**Chemistry & Biology, Volume *22***

## Supplemental Information

# Cobalt(III) Protoporphyrin Activates the DGCR8 Protein and Can Compensate microRNA Processing Deficiency

**Ian Barr, Sara H. Weitz, Talia Atkin, PeiKen Hsu, Maria Karayiorgou, Joseph A. Gogos, Shimon Weiss, and Feng Guo**

**Supplemental Data**

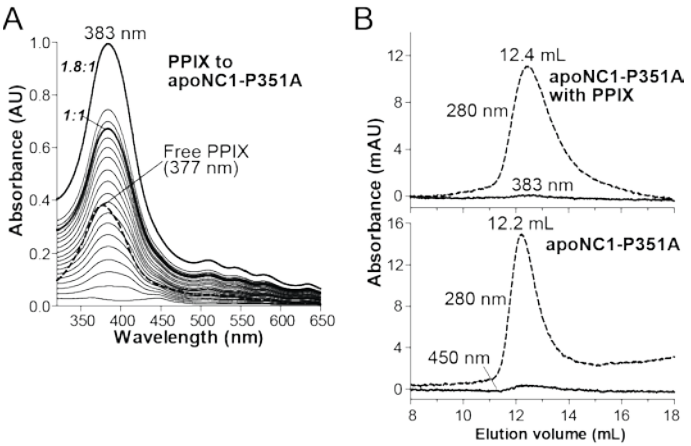


**Figure S1, related to Figure 2.** PPIX does not stably bind to apoNC1-P351A.
(A) Electronic absorption spectra of titration of PPIX into 7.5 μM apoNC1-P351A dimer at 0.5 μM intervals. After the 8.5 μM step, the PPIX concentration was increased directly to 13.5 μM. Select molar ratios of PPIX and apoNC1-P351A are indicated in bold italic. The spectrum of 5.0 μM protein-free PPIX is shown in dash. (B) SEC analyses of 4 μM apoNC1-P351A and its 1:1 molar mixture with PPIX.

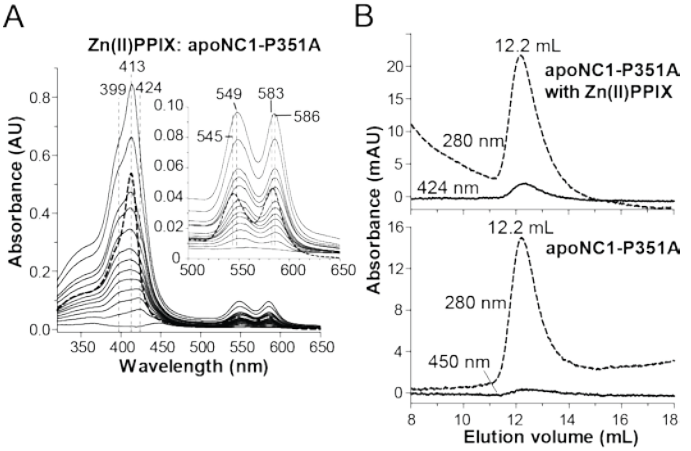


**Figure S2, related to Figure 3.** Zn(II)PPIX weakly binds apoNC1-P351A.
(A) Titration of Zn(II)PPIX into 4 μM apoNC1-P351A. The Zn(II)PPIX concentrations are 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 5.5, 6.5, 8.5, and 10.5 μM. The spectrum of 5 μM free Zn(II)PPIX is shown in dash, with a Söret peak at 413 nm. Early during the titration, a broad slanted peak with a maximum at 424 nm appeared. As the Zn(II)PPIX concentration increased further, an additional Söret peak at ~399 nm gradually rose to approximately even height as $A_{424}$. As Zn(II)PPIX became excess, a 413-nm peak started to accumulate and eventually became dominant. In the titration, the α/β bands red-shifted from 545 and 583 nm to 549 and 586 nm, respectively, but did not show any noticeable transitions at 1:1 stoichiometry.
(B) SEC analyses of 4 μM apoNC1-P351A and its 1:1 molar mixtures with Zn(II)PPIX. apoNC1-P351A eluted with a low level of 424-nm absorption, indicating that its complex with Zn(II)PPIX is unstable.

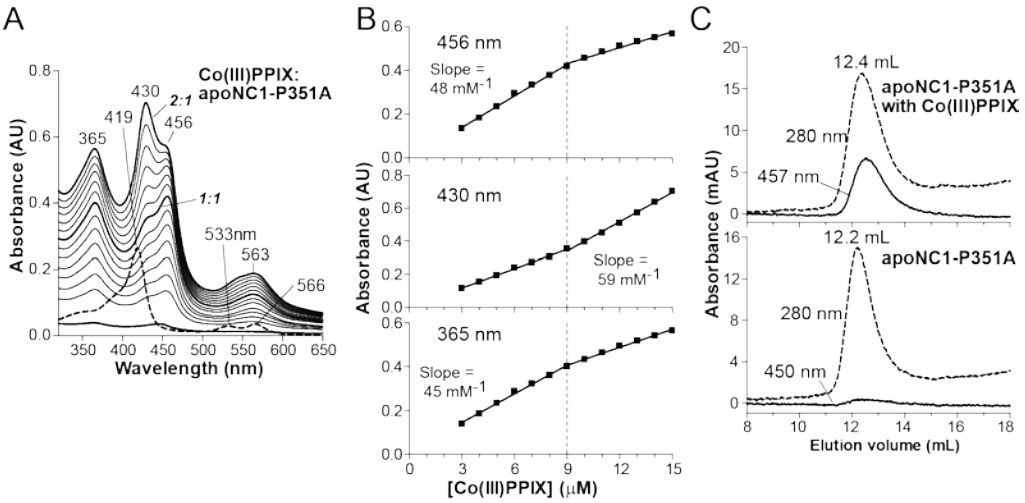


**Figure S3, related to Figure 3.** Co(III)PPIX specifically and stably binds apoNC1-P351A. (A) Titration of Co(III)PPIX into 7.5 μM apoNC1-P351A at 1.0 μM intervals. (B) Absorbance at peak wavelengths are plotted against Co(III)PPIX concentration. (C) SEC analyses of 4 μM apoNC1-P351A and its 1:1 molar mixtures with Co(III)PPIX.

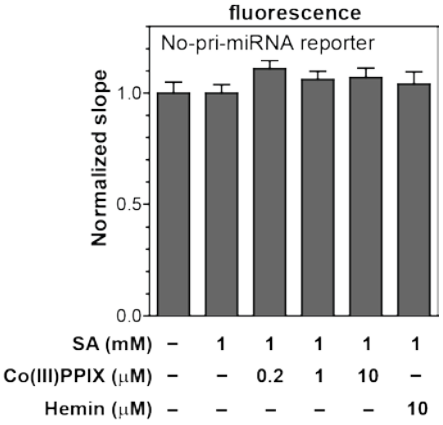


**Figure S4, related to Figure 5.** A reporter containing no pri-miRNA sequences does not respond to Co(III)PPIX or hemin treatment. Similar to the experiments in Figure 5A, Hela cells were cultured in heme-depleted media, transfected with the no-pri-miRNA reporter, treated for 10 h with succinylacetone (1 mM) either alone or together with Co(III)PPIX or hemin at the indicated concentrations. Shown are normalized eYFP/mCherry fluorescence slopes (± 95% CI).

**Table S1, related to Figure 3.** Extinction coefficients of metalloporphyrins used in this study.

| Porphyrin | Solvent | λ (nm) | ε ($mM^{-1}cm^{-1}$) | Reference |
|---|---|---|---|---|
| Co(III)PPIX | 0.1 M Tris-Acetate pH 7.9 | 416 | 93 | (Ozols and Strittmatter, 1964) |
| Ni(II)PPIX | 0.1 M Tris-Acetate pH 7.9 | 385 | 52 | |
| Cu(III)PPIX | 0.1 M Tris-Acetate pH 7.9 | 388 | 64 | |
| Zn(II)PPIX | NaOH (aq.) | 412 | 87.4 | (Leonard, et al., 1974) |
| MnPPIX | 0.1 M NaOH | 462 | 25 | (Yonetani and Asakura, 1968) |
| Sn(III)PPIX | 0.5% Pyridine, $NH_4OH$ | 406 | 164 | (Rish, et al., 2007) |
| Ga(III)PPIX | DMSO | 413 | 249 | |
| Fe(III)PPIX | 0.1 M NaOH | 385 | 58.44 | (Dawson, et al., 1986)<br>(Collier, et al., 1979) |

## Supplemental References


Collier, G.S., Pratt, J.M., De Wet, C.R., and Tshabalala, C.F. (1979). Studies on haemin in dimethyl sulphoxide/water mixtures. Biochem. J. *179*, 281-289.

Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M. (1986). Data for Biochemical Research (Oxford University Press).

Leonard, J.J., Yonetani, T., and Callis, J.B. (1974). A fluorescence study of hybrid hemoglobins containing free base and zinc protoporphyrin IX. Biochemistry *13*, 1460-1464.

Ozols, J., and Strittmatter, P. (1964). The Interaction of Porphyrins and Metalloporhyrins with Apocytochrome Beta-5. J Biol Chem *239*, 1018-1023.

Rish, K.R., Swartzlander, R., Sadikot, T.N., Berridge, M.V., and Smith, A. (2007). Interaction of heme and heme-hemopexin with an extracellular oxidant system used to measure cell growth-associated plasma membrane electron transport. Biochim. Biophys. Acta *1767*, 1107-1117.

Yonetani, T., and Asakura, T. (1968). Studies on cytochrome c peroxidase. XI. A crystalline enzyme reconstituted from apoenzyme and manganese protoporphyrin IX. J Biol Chem *243*, 3996-3998.