Supporting Information: QUESP and QUEST revisited – fast and accurate quantitative CEST experiments

Part I: single far off-resonant pool, cw saturation

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Revised QUESP formulae

For referencing within this document we recall the revised equations [4) and [12) from the main text (here equations [1] and [2), respectively):

$$MTR_{asym}(B_1,t_p)=\frac{f_b k_b\cdot\alpha}{R_{1a}+f_b k_b\cdot\alpha}(1-e^{-(R_{1a}+f_b k_b\cdot\alpha)t_p}) \tag{1}$$

$$MTR_{asym}=\frac{f_b k_b\cdot\alpha}{R_{1a}+f_b k_b\cdot\alpha}+(Z_i-1)e^{-R_{1a}t_p}-(Z_i-\frac{R_{1a}}{R_{1a}+f_b k_b\cdot\alpha})e^{-(R_{1a}+f_b k_b\cdot\alpha)t_p} \tag{2}$$

with

$$\alpha=\frac{\omega_1^2}{\omega_1^2+k_b(k_b+R_{2b})}.$$

Normalization with magnetization other than M_0 in steady state

Normalization with magnetization other than M_0 (i.e. $M_{offres}<<M_0$) increases the CEST effect in MTR_{asym} by the factor $\frac{M_0}{M_{offres}}$:

$$\frac{Mz(-\delta\omega_b,t_p)}{M_{offres}}-\frac{Mz(+\delta\omega_b,t_p)}{M_{offres}}=MTR_{asym}\cdot\frac{M_0}{M_{offres}}$$

This is a crucial issue when comparing the MTR_{asym} of differently normalized data. Namely, the reported CEST effect of 5% relates only to 5% of the value that was used for normalization. If this is not M_0 but M_{offres}, it can depend on the actual size of M_{offres} due to relaxation, in which case the 5% CEST effect is just relative to competing saturation transfer effects and not relative to M_0. The quantification methods described are very sensitive to this as discussed below.

CEST simulation system

In the following, the same paraCEST system as studied in the manuscript is assumed, neglecting spillover and MT effects. A cw irradiation of amplitude B_1 and duration t_p was used. The simulation parameters were:

- R_{1a}=0.3 Hz R_{2a}=0.5 Hz, R_{1b}=1 Hz, R_{2b}=50 Hz
- k_b=1-10000 Hz, f_b= 0.000135, $\delta\omega_b$=50 ppm
- B_0=7T, B_1=1-30 µT (standard 10 µT), t_p=0-10 s (standard 3 s)
- The initial magnetization before saturation was set to Z_i=0.3.
- We assume that a far off-resonant saturated signal is used as a reference, e.g $M_{offres}=M_{sat}$(-80 ppm).

For a paraCEST agent in water (no MT, no spillover), the condition $M_{offres} = M_0$ is valid for the case of a steady-state experiment. Also the condition $M_{offres} = M_i = M_0$ is valid in the case of fully relaxed initial magnetization $M_i = M_0$. However, in the case of short saturation and $M_i < M_0$, the value used for normalization $M_{offres} < M_0$. In addition, if saturation times are varied the M_{sat} values and also the normalization $M_{offres}(t_p)$ value will depend on the actual saturation time, thus each Z-spectrum for a different t_p will have a different normalization value. Thus two types of normalization can be performed: (i) each Z-spectrum is normalized by the same $M_{offres,single}$ value, or (ii) each Z-spectrum is normalized by the $M_{offres,int}$ measured for each saturation time/power. Whereas case (ii) might be the most common case that users perform, we first focus on the simpler case (i).

Case (i): Z_i=0.3, Normalization by a single M_{offres}

Initially, the *MTR was normalized by M_0* for Z_i=0.3 and simulated for different saturation times, saturation powers and exchange rates (circles in Figure S1a-c). The revised analytical solutions including Z_i (equation [2]) were used to describe the data perfectly (solid lines in Figure S1a-c). The same experiment was repeated using the $M_{offres} = M_{sat}$(-80 ppm) after 3 s irradiation as a value for normalization, which specifically is $M_{offres} = 0.71 \times M_0$ for al B_1. As expected, the simulated MTR values (circles in Figure S1d-f) were higher by the factor 1/0.71 than estimated by the analytical solution. However, if the off-resonant normalization M_{offres}/M_0 is known, this factor can be incorporated in the analytic formula (equation [2]) to result in :

$$MTR_{asym} \cdot \frac{M_0}{M_{offres}} = \frac{Mz(-\delta\omega_b, t_p)}{M_{offres}} - \frac{Mz(+\delta\omega_b, t_p)}{M_{offres}} = \left(\frac{R_{ex}}{R_{1a}+R_{ex}} + (Z_i - 1)e^{-R_{1a}t_p} - (Z_i - \frac{R_{1a}}{R_{1a}+R_{ex}})e^{-(R_{1a}+R_{ex})t_p} \right) \cdot \frac{M_0}{M_{offres}} \tag{3}$$

In this case, the revised equation [3] with the appropriate normalization factor is able to fit the data correctly (Figure S3g-i). Without it, the fitted concentration values would be higher by the factor of 1/0.71 as well.

Note: The Z_i used in the BM simulation and required by equations [2] and [3] is the initial value normalized by M_0. If Z_i was calculated by M_i/M_{offres}, it would be also higher for the factor M_0/M_{offres}, in which case it should be adjusted for correct evaluation.

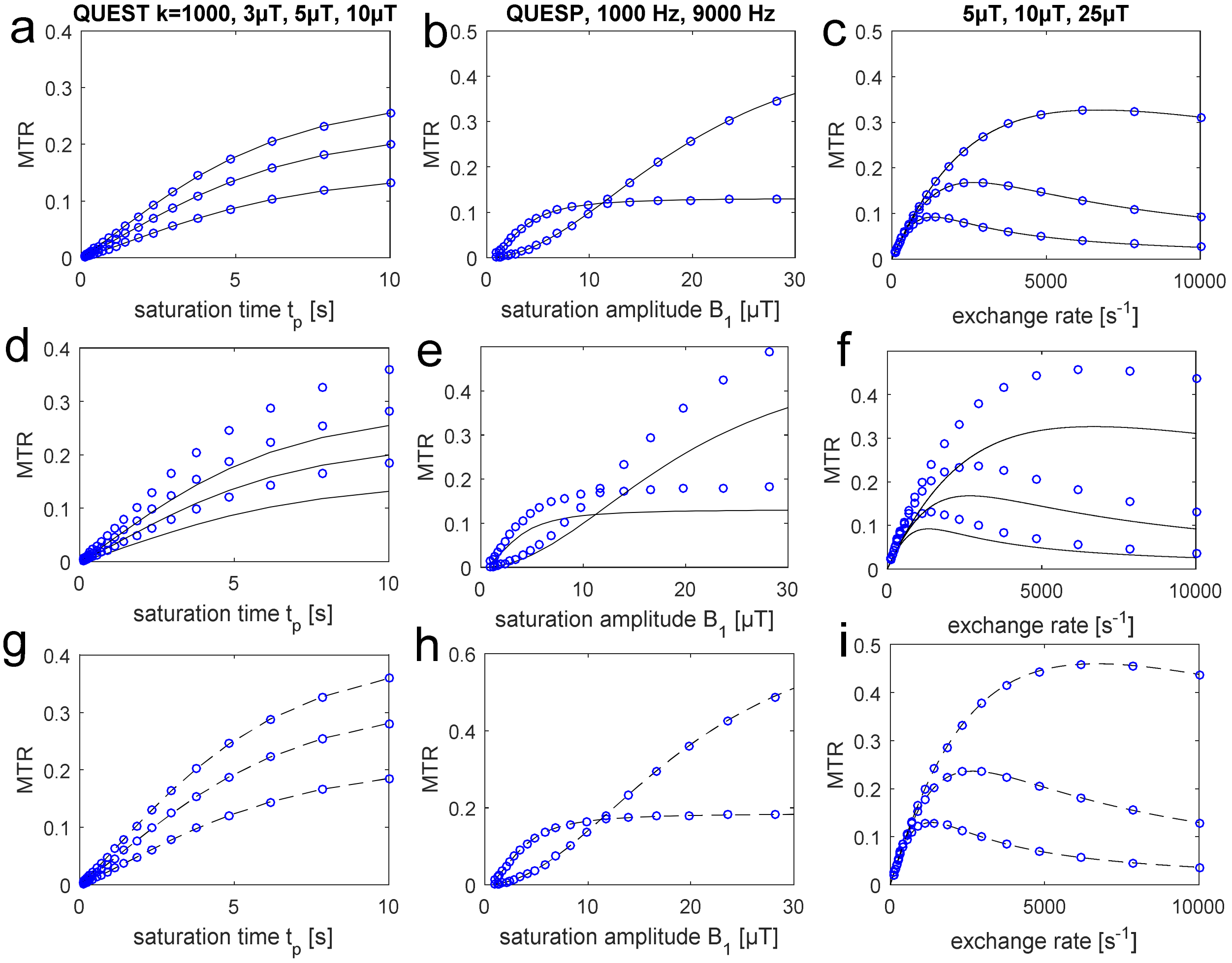

Figure S1: MTR_{asym} of simulated data (circles) together with analytic solutions of eq.[2] (solid lines) in the case of normalization by M_0 (a,b,c) and normalization by $M_{offres}=0.71\times M_0$ (d,e,f). In figures (g,h,i) both the data and the analytical formula (dashed line, eq.[3]) were normalized by M_{offres}.

Case (ii): Z_i=0.3, Normalization by each individual M_{offres}

Here, each spectrum was normalized by the reference scan $M_{offres} = M_{sat}(-80\text{ ppm}, t_p)$. In such case, each Z-spectrum was normalized with a different value of M_{offres} depending on the t_p, assuming again knowledge of $Z_i=M_i/M_0$. Under the assumption of negligible spillover, M_{offres}/M_0 can be calculated by $\mathrm{M}_{\text{offres}}(t_p)/\mathrm{M}_0 = 1+(Z_i-1)e^{-(R_{1a})t_p}$ and incorporated into the model to give :

$$MTR_{asym}\cdot\frac{M_0}{M_{offres}(t_p)} = \frac{\left(\frac{R_{ex}}{R_{1a}+R_{ex}}+(Z_i-1)e^{-R_{1a}t_p}-(Z_i-\frac{R_{1a}}{R_{1a}+R_{ex}})e^{-(R_{1a}+R_{ex})t_p}\right)}{1+(Z_i-1)e^{-R_{1a}t_p}}. \tag{4}$$

The MTR_{asym} simulation data normalized by the individual $M_{offres}(t_p)$ is again higher than expected by equation [2] normalized by M_0 (Figure S2a-c). If equation [3] for $M_{offres}(t_p=3) = 0.71$ is used, the curve deviates for other saturation times (Figure S2d) and only matches the data with the identical timing of t_p=3s (Figure S2e,f). Finally, if the equation [4] with incorporated individual normalization is used, the data is described perfectly for all cases.

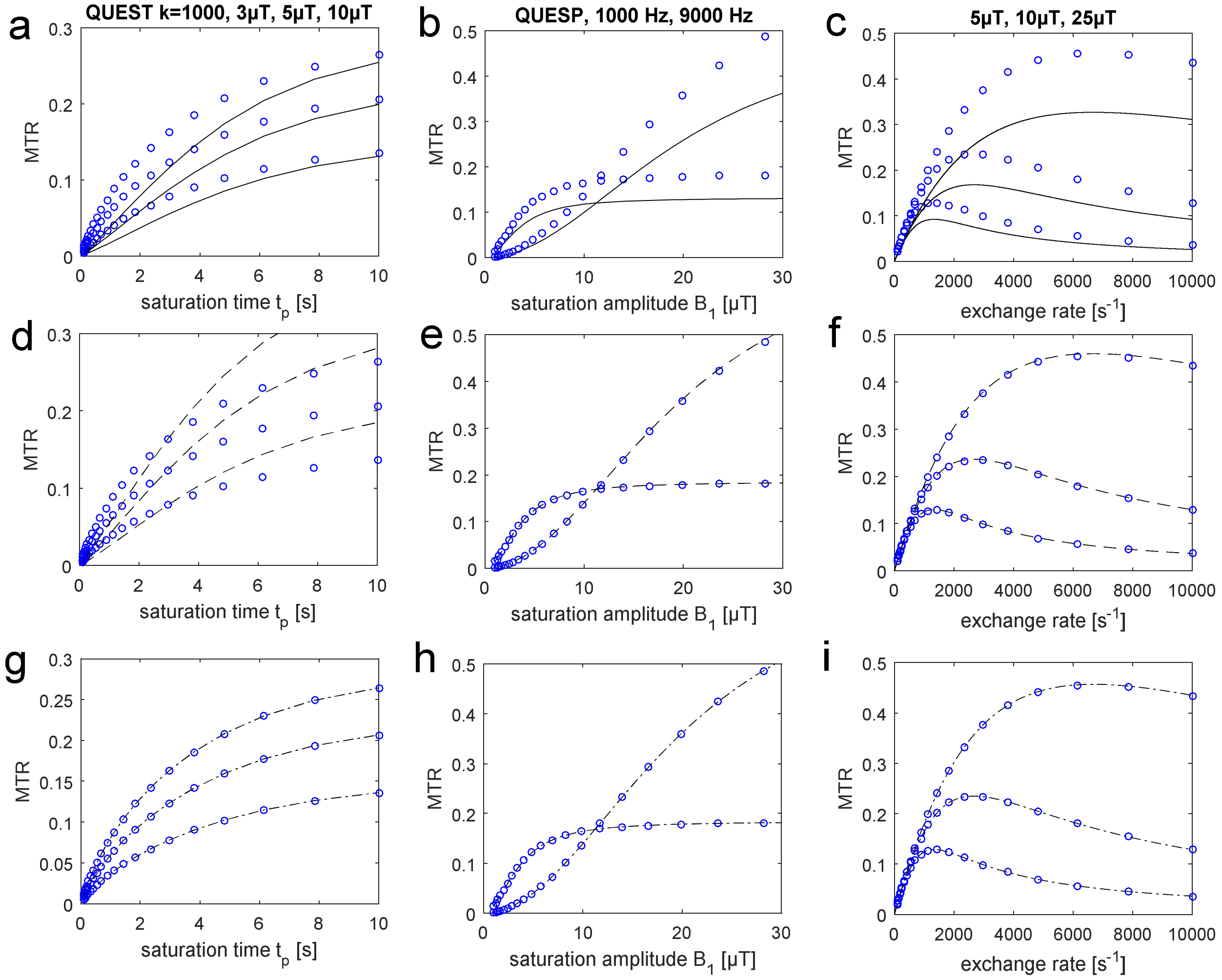

Figure S2: MTR_{asym} of simulated data (circles) normalized by individual $M_{offres}(t_p)$, together with differently normalized analytic solutions (lines). (a,b,c) Analytic solutions according to equation [2] and normalized with M_0 (solid lines). (d,e,f) Analytic solutions according to equation [3] and normalized with $M_{offres}(3\ s)=0.71\times M_0$ (dashed lines). (g,h,i) Analytic solutions according to equation [4] normalized with individual $M_{offres}(t_p)$ values (dash-dotted lines).

These two cases lead to the following conclusion: full quantification in the case of speed-up experiments requires the same type of normalization in the theory, that is used in the data analysis. Additionally, the initial Z-value Z_i must also be incorporated. These rules are not only valid for analytic evaluations, but also for full BM fitting, or Lorentzian-based evaluations.

To incorporate the normalization, both M_0 and M_{offres} must be known, as well as $Z_i = M_i/M_0$. However, since M_i is often not measured directly, a crude estimation for M_i can be made from M_0 and M_{offres} and the measured T_1 value, according to the relation :

$$M_{offres} = M_0 + (M_i - M_0)e^{-R_{1a}t_p} \tag{5}$$

$$\Leftrightarrow M_i = M_0 - (M_0 - M_{offres})e^{+R_{1a}t_p}$$

However, this estimation can be very instable, especially if M_{offres} is close to M_0. If the magnetization after the readout is known, one can also use this for Z_i estimation. For instance, for a 90° pulse, readout $M_{pre} = M_{after90°readout}=0$ and Z_i is given by :

$$Z_i = 1 - e^{-R_{1a} \cdot t_{rec}} \tag{6}$$

In the presented experimental data, $Z_i=0.865$ and $Z_i=0.769$ were measured for T=13.4 °C and 24.8 °C, respectively. However, if the measured R_1 relaxation rates of 0.423 Hz and 0.33 Hz were used in equation (6), we obtain $Z_i=0.47$ and $Z_i=0.39$ for T=13.4 °C and 24.8 °C, respectively. Thus, this estimation is crude and can often lead to incorrect results as the magnetization directly after the excitation pulse might not be exactly 0.

In order to avoid any instabilities and assumptions, both M_0 and M_i should be measured for full quantification. To measure M_i, the CEST sequence must be modified by removing the saturation block, but keeping the recovery time t_{rec} the same.

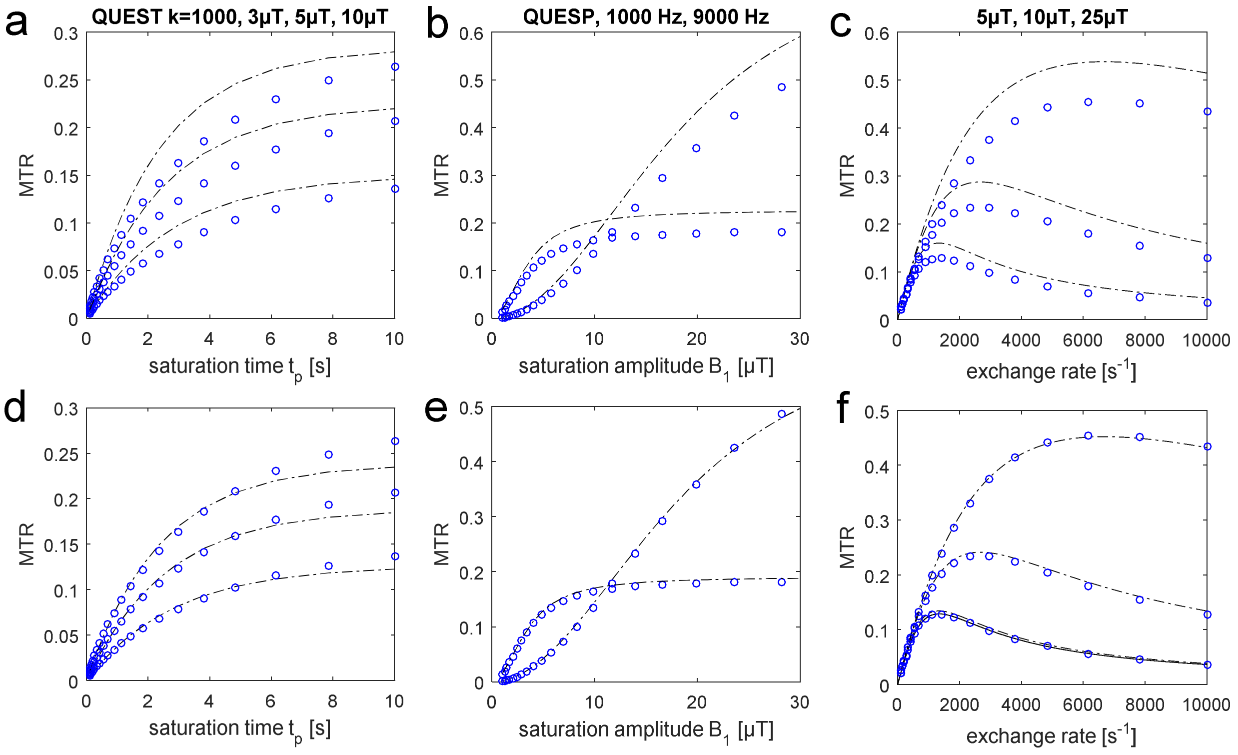

Figure S3: MTR_{asym} of simulated data (circles), normalized by individual $M_{offres}(t_p)$ together with the original equation [1] without Z_i (a,b,c). If equation [1] was normalized manually by a factor 1.19, the analytic solution yields a relatively good match to the data (d,e,f).

Effect of neglecting initial magnetization on parameter estimation

If Z-spectral data is normalized by individual M_{offres} (case(ii)) and plotted together with the original equation [1], the theory indicates existence of a factor that results in higher values obtained (Figure S3a,b,c). This factor was estimated to be 1.19; when used again to normalize the equation [1], there is a coarse match between calculations and data for varying saturation times (Figure S3d), while the curves for varying B_1 and k match the data relatively well (Figure S3e,f). The interpretation of these results is the following: if data is normalized by individual $M_{offres}(t_p)$, its main difference to the original theory (equation [1], normalized by M_0, Zi=1) is only a constant factor. This means that with this normalization, QUESP quantification would still yield a good estimation of the exchange rate, even if the normalization and Z_i is not taken into account (equation [1]); the deviation would be mostly reflected on the concentration. Similarly, QUEST yields reasonable estimations with this normalization for shorter saturation times, showing deviations for longer saturation times. In conclusion, correct QUEST requires knowledge of Z_i in any case, whereas QUESP might still yield relatively good estimations of exchange rates with the individual normalization, but wrong concentration values.

Radiation damping

In a first attempt, we could not perform the speed up experiment, as the dynamic behavior was disturbed due to radiation damping. This effect was significant with a 5 mm NMR tube (Figure S6a), and could be solved by using a smaller NMR tube with the inner diameter 1.6 mm inserted in a 5 mm NMR tube filled with D_2O (Figure S6b). In conclusion, using a smaller NMR tube to eliminate radiation damping is necessary for a transient CEST measurement, as no gradients can be applied during selective saturation.

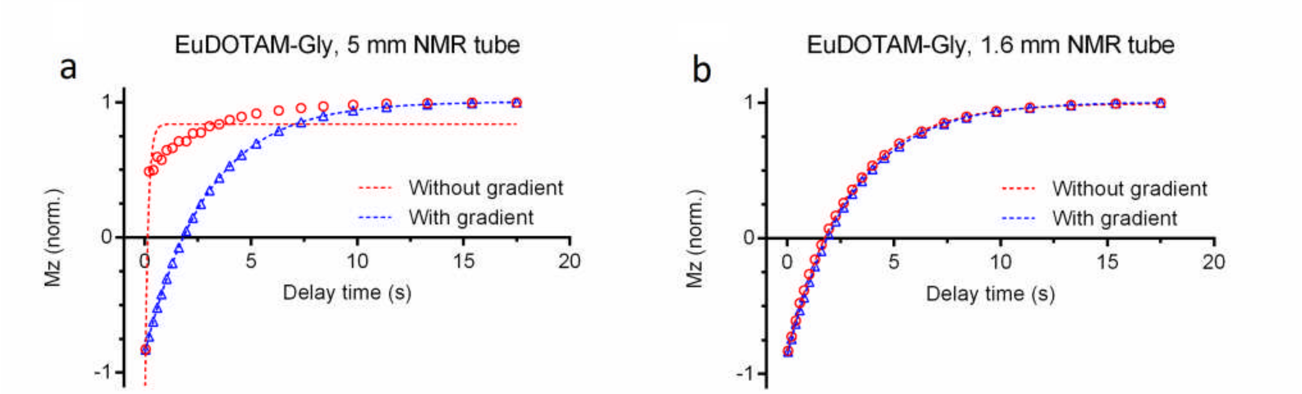

Figure S4: T_1 inversion recovery data without (red) and with gradient (blue) applied during the inversion time. Radiation damping is avoided by applying the gradient, or if replacing 5 mm (a) for 1.6 mm NMR tube (b).

Labeling efficiency in the case of paraCEST

In the original proposal of the labeling efficiency α (1,2) a simplified quantitative description of this parameter is given, that shows to be invalid in the case of paraCEST.

The original α reads on resonance:

$$\alpha = \frac{\omega_1^2}{\omega_1^2 + pq} \qquad [7]$$

$$with \quad p = R_{2b} + \frac{R_{2a} k_b}{R_{2a} + k_b f_b}; \quad q = R_{1b} + \frac{R_{1a} k_b}{R_{1a} + k_b f_b};$$

For exchange rates k_b in the range of R_{2a}/f_b and R_{1a}/f_b (for R_{1a}=0.3 Hz and f_b=10^-4 this is already at k_b=3000 Hz) this equation deviates strongly from the Bloch-McConnell simulations. The violation can be seen clearly if the limit $k_b >> R_{2a}/f_b > R_{1a}/f_b$ is observed then $p = R_{2b} + \frac{R_{2a}}{f_b}; \quad q = R_{1b} + \frac{R_{1a}}{f_b};$ and thus the labeling efficiency would become independent of the exchange rate k_b.

Also the description of R1B in equation (7) does not seem to be correct then.

The best formula for α in this regime including R_{2b} and R_{1b} we found heuristically was

$$\alpha = \frac{\omega_1^2}{\omega_1^2 + (k_b + R_{2b})(k_b + R_{1b})}$$

(1) Snoussi K, Bulte JWM, Guéron M, van Zijl PCM. Sensitive CEST Agents Based on Nucleic Acid Imino Proton Exchange: Detection of Poly(RU) and of a Dendrimer-Poly(RU) Model for Nucleic Acid Delivery and Pharmacology. Magn. Reson. Med. 2003;49:998–1005.

(2) Zhou J, Wilson DA, Sun PZ, Klaus JA, Zijl PCMV. Quantitative description of proton exchange processes between water and endogenous and exogenous agents for WEX, CEST, and APT experiments. Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med. 2004;51:945–952.