

Extended Bloch-McConnell equations for mechanistic analysis of hyperpolarized ^{13}C magnetic resonance experiments on enzyme systems

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This article is dedicated to Geoffrey Bodenhausen on the occasion of his 70th Birthday.

Abstract. We describe an approach to formulating the kinetic master equations of the time evolution of NMR signals in reacting (bio)chemical systems. Special focus is given to studies that employ signal enhancement (hyperpolarization) methods such as dissolution dynamic nuclear polarization (dDNP) and involving nuclear spin-bearing solutes that undergo reactions mediated by enzymes and membrane transport proteins. We extend the work given in a recent presentation on this topic (Kuchel and Shishmarev, 2020) to now include enzymes with two or more substrates and various enzyme reaction mechanisms as classified by Cleland, with particular reference to non-first order processes. Using this approach, we can address some pressing questions in the field from a theoretical standpoint. For example, why does binding of a hyperpolarized substrate to an enzyme *not* cause an appreciable loss of the signal from the substrate or product? Why does the concentration of an unlabelled pool of substrate, for example ^{12}C lactate, cause an increase in the rate of exchange of the ^{13}C labelled pool? To what extent is the equilibrium position of the reaction perturbed during administration of the substrate? The formalism gives a full mechanistic understanding of the time courses derived and is of relevance to ongoing clinical trials using these techniques.

1 Introduction

Nuclear magnetic resonance (NMR) spectroscopy and imaging (MRI) are widely employed techniques with far-reaching applications in physics, chemistry, medicine and the life sciences. NMR and MRI provide a wealth of information from structure elucidation, protein dynamics and metabolic profiling through to disease diagnostics in oncology, cardiology and neurology among others. The technique's low sensitivity is one of the primary concerns in the magnetic resonance community and is often a limiting factor in experiments from solid-state NMR to medical imaging. Recent work has shown that the sensitivity of NMR experiments can be improved by using non-equilibrium hyperpolarization techniques such as dissolution dynamic nuclear polarization (dDNP) to boost signal intensities by many orders of magnitude (Ardenkjaer-Larsen et al., 2003). Such techniques have led to new applications (Golman et al., 2003; Golman et al., 2006; Keshari and Wilson, 2014) and necessitated the development of acquisition strategies to exploit the hyperpolarized magnetization in a time efficient manner (Yen

38 et al., 2009); as well as new tools for signal processing and image reconstruction (Hu et al., 2010). A challenge
39 with the interpretation of these recordings is that, unlike radiotracers, hyperpolarized MR is a non-tracer technique
40 requiring the injection of physiological or even supra-physiological concentrations of substrate.

41 To date there have been many mathematical methods devised for analyzing the kinetic time courses in
42 dDNP NMR studies (Zierhut et al., 2010; Hill et al., 2013b; Pagès and Kuchel, 2015; Daniels et al., 2016).
43 However, until recently there has been little consensus on the best methods for analyzing and then interpreting
44 reaction kinetics measured therein. A theoretical framework has only recently appeared to fully elucidate the
45 underlying mechanisms (Kuchel and Shishmarev, 2020). One challenge is that the widely used Bloch-McConnell
46 equations describe the exchange of magnetization of only the MR active nuclei while the reaction kinetics are
47 subject to a plethora of molecular interactions in a (bio)chemical milieu. Furthermore, in a typical hyperpolarized
48 MR experiment the initial injection of a non-tracer concentration of substrate causes the reaction system to be
49 perturbed from its equilibrium state, or quasi-steady state, and therefore the concentrations of the reactants are
50 time dependent. In this regard, challenges relate to the description of non-linear kinetics, for example second order
51 reactions, and the involvement of un-observable (non-labelled) metabolites to the overall kinetics, *e.g.*, enzyme
52 cofactors, co-substrates and natural abundance ^{12}C -containing metabolites (Hill et al., 2013a); as well as explicit
53 descriptions of enzyme mechanisms *e.g.*, sequential ordered, sequential random, double displacement (ping-pong)
54 reactions, and allosteric interactions that occur on an enzyme far from its active site. Enzyme activity is also
55 influenced by inhibitors that can be competitive, non-competitive, or uncompetitive (Cleland, 1967; Cook and
56 Cleland, 2007). Mathematical models of enzyme systems should agree with standard descriptions of (bio)chemical
57 kinetics while remaining capable of describing the time evolution of magnetization that is described by the Bloch-
58 McConnell equations (McConnell, 1958).

59 Here we address these issues in a stepwise manner, by developing a mechanistic approach that combines
60 the MR interactions with the chemical and/or enzyme mediated reactions described by the Bloch-McConnell
61 equations. These equations are grounded in the concept of conservation of mass of the species responsible for the
62 hyperpolarized signal plus its non-hyperpolarized counterpart and the various products; this was recently
63 highlighted (Kuchel and Shishmarev, 2020) where the MR visible signal decays to produce an MR invisible one.
64

65 **1.1 Basic concepts – sensitivity**

66 We begin addressing the problem by defining the signal-to-noise ratio (SNR) in MR. In its most basic form,
67 sensitivity is described by the ratio of the signal amplitude divided by the root mean square of the amplitude of
68 the noise. When a signal $S(t)$ is detected in the NMR receiver coil that surrounds the sample, the magnitude of
69 the induced current is a function of: (i) the perturbation of nuclear spin populations from thermal equilibrium
70 $S_{sample}(t)$; plus (ii) a random contribution from the noise in the electronic circuitry $S_{electronics}(t)$. Hence:

71

$$S(t) = S_{sample}(t) + S_{electronics}(t) \quad . \quad (1)$$

72

73 The current induced in the coil is time-dependent and proportional to the magnetization that precesses in the x,y -
74 plane. In other words, the signal $S(t)$ is recorded until decoherence renders $S_{sample}(t)$ undetectable against the

75 noise, $S_{electronics}(t)$. The latter is mainly attributed to the radiofrequency (RF) circuitry in the probe head and the
 76 preamplifier(s) (*e.g.*, Johnson noise (Johnson, 1928)) of the spectrometer. If the NMR signal (free induction decay;
 77 FID) that is detected in a subsequent experiment is indistinguishable from the first, and the two are added together,
 78 then the signal amplitude (peak area) will scale linearly with the number of added FIDs, N . The noise associated
 79 with each experiment is random, and assuming its source remains fixed over time, *i.e.*, stationary noise, then the
 80 amplitude scales with the square root of the number of FIDs, $N^{1/2}$. Hence signal summation enhances the SNR
 81 of an NMR experiment in proportion to the square root of the number of FIDs. In other words, to achieve an
 82 enhancement by a factor ξ requires an increase in experiment duration of ξ^2 . Therefore, unavoidably, FID
 83 summation is a slow process and experiments can sometimes take days or weeks to achieve a sufficient SNR from
 84 a sample of a low sensitivity nuclide or one with a long relaxation time. The amount of attainable signal averaging
 85 is constrained when monitoring dynamic processes by NMR spectroscopy; and an inherently good SNR is
 86 required from the outset for a time course experiment.

87

88 1.2 Thermal effects

89 The usual way to proceed when calculating the NMR response of a spin system to RF pulse sequences is to solve
 90 the ordinary quantum mechanical master equation that describes the evolution of the spin density operator (Hore
 91 et al., 2015). This is the Liouville-von Neumann equation, that has been extended to include non-coherent
 92 interactions (predominantly relaxation phenomena) (Ernst et al., 1987):

93

$$\frac{d}{dt}\rho = -i\hat{H}\rho - \hat{T}(\rho - \rho_0) \quad , \quad (2)$$

94

95 where \hat{H} is the commutation superoperator of the coherent Hamiltonian H given by $\hat{H}\rho = [H, \rho]$, which contains
 96 information on all spin-spin and field-spin interactions; while \hat{T} is the relaxation superoperator that describes all
 97 longitudinal (T_1) and transverse (T_2) relaxation processes, as well as any cross-relaxation or cross-correlation
 98 interactions. Note, that in the interests of reducing clutter in equations (for which the operator context should be
 99 clear) hereafter we have omitted carets denoting operators and only used them to denote superoperators.

100 Our aim here is to describe the kinetics of exchange between different solutes that contain hyperpolarized
 101 nuclei *e.g.*, $A \leftrightarrow B$, in which the relaxation times are constant. In this quest, the first simplifying assumption that
 102 is worth exploring is that all intermolecular interactions, notably, scalar coupling, dipolar coupling, cross-
 103 relaxation and cross-correlation between species A and B can be ignored. This applies to non-interacting solute
 104 molecules in solution in which motional averaging occurs; and we focus on thermal effects on the evolution of
 105 the FID.

106 The so-called Zeeman polarization term describes the sensitivity of $S_{sample}(t)$ in Eq. (1) to temperature
 107 and magnetic field in an NMR experiment. Magnetic polarization is described by the equilibrium density operator
 108 ρ_0 that specifies the probability distribution of states. Zeeman polarization corresponds to the magnitude of
 109 normalized longitudinal spin order I_z that is contained in ρ_0 . Specifically, for an ensemble of spin- $1/2$ nuclei this
 110 is given by (Ernst et al., 1987):

111

$$\rho_0 = \frac{\exp(-\hbar H_0/kT)}{\text{Tr}\{\exp(-\hbar H_0/kT)\}} \quad (3)$$

112 where k is the Boltzmann constant and T is the temperature (Kelvin). The Zeeman Hamiltonian H_0 describes the
 113 interaction of the spins with the static magnetic field of magnitude B_0 , given by $H_0 = \omega_0 I_z$, where ω_0 is the
 114 Larmor frequency (rad s⁻¹). In the basis of the two eigenstates $|\alpha\rangle$ (“spin-up”) and $|\beta\rangle$ (“spin-down”), the
 115 equilibrium density operator is written in matrix form as:
 116

$$\rho_0 = \frac{1}{Z} \begin{bmatrix} \exp(\hbar\omega_0/2kT) & 0 \\ 0 & \exp(-\hbar\omega_0/2kT) \end{bmatrix} \quad (4)$$

117
 118 where Z is the partition function, given by $Z = \sum_{i=1}^M \exp(-\varepsilon_i/kT)$, and M is the number of states ($M = 2$ for an I
 119 = $\frac{1}{2}$ nucleus). In the case of a spin- $\frac{1}{2}$ system, the partition function is the sum of the populations $Z =$
 120 $\exp(\hbar\omega_0/2kT) + \exp(-\hbar\omega_0/2kT) \approx 2$ when ε_i is very small, as is typically the case at thermal equilibrium
 121 in NMR systems. The Zeeman polarization is proportional to the projection of the spin density operator onto the
 122 angular momentum operator. In other words, it is proportional to the expectation value of $\langle I_z \rangle$, and is given by
 123 (Keeler, 2010):
 124

$$\langle I_z \rangle = \text{Tr}[\rho_0 I_z] = \frac{1}{2Z} [\exp(\hbar\omega_0/2kT) - \exp(-\hbar\omega_0/2kT)] \quad (5)$$

125
 126 Hence, the Zeeman polarization for an ensemble of nuclear spins is the normalized *imbalance* between the
 127 populations of the $|\alpha\rangle$ and $|\beta\rangle$ states, p_α and p_β , respectively; in other words, it is the normalized net population
 128 difference that is given by:
 129

$$P = \frac{p_\alpha - p_\beta}{p_\alpha + p_\beta} \quad (6)$$

130
 131 This normalization is carried out with respect to the total population of the nuclear ensemble such that $p_\alpha + p_\beta =$
 132 1. Therefore, the bounds on the polarization are $-1 < P < +1$. At room temperature (~ 298 K), and in a field of
 133 11.75 T (500 MHz for ^1H nuclei), the thermal equilibrium Zeeman polarization, $P_{z,eq}$, is a mere $\sim 4 \times 10^{-5}$. Thus,
 134 there is only a tiny population difference between the spin states of a nuclear ensemble that implies inherently
 135 weak polarization. It is this small population imbalance which is manipulated in NMR experiments under thermal
 136 equilibrium conditions. This weak polarization is a consequence of the small difference in energy (~ 0.1 J mol⁻¹)
 137 between nuclear spin energy levels at room temperature (~ 2.5 kJ mol⁻¹); and it implies only weak alignment of
 138 nuclear spins in the static magnetic field of all contemporary superconducting magnets.
 139

140 In the usual quantum mechanical analysis of multiple spin systems, the density operator (that describes
 141 the probability density of states) is normalized to 1, meaning that the summed (total) probability density of all
 142 states is 1. This is expressed mathematically as $\text{Tr}[\rho] = 1$, where Tr denotes the trace of the matrix (Hore et al.,
 143 2015). To describe non-equilibrium reactions in terms of solute concentrations requires a scaled density operator
 144 (Kuhne et al., 1979):

145

$$\sigma_i = [A_i]\rho_i \quad , \quad (7)$$

146

147 where σ_i is now proportional to $[A_i]$. Differentiation of Eq. (7) leads to:

148

$$\frac{d\sigma_i}{dt} = [A_i]\frac{d\rho_i}{dt} + \frac{d[A_i]}{dt}\rho_i \quad . \quad (8)$$

149

150 Therefore, it follows that for a system at chemical equilibrium $d[A_i]/dt = 0$, so the scaled density operator is
 151 directly proportional to the normalised density operator. For non-equilibrium systems the concentrations are time
 152 dependent *viz.*, $d[A_i]/dt \neq 0$ so the two no longer scale in a straightforward manner.

153 On the other hand, equilibrium magnetization ($M_{z,eq}$) is a bulk property that is the net magnetic dipole
 154 moment per unit volume; and is proportional to $\langle I_z \rangle$ where the proportionality factor is $N\hbar\gamma$. From Eq. (5) this
 155 yields the expression for the magnetization in terms of magnetic field strength, temperature and number of spins
 156 in the sample (or more specifically in the detection volume of the NMR spectrometer):

157

$$M_{z,eq} = \frac{N\hbar\gamma}{2} \tanh\left(\frac{\hbar\gamma B_0}{2kT}\right) \quad . \quad (9)$$

158

159 In the so-called ‘high temperature limit’ (room temperature, in the cases addressed here) Eq. (9) simplifies to:

160

$$M_{z,eq} = \frac{N\hbar^2\gamma^2 B_0}{4kT} \quad . \quad (10)$$

161

162 In words, ‘thermal magnetization’ is proportional to the magnitude of the external magnetic field strength, B_0 ,
 163 and is inversely proportional to the temperature, T , while being proportional to the number of spins, N . Therefore,
 164 it is *proportional* to the concentration $[A_i]$ of the solute that bears the NMR-active nucleus.

165

166 2 Equation of motion – the Bloch equations

167 In the absence of intermolecular binding (however transient), or scalar couplings, the motion (time evolution) of
 168 magnetizations is described by the Bloch equations. Magnetization is explicitly declared to be proportional to
 169 reactant concentrations $[A]$ and $[B]$, as has recently been discussed (Kuchel and Shishmarev, 2020). To explore
 170 this situation, we start with the basic Bloch equations for a single spin- $1/2$ ensemble. The equation describes the
 171 time evolution of x , y and z magnetization in the rotating frame, and includes the influence of chemical shift, RF
 172 fields, and transverse (T_2) and longitudinal relaxation (T_1) time constants. The Bloch equations in their complete
 173 form are described as being inhomogeneous, and they can be written using a matrix and vectors:

174

$$\frac{d}{dt} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = - \begin{bmatrix} R_2 & \Omega & -\omega_y \\ -\Omega & R_2 & \omega_x \\ \omega_y & -\omega_x & R_1 \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ R_1 M_{z,eq} \end{bmatrix} \quad , \quad (11)$$

175

176 where $\Omega = \omega_0 - \omega_{RF}$ is the ‘offset frequency’ in the rotating frame; ω_0 (rad s⁻¹) is the Larmor frequency; ω_{RF}
 177 (rad s⁻¹) is the RF frequency; the x component of the RF field (rad s⁻¹) is $\omega_x = -\gamma B_1 \cos(\omega_{RF}t + \varphi)$; and the y
 178 component is $\omega_y = -\gamma B_1 \sin(\omega_{RF}t + \varphi)$, where the magnitude of the field strength is B_1 , and the phase of the
 179 wave form relative to an internal reference source is φ . The longitudinal relaxation rate constant is denoted by
 180 $R_1 = 1/T_1$; the transverse one by $R_2 = 1/T_2$; and the equilibrium magnetization by $M_{z,eq}$.

181 Equation (11) is tedious to solve analytically, but it is readily solved numerically (Allard et al., 1998;
 182 Helgstrand et al., 2000). On the other hand, by including the identity operator in the basis set and adding a constant
 183 to the equilibrium magnetization (Levitt and Dibari, 1992), we obtain a much more compliant (to analysis) matrix
 184 equation:

185

$$\frac{d}{dt} \begin{bmatrix} \frac{E}{2} \\ M_x \\ M_y \\ M_z \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & R_2 & \Omega & -\omega_y \\ 0 & -\Omega & R_2 & \omega_x \\ -2\theta & \omega_y & -\omega_x & R_1 \end{bmatrix} \begin{bmatrix} \frac{E}{2} \\ M_x \\ M_y \\ M_z \end{bmatrix}, \quad (12)$$

186

187 where E is equal to 1 and the factor $\theta = R_1 M_{z,eq}$ describes the equilibrium magnetization.

188

189 2.1 Chemical exchange kinetics of systems prior to and at equilibrium – the Bloch-McConnell equations

190 We can extend the system of equations from describing an ensemble of single spins to two or more exchanging
 191 spins. The system of equations now accounts for the magnetization interaction with the lattice and exchange via
 192 the forward and reverse chemical reactions. These are the Bloch-McConnell equations (McConnell, 1958).

193 First, consider the rate expressions for a simple bi-directional chemical reaction. The coupled differential
 194 equations describing first-order reaction kinetics of solute A becoming solute B and back again, $A \leftrightarrow B$, are
 195 typically expressed in terms of molar concentrations:

196

$$\frac{d[A(t)]}{dt} = -k_1[A(t)] + k_{-1}[B(t)] \quad , \quad (13)$$

$$\frac{d[B(t)]}{dt} = k_1[A(t)] - k_{-1}[B(t)] \quad , \quad (14)$$

197

198 that can be expressed in matrix form:

199

$$\frac{d}{dt} \begin{bmatrix} [A(t)] \\ [B(t)] \end{bmatrix} = \begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix} \begin{bmatrix} [A(t)] \\ [B(t)] \end{bmatrix} \quad . \quad (15)$$

200

201 The rate constant for the forward reaction is denoted by k_1 while for the reverse reaction it is k_{-1} . The time
 202 dependent concentrations are given by $[A(t)]$ and $[B(t)]$. As required by the fact that this is a closed system, the
 203 equations must conform to the *principle of conservation of mass*. Specifically, the sum of the rates of change of

204 $[A(t)]$ and $[B(t)]$ given by $d[A(t)]/dt + d[B(t)]/dt$, is zero. We return to this point below. In other words,
 205 mass is neither created nor destroyed during the reaction in such a closed system.

206 For the simplest case of two magnetically active solutes, each possessing a single spin- $\frac{1}{2}$ nuclide, in
 207 chemical exchange, $A \leftrightarrow B$, the direct product (a mathematical operation used in quantum mechanics to generate
 208 the necessary combinations of states) of the chemical (solute) space $\{[A], [B]\}$ and the magnetization vector space
 209 $\{M_x, M_y, M_z\}$ for each of A and B is given by:

$$\begin{bmatrix} 1 \\ 1 \end{bmatrix} \otimes \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} M_x^A \\ M_y^A \\ M_z^A \\ M_x^B \\ M_y^B \\ M_z^B \end{bmatrix}. \quad (16)$$

210

211 A new exchange matrix in the basis of the new magnetization space $\{M_x^A, M_y^A, M_z^A, M_x^B, M_y^B, M_z^B\}$ is calculated by
 212 taking the direct product of the exchange matrix with the identity operator I that is chosen to have the same
 213 dimensions as the magnetization space. The direct product is given by:

214

$$\begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix} \otimes \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} = \begin{bmatrix} -k_1 & 0 & 0 & k_{-1} & 0 & 0 \\ 0 & -k_1 & 0 & 0 & k_{-1} & 0 \\ 0 & 0 & -k_1 & 0 & 0 & k_{-1} \\ k_1 & 0 & 0 & -k_{-1} & 0 & 0 \\ 0 & k_1 & 0 & 0 & -k_{-1} & 0 \\ 0 & 0 & k_1 & 0 & 0 & -k_{-1} \end{bmatrix}. \quad (17)$$

215

216 Likewise, the matrix describing coherent and incoherent magnetization interactions can be recast in a similar
 217 fashion to give:

$$\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \otimes \begin{bmatrix} R_2 & \Omega & -\omega_y \\ -\Omega & R_2 & \omega_x \\ \omega_y & -\omega_x & R_1 \end{bmatrix} = \begin{bmatrix} R_2^A & \Omega^A & -\omega_y & 0 & 0 & 0 \\ -\Omega^A & R_2^A & \omega_x & 0 & 0 & 0 \\ \omega_y & -\omega_x & R_1^A & 0 & 0 & 0 \\ 0 & 0 & 0 & R_2^B & \Omega^B & -\omega_y \\ 0 & 0 & 0 & -\Omega^B & R_2^B & \omega_x \\ 0 & 0 & 0 & \omega_y & -\omega_x & R_1^B \end{bmatrix}. \quad (18)$$

218

219 The inhomogeneous form of the Bloch equations can now be constructed to take into account both the coherent
 220 and incoherent interactions, *as well as* chemical exchange. This yields the inhomogeneous form of the Bloch-
 221 McConnell equations, which are written (again in matrix form) as:

222

$$\frac{d}{dt} \begin{bmatrix} M_x^A \\ M_y^A \\ M_z^A \\ M_x^B \\ M_y^B \\ M_z^B \end{bmatrix} = - \begin{bmatrix} R_2^A + k_1 & \Omega^A & -\omega_y & -k_{-1} & 0 & 0 \\ -\Omega^A & R_2^A + k_1 & \omega_x & 0 & -k_{-1} & 0 \\ \omega_y & -\omega_x & R_1^A + k_1 & 0 & 0 & -k_{-1} \\ -k_1 & 0 & 0 & R_2^B + k_{-1} & \Omega^B & -\omega_y \\ 0 & -k_1 & 0 & -\Omega^B & R_2^B + k_{-1} & \omega_x \\ 0 & 0 & -k_1 & \omega_y & -\omega_x & R_1^B + k_{-1} \end{bmatrix} \begin{bmatrix} M_x^A \\ M_y^A \\ M_z^A \\ M_x^B \\ M_y^B \\ M_z^B \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ R_1^A M_{z,eq}^A \\ 0 \\ 0 \\ R_1^B M_{z,eq}^B \end{bmatrix}, \quad (19)$$

224

225 where $M_{z,eq}^A$ and $M_{z,eq}^B$ denote the respective equilibrium magnetizations (hence the subscript *eq*).

226 The inhomogeneous form of the Bloch-McConnell equations can similarly be modified by incorporating
 227 the equilibrium magnetization to create a homogeneous form of this master equation:

228

$$\frac{d}{dt} \begin{bmatrix} \frac{E}{2} \\ M_x^A \\ M_y^A \\ M_z^A \\ M_x^B \\ M_y^B \\ M_z^B \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & R_2^A + k_1 & \Omega^A & -\omega_y & -k_{-1} & 0 & 0 \\ 0 & -\Omega^A & R_2^A + k_1 & \omega_x & 0 & -k_{-1} & 0 \\ -2\theta^A & \omega_y & -\omega_x & R_1^A + k_1 & 0 & 0 & -k_{-1} \\ 0 & -k_1 & 0 & 0 & R_2^B + k_{-1} & \Omega^B & -\omega_y \\ 0 & 0 & -k_1 & 0 & -\Omega^B & R_2^B + k_{-1} & \omega_x \\ -2\theta^B & 0 & 0 & -k_1 & \omega_y & -\omega_x & R_1^B + k_{-1} \end{bmatrix} \begin{bmatrix} \frac{E}{2} \\ M_x^A \\ M_y^A \\ M_z^A \\ M_x^B \\ M_y^B \\ M_z^B \end{bmatrix}. \quad (20)$$

229

230 Again, the factors $\theta^A = R_1^A M_{z,eq}^A$ and $\theta^B = R_1^B M_{z,eq}^B$ account for the respective equilibrium magnetizations.

231

232 2.1.1 Simulations of thermal kinetics using Eq. (19)

233 Next, consider Eq. (19) for simulating the evolution of the *x*, *y*, and *z* components of the magnetization of a
 234 ‘thermal magnetization’ (*non-hyperpolarized*) sample. We seek the NMR spectrum that results from a two-site
 235 exchange reaction between solutes A and B, Fig. 1(a), as conventionally observed in room temperature NMR
 236 experiments.

237 Simulations were performed in *MatLab* with equilibrium *z* magnetizations $M_{z,eq}^A = 1.0$ and $M_{z,eq}^B = 0.8$
 238 and an initial magnetization vector given by $\mathbf{M}_0 = [0.0, 1.0, 0, 0, 0.8]$. Chemical shifts offsets were $\Omega^A = 10 \times$
 239 $2 \pi \text{ rad s}^{-1}$ and $\Omega^B = 10 \times 2 \pi \text{ rad s}^{-1}$. Relaxation rate constants were $R_1^A = R_1^B = 1 \text{ s}^{-1}$ and $R_2^A = R_2^B = 1 \text{ s}^{-1}$.
 240 The influence of an RF_y pulse was then calculated with $\omega_x = -\gamma B_1 \cos(\pi/2)$ and $\omega_y = -\gamma B_1 \sin(\pi/2)$ and with
 241 a field strength of 1.5 kHz, corresponding to $\omega_y = -\gamma B_1 = -1500 \times 2\pi \text{ rad s}^{-1}$ and $\omega_x = 0$. For a 90° RF
 242 nutation (flip) angle the pulse duration is $t_p = \pi/2\omega_y$, which gave a transformed magnetization vector after the
 243 pulse of $M(t) = [0.999, 0.007, 0.000, 0.800, -0.005, 0.000]$; this was composed mostly of $M_x^A + M_x^B$ with a
 244 residual contribution from $M_y^A + M_y^B$ arising from evolution of the chemical shift during the RF pulse; and a small
 245 contribution from $M_z^A + M_z^B$ due to return of the magnetization to the equilibrium state.

246 The observable signal (the FID, which is a function of time) is proportional to the complex signal $S(t) =$
 247 $M_x^A(t) - iM_y^A(t) + M_x^B(t) - iM_y^B(t)$. Noise was simulated by adding to the FID a normally distributed complex
 248 random vector with mean = 0 and standard deviation (SD) = 0.1. The spectrum $s(\omega)$ was then calculated by taking
 249 the Fourier transform of $S(t)$. Simulated FIDs $S(t)$ are shown in Figs. 1(b-e) left panel, the corresponding spectra
 250 $s(\omega)$ in Figs. 1(b-e) middle panel, and the recovery of the *z* magnetizations $M_z^A(t)$ and $M_z^B(t)$ are shown in Figs.
 251 1(b-e), right panel. Spectra were simulated for a range of rate constants, where exchange was either absent $k_1 =$
 252 $k_{-1} = 0$, Fig. 1(b); or for increasing rates of exchange. Thus, (c) $k_1 = 2 \text{ s}^{-1}$, $k_{-1} = 1 \text{ s}^{-1}$; (d) $k_1 = 20 \text{ s}^{-1}$,
 253 $k_{-1} = 10 \text{ s}^{-1}$; and (e) $k_1 = 2000 \text{ s}^{-1}$, $k_{-1} = 1000 \text{ s}^{-1}$, corresponding to the slow, intermediate and fast
 254 regimes, respectively.

255 The equilibrium constant was fixed so that $K = k_1/k_{-1} = 2$; hence the system was not at chemical
 256 equilibrium at $t = 0$ s. The simulations highlight an important point: In the absence of exchange the Bloch-
 257 McConnell equations predict the recovery of the z magnetizations back to their magnetic equilibrium values $M_{z,eq}^A$
 258 and $M_{z,eq}^B$ while under conditions of fast exchange this no longer takes place during the experiment. A non-
 259 equilibrium system will rapidly recover to its chemical equilibrium but not to its initial thermal equilibrium $M_{z,eq}^A$
 260 and $M_{z,eq}^B$; again in other words, this does not take place within the timescale of the NMR experiment; which is
 261 typically within five T_1 values.
 262

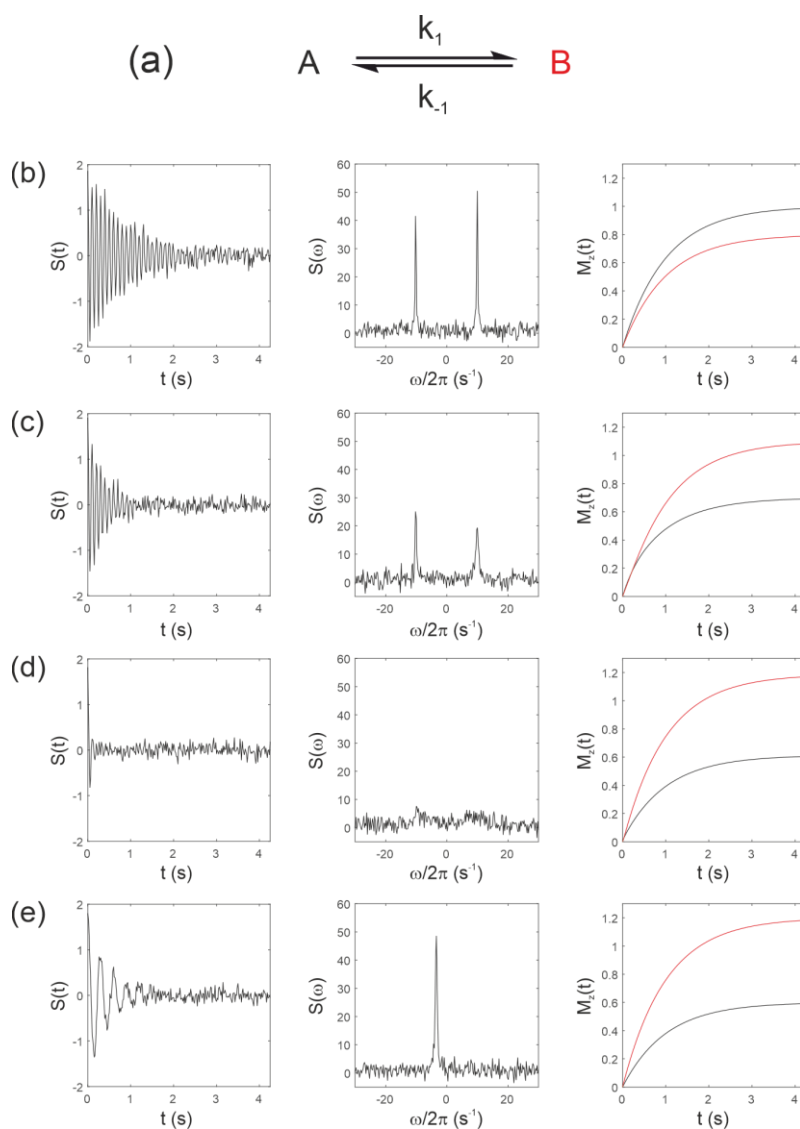


Figure 1 Simulated NMR spectra resulting from a two-site exchange process between *thermally polarized* solutes, $A \leftrightarrow B$, shown schematically in (a). Simulated FIDs $S(t)$ are shown in (b-e) left panel, with corresponding spectra $s(\omega)$, middle panel, and the recovery of z magnetizations, $M_z^A(t)$ and $M_z^B(t)$, right panel. Spectra were simulated with rate constants, (b) $k_1 = k_{-1} = 0$; (c) $k_1 = 2 \text{ s}^{-1}$, $k_{-1} = 1 \text{ s}^{-1}$; (d) $k_1 = 20 \text{ s}^{-1}$, $k_{-1} = 10 \text{ s}^{-1}$; and (e) $k_1 = 2000 \text{ s}^{-1}$, $k_{-1} = 1000 \text{ s}^{-1}$, corresponding to no exchange, slow, intermediate, and fast exchange regimes, respectively.

263 2.2 Describing hyperpolarized kinetics with the Bloch-McConnell equations

264 We now consider the predictions made by using Eq. (19) when simulating the evolution of the x , y , and z
265 components of the magnetization of a hyperpolarized sample and the resulting spectrum for a two-site exchange
266 reaction between solutes A and B. In the previous example the initial condition was $M_z^A(0) = 1.0$ and $M_z^B(0) =$
267 0.8 . To extend the Bloch-McConnell formalism to be able to predict the dynamics of a hyperpolarized experiment
268 we recognize that for the same magnitude of noise in the receiver circuit (although this may not be true for a
269 hyperpolarized sample) the initial hyperpolarized magnetization is given by:

270

$$M_{z,hy p} = \eta M_{z,eq} \quad , \quad (21)$$

271

272 where η is the enhancement factor that varies from one hyperpolarization experiment to another. In the case of
273 dDNP experiments $\eta \cong 10^4$ is typical, although this depends on the method of hyperpolarization, the solute(s) in
274 question and a set of physicochemical parameters that are described in detail in e.g., (Ardenkjaer-Larsen et al.,
275 2015).

276

277 2.2.1 Simulations of hyperpolarized kinetics using Eq. (19)

278 These were performed with equilibrium z magnetizations $M_{z,eq}^A = 1.0$ and $M_{z,eq}^B = 0.8$, as used above, but now
279 with an initial magnetization vector $\mathbf{M}(0) = [0, 0, 1.0 \times 10^4, 0, 0, 0]$. This situation corresponds to an initial
280 hyperpolarized magnetization $M_{z,hy p}^A(0)$ of only solute A and an enhancement factor of $\eta = 10^4$. Chemical shifts
281 were $\Omega^A = 10 \times 2\pi \text{ rad s}^{-1}$ and $\Omega^B = -10 \times 2\pi \text{ rad s}^{-1}$, while relaxation times were increased to represent a
282 hyperpolarized ^{13}C substrate, $R_{1A} = R_{1B} = 1/60 \text{ s}^{-1}$ and $R_{2A} = R_{2B} = 1 \text{ s}^{-1}$ with the rate constants representing
283 an enzyme mediated cell reaction $k_1 = k_{-1} = 0.005 \text{ s}^{-1}$. Figure 2(a) shows the time evolution of the z -
284 components of the magnetization, displaying the familiar (Day et al., 2007) bi-exponential time dependence of
285 $M_{z,hy p}^A(t)$ and $M_{z,hy p}^B(t)$ magnetizations.

286

287 We next simulate the effect of applying the pulse sequence shown in Fig. 2(b) corresponding to a time
288 course type of experiment with multiple sampling of the magnetization and acquisition of an FID at each time-
289 point. This is representative of real experiments that have been presented in the literature (Gabellieri et al., 2008;
Hill et al., 2013b). The time delays correspond to a pre-scan delay τ , the duration of the pulse t_p and the duration

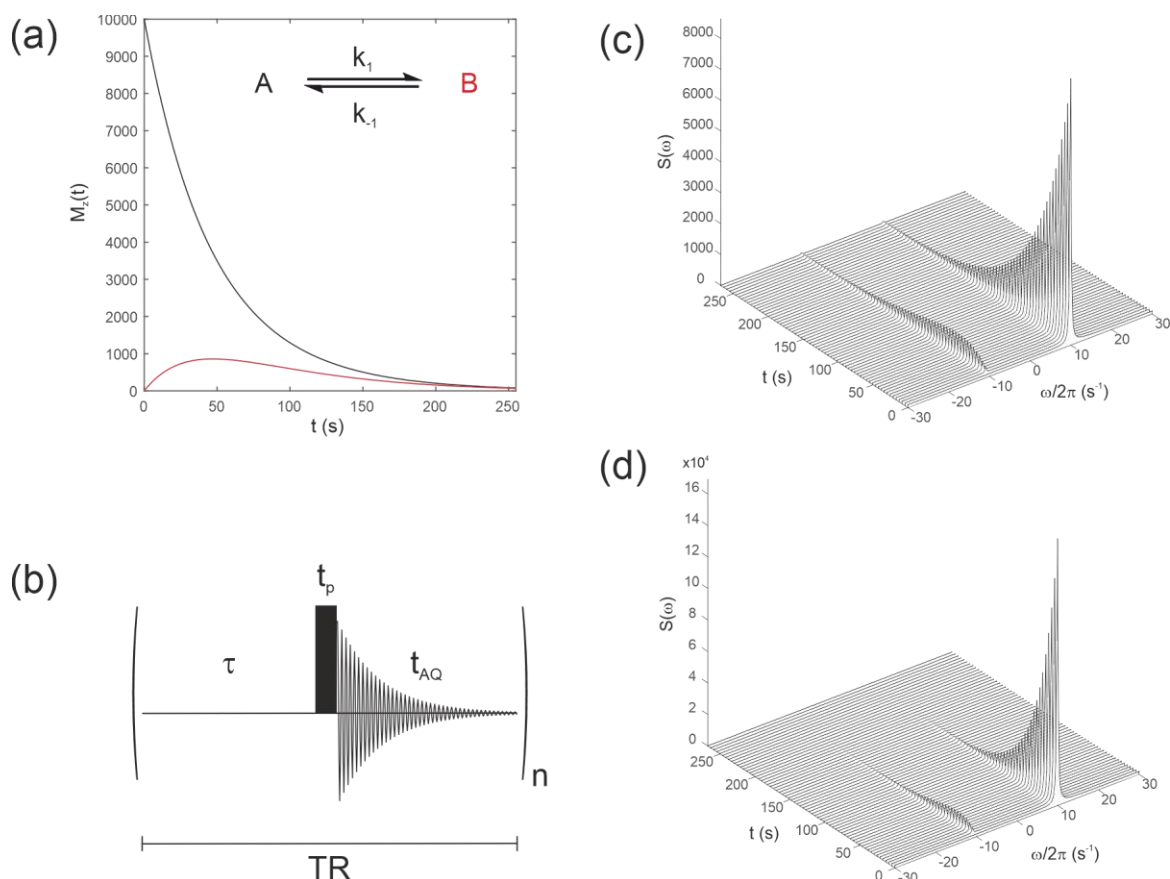


Figure 2 (a) Simulated evolution of the z-components of the magnetization M_z^A and M_z^B for a hyperpolarized solute $M_z^A(0) = 1 \times 10^4$ undergoing a two-site exchange reaction, $A \leftrightarrow B$. Longitudinal relaxation rate constants were $R_{1A} = R_{1B} = 1/60 \text{ s}^{-1}$ and $R_{2A} = R_{2B} = 1 \text{ s}^{-1}$. Rate constants were $k_1 = k_{-1} = 0.005 \text{ s}^{-1}$. (b) Simple pulse sequence for acquiring a time course experiment with multiple sampling of the magnetization and acquisition of an FID at each timepoint. (c-d) Waterfall plots of simulated spectra resulting from sequential application of the pulse sequence in (b) for an initial hyperpolarized solute A undergoing two-site exchange with solute B, calculated with a flip angles: (c) $\beta = 1^\circ$; and (d) $\beta = 20^\circ$.

290 of the FID t_{aq} . The experiment is repeated n times to sample the entire time course where the temporal resolution
 291 is then given by the total repetition time $TR = \tau + t_p + t_{aq}$ and the total duration of the experiment is given by
 292 nTR . In this experiment we make the assumption that the transverse magnetization from one experiment to the
 293 next is not recovered by the application of a subsequent pulse. This assumption is reasonable provided the
 294 acquisition time is much longer than the time taken for the FID to decay to zero, namely, $t_{aq} \gg T_2^*$.

295 The influence of this pulse sequence was then calculated, accounting for multiple sampling of the
 296 magnetization. The RF pulse was again specified by $\omega_x = -\gamma B_1 \cos(\pi/2)$ and $\omega_y = -\gamma B_1 \sin(\pi/2)$ with a field
 297 strength of 1.5 kHz, which corresponds to $\omega_y = -\gamma B_1 = -1500 \times 2\pi \text{ rad s}^{-1}$. Application of an RF pulse tilts
 298 the hyperpolarized magnetization away from the z axis by an angle of β radians. The magnitude of the observable
 299 transverse magnetization is proportional to $\sin(\beta)$, and the remaining longitudinal magnetization is proportional
 300 to $\cos(\beta)$.

301 Simulations were performed with the same magnitude of noise as in Fig. 1. The time evolution of the
 302 magnetization was recorded for the pulse sequence shown in Fig. 2(b) with sequential acquisition of 64 spectra,
 303 and a repetition time of $TR = 4.25$ s. The effect of acquiring a time series of spectra with either a flip angle $\beta =$
 304 1° , Fig. 2(c), or $\beta = 20^\circ$, Fig. 2(d), are seen in the stack plots. The pulse length (duration) was $t_p = \beta \pi / 180 \omega_y$.
 305 After a single $\beta = 1^\circ$ pulse applied to $\mathbf{M}(0)$ the magnetization vector was tilted to become $\mathbf{M}(t) =$
 306 $[0.174, 0.000, 9.998, 0.000, 0.000, 0.000] \times 10^3$ prior to acquisition of the FID. This was composed mostly of
 307 M_z^A with a small contribution from M_x^A that arose from excitation by the $\beta = 1^\circ$ pulse; or following a $\beta = 20^\circ$ pulse
 308 the magnetization vector was tilted to become $\mathbf{M}(t) = [3.420, 0.004, 9.397, 0.000, 0.000, 0.000] \times 10^3$, again
 309 comprised mostly of M_z^A but with a greater contribution from M_x^A due to excitation by a pulse with larger value of
 310 β . Since the magnetization relaxed to its thermal equilibrium state, the hyperpolarized magnetization was
 311 effectively destroyed during application of the RF (sampling) pulse, and it was not re-generated. This may not be
 312 the outcome when non-linear effects such as radiation damping cause recovery of the hyperpolarized signal
 313 (Weber et al., 2019).

314 The z magnetization after the application of a single RF pulse and delay TR is therefore given by:
 315

$$S(TR) = S(0) \cos(\theta) \exp(-R_1 TR) \quad . \quad (22)$$

316
 317 Following the application of a series of n RF pulses with a total delay $n TR = t$ the signal is given by (Kuchel
 318 and Shishmarev, 2020):
 319

$$S(t) = S(0) \cos^n(\theta) \exp(-R_1 t) \quad . \quad (23)$$

320
 321 The apparent relaxation time constant of the hyperpolarized signal, including the influence of both the intrinsic
 322 T_1 and flip angle correction, is given by (Hill et al., 2013b; Kuchel and Shishmarev, 2020):
 323

$$\exp(-R_{1,app} t) = \cos^n(\theta) \exp(-R_1 t) \quad , \quad (24)$$

$$R_{1,app} = R_1 - \frac{1}{TR} \ln \cos(\theta) \quad . \quad (25)$$

324
 325 In the previous examples in Figs. 2(c) and 2(d), with a typical $T_1 = 60$ s (Keshari and Wilson, 2014)
 326 corresponding to $R_1 = 1.67 \times 10^{-2} \text{ s}^{-1}$ and a $TR = 4.25$ s, the flip angle correction for a $\beta = 1^\circ$ pulse was 3.58
 327 $\times 10^{-5}$, which ‘for all intents and purposes’, is negligible, giving $R_{1,app} = 1.67 \times 10^{-2} \text{ s}^{-1}$ and $T_{1,app} = 59.87$ s.
 328 Hence, the time dependence of the signal shown in Fig. 2(c) is a robust reflection of the $M_z(t)$ seen in Fig. 2(a).
 329 For $\beta = 20^\circ$ the flip angle correction was 1.46×10^{-2} giving $R_{1,app} = 3.13 \times 10^{-2} \text{ s}^{-1}$ and $T_{1,app} = 31.95$ s.
 330 Therefore, for the larger flip angle there was a tradeoff between the increased sensitivity and the corresponding
 331 reduction in $T_{1,app}$ with the more rapid decay of the NMR signal. The time dependence seen in Fig. 2(d) is no
 332 longer a good reflection of the $M_z(t)$ shown in Fig. 2(a). We conclude that when the RF flip angle is small, $< 1^\circ$,

333 and the magnetization is sampled many times, the flip angle correction is negligible; accordingly, it is ignored in
334 the next sections.

335

336 **3 Relaxation of hyperpolarized magnetization in ^{13}C substrates**

337 We now take a detour into relaxation theory to give an overview of the factors that determine the values of $R_1 =$
338 $1/T_1$ of hyperpolarized ^{13}C solutes in a (bio)chemical system taking into account the main relaxation mechanisms
339 responsible for the decay of the nuclear magnetization in solution state at temperatures between ~ 20 to 180°C and
340 static magnetic field strengths between 1 mT to 23.5 T. The spin interactions discussed here are relevant to the
341 outcome of numerous dissolution-dynamic nuclear polarization (dDNP) experiments.

342 A master equation for spin systems far from equilibrium based on a Lindblad dissipator formalism has
343 recently been presented and shown to correctly predict the spin dynamics of hyperpolarized systems (Bengs and
344 Levitt, 2020). In brief, Eq. (2) is only valid for the high temperature limit and weak order approximation of a spin
345 system at thermal equilibrium, and therefore the theory accounts for a dependence of relaxation rate constants on
346 the extent of hyperpolarization. However, we do not pursue this line of enquiry here because for the enzyme
347 systems studied thus far with dDNP a constant value of T_1 has been statistically satisfactory in regression analyses
348 of the data (Pagès et al., 2013; Shishmarev et al., 2018b).

349 Once a sufficiently high level of nuclear spin polarization has been achieved by implementing dDNP
350 methodologies (often for ^{13}C nuclei $P_C > 60\%$) a jet of superheated solvent (*e.g.*, H_2O and/or D_2O at $150\text{-}180^\circ\text{C}$)
351 is injected directly onto the hyperpolarized sample (Ardenkjaer-Larsen et al., 2003; Wolber et al., 2004). Upon
352 contact with the warm solvent, the frozen sample rapidly dissolves and is then transferred under the pressure of
353 helium gas (6-9 bar) to a separate NMR/MRI spectrometer for the detection of hyperpolarized MRS signals, or to
354 a collection/quality control point for use in biological applications (Comment and Merritt, 2014). There are several
355 potential issues related to spin relaxation during these processes; and we focus on nuclear spin relaxation in
356 solution during the sample transfer stage (*i.e.*, subject to changes in magnetic field strength) or situations where a
357 solute has an altered rotational correlation time (*i.e.*, dependence on temperature or when bound to a protein). This
358 requires an understanding of the (potentially) large variety of molecular interactions that give rise to nuclear spin
359 relaxation.

360 ***Dipole-Dipole Couplings (DD)***. The dominant mechanism for the relaxation of nuclear spin
361 magnetization is often the stochastic modulation of dipole-dipole interactions (couplings) to other nuclei, either
362 in the same molecule or other molecules, including the solvent, as the molecule re-orientates in solution by
363 molecular tumbling.

364 ***Chemical Shift Anisotropy (CSA)***. Nuclear spins resonate at different frequencies depending on the
365 chemical shielding imparted by the local electronic environment and its orientation (a tensor property). The
366 modulation of the chemical shift tensor by molecular tumbling in solution has a quadratic dependence on the
367 strength of the static magnetic field and therefore increases markedly with \mathbf{B}_0 (Kowalewski and Maler, 2019).

368 ***Paramagnetic Sites***. Dissolved paramagnetic solutes (often impurities, but they can be purposely added
369 as required by the experimental design), such as radical agents that remain in the dissolution solvent, molecular
370 oxygen, and metal ions, which can be deleterious to the nuclear-spin relaxation, particularly in regions of low

371 magnetic field (Blumberg, 1960; Pell et al., 2019). However, all species can be easily scavenged by co-dissolving
 372 chelating agents in the dissolution medium (Mieville et al., 2010).

373 **Scalar Relaxation of the Second Kind.** This mechanism operates when the nuclei of interest have scalar
 374 couplings to neighbouring nuclei that also relax rapidly (Pileio, 2011; Kubica et al., 2014; Elliott et al., 2019). In
 375 dDNP NMR experiments this relaxation mechanism is often enhanced during sample transfer steps through areas
 376 of low magnetic field (Chiavazza et al., 2013; Kubica et al., 2014).

377 **Spin Rotation.** The coupling of nuclear magnetization to that of a whole molecule or to mobile parts of
 378 a molecule, *e.g.*, methyl groups, can act as an efficient relaxation mechanism. This mechanism has an unusual
 379 dependence on temperature with the relaxation rate usually increasing at higher temperatures (Matson, 1977).

380 **Quadrupolar.** Many molecules of interest in dDNP experiments contain either ^2H or ^{14}N nuclei. NMR
 381 relaxation times of such nuclei are often <1 s, and therefore not sufficiently long to be relevant for dDNP
 382 experiments. However, there are two notable exceptions in $^6\text{Li}^+$ and $^{133}\text{Cs}^+$ which have small nuclear quadrupole
 383 moments and therefore have intrinsically long T_1 values (van Heeswijk et al., 2009; Kuchel et al., 2019).

384 Derivations of relaxation rate expressions are well established and based on plausible physical models.
 385 For simplicity, we skip the majority of these since they are comprehensively presented by several authors
 386 (Kowalewski and Maler, 2019), and instead we focus on the main results of their analyses. Assuming a two spin
 387 system composed of a ^{13}C and ^1H , equations for the ^{13}C - ^1H dipole-dipole and the ^{13}C CSA contributions to the
 388 ^{13}C longitudinal relaxation rate constant (R_1) are given by Keeler (Keeler, 2010):

389
 390
$$R_{1,DD} = b_{HC}^2 \left[\frac{3}{20}J(\omega_C) + \frac{1}{20}J(\omega_H - \omega_C) + \frac{3}{10}J(\omega_H + \omega_C) \right] , \quad (26)$$

391
 392
$$R_{1,CSA} = c^2 \left[\frac{1}{15}J(\omega_C) \right] , \quad (27)$$

393
 394 where b_{HC} is the dipole-dipole coupling constant, defined as:

395
 396
$$b_{HC} = \frac{\mu_0 \gamma_H \gamma_C \hbar}{4\pi r_{HC}^3} , \quad (28)$$

397
 398 and c is the magnitude of the CSA assuming an axially symmetric(al) tensor given by:

399
 400
$$c = \gamma_C B_0 (\sigma_{\parallel} - \sigma_{\perp}) , \quad (29)$$

401
 402 where γ_H and γ_C are the magnetogyric ratios of the ^1H and ^{13}C spins, respectively, r_{HC} is the internuclear distance
 403 between the ^1H and ^{13}C atoms and σ_{\parallel} and σ_{\perp} are the parallel and perpendicular components of the axially
 404 symmetric(al) CSA tensor, respectively.

405 The so-called spectral density function that is a function of the Larmor frequency, ω , is:

406
 407
$$J(\omega) = \frac{2\tau_c}{1+\omega^2\tau_c^2} , \quad (30)$$

408

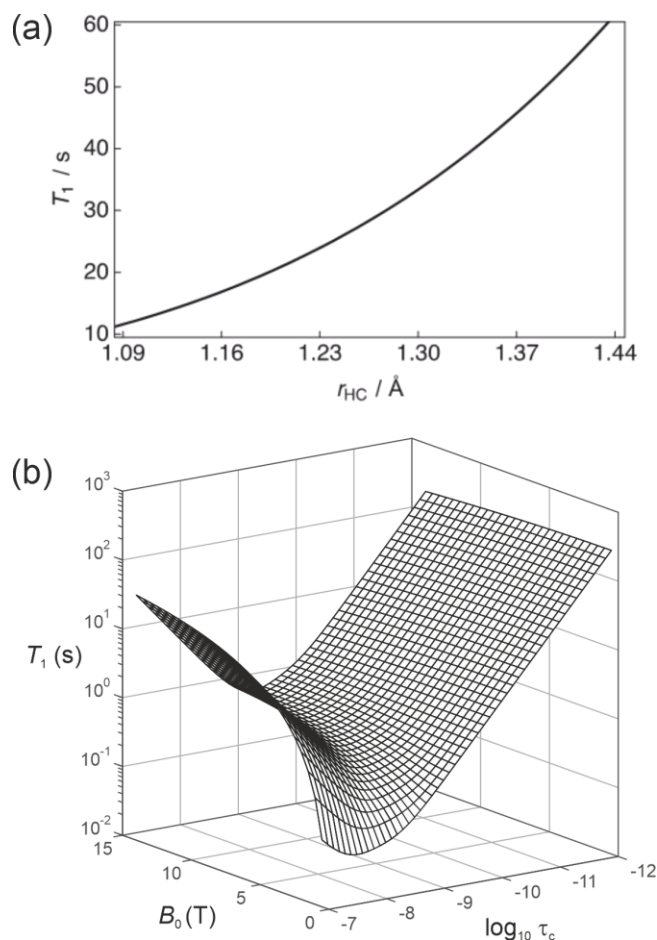


Figure 3 (a) Simulation of the ^{13}C nuclear T_1 for a two-spin ^1H - ^{13}C system as a function of the internuclear distance (r_{HC}) with a rotational correlation time $\tau_c = 0.4 \times 10^{-11}$ s, ^{13}C CSA $\sigma_{\parallel} - \sigma_{\perp} = -98\text{ppm}$ and at a magnetic field strength $B = 7$ T. (b) Dependence of the ^{13}C nuclear T_1 as a function of the magnetic field B and the rotational correlation time τ_c .

409 where τ_c is the rotational correlation time (tumbling motion) of the re-orientating spin-bearing molecule in
 410 solution. The overall longitudinal relaxation rate constant is the sum of these two dominant contributions and is
 411 given by:

$$412 \quad R_1 = R_{1,DD} + R_{1,CSA} \quad . \quad (31)$$

413 3.1 Relaxation Analysis

414 It is important (for experimental design purposes) to note the influence that a nearby ^1H spin has on the ^{13}C nuclear
 415 T_1 . Figure 3(a) shows the calculated ^{13}C T_1 for a fixed rotational correlation time of $\tau_c = 0.4 \times 10^{-11}$ s (previously
 416 reported for glycine in saline at 310 K (Endre et al., 1983)), ^{13}C CSA $\sigma_{\parallel} - \sigma_{\perp} = -98\text{ppm}$ (previously reported for
 417 phosphoenolpyruvate (Bechmann et al., 2004)) and a magnetic field strength of $B_0 = 7$ T as a function of the ^1H -
 418 ^{13}C internuclear distance r_{HC} . Biaxiality of the CSA interaction has been ignored here. A rapid rise occurs in T_1 as
 419 the ^1H - ^{13}C internuclear separation increases. In the case of $r_{\text{HC}} = 1.09$ Å, which is typical of a ^1H - ^{13}C single bond,
 420 the ^{13}C nuclear T_1 is predicted to be ~ 11.4 s. The ^1H - ^{13}C dipole-dipole coupling constant scales with
 421 r_{HC}^{-3} , consequently, the presence of a directly bonded proton significantly shortens the relaxation time constant of
 422 the ^{13}C magnetization. Small molecules containing ^{13}C atoms that do not have directly bonded ^1H , or at least ^1H

423 spins located at significant internuclear distances, are required. Such moieties include the carboxyl group that is
 424 present in many low molecular weight metabolites such as pyruvate, lactate, and methylglyoxal (Shishmarev et
 425 al., 2018a). At the longer ^1H - ^{13}C internuclear distance of 1.45 Å, implying a ^1H - ^{13}C dipole-dipole coupling
 426 constant of $b_{\text{HC}}/2\pi = -10.2$ kHz, a ^{13}C nuclear T_1 of ~60 s is predicted. At very long distances, the ^{13}C relaxation
 427 time constant will tend to that of the CSA relaxation contribution alone.

428 The dependence of R_1 on temperature and molecular size (*e.g.*, due to binding) scales with the rotational
 429 correlation time. Figure 3(b) shows the dependence of the ^{13}C nuclear T_1 ($1/R_1$) as a function of τ_c and B_0 for this
 430 2-spin-1/2 system with $r_{\text{HC}} = 1.45$ Å and $\sigma_{\parallel} - \sigma_{\perp} = -98$ ppm. In the extreme narrow limit, *i.e.*, $\omega^2\tau_c^2 \ll 1$, the
 431 following familiar equations describe the relaxation of ^{13}C spins under the dipole-dipole and CSA relaxation
 432 mechanisms (Kowalewski and Maler, 2019):

433
 434
$$R_{1,DD} = b_{\text{HC}}^2\tau_c \quad , \quad (32)$$

435
$$R_{1,CSA} = \frac{2}{15}c^2\tau_c \quad . \quad (33)$$

436
 437 In the extreme narrowing regime the ^{13}C nuclear T_1 becomes shorter with increasing magnetic field strength due
 438 to the B_0^2 dependence of $R_{1,CSA}$. At low field strengths, the magnitude of T_1 will mostly be attributed to dipole-
 439 dipole relaxation with the nearby ^1H spin. It is also worth noting that the ^{13}C T_1 follows the usual Lorentzian
 440 spectral density functional dependence on the rotational correlation time. This is clearly seen at high magnetic
 441 field.

442

443 3.2 Molecular Considerations

444 The majority of dDNP experiments used to study biological systems employ $\text{H}_2\text{O}/\text{D}_2\text{O}$ as the dissolution solvent.
 445 Detection of hyperpolarized NMR/MRI signals typically occurs in a magnetic field range of 1.5-9.4 T, thus Fig.
 446 3(b) indicates a ^{13}C nuclear T_1 of the order of ~60 s for a carbonyl group, and this is commonly seen in practice
 447 (Shishmarev et al., 2018a). It is important to remember that Eqs. (26-31) provide a greatly simplified picture of
 448 the problem in hand; in reality there are many magnetic nuclei (often within the same molecule) which contribute
 449 to the relaxation of ^{13}C magnetization. The additional dipole-dipole interactions are likely to be responsible for
 450 differences between predicted and measured ^{13}C relaxation times, along with the other (more exotic) signal
 451 attenuation mechanisms that are described above.

452 In a dDNP experiment the dissolution and transfer process can take as long as 15 s; it depends on the
 453 distance to the point of use from the polarizing source; and in clinical applications an additional 30 s can easily
 454 be added for quality control processes. Such requirements place a bound on the usable time in which
 455 hyperpolarized ^{13}C magnetization must be maintained; and it is typical to expect 45 s to be this limit. Given that
 456 the magnetic field strength “felt” by the hyperpolarized sample can be controlled (to a reasonable extent)
 457 throughout its voyage between the dDNP polarizer and the point of use (Milani et al., 2015), the rotational
 458 correlation time becomes the most important factor that impacts upon the ^{13}C nuclear T_1 . Figure 3(b) indicates
 459 that even for a rotational correlation time on the order of $\tau_c = 1 \times 10^{-10}$ s, such as found in proteins in solution

460 (Wilbur et al., 1976), Eq. (26-31) yields ^{13}C nuclear T_1 relaxation times which are too short to allow practical use
461 of such samples, *i.e.*, $5 \times T_1 \ll 45$ s, in comparison to the overall time required by a dDNP experiment.

462 A major parameter that controls the magnitude of the rotational correlation time of a spin-bearing
463 molecule is its molecular weight (M_w). Since $\tau_c \propto M_w$ the rotational correlation time has a noticeable impact on
464 the ^{13}C nuclear T_1 with even the smallest increase in molecular weight. In order to achieve ^{13}C nuclear T_1 relaxation
465 times that are sufficiently long to enable hyperpolarized ^{13}C magnetization to survive the dissolution and transfer
466 process the ^{13}C NMR signals must be detectable above the spectral noise for ~ 45 s. Hence, dDNP samples used
467 in biological experiments are currently restricted to small molecules (or ions (van Heeswijk et al., 2009; Kuchel
468 et al., 2019)). For example, the estimate of ~ 60 s for the ^{13}C nuclear T_1 of the model system described above was
469 predicted with a rotational correlation time of $\tau_c = 0.4 \times 10^{-11}$ (Endre et al., 1983), and this is sufficiently long for
470 dDNP experiments.

471

472 3.3 Enzyme Binding

473 The worst-case scenario for the model system described in Fig. 3(b) would be a moderate rotational correlation
474 time of the order of $\tau_c = 1 \times 10^{-8} - 1 \times 10^{-10}$ s for which ^{13}C nuclear T_1 relaxation times in the millisecond regime
475 are predicted. Such correlation times correspond to a system with a molecular weight comparable to that of an
476 enzyme. If the small molecule (ligand) or ion becomes bound to the enzyme, then it will assume the rotational
477 correlation time of the higher mass binding partner. In the case of $\tau_c = 1 \times 10^{-9}$ for an enzyme-ligand complex, a
478 ^{13}C substrate will have a predicted nuclear T_1 of ~ 276.4 ms at a static magnetic field strength of 7 T. Such a stark
479 variation in ^{13}C nuclear T_1 values provides good contrast in relaxation-based ligand-protein binding experiments
480 (Valensin et al., 1982).

481

482 4 Mechanistic description of reaction kinetics of hyperpolarized substrates

483 We now consider the interpretation of hyperpolarized dynamics for complex chemical reactions. To help tease
484 apart the key features of the analysis we begin with some simplifying assumptions. First, in the absence of an RF
485 pulse Eq. (20) becomes block diagonal, since transverse and longitudinal magnetization are not interconverted.
486 The evolution of the z magnetization is then dependent only on the initial conditions, T_1 , and the rate constants
487 that characterize the chemical exchange. Second, we assume that the z magnetization is sampled many times with
488 an infinitesimally small flip angle ($\ll 1^\circ$) so the longitudinal magnetization decays with its intrinsic T_1 value
489 rather than an apparent $T_{1,app}$ value. Finally, the hyperpolarized magnetization decays to zero, *i.e.*, the
490 enhancement factor η (Eq. (21)) is such that \mathbf{M}_0 is greater than \mathbf{M}_{eq} by many orders of magnitude. Thus, the
491 equilibrium magnetization at $t = \infty$ is effectively zero and it can be ignored in the analysis of real experimental
492 data.

493 To reduce clutter in the equations, for all the discussions that now follows, we drop the subscript z since
494 we hereafter deal only with longitudinal magnetization and denote $M_{z,hyp}^A$ and $M_{z,hyp}^B$ as $A^*(t)$ and $B^*(t)$
495 corresponding to hyperpolarized magnetization (identified with an asterisk *).

496

497 **4.1 Simple first order exchange kinetics of hyperpolarized substrates**

498 Confining our analysis to the physical subspace that is composed of longitudinal magnetizations, which
 499 describe first-order kinetics of a two-site exchange reaction of hyperpolarized substrates, $A^* \leftrightarrow B^*$, Eq. (20)
 500 simplifies to:

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \end{bmatrix} = \begin{bmatrix} -k_1 - R_1^A & k_{-1} \\ k_1 & -k_{-1} - R_1^B \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \end{bmatrix} . \quad (34)$$

502

Equivalently, Eq. (34) can be expanded to give:

$$\frac{dA^*(t)}{dt} = -k_1 A^*(t) + k_{-1} B^*(t) - R_1^A A^*(t) , \quad (35)$$

$$\frac{dB^*(t)}{dt} = k_1 A^*(t) - k_{-1} B^*(t) - R_1^B B^*(t) , \quad (36)$$

503

504 where k_1 and k_{-1} denote first-order rate constants, and $R_1^A = 1/T_1^A$ and $R_1^B = 1/T_1^B$ are the longitudinal relaxation
 505 rate constants of A and B, respectively.

506 Since Eqs. (35) and (36) describe the time evolution of the z magnetizations (that is proportional to
 507 concentration/mass) they do not satisfy the conservation of mass requirement because $d[A^*(t) + B^*(t)]/dt =$
 508 $-R_1^A A^*(t) - R_1^B B^*(t)$ and this tends to zero with time. However, the equations can be recast to specify that the
 509 pools of hyperpolarized substrates relax to form pools of non-polarized substrates $A \leftrightarrow B$. These pools are denoted
 510 simply by $A(t)$ and $B(t)$ (without the asterisks) as shown in Fig. 4(a). The analogy with radioactive tracers is a
 511 useful one here. A ‘hot’ pool of radioactive material decays with first order kinetics (half-life) to form a ‘cold’
 512 pool of non-radioactive material with the sum of ‘hot’ and ‘cold’ being constant.

513 The kinetics of the non-polarized pools are described by:

514

$$\frac{dA(t)}{dt} = -k_1 A(t) + k_{-1} B(t) + R_1^A A^*(t) , \quad (37)$$

$$\frac{dB(t)}{dt} = k_1 A(t) - k_{-1} B(t) + R_1^B B^*(t) . \quad (38)$$

515

516 Equations (37) and (38) now satisfy conservation of mass, since the rate of change $d[A^*(t) + A(t) + B^*(t) +$
 517 $B(t)]/dt$ is always zero. Note that $A(t)$ and $B(t)$ are not observed in the dDNP NMR experiment; but they are
 518 the counterparts of real concentrations of solute that would be assayable (bio)chemically.

519 Equations (35-38) can be written as:

520

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A(t) \\ B(t) \end{bmatrix} = \begin{bmatrix} -k_1 - R_1^A & k_{-1} & 0 & 0 \\ k_1 & -k_{-1} - R_1^B & 0 & 0 \\ R_1^A & 0 & -k_1 & k_{-1} \\ 0 & R_1^B & k_1 & -k_{-1} \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A(t) \\ B(t) \end{bmatrix} . \quad (39)$$

521 Equation (39) can be written:

522

$$\frac{dM(t)}{dt} = LM(t) \quad . \quad (40)$$

523

524 We can apply a similarity transform given by:

525

$$U = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 \end{bmatrix} \quad . \quad (41)$$

526

527 To yield an equation of motion in a transformed basis vector:

528

$$\frac{dM'(t)}{dt} = ULU^{-1}M'(t) \quad . \quad (42)$$

529

530 Given by:

531

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A^*(t) + A(t) \\ B^*(t) + B(t) \end{bmatrix} = \begin{bmatrix} -k_1 - R_1^A & k_{-1} & 0 & 0 \\ k_1 & -k_{-1} - R_1^B & 0 & 0 \\ 0 & 0 & -k_1 & k_{-1} \\ 0 & 0 & k_1 & -k_{-1} \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A^*(t) + A(t) \\ B^*(t) + B(t) \end{bmatrix} \quad . \quad (43)$$

532

533 We can now appreciate the equivalence between this formalism and conventional chemical reaction kinetics
 534 written in terms of molecular concentrations. For first order reactions, the hyperpolarized magnetization evolves
 535 according to the Bloch McConnell equations while the concentrations given by the sum of the ‘hot’ and ‘cold’
 536 pools evolve according to the conventional form of chemical reaction kinetics for a closed system. Therefore,
 537 $A^*(t) + A(t)$ and $B^*(t) + B(t)$ are proportional to $[A(t)]$ and $[B(t)]$, respectively, where the constant of
 538 proportionality depends on the initial experimental conditions, *viz.*, $[A]_0$ and $[B]_0$. In other words, provided
 539 $A^*(0) + A(0) = [A]_0$ and $B^*(0) + B(0) = [B]_0$ then the constant of proportionality is 1 and we can equate
 540 $A^*(t) + A(t) = [A(t)]$ and $B^*(t) + B(t) = [B(t)]$. This is a crucial point that we return to below.

541 Figure 4 shows numerical simulations of the time evolution of the system described by Eq. (39) with an
 542 initial magnetization vector $\mathbf{M}(0) = [1, 0, 0, 0]$ that corresponds to only hyperpolarized $A^*(0) = 1$ and
 543 longitudinal relaxation rate constants $R_1^A = R_1^B = 1/60s^{-1}$. The time dependence of $A^*(t)$, $A(t)$, $B^*(t)$ and $B(t)$
 544 were calculated numerically (left panel) for different rate constants: Fig. 4(b), $k_1 = 0.01 s^{-1}$, $k_{-1} = 0 s^{-1}$,
 545 corresponding to a uni-directional reaction; Fig 4(c), $k_1 = 0.01 s^{-1}$, $k_{-1} = 0.005 s^{-1}$, corresponding to bi-directional
 546 exchange with an equilibrium constant $K = 2$; and Fig. 4(d), $k_1 = 0.01 s^{-1}$, $k_{-1} = 0.01 s^{-1}$, also corresponding to bi-
 547 directional exchange with an equilibrium constant $K = 1$. The right column shows plots of the time dependence
 548 of $A^*(t) + A(t)$ and $B^*(t) + B(t)$ that reproduce conventional kinetics of $[A(t)]$ and $[B(t)]$, as required for
 549 mathematical and physical consistency.

550 The approach used here (as laid out in (Kuchel and Shishmarev, 2020)) enables us to create systems of
 551 differential equations that satisfy conservation of mass and therefore allow a study of the influence of non-
 552 hyperpolarized pools of substrates on reaction kinetics. The approach enables more complicated reaction
 553 mechanisms to be described to allow the inclusion of MR invisible pools of substrates such as ^{12}C , which are
 554 known to affect the outcome of dDNP experiments *in vivo*. We consider some of these scenarios next.
 555

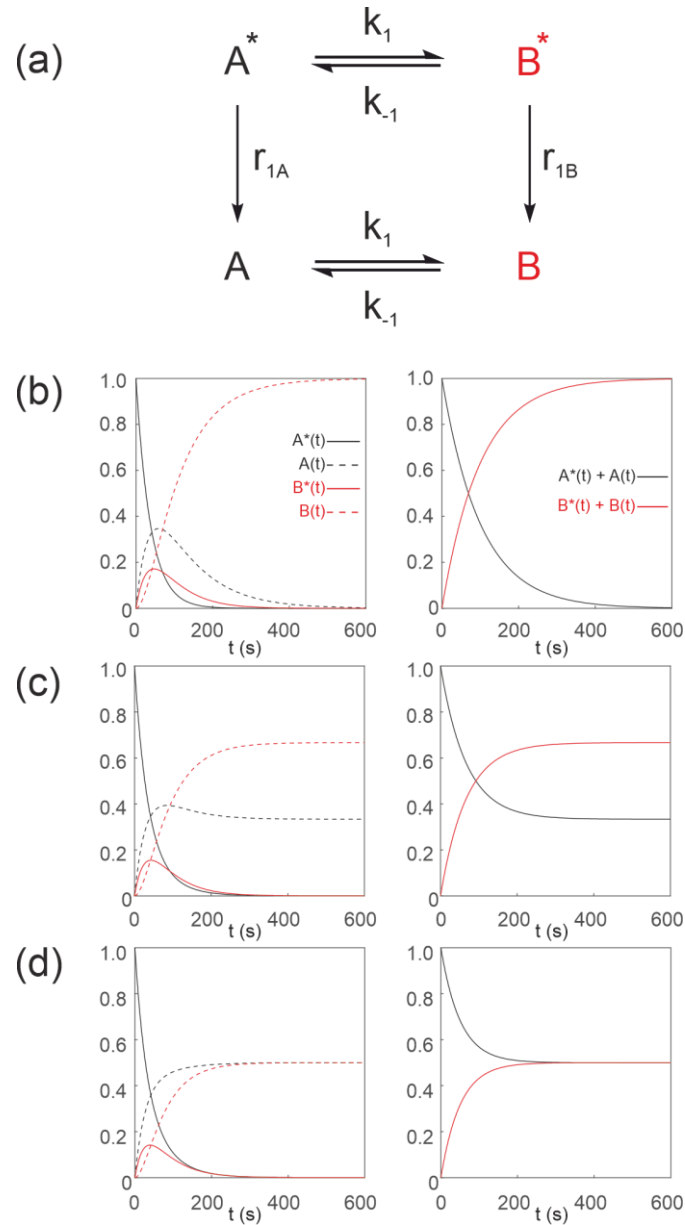


Figure 4 Simulated first order two-site exchange kinetics of hyperpolarized solutes, $A \leftrightarrow B$, conforming to conservation of mass, assuming initial hyperpolarized magnetization of only solute $A^*(0) = 1$. Longitudinal relaxation rate constants were $R_1^A = R_1^B = 1/60 \text{ s}^{-1}$. The time dependence of $A^*(t)$, $A(t)$, $B^*(t)$ and $B(t)$ (left panel) were calculated numerically using Eq. (35-38) with rate constants (b) $k_1 = 0.01 \text{ s}^{-1}, k_{-1} = 0 \text{ s}^{-1}$, corresponding to uni-directional kinetics, (c) $k_1 = 0.01 \text{ s}^{-1}, k_{-1} = 0.005 \text{ s}^{-1}$ and (d) $k_1 = 0.01 \text{ s}^{-1}, k_{-1} = 0.01 \text{ s}^{-1}$ corresponding to exchange kinetics. The right panel shows plots of the time dependence of $A^*(t) + A(t) = [A(t)]$ and $B^*(t) + B(t) = [B(t)]$.

556 **4.2 Sequential reaction kinetics of hyperpolarized substrates**

557 Equation (39) can be extended to compartmental models of arbitrary complexity: Consider a reaction scheme
 558 involving three substrates $A^* \leftrightarrow B^* \leftrightarrow C^*$ which relax through T_1 processes to form a pool of non-polarized
 559 substrates $A \leftrightarrow B \leftrightarrow C$, as shown in Fig. 5(a). This is analogous to a system where a solution of hyperpolarized
 560 solute A^* is introduced into the extracellular medium in a cell suspension, is transported into the cells where it is
 561 denoted by B^* and it is subsequently acted upon by an enzyme to form C^* . The system of differential equations
 562 that describe the kinetics of this scheme is:
 563

$$\frac{dA^*(t)}{dt} = -k_1A^*(t) + k_{-1}B^*(t) - R_1^A A^*(t) \quad , \quad (44)$$

$$\frac{dB^*(t)}{dt} = k_1A^*(t) - k_{-1}B^*(t) - k_2B^*(t) + k_{-2}C^*(t) - R_1^B B^*(t) \quad , \quad (45)$$

$$\frac{dC^*(t)}{dt} = k_2B^*(t) - k_{-2}C^*(t) - R_1^C C^*(t) \quad , \quad (46)$$

$$\frac{dA(t)}{dt} = -k_1A(t) + k_{-1}B(t) + R_1^A A^*(t) \quad , \quad (47)$$

$$\frac{dB(t)}{dt} = k_1A(t) - k_{-1}B(t) - k_2B(t) + k_{-2}C(t) + R_1^B B^*(t) \quad , \quad (48)$$

$$\frac{dC(t)}{dt} = k_2B(t) - k_{-2}C(t) + R_1^C C^*(t) \quad , \quad (49)$$

564
 565 where we have removed the square brackets that denote molar concentration to avoid some of the clutter.
 566 However, it is important to recall that there is a factor that relates magnetization to concentration, and this is
 567 estimated from the known initial experimental conditions.

568 Equations (44-49) can be recast in matrix form to give:

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \\ C^*(t) \\ A(t) \\ B(t) \\ C(t) \end{bmatrix} = \begin{bmatrix} -k_1 - R_1^A & k_{-1} & 0 & 0 & 0 & 0 \\ k_1 & -k_{-1} - k_2 - R_1^B & k_{-2} & 0 & 0 & 0 \\ 0 & k_2 & -k_{-2} - R_1^C & 0 & 0 & 0 \\ R_1^A & 0 & 0 & -k_1 & k_{-1} & 0 \\ 0 & R_1^B & 0 & k_1 & -k_{-1} - k_2 & k_{-2} \\ 0 & 0 & R_1^C & 0 & k_2 & -k_{-2} \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \\ C^*(t) \\ A(t) \\ B(t) \\ C(t) \end{bmatrix} \quad . \quad (50)$$

570
 571 It is readily verified that Eq. (50) satisfies conservation of mass, since the rate of change $(A^*(t) + A(t) + B^*(t) +$
 572 $B(t) + C^*(t) + C(t))/dt = 0$.

573 We can apply a similarity transform given by:

574
 575

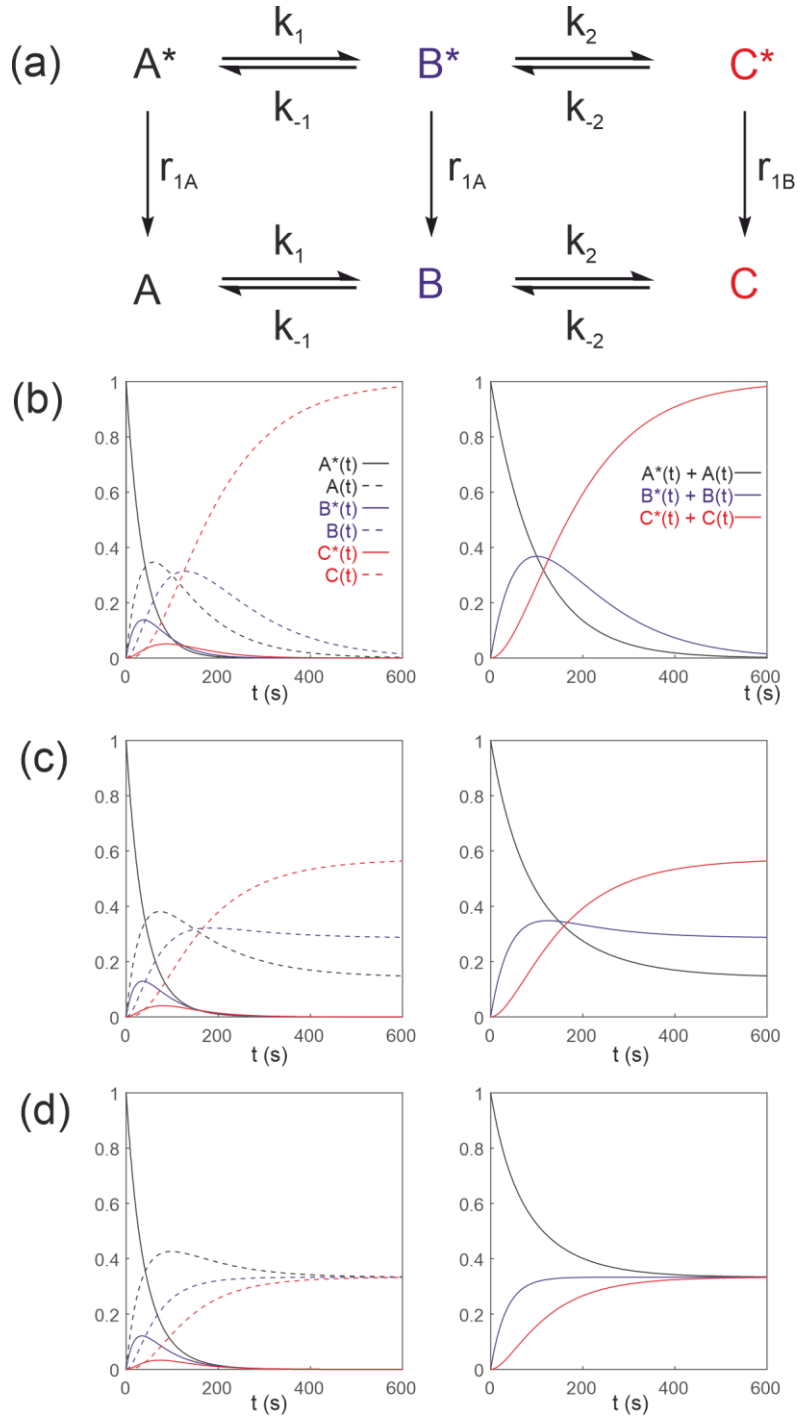


Figure 5 Simulated first order three-site exchange kinetics of hyperpolarized solutes, $A \leftrightarrow B \leftrightarrow C$, conforming to conservation of mass, assuming initial hyperpolarized magnetization of only solute $A^*(0) = 1$. Longitudinal relaxation rate constants were $R_1^A = R_1^B = R_1^C = 1/60 \text{ s}^{-1}$. The time dependence of $A^*(t)$, $A(t)$, $B^*(t)$, $B(t)$, $C^*(t)$ and $C(t)$ (left panel) were calculated numerically using Eq. (41-46) with rate constants (b) $k_1 = k_2 = 0.01 \text{ s}^{-1}$, $k_{-1} = k_{-2} = 0 \text{ s}^{-1}$, corresponding to uni-directional kinetics, (c) $k_1 = k_2 = 0.01 \text{ s}^{-1}$, $k_{-1} = k_{-2} = 0.005 \text{ s}^{-1}$ and (d) $k_1 = k_2 = k_{-1} = k_{-2} = 0.01 \text{ s}^{-1}$, corresponding to exchange kinetics. The right panel shows plots of the time dependence of $A^*(t) + A(t) = [A(t)]$, $B^*(t) + B(t) = [B(t)]$ and $C^*(t) + C(t) = [C(t)]$.

$$U = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 \end{bmatrix}. \quad (51)$$

577

578 To yield an equation of motion in the transformed basis vector given by:

579

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \\ C^*(t) \\ A^*(t) + A(t) \\ B^*(t) + B(t) \\ C^*(t) + C(t) \end{bmatrix} = \begin{bmatrix} -k_1 - R_1^A & k_{-1} & 0 & 0 & 0 & 0 \\ k_1 & -k_{-1} - k_2 - R_1^B & k_{-2} & 0 & 0 & 0 \\ 0 & k_2 & -k_{-2} - R_1^C & 0 & 0 & 0 \\ 0 & 0 & 0 & -k_1 & k_{-1} & 0 \\ 0 & 0 & 0 & k_1 & -k_{-1} - k_2 & k_{-2} \\ 0 & 0 & 0 & 0 & k_2 & -k_{-2} \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \\ C^*(t) \\ A^*(t) + A(t) \\ B^*(t) + B(t) \\ C^*(t) + C(t) \end{bmatrix}. \quad (52)$$

581

582 The hyperpolarized magnetization evolves according to the Bloch McConnell equations while the concentrations
 583 given by the sum of the ‘hot’ and ‘cold’ pools evolve according to the conventional form of chemical reaction
 584 kinetics for a closed system. Therefore, provided $A^*(0) + A(0) = [A]_0$, $B^*(0) + B(0) = [B]_0$ and $C^*(0) +$
 585 $C(0) = [C]_0$, then $A^*(t) + A(t) = [A(t)]$, $B^*(t) + B(t) = [B(t)]$ and $C^*(t) + C(t) = [C(t)]$, respectively.

586 Figure 5 shows the results of numerical integration of Eq. (50) with initial magnetization vector $\mathbf{M}(0) =$
 587 $[1, 0, 0, 0, 0, 0]$ that corresponds to having only hyperpolarized $A^*(0) = 1$ and longitudinal relaxation rate constants
 588 $R_1^A = R_1^B = R_1^C = 1/60s^{-1}$. The time dependence of $A^*(t)$, $A(t)$, $B^*(t)$, $B(t)$, $C^*(t)$ and $C(t)$ were calculated
 589 (left panel) for different rate constants: Fig. 5(b), $k_1 = k_2 = 0.01 s^{-1}$, $k_{-1} = k_{-2} = 0 s^{-1}$, corresponding to uni-
 590 directional kinetics; Fig. 5(c), $k_1 = k_2 = 0.01 s^{-1}$, $k_{-1} = k_{-2} = 0.005 s^{-1}$, corresponding to bi-directional
 591 exchange kinetics; and Fig. 5(d), $k_1 = k_2 = k_{-1} = k_{-2} = 0.01 s^{-1}$, also corresponding to bi-directional
 592 exchange kinetics. The right column shows plots of the time dependence of $A^*(t) + A(t)$, $B^*(t) + B(t)$ and
 593 $C^*(t) + C(t)$, which reproduce the conventional chemical kinetics of $[A(t)]$, $[B(t)]$ and $[C(t)]$, as required for
 594 mathematical and physical consistency.

595

596 4.3 Second-order kinetics of hyperpolarized substrates

597 We now describe hyperpolarized substrates $A^*(t)$ and $B^*(t)$ reacting with non-hyperpolarized substrates $[C(t)]$
 598 and $[D(t)]$. The system of differential equations that describes these second-order kinetics of $A^* + C \leftrightarrow B^* + D$
 599 with only the hyperpolarized pools relaxing through T_1 processes to form a pool of non-polarized substrates $A +$
 600 $C \leftrightarrow B + D$. The reactant concentrations $[C(t)]$ and $[D(t)]$ are common to both pools, as shown in Fig. 6(a). The
 601 relevant system of differential equations (again omitting the square brackets that denote concentration) is:

602

$$\frac{dA^*(t)}{dt} = -k_1 C(t) A^*(t) + k_{-1} D(t) B^*(t) - R_1^A A^*(t) \quad , \quad (53)$$

$$\frac{dB^*(t)}{dt} = k_1 C(t) A^*(t) - k_{-1} D(t) B^*(t) - R_1^B B^*(t) \quad , \quad (54)$$

$$\frac{dA(t)}{dt} = -k_1 C(t)A(t) + k_{-1}D(t)B(t) + R_1^A A^*(t) \quad , \quad (55)$$

$$\frac{dB(t)}{dt} = k_1 C(t)A(t) - k_{-1}D(t)B(t) + R_1^B B^*(t) \quad , \quad (56)$$

$$\frac{d[C(t)]}{dt} = -k_1(A^*(t) + A(t))C(t) + k_{-1}(B^*(t) + B(t))D(t) \quad , \quad (57)$$

$$\frac{d[D](t)}{dt} = k_1(A^*(t) + A(t))C(t) - k_{-1}(B^*(t) + B(t))D(t) \quad . \quad (58)$$

603

604 Again, mass is conserved as seen by the fact that $d((A^*(t) + A(t) + B^*(t) + B(t))/dt = 0$ and $d(C(t) +$
 605 $D(t))/dt = 0$. Also, recall that provided $A^*(0) + A(0) = [A]_0$, $B^*(0) + B(0) = [B]_0$, $C(0) = [C]_0$ and $D(0) =$
 606 $[D]_0$, then we can make use of the equalities $A^*(t) + A(t) = [A(t)]$, $B^*(t) + B(t) = [B(t)]$, $C(t) = [C(t)]$ and
 607 $D(t) = [D(t)]$, respectively. It is now very evident why we must equate the initial signal with the concentration
 608 via an experimentally estimated scaling factor.

609 Equations (53-58) can be written in matrix vector form as:

610

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A(t) \\ B(t) \\ C(t) \\ D(t) \end{bmatrix} = \begin{bmatrix} -k_1 C(t) - R_1^A & k_{-1} D(t) & 0 & 0 & 0 & 0 \\ k_1 C(t) & -k_{-1} D(t) - R_1^B & 0 & 0 & 0 & 0 \\ R_1^A & 0 & -k_1 C(t) & k_{-1} D(t) & 0 & 0 \\ 0 & R_1^B & k_1 C(t) & -k_{-1} D(t) & 0 & 0 \\ -k_1 C(t) & k_{-1} D(t) & -k_1 C(t) & k_{-1} D(t) & 0 & 0 \\ k_1 C(t) & -k_{-1} D(t) & k_1 C(t) & -k_{-1} D(t) & 0 & 0 \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A(t) \\ B(t) \\ C(t) \\ D(t) \end{bmatrix} \quad . \quad (59)$$

611

612 We can apply a similarity transform given by:

613

$$U = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad . \quad (60)$$

614

615 To yield an equation of motion in the transformed basis vector given by:

616

$$\frac{d}{dt} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A^*(t) + A(t) \\ B^*(t) + B(t) \\ C(t) \\ D(t) \end{bmatrix} = \begin{bmatrix} -k_1 C(t) - R_1^A & k_{-1} D(t) & 0 & 0 & 0 & 0 \\ k_1 C(t) & k_{-1} D(t) - R_1^B & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_1 C(t) & k_{-1} D(t) & 0 & 0 \\ 0 & 0 & k_1 C(t) & -k_{-1} D(t) & 0 & 0 \\ 0 & 0 & -k_1 C(t) & k_{-1} D(t) & 0 & 0 \\ 0 & 0 & k_1 C(t) & -k_{-1} D(t) & 0 & 0 \end{bmatrix} \begin{bmatrix} A^*(t) \\ B^*(t) \\ A^*(t) + A(t) \\ B^*(t) + B(t) \\ C(t) \\ D(t) \end{bmatrix} \quad . \quad (61)$$

618

619

620 Figure 6 shows numerical simulations of the time evolution of the system of Eqs. (53-58) with initial
621 magnetization corresponding to the hyperpolarized signal $A^*(0) = 1$ and non-polarized substrates $C(0) = 0.95$
622 and $D(0) = 0.05$. The longitudinal relaxation rate constants were $R_{1A} = R_{1B} = 1/60s^{-1}$. The time dependence
623 of $A^*(t)$, $A(t)$, $B^*(t)$ and $B(t)$ are subject to second order kinetics and were calculated numerically (left panel)
624 for different rate constants: Fig. 6(b), $k_1 = 0.01 M^{-1}s^{-1}$, $k_{-1} = 0 M^{-1}s^{-1}$, corresponding to unidirectional
625 kinetics; Fig. 6(c), $k_1 = 0.01 M^{-1}s^{-1}$, $k_{-1} = 0.005 M^{-1}s^{-1}$, corresponding to bi-directional exchange kinetics
626 with an equilibrium constant $K = 2$; and Fig. 6(d) $k_1 = k_{-1} = 0.01 M^{-1}s^{-1}$, with an equilibrium constant $K = 1$,
627 also corresponding to bi-directional exchange kinetics. The right column shows plots of the time dependence of
628 $A^*(t) + A(t)$, $B^*(t) + B(t)$, which capture conventional chemical kinetics of the concentrations of $[A(t)]$ and
629 $[B(t)]$, as required, as well as the kinetics of the non-polarized reactants $[C(t)]$ and $[D(t)]$.

630

631 4.3.1 An Ersatz solution

632 The system of differential equations in Eq. (59), describing a second order reaction can be reduced to one with
633 pseudo first order kinetics by introducing time-dependent rate constants $k'_1(t) = k_1C(t)$ and $k'_{-1}(t) = k_{-1}D(t)$.
634 Importantly, the pseudo first order rate constants $k'_1(t)$ and $k'_{-1}(t)$ are now time dependent. This approach has
635 been used previously (Mariotti et al., 2016) but it constitutes a special case of the more general method described
636 here, which we advocate.

637 However, we now encounter a problem. The pseudo first order rate constants for the reactions of $[C(t)]$
638 and $[D(t)]$ are now given by $k'_1(t) = k_1(A^*(t) + A(t))$ and $k'_{-1}(t) = k_{-1}(B^*(t) + B(t))$, respectively. The time-
639 dependent pseudo first order rate constants are dependent on the concentrations of both 'hot' and 'cold' pools. In
640 turn the pseudo first order rate constants for $A^*(t)$ and $B^*(t)$ are $k'_1(t) = k_1C(t)$ and $k'_{-1}(t) = k_{-1}D(t)$. Thus,
641 the kinetics of the 'hot' pools $A^*(t)$ and $B^*(t)$ become dependent on the kinetics of the 'cold' pools $A(t)$ and
642 $B(t)$. This is of particular relevance (as highlighted by Kuchel and Shishmarev, 2019) when extending the
643 equations to describe enzyme kinetics. It is this that we turn our attention to next.

644

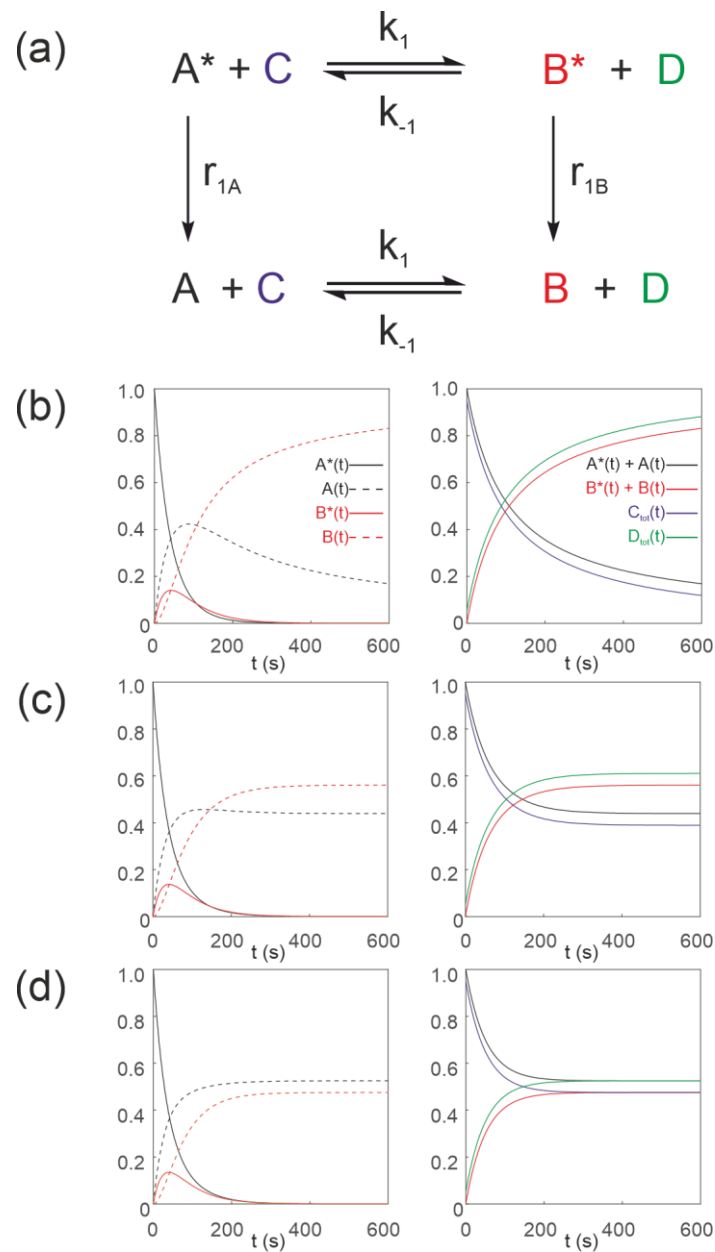


Figure 6 Simulated second order exchange kinetics of hyperpolarized solutes, $A^* + C \leftrightarrow B^* + D$, conforming to conservation of mass, assuming initial hyperpolarized magnetization of only solute $A^*(0) = 1$. Longitudinal relaxation rate constants were $R_1^A = R_1^B = 1/60 \text{ s}^{-1}$. The time dependence of $A^*(t)$, $A(t)$, $B^*(t)$ and $B(t)$ were simulated (left panel) using Eqs. (53-58) with rate constants (b) $k_1 = 0.01 \text{ M}^{-1}\text{s}^{-1}$, $k_{-1} = 0 \text{ M}^{-1}\text{s}^{-1}$, corresponding to uni-directional kinetics (c) $k_1 = 0.01 \text{ M}^{-1}\text{s}^{-1}$, $k_{-1} = 0.005 \text{ M}^{-1}\text{s}^{-1}$ and (d) $k_1 = k_{-1} = 0.01 \text{ M}^{-1}\text{s}^{-1}$, corresponding to exchange kinetics. The right panel shows plots of the time dependence of $A^*(t) + A(t) = [A(t)]$, $B^*(t) + B(t) = [B(t)]$ and non-polarized reactants $[C(t)]$ and $[D(t)]$.

647 **5 Michaelis-Menten equation for a hyperpolarized substrate**

648 Next consider an enzyme catalysed reaction with a hyperpolarized substrate. The simplest model involves a
 649 hyperpolarized substrate $S^*(t)$ that is in equilibrium with a free enzyme of concentration $[E]_0$ to form a
 650 hyperpolarized enzyme substrate complex $ES^*(t)$, which then reacts to form a hyperpolarized product $P^*(t)$. This
 651 is followed by release of the free enzyme that is then available for further reactions: $E + S^* \leftrightarrow ES^* \leftrightarrow P^* + E$. All
 652 hyperpolarized substrates relax through T_1 processes to form non-polarized pools of substrates $E + S \leftrightarrow ES \leftrightarrow P$
 653 + E as shown in Fig. 7(a). The differential equations (again omitting the square brackets denoting concentration)
 654 that describe the reaction kinetics are:

655

$$\frac{dS^*(t)}{dt} = -k_1E(t)S^*(t) + k_{-1}ES^*(t) - R_1^S S^*(t) \quad , \quad (62)$$

$$\frac{dES^*(t)}{dt} = k_1E(t)S^*(t) - k_{-1}ES^*(t) - k_2ES^*(t) + k_{-2}E(t)P^*(t) - R_1^{ES} ES^*(t) \quad , \quad (63)$$

$$\frac{dP^*(t)}{dt} = k_2ES^*(t) - k_{-2}E(t)P^*(t) - R_1^P P^*(t) \quad , \quad (64)$$

$$\frac{dS(t)}{dt} = -k_1E(t)S(t) + k_{-1}ES(t) + R_1^S S^*(t) \quad , \quad (65)$$

$$\frac{dES(t)}{dt} = k_1E(t)S(t) - k_{-1}ES(t) - k_2ES(t) + k_{-2}E(t)P(t) + R_1^{ES} ES^*(t) \quad , \quad (66)$$

$$\frac{dP(t)}{dt} = k_2ES(t) - k_{-2}E(t)P(t) + R_1^P P^*(t) \quad , \quad (67)$$

$$\frac{dE(t)}{dt} = -k_1E(t)(S^*(t) + S(t)) + (k_{-1} + k_2)(ES^*(t) + ES(t)) - k_{-2}E(t)(P^*(t) + P(t)) \quad , \quad (68)$$

656

657 where $E(t)$ is the free enzyme, $ES(t)$ is the enzyme-substrate complex, $S(t)$ is the free substrate and $P(t)$ is the
 658 free product, with relaxation rate constants R_1^S , R_1^{ES} and R_1^P , respectively. Note the appearance of the free enzyme
 659 $E(t)$ as both a reactant and product; it is regenerated through the reactions that are characterized by the rate
 660 constants k_1 and k_{-1} , and also k_2 and k_{-2} , thereby being recycled.

661

662 Mass is conserved as confirmed by the fact that $d(S^*(t) + S(t) + ES^*(t) + ES(t) + P^*(t) + P(t))/$
 663 $dt = 0$ and $d(ES^*(t) + ES(t) + E(t))/dt = 0$. Therefore, provided $S^*(0) + S(0) = [S]_0$, $ES^*(0) + ES(0) =$
 664 $[ES]_0$ and $P^*(0) + P(0) = [P]_0$ then $S^*(t) + S(t) = [S(t)]$, $ES^*(t) + ES(t) = [ES(t)]$ and $P^*(t) + P(t) =$
 $[P(t)]$, respectively.

Equations (62-68) can be written in matrix vector form as:

$$\frac{d}{dt} \begin{bmatrix} S^*(t) \\ ES^*(t) \\ P^*(t) \\ S(t) \\ ES(t) \\ P(t) \\ E(t) \end{bmatrix} = \begin{bmatrix} -k_1 E(t) - R_1^S & k_{-1} & 0 & 0 & 0 & 0 & 0 \\ k_1 E(t) & -k_{-1} - k_2 - R_1^{ES} & k_{-2} E(t) & 0 & 0 & 0 & 0 \\ 0 & k_2 & -k_{-2} E(t) - R_1^P & 0 & 0 & 0 & 0 \\ R_1^S & 0 & 0 & -k_1 E(t) & k_{-1} & 0 & 0 \\ 0 & R_1^{ES} & 0 & k_1 E(t) & -k_{-1} - k_2 & k_{-2} E(t) & 0 \\ 0 & 0 & R_1^P & 0 & k_2 & -k_{-2} E(t) & 0 \\ -k_1 E(t) & k_{-1} + k_2 & -k_{-2} E(t) & -k_1 E(t) & k_{-1} + k_2 & -k_{-2} E(t) & 0 \end{bmatrix} \begin{bmatrix} S^*(t) \\ ES^*(t) \\ P^*(t) \\ S(t) \\ ES(t) \\ P(t) \\ E(t) \end{bmatrix}. \quad (69)$$

665

666 We can apply a similarity transform given by:

$$U = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}. \quad (70)$$

667

668 To yield an equation of motion in the transformed basis vector given by:

669

$$\frac{d}{dt} \begin{bmatrix} S^*(t) \\ ES^*(t) \\ P^*(t) \\ S^*(t) + S(t) \\ ES^*(t) + ES(t) \\ P^*(t) + P(t) \\ E(t) \end{bmatrix} = \begin{bmatrix} -k_1 E(t) - R_1^S & k_{-1} & 0 & 0 & 0 & 0 & 0 \\ k_1 E(t) & -k_{-1} - k_2 - R_1^{ES} & k_{-2} E(t) & 0 & 0 & 0 & 0 \\ 0 & k_2 & -k_{-2} E(t) - R_1^P & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -k_1 E(t) & k_{-1} & 0 & 0 \\ 0 & 0 & 0 & k_1 E(t) & -k_{-1} - k_2 & k_{-2} E(t) & 0 \\ 0 & 0 & 0 & 0 & k_2 & -k_{-2} E(t) & 0 \\ 0 & 0 & 0 & -k_1 E(t) & k_{-1} + k_2 & -k_{-2} E(t) & 0 \end{bmatrix} \begin{bmatrix} S^*(t) \\ ES^*(t) \\ P^*(t) \\ S^*(t) + S(t) \\ ES^*(t) + ES(t) \\ P^*(t) + P(t) \\ E(t) \end{bmatrix}. \quad (71)$$

670

671 **5.1 Steady state of ES complex**

672 A simplified uni-directional enzyme catalysed reaction is described by setting the reverse rate constant $k_{-2} = 0$
 673 (see Fig. 7(a)). If it is assumed that a steady-state of $[ES]$ is attained very rapidly then $d(ES^*(t) + ES(t))/dt =$
 674 0 and we obtain (reverting to using square brackets to denote molar concentration):

675
$$k_1[E(t)][S^*(t) + S(t)] = (k_{-1} + k_2)[ES^*(t) + ES(t)] \quad . \quad (72)$$

676
 677 Rearranging Eq. (72) yields the Michaelis constant in terms of hyperpolarized and non-polarized pools of
 678 substrate:

679
$$K_M = \frac{(k_{-1} + k_2)}{k_1} = \frac{[E(t)][S^*(t) + S(t)]}{[ES^*(t) + ES(t)]} \quad . \quad (73)$$

680
 681 Calibrating the signals to molar concentrations is important since the signals now relate to a real parameter (K_M)
 682 of the enzyme that has units of concentration (typically mM).

683 Thus, using conservation of enzyme mass, the free enzyme concentration is given by:

684
$$[E(t)] = [E]_0 - [ES^*(t) + ES(t)] \quad . \quad (74)$$

685
 686 Then

$$\frac{d([P^*(t) + P(t)])}{dt} = \frac{k_2[E]_0 [S^*(t) + S(t)]}{K_M + [S^*(t) + S(t)]} \quad . \quad (75)$$

687
 688 In other words, this is the standard form of the Michaelis-Menten equation written as a function of both polarized
 689 and unpolarized pools of substrate.

690

691 **5.2 Simulations of Michaelis-Menten reaction**

692 Figure 7(b-c) shows the results of numerical integration of Eqs. (62-68) with an initial hyperpolarized signal
 693 $S^*(0) = 0.001$ (corresponding to a concentration $[S]_0 = 1$ mM via the experimentally determined scaling factor,
 694 which here was set to 1) and enzyme concentration $[E]_0 = 1 \times 10^{-9}$ M. The assigned longitudinal relaxation rate
 695 constants were $R_{1S} = R_{1ES} = R_{1P} = 1/60s^{-1}$. In the first instance, we set the longitudinal relaxation times of
 696 substrate, enzyme-substrate complex and product to be equal (this is discussed further below). The reaction rate
 697 constants were $k_1 = 1 \times 10^7 M^{-1}s^{-1}$, $k_{-1} = 1 \times 10^2 s^{-1}$, $k_2 = 5 \times 10^3 s^{-1}$, $k_{-2} = 0 M^{-1}s^{-1}$, such that $K_M =$
 698 $5.1 \times 10^{-4} M$ and $V_{max} = 5 \times 10^{-6} M s^{-1}$. The time dependences of $S^*(t)$, $S(t)$, $P^*(t)$ and $P(t)$ are shown in
 699 Fig. 7(b), left panel, subject to standard uni-directional Michaelis-Menten kinetics; and in Fig. 7(c), left panel, the
 700 time dependence of $ES^*(t)$ and $ES(t)$. The time dependence of $S^*(t) + S(t) = [S(t)]$ and $P^*(t) + P(t) = [P(t)]$
 701 are shown in Fig. 7(b), right panel, and $ES^*(t) + ES(t) = [ES(t)]$ and $[E(t)]$ are shown in Fig. 7(c), right panel,

702 which recapture conventional chemical kinetics of $[S(t)]$, $[ES(t)]$, $[P(t)]$ and $[E(t)]$, as required for
 703 mathematical and physical consistency.
 704

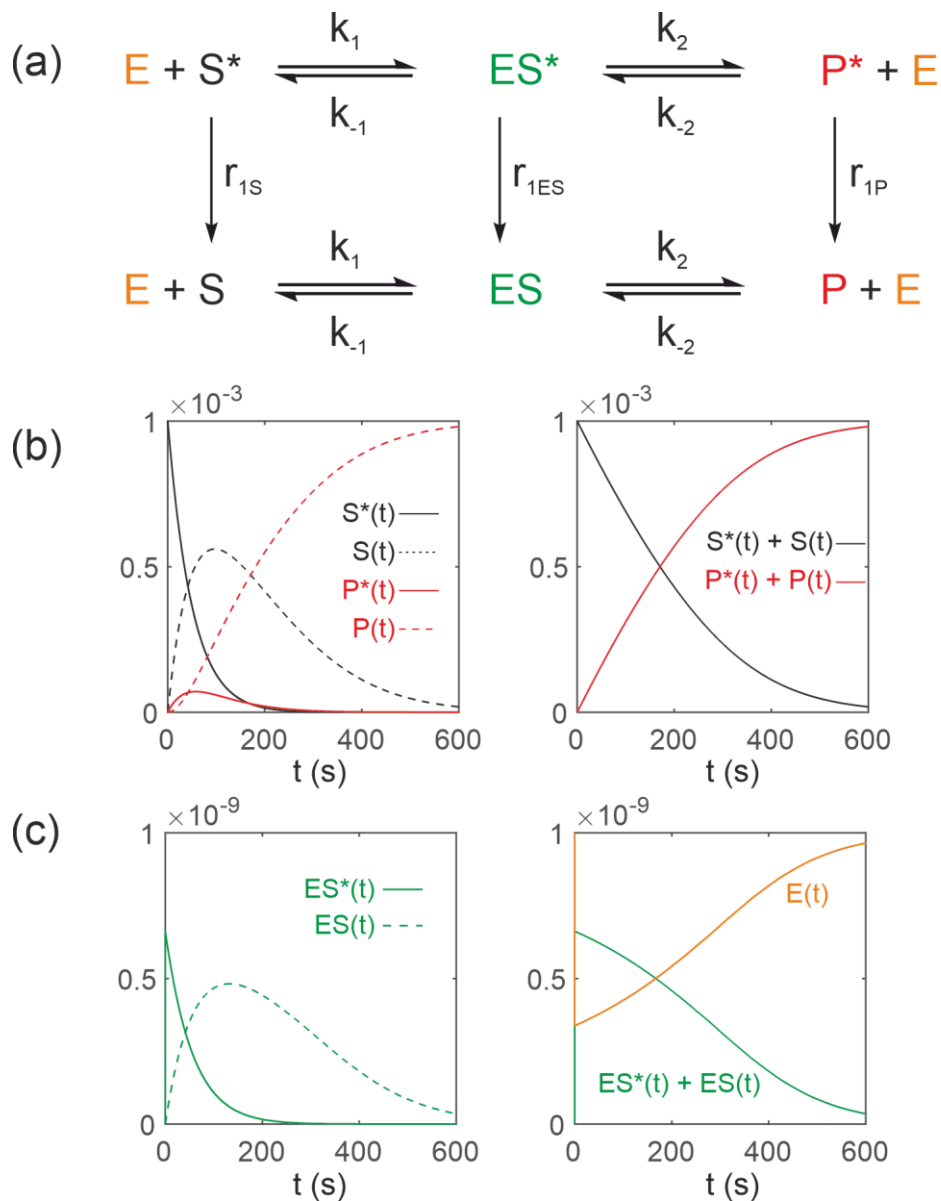


Figure 7 Simulated Michaelis-Menten kinetics for exchange of hyperpolarized solutes $E + S^* \leftrightarrow ES^* \leftrightarrow P^* + E$ conforming to conservation of mass, assuming initial hyperpolarized magnetization of only solute $S^*(0) = 0.001$ and $[E]_0 = 1 \times 10^{-9}$ M. Longitudinal relaxation rate constants were $R_{1S} = R_{1ES} = R_{1P} = 1/60s^{-1}$. The reaction rate constants were $k_1 = 1 \times 10^7 M^{-1}s^{-1}$, $k_{-1} = 1 \times 10^2 s^{-1}$, $k_2 = 5 \times 10^3 s^{-1}$ and $k_{-2} = 0 M^{-1}s^{-1}$, such that $K_M = 5.1 \times 10^{-4}$ M and $V_{max} = 5 \times 10^{-6} M s^{-1}$. Left panels: (b) Simulated time dependence of $S^*(t)$, $S(t)$, $P^*(t)$ and $P(t)$; and (c) simulated time dependence of $ES^*(t)$ and $ES(t)$. Right panels: (b) simulated time dependence of $S^*(t) + S(t) = [S(t)]$ and $P^*(t) + P(t) = [P(t)]$; and (c) $ES^*(t) + ES(t) = [ES(t)]$ and $[E(t)]$.

705 It is worth considering some of the consequences of Eq. (75) when studying enzyme mediated reactions
706 with hyperpolarized substrates. When the substrate concentration $[S^*(t) + S(t)]$ is much greater than K_M then the
707 rate of product formation $d([P^*(t) + P(t)]/dt)$ is given by $v = k_2[E]_0 = V_{max}$, which is constant (*i.e.*, it is
708 effectively a zero order reaction with respect to substrate concentration). The enzyme is said to be saturated; its
709 rate is independent of substrate concentration but V_{max} is proportional to the enzyme concentration $[E]_0$. When
710 the substrate concentration $[S^*(t) + S(t)]$ is much less than K_M then the rate of product formation
711 $d([P^*(t) + P(t)]/dt)$ is given by $V = k_2[E]_0[S^*(t) + S(t)]/K_M$ and the reaction is effectively first order with
712 respect to substrate concentration. Nevertheless, the rate is still proportional to $[E]_0$. The kinetics of enzyme
713 systems, and indeed enzyme kinetics in general, are a composite of the two parameters K_M and V_{max} . The influences
714 on one cannot be distinguished from the other on the basis of time-course experiments alone; separate
715 measurements that are needed to estimate the total enzyme concentration.

716 Further simulations were performed to explore the influence of a much shorter value of T_1^{ES} for the
717 enzyme substrate complex, while T_1^S and T_1^P were unchanged. Even if it were assumed to be very small *viz.*,
718 $T_1^{ES} = 276.4$ ms the time evolution was indistinguishable from that presented in Fig. 7; the corresponding curves
719 were superimposable. The signal that resided on the enzyme substrate complex ES^* was 6 orders of magnitude
720 lower than that of the substrate S^* and product P^* . Therefore, the kinetics of signal evolution were dominated by
721 T_1^S and T_1^P while changes in T_1^{ES} could be ignored. An exception to this analysis might occur if the active site were
722 next to a paramagnetic centre, such as is found in metalloproteins for which T_1^{ES} could be very much shorter than
723 predicted (see the relaxation theory section above).
724

725 5.3 Enzyme inhibition and hyperpolarized substrate kinetics

726 Our formalism can be readily extended to account for the influence of a ligand/solute to inhibit an enzyme. The
727 simplest case is when a solute binds reversibly to the free enzyme E to form an enzyme inhibitor complex EI;
728 hence, the enzyme becomes unable to bind and react with its substrate S. To describe this scenario, Eq. (68) is
729 modified to include an additional pathway for the loss of free enzyme:
730

$$\begin{aligned} \frac{d[E(t)]}{dt} = & -k_1[E(t)][S^*(t) + S(t)] + (k_{-1} + k_2)[ES^*(t) + ES(t)] - k_{-2}[E(t)][P^*(t) + P(t)] \\ & - k_3[E(t)][I(t)] + k_{-3}[EI(t)] \quad . \end{aligned} \quad (76)$$

731
732 The model is now extended to include differential equations describing the concentration of the inhibitor $[I(t)]$
733 and the enzyme-inhibitor complex $[EI(t)]$:
734

$$\begin{aligned} \frac{d[I(t)]}{dt} = & -k_3[E(t)][I(t)] + k_{-3}[EI(t)] \quad , \\ \frac{d[EI(t)]}{dt} = & k_3[E(t)][I(t)] - k_{-3}[EI(t)] \quad . \end{aligned} \quad (77)$$

Such equations can be incorporated into the Michaelis-Menten equations and we develop this next.

735 **5.3.1 Types of enzyme inhibition**

736 There are three commonly encountered types of reversible enzyme inhibition (Kuchel, 2009): (i) a *competitive*
 737 inhibitor is structurally similar to the substrate and binds preferentially in the active site of the free enzyme, E,
 738 thus preventing the substrate from binding and reacting; (ii) an *uncompetitive* inhibitor binds only to the enzyme-
 739 substrate complex and therefore causes substrate-concentration dependent inhibition; and (iii), a *non-competitive*
 740 inhibitor binds to both the free enzyme and to the enzyme-substrate complex; it causes a conformational change
 741 at the active site that inhibits (or even enhances) the reaction. Such an effect is referred to as allosteric inhibition
 742 (or activation).

743 Accounting for all three scenarios, the free enzyme concentration is given by:

744

$$[E(t)] = [E]_0 - [EI(t)] - [ES^*(t) + ES(t)] - [ESI^*(t) + ESI(t)] \quad (78)$$

745

746 Substituting:

747

$$\alpha = 1 + \frac{[I(t)]}{K_I} \quad \text{and} \quad \alpha' = 1 + \frac{[I(t)]}{K_I'} \quad (79)$$

748

749 where $K_I = [E(t)][I(t)]/[EI(t)]$ and $K_I' = [ES(t)][I(t)]/[ESI(t)]$, yields:

750

$$\frac{d([P^*(t) + P(t)])}{dt} = \frac{k_2[E]_0[S^*(t) + S(t)]}{\alpha K_M + \alpha'[S^*(t) + S(t)]} \quad (80)$$

751

752 The three types of enzyme inhibition can be distinguished by their influence on the kinetic parameters that are
 753 estimated in specially designed experiments performed on the enzyme over a range of substrate and inhibitor
 754 concentrations (Kuchel, 2009): (i) competitive inhibitors cause an increase in apparent K_M value while V_{max} is
 755 unchanged; (ii) uncompetitive inhibitors cause a reduction in V_{max} while the apparent K_M is unchanged; and (iii)
 756 non-competitive inhibitors cause both a reduction in V_{max} and an increase in apparent K_M .

757 An additional effect that can be considered is where either the substrate of the reaction $[S(t)]$, or the
 758 product of the reaction, $[P(t)]$, acts as the inhibitor, called unsurprisingly ‘substrate inhibition’ and ‘product
 759 inhibition’, respectively. The relevant enzyme kinetic equations are composed by substituting $[I(t)] =$
 760 $[S^*(t) + S(t)]$ or $[I(t)] = [P^*(t) + P(t)]$ in the above equations.

761

762

763 **6 Cofactors and unlabelled pools – Lactate Dehydrogenase**

764 We now consider a real system that is of contemporary interest for *in vivo* clinical studies using dDNP. It is lactate
 765 dehydrogenase (E.C. 1.1.1.27). Consider the LDH catalysed reaction of a hyperpolarized substrate; it follows an
 766 ordered sequential reaction in which $E + NADH \leftrightarrow E \cdot NADH + Pyr^* \leftrightarrow E \cdot NAD + Lac^* \leftrightarrow E + NAD^+$. Again, we
 767 assume that relaxation of magnetization occurs through T_1 processes to form a pool of reactants $E + NADH \leftrightarrow$
 768 $E \cdot NADH + Pyr \leftrightarrow E \cdot NAD + Lac \leftrightarrow E + NAD^+$ as shown in Fig. 8(a). The relevant differential equations used to
 769 describe the kinetics are (omitting the square brackets that denote concentration):

770

$$\frac{dPyr^*(t)}{dt} = -k_2 E \cdot NADH(t) Pyr^*(t) + k_{-2} E \cdot NAD(t) Lac^*(t) - R_1^P Pyr^*(t) \quad , \quad (81)$$

$$\frac{dLac^*(t)}{dt} = k_2 E \cdot NADH(t) Pyr^*(t) - k_{-2} E \cdot NAD(t) Lac^*(t) - R_1^L Lac^*(t) \quad , \quad (82)$$

$$\frac{dPyr(t)}{dt} = -k_2 E \cdot NADH(t) Pyr(t) + k_{-2} E \cdot NAD(t) Lac(t) + R_1^P Pyr^*(t) \quad , \quad (83)$$

$$\frac{dLac(t)}{dt} = k_2 E \cdot NADH(t) Pyr(t) - k_{-2} E \cdot NAD(t) Lac(t) + R_1^L Lac^*(t) \quad , \quad (84)$$

$$\frac{dNADH(t)}{dt} = -k_1 E(t) NADH(t) + k_{-1} E \cdot NADH(t) \quad , \quad (85)$$

$$\frac{dNAD(t)}{dt} = k_3 E \cdot NAD(t) - k_{-3} E(t) NAD(t) \quad , \quad (86)$$

$$\begin{aligned} \frac{dE \cdot NADH(t)}{dt} &= k_1 E(t) NADH(t) - k_{-1} E \cdot NADH(t) - k_2 E \cdot NADH(t) (Pyr^*(t) + Pyr(t)) \\ &\quad + k_{-2} E \cdot NAD(t) (Lac^*(t) + Lac(t)) \quad , \end{aligned} \quad (87)$$

$$\begin{aligned} \frac{dE \cdot NAD(t)}{dt} &= k_2 E \cdot NADH(t) (Pyr^*(t) + Pyr(t)) - k_{-2} E \cdot NAD(t) (Lac^*(t) + Lac(t)) \\ &\quad - k_3 E \cdot NAD(t) + k_{-3} E(t) NAD(t) \quad , \end{aligned} \quad (88)$$

$$\frac{dE(t)}{dt} = -k_1 E(t) NADH(t) + k_{-1} E \cdot NADH(t) + k_3 E \cdot NAD(t) - k_{-3} E(t) NAD(t) \quad , \quad (89)$$

771

772 where $E(t)$ is the concentration of free enzyme, $NAD(t)$ and $NADH(t)$ are the concentrations of the free co-
 773 factors, $E \cdot NAD(t)$ and $E \cdot NADH(t)$ are the concentrations of the enzyme-cofactor complexes and $Pyr(t)$ and
 774 $Lac(t)$ are the free substrates with relaxation rate constants R_1^P and R_1^L , respectively.

775 Mass is conserved as is confirmed by the fact that $d(Pyr^*(t) + Pyr(t) + Lac^*(t) + Lac(t))/dt = 0$.
 776 Enzyme concentration is conserved as is confirmed by $d(E \cdot NADH(t) + E \cdot NAD(t) + E(t))/dt = 0$ and
 777 cofactor pools are conserved as is confirmed by $d(NADH(t) + NAD(t) + E \cdot NADH(t) + E \cdot NAD(t))/dt = 0$.
 778 Therefore, provided $Pyr^*(0) + Pyr(0) = [Pyr]_0$ and $Lac^*(0) + Lac(0) = [Lac]_0$ then $Pyr^*(t) + Pyr(t) =$
 779 $[Pyr(t)]$ and $Lac^*(t) + Lac(t) = [Lac(t)]$, respectively.

Equations (81-89) can be written in matrix vector form as:

$$\frac{d}{dt} \begin{bmatrix} Pyr^*(t) \\ Lac^*(t) \\ Pyr(t) \\ Lac(t) \\ NADH(t) \\ NAD(t) \\ E.NADH(t) \\ E.NAD(t) \\ E(t) \end{bmatrix} = \begin{bmatrix} -k_2E.NADH(t) - R_1^P & k_{-2}E.NAD(t) & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ k_2E.NADH(t) & -k_{-2}E.NAD(t) - R_1^L & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ R_1^P & 0 & -k_2E.NADH(t) & k_{-2}E.NAD(t) & 0 & 0 & 0 & 0 & 0 \\ 0 & R_1^L & k_2E.NADH(t) & -k_{-2}E.NAD(t) & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -k_1E(t) & 0 & k_{-1} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -k_{-3}E(t) & 0 & k_3 & 0 \\ -k_2E.NADH(t) & k_{-2}E.NAD(t) & -k_2E.NADH(t) & k_{-2}E.NAD(t) & k_1E(t) & k_{-3}E(t) & -k_{-1} & 0 & 0 \\ k_2E.NADH(t) & -k_{-2}E.NAD(t) & k_2E.NADH(t) & -k_{-2}E.NAD(t) & 0 & 0 & 0 & -k_3 & 0 \\ 0 & 0 & 0 & 0 & k_1E(t) & -k_{-3}E(t) & k_{-1} & k_3 & 0 \end{bmatrix} \begin{bmatrix} Pyr^*(t) \\ Lac^*(t) \\ Pyr(t) \\ Lac(t) \\ NADH(t) \\ NAD(t) \\ E.NADH(t) \\ E.NAD(t) \\ E(t) \end{bmatrix}. \quad (90)$$

780

781 We can apply a similarity transform given by:

$$U = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}. \quad (91)$$

782

783 To yield an equation of motion in the transformed basis vector given by:

784

$$\frac{d}{dt} \begin{bmatrix} Pyr^*(t) \\ Lac^*(t) \\ Pyr^*(t) + Pyr(t) \\ Lac^*(t) + Lac(t) \\ NADH(t) \\ NAD(t) \\ E.NADH(t) \\ E.NAD(t) \\ E(t) \end{bmatrix} = \begin{bmatrix} -k_2E.NADH(t) - R_1^P & k_{-2}E.NAD(t) & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ k_2E.NADH(t) & -k_{-2}E.NAD(t) - R_1^L & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2E.NADH(t) & k_{-2}E.NAD(t) & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & k_2E.NADH(t) & -k_{-2}E.NAD(t) & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -k_1E(t) & 0 & k_{-1} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -k_{-3}E(t) & 0 & k_3 & 0 \\ -k_2E.NADH(t) & k_{-2}E.NAD(t) & -k_2E.NADH(t) & k_{-2}E.NAD(t) & k_1E(t) & k_{-3}E(t) & -k_{-1} & 0 & 0 \\ 0 & 0 & k_2E.NADH(t) & -k_{-2}E.NAD(t) & 0 & 0 & 0 & -k_3 & 0 \\ 0 & 0 & 0 & 0 & k_1E(t) & -k_{-3}E(t) & k_{-1} & k_3 & 0 \end{bmatrix} \begin{bmatrix} Pyr^*(t) \\ Lac^*(t) \\ Pyr^*(t) + Pyr(t) \\ Lac^*(t) + Lac(t) \\ NADH(t) \\ NAD(t) \\ E.NADH(t) \\ E.NAD(t) \\ E(t) \end{bmatrix}. \quad (92)$$

785

787 Figure 8(b) shows numerical simulations of the time evolution of the system that is described by Eqs.
788 (81-89) with initial hyperpolarized signal/concentration (see above for a comment on this aspect) $Pyr^*(t) =$
789 0.001 and longitudinal relaxation rate constants $R_1^p = R_1^l = 1/60s^{-1}$. The kinetic parameters used for lactate
790 dehydrogenase were as previously published (Zewe and Fromm, 1962; Witney et al., 2011) for the rabbit muscle
791 enzyme. Enzyme concentration was $[E]_0 = 1.2 \times 10^{-9} M$ and rate constants $k_1 = 1.03 \times 10^8 M^{-1}s^{-1}$, $k_{-1} =$
792 $549 s^{-1}$, $k_2 = 6.72 \times 10^6 M^{-1}s^{-1}$, $k_{-2} = 3.44 \times 10^4 M^{-1}s^{-1}$, $k_3 = 842 s^{-1}$, and $k_{-3} = 9.12 \times 10^5 M^{-1}s^{-1}$.

793 The computed time dependence of polarized and unpolarized pools $Pyr^*(t)$, $Pyr(t)$, $Lac^*(t)$ and
794 $Lac(t)$ are shown in Fig. 8(b), left panel. The time dependence of $[E(t)]$, $[E.NAD(t)]$ and $[E.NADH(t)]$ are
795 shown in Fig. 8(b), middle panel. The time dependence of $Pyr^*(t) + Pyr(t) = [Pyr(t)]$, $Lac^*(t) + Lac(t) =$
796 $[Lac(t)]$, $[NAD(t)]$ and $[NADH(t)]$ are shown in Fig. 8(b), right panel. Several interesting features are evident.
797 First, the model predicted the expected time dependences of both hyperpolarized pyruvate $Pyr^*(t)$ and its
798 conversion to $Lac^*(t)$. Under the conditions of the simulation, the free enzyme $[E(t)]$ was rapidly depleted to
799 form an equilibrium of $[E.NAD(t)]$ and $[E.NADH(t)]$. During the reaction with $Pyr^*(t)$, the equilibrium
800 position of the enzyme was altered to give a final equilibrium position that could then be appreciated from the
801 total pools of $Pyr^*(t) + Pyr(t) = [Pyr(t)]$ and $Lac^*(t) + Lac(t) = [Lac(t)]$, which predicts a net conversion
802 of $[Pyr(t)]$ to $[Lac(t)]$ of ~10%. Also note, from this simulation, the activity of the LDH switches off at $t = 200s$
803 since the concentration of $[NADH(t)]$ is limiting in this simulation i.e. it becomes depleted. This does not happen
804 if $[NADH(t)]$ is increased. In a normal cellular context NADH would be regenerated by glyceraldehyde 3-
805 phosphate dehydrogenase during glycolysis.

806 Finally, we consider real case scenarios that are reported in the literature i.e., measurement of
807 hyperpolarized $[1-^{13}C]$ pyruvate kinetics in living cells (Andersson et al., 2007; Day et al., 2007; Karlsson et al.,
808 2007; Hill et al., 2013a; Hill et al., 2013b; Lin et al., 2014; Pagès et al., 2014; Belouche-Babari et al., 2017).
809 Figure 8(c) shows the situation where the LDH expression level is altered, e.g., by the progression of disease
810 (LDH expression is known to be upregulated in more aggressive cancer phenotypes (Albers et al., 2008) or down
811 regulated during therapy (Ward et al., 2010), which can be explored through the value of $[E]_0$. Figure 8(c) shows
812 simulations of the $Lac^*(t)$ signal under the conditions that: $[E]_0 = (i) 0.6 \times 10^{-9} M$; $(ii) 1.2 \times 10^{-9} M$; and (iii)
813 $2.4 \times 10^{-9} M$, while all other parameters remained unchanged, relative to those used for Fig. 8(b). It is apparent
814 that increased enzyme expression leads to an increase in the apparent rate of conversion of $Pyr^*(t)$ to $Lac^*(t)$
815 even in the absence of a change in enzyme activity, as seen in real experiments. Another situation that is frequently
816 encountered is the change in the pool size of endogenous lactate, for example in response to hypoxia, which can
817 be explored through the parameter $Lac(0)$. Figure 8(d) shows simulations of the $Lac^*(t)$ signal under the
818 conditions that: $Lac(0) = (i) 0 mM$; $(ii) 20 mM$; and $(iii) 40 mM$, while all other parameters remained unchanged,
819 relative to those used to generate Fig. 8(b). The model therefore predicts that an increased pool of endogenous
820 unpolarized lactate leads to an increase in the rate of conversion of $Pyr^*(t)$ to $Lac^*(t)$, as reported widely in the
821 literature (Day et al., 2007).

822

823

824 **7 Conclusions**

825 We have described an approach to formulating the kinetic master equations that describe the time evolution of
826 hyperpolarized ^{13}C NMR signals in reacting (bio)chemical systems, including enzymes with two or more
827 substrates, and various enzyme reaction mechanisms as classified by Cleland. The modelling can be the basis of
828 simulating many pertinent features that are seen in dDNP experiments. Derivation of the Michaelis-Menten
829 equation in the context of dDNP experiments illustrates why formation of a hyperpolarized enzyme-substrate
830 complex does *not* cause an appreciable loss of the signal from the substrate or product. It was also able to answer
831 why the concentration of an unlabelled pool of substrate, for example ^{12}C lactate, causes an increase in the rate of
832 exchange of the ^{13}C labelled pool, and to what extent the equilibrium position of an enzyme-catalyzed reaction,
833 for example LDH, is altered upon adding hyperpolarized substrate. The formalism described here should
834 contribute to a fuller mechanistic understanding of the time courses derived from dDNP experiments and will be
835 relevant to ongoing clinical applications using dDNP.

836

837

838 **Code/Data availability**

839 All Matlab codes are reproduced in the Supplementary Information and free to adapt for personal use. For any
840 further clarification please contact the Corresponding author directly.

841

842

843 **Author contributions**

844 All authors planned the research, conducted the research and wrote the paper.

845

846 **Competing interests**

847 The authors declare that they have no conflict of interest.

848

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856

857

858 **References**

859

860 Albers, M. J., Bok, R., Chen, A. P., Cunningham, C. H., Zierhut, M. L., Zhang, V. Y., Kohler, S. J., Tropp, J.,
861 Hurd, R. E., Yen, Y. F., Nelson, S. J., Vigneron, D. B., and Kurhanewicz, J.: Hyperpolarized ^{13}C lactate, pyruvate,
862 and alanine: noninvasive biomarkers for prostate cancer detection and grading, *Cancer Res.*, 68, 8607-8615,
863 doi:10.1158/0008-5472.Can-08-0749, 2008.

864 Allard, P., Helgstrand, M., and Hard, T.: The complete homogeneous master equation for a heteronuclear two-
865 spin system in the basis of cartesian product operators, *J. Magn. Reson.*, 134, 7-16, doi:10.1006/jmre.1998.1509,
866 1998.

867 Andersson, L., Karlsson, M., Gisselsson, A., Jensen, P., Hansson, G., Månsson, S., in 't Zandt, R., and Lerche,
868 M.: Hyperpolarized ^{13}C -DNP-NMR allow metabolic in vitro studies over minutes, *Proc. Intl. Soc. Mag. Reson.*
869 *Med.*, 2007, 542,

870 Ardenkjaer-Larsen, J. H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M. H., Servin, R., Thaning,
871 M., and Golman, K.: Increase in signal-to-noise ratio of $> 10,000$ times in liquid-state NMR, *Proc. Natl. Acad.*
872 *Sci. U. S. A.*, 100, 10158-10163, doi:10.1073/pnas.1733835100, 2003.

873 Ardenkjaer-Larsen, J. H., Boebinger, G. S., Comment, A., Duckett, S., Edison, A. S., Engelke, F., Griesinger, C.,
874 Griffin, R. G., Hilty, C., Maeda, H., Parigi, G., Prisner, T., Ravera, E., van Bentum, J., Vega, S., Webb, A.,
875 Luchinat, C., Schwalbe, H., and Frydman, L.: Facing and overcoming sensitivity challenges in biomolecular NMR
876 spectroscopy, *Angew. Chem. Int. Ed. Engl.*, 54, 9162-9185, doi:10.1002/anie.201410653, 2015.

877 Bechmann, M., Dusold, S., Sebald, A., Shuttleworth, W. A., Jakeman, D. L., Mitchell, D. J., and Evans, J. N. S.:
878 ^{13}C chemical shielding tensor orientations in a phosphoenolpyruvate moiety from ^{13}C rotational-resonance MAS
879 NMR lineshapes, *Solid State Sci.*, 6, 1097-1105, doi.org/10.1016/j.solidstatesciences.2004.04.021, 2004.

880 Belouche-Babari, M., Wantuch, S., Casals Galobart, T., Koniordou, M., Parkes, H. G., Arunan, V., Chung, Y.
881 L., Eykyn, T. R., Smith, P. D., and Leach, M. O.: MCT1 inhibitor AZD3965 increases mitochondrial metabolism,
882 facilitating combination therapy and noninvasive magnetic resonance spectroscopy, *Cancer Res.*, 77, 5913-5924,
883 10.1158/0008-5472.CAN-16-2686, 2017.

884 Bengs, C., and Levitt, M. H.: A master equation for spin systems far from equilibrium, *J. Magn. Reson.*, 310,
885 106645, doi:10.1016/j.jmr.2019.106645, 2020.

886 Blumberg, W. E.: Nuclear spin-lattice relaxation caused by paramagnetic impurities, *Phys. Rev.*, 119, 79-84,
887 doi.org/10.1103/PhysRev.119.79, 1960.

888 Chiavazza, E., Kubala, E., Gringeri, C. V., Duwel, S., Durst, M., Schulte, R. F., and Menzel, M. I.: Earth's
889 magnetic field enabled scalar coupling relaxation of ^{13}C nuclei bound to fast-relaxing quadrupolar ^{14}N in amide
890 groups, *J. Magn. Reson.*, 227, 35-38, doi:10.1016/j.jmr.2012.11.016, 2013.

891 Cleland, W. W.: Enzyme kinetics, *Annu. Rev. Biochem.*, 36, 77-112, doi:10.1146/annurev.bi.36.070167.000453,
892 1967.

893 Comment, A., and Merritt, M. E.: Hyperpolarized magnetic resonance as a sensitive detector of metabolic
894 function, *Biochemistry*, 53, 7333-7357, doi:10.1021/bi501225t, 2014.

895 Cook, P. F., and Cleland, W. W.: Enzyme kinetics and mechanism, Taylor & Francis Group, New York, 2007.

896 Daniels, C. J., McLean, M. A., Schulte, R. F., Robb, F. J., Gill, A. B., McGlashan, N., Graves, M. J., Schwaiger,
897 M., Lomas, D. J., Brindle, K. M., and Gallagher, F. A.: A comparison of quantitative methods for clinical imaging
898 with hyperpolarized ^{13}C -pyruvate, *NMR Biomed.*, 29, 387-399, 10.1002/nbm.3468, 2016.

899 Day, S. E., Kettunen, M. I., Gallagher, F. A., Hu, D. E., Lerche, M., Wolber, J., Golman, K., Ardenkjaer-Larsen,
900 J. H., and Brindle, K. M.: Detecting tumor response to treatment using hyperpolarized ^{13}C magnetic resonance
901 imaging and spectroscopy, *Nat. Med.*, 13, 1382-1387, doi:10.1038/nm1650, 2007.

902 Elliott, S. J., Bengs, C., Brown, L. J., Hill-Cousins, J. T., O'Leary, D. J., Pileio, G., and Levitt, M. H.: Nuclear
903 singlet relaxation by scalar relaxation of the second kind in the slow-fluctuation regime, *J. Chem. Phys.*, 150,
904 064315, 10.1063/1.5074199, 2019.

905 Endre, Z. H., Chapman, B. E., and Kuchel, P. W.: Intra-erythrocyte microviscosity and diffusion of specifically
906 labeled [*glycyl*- α - ^{13}C]glutathione by using ^{13}C NMR, *Biochem. J.*, 216, 655-660, doi:10.1042/bj2160655, 1983.

907 Ernst, R. R., Bodenhausen, G., and Wokaun, A.: Principles of nuclear magnetic resonance in one and two
908 dimensions, Clarendon Press, Oxford, 1987.

909 Gabellieri, C., Reynolds, S., Lavie, A., Payne, G. S., Leach, M. O., and Eykyn, T. R.: Therapeutic target
910 metabolism observed using hyperpolarized ^{15}N choline, *J. Am. Chem. Soc.*, 130, 4598-4599,
911 doi:10.1021/ja8001293, 2008.

912 Golman, K., Ardenkjaer-Larsen, J. H., Petersson, J. S., Mansson, S., and Leunbach, I.: Molecular imaging with
913 endogenous substances, *Proc. Natl. Acad. Sci. U. S. A.*, 100, 10435-10439, doi:10.1073/pnas.1733836100, 2003.

914 Golman, K., in't Zandt, R., Lerche, M., Pehrson, R., and Ardenkjaer-Larsen, J. H.: Metabolic imaging by
915 hyperpolarized ^{13}C magnetic resonance imaging for in vivo tumor diagnosis, *Cancer Res.*, 66, 10855-10860,
916 doi:10.1158/0008-5472.CAN-06-2564, 2006.

917 Helgstrand, M., Hard, T., and Allard, P.: Simulations of NMR pulse sequences during equilibrium and non-
918 equilibrium chemical exchange, *J. Biomol. NMR*, 18, 49-63, doi:10.1023/a:1008309220156, 2000.

919 Hill, D. K., Jamin, Y., Orton, M. R., Tardif, N., Parkes, H. G., Robinson, S. P., Leach, M. O., Chung, Y. L., and
920 Eykyn, T. R.: ^1H NMR and hyperpolarized ^{13}C NMR assays of pyruvate-lactate: a comparative study, *NMR*
921 *Biomed.*, 26, 1321-1325, 10.1002/nbm.2957, 2013a.

922 Hill, D. K., Orton, M. R., Mariotti, E., Boulton, J. K., Panek, R., Jafar, M., Parkes, H. G., Jamin, Y., Miniotis, M.
923 F., Al-Saffar, N. M., Belouche-Babari, M., Robinson, S. P., Leach, M. O., Chung, Y. L., and Eykyn, T. R.: Model
924 free approach to kinetic analysis of real-time hyperpolarized ^{13}C magnetic resonance spectroscopy data, *Plos One*,
925 8, e71996, doi:10.1371/journal.pone.0071996, 2013b.

926 Hore, P. J., Jones, J., and Wimperis, S.: *NMR: The Toolkit*, Second Edition ed., Oxford Chemistry Primers,
927 Oxford University Press, 2015.

928 Hu, S., Lustig, M., Balakrishnan, A., Larson, P. E. Z., Bok, R., Kurhanewicz, J., Nelson, S. J., Goga, A., Pauly, J.
929 M., and Vigneron, D. B.: 3D compressed sensing for highly accelerated hyperpolarized ^{13}C MRSI with in vivo
930 applications to transgenic mouse models of cancer, *Magn. Reson. Med.*, 63, 312-321, 10.1002/mrm.22233, 2010.

931 Johnson, J.: Thermal agitation of electricity in conductors, *Phys. Rev.*, 32, 97-109, doi:10.1103/physrev.32.97,
932 1928.

933 Karlsson, M., Andersson, L., Jensen, P., Hansson, G., Gisselsson, A., Månsson, S., in 't Zandt, R., and Lerche,
934 M.: Kinetic data From cellular assay using hyperpolarized ^{13}C -DNP-NMR, *Proc. Intl. Soc. Mag. Reson. Med.*,
935 2007, 1314,

936 Keeler, J.: *Understanding NMR spectroscopy*, Second Edition ed., John Wiley & Sons, Ltd, 2010.

937 Keshari, K. R., and Wilson, D. M.: Chemistry and biochemistry of ^{13}C hyperpolarized magnetic resonance using
938 dynamic nuclear polarization, *Chem. Soc. Rev.*, 43, 1627-1659, doi:10.1039/C3cs60124b, 2014.

939 Kowalewski, J., and Maler, L.: Nuclear spin relaxation in liquids: theory, experiments and applications, 2nd
940 Edition ed., CRC Press, Taylor & Francis, Boca Raton, FL, 2019.

941 Kubica, D., Wodynski, A., Kraska-Dziadecka, A., and Gryff-Keller, A.: Scalar relaxation of the second kind. A
942 potential source of information on the dynamics of molecular movements. 3. A ^{13}C nuclear spin relaxation study
943 of CBrX_3 ($\text{X} = \text{Cl}, \text{CH}_3, \text{Br}$) molecules, *J. Phys. Chem. A*, 118, 2995-3003, doi:10.1021/jp501064c, 2014.

944 Kuchel, P. W.: Schaum's outline of biochemistry, 3rd Edition ed., McGraw-Hill Companies, Inc, 2009.

945 Kuchel, P. W., Karlsson, M., Lerche, M. H., Shishmarev, D., and Ardenkjaer-Larsen, J. H.: Rapid zero-trans
946 kinetics of Cs^+ exchange in human erythrocytes quantified by dissolution hyperpolarized $^{133}\text{Cs}^+$ NMR
947 spectroscopy, *Sci. Rep.*, 9, 19726, doi:10.1038/s41598-019-56250-z, 2019.

948 Kuchel, P. W., and Shishmarev, D.: Dissolution dynamic nuclear polarization NMR studies of enzyme kinetics:
949 Setting up differential equations for fitting to spectral time courses, *J. Magn. Reson. Open*, 1, 100001,
950 doi.org/10.1016/j.jmro.2020.100001, 2020.

951 Kuhne, R. O., Schaffhauser, T., Wokaun, A., and Ernst, R. R.: Study of transient-chemical reactions by NMR -
952 fast stopped-flow Fourier-transform experiments, *J. Magn. Reson.*, 35, 39-67, doi:10.1016/0022-2364(79)90077-
953 5, 1979.

954 Levitt, M. H., and Dibari, L.: Steady-state in magnetic-resonance pulse experiments, *Phys. Rev. Lett.*, 69, 3124-
955 3127, DOI 10.1103/PhysRevLett.69.3124, 1992.

956 Lin, G., Hill, D. K., Andrejeva, G., Boulton, J. K. R., Troy, H., Fong, A. C. L. F. W. T., Orton, M. R., Panek, R.,
957 Parkes, H. G., Jafar, M., Koh, D. M., Robinson, S. P., Judson, I. R., Griffiths, J. R., Leach, M. O., Eykyn, T. R.,
958 and Chung, Y. L.: Dichloroacetate induces autophagy in colorectal cancer cells and tumours, *Br. J. Cancer*, 111,
959 375-385, 10.1038/bjc.2014.281, 2014.

960 Mariotti, E., Orton, M. R., Eerbeek, O., Ashruf, J. F., Zuurbier, C. J., Southworth, R., and Eykyn, T. R.: Modeling
961 non-linear kinetics of hyperpolarized $[1-^{13}\text{C}]$ pyruvate in the crystalloid-perfused rat heart, *NMR Biomed.*, 29,
962 377-386, 10.1002/nbm.3464, 2016.

963 Matson, G. B.: Methyl NMR relaxation: The effects of spin rotation and chemical shift anisotropy mechanisms,
964 *J. Chem. Phys.*, 67, 5152-5161, doi.org/10.1063/1.434744, 1977.

965 McConnell, H. M.: Reaction rates by nuclear magnetic resonance, *J. Chem. Phys.*, 28, 430-431,
966 doi:10.1063/1.1744152, 1958.

967 Mieville, P., Ahuja, P., Sarkar, R., Jannin, S., Vasos, P. R., Gerber-Lemaire, S., Mishkovsky, M., Comment, A.,
968 Gruetter, R., Ouari, O., Tordo, P., and Bodenhausen, G.: Scavenging free radicals to preserve enhancement and
969 extend relaxation times in NMR using dynamic nuclear polarization, *Angew. Chem. Int. Ed. Engl.*, 49, 6182-
970 6185, doi:10.1002/anie.201000934, 2010.

971 Milani, J., Vuichoud, B., Bornet, A., Mieville, P., Mottier, R., Jannin, S., and Bodenhausen, G.: A magnetic tunnel
972 to shelter hyperpolarized fluids, *Rev. Sci. Instrum.*, 86, 024101, doi:10.1063/1.4908196, 2015.

973 Pagès, G., Puckeridge, M., Guo, L. F., Tan, Y. L., Jacob, C., Garland, M., and Kuchel, P. W.: Transmembrane
974 exchange of hyperpolarized ^{13}C -urea in human erythrocytes: Subminute timescale kinetic analysis, *Biophys. J.*,
975 105, 1956-1966, doi:10.1016/j.bpj.2013.09.034, 2013.

976 Pagès, G., Tan, Y. L., and Kuchel, P. W.: Hyperpolarized $[1-^{13}\text{C}]$ pyruvate in lysed human erythrocytes: Effects
977 of co-substrate supply on reaction time courses, *NMR Biomed.*, 27, 1203-1210, 10.1002/nbm.3176, 2014.

978 Pagès, G., and Kuchel, P. W.: FmR α analysis: Rapid and direct estimation of relaxation and kinetic parameters
979 from dynamic nuclear polarization time courses, *Magn. Reson. Med.*, 73, 2075-2080, doi:10.1002/mrm.25345,
980 2015.

981 Pell, A. J., Pintacuda, G., and Grey, C. P.: Paramagnetic NMR in solution and the solid state, *Prog. Nucl. Magn.*
982 *Reson. Spectrosc.*, 111, 1-271, doi:10.1016/j.pnmrs.2018.05.001, 2019.

983 Pileio, G.: Singlet state relaxation via scalar coupling of the second kind, *J. Chem. Phys.*, 135, 174502,
984 doi:10.1063/1.3651479, 2011.

985 Shishmarev, D., Kuchel, P. W., Pagès, G., Wright, A. J., Hesketh, R. L., Kreis, F., and Brindle, K. M.: Glyoxalase
986 activity in human erythrocytes and mouse lymphoma, liver and brain probed with hyperpolarized ^{13}C -
987 methylglyoxal, *Commun. Biol.*, 1, 232, doi:10.1038/s42003-018-0241-1, 2018a.

988 Shishmarev, D., Wright, A. J., Rodrigues, T. B., Pileio, G., Stevanato, G., Brindle, K. M., and Kuchel, P. W.: Sub-
989 minute kinetics of human red cell fumarase: ^1H spin-echo NMR spectroscopy and ^{13}C rapid-dissolution dynamic
990 nuclear polarization, *NMR Biomed.*, 31, e3870, doi:10.1002/nbm.3870, 2018b.

991 Valensin, G., Kushnir, T., and Navon, G.: Selective and non-selective proton spin-lattice relaxation studies of
992 enzyme-substrate interactions, *J. Magn. Reson.*, 46, 23-29, doi:10.1016/0022-2364(82)90159-7, 1982.

993 van Heeswijk, R. B., Uffmann, K., Comment, A., Kurdzesau, F., Perazzolo, C., Cudalbu, C., Jannin, S., Konter,
994 J. A., Hautle, P., van den Brandt, B., Navon, G., van der Klink, J. J., and Gruetter, R.: Hyperpolarized lithium-6
995 as a sensor of nanomolar contrast agents, *Magn. Reson. Med.*, 61, 1489-1493, doi:10.1002/mrm.21952, 2009.

996 Ward, C. S., Venkatesh, H. S., Chaumeil, M. M., Brandes, A. H., VanCrickinge, M., Dafni, H., Sukumar, S.,
997 Nelson, S. J., Vigneron, D. B., Kurhanewicz, J., James, C. D., Haas-Kogan, D. A., and Ronen, S. M.: Noninvasive
998 detection of target modulation following phosphatidylinositol 3-kinase inhibition using hyperpolarized ^{13}C
999 magnetic resonance spectroscopy, *Cancer Res.*, 70, 1296-1305, doi:10.1158/0008-5472.Can-09-2251, 2010.

1000 Weber, E. M. M., Kurzbach, D., and Abergel, D.: A DNP-hyperpolarized solid-state water NMR MASER:
1001 observation and qualitative analysis, *Phys. Chem. Chem. Phys.*, 21, 21278-21286, doi:10.1039/c9cp03334c, 2019.

1002 Wilbur, D. J., Norton, R. S., Clouse, A. O., Addleman, R., and Allerhand, A.: Determination of rotational
1003 correlation times of proteins in solution from carbon-13 spin-lattice relaxation measurements. Effect of magnetic
1004 field strength and anisotropic rotation, *J. Am. Chem. Soc.*, 98, 8250-8254, doi:10.1021/ja00441a059, 1976.

1005 Witney, T. H., Kettunen, M. I., and Brindle, K. M.: Kinetic modeling of hyperpolarized ^{13}C label exchange
1006 between pyruvate and lactate in tumor cells, *J. Biol. Chem.*, 286, 24572-24580, doi:10.1074/jbc.M111.237727,
1007 2011.

1008 Wolber, J., Ellner, F., Fridlund, B., Gram, A., Johannesson, H., Hansson, G., Hansson, L. H., Lerche, M. H.,
1009 Mansson, S., Servin, R., Thaning, M., Golman, K., and Ardenkjaer-Larsen, J. H.: Generating highly polarized
1010 nuclear spins in solution using dynamic nuclear polarization, *Nucl. Instrum. Meth. A*, 526, 173-181,
1011 doi:10.1016/j.nima.2004.03.171, 2004.

1012 Yen, Y. F., Kohler, S. J., Chen, A. P., Tropp, J., Bok, R., Wolber, J., Albers, M. J., Gram, K. A., Zierhut, M. L.,
1013 Park, I., Zhang, V., Hu, S., Nelson, S. J., Vigneron, D. B., Kurhanewicz, J., Dirven, H. A. A. M., and Hurd, R. E.:
1014 Imaging considerations for in vivo ^{13}C metabolic mapping using hyperpolarized ^{13}C pyruvate, *Magn. Reson. Med.*,
1015 62, 1-10, 10.1002/mrm.21987, 2009.

1016 Zewe, V., and Fromm, H. J.: Kinetic studies of rabbit muscle lactate dehydrogenase, *J. Biol. Chem.*, 237, 1668-
1017 1675, doi.org/10.1016/S0021-9258(19)83760-2, 1962.

1018 Zierhut, M. L., Yen, Y. F., Chen, A. P., Bok, R., Albers, M. J., Zhang, V., Tropp, J., Park, I., Vigneron, D. B.,
1019 Kurhanewicz, J., Hurd, R. E., and Nelson, S. J.: Kinetic modeling of hyperpolarized $^{13}\text{C}_1$ -pyruvate metabolism in
1020 normal rats and TRAMP mice, *J. Magn. Reson.*, 202, 85-92, 10.1016/j.jmr.2009.10.003, 2010.

1021