

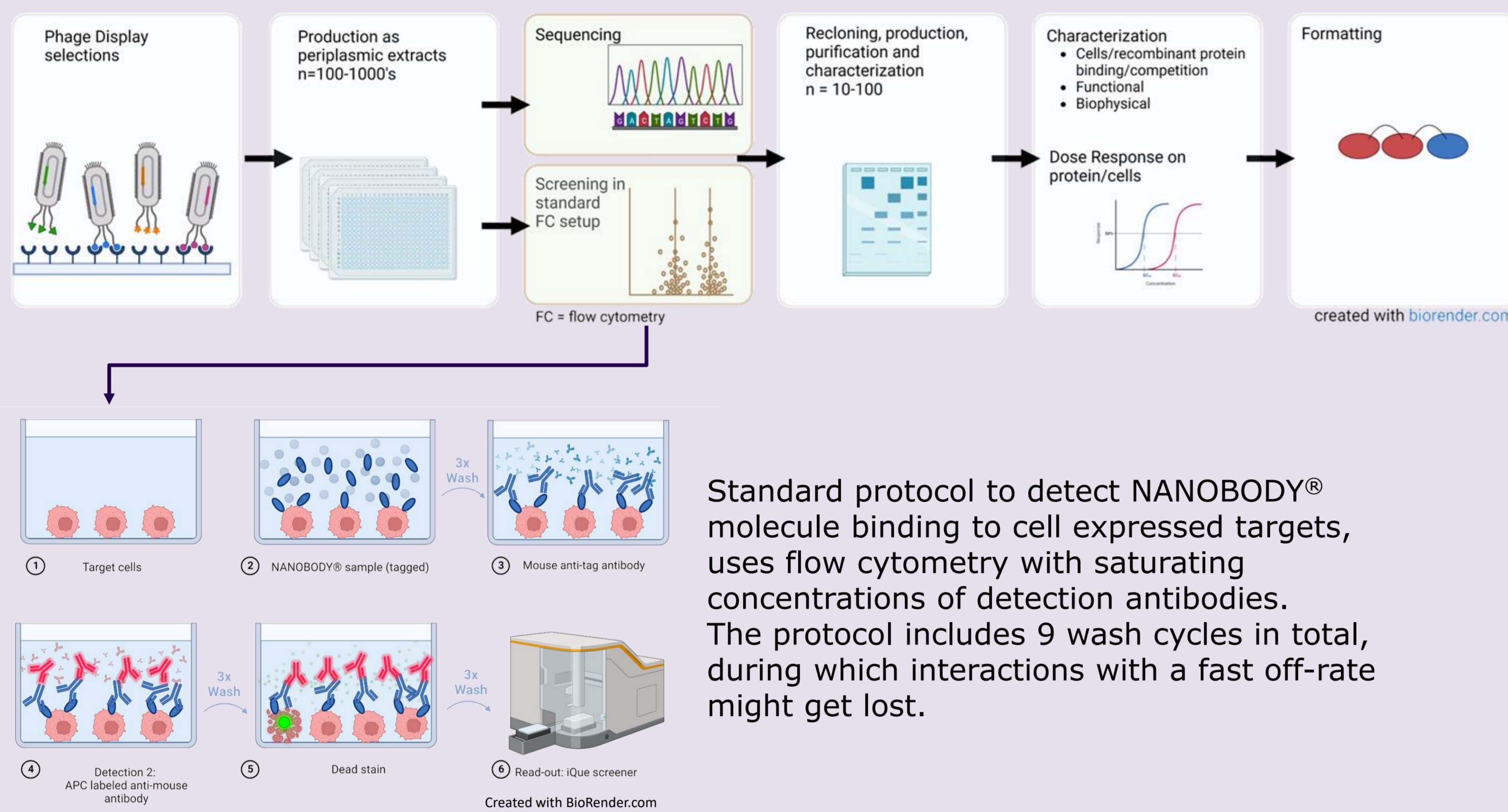
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Introduction

During the discovery of NANOBODY® molecules, thousands of clones are evaluated in screening assays that are optimized to detect high-affinity binding to cell expressed targets. A brief introduction to the conventional NANOBODY® Discovery workflow is depicted in the figure below.

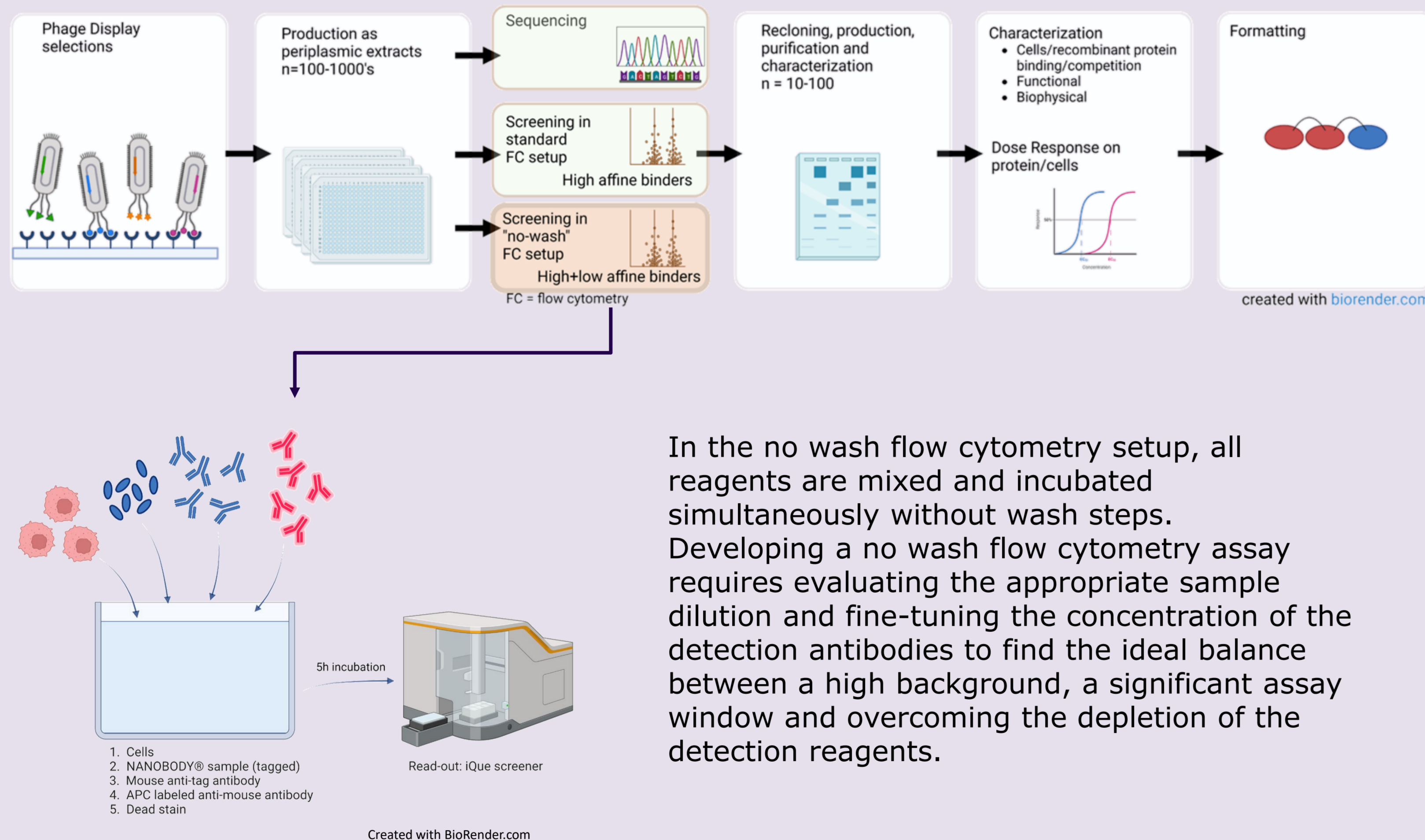
Conventional Workflow (focussed on high affine molecules)



Standard protocol to detect NANOBODY® molecule binding to cell expressed targets, uses flow cytometry with saturating concentrations of detection antibodies. The protocol includes 9 wash cycles in total, during which interactions with a fast off-rate might get lost.

For the optimal design of modalities that can explore new modes of action, there is an increased demand for NANOBODY® molecules with diverse affinities. For projects where no recombinant protein is available or the conformation on the cellular membrane is of high importance, a no wash flow cytometry based high-throughput screening method was developed that also enables the detection of NANOBODY® molecules with a fast off-rate to cell expressed targets. Conducting this method in parallel to the standard protocol, allows the differentiation between NANOBODY® molecules with slow and fast off-rates, which gives an indication of high and low affinities ($KD=K_{on}/K_{off}$).

Alternative workflow (focusses on both low and high affinity molecules)



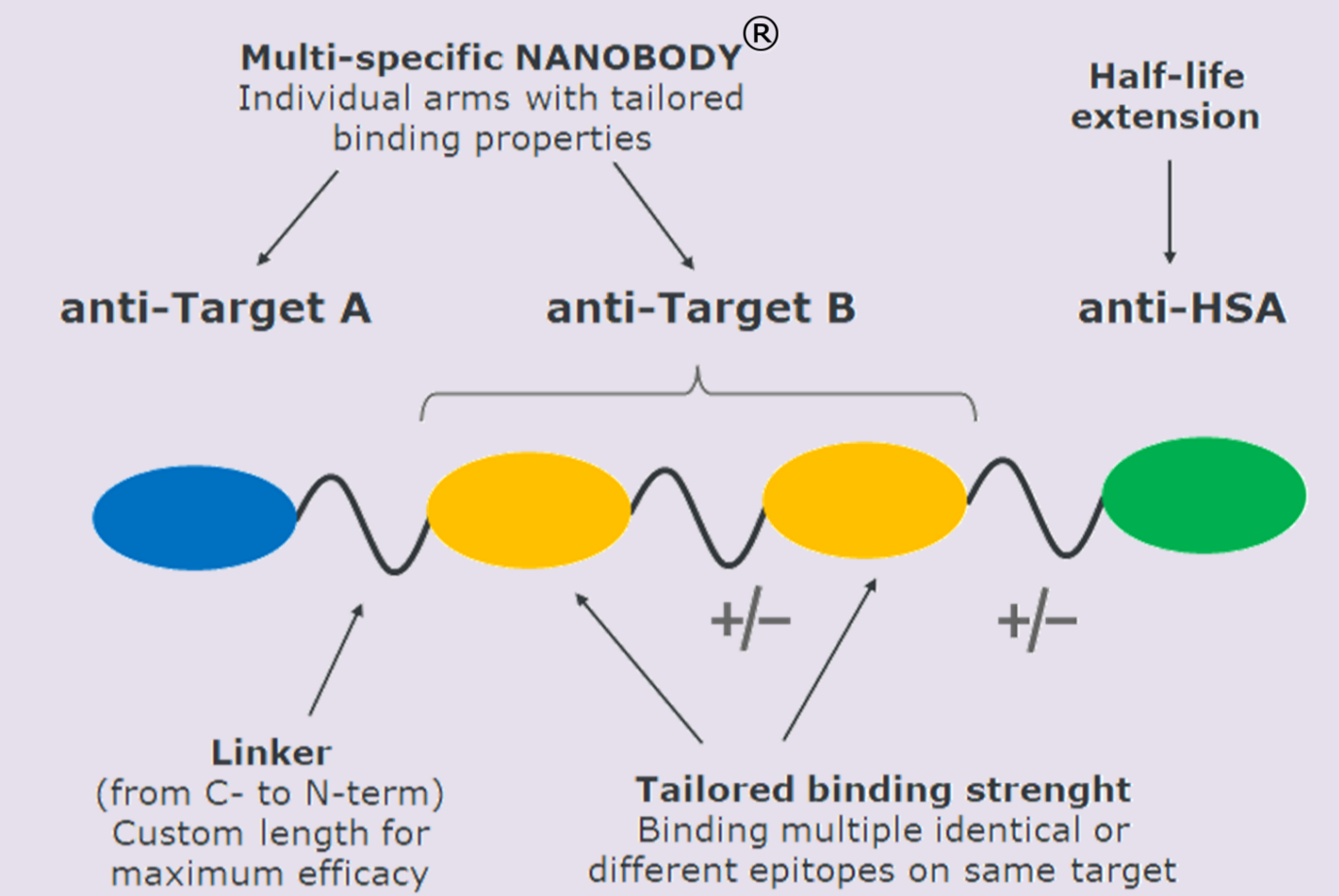
In the no wash flow cytometry setup, all reagents are mixed and incubated simultaneously without wash steps. Developing a no wash flow cytometry assay requires evaluating the appropriate sample dilution and fine-tuning the concentration of the detection antibodies to find the ideal balance between a high background, a significant assay window and overcoming the depletion of the detection reagents.

The no wash flow cytometry setup can qualitatively determine hits with slow and fast off-rates. However, when it is desired to have granularity in the data, the assay should be performed in parallel with the standard wash setup to differentiate between NANOBODY® molecules with a fast and slow off-rate.

NANOBODY® research platform

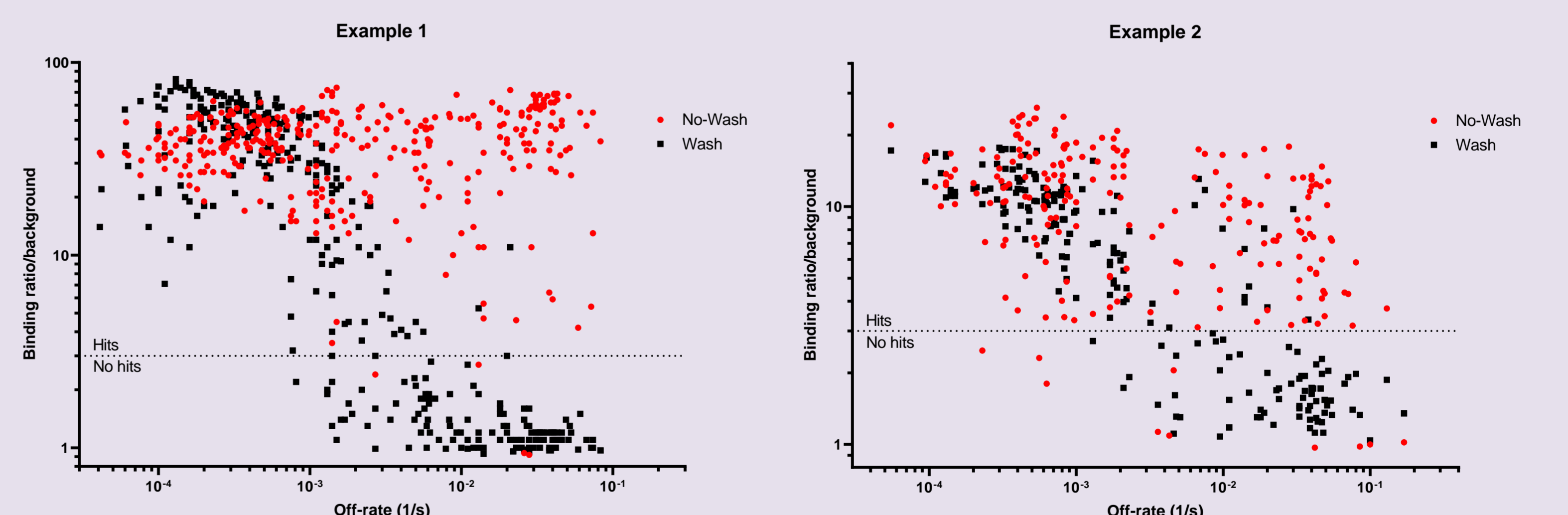
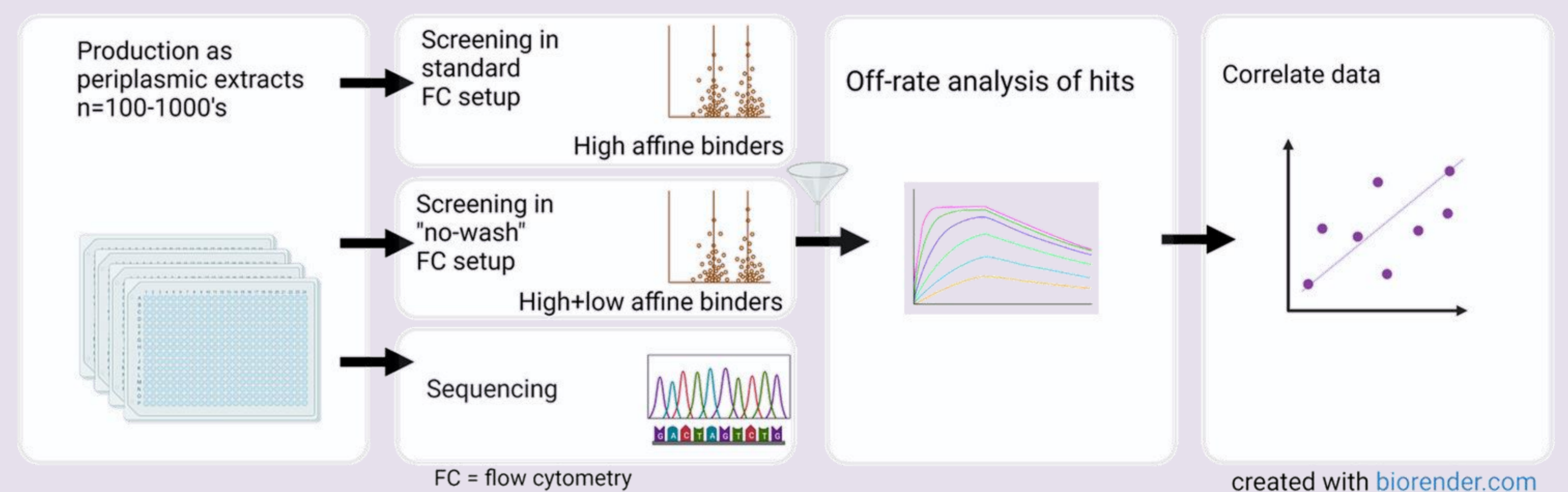
As part of Sanofi R&D the NANOBODY® Research Platform (NRP) is involved in the discovery and development of NANOBODY® molecules against disease relevant targets in various therapeutic areas (including immuno-oncology, inflammation, and rare diseases).

NANOBODY® molecules are derived from "heavy-chain-only" antibodies from llamas and alpacas. They are around a tenth the size of conventional antibodies, easily expressed as single domains and allow flexible formatting to multi-valent/specific/paratopic formats to tailor project specific needs.



Concept validation

This alternative workflow was applied in 2 projects (examples 1 and 2). The projects were interested in finding both low and high affinity NANOBODY® molecules to cell expressed target. The workflow was conducted as described in the introduction. After determining the hits in both wash and no wash assays, off-rates were determined using SPR on recombinant protein, and results were correlated.



Correlation for 2 projects (example 1 and 2) between off-rate (x-axis, measured by SPR) and ratio over background (y-axis, measured by flow cytometry) for the wash (black) and no-wash (red) assay setup

- By applying the alternative workflow, also the NANOBODY® molecules with a fast off-rate ($>1E-02$) were picked up as hits, that would have been lost using the conventional workflow.
- The alternative workflow allows high-throughput screening for binding of NANOBODY® molecules and can differentiate between fast and slow off-rate on cell expressed targets, which can be critical for projects where no recombinant protein is available or where the conformation on the cellular membrane is of high importance.

