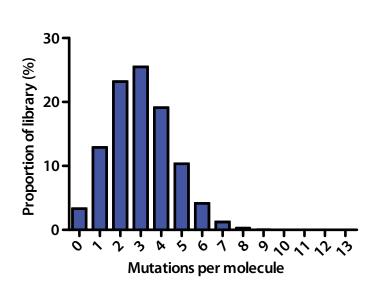
Inhibiting HER3-mediated tumor cell growth with Affibody molecules engineered to low picomolar affinity by position-directed error-prone PCR-like diversification; PLoS One; Magdalena Malm, Nina Kronqvist, Hanna Lindberg, Lindvi Gudmundsdotter, Tarek Bass, Fredrik Y Frejd, Ingmarie Höidén-Guthenberg, Zohreh Varasteh, Anna Orlova, Vladimir Tolmachev, Stefan Ståhl and John Löfblom KTH Royal Institute of Technology, Stockholm, Sweden. E-mail: lofblom@kth.se

Supporting information 1







- 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

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

Combinations of 3 mutations per Affibody molecule with 13 randomized positions

$$C = R^{N} \cdot \frac{P!}{(P - N)! \cdot N!} = 16^{3} \cdot \frac{13!}{(13 - 3)! \cdot 3!} = 1171456$$

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

Library size to cover all combinations of 3 mutations per molecule = $\frac{1171456}{0.255}$ = 4593945 = 4.6 \cdot 10⁶

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).