Jena Bioscience

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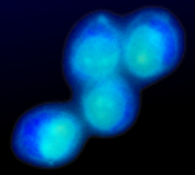
FB3 Fluorescent Biomolecules and Their Building Blocks – Design and Applications



Fluorescent Nucleotides:

A powerful toolbox for labeling of biological macromolecules

July 5th – 8th 2012

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Many biological macromolecules are accessible via nucleotides

DNA & RNA

- *made of* nucleotides (deoxy- or ribonucleotides)
- *hybridize* with complementary oligo probes

Proteins / Enzymes

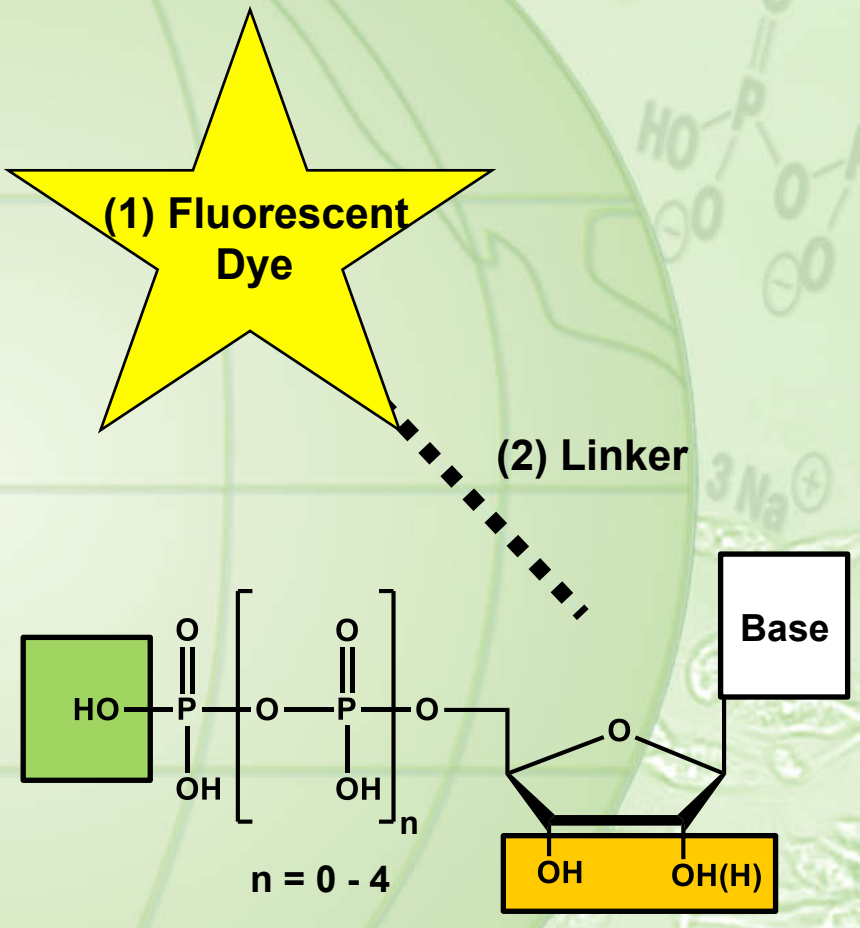
Several thousands *interact* with nucleotides or DNA/ RNA:

- Major signal transduction pathways require NTPs as cofactors
  - GPCR/G-protein ←→ GTP ← > 60% of current pharmaceutical drugs[1]
  - Protein Kinases ←→ ATP
- Polymerases ← → dNTPs
- Motor proteins, chaperones, transcription factors, small molecule kinases, …

Fluorescent nucleotides facilitate monitoring physiological processes and molecular mechanisms of disease

(1) Robinson *et al.* (2007) *Biotechnol. Prog.* 23:540

Conservative estimate: There are millions of fluorescent nucleotides possible



(1) >100 fluorescent dyes commercially available

(2) approx.10 linkers attachable to ~5 positions of nucleotide

(3) 5 natural bases, each with >10 known modifications

(4) 2 natural sugars, each with >10 known modifications

(5) 3 natural phosphate states (mono-, di-, triphosphate) ignoring higher phosphates and substitutions of oxygen with sulfur or –NH– / – CH_2–

15 million structures of fluorescent nucleotides

Do we really need so many…?

No, we don‘t…we only need two general types of fluorescent nucleotide probes

A set of pre-designed probes

available from shelf for common applications

- presumably a few hundred…thousand are sufficient -

&

Tailor–made probes

for particular, special purposes

Agenda: Fluorescent nucleotides for labeling biomolecules

1. Site (position) of label attachment
2. Linkers: More than just a handle
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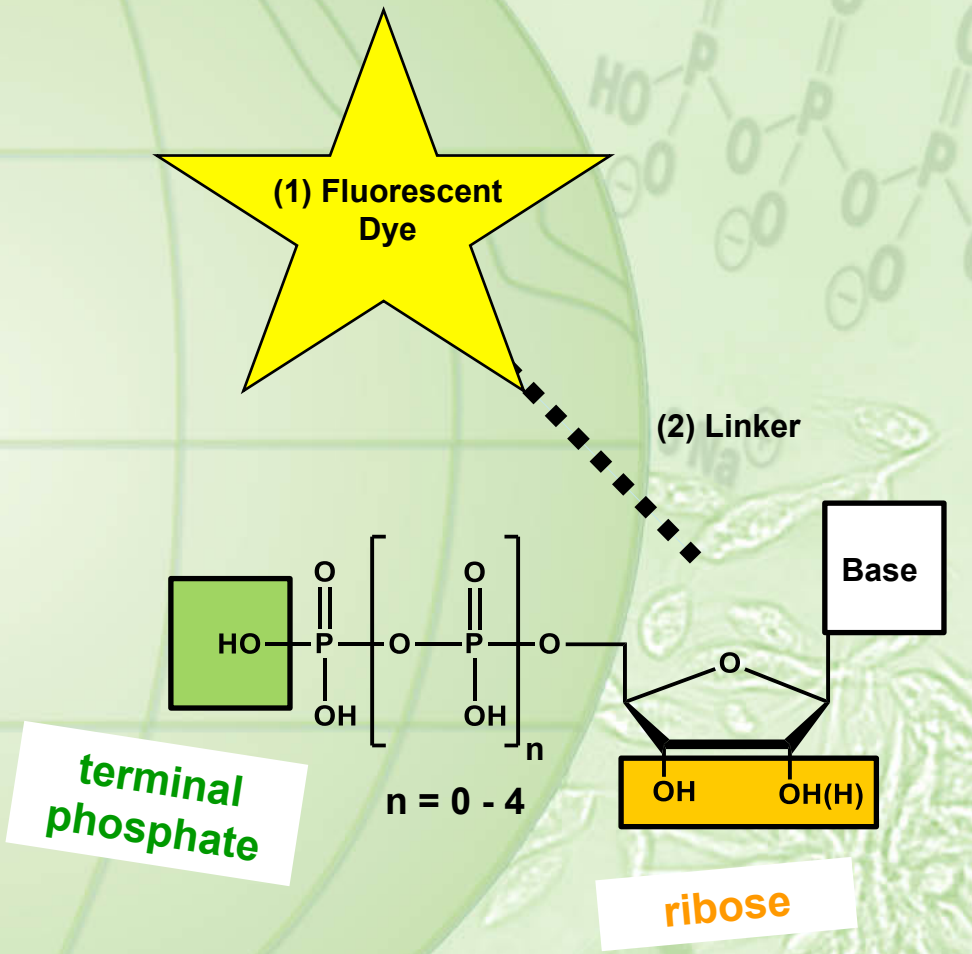
Label position: Base, sugar or terminal phosphate

For base modification purines offer more options than pyrimidines



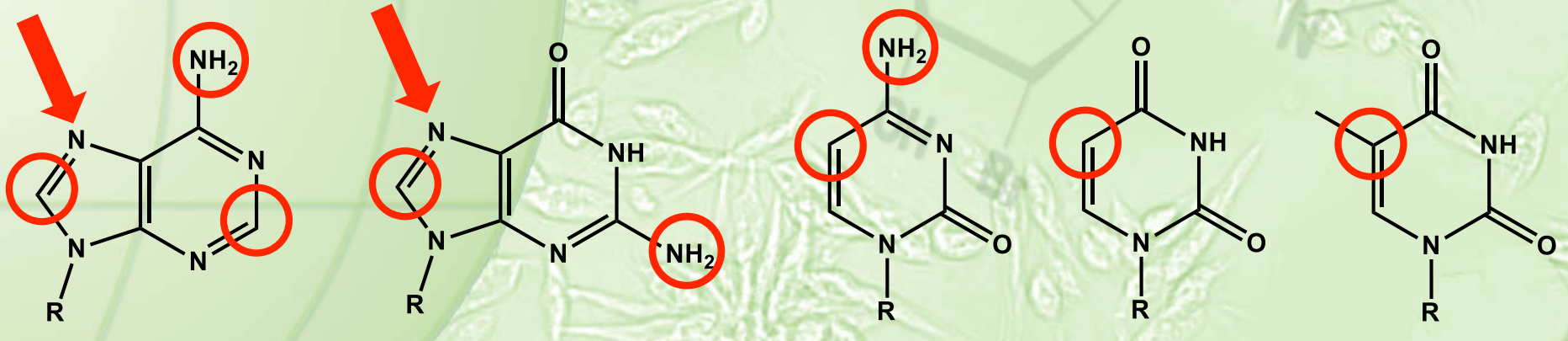
Base	Position
A	2; N^6; 7-Deaza; 8; RE
G	N^2; 7-Deaza; 8; RE
C	N^4; 5; RE
U + T	5; RE

RE = ring extension

A has 4 labeling sites + ring extensions, G has 3 +1

Base	Position
A	2; N^6; 7-Deaza; 8; RE
G	N^2; 7-Deaza; 8; RE
C	N^4; 5; RE
U + T	5; RE

RE = ring extension



A G C U T

Rule of thumb: Synthetically labeld pyrimidines easier than purines

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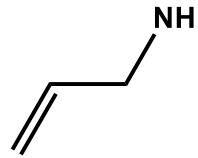
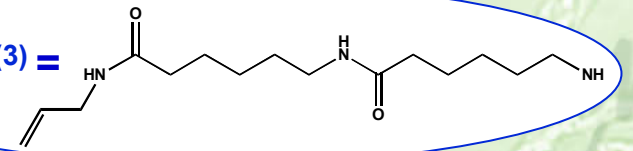
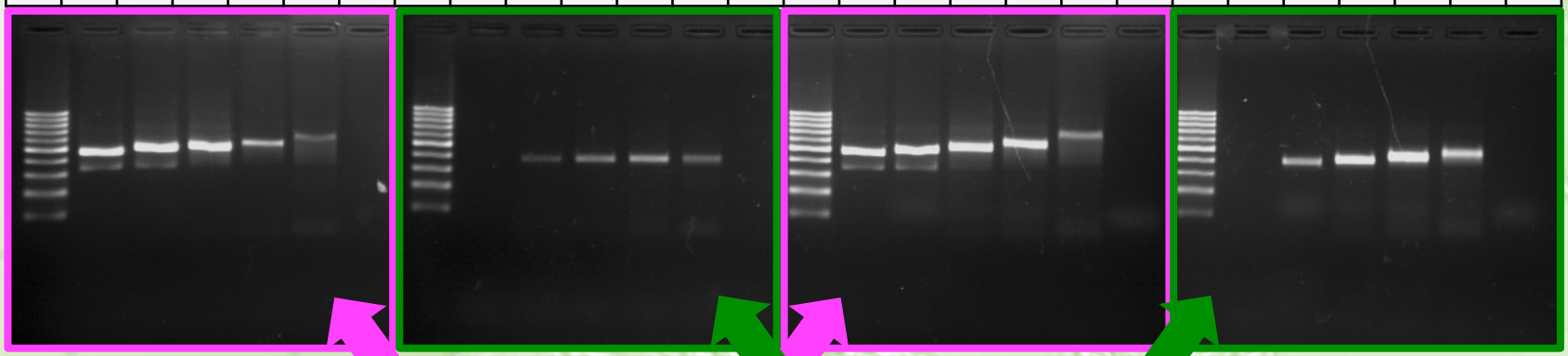
PCR with fluorescent dUTP:The longer the linker the higher the labeling density

Best results with 40…60% replacement of TTP by fluorescent dUTP

AA[2] = 

AADAH[3] = 

	Atto488-AA-dUTP							Atto488-AADAH-dUTP						
	PCR product formation (EvaGreen stained gel)							Analog incorporation (Fluorescence of unstained gel)						
(1)	M	0%	20%	40%	60%	80%	100 %	M	0%	20%	40%	60%	80%	100 %
	PCR product formation (EvaGreen stained gel)							Analog incorporation (Fluorescence of unstained gel)						
	M	0%	20%	40%	60%	80%	100 %	M	0%	20%	40%	60%	80%	100 %



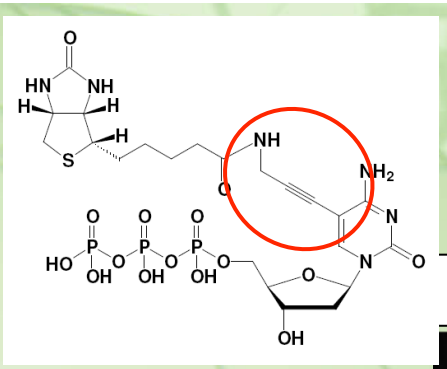
Similar amounts of PCR product formed…

…but better Atto488-dUTP incorporation with long linker

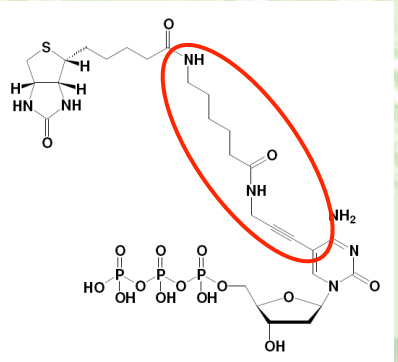
(1) Percentage of TTP-replacement with fluorescent dUTP. 0%: no fluorescent dUTP…100%: no TTP present in PCR

(2) AA = aminoallyl
(3) AADAH = aminoallyl di-diaminohexyl

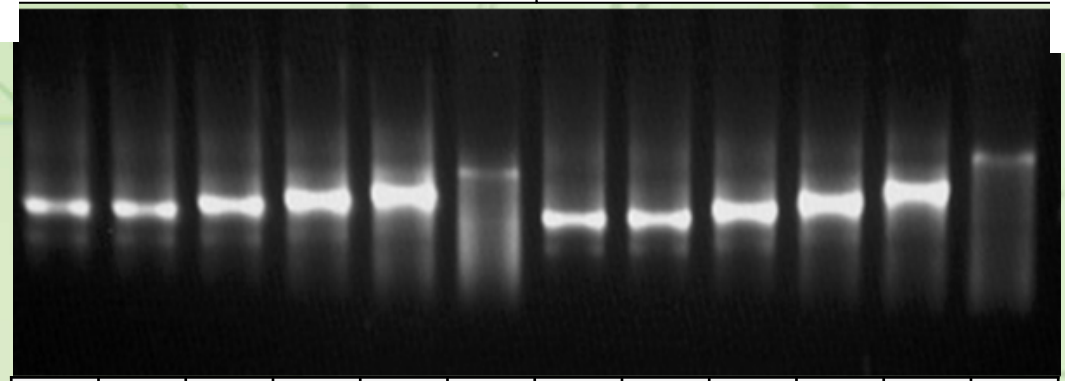
(in agreement with Zhu *et al.* (1994) *NAR* 22:3418)



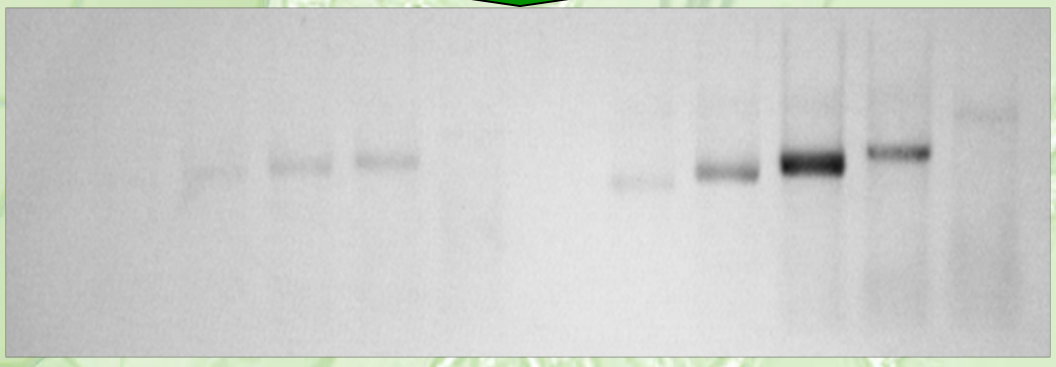
Length does matter, too for Biotin-dCTP



	4-atom-linker						11-atom-linker					
(1)	0 %	20 %	40 %	60 %	80 %	100 %	0 %	20 %	40 %	60 %	80 %	100 %
	Biotin-4-dCTP						Biotin-11-dCTP					



Southern Blot



(1) Percentage of dCTP-replacement with biotin-dCTP. 0%: no biotin-dCTP…100%: no dCTP present in PCR

Conclusion: Long linkers beneficial for PCR-incorporation but – whenever possible – for optimization different linkers shall be tried

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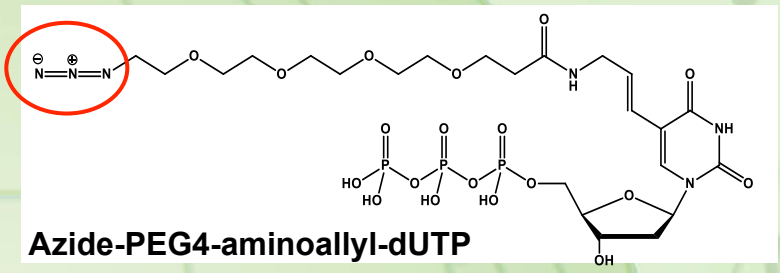
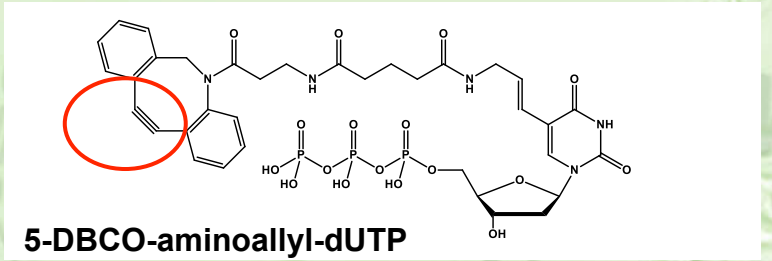
Classic Amine-NHS-ester chemistry can be replaced by CLICK reactions

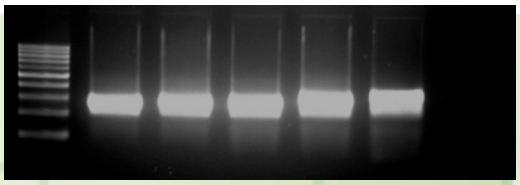


Azide-PEG4-aminoallyl-dUTP

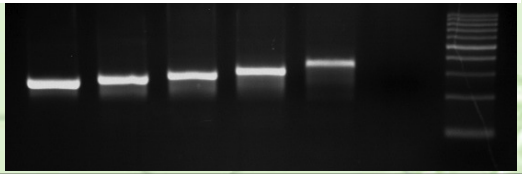


5-DBCO-aminoallyl-dUTP

(1) M 0 20 40 60 80 100%

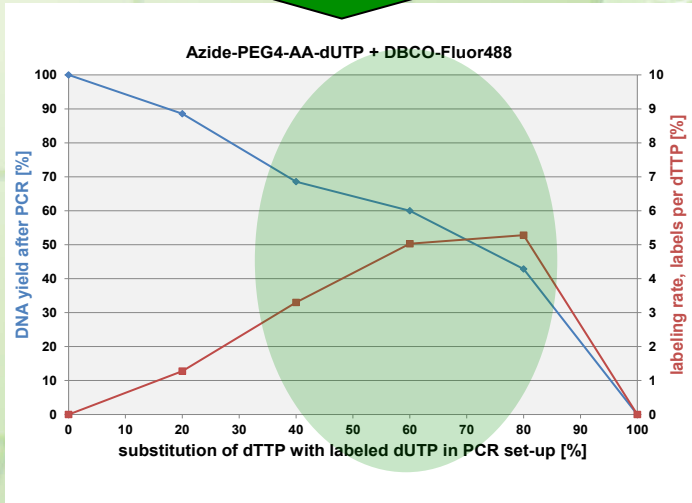


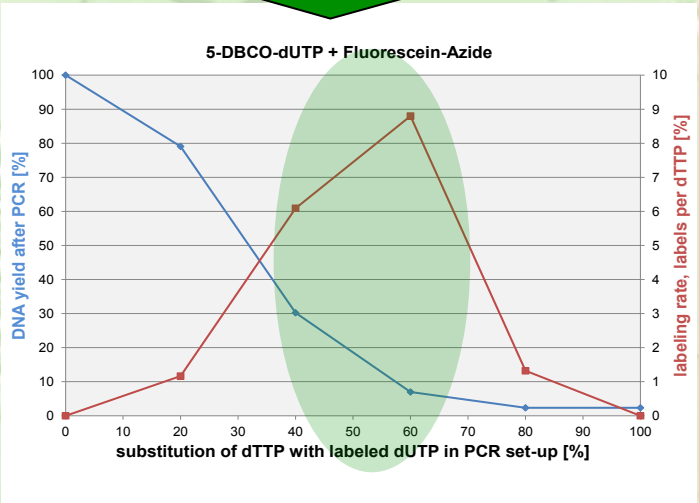
0 20 40 60 80 100% M



CLICK with DBCO-Fluorescein

CLICK with Azide-Fluorescein





(1) Percentage of TTP-replacement with dUTP analog. 0%: no dUTP analog … 100%: no TTP present in PCR

DBCO = Dibenzylcyclooctyne, field pioneered by Carolyn Bertozzi
http://www.cchem.berkeley.edu/crbgrp/

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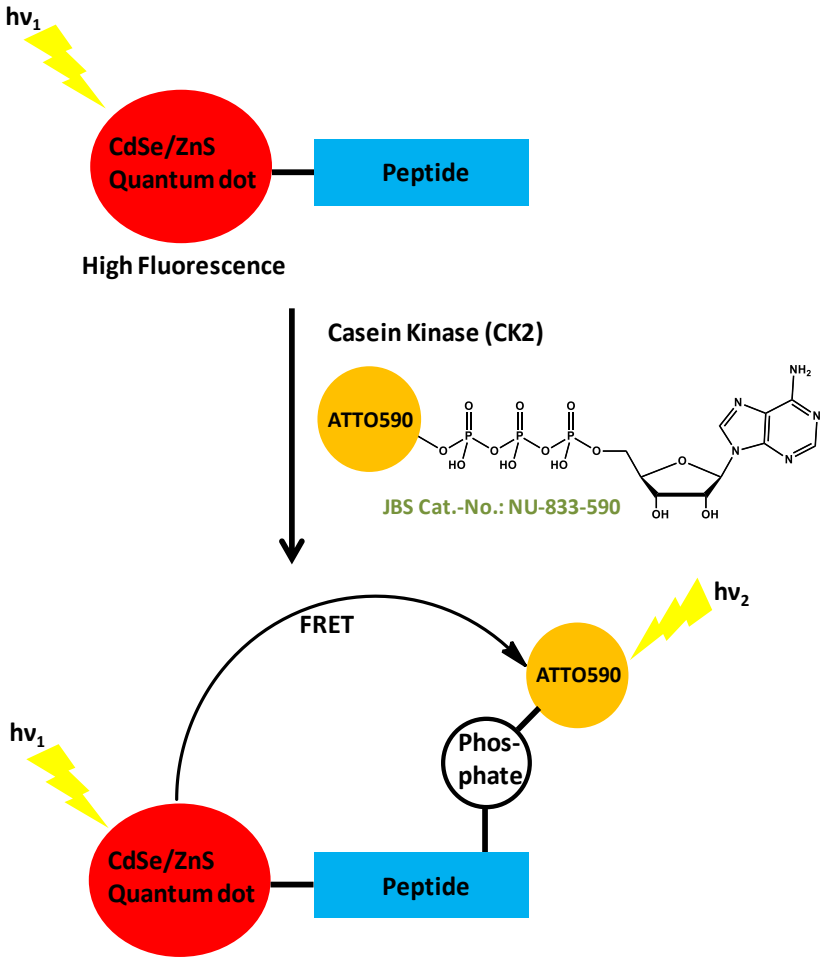
Protein kinases transfer modified γ–phosphate of ATP to their substrates…

…allowing convenient fluorescent labeling and monitoring

One-step fluorescent labeling

Freeman *et al.* (2010) *Nano Lett.* 10:2192.

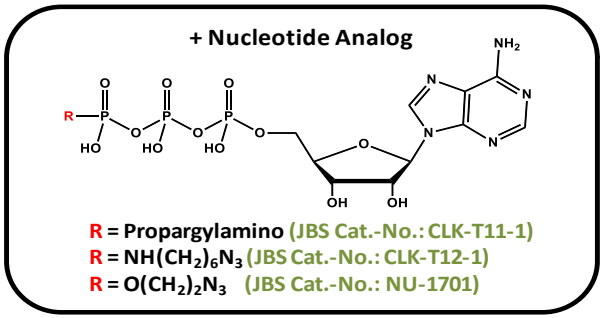


Decrease of QD fluorescence with progress of phosphorylation reaction

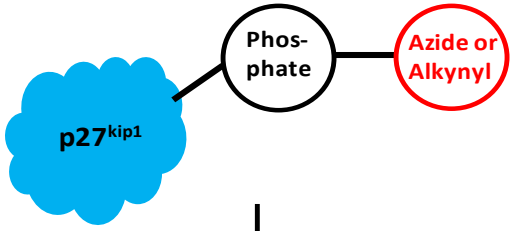
Increase of ATTO fluorescence with progress of phosphorylation reaction

Two-step (fluorescent) labeling

Recombinant protein $p27^{kip1}$



Protein Kinase cdk2



Lee *et al.* (2009) *Bioorg. Med. Chem. Lett.* 19:3804.

Labeling via „CLICK-REACTION“

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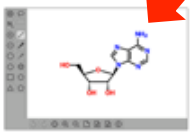
Nucleotides and their analogs is largest of eight lines of business

Roughly 2.000 nucleotides in stock & custom projects (lead discovery/optimization for big pharma but also academia-compatible custom syntheses)

Easy navigation by structure, application & structural formula



Structure Search[beta]
Search all our small molecule products (including all nucleosides and nucleotides) by sub-structure.

Nucleotides by Structure

Fluorescent Nucleotides
- Dye-/Quencher-labeled Nucleotides
- Intrinsically Fluorescent Analogs
- Nucleotide Probes Trove

Non-hydrolyzable Nucleotides
- α-Phosphate modified Nucleotides
- α,β non-hydrolyzable Nucleotides
- β-Phosphate modified Nucleotides
- β,γ non-hydrolyzable Nucleotides
- γ-Phosphate modified Nucleotides
- Non-hydrolyzable Di-Nucleotides
- Non-hydrolyzable Nucleotide Kits

Nucleotides labeled with...
- Biotin
- Digoxigenin
- Photo-labile groups ("Caged")
- Free amino group (-NH_2)
- Redox Dyes
- Halogen atoms (F, Cl, Br, I)
- Mercury (Hg)
- Selen (Se)

Analogs and Derivatives of...
- Cap
- Puromycin
- Coenzyme A (CoA)
- NAD

Unmodified (natural) Nucleotides
- dNTPs
- dNDPs
- NTPs
- NDPs
- NMPs

Important Structure Motifs
- Cyclic Nucleotides
- Di-nucleoside Phosphates
- 6-Thio Guanosines and Inosines
- 7-Deaza Purines
- 7-Methyl Guanosines
- 4-Substituted Pyrimidines
- 5-Methyl Cytidines and related
- Unmodified and Modified ddNTPs

Nucleotide Trove
- 6-Modified Purines
- 8-Oxo Guanosines
- 2'-Deoxy Uridines
- 3'-Deoxy Nucleotides
- Nucleoside Bisphosphates
- *ara*-Nucleotides
- Unmodified Purines
- Modified dNTPs
- Miscellaneous

Custom Synthesis
Didn't find the nucleotide analog you're looking for? Contact us at nucleotides@jenabioscience.com for a custom synthesis!
With our pre-made building blocks and in-house expertise we manufacture even the most exotic nucleotide analog from the mg to kg scale - find more information in the PDF file above.

Nucleotides by Application

...on DNA
- PCR & Co.
- Sequencing & Genotyping
- Mutagenesis
- Crosslinking

...on RNA
- *in vitro* Synthesis
- 5'-Capping

...on Proteins & Enzymes
- Co-translational Protein Labeling
- Kinase Signaling
- G-Protein Signaling
- Tubulin Assembling
- Structural Biology

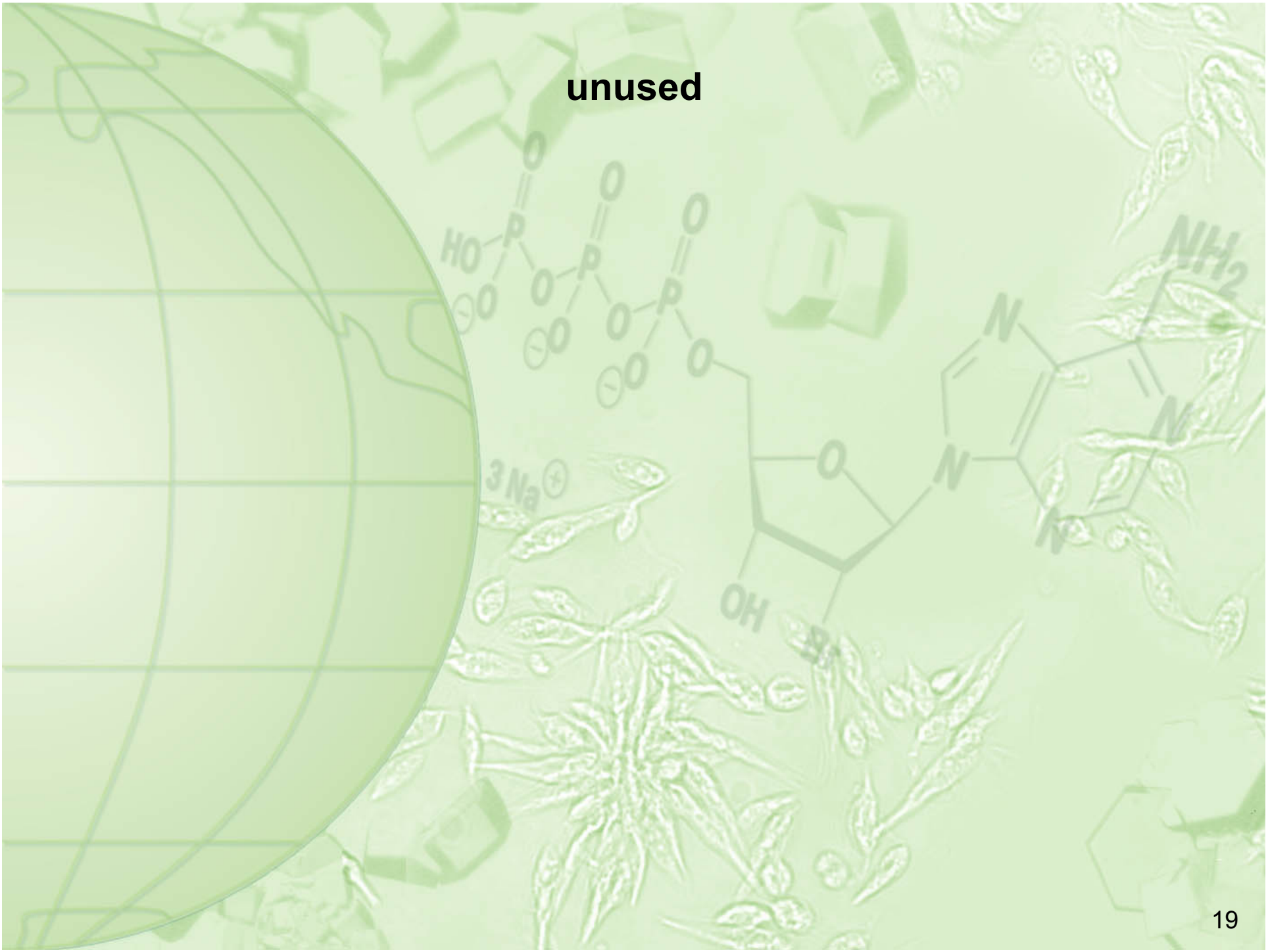
...in Cell Biology
- Cell Cycle & Proliferation
- Apoptosis
- Protein-DNA/-RNA Interaction (EMSA)
- Epigenetics

...in Drug Discovery
- SELEX/Aptamer Modification
- mRNA Modification
- Antiviral Nucleotides
- Cytostatic Nucleotides

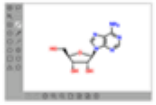
Thank you!

1998: Spin-Off from Max-Planck-Institute for molecular Physiology in Dortmund, 2012: Sales to 4.000 customers in 50+ countries

unused



Navigation



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- Puromycin
- Coenzyme A (CoA)
- NAD

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