Introduction:

The term "Click Chemistry" describes chemical reactions that are able to quickly and reliably generate substances by joining together small units. One of the most popular reactions within the Click Chemistry concept is the copper (I) catalyzed Huisgen azide alkyne cycloaddition forming a covalent linking unit (triazole) between the label and the target molecule (Figure 1).

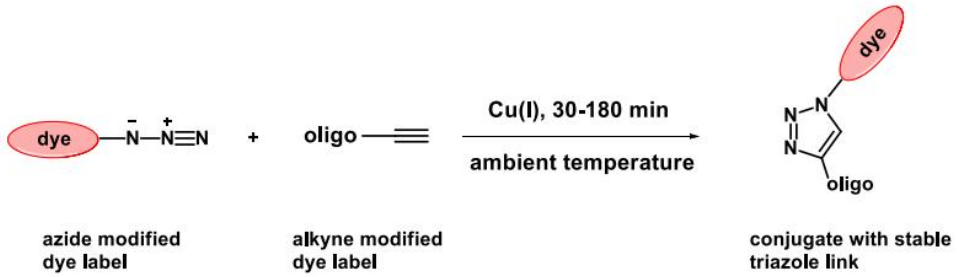

Figure 1: Cu(I) catalysed alkyne-azide "Click Reaction". The azide and the alkyne residues are interchangeable e.g. the fluorophore can be labeled with an azide moiety and the target molecule could carry the alkyne functionality.

The alkyne and azide groups are biologically unique and therefore the click reaction is very selective and specific. The fluorescent labeled click conjugates can be easily detected with high sensitivity and low background, unlike conventional labeling reactions using succinimidyl ester or maleimides which react with amines and sulfhydryls, which are common functionalities in biological environment.

ATTO-TEC offers a large variety of alkyne- or azide-modified fluorophores for Clikck Chemistry.

Protocol for Oligonucleotide Labeling via Click Chemistry

General Remarks

The following protocol describes the labeling procedure for 5 nmol of a single alkyne modified oligonucleotide.

The reaction is most efficient if the azide and alkyne are dissolved in a minimal amount of solvent and the solutions are of high concentration.

The reaction can be accelerated by raising the temperature and is generally finished in 30 min at around 40 - 45 °C.

Required Materials

- **Solution A:** Dissolve the azide- or alkyne-modified oligonucleotide in the appropriate amount of water to obtain a 2 mM solution and centrifuge shortly.

- **Solution B:** Dissolve 1.0 mg of the click reagent (azide- or alkyne-modified ATTO-dye) in the appropriate amount of DMSO/t-BuOH-solution 1:1 to obtain a 50 mM stock-solution. In case of azide or alkyne of ATTO 725, ATTO 740, ATTO 647, ATTO 610, and ATTO MB2 we recommend using ACN/t-BuOH.
- **Solution C:** Click Solution: Dissolve 54 mg TBTA (tris[(1-benzyl-1H-1,2,3-triazole-4- yl)methyl]amine) in 1 ml DMSO/t-BuOH 3:1 for a 0.1 M solution. The solution can be stored at -20 ° C.
- **Solution D:** Dissolve 1 mg CuBr in 70 µl DMSO/t-BuOH 3:1 to obtain a 0.1 M solution. NOTE: This solution must be freshly prepared and cannot be stored!
- **Solution E:** The final click solution is prepared by quickly adding 1 volume of Solution D to 2 volumes of Solution C.

Conjugate Preparation

In general, the labeling reaction works more efficiently with concentrated solutions of alkynes (e.g. oligo) and azides (dye label). In the case the reaction does not work in water, rising the pH by performing the reaction in Tris-HCl (50 mM) at pH 8.3 might be helpful.

- Pipette 5 µl of Solution A (10 nmol of oligonucleotide) in a 0.5 reaction vial.
- Add 1 - 2 µl of Solution B (50 - 100 nmol; 5 - 10 eq.) to the reaction vial.
- Pipette the correct amount of Solution B corresponding to a 2 - 10 times molar excess of the alkyne-or azide modified ATTO-dye into the reaction vial.
- 3 µl of freshly prepared Solution E is added and the reaction vial is thoroughly mixed by shaking at 25 ° C for 3 h. As previously mentioned, by rising the temperature to 40 - 45 ° C the reaction is generally finished in 30 minutes.

Conjugate Purification – Removal of Excess Reagents

- Add 100 µl of a 0.3 M NaOAc solution and precipitate the oligonucleotide by adding 1 ml cold ethanol (containing 5 % diethylether; -20 ° C). Centrifuge for at least 20 min at 6000 rpm or higher. Remove the supernatant and wash the residue with 100 µl cold ethanol (containing 5 % diethyl ether; -20 ° C). Centrifuge again for at least 10 min and remove the supernatant. Dry the residue on air.

Storage of the Conjugate

In general, conjugates should be stored under the same conditions used for the unlabeled oligonucleotide. For long-term storage we recommend freezing at -20 ° C. Protect dye conjugates from light as much as possible.

Table 1: Properties of ATTO-dye labeled azides:

Dye	MW	M*	λ abs	λ em	ε max	CF260	CF280
ATTO 390	544	544	390	476	24000	0.46	0.09
ATTO 425	602	602	439	485	45000	0.19	0.17
ATTO 430LS	789	767	436	545	32000	0.32	0.22
ATTO 465	610	496	453	506	75000	1.09	0.48
ATTO 488	828	790	500	520	90000	0.22	0.09
ATTO 490LS	896	874	498	658	40000	0.39	0.21
ATTO Rho110	744	630	507	531	100000	0.21	0.14
ATTO 514	1068	954	511	532	115000	0.21	0.07
ATTO 520	681	567	517	538	110000	0.16	0.2
ATTO 532	884	846	532	552	115000	0.2	0.09
ATTO Rho6G	828	714	533	557	115000	0.19	0.16
ATTO 540Q	873	759	543		105000	0.27	0.26
ATTO 542	1228	1114	542	562	120000	0.18	0.08
ATTO 550	908	794	554	576	120000	0.23	0.1
ATTO 565	811	711	564	590	120000	0.27	0.12
ATTO Rho12	964	851	577	600	120000	0.26	0.09
ATTO Thio12	802	702	582	607	110000	0.11	0.37
ATTO Rho101	890	790	587	609	120000	0.18	0.17
ATTO 590	891	791	593	622	120000	0.39	0.43
ATTO 594	1119	1006	603	626	120000	0.22	0.5
ATTO 620	812	712	620	642	120000	0.04	0.06
ATTO 633	866	752	630	651	130000	0.04	0.05
ATTO 643	1058	1036	643	665	150000	0.05	0.04
ATTO 647N	960	846	646	664	150000	0.06	0.05
ATTO 655	842	728	663	680	125000	0.24	0.08
ATTO 665	937	823	662	680	160000	0.07	0.06
ATTO 680	839	726	681	698	125000	0.3	0.17
ATTO 700	880	766	700	716	120000	0.26	0.41
ATTO 725	729	616	728	751	120000	0.08	0.06
ATTO 740	782	668	743	763	120000	0.07	0.07
ATTO MB2	670	556	668		110000	0.08	0.24

Table 2: Properties of ATTO-dye labeled alkyne:

Dye	MW	M^+	λ abs	λ em	ε max	CF260	CF280
ATTO 390	495	381	390	476	24000	0.46	0.09
ATTO 488	741	627	500	520	90000	0.22	0.09
ATTO 514	905	791	511	532	115000	0.21	0.07
ATTO 532	797	683	532	552	115000	0.2	0.09
ATTO Rho6G	651	551	533	557	115000	0.19	0.16
ATTO 550	731	631	554	576	120000	0.23	0.1
ATTO 565	648	548	564	590	120000	0.27	0.12
ATTO 590	742	628	593	622	120000	0.39	0.43
ATTO 594	956	843	603	626	130000	0.22	0.5
ATTO 633	703	589	630	651	130000	0.04	0.05
ATTO 643	987	873	643	665	150000	0.05	0.04
ATTO 647N	783	683	646	664	150000	0.04	0.03
ATTO 655	679	565	663	680	125000	0.24	0.08
ATTO 680	677	563	681	698	125000	0.3	0.17
ATTO 700	717	603	700	716	125000	0.26	0.41
ATTO MB2	507	393	668		100000	0.08	0.24

MW: molecular weight of the dye including counterions in g/mol; M^+ : molecular weight of dye cation (HPLC_MS acetonitrile/water 0.1 vol- % trifluoroacetic acid); λ abs: longest wavelength absorption maximum in nm; λ em: fluorescence maximum in nm; max: molar decadic extinction coefficient at the longest-wavelength absorption maximum in $M^{-1}cm^{-1}$; CF260 = ε 260/ ε max; CF280 = ε 280/ ε max;