Fig. S1. The D-ala-D-ala (green) and D-ala-D-lac (cyan) ligand models used in ROSETTALigand design computations . Only the substitution of a oxygen in place of the C-terminal amide nitrogen distinguishes the two. The remainder of the glycopeptide precursor denoted by grey dashed line, was left unmodeled.

Fig. S2. Experimental protein synthetic strategy and sequence alignment. (A) Chart of sequence mutations from wild-type, synthetic methods and predicted binding properties. Diagram shows at each step in the gene synthetic process by which method mutations were introduced. White-filled circle and box indicate synthesis by gene assembly, beginning with the wild-type sequence at top. Solid boxes indicate mutagenesis by PCR. Italicized text indicates which residues were mutated at each stage. Bold text at line termini denote completed ROSETTA designed proteins. Table at right of diagram shows number of mutations at each synthetic step, approximate predicted energy of binding (in r.e.u.) and synthetic method used. (B) Sequence alignment of wild-type 1m4w (top, grey type) and the nine ROSETTA designed proteins designated 1m4w_1 through 1m4w_9. Mutations from wild-type are indicated by grey boxes and secondary structure is below.

Fig. S3. CD and binding assay plots for representative designed proteins. (A) CD spectra for the wild-type 1m4w, designed 1m4w_6 and re-designed 1m4w_6w20v48 proteins demonstrating similar tertiary structure composition. (B) FA binding assay plots for several of the designed mutants titrated with danslyated EKdAdA peptide. (C) FA plots for re-designed proteins titrated with danslyated EKdAdA peptide.

Fig. S4. Morphology of the 1m4w_6 crystals. This crystal was grown in sitting drop, 24-well plate in buffer containing 0.1 M NaCl, 1.125 M ammonium sulfate, 0.1 M Bis-Tris pH 5.5, 3% Jeffamine M600 pH 7.0 and grown at 20°C. Dimensions of the crystal were ~150μm by 450μm. All 1m4w_6 derivatives had similar crystal morphologies.

Table SI. ROSETTALIGAND pair-wise interface energies between protein and ligand residues for the predicted 1m4w_6 model and X-ray determined structure. Sequence numbers of interface residues at left. Column headings: *atr*=attractive; *rep*=repulsive; *sol*=desolvation penalty; *hb*=hydrogen bond; *cou*=coulombic. At bottom: sums and standard deviations for each column, multiplied by ROSETTA weightings, give total ligand interaction energy at bottom right.