ISSN: 1391-8796
Proceedings of 10th Ruhuna International Science & Technology Conference
University of Ruhuna, Matara, Sri Lanka
January 18, 2023

Keynote Speech

The wisdom beyond chemistry: Novel technologies to expedite the modern drug-lead discovery process

Professor Gomika Udugamasooriya

Department of Pharmacological & Pharmaceutical Sciences, University of Houston, 4349 Martin Luther King Blvd, Health Building 2, Houston, TX 77204-5037, USA.
Department of Cancer Systems Imaging, MD Anderson Cancer Center, 1881 East Road, Houston, TX 77030-4009, USA.

The conventional western drug discovery process is streamlined as biologist first defining disease specific biomarkers and then chemists developing drugs to target those biomarkers to attack the disease. However, the statistics indicates that only 1 out of initial 10,000 drug-leads become a drug and the process takes about 7-15 years with spending 1–2 billion dollars for the overall process. These statistics simply indicate major draw backs of this conventional approach. Vast majority of initially developed active drug-leads are thrown away during this long drug discovery process due to off target effects and also multiple other reasons. Therefore, novel approaches and technologies are needed to reduce the cost and further expedite this current drug discovery process.

The standard approach in drug development is to target biomarkers that have known functions related to a given disease state. The majority of these biomarkers are proteins such as enzymes, hormones, receptors, or signaling molecules. There are 3 major ways utilized in the initial drug-lead discovery, namely: (I) natural products, (II) structure based or rational drug discovery, and (III) combinatorial high throughput drug discovery. The drug leads are first developed or identified targeting those disease specific biomarkers at laboratory level, testing only on the targeted biomarker alone. These active drug-leads are then tested on cellular level, pre-clinical animal level and finally validated through 3 phases of human trials. As the exposure become more and more complex going through above stages, initial drug-leads start to show off targeted effects because those drug leads are first identified only targeting the intended biomarker alone. Therefore, one way of reducing this risk is to incorporate features of complex biological system into the very initial steps of the drug discovery. The latter part of this article describes an innovative technology developed for this purpose.

When look at the well-known molecular classes in the modern drug development arena, small organic molecules, antibodies, peptides, aptamers and nanoparticles, carry both useful as well as undesired properties as drugs.[1] Years ago, small organic molecules exclusively represented therapeutics as easy to handle and orally available chemical compounds. However, the synthesis of majority of biologically active complex organic compounds remains a challenge to date. In addition, these molecules clear through the kidneys and do not have adequate affinity and contact time for effective imaging or therapy of the target. During the last two decades, antibodies have been developed as high affinity and specificity drugs, but this is a costly approach and also antibodies have intermediate to poor biodistribution and tumor penetration. Other macromolecules such as nanoparticles virtually have no clearance from the body. Although the use of biodegradable materials in building nanoparticles addressed this issue to some extent, very large molecular size still causes various pharmacokinetic issues in biological systems. In the meantime, peptides as intermediate size molecules emerged as great therapeutics, but current development of peptide-based pharmaceuticals is hindered by their rapid *in vivo* degradation and the initiation of immune responses. Researchers have recently investigated alternative peptide-like constructs that may be able to circumvent such complications. This is the point where peptidomimetics molecular developments were initiated, and peptoids are emerged as one such a promising molecular class for both therapeutic and diagnostic applications.

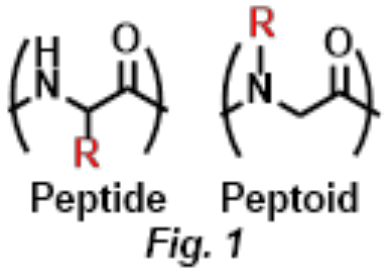

Fig. 1 Peptide vs peptoid.

Peptoids were originally invented in the early 1990s by Prof. Ronald Zukermann and major biological applications were begun around early 2000's, primarily spearheaded by Prof. Thomas Kodadek's group.[2, 3] Peptoids comprise a peptide-based backbone and N-substituted glycines (**Fig. 1**). This means the side chain ('R' group) is placed on the nitrogen atom of the amide bond in peptoids as compared to the alpha-carbon in peptides, bringing unique and favorable characters over peptides and other conventional drug classes. The solid-phase (on resin beads) submonomer peptoid synthesis is very efficient, rapid, economical and straightforward.[4] In order to add one residue (equivalent to an amino acid of a peptide), it needs only two chemical steps and each of these steps can be completed by 2 x 15 second microwave pulses.[5] Bromoacetic acid coupling

brings the two carbon units and the Br can be replaced by *any amine* group, which dramatically expands the repertoire of chemical space. In peptides there are only 20 side chains available through natural amnio acids, but in peptoids, virtually any organic moiety ('R' groups) can be incorporated into the backbone, thereby tremendously increasing the target biomarker recognition capacity. These oligomers are protease resistant, more cell permeable, non-immunogenic, achiral and adopt different conformations than peptides, yet retain the same density of functionality and backbone polarity.[3, 6] Synthesis of peptoid sequences up to about 50 units in length allows for controlled sequence composition and incorporation of diverse side chain chemistries. It became clear very quickly that the most significant hurdle to compete in the drug discovery race was to access to large collections of compounds for high throughput screens. Large combinatorial libraries of peptoids (in millions) can be synthesized easily, inexpensively, and rapidly (less than one week).[7-11] Peptoid sequences can be deduced sensitively by Edman degradation[8, 9] or mass spectrometry [10, 12].

Now a days the drug-lead discovery is mainly relying on structure based/rational approach or high throughput screenings of large combinatorial libraries. Peptoid libraries are synthesized through the "split-pool" approach, and these split-pool cycles will lead to development of 'one-bead one-compound' (OBOC) combinatorial libraries with huge diversity.[13] For example, the Kodadek group initially developed several number of such peptoid libraries with diversities varied up to millions of permutations.[3, 8-11, 14] These peptoid libraries can be developed in less than one week, using the very efficient and rapid microwave synthesis method. All other molecular classes are needed much more time and effort in order to develop similar size libraries and peptoids clearly display huge advantage in this initial development levels.

Once developed, these OBOC libraries can be utilized to rapidly identify peptoids for our favorite biological target (biomarker) that is important in the relevant disease applications. In protein screens, the interested protein is equilibrated with OBOC library beads and the protein is allowed to 'pick' the best binding sequences. Those protein-binding peptoid carrying beads ('hits') are identified via having a fluorescein tag (e.g. GFP) on the protein or by employing a secondary identification system (e.g. GST- or Fc-recognizing fluorescein/ quantum dot labeled antibodies). The peptoid sequence on that 'hit' bead is subsequently identified via Edman degradation or mass spectrometry.

As already mentioned, above high throughput screens also identify the initial drug-lead by only exposing to the intended targeted biomarker (e.g. protein). The drug-lead found here may bind to thousands of other biomolecules in cells and human body, and these off targets will be fond later of the drug discovery process after spending years of time and money, as mentioned above. In order to expedite the drug discovery process, Dr. Udugamasooriya developed a unique on-beat two-color (OBTC) high throughput screen to identify the most selective drug-lad over rest of the biomarkers present in a cell surface. This OBTC combinatorial cell screen can directly identify peptoids that are highly selective for a particular cell-surface receptor, over all other shared cell surface proteins [9, 15].

The technology principle can be explained as follows: Two identical cell groups, which differ only by the presence (red stained) or absence (green stained) of a particular protein (e.g. VEGFR2), are exposed to one-bead one-compound peptoid library beads (Fig. 2). Each bead bears a unique peptoid sequence in many copies. If a bead is bound only by the red stained, targeted-biomarker expressing cells, the peptoid on this bead binds only to the intended target (e.g. VEGFR2) and not to all other shared cell-surface proteins (Fig. 2A and B). These beads are picked and mass spectroscopy sequencing is used to identify the peptoid sequence, which will be validated subsequently for binding, selectivity, and activity in many different assays. If the peptoid on a given bead binds to other shared cell-surface molecules non-specifically, it will be bound by green cells too and these are discarded (Fig. 2C).

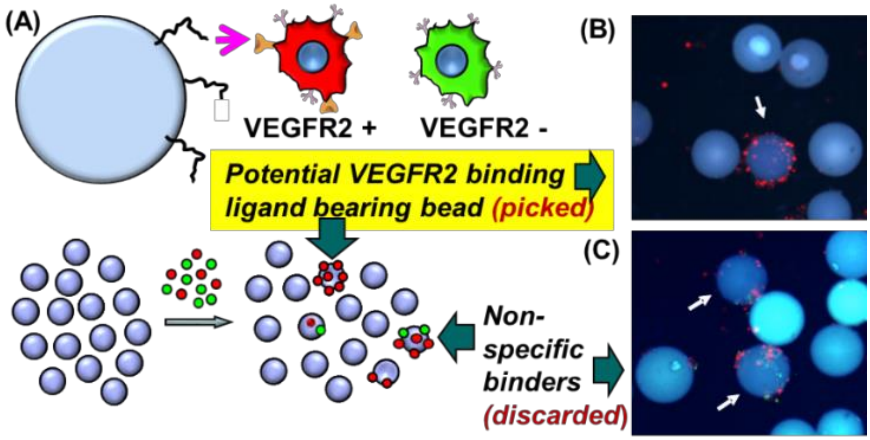

Fig. 2 On-Bead Two-Color (OBTC) high throughput cell screen technology. (A) the outline of the assay principle. (B) fluorescence microscopy image of a 'hit' bead with only red stained cells bound carrying highly specific peptoid drug-lead for VEGFR2 receptor. (C) beads that bound both red and green indicates non-specific peptoids carrying beads (discarded).

By applying this OBTC methodology, Udugamasooriya research group has thus far successfully identified and validated peptoid compounds for: VEGFR2,[9] T-cell receptors,[16] IL-15, transferrin, lipid-phosphatidylserine,[17] plectin,[18] vimentin,[19] and ACE2 (for COVID19)[20]. All those novel compounds have been patented or patent applied. So far, these drug-leads have been validated all the way to pre-clinical studies on both therapeutic and imaging applications. Recent "unbiased" and improved cell screening technology developed using this OBTC assay provides a new approach for targeting various cancer cell sub-populations, capturing new biomarkers uniquely appear over spatial and times scales, simultaneously identifying high specific ligands to target those markers. One of these "unbiased" cell screens identified plectin protein as a new biomarker for cancer stem cells. On another study, high specific peptoids were identified targeting lipid-phosphatidylserine, which is uniquely exposed on cancer cell surface as compared to located in the inner cell membrane of normal cells.

In comparison to peptides, reported data indicates that peptoids have higher tissue accumulation, moderate excretion, and higher *in vivo* stability. Remarkably longer passage through the gastrointestinal (GI) tract without rapid digestion was observed for peptoids confirming the great *in vivo* stability. As already mentioned, peptoid synthesis and further optimizations are extremely versatile and economical to handle. As protease-resistant isomers of peptides, peptoids are being developed as useful molecular tools in biochemistry, and are becoming attractive candidates for therapeutic, diagnostic and many other applications in cancer. Peptoids have thus far demonstrated very promising bioactivities in oncology and also various other disease areas as peptide mimics. Therefore, peptoids can be considered as better alternative for small molecular, antibody and peptide drugs in the future.

The OBTC technology has also been evolved as a powerful tool to identify highly selective drug-leads for various biomarkers important in many different disease states. In particular, the technology is now capable of identifying new biomarkers and a drug-lead to target that biomarker at the same time. This OBTC technology has already been applied in disease types such as cancer, COVID19, macular degeneration, cardiovascular, autoimmune, rheumatoid arthritis, Alzheimer's, and Parkinson's etc. Utilization of peptoids in OBTC technology to identify highly specific, economical and biologically amenable drug-leads along with identifying new biomarkers together allows to expedite the modern drug-lead discovery process effectively.