Literature Report

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COMMUNICATION

Environmentally Sensitive Color-Shifting Fluorophores for Bioimaging

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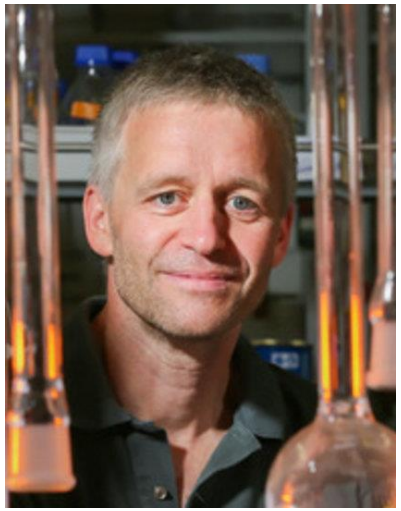
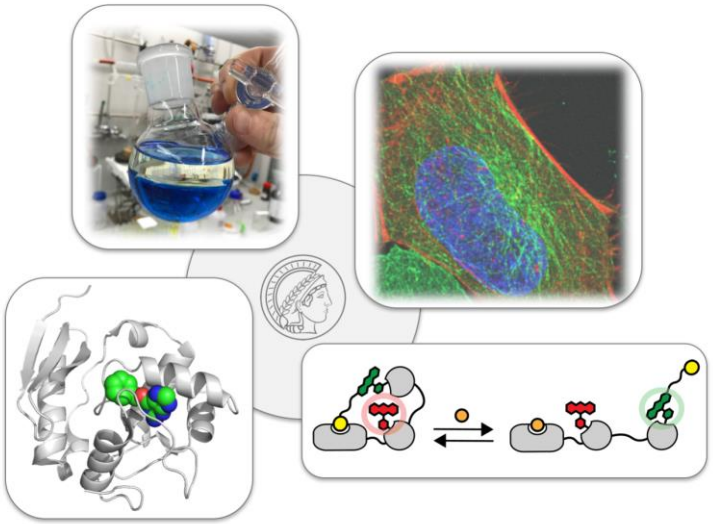


Kai Johnsson



- Development of semisynthetic fluorescent sensor proteins to measure key metabolites in living cells.
- Development and application of methods for characterizing protein-protein interactions.
- Generation of small molecules for controlling protein function in living cells.
- Engineering of new protein functions for applications in functional proteomics.
- Synthesis of new spectroscopic probes for applications in cell biology.
- Mechanistic studies on drug candidates

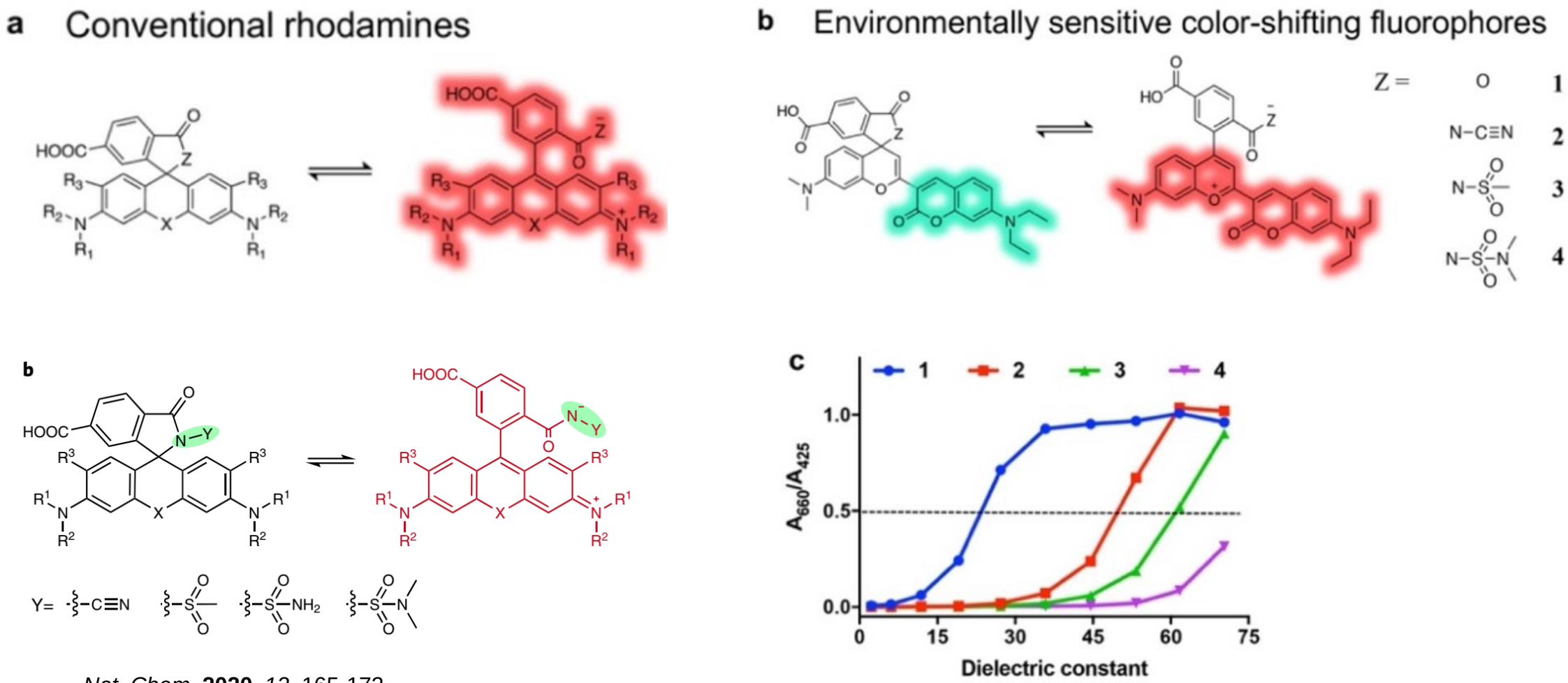


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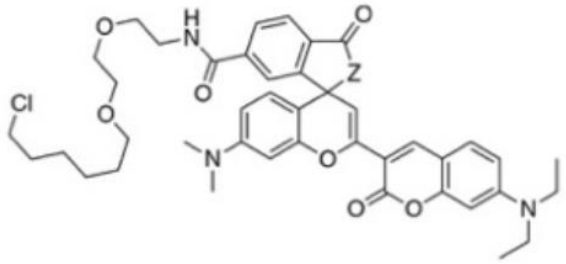
Figure 1. (**b**) Structure of the color-shifting fluorophores in equilibrium between green fluorescent spirocyclic and red fluorescent zwitterionic forms. (**c**) Normalized ratio of absorbance in red/green channels (A650 / A420) of **1**-**4** (10 μM) as a function of dielectric constant (water–dioxane mixture). The ratios of **1**-**4** were normalized to the maximum ratio of **1**

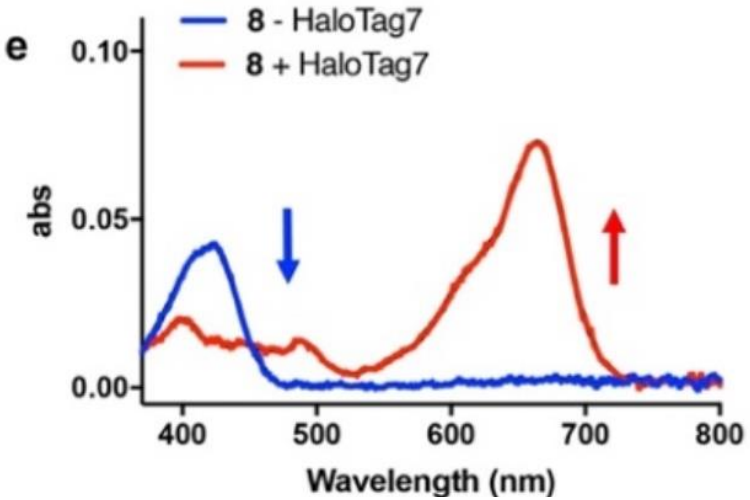
d



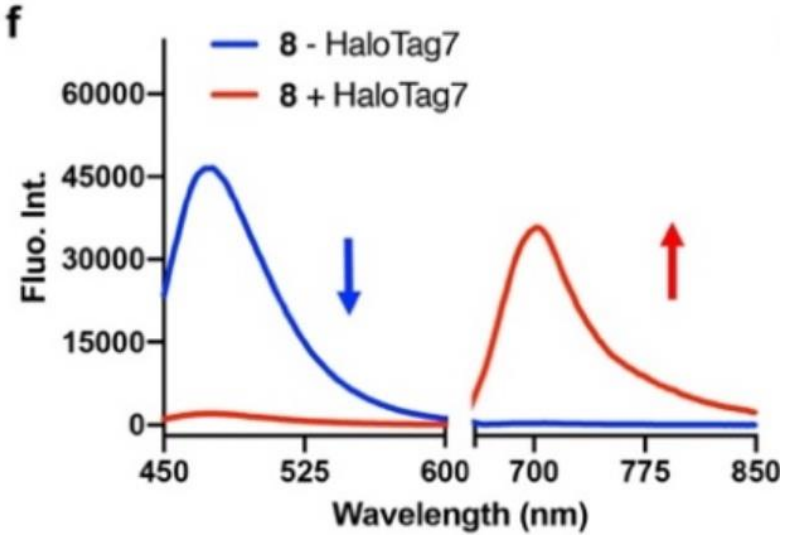
5 Z = O; **6** Z = NCN

7 Z = NSO_2CH_3; **8** Z = $NSO_2N(CH_3)_2$

e



f



g

Probe	λ_{ex} (green/red)	$\varepsilon(M^{-1}cm^{-1})$ (green/red)	λ_{em} (green/red)	ϕ (green/red)	Ratio Increase
5	-[a]/665[b]	-[a]/72000[b]	-[a]/705[b]	-[a]/0.12[b]	2.9 ± 0.8
6	426[a]/665[b]	28000[a]/75000[b]	476[a]/706[b]	0.1[a]/0/1[b]	116.5 ± 5.8
7	428[a]/666[b]	33000[a]/76000[b]	477[a]/707[b]	0.15[a]/0.11[b]	254.4 ± 4.2
8	428[a]/667[b]	41000[a]/72000[b]	476[a]/706[b]	0.1[a]/0.13[b]	2391.9 ± 34

Figure 1.(**d**) Chemical structures of **5**-**8** for HaloTag7 labeling. (**e**, **f**) Absorption (**e**) and fluorescence emission (**f**) spectra of **8** (1 μM) measured in the absence (blue line) and presence of HaloTag7 (5 μM, red line). (**g**) Photophysical properties of **5**-**8** and fluorescence ratio (F707/ F475) change induced upon HaloTag7 labeling. Data werecollected in absence (*a*) and presence (*b*) of HaloTag7.

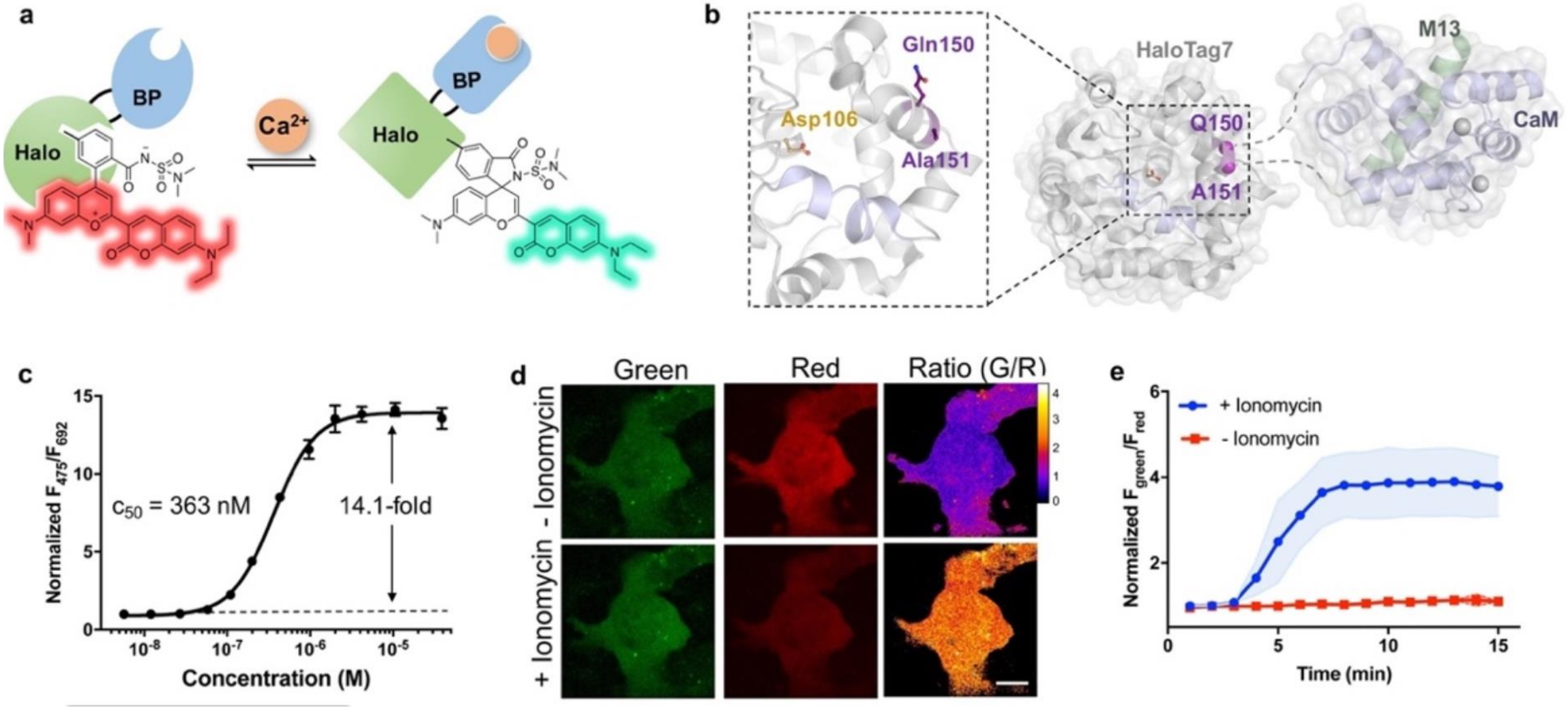
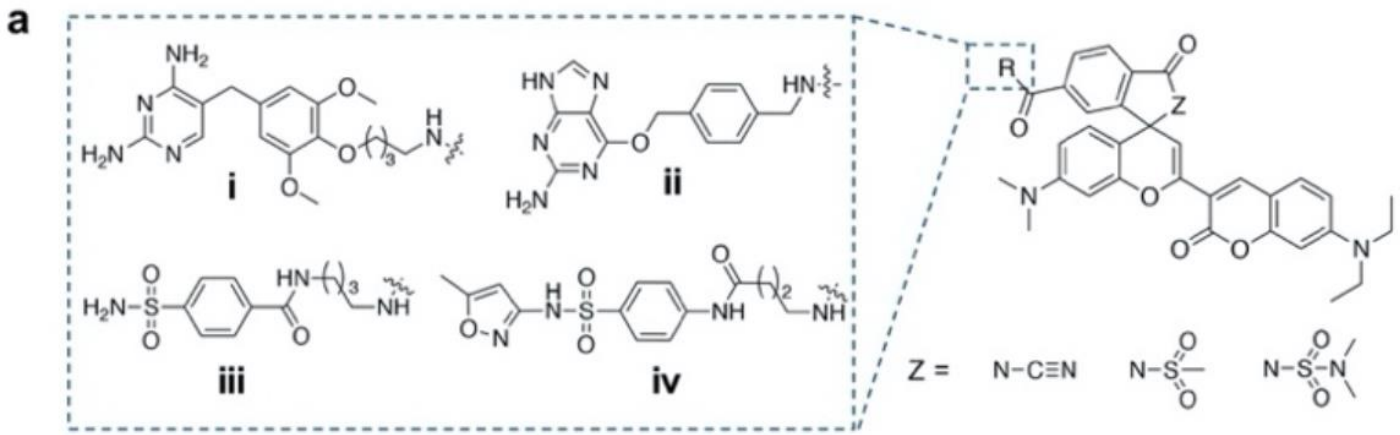


Figure 2. CSF-based Ca2+ biosensor. (**a**) Cartoon of CSF-based semisynthetic Ca2+ biosensor. Ca2+ binding induces a conformational change, thereby shifting the equilibrium between red and green fluorescent forms. (**b**) Schematic representation of rHCaMP. The HaloTag7 crystal structure (4kaj) is represented as grey cartoon. The CaM-M13 domains were extracted from the GCaMP2 crystal structure (3evr) and are represented as blue (CaM) and green (M13) cartoons. Both HaloTag7 and CaM/M13 were positioned to fuse of CaM/M13 to rHCaMP via Q150 and A151 of HaloTag7 (α-carbons represented as purple spheres). (**c**) rHCaMP-**8** sensor titration with Ca2+. (**d**) Live-cell Ca2+ imaging with rHCaMP-**8**. U2OS cells expressing rHCAMP **8** and treated with ionomycin (1 μM) prior to imaging. Scale bar: 20 μm. (**e**) Time course analysis of fluorescence ratio Fgreen/Fred upon ionomycin treatment. The ratio was normalized to the value at 0 min. s.d. is represented as shade area. N = 20 cells from 5 independent experiments.



b

Probe	R + CSF (Z)	Protein Target	Ratio Increase
9	i + **4** ($NSO_2N(CH_3)_2$)	eDHFR	2342 ± 175
10	ii + **2** (NCN)	SNAP	7.3 ± 0.2
11	Iii + **3** (NSO_2CH_3)	HCAII	41.0 ± 3.5
12	iv + **3** (NSO_2CH_3)	hSPR	13.8 ± 0.3

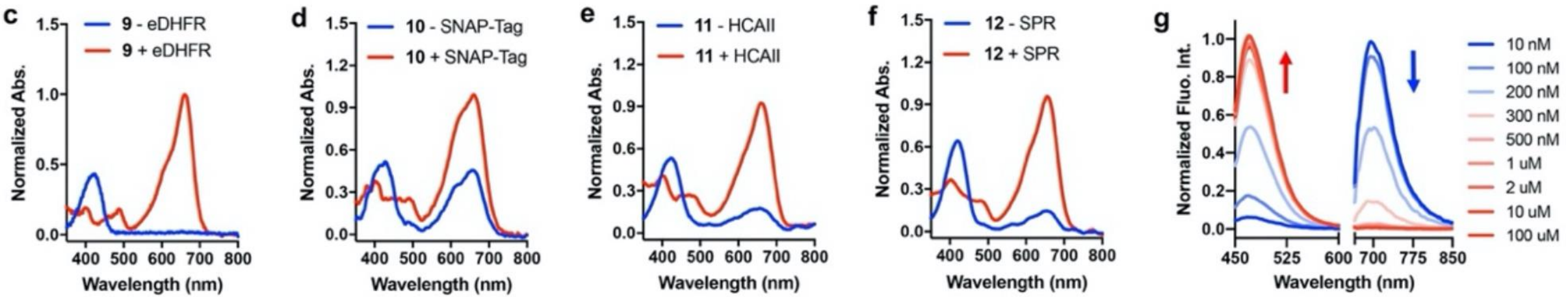


Figure 3. Probes for proteins and metabolites based on CSFs. (**a**) General structure of CSF-based probes for eDHFR (i; based on trimethoprim); SNAP-tag (ii; based on benzylguanine); HCAII (iii; based on benzenesulfonamide); hSPR (iv; based on sulfamethoxazole). (**b**) CSF-based probes **9**-**12** and fluorescence ratio change (F707 / F475) induced upon binding to target proteins. (**cf**) Normalized absorbance of CSF **9** (**c**), **10** (**d**), **11** (**e**), **12** (**f**) in absence and presence of protein target. (**g**) Normalized fluorescence emission spectra of **9** in the presence of eDHFR and various concentrations of the competitor methotrexate (MTX).

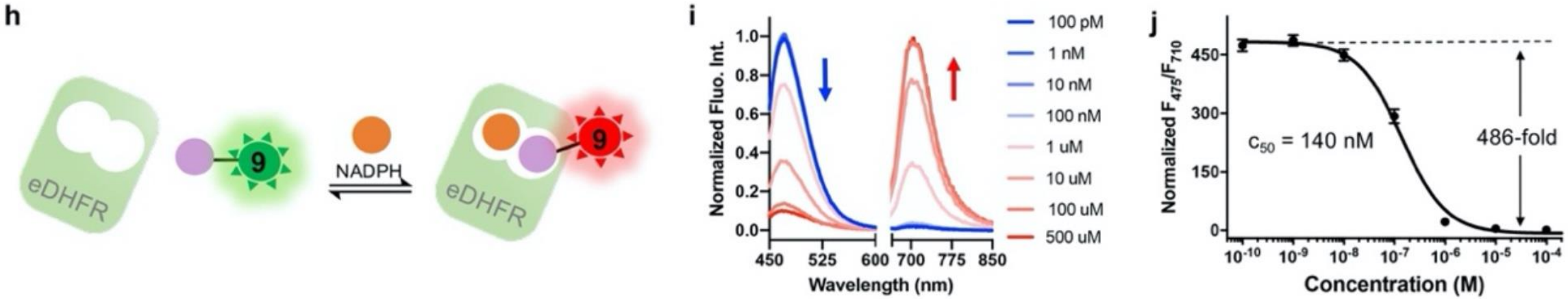


Figure 3.(**h**) A CSF-based ratiometric sensor for NADPH. Binding of **9** to the eDHFR mutant is strictly dependent on NADPH, offering a ratiometric fluorescent readout. (**i**) Normalized emission spectra of **9** in presence of eDHFR mutant and varying concentrations of NADPH. (**j**) Normalized fluorescence ratio of **9** in presence of eDHFR mutant and varying concentrations of NADPH. Error bars show ± s.d. from triplicate experiment

Thanks !