Developing robust biosensors with high specificity and sensitivity for certain biomarkers is crucial for the prevention and early-stage detection of many diseases. Moreover, being able to detect several biomarkers simultaneously reduces the time-to-result and the required amount and cost of reagents, while providing more information related to the patient's health. To achieve a high sensitivity in combination with multiplex detection, often an amplification system is combined with a target-specific labelling system [1].

In contrary, the innovative microfluidic platform Evalution relies on uniquely barcoded microparticles to create a high-throughput multiplex environment compatible with DNA as well as protein target molecules [2] (Figure 1). Although the binary code enables an unprecedented encoding capacity compared to conventional methods relying on spectral discrimination, the ability to detect low amounts of target currently remains unexplored. Here, hybridization chain reaction (HCR) is explored as signal amplification tool for the sensitive detection of three different microRNA precursor-based targets related to cancer (miR-16, miR-18, miR-30). HCR is a robust, isothermal and enzyme-free amplification method of which the amplified signal can be contained on the surface area of each of the barcoded microparticles. This last feature is a prerequisite for amplification systems suited for the Evalution platform as, in order to maintain the multiplexing capacity, the amplification product should remain attached to the microparticle surface. In a typical HCR, the target initiates a type of toehold-mediated strand displacement resulting in the cross-opening of two metastable DNA hairpins (H1 & H2) that can carry a fluorescent label. This results in the self-assembly of a nicked double-helix construct [3] (Figure 1).

For the miR-16 target, HCR signal amplification of different durations was compared to the reference signal resulting from a single HCR cycle (k=1). In Figure 2A a comparison of the resulting signal intensities for a target concentration of 2.5 nM for different durations of HCR are shown together with the LOD values obtained from calibration curves ranging from 2 to 250 nM and from 0.25 to 250 pM for the reference system and the HCR amplified signal, respectively. Depending on the assay time, not only was the sensitivity increased 10,000-fold, but the bioassay could also be tuned for total assay time and sensitivity, as desired. In addition the multiplex potential was shown by detecting simultaneously two additional microRNA precursor-based targets (miR-18 & miR-30) (Figure 2B). The results at 37°C after 60 minutes of HCR were fitted with a 4-parameter logistic model in order to obtain the corresponding LOD values of 120, 381 and 184 fM for miR-16, miR-18 and miR-30, respectively.

In conclusion, the reported amplification strategy can be directly implemented as a general approach for the sensitive and specific multiplex detection of various target molecules. Moreover, HCR allows the bioassay to be tuned for the desired time and sensitivity. In combination with the high-throughput capacity and reduced reagent consumption, the Evalution demonstrates an immense potential in the next generation of diagnostic tools towards more personalized medicine.