Supporting Information

Recombinant Protein-Stabilized Monodisperse Microbubbles With Tunable Size Using a Valve-based Microfluidic Device

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1. Sequence of Oleosin

1.1. 42-30G-63 sequence

GSATTTYDRHHVTTTQPQYRHDQHTGDRLTHPQRQQQGPSTGKLALGATPLFGVIGF
SPVIVPAMGIAIGLAGVTGFQRDYVKGKLQDVGEYTGQKTKDLGQKIQHTAHEMGDQG
QGQGQGGGKEGRKEGGKLEHHHHHH

1.2. eGFP-42-30G-63 sequence

VSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWP
TLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG
DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV
QLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMD
ELYKGSATTTYDRHHVTTTQPQYRHDQHTGDRLTHPQRQQQGPSTGKLALGATPLFGV
IGFSPVIVPAMGIAIGLAGVTGFQRDYVKGKLQDVGEYTGQKTKDLGQKIQHTAHEMG
DQGQGQGQGGGKEGRKEGGKLEHHHHHH

1.3. Detailed cloning information

The sunflower seed oleosin gene was provided as a gift from Dr. Beaudoin at Rothamsted Research, Hampshire, England. Multiple rounds of PCR were used to create the oleosin gene 42-30G-63 and eGFP-30G-63. The following PCR primers were used to create the three domains, which were combined in a single PCR step: N-terminal hydrophilic S 5’ – AAGGAGATAGGATCCACCACAACCTACGACC – 3’, N-terminal hydrophilic AS 5’ – GCACCGAGAGCGAGCTTGCCGGTTGAGG – 3’, hydrophobic S 5’ – CCTCAACCGGCAAG CTCGCTCTCGGTGC – 3’, hydrophobic AS 5’ –

CCTTCACATAATCCCTCTGAAACCCGGTAACACC – 3', C-terminal hydrophilic S 5' – GGTGTTACCGGGTTTCAG AGGGATTATGTGAAGG – 3', C-terminal hydrophilic AS 5' – TATATGAATCTCGAGTTTCCCCCCTTCTTTTCG – 3'. The PCRs to create the hydrophilic portions were run with the soluble oleosin gene as the template[1] and the hydrophobic domain PRC was run with the following oligo as the template: 5' – CTCGCTCTCGGTGCGACTCCGCTGTTTGGTGTTATAGGTTTCAGCCCTGTTATTGTTC CAGCGATGGGTATAGCGATTGGGCTTGCGGGTGTTACCGGGTTTCAG – 3'. PCR was used to create the eGFP mutants using the following primers: eGFP S 5' – ATCGGTATACATATGGTGAGCAAGGGCGAGG – 3' and eGFP AS 5' – ATCTAAAATGGATCCCTTGTACAGCTCG – 3' with pBamUK-eGFP as a template. The genes were inserted into the expression vector pBamUK, a pET series derivative constructed by the Duyne Laboratory (SOM, Penn). Mutants were confirmed through DNA sequencing prior to protein expression. pBamUK adds a 6-Histidine tag to the C-terminus of the protein for IMAC purification.

1.4. Protein Purification

B-PER protein extraction agent (Fisher Scientific) was used for protein purification. Briefly, pellets were resuspended in B-PER (30 ml B-PER per liter of culture) and DNAse was added to a final concentration of 1 μg/ml. The resuspended pellets were centrifuged at 15,000 g for 15 minutes. The 42-30G-63 supernatant was discarded and the eGFP-42-30G-63 supernatant was applied to an equilibrated column and allowed to bind for >1 hour. The remaining inclusion body pellet of 42-30G-63 was suspended in denaturing buffer (8M urea, 50 mM phosphate buffer, 300 mM NaCl). The solution was centrifuged at 15,000 g for 15 minutes and the supernatant was added to an equilibrated Ni-NTA column (Hispur Ni-NTA resin, Thermo

Scientific). The denatured 42-30G-63 was allowed to bind to the column for >1 hours and washed three times with denaturing wash buffer (denaturing buffer with 20 mM imidazole). 42-30G-63 refolding was accomplished by diluting the column 50 times with refolding buffer (50 mM phosphate buffer, 300 mM NaCl, 5% by volume glycerol, 4°C) and rocked at 4°C for >1hr. Both mutants was washed extensively with wash buffer (50 mM phosphate buffer, 300 mM NaCl, 20 mM imadzole) and eluted in fractions with elution buffer (50 mM phosphate buffer, 300 mM NaCl, 300 mM imidazole).

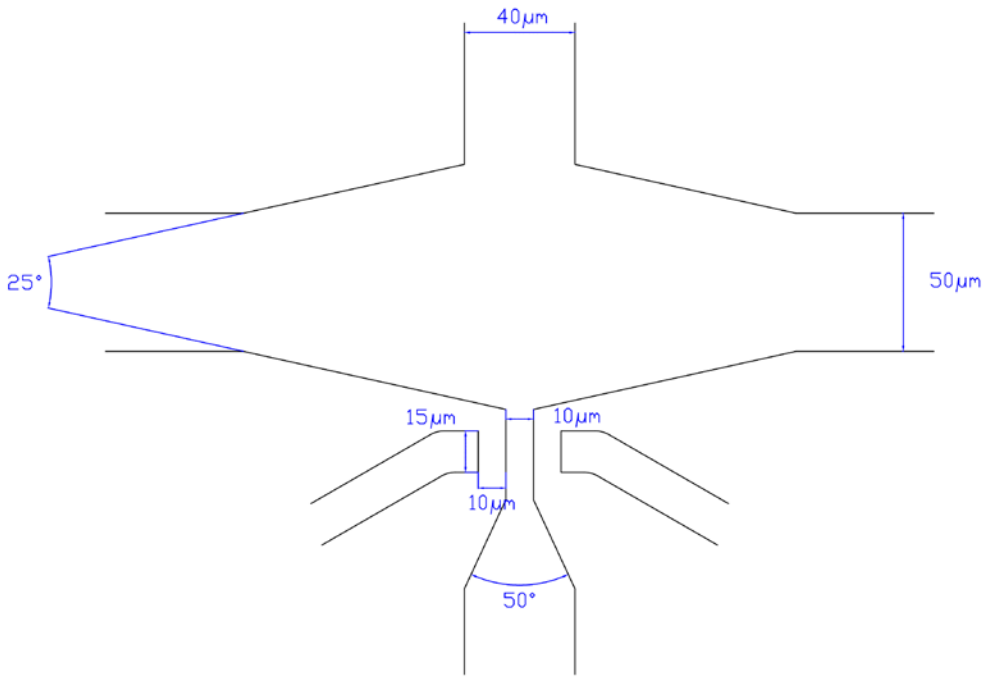

Figure S1. Microfluidic device channel and junction dimensions.

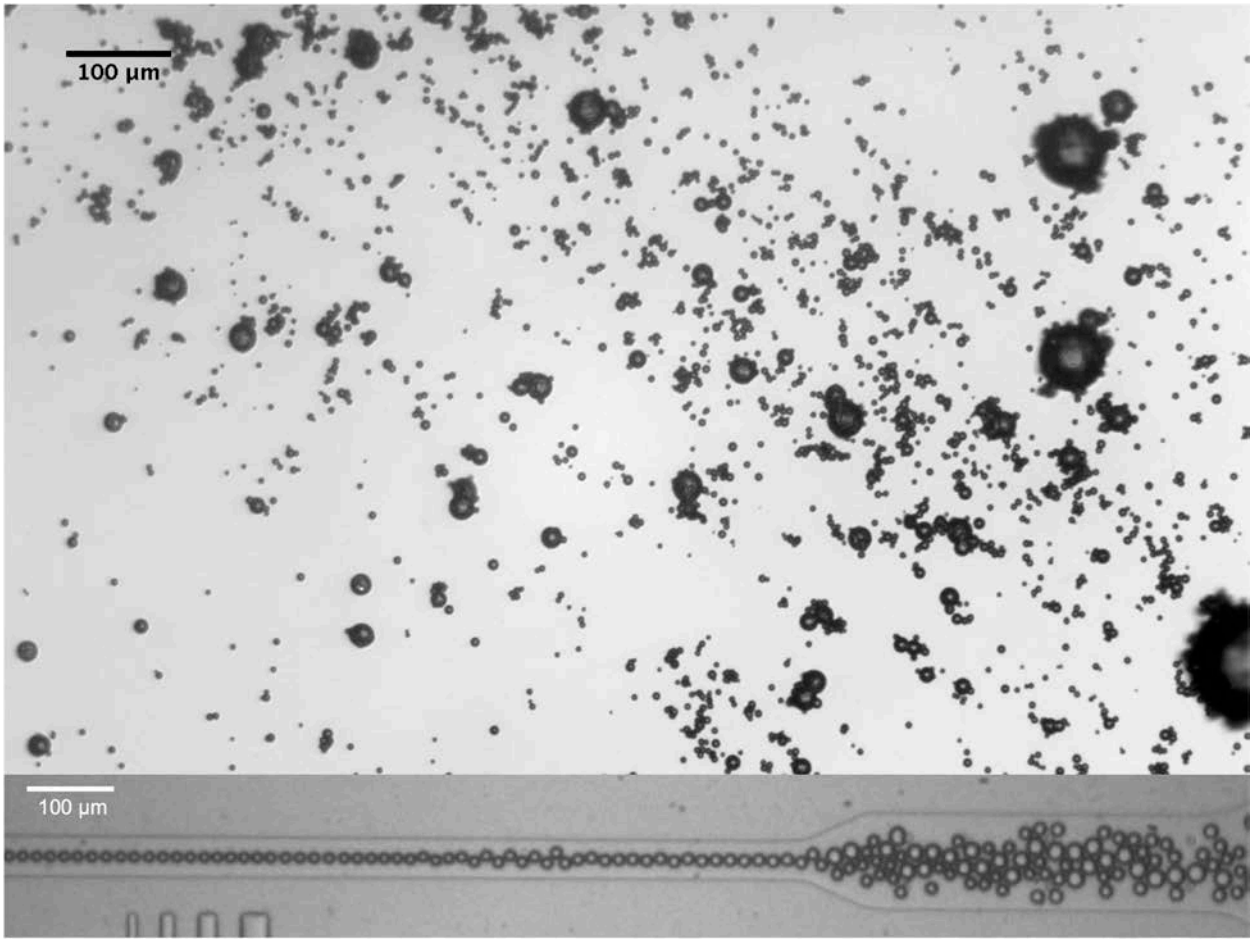

Figure S2. Micrographs of microbubbles produced using a solution containing 1mg mL^{-1} oleosin protein 42-30G-63 showing coalescence and subsequent polydispersity after collection. The inset is a micrograph of the same microbubbles coalescing upon generation in the microfluidic chip.

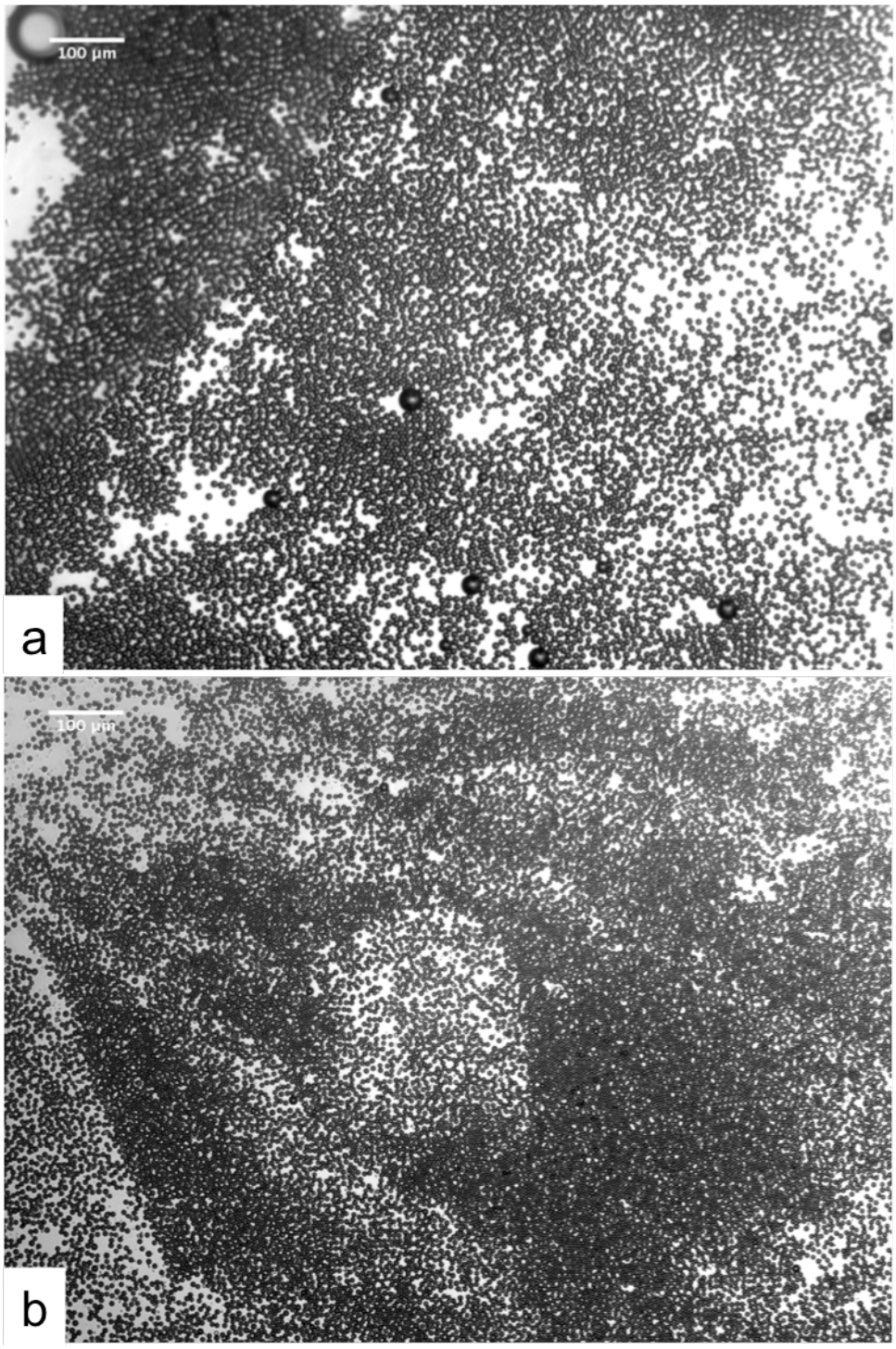

Figure S3. Microscope images of microbubbles (a) upon collection and (b) 7 days after collection.

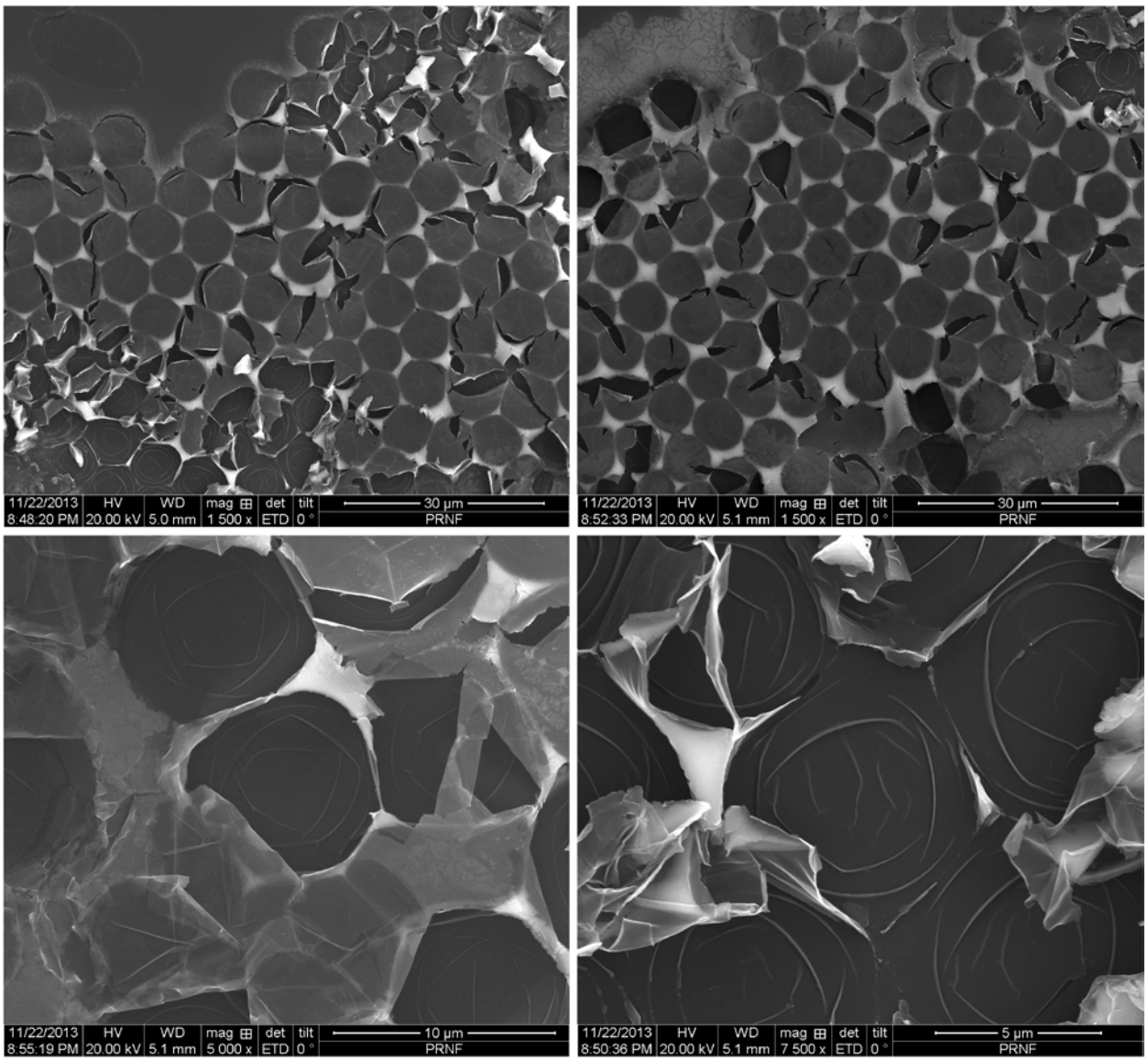

Figure S4. SEM images of dried microbubbles generated with a solution containing 1mg mL^{-1} oleosin and 10 mg mL^{-1} $(PEO)_{78}$ –$(PPO)_{30}$ –$(PEO)_{78}$.

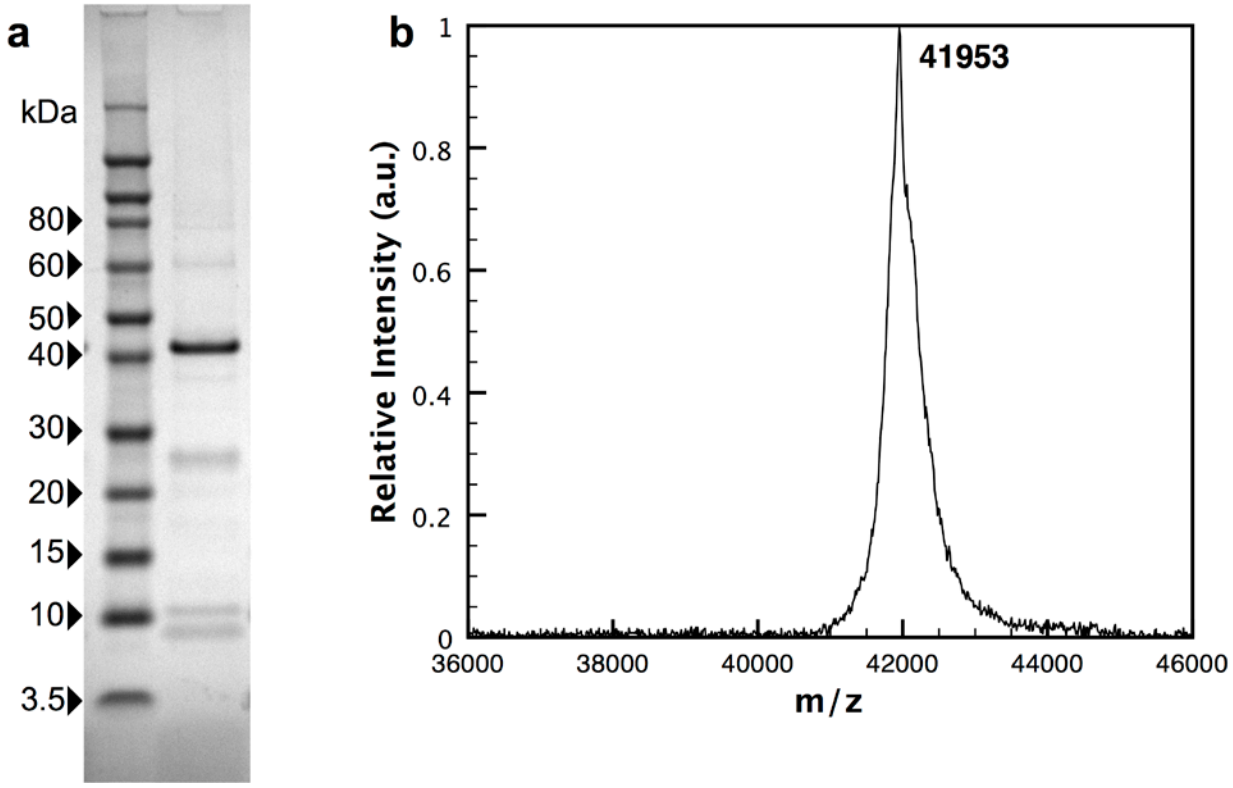

Figure S5. (a) SDS-PAGE gel showing >90 % purity for eGFP-42-30G-63. (b)MALDI-TOF spectra confirming the molecular weight for eGFP-42-30G-63 (Expected: 41890, measured: 41953).

Quantitative analysis of the CD spectra

The secondary structure of the proteins is analyzed by fitting the CD spectra using Dichroweb with the CDSSTR analysis method.[1-3]

	Helix	**Sheet**	**Turn**	**Unordered**
Wild Type Oleosin	0.05	0.4	0.13	0.41
42-30G-63	0.04	0.06	0.06	0.84

REFERENCES

1. Sreerama, N.; Venyaminov, S. Y.; Woody, R. W. Estimation of protein secondary structure from circular dichroism spectra: Inclusion of denatured proteins with native proteins in the analysis. *Anal. Biochem.* **2000**, *287*, 243-251.
2. Whitmore, L.; Wallace, B. A. DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Res.* **2004**, *32*, W668-W673.
3. Sreerama, N.; Woody, R. W. Estimation of protein secondary structure from circular dichroism spectra: Comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal. Biochem.* **2000, *87***, 252-260.