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## ***Applied Biosystems 2D Nano LC system***

Automated nanoscale 2-dimensional chromatography enhances peptide identification

### **INTRODUCTION**

The NanoLC-2D system includes all of the capabilities and benefits of the NanoLC-1D system – direct pumping for precise nanoscale gradients, and automated peak parking - with the added capability of fully automated 2-dimensional separations. The NanoLC-2D system is based on microfluidic flow control (MFC) technology, which uses continuous feedback to an electronically controlled pressure source to maintain precise nanoscale flow rates as low as 20 nL/min without flow splitting. Additionally, the system includes dynamic flow control (DFC) capability, which allows the flow rate to be changed rapidly for higher speed sample loading or for extended MS/MS analysis (peak parking) to identify co-eluting and low abundance peptides.

### **FULL INTEGRATION AND FLEXIBILITY**

The NanoLC-2D system includes two binary gradient pump systems, autosampler, and injection valves for the first and second dimensions. The 10-port valve for the second dimension accommodates 2 trap columns. The NanoLC software provides complete control of the pumps, valves, and autosampler and provides flexible sample injection modes. The system can be configured to run in either automatic 2-dimensional separation mode (for example, MudPIT), or it can be configured to operate as 2 parallel, completely independent binary gradient HPLC systems, providing ultimate flexibility.

### **SIMPLE OPERATION**

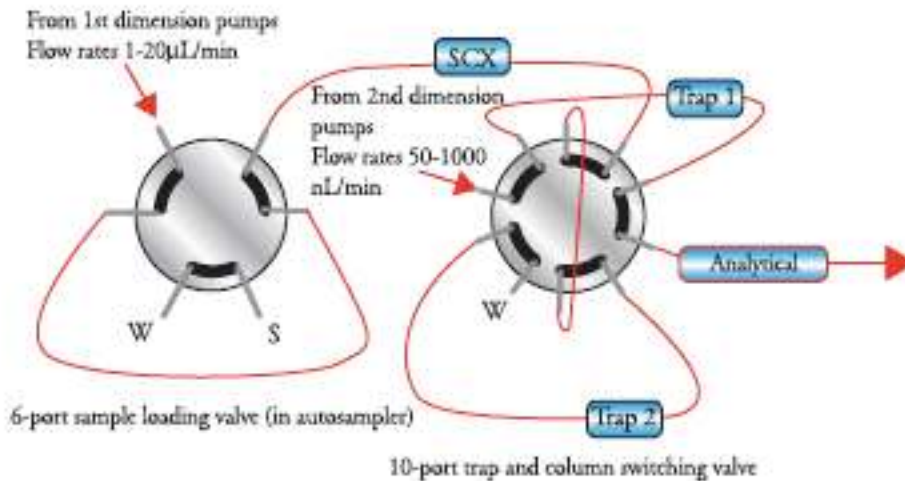
Fully automated 2-dimensional separations coupled with the intuitive NanoLC software make setting up 2D experiments fast and easy. In addition to minimized mobile phase preparation and waste generation, direct pumping allows experimental conditions (e.g. column format) to be changed easily without the need to reconfigure flow splitting hardware.



### **NANOLC-2D SYSTEM FEATURES**

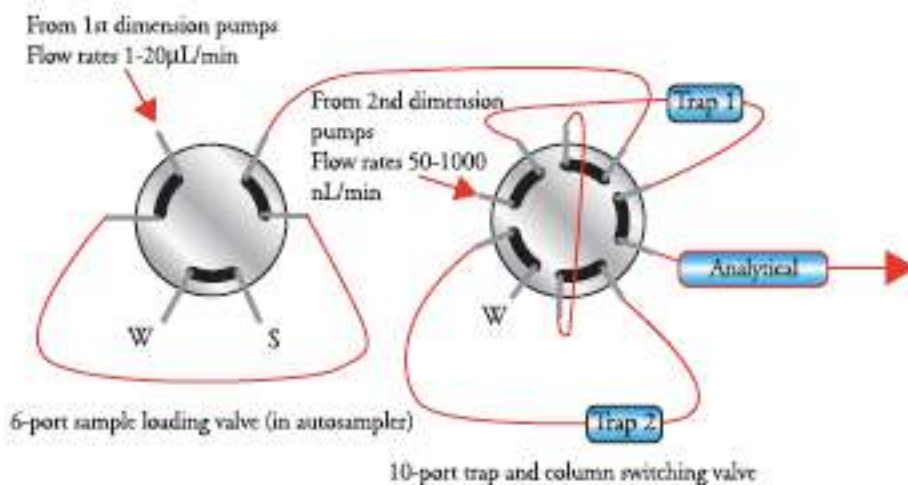
- Flexibility - 2 fully independent binary gradient systems allow operation in a variety of modes, including automated 2D, parallel, and rapid sample loading
- Precision - Direct pumping without flow splitting provides increased flow precision, resulting in improved protein identification
- Increased sensitivity - The NanoLC-2D system incorporates unique DFC capability, enabling peak parking, which improves the ability to identify lowabundance peptides
- Simple operation - The intuitive NanoLC control software allows rapid setup of 2D experiments. No flow splitters eliminates time-consuming hardware reconfiguration

## TYPICAL 2-DIMENSIONAL SYSTEM CONFIGURATION



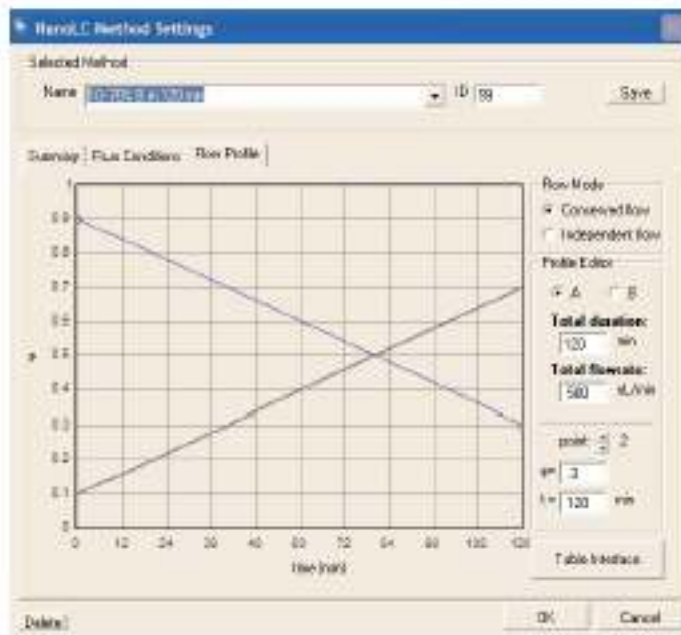
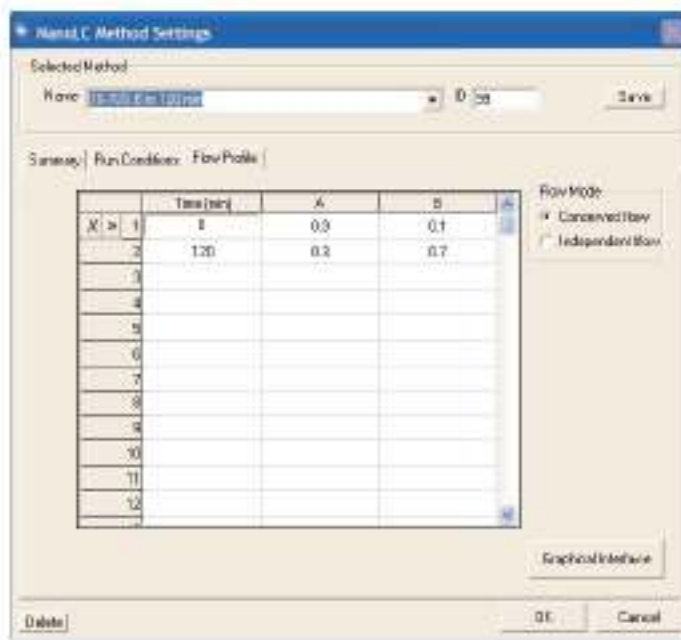
The first dimension separation consists of salt steps or gradients of progressively increasing concentration through a strong cation exchange column. Each step elutes a fraction of peptides to a C-18 trap column. The use of a trap column allows rapid loading and flushing to desalt prior to the reversed phase separation in the second dimension. The 10-port valve accommodates 2 trap columns, increasing throughput by permitting one trap column to be loaded while the other is eluted onto the analytical column. A separate binary pump system is used to control the reversed phase separations, which are usually long gradients of 1 to 2 hours, at flow rates of 100-400  $\text{nL}/\text{min}$ .

## TYPICAL RAPID SAMPLE LOADING CONFIGURATION

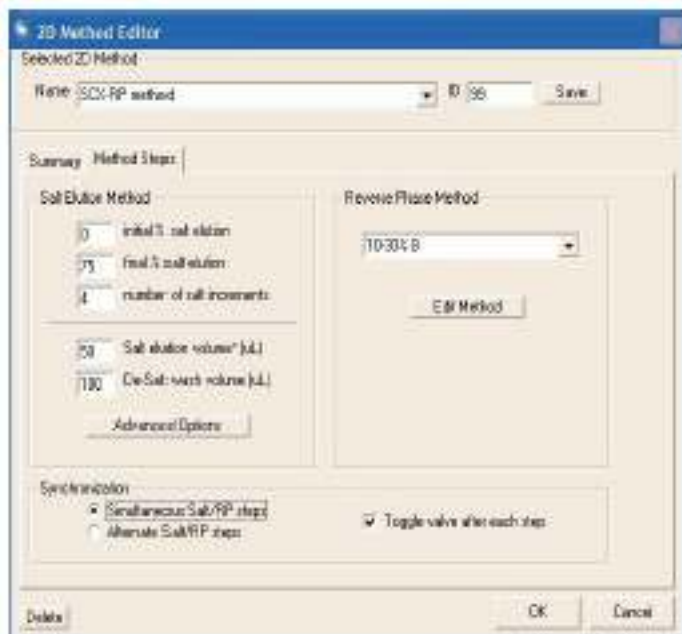


The first dimension pump system is used for rapid loading of sample and flushing to desalt onto either of two trap columns accommodated by the 10-port valve. The second binary pump system is used to elute the sample from the trap column onto the reversed phase column and to control the reversed phase separations, which are usually long gradients of 1 to 2 hours, at flow rates of 100-400  $\text{nL}/\text{min}$ .

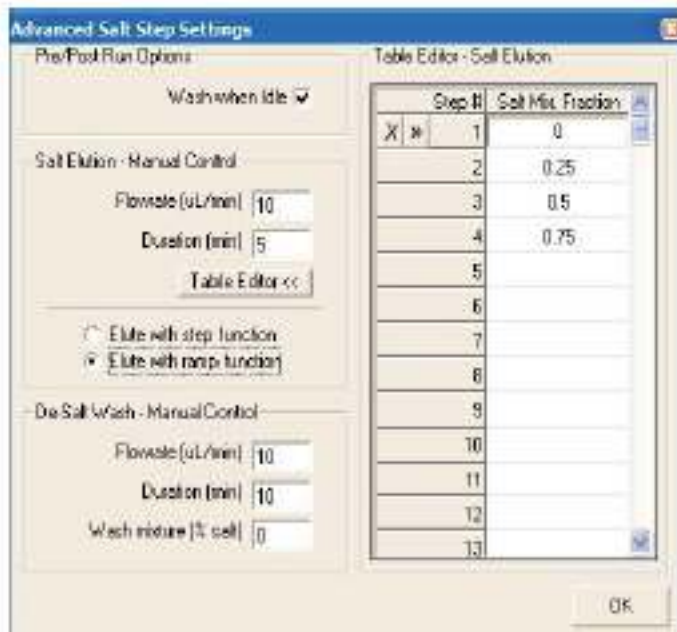
## INTUITIVE NANOLC CONTROL SOFTWARE SIMPLIFIES PROGRAMMING 2D EXPERIMENTS



Flow profiles can be programmed graphically by clicking and dragging points. Alternatively, flow profiles can be programmed using a table interface.



The 2D Method Editor makes it easy to program both channels for a 2D separation. A 2D method can be created simply by specifying the desired initial and final percentages of salt and the total number of steps, as well as the method to be used for the reversed phase separation in the second dimension.



The Advanced Salt Step Settings window includes options for specifying salt elution. The user can choose steps or ramped increases in concentration and fine tune the compositions of each fraction.



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