文部科学省事業　私立大学研究ブランディング事業シンポジウム2020

プロスタグランジン受容体の立体構造解析

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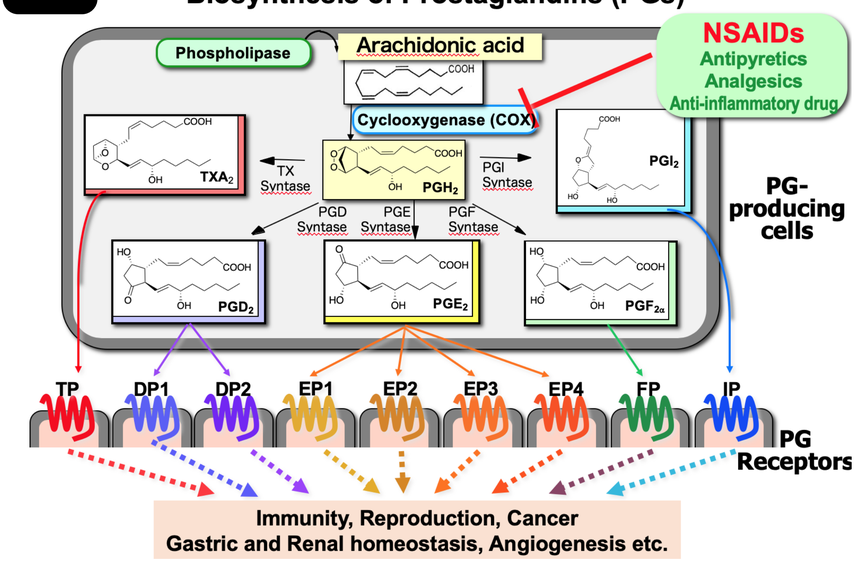
1

Biosynthesis of Prostaglandins (PGs)



Immunity, Reproduction, Cancer
Gastric and Renal homeostasis, Angiogenesis etc.

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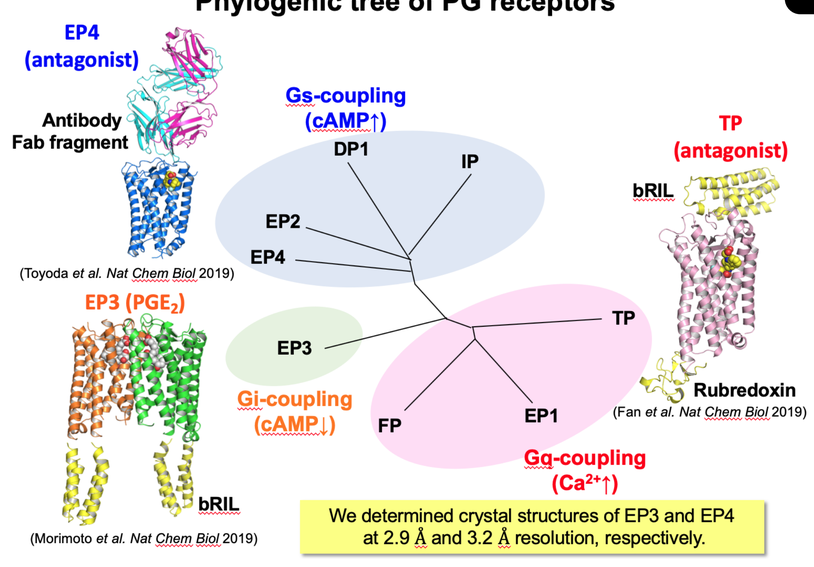
Phylogenic tree of PG receptors



We determined crystal structures of EP3 and EP4 at 2.9 Å and 3.2 Å resolution, respectively.

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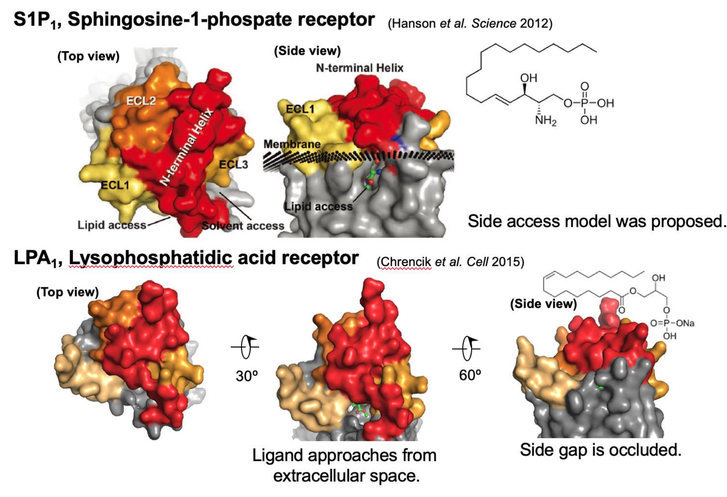
Ligand access pathway of lipid receptors



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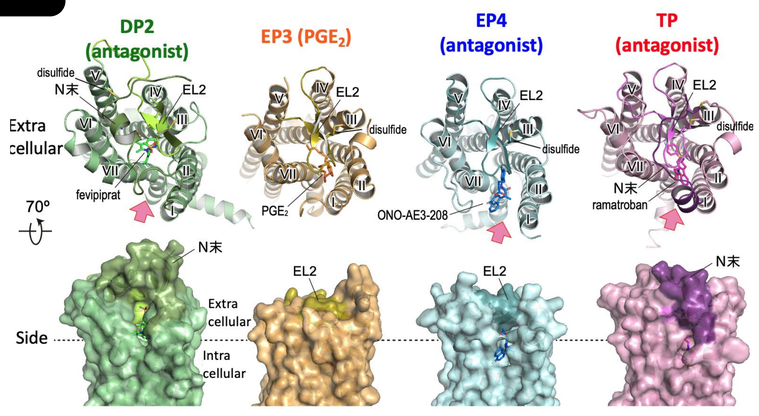
Extracellular lid of PG receptors



Gaps exist between TM1 and TM7 of antagonist-bound PG receptors.

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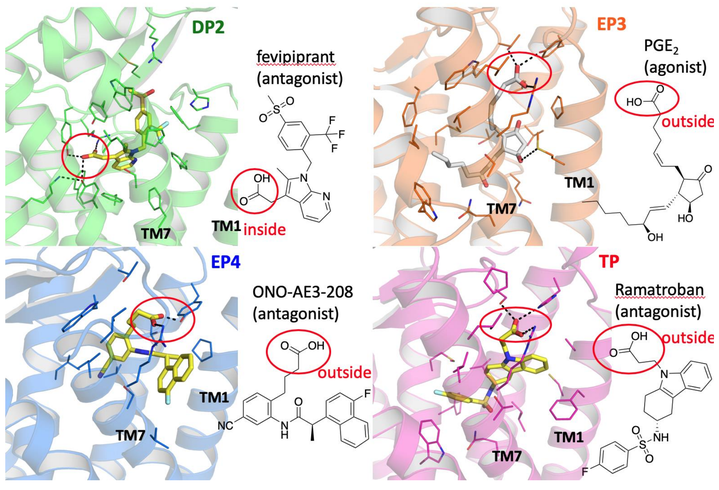
Ligand-binding mode of PG receptors



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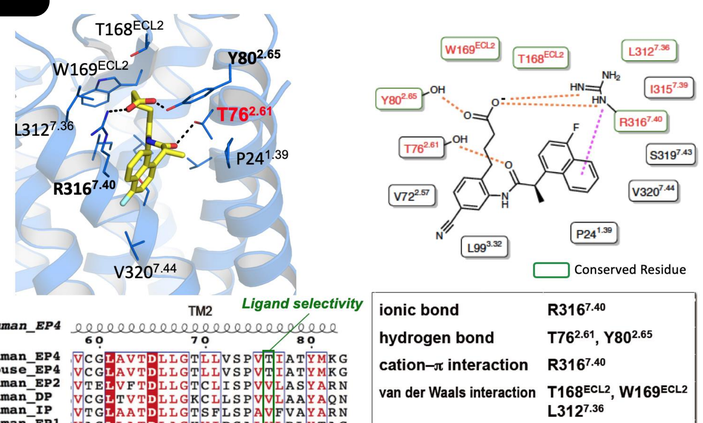
Binding mode of EP4 antagonist



Ligand selectivity

ionic bond	R316 7.40
hydrogen bond	T76 2.61, Y80 2.65
cation–π interaction	R316 7.40
van der Waals interaction	T168 ECL2, W169 ECL2 L312 7.36

T76 2.61 is not conserved.

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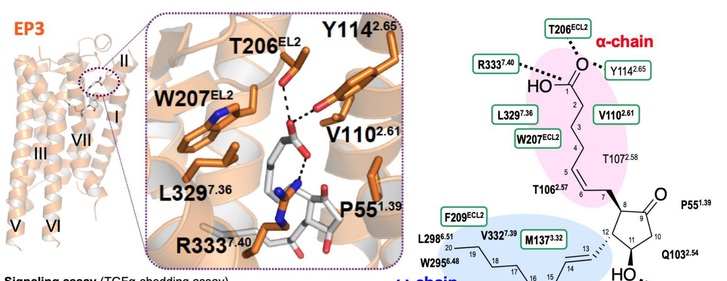
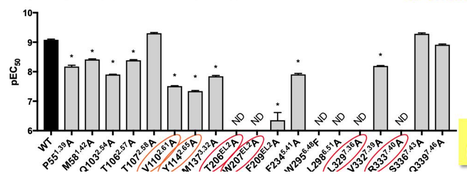
Binding mode of PGE_2



Signaling assay (TGFα-shedding assay)



Conserved Residue

Carboxyl group of α-chain is recognized by Y114・T206・R333.

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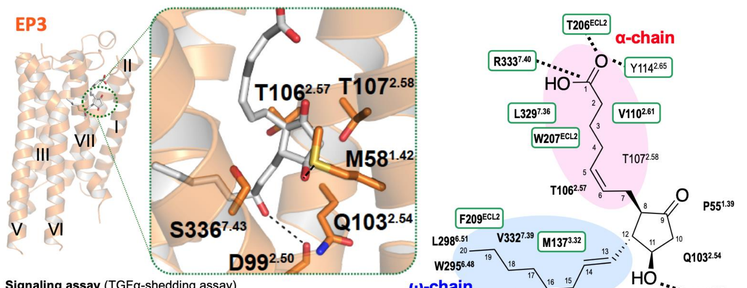
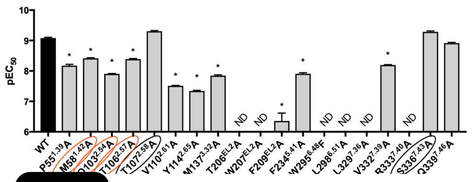
Binding mode of PGE_2



Signaling assay (TGFα-shedding assay)



Conserved Residue

M58 interact with 11-OH of PGE_2.

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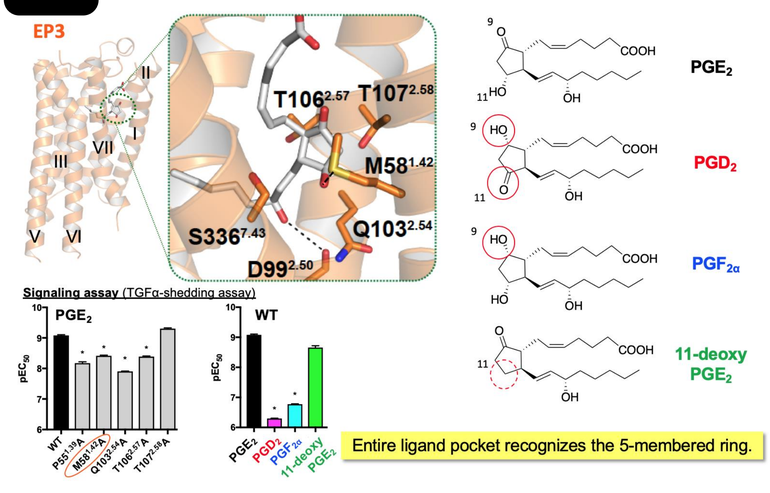
Selectivity of PGs



Entire ligand pocket recognizes the 5-membered ring.

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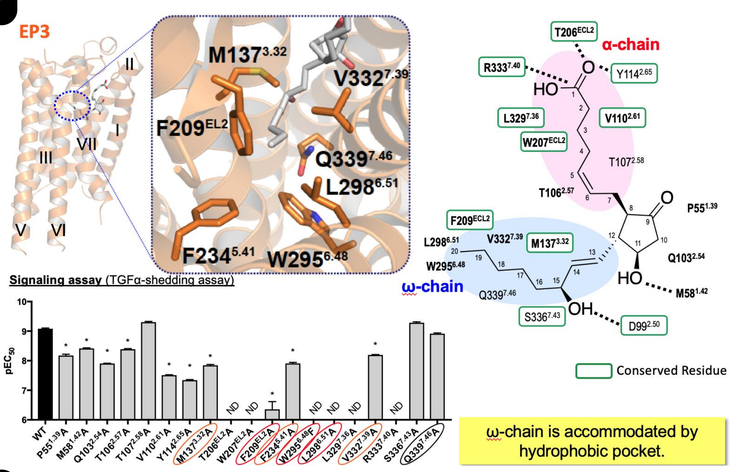
Binding mode of PGE_2



ω-chain is accommodated by hydrophobic pocket.

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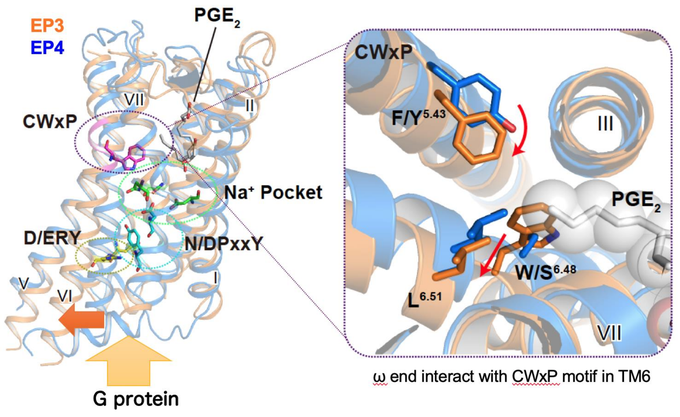
Putative activation mechanism

Conserved motifs important for GPCR activation



ω end interact with CWxP motif in TM6

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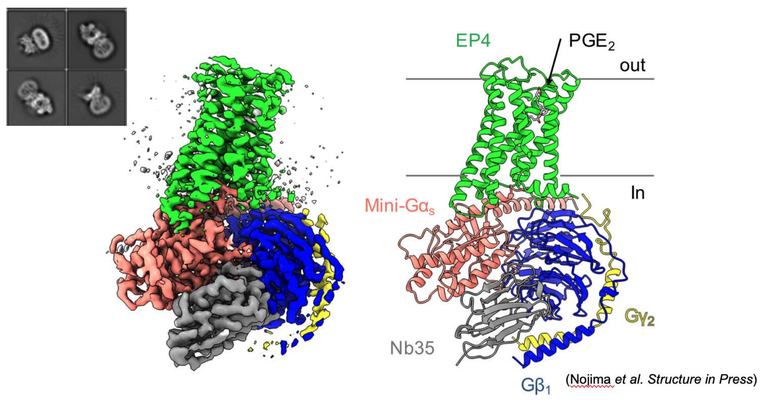
Cryo-EM single particle analysis of EP4



We determined cryo-EM structure of EP4–Gs complex at 3.3 Å resolution.

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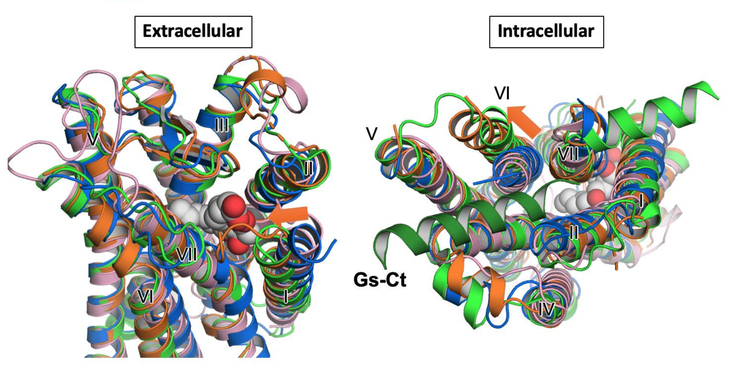
Comparison between antagonist- and agonist-bound forms

EP4, TP (agtagonist)
EP3, EP4–Gs (PGE_2)



Extracellular side of TM1 displaces inward.

Extracellular side of TM6 displaces outward.

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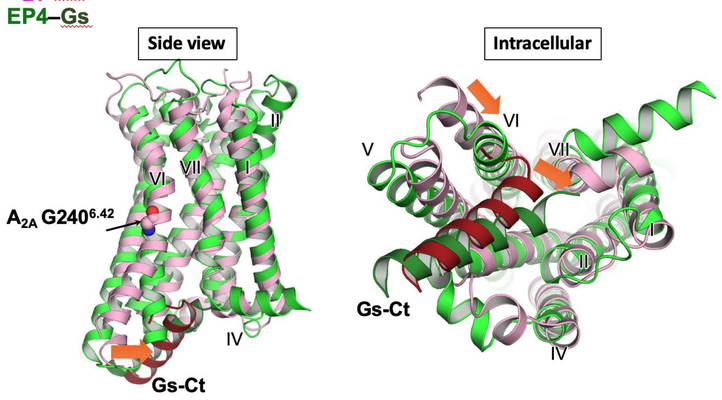
Comparison with another Gs-bound GPCR

A_{2A}–Gs
EP4–Gs



Displacement of TM6 is smaller than other Gs-bound GPCRs.
C-terminal tip of Gs points in a different orientation.

Summary

- Crystal structures of antagonist bound-EP4 and PGE_2-bound EP3 were determined at 3.2 Å and 2.9 Å resolution, respectively.
- Cryo-EM structure of EP4–Gs complex was determined at 3.3 Å.
- ECL2 forms β-hairpin loop, which occludes the extracellular side.
- Ligand binds via the gap between TM1 and 7.
- The carboxyl moiety of PGE_2 is recognized by three conserved residues (Y114 2.65・T206 EL2・R333 7.40).
- The interaction of PGE_2 ω-chain and TM6 of the receptor appears to contribute to the receptor activation.
- Displacement of TM6 is smaller than other Gs-bound GPCRs, and C-terminal tip of Gs points in a different orientation.

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