SUPPLEMENTARY INFORMATION

Peptide Arrays for Detecting Naphthenic Acids in Oil Sands Process Affected Water

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Materials and Methods

Synthesis of Immobilized Peptides (Peptide Array)

Peptide array (1st library) was synthesized in duplicates on a cellulose membrane using AutoSpot ASP222 (Intavis AG, Germany) as described previously.[1,2] Each peptide was synthesized at a concentration of ~50 nmoles, which was spread on the membrane in a spot with a diameter of 4 mm and each spot was separated from the next peptide (spot) by 8 mm. Briefly, amino functionalized amino-PEG500 cellulose membrane-UC540 (Intavis, Germany) was used to build the peptide arrays following Fmoc solid phase peptide synthesis method or the SPOT synthesis method.[3] The peptides were synthesized from the C-terminal and a β-alanine linker was anchored to the surface of the amino-PEG500 cellulose membrane to act as a spacer between the membrane and the peptide. Fmoc amino acids activated with HOBt and DIC for 15 min were spotted on the membrane in 60 nL aliquots per spot by a robotic syringe. The concentration of each peptide (spot) was controlled by the amount of the liquid delivered during the reaction steps. By delivering 60 nL of the activated amino acids (0.25 mM/mL) at intervals of 8.0 mm, a loading of 0.4 μmol/cm^2 was achieved. After coupling of the Fmoc amino acid, the membrane was removed from the synthesizer and was treated with acetic anhydride (2%) to cap any free remaining amino groups. The membrane was then washed and treated with 20% piperidine in DMF for the Fmoc group deprotection. After washing with DMF and IPA, the membrane was air-dried and carefully repositioned on the robotic synthesizer to repeat the next coupling cycle. These steps were repeated for each amino acid until the end of the sequence. At the end, all peptides were N-terminally acetylated. Finally, the side-chain protecting groups were removed using a cocktail of reagents comprised of TFA (15 mL), DCM (15 mL), triisopropylsilane (0.9

mL), and water (0.6 mL). The membrane was allowed to react with the cocktail solution in a polypropylene box with a lid for about 3 h. After extensive washing with DCM, DMF, and ethanol, the membrane was dried with cold air and stored in a sealed bag at -20 °C until use.

Synthesis of EDAN-NAs

Commercially available NAs (Aldrich, Technical grade) were used to prepare their fluorescent adduct with EDAN. NAs consist of a complex mixture of a variety of aliphatic acids. EDAN is a fluorescent amine reagent that has been used previously to analyze volatile fatty acids (VFA) through spectrofluorometric measurement of EDAN-VFA adducts.[4] First cyclohexanepentanoic acid (CHPA) was used as a single component of NAs to synthesize a representative EDAN-CHPA adduct. Following successful tests of the conjugation of EDAN-CHPA (vide infra), EDAN-NAs conjugation was performed using commercial NAs. The conjugation reaction for the preparation of adducts involved use of coupling agents HCTU and HOBt in an organic solvent in the presence of NMM base (**Figure S1**, supporting information). Briefly, the free carboxyl group of NAs (1 mmol) was activated using HCTU (1 equiv.) in the presence of HOBt (1 equiv.) and NMM (2 equiv) in DMF for 5 min to give OBt active esters of the corresponding acids. Ethanolic solution of EDAN (1.2 mmol) was added to the activated OBt ester and the reaction was monitored using TLC in 30% ethylacetate/hexane.[4,5] Conjugation reaction was monitored from 2 min up to 24 hr using thin layer chromatography (TLC). Starting at 2 min 52% conjugation was detected after EDAN addition, with no further improvement in adduct yield up to 24 hr. The reaction was stopped by the addition of ter-butyl ether and the reaction mixture was washed with phosphate buffer (50 mM, pH 8) several times. The ether layer was collected and concentrated. The residue was dissolved in 50% IPA/water and the fluorescent

adduct was purified using semi-preparative reversed-phase (RP) HPLC column (Vydac C18, 1 × 25 cm, 5 μm) on Varian Prostar 210 HPLC system (Walkersville, USA) using 15-95% (ACN/H_2O) in 50 min with 1.3 mL/min flow rate. The fluorescent adducts were confirmed using mass spectrometry and analytical HPLC with final purified yield of 35% (**Figure S2**). The reserved-phase HPLC chromatogram of EDAN-NAs shows that the adducts elute as multiple peaks over a period of 10 minutes (**Figure S2A**). The HPLC peaks corresponding to adducts were collected and analyzed using electrospray mass spectrometry (Waters micromass ZQ). The mass spectrum of the adducts show the expected $[M+H]^+$ of EDAN-NAs at 310 - 510. A shift in the molecular weight of the EDAN-NAs with respect to NAs mixture (m/z 150-350) confirmed the addition of EDAN (m/z 168) to the NAs.

Synthesis of Soluble Peptides

Peptides **17** and **31** (each 14-mer) were synthesized using automated synthesizer (Tribute, Protein Technology Inc. USA) utilizing solid phase method.[6] Peptide syntheses were done on Rink Amide MBHA resin at 0.1 mmol scale, exploiting Fmoc chemistry, employing four equivalent of Fmoc protected amino acids and coupling agents like NMM and HCTU. After completion of synthesis each peptide was cleaved from the resin using mixture of TFA, triisopropylsilane and water in ratio 8.5:1:0.5 and precipitated using chilled diethyl ether. Both peptides were purified on RP-HPLC using semi–preparative C18 (Vydac) column using gradient of 15-55% acetonitrile/water (0.05%TFA) for 55 min with flow rate of 2 mL/min. Approximately 95% purity was gained for purified peptides, which was assessed using Vydac analytical C18 HPLC column (**Figure S6**). Molecular mass of each peptide was determined by MALDI-TOF mass spectrometry (**Figure S7**) and the data was in good agreement with the

molecular mass predicted from the amino acid sequence. For Peptide 17, [M+H]+ value is 1518.7 (calculated) and 1518.7 (observed); and for Peptide 31, [M+H]+ value is 1608.7 (calculated) and 1609.6 (observed)

References

(1) Falsey, J. R.; Renil, M.; Park, S.; Li, S.; Lam, K. S., *Bioconjugate Chem.* **2001**, *12*, 34-353.

(2) Ahmed, S.; Mathews, A. S.; Byeon, N.; Lavasanifar, A.; Kaur, K. *Anal. Chem.* **2010**, *82*, 7533-7541.

(3) Frank, R. *J. Immunol. Methods* **2002**, *267*, 13-26.

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(5) Kobayashi, M.; Chiba, Y. *Anal. Biochem.* **1994**, *219*, 189-94.

(6) Hood, C. A.; Fuentes, G.; Patel, H.; Page, K.; Menakuru, M.; Park, J. H., *J. Pept. Sci.* **2008**, *14*, 97.

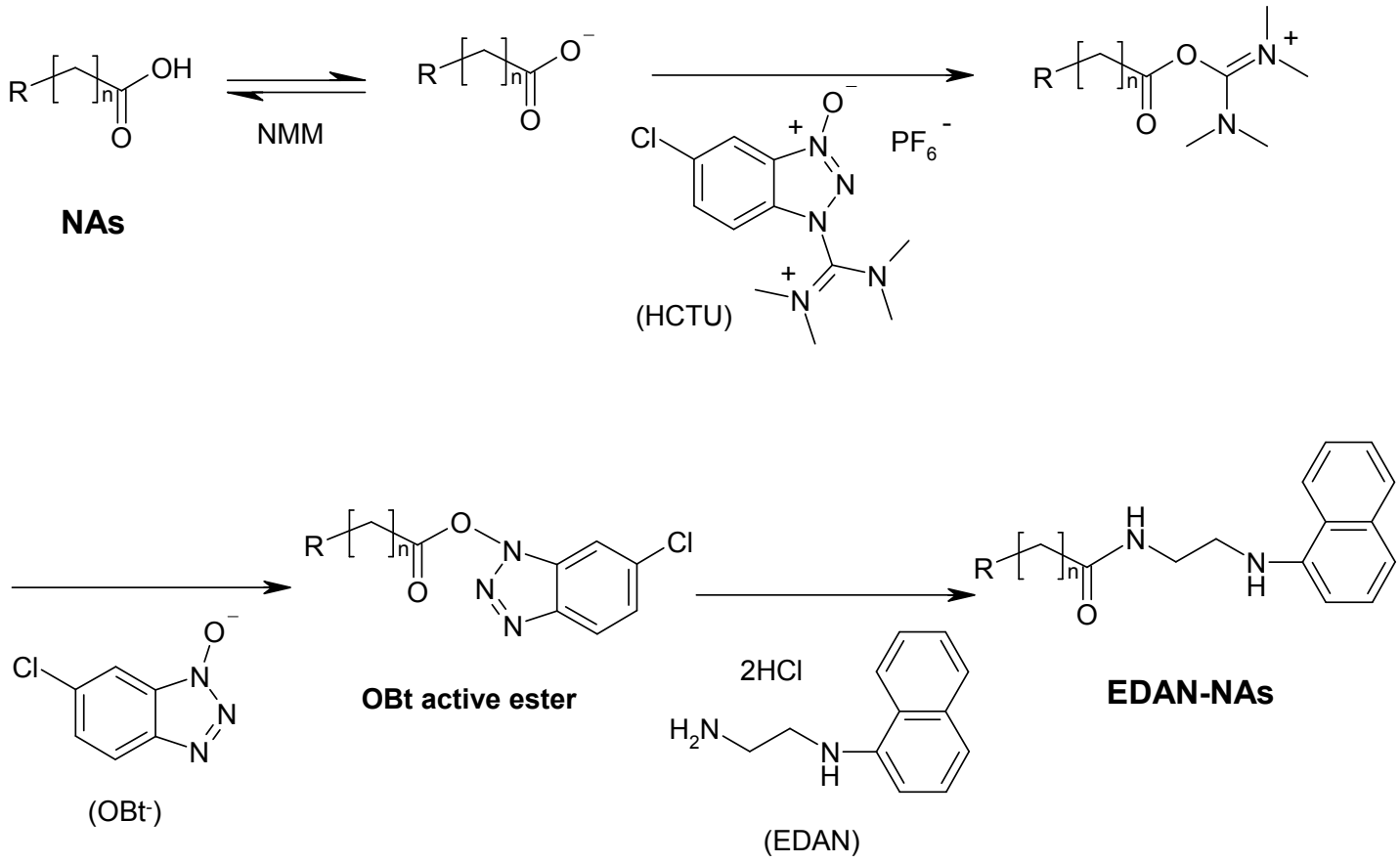
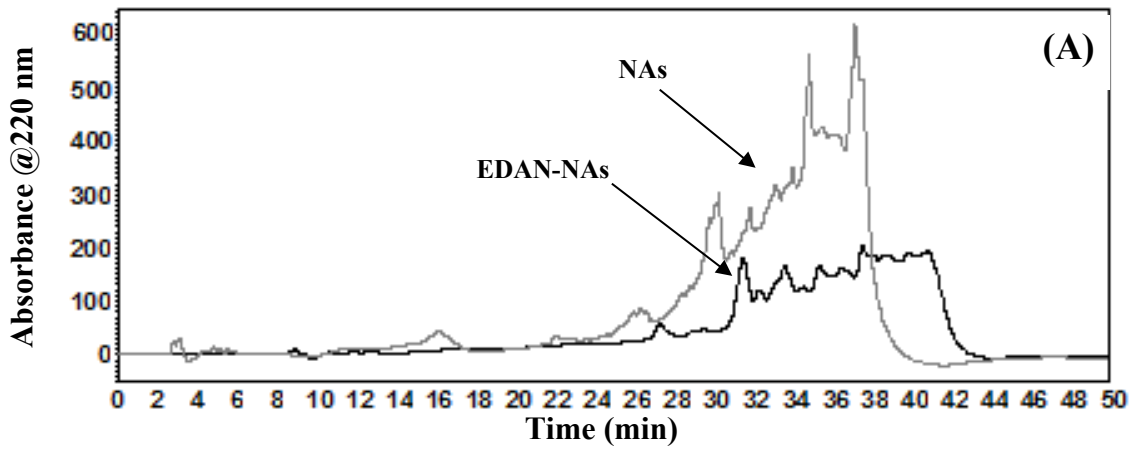
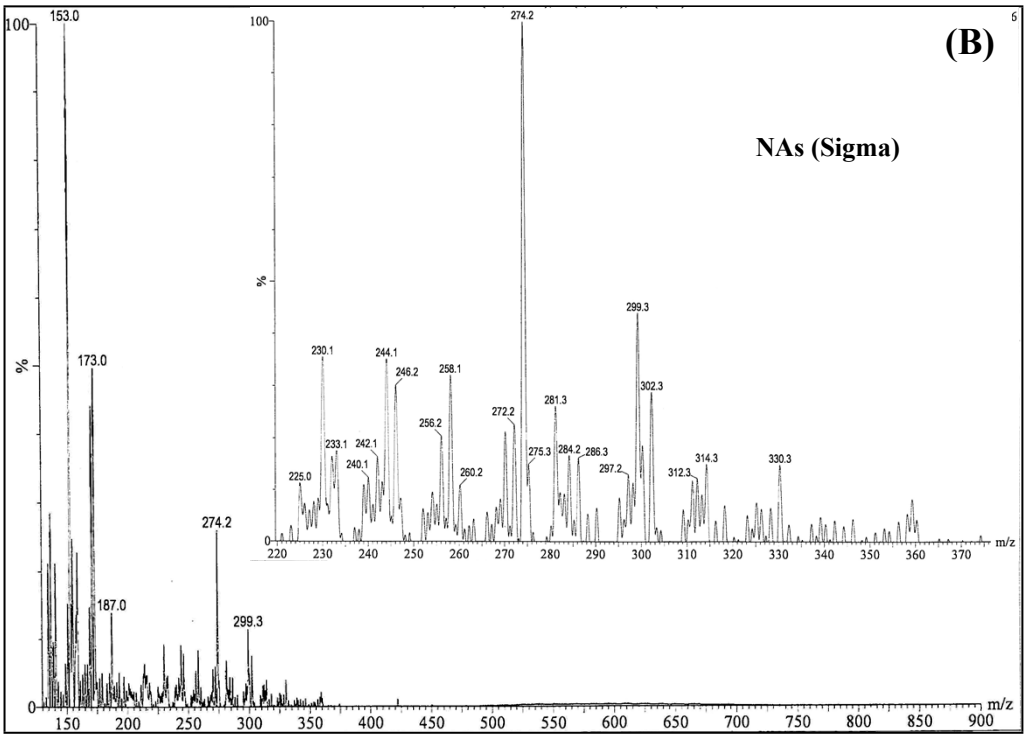


Figure S1. Reaction pathway of the proposed mechanism for the synthesis of EDAN-NAs adducts. First step: Pre-activation of carbon bearing-OH group with N-methylmorpholine (NMM). Second step: Activation of pre-activated carbon bearing-OH group using HCTU followed by the formation of the more stable OBt active ester. The final step: is the fluorescent labelling of pre-activated carboxylic acid group of NAs with the fluorescent EDAN (N-(1-naphthyl) ethylenediamine).


(A)
NAs
EDAN-NAs
Absorbance @220 nm
Time (min)


(B)
NAs (Sigma)
m/z

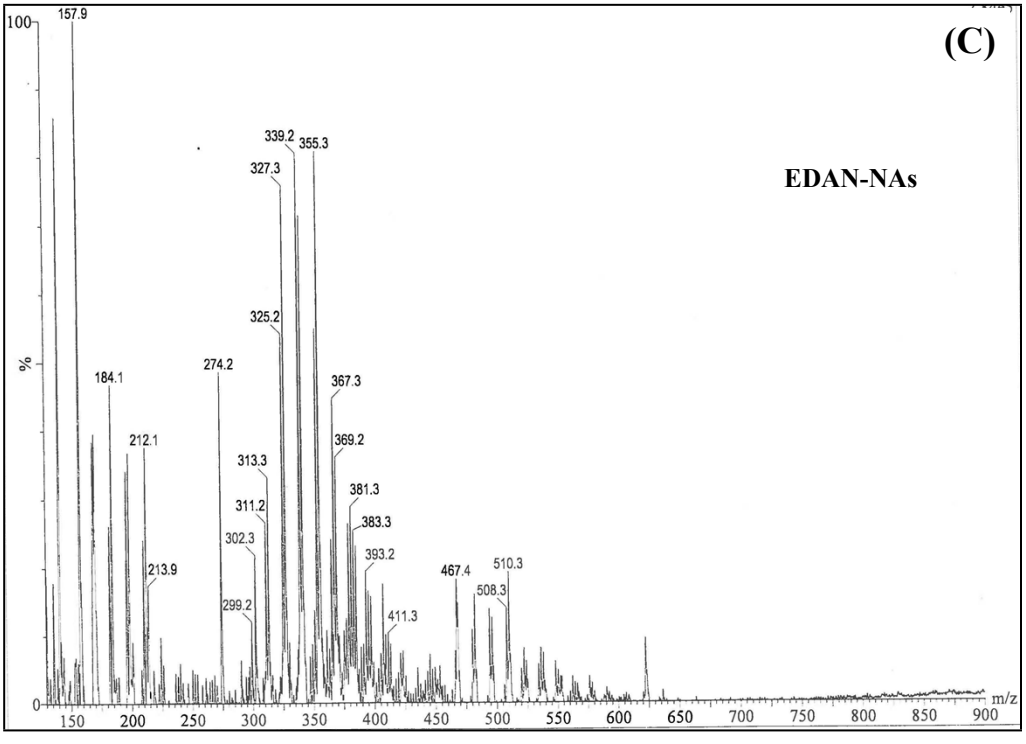

(C)
EDAN-NAs
m/z

Figure S2. (A) Reversed phase-HPLC chromatogram showing overlay of NAs and EDAN-NAs. The HPLC gradient used for elution was 30-95% ACN/H_2O over 30 min with a flow rate of 1.3 mL/min on a Vydac C18 semi-preparative (1 x 25 cm, 5 μm) column. (B and C) Electrospray mass spectra (ESI-MS) for commercial NAs (Sigma) and EDAN-NAs showing their $[M+H]^+$, respectively. The mass spectra shows that $[M+H]^+$ for EDAN-NAs adducts and NAs ranges at 310-510 and 150-350, respectively, confirming the addition of EDAN (m/z 186) to the NAs with a net increase in m/z of 168 with the elimination of water molecules (see the reaction, Figure S1).

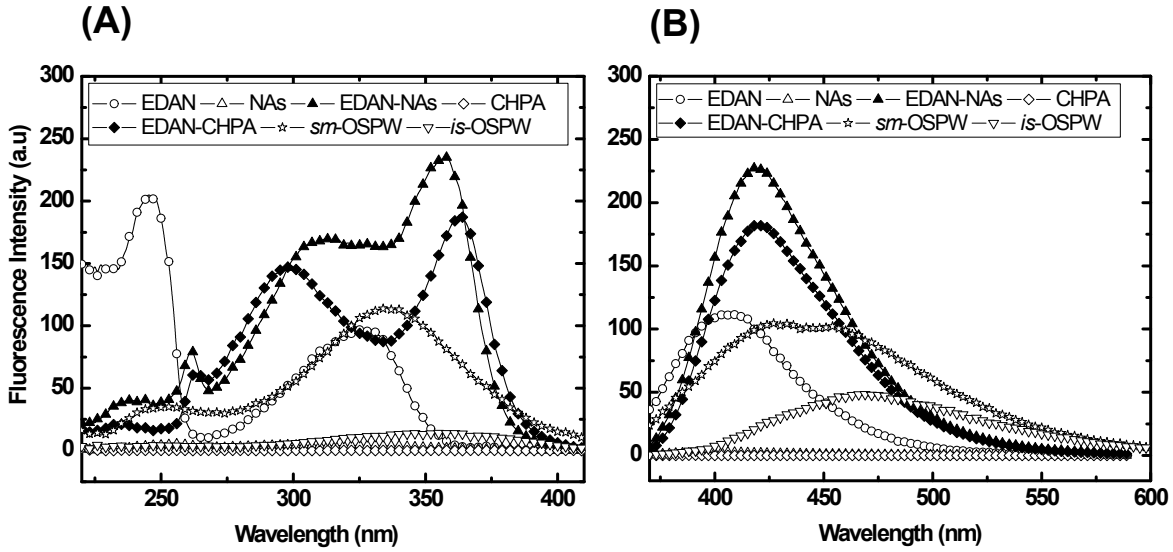


Figure S3. Excitation (A) and emission (B, λex 350 nm) spectra of EDAN, NAs, EDAN-NAs, CHPA, EDAN-CHPA, *sm*-OSPW and *is*-OSPW.

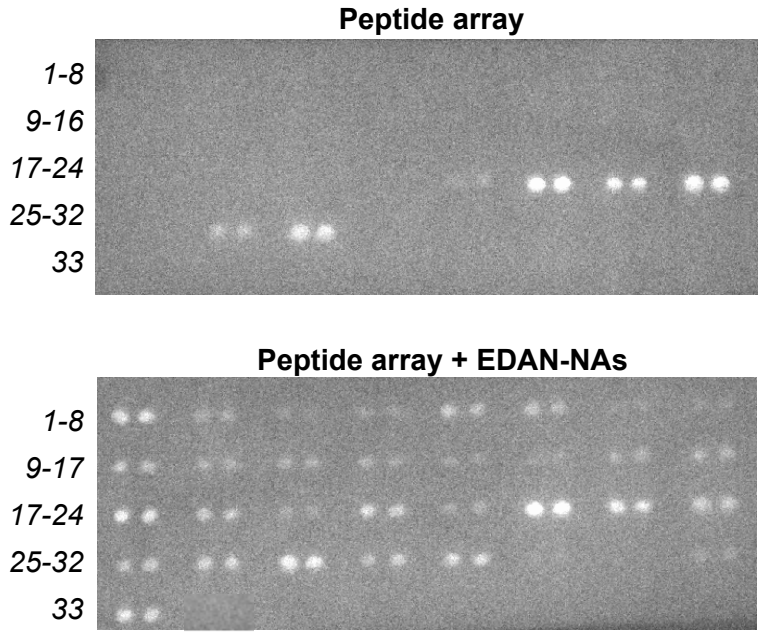


Figure S4. Images of peptide array before and after incubation with EDAN-NAs. Each peptide is spotted in duplicates. The peptide array was imaged using Kodak Fluorescence Imager at λex/em 353/440 nm.

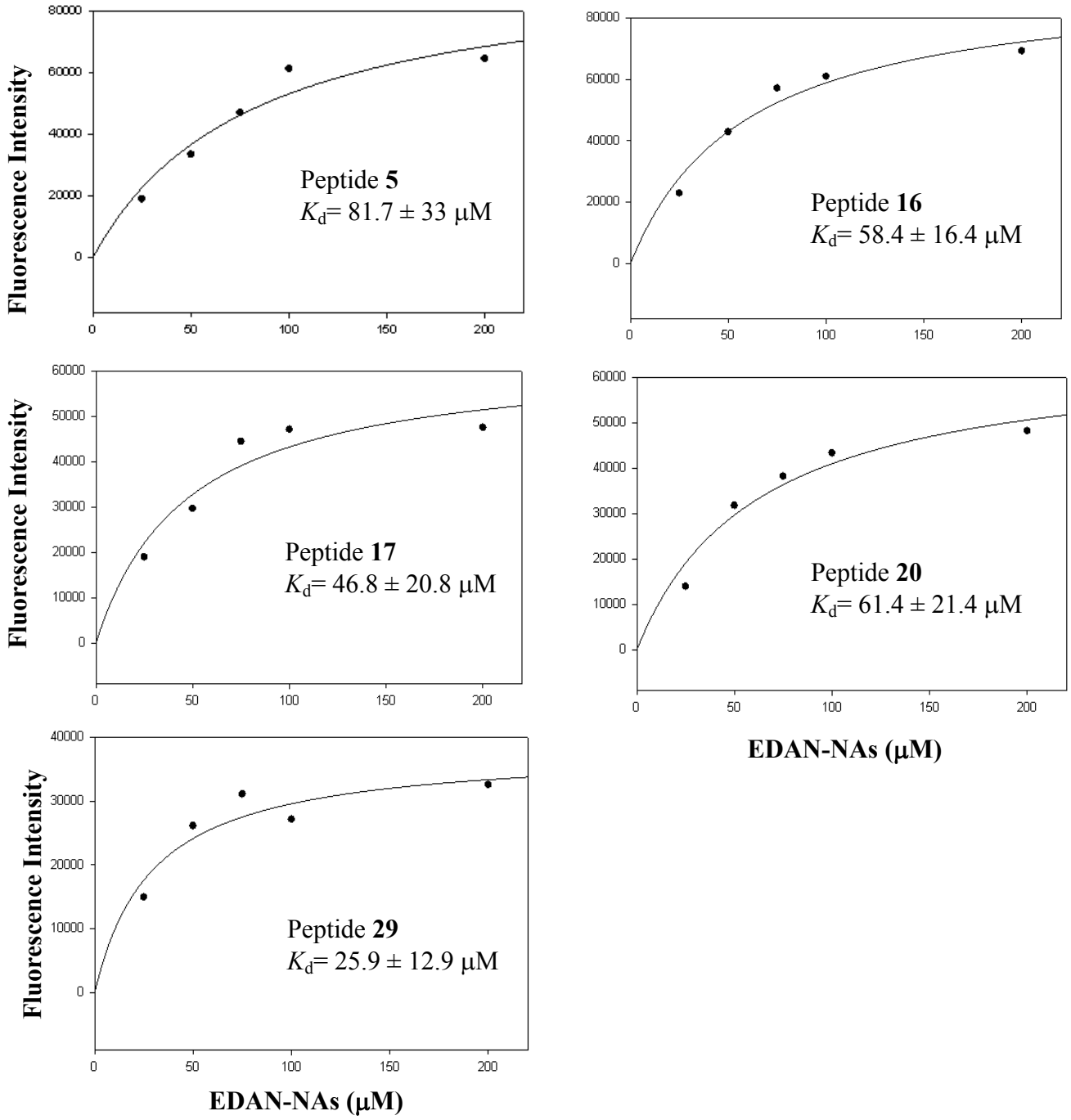


Figure S5. Binding curves for a few representative peptides from the 2nd peptide array library as shown in Figure 2a. Shown is the fluorescence intensity (λex/em 353/440 nm) of peptide bound EDAN-NAs as a function of EDAN-NAs concentrations (0-200 μM). The symbols are experimental data points whereas the line was calculated by fitting data to one binding site equation f = Bmax*abs(x)/(Kd + abs(x)), where Kd is the apparent binding constant.

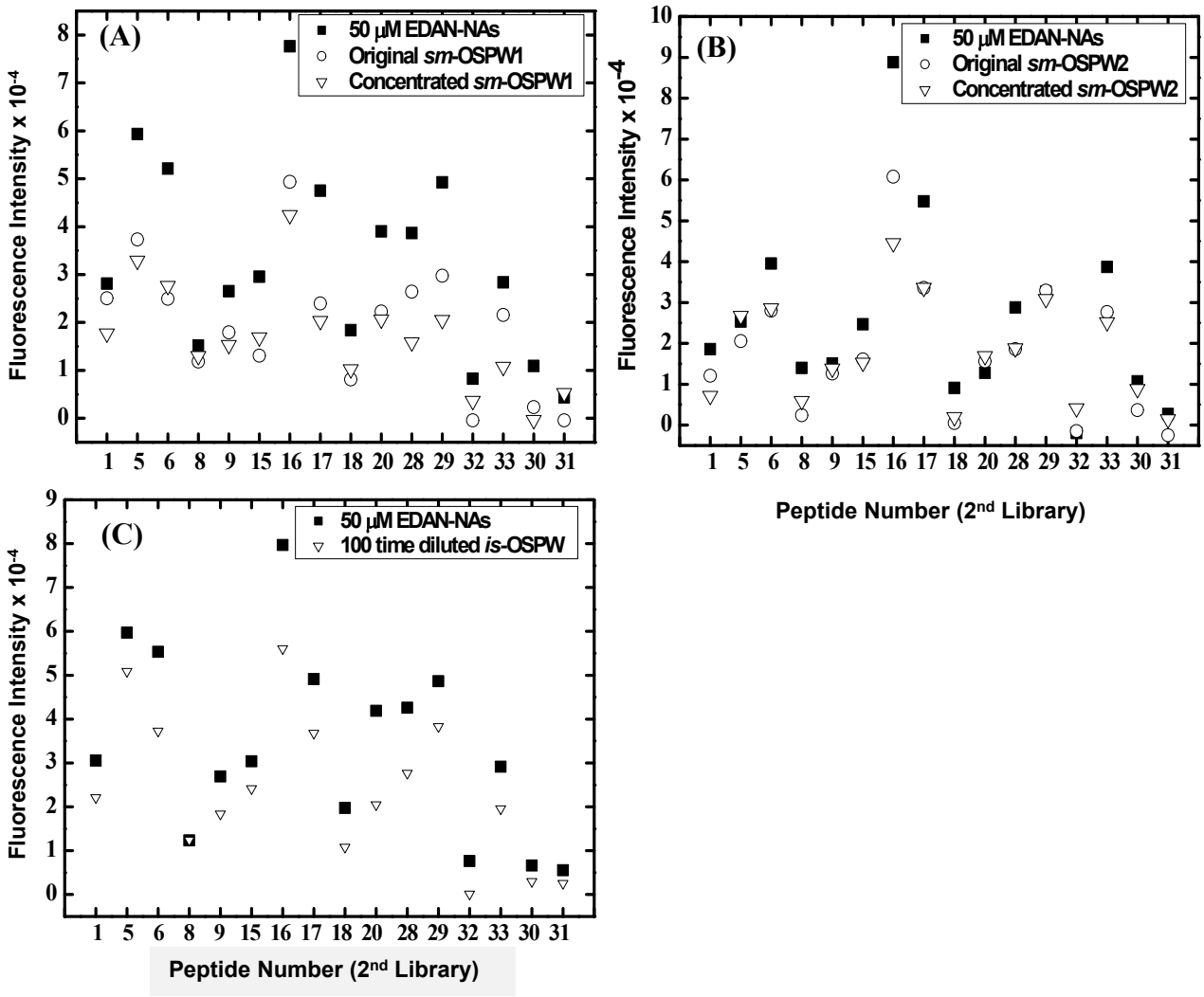


Figure S6. Competitive binding of EDAN-NAs to the peptide array in the presence of *sm*-OSPW and *is*-OSPW. EDAN-NAs (50 μM) was incubated (30 min, r.t.) with the peptide array in the presence of (A) sm-OSPW1 at two concentrations (original, TOC 40 mg/L and 10x concentrated), (B) sm-OSPW2 at two concentrations (original, TOC 36 mg/L and 10x concentrated), and (C) 100 times diluted is-OSPW for 30 min at room temperature. The net fluorescence intensity was assessed using a Kodak imager at λex/em 353/440 nm and the data were normalized to the background.

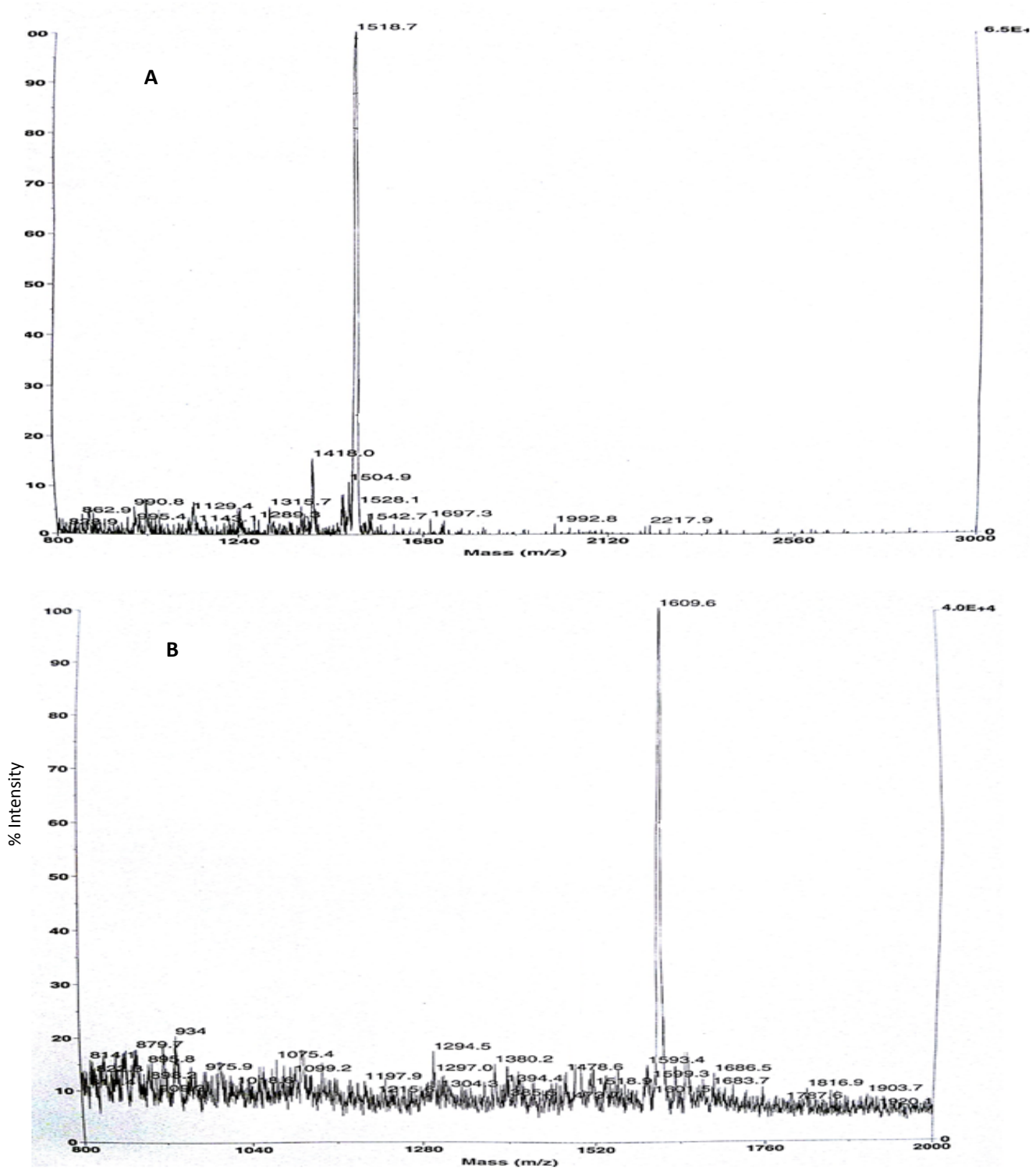


Figure S7. MALDI-TOF mass spectra for soluble peptides. (A) shows mass of peptide **17** with $[M+H]^+$ 1518.7 (cald. $[M+H]^+$ 1518.7) and (B) shows mass of peptide **31** with $[M+H]^+$ 1609.6 (calcd. $[M+H]^+$ 1608.7).

Table S1. Concentration of soluble peptides estimated using UV Spectroscopy for ITC experiments

Trial No.	Sample details	Filename	Test solution in IPA/water	Absorbance (in quartz cuvette)	Concentration (mM)*	Background spectra
1	Peptide 17, High binding, Stock	PD17T1	1 ml PD17	1.010 at 257 nm	7.656	IPA/water
2	Peptide 31, Low binding, Stock	PD31T1	1 ml PD31	2.211 at 275 nm	2.591	IPA/water
3	Peptide 31, Low binding, Standard	PD31T2	1 ml PD31	0.745 at 275 nm	0.873	IPA/water

*The concentration of the soluble peptides in IPA/water solution was estimated from UV spectroscopy. The peptide solutions for UV spectroscopy measurements were prepared by dissolving the corresponding peptide (after lyophilization) in 35% v/v IPA/water solution. For accurate measurements, the peptide solutions were diluted to get absorbance values in 0.2-1.0 range.

[Peptide concentration] mg/ml in the test solution = (A x DF x MW) / ε

Here,

A = Absorbance at 257 nm or 275 nm

DF = Dilution factor, if any

MW = Molecular weight of peptide (1516 g/mol for peptide **17** and 1608 g/mol for peptide **31**)

ε = molar absorptivity (200 L mol^{-1} cm^{-1} for phenylalanine, in peptide **17** and 1372 L mol^{-1} cm^{-1} for tyrosine, in peptide **31**)