PNA Oligomer
Quick Reference Card

Using PNA Oligomers

Handling and Storage

Dissolve the PNA in 200-1000µL plain water (or occasionally 0.1% aqueous trifluoroacetic acid (TFA)) and divide the solution into aliquots. Aliquots that are not going to be used the same day should be dried down, for example using a speed-vac concentrator.

Typically PNA oligomers (and peptides) are purified by reverse phase HPLC with (TFA) present and as a result the purified PNA will be protonated at all the basic amino groups (at A, C and the amino terminus) and hence carry trifluoroacetate counter-ions when dissolved in plain water. This is to your advantage as this will help keep the PNA in solution at the high concentration of your stock solution.

Two serious problems can potentially arise from the TFA. Addition of a concentrated stock solution of PNA in plain water directly into a cell culture, for example, could lower the pH. Also, the high concentration of TFA may have an adverse effect on the system tested. However, these problems are easily avoided by using properly buffered solutions.

Alternatively, the PNA can be dissolved in the appropriate buffer and the aliquots kept frozen until they are going to be used. This is not recommended and will only work for certain PNA sequences, i.e. short PNAs with few purines.

Occasionally, it may be difficult to dissolve a particular sequence, for example a long oligomer having a fluorescein label. If you encounter this problem, add 10-20% acetonitrile to the aqueous solution and eventually heat the sample to 50°C for about 10 minutes.

PNA oligomers have a high affinity for glass surfaces and polystyrene. When working with low (sub-micro molar) concentrations of PNA a majority of the PNA may become bound to the container. Whenever possible, use polypropylene or polyethylene materials during handling and storage of PNA.

Buffers and Ionic Strength

In contrast to DNA•DNA duplex formation – no salt is necessary to facilitate and stabilize the formation of a PNA•DNA (or RNA) duplex. For this reason, the T_m of a PNA•DNA-duplex is almost independent of ionic strength. For example, the T_m of a 15-mer duplex has been shown to decrease by only 5°C as the NaCl concentration was raised from 10 mM to 1 M. Thus a PNA, when used at low ionic strength (e.g. 10 mM phosphate buffer with no NaCl), will effectively bind to a target under low salt conditions in the presence of a competing DNA strand because the stability of the DNA•DNA duplex is relatively small.

It is important, however, NOT to perform the hybridization in the absence of a buffer since PNA/DNA duplexes precipitate at low pH (see above) or when no counter-ions are present.

In the case of RNA, low salt conditions serve to destabilize intramolecular hybridization (tertiary structure) favoring hybridization of the PNA. Vice versa, hybridization conditions can be chosen so that DNA or RNA competes better with the PNA for hybridization to the target.

Quantification

Extinction coefficients have been determined for three of the four PNA monomer units (C = 6.6, T = 8.6, and A = 13.7 $mL\mu mol^{-1}cm^{-1}$). These values are very close to those determined for the corresponding natural nucleotides. Thus a value of 11.7 $mL\mu mol^{-1}cm^{-1}$ can be assumed for the G monomer.

Hypochromic effects must also be considered when quantifying PNA oligomers based on UV absorption at 260 nm at room temperature. Single stranded PNA is highly stacked leading to significant reduction in observed extinction coefficients of the oligomers relative to the sum of the extinction coefficients of the individual bases that comprise the oligomer. Melts performed on single stranded PNAs show a sigmoidal curve with a "T_m" of 40-45¡C and an observed hyperchromicity of 15-25% practically independent of the sequence and length of the PNA. For most purposes, one should include a 20% Hypochromicity factor when calculating PNA concentration.

When a more accurate determination of concentration is necessary, measure the absorption at 260 nm at a temperature higher than 50°C.

Melting Experiments

The above considerations have an impact on UV behavior in connection with hybridization. Hypochromicity associated with PNA•DNA duplex formation at room temperature is small, typically only 3-5%, and is primarily due to an increase in the stacking of the DNA upon binding (remember the PNA is already highly stacked prior to binding). This has little impact, however, on the magnitude of the melting curves of PNA•DNA duplexes, as they will typically melt at temperatures above 45¡C (i.e. above the “melting temperature” of the single stranded PNA). PNA-DNA duplexes will typically display the same net gain in absorbance as DNA•DNA duplexes upon melting.

Trouble Shooting

If you suspect that your PNA oligomer is aggregating it is easily determined. Spin the solution and thereafter determine the absorbance at 260 nm. Using the extinction coefficients listed above calculate the current concentration of PNA. Do this routinely to ensure that the PNA oligomer concentration is what you expect it to be.

General Rules for Working with PNA Oligomers

About Sequence Design

There are more restrictions on the design of PNA sequences than on the design of DNA sequences. However, it is easier to accommodate the requirements for the PNA oligomer because you only need a probe length of only 12–15 bases (compared to 25–40 bases, which is the typical length for an oligonucleotide probe).

Orientation

PNAs can form duplexes in either orientation, but the **ANTI PARALLEL orientation** is strongly preferred. This will be the orientation for all antisense and DNA probe type applications. The N-terminal of the PNA oligomer is equivalent to the 5'-end of an oligonucleotide and is often referred to as "the 5'-end of the PNA".

Melting Temperature

Because the PNA strand is uncharged a PNA•DNA-duplex will have a higher Tm than the corresponding DNA•DNA-duplex. Typically there will be an increase in Tm of about 1°C per basepair at 100 mM NaCl depending on the sequence. At lower salt the Tm differences are going to be even more dramatic. A 10-mer PNA will typically have a Tm of about 50°C, and a 15-mer typically a Tm of 70° with the anti parallel complementary oligonucleotide (DNA or RNA).

Due to this higher affinity it is NOT necessary to prepare long PNA-oligomers, as opposed to the 25-40 units, which is the typical length for an oligonucleotide probe. Bear in mind that the shorter a probe the more specific it is. The impact of a mismatch is greater the shorter the sequence is. The primary concern regarding length of the PNA-probe should be the warranted uniqueness, i.e. what length is unique for the particular application. For most applications an oligomer length of 12-15 is optimal, and in many cases even shorter probes will work well. Longer PNA oligomers, depending on the sequence, tend to aggregate and are difficult to purify and characterize.

Aggregation

Purine rich PNA oligomers tend to aggregate, with G-rich oligomers being the worst. As a rule never have more than 7 purines in any stretch of 10 units. Observing this rule will dramatically reduce the likelihood that your PNA oligomer will aggregate. The shorter the sequence the less attention needs to be paid to the sequence.

Self-Complementary Sequences

As PNA/PNA interactions are even stronger than PNA/DNA interactions try to avoid self-complementary sequences.