Fig. S1. History of Fluorescent Compounds

Number of Publications with "fluorescent dye"

Fig. S1. History of fluorescent compounds. Growing interest of fluorescent compound was graphically demonstrated by histogram. Since the discovery of the first fluorescent compound in 1840s, number of publications for selected range of year is demonstrated. Number of publications were obtained by Key word searching for “fluorescent dye” on Scifinder database.1 Microscope history timeline and the application of the super-resolution technology are collected as well.2
**Fig. S2. Tagging Fluorophores via Chemical or Biological Handles**

**Top)** Chemically labeled fluorescent tags utilize azide-alkyne cycloaddition, known as click chemistry, or maleimide-cysteine biorthogonal conjugation. Shapes filled with red indicate chemical moiety. Black wavy line indicates target biomolecule such as protein or peptide engineered with chemical handle. **Bottom)** Biologically labeled technique involves engineering cell line or protein of interest with HaloTag or SNAP-tag. Fluorescent reporter with corresponding chemical handles (HaloTag ligand or SNAP tag ligand) will form a highly specific covalent bond upon labeling to those engineered Halotag or SNAP-tag, respectively.

**Fig. S3. Activity Based Protein Profiling Using a Fluorescent Probe**

**Fig. S3** A schematic view of general applications of fluorescent probe in activity-based protein profiling (ABPP). Activity based probe (ABP) containing “click”able moiety is added to cells and covalently linked to target protein via photo-crosslinking. Fluorescent probe is then added and react with ABP-labeled target proteins. Changes in fluorescence and mass of treated cells are analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and mass spectrometry to detect the target protein.
**Fig. S4.** Number of Publications for Common Fluorescent Cores

<table>
<thead>
<tr>
<th>Core Scaffold</th>
<th># of Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescein</td>
<td>15638</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>12950</td>
</tr>
<tr>
<td>Cyanine</td>
<td>5871</td>
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<tr>
<td>Xanth</td>
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<tr>
<td>Coumarin</td>
<td>3144</td>
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<tr>
<td>BODIPY</td>
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<tr>
<td>Pyrene</td>
<td>1764</td>
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<td>Styryl</td>
<td>1047</td>
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<tr>
<td>Styrene</td>
<td>1022</td>
</tr>
<tr>
<td>Quinoline</td>
<td>735</td>
</tr>
</tbody>
</table>

**Publication by Core Scaffold**

- Fluorescein: 32%
- Rhodamine: 7%
- Cyanine: 12%
- Xanth: 6%
- Coumarin: 7%
- BODIPY: 4%
- Pyrene: 2%
- Styryl: 2%
- Styrene: 2%
- Quinoline: 2%

**FDA approved synthetic dyes**

- Fluorescein Sodium
- Indocyanine Green (ICG)

**Fig. S4.** List of core fluorescent scaffolds are shown in the box (top). R groups indicate commonly modified and/or functionalized sites. The number of publications for each fluorescent core was collected from Scifinder database.1 ‘research topic’ (left). A pie-chart shows relative percentages of each core to visualize the most developed scaffold and the least developed scaffolds. Chemical structure and name of FDA approved synthetic dyes (right).
Fig. S5. Process of Target-oriented and Diversity-oriented Fluorescent Library Screening

- **Target-oriented fluorescent sensor discovery**
  - Target determination
  - Target recognition motif search
  - Sensor Design (dye-linker-recognition motif)
  - Case-by-case single target sensor discovery

```
Ca^{2+}  Hg^{2+}
e.g. BAPTA
```

- **Diversity-oriented fluorescent sensor discovery**
  - Fluorophore determination
  - Fluorescent probe-library (combinatorial)
  - High-throughput multi-analyte screening
  - multiple target sensor discovery

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**Fig. S5.** Workflow for target-oriented fluorescent sensor discovery and diversity-oriented fluorescent sensor discovery. In target-oriented strategy, target is first defined, and the recognition motifs are screened in combinatorial manner to discover fluorescent probe with high selectivity to defined target (top). Diversity-oriented probe discovery involves an undefined target. First, structurally flexible and tunable fluorescent scaffold is defined to generate diversity-oriented fluorescent libraries (DOFL). With DOFLs, multiple targets/analytes are screened to discover highly selective probe (bottom).
**Fig. S6. Diversity-oriented fluorescent library with coumarin core scaffold**

Generated libraries of fluorescent dyes can be efficiently analyzed in a high-throughput manner to discover highly selective probes for in vivo and live cells. Furthermore, libraries of fluorescent compounds can be utilized for structure–activity relationship (SAR) studies. The SAR studies to understand the effect of subtle changes to the chemical and optical properties of the fluorophore will provide additional insights for rational design.

**Fig. S7. Probes developed by diversity-oriented probe discovery strategies**

A series of cell-permeable and highly selective probes were successfully discovered through diversity-oriented fluorescent sensor discovery.
References