BioCode Gold-Nanobeacon for the detection of fusion transcripts causing chronic myeloid leukemia

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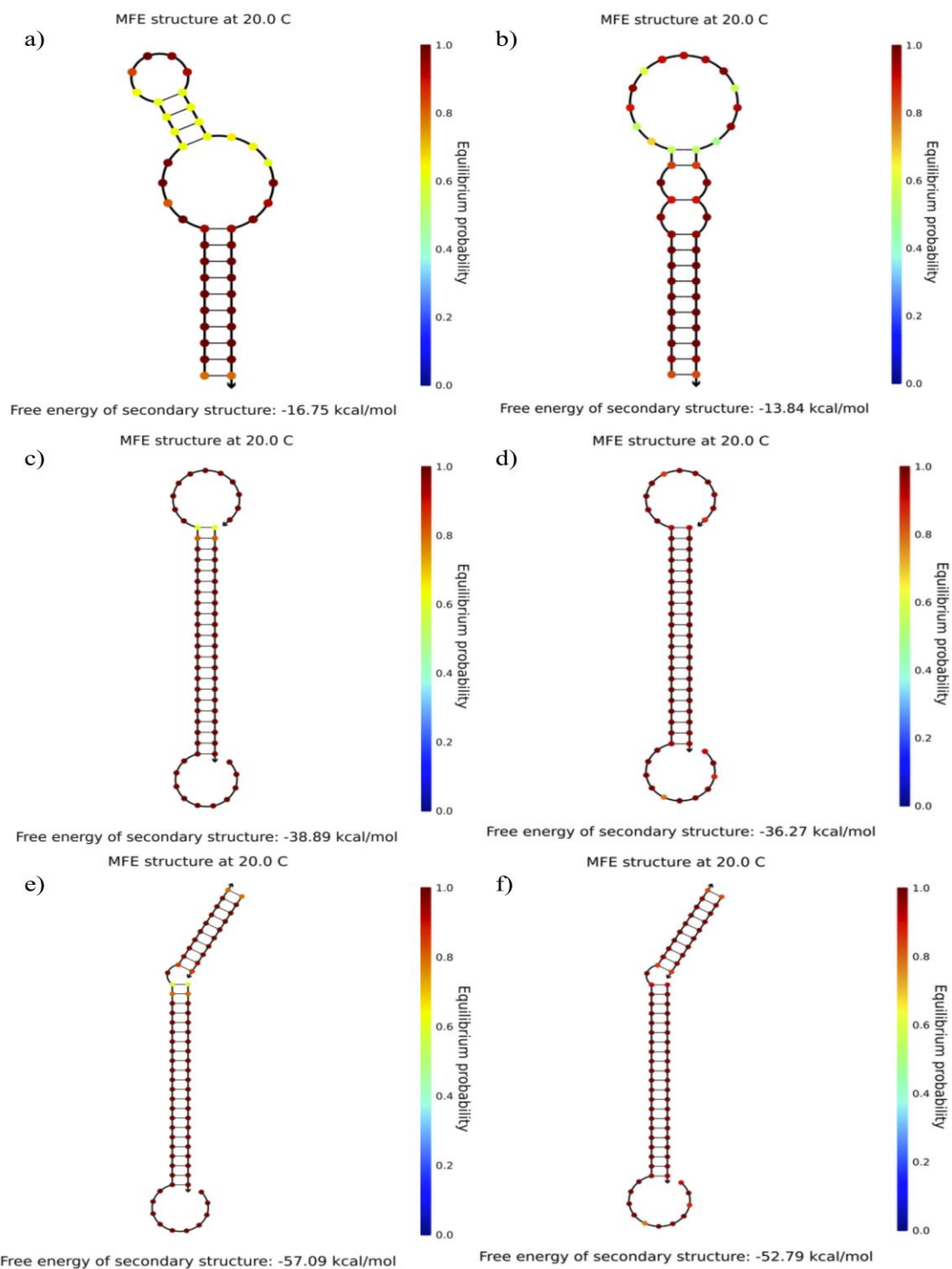* Corresponding Author. Email: pmvb@fct.unl.pt; Phone/Fax: +351 21 294 8530.

Figure S1. *in silico* verification of hybridization of the designed sequences using the software NUPACK; a) e13a2-Hairpin in absence of target; b) e14a2-Hairpin in absence of target; c) e13a2-Hairpin in the presence of complementary target; d) e14a2-Hairpin in presence of complementary target; e) e13a2-Hairpin in presence of complementary target and of complementary acceptor labeled oligonucleotide; f) e14a2-Hairpin in presence of complementary target and of complementary acceptor labeled oligonucleotide.
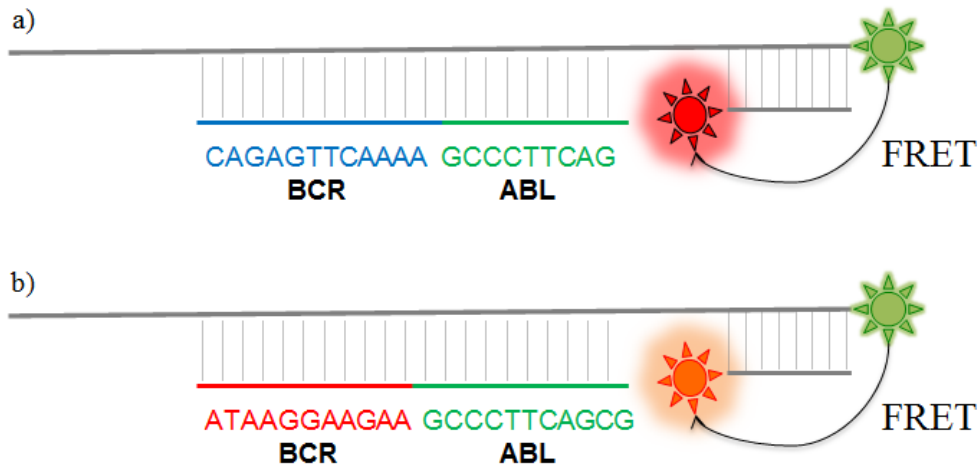
Figure S2. Schematic representation of specific target recognition by the designed hairpins; a) e14a2-hairpin hybridized to the e14a2 target; b) e13a2-hairpin hybridized to the e13a2 target;
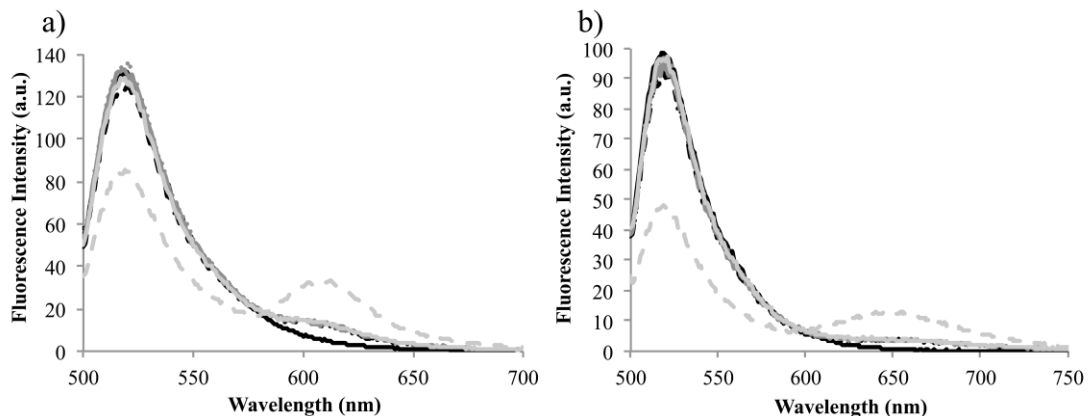

Figure S3. Experimental validation of hybridization of the designed sequences; a) e13a2-hairpin characterization; b) e14a2-hairpin characterization. Emission spectra of: donor blank reaction (solid black line); reaction blank (dashed black line); non-complementary reaction (dotted black line); ABL reaction (solid dark grey); BCR reaction (dashed dark grey line); BCR+ABL reaction (dotted dark grey line); cross template reaction (solid light grey line); positive reaction (dashed light grey line).
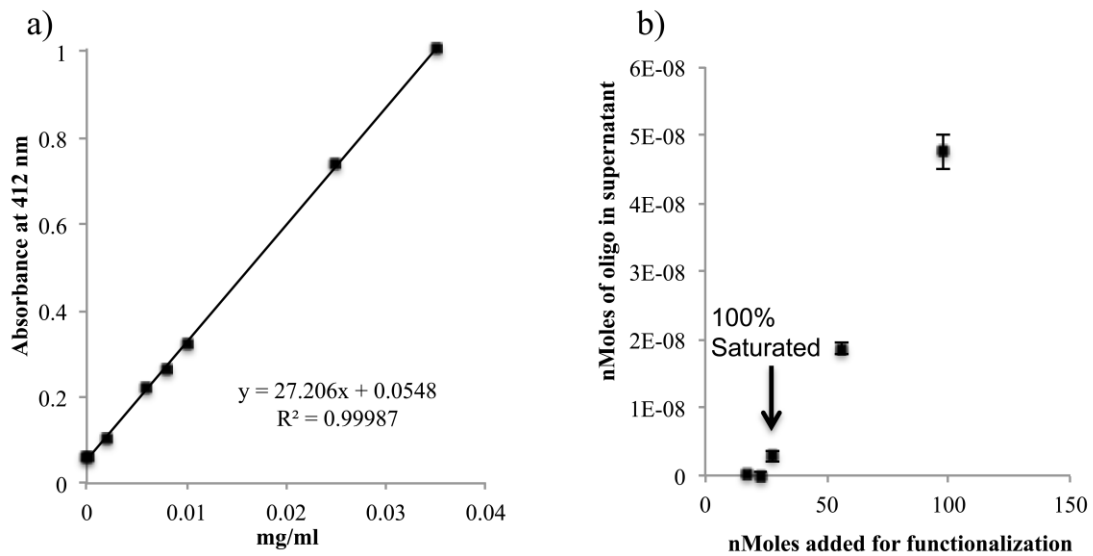
Figure S4. Calibration of PEG functionalization of AuNP surface.
a) Calibration curve used for the quantification of thiolated PEG in the supernatant; b) Quantification of PEG in supernatant as a function of the added PEG, corrected for the residual absorbance of DTNB. The error bars represent 3 independent assays;
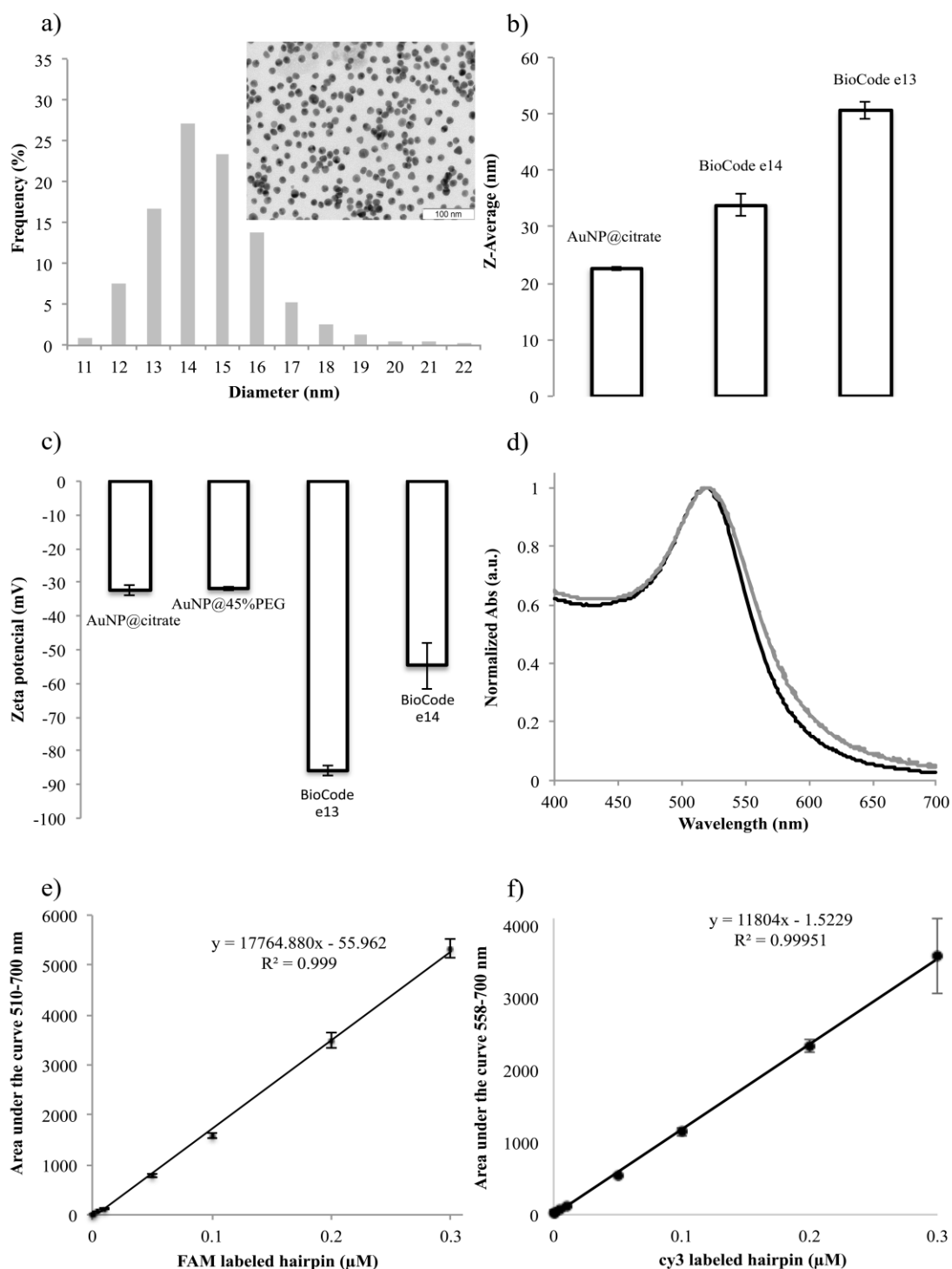
Figure S5. Characterization of the synthetized BioCodes and gold nanoparticles;
a) Size distribution of the synthetized AuNP, Inset: TEM image of the AuNP (scale bar: 100nm); b) Hydrodynamic diameter of the AuNP, BioCode-e13 and BioCode-e14; c) zeta potential of citrate capped AuNP, PEG and Au-nanobeacons d) UV-VIS spectra of the AuNP (solid black line), BioCode-e13 (solid grey line) and BioCode-e14 (dashed grey line); e) calibration curve for the quantification of hairpins per AuNP for the BioCode-e13; f) calibration curve for the quantification of hairpins per AuNP for the BioCode-e14; error bars correspond to 3 independent assays
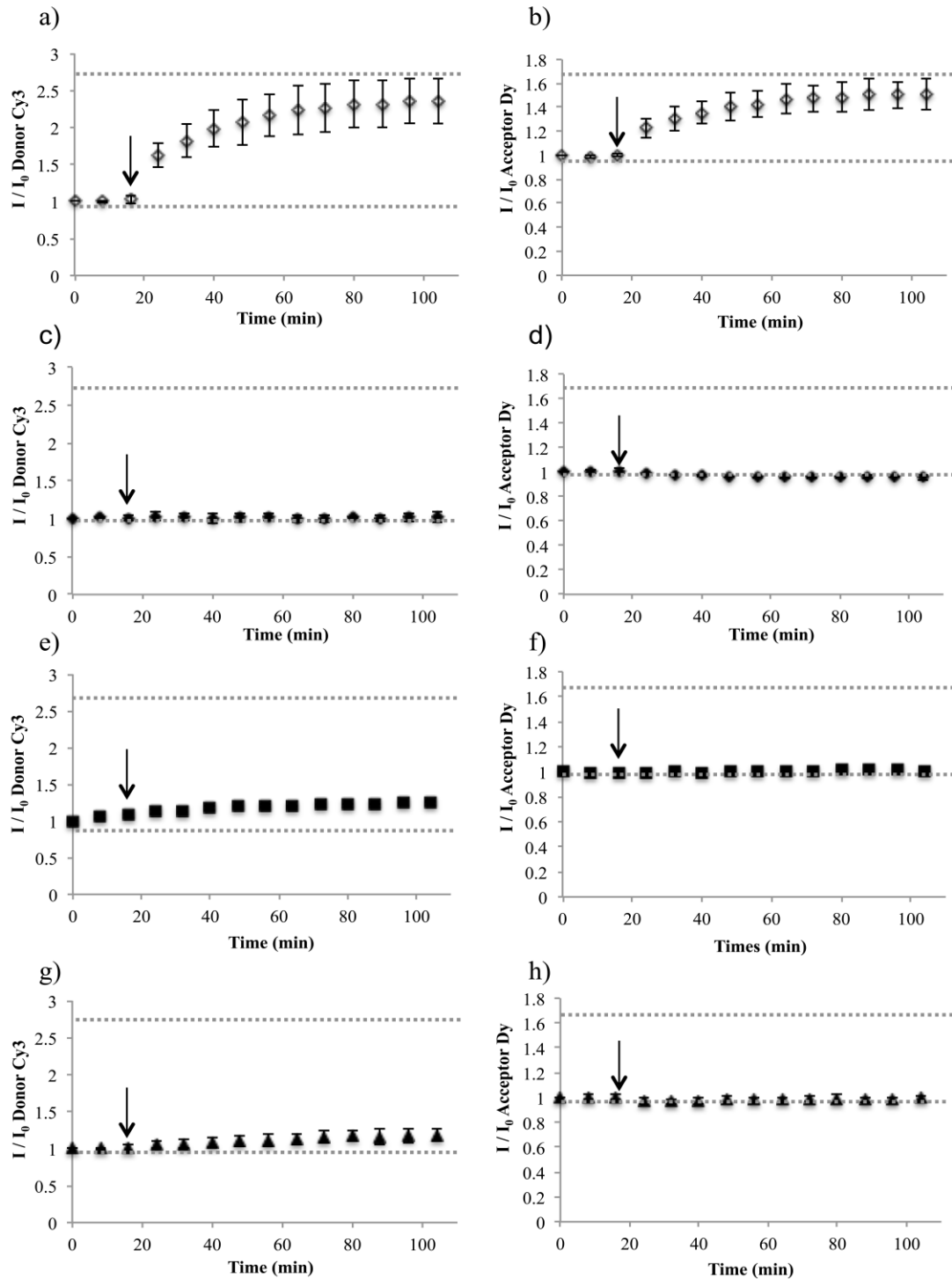
Figure S6. Hybridization assays of BioCode e14.
a) BioCode-e14 donor emission in presence of e14a2 complementary target (white diamonds); b) Acceptor-Dy emission in presence of e14a2 complementary target; c) BioCode-e14 donor emission in presence of non-complementary target (black diamonds); d) Acceptor-Dy emission in presence of non-complementary target (black diamonds); e) BioCode-e14 donor emission in presence of exon 14 BCR derived target (black squares); f) Acceptor-Dy emission in presence of exon 14 BCR derived target (black squares) g) BioCode-e14 donor emission in presence of ABL target (Black diamonds); h) Acceptor-Dy emission in presence of ABL target (Black diamonds). The error bars represent at least 3 independent assays. The black arrow represents the addition of the target sequence.
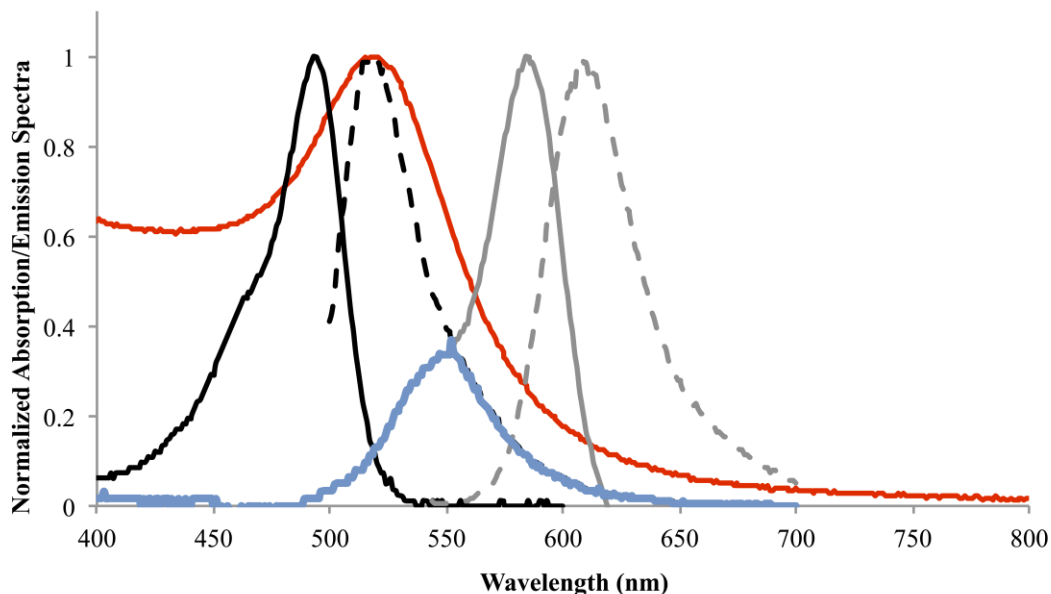
Figure S7. Normalized absorption and emission spectra of the fluorophores used for BioCode-e13 and absorption spectra of AuNP.

Solid black line –FAM Absorption, dashed black line- FAM emission, solid grey line –ROX Absorption, dashed grey line –ROX Emission; Solid red line- AuNP Absorption; Solid blue line – overlap between emission spectra of FAM and absorption spectra of ROX;
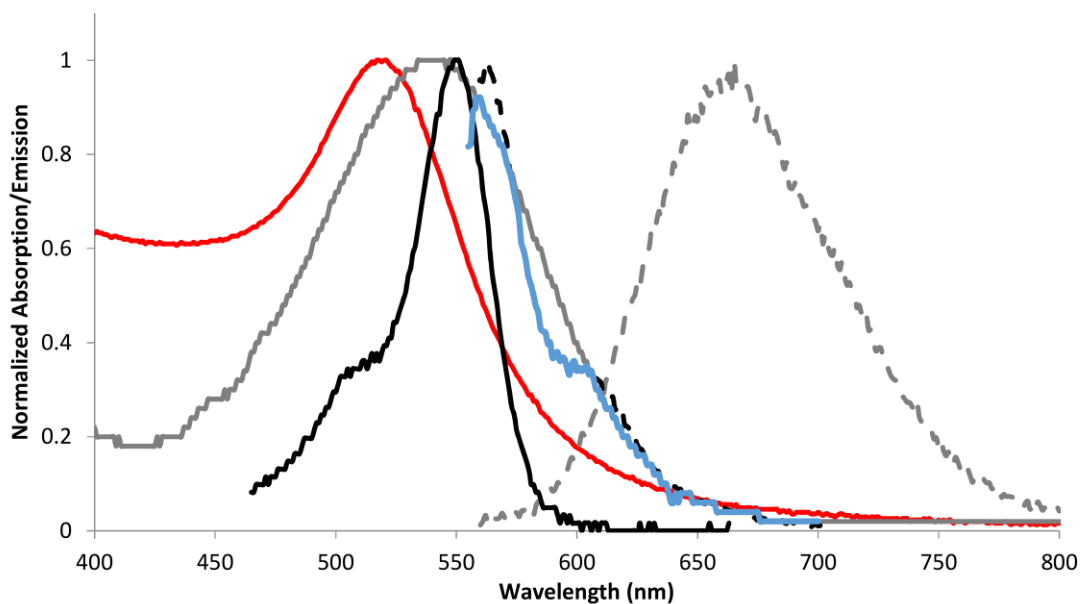

Figure S8. Normalized absorption and emission spectra of the fluorophores used for BioCode-e14 and absorption spectra of AuNP.

Solid black line – Cy3 Absorption, dashed black line – Cy3 emission, solid grey line – Dy-520XL megastockes Absorption, dashed grey line – Dy-520XL megastockes Emission; Solid red line- AuNP Absorption; Solid blue line – Overlap between emission spectra of Cy3 and absorption spectra of Dy-520XL megastockes;