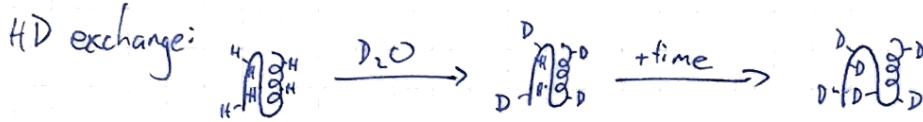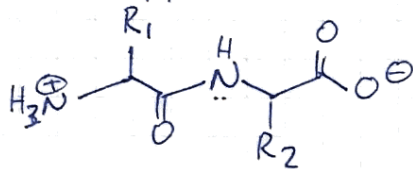# H/D exchange

NMR + protein dynamics — have focussed on τ_c timescale + R_{ex} & EXSY
— what about slower processes? ms — years
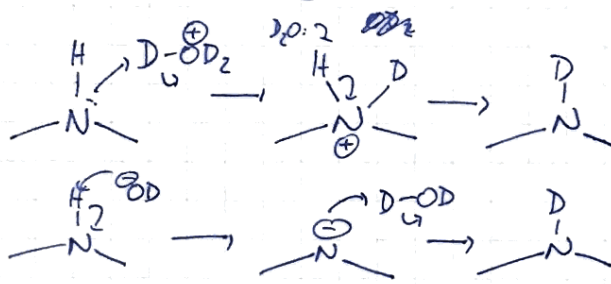
HD exchange:

Monitor peak intensity over time:

$$I \propto \exp(-k_{ex} t)$$

Physical interpretation?

Consider a dipeptide:

What does rate depend on?
- pH
- temperature
- sequence
- isotope ($H \to D$, $D \to H$, $H \to H$...)

acid catalysis

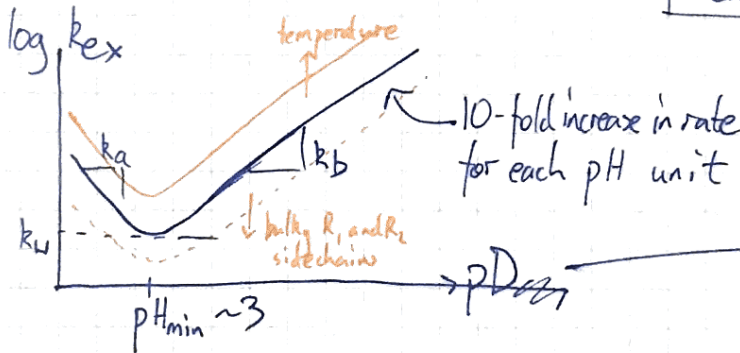+ water catalysis

base catalysis

$$\boxed{k_{ex} = k_a [D^+] + k_b [OD^-] + k_w}$$

10-fold increase in rate for each pH unit

$$\to pH \text{ vs } pD \quad (?)$$

$$pD = -\log_{10} [D^+] \quad \text{(by definition)}$$

but this is not measured directly by pH meter! $pH^* = pD - 0.4$
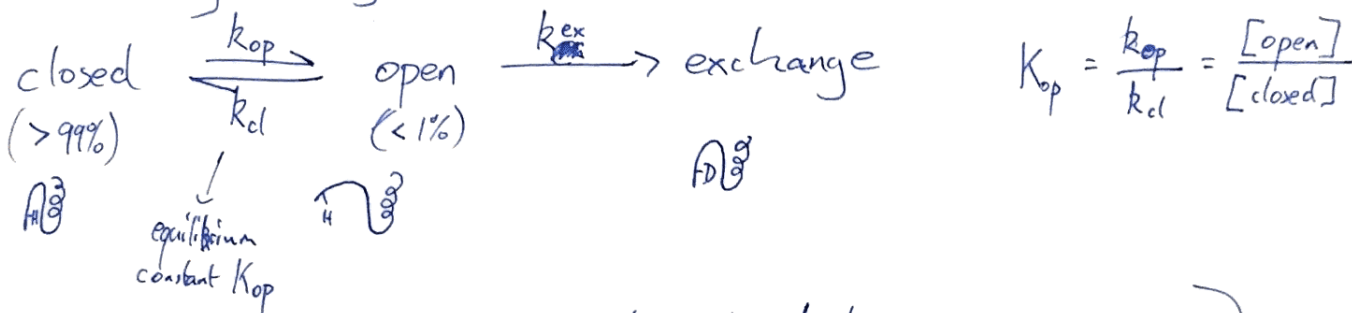
Temperature effect well characterised

Effect of sidechains also well studied (measured for all pairs)

∴ We can calculate 'random coil' exchange rates using database values → SPHERE webserver.

What about HX within proteins? At a minimum, hydrogen bonding protects amide protons from exchange.
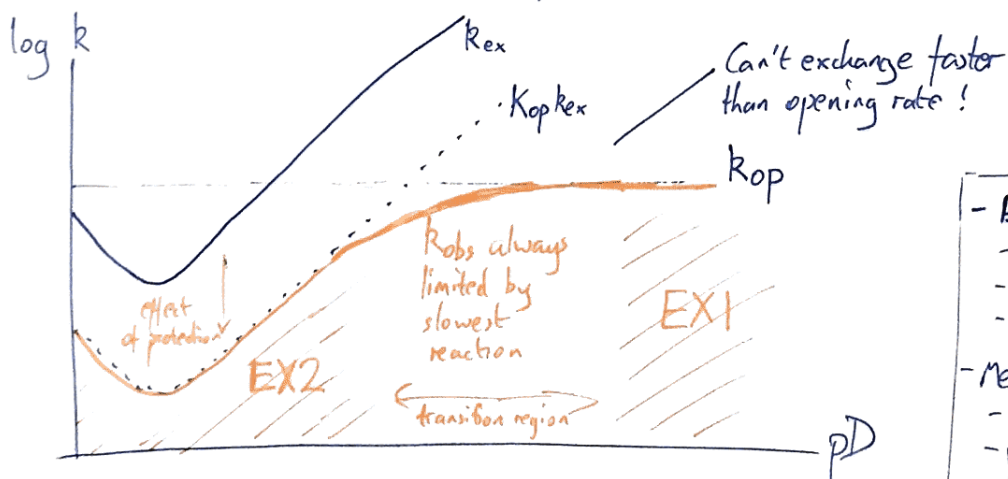
Linderstrøm-Lang exchange model:

closed $\xrightarrow{k_{op}}$ open $\xrightarrow{k_{ex}}$ exchange $K_{op} = \dfrac{k_{op}}{k_{cl}} = \dfrac{[open]}{[closed]}$
(>99%) $\xleftarrow{k_{cl}}$ (<1%)

equilibrium constant K_{op}

Two limits: Exchange of the open state is fast
— spend most of time waiting for rare opening events ⎤ EX 1
— opening rate is RDS $\Rightarrow k_{obs} \approx k_{op}$ ⎦

OR — Equilibrium of open ⇌ closed is rapid — pre-equilibrium ⎤
Rate ∝ [~~open~~] % open $\approx K_{op}$ × exchange rate ⎥ EX2
$k_{obs} \approx K_{op} k_{ex} = \dfrac{k_{op} k_{ex}}{k_{cl}}$ ⎦

From perspective of protein: so I've reached the open state... now what?

General solution: $k_{obs} = \dfrac{k_{op} k_{ex}}{k_{cl} + k_{ex}}$

Information content? EX 1 — kinetic! Tells you about k_{op}
EX 2 — thermodynamic! Tells you %open
or 'protection factor' $P = 1/K_{op}$

log k / pD plot: k_{ex}, $K_{op} k_{ex}$, k_{op}. "Can't exchange faster than opening rate!" k_{obs} always limited by slowest reaction. EX2, EX1, effect of protection, transition region

SS patterns:

log P — helix fraying ; solvent exposed β sheet

- Examples:
  - IgS — N.B. PFs not pathway info
  - YFP
  - amyloid (quenched HX)
- Measurement strategies
  - sample prep (conc w/ lyophilisation)
  - why $H_2O \rightarrow D_2O$?
  - dead time — SOFAST / stopped flow
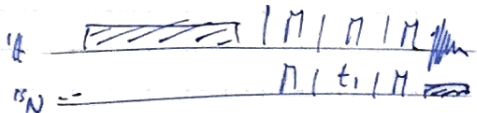 - sample incubation / stability
- Isotope effects
- H-H exchange?
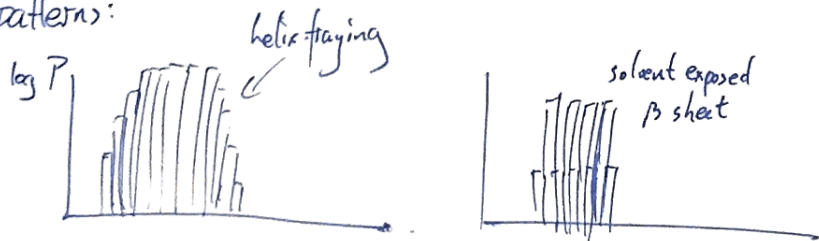
- EXSY (NOESY)
  - ↳ Saturation transfer
- CLEANEX
- Diffusion
- Scalar coupling
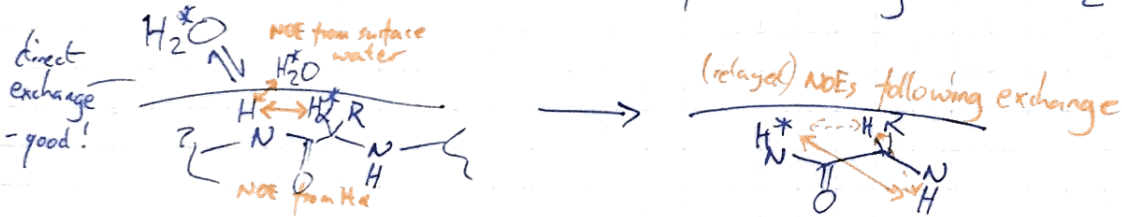- SOLEXSY

General:
$$k_{ex} > \frac{1}{T_1(H_2O)}$$

BRUKER

H-H exchange:

SATURATION TRANSFER:

- Long presaturation period during which saturated H_2O protons exchange onto protein & 2do?

- Loss of intensity for labile residues

- Temperature picture — heat diffuses from saturated spins
  — saturation of $H\alpha$ along with H_2O

- direct exchange - good!
- H_2^*O
- NOE from surface water
- NOE from $H\alpha$
- (relayed) NOEs following exchange

- Hard to quantify — depends on T_1, degree of saturation, length of sat. pulse

MAGNETISATION TRANSFER:
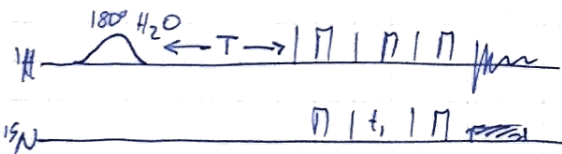
- Short selective inversion pulse followed by well defined mixing time
- Can observe buildup at small times
- Still potential for NOE effects
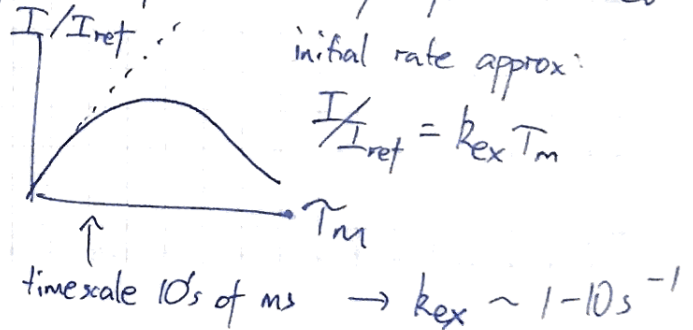
How to eliminate NOE effects? Recall NOE vs ROE:

longitudinal cross-relaxation < 0 in macromolecular limit $(\omega_0 \tau_c \gg 1)$

transverse cross-relaxation always > 0

$$\sigma_{tr} = -2\sigma_{long}$$

Solution — lock Magnetisation at $35°$ angle to cancel NOE and ROE contributions
$\longrightarrow$ CLEANEX experiment

- Doesn't cancel NOEs from rapidly rotating molecules eg. hydration water

initial rate approx:
$$\frac{I}{I_{ref}} = k_{ex} T_m$$

timescale 10's of ms $\longrightarrow k_{ex} \sim 1 - 10 s^{-1}$
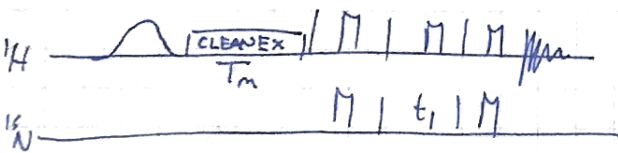
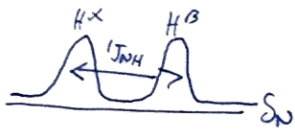## DIFFUSION: D_{obs} = population weighted average of protein + solvent

$D_{protein} \sim 10^{-10}$ to $10^{-11} \, m^2 s^{-1}$
$D_{water} \sim 10^{-9} \, m^2 s^{-1}$ $\Bigg] \rightarrow$ residue resolved diffusion measurements
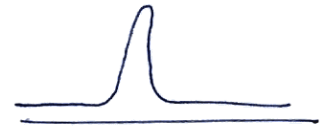
$k_{ex} \sim 1 - 10^4 \, s^{-1}$

SCALAR COUPLING: $^1J_{NH} \approx 92 \, Hz$

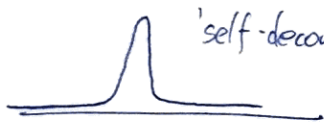^1H coupled ^{15}N spectra:

in absence of exchange $\xrightarrow{decoupling}$

α / β states rapidly interconverting

effect of rapid exchange: $k_{ex} \gg J$

'self-decoupling' as exchanging spins have random states

Intermediate exchange:

  $k_{ex} \sim {}^1J_{NH}$

Problem — broad signal, no INEPT transfers!
 — use a different detection scheme eg. HACAN
 — compare signal $\pm$ ^1H decoupling, which gives reference signal
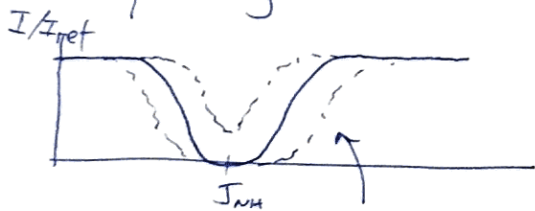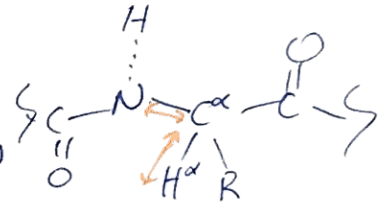
 — magnitude of signal dip depends on time spent as ^1N_x magnetisation

k_{ex} up to $10^5 \, s^{-1}$ (sidechains)

SOLEXSY

· Observe that ^{15}N chemical shifts have an isotope effect
  — shift upfield of $\sim 0.7 \, ppm$ in ND vs NH
  — just not commonly observed!

· 50% H_2O /50% D_2O : $\underbrace{HA \longrightarrow CA \rightarrow N_x \xrightarrow{t_1} N_z}$ $\xrightarrow{T_m} H_N$ observe

can be NH or ND here
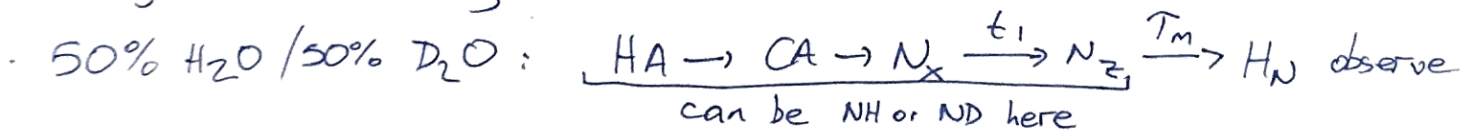
· Observe 2 peaks as function of mixing time :

limited by $^{15}N \, T_1$
$\sim 3 \, sec$

initial slope $\sim k_{ex}$

$k_{ex} \sim 0.2 - 20 \, s^{-1}$

Bimolecular rxns

$$P_{free} + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} P_{bound} \qquad \Longrightarrow \qquad P_{free} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P_{bound}$$
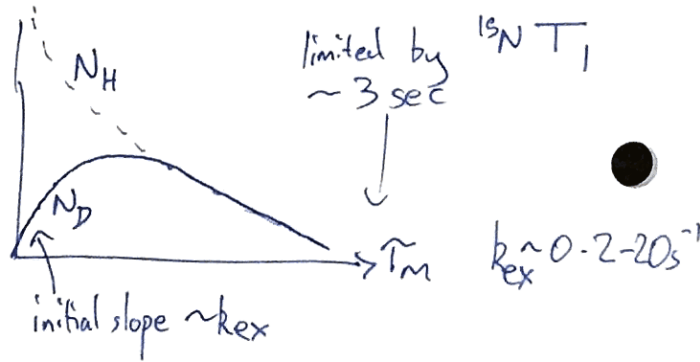
$$k_{-1} = k_{off} \qquad k_1 = L_{free} \cdot k_{on}$$

$$K_d = k_{off}/k_{on} \quad \Rightarrow \quad k_1 = k_{off} \cdot \frac{L_{free}}{K_d}$$

$$\left(L_{free} = K_d , \quad k_1 = k_{-1} \quad OK \right)$$

Calculating L_{free}: $\quad K_d = \frac{P_{free} \cdot L_{free}}{PL} = \frac{(P_0 - PL)(L_0 - PL)}{PL}$

$$\Rightarrow K_d \cdot PL = PL^2 - P_0 PL - L_0 PL + P_0 L_0 - K_d PL$$

$$\Rightarrow PL = \frac{1}{2}\left[K_d + P_0 + L_0 + \sqrt{(K_d + P_0 + L_0)^2 - 4 P_0 L_0} \right] \quad \text{and} \quad L_{free} = L_0 - PL$$
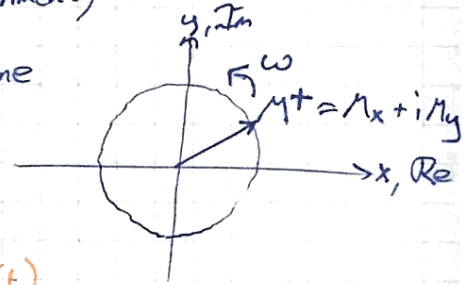

Bloch-McConnell equations $\quad$ (single pulse experiment)

Free precession of single spin in complex plane

$$M^+(t) = M^+(0) e^{i\omega t} e^{-R_2 t}$$

$$\frac{dM^+}{dt} = i\omega M^+(0) e^{i\omega t} = i\omega M^+(t)$$

$$(i\omega - R_2) M^+(0) e^{i\omega t} e^{-R_2 t} = (i\omega - R_2) M^+(t)$$

$$M^+ = M_x + i M_y$$

Two isolated spins M_a^+ and M_b^+ $\Rightarrow$ 2 ODEs – write in matrix form

$$\frac{d}{dt}\begin{pmatrix} M_a^+ \\ M_b^+ \end{pmatrix} = \begin{pmatrix} i\omega_a - R_{2a} - k_{ab} & 0 \; k_{ba} \\ 0 \; k_{ab} & i\omega_b - R_{2b} - k_{ba} \end{pmatrix} \cdot \begin{pmatrix} M_a^+ \\ M_b^+ \end{pmatrix} \equiv \begin{pmatrix} [i\omega_a - R_{2a}] M_a^+ \\ [i\omega_b - R_{2b}] M_b^+ \end{pmatrix}$$

no off-diagonal terms – spins are uncoupled (not in J coupling sense!)

Exchange couple the spins evolution together

$$\frac{d}{dt}\vec{M} = \Lambda \cdot \vec{M} \quad \text{is solved by combinations of } e^{\lambda_i t} \vec{u}_i$$
$$\text{where } \{\lambda_i\} \text{ are eigenvalues of } \Lambda.$$

Eigenvalues $A\vec{x} = \lambda\vec{x} \Rightarrow (A - \lambda I)\vec{x} = 0 \Rightarrow |A - \lambda I| = 0$ (characteristic equation)

$$\vec{M} = e^{\Lambda t} \cdot \vec{M}_0$$

Simpler case: Symmetric 2-site exchange without relaxation $\left(k_{ab}=k_{ba}=k\right)$

$\omega_a = 0$ (choice of reference)

$$\Lambda = \begin{pmatrix} \cancel{\omega_a} -k & +k \\ +k & i\omega_A -k \end{pmatrix}$$

$$|\Lambda - \mathbb{1}\lambda| = \begin{vmatrix} -k-\lambda & k \\ k & i\omega-k-\lambda \end{vmatrix} = -(k+\lambda)(i\omega-k-\lambda) - k^2$$

$$= \lambda^2 + (2k-i\omega)\lambda + \cancel{k^2} - k^2 - ik\omega = 0$$

$$\Rightarrow \lambda = \frac{i\omega - 2k \pm \sqrt{(i\omega-2k)^2 + 4i k\omega}}{2} = \frac{1}{2}i\omega - k \pm \frac{1}{2}\sqrt{\cancel{4k^2} - \omega^2 + 4k^2}$$

$$= \frac{1}{2}i\omega - k \pm \frac{1}{2}i\omega\sqrt{1 - \frac{4k^2}{\omega^2}}$$

Slow EX: $k \ll \omega$, $1 - \frac{4k^2}{\omega^2} \approx 1$

$$\Rightarrow \lambda = \left(\frac{1}{2} \pm \frac{1}{2}\right)i\omega - k \quad \leftarrow \text{lifetime broadening}$$

$$\swarrow \text{precession at } 0 \text{ and } \omega \text{ frequencies}$$

Fast EX: $k_m \gg \omega$, $4k^2 - \omega^2 \approx 4k^2$

$$\Rightarrow \lambda = \frac{1}{2}i\omega - k \pm k = \frac{1}{2}i\omega, \quad \frac{1}{2}i\omega - 2k$$

$$\underset{\text{peak at average shift}}{\uparrow} \qquad \underset{\substack{\text{zero amplitude,} \\ \text{not observed.}}}{\uparrow}$$

$$\lambda = \frac{1}{2}i\omega - k \pm \cancel{\cancel{2}}k\sqrt{1 - \frac{\omega^2}{4k^2}} \qquad \text{Expand } \sqrt{1-x} \approx 1 - \frac{1}{2}x + O(x^2)$$

$$\approx \frac{1}{2}i\omega - k \pm k\left(1 - \frac{\omega^2}{8k^2}\right)$$

+ve root: $\lambda \rightarrow \frac{1}{2}i\omega - \underbrace{\frac{\omega^2}{8k}}_{R_{ex}}$. Fast intermediate.

cf. $R_{ex} = \dfrac{p_A\, p_B\, \Delta\omega^2}{k_{ex}}$ $\qquad p_A = p_B = \frac{1}{2}$

$$k_{ex} = k_{-1} + k_1 = 2k \qquad \underline{\underline{OK}}$$

EXSY

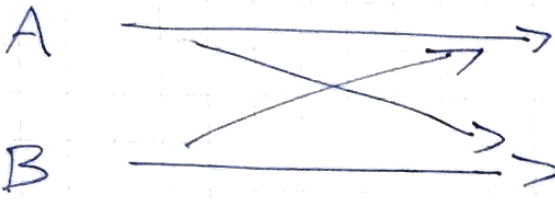$t = 0$ $t = T$

A   A $\vec{M_0} = \begin{pmatrix} P_A \\ P_B \end{pmatrix}$

B   B $\vec{M_T} = \begin{pmatrix} P_A \\ P_B \end{pmatrix}$

$$\vec{M_T} = \begin{pmatrix} A \to A & B \to A \\ A \to B & B \to B \end{pmatrix} \cdot \vec{M_0}$$

$\underbrace{\qquad\qquad\qquad\qquad}_{A(t)}$ How to find matrix entries?

$$A(0) = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$$

$$\frac{d\vec{M}}{dt} = (K+R)\vec{M}$$

$$K = \begin{pmatrix} -k_{ab} & k_{ba} \\ k_{ab} & -k_{ba} \end{pmatrix} \qquad R = \begin{pmatrix} -R_{1a} & 0 \\ 0 & -R_{1b} \end{pmatrix}$$

$$\vec{M}(t) = e^{(K+R)t} \vec{M}(0)$$

$$\Rightarrow A = e^{(K+R)T}$$

Matrix exponential: $e^X = 1 + X + \frac{1}{2}X^2 + \cdots$

For small T, $A \approx \begin{pmatrix} 1 - k_{ab}t - R_{1a}t & k_{ba}t \\ k_{ab}t & 1 - k_{ba}t - R_{1b}t \end{pmatrix}$

General solution (simplified, for $k_{ab} = k_{ba}$, $R_{1a} = R_{1b}$):

$$A(t) = \begin{pmatrix} \frac{1}{2}e^{-Rt}[1 + e^{-2kt}] & \frac{1}{2}e^{-Rt}[1 - e^{-2kt}] \\ \frac{1}{2}e^{-Rt}[1 - e^{-2kt}] & \frac{1}{2}e^{-Rt}[1 + e^{-2kt}] \end{pmatrix}.$$