Biophysical Journal, Volume *113*

Supplemental Information

Charge Influences Substrate Recognition and Self-Assembly of Hydrophobic FG Sequences

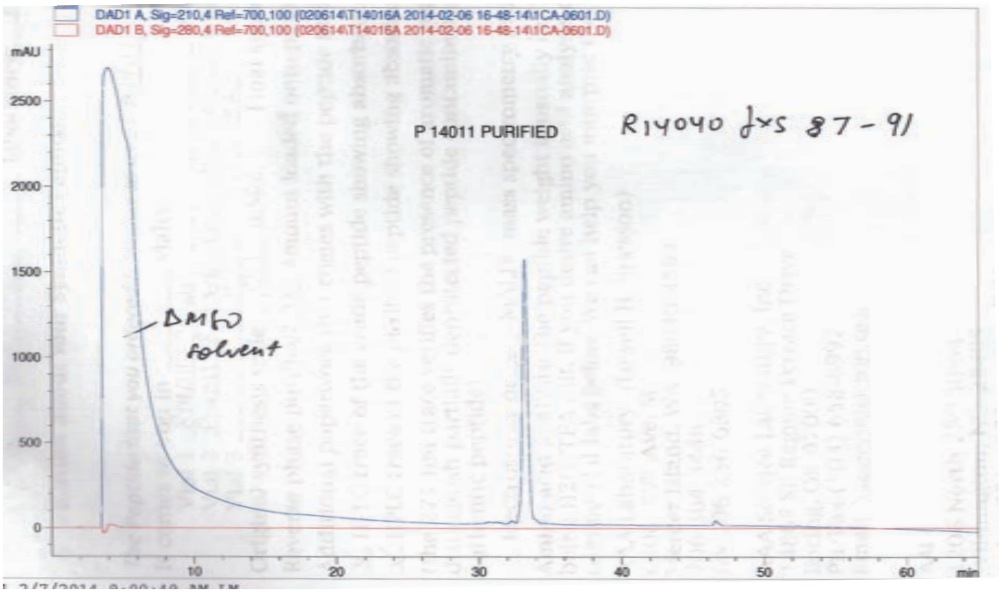
Wesley G. Chen, Jacob Witten, Scott C. Grindy, Niels Holten-Andersen, and Katharina Ribbeck

Supplementary Data S1

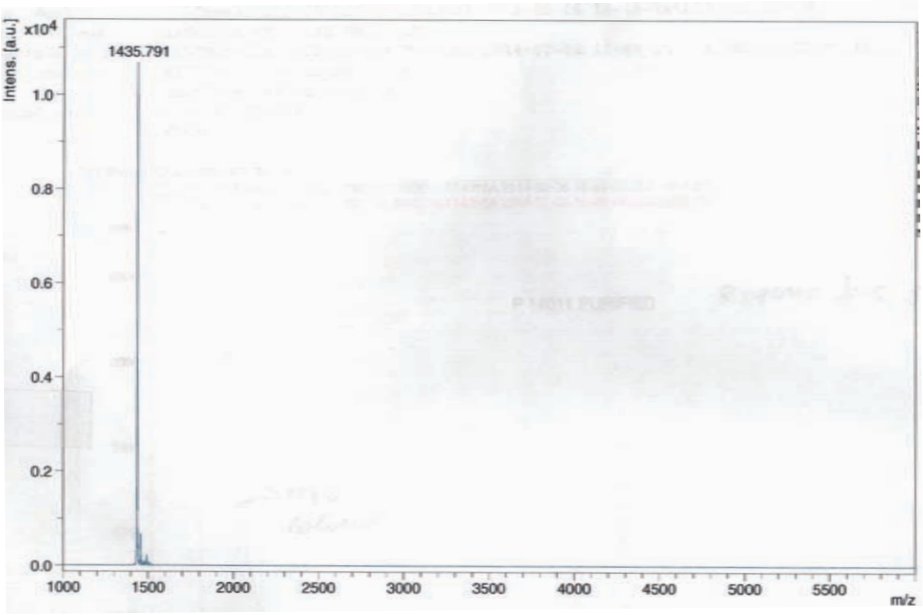
Purity of synthesized peptides assessed by analytical HPLC and mass spectrometry

FGAK Peptide

Analytical HPLC Nspgdga_rgm l



Mass spectrometry analysis



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Reflector detector voltage: 1.59 kV
Ion source voltage 1: 20 kV
Ion source voltage 2: 18.55 kV
Ion source lens voltage: 6.5 kV
Number of shots: 400

Analysis Report

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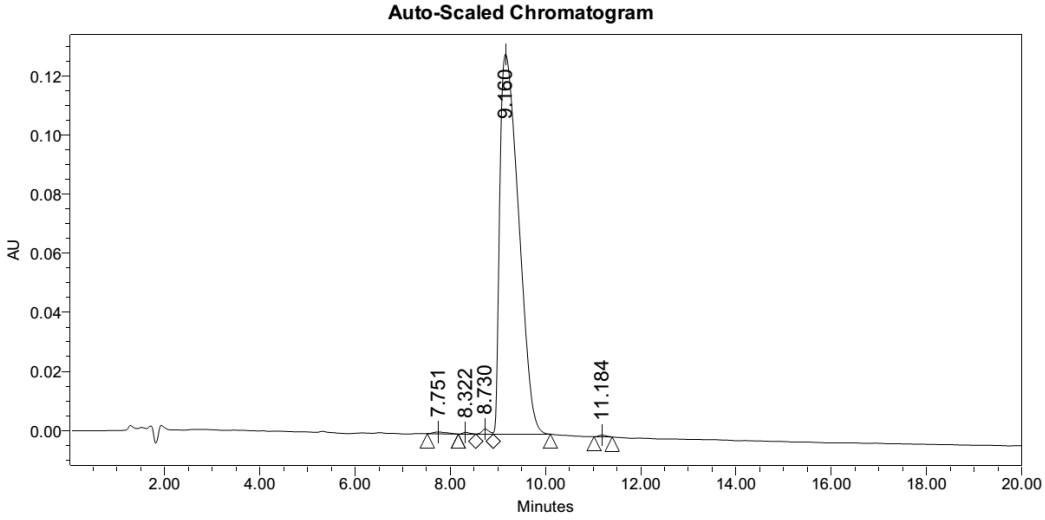
Product Name: P155934 Lot: QP102815KZ1E
Mobile Phase: A: 0.05% TFA in H2O
B: 0.05% TFA in 100% ACN
Grads: 21%-31%B in 20 min Flow : 1.0 ml/min
Column : Agilent ZORBAX 300SB-C18 5um 4.6*150mm 220nm



Peak Results

	Retention Time (min)	Int Type	Area (µV*sec)	Height (µV)	Width (sec)	% Area
1	7.751	BB	12262	590	39.000	0.34
2	8.322	BV	6094	572	22.000	0.17
3	8.730	VV	18379	1727	22.000	0.52
4	9.160	VB	3515511	128499	72.000	98.78
5	11.184	BB	6614	609	23.000	0.19

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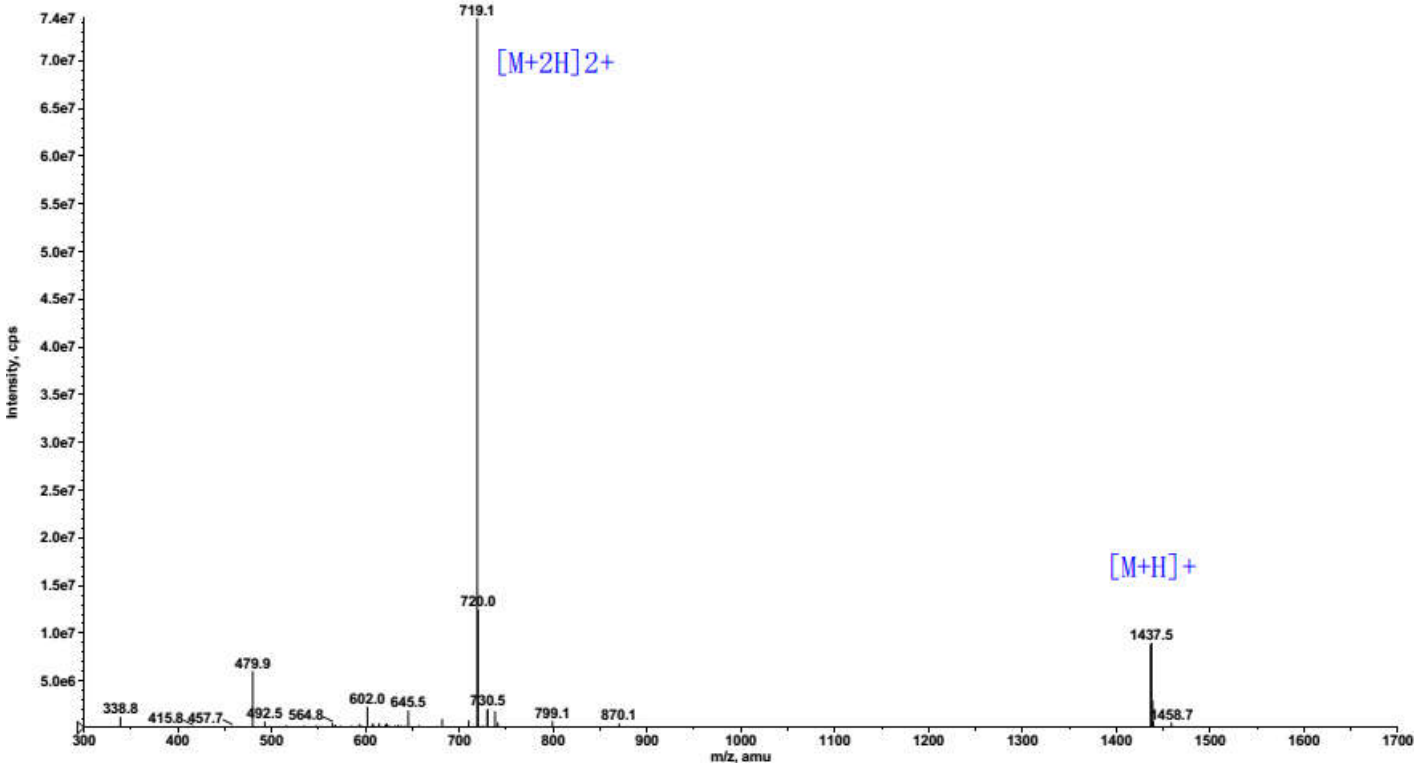
Email: contactus@bostonopenlab.com•www.bostonopenlabs.com

Product Name: P155934
Lot: QP102815KZ1E
MW: 1435.64
Date:2015-11-16



763-D Concord Avenue, Cambridge, MA 02138•Phone: (877) 299-8500•Fax:(617) 800-0997
Email: contactus@bostonopenlab.com•www.bostonopenlabs.com

Analysis Report

Inj. Date: 11/16/2015 11:28:06 Operator: Aiqin Wang
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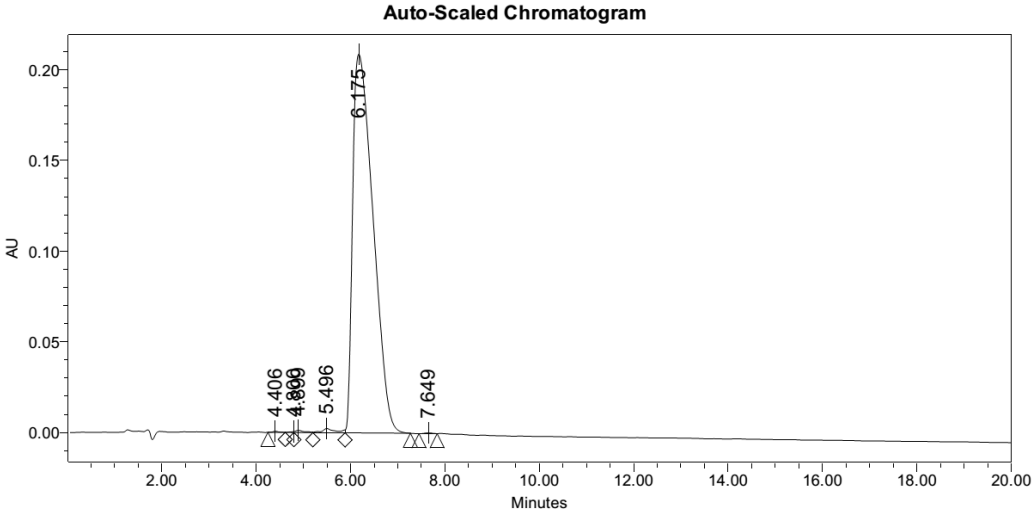
Column : Agilent ZORBAX 300SB-C18 5um 4.6*150mm 220nm



Peak Results

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2	4.800	VV	2555	496	11.000	0.04
3	4.899	VV	15792	1193	24.000	0.25
4	5.496	Vv	45962	2333	41.000	0.73
5	6.175	vB	6256502	208887	83.000	98.83
6	7.649	BB	4769	436	23.000	0.08

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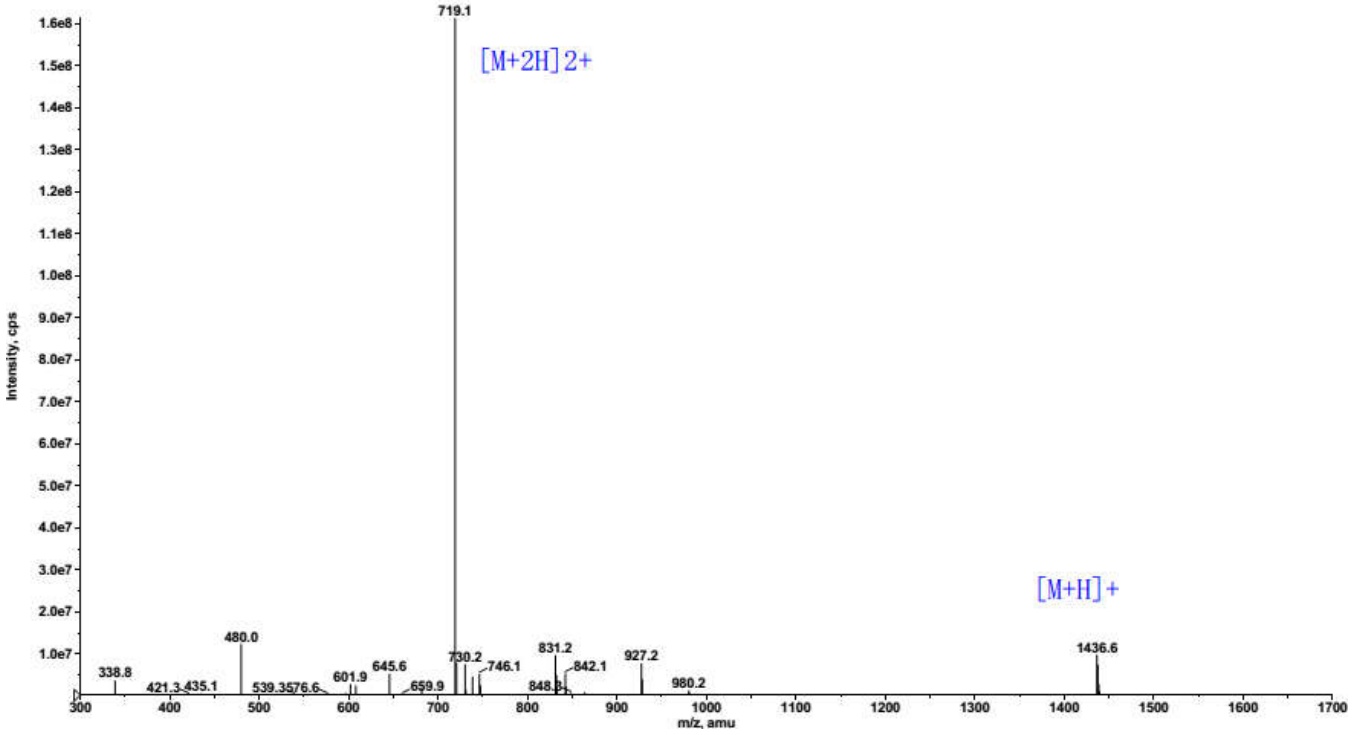
Lot: QP102815KZ1F
MW: 1435.64
Date:2015-11-16



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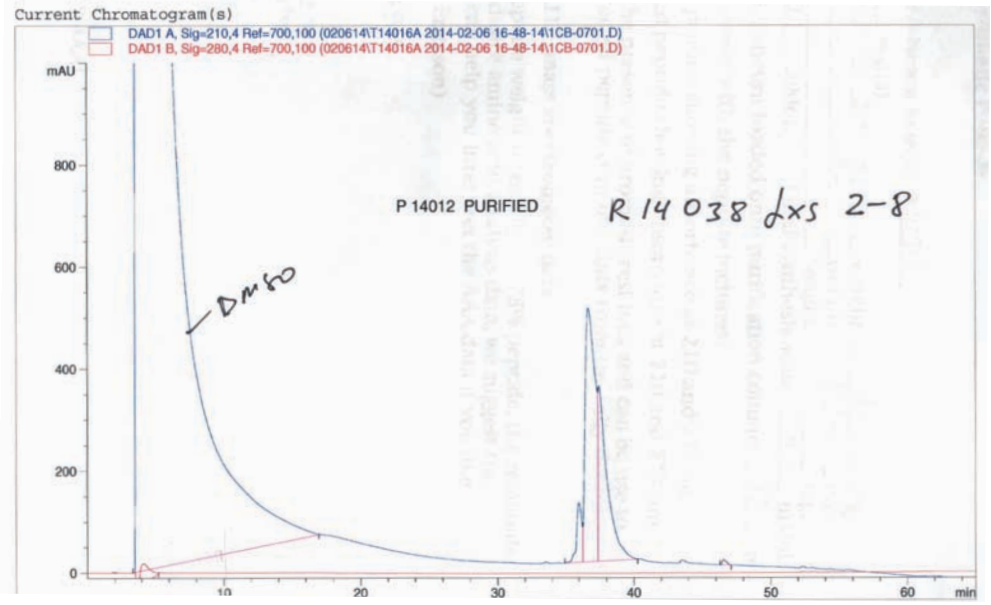
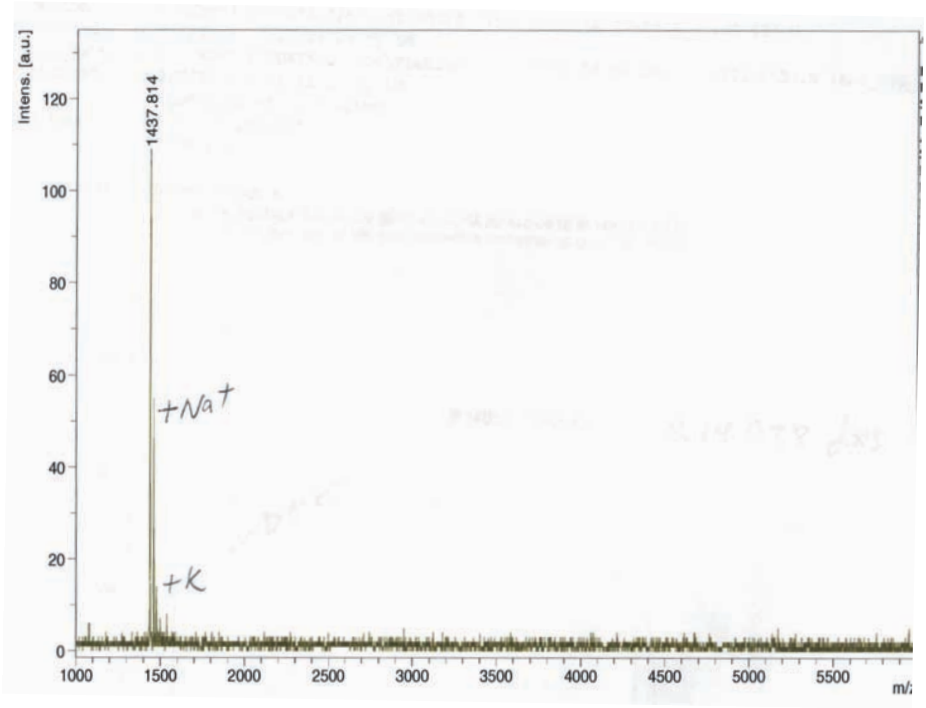
Email: contactus@bostonopenlab.com•www.bostonopenlabs.com

FGAE Peptide

Analytical HPLC



Mass spectrometry analysis



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Linear detector voltage: 2.691 kV
Reflector detector voltage: 1.59 kV
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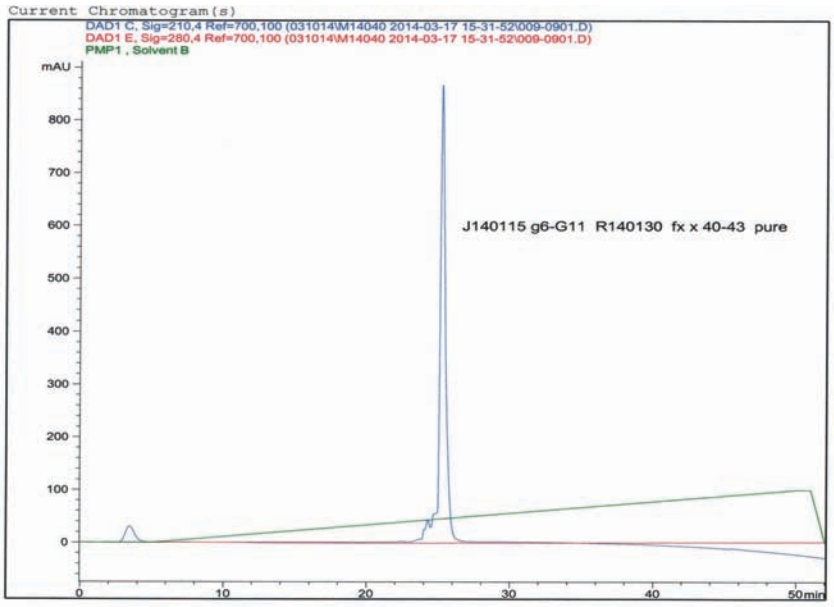
Ion source voltage 2: 18.55 kV
Ion source lens voltage: 6.5 kV
Number of shots: 400

FGAR Peptide

Analytical HPLC



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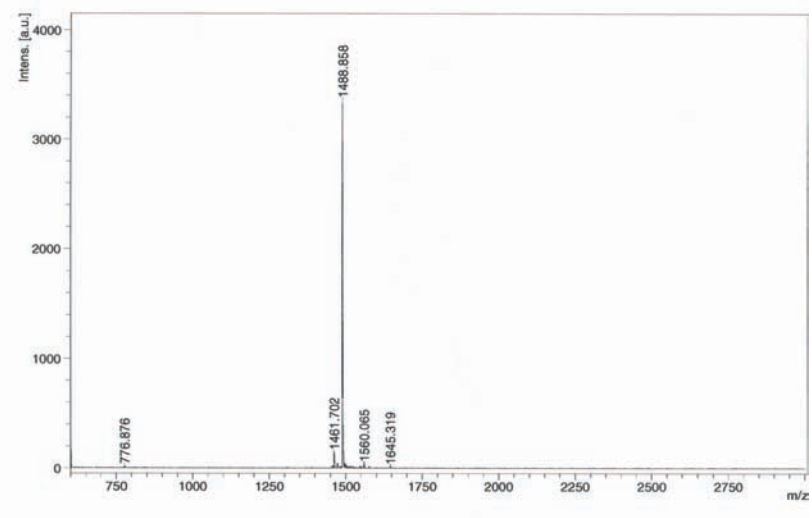

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Reflector detector voltage: 1.59 kV
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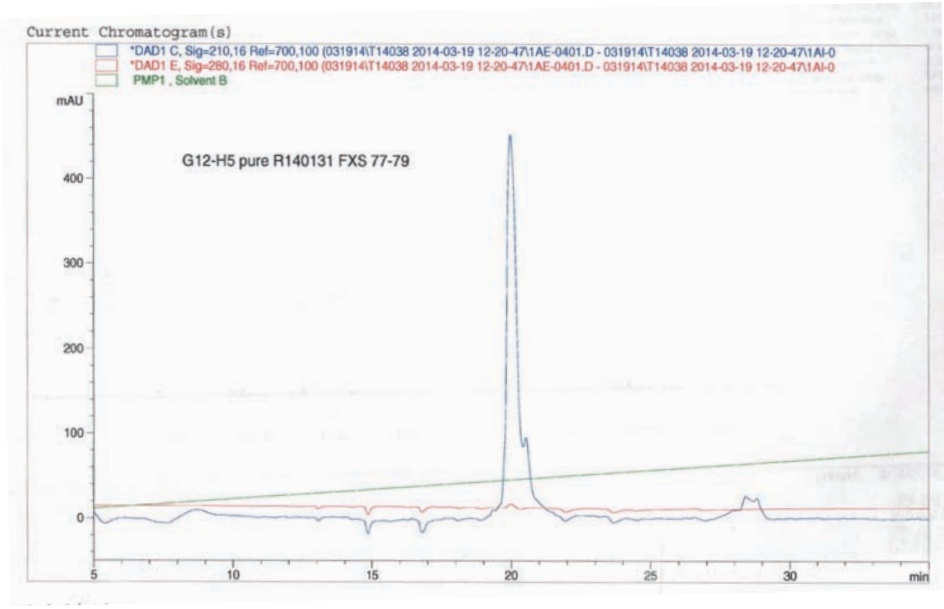
Ion source voltage 2: 18.55 kV
Ion source lens voltage: 6.5 kV
Number of shots: 400

FGAD peptide

Analytical HPLC



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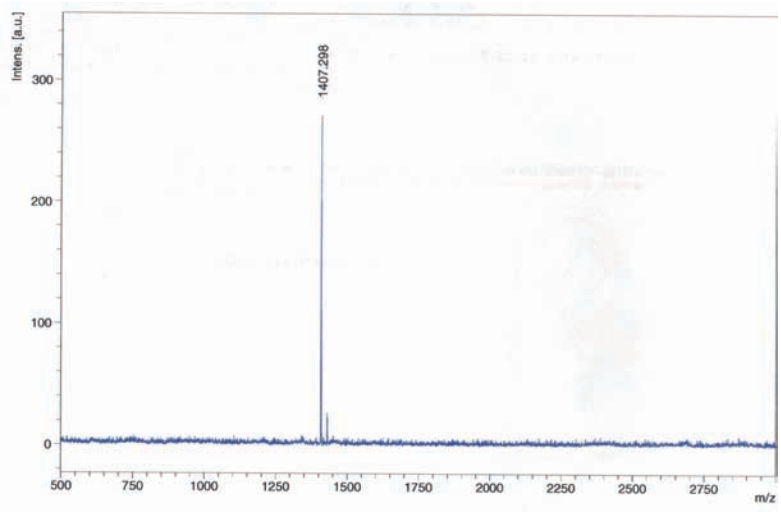

Laser repetition rate in Hz: 60 Hz
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Reflector detector voltage: 1.59 kV
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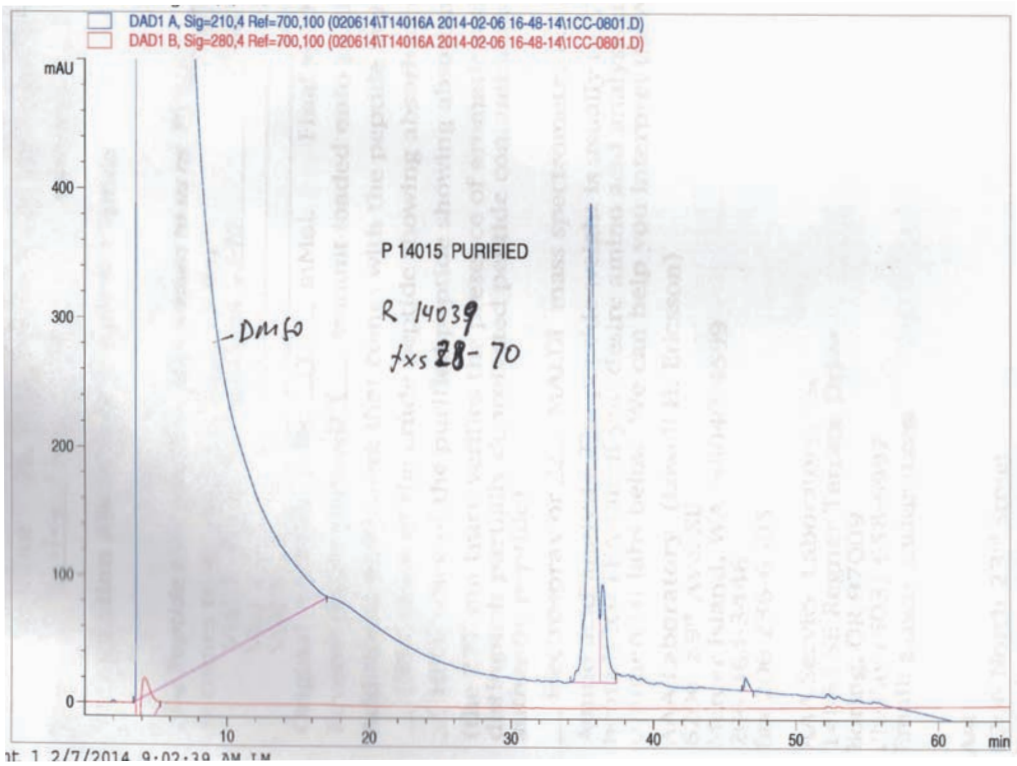
Ion source voltage 2: 18.55 kV
Ion source lens voltage: 6.5 kV
Number of shots: 400

FGAS Peptide

Analytical HPLC



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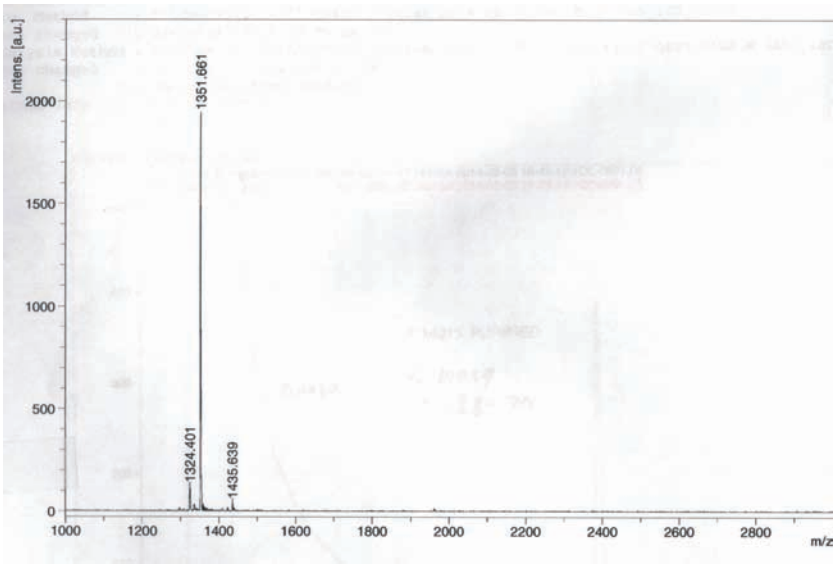

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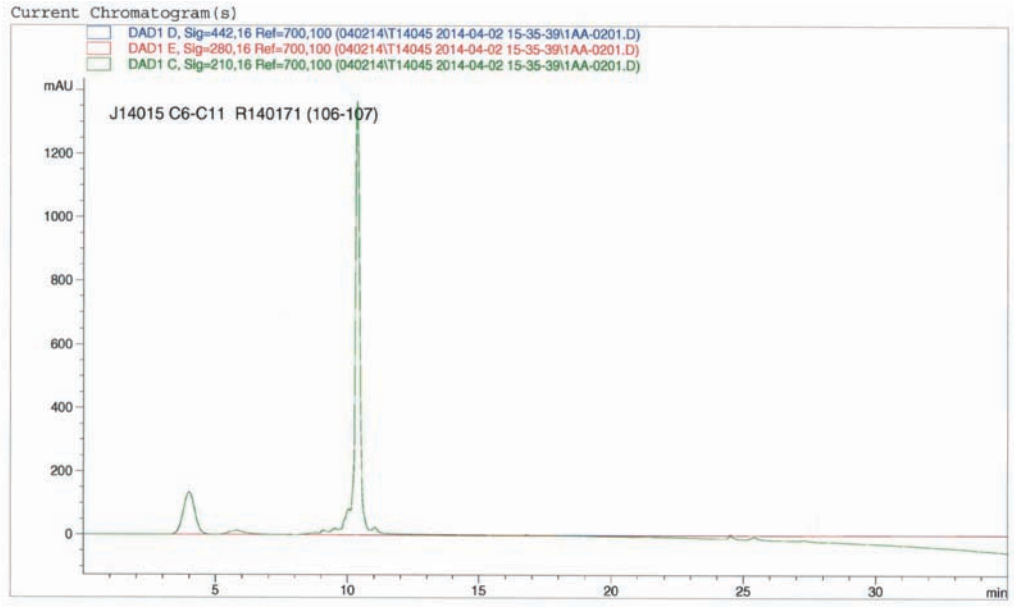
Reflector detector voltage: 1.59 kV
Ion source voltage 1: 20 kV
Ion source voltage 2: 18.55 kV
Ion source lens voltage: 6.5 kV
Number of shots: 400

SGAK Peptide

Analytical HPLC



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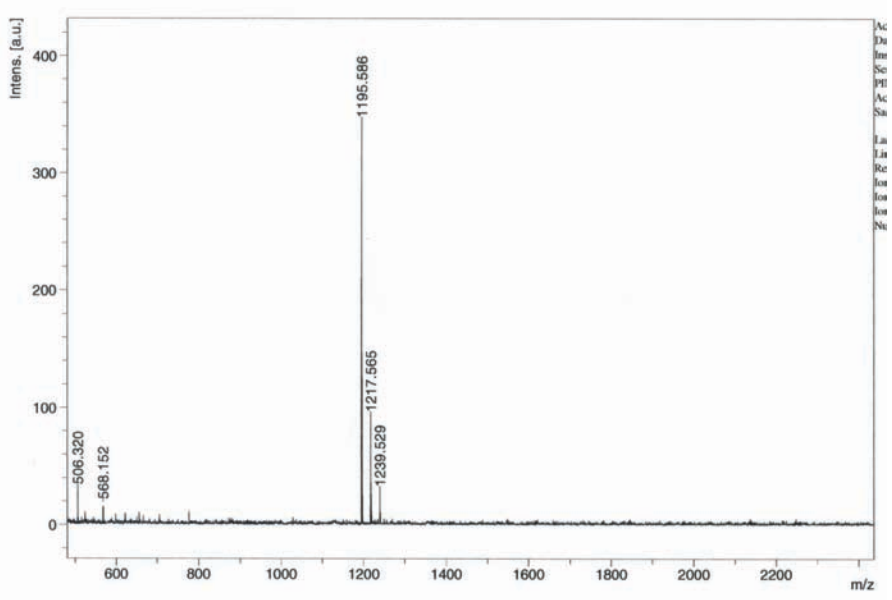

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Reflector detector voltage: 1.59 kV
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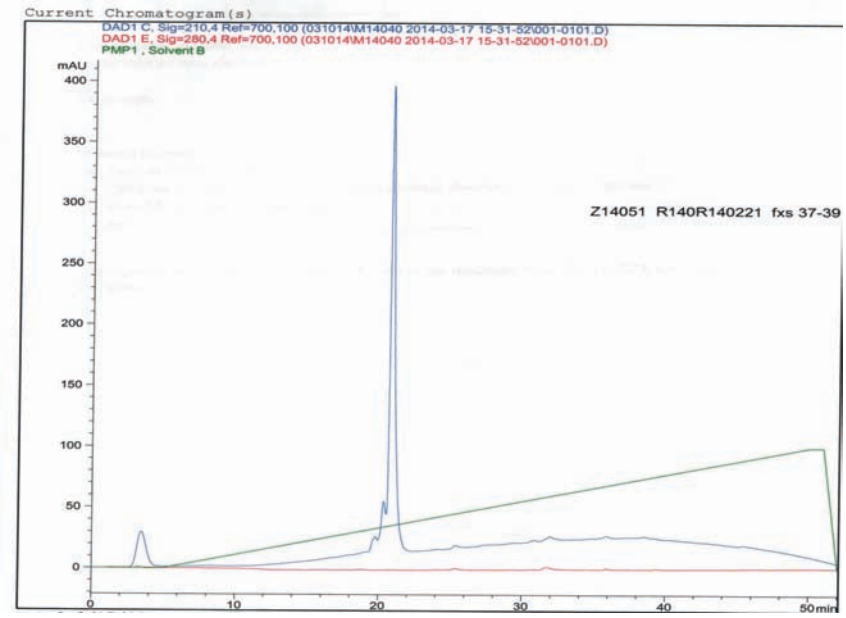
Ion source lens voltage: 6.5 kV
Number of shots: 400

Nsp1 Consensus Peptide

Analytical HPLC



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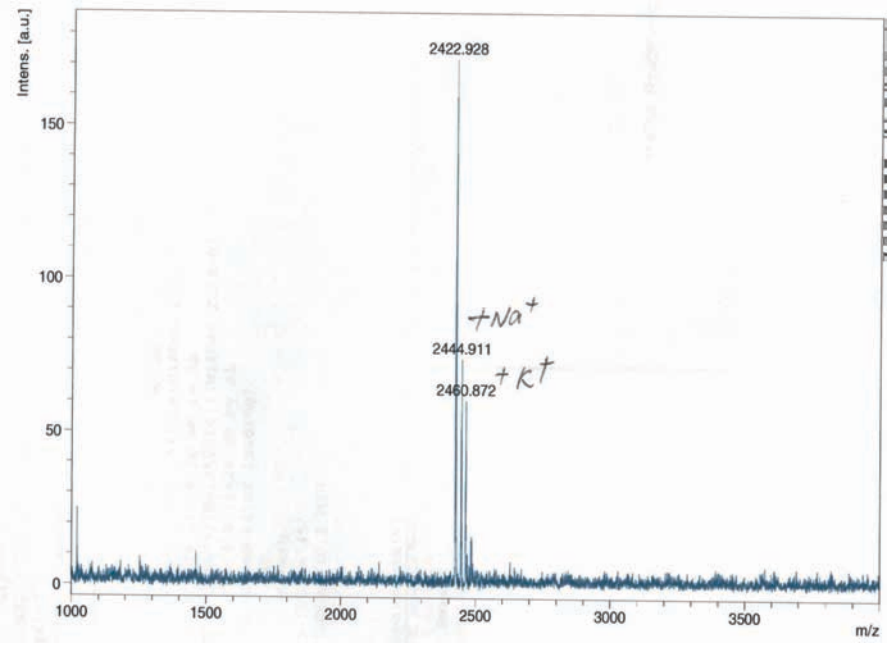

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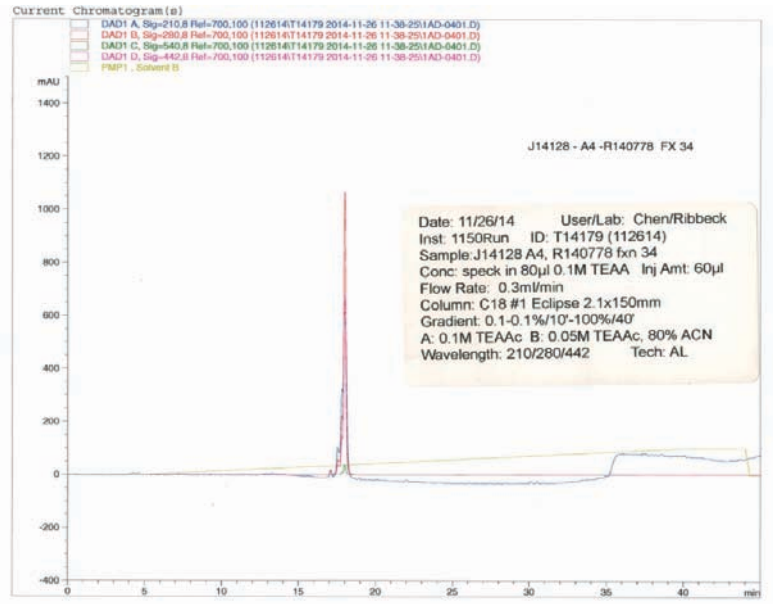
Reflector detector voltage: 1.59 kV
Ion source voltage 1: 20 kV
Ion source voltage 2: 18.55 kV
Ion source lens voltage: 6.5 kV
Number of shots: 400

Hydrophilic (-) peptide reporter with N-terminal FAM label

Analytical HPLC



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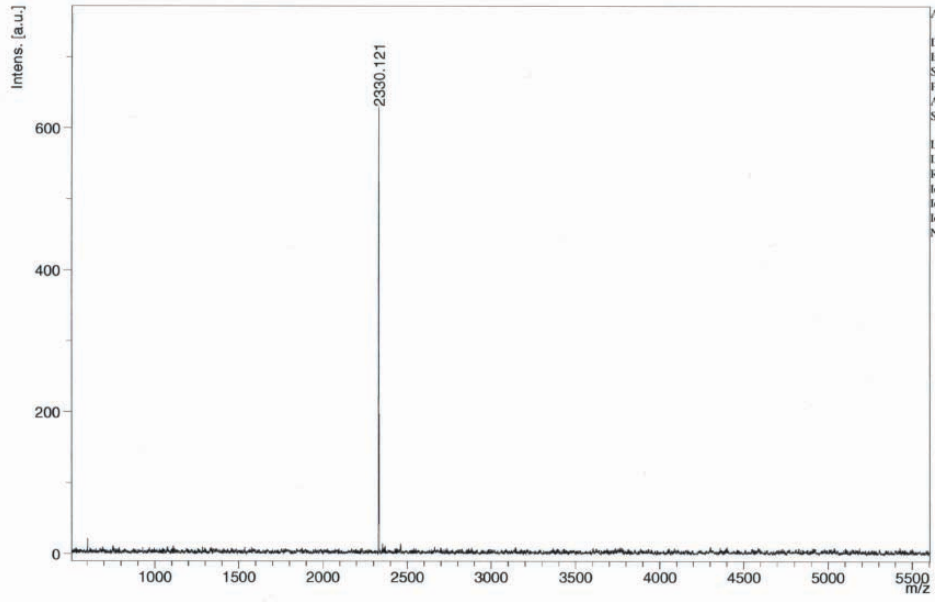

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Reflector detector voltage: 1.59 kV
Ion source voltage 1: 20 kV
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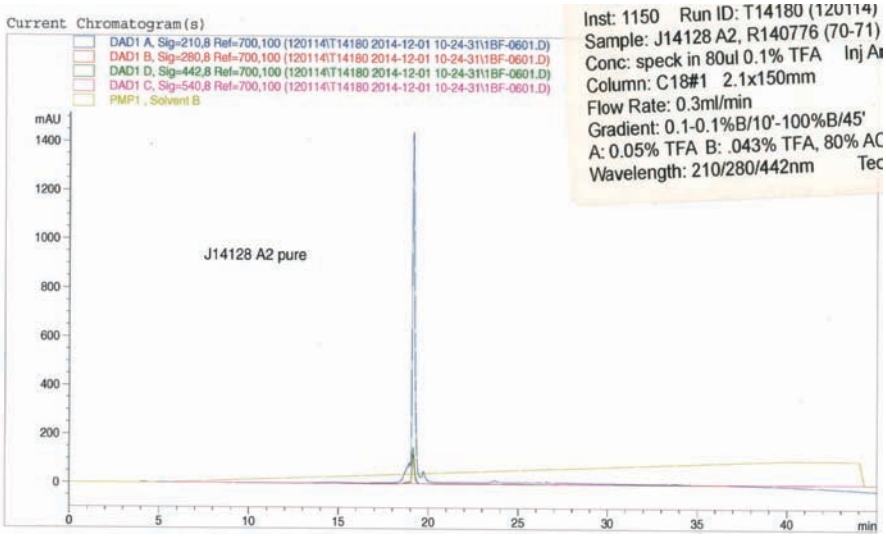
Ion source lens voltage: 6.5 kV
Number of shots: 400

Hydrophilic (+) Peptide with N-terminal FAM labeling

Analytical HPLC



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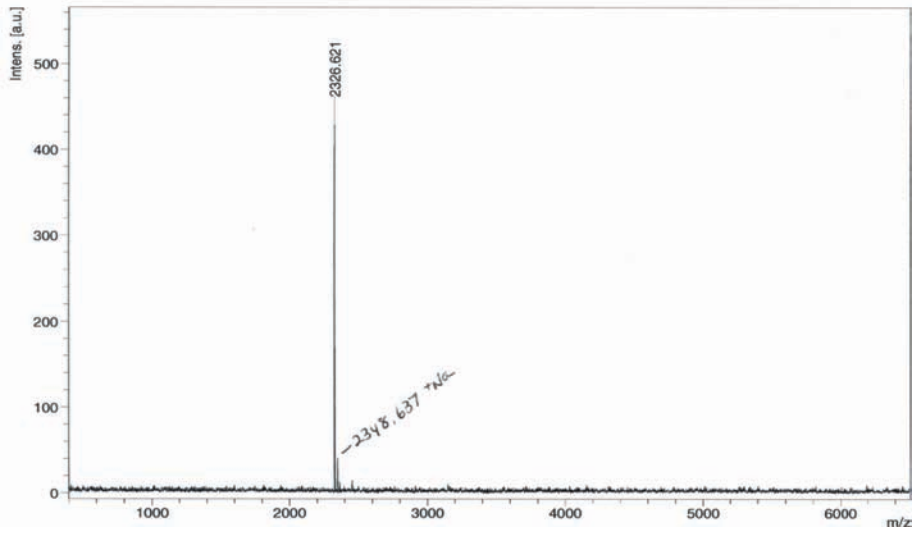

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Ion source voltage 1: 20 kV
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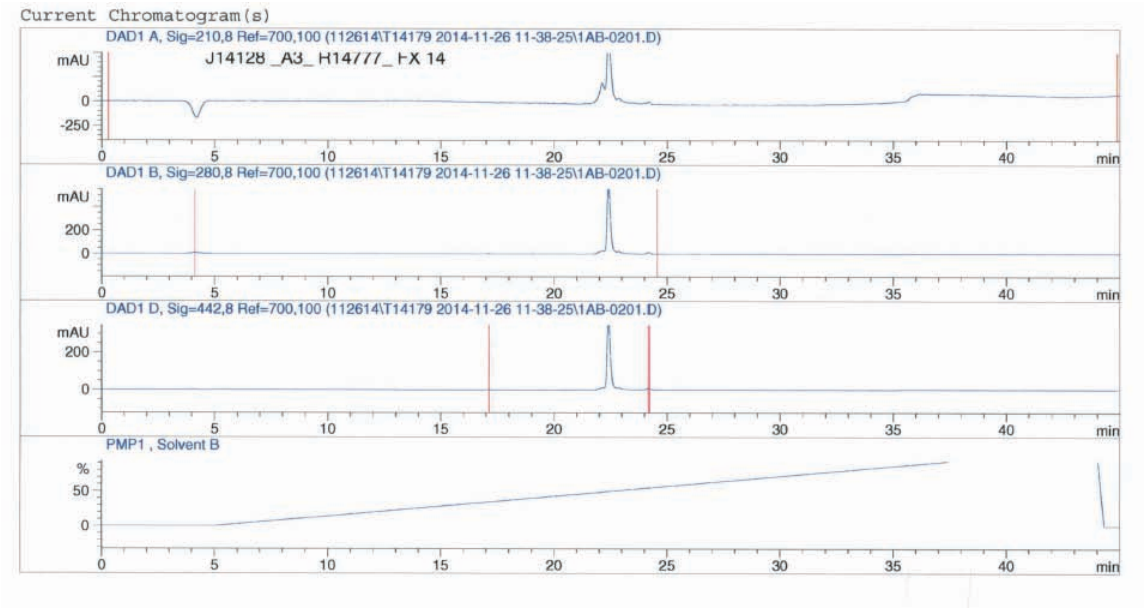
Ion source lens voltage: 6.5 kV
Number of shots: 400

Hydrophobic (-) Reporter Peptides with N-terminal FAM

Analytical HPLC



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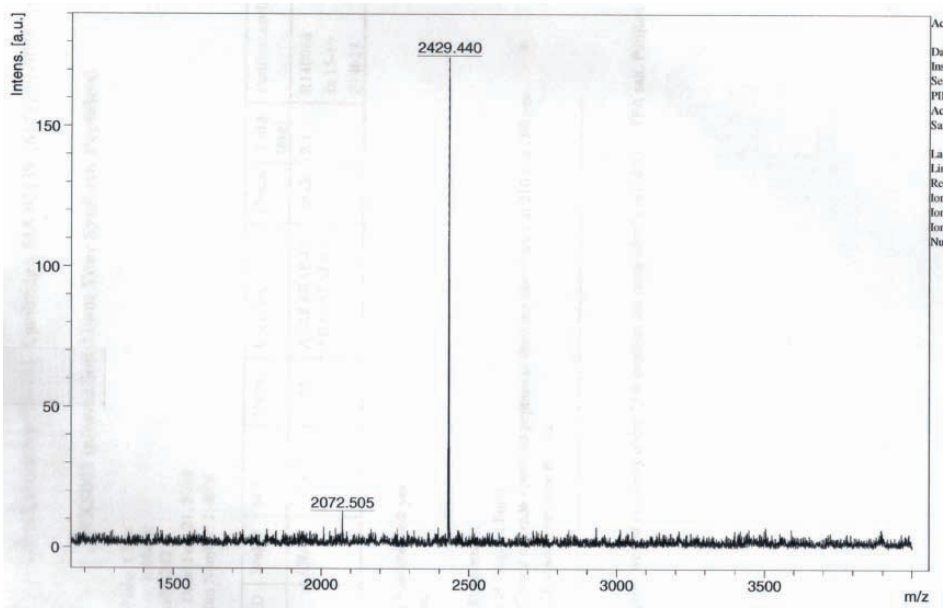

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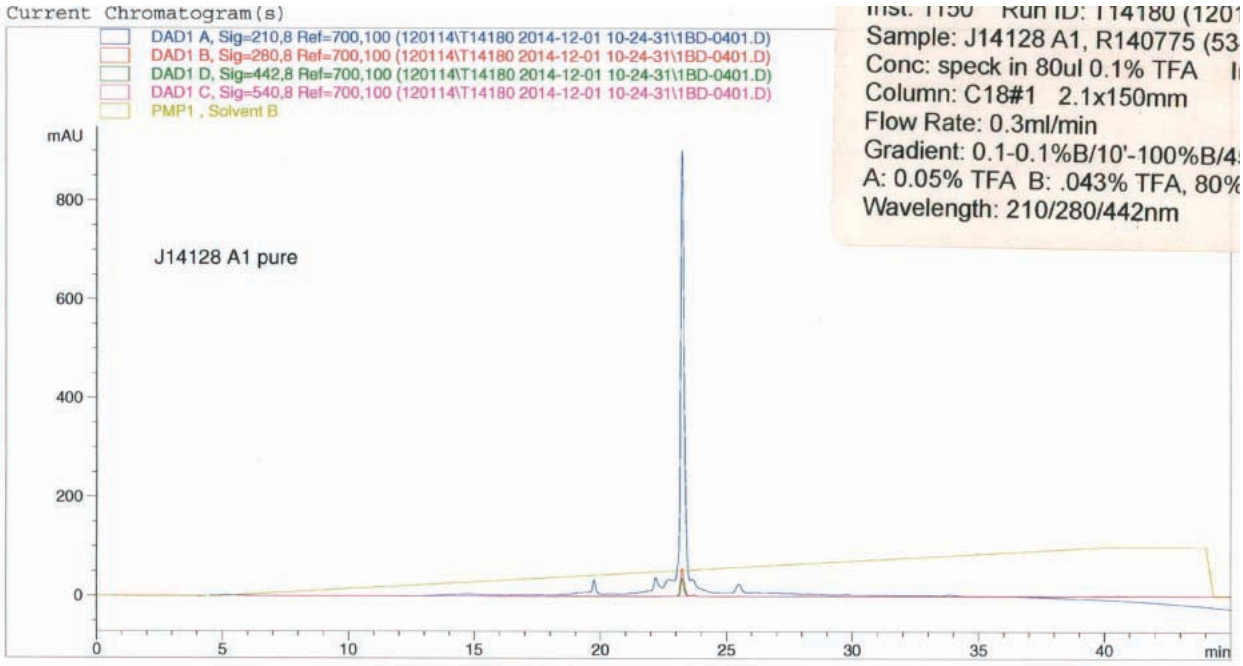
Ion source lens voltage: 6.5 kV
Number of shots: 400

Hydrophobic (+) Reporter Peptide with N-terminal FAM

Analytical HPLC



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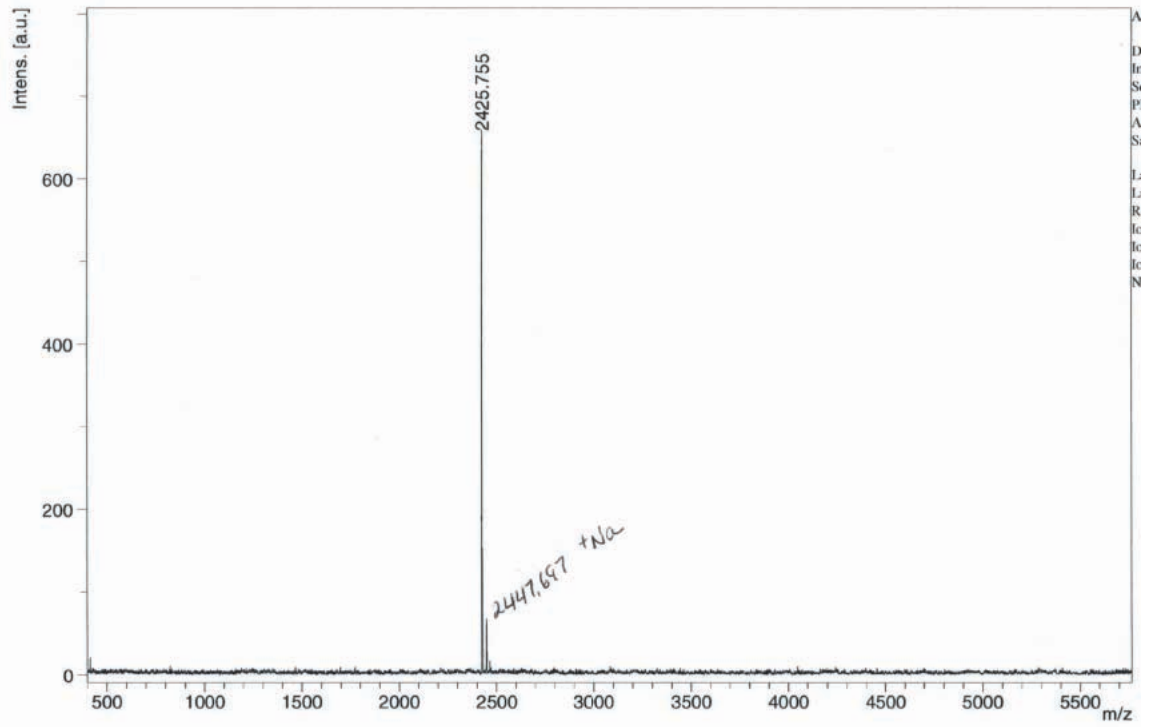
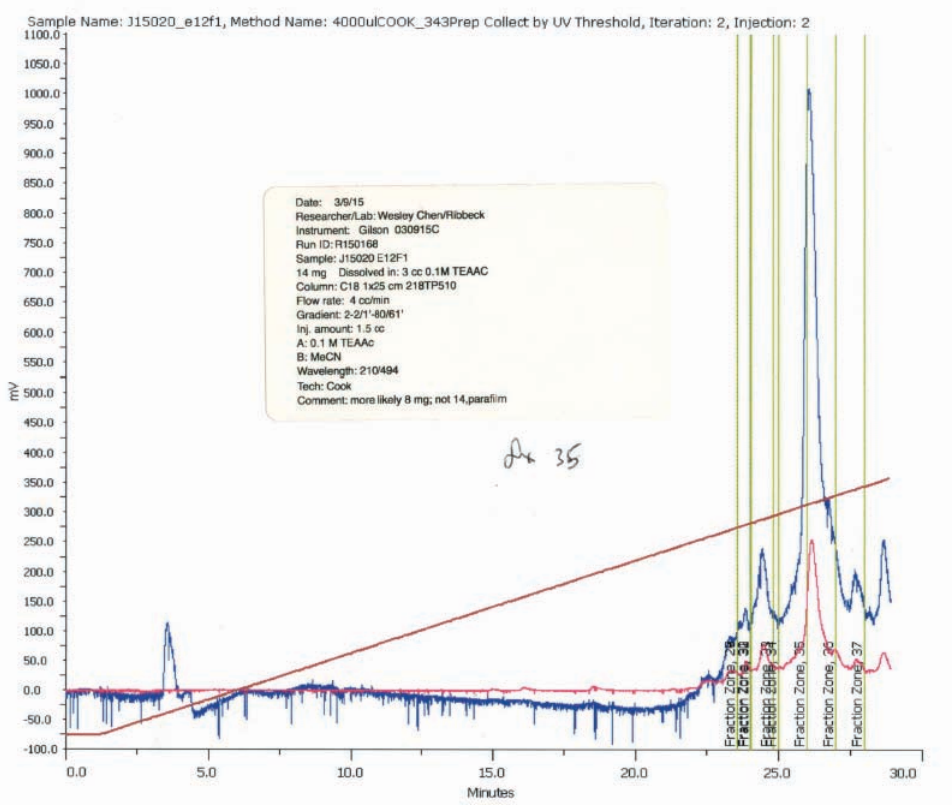
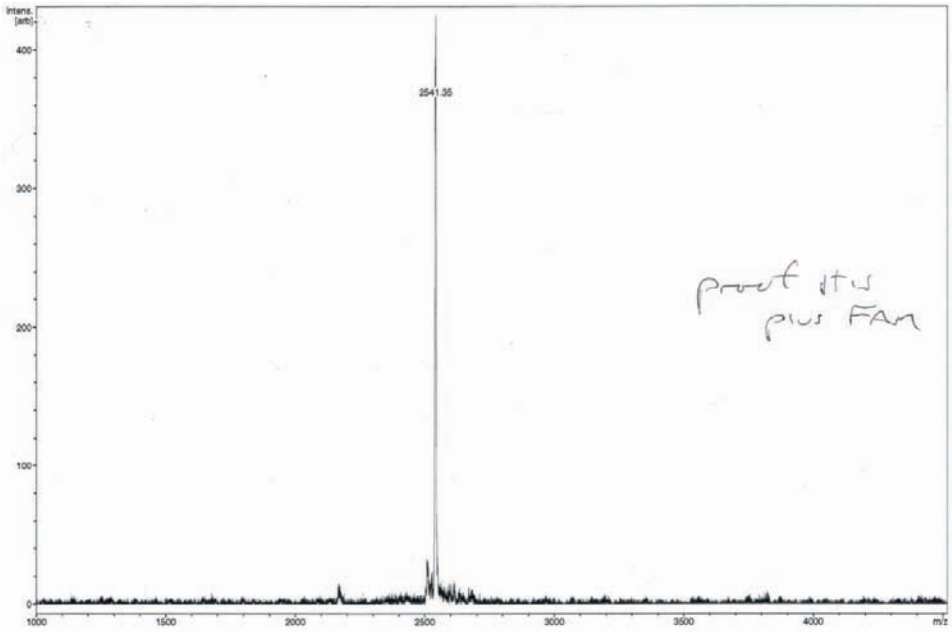


Hydrophobic (-W) peptide with N-terminal FAM labeling

HPLC Purification. Fraction 35 was collected for mass spectrometry and diffusion analysis.



Mass spectrometry analysis



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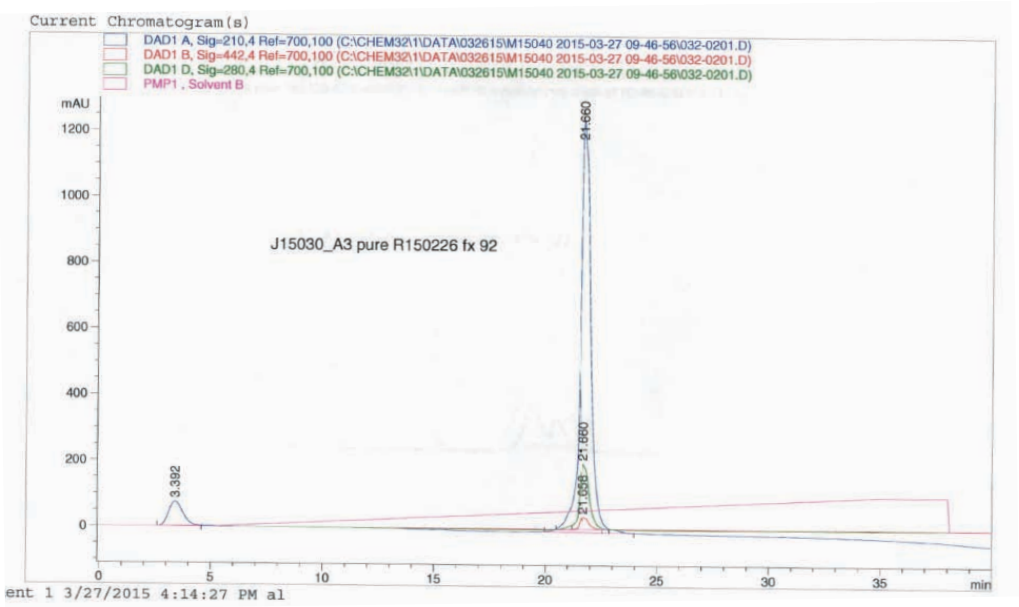
Ion source lens voltage: 6.5 kV
Number of shots: 400

Hydrophobic (+W) peptide with N-terminal FAM labeling

Analytical HPLC



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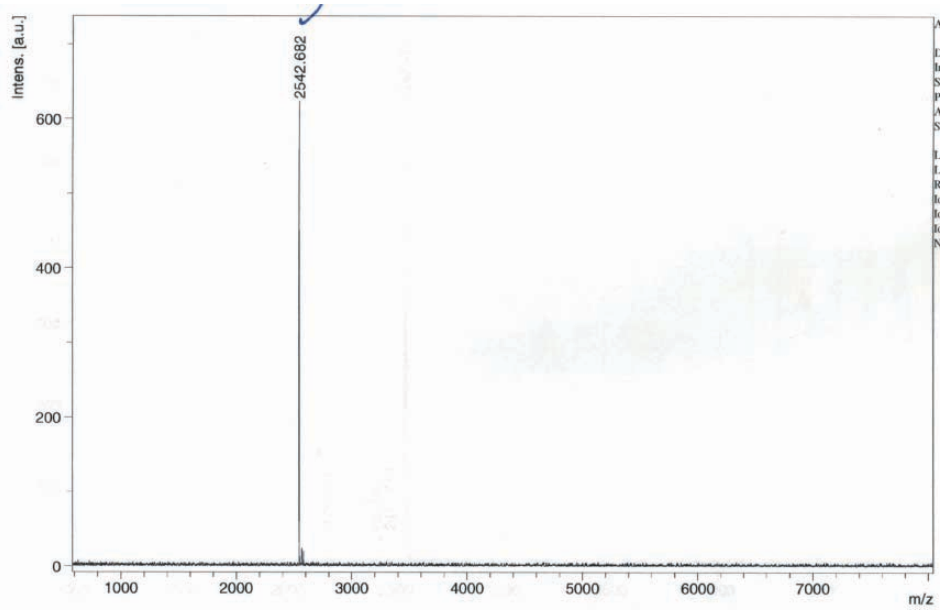

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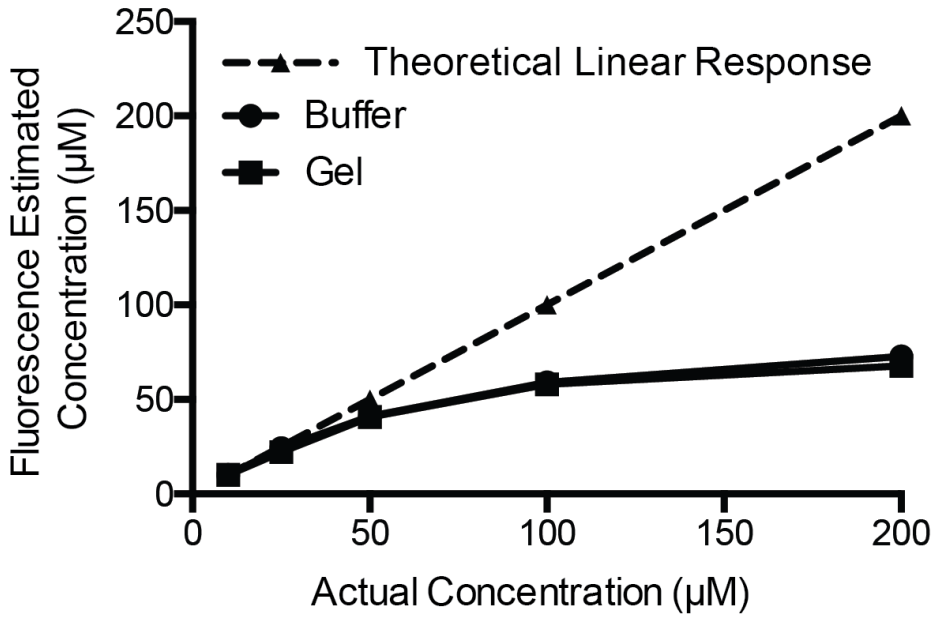
Number of shots: 400

Supplementary Figure S2

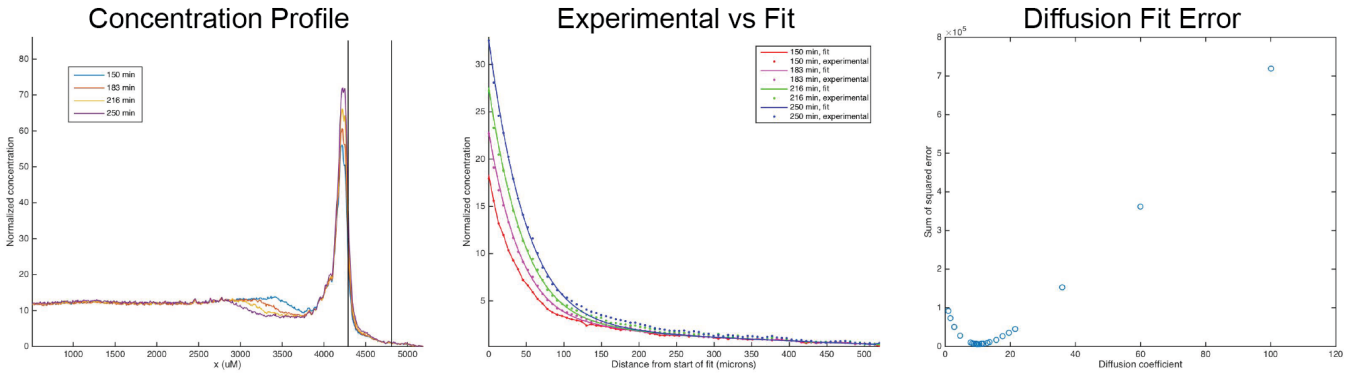


Supplementary Figure S2: Quantification of fluorescence signal as a function of fluorophore concentration.

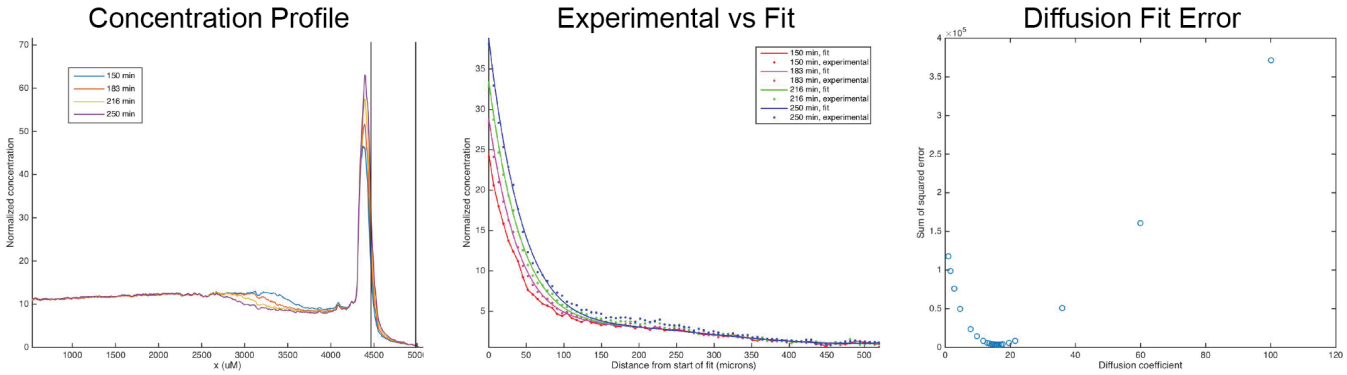
Fluorescence signal is approximately linear up to 50 μM and saturates by 100 μM in buffer and gel conditions. Dashed lines represent the theoretical linear response of fluorescence as a function of concentration. The gels and buffer calibration curves overlap in their associations. All concentrations are reported according to the experimental curve developed and represent lower values of the actual concentrations for values >100 μM.

Supplementary Figure S3

A



B



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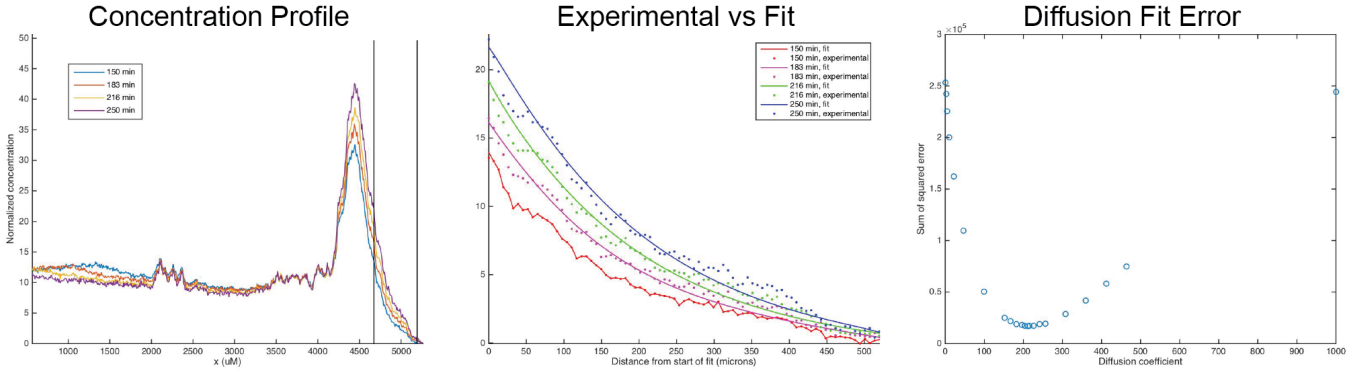

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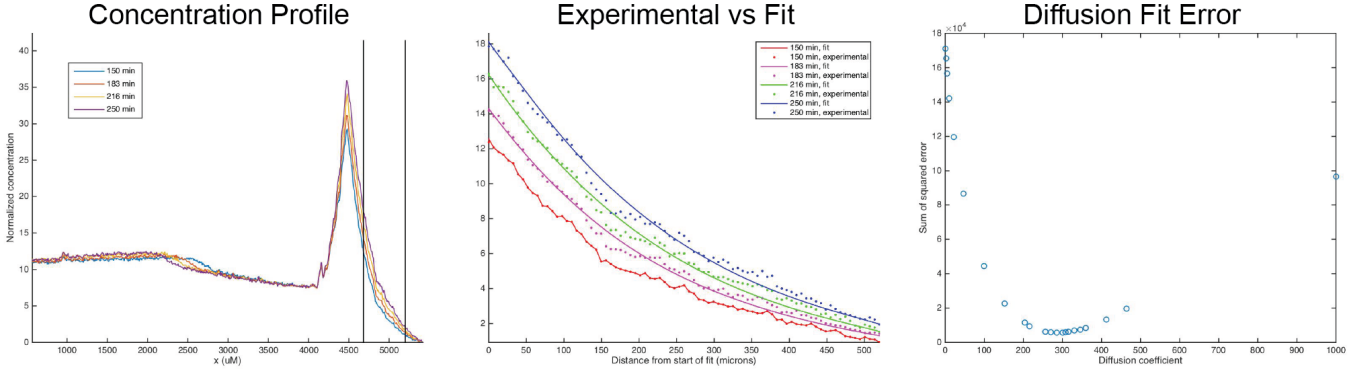
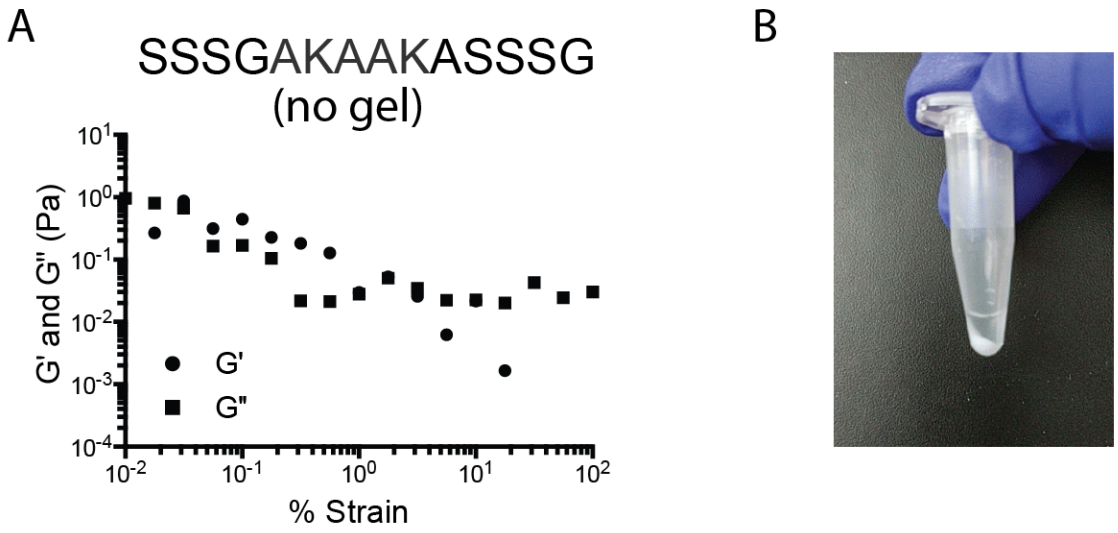


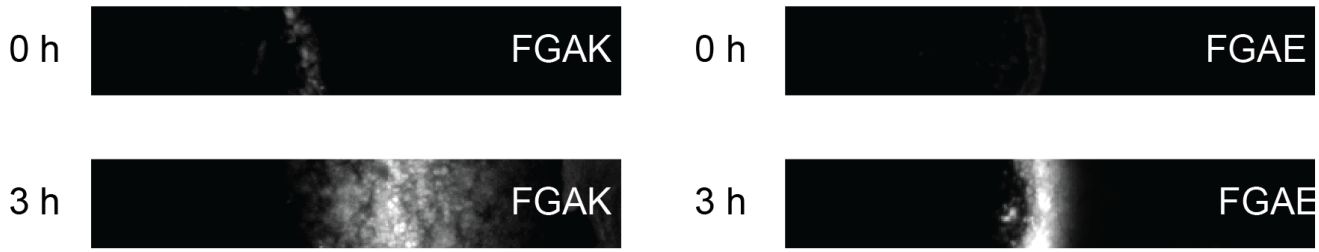
Supplementary Figure S3: Analytical process for calculating effective diffusion coefficients. Examples are given for A) NTF2 diffusion into FGAK, B) W7A diffusion into FGAK, C) NTF2 diffusion into FGAE, and D) W7A diffusion into FGAE to show the reliability of the analytical process across multiple gels. The first column represents the region of the concentration profile where the fitting is implemented. The second column contains the actual data (circles) vs. fit (solid line) at four evenly spaced time points. The third column contains the error of the fit as a function of iterated effective diffusion coefficients.

Supplementary Figure S4



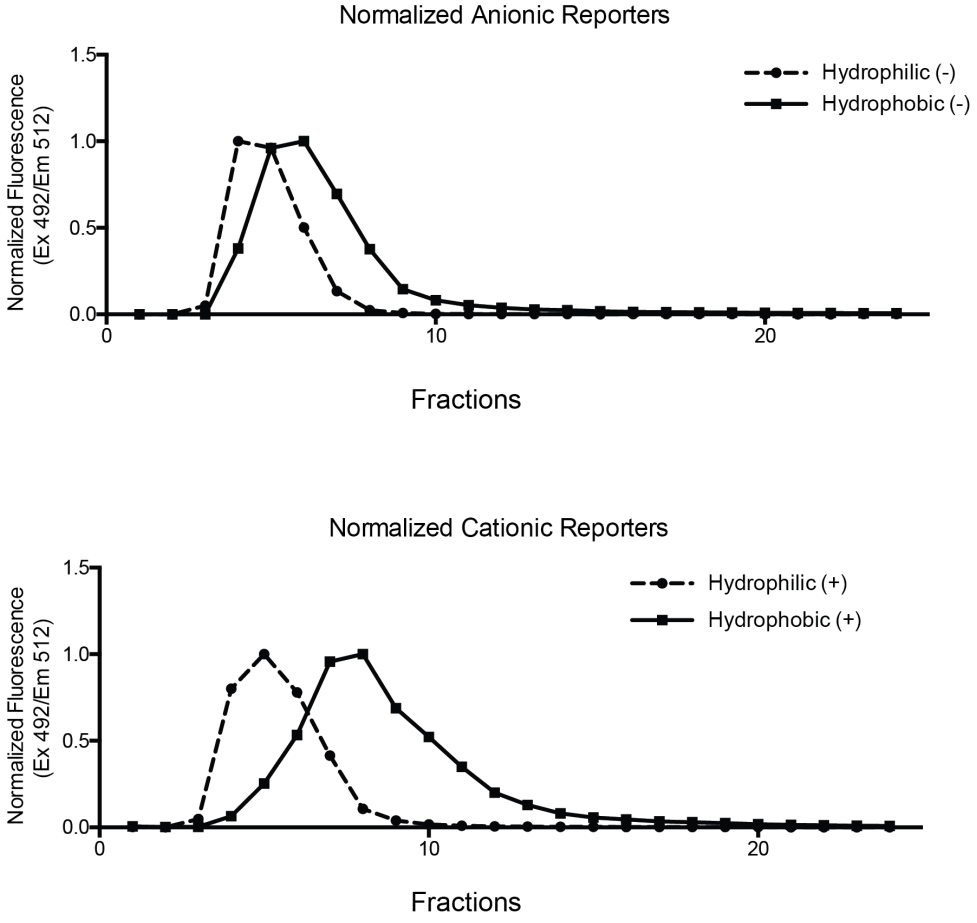
Supplementary Figure S4: Verification of F as essential amino acid for self-assembly in FGAK peptides.
A) Frequency sweep of the F→S substitution (FGAK → SGAK) to determine the effect of F on the self-assembly of peptides. The elastic modulus (G') and loss modulus (G") are reported. Note that the measured values are below the sensitivity of the rheometer using the specified cone-plate geometry due to the viscous nature of SGAK peptide solutions. B) Precipitated FGAS peptides in 20 mM NaCl, 20 mM HEPES [pH 7] after gentle centrifugation.

Supplementary Figure S5



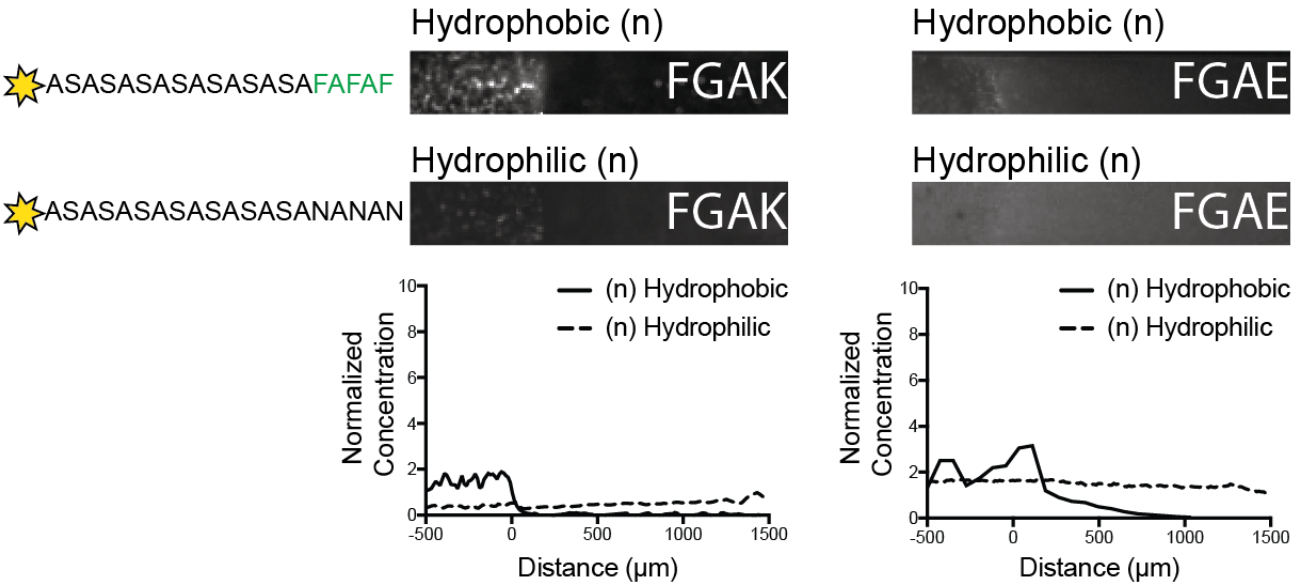
Supplementary Figure S5: Verification of hydrophobic domain availability in FG peptide gels. Transport of Nile Red into FGAK and FGAE gels after 0 h and 3 h of incubation. Fluorescence indicates that the dye is able to access hydrophobic environments created by FG domains within the gels. Images are of representative gels from three independent replicates.

Supplementary Figure S6



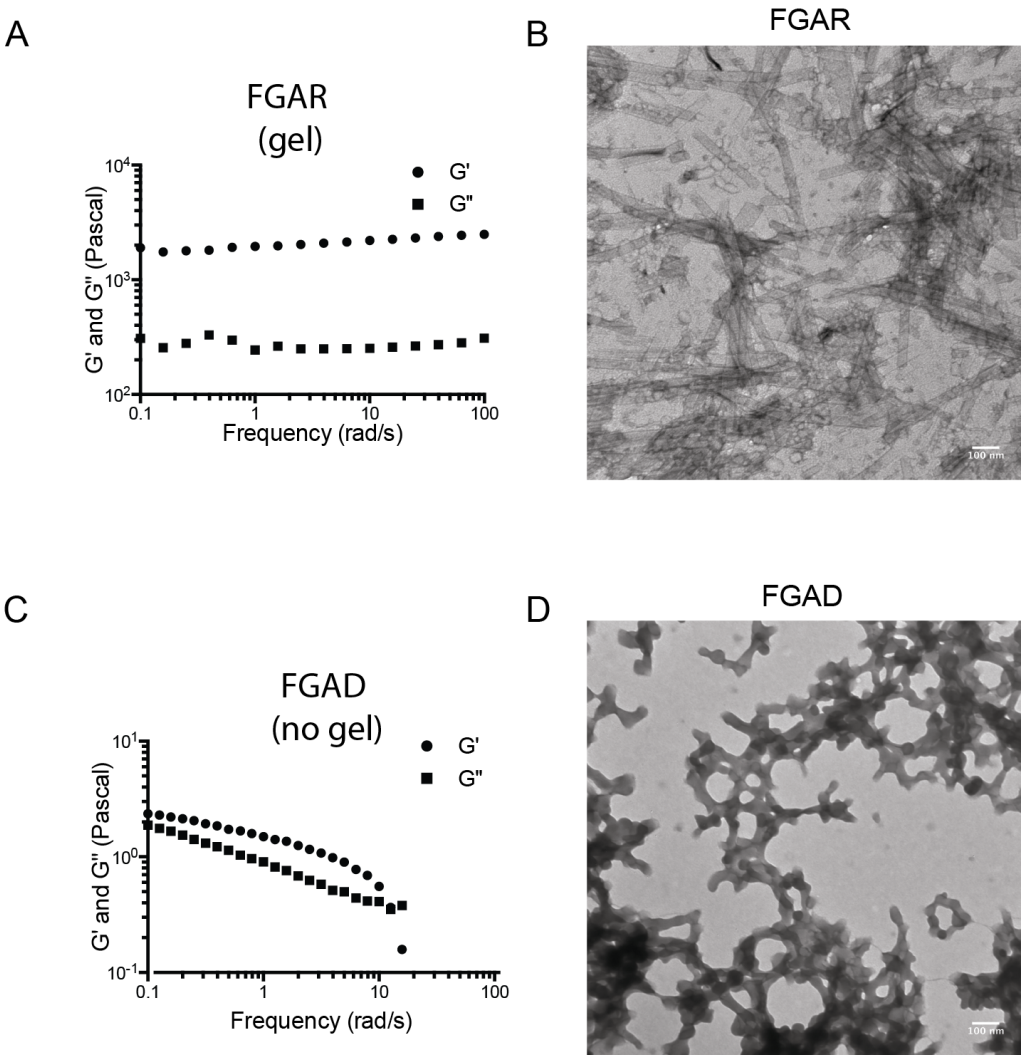
Supplementary Figure S6: Fractionation of hydrophilic reporters and their hydrophobic counterparts in phenyl-sepharose columns.
Fluorescence signals from each fraction were collected and normalized to the signal with the highest intensity of emission. For both cationic and anionic reporters, the hydrophobic reporters eluted later. This increased retention time reflects stronger binding to phenyl-sepharose beads.

Supplementary Figure S7



Supplementary Figure S7: Diffusion of neutrally charged Hydrophilic (n) and Hydrophobic (n) reporters into cationic FGAK and anionic FGAE gels. Purely neutral reporters interact minimally with the FGAK and FGAE gels regardless of overall hydrophobicity.

Supplementary Figure S8



Supplementary Figure S8: Affect of amino acid sidechain chemistry on self-assembly and mechanical properties of FG-containing peptides.
A) Frequency sweep of FGAR gel with G' (storage) and G" (loss) moduli reported at 2% (w/v) showing the stable self-assembled matrix is maintained when converting from K to R. B) Corresponding image from transmission electron microscopy showing the structural variation of FGAR peptide self-assembly when compared to that of FGAK peptides. C) Frequency sweep of FGAD peptide solution with G' (storage) and G" (loss) moduli reported at 2% (w/v) showing that FGAD does not form a gel. D) Corresponding image from transmission electron microscopy showing the amorphous structure of FGAD peptide aggregates.