On-bead screening of a library to detect host–guest complexation by an aniline reporter†

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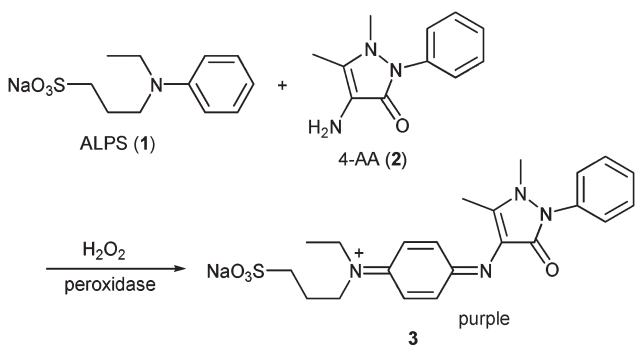
We developed a new labeling reagent and a color assay system in water to detect binding between target molecules and library members on beads, which is free of label-induced artifacts that can cause misleading results.

Combinatorial chemistry is a powerful tool for rapidly discovering desired molecules such as new drugs and materials.[1] The "one-bead-one-compound" (OBOC) combinatorial library method can easily generate thousands to millions of compounds using a "split-mix" synthesis procedure,[2–4] which can be assayed against a wide variety of labeled targets to discover their ligands[2–8] or synthetic receptors.[9–14] The on-bead color assay for the OBOC library is a very rapid screening method and can be used to easily assess qualitative binding abilities. Wennemers and Still, however, reported that many simple dyes bind certain peptides in water with significant selectivity.[15] Great care should thus be taken to avoid label-induced artifacts.[9,11,13–15] Recently, we reported that the addition of a non-ionic surfactant, Triton-X, can suppress the binding interaction.[16] It is possible, however, that the additive also affects target–ligand interactions. An additive-free screening environment is ideal for estimating real interactions. Trinder reagents[17] such as ALPS (**1**)[18] are chromogenic reagents for colorimetric determination of hydrogen peroxide activity. The reagents (hydrogen donors) are oxidatively condensed with 4-aminoantipyrine (**2**, 4-AA) in the presence of H_2O_2 and peroxidase (shown in Scheme 1). This system is widely used for diagnostic assays and biochemical examinations because hydrogen peroxide is produced by enzymatic oxidation of substrates such as glucose. We focused on the Trinder reagent for labeling targets, since it is expected not to bind certain peptides because the reagent is small and less polar than typical dyes and fluorophores.



Scheme 1 Trinder reaction.

At first, some aniline linked carboxylic acids **4–10** (Fig. 1) were treated with 4-AA in the presence of H_2O_2 and horseradish peroxidase (HRP) in water at pH 6.86 to explore the highly sensitive aniline derivatives. The absorption maxima and molar absorption coefficients of the chromogens are shown in Table 1. The chromogen from *N*-phenylglycine (**4**) had less than half the intensity of that from the commercially available Trinder reagent ALPS (**1**). The color intensity of chromogens was decreased by the introduction of methoxy groups on the aromatic ring[19] (compounds **5** and **6**). Unexpectedly, the intensity was drastically decreased when a spacer group was connected to **4** by an amide linkage (compound **7**). On the other hand, the intensity of the chromogen from 4-(ethylphenylamino)butyric acid (**8**) was 1.6 times higher than that from **4**. Although the introduction of a spacer group decreased the intensity, the molar absorption coefficient of the chromogen remained at approximately 1800 (compound **9**). The intensity of the chromogen from the

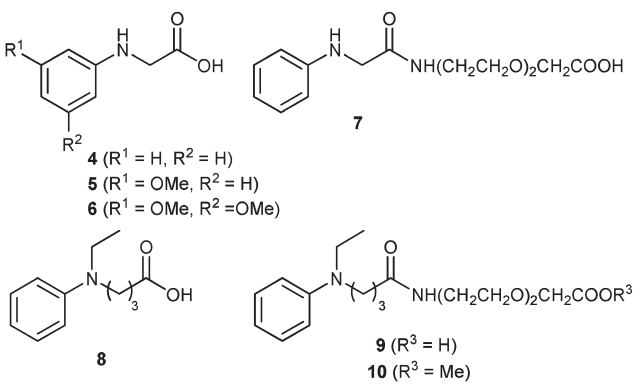


Fig. 1 Hydrogen donors in the Trinder reaction.

Table 1 Wavelengths of maximum absorption (λ_{max}) and molar absorption coefficients (ε) of the chromogens from hydrogen donors **1** and **4–10**[a]

Compound	λ_{max}/nm	ε
1	560	14499
4	534	6879
5	526	2509
6	582	2104
7	530	71
8	558	11042
9	562	1829
10	560	9861

[a] The data were recorded in pH 6.86 phosphate buffer.

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corresponding methyl ester **10** was five times higher than that from **9**. The reason for the drastic change in absorbance is unclear. As shown in Fig. 2, absorbance of chromogen from methyl ester **10** reached a maximum within 5 min and then gradually decreased.

Next, the carboxylic acid **9** was loaded on Novasyn® TG resin[20] to investigate the coloration on a solid support. Resin **11** immediately displayed a striking purple color following treatment with 4-AA, H_2O_2, and HRP in pH 6.86 buffer (Fig. 3). The color of the control resin **12** was invariant with the same treatment.

A previous study indicated that the resin-bound peptidocalix[4]-arene **13b** binds dye-labeled tripeptide **14a**.[16] Based on this result, tripeptide **16a**, which had the same peptide sequence as **14a**, was synthesized. The mixture of resin-bound peptidocalix[4]arene **13b** and *N*-acetylaminomethylated resin (control resin) **12** was incubated with a labeled peptide **16a** in pH 6.86 phosphate buffer. After agitation for 12 h, the beads were rinsed several times with the buffer, and subsequently treated with 4-AA, HRP, and H_2O_2. The mixture was incubated at 38 °C for 5 min and inspected under a low-power microscope. Large beads stained purple and small beads had no color, which indicated that the present method can be applied to an on-bead binding assay. The detection limit for **16a** was less than 13 μmol L^{-1} (Fig. 4).

Prior to screening of the library, we confirmed that the labeling group did not bind any library members. No colored beads were observed in the screening of a peptidocalix[4]arene library **13a**, consisting of 1000 members, even with 1.3 mmol L^{-1} of **17** (Fig. 5A). In contrast , almost 10% of the beads were stained in the

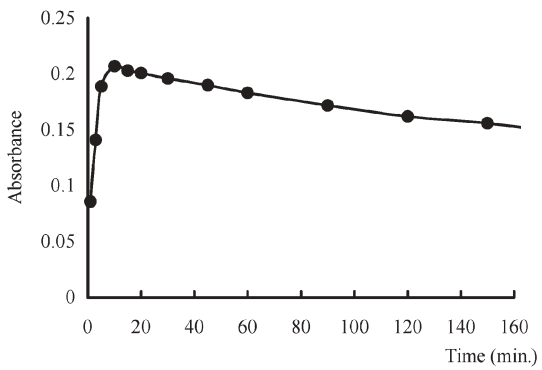


Fig. 2 Time dependence of color development of the chromogen from **10**. The absorbance was recorded at 560 nm.

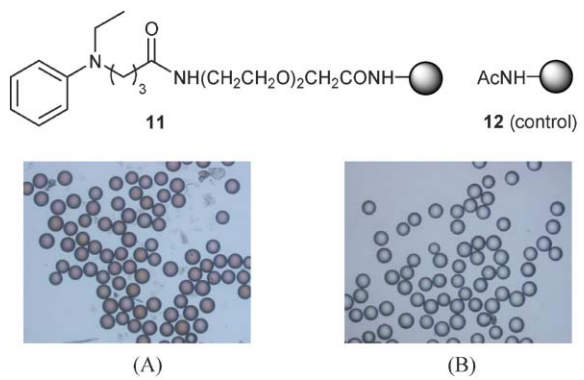
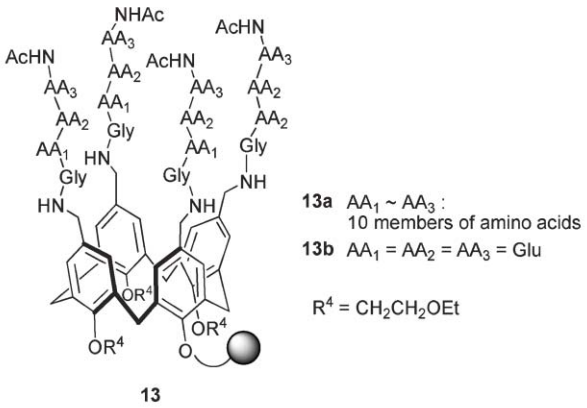
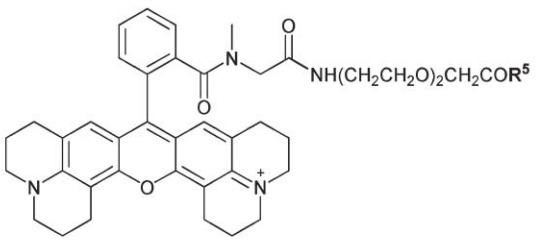
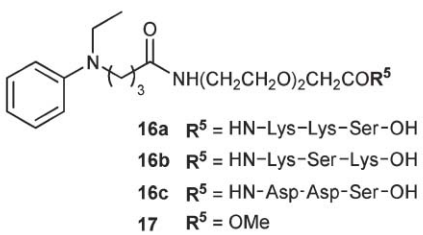


Fig. 3 Coloration by the Trinder reaction on a solid support **11** (A) and control experiment (B, resin **12**).







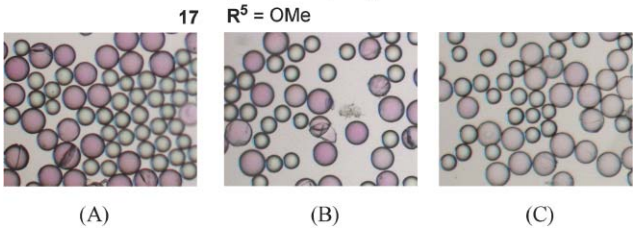


Fig. 4 Detection of host (**13b**, large beads)–guest (**16a**) complexation on beads by the Trinder reaction in various guest concentrations with control resin (**12**, small beads). Guest concentration: (A) 1.3 mmol L^{-1}, (B) 130 μmol L^{-1}, (C) 13 μmol L^{-1}.

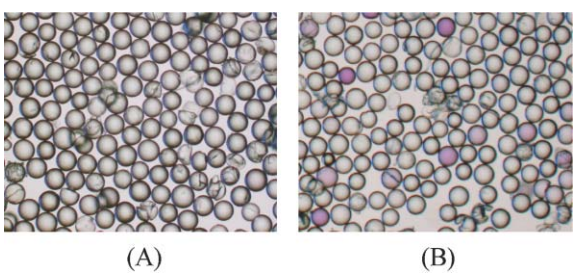


Fig. 5 Screening of the library **13a** for binding to **17** (1.3 mmol L^{-1}) by the Trinder reaction (A) and for binding to **15** (5 μmol L^{-1}) by color assay (B) in pH 6.86 phosphate buffer.

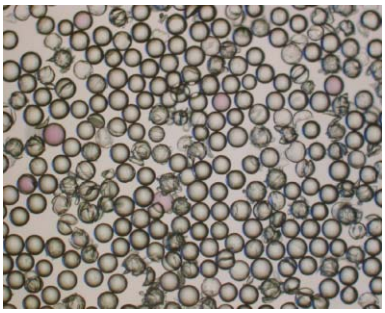


Fig. 6 Screening of the library **13a** for binding to **16a** (13 μmol L^{-1}) by the Trinder reaction in pH 6.86 phosphate buffer.

Table 2 Peptide sequences of the library members for binding to a guest peptide **16a**

Entry	AA_1	AA_2	AA_3	Frequency[a]
1	Glu	Glu	Glu	4
2	Glu	Glu	Tyr	4
3	Glu	Leu	Glu	4
4	Glu	Pro	Glu	3
5	Glu	Glu	Pro	2
6	Ser	Glu	Glu	2
7	Glu	Asn	Glu	1
8	Glu	Glu	Ser	1
9	Glu	Ser	Glu	1
10	Glu	Tyr	Glu	1
11	Tyr	Glu	Glu	1
Total				24

[a] Number of beads having the indicated sequences.

screening of **13a** with corresponding dye-labeled compound **15** (5 μmol L^{-1}, Fig. 5B). Thus, the present method avoids misleading results caused by label-induced artifacts. Application to an actual screening of **13a** with an aniline-labeled tripeptide **16a** is shown in Fig. 6. There were only a few colored beads. All colored beads were isolated and decoded to identify their amino acid sequences. The sequences on the beads mainly consisted of two or three Glu (Table 2). The preference for an acidic amino acid was consistent with that obtained for dye-labeled tripeptide **14a**, which has the same peptide sequence as **16a**.[16]

Finally, screening of the library **13a** for binding with some tripeptides **16** was performed to compare with the results of the conventional dye-labeled method by compounds **14**, reported previously.[16] In the screening for tripeptide **14a** and **16a**, some colored beads were found, in both cases as described above. There were no colored beads found, however, for binding to **16b**, which has a different peptide sequence order than **16a**. In contrast, in the corresponding screening of **14b** by the dye-labeled method, there were faintly colored beads. These were detectable under a fluorescence microscope. Similar results were obtained for peptides **14c** and **16c**. These observations indicated that the present method is comparable in sensitivity with a color assay, but less sensitive than fluorescence detection (Fig. 7).

In summary we have developed a novel color assay system in water without additives for detecting host–guest complexation on beads. This assay system is free of label-induced artifacts that might lead to misleading results.

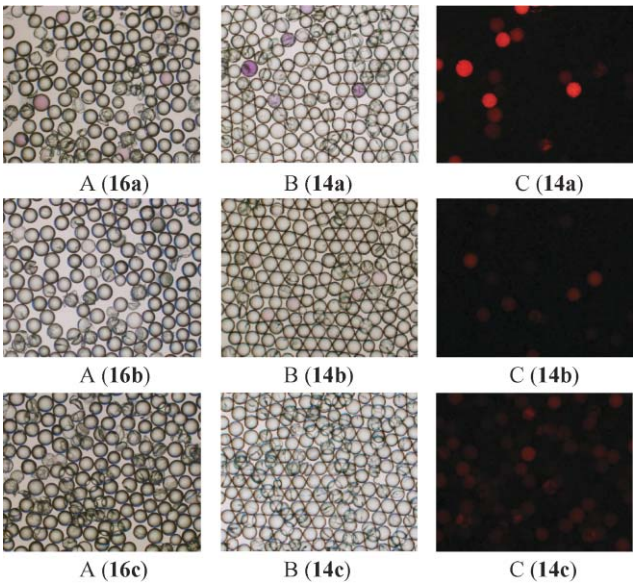


Fig. 7 Screening of the library **13a** for binding to some tripeptides (compounds shown in parentheses under each picture). A series: detection by the Trinder reaction (13 μmol L^{-1} of peptides in pH 6.86 phosphate buffer) B and C series: color detection (B) and fluorescence detection (C) (5 μmol L^{-1} of peptides in pH 6.86 phosphate buffer containing 5% Triton X).

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