Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information

A metal carbonyl-protein needle composite designed for intracellular CO delivery to modulate NF-κB activity

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Materials. Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. DMEM (Sigma, D5796) containing 5% FBS, 1.8 mM L-glutamine, 0.9 mM sodium pyruvate, 0.1% sodium bicarbonate, 1% MEM non-essential amino acid solution (Sigma, M7145), 90 U/mL penicillin and 90 μg/mL streptomycin was used as a cell culture medium. PBS tablets (Takara Bio, T900) were used to make 10 mM PBS pH 7.4. Expression and purification of β-PN was performed as described previously.[1] RuCO·apo-E45C/C48A-rHLFr was prepared as described previously,[2] CORM-3 and $Ru(DMSO)_4Cl_2$ were synthesized as described previously.[3,4] CORM-3 was dissolved in distilled water and used immediately to each experiment. Sperm whale myoglobin was expressed and purified for myoglobin (Mb) assay according to the procedure.[5] COP-1 was synthesized according to the reported procedure.[6] HEK293/κB-Fluc cells were isolated as previously descried.[2] Amino acid sequences of monomers of β-PN and β-PN(ΔHis-tag) are GSMAISDPNSSSVPPLEGNGTILVKGNVTIIVEGNADITVKGDATTLVEGNQTNT VNGNLSWKVAGTVDWDVGGDWTEKMASMSSISSGQYTIDGSRIDIGSVEGYIP EAPRDGQAYVRKDGEWVLLSTFLVEHHHHHH and GSHMLEGNGTILVKGNVTIIVEGNADITVKGDATTLVEGNQTNTVNGNLSWKV AGTVDWDVGGDWTEKMASMSSISSGQYTIDGSRIDIGSVEGYIPEAPRDGQAY VRKDGEWVLLSTFLVE, respectively.

Physical measurement. UV-vis spectra were recorded on a JASCO V-670 spectrometer. MALDI-TOF mass spectra were recorded on an Autoflex III mass spectrometer (Bruker Daltonics). ATR-IR measurements were conducted using a

FT-IR4200 instrument (JASCO). ICP-MS measurements were conducted using an Elan DRC-e instrument (PerkinElmer). Luminescence measurements for NF-κB reporter assay were conducted using a kit (Promega, E1500) and a Modulus Single Tube Luminometer (Turner BioSystems). Luminescence measurements for ROS assay were conducted using a 96-well plate reader (GloMax-Multi + Detection System). An MTT assay was conducted using model 680 Microplate Reader (BIO-RAD). Dynamic light scattering measurements were performed using a Zetasizer μV system (Malvern, UK).

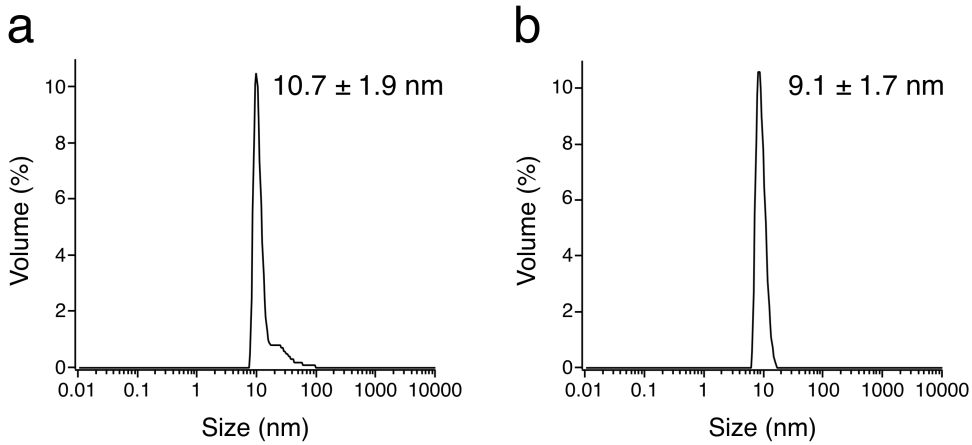

Fig. S1. Dynamic light scattering spectra of (a) **β-PN_Ru** and (b) **β-PN_iRu**. The measurement was performed using 10 μM proteins in 0.1 M sodium phosphate pH 7.0 at 25°C.

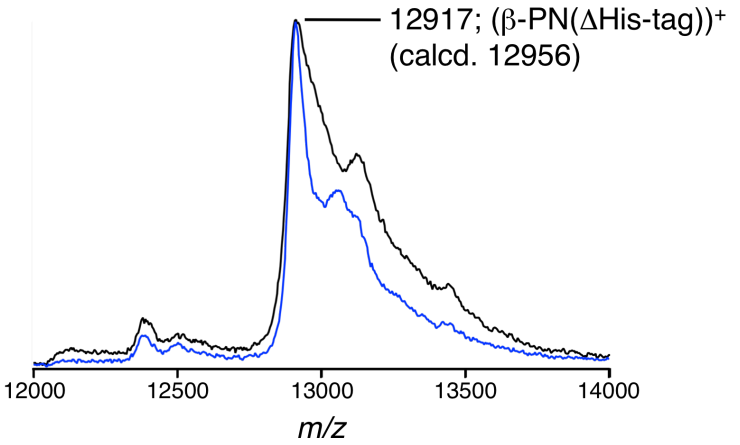

Fig. S2. MALDI-TOF mass spectra of monomer of β-PN(ΔHis-tag) (black) and after reaction of β-PN(ΔHis-tag) with CORM-2 (blue). In the reaction, a methanol solution of CORM-2 (30 μM) was slowly added to an aqueous solution of β-PN(ΔHis-tag) (5.0 μM in 20 mM Tris/HCl pH 8.0) and the mixture (final concentration of 20% methanol) was stirred at 25°C for 3 h in the dark.

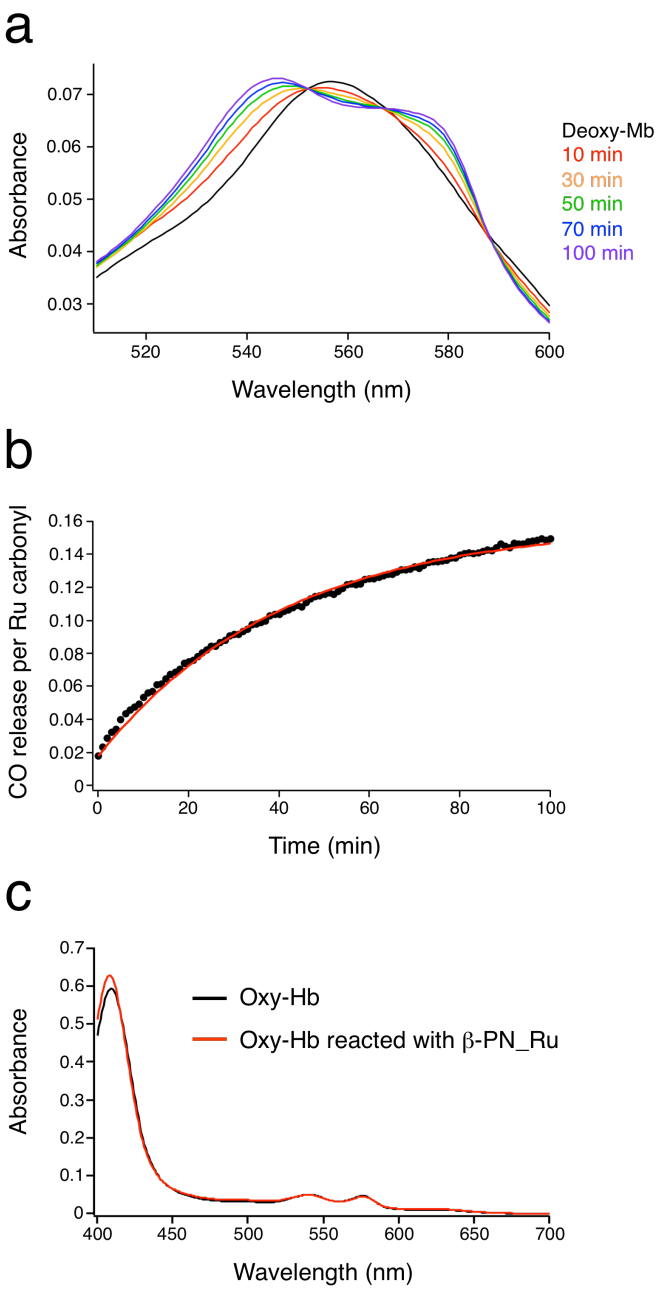

Fig. S3. CO release of **β-PN_Ru** evaluated by myoglobin (Mb) and oxy-hemoglobin (Hb) assay. (a) Absorption spectra of carbonmonoxy Mb (MbCO) formed over time after addition of **β-PN_Ru**. **β-PN_Ru** (18 μM Ru carbonyl) was added to deoxy-Mb (6.9 μM) in 10 mM PBS containing 6.9 mM $Na_2S_2O_4$ under Ar atmosphere. Absorbance of 250-700 nm was recorded every 1 min after addition of **β-PN_Ru**. (b) Equivalent of released CO per Ru carbonyl of **β-PN_Ru** (filled circle) over time. Conversion of

deoxy-Mb to MbCO was calculated according to the reported procedure.[7] The data were fitted using Excel and Solver to pseudo first order kinetics as shown in red line.[8] Average of three independent measurements is shown. (c) Absorption spectra of oxy-Mb (black line) and oxy-Hb reacted with **β-PN_Ru** for 90 min (red line). **β-PN_Ru** (12 μM Ru carbonyl) was added to the oxy-Hb (5.0 μM) in 10 mM PBS. After 90 min incubation, absorbance of 400-700 nm was recorded.

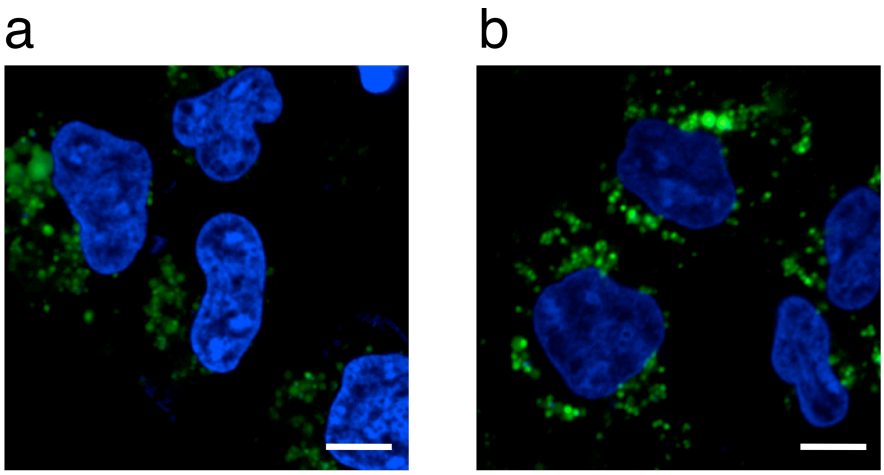

Fig. S4. Confocal fluorescence images of (a) ATTO520-modified β-PN and (b) ATTO520-modified **β-PN_Ru** in HEK293 cells (scale bars, 10 μm). Each needle (0.83 μM) was incubated with HEK293 cells for 12 h in the medium at 37°C under 5% CO_2. Cell nuclei were labeled with blue fluorescent Hoechst 33342.

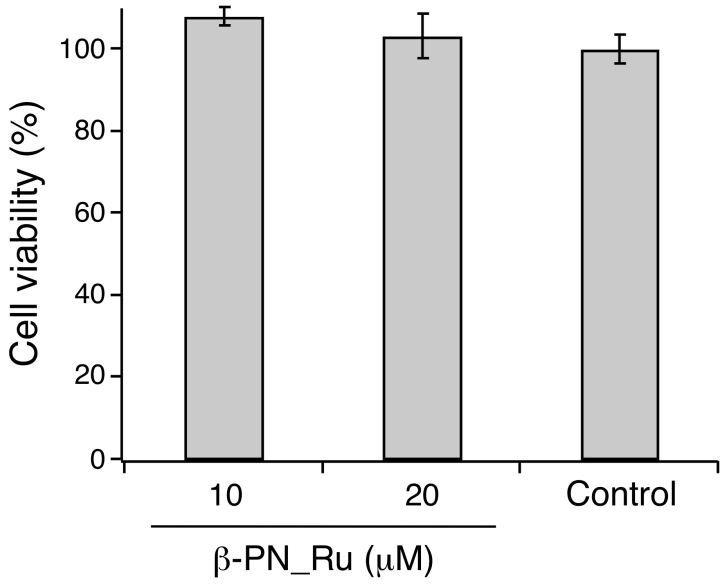

Fig. S5. Cell viability in the presence of **β-PN_Ru** evaluated using an MTT assay. HEK293/κB-Fluc cells were incubated with **β-PN_Ru** (10 and 20 μM Ru carbonyl) for 25 h at 37°C under 5% CO_2. A sample of untreated cells was used as a control. Each experiment was performed three times and the data represent mean ± SEM.

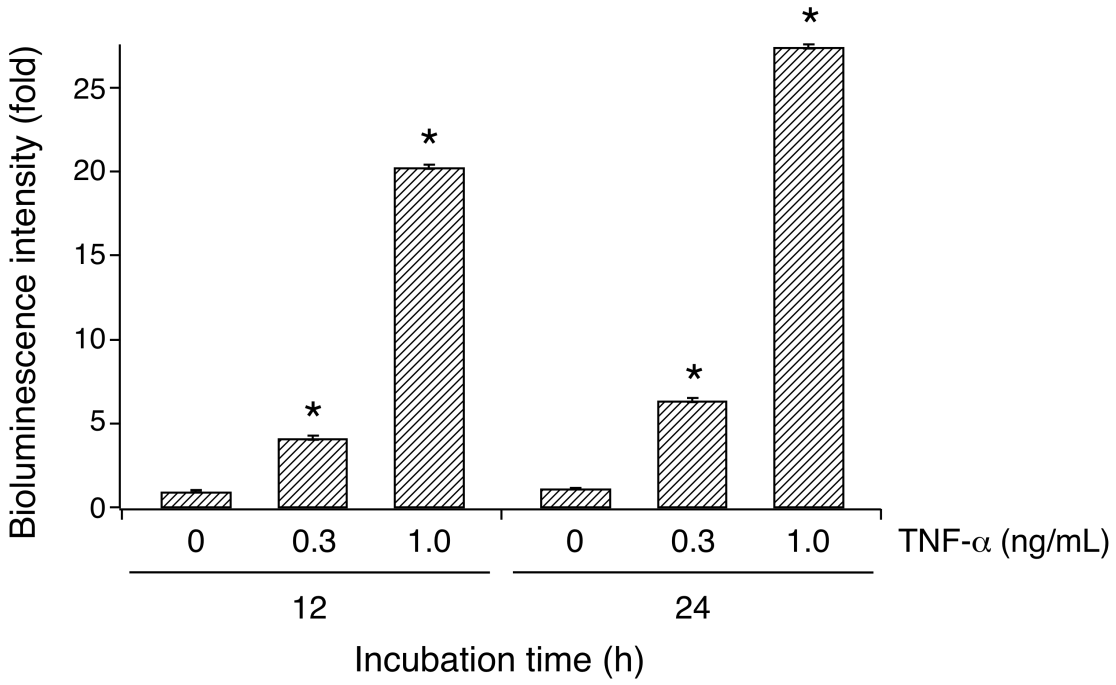

Fig. S6. Fluc reporter activity in HEK293/κB-Fluc cells in response to TNF-α treatments. HEK293/κB-Fluc cells (1.0×10^4 cells) were seeded in a 96-well plate and cultured overnight. The cells were lysed 12 or 24 h after 0-1.0 ng/mL TNF-α treatments and then Fluc activity was measured. The value after treatment of 0.3 ng/mL TNF-α is same in Fig. 4b. All the data shows the subtracted bioluminescence intensity of the cells that were treated with TNF-α from that of the negative control cells treated with buffer (0.1 M sodium phosphate pH 7.0). *$P < 0.05$ compared to the negative control cells at each incubation time point.

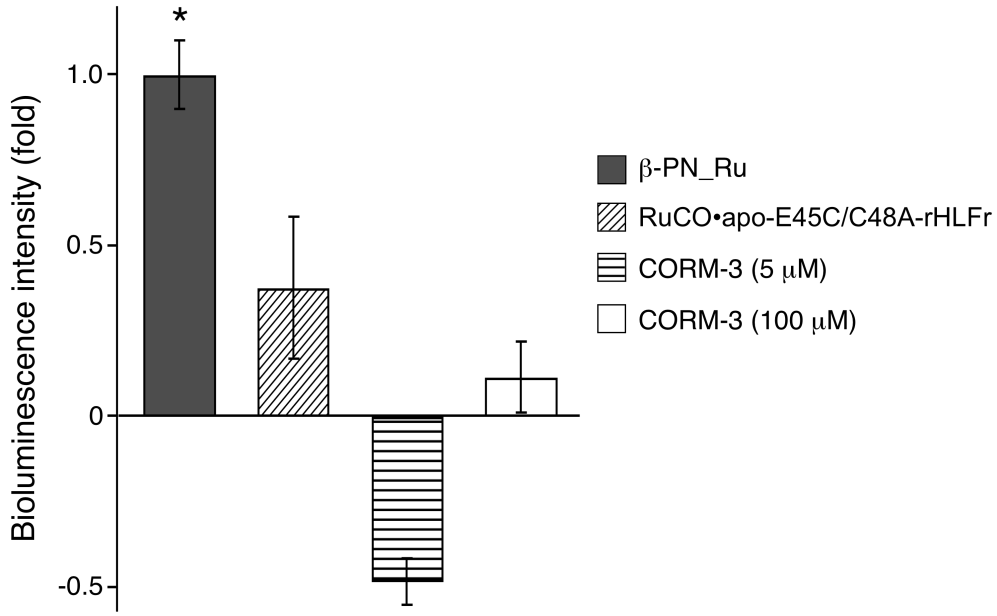

Fig. S7. Comparison to the effect on NF-κB activity. HEK293/κB-Fluc cells (1.0×10^4 cells) were seeded in a 96-well plate and cultured overnight. The cells were preincubated with **β-PN_Ru** (5 μM Ru carbonyl), RuCO•apo-E45C/C48A-rHLFr (5 μM Ru carbonyl),[2] or CORM-3 (5 or 100 μM Ru carbonyl) for 1 h. Subsequently the cells were cultured for 24 h. The NF-κB activity was assessed by bioluminescence intensity from luciferase activity according to reported procedure.[2] All the data shows the subtracted bioluminescence intensity of the cells that were treated with each sample from that of the negative control cells treated with buffer (0.1 M sodium phosphate pH 7.0). *$P < 0.05$ compared to the negative control cells at each incubation time point. Each experiment was performed three times and the data represent mean ± SEM.

Table S1. Equivalent of released CO per mole of Ru carbonyl and half-life ($t_{1/2}$) for CO release with and without glutathione and KCl.

Composite	Additive	Equivalent of CO per Ru	$t_{1/2}$ (min)
β-PN_Ru	−	0.15	10.6
	Glutathione, KCl	0.10	18.0
CORM-3	−	0.64	0.47
	Glutathione, KCl	0.35	0.32

β-PN_Ru and CORM-3 (20 μM Ru carbonyl) were added to the Mb (26 μM) in 10 mM PBS containing 17.3 mM $Na_2S_2O_4$ with or without 5 mM glutathione and 150 mM KCl under Ar atmosphere. Equivalent of released CO per Ru carbonyl and $t_{1/2}$ were determined by same methods shown in Fig. S3a and S3b. The concentrations of Ru, Mb, and $Na_2S_2O_4$ were higher than those of the experiment in Fig. S3 to detect clearly the spectral change of deoxy-Mb to MbCO in the presence of glutathione and KCl. Under the conditions, the $t_{1/2}$ values of β-PN_Ru and CORM-3 in the absence of glutathione and KCl were shorter than those shown in Fig. S3 because $Na_2S_2O_4$ with higher concentration enhances the CO release rates.[9,10]

References

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