1 Spiegelmer Technology Platform

Spiegelmers are biostable oligonucleotide therapeutics. Like aptamers and monoclonal antibodies, Spiegelmers bind their targets with high selectivity and specificity and can inhibit pathophysiological protein-protein interactions. Spiegelmers differ from aptamers by virtue of their mirror image configuration, an attribute that renders them resistant to the body's enzymatic degradation system. This innate biostability allows the drug to be available in the patient for longer, overcoming the susceptibility to degradation that has hampered the clinical success of natural oligonucleotide therapeutics. As a substance class Spiegelmers seem to be not immunogenic, reducing the possibility of side-effects due to the patient generating an immune response to the treatment. In addition, the L-configuration does not allow for potentially unwanted interference with native oligonucleotides or by complementary binding or incorporation. In sum, Spiegelmers display highly attractive qualities that make them an interesting substance class for development into drugs.

1.1 Spiegelmers: Next Generation Aptamers

To understand what a Spiegelmer is, it is useful to first explain the concept of aptamers. An aptamer is a nucleic acid structure that can bind to a target molecule in a manner similar to an antibody recognizing an antigen.[1] Based on the SELEX (Systematic Evolution of Ligands by EXponential enrichment) process[2], aptamers can be identified from huge combinatorial nucleic acids libraries of more than 10^{15} different sequences. The sequences in the library are comprised of a central randomized region flanked by fixed sequences that permit amplification by polymerase chain reaction (PCR). The library size depends on the length of the random portion of the molecule, creating a diverse universe of molecules ready for screening. Specific aptamers are selected by incubating the library with the target and eluting the bound aptamers. After amplification by PCR, these binding molecules are re-selected, eluted and amplified again. By repeating this procedure multiple times, and eluting bound aptamers under progressively more stringent conditions, the molecules with greatest affinity and specificity are selected.

Aptamers have binding characteristics similar to peptides or antibodies, with affinities in the low nanomolar to picomolar range. However, there is one major drawback to aptamers as useful therapeutic products: As natural nucleic acid polymers they are prone to rapid degradation by nucleases that are present in all tissues in the body. To overcome this problem, methods of creating nuclease-resistant molecules that retain the binding capabilities of aptamers needed to be discovered. The ideal answer to this problem are Spiegelmers.[3,4] Spiegelmers are biostable aptamers which have all of the diversity characteristics of aptamers but possess a structure that prevents enzymatic degradation.

1.1.1 Identification of Spiegelmer Products

The name 'Spiegelmer' comes from the German '*Spiegel*', meaning mirror, and reflects the molecules' mirror image configuration. This reversed configuration is the key to Spiegelmers' biostability and is achieved by the use of non-natural L-nucleotides rather than the natural D-nucleotides for their synthesis. The L-form cannot be recognized by nucleases and therefore the molecule remains intact in

biological environments. NOXXON has developed the methods to form high affinity L-oligonucleotides that possess outstanding binding affinities and functionality.

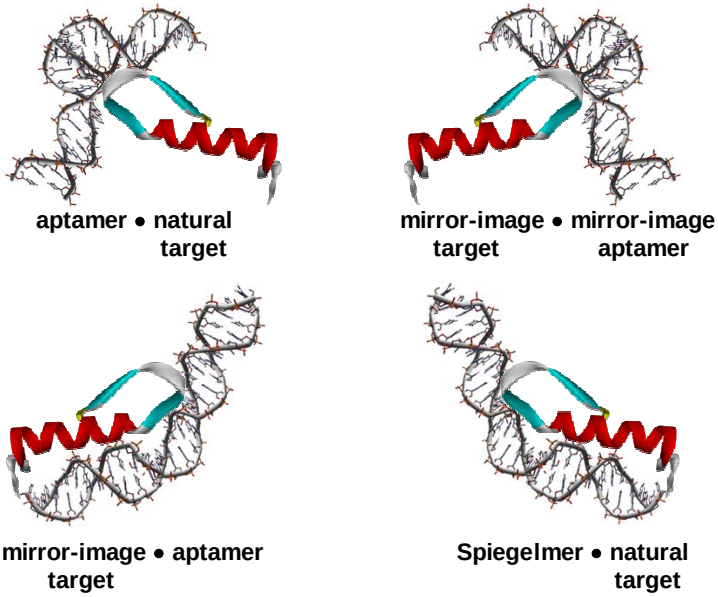

Fig. 1 Reciprocal Specificities of aptamers (D-RNA) and Spiegelmers (L-RNA).

The identification of a Spiegelmer is based on the reciprocal specificities of mirror image binder pairs (see Fig. 1). If an aptamer binds a specific configuration of a target, the mirror-image of the aptamer will identically bind the mirror image of the target (Fig. 1). NOXXON carries out the process of aptamer selection against the mirror-image target and isolates a highly specific aptamer against this mirror-image. The corresponding mirror-image nucleic acid (L-oligonucleotide) of this aptamer, the Spiegelmer, will now bind to the natural target with the same binding characteristics. More important, this Spiegelmer is resistant to nuclease degradation. This mirror image selection process is NOXXON's patented Spiegelmer technology platform (Fig. 2) that generates any number of stable oligonucleotides possessing outstanding binding and functional characteristics.

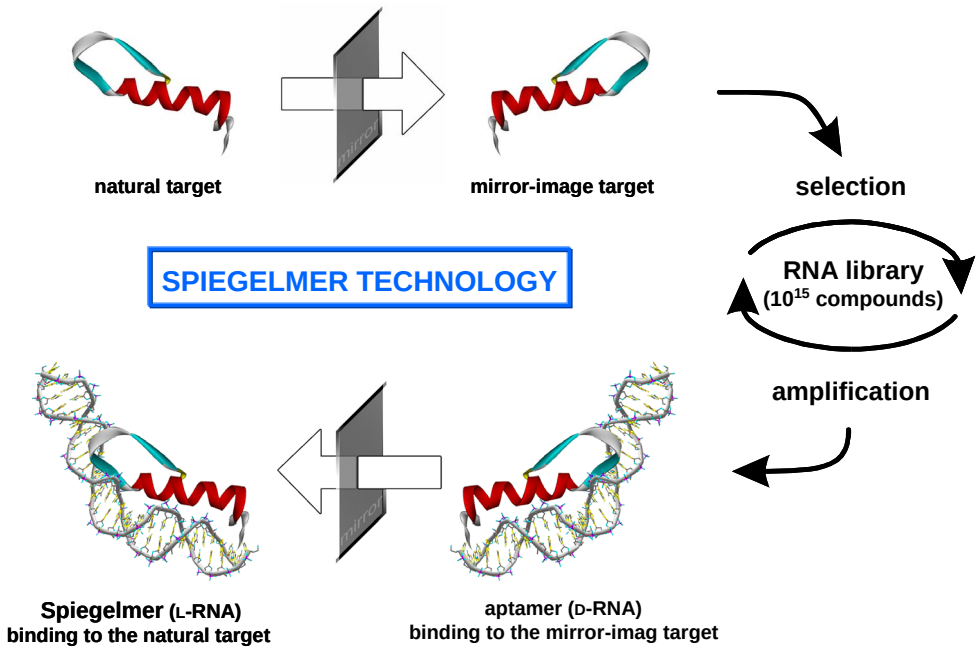

Fig. 2 Spiegelmer identification process.

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2. Tuerk, C. & Gold, L. (1990), Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* Vol. **249**, 505-510.
3. Klussmann, S., Nolte, A., Bald, R., Erdmann, V. A. & Furste, J. P. (1996), Mirror-image RNA that binds D-adenosine. *Nat Biotechnol* Vol. **14**, 1112-1115.
4. Vater, A. & Klussmann, S. (2003), Toward third-generation aptamers: Spiegelmers and their therapeutic prospects. *Curr Opin Drug Discov Devel* Vol. **6**, 253-261.