## Supplementary materials



Scheme 1. Synthesis of $\left[{ }^{13} \mathrm{C}, \gamma^{18} \mathrm{O}_{3}\right] \mathrm{GTP}$. G stands for guanosine. $\mathrm{O}^{*}$ indicates ${ }^{18} \mathrm{O}$ labeling.



$\downarrow\left[{ }^{18} \mathrm{O}\right]$ phosphate


Scheme 2. Synthesis of $\left[{ }^{13} \mathrm{C}, \gamma^{18} \mathrm{O}_{4}\right]$ GTP. G stands for guanosine. $\mathrm{O}^{*}$ indicates ${ }^{18} \mathrm{O}$ labeling.


Scheme 3. Synthesis of $\left[{ }^{13} \mathrm{C}, \beta^{18} \mathrm{O}_{3}\right]$ GTP. G stands for guanosine. $\mathrm{O} *$ indicates ${ }^{18} \mathrm{O}$ labeling.






Scheme 4. Synthesis of $\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}, \beta^{18} \mathrm{O}_{2}\right]$ GTP. G stands for guanosine. $\mathrm{O}^{*}$ indicates ${ }^{18} \mathrm{O}$ labeling.
A. Synthesis of $\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}, \beta \mathrm{~S}\right]$ GDP


B. Synthesis of $\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}\right.$, pro-R $\left.\beta^{18} \mathrm{O}\right]$ GTP

$\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}\right.$, pro-R $\left.\beta^{18} \mathrm{O}\right] \mathrm{GTP} \longleftarrow[\mathrm{S}(\mathrm{p}), \beta \mathrm{BS}] \mathrm{GTP}$
C. Synthesis of $\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}\right.$, pro-S $\left.\beta^{18} \mathrm{O}\right] \mathrm{GTP}$
$\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}, \beta \mathrm{~S}\right] \mathrm{GDP} \xrightarrow{\text { acetate kinase, acetyl phosphate }}[\mathrm{S}(\mathrm{p}), \beta \mathrm{BS}] \mathrm{GTP}+[\mathrm{R}(\mathrm{p}), \beta \mathrm{BS}] \mathrm{GTP}$
$\downarrow$ myosin
$\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}\right.$, pro-S $\left.\beta^{18} \mathrm{O}\right] \mathrm{GTP} \quad[\mathrm{R}(\mathrm{p}), \beta \mathrm{S}] \mathrm{GTP}$

Scheme 5. Synthesis of $\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}\right.$, pro-S, $\left.\beta^{18} \mathrm{O}\right] \mathrm{GTP}$ and $\left[{ }^{13} \mathrm{C},{ }^{15} \mathrm{~N}\right.$, pro-R, $\left.\beta^{18} \mathrm{O}\right]$ GTP. G stands for guanosine. $\mathrm{O}^{*}$ indicates ${ }^{18} \mathrm{O}$ labeling. CDI and PEP stand for carboyldiimidazole and phosphoenolpyruvate.

