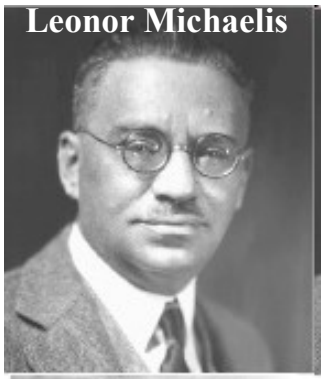
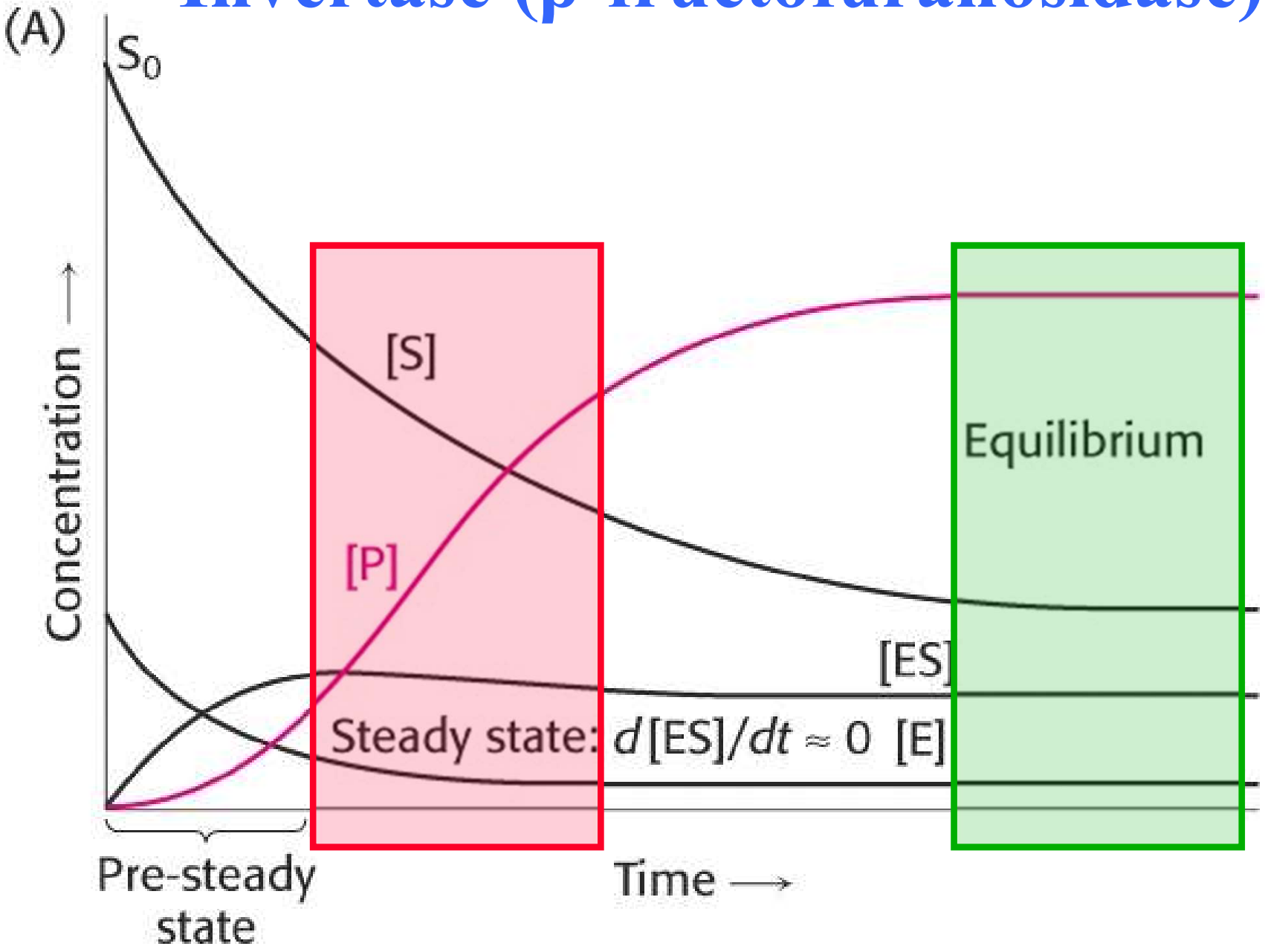
Applying our knowledge to enzymes: Invertase (β-fructofuranosidase)





What about (common) reversible chemistry?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E$$

$$\Rightarrow \quad v = \frac{\dfrac{V^f_{max}[S]}{K^S_M} - \dfrac{V^r_{max}[P]}{K^P_M}}{1 + \dfrac{[S]}{K^S_M} + \dfrac{[P]}{K^P_M}}$$

And for initial $v_0 \Rightarrow$ [P] = 0: Michaelis Menten!

$$V^f_{max} = k_2[E]_T \qquad V^r_{max} = k_{-1}[E]_T$$

$$K^S_M = \frac{k_{-1} + k_2}{k_1} \qquad K^P_M = \frac{k_{-1} + k_2}{k_{-2}}$$

$$[E]_T = [E] + [ES]$$

@ equilibrium: v = 0

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V^f_{max} K^P_M}{V^r_{max} K^S_M}$$

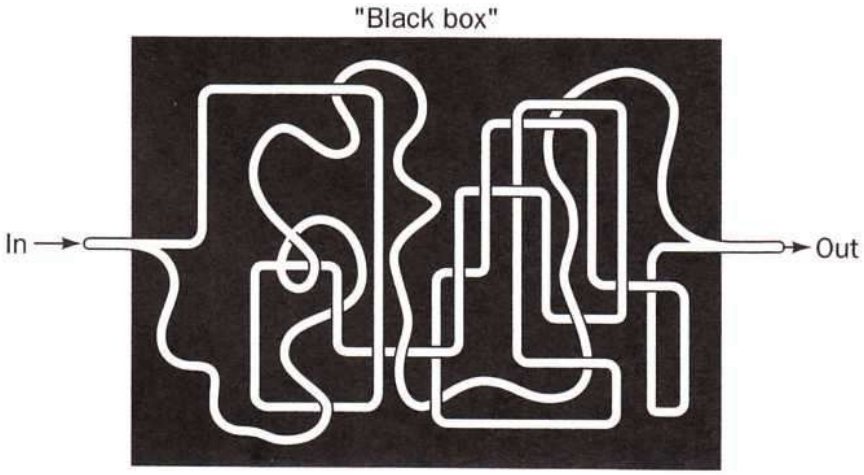
Haldane relationship

What if there are still more reversible steps?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} P + E$$

While $[S]_0$ and $[P]_0$ can be manipulated under steady-state conditions, the resulting equations do not allow to solve for all six (or even more) unknown constants

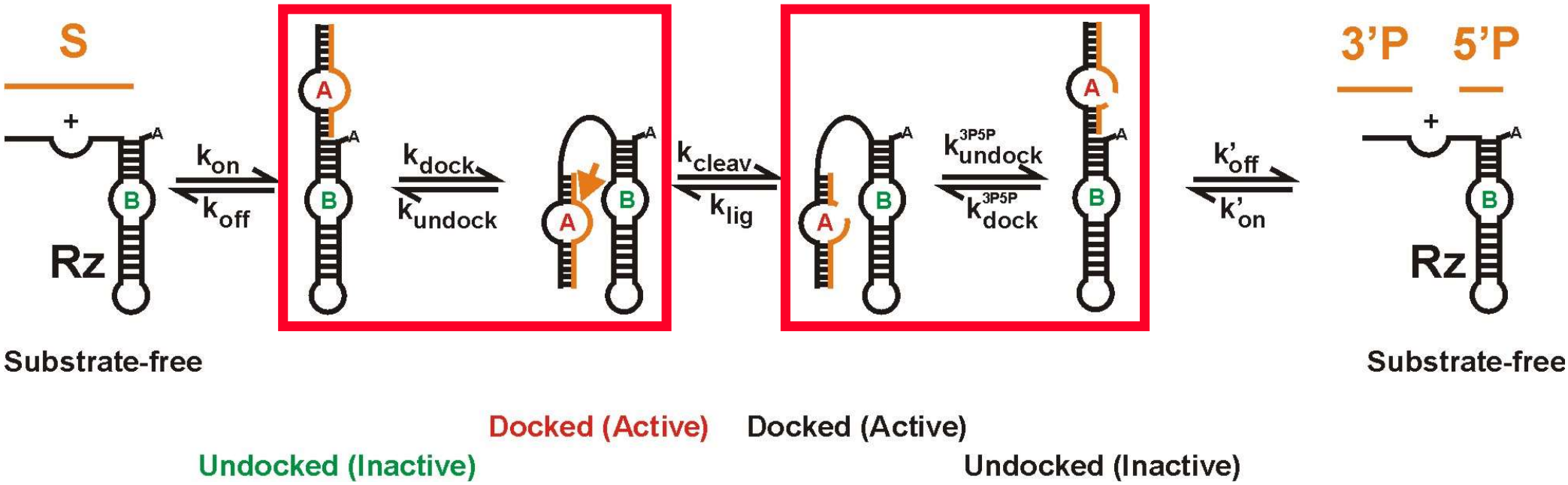
$\Rightarrow$ we have to look into the black box!



In addition, steady-state kinetic measurements:

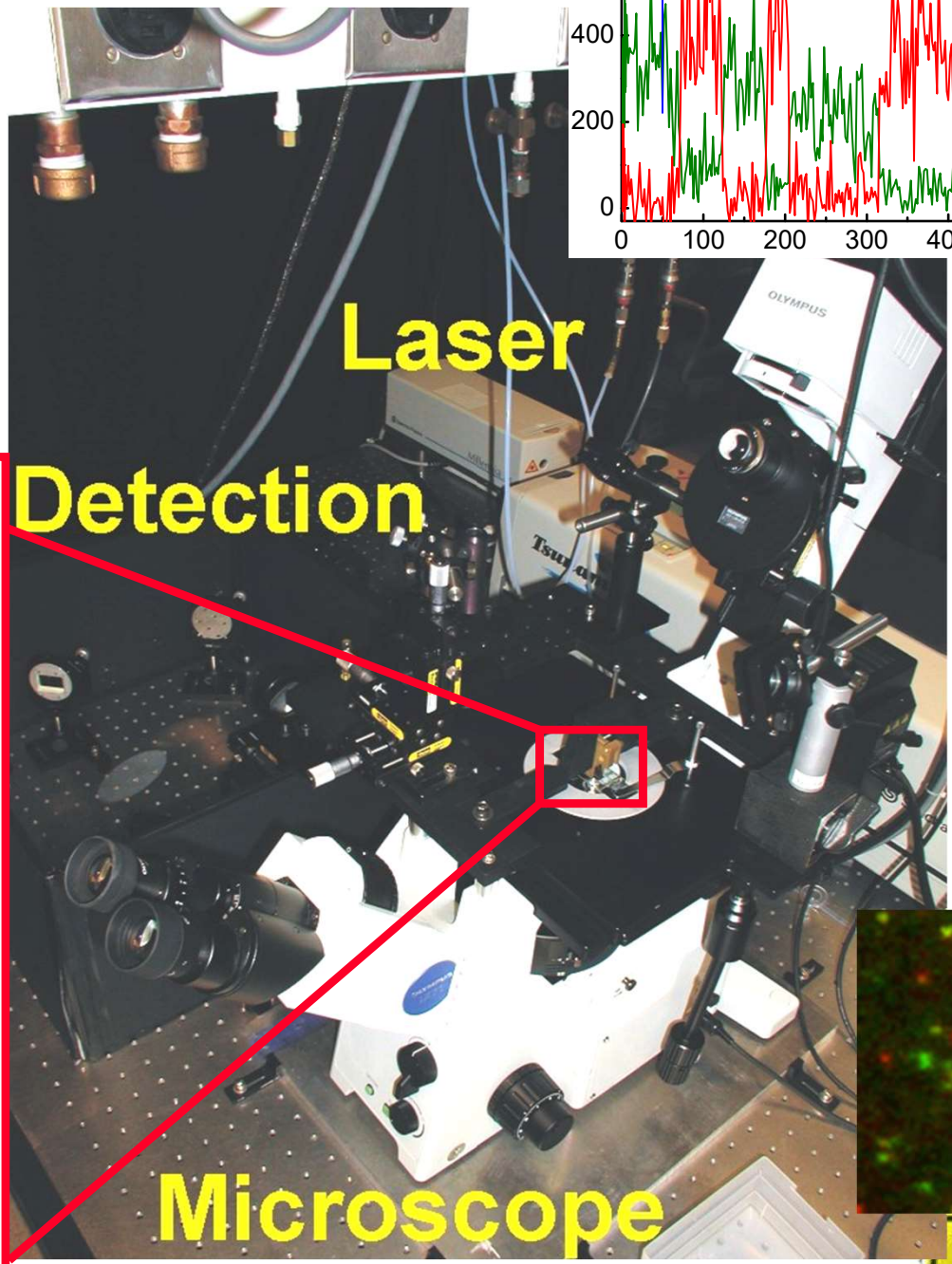
- **only yield phenomenology, not mechanism**
- **cannot unambiguously establish a mechanism**
- **can only rule out some alternative mechanisms**

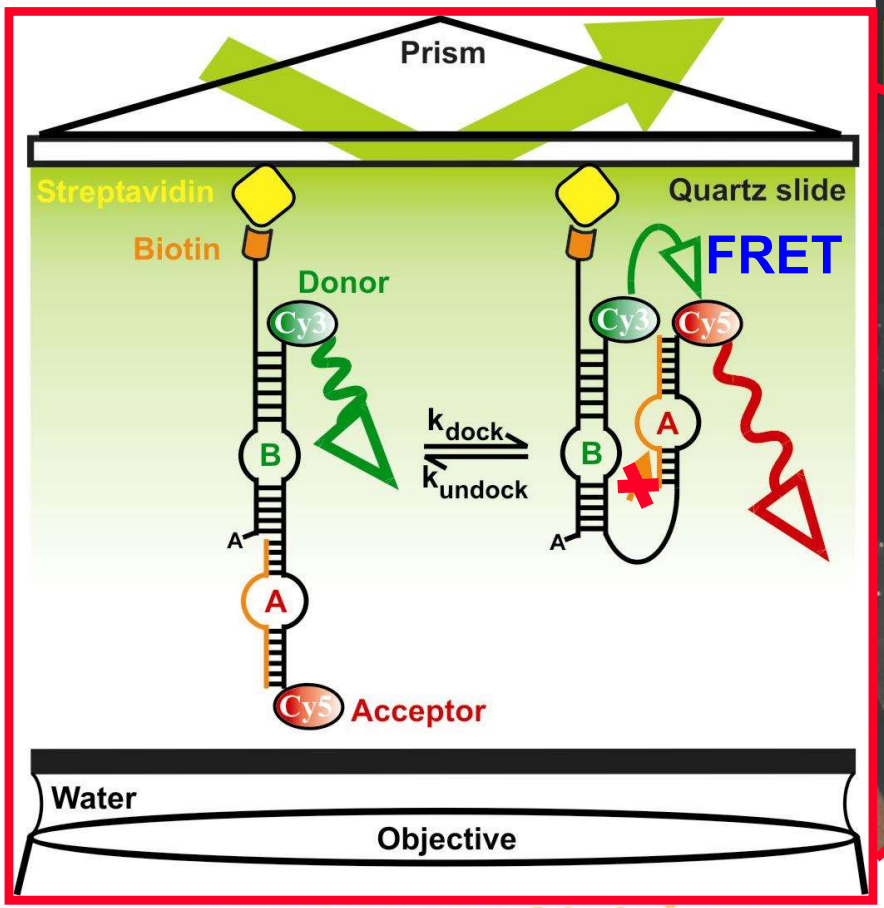
Case in point -- The hairpin ribozyme: Reversible domain docking is a crucial step on the reaction pathway

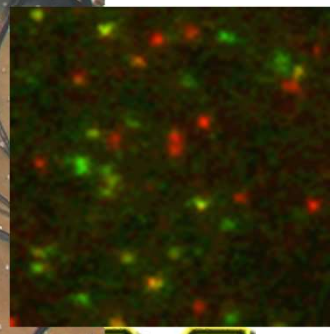


Rueda, D., Bokinsky, G., Rhodes, M.M., Rust, M.J., Zhuang, X. and Walter, N.G. *Proc. Natl. Acad. Sci. USA* 101 (2004) 10066-10071

We measured docking rate constants by single molecule FRET

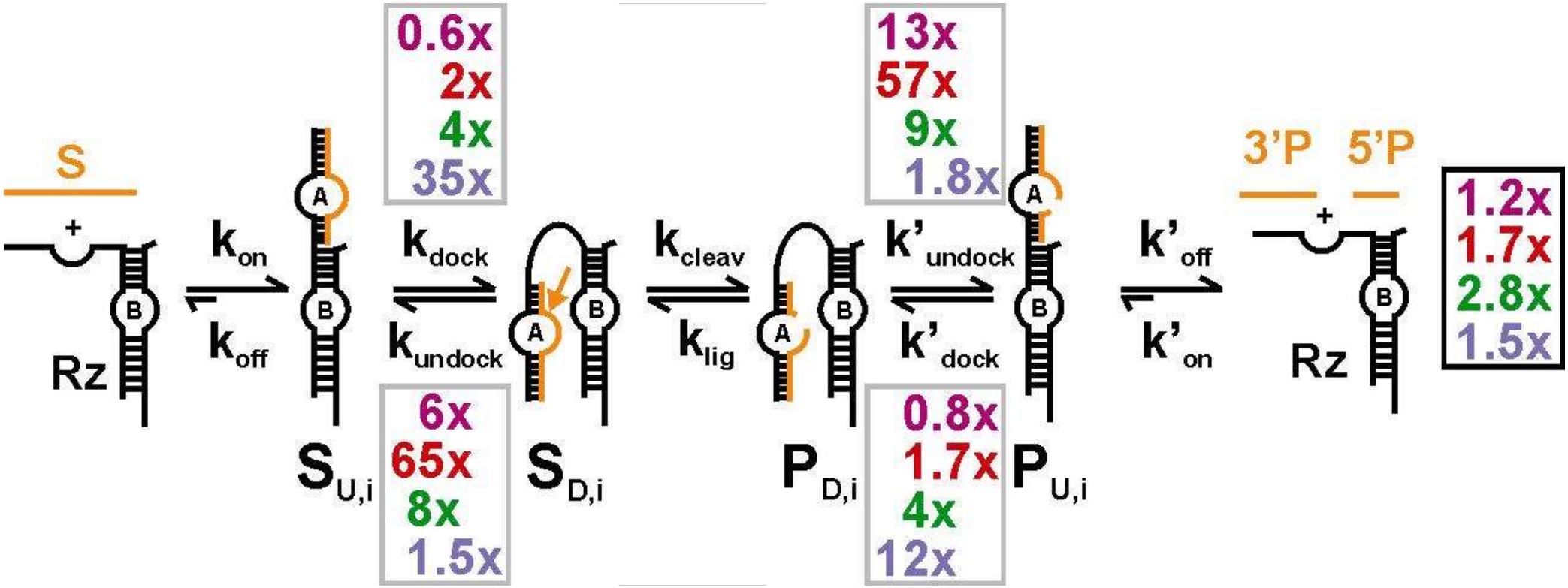






Zhuang, X., Kim, H., Pereira, M.J.B., Babcock, H.P., Walter, N.G. and Chu, S. *Science* 296 (2002) 1473-1476

Kinetic modeling then allowed us to extract the unobservable reversible chemistry rate constants



Rueda, D., Bokinsky, G., Rhodes, M.M., Rust, M.J., Zhuang, X. and Walter, N.G. *Proc. Natl. Acad. Sci. USA* 101 (2004) 10066-10071