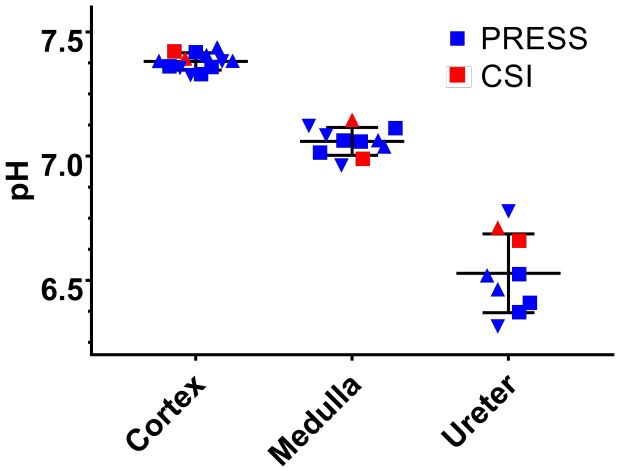
Kidney spectrum (PRESS)



Average pH map



pH compartmentation



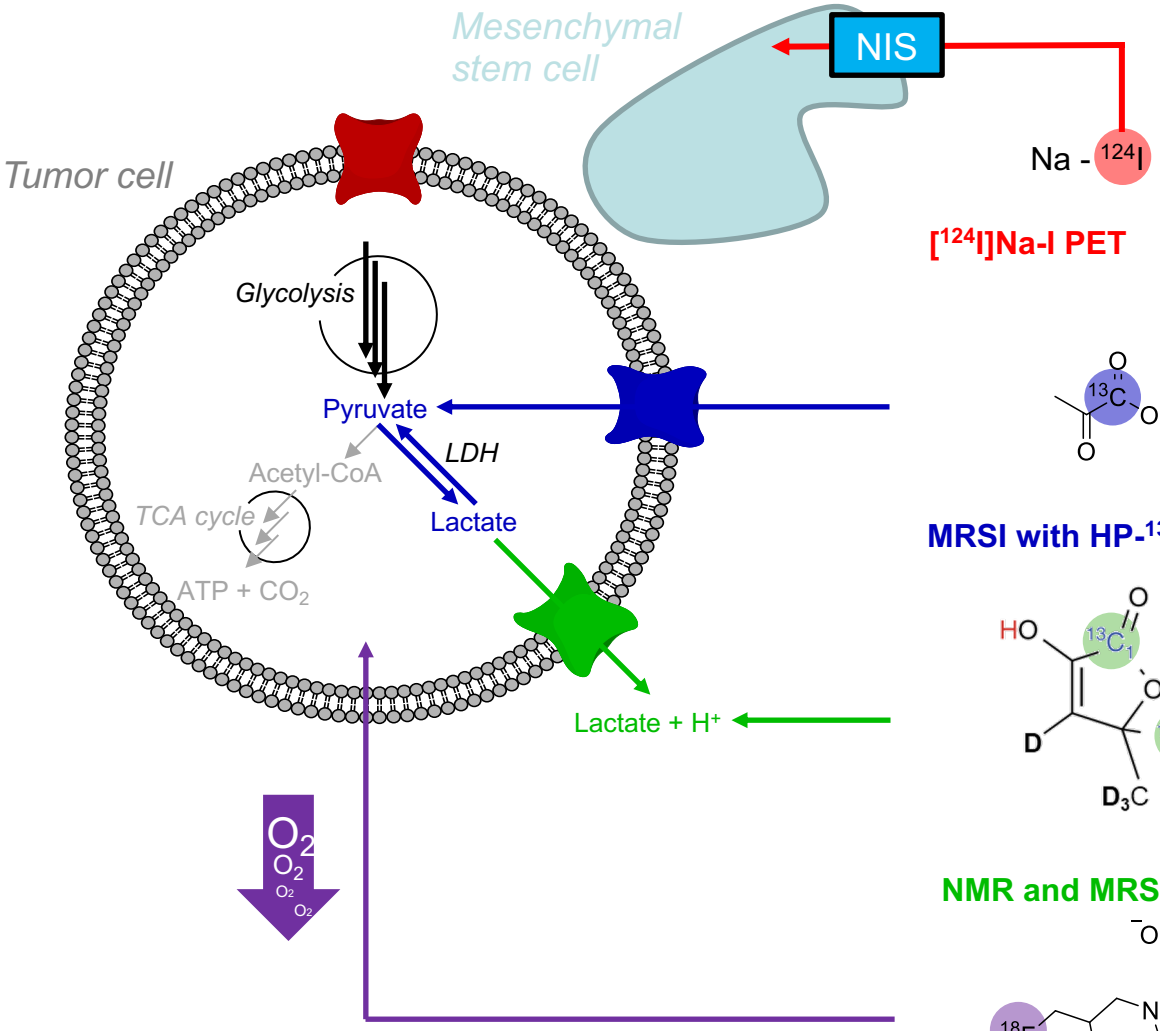
→ 3 pH compartments in kidney visible

✓ Equivalent absolute pH values compared to rats

→ pH compartment detection by **deep learning** show equal or superior performance to conventional fitting (submitted to EJNMMI Research)

Metabolic characterization of PDX glioblastoma in mice treated with NIS-expressing MSCs

joint project with B2, C8



Iodide uptake

[^{124}I]Na-I PET

LDH activity

MRSI with HP-^{13}C-Pyruvate

Acidification

NMR and MRSI with zymonic acid

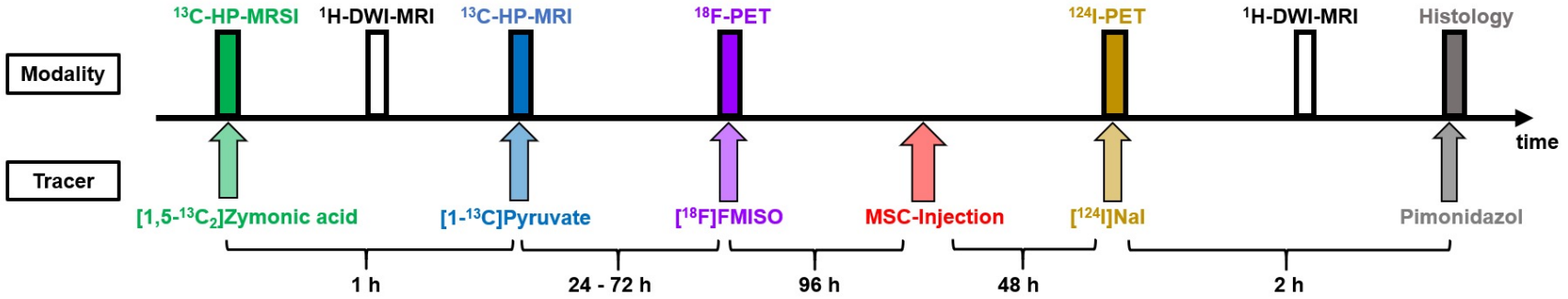
Hypoxia

FMISO-PET

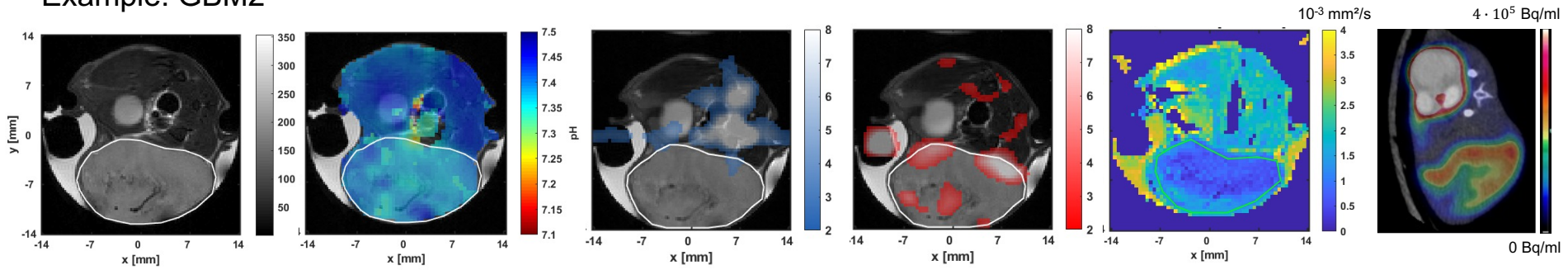
Metabolic characterization of PDX glioblastoma in mice treated with NIS-expressing MSCs (with B2 and C8)

Study design:

- Multimodal imaging before stem cell injection PET/CT / ^{1}H-MRI / ^{13}C-HP-MRI
- Two different metabolic phenotypes
- Goal: Metabolic stratification of PDX glioblastoma model



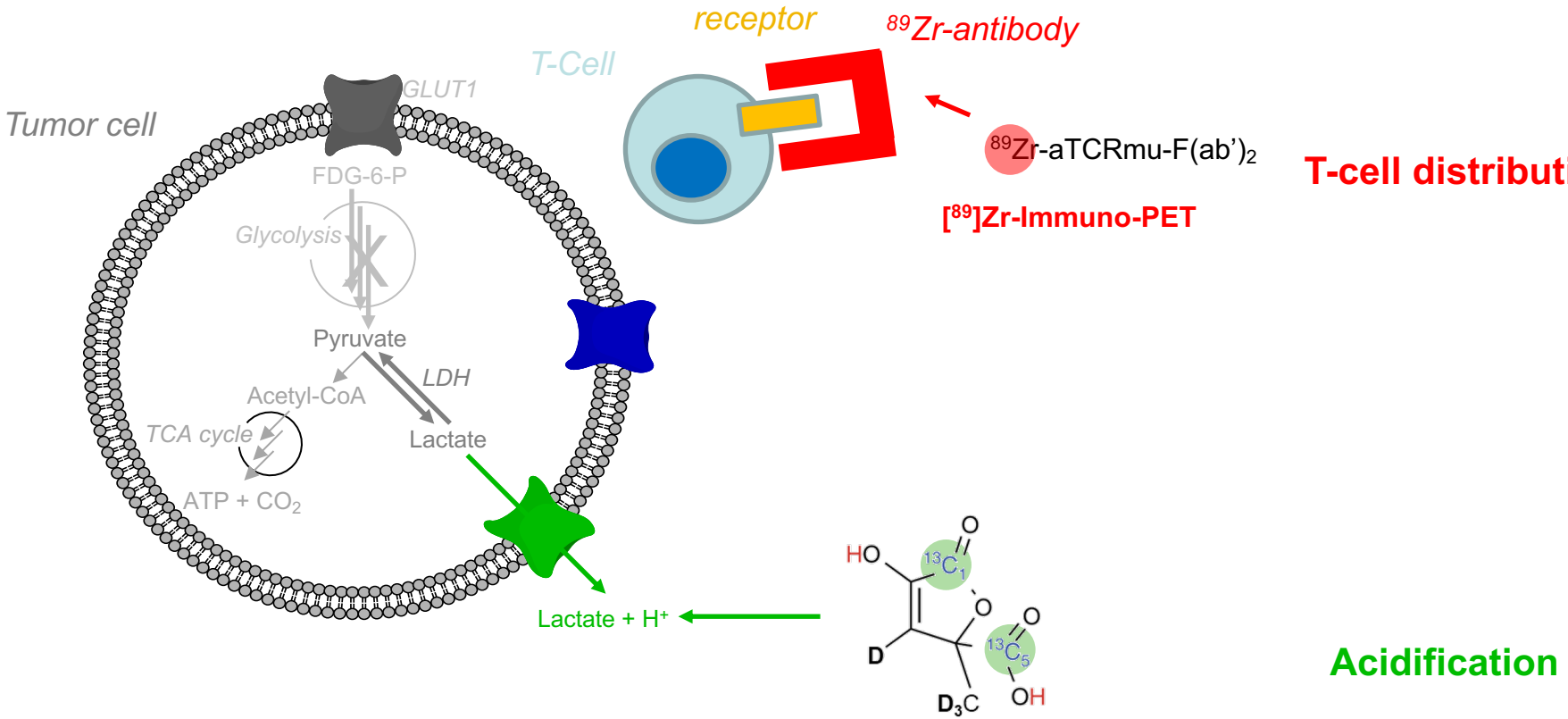
Example: GBM2



T_{2w} Anatomical | pH | [1-^{13}C]Pyruvate | [1-^{13}C]Lactate | DWI | [^{18}F]FMISO-PET

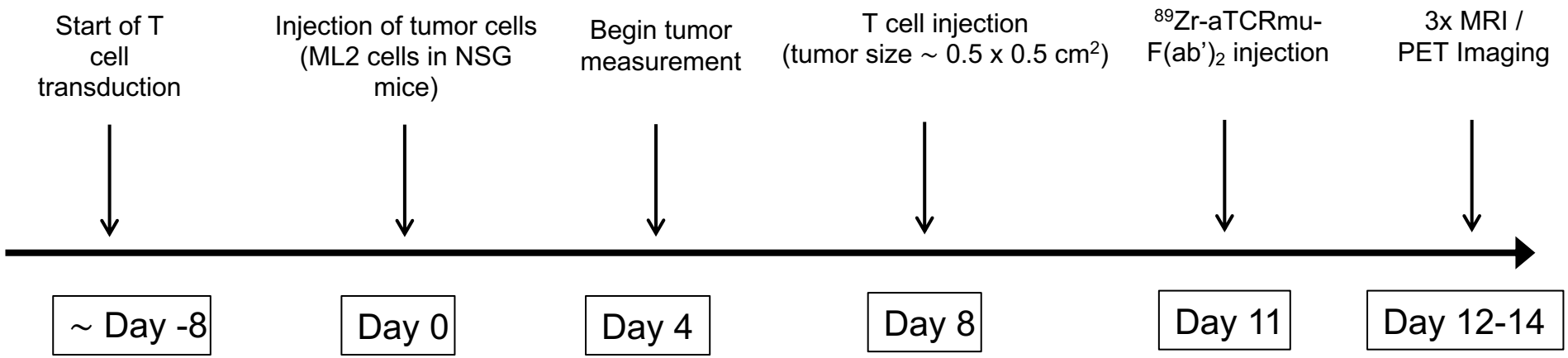
Influence of tumor pH on T-cell therapies

joint project with C10



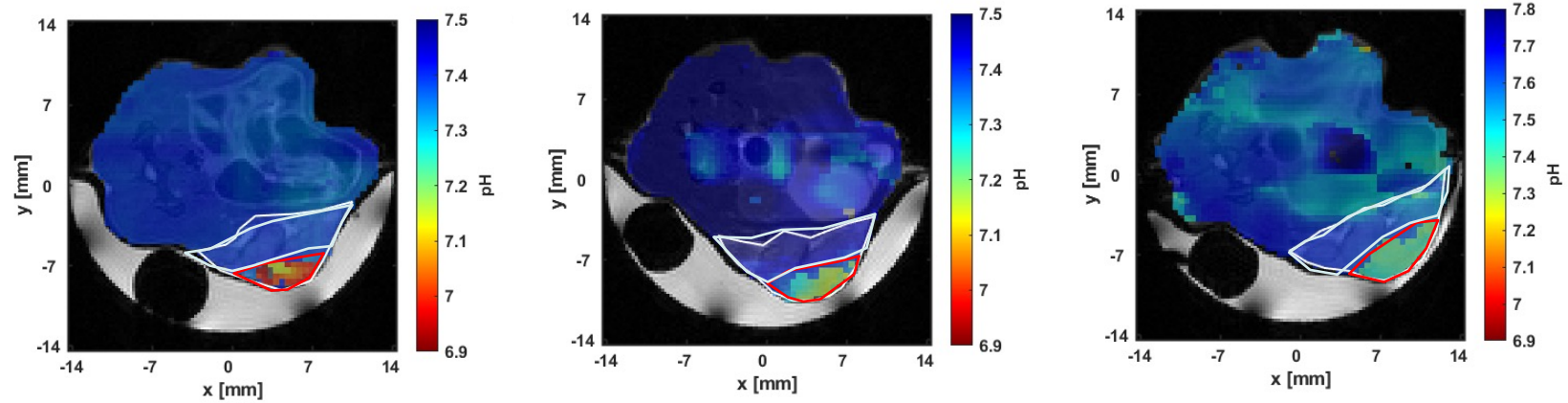
Influence of tumor pH on T-cell therapies

Timeline:



24h post Fab-Inj. 48h post Fab-Inj. 72h post Fab-Inj.

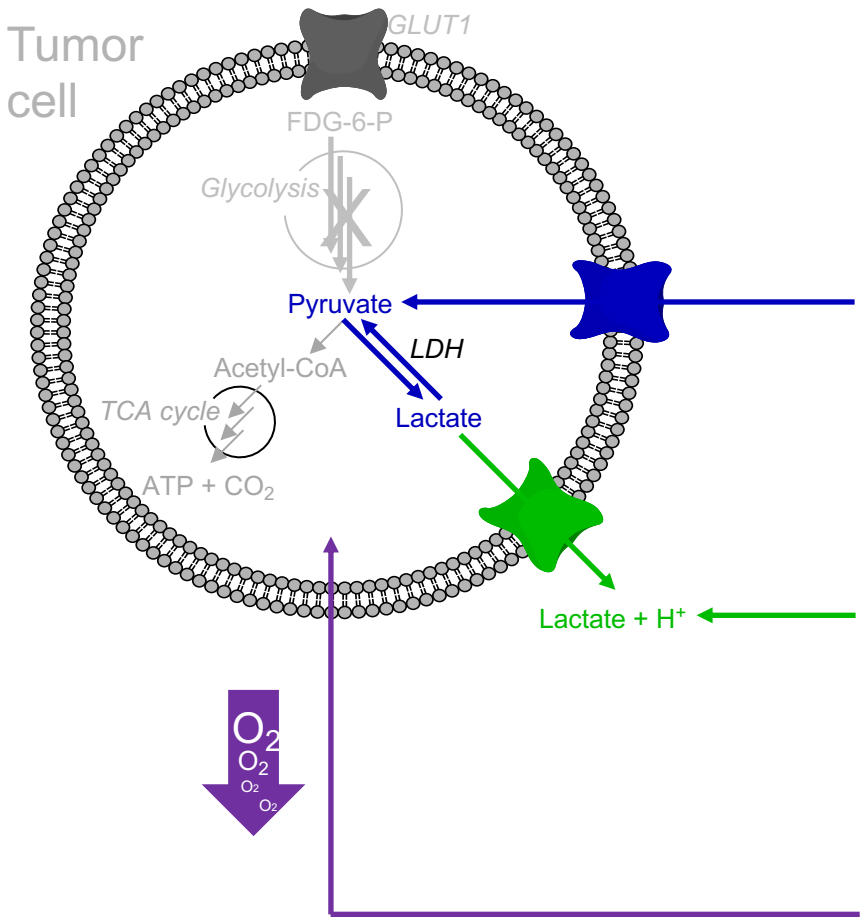
pH map

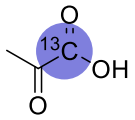


→ pH maps allow tumor segmentation in 2 regions: physiologic (yellow) and acidic (red)

Metabolic heterogeneity in feline fibrosarcoma

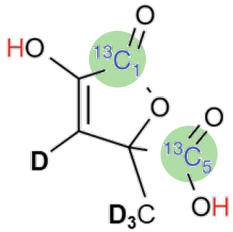
joint project with Z2





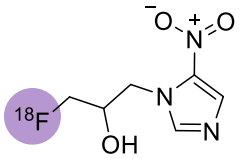
LDH activity

MRSI with HP-^{13}C-Pyruvate



Acidification

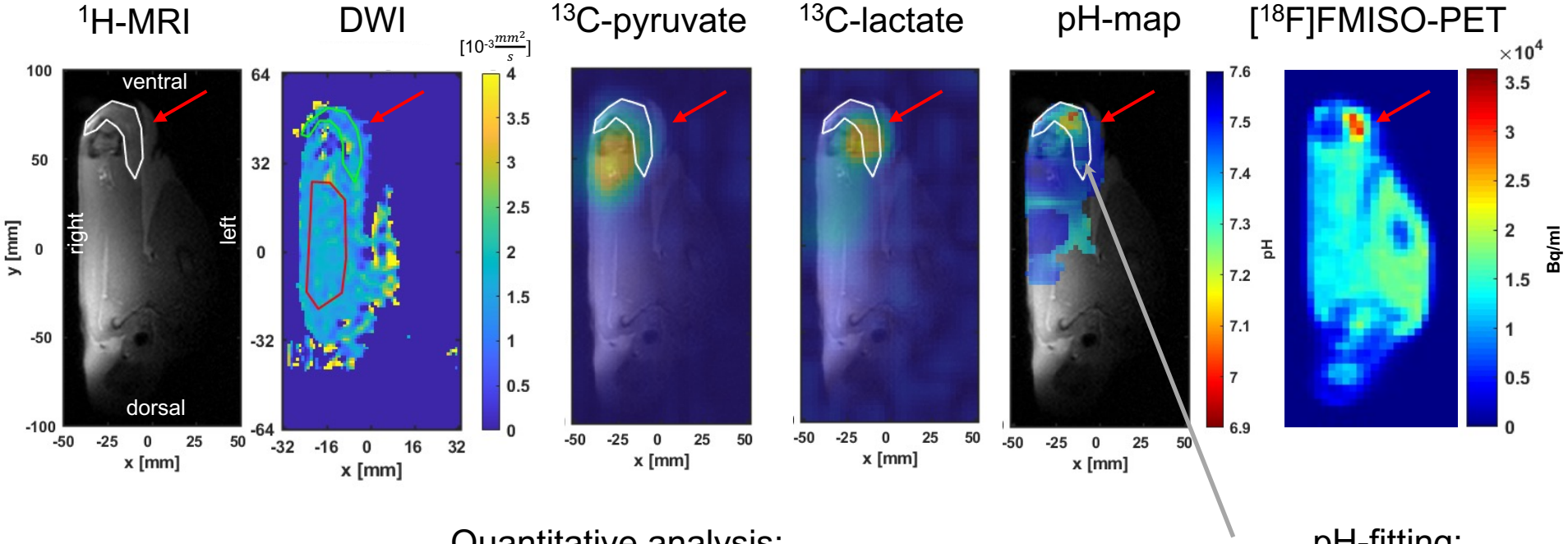
NMR and MRSI with zymonic acid



Hypoxia

FMISO-PET

Metabolic heterogeneity in feline fibrosarcoma



Quantitative analysis:

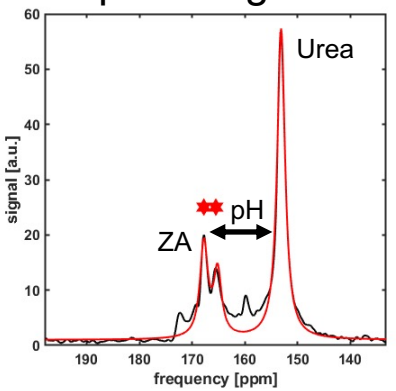
DWI: $ADC_{ROI} = 1.29 \pm 0.71 \cdot 10^{-3} \frac{mm^2}{s}$

^{13}C-MRSI: $AUC_{(Pyr/Lac)} = 0.17$

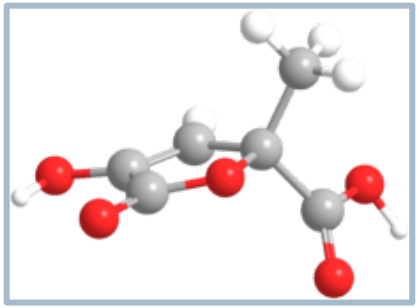
$pH_{ROI} = 7.35$

→ Metabolic heterogeneity both for AUC and pH

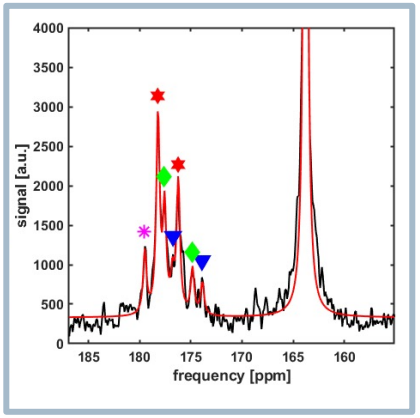
pH-fitting:



Summary Project A7



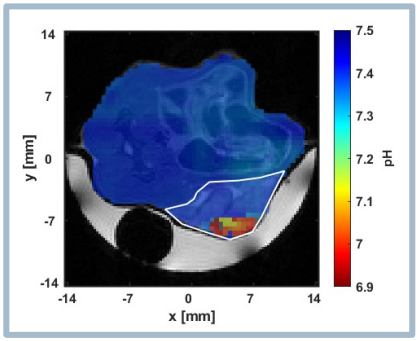
1. Several hyperpolarized MRI pH sensors were developed and validated *in vivo* including
    - zymonic acid
    - Z-OMPD



2. Spectral pH analysis allows to analyze
    - sub-voxel pH heterogeneity

3. pH imaging was applied within our consortium for therapy

    a) **selection**: Metabolic differentiation in patient-derived glioblastoma in mice treated with NIS-expressing MSCs (with B2 and C8)
    b) **monitoring**: T-cell activity in human myeloid sarcoma xenografts in mice (C10)
    c) **individualization**: Metabolic heterogeneity in feline fibrosarcoma (Z2)



Acknowledgements

Nuclear Medicine

- **Prof. Dr. M. Schwaiger**
- **Prof. Dr. W. Weber**
- **M. Grashei**
- **Dr. C. Hundshammer**
- **Dr. S. Düwel**
- **P. Wodtke**
- **S. Sühnel**
- **S. Reder**
- M. Herz
- Dr. Geoffrey Topping
- Frits van Heijster
- Dr. Jason Skinner
- Dr. B. Feuerecker
- Dr. M. Bräuer
- P.D. Dr. S. Nekolla
- Dr. J. Cabello
- M. Schillmaier
- E. Bliemsrieder
- L. Nagel
- M. Aigner
- S. Schachoff
- M. Mittelhäuser
- H. Rolbieski
- B. Blechert
- M. Michalik

SFB824

Z2
- Prof. Dr. W. Weichert
- **PD Dr. K. Steiger**
- N. Wirges
- O. Seelbach
- M. Mielke

B2
- **Prof. Dr. R. Glaß**
- **R. Kälin**

C8
- **Prof. Dr. C. Spitzweg**
- **C. Kitzberger**
- R. Spellerberg

C10
- **Prof. Dr. A. Krackhardt**
- **PD Dr. C. D'Alessandria**
- **L. Russelli**
- **D. Gosmann**

MTK, CTK

- Prof. Dr. J. Hirschberger
- Prof. Dr. A. Meyer-Lindenberg
- S. Kahl

University Hospital Freiburg

- Prof. Dr. J.B. Hövener
- C. Müller

ZPF

- PD Dr. C. Baumgartner
- Dr. J. Fischer
- S. Baer

Chemistry

- Prof. Dr. S. Glaser
- Prof. Dr. B Sieber
- Prof. Dr. B Reif
- Prof. Dr. W. Eisenreich
- Dr. R. Marx
- Dr. M. Gersch
- Dr. S. Köcher
- C. Schwarz
- D. Ruseckas
- A. Greiner
- A. Schröder

BioEngineering

- Prof. Dr. A. Haase
- PD Dr. M. Menzel
- J. Hintermair


Thank you for a wonderful SFB!