sirius fine chemicals

SiChem manual

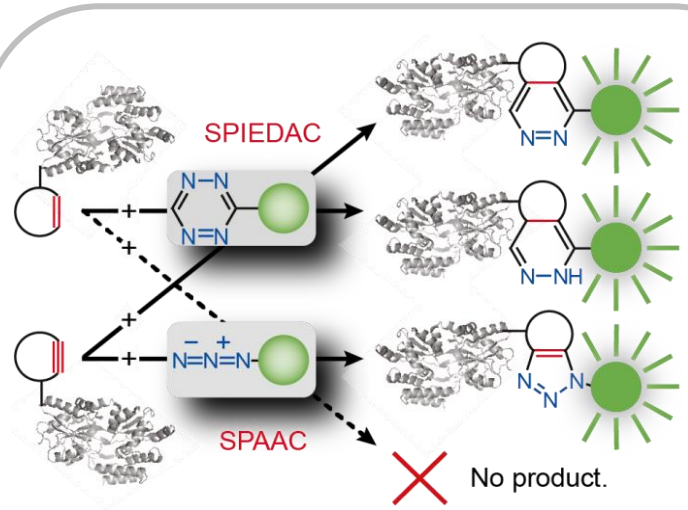
Sirius Fine Chemicals
SiChem GmbH
Fahrenheitstr. 1
D-28359 Bremen
Tel.: ++49-421-2208-228
FAX: ++49-421-2208-226
email: info@sichem.de
www.sichem.de

Si-Click - (Copper-free) Click Chemistry

Mounting labels on proteins in intact cells allows observing and tracking them in their natural environment and represents a powerful tool for studying cell biology and developing drugs. Nowadays, a large variety of approaches exists for protein labeling – differing in size of modification, general scope, as well as the ease of usability and exchangeability.

A major drawback of many genetically encoded tags represents their large size which can be overcome by making use of the **fascinating potential of specifically labeling single amino acid residues with small molecule dyes**. A set of genetically encodable **unnatural amino acids (UAAs)** is now commercially **available from SiChem*** allowing for biocompatible and even bioorthogonal site-specific labeling of proteins with (fluorogenic) dyes.

The new L-lysine-based compounds have (bi)cyclooctyne, (trans)-cyclooctene or propargyl units that can be grafted into proteins – amongst other of *E. coli* and mammalian cells. **Copper(I)-catalyzed (CuAAC)** click chemistry between azides and linear alkynes as well as the two most potent representatives of in vivo bioorthogonal chemical reactions,



Orthogonality and cross-reactivity of **SPIEDAC** and **SPAAC** reaction with fluorogenic tetrazine (Tet) - and azide-functionalized dyes (T.Plass)

SPIEDAC: Strain-**P**romoted **I**nverse **E**lectron-Demand **D**iels-**A**lder **C**ycloaddition
SPAAC: Strain-**P**romoted **A**lkyne-**A**zide **C**ycloaddition

SPAAC and **SPIEDAC**, can be used to efficiently and rapidly attach fluorophores conjugated to azide and tetrazine moieties, respectively. Due to the modularity of the technique, any imaginable probe fused to azide or tetrazine can be attached to any protein modified with a corresponding unnatural side chain with the maximum freedom of placement within the protein under investigation.

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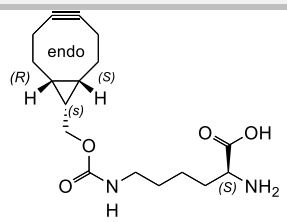
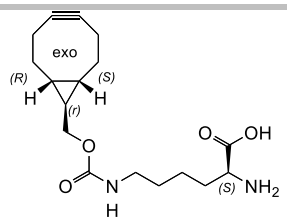
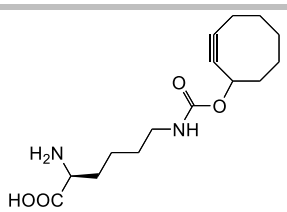
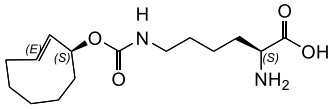
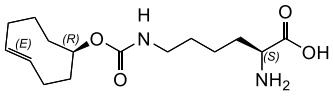
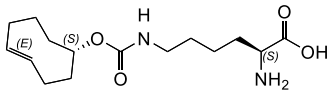
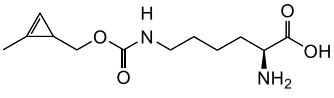
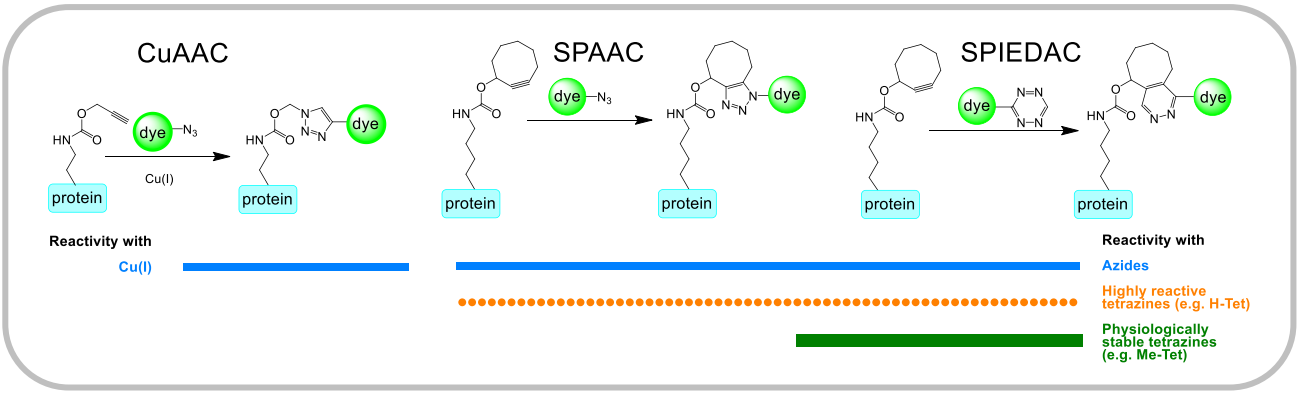
The following Unnatural Amino Acids (UAAs) are available from SiChem:

	reacts with ►	Azides	H-Tetrazines	Me-Tetrazines
endo-BCN **SC-8014**		**yes** (slow) ►	**yes** (very fast) ►►►►	**yes** (middle) ►►
exo-BCN **SC-8016**		**yes** (slow) ►	**yes** (very fast) ►►►►	**yes** (middle) ►►
SCO **SC-8000**		**yes** (slow) ►	**yes** (fast) ►►►	**no** ✕
TCO*A **SC-8008**		**no** ✕	**yes** (very fast) ►►►►	**yes** (very fast) ►►►►
TCO4-AX **SC-8004**		**no** ✕	**yes** (very fast) ►►►►	**yes** (very fast) ►►►►
TCO4-EQ **SC-8060**		**no** ✕	**yes** (very fast) ►►►►	**yes** (very fast) ►►►►
CypK (CP) **SC-8017**		**no** ✕	**yes** (fast) ►►►	**no** ✕

Which UAA do I need for my experiments ?
Reaction scheme for choosing the right UAA:



Preparation of stock solutions for protein expression experiments

For standard experiments in ***E. coli***, **a final UAA-concentration of around 1 mM** is sufficient for optimal protein expression. Ideally, the UAA is added from a **100 mM stock solution in 0.2 M NaOH** after inoculation and initial growth of the cell culture (at around OD_{600} 0.2–0.4). One-time addition of UAA is sufficient. If necessary, the volume of 0.2 M NaOH can be neutralized by adding equal amounts of 0.2 M HCl to maintain exactly the same buffer capacity of the growth medium.

For experiments in **mammalian cells** (HEK 293, U2OS, HeLa Kyoto etc.), **a final UAA-concentration of around 250-500 µM** is sufficient for optimal protein expression.

UAAs can be added from a 100 mM stock 15% (vol/vol) DMSO / 0.2 M NaOH
(if necessary: neutralization with equal amounts of 0.2 M HCl)

Freshly before adding to cells:
Prepare a 1:4 dilution into 1M HEPES
(► 25 µM). Use this dilution immediately, and do not store !
Further dilute to
1 mM for E. coli-experiments (1:25) or to
250 µM for cell-experiments (1:100)

However, **the usage of small aliquots is recommended**.

Please note that for synthetic reasons, some UAAs are delivered as free acid or salt (e.g. HCl, formic acid).
To speed up the process of dissolving, measures such as ultrasound, vortexing, overhead shaking, warming to 37° C etc. can be applied.

All UAAs stock solutions can be stored at -20° C up to several months.

► **stock solution:**	**100 mM in 15% (vol/vol) DMSO / 0.2 M NaOH**
► **final conc.: *E. coli:***	**1 mM**
► **final conc.: mam. cells*:***	**250-500 µM**
► **storage:**	**at - 20° C up to several months**

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