August 27, 2018

Charles Springer, Narrative of Scientific Contributions

The following are highlights of our contributions – presented generally from more recent to older.

1. Active Trans-Membrane Water Cycling [AWC] / High-Resolution Mapping of Na^+,K^+-ATPase Activity *In Vivo*

Application of our shutter-speed concept [Sec. 3, below] to the results of genetic and enzymatic manipulations spanning a number of different cell types and models [from cell suspensions, to perfused (heart and brain) tissues, to animals, to humans] has recently led us to discover a fundamental aspect of the biology of water .[a-g] A metabolically active trans-membrane water cycling [cellular water efflux rate constant, $k_{io}(a)$] has not been previously described. It seems likely this phenomenon dominates steady-state trans-cytolemmal water molecule exchange in all cells.[a-g] This process was previously thought completely passive, but our work shows it driven by the homeostatic activity of the cell membrane P-type ATPase. In mammalian cells, this is the Na^+,K^+-ATPase [NKA; *The Sodium Pump*], arguably biology's most crucial enzyme: it maintains ion concentration gradients and the membrane potential, and is usually the cell's most significant ATP consumer. Active water cycling [AWC] enables high-resolution metabolic imaging – k_{io} mapping – of tumor,[b,c,f] multiple sclerosis (normal-appearing brain and lesions),[d] normal brain,[d] and myocardial (unpublished) tissue *in vivo*. It was not previously possible to even measure NKA turnover *in vivo*. The rigorous studies of AWC employed paramagnetic Gd(III) chelate contrast agents.[a-g] However, a new analysis of diffusion-weighted ^1H_2O data promises a completely non-invasive method for accomplishing this.[f] Since the vast majority of MRI arises from the ^1H_2O signal, this new finding has the possibility of transforming a large fraction of all MRI studies. In the brain, this could even herald a new, quantitative form of fMRI.[e-g] Our most recent results show that $k_{io}(a)$ correlates with neuronal firing.[g] Recent results from Aime's Turin group and Glickson's UPenn group show that $k_{io}(a)$ correlates with metastatic potential in breast cancer and melanoma cell lines, respectively.

a. Y. Zhang, M. Poirier-Quinot, C.S. Springer, J.A. Balschi, Active Trans-Plasma Membrane Water Cycling in Yeast is Revealed by NMR, *Biophys. J.* **101**, 2833-2842 (2011). **[doi:10.1016/j.bpj.2011.10.035]**

b. C.S. Springer, X. Li, L.A. Tudorica, K.Y. Oh, N. Roy, S.Y-C. Chui, A.M. Naik, M.L. Holtorf, A. Afzal, W.D. Rooney, W. Huang, Intra-Tumor Mapping of Intra-Cellular Water Lifetime: Metabolic Images of Breast Cancer?, *NMR Biomed* **27**, 760-773 (2014). **[doi:10.1002/nbm.3111]**

c. A. Tudorica, K. Y. Oh, S. Y-C. Chui, N. Roy, M. L. Troxell, A. Naik, K. A. Kemmer, Y. Chen, M. L. Holtorf, A. Afzal, C. S. Springer, X. Li, W. Huang, "Early Prediction and Evaluation of Breast Cancer Response to Neoadjuvant Chemotherapy Using Quantitative DCE-MRI," *Trans. Oncol.* **9**, 8-17 (2016). [**doi:10.1016/j.tranon.2015.11.016**]

d. W.D. Rooney, X. Li, M.K. Sammi, D.N. Bourdette, E.A. Neuwelt, C.S. Springer, Mapping Human Brain Capillary Water Lifetime: High-Resolution Metabolic Neuroimaging, *NMR Biomed* **28**, 607-623 (2015). **[doi:10.1002/nbm.3294]**

e. R. Bai, C. S. Springer, D. Plenz, P. J. Basser, "Fast, Na^+/K^+ Pump Driven, Steady-State Transcytolemmal Water Exchange Found in Neuronal Tissue: A Study of Rat Brain Cortical Cultures," *Magn. Reson. Med.*, **79**,3207-3217 (2018). **[doi:10.1002/mrm.26980]**

f. C. S. Springer, "Using ^1H_2O to Measure and Map Sodium Pump Activity *in Vivo*," *J. Magn. Reson.*, **291**,110-126 (2018). **[doi.org/10.1016/j.jmr.2018.02.018]**

g. R. Bai, C. S. Springer, D. Plenz, P. J. Basser, "Brain Active Trans-Membrane Water Cycling Measured by MR is Associated with Neuronal Activity," *Magn. Reson. Med.*, **80**,0000-0000 (2018). **[DOI:10.1002/mrm.27473]**

2. The Shutter-Speed DCE-MRI Pharmacokinetic Paradigm

As indicated below, our shutter-speed concept has found particular application in pharmacokinetic analyses of [Dynamic-Contrast-Enhanced] DCE-MRI data. Although tracer pharmacokinetic models are almost exclusively used in DCE-MRI, that represents an inherent error because of the fundamental tracer limitation that its compartmentalization is not encoded in its signal. The tissue ^1H_2O signals do report the varying compartmental contrast reagent, CR, concentrations. Among our recent publications is a comprehensive overview.[a] The initial applications of shutter-speed DCE-MRI emphasized elimination of systematic errors in tracer pharmacokinetic parameters, because this greatly increases the discrimination of malignant from benign tumors.[a-d] However, recent work [elaborated above] reveals that shutter-speed DCE-MRI also allows access to a completely new imaging biomarker measuring the on-going activity of the vital cell membrane enzyme Na^+,K^+-ATPase, which is very sensitive to the cellular energy state. This is precluded from all tracer models. We have shown that any tracer paradigm model is the vanishing shutter-speed limit of its analogous shutter-speed model:[e] the tracer paradigm is but a singularity of the shutter-speed paradigm – a special case. Thus, we have generalized the old, and well-established, field of pharmacokinetics.

a. X. Li, R.A. Priest, W.J. Woodward, F. Siddiqui, T.M. Beer, M.G. Garzotto, W.D. Rooney, C.S. Springer, Cell Membrane Water Exchange Effects in Prostate DCE-MRI, *J. Magn. Reson.* **218**, 77-85 (2012). **[doi:10.1016/j.jmr.2012.03.019]**

b. X. Li, R.A. Priest, W.J. Woodward, I.J. Tagge, F. Siddiqui, W. Huang, W.D. Rooney, T.M. Beer, M.G. Garzotto, C.S. Springer, Feasibility of Shutter-Speed DCE-MRI for Improved Prostate Cancer Detection, *Magn. Reson. Med.* **69**, 171-178 (2013). **[doi:10.1002/mrm.24211]**

c. W. Huang, L.A. Tudorica, X. Li, S.B. Thakur, Y. Chen, E.A. Morris, I.J. Tagge, M. Korenblit, W.D. Rooney, J.A. Koutcher, C.S. Springer, Discrimination of Benign and Malignant Breast Lesions by Using Shutter-Speed Dynamic Contrast-enhanced MR Imaging, *Radiology* **261**, 394-403 (2011). **[doi:10.1148/radiol.11102413]**

d. X. Li, W.D. Rooney, C.G. Várallyay, S. Gahramanov, L.L. Muldoon, J.A. Goodman, I.J. Tagge, A.H. Selzer, M.M. Pike, E.A. Neuwelt, C.S. Springer, Dynamic-Contrast-Enhanced-MRI with Extravasating Contrast Reagent: Rat Cerebral Glioma Blood Volume Determination, *J. Magn. Reson.* **206**, 190-191 (2010). **[doi:10.1016/j.jmr.2010.07.004]**

e. G. W. Wilson, M. Woods, C. S. Springer, S. Bastawrous, P. Bhargava, J. H. Maki, "Human Whole-Blood ^1H_2O Longitudinal Relaxation with Normal and High-Relaxivity Contrast Reagents: Influence of Trans-Cell-Membrane Water Exchange," *Magn. Reson. Med.* **72**, 1746-1754 (2014). [**doi:10.1002/mrm.25064**]

3. The NMR Shutter-Speed Concept

In 1999, our group coined the metaphor "NMR Shutter-Speed," a generalization of the "timescale" reciprocal.[a] Subsequently, we have shown the power of this concept for understanding and defining the molecular exchange condition of an NMR signal, and developed the laws of shutter-speed.[b,c] The germ of this concept goes back even further in our work.[d] Since almost all NMR signals exhibit manifestations of molecular exchange, this is of extremely wide-ranging application. We have used it extensively to enable determination of the equilibrium exchange kinetics of water molecules across the cell membrane from DCE-MRI pharmacokinetic data.[a-c] Incorporating the shutter-speed concept into pharmacokinetic analysis greatly increases the discrimination of malignant from benign tumors.[b,c]

a. C.S. Landis, X. Li, F.W. Telang, P.E. Molina, I. Palyka, G. Vetek, and C.S. Springer, "Equilibrium Transcytolemmal Water Exchange Kinetics in Skeletal Muscle *In Vivo*," *Magn. Reson. Med.* **42**, 467-478 (1999).

b. X. Li, W. Huang, E.A. Morris, L.A. Tudorica, V.E. Seshan, W.D. Rooney, I. Tagge, Y. Wang, J. Xu, and C.S. Springer, "Dynamic NMR Effects in Breast Cancer Dynamic-Contrast-Enhanced MRI," *Proc. Natl. Acad. Sci., USA* **105**, 17937-17942 (2008).

c. W. Huang, X. Li, E.A. Morris, L.A. Tudorica, V.E. Seshan, Y. Wang, J. Xu, and C.S. Springer, "The Magnetic Resonance Shutter-Speed Discriminates Vascular Properties of Malignant and Benign Breast Tumors," *Proc. Natl. Acad. Sci., USA* **105**, 17943-17948 (2008).

d. S.R. Tanny, M. Pickering, and C.S. Springer, "Increasing the Time Resolution of Dynamic Nuclear Magnetic Resonance Spectroscopy Through the Use of Lanthanide Shift Reagents," *J. Am. Chem. Soc.*, **95**: 6227-6232 (1973).

4. Bulk Magnetic Susceptibility (BMS) Manifestations in *In Vivo* MR and MRI

Biological tissue is compartmentalized. Since the compartments can have different magnetic susceptibilities, intrinsic magnetic field gradients can exist within tissue present in even a homogeneous MR magnetic field. Our group systematized the principles of bulk magnetic susceptibility (BMS) manifestation in *in vivo* spectroscopy,[a] and imaging,[b] emphasizing the crucial role of Lorentz cavity demagnetization.[c] Soon after the discovery of BOLD-based fMRI, we showed we could mimic the BOLD effect,[d] thereby confirming its BMS origins. We also demonstrated the first murine fMRI.[e] Our principles allowed interpretation of transverse blood $^{1}H_2O$ relaxation,[c] and the spectroscopic discrimination of intra- and extramyocellular lipid signals in oriented muscle tissue *in vivo* (Boesch, Kreis, *et al*). High-field BMS mapping is now a very active area of neuroimaging research.

a. S.C-K. Chu, Y. Xu, J.A. Balschi, and C.S. Springer, "Bulk Magnetic Susceptibility Shifts in NMR Spectra of Compartmentalized Samples: Use of Paramagnetic Reagents," *Magn. Res. Med.,* **13**: 239-262 (1990).

b. Y. Xu, J.A. Balschi, and C.S. Springer, "Magnetic Susceptibility Shift Selected Imaging: MESSI," *Magn. Res. Med.,* **16**: 80-90 (1990).

c. G. J. Wilson, C. S. Springer, S. Bastawrous, J. H. Maki, "Human Whole Blood $^{1}H_2O$ Transverse Relaxation with Gadolinium-based Contrast Reagents: Magnetic Susceptibility and Transmembrane Water Exchange," *Magn. Reson*. *Med.*, **77**,2015-2027 (2017). [**doi:10.1002/mrm.26284**]

d. M.S. Albert, W. Huang, J-H. Lee, C.S. Patlak, and C.S. Springer, "Susceptibility Changes Following Bolus Injections," *Magn. Res. Med.*, **29**, 700-708 (1993).

e. W. Huang, I. Pálya, H-F. Li, E.M. Eisenstein, N.D. Volkow, and C.S. Springer, "Magnetic Resonance Imaging (MRI) Detection of the Murine Brain Response to Light: Temporal Differentiation and Negative Functional MRI Changes," *Proc. Nat. Acad. Sci. USA*, **93**, 6037-6042 (1996).

5. *In Vivo* ^{23}Na MR and Hyperpolarized ^{129}Xe MRI

Though the ^{23}Na MR signal is biological tissue's second strongest, the ^{23}Na nucleus is quadrupolar, with a spin quantum number of 3/2 – indicating four levels and three transitions. This means its spin dynamics, especially in the complicated microenvironments *in vivo*, can be quite complex: two different signals do not necessarily imply two different molecular environments. Our group systematized the molecular structural and dynamic requirements for the four different conditions the ^{23}Na resonance can enjoy, and how the frequencies and relaxation rate constants for the single, double, and triple quantum coherences are manifest in these.[a-c] We showed in cell suspensions the intra- and extracellular ^{23}Na signals can be discriminated without need of a shift reagent [Sec. 6, below], and possibly even the rate constant for homeostatic trans-cytolemmal Na^+ exchange be determined.[d] In the same time period, we demonstrated the first hyperpolarized ^{129}Xe MR images of biological tissue.[e] Single quantum, multi-quantum filtered, and multiexponential *in vivo* ^{23}Na MRI is now a very active field of research, especially at ultrahigh field, as is hyperpolarized ^{3}He, ^{13}C, and ^{129}Xe MRI and MRSI.

a. W.D. Rooney, T.M. Barbara, and C.S. Springer, "Two Dimensional Double Quantum NMR Spectroscopy of Isolated Spin 3/2 Systems: ^{23}Na Examples," *J. Am. Chem. Soc.*, **110**: 674-681 (1988).
b. W.D. Rooney and C.S. Springer, "A Comprehensive Approach to the Analysis and Interpretation of the Resonances of Spins 3/2 from Living Systems," *NMR Biomed.,* **4**, 209-226 (1991).
c. W.D. Rooney and C.S. Springer, "The Molecular Environment of Intracellular Sodium: ^{23}Na NMR Relaxation," *NMR Biomed.*, **4**, 227-245 (1991).
d. Y. Zhang, M. Poirer-Quinot, C.S. Springer, and J.A. Balschi, "Discrimination of Intra- and Extracellular $^{23}Na^{+}$ Signals in Yeast Cell Suspensions Using Longitudinal Magnetic Resonance Relaxography," *J. Magn. Reson.* **205**, 28-37 (2010). [DOI:10.1016/j.jmr.2010.03.018]
e. M.S. Albert, G.D. Cates, B. Driehuys, W. Happer, B. Saam, C.S. Springer, and A. Wishnia, "Biological Magnetic Resonance Imaging Using Laser-Polarized ^{129}Xe," *Nature*, **370**, 199-201 (1994).

6. Frequency Shift Reagents for Biological Cation MR

Inorganic cations (Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}) are crucial in biology. Among their most important biochemical aspects are their trans-membrane concentration gradients, which store free energy from ATP hydrolysis. However, their NMR resonances do not have compartmental information encoded. The ^{23}Na signal is the second strongest from tissue, but changes in its *in vivo* intensity are confounded by compartmental volume changes (edema), ion gradient changes, or both. Our group introduced the use of paramagnetic frequency shift reagents for cation MR, and pioneered their utility for spectroscopic visualization of gradients across vesicle[a] and cell[b] membranes, in perfused tissue,[c] and *in vivo*.[d] This became a significant area for biophysical research in perfused tissues and in animal models.

a. M. M. Pike, S.R. Simon, J.A. Balschi, and C.S. Springer, "High-Resolution Nuclear Magnetic Resonance Studies of Transmembrane Cation Transport: Use of an Aqueous Shift Reagent for ^{23}Na," *Proc. Natl. Acad. Sci., USA*, **79**: 810-814 (1982).
b. M.M. Pike, E.T. Fossel, T.W. Smith, and C.S. Springer, "High-Resolution ^{23}Na NMR Studies of Human Erythrocytes: Use of Aqueous Shift Reagents," *Amer. J. Physiol.,* **246**, C528-C536 (1984).
c. M.M. Pike, J.C. Frazer, D.F. Dedrick, J.S. Ingwall, P.D. Allen, C.S. Springer, and T.W. Smith, "^{23}Na and ^{39}K NMR Studies of Perfused Rat Hearts: Discrimination of Intra- and Extracellular Ions Using a Shift Reagent," *Biophys. J.,* **48**: 159-173 (1985).
d. J.A. Balschi, J.A. Bittl, C.S. Springer, and J.S. Ingwall, "^{31}P and ^{23}Na NMR of Normal and Ischemic Rat Skeletal Muscle: Use of a Shift Reagent *In Vivo*," *NMR Biomed.*, **3**: 47-58 (1990).