Supporting Information

Self-Assembly of a Catalytic Multivalent Peptide-Nanoparticle Complex
Davide Zaramella, Paolo Scrimin,* Leonard J. Prins*
Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy

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1. Instrumentation and reagents

Peptide synthesis was performed on a Zinsser Sophas synthesizer starting from Wang resin (0.8 mmol/g; 100-200 mesh) using standard Fmoc-chemistry. All commercially available reagents and solvents were used without further purification.

HPLC purifications were performed on a preparative HPLC Shimadzu LC-8A equipped with a Shimadzu SPD-20A UV detector. The column used for separation was a Jupiter Proteo 4μ 90Å 250 x 21.2 mm, flow: 17 mL/min, eluents: H₂O + 0.1% HCOOH (A), CH₃CN + 0.1% HCOOH (B), gradient: 0-30 min 5-35% B, λdet = 226 nm. The chromatographic column used for separations in UHPLC analysis was a Zorbax RRHP Eclipse Plus C18 2.1 x 100 mm, 1.8 μm, flow 0.2 mL/min, eluents: H₂O + 0.1% HCOOH (A), CH₃CN + 0.1% HCOOH (B), gradient: 0-5 min 10-90% B. Aqueous phases were concentrated at reduced pressure using a Genevac EZ-2 Plus centrifuge used.

The UPLC/MS analysis were performed using an Agilent 1290 Infinity UPLC equipped with diode array and ESI-MS detector The chromatographic column used was an Agilent RRHD Zorbax Eclipse Plus C18 (2.1x150mm 1.8 micron), flow 0.2 ml/min, from 2% to 52% ACN+0.1% HCOOH in 5 minutes. High resolution mass-spectra were recorded on a Mariner ESI-TOF spectrometer (Perceptive BioSystems) in positive (M+H) ion mode, as indicated.

Kinetic experiments were performed on a Varian Cary 100 UV/Vis spectrophotometer equipped with thermostatted multiple cell holders.

Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a thermostatted cell holder.
2. Synthesis and characterization of $H_0 - H_3$

The synthesis of peptides $H_0 - H_3$ was performed on a Zinsser Sophas synthesizer starting from Wang resin resin (0.8 mmol/g; 100-200 mesh) using standard Fmoc-chemistry using DIC/HOBt as coupling agents. After TFA cleavage, the peptides were precipitated using $\text{Et}_2\text{O}$ and purified with preparative RP-HPLC (Jupiter Proteo 4μ, 90 Å, 250 x 21.2 mm, flow: 17 mL/min, eluents: $\text{H}_2\text{O} + 0.1\%$ $\text{HCOOH}$ (A), $\text{CH}_3\text{CN} + 0.1\%$ $\text{HCOOH}$ (B), gradient: 0-30 min 5-35% B, $\lambda_{\text{det}} = 226$ nm). The purified products were analyzed with UPLC/MS (Agilent Zorbax RRHD Eclipse C18, 1.8 μ, 2.1x150 mm, flow: 0.2 ml/min, eluents: : $\text{H}_2\text{O} + 0.1\%$ $\text{HCOOH}$ (A), $\text{CH}_3\text{CN} + 0.1\%$ $\text{HCOOH}$ (B), gradient: 0-5 min 2-52% B, $T = 40$ °C, $\lambda_{\text{det}} = 240$-350 nm. In these samples Ac-Trp-OEt was co-injected ($[\text{C}]_t = 180$ μM) in order to quantify the concentration of the peptide solutions. The exact mass of the peptides was determined on a Mariner ESI-TOF spectrometer.

![Fluorescence emission spectra of $H_0 - H_3$](image)

**Figure SI-1.** Fluorescence emission spectra of $H_0 - H_3$ ($\lambda_{\text{ex}} = 280$ nm, slits: 5/20 nm ). Conditions: $[\text{peptide}] = 2.0$ μM, pH = 7.0; [HEPES] = 10 mM, $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 90:10$, $T = 37$ °C.
Figure SI-2. UPLC-Chromatogram of H₀. Ac-Trp-OEt was added as a reference.

Figure SI-3. HR-MS of H₀ (m/z: 592.1942 [M+H]⁺, calcd. 592.1891)
**Figure SI-4.** UPLC-Chromatogram of H1. Ac-Trp-OEt was added as a reference.

**Figure SI-5.** HR-MS of H1 (m/z: 729.2523 [M+H]⁺, calcd. 729.2480)
Figure SI-6. UPLC-Chromatogram of \( \text{H}_2 \). Ac-Trp-OEt was added as a reference.

Figure SI-7. HR-MS of \( \text{H}_2 \) (\( m/z \): 866.3366 [M+H]\(^+\), calcd. 866.3069; \( m/z \): 433.6649 [M+2H]\(^2+\), calcd. 433.6535).
**Figure SI-8.** UPLC-Chromatogram of $\text{H}_3$. Ac-Trp-OEt was added as a reference.

**Figure SI-9.** HR-MS of $\text{H}_3$ ($m/z$: 1003.4070 $[\text{M+H}]^+$, calcd. 1003.3658; $m/z$: 502.1976 $[\text{M+2H}]^{2+}$, calcd. 502.1829).
3. Kinetic experiments

Hydrolysis of $N$-d-CBZ-Phe-ONP by $H_0$ - $H_3$/Au MPC 1 (Figure 3a, manuscript)

Peptides $H_0$ – $H_3$ were added at their respective saturating concentrations (11 µM for $H_0$ and $H_1$; 8.5 µM for $H_2$ and $H_3$) to distinct cuvettes containing Au MPC 1 ([head group] = 60 µM)$^1$ and HEPES (10 mM, pH 7.0) in H$_2$O:CH$_3$CN = 9:1 at 37 °C. After having recorded the initial absorbance (blank), substrate $N$-CBZ-d-Phe-ONP was added to obtain a final concentration of 10 µM. The absorbance was recorded at 400 nm for 50 minutes. The obtained curves were fitted to a model in order to obtain the (pseudo) first-order rate constants. All concentrations mentioned refer to the final values.

Turn-over experiment (Figure 4, manuscript)

Peptide $H_1$ (5 µM) was added to 2 ml of Au MPC 1 ([head group] = 60 µM) and HEPES (10 mM, pH 7.0) in H$_2$O:CH$_3$CN = 9:1 at 37 °C. After having recorded the initial absorbance at 400 nm $N$-CBZ-d-Phe-ONP (1 mM) in CH$_3$CN was added to obtain a final concentration of 10 µM and the absorbance at 400 nm was measured until a constant level was obtained. Subsequently, an additional 10 µM of $N$-CBZ-d-Phe-ONP was added and the measurement was continued. This was repeated for other 2 times. All concentrations mentioned refer to the final values.

Activating/deactivating of Au MPC 1 by peptides $H_0$ and $H_1$ (Figure 5a, manuscript)

Three cuvettes were prepared containing Au MPC 1 ([head group] = 60 µM) and HEPES (10 mM, pH 7.0) in H$_2$O:CH$_3$CN = 9:1 at 37 °C. After having recorded the initial absorbance at 400 nm, 20 µL of a stock solution of $N$-CBZ-d-Phe-ONP (1 mM) in CH$_3$CN was added and the absorbance at 400 nm was measured for 300 seconds. After 300 seconds, peptides $H_1$ (11 µM) or $H_0$ (11 µM) were added to separate cuvettes and measurement of the absorbance at 400 nm was continued. All concentrations mentioned refer to the final values.

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4. **Background rate and control experiments**

**Figure SI-10.** Changes in the absorbance at 400 nm upon the addition of \(\text{Cbz-Phe-ONP} \) (10 µM) to a solution of \(\text{H}_0 \) (■, 11 µM), \(\text{H}_1 \) (■, 11 µM), \(\text{H}_2 \) (■, 8.5 µM), \(\text{H}_3 \) (■, 8.5 µM). Included is also the background hydrolysis of \(\text{Cbz-Phe-ONP} \) (*) at the same concentration. Conditions: [HEPES] = 10 mM, \(\text{H}_2\text{O:CH}_3\text{CN} = 90:10\), pH = 7.0; T= 37 °C.

**Figure SI-11.** Changes in the absorbance at 400 nm upon the addition of \(\text{Cbz-Phe-ONP} \) (10 µM) to a solution of \(\text{H}_1 \) (11 µM) in the presence (black line) and absence (■, see Fig. SI-10) of tetramethyl ammonium chloride (60 µM). Conditions: [HEPES] = 10 mM, \(\text{H}_2\text{O:CH}_3\text{CN} = 90:10\), pH = 7.0; T= 37 °C.
5. Titration bromothymol blue (BTB)

Figure SI-12. a) Absorption spectra of BTB (30 µM) at various pH values. Buffers: 10 mM MES pH 6.0; 10 mM PIPES pH 6.5; 10 mM HEPES pH 7.0, 7.5, 8.0; T= 37°C. b) Absorption maximum of BTB at 618 nm as a function of pH. c) Absorption spectra of BTB (30 µM) in the presence (red line) and absence (black line) of Au MPCs 1. ([head group] = 60 µM; 10 mM HEPES pH 7.0; T= 37°C).
6. Reaction rates at pH 6.5 and 6.0

**Figure SI-13.** Changes in the absorbance at 320 nm upon the addition of Cbz-Phe-ONP (10 µM) to a solution of H1/Au MPC 1 with a) \([H_1] = 11 \mu M\) or b) 5 µM for both pH = 6.5 and pH = 6.0. Conditions: [head group] = 60 µM; 10 mM MES pH 6.0 and 10 mM PIPES pH 6.5; 10% ACN; T= 37°C. It is emphasized that in these kinetics the absorbance of \(p\)-nitrophenol was followed (at 320 nm) instead of the absorbance of the \(p\)-nitrophenolate ion (at 400 nm). c) Plot of the observed rate constant, \(k_{obs}\), as a function of pH for \(H_1 = 5\) or 10 µM. The value for pH 7.0 is obtained from the experiments reported in Figure 3 of the manuscript).