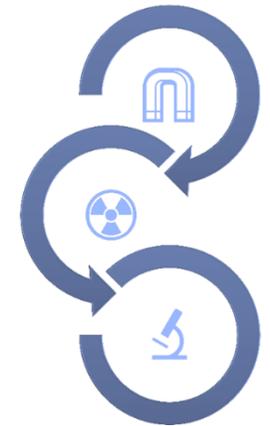
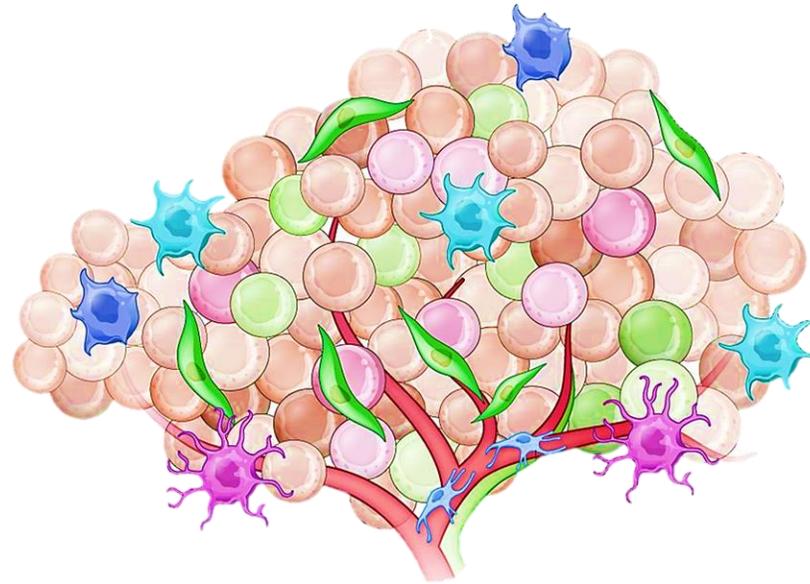


16th European Molecular Imaging Meeting | EMIM 2021

24-28 August 2021 in Göttingen, Germany

Declaration of Financial Interests or Relationships

I or one of my co-authors have **no financial interests or relationships** to disclose with regard to the subject matter of this presentation.

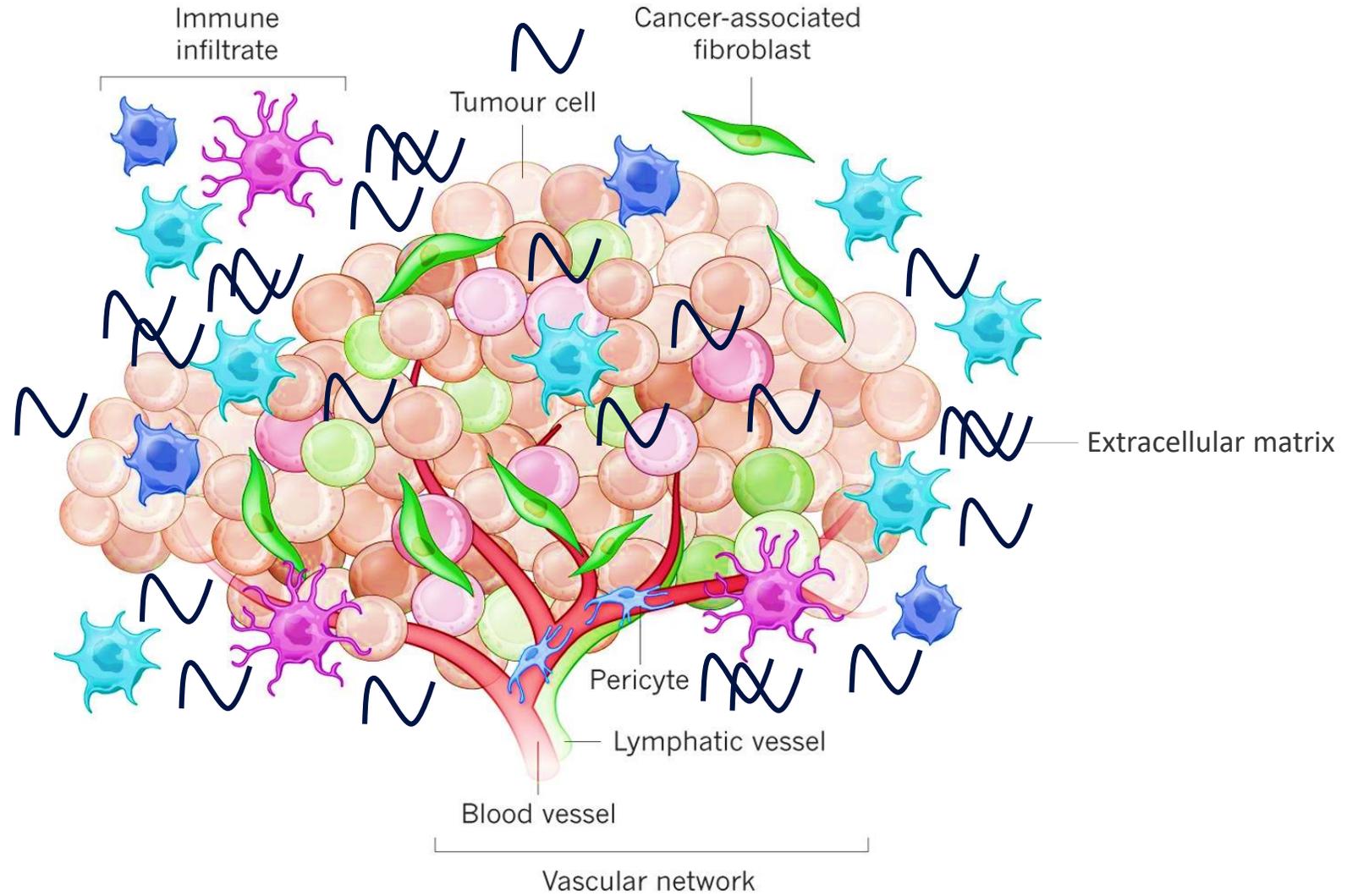


TUMOR MICROENVIRONMENT IMAGING: MULTIMODALITY BENEFIT TO STUDY CHONDROSARCOMA

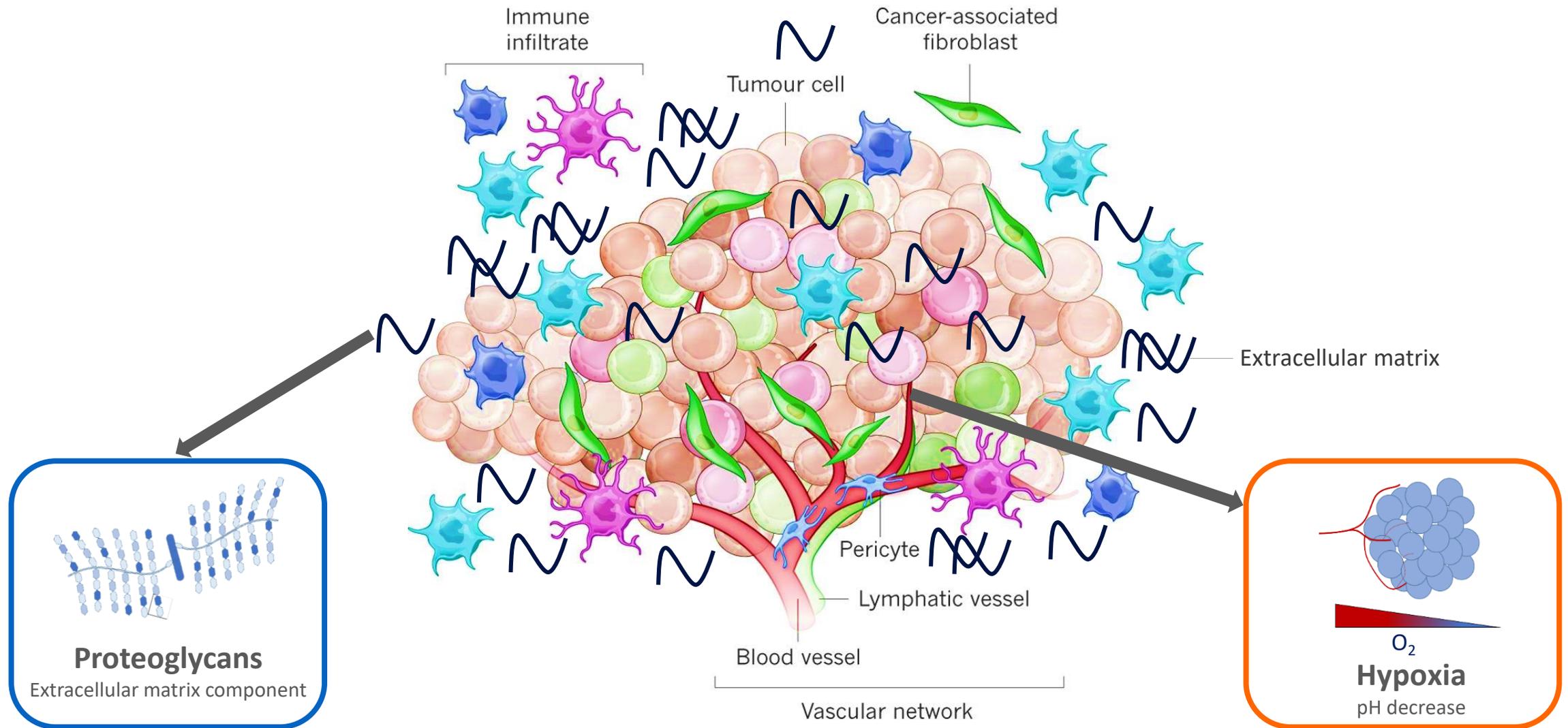
*Roxane Autissier ; Leslie Mazuel ; Elise Maubert ; Jean-Marie Bonny ; Philippe Auzeloux ; Sébastien Schmitt ;
Amidou Traoré ; Caroline Peyrode ; Elisabeth Miot-Noirault ; Guilhem Pagés*

16th European Molecular Imaging Meeting | EMIM 2021 – August 25th

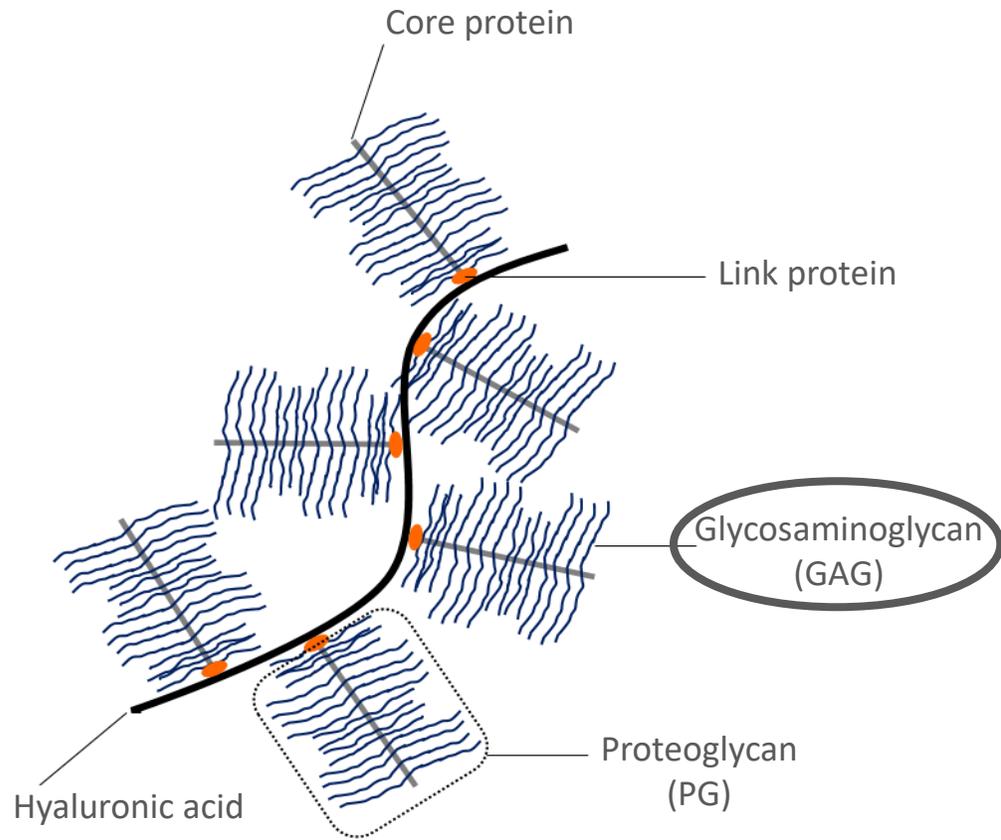
Context: Tumor microenvironment



Context: Tumor microenvironment

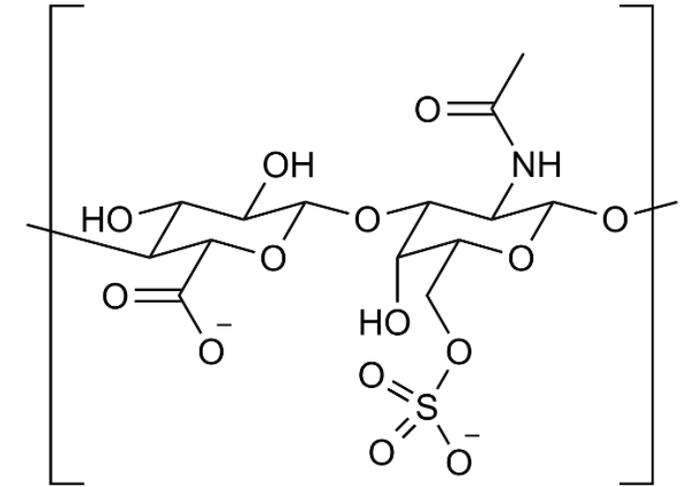
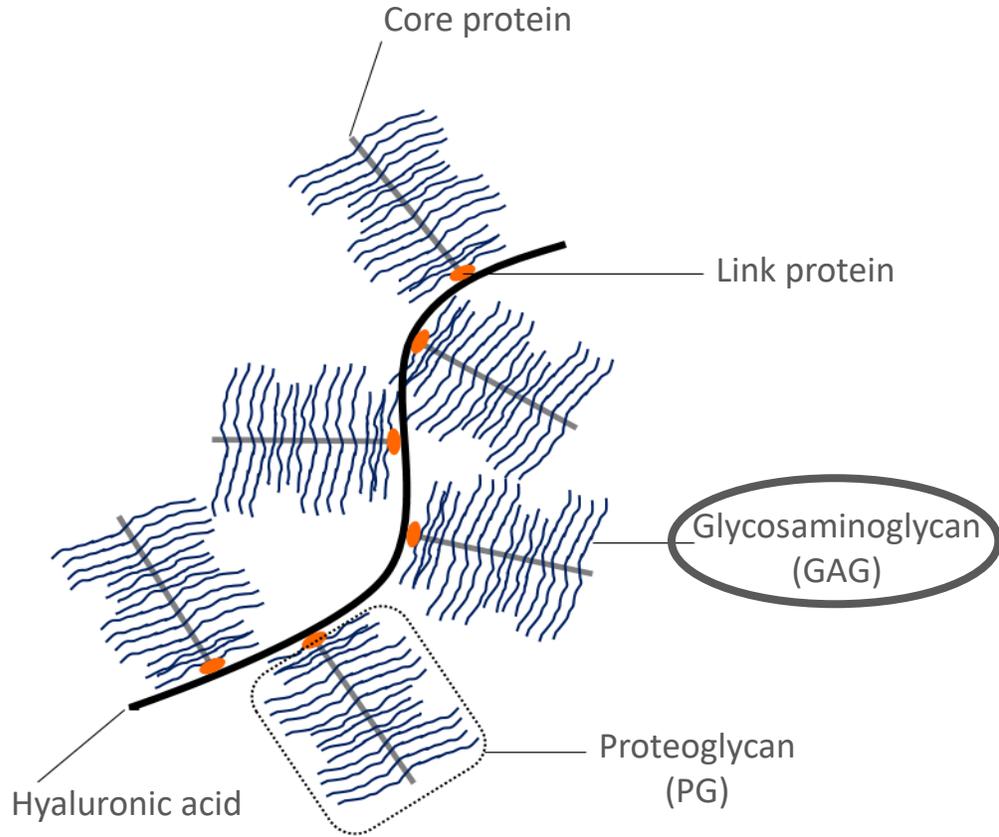


Context: Proteoglycans



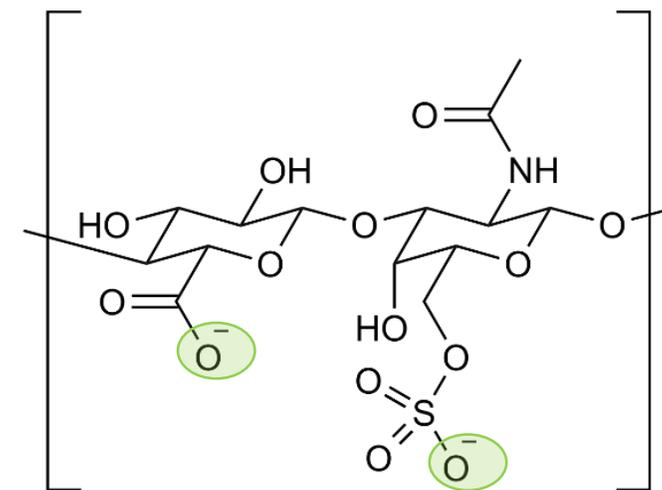
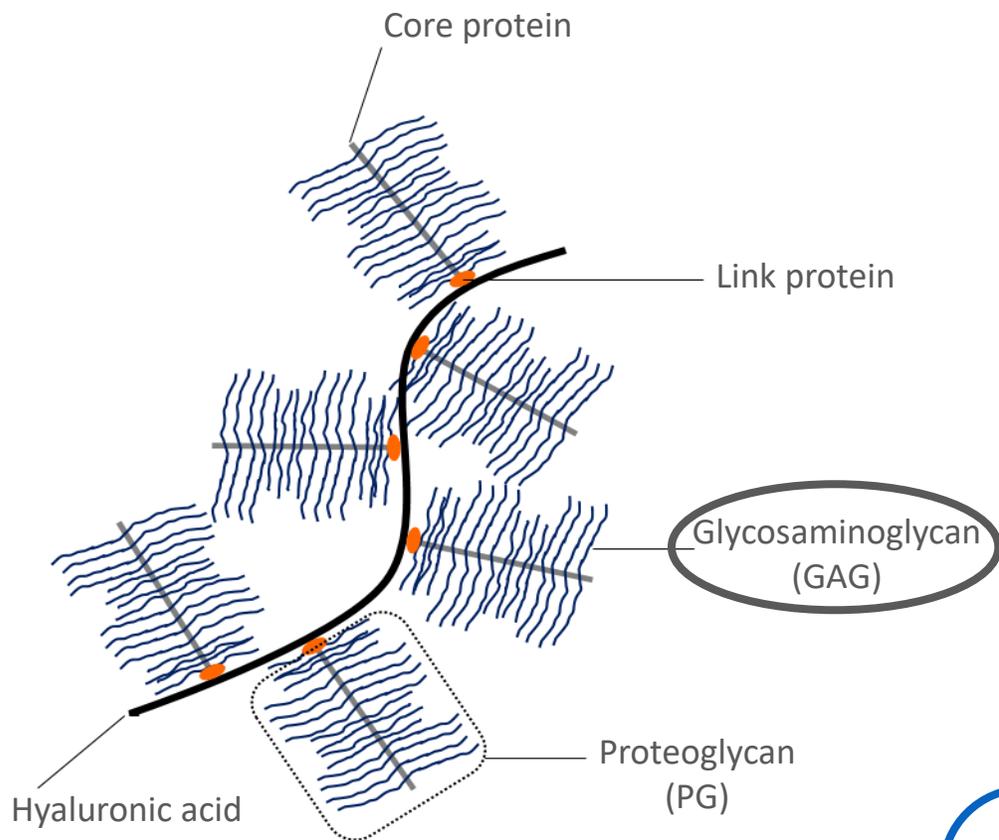
- Aigner T. et al., 2002, *Cancer*
- Vidal A. et al., 2015, *Appl. Radiat. Isot.*
- Ling W. et al., 2008, *PNAS*

Context: Proteoglycans



Chondroitin sulfate

Context: Proteoglycans



In vivo Imaging

Nuclear imaging

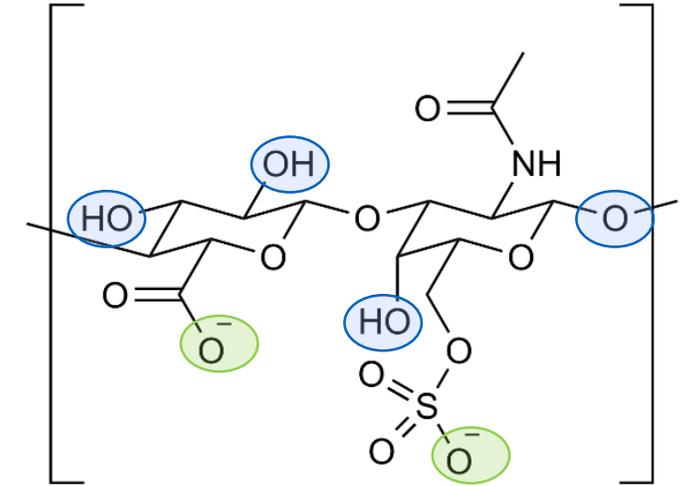
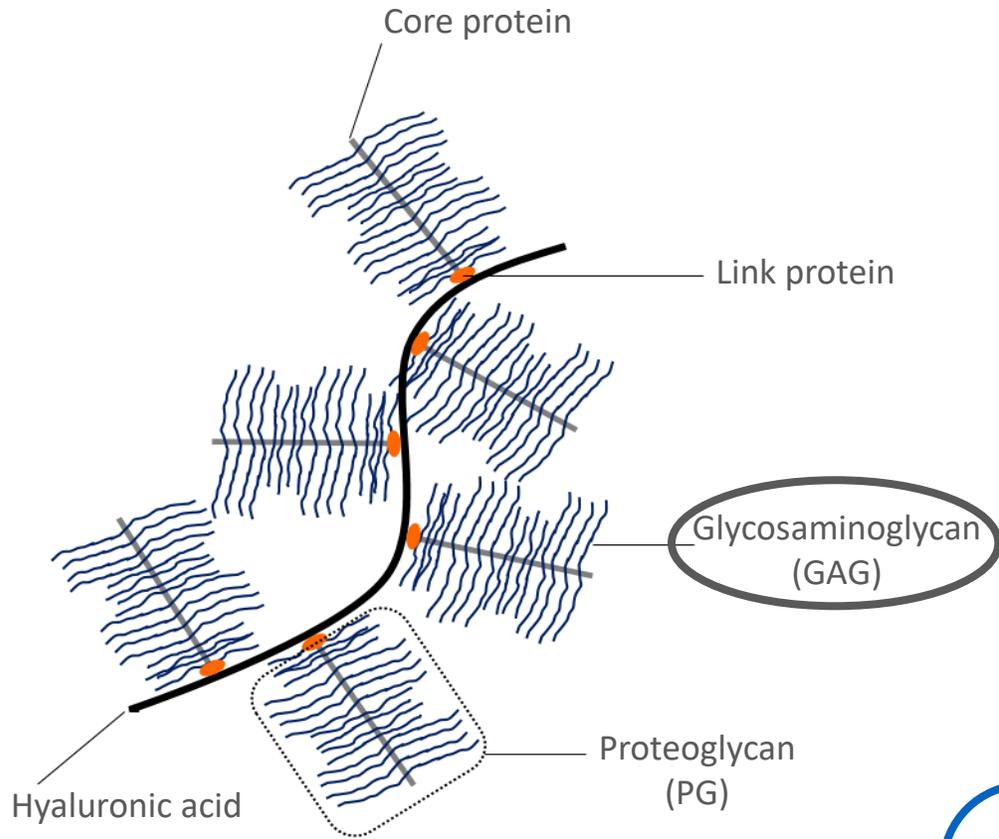
^{99m}Tc -NTP 15-5

Negative charges targeting



- Aigner T. et al., 2002, Cancer
- Vidal A. et al., 2015, Appl. Radiat. Isot.
- Ling W. et al., 2008, PNAS

Context: Proteoglycans



In vivo Imaging

Nuclear imaging

^{99m}Tc-NTP 15-5

Negative charges targeting



Magnetic resonance imaging

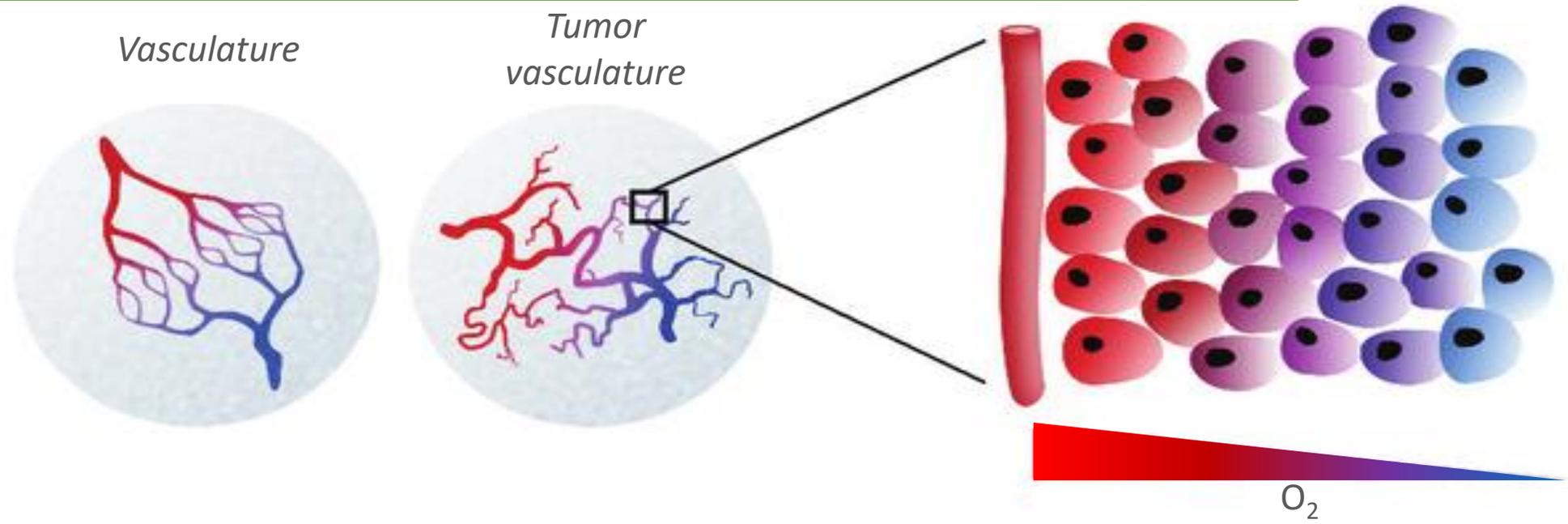
11,7 T
GAG CEST

Hydroxyl moieties signal

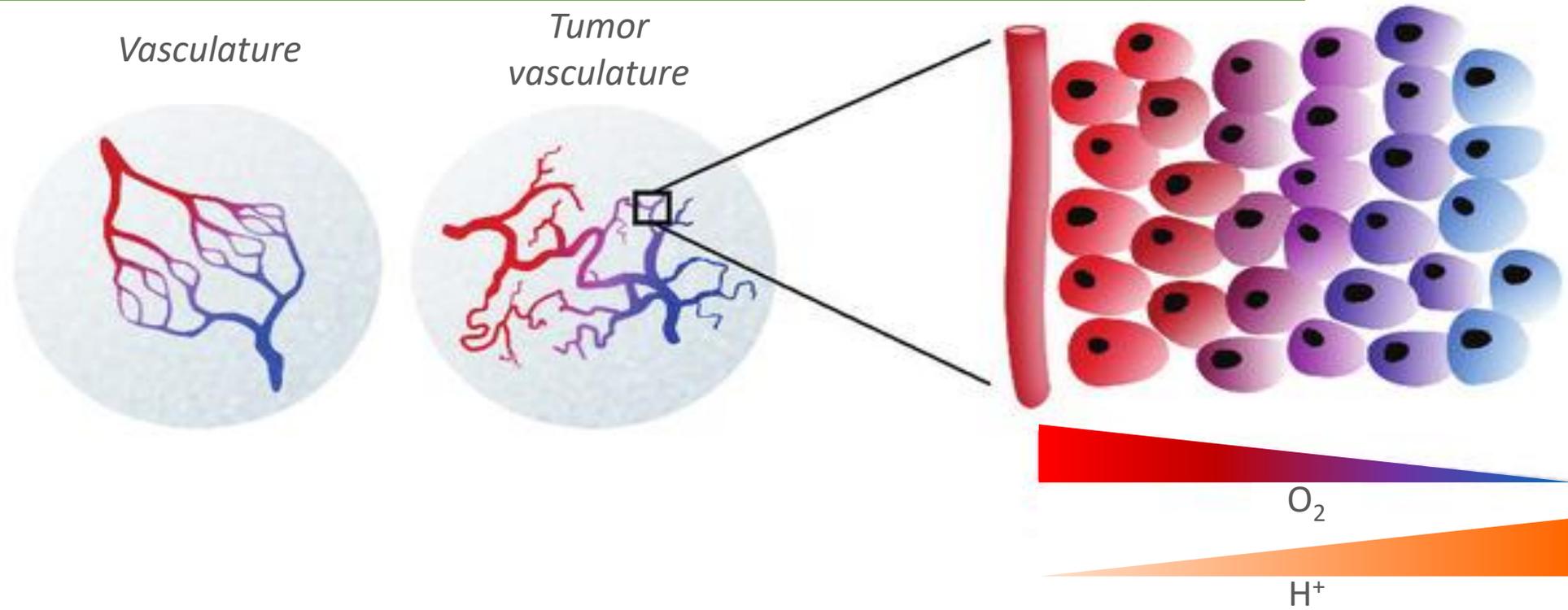


Aigner T. et al., 2002, Cancer
 Vidal A. et al., 2015, Appl. Radiat. Isot.
 Ling W. et al., 2008, PNAS

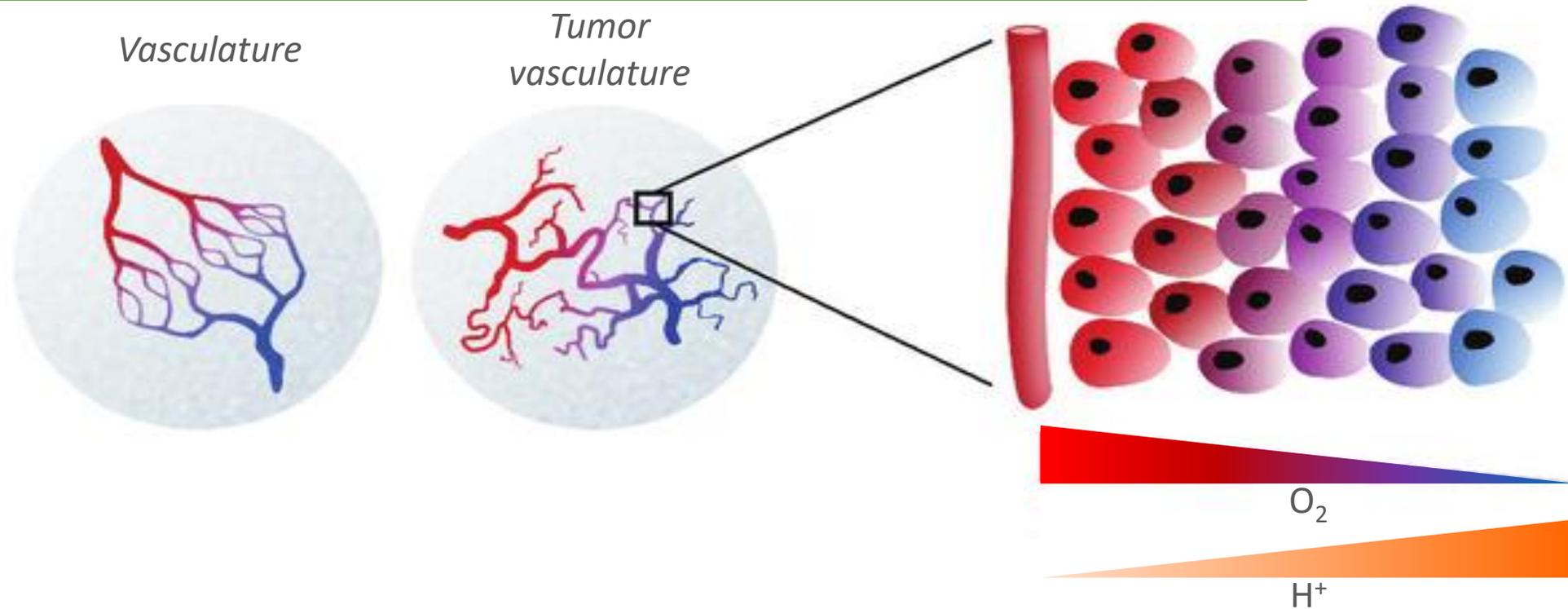
Context: Hypoxia and pH



Context: Hypoxia and pH



Context: Hypoxia and pH



In vivo Imaging

Nuclear imaging

^{18}F -FMISO

Redox potential



Magnetic resonance imaging

11,7 T

Guanidyl CEST & APT CEST

Amine & Amide moieties signal both affected by pH variations

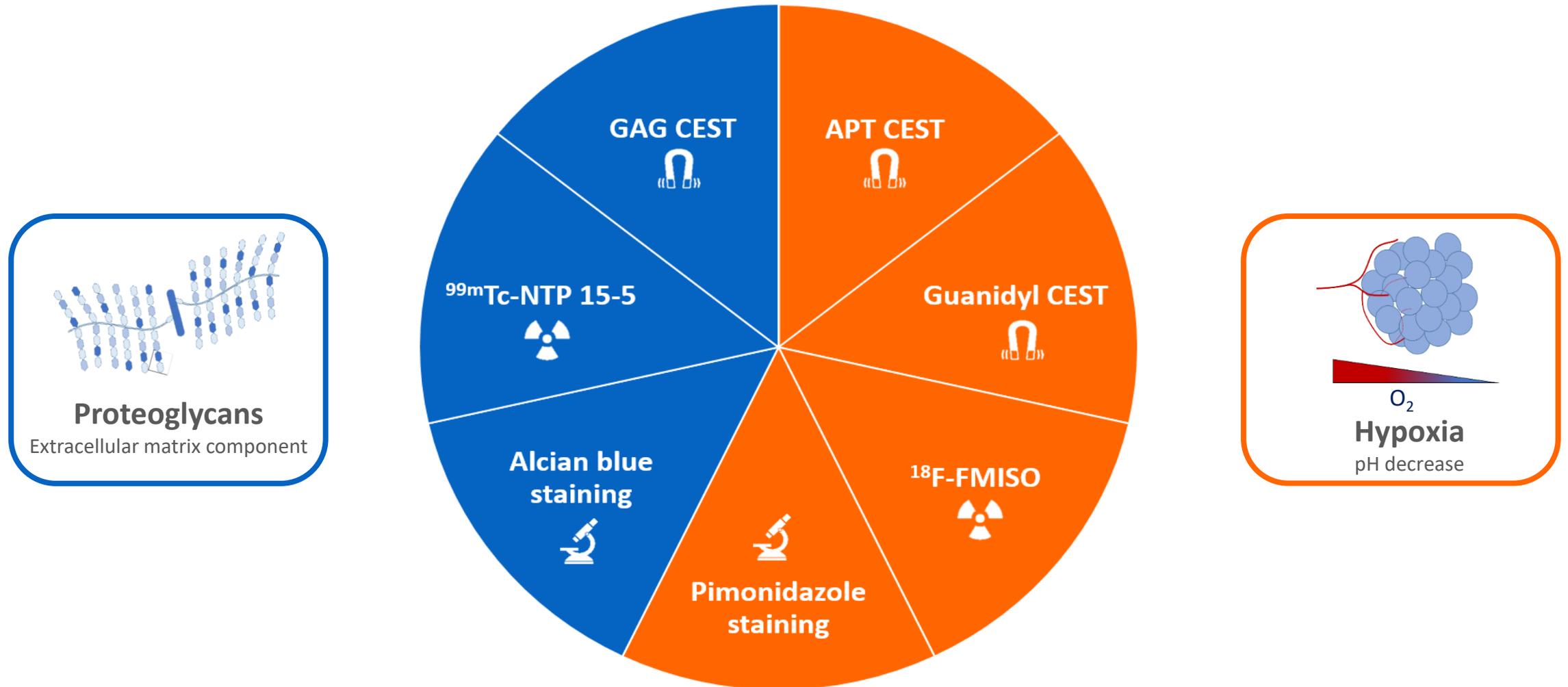


Methods: Multimodal imaging

To characterize and map Swarm chondrosarcoma microenvironment by multimodal imaging...

Methods: Multimodal imaging

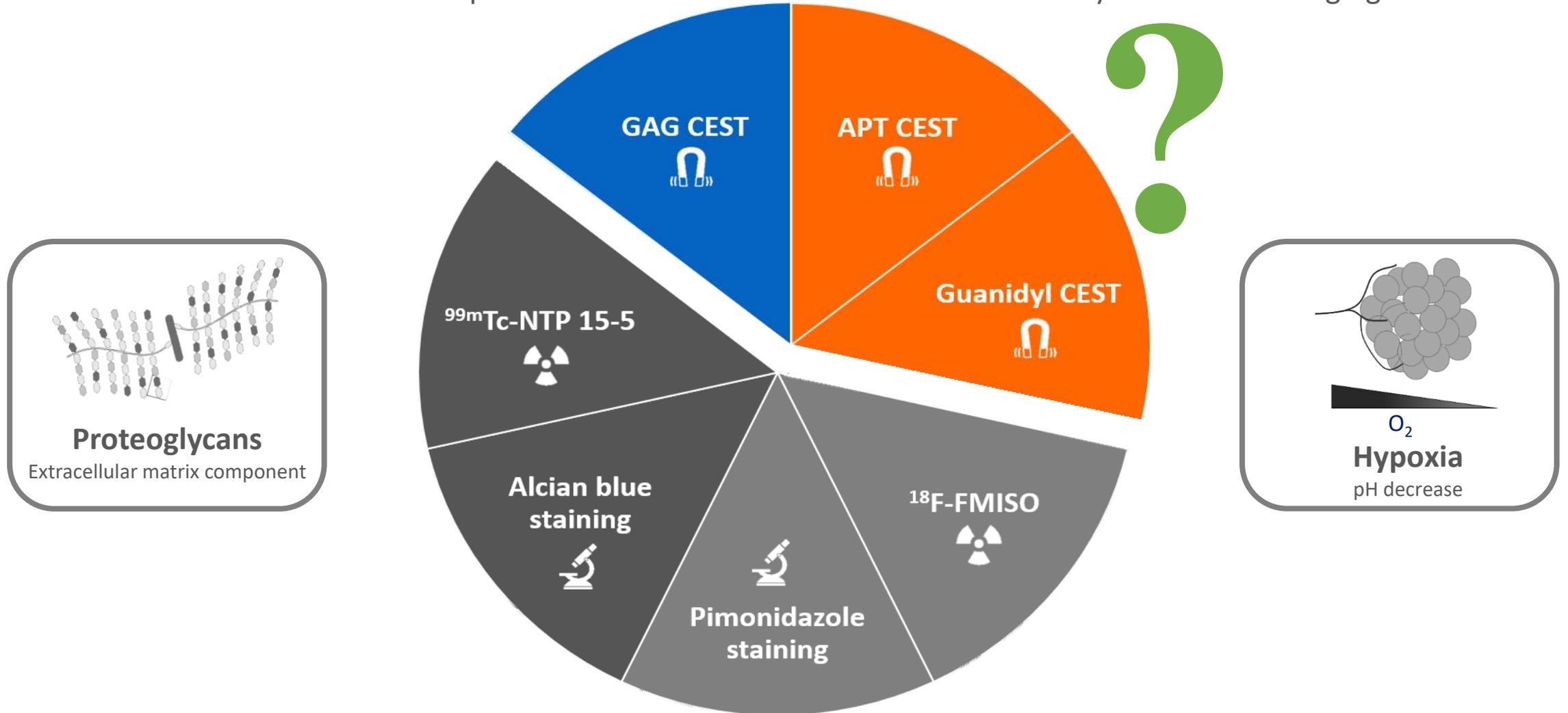
To characterize and map Swarm chondrosarcoma microenvironment by multimodal imaging...



... on female NMRI Nude mice (n=10) with SWARM tumor subcutaneous transplant (Grade II chondrosarcoma)

Methods: Multimodal imaging

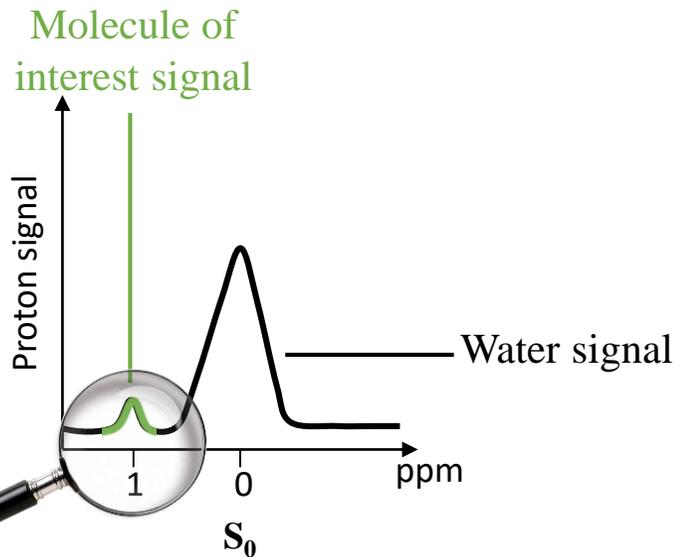
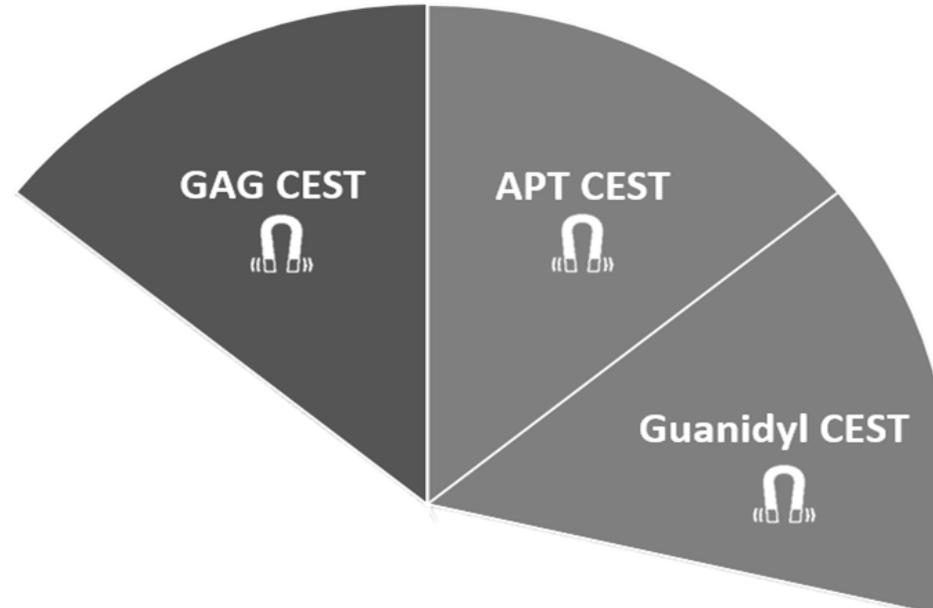
To characterize and map Swarm chondrosarcoma microenvironment by multimodal imaging...



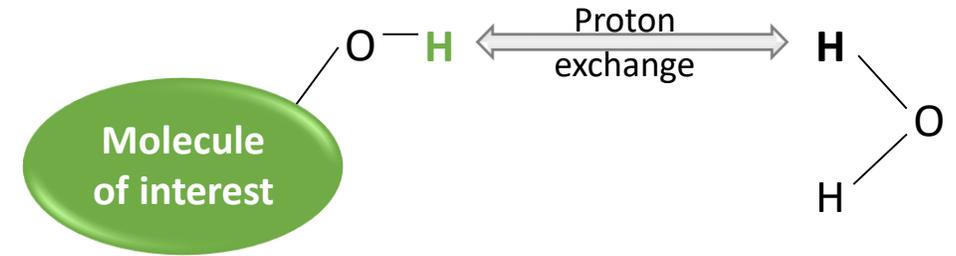
... on female NMRI Nude mice (n=10) with SWARM tumor subcutaneous transplant (Grade II chondrosarcoma)

Methods: Chemical Exchange Saturation Transfer MRI

CEST MRI principle

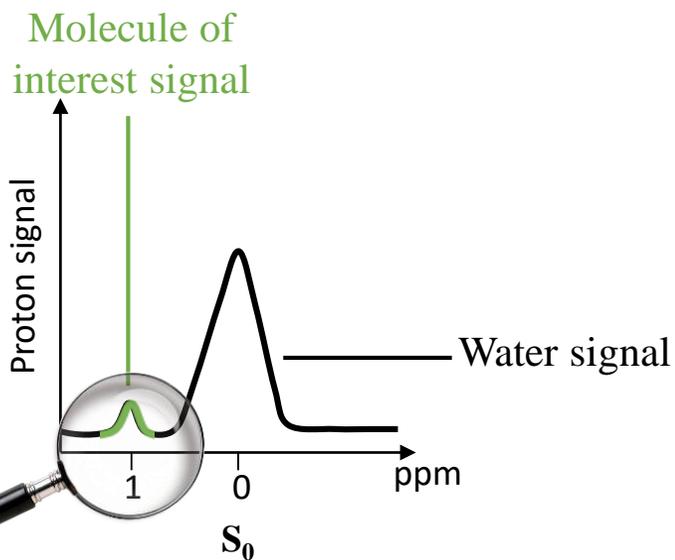
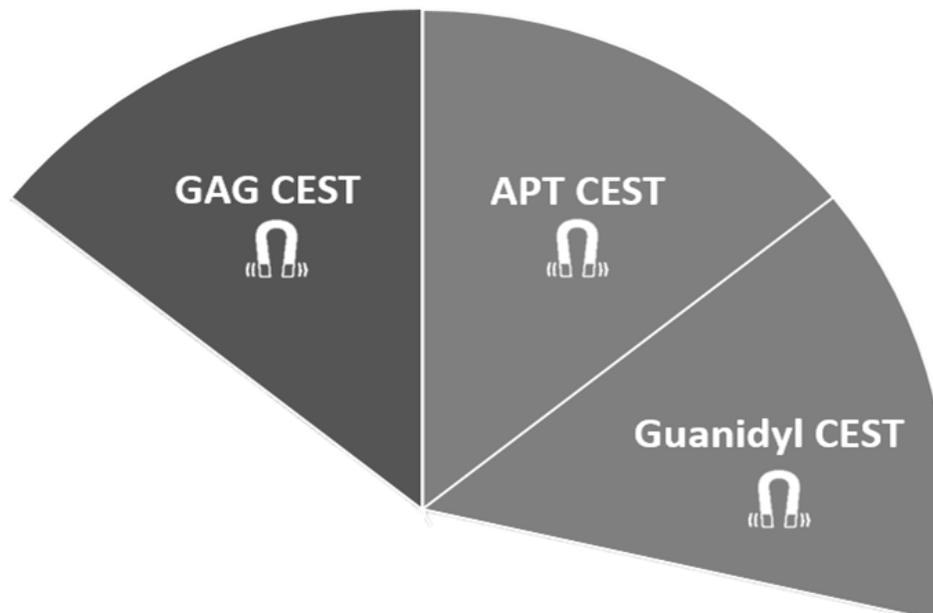


Without saturation

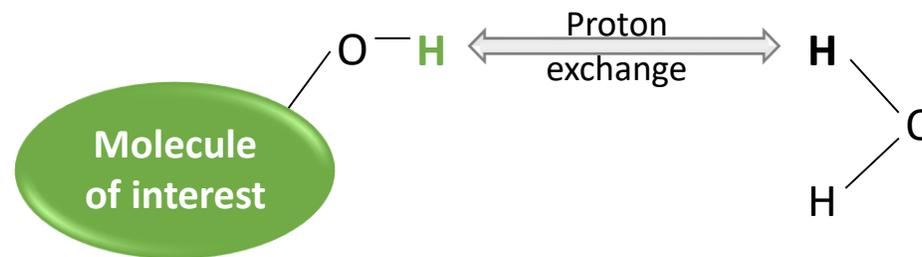


Methods: Chemical Exchange Saturation Transfer MRI

CEST MRI principle

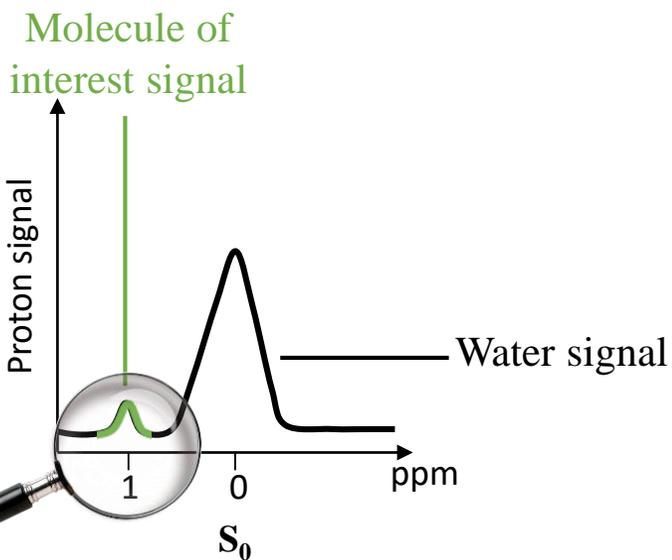
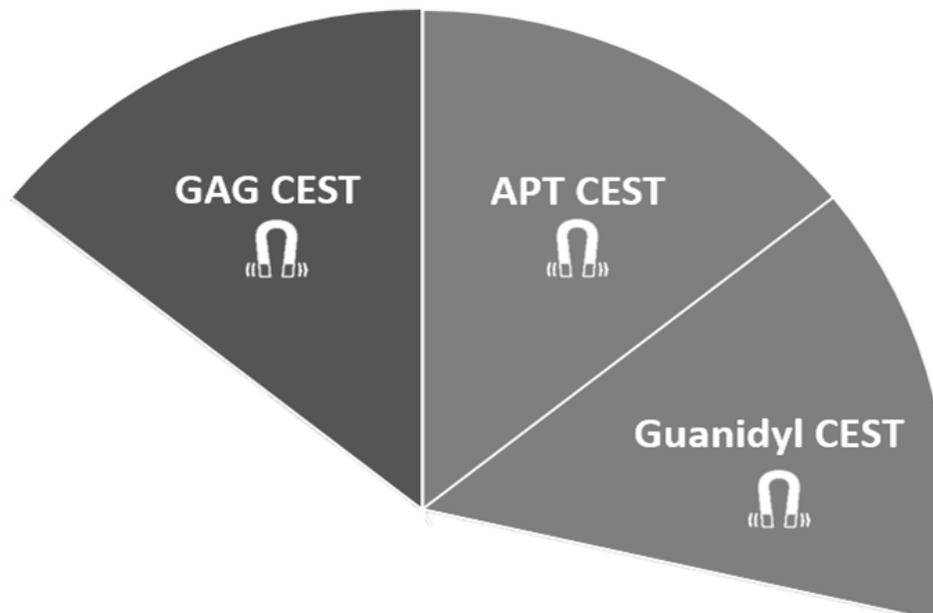


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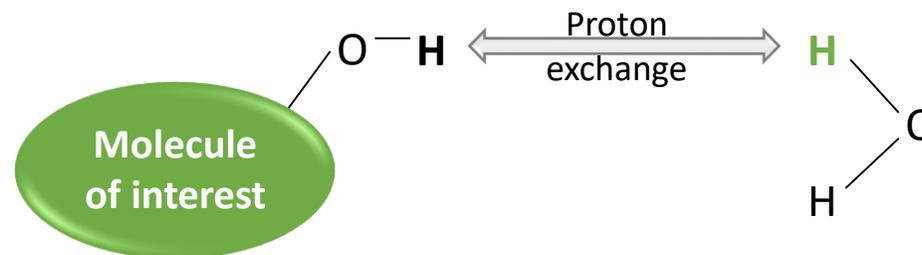


Methods: Chemical Exchange Saturation Transfer MRI

CEST MRI principle

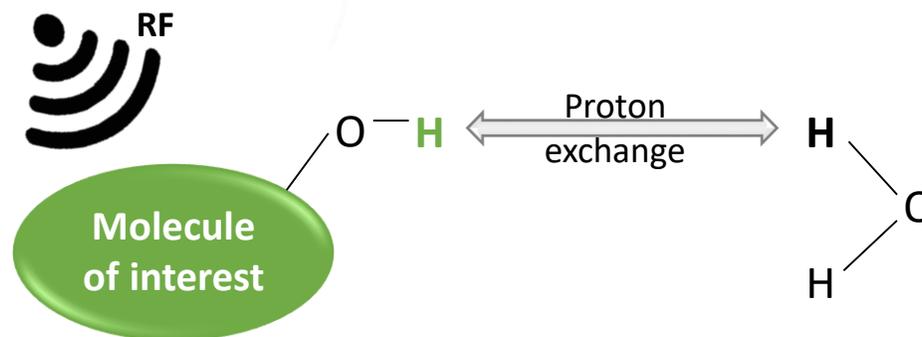
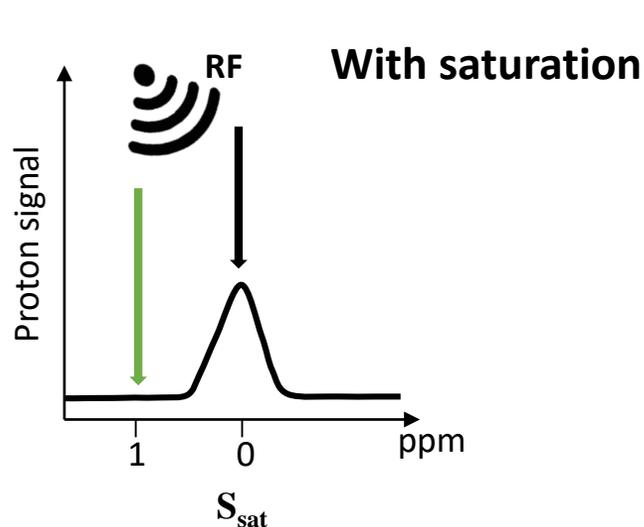
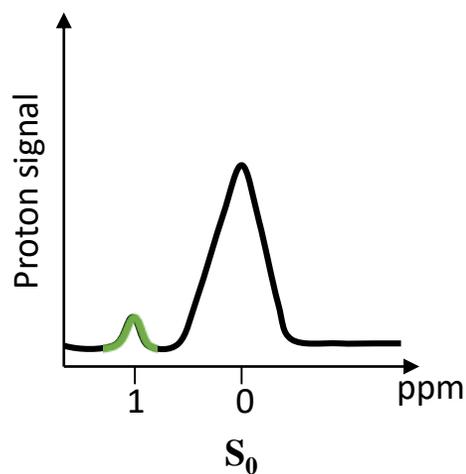
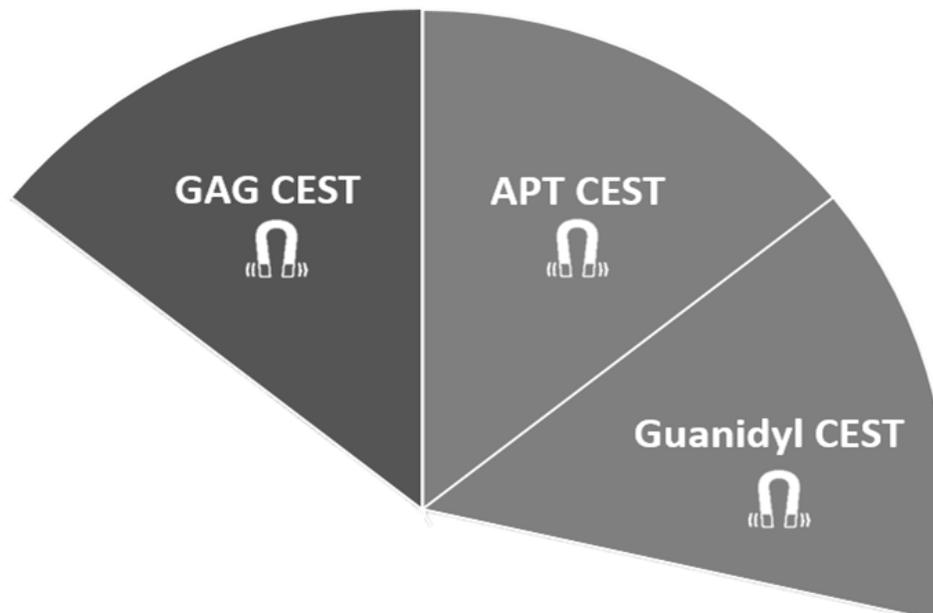


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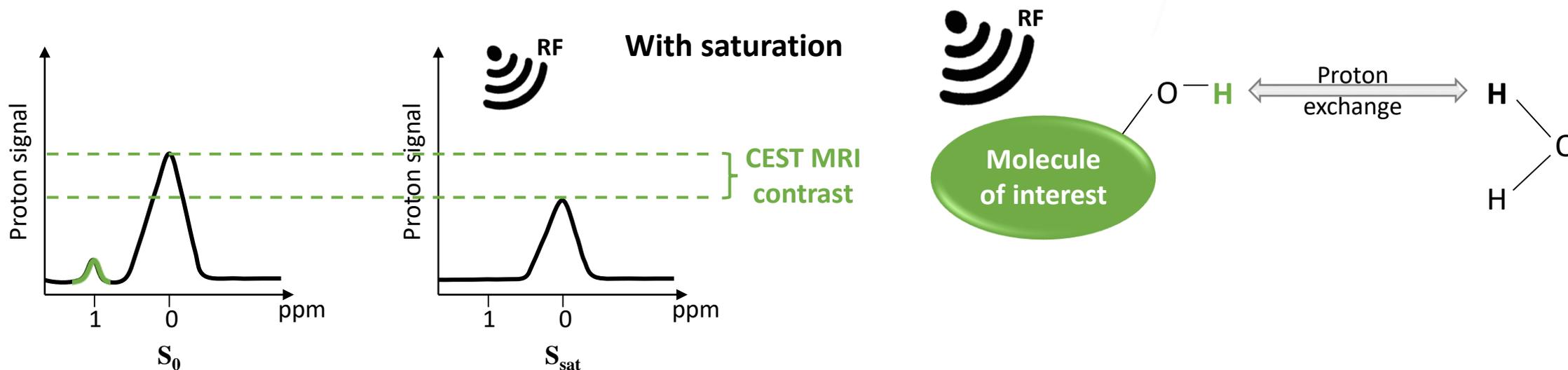
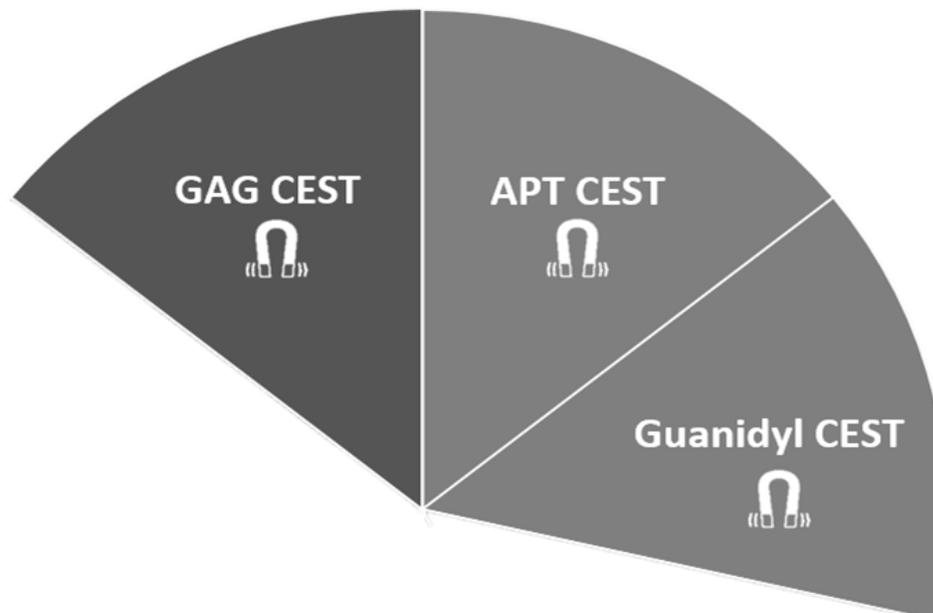
Methods: Chemical Exchange Saturation Transfer MRI

CEST MRI principle

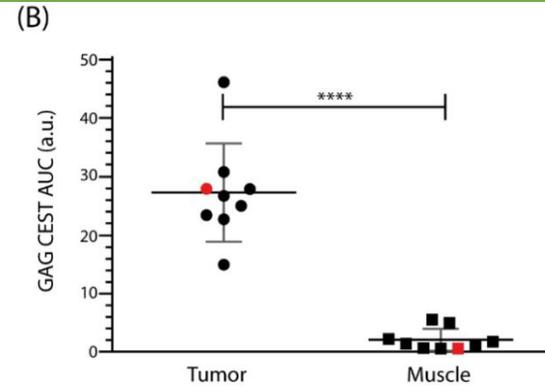
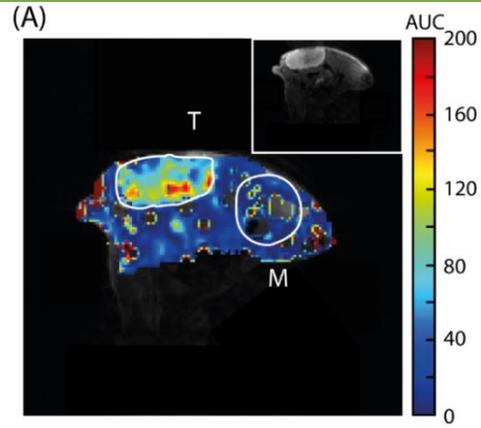
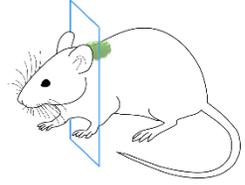


Methods: Chemical Exchange Saturation Transfer MRI

CEST MRI principle

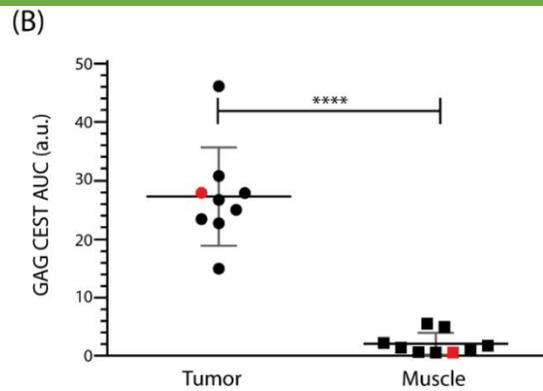
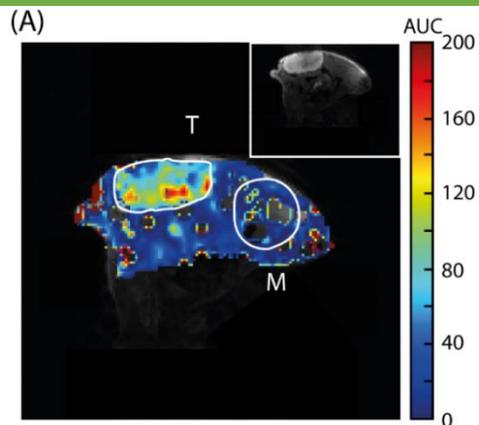
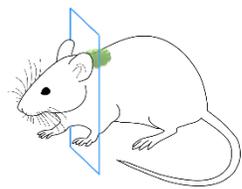


Results: Proteoglycan component

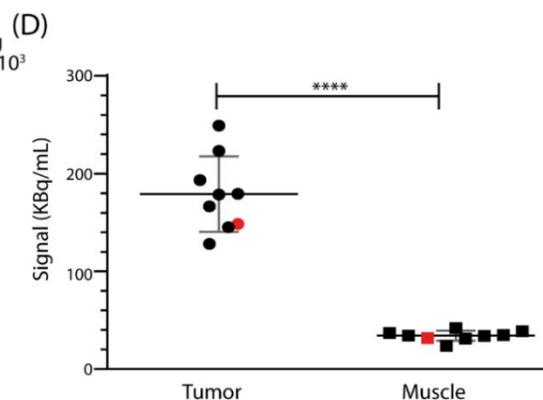
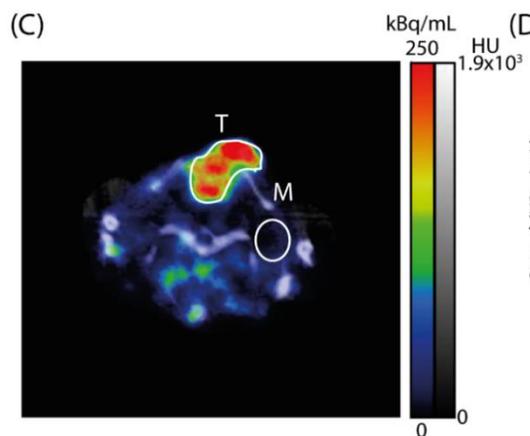
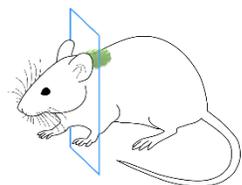


CEST MRI

Results: Proteoglycan component

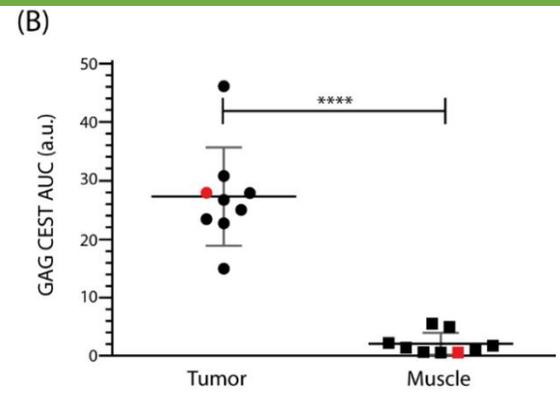
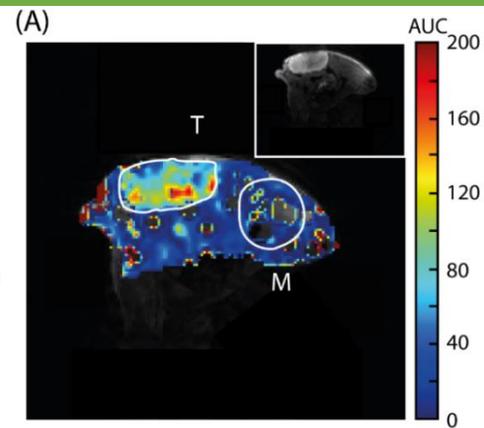
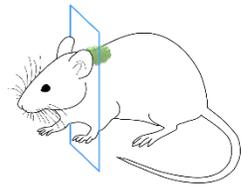


CEST MRI

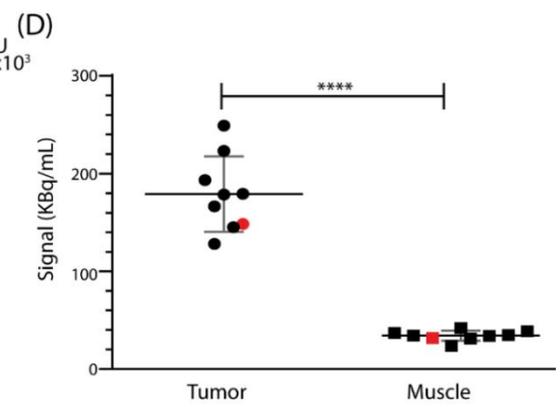
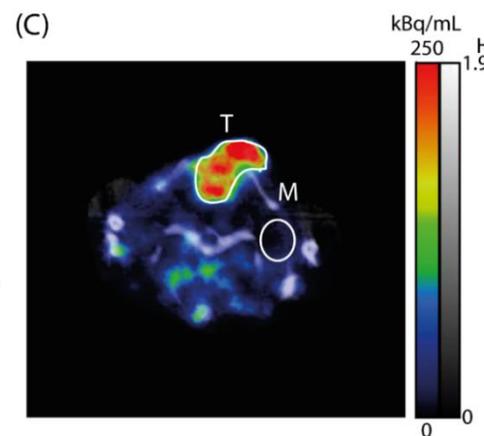
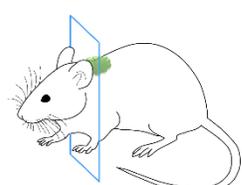


$^{99m}\text{Tc-NTP 15-5}$

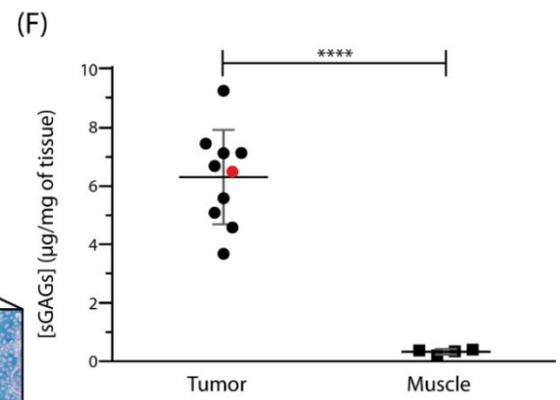
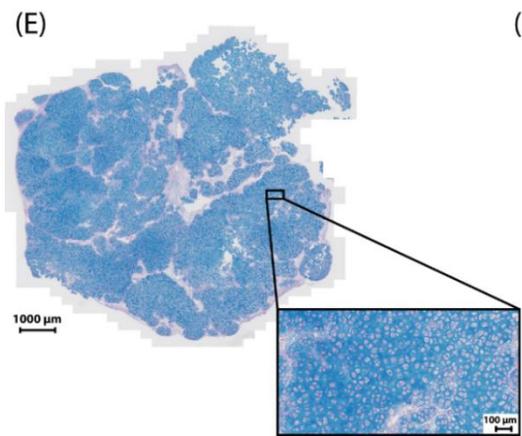
Results: Proteoglycan component



CEST MRI

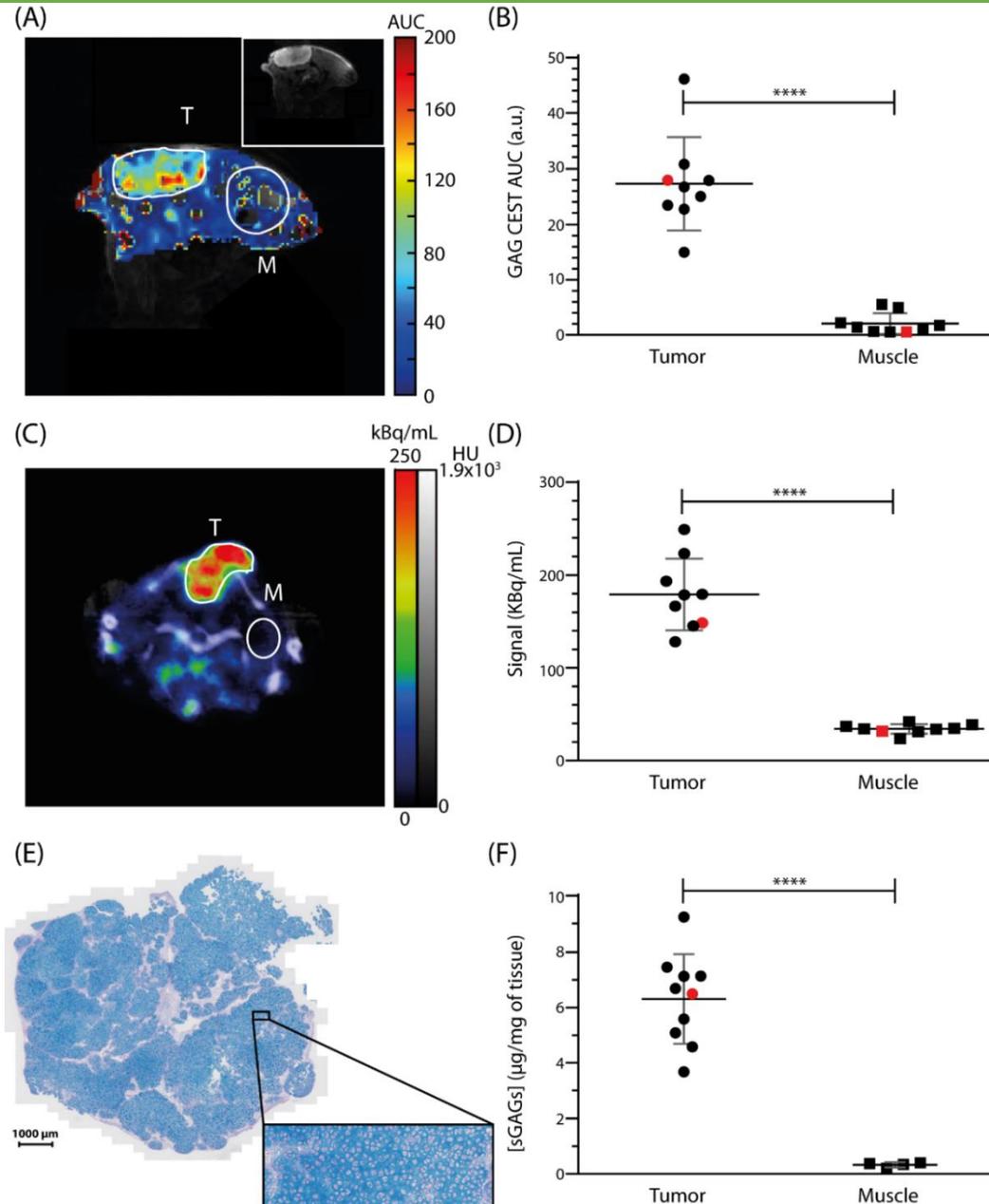


^{99m}Tc -NTP 15-5



Alcian blue staining & sGAG assay

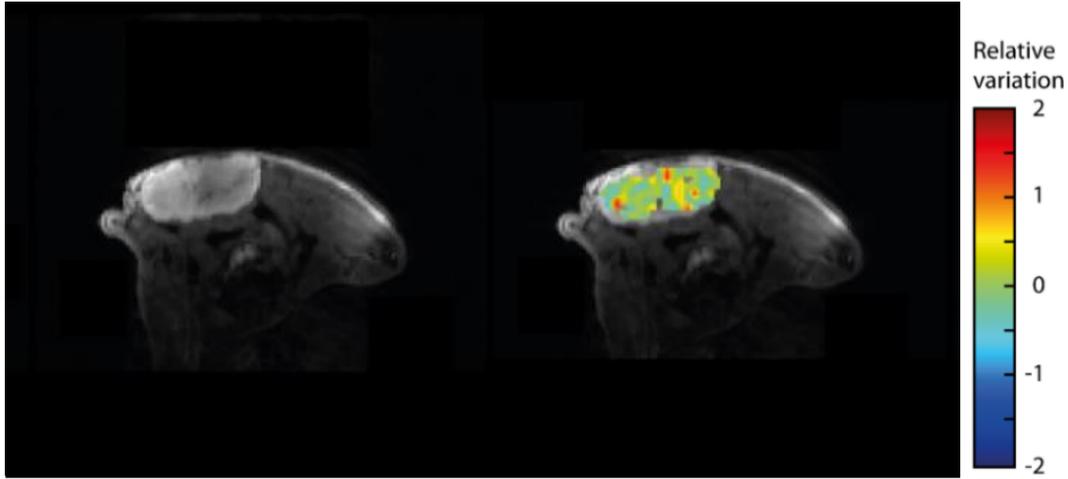
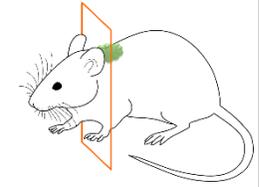
Results: Proteoglycan component



- Both *in vivo* imaging methods demonstrate that proteoglycan content of tumor extracellular matrix is higher than muscle
- High resolution cartography provided by CEST MRI
- Specificity provided by $^{99\text{m}}\text{Tc}$ -NTP 15-5
- Final validation with *ex vivo* analyses

Results: Hypoxia and pH component

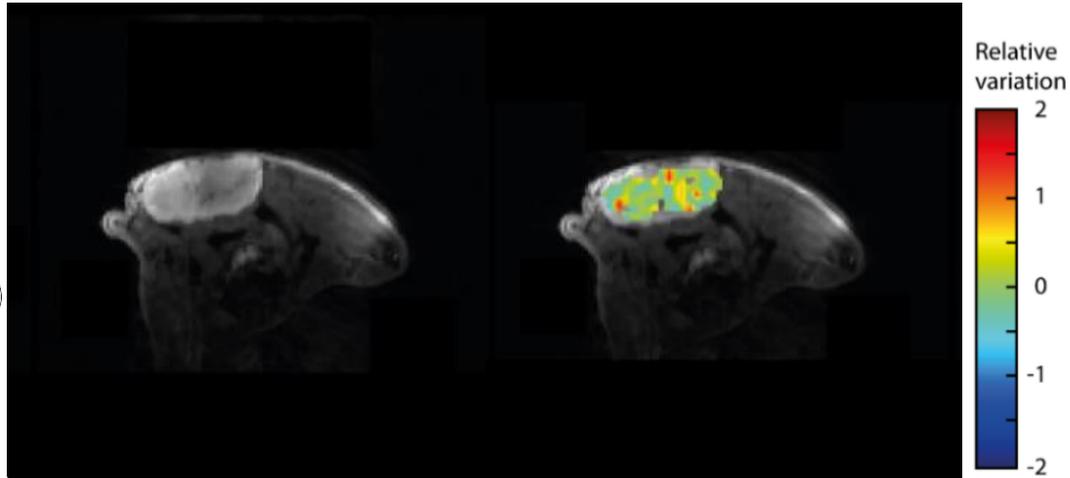
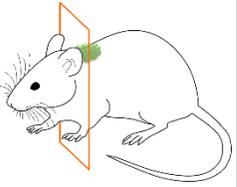
(A)



CEST MRI

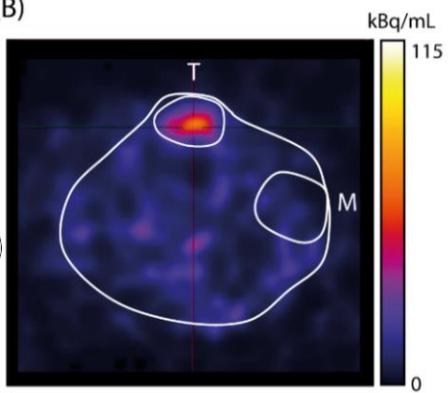
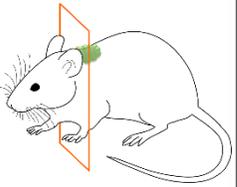
Results: Hypoxia and pH component

(A)

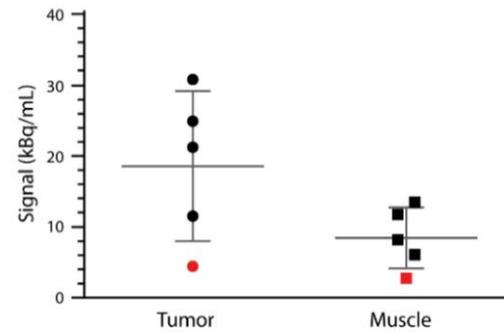


CEST MRI

(B)



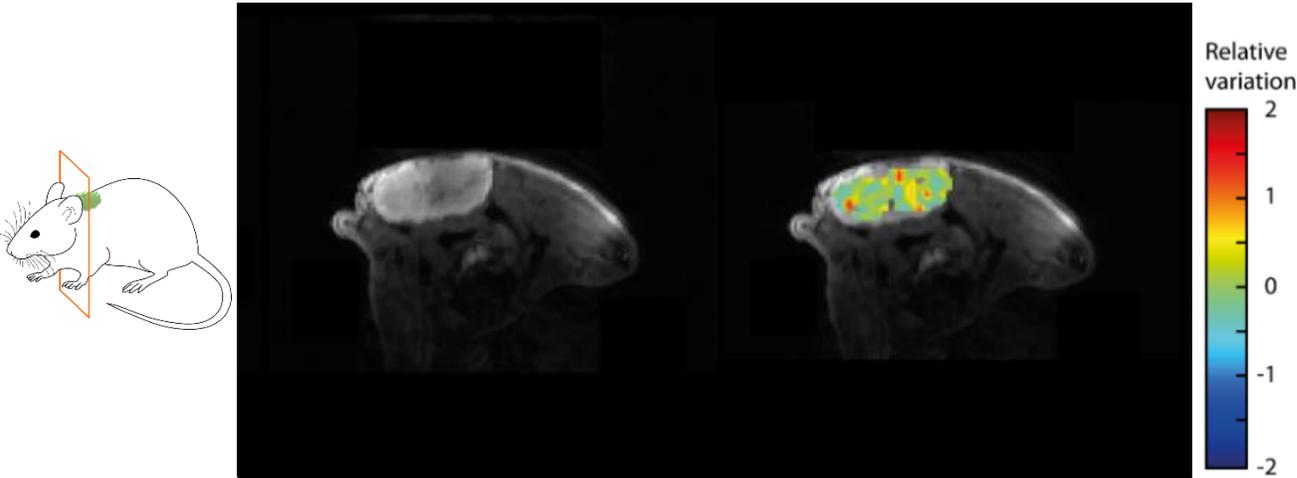
(C)



^{18}F -FMISO

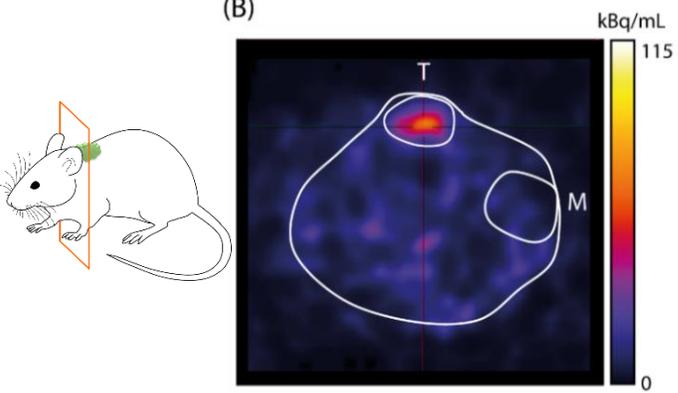
Results: Hypoxia and pH component

(A)



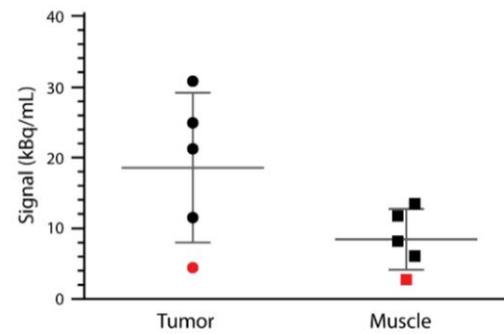
CEST MRI

(B)

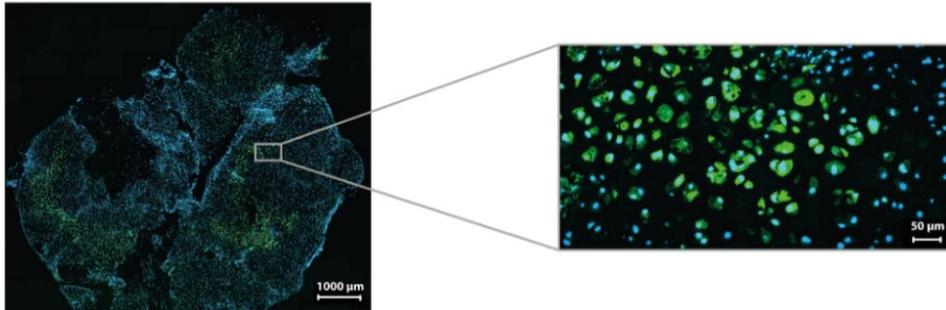


^{18}F -FMISO

(C)



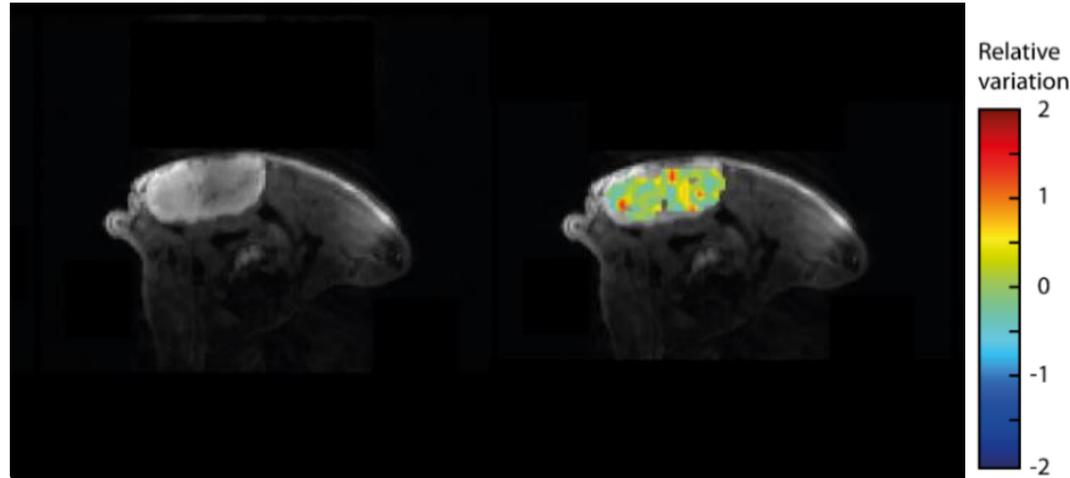
(D)



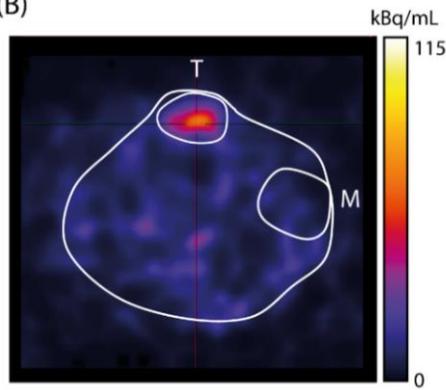
Pimonidazole
immunofluorescence

Results: Hypoxia and pH component

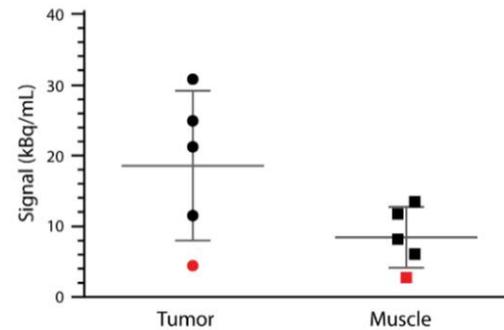
(A)



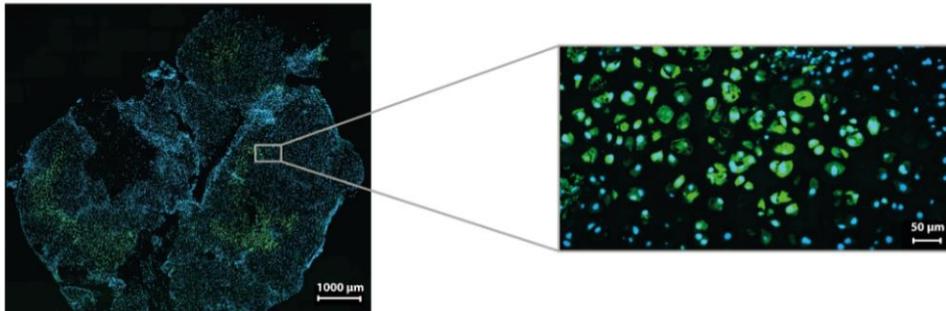
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(C)

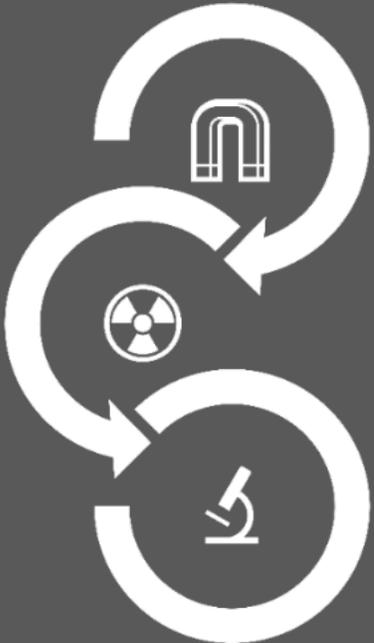


(D)



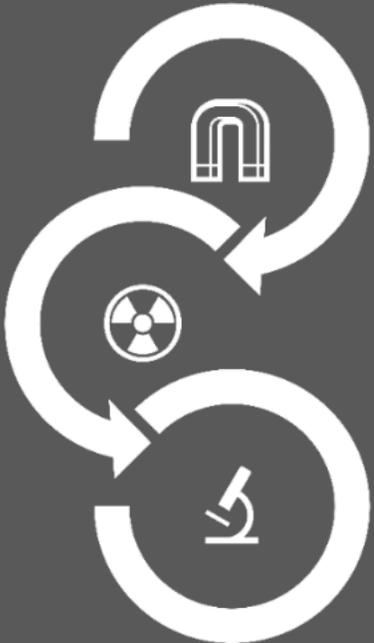
- Both *in vivo* imaging methods demonstrate that tumor has an hypoxic fraction with acidic areas
- High resolution cartography provided by CEST MRI
- Specificity provided by ^{18}F -FMISO
- Final validation with *ex vivo* analyses

Conclusions



Tumor microenvironment exploration by using multimodal imaging to study...	
1) ...proteoglycans	2) ...hypoxia and pH
^{99m}Tc -NTP 15-5 ✓	^{18}F -FMISO ✓
GAG CEST ✓	Guanidyl / APT CEST ✓
Blue Alcian ✓	Pimonidazole ✓
sGAG assay ✓	

Conclusions



Tumor microenvironment exploration by using multimodal imaging to study...	
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^{99m}Tc -NTP 15-5 ✓	^{18}F -FMISO ✓
GAG CEST ✓	Guanidyl / APT CEST ✓
Blue Alcian ✓	Pimonidazole ✓
sGAG assay ✓	

Simultaneous proteoglycans and hypoxia mapping of chondrosarcoma environment by frequency selective CEST MRI

Autissier R, Mazuel L, Maubert E, Bonny J-M, Auzeloux P, Schmitt S, Traoré A, Peyrode C, Miot-Noirault E, Pagés G. *Magn Reson Med*. 2021 Aug;86(2):1008-1018. doi: 10.1002/mrm.28781.

Acknowledgments

Thank you for your attention
Thanks to EMIM 2021 organization team

My supervisors:

Dr. Elisabeth Miot Noirault
Dr. Guilhem Pagès
Dr. Leslie Mazuel

Radiochemists:

Dr. Philippe Auzeloux
Dr. Sebastien Schmitt

My lab's coworkers:

IMoST & AgroResonance teams

Facilities:

IVIA - In Vivo Imaging in Auvergne
Anipath (Christelle Damon-Soubeyrand)

Grants:

**Ministère français de l'Enseignement supérieur,
de la Recherche et de l'Innovation**
La Ligue contre le cancer

Doctoral school SVSAE & Université Clermont Auvergne



Future work

To continue...

Is multimodal imaging able to detect tumor microenvironment response during therapy ?

Paratibial Swarm rats follow-up during 5 weeks (n=20) with treatment*

* Zoledronic acid (Aclasta®): inhibitor of bone resorption (osteoclast inhibition)

* TH-302 (Evofofosfamide): hypoxia-activated prodrug

Future work

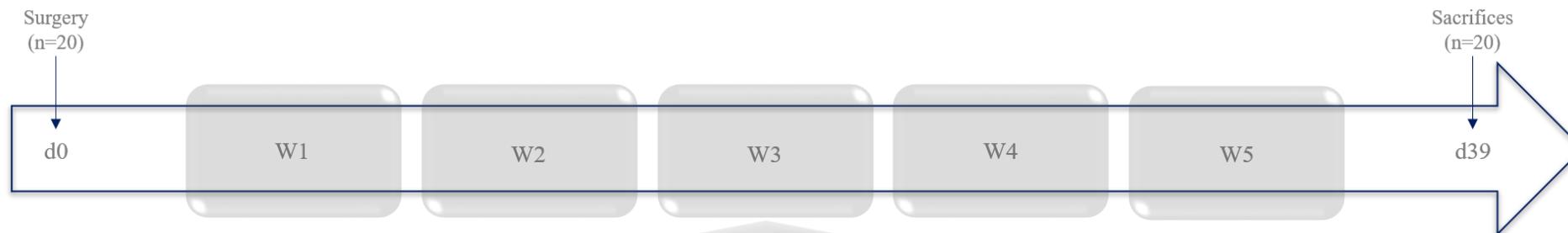
To continue...

Is multimodal imaging able to detect tumor microenvironment response during therapy ?

Paratibial Swarm rats follow-up during 5 weeks (n=20) with treatment*

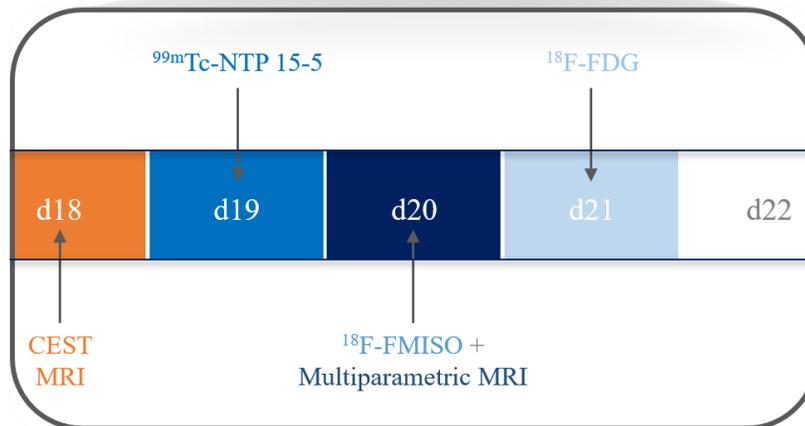
* Zoledronic acid (Aclasta®): inhibitor of bone resorption (osteoclast inhibition)

* TH-302 (Evofofosfamide): hypoxia-activated prodrug



- ❖ **Measurements:**
 - Twice a week
- ❖ **Zol. A. treatment :**
 - 0.1mg/kg
 - SC
 - Twice a week

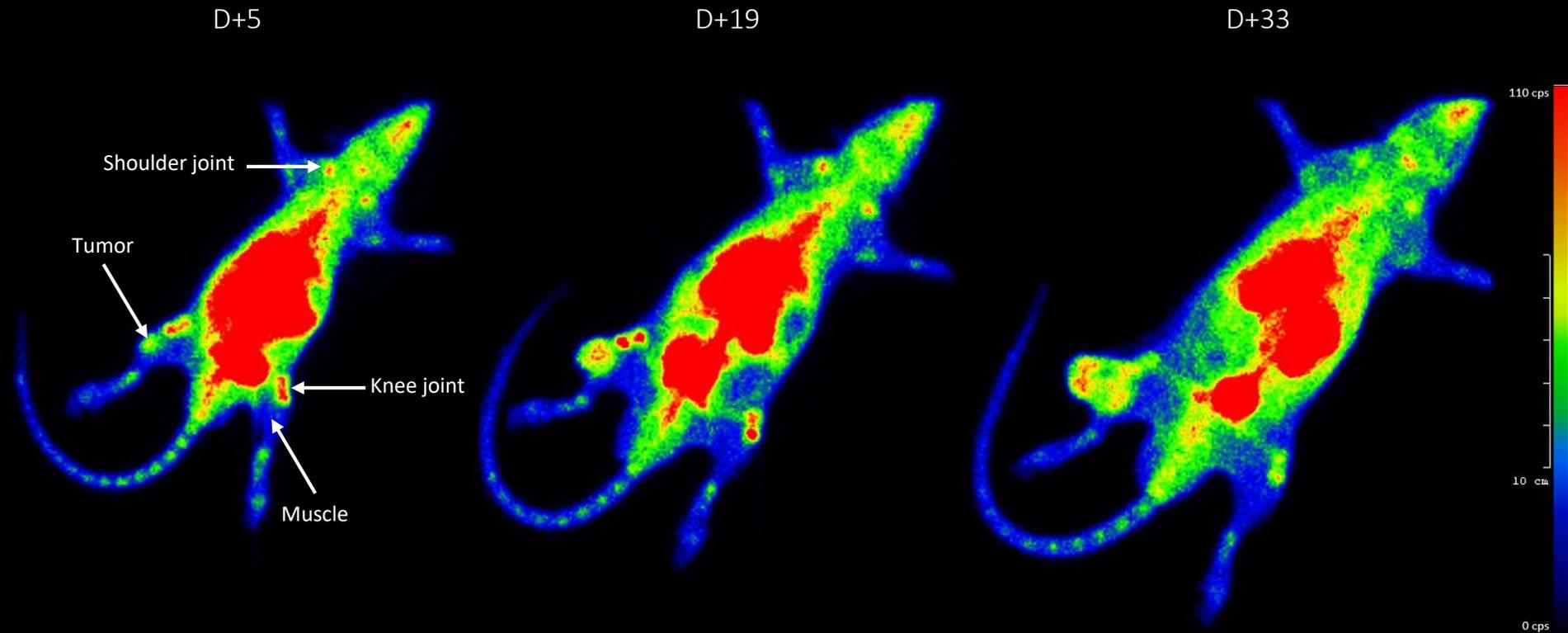
📖 Miot-Noirault E. et al., 2013, EJNMMI



- ❖ **Measurements:**
 - Twice a week
- ❖ **TH-302 treatment :**
 - 50mg/kg
 - IP
 - Five times a week

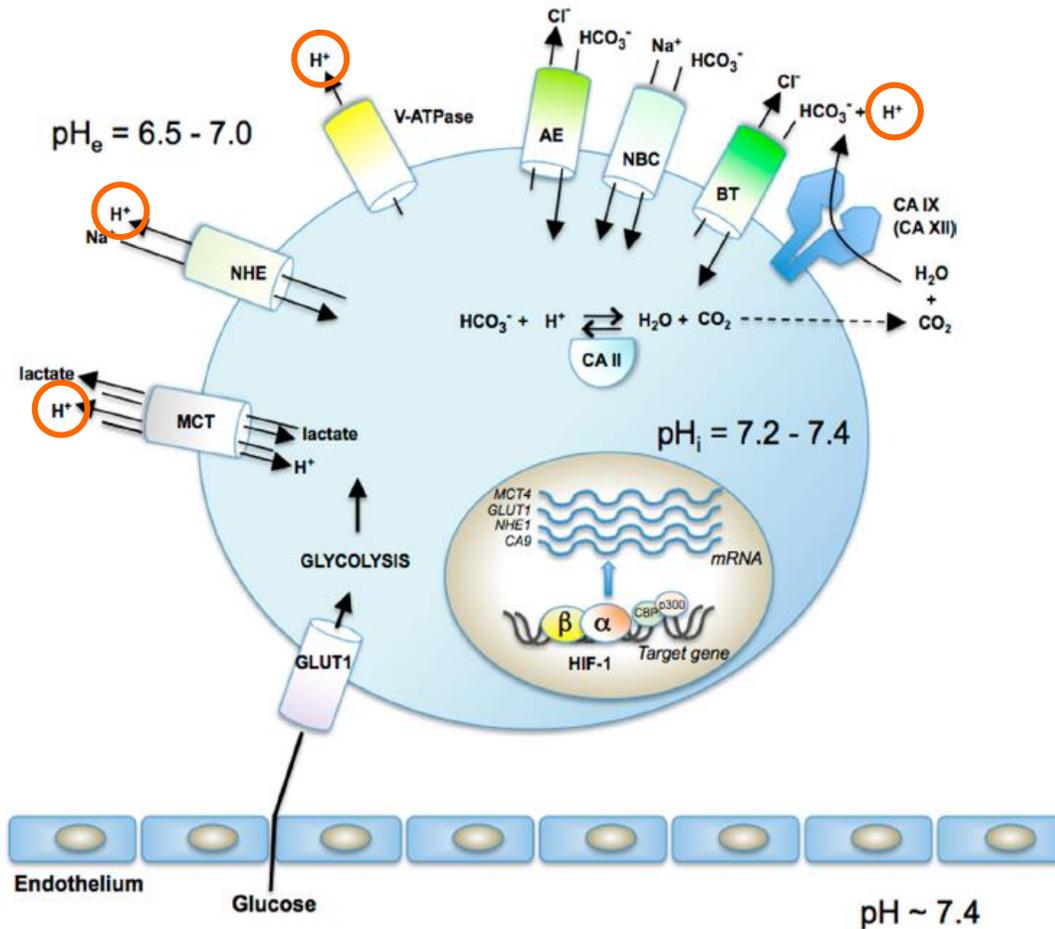
📖 Harms J. et al., 2019, Cells

Preliminary results: ^{99m}Tc -NTP 15-5



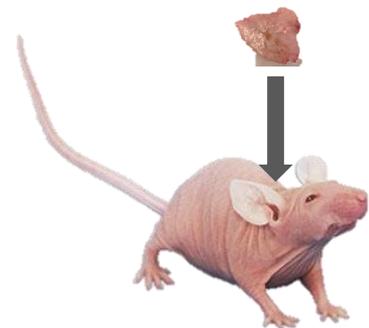
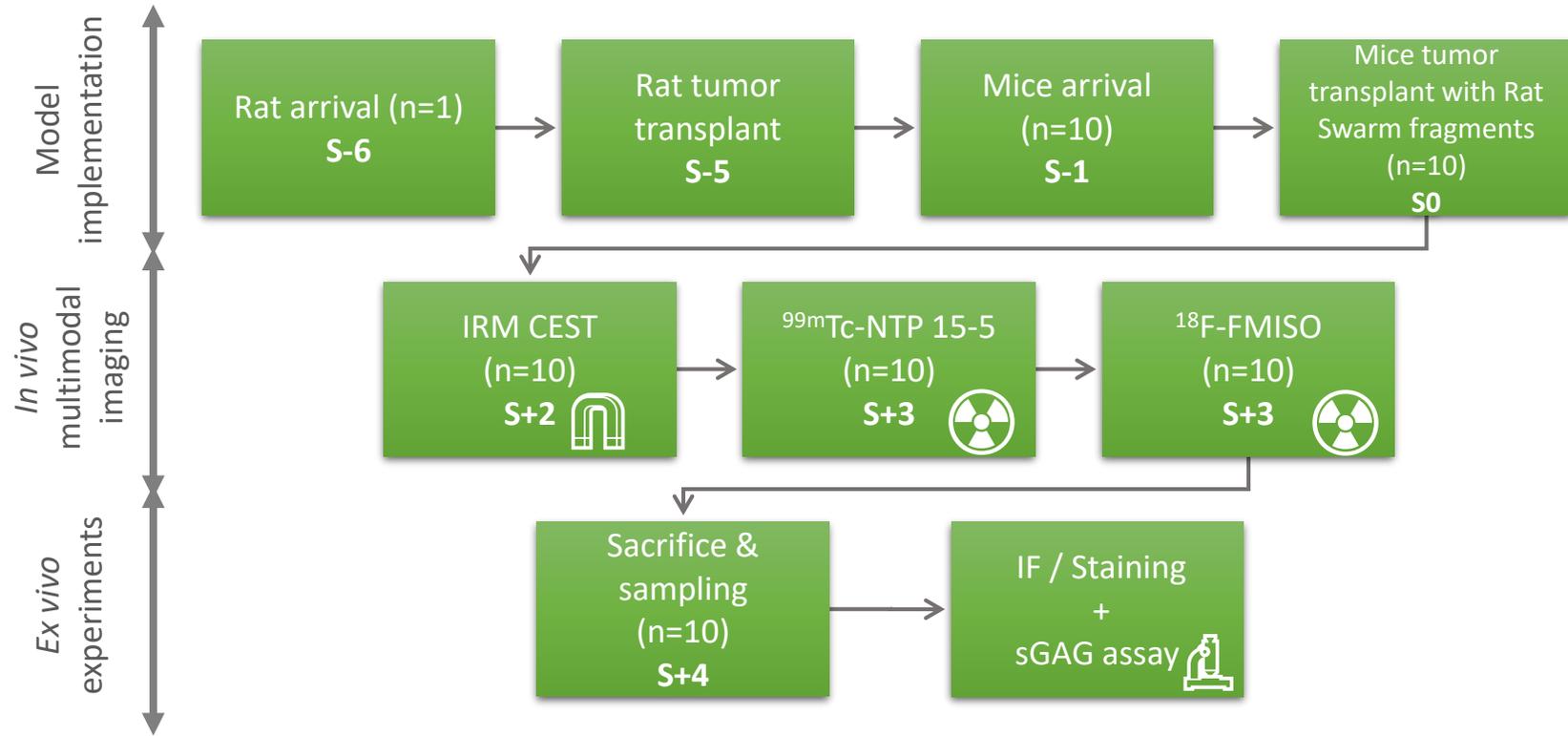
Control rat OR cage C
Anesthesia: Isoflurane 2,5%
IV injected mid-dose: 30 MBq
Scan time: 30 min post-injection
SPECT imaging duration: 20 min

Context: Hypoxia and pH



Hypoxia = $\nearrow pH_i$ and $\searrow pH_e$
 Hypoxia + HIF = oncogene transcription

Methods: Study design and animal model



Female NMRI Nude mice (n=10)
SWARM tumor with subcutaneous growth (Grade II chondrosarcoma)

Ethical commission approval:
#15991

^{99m}Tc -NTP 15-5

^{99m}Tc -1-[(triethylammonio)propyl]-1,4,7,10,13-pentaazacyclopentadecan

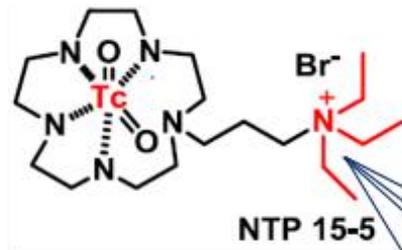
Patent WO/2001/000621/PCT/FR2000/001731

γ radiations

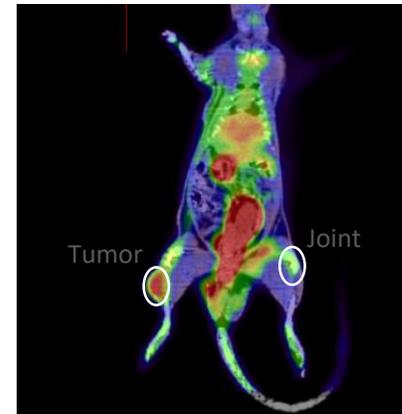
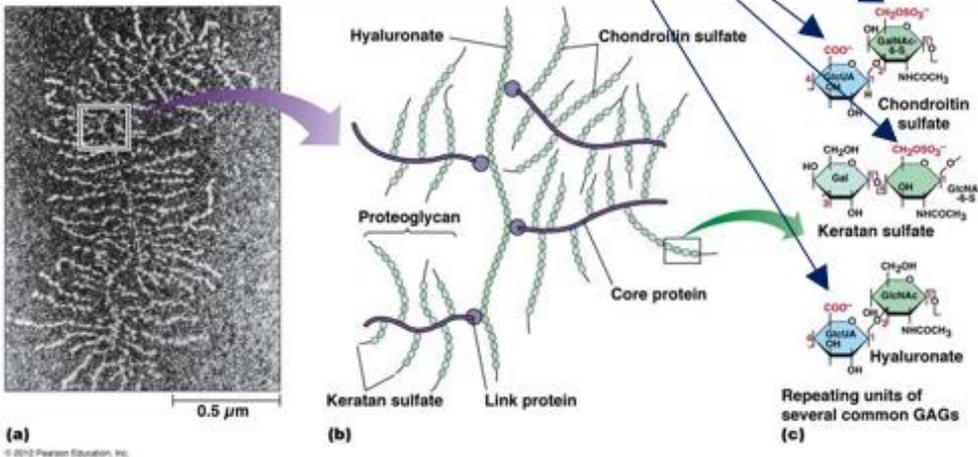
Principle

bi-fonctionnal:

- One macrocycle ^{99m}Tc for imaging
- One quaternary ammonium for proteoglycans targeting



$T_{1/2} = 6,0058 \text{ h}$
Scan time : 30min post injection
10 sec / projection



- Selective localization for healthy joints and tumor
- Excellent contrast for imaging

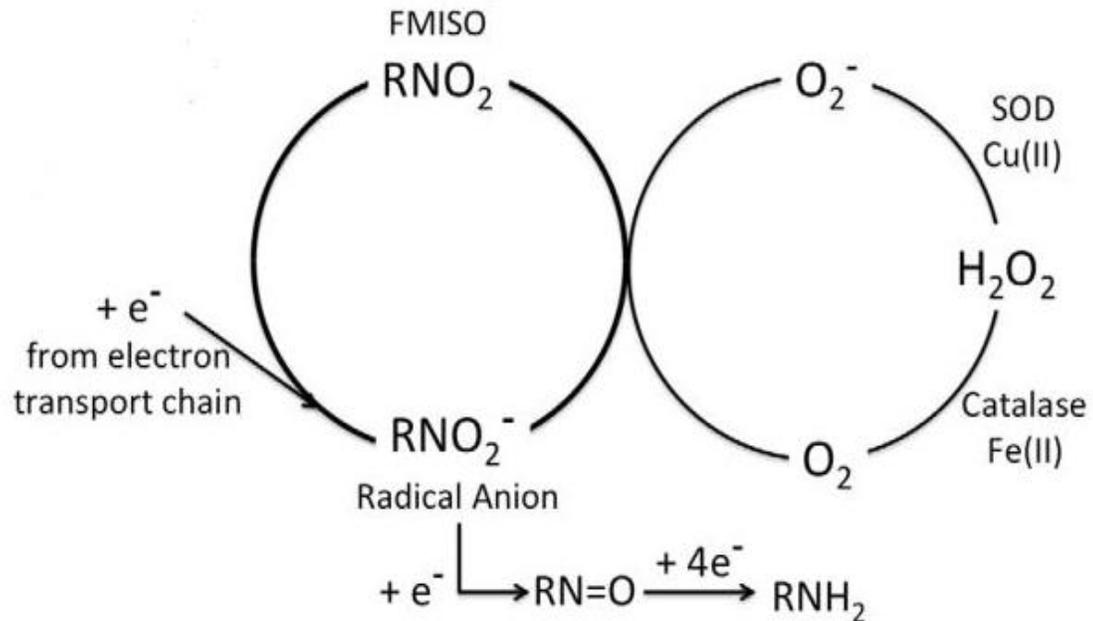
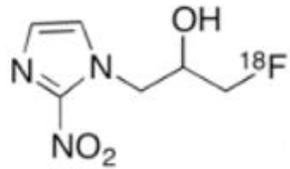


Miot-Noirault *et al.* 2009

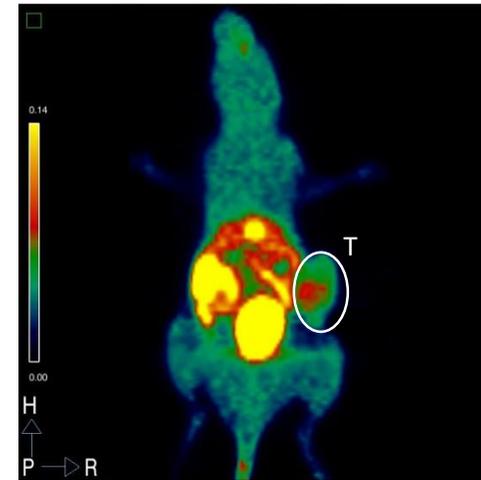
^{18}F -Fluoromisonidazole

β^+ radiations

Principle



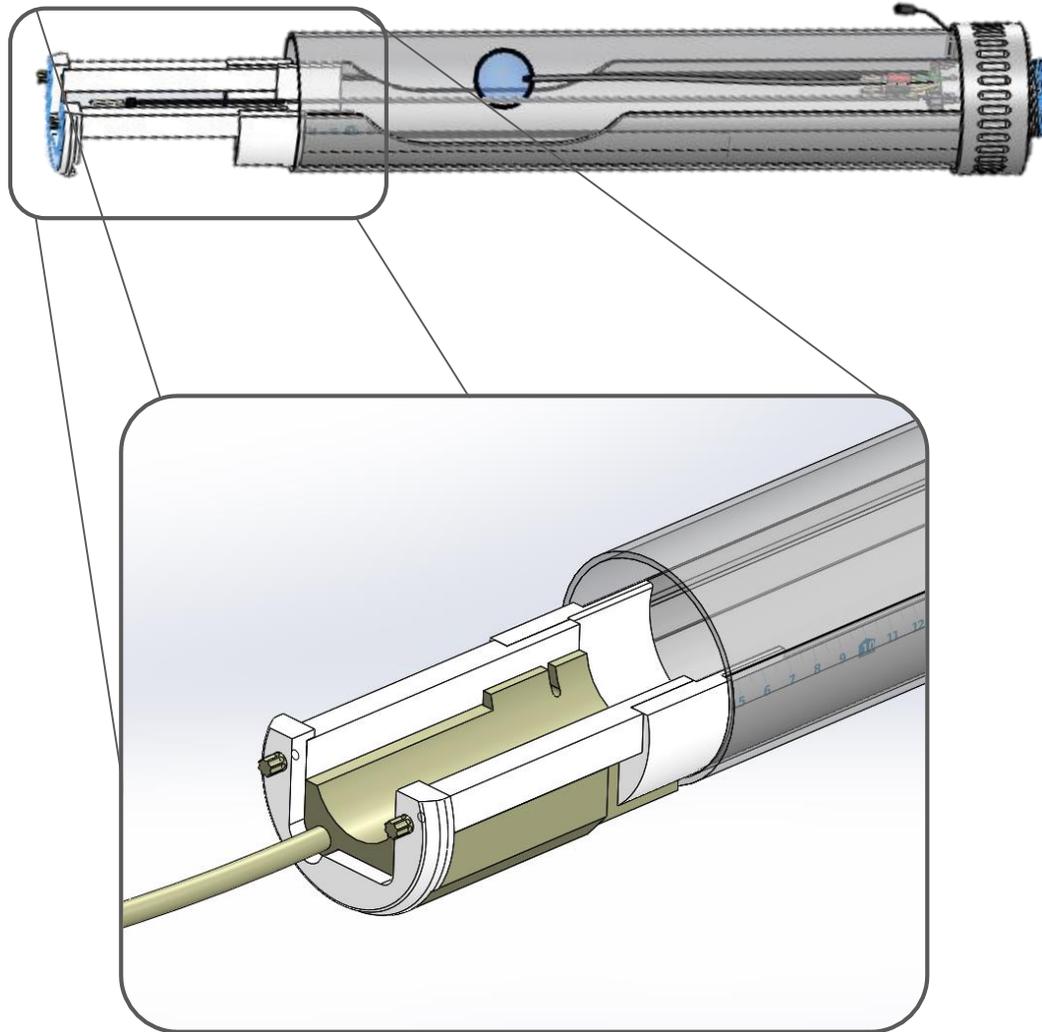
$T_{1/2} = 109$ min
Scan time : 4h post injection
15 min / bed position



Chondrosarcoma H-EMC-SS on NMRI Nude mouse

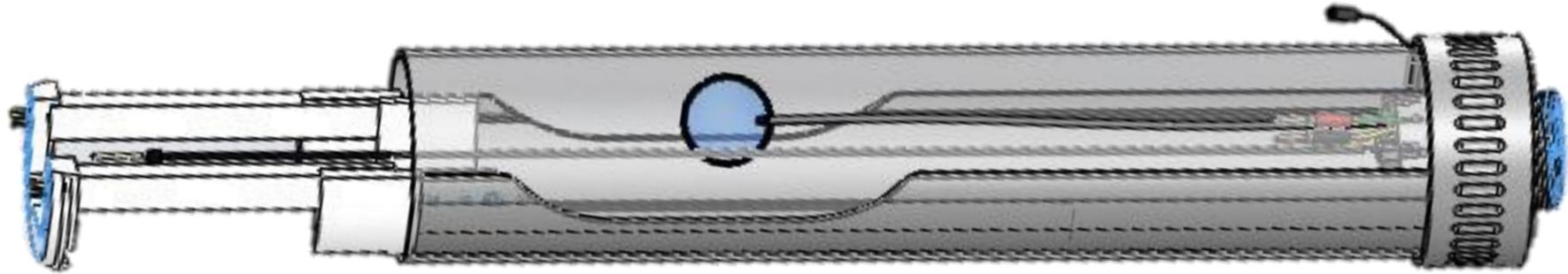
CEST MRI

Chemical Exchange Saturation Transfer MRI



CEST MRI

Chemical Exchange Saturation Transfer MRI



Receiving antenna

CEST MRI

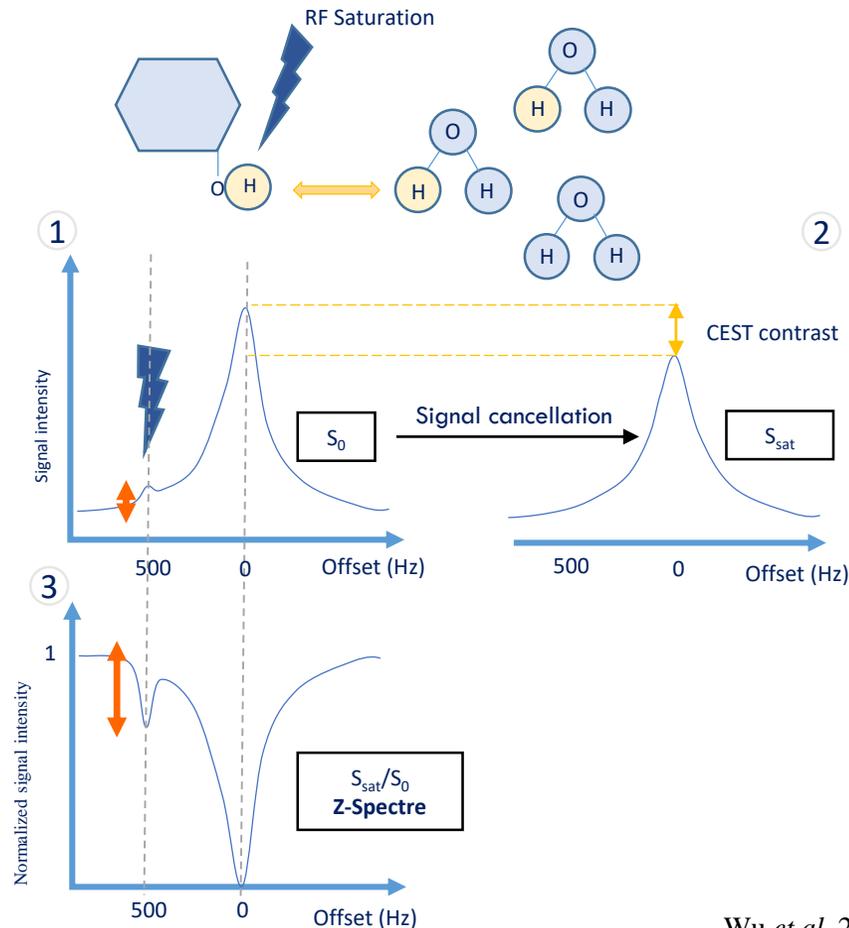
Chemical Exchange Saturation Transfer MRI

Sequences:

RARE WASSR: $B_1 = 0,1 \mu\text{T}$ for 1 sec, $\Delta\omega_{\text{sat}} = \pm 1000\text{Hz}$, $\Delta 20\text{Hz}$

RARE CEST Z-spectra: $B_1 = 1.5 \mu\text{T}$ for 4s, $\Delta\omega_{\text{sat}} = \pm 2500\text{Hz}$, $\Delta 50\text{Hz}$

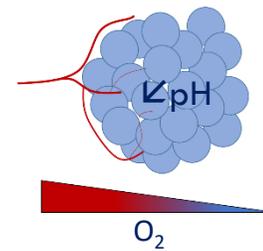
Principe



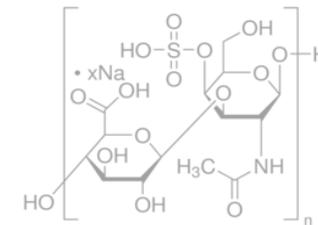
Moieties with exchangeable protons

Moieties	Frequencies
Hydroxyles (-OH)	10 - 600 Hz
Guanidyl (-NH ₂)	800 - 1200 Hz
Amides (-NH)	1700 - 1900 Hz

Hypoxia



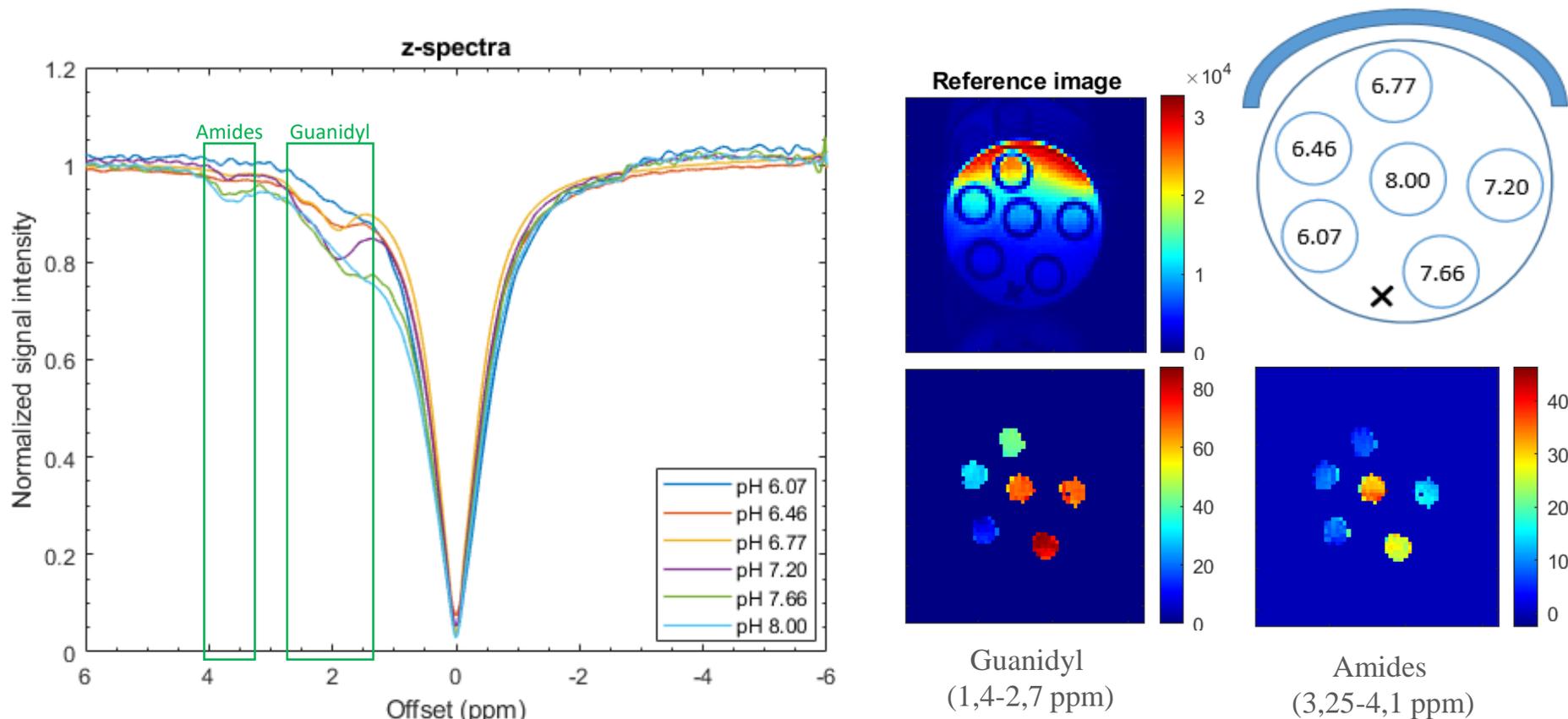
Proteoglycans



All contrasts in one acquisition
None injection

CEST MRI : *In vitro* on protamine phantom

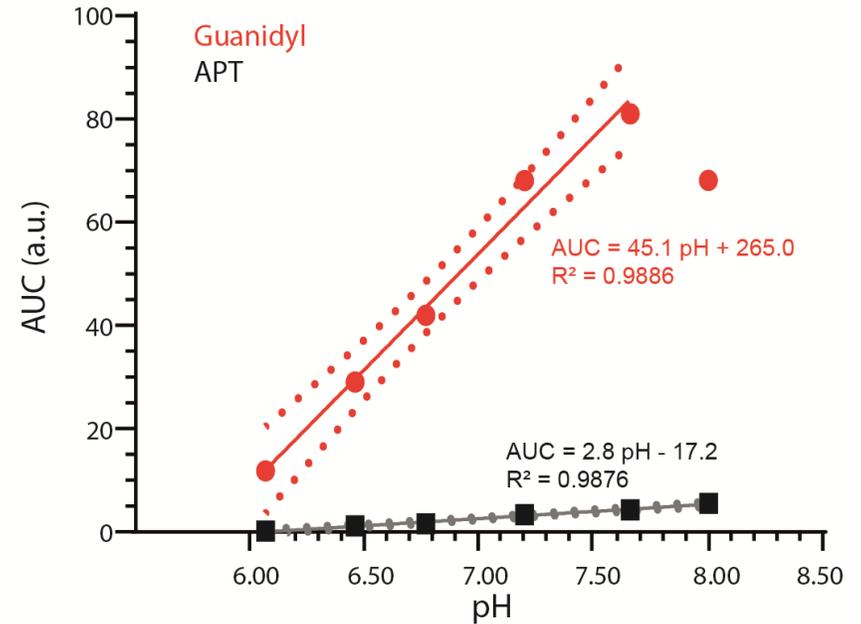
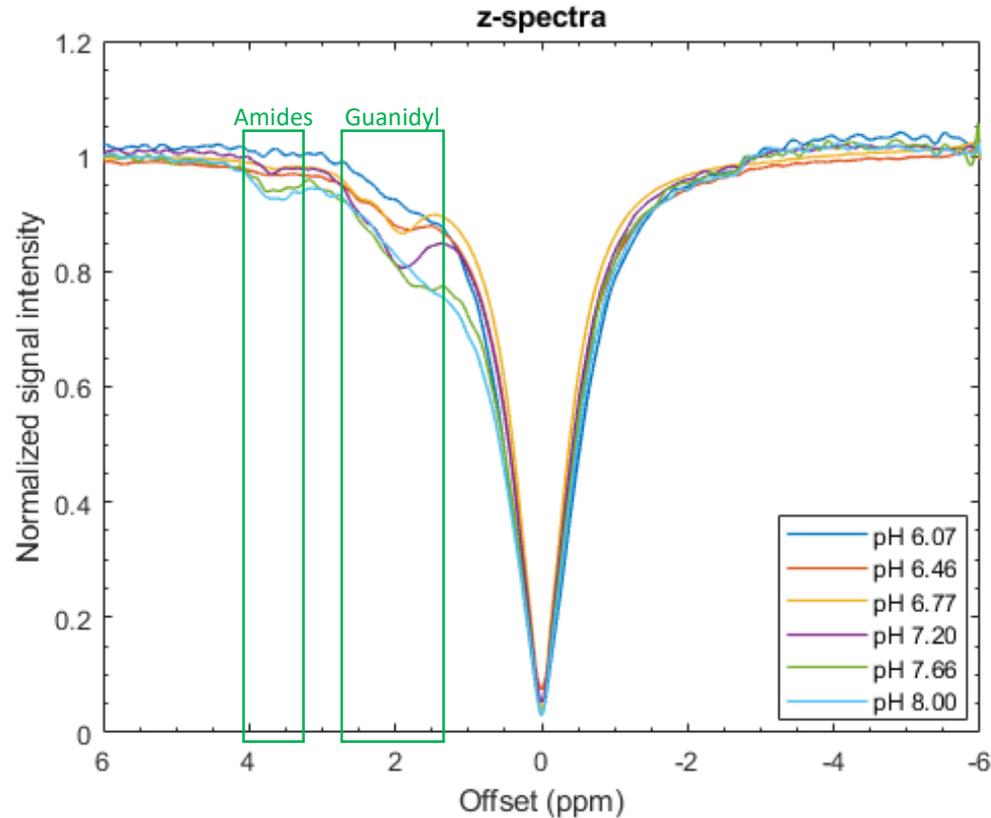
15mg/mL + 0,15 mM de $MnCl_2$ – different pH



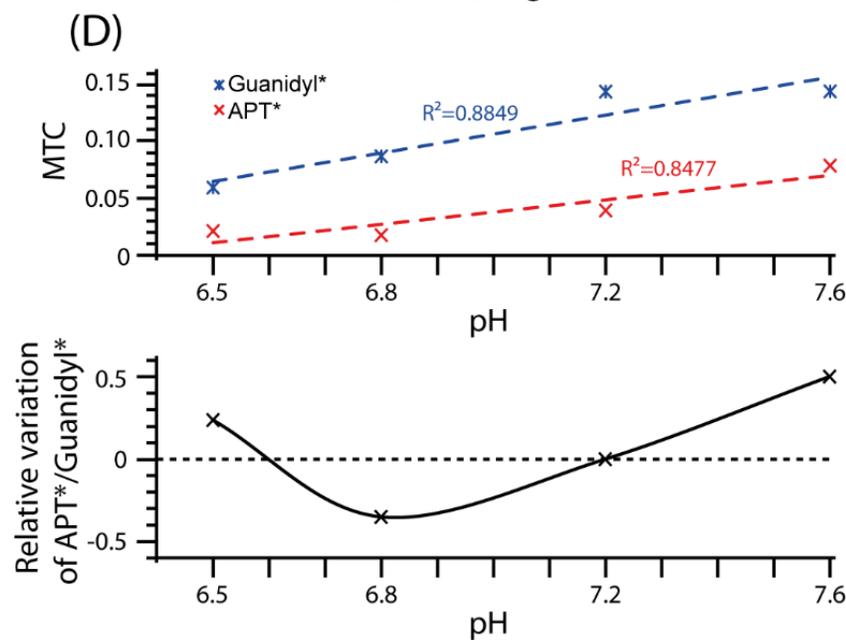
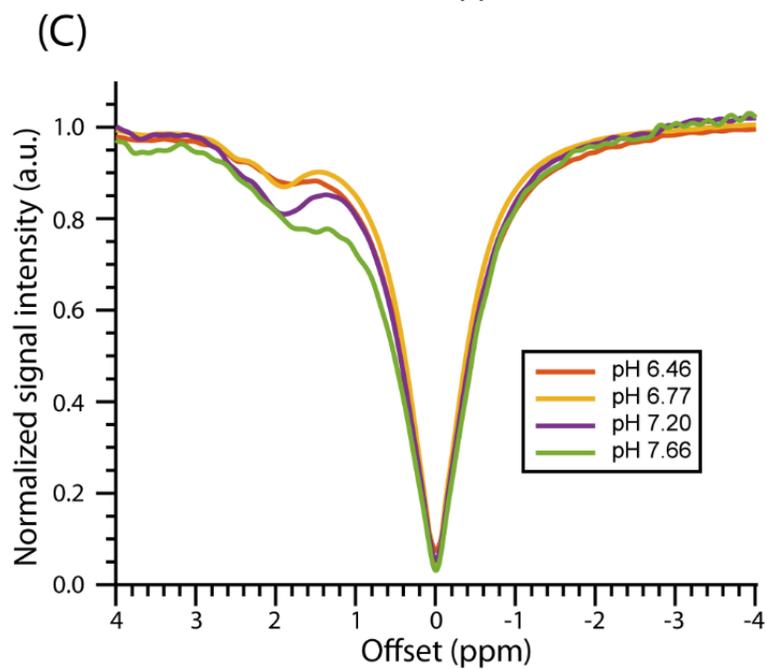
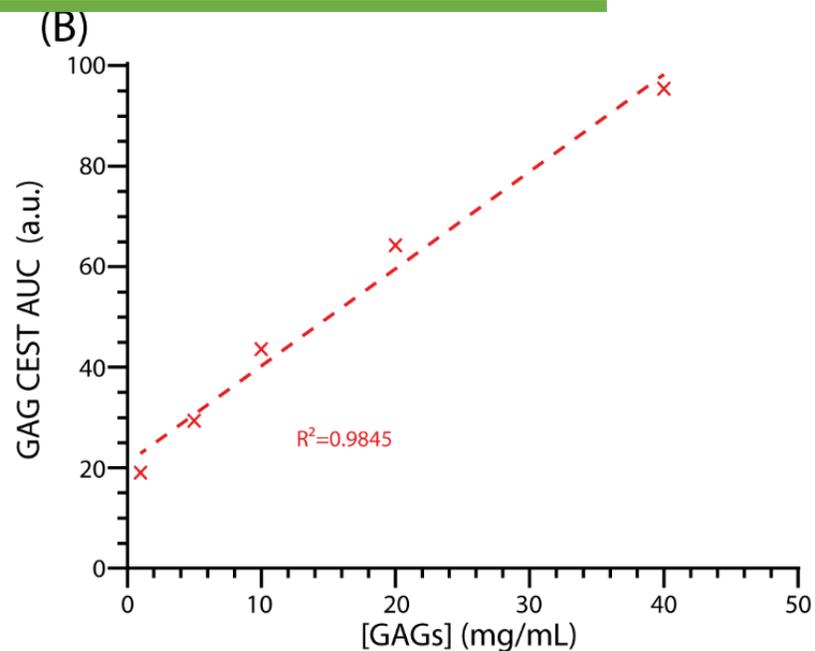
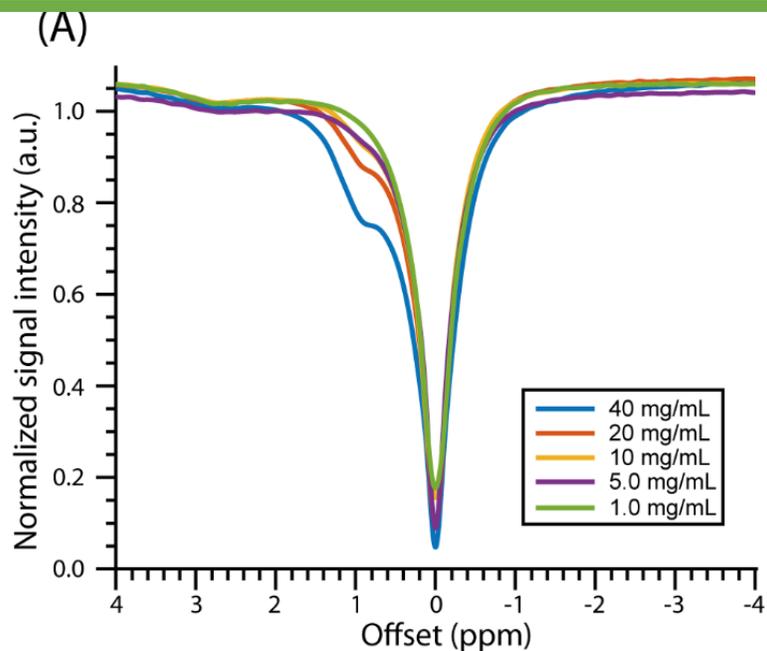
RARE CEST : Intensité 3 μT ; ± 3000 Hz ; $\Delta 50$ Hz ; TE: 4,756 ms ; TR: 4500 ms ; TS: 3948.651 ms et RARE factor: 16

CEST MRI : *In vitro* on protamine phantom

15mg/mL + 0,15 mM de $MnCl_2$ – different pH

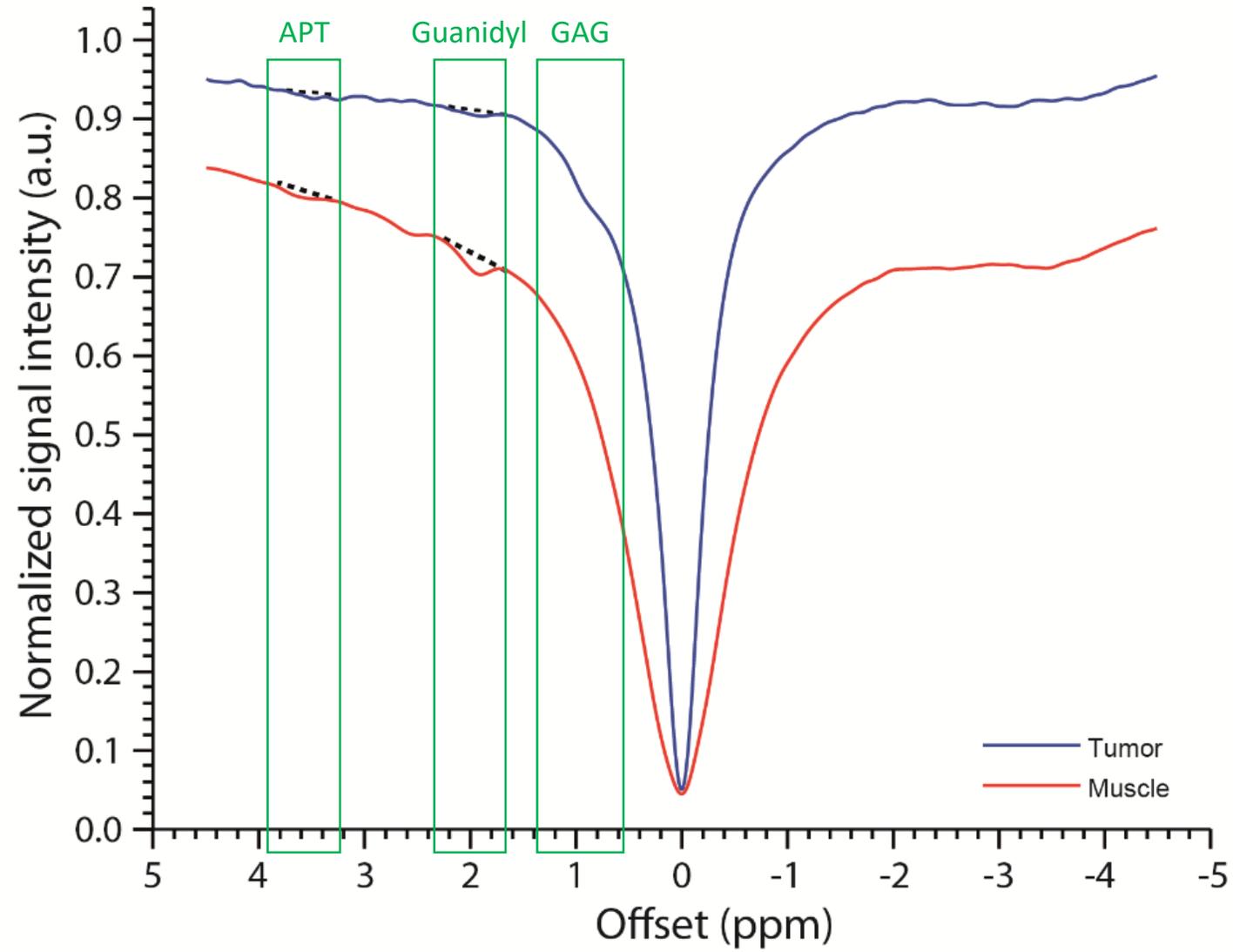


CEST MRI : *In vitro* on phantom

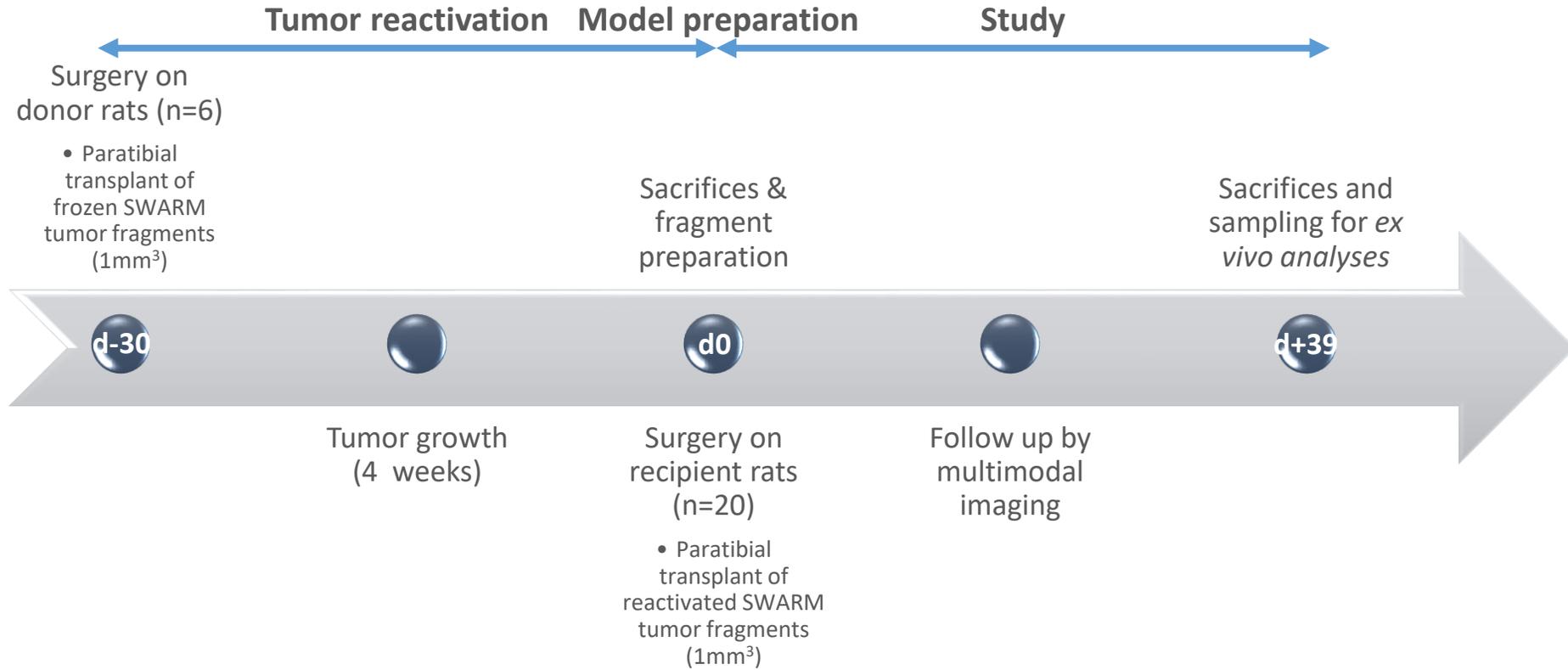


CEST MRI : *In vivo*

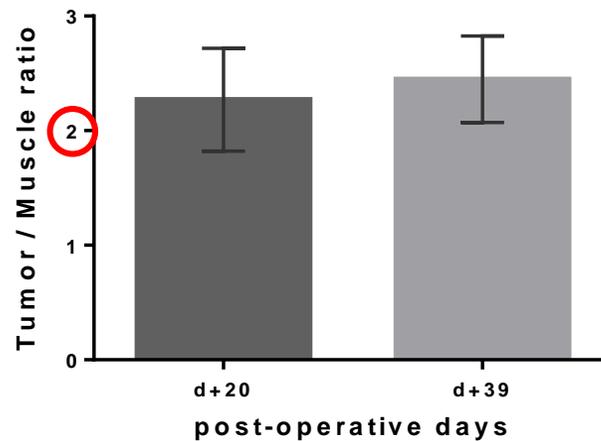
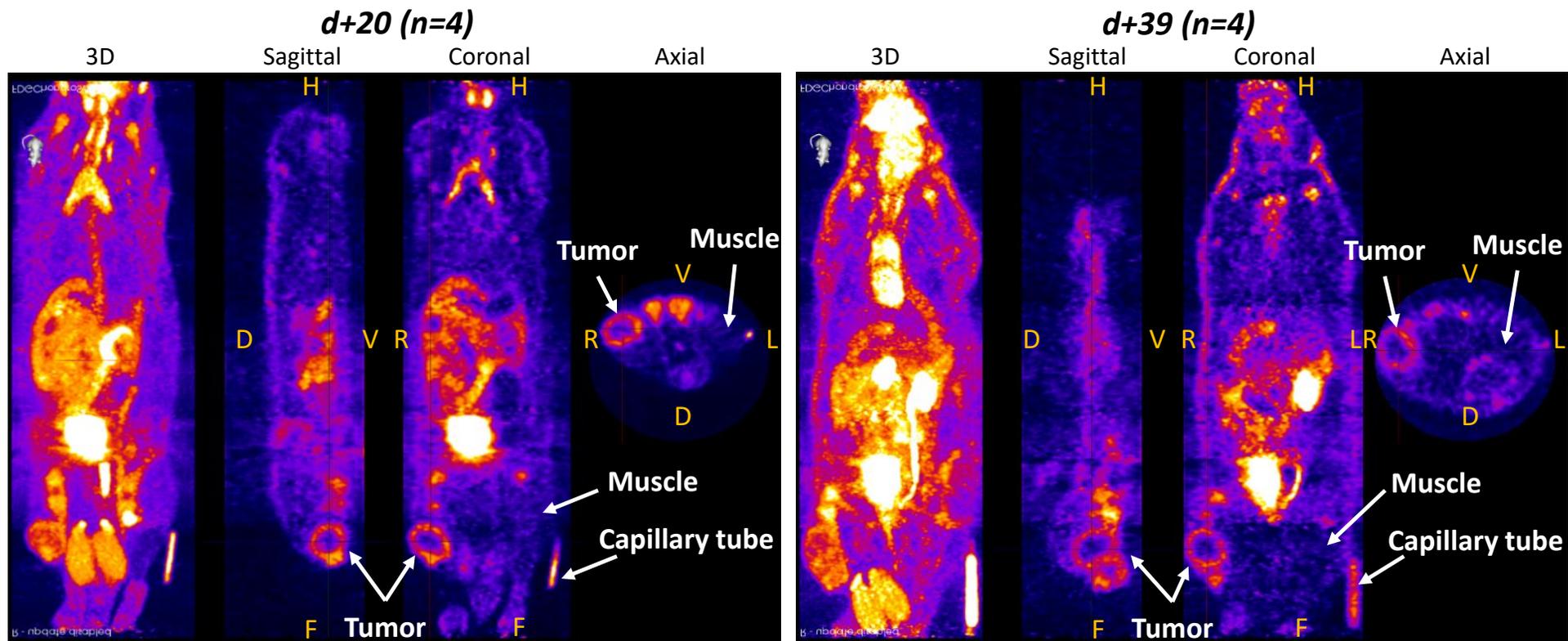
(A)



Paratibial Swarm rat model

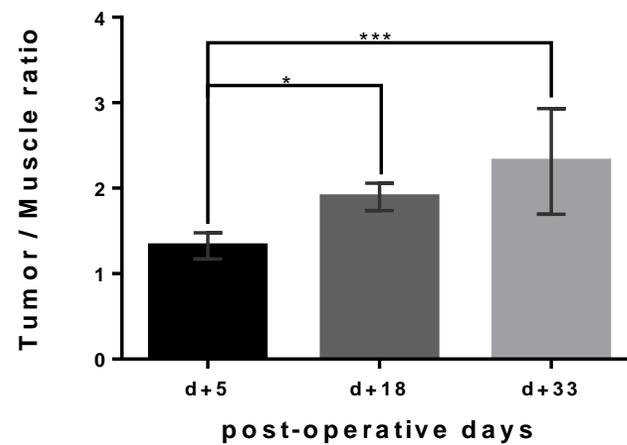
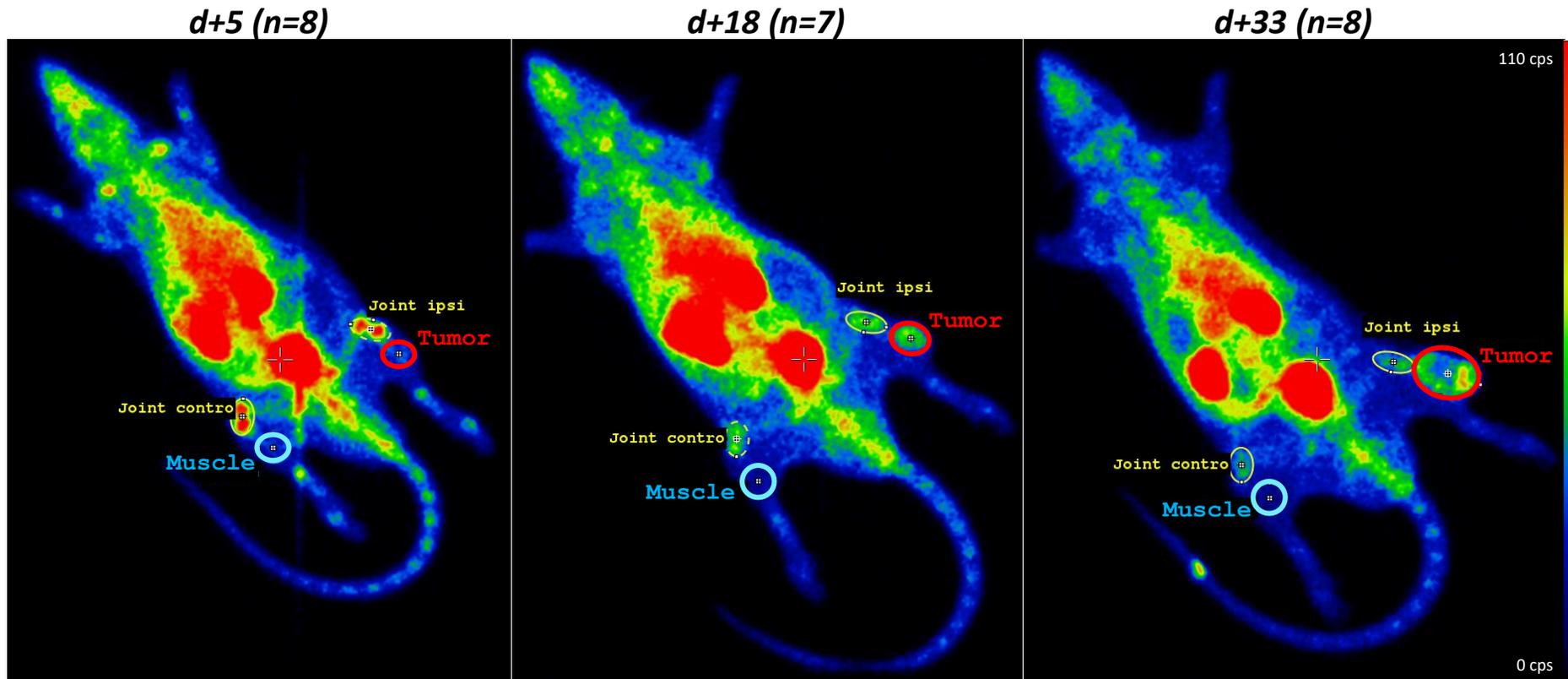


Previous results: ^{18}F -FDG



Fasting time: 12h
Anesthesia: Isoflurane 2,5%
IV injected mid-dose: 20 MBq
Scan time: 60 min post-injection
PET imaging duration: 20 min

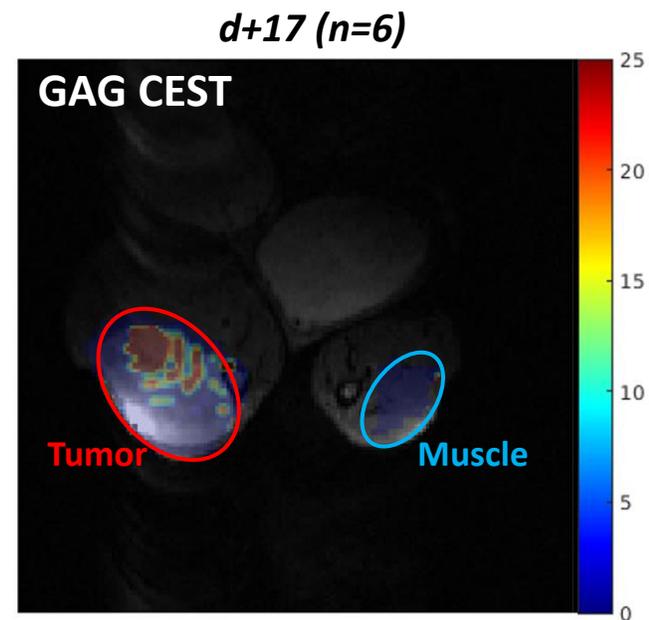
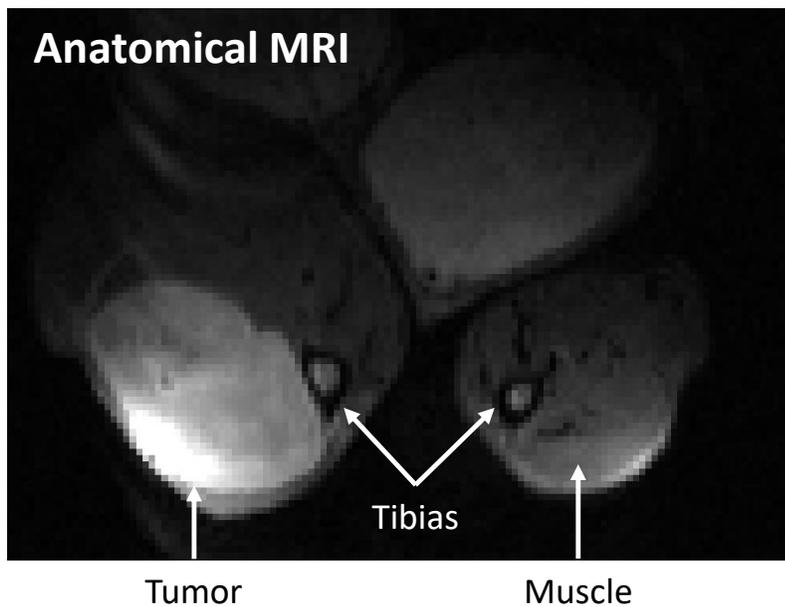
Previous results: ^{99m}Tc -NTP 15-5



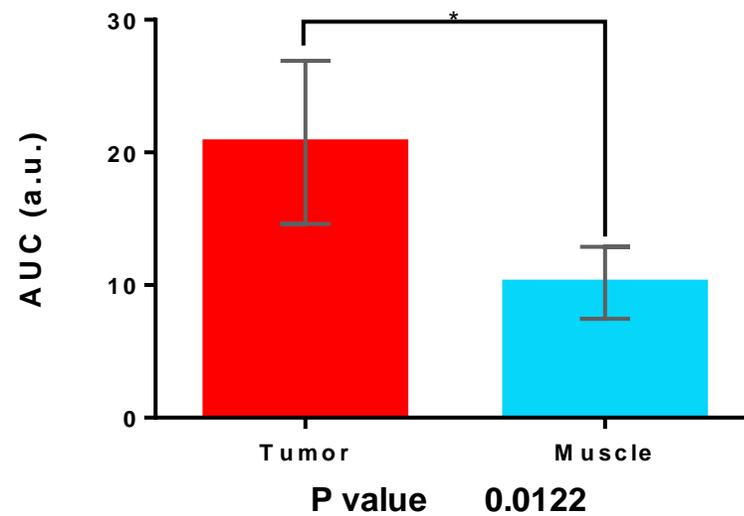
Anesthesia: Isoflurane 2,5%
IV injected mid-dose: 30 MBq
Scan time: 30 min post-injection
SPECT imaging duration: 20 min

One way ANOVA then Tukey's test

Previous results: CEST MRI



GAG CEST

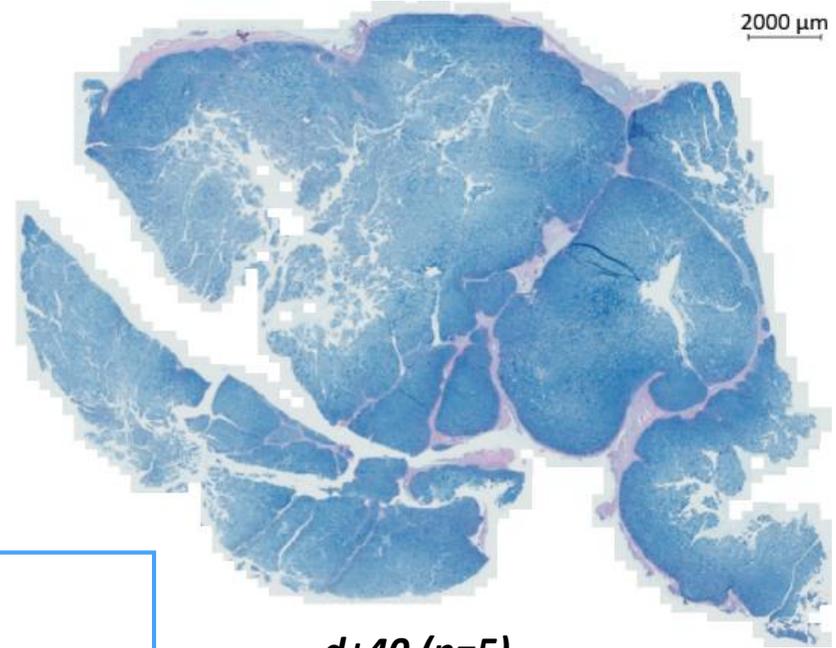
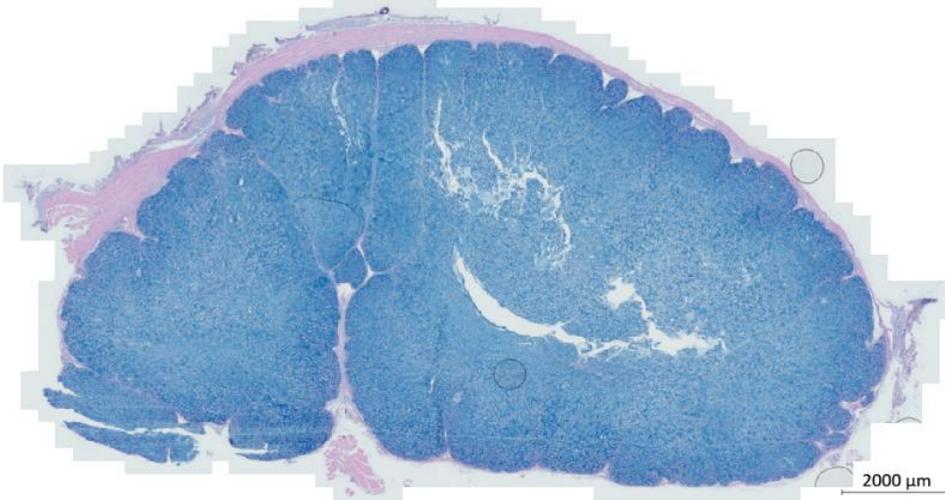


Anesthesia: Isoflurane 2,5%
None injection
CEST MRI duration: 45 min

Paired *t*-test

Previous results: Alcian blue staining

Tumor

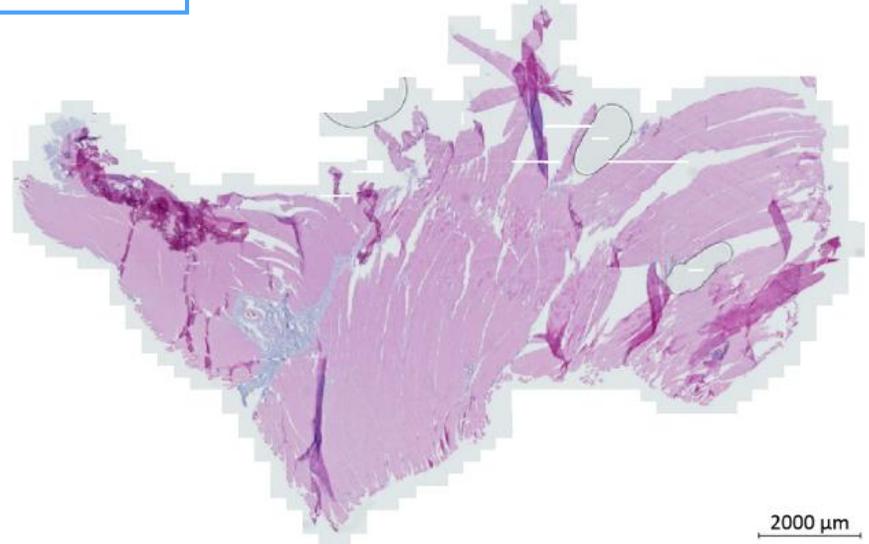


d+26 (n=3)

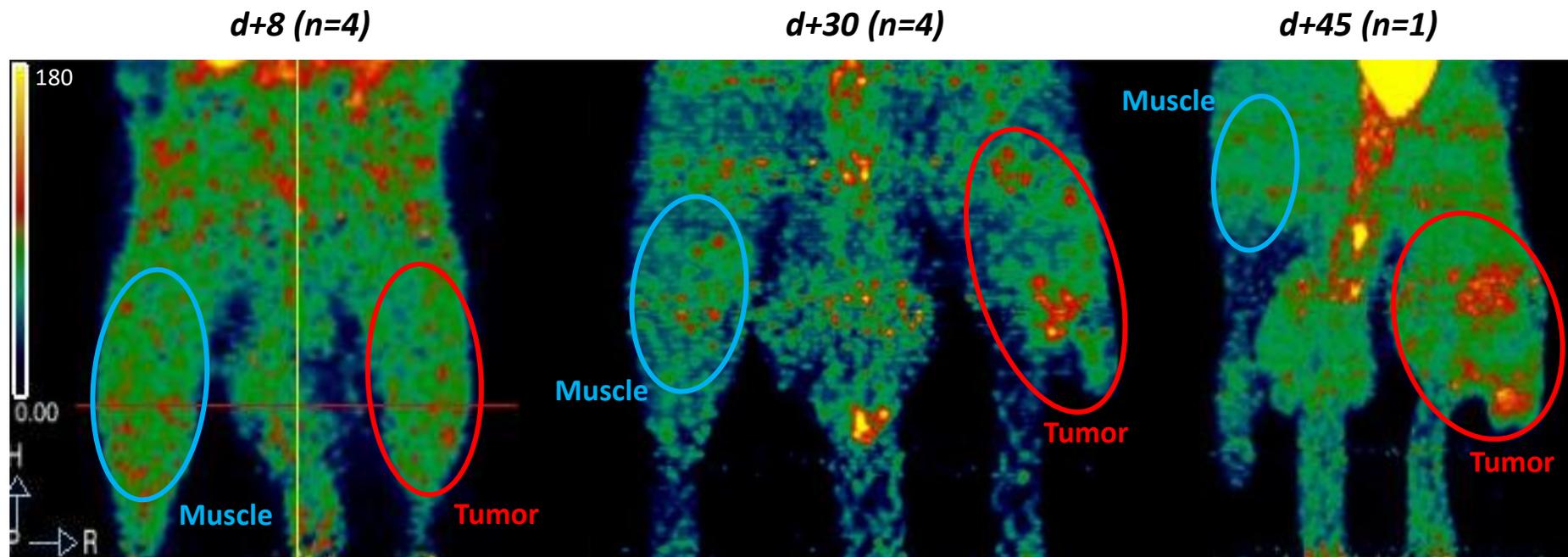
Alcian Blue
Nuclear red
X20, Zeiss AxioScan Z1

d+40 (n=5)

Muscle



Previous results: ^{18}F -FMISO

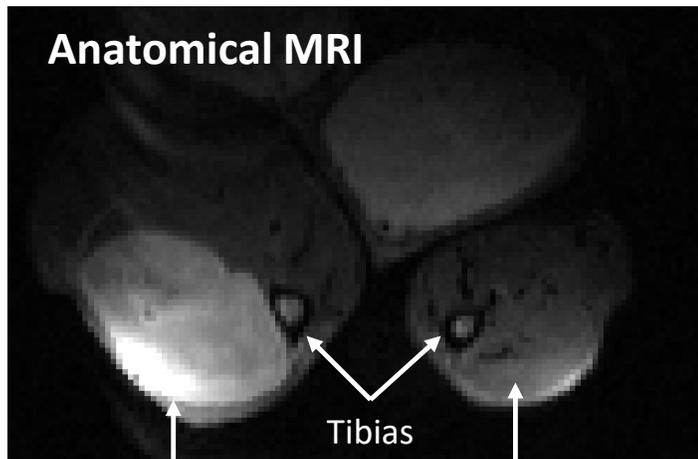


^{18}F -FMISO

Anesthesia: Isoflurane 2,5%
IV injected mid-dose: 40 MBq
Scan time: 150 min post-injection
PET imaging duration: 20 min

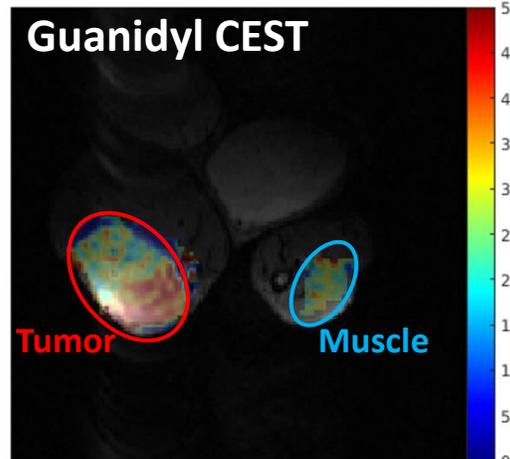
Tumor / Muscle ratio = 1,83

Previous results: CEST MRI



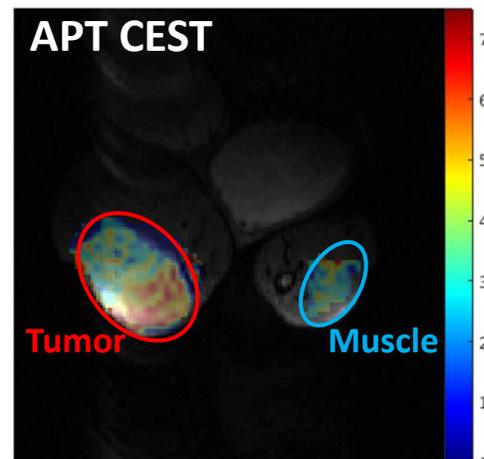
Tumor

Muscle



Tumor

Muscle



Tumor

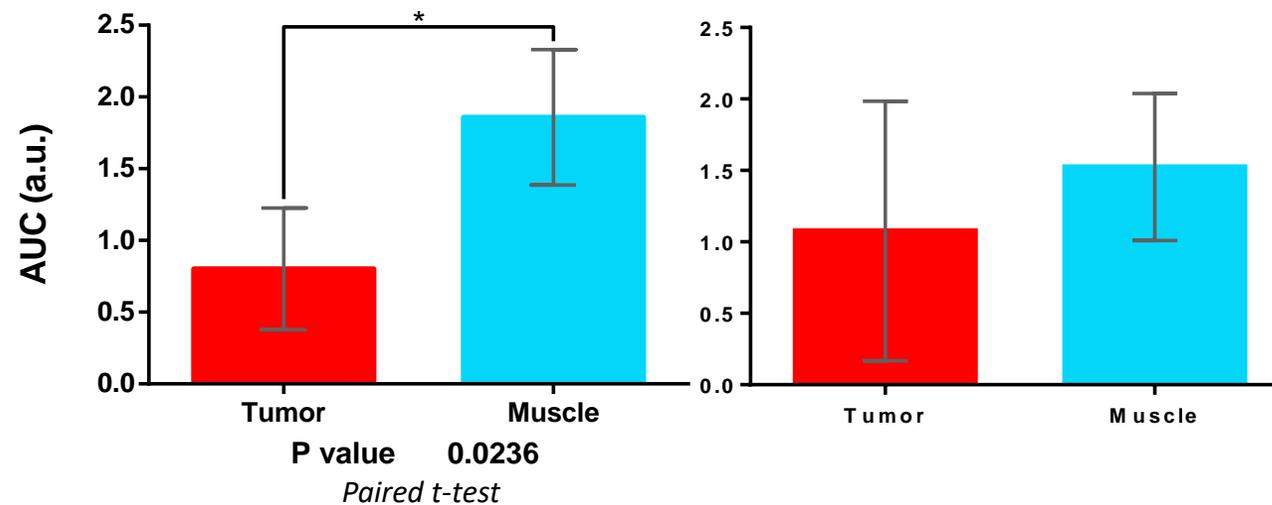
Muscle

d+17 (n=6)

Anesthesia: Isoflurane 2,5%
None injection
CEST MRI duration: 45 min

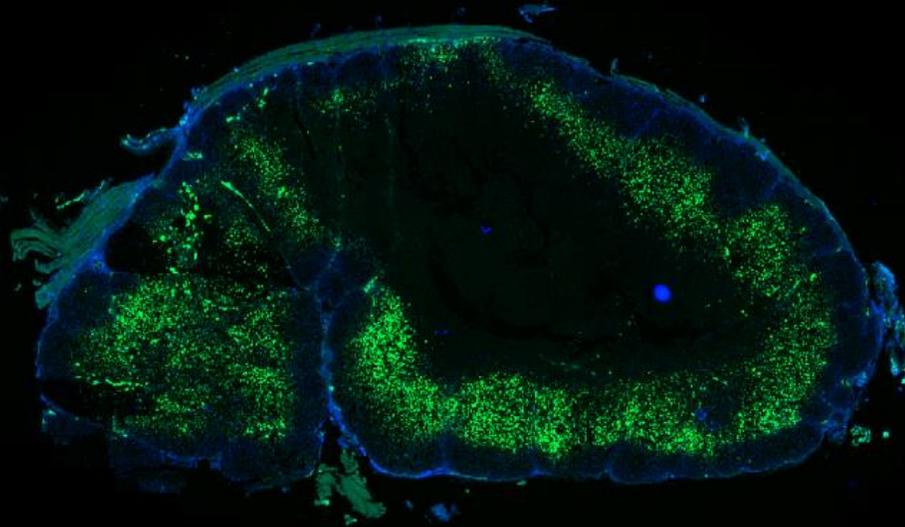
Guanidyl CEST

APT CEST



Previous results: Pimonidazole immunofluorescence

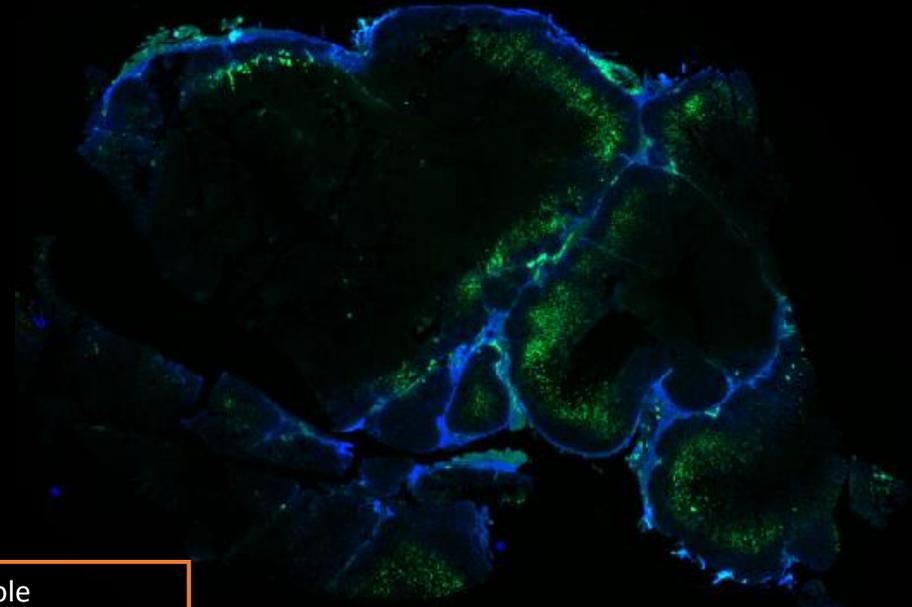
Tumor



d+26 (n=3)

2000 μm

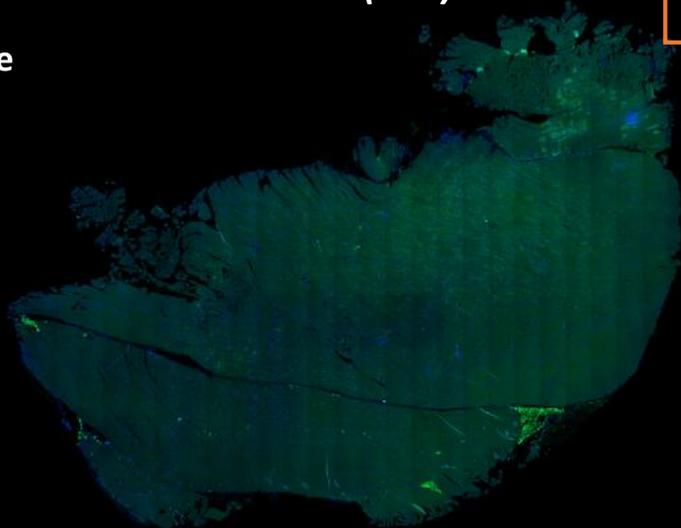
Pimonidazole
Hoechst
X20, Zeiss AxioScan Z1



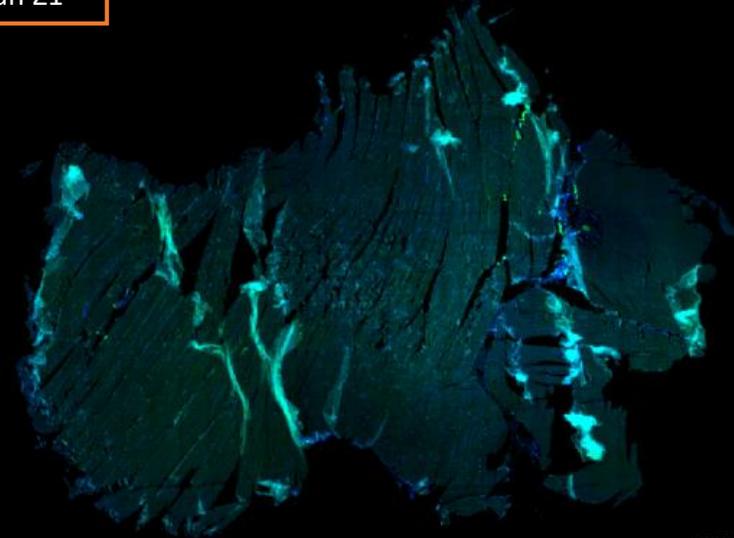
d+40 (n=5)

2000 μm

Muscle



2000 μm



2000 μm