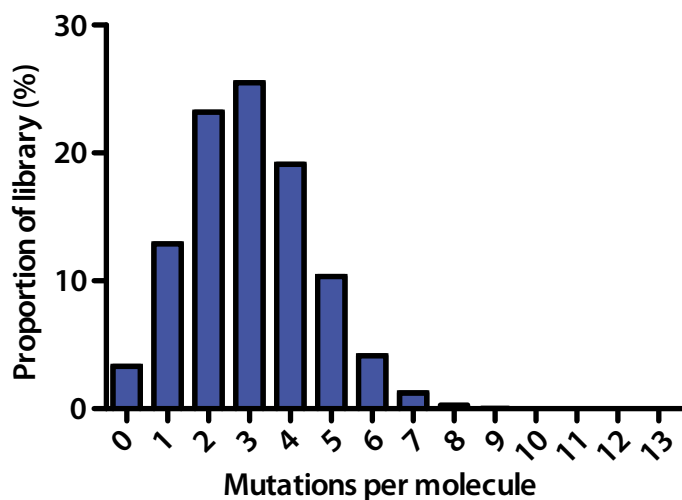# Supporting information 1

A

B

P   = Number of randomized positions
R   = Number of different possible codons in the randomized positions
N   = Mutations per molecule
C   = Number of possible combinations of N mutations per molecule

$$C = R^N \cdot \text{binomial coefficient} \begin{pmatrix} P \\ N \end{pmatrix} = R^N \cdot \frac{P!}{(P-N)! \cdot N!}$$

Combinations of 3 mutations per Affibody molecule with 13 randomized positions

$$C = R^N \cdot \frac{P!}{(P-N)! \cdot N!} = 16^3 \cdot \frac{13!}{(13-3)! \cdot 3!} = 1\,171\,456$$

Proportion of Affibody library containing 3 mutations per molecule (see Online Resource 1a) = 25.5 %

$$\text{Library size to cover all combinations of 3 mutations per molecule} = \frac{1\,171\,456}{0.255} = 4\,593\,945 = 4.6 \cdot 10^6$$

Online Resource 1. Theoretical binomial distribution of mutation frequencies in the library and theoretical calculations on combinatorics and library coverage. a Distribution of mutation frequencies in a library that is randomized in 13 positions with a mutation frequency of 23 % in each position (3/13), assuming that the incorporation of codons follow a binomial distribution. **b** Theoretical calculation of the number of combinations of 3 mutations per molecule in a library design that is intended to randomize 13 positions with 16 different codons and a mutation frequency of 23 % in each position (3/13).