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Robust in vitro affinity maturation strategy based on interface-focused high-throughput mutational scanning

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Abstract
Development of protein therapeutics or biosensors often requires in vitro affinity maturation. Here we report a robust affinity engineering strategy using a custom designed library. The strategy consists of two steps beginning with identification of beneficial single amino acid substitutions then combination. A high quality combinatorial library specifically customized to a given binding-interface can be rapidly designed by high-throughput mutational scanning of single substitution scanning libraries. When applied to the optimization of a model antibody Fab fragment, the strategy created a diverse panel of high affinity variants. The most potent variant achieved a 2110-fold affinity improvement to an equilibrium dissociation constant (Kd) of 3.45 pM with only 7 amino acid substitutions. The method should facilitate affinity engineering of a wide variety of protein-protein interactions due to its context-dependent library design strategy.

[1] Fujino,Y., Fujita, R., Wada, K., Fujishige, K., Kanamori, T., Hunt, L., Yoshihiro, S., Takuya, U., Robust in vitro affinity maturation strategy based on interface-focused high-throughput mutational scanning. Biochem. Biophys. Res. Commun. 428:395-400, 2012