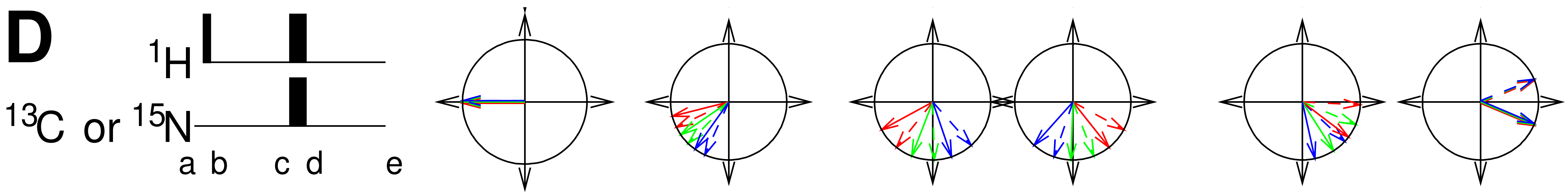
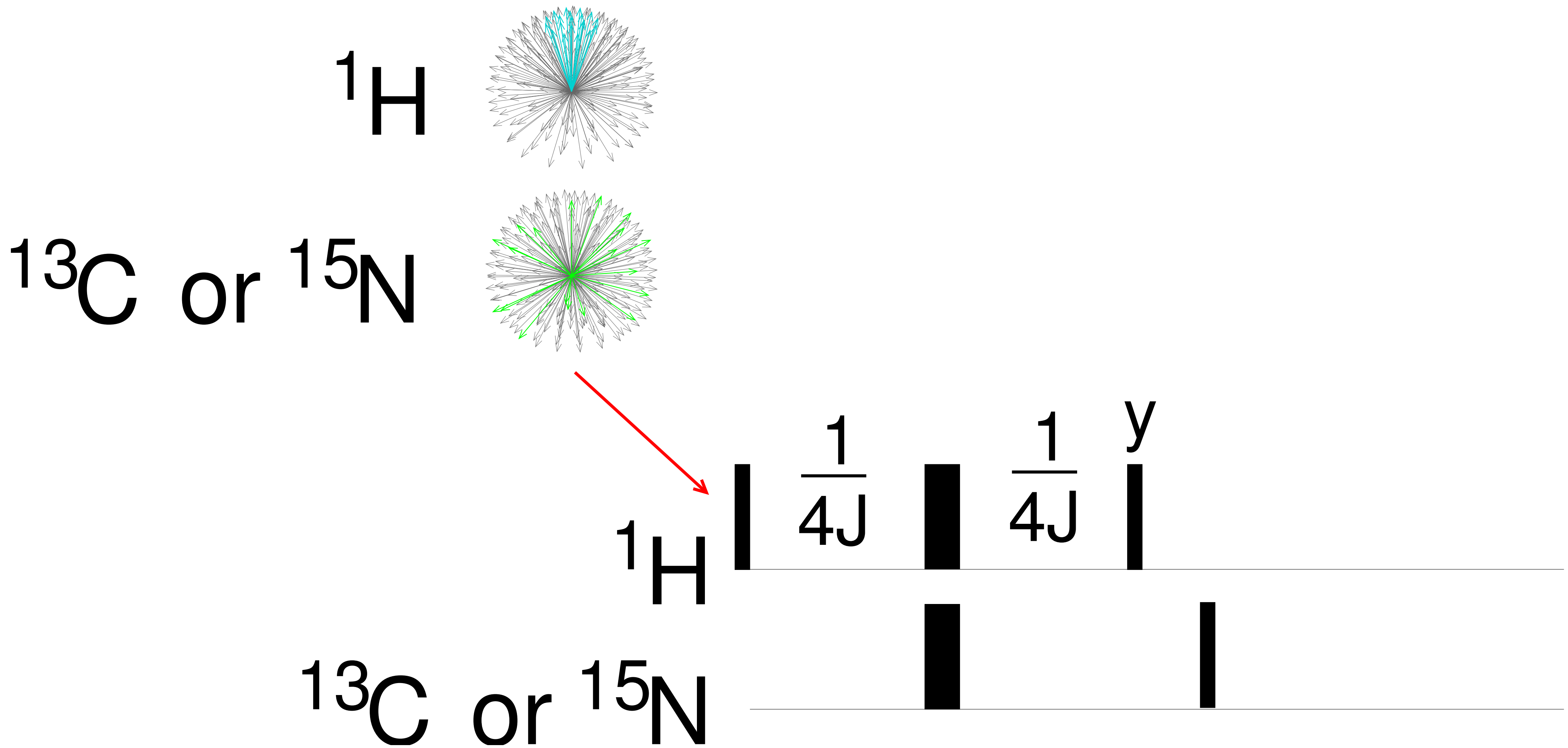
Lecture 11: INEPT, HSQC

Simultaneous spin echo

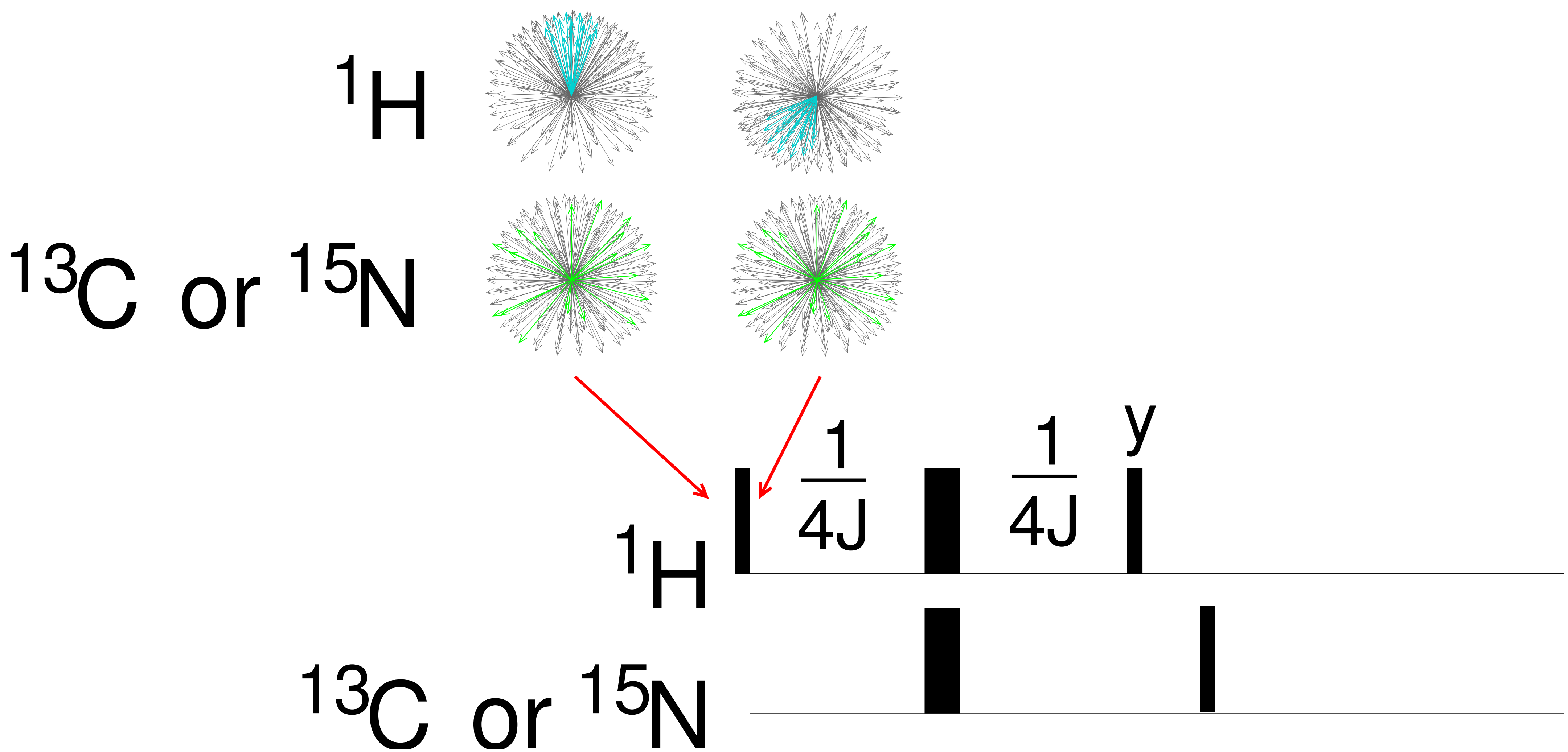


INEPT



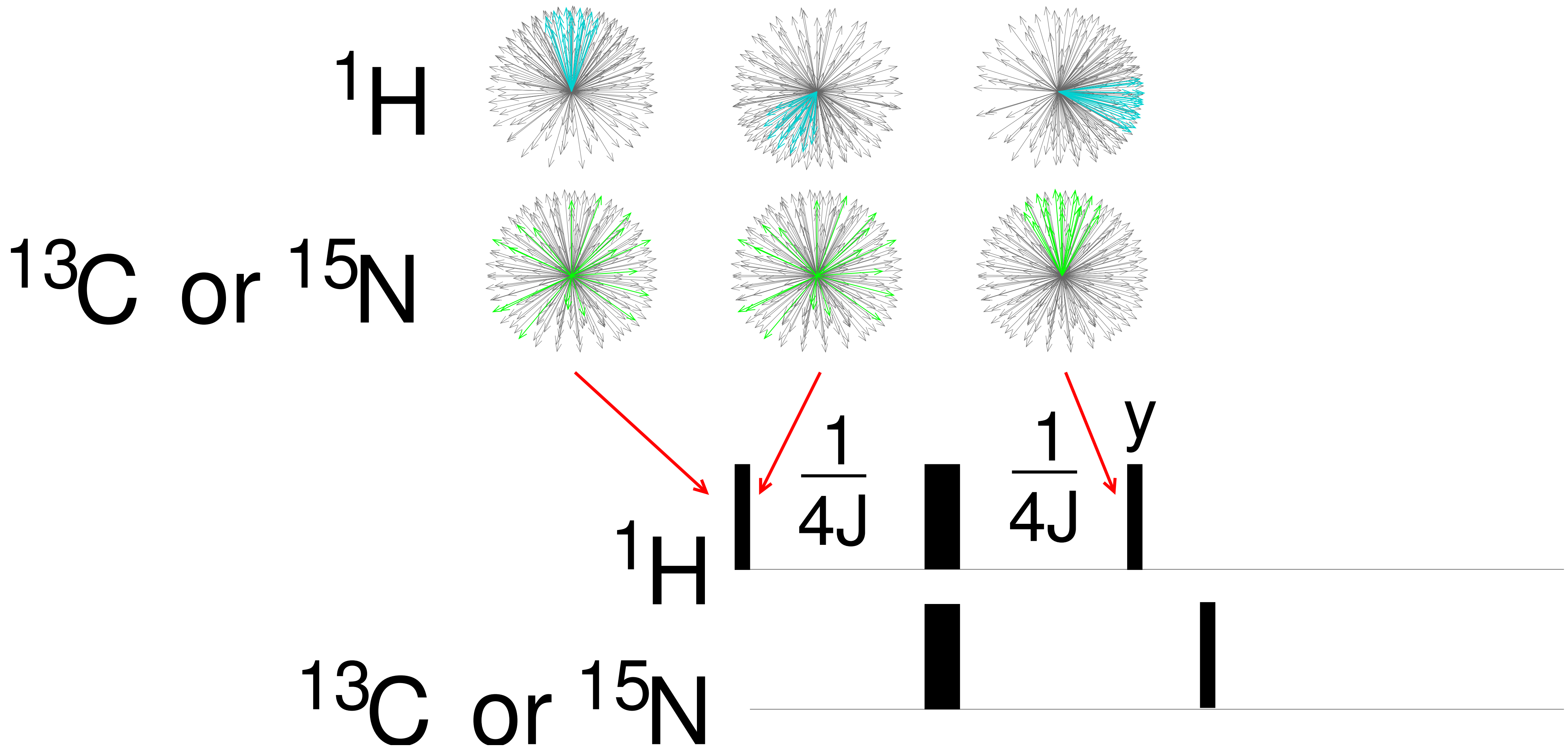
$$\hat{\rho}(\mathrm{a}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\mathscr{I}_z + \frac{1}{2}\kappa_2\mathscr{S}_z$$

INEPT



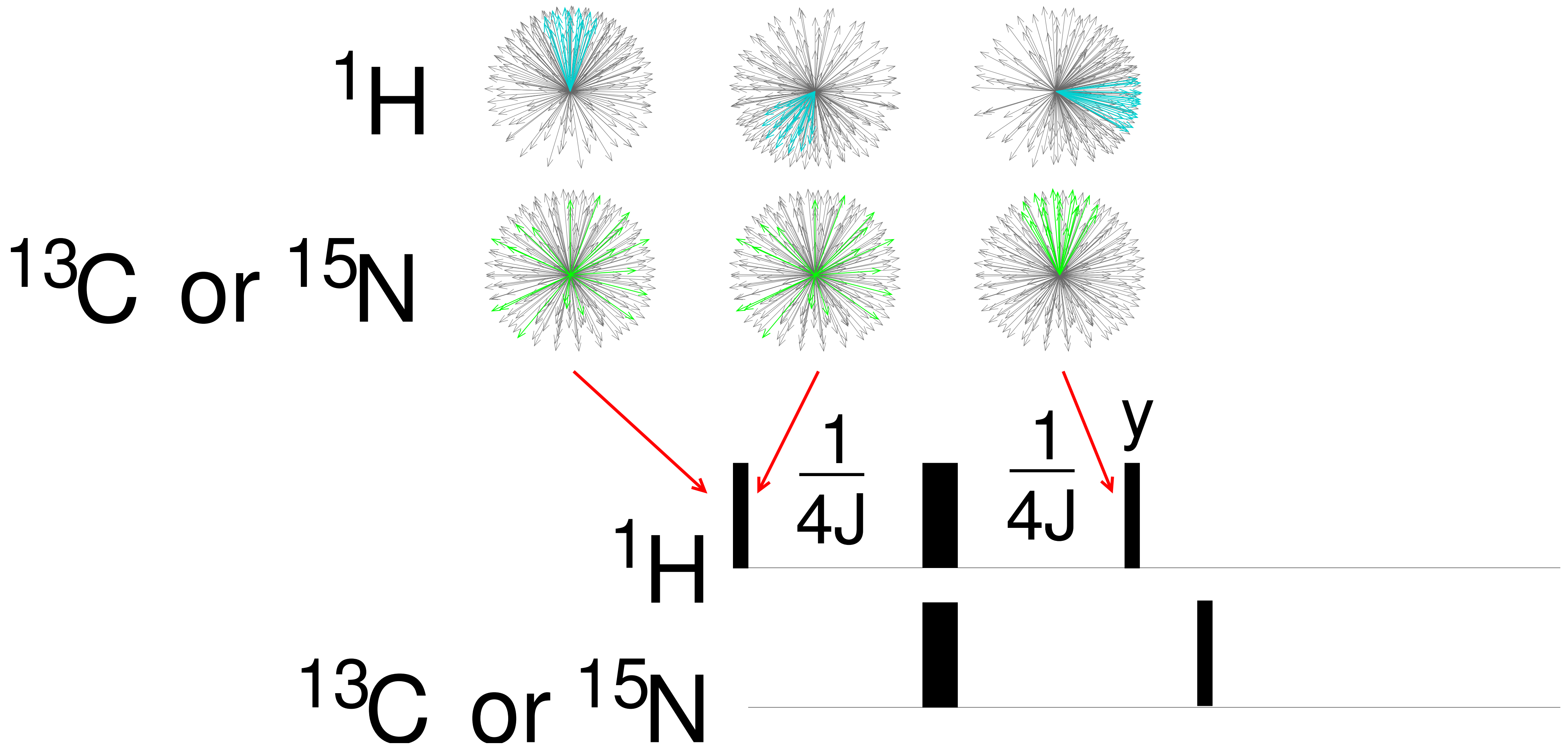
$$\widehat{\rho}(\mathrm{b}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\mathscr{I}_y + \frac{1}{2}\kappa_2\mathscr{S}_z$$

INEPT



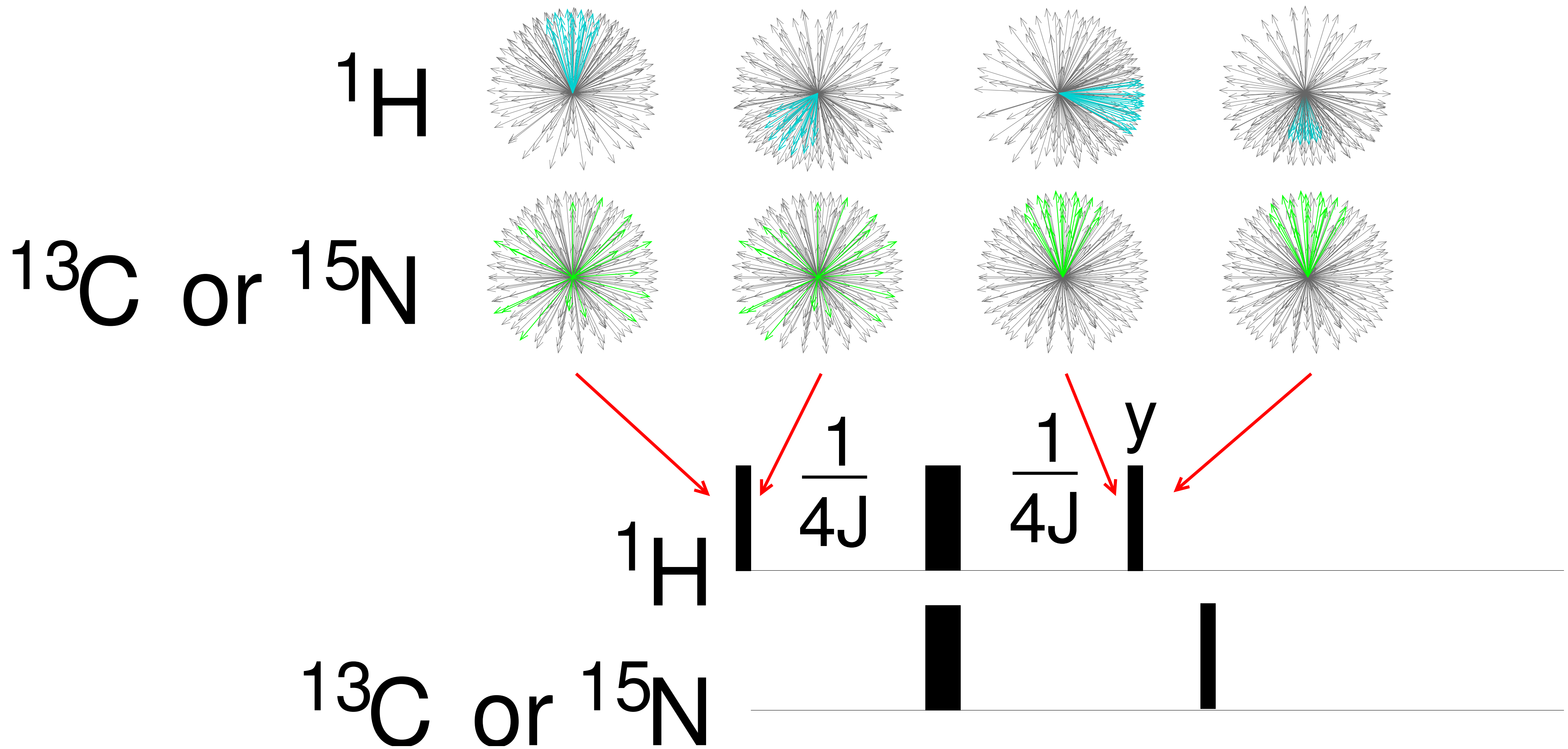
$$\widehat{\rho}(\mathrm{e}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1 \cos\frac{\pi J}{2J}\mathscr{I}_y - \frac{1}{2}\kappa_1 \sin\frac{\pi J}{2J}\left(2\mathscr{I}_x\mathscr{S}_z\right) - \frac{1}{2}\kappa_2\mathscr{S}_z$$

INEPT



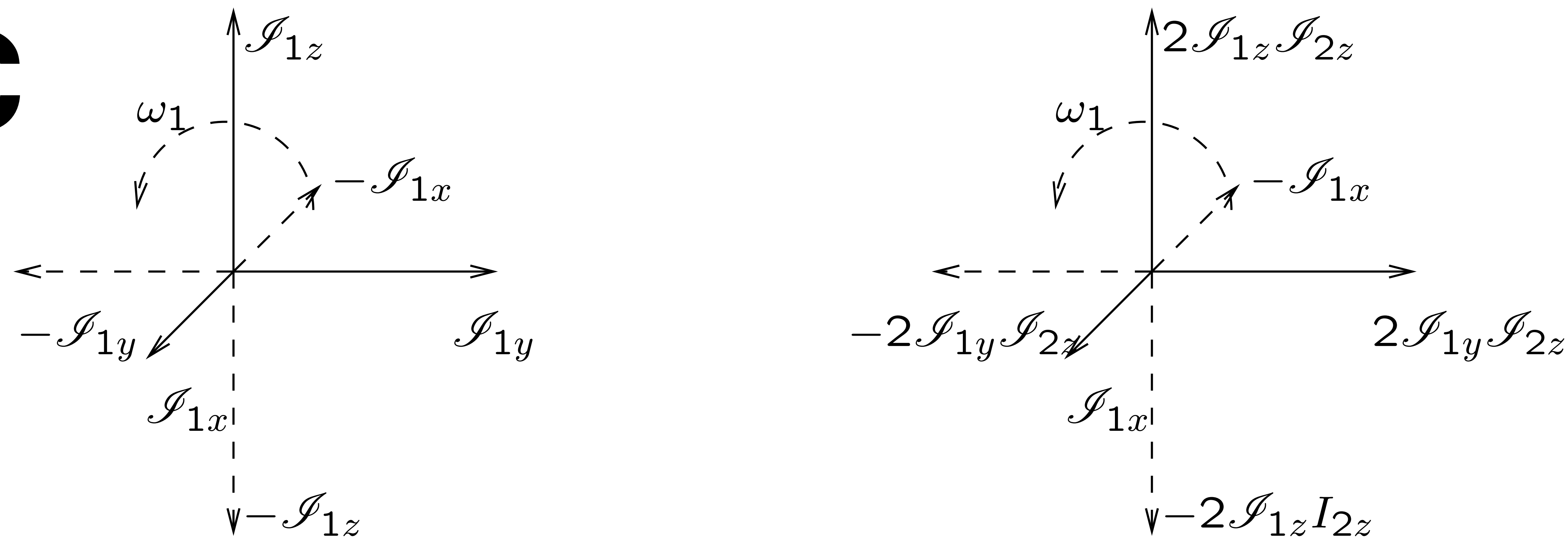
$$\widehat{\rho}(\mathrm{e}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(2\mathscr{I}_x\mathscr{S}_z\right) - \frac{1}{2}\kappa_2\mathscr{S}_z$$

INEPT



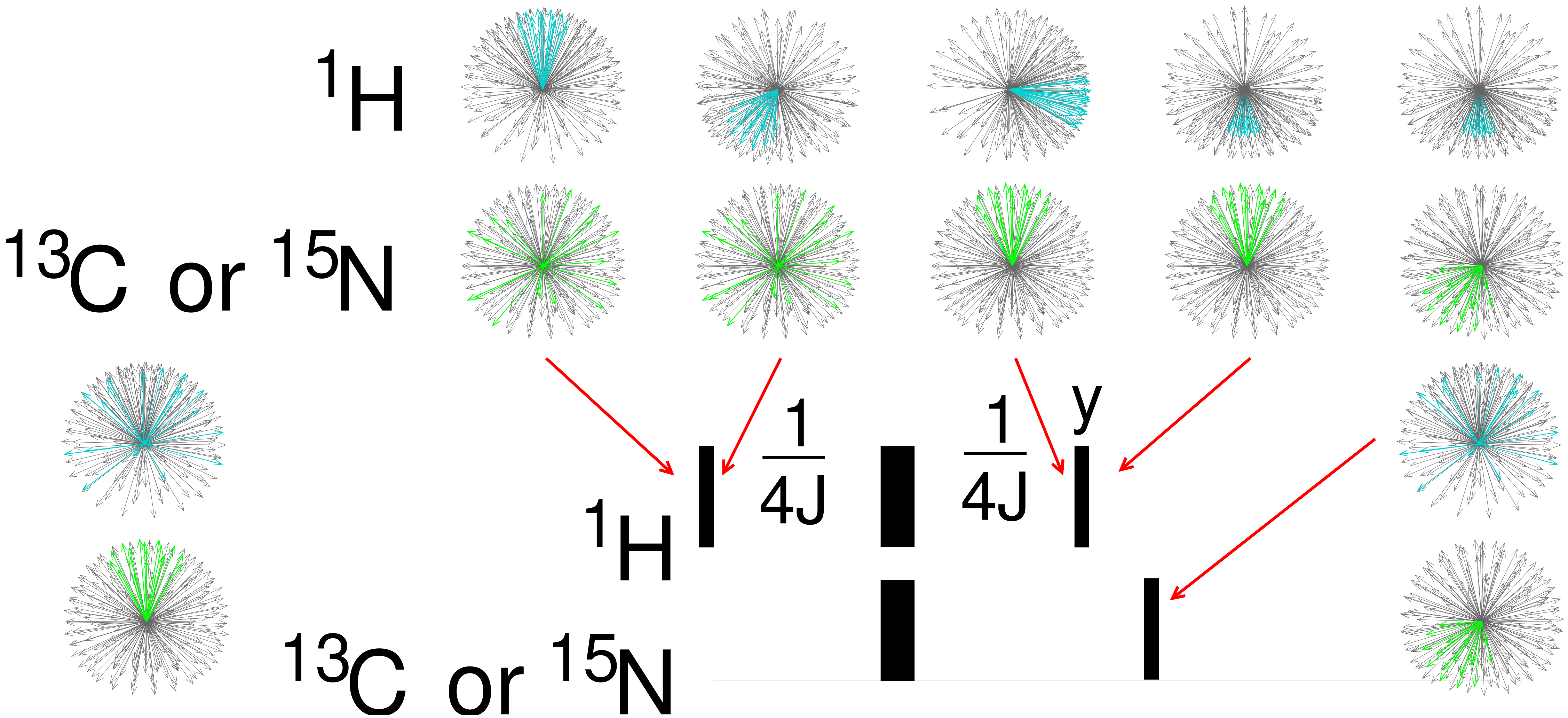
$$\widehat{\rho}(\mathrm{f}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(2\mathscr{I}_z\mathscr{S}_z\right) - \frac{1}{2}\kappa_2\mathscr{S}_z$$

C



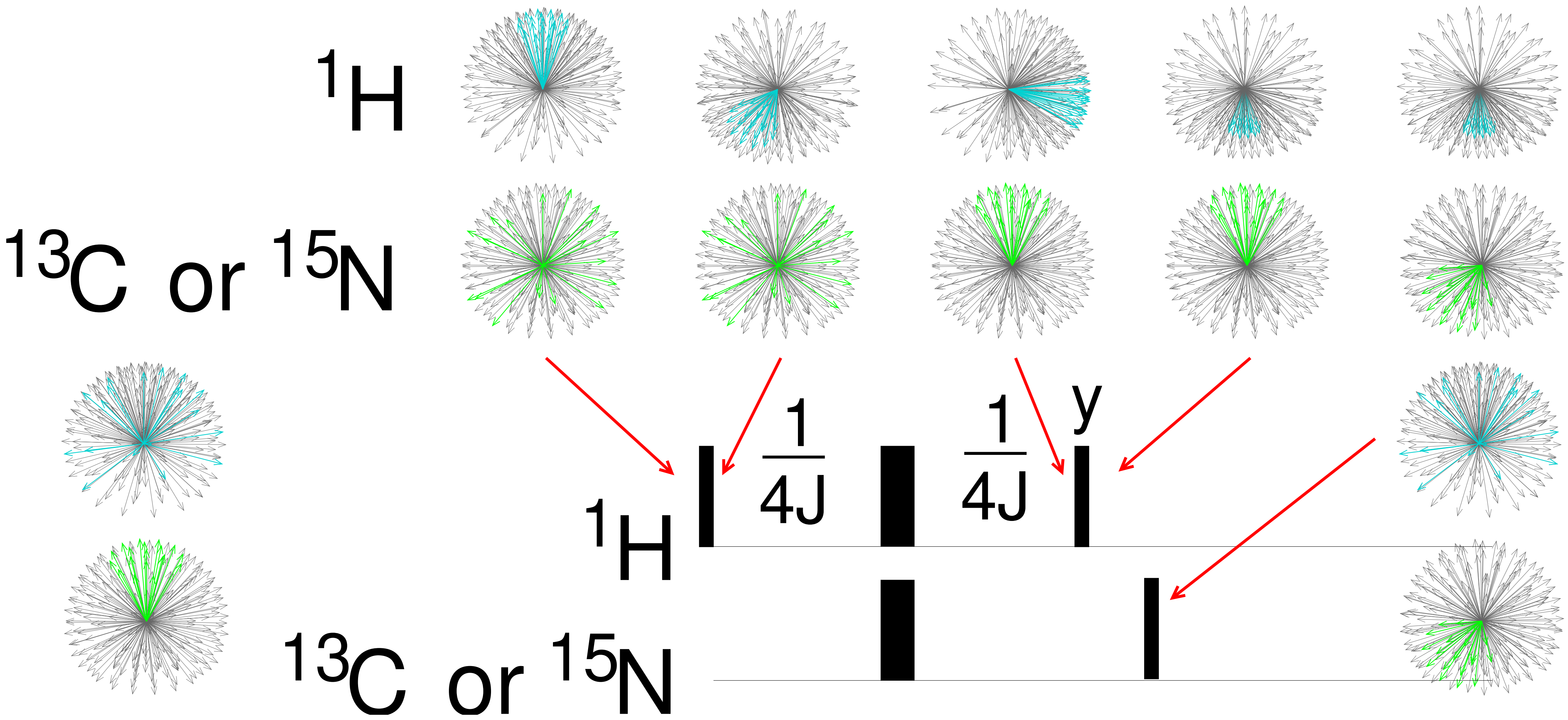
$\mathscr{I}_{1z}$
ω_1
$-\mathscr{I}_{1x}$
$-\mathscr{I}_{1y}$
$\mathscr{I}_{1y}$
$\mathscr{I}_{1x}$
$-\mathscr{I}_{1z}$
$2\mathscr{I}_{1z}\mathscr{I}_{2z}$
ω_1
$-\mathscr{I}_{1x}$
$-2\mathscr{I}_{1y}\mathscr{I}_{2z}$
$2\mathscr{I}_{1y}\mathscr{I}_{2z}$
$\mathscr{I}_{1x}$
$-2\mathscr{I}_{1z}I_{2z}$

INEPT



$$\hat{\rho}(\mathrm{g}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(2\mathscr{I}_z\mathscr{S}_y\right) + \frac{1}{2}\kappa_2\mathscr{S}_y$$

INEPT



$$\hat{\rho}(\mathrm{g}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(2\mathscr{I}_z\mathscr{S}_y\right) + \frac{1}{2}\kappa_2\mathscr{S}_y$$

INEPT

$$
\begin{aligned}
\mathscr{I}_t &\longrightarrow \mathscr{I}_t \longrightarrow \mathscr{I}_t \\
-2\mathscr{I}_z\mathscr{S}_y &\longrightarrow \begin{cases} -c_2\, 2\mathscr{I}_z\mathscr{S}_y \longrightarrow \begin{cases} -c_2 c_J\;\; 2\mathscr{I}_z\mathscr{S}_y \\ +c_2 s_J\;\; \mathscr{S}_x \end{cases} \\ +s_2\, 2\mathscr{I}_x\mathscr{S}_z \longrightarrow \begin{cases} +s_2 c_J\;\; 2\mathscr{I}_z\mathscr{S}_x \\ +s_2 s_J\;\; \mathscr{S}_y \end{cases} \end{cases} \\
\mathscr{S}_y &\longrightarrow \begin{cases} +c_2\mathscr{S}_y \longrightarrow \begin{cases} +c_2 c_J\;\; \mathscr{S}_y \\ -c_2 s_J\;\; 2\mathscr{S}_x\mathscr{I}_z \end{cases} \\ -s_2\mathscr{S}_x \longrightarrow \begin{cases} -s_2 c_J\;\; \mathscr{S}_x \\ -s_2 s_J\;\; 2\mathscr{S}_y\mathscr{I}_z \end{cases} \end{cases}
\end{aligned}
$$

Relaxation with J-coupling

- $\hat{H}_J$: $\mathscr{I}_{1x} \to 2\mathscr{I}_{1y}\mathscr{I}_{2z}$ $\mathscr{I}_{1y} \to -2\mathscr{I}_{1x}\mathscr{I}_{2z}$
  $\Rightarrow$ $\mathscr{I}_{1+} = \mathscr{I}_{1x} + \mathrm{i}\mathscr{I}_{1y} \to -\mathrm{i}2\mathscr{I}_{1+}\mathscr{I}_{2z}$ different R_2

- $\mathscr{I}_{1+} \leftrightarrow 2\mathscr{I}_{1+}\mathscr{I}_{2z}$ $\Rightarrow$ $\overline{R}_2$

- relaxation of $\mathscr{I}_{1+}$ depends on $2\mathscr{I}_{1+}\mathscr{I}_{2z}$
  relaxation of $2\mathscr{I}_{1+}\mathscr{I}_{2z}$ depends on $\mathscr{I}_{1+}$
  cross-correlated cross-relaxation (ingnored here)
  cf. cross-relaxation of $\Delta\langle M_{1z}\rangle$ and $\Delta\langle M_{2z}\rangle$ (NOE)

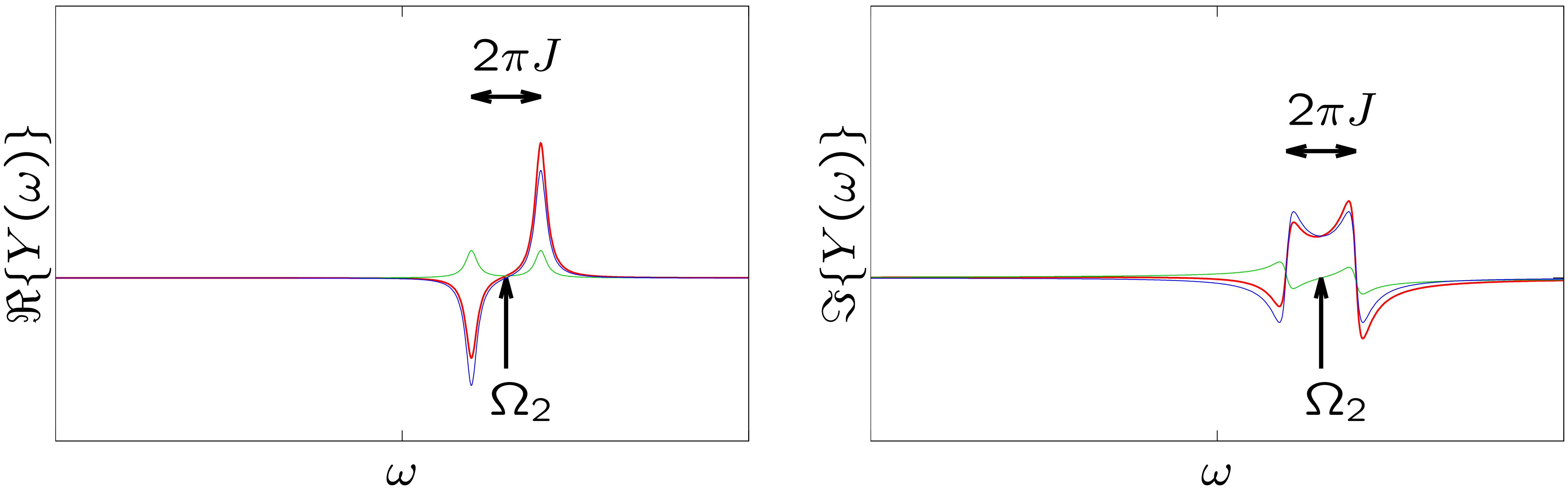
INEPT

$$\langle M_+ \rangle \;\propto\; \frac{\kappa_1}{4}\mathrm{e}^{-\overline{R}_2 t}\left(\mathrm{e}^{\mathrm{i}(\Omega_2-\pi J)t} - \mathrm{e}^{\mathrm{i}(\Omega_2+\pi J)t}\right) + \frac{\kappa_2}{4}\mathrm{e}^{-\overline{R}_2 t}\left(\mathrm{e}^{\mathrm{i}(\Omega_2-\pi J)t} + \mathrm{e}^{\mathrm{i}(\Omega_2+\pi J)t}\right)$$

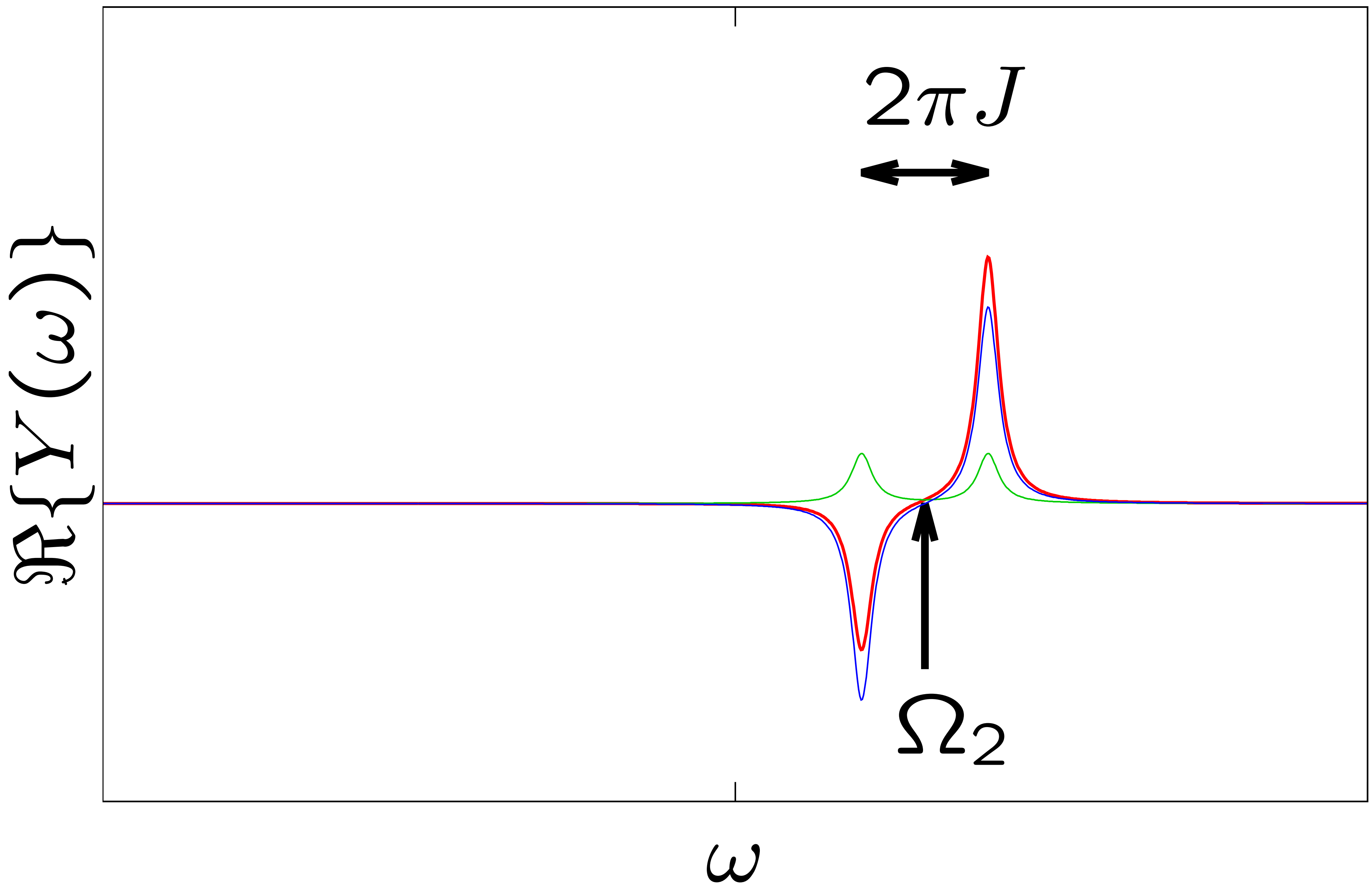
$$\Re\{Y(\omega)\} =$$

$$\frac{\mathcal{N}\gamma_1\gamma_2\hbar^2 B_0}{16 k_\mathrm{B} T}\left(\frac{\overline{R}_2}{\overline{R}_2^2 + (\omega - \Omega_2 + \pi J)^2} - \frac{\overline{R}_2}{\overline{R}_2^2 + (\omega - \Omega_2 - \pi J)^2}\right) + \frac{\mathcal{N}\gamma_2\gamma_2\hbar^2 B_0}{16 k_\mathrm{B} T}\left(\frac{\overline{R}_2}{\overline{R}_2^2 + (\omega - \Omega_2 + \pi J)^2} + \frac{\overline{R}_2}{\overline{R}_2^2 + (\omega - \Omega_2 - \pi J)^2}\right)$$

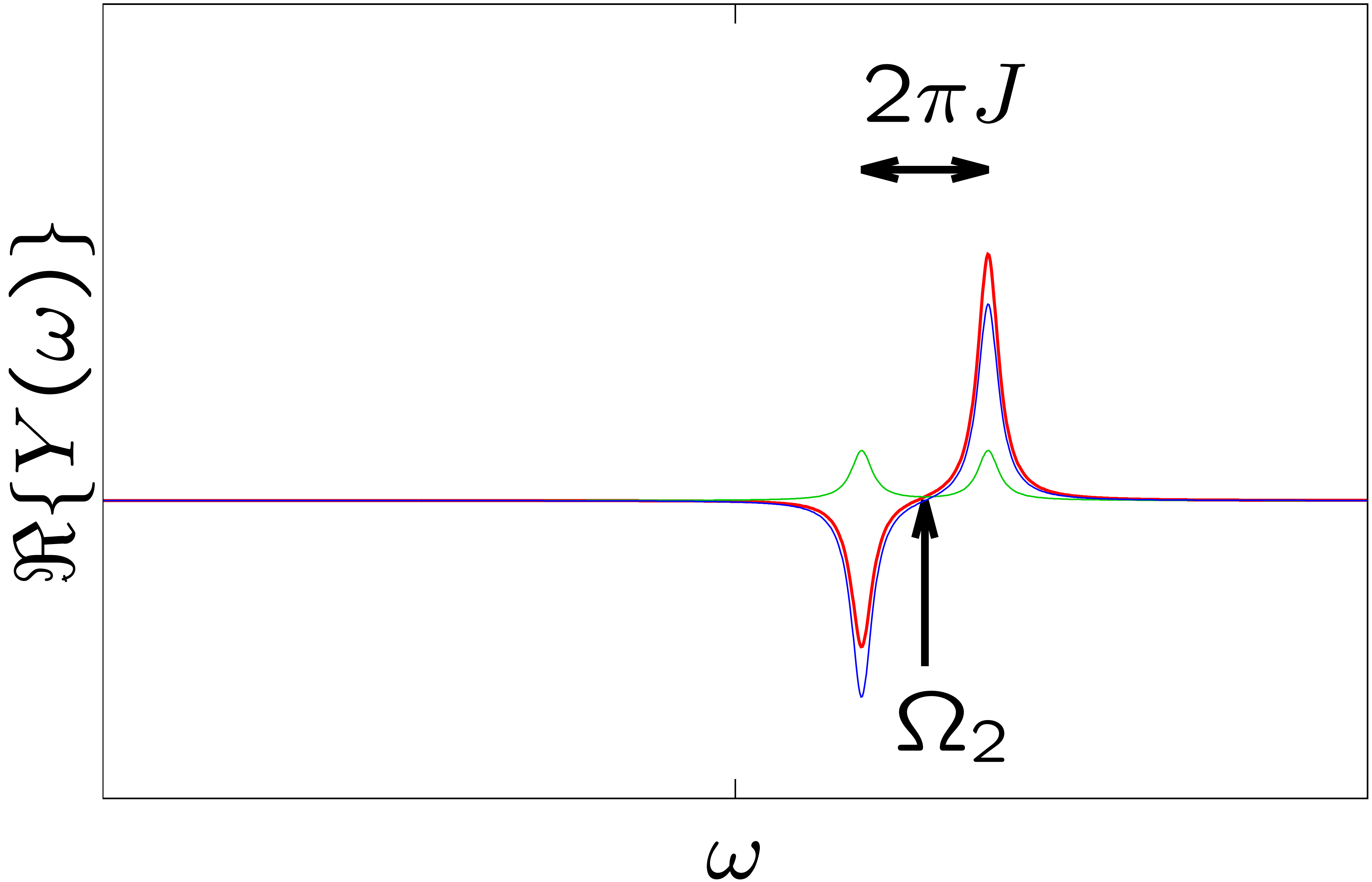
INEPT



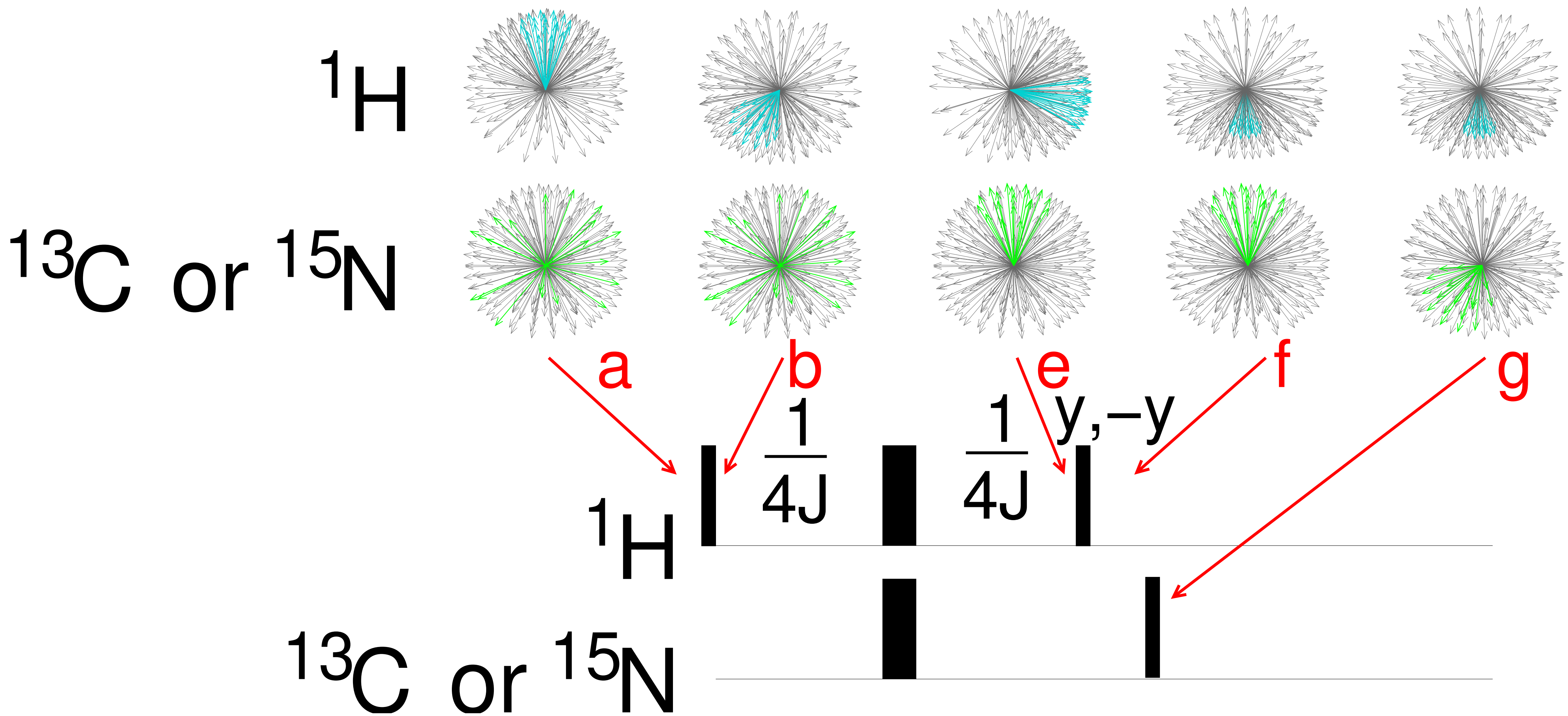
INEPT:



anti-phase vs. in phase coherences



Phase cycling

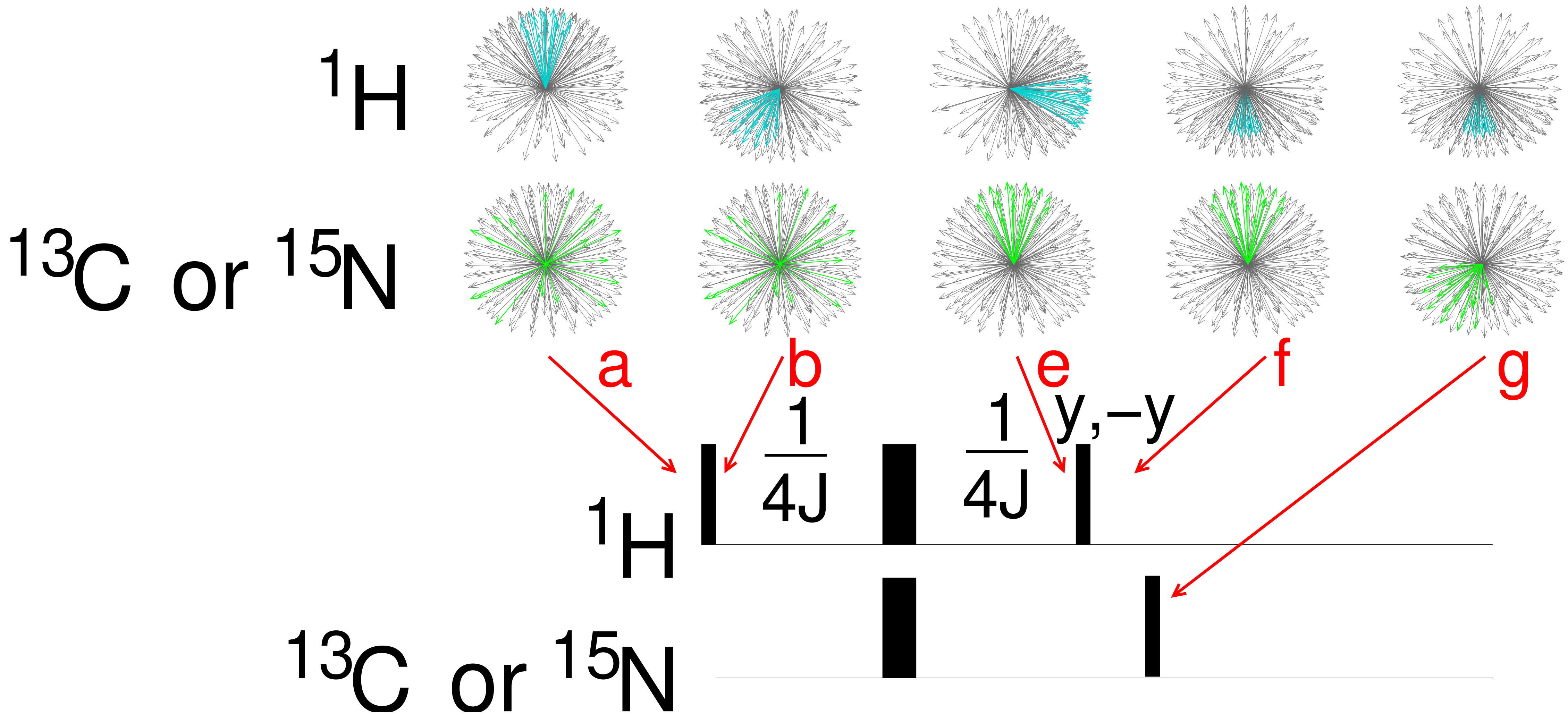


$$\phi = +90^\circ,\quad y: \quad \hat{\rho}(\mathrm{g}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1(2\mathscr{I}_z\mathscr{S}_y) + \frac{1}{2}\kappa_2\mathscr{S}_y$$

$$\phi = -90^\circ,\ -y: \quad \hat{\rho}(\mathrm{g}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1(2\mathscr{I}_z\mathscr{S}_y) + \frac{1}{2}\kappa_2\mathscr{S}_y$$

$$\text{difference}: \quad \hat{\rho}(\mathrm{g}) = \quad - \quad \kappa_1(2\mathscr{I}_z\mathscr{S}_y)$$

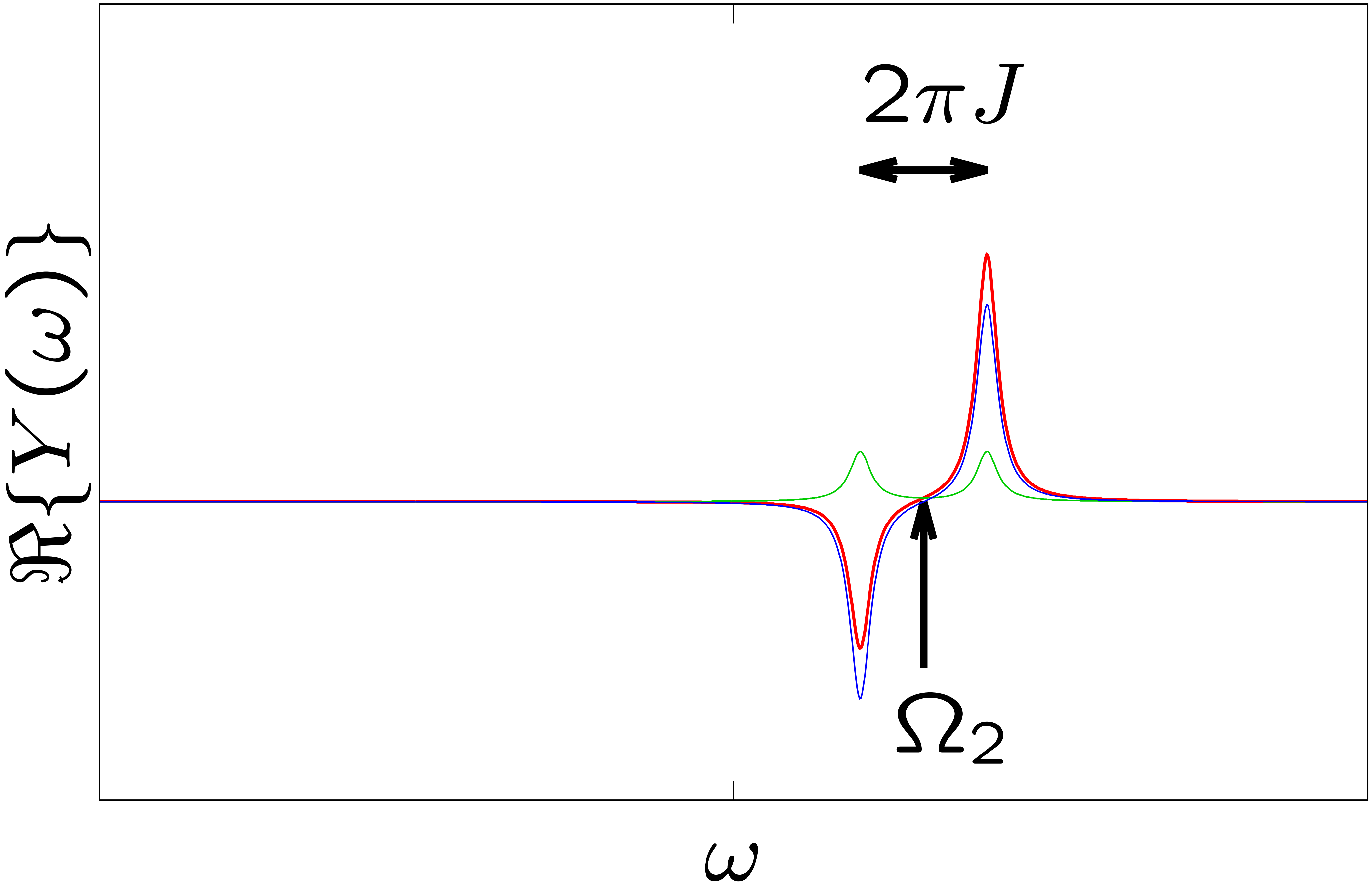
Phase cycling



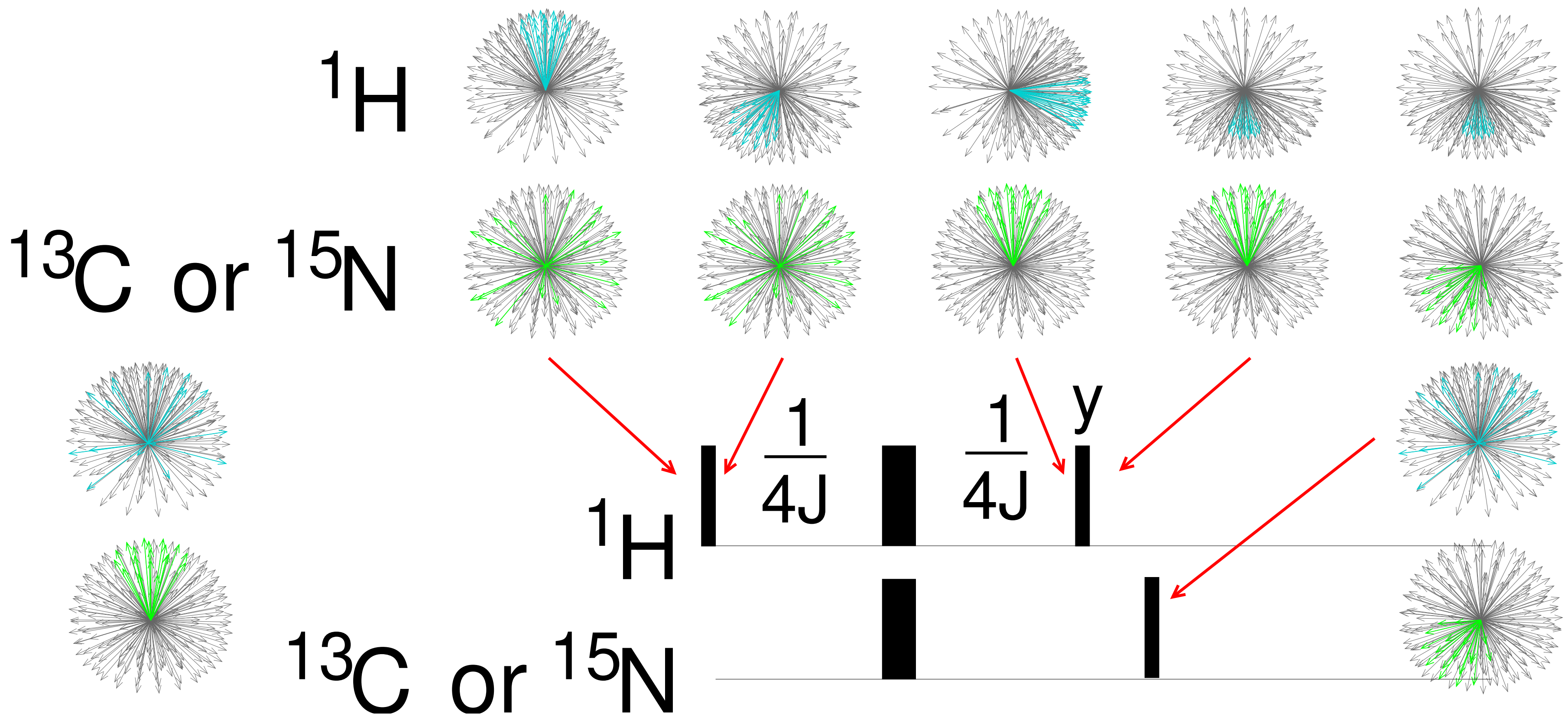
$$\Re\{Y(\omega)\} =$$

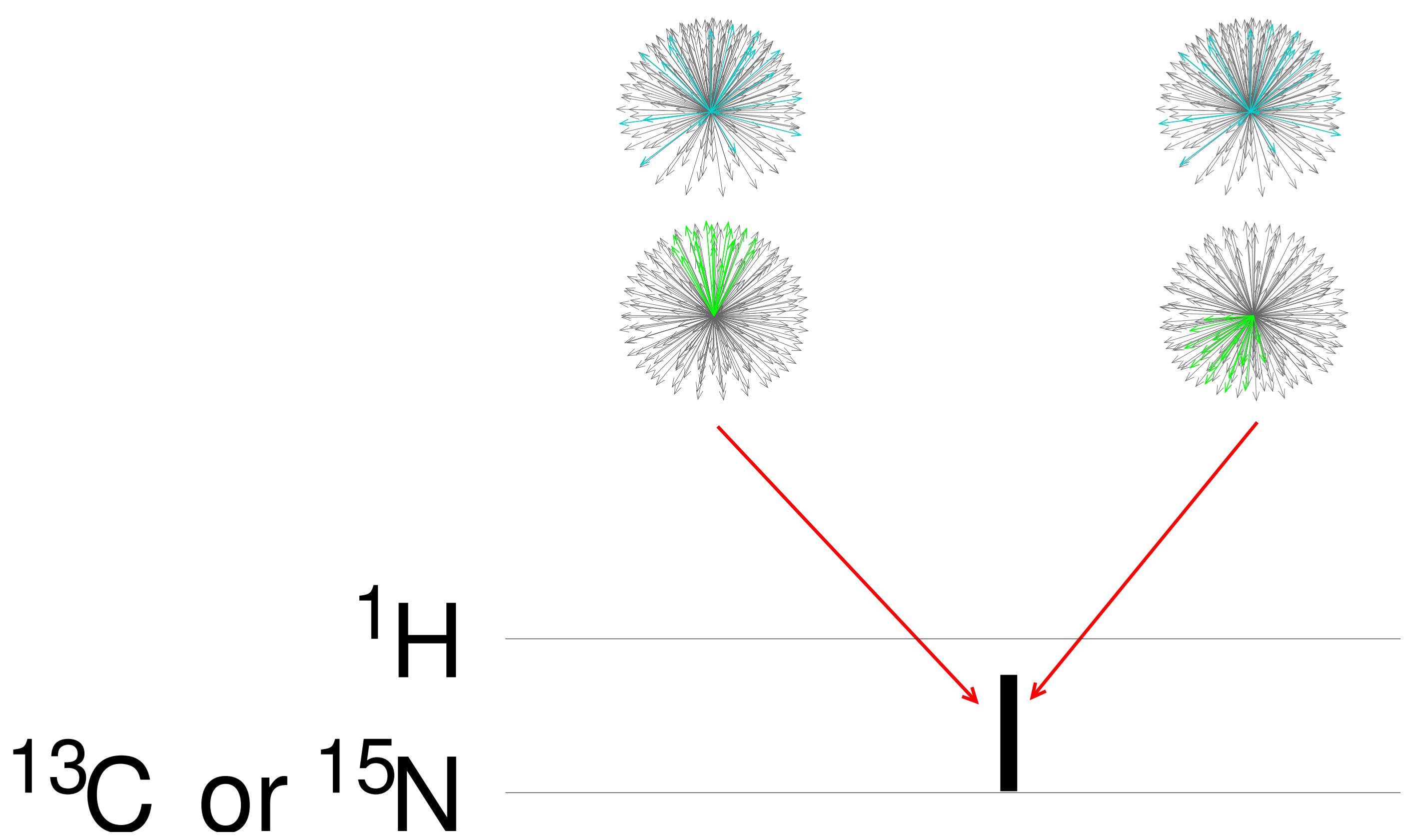
$$\frac{\mathcal{N}\gamma_1\gamma_2\hbar^2 B_0}{16k_\mathrm{B}T}\left(\frac{\overline{R}_2}{\overline{R}_2^2+(\omega-\Omega_2+\pi J)^2}-\frac{\overline{R}_2}{\overline{R}_2^2+(\omega-\Omega_2-\pi J)^2}\right)$$

INEPT with phase cycle:



INEPT vs. direct excitation





INEPT vs. direct excitation

INEPT (phase cycled): $\Re\{Y(\omega)\} =$

$$\frac{\mathcal{N}\gamma_1\gamma_2\hbar^2 B_0}{16k_{\mathrm{B}}T}\left(\frac{\overline{R}_2}{\overline{R}_2^2+(\omega-\Omega_2+\pi J)^2}-\frac{\overline{R}_2}{\overline{R}_2^2+(\omega-\Omega_2-\pi J)^2}\right)$$

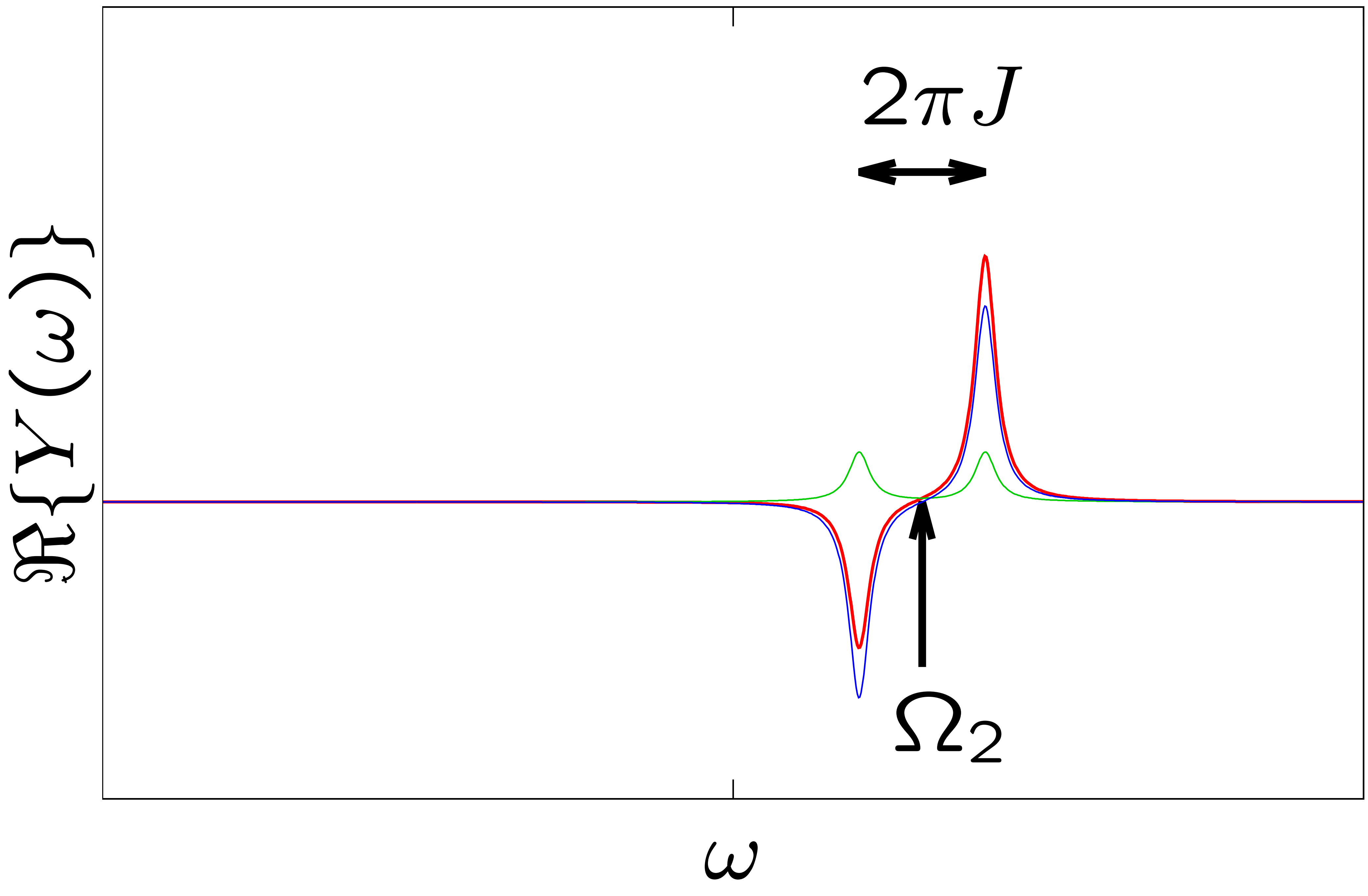
Direct excitation: $\Re\{Y(\omega)\} =$

$$\frac{\mathcal{N}\gamma_2\gamma_2\hbar^2 B_0}{16k_{\mathrm{B}}T}\left(\frac{\overline{R}_2}{\overline{R}_2^2+(\omega-\Omega_2+\pi J)^2}+\frac{\overline{R}_2}{\overline{R}_2^2+(\omega-\Omega_2-\pi J)^2}\right)$$

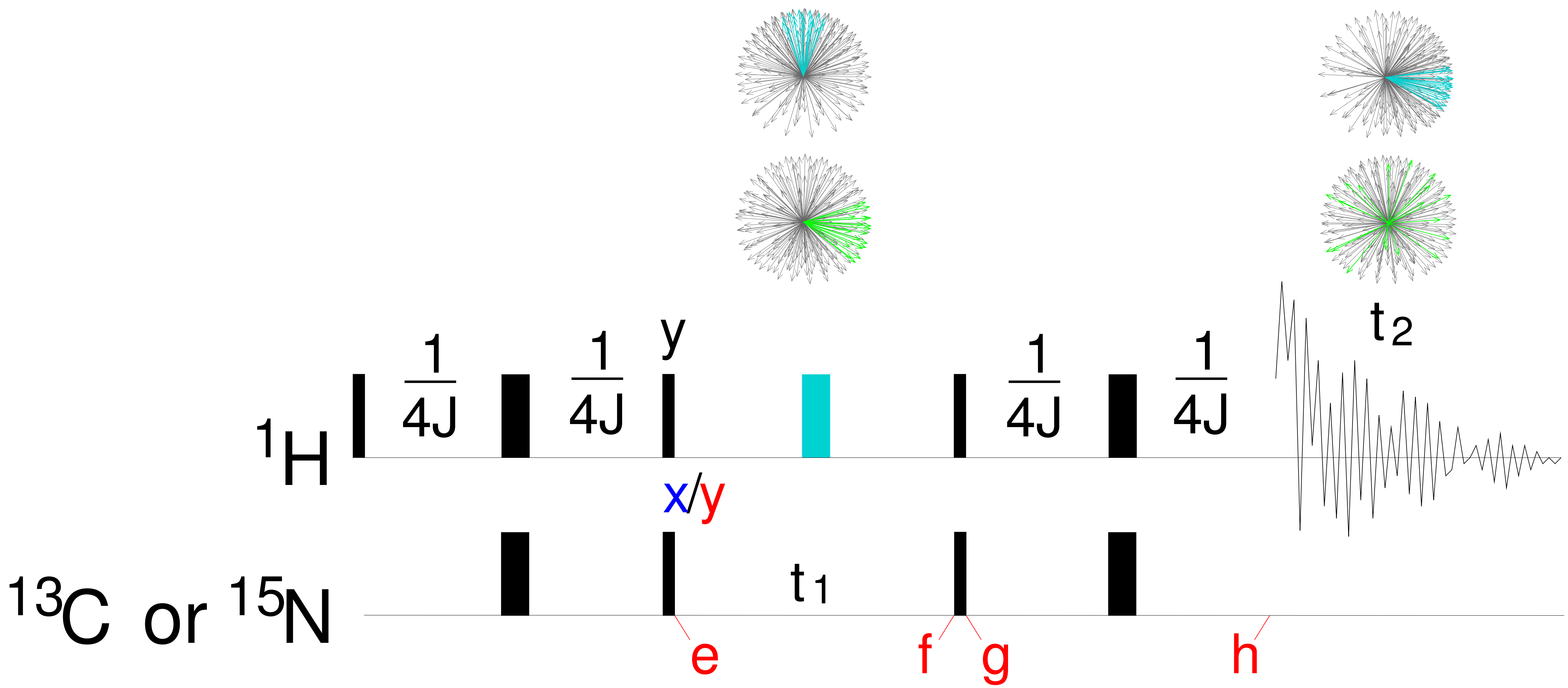
$\gamma_1/\gamma_2 \approx$ **4** for ^{13}C $\qquad$ $\gamma_1/\gamma_2 \approx$ **10** for ^{15}N

Insensitive **N**uclei **E**nhanced by **P**olarization **T**ransfer

INEPT vs. direct excitation:



HSQC Spectroscopy
(**H**eteronuclear **S**ingle-**Q**uantum **C**oherence)



COMPLEX EXPERIMENT

ANALYSIS FACILITATED BY SIMPLIFICATIONS

Using results of already analyzed building blocks (echoes)

Ignoring components of $\hat{\rho}$ that cannot produce signal

HSQC Spectroscopy

Measured quantity: M_{1+}

(M_{2+} does not pass the frequency filters)

Only $\mathscr{I}_x \hat{M}_{1+}$ and $\mathscr{I}_y \hat{M}_{1+}$ have non-zero traces:

$$\begin{aligned} \mathrm{Tr}\left\{\mathscr{I}_x(\mathscr{I}_{1x} + \mathrm{i}\mathscr{I}_{1y})\right\} &= 1 \\ \mathrm{Tr}\left\{\mathscr{I}_y(\mathscr{I}_{1x} + \mathrm{i}\mathscr{I}_{1y})\right\} &= \mathrm{i} \end{aligned}$$

Directly measurable: $\mathscr{I}_x$, $\mathscr{I}_y$ (in-phase single-quantum of nucleus 1)

Evolve to measurable due to J coupling:

$2\mathscr{I}_x\mathscr{S}_z$, $2\mathscr{I}_y\mathscr{S}_z$ (anti-phase single-quantum of nucleus 1)

Need 90° pulse + J coupling:

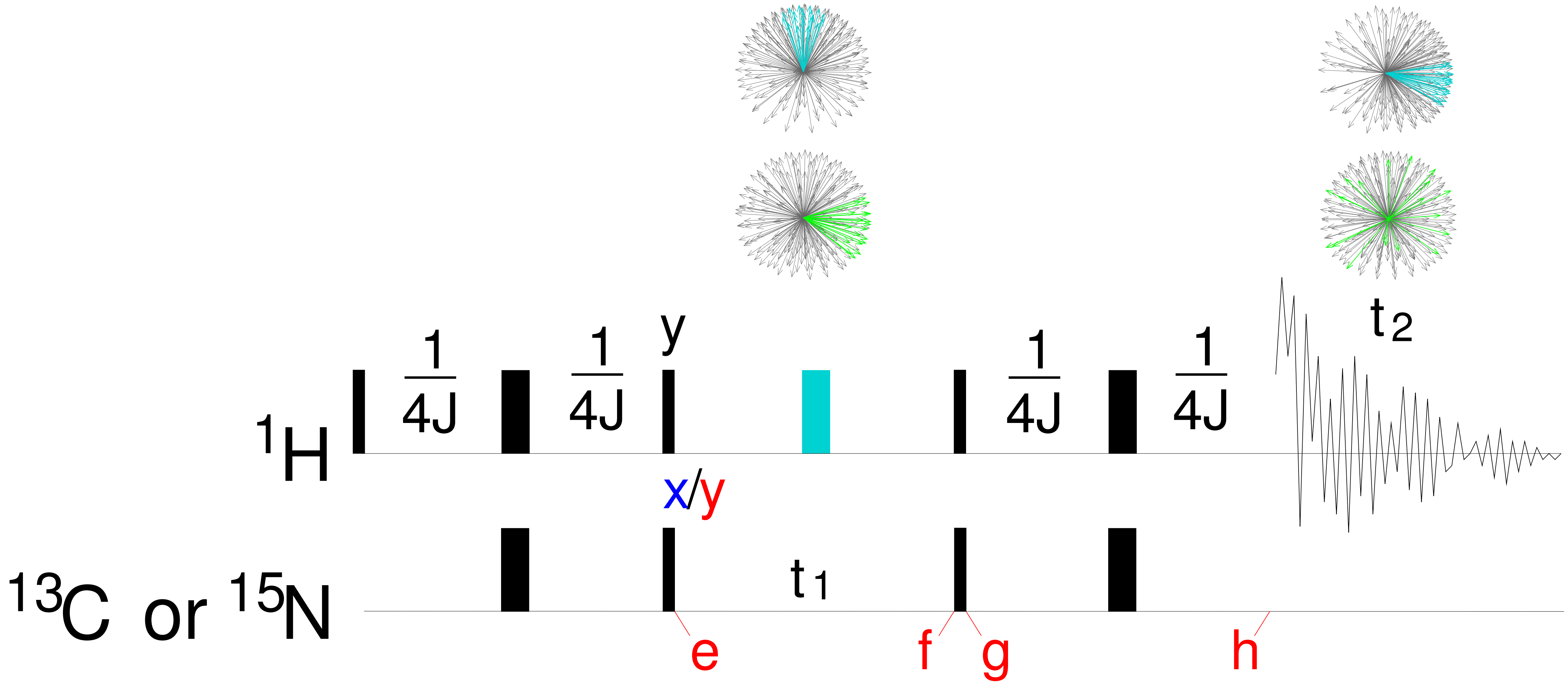
$\mathscr{I}_z$ (90° pulse), $\mathscr{S}_z$, $2\mathscr{I}_z\mathscr{S}_z$ (populations, longitudinal polarization)

$\mathscr{S}_x$, $\mathscr{S}_y$, $2\mathscr{I}_z\mathscr{S}_x$, $2\mathscr{I}_z\mathscr{S}_y$ (single-quantum of nucleus 2)

$2\mathscr{I}_x\mathscr{S}_x$, $2\mathscr{I}_y\mathscr{S}_y$, $2\mathscr{I}_x\mathscr{S}_y$, $2\mathscr{I}_y\mathscr{S}_x$ (multiple-quantum)

Never measurable: $\mathscr{I}_t$ (unit matrix)

HSQC Spectroscopy

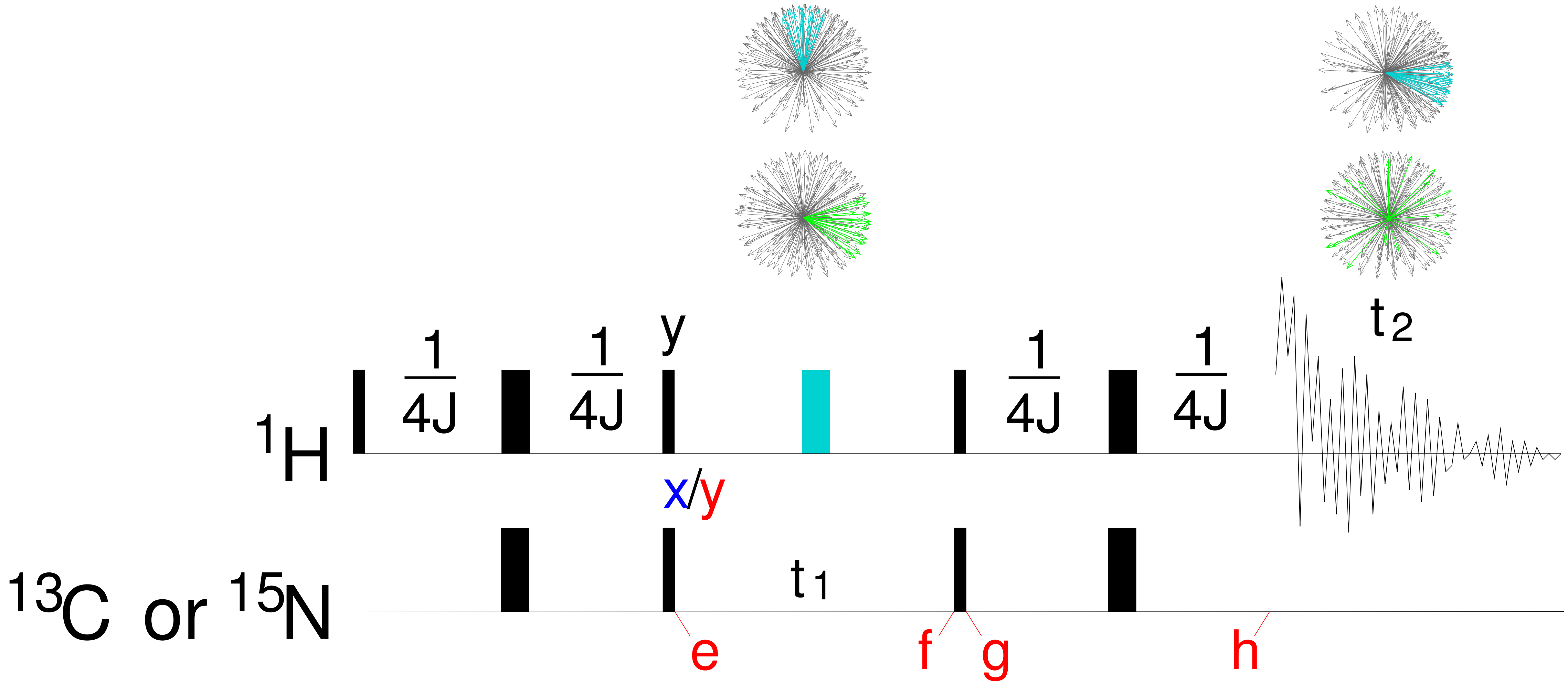


BLOCK 1: INEPT

$$\hat{\rho}(\mathrm{a}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(\mathscr{I}_z\right) + \frac{1}{2}\kappa_2\mathscr{S}_z \rightarrow$$

$$\hat{\rho}(\mathrm{e}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(2\mathscr{I}_z\mathscr{S}_y\right) + \frac{1}{2}\kappa_2\mathscr{S}_y$$

HSQC Spectroscopy

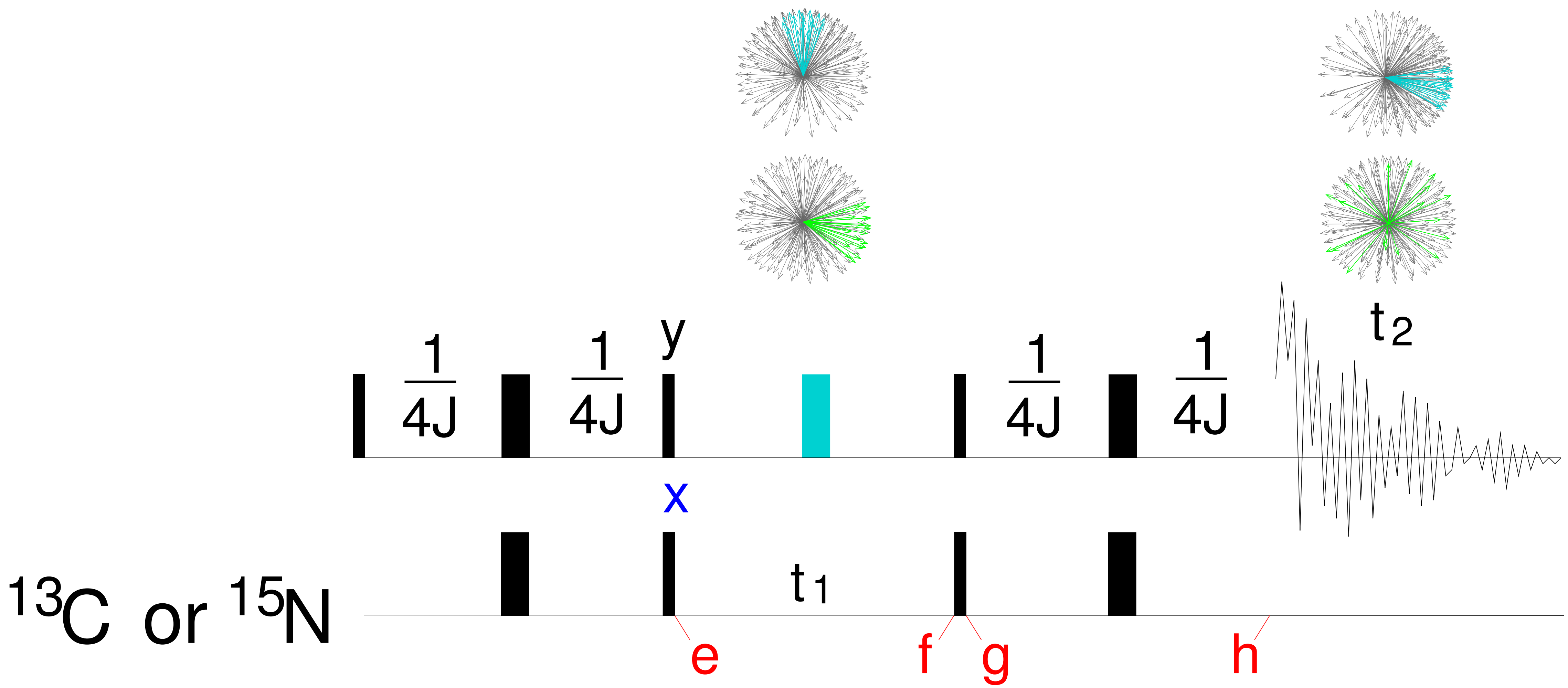


BLOCK 2: DECOUPLING ECHO, INCREMENTED t_1

$$\hat{\rho}(\mathrm{e}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(2\mathscr{I}_z\mathscr{S}_y\right) + \frac{1}{2}\kappa_2\mathscr{S}_y \rightarrow$$

$$\hat{\rho}(\mathrm{f}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(c_{21}2\mathscr{I}_z\mathscr{S}_y - s_{21}2\mathscr{I}_z\mathscr{S}_x\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_y - s_{21}\mathscr{S}_x\right)$$

HSQC Spectroscopy – Real

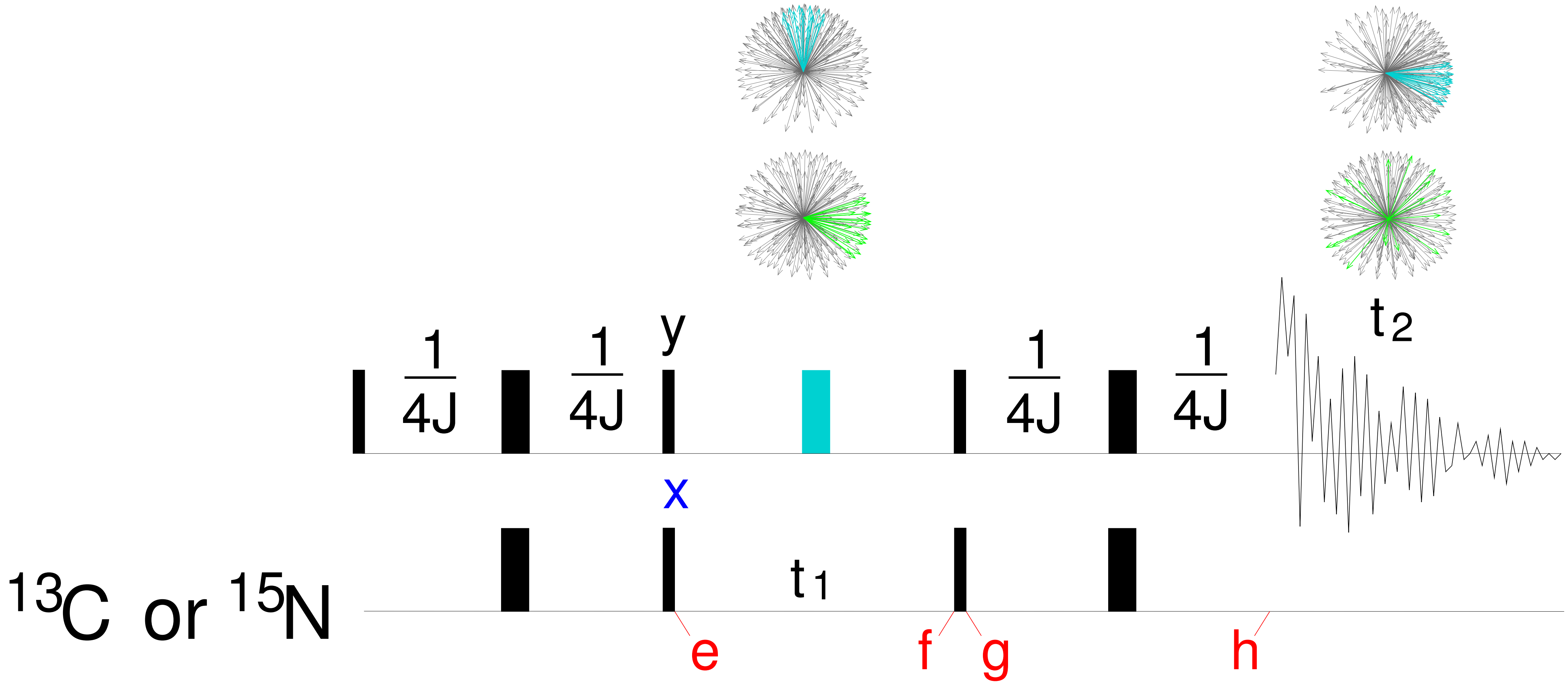


BLOCK 3: TWO 90° PULSES, PHASE x (^{13}C or ^{15}N)

$$\hat{\rho}(\mathrm{f}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(c_{21}2\mathscr{I}_z\mathscr{S}_y - s_{21}2\mathscr{I}_z\mathscr{S}_x\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_y - s_{21}\mathscr{S}_x\right)$$

$$\hat{\rho}(\mathrm{g}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(c_{21}2\mathscr{I}_y\mathscr{S}_z - s_{21}2\mathscr{I}_y\mathscr{S}_x\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_z - s_{21}\mathscr{S}_x\right)$$

HSQC Spectroscopy – Real

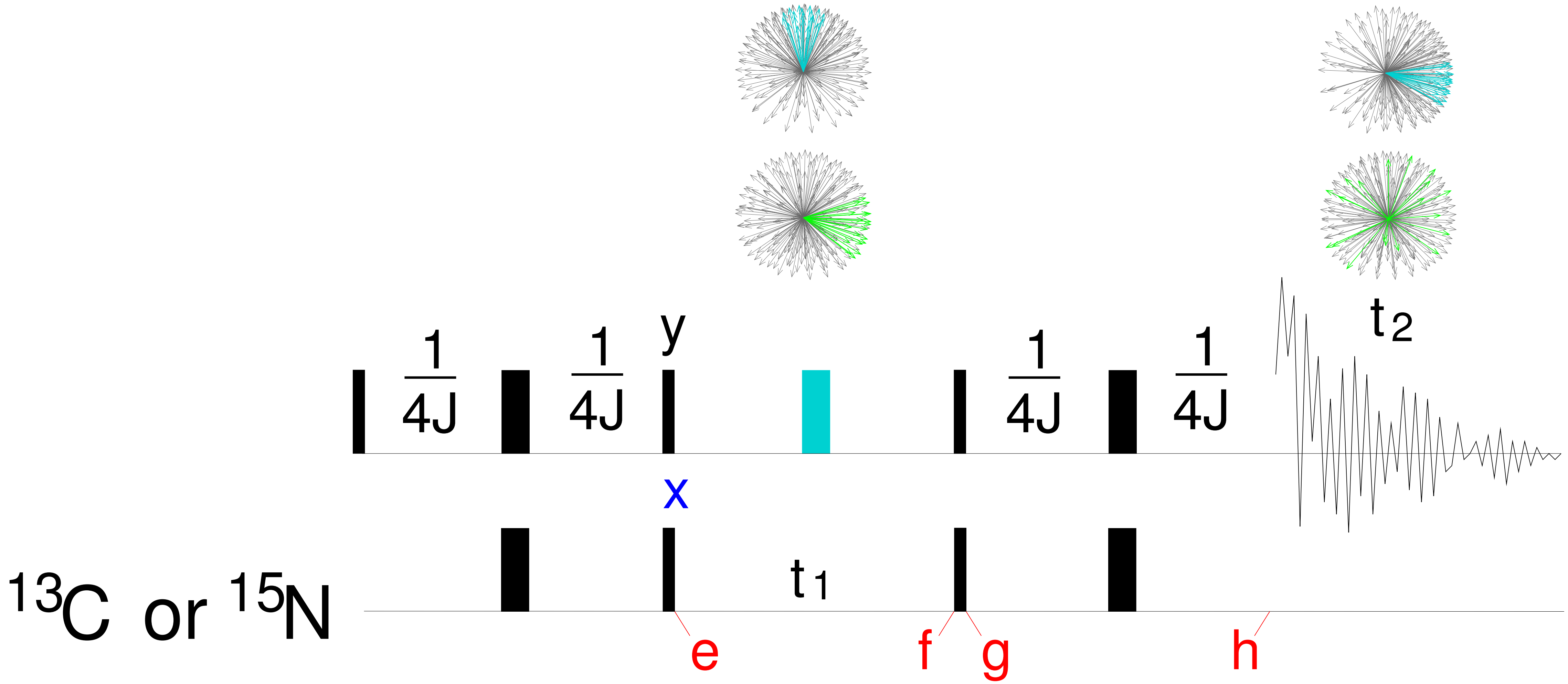


BLOCK 3: TWO 90° PULSES, PHASE x (^{13}C or ^{15}N)

$$\hat{\rho}(\mathrm{f}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(c_{21}2\mathscr{I}_z\mathscr{S}_y - s_{21}2\mathscr{I}_z\mathscr{S}_x\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_y - s_{21}\mathscr{S}_x\right)$$

$$\hat{\rho}(\mathrm{g}) = -\frac{1}{2}\kappa_1 c_{21}2\mathscr{I}_y\mathscr{S}_z + \text{ unmeasurable (no more 90° pulses)}$$

HSQC Spectroscopy − Real

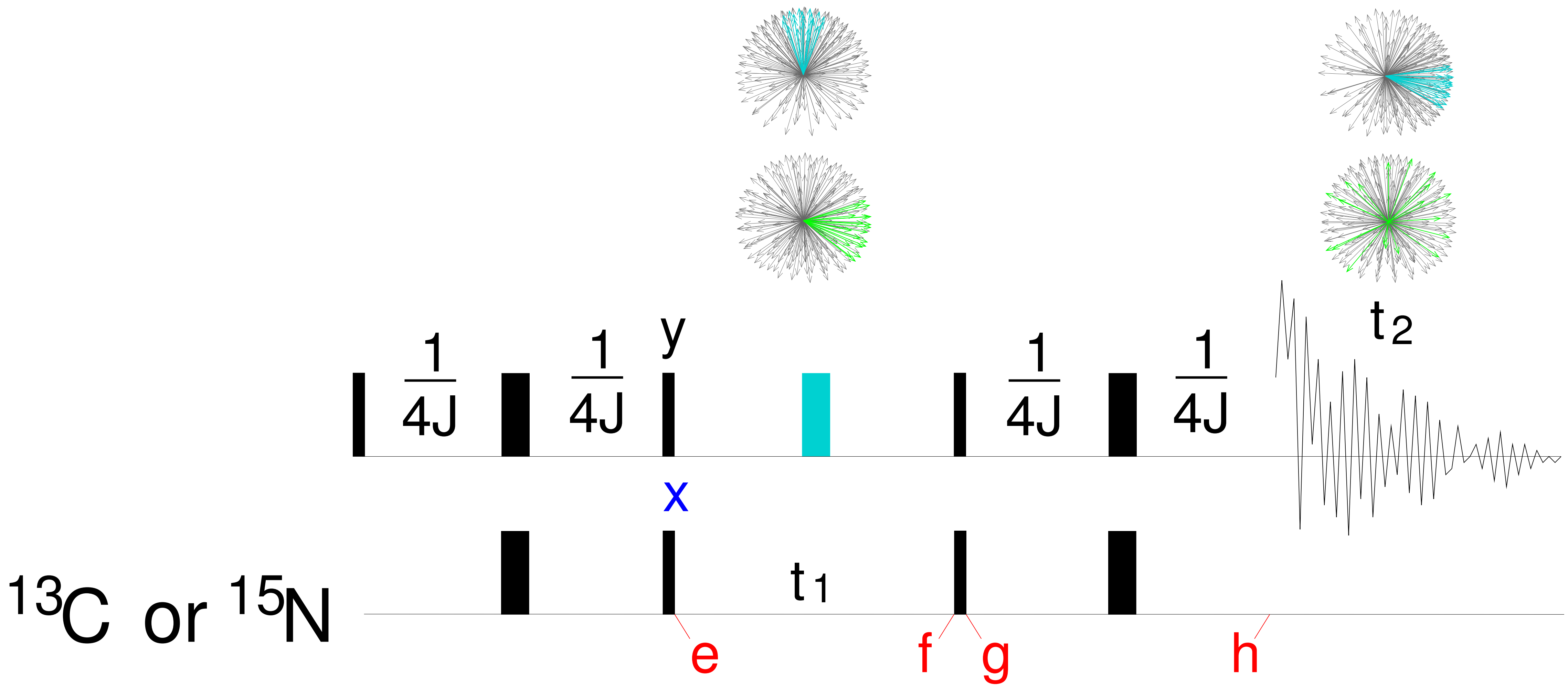


BLOCK 4: SIMULTANEOUS ECHO

$\hat{\rho}(\mathrm{g}) = -\frac{1}{2}\kappa_1 c_{21} 2\mathscr{I}_y\mathscr{S}_z +$ unmeasurable $\rightarrow$

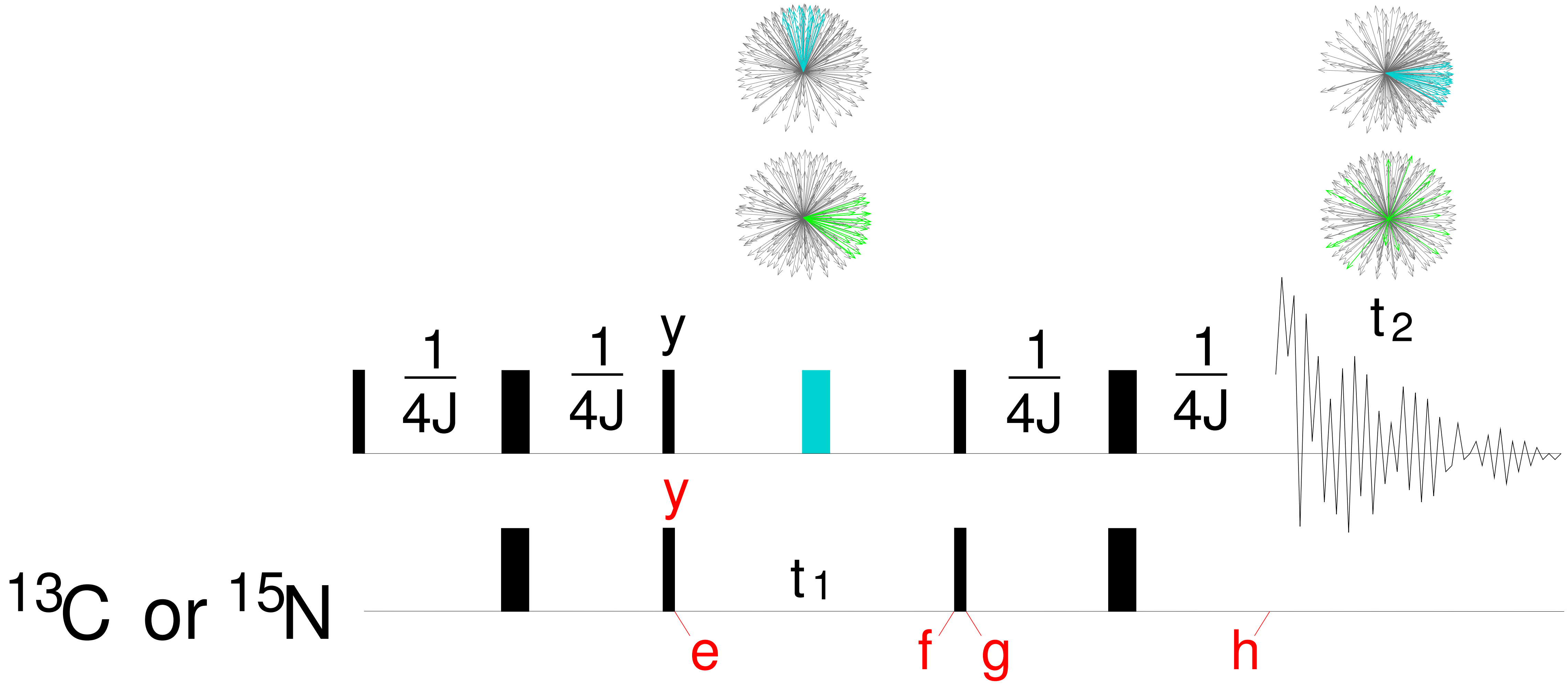
$\hat{\rho}(\mathrm{h}) = \frac{1}{2}\kappa_1 c_{21} \mathscr{I}_x +$ unmeasurable

HSQC Spectroscopy – Real



$$\frac{1}{2}\kappa_1 c_{21}\mathscr{I}_x \rightarrow \begin{cases} \frac{1}{2}\kappa_1 c_{21} c_{12}\,\mathscr{I}_x \rightarrow \begin{cases} +\frac{1}{2}\kappa_1 c_{21} c_{12} c_J \;\; \mathscr{I}_x \\ +\frac{1}{2}\kappa_1 c_{21} c_{12} s_J \;\; 2\mathscr{I}_y\mathscr{S}_z \end{cases} \\ \frac{1}{2}\kappa_1 c_{21} s_{12}\,\mathscr{I}_y \rightarrow \begin{cases} +\frac{1}{2}\kappa_1 c_{21} s_{12} c_J \;\; \mathscr{I}_y \\ -\frac{1}{2}\kappa_1 c_{21} s_{12} s_J \;\; 2\mathscr{I}_x\mathscr{S}_z \end{cases} \end{cases}$$

HSQC Spectroscopy – Imaginary

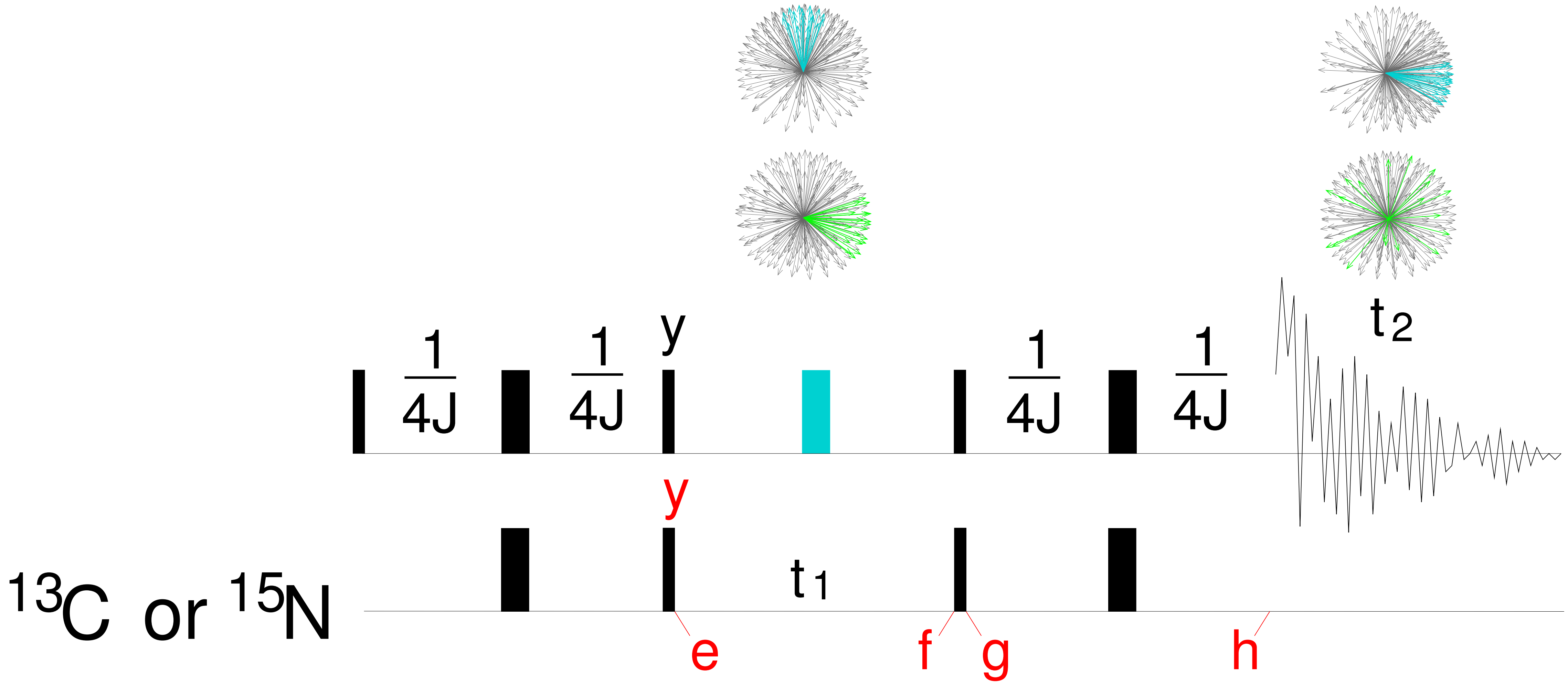


BLOCK 3: TWO 90° PULSES, PHASE y (^{13}C or ^{15}N)

$$\hat{\rho}(\mathrm{f}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(c_{21}2\mathscr{I}_z\mathscr{S}_y - s_{21}2\mathscr{I}_z\mathscr{S}_x\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_y - s_{21}\mathscr{S}_x\right)$$

$$\hat{\rho}(\mathrm{g}) = \frac{1}{2}\mathscr{I}_t - \frac{1}{2}\kappa_1\left(c_{21}2\mathscr{I}_y\mathscr{S}_y + s_{21}2\mathscr{I}_y\mathscr{S}_z\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_y + s_{21}\mathscr{S}_z\right)$$

HSQC Spectroscopy – Imaginary

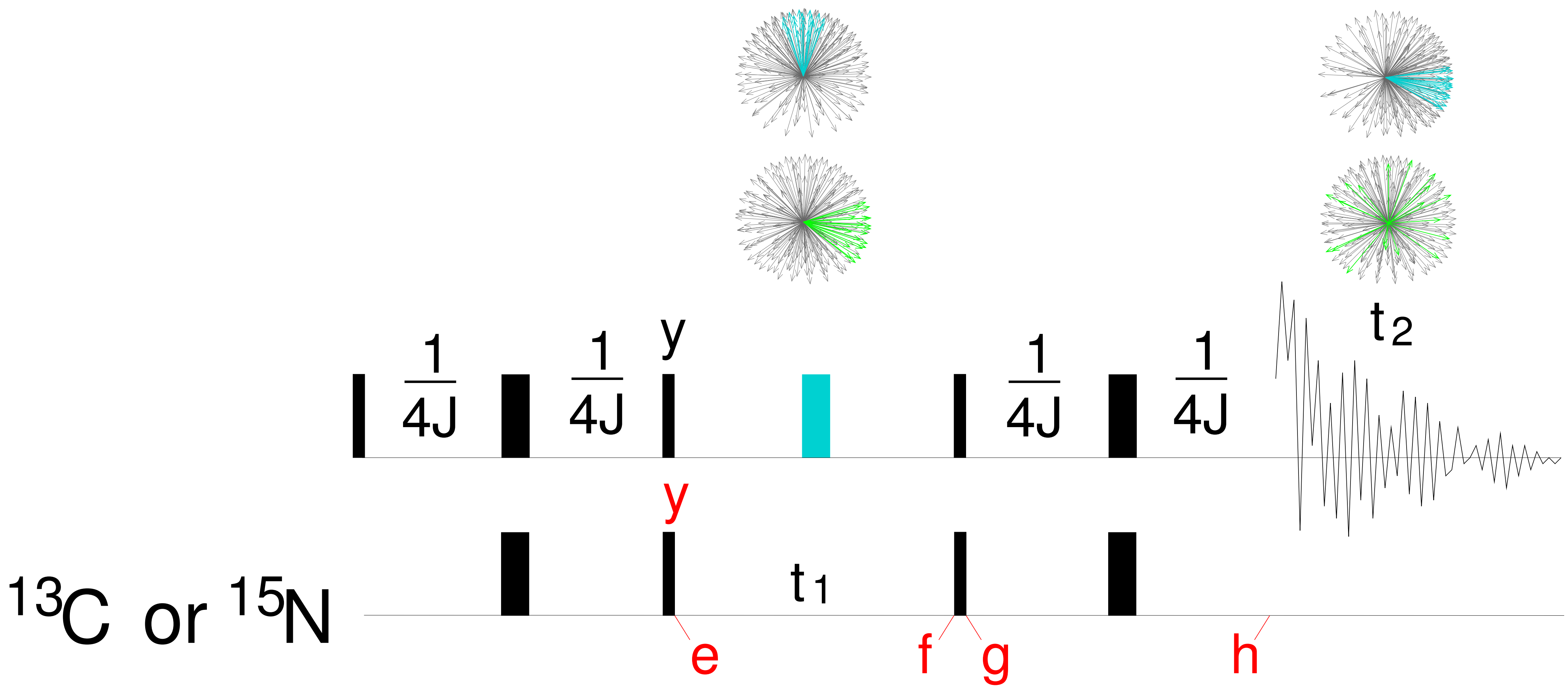


BLOCK 3: TWO 90° PULSES, PHASE y (^{13}C or ^{15}N)

$\hat{\rho}(\mathrm{f}) = \frac{1}{2}\mathscr{I}_t + \frac{1}{2}\kappa_1\left(s_{21}2\mathscr{I}_z\mathscr{S}_y - s_{21}2\mathscr{I}_z\mathscr{S}_x\right) + \frac{1}{2}\kappa_2\left(c_{21}\mathscr{S}_y - s_{21}\mathscr{S}_x\right)$

$\hat{\rho}(\mathrm{g}) = -\frac{1}{2}\kappa_1 s_{21}2\mathscr{I}_y\mathscr{S}_z +$ unmeasurable (no more 90° pulses)

HSQC Spectroscopy – Imaginary

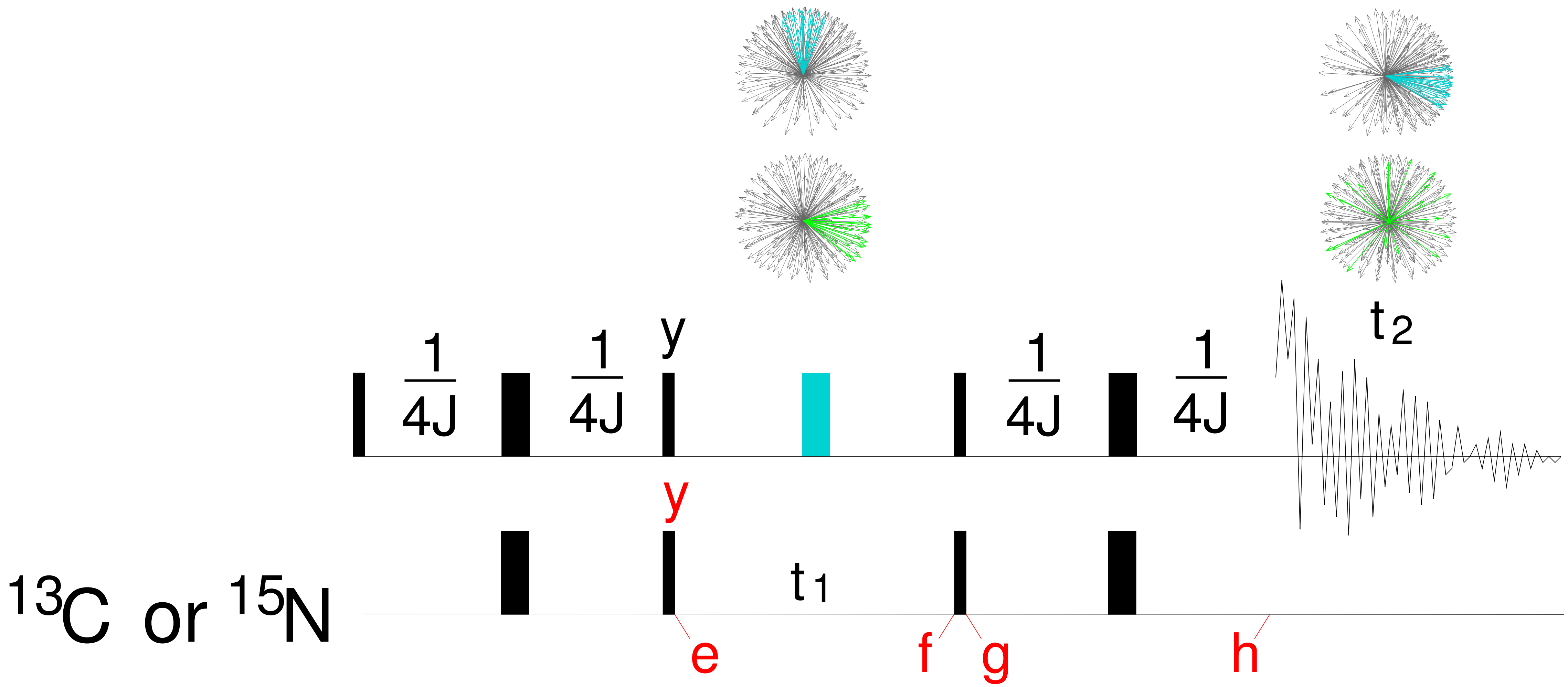


BLOCK 4: SIMULTANEOUS ECHO

$\widehat{\rho}(\mathrm{g}) = -\frac{1}{2}\kappa_1 s_{21} 2\mathscr{I}_y\mathscr{S}_z +$ unmeasurable $\rightarrow$

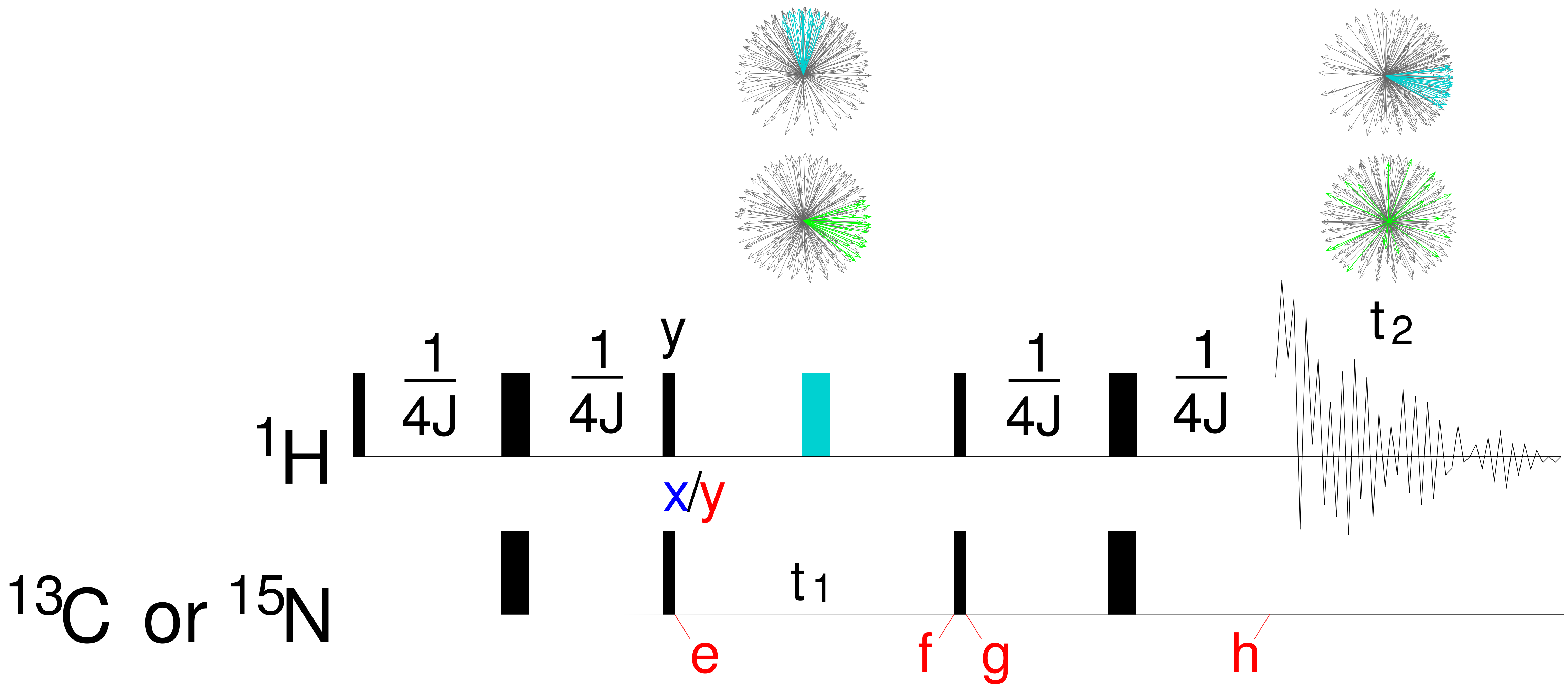
$\widehat{\rho}(\mathrm{h}) = \frac{1}{2}\kappa_1 s_{21}\mathscr{I}_x +$ unmeasurable

HSQC Spectroscopy – Imaginary



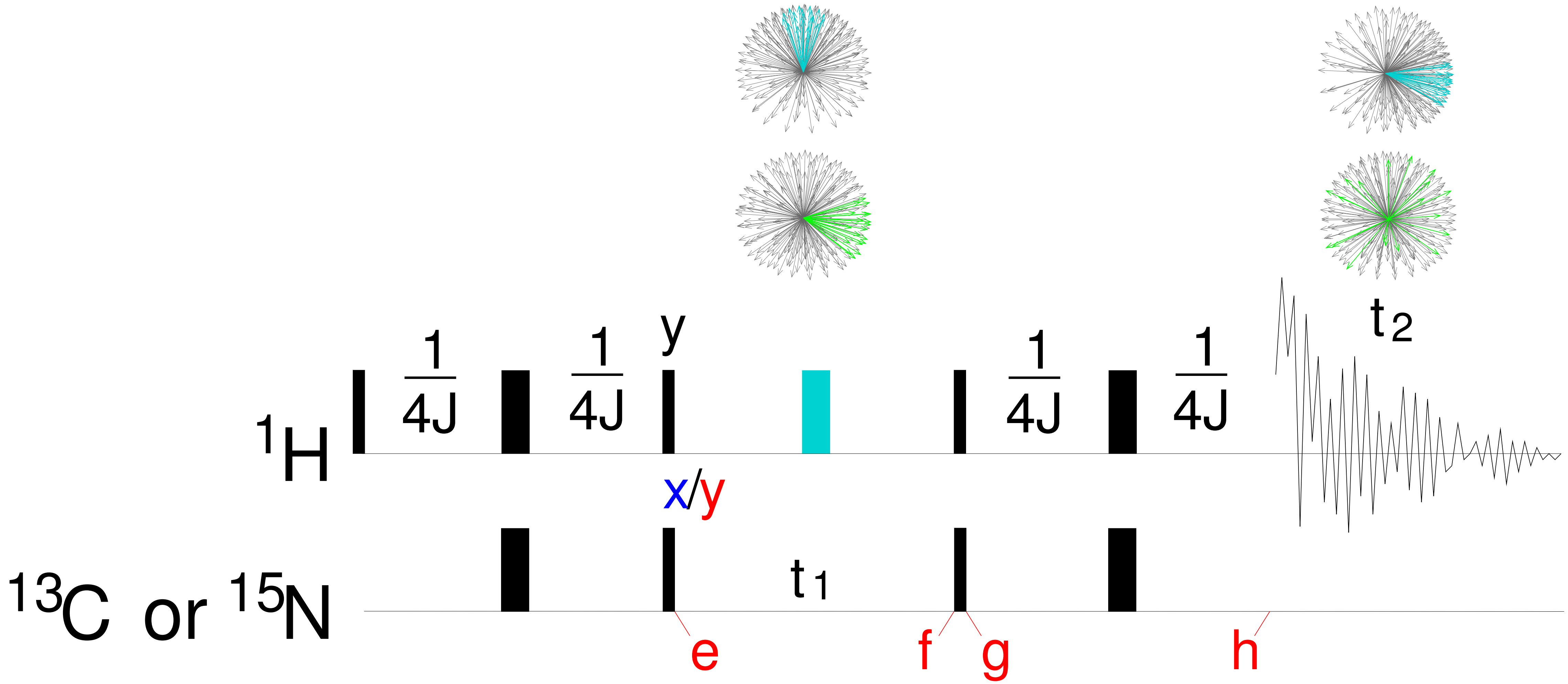
$$\frac{1}{2}\kappa_1 s_{21}\mathscr{I}_x \rightarrow \begin{cases} \frac{1}{2}\kappa_1 s_{21} c_{12}\,\mathscr{I}_x \rightarrow \begin{cases} +\frac{1}{2}\kappa_1 s_{21} c_{12} c_J\;\mathscr{I}_x \\ +\frac{1}{2}\kappa_1 s_{21} c_{12} s_J\;2\mathscr{I}_y\mathscr{S}_z \end{cases} \\ \frac{1}{2}\kappa_1 s_{21} s_{12}\,\mathscr{I}_y \rightarrow \begin{cases} +\frac{1}{2}\kappa_1 s_{21} s_{12} c_J\;\mathscr{I}_y \\ -\frac{1}{2}\kappa_1 s_{21} s_{12} s_J\;2\mathscr{I}_x\mathscr{S}_z \end{cases} \end{cases}$$

HSQC Spectroscopy – Hypercomplex



$$\frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1}\mathscr{I}_x \rightarrow \begin{cases} \frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1} c_{12}\,\mathscr{I}_x \rightarrow \begin{cases} +\frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1} c_{12} c_J & \mathscr{I}_x \\ +\frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1} c_{12} s_J & 2\mathscr{I}_y\mathscr{S}_z \end{cases} \\ \frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1} s_{12}\,\mathscr{I}_y \rightarrow \begin{cases} +\frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1} s_{12} c_J & \mathscr{I}_y \\ -\frac{1}{2}\kappa_1 \mathrm{e}^{\mathrm{i}\Omega_2 t_1} s_{12} s_J & 2\mathscr{I}_x\mathscr{S}_z \end{cases} \end{cases}$$

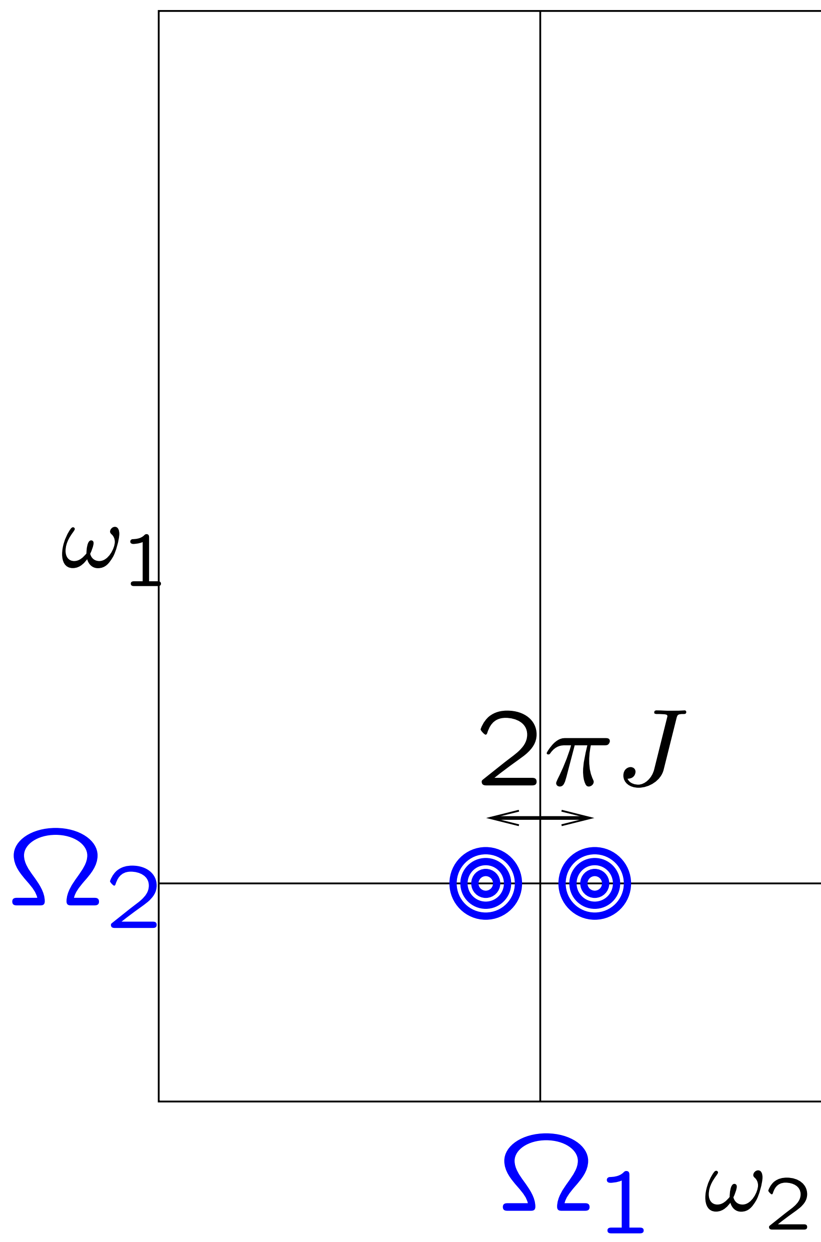
HSQC Spectroscopy – Hypercomplex



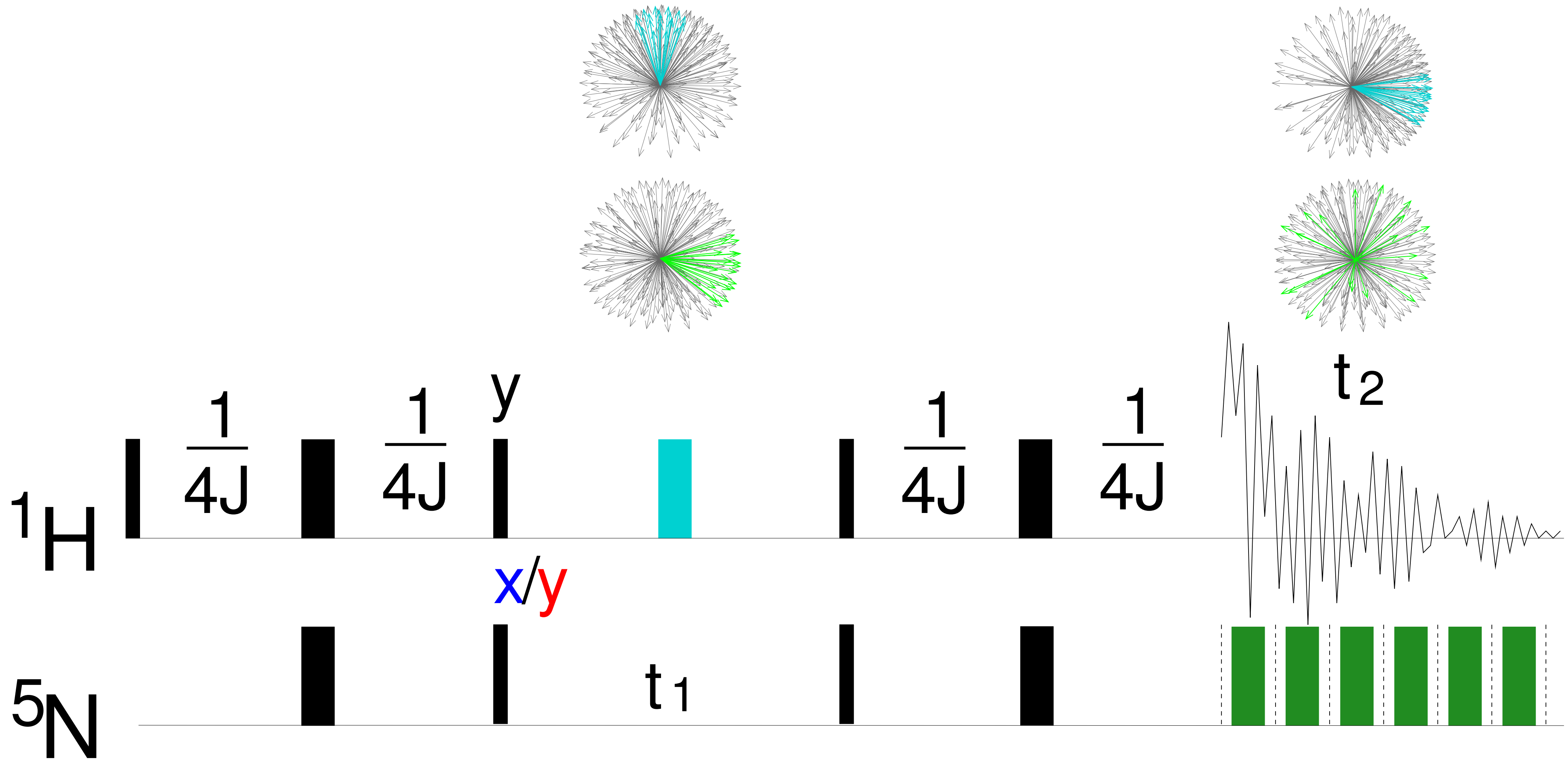
$$\Re\{Y(\omega)\} = \frac{\mathcal{N}{\gamma_1}^2\hbar^2 B_0}{16 k_{\mathrm{B}} T} \times$$

$$\frac{\overline{R}^2_{2,2}}{\overline{R}^2_{2,2} + (\omega - \Omega_2)^2}\left(\frac{\overline{R}^2_{2,1}}{\overline{R}^2_{2,1} + (\omega - \Omega_1 + \pi J)^2} + \frac{\overline{R}^2_{2,1}}{\overline{R}^2_{2,1} + (\omega - \Omega_1 - \pi J)^2}\right)$$

Decoupling in direct dimension

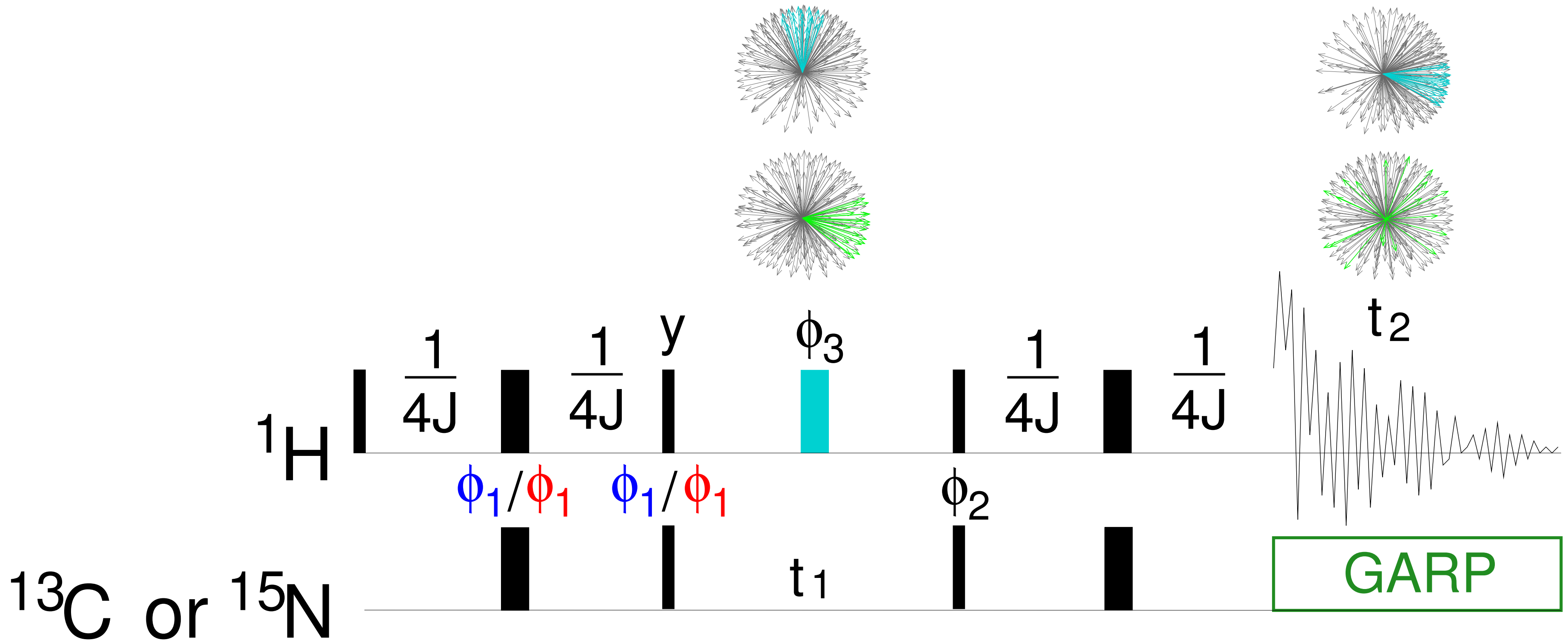


Decoupling in direct dimension



$$\Re\{Y(\omega)\} = \frac{\mathcal{N}\gamma_1{}^2\hbar^2 B_0}{8k_\mathrm{B}T}\,\frac{\overline{R}^2_{2,2}}{\overline{R}^2_{2,2}+(\omega-\Omega_2)^2}\,\frac{R^2_{2,1}}{R^2_{2,1}+(\omega-\Omega_1)^2}$$

Decoupling in direct dimension



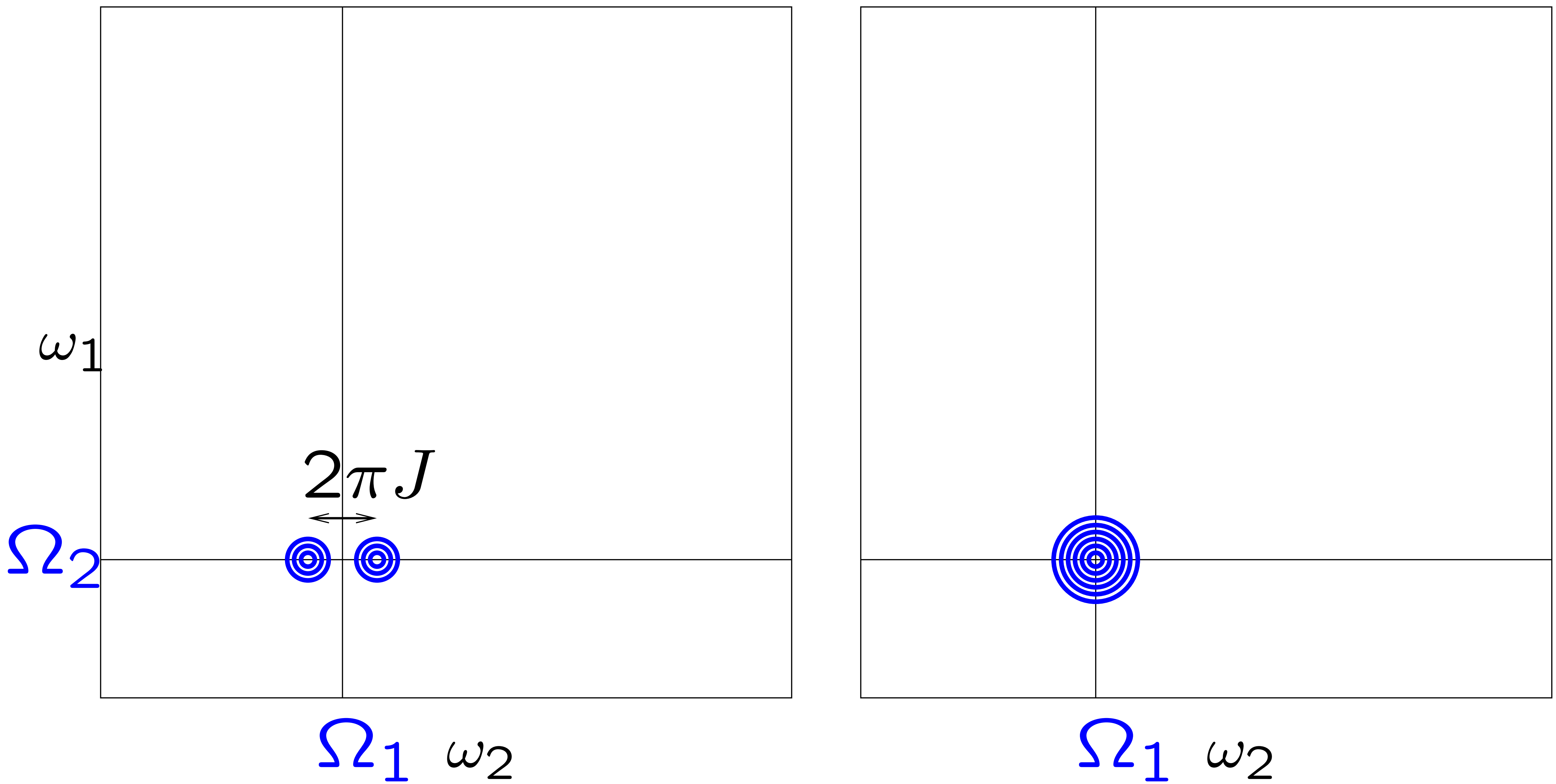
ϕ_1=x,−x,x,−x,x,−x,x,−x ϕ_2=x,x,−x,−x,x,x,−x,−x

ϕ_1=y,−y,y,−y,y,−y,y,−y ϕ_3=x,x,x,x,−x,−x,−x,−x

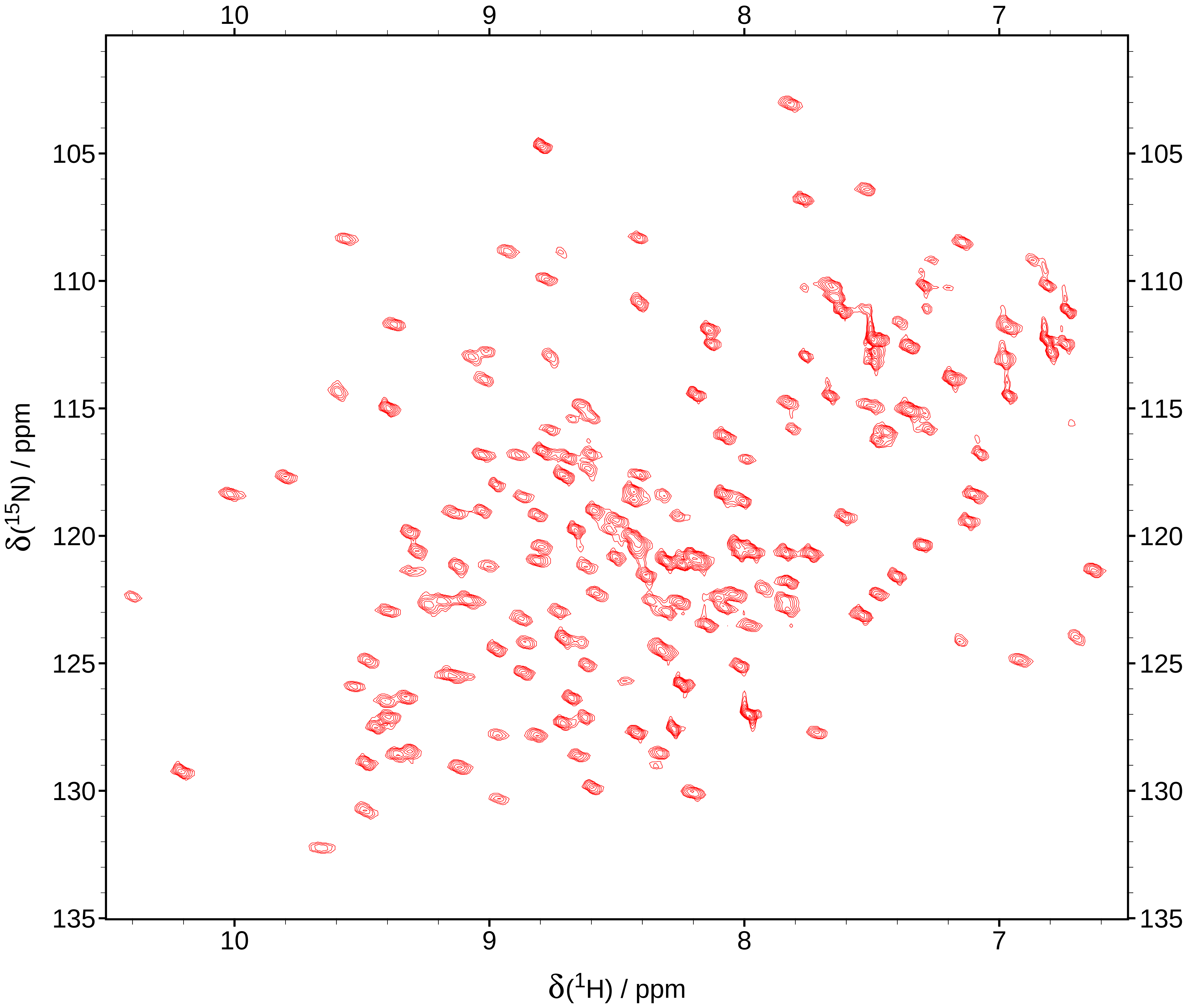
signal multiplied by: 1,−1,−1,1,1,−1,−1,1

$$\Re\{Y(\omega)\} = \frac{\mathcal{N}{\gamma_1}^2\hbar^2 B_0}{8k_\mathrm{B}T}\,\frac{\overline{R}_{2,2}^2}{\overline{R}_{2,2}^2+(\omega-\Omega_2)^2}\,\frac{R_{2,1}^2}{R_{2,1}^2+(\omega-\Omega_1)^2}$$

Decoupling in direct dimension



HSQC spectrum of a 20 kDa protein



Benefits of HSQC

- *High sensitivity* for ^{13}C or ^{15}N

  (higher by $(\gamma_1/\gamma_2)^{5/2}$ than by the direct detection

- *High resolution*

  Second dimension and less peaks in spectrum

  (only $^{13}C/^{15}N$-bonded protons and protonated $^{13}C/^{15}N$ visible)

- *Important structural information*

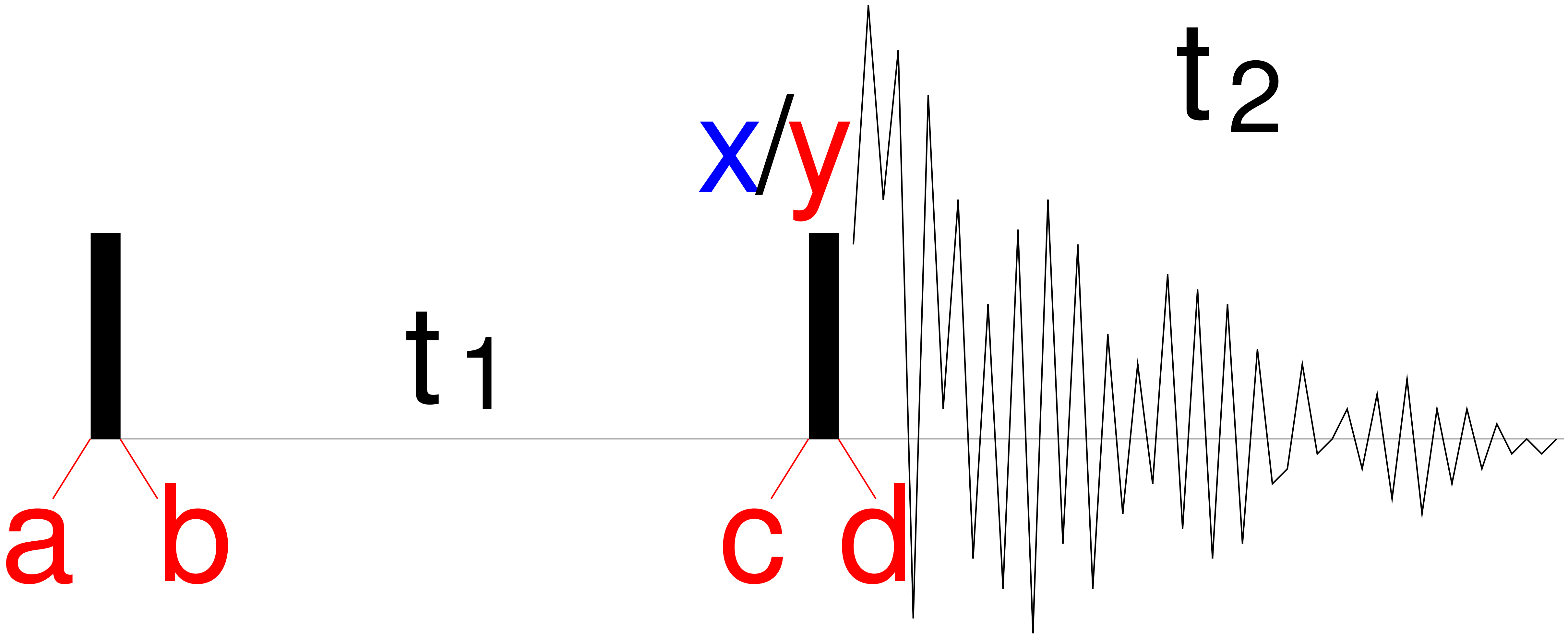
  ^{1}H-^{13}C and ^{1}H-^{15}N correlation

  (it tells us which proton is attached to which ^{13}C or ^{15}N).

HOMEWORK:

COSY

Section 11.3



x/y
t 2
t 1
a
b
c
d