Supporting Information for

Boronic Acid Converters for Reactive Hydrazide Amplifiers: Polyphenol Sensing in Green Tea with Synthetic Pores

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1. General. Synthetic pore **T** (Figure S1)[S1] and amplifiers $\mathbf{A_n}$ (Figure S2)[S2] were prepared as described previously. Reagents, buffers, enzymes and salts were purchased from Sigma, Aldrich, or Fluka. Polyphenon was from Sigma. Tea leaves (Shincha from Shizuoka) and tea bag were purchased from supermarkets in Japan and Switzerland, respectively. Egg yolk phophatidylcholine (EYPC) was obtained from Avanti polar lipids. Fluorescence measurements were performed on either a FluoroMax-2 or a FluoroMax-3, Jobin Yvon-Spex.

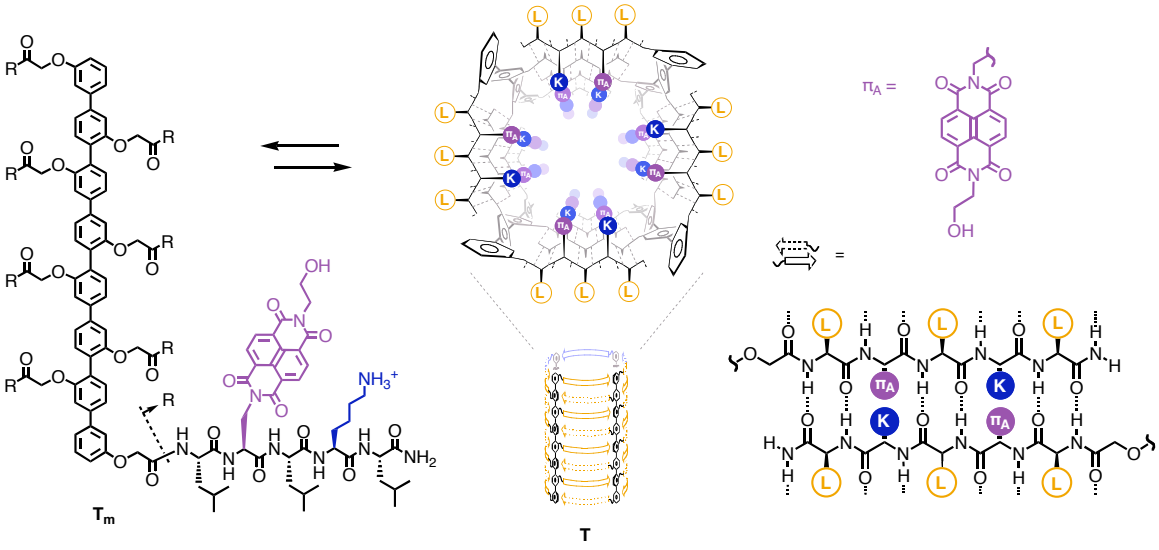

Figure S1. Structure of monomer $\mathbf{T_m}$ that self-assembles into pore **T** (from ref. S2).

2. Carboxyfluorescein(CF)-Loaded EYPC-LUVs. CF-loaded EYPC-LUVs were prepared as previously described. Solutions of EYPC (25 μl) in 2 ml 1:1 $CHCl_3$:MeOH were dried under vacuum (>2 hrs) to form thin films. The resulting films were hydrated with buffer (50 mM CF, 10 mM NaCl, 10 mM HEPES, pH 7.4) for more than 30 min and subsequently subjected to freeze-thaw cycles (5x) and extrusions (10x, Mini-Extruder with a stacked polycarbonate membrane of pore size 0.1μm). 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. The final concentration of the CF-loaded vesicle solutions were ~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).

3. Polyphenol Sensing with Pore 1

A. Preparation of Boronate Amplifiers. Hydrazide amplifiers $\mathbf{A_1}$ (3 μmol) in 5.0 mM formylphenyl boronic acid $\mathbf{C_1}$ in DMSO (600 μl, 3 μmol) was stirred for 2 hrs at 50 °C, and the solution of $\mathbf{A_1C_1}$ was used for pore blockage experiments without further workup. Boronate amplifiers $\mathbf{A_nC_n}$ (Figure S2) were prepared from $\mathbf{A_1}$-$\mathbf{A_4}$ and $\mathbf{C_1}$-$\mathbf{C_4}$ following the same procedure.

B. Pore Blockage Experiments with Boronate Amplifiers. 100 μl of the CF-loaded EYPC-LUVs were added to 1900 μl of gently stirred buffer (10 mM HEPES, 107 mM NaCl, pH 6.5) containing various concentrations ($c_{blocker}$ 0-300 μM) of boronate amplifiers $\mathbf{A_nC_n}$. Fluorescence emission intensities F_t (λ_{ex} = 492 nm; λ_{em} = 517 nm) were monitored as a function of time over 400 s. Pore **T** was added (20 μl from a 37.5 μM DMSO stock solution of $\mathbf{T_m}$, giving a final pore concentration of 375 nM $\mathbf{T_m}$ or ≤ 94 nM **T** in the cuvette) after 30 s and 1.2% Triton X-100 (40 μl) after 300 s (Figure S3). The fluorescence time courses were normalized to fractional emission intensities, *I*, according to:

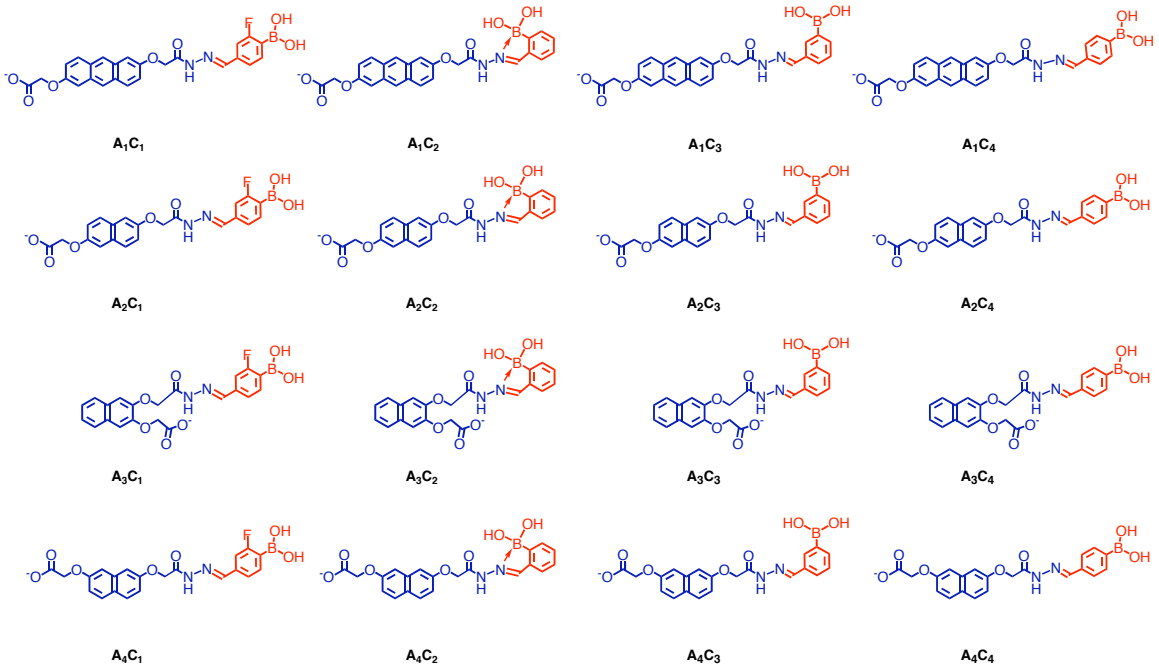

Figure S2. Structure of boronate amplifiers $\mathbf{A_nC_n}$ used in this study. Boronic acids are shown in trigonal acid form, the tetrahedral conjugate bases are omitted (pK_a between ~5 and ~9, compare Figure S5).

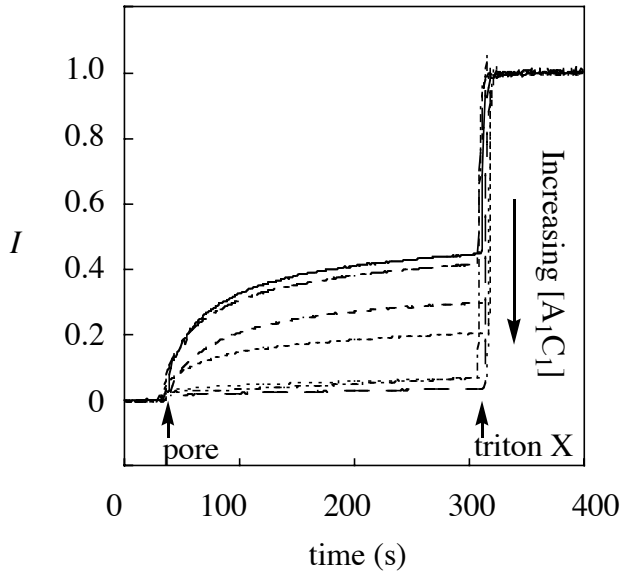

Figure S3. Representative normalized kinetics of pore **T**-mediated release of CF from CF-loaded EVPC-LUVs, demonstrating pore blockage with increasing concentration of boronate amplifier $\mathbf{A_1C_1}$.

$$I = (F_t\text{-}F_0)/(F_\infty\text{-}F_0) \qquad \text{(S1)}$$

where $F_0 = F_t$ before addition of pore **1**, $F_\infty = F_t$ after lysis with Triton X-100. The obtained fluorescence intensities just before membrane lysis (~295 s) were plot against blocker concentration and further normalized into pore activities, Y, according to:

$$Y = (I^F - I^F_{MIN})/(I^F_{MAX} - I^F_{MIN}) \qquad \text{(S2)}$$

where I^F is I just before lysis, I^F_{MIN} is the minimal I^F in the dose-response curve, and I^F_{MAX} is the maximal I^F in the dose-response curve. To obtain IC_{50} values, activities were then fit to the Hill equation according to:

$$Y = Y_\infty + (Y_0 - Y_\infty)/\{1 + (c_{blocker}/IC_{50})^n\} \qquad \text{(S3)}$$

where Y_0 is Y in the absence of pore blocker, Y_∞ is Y with excess blocker, IC_{50} is the blocker concentration required for 50% inactivation, and n is the Hill coefficient (Figure S4). Results are listed in Figure 2, schematic possible explanations of the found differences are given in Figure S5.

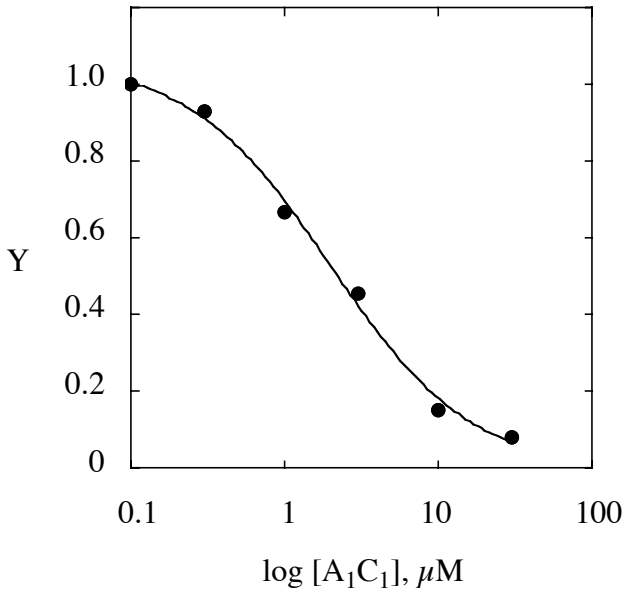

Figure S4. Dose-response curve for $\mathbf{A_1C_1}$ blockage of pore **T** with fit to Hill equation.

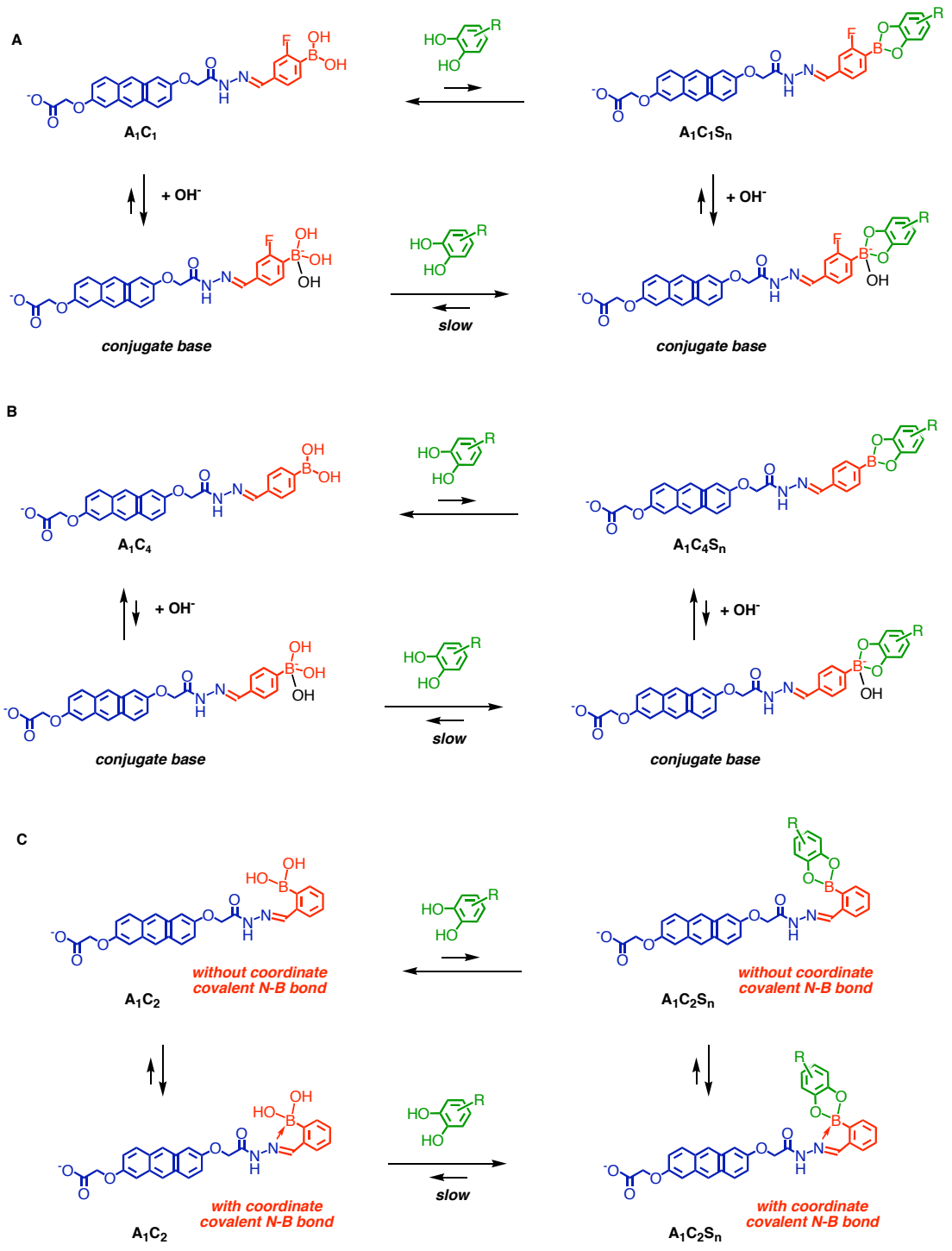

Figure S5. Schematic possible explanation why $\mathbf{A_1C_1}$ (A, more acidic, ~2-) is a better inactivator than $\mathbf{A_1C_4}$ (B, less acidic, ~1-), and why esters $\mathbf{A_1C_1S_n}$ (A) and $\mathbf{A_1C_2S_n}$ (C) are more stable than $\mathbf{A_1C_4S_n}$ (B); see, e.g., ref. S3.

C. Pore activation with catechin. Various concentrations (0-3000 μM) of catechin $\mathbf{S_1}$ and 20 μl of boronate amplifiers $\mathbf{A_nC_1}$ (50 μM) were mixed in a fluorescent cuvette and diluted with buffer (10 mM HEPES, 107 mM NaCl, pH 6.5) to 1900 μl. 100 μl of the CF-loaded EYPC-LUVs were added to the solution, and fluorescence emission intensities were monitored as a function of time over 400 s. Pore **T** was added (20 μl from a 37.5 μM DMSO stock solution of $\mathbf{T_m}$, giving a final pore concentration of 375 nM $\mathbf{T_m}$ or ≤ 94 nM **T** in the cuvette) after 30 s and 1.2% Triton X-100 (40 μl) after 300 s (Figure S6). The data was analyzed by same procedure as pore blockage experiment to obtain EC_{50} values instead of IC_{50}.

D. Tea sample preparation. 10 mg of dry tea leaves were incubated in 1 ml of hot water at 100 ˚C for 10 min, and the supernatant was collected. 100 μl of the extract or 100 μl of polyphenon solution (0.2 mg/ml) in buffer (10 mM HEPES, 107 mM NaCl, pH 6.5) was added to 850 μl of buffer (10 mM HEPES, 107 mM NaCl, pH 6.5) containing 40 μl of activated thiol-sepharose® 4B. The solution was incubated at 37˚C for 1 h in the absence or the presence of *tyrosinase from mushroom* (EC 1.14.18.1, final 10 μg/ml, ≥2 units/μg). The sample was then centrifuged and the supernatant was used for polyphenol sensing experiments.

E. Tea polyphenol sensing. A mixture of 100 μl of the tea sample and 20 μl of boronate amplifier A_1C_1 (5 mM in DMSO) in a fluorescence cuvette was diluted with 1780 μl of buffer (10 mM HEPES, 107 mM NaCl, pH 6.5). 100 μl of CF-loaded EYPC-LUVs were added to the fluorescence cuvette and the time-dependant release of CF dye from the vesicle was monitored fluorometrically at 517 nm as a function of time after addition of pore **1** (20 μl, 375 nM) after 30 s and triton X-100 after 300 s (Figure S7). Fluorescence intensities were normalized as described in section 3B and activities, Y, just before membrane lysis were collected.

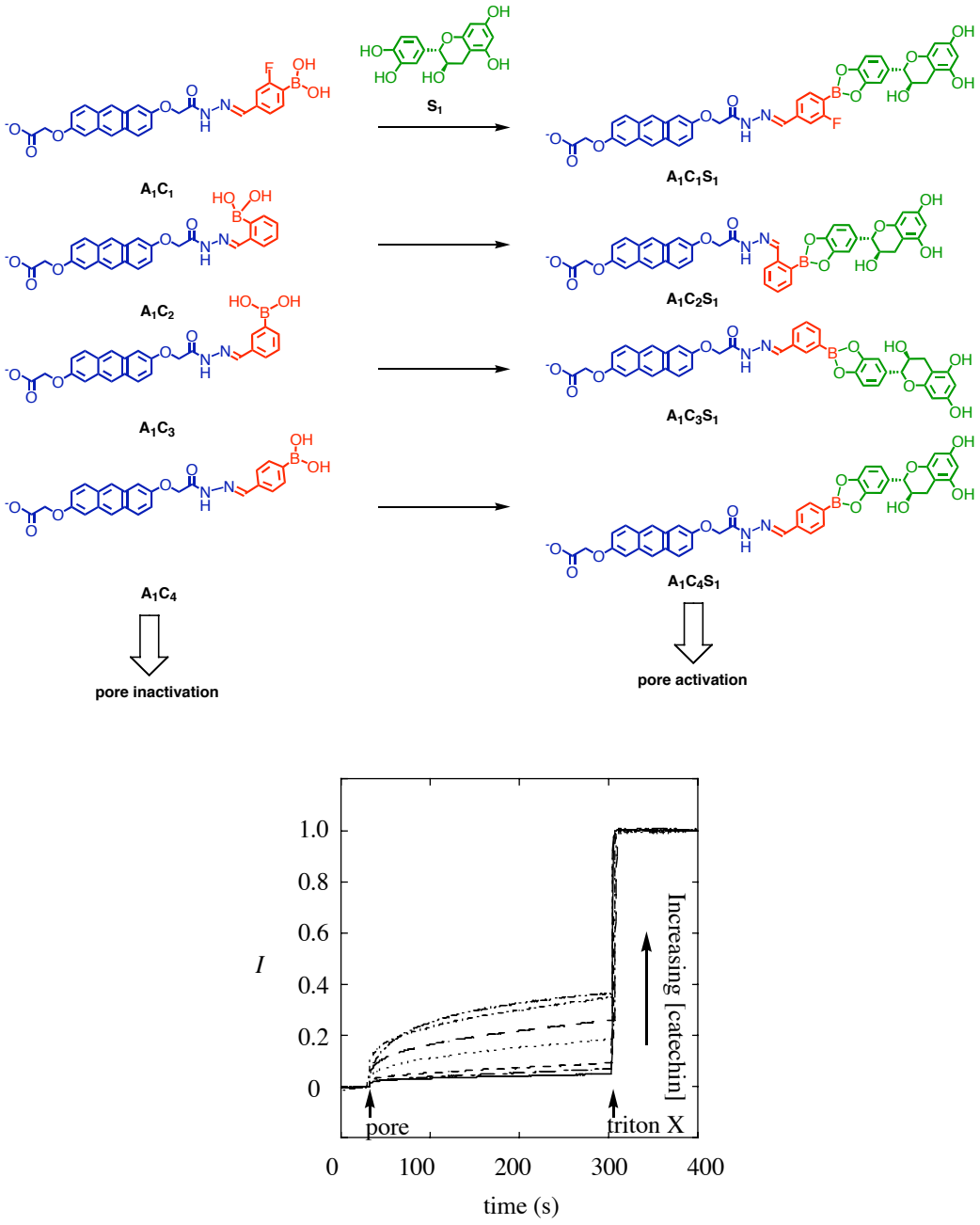

Figure S6. Representative normalized kinetics of catechin-mediated activation of pore **T**, demonstrating increase of pore activity with increasing concentration of (+)-catechin $\mathbf{S_1}$ at constant concentration of $\mathbf{A_1C_1}$ (50 μM) with structures of inactivators $\mathbf{A_1C_n}$ and activators $\mathbf{A_1C_nS_1}$ used. Boronic acids and esters are shown in trigonal acid form, the tetrahedral conjugate bases are omitted (pK_a between ~5 and ~9, esters are more acidic than acids, compare Figure S5).

The pore activation corresponding to polyphenols ($Y_{polyphenol}$) was calculated according to:

$$Y_{polyphenol} = Y_{tyrosinase(-)} - Y_{tyrosinase(+)} \qquad (S4)$$

Where $Y_{tyrosinase(+)}$ is Y value for the sample treated with tyrosinase, and $Y_{tyrosinase(-)}$ is Y value for the sample without tyrosinase treatment. The obtained $Y_{polyphenol}$ was applied to the Hill equation (S3) together with Y_0, Y_∞, n and EC_{50} values of catechin to give the catechin equivalent polyphenol concentration in cuvette ($c_{catechin}$). In order to translate this value into a total concentration of polyphenols in green tea, $c_{catechin}$ was converted to the mass of polyphenol extracted as catechin, $M_{catechin}$, according to equation (S5), where 2 ml is the total sample volume in cuvette, 290.3 g/mol is the MW of catechin, and multiplication by 100 accounts for the dilution factor.

$$M_{catechin} = c_{catechin}\,(2ml)(290.3g/mol)(100) \qquad (S5)$$

$M_{catechin}$ was then divided by the total mass of tea leaves used, M_{sample} (10 mg), by equation (S6) to obtain the final polyphenol content, PC_{sample}, in the tea leaves. The value was determined by averaging three independent experiments.

$$PC_{sample} = M_{catechin}/M_{sample} \qquad (S6)$$

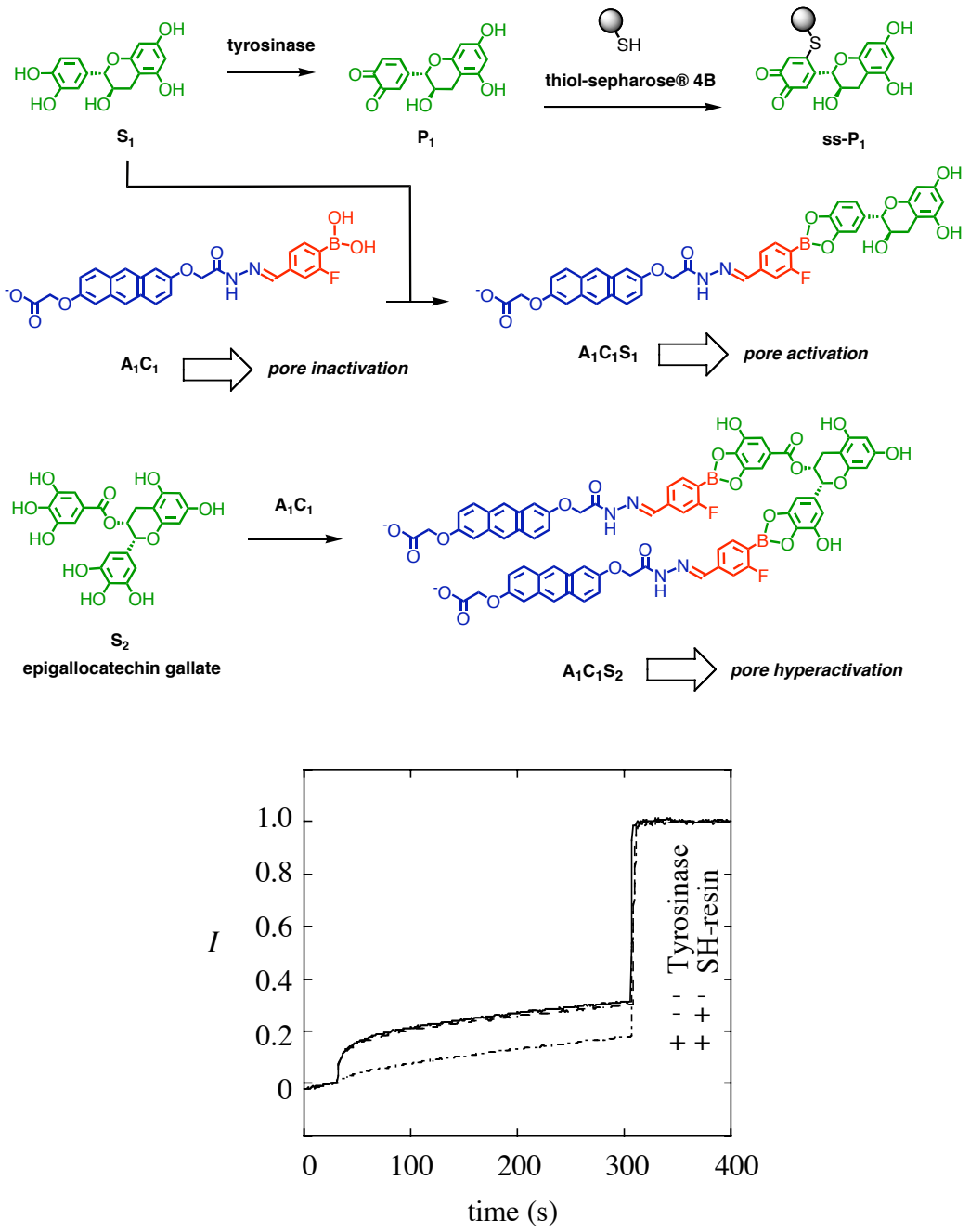

Figure S7. Signal degeneration for polyphenols in green tea extract by tyrosinase oxidation coupled with conjugate addition to thiol-resin following procedures D and E, with simplified sensing scheme for **S**$_1$ and speculative example for the possibly divalent pore "hyperactivation" with epigallocatechin gallate (**S**$_2$). Boronic acids and esters are shown in trigonal acid form, the tetrahedral conjugate bases are omitted (pK_a between ~5 and ~9, esters are more acidic than acids).

(S1) Tanaka, H.; Litvinchuk, S.; Bollot, G.; Mareda, J.; Tran, D.-H.; Sakai, N.; Matile, S. *J. Am. Chem. Soc*. **2006**, *128*, 16000-16001.

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

(S3) (a) James, T. D.; Phillips, M. D.; Shinkai, S. *Boronic Acids in Saccharide Recognition.* Royal Society of Chemistry: London, 2006. (b) James, T. D. *Top. Curr. Chem.* **2007**, *277*, 107-154. (c) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291-5300.