## Title of Talk: Chemical Exchange Saturation Transfer: The Promise

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**Highlights:**

1) Performing contrast studies with imaging sensitivity and spectroscopic specificity, while still allowing standard anatomical MRI to be performed without interference.
2) Unlimited potential for the generation of novel paramagnetic and diamagnetic contrast agents
3) Possibility of designing pulse sequences specifically for certain agents
4) Biodegradable non-paramagnetic contrast agents are possible, for instance sugars.
5) Endogenous contrast for imaging of pH, tumor grade, and tissue metabolites.
6) Fast translation to the clinic for many agents

**Brief history of the field:** Chemical exchange saturation transfer or "CEST" MRI was first proposed by Balaban and coworkers.[1,2] It was then quickly picked up by others[3-6] and has, in the last decade evolved into a promising novel technology with great potential for clinical translation. In December of 2013, the original 2000 CEST paper had been cited 449 times (google scholar). For some recent reviews, see[7-14]

**The contrast explained:**[12] MRI has great sensitivity (55 M water concentration and thus 110 M protons) and allows high-resolution anatomical imaging. However, it often lacks specificity. MR spectroscopy, on the other hand, has great specificity (different chemicals have multiple and different resonances), but low sensitivity (mM concentrations). CEST allows some of the specificity of MRS to be combined with the sensitivity of MRI. CEST can detect low concentration molecules if they contain groups with exchangeable protons, such as for instance amide (-NH), amine (NH2), imino (-N(H)-) and hydroxyl units. These protons can be labeled magnetically using frequency-specific saturation or selective pulsed approaches, and this label is subsequently transferred to the water via exchange. Due to the large size of the water pool, an unlabeled proton will come back in place and the process is continuously repeated. If this labeling and transfer can be performed efficiently, this will lead to large signal enhancements that become visible in the water signal intensity and thus can be imaged. These generally are enhancements by a factor of 100 -1000. Currently, the largest enhancements achieved have been for paramagnetic CEST (paraCEST) shift agents in liposomes, for which micromolar to nanomolar concentrations can be detected via the water signal.[15,16]

**Exogenous CEST agents:** Current MRI contrast agents are relaxation based and often distort or interfere with the anatomical contrast. Because CEST agents are of low concentration, their presence without labeling does not affect the normal MRI signal and all normal imaging approaches can be applied without agent-based artifacts/interference because the effect of the agent can be turned on and off with radiofrequency (RF) labeling. In addition, contrary to commonly used agents for MRI that are paramagnetic, CEST allows the design of both paramagnetic and diamagnetic (diaCEST) agents. For paraCEST agents, lanthanides as well as other metals[17-19] are being studies. This greatly expands the possibility for designing new agents with many different functional properties. Such compounds can for instance have exchangeable proton groups with one or more different frequencies. Alternatively, multiple agents (agent cocktails) with different frequency shifts with respect to the water frequency can be used. The possibility of frequency-specific labeling increases the specificity of the agents and can be seen as analogous to optical spectroscopy. Colors can be assigned to the resonances[20,21] to exploit

this in the contrast display. In addition to frequency-specific contrast, one can also design agents based on a certain exchange rate of the protons. Since the proton exchange rate often depends on pH and temperature, such agents can then be used to image these parameters. The fact that multiple parameters (frequency shift, exchange rate) cause contrast and the fact that the contrast can be turned on and off or built up as a function of time can be used to design specific pulse sequences to detect specific type agents or to separate the signal of the agents from the background. Efforts to design such sequences are only just starting.[22-26]

Over the last decade a large group of CEST agents with different functional properties have been designed,[7-14] including some sensitive to metabolism, enzyme kinetics,[27] ion binding, etc. Reporter genes based on CEST have also been proposed.[28] However, this is just the beginning of limitless possibilities. A great strength of being able to use diamagnetic compounds is that simple biodegradable agents such as sugars and peptides suddenly become potential contrast agents. For instance, recent studies have shown the potential of using normal D-glucose as an MRI contrast agent for imaging tumor glucose uptake.[29,30]

**Endogenous CEST contrast:**
Tissue contains metabolites, proteins/peptides and carbohydrates. Basically, it therefore contains a collection of CEST agents that can be activated using RF. The first endogenous agent detected in vivo was urea.[31] Soon after, Zhou et al. showed the possibility to use the signal of water-accessible amide protons in tissue for CEST detection.[6] This so-called amide-proton transfer (APT) weighted MRI can be used to image pH changes during ischemia due to the strong sensitivity of the amide exchange rate on pH in the physiological pH range.[6,32] Subsequently, APT-weighted MRI was shown to allow the imaging of tumor tissue without interference by edema.[33] Other endogenous tissue contrast reported has been for glucosaminoglycans in the knee,[34] glycogen in the liver[35] and glutamate, myoinositol and creatine in brain and muscle[36-38]. In addition, bacteria and viruses also contain many CEST compounds, possibly allowing them to be imaged,[39] e.g. during infection. However, care has to be taken with assignments to specific compounds, because the signals of amide, amine and hydroxyl groups overlap and also may interfere with direct water saturation and conventional semi-solid magnetization transfer contrast (MTC). For instance, in the muscle, glycogen and creatine signals contribute. Fortunately, in the case of tumors MTC and APT/CEST effects seem to be additive allowing strong contrast to be generated. This contrast also can be used to separate recurrent tumor from treatment necrosis,[40] which has not been possible before. The use of frequency and exchange rate specific pulse sequences is expected to allow more specific detection of different endogenous agents in the near future.

**Fast translation to the clinic:** The availability of endogenous agents and biodegradable agents leads to great potential for fast clinical translation of CEST technology. Some already being applied are based on endogenous CEST contrast. The first is the study of brain tumors using APTw contrast, which is already being studied by several groups.[41-44] With respect to exogenous agents, there is potential for using previously approved agents from other imaging modalities (CT)[45,46] or from clinical testing for other purposes such as the glucose tolerance test (glucose, dextran).

**Conclusion:** The new CEST contrast has unlimited potential for the generation of new contrast agents with multiple functionalities. Both paramagnetic and diamagnetic exogenous agents can be designed and the contrast can be turned on and off using RF. Many FDA approved compounds contain exchangeable protons and have potential as CEST MRI agents, including several that are biodegradable. Endogenous CEST contrast is now already being tested in the clinic. The field is just starting and what we are seeing is the tip of the iceberg.

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