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Folding of FluoroCubes

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1 Works for me dx.doi.org/10.17504/protocols.io.8k2huye

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ABSTRACT

Photobleaching limits extended imaging of fluorescent biological samples. Here, we developed DNA based “FluoroCubes” that are similar in size to the green fluorescent protein (GFP), have single-point attachment to proteins, have a ~60-fold higher photobleaching lifetime and emit ~50-fold more photons than single organic dyes. We demonstrate that DNA FluoroCubes provide outstanding tools for single-molecule imaging, allowing the tracking of single motor proteins for >800 steps with nanometer precision.

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ATTACHMENTS

Supplementary-Tables.pdf

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Fluorescence, Dyes, Single-molecule, TIRF microscopy

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MATERIALS

NAME	CATALOG #	VENDOR
Freeze N Squeeze™ DNA Gel Extraction Spin Columns	732-6165	BioRad Sciences
Magnesium chloride hexahydrate	M9272	Sigma – Aldrich
Trizma® base	T1503	Sigma – Aldrich
Trizma® hydrochloride	T3253	Sigma – Aldrich
Boric acid	B6768	Sigma – Aldrich
Agarose LE	RBA-500	Thomas Scientific
Ethylenediaminetetraacetic acid disodium salt dihydrate	E5134	Sigma – Aldrich

Introduction 5m

1 5m
This protocol shows how the six dye FluoroCubes (described on BioRxiv) are designed, folded and purified. This protocol can not only be applied to the six dye FluoroCubes but also to all other DNA nanostructures described in the manuscript such as the Single Dye Cubes (FluoroCube with one dye), the double-stranded DNA constructs with one dye as well as the Compact Cubes with one or six dyes. The only differences between these designs will be the oligonucleotides used to assemble each construct (as described in detail in Step 3 and the attached Supplementary Tables).
For simplicity, we will only use the six dye Fluorocube as an example throughout the protocol.

Design 1h 30m

2 30m
Pick a dye that you would like to use for the six dye FluoroCube. We have tested ATTO 488, ATTO 565, ATTO 647N, Cy3, Cy3N (sulfonated Cy3), and Cy5. Based on our experience, ATTO 647N and Cy3N work very well as six dye FluoroCubes and we recommend using these two dyes.

3 1h
Order the modified oligonucleotides from your favorite oligonucleotide synthesis company. Note, that different companies may use different linkers to attach the dyes to the oligos. This in turn may result in a different performance of the FluoroCubes because the linker length (and attachment chemistry) likely plays a role in achieving high photostability. We list all modified oligonucleotides used in this study and their respective manufacturer in Supplementary Table 1. To pick the right sequences for your desired FluoroCube, look at Supplementary Table 2. In Supplementary Table 2 we list which modified oligonucleotides from Supplementary Table 1 you need to purchase in order to create the desired FluoroCube.

Supplementary-Tables.pdf

Folding 15h 30m

4 15m
Dissolve each oligo in deionized water to a final concentration of 100 µM. Make 5 µl aliquots for each oligo and store at -20° C.

5 15m
For a 50 µl folding reaction:
Take 5 µl of each of the four oligos required to fold the desired six dye FluoroCube (10 µM final) and add it to a PCR tube. Then add 25 µl of deionized water, and 5 µl of the 10x folding buffer (50 mM Tris pH 8.5, 10 mM EDTA and 400 mM $MgCl_2$).

6 15h
Run your samples in a thermocycler to fold the six dye FluoroCubes with the following temperature ramp (it takes about 15 hours total):

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1. Denaturation at 85° C for 5 min
2. Cooling from 80° C to 65° C with a decrease of 1° C per 5 min
3. Further cooling from 65° C to 25° C with a decrease of 1° C per 20 min
4. Hold at 4° C.

Purification 4h

7 Folding products are analyzed by 2.0% agarose gel electrophoresis in TBE (45 mM Tris-borate and 1 mM EDTA) with 12 mM $MgCl_2$ at 70 V for 3 hours. The gel box should be placed in an ice bath to minimize potential unfolding. After the gel finished running, it can be analyzed by any multicolor gel imager (e.g. Typhoon scanner). Note, do not add any DNA-stain such as ethidium bromide as it will bind to the DNA and therewith might interfere with absorbance and emission pattern of the FluoroCubes in downstream imaging applications. 3h 30m

8 The desired band can then be extracted from the gel using a razor blade. It is typically the top band (See Figure 1c in the BioRxiv manuscript). Afterwards the excised band is placed in a Freeze 'N Squeeze column (BioRad: 732-6165) and centrifuged for 3 min at 12,000 rcf at 4° C. The flow-through contains the desired FluoroCube. The FluoroCubes can be stored at 4° C for a couple of weeks. 30m

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