Supporting Information

Methods

Cloning of aECM-N_3 constructs. Synthetic oligonucleotides encoding the CS5 cell-binding domain were annealed, phosphorylated, and ligated into pEC2[1] to produce pEC2-CS5. An oligonucleotide encoding the elastin-like repeat $(VPGVG)_2VPGFG(VPGVG)_2$ was similarly ligated into pUC19 (New England Biolabs, Ipswich, MA) between the *Eco*RI and *Bam*HI sites. The insert was excised with *Ban*I and self-ligated to form multimers (see Supplementary Figure 1). The multimerization mixture was ligated into *Ban*I-linearized pEC2-CS5. Transformants with the pentamer sequence were selected, digested with *Xho*I and *Sal*I, and ligated into a modified pET28a plasmid (Novagen, San Diego, CA). This step was repeated twice to obtain the final $[CS5(ELF)_5]_3$ construct under control of the T7 promoter. A similar cloning strategy was used for the RGD and RDG constructs. The *pheRS** gene encoding the alpha subunit of the A294G mutant of *E. coli* phenylalanyl-tRNA synthetase was subcloned into the *Sph*I site from the pKSS vector kindly provided by Dr. Peter Kast.[2] The final plasmids were designated pNS-CS5-ELF[3], pSM-RGD-ELF, and pSM-RDG-ELF.

Protein expression and purification. aECM-N_3 proteins were expressed in a phenylalanine auxotrophic *E. coli* strain, termed AF-IQ,[4] harboring either pNS-CS5-ELF, pSM-RGD-ELF, or pSM-RDG-ELF. To express proteins from these strains, a culture was grown overnight in 2 x YT medium and used to inoculate 1L of M9AA medium supplemented with antibiotics kanamycin and chloramphenicol (Sigma). At an OD_{600} of 1.0, expression of the target proteins and the T7 RNA polymerase was induced by adding

1 mM isopropyl-β-D-thiogalactoside (IPTG). After 10 minutes, the cells were washed twice with 0.9% NaCl and resuspended in M9 medium containing 19 amino acids (excluding phenylalanine) to a final volume of 1 L. The cultures were supplemented with either 25 mg/L phenylalanine (positive control) or up to 350 mg of *p*N_3Phe and grown for 4 hours. Protein expression was monitored by SDS-PAGE and Western blotting using an anti-T7 tag horseradish peroxidase conjugated antibody (Novagen). Cells were harvested after 4 hours by centrifugation (10,000g, 10 min, 25°C), and the cell pellets were resuspended in 20 mL of TEN buffer (10 mM Tris, 1 mM EDTA, 0.1 M NaCl) by sonication and subsequently frozen. The frozen lysate was treated with 1 mM phenylmethylsulfonylfluoride and 10 μg/mL of both DNase and RNase. The resulting mixture was agitated for 4 hours at 37°C and later centrifuged at a temperature above the expected lower critical solution temperature (LCST) of the protein (22000g, 60 min, 25°C). The target protein was extracted from the pellet into 4 M urea at 4°C. The resulting suspension was clarified by centrifugation (22000g, 60 min, 2°C), and the supernatant was dialyzed in cold (4°C) distilled water for 3 days, frozen, and lyophilized. Typical experiments yielded 40 mg of protein per liter of culture.

Mechanical testing. Samples were prepared in Teflon molds by drying 10% aECM-N_3 solutions in DMSO overnight at 50°C followed by irradiation with a 100 W mercury lamp for 30 minutes. Samples were removed from the molds, swollen in 4°C water overnight, cut into test strips, and finally equilibrated in PBS at 37°C. Films were approximately 3 mm x 10 mm. Tensile testing of equilibrated films was performed at 37°C in phosphate buffered saline at pH 7.4. Films were extended at a rate of 10%

length/minute using an Instron-5542 (Instron Corporation, Canton, MA) with a 5N load cell. The data was acquired using the Instron Series IX Software for Windows (Merlin version).

Photolithographic patterning of aECM-N_3. Passivated glass slides were prepared by treatment with a PEO-silane reagent. Standard glass microscope slides (Corning) were immersed in a solution of concentrated H_2SO_4 for 1 hour. After a thorough washing with water, slides were placed into a boiling solution of $NH_4OH/H_2O_2/H_20$ (1:1:5) for 30 minutes. The slides were gently shaken in a 1% (v/v) solution of 2-[methoxy(polyethylenoxy)propyl] trimethoxysilane (Gelest) in toluene for 30 minutes. The slides were rinsed immediately with toluene, followed by a stream of methanol and water. The functionalized slides were then cured at 100ºC for 30 minutes. Each PEO-modified slide was cut into 4 similarly sized pieces using a diamond cutter, and individual substrate pieces were covered dropwise with 10 μL of a 12.5 mg/mL solution of aECM-N_3 in DMSO that had been centrifuged for 1 minute at 14,000 rpm to remove particulates. Substrates were spun for 30 seconds at 1000 rpm on a Specialty Coating Systems model P-6000 spin coater. Protein-coated slides were dried at 50°C for 4 hours. Exposure of protein to sunlight was avoided until photolithography was complete. Protein-coated slides were irradiated for 60 seconds at 365nm using a Karl Suss mask aligner under a chrome-on-quartz mask prepared by Dr. Michael Diehl via chrome deposition and stripping from a 3000 dpi transparency. Irradiated substrates were washed for 4.5 hours in 6 M guanidine hydrochloride to remove soluble protein from the masked

regions. Protein-patterned substrates were then rinsed for 5 minutes in filtered water and sterilized by an ethanol rinse before use.

Cell culture. Rat-1 fibroblasts (ATCC, Manassas, VA) were maintained in a 37°C, 5% CO_2 humidified environmental chamber. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM, 10% fetal calf serum, Gibco), which was replaced every two days. Near-confluent Rat-1 cultures were passaged by treatment with trypsin (0.05% trypsin/EDTA) at 37°C for 2-5 minutes (Invitrogen) and resuspended in 2 mL of serum-free DMEM. Cells were then centrifuged (1050 rpm, 3 min), and resuspended in fresh serum-free DMEM at a concentration of 1.0×10^6 cells/mL.

Cell patterning. Patterned substrates were placed in a 6-well plate, and Rat-1 fibroblasts (6.0×10^5 cells/substrate, ~ 1.0×10^5 cells/cm^2) were deposited on the patterned surfaces in a total of 3 mL of serum-free DMEM. After 4 hours of incubation, phase contrast images were captured with a Nikon Eclipse TE 300 microscope. Fluorescence images were acquired using a laser scanning confocal microscope consisting of a confocal system (Nikon C-1) combined with an inverted optical microscope (Nikon TE-2000-U). To fix and fluorescently label cell patterns, the substrates were washed three times with PBS before treatment with chilled acetone at -20°C for 5 minutes. The substrates were again rinsed with PBS and blocked with a 10% BSA solution for 30 minutes at room temperature. Afterwards, 1 μL of a monoclonal anti-T7 tag primary antibody (Novagen) was added and allowed to incubate at room temperature for 1.5 hours. Individual substrates were then transferred to a standard Petri dish (VWR) containing a solution of 0.1% BSA and placed on a waver to wash for 10 minutes. A secondary

antibody/phalloidin solution composed of 425 μL PBS, 62.5 μL secondary antibody (Cy2-labeled anti-mouse, 0.5 mg/mL, Chemicon) and 12.5 μL rhodamine phalloidin (6.6 μM, Invitrogen) was added per sample for 1 hour in the dark. Labeled samples were washed in 0.1% BSA for 10 minutes on a waver. The samples were then rinsed three times with PBS without agitation, and mounted with a glass coverslip using 25 μL of Biomeda gel/mount (Biomeda Corporation, Foster City, CA) solution. The samples were dried at room temperature for 1 hour and sealed with clear fingernail polish.

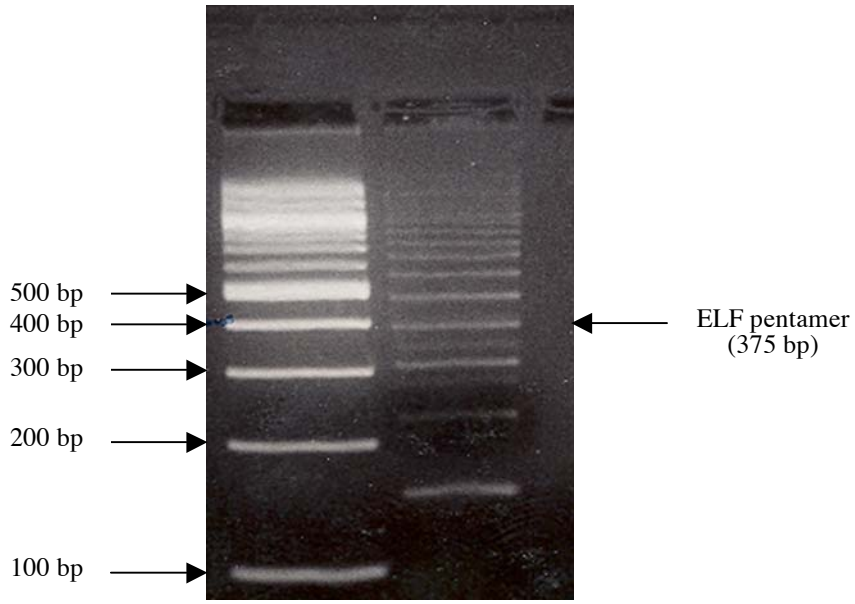
Cell Spreading. Cell spreading experiments were performed on uniformly crosslinked films to determine whether the nature of cell attachment to protein-coated surfaces is sequence-specific to the RGD cell-binding domain. RGD-N_3 and RDG-N_3 substrates were prepared by spin coating 10 μL of a 12.5 mg/mL protein solution at 1000 rpm for 30 seconds onto base-cleaned (saturated potassium hydroxide in ethanol) coverslips (12 mm diameter). Substrates were dried at 50ºC overnight and uniformly irradiated for 60 seconds using an unfiltered Oriel 100W medium pressure mercury lamp. Substrates were rinsed with water for several minutes before being sterilized by an ethanol rinse.

For the positive control, 1 mL of a 10 μg/mL fibronectin solution in PBS was adsorbed overnight in a 6-well plate at 4ºC. The wells were rinsed three times with PBS, and blocked with a 2 mg/mL BSA solution for 30 minutes at room temperature and rinsed three times. For the negative control, 1 ml of a 2 mg/mL BSA solution in PBS was adsorbed overnight in a 6-well plate at 4ºC. The wells were rinsed three times with PBS, and blocked with a 2 mg/mL BSA solution for 30 minutes at room temperature and rinsed three times. RGD-N_3 and RDG-N_3 substrates were adhered to the bottom of a

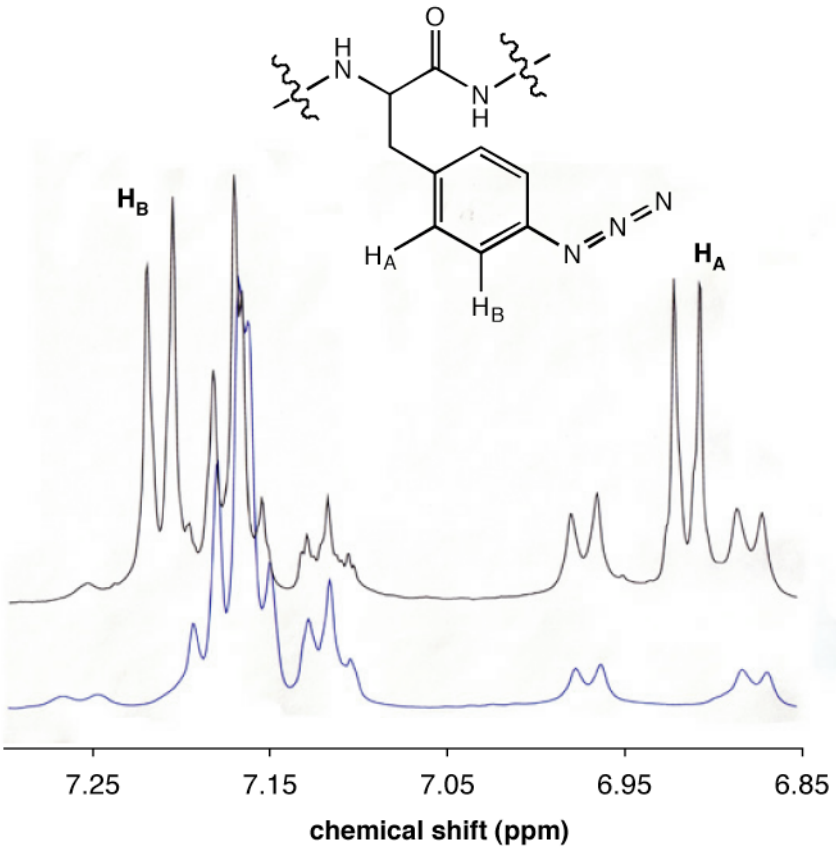
BSA-blocked well by applying sterilized vacuum grease around the edge of the coverslips. Rat-1 cells were resuspended in serum-free media and seeded in a total volume of 3 mL per well at a density of 2.0 x 10^3 cells/cm^2. For quantification of spread area, pictures were obtained 4 hours post-seeding using a Nikon Eclipse TE 300 microscope. Cell areas were manually traced using ImageJ v. 1.33q (National Institutes of Health, Bethesda, MD). For each substrate, at least 200 cells in total were examined in at least 3 independent experiments.

Atomic Force Microscopy. Topographical scans of RGD-N_3 protein patterns were obtained with an AutoProbe M5 atomic force microscope (Park Scientific Instruments, Woodbury, NY) in a constant-force contact mode, using pyramidal tips (0.58 N/m, Veeco DNP-S). Imaging was performed dry or in water. When imaging in water, a glass slide was affixed to the back of the cantilever mount in the path of the laser, and the space between the sample and the slide was filled with water to provide a smooth and constant optical interface.

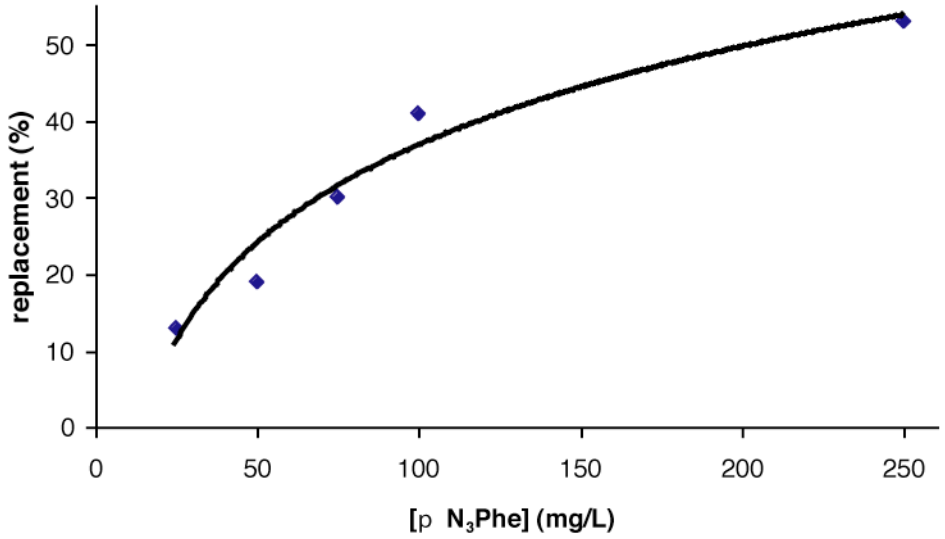
Supplementary Figures



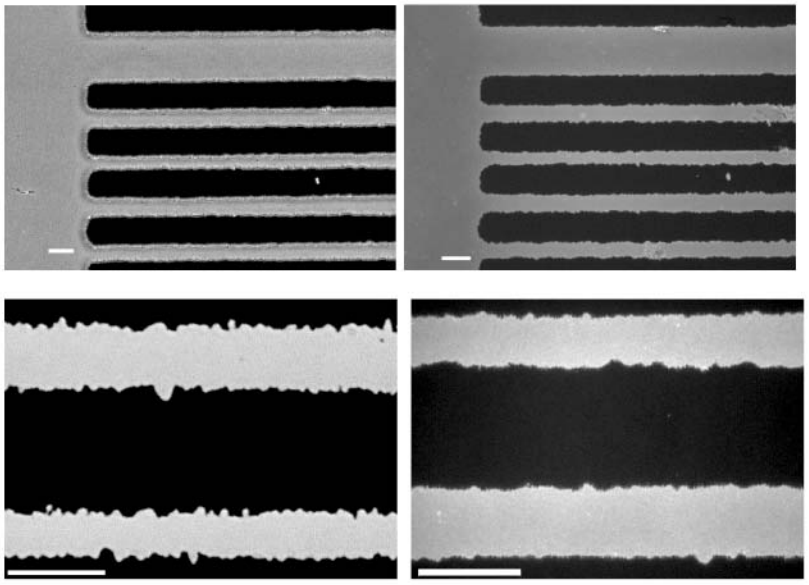
Supplementary Figure 1. Representative 3% agarose gel of a multimerization reaction. ELF monomer DNA (*Ban*I sticky ends) was incubated with T4 DNA ligase for 25 minutes at room temperature before being loaded into the gel. Lane 1 (left) is the 100 bp molecular weight ladder and Lane 2 (right) is the reaction mixture. The desired pentamer repeat is 375 bp.



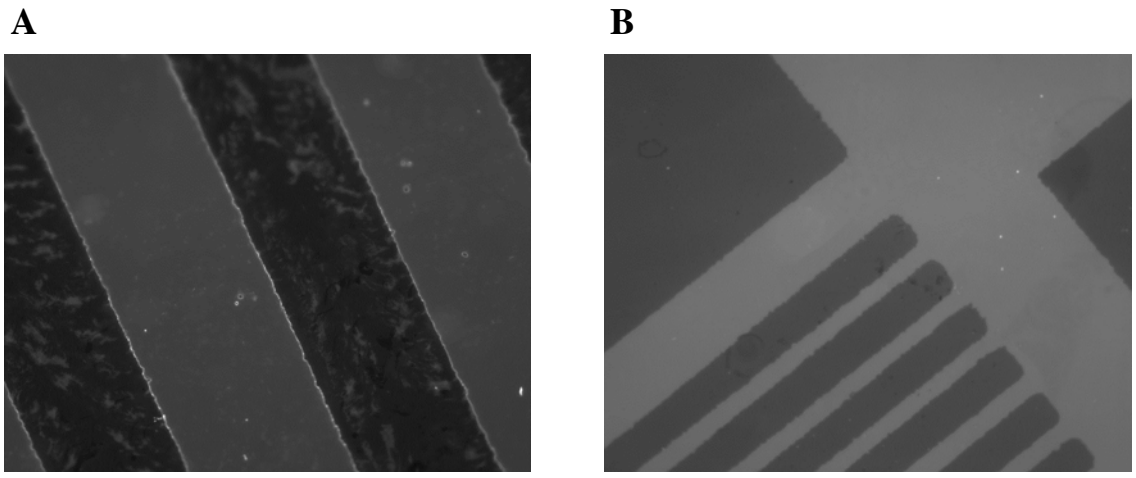
Supplementary Figure 2. Representative ^{1}H NMR spectra (6.85 - 7.30 ppm region) of an aECM-N_3 construct expressed in media supplemented with phenylalanine (bottom spectrum) or with 250 mg/L *p*N_3Phe (top spectrum). Spectra are identical except for two additional doublets in the top spectrum assigned to the aromatic protons of *p*N_3Phe; integration indicates replacement of 53% of the phenylalanine residues by *p*N_3Phe. 600 MHz spectra were taken on 1 mM samples in DMSO-d_6 at 23°C.



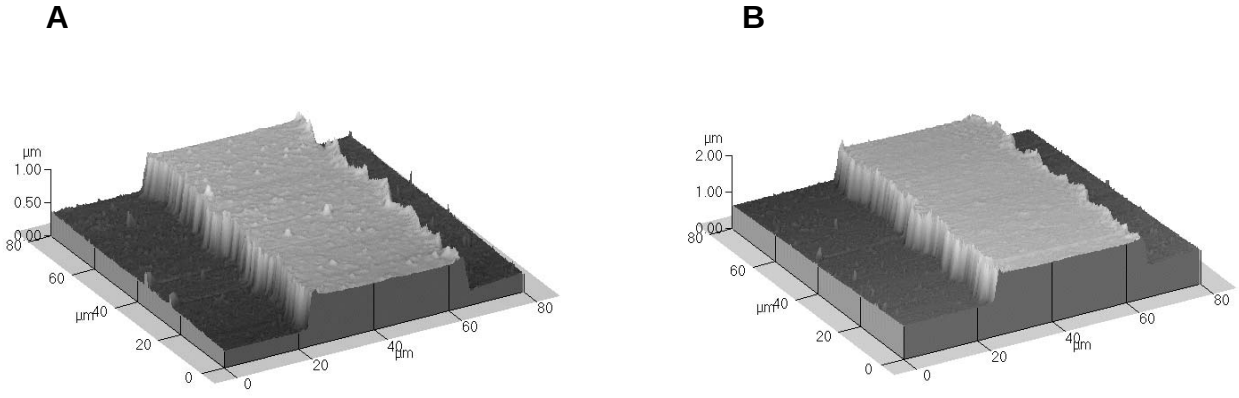
Supplementary Figure 3. Incorporation of *p*N_3Phe into the CS5-N_3 protein as a function of concentration in the expression medium.



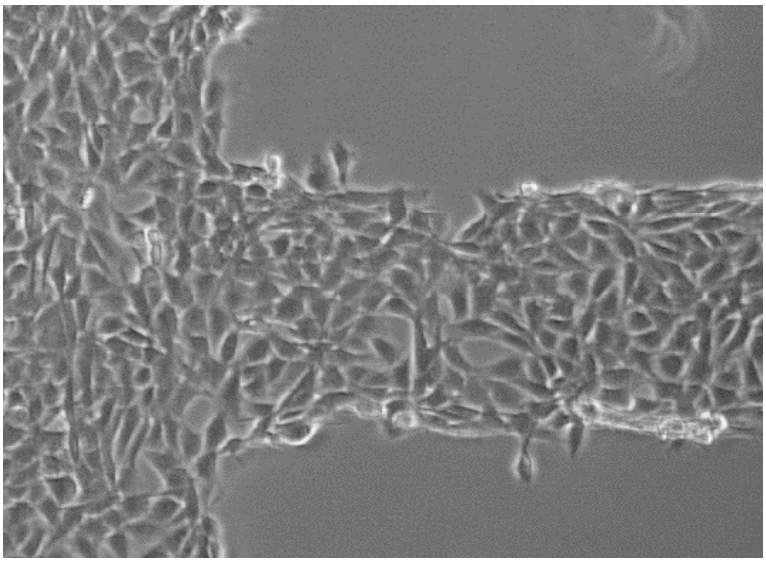
Supplementary Figure 4. Phase contrast microscopy images of the chrome mask used in photopatterning (left panels) compared with fluorescence microscopy images of the protein patterns (CS5-N_3, right panels). Scale bars represent 50 μm. In the top panels (lower magnification), the protein pattern could be visually matched to precisely the region on the mask that created it. The bottom panels (higher magnification) show two separate regions with features of similar sizes. Thin lines (50 μm spacing) were designed to pattern individual cells or a small number of cells. Thicker lines allow patterning of a larger cell population (up to 500 μm spacing).



Supplementary Figure 5. Fluorescence microscopy images of protein patterns after washing. The bright regions represent immunostained RGD-N_3 protein and the dark regions represent the masked region of the substrate. It is crucial that uncrosslinked, soluble protein be effectively removed from the surface to prevent the undermining of cell patterns. The surface shown in (A) was deemed unacceptable for cell patterning experiments, as uncrosslinked protein was not fully removed from the masked regions. A thoroughly rinsed surface as shown in (B) was considered acceptable for cell patterning experiments.



Supplementary Figure 6. AFM images of patterned RGD-N_3. The images were recorded on (A) dried or (B) hydrated patterned films in constant-force contact mode with an autoprobe M5 atomic force microscope (Park Scientific).



Supplementary Figure 7. Phase contrast microscopy of Rat-1 fibroblasts attached to photopatterned RGD-N_3. Cells positioned along the RGD-N_3 pattern edges were elongated parallel to the pattern border.

References

(1) Cantor, E. J. Ph.D. Dissertation, University of Massachusetts, Amherst, MA, 1997.
(2) Kast, P. *Gene*, **1994**; Vol. 138, pp 109-114.
(3) Sharma, N. Ph.D. Dissertation, University of Massachusetts, Amherst, MA, 2001.
(4) Yoshikawa, E.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules*, **1994**; Vol 27, pp 5471-5475.