Discussion Questions (Q10) for

- What background or context led the authors to pursue this detailed kinetic analysis of Ras?

- Major focus here – understand the thinking behind the designs of the kinetic experiments and the analysis to extract desired rate constants in model in Scheme 1.

  - Sketch design of experiments to measure kinetics of GTP and GDP binding and state justification and caveats:
    - expected eqns for kobs for each
    - why might this not be valid for GTP, i.e., think substrate?
    - explain behavior observed in Fig. 2B – what values are obtained, what is expected, but not detectable?

  - Sketch design of the experiment to measure \( k_2 \) and \( k_{-2} \) and state justification and caveats:
    - key observations from Fig. 3 (\( k_2 \)) and Table 3
    - What evidence do they give that \( k_{-2} < k_2/20 \)?

  - Sketch design of the experiment to measure \( k_2 \) and \( k_{-1} \) and state justification and caveats:
    - Cold-chase experiments in Fig. 4 (\( k_2 \) and \( k_{-1} \))
    - Write the rate law for the decay of \( R\cdot\text{GTP} \)
    - From that explain eqns for expected \( k_{\text{obs}} \) and ratio of \(^3\text{H-GDP}/^3\text{H-GTP} \)
    - Looking at data in Fig. 4, what can you state about behavior of “Normal” vs “Mutant”

  - Sketch design of experiments to measure \( k_3 \) directly and state justification and caveats
    - why can’t \( k_{-1} \) be measured in the same way?

- Interpretations
  - Rate constant for hydrolysis – how good as an enzyme?
  - How do they arrive at Scheme 2 to describe the in vivo states of Ras?
    - Consider: what are the Kd values for GTP and GDP (they give Kassoc)?
    - Look up estimates for cellular [GTP] & [GDP]
  - Consider the value of \([R\cdot\text{GTP}]/[R\cdot\text{GDP}]\) they estimate from Scheme 2 – what would this suggest about the “switchability” of Ras? i.e., If Ras establishes steady-state by this basal mechanism, would it ever be totally “off” or “on”?
  - Do the differences in rate constants measured for the G12D mutant really seem like they would make a difference in the ability of this mutant to remain “activated” if the basal mechanism stands alone?
  - other thoughts?