Points to Keep in Mind When Choosing an FP

1. **Excitation & Emission (ex/em):**
    - Each FP has its unique ex/em peak. Therefore, choose FPs that your system can excite, and detect the emission. For example, if your microscope has only two lasers, at 488nm and 561nm, you will not be able to use far red-FPs. If you do not have a filter that will pass blue light to the detector/camera, then BFPs are of no use to you.

    - When using more than one FP, make sure their emission light does not overlap in wavelength. In many microscopes the filters are not narrow enough to distinguish between closely related colors. Furthermore, most FPs have a broad range of emission which will be detected by longer-wavelength filters (e.g. GFP also emits yellow light).

2. **Oligomerization:** The first generations of FPs were prone to oligomerize. This may affect the biological function of the FP-fusion protein. Therefore, it is recommended to use monomeric FPs (usually denoted by a "m" as the first letter in the protein name, e.g. mCherry).

3. **Oxygen:** The maturation of the chromophore on many FPs (particularly those derived from GFP) requires oxygen. Therefore, these FPs cannot be used in oxygen deprived environment. Recently, a new GFP isolated from the Unagi eel was shown to mature independently of oxygen, making suitble for use in anaerobic conditions.

4. **Maturation Time:** Maturation time is the time it takes the FP to correctly fold and create the chromophore. This can be from a few minutes after it is translated to a few hours. For example, superfolder GFP (sfGFP) and mNeonGFP can fold in <10min at 37°C, mCherry takes ~15min, TagRFP ~100min and DsRed ~10hours.

5. **Temperature:** FPs maturation times and fluorescent intensity can be affected by the temperature. For instance, enhanced GFP (EGFP) was optimized for 37°C, and is therefore most suited for mammalian or bacteria studies, whereas GFP^{S65T} is better suited for yeast studies (24-30°C).

6. **Brightness:** The brightness is a measure of how bright is the emission. Brightness is calculated as the product of extinction coefficient and quantum yield of the protein, divided by 1000. In many cases the brightness is compared to that of EGFP which is set as 1. Some proteins are very dim (e.g. TagRFP657, which has a brightness of 0.1) and this should be taken into account.

7. **Photostability:** Fluorescent molecules gets bleached (i.e. lose the ability to emit light) after prolonged exposure to excitation light. Photostability can be as short as 100ms (EBFP) or as long as 1 hour (mAmetrine1.2). However, for most FPs it is a few seconds to a few minutes.

8. **pH Stability:** This parameter is important if you are planning to express the FP in acidic environments (e.g. yeast cytosol, which is slightly acidic, or synaptic vesicles). Some FPs have different ex/em spectra (e.g. mKeima) or change fluorescent intensity upon pH changes (e.g. pHluorin, pHTomato).

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