PROTEIN ENGINEERING

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SUMMARY & MISSION STATEMENT

We use protein engineering to develop new protein therapeutics especially for cancer, with an emphasis of solving problems that cannot be addressed by more traditional approaches. We exploit the interdisciplinary nature of our laboratory, bridging protein design, structural biology, protein biochemistry, cell signaling and animal work.

OVERVIEW

We have developed the ability to rapidly generate highly specific binding molecules to essentially any target, using selection and directed evolution from synthetic libraries of Designed Ankyrin Repeat Proteins. The ease of engineering many multispecific formats, tunable half-lives, or chemical derivatization, greatly extends what can be achieved with traditional antibody formats, leading to, e.g., selective induction of apoptosis by several mechanisms as well as other new modes of action. DARPins are easy to produce, extremely robust and have shown good tolerability in clinical trials. Such projects are carried out from the protein design stage to in vivo efficacy testing.

We have also developed adenovirus as a platform, in which the virus carries no viral genes but genes for multiple secreted therapeutic payloads, in order to produce therapeutics *in situ* in the tumor. The constructed viruses are shielded from the cellular and humoral immune system by an engineered protein shield and equipped with receptor-specific uptake mechanisms. This strategy allows us to simultaneously deliver multiple genes *in vivo*, for, e.g., producing therapeutic antibodies for checkpoint blockade, receptor inhibition, or producing cytokines, at the location where they are needed.

SELECTED CANCER RELATED PUBLICATIONS

Adenoviral vector with shield and adapter increases tumor specificity and escapes liver and immune control. Schmid, M., Ernst, P., Honegger, A., Suomalainen, M., Zimmermann, M., Braun, L., Stauffer, S., Thom, C., Dreier, B., Eibauer, M., Kipar, A., Vogel, V., Greber, U. F., Medalia, O., and Plückthun, A. **Nature Comm.** 2018; 9: 450.

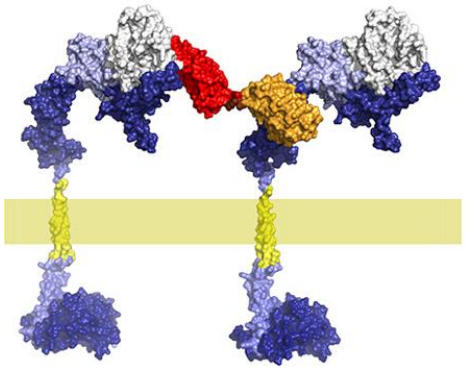
Intermolecular biparatopic trapping of ErbB2 prevents compensatory activation of PI3K/AKT via RAS–p110 crosstalk. Tamaskovic, R., Schwill, M., Nagy-Davidescu, G., Jost, C., Schaefer, D. C., Verdurmen, W. P. R., Schaefer, J. V., Honegger, A., and Plückthun, A. **Nature Comm.** 2016; 7: 11672.

Designed ankyrin repeat proteins (DARPins): binding proteins for research, diagnostics, and therapy. Plückthun, A. **Annu. Rev. Pharmacol. Toxicol.** 2015; 55: 489-511.

Structural basis for eliciting a cytotoxic effect in HER2-overexpressing cancer cells via binding to the extracellular domain of HER2. Jost, C., Schilling, J., Tamaskovic, R., Schwill, M., Honegger, A., and Plückthun, A. **Structure** 2013; 21: 1979-1991.

Development of a generic adenovirus delivery system based on structure-guided design of bispecific trimeric DARPin adapters. Dreier, B., Honegger, A., Hess, C., Nagy-Davidescu, G., Mittl, P. R., Grütter, M. G., Belousova, N., Mikheeva, G., Krasnykh, V., and Plückthun, A. **Proc. Natl. Acad. Sci. U. S. A.** 2013; 110: E869-877.

Efficient tumor targeting with high-affinity designed ankyrin repeat proteins: effects of affinity and molecular size. Zahnd, C., Kawe, M., Stumpp, M. T., de Pasquale, C., Tamaskovic, R., Nagy-Davidescu, G., Dreier, B., Schibli, R., Binz, H. K., Waibel, R., and Plückthun, A. **Cancer Res.** 2010; 70: 1595-1605.



Biparatopic DARPins have been designed that bend and link the HER2 receptor in an inactive conformation, completely uncoupling it from the intracellular signaling machinery, and rendering the kinase domains inactive. This is necessary and sufficient to drive HER2-addicted tumors to apoptosis in vivo, eliciting a much stronger response than, e.g. trastuzumab and pertuzumab. In the construct shown, the red and orange DARPins must be linked in exactly this manner to arrive at such a strong apoptotic response. This example illustrates the power of protein engineering in arriving at molecules with highly selective yet powerful biological responses.