

Darren A. Thompson, Ph.D. - Research Summary

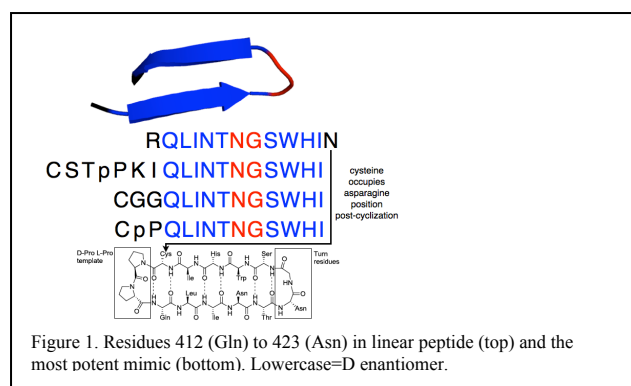
Synopsis: My experiences in both industry and academia are well suited for a staff scientist position. Going directly to industry following my undergraduate degree exposed me to the machinations of first-rate scientific leaders and their encouragement sent me back to graduate school. I have 38 published articles in a multitude of fields (e.g. Medicinal Chemistry, NMR and X-ray Crystal structures, EPR biophysics, Protein & Peptide Chemistry, etc.) and the sheer breadth of my bio-technological knowledge impresses even the most skeptical critics.

Post-Doctoral Research

The Scripps Research Institute
Department of Chemistry
Advisor: Philip E. Dawson

Hepatitis C Envelope Glycoprotein 2 nAb Epitope Peptidomimetics

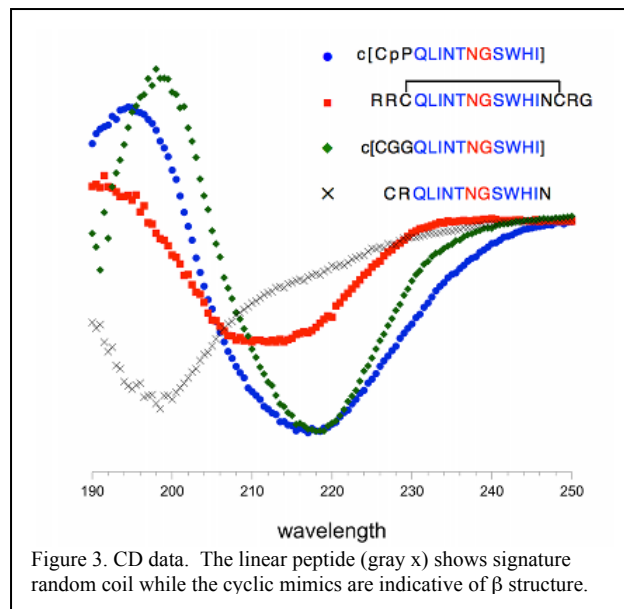
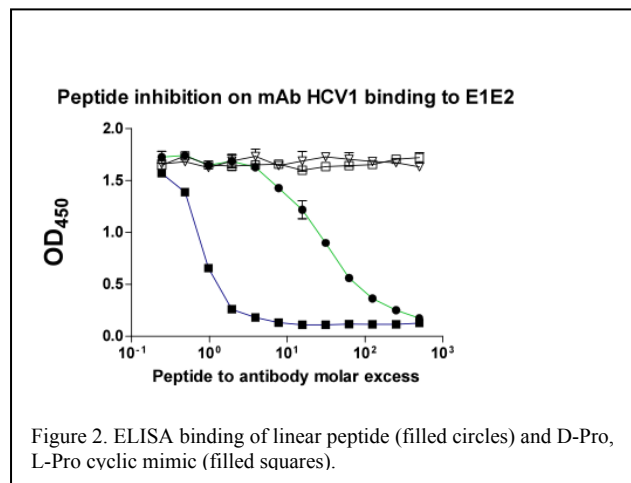
Antibodies capable of neutralizing HCV infection bind to a conserved linear epitope from the E2 envelope glycoprotein that has been shown to adopt a β hairpin conformation when bound to neutralizing antibodies. To translate this epitope into a vaccine candidate, it is necessary to make analogs



that are structured in solution. A series of circular peptides were designed and synthesized by an intramolecular native chemical ligation strategy, affording peptide mimics such as those shown in figure 1.

Inclusion of a β -turn promoting element (e.g. the D-Pro-L-Pro dipeptide) and head to tail cyclization of this peptide dramatically enhances affinity for the neutralizing antibody, (figure 2), consistent with the preorganization of the turn structure.

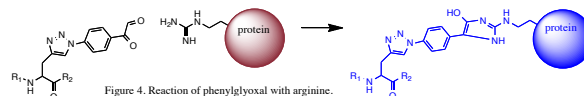
The induction of beta hairpin structure in the



analogous was confirmed by Circular Dichroism and NMR. CD spectra of the peptidomimetics in phosphate buffered water reveal the characteristic β sheet minimum at 217 nm which is absent in the linear peptide (gray) (figure 3). 2-D ROESY NMR studies of the mimic in isolation, as well as co-crystallization with the antibody FAB region, confirm the existence of the hairpin fold in the mimics.

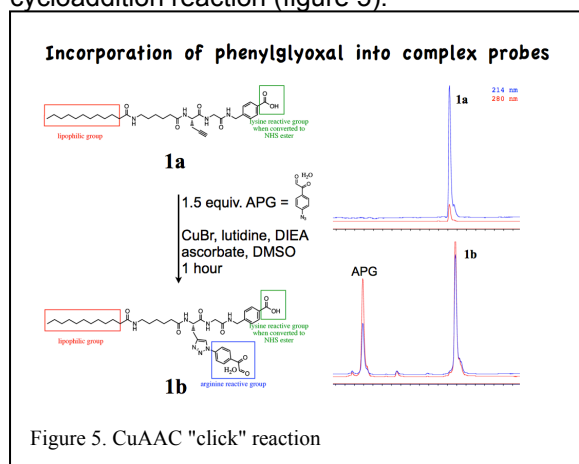
Arginine Bioconjugation

The guanidinium group of Arginine is highly abundant on the surface of proteins due to its positive charge (pKa \sim 12). As a result, a straightforward method for synthesizing reagents to selectively and efficiently modify Arg residues would be broadly applicable to proteins. Arg residues are known to react with dicarbonyl reagents. As early as 1916 chemist Otto Diels recognized vicinal dicarbonyl compounds (e.g. 2,3-butanedione) would selectively react with terminal amidines. Fifty years later the dicarbonyl reagent phenylglyoxal was shown to modify proteins at arginine residues (figure 4). However, these reactions simply modify the Arg residue and the introduction of other functionality (biotin, fluorophores, PEG, drugs) has been only narrowly explored. Since the hydroxyimidazole conjugation product is hydrolytically stable, conjugation strategies based on the phenylglyoxal group would be compatible with most medical and

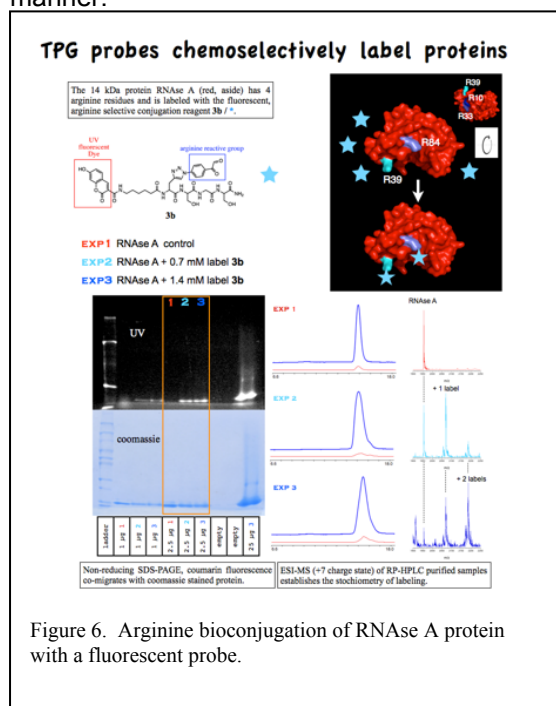


materials applications.

One reason for the lack of phenylglyoxal conjugation reagents may be that the synthesis of more complex arginine directed conjugates is hampered by the few commercially available bifunctional phenylglyoxal starting materials and sensitivity of the dicarbonyl group during synthetic manipulation. One readily available derivative, *para*-azidophenylglyoxal (APG), was designed as a photocrosslinking agent to establish tertiary structure of the ribosome. Here we have co-opted this reagent to participate in a copper catalyzed azide alkyne cycloaddition reaction (figure 5).



The high chemoselectivity of this click reaction allows the phenylglyoxal functional group to be introduced onto probe molecules in a straightforward manner.



The resulting new class of - triazolyl-phenylglyoxal conjugation reagents efficiently modify peptides and proteins at Arg residues and are compatible with a wide range of labels and reactive groups (figure 6).

Graduate Research

University of California at Santa Cruz

Department of Chemistry

Advisor: Glenn L. Millhauser

Peptoid Mimics of Agouti Related Protein

Studies implicate 7TM-GPCR Melanocortin 4 Receptor (MC4R) mutations in human morbid obesity, and knockouts of the MC4R in mice produce a hyperphagic, overweight phenotype. The endogenous antagonist of MC4R is Agouti Related Protein (AGRP). In AGRP, binding was eliminated by replacement of amino acids 111-114 (RFFN) with alanine and AGRP (87-132, R111A) fails to antagonize NDP-MSH (a potent α -Melanocortin Stimulating Hormone analogue) stimulated cAMP production at MC4R. The principal binding pharmacophore of AGRP is the RFF triplet. A cyclic peptide corresponding to AGRP (110-117), cyclo[CRFFNAFC], antagonizes MC4R, albeit with 1 - 2 logs less potency than protein. Interestingly, the linear analogue SRFFNAFS has no detectable MC4R affinity. These findings suggest that activity of the RFF triplet requires that its backbone take on a turn configuration.

Two peptoid-like scaffolds designed to

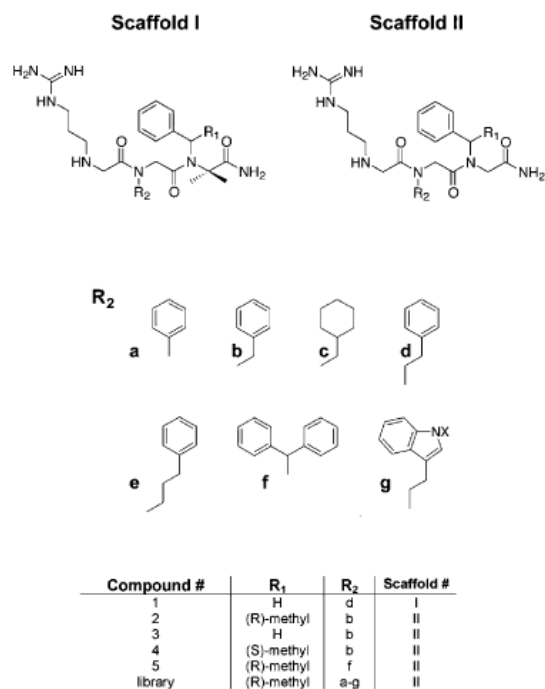


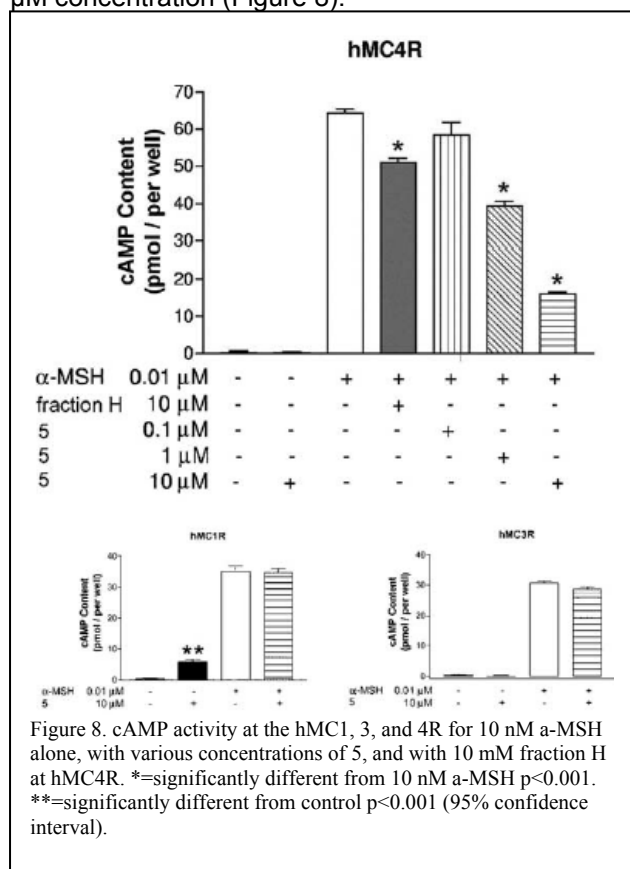
Figure 7. Two dimensional drawings of compounds used to mimic agouti related protein.

mimic the RFF triplet, each containing a steric restraint, were developed (figure 7).

One of these scaffolds exhibited antagonist function at MC4R. Using this scaffold, we then rationally designed a seven-member library and, within this library, identified an antagonist that displaced both agonist and antagonist at MC4R with micromolar

IC50 values. These findings enhance the understanding of structure activity relationships involved in MCR antagonism, and may yield insight into the molecular determinants of melanocortin receptor signaling and drugs aimed at treating disorders related to energy balance.

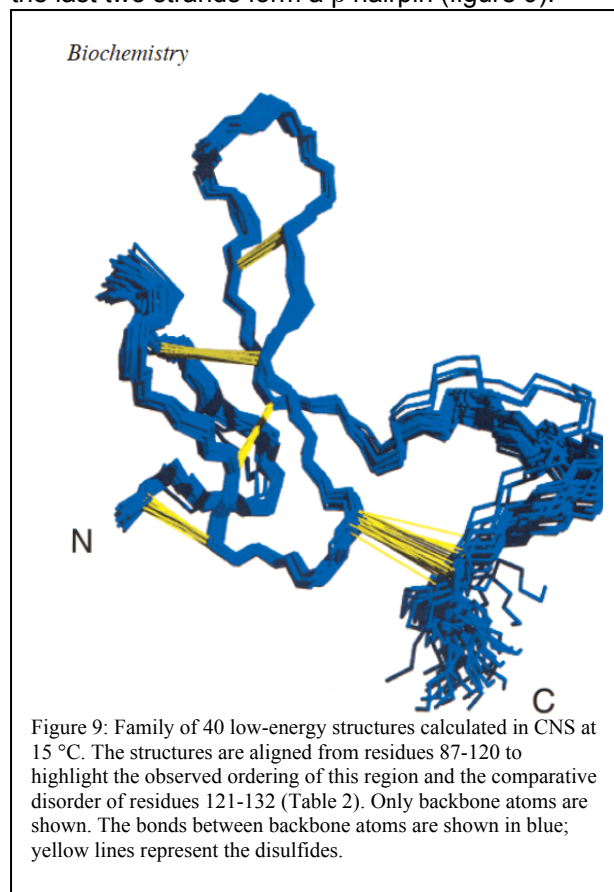
ESI-MS of library fraction H, displaying prominent MC4R binding, showed a major component of 558 g/mol consistent with the MH+ of compound 5. This molecule was synthesized separately, product confirmed by ESI-MS, and purified by HPLC. Although the peptoid showed no significant hMC4R cAMP inhibition at 100 nM, there is significant inhibition at 1.0 μM. In addition, 5 is an improved antagonist over fraction H at 10 μM. Partial agonist activity is seen with 10 μM peptoid at hMC1R. At high dosage 5 is also a partial agonist at hMC4R (data not shown). No significant antagonist activity is detected for 5 at hMC1R or hMC3R at 10 μM concentration (Figure 8).



High-Resolution NMR Structure of the Chemically-Synthesized Melanocortin Receptor Binding Domain AGRP(87-132) of the Agouti-Related Protein.

The agouti-related protein (AGRP) is an endogenous antagonist of the melanocortin receptors MC3R and MC4R found in the hypothalamus and exhibits potent orexigenic (appetite-stimulating) activity. The cysteine-rich C-terminal domain of this protein, corresponding to AGRP(87-132), contains five disulfide bonds and exhibits receptor binding affinity and antagonism equivalent to that of the full-length protein. The

three-dimensional structure of this domain has been determined by 1H NMR at 800 MHz. The first 34 residues of AGRP(87-132) are well-ordered and contain a three-stranded antiparallel β sheet, where the last two strands form a β hairpin (figure 9).



The relative spatial positioning of the disulfide cross-links demonstrates that the ordered region of AGRP(87-132) adopts the inhibitor cystine knot (ICK) fold previously identified for numerous invertebrate toxins. Interestingly, this may be the first example of a mammalian protein assigned to the ICK superfamily. The hairpin's turn region presents a triplet of residues (Arg-Phe-Phe) known to be essential for melanocortin receptor binding. The structure also suggests that AGRP possesses an additional melanocortin-receptor contact region within a loop formed by the first 16 residues of its C-terminal domain. This specific region shows little sequence homology to the corresponding region of the agouti protein, which is an MC1R antagonist involved in pigmentation. Consideration of these sequence differences, along with recent experiments on mutant and chimeric melanocortin receptors, allows us to postulate that this loop in the first 16 residues of its C-terminal domain confers AGRP's distinct selectivity for MC3R and MC4R.

Industrial Research

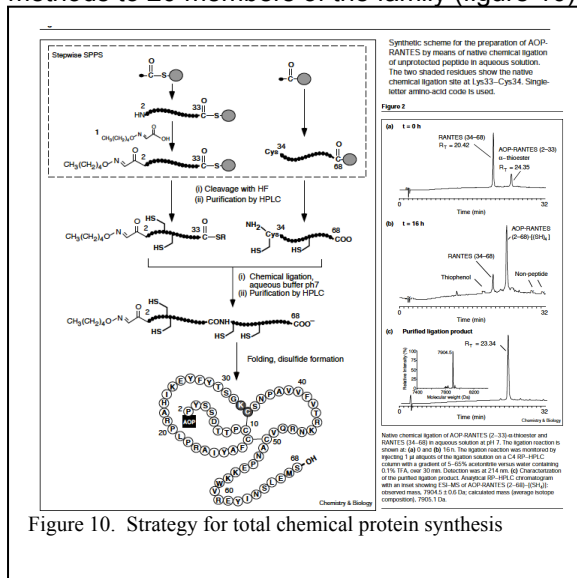
Gryphon Sciences

Department of Chemistry

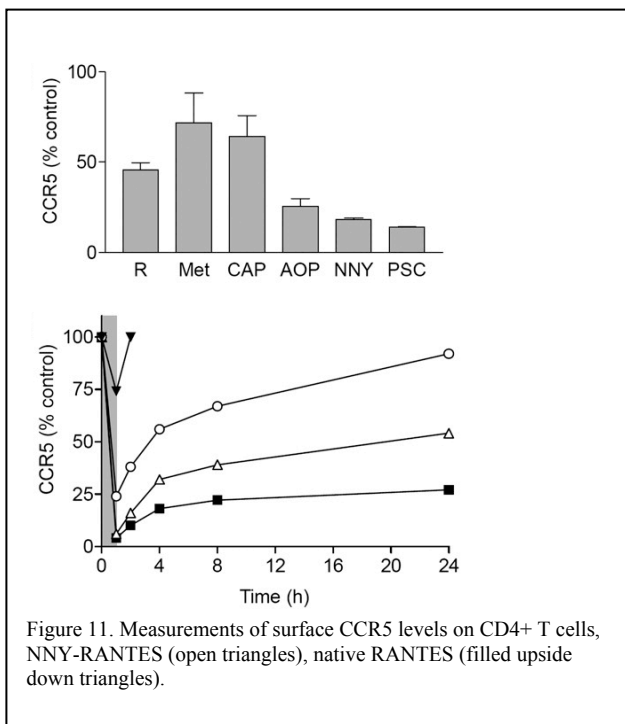
Direct Supervisor: Stephen B.H. Kent

Chemical Protein Synthesis

Gryphon Sciences was a biotech start-up dedicated to the total chemical synthesis of proteins, opening in 1995, and ultimately folding in 2004, the IP now belongs to Amylin Pharmaceuticals / BMS. My particular focus was a family of small (~70 amino acid) extracellular cytokines called chemokines. At Gryphon, I optimized the synthesis of this important emergent class of bio-molecules and applied the methods to 20 members of the family (figure 10).



Gryphon sciences aimed to apply medicinal chemistry approaches to the development of protein therapeutics. Through this process I generated an important lead compound, NNY-RANTES, in the race to find inhibitors of Env binding with CCR5, the



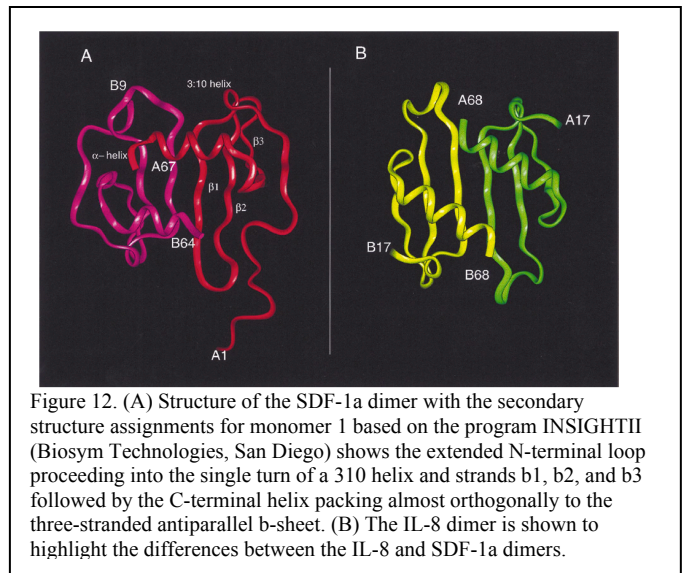
co-receptor for HIV fusion to T-cells. (figure 11) --

In addition the chemokines I designed and synthesized directly contributed to several key

biological discoveries including lymphocyte arrest in circulating blood, influence of fibronectin on chemotaxis gradients, and the crystal structure of stromal derived factor 1 alpha (SDF-1 α) (figure 12).

In just two weeks I could be presented with a DNA sequence and produce fully functional, folded protein in tens of milligram quantities.

I was included as co-author on a series of ~20 manuscripts describing these studies including a



science paper and three PNAS publications.