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Repeated 100fold Affinity Improvements with AbAccel

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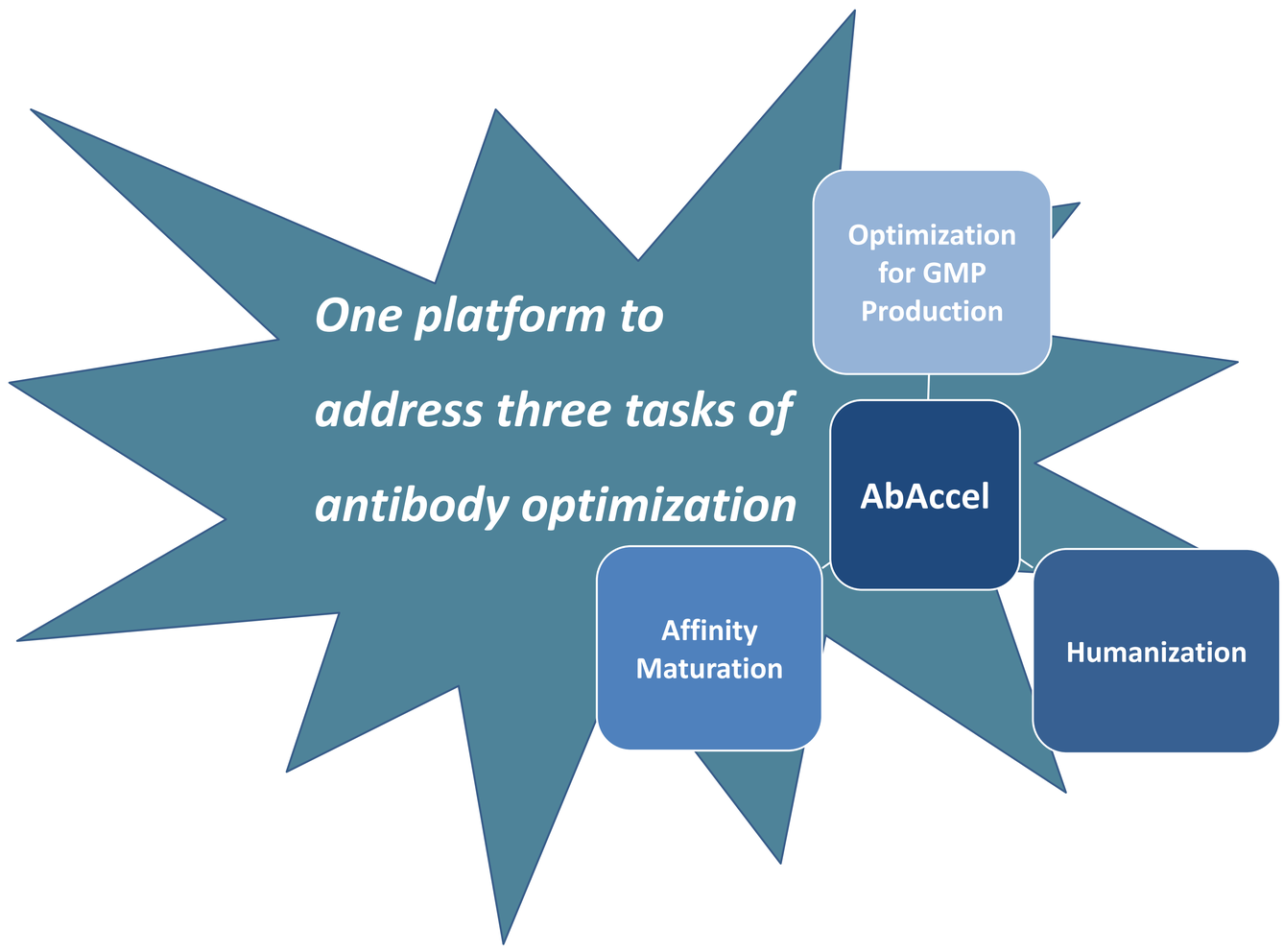
Introduction:

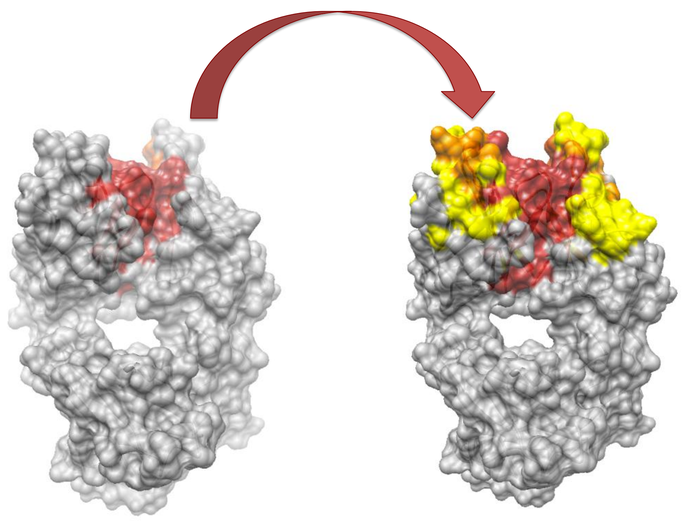
A 100fold affinity improvement with AbAccel was reached in two separate affinity maturation projects with just one round of CDR-directed mutagenesis. Key properties of the parental antibodies, like species cross-reactivity, specificity and stability, were retained.

AbAccel is based on a special developability focused library design. Selected AbAccel frameworks, removal of sequences with biochemical liabilities from the library and a focus on the close to germline sequence space replaces subsequent engineering work.



AbAccel Libraries:

Transfer principle specificity determining elements



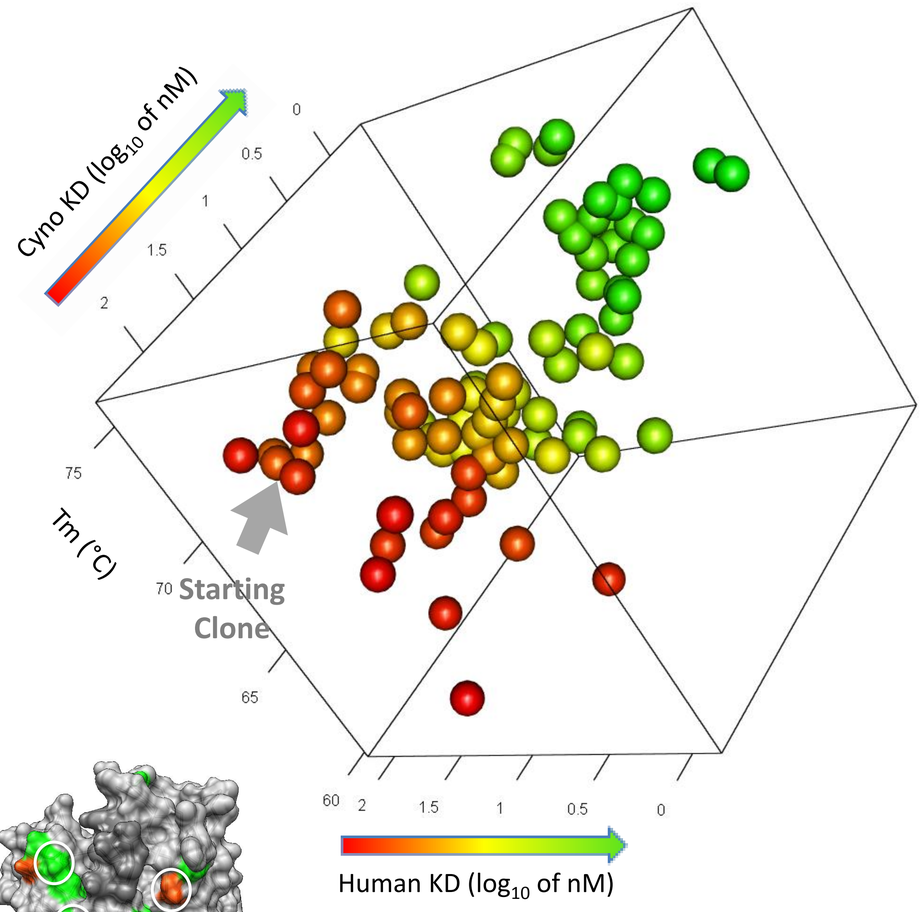
Candidate antibody **Affinity maturation landscape**

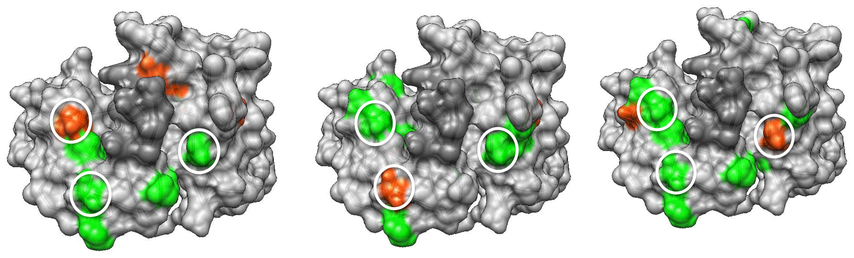
Developability focused library design:

- Mutations are restricted to CDR positions
- Library design excludes biochemical liabilities and T cell epitopes
- Framework-specific defined nature-like distributions of amino acids in ~50 randomized CDR positions calculated from analyzed NGS data
- Library is biased to very few deviations from the germline per sequence

Example 1:

- Two logs improvement of both:
  - Binding to Human antigen
  - Binding to Cyno antigen
- ScFv Tm of 70°C retained during affinity maturation
- Paratope delineation revealed differential influence on Human/Cyno affinity and stability





Stability (Tm) | Anti-human affinity | Anti-cyno affinity

Properties of selected mutants:

Example 2:

- EGFRvIII is a tumor-specific deletion mutant of EGFR
- Selective binding and stability of an EGFRvIII specific antibody was retained after 100fold affinity improvement

	Binding to EGFRvIII	Binding to EGFR wt	Thermal Stability
	K_D (SPR) [nM]	K_D (SPR) [nM]	Tm [°C]
Starting scFv	7, 8	Not detected	64
scFv-improved-A	0,08	Not detected	61,4
scFv-improved-B	0,16	Not detected	65,6

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