

# Crystal Structure of the Conserved Subdomain of Human Protein SRP54M at 2.1 Å Resolution: Evidence for the Mechanism of Signal Peptide Binding

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Protein SRP54 is an integral part of the mammalian signal recognition particle (SRP), a cytosolic ribonucleoprotein complex which associates with ribosomes and serves to recognize, bind, and transport proteins destined for the membrane or secretion. The methionine-rich M-domain of protein SRP54 (SRP54M) binds the SRP RNA and the signal peptide as the nascent protein emerges from the ribosome. A focal point of this critical cellular function is the detailed understanding of how different hydrophobic signal peptides are recognized efficiently and transported specifically, despite considerable variation in sequence. We have solved the crystal structure of a conserved functional subdomain of the human SRP54 protein (hSRP54m) at 2.1 Å resolution showing a predominantly alpha helical protein with a large fraction of the structure available for binding. RNA binding is predicted to occur in the vicinity of helices 4 to 6. The N-terminal helix extends significantly from the core of the structure into a large but constricted hydrophobic groove of an adjacent molecule, thus revealing molecular details of possible interactions between alpha helical signal peptides and human SRP54.

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## Introduction

Signal recognition particle (SRP) is a ribonucleoprotein complex which binds the signal peptide as it appears on the surface of translating ribosomes. This step is followed by the association of SRP with a receptor in the membrane of the endoplasmic reticulum and subsequent direction of the secretory protein to its proper cellular location (for review see, Walter & Johnson, 1994; Lütcke, 1995; Bovia & Strub, 1996; Strub & Bui, 1999). SRP-like particles have been identified in all organisms (Samuelsson & Zwieb, 1999) and typically are composed of one RNA molecule and six proteins. Despite some variation in the phylogenetic conservation of certain SRP components, the portion of

the SRP RNA which corresponds to helix 8 is always present (Larsen & Zwieb, 1991; Samuelsson & Zwieb, 1999). Another essential component of every SRP is protein SRP54, named ffh in bacteria. Human protein SRP54 is a polypeptide of 504 amino acid residues with three domains: the N-terminal domain, a GTP binding site in the center, and the methionine-rich M-domain (SRP54M) in the C-terminal region (Bernstein *et al.*, 1989; Gowda *et al.*, 1998; Römisch *et al.*, 1989). Comparative sequence analysis and site-directed mutagenesis studies provide evidence that SRP54M interacts with helix 8 of SRP RNA (Gowda *et al.*, 1997). Furthermore, chemical cross-linking experiments have implicated SRP54, and in particular SRP54M, in the recognition of signal peptides (Kurzchalia *et al.*, 1986; Lütcke *et al.*, 1992), thus giving the SRP54M domain a unique functional role in protein targeting.

For many years, the observation that numerous hydrophobic signal peptides are recognized efficiently and targeted specifically despite their considerable variation in sequence has been puzzling.

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Abbreviation used: SRP, signal recognition particle.

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Certain common properties of signal peptides have been determined (von Heijne, 1985), but the molecular details of how signal peptides are recognized by SRP have remained elusive. To approach an answer to this important biological question, we have crystallized a functional subdomain of the human SRP54 protein (hSRP54m) and have determined its structure at 2.1 Å resolution. The structure of the hSRP54m dimer provides clues for which region is likely to be in contact with RNA, and reveals a possibility for the interaction with a wide variety of alpha helical signal peptides. There are structural similarities but also significant differences between the human SRP54m and its bacterial homologue, the *Thermus aquaticus* ffh (Keenan *et al.*, 1998). In both structures interactions occur between neighboring molecules. However, in ffh, the groove proposed for signal peptide interaction is wide and short and binds a loop, whereas in hSRP54m the groove is deep and elongated and binds alpha helices.

## Results and Discussion

### Crystal structure of hSRP54m

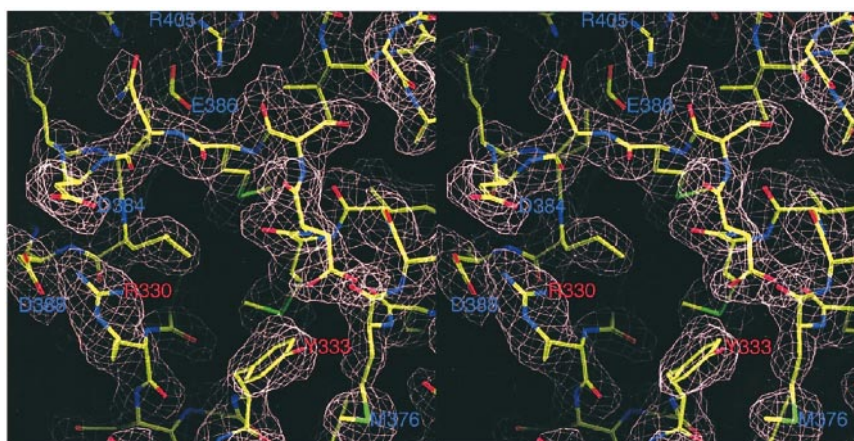
Because of its molten-globule state (Zheng & Gierasch, 1997) the M-domain of the mammalian SRP54 was considered to be difficult to crystallize (Keenan *et al.*, 1998). However, we recently expressed human SRP54m (120 residues;  $M_r = 13,584.8$ ), a functionally active polypeptide that corresponds to positions 322 to 441 of the human protein (Gowda *et al.*, 1998; Samuelsson & Zwieb, 1999), and have produced well-ordered crystals (Gowda *et al.*, 1999). The human SRP54m structure was solved at a resolution of 2.1 Å by multiwavelength anomalous diffraction (MAD) (Hendrickson, 1991) from crystals of the selenomethionine derivative. A section of the experimen-

tal electron density contoured at one standard deviation is shown in Figure 1, and details of the crystallographic analysis are presented in Table 1 and Materials and Methods.

The structure of the hSRP54m monomer consists of seven alpha helices, h1 to h7 (Figure 2). Helices 2 to 7 are arranged in a core with dimensions of 46 Å × 27 Å × 24 Å, while helix 1 extends 26 Å from the core. The structure of the core is stabilized by several hydrophobic residues which include the phylogenetically invariant methionine at position 382, but also hydrogen bonding networks and salt bridges which involve the relatively conserved Glu386, Arg402, and Arg405 (Figure 3). A highly structured loop of 17 amino acid residues (positions 349 to 365) is maintained between helix 2 and helix 3, which includes residues Met-Ile-Pro-Gly (positions 351 to 354) and a stacking interaction between Phe355 and Phe359. A distinct feature of the structure is the deep elongated groove which is formed with contributions of helices 2, 3, 4, including the connecting loops. In the hSRP54m crystal, this groove is occupied by helix 1 from a neighboring molecule (Figure 4), suggesting that the groove is the site of signal peptide recognition.

### Signal peptide recognition in the hSRP54m dimer

The possibility that the interaction of a neighboring helix 1 with the deep groove mimics signal peptide binding is suggested not only by the hydrophobic character of the groove, but also by its well-defined size and shape. The groove is wide at both ends, but more constricted near the center (Figures 4 and 5), thus having the potential to accommodate a variety of signal peptides which, in general, possess residues of lesser steric bulk near the center, exactly where the groove is constricted. Furthermore, the overall dimensions of the



**Figure 1.** Stereo view of the electron density map contoured at  $1\sigma$  with the final refined structure superimposed. The region shown corresponds to the N-terminal portion of the signal peptide-binding pocket. Residues are labeled blue for the A monomer and red for the B monomer. Indicated are the completely conserved salt bridge between Glu386 and Arg405 as well as the aspartate residues likely to be responsible for accepting the positively charged N terminus of the signal peptide. The Figure was created using the program O (Jones *et al.*, 1991).

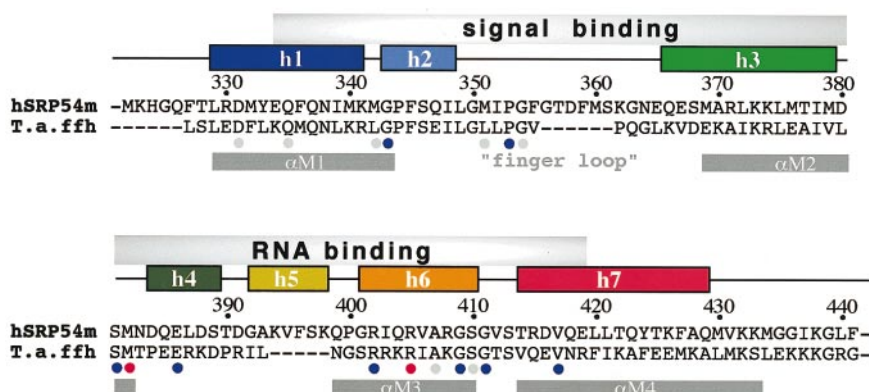
**Table 1.** Crystallographic statistics for the selenomethionine derivative of hSRP54m

A. Data collection statistics						
X-ray source <sup>a</sup>	Wavelength (Å)	Resolution (Å)	Number of reflections observed/unique	Data coverage (%) overall/last shell	$\langle I \rangle / \langle \sigma(I) \rangle$ overall/last shell	$R_{\text{sym}}$ (%) <sup>b</sup> overall/last shell
X	250.9796 ( $\lambda_1$ )	20 - 2.09	275,461/27,252	99.9/99.3	31.8/5.60	6.5/23.7
	0.9793 ( $\lambda_2$ )	20 - 2.09	276,180/27,381	99.9/99.4	31.6/5.76	5.5/22.0
	0.9300 ( $\lambda_3$ )	20 - 1.98	169,527/22,823	80.0/77.8	22.4/5.08	4.7/23.0
X12C	0.9300 ( $\lambda_3$ )	50 - 2.10	165,944/24,001	98.0/94.0	22.1/2.60	4.8/29.6
B. Phasing (solve)						
$R_{\text{cullis}}$ <sup>c</sup>	0.59	Phasing power <sup>d</sup>	Centric	1.02		
FOM	0.66		Acentric	1.37		
Map correlation coefficient <sup>e</sup>		0.7426				
C. Refinement (X-plor)						
Resolution range (Å)		50 - 2.1				
I/s cutoff		0.0				
Number of residues		215				
Number of water molecules		92				
Total number of non-hydrogen atoms		1792				
R-factor (%) <sup>f</sup>		24.3 (for 22,839 reflections)				
$R_{\text{free}}$ (%) <sup>g</sup>		31.4 (for 2529 reflections)				
r.m.s. deviation						
Bond length (Å)		0.008				
Bond angles (deg.)		1.065				
Average B value						
Overall		46.1				
Backbone		42.9				

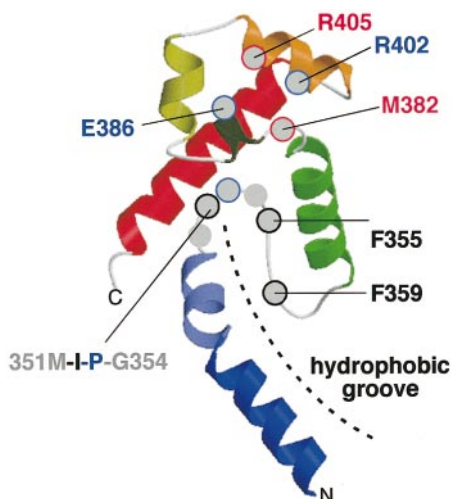
<sup>a</sup> All data sets were collected at Brookhaven National Laboratories at the National Synchrotron Light Source.  
<sup>b</sup>  $R_{\text{sym}} = \sum_i \sum_l |I_{ih} - \langle I_h \rangle| / \sum_i \sum_l \langle I_h \rangle$ , where  $\langle I_h \rangle$  is the mean of the observations  $I_{ih}$  of reflection  $h$ .  
<sup>c</sup>  $R_{\text{cullis}} = \sum |E| / \sum_j |F_1| - |F_{10}|$ , where  $E$  is the lack of closure.  
<sup>d</sup> Phasing power =  $\langle |F_H(\text{calc})| / |E| \rangle$ , where  $F_H(\text{calc})$  is the calculated anomalous difference and  $E$  is the lack of closure.  
<sup>e</sup> Map correlation coefficient was calculated between the calculated map for the final refined model and the experimental maps after solvent flattening using SOLOMON.  
<sup>f</sup>  $R = \sum (|F_p(\text{obs})| - |F_p(\text{calc})|) / \sum |F_p(\text{obs})|$ .  
<sup>g</sup>  $R_{\text{free}} = R$ -factor for a selected subset (10%) of the reflections that was not included in prior refinement calculations.

groove are consistent with the nature of archetypal cleaved signal sequences which are characterized by an N-terminal region with a weak net-

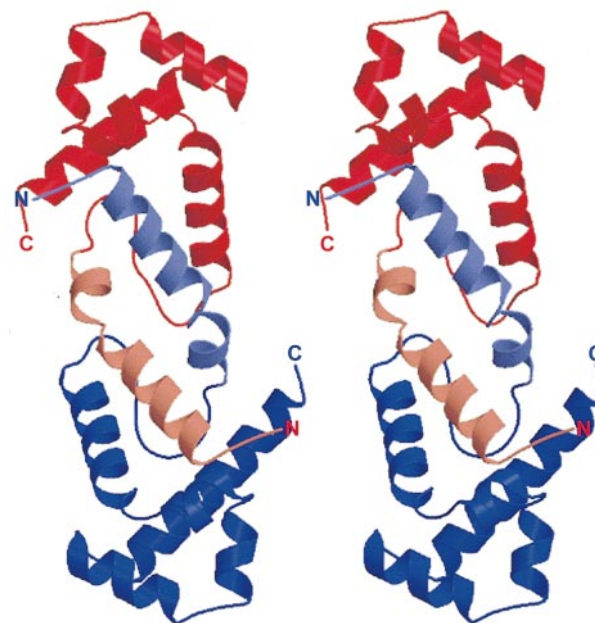
positive charge, a hydrophobic (h) region of approximately 12 residues, followed by five to seven residues with higher average polarity (see



**Figure 2.** Secondary structure of human SRP54m. Alpha helices (h1 to h7) as found in the crystal structure of the protein are indicated as colored rectangles above the sequence. Gray dots below the sequence indicate residues which are conserved to 80% or more in 37 representative aligned SRP54 sequences (Samuelsson & Zwieb, 1999), blue dots mark residues that are conserved to a degree of 90% or more, and red dots label invariant residues. Numbering is according to the sequence of the full-length human SRP54 protein (Gowda *et al.*, 1998). Gray rectangles below the aligned sequence of *T. aquaticus* fhh indicate helices and position of the finger loop which interacts with a neighboring molecule in the fhh crystal structure as determined by Keenan *et al.* (1998). The approximate regions that interact with the signal peptide or the SRP RNA are shown on top of each panel.



**Figure 3.** Ribbon view of the hSRP54m monomer. Helices and amino acid residues are color-coded as described in the legend to Figure 2. Indicated are the positions of the invariant M382 and R405, the conserved E386 and R402, as well as several residues in the loop between helices 2 and 3. F355 and F359 are in a stacked configuration. The broken line indicates the location of the large hydrophobic groove formed by the loop and the flanking helices. The Figure was created using the program MOLSCRIPT (Kraulis, 1991).



**Figure 4.** Ribbon stereo view of the hSRP54m dimer as found in the crystal. The monomers are colored red for A and blue for B, respectively. The N and C termini are labelled as such. The Figure was created using the program MOLSCRIPT (Kraulis, 1991).

Figure 6(a)). According to this general signal peptide design, helix 1 of hSRP54m appears to be equivalent to the h-region of the signal peptide (13 residues from Leu329 to Lys341), whereas helix 2 is located at the opposing side of the groove constriction.

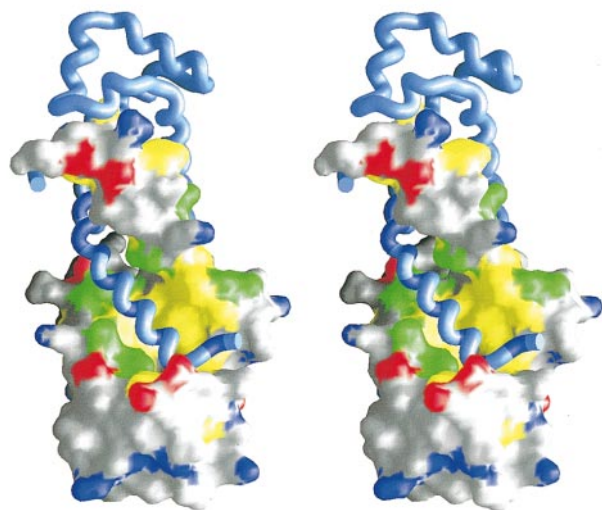
Figure 7 indicates that the overall electrostatic potential of the signal peptide-binding groove is negative, thereby suggesting a gross mechanism of signal peptide binding by simple field effects. We hypothesize that upon capture of the signal sequence, strong hydrophobic interactions would occur along the entire length of the signal peptide. In keeping with the described design, a relatively precise longitudinal positioning of the signal peptide would be controlled by the constriction of the groove and by introducing a slight bend (Figure 6(b)). Interestingly, the kinked shape of signal peptides was proposed to be a factor in their recognition by SRP (Matoba & Ogrydziak, 1998). In our model of signal peptide recognition, the alpha-helical signal peptide is expected to be oriented in the same N-to-C-direction as helix 1, because the position of the acidic residues Asp384 and Asp388 of the neighboring molecule would form favorable interactions with the positively charged N terminus of the protein as it emerges from the ribosome (Figure 5). It should be noted that helix 1 consists of 13 residues which is near the maximum size of the h-region ( $10(\pm 3)$ , Figure 6(a)). Thus, smaller signal peptides could be accommodated in the same groove, although

structural details of the interaction may be somewhat different.

It is important to note that helix 1 of hSRP54m is amphipathic in character, whereas signal peptides are hydrophobic with little or no moment. In the hSRP54m monomer the surface of the hydrophobic groove could be exposed to solvent, or it might bind the hydrophobic signal peptide, or it might interact with a differently oriented helix 1 from the same molecule (discussed below). In all cases, as we have crystallized only a portion of SRP54, hydrophobicity could be contributed by other portions of SRP54 (Newitt & Bernstein, 1997) or additional proteins of the large SRP domain.

#### Consideration of a hypothetical hSRP54m structure

As discussed above, helix 1 of a neighboring molecule of hSRP54m is inserted into the hydrophobic groove. This interaction might be expected to occur in the hSRP54m dimer to protect the hydrophobic pocket that would otherwise be exposed, and it is possible that binding to the signal sequence involves a switch in which a protecting helix is displaced by an authentic signal peptide. Sedimentation velocity centrifugation and gel filtration demonstrate that a substantial amount of hSRP54m dimers are present in solution (not shown). Thus, the formation of the dimer is not crystallographically induced. Nevertheless, we cannot exclude the possibility that the dimer

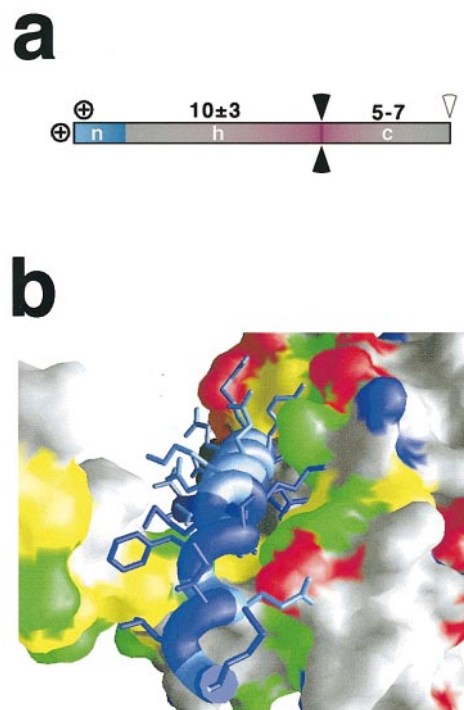


**Figure 5.** Stereo view of the interactions in the signal peptide binding pocket with the dimer arranged as shown in Figure 4. The A monomer is shown in a tubular representation in which the hydrophobic side-chains are colored dark blue and other side-chains are shown in light blue. The B monomer is shown in a surface representation in which the methionine residues are shown green; other hydrophobic residues, Ala, Leu, Ile, Phe, Pro, Tyr, and Val, are colored yellow; acidic and basic amino acid residues are indicated in red and blue, respectively.

represents an artificial structure that arises as a result of constructing this particular truncation of SRP54. A more compact hSRP54m monomer in which helix 1 folds back on its own molecule could be formed by swapping helices 1 and 2 of the A-monomer with the same two helices of the B-monomer (Figure 4). However, the formation of this hypothetical version of hSRP54m would require a substantial rearrangement of the hydrophobic groove. The loop between helices 2 and 3 would have to move significantly, yet this loop is a highly structured part of the groove. Furthermore, several conserved loop residues and the stacking of Phe355 onto Phe359 (Figure 3) demonstrate that the three-dimensional structure of the loop is well defined, and indicates that the experimentally observed conformation of the groove is preferred. In summary, regardless of whether the particular dimer is an artificial structure or not, the arguments provided above support the view that the interaction between helix 1 and the hydrophobic pocket provides a valid model for signal peptide interaction.

### SRP RNA binding site

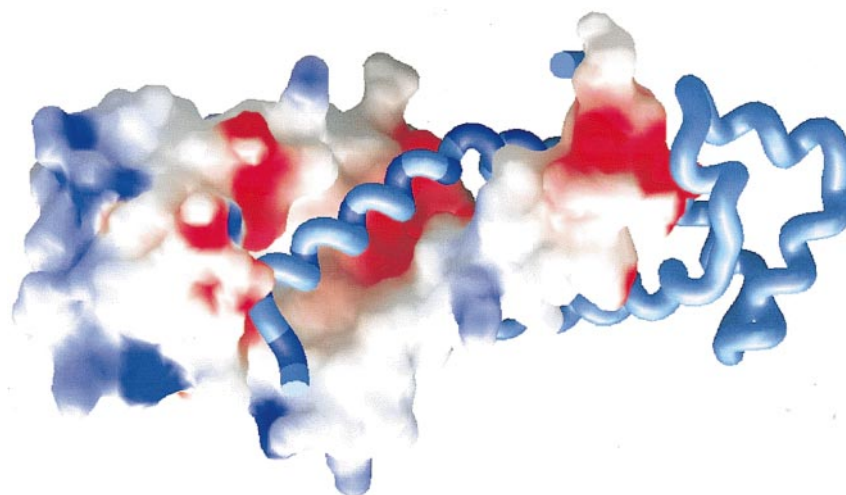
The electrostatic surface potential of hSRP54m suggests that the RNA-binding activity involves residues from helices 5, 6, and, in part, helices 4 and 7 (Figure 7). This proposal is supported by the



**Figure 6.** (a) Schematic view of an archetypical signal peptide (von Heijne, 1985). The net-positive charges at the N terminus (n-region, blue) are indicated, as are the number of residues typically found in the h and c-regions. The open arrow head indicates the site of signal peptide cleavage; the filled arrow heads mark the center of a region of less steric bulk. (b) View of the signal peptide binding site in the crystal structure of the hSRP54 m dimer seen approximately along the long axis from helix 2 to helix 1. The coloring scheme is as described in the legend to Figure 5, with the side-chains of the N-terminal helix 1 drawn in. (b) Created using the program GRASP (Nicholls *et al.*, 1991).

analysis of deletions which abolish the interaction with SRP RNA (Gowda *et al.*, 1998) as well as systematic site-directed mutagenesis experiments in which certain individual residues in this region cause the loss of the RNA binding activity (data not shown). Furthermore, the arginine-rich helix-turn-helix motif formed by helices  $\alpha$ M3 and  $\alpha$ M4 proposed to bind RNA (Keenan *et al.*, 1998) is also present in the human structure between h6 and h7 (Figure 2). The importance of the structural integrity of the core for RNA binding is supported by biochemical studies of the *ffh* from *Bacillus subtilis* in which a larger segment, corresponding to residues 351 to 452 of human SRP54, bound to RNA, but a smaller peptide equivalent to residues at 391 to 420 was inactive (Kurita *et al.*, 1996).

Surprisingly, the hSRP54m crystal structure shows that several conserved basic residues such as Arg402 and Arg405, are required for maintaining a portion of the core of the folded molecule and are not available for direct contact with the RNA (Figure 8). It is possible that conformational



**Figure 7.** Electrostatic surface potential in the hSRP54m dimer. The A monomer is shown in a tubular representation with the hydrophobic side-chains colored dark blue and other residues shown in light-blue. Negative charge, as calculated by GRASP (Nicholls *et al.*, 1991) for the B monomer, is shown by red and positive charge is shown by blue shading, respectively.

changes in SRP54 as mediated by GTP or SRP RNA regulate the release of signal sequences (Miller *et al.*, 1993) and expose the conserved positively charged residues. In the structure of hSRP54m, this idea is enforced by the tight spatial linkage of the RNA-binding site with the site of signal peptide recognition (Figures 2 and 3).

#### Comparison of human SRP54m with *T. aquaticus* ffh

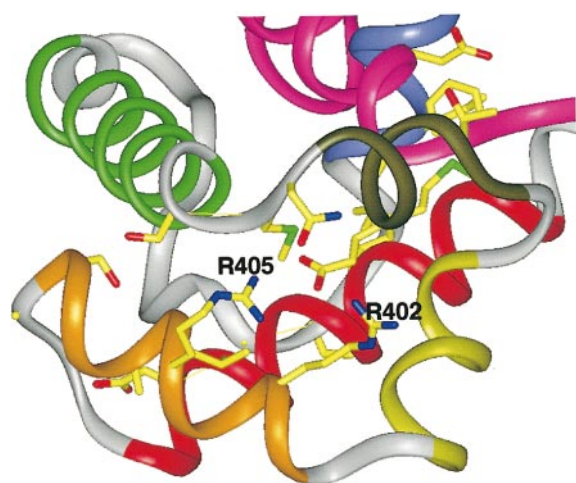
As would be expected from the degree of sequence homology among the members of the SRP54 family of proteins (Samuelsson & Zwieb, 1999), the structure of the human SRP54m is similar but also considerably different from the recently obtained 3.2 Å crystal structure of *T. aquaticus* ffh

(Keenan *et al.*, 1998). Both structures contain a hydrophobic pocket proposed to be the site of signal peptide binding. However, in the ffh crystal structure, a very large groove is occupied by a hydrophobic loop from a neighboring molecule (Keenan *et al.*, 1998), whereas in hSRP54m a deep elongated groove binds to a helix which provides a model for alpha-helical signal peptide binding. Significant differences between the two structures include, as indicated above, the extended orientation of helix 1, the addition of helix 2 which precedes the large internal loop, the highly structured conformation of this loop, and the presence of two additional helices (helices 4 and 5) in the C-terminal region of the molecule.

It appears that the region of the *T. aquaticus* ffh "finger loop" (Keenan *et al.*, 1998) would be in part occupied by helix 2 in the human hSRP54m (compared in Figure 2). If this portion of the bacterial structure is indeed alpha helical, the loop would be reduced by six amino acid residues when compared to the equivalent loop of hSRP54m. If, on the other hand, helix 2 is absent in ffh, the sizes of the human and bacterial loop would be comparable, although their conformations would still be very different.

Helices 4 and 5 are situated in the suggested RNA-binding region and are clearly defined in the human hSRP54m. At the relatively low resolution, these helices might be invisible in the *T. aquaticus* structure or could in fact be absent. However, regardless of this consideration, at least one of the two helices (h4 or h5) is likely to be absent in ffh, because this region contains an alignment gap (see Figure 2) in the sequences of the bacteria and lower eukaryotes (Samuelsson & Zwieb, 1999).

The three-dimensional structure of the human hSRP54m dimer supports results from biophysical studies which indicate that signal peptides are alpha helical in non-polar surroundings (Gierasch, 1989). In the *T. aquaticus* ffh structure, a flexible finger loop from a neighboring molecule inserts



**Figure 8.** Representation of the putative SRP RNA binding interface. Helices are colored as shown in Figures 2 and 3. The Figure shows the side-chains of the conserved residues, including the labelled R402 and R405.

into a wide groove proposed to accommodate the signal peptide, but no specific predictions about the extended or alpha-helical conformation of the signal peptide were made (Keenan *et al.*, 1998). Furthermore, in the crystal structure of the ffh trimer, the region which corresponds to helix 1 of the human SRP54m is not available to mimic the signal peptide. Finally, the bacterial signal peptide binding groove is wide and short and formed by another finger loop, whereas in hSRP54m the groove is deep and elongated to bind alpha helices 1 and 2. Future studies will be required to address the question if the helical arrangement in the human hSRP54m dimer and the finger loop of the bacterial ffh trimer are representations of the signal peptide binding mechanism at different stages of the interaction, or if these differences reflect distinct mechanisms of signal peptide recognition between eukaryotes and bacteria.

### Significance of the abundant methionine residues

In the so-called methionine bristle hypothesis, recognition of signal peptides was proposed to occur through the side-chains of abundant methionine residues which protrude from the hydrophobic faces of several amphipathic helices thus forming a flexible signal peptide binding pocket (Bernstein *et al.*, 1989). More recently, comparative sequence analysis and high-stringency secondary structure prediction of SRP54 indicated that methionine residues cluster near helix caps or within loops but not within helices (Gowda *et al.*, 1998). This assertion was supported by the structure of *T. aquaticus* ffh (Keenan *et al.*, 1998) where only three of the 21 hydrophobic groove residues are methionine residues. Although methionine residues contribute to the hydrophobic character of the groove in the human SRP54m structure (labelled green in Figure 5), four (Met342, Met351, Met360, and Met382) of the nine methionine residues do not protrude from helical regions but are located within loops. Two other methionine residues (Met340 in helix 1, and Met379 in helix 3) are very close to helix termini (Figure 2). Furthermore, in the three-dimensional structure of hSRP54m Met379 is close to the invariant Met382 as part of the hydrophobic core structure of the protein. This leaves only three methionine residues (Met332, Met369, and Met376) located within helical regions, but these residues are present only in a subset of eukaryotic sequences (Samuelsson & Zwieb, 1999) and are surrounded by low methionine scores (Gowda *et al.*, 1998), thus providing little support for the idea that these methionine residues have a particular function in signal peptide recognition. In keeping with the arguments presented above, a general role for methionine side-chains in signal peptide recognition appears to be unsupported. Instead, our model relies on the overall hydrophobic character and shape of a wide but constricted groove (described above), and this

groove is created by a variety of amino acid residues.

### Phylogenetic differences in signal peptide recognition

Signal sequences are characterized by a hydrophobic h-region of approximately 12 residues (Figure 6(a)) and are functionally exchangeable between distant phylogenetic groups. Recently, however, differences in the efficiency of protein targeting between remote phylogenetic groups have been emphasized (Zheng & Gierasch, 1996). For example, mammalian cells were unable to process the signal of the *Saccharomyces cerevisiae* vacuolar proteinase Y (CPY), but a change of a single glycine residue to leucine in the h-region of CPY resulted in enhanced function (Bird *et al.*, 1990). If, as we suggest, the hSRP54m dimers provide a model for the interaction of SRP54 with signal peptides, it may be possible to predict species-specific discriminatory properties of signal peptides based on the crystal structure. As an example, comparative sequence analysis demonstrates that groove residue at position 376 is a methionine residue only in the higher eukaryotes (Figure 2; Samuelsson & Zwieb, 1999) and might be used to distinguish signals of higher eukaryotes from yeast or bacterial signal peptides. We anticipate that knowledge of the atomic coordinates of hSRP54m will help in the future to carry out rational site-directed mutagenesis experiments aimed to improve the efficiency of protein secretion in numerous heterologous recombinant systems.

### Conclusion

The high-resolution structure of the human SRP54m provides a detailed view of the most conserved portion of the human SRP. The structure of the hSRP54m dimer indicates how SRP54 recognizes signal peptides, which region is in contact with SRP RNA, and demonstrates a close spatial link between the two functions. For the first time, the molecular interactions at the homodimer interface provide an elegant model for the mechanism by which alpha-helical signal peptides bind to SRP54 at atomic resolution. A similarly shaped constricted hydrophobic groove is likely to bind secretory signal peptides in all organisms, although it is unclear if there are alternate methods for the interaction with signal peptides. Clearly, the model does not explain details of the dynamic aspects of SRP-mediated signal peptide recognition which might involve SRP RNA and other components of SRP and the ribosome. Nevertheless, the structure paves the way for a more precise molecular understanding of signal peptide recognition. Furthermore, the structure of the SRP54m dimer sheds light on a potential mechanism for species-specific differences in protein targeting and secretion.

## Materials and Methods

### Protein purification

The gene for hSRP54m (Gowda *et al.*, 1999) was introduced into the T7-based expression vector pET-29 and overexpressed in the methionine deficient strain of *Escherichia coli* B834(DE3). Cells were grown in a selenomethionine-containing medium as described (Ramakrishnan *et al.*, 1993). The cells were induced at an  $A_{600}$  of 0.6 with 0.4 mM isopropyl- $\beta$ -D-thiogalactoside and were harvested three hours after induction. Human SRP54 m protein was purified by ion-exchange, hydroxylapatite, and gel-filtration chromatography using procedures similar to those described (Clemons *et al.*, 1998). For crystallization, the selenomethionine modified protein was concentrated to 25 mg/ml in 0.15 M NaCl, 50 mM Tris-HCl (pH 8.0).

### Crystallization

Crystallization was carried out at 4 °C using the sitting drop method by mixing 10 ml of protein with 10 ml of well solution consisting of 25% (w/v) PEG 2000 MME (monomethyl-ether), 0.2 M ammonium sulfate, and 0.1 M sodium acetate (pH 4.6) (Gowda *et al.*, 1999). Crystals were equilibrated with the well solution containing 15% (v/v) glycerol (a cryoprotectant) in a stepwise fashion. The crystals were in the orthorhombic space group  $P2_12_12_1$  with cell dimensions  $a = 29.0 \text{ \AA}$ ,  $b = 61.5 \text{ \AA}$ ,  $c = 129.5 \text{ \AA}$ ,  $\alpha\beta\gamma = 90^\circ$ .

### Data collection

All data were collected in images corresponding to one degree rotations from a single, flash-cooled selenomethionyl crystal in a cryostream at 100 K. We used the wiggler beamline X25 at the NSLS (Brookhaven National Laboratory) at wavelengths corresponding to the inflection point, the "white line", and a remote point of the K-edge of selenium. A fourth data set, obtained at the same remote wavelength, was collected at beamline X12-C at NSLS. All images were integrated and scaled using the programs DENZO and SCALEPACK (Otwinowski & Minor, 1997).

### Phase determination and refinement

Automatic determination of the selenium sites and subsequent MAD phasing was carried out using the three wavelengths collected at X25 (see Table 1) and the program SOLVE (Terwilliger, 1997) available at <http://www.solve.lanl.gov>. The program identified 13 of the 22 possible selenium sites, and the resulting map was solvent flattened using the program SOLOMON (Abrahams & Leslie, 1996) assuming a solvent content of 55%. The resulting map was of excellent quality and the protein model was built into this map using the program O (Jones *et al.*, 1991). Refinement was carried out with the data set collected on X12-C, corresponding to a wavelength of 0.9300 ( $\lambda_3$ ), from 50 to 2.1 Å using the X-PLOR program (Brünger, 1988). The free  $R$ -factor (Brünger, 1992) was used to guide refinement and all measured reflections were used.

### Protein Data Bank accession number

The hSRP54m coordinates have been deposited in the Brookhaven Protein Data Bank under the accession code 1QB2 and are available immediately at the signal recognition particle database (URL: <http://psyche.uthct.edu/dbs/SRPDB/SRPDB.html>).

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