Supporting Text

Peptide Synthesis. DapBz was incorporated as Fmoc-Dap(alloc)-OH (Bachem), deprotected on the solid phase resin, and side chain capped with benzoic acid using standard HOBt/HBtU chemistry. SeMet was incorporated as Fmoc-SeMet-OH (Anaspec). HPLC was performed by using a Waters Prep LC 4000 System equipped with a Waters 2487 detector (set at 228 and 280 nm), a C_{18} reverse-phase analytical column (Beckman Ultrasphere, 5 μm, 4.6 × 150 mm) and a C_{18} reverse-phase preparative column (YMC-pack, 250 × 20 mm). A standard gradient of 80:20 to 20:80 (water:acetonitrile, 0.1% TFA) over 25 min (1 ml/min on the analytical column, and 15 ml/min on the preparative column) was used in all cases. Identity was confirmed by electrospray mass spectroscopy (PerSeptive Biosystems Mariner BioSpectrometry Workstation, using the Turbo Ion Source) and by analytical HPLC. Retention times for peptides **1**, **2**, and **3** on the analytical column are 28.5, 26.0, and 26.5 min respectively.

Analytical Ultracentrifugation. Stock solutions of peptides were prepared in 50 mM phosphate buffer with 100 mM NaCl at pH 7.2. Each solution was then loaded onto a 1.0 kDa MWCO DispoDialyzer (Harvard Biosciences) and dialyzed at room temperature. Dilutions were made from the dialyzed stock solution. Peptide solutions were loaded into standard two-sector epon centerpieces and spun, at 25°C, in a Beckman XL-I analytical ultracentrifuge at 40,000, 45,000, and 50,000 rpm for ~24 h at each speed. The contents of each cell were confirmed to be at equilibrium by using WINMATCH before increasing the speed. Data were analyzed by using the programs NONLIN (1) and SEDPHAT (2). Molecular weights were determined by using a partial specific volume, ν , calculated by using SEDNTERP (3) or determined by densitometry.

Partial Specific Volume Determination. Peptide **1** was dissolved in 10 mM phosphate buffer (pH 7.2), filtered through a 0.45 μM syringe filter, and extensively dialyzed in MWCO 500 dialysis tubing (Spectrum Laboratories). The concentration of the dialyzed solution was determined based on absorbance at 280 nm. The dialyzed solution of **1** and a sample of dialysate were immediately brought to the National Analytical

Ultracentrifugation Facility (University of Connecticut, Storrs), and the densities of the stock solution and four dilutions thereof (80%, 60%, 40%, and 20% of the stock solution) were measured at 20°C by using a DMA 60 Density Meter (Anton Paar).

Crystallization. Peptides were dissolved at ≥8 mg/ml in 10 mM phosphate (pH 7.2). Initial crystallization conditions were obtained from a sparse-matrix screen (Hampton Research). Crystals were grown by using vapor diffusion with hanging-drop geometry by mixing 1.5 μl of protein with an equal volume of reservoir solution. Peptides **1**, **2**, and **3** grew from 100 mM Hepes-Na (pH 7.5)/10% vol/vol *i*-propanol/20% wt/vol PEG 4000. Peptides crystallize overnight as small rods. Peptide **1** also crystallizes from 25% *t*-butanol in 0.1M Tris buffer, pH 8.5, as small rods in 1-2 weeks. The same unit cell is obtained for crystals of **1** grown from either condition.

Data Collection and Phasing. ***Peptides 2 and 3.*** MAD data sets were collected at beamline X12C at the National Synchrotron Light Source, Brookhaven National Laboratory. Crystals were frozen in a stream of N_2 gas cooled to –180°C by using FMS oil (Hampton Research) as a cryoprotectant. The DENZO and SCALEPACK package (4) was used for data indexing, reduction, and scaling (see data collection statistics in Table 3). The data for both peptides exhibited twofold and fourfold noncrystallographic symmetry, as determined by a global locked rotation function in the program GLRF (5). Heavy-atom sites were located and starting phases obtained by using the automated heavy atom solution program SOLVE (6) with an initial figure of merit of 0.62 and 0.56 for the data sets from peptides **2** and **3**, respectively. The initial electron density maps showed connected helical density.

Peptide 1. A 1.2-Å data set was collected at the 14-BM-C beamline at the BIOCARS facility (Argonne National Laboratory, Argonne, IL). Crystals were frozen in a stream of N_2 gas cooled to –180°C by using Paratone- N (Hampton Research) as a cryoprotectant. Data reduction, scaling and assessment of noncrystallographic symmetry were performed as for **2** and **3**. Starting phases were obtained by molecular replacement with the program MOLREP (7) by using the refined model of the tetramer of **3**, including side chains, as a

search model, resulting in an initial correlation coefficient of 0.53 and an initial *R* factor of 0.43.

Refinement. Iterative rounds of minimization and simulated annealing by using slow-cool torsional molecular dynamics, individual B-factor refinement, and manual rebuilding were used to refine all structures with the program CNS (8) by using an MLHL target (for **2** and **3**) or MLF target (for **1**). Topology and parameter files were created for the nonstandard groups acetyl (9, 10), amide (9–12), DapBz (13–19), and methionine selenoxide (20–24) by using bond lengths and angles from the literature. Values for D-Ala and D-Pro were identical to CNS defaults for their L-enantiomers with an inversion of chirality. S chirality was arbitrarily assigned at the oxidized selenium. For statistical cross-validation purposes 10% of the data were excluded from refinement (25, 26). Manual fitting was done by using SigmaA-weighted $2F_o - F_c$ and $F_o - F_c$ electron density maps (27) and $2F_o - F_c$ composite-omit map in the graphics program O (28). Waters were added by using the automated water-picking program in CNS. The refinement for peptide **1** was continued by using SHELXL (29), extending the resolution from 1.5 Å to 1.2 Å. Anisotropic B-factor refinement resulted in a drop in both *R* and R_{free}.

We attribute the relatively high value of R_{free} in the structure of peptide **1** to an inability to overcome the model bias due to a lack of independent phase information inherent in a molecular replacement solution. The value of R_{free} does indicate that the model of peptide **1** is correct in its essential features. Together with the two selenomethionine structures, the structure of peptide **1** demonstrates that the selenomethionine derivatives do indeed share the native structure.

Several attempts were made to discover and address the source of the discrepancy between the values of R_{work} and R_{free}. The high value of R_{free} most likely indicates a number of small deviations of the model from the actual structure, rather than any single large flaw. The following points address this discrepancy:

(*i*) The discrepancy does not appear to stem from an incorrect space group. Further rounds of molecular replacement in the eight possible orthorhombic space groups confirmed the assignment of the correct space group. Several cycles of refinement were performed in the lower-symmetry space group $P2_1$, in case the data were of

lower symmetry. Refinement in $P2_1$ did not lead to any improvement in R_{free}. Moreover, the data in $P2_1$ were checked to exclude the possibility of twinning by merohedry.

(*ii*) The discrepancy does not appear to be due to poor data at high resolution. Data quality does not deteriorate in the higher resolution ranges or at high resolutions. Moreover, refinement at lower resolution does not improve the difference between R_{free} and R_{work}.

(*iii*) The discrepancy does not appear to be due to poor data quality. The images were re- processed and re-scaled so as to eliminate any possible overloads in the low-resolution bins. Several rounds of refinement were performed with the newly processed data without any improvement in R_{free}. A new data set was collected from crystals of **1**. These data were of high quality but lower resolution. Molecular replacement followed by refinement and rebuilding of these data also did not converge to a lower R_{free}.

(*iv*) The discrepancy does not appear to be due to a few bad reflections in the test set. An examination of the test set did not reveal any overloaded or otherwise suspicious reflections. Reducing the test set to 5% did not have any effect, nor did selecting a new 10% test set.

(*v*) The discrepancy does not appear to be due to an incorrect register of side chains. Maps generated from a polyalanine model, debiased by simulated annealing, and thus free of bias from the side chains, showed no slipping of register of the side chains.

(*vi*) The model does not appear to be caught in a local minimum. The high resolution limit was cut back to 2.0 Å on two occasions so as to increase the radius of convergence and give the model more freedom to shift during simulated annealing steps. Resolution was gradually increased in subsequent refinement steps. This procedure did not reduce R_{free}.

(*vii*) The discrepancy does not appear to be due to improperly placed waters, which can have a significant effect in a structure of such a small size. Waters of crystallization were located from scratch in a map generated without any waters on multiple occasions. No significant difference in R_{free} was found.

(*viii*) The discrepancy may be due to a number of small differences between the partially refined working model and the true structure. However, we were unable to determine what those changes might be, as both the SigmaA-weighted $2F_o - F_c$ and composite omit maps, beyond a certain stage of refinement, reflected the partially refined model used to calculate phases. Attempts to improve the maps by using the Prime-and-Switch (30) protocol in SOLVE (6) and SHELXE (31, 32) were unsuccessful because of the relatively low solvent content of crystals of **1**.

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