Construction of higher efficient systems, Novel PSP-5200 for larger scale syntheses and rapid characterization for small peptide libraries

Kiyoshi Nokihara*, Yuki Tominaga, Takeshi Kasama, Haruyuki Fujino, Atsushi Kitagawa, Toru Sasaki, and Shun Nokihara

Correspondence: Dr. Kiyoshi NOKIHARA
CEO & CSO HiPep Laboratories, Kyoto, Japan
Professor, School of the 1st Clinical Medicine, Affiliated Hospital with Nanjing Medical University, Jiangsu, China

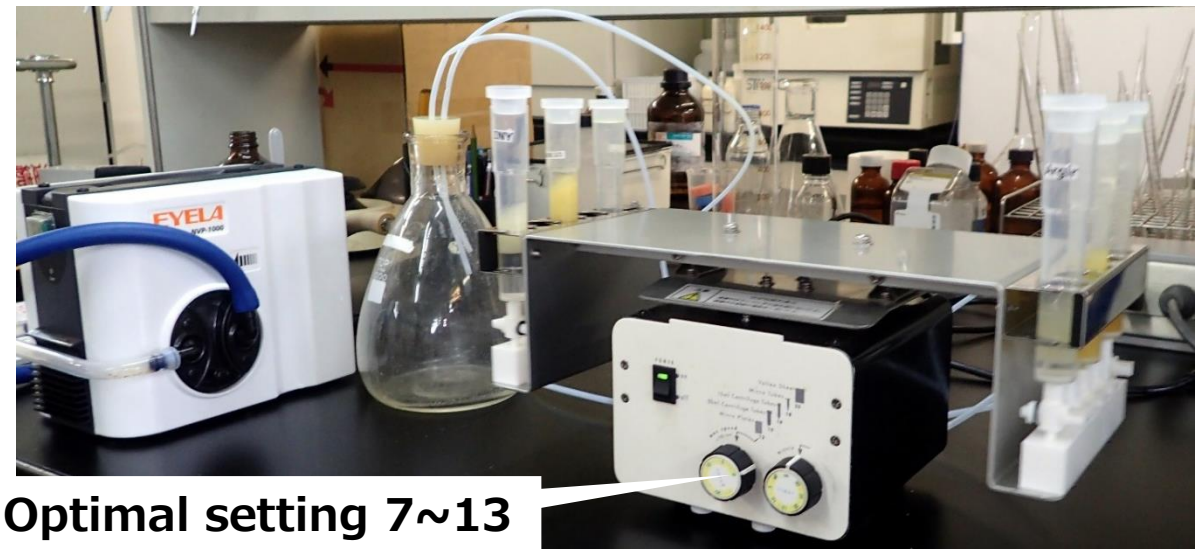
email: PepTenChip@hipep.jp URL: http://www.hipep.jp

Due to the cell-based assays demands on synthetic peptides are increasing.

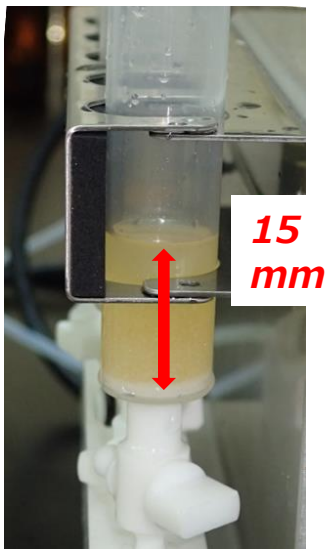
The present paper describes higher efficient systems for library construction and characterization with energy-saving manner.

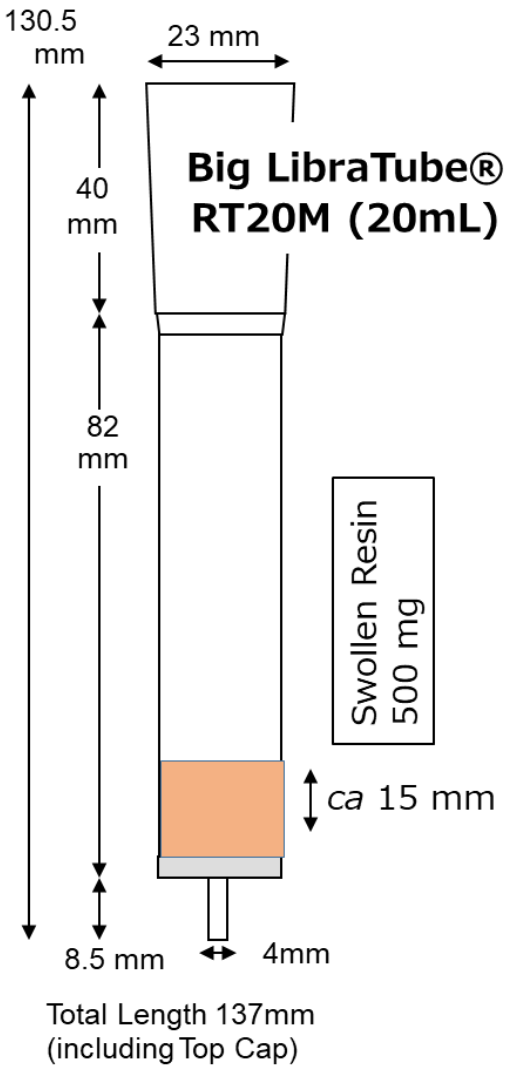
(1) Larger scale by a novel PSP5200 for RT20M

- Previously we have developed personal synthesizers with simultaneous multiple mode (PetiSyzer®) and disposable reactors, RT3M, RT5M and RT20M (3, 5 and 20 mL, respectively). RT20M has been often used as columns for separation.
- The PSP5200 has a larger attachment on a shaking device having 5 x 2 positions.



Optimal setting 7~13





- The mixing principle is an oval-rotation. More rotational moment was required for complete mixing. The shaking speed and total reaction volume have been optimized by observation.
- Larger filtration area of RT20 allowed **rapid removal of solvents**.
- The optimal reaction scale: 300-500 mg polymer supports/reactor.
- Target peptides with molecular weight around 1 kDa ➡ conventional polystyrene-resin (substitution is 0.6-0.7 mmol/g) can generate 2-300 mg of crude peptide per reactor. TentaGel® (substitution is ca 0.25 mmol/g) give ca 75 mg of crude peptide per reactor.
- Generally, reaction time is a little bit longer than that of smaller reactors, although the total consumption of solvent was ca **30 mL for one coupling** (including washing after coupling and deprotection), hence mixing with larger volume was ineffective.
- After cleavage the **purity of synthetic peptides was >90%** and could be used without purification for further applications.

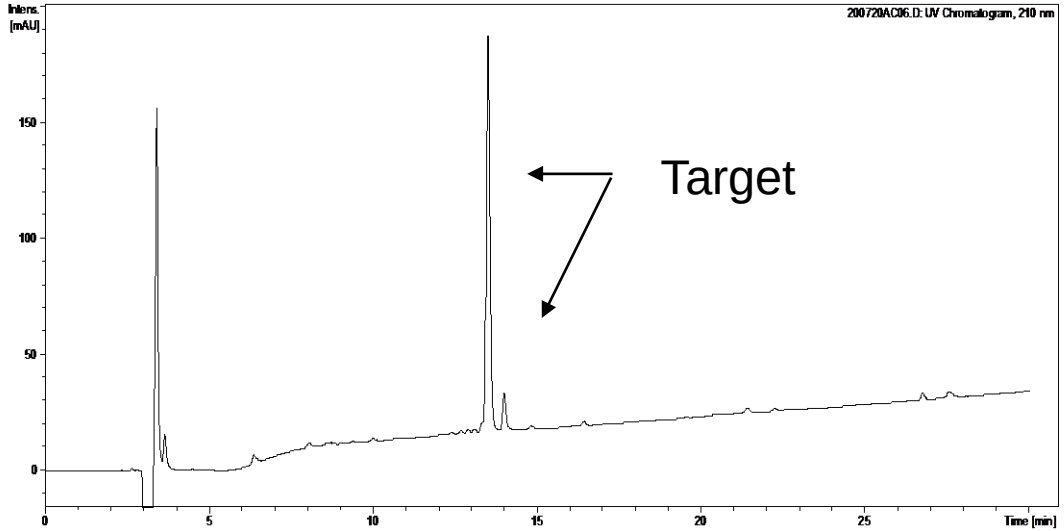
Example
Assembly: PSP5200 + RT20

Resin	TentaGel®S RAM 300 mg, 0.20 mmol/g
Fmoc removal	5 min X2 with 20% PIP in DMF
Coupling	4 eq. excess acyl component with HBTU-HOBt-DIEA in DMF (*ca* 2 mL/each)
Acetylation	AcOH/HBTU/HOBt/NMM
Cleavage	TFA/TIS/H2O/EDT = 85/5/5/5 v/v% RT.2 hr
Crude Yield	*ca* 100%

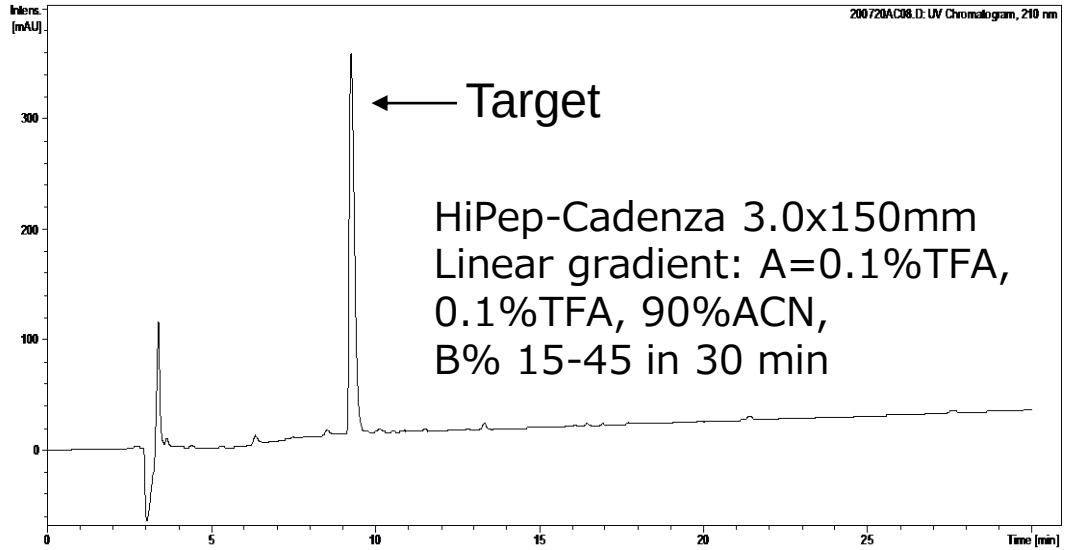
Solvent consumption
ca 2-3 mL/each coupling & washing

HPLC Profiles of he Crude Peptides

Ac-Arg-Phe-Ala-Ala-**Cys**-Ala-Ala-NH_2



Ac-Arg-Phe-Ala-Ala-**Lys**-Ala-Ala-NH_2



A standard operation protocol for solid-phase synthesis

#	Operation	Time	Cycle
1	Resin Preparation for swelling	Over Night	X 1
2	Fmoc-removal with piperidine in DMF	5 min	X 2
3	Resin-wash: 30 sec mixing with DMF or 30-50% DCM in DMF Solvent removal: @2 mL/RT20M	1 min	X 5
4	Coupling: addition of acyl component (4 eq. excess with HBTU-HOBt-DIEA in DMF), ca 2 mL/each and mixing	60 min	X 1
5	Resin-wash: 30 sec mixing, Solvent removal	1 min	X 5
6	#2	5 min	X 2
7	#3	1 min	X 5
8	#4	60 min	X 1
9	#5	1 min	X 5
	#2 ~#5 repeat, to complete target sequence		
100	Fmoc-removal with piperidine in DMF	5 min	X 2
101	Resin-wash (DMF ➡ DCM ➡ MeOH ➡ tert-butyl methyl ether) : 30 sec mixing/each, Removal of solvent ➡ dried *in vacuo*	1 min	X 5
201	Cleavage by TFA cocktail	60 min	X 1

Solvent consumption was ca 30 mL/cycle, a hexa-peptide could be assembled in one day.
Conventional abbreviations were used.

email: PepTenChip@hipep.jp URL: http://www.hipep.jp

The peptidyl-resin generated from TentaGel® without linker in RT20M can also be used directly as **an affinity column** after cleavage (Fig right) for this application high quality peptides on resin is particularly important since the peptide immobilized on resin cannot purify by HPLC. http://hipep.jp/?p=1038

[Nokihara, K., and Ando, E., Peptide Chemistry 1993, ed., Okada, Y., 25-28, 1994],

(2) Rapid characterization for the large number of library

For statistical analyses ultra-rapid analyses system for oligopeptides has been developed. The allover run-time could be dramatically reduced up to: **8 min/analysis including column wash.** This was required and performed >2500 analyses for statistical data handling purpose, hence this system was very effective **for higher quality of crude peptides**.

Assembly of two model peptides using PSP5200+RT20M

Inyline
Ac-Glu-Asp-Tyr-Tyr-Arg-Leu-NH_2
Argireline
Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2

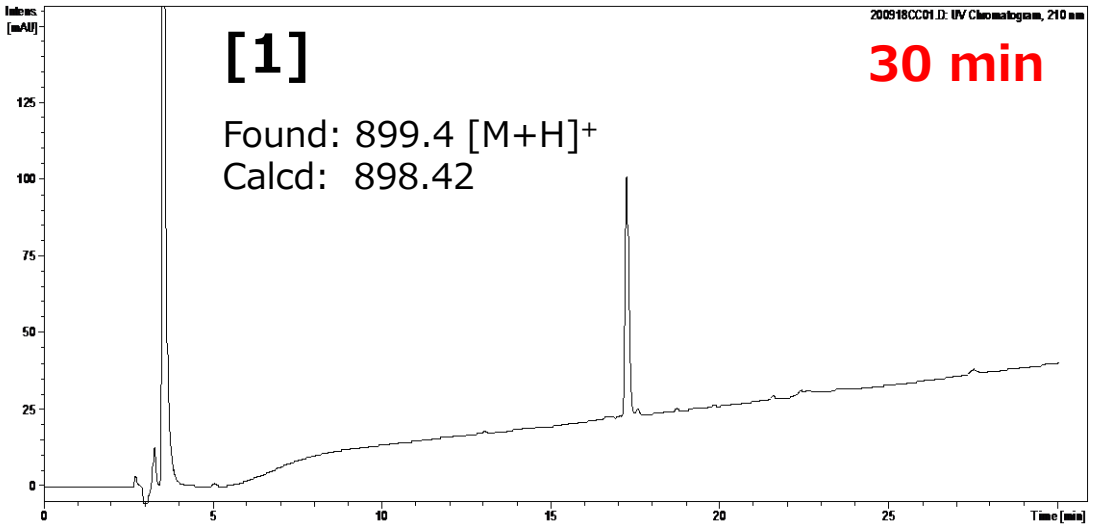
Peptidyl resin yielded ca 100%
Crude Peptides >90-95% purity

Resin 0.5 g	RinkAmide MBHA 0.67 mmol/g
Fmoc removal	5 min X 2 with 20% PIP in DMF @ 2 mL
Coupling	Acyl comp. 4 eq. excess with HBTU/HOBt
Acetylation	AcOH/HBTU/HOBt/NMM
Cleavage	TFA/TIS/H2O/EDT= 85/5/5/5 v/v% RT 5h

Comparison of the [1] conventional *vs* [2] rapid HPLC

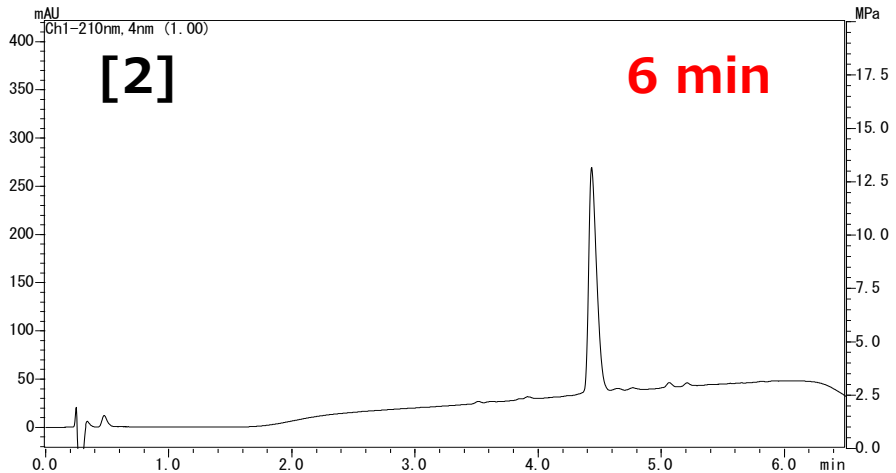
Ac-Glu-Asp-Tyr-Tyr-Arg-Leu-NH_2

[1] HiPep-Cadenza C18 3.0 i.d. x 150 mm



Injection: 10 μL
Flow: 0.3 mL/min
A=0.1%TFA,
B=0.1%TFA in 90% ACN,
B%=15-45 in 30 min

[2] HiPep-Cadenza C18 2.0 i.d. x 30 mm



Injection: 1 μL
Flow: 0.4 mL/min
A: 0.1% HCOOH
B: 0.1% HCOOH in ACN
B%=7 (4min) ～ 27 (2 min)
at 40℃

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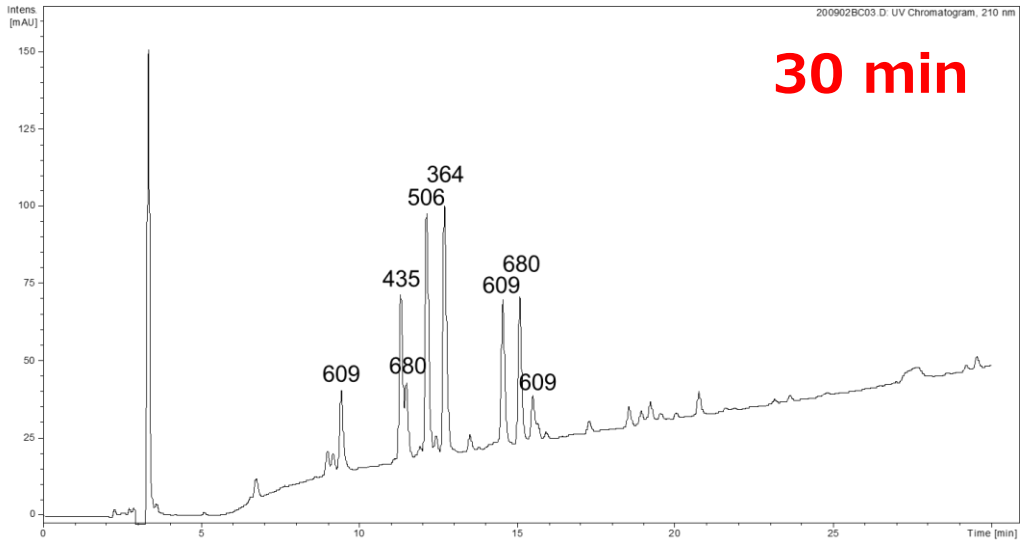
Comparison of the [1] conventional *vs* [2] rapid HPLC

[1] HiPep-Cadenza C18 3.0 id x 150 mm



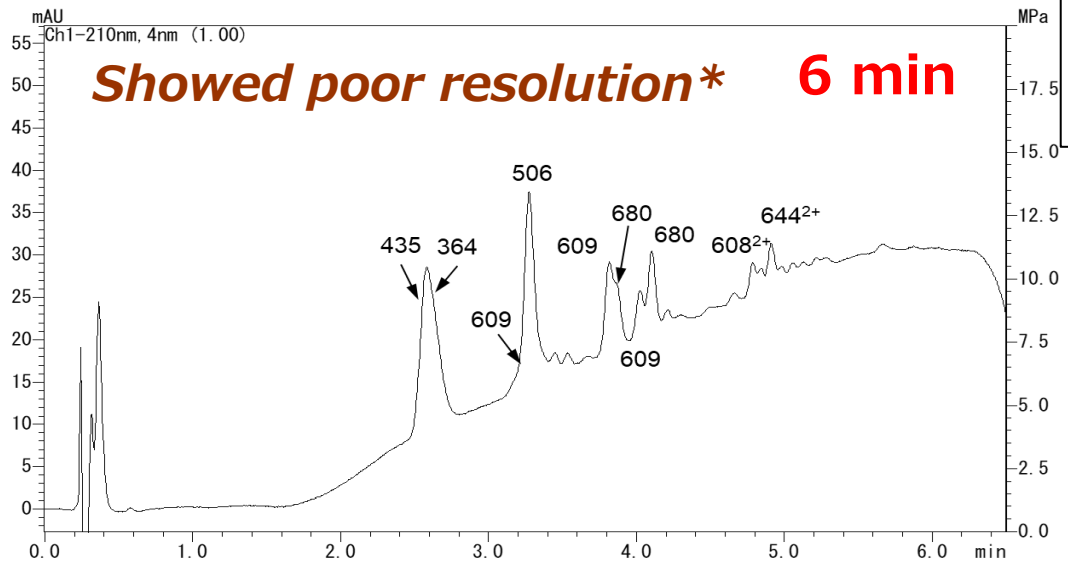
30 min

Inject 10 μL; Flow: 0.3 mL/min
A=0.1%TFA, B=0.1%TFA in 90% ACN,
B%=15-45 in 30 min

m/z 680: Ac-RFAACA
m/z 609: Ac-RFAAC
m/z 506: Ac-RFAA
m/z 435: Ac-RFA
m/z 364: Ac-RF

Analyte containing deletion peptides

[2] HiPep-Cadenza C18 2.0 id x 30 mm



Showed poor resolution* **6 min**

Inject 1 μL; Flow: 0.4 mL/min
A=0.1% HCOOH, B=0.1% HCOOH in ACN
B%=7 (4 min) – 27 (2 min) at 40℃

m/z 680: Ac-RFAACA
m/z 609: Ac-RFAAC
m/z 506: Ac-RFAA
m/z 435: Ac-RFA
m/z 364: Ac-RF

➡ ***although poor resolution assignment could be performed for evaluation of syntheses.***

Summary

① Simultaneous multiple assembly by **PSP-5200** with **RT20 [less solvent consumption]** & a rapid characterization system for libraries were presented.

② Resolution is not crucial for high quality crude peptides.

③ The system is particularly useful for protected fragments having stronger retention .

➡ Saving energy: Lower costs Rapidness with less skill

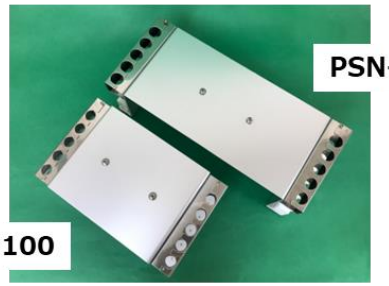
新製品　PetiSyzer PSN-5200　PSP5200

RT20M用のラック

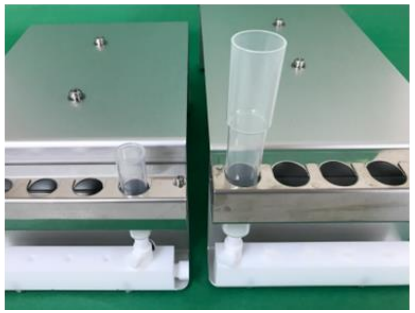
濾過面積が大きく、合成がスピードアップ！

創薬関連でペプチドの需要が増加し、研究効率を上げるため、LibraTube® RT20Mを左右5本ずつ，同時振とう撹拌を行うためのアタッチメントを製品化しました。

カップリング反応の状況にも依存しますが、目安としてRT20M 1本につきレジン仕込み量300 ~ 500 mgの合成が可能です。



本製品は、既製品 LibraTube® RT20M (20 mL)用のラックです
PSP-5100 を既にお持ちの場合、そのまま振とう機に付け替えて使用できます



当該製品の振とう撹拌はオーバル回転（偏遠心）です。リアクターへの充填量、溶媒や回転数を観察しながら調整し、目視であふれることなく、撹拌・混合されていることをよくご確認ください。PSN-5200は加熱ユニットがありません。

左：PSN-5100　と　RT5M
右：PSN-5200　と　RT20M

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