Supplementary Information for

# *Epac-Inhibitors: Facts and Artefacts*

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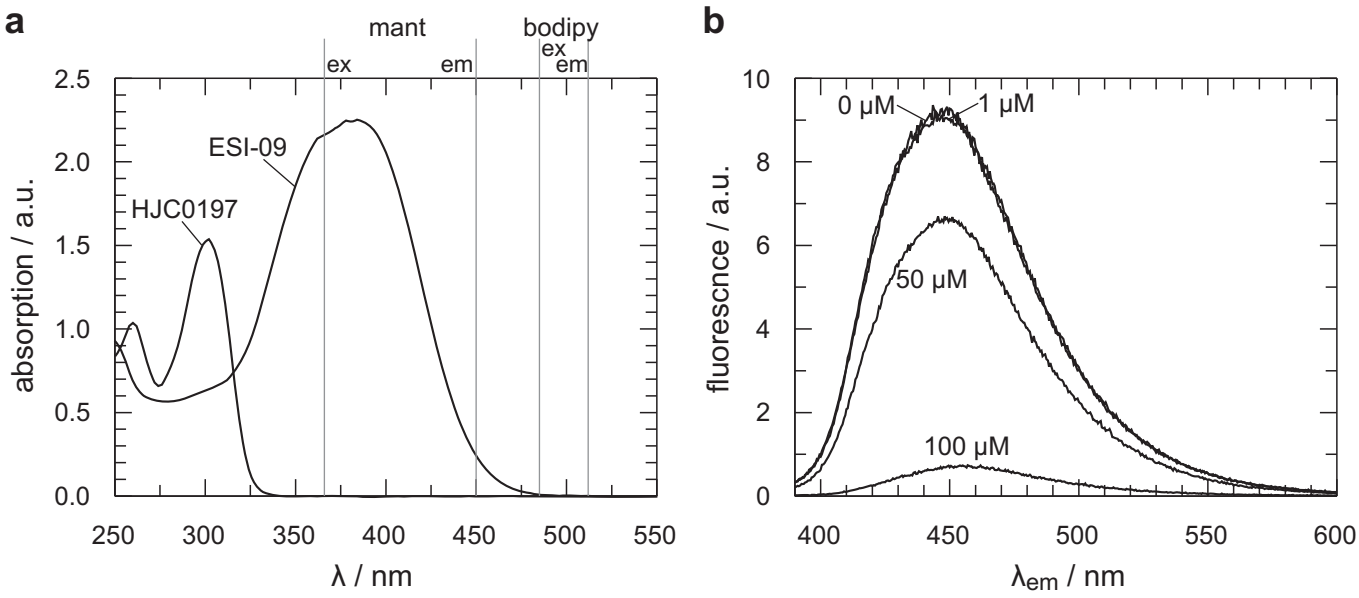
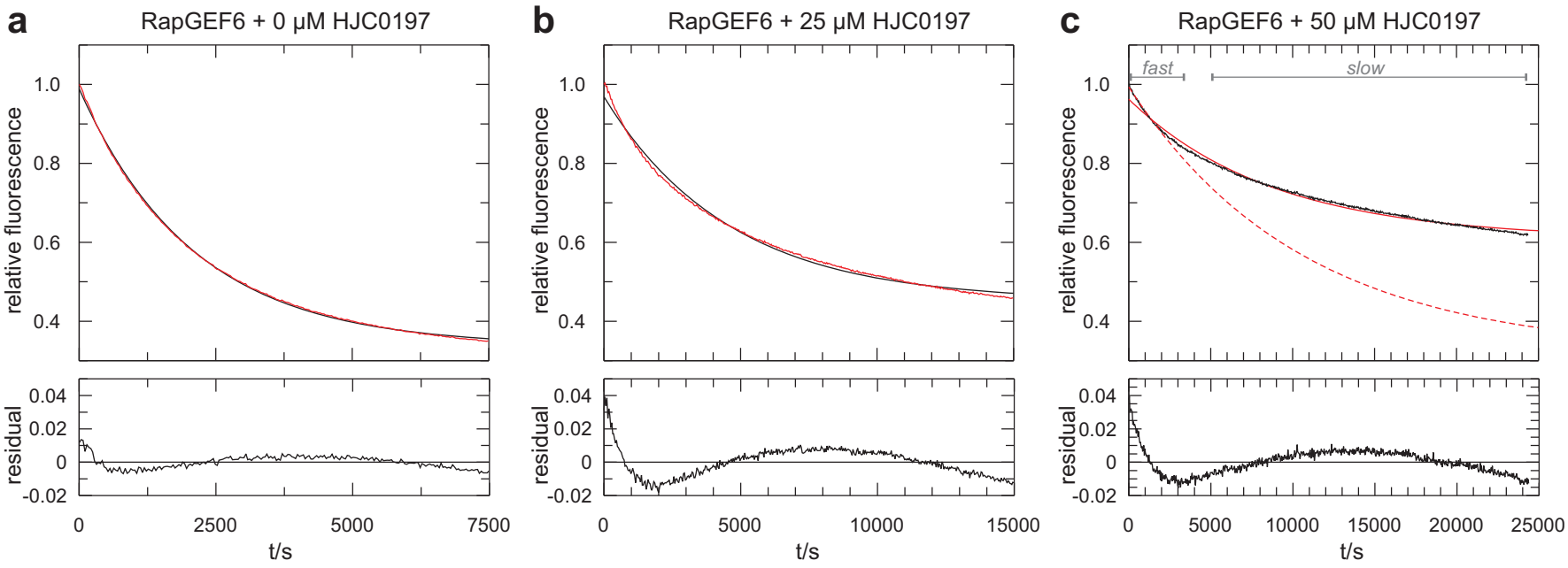
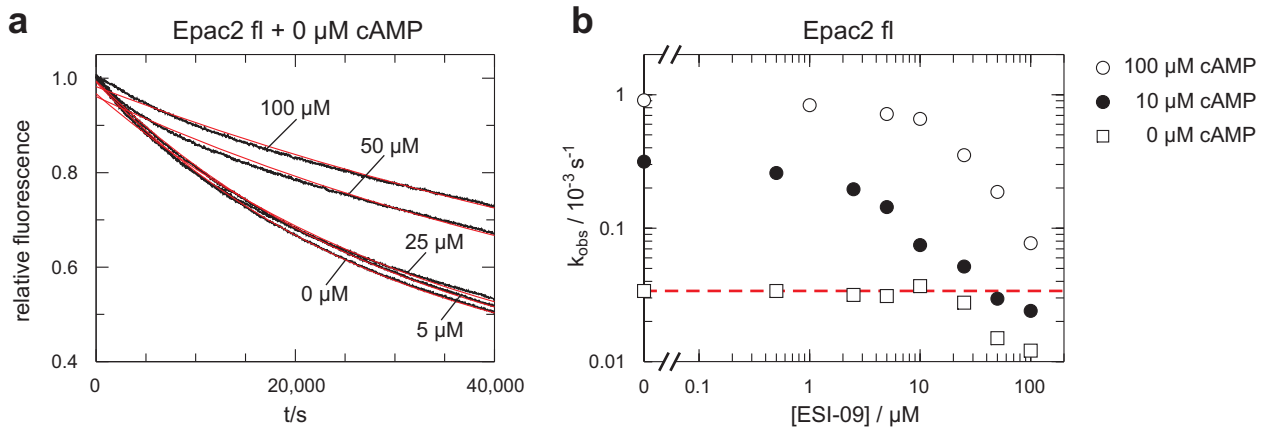


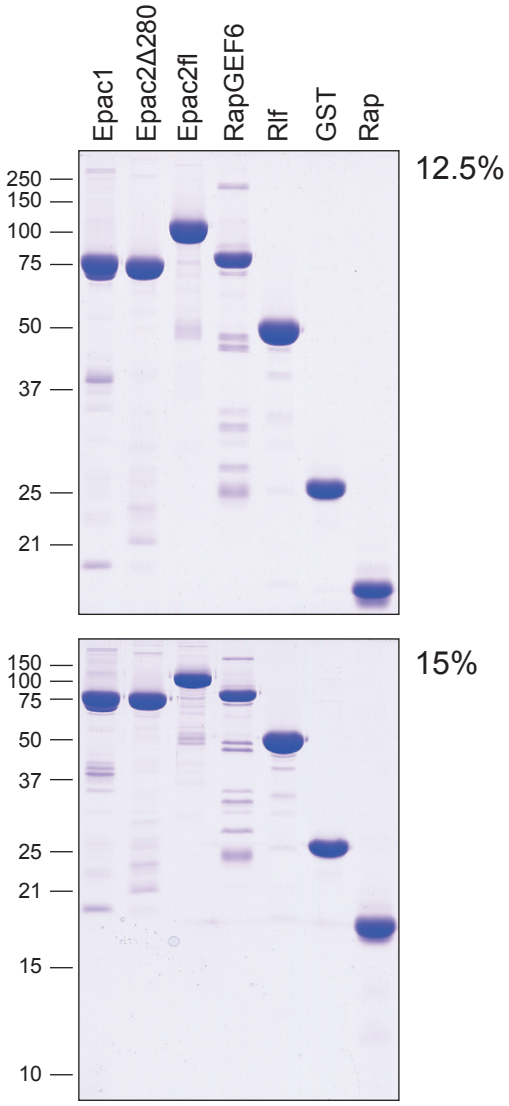

**Supplementary Figure 1** | (a) Absorption spectra of 100 µM ESI-09 and HJC0197 in 50 mM Tris-HCl, pH 7.5. The grey lines indicate the optimal excitation and emission wavelengths to record fluorescence of mGDP ($\lambda_{ex}$ = 366 nm; $\lambda_{em}$ = 450 nm) and GDP-bodipy ($\lambda_{ex}$ = 485 nm; $\lambda_{em}$ = 512 nm). (b) Fluorescence emission spectra of mGDP in 50 mM Tris-HCl, pH 7.5 upon excitation at 366 nm in the precense of the indicated concentrations of ESI-09.




**Supplementary Figure 2 |** Nucleotide exchange activity of RapGEF6 in the presence of 0 µM HJC0197 (a) 25 µM HJC0197 (b) and 50 µM HJC0197 (c). Data are taken from Fig. 2f and fitted as single exponential decay with offset (red) line. The residuals, defined as the difference between measured and calculated value, are shown underneath the graphs. Whereas the activity of RapGEF6 in the absence of HJC0197 in (a) is in good agreement with the fit, systematic deviations are observed in the presence of HJC0197 in (b) and (c). As indicated in (c) a phase of fast decay is followed by a phase of slow decay. The initial fast decay is denoted by the dashed line. Note, nucleotide exchange is not complete at the end of the reactions in (b) and (c), which is evident from the higher offset compared to (a). If extrapolated, the fit is not reaching the expected “real” offset, as it tries to “compensate” for the biphasic character of the data.




**Supplementary Figure 3 |** Nucleotide exchange activity of Epac2$^{fl}$ in the presence of ESI-09. (a) Nucleotide exchange activity of Epac2$^{fl}$ in the absence of cAMP and in the presence of various concentrations of ESI-09 as indicated. The data are fitted as single exponential decay with off-set (red lines) to obtain the rate constants $k_{obs}$. Note, at higher concentrations of ESI-09 deviations of the measured data from the fit are observed. (b) $k_{obs}$ of Epac2$^{fl}$ catalysed exchange activity in the presence of 100 µM cAMP (open circles), 10 µM cAMP (filled circles) or 0 µM cAMP (open squares) were plotted against the concentration of ESI-09. For the determination of $k_{obs}$ note, with increasing concentrations of ESI-09 the experimental data systematically deviated from the fit (see also (a)). $k_{obs}$ values were plotted irrespective of this. The red dotted line corresponds to the exchange activity in the absence of cAMP and ESI-09.




**Supplementary Figure 4** | Analysis of recombinant proteins used in this study. 4 µg of purified protein were loaded to a 12.5 % (upper panel) or to a 15 % (lower panel) SDS-PAGE. Gels were stained with Coomassie Brillant Blue.