provides an accessible alternative that can provide genome-wide information using living systems.

The major findings of the study herein can be divided into *in vitro* and *in vivo* findings (**Fig. 1**). *In vitro*, the authors observed loss of reactivity of key purines in the *S*-adenosylmethionine (SAM)-I riboswitch upon titration with the SAM ligand, owing to ligand-induced tertiary structure formation, as confirmed by a measured strong correlation between LASER reactivity and C8 solvent accessibility. These findings support LASER as a probe of RNA tertiary structure. *In vivo*, results show reduced RNA reactivity due to protein protection of the C8, as assayed in diverse RNA–protein complexes. LASER detects *in vivo* protein-induced protections that current probes are blind to. Excitingly, LASER data thus provide evidence that *in vivo* protections caused by proteins can be detected over protections resulting from base pairing.

LASER joins a growing set of tools[7] now available for querying the dynamic *in vivo* RNA structurome, but is special in that it provides information on tertiary structure formation and protein binding. It is unclear the extent to which all tertiary structures and RNA–protein complexes will be sensitive to LASER, or whether protections from tertiary structure and ribonucleoprotein complexes can be differentiated. Nonetheless, combined application of structure-probing tools has enormous potential to improve RNA structure prediction at the secondary and tertiary levels *in vivo*. Given the dynamic nature of RNA structure, changes in RNA folding as a result of environmental or cellular conditions are of special interest, and LASER may be particularly enlightening in this regard. ■

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Competing financial interests

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GPCRs

Lock and key become flexible

G-protein-coupled receptors (GPCRs) are critically involved in signal transduction. Structural views of several GPCRs have recently been obtained, but the structural principles determining subtype selectivity are still mostly elusive. Now, a combined solid-state NMR and molecular-modeling approach reveals how bradykinin GPCRs distinguish between closely related peptide ligands.

Marc Baldus

GPCRs regulate numerous physiological processes and have key functional sites that are accessible at the cell surface, making them highly attractive pharmacological targets[1]. Yet, we have only begun to understand an intricate phenomenon in the pharmacology of GPCR ligands, namely that seemingly minor chemical ligand modifications can have profound consequences for GPCR activation and specificity[1]. In the current issue, Joedicke *et al.*[2] investigate this problem for the two kinin peptides kallidin (KD) and bradykinin (BK), which interact with the BK GPCRs B_1R and B_2R.

KD (as well as the *in vivo* variant desArg-kallidin, DAKD, in which the C-terminal arginine is removed) and BK differ only by their N- and C-terminal residues. Yet they are highly specific for the human bradykinin GPCRs B_1R and B_2R, respectively, which exhibit a high sequence conservation. Using an innovative combination of high-sensitivity solid-state NMR and molecular modeling (**Fig. 1a**), Joedicke *et al.*[2] report data revealing that the peptides (**Fig. 1b**) have a remarkable structural flexibility, and show that receptors B_1R and B_2R discriminate between the C-terminal parts of their respective peptide ligands via specific peptide conformations and peptide–receptor interactions. These results suggest that not only does receptor plasticity play a substantial role along the activation pathway of GPCRs, but the intrinsic flexibility of peptide ligands critically contributes to subtype selectivity as well.

Rather than attempting to trap these subtle structural aspects in GPCR crystals, the authors used the fact that solid-state NMR chemical shifts are highly sensitive reporters of molecular conformation and the local molecular environment in solution, membrane and even cell settings. Compared to earlier work that combined solid-state NMR and molecular modeling to study free and GPCR-bound ligands[3–5] and entire 3D structures of a (bacterial) GPCR[6], Joedicke *et al.*[2] here employed high-sensitivity (so-called dynamic nuclear polarization, DNP[7]) experiments that greatly reduced the amount of receptor needed. Moreover, they made use of state-of-the art docking and modeling methods that capitalize on the growing arsenal of GPCR structures[1]. Taken together, their combined spectroscopic and modeling approach provides remarkable insight into the conformational landscape of the two free peptide ligands (**Fig. 1c**) that may be relevant for explaining basal activities, and elucidates binding epitopes that are characterized by a highly optimized ligand conformation (**Fig. 1d**) and a distinct molecular network that establishes ligand–receptor interactions (**Fig. 1e**).

These results complement growing evidence that, rather than a set of well-defined structural states, the functional profile of many membrane proteins including GPCRs or receptor tyrosine kinases may best be described by a conformational landscape consistent with protein dynamics seen in NMR[8,9]. In this notion, conformational flexibility of ligand

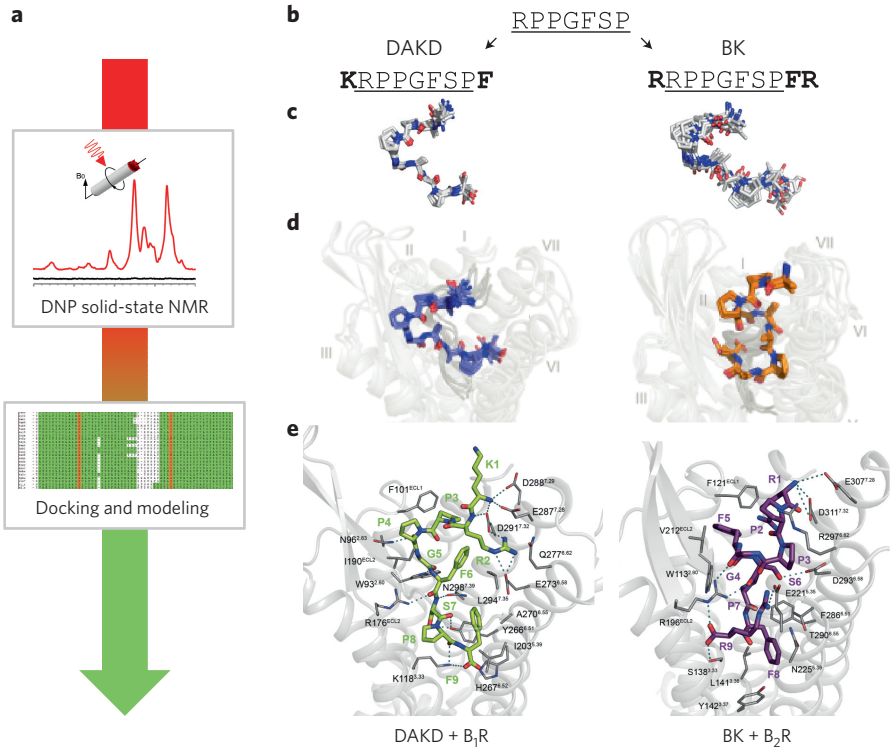

Figure 1 | Novel insight into subtype selectivity of bradykinin GPCRs. (**a**,**b**) Joedicke *et al.*[2] combined DNP solid-state NMR and docking/modeling (**a**) to study the BK ligands DAKD and BK, which differ only by N- and C-terminal amino acids (**b**). (**c**–**e**) The authors determined the conformation of kinin peptides in free (**c**) and receptor-bound (**d**,**e**) forms. In the case of DAKD, free and B_1R-bound conformations are similar, both featuring a V-shaped fold. In contrast, the BK structure significantly changes from being embedded in free solution to having a β-turn-like structure upon binding to B_2R. In both cases, the bound ligand state is characterized by distinct peptide–receptor interactions in which local charges are critical (**e**).

peptides and peptide analogs could be a critical additional degree of freedom that helps to establish subtype selectivity and offers, in a pharmacological context, novel routes for designing GPCR subtype-selective biochemical tools and drugs.

Clearly, additional work will be needed to establish whether and how such ligand–receptor binding profiles are linked to other protein regions that are potentially involved in establishing subtype specificity, including transmembrane helices, loops or secondary binding sites examined in recent X-ray structures[1,10]. In addition, the surrounding membrane environment[11] may need to be considered to fully understand how conformational and chemical variations in receptor or ligand profiles contribute to the energetics of GPCR functioning. Given the progress in NMR for studying membrane proteins in a eukaryotic[9,10] and potentially (native) membrane setting[10], new insight may again come from combining state-of-the-art X-ray and cryo-EM data with NMR and modeling approaches.

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The author declares no competing financial interests.

PROTEIN EVOLUTION

Hacking an enzyme

Early stages of protein evolution are inherently difficult to study. Genetic selection in *Escherichia coli* has now identified a life-sustaining *de novo* enzyme arising from a simple scaffold that is completely different from the native enzyme.

Kristoffer E Johansson & Jakob R Winther

One of the intriguing mysteries of nature is how protein-based functionalities, including enzymatic activity, emerge. We know that, in general, proteins evolve from other proteins by genetic selection, but early events in this process are obscure. The study of protein evolution is tightly linked to the evolution of life, but it also inspires targeted *de novo* design of protein structures with desired properties. In this issue, Donnelly *et al.* describe, for the first time, the *de novo* formation of enzymatic activity capable of sustaining life[1]. Furthermore, the authors identified this new enzyme not from careful design and theoretical considerations, but from a semi-random sequence library. This finding has implications for how we think about early events in evolution and the generation of novel enzymatic activities, both by design and by selection.

In previous work, a semi-random library of DNA sequences was developed for encoding protein structures that were prone to the formation of four-helix bundles. To explore the potential of random sequences to evolve enzymatic activity, this