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Peptide-Based MRI-CEST Probes for Real-Time Tumor pH Imaging

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FACTS & FIGURES

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Project website:	https://www.ihi-illuminate.org/
Social media:	

https://www.linkedin.com/company/ihi-illuminate-project/

CHALLENGE

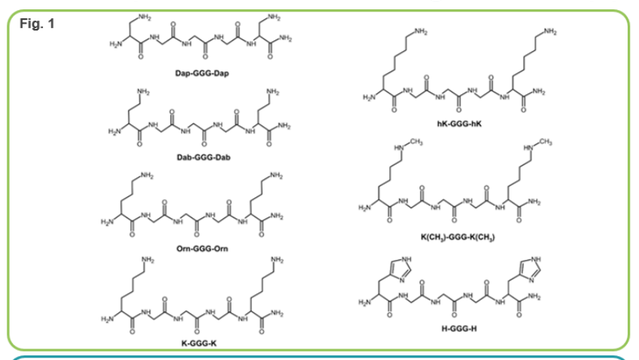
Personalized cancer treatment requires reliable, non-invasive biomarkers to characterize tumor metabolism and monitor treatment response. While **metabolic Magnetic Resonance Imaging (metabolic-MRI)** provides valuable information on tumor metabolic activity, complementary imaging of the tumor microenvironment may further improve tumor characterization. **Chemical Exchange Saturation Transfer MRI (CEST-MRI)**, particularly using peptide-based contrast agents, offers a promising approach for mapping physiological parameters such as tumor pH. However, the development of efficient, sensitive, and biocompatible CEST agents remains challenging. In this context, we developed a **novel peptide-based CEST contrast agent** for *in vivo* tumor pH mapping, aiming to complement metabolic-MRI with additional information on tumor physiology and ultimately support more precise, personalized theranostic strategies.

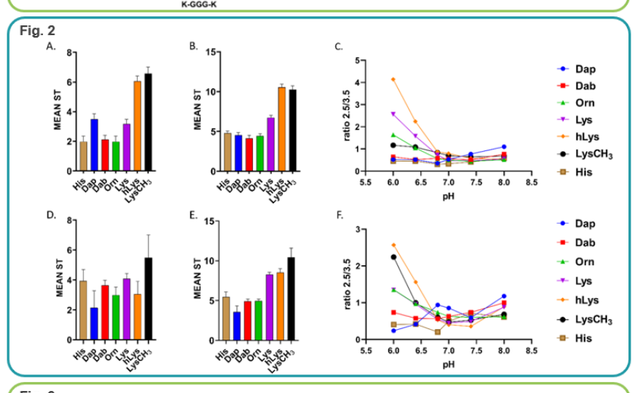
APPROACH & METHODOLOGY

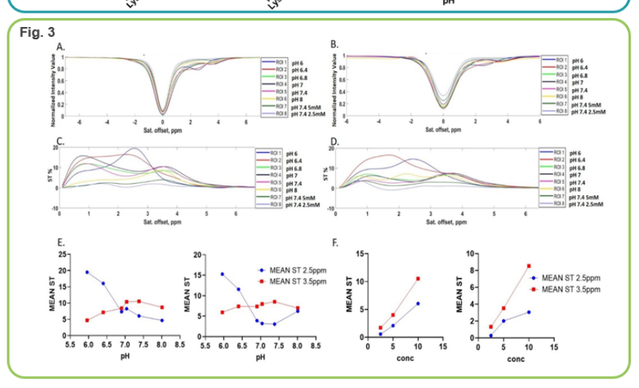
Objective: To investigate whether simple peptides containing amide and amine groups can act as efficient CEST-MRI contrast agents at molar concentrations comparable to Iopamidol, while requiring a substantially lower administered mass. ***Peptide design & synthesis:*** Peptides were designed around a central Gly-Gly-Gly (GGG) motif and functionalized with terminal amine, amide, or imidazole groups. Side-chain length and composition were systematically varied to modulate spatial and electronic properties and promote intramolecular hydrogen bonding, thereby tuning proton exchange rates and CEST performance. ***In vitro characterization:*** CEST properties and pH responsiveness were evaluated to identify the peptide with the most favorable exchange kinetics and contrast efficiency. ***In vivo validation:*** The hLys-containing pentapeptide was selected for *in vivo* CEST-MRI in a murine tumor model. Tumor pH mapping and contrast efficiency were evaluated and compared with Iopamidol at a substantially lower administered mass.

RESULTS

The CEST properties of a series of **pentapeptides** were evaluated as MRI-CEST pH reporters. Among the synthesized compounds, the **hLys-containing** peptide exhibited the highest pH sensitivity in the 6.0-8.0 range, owing to its enhanced CEST response at 2.5 and 3.5 ppm. *In vivo* MRI-CEST studies in a murine tumor model demonstrated **accurate mapping of acidic tumor pH values**, in agreement with results obtained using Iopamidol. Notably, comparable pH maps were achieved with an approximately **eight-fold lower administered dose**. Combined with its excellent chemical and biological stability, these findings identify the hLys-containing peptide as a promising candidate for MRI-based pH imaging and future personalized diagnostic applications.







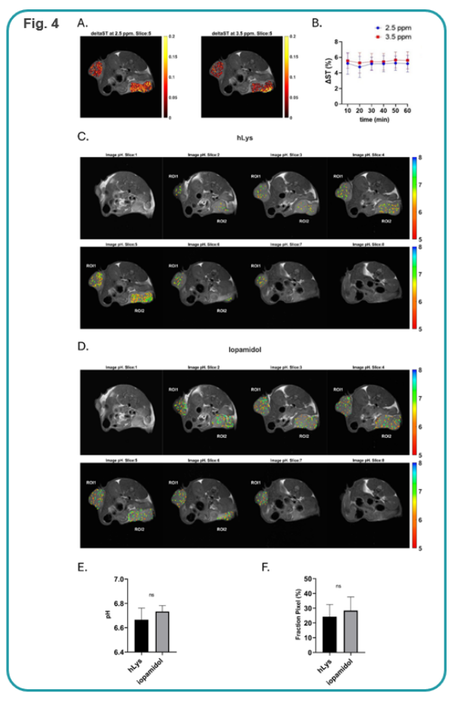


Fig. 1. Chemical structures of the investigated peptides.

Fig. 2. Mean ST effects at 2.5 and 3.5 ppm (A,B,D,E) and corresponding ratiometric curves (2.5/3.5 ppm) at different pH values (C,F) for the investigated peptides at 21°C (A-C) and 33°C (D-F). Peptide concentration: 10 mM; pH 7.4; saturation power: 1.5 μT; duration: 3 s.

Fig. 3. CEST response of hLys peptide (10 mM) at 21°C (A,C) and 33°C (B,D), showing Z-spectra, ST curves, pH dependence (E), and concentration dependence (F). Saturation power: 1.5 μT; duration: 3 s.

Fig. 4. *In vivo* CEST-MRI of hLys in tumor-bearing mice. (A,B) CEST contrast maps and time evolution at 2.5 and 3.5 ppm following i.v. injection of hLys (1 mmol/kg). (C) Comparison of tumor extracellular pH maps obtained with hLys (1 mmol/kg) and Iopamidol (10 mmol/kg). (D) Mean tumor pH values for hLys and Iopamidol (n=6 tumors). (E) Mean tumor extracellular pH for hLys vs Iopamidol (n=6). (F) Tumor contrast agent extravasation fraction.

VALUE OF IHI COLLABORATION

The IHI collaboration brings together complementary expertise in imaging, cancer biology and theranostics to accelerate the development of **personalised cancer care**. Within ILLUMINATE, this multidisciplinary framework supports the integration of advanced imaging technologies to improve patient stratification, treatment monitoring and clinical decision-making. Ultimately, IHI enables the **translation of innovative technologies into integrated, personalised theranostic strategies**.

CAREER IMPACT

ILLUMINATE fosters an **interdisciplinary and translational research environment**, connecting fundamental research with technological development and clinical needs. For researchers this represents an opportunity to engage more closely with the clinical setting and gain a deeper understanding of **patients' needs and healthcare challenges**. This cross-disciplinary perspective supports the development of a more integrated and patient-centred approach to cancer research.

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