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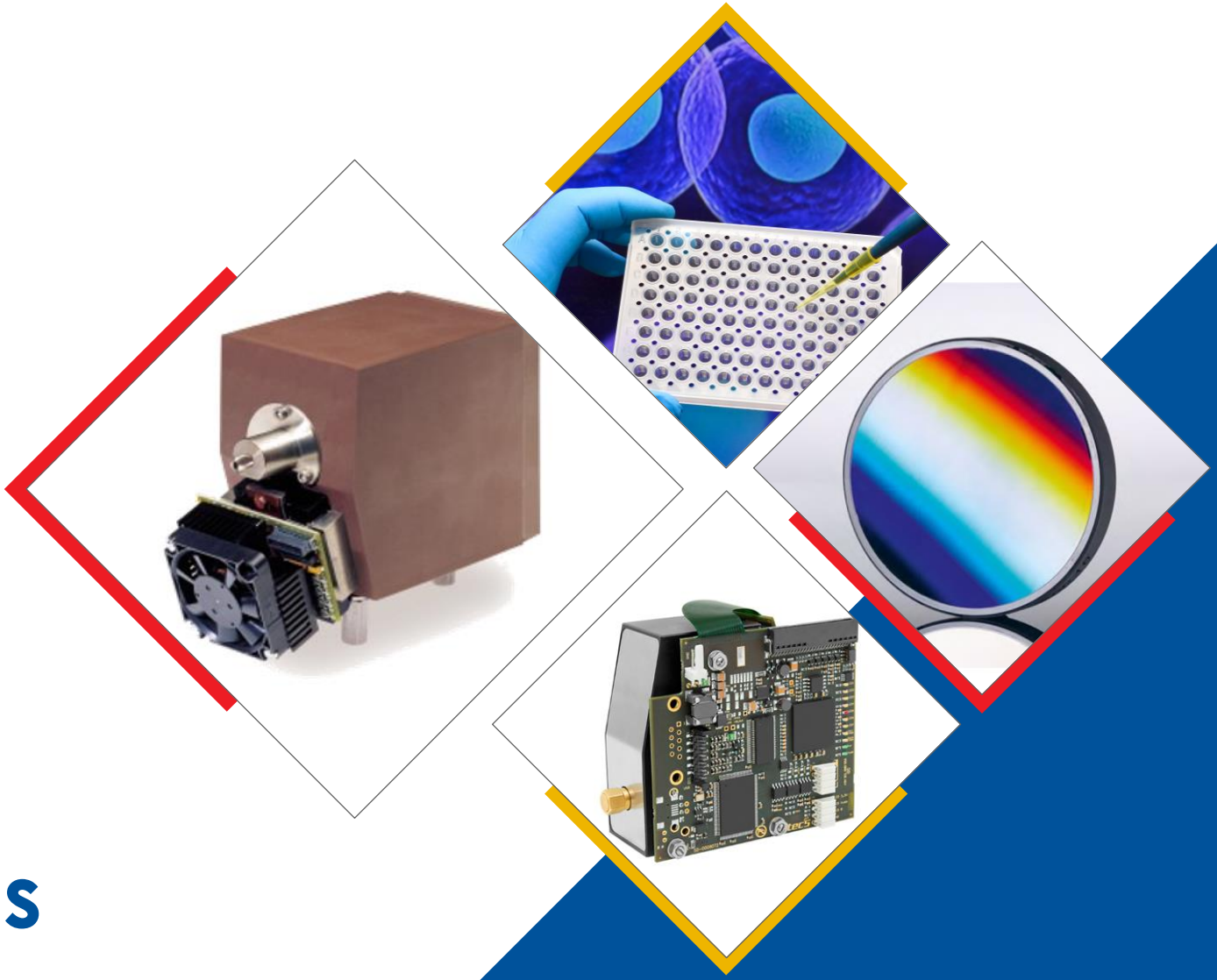
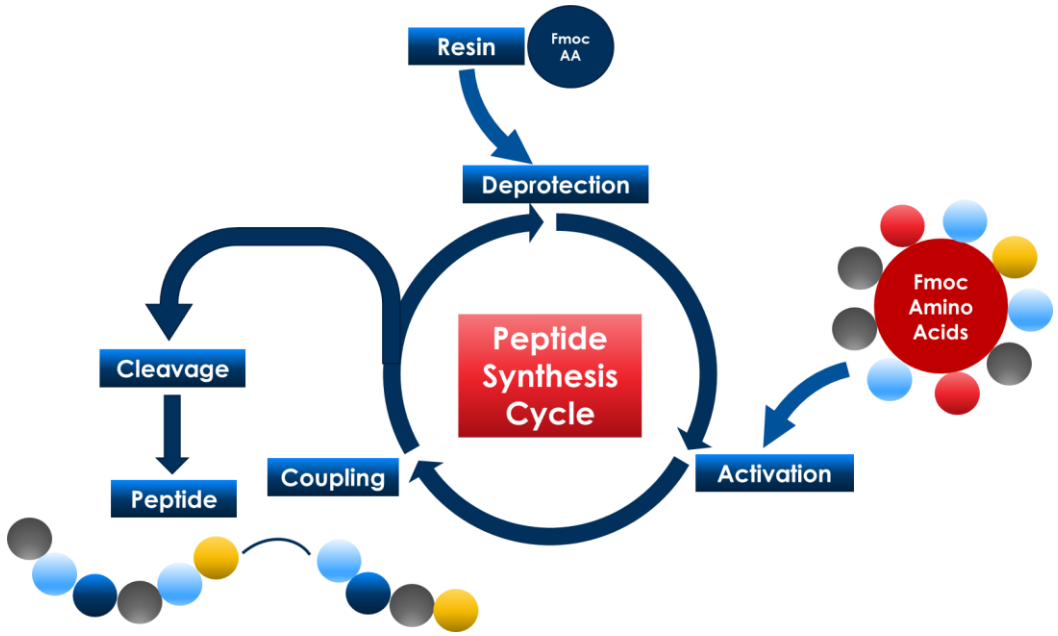


Spectroscopy & OEM Solutions for Peptide Synthesis

Rev. June 2021

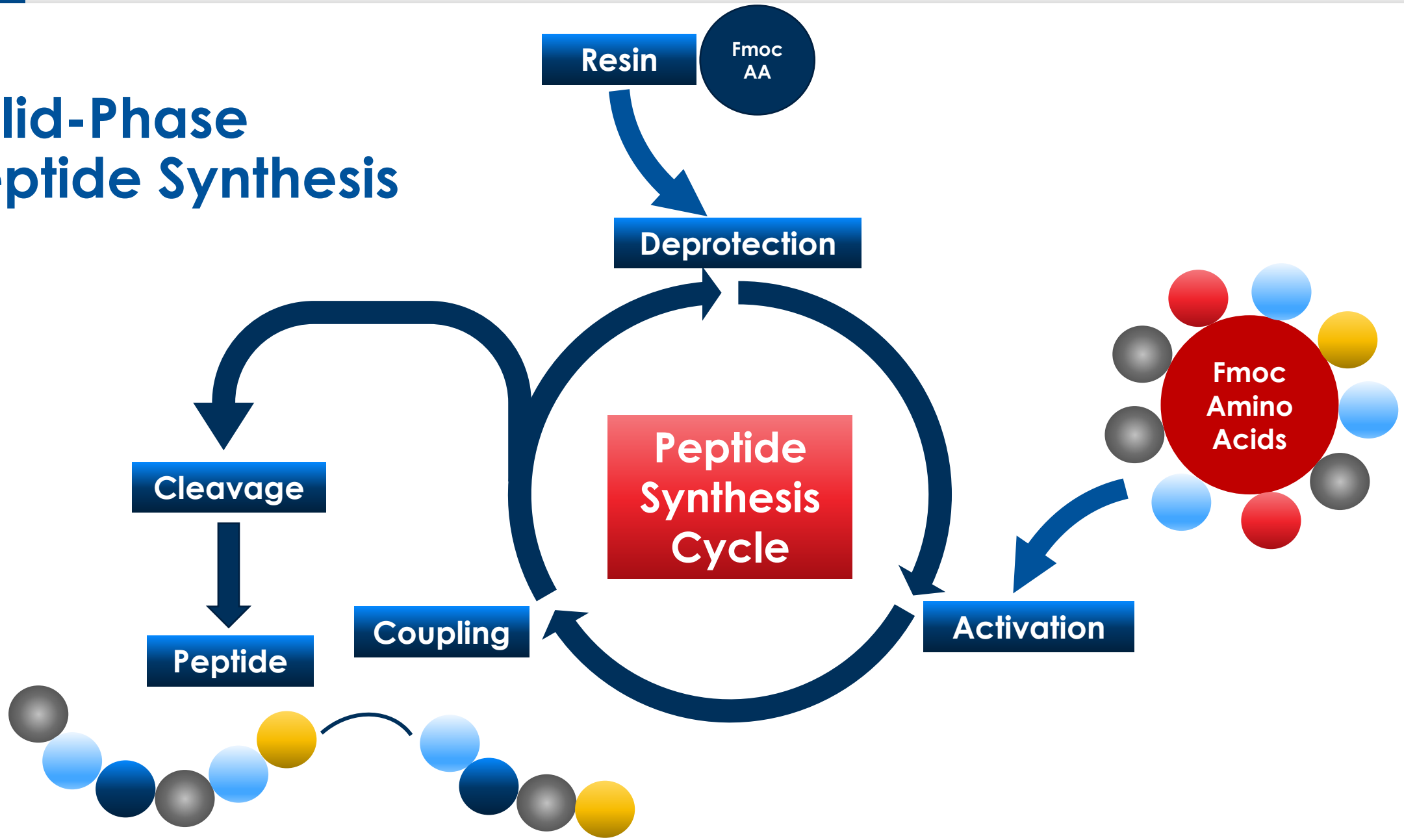
What is Peptide Synthesis?

- Peptide synthesis, the production of peptides, is characterized as the formation of a peptide bond between 2 amino acids, resulting in chains of up to 30 to 50 amino acids.
- Peptides are used to prepare antibodies, map antibody epitopes and enzyme binding sites and to design enzymes, drugs and vaccines.
- Peptides are chemically synthesized by the condensation reaction of the carboxyl group of one amino acid to the amino group of another. Protecting group strategies are necessary to prevent undesirable side reactions with the various amino acid side chains.
- Peptide purity is measured as a percentage of the target peptide to impurities that absorb at the peptide bond absorption wavelengths (190nm and 210-220nm).

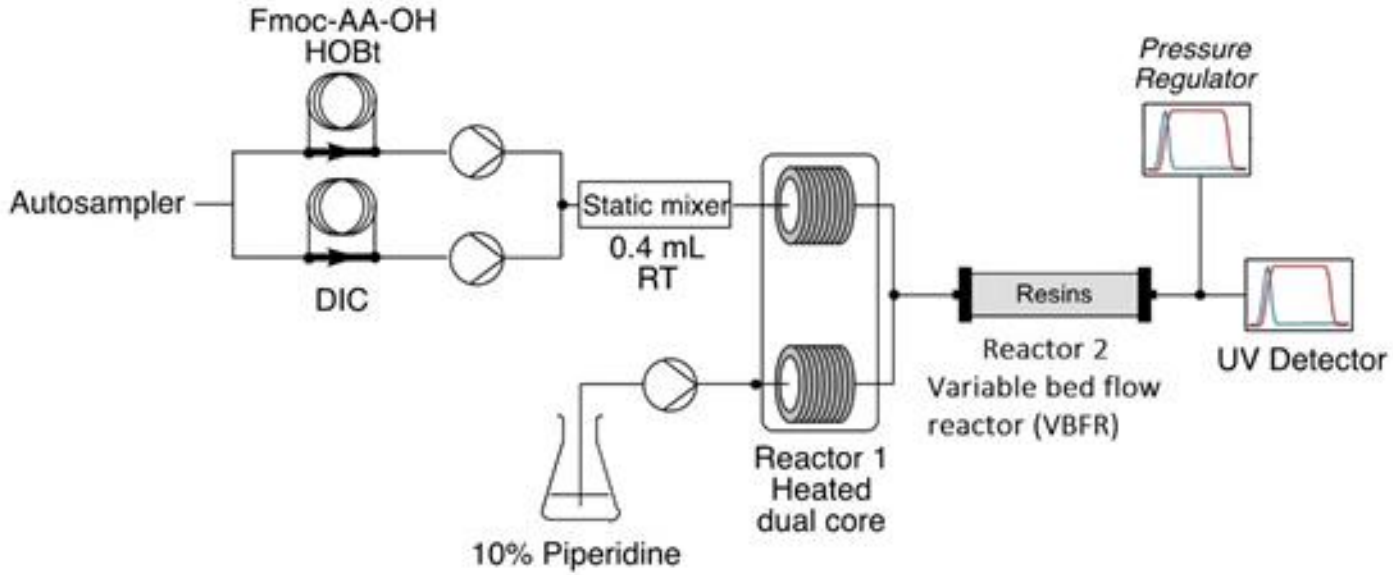


Peptide Synthesis Cycle

Solid-Phase Peptide Synthesis



Monitoring of Fmoc Deprotection by UV Absorbance



The use of N-terminal Fmoc protection allows for a milder deprotection scheme. Fmoc deprotection utilizes a base, typically 20–50% piperidine in DMF. The exposed amine is therefore neutral, and consequently no neutralization of the peptide-resin is required.

Because the liberated fluorenyl group is a chromophore, **Fmoc deprotection can be monitored by UV absorbance of the reaction mixture, a strategy which is employed in automated peptide synthesizers.**

Example of UV Absorbance Spectroscopy of Fmoc-Glycine

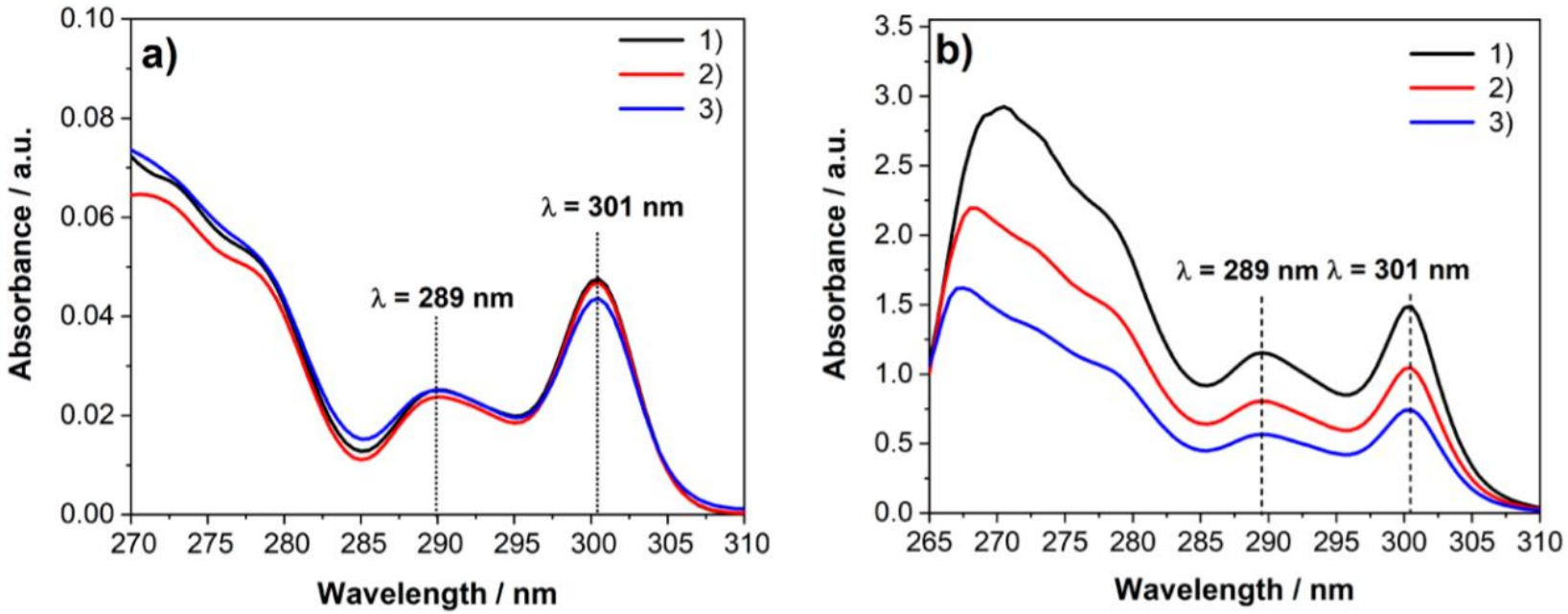
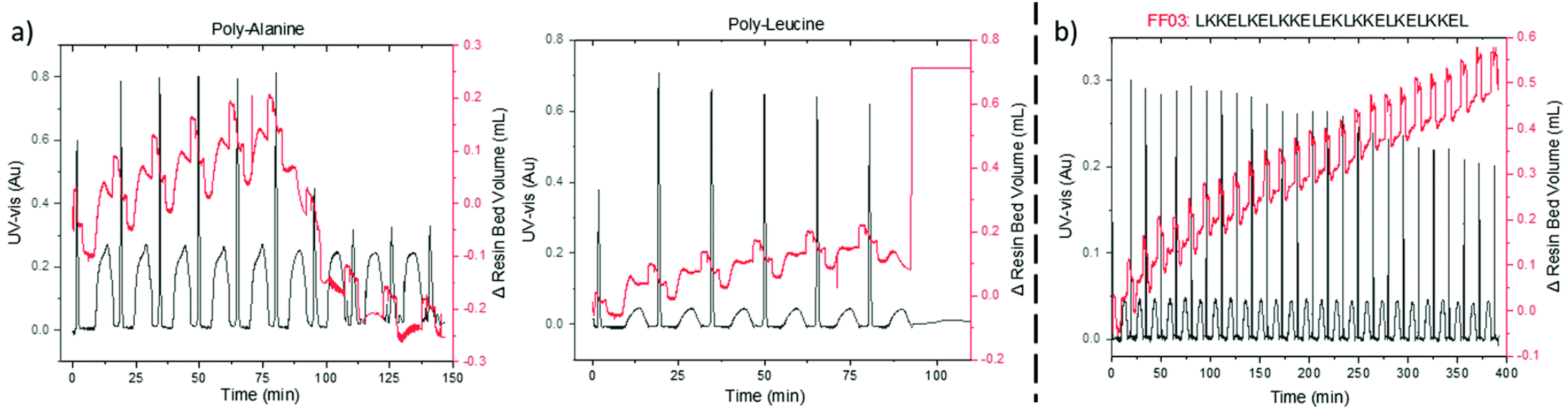


Figure 7. Absorption spectra with the concentrations in mol/dm^3 of (**a**) TiO_2@SiO_2–$(CH_2)_3$–NH–Gly–Fmoc, (**b**) Fmoc–Gly–OH used as the model sample.

Efficient method for the concentration determination of Fmoc groups incorporated in the core-shell materials by Fmoc–Glycine

El ˙zbieta Szczepa ´nska 1 , Beata Grobelna 1 , Jacek Ryl 2 , Amanda Kulpa 1 , Tadeusz Ossowski 1 and Paweł Niedziałkowski 1

In-line UV-VIS monitoring of Solid-phase Peptide Synthesis



Real-time monitoring of solid-phase peptide synthesis using a variable bed flow reactor
Eric T. Sletten, Manuel Nuño, Duncan Guthrie and Peter H. Seeberger

What are the critical requirements for the UV-VIS Detector?

Key parameters for the proper selection of an Optical Spectrometer Platform for Peptide Synthesis:

Stray Light

- UV Absorbance signals are weak. In order to maintain an adequate SNR and, more importantly, an excellent linearity, it is essential to select an optical spectrometer platform with very low stray light.
- Concave gratings, by their design, correction of the aberrations and simplicity of use (only one piece of optics), deliver the required ultimate performance.

Throughput

- A high-throughput spectral engine is required to ensure the maximum collection of weak absorbance signals. Conservation of the Optical Etendue and aperture matching are two parameters, among many others, to pay attention to. Selection of ion-etched, blazed holographic gratings to deliver the highest diffraction efficiency over the spectral range of interest is also critical.

Wavelength Accuracy

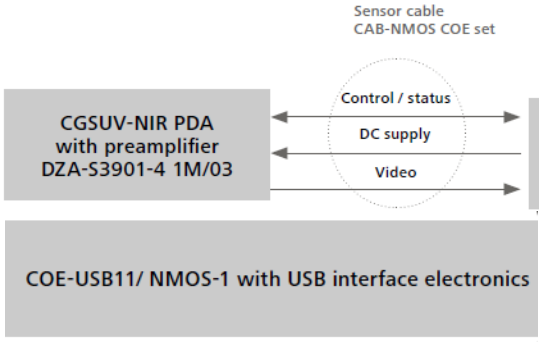
- In order to properly calibrate and reference the absorbance signals and assign the proper coefficients of extinction in the post-processing of the Fmoc data, it is important to maintain a high absolute wavelength accuracy over a wide range of temperatures and over the lifetime of the instrument.
- Breathing designs, with the right selection of material (ceramic), guarantee the lowest thermal and long-term drifts possible.

What solution can tec5USA propose? → CGS UV-NIR PDA

The CGS Spectrometer is built around a blazed, flat-field, imaging grating, with excellent stray light rejection performance, configured with a silicon PDA detector.
The sensor, designed for Absorbance applications, delivers an extremely low noise, ensuring a high SNR ratio – even in low signal conditions.
The configuration results in a spectral pixel distance of 0.7 nm / pixel, and can achieve a spectral resolution of less than 3nm.
All optical components are mounted in a housing made of aluminum.
The spectrometer modules are compact, thermally stable, making them ideal for industrial applications.

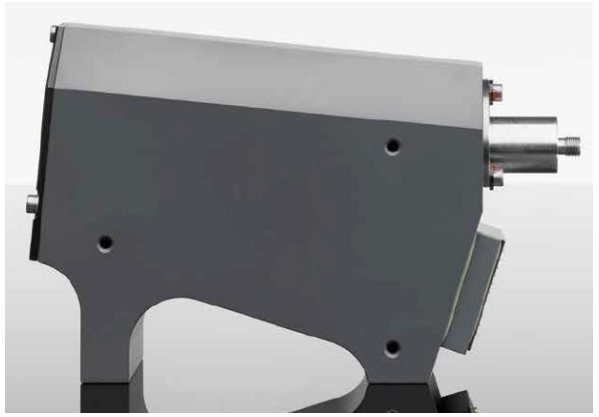
CGS UV-NIR PDA
Technical Data

Optical entrance	SMA connector 40 µm optical slit (can be varied upon request) NA = 0.22 (homogeneous illumination of the acceptance angle) 600 µm mono-fiber interface recommended for customer
Grating	Flat field 534 l/mm (in the center), blazed for approx. 230 nm
Spectral range	190 nm – 935 nm
Resolution (FWHM) with 50 µm slit	UV-VIS < 2.0 nm NIR < 2.0 nm
Stray light (ASTM 387-04)	3 AU at 240 nm with deuterium lamp (absorption A_{10} of NaI)
Integration time (dependent on on-site electronics)	min. 500 µs
Sensor	Hamamatsu S3903, 1024 pixels
Housing size L x W x H	78 x 30 x 75 mm³



What solutions can tec5USA propose? → MCS FLEX PDA

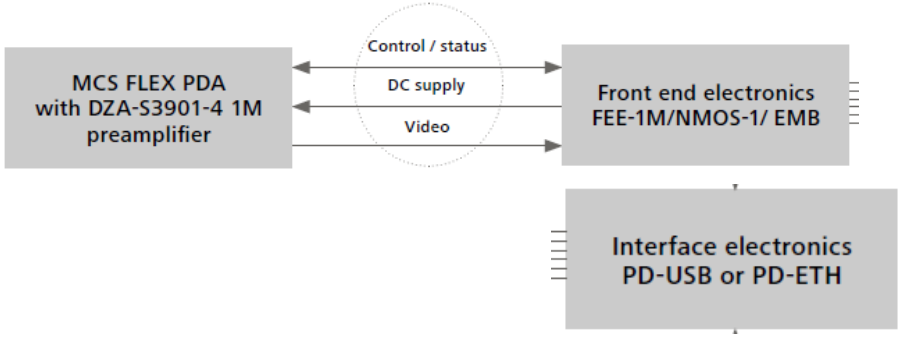
The MCS FLEX spectrometer, a high-end platform in tec5USA's product offering, is designed with a central housing made of a special Aluminum alloy to ensure outstanding thermal stability. The spectral engine is then configured with a PDA detector for low noise and very high dynamic range for Absorbance applications. Offered with a family of imaging holographic blazed aberration-corrected gratings, the throughput of the spectrometer is further improved with the use of a cross-section fiber converter as the input. The specifications of the cross converter (number of fibers and fiber diameter) are determined by the height of the PDA sensor selected (0.5mm or 2.5mm) as well as by the desired spectral resolution to be achieved.



MCS FLEX PDA

Technical Data

Optical entrance	Cross-section converter
Cross-section converter	Diameter: 0.5 mm NA = 0.22 (consistent illumination of the acceptance angle) Mounted in an SMA connector
Grating	Flat field 248 l/mm (in the center), blazed for approx. 250 nm
Diode array	Manufacturer: Hamamatsu Type: S 3904-1024Q Number of pixels: 1024
Spectral range	190 – 1015 nm
Wavelength accuracy	0.5 nm
Temperature drift	≤ 0.009 nm/K
Spectral pixel distance	$\Delta\lambda_{Pixel} \approx 0.8$ nm
Resolution	$\Delta\lambda_{FWHM} \approx 3-4$ nm
Stray light	≤ 0.1 % at 340 nm with deuterium lamp (Transmission of $NaNO_2$ solution, 50 g/l, 1cm)
Housing size L x W x H	160.3 x 62 x 122.2 mm



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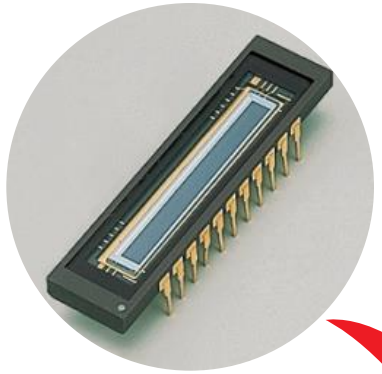
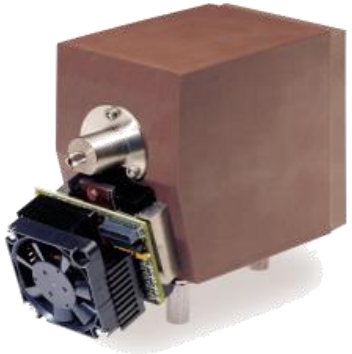


Sensors

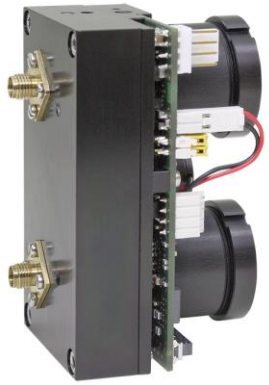
Electronics

Sample Interface

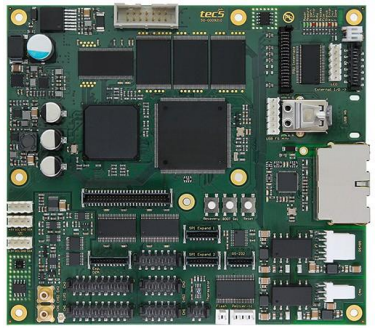
Gratings



tec5 Custom
Spectral Solutions

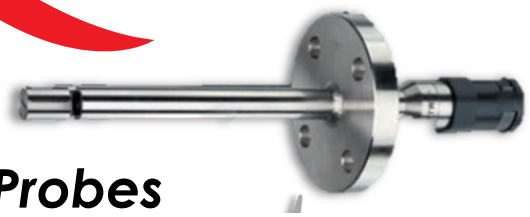


Light Sources



Data Processing & Analysis

For Life Sciences, Medical, Biotechnology, Analytical and Metrology applications.

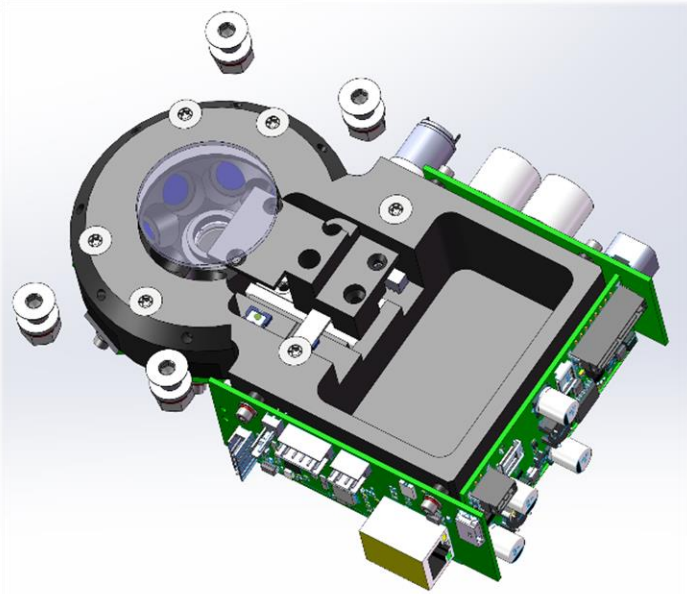


Probes

Custom OEM Spectral Engines & Spectrometers

Experts in Spectroscopy Solutions for OEM Applications

- Customization and Custom Spectrometer and Camera Solutions available to qualified OEM Customers.
- Strong knowledge of Spectroscopy, sensors, custom electronics designs, firmware and software solutions.
- Dedicated Teams of Engineers (OE, ME, EE, FW, SW) available for the definition, design, engineering and release to high-volume production of exclusive spectrometer and sensor solutions.
- Ability to gather novel Technology tools within the different segments of the Nynomic Group, with the financial strength of 425+ employees, yet with the flexibility and response time of smaller, more dynamic companies.
- Design review, Performance improvement and Contract Manufacturing of Customer's sub-systems are added-value offered by tec5 USA for a total system cost-reduction campaign.

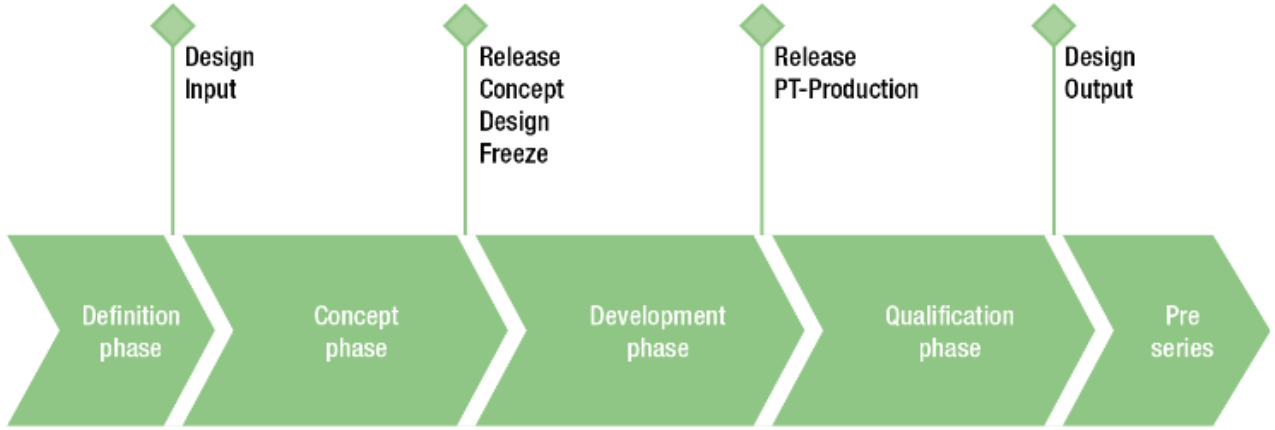


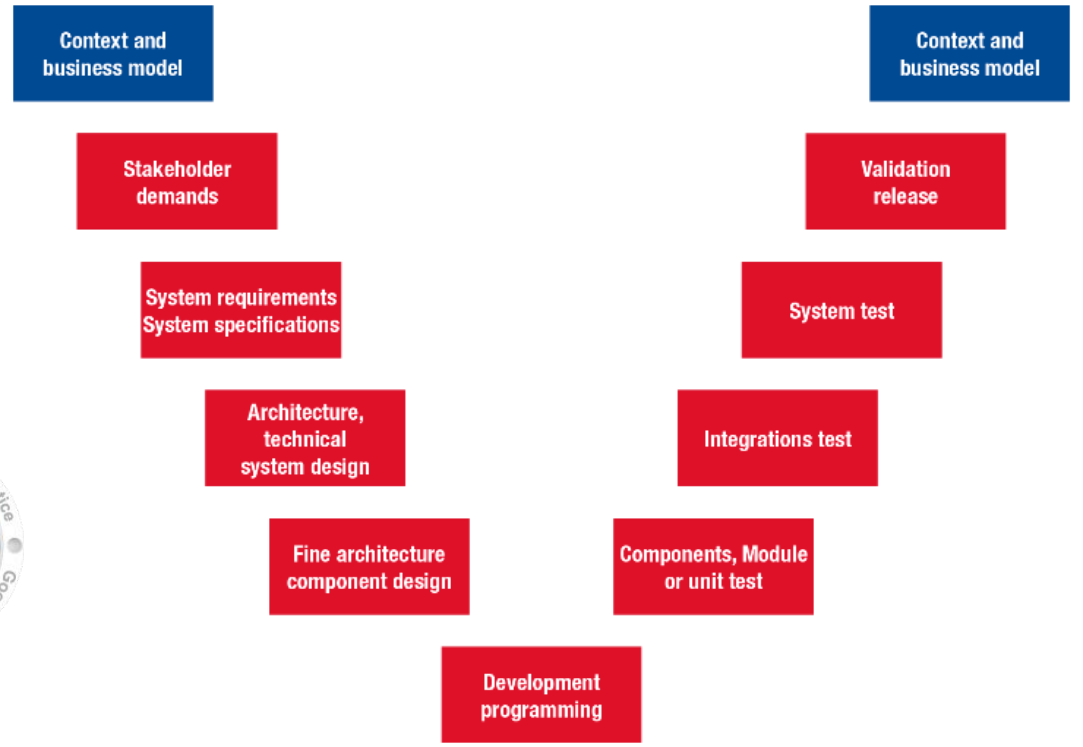
Skills & Excellence

Beyond Well-Resolved Spectra….

…every production process has its own regulative and normative contexts.

- **tec5 operates in R&D, Engineering and Manufacturing environments according to standardized norms.**
- **tec5 is compliant to most normative requirements prevailing in critical Process Analytical Technology (PAT) conditions.**





Thank you.

Contact tec5USA today to discuss your requirements to design your next-generation of automated peptide synthetizers!

Enabling real-time process verification through rapid inline spectroscopy

Contact US

516 – 653 - 2000

80 Skyline Drive Unit 1
Plainview, NY 11803
USA

sales@tec5usa.com

http://www.tec5usa.com