Once Blind, Now We See GLP-1 Molecular Action

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The macromolecular mechanics of GLP-1 with its cell surface receptor came into focus as two landmark publications recently published in *Nature* collectively herald advancement in structure-based design for a receptor class of great therapeutic importance (Jazayeri et al., 2017; Zhang et al., 2017).

Two new reports from independent research groups (Jazayeri et al., 2017; Zhang et al., 2017) have significantly added to our understanding of the structural interactions that initiate biochemical signaling within class B GPCRs, specifically the glucagon-like peptide receptor (GLP-1R). These reports reinforce structural results involving additional members of the class B GPCR family, glucagon (GCGR), calcitonin, and corticotropin-releasing factor 1 (CRF-1). This Preview focuses on the medicinal ramifications of these landmark achievements and is intended to complement the insightful review by Schwartz and Frimurer (2017), which highlighted the technical aspects of these accomplishments with emphasis on specific ligand-receptor structural contacts.

The emergence of peptide-based GLP-1 therapy is proving transformative in the treatment of adult-onset type 2 diabetes. In most patients, these agents provide a sizable improvement in glycemic control, little risk of hypoglycemia, a modest lowering of body weight, and a reduction in cardiovascular mortality (Drucker, 2016). Furthermore, in insulin-dependent diabetes, GLP-1 agonists lessen the need for insulin, promoting body weight loss while reducing the risk for hypoglycemic events. Similarly, remarkable benefits have been realized in the case of other peptide-based agonists signaling through class B GPCRs. Examples include glucagon, used primarily for the treatment of life-threatening hypoglycemic coma; parathyroid hormone, used for regenerative therapy in the management of severe osteoporosis; and GLP-2, as treatment for short bowel syndrome. The miraculous medicinal performance of these agents, currently administered only by injection, has stimulated the search for more convenient, orally administered drugs. In this regard, structural insights provide detailed information that might empower their discovery. Historically, the *de novo* identification of class A and B GPCR mimetics of natural peptide ligands has proven problematic, whether conducted by high-throughput screening (HTS), rational design, or traditional medicinal chemistry (Bortolato et al., 2014). The relatively few successes largely pertained to oral antagonists of class A GPCRs, notably angiotensin, endothelin, and neurokinin, where leads originating from HTS required extensive optimization by traditional medicinal chemistry.

Zhang et al. (2017) employed a cryo-electron microscopy technique to study a full-length GLP-1(*7-37*) hormone, bound in an active conformation to rabbit GLP-1R and its G protein signaling domain. The results enabled Zhang et al. (2017) to deduce the sequence of ligand-receptor interactions underlying signal transduction and map the key contact points at the interface, which mainly comprise conserved residues. These intermolecular interactions include the N-terminal His^{7} with R299; Glu^{9} with L388, S392, R190, and Y145; Thr^{13} with 197; the triad of $Ser^{14,17,18}$ with W297 and R299; and Trp^{31} with Q211 and H212. Jazayeri et al. (2017) use a thermo-stable GLP-1R construct encompassing the extracellular and trans-membrane domains complexed with labeled peptide ligands, exhibiting multiple non-native substitutions discovered at Bristol-Myers Squibb. In contrast to the approach by Zhang et al. (2017), these ligands lack two-thirds of native GLP-1 (residues 18–37) and consequently employ alternative receptor contacts (Figure 1). For example, the imidazole in the surrogate N-terminal-capping group, termed "Cap-1," substitutes for the native His7 and Ala8 that interacts with E387 and is further stabilized by K383. Similarly, the geminal dimethyl group of Cap-1 interacts with a hydrophobic pocket formed by two leucine residues at L384 and L388. The tetrazole mimic of the Glu^{9} carboxylate maps to R190, in agreement with the findings using native GLP-1(7-37). Jazayeri et al. (2017) employed the structural insights to refine the truncated agonist to site-selectively insert a much larger polyethylene glycol, which prolonged the duration of action at competitive potency relative to short-acting exendin-4, as demonstrated in mouse oral glucose tolerance tests. A more challenging test will be whether accelerated agonist refinement can be similarly made to identify an oral drug candidate, as the current truncated leads are considerably larger with enhanced hydrogen-bonding sites when compared to conventional drugs. Perhaps the most profound insight is the demonstration that the two differing length GLP-1 ligands, while interacting at distinct receptor contact sites, both lead to receptor activation. At the minimum, this realization expands the purist's definition of a "peptidomimetic" as a surrogate ligand that precisely replicates the topology of the native peptide.

Collectively, these findings elegantly confirm and reconcile empirical observations derived from traditional peptide structure-function studies using full-length GLP-1 agonists (de Graaf et al., 2016; Finan et al., 2015). The sheer number of discontinuous contacts at the ligand-receptor interface also plausibly illustrates why it has proven so difficult

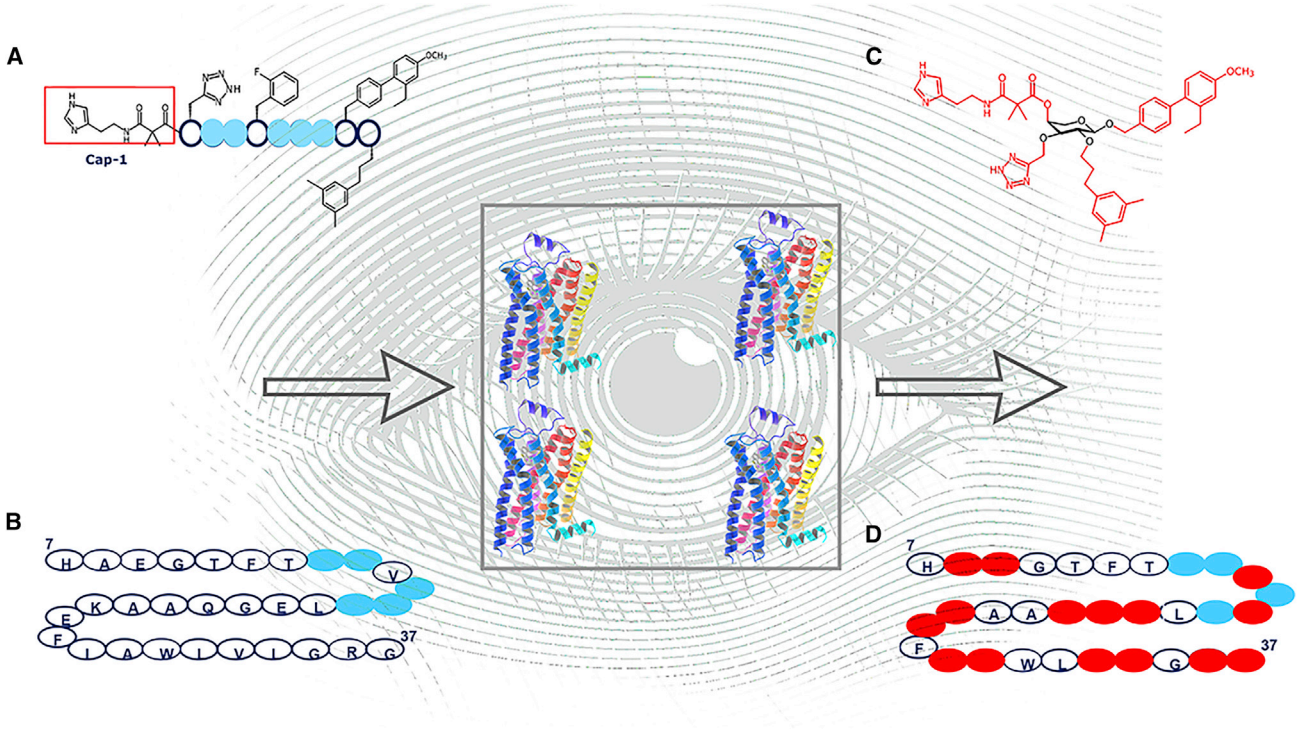

Figure 1. Macromolecular Structure-Based Drug Design
Translation of structural information to envisioned drug candidates starting with truncated (A) and full-length (B) GLP-1 agonists as respectively employed in the reports of Jazayeri et al. (2017) and Zhang et al. (2017). The blue circles represent conserved amino acids among these two differing, high-potency agonists. Compound (C) represents a hypothetical drug lead employing a carbohydrate scaffold to display key chemical functionalities in a form more suitable for oral administrations (Hirschmann et al., 1993). Compound (D) is a putative peptide where structural information would be used to accelerate the discovery of amino acid substitutions (red circles) to achieve balanced, full agonism at multiple receptors.

to identify small-molecule ligands by HTS and the daunting challenge of *de novo* drug design. Inevitably, such information will be used to more intelligently enrich HTS libraries with pharmacophores that might increase the chance for success. In library design, an acidic group interacting at R190 in concert with a soft base, such as an imidazole, could increase the odds of identifying a high-affinity lead at the orthostatic receptor-binding site. Such drug leads, as well as ligands associating at an allosteric site, could reduce the challenge in oral drug design to something considerably less complex (Bortolato et al., 2014; Song et al., 2017). A more immediate practical application is the employment of these structures to accelerate the discovery of peptides that simultaneously signal at more than one receptor across multiple species. This has been achieved largely by an experimental approach for GLP-1 agonists that are balanced in activity at the related glucagon and GIP receptors (Finan et al., 2015; Tschöp and DiMarchi, 2017). Finally, the quest for traditional small molecules should be viewed in the context of the advancements in peptide optimization, which have provided highly potent GLP-1 agonists with no apparent off-target toxicity that no longer require daily injection and, more recently, the promise for oral administration (Lau et al., 2015).

It is clear that the work by Jazayeri et al. (2017) and Zhang et al. (2017) has a wide range of implications for basic science, as well as drug discovery, much of which cannot be predicted. However, in the immediate term, it would be best to maintain some awareness of the degree that the reported structures are specific to the macromolecular reagents and the experimental conditions under which the observations were obtained. The technology will undoubtedly improve and provide structures with enhanced speed. Whether we can adapt the methods to define the mechanism by which certain ligands demonstrate partial or biased downstream signaling will surely be determined. Is this a transformative advance in *de novo* design to identify ligands, of whatever molecular size but of suitable affinity and specificity, at orphan receptors to allow pharmacological interrogation of biological function? Time will tell, but it seems intuitively obvious that this and similar work are advancing our ability to conduct structure-based drug design at the macromolecular level in a family of receptors seminal to modern medicine.

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