Signal Amplification by Conjugate Addition for Differential Polyphenol Sensing: Accessing the "French Paradox" with Synthetic Pores

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Supporting Online Material

Materials and Methods. As in (*S1*) - (*S3*), Supporting Information. CF was from Fluka/Aldrich, EYPC from Avanti Polar Lipids, buffers and inorganic salts were of the best grade available from Sigma. Triton X-100 and analytes were from Sigma/Aldrich. Large unimellar vesicles (LUVs) were prepared by the Mini-Extruder with polycarbonate membrane, pore size 100 nm, from Avanti. Fluorescence spectra were recorded on either a Fluoromax 2 or Fluoromax 3 from Jobin Yvon-Spex equipped with an injector port, a magnetic stirrer and a temperature controller (25 ^{o}C).

Abbreviations. CF, 5(6)-carboxyfluorescein; DAA: Dialkoxyanthracene; DMF: *N*,*N*-Dimethylformamide; DMSO: Dimethylsulfoxide; EYPC LUVs: Egg yolk phosphatidylcholine large unilamellar vesicles; HEPES: N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); His, H: *L*-Histidine; Leu, L: *L*-Leucine; Lys, K: *L*-Lysine; MES: 2-

Morpholinoethanesulfonic acid monohydrate; NDI: 1,4,5,8-Naphthalenediimide; Ndi, π_A: Artificial amino acid in pore **10**.

$1^3,2^3,3^2,4^3,5^2,6^3,7^2,8^3$-Octakis(*Gla*-Leu-Ndi-Leu-Lys-Leu-NH_2)-*p*-Octiphenyl 9. Monomer **9** for self-assembly into pore **10** (Figure 2) was prepared in 24 steps following previously reported procedures (*S1*).

DAA Hydrazide 1. Hydrazide **1** (Figure 1) was prepared in 7 steps following previously reported procedures (*S2*).

EYPC-LUVs⊃CF. As in refs (*S1*) - (*S3*). Solutions of EYPC (25 mg) in $CHCl_3$/MeOH 1:1 (1 ml) were dried under a stream of nitrogen and then under vacuum (>2 h) to form thin films. The resulting films were hydrated with 1 ml buffer (50 mM CF, 10 mM HEPES, 10 mM NaCl, pH 7.4) for more than 30 min, subjected to freeze-thaw cycles (5x) and extrusions (15x, Mini-Extruder with a polycarbonate membrane, pore size 100 nm). Extravesicular CF was removed by gel filtration (Sephadex G-50) with buffer (107 mM NaCl, 10 mM HEPES, pH 7.4). The LUV fractions were combined and diluted to 6 ml with the corresponding buffer. Lipid concentrations were estimated from the amount of entrapped dye; the estimated values were consistent with earlier results from phosphate analysis. The final stock solutions had the following characteristics: ~2.5 mM EYPC; *inside*, 50 mM CF, 10 mM NaCl, 10 mM HEPES, pH 7.4; *outside*, 107 mM NaCl, 10 mM HEPES, pH 7.4.

Detection of diphenolase and monophenolase activity of tyrosinase for various substrates. Analytes **4a**, **4b**, **4d**, **4e**, **4f**, **4g**, **4h**, **4i** and **6j** (various concentrations) were

incubated at 25˚C with *tyrosinase from mushroom* (EC 1.14.18.1, final 10 μg/ml, ≥2 units/μg, 10 mM HEPES, 107 mM NaCl, pH 6.5) in the presence of amplifier **1** (50 μM). To the resulting mixture, EYPC-LUVs⊃CF (100 μl from above stock solutions) were added. Fluorescence emission intensity F_t (λ_{ex} 492 nm, λ_{em} 517 nm) was monitored as a function of time during addition of monomer **9** (20 μl stock solution in DMSO, usually 375 nM final monomer concentration) and 40 μl 1.2% aq triton X-100 for final calibration. Fluorescence kinetics were normalized to fractional intensity I_F applying equation [S1]

$$I_F = [(I_t - I_0) / (I_\infty - I_0)] / [(I_t^{MAX} - I_0) / (I_\infty - I_0)] \quad [S1],$$

where $I_0 = I_t$ at pore addition, $I_\infty = I_t$ at saturation after lysis, and $I_t^{MAX} = I_t$ at maximal emission intensity before lysis. From the obtained curves, $I_F^{MAX} = I_F$ at maximal fractional emission intensity before lysis was obtained for each measurement and converted into fractional pore activity Y applying equation [S2]

$$Y = [(I_F^{MAX} - I_F^{MAX(0)}) / (I_F^{MAX(\infty)} - I_F^{MAX(0)})] \quad [S2],$$

where $I_F^{MAX(0)}$ is $I_F^{MAX(0)}$ obtained under the conditions giving rise to lowest pore activity and $I_F^{MAX(\infty)}$ is I_F^{MAX} of the highest activity (= 1). For dose response curves, the obtained fractional pore activities Y were plotted as a function of blocker concentration $c_{BLOCKER}$ and fitted to the Hill equation [S3]

$$Y = Y_\infty + (Y_0 - Y_\infty) / \{1 + (c_{BLOCKER} / IC_{50})^n\} \quad [S3],$$

where Y_0 is Y without blocker, Y_∞ is Y with excess blocker, IC_{50} the concentration for 50% pore blockage and n the Hill coefficient.

References

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S2. S. Hagihara, L. Gremaud, G. Bollot, J. Mareda and S. Matile, *J. Am. Chem. Soc.*, 2008, **130**, 4347-4351.

S3. S. Hagihara, H. Tanaka and S. Matile, *J. Am. Chem. Soc.*, in press.