Electronic Supplementary Material (ESI) for Analytical Methods.

Green fluorescent protein-based assays for high-throughput functional characterization and ligand-binding studies of biotin protein ligase

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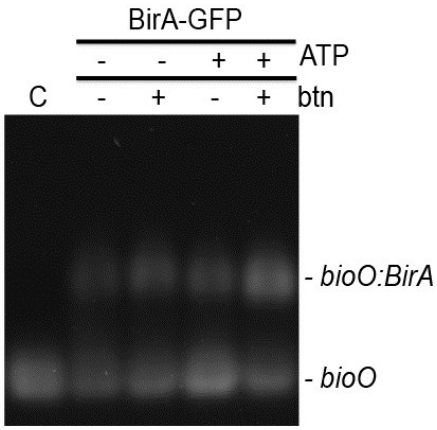
SUPPLEMENTARY TABLES AND FIGURES

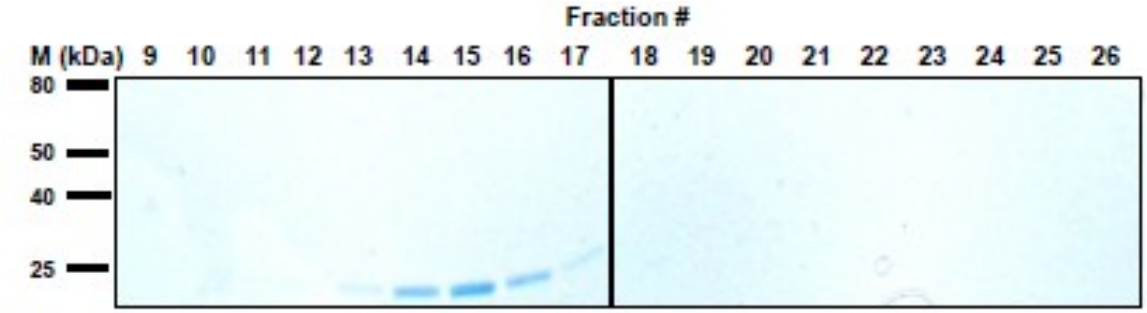
Supplementary Table S1: Determination of K_{obs} by linear regression of ΔT_m *vs* log[ligand]

	biotin	**biotin/ATP**	biotin/**ATP**
Best fit x-intercept	-6.636	-11.48	-11.13
R square	0.9964	0.9849	0.9638
Low range x-intercept*	-6.71	-11.97	-11.82
High range x-intercept*	-6.566	-11.05	-10.55
K_{obs} best fit (M)	2.31E-07	3.31E-12	7.41E-12
K_{obs} low range* (M)	1.95E-07	1.07E-12	1.51E-12
K_{obs} high range* (M)	2.72E-07	8.91E-12	2.82E-11

* range 95% confidence interval



Supplementary Figure S1: *bioO*-binding activity of BirA. All reactions were performed identically and represented as for BirA-GFP (cf Figure 2A).



Supplementary Figure S2: Identification of BirA proteins in the presence of *bioO*, biotin, ATP and $MgCl_2$ following SEC. Fraction numbers correspond to elution volumes (ml) of Figure 2B.