Covalent Drugs

LETTER

doi:10.1038/nature12796

K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions

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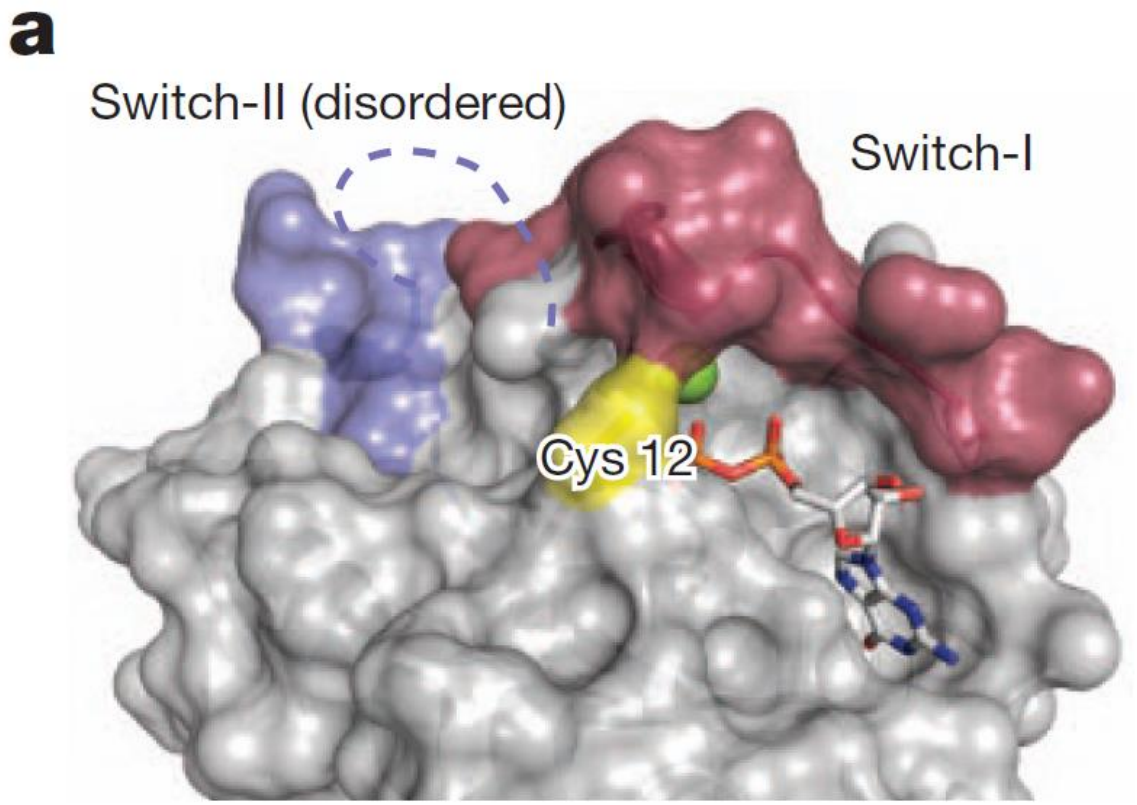
Somatic mutations in the small GTPase K-Ras are the most common activating lesions found in human cancer, and are generally associated with poor response to standard therapies[1–3]. Efforts to target this oncogene directly have faced difficulties owing to its picomolar affinity for GTP/GDP[4] and the absence of known allosteric regulatory sites. Oncogenic mutations result in functional activation of Ras family proteins by impairing GTP hydrolysis[5,6]. With diminished regulation by GTPase activity, the nucleotide state of Ras becomes more dependent on relative nucleotide affinity and concentration. This gives GTP an advantage over GDP[7] and increases the proportion of active GTP-bound Ras. Here we report the development of small molecules that irreversibly bind to a common oncogenic mutant, K-Ras(G12C). These compounds rely on the mutant cysteine for binding and therefore do not affect the wild-type protein.

mutant over wild-type K-Ras. Notably, the mutant Cys 12 sits in close proximity to both the nucleotide pocket and the switch regions involved in effector interactions (Fig. 1a). To identify a chemical starting point, we used a disulphide-fragment-based screening approach called tethering[8]. We screened a library of 480 tethering compounds against K-Ras(G12C) in the GDP state using intact protein mass spectrometry[9,10] (see Methods and Extended Data Table 1). Fragments 6H05 (94 ± 1% (mean ± s.d.)) and 2E07 (84.6 ± 0.3%) gave the greatest degree of modification (Fig. 1b, c). Reaction with wild-type K-Ras, which contains three native cysteine residues, was not detected. Conversely, both compounds modify the oncogenic G12C mutant of the highly homologous protein H-Ras[11,12] (Fig. 1b). Binding was not diminished by 1 mM GDP in the presence of EDTA, suggesting that the compounds bind in an allosteric site not overlapping with GDP. Pre-loading of K-Ras with GTP significantly

Relevant for exam: Figures 1 to 4

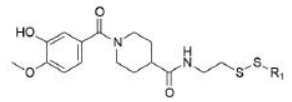
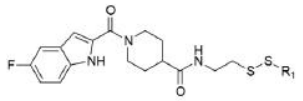
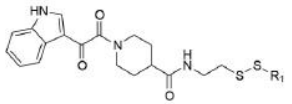
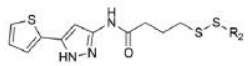
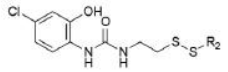
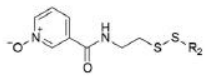
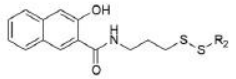
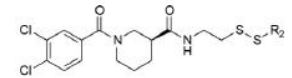
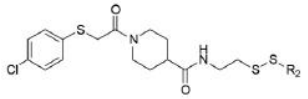
Figure 1a

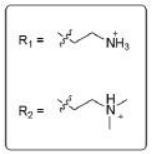


- Why is Cys12 highlighted (yellow)?
  Are there other cysteines in K-RAS?
  Is Cys12 found in wild-type K-RAS?
- What is the small molecule bound to the pocket of K-RAS?
- What is Switch-I and Switch-II?
- Which strategy is proposed to inhibit K-RAS?

Extended Table 1

Extended Data Table 1 | Hit fragments and percentage modification from the primary tethering screen

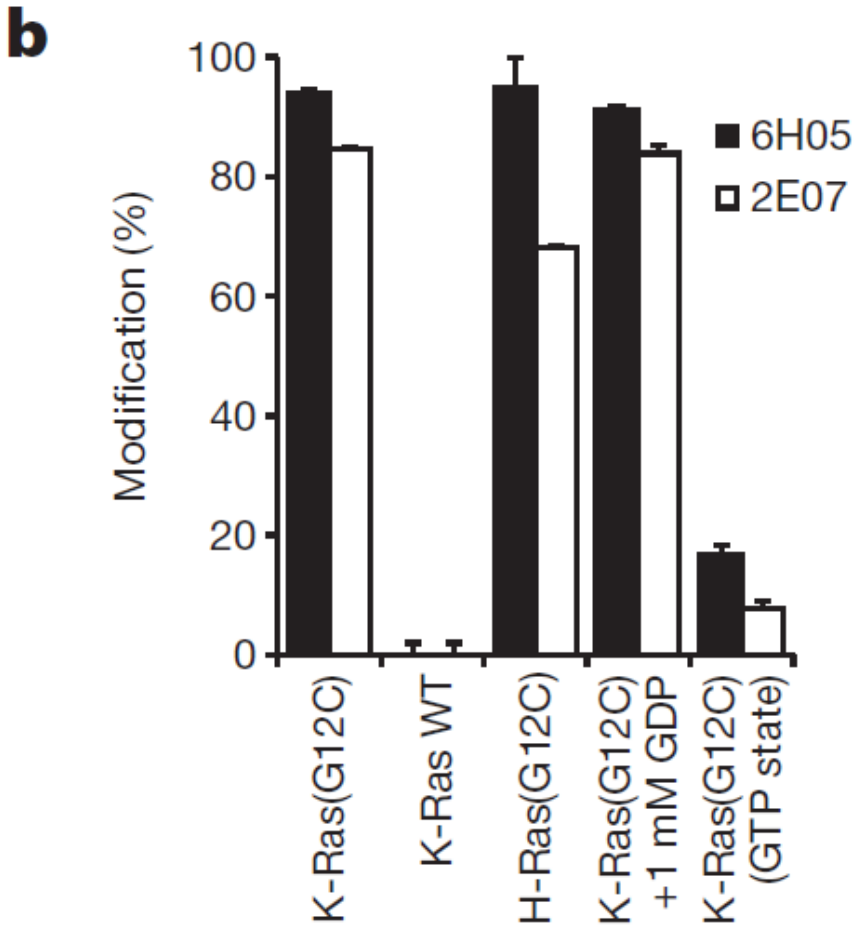
Fragment structure	Fragment number	Percent Modification
	2C10	60%
	2D04	60%
	2D05	60%
	2E07	70%
	3C09	60%
	4C09	60%
	5B03	60%
	5F10	65%
	6H05	95%



480 chemical compounds were screened against K-RAS (loaded with GDP).

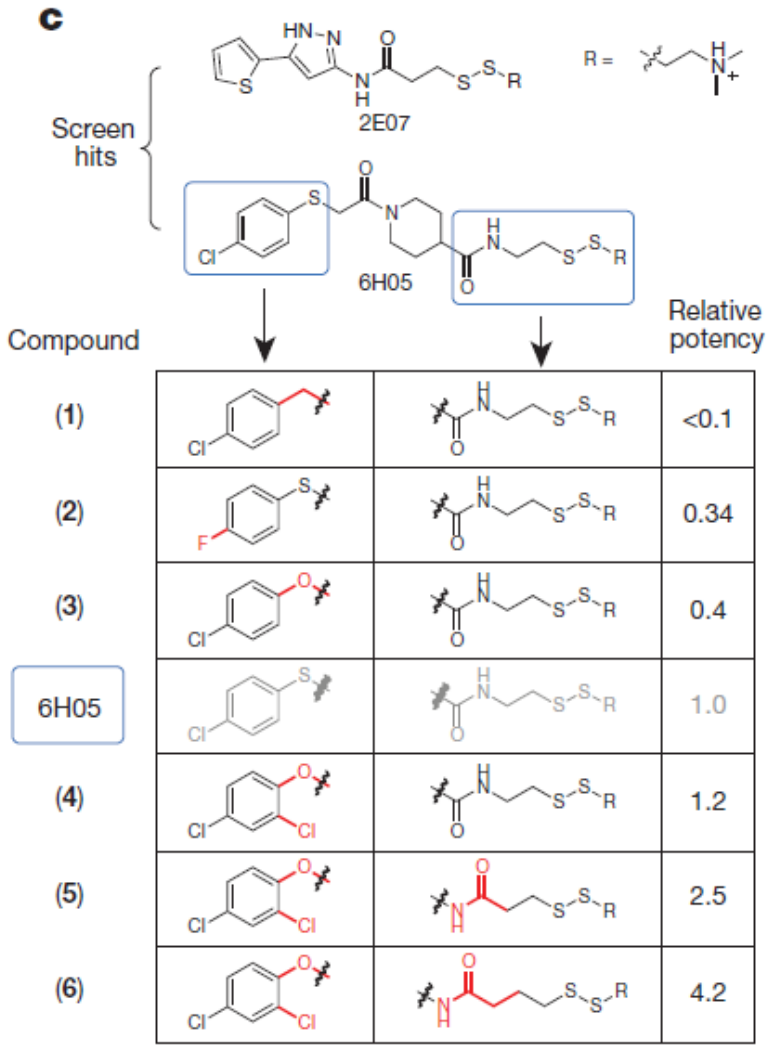
- What do the chemical compounds have in common? Why?
- How was the screen performed?
- Which two compounds performed best?

Figure 1b



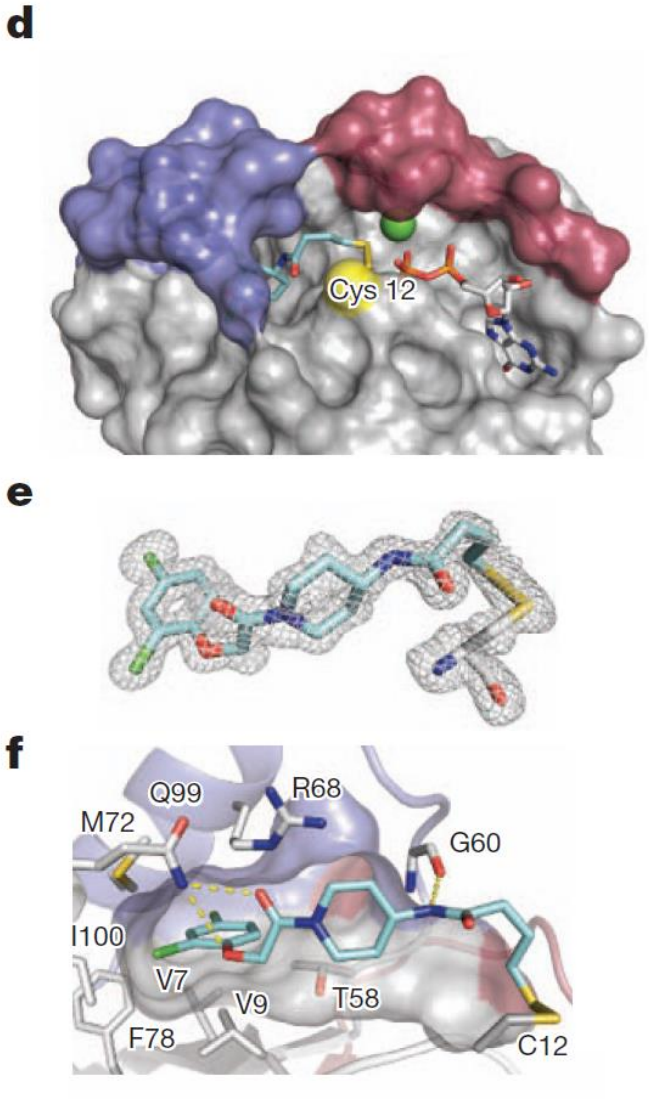
- What does «Modification (%)» mean?
- Do the compounds bind to wild-type K-RAS?
- Do they bind to H-RAS?
- Do they bind in presence of GDP? Or GTP?

Figure 1c



- Which parts of compounds 6H05 were modified to improve the binding/activity?
- Which modification improved the binding/activity? How much?

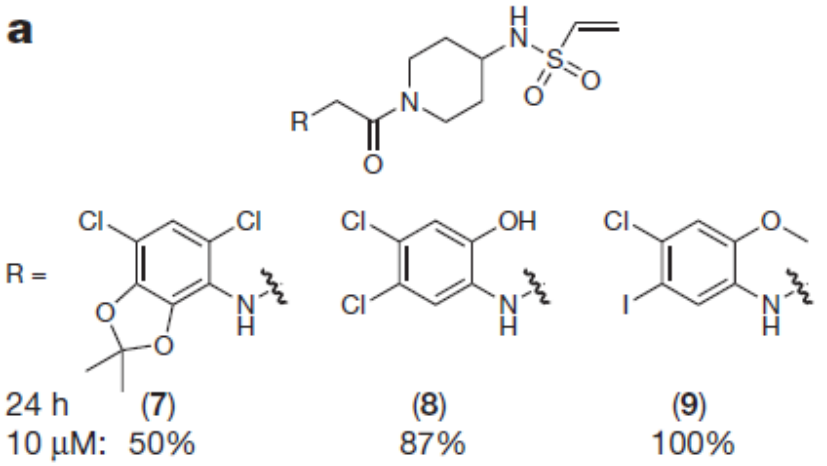
Figure 1d



The X-ray structure of compound **6** bound to K-RAS/GDP was solved.

- Where did compound **6** bind?
- Does it overlap with GDP binding?
- Does it change the comformation of K-RAS Switch-I or Switch-II?

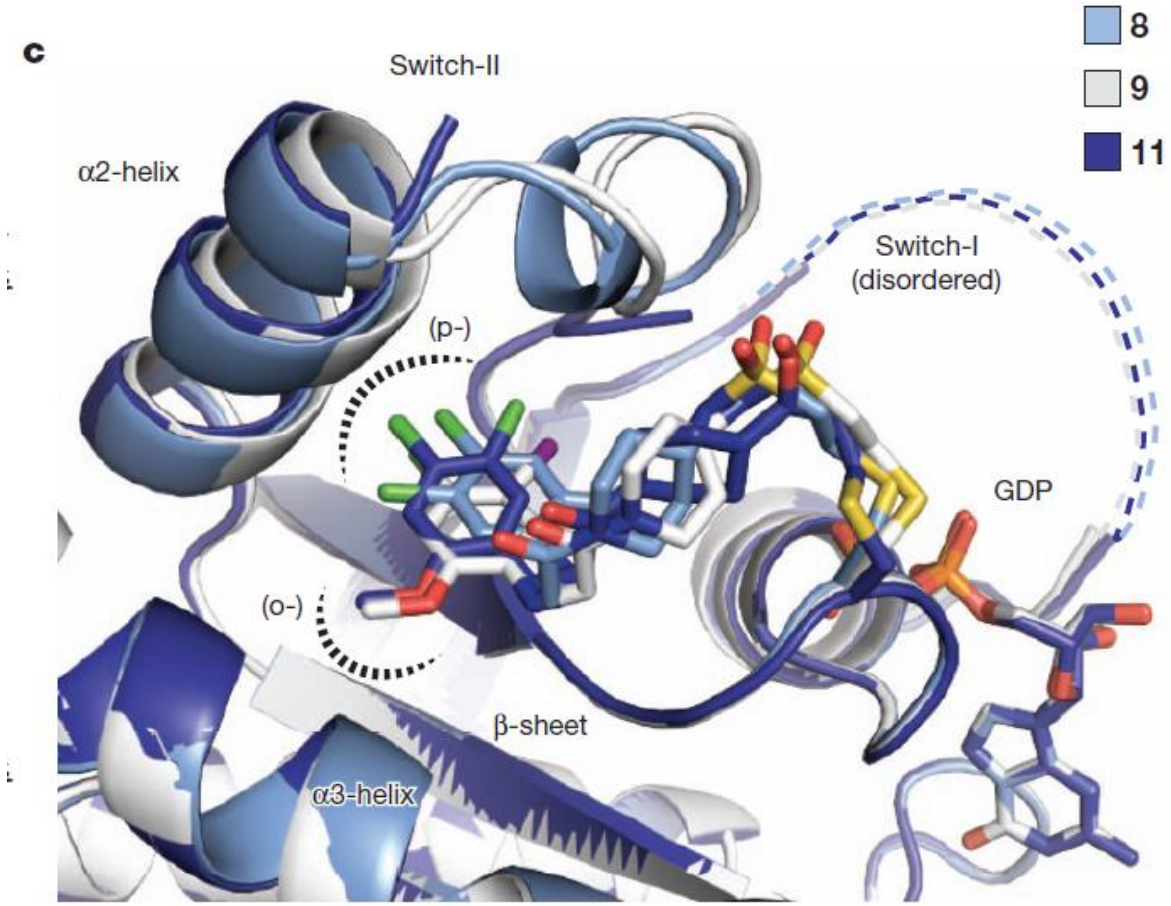
Figure 2a and 2b



Compound **6** was chemically modified by introducing vinylsulfonamide or acrylamide groups.

- Why were these groups introduced?
- Which method was used to measure the binding/activity of the new compounds?
- Which one of the compounds in Figure 2a/b worked best?

Figure 2c



The irreversible ligands **8**, **9** and **11** bind to the Switch-II pocket (S-IIP):

- Do they introduce a conformational change in K-RAS? Where?

Figure 2d-f

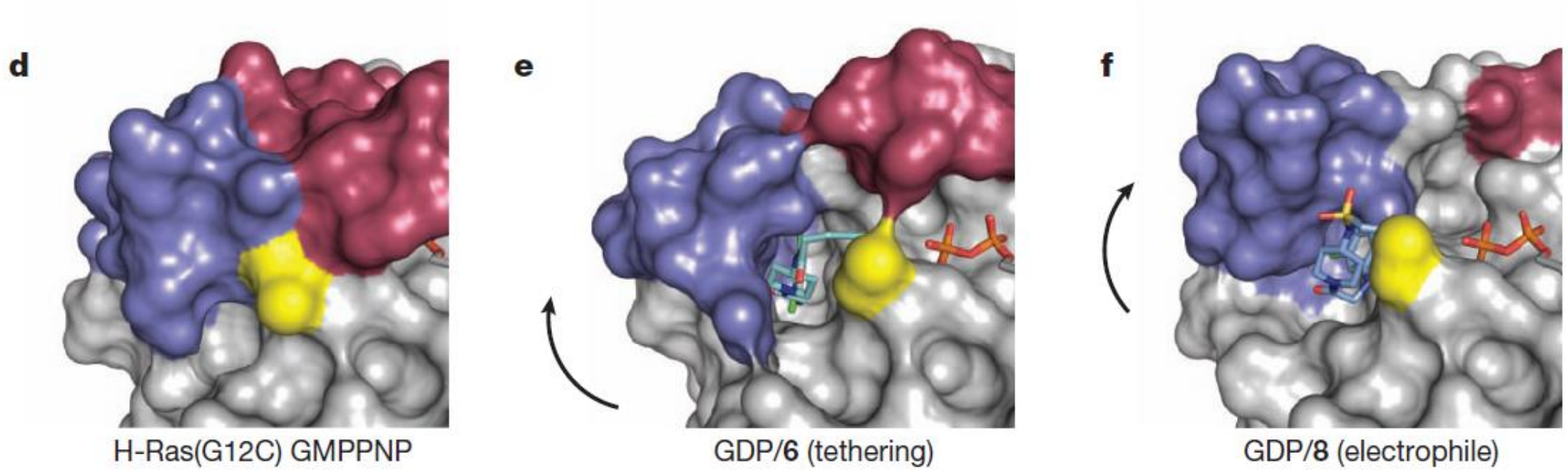
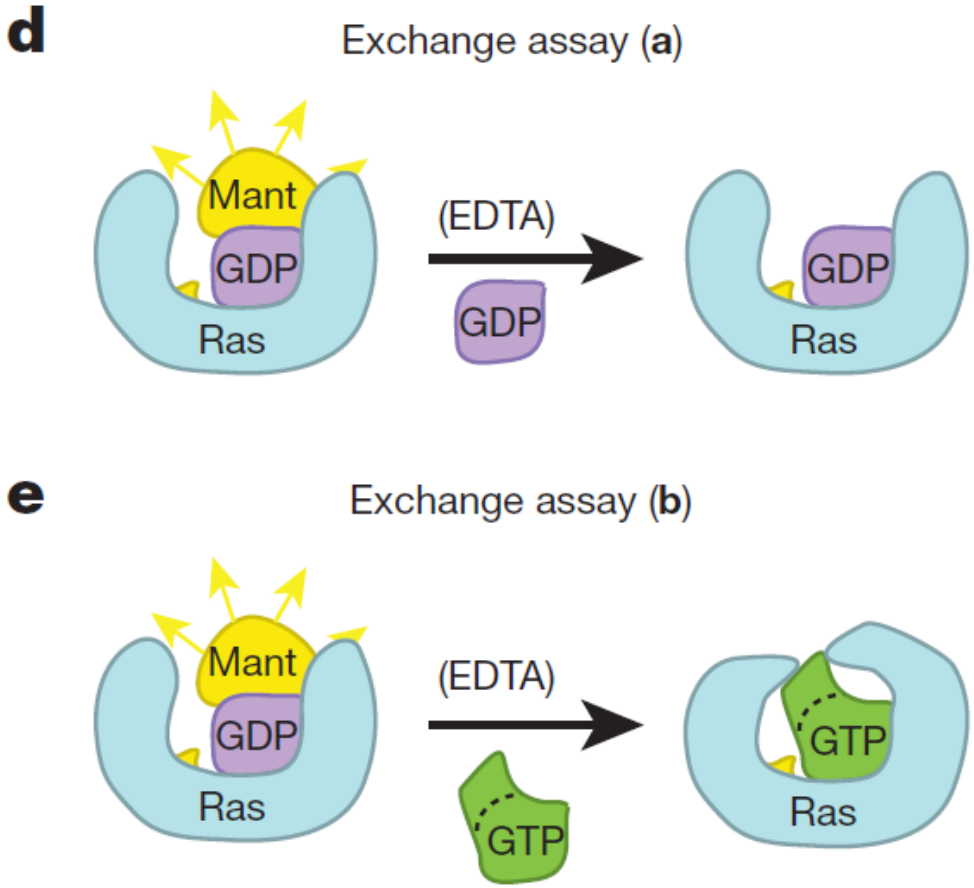
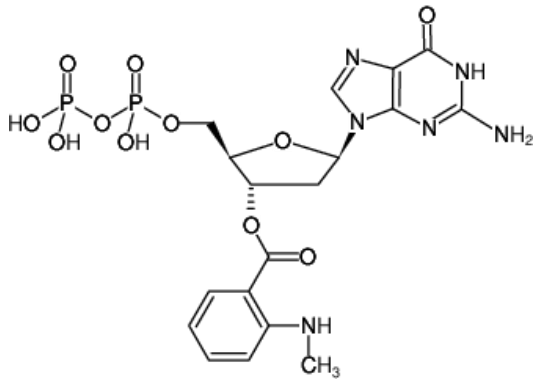


Figure 3d and 3e



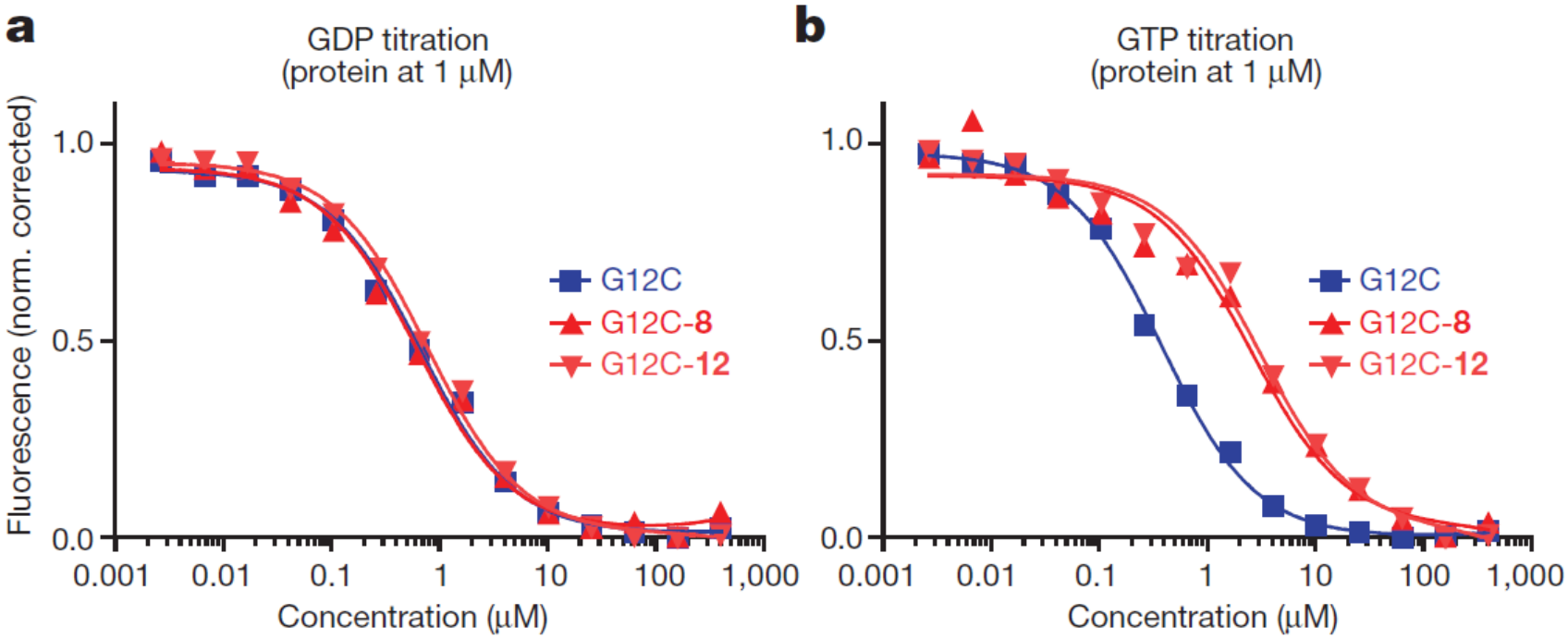
Mant GDP =



They next tested if the covalent ligands change the affinity of K-RAS for the nucleotides GDP and GTP.

- Which assay did they use and how did it work?

Figure 3a and 3b



- Do the covalent ligands increase of lower the affinity for GDP and GTP?

Figure 3c

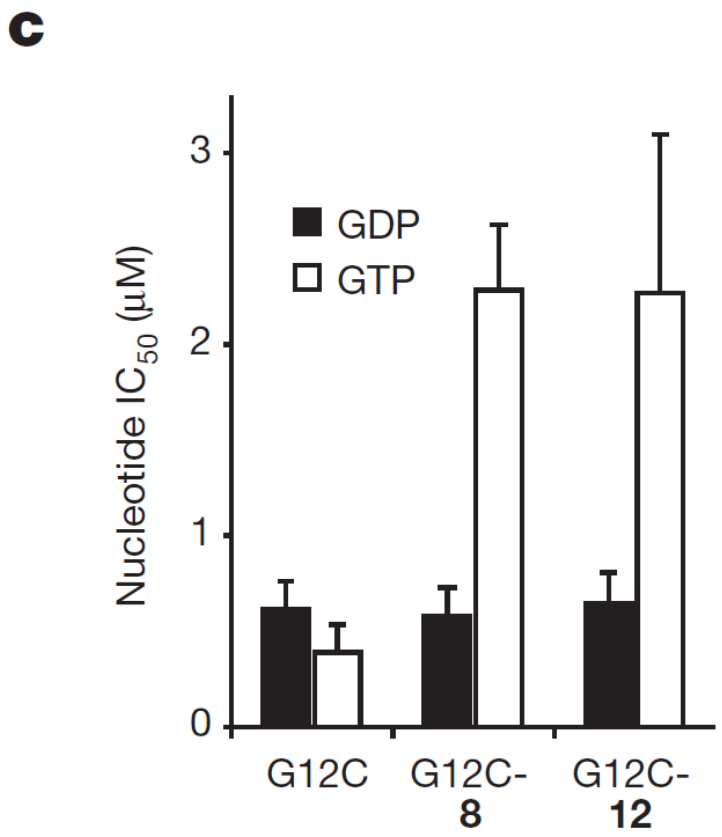
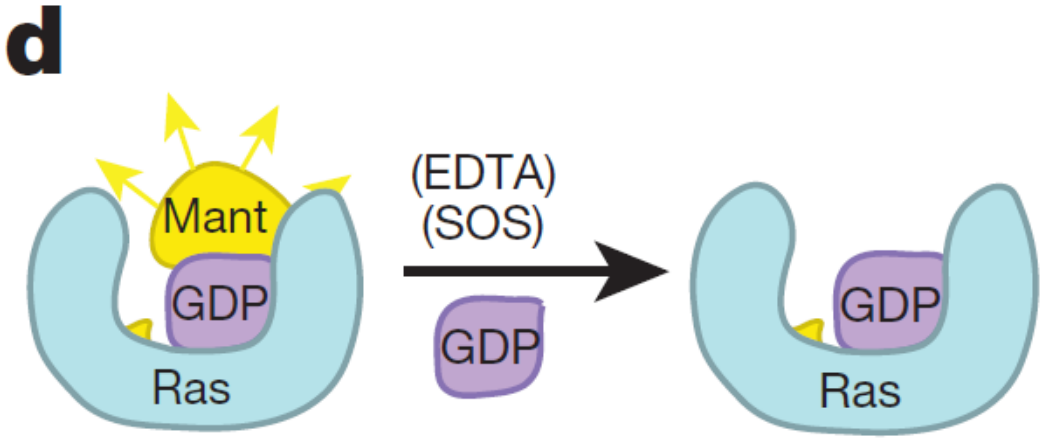


Figure 4d


d
Mant
GDP
Ras
(EDTA)
(SOS)
GDP
GDP
Ras

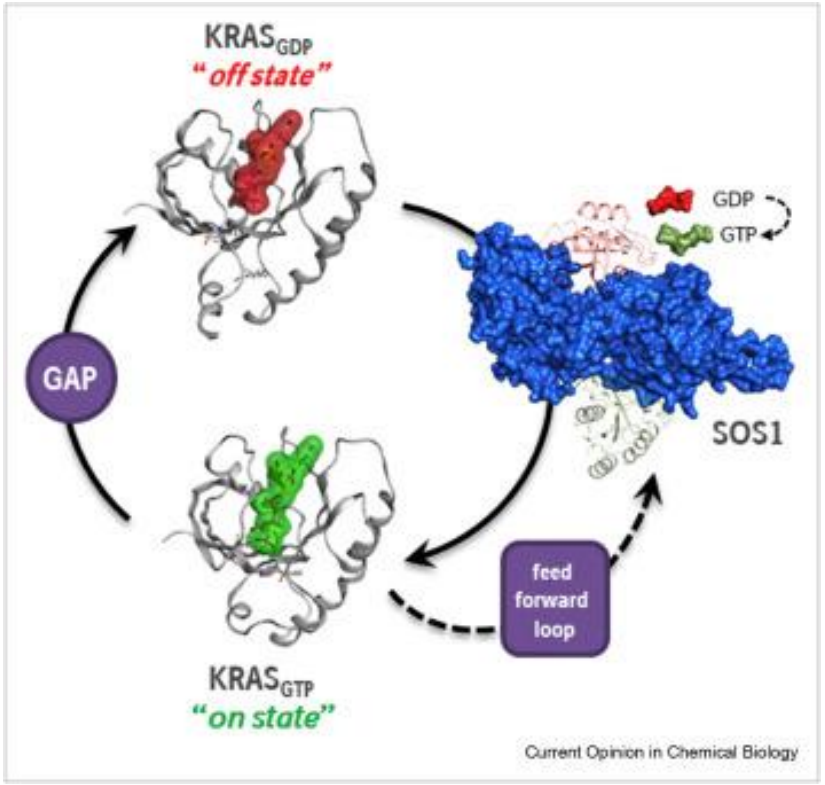
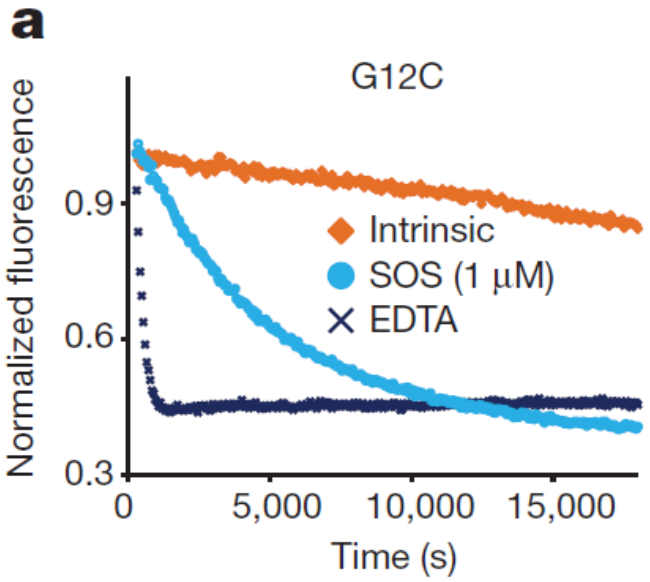
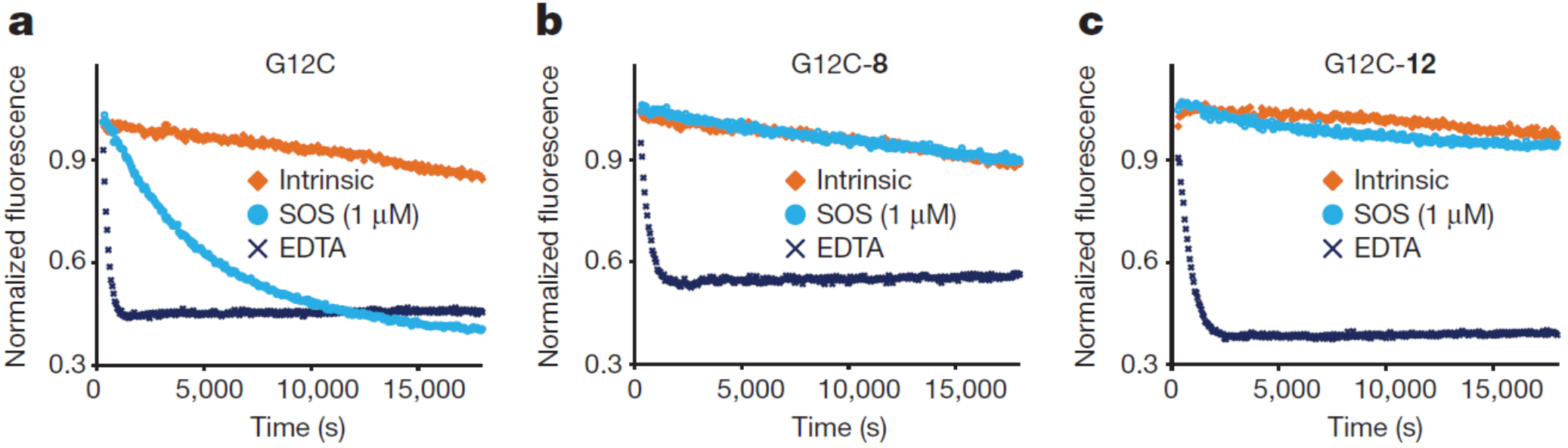

KRAS$_{GDP}$
"off state"
GDP
GTP
GAP
SOS1
feed
forward
loop
KRAS$_{GTP}$
"on state"
Current Opinion in Chemical Biology

Figure 4a



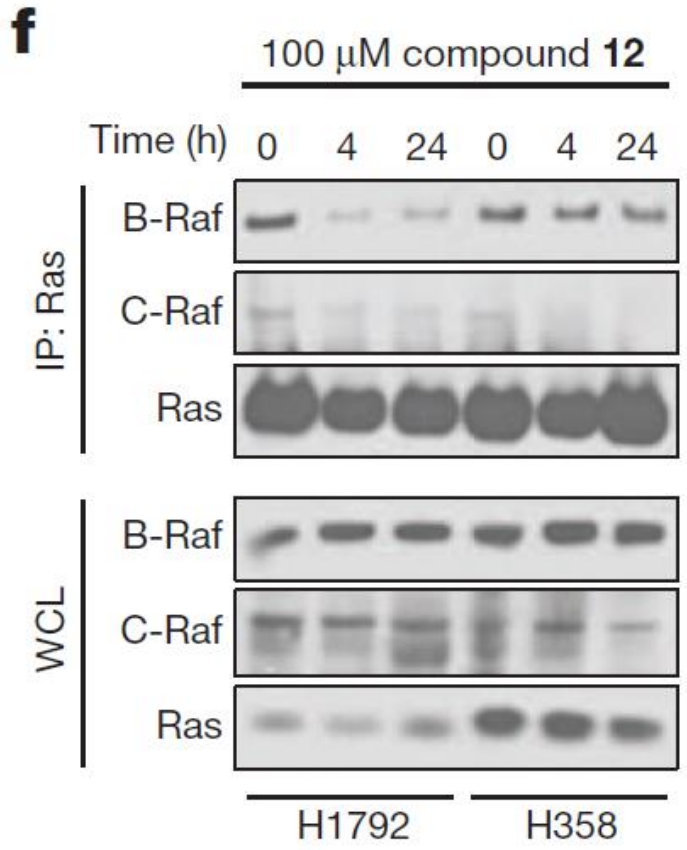
- What is measured here (i.e. which fluorescence)?
- Why is fluorescence lowered so fast with EDTA?
- Do inhibitors **8** and **12** affect SOS binding?

Figure 4a-c



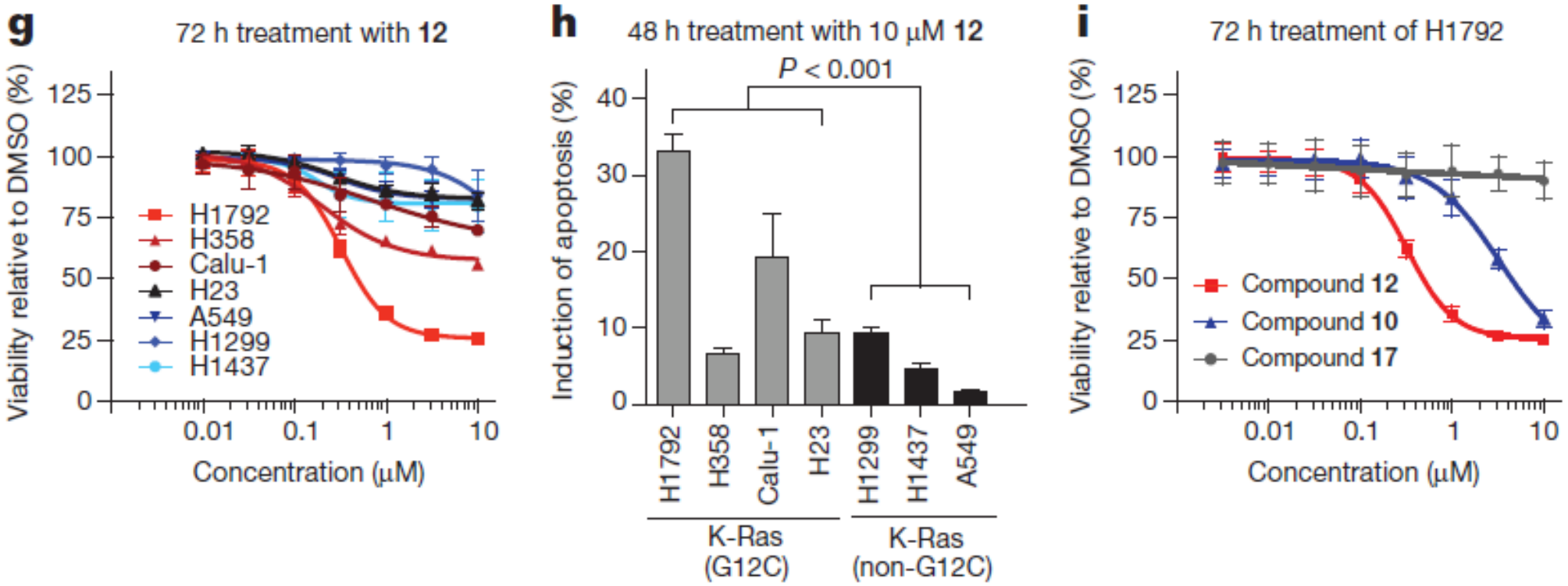
- What is measured here (i.e. which fluorescence)?
- Why is fluorescence lowered so fast with EDTA?
- Do inhibitors **8** and **12** affect SOS binding?

Figure 4f



- What was tested with this experiment?
- Does compound **12** affect binding between K-RAS and B-Raf/C-Raf?

Figure 4g-i



- Did compound 12 affect tumor cell growth?

Sotorasib: first approved KRAS inhibitor drug

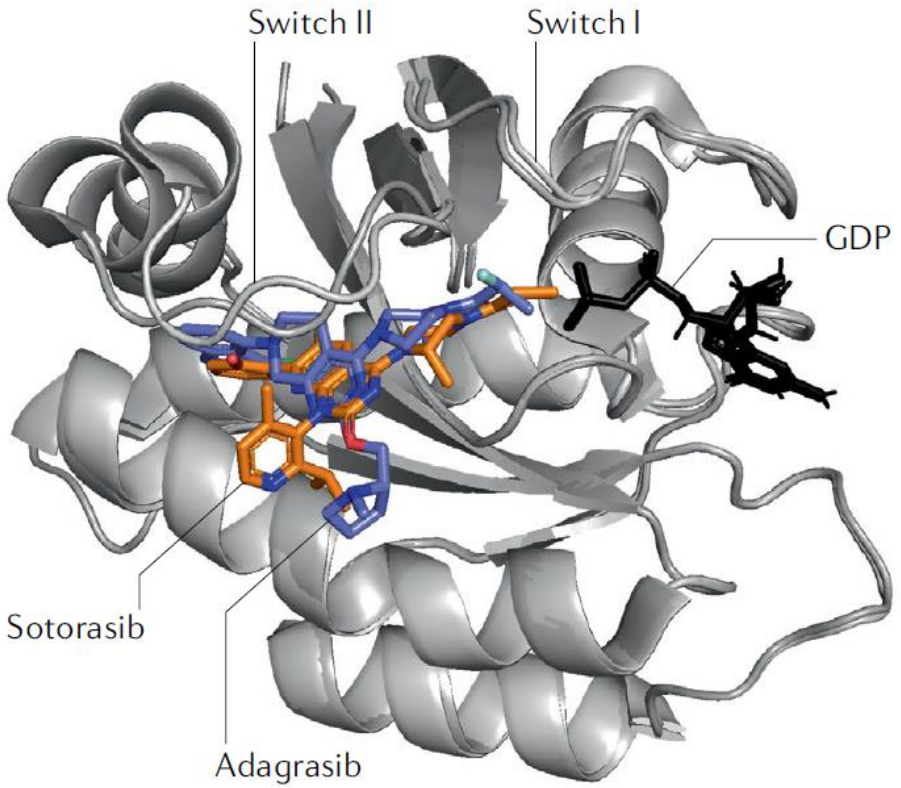


Fig. 3 | **Aligned structures of KRAS(G12C) co-crystallized with adagrasib (MRTX849) and sotorasib (AMG-510).** The covalent inhibitors adagrasib (PDB ID: 6UT0) and sotorasib (PDB ID: 6OIM) are bound to the switch II pocket, which is adjacent to the GDP-binding pocket[93,96].