Protein engineers navigate toward more targeted therapeutics

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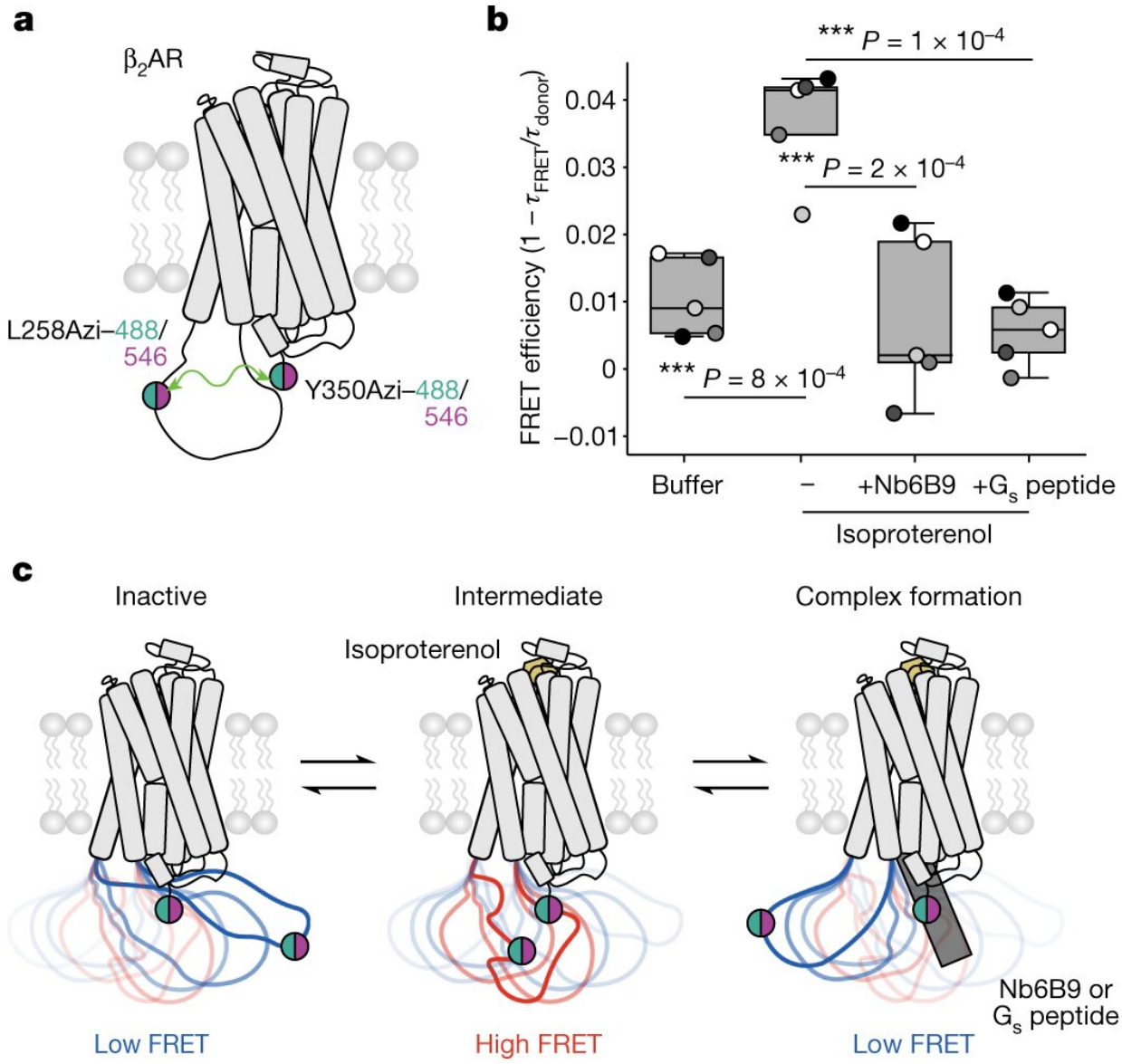

Agonist- and cytoplasmic effector-binding proteins drive conformational

changes in ICL3. a, Schematic of the β2AR ICL3 FRET sensor. Membrane extracts of cells expressing β2AR(L258Azi/Y350Azi/Δ351–413) were labeled with Alkyne-AZDye488 and Alkyne-AZDye546 to generate the sensor. b, FRET efficiency of untreated sensor (buffer), sensor treated with isoproterenol (100 μM), sensor treated with isoproterenol and nanobody Nb6B9 (500 nM) or sensor treated with isoproterenol and 10 μM G_s peptide (10 μM). FRET efficiency is defined as $1 - {}_{\tau FRET/\tau donor}$, where ${}_{\tau FRET}$ is the average lifetime of the FRET sensor (Extended Data Fig. 4p, gray bars) and ${}_{\tau donor}$ is the average lifetime of an AZDye488-only-labeled control sample (Extended Data Fig. 4p, white bars). Box edges delineate the 1st and 3rd quartiles of the data, the center line represents the median, whiskers represent the furthest points within 1.5× the interquartile rangean and points represent five independent experiments. One-way ANOVA followed by Tukey's post hoc significance test; ***P is less than 0.001 ($F = 15.2$, $P = 6 \times 10^{-6}$, 16 d.f.). c, Proposed sensor readout of ICL3 conformational equilibrium. Left, in the receptor's inactive state, the donor and acceptor probes are further apart, resulting in low FRET. Center, agonist (isoproterenol) binding increases probe proximity, thereby increasing FRET efficiency (intermediate). Right, formation of agonist–receptor–effector (with Nb6B9 or G_s peptide) complex displaces ICL3 from the intracellular cavity, extending the distance between donor and acceptor probes and quenching the FRET readout. Credit: *Nature* (2023). DOI: 10.1038/s41586-023-05789-z

More than a third of FDA-approved drugs work by targeting a G protein-coupled receptor, or GPCR. The human body has more than 800 types of GPCRs that provide cells with information about the external environment to calibrate responses. Drugs that either block or activate GPCRs are used to treat a wide range of diseases including hypertension, pain and inflammation. Most drugs bind to the outside of the receptor, but this can result in adverse side effects since receptors often resemble one another.

In a new study published in *Nature*, Sivaraj Sivaramakrishnan, a professor in the University of Minnesota College of Biological Sciences,

along with graduate student Fred Sadler and co-authors Michael Ritt and Yatharth Sharma, uncovered the role of the third intracellular loop in the GPCR's signaling mechanism, suggesting the possibility of a more targeted approach to drug discovery and a paradigm shift for new therapeutics.

"Typical GPCR drugs act as on or off switches for cellular signaling outcomes," said Sivaramakrishnan. "Drugs that leverage the loop effectively can act as signaling dimmer switches to more precisely control drug responses."

The authors developed new biochemical and biophysical tools, combined with computational measurements by collaborators Ning Ma and Nagarajan Vaidehi at the City of Hope Cancer Center. They tracked how the third intracellular loop changes in shape, or conformation, through the receptor signaling process. In a breakthrough for the field, their data show that the loop acts as a kind of gate to ensure that receptors activate the correct type of G protein signaling at the right intensity.

"A key advantage of this loop is that it is highly unique, even among closely related receptors, making it an outstanding drug target," said Sadler. "Developing drugs through this newly discovered mechanism would allow for far more targeted therapeutics."

More information: Fredrik Sadler et al, Autoregulation of GPCR signalling through the third intracellular loop, *Nature* (2023). DOI: 10.1038/s41586-023-05789-z

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