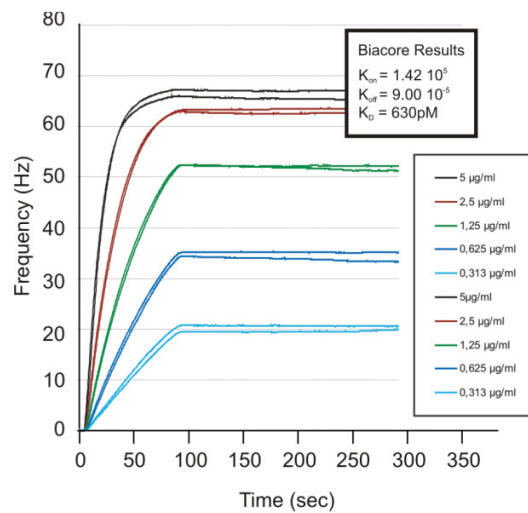
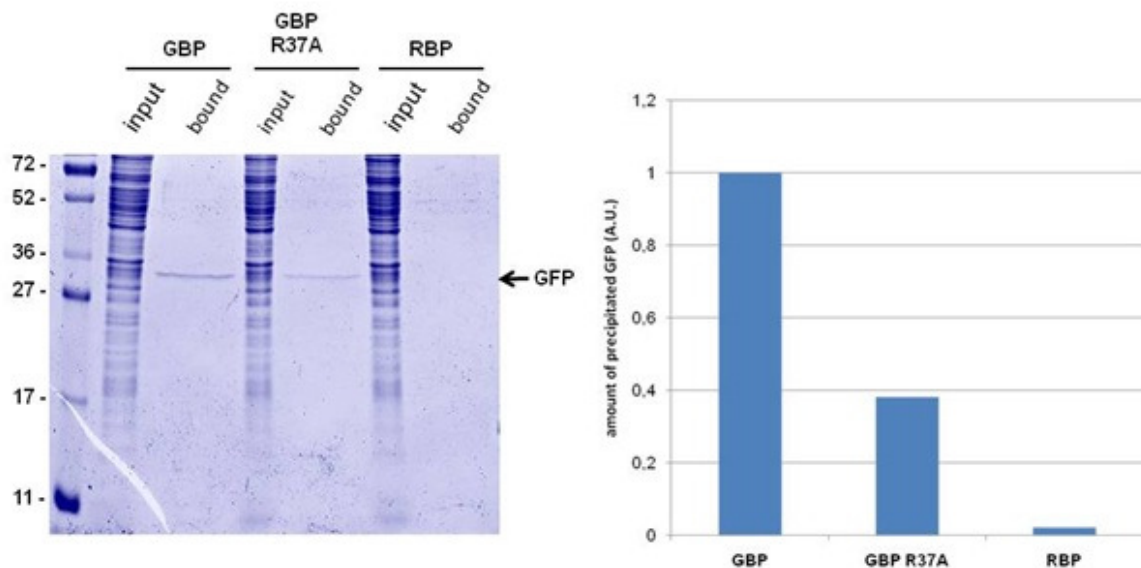


Supplementary Figures



Supplementary Figure S1 Determination of the GFP binding affinity of the GFP-binder. GFP-Binder was immobilized on an Attana-Carboxyl Chip by amine-coupling. Binding kinetics were determined by injection of increasing concentrations of GFP (0.313 µg, 0.625 µg, 1.25 µg, 2.5 µg and 5 µg).



Supplementary Figure S2 *Left panel:* Immunoprecipitation of GFP. Comparison of GBP with GBP R37A and RBP (red fluorescent protein). Protein extracts of GFP producing HEK 293T cells were subjected to immunoprecipitation with GBP, GBP R37A or RBP. Aliquots of input and bound fraction were separated by SDS-PAGE and visualized by Coomassie Blue. Precipitated GFP is marked by an arrows. *Right panel:* Quantification of GFP precipitated with GBP, GBP R37A and RBP. Relative amounts were determined by densitometry of Coomassie Blue stained gels (blue bars) using ImageJ software (Version 1.34, <http://rsb.info.nih.gov/ij/>). For comparison amount of precipitated GFP by GBP was set to 1.

Supplementary Methods

Control experiments for GFP binding

For reference affinity measurements purified GFP-binding protein (GFP-binder) was immobilized on an Attana Carboxyl Chip by amine-coupling according to manufacturer's instructions. Binding kinetic of GFP to the immobilized GFP-binder were performed on an Attana A 100 Quartz Crystal Microbalance (Attana AB, Sweden) by injection of 5 different concentrations of GFP (0.313 µg, 0.625 µg, 1.25 µg, 2.5 µg and 5 µg, two times replicates). The binding kinetics were evaluated with the ClampXP software (Attana AB, Sweden) which resulted in a Kon rate of 1.42×10^5 [1/Ms] and a Koff rate of 9.00×10^{-5} [1/Ms]. Taken together this measurement lead to a dissociation constant K_D of 0.63 nM.

Binding studies of a mutant version of the GFP-binding protein (GBP R37A)

Recently the crystal structure of the GBP-GFP complex was resolved (38). Based on the crystal structure a contact side between an arginin residue of the GBP at position 37 (Arg37) and a glutamat residue of GFP at position 142 (Glu142) was determined. To evaluate the impact of this contact side for the binding affinity of the GBP we generated a mutated version of the GBP by replacing the Arg37 at position 37 with an Ala (GBP R37A).

In a first experiment the GBP R37A was expressed and purified from *E.coli* and coupled to an immobilized matrix as described previously(39). Subsequently immunoprecipitation analysis comparing GBP and the mutated version GBP R37A was performed. Therefore 1×10^7 HEK293T cells transiently transfected with an expression vector coding for eGFP were treated as described (39). After cell lysis equal amounts of the soluble supernatant were either subjected to GBP- or GBP R37A- or RBP- (red fluorescent binding protein used as a negative control) sepharose beads and incubated for 30 min at room temperature. Subsequently beads were sedimented by centrifugation, washed two times in 1 ml of dilution buffer containing 300 mM NaCl. After the last washing step the beads were resuspended in 2x SDS-containing sample buffer and boiled for 10 min at 95°C. Precipitated proteins were separated by SDS-PAGE and visualized by Coomassie Blue (supplementary Figure S2, left panel)

The quantitative comparison of precipitated GFP by the GBP in relation to GBP R37A showed that GBP has a ~ 2.5 fold higher efficiency whereas no binding was detectable with the RBP as negative control (supplementary Figure S2, right panel).

Supplementary References

38. Kirchhofer, A. et al. Modulation of protein properties in living cells using nanobodies. *Nat. Struct. Mol. Biol.* **17**, 133-138 (2010)
39. Rothbauer, U. et al. A versatile nanotrap for biochemical and functional studies with fluorescent fusion proteins. *Mol. Cell. Proteomics* **7**, 282–289 (2008)