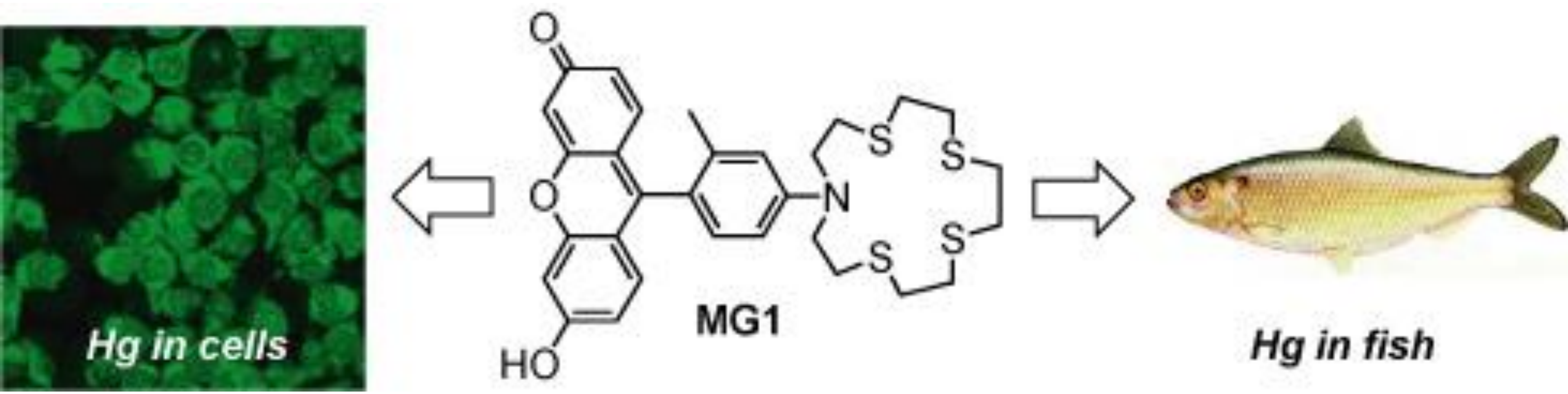
Ion selective synthetic fluorescent chemosensors



General issues

- Cation sensing by chemosensors *in vivo*
- Fluorescent signal is much more sensitive than coloring (histochemical dyes)
- Small sized sensor can diffuse through membranes
- At present: calcium, magnesium, sodium, potassium, zinc, copper, iron, cadmium, mercury
- Sensor design: as discussed before
- Detection: translate binding event into a readable signal

Binding / dissociation constants

- $K_a = \dfrac{[M_x L_y]}{[M]^x [L]^y}$

- Many factors can affect the ligand coordination sites (e.g. protonation etc.) → apparent stability constant: $K_a^{app} = \dfrac{[ML]}{[M'][L']}$

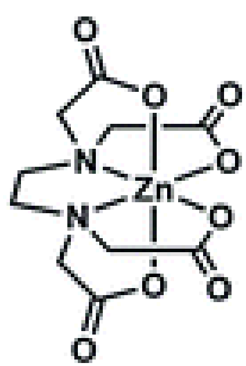
- Here M’ and L’ refers to all kinds of M and L species (free, protonated etc.)

Binding / dissociation constants

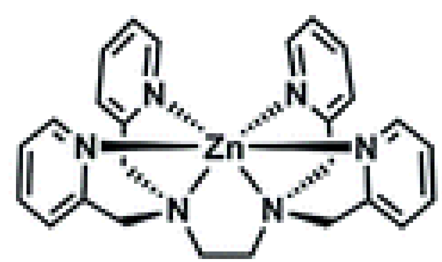
- The apparent K_a can be altered in cellular environment (eg. Fura-2 and Ca K_d = 145 nM in vitro and 371 nM in cells)
- The presence of complex equilibria makes it difficult to determine selectivity of one ion over another
- pM values refer to the –lg[M], where [M] is the free aqueous metal ion concentration under given conditions (e.g. pH, $[M]_{tot}$; $[L]_{tot}$)

pM relevancy

- If pKa of the ligand's donor atom is higher than pH of solution, protonation can efficiently compete with binding
- EDTA has larger K_a for Zn than TPEN
- EDTA (pK_{a1} = 10.19) TPEN (pK_{a1} = 7.12)
- The apparent K_a is more affected for EDTA, pM is lower (free Zn is higher)
- To judge ion selectivities the use of pM is highly recommended



$[Zn(EDTA)]^{2}$



$[Zn(TPEN)]^{2+}$

Signal transduction strategies

- Internal charge transfer (ICT) systems

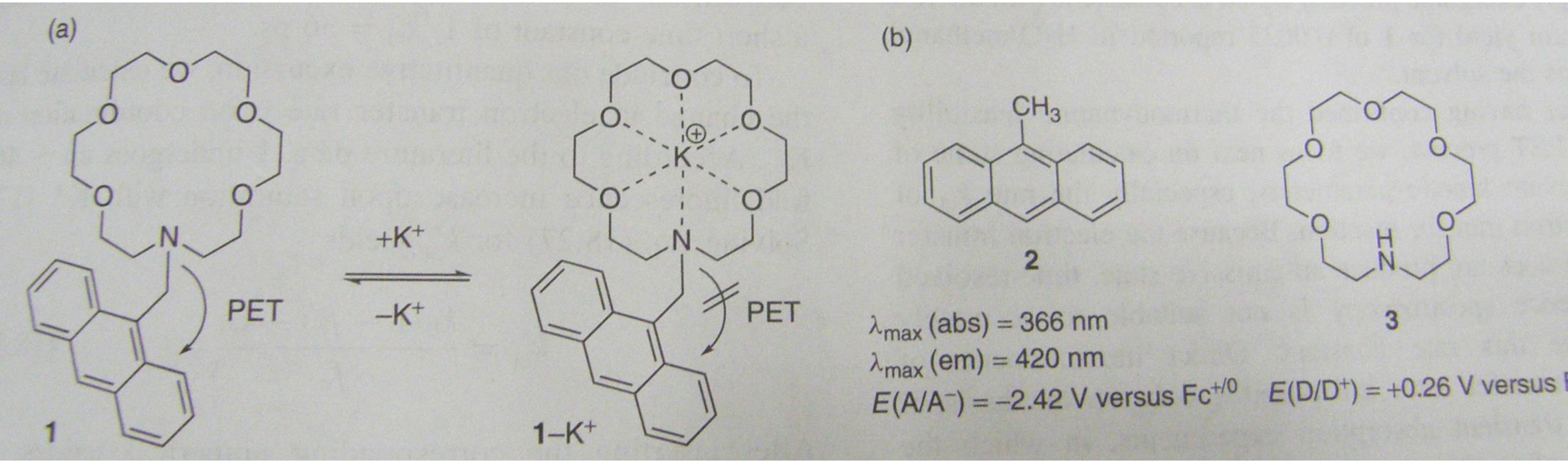
  - Enable two-point (ratiometric) detection
- Photoinduced electron transfer (PET) systems
  - Signal evolution can be detected very sensitively
- Excimers
  - Mainly for hybridization studies
- Energy transfer systems
  - Hybridization
  - Distance measurements
  - Ca-sensing protein, protein-protein interactions

Photoinduced electron transfer systems

- Spacer separated receptor – fluorophore (donor-acceptor)
- Driving force of PET can be estimated: Rehm-Weller equation $\Delta G_{ET} = E(D^{+}/D) - E(A/A^{-}) - \Delta E_{00} + \omega_{p}$

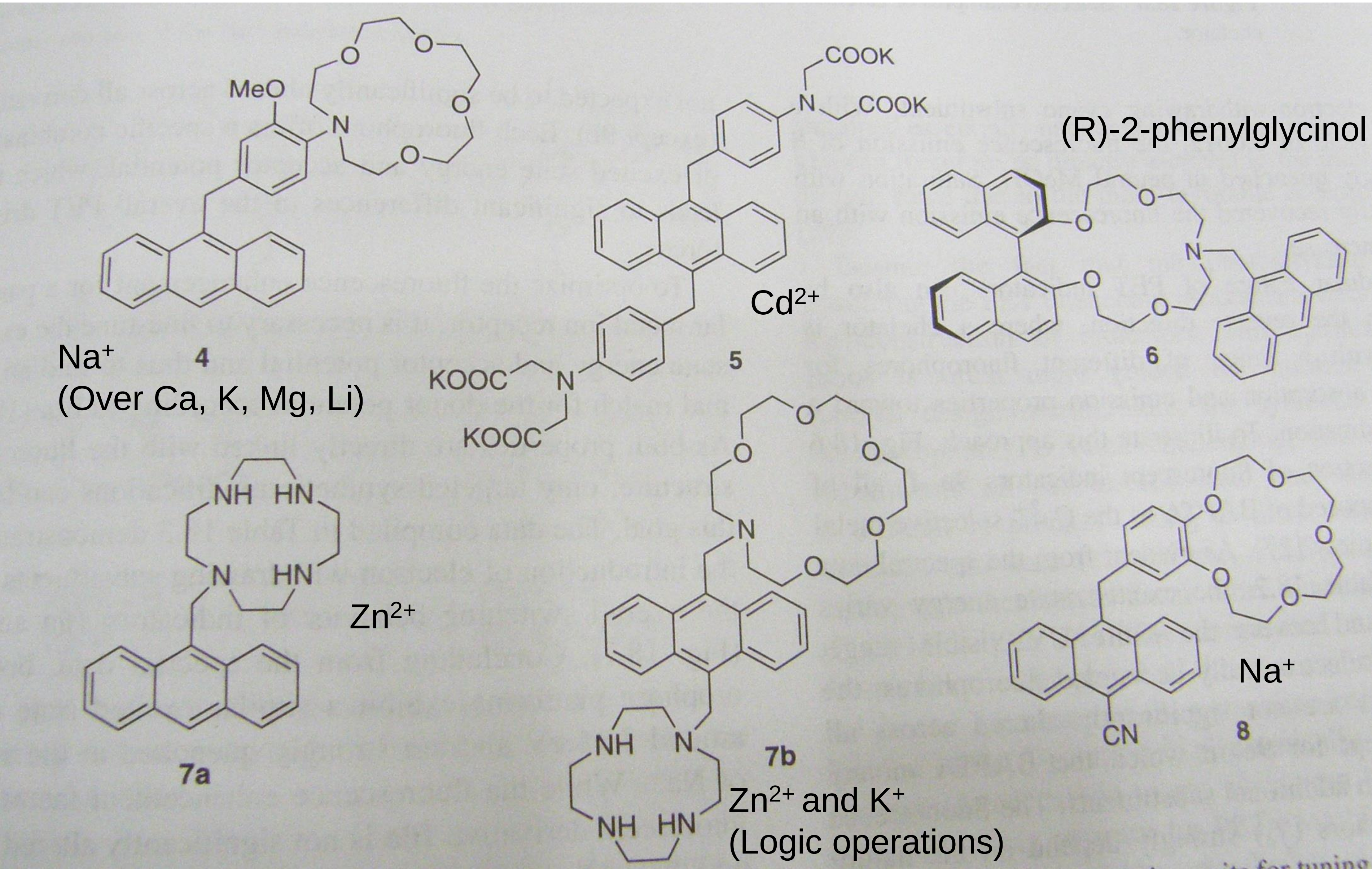
  Redox potentials of A, D, energy of the 0-0 transition and work related to coulombic stabilization
- Coordination changes donor potential therefore increases ΔG_{ET}
- FE = ϕ_f' / ϕ_f

Prediction



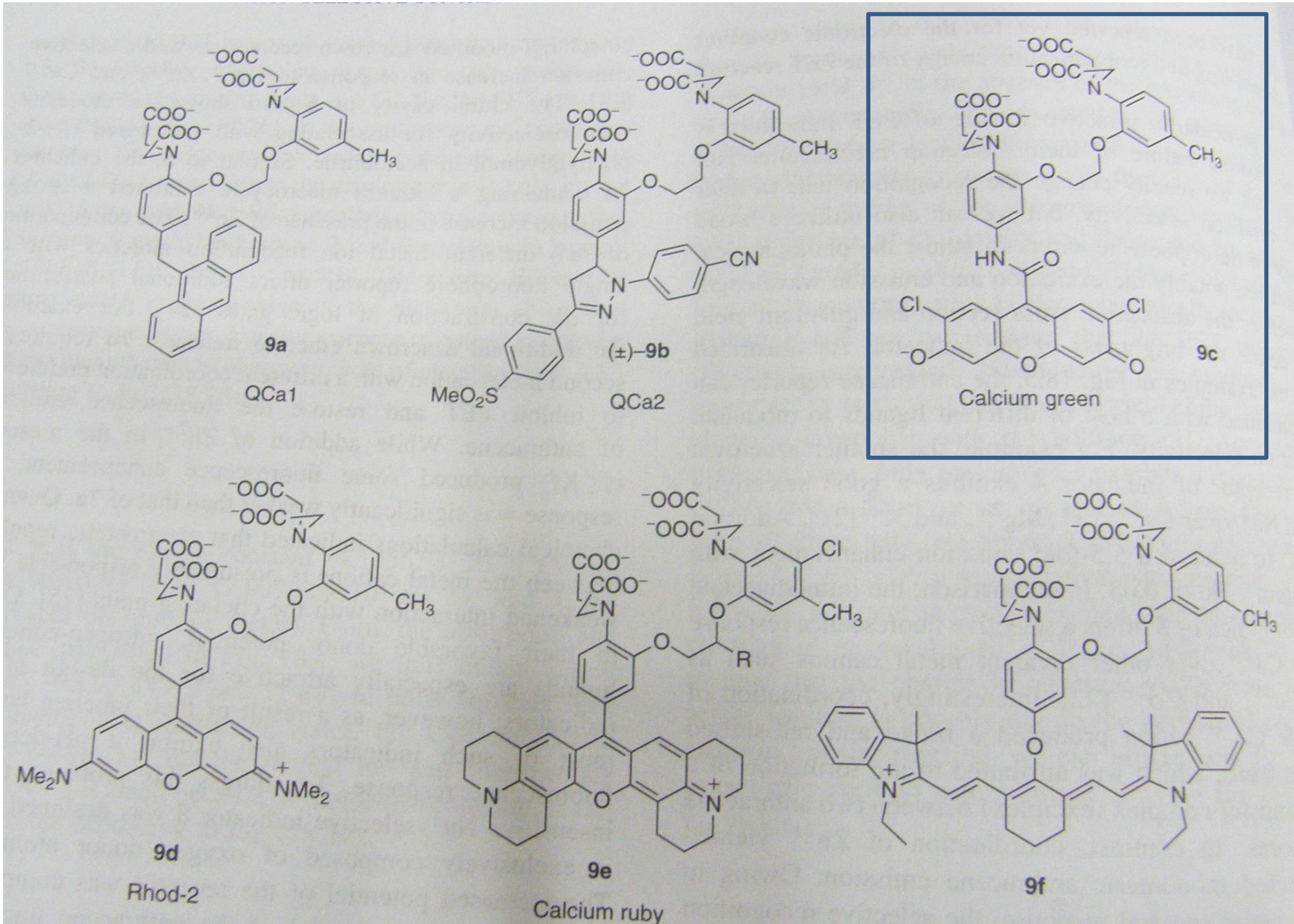
(a)
+K+
–K+
PET
PET
1
1–K+
(b)
CH3
2
3
λmax (abs) = 366 nm
λmax (em) = 420 nm
E(A/A⁻) = –2.42 V versus Fc+/0
E(D/D+) = +0.26 V versus

Examples



4
Na+
(Over Ca, K, Mg, Li)
5
Cd2+
(R)-2-phenylglycinol
6
7a
Zn2+
7b
Zn2+ and K+
(Logic operations)
8
Na+

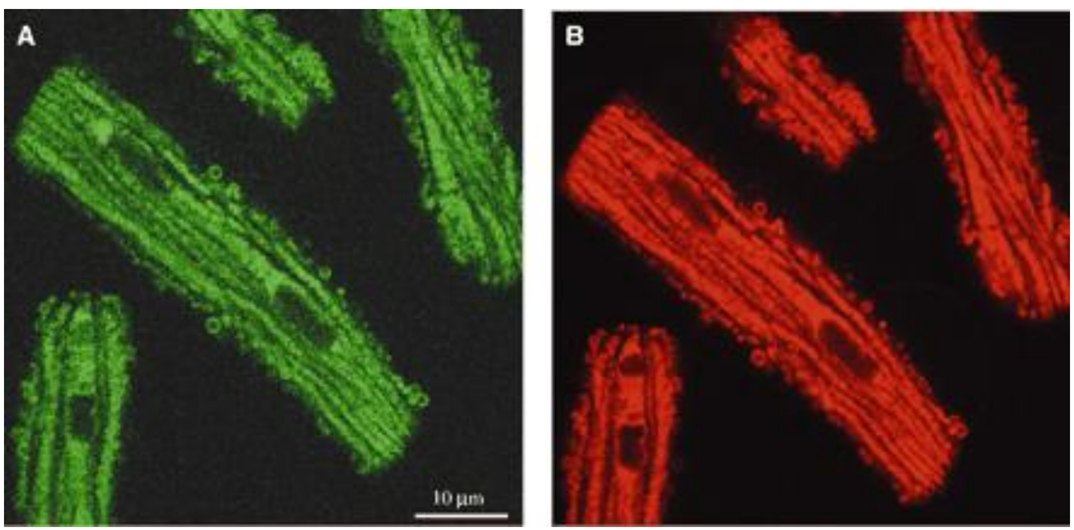
Calcium selective indicators - modularity



Calcium selective indicators - modularity

TABLE 18.2 Photophysical Data for Ca^{2+}-Responsive Fluorescent Indicators

Compound	λ_{abs} (nm)[a]	λ_{em} (nm)	E_{00} (eV)[b]	Φ_f[c]	$\Phi_f(Ca^{2+})$[d]	f_e[e]	log K[f]
9a	368 (3.92)	440	3.09	0.0014	0.023	16	6.6
9b	388 (4.43)	489	2.87	0.0011	0.1	92	6.5
9c	506 (5.17)	526	2.40	0.0051	0.183	36	6.4
9d	553 (4.98)	576	2.20	0.03	0.10	3.4	6.0
9e	579 (5.00)	598	2.11	0.026	0.42	32	4.5
9f	766 (5.30)	782	1.60	0.05	0.12	2.4	6.6

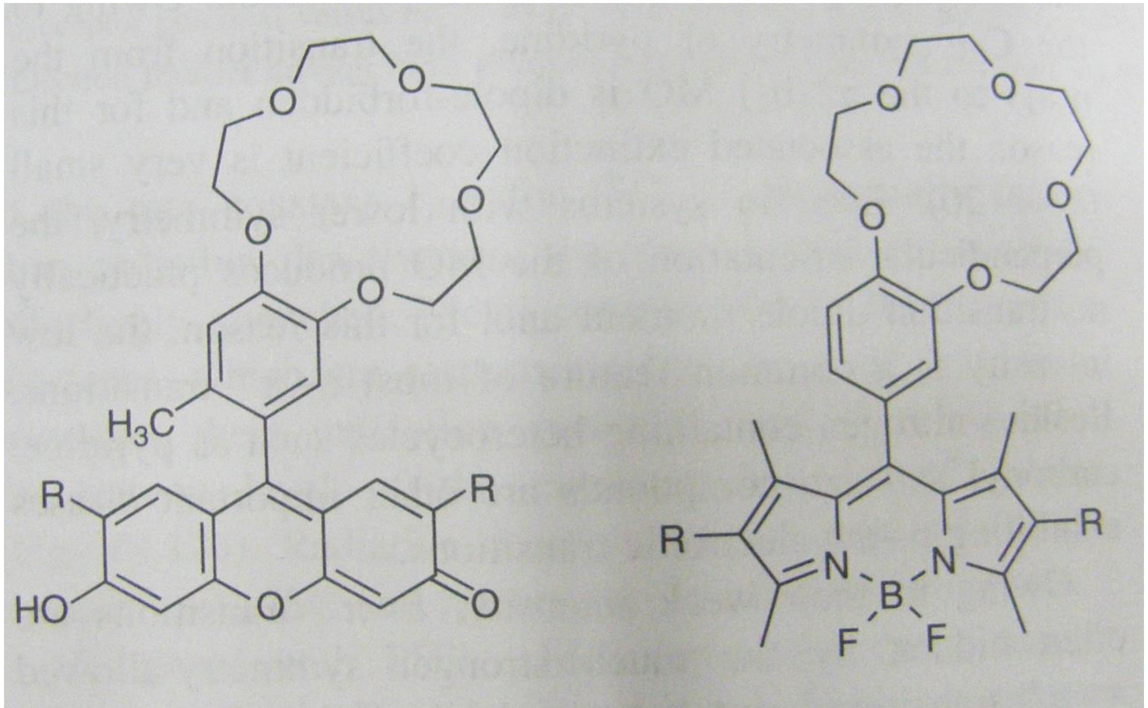


Green: Autofluorescence of flavoproteines (in mitochondria)

Red: Rhod-2 (free calcium)

Co-localization

Sodium selective indicators



10a R = H
10b R = Cl

11a R = H
11b R = COOEt

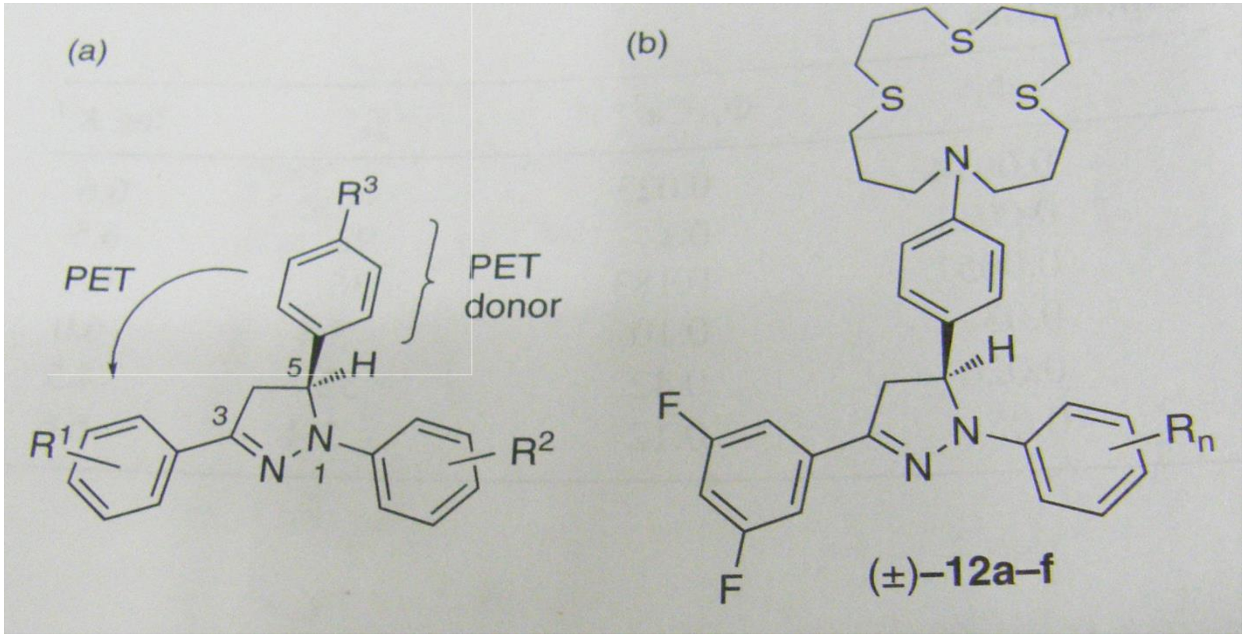
Find optimal D/A match

Tuning with different EWGs (11b)

TABLE 18.3 Effect of Structural Modifications on the Spectral Properties and Fluorescence Switching Behavior of Two Na^+-Selective Indicators Utilizing the Same Crown Ether as Metal Ion Receptor Moiety

Compound	λ_{abs} (nm)	λ_{em} (nm)	E_{00} (eV)[a]	Φ_f[b]	$\Phi_f(Na^+)$[c]	f_e[d]	log K[e]
10a	492	525	2.44	0.008	0.041	5.3	0.42
10b	506	515	2.43	0.005	0.025	4.6	0.35
11a	499	508	2.46	0.64	0.72	1.13	n.d.
11b	497	507	2.47	0.0027	0.10	37	2.50

Copper selective indicators



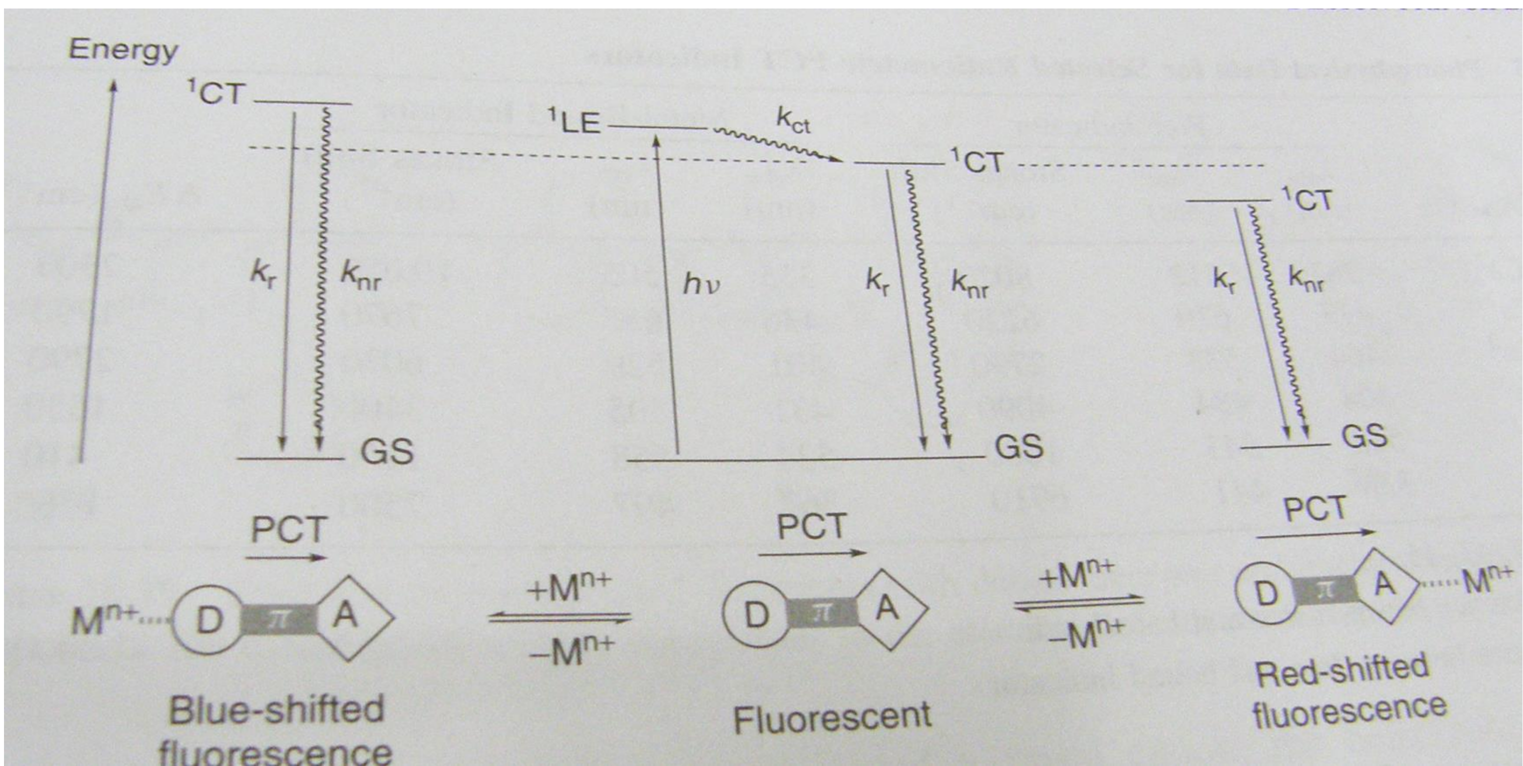
Copper is a soft LA that does not alter the donor's potential very much – fine tuning is necessary

TABLE 18.4 Photophysical Data for Cu^+-Responsive Fluorescent Indicators 12a–

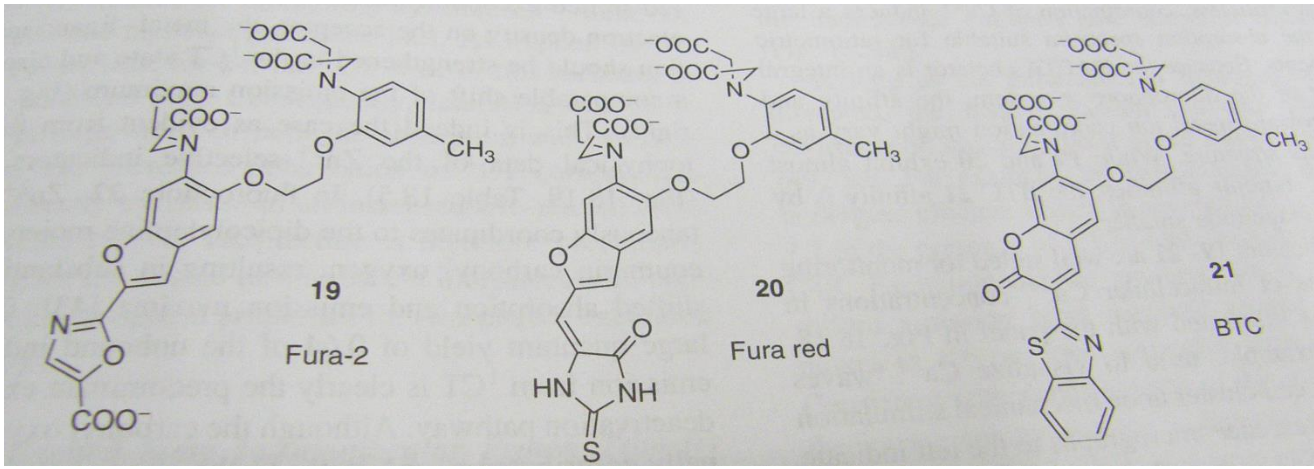
Compound	R_n	λ_{abs} (nm)	λ_{em} (nm)	$\Phi_f{}^a$	$f_e{}^b$	E
12a	—	371	476	0.20	3	
12b	3-F	366	461	0.19	7	
12c	2,5-F_2	355	451	0.12	14	
12d	2,3,5-F_3	350	436	0.07	50	
12e	2,3,5,6-F_4	330	420	0.024	—	
12f	2,3,4,5,6-F_5	323	423	0.0063	—	

Internal charge transfer (ICT) sensors

- Receptor and signaling units are integrated
- Guest induces shifted emission maximum
- Ratiometric sensing



Calcium ICT sensors



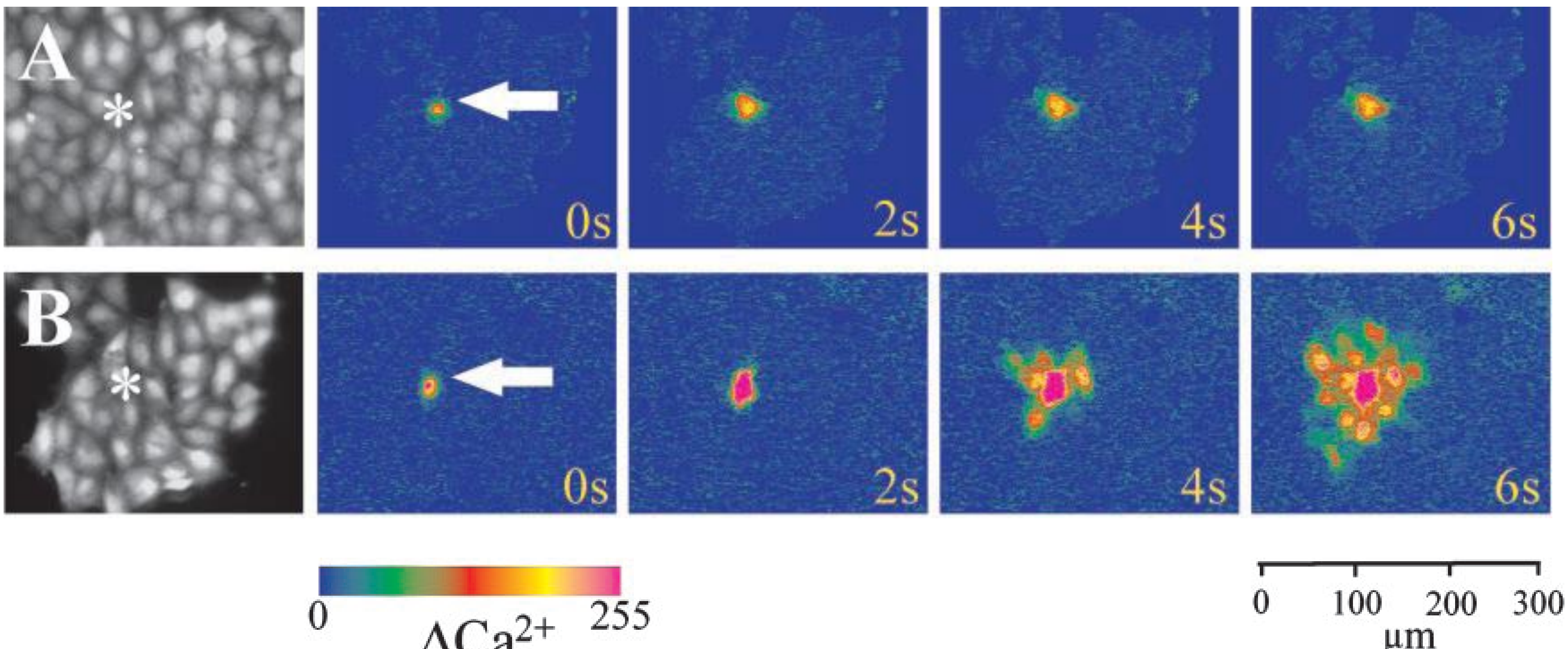
Abs and em are shifted

EM shift is much weaker
Due to dumpening of Ca

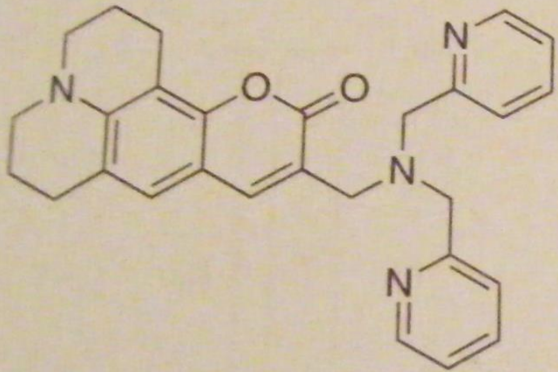
TABLE 18.5 Photophysical Data for Selected Ratiometric PCT Indicators

		Free indicator			Metal-Bound Indicator		
Compound	Analyte	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (cm^{-1})	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (cm^{-1})
19	Ca^{2+}	363	512	8020	335	505	10,050
20	Ca^{2+}	473	670	6220	436	655	7670
21	Ca^{2}	464	533	2790	401	529	6030
22	Zn^{2}	404	484	4090	431	505	3400
23	Zn^{2}	513	543	1080	524	558	1160
24	Zn^{2}	338	441	6910	362	497	7500

Fura-2 in cells



Zinc ICT sensors



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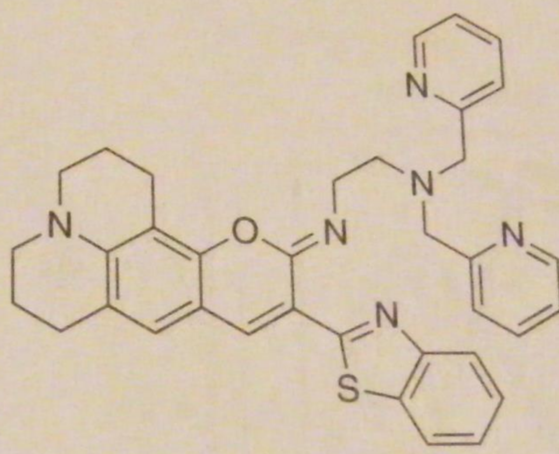

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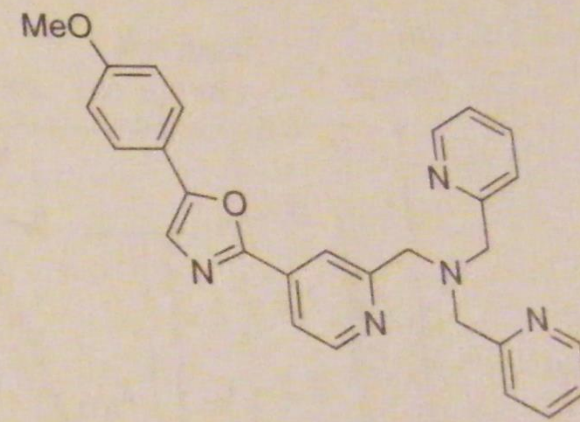

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TABLE 18.5 Photophysical Data for Selected Ratiometric PCT Indicators

Compound	Analyte	Free indicator λ_{abs} (nm)	Free indicator λ_{em} (nm)	Free indicator Stokes Shift (cm^{-1})	Metal-Bound Indicator λ_{abs} (nm)	Metal-Bound Indicator λ_{em} (nm)	Metal-Bound Indicator Stokes Shift (cm^{-1})
19	Ca^{2+}	363	512	8020	335	505	10,050
20	Ca^{2+}	473	670	6220	436	655	7670
21	Ca^{2}	464	533	2790	401	529	6030
22	Zn^{2}	404	484	4090	431	505	3400
23	Zn^{2}	513	543	1080	524	558	1160
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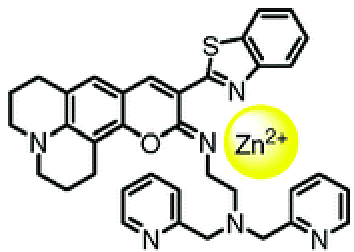
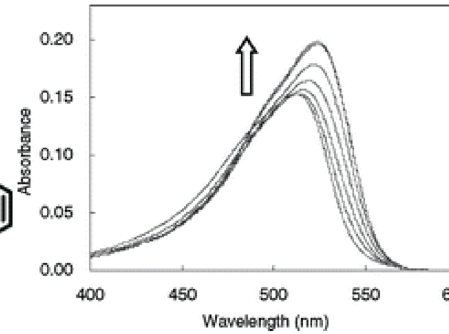
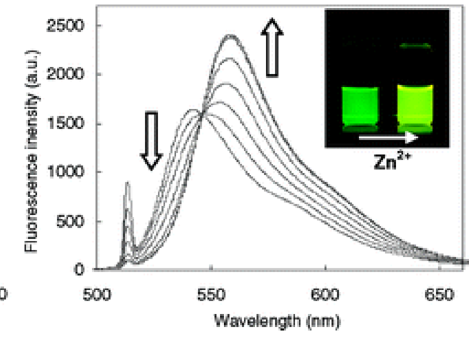
Zn coordinates to A → red shifted emission

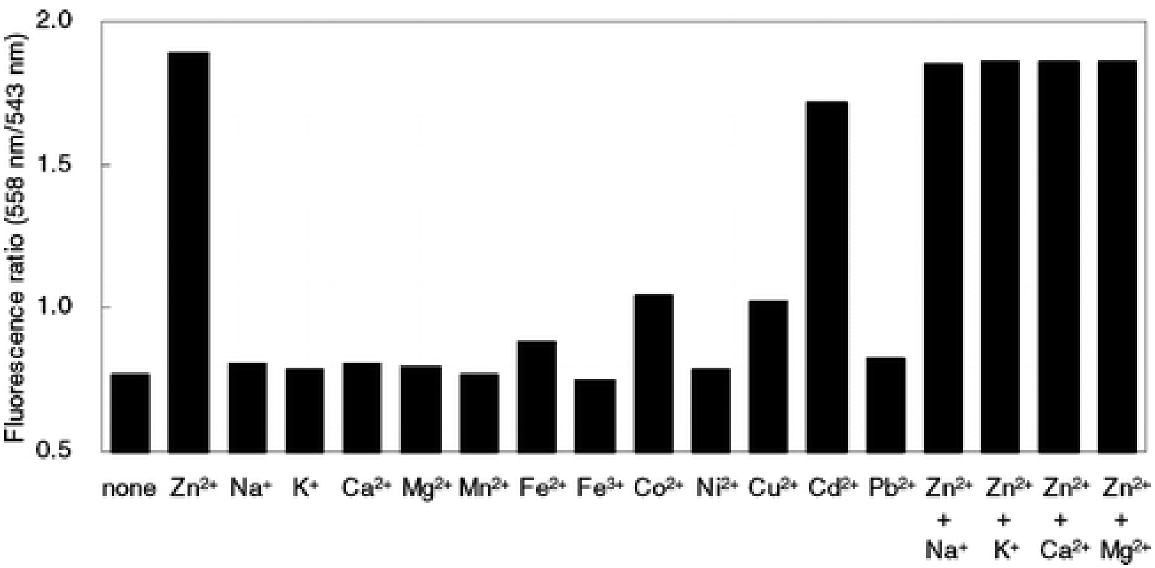
(Zn in neurobiology)

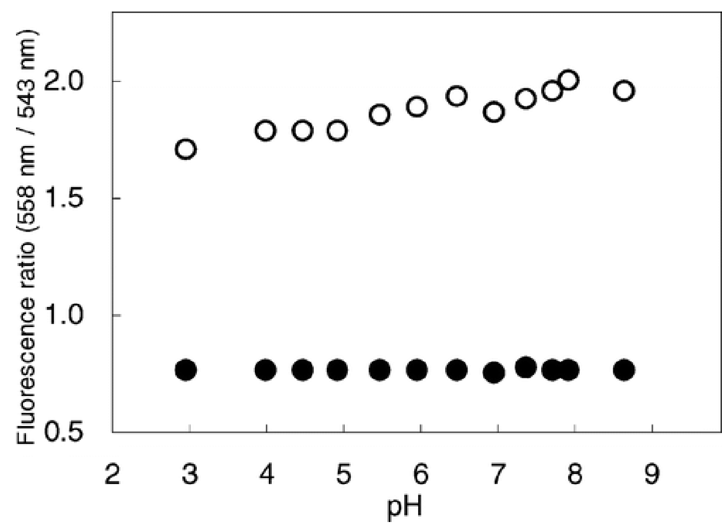
Zinc ICT sensors



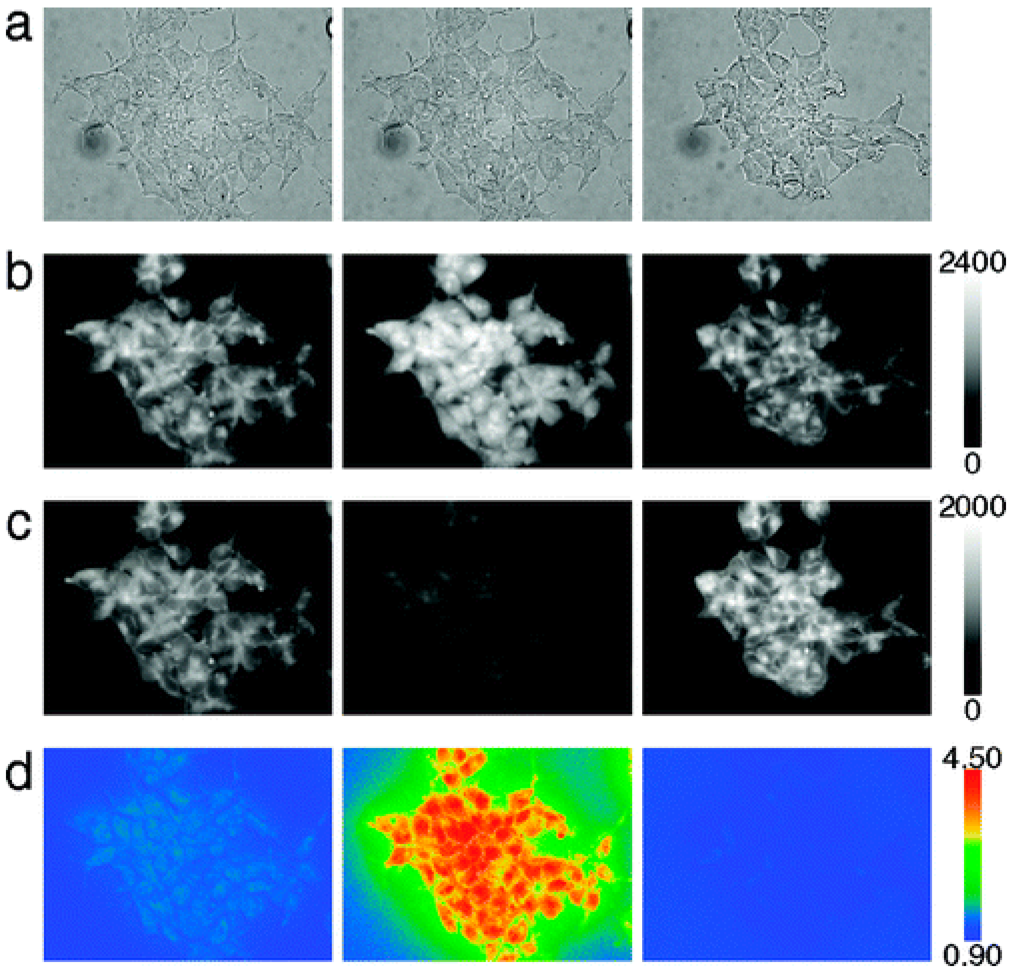




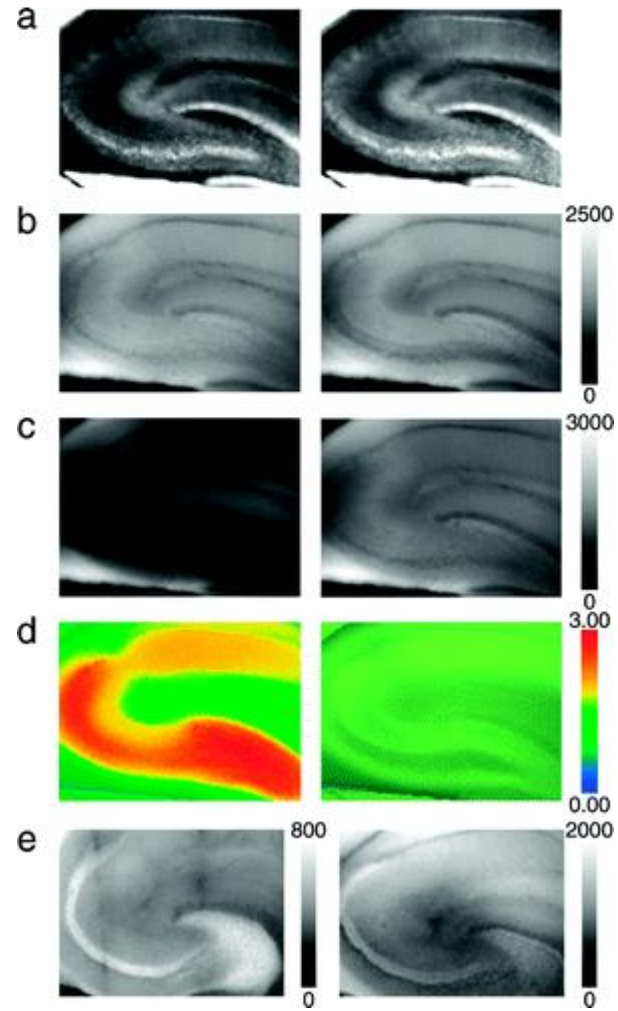




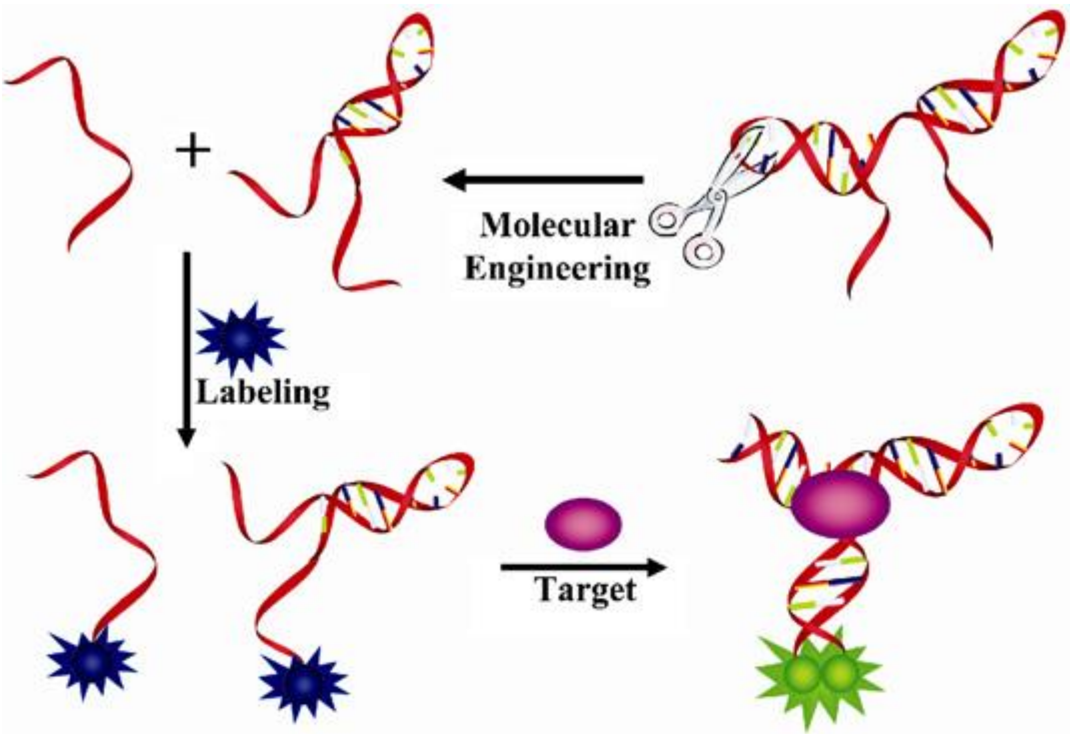
Zinc ICT sensors



510 580/510 580

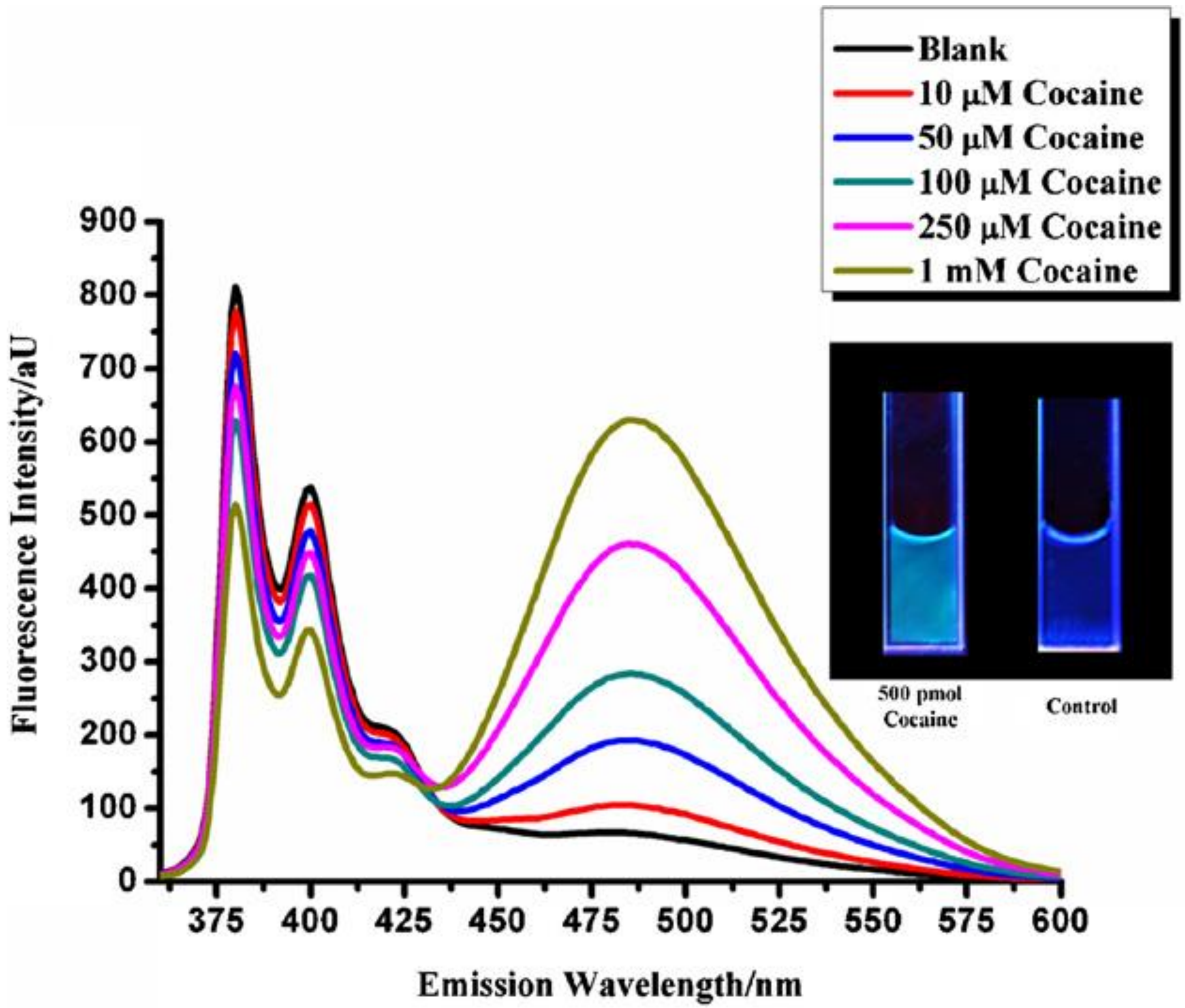


Excimer-based aptamer sensors



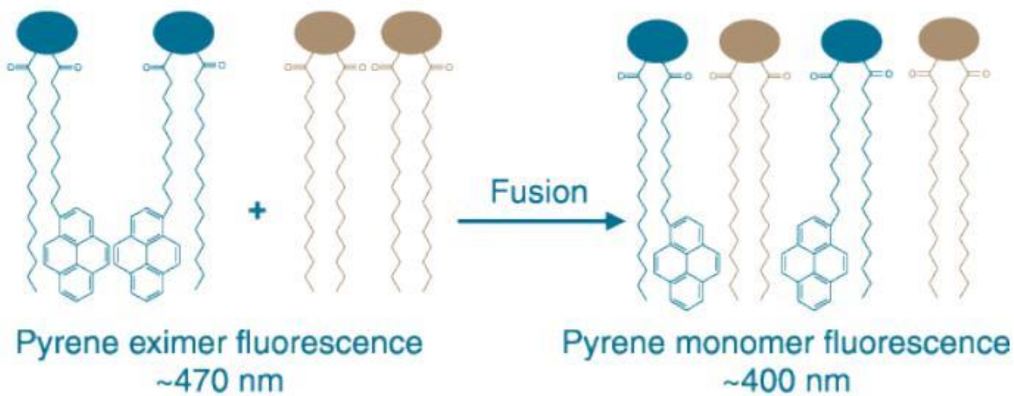
Aptamer: A DNA strand that recognizes an analyte (cocaine)

Excimer sensors



Excimer sensors

Pyrene excimer



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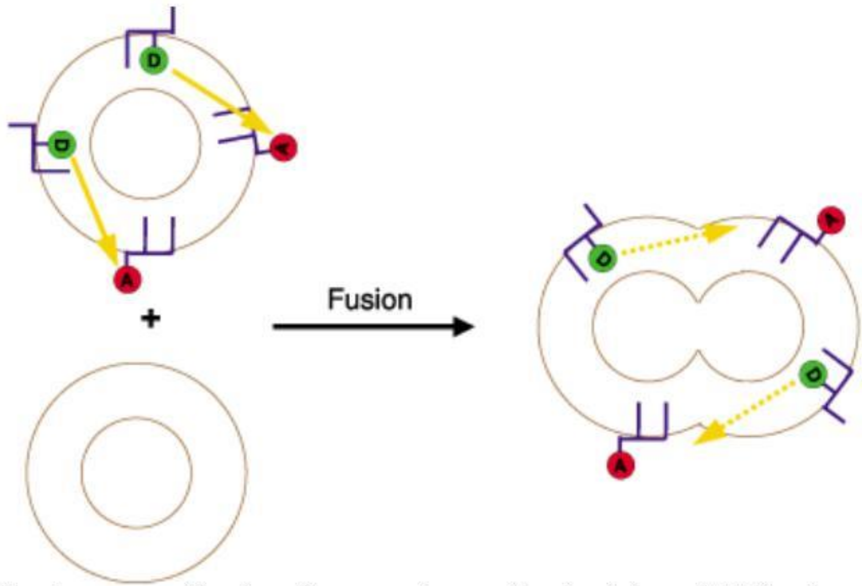
FRET sensors

Lipid mixing - relief of FRET



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FRET sensors – base pair separation