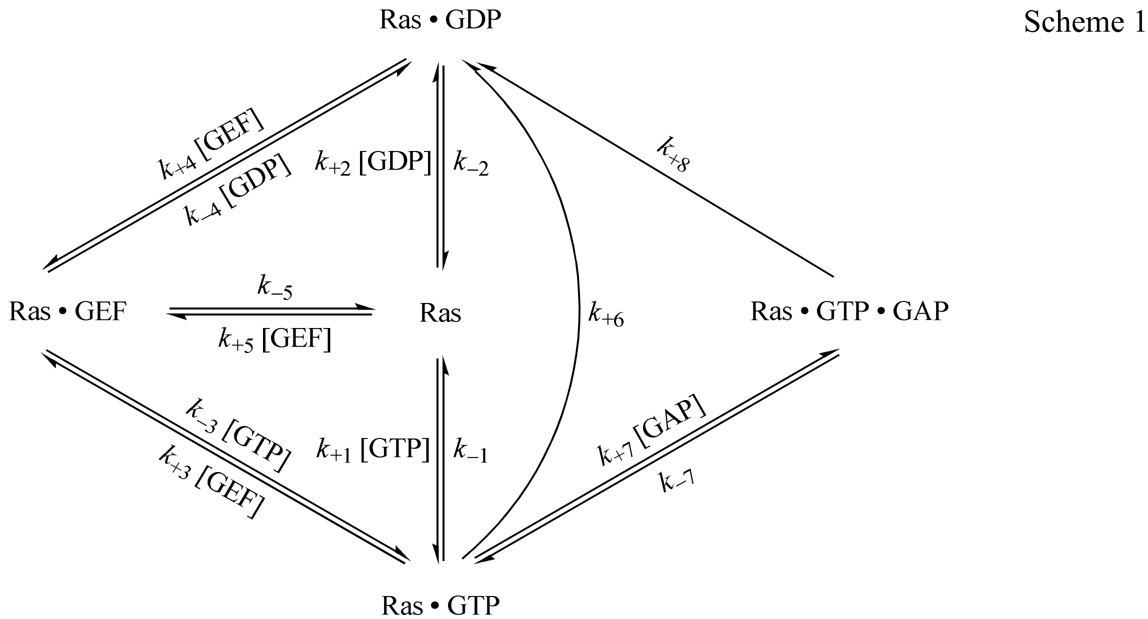
Supporting Information

Derivation of the expression of the fraction of the GTP-bound form of Ras in terms of the kinetic parameters of the intrinsic Ras GNE and GTP hydrolysis and of GEF and GAP with Ras.



As shown in Scheme 1, Ras has a variety of forms that can be referred to as nodes. Each node will be assigned a corresponding letter for simplicity.

Ras → A
Ras • GTP → B
Ras • GDP → C
Ras • GEF → D
Ras • GTP • GAP → E

The notation of an intermediate form/node enclosed in parentheses equals the sum of all arrows leading away from the node. Each arrow is represented by a notation of two letters in parentheses in which the first letter is the origin and the second the destination.

$(A) = (AB) + (AC) + (AD) = k_{+1}[GTP] + k_{+2}[GDP] + k_{+5}[GEF]$
$(B) = (BA) + (BC) + (BD) + (BE) = k_{-1} + k_{+6} + k_{+3}[GEF] + k_{+7}[GAP]$
$(C) = (CA) + (CD) = k_{-2} + k_{+4}[GEF]$
$(D) = (DA) + (DB) + (DC) = k_{-5} + k_{-3}[GTP] + k_{-4}[GDP]$
$(E) = (EB) + (EC) = k_{-7} + k_{+8}$

Using the "one-branch" approach {Huang, 1979 #1942}, the determinant of any enzyme species is the summation of the products of the nearest paths leading toward the node and all other remaining nodes.

$[Ras \bullet GTP] = (AB)(C)(D)(E) + (DB)(A)(C)(E) + (EB)(A)(C)(D)$

Applying the "consecutive branch" approach {Huang, 1979 #1942}, the (EB) term is canceled because no path connects the remaining nodes to (EB).

$[Ras \bullet GTP] = (AB)(C)(D)(E) + (DB)(A)(C)(E)$

Ras•GDP has been chosen as the common node for simplicity in the resulting equation. The "consecutive branch" approach is continued further until C is the origin of all paths.

$[Ras \bullet GTP] = (AB)[(CA)(D)(E) + (CD)(DA)(E)] + (DB)[(CD)(A)(E) + (CA)(AD)(E)]$

The (E) term is then factored out.
$[\text{Ras} \bullet \text{GTP}] = (\text{E})\big((\text{AB})[(\text{CA})(\text{D}) + (\text{CD})(\text{DA})] + (\text{DB})[(\text{CD})(\text{A}) + (\text{CA})(\text{AD})]\big)$

The rate constants are now substituted into the equation.

$$[\text{Ras} \bullet \text{GTP}] = (k_{-7} + k_{+8})\Big(k_{+1}[\text{GTP}]\big(k_{-2}(k_{-5} + k_{-3}[\text{GTP}] + k_{-4}[\text{GDP}]) + (k_{+4}[\text{GEF}])(k_{-5})\big) + k_{-3}[\text{GTP}]\big(k_{+4}[\text{GEF}](k_{+1}[\text{GTP}] + k_{+2}[\text{GDP}] + k_{+5}[\text{GEF}]) + (k_{-2})(k_{+5}[\text{GEF}])\big)\Big)$$

We assume that k_{+1} and k_{+2} are equivalent and that k_{-3} and k_{-4} are equivalent because Ras and Ras•GEF have almost equal affinities for GTP and GDP.
if $k_{+1} = k_{+2} = k'$ and $k_{-3} = k_{-4} = k''$

$$[\text{Ras} \bullet \text{GTP}] = (k_{-7} + k_{+8})\big(k'[\text{GTP}](k_{-2}(k_{-5} + k''[\text{GTP}] + k''[\text{GDP}]) + k_{+4}k_{-5}[\text{GEF}]) + k''[\text{GTP}](k_{+4}[\text{GEF}](k'[\text{GTP}] + k'[\text{GDP}] + k_{+5}[\text{GEF}]) + k_{-2}k_{+5}[\text{GEF}])\big)$$

[GTP] is factored out, and the remaining terms are distributed.

$$[\text{Ras} \bullet \text{GTP}] = (k_{-7} + k_{+8})[\text{GTP}](k'k_{-2}k_{-5} + k'k''k_{-2}([\text{GTP}] + [\text{GDP}]) + k'k_{+4}k_{-5}[\text{GEF}] + k'k''k_{+4}[\text{GEF}]([\text{GTP}] + [\text{GDP}]) + k''k_{+4}k_{+5}[\text{GEF}]^2 + k''k_{-2}k_{+5}[\text{GEF}])$$

The term ($k_{-2} + k_{+4}$[GEF]) is then factored out of the equation.

$$[\text{Ras} \bullet \text{GTP}] = (k_{-7} + k_{+8})[\text{GTP}]\big(k'k_{-5}(k_{-2} + k_{+4}[\text{GEF}]) + k'k''([\text{GTP}] + [\text{GDP}])(k_{-2} + k_{+4}[\text{GEF}]) + k''k_{+5}[\text{GEF}](k_{-2} + k_{+4}[\text{GEF}])\big)$$
$$= (k_{-7} + k_{8})(k_{-2} + k_{+4}[\text{GEF}])[\text{GTP}]\big(k'k_{-5} + k''k_{+5}[\text{GEF}] + k'k''([\text{GTP}] + [\text{GDP}])\big)$$

The same approach is applied to Ras•GDP, but with B used as the common node.

$$[\text{Ras} \bullet \text{GDP}] = (\text{AC})(\text{B})(\text{D})(\text{E}) + (\text{DC})(\text{A})(\text{B})(\text{E}) + (\text{EC})(\text{A})(\text{B})(\text{D}) + (\text{BC})(\text{A})(\text{D})(\text{E})$$
$$= (\text{AC})[(\text{BA})(\text{D})(\text{E}) + (\text{BD})(\text{DA})(\text{E})] + (\text{DC})[(\text{BD})(\text{A})(\text{E}) + (\text{BA})(\text{AD})(\text{E})] + (\text{EC})(\text{BE})(\text{A})(\text{D}) + (\text{BC})(\text{A})(\text{D})(\text{E})$$
$$= (\text{E})\big((\text{AC})[(\text{BA})(\text{D}) + (\text{BD})(\text{DA})] + (\text{DC})[(\text{BD})(\text{A}) + (\text{BA})(\text{AD})]\big) + (\text{A})(\text{D})[(\text{EC})(\text{BE}) + (\text{BC})(\text{E})]$$
$$= (k_{-7} + k_{+8})\Big(k_{+2}[\text{GDP}]\big(k_{-1}(k_{-5} + k_{-3}[\text{GTP}] + k_{-4}[\text{GDP}]) + (k_{+3}[\text{GEF}])(k_{-5})\big) + k_{-4}[\text{GDP}]\big(k_{+3}[\text{GEF}](k_{+1}[\text{GTP}] + k_{+2}[\text{GDP}] + k_{+5}[\text{GEF}]) + (k_{-1})(k_{+5}[\text{GEF}])\big)\Big) + (k_{+1}[\text{GTP}] + k_{+2}[\text{GDP}] + k_{+5}[\text{GEF}])(k_{-5} + k_{-3}[\text{GTP}] + k_{-4}[\text{GDP}])\big((k_{+8})(k_{+7}[\text{GAP}]) + (k_{+6})(k_{-7} + k_{+8})\big)$$

Once again the assumption that Ras and Ras • GEF have almost equal affinities for GTP and GDP is applied.
if $k_{+1} = k_{+2} = k'$ and $k_{-3} = k_{-4} = k''$

$$[\text{Ras} \bullet \text{GDP}] = (k_{-7} + k_{+8})\Big(k'[\text{GDP}]\big(k_{-1}(k_{-5} + k''[\text{GTP}] + k''[\text{GDP}]) + (k_{+3}[\text{GEF}])(k_{-5})\big) + k''[\text{GDP}]\big(k_{+3}[\text{GEF}](k'[\text{GTP}] + k'[\text{GDP}] + k_{+5}[\text{GEF}]) + (k_{-1})(k_{+5}[\text{GEF}])\big)\Big) + (k'[GTP] + k'[GDP] + k_{+5}[GEF])(k_{-5} + k''[GTP] + k''[GDP])\big((k_{+8})(k_{+7}[GAP]) + (k_{+6})(k_{-7} + k_{+8})\big)$$

[GDP] is factored out, and the remaining terms are distributed.

$$
\begin{aligned}
[\text{Ras} \bullet \text{GDP}] = {} & (k_{-7} + k_{+8})[\text{GDP}](k'k_{-1}k_{-5} + k'k''k_{-1}([\text{GTP}] + [\text{GDP}]) + k'k_{+3}k_{-5}[\text{GEF}] \\
& + k'k''k_{+3}[\text{GEF}]([\text{GTP}] + [\text{GDP}]) + k''k_{+3}k_{+5}[\text{GEF}]^2 + k''k_{-1}k_{+5}[\text{GEF}]) \\
& + (k'([\text{GTP}] + [\text{GDP}]) + k_{+5}[\text{GEF}])\big(k_{-5} + k''([\text{GTP}] + [\text{GDP}])\big)\big(k_{+7}k_{+8}[\text{GAP}] \\
& + k_{+6}(k_{-7} + k_{+8})\big)
\end{aligned}
$$

After distributing the $k_{+5}k_{-5}$[GEF] term is removed because it is a cyclic term.

$$
\begin{aligned}
[\text{Ras} \bullet \text{GDP}] = {} & (k_{-7} + k_{+8})[\text{GDP}]\big(k'k_{-5}(k_{-1} + k_{+3}[\text{GEF}]) + k'k''(k_{-1} + k_{+3}[\text{GEF}])([\text{GTP}] + [\text{GDP}]) \\
& + k''k_{+5}[\text{GEF}](k_{-1} + k_{+3})\big) \\
& + \big(k'k_{-5}([\text{GTP}] + [\text{GDP}]) + k'k''([\text{GTP}] + [\text{GDP}])^2 \\
& + k''k_{+5}[\text{GEF}]([\text{GTP}] + [\text{GDP}])\big)\big(k_{+7}k_{+8}[\text{GAP}] + k_{+6}(k_{-7} + k_{+8})\big)
\end{aligned}
$$

The term (k_{-1} + k_{+3}[GEF]) is then factored out of the equation.

$$
\begin{aligned}
[\text{Ras} \bullet \text{GDP}] = {} & (k_{-7} + k_{+8})(k_{-1} + k_{+3}[\text{GEF}])[\text{GDP}](k'k_{-5} + k'k''([\text{GTP}] + [\text{GDP}]) + k''k_{+5}[\text{GEF}]) \\
& + (k'k_{-5} + k'k''([\text{GTP}] + [\text{GDP}]) + k''k_{+5}[\text{GEF}])\big(k_{+7}k_{+8}[\text{GAP}] \\
& + k_{+6}(k_{-7} + k_{+8})\big)([\text{GTP}] + [\text{GDP}])
\end{aligned}
$$

The term $(k'k_{-5} + k'k''([\text{GTP}] + [\text{GDP}]) + k''k_{+5}[\text{GEF}])$ is then factored out of the equation.

$$
\begin{aligned}
[\text{Ras} \bullet \text{GDP}] = {} & \Big((k_{-7} + k_{+8})(k_{-1} + k_{+3}[\text{GEF}])[\text{GDP}] \\
& + \big(k_{+7}k_{+8}[\text{GAP}] + k_{+6}(k_{-7} + k_{+8})\big)([\text{GTP}] + [\text{GDP}])\Big)(k'k_{-5} \\
& + k'k''([\text{GTP}] + [\text{GDP}]) + k''k_{+5}[\text{GEF}])
\end{aligned}
$$

The fraction of GTP-bound Ras can be defined as,

$$
= \frac{[\text{Ras} \bullet \text{GTP}]}{[\text{Ras} \bullet \text{GTP}] + [\text{Ras} \bullet \text{GDP}]}
$$

After substituting the values of Ras•GTP and Ras•GDP, the fraction of GTP-bound Ras is

$$
= \frac{(k_{-7} + k_8)(k_{-2} + k_{+4}[\text{GEF}])[\text{GTP}]\big(k'k_{-5} + k''k_{+5}[\text{GEF}] + k'k''([\text{GTP}] + [\text{GDP}])\big)}{\Big((k_{-7} + k_{+8})(k_{-1} + k_{+3}[\text{GEF}])[\text{GDP}] + \big(k_{+7}k_{+8}[\text{GAP}] + k_{+6}(k_{-7} + k_{+8})\big)([\text{GTP}] + [\text{GDP}])\Big)(k'k_{-5} + k'k''([\text{GTP}] + [\text{GDP}]) + k''k_{+5}[\text{GEF}])}
$$

Divide by the terms $(k_{-7} + k_{+8})(k'k_{-5} + k'k''([\text{GTP}] + [\text{GDP}]) + k''k_{+5}[\text{GEF}])$, and the resulting Equation 1 defines the comprehensive fraction of the GTP-bound Ras (the comprehensive $f_{\text{Ras•GTP}}$).

$$
= \frac{(k_{-2} + k_{+4}[\text{GEF}])[\text{GTP}]}{(k_{-2} + k_{+4}[\text{GEF}])[\text{GTP}] + (k_{-1} + k_{+3}[\text{GEF}])[\text{GDP}] + \left(\frac{k_{+7}k_{+8}}{k_{-7} + k_{+8}}[\text{GAP}] + k_{+6}\right)([\text{GTP}] + [\text{GDP}])} \qquad \text{Equation 1}
$$

To account for only the intrinsic turnover of GTP to GDP by Ras, all terms relating to GEF and GAP can be canceled to yield Equation 2 for the intrinsic fraction of the GTP-bound Ras (the intrinsic $f_{\text{Ras•GTP}}$).

$$
= \frac{k_{-2}[\text{GTP}]}{k_{-2}[\text{GTP}] + k_{-1}[\text{GDP}] + k_{+6}([\text{GTP}] + [\text{GDP}])} \qquad \text{Equation 2}
$$

The same principle can be applied to GEF- and/or GAP-only situations, thus resulting in equations for the fraction of GTP-bound Ras as follows. In a case in which only GEF is active, Equation 3 for the GEF-mediated fraction of the GTP-bound Ras (the GEF-mediated $f_{\text{Ras•GTP}}$) is

$$
= \frac{(k_{-2} + k_{+4}[\text{GEF}])[\text{GTP}]}{(k_{-2} + k_{+4}[\text{GEF}])[\text{GTP}] + (k_{-1} + k_{+3}[\text{GEF}])[\text{GDP}] + k_{+6}([\text{GTP}] + [\text{GDP}])} \qquad \text{Equation 3}
$$

Similarly, if only GAP is active, Equation 4 for the GAP-mediated fraction of the GTP-bound Ras (the GAP-mediated $f_{\text{Ras•GTP}}$) is

$$= \frac{k_{-2}[\text{GTP}]}{k_{-2}[\text{GTP}] + k_{-1}[\text{GDP}] + \left(\frac{k_{+7}k_{+8}}{k_{-7}+k_{+8}}[\text{GAP}] + k_{+6}\right)([\text{GTP}] + [\text{GDP}])} \qquad \text{Equation 4}$$