

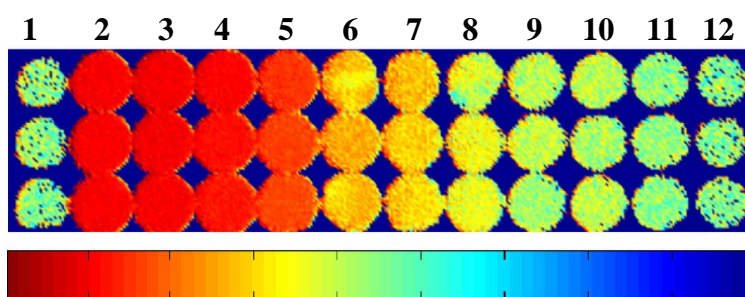
Model Abstract: Europium Tetracycline as a Luminescent Probe for Phosphonucleosides (Times Roman, 14-pt letters)

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A broad variety of enzymatic reactions is associated with the consumption of ATP. These include, among many others, (a) various phosphorylation reactions catalyzed by kinases, (b) the formation of cAMP by adenylate cyclases, and (c) the decomposition of ATP by ATPase. We have studied the effect of a series of adenosine (ATP, ADP, AMP, cAMP), and guanosine (GTP, GDP) phosphoric esters, and of pyrophosphate (PP) on the luminescence of the europium tetracycline (EuTC) complex. It is found that phosphonucleosides display highly different quenching effects on the luminescence of EuTC that is typical for lanthanide-ligand complexes.^[1-3] The triphosphates ATP and GTP act as strong quenchers by reducing luminescence by up to 25% of its initial intensity. This is attributed to the formation of a ternary 1:1:1 complex. All other phosphates caused weak quenching only. The effect was used to determine the activity of the enzyme creatine kinase (acting as a model for a non-membrane bound kinase).

Pseudo-color images of various activities of the enzyme that were obtained by time-resolved fluorometry (40 μ s gating time) along with rapid lifetime imaging of the luminescence of the probe. All experiments were performed in triplicate (3 rows). Enzyme activities ranged from 0 (nos. 1 and 12) to 135 milliunits per mL (no. 2). (one graph maximally)



Compared to other methods, this approach does not require the application of radio-labelled substrates for ATP, nor of immunoassays. In fact, it represents the first direct fluorimetric method for monitoring the conversion of ATP to its reaction products (mainly ADP, cAMP or PP). It may also be used for monitoring the respective conversions of guanosine phosphates. The method is affordable, straightforward, and versatile, and does not require the use of additional enzymes (such as luciferase in bioluminescent ATP assays), or of fluorescently labeled antibodies (like anti-phospho tyrosine or anti-cAMP). On the other side, the probe is not specific since it responds to almost any phosphate. Thus, applications will be limited to specific and well-controlled situations such as pharmaceutical screening of enzyme regulators, rather than in cellular assays or diagnosis. Moreover, the probe is highly sensitive to temperature and pH. An inhibition assay performed with nitrate anions demonstrated that the method also is applicable to the screening of enzyme regulators.

References: [1] D. Parker, R. S. Dickins, *Chem. Rev.* 102 (2002) 977. [2] H. Tsukube, S. Shinoda, *Chem. Rev.* 102 (2002) 2389. [3] M. Schaeferling et al., *Chem. – Eur. J.* 13 (2007), in press. [4] B. Valeur, in: *Fluorescence Spectroscopy in Biology*, M. Hof et al. (eds.), Springer, Berlin, 2004, p.30 ff. (up to 4 refs; all in 10-pt lettering; note the citation format; in case of >2 authors write "et al." after the first author).

Additional hints:

- * Use single spacing and 12-pt letters throughout, except for the title (14-pt);
- * use 1.5 cm margins throughout (as used in this model abstract); ll text left-aligned;
- * no fonts other than Times Roman and Greek symbols;
- * one page maximally;
- * do not paginate the Abstract.